SUPPLEMENTAL FIGURES

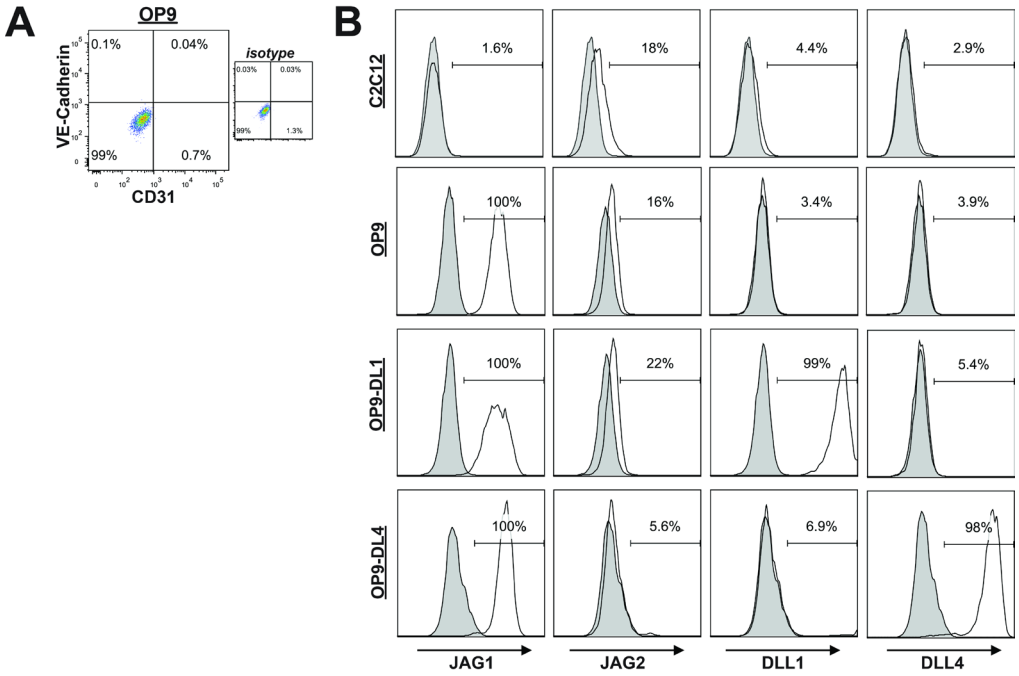

Figure S1. VE-Cadherin, CD31, and NOTCH ligand antibody control staining. (A) Staining with antibodies for endothelial markers VE-Cadherin and CD31, showing absence of expression on the non-endothelial stromal line OP9. (B) Staining with antibodies for NOTCH ligands, JAG1, JAG2, DLL1, and DLL4, showing relative absence of ligand expression on murine C2C12 myoblast cell line, endogenous JAG1 expression on OP9 stromal cells, and specific DLL1 and DLL4 expression on OP9 cells engineered to express DLL1 (OP9-DL1) or DLL4 (OP9-DL4), respectively.

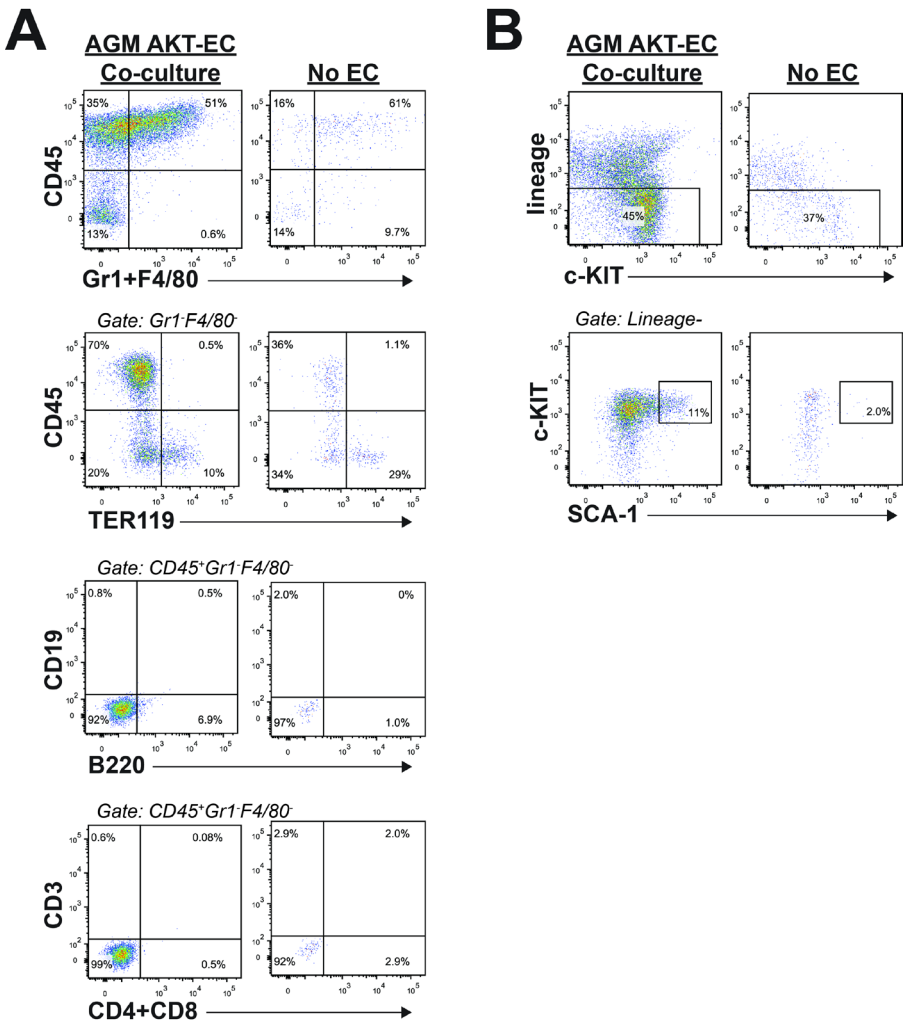

Figure S2. Hematopoietic surface markers expressed by cells generated following co-culture of E9.5-10 P-Sp/AGM-derived VE-Cadherin^{+} precursors. (A) Surface expression of CD45 and myeloid (Gr1 and F4/80), erythroid (TER119), B-lymphocyte (CD19 and B220), or T-lymphocyte (CD3, CD4, and CD8) markers, gated as indicated, following co-culture on AGM AKT-EC or without EC. (B) Surface expression for markers of LSK stem/progenitor cells (c-KIT and SCA-1, following exclusion of lineage markers Gr1, F4/80, TER119, B220), following co-culture on AGM AKT-EC or without EC. For all analysis, AGM AKT-EC are excluded by staining for flk1 and/or VE-Cadherin.

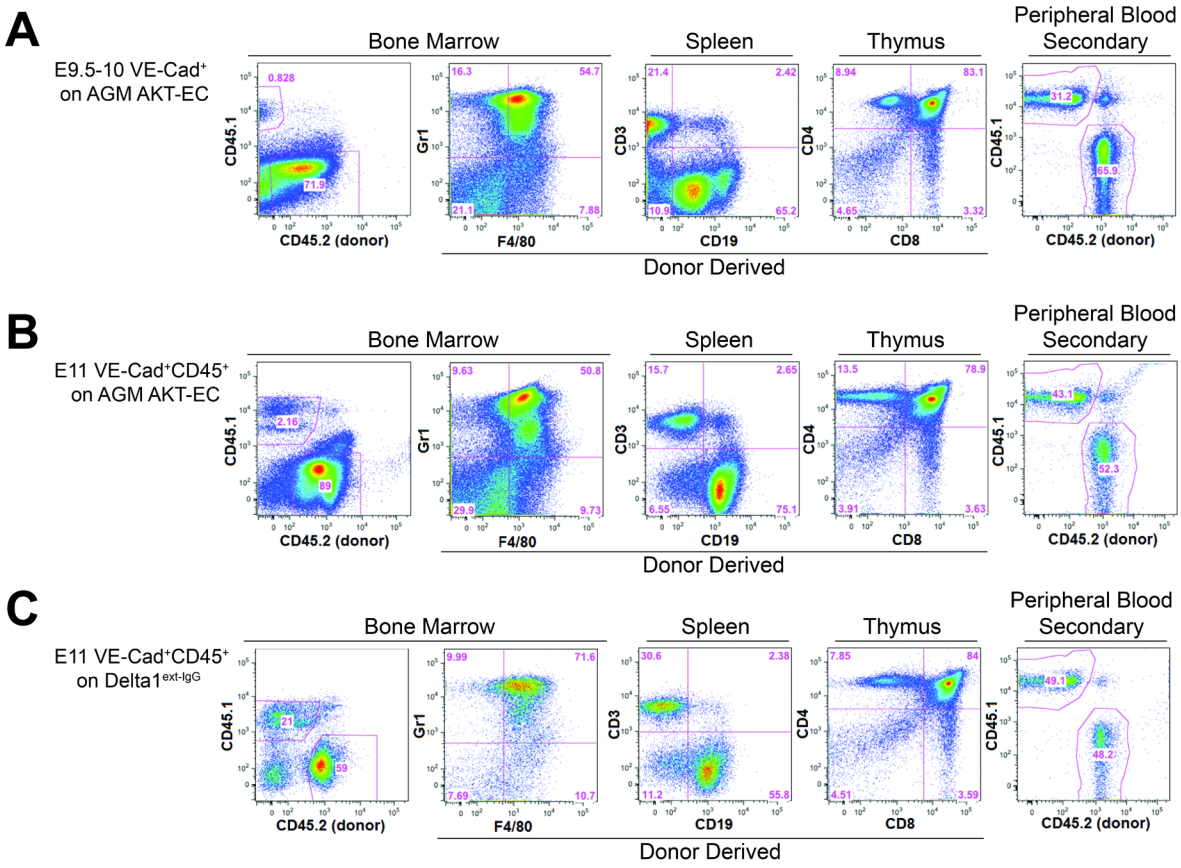

Figure S3. Sample flow cytometry plots of donor engraftment in primary and secondary recipients. Sample flow cytometry analysis of donor (CD45.2) engraftment in bone marrow, spleen and thymus of primary recipients, including myeloid (Gr1 and F4/80), lymphoid (CD3 and CD19), and T-cell (CD4 and CD8), and PB engraftment in corresponding secondary recipients, of (A) E9.5-10 VE-Cadherin^{+} cells co-cultured on AGM AKT-EC, (B) E11 VE-Cadherin^{+}CD45^{+} cells co-cultured on AGM AKT-EC, or (C) E11 VE-Cadherin^{+}CD45^{+} cells cultured on Delta1$^{ext\text{-}IgG}$ with 4GF+SB.

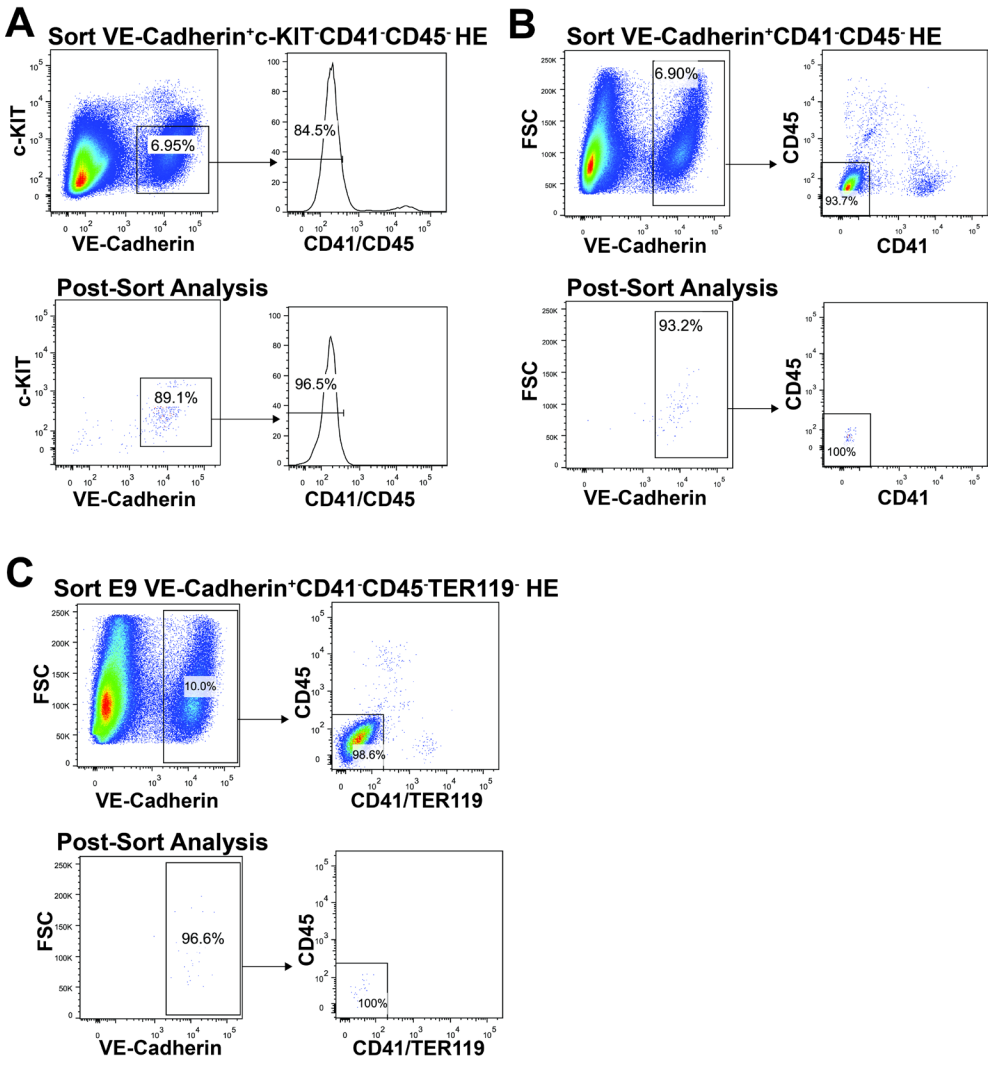

Figure S4. Sample sorting strategies and post-sort analysis for VE-Cadherin$^+$ endothelial cell population containing hemogenic endothelium (HE). (A) HE sorted from E9.5-10 P-Sp VE-Cadherin$^+$c-KIT$^-$ population by further exclusion of CD41 and CD45 hematopoietic markers. (B) HE sorted from E9.5-10 P-Sp VE-Cadherin$^+$ population by exclusion of hematopoietic markers CD41 and CD45. (C) HE sorted from E9 P-Sp VE-Cadherin$^+$ population by exclusion of hematopoietic markers CD41, TER119, and CD45.

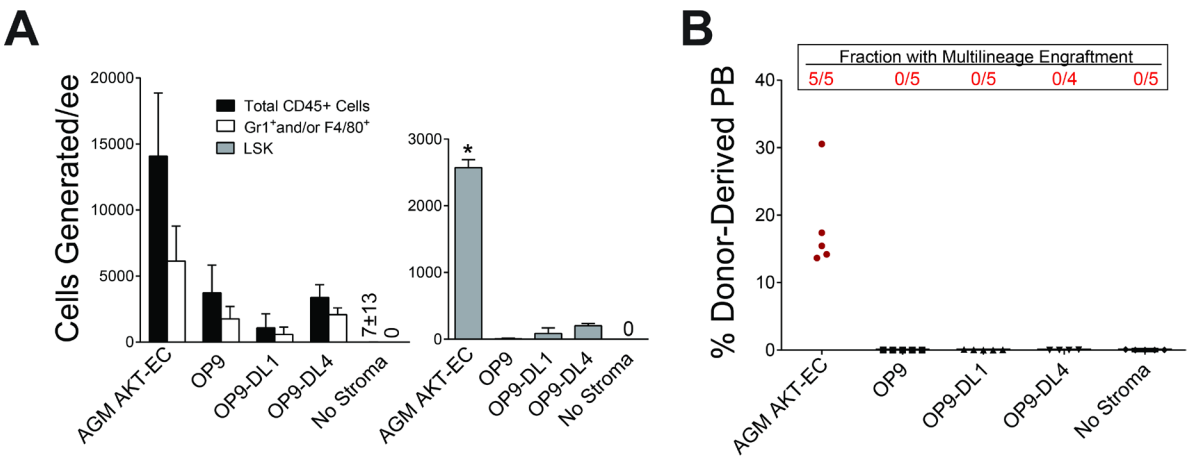

Figure S5. Co-culture of P-Sp/AGM-derived hemogenic endothelium generates non-engrafting hematopoietic cells following co-culture on OP9 stromal lines. (A) Total $CD45^+$, myeloid ($Gr1^+$ and/or $F480^+$), and LSK cells generated per embryo equivalent (ee) of starting E9.5 VE-$Cadherin^+CD45^-CD41^-$ HE cells, following co-culture on AGM AKT-EC, OP9 stroma, OP9 stroma engineered to express Notch ligands DLL1 (OP9-DL1) or DLL4 (OP9-DL4), or no stroma. Shown is mean±s.d. from replicate samples (n=3), from representative experiment (n=2). (for LSK cells, * $P<0.01$ for AGM AKT-EC vs each of OP9, OP9-DL1, OP9-DL4, and no stroma, unpaired Student's t-test). (B) Engraftment in peripheral blood at 2 weeks post-transplant of mice transplanted with 1 ee of HE following co-culture with AGM AKT-EC, OP9 stromal lines, or no stoma. Numbers above indicate fraction of mice with multilineage engraftment (B-lymphoid and myeloid at two weeks), designated by data points in red.

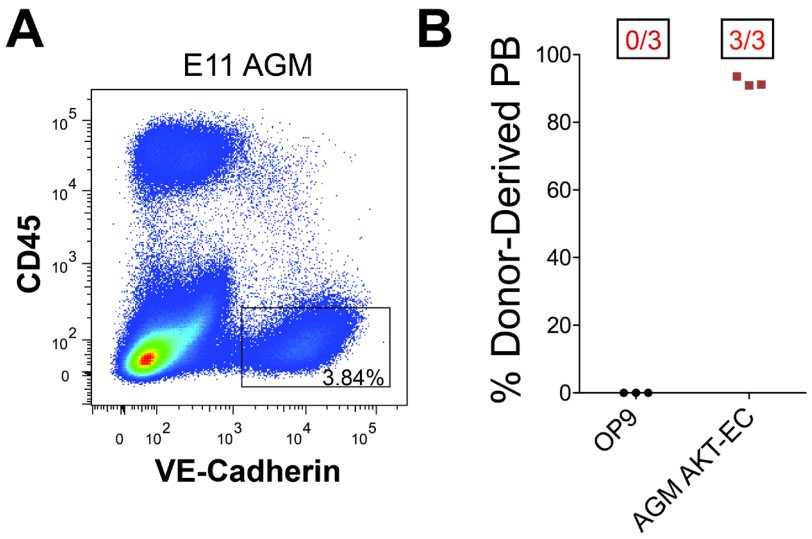

Figure S6. AGM AKT-EC Co-culture generates HSC from E11 VE-Cadherin^{+}CD45^{-} precursor cells. (A) Sort window for E11 AGM-derived VE-Cadherin^{+}CD45^{-} cells. (B) Engraftment in peripheral blood at ≥16 weeks post-transplant from VE-Cadherin^{+}CD45^{-} precursors following co-culture on AGM AKT-EC or OP9 stroma, each transplanted with 1 ee of cells. Numbers above indicate fraction of mice with multilineage engraftment, designated by data points in red.

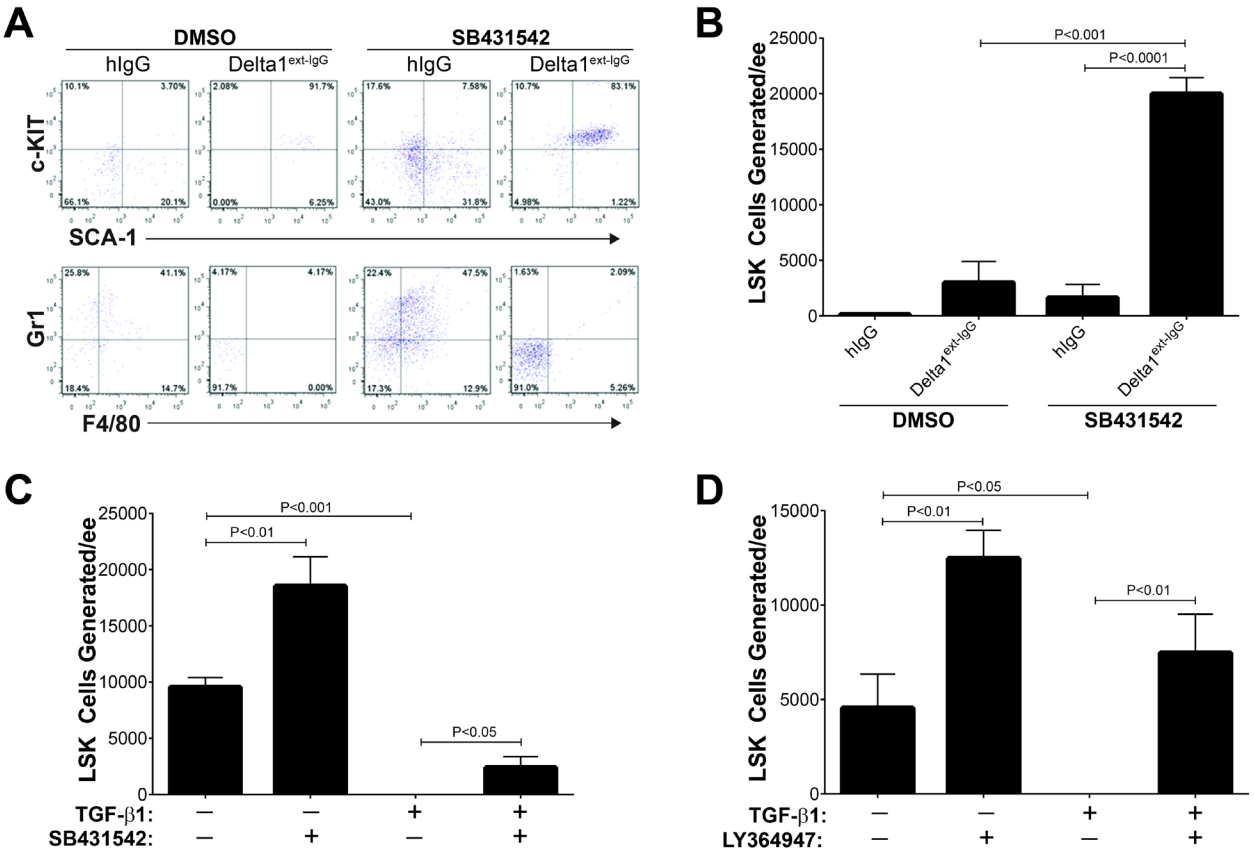

Figure S7. Notch pathway activation by immobilized Notch ligand Delta1$^{ext\text{-}IgG}$ synergizes with TGF-β inhibition to generate phenotypic hematopoietic stem/progenitor cells. (A) Flow cytometry analysis of cells generated by culture on Delta1$^{ext\text{-}IgG}$ or control (hIgG) showing stem/progenitor markers (SCA-1 and c-KIT) and myeloid markers (Gr1 and/or F4/80) with and without addition of TGF-β inhibitor (10μM SB431542). (B) Numbers of phenotypic LSK (SCA-1^{+}c-KIT^{+}Gr1^{-}F4/80^{-}) cells generated following 5 days culture in serum-containing media on Delta1$^{ext\text{-}IgG}$ or control (hIgG) with and without addition of TGF-β inhibitor (SB431542). (C) Numbers of LSK cells generated following 5 days culture in serum-free media on Delta1$^{ext\text{-}IgG}$ with or without recombinant TGF-β1 (10 ng/ml) and/or TGF-β inhibitor (SB431542). (D) Numbers of LSK cells generated following 5 days culture in serum-free media on Delta1$^{ext\text{-}IgG}$ with or without recombinant TGF-β1 (1 ng/ml) and/or TGF-β inhibitor (10μM LY364947). Data expressed as mean ±s.d., from replicate samples (n=3). P values are indicated, unpaired Student's t-test .

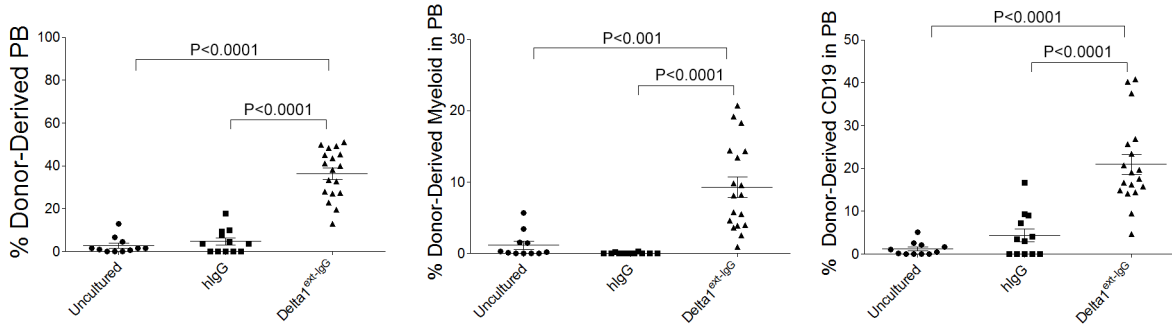

Figure S8. Delta1$^{ext\text{-}IgG}$ increases rapidly repopulating progenitors from E11 AGM-derived CD45^{+}VE-Cadherin^{+} cells. PB engraftment at 2 weeks post-transplant of E11 AGM CD45^{+}VE-cadherin^{+} cells directly transplanted after sort (uncultured) or transplanted following 5 days culture on Delta1$^{ext\text{-}IgG}$ or control (hIgG), at 1 embryo equivalent (ee). Shown is pooled analysis from two experiments plotting engraftment from individual mice, including total donor engraftment (CD45.2), donor myeloid (Gr1 and/or F480), and donor B-cell (CD19) in the PB. Error bars show mean±sem. P values are indicated, unpaired Student's t-test.