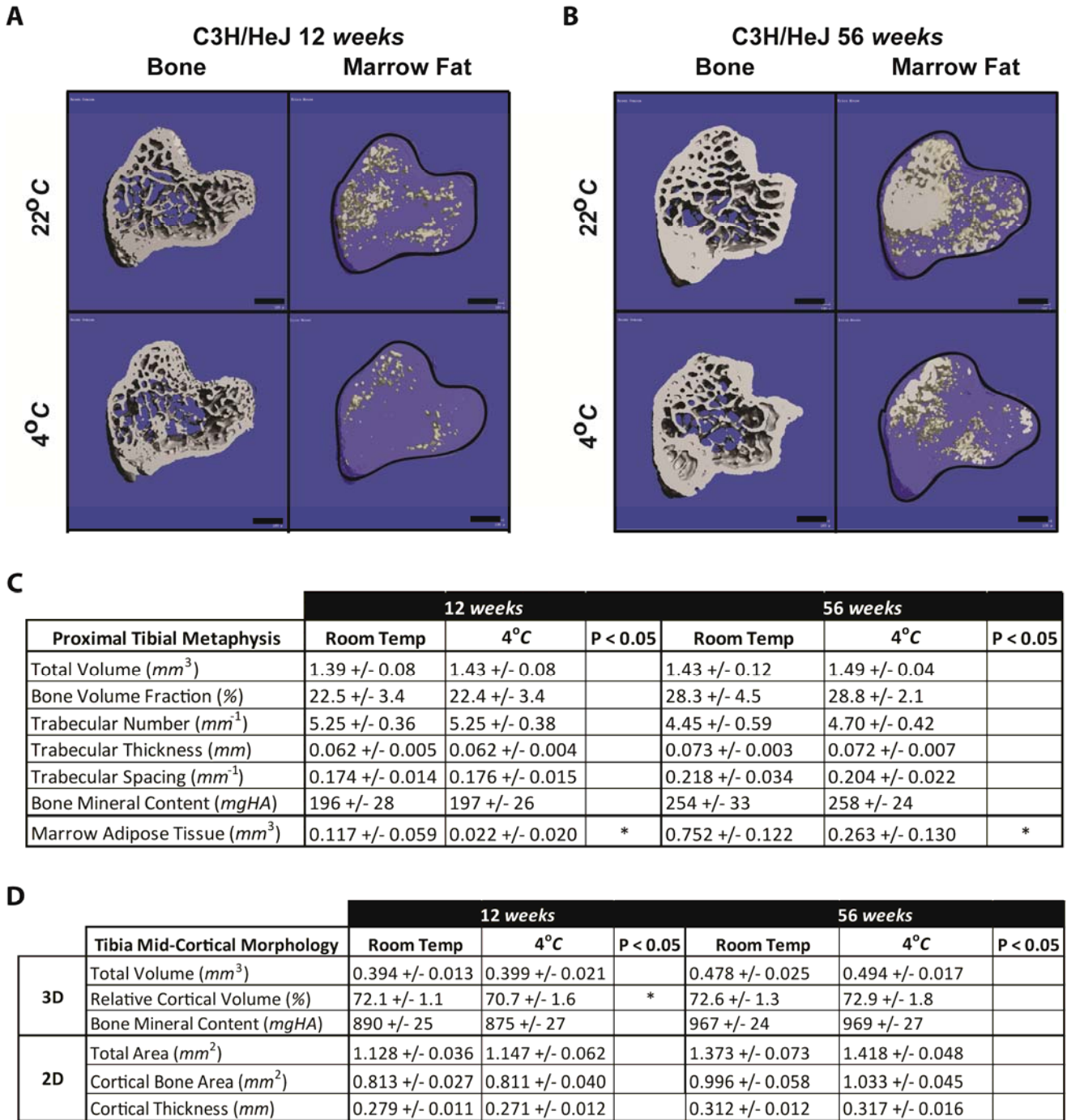
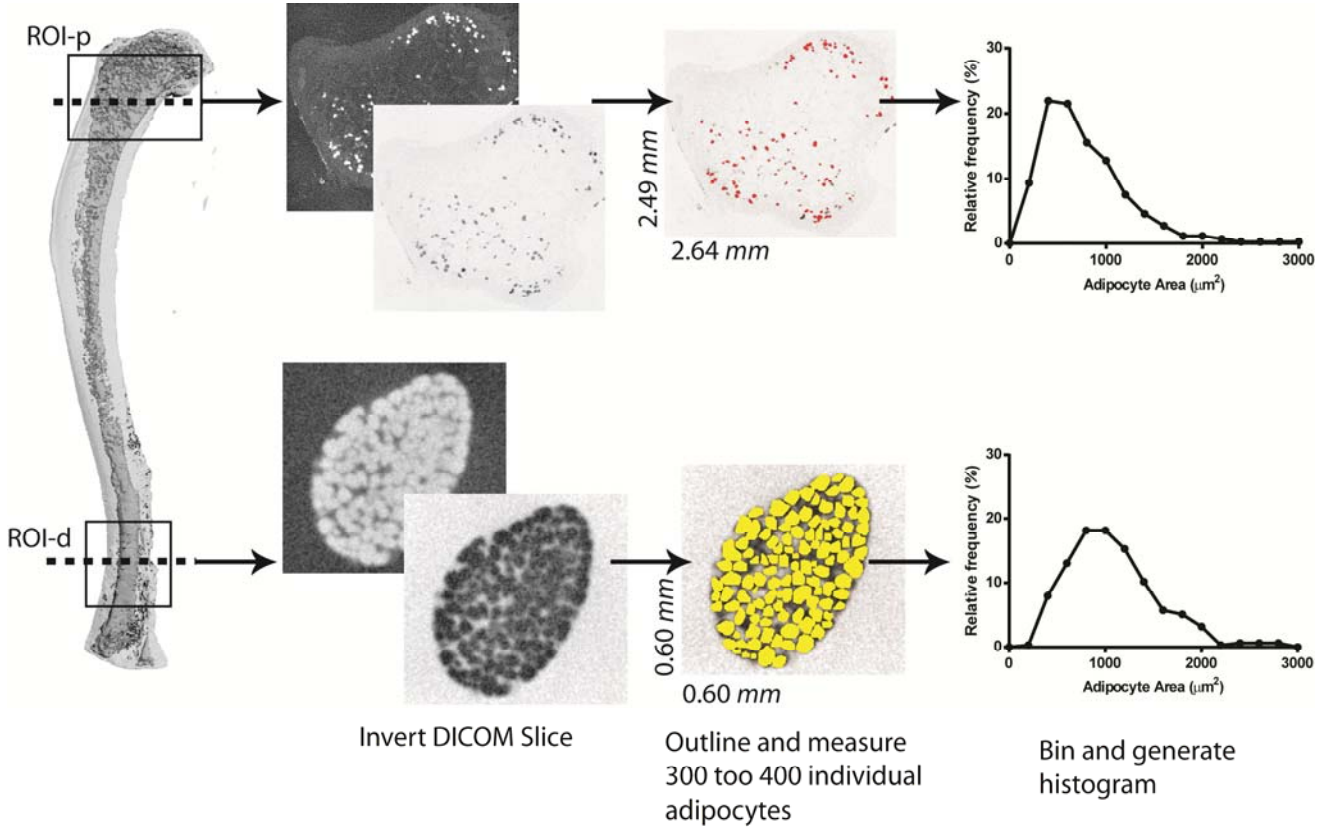


**SUPPLEMENTARY FIGURE 1**



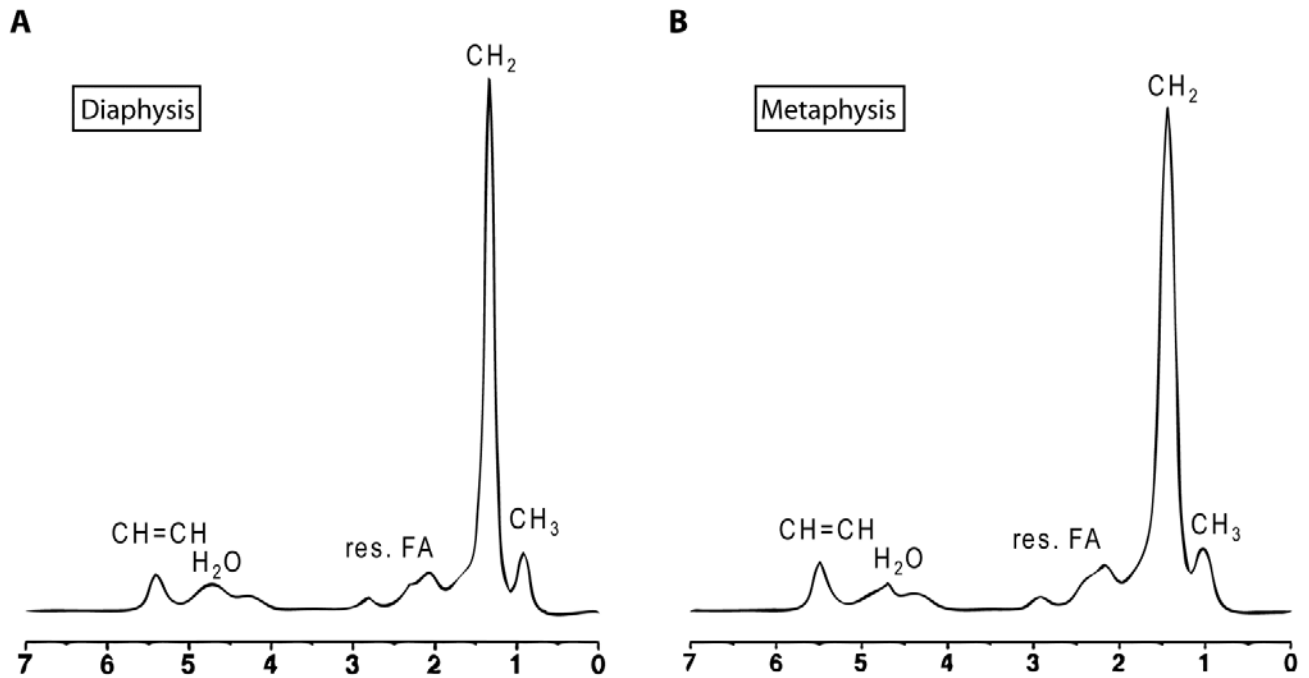
**Supplementary Figure 1. Trabecular and cortical analyses with cold exposure.** (A,B) Representative images of the proximal tibial metaphysis both prior to decalcification and after osmium staining of the data presented in (C). Marrow fat is in white. Scale bar = 500  $\mu m$ . (C) Quantification of 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**



**Supplementary Figure 2. NanoCT for quantification of adipocyte size.** To quantify tibial adipocyte size using nano-computed-tomography, two regions of interest were scanned at 2  $\mu\text{m}$  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  $\mu\text{m}$  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.

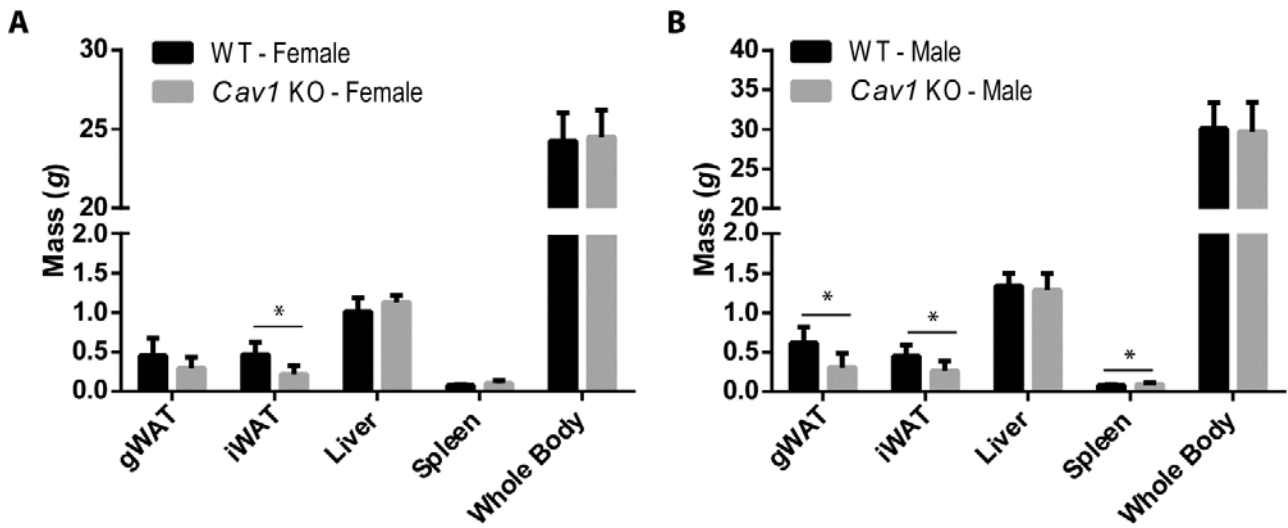
### SUPPLEMENTARY FIGURE 3



**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 [(-CH<sub>2</sub>-)<sub>n</sub>], methyl protons at 0.9 ppm (-CH<sub>3</sub>), 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 (H<sub>2</sub>O) at 4.7 ppm.



**SUPPLEMENTARY FIGURE 5**



**C**

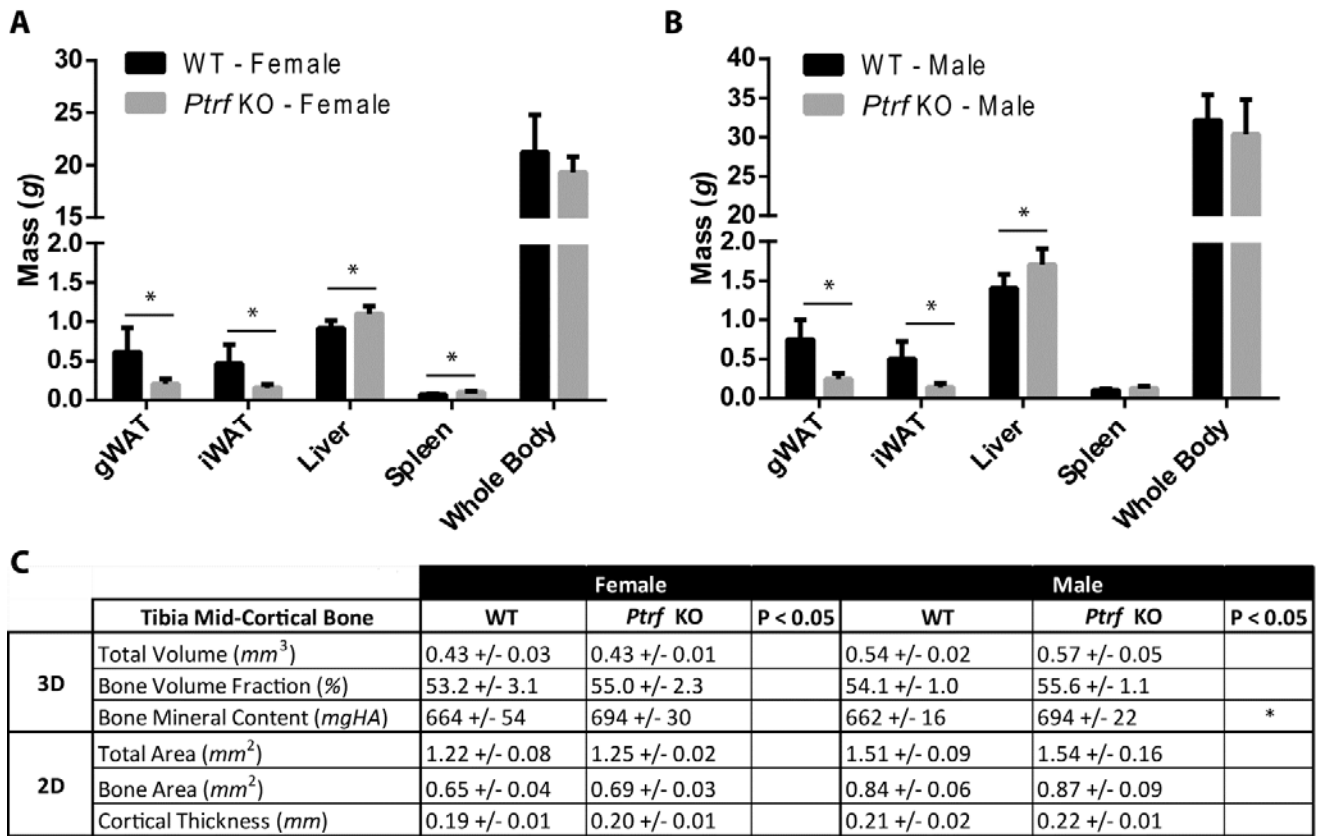
Proximal Tibial Metaphysis	Female			Male		
	WT	Cav1 KO	P < 0.05	WT	Cav1 KO	P < 0.05
Total Volume ( $mm^3$ )	1.09 +/- 0.41	0.95 +/- 0.43		1.43 +/- 0.25	1.36 +/- 0.12	
Bone Volume Fraction (%)	37.8 +/- 8.3	44.9 +/- 8.7		41.9 +/- 5.2	42.8 +/- 7.7	
Trabecular Number ( $mm^{-1}$ )	5.61 +/- 0.68	5.93 +/- 0.58		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 ( $mm^{-1}$ )	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 ( $mm^3$ )	0.050 +/- 0.059	0.036 +/- 0.025		0.039 +/- 0.063	0.029 +/- 0.053	

**D**

Tibia Mid-Cortical Morphology	Female			Male			
	WT	Cav1 KO	P < 0.05	WT	Cav1 KO	P < 0.05	
<b>3D</b>	Total Volume ( $mm^3$ )	0.35 +/- 0.03	0.37 +/- 0.05		0.41 +/- 0.05	0.44 +/- 0.06	
	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	
<b>2D</b>	Total Area ( $mm^2$ )	1.02 +/- 0.08	1.08 +/- 0.13		1.18 +/- 0.14	1.24 +/- 0.19	
	Cortical Bone Area ( $mm^2$ )	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 ± standard deviation. \*Two-tailed t-Test, p<0.05.

**SUPPLEMENTARY FIGURE 6**



**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	
<i>Scd1</i>	NM_139192	Forward	CTCAGCGCTGGGAAAGTG
		Reverse	GAAGTGGAGATCTCTTGGAGCA
<i>Scd2</i>	NM_031841.1	Forward	GCAGATGTTCCGCCCTGAAATTA
		Reverse	CAAATATGCAAAGAGGCAGGTGTAG
<i>Fads1</i>	NM_053445.2	Forward	CACTACGCTGGTCAGGATGC
		Reverse	AGTGAGCGCCTTATTCTTGGT
<i>Fads2</i>	NM_031344.2	Forward	GACATTTCCAGCACCATGCG
		Reverse	TTCTTGCCATACTCGAGGGG
<i>Fads3</i>	NM_173137.1	Forward	CCCTGGTCAAGGCATTCTGT
		Reverse	AGACTTCTTCAGGGACCCGAT
<i>Gpam</i>	NM_017274.1	Forward	CTGTCTGGGGATAGCTTTGCT
		Reverse	ACAAAACATGAGCTGTATCCTTGA
<i>Tbp</i>	NM_001004198.1	Forward	AGTGCCAAGTGTGAGCCTCT
		Reverse	ACAGTGATGTGGGGACAAAACG
<i>Srebf1</i>	NM_001276707.1	Forward	CCATGGACGAGCTACCCTTC
		Reverse	AGCATGTCTTCGATGTTCGGT
<i>Pparg</i>	NM_013124.3	Forward	AACTCTGGGAGATCCTCCTGT
		Reverse	ATGGTAATTTCTTGTGAAGTGCTCA
<i>Cebpa</i>	NM_001287577.1	Forward	TGCGCAAGAGCCGAGATAAA
		Reverse	GCGGTCATTGTCACTGGTCA
<i>Cebpb</i>	NM_024125.5	Forward	TTCCTTTCCGACCTCTTCGC
		Reverse	CACGTAACCGTAGTCGGACG

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