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-	12 weeks		56 weeks			
Proximal Tibial Metaphysis	Room Temp	4°C	P < 0.05	Room Temp	4° <i>C</i>	P < 0.05
Total Volume ( <i>mm</i> <sup>3</sup> )	1.39 +/- 0.08	1.43 +/- 0.08		1.43 +/- 0.12	1.49 +/- 0.04	
Bone Volume Fraction (%)	22.5 +/- 3.4	22.4 +/- 3.4		28.3 +/- 4.5	28.8 +/- 2.1	
Trabecular Number ( <i>mm</i> <sup>-1</sup> )	5.25 +/- 0.36	5.25 +/- 0.38		4.45 +/- 0.59	4.70 +/- 0.42	
Trabecular Thickness (mm)	0.062 +/- 0.005	0.062 +/- 0.004		0.073 +/- 0.003	0.072 +/- 0.007	
Trabecular Spacing ( <i>mm</i> <sup>-1</sup> )	0.174 +/- 0.014	0.176 +/- 0.015		0.218 +/- 0.034	0.204 +/- 0.022	
Bone Mineral Content (mgHA)	196 +/- 28	197 +/- 26		254 +/- 33	258 +/- 24	
Marrow Adipose Tissue (mm <sup>3</sup> )	0.117 +/- 0.059	0.022 +/- 0.020	*	0.752 +/- 0.122	0.263 +/- 0.130	*

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-		12 weeks			56 weeks		
	Tibia Mid-Cortical Morphology	Room Temp	4°C	P < 0.05	Room Temp	4° <i>C</i>	P < 0.05
	Total Volume ( <i>mm</i> <sup>3</sup> )	0.394 +/- 0.013	0.399 +/- 0.021		0.478 +/- 0.025	0.494 +/- 0.017	
3D	Relative Cortical Volume (%)	72.1 +/- 1.1	70.7 +/- 1.6	*	72.6 +/- 1.3	72.9 +/- 1.8	
	Bone Mineral Content (mgHA)	890 +/- 25	875 +/- 27		967 +/- 24	969 +/- 27	
	Total Area ( <i>mm</i> <sup>2</sup> )	1.128 +/- 0.036	1.147 +/- 0.062		1.373 +/- 0.073	1.418 +/- 0.048	
2D	Cortical Bone Area (mm <sup>2</sup> )	0.813 +/- 0.027	0.811 +/- 0.040		0.996 +/- 0.058	1.033 +/- 0.045	
	Cortical Thickness (mm)	0.279 +/- 0.011	0.271 +/- 0.012		0.312 +/- 0.012	0.317 +/- 0.016	

**Supplementary Figure 1. Trabecular and cortical analyses with cold exposure.** (**A**,**B**) Representative images of the proximal tibial metaphysis both prior to decalcification and after osmium staining of the data presented in (C). Marrow fat is in white. Scale bar =  $500 \mu m$ . (C) Quantification of trabecular parameters and MAT volume in the proximal tibial metaphysis (biological replicate N = 9 (C3H 12-week-old), and 11 (B6 12-week-old, 56-week-old)). (D) Quantification of cortical parameters (biological replicate N = 9 (C3H 12-week-old), and 11 (B6 12-week-old, 56-week-old)). All values represent mean  $\pm$  standard deviation. \*Two-tailed *t*-Test, p<0.05.



Supplementary Figure 2. NanoCT for quantification of adipocyte size. To quantify tibial adipocyte size using nanocomputed-tomography, two regions of interest were scanned at 2  $\mu$ m resolution. To measure rMAT adipocytes we scanned at the proximal metaphysis (ROI-p). To measure cMAT adipocytes we scanned the mid-portion of the distal tibia (ROI-d). Three to five DICOM images, each at least 100  $\mu$ m apart were selected for analysis from each sample. The DICOM slice was opened in ImageJ and inverted to make visualization of the adipocytes easier. Most of the proximal adipocytes could be selected for analysis by the program, but the distal adipocytes were drawn by hand. With either method, each adipocyte outline was checked against the un-rendered image to confirm its accuracy. The area within each outline was then measured. After 300 to 400 adipocytes had been identified for analysis the frequency of each adipocyte area, with a bin size as indicated in the figures, was calculated. This size distribution was then represented as a histogram.



**Supplementary Figure 3. Human** <sup>1</sup>**H-MRS.** Spectra at the (**A**) mid-tibial diaphysis and (**B**) distal tibial metaphysis demonstrate olefinic protons at 5.3 ppm (-CH=CH-), an estimate of fatty acid (FA) unsaturated bonds, methylene protons at 1.3 ppm [(-CH2-)n], methyl protons at 0.9 ppm (-CH3), and residual (res.) FA including allylic and diallylic methylene protons. Total marrow lipid content was determined by combining all lipid peaks. Unsaturation index (UI) was determined by obtaining a ratio between the olefinic resonance at 5.3 ppm and total lipid content. Lipid resonances were scaled to unsuppressed water peak (H2O) at 4.7 ppm.



Supplementary Figure 4. Differential loss of MAT in female mice with knock-out of *Cav1* or *Ptrf*. (A,B) Region-specific quantification of tibial MAT volume by  $\mu$ CT (biological replicate N = 4-6 as indicated in the graph). Box plot center line represents median, box extends from the 25th to 75th percentile, whiskers indicate range. \*(A) Non-parametric Mann-Whitney test, (B) two-tailed *t*-Test, p<0.05.



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	Female			Male		
Proximal Tibial Metaphysis	WT	Cav1 KO	P < 0.05	WT	Cav1 KO	P < 0.05
Total Volume ( <i>mm</i> <sup>3</sup> )	1.09 +/- 0.41	0.95 +/- 0.43		1.43 +/- 0.25	1.36 +/- 0.12	
Bone Volume Fraction (%)	37.8 +/- 8.3	44.9 +/- 8.7		41.9 +/- 5.2	42.8 +/- 7.7	
Trabecular Number ( <i>mm</i> <sup>-1</sup> )	5.61 +/- 0.68	5.93 +/- 0.58		6.25 +/- 0.55	6.74 +/- 0.66	
Trabecular Thickness (mm)	0.078 +/- 0.009	0.085 +/- 0.009		0.077 +/- 0.005	0.074 +/- 0.007	
Trabecular Spacing (mm <sup>-1</sup> )	0.17 +/- 0.03	0.15 +/- 0.03		0.14 +/- 0.02	0.13 +/- 0.02	
Bone Mineral Content (mgHA)	332 +/- 76	400 +/- 71		356 +/- 38	354 +/- 65	
Marrow Adipose Tissue (mm <sup>3</sup> )	0.050 +/- 0.059	0.036 +/- 0.025		0.039 +/- 0.063	0.029 +/- 0.053	

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_			Female			Male	
	Tibia Mid-Cortical Morphology	wт	Cav1 KO	P < 0.05	WТ	Cav1 KO	P < 0.05
	Total Volume ( <i>mm</i> <sup>3</sup> )	0.35 +/- 0.03	0.37 +/- 0.05		0.41 +/- 0.05	0.44 +/- 0.06	
3D	Relative Cortical Volume (%)	63.1 +/- 3.9	65.4 +/- 1.6		66.0 +/- 3.7	63.5 +/- 3.6	
	Bone Mineral Content (mgHA)	825 +/- 54	854 +/- 21		848 +/- 46	812 +/- 56	
	Total Area ( <i>mm</i> <sup>2</sup> )	1.02 +/- 0.08	1.08 +/- 0.13		1.18 +/- 0.14	1.24 +/- 0.19	
2D	Cortical Bone Area (mm <sup>2</sup> )	0.64 +/- 0.06	0.70 +/- 0.09		0.78 +/- 0.11	0.79 +/- 0.10	
	Cortical Thickness (mm)	0.22 +/- 0.02	0.24 +/- 0.02		0.25 +/- 0.03	0.24 +/- 0.02	

Supplementary Figure 5. Body mass, tissue mass, and bone parameters of wild-type and *Cav1* knock-out mice. Male and female mice at 16 weeks of age. (A,B) Body and tissue mass (biological replicate N = 8 (Female); 9 (Male KO); 14 (Male WT)). Gonadal white adipose tissue (gWAT), inguinal WAT (iWAT). (C) Quantification of trabecular parameters and MAT volume in the proximal tibial metaphysis (biological replicate N = 8 (Female); 13 (Male WT); 9 (Male KO)). (D) Quantification of cortical parameters at the mid-diaphysis (biological replicate N = 8 (Female); 13 (Male WT); 9 (Male KO)). Presented as mean  $\pm$  standard deviation. \*Two-tailed t-Test, p<0.05.



<u>ر</u>		Female			Male			
	Tibia Mid-Cortical Bone	WT	Ptrf KO	P < 0.05	WТ	Ptrf KO	P < 0.05	
	Total Volume ( <i>mm</i> <sup>3</sup> )	0.43 +/- 0.03	0.43 +/- 0.01		0.54 +/- 0.02	0.57 +/- 0.05		
3D	Bone Volume Fraction (%)	53.2 +/- 3.1	55.0 +/- 2.3		54.1 +/- 1.0	55.6 +/- 1.1		
	Bone Mineral Content (mgHA)	664 +/- 54	694 +/- 30		662 +/- 16	694 +/- 22	*	
	Total Area (mm <sup>2</sup> )	1.22 +/- 0.08	1.25 +/- 0.02		1.51 +/- 0.09	1.54 +/- 0.16		
2D	Bone Area (mm <sup>2</sup> )	0.65 +/- 0.04	0.69 +/- 0.03		0.84 +/- 0.06	0.87 +/- 0.09		
	Cortical Thickness (mm)	0.19 +/- 0.01	0.20 +/- 0.01		0.21 +/- 0.02	0.22 +/- 0.01		

**Supplementary Figure 6. Body mass, tissue mass, and cortical parameters of wild type and** *Ptrf* **knock-out mice.** (A) Tissue and body mass from female mice at 12-15 weeks of age (biological replicate N = 5). (B) Tissue and body mass from male mice at 12-16 weeks of age (biological replicate N = 7 (WT); 8 (KO)). (C) Quantification of cortical parameters at the mid-tibial diaphysis in male and female mice at 16-17 weeks of age (biological replicate N = 5 (Female KO); 6 (Female WT); 7 (Male KO); 9 (Male WT)). Gonadal white adipose tissue (gWAT), inguinal WAT (iWAT). Presented as mean  $\pm$  standard deviation. \*Two-tailed t-Test, p<0.05.

# SUPPLEMENTARY TABLE 1

Transcript	NCBI RefSEQ	Primers		
Sed1	NNA 120102	Forward	CTCAGCGCTGGGAAAGTG	
5001	NIVI_139192	Reverse	GAACTGGAGATCTCTTGGAGCA	
Sed 2	NINA 021841 1	Forward	GCAGATGTTCGCCCTGAAATTA	
5002	NIN_051641.1	Reverse	CAAATATGCAAAGAGGCAGGTGTAG	
Eads1		Forward	CACTACGCTGGTCAGGATGC	
Fuusi	10101_055445.2	Reverse	AGTGAGCGCCTTATTCTTGGT	
Eads?	NINA 021244 2	Forward	GACATTTCCAGCACCATGCG	
Fuusz	NIVI_051544.2	Reverse	TTCTTGCCATACTCGAGGGG	
Eads2	NM_173137.1	Forward	CCCTGGTCAAGGCATTCTGT	
ruus5		Reverse	AGACTTCTTCAGGGACCCGAT	
Gpam	NM_017274.1	Forward	CTGTCTGGGGATAGCTTTGCT	
		Reverse	ACAAAACATGAGCTGTATCCTTGA	
Thn	NINA 001004109 1	Forward	AGTGCCAAGTGTGAGCCTCT	
тор	NIM_001004198.1	Reverse	ACAGTGATGTGGGGACAAAACG	
Srahf1		Forward	CCATGGACGAGCTACCCTTC	
5160/1	NIM_001270707.1	Reverse	AGCATGTCTTCGATGTCGGT	
Doorg		Forward	AACTCTGGGAGATCCTCCTGT	
Ppuig	NIM_013124.3	Reverse	ATGGTAATTTCTTGTGAAGTGCTCA	
Cebpa	NM_001287577.1	Forward	TGCGCAAGAGCCGAGATAAA	
		Reverse	GCGGTCATTGTCACTGGTCA	
Cehnh	NIM 02/125 5	Forward	TTCCTTTCCGACCTCTTCGC	
Севри	11101_024125.5	Reverse	CACGTAACCGTAGTCGGACG	

Supplementary Table 1. Primer Sequences. Rat primer sequences for qPCR.