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Supplemental Information

A Common Origin for B-1a and B-2 Lymphocytes in Clonal Pre- Hematopoietic Stem Cells

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SUPPLEMENTAL FIGURES

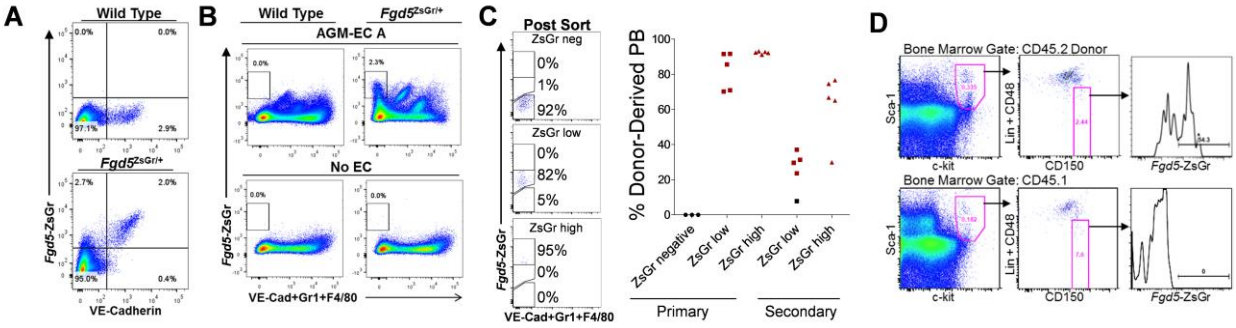

Figure S1. *Fgd5*-ZsGr expression tracks HSC potential following AGM-EC co-culture of VE-Cadherin^{+}CD45$^{+/lo}$ pre-HSC isolated from E11.5 *Fgd5*$^{ZsGr/+}$ embryo AGM, Related to Figures 1 and 2. A) Co-expression of *Fgd5*-ZsGr and VE-Cadherin in cells from dissected AGM regions of E11.5 *Fgd5*$^{ZsGr/+}$ embryos and wild type littermates. B) Expression of *Fgd5*-ZsGr in cells following 5 day culture of VE-Cadherin^{+}CD45$^{+/lo}$ pre-HSC from AGM regions of E11.5 *Fgd5*$^{ZsGr/+}$ embryos and wild type littermates with either AGM-EC or in the absence of EC stroma. Cells expressing VE-Cadherin, Gr1, or F4/80 are gated out to exclude autofluorescent myeloid and endothelial cells. C) Donor-derived peripheral blood engraftment in primary mice (at 24 weeks) and secondary mice (at 16 weeks) transplanted with cells sorted following 5 day culture of VE-Cadherin^{+}CD45$^{+/lo}$ pre-HSC from AGM regions of E11.5 *Fgd5*$^{ZsGr/+}$ embryos, based on expression of *Fgd5*-ZsGr expression (negative, low and high *Fgd5*-ZsGr expression, after gating out cells expressing VE-Cadherin, Gr1, or F4/80). D) Bone marrow engraftment from progeny of single cell (Figure 1B-C), demonstrating *Fgd5*-ZsGr expression in donor-derived (CD45.2) Lineage^{-}Sca1^{+}c-kit^{+}CD150^{+}CD48^{-} phenotypic HSC.

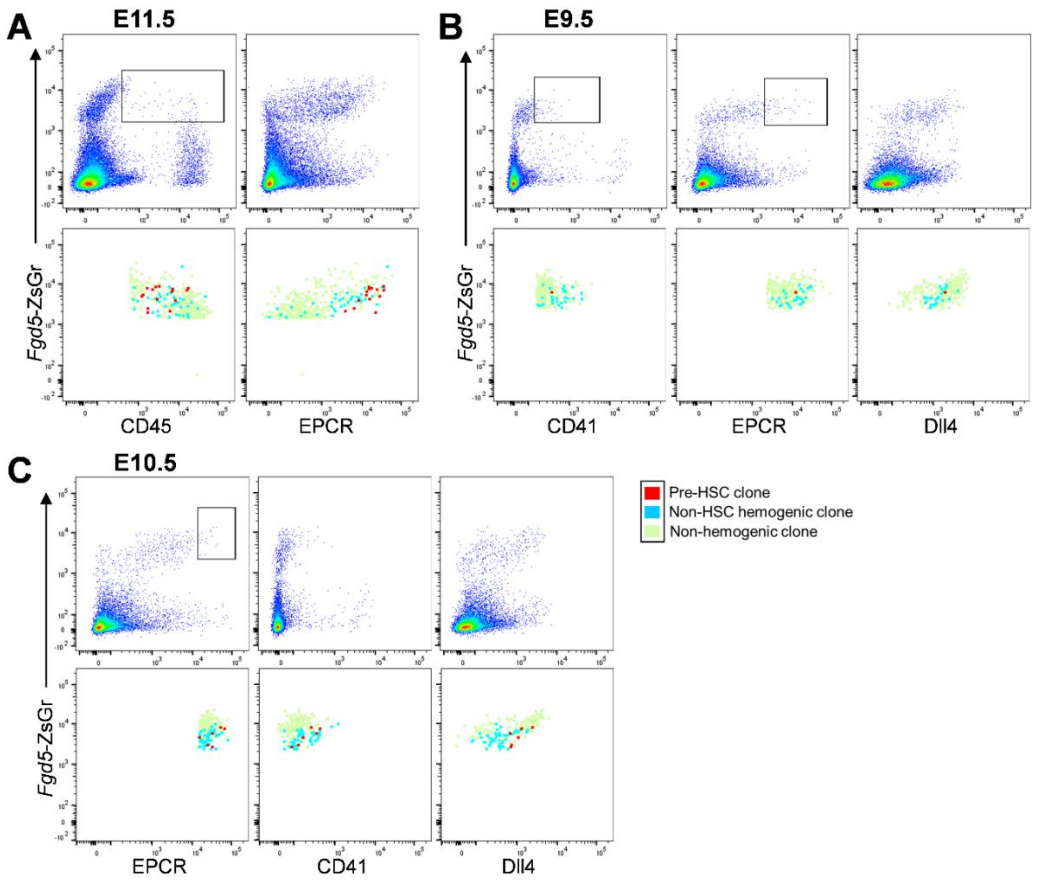

Figure S2. Index sorting parameters of functionally distinct hemogenic precursors, Related to Figure 3. Representative FACS analysis of the entire P-Sp/AGM population (top panels, boxes indicate windows used for sorting) and corresponding index sorting analysis (bottom panels), identifying each single cell with HSC potential (pre-HSC clone), hematopoietic colony formation without HSC potential (non-HSC hemogenic clone), or no hematopoietic colony formation (non-hemogenic clone) based on functional potential following AGM-EC co-culture. A) E11.5 AGM (corresponds to Figure 3A), B) E9.5 P-Sp (corresponds to Figure 3B, left panel), and C) E10.5 AGM (corresponds to Figure 3B, right panel).

Table S1. B cell engraftment from E9.5 and E11.5 pre-HSC clones, Related to Figures 1 and 2

Stage	# of Clones Analyzed	% Donor PB Engraftment Mean±SEM (range)	% Donor Peritoneal Engraftment Mean±SEM (range)	# of Clones with B-1a Engraftment	# of Clones with B-2 Engraftment	%B-1a Cells in Peritoneum[a]	%B-2 Cells in Peritoneum[a]
E9.5	6	40±15 (8.9-88)	55±10 (31-86)	6	6	28±8	42±8
E11.5	27	76±0.8 (10-91)	80±1.1 (18-92)	27	27	*7.5±0.9	*71±2

[a]%B-1a and B-2 calculated as percent of total donor-derived IgM positive B cells detected in peritoneal washes. Shown is mean ± SEM; *$p<0.001$, E9.5 vs E11.5 (unpaired student t-test). Data for E9.5 is pooled from 4 independent experiments and for E11.5 from 2 independent experiments.

Table S2. List of Antibodies

Antigen	Clone	Fluorochrome(s)	Source	Catalog No.
CD45	30-F11	FITC, APC	eBioscience	11-0451
CD41	eBioMWReg30	PE, APC, APC-eFluor780	eBioscience	12-0411
VE-Cadherin	11D4.1	AlexaFluor647, PE	BD Biosciences	562242
VE-Cadherin	eBioBV13	PE/Cy7	eBioscience	25-1441
c-kit	2B8	APC	BD Biosciences	553356
Sca-1	D7	APC, PE/Cy7	eBioscience	17-5981
F4/80	BM8	APC-eFluor780, PE	eBioscience	47-4801
Gr-1/Ly6-G	RB6-8C5	APC-eFluor780, PerCP	eBioscience	47-5931
CD19	ID3	APC, PE	BD Biosciences	550992
B220/CD45R	RA3-6B2	PerCP	BD Biosciences	561086
TER-119	TER-119	PE	eBioscience	12-5921
CD3	17A2	FITC, PE	BD Biosciences	555274
CD4	RM4-5	PE	BD Biosciences	553049
CD8	53-6.7	PerCP	BD Biosciences	553036
CD150	TC15-12F12.2	PerCP/Cy5.5	BioLegend	115922
CD48	HM48-1	PE/Cy7, PE	BioLegend	103424
Delta-like-4	HMD4-1	APC	Biolegend	130814
EPCR/CD201	eBio1560	PE, PerCP-eFluor710	eBioscience	12-2012
CD45.1/Ly5.1	A20	PE/Cy7, eFluor450	eBiosciences	25-0453
CD45.2/Ly5.2	104	APC-eFluor780, PE/Cy7	eBiosciences	47-0454
CD11b/Mac1	M1/70	FITC, APC-eFluor780	eBiosciences	11-0112
IgM	R6-60.2	PerCP-Cy5.5	BD Biosciences	562034
CD23	B3B4	APC	Invitrogen	MCD2305
CD5	53-7.3	PE	BD Biosciences	553023
AA4.1	AA4.1	FITC	eBiosciences	11-5892
CD21	7G6	PE	BD Biosciences	552957