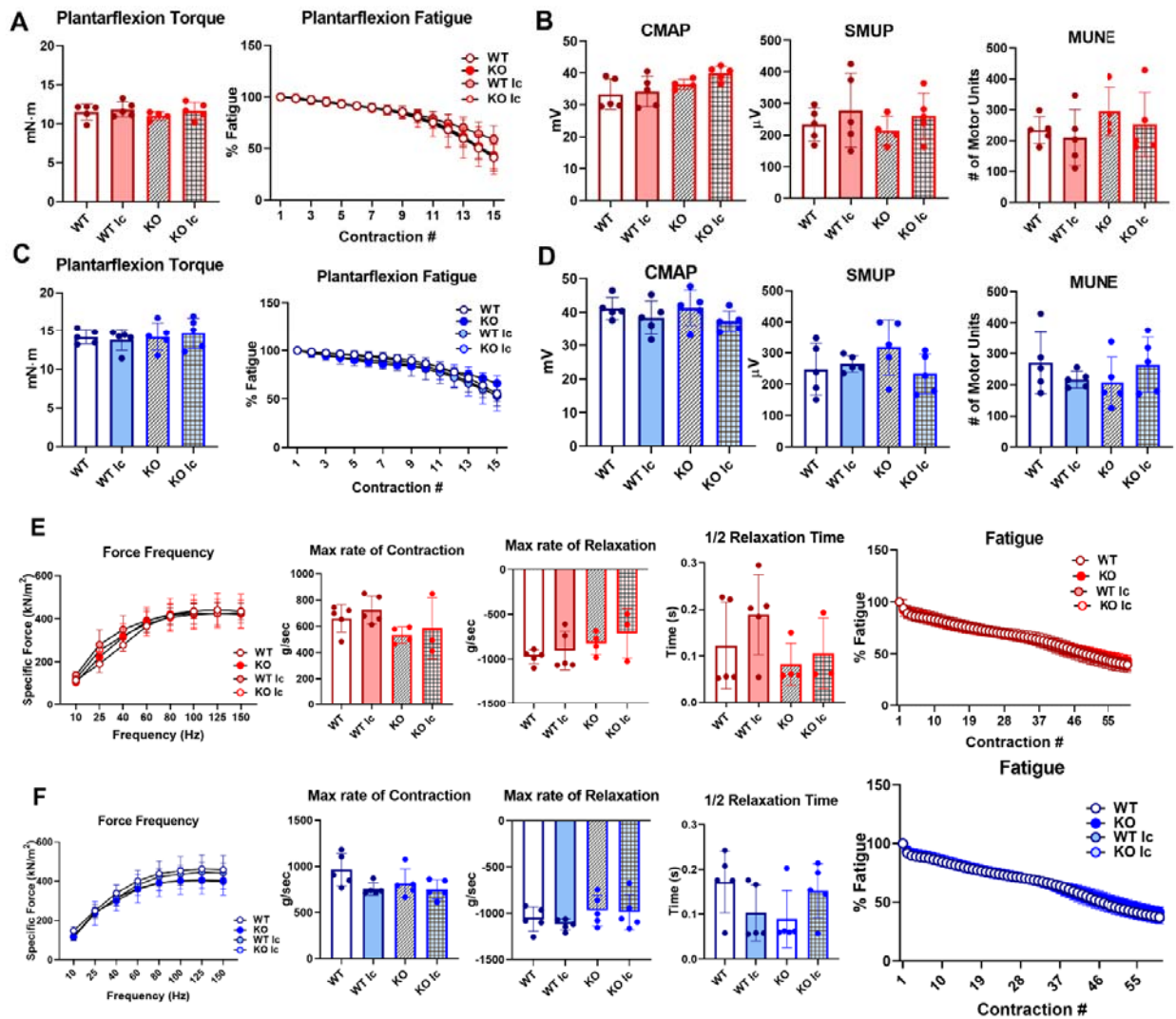


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1149 **Supplementary Figure 1: Pup numbers for the lactation experiment and**
1150 **body weight measurements for the low-calcium-diet experiment**

1151 Panel A shows total pup numbers in WT and KO female mice that underwent
1152 pregnancy and 2 weeks of lactation. There is no significant differences in the pup
1153 numbers between genotypes. Students t-test was performed for statistical analysis. n=
1154 8/group.

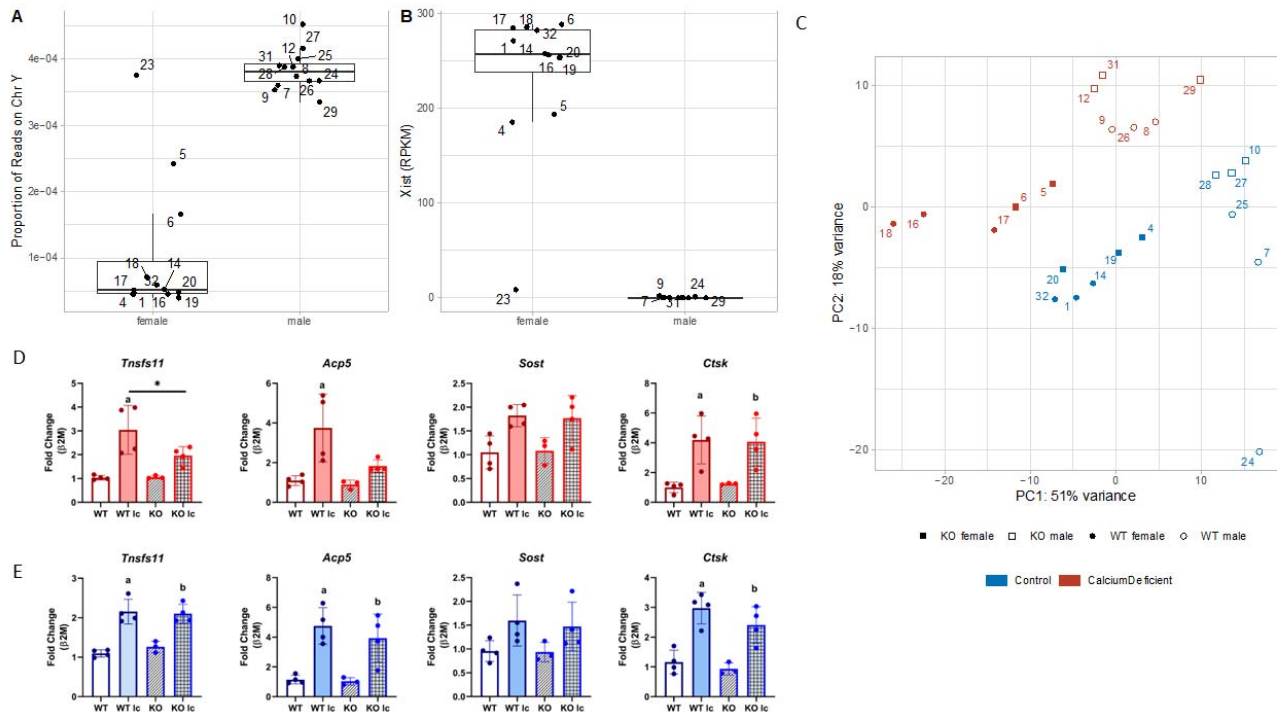
1155 Panels B and C show total body weight of WT and KO female (B) and male (C)
1156 mice. No statistically significant difference was found among the groups, regardless of
1157 genotype or diet. 2-way ANOVA was performed. n= 4-5/group. As depicted here, red is
1158 female, and blue is male.



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1160 **Supplementary Figure 2: Neither genotype nor dietary calcium alters**
1161 **muscle functions *in vivo* or *ex vivo***

1162 Panels A and C show *in vivo* muscle plantarflexion force (reported as
1163 plantarflexion torque and plantarflexion fatigue) in WT and KO female (A) and male (C)
1164 mice on a control or a low calcium diet, panels B and D show muscle electrophysiology
1165 parameters of CMAP, SMUP, and MUNE in WT and KO female (B) and male (D) mice,
1166 and panels E and F show *ex vivo* EDL functional measurement (reported as specific
1167 force frequency, maximum rate of contraction, maximum rate of relaxation, half-
1168 relaxation time, and % fatigue) in WT and KO female (E) and male (F) mice

1169 2-way ANOVA was performed. n= 4-5/group. As depicted here, red is female,
1170 and blue is male.



Supplementary Figure 3: Quality control and validation of RNA sequencing

Sanity check of data on the sample's sex. A: Boxplot of proportional of reads on chromosome Y. Male should have a higher value than female. B: Boxplot of RPKM of *Xist*. Males should have very low expression of *Xist*.

C: Scatter plot of PC1 and PC2 from Principal Component Analysis (PCA) of gene expression data.

D: qPCR analysis of *Tnsfs11*, *Acp5*, *Sost*, and *Ctsk* genes from osteocyte-enriched bone chips from female 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, *= p < 0.05.

E: qPCR analysis of *Tnsfs11*, *Acp5*, *Sost*, and *Ctsk* genes from osteocyte-enriched bone chips from male 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, *= p < 0.05.

Bone Parameters	Virgin		Lactation	
	WT	KO	WT	KO
Femoral cortical bone parameters				
Ct. B. Ar/T. Ar (%)	47.4 ± 1.2	48 ± 1	35.2 ± 1.8 ^a	37.5 ± 1.8 ^{b, c}
Ct. Th (mm)	0.18 ± 0.004	0.19 ± 0.005	0.13 ± 0.004 ^a	0.14 ± 0.01 ^{b, c}
Ps. Pm (mm)	5.16 ± 0.2	5.2 ± 0.06	5.18 ± 0.16	5.2 ± 0.14
Es. Pm (mm)	3.95 ± 0.1	4 ± 0.13	4.4 ± 0.11 ^a	4.3 ± 0.09 ^b
Marrow cavity area (mm ²)	0.93 ± 0.1	0.93 ± 0.04	1.16 ± 0.05 ^a	1.13 ± 0.05 ^b
Femoral trabecular bone parameters				
BV/TV (%)	3.7 ± 1	4.5 ± 0.8	3.1 ± 0.7	4 ± 1.1
Tb. Th (mm)	0.043 ± 0.002	0.044 ± 0.001	0.039 ± 0.002 ^a	0.039 ± 0.001 ^b
Tb. Sp (mm)	0.37 ± 0.05	0.36 ± 0.03	0.57 ± 0.15 ^a	0.44 ± 0.09
Tb. N (1/mm)	0.85 ± 0.2	1.06 ± 0.2	0.8 ± 0.2	1.04 ± 0.25

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Bone parameters	Change	% Change	
		WT	KO
Cortical Bone Area Fraction	Decrease	26%	22% *
Cortical Thickness	Decrease	29%	24% *
Ultimate Force	Decrease	38%	31% *
Osteoclast Number/ bone parameter	Increase	141%	129%
TRAP-positive osteocytes	Increase	101%	175% *
Lacunar Area	Increase	26%	15% *
Serum RANKL	Increase	170%	80% *

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Supplementary Table 1: FNDC5 KO mice femurs are partially resistant to lactation-induced bone loss.

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Femoral cortical and trabecular bone parameters of WT and FNDC5 KO female virgin and lactation mice. n = 5-8/group. Data presented as mean ± standard deviation.

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a= significant compared to WT control, b= significant compared to KO control, c= significant compared to WT low Ca diet, 2-way ANOVA, significance <0.05, n= 8/group.

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Percentage change in different bone and serum parameters in WT and FNDC5 KO female mice with lactation. * = p<0.05 compared to WT.

Bone Parameters	Female Normal Diet		Female Low Ca Diet		Male Normal Diet		Male Low Ca Diet	
	WT	KO	WT	KO	WT	KO	WT	KO
Ex vivo femur DXA								
BMD (mg/cm ²)	75.4± 2.4	76.6± 1.5	65.4± 4.3 ^a	71.4± 3.4 ^c	74.6± 1.5	78.3± 3 ^a	68.2± 3	68.1± 2 ^b
BMC (g)	0.03± 0.002	0.03± 0.001	0.024± 0.002 ^a	0.027± 0.002 ^{b,c}	0.029± 0.002	0.032± 0.004	0.026± 0.002	0.025± 0.003 ^b
Femoral cortical bone parameters								
Ct.	47.8±	48.4±	41.6±	45.2±	40.1±	43.6±	38.3±	39.1±
B.Ar/T.Ar%	1.6	0.4	1.1 ^a	1.4 ^{b,c}	1.4	0.6 ^a	0.9	1.2 ^b
Ct. Th (mm)	0.2± 0.01	0.2± 0.01	0.15± 0.01 ^a	0.17± 0.01 ^{b,c}	0.15± 0.01	0.2± 0.01 ^a	0.14± 0.01	0.14± 0.01 ^b
Marrow Cavity Area	0.92 ± 0.04	0.86± 0.02	1.02 ± 0.06 ^a	0.9 ± 0.02 ^c	1.1 ± 0.04	1.03 ± 0.06 ^a	1.2 ± 0.03	1.08 ± 0.03 ^c
Femoral trabecular bone parameters								
BV/TV (%)	3.6 ± 1.2	4.3 ± 1	3.2 ± 1	3.9 ± 1	6.1 ± 1.1	8.7 ± 1.9	5.3 ± 1.2	6.4 ±0.6
Tb. Th (mm)	0.059± 0.002	0.059± 0.004	0.056± 0.002	0.055± 0.001	0.036 ±0.001	0.035 ± 0.001	0.035±0. 001	0.035 ± 0.002
Tb. Sp (mm)	0.38 ± 0.03	0.35 ± 0.02	0.51 ± 0.12 ^a	0.48 ± 0.08 ^b	0.274± 0.025	0.235 ± 0.021	0.278 ± 0.027	0.265 ± 0.01
Tb. N (1/mm)	0.81 ± 0.2	0.95 ± 0.14	0.7 ± 0.02	0.91 ±0.13	1.7 ± 0.34	2.5 ^a ± 0.5	1.5 ± 0.3	1.8 ± 0.1
Femoral mechanical properties								
Ultimate Force (N)	19±1	19.4± 1.15	14.8± 0.7 ^a	16.4± 0.5 ^b	18.3± 1	17.6± 0.9	15± 1.3 ^a	12.7± 1.5 ^{b,c}
Stiffness (N/mm)	78.6± 3.2	79.1± 4.9	56.8± 5 ^a	67± 4.3	76.7± 5.6	56.4± 4.75 ^a	56± 10.2 ^a	48.5± 4.9 ^{b,c}
Energy to Failure (N)	2.9± 0.3	3.1± 0.6	1.8± 0.5 ^a	2± 0.3 ^b	3.6± 0.9	3.01± 0.6	2.5± 0.3 ^a	2.35± 0.14

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Supplementary Table 2: WT and FNDC5 KO female and male mice bone responds differently to a low-calcium diet

1200 Femoral BMD, BMC, cortical and trabecular bone parameters, and mechanical
1201 properties of 4-5-month-old WT and KO female and male mice under a normal diet or a 2-week
1202 low calcium diet. n = 5/group. Data presented as mean \pm standard deviation.

1203 a= significant compared to WT control, b= significant compared to KO control, c=
1204 significant compared to WT low Ca diet, 2-way ANOVA, significance <0.05 , n= 4-5/group.

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