Supplementary data

The antibodies and panel design used in this study. A panel consisted of 1 or more staining tubes

Panel Staining tube Cell target	Conjugate	Titer	Stock (µg/µL)	Clone	Company
Lymphocytes					
Staining tube 1					
T-cells	CD3-PE	1:200	0.2	17A2	BioLegend
T-killer	CD8-PacOr	1:100	0.1	5H10	Life Technologies
Myeloid cells	Gr-1-PECy5	1:640	0.2	RB6-8C5	BioLegend
Signaling	CD200-APC	1:100	0.2	OX-90	BioLegend
Leukocytes	CD45-FITC	1:200	0.5	30-F11	BioLegend
B-cells	CD19-APC-Cy7	1:100	0.2	6D5	BioLegend
Staining tube 2					
Activated T-helper	CD25-PECy7	1:200	0.2	PC61	BioLegend
T-cells	CD3-V500	1:200	0.2	500A2	BD
T-helper	CD4-PERCPCy5.5	1:200	0.2	RM4-5	BioLegend
Leukocytes	CD45-APC	1:100	0.2	30-F11	BioLegend
NK-cell	NK1.1-FITC	1:400	0.5	PK136	BioLegend
Macrophages					
Staining tube 1					
Leukocytes	CD45-APC	1:100	0.2	30-F11	BioLegend
T-cells	CD3-PE	1:200	0.2	17A2	BioLegend
Myeloid cells	CD11b-V500	1:200	0.2	M1/70	BD
M1	CCR7-PERCPCy5.5	1:20	0.5	4B12	BioLegend
CD200 receptor	CD200R-FITC	1:50	0.5	OX-110	BioLegend
Staining tube 2					
Leukocytes	CD45-FITC	1:200	0.5	30-F11	BioLegend
B-cells	CD19-APCCy7	1:100	0.2	6D5	BioLegend
M2	CD206-AF467	1:100	0.5	C068C2	BioLegend
Myeloid cells	CD11b-V500	1:200	0.2	M1/70	BD
Myeolid cells	Gr-1-PacOr	1:320	0.1	RB6-8C5	Life Technologies
Staining tube 3					
Leukocytes	CD45-FITC	1:200	0.5	30-F11	BioLegend
Myeloid cells	CD11b-V500	1:200	0.2	M1/70	BD
Monocytes	CD14-APC-Cy7	1:40	0.2	Sa14-2	BioLegend
M1	CD86-PE-Cy5	1:100	0.2	GL-1	BioLegend
Sham					
Staining tube 1					
Leukocytes	CD45-FITC	1:200	0.5	30-F11	BioLegend
T-cells	CD3-PE	1:200	0.2	17A2	BioLegend
Myeloid cells	CD11b-V500	1:200	0.2	M1/70	BD
T-helper	CD4-PERCPCy5.5	1:200	0.2	RM4-5	BioLegend
B-cells	CD19-PE-Cy5	1:100	0.2	RM4-5	BioLegend
T-killer	CD8-PacOr	1:100	0.1	5H10	Life Technologies
Monocytes	CD14-APC-Cy7	1:40	0.2	Sa14-2	BioLegend
NK-cell	NK1.1-AF700	1:400	0.5	PK136	BioLegend

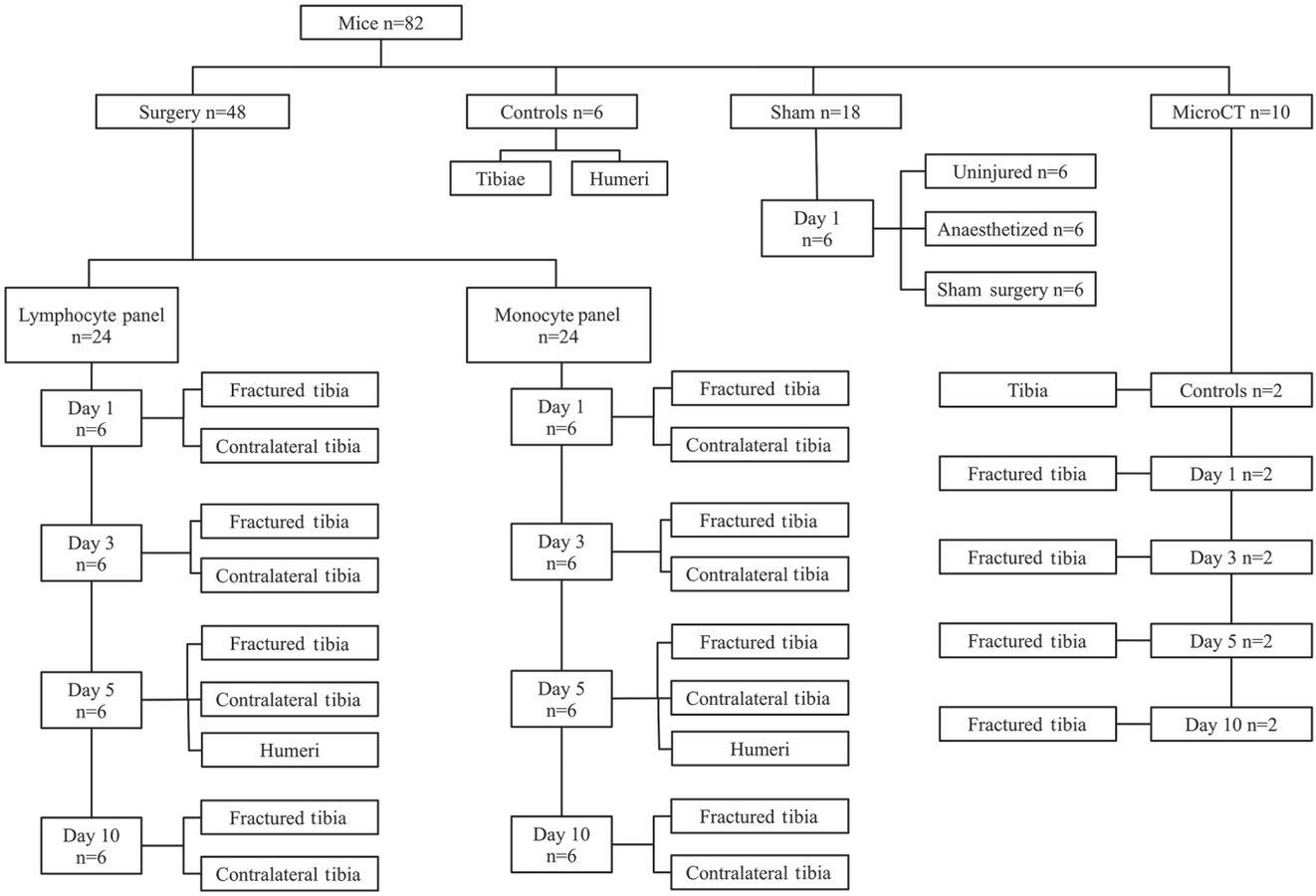
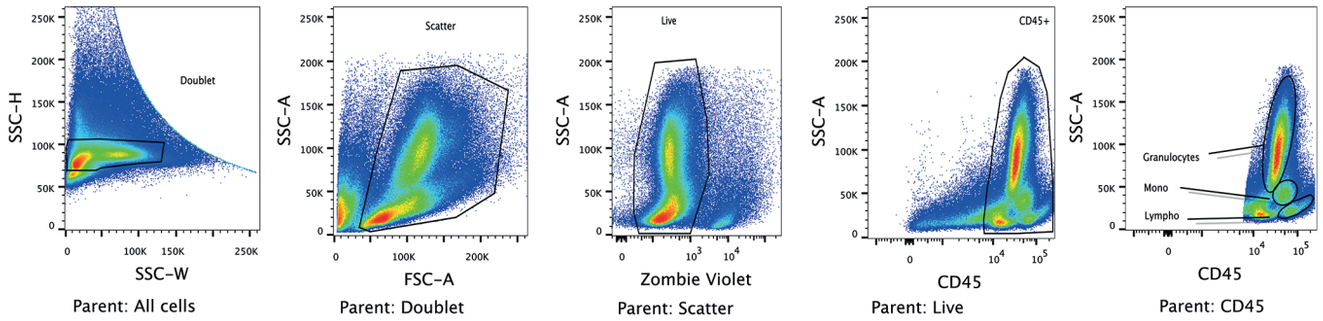
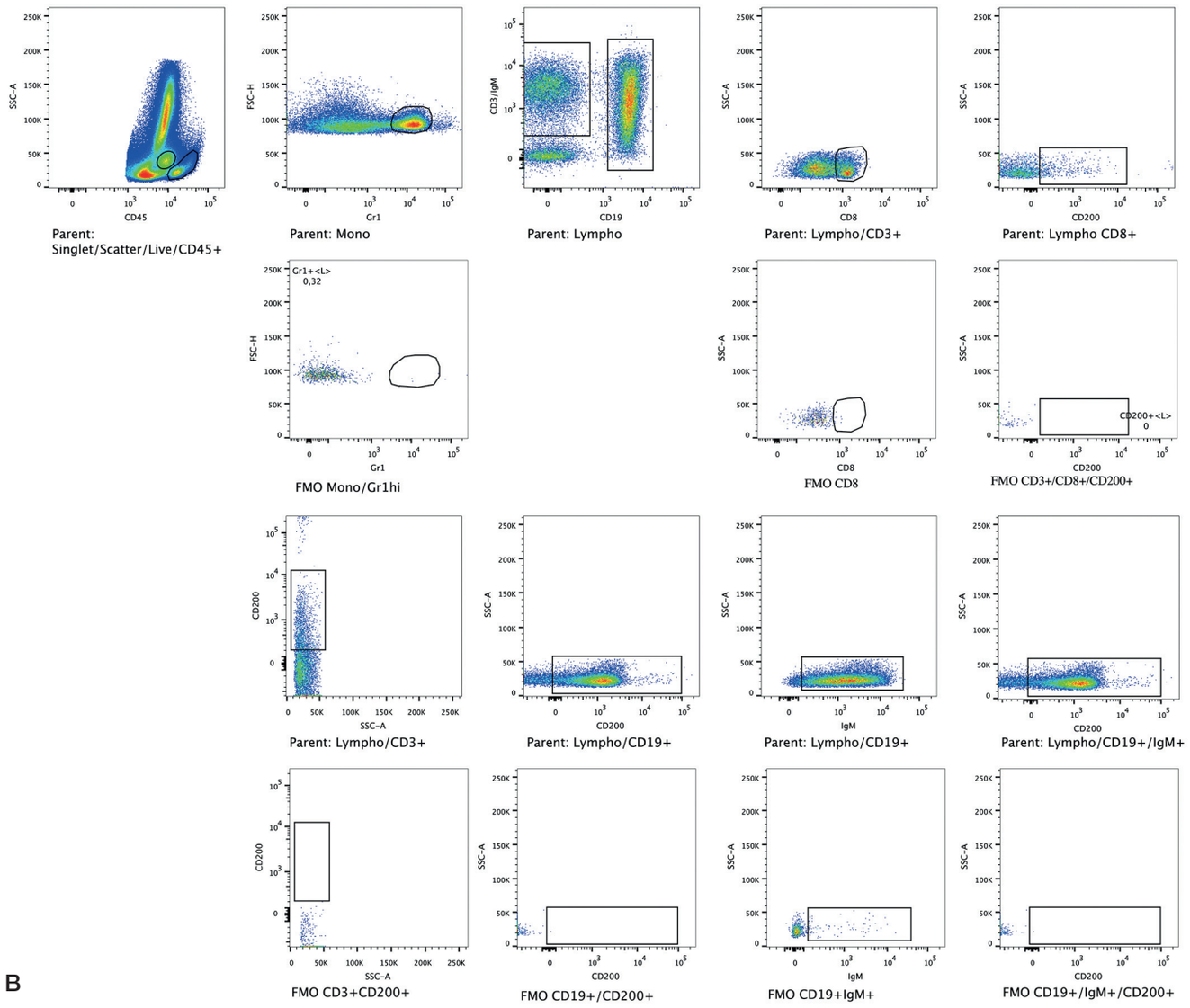


Figure S1. Flow diagram of study design. The panels refer to panels of antibodies as disclosed in Table 1.

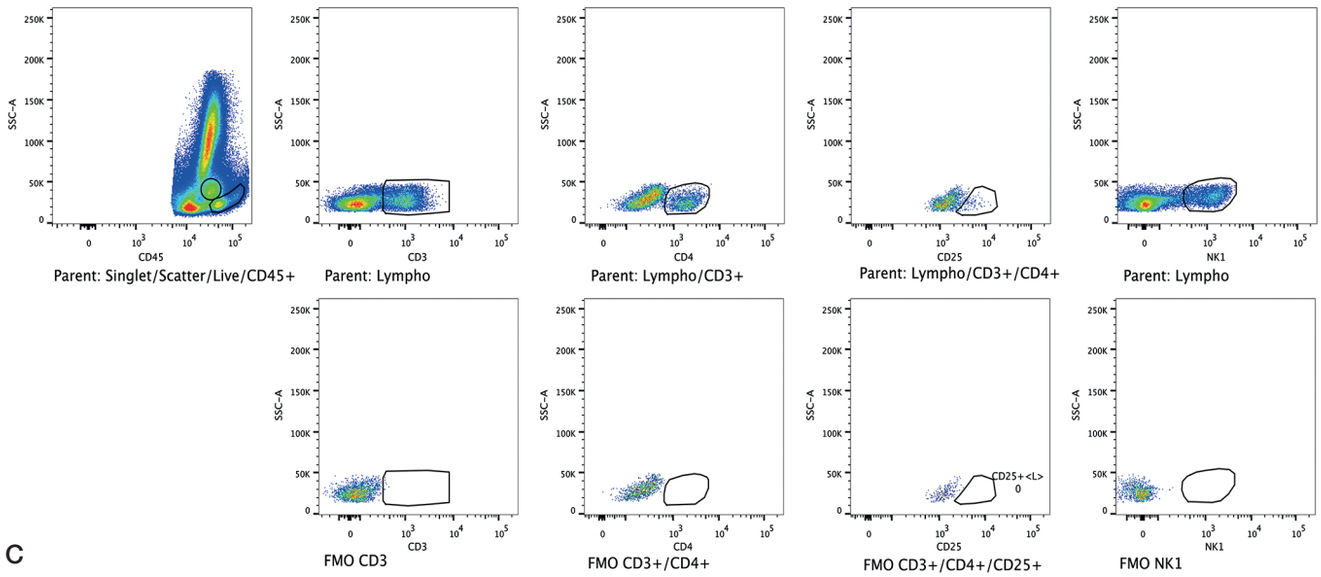

Doublet
SSC-H
SSC-W
Parent: All cells
Scatter
SSC-A
FSC-A
Parent: Doublet
Live
SSC-A
Zombie Violet
Parent: Scatter
CD45+
SSC-A
CD45
Parent: Live
Granulocytes
Mono
Lympho
SSC-A
CD45
Parent: CD45

A

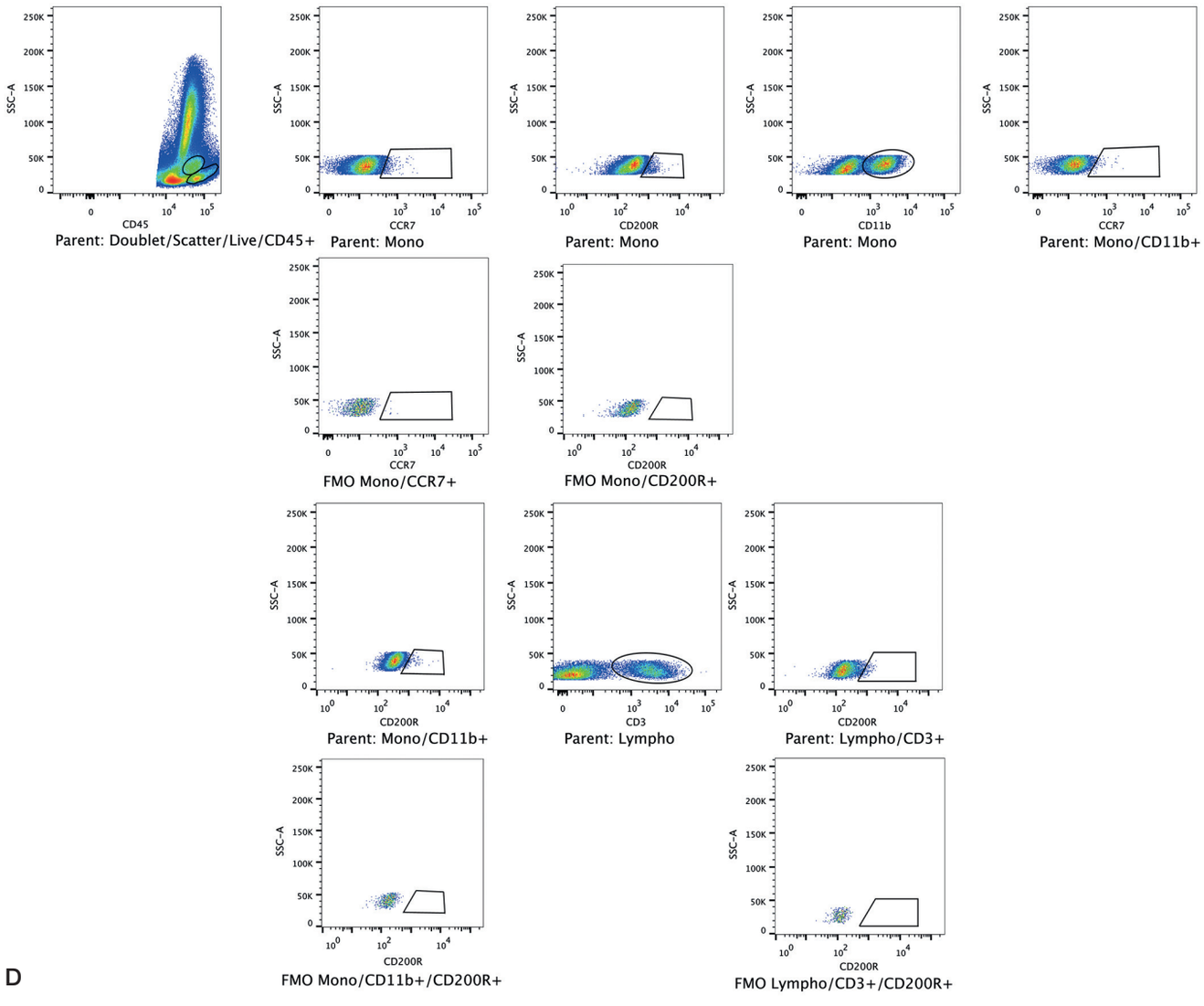

SSC-A
CD45
Parent: Singlet/Scatter/Live/CD45+
FSC-H
Gr1
Parent: Mono
CD3/IgM
CD19
Parent: Lympho
SSC-A
CD8
Parent: Lympho/CD3+
SSC-A
CD200
Parent: Lympho CD8+
Gr1+<L>
0,32
FSC-H
Gr1
FMO Mono/Gr1hi
SSC-A
CD8
FMO CD8
SSC-A
CD200+<L>
0
CD200
FMO CD3+/CD8+/CD200+
CD200
SSC-A
Parent: Lympho/CD3+
SSC-A
CD200
Parent: Lympho/CD19+
SSC-A
IgM
Parent: Lympho/CD19+
SSC-A
CD200
Parent: Lympho/CD19+/IgM+
CD200
SSC-A
FMO CD3+CD200+
SSC-A
CD200
FMO CD19+/CD200+
SSC-A
IgM
FMO CD19+IgM+
SSC-A
CD200
FMO CD19+/IgM+/CD200+

B

C


SSC-A
CD45
Parent: Singlet/Scatter/Live/CD45+
CD3
Parent: Lympho
CD4
Parent: Lympho/CD3+
CD25
Parent: Lympho/CD3+/CD4+
NK1
Parent: Lympho
FMO CD3
FMO CD3+/CD4+
CD25+ <L>
0
FMO CD3+/CD4+/CD25+
FMO NK1

D


SSC-A
CD45
Parent: Doublet/Scatter/Live/CD45+
CCR7
Parent: Mono
CD200R
Parent: Mono
CD11b
Parent: Mono
CCR7
Parent: Mono/CD11b+
FMO Mono/CCR7+
FMO Mono/CD200R+
CD200R
Parent: Mono/CD11b+
CD3
Parent: Lympho
CD200R
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CD200R
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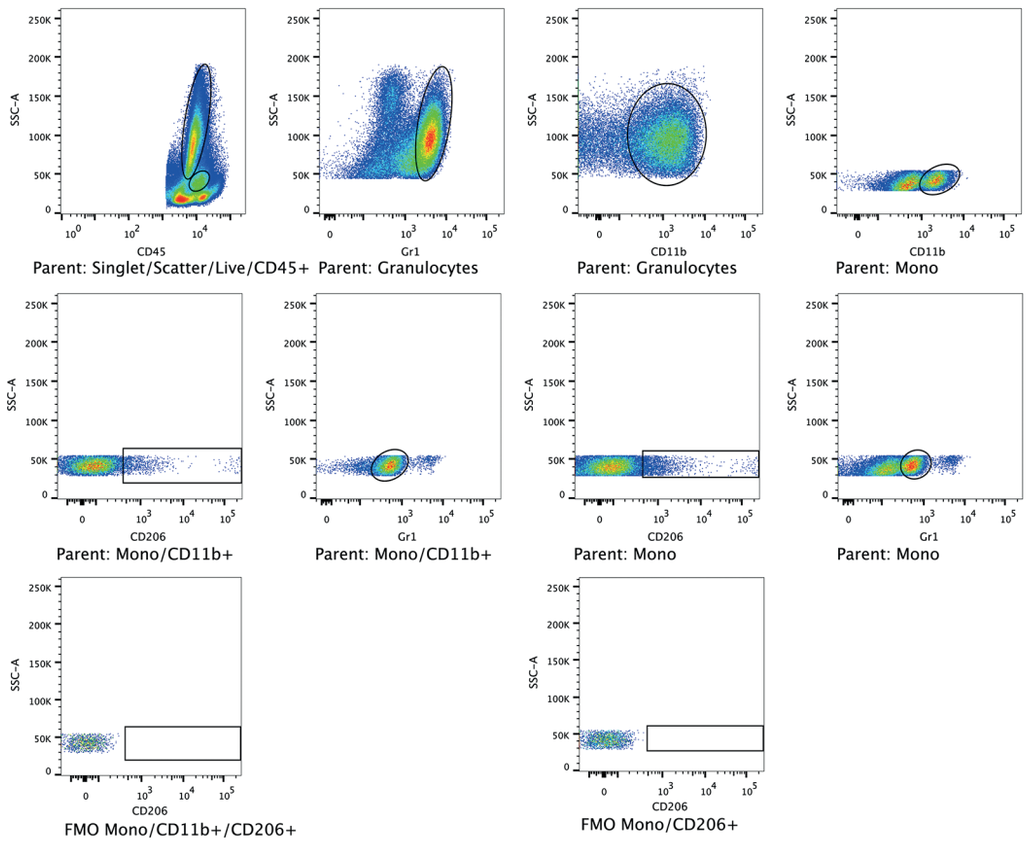
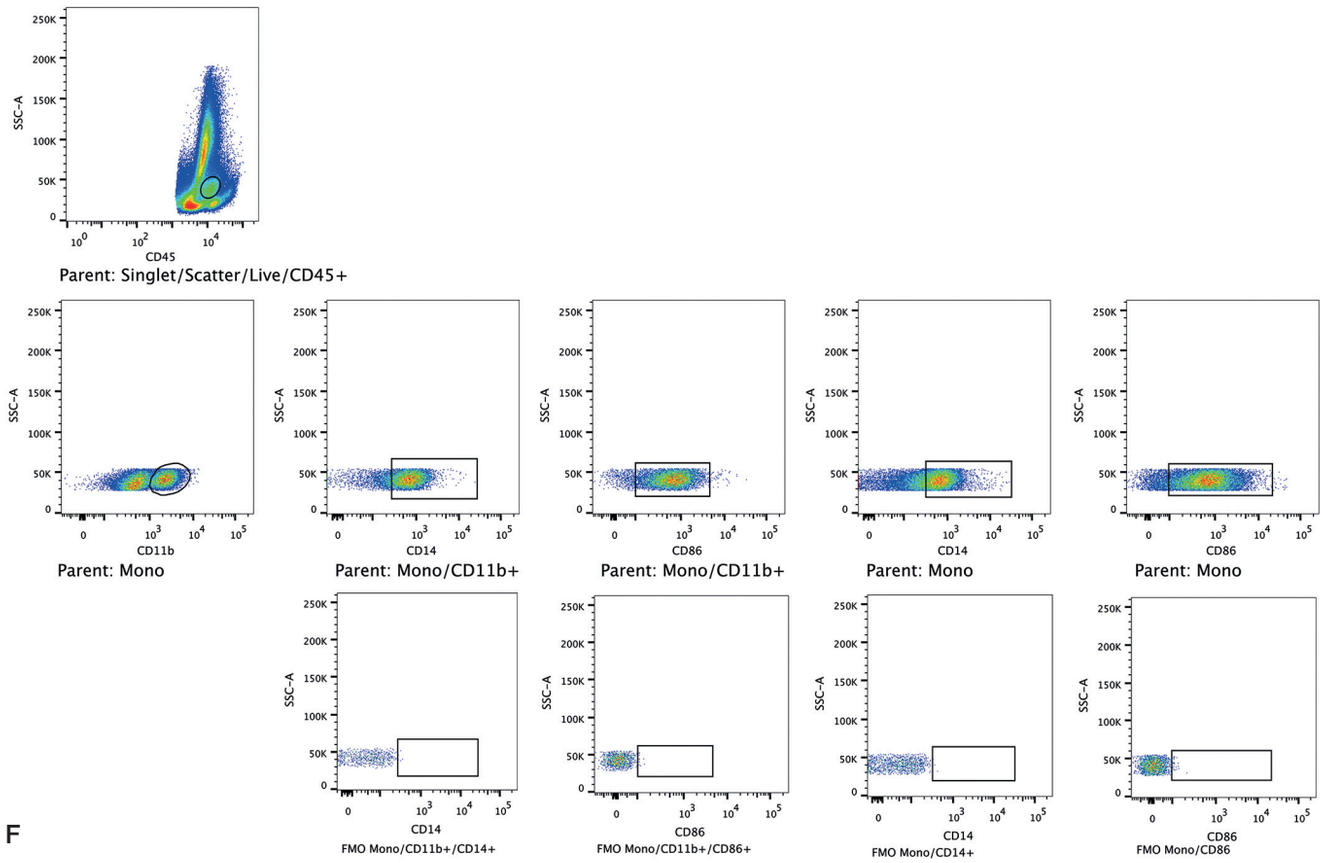
CD200R
FMO Lympho/CD3+/CD200R+

E


SSC-A
CD45
Parent: Singlet/Scatter/Live/CD45+
Gr1
Parent: Granulocytes
CD11b
Parent: Granulocytes
CD11b
Parent: Mono
CD206
Parent: Mono/CD11b+
Gr1
Parent: Mono/CD11b+
CD206
Parent: Mono
Gr1
Parent: Mono
CD206
FMO Mono/CD11b+/CD206+
CD206
FMO Mono/CD206+



F

Figure S2 A-F. Dot plots from one tissue sample from an uninjured mouse. The staining design included five different staining tubes as can be seen in the table of antibodies and the flow diagram. Beneath the sample dot plots are the corresponding FMOs A) All samples were first gated for singlet cells, scatter gate and living cells. This approach was the same for all samples. B) Lymphocyte panel, staining tube 1. C) Lymphocyte panel, staining tube 2. D) Monocyte panel, staining tube 1. E) Monocyte panel, staining tube 2. F) Monocyte panel, staining tube 3.

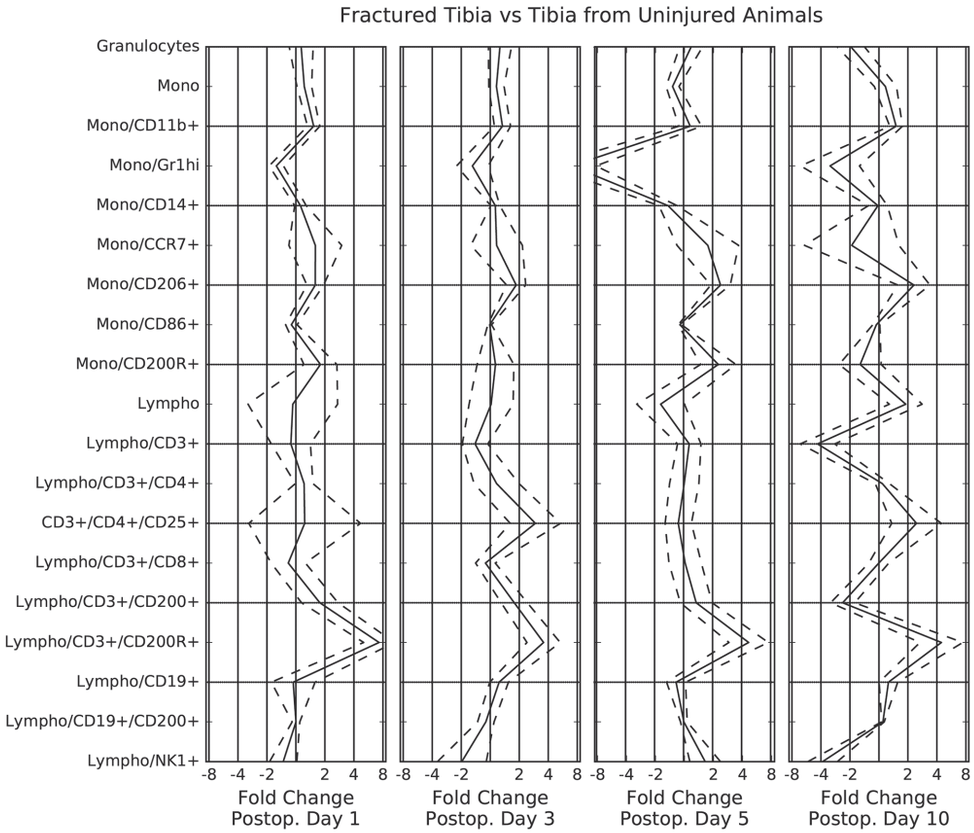


Figure S3. Same as figure 5 but with Bonferroni correction, meaning that the confidence intervals covers 99.8 % of the probability distribution of the mean.

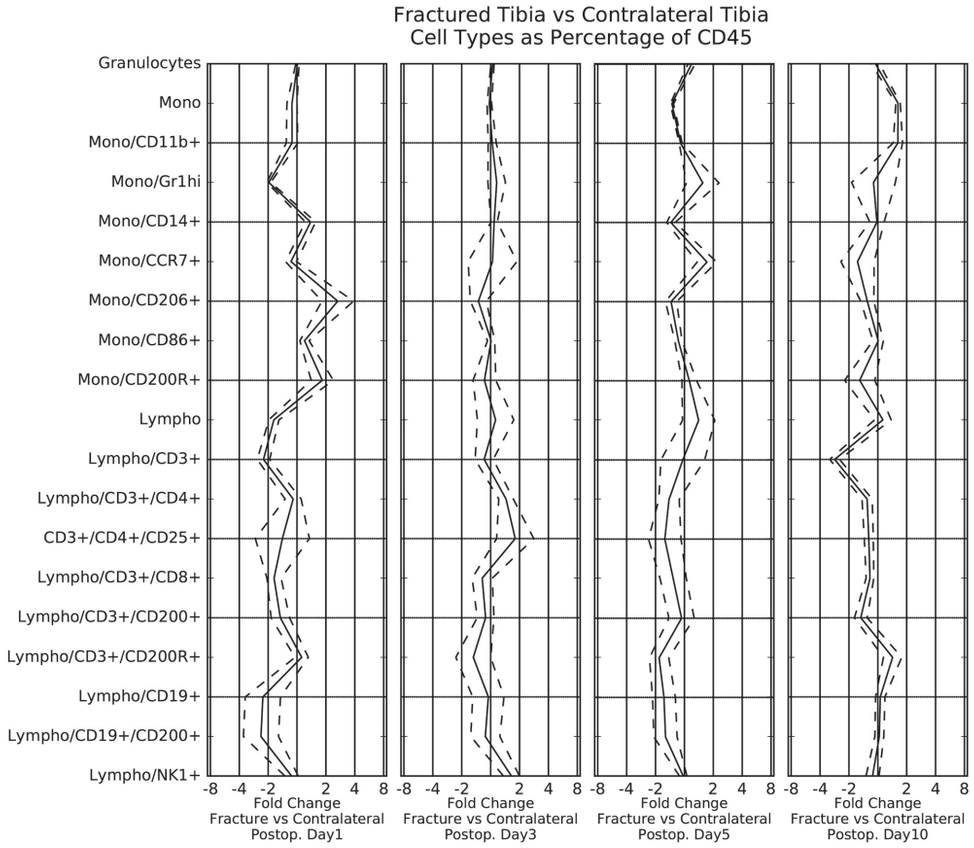


Figure S4. Injured tibia vs contralateral tibia with cell types expressed as percentage of CD45+ cell count. The straight line marks the mean value of six observations from each mouse, and the dashed lines mark the 95% confidence interval.

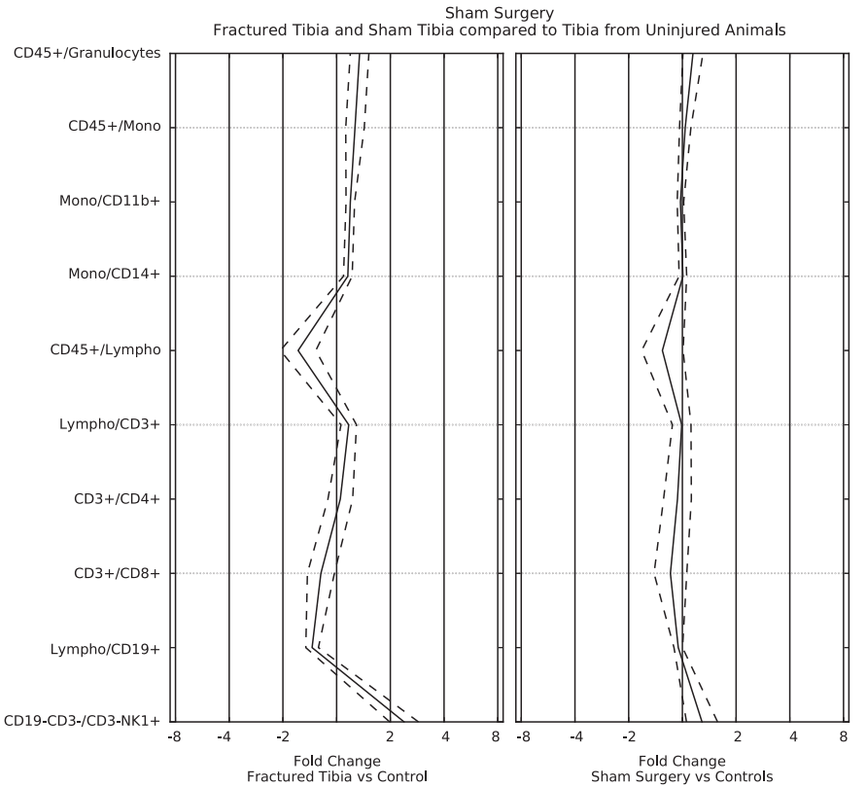


Figure S5. Effect of sham surgery on immune populations 5 days after surgery. A smaller panel was used than in other experiments, but all major cell populations were studied.