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

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

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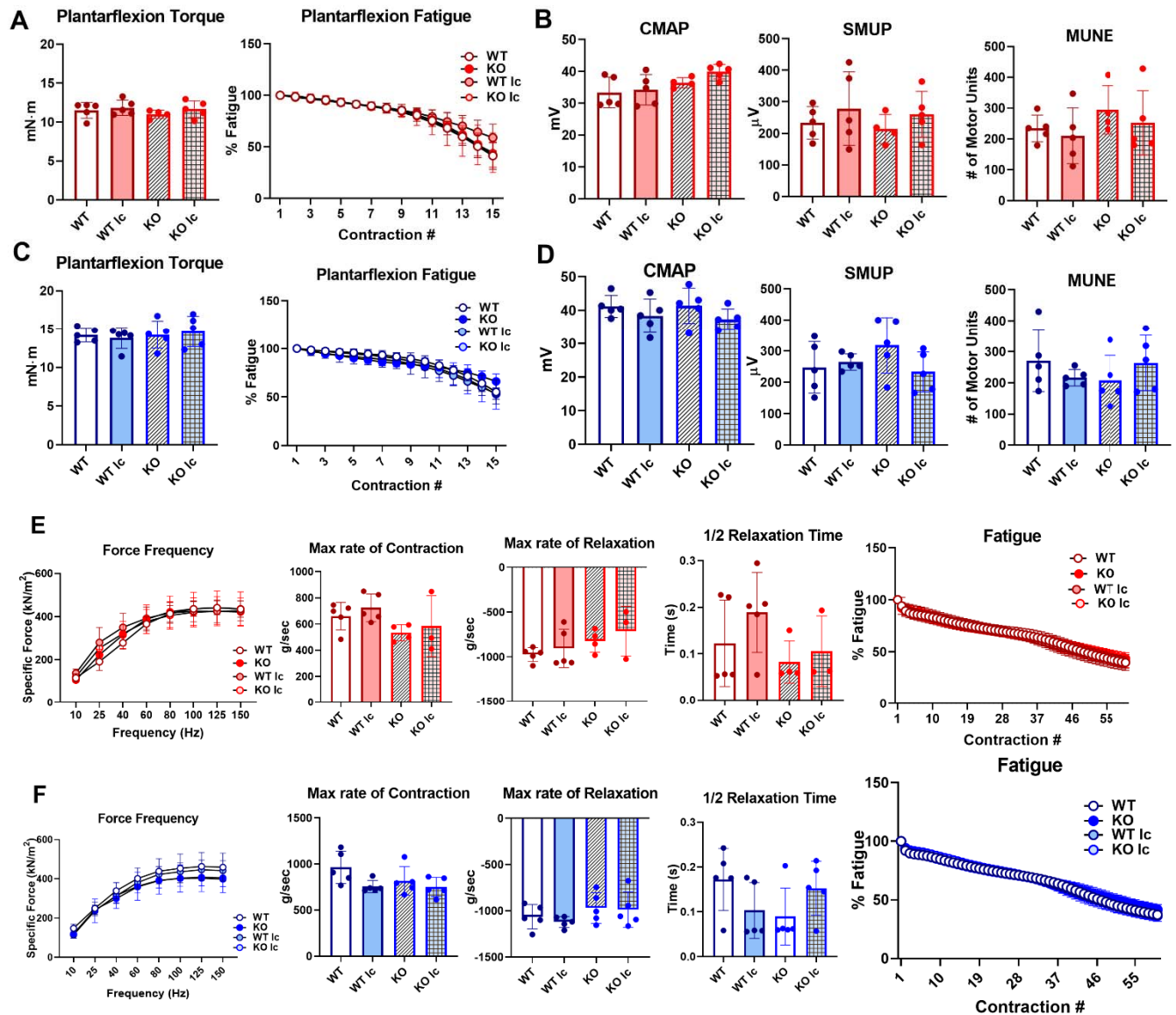
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panels **B** and **C** show total body weight of WT and KO female (**B**) and male (**C**) mice. No statistically significant difference was found among the groups, regardless of genotype or diet. 2-way ANOVA with Tukey's post hoc test was done. n= 4-5/group. As depicted here, red is female, and blue is male.

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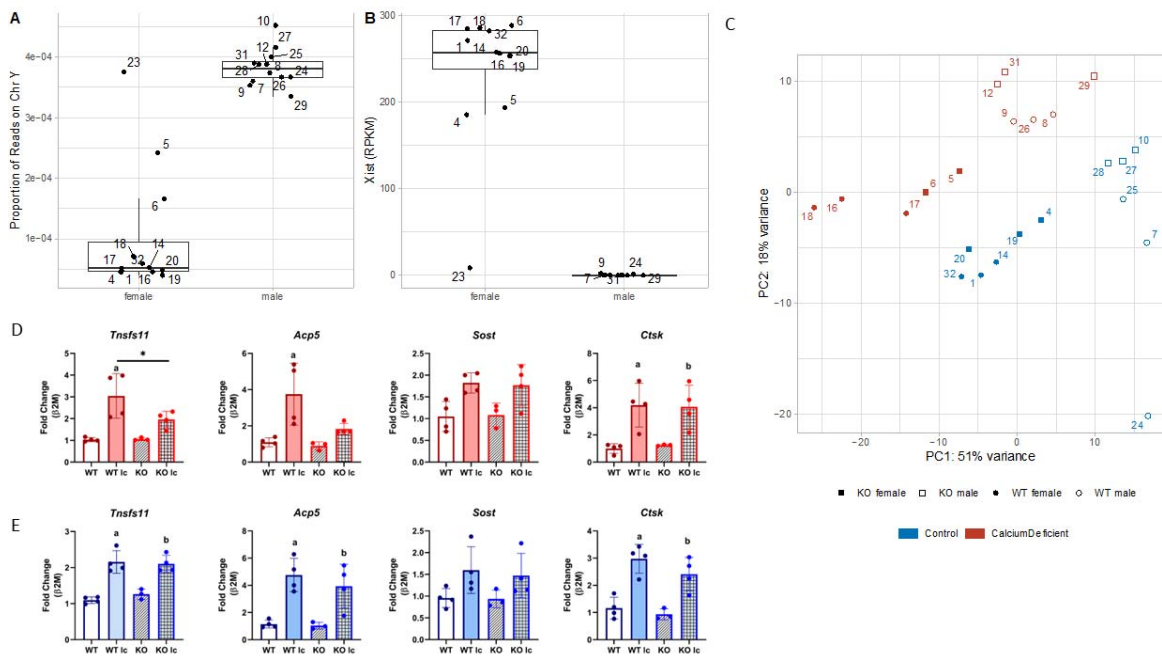
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Supplementary Figure 2: Neither genotype nor dietary calcium alters muscle functions *in vivo* or *ex vivo*

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

2-way ANOVA was performed. n= 4-5/group. As depicted here, red is female, 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 *Tnfsf11*, *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 *Tnfsf11*, *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
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% *	

1034 **Supplementary Table 1: FNDC5 KO mice femurs are partially resistant to lactation-induced bone loss.**

1035 Femoral cortical and trabecular bone parameters of WT and FNDC5 KO female virgin and lactation mice. n
1036 = 5-8/group. Data presented as mean ± standard deviation. a= significant compared to WT control, b= significant
1037 compared to KO control, c= significant compared to WT low Ca diet, 2-way ANOVA, significance <0.05, n= 8/group.

1038 Percentage change in different bone and serum parameters in WT and FNDC5 KO female mice with
1039 lactation. *= p<0.05 compared to WT.
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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	6.6± 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

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

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1043 Femoral BMD, BMC, cortical and trabecular bone parameters, and mechanical properties of
1044 4-5-month-old WT and KO female and male mice under a normal diet or a 2-week low calcium diet.
1045 n = 5/group. Data presented as mean \pm standard deviation.
1046 a= significant compared to WT control, b= significant compared to KO control, c= significant
1047 compared to WT low Ca diet, 2-way ANOVA, significance <0.05, n= 4-5/group.
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