

Supplemental Figures

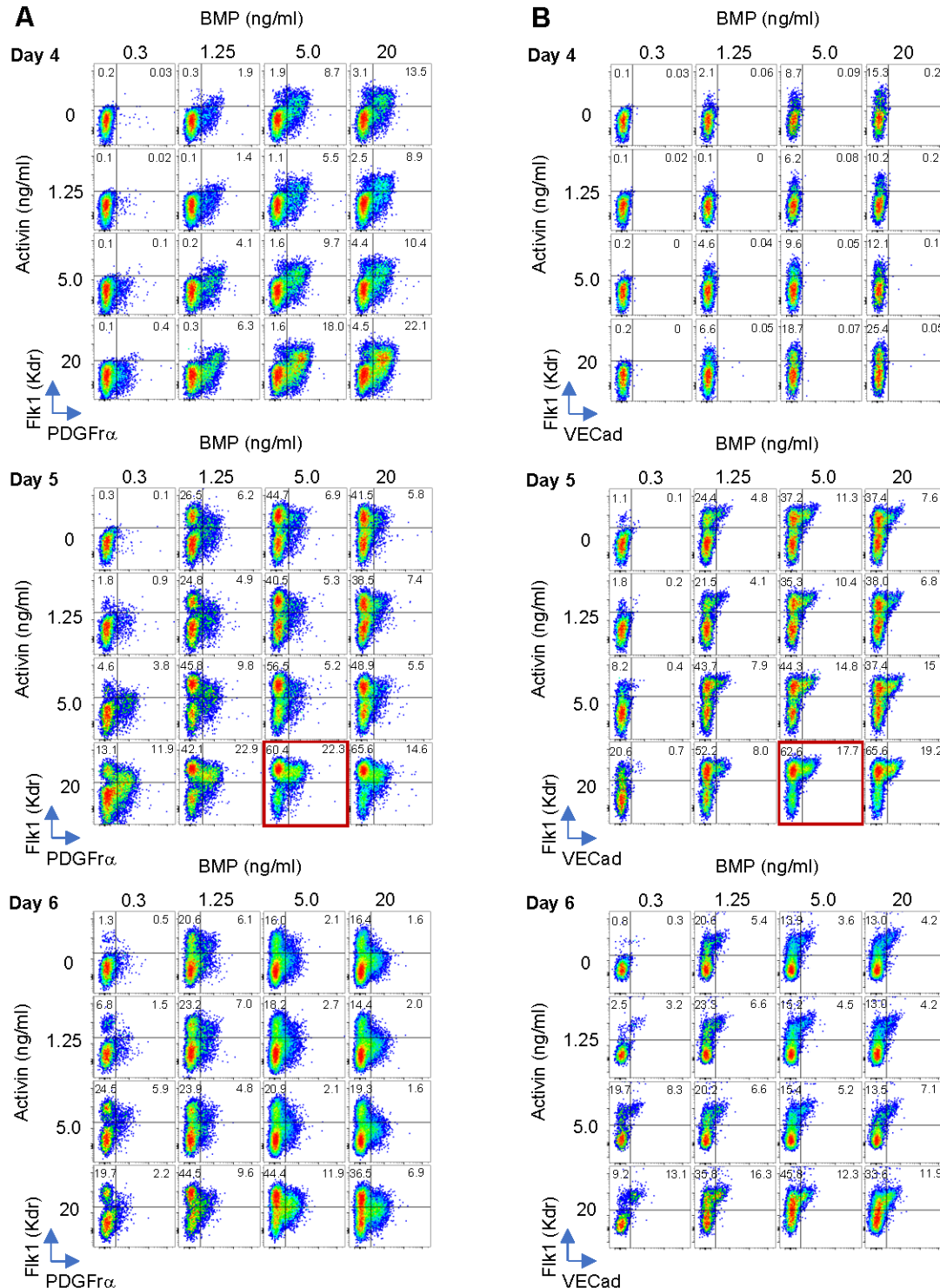


Figure S1: Phenotypic analysis of sci-Plex conditions. Expression of Flk1 (Kdr) vs Pdgfr α (A) or VECadherin (Cdh5) (B). Gates are drawn based off isotype controls. Red boxes indicate chosen conditions for optimal lateral plate mesoderm production and subsequent hematopoietic output.

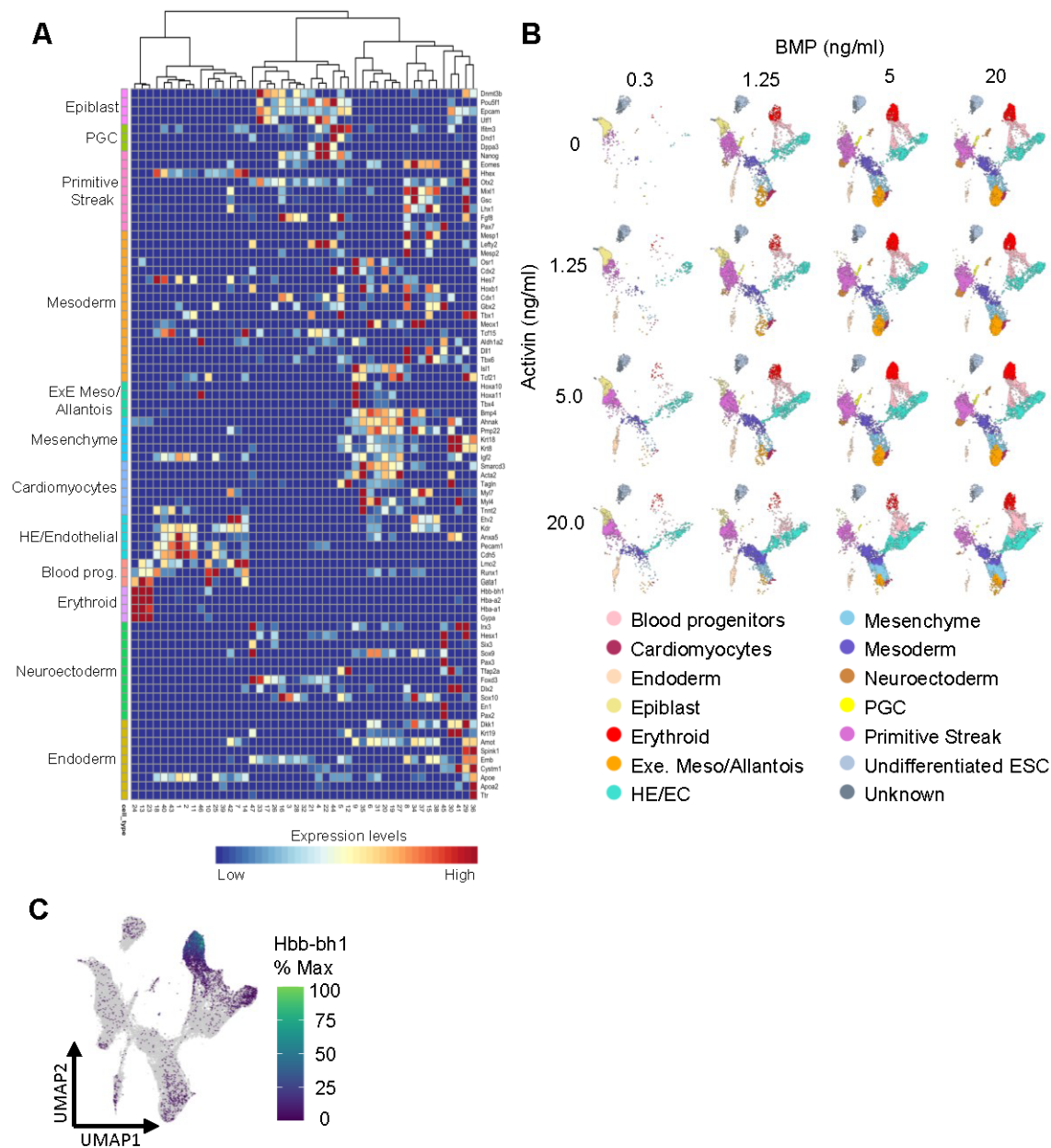


Figure S2: Cell type classification of sci-Plex dataset. A: Heatmap of diagnostic marker expression for cell type based on cluster. B: Cell type classification based on diagnostic markers subset for Activin/BMP conditions. C: Gene expression heatmap for primitive erythrocyte marker hemoglobin Z, beta-like embryonic chain (*Hbb-bh1*) within the full EB sci-Plex dataset.

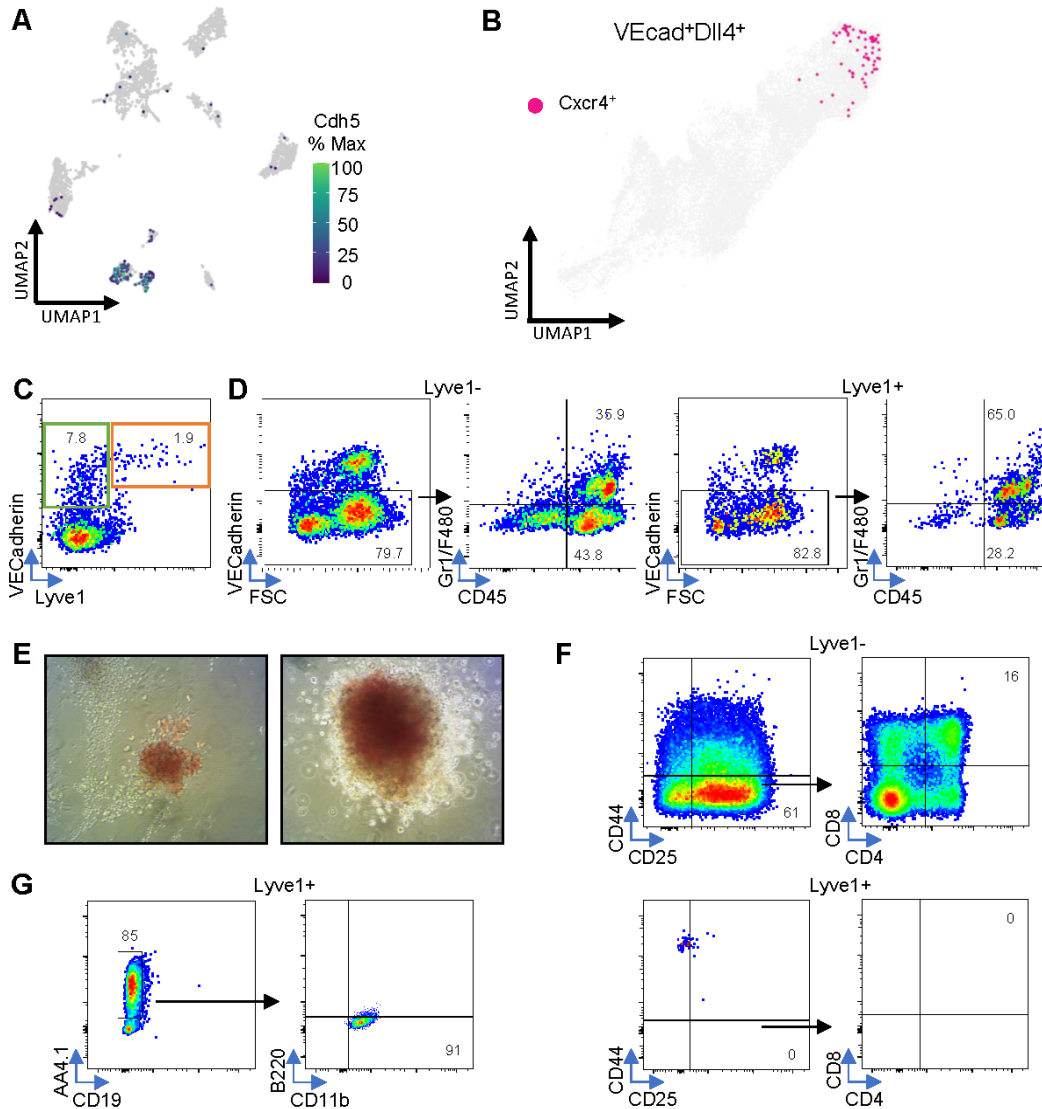


Figure S3: Functional assessment of hematopoietic activity for Lyve1^{+/-} populations generated from EB differentiation. A: Gene expression heatmap for VECadherin (*Cdh5*) within embryo dissection sci-Plex dataset. B: Gene expression of *Dll4*⁺*VECadherin*⁺*Cxcr4*⁺ within the HE/EC subset of EB sci-Plex data. C: Expression of VECadherin and Lyve1 from EBs collected for sorting on Day 7 of differentiation. Green and orange boxes indicate the sorted populations. D: Phenotypic expression of Lyve1⁻ (left) and Lyve1⁺ (right) cells sorted from (C) for the pan-hematopoietic marker CD45 and myeloid markers Gr1 and F480 within the VECadherin⁻ population of cells after 13 days of co-culture. E: Images of CFU-E (left) and CFU-GEMM (right) colonies generated in CFU assay for cells collected from (D). F: Phenotypic expression of Tcell markers CD44⁻CD25⁺CD8⁺CD4⁺ for Lyve1⁻ (top) and Lyve1⁺ (bottom) cells collected from (D) and subjected to co-culture on OP9-Dll4 stromal cells. G: Phenotypic expression of Bcell markers CD19-AA4.1⁺B220⁻CD11b⁺ for Lyve1⁺ cells collected from (D) and subjected to co-culture on OP9 stromal cells. For all dot plots in C-G, gates are drawn based off isotype controls.

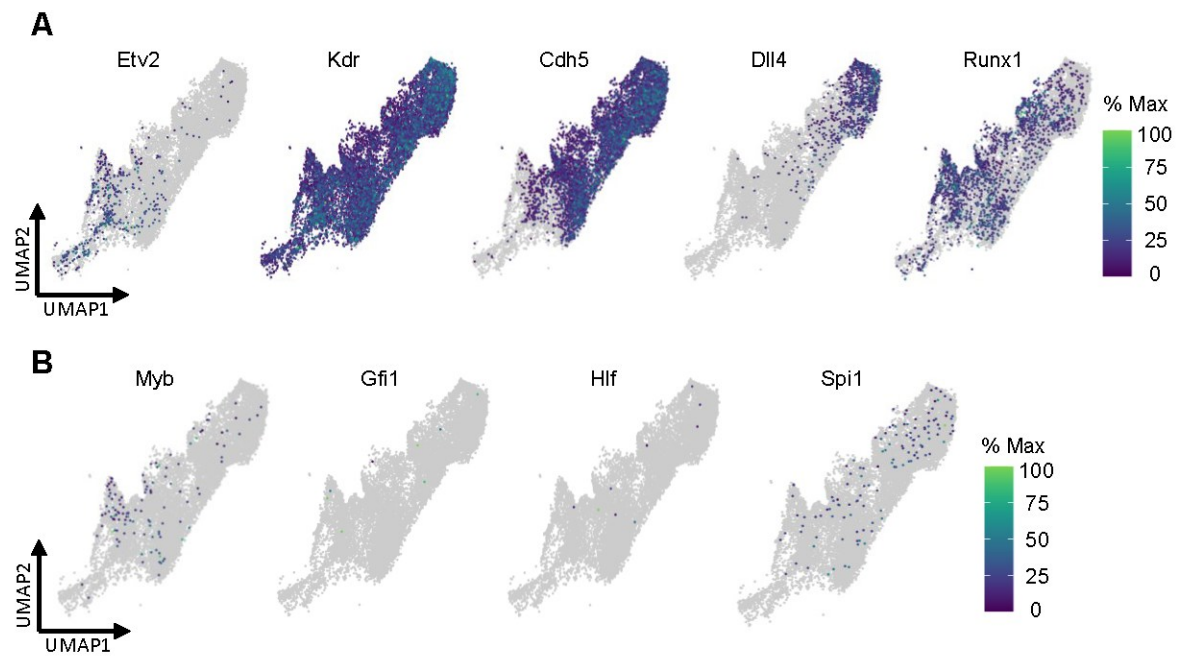


Figure S4: Expression of key hematopoietic development markers from sci-Plex data. Gene expression heatmaps for hematovascular (A) and EHT (B) markers within the HE/EC subset of EB sci-Plex data.

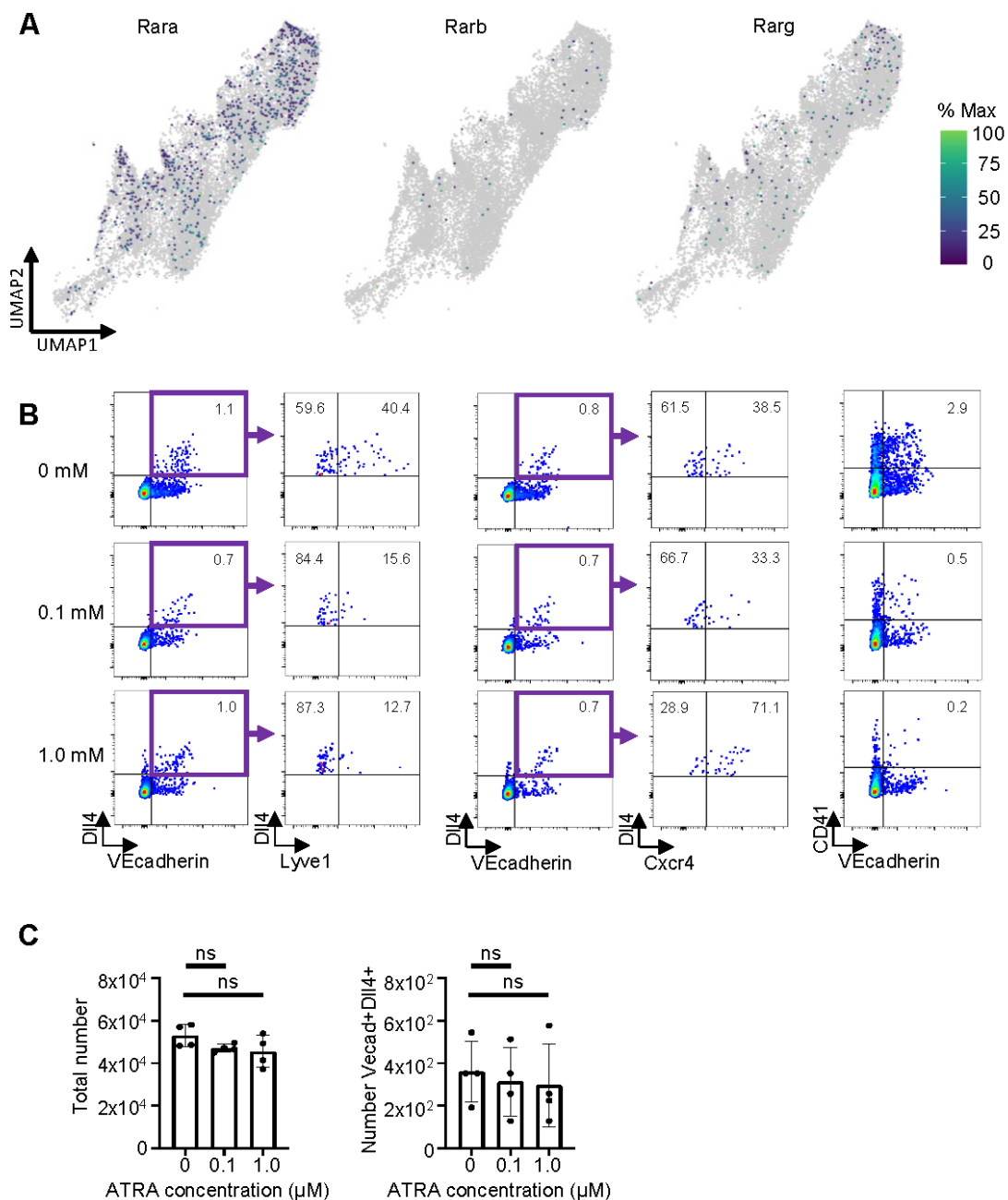


Figure S5: Retinoic acid signaling influences HE fate in EB differentiation protocol. A: Gene expression heatmaps retinoic acid receptors within the HE/EC subset of EB sci-Plex data. B: Phenotypic expression of Lyve1 (left) and Cxcr4 (middle) within the Dll4⁺VECadherin⁺ population, and CD41 x VECadherin (right). Gates are drawn based off isotype controls. C: Total (left) or VECadherin⁺Dll4⁺ number of cells across ATRA concentrations. Error bars represent standard deviation. Comparisons to 0 μ M were non-significant, as listed.