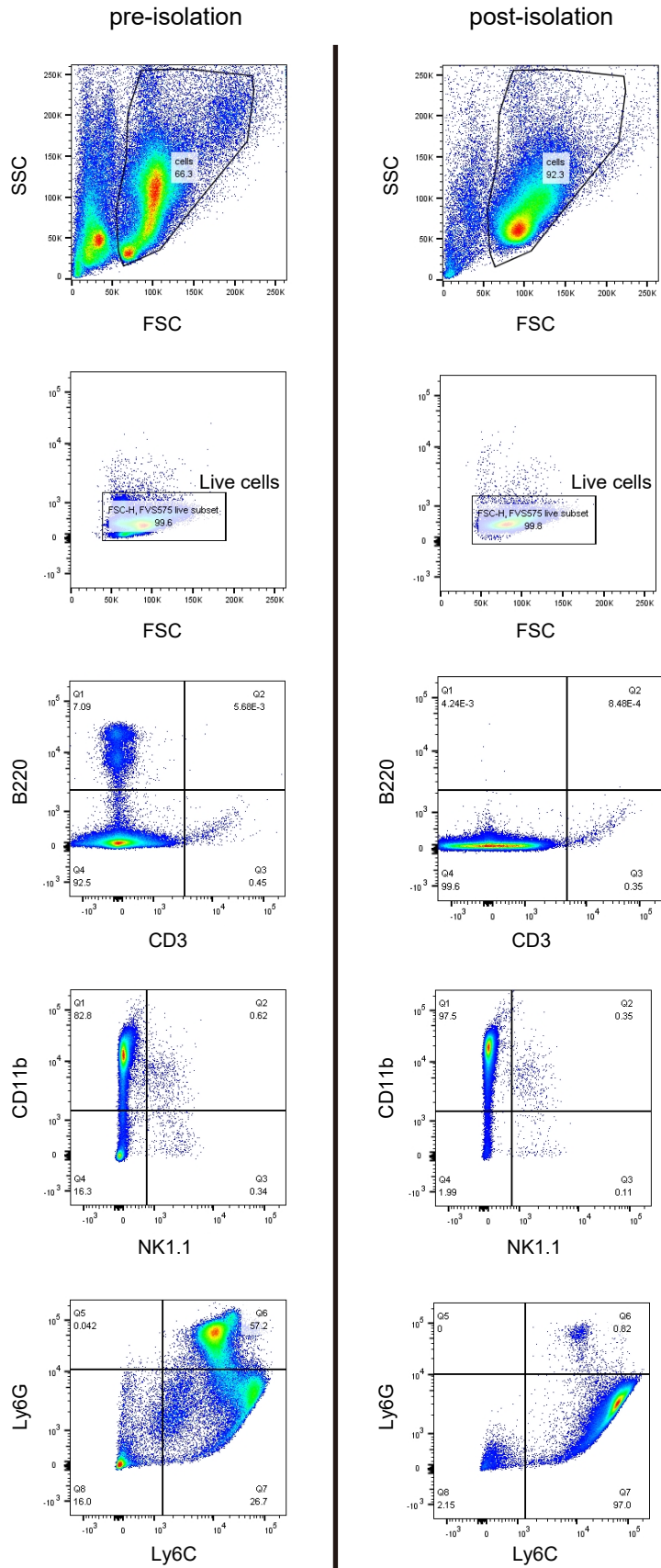


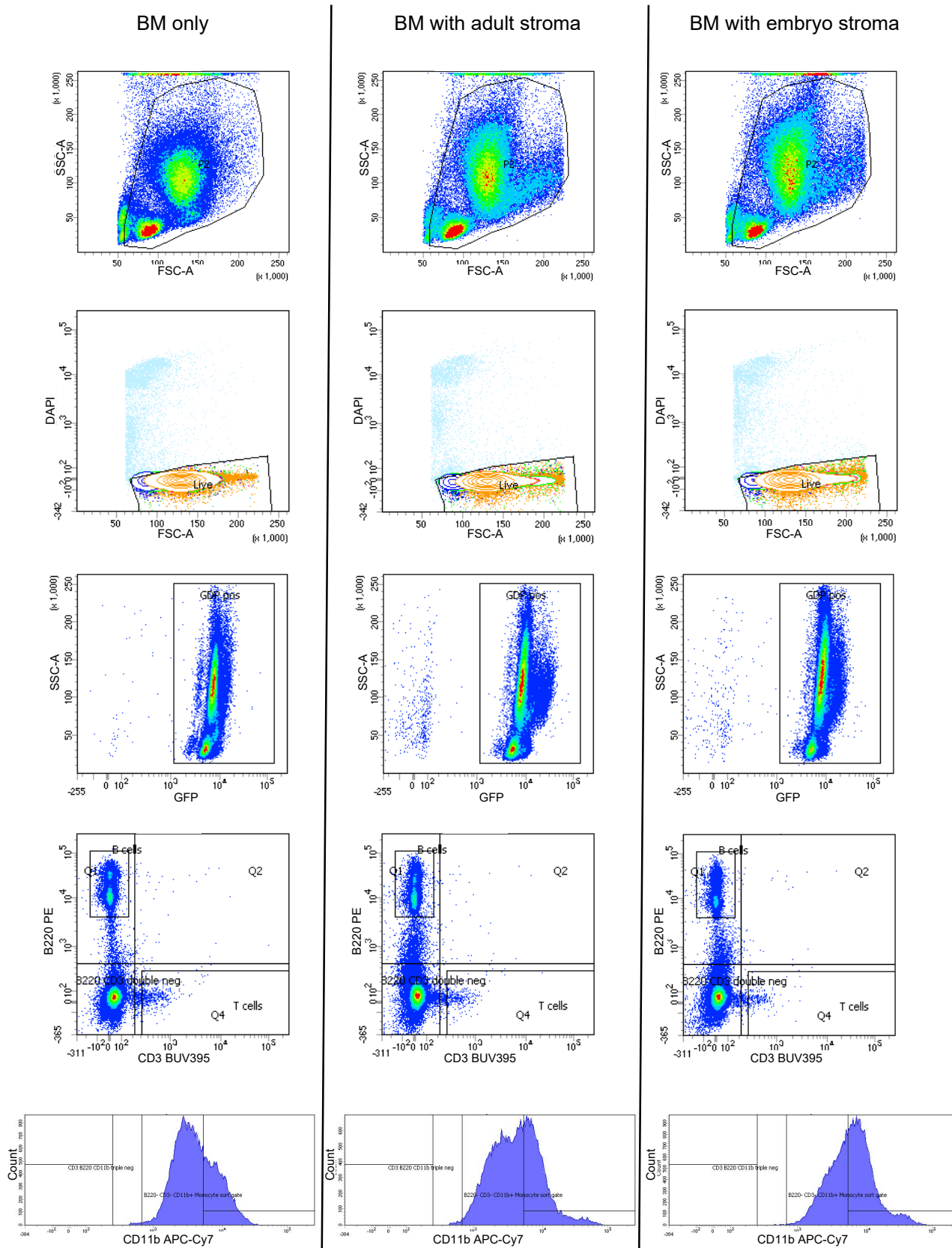
**Supplementary Files-** Delineating proinflammatory microenvironmental signals by ex vivo modeling of the immature intestinal stroma.

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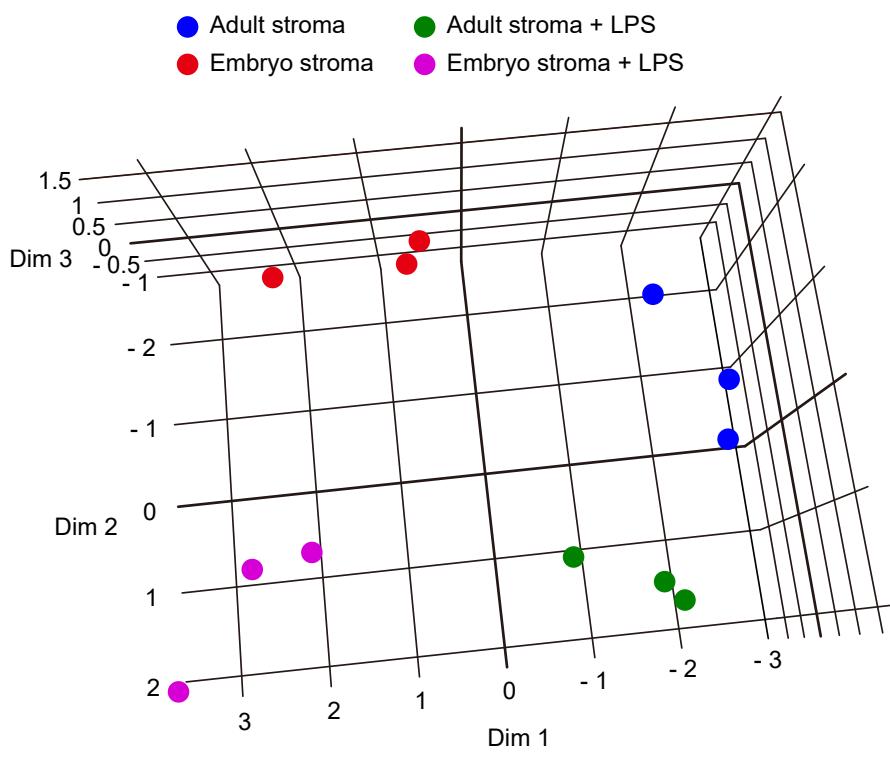


Suppl Fig.1 FACS validation of MACS enrichment of CD3-B220-NK1.1-CD11b+Ly6G-Ly6C+ monocytes from mouse bone marrow cells.



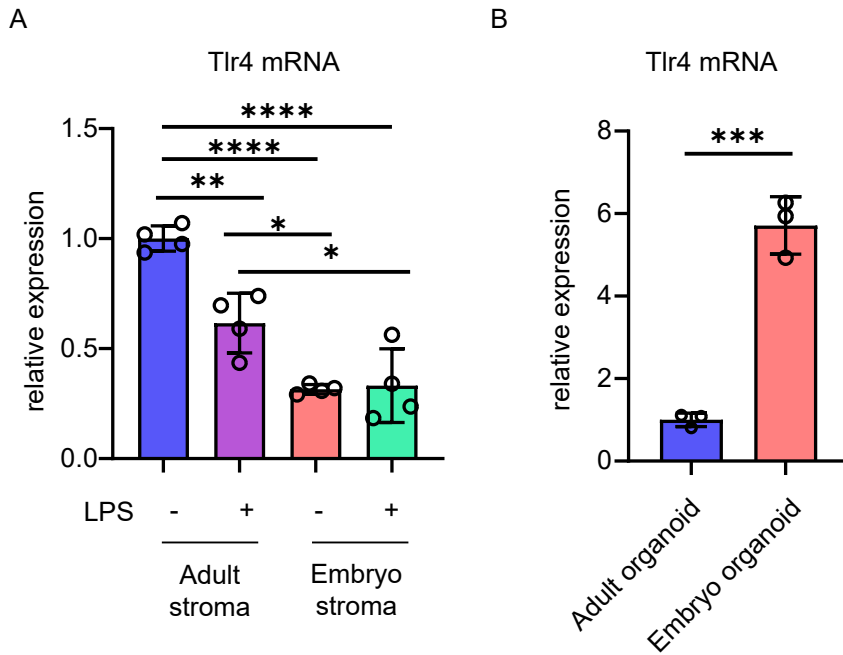
Suppl Fig.2 FACS gating strategy to identify the proportion of T cells, B cells, and myelomonocytic cells in co-cultures of wild-type intestinal stromal cells and GFP+ bone marrow derived cells.

DAPI- GFP+ live bone marrow cells were classified into CD3+ T cells, B220+ B cells, and CD3-B220-CD11b+ myelomonocytic cells. The proportion of each cell population is shown in Fig.1A.



Suppl Fig.3 Multidimensional scaling plot comparison of transcriptome of LPS or control vehicle treated embryonic and adult stromal cells ex vivo.

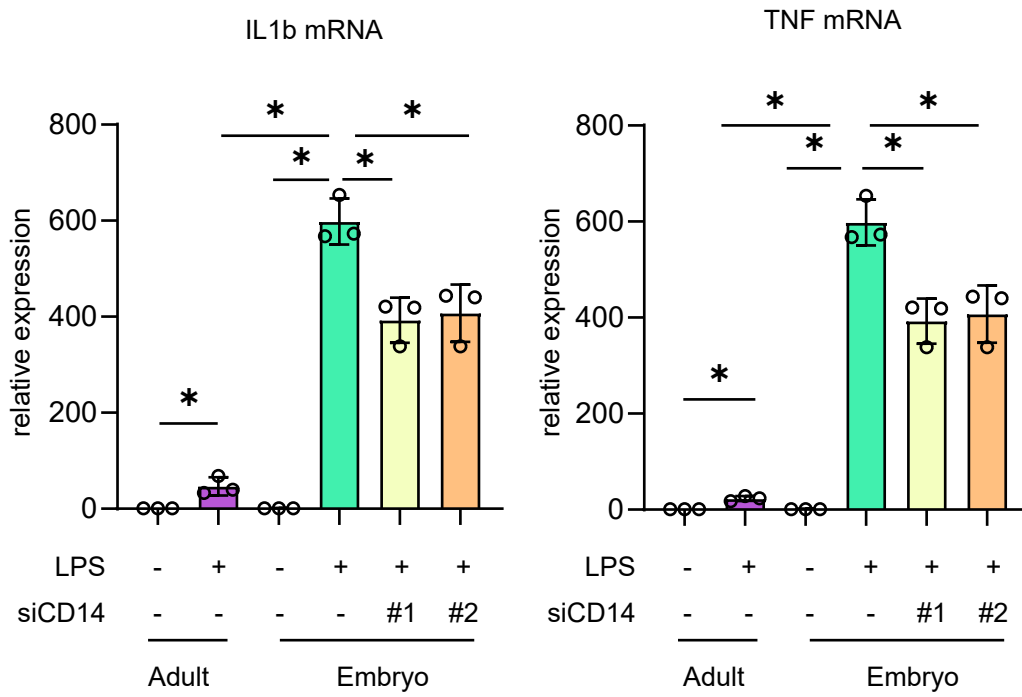
# Ichinose et al., Suppl Figure4



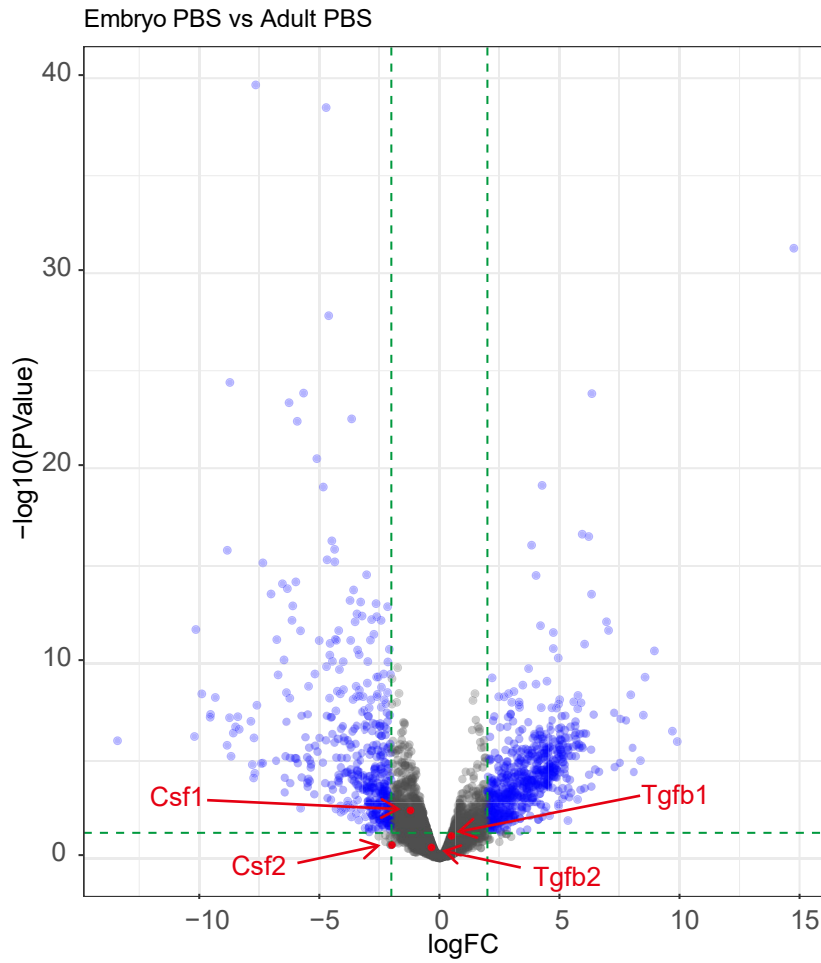
Suppl Fig.4 TLR4 expression is not induced in the embryonic or adult intestinal stroma following LPS stimulation, but is upregulated in embryonic intestinal epithelium.

(A) TLR4 mRNA expression normalized to Gapdh in adult and embryonic stromal cells treated with LPS or vehicle by qPCR. N=4 biological replicates. t-test. \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001

(B) TLR4 mRNA expression normalized to Gapdh in adult and embryonic small intestinal organoids (epithelial cells) by qPCR. N=3 biological replicates. t-test. \*\*\*p<0.001



Suppl Fig.5 CD14 regulates inflammatory cytokine production from monocyte derived cells. IL1b and TNF mRNA expression normalized to Gapdh in LPS or vehicle treated adult and embryonic intestinal stromal cells, with the addition of control or CD14 siRNA knockdown in embryonic stromal cell cultures. N=3 biological replicates. t-test. \*p<0.05



Suppl Fig.6 Volcano plot of differentially expressed transcripts between vehicle treated adult and embryonic intestinal stromal cells. Colony-stimulating factor (CSF)-1,2 and transforming growth factor beta (TGF $\beta$ )-1,2 were not significantly differently expressed between embryonic and adult intestinal stromal cells. Absolute value of  $\log_{2}$  fold change  $\geq 2.0$ , FDR $<0.01$