SUPPLEMENTAL DATA

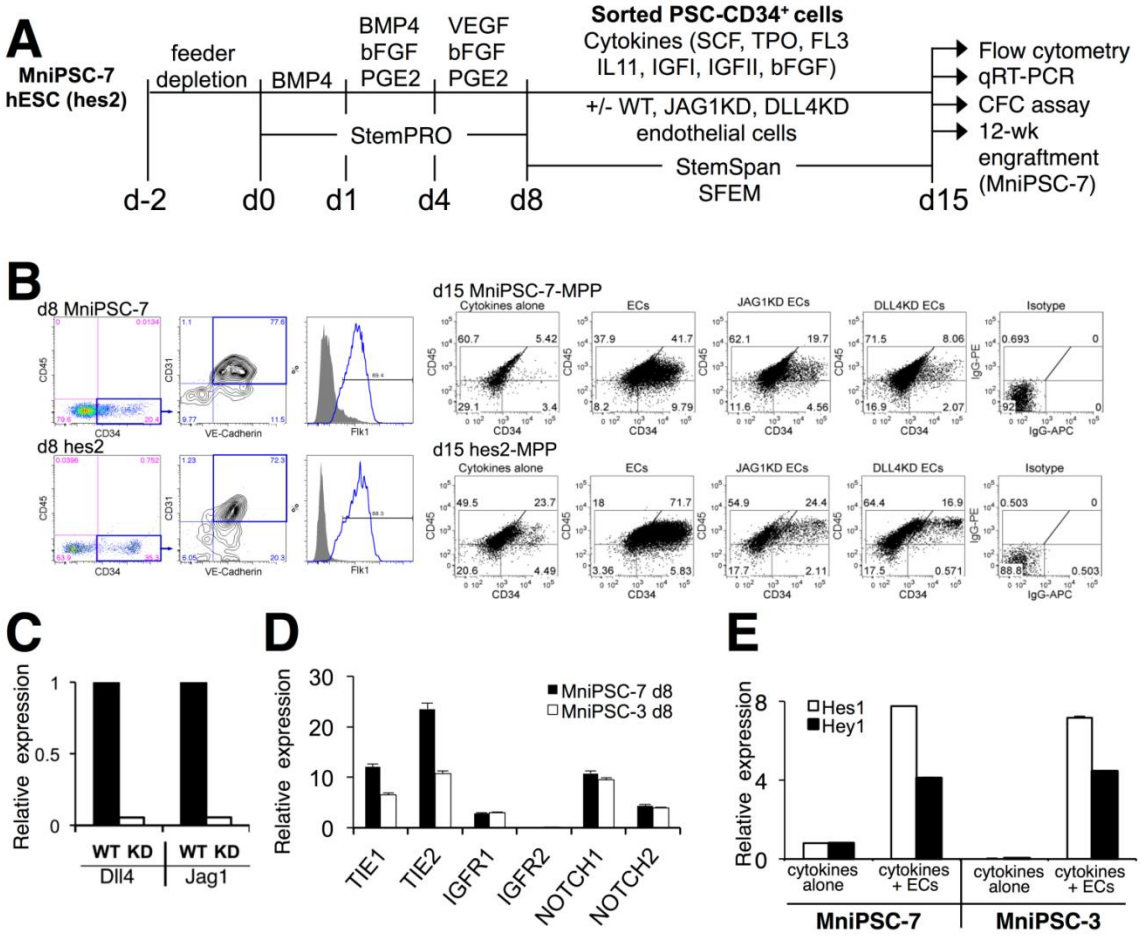


Figure S1

Supplemental Figure 1. MniPSC-MPP express angiocrine receptors and upregulate downstream targets of Notch activation upon EC co-culture. (**A**) Experimental schematic for Figure 1 experiments. MniPSC-7 and hes2 were induced toward hemogenic mesoderm in StemPRO with cytokines. Day 8 $CD34^+$ cell cells were plated in StemSpan with cytokines ± co-culture with endothelial cells (EC) that express Notch ligands (JAG1, DLL4) or EC with ligand knockdown (JAG1KD, DLL4KD). (**B**) Flow cytometry of *Left:* day 8 precursors and *Right:* day 15 MPP after co-culture with cytokines ± the indicated EC. **(C)** qRT-PCR analysis JAG1 and DLL4 expression in WT endothelial cells, JAG1KD, and DLL4KD endothelial cells. **(D)** Upregulation of angiocrine responsive receptors after hemogenic induction of MniPSC. Data are normalized to β-actin and calibrated to undifferentiated MniPSC. (**E**) Day 15 MniPSC-MPP upregulate downstream targets of Notch1/2 activation after direct co-culture of 2 different MniPSC lines 3 and 7 with endothelial cells.

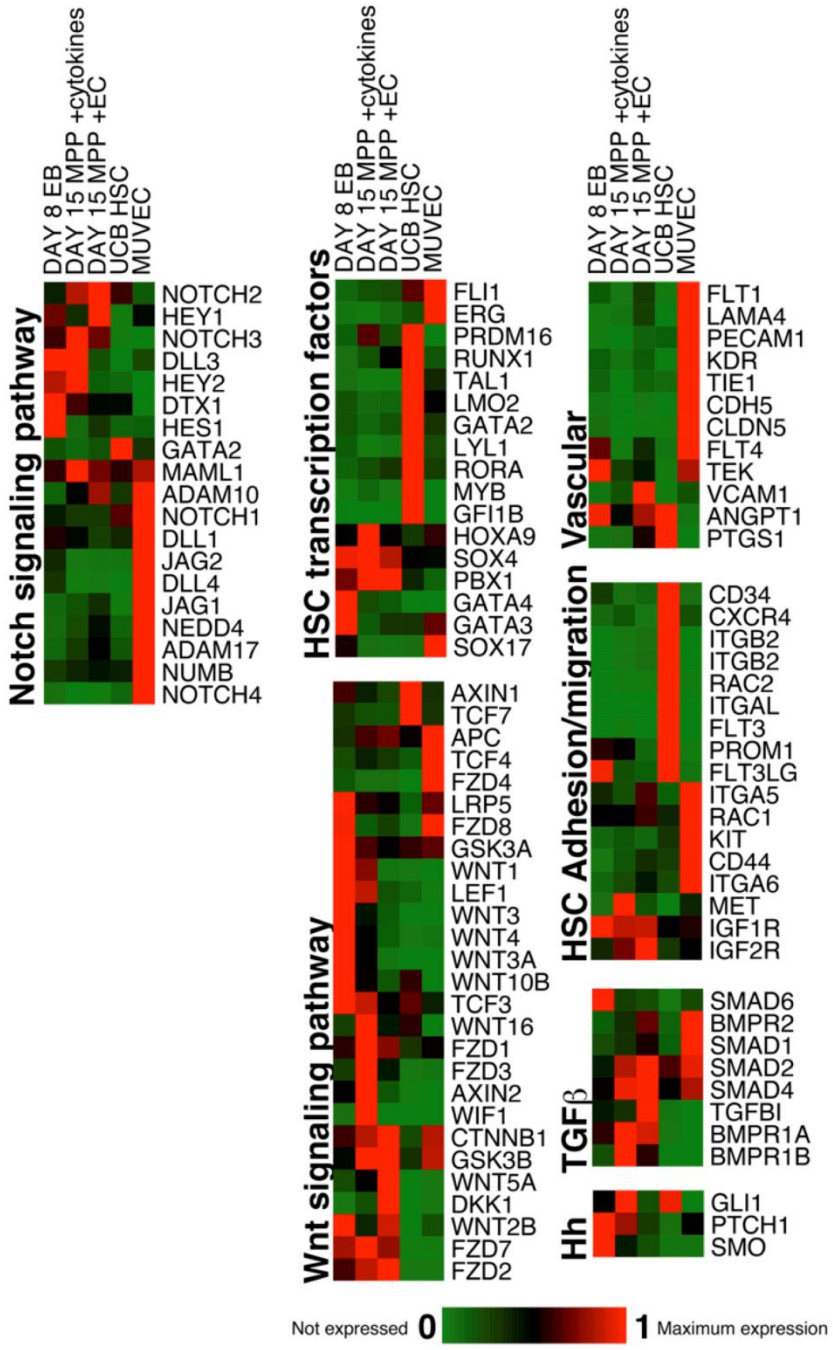


Figure S2

Supplemental Figure 2. RNA sequencing analysis of MniPSC-derived hematopoietic cells. Whole transcriptome (RNAseq) analysis of MniPSC-CD34^{+} cells before (day 8 EBs) and after induction with cytokines alone (-EC) or with EC and cytokines. UCB HSCs: umbilical cord blood hematopoietic stem cells. MUVEC: monkey umbilical vein endothelial cells.

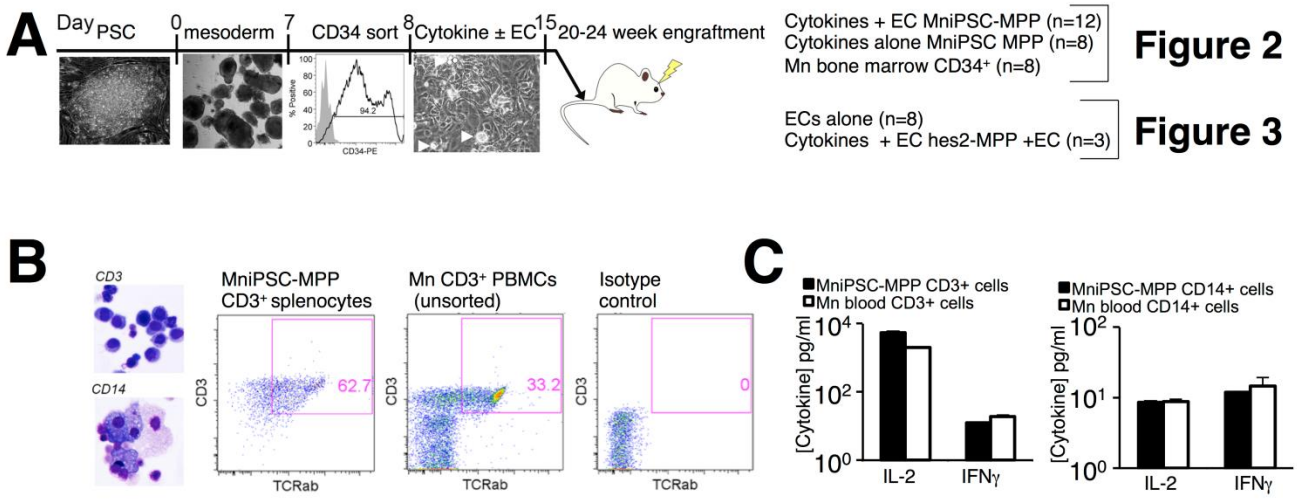


Figure S3

Supplemental Figure 3. Long-term engrafting MniPSC-MPP differentiate into functional myeloid and T lymphoid cells *in vivo* (A) Experimental schematic for experiments shown in Figures 2 and 3. (**B**) Lymphoid (CD3) and myeloid (CD14) cells from EC-induced MniPSC-MPP transplanted mice (10X objective). *Left:* Morphology of $CD3^+$ and $CD14^+$ cells from spleen and marrow of EC-induced MniPSC-MPP transplanted mice, respectively. *Right:* CD3 and TCRαβ expression by cells from spleen EC-induced MniPSC-MPP transplant recipient and monkey blood mononuclear cells (PBMCs). (**C**) T_h1 cytokine production by $CD3^+$ and $CD14^+$ cells.

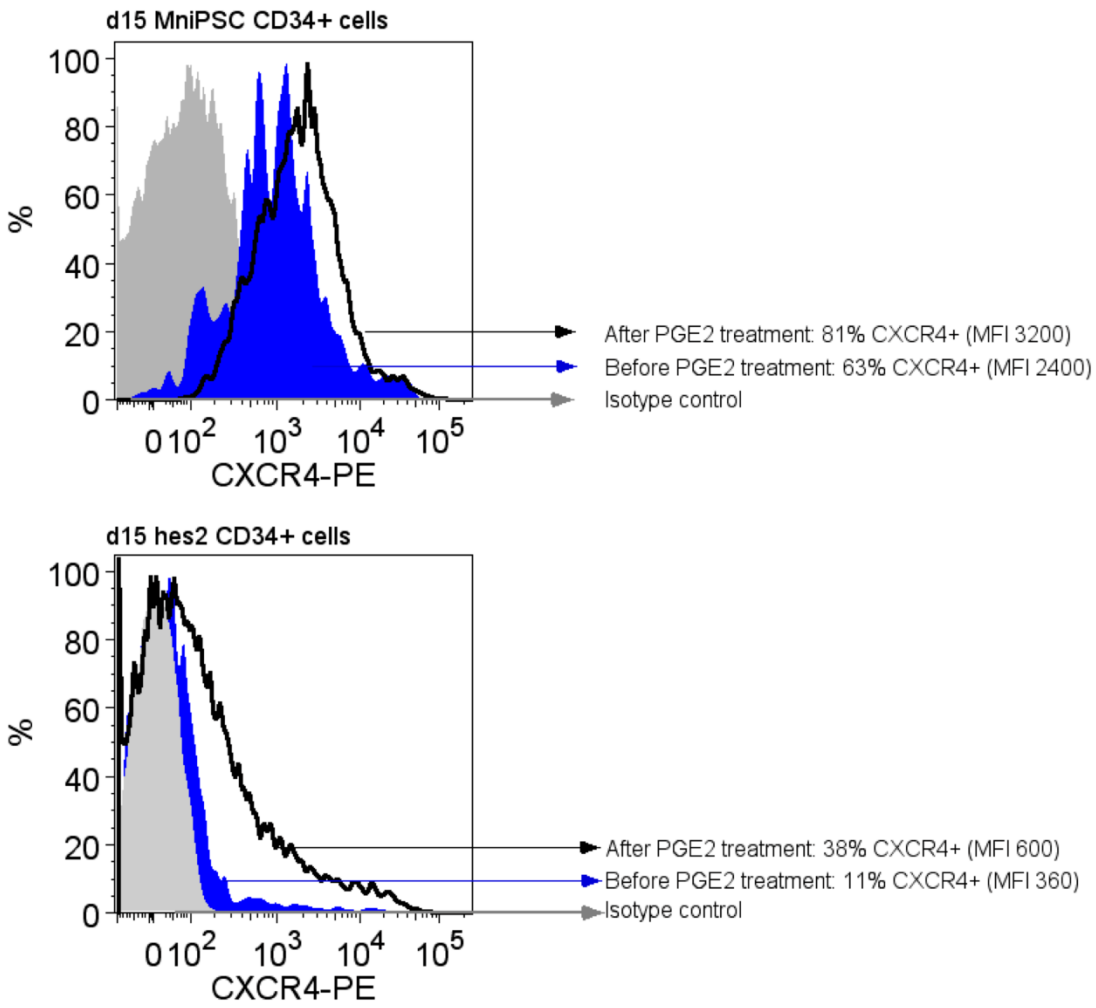


Figure S4

Supplemental Figure 4. Prostaglandin E2 increases expression of CXCR4 on hematopoietic progenitor cells differentiated from MniPSC and hes2 hESC. Flow cytometry analysis of CXCR4 before and after treatment with prostaglandin E2 (PGE2).

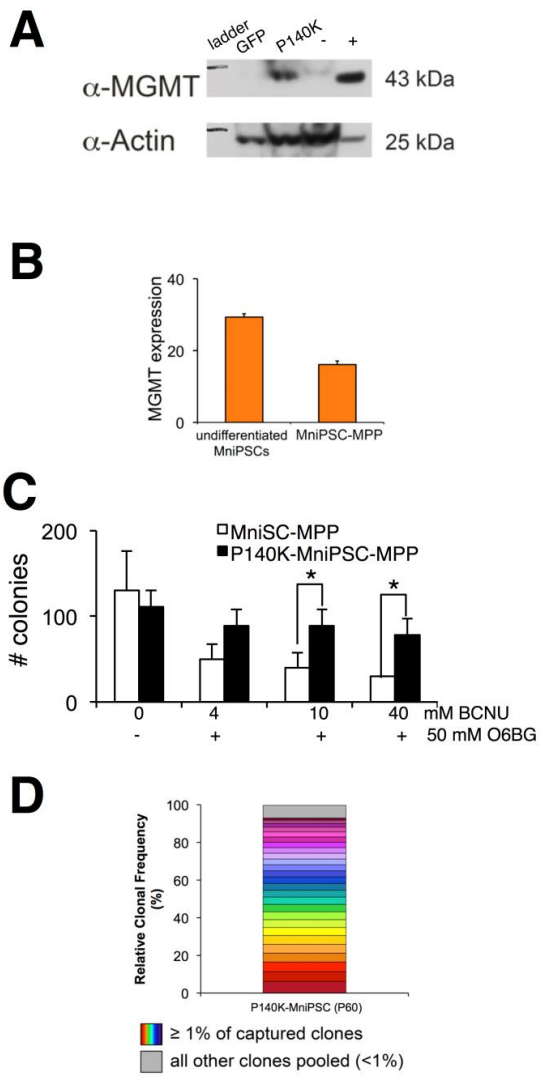


Figure S5

Supplemental Figure 5. P140K-MGMT expression, chemotherapy resistance and polyclonality in lentivirus transduced MniPSC. Stable P140K-MGMT expression in MniPSC. Western blot of MGMT and β–actin in *L-R:* GFP-transduced MniPSC (Passage 41), P140K-transduced MniPSC (Passage 41), untransduced MniPSC (Passage 19, (-)), and positive control (+), which corresponds to PBMCs from monkey with high level P140K^{+} gene marking. **(B)** MGMT expression in P140K-modified MniPSC and MniPSC-MPPs. Expression normalized to GAPDH and calibrated to untransduced MniPSC. Mean ± S.D (3 biologic replicates). **(C)** CFUs from day 8 P140K^{+}MniPSC-MPP and P140K^{-}MniPSC-MPP after treatment with BCNU chemotherapy and wild-type MGMT inhibitor O6-benzylguanine (O6BG). **(D)** Clonality of P140K- MniPSC in culture. Clones constituting ≥1% of all gene modified genomes detected are designated with colored boxes. All other clones are pooled into a single gray shaded box.

Supplemental Table 1. RIS analysis of hematopoietic tissues from 2˚ transplant recipient mice.

See also Supplemental Table 2.

Expansion Method*	**Tissue**	**No. Unique RIS Identified**	**No. of in vivo RIS Overlapping with ex vivo RIS (passage)**	**No. of in vivo RIS Overlapping with Other Tissues**
Cytokines alone	right femur	10	2 (60,61)	2
	left femur	2	0	2
	peripheral blood	3	0	2
	spleen	2	0	2
$E4^{+}EC$	right femur	5	1 (60,61)	1
	left femur	5	3 (60,61)	2
	peripheral blood	3	0	2
	spleen	6	1 (60,61)	1

*Expansion method pertains to preparation of cells prior to infusion into 1˚ recipient mice only.

Supplemental Table 2. PCR primer sequences related to experimental procedures.

Gene	Forward Primer	Reverse Primer
B-ACTIN	TGAGGGGTATGCCCTCCCCCAT	AGGACTCCATGCCCAGGAAGGA
B-ACTIN[*]	TGACCCAGATCATGTTTGAGACC	GCTTCTCCTTAATGTCACGCAC
B-ACTIN[†]	TCCTGTGGCACTCACGAAACT	GAAGCATTTGCGGTG GACGAT
BSG[‡]	CGTAAGGGCCACGGTGTATT	CTGTGGCGCTGTCATTCAAG
BSG[§]	ACTGCTGAGGACACCAGCTT	CTCTGCCTTCTGCCCAATAG
IGFR1	AAACCGCTGCCAGAAAATGTG	GACCTTCACAAGGGATGCAGTA
IGFR2	CTCCGATATTCGGATGGAGACC	TGCTTCTTCTCTGTTTCCGTCT
HES1	GTCTACCTCTCTCCTTGGTCCT	GGCTTTGATGACTTTCTGTGCT
HEY1	TTTTAACCAGAGGCAAAGCGTG	TCGGCGCTTCTCAATTATTCCT
NOTCH1	TTTGTGCTTCTGTTCTTCGTGG	CTCCTCGAACCGGAACTTCTTG
NOTCH2	CCAGGGCCTGAGCCTTTGAA	GGACATTTGCAGTATCCTGTGCC
TIE1	ACTGTGAGAAGTCAGACCGGAT	TCCATCCCCAGAGAACGAGAC
TIE2	ACAGTGCTCCATCCAAAAGACT	ACCAGTTGCACACAGAGTTCATA

[*] PCR primers specific for detection of nonhuman primate specific cDNA (mRNA); no cross-reactivity with mouse or human sequences

[†] PCR primers for detection of human and nonhuman primate genomic DNA

[‡] PCR primers for detection of human genomic DNA; no cross-reactivity with mouse or nonhuman primate sequences

[§]PCR primers for detection of macaque genomic DNA; no cross-reactivity with mouse or human sequences

Supplemental Table 3. Identified sites of lentivirus integration in tissues from secondary transplant recipients.

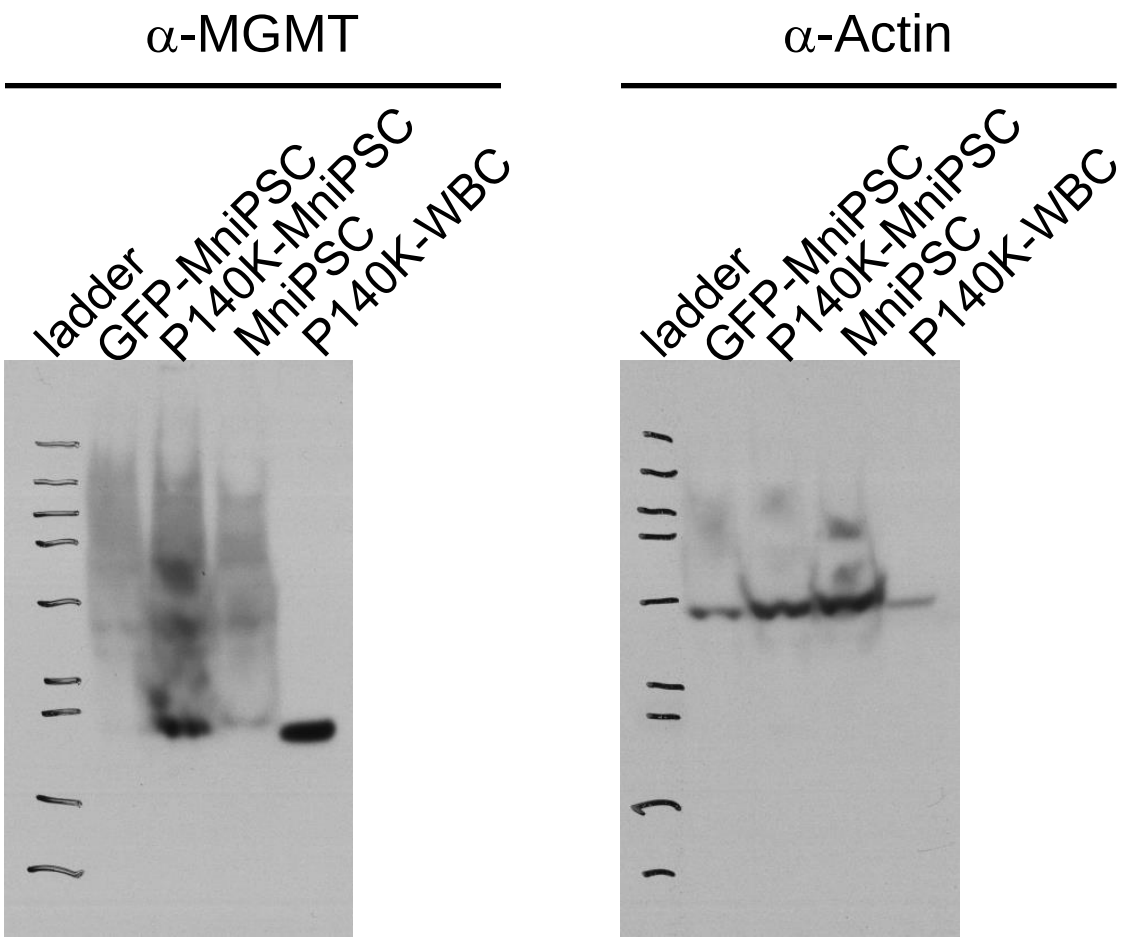
Cytokine-Only Expanded			
Right Femur	**Left femur**	**Blood**	**Spleen**
Chr5:103698085	Chr5:103698085	Chr5:103698085	Chr5:103698085
Chr20:87710990	Chr2:162249731	Chr2:162249731	Chr19:59418572
Chr19:59418572	Chr7:51749247		
Chr19:43323666			
Chr11:86007596			
Chr7:20312665			
Chr13:16041390			
Chr13:84521123			
Chr2:21755669			
Chr1:30696395			
E4EC-Expanded			
Right Femur	**Left femur**	**Blood**	**Spleen**
Chr5:103698085	Chr5:103698085	Chr5:103698085	Chr5:103698085
Chr12:53336416	Chr19:59418572	Chr19:59418572	Chr6:178650209
Chr1:134305327	Chr19:10736750	Chr8:125097876	Chr11:49663495
Chr15:98736817		Chr8:58168205	Chr16:72876574
Chr15:41231023		Chr13:16041390	Chr5:149403184
			Chr9:17589998

NOTE: All loci listed correspond to the October 2010 (BGI CR_1.0/rheMac3) Rhesus (*Macaca mulatta*) genome alignment.

*****Shaded boxes indicate loci detected in both ex vivo culture and in vivo recipient tissue. Colors correspond with clonal notation in Supplemental Figure 1D.

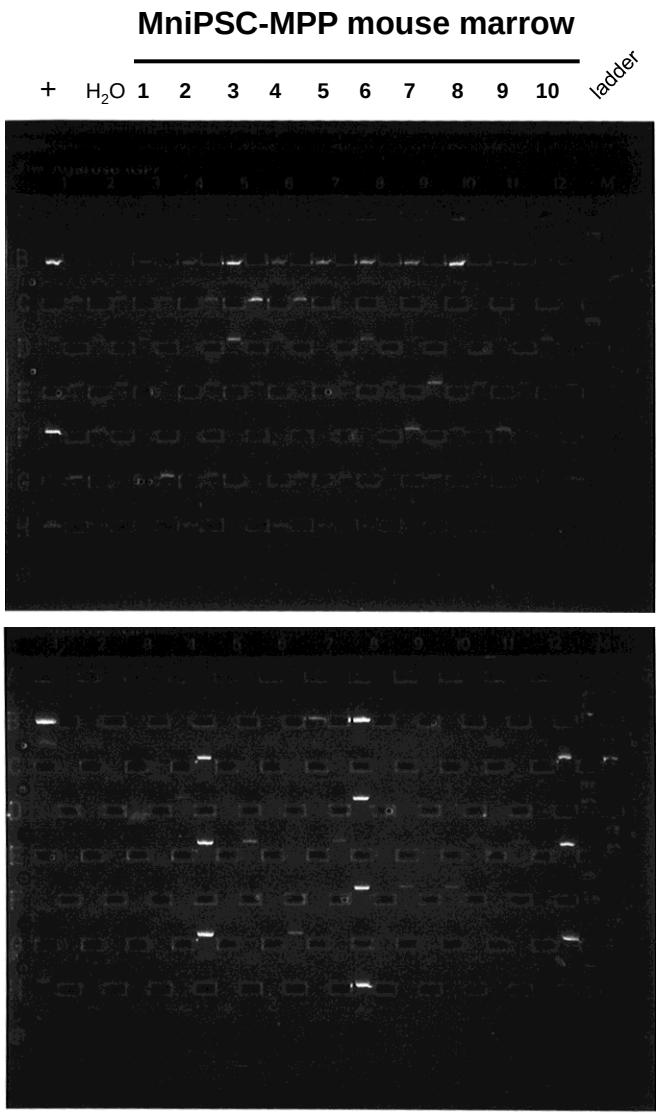
[#]Colored text indicates loci detected in multiple recipient tissues, but not in ex vivo cultures.

Supplemental Uncut Gel 1, pertaining to Supplemental Figure 5.



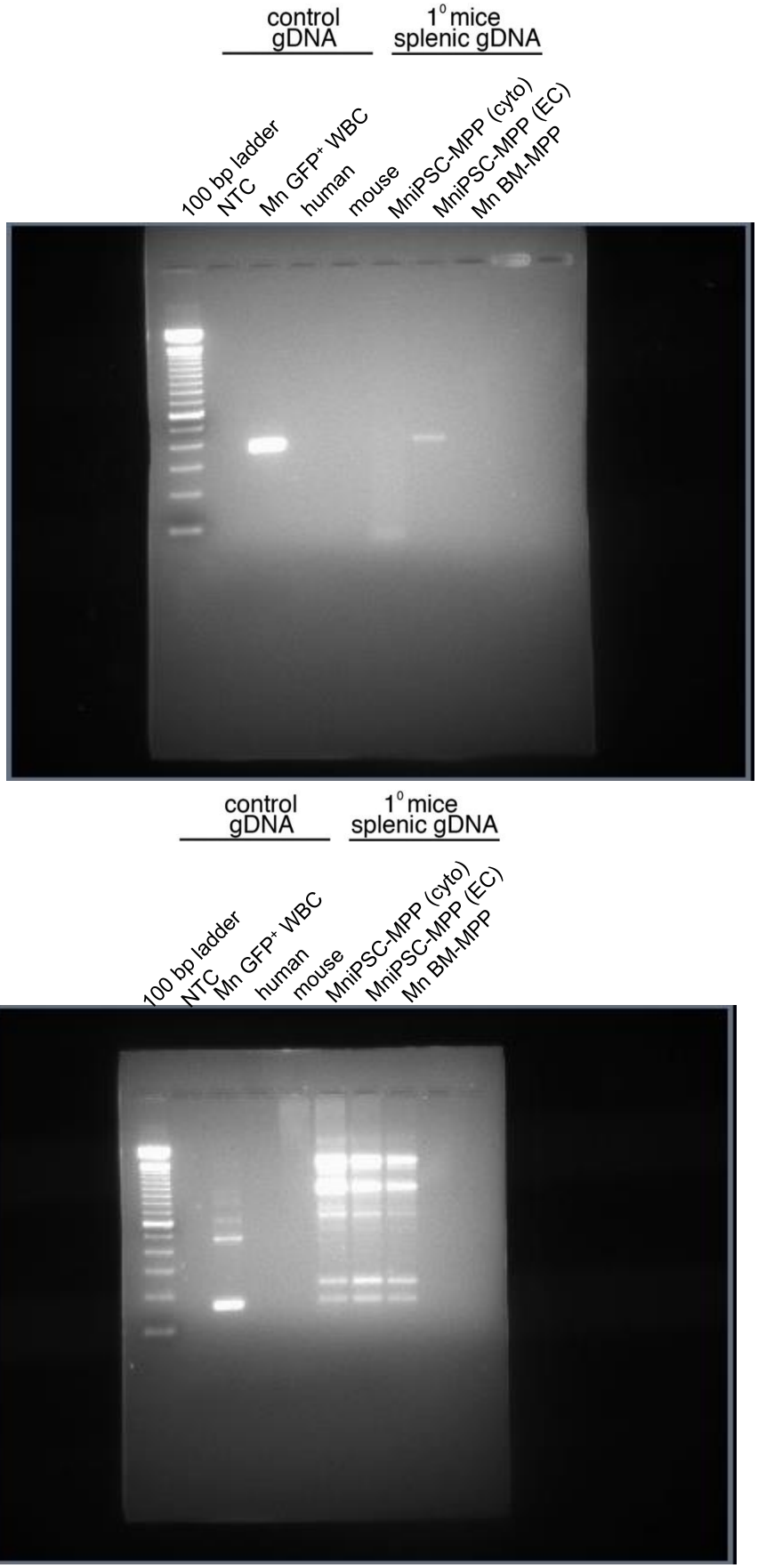
Full unedited gel image of Western blot for Supplemental Figure 5. All lanes shown correspond to the lanes shown in Supplemental Figure 5, panel A. Images were cropped to show the specific band that corresponds to detection of the protein of interest. Lane 4, designated as MGMT negative control (-) in the Figure image corresponds to untransduced MniPSC. MGMT positive control (+) corresponds to P140K-GFP transduced monkey white blood cells (WBC) that were sorted for GFP+ fraction to obtain a purified population of P140K-MGMT+ cells as a positive control for the assay.

Supplemental Uncut Gel 2, pertaining to Figure 5., Panel A.



Full unedited gel image of gel containing CFU PCR products as shown in Figure 5A. Representative PCR analysis for detection of GFP (top gel) and the lentivirus long terminal repeat (LTR, bottom gel) on gDNA isolated from hematopoietic colonies that were generated from the bone marrow of a mouse transplanted with EC-induced MniPSC-MPP (Top lane of 96-well lane gel only).

Supplemental Uncut Gel 3, pertaining to Figure 5., Panel B.



Full unedited gel images of gels containing PCR products as shown in Figure 5B. Representative PCR analysis for detection of GFP (top gel) and Macaque Basigin (MnBSG) (bottom gel) on gDNA isolated from control gDNA samples (L-R: No template control PCR (NTC, Mn GFP+ WBC, human cells, untransplanted mouse spleen cells) or from the spleens of mice transplanted with no cells or transplanted with the indicated cell populations (L-R cytokine induced MniPSC-MPP, EC-induced MniPSC-MPP, or Mn BM-MPP).