

Stem Cell Reports, Volume 11

Supplemental Information

Generation of Vascular Endothelial Cells and Hematopoietic Cells by Blastocyst Complementation

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SUPPLEMENTAL INVENTORY

Generation of Vascular Endothelial Cells and Hematopoietic Cells by Blastocyst Complementation

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Inventory of supplemental information

Figure S1: This figure is related to Figure 1.

Figure S2: This figure is related to Figure 2.

Figure S3: This figure is related to Figure 3.

Figure S4: This figure is related to Figure 4

Table S1: This table is related to Table 1.

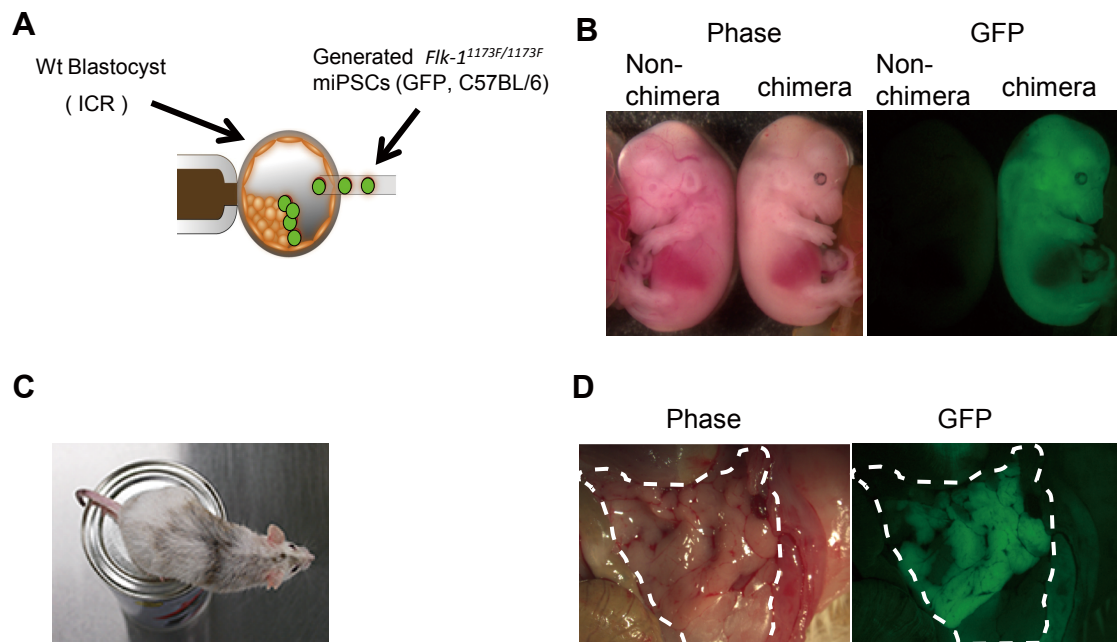


Figure S1. Generation of *Flk-1*^{1173/1173} chimeric mice. Related to Figure 1.

(A) *Flk-1*^{1173/1173} chimera generated by injection of *Flk-1*^{1173/1173} EGFP-expressing miPSCs into ICR wildtype mouse blastocysts.

(B) E14.5 mice have systemic chimera.

(C) Representative 26-week-old *Flk-1*^{1173/1173} chimera mouse was shown. Black eyes and black/brown coat were derived from *Flk-1*^{1173/1173} EGFP-expressing miPSCs.

(D) Analysis of chimerism in the pancreas of an adult chimera.

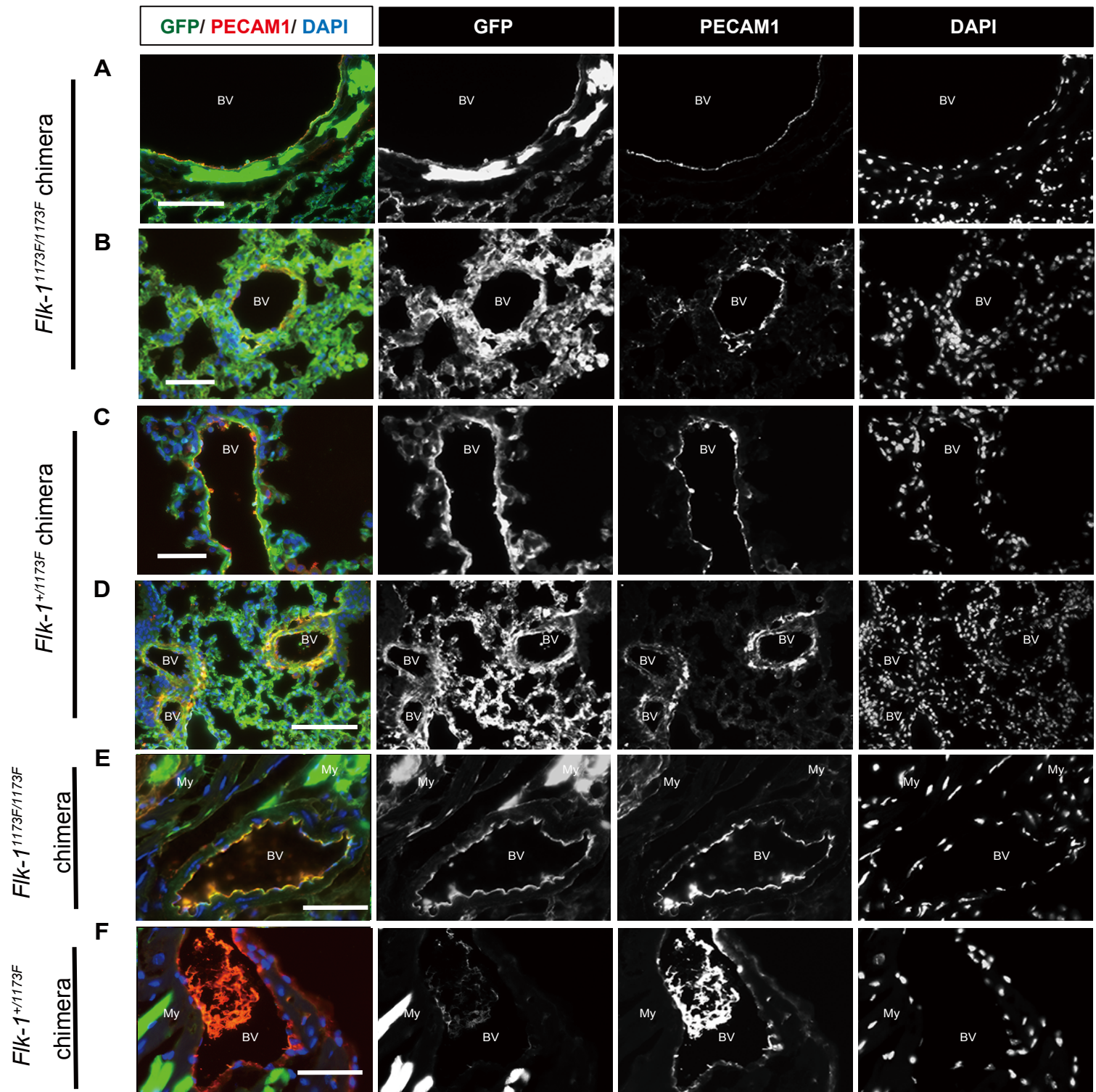


Figure S2. Analysis of chimerism tissues of adult chimeric mice generated from *Flk-1*^{1173F/1173F} blastocysts and GFP- labelled miPSCs. Related to Figure 2.

(A to D) Section of lung, and heart (E and F) were immunostained with antibodies against GFP and PECAM1. Nuclei were counterstained with DAPI.

BV, blood vessel; My, myocardium. Scale bars, 50 μ m (B, C, E and F) and 100 μ m (A and D).

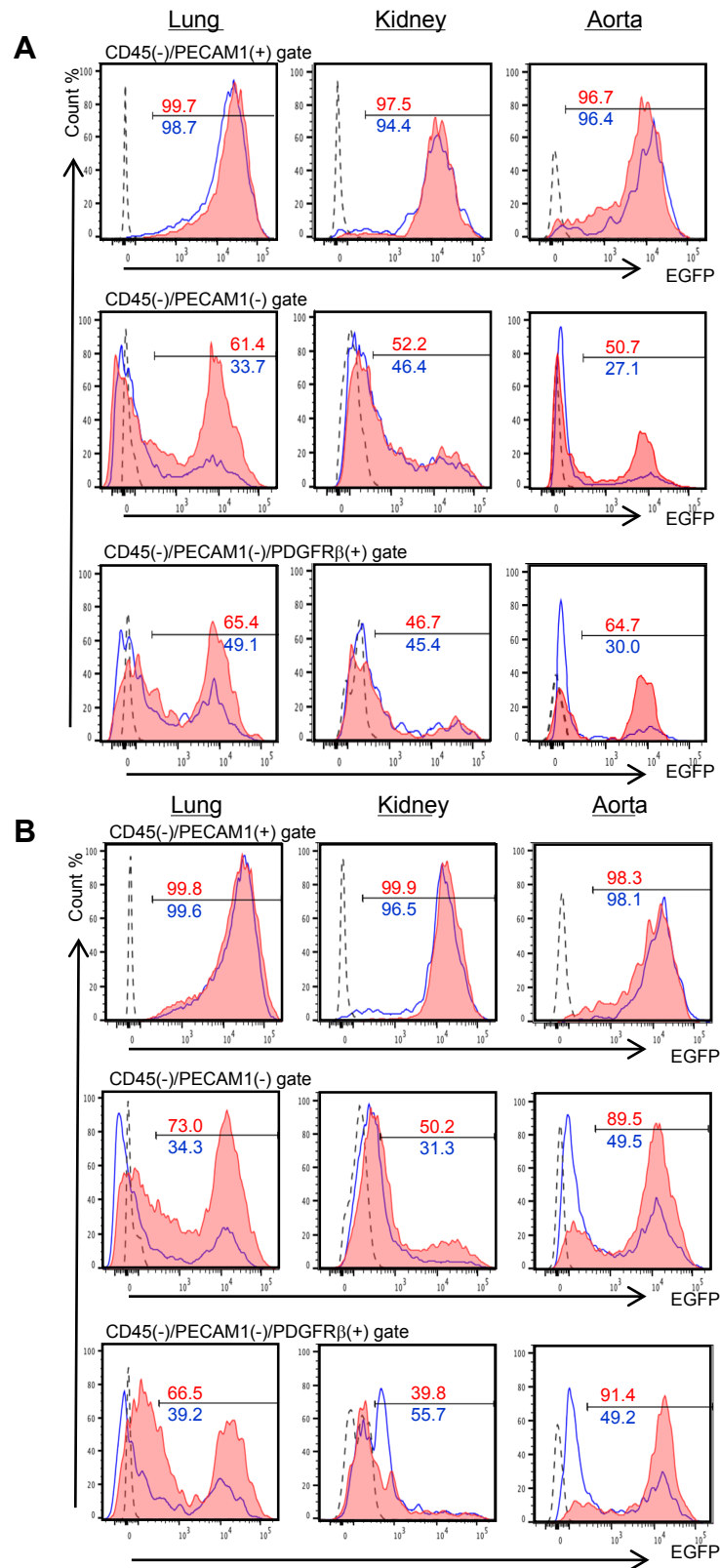


Figure S3. Flow cytometry analysis of the EGFP-expressing mESC-derived cells in the vascular endothelium and pericytes of each organ. Related to Figure 3.

(A and B) Ratio of donor cell contribution in lung, kidney and aorta of *Flk-1*^{1173F/1173F} or *Flk-1*^{+/1173F} chimeric mice were analyzed using antibodies against CD45, PECAM1, and PDGFRβ. Vascular endothelial cells (CD45⁺/PECAM1⁺), non-vascular endothelial cells (CD45⁺/PECAM1⁻) and pericytes (CD45⁻/PECAM1⁻/PDGFRβ⁺) were gated and analyzed for EGFP expression. Red line, *Flk-1*^{1173F/1173F} chimeric mouse; blue line, *Flk-1*^{+/1173F} chimeric mouse; gray dashed-line, C57BL/6 mouse shown as a control.

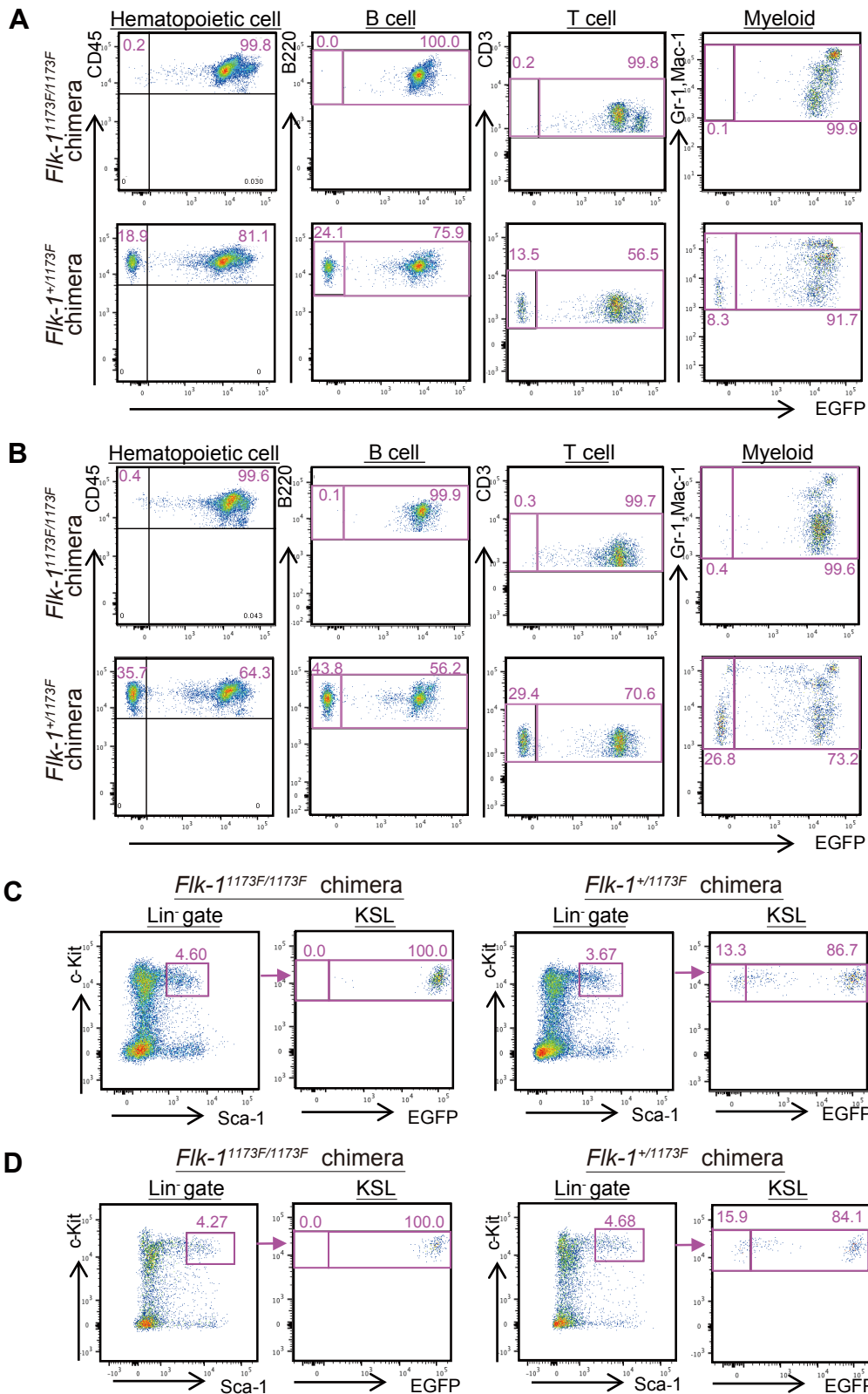


Figure S4. Flow cytometry analysis of chimerism in mESC-derived hematopoietic cells.

Related to Figure 4.

(A and B) Ratio of EGFP and hematopoietic lineage marker expression in peripheral blood from *Flk-1* mutant chimeras. (C and D) Flow cytometric plots and gating for the KSL HSCs fraction within the BM of *Flk-1* mutant chimeras.

Supplemental Table

Table S1. Results of *Flk-1*^{1173F/1173F} mouse-rat interspecies blastocyst complementation. Related to Table 1.

Embryo- nic Stage	Injected Cell Lines	Injection/ Transfer	No. (%) of Embryo		<i>Flk-1</i> Genotype (%)		
			All	Chimeras	+/+	+/ <i>1173F</i>	<i>1173F</i> / <i>1173F</i>
E13.5	riPST1-3	90	11 (12)	11 (100)	4 (36)	7 (64)	0 (0)
	Fri6.1	70	13 (18)	11 (85)	4 (36)	7 (64)	0 (0)
	Total	160	24 (15)	22 (92)	8 (36)	14 (64)	0 (0)
E9.5	riPST1-3	137	73 (55)	73 (100)	19 (26)	37 (51)	17 (23)
	total				(*4)	(*16)	(*10)

(*delay or small)