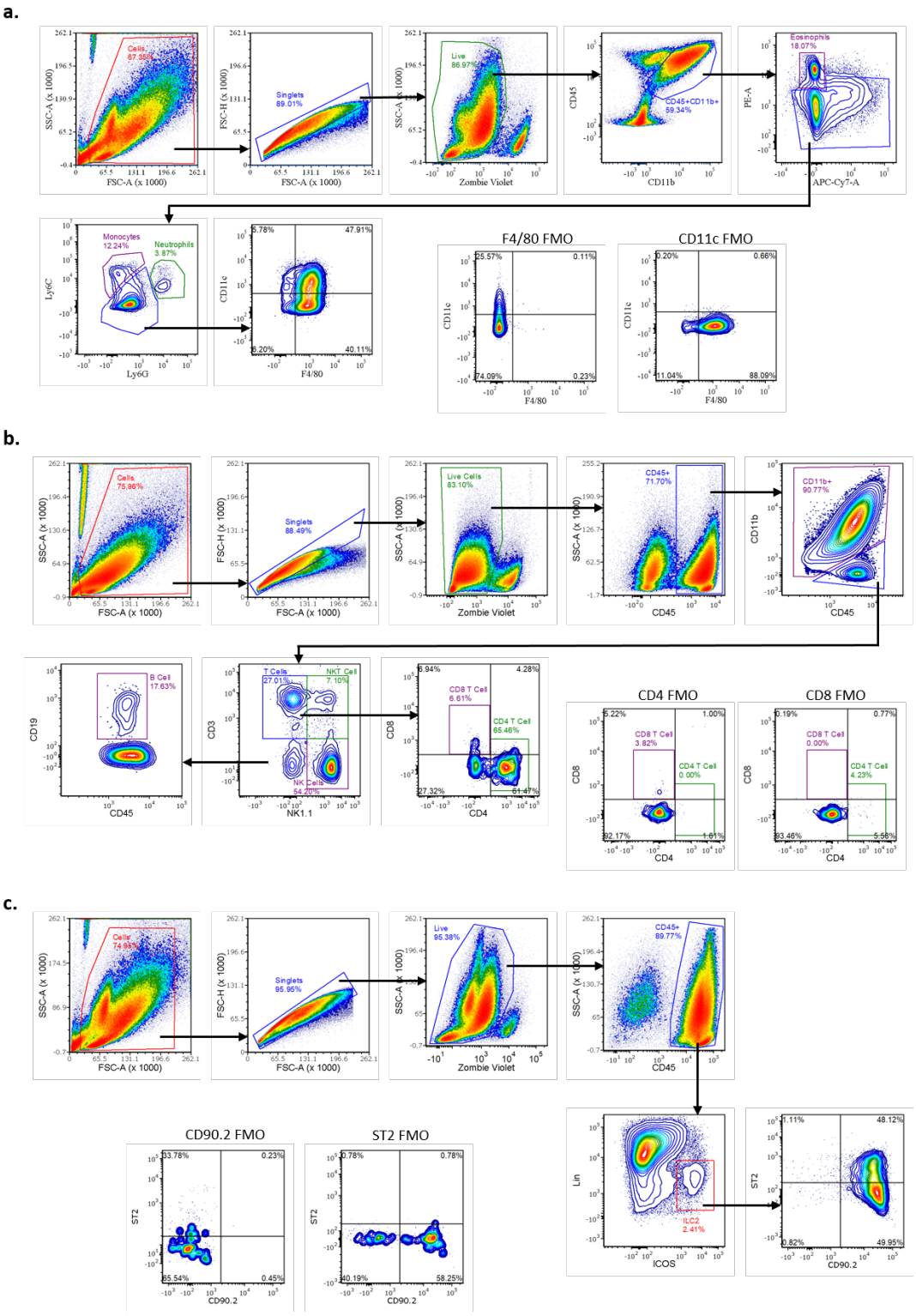
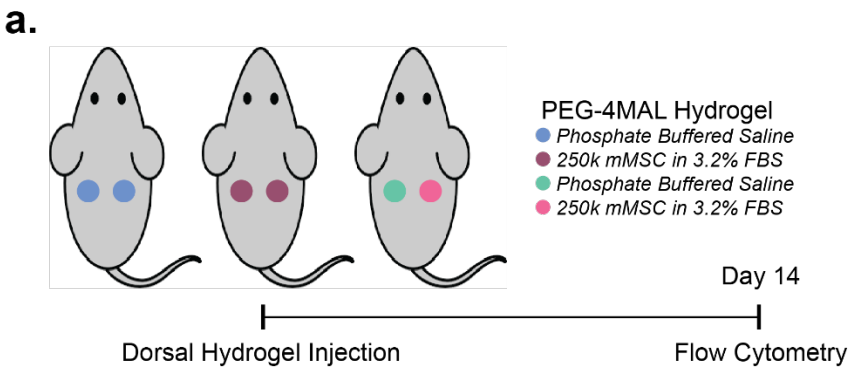
Supplementary Material

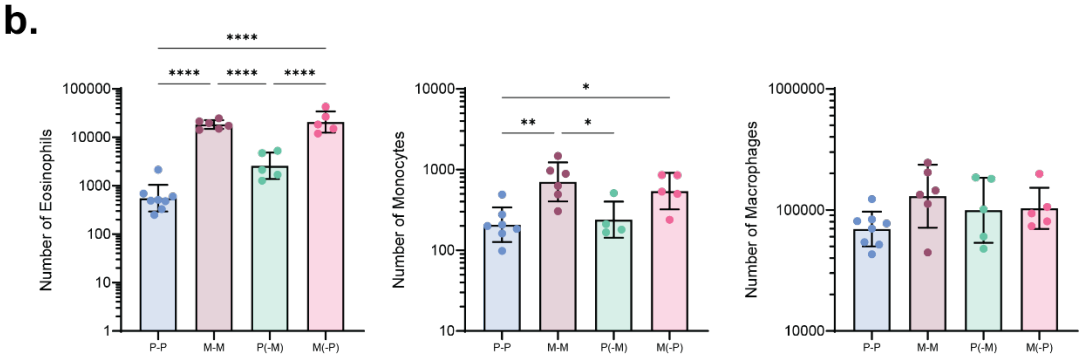
Host type 2 immune response to xenogeneic serum components impairs biomaterial-directed osteo-regenerative therapies

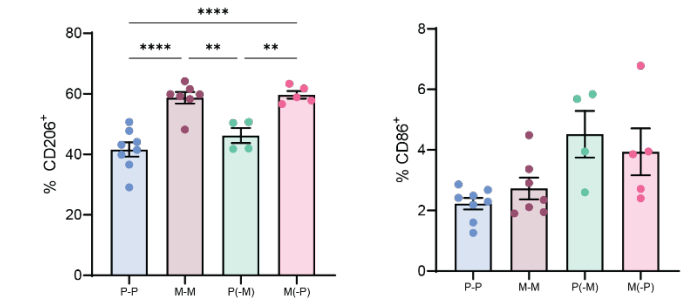
Martin et al.



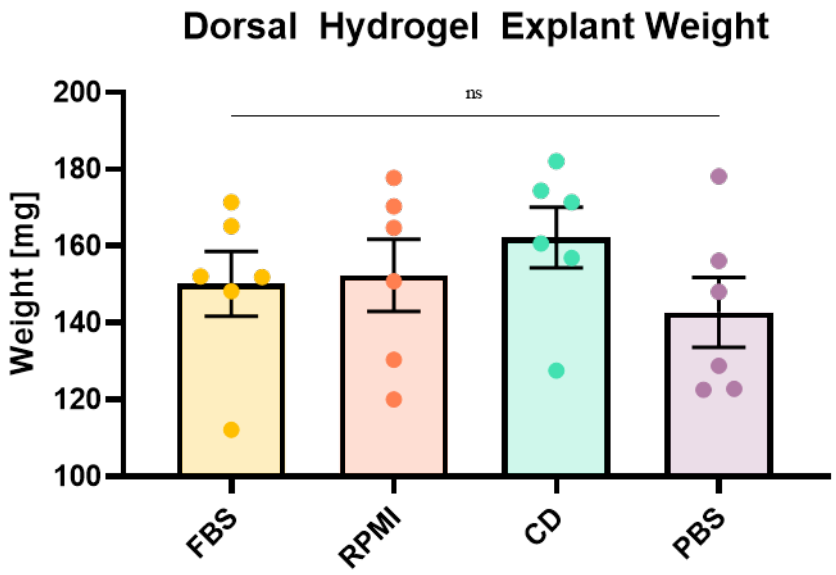
Supplementary Figure 1. Flow cytometry gating strategies. (**a**) Myeloid panel. (**b**) Lymphoid panel. (**c**) Type 2 Innate Lymphoid Cell (ILC2) panel.





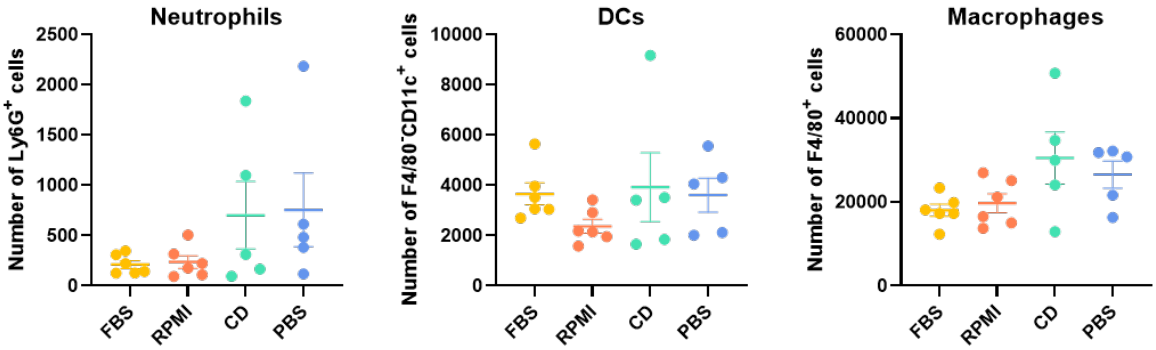


Supplementary Figure 2. Neighbor effects study. (**a**) Schematic of experimental design. Mice were subcutaneously injected with PEG-4MAL hydrogels containing either PBS or 250k mMSCs in 3.2% MSC-qualified FBS. Mice were split into 3 groups in which: (1) both gels injected contained PBS (P-P; blue), (2) both gels injected contained mMSCs + FBS (M-M; purple), (3) 1 gel of each condition were delivered: PBS with neighboring mMSC gel (P(-M); green) and mMSCs with neighboring PBS gel (M(-P); pink). (**b**) Number of infiltrating myeloid cells. Similar results were obtained for PBS-containing and mMSCs + FBS-containing gels regardless of whether the other gel in the mouse contained the same condition or a different condition; mean ± SEM. (**c**) Macrophage surface marker characterization as a percent of all F4/80^{+} cells; mean ± SEM. A one-way ANOVA was used to detect statistical differences followed by Tukey's multiple comparisons test with adjustment for multiple comparisons. *p<0.05, **p<0.01, ****p<0.0001.

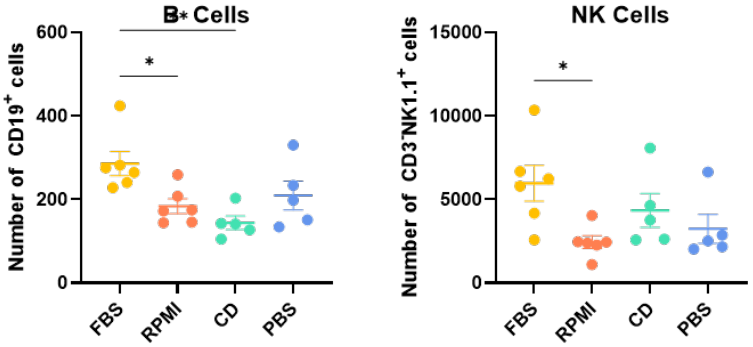


Supplementary Figure 3. Mass of dorsal gels explants. This is the wet weight of the dorsal hydrogels immediately following explanation; mean ± SEM. A one-way ANOVA was used to detect statistical differences followed by Tukey's multiple comparisons test with adjustment for multiple comparisons. No differences were detected between any groups.

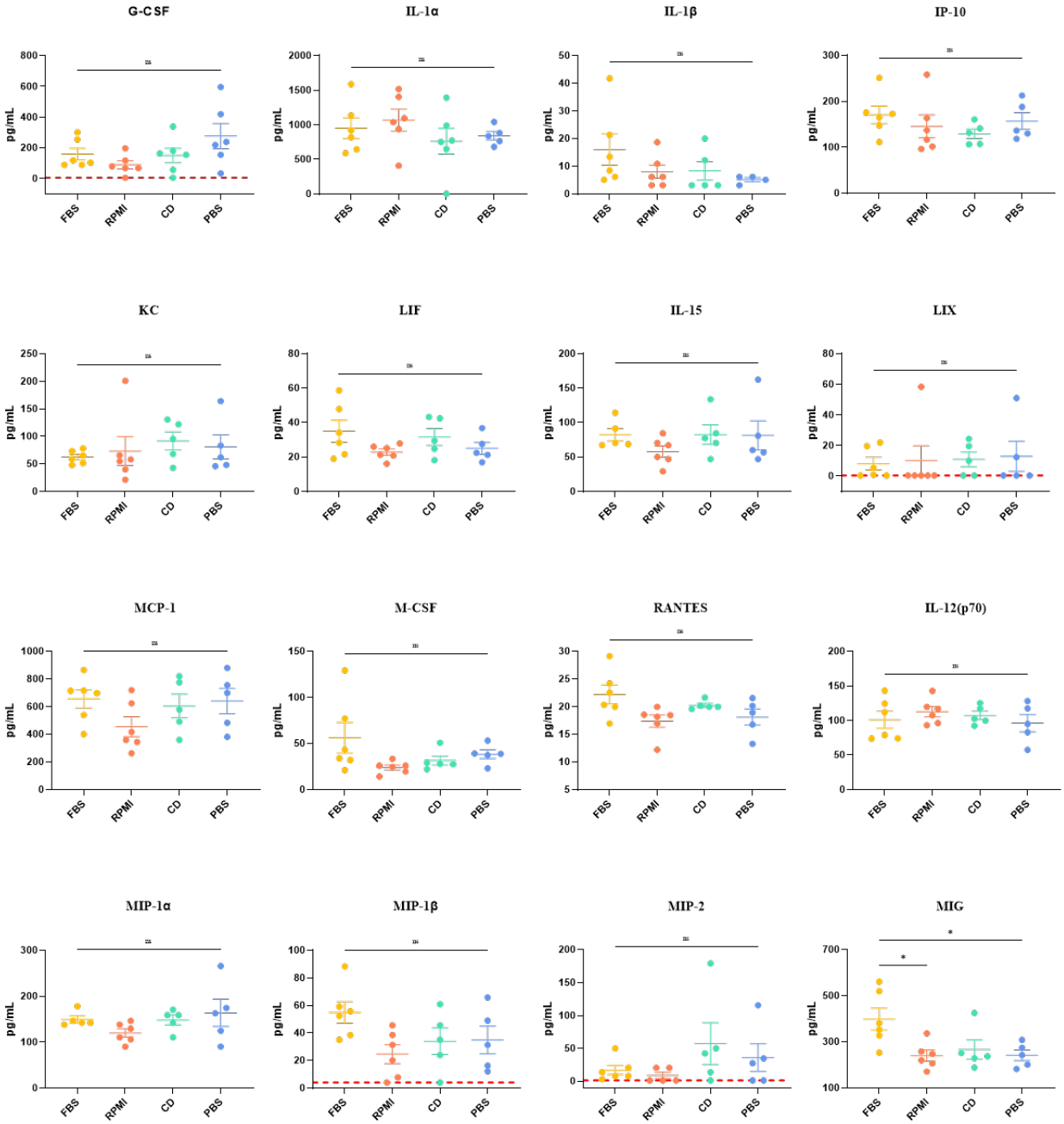
a.



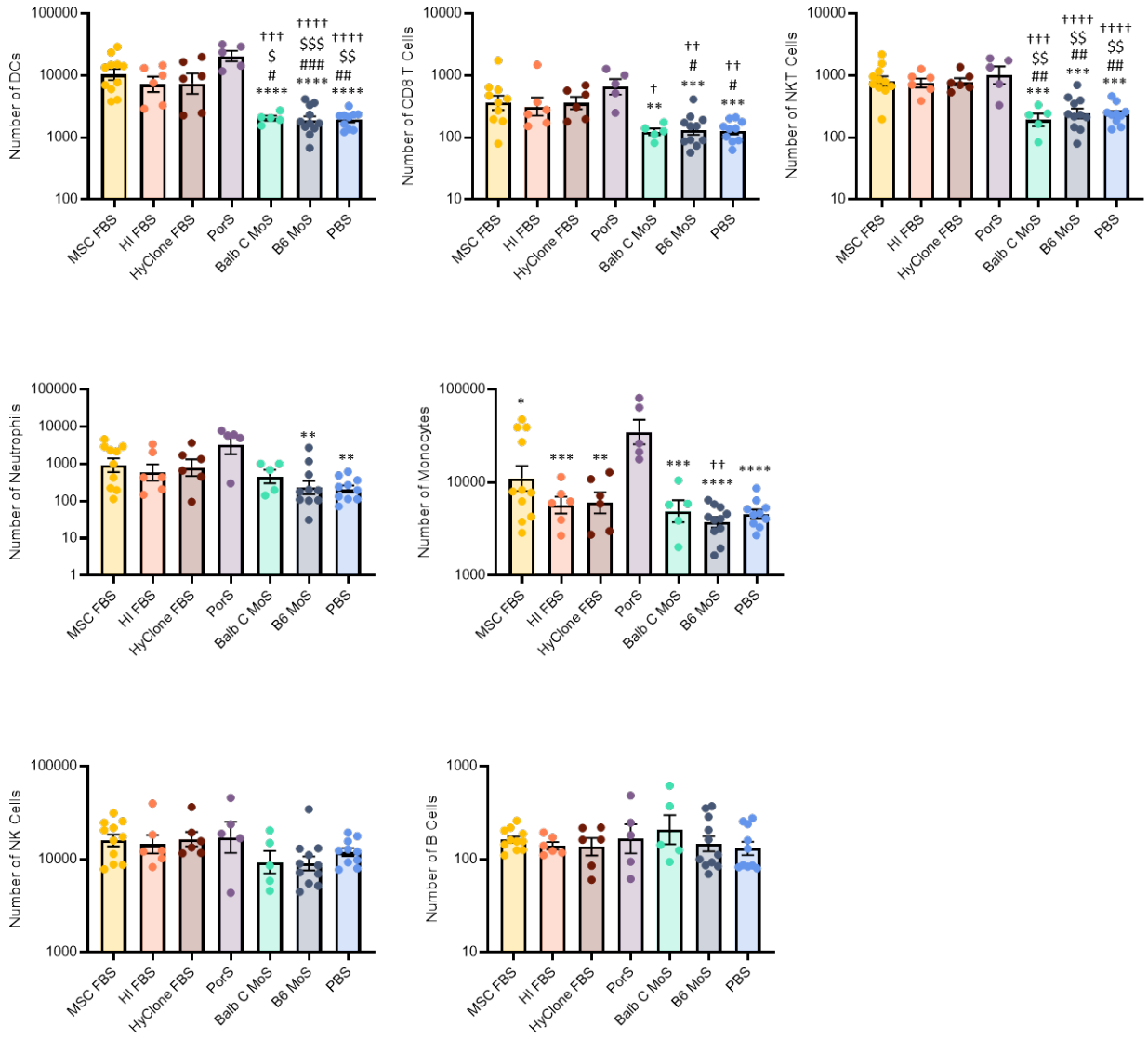
b.



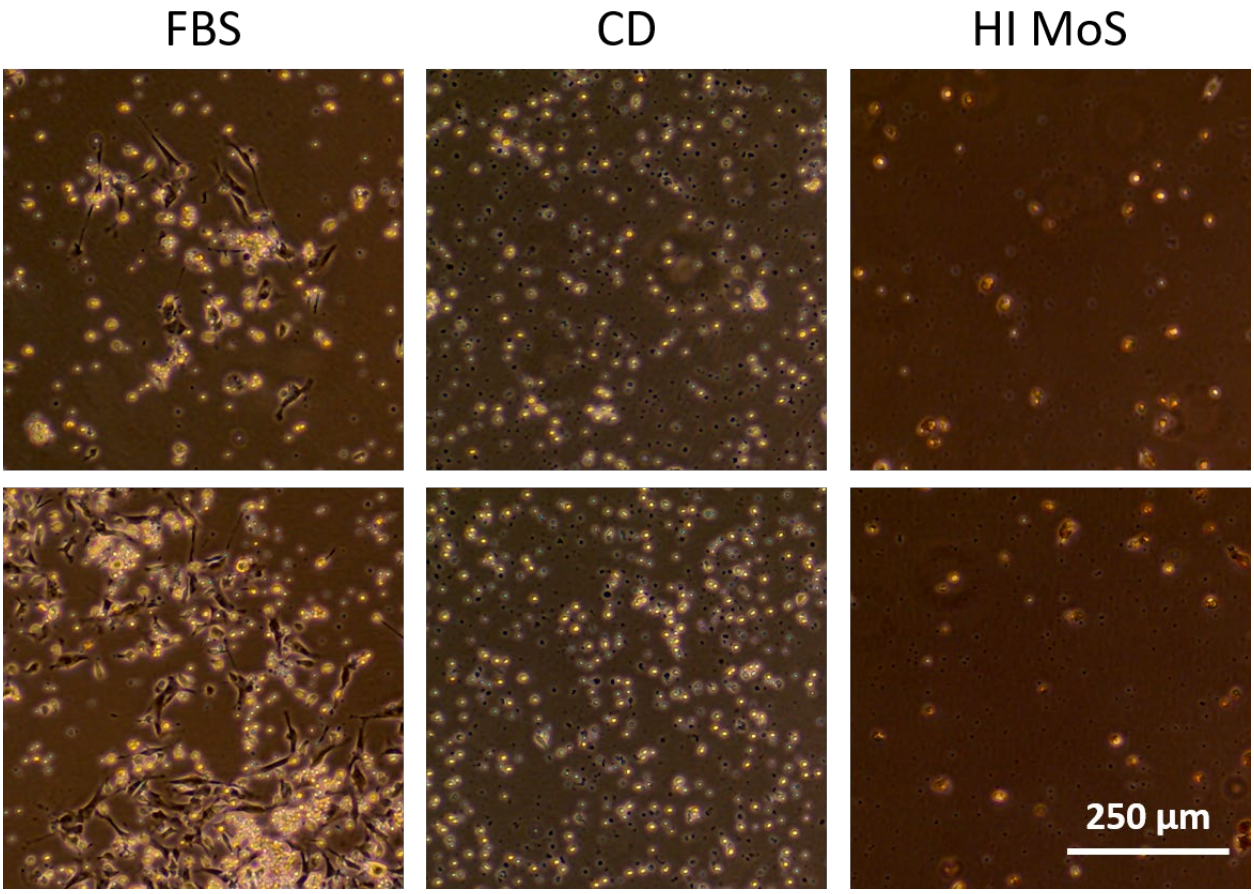
Supplementary Figure 4. Immune cell populations with no significant differences between FBS containing gels and PBS. PEG-4MAL hydrogels containing RPMI 1640 + 3.2% MSC-qualified FBS (Complete Media), RPMI 1640 (Base Media), Xcell Chemically Defined Media, or PBS were injected subcutaneously into the mouse dorsum. On day 7, hydrogels were explanted and the immune response was analyzed via flow cytometry. Number of immune cells in (**a**) $CD11b^+$ myeloid and (**b**) $CD11b^-$ lymphoid populations with no significant differences between hydrogels containing FBS and those without (n=5-6); mean ± SEM. A one-way ANOVA was used to detect statistical differences followed by Tukey's multiple comparisons test with adjustment for multiple comparisons. *p<0.05, **p<0.01.



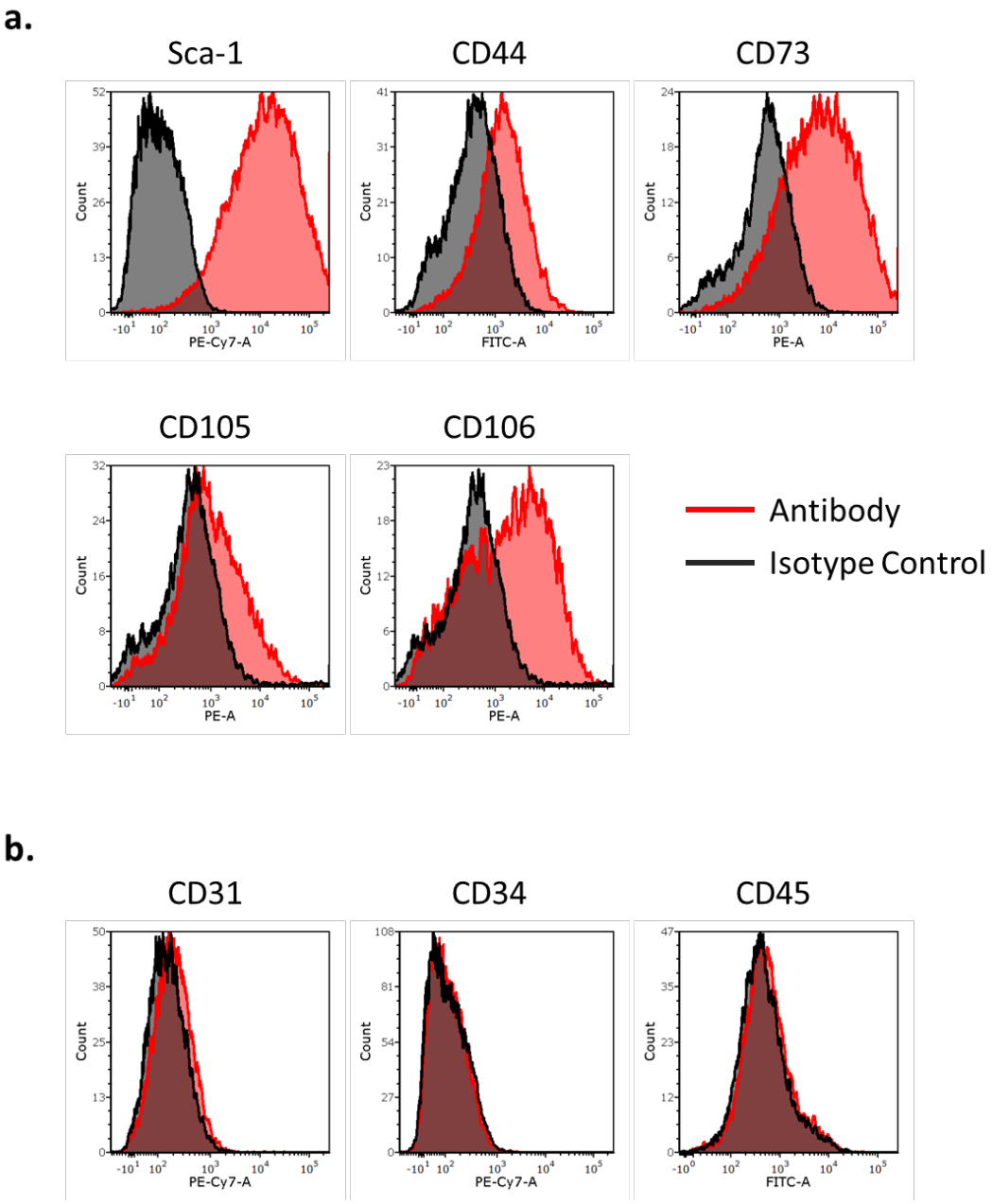
Supplementary Figure 5. Cytokines with no significant differences between groups. PEG-4MAL hydrogels containing RPMI 1640 + 3.2% MSC-qualified FBS (Complete Media), RPMI 1640 (Base Media), Xcell Chemically Defined Media, or PBS were injected subcutaneously into the mouse dorsum. On day 7, hydrogels were explanted and the immune response was analyzed by Luminex multiplex ELISA. Red, dashed line indicates the limit of detection of the Luminex MAGPIX System. A one-way ANOVA was used to detect statistical differences followed by Tukey's multiple comparisons test. *p<0.05 for MIG. There were no significant differences between any groups for any of the other cytokines listed here. VEGF was above the limit of detection for all samples. IL-17A was below limit of detection for all samples.



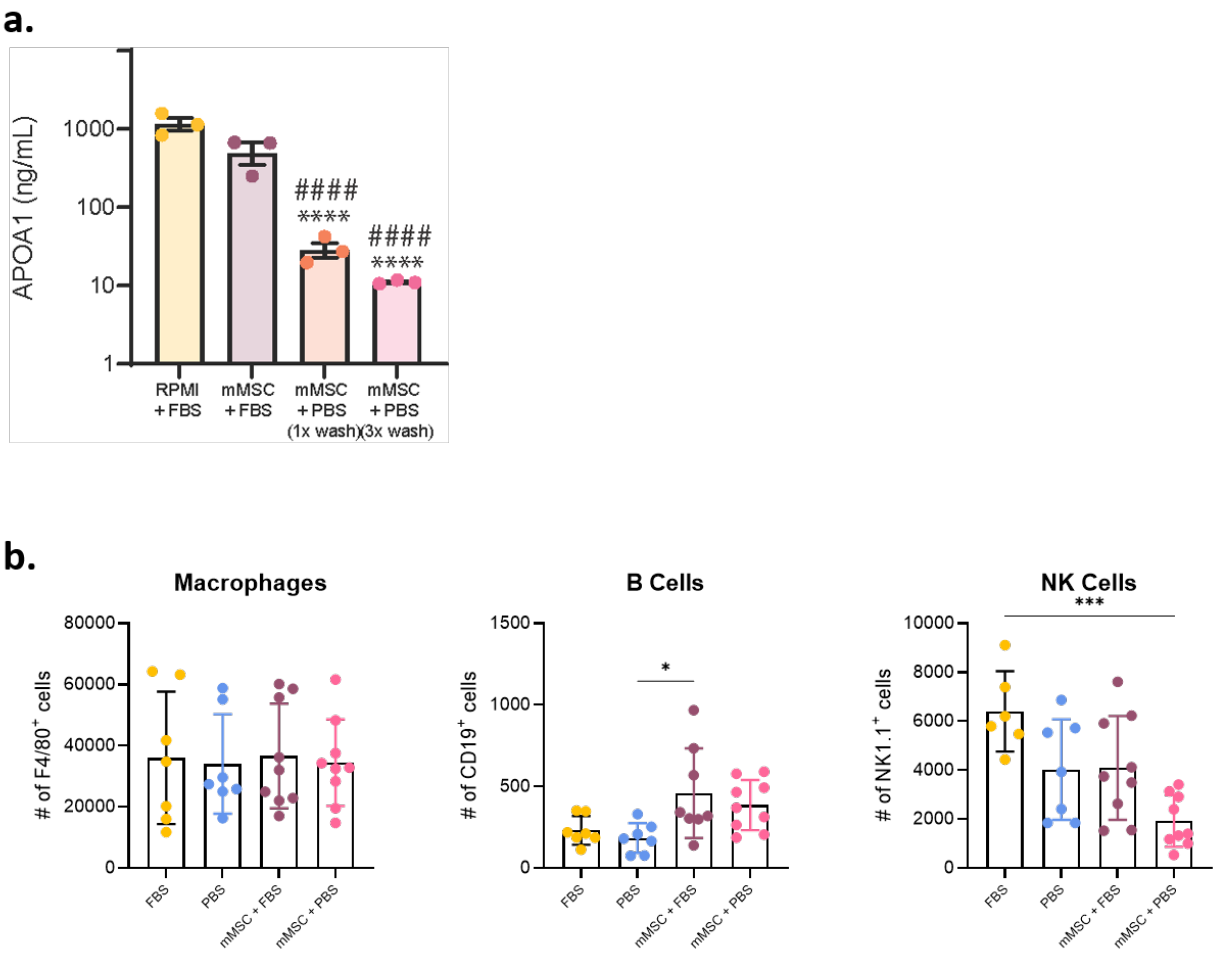
Supplementary Figure 6. Number of infiltrating DCs, CD8 T cells, NKT cells, and immune cell populations with no significant differences between FBS and PBS containing gels in the serum comparison study. A one-way ANOVA was used to detect statistical differences followed by Tukey's multiple comparisons test with adjustment for multiple comparisons. Data was log transformed prior to ANOVA analysis. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$, $^{\dagger\dagger\dagger\dagger}p<0.000$ vs. MSC FBS; $^{\$}p<0.05$, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$, $^{\$\$\$\$}p<0.000$ vs. HI FBS; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.000$ vs. HyClone FBS; *p<0.05, **p<0.01, ***p<0.001, ****p<0.000 vs. PorS.



Supplementary Figure 7. Bright-field images of mMSCs after 2 days in culture and one feeding. mMSCs were cultured in media supplemented with 16% FBS as usual, 16% heat inactivated mouse serum (Rockland), or cultured in chemically defined media intended for use in culturing human MSCs. Representative images of n=2 independent studies.



Supplementary Figure 8. mMSC surface marker characterization. Histograms from flow cytometry data of bone marrow derived mMSCs stained for (**a**) positive markers: Sca-1, CD44, CD73, CD105, and CD106 and (**b**) negative markers: CD31, CD34, and CD45



Supplementary Figure 9. Immune response to washed and unwashed mMSCs. (**a**) Concentration, as measured by ELISA, of serum component APOA1 in cell suspensions of *ex vivo* expanded mMSCs following washing the cell pellet either once or 3 times with 15 mL of PBS. Unwashed cell pellet in complete media (RPMI + 3.2% MSC-qualified FBS) and complete media without cells were included as controls. Data was log-transformed and a one-way ANOVA was used to detect statistical differences followed by Tukey's multiple comparisons test with adjustment for multiple comparisons. ****$p<0.0001$ vs. RPMI + FBS; $^{\#\#\#\#}p<0.0001$ vs. mMSC + FBS. (**b**) Immune cell populations with no significant differences between mMSC-laden hydrogels containing FBS and PBS. A one-way ANOVA was used to detect statistical differences followed by Tukey's multiple comparisons test with adjustment for multiple comparisons. *$p<0.05$, ***$p<0.001$.