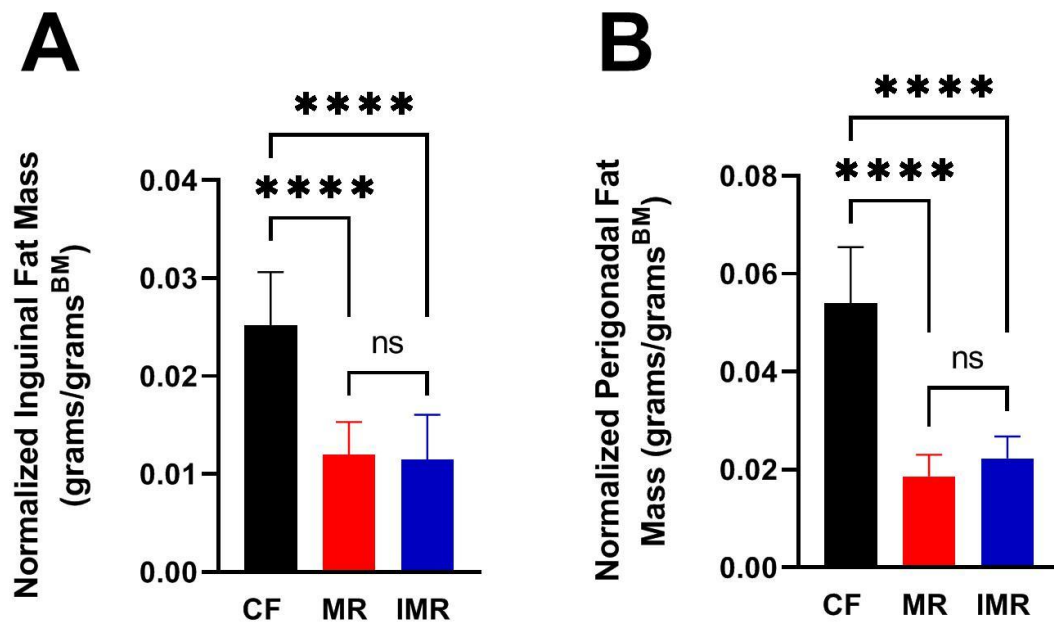


## SUPPLEMENTARY FIGURE 1

### Fat Depot Sizes Normalized to Total Body Mass for Mice Subjected to IMR and Continuous MR

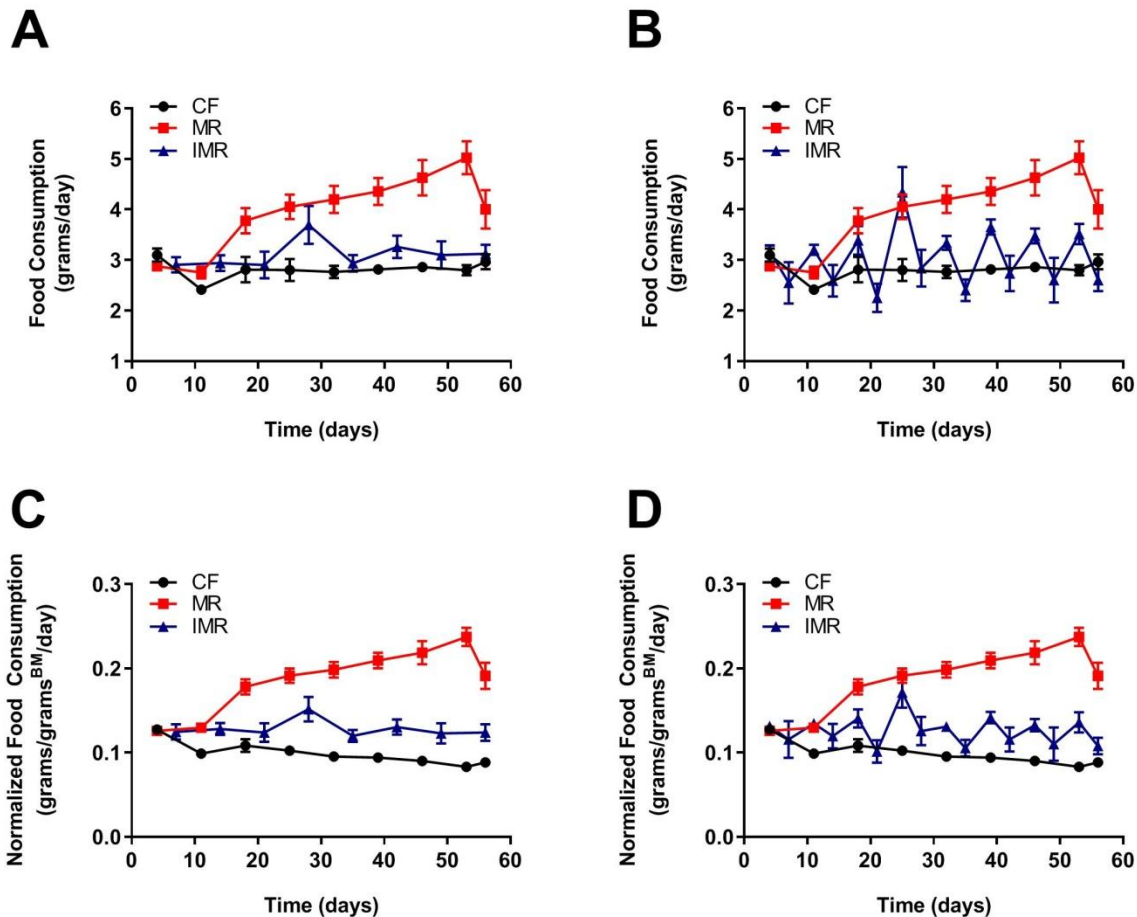
Normalized average values at the conclusion of the experiment are shown for both (A) the mass of inguinal fat pads and (B) the mass of perigonadal fat pads from mice fed the indicated diets. Bars denote standard error of the mean (SEM). Statistically significant differences are indicated (\*\*\*\*,  $p < 0.0001$ ). N=8 for all groups.



## SUPPLEMENTARY FIGURE 2

### Food Consumption Measurements for Mice Subjected to IMR and Continuous MR

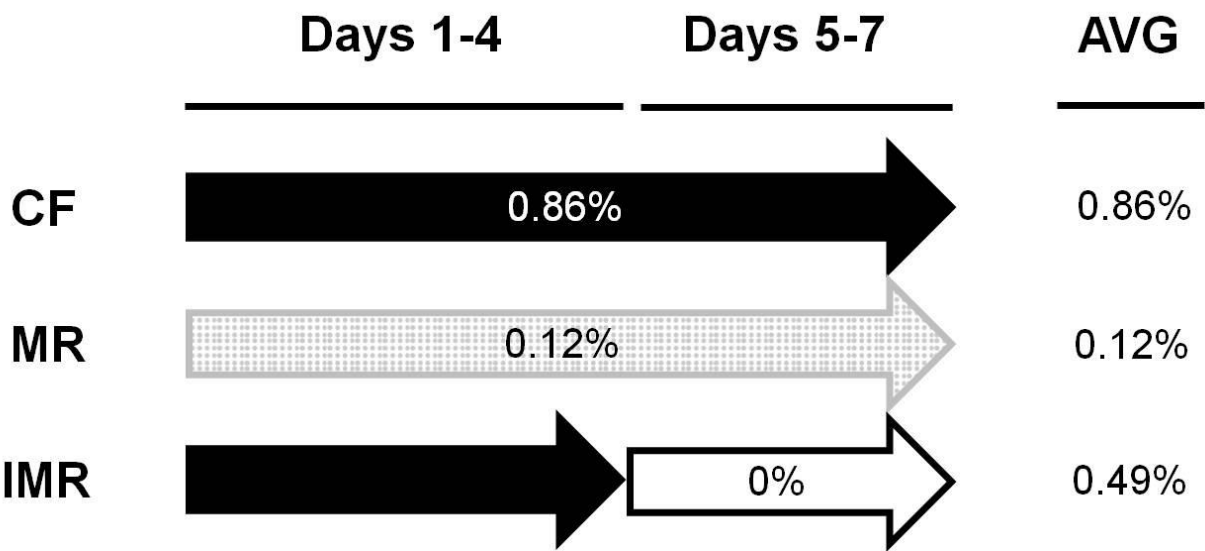
Comparisons over time of average values for (A-B) food consumption, and (C-D) food consumption normalized to total body mass for control-fed (CF; black circles) or continuously methionine-restricted (MR; red squares) mice, as well as animals subjected to IMR (blue triangles). For panels A and C, each value represents the average daily food consumption over a given week. In contrast, panels B and D are expanded versions of these graphs that show all IMR values for food consumption (*i.e.*, methionine-replete and methionine-restricted periods). For all panels, bars denote SEM. N=8 for all groups.



**SUPPLEMENTARY FIGURE 3**

**Dietary Regimens**

A pictorial representation of one week of control feeding (CF), continuous methionine restriction (MR), and intermittent methionine restriction (IMR) is shown. Percent values indicate the concentration of methionine present in each diet, as well as the 7-day average (AVG) for each regimen. Black arrows indicate intervals of methionine-replete feeding (0.86% methionine), gray arrows indicate intervals of low methionine feeding (0.12% methionine), and white arrows indicate intervals of methionine-free feeding (0% methionine).



## SUPPLEMENTARY TABLE 1

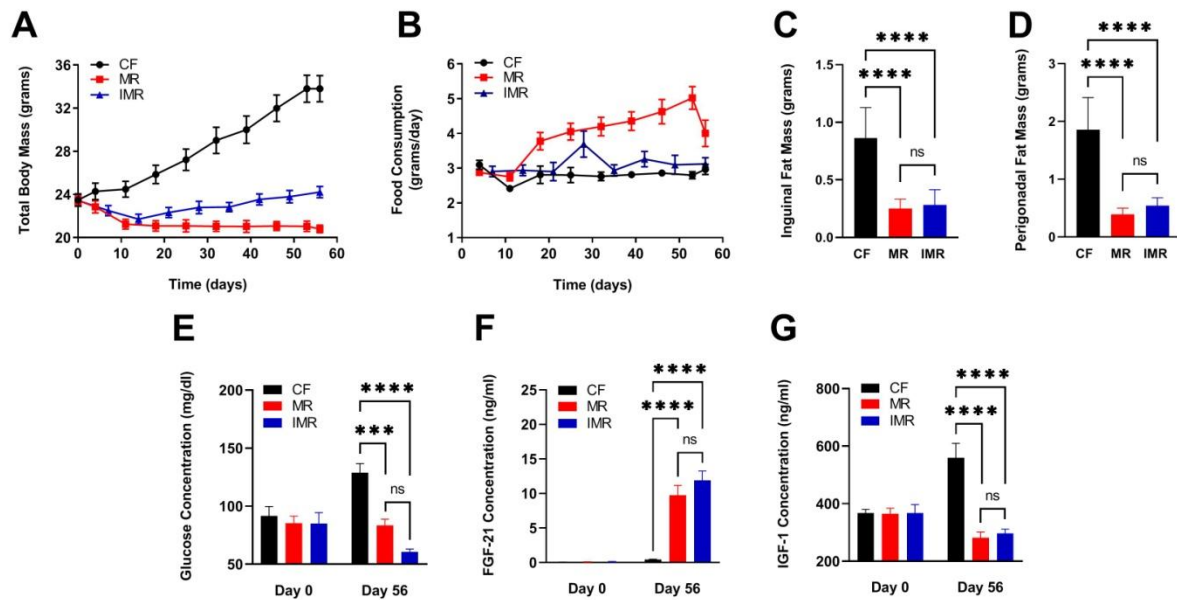
### Composition of Mouse Diets

	Diet 1 (CF; 0.86%) Cat No: A18072401		Diet 2 (MR; 0.12% MET) Cat No: A19022801		Diet 3 (0% MET) Cat No: A19022802	
	g%	kcal%	g%	kcal%	g%	kcal%
Protein	15	12	15	12	15	12
Carbohydrate	41	31	41	31	41	31
Fat	34	57	34	57	34	57
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>kcal/g</b>		<b>5.3</b>		<b>5.3</b>		<b>5.3</b>
Ingredient	G	kcal	G	kcal	g	kcal
L-Arginine	11.2	45	11.2	45	11.2	45
L-Histidine-HCl-H <sub>2</sub> O	3.3	13	3.3	13	3.3	13
L-Isoleucine	8.2	33	8.2	33	8.2	33
L-Leucine	11.1	44	11.1	44	11.1	44
L-Lysine	14.4	58	14.4	58	14.4	58
DL-Methionine	8.86	35	1.24	5	0	0
L-Phenylalanine	11.6	46	11.6	46	11.6	46
L-Threonine	8.2	33	8.2	33	8.2	33
L-Tryptophan	1.8	7	1.8	7	1.8	7
L-Valine	8.2	33	8.2	33	8.2	33
L-Glutamic Acid	27.83	111	35.5	142	35.5	142
L-Glutamine	0	0	0	0	1.24	5
Glycine	23.3	93	23.3	93	23.3	93
Corn Starch	35	140	35	140	35	140
Maltodextrin	125	500	125	500	125	500
Dextrose	50	200	50	200	50	200
Sucrose	150	600	150	600	150	600
Cellulose	50	0	50	0	50	0
Corn Oil	46	414	46	414	46	414
Lard	257	2313	257	2313	257	2313
Mineral Mix S10001	35	0	35	0	35	0
Vitamin Mix V10001	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0
Dye	0.05	0	0.05	0	0.05	0
<b>Total</b>	<b>898</b>	<b>4759</b>	<b>898</b>	<b>4759</b>	<b>898</b>	<b>4759</b>

## SUPPLEMENTARY FILE 1

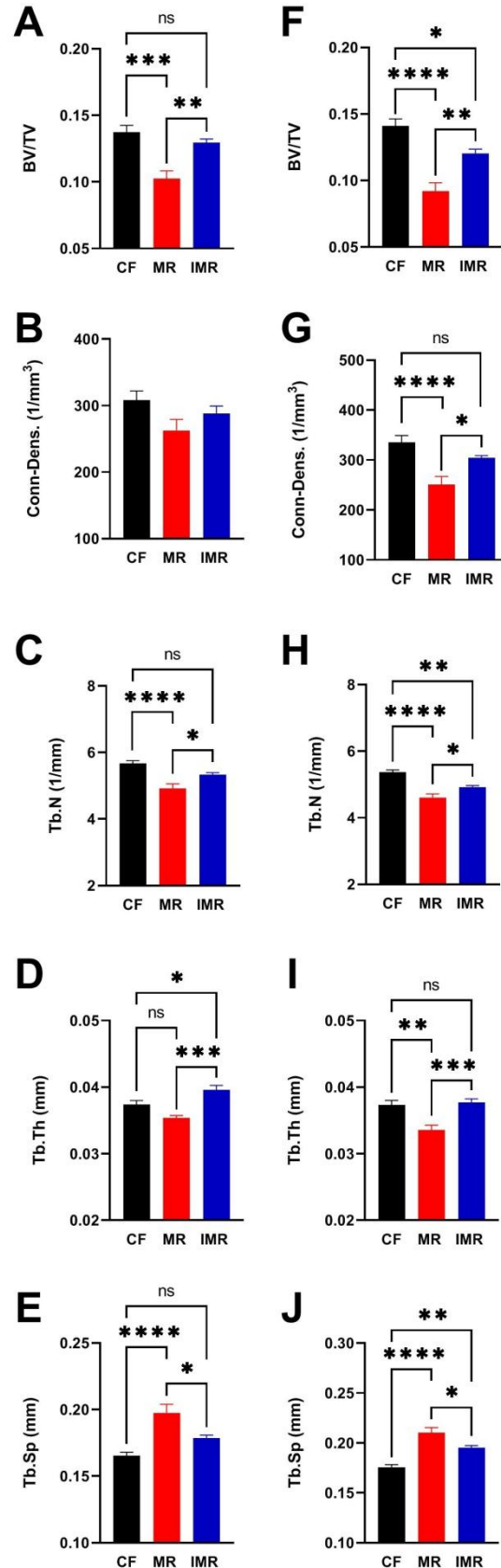
### Figure 1. Both IMR and Continuous MR Confer Metabolic Health Benefits to Mice

Comparisons over time of average values are shown for (A) total body mass and (B) food consumption for control-fed (CF; black circles), continuously methionine-restricted (MR; red squares), and intermittently methionine-restricted (IMR; blue triangles) mice. Also shown are average values at the conclusion of the experiment (8 weeks) for the mass of (C) inguinal and (D) perigonadal fat pads. In addition, average values for the circulating levels of (E) fasting glucose, (F) plasma FGF-21, and (G) plasma IGF-1 are also depicted. For panels E-G, IMR values were obtained following a period of methionine-restricted feeding. For all panels, error bars denote SEM. For panels C-G, statistically significant differences are either indicated (\*\*,  $p < 0.001$ ; \*\*\*,  $p < 0.0001$ ) or absent (ns).  $N=8$  for all groups.



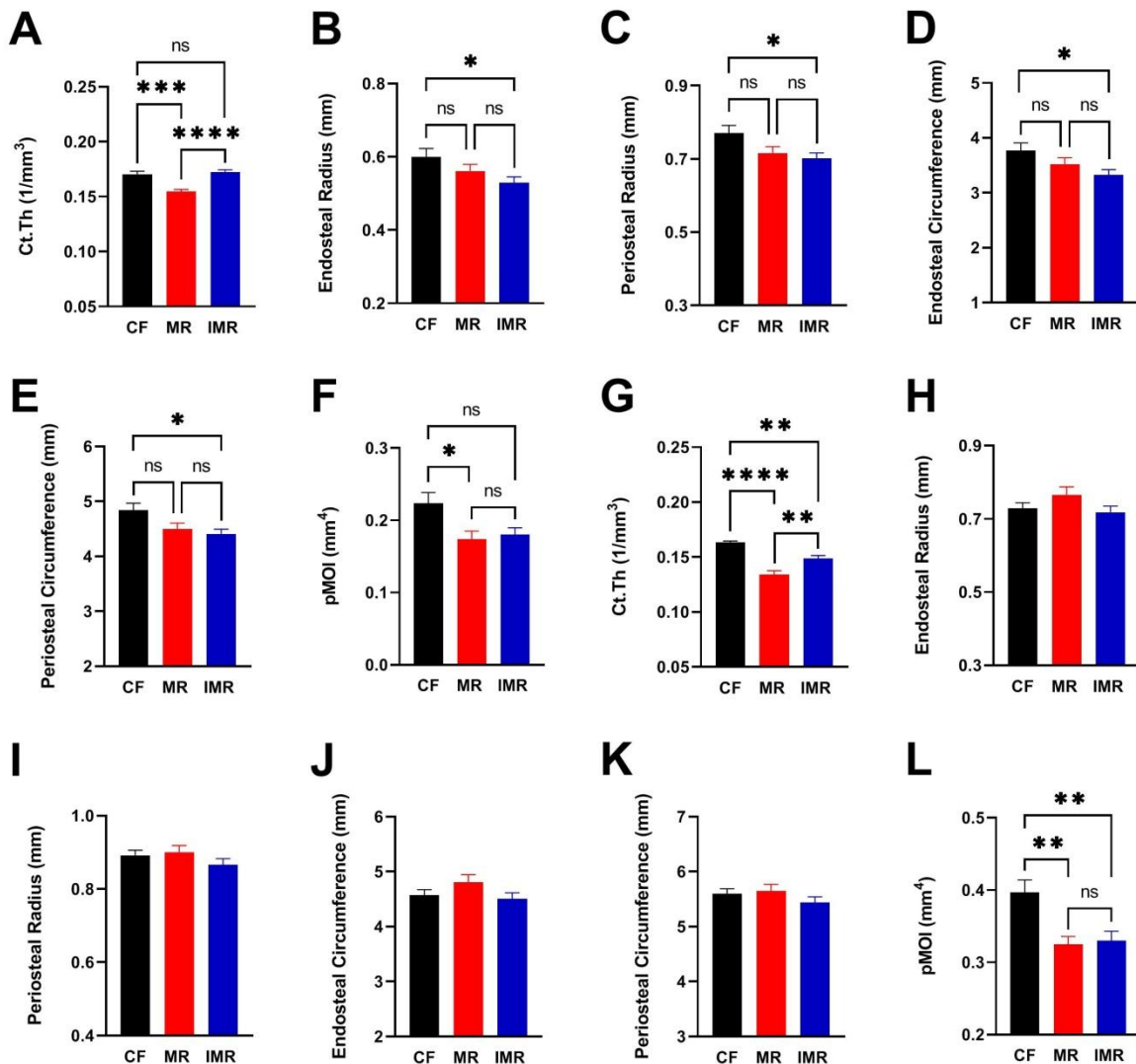
### Figure 3. IMR Preserves Trabecular Bone

Average values at the conclusion of the experiment (8 weeks) are shown for (A, F) trabecular bone volume/total volume (BV/TV), (B, G) connective density, (C, H) trabecular number (Tb. N), (D, I) trabecular thickness (Tb. Th), and (E, J) trabecular spacing (Tb. Sp). Values were determined by micro-CT analyses of tibiae (A-E) and femurs (F-J), respectively. Bars denote SEM. Statistically significant differences are either indicated (\*,  $p<0.05$ ; \*\*,  $p<0.01$ ; \*\*\*,  $p<0.001$ ; \*\*\*\*,  $p<0.0001$ ) or absent (ns). N=8 for all groups.



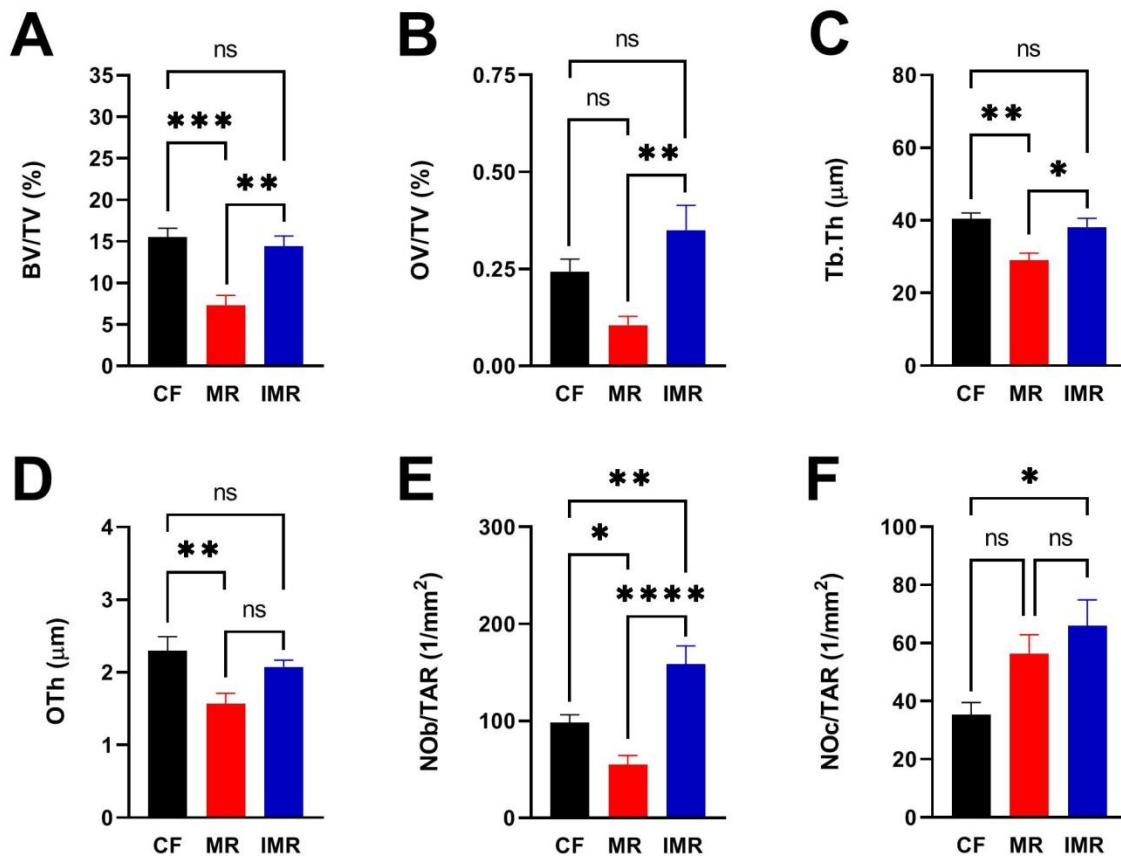
## Figure 5. IMR Preserves Cortical Bone

Average values at the conclusion of the experiment (8 weeks) are shown for (A, G) cortical thickness (Ct.Th), (B, H) endosteal radius, (C, I) periosteal radius, (D, J) endosteal circumference, (E, K) periosteal circumference, and (F, L) polar moment of inertia (pMOI). Values were determined by micro-CT analyses of tibiae (A-F) and femurs (G-L), respectively. Bars denote SEM. Statistically significant differences are either indicated (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ) or absent (ns). N=8 for all groups.



## Figure 6. IMR Results in Both Improved Osteoid Formation and Bone Resorption

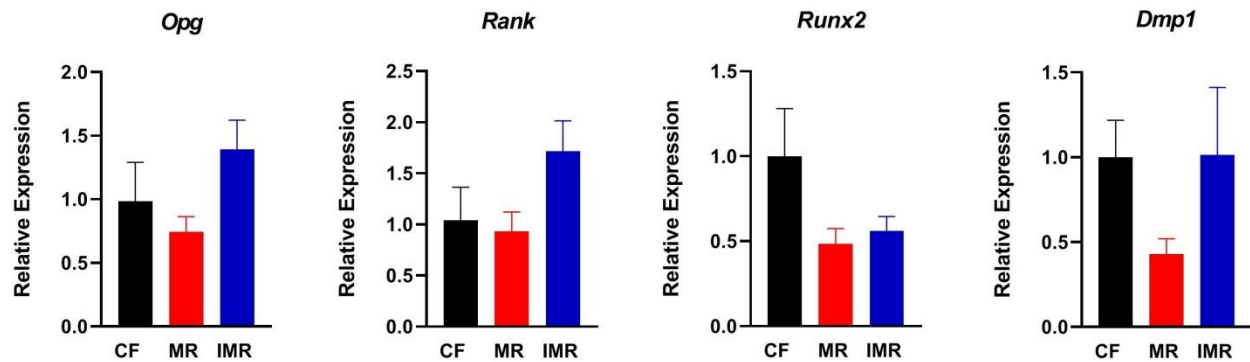
Average values at the conclusion of the experiment (8 weeks) are shown for (A) trabecular bone volume/total volume (BV/TV; %), (B) osteoid volume/total volume (OV/TV; %), (C) trabecular thickness (Tb. Th), (D) osteoid thickness (OTh), (E) number of osteoblasts/total area (NOb/TAR), and (F) number of osteoclasts/total area (NOc/TAR). Measurements were obtained by static histomorphometric analyses of tibiae. Bars denote SEM. Statistically significant differences are either indicated (\*,  $p<0.05$ ; \*\*,  $p<0.01$ ; \*\*\*,  $p<0.001$ ; \*\*\*\*,  $p<0.0001$ ) or absent (ns). N=8 for all groups.





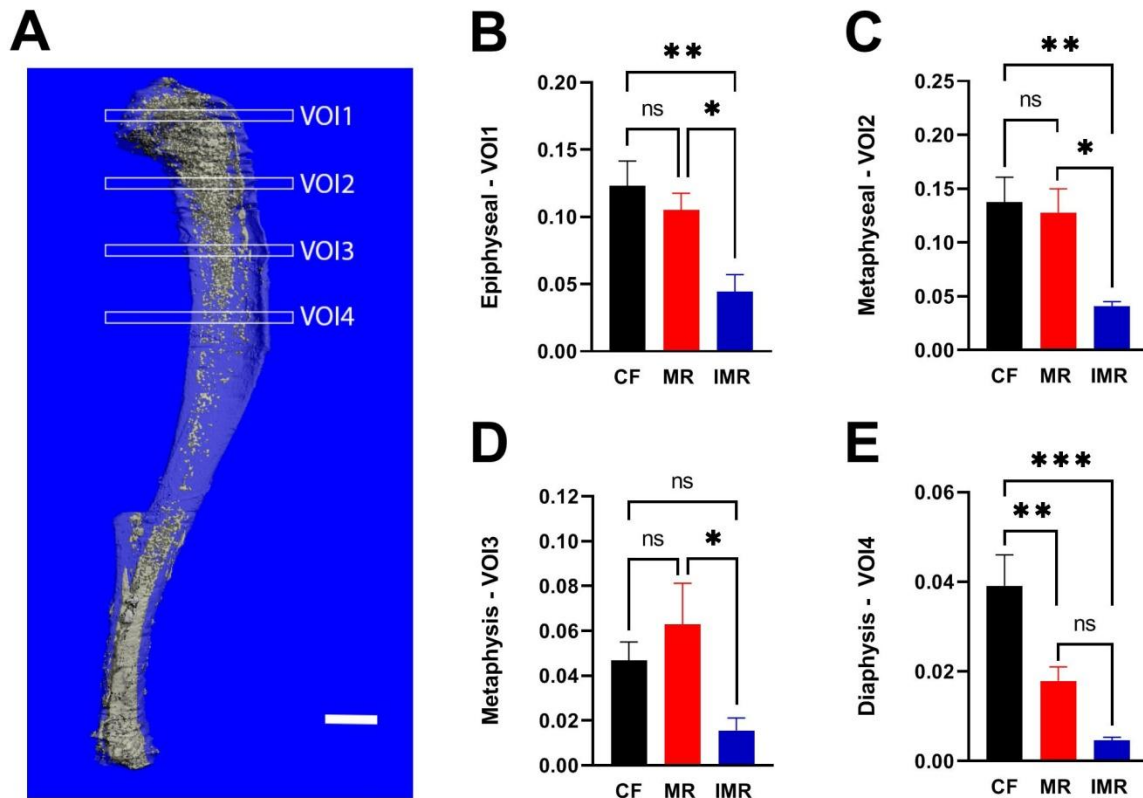
**Figure 7. Relative Expression of Factors Involved in the Differentiation and Activity of Osteoblasts and Osteoclasts**

Average relative expression is shown for genes encoding factors that regulate the differentiation and activity of osteoclasts (*Opg* and *Rank*) and osteoblasts (*Runx2* and *Dmp1*). Values represent the relative abundance of these transcripts in humeral bone marrow at the conclusion of the experiment (8 weeks) for animals fed the indicated diets. Bars denote SEM. N=8 for all groups.



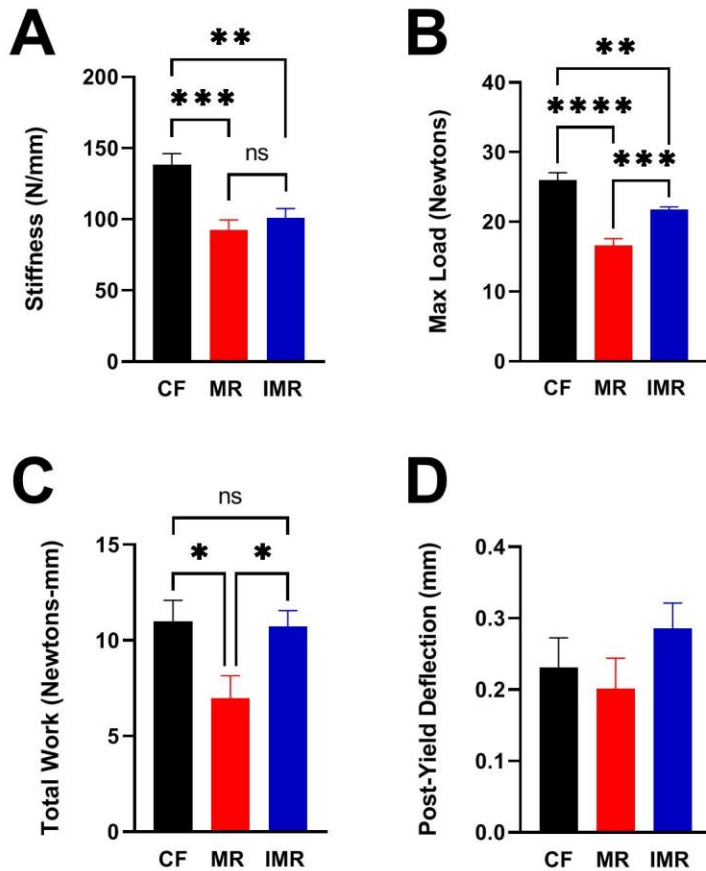
## Figure 8. IMR Prevents Bone Marrow Adipogenesis

(A) Positioning of the volumes of interest (VOIs) in a representative micro-CT image of an osmium-stained tibia from a control-fed mouse at the conclusion of the experiment (8 weeks; light blue, bone; grayscale, marrow fat). Average values are shown for the proportions of marrow fat present in the tibiae of mice fed the indicated diets. Values are for fat located in the (B) epiphyseal (VOI1), (C) metaphyseal (VOI2), (D) metaphysis (VOI3), and (E) diaphysis (VOI4). For panel A, bar denotes 1 mm. For panels B-D, bars denote SEM. Statistically significant differences are either indicated (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ) or absent (ns). N=8 for all groups.



## Figure 9. IMR Preserves Bone Strength

Average values at the conclusion of the experiment (8 weeks) are shown for (A) bone stiffness, (B) maximum load, (C) total work required to fracture bone, and (D) post-yield deflection. Values were determined by four-point bending tests of femurs from mice fed the indicated diets. Bars denote SEM. Statistically significant differences are either indicated (\*,  $p<0.05$ ; \*\*,  $p<0.01$ ; \*\*\*,  $p<0.001$ ; \*\*\*\*,  $p<0.0001$ ) or absent (ns). N=8 for all groups.



**Figure S1. Fat Depot Sizes Normalized to Total Body Mass for Mice Subjected to IMR and Continuous MR**

Normalized average values at the conclusion of the experiment are shown for both (A) the mass of inguinal fat pads and (B) the mass of perigonadal fat pads from mice fed the indicated diets. Bars denote standard error of the mean (SEM). Statistically significant differences are indicated (\*\*\*\*,  $p < 0.0001$ ). N=8 for all groups.

