Supplementary Information for

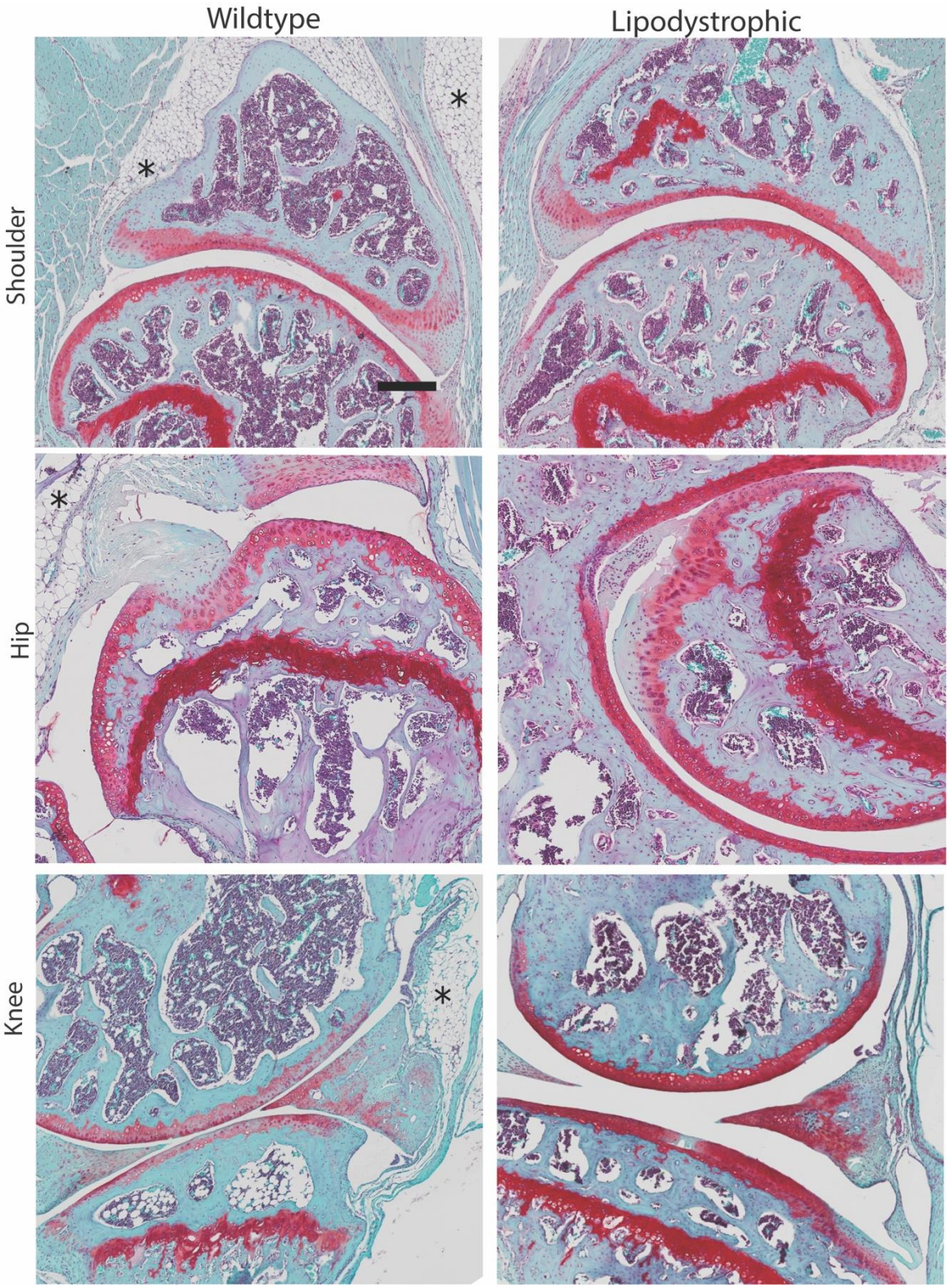
Adipose Tissue is a Critical Regulator of Osteoarthritis

Kelsey H. Collins, Kristin L. Lenz, Eleanor N. Pollitt, Daniel Ferguson, Irina Hutson, Luke E. Springer, Arin K. Oestreich, Ruhang Tang, Yun-Rak Choi, Gretchen A. Meyer, Steven L. Teitelbaum, Christine T.N. Pham, Charles A. Harris, Farshid Guilak

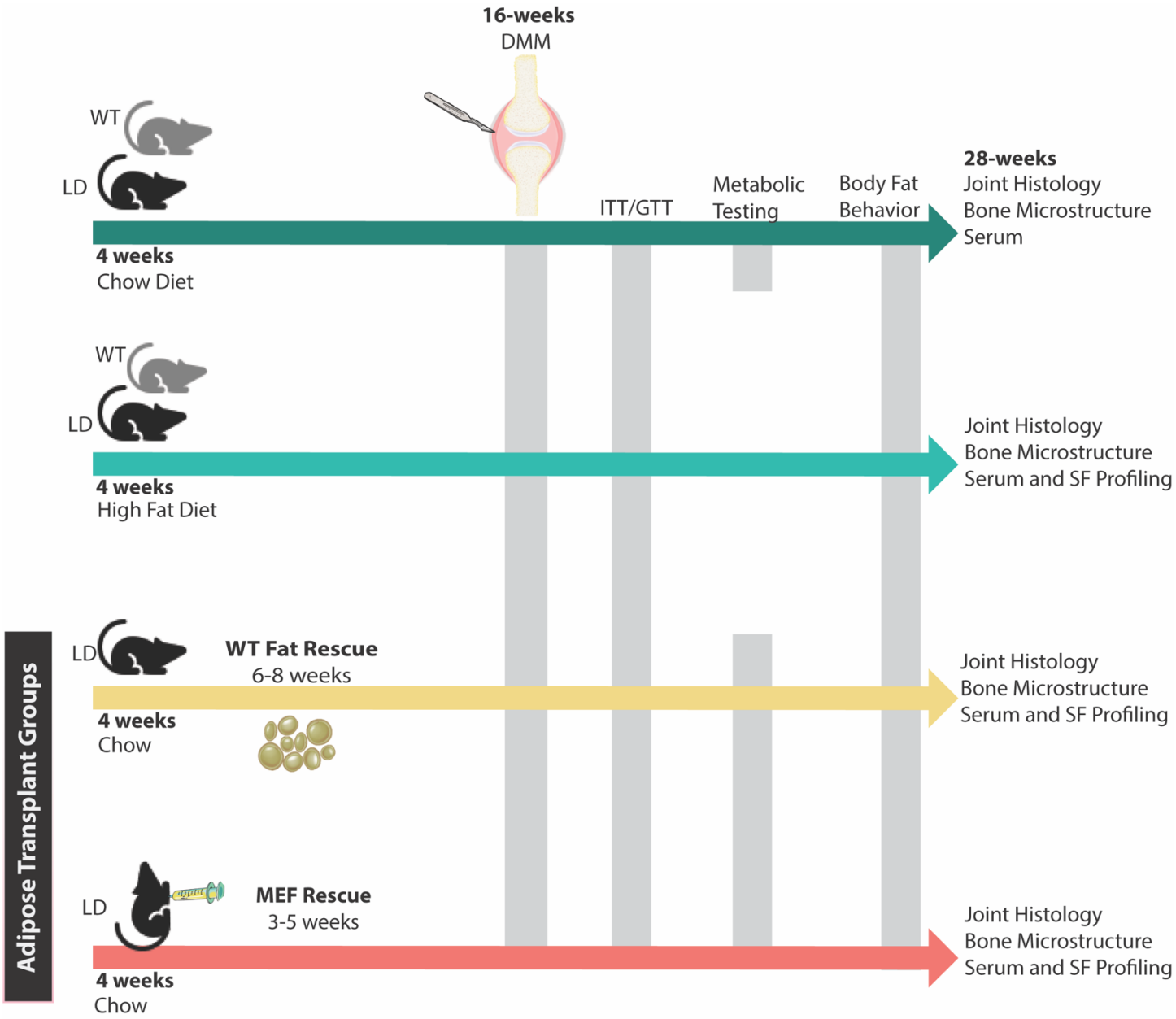
Correspondence to:
*Farshid Guilak, PhD
Email: guilak@wustl.edu

This PDF file includes:

Supplementary Figures S1-S2
Supplementary Tables S1-S5



Supplementary Figure 1. Fat pads are absent in Shoulder, Hip, and Knee Joints of LD mice. LD mice do not demonstrate fat pads adjacent to shoulder, hip, or knee joints, indicated with black asterisks in the left panels. Scale bar indicates 100μm.



Supplementary Figure 2. Timeline for study. First, male and female LD mice and WT littermate controls were fed a chow-control diet from weaning (4 weeks of age) and were challenged with DMM surgery at 16 weeks of age. Metabolic profiles were determined using insulin tolerance tests (ITT) and glucose tolerance tests (GTT) at 20 weeks of age, 4-weeks post DMM. At 22-24 weeks of age, metabolic testing using indirect calorimetry assessments were completed using a Phenomaster device. At 27-weeks of age, one week prior to sacrifice, body composition and behavior assays were completed. Animals were euthanized at 28-weeks of age, 12-weeks post DMM surgery. Tissues were preserved for joint histology and bone microstructure, and serum, synovial fluid, and fecal matter was stored at -80°C for profiling. The same timeline was followed for male and female LD and WT HFD-fed mice, with the exception of indirect calorimetry metabolic testing – these animals were not evaluated using the Phenomaster. In the transplant groups, LD host mice either received wild type fat rescue (WT Fat-rescue) at 6-8 weeks of age, or a cell-based injection of preadipocyte mouse embryonic fibroblasts (MEF-rescue) at 3-5 weeks of age. Both transplant groups were maintained on chow diets, and followed the same protocol as the chow-fed LD and WT mice. The same euthanization and tissue preparation procedures were employed to all groups of mice at 28-weeks of age.

Supplementary Table 1. Serum Changes in Chow and HFD Male Mice by Genotype, data are shown as Mean ± SEM.

	Chow		**HFD**		**Main Effects (p<0.05)**
Pro-Inflammatory Mediators	WT	LD	WT	LD	
IL-1α (pg/mL)	140.7±20.9	368.7±31.6[a]	294.0±28.2[a]	243.4±39.5[a]	Genotype, Diet*Genotype
IL-1β (pg/mL)	5.4±1.1	38.3±13.9[a]	8.3±3.5	23.4±8.0[a]	Genotype
IL-6 (pg/mL)	3.8±1.3	14.3±4.2[a]	2.7±0.9	11.4±2.65[a]	Genotype
TNF-α (pg/mL)	9.1±1.8	25.6±5.7[a]	6.5±2.2	17.8±3.7[a]	Genotype
IP-10 / CXCL-10 (pg/mL)	114.4±11.5	155.2±20.0[a]	127.1±10.0	157.5±11.1[a]	Genotype
MCP-1 (pg/mL)	64.5±9.4	141.9±27.2[a]	89.7±20.3	117.5±35.5[a]	Genotype
IL-12p70 (pg/mL)	83.2±22.9	310.4±136.2[a]	80.9±22.7	250.8±77.3[a]	Genotype
IL-15 (pg/mL)	70.6±6.0	75.3±14.6	154.9±31.0	1140.9±415.8[ab]	Diet, Genotype, Diet*Genotype
IL-17 (pg/mL)	5.8±1.1	7.6±1.6	6.7±1.2	14.0±4.4	NS
IFN β-1 (pg/mL)	157.2±10.4	166.9±30.9	244.5±36[a]	212.8±32.3[a]	Diet
TARC / CCL-17 (pg/mL)	19.0±3.0	35.0±6.3[a]	21.7±2.4	18.3±2.3	Diet p=0.09, Diet*genotype
Anti-Inflammatory Mediators					
IL-10 (pg/mL)	7.2±1.8	81.0±27.9[a]	11.4±4.0	74.4±16.2[a]	Genotype
IL-4 (pg/mL)	1.3±0.3	5.7±1.5[a]	1.7±0.4	3.7±1.5[a]	Genotype
TIMP-1(ng/mL)	0.8±0.1	2.1±0.2[a]	1.6±0.[a]	3.7±0.3 [ab]	Diet, Genotype

Sidak's Post-hoc Test
[a]p<0.05 compared to WT Chow
[b]p<0.05 compared to WT HFD
n=10-14/group

Supplementary Table 2. MicroCT outcomes for all groups: Bone Mineral Density (BMD), Bone Volume/Total Volume (BV/TV), Trabecular Number, and Trabecular Thickness. Data were compared in 3 groups: Chow Male and Female LD and WT) by 3-way ANOVA (limb, sex, genotype), HFD Male and Chow Male LD and WT (limb, diet, genotype), and MEF rescue (MEF-R) and WT fat rescue (WF-R) vs. Chow LD and WT, 2-Way ANOVA (limb, group). Data are separated into contralateral non-surgical limbs (2a) and DMM surgical limbs (2b) for viewing purposes.

2a. Contralateral Non-Surgical Limbs

		Chow				Main Effects	**HFD**		Main Effects	**Transplant**		Main Effects
		Female		**Male**		M vs. F P<0.05			HFD M vs. Chow M P<0.05			Transplant vs. Chow P<0.05
Outcome	**Region**	**WT**	**LD**	**WT**	**LD**		**WT**	**LD**		**WF-R**	**MEF-R**	
BMD (gCaHA/cm³)	LTP	0.62±0.02	0.84±0.02[ab*]	0.66±0.02	0.72±0.02	Sex, Genotype, Sex*Genotype	0.66±0.02	0.71±0.04	Genotype	0.75±0.03	0.71±0.4	Group
	LFC	0.52±0.01	0.74±0.04[a]	0.58±0.01	0.68±0.04	Genotype	0.58±0.13	0.64±0.02	Genotype	0.72±0.04[a]	0.70±0.04	Group
	MFC	0.51±0.02	0.75±0.05[a]	0.63±0.02	0.71±0.04	Genotype, Sex*Genotype	0.67±0.02	0.70±0.03	Genotype	0.77±0.03[a]	0.75±0.05	Group
BV/TV (%)	LTP	49.2±1.0	85.8±2.1[a*]	61.6±1.9	70.0±2.7	Genotype, Sex*Genotype, Limb*Sex*Genotype	56.1±2.1	67.1±2.6[c]	Diet, Genotype	69.4±4.0	70.5±5.2	Group
	LFC	41.5±0.3	73.0±4.3[a]	52.9±1.4*	58.4±2.3	Genotype, Sex*Genotype, Limb*Sex*Genotype	48.7±1.3	60.0±1.8[c]	Genotype	64.4±4.4	64.9±4.6	Group
	MFC	42.4±0.4	77.3±4.5[a]	58.2±2.5	63.0±2.5[a]	Genotype, Sex*Genotype	58.9±1.6	66.1±2.1	Genotype, Limb*Genotype	68.2±3.2	70.8±4.8[a]	Group
Trabecular Thickness (mm)	MTP	0.08±0.01	0.08±0.01	0.09±0.01	0.08±0.01	None	0.09±0.01	0.08±0.01	None	0.09±0.01	0.07±0.01	None
	LTP	0.09±0.001	0.09±0.007	0.09±0.005	0.09±0.006	Sex*Genotype	0.08±0.005	0.09±0.004	Diet, Genotype	0.11±0.004	0.09±0.009	Group
	LFC	0.10±0.01	0.10±0.01	0.10±0.01	0.10±0.01	Genotype, Sex*Limb	0.10±0.01	0.10±0.01	Genotype, Limb*Diet	0.10±0.01	0.10±0.01	Limb* Group
	MFC	0.10±0.01	0.11±0.01	0.10±0.01	0.11±0.01	Genotype	0.11±0.01	0.11±0.01	Limb, Genotype, Limb*Diet, Limb*Genotype	0.12±0.01	0.11±0.01	Limb
	TM	0.05±0.01	0.07±0.01*	0.06±0.01	0.05±0.01	Limb, Genotype, Limb*Sex, Limb*Sex*Genotype	0.06±0.01	0.06±0.01	Limb, Limb*Diet, Limb*Genotype, Limb*Diet* Genotype	0.07±0.01[b]	0.07±0.01[b]	Limb* Group
Trabecular Number (1/mm)	MTP	6.9±0.3	11.6±1.3[a]	7.4±0.4	8.9±0.5	Genotype, Sex*Genotype	7.3±0.2	9.7±1.0	Diet	10.0±1.0	11.5±1.3	Group
	LTP	6.6±0.6	9.1±0.7	6.5±0.2	8.0±0.7	Limb, Genotype	7.6±0.6	6.8±0.2	Genotype	6.5±0.2	8.6±0.8 [Ad]	Group
	LFC	4.4±0.1	6.8±0.4[a*]	5.6±0.2*	5.7±0.2	Genotype, Sex*Genotype, Limb*Sex*Genotype	5.1±0.1	5.7±0.1[c]	Genotype, Limb*Diet* Genotype	6.2±0.5	5.9±0.3	Limb* Group
	MFC	4.6±0.2	6.8±0.4[a*]	5.5±0.2	5.9±0.1	Genotype Limb*Genotype Sex*Genotype Limb*Sex*Genotype	5.6±0.1	5.8±0.1	Genotype	5.6±0.2	6.7±1.0[a]	Group P=0.07
	TM	1.2±0.3	3.5±0.8[a*]	0.8±0.1	1.8±0.3	Genotype, Limb*Sex, Limb*Sex*Genotype	1.0±0.2	1.8±0.3	Limb, Genotype, Limb*Diet, Limb*Genotype	2.0±0.2	2.2±0.4	Limb, Group

2b. DMM Surgical Limbs MicroCT Outcomes.

		Chow				Main Effects	High-Fat Diet Males		Main Effects	Fat Transplant		Main Effects (P<0.05)
		Female		Male		M V. F P<0.05	Genotype		HFD M V. Chow M P<0.05			Transplant V. Chow M
Outcome	**Region**	**WT**	**LD**	**WT**	**LD**		**WT**	**LD**		**WF-R**	**MEF-R**	
BMD (gCaHA/cm³)	LTP	0.61±0.03	0.90±0.02[ab*]	0.64±0.03	0.74±0.04	Sex, Genotype, Sex*Genotype	0.63±0.02	0.71±0.03	Genotype	0.75±0.04	0.70±0.04	Group
	LFC	0.52±0.01	0.70±0.06[a]	0.56±0.02	0.67±0.04	Genotype	0.56±0.01	0.66±0.02[c]	Genotype	0.64±0.04	0.69±0.05	Group
	MFC	0.56±0.03	0.79±0.08[a]	0.61±0.03	0.75±0.06	Genotype, Sex*Genotype	0.64±0.02	0.77±0.03	Genotype	0.70±0.02	0.77±0.06[a]	Group
BV/TV (%)	LTP	53.5±2.9[b]	74.8±11.1[a]	61.3±2.5[b]	73.5±1.8[a]	Genotype, Sex*Genotype, Limb*Sex*Genotype	55.0±2.1	66.1±2.1[c]	Diet, Genotype	70.0±4.9	69.1±4.4	Group
	LFC	43.0±0.1	66.5±8.6[a]	52.5±1.8	62.5±2.4	Genotype, Sex*Genotype, Limb*Sex*Genotype	48.7±1.0	59.7±2.2[c]	Genotype	55.2±5.0	64.0±4.9	Group
	MFC	43.7±1.7	71.4±9.4[a]	56.9±1.7*	71.0±3.0[a]	Genotype, Sex*Genotype	55.2±1.5	70.1±2.2[c]	Genotype, Limb*Genotype	61.8±2.6	73.1±5.3[a]	Group
Trabecular Thickness (mm)	MTP	0.09±0.01	0.08±0.01	0.08±0.01	0.08±0.01	None	0.08±0.01	0.07±0.06	None	0.08±0.01	0.07±0.01	None
	LTP	0.10±0.003	0.08±0.001	0.09±0.002	0.10±0.003	Sex*Genotype	0.09±0.003	0.09±0.004	Diet, Genotype	0.09±0.001	0.08±0.01[b]	Group
	LFC	0.10±0.01	0.10±0.01	0.10±0.01	0.11±0.01 [A]†	Genotype, Sex*Limb	0.09±0.01	0.10±0.01[c]	Genotype, Limb*Diet	0.10±0.01	0.09±0.01[b]	Limb*Group
	MFC	0.10±0.01	0.12±0.01	0.11±0.01	0.13±0.01[a]†	Genotype	0.11±0.01	0.12±0.01[c]	Limb, Genotype, Limb*Diet, Limb*Genotype	0.12±0.01	0.12±0.01	Limb
	TM	0.06±0.01	0.08±0.01	0.06±0.01	0.07±0.01[a]†	Limb, Genotype, Limb*Sex, Limb*Sex*Genotype	0.06±0.01	0.06±0.01[b]	Limb, Limb*Diet, Limb*Genotype, Limb*Diet*Genotype	0.07±0.01	0.06±0.01[b]	Limb*Group
Trabecular Number (1/mm)	MTP	6.2±0.4	11.1±2.4[a]	7.5±0.4	9.5±0.6	Genotype, Sex*Genotype	8.7±0.4	12.5±1.7	Diet	12.5±3.1	13.0±3.4	Group
	LTP	5.3±0.1	7.4±0.4	6.7±0.4	6.9±0.4	Limb, Genotype	6.0±0.1	7.1±0.4	Genotype	6.8±0.4	8.2±0.7	Group
	LFC	4.7±0.1	6.3±0.3[a]	5.3±0.2	5.7±0.2	Genotype, Sex*Genotype, Limb*Sex*Genotype	5.3±0.1	5.7±0.1	Genotype, Limb*Diet* Genotype	5.3±0.4	6.6±0.4[ad]	Limb*Group
	MFC	4.9±0.2	6.1±0.3†	5.3±0.2	6.0±0.1[a]	Genotype Limb*Genotype Sex*Genotype Limb*Sex*Genotype	5.2±0.1	5.7±0.1	Genotype	5.2±0.2	6.0±0.3	Group P=0.07
	TM	1.0±0.2	3.1±0.8	0.9±0.1	3.1±0.4 [A]†	Genotype, Limb*Sex, Limb*Sex*Genotype	0.9±0.2	2.0±0.2[cb]	Limb, Genotype, Limb*Diet, Limb*Genotype	2.1±0.3	3.0±1.0[a]	Limb, Group

Sidak's Post hoc for 3-way ANOVAS, Tukey's post-hoc for 2-way ANOVA
[a]p<0.05 compared to WT chow control,
[b]p<0.05 vs. LD-M chow,
[c]p<0.05 compared to WT HFD control,
[d]p<0.05 WT fat transplant
*p<0.05 by sex,
†p<0.05 within group by limb,
male n= 9-17/group, female n=5-12
Abbreviations: LTP: lateral tibial plateau, LFC: lateral femoral condyle, MFC: Medial femoral condyle; MTP: Medial tibial plateau, TM: Tibial Metaphysis; BMD: Bone Mineral Density, BV/TV: Bone Volume/Total Volume

Supplementary Table 3. Male Synovial Fluid Profiles from Chow and High Fat Diet-fed LD and WT Mice.

	Chow				**HFD**				Main Effects ($p<0.05$)
Genotype	WT		LD		WT		LD		
(pg/mL)	Con	DMM	Con	DMM	Con	DMM	Con	DMM	
IL-7	67.2±13.0	30.2±9.7	50.0±12.3	39.7±9.0	57.2±19.5	28.7±8.7	37.2±9.4	30.4±8.7	Limb
Fractalkine	8.7±0.3	9.3±1.0	8.5±0.5	8.0±0.4	11.4±1.0	11.3±1.0	10.2±0.8	9.5±0.8	Limb, Diet
G-CSF	44.7±6.3	36.4±8.1	63.5±10.3	63.1±12.2	32.7±7.1	40.5±9.3	38.8±7.4	54.4±9.3	Genotype
IL-6	287.4±102.2	176.4±96.2	230.1±89.7	978.5±427.4	622.8±257.9	441.4±155.3	1176.4±615.1	1859.6±770.0	Genotype
IL-12p40	226.8±78.0	259.9±86.0	189.4±52.3	103.5±33.8	313.1±66.9	305.6±55.0	138.6±27.0	102.0±31.3	Genotype
IL-15	489.1±78.6	472.6±59.1	357.9±78.0	405.2±70.3	549.5±58.7	415.3±64	311.7±43.4	408.6±53.6	Genotype
IFN-γ	87.3±24.7	105.8±28.8	77.7±17.2	91.2±19.9	77.5±14.5	75.3±23.2	23.7±9.3	31.7±12.9	Diet, Genotype
IL-16	3.1±0.6	4.2±0.6	5.9±1.2[a]	4.4±0.6	1.8±0.5	2.2±0.5	3.0±0.3	3.3±0.5	Diet, Genotype
MIP-2	9780±529	10059±584	9913±601	9962±334	10028±442	8468±615	7468±642	7720±436	Diet, Genotype, Diet*genotype
IL-1β	146.7±28.6	115.4±28.5	85.1±20.0	117.3±25.9	140.5±9.0	73.5±19.3	85.1±19.1	92.0±23.0	Limb*Genotype
MCP-5	101.3±22.3	110.0±4.4	150.8±19.5	105.2±12.0	48.7±7.2	147.0±35.1	97.4±13.0	105.9±20.6	Limb*Genotype, Diet*Genotype
MDC	20.5±4.0	40.1±7.7	82.2±14.0[a]	75.7±23.0[a]	29.6±6.6	100.0±22.7[ab]	21.4±4.8	27.8±4.7	Limb, Limb*Genotype, Diet*Genotype
TNF-α	9.4±1.0	14.0±2.3	11.0±2.2	9.3±1.5	6.6±0.7	6.7±1.3	5.6±1.0	5.7±1.1	Diet
Eotaxin	556.9±67.3	541.3±54.0	521.7±69.2	431.0±50.0	647.4±163.4	639.0±94.0	679.9±115.0	860.4±236.0	Diet
LIX	618.3±169.0	1268.9±292.1	634.3±151.0	1018.0±261.0	520.7±164.0	445.7±121.4	167.8±58.3	365.6±125.0	Diet
IL-1α	18.2±8.5	263.2±75.0	286.15±90.3	109.1±30.0	881.7±163.9	612.7±232.0	1135.9±347.0[a]	1475.7±478.6[a]	Diet
IP-10	20.2±5.1	18.4±3.3	31.8±6.2	32.2±5.8	53.3±10.8[a]	70.0±17.0[a]	51.3±6.1[a]	70.3±11.2[a]	Diet

Dunnet's Post-hoc Test
[a]$p<0.05$ compared to WT chow contralateral
[b]$p<0.05$ compared to WT HFD contralateral
n= 10-12/group

Supplementary Table 4. Serum Changes in WT Fat rescue (WF-R) and MEF rescue (MEF-R) Corrected Mice by Genotype, data are shown as Fold Change relative to WT contralateral, Mean ± SEM.

	Chow		Fat-Transplant		1-way ANOVA p-value
(Fold Change)	WT	LD	WF-R	MEF-R	
MCP-5	1.1±0.1	1.0±0.2	2.2±0.3[a]	1.9±0.3[a]	0.002
IL-15	1.0±0.2	1.0±0.2	1.9±0.6	3.6±1.1[a]	0.015
IFN β-1	1.0±0.3	0.8±0.1	21.5±8.8[a]	7.4±2.3	0.011
TARC / CCL-17	1.0±0.2	1.9±0.4[a]	1.9±0.2[a]	1.3±0.1	0.0187
IL-4 (pg/mL)	1.0±0.3	2.5±1.0	0.6±0.1	0.6±0.1	0.0420
MCP-1	1.1±0.2	1.7±0.4	0.9±0.2	0.9±0.1	0.094
IL-12p70	1.0±0.1	1.8±0.5	1.1±0.2	1.1±0.2	0.094
LIF	0.9±0.3	1.6±0.2	2.0±0.8	2.1±0.7[a]	0.167
IL-1β	1.0±0.2	3.0±1.2[a]	0.5±0.2	0.5±0.2	0.187
IP-10 / CXCL-10	1.0±0.1	1.8±0.5	1.0±0.3	1.1±0.2	0.181

Dunnet's Post-hoc Test
[a]p<0.05 compared to WT
[b]p<0.05 compared to
n=10-14/group

Supplementary Table 5. Synovial Fluid Changes in MEF/WT expressed as fold change relative to WT contralateral (Mean ± SEM).

	Chow				**Transplant**				Main Effects (p<0.05)
Genotype	WT		LD		WT fat		MEF		
Limb	Con	DMM	Con	DMM	Con	DMM	Con	DMM	
IL-7	1.1±0.2	0.5±0.1	0.8±0.2	0.7±0.1	1.4±0.1[c]	1.6±0.1[ab]	1.2±0.1	1.7±0.1[ab]	Limb*group, group
Fractalkine	1.0±0.1	1.0±0.1	0.9±0.1	0.9±0.1	1.4±0.1	1.3±0.2	1.7±0.1[cd]	1.9±0.2[ab]	Group
G-CSF	1.0±0.2	0.8±0.2	1.4±0.3	1.6±0.3	1.6±0.2	2.1±0.3[a]	1.2±0.1	1.4±0.1	Group
IL-12p40	1.0±0.4	1.1±0.4	1.0±0.3	0.9±0.4	1.5±0.1	1.8±0.1	1.3±0.1	1.6±0.1	Group
IL-15	1.0±0.2	1.0±0.1	0.7±0.2	0.8±0.1	1.2±0.1	1.3±0.2	1.0±0.1	1.4±0.1[b]	Group
IFN-γ	1.0±0.3	0.7±0.2	0.7±0.2	0.7±0.2	0.8±0.1	0.9±0.1	0.8±0.1	1.0±0.1	NS
IL-16	1.0±0.2	1.3±0.2	1.9±0.4	1.6±0.3	0.6±0.3[c]	0.3±0.1[ab]	0.5±0.2[c]	0.4±0.1[b]	Group
MIP-2	1.0±0.1	1.0±0.1	1.0±0.1	1.1±0.1	1.6±0.1[cd]	1.7±0.1[ab]	1.6±0.1[cd]	1.7±0.1[ab]	Group
IL-1β	1.0±0.2	12.3±3.1	0.7±0.2	6.0±1.6[a]	1.6±0.3	1.9±0.3[a]	1.0±0.3	1.4±0.4[ab]	Limb, Group, Limb*group
MCP-5	0.9±0.3	0.7±0.1	0.9±0.2	0.8±0.2	0.6±0.1	0.6±0.1	0.8±0.1	0.9±0.1	NS
MDC	1.0±0.2	2.0±0.4	3.7±0.7[d]	3.7±1.2	0.2±0.1[c]	0.3±0.1[b]	0.2±0.1[c]	0.4±0.2[b]	Group
TNF-α	1.0±0.2	1.2±0.2	1.2±0.3	0.9±0.1	1.3±0.2	1.6±0.1[b]	1.1±0.2	1.4±0.1	Group p=0.086
Eotaxin	1.0±0.1	1.0±0.1	0.9±0.1	0.7±0.1	0.1±0.1[cd]	0.1±0.1[ab]	0.2±0.1[cd]	0.2±0.1[ab]	Group
LIF	1.0±0.2	1.0±0.2	0.9±0.3	0.2±0.1	0.9±0.1	1.4±0.2[b]	1.4±0.3	1.5±0.2[b]	Group
TARC	1.0±0.3	1.6±0.3	1.4±0.2	0.8±0.2[a]	0.4±0.1[c]	0.5±0.1[a]	0.5±0.1[c]	0.7±0.3[a]	Group, Limb*Group
IL-1α	1.0±0.5	14.4±4.0	15.7±5.0[d]	5.3±1.6	17.9±2.1[d]	23.3±2.2[b]	18.0±0.9[d]	21.6±2.06[b]	Group, Limb*Group

Sidak's Post-hoc Test
[a]p<0.05 vs WT DMM
[b]p<0.05 vs LD DMM
[c]p<0.05 vs LD Con
[d]p<0.05 vs WT Con
n= 10-12/group