Supplement S1. Technical Details for Isolation of Synovial Fluid Cells from Mouse Knee

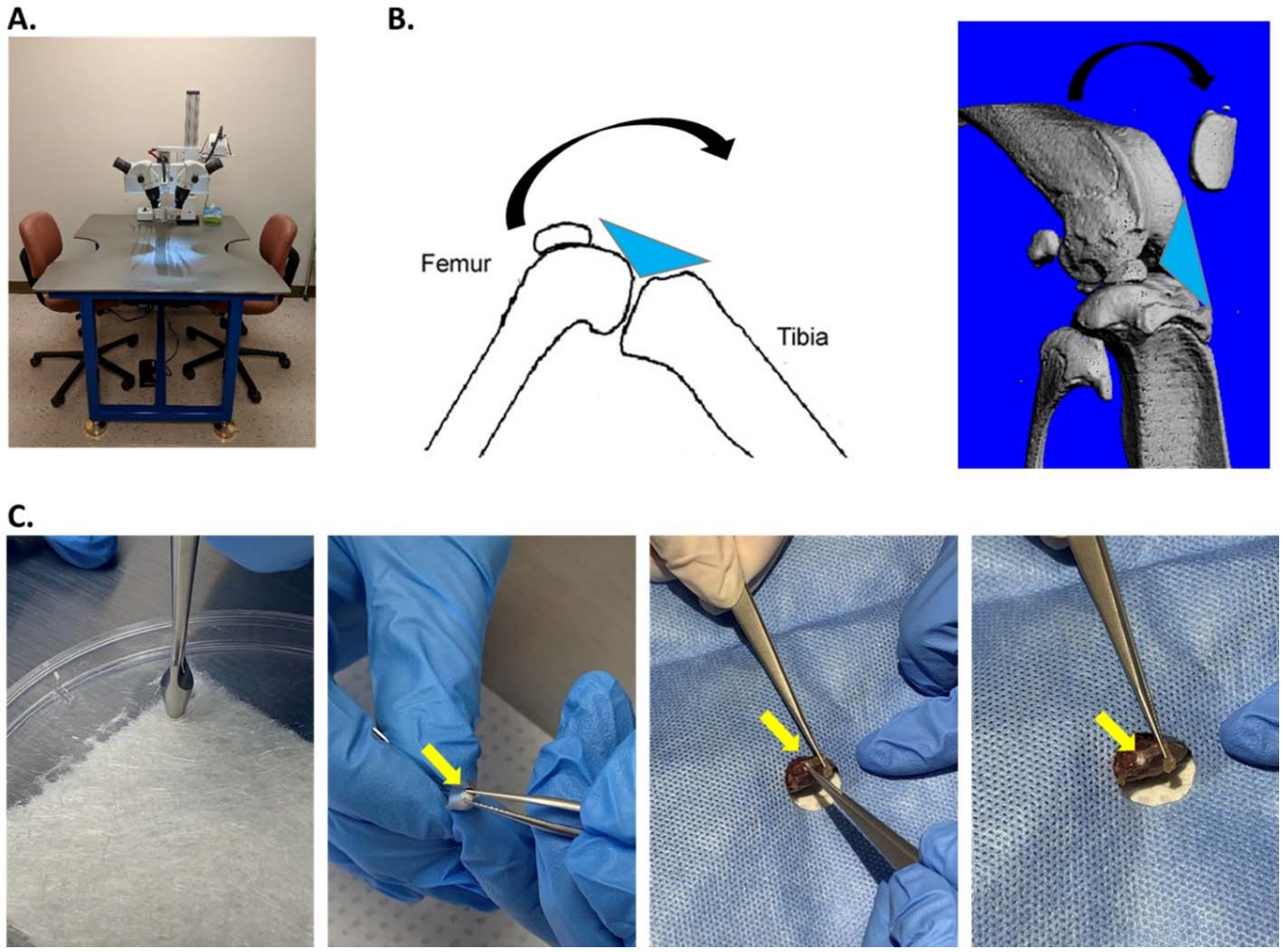
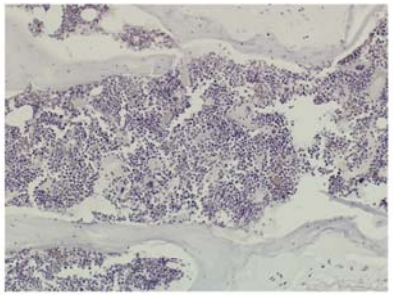


Figure S1. Technical Details for Isolation of Synovial Fluid Cells from Mouse Knee. **A**) Olympus custom dual-view Olympus SZ61 stereomicroscopes with motorized focus **B**) Diagram and MicroCT image demonstrating anatomy of mouse knee with resection of patella tendon to open knee joint to access synovial fluid at articular joint space (blue triangle). **C**) 2mm punch of Melgisorb (yellow arrow) being place in knee joint to absorb synovial fluid and cells with patellar tendon pulled distally to open joint space.

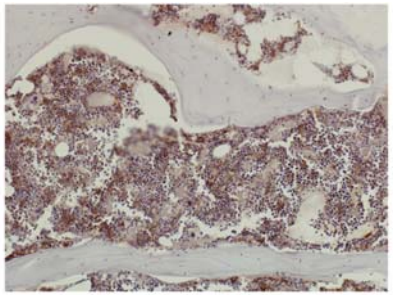
Figure S2. Representative images of IHC staining of mouse limbs. Standardized images (brown= + stain, blue=hematoxylin counter stain) at Day 7 (**A,B**) and Day 14 (**C,D**) post-fracture (20x)**. A,C)** Bone marrow used as internal control for each limb with negative and primary antibody using ImmPRESS HRP Goat Anti-Rat IgG, mouse adsorbed polymer detection kit, MP-7444-15, for rat anti-mouse F4/80 antibody, MCA497G (isotype IgG), and ImmPRESS HRP Horse Anti-Rabbit IgG polymer detection kit, MP-7401, for both rabbit anti-mouse CD4 antibody, ab183685 (isotype IgG) and for rabbit anti-mouse CD8 antibody, ab209775 (isotype IgG). **B,D)** Staining of synovium in contralateral control limbs and fractured limbs from mice with no treatment.

Day 7

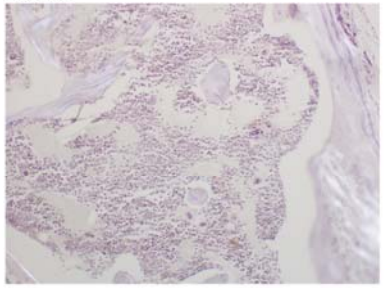
A.



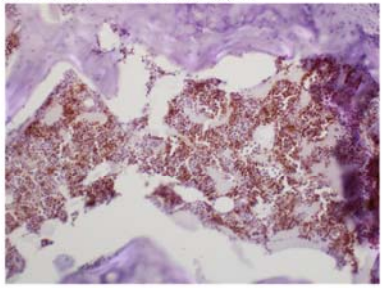
F4/80 Bone marrow Control limb No Tx – negative



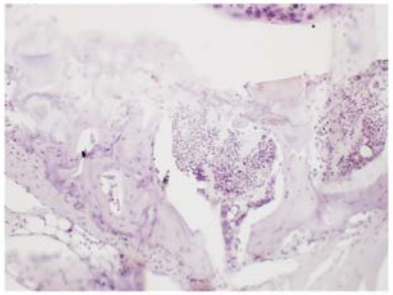
F4/80 Bone marrow Control limb No Tx – primary anybody conc. 3.3 µg/µl



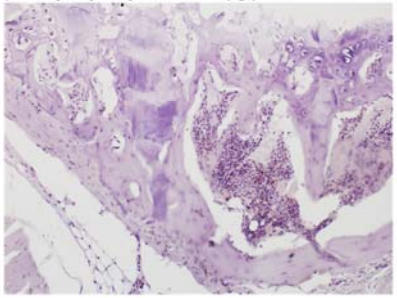
CD4 Bone marrow Control limb No Tx – negative



CD4 Bone marrow Control limb No Tx – primary anybody conc. 6.95 µg/µl

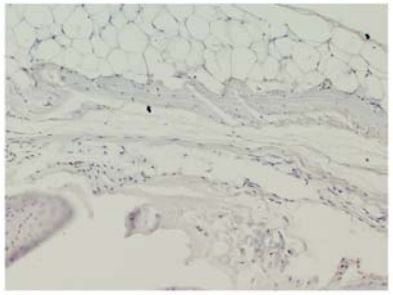


CD8 Bone marrow Control limb No Tx – negative

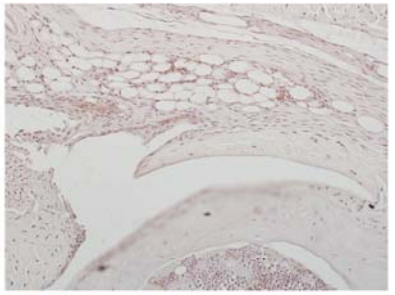


CD8 Bone marrow Control limb No Tx – primary anybody conc. 6.3 µg/µl

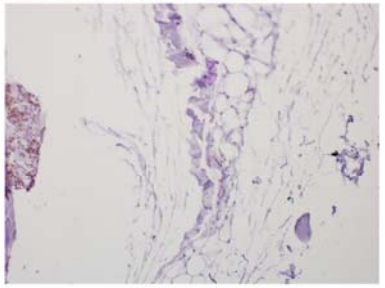
B.



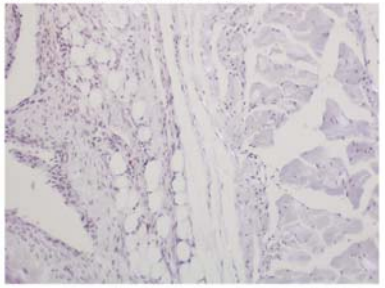
F4/80 Control limb No Tx – primary anybody conc. 3.3 µg/µl



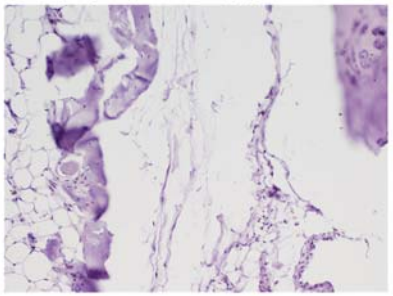
F4/80 Fracture limb No Tx – primary anybody conc. 3.3 µg/µl



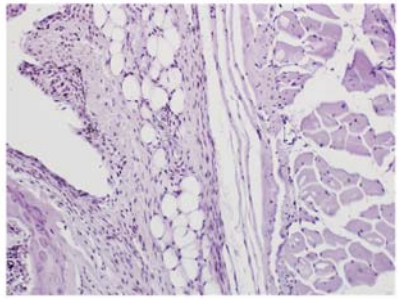
CD4 Control limb No Tx – primary anybody conc. 6.95 µg/µl



CD4 Fractured limb No Tx – primary anybody conc. 6.95 µg/µl



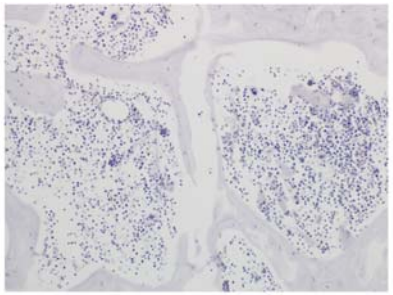
CD8 Control limb No Tx – primary anybody conc. 6.3 µg/µl



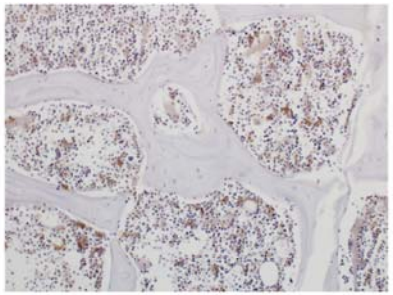
CD8 Fractured limb No Tx – primary anybody conc. 6.3 µg/µl

Day 14

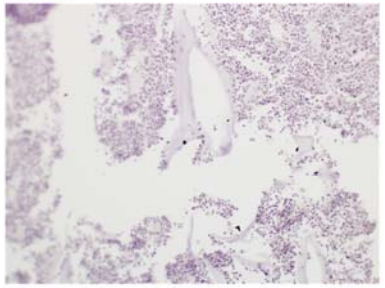
C.



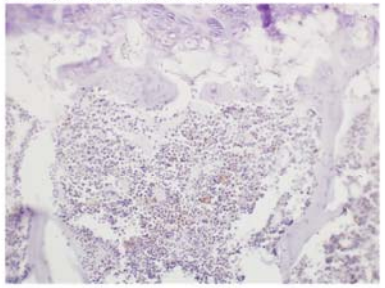
F4/80 Bone marrow Control limb No Tx – negative



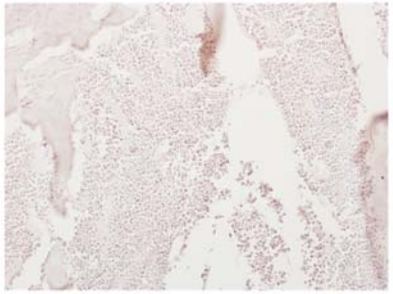
F4/80 Bone marrow Control limb No Tx – primary anybody conc. 3.3 µg/µl



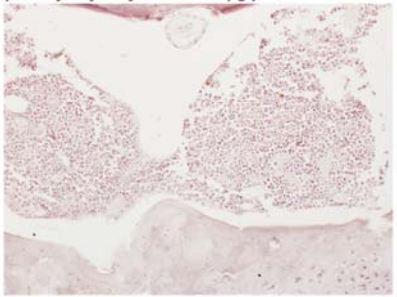
CD4 Bone marrow Control limb No Tx – negative



CD4 Bone marrow Control limb No Tx – primary anybody conc. 6.95 µg/µl

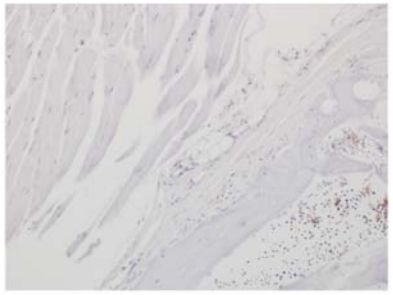


CD8 Bone marrow Control limb No Tx – negative

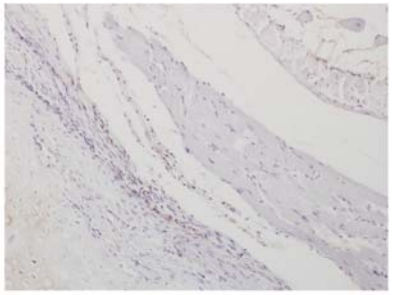


CD8 Bone marrow Control limb No Tx – primary anybody conc. 6.3 µg/µl

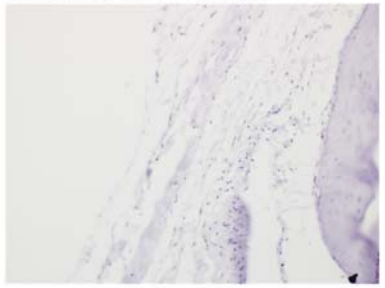
D.



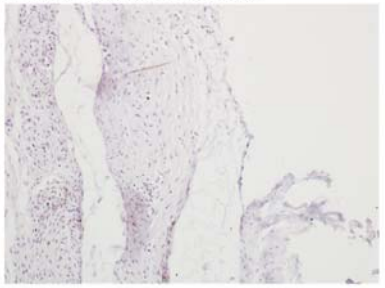
F4/80 Control limb No Tx – primary anybody conc. 3.3 µg/µl



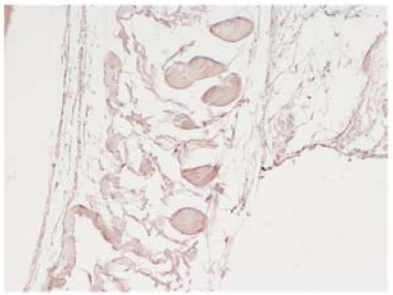
F4/80 Fracture limb No Tx – primary anybody conc. 3.3 µg/µl



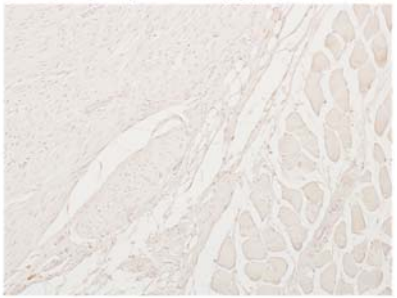
CD4 Control limb No Tx – primary anybody conc. 6.95 µg/µl



CD4 Fractured limb No Tx – primary anybody conc. 6.95 µg/µl



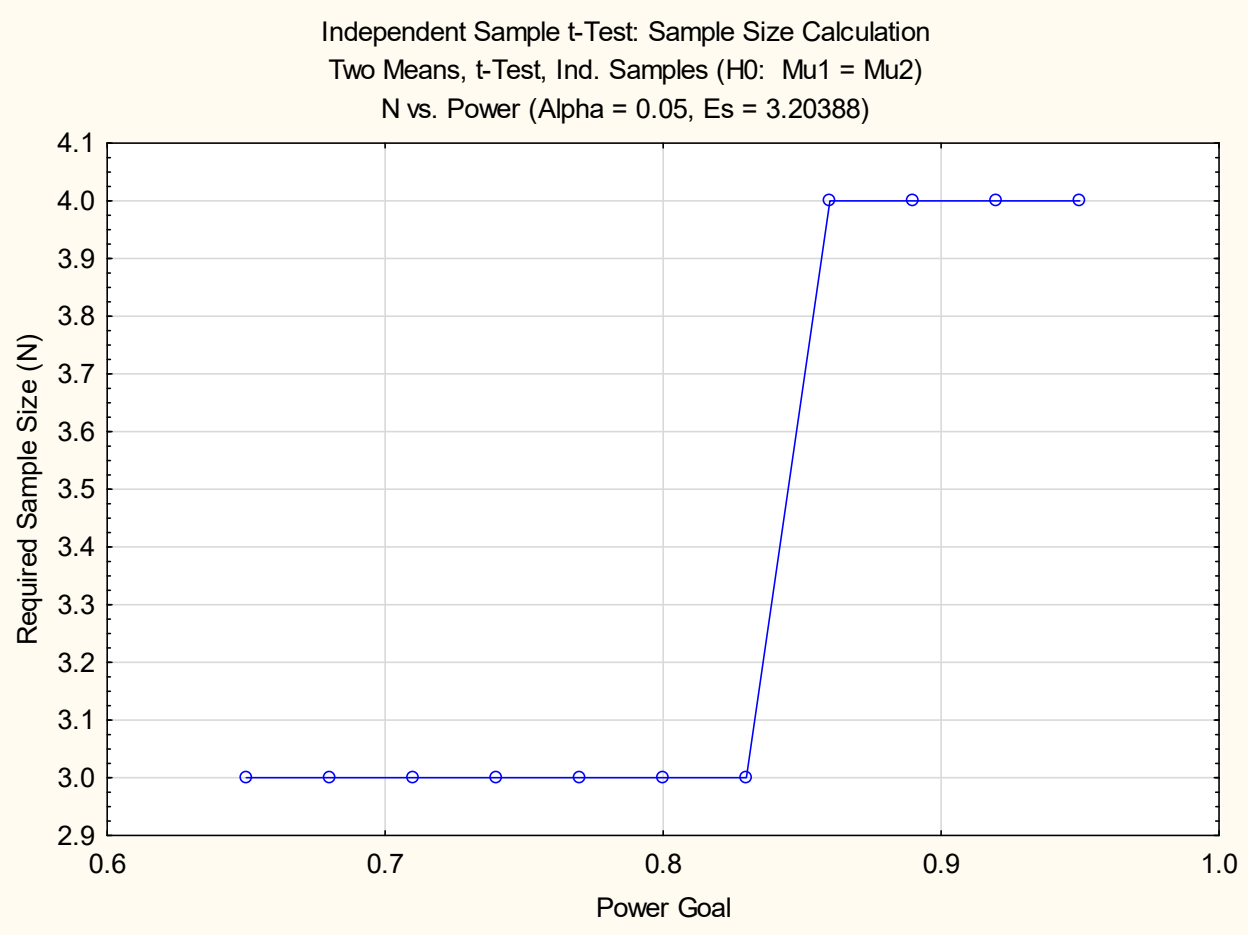
CD8 Control limb No Tx – primary anybody conc. 6.3 µg/µl



CD8 Fractured limb No Tx – primary anybody conc. 6.3 µg/µl

Supplement S3.

These findings are valuable in powering future studies to identify immune cell signatures following articular fracture. Based on these data, we determined that a sample size of three synovial fluid samples per group, each sample representing a pool of three animals for a total of 9 mice required, would be needed for 80% power to detect a 20% difference in cell subsets at alpha=0.05.



Supplemental Table S1.

Group	Time post-fx (days)	SF (n)	Pooled SF Fx Limb B cells (%)	Pooled SF Fx Limb T cells (%)	Pooled SF Fx Limb Non-B&T cells (%)	Pooled SF Fx Limb NK cells (%)	Pooled SF Fx Limb Dendritic cells (%)	Pooled SF Fx Limb Macrophages (%)	Pooled SF Fx Limb Monocytes (%)	WB (n)	WB B cells (%)	WB T cells (%)	WB Non-B&T cells (%)	WB NK cells (%)	WB Dendritic cells (%)	WB Macrophages (%)	WB Monocytes (%)
No Tx	7	n=1 (3 pooled)	34.7%	13.2%	38.9%	10.9%	18.6%	30.6%	3.83%	n=3	55.0% ± 6.3%	25.3% ± 4.6%	16.5% ± 2.7%	34.5% ± 12.4%	7.5% ± 4.0%	6.0% ± 2.2%	2.6% ± 1.0%
No Tx	7																
No Tx	7																
IL-1Ra	7	n=1 (3 pooled)	28.1%	16.3%	41.8%	11.1%	18.4%	34.5%	0.45%	n=3	54.9% ± 6.6%	20.9% ± 4.1%	20.7% ± 4.3%	35.3% ± 3.5%	3.6% ± 1.0%	5.4% ± 2.7%	2.6% ± 0.5%
IL-1Ra	7																
IL-1Ra	7																
										Day 7 Treatment p-value	p=0.83	p=0.28	p=0.13	p=0.83	p=0.28	p=0.83	p=0.51
No Tx	14	n=2 (4 pooled per sample)	N/A	N/A		N/A	N/A	N/A	N/A	n=8	60.3% ± 2.1%	23.6% ± 3.1%	11.5% ± 3.3%	54.1% ± 13.3%	13.0% ± 4.1%	12.7% ± 3.9%	5.5% ± 1.2%
No Tx	14																
No Tx	14																
No Tx	14		N/A	N/A		N/A	N/A	N/A	N/A								
No Tx	14																
IL-1Ra	14	n=2 (4 pooled per sample)	N/A	N/A		N/A	N/A	N/A	N/A	n=8	57.8% ± 6.3%	25.7% ± 7.7%	11.6% ± 3.8%	62.9% ± 13.2%	13.4% ± 4.5%	13.4% ± 5.9%	5.3% ± 2.9%
IL-1Ra	14																
IL-1Ra	14																
IL-1Ra	14		N/A	N/A		N/A	N/A	N/A	N/A								
IL-1Ra	14																
IL-1Ra	14																
										Day 14 Treatment p-value	p=0.60	p=0.67	p=0.96	p=0.11	p=0.92	p=0.83	p=0.34
										No Tx Day 7 vs 14 p-value	p=0.15	p=0.31	p=0.07	p=0.04	p=0.15	p=0.02	p=0.02
										IL-1Ra Day 7 vs 14 p-value	p=0.54	p=0.41	p=0.02	p=0.01	p=0.01	p=0.01	p=0.02

Table S1. Frequencies of immune cells in mouse knee-derived synovial fluid (SF) and whole blood (WB) following articular fracture. Comparison of immune cells subsets in synovial fluid and whole blood (mean ± st dev) with and without intra-articular IL-1Ra treatment (Tx) gated on CD45+ for T cells and B cells and Non-B and T cells for remaining cell subsets; N/A indicates cell count, as determined by detectable events, was too low for subset analysis. P-values reported for Mann-Whitney U test between treatment groups and time, independently.