

Fig. S1. Generation and characterization of hPSC-derived macrophages. A. Representative flow cytometric analyses of CD34 and CD43 expression in WNTi and WNTd hematopoietic progenitor cultures on day 8 of differentiation, as well as CD34 expression in human cord blood. B. Erythroid and myeloid potential of CD34+CD43+ and CD34-CD43+ WNTi cell fractions, as determined from numbers of EryP-CFC and CFU-GM colonies, respectively, forming in MethoCult H4034. Percentages are averaged from 3 experiments. C. Representative forward scatter (FSC) and side scatter (SSC) flow cytometry plots for quantification shown in Fig. 1C. D. Median fluorescence intensity of pHrodoRed in WNTd and WNTi macrophages following 1 hour of incubation with pHrodoRed *E. coli* particles. Results are from three independent macrophage differentiations. Two-tailed Student's t-test.

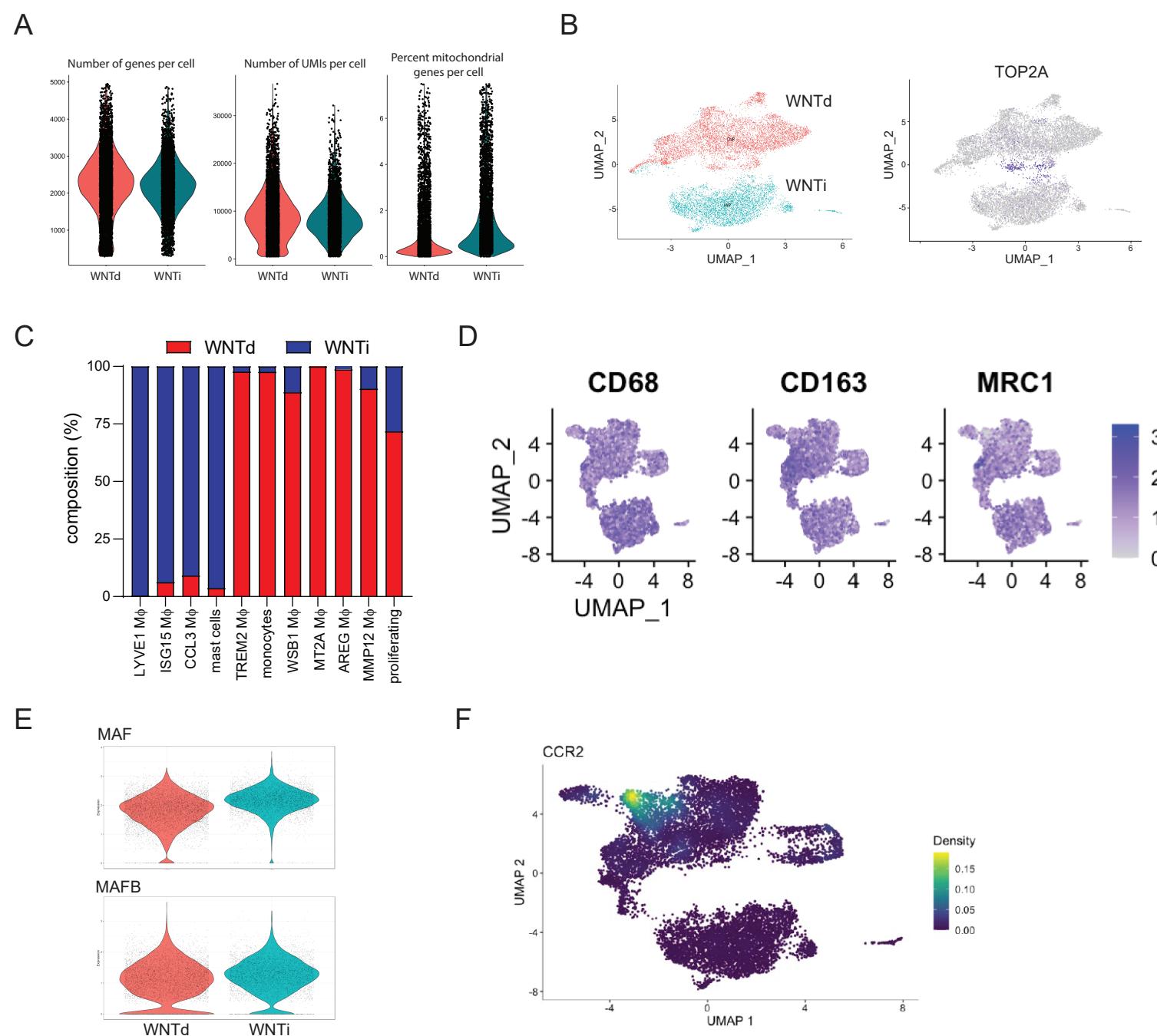


Fig. S2. scRNAseq analysis of hPSC-derived macrophages. A. scRNAseq parameters for WNTd and WNTi Day 14 macrophage cultures in Fig. 2. B. UMAP plots of WNTi and WNTd Day 14 macrophage cultures with cell cycle regression, showing that many TOP2A positive cells are still forming a separate cluster after regression. For this reason, this dataset was analyzed without cell cycle regression. C. The percentage of WNTi and WNTd cells making up each cluster in Fig. 2B. D. Feature plots of expression of common macrophage genes in the WNTi and WNTd macrophages. E. Violin plots of expression of macrophage transcription factors *MAF* and *MAFB* in WNTd and WNTi macrophages. F. Density plot of *CCR2* gene expression in the Day 14 macrophage cultures in Fig. 2.

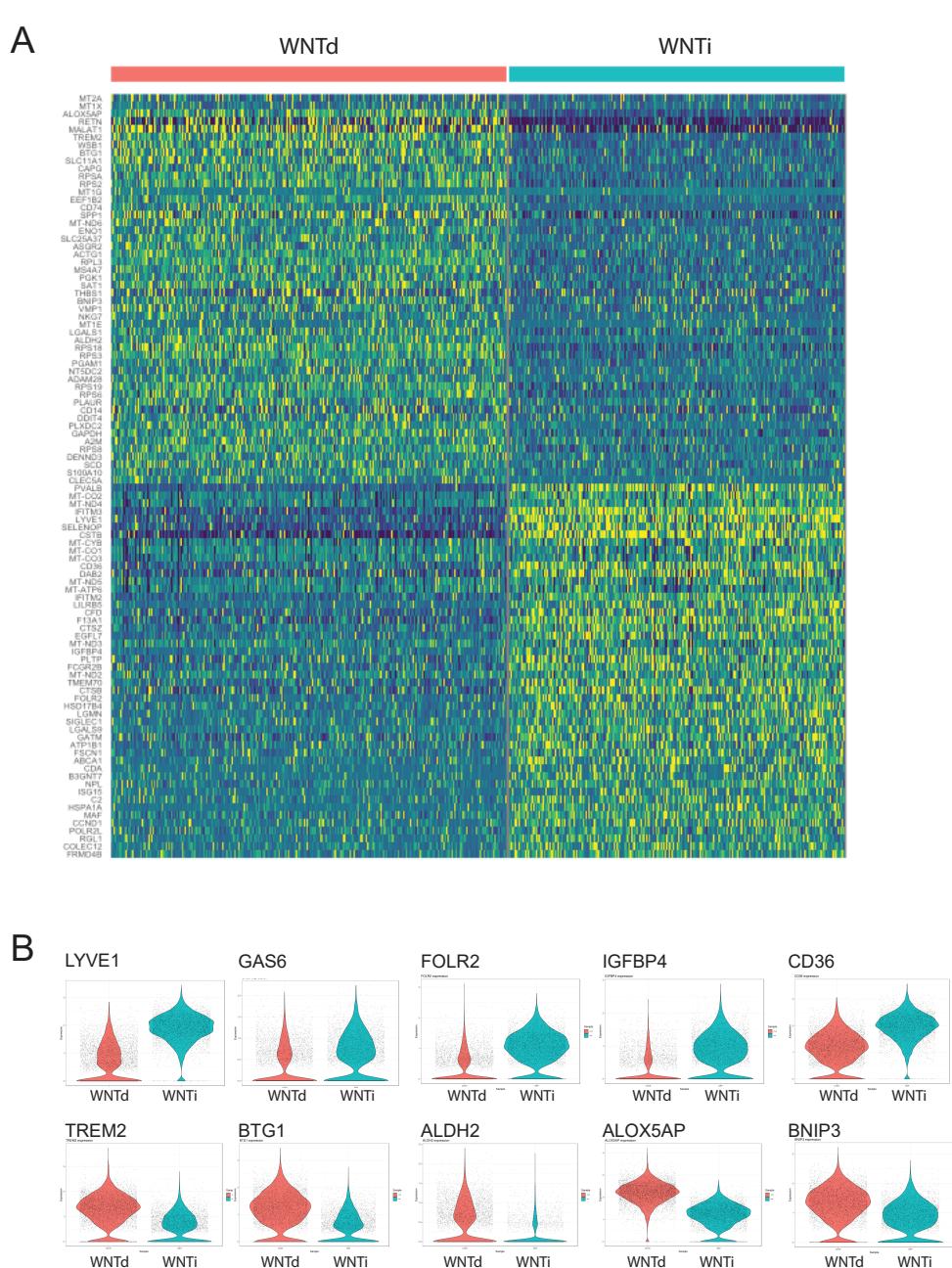


Fig. S3. WNTd and WNTi macrophage gene signatures. A. Heat map of the genes with log₂ expression difference greater than 0.5 in the WNTd and WNTi macrophage clusters in Fig. 2E. B. Violin plots of the individual genes that make up the WNTi and WNTd macrophage signatures in Fig. 2E.

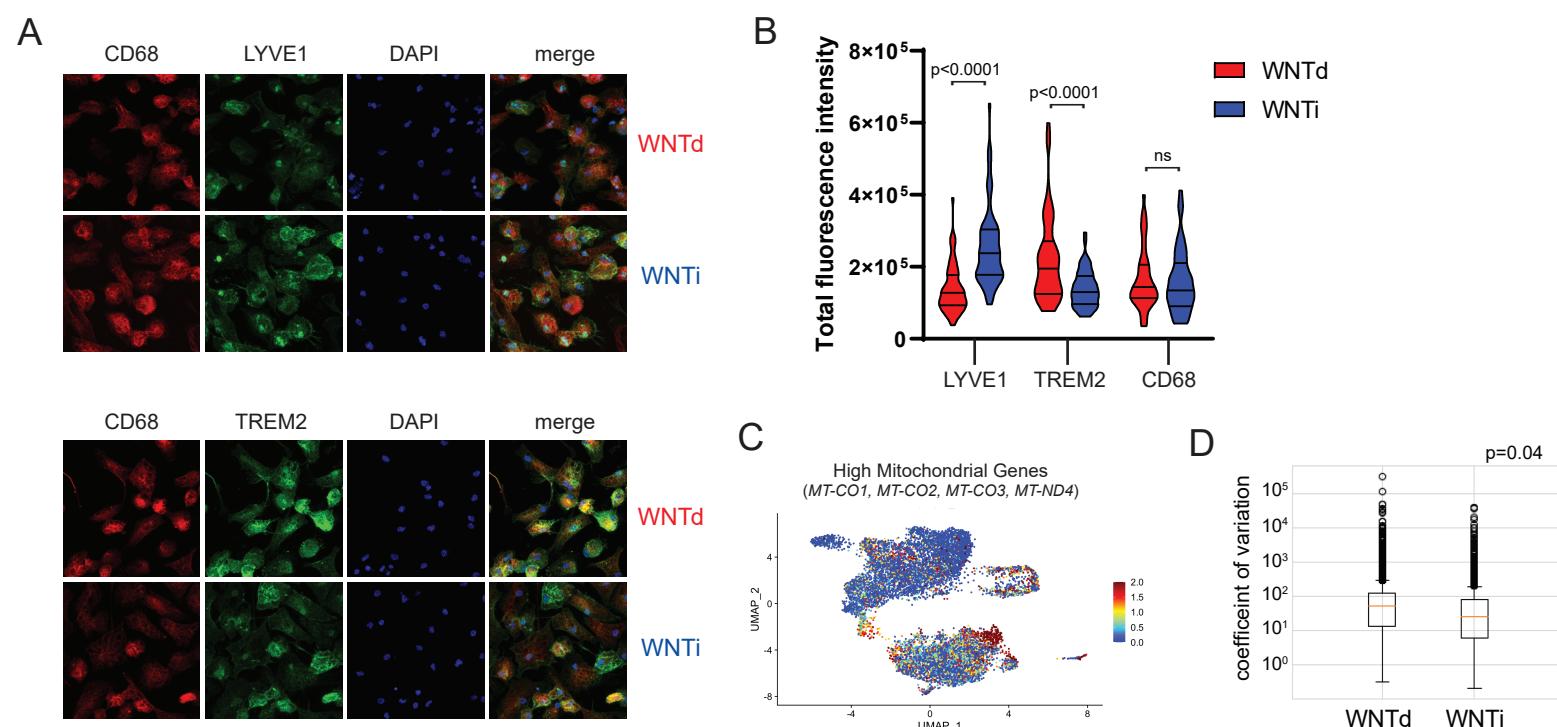


Fig. S4. Gene and protein expression differences in WNTd and WNTi macrophages.

A. Immunofluorescence images (maximum intensity z-stack projections) of LYVE1 and TREM2 protein expression in WNTi and WNTd macrophages. B. Quantitation of total fluorescence intensity per cell (using sum z-stack projection) for LYVE1, TREM2 and CD68 immunofluorescence. Results are from three independent differentiations, with at least 15 cells per differentiation. Significance calculated using two-tailed student's t test. C. Z-score feature plot of mitochondrial genes overexpressed in the MT-CO2 M ϕ cluster shown in Fig. 2. D. Coefficient of variation (CV) of gene expression for all genes in the WNTd and WNTi macrophages, calculated as $CV = \text{StDev expression} / \text{mean expression}$ for each gene. The lower CV in the WNTi macrophages indicates that expression of individual genes has a lower dispersion in these cells than in the WNTd macrophages.

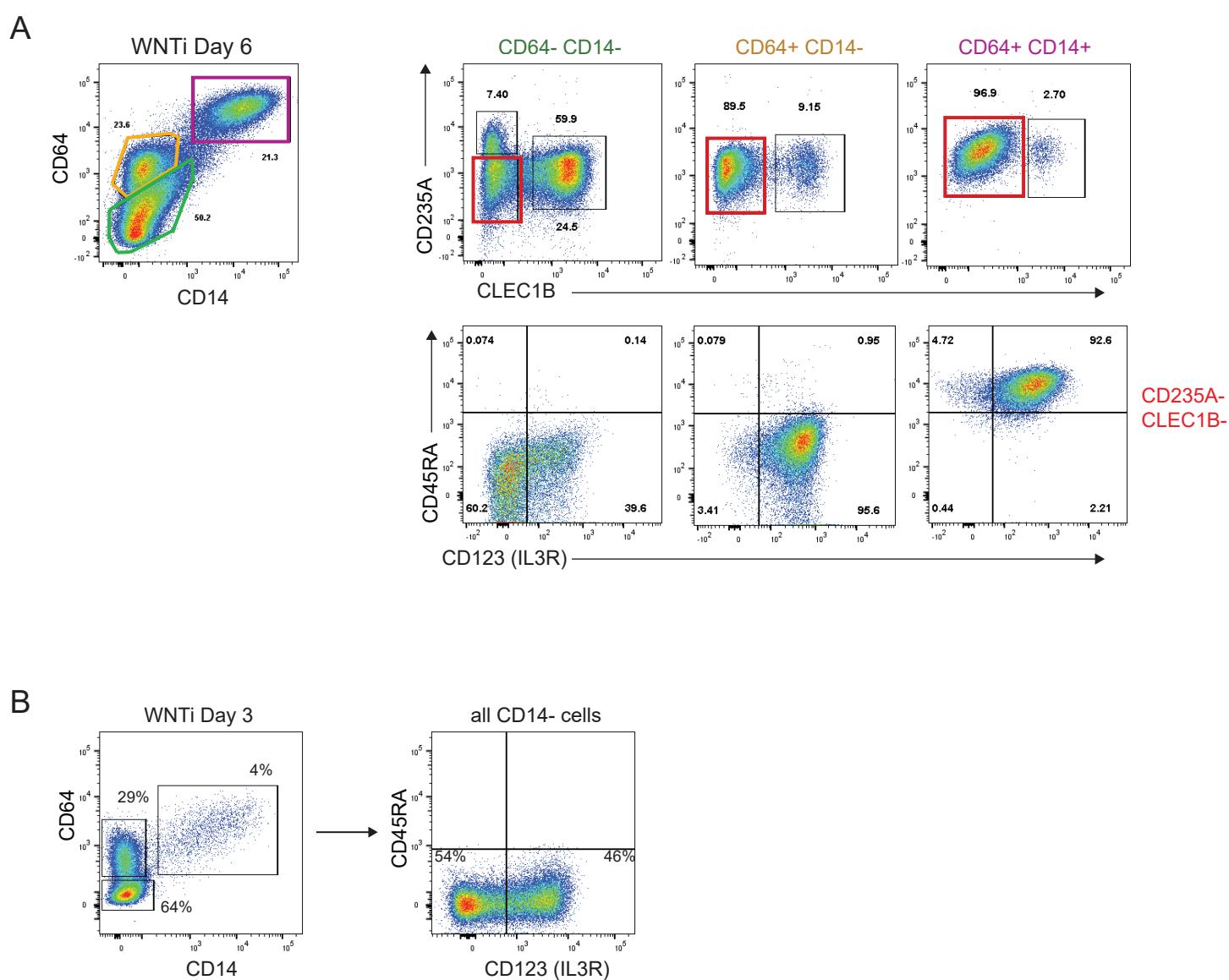


Fig. S5. WNTi macrophage progenitor analysis. A. Flow cytometric analysis of IL3R and CD45RA expression on CD235A-CLEC1B- cells in macrophage Day 6 WNTi culture. B. Flow cytometric analysis of IL3R and CD45RA expression in macrophage Day 3 WNTi culture. All plots are representative of 3 independent experiments.

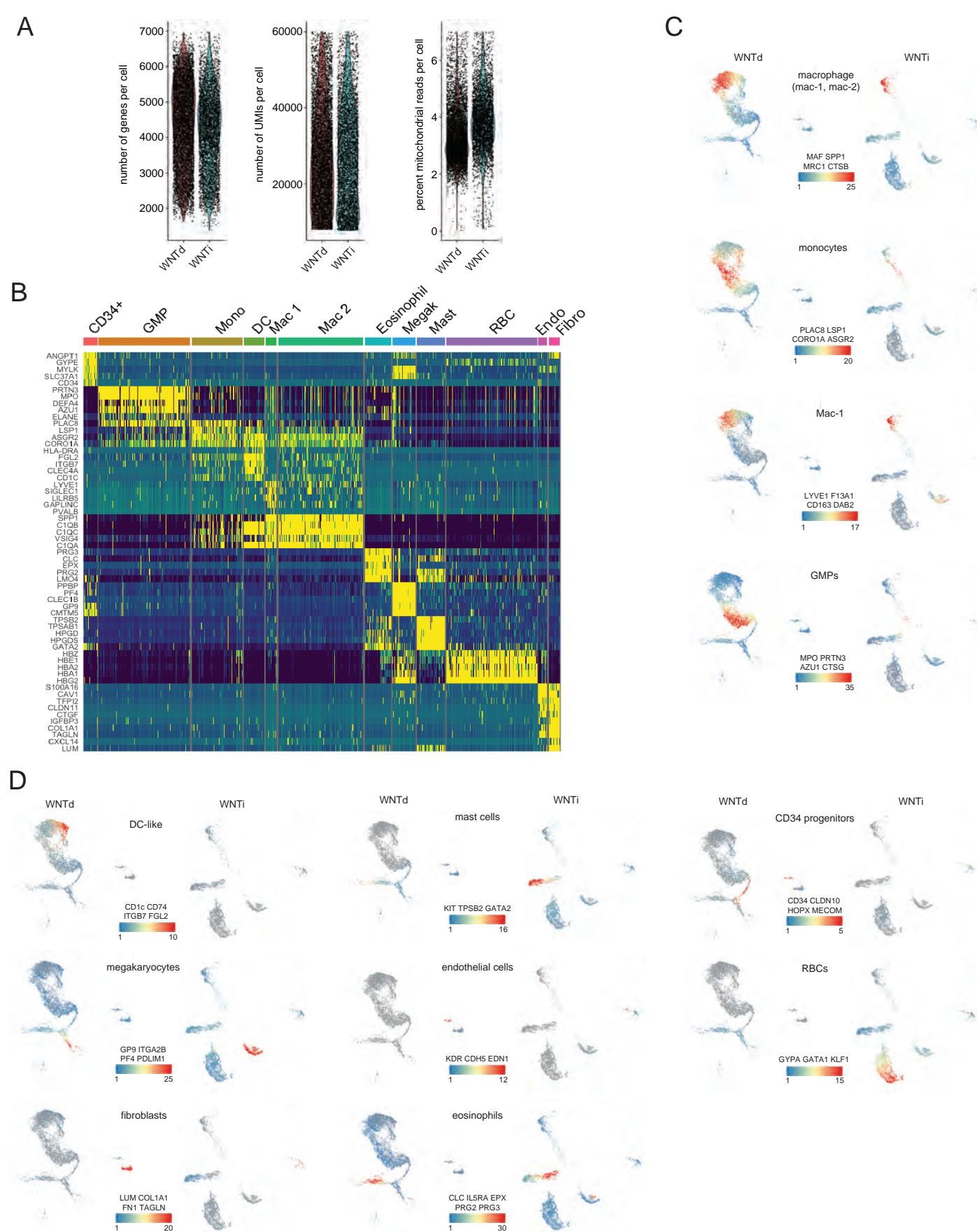


Fig. S6. WNTd and WNTi macrophage progenitor scRNAseq clusters. A. Comparison of scRNAseq parameters for WNTd and WNTi macrophage progenitor samples. B. Heat map of top 5 differentially expressed genes for each scRNAseq cluster in Fig. 3D. C. Z-score feature plots of cell type characteristic genes for macrophage, monocyte and GMP cell clusters in WNTd and WNTi macrophage progenitor scRNAseq analysis shown in Fig. 3. Genes used to calculate the z-score are indicated below each plot. D. Z-score feature plots for additional cell type clusters shown in Fig. 3. Genes used to calculate the z-score are indicated below each plot.

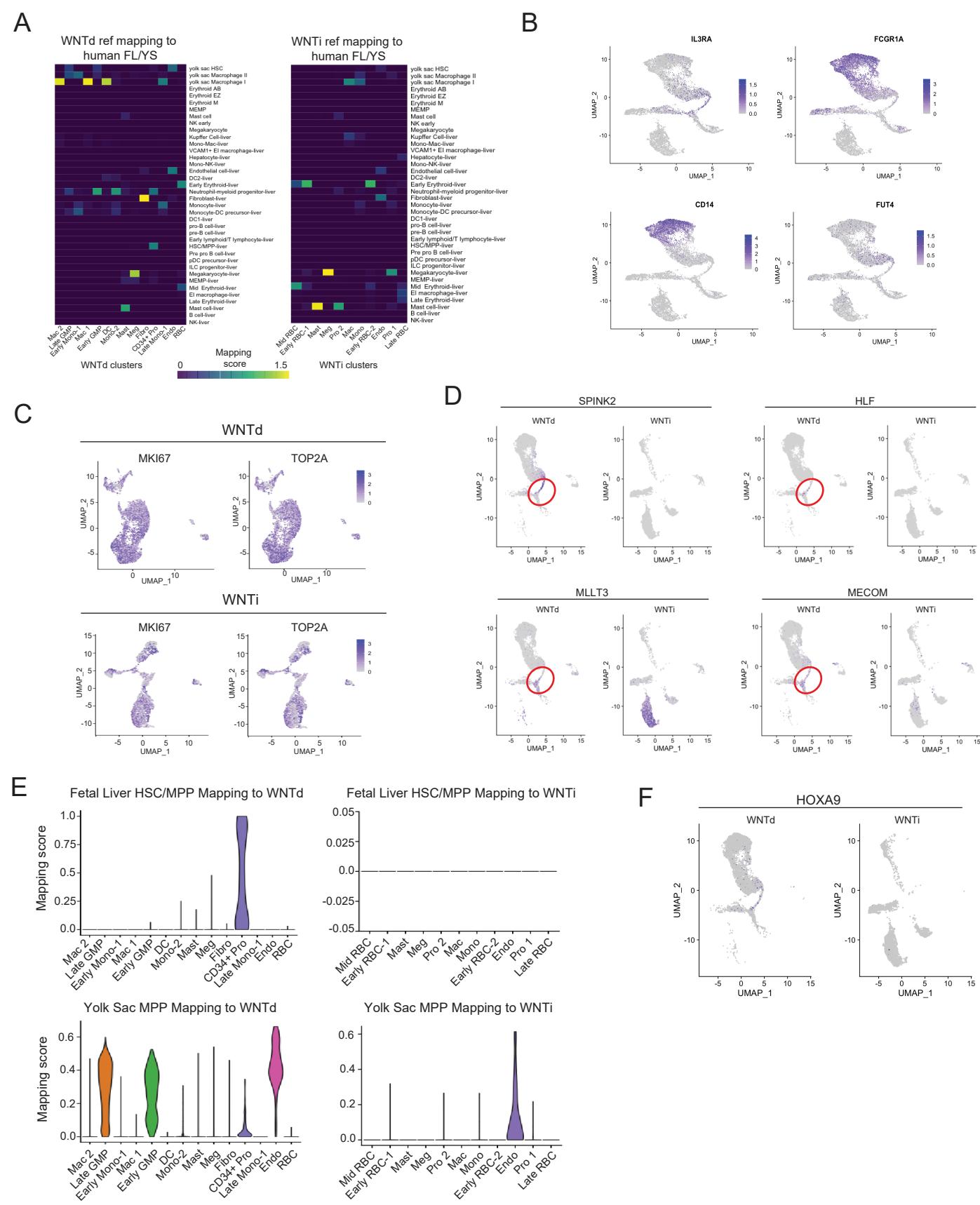


Fig. S7. Comparison of WNTd and WNTi progenitors to *in vivo* hematopoiesis. A. Reference mapping of WNTd and WNTi progenitors to integrated human fetal liver and yolk sac dataset from Popescu, et. al. B. scRNASeq feature plots of cell surface markers used in Fig. 3A and B (*IL3RA*, *FCGR1A*, *CD14*), and for neutrophil marker *CD15* (*FUT4*). C. Feature plots of expression of S/G2 markers *MKI67* and *TOP2A* in WNTi and WNTd progenitor cultures. D. Feature plots of expression of *SPINK2*, *HLF*, *MLLT3* and *MECOM*. Red circles indicate location of CD34+ WNTd progenitors. E. Reference mapping of human fetal liver HSC/MPPs and yolk sac MPPs onto WNTd and WNTi progenitor populations. F. Feature plots of *HOXA9* expression in WNTd and WNTi progenitor cultures.

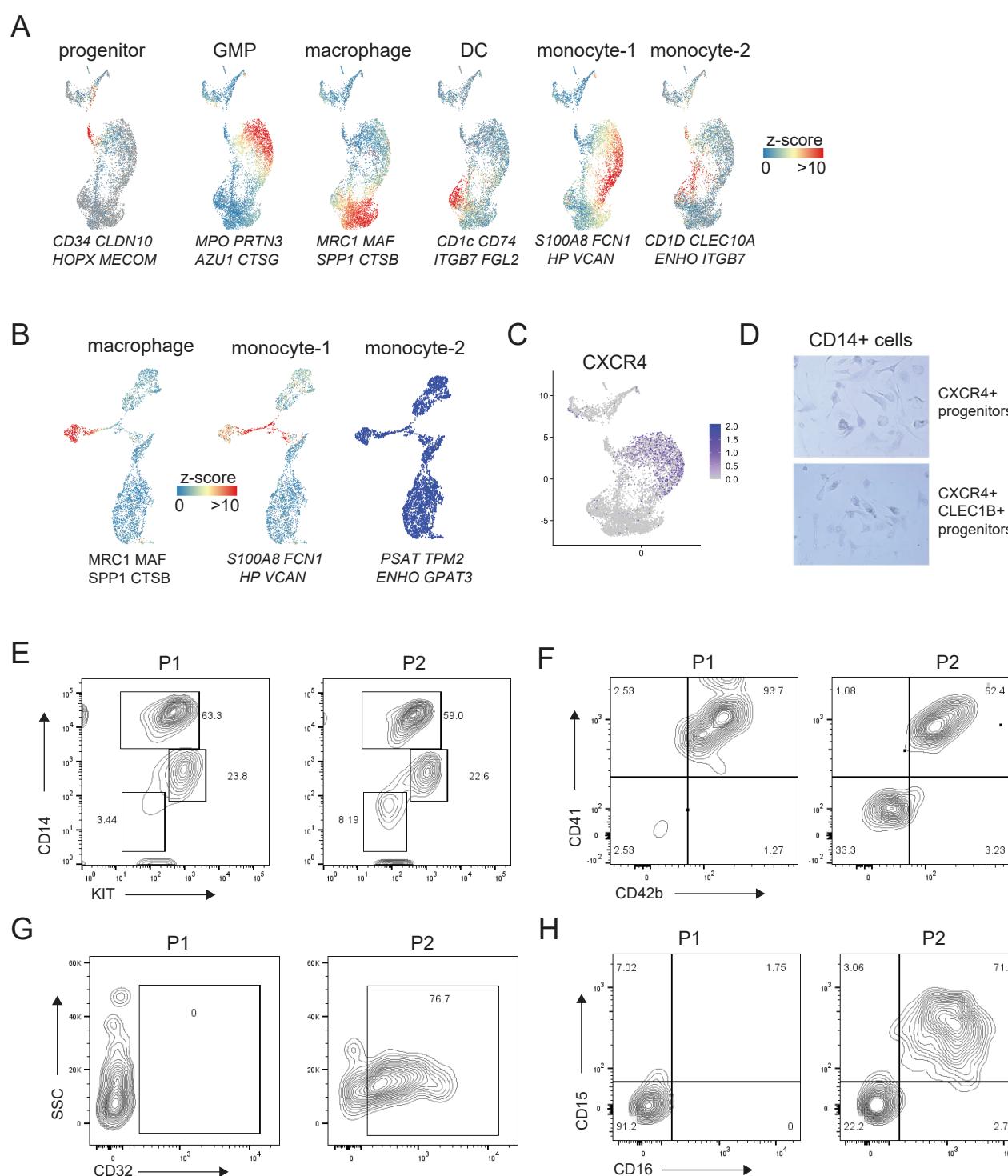


Fig. S8. WNTd and WNTi progenitor populations. A. Z-score feature plots of monocyte/macrophage lineage clusters in the WNTd macrophage progenitor scRNAseq analysis shown in Fig. 4. Genes used to calculate the z-scores are indicated below each plot. Genes are the same cell-type characteristic genes shown in Fig. S4, except for the genes used to differentiate monocyte-1 and monocyte-2, which are the top differentially expressed genes in each cluster that are not shared between the two clusters. B. Z-score feature plots of monocyte and macrophage clusters in the WNTi macrophage progenitor scRNAseq analysis shown in Fig. 4. Genes used to calculate the z-scores are indicated below each plot. The monocyte-1 and monocyte-2 genes are the same as in (A), showing that the monocytes in the WNTi culture share a gene expression pattern with the monocyte-1 cluster in the WNTd culture, and do not express the genes characteristic of monocyte-2. C. Feature plot of CXCR4 gene expression in the WNTd macrophage progenitor culture. D. Brightfield images (representative of two experiments) of CD14+ cells differentiated from CXCR4+ and CXCR4+CLEC1B+ progenitors. E-F. Flow cytometric analysis of progenitor 1 (P1) and progenitor 2 (P2) cultures after 7 days in (E) mast cell and (F) megakaryocyte promoting conditions. Plots in F show only CD14- cells. G-H. Flow cytometric analysis of granulocyte markers CD32 (F) and CD15 and CD16 (G) on CD14- cells isolated from MethoCult H4034. All flow cytometric analyses are representative of two independent experiments.

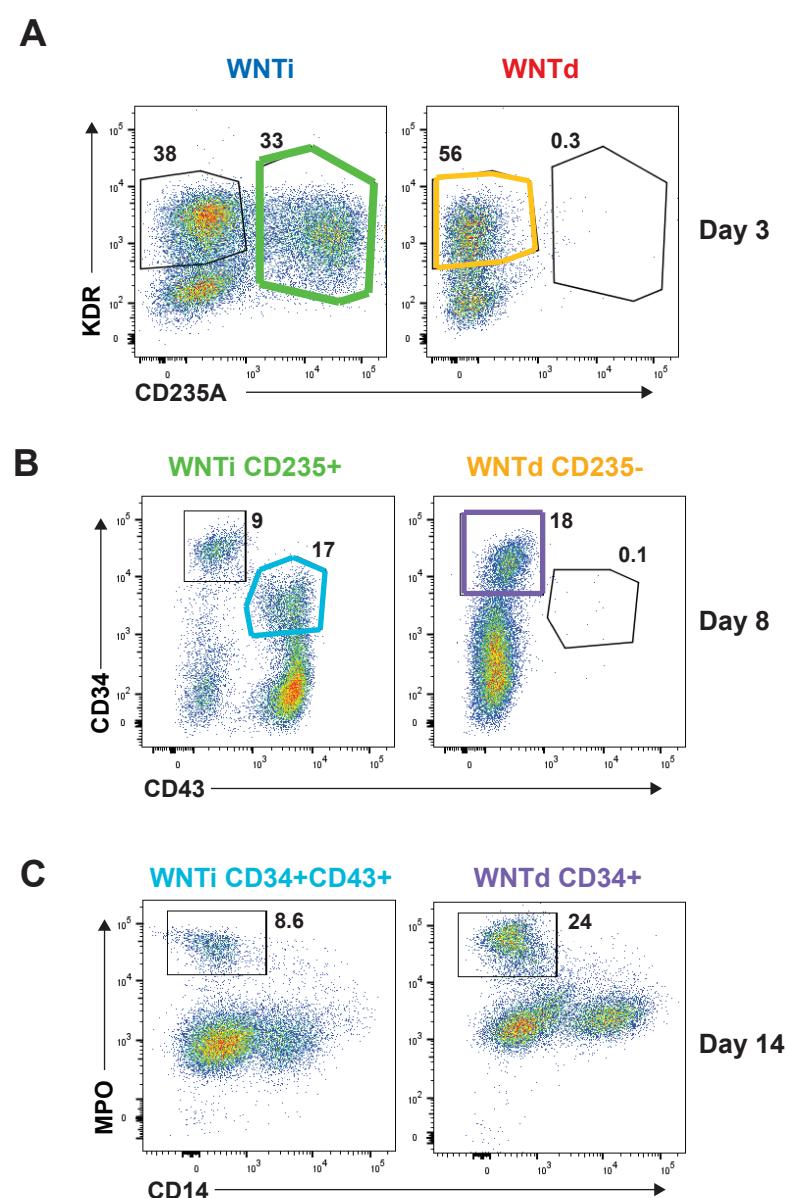


Fig. S9. MPO expression analysis in WNTi macrophage progenitors. A. Mesoderm progenitor analysis on Day 3 of hematopoietic progenitor differentiation. WNTd KDR+CD235A+ and WNTi KDR+CD235A+ cells were isolated by FACS sorting, reaggregated into embryoid body clusters, then cultured as usual until Day 8. B. Analysis of hematopoietic progenitors derived from populations sorted in (A) on Day 8 of hematopoietic progenitor differentiation. WNTd CD34+CD43- and WNTi CD34+CD43+ cells were FACS sorted and then cultured in macrophage media. C. Analysis of MPO expression in WNTd and WNTi cultures 6 days after populations in (B) were placed into macrophage media.

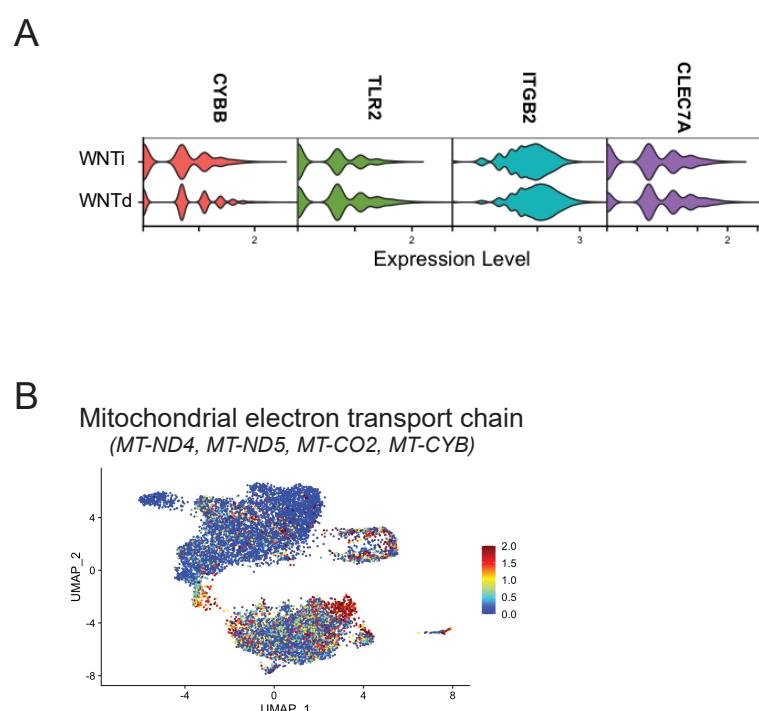


Fig. S10. Expression levels of potential ROS-altering genes in WNTd and WNTi macrophages.

A. Violin plots of *CYBB*, *TLR2*, *ITGB2* and *CLEC7A* expression in WNTd and WNTi macrophages in the macrophage day 14 scRNAseq dataset. B. Z-score feature plot showing mitochondrially encoded genes that have higher expression in WNTi macrophages than WNTd macrophages.

Table S1. scRNAseq data for combined Day 14 WNTd and WNTi mature macrophages (shown in Figure 2)

[Click here to download Table S1](#)

Table S2. scRNAseq data for combined WNTd and WNTi macrophage progenitors (shown in Figure 3)

[Click here to download Table S2](#)

Table S3. Gene signatures for EMP, pre-macrophage (pMac), and macrophage adapted from Mass, et al, Science, 2016.

[Click here to download Table S3](#)