# Deletion of FNDC5/Irisin modifies murine<br>
2 Osteocyte function in a sex-specific manner<br>
Anika Shimonty<sup>1</sup>, Fabrizio Pin<sup>2</sup>, Matt Prideaux<sup>2</sup>, Gang Peng<sup>3</sup>, Joshua R Huot<sup>2</sup>, Hyeonw<br>
4 Clifford J Rosen<sup>5</sup>, Bruce M Spiegelm

# 2 **OSteocyte function in a sex-specific manner**<br><sup>3</sup> Anika Shimonty<sup>1</sup>, Fabrizio Pin<sup>2</sup>, Matt Prideaux<sup>2</sup>, Gang Peng<sup>3</sup>, Joshua R Hu<br><sup>4</sup> Clifford J Rosen<sup>5</sup>, Bruce M Spiegelman<sup>6</sup>, Lynda F Bonewald<sup>7\*</sup><br><sup>1</sup>Indiana Center for Anika Shimonty<sup>1</sup>, Fabrizio Pin<sup>2</sup>, Matt Prideaux<sup>2</sup>, Gang Peng<sup>3</sup>, Joshua R Huot<sup>2</sup>, Hyeonwoo Kim<sup>4</sup>, 2.11 Anika Shimonty , Fabrizio Fili , Matt Fridedix , Galig Felig , Sosida K Haot , Hyeonwoo Kili ,<br>3  $\cdot$  Clifford J Rosen<sup>5</sup>, Bruce M Spiegelman<sup>6</sup>, Lynda F Bonewald<sup>7\*</sup><br><sup>3</sup> <sup>1</sup>Indiana Center for Musculoskeletal Health, Clifford J Rosen<sup>5</sup>, Bruce M Spiegelman<sup>6</sup>, Lynda F Bonewald<sup>7\*</sup> 5<br>6<br>7<br>8 <sup>1</sup>Indiana Center for Musculoskeletal Health, School of Medicine, Indiana University, IN, 46202, Indianapolis.<br>
19 Indiana Center for Musculoskeletal Health, Department of Anas<br>
19 School of Medicine, Indiana University, IN, 46202, Indianapolis.<br>
19 <sup>3</sup>Indiana Center for Muscul <sup>2</sup>Indiana Center for Musculoskeletal Health, D.<br>
<sup>2</sup>Indiana Center for Musculoskeletal Health, D.<br>
<sup>3</sup>Indiana Center for Musculoskeletal Health, D.<br>
<sup>3</sup>Indiana Center for Musculoskeletal Health, D.<br>
and Molecular Genetics <sup>2</sup>Indiana Center for Musculoskeletal Health, Department of Anatomy, School of Medicine, Indiana University, IN, 46202, Indianapolis.<br>
<sup>3</sup>Indiana Center for Musculoskeletal Health, Department of Medicine<br>
and Molecular Genetics, School of Medicine, Indiana University, IN, 46202,<br>
<sup>3</sup>Indiana <sup>3</sup>Indiana Center for Musculoskeletal Health, Departmer<br>
and Molecular Genetics, School of Medicine, Indiana Univers<br>
1<br>
<sup>3</sup> Maine Medical Center Research Institute, ME, 04074,<br>
<sup>5</sup> Maine Medical Center Research Institute, <sup>3</sup>Indiana Center for Musculoskeletal Health, Department of Medicine and Molecular Genetics, School of Medicine, Indiana University, IN, 46202,<br>
1 Indianapolis.<br>
<sup>9</sup> Indianapoli 11 Indianapolis.<br>
<sup>4</sup>Department of Biological Sciences, Korea Advanced Institute of<br>
<sup>5</sup>Naine Medical Center Research Institute, ME, 04074, Scarborough,<br>
<sup>5</sup>Naine Medical Center Research Institute, ME, 04074, Scarborough,<br> 12 <sup>4</sup>Depart<br>
<sup>4</sup>Depart<br>
<sup>4</sup>Depart<br>
<sup>5</sup> Maine<br>
<sup>5</sup> Maine<br>
<sup>5</sup> Maine<br>
<sup>5</sup> Maine<br>
<sup>5</sup> Maine<br>
<sup>5</sup> Maine<br>
<sup>7</sup> Department<br>
<sup>17</sup> Department<br>
<sup>7</sup> Department<br>
<sup>7</sup> Department<br>
<sup>7</sup> Department<br>
<sup>7</sup> Department<br>
<sup>7</sup> Department<br>
<sup>7</sup> Dep <sup>4</sup>Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, South Korea.<br>
<sup>5</sup> Maine Medical Center Research Institute, ME, 04074, Scarborough,<br>
<sup>5</sup> Maine Medical Center Research Institute, ME, 04074, Scarborough,<br>
<sup>5</sup> Department of Cancer Biology, D Frame Pauli et and Technology, Pergering Science and SMaine Medical Center Research Institute<br>15 SA.<br>
6 Separtment of Cancer Biology, Dana<br>
17 Department of Cell Biology, Harvard University<br>
8 Boston, USA.<br>
7 Tepartment of Maine Medical Center Research Institute, ME, 04074, Scarborough,<br>15<br>16 <sup>6</sup>Department of Cancer Biology, Dana Farber Cancer Institute and<br>17 Department of Cell Biology, Harvard University Medical School, MA, 02115,<br>18 <sup>7</sup>De 16<br>
17 Depa<br>
18 Bosto<br>
19<br>
20 Surge<br>
21 Indian<br>
22 Indian<br>
23<br>
24<br>
25 <sup>6</sup>Department of Cancer Biology, Dana Farber Cancer Institute and 17 Department of Cell Biology, Harvard University Medical School, MA, 02115,<br>
16 Boston, USA.<br>
<sup>7</sup>Department of Anatomy, Cell Biology and Physiology, Orthopaedic<br>
<sup>7</sup>Department of Anatomy, Cell Biology and Physiology, Orth 18 Boston, USA.<br>
<sup>7</sup>Department of Anatomy, Cell Biology and Physiology, Orthopaedic<br>
<sup>7</sup>Department of Anatomy, Cell Biology and Physiology, Orthopaedic<br>
Surgery, School of Medicine, Indiana Center for Musculoskeletal Healt The Terms, The Terms, The Terms, Terms<br>
20 Surgery, School<br>
21 Indiana Center<br>
22 Indianapolis.<br>
23<br>
24 \*Forcorr<br>
25 Ifbonews<br>
26<br>
27 <sup>7</sup>Department of Anatomy, Cell Biology and Physiology, Orthopaedic Surgery, School of Medicine, Indiana Center for Musculoskeletal Health,<br>
19 Department of Musculoskeletal Health, Indiana University, IN, 46202,<br>
19 Department of Musculoskeletal Health, Indiana University, IN, 46202,<br>
19 21 Surgery, School of Musculoskeletal Health, Indiana University, IN, 46202,<br>
22 Indianapolis.<br>
23 Torcorrespondence:<br>
25 Toppeval@iu.edu(LB)<br>
26 Toppeval@iu.edu(LB)<br>
27 Toppeval. 22 Indiana polis.<br>23<br>24 \* Forcorrespondence:<br>25 for Musculoskeletal Health, Indiana University, Indiana University, Indiana University, IN, 46202, IN, 47<br>27<br>27 22 Indianapolis. 24<br>25<br>26<br>27 24 \*Forcorrespondence:<br>25 Honewal@iu.edu(LB)<br>26 27 25 let the control con  $\frac{27}{1}$

2<br>|
| numerol<br>| differend<br>| without bus tissues but its effects on bone are unclear. We found significant sex and genotype<br>
reces in bone from wildtype (WT) mice compared to mice lacking *Fndc5* (KO), with and<br>
t calcium deficiency. Despite their bone being differences in bone from wildtype (WT) mice compared to mice lacking *Fndc5* (KO), with and<br>without calcium deficiency. Despite their bone being indistinguishable from WT females, KO<br>female mice were partially protected fr anticulated in bone from wildtype (WT) mice compared to mice lacking Fndc5 (KO), with and<br>without calcium deficiency. Despite their bone being indistinguishable from WT females, KO<br>female mice were partially protected from when the male mice were partially protected from osteocytic osteolysis and osteoclastic bone<br>resorption when allowed to lactate or when placed on a low-calcium diet. Male KO mice have<br>more but weaker bone compared to WT ma Fesorption when allowed to lactate or when placed on a low-calcium diet. Male KO mice have<br>more but weaker bone compared to WT males, and when challenged with a low-calcium diet<br>lost more bone than WT males. To begin to un resorption weaker bone compared to WT males, and when challenged with a low-calcium diet<br>lost more bone than WT males. To begin to understand responsible molecular mechanisms,<br>osteocyte transcriptomics was performed. Osteo lost more bone than WT males. To begin to understand responsible molecular mechanisms, osteocyte transcriptomics was performed. Osteocytes from WT females had greater expression of genes associated with osteocytic osteolys osteocyte transcriptomics was performed. Osteocytes from WT females had greater expression<br>of genes associated with osteocytic osteolysis and osteoclastic bone resorption compared to<br>WT males which had greater expression o of genes associated with osteocytic osteolysis and osteoclastic bone resorption compared to<br>WT males which had greater expression of genes associated with steroid and fatty acid<br>metabolism. Few differences were observed be of males which had greater expression of genes associated with steroid and fatty acid metabolism. Few differences were observed between female KO and WT osteocytes, but with a low calcium diet, the KO females had lower exp metabolism. Few differences were observed between female KO and WT osteocytes, but with a<br>low calcium diet, the KO females had lower expression of genes responsible for osteocytic<br>osteolysis and osteoclastic resorption tha metabolism. Few differences were observed by calcium diet, the KO females had lower expression of genes responsible for osteocytic osteolysis and osteoclastic resorption than the WT females. Male KO osteocytes had lower ex osteolysis and osteoclastic resorption than the WT females. Male KO osteocytes had lower<br>expression of genes associated with steroid and fatty acid metabolism, but higher expression of<br>genes associated with bone resorption expression of genes associated with steroid and fatty acid metabolism, but higher expression of genes associated with bone resorption compared to male WT. In conclusion, irisin plays a critical role in the development of t expressessociated with bone resorption compared to male WT. In conclusion, irisin plays a critical<br>role in the development of the male but not the female skeleton and protects male but not<br>female bone from calcium deficien role in the development of the male but not the female skeleton and protects male but not<br>female bone from calcium deficiency. We propose irisin ensures the survival of offspring by<br>targeting the osteocyte to provide calci From the development of the male but not the male but not the protect material but not the female bone from calcium deficiency. We propose irisin ensures the survival of offspring by targeting the osteocyte to provide calc female bone from calcium deficiency. We propose irisin ensures the survival of offspring by text word count (Introduction, Results, and Discussion): 12270<br>Number of data elements: 1 table, 8 figures.

- rext word<br>Number c<br>Number c --<br>30<br>31 30 In-text word count (Introduction, Results, and Discussion): 12270<br>
Number of data elements: 1 table, 8 figures.<br>
2
- 

29

31 Number of data elements: 1 table, 8 figures.

33 It is wide<br>34 skeleton by musc<br>35 muscle and bone<br>36 (2019). Muscle p of the skeleton by muscle is essential for life. Less well-known but becoming more generally accepted is that<br>
muscle and bone can communicate through secreted factors *Brotto and Bonewald (2015)*; *Bonewald*<br>
(2019). Muscle prod muscle and bone can communicate through secreted factors *Brotto and Bonewald (2015)*; *Bonewald* (2019). Muscle produces factors such as *B*-aminoisobutyric Acid (BAIBA) and irisin with exercise, that have positive effect

35 muscle and bone can communicate through secrecta ractors Brotto and Bonewald (2015), bonewald (2019). Muscle produces factors such as  $\theta$ -aminoisobutyric Acid (BAIBA) and irisin with exercise, that have positive effect (2019). Muscle produces factors such as *δ*-aminoisobutyric Acti (BAIBA) and irism with exercise, that<br>
have positive effects on bone, adipose tissue, brain, and other organs, whereas sedentary muscle<br>
produces factors su 33 produces factors such as myostatin that has negative effects on both bone and muscle *Brotto and*<br>39 Bonewald (2015); *Karsenty and Mera* (2018); *Kitase et al.* (2018); *Bostrom et al.* (2012); *Hamrick et al.* (2006) produces factors such as myostatin that has negative enects on both bone and muscle Brotto and<br>Bonewald (2015); Karsenty and Mera (2018); Kitase et al. (2018); Bostrom et al. (2012); Hamrick et<br>al. (2006).<br>Many of the fac Solution (2015); Karsenty and Meta (2016); Kitase et al. (2016), Bostrom et al. (2012), Hamrick et al. (2016).<br>
40 any of the factors secreted by bone are produced by osteocytes, the most abundant and<br>
42 the longest-livi 41 Ma<br>
42 the longes<br>
43 terminally<br>
44 matrix Dc<br>
mechanos<br>
45 mechanos<br>
46 Under unlo<br>
47 and Recep<br>
48 and activa<br>
49 et al. (201<br>
factors su<br>
51 function M<br>
65 continues 42 the longest-living bone cell *Bonewald* (2011); *Dallas et al.* (2013). These cells are derived from<br>43 terminally differentiated osteoblasts that become surrounded by the newly mineralizing bone<br>44 eminizing bone con Etherminally differentiated osteoblasts that become surrounded by the newly mineralizing bone<br>
matrix *Dallas et al.* (2013). Osteocytes are multifunctional and appear to be the major<br>
mechanosensory cell in bone *Bonewa* matrix *Dallas et al.* (2013). Osteocytes are multifunctional and appear to be the major<br>mechanosensory cell in bone *Bonewald* (2011); Temiyasathit and Jacobs (2010); Uda et al. (2017).<br>Under unloaded conditions, these c mechanosensory cell in bone *Bonewald* (2011); Temiyasathit and Jacobs (2010); Uda et al. (2017).<br>
Under unloaded conditions, these cells produce sclerostin, a negative regulator of bone formation<br>
and Receptor Activator Under unloaded conditions, these cells produce sclerostin, a negative regulator of bone formation<br>
and Receptor Activator of Nuclear factor Kappa β ligand (RANKL), the major factor that recruits<br>
and activates osteoclasts and Receptor Activator of Nuclear factor Kappa  $\beta$  ligand (RANKL), the major factor that recruits<br>and activates osteoclasts to resorb bone *Nakashima et al.* (2011); *Xiong and O'Brien* (2012); *Xiong<br>et al.* (2015); *On* 47 and Receptor Activator of Nuclear factor Rappa β ligand (MANKL), the major factor that recruits<br>
and activates osteoclasts to resorb bone *Nakashima et al.* (2011); *Xiong and O'Brien* (2012); *Xiong*<br> *et al.* (2015) et al. (2015); Ono et al. (2020). In contrast, with anabolic mechanical loading, these cells produce<br>factors such as prostaglandin E2 (PGE<sub>2</sub>) that have positive effects on myogenesis and muscle<br>function *Mo* et al. (2015) factors such as prostaglandin E2 (PGE<sub>2</sub>) that have positive effects on myogenesis and muscle<br>function *Mo et al.* (2015). Osteocytes play a major role in mineral metabolism, through regulation<br>of both calcium and phospha 51 function *Mo et al.* (2015). Osteocytes play a major role in mineral metabolism, through regulation<br>
52 for both calcium and phosphate homeostasis. Osteocytes secrete Fibroblast Growth Factor 23 to<br>
53 target the kidne 52 of both calcium and phosphate homeostasis. Osteocytes secrete Fibroblast Growth Factor 23 to<br>
51 target the kidney to regulate phosphate excretion. Both Parathyroid Hormone (PTH) and<br>
54 Parathyroid related peptide (PT East the kidney to regulate phosphate excretion. Both Parathyroid Hormone (PTH) and<br>
Farathyroid related peptide (PTHrP) regulate calcium homeostasis via the PTH type 1 receptor on<br>
osteocytes *Feng et al.* (2009); Teti a Parathyroid related peptide (PTHrP) regulate calcium homeostasis via the PTH type 1 receptor on<br>
osteocytes *Feng et al.* (2009); Teti and Zallone (2009). Under the physiological calcium-<br>
demanding condition of lactation, osteocytes *Feng et al.* (2009); *Teti and Zallone* (2009). Under the physiological calcium-<br>demanding condition of lactation, osteocytes respond to PTHrP by removing their surrounding<br>perilacunar matrix to provide calcium 57 perilacunar matrix to provide calcium for offspring, and upon weaning this perilacunar matrix is<br>
58 rapidly replaced, a process referred to as perilacunar remodeling Qing and Bonewald (2009); Qing<br>
59 et al. (2012); W perilacunar matrix to provide calcium for offspring, and upon weaning this perilacunar matrix is<br>
rapidly replaced, a process referred to as perilacunar remodeling Qing and Bonewald (2009); Qing<br>
et al. (2012); Wysolmerski 57 perilacunar matrix periodic as perilacunar matrix empedding *Qing and Bonewald (2009)*; *Qing*<br>
57 et *al. (2012); Wysolmerski (2013).* However, under pathological conditions such as ovariectomy,<br>
57 hyperparathyroidism Figure 1. (2012); Wysolmerski (2013). However, under pathological conditions such as ovariectomy,<br>
ter al. (2012); Wysolmerski (2013). However, under pathological conditions such as ovariectomy,<br>
hyperparathyroidism, hypop 59 et al. (2012); Wysolmerski (2012); However, under pathological conditions such as ovariectomy,<br>
hyperparathyroidism, hypophosphatemic rickets, and cancer, excessive removal of their<br>
perilacunar matrix occurs through os

61 perilacunar matrix occurs through osteocytic osteolysis Tsourdi et al. (2018); Jähn-Rickert and<br>62 Zimmermann (2021); Pin et al. (2021); Shimonty et al. (2023).<br>63 Bone is the largest calcium reservoir in the body and h Example 1918 perilary in the teal of the teal (2021); Shimonty et al. (2023).<br>
Bone is the largest calcium reservoir in the body and human mothers can lose an average<br>
of 250 mg/day of calcium in milk, emphasizing the need Solution (2022); Pin et al. (2022); Pin et al. (2021)<br>
64 of 250 mg/day of calcium in milk, emphasizing the need for a<br>
10ss Qing et al. (2012); Wysolmerski (2002); Kalkwarf (2004). 64 of 250 mg/day of calcium in milk, emphasizing the need for a calcium-replete diet to prevent bone<br>
loss Qing et al. (2012); Wysolmerski (2002); Kalkwarf (2004). During lactation, PTHrP targets the<br>
3 65 loss Qing et al. (2012); Wysolmerski (2002); Kalkwarf (2004). During lactation, PTHrP targets the  $\frac{3}{4}$ 65 loss Qing et al. (2012); Wysolmerski (2002); Kalkwarf (2004). During lactation, PTHrP targets the

For perilacunar matrix and to increase RANKL as an activator of osteoclasts *Kovacs* (2001). During<br>
factation, RANKL targets osteoclasts, thereby driving osteoclastic bone resorption. Osteocytic<br>
osteolysis is accomplish 68 lactation, RANKL targets osteoclasts, thereby driving osteoclastic bone resorption. Osteocytic osteolysis is accomplished through the expression of 'osteoclast-specific' genes such as cathepsin K (Crsk), tartrate-resis

69 osteolysis is accomplished through the expression of 'osteoclast-specific' genes such as cathepsin K<br>(Cfsk), tartrate-resistant acid phosphatase (TRAP, gene *Acp5*), and carbonic anhydrase 1 (Car 1) Qing<br>and Bonewald ( (Ctsk), tartrate-resistant acid phosphatase (TRAP, gene *Acp5*), and carbonic anhydrase 1 (Car 1) Qing<br>
and Bonewald (2009); Qing et al. (2012). In addition, there is an increase in genes coding for the<br>
proton pumps, ATP The proton pumps, ATPase H<sup>+</sup> Transporting V1 Subunit G1 (Atp6vJg1) and Carbonic anny disse of the proton pumps, ATPase H<sup>+</sup> Transporting V1 Subunit G1 (Atp6vJg1) and ATPase H<sup>+</sup> Trans- porting V0<br>Subunit D2 (Atp6v0d2) ne 27 and Bonewald (2009); Qing et al. (2012). In dedictor, there is an increase in genes coding for the proton pumps, ATPase H\* Transporting V1 Subunit G1 (Atp6v0d2) and ATPase H\* Trans- porting V0<br>Subunit D2 (Atp6v0d2) nec proton pumps, ATPase H<sup>2</sup> Transporting V1 Subunit G1 (At*pbv1g1*) and ATPase H<sup>2</sup> Trans- porting V0<br>
Subunit D2 (At*p6v0d2*) necessary to dissolve and remove calcium from bone collagen Jahn (2017).<br>
Systemic calcium defic Subunit D2 (Atp6v0d2) necessary to dissolve and remove calcularition bone conagen Jahn (2017).<br>
Systemic calcium deficiency such as a decrease in dietary calcium triggers an increase<br>
PTH, acting to mobilize calcium from b PTH, acting to mobilize calcium from bones to maintain normal homeostatic circulating calcium<br> *Goltzman* (2008). Worldwide, over 3.5 billion people suffer from dietary calcium deficiency, and<br>
women are at a higher risk Goltzman (2008). Worldwide, over 3.5 billion people suffer from dietary calcium deficiency, and<br>women are at a higher risk of this condition *Kumssa et al.* (2015); *Body et al.* (2016). Aging often<br>results in hypocalcemia

Frame are at a higher risk of this condition *Kumssa et al.* (2015); *Body et al.* (2016). Aging often<br>
results in hypocalcemia and bone loss due to low vitamin D, hypoparathyroidism, genetic<br>
abnormalities, medications de The results in hypocalcemia and bone loss due to low vitamin D, hypoparathyroidism, genetic<br>
abnormalities, medications decreasing dietary calcium absorption, and menopause in women.<br>
Calcium deficiency can lead to osteope 39 abnormalities, medications decreasing dietary calcium absorption, and menopause in women.<br>
31 Calcium deficiency can lead to osteopenia, osteoporosis, and increased fracture risk, primarily due to<br>
31 secondary hyperpa Calcium deficiency can lead to osteopenia, osteoporosis, and increased fracture risk, primarily due to<br>
secondary hyperparathyroidism *Kumssa et al.* (2015); *Body et al.* (2016).<br>
Irisin is a recently discovered myokine secondary hyperparathyroidism *Kumssa et al.* (2015); *Body et al.* (2016).<br>
Irisin is a recently discovered myokine generated in response to exercise when Fibronectin type<br>
III Domain Containing protein 5 (FNDC5) is prote Figure 2018 Strategy and Controlline Conduction (Secondary Strategy Premium Containing protein 5 (FNDC5) is proteolytically cleaved by a yield Bostrom et al. (2012). FNDC5 is expressed in the heart, kidney, testes, however III Domain Containing protein 5 (FNDC5) is proteolytically cleaved by a yet undetermined protease<br> *Bostrom et al.* (2012). FNDC5 is expressed in the heart, kidney, testes, brain, and other tissues;<br>
however, skeletal musc Bastrom et al. (2012). FNDCS is expressed in the heart, kidney, testes, brain, and other tissues;<br>
however, skeletal muscle appears to be the primary producer *Erickson* (2013); *Maak et al.* (2021);<br>
Tsourdi et al. (2022) Bostrom et al. (2022). Tribes is expressed in the heart, kidney, testes, brain, and other tissues,<br>
Bostrom et al. (2022). Cleaved irisin circulates to distant organs, such as adipose tissue where irisin<br>
increases a therm Sourdi et al. (2022). Cleaved irsin circulates to distant organs, such as adipose tissue where irisin increases a thermogenic gene program, including the expression of uncoupling protein 1 (UCP1) in a process referred to a Fourier al. (2022). Cleaved irisin circulates to distant organs, such as adipose tissue where irisin increases a thermogenic gene program, including the expression of uncoupling protein 1 (UCP1) in a process referred to as Bracess referred to as browning. This is associated with increased energy expenditure and<br>
87 improvement in glucose tolerance, both of which are important for the prevention of Type 2<br>
48 diabetes and the reduction of co 88 improvement in glucose tolerance, both of which are important for the prevention of Type 2<br>
88 improvement in glucose tolerance, both of which are important for the prevention of Type 2<br>
48 diabetes and the reduction o 89 diabetes and the reduction of complications from obesity *Perakakis et al.* (2017); *Korta et al.* (2019). Irisin can also regulate glucose uptake in skeletal muscle *Lee et al.* (2015), and increases myogenesis and oxi (2019). Irisin can also regulate glucose uptake in skeletal muscle *Lee et al.* (2015), and increases<br>myogenesis and oxidative metabolism, responsible for increasing skeletal muscle mass *Colaianni*<br>and Grano (2015). Iris myogenesis and oxidative metabolism, responsible for increasing skeletal muscle mass Colaianni<br>
and Grano (2015). Irisin also plays an important positive role in cognitive functions with exercise,<br>
aging, and degenerative 93<br>
93 and Grano (2015). Irisin also plays an important positive role in cognitive functions with exercise,<br>
94 aging, and degenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD)<br>
95 Islam et al 94 and degenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD)<br>95 *Islam et al.* (2021). Using the tail-vein injection method to deliver exogenous irisin, it was shown<br>96 that irisin can cross t

95 *Islam et al.* (2021). Using the tail-vein injection method to deliver exogenous irisin, it was shown<br>
96 that irisin can cross the blood-brain barrier *Islam et al.* (2021).<br>
97 Results from studies regarding the effec that irisin can cross the blood-brain barrier *Islam et al.* (2021).<br>
97 Results from studies regarding the effects of irisin on the skeleton are complex and somewhat<br>
198 contradictory. Colaianni et al have shown that rec Results from studies regarding the effects of irisin on the<br>contradictory. Colaianni et al. have shown that recombinant irisin<br>bone in young male mice by reducing the secretion of osteoblast<br>4 98 contradictory. Colaianni et al have shown that recombinant irisin exerts a beneficial effect on cortical<br>99 bone in young male mice by reducing the secretion of osteoblast inhibitors and increasing the activity<br>99 4 bone in young male mice by reducing the secretion of osteoblast inhibitors and increasing the activity<br>4 99 bone in young male mice by reducing the secretion of osteoblast inhibitors and increasing the activity.<br>4

2008 of osteogenic cells colatains et al. (2013); However, another study has shown that recombinant irisin treatment of MLO-Y4 osteocyte-like cells induces gene and protein level expression of Sost/sclerostin, anegative r 101<br>
10203). Rosen et al. have shown using female FNDC5 overexpression of Sost/sclerostin,<br>
10203). Rosen et al. have shown using female FNDC5 overexpressing female mice that irisin acts<br>
10203). Rosen et al. have shown t 102 a negative regulator of both chinatation white maintaining cell viability anter calculate stress that its in acts (2018). Kosen et al. have shown using female FNDC5 overexpressing female mice bone resorption Estell et 103 (2019), nosen et al. have shown dang female THOCS Overexpressing female that irisin acts<br>
104 directly acts on ostecolast progenitors to increase differentiation and promote bone resorption<br>
105 are protected against Estell et al. (2020). Kim et al. have shown that 9-month-old ovariectomized FNDCS global KO mice<br>
are protected against ovariectomy-induced trabecular bone loss through the inactivation of<br>
osteocytic osteolysis and osteoc

are protected against ovariectomy-induced trabecular bone loss through the inactivation of osteocytic osteolysis and osteoclastic bone resorption *Kim et al.* (2028). The majority of these studies used only male or female osteocytic osteolysis and osteoclastic bone resorption *Kim et al.* (2018). The majority of these studies used only male or female mice, suggesting a sex-dependent response may be responsible for these seemingly opposing f <sup>108</sup> studies used only male or female mice, suggesting a sex-dependent response may be responsible<br>
109 for these seemingly opposing findings *Estell et al.* (2020); *Colaianni et al.* (2017); *Kawao et al.* (2018);<br>
110 109 for these seemingly opposing findings *Estell et al.* (2020); *Colaianni et al.* (2017); *Kawao et al.* (2018);<br>
110 *Ma et al.* (2018); *Colucci et al.* (2019); *Posa et al.* (2021).<br>
111 As shown previously, FNDCS de 109 for these seemingly opposing findings Estell et al. (2021); Colaidim et al. (2021); As shown previously, FNDC5 deletion has a protective effect against ovariectomy- induced<br>
110 As shown previously, FNDC5 deletion has Ma et al. (2018); Colucci et al. (2019); Posa et al. (2021).<br>
111 As shown previously, FNDC5 deletion has a pro<br>
112 bone loss via a reduction of osteocytic osteolysis and o<br>
113 therefore, hypothesized that FNDC5 deletion The loss via a reduction of osteocytic osteolysis and osteoclastic resorption *Kim et al.* (2018). We, therefore, hypothesized that FNDC5 deletion would also be protective against bone loss due to calcium deficiency that o therefore, hypothesized that FNDC5 deletion would also be protective against bone loss due to calcium deficiency that occurs with lactation and a calcium-deficient diet. Our data show that the female skeleton in FNDC5 null 114 calcium deficiency that occurs with lactation and a calcium-deficient diet. Our data show that the<br>115 female skeleton in FNDC5 null female mice was resistant to bone loss due to both lactation and<br>116 female skeleton female skeleton in FNDC5 null female mice was resistant to bone loss due to both lactation and<br>116 low calcium. However, for FNDC5 null males, deletion not only failed to protect but exacerbated<br>117 bone loss in response t lactation in Follow Control of the skeleton in the skeletion in the spot of the skeleton in the skeleton in the skeleton in the skeleton in Figure was respond to irisin differently under calcium-demanding conditions based 117 bone loss in response to low calcium. We propose that male and female osteocytes respond to irisin differently under calcium-demanding conditions based on the divergence of the male and female osteocyte transcriptome w 118 irisin differently under calcium-demanding conditions based on the divergence of the male and female osteocyte transcriptome with sexual maturity when the female osteocyte must serve a critical role in reproduction and 119 female osteocyte transcriptome with sexual maturity when the female osteocyte must serve a<br>119 female osteocyte transcriptome with sexual maturity when the female osteocyte must serve a<br>121 a critical role in reproduct 119 female osteory of an experimental material material material material material material material material<br>121<br>121 121<br>121<br> critical role in reproduction and lactation.<br>
121

123 Wi<br>
124 No<br>
125 between<br>
126 showing 123 With lactation, FNDC5 global KO mice lose less bone and are mechanically stronger compared to WT<br>
124 No significant differences were observed in either bone composition or morphometry<br>
125 between 4-5-month-old virgin 125 between 4-5-month-old virgin WT and FNDC5 global KO female mice [Fig 1A, 1B, 1C, sup table 1),<br>
126 between 4-5-month-old virgin WT and FNDC5 global KO female mice [Fig 1A, 1B, 1C, sup table 1),<br>
126 showing that the a showing that the absence of FNDC5/irisin does not affect female bone development. It has been<br>previously shown that during lactation, maternal bones release calcium to supplement milk,<br>especially in response to the large c previously shown that during lactation, maternal bones release calcium to supplement milk,<br>
especially in response to the large calcium demand induced by large litter size or a calcium-deficient<br>
diet *Wysolmerski* (2002) especially in response to the large calcium demand induced by large litter size or a calcium- deficient<br>
diet *Wysolmerski* (2002); *Ardeshirpour et al.* (2015). Similar to previous studies, 2 weeks of lactation<br>
resulted 213 diet Wysolmerski (2002); Ardeshirpour et al. (2015). Similar to previous studies, 2 weeks of lactation resulted in bone loss in both WT and KO mice, with a significant reduction in cortical bone area (Ct. B.Ar), cortic 139 diet November 12002); The studies of the MDC mice, with a significant reduction in cortical bone area (Ct.<br>
131 B.Ar), cortical bone area fraction percentage (Ct.B.Ar/T.Ar%), and cortical thickness (Ct. Th) (Fig 1A,<br>
1 B.Ar), cortical bone area fraction percentage (Ct.B.Ar/T.Ar%), and cortical thickness (Ct. Th) (Fig 1A,<br>18) as well as bone mineral density, BMD (Fig 1C). However, the KO mice lost less bone compared to<br>1833 the WT mice, a 132 B. Is well as bone mineral density, BMD (Fig 1C). However, the KO mice lost less bone compared to the WT mice, as evidenced by the significantly higher bone area fraction percent, cortical thickness, and BMD (Fig. 1A, 133 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11.1 140 11 and BMD (Fig. 1A, 1B, 1C) as well as the lower percentage of bone loss (Sup Table 1). These data<br>suggest that the FNDCS KO mice are more resistant to the effects of calcium demand. Analysis of<br>trabecular bone parameters in

suggest that the FNDCS KO mice are more resistant to the effects of calcium demand. Analysis of<br>
trabecular bone parameters including trabecular bone volume fraction (BV/TV), trabecular thickness<br>
(Tb. Th), trabecular spac 135 trabecular bone parameters including trabecular bone volume fraction (BV/TV), trabecular trickness<br>
135 (Tb. Th), trabecular spacing (Tb. Sp), and trabecular number (Tb. N) showed no significant difference<br>
138 in bone 137 (Tb. Th), trabecular spacing (Tb. Sp), and trabecular number (Tb. N) showed no significant difference<br>
138 in bone loss between lactating WT and lactating KO mice (Sup table 1). There was no significant<br>
139 difference 138 in bone loss between lactating WT and lactating KO mice (Sup table 1). There was no significant difference in the pup numbers between WT and KO females (Sup fig 1A).<br>
140 Bone loss can have significant effects on bone 139 difference in the pup numbers between WT and KO females (Sup fig 1A).<br>
139 difference in the pup numbers between WT and KO females (Sup fig 1A).<br>
140 Bone loss can have significant effects on bone mechanical properties 140 Bone loss can have significant effects on bone mechanical<br>
141 strength, stiffness, and fragility. To determine mechanical properties, i<br>
142 performed on mice femurs. There was no significant difference between<br>
143 t 141 strength, stiffness, and fragility. To determine mechanical properties, 3-point bending tests were<br>
142 performed on mice femurs. There was no significant difference between virgin WT and KO mice in<br>
143 terms of ultim 142<br>
142 performed on mice femurs. There was no significant difference between virgin WT and KO mice in<br>
143 terms of ultimate force and stiffness (Fig. 1D). However, femurs from the lactating KO mice were<br>
144 strenger th There is the microsofter and stiffness (Fig. 1D). However, femurs from the lactating KO mice were<br>tronger than lactating WT, as evidenced by the higher stiffness and significantly higher ultimate<br>force needed to break the

144 stronger than lactating WT, as evidenced by the higher stiffness and significantly higher ultimate<br>
145 force needed to break the bone (Fig 1D, Sup Table 1, Table 2). This data indicates that lactating KO<br>
146 female b 145 force needed to break the bone (Fig 1D, Sup Table 1, Table 2). This data indicates that lactating KO<br>
146 female bone retains greater resistance to fracture than lactating WT mice by less lactation-induced<br>
147 bone lo Framele bone retains greater resistance to fracture than lactating WT mice by less lactation-induced<br>
145 formale bone loss.<br>
148 With lactation, FNDC5 global KO mice have fewer TRAP-positive osteo-clasts and osteocytes as 147 bone loss.<br>
146 With lactation, FNDC5 global KO mice have fewer TRAP-positive osteo- clasts and osteocytes as well as<br>
149 smaller osteocyte lacturar area compared to WT mice<br>
150 Previously it was shown that lactation 148 Wit<br>
149 smaller oste<br>
150 Pre<br>
151 bone resc<br>
152 contributic<br>
153 resistant<br>
154 osteocytes<br>
155 Virg maller osteocyte lacunar area compared to WT mice<br>
149 Smaller osteocyte lacunar area compared to WT mice<br>
150 Previously it was shown that lactation-induced bone loss occurs via not only osteoclastic<br>
151 bone resorption 149 smaller osteocyte lacular area compared to WT mice<br>150 Previously it was shown that lactation-<br>151 bone resorption but also osteocytic osteoly:<br>152 contribution of each means of resorption, tik<br>153 resistant acid phosp 151 bone resorption but also osteocytic osteolysis *Qing et al (2012)*. To determine the relative<br>
151 contribution of each means of resorption, tibial longitudinal sections were stained for tartrate-<br>
153 resistant acid p 152 contribution of each means of resorption, tibial longitudinal sections were stained for tartrate-<br>153 resistant acid phosphatase TRAP-positive multinucleated osteoclasts as well as TRAP-positive<br>155 Virgin FNDC5 KO fem 154 osteocytes.<br>
155 Virgin FNDC5 KO female mice had fewer TRAP-positive osteocytes compared to virgin WT<br>
6

155 Virgin<br>
Virgin<br>
154 Osteopolis<br>
154 Osteopolis<br>
154 Osteopolis<br>
154 Osteopolis<br>
154 Osteopolis<br>
164 Osteopolis<br>
164 Osteopolis<br>
165 Osteopolis<br>
165 Osteopolis<br>
165 Osteopolis<br>
165 Osteopolis<br>
165 Osteopolis<br>
165 Osteop

 $155$  Virgin FNDCs  $k$  female microscopy of  $\frac{1}{2}$ 

152 contribution of each means of resorption, tibial longitudinal sections were stained for tartrate-

157 mice and suggests that the osteocytes in the female KO mice are less 'primed' to initiate osteocytic<br>
158 mice and suggests that the osteocytes in the female KO mice are less 'primed' to initiate osteocytic<br>
158 16, Su 158 osteolysis. With lactation, TRAP-positive osteocytes significantly increased in both WT and KO mice (Fig<br>159 16, Sup Table 2). Virgin KO mice started with a lower number of TRAP-positive osteocytes compared to<br>160 virg 159 16, Sup Table 2). Virgin KO mice started with a lower number of TRAP-positive osteocytes compared to virgin WT, and with lactation, their number of TRAP-positive osteocytes was still significantly lower compared to la 159 1G, Sup Table 2). Virgin KO mice started with a lower number of TRAP- positive osteocytes compared to 1616 compared to lactation, in response to calcium demand, osteocytes can remove their perilacunar<br>
1616 During lactation, in response to calcium demand, osteocytes can remove their perilacunar<br>
1616 matrix. This process i 162 During lactation, in respon<br>
163 matrix. This process is similar but<br>
164 Bélanger (1969); Wysolmerski (20<br>
166 lacunar area and found no significa<br>
167 even though the KO females have fe<br>
168 area increased in both gr matrix. This process is similar but not identical to osteoclastic bone resorption *Tsourdi et al.* (2018);<br> *Bélanger* (1969); *Wysolmerski* (2012) as osteoclasts generate resorption pits, whereas osteocytes<br>
increase thei *Bélanger* (1969); *Wysolmerski* (2012) as osteoclasts generate resorption pits, whereas osteocytes<br>increase their lacunar size Qing et al. (2012); *Wysolmerski* (2013). We measured the osteocyte<br>lacunar area and found no 165<br>
165 increase their lacunar size Qing et al. (2012); Wysolmerski (2013). We measured the osteocyte<br>
1646 lacunar area and found no significant difference between virgin WT and KO female mice (Fig. 1F, 1H)<br>
167 even tho Islamar area and found no significant difference between virgin WT and KO female mice (Fig. 1F, 1H)<br>
even though the KO females have fewer TRAP-positive osteocytes (Fig. 1G). With lactation, the lacunar<br>
area increased in even though the KO females have fewer TRAP-positive osteocytes (Fig. 1G). With lactation, the lacunar<br>area increased in both groups; however, KO mice had significantly smaller average lacunar area<br>compared to WT (Fig. 1H). 168 area increased in both groups; however, KO mice had significantly smaller average lacunar area<br>
169 compared to WT (Fig. 1H). We did not observe any difference in the osteocyte density among any of<br>
170 the groups (WT=

- 168 compared to WT (Fig. 1H). We did not observe any difference in the osteocyte density among any of<br>
168 compared to WT (Fig. 1H). We did not observe any difference in the osteocyte density among any of<br>
170 the groups 179 the groups (WT= 258.2±51.46, WT L= 274.6±57.37, KO= 254.8±47.66, and KO L= 273.4±59.75). These<br>
171 dta show that female lactating FNDCS KO mice undergo less osteocytic osteolysis compared to WT<br>
172 females under the during lactation to induce osteoclastic bone emdergo less osteocytic osteolysis compared to WT<br>172 data show that female lactating FNDCS KO mice undergo less osteocytic osteolysis compared to WT<br>173 In virgin mice, there w
- 172 females under the calcium-demanding condition of lactation.<br>
173 females under the calcium-demanding condition of lactation.<br>
173 lowing mice, there were no significant differences in osteoclast number per bone perimet 173 In virgin mice, there were no significant differences in<br>
174 (Oc/B.Pm) between WT and KO female mice (Fig 11). With la<br>
175 both groups, however, KO mice had significantly fewer oste<br>
176 percentage increase in the nu 174 (Oc/B.Pm) between WT and KO female mice (Fig 1!). With lactation, osteoclast number increased in both groups, however, KO mice had significantly fewer osteoclasts (Fig 1!) and a significantly lower percentage increase 175 both groups, however, KO mice had significantly fewer osteoclasts (Fig 1I) and a significantly lower percentage increase in the number of osteoclasts compared to WT (Sup Table 1). This suggests that with lactation, few The percentage increase in the number of osteoclasts compared to WT (Sup Table 1). This suggests that with lactation, fewer osteoclasts are activated in the KO as compared to the WT mice.<br>
RANKL, another major factor in bo with lactation, fewer osteoclasts are activated in the KO as compared to the WT mice.<br>
RANKL, another major factor in bone resorption *Xiong and O'Brien* (2012), is also increased<br>
during lactation to induce osteoclastic b RANKL, another major factor in bone resorption *Xiong and O'Brien* (2012), is<br>
179 during lactation to induce osteoclastic bone resorption *Ardeshirpour et al.* (2015) by<br>
180 major source of RANKL *Nakashima et al.* (2011 dring lactation to induce osteoclastic bone resorption *Ardeshirpour et al.* (2015) by osteocytes, the<br>major source of RANKL *Nakashima et al.* (2011); *Xiong and O'Brien* (2012); *Ono et al.* (2020). Virgin<br>WT and KO mice 180 major source of RANKL *Nakashima et al.* (2011); *Xiong and O'Brien* (2012); *Ono et al.* (2020). Virgin WT and KO mice had comparable serum RANKL levels (Fig. 1I). With lactation, the increase in serum RANKL was signi 181 WT and KO mice had comparable serum RANKL levels (Fig. 1J). With lactation, the increase in serum<br>
RANKL was significant in the WT mice, but not in the KO mice (Fig. 1I, Sup Table 1).<br>
183<br>
7 182 RANKL was significant in the WT mice, but not in the KO mice (Fig. 1I, Sup Table 1).<br>183  $\frac{183}{7}$
- 



184 Fig 1: With lactation, FNDC5 global KO mice lose less bone and are mechanically stronger co ompared



187 (WT L), and KO lactation (KO L) mice.<br>
188 B:  $\mu$ CT analysis of femoral cortical bone parameters of virgin and lactating WT and KO mice reported as cortical bone area (Ct. B.Ar), cortical bone area fraction (Ct. B.Ar 187 (WT L), and RO lactation (RO L) line.<br>188 **B**:  $\mu$ CT analysis of femoral co<br>189 mice reported as cortical bone are 189 B: *pc* analysis of femoral cortical bone parameters of virgin and lactating WT and KO<br>189 mice reported as cortical bone area (Ct. B.Ar), cortical bone area fraction (Ct. B.Ar/ T.Ar 9 189 mice reported as cortical bone area (Ct. B.Ar), cortical bone area fraction (Ct. B.Ar) T.Ar % female<br>%), and<br>%), and  $6)$ , and<br> $\overline{\phantom{a}}$  $\sim$ <sup>0</sup>, and  $\sim$ 

- 
- 
- 
- C: Ex vivo DXA analy<br>
191 C: Ex vivo DXA analy<br>
192 mice.<br> **D:** 3-point bending<br>
194 and stiffness.<br> **E: Representative TF**<br>
196 KO virgin (KO), and KO lacta<br>
197 F: Representative ba<br>
198 virgin (KO), WT lactation (M<br>
199 1912<br>
1922 mice.<br>
1913 Design the Balaysis of WT and KO virgin and lactating mice reported as ultimate force<br>
1914 and stiffness.<br>
1914 E: Representative TRAP-stained images of cortical bone from WT virgin (WT), WT lactati 193<br>194 and st<br>195<br>196 KO vir<br>197<br>198 virgin<br>199<br>200<br>201<br>202
- 
- 
- 
- 
- 203<br>
1934 and stiffness.<br>
1935 **E:** Representative TRAP-stained images of cortical bone from WT virgin (WT), WT lactation (WT L),<br>
1936 **KO virgin (KO)**, and KO lactation (KO L) mice.<br>
1937 **F:** Representative backscatter 195 **E:** Repr<br>
195 **E:** Repr<br>
196 KO virgin (KO),<br>
197 **F:** Repr<br>
198 virgin (KO), W<br>
199 **G:** Perc<br>
200 H: Oste<br>
201 I: Oste<br>
202 J: Serur<br>
203 4-5-mc<br>
204 WT, b= Signif<br>
205 performed for 195 Expresentative Trial scaling electron microscope (BSEM) images of WT virgin (WT), KO<br>
195 KO virgin (KO), and KO lactation (KO L) mice.<br>
195 Expresentative backscatter scanning electron microscope (BSEM) images of WT v 197 F: Representative backscatter scannir<br>
198 virgin (KO), WT lactation (WT L), and KO lactation<br>
199 G: Percent TRAP-positive osteocytes (<br>
199 H: Osteocyte lacunar area in femurs f<br>
199 I: Osteoclast number per bone per 1978<br>
1978<br>
1978<br>
1978<br>
1978<br>
1979<br>
1979<br>
1979 Fercent TRAP-positive osteocytes (TRAP +ve) in tibia from virgin and lactating WT and KO mice.<br>
1979 G: Percent TRAP-positive osteocytes (TRAP +ve) in tibia from virgin and la
- 

198 **198 virgin (KO), WE are the COND** of the Control of 1999<br>
1999 G: Percent TRAP-positive osteocytes (TRAP +ve) in the finding WT and nice.<br>
1999 H: Osteoclast number per bone perimeter in tibia from virgin and lactating WT and KO mice.<br>
1999 - Significantly different from KO 200 H: Osteocyte Ideam area in Femaris Shown virgin and lactating WT and lactating<br>202 J: Serum RANKL levels in virgin and lactating WT and KO mice.<br>203 4-5-month-old WT and KO virgin and lactating mice, n= 5-8/group. a= 202 I: Serum RANKL levels in virgin and lactating WT and KO mice.<br>
202 I: Serum RANKL levels in virgin and lactating mice, n= 5-8/group, a= Significantly different f<br>
201 4-5-month-old WT and KO virgin and lactating mice, 202 3 3 3 3 3 3 3 3 3 4 4 5 4 month-old WT and KO virgin and lactating with an NO mice.<br>
203 4 -5 -month-old WT and KO virgin and lactating mice, n= 5-8/g<br>
204 WT, b= Significantly different from KO,  $* = p < 0.05$ ,  $* = p < 0.$ WT, b= Significantly different from KO,  $* = pc$  0.05,  $* = pc$  0.01,  $* * = pc$  0.001. 2-way ANOVA was<br>
performed for statistical analysis. The interaction was not significant.<br> **EXEC Virgin and MOVE AD virgin and Significant**<br> **E** 204 WT, b= Significantly different from KO, \*= p< 0.05, \*\*= p< 0.01, \*\*\*= p< 0.001. 2-way ANOVA was **EXECUTE:** The interaction of the statistical and the interaction and the interaction of the groups of the groups (Sup fig. 18, C), or in food intake (Per day average females on a normal diet, 3.341.01g for KO females on a 206 FNDC5 KO female and male bone have opposite responses to a low- calcium diet<br>207 After observing that bones are partially protected against lactation-i<br>208 FNDC5/irisin KO female mice, we sought to determine if FNDC5/i FNDC5/irisin KO female mice, we sought to determine if FNDC5/irisin null (KO) male bone is protected<br>
from calcium deficiency. Therefore, both female and male mice were placed on a calcium-deficient<br>
diet for 2 weeks to in 209 from calcium deficiency. Therefore, both female and male mice were placed on a calcium-deficient<br>
210 det for 2 weeks to induce bone loss. We do not see any significant difference in body weight in any<br>
211 of the grou 210<br>
210 diet for 2 weeks to induce bone loss. We do not see any significant difference in body weight in any<br>
211 of the groups (Sup fig 1B, C), or in food intake (Per day average food intake was 3.9±0.9g for WT<br>
2122 fem

211 of the groups (Sup fig 1B, C), or in food intake (Per day average food intake was 3.9±0.9g for WT<br>
2122 females on a normal diet, 3.74±1.01g for KO females on a normal diet, 3.66±1.1g for WT females on<br>
2123 a low-calc 212 females on a normal diet, 3.74±1.01g for KO females on a normal diet, 3.66±1.1g for WT females on<br>
a low-calcium-diet, 3.8±0.7g for KO females on a normal diet, 3.66±1.1g for WT males on a<br>
normal diet, 4.3±1.5g for KO 213 a low-calcium-diet, 3.8±0.7g for KO females on a low-calcium-diet, 4.2±1.3g for WT males on a<br>214 normal diet, 4.3±1.5g for KO females on a normal diet, 3.94±1.8g for WT males on a normal diet, 4.3±1.5g for KO males on normal diet, 4.3±1.5g for KO males on a normal diet, 3.94±1.8g for WT males on a low-calcium-diet,<br>
215 and 4.4±1.2g for KO males on a low-calcium-diet).<br>
216 With regards to the female mice, similar results were observed and 4.4±1.2g for KO males on a low-calcium-diet).<br>
215 with regards to the female mice, similar results were observed with the low calcium diet as was<br>
217 observed with lactation. At baseline, WT and KO female mice showed 216 With regards to the female mice, similar restands to the female mice, similar restands to the female mice, similar restands and BMC (Sup Table 2), as well as no differe parameters (Sup Table 3). After 2 weeks of a lowobserved with lactation. At baseline, WT and KO female mice showed no significant differences in their<br>
218 BMD and BMC (Sup Table 2), as well as no differences in either cortical (Fig 2B) or trabecular bone<br>
219 parameter 218 BMD and BMC (Sup Table 2), as well as no differences in either cortical (Fig 2B) or trabecular bone<br>
219 parameters (Sup Table 3). After 2 weeks of a low calcium diet, both WT and KO female mice lost<br>
220 bone as can b parameters (Sup Table 3). After 2 weeks of a low calcium diet, both WT and KO female mice lost<br>
220 bone as can be evidenced by decreased BMD (Sup Table 2) and bone area fraction (Fig 2B).<br>
221 However, similar to the lact bone as can be evidenced by decreased BMD (Sup Table 2) and bone area fraction (Fig 2B).<br>
221 However, similar to the lactation experiment, the KO female mice were partially resistant to bone loss<br>
222 compared to the fema 221 However, similar to the lactation experiment, the KO female mice were partially resistant to bone loss<br>222 compared to the female WT mice given a low calcium diet (Fig 2A, B). Interestingly a higher marrow<br>223 cavity a 222 compared to the female WT mice given a low calcium diet (Fig 2A, B). Interestingly a higher marrow<br>223 cavity area was observed in the WT compared to the KO, unlike the lactation experiment (Sup Table 2).<br>9 223 cavity area was observed in the WT compared to the KO, unlike the lactation experiment (Sup Table 2).<br>9

223 cavity area was observed in the WT compared to the KO, unlike the lactation experiment (Sup Table 2).<br>g

225 break, and thus were stronger com- pared to WT females given a low calcium diet (Fig 2C).<br>
225 break, and thus were stronger com- pared to WT females given a low calcium diet, the female<br>
227 Kbene is more resistant to 226 Therefore, similar to the calcium-demanding conditions of lactation, on a low calcium diet, the female<br>
226 Therefore, similar to the calcium-demanding conditions of lactation, on a low calcium diet, the female<br>
227 KO 227 CO bone is more resistant to bone loss than WT.<br>
228 Unlike female bone, significant differences were observed between WT and KO male bone<br>
229 at baseline. KO male mice on a normal diet had a significantly higher BMD, Unlike female bone, significant differenc<br>
229 at baseline. KO male mice on a normal diet had<br>
230 bone area fraction compared to WT males of the<br>
1231 had significantly lower stiffness than WT (Fig 2F),<br>
232 the bone. The 229 at baseline. KO male mice on a normal diet had a significantly higher BMD, BMC (Sup Table 2), and<br>
229 at bone area fraction compared to WT males of the same age (Fig 2E). However, femurs from KO mice<br>
231 hone area fr bone area fraction compared to WT males of the same age (Fig 2E). However, femurs from KO mice<br>
had significantly lower stiffness than WT (Fig 2F), indicating a difference in the material properties of<br>
the bone. Therefor 233 bond significantly lower stiffness than WT (Fig 2F), indicating a difference in the material properties of<br>
232 had significantly lower stiffness than WT (Fig 2F), indicating a difference in the material properties of<br> 232 the bone. Therefore, the KO males have larger, denser, but weaker bones compared to WT males. To<br>
232 the bone. Therefore, the KO males have larger, denser, but weaker bones compared to WT males. To<br>
233 determine the determine the effect of calcium deficiency on male mice, KO and WT mice were subjected to a low-<br>
calcium diet for 2 weeks. Unlike the female KO mice which were protected from the effects of a low<br>
calcium diet, the KO mal

significantly less stiff and therefore weaker compared to the WT males on a low calcium diet (Fig 2F).<br>
239 These data confirm a sex-specific response to a low-calcium diet.<br>
240 To ensure that the effects observed in the 235 calcium diet, the KO male mice had an opposite response. The male KO mice had greater bone loss<br>
235 compared to the WT male mice (Fig 2D, E, Table 2), the trabecular bone loss followed the same<br>
237 tends but was not compared to the WT male mice (Fig 2D, E, Table 2), the trabecular bone loss followed the same<br>trends but was not statistically significant (Sup Table 2), and the femurs from the KO male mice were<br>significantly less stiff a trands but was not statistically significant (Sup Table 2), and the femurs from the KO male mice were<br>
significantly less stiff and therefore weaker compared to the WT males on a low calcium diet (Fig 2F).<br>
These data conf is a significantly less stiff and therefore weaker compared to the WT males on a low calcium diet (Fig 2F).<br>
239 These data confirm a sex-specific response to a low-calcium diet.<br>
240 To ensure that the effects observed in These data confirm a sex-specific response to a low-calcium diet.<br>
To ensure that the effects observed in the KO mice were due to circulating irisin, and not<br>
FNDC5 deletion, we injected AAV8- irisin in KO male mice, with To ensure that the effects observed in the KO mice were<br>
241 FNDC5 deletion, we injected AAV8-irisin in KO male mice, with A,<br>
242 them on the same low Ca diet. We chose male mice due to the h<br>
243 and strength we saw in t <sup>21</sup><br>
241 FNDC5 deletion, we injected AAV8-irisin in KO male mice, with AAV8-GFP as the control, and placed<br>
242 them on the same low Ca diet. We chose male mice due to the highly significant effect on bone mass<br>
243 and s 242 them on the same low Ca diet. We chose male mice due to the highly significant effect on bone mass<br>243 and strength we saw in the KO males compared to WT males on a low-calcium diet. The irisin injection<br>244 rescued th 243 and strength we saw in the KO males compared to WT males on a low-calcium diet. The irisin injection rescued the skeletal phenotype in KO male mice, shown by the higher cortical bone area fraction and the lower endoste examed the skeletal phenotype in KO male mice, shown by the higher cortical bone area fraction<br>245 and the lower endosteal perimeter (Fig 2G). There was a tendency for higher ultimate force and<br>246 strength we saw in the K 245 and the lower endosteal perimeter (Fig 2G). There was a tendency for higher ultimate force and stiffness in the KO males that received the AAV8-irisin injection, however, this did not reach statistical significance (Fi 246 stiffness in the KO males that received the AAV8-irisin injection, however, this did not reach statistical significance (Fig 2H). These data show that the observed effects in the FNDC5 null animals are due to an absenc 247 statistical significance (Fig 2H). These data show that the observed effects in the FNDC5 null animals<br>248 are due to an absence of irisin. 248 statistical significance (Fig 2H). These data show that the observed effects in the observed effects in the FNDC5 number of first i



249

250<br>251<br>252<br>253<br>254 250 Fig 2: FNDC5 KO female and male mice have opposite responses to a low-calcium diet with regard to bone<br>251 composition, structure, and mechanics, and irisin injection rescues FNDC5 KO male mice phenotype under a low-<br>2

251 composition, structure, and mechanics, and irisin injection research MDC5 KO male mice phenotype under a low-<br>253 A: Representative µCT images of femoral midshaft cortical bones from WT low-calcium diet female mouse<br>25

252 calcium dict<br>
253 **A**: Re<br>
254 (WT lc) and K<br>
255 **B**: Fe<br>
256 control (KO), 253 A: Representative µCT images of femoral midshaft cortical bones from WT low- calcium diet female mouse<br>254 (WT lc) and KO low-calcium diet female mouse (KO lc).<br>255 B: Female femoral midshaft cortical bone parameters o 255 **B:** Female femoral midshaft cortical bone para<br>256 control (KO), and KO low-calcium diet (KO lc) mice recortical thickness (Ct.Th).<br>258 C: Mechanical properties of femurs from fem 255 B: Female femoral midshaft cortical bone parameters of WT control (WT), WT low calcium diet (WTTe), KO<br>256 control (KO), and KO low-calcium diet (KO lc) mice reported as cortical bone area fraction (Ct. B.Ar/T.Ar%) and control (KO), and KO low-calcium diet (KO lc) mice reported as cortical bone area fraction (Ct. B.Ar/T.Ar%) and<br>257 cortical thickness (Ct.Th).<br>258 **C:** Mechanical properties of femurs from female WT and KO control and low

258 **c**: Mechanical property of the contract of the corresponding to the corresponding to the corresponding to the corresponding of the corresponding to the corresponding of the corresponding of the corresponding of the co 258 C: Mechanical properties of femurs from female WT and KO control and low- calcium diet reported as

260 **D:** Representative μ<br>261 Ic) and KO low-calcium diet r<br>262 **E:** Male femoral mic<br>263 control (KO), and KO low-ca 260 D: Representative per images of remoral midshaft cortical bones from WT low-calcium diet male mice (WT<br>262 E: Male femoral midshaft cortical bone parameters of WT control (WT), WT low-calcium diet (WT lc), KO<br>263 contr 262 **E:** Male femoral midshaft cortical bor<br>263 control (KO), and KO low-calcium diet (KO lc<br>264 cortical thickness (Ct. Th).<br>265 **F:** Mechanical properties of femurs 262 E: Male femoral midshaft cortical bone parameters of WT control (WT), WT low-calcium diet (WT lc), KO<br>263 control (KO), and KO low-calcium diet (KO lc) mice reported as cortical bone area fraction (Ct. B.Ar/T.Ar%) and<br>

264 contical thickness (Ct. Th).<br>
265 F: Mechanical properties of femurs from male WT and KO control and low-calcium diet reported as<br>
266 ultimate force and stiffness.<br>
267 n= 4-5/group. a= Significantly different from WT

265 F: Mechanical production of the United States (Ct. Th).<br>266 ultimate force and stiffnes<br>267 n= 4-5/group. a=<br>268 0.01. 2-way ANOVA was pe 265 It is mechanical properties of femurs from male WT and KO control and low- calcium diet reported as<br>267  $n= 4-5/group$ . a= Significantly different from WT, b= Significantly different from KO,  $* = p < 0.05$ ,  $* = p < 0.01$ .<br>268

- 267  $n= 4-5/$ group. a= Sig<br>
268 0.01. 2-way ANOVA was perf<br>
269 **G**:  $\mu$ CT measuremen<br>
270 week low calcium diet, rep 268 0.01. 2-way ANOVA was performed. As depicted here, red is female, and blue is male.<br>
269 G: µCT measurement of femoral cortical bone of AAV8-GFP or AAV8-irisin injected male KO mice after a 2-<br>
270 week low calcium die 269 **6:** μCT measurement of femoral cortical bone of AAV8-GFP or AAV8-irisin inje<br>270 week low calcium diet, reported as cortical bone area fraction (Ct. B.Ar/T.Ar%),<br>271 periosteal parameter (Ps.Pm), and endosteal parame 269 G: µCT measurement of remoral cortical bone of AAV8-GFT of AAV8-histin injected male KO line after a 2-<br>270 week low calcium diet, reported as cortical bone area fraction (Ct. B.Ar/T.Ar%), cortical thickness (Ct. Th),<br>
- 271 week low calcium diet, reported as under the state of temperature (Ct. B.Ar.), and properties of femurs from male KO low-calcium diet mice injected with AAV8-GFP or AAV8-<br>273 irisin reported as ultimate force and stiff
- periosteal parameter (Ps.Pm), and endosteal parameter (Es.Pm).<br>
272 **H.** Mechanical properties of femurs from male KO low-calcium diet mice injected with AAV8-GFP or AAV8-<br>
273 irisin reported as ultimate force and stiffne 272 F. Mechanical properties of femals from male Re fow eaction diet mice injected with AAV8-GFP or AAV8-<br>274 n= 5-7/group, \*= p< 0.05. Student's t-test was performed for statistical analysis between male KO GFP vs<br>275 iri 274 n= 5-7/group, \*= p< 0.05. Student's t<br>275 irisin-injected mice. As depicted here, green sk<br>276 **Osteocytesfromfemale and male K**<br>277 To investigate if the bone loss w
- 

275 irisin-injected mice. As depicted here, green shaded bars represent GFP-injected mice.<br>276 **Osteocytes from female and male KO mice respond differently to a low-calcium diet**<br>277 To investigate if the bone loss was due 276 **275 276 276 276 276 276 277 277 277 277 278 278 278 278 278 278 278 278 278 278 278 279 34**) **276 278 279 279 279 279 279 279 279 279 288 279 288 2** 277 To investigate if the bone loss was due to osteoclast or osteocyte activation,<br>278 the groups were TRAP-stained. Under a normal control diet, the tibia from both KO female<br>279 3A) mice had fewer TRAP-positive osteocyte

the groups were TRAP-stained. Under a normal control diet, the tibia from both KO female and male (Fig.<br>
279 3A) mice had fewer TRAP-positive osteocytes compared to their WT counterparts. This indicates that<br>
280 their ost 279 3.0 The groups were less 'primed' or 'activated' for resorption.<br>
279 3.0 Their osteocytes were less 'primed' or 'activated' for resorption.<br>
281 Under a low calcium diet, the number of TRAP-positive osteocytes increas 279 1299 Similiarly lower number of osteoclasts compared to WT females on a low-calcium-diet (Fig 3A) miliarly lower number was still significantly lower in the KO females than the WT females. The low calcium diet increase 281 Under a low calcium diet, the number of TRAP-positive of the in the KO females than the WT females. The low calcium diet in WT and KO male mice. The KO male mice had a significantly higher than the WT females. The low

Framale mice, similar to lactation (Fig 3A, Table 1); however, the total number was still significantly lower<br>
2823 in the KO females than the WT females. The low calcium diet increased TRAP-positive osteocytes in both<br>
28 in the KO females than the WT females. The low calcium diet increased TRAP-positive osteocytes in both<br>
284 WT and KO male mice. The KO male mice had a significantly higher level of increase (Fig 3A, Table 1), and<br>
285 had 283 Initiantly higher TRAP-positive osteocytes compared to WT. This indicates an increased activation of osteocytes in the KO males and suggests higher osteocytic bone resorption.<br>
285 In the KO males and suggests higher o 285 had significantly higher TRAP-positive osteocytes compared to WT. This indicates an increased activation<br>286 of osteocytes in the KO males and suggests higher osteocytic bone resorption.<br>287 There was no significant di 286 of osteocytes in the KO males and suggests higher osteocytic bone resorption.<br>
287 There was no significant difference between WT and KO mice in osteoclast numbers per bone<br>
288 perimeter for both females and males (Fi There was no significant difference between WT and KO mice in ostenties and males and males (Fig. 3B). Both WT and KO females multinucleated TRAP-positive osteoclast number with a low-calcium diet, higher originalizative l perimeter for both females and males (Fig. 3B). Both WT and KO females had an increase in their<br>multinucleated TRAP-positive osteoclast number with a low-calcium diet, however, KO females had a<br>significantly lower number o multinucleated TRAP-positive osteoclast number with a low-calcium diet, however, KO females had a<br>
290 significantly lower number of osteoclasts compared to WT females on a low-calcium-diet (Fig 3B).<br>
291 Similarly, under 290 significantly lower number of osteoclasts compared to WT females on a low-calcium-diet (Fig 3B).<br>291 Similarly, under a normal diet, there was no difference in the number of osteo- clasts between male WT<br>292 and KO. Un 291 Similarly, under a normal diet, there was no difference in the number of osteo- clasts between male WT and KO. Under a low-calcium diet, osteoclast numbers increased in both groups, however, there was no<br>292 and KO. Un 292 and KO. Under a low-calcium diet, osteoclast numbers increased in both groups, however, there was no difference in the number of osteo- class between male WT and KO. Under a low-calcium diet, osteoclast numbers increas  $22$ 

293<br>
294 bone perimeter. There was no difference in osteoblast numbers in either female or male normal or low-<br>
295 calcium diet mice groups (data not shown).<br>
296 lacunar area compared to WT males (Fig 3C, 3D). There was control conditions, female osteocytes have more resorptive activity. On a low calcium diet, all the<br>groups have increased osteocyte lacunar area, indicating an increased level of osteocytic osteolysis<br>(Fig 3E). However, in 296 Under normal control diet conditi<br>
297 lacunar area compared to WT males (Fi<br>
298 FNDC5 KO female and male mice with rea<br>
299 control conditions, female osteocytes have<br>
200 groups have increased osteocyte lacunar<br>
201 Iacunar area compared to WT males (Fig 3C, 3D). There was no significant difference between<br>
FNDC5 KO female and male mice with regards to osteocyte lacunar area. This indicates that under<br>
control conditions, female osteo 298 FNDCS KO female and male mice with regards to osteocyte lacunar area. This indicates that under<br>
298 FNDCS KO female and male mice with regards to osteocyte lacunar area. This indicates that under<br>
2010 control conditi control conditions, female osteocytes have more resorptive activity. On a low calcium diet, all the<br>
groups have increased osteocyte lacunar area, indicating an increased level of osteocytic osteolysis<br>
(Fig 3E). However, 299 compositions, filications, filicating an increased level of osteocytic osteolysis<br>
200 groups have increased osteocyte lacunar area, indicating an increased level of osteocytic osteolysis<br>
202 frig 3E). However, in fem 300 (Fig 3E). However, in female KO mice, the average lacunar area is significantly less than in WT female<br>302 (Fig 3E). However, in female KO mice, the average lacunar area is significantly less than in WT female<br>302 mice 302 (Fig 3F). There was no significantly to the control diet (Fig 3G) however, the KO mice, similar to what was observed with the lactation response. The male KO mice, on the other<br>
303 increased osteocytic osteolysis. Tog

303 hand, have significantly larger lacunar areas compared to WT males on a low calcium diet, suggesting<br>
304 in-creased osteocytic osteolysis. Together these data show that bones from female KO mice are<br>
305 more resistan 303<br>
304 in- creased osteocytic osteolysis. Together these data show that bones from female KO mice are<br>
305 more resistant to calcium-demanding conditions, but the deletion of FNDCS/irisin from males makes<br>
306 them more 313 313 313 in- creased osteolyne are all the show the deletion of FNDCS/irisin from males makes<br>306 them more susceptible to bone loss under calcium-demanding conditions. This also shows that male<br>304 and female KO mice r 306 them more susceptible to bone loss under calcium-demanding conditions. This also shows that male<br>307 and female KO mice respond completely differently to the challenge of calcium deficiency.<br>308 Serum RANKL levels incr 307 and female KO mice respond completely differently to the challenge of calcium deficiency.<br>
308 Serum RANKL levels increased in all the low calcium diet groups compared to control diet<br>
309 groups (Fig 3F). There was no Serum RANKL levels increased in all the low calcium diet groups compared to co<br>
309 Serum RANKL levels increased in all the low calcium diet groups compared to co<br>
309 groups (Fig 3F). There was no significant difference b 309 Seroups (Fig 3F). There was no significant difference between WT and KO female mice and between WT and KO male mice. Serum PTH was measured because decreases in serum calcium stimulate the parathyroid gland to release 319 WT and KO male mice. Serum PTH was measured because decreases in serum calcium stimulate the parathyroid gland to release PTH to remove calcium from bone to maintain normal calcium levels *Jahn* et al (2017); *Matikai* 311 parathyroid gland to release PTH to remove calcium from bone to maintain normal calcium levels *Jahn* et al (2017); *Matikainen et al (2021)*. PTH levels significantly increased in WT females and WT and KO males when

Barathyroid gland to release 1 First definition bone to maintain normal calcium releases and to relation of ranks when subjected to a low calcium diet compared to the control diet (Fig 3G), however, the KO female group did 313 males when subjected to a low calcium diet compared to the control diet (Fig 3G), however, the KO<br>314 male group did not have a statistically significant increase in PTH levels. There was no significant<br>315 difference Framate group did not have a statistically significant increase in PTH levels. There was no significant difference in serum calcium levels in any of the groups (8-10 mg/dL range for all groups), which indicates that the el 315 difference in serum calcium levels in any of the groups (8-10 mg/dL range for all groups), which<br>316 indicates that the elevated PTH is maintaining normal circulating calcium levels in these mice (Fig 3H).<br>317 Since FN 316 indicates that the elevated PTH is maintaining normal circulating calcium levels in these mice (Fig 3H).<br>317 Since FNDCS/irisin is robustly produced in skeletal muscle, we wanted to determine if the<br>318 deletion of FND 317 Since FNDC5/irisin is robustly produced in skeletal muscle, we wanted to determine if the deletion of FNDC5/irisin affects muscle function, under either a normal or a low calcium diet. *In vi* and ex *vivo* muscle cont 318 deletion of FNDC5/irisin affects muscle function, under either a normal or a low calcium diet. *In vivo* and *ex vivo* muscle contractility functions were performed in these mice. No difference was found between WT and 319 deletorior FNDC5/irisin articles muscle function, under either a normal or a low catedrin delt. In vivo<br>318 and ex vivo muscle contractility functions were performed in these mice. No difference was found<br>321 of FNDC5 320 and ex vivo muscle contractintly functions were performed in these infect. No difference was found<br>321 between WT and KO mice on either a normal or a low calcium diet (Sup Fig 2). This indicates deletion<br>322 into the c 321 of FNDC5 is not affecting muscle function and that bone resorption is releasing sufficient calcium<br>322 into the circulation to maintain calcium homeostasis and supplying sufficient calcium for skeletal<br>323 muscle funct 322 into the circulation to maintain calcium homeostasis and supplying sufficient calcium for skeletal<br>323 muscle function.<br>323 13 323 into the circulation to maintain calcium homeostasis and supplying sufficient calcium for sufficient calcium for<br>323 into the circulation for skeletal calcium for skeletal calcium for skeletal calcium for skeletal calc



- 324
- 

- 325<br>326<br>327<br>328<br>329 1.g. Sicocytes from final and male KO mice respond differently to a low-calcium diet.<br>
325 A: Percentage of TRAP-positive (+-ve) osteocytes in female and male WT and KO m<br>
327 B. Osteoclast number (N.Oc/B.Pm) in WT and KO
- 327 **A:** Percentage of TRAP-positive (+-ve) osteocytes in female and male WT and KO mice given a normal or a low-<br>328 **B.** Osteoclast number (N.Oc/B.Pm) in WT and KO female and male mice given a normal or a low-<br>339 calciu **B. Osteoclast number (1988)**<br>
329 calcium diet.<br> **C: Representative BSE (1998)**<br>
531 F) and WT male (WT M) given<br> **D. Osteocyte lacunar a**
- 329 Calcium diet.<br>329 Calcium diet.<br>330 C: Representative BSEM images depicting osteocyte lacunar area in femurs from WT female (WT<br>331 F) and WT male (WT M) given a normal diet at 450X magnification.<br>332 D. Osteocyte lacu 330 **C:** Rep<br>331 F) and WT ma<br>332 **D.** Ost 330 C: Representative BSEM images depicting osteocyte lacunar area in terminals from WT female (WT<br>331 F) and WT male (WT M) given a normal diet at 450X magnification.<br>332 D. Osteocyte lacunar area in WT and KO female and
- 1332 **D.** Osteocyte lacunar area in WT and KO female and male m 332 D. Osteocyte lacunar area in WT and KO female and male mice given a normal diet.

- 
- 
- 
- 
- 



343

345 WT female and male mice where female KO mice are protected but male KO mice have greater bone loss than WT.<br>346 WT.<br>347 Percentage changes in different bone and serum parameters of WT and KO female and male mice with a

- 345 WT.<br>346 WT.<br>347 Percentage changes in different bone and serum parameters of WT and KO female and male mice with a<br>348 2-week low-calcium diet. \*= p<0.05 compared to WT.<br>**Female and male osteocyte transcriptomes are di** 347<br>347<br>348 2-we<br>349<br>350
- 

Percentage changes in different bone and serum parameters of WT and KO female and male mice with a<br>
2-week low-calcium diet. \*= p<0.05 compared to WT.<br> **Female and male osteocyte transcriptomes are distinctly different**<br>
3 Example and male osteocyte transcriptomes<br>350 Total RNA sequencing of osteocyte-enrich<br>351 significant sex-dependent differences in the osteo<br>352 F). The major differentially expressed genes we Total RNA sequencing of osteocyte-enriched bone chips from fer<br>350 Total RNA sequencing of osteocyte-enriched bone chips from fer<br>352 F). The major differentially expressed genes were involved in the ster<br>353 transport, an significant sex-dependent differences in the osteocyte transcriptome under normal conditions (Fig. 4A, C,<br>
F). The major differentially expressed genes were involved in the steroid, fatty acid, cholesterol, lipid<br>
transpor 352 F). The major differentially expressed genes were involved in the steroid, fatty acid, cholesterol, lipid<br>353 transport, and metabolic processes. Compared to male WT mice, female WT mice had an approximately<br>354 2-3-f 353 transport, and metabolic processes. Compared to male WT mice, female WT mice had an approximately<br>
354 2-5-fold higher expression of very low-density lipoprotein receptor (*VIdIr*), voltage-dependent calcium<br>
355 chan 2-3-fold higher expression of very low-density lipoprotein receptor (*VldIr*), voltage-dependent calcum<br>
2-3-fold higher expression of avery low-density lipoprotein receptor (*VldIr*), voltage-dependent calcum<br>
2-3-fold l

2-3-fold higher expression of very low-density lipoprocem receptor (*viviii*), otinge-dependent calcium<br>
channel T type alpha 1H subunit (*Cacna1h*), aldehyde dehydrogenase (*Aldh1l2*), and a 2-3-fold lower<br>
expression of stantier T type alpha 1 H substitute to the them, alderly deliver expression of apolity order expression of apolity and the type alpha 1 Apod2, Apod4, Apoc3 and others involved in steroid and falty acid<br>
are tabolic proces Expression of apolipoproteins Apoux, Apoux, Apoca, Apoca, Apoca, apoca, and called in steroid and latty acted<br>
357 expression of several ligid and solute carrier genes<br>
358 and apolipoprotein genes in female WT compared to 358 and apolipoprotein genes in female WT compared to male WT. This suggests that male osteocytes may<br>359 be greater regulators and utilizers of these sources of energy than female osteocytes.<br>360 offerences were also obs 359 be greater regulators and utilizers of these sources of energy than female osteocytes.<br>
360 Differences were also observed in genes involved in extracellular matrix organization<br>
361 pathways, bone development, ossifi 360 Differences were also observed in genes involved in extracellular manufold parthways, bone development, ossification, bone remodeling, and re-sorption path<br>362 osteocytes have higher expression of genes shown to be hi 361 pathways, bone development, ossification, bone remodeling, and re-sorption pathways. Female WT<br>362 observed is a very essent of genes shown to be highly expressed in osteocytes during<br>363 lactation compared to male WT osteocytes have higher expression of genes shown to be highly expressed in osteocytes during<br>
lactation compared to male WT osteocytes. These include Tnfsf11 (RANKL, 2.7-fold), Ctsk (2.5-fold),<br>
Acp5 (TRAP, 2.2- fold), *Mm* 363 Iactation compared to male WT osteocytes. These include  $Tnf5f11$  (RANKL, 2.7-fold), Ctsk (2.5-fold),  $Acp5$  (TRAP, 2.2- fold),  $Mmp13$  (2.7-fold), osteoclast associated receptor (*Oscar*, 4.6-fold), macrophage stimulati 363 Incurrence of the term of the male with the male to the male to the male to the male that it is the male to male the second the second of the male simulating 1 receptor (*Mst1r*, 3-fold), as well as several collagen ge 364<br>
365 Stimulating 1 receptor (*Mst1r*, 3-fold), as well as several collagen genes and bone formation and<br>
366 mineralization genes including alkaline phosphatase (*Alpl*, 2.4-fold), periostin (*Postn*, 2.6-fold), and<br>
3 stimulating 1 receptor (*WSLI*, 3-fold), as well as several collagen genes and bone formation and<br>mineralization genes including alkaline phosphatase (*Alpl*, 2.4-fold), periostin (*Postn*, 2.6-fold), and<br>significant diffe Source interalization genes including alkaline phosphatase (Alpl, 2.4-fold), periostin (Postn, 2.6-fold), tale<br>
366 and males. This suggests that the higher in the WT females compared to WT males, but no<br>
366 and males. Th Box Dingt (2.2-fold). Tor 50 was expressed ingited in the WT females compared to WT males, but no<br>
significant difference was found in either *TGFβ1* or *TGFβ2* expression levels between WT females<br>
and males. This sugges

369 significant difference was found in either TGFθ1 or TGFβ2 expression levels between WT females<br>369 accommodate the rapid replacement of the perilacunar matrix with weaning. The upregulated and<br>371 downregulated pathwa 370 accommodate the rapid replacement of the perilacunar matrix with weaning. The upregulated and<br>371 downregulated pathways in WT females compared to WT males are depicted in Fig. 4.<br>372 **Female and male KO osteocyte tra** 371 downregulated pathways in WT females compared to WT males are depicted in Fig. 4.<br> **Example and male KO osteocyte transcriptomes have fewer differences compared to WT female and<br>
male transcriptomes<br>
XO female and KO** Female and male KO osteocyte transcriptomes have fewer differences compared to<br>
373 male transcriptomes<br>
374 KO female and KO male osteocyte transcriptomes significantly differed<br>
4. fold greater compared to KO males. Bone male transcriptomes<br>373 male transcriptomes<br>374 KO female and KO male osteocyte transcriptomes significantly differed in pathways<br>375 facilitating ossification and bone mineralization, and extracellular structure and matri 373 male transcriptomes<br>374 KO female<br>375 facilitating ossificati<br>376 (Fig. 4B, F). In KO fen<br>377 4-fold greater comp 375 facilitating ossification and bone mineralization, and extracellular structure and matrix organization<br>376 (Fig. 4B, F). In KO females, several collagen genes such as *Col2a1*, *Col5a2*, *Col8a2*, and *Col11a1* were 2-1376 (Fig. 4B, F). In KO females, several collagen genes such as *Col2a1, Col5a2, Col8a2*, and *Col11a1* were 2-<br>377 4-fold greater compared to KO males. Bone formation genes including *Alpl* (2.5-fold), osteocalcin<br>16 376 (Fig. 4B, F). In KO females, several conagen genes such as Col2a1, Col5a2, Col6a2, and Col11a1 were 2-<br>377 4-fold greater compared to KO males. Bone formation genes including Alpl (2.5-fold), osteocalcin<br>16 377 4-fold greater compared to KO males. Bone formation genes including Alpl (2.5-fold), osteocalcin

(Byn), 2.7-fold), Fostn (2.9-fold), and White (2.4-fold) were also increasing AcpS and Cfsk were not<br>
significantly different between KO female and KO male osteocytes. TGF63 was expressed higher in<br>
the KO females compared 389 significantly different between KO female and KO male osteocytes. TGFB3 was expressed higher in<br>379 significantly different between KO female and KO male osteocytes. TGFB3 was expressed higher in<br>382 The transcriptomes 381 significantly uncertificantly to female and KO finale and KO male osteocytes. TGFB3 was expressed injection<br>382 The transcriptomes of WT and KO male osteocytes differed significantly, with much lower<br>383 expression of 382 The transcriptomes of WT and KO male osteocytes<br>
282 expression of genes in pathways involving steroid, fatty<br>
384 metabolic processes in the KO males compared to WT males<br>
285 genes coding for solute carriers, aldehy 383 expression of genes in pathways involving steroid, fatty acid, lipid, and cholesterol transport and<br>
384 expression of genes in the KO males compared to WT males (Fig. 4C, F). A 2-4-fold downregulation of<br>
385 genes c 383 expression of genes coding for solute carriers, aldehyde oxidase, and fatty acid binding proteins was observed in KO<br>
385 genes coding for solute carriers, aldehyde oxidase, and fatty acid binding proteins was observe

385 genes coding for solute carriers, aldehyde oxidase, and fatty acid binding proteins was observed in KO<br>
386 males, while *Oscar* and *Mst1r* are 2-3-fold higher in KO males compared to WT males. In contrast, a<br>
288 re 385 males, while *Oscar* and *Mst1r* are 2-3-fold higher in KO males compared to WT males. In contrast, a<br>
387 relatively small number of genes, 40, were differentially expressed between WT female and KO<br>
588 female osteo 387 relatively small number of genes, 40, were differentially expressed between WT female and KO<br>
388 female osteocytes which reflects the lack of differences in bone morphology and bone mechanical<br>
389 poperties (Fig. 4D, 388 female osteocytes which reflects the lack of differences in bone morphology and bone mechanical<br>
399 properties (Fig. 4D, F).<br>  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{2}$  and  $\frac{1}{$ 389 properties (Fig. 4D, F).<br>
390  $\frac{1}{2}$  and  $\frac{1$ 390 **A** WTF: WT<br>  $\frac{1}{3}$  and  $\frac{1}{3}$  and  $\frac{1}{3}$ <br>  $\frac{1}{3}$  and  $\frac{1}{3}$ <br>  $\frac{1}{3}$ carboxylic acid catabolic process alcohol metabolic process ● Count equiation of peptidase activity  $\bullet$  $• 10$ ۵ lipid catabolic process  $\bullet$  20 small molecule catabolic process ó  $30$ bone mineralization  $40$ chondrocyte differentiation  $\bullet$ negative regulation of hydrolase activity  $\bullet$ -log10(p.adju fatty acid metabolic process  $\bullet$ 20  $\bullet$ lipid transport  $15$ linid localization  $\bullet$ extracellular matrix organization  $\bullet$ 10 skeletal system morphogenesis  $\bullet$  $\bullet$ extracellular structure organization cartilage development ۰ external encapsulating structure organization limb development appendage development connective tissue development regulation of peptidase activity ۵ ossification SMAD protein signal transduction glycoprotein biosynthetic process  $0.04$  $0.08$   $0.12$  $0.04$  $0.08$  $0.12$  $0.04$   $0.08$   $0.12$  $0.04$  $0.08$   $0.12$ WTF WTM<br>KOF KOM -2  $\overline{\mathbf{0}}$ Gene Ratio

391

393 and male KO osteocyte transcriptomes have fewer differences compared to WT female and male<br>395 and male KO osteocyte transcriptomes have fewer differences compared to WT female and male<br>396 A: Volcano plot showing the

- 395 transcriptomes.<br>396 **a**: Volcano plot showing the significantly regulated genes between WT female control (WT F) and<br>397 MT male control (WT M) osteocyte transcriptome.<br>398 **B:** Volcano plot showing the significantly r
- 395 transcriptomes:<br>396 A: Volcar<br>397 WT male contro<br>398 B: Volca<br>399 and KO male co
- 
- 
- 
- 397 MT male control (WT M) osteocyte transcriptome.<br>
396 B: Volcano plot showing the significantly regulated genes between KO female control (KO F)<br>
398 B: Volcano plot showing the significantly regulated genes between WT B: Volcano plot showing the significantly and KO male control (KO M) osteocyte transcriptome.<br>
400 C: Volcano plot showing the significantly re<br>
401 KO male control (KO M) osteocyte transcriptome.<br>
402 D: Volcano plot show 399 and KO male control (KO M) osteocyte transcriptome.<br>
399 and KO male control (KO M) osteocyte transcriptome.<br>
399 and KO male control (KO M) osteocyte transcriptome.<br>
399 b: Volcano plot showing the significantly regul C: Volcano plot showing the significantly regulat<br>
399 and KO male control (KO M) osteocyte transcriptome.<br>
399 b: Volcano plot showing the significantly regulat<br>
399 and KO female control (KO F) osteocyte transcriptome.<br> 400<br>
400 C: Volcano plot showing the significantly regulated genes between WT female control (WT F) and<br>
402 D: Volcano plot showing the significantly regulated genes between WT female control (WT F) and<br>
403 KO female con 102 **D**: Volcano plot showing the significantly re<br>
103 KO female control (KO F) osteocyte transcriptome<br>
104 E: Heat map showing the differentially ex<br>
105 male control (WT M), KO female control (KO F), an<br>
105 F: Gene se 403<br>
402 D: Volcano plot showing the signincarity regulated genes around (WT F), WT<br>
402 E: Heat map showing the differentially expressed genes among WT female control (WT F), WT<br>
405 male control (WT M), KO female control E: Heat map showing the differentially exp<br>
404 E: Heat map showing the differentially exp<br>
405 F: Gene set enrichment analysis of Gene (<br>
407 genes between WT female control (WT F) and<br>
408 between KO female control (KO F 4045<br>
4046 E: Green set enrichment analysis of Gene Ontology (GO) analysis of the significantly regulated<br>
405 male control (WT M), KO female control (KO F), and KO male control (KO M) osteocyte transcriptome,<br>
406 E: Gree F: Gene set enrichment analysis of Gene Ontology (GO) analysis of the significantly regular<br>genes between WT female control (WT F) and WT male control (WT M) osteocyte transcriptome<br>between KO female control (KO F) and KO enes between WT female control (WT F) and WT male control (WT M) osteocyte transcriptome,<br>
408 between KO female control (KO F) and KO male control (KO M) osteocyte transcriptome, WT male<br>
409 control (WT M) and KO male co between KO female control (KO F) and KO male control (KO M) osteocyte transcriptome, WT male<br>
409 control (WT M) and KO male control (KO M) osteocyte transcriptome, and WT female control (WT F)<br>
410 and KO female control ( control (WT M) and KO male control (KO M) osteocyte transcriptome, and WT female control (WT F)<br>
and KO female control (KO F) osteocyte transcriptome. The figure shows the union of the top 10 GO<br>
terms of each analysis. If
- 
- 

and KO female control (KO F) osteocyte transcriptome. The figure shows the union of the top 10 GO<br>
terms of each analysis. If a term in the union, besides the top 10, is also significant (adjusted p-value<br>
less than 0.05 w that the mass of each analysis. If a term in the union, besides the top 10, is also significant (adjusted p-value<br>
412 less than 0.05 was used for GO analysis) in an analysis, it is also included in the figure.<br>
413 The la 412 dess than 0.05 was used for GO analysis) in an analysis, it is also included in the figure.<br>
413 The latter group in the figure's title is the reference group. n=3/group. For DEG analyonal<br>
414 unadjusted p-value <0.0 The latter group in the figure's title is the reference group. n=3/group<br>
414 unadjusted p-value <0.01 was used.<br>
415 With calcium deficiency, genes responsible for osteocytic osteolysis are lower in<br>
415 With calcium defi unadjusted p-value <0.01 was used.<br>
With calcium deficiency, genes responsible for osteocytic osteolysis are lower in the female KO<br>
compared to the female WT osteocyte transcriptome<br>
413 Calcium deficiency in WT female mi With calcium deficiency, genes<br>
empared to the female WT osteocyte<br>
417 Calcium deficiency in WT fem<br>
genes compared to WT females on a i<br>
419 2-4-fold in the calcium-deficient WT<br>
420 increase in *Tnsfs11, Acp5*, and *Cts* with calcium deficiency, genes responsible for osteocytic osteolysis are lower in the female KO<br>compared to the female WT osteocyte transcriptome<br>417 Calcium deficiency in WT female mice induced higher expression of osteoc 416 compared to the remain WT osteocyte transcriptome<br>417 Calcium deficiency in WT female mice indu<br>418 genes compared to WT females on a normal diet (Fi<br>419 2-4-fold in the calcium-deficient WT females. Rea<br>420 increase i expansive of the material on a normal diet (Fig. 5A, E). *Acp5*, *Ctsk, Pth1r*, and *Mst1r* were elevated<br>
419 Cal-fold in the calcium-deficient WT females. Real-time PCR analysis of osteocytes also showed an<br>
420 increase 418 genes compared to WT females on a normal diet (Fig. 3A, E). Acp3, Ctsk, Pth1r, and Mst1r were elevated<br>419 and the calcium-deficient WT females. Real-time PCR analysis of osteocytes also showed an<br>420 increase in *Tnsf* increase in *Tnsfs11, Acp5,* and *Ctsk gene* expression levels in the calcium-deficient WT females compared<br>to WT females on a normal diet. There was no difference in *Sost* expression (Sup Fig 2D). Additionally,<br>five diff 420 Increase in *majaar, Acpb*, and Cas gene expression levels in the calculativenent with emiales compared<br>421 to WT females on a normal diet. There was no difference in *Sost* expression (Sup Fig 2D). Additionally,<br>422 f 422 to WT females on a normal diet. There was no difference in Sost expression (Sup Fig 2D). Additionally,<br>422 tive different Mmps (*Mmp13*, *Mmp15*, *Mmp2*, *Mmp16*, and *Mmp14*) were upregulated 2-3.5-fold in the<br>424 ov 422 five different Mintys (Mmp23, Minty23, Minty2, Minty2, and Minty24) were upregulated 2-3.5-fold in the<br>423 WT calcium-deficient females. These are genes thought to play a role in osteocytic osteolysis. Bone<br>424 formati 424 formation and remodeling genes including *Bglap, Bglap2, Alpl, Wnt 5a*, and *Wnt 2b* were upregulated 2-<br>425 5-fold in the WT low calcium diet group compared to WT female normal diet group as well. These genes<br>426 may 424 formation and remodeling genes including *Dylap, Dylapz, Alpli, Wht* 5a, and Whit 2b were upregulated 2-<br>425 5-fold in the WT low calcium diet group compared to WT female normal diet group as well. These genes<br>426 may 426 may be increased to provide quick bone formation upon return to normal calcium demand.<br>18

426 may be increased to provide quick bone formation upon return to normal calcium demand.

427 428 osteoclast and resorption genes including Ctsk (2.8-fold), *Mmp13* (3- fold), and *Oscar* (2.6-fold) in<br>429 osteoclast and resorption genes including Ctsk (2.8-fold), *Mmp13* (3- fold), and *Oscar* (2.6-fold) in<br>429 c

Example to the Context and resorption genes including Ctsk (2.8-fold), *Mmp2* (3- fold), and Oscar (2.8-fold) in<br>
comparison levels of *Acp5* and *Pth1r* were not different in osteocytes from *KO* female mice on a<br>
anormal expression levels of *Acp5* and *Pth1r* were not different in osteocytes from KO female mice on a<br>normal diet or a low calcium-deficient KReal-time PCR analysis also showed an increase in *Ctsk* gene<br>expression level in t expression levels of Acp5 and Particle in the concludent in osteocytes from No female thee on a<br>normal diet or a low calcium-deficient KO females compared to KO females on a normal diet, with no<br>significant difference in t Formal diet or a low calcium diet. Real-time PCN analysis also showed an increase in Ctsk genes<br>expression level in the calcium-deficient KO females compared to KO females on a normal diet, with no<br>significant difference i significant difference in the expression levels of *Tnfsf11*, *Acp5*, and *Sost* genes (Sup fig 2D).<br>
Next, we compared KO female mice on a low-calcium diet to WT female mice on a low-calcium<br>
diet (Fig. 5C, E). Several bo Maximizant difference in the expression levels of Trigging, Acpb, and Sost genes (Sup fig 2D).<br>
Next, we compared KO female mice on a low-calcium diet to WT female mice on<br>
diet (Fig. 5C, E). Several bone resorption genes diet (Fig. 5C, E). Several bone resorption genes were lower by 2-fold in KO females, including Tnsfs11 and<br>436 Mmp15. Real-time PCR analysis also showed a significantly lower expression of the Tnsfs11 gene in the<br>437 calc Analysis diet (Fig. 5C, E). Several bone resorption genes were lower by 2-fold in KO females, including *Mpp15*. Real-time PCR analysis also showed a significantly lower expression of the *Tnsfs11* gene in the calcium - d Mmp15. Real-time PCR analysis also showed a significantly lower expression of the Thissand Colcum-deficient KO females compared to calcium-deficient WT females (Sup Fig 2D). Additionally, bone formation genes including Alp 438 bone formation genes including *Alpl, Bglap, Wht2b, Col1a1, Col1a2,* and *Postn* were also approximately 2-fold lower in the KO low calcium females compared to WT low calcium females. This suggests that female KO osteo 439 bone formation genes including Alpl, bylap, Whizb, Col1a1, Col1a2, and Postn were also<br>approximately 2-fold lower in the KO low calcium females compared to WT low calcium females. This<br>suggests that female KO osteocyte out also approximately 2-fold of the KO osteocytes are less responsive to calcium deficiency than female WT osteocytes. This is approximately compared to MT osteocytes. This is approximately compared to MT osteocytes. This osteocytes.<br>441 osteocytes are less responsive to calcium definition of calcium definitions of calcium definitions of the<br>Alternative to calcium definitions of the material was also calcium definitions of the material of t 441 osteocytes.



442

- 443<br>444<br>445<br>446 Fig 5: The Osteocyte transcriptomes from female WT and KO mice are distinct when challenged with<br>
444 **A:** Volcano plot showing the significantly regulated genes between WT female control (WT C) and<br>
446 **B:** Volcano plot
- 445 **a low-calcium dict**<br>445 **A:** Volcano<br>447 **B:** Volcano<br>448 KO female low-calc
- 
- Framale low-calcium diet-fed mice (WT lc) osteocyte transcriptome.<br>
8: Volcano plot showing the significantly regulated genes between KO female control (KO C) and<br>
445 KO female low-calcium diet-fed mice (KO lc) osteocyte B: Volcano plot showing the significantly regulated genes betw<br>
KO female low-calcium diet-fed mice (KO lc) osteocyte transcriptome.<br>
C: Volcano plot showing the significantly regulated genes betw<br>
mice (WT lc) and KO fema 448 Ko female low-calcium diet-fed mice (KO lc) osteocyte transcriptome.<br>448 Ko female low-calcium diet-fed mice (KO lc) osteocyte transcriptome.<br>449 c: Volcano plot showing the significantly regulated genes between WT fem C: Volcano plot showing the significantly regulated genes bet<br>mice (WT lc) and KO female low-calcium diet-fed mice (KO lc) osteo<br>D: Heat map showing the differentially expressed genes an<br>WT female low-calcium diet-fed mice 449 C: Volcano plot showing the significantly regulated genes between WT female low-calcium diet-fed<br>450 mice (WT lc) and KO female low-calcium diet-fed mice (KO lc) osteocyte transcriptome.<br>451 D: Heat map showing the dif 150 mice (WT in the state of the differentially expressed genes among WT female compared MT female low-calcium diet-fed mice (WT lc), KO female control (KO C), and KO female diet-fed mice (KO lc) osteocyte transcriptome.<br>
- WT female low-calcium diet-fed mice (WT Ic), KO female control (KO C), and KO female low-calcium<br>diet-fed mice (KO Ic) osteocyte transcriptome.<br>E: Gene set enrichment analysis of Gene Ontology (GO) analysis of the signific diet-fed mice (KOlc) osteocyte transcriptome.<br>
E: Gene set enrichment analysis of Gene Ontology (GO) analysis of the significantly regulated<br>
genes between WT female control (WT C) and WT female low-calcium diet-fed mice ( 454 **E:** Gene set enrichment analysis of Ge<br>455 genes between WT female control (WT C)<br>456 osteocyte transcriptome, between KO femal dentificant and the state of Gene Ontrology (CD) and the significantly regulated<br>455 genes between WT female control (CO) and WT female low-calcium diet-fed mice (WT Ic)<br>456 osteocyte transcriptome, between KO female contr osteocyte transcriptome, between KO female control (KO C) and KO female low-calcium diet-fed<br>20  $456$  female control (Ko C) and Ko female control (KO  $\geq$ ) and KO female low-calcium dietarial low-calcium dietarial di

- Figure 1578 male low-calcium diet-fed mice (KO lc) osteocyte transcriptome. The figure shows the union of<br>
4578 female low-calcium diet-fed mice (KO lc) osteocyte transcriptome. The figure shows the union of<br>
461 the top 1 Female low- calcium diet-fed mice (KO ic) osteocyte transcriptome. The figure shows the union of<br>the top 10 GO terms of each analysis. If a term in the union, besides the top 10, is also significant<br>(adjusted p-value less (adjusted p-value less than 0.05 was used for GO analysis) in an analysis, it is also included in the figure.<br>
The latter group in the figure's title is the reference group. n=2-3/group. For DEG analy<br>
unadjusted p-value The latter group in the figure's title is the reference group. n=2-3/group. For DEG anal<br>
462 unadjusted p-value <0.01 was used.<br>
465 With calcium deficiency, genes responsible for bone resorption, bone formation, and lipi unadjusted p-value <0.01 was used.<br>
With calcium deficiency, genes responsible for bone resorption, bone formation, and lipid metabolism<br>
are differentially regulated in the osteocyte transcriptome in male KO mice compared With calcium deficiency, genes r<br>
464 are differentially regulated in the osted<br>
465 Calcium deficiency in WT mal<br>
466 Ctsk, Oscar, and Mst1r in their osteod<br>
467 6A, E). Real-time PCR validation als<br>
expression levels in 463 are differentially regulated in the osteocyte transcriptome in male KO mice compared to male WT mice<br>465 Calcium deficiency in WT male mice caused a 2-7-fold increased expression of *Tnsfs11, Acp5*,<br>466 Ctsk, Oscar, an 464 are differentially regulated in the osteocyte transcriptome in male NO increased expression of Triangle 1465 Calcium deficiency in WT male microscriptome compared to WT males on a normal diet (647 B. Real-time PCR vali 265 Cfsk, Oscar, and Mstfr in their osteocyte transcriptome compared to WT males on a normal diet (Fig.<br>
64, E). Real-time PCR validation also showed a similar increase in Tnsfs11, Acp5, and Ctsk gene<br>
26, E). Real-time PC
- East, Oscur, and Mstr in their osteocyte transcriptome compared to WT males on a normal diet (Sup Fig.<br>
64, E). Real-time PCR validation also showed a similar increase in Tnsfs11, Acp5, and Ctsk gene<br>
expression levels in 468 expression levels in the calcium-deficient WT males compared to WT males on a normal diet (Sup Fig<br>
468 expression levels in the calcium-deficient WT males compared to WT males on a normal diet (Sup Fig<br>
469 2E). Bone 269 21. Bone formation and remodeling genes including *Postn, Col1a1, Col1a2, Bglap,* and *Wnt4* were also<br>
2470 elevated 2-4-fold in the WT male low calcium diet compared to the WT normal diet control group.<br>
2471 Multipl
- 2E). Boncelorington and remodeling genes including Postn, Coltaz, Coltaz, Pograp, and Whenevele also<br>
470 elevated 2-4-fold in the WT male low calcium diet compared to the WT normal diet control group.<br>
471 Multiple genes Multiple genes involved in the steroid and fatty acid metabolic process pathways as well<br>
471 lipid catabolic processes were downregulated 2-7-fold in the calcium-deficient WT males compare<br>
473 to WT males on a normal die Hipple catabolic processes were downregulated 2-7-fold in the calcium-deficient WT males compared<br>to WT males on a normal diet. These genes include several solute carrier family protein genes<br>stezZa2 and *Ste2Ta5*, severa to WT males on a normal diet. These genes include several solute carrier family protein genes<br>
SIc27a2 and SIc27a5, several apolipoprotein genes including Apoa1, ApoB, and Apoc1, several cyp<br>
quenes including Cyp2e1 and Cy SIEC27a2 and SIEC27a5, several apolipoprotein genes including Apoa1, ApoB, and Apoc1, several cyp<br>
475 senes including Cyp2e1 and Cyp7a1, and Plin1.<br>
476 similarly, osteocytes from KO males on a low calcium diet had a 2-4 Exercise and Sicz7a3, several apompoprocum genes including Apoc2, Apoc, and Apoc2, several cyp<br>genes including Cyp2e1 and Cyp7a1, and Plin1.<br>Similarly, osteocytes from KO males on a low calcium diet had a 2-4-fold higher e Ecrics including Cyp2e1 and Cyp7a1, and Plin1.<br>
476 Similarly, osteocytes from KO males on a<br>
477 such as *Col1a1*, *Col1a2*, *Alpl, Bglap*, and *Postn*<br>
479 diet (Fig. 6B, E). Therefore, genes responsible for<br>
480 in both osteoclast genes such as *Trafs11, Oscar,* and *Car3* and a 2-5-fold upregulation of bone formation genes<br>such as *Col1a1, Col1a2, Alpl, Bglap,* and *Postn* compared to osteocytes from KO males on a normal<br>diet (Fig. 6B, E
- 477 osteoclast genes such as TriayJ.11, Oscar, and Car3 and a 2-5-fold upregulation of bone formation genes<br>478 such as Col1a1, Col1a2, Alpl, Bglap, and Postn compared to osteocytes from KO males on a normal<br>479 diet (Fig. 36 such as Collaz, Alpl, Bglap, and Y sold contened to Osteocytes from No males on a normal<br>
479 diet (Fig. 6B, E). Therefore, genes responsible for bone resorption and bone formation were increased<br>
480 in both WT and KO 489 diet (Fig. 6C, E), there expression levels in the calcium-deficient KO males compared to KO males on a normal<br>449 diet, validating the RNA sequencing data (Sup Fig. 2E).<br>449 when KO males were compared to WT males on a 480 and Ctsk gene expression levels in the calcium-deficient KO males compared to KO males on a normal<br>482 diet, validating the RNA sequencing data (Sup Fig 2E).<br>483 when KO males were compared to WT males on a low calciu 482 and Ctsk gene expression levels in the calcium-deficient NO males compared to NO males on a riomination diet<br>482 diet, validating the RNA sequencing data (Sup Fig 2E).<br>484 bighter expression of bonne resorption genes When KO males were compared to WT males or<br>fold higher expression of bone resorption genes include<br>ass males compared to WTs. Several collagen formation<br>colsa2, Tnn, Aspn, and lgfbp6 were also significantly de<br>diet compare fold higher expression of bone resorption genes including *Oscar* and *Mst1r* in the KO low calcium diet<br>
males compared to WTs. Several collagen formation genes and ossification genes including *Col3a1*,<br> *Col8a2*, *Tnn*, Fold migher expression of bone resorption genes including Oscar and Mst1r in the KO low calcium det<br>
males compared to WTs. Several collagen formation genes and ossification genes including *Col3a1*,<br>
486 *Col3a2*, *Tnn*, Fraction genes and ossificant genes including colour,<br>
Fraction dist compared to WTs on a low-calcium diet. It is not clear whether these also play a role in the increased<br>
bone resorption observed with calcium deficiency Ecology, Thi, Asph, and Igfbp6 were also significantly downregulated in the Ko males on a low calciant<br>diet compared to WTs on a low-calcium deficiency in KO males. Real-time PCR analysis showed no<br>significant difference i Mone resorption observed with calcium deficiency in KO males. Real-time PCR analysis showed no<br>a significant difference in expression levels of *Tnsfs11*, *Acp5*, *Sost*, and *Ctsk* genes between calcium-<br>deficient KO male 1489 significant difference in expression levels of *Tnsfs11*, *Acp5*, *Sost*, and *Ctsk* genes between calcium-<br>1490 deficient KO males and calcium-deficient WT males, reflecting the RNA sequencing data (Sup Fig 2E). No<br>1 489 significant difference in expression levels of *majali*, Acp3, 3031, and Ctsk genes between calcium-<br>deficient KO males and calcium-deficient WT males, reflecting the RNA sequencing data (Sup Fig 2E). No<br>significant di 491 significant difference was observed in expression levels of genes involved in the lipid catabolic process  $\frac{21}{\sqrt{2}}$  $\frac{49}{21}$  significant difference was observed in expression levels of genes involved in the lipid catabolic process



## 492



494

- ---<br>494<br>495<br>496<br>497 495<br>496<br>497<br>498 Fig 6: The Osteocyte transcriptomes from male WT and KO mice are dis- tinct when challenged with a<br>
495 A: Volcano plot showing the significantly regulated genes between WT male control (WT C) and<br>
498 MT male low-calcium 497 **A**: Volcan<br>
498 WT male low-cal<br>
499 **B**: Volcan<br>
500 male low-calcium
- 
- 497 A: Volcano plot showing the significantly regulated genes between WT male control (WT C) and KO<br>499 B: Volcano plot showing the significantly regulated genes between KO male control (KO C) and KO<br>499 male low-calcium d 499 B: Volcano plot showing the significantly regulated genes between alle low-calcium diet-fed mice (KO lc) osteocyte transcriptome.<br>
501 C: Volcano plot showing the significantly regulated genes between mice (WT lc) and 499 B: Volcano plot showing the significantly regulated genes between KO male control (KO C) and KO male low-calcium diet-fed mice (KO lc) osteocyte transcriptome.<br> **C:** Volcano plot showing the significantly regulated gen 501 **C**: Volcano plot showing the significantly regulated genes<br>502 mice (WT lc) and KO male low-calcium diet-fed mice (KO lc) oste<br>503 **D**: Heat map showing the differentially expressed genes a<br>22 E: Volcano plot showing the significantly regulated genes between WT male low-calcium diet-fed<br>
mice (WT lc) and KO male low-calcium diet-fed mice (KO lc) osteocyte transcriptome.<br>
D: Heat map showing the differentially ex
- 503 **b**: Heat map showing the differentially expressed genes among WT male control<br>22 503 D: Heat map showing the differentially expressed genes among WT male control (WT C), WT male

504

1505 Ic) osteocyte transcriptome.<br>
506 E: Gene set enrichment analysis of Gene Ontology (GO) analysis of the significantly regulated<br>
507 E: Gene set enrichment analysis of Gene Ontology (GO) analysis of the significantly E: Gene set enrichment<br>
505 E: Gene set enrichment<br>
507 genes between WT male comments<br>
509 osteocyte transcriptome, a<br>
diet-fed mice (KO Ic) osteoc<br>
511 each analysis. If a term in th<br>
512 0.05 was used for GO analys<br>
513 software that the control (WT C) and WT male low-calcium diet-fed mice (WT lc) osteocyte<br>
stanscriptome, between KO male control (KO C) and KO male low-calcium diet-fed mice (KO lc)<br>
osteocyte transcriptome, and WT male lo transcriptome, between KO male control (KO C) and KO male low-calcium diet-fed mice (KO lc)<br>
steocyte transcriptome, and WT male low-calcium diet-fed mice (WT lc) and KO male low-calcium<br>
diet-fed mice (KO lc) osteocyte tr

osteocyte transcriptome, and WT male low-calcium diet-fed mice (WT lc) and KO male low-calcium<br>diet-fed mice (KO lc) osteocyte transcriptome. The figure shows the union of the top 10 GO terms of<br>each analysis. If a term in 510 osteocyte transcriptome. The figure shows the union of the top 10 GO terms of<br>511 eit-fed mice (KO lc) osteocyte transcriptome. The figure shows the union of the top 10 GO terms of<br>512 ech analysis, if a term in the u Fiam analysis. If a term in the union, besides the top 10, is also significant (adjusted p-value less than<br>
512 each analysis, if a term in the union, besides the top 10, is also significant (adjusted p-value less than<br>
51 512 0.05 was used for GO analysis) in an analysis, it is also included in the figure.<br>
512 0.05 was used for GO analysis) in an analysis, it is also included in the figure.<br>
515 The latter group in the figure's title is t 513 The latter group in the figure's title is the reference group. n=<br>514 unadjusted p-value <0.01 was used.<br>515 Male and female osteocytes respond differently to calcium deficiency in a<br>516 In response to 2 weeks of calci unadjusted p-value <0.01 was used.<br>
Male and female osteocytes respond differently to calcium deficiency in a genotype-specific manner<br>
In response to 2 weeks of calcium deficiency, WT female mice had higher expression of 515 **Male and female osteocytes res**<br>516 In response to 2 weeks of cal<br>517 involved in extracellular matrix and<br>518 male mice with calcium deficiency<br>529 increased expression of bone form<br>520 genes such as *Col2a1*, *Col6a* In response to 2 weeks of calcium deficiency, WT female mice had higher expression of geness<br>
involved in extracellular matrix and structure organization as well as ossification compared to WT<br>
male mice with calcium defic 517 involved in extracellular matrix and structure organization as well as ossification compared to WT<br>
518 male mice with calcium deficiency. Calcium deficiency in WT female mice caused significantly<br>
519 increased expre male mice with calcium deficiency. Calcium deficiency in WT female mice caused significantly<br>
increased expression of bone formation genes compared to WT males including several collagen<br>
genes such as  $Col2o1$ ,  $Col6o3$ ,  $Col4$ increased expression of bone formation genes compared to WT males including several collagen<br>genes such as  $Col2o1$ ,  $Col6a3$ ,  $Col4a2$ , as well as *Postn*, and  $Bglap2$ . This was accompanied by an<br>increased expression of bone re genes such as *Col2o1, Col6o3, Col4o2,* as well as *Postn,* and *Bglop2*. This was accompanied by an<br>
fincreased expression of bone resorbing genes in WT females including several *Car* genes, *Mmp13*,<br> *Mmp16, Tnsfs11,* Section as Col2a1, Coloca, Colemation as Postn, and Bglap2. This was accompanied by an increased expression of bone resorbing genes in WT females including several Car genes, *Mmp13*, diet (Fig. 7A, C, D). This suggests th

mercused expression of bone resorbing genes in WT females including several Car genes, immples, Mmp16, Thafs11, and Mst1r in their osteocyte transcriptome compared to WT males on a low-calcium delt (Fig. 7A, C, D). This s The mumples, Traying, The Mster in their osteocyte transcriptome compared to WT minics on a low caretain diet (Fig. 7A, C, D). This suggests that both bone formation and bone resorption are upregulated in WT females compa WT females compared to WT males in response to calcium deficiency, and WT females undergo<br>higher bone remodeling compared to WT males.<br>On the other hand, in response to calcium deficiency, KO female and male mice have less 525 higher bone remodeling compared to WT males.<br>
525 higher bone remodeling compared to WT males.<br>
527 significantly differently expressed genes compared to WT females and males (Fig &B, C, D). The<br>
528 major upregulated 526 On the other hand, in response to calciu<br>527 significantly differently expressed genes compain<br>528 major upregulated bone formation genes in K<br>6529 collagen genes such as *Col2a1* and *Col8a2*. The r<br>530 in KO females 527 significantly differently expressed genes compared to WT females and males (Fig &B, C, D). The<br>528 major upregulated bone formation genes in KO females compared to KO males include several<br>529 collagen genes such as 528 major upregulated bone formation genes in KO females compared to KO males include several<br>
stagen genes such as *Col2o1* and *Col8o2*. The major bone resorption gene that were upregulated<br>
in KO females compared to KO 529 collagen genes such as *Col2a1* and *Col8a2*. The major bone resorption gene that were upregulated in KO females compared to KO males were *Mmp13* and *Dcstamp*.<br>530 collagen genes such as *Col2a1* and *Col8a2*. The ma Follagen genes such as Col2a1 and Colouz. The major bone resorption gene that were upregulated<br>in KO females compared to KO males were *Mmp13* and *Destamp*.<br>23 m KO females compared to KO males were *Mmp13* and *Dcstamp*.



531

- ---<br>532<br>533<br>534<br>535 Fig 7: The Osteocyte transcriptomes from male and female line are distinct when challenged with a<br> **EXECUT A**: Volcano plot showing the significantly regulated genes between WT female low-calcium diet-<br> **EXECUT A**: Volcano
- 533 **Iow calcium dict**<br>535 fed (WTF) and W<br>535 fed (WTF) and W<br>536 **B**: Volcar<br>537 fed (KOF) and K
- 
- For extend with the significantly regulated genes between WT female low-calcium diet-<br>
B: Volcano plot showing the significantly regulated genes between KO female low-calcium diet-<br>
Fed (KO F) and KO male low-calcium diet-B. Volcano plot showing the significantly regulated genes between KO fema<br>
535 fed (KO F) and KO male low-calcium diet-fed mice (KO M) osteocyte transcriptome.<br>
538 C: Heat map showing the differentially expressed genes am 537 fed (KO F) and KO male low-calcium diet-fed mice (KO M) osteocyte transcriptome.<br>
538 fed (KO F) and KO male low-calcium diet-fed mice (KO M), WT female low-calcium diet-fed<br>
1539 mice (WT M), KO male low-calcium diet-538 C: Heat map showing the differentially expressed genes among WT male<br>539 mice (WT M), KO male low-calcium diet-fed mice (KO M), WT female low-calcium<br>540 KO female low-calcium diet-fed (KO F) osteocyte transcriptome.<br>5 C: Heat map showing the differentially expressed genes among WT male low-calcium diet-fed<br>
S39 mice (WT M), KO male low-calcium diet-fed mice (KO M), WT female low-calcium diet-fed (WT F), and<br>
S40 KO female low-calcium di KO female low-calcium diet-fed (KO F) osteocyte transcriptome.<br>
541 D: Gene set enrichment analysis of Gene Ontology (GO) analysis of the significantly regulated<br>
542 genes between WT female low-calcium diet-fed (WT F) and 541 **D:** Gene set enrichment analysis of Gene Ontology (GC<br>
542 genes between WT female low-calcium diet-fed (WT F) and WT<br>
543 osteocyte transcriptome, and between KO female low-calcium<br>
544 diet-fed mice (KO M) osteocyte S42 genes between WT female low-calcium diet-fed (WT F) and WT male low-calcium diet-fed mice (WT M) osteocyte transcriptome, and between KO female low-calcium diet-fed (KO F) and KO male low-calcium diet-fed mice (KO M) o 543 osteocyte transcriptome, and between KO female low-calcium diet-fed (KO F) and KO male low-calcium diet-fed mice (KO M) osteocyte transcriptome. The figure shows the union of the top 10 GO terms of each analysis. If a 544 diet-fed mice (KO M) osteocyte transcriptome. The figure shows the union of the top 10 GO terms of<br>545 each analysis. If a term in the union, besides the top 10, is also significant (adjusted p-value less than<br>24 545 each analysis. If a term in the union, besides the top 10, is also significant (adjusted p-value less than  $\frac{24}{\sqrt{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{1}{1-\frac{$  $\frac{1}{24}$

- 
- 547 The latter group in the figure's title is the reference group. n=2<br>548 unadjusted p-value <0.01 was used.
- $548$  unadjusted p-value <0.01 was used. 548 unadjusted p-value <0.01 was used.

Example 18<br>
550 Irisin ha<br>
551 working mainly<br>
552 bone, and brai<br>
553 al. (2021); Kin working mainly through its receptor  $\alpha V65$  integrin, has been shown to have powerful effects on fat,<br>
bone, and brain tissues *Bostrom et al.* (2012); *Tsourdi et al.* (2022); *Korta et al.* (2019); *Islam et<br>
al.* (2021 bone, and brain tissues *Bostrom et al.* (2012); Tsourd *et al.* (2022); Korta et al. (2019); Islam et al. (2012); Kinn et al. (2013); Kinn et al. (2014); Kinn et al. (2014); Kinn et al. (2014); Wirann et al. (2014); Wir S52<br>
S52 bone, and brain essays of the U. (2012); *Wann* et al. (2013); *Xin et al.* (2016); *Wann*<br>
et al. (2012); *Bao et al.* (2022); *Zhang et al.* (2022). With regard to bone, studies have generated to<br>
complex and ev East, the matrix (2021); Kim et al. (2021); Colaimm et al. (2021); Wrami et al. (2021); Wrami et al. (2021); With  $\theta$  al. (2021); Colaimni et al. (2018); Colaimni et al. (2018); Colaimni et al. (2018); Colaimni at al. (2 554<br>
Solution (2013); Kim et al. (2021); Laming et al. (2021); With regard to bone, studies have generated<br>
complex and even contradictory results *Erickson* (2013); *Mark et al.* (2021); *Colaianni and Grano*<br>
(2015); *Ki* 

Source and even contradictory results Erickson (2013); Matak et al. (2014); Colaidmin and Granou<br>
(2015); Kim et al. (2018); Estell et al. (2020); Colaianni et al. (2014); Zhang et al. (2018). Of note,<br>
the majority of bon Extern, hinnet al. (2019), stell et al. (2029), collatimized ant (2019), zalong et al. (2019). Or loce,<br>the majority of bone studies have been performed either exclusively on males, or females, but few<br>on both. Most studie 558 on both. Most studies have used recombinant irisin treatment whereas we have focused on the effects of deleting irisin. Other studies have mainly examined the effects on osteoblasts and osteoclasts, whereas our studies effects of deleting irisin. Other studies have mainly examined the effects on osteoblasts and<br>
steoclasts, whereas our studies have focused on osteocytes *Kim et al.* (2018).<br>
Global deletion of FNDCS on a normal diet had osteoclasts, whereas our studies have focused on osteocytes *Kim et al.* (2018).<br>
Show osteoclasts, whereas our studies have focused on osteocytes *Kim et al.* (2018).<br>
Show this bone has impaired mechanical properties. Th Signal of these osteoclasts, whereas our statics have focused on osteocytes Kim et al. (2018).<br>
Signal deletion of FNDC5 on a normal diet had essentially no effect court but in contrast, the null male mice have significant but in contrast, the null male mice have significantly more bone compared to wildtype males, but<br>this bone has impaired mechanical properties. This suggests that the lack of FNDC5 is having no<br>effect on the development or this bone has impaired mechanical properties. This suggests that the lack of FNDCS is having no<br>
effect on the development or growth of the female skeleton, but does affect the male skeleton,<br>
increasing the size yet impai

effect on the development or growth of the female skeleton, but does affect the male skeleton,<br>
increasing the size yet impairing matrix properties responsible for strength. Examination of their<br>
osteocytes showed that bot increasing the size yet impairing matrix properties responsible for strength. Examination of their<br>stecocytes showed that both female and male null mice have significantly fewer TRAP-positive<br>osteocytes compared to their s 556 osteocytes showed that both female and male null mice have significantly fewer TRAP-positive<br>
566 osteocytes compared to their sex-matched wildtype controls suggesting that their osteocytes<br>
367 osteocytic osteolysis a osteocytes compared to their sex-matched wildtype controls suggesting that their osteocytes<br>
ser more quiescent or less primed for bone resorption.<br>
Challenging the null animals with calcium deficiency revealed dramatic di For a more quiescent or less primed for bone resorption.<br>
Sesenting the null animals with calcium deficiency revealed dramatic differences in<br>
SFO osteocytic osteolysis and osteoclast activation, two major functions of ost Challenging the null animals with calcium deficent<br>569 Challenging the null animals with calcium deficent<br>571 FNDC5 in females is partially protective against calc<br>4572 accelerates both of these osteocyte functions result<br> 559 osteocytic osteolysis and osteoclast activation, two major functions of osteocytes. Deletion of<br>
571 FNDC5 in females is partially protective against calcium deficiency, but deletion in males<br>
3672 accelerates both of **EXECT EXECT THE EXECT STATE STATE** accelerates both of these osteocyte functions resulting in greater bone loss compared to<br>
straction, osteocytes express genes previously that under calcium-demanding conditions such as<br>
lactation, osteocytes express genes 573 controls. We have shown previously that under calcium-demanding conditions such as<br>
1674 lactation, osteocytes express genes previously thought only to be specific for osteoclasts<br>
1675 including cathepsin K, TRAP, ca 574 lactation, osteocytes express genes previously thought only to be specific for osteoclasts<br>
including cathepsin K, TRAP, carbonic anhydrase, the proton pump V-ATPase, and others Qing et<br>
575 controls. We have that ost For including cathepsin K, TRAP, carbonic anhydrase, the proton pump V-ATPase, and others Qing et al. (2012) and shown that osteocytes are the major source of RANKL *Nakashima et al.* (2011);<br> *Xiong and O'Brien* (2012); 575 *al.* (2012) and shown that osteocytes are the major source of RANKL *Nakashima et al.* (2011);<br>
577 *Xiong and O'Brien* (2012); *Xiong et al.* (2015). In this study, lactating females lacking FNDC5<br>
578 were partially Xiong and O'Brien (2012); Xiong et al. (2015). In this study, lactating females lacking FNDC5<br>
shown that of al. (2018). To determine the effects of calcium deficiency on males, mice were given a<br>
low-calcium diet for 2 we Example 19 Kim et al. (2018). To determine the effects of calcium deficiency on males, mice were given a<br>
S79 Kim et al. (2018). To determine the effects of calcium deficiency on males, mice were given a<br>
low-calcium diet Kim et al. (2018). To determine the effects of calcium deficiency on males, mice were given a<br>
sach to bone loss was exacerbated in null males compared to controls on a low calcium diet.<br>
With two weeks of lactation and li For the effects of the effects of FNDC5/irisin deletion in females,<br>
bone loss was exacerbated in null males compared to controls on a low calcium diet.<br>
With two weeks of lactation and litter size comparable to wildtype c 581 bone loss was exacerbated in null males compared to controls on a low calcium diet.<br>
582 With two weeks of lactation and litter size comparable to wildtype controls, the null<br>
26

582 bone loss with two weeks of lactation and litter size comparable to wildtype controls on a low calcium diet.<br>26 582 With two weeks of lactation and litter size comparable to wildtype controls, the null

583 osteocytes, and smaller lacunar size. Our observation that the deletion of FNDC5/ irisin makes<br>
state inctating mice partially resistant to bone loss has an important implication with regard to the<br>
purpose of lactation. L Examples are and smaller than the bone loss has an important implication with regard to the<br>
purpose of lactation. Lactation is a critical period for pups as they obtain essential nutrients,<br>
especially calcium, from the m purpose of lactation. Lactation is a critical period for pups as they obtain essential nutrients,<br>especially calcium, from the mother's milk for their proper growth. Calcium lost by the<br>mother's bone during lactation is ra specially calcium, from the mother's milk for their proper growth. Calcium lost by the<br>stass mother's bone during lactation is rapidly replaced upon weaning with complete recovery of bone<br>mass within a week Qing et al. (20

588 mother's bone during lactation is rapidly replaced upon weaning with complete recovery of bone<br>
588 mother's bone during lactation is rapidly replaced upon weaning with complete recovery of bone<br>
589 mass within a week mass within a week Qing et al. (2012); Wysolmerski (2002); Kalkwarf (2004); Kovacs (2001);<br>
589 motes within a week Qing et al. (2012); Wysolmerski (2002); Kalkwarf (2004); Kovacs (2001);<br>
589 moternal bones to fulfill the 589 *Musim* a week canglet al. (2012); Wysolmerski (2002); Rahkwarf (2004); Novacs (2001);<br>589 *Mysolmerski* (2012). Our data suggest that FNDC5/irisin acts as a regulator of calcium release from<br>589 maternal bones to fulf MySolineski (2012). Our data suggest that FNOC5/irisin acts as a regulator of calcium release from<br>
maternal bones to fulfill the offspring survival and consequently, successful reproduction.<br>
To determine if low calcium w beneficial role in ensuring offspring survival and consequently, successful reproduction.<br>
To determine if low calcium would have a similar effect on male FNDC5 null bone, both<br>
males and females were subjected to a low-ca 593 To determine if low calcium would have a similar effect on male FNDC5 null<br>
594 males and females were subjected to a low-calcium diet for 2 weeks. The effects of a<br>
595 diet on female osteocytes and bone loss were ess males and females were subjected to a low-calcium diet for 2 weeks. The effects of a low-calcium<br>diet on female osteocytes and bone loss were essentially identical to the effects of lactation, with<br>two exceptions. First, s diet on female osteocytes and bone loss were essentially identical to the effects of lactation, with<br>two exceptions. First, serum RANKL levels were not significantly different between virgin and<br>lactating null females, whi two exceptions. First, serum RANKL levels were not significantly different between virgin and<br>
space to the suggesting that RANKL plays less of a role in lactation compared to acalcium<br>
diet suggesting that RANKL plays les <sup>597</sup><br> **Example 1968** is they were between null females on a normal compared to a calcium<br>
diet suggesting that RANKL plays less of a role in lactation compared to calcium deficiency.<br>
Secondly, the medullary cavity and en diet suggesting that RANKL plays less of a role in lactation compared to calcium deficiency.<br>
Secondly, the medullary cavity and endosteal bone in the low-calcium females were completely<br>
protected in the FNDC5 null female Secondly, the medullary cavity and endosteal bone in the low-calcium females were completely<br>protected in the FNDC5 null females but were not in the lactating FNDC5 null mice. Bone loss due to<br>lactation or due to dietary c From the medulary calcium deficiency may target different bone iss due to food increase to food increase that endosteal bone is removed faster than periosteal bone isse. Our unpublished observations suggest that endosteal Follow and the to dietary calcium deficiency may target different bone sites. Our unpublished<br>
602 observations suggest that endosteal bone is removed faster than periosteal bone with lactation, but<br>
this remains to be car 602 observations suggest that endosteal bone is removed faster than periosteal bone with lactation, but<br>
this remains to be carefully validated. This difference may also be due to elevated PTHrP during<br>
for due to differen this remains to be carefully validated. This difference may also be due to elevated PTHrP during<br>
foos this remains to be carefully validated. This difference may also be due to elevated PTHrP during<br>
lactation *Kovacs* (2 For idea is not clear if hormones target distinct bone sites. Similar to the lactating PTH levels Goltzman (2008),<br>
and it is not clear if hormones target distinct bone sites. Similar to the lactating FNDCS null mice,<br>
th External increases interests. Similar to the lactating PNDCS null mice,<br>the null firmals placed on the low calcium diet had fewer TRAP-positive ostecolasts, fewer TRAP-<br>positive ostecolasts and analler lactarian size. Seru the null females placed on the low calcium diet had fewer TRAP-positive osteoclasts, fewer TRAP-positive osteocytes, and smaller lacunar size. Serum RANKL levels increased in both wildtype and null mice with dietary calciu mull mice with dietary calcium deficiency, therefore, serum RANKL alone is not enough to explain<br>
for the partial protective effect of FNDCS deletion against bone loss. In summary, female null mice<br>
are not only resistant

(2018) but are also resistant to calcium deficiency either due to an increase in PTHrP as with<br>612 lactation, or an increase in PTH as with a low calcium diet.<br>613 Osteoporosis manifests earlier in females due to menopause the partial protective effect of FNDCS deletion against bone loss. In summary, female null mice<br>
for are not only resistant to bone loss due to estrogen deficiency as we showed previously *Kim*<br>
(2018) but are also resista Figure 1009 are not only resistant to bone loss due to estrogen deficiency as we showed previously *Kim*<br>
610 are not only resistant to calcium deficiency either due to an increase in PTHrP as with<br>
12018) but are also res (2018) but are also resistant to calcium deficiency either due to an increase in PTHrP as with<br>
fatation, or an increase in PTH as with a low calcium diet.<br>
613 but previously manifests earlier in females due to menopause Figure 12018) but are also seen in PTH as with a low calcium diet.<br>
Costeoporosis manifests earlier in females due to menopause, but males also develop<br>
osteoporosis but at an older age *Johannesdottir et al.* (2013); *Joh* 613 Osteoporosis manifests earlier in females due to<br>614 osteoporosis but at an older age *Johannesdottir et al.* (20<br>615 the elderly are known to suffer from calcium deficiency wh<br>616 al. (2015); *Body et al.* (2016). Die 614 osteoporosis but at an older age *Johannesdottir et al.* (2013); *Johnston and Dagar* (2020), and<br>615 the elderly are known to suffer from calcium deficiency which accelerates bone loss Kumssa et<br>616 al. (2015); *Body* 615 the elderly are known to suffer from calcium deficiency which accelerates bone loss Kumssa et al. (2015); Body et al. (2016). Dietary calcium deficiency has been shown previously to affect female and male bone differen and the enderly are the elder to suffer from calcium deficiency has been shown previously to affect<br>
female and male bone differently where female rat bones are more sensitive to a low calcium diet<br>
27 for the first of the property of all (2021). Dietary calculations are more sensitive to a low calcium diet<br>for the property where female rat bones are more sensitive to a low calcium diet<br>27 617 female and male bone differently where female rat bones are more sensitive to a low calcium diet

618 Figure 1998<br>
619 females were more affected by calcium deficiency and lost more bone compared to wildtype<br>
620 male mice. However, the opposite was observed for the FNDC5/irisin null mice, where female<br>
621 null mice were male mice. However, the opposite was observed for the FNDC5/irisin null mice, where female<br>
fo20 male mice. However, the opposite was observed for the FNDC5/irisin null mice, where female<br>
null mice were partially resistan null mice were partially resistant, and male null mice were more susceptible to bone loss with<br>calcium deficiency compared to their wildtype counterparts. Despite starting with more bone<br>623 volume compared to wildtype, th 622 calcium deficiency compared to their wildtype counterparts. Despite starting with more bone<br>623 volume compared to wildtype, the FNDC5 null males had increased osteocyte lacunar area and<br>624 lost more bone with dietary For the their latter of ottoor is a sexually direct the theorem of the theorem and their wildtype, the FNDC5 null males had increased osteocyte lacunar area and<br>lost more bone with dietary calcium deficiency compared to wi

624 lost more bone with dietary calcium deficiency compared to wild-type males. This greater bone<br>625 loss can be explained through the dramatic increase of TRAP-positive osteocytes and TRAP-<br>626 positive osteoclasts, but 624 lost more bone with dietary calcium deficiency compared to wild-type males. This greater bone<br>625 loss can be explained through the dramatic increase of TRAP-positive osteocytes and TRAP-<br>626 positive osteoclasts, but Here we report that osteocyte lacunar size is significantly larger in virgin wildtype female mice<br>
compared to same-age wildtype males. This difference in lacunar area indicates a distinction<br>
between female and male osteo 627 difference indicates that FNDC5/irisin may be involved in the regulation of calcium release from<br>628 bone via osteocytes in a sex-dependent manner.<br>629 Lacunar area is an indicator of osteocyte regulation of their lac 628 bone via osteocytes in a sex-dependent manner.<br>
629 Lacunar area is an indicator of osteocyte regulation of their lacunar microenvironment.<br>
630 Here we report that osteocyte lacunar size is significantly larger in vi Example 12 bond and male of osteocyte<br>
629 Lacunar area is an indicator of osteocyte<br>
630 Here we report that osteocyte lacunar size is sig<br>
compared to same-age wildtype males. This dif<br>
632 between female and male osteoc For that osteocyte lacunar size is significantly larger in virgin wildtype female mice<br>
630 Here we report that osteocyte lacunar size is significantly larger in virgin wildtype female mice<br>
631 compared to same-age wildty compared to same-age wildtype males. This difference in lacunar area indicates a distinction<br>
between female and male osteocyte function. The mammalian skeleton is a sexually dimorphic<br>
organ *Sharma* (2023), and female an between female and male osteocyte function. The mammalian skeleton is a sexually dimorphic<br>
organ *Sharma* (2023), and female and male bones respond differently to circulating factors,<br>
hormones, and myokines as well as o For example and male bones respond differently to circulating factors,<br>
hormones, and myokines as well as other challenges *Kurapaty and Hsu* (2022); *Lu et al.* (2022);<br>
Cosipov et al. (2022). As osteocytes are regulator 634 hormones, and myokines as well as other challenges *Kurapaty and Hsu* (2022); *Lu et al.* (2022);<br>635 *Osipov et al.* (2022). As osteocytes are regulators of bone formation and resorption *Bonewald*<br>(2011); *Dallas et* 635 *Osipov et al.* (2022). As osteocytes are regulators of bone formation and resorption *Bonewald* (2011); *Dallas et al.* (2013); *Robling and Bonewald* (2020), this sex difference may be due to differences in male and G35 Osipov et al. (2022). As osteocytes are regulators of bone formation and resorption *Bonewald*<br>
(2011); *Dallas et al.* (2013); *Robling and Bonewald* (2020), this sex difference may be due to<br>
differences in male and 637 differences in male and female osteocytes. A recent study by Youlten and colleagues has<br>638 shown that male and female osteocyte transcriptomes are distinctly different *Youlten et al.*<br>(2021). At 4 weeks of age, the 638 shown that male and female osteocyte transcriptomes are distinctly different *Youlten et al.* (2021). At 4 weeks of age, the female osteocyte transcriptome diverges from the male osteocyte transcriptome and these diff (2021). At 4 weeks of age, the female osteocyte transcriptome diverges from the male osteocyte<br>
640 transcriptome and these differences continue with age. A cluster of genes more highly<br>
expressed in female osteocytes com Example 1921). This suggests of the set age, the female osteocytes are those involved in bone<br>
fe40 transcriptome and these differences continue with age. A cluster of genes more highly<br>
expressed in female osteocytes com expressed in female osteocytes compared to male osteocytes are those involved in bone<br>641 expressed in female osteocytes compared to male osteocytes are those involved in bone<br>643 include genes necessary for osteocytic per

Expression, the same ones elevated in osteocytes in response to lactation. These transcripts<br>
for espression, the same ones elevated in osteocytic perilacunar remodeling and reduction in pH, which are<br>
essential for calci include genes necessary for osteocytic perilacunar remodeling and reduction in pH, which are<br>
644 essential for calcium removal Qing et al. (2012). This suggests that the larger lacunar area in<br>
645 female osteocytes comp 644 essential for calcium removal Qing et al. (2012). This suggests that the larger lacunar area in<br>645 female osteocytes compared to male osteocytes may be due to the higher expression of bone<br>646 resorption genes.<br>The m From the larger than the larger that the larger that the larger term of the removal of the resorption genes.<br>
The magnitude of the effect size due to FNDC5 deficiency appears modest with regards<br>
to the quantitative cortic From the magnitude of the effect size due to FNDCS deficiency appears modest with regards<br>
for the quantitative cortical bone parameters. However, if one examines the changes in<br>
osteocyte lacunar size and the mechanical p 647 The magnit<br>648 to the quantitativ<br>649 osteocyte lacunar<br>650 greater. As shown<br>651 increases by ove<br>652 approximately 389 to the quantitative cortical bone parameters. However, if one examines the changes in<br>
osteocyte lacunar size and the mechanical properties of these bones, the differences are<br>
greater. As shown in Figure 3 E, the lacunar 649 osteocyte lacunar size and the mechanical properties of these bones, the differences are<br>650 greater. As shown in Figure 3 E, the lacunar area of the wildtype females on a low calcium diet<br>651 increases by over 30% and exted by the mechanical properties and the mechanical properties of the wildtype females on a low calcium diet<br>
increases by over 30% and the FNDC5-null by less than 20%, while in the males it is<br>
approximately 38% in wild 651 increases by over 30% and the FNDC5-null by less than 20%, while in the males it is<br>approximately 38% in wildtype compared to 46% in null. According to Sims and Buenzli *Buenzli*<br>28 approximately 38% in wildtype compared to 46% in null. According to Sims and Buenzli *Buenzli*<br>28  $\begin{array}{c} \hline \text{1} \end{array}$  38

and sims (2015), a potential total loss of  $^{\circ}$ 16,000 mm (16 mL) of bone occurs through lactation<br>
in the human skeleton. This was based on our measurements in lactation-induced murine<br>
osteocytic osteolysis Qing et al. 655 osteocytic osteolysis *Qing et al.* (2012). They used our 2D section of tibiae from lactating mice<br>655 osteocytic osteolysis *Qing et al.* (2012). They used our 2D section of tibiae from lactating mice<br>656 showing an 656 showing an increase in lacunar size from 38 to 46  $\mu$ m<sup>2</sup>. In that paper we also showed that<br>657 canalicular width is increased with lactation. Therefore, this suggests dramatically lower<br>intracortical porosity due t showing an increase in lacunar size from 38 to 46 µm<sup>2</sup>. In that paper we also showed that<br>canalicular width is increased with lactation. Therefore, this suggests dramatically lower<br>intracortical porosity due to the osteoc intracortical porosity due to the osteocyte lacunocanalicular system in female null mice<br>
compared to female wild-type mice either with lactation or a low calcium diet and a dramatic<br>
increase in intracortical porosity in

659 compared to female wild-type mice either with lactation or a low calcium diet and a dramatic<br>660 increase in intracortical porosity in null males compared to wild-type males on a low calcium<br>661 diet. Based on these d For increase in intracortical porosity in null males compared to wild-type males on a low calcium<br>diet. Based on these data, using the FNDCS null animals, we would speculate that the product<br>of FNDCS, irisin, is having a s diet. Based on these data, using the FNDC5 null animals, we would speculate that the product<br>of FNDC5, irisin, is having a significant effect on the ultrastructure of bone in both males and<br>females challenged with a low ca of FNDC5, irisin, is having a significant effect on the ultrastructure of bone in both males and<br>
females challenged with a low calcium diet.<br>
To begin to understand the molecular mechanisms responsible for the sex and gen for formales challenged with a low calcium diet.<br>
To begin to understand the molecular mechanisms responsible for the sex and genotype<br>
differences, we compared the osteoxyte transcriptomes of 5-month-old female and male, To begin to understand the molecula<br>664 To begin to understand the molecula<br>665 and null mice. Our results show that the ost<br>667 mice are significantly different under norm<br>668 but not described in the Youlten paper Yo<br>669 differences, we compared the osteoxyte transcriptomes of 5-month-old female and male, wildtype<br>and null mice. Our results show that the osteocyte transcriptomes of female and male, wildtype<br>mice are significantly different and null mice. Our results show that the osteocyte transcriptomes of female and male wildtype<br>
mice are significantly different under normal conditions. A surprising difference we observed<br>
but not described in the Youlten mice are significantly different under normal conditions. A surprising difference we observed<br>but not described in the Youlten paper *Youlten et al.* (2021) was that compared to wildtype<br>female osteocytes, wildtype male os but not described in the Youlten paper *Youlten et al.* (2021) was that compared to wildtype<br>female osteocytes, wildtype male osteocytes have much higher expression of genes involved in<br>steroid, lipid, and cholesterol meta 669 female osteocytes, wildtype male osteocytes have much higher expression of genes involved in steroid, lipid, and cholesterol metabolism and transport pathways, lipid and solute carrier genes, and apolipoprotein genes. steroid, lipid, and cholesterol metabolism and transport pathways, lipid and solute carrier<br>genes, and apolipoprotein genes. This suggests that osteocyte metabolism and bioenergetics<br>are distinctly different between wildty 671 steroid, and apolipoprotein genes. This suggests that osteocyte metabolism and bioenergetics<br>
are distinctly different between wildtype females and wildtype males. We hypothesize that the<br>
differentially expressed gene For an edistinctly different between wildtype females and wildtype males. We hypothesize that the differentially expressed genes in these bioenergetic and metabolic pathways modulate bone mass and formation and may shed l 673 differentially expressed genes in these bioenergetic and metabolic pathways modulate bone<br>674 mass and formation and may shed light on the sexual dimorphism of bones. As these<br>675 differentially regulated pathways wer mass and formation and may shed light on the sexual dimorphism of bones. As these<br>differentially regulated pathways were not previously reported by Youlten and colleagues<br>Youlten et al. (2021), this may be due to differenc

differentially regulated pathways were not previously reported by Youlten and colleagues<br>
for *soulten et al.* (2021), this may be due to differences in strain, housing, diet, or microbiome.<br>
Another explanation is the gr For the state of the state Another explanation is the greater osteocyte purity in our study as we used a series of collagenase<br>
digestions and EDTA chelation to remove any surface cells which was not performed in the<br>
Youlten paper *Youlten et al.* For difference of the greater and the greater of collagen matrix of the difference cells which was not performed in the Youlten paper *Youlten et al.* (2021).<br>
A second major difference between female and male wildtype ost For the paper Youlten et al. (2021).<br>
Second major difference between female and male wildtype osteocytes was the higher<br>
expression of genes involved in collagen matrix formation, bone mineralization, remodeling,<br>
resorp 680 A second major difference be<br>
681 expression of genes involved in co<br>
682 resorption, and osteocytic osteolysis<br>
683 expressed bone resorption genes<br>
684 elevated during lactation *Qing et*<br>
685 osteocytic osteolysis. expression of genes involved in collagen matrix formation, bone mineralization, remodeling,<br>resorption, and osteocytic osteolysis pathways in females compared to males. Many of the highly<br>expressed bone resorption genes in Fraction, and osteocytic osteolysis pathways in females compared to males. Many of the highly<br>expressed bone resorption genes in wildtype female osteocytes have been shown to be<br>elevated during lactation *Qing et al.* (20 expressed bone resorption genes in wildtype female osteocytes have been shown to be<br>elevated during lactation *Qing et al.* (2012) including *Acp5*, *Ctsk*, and *Mmp13*, all involved in<br>osteocytic osteolysis. This further elevated during lactation *Qing et al.* (2012) including *Acp5*, *Ctsk*, and *Mmp13*, all involved in<br>685 osteocytic osteolysis. This further supports our hypothesis that wildtype female osteocytes are<br>686 more primed for ocal elevated during lactation Qing et al. (2012) including Acp5, CEsk, and Minp13, an involved in<br>685 osteocytic osteolysis. This further supports our hypothesis that wildtype female osteocytes are<br>686 more primed for res more primed for resorption compared to wildtype males, presumably to meet the increased<br>calcium demand during lactation, and correlates with the observed larger lacunae compared to<br>29 calcium demand during lactation, and correlates with the observed larger lacunae compared to<br>29  $\frac{1}{29}$  calcium demand during lactation, and correlates with the observed larger lacunas compared to  $\frac{1}{29}$ 

688 males.

Example 18<br>
689<br>
690<br>
691 recept<br>
692 signific<br>
693 betwee<br>
694 be resi:<br>
695 investig<br>
696 in the p<br>
697 the ost<br>
698 showed<br>
698 showed<br>
699 necess:<br>
700 0 Alliston and colleagues generated transgenic mice with reduced expression of the TGFβ Type II<br>
receptor in mice expressing Dmp1-Cre *Dole et al.* (2020) (PMID: 32282961) and found a<br>
significant difference in bone paramet For the microsomorphism and markers and markers of osteocyte perilacunar remodeling<br>
significant difference in bone parameters and markers of osteocyte perilacunar remodeling<br>
between the sexes. The females were subjected 692 significant difference in bone parameters and markers of osteocyte perilacunar remodeling<br>692 significant difference in bone parameters and markers of osteocyte perilacunar remodeling<br>694 be resistant to osteocytic o EGB3<br>
between the sexes. The females were subjected to lactation and the transgenics were found to<br>
be resistant to osteocytic osteolysis compared to controls. However, these investigators did not<br>
investigate the lacunar be resistant to osteocytic osteolysis compared to controls. However, these investigators did not<br>investigate the lacunar remodeling process in males as compared to females as was performed<br>in the present study using a low Examples the lacunar remodeling process in males as compared to females as was performed<br>
in the present study using a low calcium diet. Their study does suggest that TGFB is involved in<br>
the osteocytic osteolysis that oc in the present study using a low calcium diet. Their study does suggest that TGFB is involved in<br>
the osteocytic osteolysis that occurs with lactation, however, even though the transgenic males<br>
showed a disrupted lacunoc 697 the osteocytic osteolysis that occurs with lactation, however, even though the transgenic males<br>698 showed a disrupted lacunocanlicular network compared to wildtype males, this does not<br>699 necessarily indicate a defe showed a disrupted lacunocanlicular network compared to wildtype males, this does not<br>necessarily indicate a defect in perilacunar remodeling. It is more likely that the defect<br>occurred during bone formation when osteoblas meeting indicate a defect in perilacunar remodeling. It is more likely that the defect<br>occurred during bone formation when osteoblasts were differentiating into osteocytes. In our<br>study, we observed a higher expression of

occurred during bone formation when osteoblasts were differentiating into osteocytes. In our<br>
study, we observed a higher expression of *TGF63* in wildtype female mice compared to<br>
wildtype male mice, with no significant d 201 study, we observed a higher expression of *TGF63* in wildtype female mice compared to wildtype male mice, with no significant differences in *TGF61* or *TGF62* expression. This suggests that *TGF63* may play a role in 3004, we observed a migher expression of 19075 in wildtype innale interecting<br>2020 wildtype male mice, with no significant differences in *TGFθ1* or *TGFθ2* expression. This<br>301 study, the valificant of transformalism of Wildtype male mice, with no significant differences in TGFB1 or TGFB2 expression. This<br>suggests that TGFβ3 may play a role in generating the larger lacunar area in wildtype females<br>compared to wildtype males through incre Suggests that FGFβ3 may play a role in generating the larger lacunar area in windype remates<br>
compared to wildtype males through increased matrix related signaling.<br>
Few differences were observed between wildtype female a For differences were observed between wildtype female and<br>
To transcriptomes as would be expected for bone morphometry and the or<br>
was the number of TRAP-positive osteocytes. In contrast, osteocytes fr<br>
null males are sign The transcriptomes as would be expected for bone morphometry and the only difference observed<br>
The number of TRAP-positive osteocytes. In contrast, osteocytes from wildtype males and<br>
The null males are significantly diffe The matter as the number of TRAP-positive osteocytes. In contrast, osteocytes from wildtype males and<br>
roll males are significantly different with regards to fatty acid and lipid metabolism pathways<br>
whereas null male mice To mull males are significantly different with regards to fatty acid and lipid metabolism pathways<br>
This suggests a role for irisin in lipid metabolism and bioenergetics in male osteocytes. Lower<br>
This suggests a role for Whereas null male mice have lower expression of these genes compared to wildtype males.<br>
This suggests a role for irisin in lipid metabolism and bioenergetics in male osteocytes. Lower<br>
expression in the null male mice may

The Superstian of the initial metabolism and bioenergetics in male osteocytes. Lower<br>This suggests a role for irisin in lipid metabolism and bioenergetics in male osteocytes. Lower<br>expression in the null male mice may be r expression in the null male mice may be responsible for the higher bone mass and inferior<br>
712 Diomechanical properties compared to wildtype males suggesting these pathways mediate the<br>
715 Osteocytes from null females ha For the null males are particles in the null males suggesting these pathways mediate the effects of FNDCS/irisin on male bone.<br>
Osteocytes from null females have higher expression of genes and pathways involved in collage The effects of FNDC5/irisin on male bone.<br>
Osteocytes from null females have higher expression of genes and pathways involved in<br>
collagen matrix organization, ossification, and mineralization compared to null males. Unlik The Contract of Figure of Figure 1974.<br>
715 collagen matrix organization, ossifica<br>
715 wildtype males and females, there was<br>
717 acid metabolism genes in null male<br>
718 FNDC5/irisin regulates male bone through<br>
719 Lacta For the matrix organization, ossification, and mineralization compared to null males. Unlike<br>
wildtype males and females, there was no difference in expression of lipid, cholesterol, and fatty<br>
acid metabolism genes in nu 216 wildtype males and females, there was no difference in expression of lipid, cholesterol, and fatty<br>217 acid metabolism genes in null males compared to null females. Again, this indicates that<br>218 FNDC5/irisin regulates

acid metabolism genes in null males compared to null females. Again, this indicates that<br>
FNDC5/irisin regulates male bone through these lipid-related pathways.<br>
Lactation and calcium deficiency induce the same changes in FINDC5/irisin regulates male bone through these lipid-related pathways.<br>
Lactation and calcium deficiency induce the same changes in females. Similar to that<br>
reported previously for lactation *Qing et al.* (2012), osteocy FRD FRD FRD CHERR INTERTION INTERTAINS 19<br>
The Lettation and calcium deficiency induce the same changes in f<br>
reported previously for lactation *Qing et al.* (2012), osteocytes from wildty<br>
calcium diet exhibited an increa The reported previously for lactation *Qing et al.* (2012), osteocytes from wildtype female mice on a low<br>
calcium diet exhibited an increase of several osteo- clast/resorption/lactation genes including Acp5,<br>
30 reported previously for lactation Qing et al. (2012), osteocytes from wildtype female linee on a low<br>calcium diet exhibited an increase of several osteo- clast/resorption/lactation genes including *Acp5*,<br>30 Fraction diet exhibited an increase of several osteo- clast/resorption/lactation genes including Acp5,

East, Oscar, Mistar, and Parti compared to wheely lemnes on a normal diet. Surprisingly, we associeted an increase in bone formation genes including Collad, Alpl, and Bglap. As osteocytic osteolysis is rapidly reverse bone

observed an increase in othe formation genes including Col1a1, Alpl, and Bglap. As osteocyte<br>osteolysis is rapidly reverse bone loss. We propose that once calcium is replemished, shutting off the proton pump will<br>rapidly r reverse bone loss. We propose that once calcium is replenished, shutting off the proton pump will<br>
rapidly reverse the pH within the osteocyte lacunae, allowing bone-forming proteins such as<br>
alkaline phosphatase to become rapidly reverse the pH within the osteocyte lacunae, allowing bone-forming proteins such as<br>alkaline phosphatase to become active to rapidly replace the osteocyte perilacunar matrix *Jahn* et<br>al. (2017); *Andersson et al.* 727 alkaline phosphatase to become active to rapidly replace the osteocyte perilacunar matrix *Jahn* et *al.* (2017); *Andersson et al.* (2003); *Silver et al.* (1988); *Kaplan* (1972); *Farley and Baylink* (1986).<br>
728 Th 227 and (2017); *Andersson et al.* (2003); *Silver et al.* (1988); *Kaplan* (1972); *Farley and Baylink* (1986).<br>
The main molecular mechanism responsible for the resistance of null female mice to<br>
calcium deficiency comp 228 allevative materials are considered at the significant control in the main molecular mechanism responsible for the resistance of null fermale mice<br>
228 calcium deficiency compared to wildtype female mice is lower expre calcium deficiency compared to wildtype female mice is lower expression of genes such as<br> *Thfsf11*, responsible for osteoclastic resorption. A correspondingly lower expression of bone<br>
formation genes including *Col1a1*,

*The The The The The The The The The Compared to selection* and *Fnfsf11*, responsible for osteoclastic resorption. A correspondingly lower expression of bone<br>formation genes including *Col1o1*, *Alpl*, and *Bglap* compar *Thisyax*, responsible for osteoclastic resorption. A correspondingly lower expression of bone<br>formation genes including *Coltat*, *Apl*, and *Bglap* compared to wildtype females on a low-<br>calcium diet was observed. The l Formation genes including Collat, Alpl, and Bglap compared to which entired on a low-<br>calcium diet was observed. The lower expression of both formation and resorption genes<br>suggests a coupling of resorption with formation 1334 suggests a coupling of resorption with formation. Irisin appears to regulate calcium release in<br>
1345 the female skeleton.<br>
235 the female skeleton.<br>
235 observed. Osteocytes from wildtype male mice on a low calcium d The fermale skeleton.<br>
Osteocytes from wildtype male mice on a low calcium diet expressed higher levels of<br>
bone resorption genes including *Tnsfs11*, *Acp5*, *Ctsk*, *Oscar*, and *Mst1r* compared to wildtype<br>
male mice o 0steocytes fr<br>
737 bone resorption gen<br>
738 male mice on a norm<br>
739 genes as there is also<br>
740 rapidly replace their<br>
741 calcium deficiency sk<br>
742 *Car3*, as well as an ir<br>
743 mice on a normal<br>
744 deficiency and FNDC For the secondary of the male including The state of the females, there is a coupling with bone formation<br>
The male mice on a normal diet as expected. Like the females, there is a coupling with bone formation<br>
genes as th For the test including The state of the term and the separation genes as there is also an increase in *Bglap* and *Colta1*, suggesting the potential for osteocytes to rapidly replace their perilacunar matrix with calcium genes as there is also an increase in *Bglap* and *Col1o1*, suggesting the potential for osteocytes to<br>
rapidly replace their perilacunar matrix with calcium repletion. Similarly, the male null mice with<br>
calcium deficien The calcular deficiency showed an increase in bydepthat collarly, the male mill mice with<br>
calcium deficiency showed an increase in bone resorption genes including Tnsfs11, Oscar, and<br>
car3, as well as an increase in bone 741 rapidly and the matrix or interest in bone entropy perioding *Tosfs11, Oscar,* and Car3, as well as an increase in bone formation genes such as *Alpl* and *Bglap* compared to null mice on a normal diet. The major diff Calcular dentency showed an increase in bone resorption genes including *insysts*, oscar, and<br>
Car3, as well as an increase in bone formation genes such as *Alpl* and *Bglap* compared to null<br>
mice on a normal diet. The m

242 Cars, as well as an increase in bone formation genes such as Alpl and Bglap compared to null<br>mice on a normal diet. The major differences between wildtype male mice with calcium<br>deficiency and FNDC5-null male mice with 2744<br>
2744 deficiency and FNDC5-null male mice with calcium deficiency were the lower expression of<br>
2745 genes involved in the extracellular matrix organization, ossification, and bone development<br>
2745 pathways in the n 745<br>
The senes involved in the extracellular matrix organization, ossification, and bone development<br>
pathways in the null male mice compared to wildtype males. This suggests a mechanism for how<br>
null male mice lose more 246 pathways in the null male mice compared to wildtype males. This suggests a mechanism for how<br>
247<br>
248 null male mice lose more bone with calcium deficiency compared to wildtype males.<br>
248 lissue *Bostrom et al.* (20 2747 pathways in the numeral metallian deficiency compared to wildtype males.<br>
2748 lissue Bostrom et al. (2012); Zhang et al. (2014); Celi and Brown (2017); Luo et al. (2022), can<br>
2749 tissue Bostrom et al. (2012); Zhan Frame microtreated that microtrice there is materially constrained the time of the set of the microsoftes. It is an modulation of the discussion of the discussion of the discussion of the discussion of the material of bon tissue *Bostrom et al.* (2012); *2hang et al.* (2014); *Celi and Brown* (2017); *Luo et al.* (2022), can<br>potentially modulate osteogenic differentiation of bone marrow mesenchymal stem cells through<br> $\alpha$ V*BS Zhu et al.* ( For the all (2012); Zhang et al. (2014); Cell and Brown (2017); Lub et al. (2022); and potentially modulate osteogenic differentiation of bone marrow mesenchymal stem cells through  $\alpha$ V*BS Zhu et al.* (2023) and bone mar 2751 at al. (2023) and bone marrow adipose tissue can modulate bone properties Yeung et al. (2005); Rosen and Bouxsein (2006); Muruganandan and Sinal (2014); Styner et al. (2015); Schwartz et al. (2015); During (2020) as 2752 *al.* (2025); *Rosen and Bouxsein (2006); Muruganandan and Sinal (2014); Styner et al. (2015); Schwartz et al. (2015); During (2020) as well as osteocyte number and activity <i>Saedi A et al.* (2019, 2020). Irisin can m 353 Schwartz et al. (2015); During (2020) as well as osteocyte number and activity Saedi A et al.<br>
354 (2019, 2020). Irisin can modulate brain activity and signaling *Islam et al.* (2021); Wrann et al.<br>
355 (2013); Young (2019, 2020). Irisin can modulate brain activity and signaling *Islam et al.* (2021); Wrann et al.<br>
(2013); Young et al. (2019); Jo and Song (2021); Qi et al. (2022) through BDNF Wrann et al. (2013) and<br>
BDNF promotes ost  $(2013)$ ; Young et al. (2019); Jo and Song (2021); Qi et al. (2022) through BDNF Wrann et al. (2013) and<br>BDNF promotes osteogenesis in human bone mesenchymal stem cells *Liu et al.* (2018). Our data<br>31  $756$  BDNF promotes osteogenesis in human bone mesenchymal stem cells *Liu et al.* (2018). Our data  $31$  $\frac{31}{200}$ 

2578 encoding integrins are usually stable in the ell membrane with our differences between either violence of Frances in our of 512-1000, unpublished). As such, we postulate that the effect of irisin on osteocytes is not Expression of Findes in primary osteoblasts and primary osteocytes (transcriptome analysis with a<br>
The expression of Finde5 (transcriptome raw count of 512-1000, unpublished). As such, we postulate<br>
that the effect of iri expression of *Fnde5* (transcriptome raw count of 512-1000, unpublished). As such, we postulate<br>that the effect of irisin on osteocytes is not an autocrine effect, but rather due to irisin production<br>by skeletal muscle.<br> Expression of Findes (transcriptome raw count of St2-1000, unpublished). As such, we postulate<br>
that the effect of irisin on osteocytes is not an autocrine effect, but rather due to irisin production<br>
by skeletal muscle.<br> 2762 by skeletal muscle.<br>
1762 by skeletal muscle.<br>
1762 characterism of the effect of the effect of irisin must bind to aV85 integrins to function. Osteocyte-like cell line Kim et al. (2018).<br>
1765 characterism are usua 763 Irisin must b<br>
764 which was first dis<br>
765 Integrins are usually<br>
766 *al.* (2019). In our R<br>
767 encoding integrins<br>
768 male or female, ca<br>
769 Hsp90 $\alpha$  is necessar<br>
770 this heat shock pro<br>
771 with no significan Which was first discovered using the female MLO-Y4 osteocytes capited in *a female MLO-Y4* osteocyte-like cell line *Kim et al.* (2018).<br>
Integrins are usually stable in the cell membrane with a half-life of 12-24 hours Which was first discovered using the female MLO-14 osteocyte-like cell line Kim et al. (2019).<br>
Integrins are usually stable in the cell membrane with a half-life of 12-24 hours *Moreno-Layseca et*<br> *al. (2019)*. In our R Integrins are usually stable in the cell membrane with a half-life of 12-24 hours Moreho-Layset Cardin and *H*<sub>1</sub><br> *al. (2019)*. In our RNA sequencing data, we observed a stable expression of both *ITGAV* and *ITGB5*,<br>
ma 1666 and BS respectively, we observed a stable expression of both ITGAV and ITGDs,<br>
ancelong integris and BS respectively, with no differences between either wildtype or null,<br>
male or female, calcium replete or calcium d

encoding integrins av and *os i* espectively, with no differences between either wildtype or null,<br>
male or female, calcium replete or calcium deficient mice. Recently it has been published that<br>
Hsp90α is necessary to fa Hsp90 $\alpha$  is necessary to facilitate irisin-αVβ5 binding *Mu et al.* (2023). *Hsp90o*, the gene encoding<br>
T70 this heat shock protein, is very highly expressed in both wildtype and null male and female mice,<br>
with no sig 1999α is necessary to neuriate institute yo shalling Mu et al. (2023). Hsp90a, the gene encoding<br>
770<br>
770 this heat shock protein, is very highly expressed in both wildtype and null male and female mice,<br>
1771 significan 771 with no significant regulation by diet. The high expression of Hsp90α in osteocytes may explain their<br>
771 with no significant regulation by diet. The high expression of Hsp90α in osteocytes may explain their<br>
772 sh 1712<br>
1722 significant and rapid responses to irisin *Kim et al (2018)*.<br>
1732 In summary, during normal development and on a regular diet, FNDCS/irisin deletion has<br>
1732 in more but weaker bone. However, with challenge Frame of the summary, during normal development and of<br>
1773 In summary, during normal development and of<br>
1775 In more but weaker bone. However, with challeng<br>
differences were observed. Our data suggest that iri<br>
1777 In Frameterial and the female skeleton but a significant effect on the male skeleton resulting<br>
in more but weaker bone. However, with challenges, such as calcium deficiency, dramatic<br>
differences were observed. Our data sugg The more but weaker bone. However, with challenges, such as calcium deficiency, dramatic<br>
differences were observed. Our data suggest that irisin activates the osteocyte in females to<br>
initiate the removal of their perilac T76 differences were observed. Our data suggest that irisin activates the osteocyte in females to initiate the removal of their perilacunar matrix and for bone resorption through osteoclast activation, presumably to provid initiate the removal of their perilacunar matrix and for bone resorption through osteoclast activation, presumably to provide calcium for reproduction purposes. In contrast, in males, irisin protects against osteocytic ost activation, presumably to provide calcium for reproduction purposes. In contrast, in males, irisin<br>protects against osteocytic osteolysis and osteoclastic bone resorption under calcium-<br>demanding conditions. This sex-speci protects against osteocytic osteolysis and osteoclastic bone resorption under calcium-<br>demanding conditions. This sex-specific effect may be due to the sexual dimorphism of the<br>osteocyte transcriptome. We have discovered a diseases, such as osteoporosis, and lead to the development of sex- targeted therapies.<br>
diseases, such as osteoporosis, and lead to the development of sex- targeted therapies.<br>
diseases, such as osteoporosis, and lead to 781 osteocyte transcriptome. We have discovered a new novel function of irisin to ensure the<br>3782 survival of offspring and that irisin is essential for male but not female skeletal development.<br>3783 These findings could h 522 survival of offspring and that irisin is essential for male but not female skeletal development.<br>
783 These findings could have implications for understanding sex-dependent differences in bone<br>
784 diseases, such as os These findings could have implications for understanding sex-dependent differences in bone<br>diseases, such as osteoporosis, and lead to the development of sex-targeted therapies.<br>32 284 These findings could have implicated to the development of sex-targeted therapies.<br>
diseases, such as osteoporosis, and lead to the development of sex-targeted therapies.<br>
32 784 diseases, such as osteoporosis, and lead to the development of sex- targeted therapies.



**Bone Protection** 



785

Methods<br>
798 **Animal Experts<br>
799** All animal e<br>
800 Care and Use Comn<br>
801 FNDC5 Knockout (K Framman Experiments<br>
799 All animal experiments<br>
800 Care and Use Committee (I.<br>
801 FNDC5 Knockout (KO) mice Care and Use Committee (IACUC) of the Indiana University School of Medicine. Heterozygous C57BI/6J<br>
FNDC5 Knockout (KO) mice were provided by Dr. Bruce Spiegelman at Harvard University and bred in<br>
our facility to obtain h 800 ENDICES Knockout (KO) mice were provided by Dr. Bruce Spiegelman at Harvard University and bred in<br>802 ever and Use Committee (IACUC) of the Indian University and bred in<br>803 determined using a PCR reaction with primer 802 our facility to obtain homozygous global FNDCS KO and wildtype (WT) control mice. Genotype was<br>803 determined using a PCR reaction with primers targeting portions of exon 3 absent in KO (WT Forward:<br>804 GCG GCT CGA GA 803 determined using a PCR reaction with primers targeting portions of exon 3 absent in KO (WT Forward:<br>
802 GG GCT CGA GAG ATG AAG AAG AA, WT Reverse: CAG CCC ACA AGA AGT GC, KO Forward: GGA CTT<br>
805 CAA GTC CAA GGT CA, 804 GCG GCT CGA GAG ATG AAG AA, WT Reverse: CAG CCC ACA AGA AGT GC, KO Forward: GGA CTT<br>
805 CG GCT CGA GGT CA, KO Reverse: CCT AAG CCC ACC CAA ATT AC). Mice were housed in a temperature-<br>
controlled (20–22<sup>3</sup>C) room on a

CAGTIC CAA GGT CA, KO Reverse: CCT AAG CCC ACC CAA ATT AC). Mice were housed in a temperature-<br>
son CAA GTC CAA GGT CA, KO Reverse: CCT AAG CCC ACC CAA ATT AC). Mice were housed in a temperature-<br>
controlled (20–22<sup>3</sup>C) ro 810 controls. All animals were 4-5 months old at the time of sacrifice and analysis. For all lactating mice,<br>
811 the litter size ranged from 8-11 pups Qing et al. (2012).<br>
812 For the low calcium diet experiments, 4-5-mon controlled (20–22 C) room on a 12-hour light/dark cycle with ad illutum food and water. Qualified<br>veterinary staff and/or animal care technicians performed regular health check inspections.<br>For the lactation experiments, 4 808 For the lactation experiments, 4-month-old WT and FNDC5 global KO female mice we<br>
809 delivered pups, and lactated for 2 weeks before sacrifice. Virgin WT and KO mice were<br>
810 controls. All animals were 4-5 months old delivered pups, and lactated for 2 weeks before sacrifice. Virgin WT and KO mice were used as<br>
scontrols. All animals were 4-5 months old at the time of sacrifice and analysis. For all lactating mice,<br>
the litter size rang 810 controls. All animals were 4-5 months old at the time of sacrifice and analysis. For all lactating mice,<br>
811 the litter size ranged from 8-11 pups  $Qing$  et al. (2012).<br>
812 For the low calcium diet experiments, 4-5-mo 811 the litter size ranged from 8-11 pups Qing et al. (2012).<br>
812 For the low calcium diet experiments, 4-5-month-old male and female WT and FNDC5 global KO<br>
813 mice were fed either a control diet (0.6% calcium, Teklad, 812 For the low calcium diet experiments, 4-5-month-<br>
812 For the low calcium diet experiments, 4-5-month-<br>
814 calcium, 0.4% phosphorus, Teklad TD.95027) for 2 weeks<br>
water was used in place of tap water to control calciu

For the low calcium, 0.4% phosphorus, Teklad TD.95027) for 2 weeks. Food was replaced every two days. Distilled<br>
813 mice were fed either a control diet (0.6% calcium, Teklad, TD.97191) or a low calcium (Ca) diet (0.01%<br>
8 ealidium, 0.4% phosphorus, Teklad TD.95027) for 2 weeks. Food was replaced every two days. Distilled<br>water was used in place of tap water to control calcium intake. On the day of sacrifice, blood was<br>collected under anest 815 water was used in place of tap water to control calcium intake. On the day of sacrifice, blood was<br>816 collected under anesthesia, and mice were euthanized for sample collection, processing, and analysis<br>814 *Qing* (2 collected under anesthesia, and mice were euthanized for sample collection, processing, and analysis<br>
817 *Qing* (2012); Jahn et al. (2017).<br>
818 **AV8 injection**<br>
820 **AV8-irisin and AAV8-GFP constructs were obtained from** 217 *Qing* (2012); Jahn et al. (2017).<br>
818 **AV8 injection**<br>
819 AAV8 injection<br>
819 AAV8-irisin and AAV8-GFP constructs were obtained from Dr. Bruce Spiegelman at Harvard<br>
820 University. AAV8 Mouse ORF 1-140 (containing 818 **AAV8 injection**<br>819 AAV8 injection<br>819 AAV8-irisin and AAV8-(820<br>820 University. AAV8 Mouse ORF 1<br>821 amino-acid linker plus a C-term<br>822 vector (Addgene plasmid no. 1<br>823 was obtained from Addgene (10<br>824 GC per ml39 819 AAV8-irisin and<br>820 University. AAV8 Mot<br>821 amino-acid linker plus<br>822 vector (Addgene plas 820 University. AAV8 Mouse ORF 1-140 (containing the N-terminal signal peptide and irisin) plus a five-<br>
822 amino-acid linker plus a C-terminal flag tag was cloned into the pENN.AAV.CB7.Cl.pm20d1flag.WPRE-rBG<br>
822 vector either AAV8-irisin or AAV8-GFP control (1  $\times$  10<sup>10</sup> GC per mouse) in 100  $\mu$ L in PBS *Islam et al.* (2021).<br>
One week after injection with either the control virus containing GFP or the virus coding for<br>
circulating ir state of (Addgene plasmid no. 132682). AAV8-GFP (pENN.AAV.CB7.CI.eGFP.WPRE.rBG), used as control, was obtained from Addgene (105542), and packaged at the UPenn Vector Core to a titer of 2.10 × 1013<br>824 GC per mi39. FNDCS <p>\nvector (Addgene plasmid no. 132682). AAV8-GFP (pENNAAV.CB7.CI.eGFP.WPRE.rBG), used as control, was obtained from Addgene (105542), and packaged at the UPenn Vector Core to a titre of 2.10 × 1013 GC per m139. FNDCS KO male mice were placed under anesthesia and injected into the tail vein with either AAV8-risin or AAV8-GFP control (1 X 10<sup>10</sup> GC per mouse) in 100 μl in PBS <i>Islam et al.</i> (2021). One week after injection with either the control virus containing GFP or the virus coding for circulating irisin, the mice were placed on a low-calicum diet for two weeks before sacrifice. In vivo and exvivo muscle contractility andelectrophysiology measurement in GC per ml39. FNDC5 KO male mice were placed under anesthesia and injected into the tail vein with<br>either AAV8-irisin or AAV8-GFP control (1 x 10<sup>10</sup> GC per mouse) in 100  $\mu$ L in PBS *Islam et al.* (2021).<br>One week after 825 either AAV8-irisin or AAV8-GFP control (1  $\times$  10<sup>10</sup> GC per mouse) in 100  $\mu$ L in PBS *Islam et al.* (2021).<br>826 one week after injection with either the control virus containing GFP or the virus coding for<br>627 circ either AAV8-Irisin or AAV8-GFP control (1 X 10<sup>-3</sup> GC per mouse) in 100  $\mu$ L in PBS *islam et al.* (2021).<br>
826 Gne week after injection with either the control virus containing GFP or the virus coding for<br>
firculating i

Example 1827 Circulating irisin, the mice were placed on a low-calcium diet for two weeks before sacrifice.<br> **S28** *In vivo* and *exvivo* muscle contractility and electrophysiology measurement<br> *In vivo* plantarflexion to 828 **and in the micropropy in the micropropy** is the micropropy of the microsety of the wivo plant and for the mouse was assessed one day before sacrifice (Scientific Inc, described in *Pin et al.* (2022). Briefly, the mou **Sand 19 In vivo and ex vivo muscle contractility and electrophysiology measurement**<br>
829 In vivo plantarflexion torque was assessed one day before sacrifice (Scientific Inc, Canada) as<br>
830 described in *Pin et al.* (2022 830 described in *Pin et al.* (2022). Briefly, the mouse was placed under anesthesia and the left hind foot was described in *Pin et al.* (2022). Briefly, the mouse was placed under anesthesia and the left hind foot was 34 830 described in Pin et al. (2022). Briefly, the mouse was placed under anesthesia and the left film foot was<br>34

affixed to the force transducer aligned with the tibia at 90<sup>-</sup>. The tibial nerve was strimulated using<br>a monopolar electrodes (Natus Neurology, Middleton, WI). Maximum twitch torque was established by<br>assume with the siem 933 using a 0.2 ms square wave pulse. Peak plantarflexion torque was measured by using a stimulation of<br>
834 using a 0.2 ms delivered at 100Hz stimulation frequency.<br> *In vivo* electrophysiological functions were assessed 834 0.2 ms delivered at 100Hz stimulation frequency.<br>
835 *In vivo* electrophysiological functions were assessed one day before sacrifice with the Sierra<br>
835 *In vivo* electrophysiological functions were assessed one day 235 *In vivo* electrophysiological functions were<br>836 Summit 3–12 Channel EMG (Cadwell Laboratorie<br>in *Huot et al.* (2022). Briefly, peak-to-peak and b<br>638 (CMAP) were measured using supramaximal stim<br>duration, and peak-to mini 3–12 Channel EMG (Cadwell Laboratories Incorporated, Kennewick, WA, USA) as described<br>
835 Summit 3–12 Channel EMG (Cadwell Laboratories Incorporated, Kennewick, WA, USA) as described<br>
837 in *Huot et al.* (2022). Bri

in *Huot et al.* (2022). Briefly, peak-to-peak and baseline-to-peak compound muscle action potentials<br>
838 (CMAP) were measured using supramaximal stimulations of <10 mA continuous current for 0.1 ms<br>
840 duration, and pea (CMAP) were measured using supramaximal stimulations of <10 mA continuous current for 0.1 ms<br>
duration, and peak-to-peak single motor unit (SMUP) potentials were measured using an<br>
incremental stimulation technique. Motor 839 duration, and peak-to-peak single motor unit (SMUP) potentials were measured using an incremental stimulation technique. Motor unit number estimation (MUNE) was measured using the equation: MUNE = CMAP amplitude/avera EXALUTE SIMULTE SIMULTE COMPROVER THE SIMULTE SIMULTE SIMULTE SIMULTE SIMULTE SIMULTE SIMULTE SIMULTE COMP amplitude/average SMUP.<br>
Ex vivo muscle contractility was measured in the extensor digitorum longus (EDL) muscle as equation: MUNE = CMAP amplitude/average SMUP.<br> *Ex vivo* muscle contractility was measured in the extensor digitorum longus (EDL) muscle as<br>
described in *Huot et al.* (2021). EDL was collected from the mouse and mounted b Ex vivo muscle contractility was measured in<br>
843 described in *Huot et al.* (2021). EDL was collected<br>
844 transducer, and then submerged in a stimulation b<br>
845 data were collected using Dynamic Muscle Control<br>
846 Contr Ex vivo muscle contraction, was collected from the muscles were forced to contract, and the muscles of the muscles was collected from the muscles were forced to contract, and that were collected using Dynamic Muscle Contro transducer, and then submerged in a stimulation bath. The muscles were forced to contract, and<br>
844<br>
844<br>
845 data were collected using Dynamic Muscle Control/Data Acquisition (DMC) and Dynamic Muscle<br>
Control Data Analys

845 data were collected using Dynamic Muscle Control/Data Acquisition (DMC) and Dynamic Muscle<br>
846 Control Data Analysis (DMA) programs (Aurora Scientific). The EDLs were weighed for normalization<br>
847 purposes.<br>
848 Bod Control Data Analysis (DMA) programs (Aurora Scientific). The EDLs were weighed for normalization<br>
847 burposes.<br>
848 Body composition assessment by dual-energy X-ray absorptiometry (DXA)<br>
849 The right femurs from mice w Body composition assessment by dual-energy X-ray absorptiometry (DXA)<br>
849 The right femurs from mice were dissected and cleaned of so<br>
850 paraformaldehyde (PFA) for 48 hours, and then transferred to 70%<br>
851 measurements

Bayant Transferred Control Data Analysis (DMA)<br>
Bayantom assessment by dual-energy X-ray absorptiometry (DXA)<br>
The right femurs from mice were dissected and cleaned of soft tissue, fixed in 4%<br>
paraformaldehyde (PFA) for 848 Bod<br>
849 The<br>
850 paraforma<br>
851 measurem<br>
852 mineral.de<br>
853 Bon<br>
854 Rig<br>
855 (2022). Bri<br>
855 (2022). Bri<br>
856 integration<br>
857 (ROI) of th<br>
858 cortical bo example in the right femula of the right femula of the right femurs from micelane of the right femurs from Magnetic model of the right femurs were endined using a faxitron (Faxitron X-ray Corp, Wheeling, IL) to measure b measurements were obtained using a faxitron (Faxitron X-ray Corp, Wheeling, IL) to measure bone<br>
mineral density (BMD) and bone mineral content (BMC) *Essex et al.* (2022).<br> **Bone morphometry analysis by micro-computed to** mineral density (BMD) and bone mineral content (BMC) *Essex et al.* (2022).<br> **Show morphometry analysis by micro-computed tomography (** $\mu$ **CT)**<br>
Right femurs were analyzed using a Skyscan 1176  $\mu$ CT as described previousl **Bone morphometry analysis by micro-computed tomography (** $\mu$ **CT)**<br>
Right femurs were analyzed using a Skyscan 1176  $\mu$ CT as descri<br>
(2022). Briefly, specimens were scanned at 55 kV, 145  $\mu$ A, high resolution, 1<br>
integra Bone morphometry analysis by incro-computed tomography ( $\mu$ CT)<br>854 Bight femurs were analyzed using a Skyscan 1176  $\mu$ CT as d<br>855 (2022). Briefly, specimens were scanned at 55 kV, 145  $\mu$ A, high resolut<br>856 integration 865 Right Femurs were analyzed using a Skyscan 1176 (Ct as described previously Pin et al.,<br>
855 (2022). Briefly, specimens were scanned at 55 kV, 145  $\mu$ A, high resolution, 10.5 mm voxel, and 200 ms<br>
854 integration time (2022). Briefly, specifies were scanned at 55 kV, 145  $\mu$ , high resolution, 10.5 mm voxel, and 200 ms<br>integration time. For cortical parameters, three-dimensional images from a 1mm region of interest<br>(ROI) of the mid-dia 868 (Tb. Th), trabecular spacing (Tb. Sp), and connectivity density (Conn.D) *Kitase et al.* (2018).<br>858 cortical bone thickness (Ct. Th), marrow cavity area, periosteal perimeter (Ps. Pm), and endosteal perimeter (Es. Pm) 858 (Router and Euclidate total bone thickness (Ct. Th), marrow cavity area, periosteal perimeter (Ps. Pm), and endosteal<br>perimeter (Es. Pm) according to ASBMR guidelines *Bouxsein* et al. (2010). For trabecular<br>parameters bone thickness (Tb. Th) according to ASBMR guidelines *Bouxsein et al.* (2010). For trabecular parameters, three-dimensional images reconstructed within the range of 0.5 mm from the most proximal metaphysis of tibiae were parameters, three-dimensional images reconstructed within the range of 0.5 mm from the most<br>proximal metaphysis of tibiae were analyzed. Trabecular morphometry was performed by excluding<br>the cortical bone from the endocort proximal metaphysis of tibiae were analyzed. Trabecular morphometry was performed by excluding<br>the cortical bone from the endocortical borders using hand-drawn contours followed by thresholding<br>and characterized by bone vo 19862 the cortical bone from the endocortical borders using hand-drawn contours followed by thresholding<br>
863 and characterized by bone volume fraction (BV/TV), trabecular number (Tb. N), trabecular thickness<br>
(Tb. Th), tr 863 and characterized by bone volume fraction (BV/TV), trabecular number (Tb. N), trabecular thickness<br>864 (Tb. Th), trabecular spacing (Tb. Sp), and connectivity density (Conn.D) *Kitase et al.* (2018).<br>35 864 (Tb. Th), trabecular spacing (Tb. Sp), and connectivity density (Conn.D) Kitase et al. (2018).<br>35  $8644 \times 10^{10}$ , trabecular spacing (Tb. Sp), and connectivity density (Connect) and connectivity (Connectivity  $(2012)$ .

Finitude-resistant acid phosphatase (TRAP) staining<br>866 Tibiae were stripped of soft tissue, fixed in 4% P<br>867 3-4 weeks, and processed into paraffin as described p<br>868 staining for TRAP activity using the standard naphtho 886 SFA at Wields, and processed into paraffin as described previously followed by sectioning (5  $\mu$ m) and<br>868 straining for TRAP activity using the standard naphthol AS-BI phosphate post coupling method and<br>869 counters 888 STATE THAP activity using the standard naphthol AS-BI phosphate post coupling method and<br>869 conterstained with toluidine blue *Pin et al.* (2021). Briefly, after equilibration in 0.2 M sodium acetate,<br>870 mM sodium t 869 sounterstained with toluidine blue *Pin et al.* (2021). Briefly, after equilibration in 0.2 M sodium acetate, 50 mM sodium tartrate, pH 5.0, for 20 min at RT, sections were incubated at 37<sup>uc</sup> in the same buffer conta counterstained with toluidine blue Pin et al. (2021). Briefly, aree equilibration in 0.2 M sodium acetate,<br>
869 mM sodium tartrate, pH 5.0, for 20 min at RT, sections were incubated at 37<sup>ti</sup>C in the same buffer<br>
871 conta 50 so mixi sodium tartrate, pH 5.0, for 20 min at RT, sections were includated at 37°C. In the same buffer containing 0.5 mg/ml naphthol AS-MX phosphate (Sigma Chem. Co., St. Louis, MO) and 1.1 mg/ml Fast<br>
872 Red Violet L Red Violet LB salt (Sigma) and counter-stained in toluidine blue. Images were taken at 5X and 40X using<br>
an Olympus BX51 fluorescent microscope and Olympus cellSense Entry 1.2(Build 7533) imaging<br>
software. TRAP-positive o

873 an Olympus BX51 fluorescent microscope and Olympus cellSense Entry 1.2(Build 7533) imaging<br>874 software. TRAP-positive osteocytes and osteoclasts 1.5 mm distal from the growth plate were quantified<br>875 sing Osteomeasur software. TRAP-positive osteocytes and osteoclasts 1.5 mm distal from the growth plate were quantified<br>
sing Osteomeasure software (OsteoMetrics.lnc) in a blind fashion. Toluidine blue-stained osteoblasts<br>
from the same se sing Osteomeasure software (OsteoMetrics.lnc) in a blind fashion. Toluidine blue-stained osteoblasts<br>
from the same sections were quantified 1.5 mm distal from the growth plate using the same software.<br>
Osteocyte lacunar a 876 from the same sections were quantified 1.5 mm distal from the growth plate using the same software.<br>
877 Osteocyte lacunar area measurement by Backscatter Scanning Electron Microscopy (BSEM)<br>
878 Femurs were stripped o **S77 Osteocyte lacunar area measurement by Backscatter Scanning Electron Microscopy (BSEM)**<br>
Femurs were stripped of soft tissue and fixed in 4% PFA for 48 hours before proceeding to<br>
dehydration and embedding steps as p **Osteocyte lacunar area measurement by Backscatter Scanning Electron Microscopy (BSEM)**<br>
Femurs were stripped of soft tissue and fixed in 4% PFA for 48 hours before proceeding to<br>
dehydration and embedding steps as previou dehydration and embedding steps as previously described Qing et al. (2012). Briefly, femurs were<br>
880 dehydrated in graded ethanol and placed into acetone. Subsequently, the femurs were immersed in<br>
881 infiltration soluti extinguished in graded ethanol and placed into acetone. Subsequently, the femurs were immersed in infiltration solution made of 85% destabilized methyl methacrylate (MMA, Sigma), 15% dibutyl phthalate (Sigma), 1% PEG400 (S 881 infiltration solution made of 85% destabilized methyl methacrylate (MMA, Sigma), 15% dibutyl<br>882 infiltration solution made of 85% destabilized methyl methacrylate (MMA, Sigma), 15% dibutyl<br>883 PM/acetone until infiltr phthalate (Sigma), 1% PEG400 (Sigma), and 0.7% benzoyl peroxide (Polysciences, Inc., Warrington,<br>
PA)/acetone until infiltration was complete. The femurs were then placed on pre-polymerized base<br>
layers, covered with fresh 883 PA)/acetone until infiltration was complete. The femurs were then placed on pre-polymerized base<br>
884 layers, covered with freshly catalyzed MMA embedding solution (for 100 mL, 85mL MMA, 14mL<br>
885 layers, covered with Iayers, covered with freshly catalyzed MMA embedding solution (for 100 mL, 85mL MMA, 14mL<br>
885 Iayers, covered with freshly catalyzed MMA embedding solution (for 100 mL, 85mL MMA, 14mL<br>
885 MUA was polymerized. The polymer 885 dibutyl phthalate, 1mL PEG400, 0.33uL DMT, and 0.8g BPO), and incubated under vacuum until the<br>886 MMA was polymerized. The polymerized blocks were trimmed, sequentially polished to a completely<br>887 smooth surface, and MMA was polymerized. The polymerized blocks were trimmed, sequentially polished to a completely<br>
smooth surface, and coated with gold using a sputter coater (Desk V, Denton Vacuum, NJ, USA). Then<br>
BSEM (JEOL: JSM-7800F) wa 887 smooth surface, and coated with gold using a sputter coater (Desk V, Denton Vacuum, NJ, USA). Then<br>888 BEM (JEOL: JSM-7800F) was performed to image the osteocyte lacunae on the sectioned bone surface<br>at 450X magnifica 888 BSEM (JEOL: JSM-7800F) was performed to image the osteocyte lacunae on the sectioned bone surface<br>
889 BSEM (JEOL: JSM-7800F) was performed to image the osteocyte lacunae on the sectioned bone surface<br>
889 are after co

889 at 450X magnification starting 2 mm distal from the growth plate. Six fields from the endosteal and<br>890 periosteal sides of the cortical bone were taken as described previously *Qing and Bonewald* (2009).<br>891 Using Ima eriosteal sides of the cortical bone were taken as described previously Qing and Bonewald (2009).<br>
881 Using Imagel (NIH), the images were thresholded for background removal, binarized, and the lacunar<br>
882 area from each Beriosteal sides of the cortical bone were taken as described previously dang bonewald (2009).<br>
Bong Imagel (NIH), the images were thresholded for background removal, binarized, and the lacunar<br>
area from each sample quant area from each sample quantitated.<br>
893 area from each sample quantitated.<br>
895 bechanical testing using 3-point bending<br>
894 Mechanical testing was performed essentially as described in *Melville et al.* (2015). Briefly<br> Mechanical testing using 3-po<br>894 Mechanical testing was per<br>895 the left femurs were stripped of so<br>896 use. Frozen femurs were brought<br>897 mm span) of a 3-point bending p<br>898 samples were tested in monotor Mechanical testing using 3-point bending<br>
894 Mechanical testing was performed essentially as described in *Melville et al.* (2015). Briefly,<br>
895 the left femurs were stripped of soft tissue, wrapped in PBS- soaked gauze, the left femurs were stripped of soft tissue, wrapped in PBS- soaked gauze, and stored at -20<sup>n</sup>C until<br>
use. Frozen femurs were brought to room temperature and mounted across the lower supports (8<br>
mm span) of a 3- point the left femurs were stripped of soft tissue, wrapped in PBS- soaked gauze, and stored at -20-C until<br>the left femurs were brought to room temperature and mounted across the lower supports (8<br>mm span) of a 3- point bending 897 mm span) of a 3- point bending platen on a TestResources R100 small force testing machine. The<br>898 samples were tested in monotonic bending to failure using a crosshead speed of 0.05 mm/s.<br>898 36  $898$  samples were tested in monotonic bending to failure using a crosshead speed of 0.05 mm/s.<br>36 898 samples were tested in monotonic bending to failure using a crosshead speed of 0.05 mm/s.

899

Serum NANKL analysis<br>
901 The levels of RANKL<br>
902 Techne Corporation, Minne<br>
903 Serum parathyroid ho<br>
904 Serum was obtained

903 Serum parathyroid hormone (PTH) analysis<br>904 Serum was obtained from terminal cardi<br>905 using the MicroVue Bone Mouse PTH 1-84 ELISA<br>906 manufacturer's protocol.<br>907 Calcium measurement

Serum RANKL analysis<br>
890 Serum RANKL analysis<br>
899 The levels of RANKL were measured in mouse centrifuged serum by using an ELISA<br>
892 Techne Corporation, Minneapolis, MN), according to the manufacturer's protocol.<br>
893 S manufacturer's protocol.<br>
907 Calcium measurement<br>
908 Plasma calcium levels were determined using the Pointe Scientific calcium Reagent kit<br>
909 (Manufacturer and city). Briefly, diluted serum (1:4 in dH2O) was incubated Serum parathyroid hormone (PTH) analysis<br>
902 Serum was obtained from terminal cardiac puncture and serum PTH level<br>
905 using the MicroVue Bone Mouse PTH 1-84 ELISA assay (Quidel Corp., San Diego, C.<br>
906 manufacturer's p using the MicroVue Bone Mouse PTH 1-84 EUSA assay (Quidel Corp., San Diego, CA) according to the<br>906 manufacturer's protocol.<br>2017 Calcium measurement<br>908 Plasma calcium levels were determined using the Pointe Scientific c 908 Plasma calcium leve<br>909 (Manufacturer and city). Brie<br>910 color reagent for 1 min and<br>911 Synergy HTX).

manufacturer's protocol.<br>
Sof manufacturer's protocol.<br>
Calcium measurement<br>
Plasma calcium levels were determined using the Pointe Scientific calcium Reagent kit<br>
Manufacturer and city). Briefly, diluted serum (1:4 in dH2 Calcium measureme<br>
906 Plasma calcium le<br>
909 (Manufacturer and city).<br>
910 color reagent for 1 min<br>
911 Synergy HTX).<br>
912 Sample collection and<br>
913 Bulk RNA sequenc<br>
914 male and female, WT an<br>
915 after sequential dige 909 (Manufacturer and city). Briefly, diluted serum (1:4 in dH2O) was incubated with a working calcium<br>
910 color reagent for 1 min and the absorbance read at 575 nm using a spectrophotometer (BioTek<br>
911 Sample collectio 910<br>
910 color reagent for 1 min and the absorbance read at 575 nm using a spectrophotometer (BioTek<br>
911 Synergy HTX).<br>
912 Sample collection and processing for RNA sequencing<br>
913 Bulk RNA sequencing was performed on os 911 Synergy HTX).<br>
912 Sample collection and processing for RNA sequencing<br>
913 Bulk RNA sequencing was performed on osteocytes from the control and low calcium diet,<br>
914 male and female, WT and KO mice. Osteocyte RNA wa 912 **Sample**<br>
913 Bulk RM<br>
914 male and fem<br>
915 after sequentia<br>
916 previously des<br>
917 bones, the epip<br>
918 midshafts wer<br>
919 chelation/dige<br>
920 for 30 min foll<br>
921 with PBS and a<br>
922 with Trizol rea 912 Sample concedent and processing for MWA sequencing<br>913 Bulk RNA sequencing was performed on osteocyt<br>914 male and female, WT and KO mice. Osteocyte RNA was<br>915 after sequential digestion to remove surface cells includi male and female, WT and KO mice. Osteocyte RNA was extracted from tibia and femur diaphyses<br>
after sequential digestion to remove surface cells including osteoclasts, osteoblasts, and lining cells as<br>
perviously described 915 after sequential digestion to remove surface cells including osteoclasts, osteoblasts, and lining cells as<br>916 previously described *Qing et al.* (2012); *Pin et al.* (2022). Briefly, soft tissue was removed from the<br> 916 proviously described *Qing et al.* (2012); *Pin et al.* (2022). Briefly, soft tissue was removed from the bones, the epiphyses were cut off and bone marrow was removed by flushing with PBS. The remaining midshafts wer 917 bones, the epiphyses were cut off and bone marrow was removed by flushing with PBS. The remaining<br>918 midshafts were incubated at 37<sup>7</sup>C with 0.2% type 1 collagenase (Sigma) for 30 minutes, followed by<br>919 chation/ di midshafts were incubated at 37<sup>7</sup>C with 0.2% type 1 collagenase (Sigma) for 30 minutes, followed by<br>
the lation/ digestion in 0.53 mM EDTA/ 0.05% trypsin (Cellgro, Mediatech, Inc, Manassas, VA) at 37<sup>7</sup>C<br>
for 30 min follo midshafts were incubated at 37 9 With 0.2% type 1 conagenase (Sigma) for 30 minutes, followed by<br>
thelation/ digestion in 0.53 mM EDTA/ 0.05% trypsin (Cellgro, Mediatech, Inc, Manassas, VA) at 37<sup>B</sup>C<br>
for 30 min followed b chelation/ digestion in 0.53 mM EDTA/ 0.05% trypsin (Cellgro, Mediatech, Inc, Manassas, VA) at 37 = Cor 30 min followed by a second collagenase digestion. After each step, the bone chips were rinsed<br>with PBS and after the

with PBS and after the final step, flash-frozen in liquid nitrogen, and pulverized in liquid nitrogen, with Trizol reagent (Qiagen, Carlsbad, CA) added to the resulting bone powder. Total RNA was isolated with an RNA purif with Trizol reagent (Qiagen, Carlsbad, CA) added to the resulting bone powder. Total RNA was<br>
sisolated with an RNA purification kit (Qiagen miRNeasy mini kit) and DNase treatment to remove<br>
DNA contamination.<br> **Ubrary pre** 923 isolated with an RNA purification kit (Qiagen miRNeasy mini kit) and DNase treatment to remove<br>
924 DNA contamination.<br>
Ubrary preparation and RNA sequencing<br>
926 Total RNA samples were first evaluated for their quanti 924 DNA contamination.<br>
925 Isolah RNA sequencing<br>
926 Total RNA samples were first evaluated for their quantity and quality using Agilent TapeStation.<br>
927 All the samples used for the sequencing had a RIN of at least 5. 925 Library prepara<br>
926 Total RNA sam<br>
927 All the samples used<br>
928 for library preparati<br>
929 uniquely dual-indexe<br>
930 Multiple libraries we<br>
931 NovaSeq 6000 seque End of preparation and NWA sequencing<br>926 Total RNA samples were first evaluated<br>927 All the samples used for the sequencing had a<br>929 uniquely dual-indexed library was quantified 927 All the samples used for the sequencing had a RIN of at least 5. 100 nanograms of total RNA were used<br>928 for library preparation with the KAPA total RNA Hyperprep Kit (KK8581) (Roche). Each resulting<br>929 uniquely dual for library preparation with the KAPA total RNA Hyperprep Kit (KK8581) (Roche). Each resulting<br>
uniquely dual-indexed library was quantified and quality accessed by Qubit and Agilent TapeStation.<br>
Multiple libraries were p 929 uniquely dual-indexed library was quantified and quality accessed by Qubit and Agilent TapeStation.<br>930 Multiple libraries were pooled in equal molarity. The pooled libraries were sequenced on an Illumina<br>931 NovaSeq 6 930 Multiple libraries were pooled in equal molarity. The pooled libraries were sequenced on an Illumina<br>931 NovaSeq 6000 sequencer with the v1.5 reagent kit. 100 bp paired-end reads were generated.<br>937 931 NovaSeq 6000 sequencer with the v1.5 reagent kit. 100 bp paired end reads were generated.<br>37 931 NovaSeq 6000 sequencer with the v1.5 reagent kit. 100 bp paired-end reads were generated.

ma-seq data analysis<br>
933 The sequencing read<br>
934 Cambridge, UK) for quality<br>
935 genome mm10 using the<br>
936 parameter: "--outSAMmapc Cambridge, UK) for quality control. The sequence data were then mapped to the mouse reference<br>genome mm10 using the RNA-seq aligner STAR (v2.7.10a) *Dobin et al.* (2013) with the following<br>parameter: "--outSAMmapqUnique60" genome mm10 using the RNA-seq aligner STAR (v2.7.10a) *Dobin et al.* (2013) with the following<br>parameter: "--outSAMmapqUnique60". To evaluate the quality of the RNA-seq data, the number of<br>reads that fell into different a 933 genome mm20 using the RNA-seq angier STAR (v2.7.10a) Dobin et al. (2013) with the following<br>936 gammeter: "--outSAMmapqUnique60". To evaluate the quality of the RNA-seq data, the number of<br>937 reads that fell into diff exall that fell into different annotated regions (exonic, intronic, splicing junction, intergenic,<br>
gromoter, UTR, etc.) of the reference genome was assessed using bamutils *Breese and Liu* (2013).<br>
Uniquely mapped reads w

938 promoter, UTR, etc.) of the reference genome was assessed using bamutils *Breese and Liu* (2013).<br>939 Uniquely mapped reads were used to quantify the gene level ex- pression employing featureCounts<br>940 (subread v2.0.3 942 During data quality control<br>943 have a similar proportion of rea<br>944 the gene Xist, typically highly of<br>945 sample was excluded from the area

Uniquely mapped reads were used to quantify the gene level ex- pression employing featureCounts<br>
939 Uniquely mapped reads were used to quantify the gene level ex- pression employing featureCounts<br>
940 (subread v2.0.3) *Li* (subread v2.0.3) *Liao et al.* (2014) with the following parameters: "-s 2 -Q 10".<br>
Quality control of samples<br>
Puring data quality control, one of the KO female control samples (sample 23) was found to<br>
have a similar pro 941 Quality control of samples<br>
942 During data quality control, one of the KO female control samples (sam<br>
943 have a similar proportion of reads on chromosome Y as in male mice and a v<br>
944 the gene Xist, typically highl have a similar proportion of reads on chromosome Y as in male mice and a very low expression of<br>
944 the gene Xist, typically highly expressed in females (Supplementary Figure 3A, 3B), therefore this<br>
945 sample was exclud the gene Xist, typically highly expressed in females (Supplementary Figure 3A, 3B), therefore this<br>
sample was excluded from the analysis.<br>
The WT female low-calcium diet samples (samples 16, 17, and 18) had low mapping<br>
p 945 sample was excluded from the analysis.<br>
946 The WT female low-calcium diet samples (samples 16, 17, and 18) had low mapping<br>
947 percentages of 37%, 32%, and 61%, respectively. This may be due to bacterial contaminatio The WT female low-calcium d<br>
947 percentages of 37%, 32%, and 61%, re<br>
948 two possible methods to process these<br>
949 alignment or align the reads without<br>
950 before alignment may result in removin<br>
951 bacterial genome ( ercentages of 37%, 32%, and 61%, respectively. This may be due to bacterial contamination. The<br>two possible methods to process these data are to filter all the possible contaminated reads before<br>alignment or align the read 1948 two possible methods to process these data are to filter all the possible contaminated reads before alignment or align the reads without filtering. However, filtering the possible contaminated reads before alignment m alignment or align the reads without filtering. However, filtering the possible contaminated reads<br>before alignment may result in removing some reads from the mouse genome which is similar to the<br>bacterial genome (causing before alignment may result in removing some reads from the mouse genome which is similar to the bacterial genome (causing lower gene expression). In contrast, using data without filtering may result in some genes having h 955 betarial genome (causing lower gene expression). In contrast, using data without filtering may result in some genes having ligher expression levels due to reads from the bacterial genome which are aligned to mice genes 952 result in some genes having higher expression levels due to reads from the bacterial genome which are aligned to mice genes. We decided to perform a principal component analysis (PCA) using data without filtering and f are aligned to mice genes. We decided to perform a principal component analysis (PCA) using data<br>
954 without filtering and found that the samples clearly clustered into four groups: control male mice,<br>
955 control female without filtering and found that the samples clearly clustered into four groups: control male mice,<br>control female mice, low-calcium diet male mice, and low-calcium diet female mice (Supplementary<br>Figure 3C). Within each g control female mice, low-calcium diet male mice, and low-calcium diet female mice (Supplementary<br>
Figure 3C). Within each group, the separation of WT and KO mice is also clear. Due to contamination,<br>
samples 16 and 18 were Figure 3C). Within each group, the separation of WT and KO mice is also clear. Due to contamination,<br>957 samples 16 and 18 were slightly far apart from the others. However, contamination should not have<br>958 a large global 957 samples 16 and 18 were slightly far apart from the others. However, contamination should not have a large global influence on the data as samples 16, 17, and 18 are close to the non-contaminated samples 5 and 6, also

958 a large global influence when the acts samples 16, 17, and 18 are close to the non-contaminated<br>959 samples 5 and 6, also in the low-calcium diet female group. Additionally, we validated the data using<br>960 qPCR with se 959 a must be a large global in the low-calcium diet female group. Additionally, we validated the data using<br>960 a pcR with selected genes.<br>961 **Differentially expressed gene analysis**<br>962 The read counts matrix was import 960 g PCR with selected genes.<br>
961 Differentially expressed gene analysis<br>
962 The read counts matrix was imported to R *Team* (2022) and analyzed with DEseq2 *Love et al.*<br>
963 (2014). Within DESeq2, read counts data wer 961 **Differentially express**<br>962 The read counts ma<br>963 (2014). Within DESeq2, read<br>964 expressed genes (DEGs) we<br>965 DEGs between different gro Sol Bincremially expressed gene analysis<br>962 The read counts matrix was importe<br>963 (2014). Within DESeq2, read counts data<br>964 expressed genes (DEGs) were detected after<br>965 DEGs between different groups. Significant g 963 (2014). Within DESeq2, read counts data were normalized with median of ratios, and differentially<br>964 expressed genes (DEGs) were detected after independent filtering. In DEG analysis, we first detected<br>965 DEGs betwee expressed genes (DEGs) were detected after independent filtering. In DEG analysis, we first detected<br>DEGs between different groups. Significant genes were defined as genes with an unadjusted p-value less<br>38 965 DEGs between different groups. Significant genes were defined as genes with an unadjusted p-value less<br>38 965 DEGs between different groups. Significant genes were defined as genes with an unadjusted p-value less<br>38

966 967 eme sets from *Gene ontology resource: enriching a GOld mine* (2021) using R package<br>
968 clusterProfiler *Wu* (2021). p value of less than 0.05 was considered as significant for the gene<br>
969 ontology analysis. Sever

estable from Gene ontology analysis. Several RNA sequencing and pathway figures were prepared with R packages<br>
968 clusterProfiler *Wu* (2021). p value of less than 0.05 was considered as significant for the gene<br>
969 onto ontology analysis. Several RNA sequencing and pathway figures were prepared with R packages<br>
970 gplot2 Wickham (2016) and ComplexHeatmap *Gu* (2022). The data was deposited in NCBI GEO<br>
971 database (accession number GSE2 970 ggplot2 Wickham (2016) and ComplexHeatmap Gu (2022). The data was deposited in NCBI GEO<br>
971 database (accession number GSE242445).<br>
972 Real-time quantitative polymerase chain reaction (qPCR)<br>
973 Total RNA was rever 973 Galaxies (accession number GSE242445).<br>
972 Real-time quantitative polymerase chain reaction (qPCR)<br>
973 Total RNA was reverse transcribed to cDNA using the Verso cDNA Kit (Thermo Fisher Scientific).<br>
974 Total RNA was 972 Real-time quantitative polymerase characterized to Transcript levels were measured by real-time<br>975 TaqMan and SYBR Gene Expression Assa<br>976 RANKL (*Tnfsf11*, Forward primer: CCG AGC<br>977 ATC<br>978 TTG), Cathepsin K (*Ct* Freal time quantitative polymerase chain reaction (qPCR)<br>973 Total RNA was reverse transcribed to cDNA using the V<br>974 Transcript levels were measured by real-time PCR (Light Cycl<br>975 TaqMan and SYBR Gene Expression Assay Transcript levels were measured by real-time PCR (Light Cycler 96; Roche), taking advantage of the<br>
TaqMan and SYBR Gene Expression Assay System (Thermo Fisher Scientific). Expression levels for<br>
RANKL (*Tnfsf11*, Forward

975 TaqMan and SYBR Gene Expression Assay System (Thermo Fisher Scientific). Expression levels for<br>
975 TaqMan and SYBR Gene Expression Assay System (Thermo Fisher Scientific). Expression levels for<br>
976 RANKL (*Tnfsf11*,

RANKL (*Tnfsf11*, Forward primer: CCG AGC TGG TGA AGA AAT TAG, Reverse: CCC AAA GTA CGT CGC<br>
ATC<br>
TTG), Cathepsin K (Ctsk, Primer Bank ID: Mm.PT.58.9655974, IDT), TRAP (Acp5,<br>
Mm.PT.58.5755766, IDT), and sclerostin (Sost, 977<br>
978 TTG), Cathepsin K (Ctsk, Primer Bank ID: Mm.PT.58.9655974, IDT), TRAP (Acp5,<br>
979 Mm.PT.58.5755766, IDT), and sclerostin (Sost, Mm00470479\_m1, Applied Biosystems) were quantitated.<br>
980 Gene expression was normali 978<br>979 Mm.<br>980 Gen<sub>'</sub><br>981 Reve<br>982<br>983 (Gra<br>985 two<br>985 two<br>985 two<br>985 two Mm.PT.58.5755766, IDT), and selerosin (Sost, Mm00470479\_m1, Applied Biosystems) were quantitated.<br>
979 Mm.PT.58.5755766, IDT), and selerosin (Sost, Mm00470479\_m1, Applied Biosystems) were quantitated.<br>
980 Gree expression 979 Mm.FT.58.5755766, IDT), and sclerostin (Jost, Miniod+70479\_m11, Applied Biosystems) were quantitated.<br>980 Gene expression was normalized to β-2-microglobulin (*B2m*, Forward: ACA GTT CCA CCC CCA CAT T,<br>982 Statistical 980 Gene expression was normalized to β-2-microglobulin (B2m, Forward: AcA GTT CCR CCC TCA CAT T, Reverse: TAG AAA GAC C A GTC CTTG CTG AAG) levels using the standard 2-ΔΔCt method.<br>
982 Statistical Analysis<br>
983 Data are 982 Statistical Analysis<br>982 Statistical Analysis<br>983 Data are expressed as individual data points. The statistical analysis was done by<br>984 (Graph Pad Software, San Diego, CA, USA) and R 4.3.0. When comparing three or mo 982 Statistical Analysis

983 (Graph Pad Software, San Diego, CA, USA) and R 4.3.0. When comparing three or more groups with<br>
two variables, a two-way analysis of variance (ANOVA) was used. To compare between two groups,<br>
the unpaired, two-tailed S 1995 (We would like to thank the Center for Medical Genomics, the system with the unpaired, two-tailed Student's t-test was used. Differences were considered significant at  $* p < 0.05, ** p < 0.01$ , and  $*** p < 0.001$ .<br>
987 (Graph 995 the unpaired, two-tailed Student's t-test was used. Differences were considered significant at  $* p < 0.05, ** p < 0.01$ , and  $*** p < 0.001$ .<br>988 ACKNOWLEDGEMENTS:<br>989 We would like to thank the Center for Medical Genomics, th 987 0.05,  $*$  p < 0.01, and  $**$  p < 0.001.<br>
988 ACKNOWLEDGEMENTS:<br>
989 We would like to thank the Center for Medical Genomics, the Small Animal Phenotypic Core, and<br>
990 the Histology and Histomorphometry Core at the Ind 988 **ACKNOWLEDGEMENTS:**<br>989 We would like to thank the Ce<br>990 the Histology and Histomorphomet<br>991 and advice with histological sample<br>992 Sakamoto, and Carrie Zhao for their<br>993 by NIH awards P01 AG039355 (to L.<br>994 Discl Me Would like to thank the C<br>1989 Me Would like to thank the C<br>1991 And advice with histological sample<br>1992 Sakamoto, and Carrie Zhao for the 990 the Histology and Histomorphometry Core at the Indiana Center for Musculoskeletal Health for help<br>
991 and advice with histological sample preparation. We would like to thank Dr. Yukiko Kitase, Dr. Eijiro<br>
992 Sakamoto 991 and advice with histological sample preparation. We would like to thank Dr. Yukiko Kitase, Dr. Eijiro<br>992 Sakamoto, and Carrie Zhao for their help and advice with the experiments. This work was supported<br>993 by NIH awa Sakamoto, and Carrie Zhao for their help and advice with the experiments. This work was supported<br>
993 by NIH awards P01 AG039355 (to L.F.B).<br>
994 Disclosures<br>
995 The authors declare that they have no conflicts of interes

by NIH awards P01 AG039355 (to L.F.B).<br>994 Disclosures<br>The authors declare that they have no conflicts of interest.<br>996 Data Availability Statement<br>997 All data that support the findings of this study are available from th 994 **Disclosures**<br>
995 The authors declare that they have<br>
996 Data Availability Statement<br>
997 All data that support the finding<br>
998 upon reasonable request. 995 Disclosures<br>
995 The author<br>
996 Data Availa<br>
997 All data th<br>
998 upon reasonable 2003 The authors declare that they have no continue of interests.<br>
995 **Data Availability Statement**<br>
998 upon reasonable request.<br>
998 39 997 Data Avanability Statement<br>997 All data that support the fi<br>998 upon reasonable request. 998 All data that support the findings of this study are available from the corresponding authority<br>998 All data the corresponding and the corresponding and the corresponding and the corresponding and the corresponding of 998 upon reasonable request.

999 The osteocyte transcriptome data is GSE242445.<br>999 The original metric of the NCBI GEO data is GSE242445.  $1001$ 



1002<br>1003<br>1004<br>1005<br>1006<br>1007 1003 Supplementary Figure 1: Pup numbers for the lactation experiment, and body weight measurements for the low<br>
1005 Panel A shows total pup numbers in WT and KO female mice that underwent pregnancy<br>
1006 and 2 weeks of l 1004 calcium-diet experiment<br>1005 and 2<br>1007 genot<br>1008 1005 Panel A shows total pup numbers in WT and KO female mice that underwent pregnancy<br>
1005 and 2 weeks of lactation. There are no significant differences in the pup numbers between<br>
1007 genotypes. Students t-test was pe 1007 and 2 weeks tudents the significant of statistical analysis. n= 8/group.<br>
1008 band C show total body weight of WT and KO female (B) and male (C) mice. No<br>
1009 statistically significant difference was found among the 1008 panels **B** and **C** show total body weight of WT and KO female (B) ar<br>1009 statistically significant difference was found among the groups, regardless of<br>1010 way ANOVA with Tukey's post hoc test was done. n= 4-5/group panels **B** and C show total body weight of WT and KO female (B) and male (C) mice. No<br>1009 statistically significant difference was found among the groups, regardless of genotype or diet. 2-<br>1010 way ANOVA with Tukey's pos 1010 way ANOVA with Tukey's post hoc test was done.  $n = 4-5/$ group. As depicted here, red is female, and blue is male. 2009 and genotype or diet. 2011  $1011$  and blue is male.



1012

1013 Supplementary Figure 2: Neither genotype nor dietary calcium alters muscle functions in vivo or ex vivo

1014 Panels A and C show in vivo muscle plantarflexion force (reported as plantarflexion to orque and plantamexion fatigue) in WT and KO female (A) and male (C) mice on a control or a low calcium diet, panels **D** and **D** show muscle electrophysiology parameters of CMAP, SMOT, and MONE in WT and KO 1017 female (B) and male (D) mice, and panels E and F show ex vivo EDL functional measurement (re ported as 1018 specific force frequency, maximum rate of contraction, maximum rate of relaxation, half-relaxat tion time, 1019 and % fatigue) in WT and KO female (E) and male (F) mice.

1020 2-way ANOVA was performed. n= 4-5/group. As depicted here, red is female, and blue is male.



1022<br>1023<br>1024<br>1025<br>1026

Supplementary Figure 3: Quality control and validation of NNA sequencing<br>1023 Sanity check of data on the sample's sex. A: Boxplot of proportional of reads<br>1024 a higher value than female. B: Boxplot of RPKM of Xist. Males 3 Sanity check of data on the sample's sex. A: Boxplot of proportional of reads on chromosome Y. Male should have<br>
2023 a higher value than female. B: Boxplot of RPKM of *Xist*. Males should have very low expression of *Xi* 1025 C: Scatter plot of PC1 and PC2 from Principal Component Analysis (PCA) of gene expression data.<br>
1026 D: qPCR analysis of *Tnsfs11*, *Acp5*, *Sost*, and *Ctsk* genes from osteocyte-enriched bone chips f<br>
1027 samples 1025 C: Scatter plot of PC1 and PC2 from Principal Component Analysis (PCA) or gene expression data.<br>
1026 D: qPCR analysis of *Tnsfs11*, *Acp5*, *Sost*, and *Ctsk* genes from osteocyte-enriched bone chi<br>
1027 samples. n= 1027 Samples. n= 3-4/sample. Two-way ANOVA was performed for statistical analysis. Gene fold-change was<br>1028 normalized using β-2-microglobulin as the housekeeping gene. a= Significantly different from WT, b= Significant 1027 samples. n= 3-4/sample. Two-way ANOVA was performed for statistical analysis. Gene fold-change was normalized using β-2-microglobulin as the housekeeping gene. a= Significantly different from WT, b= Significantly different from KO, <math display="inline">\* = p &lt; 0.05</math>.<br/>\nE: qPCR analysis of <i>Insfs11</i>, <i>Acp5</i>, <i>Sost</i>, and <i>Ctsk</i> genes from osteocyte-enriched bone chips from male samples.<br/>\nn= 3-4/sample. Two-way ANOVA was performed for statistical analysis. Gene fold-change was normalized using β-2-microglobulin as the housekeeping gene. a= Significantly different from WT, b= Significantly different from KO.

**E:** qPCR analysis of *Tnsfs11*, *Acp5*, *Sost*, and *Ctsk* genes from osteocyte-enriched bone chips from male samples.<br>
n=3-4/sample. Two-way ANOVA was performed for statistical analysis. Gene fold-change was normalized 1030 **E:** qPCR analysis of *Tnsfs11, A*<br>1031 n= 3-4/sample. Two-way ANO<br>1032 2-microglobulin as the housek<br>1033 \*= p< 0.05. 1031 **E:** qPCR analysis of *Thispann*, Acp5, 303t, and Ctsk genes from osteocyte-enriched bone chips from male samples.<br>
1031 **n=3-4/sample.** Two-way ANOVA was performed for statistical analysis. Gene fold-change was norm

- 1033  $* = p < 0.05$ .
- 1033 \*= p<br>1033 \*= p<br>1035 \*= p<br>10



Serum RANKL<br>
Supplementary Table 1: FNDC5 KO mice femurs are partially resistant to lactation-induced bone loss.<br>
Femoral cortical and trabecular bone parameters of WT and FNDC5 KO female virgin and lactation = 5-8/group. Supplementary Table 1: FNDC5 KO mice femurs are partially resistant to lactation-induced bone loss.<br>Femoral cortical and trabecular bone parameters of WT and FNDC5 KO female virgin and lactation-<br>5-8/group. Data presented 1035 Femoral cortical and trabecular bone parameters of WT and FNDC5 KO female virgin and lactat<br>
1036 = 5-8/group. Data presented as mean ± standard deviation. a= significant compared to WT control, b=<br>
1037 compared to K 1036 = 5-8/group. Data presented as mean ± standard deviation. a= significant compared to WT control, b= significant<br>1037 compared to KO control, c= significant compared to WT low Ca diet, 2-way ANOVA, significance <0.05, 1036 = 5-8/group. Data presented as mean ± standard deviation. a= significant compared to WT control, b= significant<br>
1037 compared to KO control, c= significant compared to WT low Ca diet, 2-way ANOVA, significance <0.05, 1038 Percentage change in different bone and serum parameters in WT and FNDC5 KO female mice with<br>1039 lactation. \*= p<0.05 compared to WT.<br>1040 44 1039 Percentage in different bone and serves in different bone and  $\frac{44}{1000}$  $1039$  lactation.  $\epsilon = p<0.05$  compared to WT.<br>1040



 $b$ le  $\ddot{ }$  $\frac{1}{2}$  $\overline{e}$  a le m  $\frac{1}{2}$ diffe 1041 Supplementary Table 2: WT and TNDC5 KO female and male mice bone responds differently<br>1042 to a low-calcium diet<br>45

 $\frac{1}{\sqrt{N}}$ <br> $\frac{1}{\sqrt{N}}$ <br>to a low-ca ent<br>die<br>die 1042 to a low-calcium diet

1044 4-5-month-old WT and KO female and male mice under a normal diet or a 2-week low calcium diet.<br>
1045 n = 5/group. Data presented as mean ± standard deviation.<br>
1046 a = significant compared to WT control, b = signific 1044 4-5-month-old WT and KO female and male mice under a normal diet or a 2-week low calcium diet. 1046 a = significant compared to WT control, b = significan<br>1047 compared to WT low Ca diet, 2-way ANOVA,<br>048 1047 compared to WT low Ca diet, 2-way ANOVA, significance <0.05, n= 4-5/group.<br>048 111.<br>1048<br>1048

050 Andersson G, Ek<br>051 an osteopontin phosp<br>052 Ardeshirpour L,<br>053 Prevents Bone Loss D 2050 Andersson G, Ek-Rylander B, Hollberg K, Ljusberg-Sjölander J, Lang F, Norgård M, Wang T, Zhang SJ. TRACP as<br>
051 an osteopontin phosphatase. J Bone Miner Res. 2003; 18(10):1912–1917.<br>
2052 Ardeshirpour L, Dumitru C, D 051 an osteopontin p n o sp h a t a se . J Bone Miner Res. 2005, 10(10):1912–1917.<br>
052 Ardeshirpour L, Dumitru C, Dann P, Sterpka J, VanHouten J, Kim W, Ko<br>
053 Prevents Bone Loss During Lactation But Does Not Affect Milk 252 Ardeshirpour L, Dumitru C, Dumitri, Sterpka J, Vanhouten J, Kim W, Kostenuik I, Wysolmerski J. Ord Treatment<br>
2053 Prevents Bone Loss During Lactation But Does Not Affect Milk Production or Maternal Calcium Metabolism.

- 054 Endocrinology. 2015; 156(8):2762–73.<br>055 Bao JF, She QY, Hu PP, Jia N, Li A. Irisin, a fascinating field in our times. Trends Endocrinol Metab. 2022;<br>056 33(9):601–613.<br>Bélanger LF. Osteocytic osteolysis. Calcif Tissue 054 Endocrinology. 2015, 156(8).2762–75.<br>055 Bao JF, She QY, Hu PP, Jia<br>056 33(9):601–613.<br>057 Bélanger LF. Osteocytic osteo<br>058 Body JJ, Terpos E, Tombal B,
- 
- 655 Bao JF, She QY, Hu PP, Jia N, Li A. Irisin, a fascinating field in our times. Trends Endocrinol Metab. 2022,<br>056 Bao JF, She QY, Hu PP, Jia N, Li A. Irisin, a fascinating field in our times. Trends Endocrinol Metab. 20 056 33(9):001–013.<br>057 Bélang<br>058 Body J.<br>059 patient: A SIOG <sub>1</sub><br>060 Bonew Belanger LF. Osteocytic osteolysis. Calcif Tissue Res. 1969, 4(1):1–12.<br>
058 Body JJ, Terpos E, Tombal B, Hadji P, Arif A, Young A, Aapro M, Cole<br>
059 patient: A SIOG position paper. Cancer Treat Rev. 2016; 51:46–53.<br>
060 body J, Terpos E, Tombal B, Hadji T, Arif A, Toung A, Aapro M, Coleman R. Bone health in the elderly cancer<br>059 patient: A SIOG position paper. Cancer Treat Rev. 2016; 51:46–53.<br>800 Bonewald LF. The amazing osteocyte. J Bo
- 

Bonewald L. Use it or lose it to age: A review of bone and<br>060 Bonewald L. Use it or lose it to age: A review of bone and<br>061 Bonewald LF. The amazing osteocyte. J Bone Miner Res. 201<br>062 Bostrom P, Wu J, Jedrychowski MP, Bonewald L. Use it or lose it to age: A review of Bone and muscle communication. Bone. 2019, 120:212–216.<br>
Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011; 26(2):229–267.<br>
Bostrom P, Wu J, Jedrychowski MP, Korde Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011, 20(2):229–267.<br>
062 Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bost<br>
063 Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spieg 063 Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BM. A PGC1-alpha-dependent myokine that drives<br>064 brown-fat-like development of white fat and thermogenesis. Nature. 2012; 481(7382):463–471.<br>065 B

- 064 brown-fat-like development of white fat and thermogenesis. Nature. 2012; 481(7382):463–471.<br>
065 Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone<br>
066 microstru Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guideline<br>066 microstructure in rodents using micro-computed tomography. J Bone Miner Res. 2010; 25(7):14<br>067 Breese MR, Liu Y. NGSUtils: a software
- Bouxsell ML, Boyd Sk, Christiansen BA, Guidberg RL, Jepsen KJ, Müller R. Guidelines for assessment of bone<br>066 microstructure in rodents using micro-computed tomography. J Bone Miner Res. 2010; 25(7):1468–86.<br>067 Breese MR Breese MR, Liu Y. NGSUtils: a software suite for analyzing and manipulating next-gener<br>068 datasets. Bioinformatics. 2013; 29(4):494–500.<br>069 Brotto M, Bonewald L. Bone and muscle: Interactions beyond mechanical. Bone. 201
- 
- oor Breese MR, Ed. I. NGSUtils: a software suite for analyzing and manipulating next- generation sequencing<br>068 datasets. Bioinformatics. 2013; 29(4):494–500.<br>069 Brotto M, Bonewald L. Bone and muscle: Interactions beyond 069 Brotto M, Bonewald L. Bone and musc<br>070 Celi FS, Brown H. Adipose Tissue Pla<br>071 Spiegelman B, editor, Springer Copyright; 2017.<br>072 Colaianni G. Cuscito C. Mongelli T. Ora Corrections brotto M, Bone wald L. Bone and muscle: Interactions beyond mechanical. Bone. 2015, 80:109–114.<br>
070 Celi FS, Brown H. Adipose Tissue Plasticity: Hormonal and Environmental Manipulation, in Horr<br>
071 Spiegelman
- 071 Spiegelman B, editor, Springer Copyright; 2017.<br>
071 Spiegelman B, editor, Springer Copyright; 2017.<br>
072 Colaianni G, Cuscito C, Mongelli T, Oranger A, Mori G, Brunetti G, Colucci S, Cinti S, Grano M. Irisin enhance<br>
- 071 Spiegelman B, editor, Springer Copyright; 2017.<br>
072 Colaianni G, Cuscito C, Mongelli T, Or<br>
073 osteoblast differentiation in vitro. Int J Endocrin<br>
074 Colaianni G, Cuscito C, Mongelli T, Pi<br>
075 Benedetto A, Brunett orz Colaianni G, Cuscito C, Mongelli T, Oranger A, Mori G, Brunetti G, Colucci S, Cinti S, Grano M. Irisin emanices<br>073 osteoblast differentiation in vitro. Int J Endocrinol. 2014; p. 902186–902186.<br>075 Benedetto A, Brunet 073 osteoblast differentiation in vitro. Int J Endocrinol. 2014, p. 902186–902186.<br>
074 Colaianni G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu<br>
075 Benedetto A, Brunetti G, Yuen T, Sun L, Reseland JE, Colucci S
- Colaianni G, Cuscito C, Mongelli T, Fignataro T, Buccoliero C, Liu T, Lu T, Sartini L, Di Comite M, Mori G, Di<br>O75 Benedetto A, Brunetti G, Yuen T, Sun L, Reseland JE, Colucci S, New MI, Zaidi M, Cinti S, Grano M. The myok 076 Increases cortical bone mass. Proc Natl Acad Sci. 2015; 112(39):12157–62.<br>077 Colaianni G, Mongelli T, Cuscito C, Pignataro P, Lippo L, Spiro G, Notarnicola A, Severi I, Passeri G, Mori G, Brunett<br>078 Moretti B, Tarant 077 Colaianni G, Mongelli T, Cuscito C, Pignataro P, Lippo L, Spiro G, Nongelli T, Cuscito C, Pignataro P, Lippo L, Spiro G, Nongelli T, Cuscito C, Pignataro P, Lippo L, Spiro G, Nongelli T, Cuscito C, Pignataro P, Lippo L Colaianni G, Mongelli T, Cuscito C, Pignataro P, Lippo L, Spiro G, Notarnicola A, Severi I, Passeri G, Mori G, Brunetti (1768) Moretti B, Tarantino U, Colucci SC, Reseland JE, Vettor R, Cinti S, Grano M. Irisin prevents an 079 atrophy in hind-limb suspended mice. Sci Rep, 2017; 7(1): p. 2811.<br>47 079 atrophy in hind-limb suspended mice. Sci Rep, 2017; 7(1): p. 2811.

- 
- 
- Colatanni G, Grano M. Role of Irisin on the bone-muscle functional unit. Bonekey Rep, 2015, 4: p. 765.<br>
081 Colucci SC, Buccoliero C, Sanesi L, Errede M, Colaianni G, Annese T, Khan MP, Zerlotin R, Dicarlo I<br>
082 Kozloff K
- Coluctive, Buccoliero C, Sanesi L, Errede M, Colafami G, Annese T, Khan MP, Zerlotin R, Dicarlo M, Schipani E,<br>
082 Kozloff KM, Grano M. Systemic Administration of Recombinant Irisin Accelerates Fracture Healing in Mice. I 083 2019; p. 22–22.<br>084 Dallas SL, Prideaux M, Bonewald LF. The osteocyte: an endocrine cell ... and more. Endocr Rev. 2013; 34(5):658<br>085 90.<br>086 Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Cha 083 2019, p. 22–22.<br>084 Dallas !<br>085 90.<br>086 Dobin<br>087 universal RNA-s
- Dallas SL, Frideaux M, Bonewald LF. The osteocyte: an endocrine cell ... and more. Endocrine 2013, 34(5):658–<br>085–90.<br>Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR:
- 085 90.<br>086<br>087 univ<br>088<br>089 Sex Learn A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut F, Chaisson M, Gingeras TR. STAR: ultrafa<br>
087 universal RNA-seq aligner. Bioinformatics. 2013; 29(1):15–21.<br>
Dole NS, Yee CS, Mazur CM, Acevedo C, Alli 088 Dole NS, Yee CS, Mazur CM, Acevedo C, Alliston T. TO<br>089 Sexually Dimorphic. J Bone Miner Res. 2020; 35(8):1549-1561.<br>090 During A. Osteoporosis: A role for lipids. Biochimie. 2
- 
- O89 Sexually Dimorphic. J Bone Miner Res. 2020; 35(8):1549-1561.<br>
During A. Osteoporosis: A role for lipids. Biochimie. 2020; 178:49–55.<br>
Erickson HP. Irisin and FNDC5 in retrospect: An exercise hormone or a transmembrane During A. Osteoporosis: A role for lipids. Biochimie. 20<br>091 Erickson HP. Irisin and FNDC5 in retrospect: An exercise hori<br>092 289-93.
- Erickson HP. Irisin and FNDC5 in retrospect: An exercise hormone or a transm<br>
092 289-93.<br>
Essex AL, Huot JR, Deosthale P, Wagner A, Figueras J, Davis A, Damrath J<br>
094 Triggering Receptor Expressed on Myeloid Cells 2 (TRE Erickson HP. Irisin and FNDC5 in retrospect. An exercise hormone or a transmembrane receptor? Adipocyte, 2013, 2(4): p.<br>092 289-93.<br>094 Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) R47H Variant Causes Distinct 092 209 93.<br>093<br>094 Triggeri<br>095 Musculo<br>096 Essex AL, Huot JR, Deosthale P, Wagner A, Figueras J, Davis A, Damrath J, Pin P, Wandee J, Bonetto A, Plotkin Li<br>094 Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) R47H Variant Causes Distinct Age- and Sex-Depend
- 095 Musculoskeletal Alterations in Mice. J Bone Miner Res. 2022; 37(7):1366–1381.<br>
096 Estell EG, Le PT, Vegting Y, Kim H, Wrann C, Bouxsein ML, Nagano K, Baron R, Spiegelman BM, Rosen CJ. Irisin<br>
097 directly stimulates o 095 Musculoskeletal Alterations in Mice. J Bone Miner Res. 2022, 37(7):1366–1381.<br>096 Estell EG, Le PT, Vegting Y, Kim H, Wrann C, Bouxsein ML, Naganc<br>097 directly stimulates osteoclastogenesis and bone resorption in vitro
- Esten Ed, Le PT, Vegting P, Kim H, Wrann C, Bouxsein ML, Nagano K, Baron K, Spiegelman BM, Kosen CJ. Irisin<br>097 directly stimulates osteoclastogenesis and bone resorption in vitro and in vivo. Elife. 2020; p. 2020–2029.<br>10 098 Farley JR, Baylink DJ. Skeletal alkaline phosphatase activity as a bone formation index in vitro. M<br>099 35(6):563–71.<br>100 Feng JQ, Ye L, Schiavi S. Do osteocytes contribute to phosphate homeostasis? Curr Opin Ner<br>101 2
- Farley JR, Baylink DJ. Skeletal alkaline phosphatase activity as a bone formation index in vitro. Metabolism. 1986;<br>100 Feng JQ, Ye L, Schiavi S. Do osteocytes contribute to phosphate homeostasis? Curr Opin Nephrol Hyperte
- 099 35(6):563–71.<br>
100 Feng<br>
101 2009; 18(4):28<br>
102 Geng<br>
103 the male rat. C 101 2009; 18(4):285–91.<br>101 2009; 18(4):285–91.<br>102 Geng W, Wright GL. Skeletal sensitivity to dietary calcium deficiency is increased in the female compared with<br>103 the male rat. Can J Physiol Pharmacol. 2001; 79(5):379– 101 2009, 10(4):205 91.<br>
102 Geng W, W<br>
103 the male rat. Can J P<br>
104 The Gene O<br>
105 Goltzman I
- 
- 102 Geng W, Wright GL. Skeletal sensitivity to dietary calcium deficiency is increased in the female compared with<br>103 the male rat. Can J Physiol Pharmacol. 2001; 79(5):379–85.<br>104 The Gene Ontology Resource: enriching a 103 the male rat. Can J Physiol Pharmacol. 2001, 79(9):379–85.<br>104 The Gene Ontology Resource: enriching a GOld mi<br>105 Goltzman D. Studies on the mechanisms of th<br>106 parathyroid hormone. Arch Biochem Biophys. 2008; 473(2) 105 Goltzman D. Studies on the mechanisms of the skeletal anabolic action of endogenous and<br>106 parathyroid hormone. Arch Biochem Biophys. 2008; 473(2):218–242.<br>107 Gu Z. Complex heatmap visualization. iMeta, 2022; 1(3): p
- 
- 106 parathyroid hormone. Arch Biochem Biophys. 2008; 473(2):218–242.<br>107 Gu Z. Complex heatmap visualization. iMeta, 2022; 1(3): p. e43.<br>108 Hamrick MW, Samaddar T, Pennington C, McCormick J. Increased muscle mass with myo 107 Gu Z. Complex heatmap visualization. iMeta, 2022; 1(3): p. e43.<br>108 Hamrick MW, Samaddar T, Pennington C, McCormick J.<br>109 improves gains in bone strength with exercise. J Bone Miner Res. 2006<br>110 Huot JR, Pin F, Essex 107 Gu Z. Complex heatmap visualization. inveta, 2022, 1(3): p. e43.<br>
108 Hamrick MW, Samaddar T, Pennington C, McCormick J. I<br>
109 improves gains in bone strength with exercise. J Bone Miner Res. 2006<br>
110 Huot JR, Pin F, 109 improves gains in bone strength with exercise. J Bone Miner Res. 2006; 21(3):477–83.<br>
10 Huot JR, Pin F, Essex AL, Bonetto A. MC38 Tumors Induce Musculoskeletal Defects in Colorectal Cancer. Int J Mol<br>
18
- 110 **improves gains in bone strength with exercise.** J Bone Miner Res. 2000, 21(3):477–83.<br>110 **Huot JR**, Pin F, Essex AL, Bonetto A. MC38 Tumors Induce Musculoskeletal De 110 Huot JR, Pin F, Essex AL, Bonetto A. MC38 Tumors Induce Musculoskeletal Defects in Colorectal Cancer. Int J Mor<br>48

111 Sci. 2021, 22(3).<br>112 Huot JF<br>113 induced cachexia<br>114 Islam M<br>115 Christie BR, Schr 112 Huot JR, Pin F, Chatterjee R, Bonetto A. Poeta overexpression preserves masse mass and function in cisplatin-<br>113 Induced cachexia. J Cachexia Sarcopenia Muscle. 2022; 13(5):2480–2491.<br>114 Islam MR, Valaris S, Young MF 113 Induced cachexia. J Cachexia Sarcopenia Muscle. 2022, 13(3):2480–2491.<br>114 Islam MR, Valaris S, Young MF, Haley EB, Luo R, Bond SF, Ma<br>115 Christie BR, Schmider AB, Soberman RJ, Besnard A, Jedrychowski MP, Kir<br>116 BM, 115 Christie BR, Schmider AB, Soberman RJ, Besnard A, Jedrychowski MP, Kim H, Tu H, Kim E, Choi SH, Tanzi RE, Spiegelman<br>116 BM, Wrann CD. Exercise hormone irisin is a critical regulator of cognitive function. Nat Metab. 2

116 BM, Wrann CD. Exercise hormone irisin is a critical regulator of cognitive function. Nat Metab. 2021; (8):1058–1070.<br>117 Jahn K, Kelkar S, Zhao H, Xie Y, Tiede-Lewis LM, Dusevich V, Dallas SL, Bonewald LF. Osteocytes A

117 Jahn K, Kelkar S, Zhao H, Xie Y, Tiede-Lewis LM, Dusevich V, Dallas SL, Bonewald LF. Osteocytes Acidify<br>118 Microenvironment in Response to PTHrP In Vitro and in Lactating Mice In Vivo. J Bone Miner Res. 2017; 32(8):17 117 Jahn K, Kelkar S, Zhao H, Xie T, Tiede Eewis EM, Dusevien V, Dunas SL, Bonewald ET. Osteocytes Acidify Their<br>118 Microenvironment in Response to PTHrP In Vitro and in Lactating Mice In Vivo. J Bone Miner Res. 2017; 32(

119 Microenvironment in Response to PTHTP In Vitro and in Lactating Mice In Vivo. J Bone Miner Res. 2017, 32(8):1761–1772.<br>119 Jähn-Rickert K, Zimmermann EA. Potential Role of Perilacunar Remodeling in the Progression of 119 Jamm-Rickert R, Zimmermann EA. Potential Role of Perilacular Remodeling in the Progression of Osteoporosis<br>120 and Implications on Age-Related Decline in Fracture Resistance of Bone. Curr Osteoporos Rep. 2021; 19(4):3

120 and Implications on Age-Related Decline in Fracture Resistance of Bone. Curr Osteoporos Rep. 2021; 19(4):391–402.<br>
121 **b** D, Song J. Irisin Acts via the PGC-1α and BDNF Pathway to Improve Depression-like Behavior. Cl 122 2021; 10(4):292–302.<br>122 2021; 10(4):292–302.<br>123 Johannesdottir F, Aspelund T, Reeve J, Poole KE, Sigurdsson S, Harris TB, Gudnason VG, Sigurdsson G.<br>124 Similarities and differences between sexes in regional loss of 122 2021, 10(4):292 302.<br>
123 Johannesdot<br>
124 Similarities and differences<br>
125 AGES-Reykjavik longit<br>
126 Johnston CB, 123 Similarities and differences between sexes in regional loss of cortical and trabecular bone in the mid-femoral neck: the<br>125 AGES-Reykjavik longitudinal study. J Bone Miner Res. 2013; 28(10):2165–76.<br>126 Johnston CB, D 125 AGES-Reykjavik longitudinal study. J Bone Miner Res. 2013; 28(10):2165–76.<br>126 Johnston CB, Dagar M. Osteoporosis in Older Adults. Med Clin North Am. 2020; 104(5):873–884.<br>127 Kalkwarf HJ. Lactation and maternal bone h

125 AGES-Reykjavik longitudinal study. J Bone Miner Res. 2013, 20(10):2165–76.<br>126 Johnston CB, Dagar M. Osteoporosis in Older Adults. Med Clin North<br>127 Kalkwarf HJ. Lactation and maternal bone health. Adv Exp Med Biol<br>12

127 Kalkwarf HJ. Lactation and maternal bone health. Adv Exp Med Biol. 2004; 554:101–115.<br>
128 Kaplan MM. Alkaline phosphatase. N Engl J Med. 1972; 286(4):200–202.<br>
129 Karsenty G, Mera P. Molecular bases of the crosstalk

127 Kalkwarf HJ. Lactation and maternal bone health. Adv Exp Med Biol. 2004, 554:101–115.<br>128 Kaplan MM. Alkaline phosphatase. N Engl J Med. 1972; 286(4):200–202.<br>130 Kawao N, Moritake A, Tatsumi K, Kaji H. Roles of Irisin Kaplan MM. Alkaline phosphatase. N Engl J Med. 1972, 286(4):200–202.<br>
Karsenty G, Mera P. Molecular bases of the crosstalk between bone and<br>
Kawao N, Moritake A, Tatsumi K, Kaji H. Roles of Irisin in the Linkage fi<br>
131 Un

Raisenty G, Mera P. Molecular bases of the crosstalk between bone and muscle. Bone. 2016, 119:43–49.<br>
130 Kawao N, Moritake A, Tatsumi K, Kaji H. Roles of Irisin in the Linkage from Muscle to Bone During Med<br>
131 Unloading 130 Kawao N, Moritake A, Tatsumi K, Kaji H. Roles of Irisin in the Enkage from Muscle to Bone During Mechanical<br>131 Unloading in Mice. Calcif Tissue Int. 2018; 103(1):24–34.<br>133 Strutzenberg TS, Pascal BD, Le PT, Brooks DJ 132 Kim H, Wrann CD, Jedrychowski M, Vidoni S,<br>133 Strutzenberg TS, Pascal BD, Le PT, Brooks DJ, Roche AM<br>134 Baron R, Rosen CJ, Bonewald LF, Spiegelman BM. Irisin<br>135 Cell. 2018; 175(7):17–17. 132 Kim H, Wrann CD, Jedrychowski M, Vidoni 3, Kitase T, Nagano K, Zhou C, Chou J, Farkman VA, Novick 31,<br>133 Strutzenberg TS, Pascal BD, Le PT, Brooks DJ, Roche AM, Gerber KK, Mattheis L, Chen W, Tu H, Bouxsein ML, Griffi 134 Baron R, Rosen CJ, Bonewald LF, Spiegelman BM. Irisin Mediates Effects on Bone and Fat via alphaV Integrin Receptors.<br>135 Cell. 2018; 175(7):17–17.<br>136 Kitase Y, Vallejo JA, Gutheil W, Vemula H, Jähn K, Yi J, Zhou J, B

135 Cell. 2018; 175(7):17–17.<br>136 Kitase Y, Vallejo JA, Gutheil W, Vemula H, Jähn K, Yi J, Zhou J, Brotto M, Bonewald LF. 6-aminoisobutyric Acid, I-<br>137 BAIBA, Is a Muscle-Derived Osteocyte Survival Factor. Cell Rep. 2018;

135 Cell. 2018, 175(7):17–17.<br>136 Kitase Y, Vallejo<br>137 BAIBA, Is a Muscle-Derive<br>138 Korta P, Pocheć<br>139 Medicina (Kaunas), 2019; 136 BAIBA, Is a Muscle-Derived Osteocyte Survival Factor. Cell Rep. 2018; 22(6):1531–1544.<br>138 Korta P, Pocheć E, Mazur-Biały A. Irisin as a Multifunctional Protein: Implications for Health and Certain Diseas<br>139 Medicina 137 BAIBA, IS a Muscle-Derived Osteocyte Survival Factor. Cell Rep. 2016, 22(6):1531–1544.<br>
138 Korta P, Pocheć E, Mazur-Biały A. Irisin as a Multifunctional Protein: Implicat<br>
139 Medicina (Kaunas), 2019; 55(8).<br>
140 Kova

139 Medicina (Kaunas), 2019; 55(8).<br>
139 Medicina (Kaunas), 2019; 55(8).<br>
140 Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. The Journal of clinical endocrinology<br>
141 and metabolism. 2001; (6):86–86. 139 Medicina (Kaunas), 2015, 35(8).<br>140 Kovacs CS. Calcium ar<br>141 and metabolism. 2001; (6):86– 140 Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. The Journal of clinical endocrinology<br>141 and metabolism. 2001; (6):86–86. 141 and metabolism. 2001, (6):06–86.

- 
- Rumssa DB, Joy EJ, Ander EL, Watts MJ, Toung SD, Wanker S, Broadley MR. Dietary calcium and zinc deficiency risks are<br>143 decreasing but remain prevalent. Sci Rep, 2015; 5: p. 10974.<br>145 Rev Musculoskelet Med. 2022.<br>146 Le 143 decreasing but remain prevalent. Scritep, 2015, 5: p. 10974.<br>144 Kurapaty SS, Hsu WK. Sex-Based Difference<br>145 Rev Musculoskelet Med. 2022.<br>146 Lee HJ, Lee JO, Kim N, Kim JK, Kim HI, Lee Y\<br>147 Irisin, a Novel Myokine, Kurapaty SS, Hsu WK. Sex-Based Difference in Bone Healing: A Review of Recent Preclinical Literature. Curr<br>145 Rev Musculoskelet Med. 2022.<br>146 Lee HJ, Lee JO, Kim N, Kim JK, Kim HI, Lee YW, Kim SJ, Choi JI, Oh Y, Kim JH,
- 145 Rev Musculoskelet Med. 2022.<br>146 Lee HJ, Lee JO, Kim N,<br>147 Irisin, a Novel Myokine, Regulate<br>148 Liao Y, Smyth GK, Shi V<br>149 to genomic features. Bioinform
- 146 Lee HJ, Lee Jo, Kim N, Kim JK, Kim HI, Lee YW, Kim JJ, Choi JI, Oh Y, Kim JI, Suyeon-Hwang, Park SH, Kim HS.<br>147 Irisin, a Novel Myokine, Regulates Glucose Uptake in Skeletal Muscle Cells via AMPK. Mol Endocrinol. 2015
- 147 Irisin, a Novel Myokine, Regulates Glucose Optake in Skeletal Muscle Cells via AMPK. Mol Endocrinol. 2015, 29(6):873–81.<br>148 Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning se 149 to genomic features. Bioinformatics. 2014; 30(7):923–953.<br>150 Liu Q, Lei L, Yu T, Jiang T, Kang Y. Effect of Brain-Derived Neurotrophic Factor on the Neurogenesis and<br>151 Osteogenesis in Bone Engineering. Tissue Eng Pa
- 149 to genomic reatures. Biomormatics. 2014, 30(7):323–333.<br>150 Liu Q, Lei L, Yu T, Jiang T, Kang Y. Effect of Brai<br>151 Osteogenesis in Bone Engineering. Tissue Eng Part A. 2018; 24<br>152 Love MI, Huber W, Anders S. Moderate 150 Liu C, Lei L, Yu Y, Jiang Y, Kang Y. Effect of Brain-Derived Neurotrophic Factor on the Neurogenesis and<br>151 Osteogenesis in Bone Engineering. Tissue Eng Part A. 2018; 24:1283–1292.<br>152 Love MI, Huber W, Anders S. Mode
- 151 Osteogenesis in Bone Engineering. Tissue Eng Part A. 2010, 24:1283–1292.<br>152 Love MI, Huber W, Anders S. Moderated estimation of fold ch<br>153 DESeq2. Genome Biol. 2014; 15(12):550–550.<br>154 Lu D, Demissie S, Horowitz NB, 152 Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with<br>153 DESeq2. Genome Biol. 2014; 15(12):550–550.<br>154 Lu D, Demissie S, Horowitz NB, Gower AC, Lenburg ME, Alekseyev YO, 153 DESeq2. Genome Biol. 2014, 15(12):550–550.<br>154 Lu D, Demissie S, Horowitz NB, Gower.<br>155 Webster MZ, Schlezinger JJ, Morgan EF, Gerste<br>156 Dimorphism in Murine Postnatal Bone Aging. JBI<br>157 Luo X, Li J, Zhang H, Wang Y 154 Lu D, Demissie S, Horowitz ND, Gower Ac, Lenburg ME, Alekseyev TO, Husseln AI, Bragdon B, Liu T, Daukss D, Tage JM,<br>155 Webster MZ, Schlezinger JJ, Morgan EF, Gerstenfeld LC. Temporal and Quantitative Transcriptomic Di
- 156 Dimorphism in Murine Postnatal Bone Aging. JBMR Plus, 2022; 6(2): p. e10579.<br>157 Luo X, Li J, Zhang H, Wang Y, Shi H, Ge Y, Yu X, Wang H, Dong Y. Irisin promotes the browning of white adipocytes<br>158 tissue by AMPKα1 s Luo X, Li J, Zhang H, Wang Y, Shi H, Ge Y, Yu X, Wang H, Dong Y. Iris<br>158 tissue by AMPKa1 signaling pathway. Res Vet Sci. 2022; 152:270–276.<br>159 Ma Y, Qiao X, Zeng R, Cheng R, Zhang J, Luo Y, Nie Y, Hu Y, Yang<br>160 promote
- 157 Luo X, Li J, Zhang H, Wang H, Shi H, Ge H, Yu X, Wang H, Dong H. Insin promotes the browning of white adipocytes<br>158 tissue by AMPKa1 signaling pathway. Res Vet Sci. 2022; 152:270–276.<br>160 promotes proliferation but in
- 158 tissue by AMP Ra1 signaling pathway. Res Vet Sci. 2022, 152:270–276.<br>159 Ma Y, Qiao X, Zeng R, Cheng R, Zhang J, Luo Y, Nie Y, Hu Y<br>160 promotes proliferation but inhibits differentiation in osteoclast precur<br>161 Maak 159 Ma Y, Qiao X, Zeng R, Cheng R, Zhang J, Luo Y, Nie Y, Hu Y, Yang Z, Zhang J, Lui L, Xu W, Xu CC, Xu L. Irishi<br>160 promotes proliferation but inhibits differentiation in osteoclast precursor cells. Faseb j. 2018; p. 201 161 Maak S, Norheim F, Drevon CA, Erickson HP. Progress and Challenges in the Biology of FNDC5 and Irisin. Endoc<br>162 Rev. 2021; 42(4):436–456.<br>163 Matikainen N, Pekkarinen T, Ryhänen EM, Schalin-Jäntti C. Physiology of Cal
- 162 Rev. 2021; 42(4):436–456.<br>162 Rev. 2021; 42(4):436–456.<br>163 Matikainen N, Pekkarinen T, Ryhänen EM, Schalin-Jäntti C. Physiology of Calcium Homeostasis: An Overview.<br>164 Endocrinol Metab Clin North Am. 2021; 50(4):575–
- 162 Rev. 2021, 42(4):436–456.<br>163 Matikainen N, Pe<br>164 Endocrinol Metab Clin Nori<br>165 Melville KM, Rok<br>166 1226:99–115. 163 Matikainen N, Pekkarinen P, Ryhänen EM, Schalin-Jäntti C. Physiology of Calcium Homeostasis: An Overview.<br>164 Endocrinol Metab Clin North Am. 2021; 50(4):575–590.<br>165 Melville KM, Robling AG, Meulen MCVD. In vivo axial 164 Endocrinor Metab Clin North Am. 2021, 50(4):575–590.<br>165 Melville KM, Robling AG, Meulen MCVD. In<br>166 1226:99–115.<br>167 Mo C, Zhao R, Vallejo J, Igwe O, Bonewald L, '<br>168 skeletal muscle myoblasts via EP4 receptor activ
- 166 1226:99–115.<br>
166 1226:99–115.<br>
166 Melville KM, Nobiling Ad, Meuter MCVD. In vivo axial loading of the mouse tibia. Methods Mol Biol. 2015,<br>
167 Mo C, Zhao R, Vallejo J, Igwe O, Bonewald L, Wetmore L, Brotto M. Prosta
- 167 Mo C<br>168 skeletal muscle<br>169 More<br>170 21(2):122–133 167 Skeletal muscle myoblasts via EP4 receptor activation. Cell Cycle. 2015; 14(10):1507–1523.<br>
169 Moreno-Layseca P, Icha J, Hamidi H, Ivaska J. Integrin trafficking in cells and tissues. Nat Cell Biol. 2019;<br>
170 21(2):1
- 169 Moreno-Layseca P, Icha J, Hamidi H, Ivaska J. Integrin trafficking in cells and<br>170 21(2):122–132.<br>171 Mu A, Wales TE, Zhou H, Draga-Coletă SV, Gorgulla C, Blackmore KA, Mittenbür<br>172 Zhang Q, Wang ZF, Jedrychowski MP, moreno-Layseca 1, Icha J, Hamidi H, Iwaska J. Integrin trafficking in cells and tissues. Nat Cell Biol. 2019,<br>170 21(2):122–132.<br>171 Mu A, Wales TE, Zhou H, Draga-Coletă SV, Gorgulla C, Blackmore KA, Mittenbühler MJ, Kim C 170 21(2):122– 132.<br>171 Mu A,<br>172 Zhang Q, Wang :<br>173 Engen JR, Spiege 171 Mu A, Wales TE, Zhou H, Draga-Coletă SV, Gorgulla C, Blackmore KA, Mittenbühler MJ, Kim CR, Bogoslavski D,<br>172 Zhang Q, Wang ZF, Jedrychowski MP, Seo HS, Song K, Xu AZ, Sebastian L, Gygi SP, Arthanari H, Dhe-Paganon S, 173 Engen JR, Spiegelman BM. Irisin acts through its integrin receptor in a two-step process involving extracellular Hsp90 $\alpha$ .<br>50 173 Engen II, Spiegelman BM. Irisin acts through its integrin receptor in a two-step process involving extracellular Hsp90a.<br>50

174 Morecal. 2023, 83(11):1903–1920.<br>175 Muruganandan S, Sina<br>176 differentiation. IUBMB Life. 2014;<br>177 Nakashima T, Hayashi N<br>178 Wagner EF, Penninger JM, Takay 175 differentiation. IUBMB Life. 2014; 66(3):147–155.<br>176 differentiation. IUBMB Life. 2014; 66(3):147–155.<br>177 Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A,<br>178 Wagner EF 175 differentiation. ISBND Life. 2014, 66(3):147–155.<br>178 Wagner EF, Penninger JM, Takayanagi H. Eviden<br>179 expression. Nat Med. 2011; 17(10):1231–1235.<br>180 Ono T, Hayashi M, Sasaki F, Nakashima T. RA 177 Makashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LT, Kodama T, Wutz A,<br>178 Wagner EF, Penninger JM, Takayanagi H. Evidence for osteocyte regulation of bone homeostasis through RANKL<br>179 expres

- 179 expression. Nat Med. 2011; 17(10):1231–1235.<br>180 Ono T, Hayashi M, Sasaki F, Nakashima T. RANKL biology: bone metabolism, the immune system, and beyond. Inflamn<br>181 Regen, 2020; 40: p. 2.<br>182 Osipov B, Paralkar MP, Ema 179 Expression. Nat Med. 2011, 17(10):1231–1235.<br>180 Ono T, Hayashi M, Sasaki F, Nakashima T.<br>181 Regen, 2020; 40: p. 2.<br>182 Osipov B, Paralkar MP, Emami AJ, Cunnin<br>183 differences in systemic bone and muscle loss follo
- 181 Regen, 2020; 40: p. 2.<br>181 Regen, 2020; 40: p. 2.<br>182 Osipov B, Paralkar MP, Emami AJ, Cunningham HC, Tjandra PM, Pathak S, Langer HT, Baar K, Christiansen BA. Sex<br>183 differences in systemic bone and muscle loss follo
- 182 Osipov B, P.<br>182 Osipov B, P.<br>183 differences in system<br>184 Perakakis N<br>185 role of irisin in gluco 183 differences in systemic bone and muscle loss following femur fracture in mice. J Orthop Res. 2022; (4):878–890.<br>
184 Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, Mantzoros CS. Physiolog 184 Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, Mantzoros CS. Physic<br>185 role of irisin in glucose homeostasis. Nat Rev Endocrinol. 2017; 13(6):324–337.<br>186 Pin F, Prideaux M, Huot JR, Ess
- 185 role of irisin in glucose homeostasis. Nat Rev Endocrinol. 2017; 13(6):324–337.<br>186 Pin F, Prideaux M, Huot JR, Essex AL, Plotkin LI, Bonetto A, Bonewald LF. Non-bone metastatic cancers promote<br>187 osteocyte-induced bo
- 185 Fole of irisin in gluesse homeostasis. Nat Rev Endocrinol. 2017, 13(6):324–337.<br>186 Pin F, Prideaux M, Huot JR, Essex AL, Plotkin LI, Bonetto A, Bonewald LF.<br>187 osteocyte-induced bone destruction. Cancer Lett. 2021; 5 187 osteocyte-induced bone destruction. Cancer Lett. 2021; 520:80–90.<br>188 Pin F, Jones AJ, Huot JR, Narasimhan A, Zimmers TA, Bonewald LF, Bonetto A. RANKL Blockade Reduces<br>189 Cachexia and Bone Loss Induced by Non-Metasta 188 **Pin F, Jones AJ, Huot JR, Narasimhan A, Zimmers TA, I**<br>189 Cachexia and Bone Loss Induced by Non-Metastatic Ovarian Cancer<br>190 **Posa F, Colaianni G, Di Cosola M, Dicarlo M, Gaccione**<br>191 Promotes Osteogenic Differenti
- 189 Cachexia and Bone Loss Induced by Non-Metastatic Ovarian Cancer in Mice. J Bone Miner Res. 2022; 37(3):381–396.<br>189 Cachexia and Bone Loss Induced by Non-Metastatic Ovarian Cancer in Mice. J Bone Miner Res. 2022; 37(3)
- 189 Cachexia and Bone Loss Induced by Non-Metastatic Ovarian Cancer in Mice. 3 Bone Miner Res. 2022, 37(3):381–396.<br>191 Promotes Osteogenic Differentiation of Dental Bud-Derived MSCs. Biology. 2021; (4).<br>192 Qi JY, Yang LK 190 Promotes Osteogenic Differentiation of Dental Bud-Derived MSCs. Biology. 2021; (4).<br>191 Promotes Osteogenic Differentiation of Dental Bud-Derived MSCs. Biology. 2021; (4).<br>193 neurodegenerative diseases. Neuroscience. 191 Promotes Osteogenic Differentiation of Dental Bud-Derived MSCs. Biology. 2021, (4).<br>192 Qi JY, Yang LK, Wang XS, Wang M, Li XB, Feng B, Wu YM, Zhang K, Liu SB.<br>193 neurodegenerative diseases. Neuroscience. 2022; 498:28
- 192 Qi Jy, Yang Ek, Wang XJ, Wang M, Li XB, Teng B, Wu YM, Zhang K, Liu SB. Irisin: A promising treatment for<br>
193 neurodegenerative diseases. Neuroscience. 2022; 498:289–299.<br>
194 Qing H, Ardeshirpour L, Pajevic PD, Dusev 193 neurodegenerative diseases. Neuroscience. 2022, 498:289–299.<br>
194 Qing H, Ardeshirpour L, Pajevic PD, Dusevich V, Jähn<br>
195 of osteocytic perilacunar/canalicular remodeling in mice during<br>
196 Qing H, Bonewald LF. Oste
- 194 Cing H, Ardeshirpour L, Pajevic PD, Dusevich V, Jahn K, Kato 3, Wysolmerski J, Bone wald LP. Demonstration<br>195 of osteocytic perilacunar/canalicular remodeling in mice during lactation. J Bone Miner Res. 2012; 27(5):10 195 of osteocytic perilacular canalicular remodeling in mice during idetation. J Bone Miner Res. 2012, 27(5):1018–1047.<br>196 Qing H, Bonewald LF. Osteocyte remodeling of the perilacunar and pericanalicular matrix. Internati
- 
- 196 Cing H, Bonewald LF. Osteocyte remodeling of the perilacular and pericanalicular matrix. International Journal<br>196 Robling AG, Bonewald LF. The Osteocyte: New Insights. Annu Rev Physiol. 2020; 82:485–506.<br>199 Rosen CJ, 198 Robling AG, E<br>198 Robling AG, E<br>199 Rosen CJ, Bo<br>200 2006; 2(1):35–43.<br>201 Saedi A, Be 198 Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? Nat Cline<br>200 2006; 2(1):35–43.<br>201 Saedi A, Bermeo S, Plotkin L, Myers DE, Duque G. Mechanisms of palmitate-induc<br>202 osteocytes. Bone
- 199 Rosen CJ, Bouxsein ML. Mechanisms of disease. Is osteoporosis the obesity of bone? Nat Clin Fract Rheumatol.<br>
199 2006; 2(1):35–43.<br>
199 Saedi A, Bermeo S, Plotkin L, Myers DE, Duque G. Mechanisms of palmitate-induced
- 200 2000, 2(1):33–43.<br>201 Saedi A,<br>202 osteocytes. Bone.<br>203 Saedi A, 4<br>204 Volumes have Reg 201 Saedi A, Bermeo S, Plotkin L, Myers DL, Daque G. Mechanisms of palmitate-induced lipotoxicity in<br>202 osteocytes. Bone. 2019; 127:353–359.<br>203 Saedi A, Chen L, Phu S, Vogrin S, Miao D, Ferland G, Gaudreau P, Duque G. Ag 202 osteocytes. Bone. 2019, 127:353–359.<br>203 Saedi A, Chen L, Phu S, Vogrin !<br>204 Volumes have Regional Impacts on Bone 203 Saedi A, Chen L, Phu S, Voghir S, Miao D, Ferland G, Gaudreau P, Duque G. Age-Related Increases in Marrow Fat<br>204 Volumes have Regional Impacts on Bone Cell Numbers and Structure. Calcif Tissue Int. 2020; 107(2):126–13  $204$  Volumes have Regional Impacts on Bone Cell Numbers and Structure. Calcif Tissue Int. 2020, 107(2):126–134.

205 Schwartz AV. Marrow fat and bone: review of clinical findings. Front Endocrinol (Lausannic), 2015, 6: p. 40.<br>206 Sharma A, Michels LV, Pitsillides AA, Greeves J, Plotkin LI, Cardo V, Sims NA, Clarkin CE. Sexin<br>207 Impr 206 Sharma A, Michels Lv, Fitsillides AA, Greeves J, Flotkin Li, Cardo V, Sinis NA, Clarkin CE. Sexing Bones.<br>207 Improving Transparency of Sex Reporting to Address Bias Within Preclinical Studies. J Bone Miner Res. 2023;

207 Improving Transparency of Sex Reporting to Address Bias Within Preclinear Studies. J Bone Miner Res. 2023, 38(1):5–13.<br>208 Shimonty A, Bonewald LF, Pin F. Role of the Osteocyte in Musculoskeletal Disease. Curr Osteopor 208 21(3):303–310.<br>209 21(3):303–310.<br>210 Silver IA, Murrills RJ, Etherington DJ. Microelectrode studies on the acid microenvironment beneath adherent<br>211 macrophages and osteoclasts. Exp Cell Res. 1988; 175(2):266–76.<br>212

209 21(3):303–310.<br>210 Silver I<br>211 macrophages and<br>212 Styner<br>213 C, Rubin J.Exer 211 macrophages and osteoclasts. Exp Cell Res. 1988; 175(2):266–76.<br>212 Styner M, Pagnotti GM, Galior K, Wu X, Thompson WR, Uzer G, Sen B, Xie Z, Horowitz MC, Styner MA, Rubin<br>213 C, Rubin J.Exercise Regulation of Marrow F 211 macrophages and osteoclasts. Exp Cell Res. 1988; 179(2):266–76.<br>212 Styner M, Pagnotti GM, Galior K, Wu X, Thompson WR,<br>213 C, Rubin J.Exercise Regulation of Marrow Fat in the Setting of<br>214 Endocrinology. 2015; 156(8) Styner M, Pagnotti GM, Galior K, Wu X, Thompson WR, Uzer G, Sen B, Xie Z, Horowitz MC, Styner MA, Rubin<br>213 C, Rubin J.Exercise Regulation of Marrow Fat in the Setting of PPARµ Agonist Treatment in Female C57BL/6 Mice.<br>214

213 C, Rubin J.Exercise Regulation of Marrow Fat in the Setting of PPARµ Agonist Treatment in Pemae C57BL/6 Mice.<br>214 Endocrinology. 2015; 156(8):2753–61.<br>215 Team RC. R: A Language and Environment for Statistical Computin 214 Endocrinology. 2015; 156(8):2753–61.

215 Team RC. R: A Language and Environment for Statistical Computing. 2022, R Foundation for Statistical<br>216 Computing. In: 2022.<br>217 Temiyasathit 5, Jacobs CR. Osteocyte primary cilium and its role in bone mechanotransduc

217 Temiyasathi<br>218 Sci. 2010; 1192:422–4<br>219 Teti A, Zallo<br>220 Bone. 2009; 44(1):11 217 Temiyasathit S, Jacobs CR. Osteocyte primary cilium and its role in bone incentionalisated.<br>218 Sci. 2010; 1192:422–430.<br>220 Bone. 2009; 44(1):11–17.<br>221 Tsourdi E, Jähn K, Rauner M, Busse B, Bonewald LF. Physiological 218 Sci. 2010, 1192:422–430.<br>219 Teti A, Zallone<br>220 Bone. 2009; 44(1):11–17<br>221 Tsourdi E, Jähn<br>222 Musculoskelet Neuronal In

220 Bone. 2009; 44(1):11–17.<br>220 Bone. 2009; 44(1):11–17.<br>221 Tsourdi E, Jähn K, Rauner M, Busse B, Bonewald LF. Physiological and pathological osteocytic osteolysis. J<br>222 Musculoskelet Neuronal Interact. 2018; 18(3):292–

220 Bone. 2009, 44(1):11–17.<br>221 Tsourdi E, Jähn 1<br>222 Musculoskelet Neuronal Inte<br>223 Tsourdi E, Anasta.<br>224 Narrative Review of the Lit 222 Musculoskelet Neuronal Interact. 2018; 18(3):292–303.<br>223 Tsourdi E, Anastasilakis AD, Hofbauer LC, Rauner M, Lademann F. Irisin and Bone in Sickness and in Health: A<br>224 Narrative Review of the Literature. J Clin Med. 222 Musculoskelet Neuronal Interact. 2018, 18(3):292–303.<br>223 Tsourdi E, Anastasilakis AD, Hofbauer LC, Ra<br>224 Narrative Review of the Literature. J Clin Med. 2022;

223 Tale in Sickness and in Health. A<br>224 Narrative Review of the Literature. J Clin Med. 2022; (22):11–11.<br>225 Uda Y, Azab E, Sun N, Shi C, Pajevic PD. Osteocyte Mechanobiology. Curr Osteoporos Rep, 2017; 15(4): p. 318-32 225 Uda Y, Azab E, Sun N, Shi C, Pajevic PD. Osteocyte Mech<br>226 Wang H, Zhao YT, Zhang S, Dubielecka PM, Du J, Yano N<br>227 role to protect the heart against ischemia and reperfusion injury.<br>228 Wickham H. GGPLOT2: Elegant G 225 Uda T, Azab E, Sun N, Shi C, Pajevic PD. Osteocyte Mechanobiology. Curr Osteoporos Rep, 2017, 15(4): p. 318-325.<br>226 Wang H, Zhao YT, Zhang S, Dubielecka PM, Du J, Yano N, Chin YE, Zhuang S, Qin G, Zhao TC. Irisin play

Wang H, Zhao TT, Zhang S, Dubielecka PM, Du 3, Taho N, Chin Te, Zhuang S, Qin G, Zhao Te. Irisin plays a pivotal<br>227 role to protect the heart against ischemia and reperfusion injury. J Cell Physiol. 2017; 232(12):3775–378 227 Fole to protect the heart against ischemia and repertusion injury. J Cell Firysiol: 2017, 232(12):3775–3785.<br>228 Wickham H. GGPLOT2: Elegant Graphics for Data Analysis. New York: Springer-Verlag; 2016.<br>230 Exercise ind

228 Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, Lin JD, Greenberg ME,<br>230 Exercise induces hippocampal BDNF through a PGC-1*a*/FNDC5 pathway. Cell Metab. 2013; 18(5):649–59.<br>231 Wu T. clusterProfi 229 Wramn CD, White Jr, Salogiannins J, Lazink-Bogoslavski D, Wd J, Ma D, Lin JD, Greenberg ME, Spiegelman BM.<br>230 Exercise induces hippocampal BDNF through a PGC-1 $\alpha$ /FNDC5 pathway. Cell Metab. 2013; 18(5):649–59.<br>231 Wu 230 Exercise induces impocampal BDNF through a PGC-1α/FNDC5 pathway. Cen Metab. 2015, 18(5):849–59.<br>231 Wu T. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. Innovation (Car<br>232 100141.<br>233 Wy

232 100141.<br>232 100141.<br>233 Wysolmerski JJ. The evolutionary origins of maternal calcium and bone metabolism during lactation. J<br>234 Mammary Gland Biol Neoplasia. 2002; 7(3):267–76.<br>235 Wysolmerski JJ. Osteocytic osteolysi 232 100141.<br>233<br>234 Mamma<br>235 233 Wysolmerski JJ. The evolutionary origins of material calcium and bone metabolism during lactation. J<br>234 Mammary Gland Biol Neoplasia. 2002; 7(3):267–76.<br>235 Wysolmerski JJ. Osteocytic osteolysis: time for a second loo

234 Mammary Gland Biol Neoplasia. 2002, 7(3):267–76.<br>235 Wysolmerski JJ. Osteocytic osteolysis: time for Wysolmerski JJ. Osteocytic osteolysis: time for a second look? Bonekey Rep, 2012; 1: p. 229.

237 54(2):230–236.<br>237 54(2):230–236.<br>238 Min C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, Lee Y, Ye J, Lian K, Xu C, Zhang L, Wang Q, Liu Y, Tao L. Irisin<br>239 improves fatty acid oxidation and glucose utilization in type 2 237  $34(2)$ :230–236.<br>
238  $\frac{1}{2}$  Xin C,<br>
239 improves fatty<br>
240 Obes. 2016; 40<br>
241 Xiong

238 *XIII C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, Lee T, Te J, Lian K, Xu C, Zhang C, Wang Q, Liu T, Tao L. Irishi<br>239 improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK sign* 240 Obes 2016; 40(3):443–51.<br>241 Xiong J, Piemontese M, Onal M, Campbell J, Goellner JJ, Dusevich V, Bonewald L, Manolagas SC, O'Brien CA.<br>242 Osteocytes, not Osteoblasts or Lining Cells, are the Main Source of the RANKL R 240 Obes. 2010, 40(3):443–51.<br>241 – Xiong J, Piemonte<br>242 Osteocytes, not Osteoblas<br>243 Remodeling Bone. PLoS On<br>244 – Xiong J, O'Brien (245 – 2012: 27(3):499–505 Xiong J, Piemontese M, Onal M, Campbell J, Goellner JJ, Dusevich V, Bonewald L, Manolagas SC, O'Brien CA.<br>242 Osteocytes, not Osteoblasts or Lining Cells, are the Main Source of the RANKL Required for Osteoclast Formation

243 Remodeling Bone. PLoS One. 2015; 10(9):138189–138189.<br>244 Siong J, O'Brien CA. Osteocyte RANKL: new insights into the control of bone remodeling. J Bone Miner Res.<br>245 2012; 27(3):499–505.<br>246 Yeung DK, Griffith JF, An

243 Remodeling Bone. PLos One. 2013, 10(9).138183 138183.<br>244 Xiong J, O'Brien CA. Osteocyte RANKL: new insig<br>245 2012; 27(3):499–505.<br>246 Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J,<br>247 fat content and decreased mar 244 Xiong 3, O'Brien CA. Osteocyte RANKL: new insights into the control of bone remodeling. J Bone Miner Res.<br>245 2012; 27(3):499–505.<br>246 Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J, Leung PC. Osteoporosis is associa 245 2012, 27(3):499–503.<br>246 Yeung DK, G<br>247 fat content and decre<br>248 22(2):279–85.<br>249 Youlten SE, K

Teang DK, Griffith JF, Antonio GE, Lee FK, Wood, Leang FC. Osteoporosis is associated with increased marrow<br>247 fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study. J Magn Reson Imaging. 2005; 248 22(2):279–85.<br>248 22(2):279–85.<br>249 voulten SE, Kemp JP, Logan JG, Ghirardello EJ, Sergio CM, Dack MRG, Guilfoyle SE, Leitch VD, Butterfield NC,<br>250 Komla-Ebri D, Chai RC, Corr AP, Smith JT, Mohanty ST, Morris JA, McDo 248 22(2):279–85.<br>249 Yoult<br>250 Komla-Ebri D,<br>251 Bartonicek N,<br>252 Adams DJ, Le<br>253 Croucher PI. 1998 Komla-Ebri D, Chai RC, Corr AP, Smith JT, Mohanty ST, Morris JA, McDonald MM, Quinn JMW, McGlade AR, 251 Bartonicek N, Jansson M, Hatzikotoulas K, Irving MD, Beleza-Meireles A, Rivadeneira F, Duncan E, Richards JB, 25 251 Bartonicek N, Jansson M, Hatzikotoulas K, Irving MD, Beleza-Meireles A, Rivadeneira F, Duncan E, Richards JB,<br>252 Adams DJ, Lelliott CJ, Brink R, Phan TG, Eisman JA, Evans DM, Zeggini E, Baldock PA, Bassett JHD, Willia 252 Adams DJ, Lelliott CJ, Brink R, Phan TG, Eisman JA, Evans DM, Zeggini E, Baldock PA, Bassett JHD, Williams GR,<br>253 Croucher PI. Osteocytetranscriptome mapping identifies a molecular landscape control- ling skeletal hom 253 Croucher PI. Osteocytetranscriptome mapping identifies a molecular landscape control-ling skeletal homeostasis and<br>254 susceptibility to skeletal disease. Nat Commun. 2021; 12(1):2444–2444.<br>255 Young MF, Valaris S, Wra

- 254 Susceptibility to skeletal disease. Nat Commun. 2021; 12(1):2444–2444.<br>255 Young MF, Valaris S, Wrann CD. A role for FNDC5/Irisin in the beneficial effects of exercise on the brain and in<br>256 neurodegenerative diseases 254 susceptibility to skeletal disease. Nat Commun. 2021, 12(1):2444–2444.<br>255 Young MF, Valaris S, Wrann CD. A role for FNDC5/Irisin in the ber<br>256 neurodegenerative diseases. Prog Cardiovasc Dis. 2019; 62(2):172–178.<br>257
- 255 Nourodegenerative diseases. Prog Cardiovasc Dis. 2019; 62(2):172– 178.<br>256 neurodegenerative diseases. Prog Cardiovasc Dis. 2019; 62(2):172– 178.<br>257 Zhang D, Bae C, Lee J, Lee J, Jin Z, Kang M, Cho YS, Kim JH, Lee W,
- 256 neurodegenerative diseases. Prog cardiovase Dis. 2015, 62(2):172– 176.<br>257 **Zhang D, Bae C, Lee J, Lee J, Jin Z, Kang M, Cho YS, Kim JH, Le**<br>258 are through preferential stimulation of aerobic glycolysis. Bone. 2018<br>25 257 Zhang D, Bae C, Lee J, Lee J, Jin Z, Kang M, Cho 13, Kim JH, Lee W, Lim SK. The bone anabolic effects of irisin<br>258 are through preferential stimulation of aerobic glycolysis. Bone. 2018; 114:150–160.<br>259 Zhang H, Wu X
- 258 are through preferential stimulation of aerobic glycolysis. Bone. 2016, 114:150–160.<br>259 2hang H, Wu X, Liang J, Kirberger M, Chen N. Irisin, an exercise-induced bioact<br>260 promotion during aging process. Ageing Res Re 269 Internation during aging process. Ageing Res Rev. 2022; 80:101680–101680.<br>261 Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, Qi L, Zhang M, Wang X, Cui T, Yang LJ, Tang D. Irisin<br>262 stimulates browning of white adipo 260 promotion during aging process. Ageing Res Rev. 2022, 80:101680–101680.<br>261 **Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, Qi L, Zhang**<br>262 stimulates browning of white adipocytes through mitogen-activated p<br>263 kina 261 Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, Qi L, Zhang M, Wang X, Cui Y, Yang D, Yang D. Irisin<br>262 stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP<br>263 k
- 263 kinasesignaling. Diabetes. 2014; 63(2):514–539.<br>264 Zhu J, Li J, Yao T, Li T, Chang B, Yi X. Analysis of the role of irisin receptor signaling in regulating<br>265 osteogenic/adipogenic differentiation of bone marrow mese 263 kinase signaling. Diabetes. 2014, 63(2):514–539.<br>264 Zhu J, Li J, Yao T, Li T, Chang B, Y<br>265 osteogenic/adipogenic differentiation of bone m<br>266 24. 264 Zhu J, Li J, Yao T, Li T, Chang B, TT X. Analysis of the role of institute performance in regulating in regulating<br>265 osteogenic/adipogenic differentiation of bone marrow mesenchymal stem cells. Biotechnol Genet Eng  $266$  24.  $24.$