



Supplementary information, Figure S7 Notch signaling downregulation facilitates HSC emergence. (A) Model of the HE turning into HSPCs or non-hemogenic ECs. Red cells represent non-hemogenic ECs, yellow cells represent the HE, and green cells represent HSPCs. (B) The expression of *ch25h* and *cyp7b1* in sorted non-hemogenic ECs, HE and HSPCs. (C) Clotrimazole treatment in sorted non-hemogenic ECs, HE and HSPCs in E11 mouse embryo. The hematopoietic potential/differentiation of non-hemogenic ECs or HSPCs was

not altered after treatment with clotrimazole. While in the HE population, the hematopoietic potential was greatly attenuated. (D, E) Flow cytometry analysis of Notch1 expression in sorted Tie2⁺ cells from the E11 mouse AGM region after treatment with DMSO or 7 α -25-OHC. Upon 7 α -25-OHC stimulation, the Tie2⁺ cell number was increased, while the Notch1 level in Tie2⁺ cells was decreased. (F, G) 7 α -25-OHC treatment elevated donor derived chimerism (GFP⁺) and multi-lineage potential in bone marrow of the recipients (GFP⁻) three months after transplantation. Error bar, \pm s. d. *p<0.05, **p<0.01.