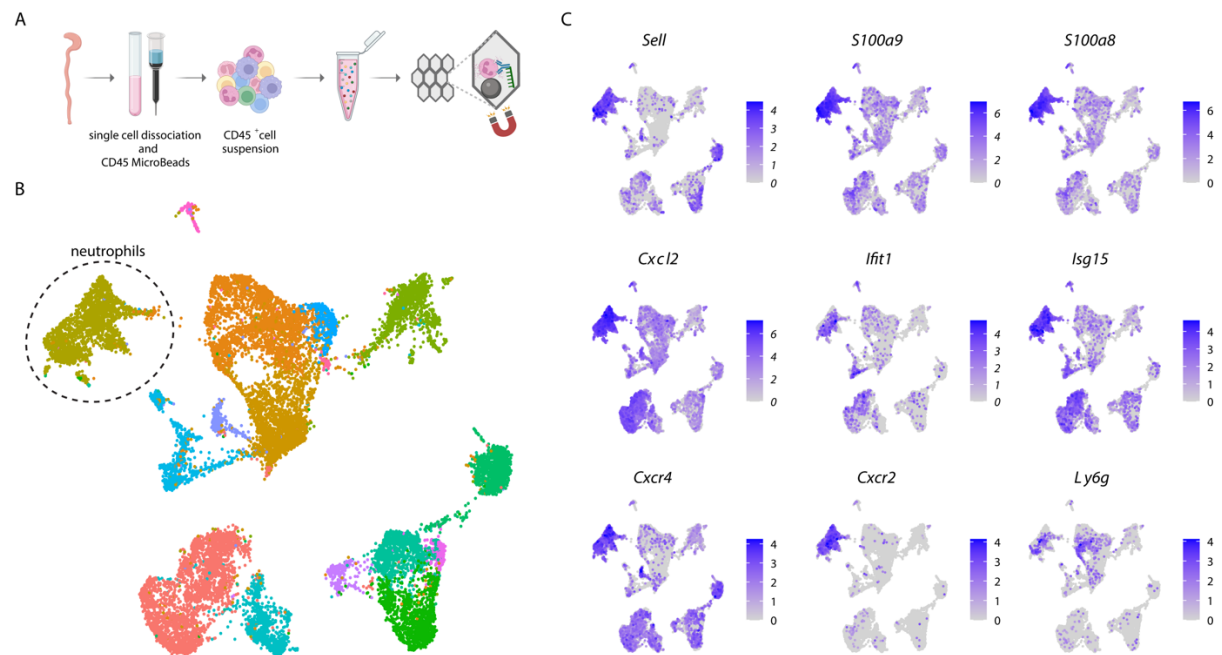


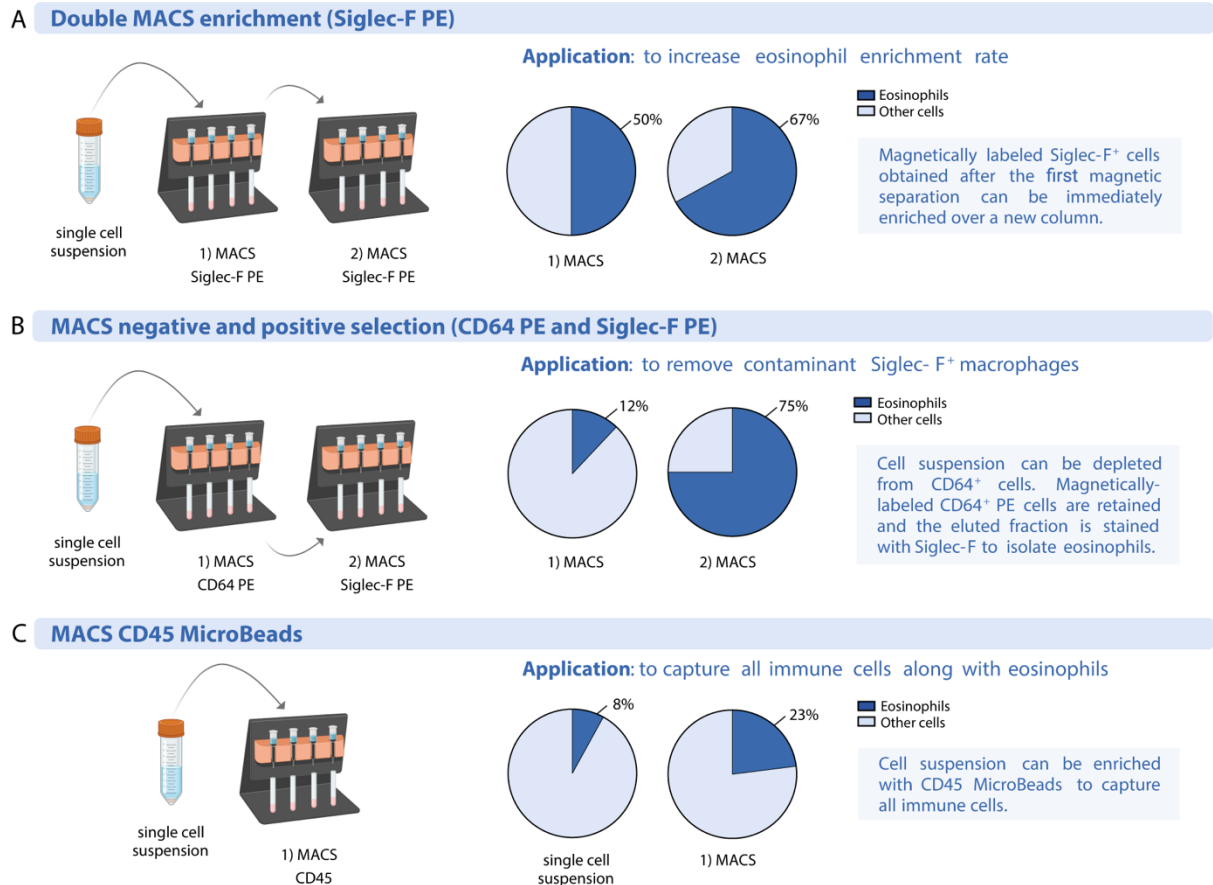
# Stress-free single-cell transcriptomic profiling and functional genomics of murine eosinophils

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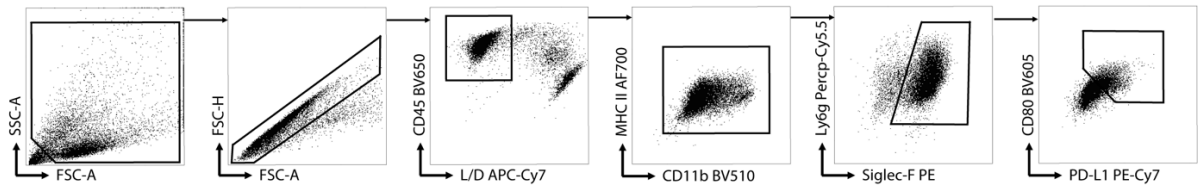
## Supplementary Figures



**Supplementary Figure 1. Murine neutrophil single-cell transcriptomes are found in magnetically enriched CD45<sup>+</sup> cells.** **A**, Experimental workflow of scRNAseq. Single cell suspension was prepared from murine colons of WT mice (n = 6, B6J). CD45 cells were enriched using CD45 MicroBeads. Single cell RNA was captured with the BD Rhapsody system. **B**, Uniform Manifold Approximation and Projection (UMAP) of all single-cell transcriptomes passing quality control. **C**, UMAPs showing the expression of neutrophil-specific marker genes<sup>62</sup> in cluster 3. This figure was created using a licensed version of Biorender.com.



**Supplementary Figure 2. Alternative strategies to magnetically enrich murine eosinophils from single cell suspensions.** **A**, Eosinophils are enriched from the colon of *I15*-tg mice ( $n = 1$ ) using Siglec-F PE antibody and anti-PE MicroBeads. To increase the purity of eosinophils, magnetically labeled cells are enriched over a second column as detailed in the manufacturer's protocol. **B**, A single cell suspension obtained from the lungs of *I15*-tg mice ( $n = 2$ ) is stained with CD64 PE antibody (X54-5/7.1, BioLegend) and anti-PE MicroBeads. Magnetically labeled cells (mostly macrophages) are retained in the column and the unlabeled cell fraction is subsequently stained with Siglec-F PE antibody and anti-PE MicroBeads to isolate eosinophils. **C**, Eosinophils are isolated from the colon of *I15*-tg mice ( $n = 1$ ) as part of the CD45<sup>+</sup> cells. CD45 MicroBeads are used for the positive selection of CD45 cells. **A-C**, Left, schematic overview of the workflow. Single cell suspensions are prepared from the organ of interest. Middle: pie charts represent the percentage of eosinophils in the single cell suspension after MACS isolation. Eosinophil purity is assessed by flow cytometry. Eosinophils are gated as live, CD45<sup>+</sup> CD11b<sup>+</sup> MHCII<sup>+</sup> Ly6G<sup>-</sup> Siglec-F<sup>+</sup> cells. Examples of when alternative strategies are required are suggested under "Application". This figure was created using a licensed version of Biorender.com.



**Supplementary Figure 3. Gating strategy to identify CD80-PD-L1<sup>+</sup> and CD80-PD-L1<sup>-</sup> eosinophils.** Sorting strategy to isolate live CD45<sup>+</sup> CD11b<sup>-</sup> MHCII<sup>+</sup> Ly6G<sup>-</sup> Siglec-F<sup>-</sup> CD80<sup>+</sup> PD-L1<sup>+</sup> (A-Eos) and CD80<sup>+</sup> PD-L1<sup>-</sup> eosinophils. The raw data used to generate this figure have previously been published in ref. <sup>10</sup>.