Persistent glycolysis defines the foreign body response to polymeric implants.

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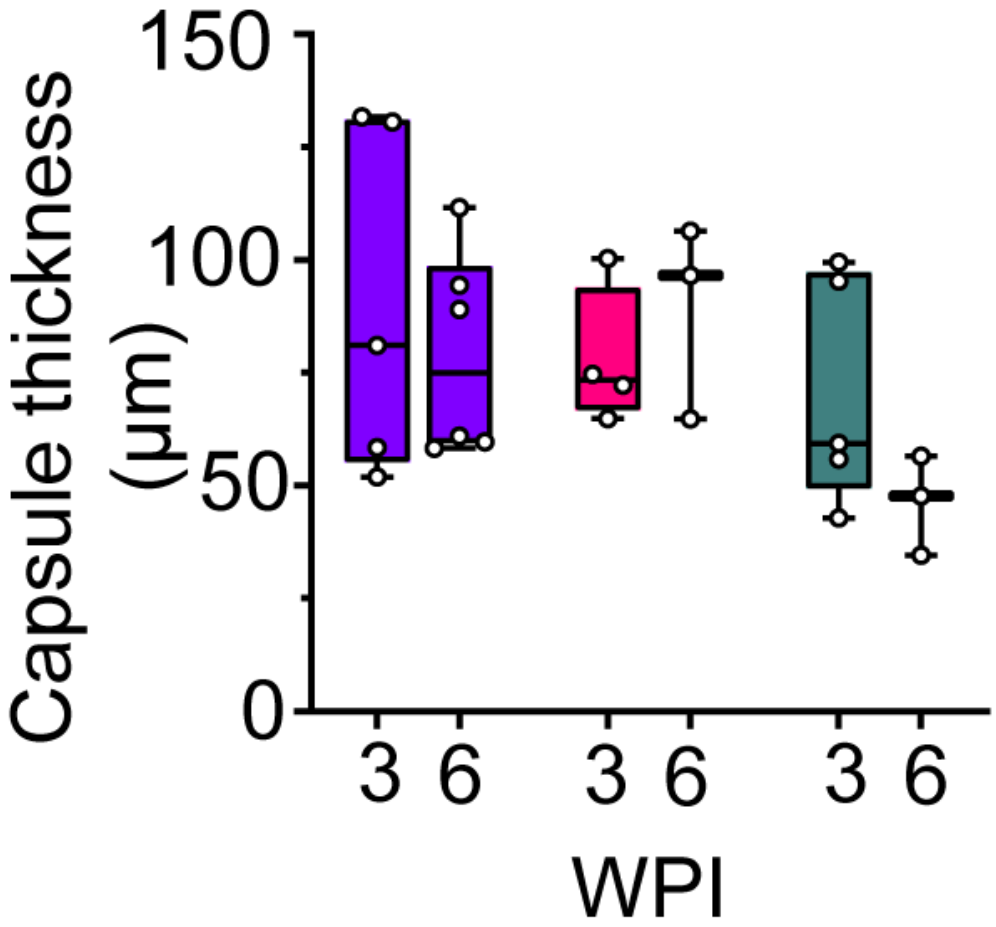
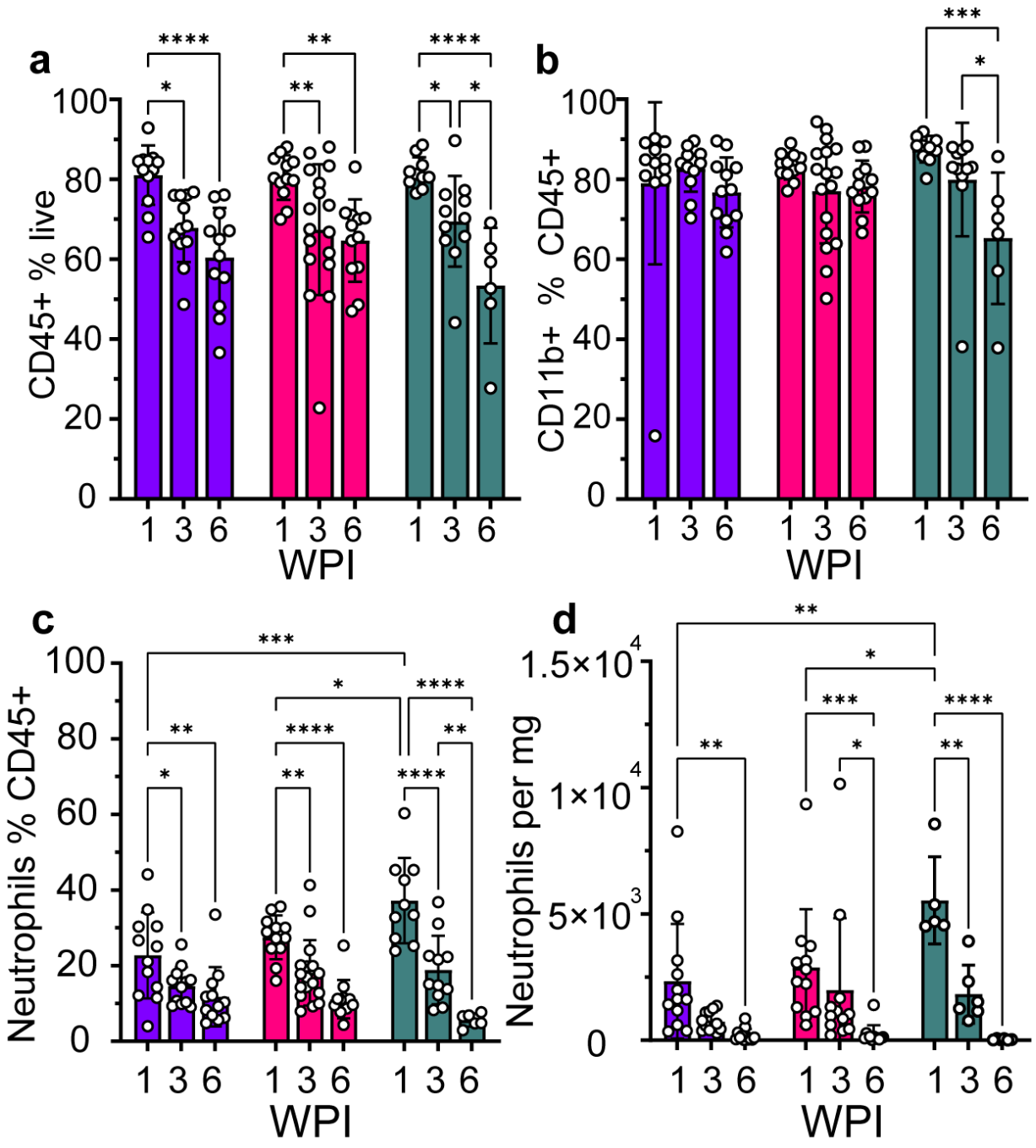
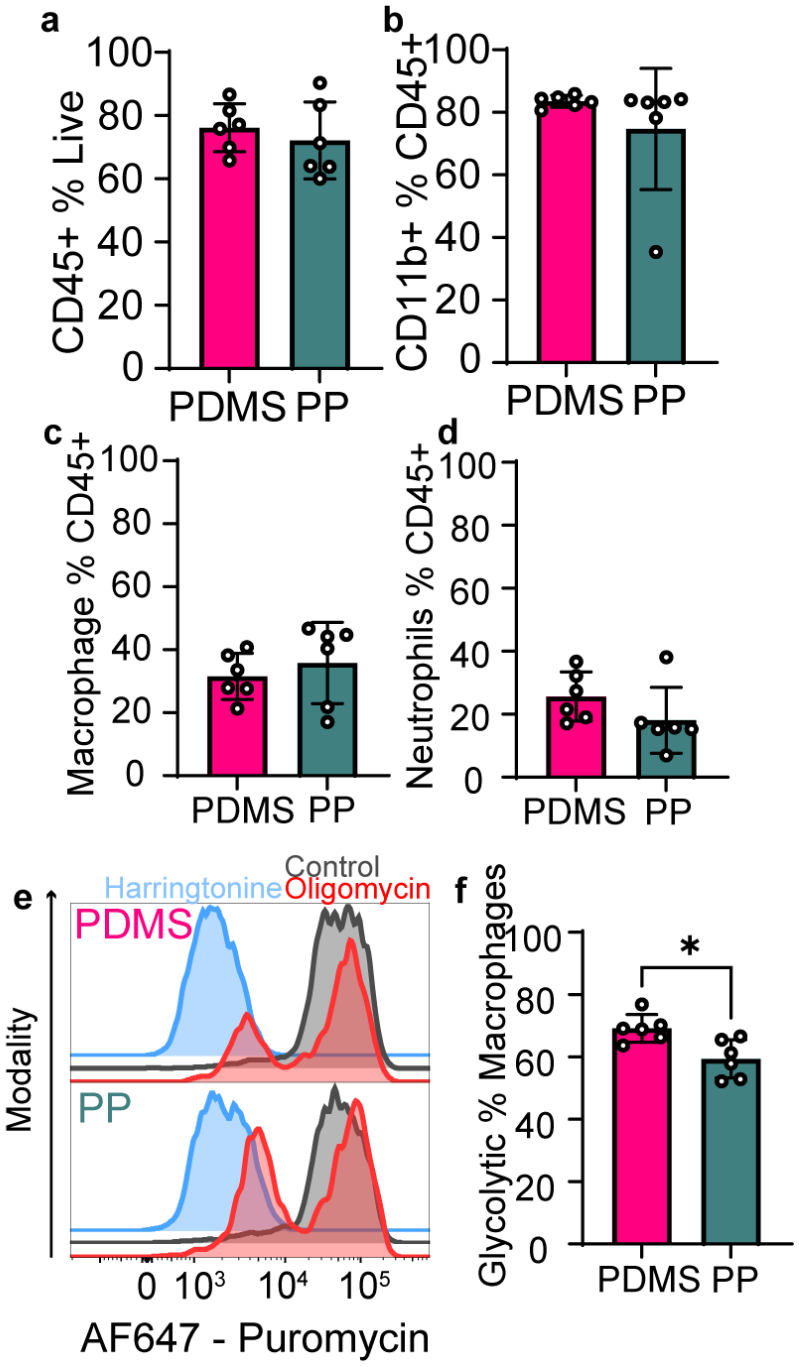


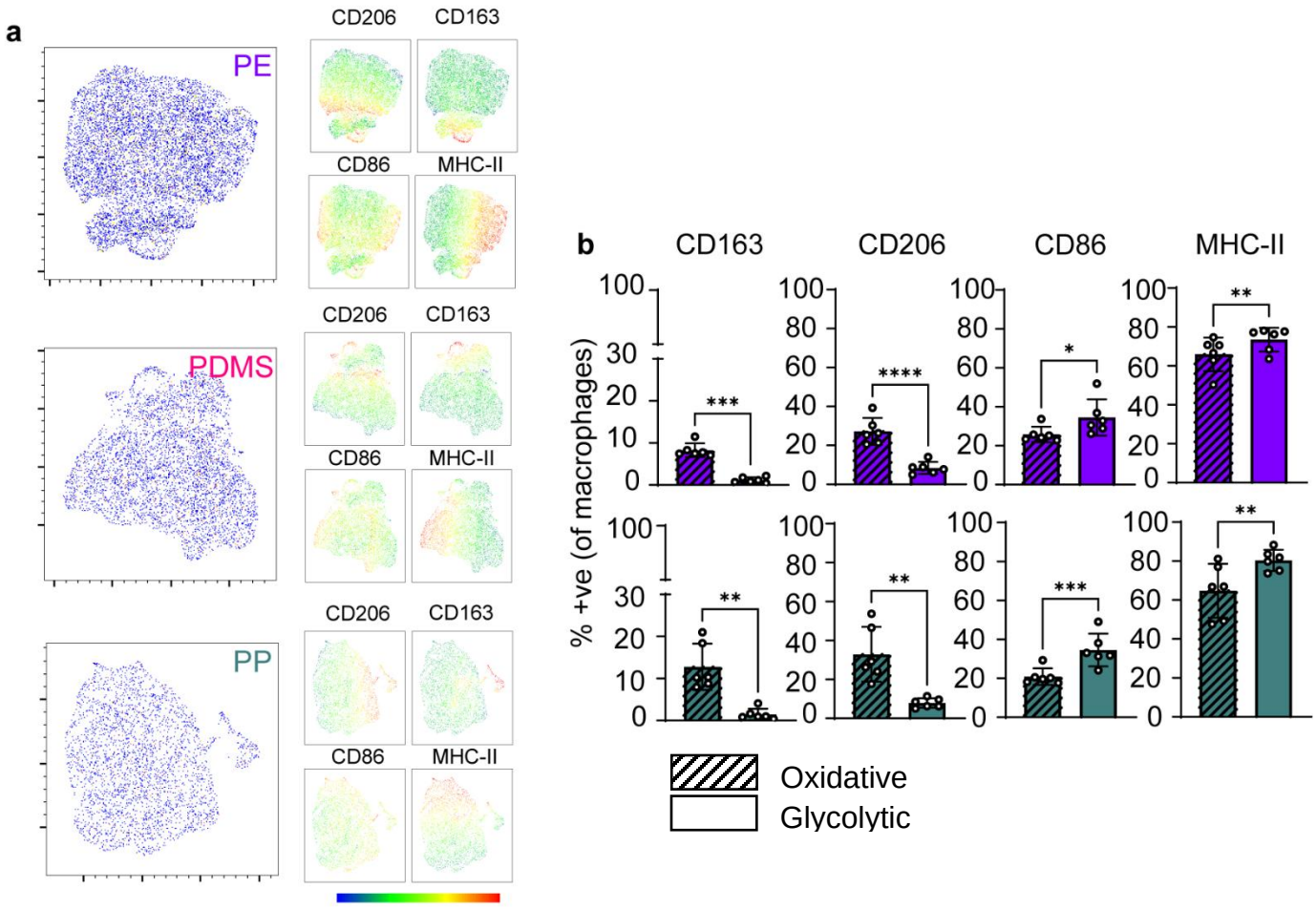
Supplemental Figure 1: Fibrotic capsule thickness associated with subcutaneous polymer implantation at 3- and 6-WPI. k = 2-3, n= 3-6. Two-way ANOVA with Fischer's LSD. *$p < 0.05$.



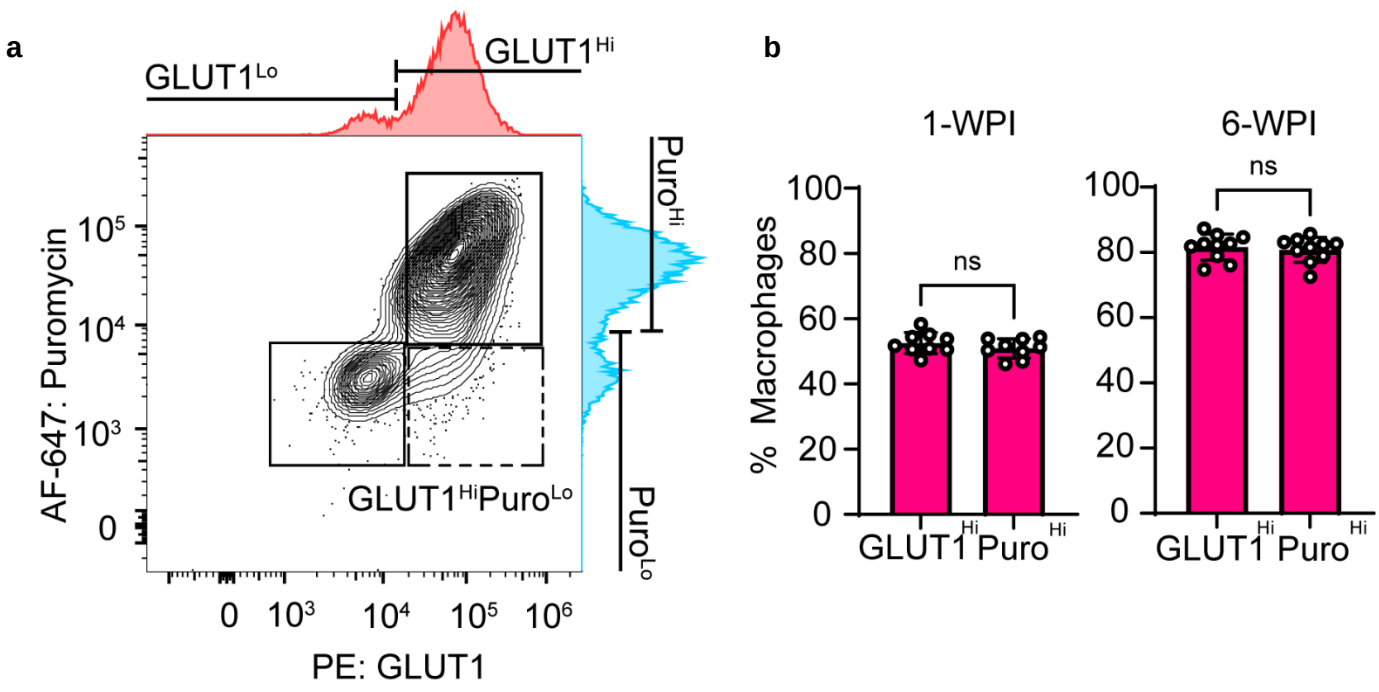
Supplemental Figure 2: Temporal leukocyte dynamics of the PIMD associated FBR. Frequency of **a,** total leukocytes (CD45+) **b,** myeloid cells (CD11b+)**,** and **c,** neutrophils (Ly6G+ Ly6C^{Mid}). **d.** Neutrophil counts per mg of explanted tissue. n = 6-17. Compared using a regular two-way ANOVA with Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.



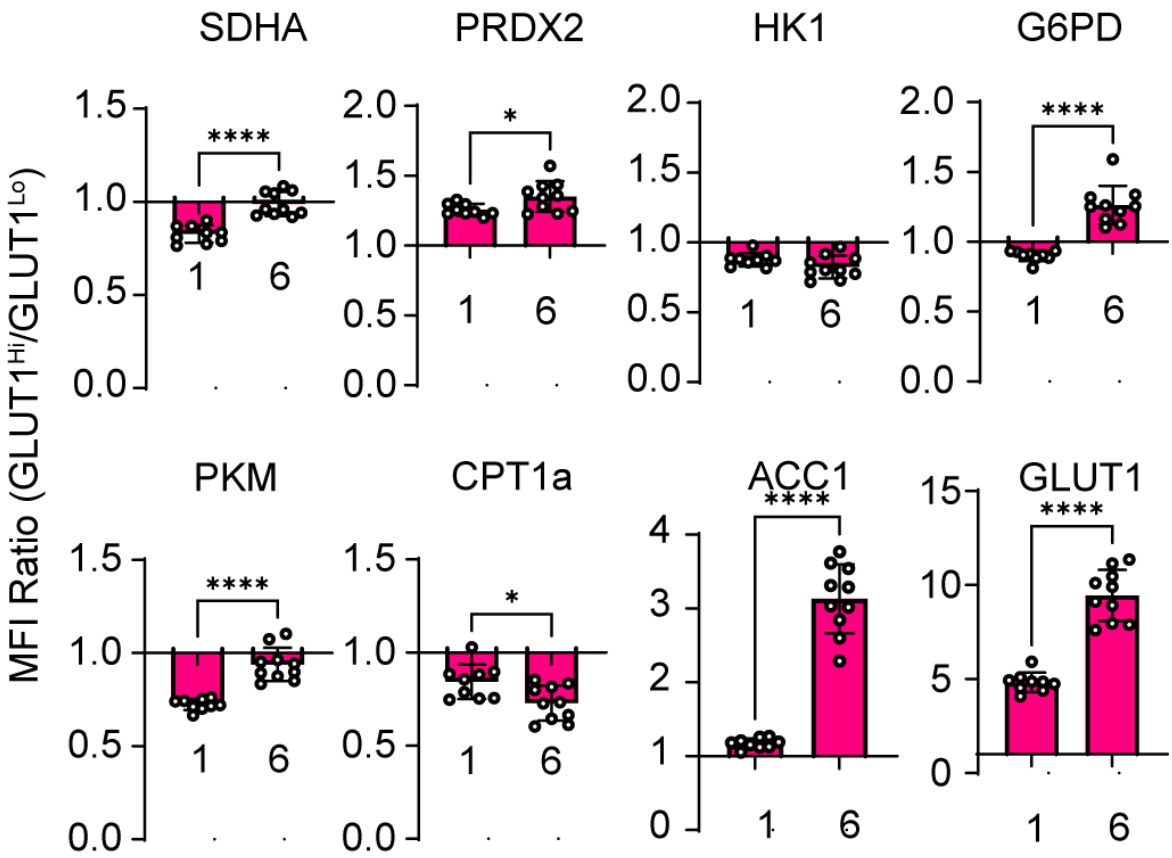
Supplemental Figure 3: Leukocyte dynamics and central carbon peri-implant macrophage metabolic utilization associated with PDMS and PP in female mice at 3-WPI. Frequency of **a,** total leukocytes (CD45+) **b,** myeloid cells (CD11b+)**, c,** macrophages (F480+), and **d,** neutrophils (Ly6G+ Ly6C^{mid}). **e,** Representative histograms of puromycin expression in peri-implant macrophages associated with PDMS and PP implants. **f,** Frequency of macrophages that are glycolytic (Puromycin^{Hi}**)**. n = 6. Compared using an unpaired t-test. *$p < 0.05$.



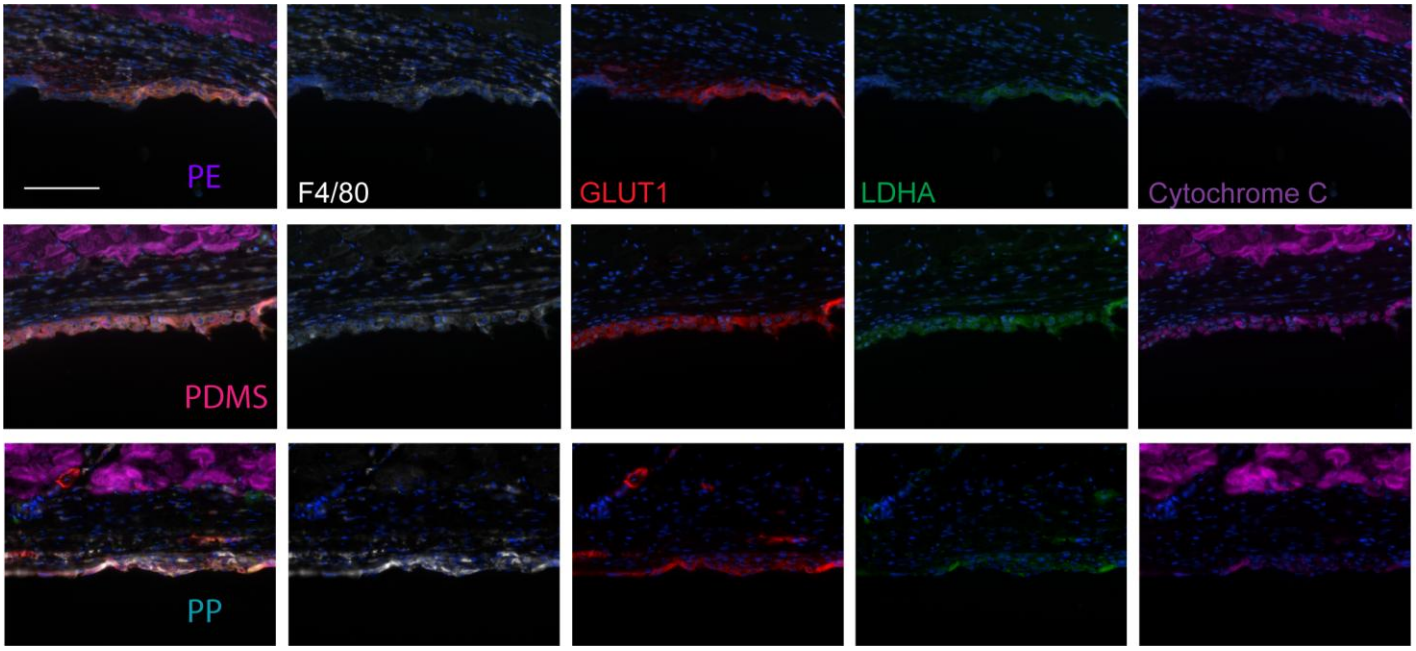
Supplemental Figure 4: Expression of canonical macrophage phenotypic markers and their correlation to metabolic utilization 6-WPI. **a.** Two-dimensional representation **(**UMAPs) of peri-implant macrophages canonical marker expression at 6-WPI. **b.** Frequency of positive expression in glycolytic and oxidative macrophages within the oligomycin A treated group for PE and PP-associated macrophages. Hashed bars represent oxidative cells, solid bars represent glycolytic cells. n = 6. Compared using a paired t-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.



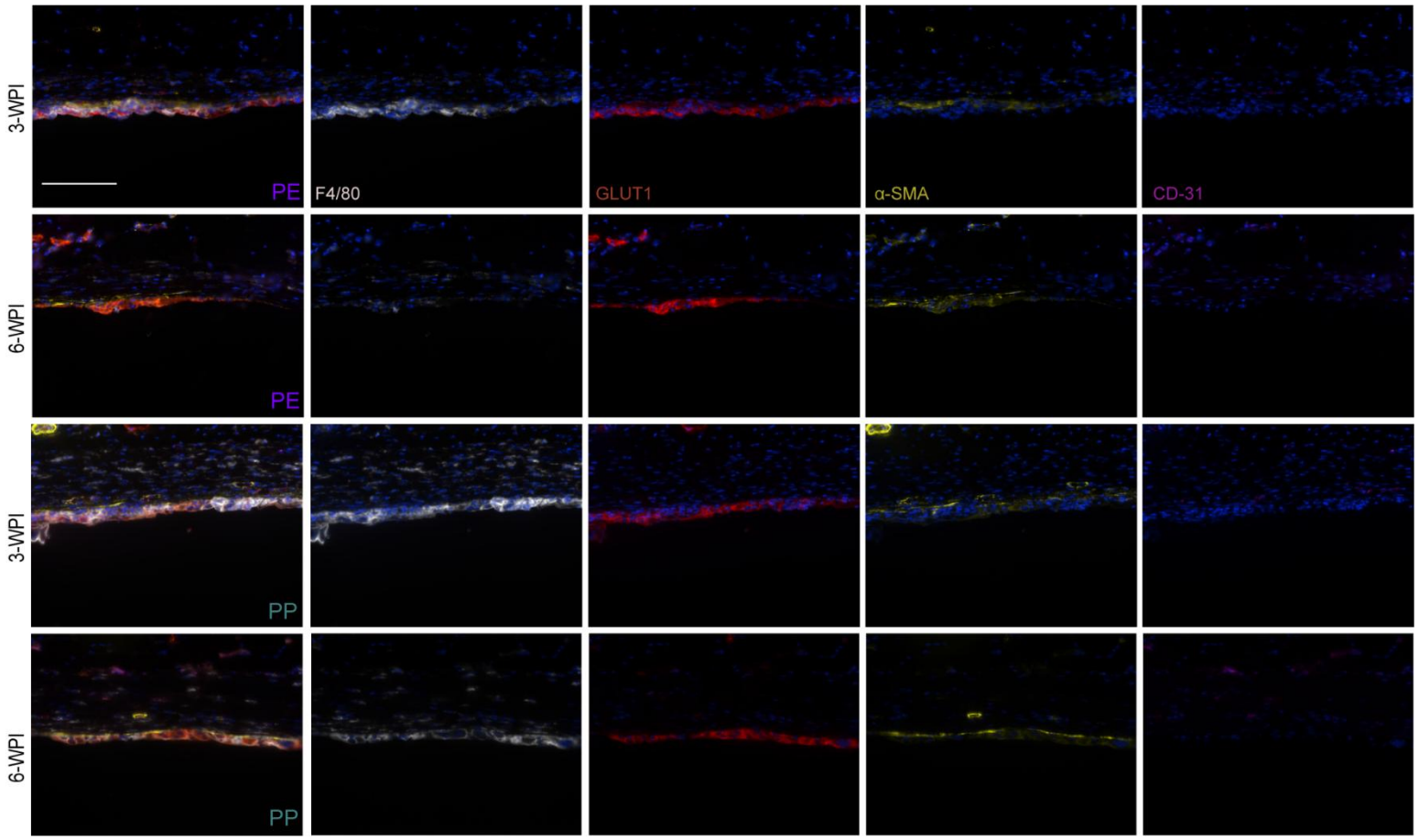
Supplemental Figure 5: GLUT1^{HI} cells are directly correlated with glycolytic utilization (Puromycin^{Hi}) in PDMS-associated macrophages at 1- and 6-WPI. **a.** Representative co-expression of Puromycin and GLUT1 in an Oligomycin A treated sample indicate glycolytic and oxidative macrophage populations with consistently high and low GLUT1 expression (1) and a population highly expressing GLUT1 but with low puromycin intensity (2), which is not present in the control-treated samples. **b.** Frequency of GLUT1^{Hi} macrophages and Puromycin^{Hi} macrophages in matched vehicle and oligomycin A treated PDMS samples at 1- and 6-WPI. n = 9-10. Compared using a paired t-test. *$p < 0.05$.



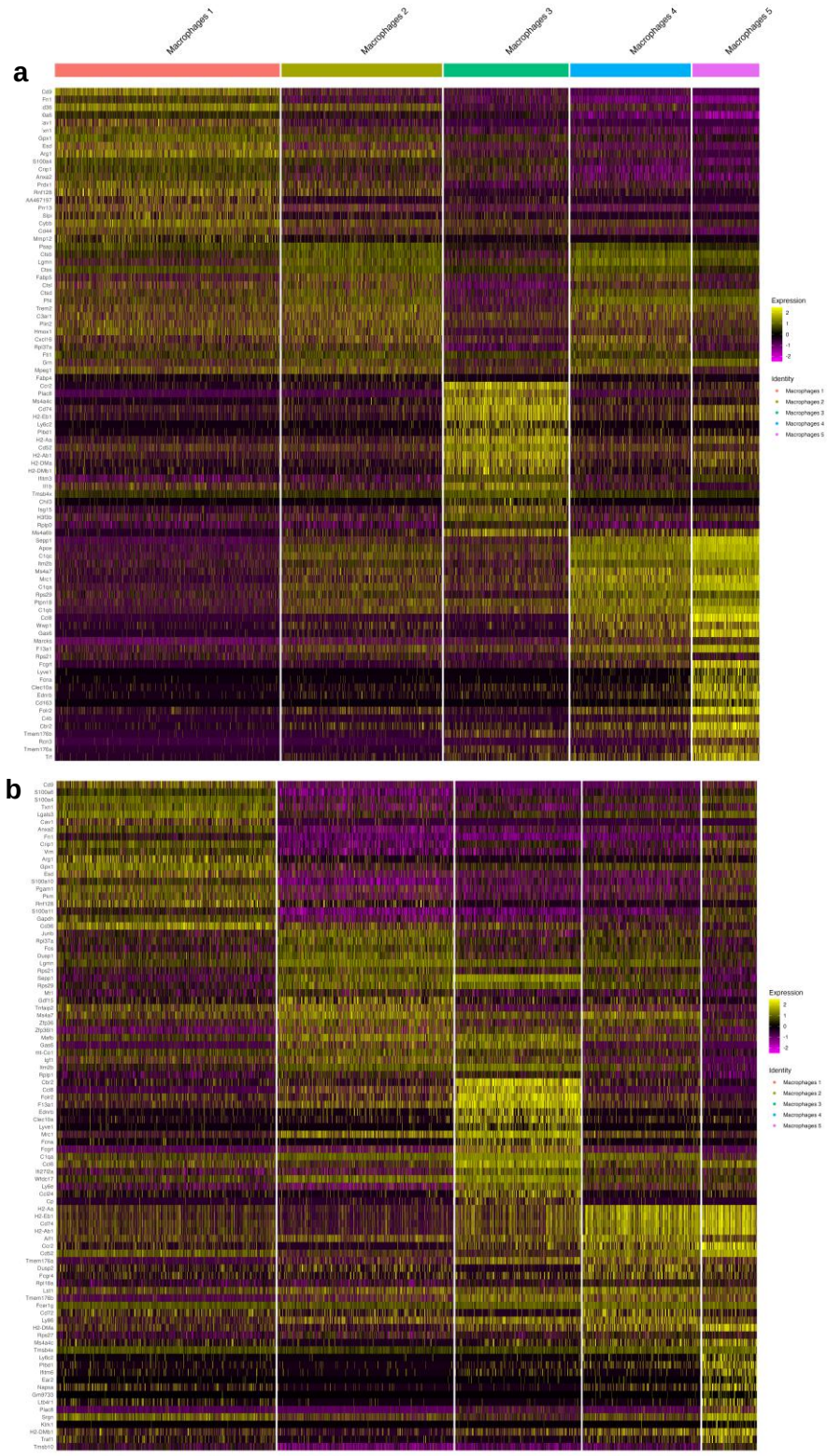
Supplemental Figure 6: Ratio of metabolic enzyme expression in GLUT1^{Hi} to GLUT1^{Lo} cells. n = 9-10. Compared using an unpaired t-test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.



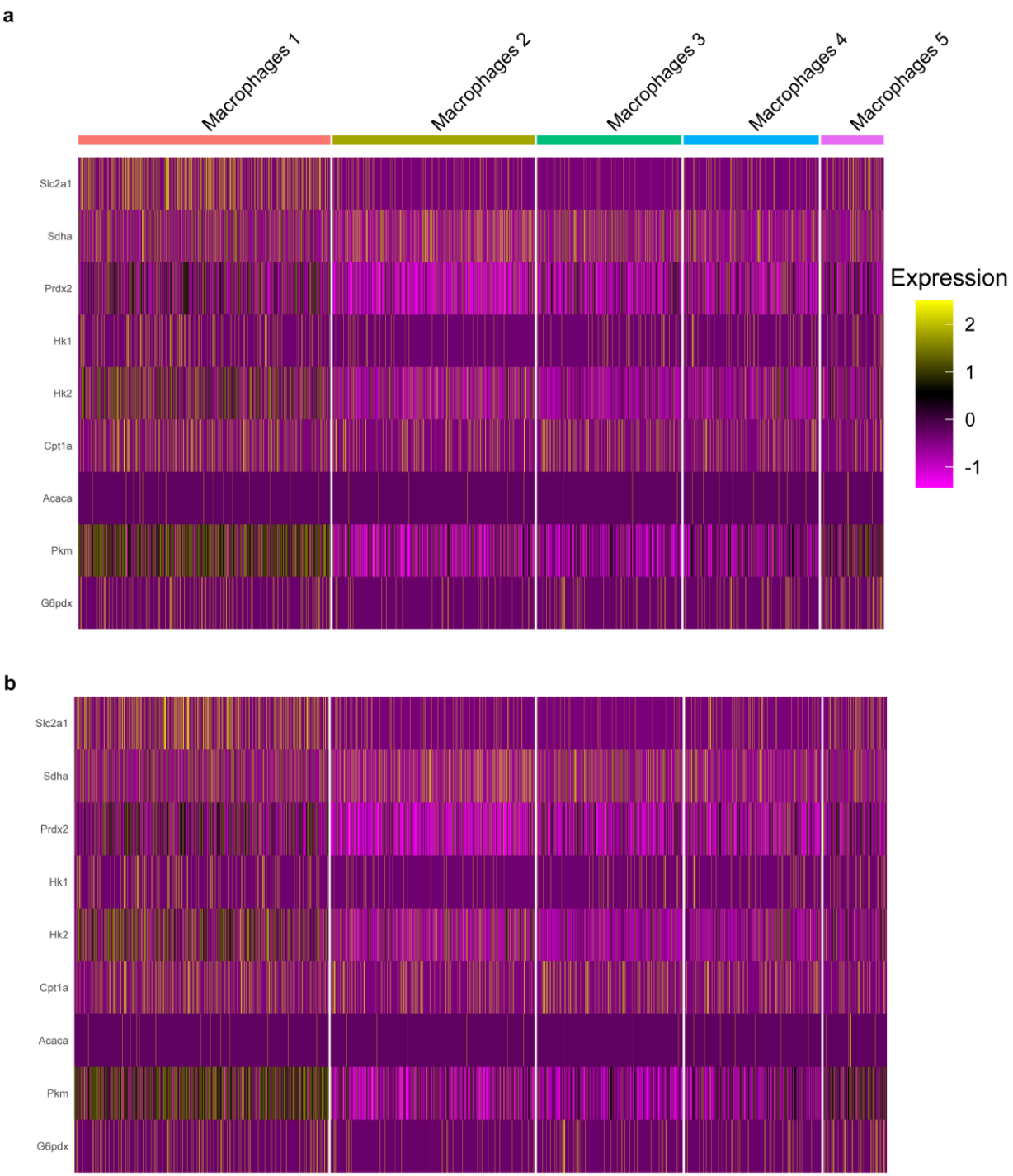
Supplemental Figure 7: Representative spatial expression of metabolic markers in implant-associated tissues 6-WPI. Composite images (left) and individual channels for PE, PDMS, and PP material image at 40X magnification. Scale bar = 100 µm.



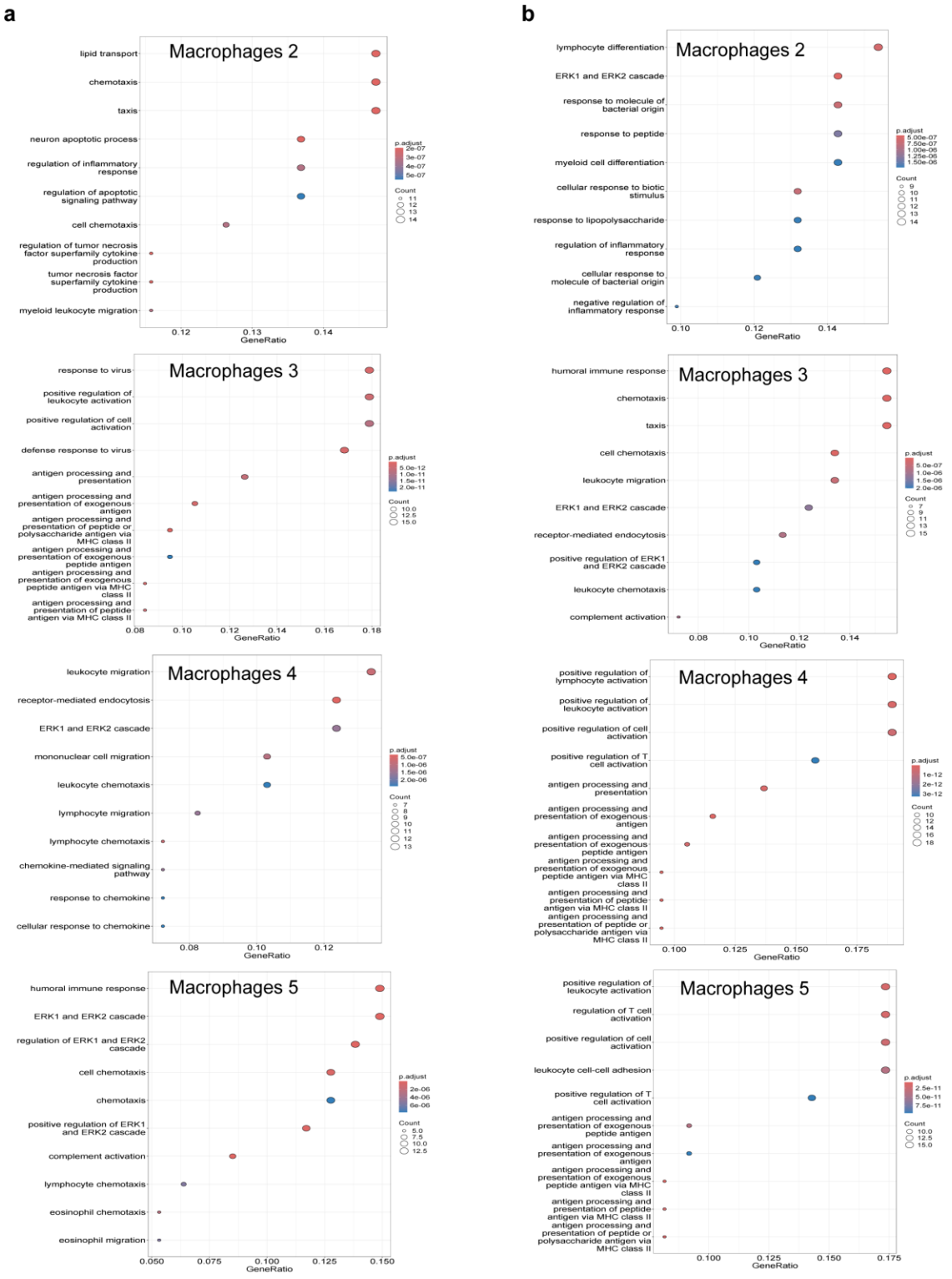
Supplemental Figure 8: Representative OPAL™ Immunohistochemical staining of fibrotic markers in PE- and PP-associated tissues 3- and 6-WPI. Presented as composite images and individual channels**.** Imaged at 40X magnification. Scale bar =100 µm.



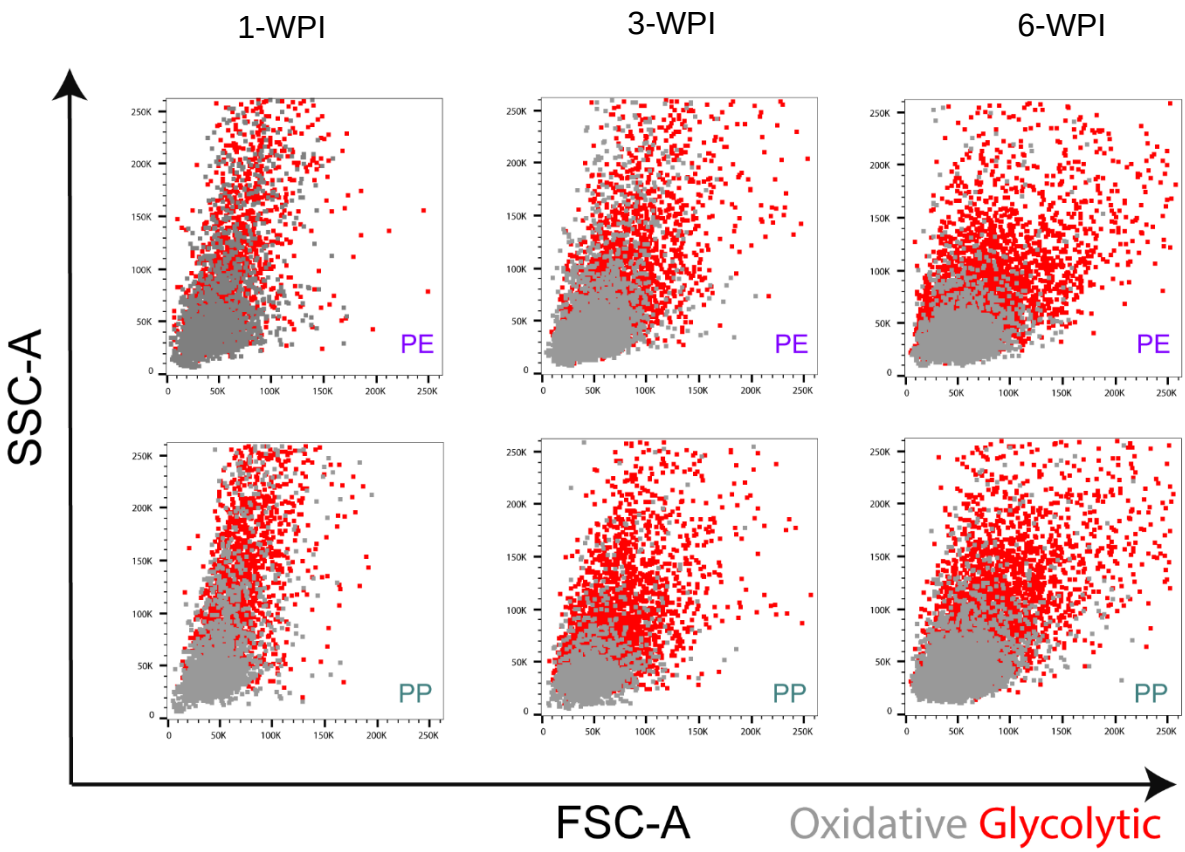
Supplemental Figure 9: Heatmap of top 20 DEGs in scRNA-seq dataset for PDMS-associated macrophages at 2-WPI (**a**) and 4-WPI (**b**). Relative expression normalized to the total number of transcripts in each cell.



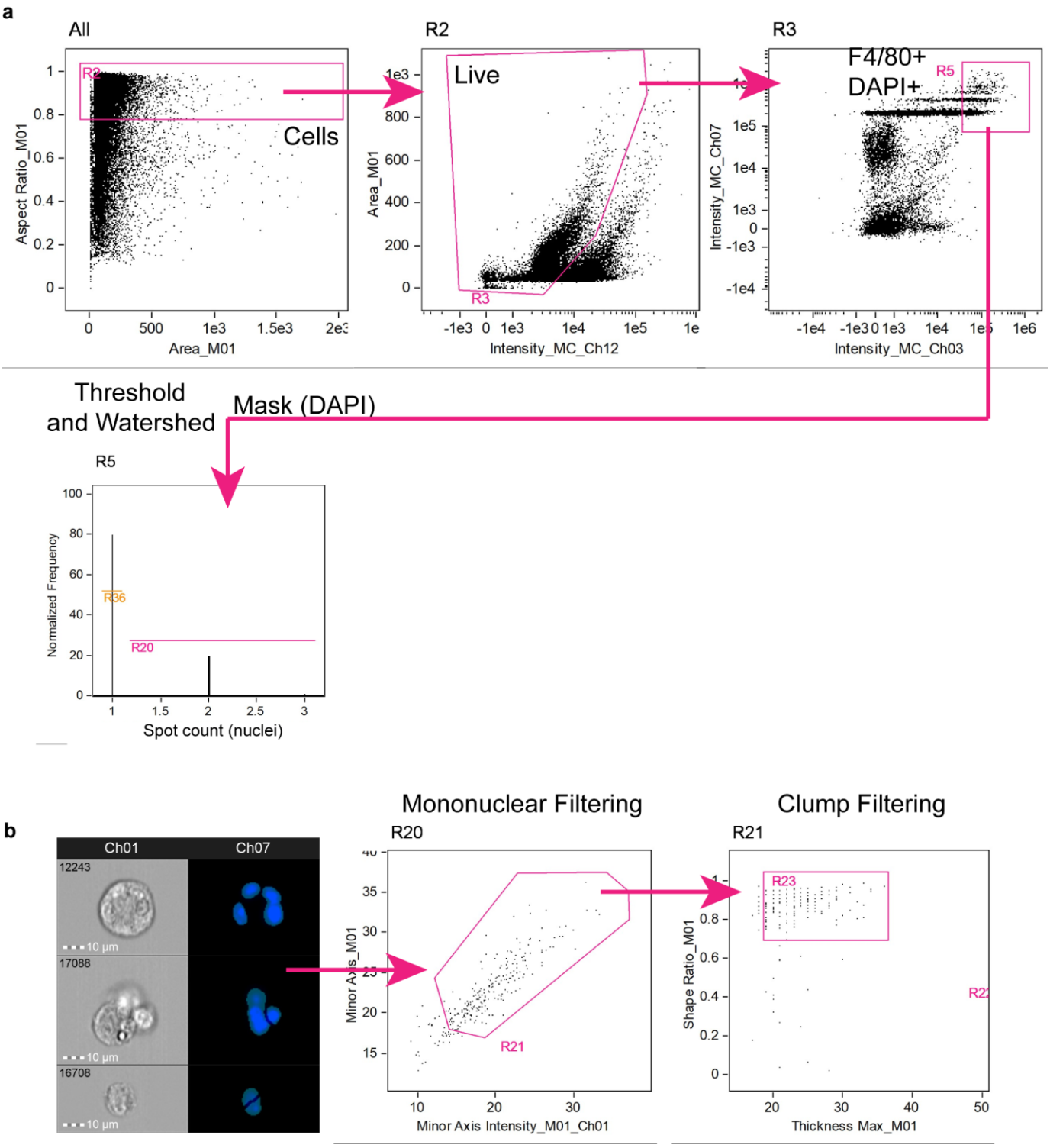
Supplemental Figure 10: Heatmap of metabolic genes from scRNA-seq dataset for PDMS-associated macrophages at 2-WPI (**a**) and 4-WPI (**b**). Relative expression normalized to the total number of transcripts in each cell



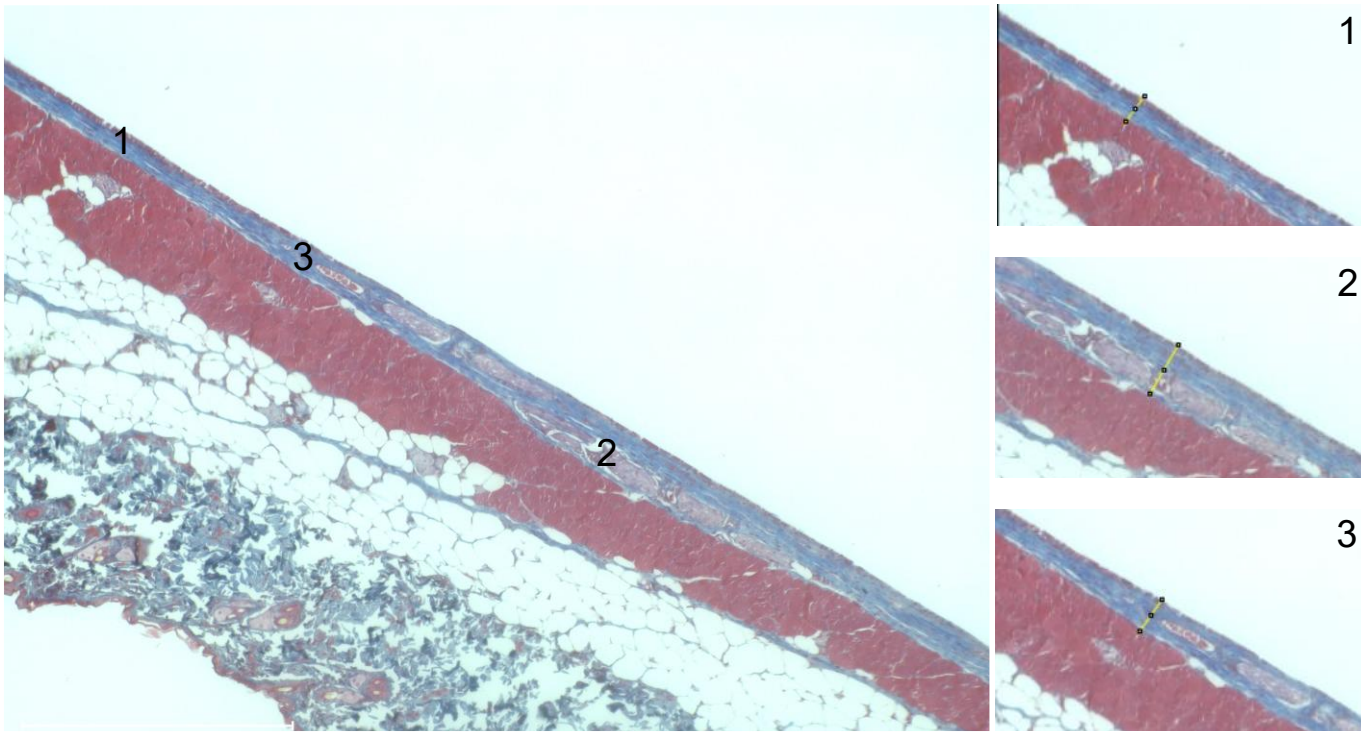
Supplemental Figure 11: Dotplots representing gene ontology (GO) analyses in macrophage clusters 2-5 at 2- (a) and 4-WPI (b). Top 10 pathways selected based on p-adjusted value and ranked based on gene ratio.



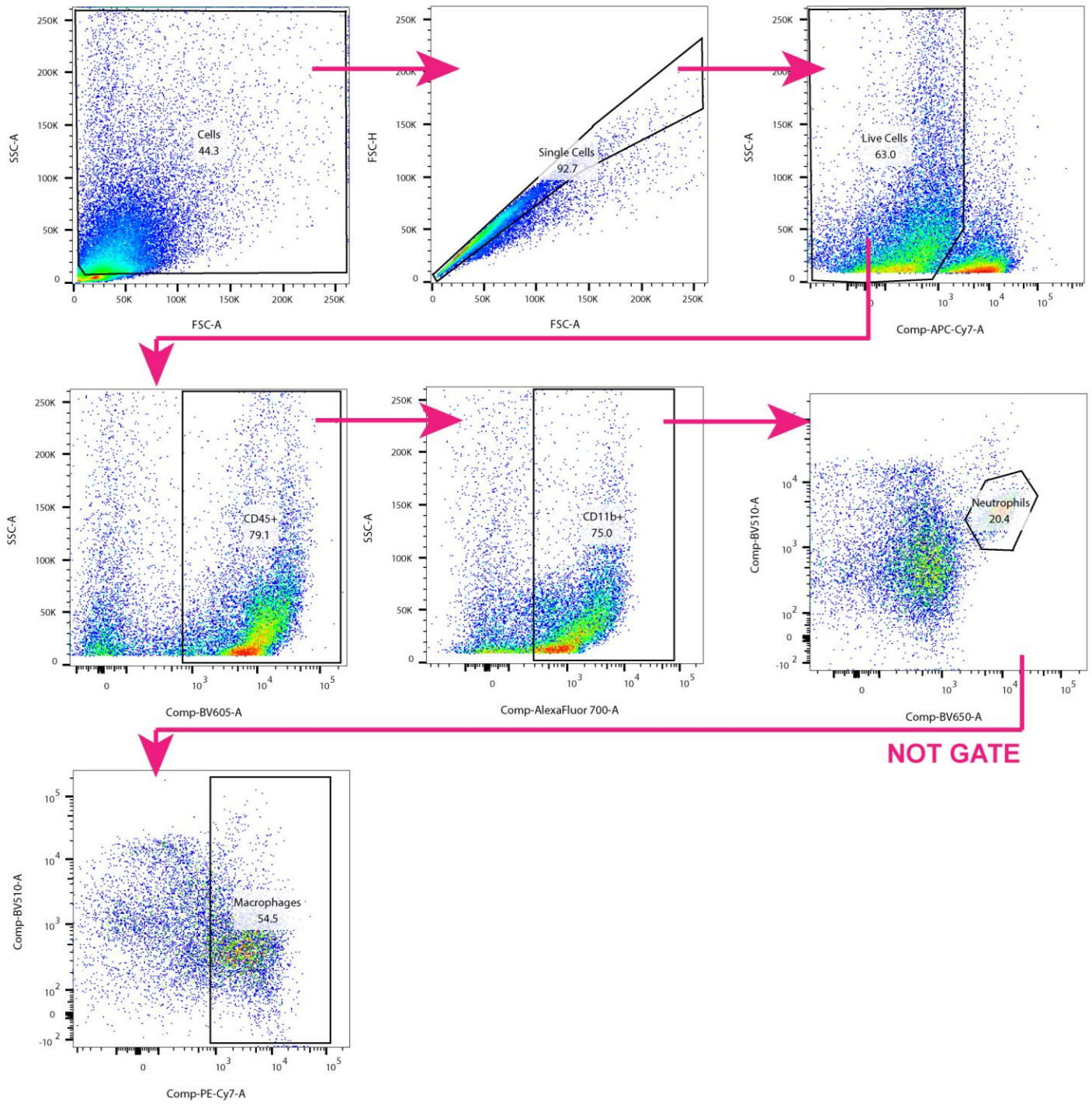
Supplemental Figure 12: Representative scatter plots of glycolytic and oxidative macrophage size at 1-, 3- and 6-WPI in for PE and PP.



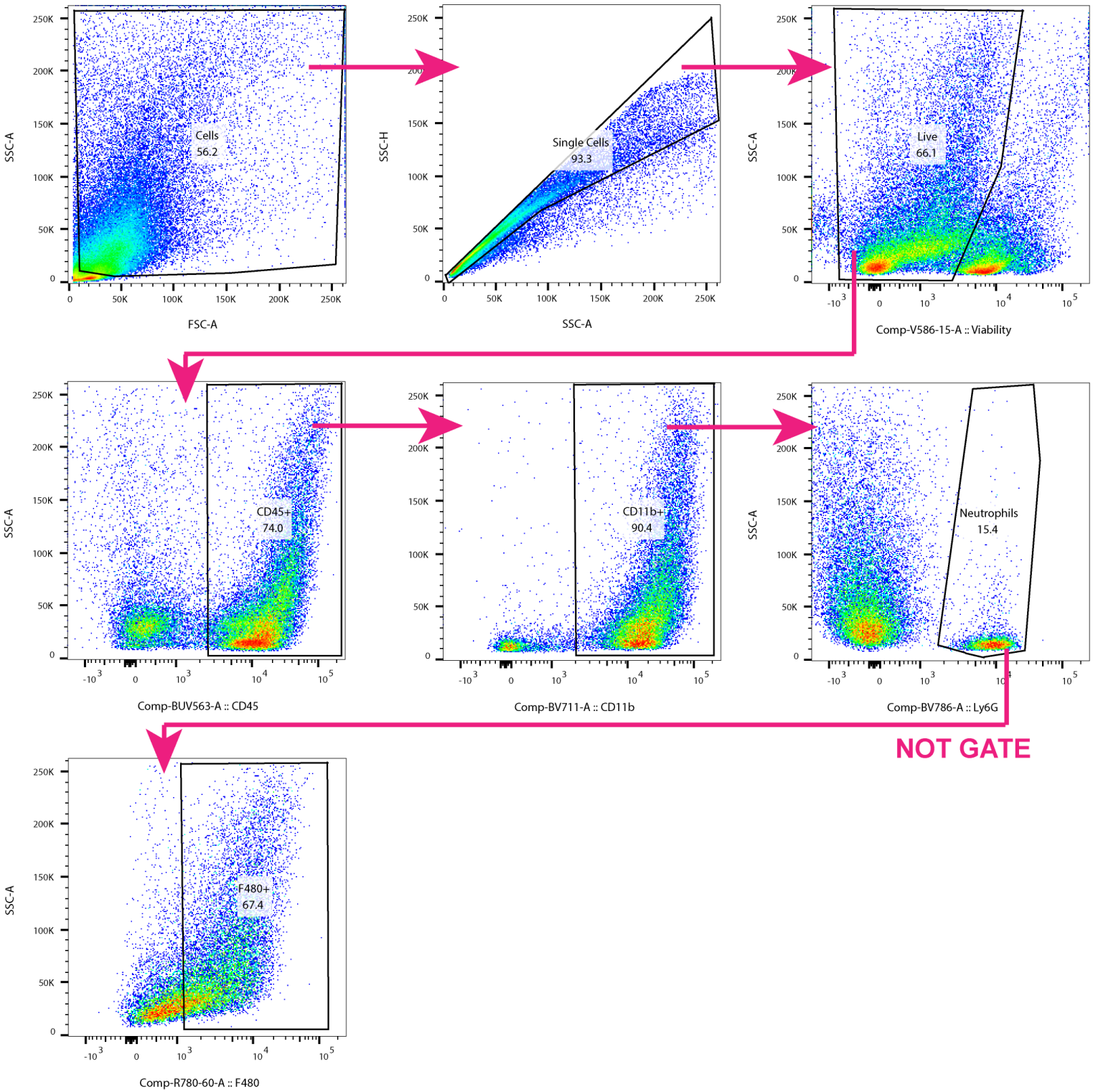
Supplemental Figure 13: Gating scheme used for ImageStream® cytometry panel. **a.** Gating scheme used to identify macrophages and multinucleated cells. **b.** Post-multinucleation gate processing to identify erroneously masked mononuclear cells and cell clumps.



Supplemental Figure 14: Representative imagery of the quantification method for Masson's trichrome stained slides. Images were captured at 10X magnification and measured using ImageJ2 at the least fibrotic (1), most fibrotic (2) and most 'representative' (3) points. Muscle tissue (red), collagen (blue) and nuclei (brown/black).



Supplemental Figure 15: Gating scheme used for leukocyte frequency flow cytometry panel.



Supplemental Figure 16: Gating scheme used metabolic enzyme expression flow cytometry panel (Met-flow).

Supplemental Table 1. Target genes and primer sequences for bulk gene expression analyses

Target Gene	Forward Sequence (5' -> 3')	Reverse Sequence (3' -> 5')	Source
Canx	ATGGAAGGGAAGTGGTTACTGT	GCTTTGTAGGTGACCTTTGGAG	Thermo Fisher Scientific
Rer1	GGCTGGACAAGTCTACCCC	GCGTAGGTCACAATGTACCAAC	Thermo Fisher Scientific
Col1a1	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG	Thermo Fisher Scientific
Col3a1	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC	Thermo Fisher Scientific
Fn1	GCTCAGCAAATCGTGCAGC	CTAGGTAGGTCCGTTCCCACT	Thermo Fisher Scientific
Il1β	AGCTCCAAGAAAGGACGAACA	GCCCTGTAGGTGAGGTTGAT	Thermo Fisher Scientific
Il6	CCACAGTCCTTCAGAGAGATACA	CCTTCTGTGACTCCAGCTTAT	Thermo Fisher Scientific
Tgfβ	CGAAGCGGACTACTATGCTAAA	TCCCGAATGTCTGACGTATTG	Thermo Fisher Scientific
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC	Thermo Fisher Scientific
Adgre1	TGACTCACCTTGTGGTCCTAA	CTTCCCAGAATCCAGTCTTTCC	Thermo Fisher Scientific
Tnf	GCCTCTTCTCATTCCTGCTT	TGGGAACTTCTCATCCCTTTG	Thermo Fisher Scientific

Supplemental Table 2. Antibodies used for flow cytometry.

Antibody Target	Clone	Fluor	Supplier	Product#	Dilutions
Anti-ACC1	EPR23235-147	Unconjugated	ABCAM	AB272704	1/1000
Anti-CD11b	M1/70	AF700	BioLegend	101222	1/500
Anti-CD11b	M1/70	BV711	BioLegend	101242	1/500
Anti-CD45	30-F11	BV605	BioLegend	103155	1/750
Anti-CD45	30-F11	BUV563	BD Biosciences	741274	1/500
Anti-CD86	GL-1	BV421	BioLegend	105032	1/200
Anti-CD163	S15049I	KB520	BioLegend	155317	1/200
Anti-CD206	C068C2	PerCP-Cy5.5	BioLegend	141715	1/200
Anti-CPT1a	EPR21842-71-2F	Unconjugated	ABCAM	AB235841	1/200
Anti-F4/80	BM8	PE	BioLegend	123110	1/750
Anti-F4/80	BM8	PE-Cy7	BioLegend	123114	1/750
Anti-F4/80	BM8	APC-Fire™	BioLegend	1213152	1/200
Anti-F4/80	BM8	BV605	BioLegend	123133	1/200
Anti-G6PD	EPR20688	Unconjugated	ABCAM	AB231828	1/1000
Anti-GLUT1	EPR3915	PE	ABCAM	AB209449	1/200
Anti-HK1	EPR10134(B)	BUV661	BD Biosciences	570557	1/50
Anti-Ly6C	HK1.4	BV510	BioLegend	128033	1/500
Anti-Ly6G	1A8	BV650	BioLegend	127641	1/500
Anti-Ly6G	1A8	BV785	BioLegend	127645	1/500
Anti-Ly6G	1A8	AF488	BioLegend	127626	1/500
Anti-MHC-II	M5/114.15.2	BV785	BioLegend	107645	1/500
Anti-SDHA	EPR9043(B)	Unconjugated	ABCAM	AB240098	1/1000
Anti-PKM	EPR10138(B)	Unconjugated	ABCAM	AB206129	1/200
Anti-PRDX2	EPR5154	BUV615	BD Biosciences	570740	1/50
Anti-Puromycin	12D10	AF647	Sigma-Aldrich	Mabe343-af647	1/1000

Supplemental Table 3. Antibodies used for OPAL™ fluorescent histology.

Antibody Target	Clone	Fluor	Supplier	Product#	Dilutions
Anti-α-SMA	1A4	Unconjugated	ABCAM	AB7817	1/500
Anti-CD31	D8V9E	Unconjugated	CellSignaling	77699S	1/200
Anti-CD68	SP251	Unconjugated	ABCAM	AB192847	1/200
Anti-Cytochrome C	EPR1326-80-5	Unconjugated	ABCAM	AB76107	1/250
Anti-F4/80	D2S9R	Unconjugated	CellSignaling	70076	1/250
Anti-GLUT1	EPR3915	Unconjugated	ABCAM	AB115730	1/250
Anti-LDHA	2E2G6	Unconjugated	Proteintech	66287-1-Ig	1/100