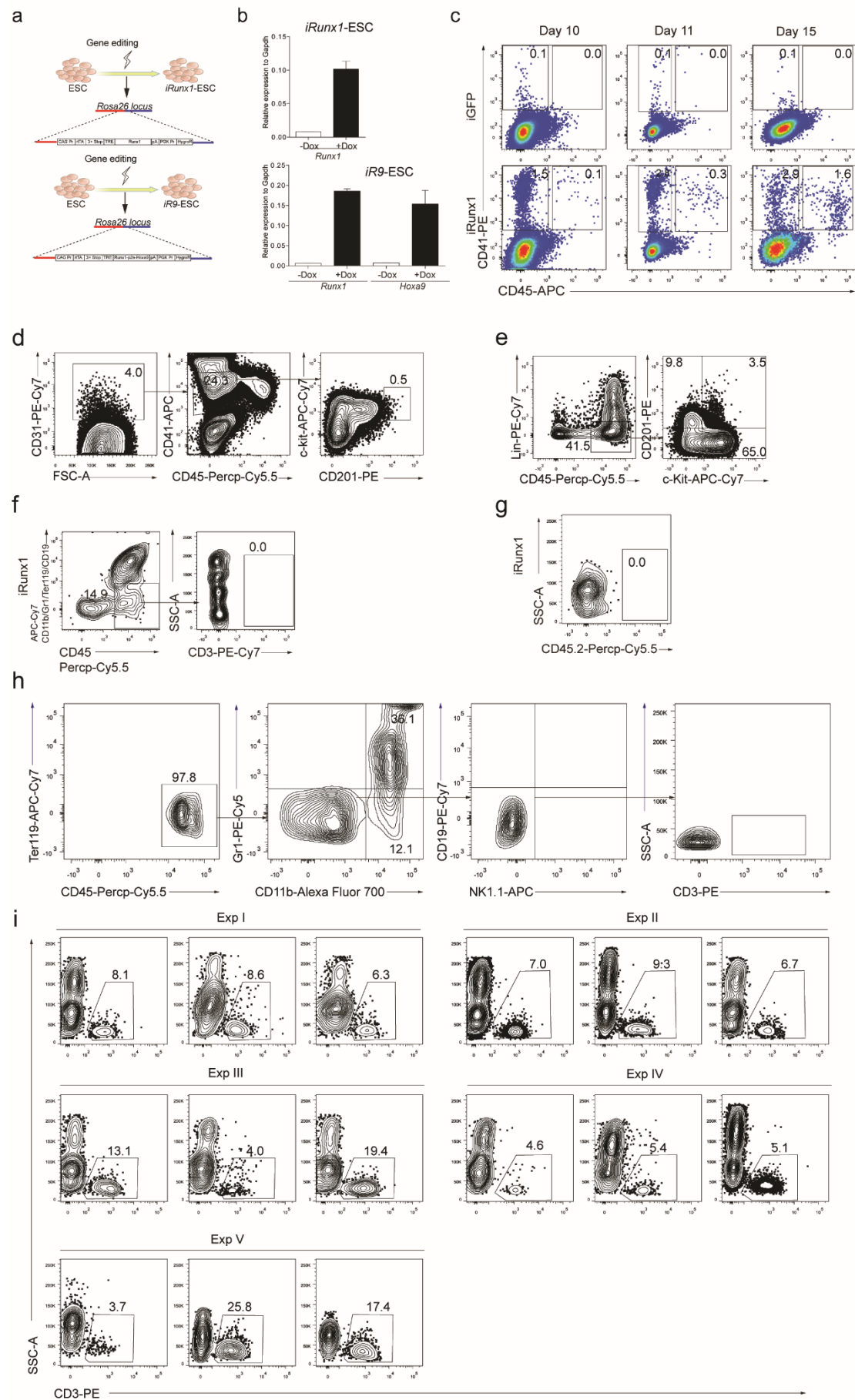


Figure S1



Supplementary Figure 1. Transcription factor Runx1 as the starting candidate for iT cell regeneration. **a** Schematic diagram of iRunx1-ESC and iRunx1-p2a-Hoxa9 (*iR9*)-ESC construction. A rtTA-TRE-Runx1-HygroR DNA cassette or rtTA-TRE-Runx1-Hoxa9-HygroR DNA cassette was inserted into the *Rosa26 locus* of CD45.2⁺ C57BL/6 mouse ESC by homologous recombination. The *iRunx1*- or *iR9*-ESC clones were selected by hygromycin B (150 µg/ml). **b** Q-PCR analysis of the expression of Runx1 in iRunx1-ESC, and the expression of Runx1 and Hoxa9 in *iR9*-ESC after doxycycline induction. **c** Runx1 expression promotes endothelial to hematopoietic transition. CD41⁺CD45⁻ and CD41⁺CD45⁺ populations of iGFP-ESC-derived and iRunx1-ESC-derived cells were analyzed at day 10, day 11 and day 15 by flow cytometry. **d** Sorting gates of iRunx1 ESC-derived iHEC population at day 11. Representative plots from three independent experiments are shown. **e** Immunophenotypes of hematopoietic progenitors from iRunx-ESC-derived iHEC after ten-day maturation. Representative plots from four independent experiments are shown. Lin was defined as CD2⁻CD3⁻CD4⁻CD8⁻Gr1⁻Ter119⁻CD19⁻NK1.1⁻TCRγδ⁻. Hematopoietic progenitors were defined as CD45⁺Lin⁻c-kit⁺CD201⁺. **f** The failure of iT cell induction from iRunx1-ESC-derived iHEC. Flow cytometry analysis of induced T cells from in vitro induction of iRunx1-ESC-derived iHEC. One thousand cells of iRunx1-ESC-derived iHEC were seeded into per well (24-well plates) pre-seeded with OP9-DL1 feeder cells (10000 cells/well) 12 hours prior maturation in EM medium with doxycycline (1 µg/ml). Doxycycline was sustained for 10 days during the maturation step. After maturation, the bulk blood cells were assessed for T lineage generation potential. For in vitro T cell induction, the medium was changed to T cell induction medium (TIM, α-MEM, 20% DFBS, and 1% GlutaMAX) supplemented with 2% conditioned medium derived from supernatants of AFT024-hFlt3L and AFT024-hIL7 cell culture for sustaining 12 days. **g** iRunx1-ESC-derived iHEC failed to contribute any hematopoietic in B-NDG mice four weeks after transplantation. One million iHEC-derived hematopoietic cells were transplanted into individual B-NDG mice (CD45.1⁺) irradiated by X-ray (2.25 Gy). One representative mouse was analyzed. **h** Lineage characterization of bulk cells collected at day 10 after coculture of *iR9*-ESC-derived iHEC and OP9-DL1. One representative plot from three independent experiments are shown. Myeloid cells were defined as CD45⁺CD11b⁺. **i** Flow cytometry analysis of pluripotent stem cell-derived T cells in PB of iHPC recipients. Plots from three mice of each independent experiment (Exp I, II, III, IV, V) are shown.