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Supplemental Information

Common Developmental Pathway for Primitive Erythrocytes and Multipotent Hematopoietic Progenitors in Early Mouse Development

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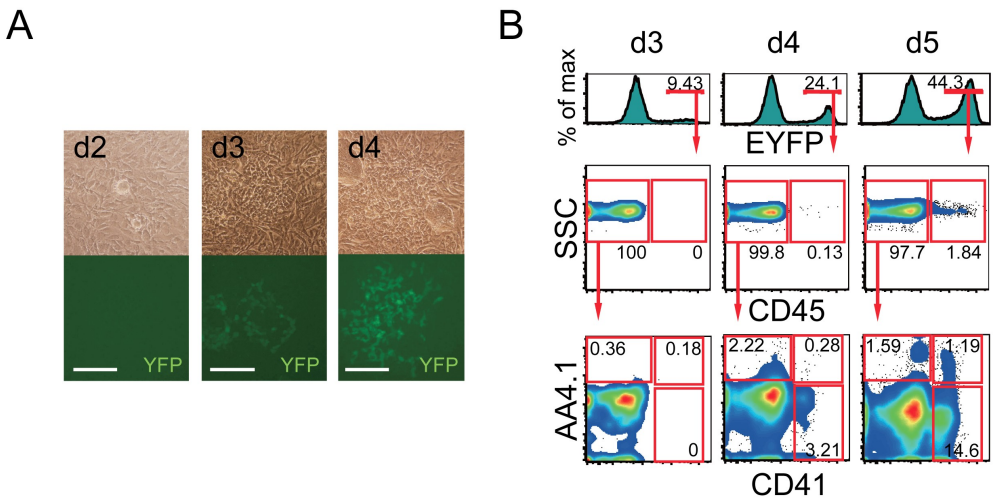

Figure S1. Appearance of distinct cell subsets during the hematopoietic differentiation of mesodermal cells, related to Figure 1. (A) The differentiation of *Mesp1-cre*/*Rosa26-eYfp* ES cells on OP9 cells. Phase contrast (upper) and fluorescent (lower) images of the culture (days 2–4) are shown. Scale bars: 200 μm. (B) Flow cytometric analysis of marker expression in the EYFP-labeled fraction from day 3 to day 5 of ES cell differentiation. Representative results from three separate experiments are shown.

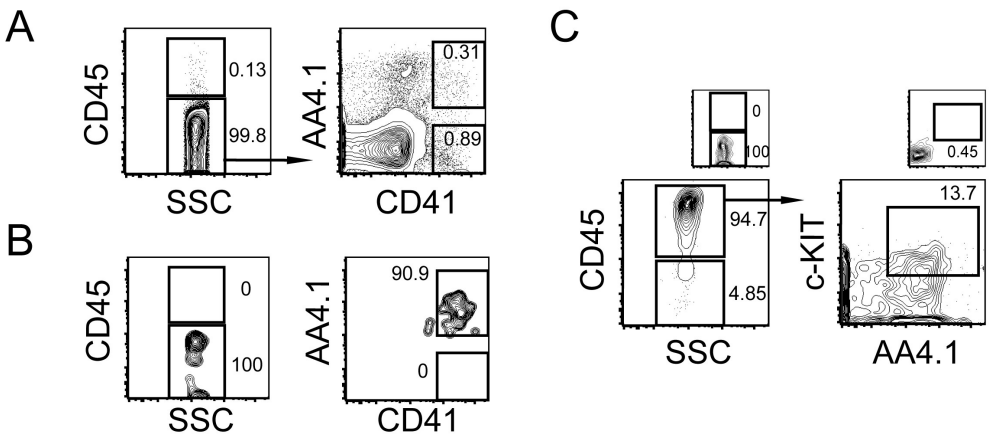

Figure S2. DP cells are immediate precursors of CD45$^+$ definitive hematopoietic cells, related to Figure 2. (A, B) Sort gates (A) and post-sort analysis (B) of DP cells. (C) Differentiation output of DP cells. After 2 days of culture on OP9 cells, the sorted DP cells were analyzed. Control staining is shown at the top right of each plot. Representative results from three separate experiments are shown.

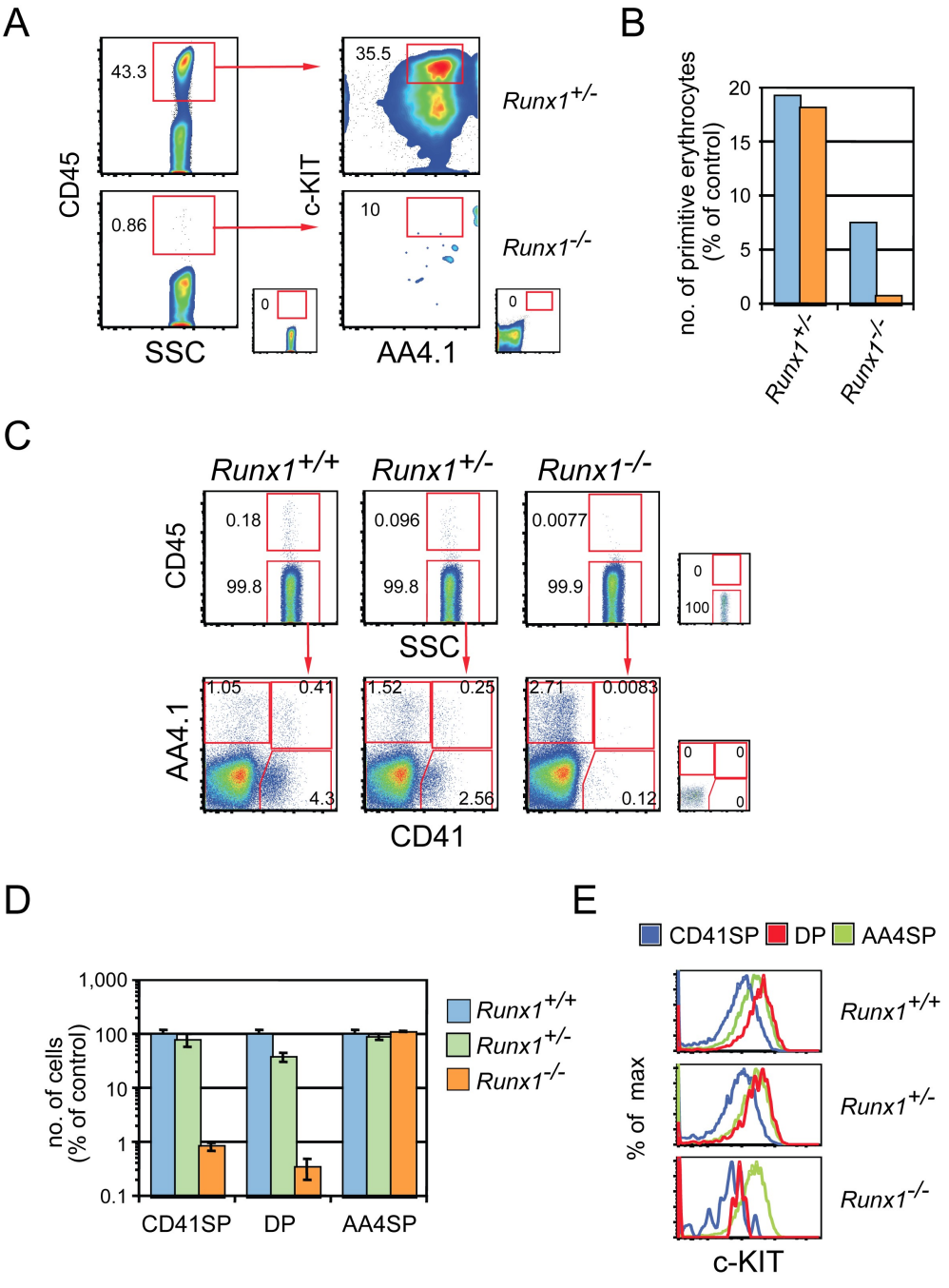

Figure S3. Deletion of Runx1 abrogates DP cell development, related to Figure 2. (A) *Runx1*$^{+/-}$ or $^{-/-}$ cells on day 7 of differentiation were analyzed for

marker expression. Small plots shown to the right of *Runx1*$^{-/-}$ data represent control staining. (B) Numbers of primitive erythrocytes (TER119^{+} cells on day 7 of differentiation) from *Runx1*$^{+/-}$ or $^{-/-}$ cells. Relative numbers compared with *Runx1*$^{+/+}$ cells (value = 100%) are shown for two separate experiments (blue and orange). (C) *Runx1*$^{+/+}$, $^{+/-}$, or $^{-/-}$ cells were analyzed on day 5 of differentiation. Small plots shown to the right of the *Runx1*$^{-/-}$ column represent control staining. (D) Numbers of CD41SP, DP, and AA4SP cells produced from *Runx1*$^{+/+}$, $^{+/-}$, or $^{-/-}$ cells (blue, green, and orange, respectively). Relative numbers compared with *Runx1*$^{+/+}$ cells (value = 100%) are shown for each cell subset. Values are expressed as mean ± S.D. of triplicate cultures. (E) c-KIT expression levels in CD41SP (blue), DP (red), and AA4SP (green) cells derived from *Runx1*$^{+/+}$, $^{+/-}$, or $^{-/-}$ ES cells. Representative results from two separate experiments are shown.

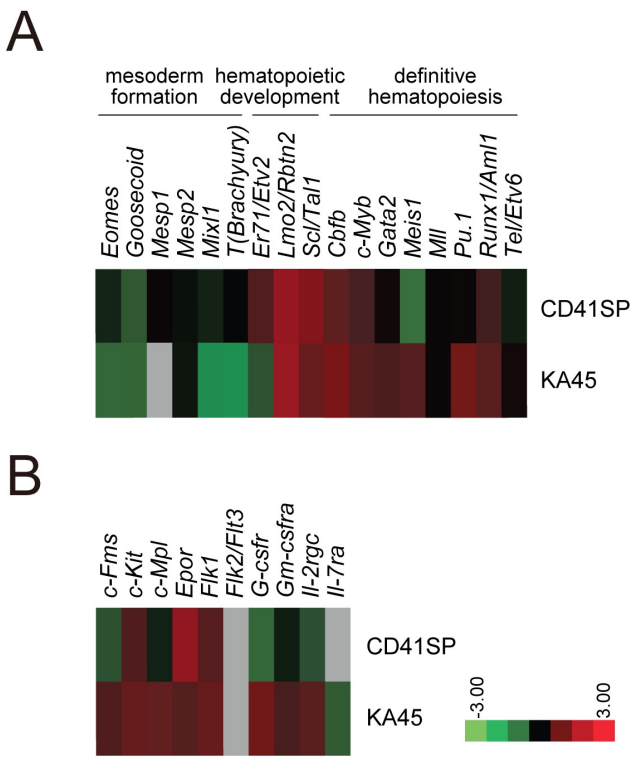

Figure S4. Gene expression profiles in CD41SP cells and the earliest hematolymphoid progenitors, related to Figure 3. (A, B) CD41SP cells and $CD45^{+}c\text{-}KIT^{+}AA4^{+}$ definitive hematopoietic progenitors (KA45) isolated on days 5 and 7, respectively, were compared for the gene expression of transcription factors (A) and hematopoietic cytokine receptors (B). Gene expression levels are displayed as a heat map. Genes shown in gray color were undetectable.

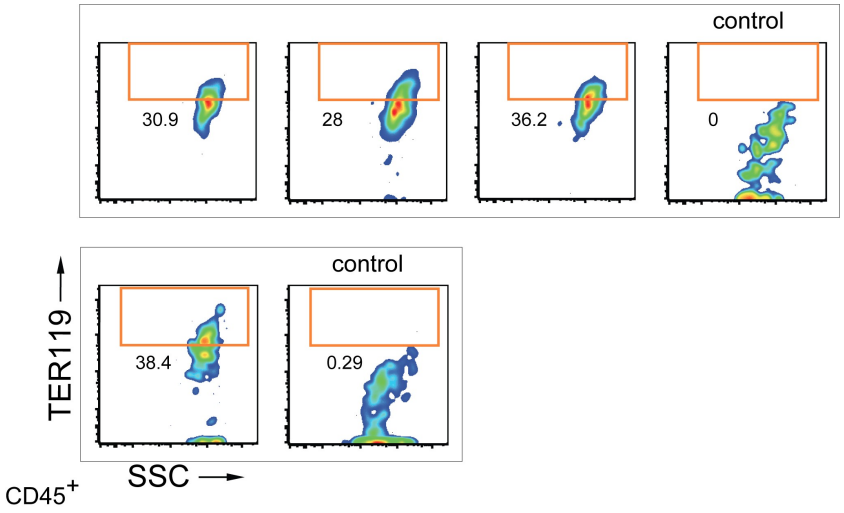

Figure S5. Coexistence of primitive and definitive erythrocytes in colonies derived from single CD41SP cells, related to Figure 6. FACS plots are shown for four CD41SP cell clones derived from ES cells that were positive for ε-*y* globin expression and contained $CD45^{+}TER119^{+}$ cells. The flow cytometry patterns for TER119 expression are shown. The plots shown are pregated for $CD45^{+}$ cells. The upper and lower panels contain clones from separate experiments. Control stainings for each experiment are shown on the right.

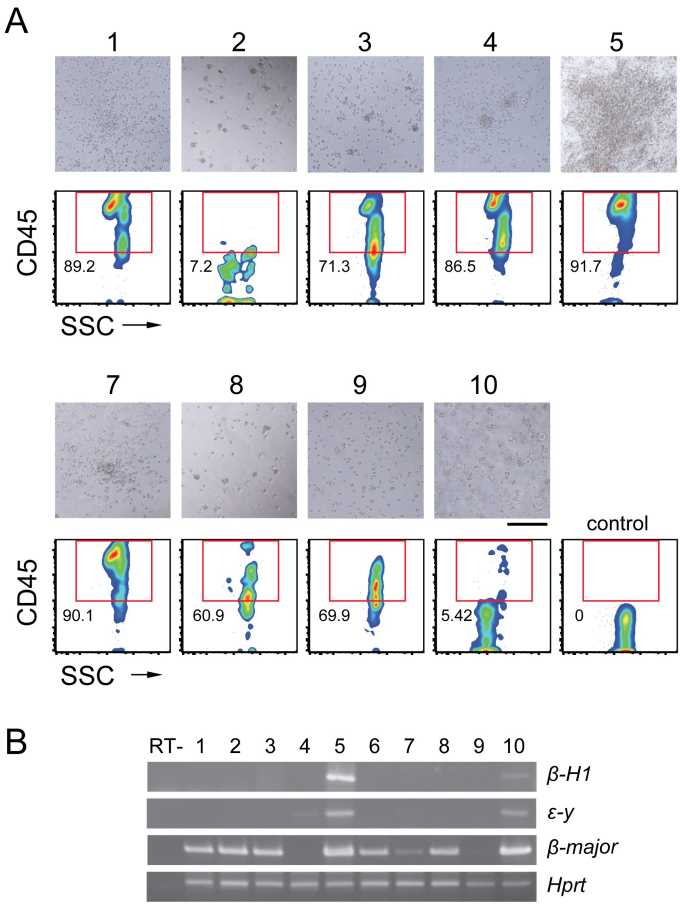

Figure S6. Bipotential differentiation capability of *in vivo*-derived CD41SP cells, related to Table 1. (A, B) E9.5 yolk-sac-derived CD41SP cells were seeded in 48-well plates at 1 cell/well. Each colony that developed from single CD41SP cells was collected and analyzed for CD45 expression by using half of the cells. Colonies consisting of both $CD45^{+}$ and $CD45^{-}$ cells (A, numbered 1–10) were further analyzed for β-globin expression by RT-PCR (B) using the remaining half of the cells. Nested RT-PCR for *Hprt* as an internal control was performed. Upper and lower panels in A show pictures of the analyzed colonies (nos. 1–10) and the flow cytometry pattern for CD45 expression, respectively. Scale bar: 200 μm. Representative results of three separate experiments are shown.