CD117^{hi} expression identifies a human fetal hematopoietic stem cell population with high proliferation and self-renewal potential

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SUPPLEMENTARY INFORMATIONS

CD117hi expression identifies a human fetal Hematopoietic Stem Cell population with high proliferation and self-renewal potential

I. Supplementary Methods

Human tissues

Human first trimester FL (6 to 12WG) were obtained after voluntary, spontaneous or therapeutic abortions. Human second trimester FL (12 to 24WG) were obtained after therapeutic termination of birth. Informed consent was obtained from the patients in compliance with French laws, and with a specific authorization of the French Biomedicine Agency for this project. Developmental age was estimated based on several anatomic criteria according to the Carnegie classification for embryonic stages (1). Fetal samples numbers and characteristics are reported on Supplemental Table S2.

Cell preparation

Fetal livers were excised sterilely using microsurgery instruments and a dissecting microscope, in phosphate-buffered saline (PBS) supplemented with 10% heat-inactived fetal calf serum (FCS) (Dominique Dutscher). Fetal livers were then disrupted mechanically through 18, 23 and

26 gauge needles successively. Cells clumps were removed on a 70µm nylon filter (Miltenyi Biotec), washed three times with Dulbecco's modified eagle medium (DMEM) (Gibco/ThermoFischer)/FCS 10% and quantified. Mononuclear cells (MNC) were isolated using Ficoll centrifugation gradient (Eurobio).

Cell analysis and sorting by flow cytometry

Cells were incubated for 30 minutes at 4°C with combination of antibodies in PBS/Bovine serum albumin (BSA) 0.5% and washed. Monoclonal antibodies and sorted panels used are indicated in Supplemental Table S3. Labeled cells were analyzed on Facs CANTO II or sorted on Facs Aria II (BD Biosciences). Flow cytometry data were analyzed with Flowjo software (FlowJo).

Cell culture and analysis

- Long term culture-initiated cells (LTC-IC) and extended LTC-IC (eLTC-IC)

$CD117^{hi}$ and $CD117^{lo/neg}$ sorted cells (Figure S1A) were cultivated in bulk on MS-5 mouse BM stromal cells as previously described with some modifications (2). Briefly, sorted cells were cultivated in limit dilutions with humans Interleukin-3 (IL-3) (10 ng/ml), Fms-related tyrosine kinase 3 ligand (Flt3-L) (50 ng/ml), Stem cell factor (SCF) (50 ng/ml) and Trombopoietin (TPO) (20 ng/ml) (Miltenyi-Biotec) for five weeks on MS-5 cells. Half of the medium was replaced weekly and presence of Cobblestone area forming colony (CAF-C) after 5 weeks of culture was quantified. The frequency of long-term culture-initiated cells (LTC-ICs) was determined after limiting dilution experiments using L-Calc software (STEMCELL Technologies). For eLTC-IC assay, after 5 weeks of culture, cells were replated every 3 weeks on fresh MS-5 cells until exhaustion and hematopoietic cells are quantified and analyzed by flow cytometry.

- Lympho-myeloid differentiation assay

Lympho-myeloid differentiation was performed as described by Alhaj Hussen et al. (3), with minor modifications. Sorted cells (10 per well) were seeded on MS-5 cells (1.5 10^4 cells/well plated 24 hours prior to co-culture) in 96-well flat-bottom plates, in 200 µl RPMI medium with 10% FCS (Dominique Dutscher), 1% Penicillin-Streptomycin (ThermoFischer), and 1/1000 β-mercaptoethanol (Gibco/ThermoFisher), with humans SCF (10 ng/ml), TPO (10 ng/ml), IL-2 (10ng/ml) and IL-7 (10ng/ml) (Miltenyi-Biotec). Fifty microliters of fresh medium with 4X cytokines were added to each well on day 7 of coculture. Lympho-myeloid differentiation was assessed at day 14 of coculture by flow-cytometry using CD45, CD19, CD56 and CD14 (monoclonal antibodies are listed in Supplemental Table S3).

NSG mouse transplantation

Nonobese diabetic/severe combined immunodeficiency gamma (NSG) mice (8 weeks-old female) were sublethally irradiated (2.25 Gy) and received sorted cells by intravenous injection 24 hours after irradiation. Human CD45+ hematopoietic engraftment in BM and human hematopoietic lineages (lymphoid CD3, myeloid CD33, erythroid CD235a) engraftment in BM, spleen, thymus was assessed 16-20 weeks after transplantation by flow cytometry analysis (monoclonal antibodies listed in Supplemental Table S3).

Gene expression analysis

RNAs were purified using RNeasy Plus Micro Kit (Qiagen) according to manufacturer's protocol and were used for transcriptomic assay or reverse transcription (RT) quantitative real-time polymerase chain reaction (q-PCR) assay. Quantity and quality of RNA were validated with

Bioanalyzer 2100 (Agilent) by Cochin Institut Genom'ic plateforme. Transcriptomic assay was performed on Affymetrix HumanGene2.0st chipset using 2 ng of total RNA reverse transcribed following the Ovation Pico WTA System V2 (Nugen). Briefly, the resulting double strand cDNA is used for amplification based on SPIA technology. After purification according to Nugen protocol, 3.6 ug of Sens Target DNA are fragmented and biotin labelled using Encore Biotin Module kit (Nugen). After control of fragmentation using Bioanalyzer 2100, cDNA is then hybridized to GeneChip® Human Gene 2.0 ST (Affymetrix) at 45°C for 17 hours.

After overnight hybridization, chips are washed on the fluidic station FS450 following specific protocols (Affymetrix) and scanned using the GCS3000 7G. The scanned images are then analyzed with Expression Console software (Affymetrix) to obtain raw data (cel files) and metrics for Quality Controls. RMA normalization is performed using R with Version 21 of Entrezgene CDF brain array.

Supervised analyses were performed to identify genes specifically up-regulated in $CD117^{hi}$ HSCs. Then, the lists of differentially expressed genes were analyzed for gene ontology (GO) with DAVID (database used for annotation, visualization and integrated discovery) (http://david.abcc.ncifcrf.gov) and enriched GO terms for biological processes (BP) were summarized by REVIGO (4). The global expression profile was also analyzed with GSEA (Gene Set Enrichment Analysis) with the functional (curated) data set present in the Molecular Signature Database of the Broad Institute (www.broadinstitute.org/gsea)(5).

For RT-qPCR assay, RNAs were reverse-transcripted in cDNA using SuperScript III Reverse Transcriptase kit (Invitrogen) according to manufacturer's protocol. qPCR was done using SYBR Green PCR master mix reagent (Applied Biosystems) on 7500 Fast RTPCR system (Applied Biosystems). Primers used are listed in Supplemental Table S4.

II. Supplementary Tables

Supplemental Table S1. Comparison of LTC-IC frequency of $CD117^{hi}$ population versus other known HSC-enriched populations

Trimester of development	Weeks of gestation (WG)	LTC-IC frequency ± SEM					
		$CD117^{hi}$	$CD144^{lo}CD45^{+}$	$CD117^{hi}CD90^{+}$	$CD117^{hi}CD90^{-}$	$GPI80^{+}CD90^{+}$	$CD143^{+}$
T1	**6-9**	0.054±0.02 (n=5)	0.023 (n=1)				
	9-12	0.125±0.02 (n=5)		0.070±0.007 (n=2)	0,053±0,00 (n=2)	0.004±0.006 (n=3)	0.043±0.017 (n=2)
T2	**12-24**	0.150±0.06 (n=10)		0,033 (n=1)	0.025±0.013 (n=2)	0.015±0.003 (n=2)	0.012±0.002 (n=2)

SEM = Standard error of the mean
n = number of samples analyzed

Supplemental Table S2. T1 and T2 FL characteristics

Experiment	Trimester (T)	Stage (WG)	Number of samples
Phenotyping	T1	6-9	31
		9-12	30
	T2	12-24	11
LTC-IC	T1	6-9	2 ($117^{lo/neg}$) or 5(117^{hi})
		9-12	5
	T2	12-24	3 ($117^{lo/neg}$) or 10 (117^{hi})
NSG injection	T1	6-12	8 ($117^{lo/neg}$) or 15 (117^{hi})
	T2	12-24	1 ($117^{lo/neg}$) or 4 (117^{hi})
Transcriptome	T1	6-12	4
	T2	12-24	2
q-PCR	T1	6-12	4
	T2	12-24	3

WG = weeks of gestation

Supplemental Table S3. Antibodies with conjugates and FACS and sorting panels

Cell analysis and sorting			
Target	**Clone**	**Fluorochrome**	**Supplier**
CD34	581	APC or APC-Cy7	Biolegend
CD38	HIT2	FITC	Biolegend
CD45	HI30	PerCP-Cy5.5 or APC	Biolegend
CD117	104D2	PE-Cy7	Biolegend
CD90	5E10	PE or PerCP-Cy5.5	Biolegend
CD143	5-369	PE	Biolegend
CD144	BV9	PE	Biolegend
GPI80	3H9	PE	Clinisciences
Reconstitution analysis			
CD34	581	APC	Biolegend
CD38	HIT2	FITC	Biolegend
CD45	HI30	PE-Cy7	Biolegend
CD117	104D2	PE	Biolegend
CD19	J4.119	PE	Beckman Coulter
CD33	D3HL60.251	FITC	Beckman Coulter
GPA	HI264	FITC	Biolegend
Lympho-myeloid differentiation analysis			
CD14	M5E2	PE	Biolegend
CD19	J4.119	PE	Beckman Coulter
CD45	HI30	PerCP-Cy5.5	Biolegend
CD56	B159	APC	BD Pharmigen

Supplemental Table S4. qPCR primers

HSCs markers		
Target	**Sens**	**Primers 5'-3'**
MYB	Forward	CTCCTACACCATTCAAACATGCA
	Reverse	CTCCTACACCATTCAAACATGCA
HLF	Forward	GATGACAAGTACTGGGCAAGG
	Reverse	GGATGGCGATCTGGTTCTCT
PROM1	Forward	CAAGGACAAGGCGTTCAC
	Reverse	GCTCTTCAAGGTGCTGTTC
ANGPT1	Forward	GAACCGGATTTCTCTTCCCAGA
	Reverse	TCTGGGCCATCTCCGACTT
cKIT	Forward	CGACGAGATTAGGCTGTTATGC
	Reverse	TCTGCCTTTTCCGTGATCCA
Lymphoid markers		
HLA-DOA	Forward	GCACCAGAGTGTAATGGCCC
	Reverse	CGCCGTAAGACTGGTAGAAGG
HLA-DQA1	Forward	GAACACCAACTGCTGAGGCT
	Reverse	CAAGTTTACACCACAAGAGGCA
CIITA	Forward	GTCCTCATGTGGAGACGCTG
	Reverse	CCAGCGTGGTTAGTGTCCTC
PIK3CD	Forward	CTGTACGCCGTGATCGAGAA
	Reverse	ACATGTAGAGGCAGCGTTCC
RUNX2	Forward	CCCTGAACTCTGCACCAAGT
	Reverse	GGCTCAGGTAGGAGGGGTAA
GAPDH	Forward	GGGAAGGTGAAGGTCGGAGT
	Reverse	GGGTCATTGATGGCAACAATA

Supplemental Table S5, included as a separate Excel file, contains Gene Ontology Biological Process (GO BP) enrichment for T1 and T2 FL CD117^{hi} up-regulated genes (*vs* CD117$^{lo/neg}$)

Supplemental Table S6: genes from JAATINEN HSC UP shared by T1 and T2 CD117^{hi}

EntrezGeneID	Symbol	Entrez Gene Name	Location	Type(s)	CD117^{hi} UP T1		CD117^{hi} UP T2	
					p-value	Fold change	p-value	Fold change
3131	**HLF**	HLF, PAR bZIP transcription factor	Nucleus	transcription reg.	0,0000031	28,0	0,000051	25,0
3815	**KIT**	KIT proto-oncogene receptor tyrosine kinase	Plasma Membrane	transmembrane R	0,0000010	14,8	0,000004	24,2
8842	**PROM1**	prominin 1	Plasma Membrane	other	0,0000610	12,3	0,000133	23,8
2322	**FLT3**	fms related tyrosine kinase 3	Plasma Membrane	kinase	0,0079412	3,1	0,000348	14,4
10863	**ADAM28**	ADAM metallopeptidase domain 28	Plasma Membrane	peptidase	0,0430905	2,5	0,001293	14,4
54360	**CYTL1**	cytokine like 1	Extracellular Space	cytokine	0,0020511	8,4	0,006418	11,7
284	**ANGPT1**	angiopoietin 1	Extracellular Space	growth factor	0,0235438	8,8	0,063574	10,7
4602	**MYB**	MYB proto-oncogene, transcription factor	Nucleus	transcription reg.	0,0005409	14,2	0,010669	9,4
3606	**IL18**	interleukin 18	Extracellular Space	cytokine	0,0018403	3,6	0,000615	8,7
9053	**MAP7**	microtubule associated protein 7	Cytoplasm	other	0,0045544	3,8	0,003126	7,5
4300	**MLLT3**	MLLT3, super elongation complex subunit	Nucleus	other	0,0010945	7,7	0,008605	7,4
83879	**CDCA7**	cell division cycle associated 7	Nucleus	other	0,0007813	5,4	0,002353	7,3
9834	**KIAA0125**	KIAA0125	Other	other	*0,1995920*	1,6	0,004067	7,3
6691	**SPINK2**	serine peptidase inhibitor, Kazal type 2	Extracellular Space	other	0,0000735	6,1	0,000421	7,3
9805	**SCRN1**	secernin 1	Cytoplasm	other	0,0452476	2,5	0,007183	7,2
124540	**MSI2**	musashi RNA binding protein 2	Cytoplasm	other	0,0003194	4,1	0,000338	7,2
2982	**GUCY1A3**	guanylate cyclase 1 soluble subunit alpha	Cytoplasm	enzyme	0,0266143	4,2	0,034032	6,8
114900	**C1QTNF4**	C1q and tumor necrosis factor related protein	Extracellular Space	other	0,0000475	7,9	0,000916	6,6
79870	**BAALC**	brain and acute leukemia, cytoplasmic	Cytoplasm	other	0,0166380	2,1	0,001463	5,4
56034	**PDGFC**	platelet derived growth factor C	Extracellular Space	growth factor	0,0391966	2,3	0,009053	5,3
4883	**NPR3**	natriuretic peptide receptor 3	Plasma Membrane	G-protein coupled R	0,0070240	3,9	0,015195	5,1
55930	**MYO5C**	myosin VC	Cytoplasm	other	0,0023645	3,3	0,003521	4,9
2769	**GNA15**	G protein subunit alpha 15	Plasma Membrane	enzyme	0,0142921	2,2	0,002636	4,7
1829	**DSG2**	desmoglein 2	Plasma Membrane	other	0,0001209	6,6	0,003799	4,7
8091	**HMGA2**	high mobility group AT-hook 2	Nucleus	enzyme	0,0011217	2,7	0,000774	4,3
84460	**ZMAT1**	zinc finger matrin-type 1	Nucleus	other	0,0420569	2,7	0,036140	4,3
53335	**BCL11A**	B-cell CLL/lymphoma 11A	Nucleus	transcription reg.	0,0092484	2,1	0,001453	4,2
84941	**HSH2D**	hematopoietic SH2 domain containing	Cytoplasm	other	0,0156856	2,5	0,009464	4,2
586	**BCAT1**	branched chain amino acid transaminase 1	Cytoplasm	enzyme	0,0116062	3,5	0,040719	3,7
253827	**MSRB3**	methionine sulfoxide reductase B3	Cytoplasm	other	0,0238415	2,5	0,024893	3,6
81615	**TMEM163**	transmembrane protein 163	Cytoplasm	other	0,0000611	4,7	0,001930	3,6
51514	**DTL**	denticleless E3 ubiquitin protein ligase homol	Nucleus	other	0,0016110	3,6	0,011089	3,6
4352	**MPL**	MPL proto-oncogene, thrombopoietin recepto	Plasma Membrane	transmembrane R	0,1341490	2,7	0,176817	3,4
91851	**CHRDL1**	chordin like 1	Extracellular Space	other	0,2399100	1,6	0,040204	3,4
1021	**CDK6**	cyclin dependent kinase 6	Nucleus	kinase	0,0010490	3,3	0,007780	3,2
55151	**TMEM38B**	transmembrane protein 38B	Nucleus	ion channel	0,0052347	2,4	0,007261	3,2
57820	**CCNB1IP1**	cyclin B1 interacting protein 1	Nucleus	enzyme	0,0000177	2,7	0,000065	3,2
8204	**NRIP1**	nuclear receptor interacting protein 1	Nucleus	transcription reg.	0,0012519	3,4	0,012008	3,2
83698	**CALN1**	calneuron 1	Cytoplasm	other	0,0077904	1,8	0,001110	3,1
10606	**PAICS**	phosphoribosylaminoimidazole carboxylase; p	Cytoplasm	enzyme	0,0011903	3,1	0,008990	3,1
9331	**B4GALT6**	beta-1,4-galactosyltransferase 6	Cytoplasm	enzyme	0,0000050	3,1	0,000077	3,1
84707	**BEX2**	brain expressed X-linked 2	Nucleus	other	0,0002656	3,1	0,003372	2,9
1789	**DNMT3B**	DNA methyltransferase 3 beta	Nucleus	enzyme	0,0113806	2,3	0,016994	2,9
23175	**LPIN1**	lipin 1	Nucleus	phosphatase	0,0228639	1,7	0,004956	2,9
5332	**PLCB4**	phospholipase C beta 4	Cytoplasm	enzyme	0,0697555	1,9	0,037875	2,9
9392	**TGFBRAP1**	transforming growth factor beta receptor assc	Other	other	0,0001856	2,4	0,000717	2,7
29128	**UHRF1**	ubiquitin like with PHD and ring finger domain	Nucleus	transcription reg.	0,0036522	2,3	0,009085	2,7
3925	**STMN1**	stathmin 1	Cytoplasm	other	0,0000994	2,0	0,000104	2,7
400464	**LOC400464**	ubiquitin conjugating enzyme E2 Q2 pseudoge	Other	other	0,1028590	3,2	*0,310743*	2,7
5325	**PLAGL1**	PLAG1 like zinc finger 1	Nucleus	transcription reg.	0,0073727	2,7	0,036134	2,6
3205	**HOXA9**	homeobox A9	Nucleus	transcription reg.	0,0048977	2,0	0,005216	2,6
55211	**DPPA4**	developmental pluripotency associated 4	Nucleus	other	0,0070503	3,3	0,078025	2,6
23107	**MRPS27**	mitochondrial ribosomal protein S27	Cytoplasm	other	0,0001222	3,0	0,002550	2,6
283742	**FAM98B**	family with sequence similarity 98 member B	Nucleus	other	0,0081154	2,3	0,021047	2,6
3206	**HOXA10**	homeobox A10	Nucleus	transcription reg.	0,0019716	3,4	0,037638	2,6
7374	**UNG**	uracil DNA glycosylase	Nucleus	enzyme	0,0003317	3,2	0,012642	2,4
4174	**MCM5**	minichromosome maintenance complex comp	Nucleus	enzyme	0,0014832	2,9	0,024908	2,4
10327	**AKR1A1**	aldo-keto reductase family 1 member A1	Cytoplasm	enzyme	0,0001576	2,2	0,000922	2,3
114112	**TXNRD3**	thioredoxin reductase 3	Cytoplasm	enzyme	0,0932166	2,1	*0,153835*	2,3
2624	**GATA2**	GATA binding protein 2	Nucleus	transcription reg.	*0,1693950*	2,5	*0,357163*	2,3
55120	**FANCL**	Fanconi anemia complementation group L	Nucleus	enzyme	0,0385400	1,9	*0,057943*	2,3
64754	**SMYD3**	SET and MYND domain containing 3	Nucleus	enzyme	0,0096300	1,9	0,020684	2,2
83871	**RAB34**	RAB34, member RAS oncogene family	Cytoplasm	enzyme	0,0095091	2,4	*0,062616*	2,2
64116	**SLC39A8**	solute carrier family 39 member 8	Extracellular Space	transporter	0,0005657	2,2	0,006197	2,1
6595	**SMARCA2**	SWI/SNF related, matrix associated, actin dep	Nucleus	transcription reg.	0,0287496	2,0	0,075743	2,1
22797	**TFEC**	transcription factor EC	Nucleus	transcription reg.	0,0488763	1,8	*0,065003*	2,1
4363	**ABCC1**	ATP binding cassette subfamily C member 1	Plasma Membrane	transporter	0,0020404	2,4	0,032471	2,1
10419	**PRMT5**	protein arginine methyltransferase 5	Cytoplasm	enzyme	0,0001884	2,3	0,005841	2,0
84650	**EBPL**	emopamil binding protein like	Cytoplasm	enzyme	0,0017533	2,0	0,012450	2,0
8936	**WASF1**	WAS protein family member 1	Nucleus	other	*0,0684272*	1,8	*0,132702*	1,9
58504	**ARHGAP22**	Rho GTPase activating protein 22	Cytoplasm	other	0,0410537	1,4	0,015046	1,9
23243	**ANKRD28**	ankyrin repeat domain 28	Cytoplasm	other	0,0001578	2,9	0,021781	1,9
51692	**CPSF3**	cleavage and polyadenylation specific factor 3	Nucleus	enzyme	0,0093597	2,2	*0,102089*	1,9
4675	**NAP1L3**	nucleosome assembly protein 1 like 3	Nucleus	other	0,0588249	1,9	*0,195256*	1,8
5136	**PDE1A**	phosphodiesterase 1A	Cytoplasm	enzyme	0,0071599	1,8	0,032515	1,8
7205	**TRIP6**	thyroid hormone receptor interactor 6	Extracellular Space	cytokine	0,0028884	2,4	*0,082326*	1,8
57465	**TBC1D24**	TBC1 domain family member 24	Cytoplasm	other	0,0125183	2,0	*0,094850*	1,8
1374	**CPT1A**	carnitine palmitoyltransferase 1A	Cytoplasm	enzyme	0,0029647	2,9	*0,154607*	1,8
84939	**MUM1**	melanoma associated antigen (mutated) 1	Nucleus	other	0,0018057	2,6	*0,095753*	1,7
148418	**SAMD13**	sterile alpha motif domain containing 13	Other	other	0,0621723	1,6	*0,123105*	1,7
79884	**MAP9**	microtubule associated protein 9	Other	other	0,0067026	2,8	*0,241069*	1,7
3033	**HADH**	hydroxyacyl-CoA dehydrogenase	Cytoplasm	enzyme	0,0270693	1,9	*0,182315*	1,6
23576	**DDAH1**	dimethylarginine dimethylaminohydrolase 1	Cytoplasm	enzyme	0,0029512	4,4	*0,352368*	1,6
55179	**FAIM**	Fas apoptotic inhibitory molecule	Plasma Membrane	other	0,0382388	1,8	*0,208798*	1,6
5321	**PLA2G4A**	phospholipase A2 group IVA	Cytoplasm	enzyme	0,0068467	2,3	*0,221053*	1,5
2653	**GCSH**	glycine cleavage system protein H	Cytoplasm	enzyme	0,0933764	1,8	*0,344977*	1,5
51477	**ISYNA1**	inositol-3-phosphate synthase 1	Cytoplasm	enzyme	0,0004006	2,7	*0,123708*	1,5
23363	**OBSL1**	obscurin like 1	Cytoplasm	other	*0,1459220*	1,5	*0,334878*	1,5
10329	**TMEM5**	transmembrane protein 5	Plasma Membrane	other	0,0104900	1,5	0,072099	*1,4*
144245	**ALG10B**	ALG10B, alpha-1,2-glucosyltransferase	Plasma Membrane	transporter	*0,1334160*	1,6	*0,425384*	*1,4*

Italics: N.S.; highlighted in grey: genes tested by q-PCR.

Supplemental Table S7, included as a separate Excel file, contains the list of genes specific for T1 CD117^{hi}, T2 CD117^{hi} and genes common for T1 and T2 CD117^{hi}, and the GO BP enrichment for each list

III. Supplementary Figures

Figure legends

Supplemental Figure S1. CD117^{hi} analysis for known FL HSC markers. FACS analysis of CD117^{hi} population for cell surface markers described as allowing HSC enrichment of FL (CD144, CD143, GPI80). A typical analysis is represented on upper panel (8.1 WG for CD144, 9 WG for CD143, and 11.9 WG for GPI80). (n): number of FL analyzed. N.D.: Not done.

Supplemental Figure S2. Long term primary hematopoietic reconstitution and secondary transplantation analyses of NSG mice injected with CD117^{hi} population. (A) Primary reconstitution. FACS analysis of BM, spleen and thymus of NSG mouse injected with T1 CD117^{hi} FL cells. Mice were tested for the presence of human CD45 in their BM, spleen and thymus 16 to 20 weeks post-transplantation, and BM multilineage engraftment was assessed by staining with specific monoclonal antibodies to CD34, CD38, and CD117 (HSC), CD19 (lymphoid), CD33 (myeloid) and GPA (erythroid) cells. A significant proportion of CD117^{hi} (CD34^{+}CD38^{-}CD45^{+}CD117^{hi}) cells was still detected in primary NSG BM. (B) Secondary transplantation. FACS analysis of BM, spleen and thymus of NSG mouse injected with 2.10^{6} human CD45^{+} cells isolated from primary reconstituted NSG BM. Mice were tested for the presence of human CD45 in their BM, spleen and thymus 12 weeks post-transplantation, and BM multilineage engraftment was assessed by staining with specific monoclonal antibodies to CD34, CD38, and CD117 (HSC), CD19 (lymphoid), CD33 (myeloid) and GPA (erythroid) cells. A

significant proportion of $CD117^{hi}$ ($CD34^{+}CD38^{-}CD45^{+}CD117^{hi}$) cells was still detected in secondary NSG BM.

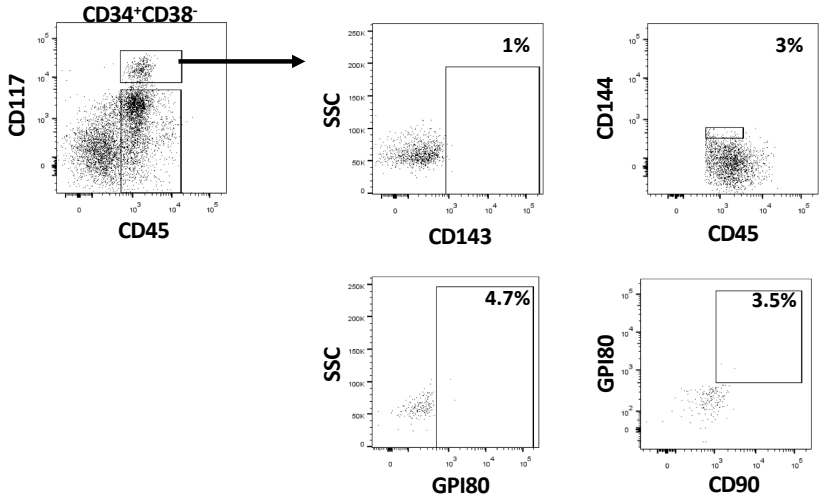
Numbers indicate percentages of positive cells in the corresponding quadrants. Data shown are representative of all NSG analyses performed.

Supplemental Figure S3. Gene set enrichment analysis (GSEA) of CD117hi compared with CD117lo/- from T1 or T2 FL. Both T1 and T2 $CD117^{hi}$ HSCs specific sets of genes are enriched in processes involved in cell cycle, DNA replication and repair. NES= normalized enrichment score.

Supplemental Figure S4. Transcriptome validation by RT-qPCR. (A) RT-qPCR for HSC related genes (MYB, HLF, PROM1, ANGPT1, and cKIT as positive control). $CD117^{hi}$ relative expression with $CD117^{lo/neg}$ used as reference populations and GAPDH as reference gene. (B) RT-qPCR for lymphopoiesis related genes (HLA-DOA, HLA-DQA1, CIITA, PI3KCD, RUNX2). $CD117^{hi}$ relative expression with $CD117^{lo/neg}$ used as reference populations and GAPDH as reference gene.

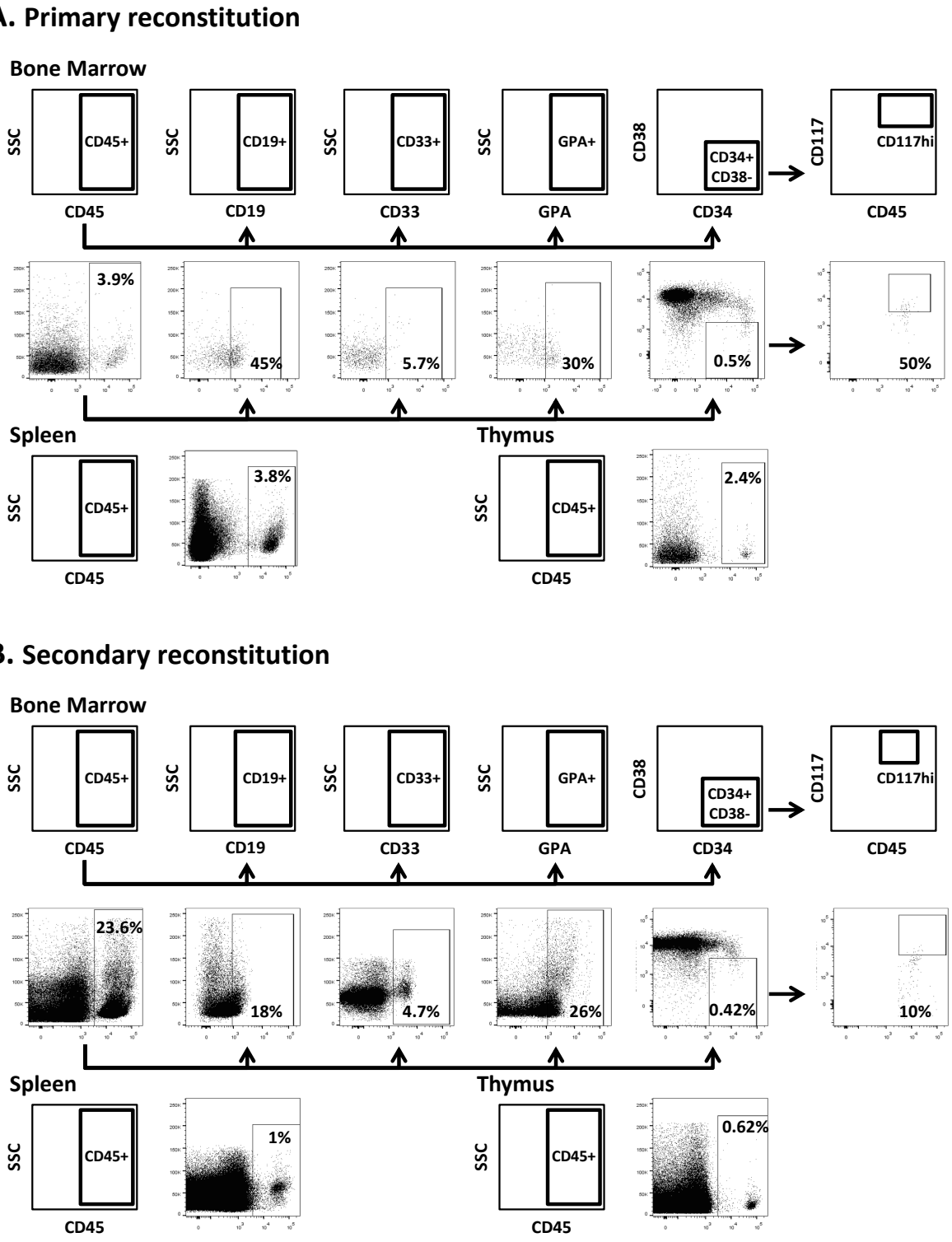
* $p<0.05$; NS: not significant

Supplementary Figure 1

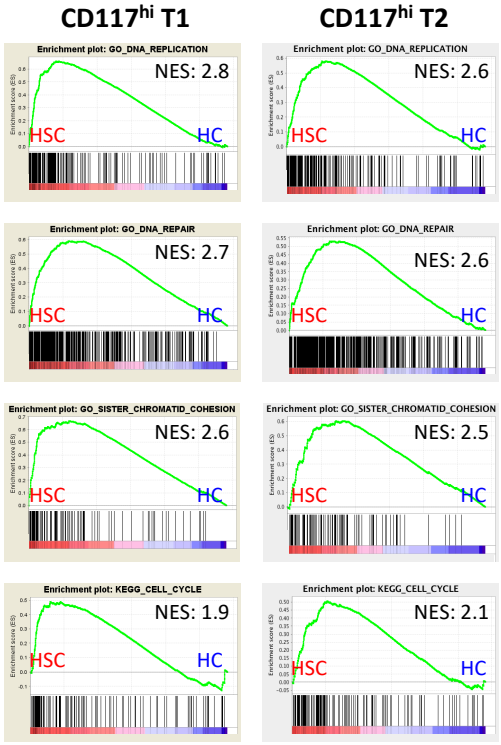


Population	Stage	% CD143^{+}	% CD45^{lo}CD144^{lo}	%CD90^{+}GPI80^{+}
CD117^{hi}	T1	0.9±0.4 (n=12)	3.3±0.9 (n=21)	4.1±1.3 (n=6)
	T2	3.6±1.6 (n=6)	N.D.	1.5±0.7 (n=7)

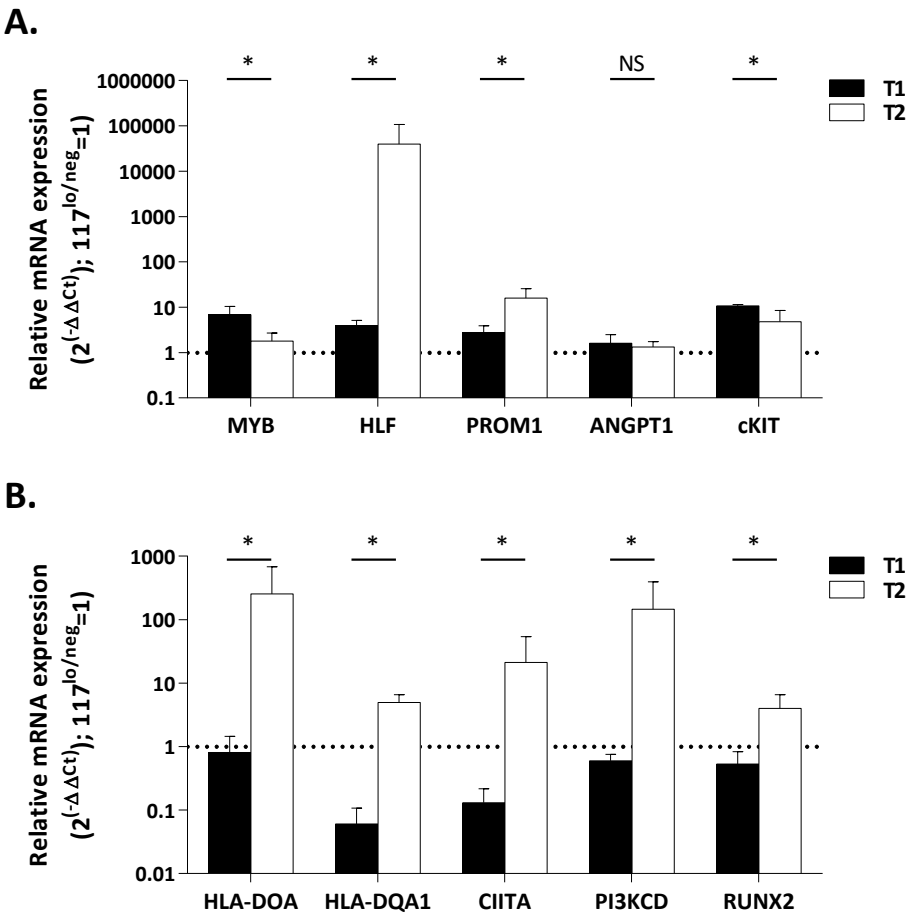
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



IV. References for the supplementary

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