

Supplemental Information

Chole-seq: a modular single-cell multi-omics framework for chemical decoding of membrane cholesterol and transcriptomes

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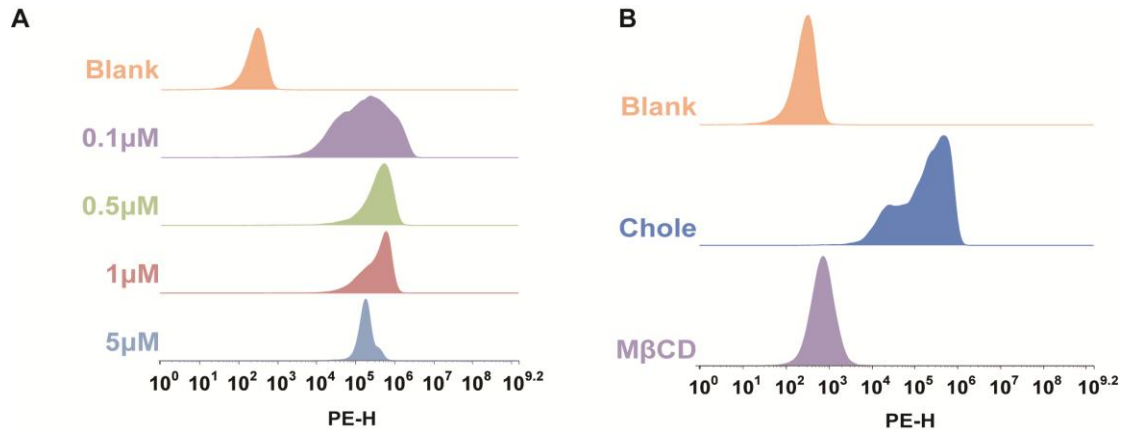
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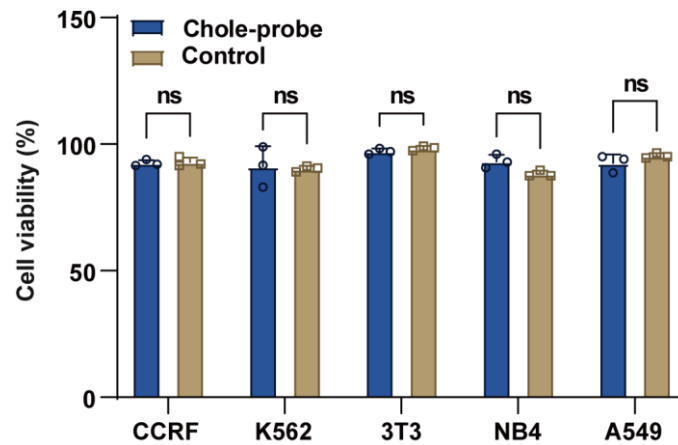
29 **Supplementary Figures**



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31 **Figure S1.** Optimization and specificity validation of Chole-seq. (A) Dose-dependent
32 analysis of Chole-probe labeling in Jurkat cells at concentrations ranging from 0.1 to 5
33 μM. (B) Specificity validation by cholesterol depletion with MβCD in Jurkat cells.

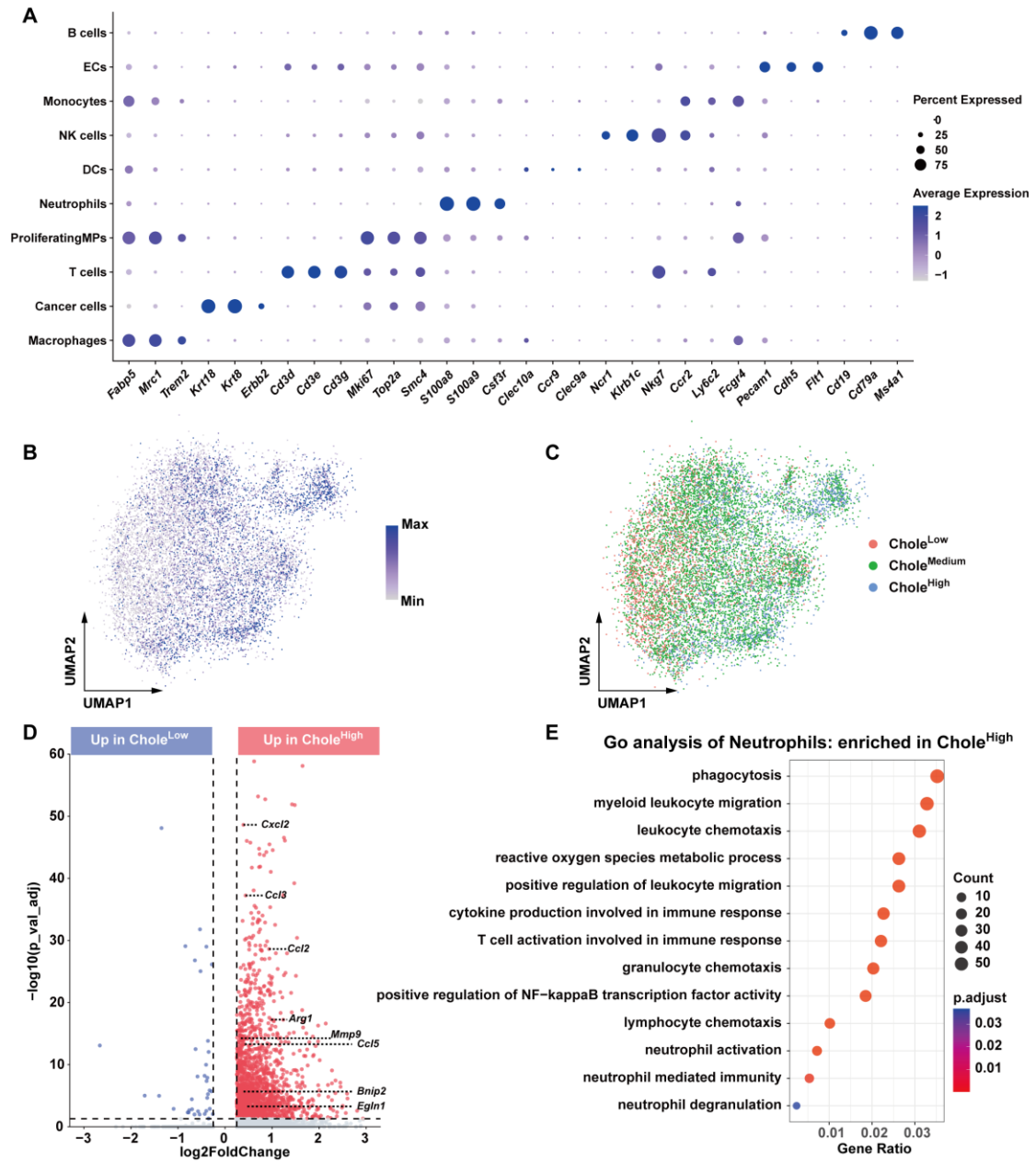
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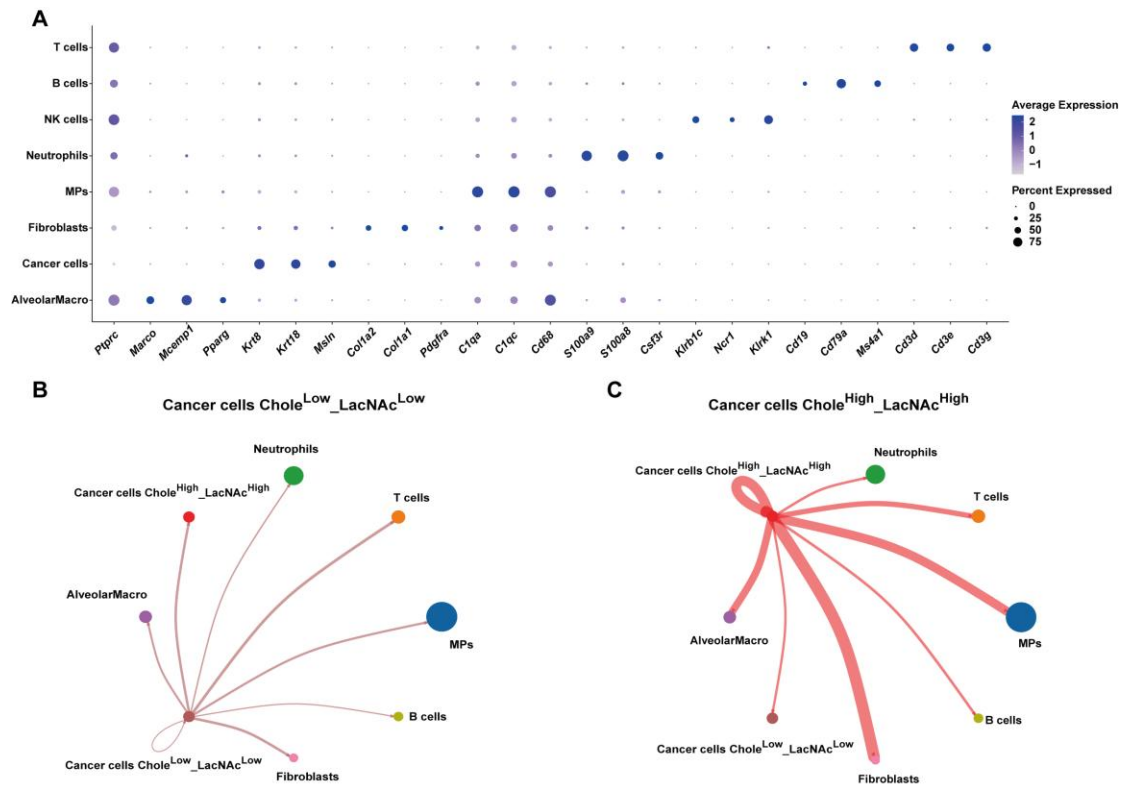
36 **Figure S2.** Biocompatibility assessment of Chole-probe labeling by AO/PI staining to
37 evaluate cell viability (n = 3). Data are presented as mean ± SD. P values were
38 determined by two-way ANOVA. ns, $P > 0.05$.

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41 **Figure S3.** Characterization of membrane cholesterol heterogeneity in neutrophils. (A)
 42 Canonical marker genes used for cell type identification in the MB49 mouse model. (B)
 43 UMAP plots showing membrane cholesterol abundance in neutrophils based on
 44 transcriptomic profiles. (C) Neutrophil subpopulations were classified into Chole^{High},
 45 Chole^{Medium}, and Chole^{Low} groups according to cholesterol abundance. (D) Differential
 46 gene expression analysis between the Chole^{High} and Chole^{Low} groups. (E) GO analysis
 47 identifying enriched biological processes in the Chole^{High} and Chole^{Low} groups.



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49 **Figure S4.** Functional validation of Chole^{High}_LacNAc^{High} and Chole^{Low}_LacNAc^{Low}
 50 tumor cells in the MB49 mouse lung metastasis model. (A) Cell type annotation
 51 markers. (B, C) Cell communication landscapes of Chole^{Low}_LacNAc^{Low} cells and
 52 Chole^{High}_LacNAc^{High} cells.

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