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Supplemental Information

In Vivo Generation of Engraftable Murine Hematopoietic Stem Cells by *Gfi1b, c-Fos*, and *Gata2* Overexpression within Teratoma

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Supplementary Information

Supplementary Tables

Supplementary Table 1: qPCR primer and probe list

Gene	Forward (5' to 3')	Reverse (3' to 5')	Roche universal probe no.
Gfi1b	gcacagagtctcccttggac	atgaggggtggagaacacc	80
cFos	gggacagcctttcctactaccc	agatctgcgcaaaagtcctg	67
Gata2	gcttcacccctaagcagaga	atctcgtcgccagagagg	76
Erg	catgagtctccggaaagcag	tgaagcacaacaccttctataaactt	53
HoxA9	tccctgactgactatgcttgtg	gttggcagccgggttatt	25
Rora	cctactgttccttcaccaacg	tgttctgggcaaggtgttc	60
FoxC1	gctttcctgctcattcgtctt	aaatatcttacaggtgagaggcaag	34
Oct3/4	aatgccgtgaagttggagaa	ccttctgcagggctttcat	95
Sox2	acagctacgcgcacatga	ggtagcccagctgctcct	19
Klf4	cgggaagggagaagacact	gagttcctcacgccaacg	62

Supplementary Table 2: Antibody staining list

Peripheral blood antibody staining	Bone marrow antibody staining
CD45.1-PE-Cy7 (#560578 BD Biosciences)	Biotinylated lineage cocktail (#130-092-613（Miltenyibiotec）
CD45.2-FITC (#109806 BioLegend)	CD45.1-PB (#553776 BD Biosciences)
CD4-APC (#100515 BioLegend)	CD45.2-FITC (#109805 BioLegend)
CD8-APC (#17-0088-42 eBioscience),	Sca1-PE-Cy7 (#558162 PharMingen)
B220-APC-Cy7 (#25-0452-82 eBioscience)	cKit-APC (#553356 PharMingen)
Gr1-Pacific Blue (PB) (#108430 BioLegend)	Streptavidin-APC-Cy7 (#554063 PharMingen)
Mac-1-PB (#101224 BioLegend)	

Supplementary Figures

Supplementary Figure and Legends:

Figure S1

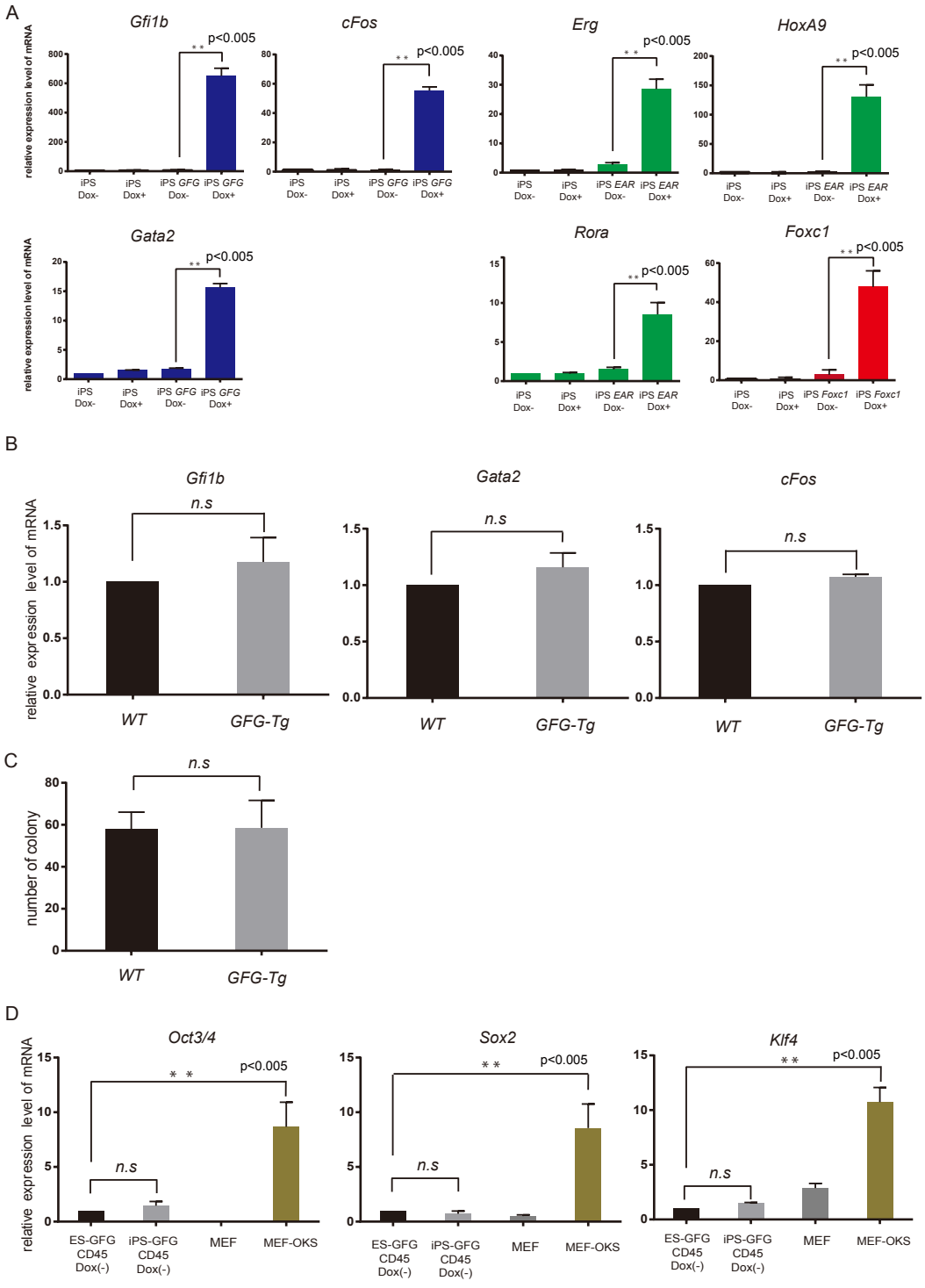

Figure S1: Related to Figure 1; establishment of inducible iPSCs from Ly5.1 mice

(A) Average relative transgene expression in iPSCs following a four-day induction by addition of Dox. Data are the mean ± SD from two independent experiments (n = 3). **P* < 0.005.

(B) Expression analysis of *Gfi1b*, *Gata2* and *cFos* in WBMCs of GFG transgenic mice (GFG-Tg) without Dox. Data are the mean ± SD from two independent experiments (n = 3). n.s : not significant.

(C) Colony formation from WBMCs isolated from wild type mice and GFG-Tg mice without Dox. Data are the mean ± SD from two independent experiments (n = 3). n.s : not significant.

(D) Analysis of reactivation of *Oct*, *Sox2* and *Klf4* genes using pluripotent stem cells derived $CD45^{+}$ cells by in vitro embryoid body differentiation and MEF. MEF-OKS group is positive control. Data are the mean ± SD from two independent experiments (n = 5). n.s : not significant.

Figure S2

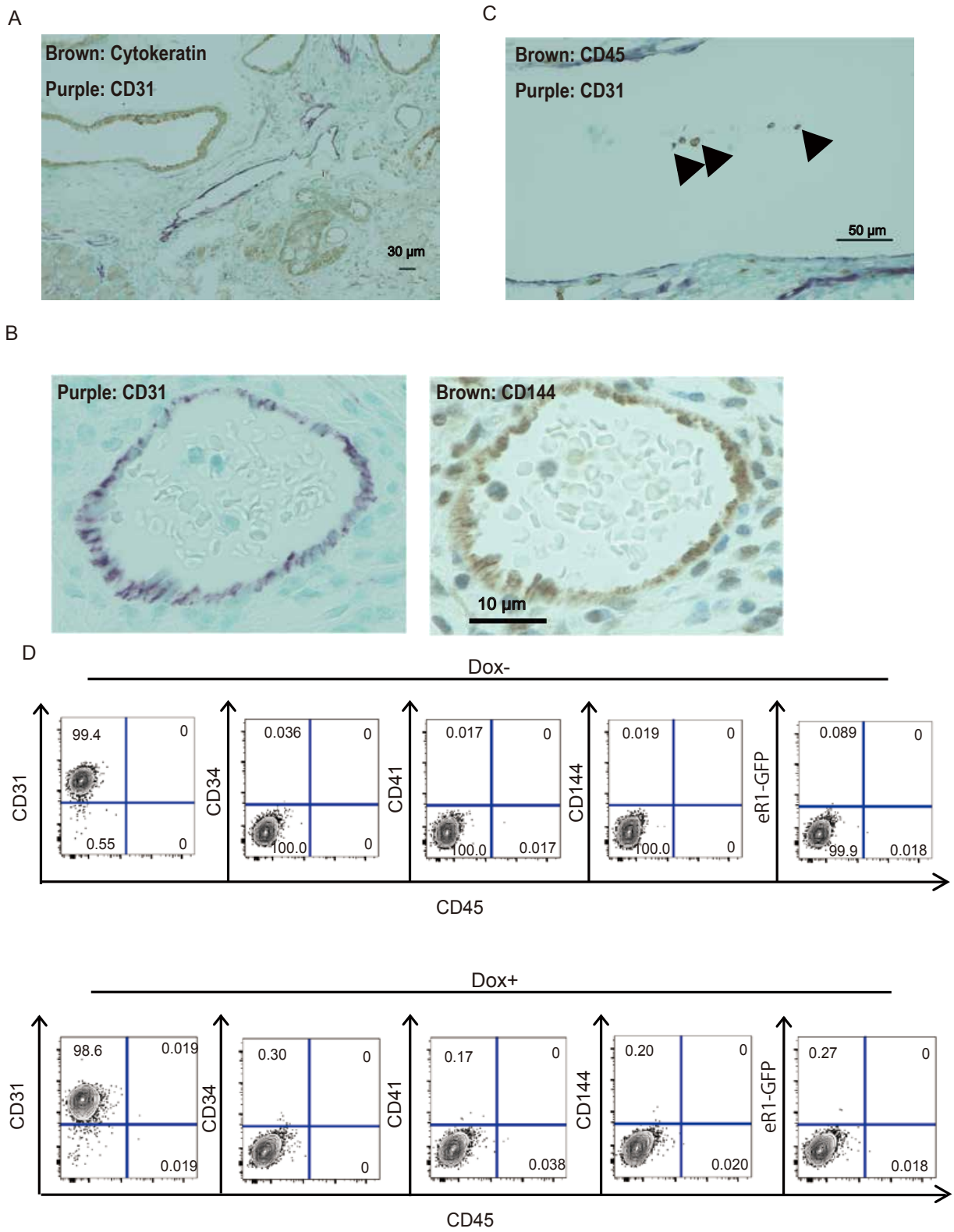

Figure S2: Related to Figure 2; analysis of hematopoietic-generating tissue in teratomas
(A) Representative image of a section of an GFG-teratoma immunostained for vascular

endothelial cells ($CD31^+$ cells; purple) and epithelial cells (Cytokeratin; brown).
(B) Serial sections of an GFG-teratoma immunostained for vascular endothelial cell markers CD31 (purple) and CD144 (brown).
(C) Representative image of a section of an GFG-teratoma immunostained for vascular endothelial cells ($CD31^+$ cells; purple) and hematopoietic cells ($CD45^+$ cells; brown).
(D) Representative flow cytometric plots displaying hematopoietic lineage marker expression on GFG-transduced eR1-iPSCs after four days cultured with or without dox.

Figure S3

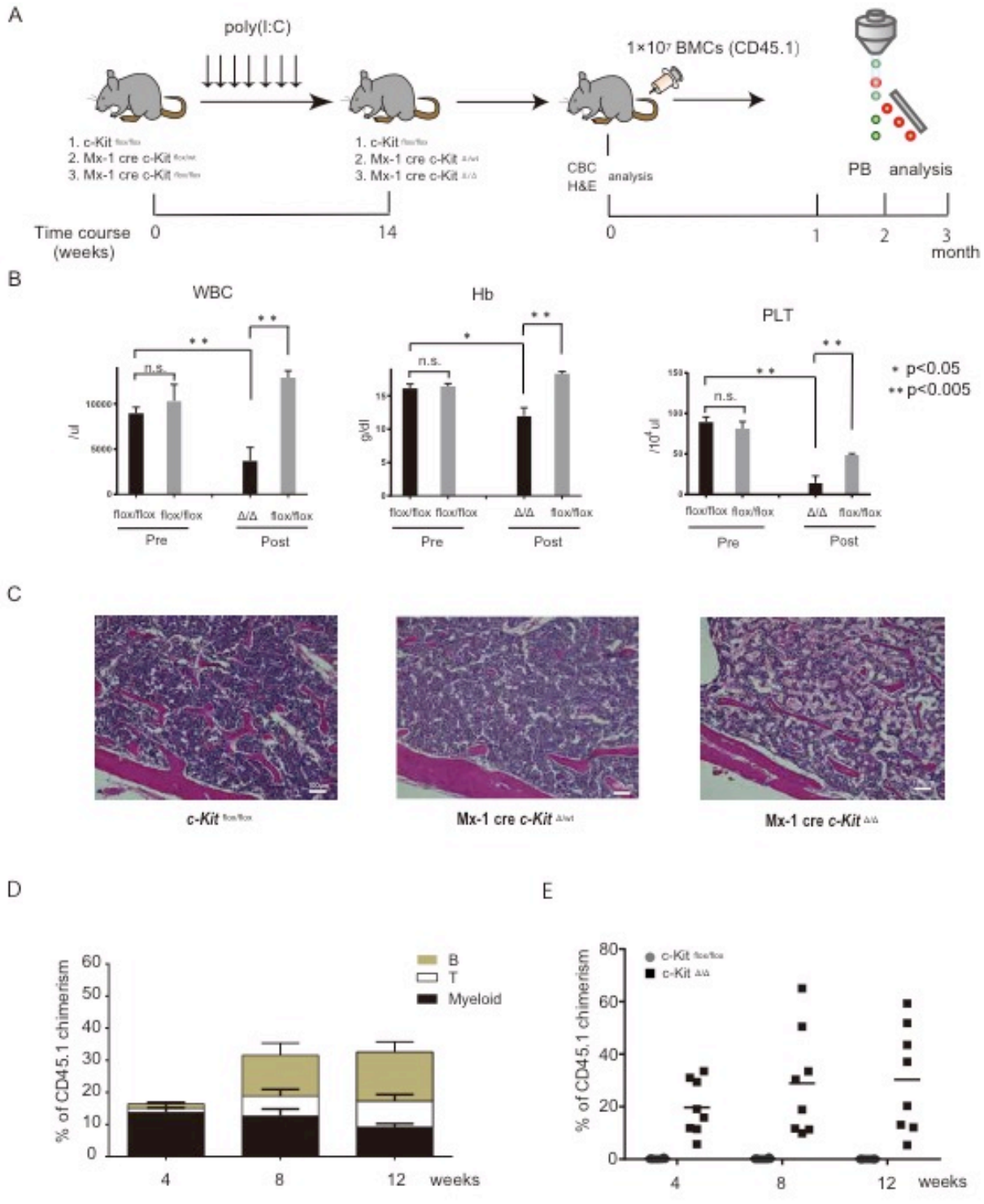

Figure S3: Related to Figure 3; analysis of hematopoiesis in *c-Kit* deficient mice

(A) Schematic of the strategy to validate HSPC-deficient host mice. CBC, histological analysis and hematopoietic reconstitution assay were undertaken following poly(I:C) administration.

(B) CBC was tested in peripheral blood (PB) from two groups of mice: poly(I:C)-treated (*c-Kit*-

deficient mice; black bars) and PBS-treated (control mice; grey bars). CBCs were tested both pre- and post-treatment. WBC: white blood cell, Hb: hemoglobin, PLT: platelet. Data are the mean ± SD from two independent experiments (n = 6 per group). $**P < 0.005$, $*P < 0.05$, n.s : not significant.

(C) *c-Kit* deficiency leads to impaired hematopoiesis. Femurs section from the control and *c-Kit*-deficient mice stained with hematoxylin-eosin.

(D, E) Donor-derived PB cell ratio was measured 12 weeks after transplantation into poly (I:C administrated recipients together with 1×10^7 bone marrow (BM) cells (CD45.1). (n=8)

Figure S4

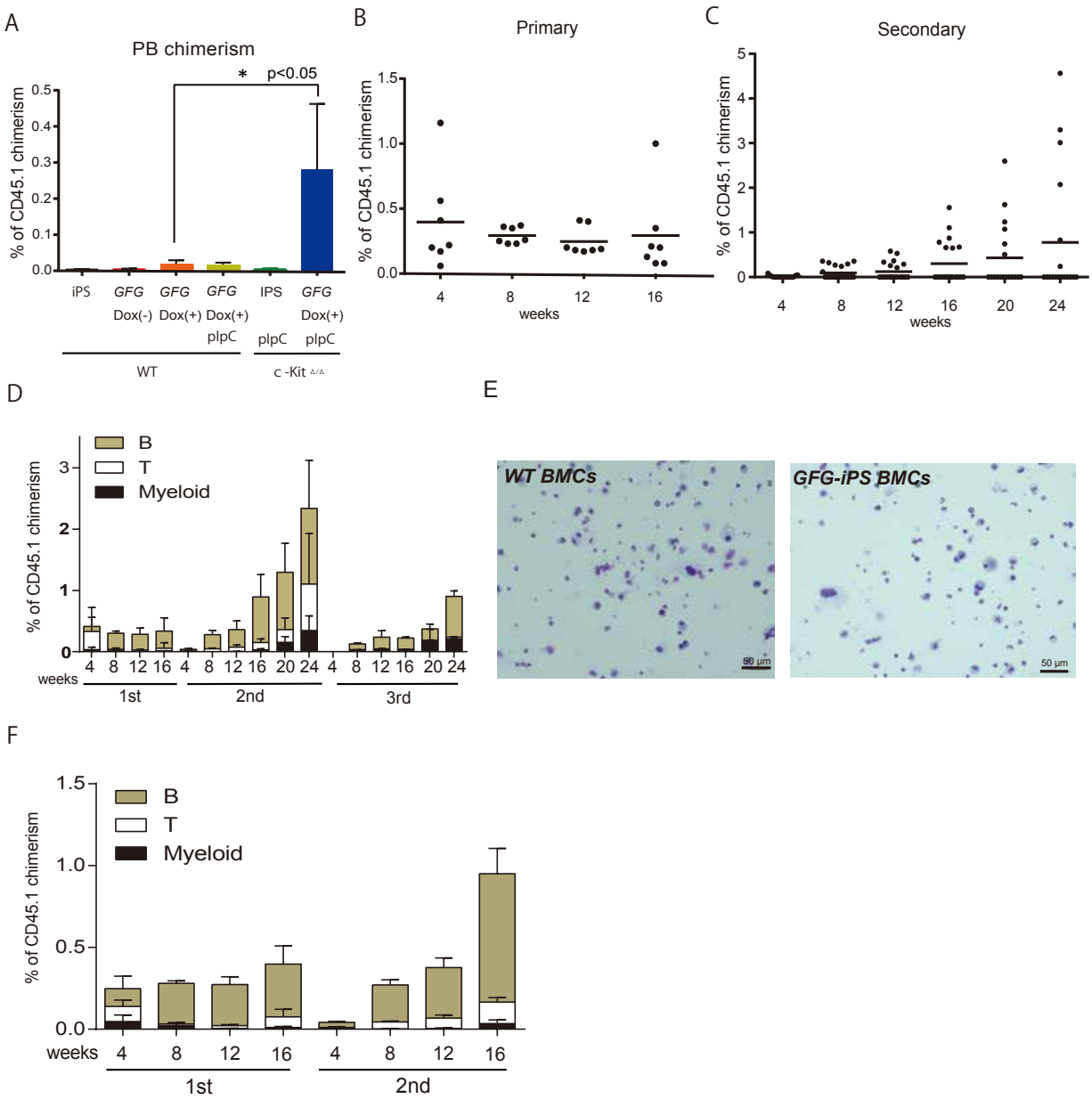

Figure S4: Related to Figure 3 and 4; *c-Kit* deficiency affords long-term HSC formation

from *GFG*-teratomas

(A) Percentage of CD45.1$^+$ PB chimerism in teratoma-bearing C57BL/6 mice and *c-Kit*-deficient mice at 14-16 weeks after iPSCs injection. Data are the mean ± SD (n = 8 *$P < 0.05$).

(B,C) Percentage of CD45.1$^+$ PB chimerisms in primary (B) and secondary (C) recipients of the bone marrow transplantation assay described in Figure 3D. Data are the mean ± SD from two independent experiments (n = 8 primary n=21 secondary)

(D) Percentage of CD45.1$^+$ PB chimerisms in primary, secondary, and tertiary recipients of BM from teratoma-bearing host mice. Data are the mean ± SD from two independent experiments. (n=5)

(E) Cytospin images of CFUs from the colony-forming assay with *GFG*-iPSC derived BMCs from recipient mice after secondary transplantation (n=5).

(F) Percentage CD45.1$^+$ PB chimerism analysis of teratoma block-derived BM cells after transplanting into primary and secondary recipient mice. Data are the mean ± SD from two independent experiments (n=5).

(E) Frequency teratomas giving rise to CD45.1$^+$ PB cells, from various origins (n=5).