Supplementary Figures

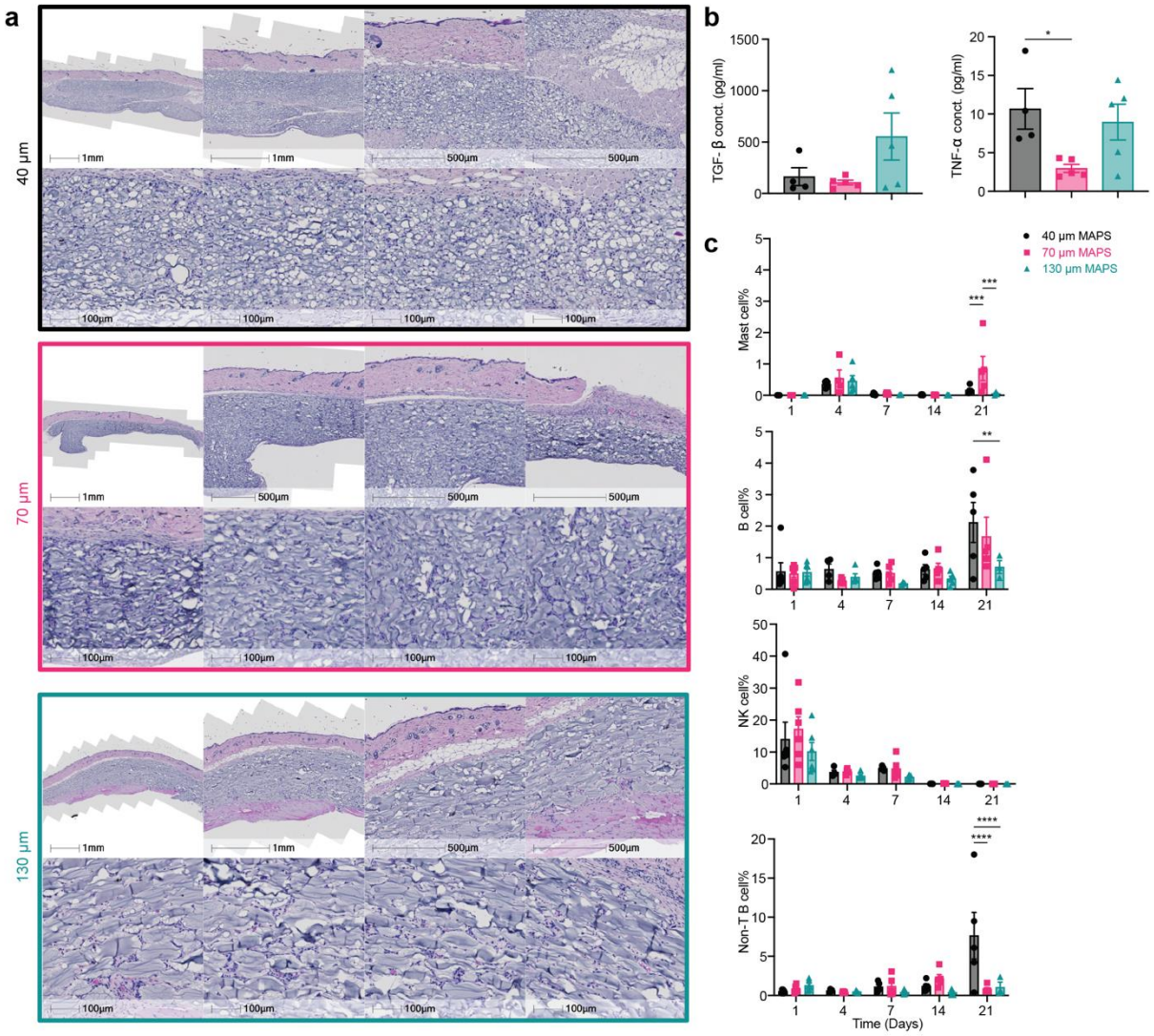


Figure S1. 40 µm, 70 µm, and 130 µm MAP scaffolds showed a difference in immune cell recruitment and FBR in the subcutaneous implantation model. a, representative pictures of Hematoxylin and eosin (H&E) staining on day 21. The top row in each panel from left to right showed pictures with objectives of 1x and 2x (implant overview, scale bar, 1 mm), 5x (skin/dorsal interface, scale bar, 500 µm), 5x (capsule/ventral interface, scale bar, 500 µm). The bottom row in each panel showed representative pictures inside the implant with objectives of 10x. b, ELISA results of selected cytokine concentrations inside the hydrogel implants. c, mast cell, B cell, NK cell, non-T/B cell percentages among all CD45+ live cells from flow cytometry. Statistical analysis: two-way ANOVA with Šídák's multiple comparisons test made between treatment groups only when there was a significance in the interaction term of treatment type x time. ∗ $p < 0.05$, ∗∗ $p < 0.001$, ∗∗∗ $p < 0.001$, ∗∗∗∗ $p < 0.0001$. Error bars, mean ± s.e.m., n = 5 mice per group with some data points removed due to experimental reasons.

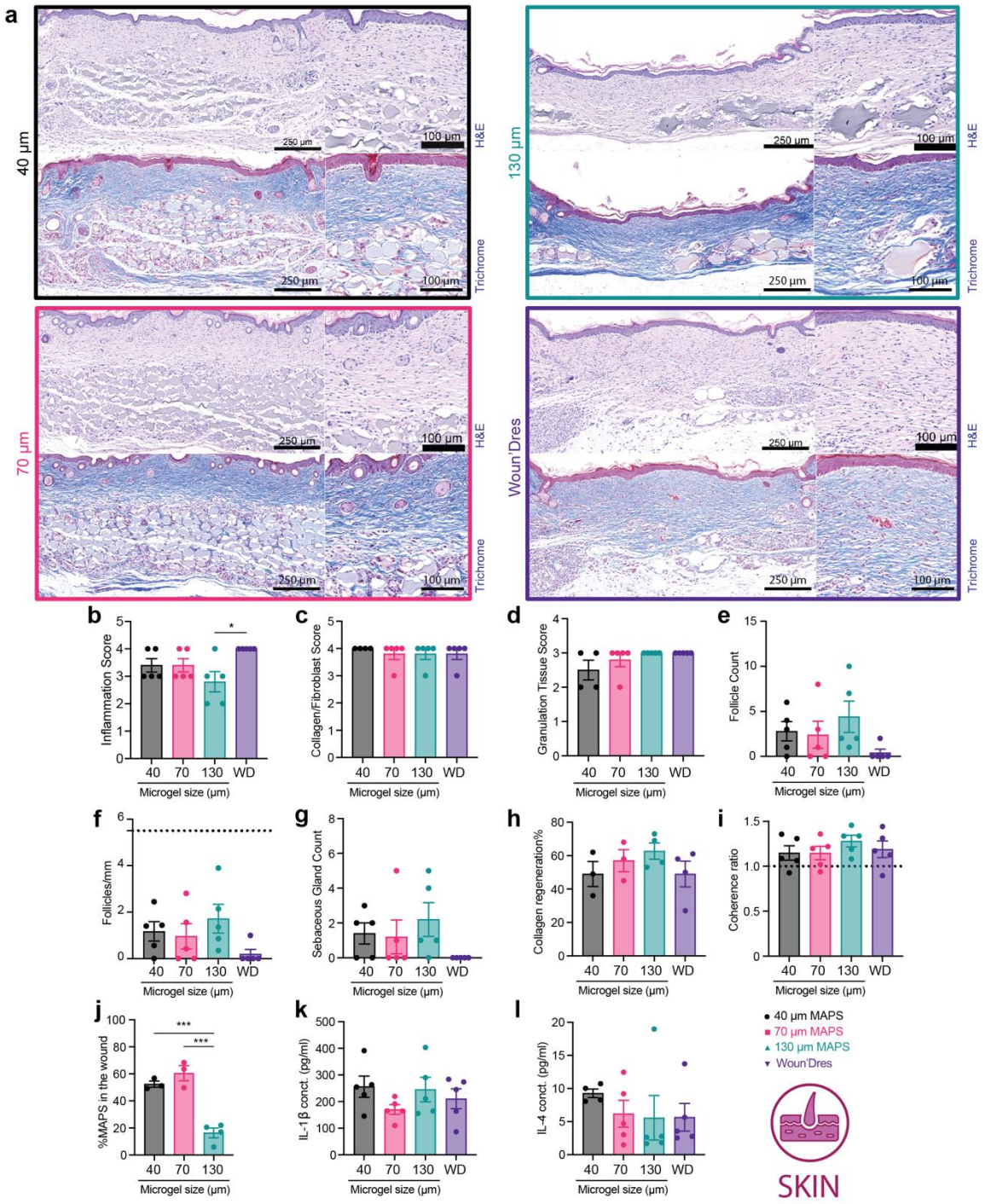


Figure S2. 130 µm MAP scaffolds induced mature collagen regeneration and reduced inflammation level in the skin wound. a, representative pictures of 21-day skin wound samples with H&E staining and Masson's trichrome staining (left panel, full wound and surrounding skin, scale bar, 250 µm; right panel, zoom-in picture at the wound site, scale bar, 100 µm). b-d, histologic assessment of inflammation, collagen/fibroblast score and granulation tissue. e, the cell nucleus area in the connective tissue. The dotted line and the grey area stand for the average number and the range for normal skin. f, the cell nucleus area in the remaining biomaterials. The

dotted line and the grey area stand for the average number and the range for normal skin. g-i, histologic quantification of follicle count, follicles/mm, and sebaceous gland count. The gray line stands for the value in normal skin. j, collagen regeneration percentage compared to the normal skin. k, the coherence ratio in collagen fibers. The dotted line stands for the level of normal skin. l, the remaining MAP scaffolds amount in the wound relative to the size of the wound. m-n, ELISA results of IL1-β and IL-4 concentrations in the MAP-treated wounds on day 21. Statistical analysis: two-way ANOVA with Šídák's multiple comparisons test made between treatment groups only when there was a significance in the interaction term of treatment type x time. Dunnet method was used to compare each treatment against normal skin baseline (gray pond). */# $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$, **** $p < 0.0001$. Error bars, mean ± s.e.m., n = 5 mice per group with some data points removed due to experimental reasons.

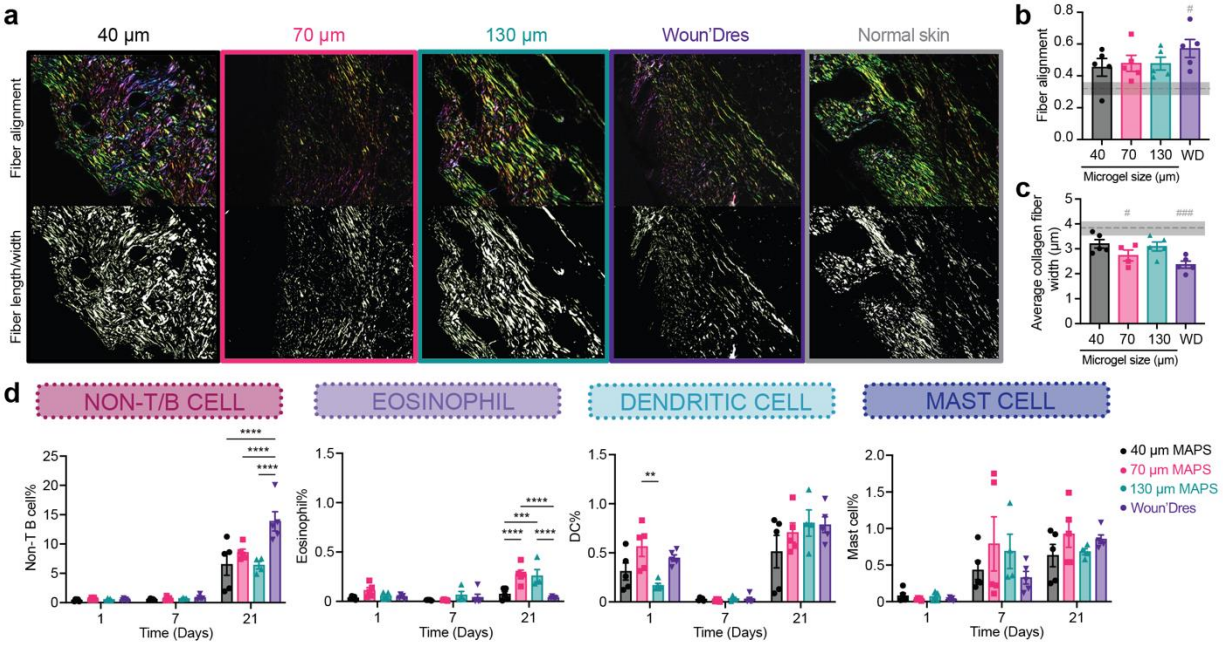


Figure S3. 130 μm MAP scaffolds induced mature collagen regeneration, and immune cell recruitment followed a size-dependent manner in the skin wound. a, representative pictures of 21-day skin wound samples with Picro-Sirius Red staining (top row, fiber alignment analysis; bottom row, fiber length/width analysis). b, fiber alignment score (calculated by ImageJ software) in the regions of interest. The dotted line and the grey area stand for the average number and the range for normal skin. c, average width of collagen fibers in the regions of interest. The dotted line and the grey area stand for the average number and the range for normal skin. d, non-T/B cell, eosinophil, dendritic cell, mast cell percentages among all CD45+ live cells from flow cytometry. Statistical analysis: two-way ANOVA with Šídák's multiple comparisons test made between 40 μm, 70 μm, and 130 μm MAP scaffolds and wound dressing groups only when there was a significance in the interaction term of scaffold type x time. ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Error bars, mean ± s.e.m., n = 5 mice per group.

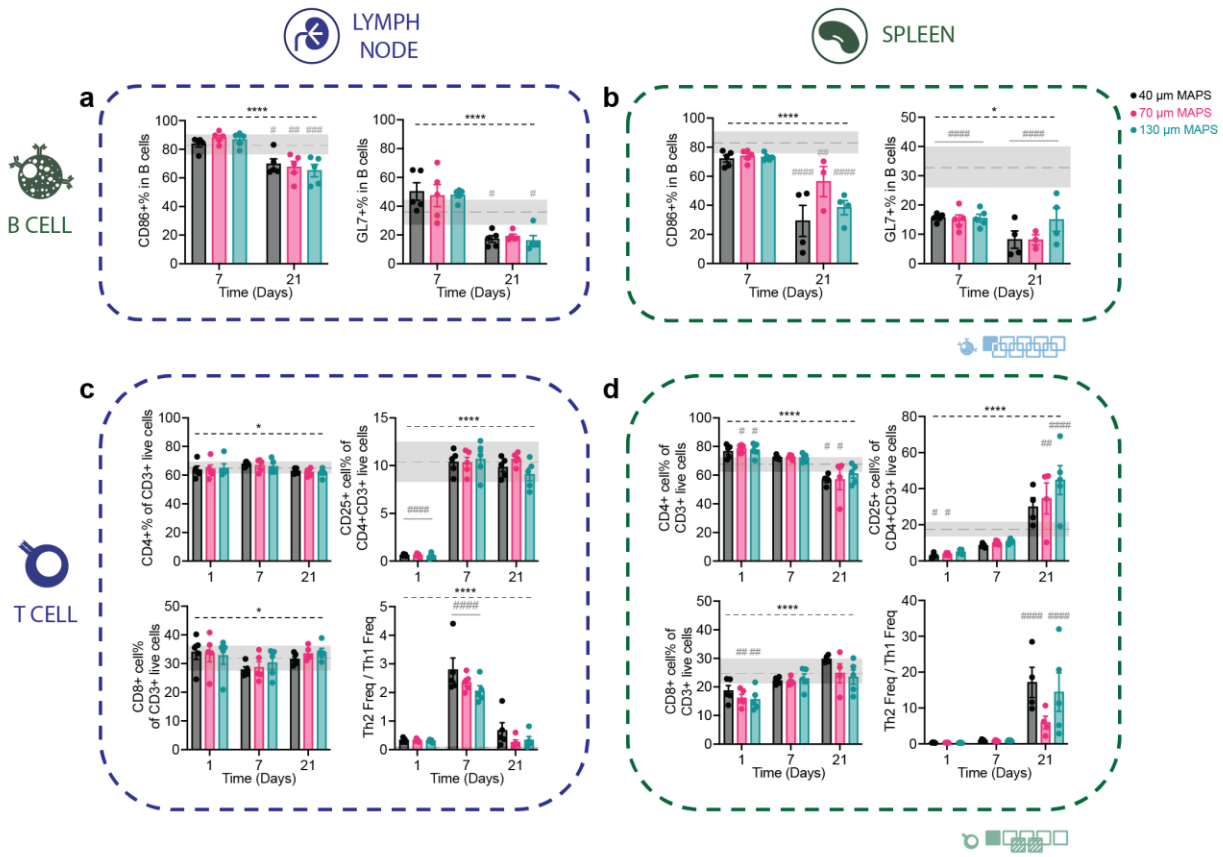


Figure S4. MAP scaffolds treatment induced phenotype switches in both B cell and T cell populations compared to baseline in draining lymph nodes and spleen of mice. a and b, B cell profiles in draining lymph nodes and spleen of mice treated with 40 µm, 70 µm, and 130 µm MAP scaffolds across 3 time points. c and d, T cell profiles in draining lymph nodes and spleen of mice treated with 40 µm, 70 µm, and 130 µm MAP scaffolds across 3 time points. Statistical analysis: two-way ANOVA with Šídák's multiple comparisons test made between 40 µm, 70 µm, and 130 µm MAP scaffolds groups only when there was a significance in the interaction term of scaffold type x time. After a two-way ANOVA, Dunnet method was used to compare the experiment groups with the baseline control group (mice without wounding). */# $p<0.05$, **/## $p<0.01$, ***/### $p<0.001$, ****/#### $p<0.0001$. Asterisks with solid line stand for comparisons between MAP scaffolds. Asterisks with dash line stand for significant difference in time. Error bars, mean ± s.e.m., n = 5 mice per group but with some data points removed due to experimental reasons. The blue symbol beneath panel b stands for the 9-color B panel used in panel a, b of this figure. The green symbol beneath panel d stands for the 7-color T cell panel used in panel c, d of this figure.

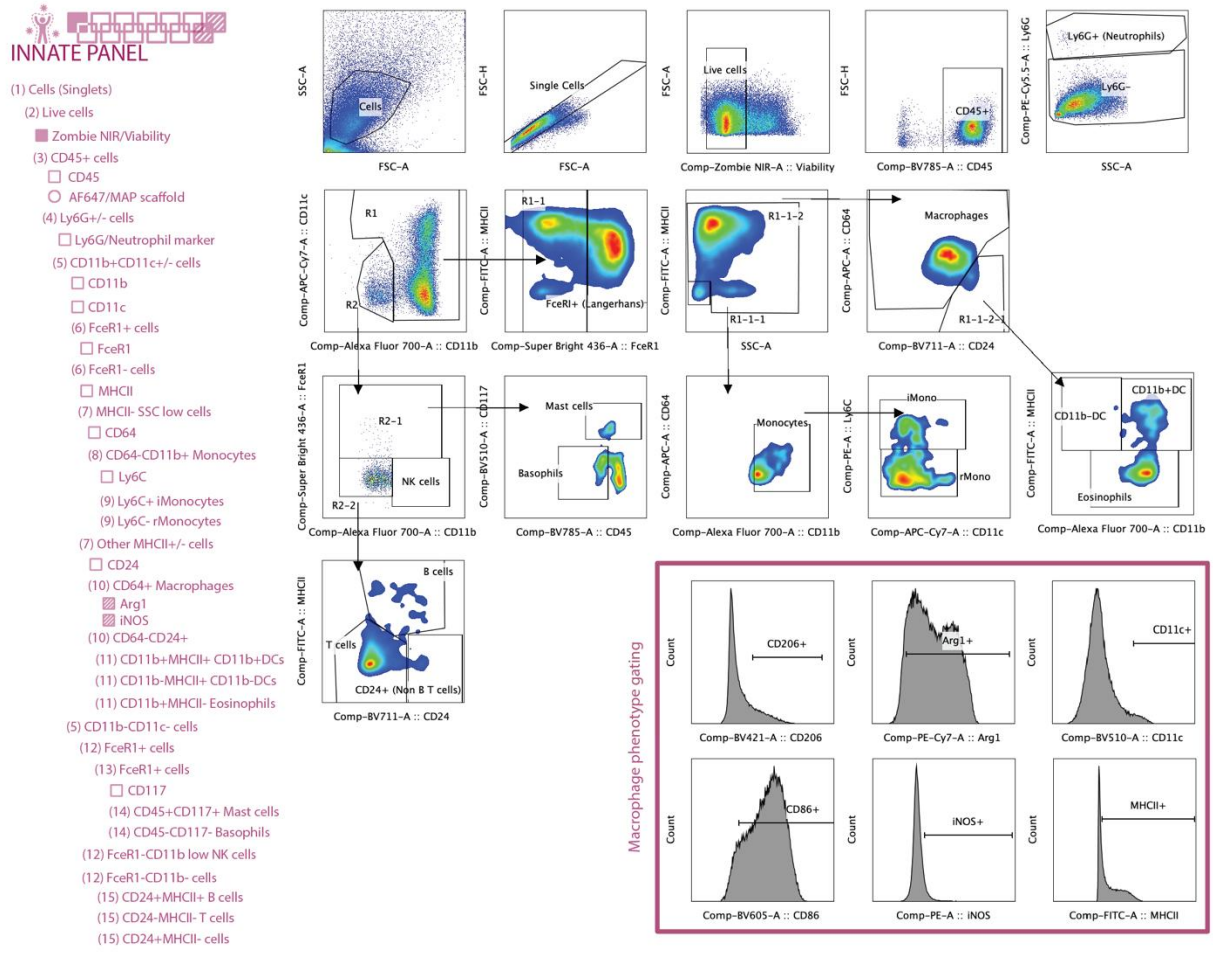


Figure S5. Gating strategy for flow cytometry analysis on myeloid cells with 13 markers.

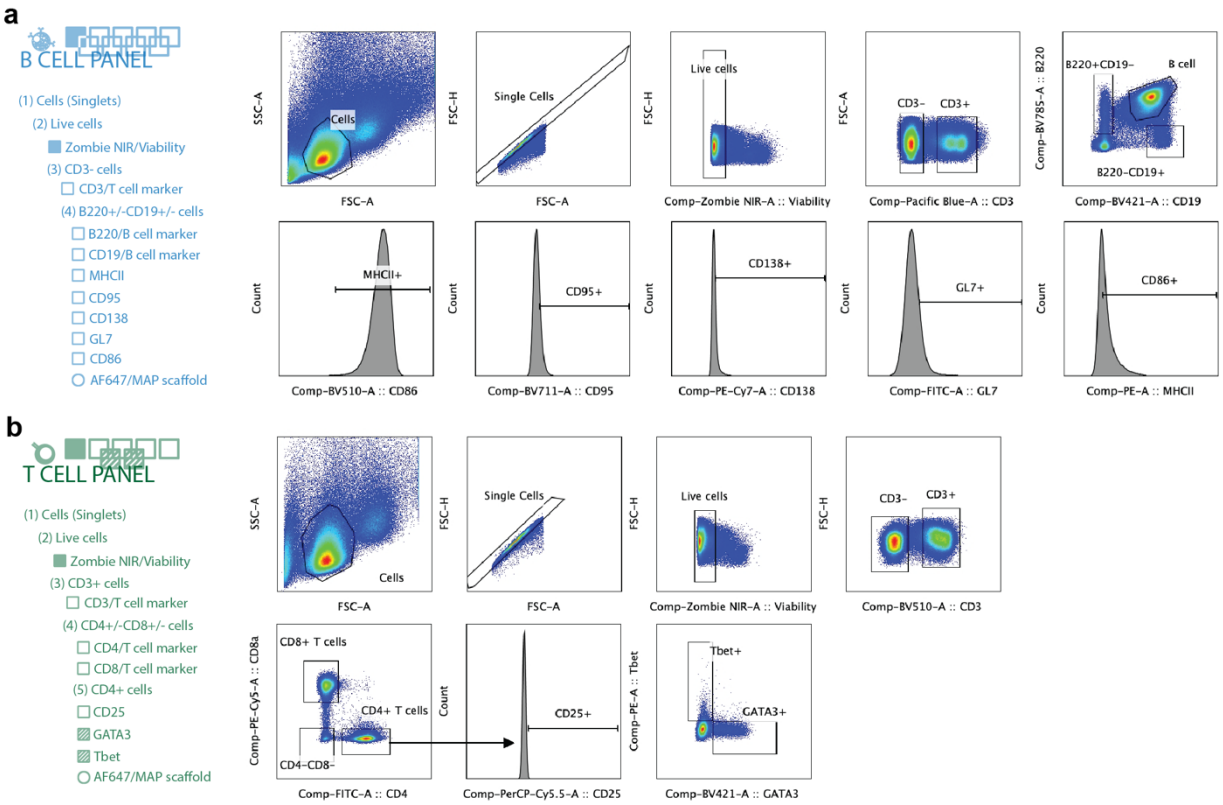


Figure S6. Gating strategy for flow cytometry analysis on: a) B cells with 9 markers. b) T cells with 7 markers.

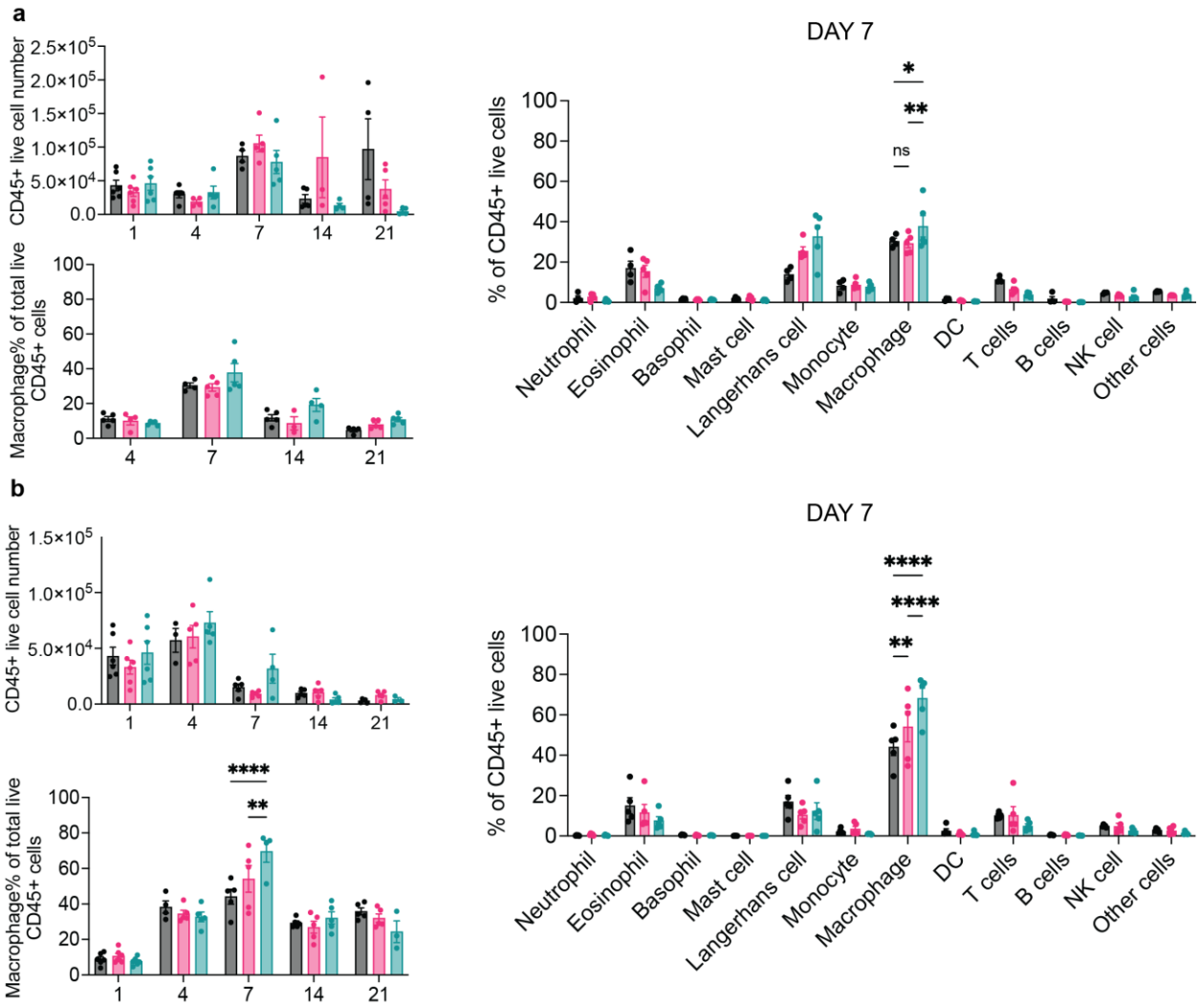


Figure S7. Immune cell recruitment and response followed a size-dependent manner in Subcutaneous model on independent experiments across time. A) Additional experiment B) Experiment use in the manuscript. Total number of live immune cells (Zombie NIR- CD45+), macrophages across five time points and myeloid cell abundancy at day 7 for 40 µm, 70 µm, and 130 µm MAP scaffolds. Statistical analysis: two-way ANOVA with Šídák's multiple comparisons test made between 40 µm, 70 µm, and 130 µm MAP scaffolds and wound dressing groups only when there was a significance in the interaction term of scaffold type x time. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Error bars, mean ± s.e.m., n = 5 mice per group.

Table S1. Scoring of Histological Sections for Total Wound Healing

Score	Criteria
1-3	None to minimal cell accumulation. No granulation tissue or epithelial travel.
4-6	Thin, immature granulation that is dominated by inflammatory cells but has few fibroblasts, capillaries, or collagen deposition. Minimal epithelial migration.
7-9	Moderately thick granulation tissue can range from being dominated by inflammatory cells to more fibroblasts and collagen deposition. Extensive neovascularization. Epithelium can range from minimal to moderate migration.

10-12	Thick, vascular granulation tissue dominated by fibroblasts and extensive collagen deposition. Epithelium partially to completely covering the wound.

Table S2. Scoring of Epidermis/re-epithelialization

Score	Criteria
0	No migration.
1	Minimal re-epithelialization (<10%).
2	Partial re-epithelialization (incomplete closure).
3	Complete re-epithelization without keratin layer formation.
4	Complete/thick re-epithelialization with keratin layer formation.

Table S3. Scoring of Granulation Tissue/Vascularization

Score	Criteria
0	No granulation tissue.
1	Early granulation tissue, no vascularization.
2	Mature granulation tissue, early vascularization.
3	Mature granulation tissue with mature blood vessel formation.

Table S4. Scoring of Collagen Deposition/Fibroplasia

Score	Criteria
0	No collagen deposition/fibroplasia.
1	Fibroblast proliferation/no collagen deposition.
2	Fibroblast proliferation with minimal collagen deposition.
3	Fibroblast proliferation with extensive haphazard collagen deposition.
4	Extensive organized collagen deposition or complete replacement of dermis with fibrous tissue (mature scar).

Table S5. Scoring of Inflammation

Score	Criteria
0	No inflammatory cells.
1	1-50 leukocytes per high power field.
2	51-100 leukocytes per high power field.
3	101-250 leukocytes per high power field.
4	>250 leukocytes per high power field or microabscesses or abscesses present.

Table S6. A list of antibodies for the innate panel

Marker	**Clone**	**Fluorophore**	**Supplier**	**Cat. #**	**Titration (tissue/cell type)**
FCeR1	MAR-1	PB	BioLegend	134314	2 in 100 (Splenocyte)
CD117	ACK2	PE	BioLegend	135105	1.25 in 100 (Splenocyte)
CD45	30-F11	BV785	BioLegend	103149	0.625 in 100 (Implant)
CD24	M1/69	BV711	BD Biosciences	563450	0.25 in 100 (Implant)
Ly6C	HK1.4	BV510	BioLegend	128033	1.25 in 100 (Splenocyte)
MHCII	M5/114.15.2	SB600	eBioscience	63-5321-82	0.5 in 100 (Splenocyte)

Ly6G	1A8	PE-Cy5	eBioscience	15-9668-82	0.3125 in 100 (Implant)
CD64	X54-5/7.1	PE-Cy7	BioLegend	139313	0.625 in 100 (Implant)
CD11b	M1/70	Alexa Fluor 700	eBioscience	56-0112-80	0.0625 in 100 (Implant)
CD11c	N418	APC-Cy7	BioLegend	117323	0.039 in 100 (Implant)
		Zombie NIR	BioLegend	423105	0.0625 in 100 (Splenocyte)

Table S7. A list of antibodies for the macrophage panel

Marker	Clone	Fluorophore	Supplier	Cat. #	Titration (cell type)
CD206	C068C2	BV421	BioLegend	141717	2 in 100 (Macrophage)
CD11c	N418	BV510	BioLegend	117338	1.25 in 100 (Macrophage)
MHCII	M5/114.15.2	FITC	eBioscience	11-5321-82	0.25 in 100 (Macrophage)
iNOS	CXNFT	PE	eBioscience	12-5920-80	1.5 in 100 (Macrophage)
F4/80	BM8	PerCP-Cy5.5	eBioscience	45-4801-80	0.5 in 100 (Macrophage)
CD86	GL-1	BV605	BioLegend	105037	0.156 in 100 (Macrophage)
Arg1	A1exF5	PE-CY7	eBioscience	17-3697-80	1.5 in 100 (Macrophage)
CD11b	M1/70	Alexa Fluor 700	eBioscience	56-0112-80	0.25 in 100 (Macrophage)
Viability		Zombie NIR	BioLegend	423105	0.0625 in 100 (Macrophage)

Table S8. A list of antibodies for the B cell panel

Marker	Clone	Fluorophore	Supplier	Cat. #	Titration (cell type)
CD3	17A2	PB	BioLegend	100214	0.25 in 100 (Splenocyte)
GL7	GL7 (RUO)	FITC	BD Pharmingen	553666	1 in 100 (Splenocyte)
CD95	Jo2 (RUO)	BV711	BD Biosciences	740716	1 in 100 (Splenocyte)
B220	RA3-6B2	BV785	BioLegend	103226	0.0625 in 100 (Splenocyte)
CD19	1D3	BV421	BioLegend	115537	0.3125 in 100 (Splenocyte)
MHCII	M5/114.15.2	BV510	BioLegend	107608	0.125 in 100 (Splenocyte)
CD138	281-2	PE-Cy7	BioLegend	142513	1 in 100 (Splenocyte)
CD86	GL-1	PE	BioLegend	105040	0.625 in 100 (Macrophage)
Viability		Zombie NIR	BioLegend	423105	0.0625 in 100 (Splenocyte)

Table S9. A list of antibodies for the T cell panel

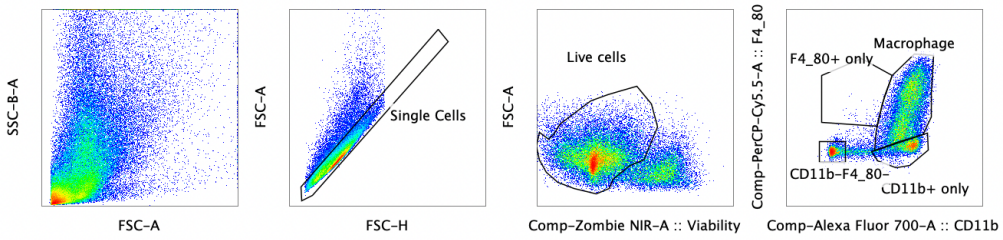
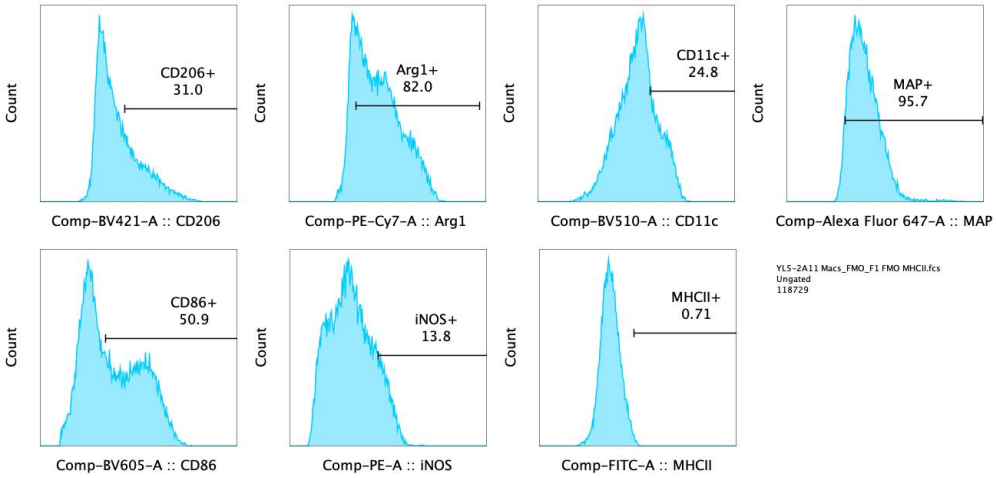
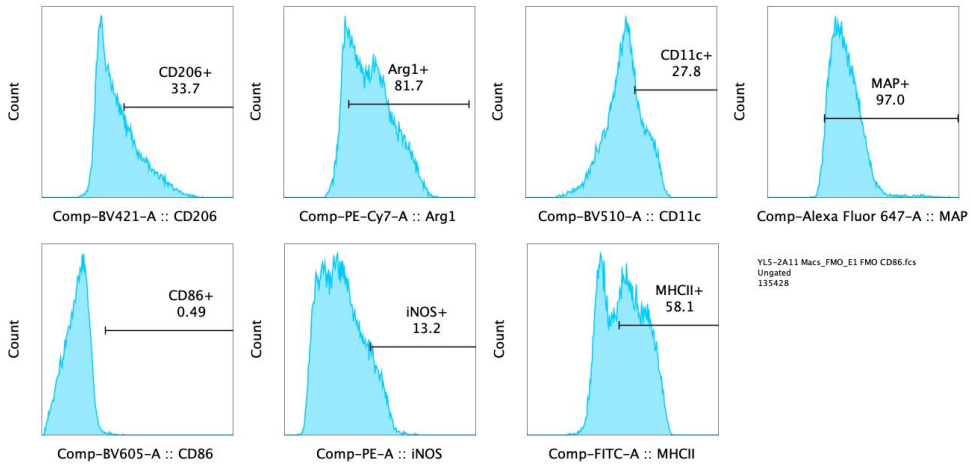
Marker	Clone	Fluorophore	Supplier	Cat. #	Titration (cell type)
CD3	17A2	BV510	BioLegend	100233	1.25 in 100 (Splenocyte)
CD4	GK1.5	FITC	BD Pharmingen	557307	0.156 in 100 (Splenocyte)
CD8a	53-6-7	PE-Cy5.5	BioLegend	100710	0.1 in 100 (Splenocyte)
CD25	PC61	PerCP-Cy5.5	BioLegend	102030	0.5 in 100 (Splenocyte)
Tbet	4B10	PE	BioLegend	644810	2.5 in 100 (Splenocyte)
GATA3	16E10A23	BV421	BioLegend	653806	2.5 in 100 (Splenocyte)
Viability		Zombie NIR	BioLegend	423105	0.0625 in 100 (Splenocyte)

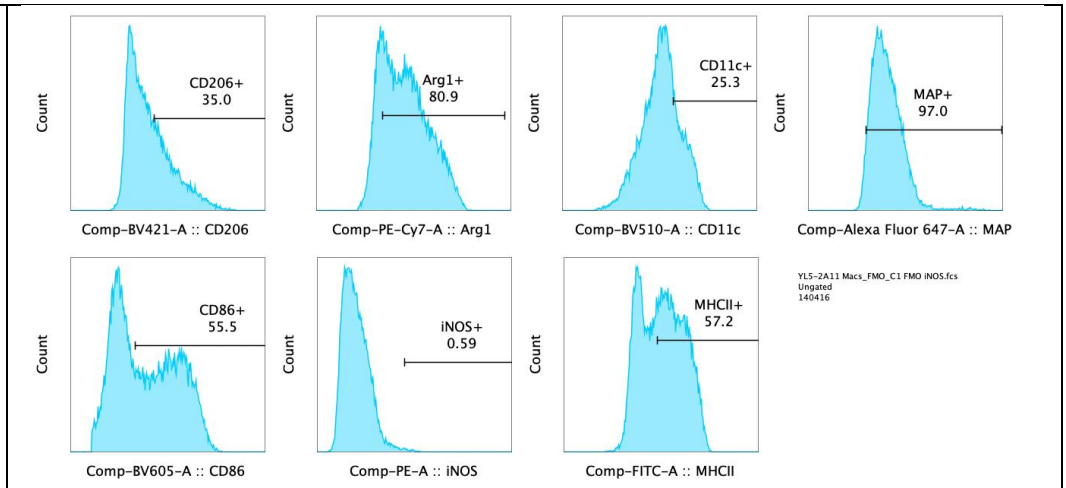
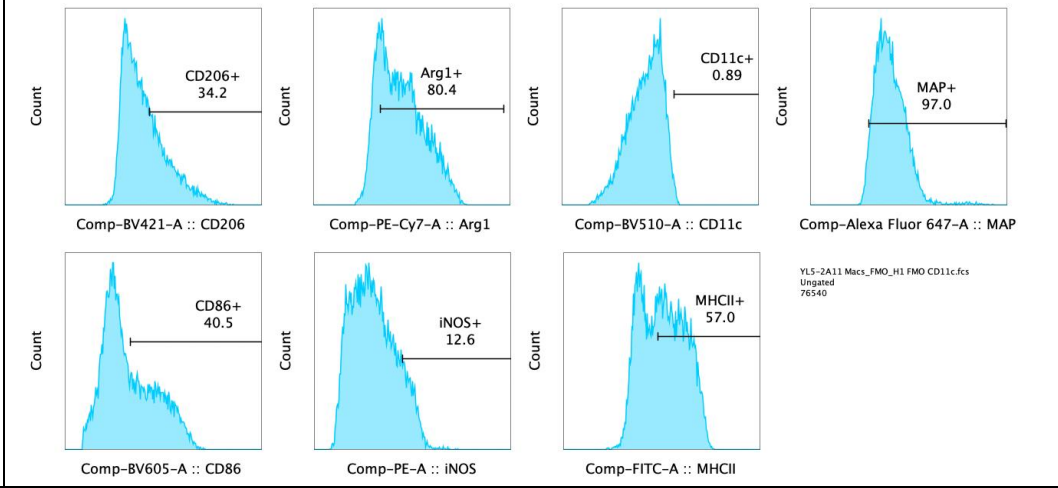
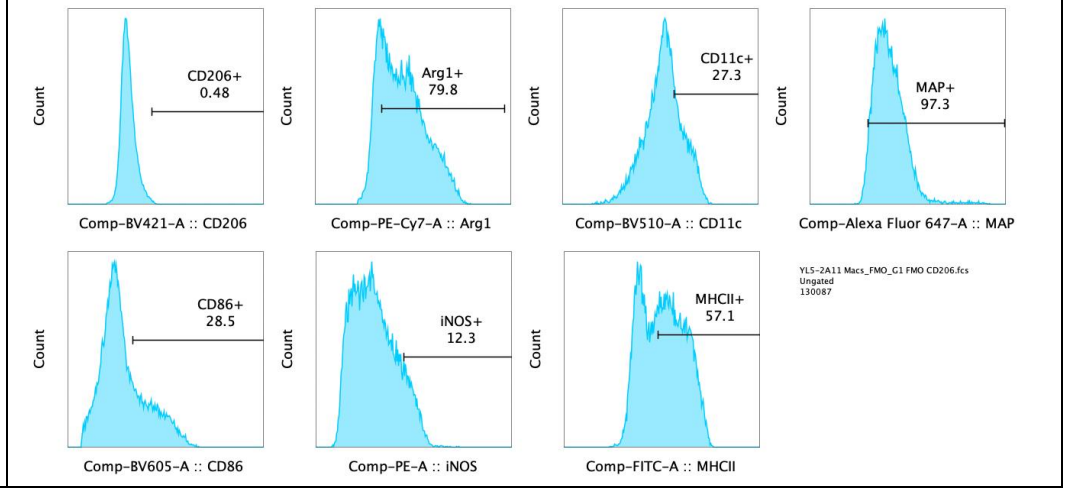
Table S10. A list of antibodies for the APC panel

Marker	Clone	Fluorophore	Supplier	Cat. #	Titration (cell type)
CD3	17A2	NovaFluor Red 685	Thermo Fisher	M002T02R02	0.0625 in 100 (Splenocyte)
CD45	30-F11	Alexa Fluor 532	Thermo Fisher	58-0451-82	0.3125 in 100 (Splenocyte)
XCR1	ZET	APC-Cy7	BioLegend	148223	0.3125 in 100 (Splenocyte)
CD64	X54-5/7.1	BV711	BioLegend	139311	0.156 in 100 (Splenocyte)
Ly6C	HK1.4	BV570	BioLegend	128030	0.625 in 100 (Splenocyte)
CD169	3D6.112	PE-Cy7	BioLegend	142412	2.5 in 100 (Splenocyte)
F4/80	BM8	PB	Thermo Fisher	MF48028	2 in 100 (Splenocyte)
CD80	16-10A1	PE-CF594	BioLegend	104737	1.25 in 100 (Splenocyte)
CD103	M290	VioBright515	Miltenyi	130-111-609	2 in 100 (Splenocyte)
CD24	M1/69	SB600	Thermo Fisher	63-0242-80	0.625 in 100 (Splenocyte)
CD11c	N418	SB780	Thermo Fisher	78-0114-82	0.625 in 100 (Splenocyte)
pDCA-1	927	BV650	BioLegend	127019	0.078 in 100 (Splenocyte)
ICAM-1	3E2	SB436	Thermo Fisher	62-0542-80	0.625 in 100 (Splenocyte)
CCR7	4B12	PE	Thermo Fisher	12-1971-80	1.25 in 100 (Splenocyte)
B220	RA3-6B2	APC/Fire810	BioLegend	103277	0.3125 in 100 (Splenocyte)
CD172a	P84	BB700	BD Bioscience	742205	1 in 100 (Splenocyte)
MHCII	M5/114	BV510	BioLegend	107635	0.25 in 100 (Splenocyte)
Ly6G	1A8	BV480	BD Bioscience	746448	0.125 in 100 (Splenocyte)
CD11b	M1/70	Alexa Fluor 700	eBioscience	56-0112-80	0.0625 in 100 (Splenocyte)

Viability	Zombie NIR	BioLegend	423105	0.03125 in 100 (Splenocyte)

Table S11. Fluorescence minus one (FMO) controls for the macrophage panel

Phenotype gating	
FMO-MHCII (Gated on macrophage population)	
FMO-CD86 (Gated on macrophage population)	

FMO-iNOS (Gated on macrophage population)	
FMO-CD11c (Gated on macrophage population)	
FMO-CD206 (Gated on macrophage population)	

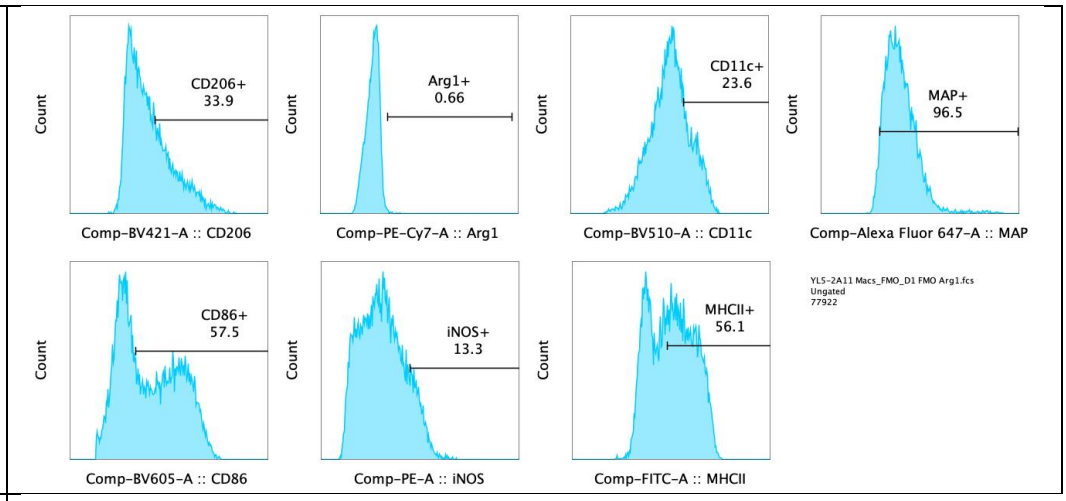
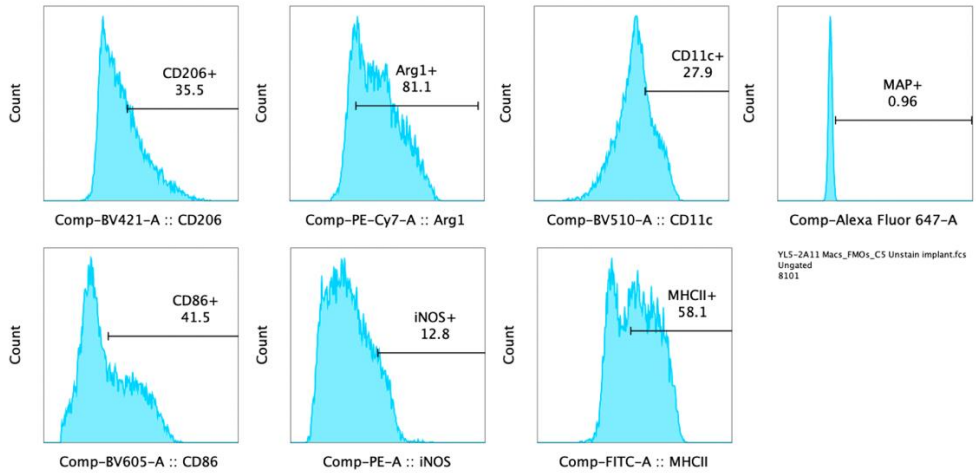
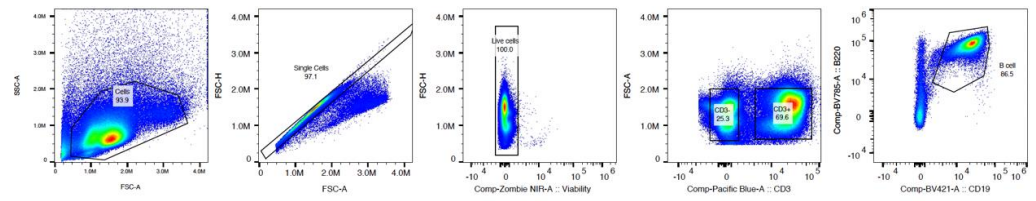
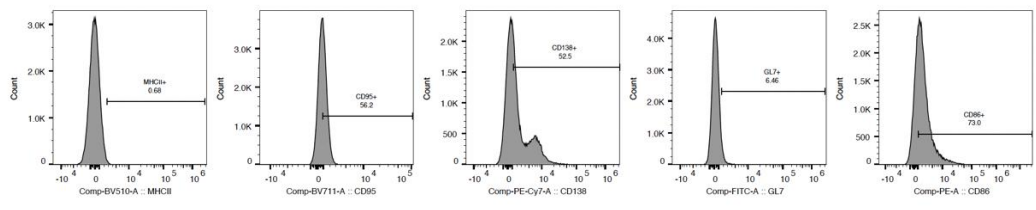
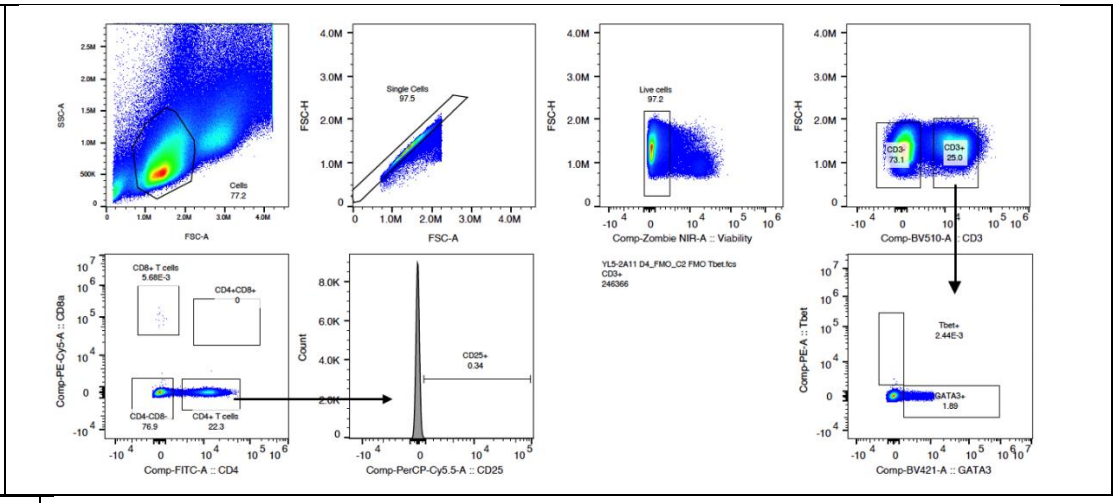
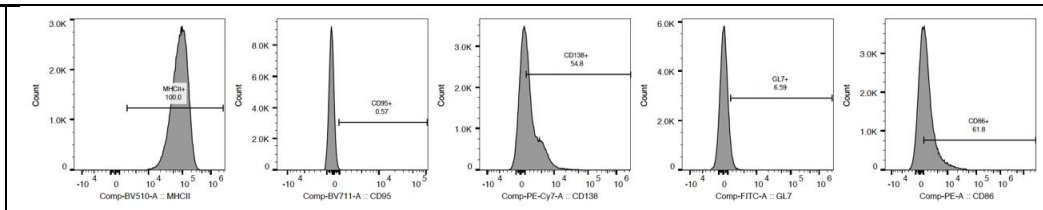
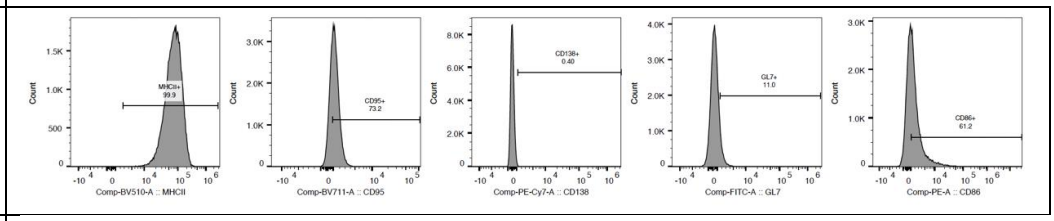
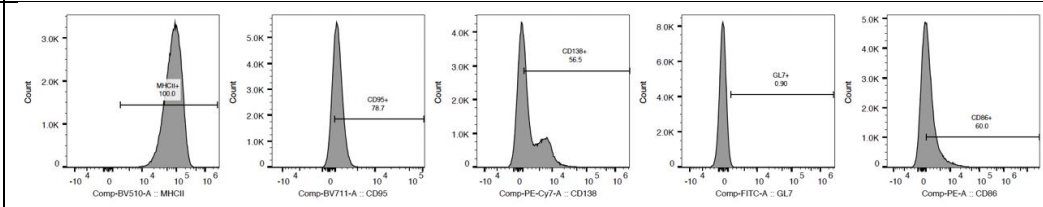
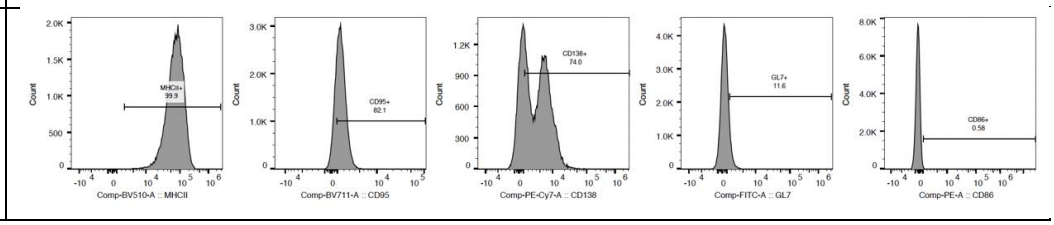
FMO-Arg1 (Gated on macrophage population)	
FMO-MAP AF647 (Gated on macrophage population, using L-MAPS implant without AF647 labeling)	

Table S12. Fluorescence minus one (FMO) controls for the B cell panel

Phenotype gating	
FMO-MHCII (Gated on B cell population)	

FMO-Tbet	
FMO-CD95 (Gated on B cell population)	
FMO-CD138 (Gated on B cell population)	
FMO-GL7 (Gated on B cell population)	
FMO-CD86 (Gated on B cell population)	

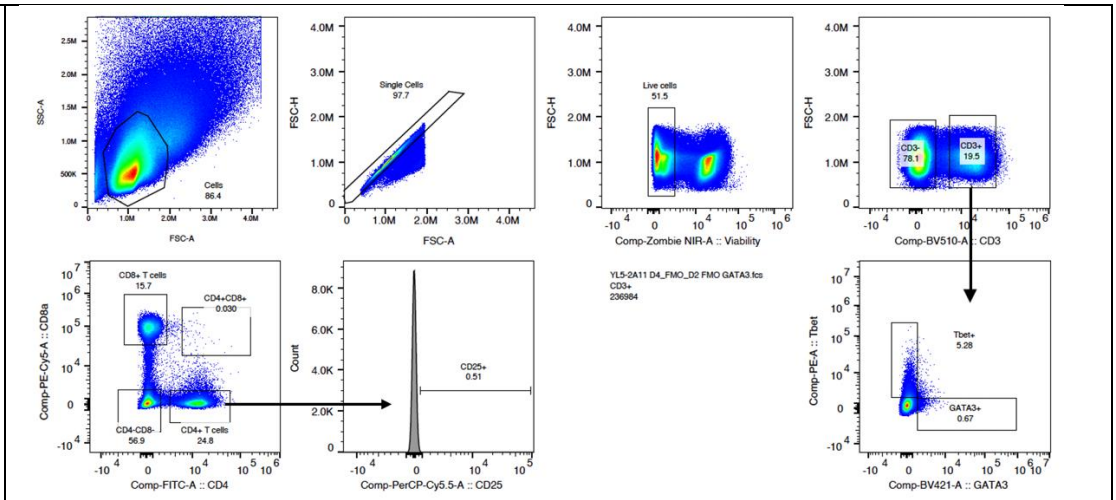
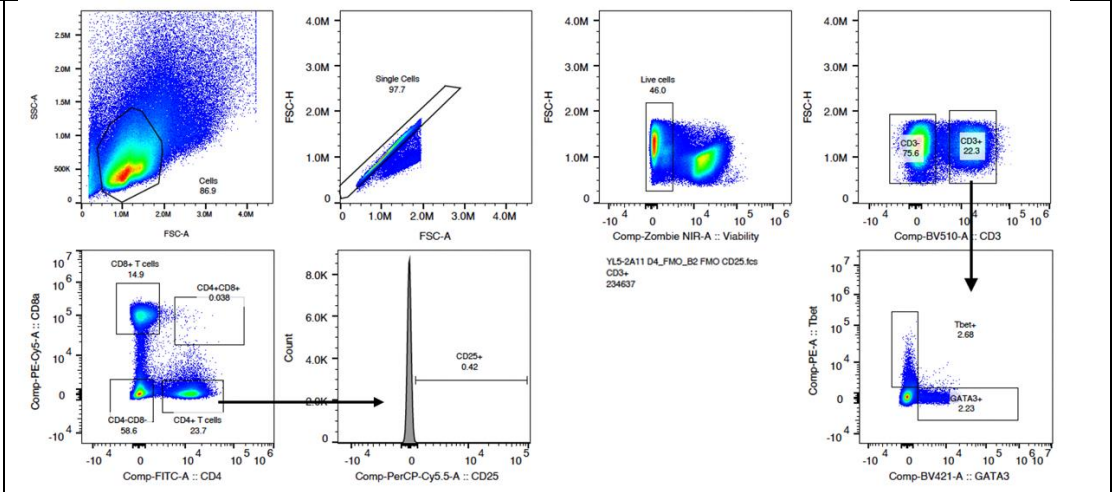
FMO-GATA3	
FMO-CD25	

Table S13. Fluorescence minus one (FMO) controls for the T cell panel