

Stem Cell Reports, Volume 11

Supplemental Information

**Studies in an Early Development Window Unveils a Severe HSC Defect
in both Murine and Human Fanconi Anemia**

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Studies in an early development window unveil a severe HSC defect in both murine and human Fanconi anemia

Carine Domenech^{1,2,3,4,5}, Loïc Maillard^{1,2,3,4}, Alix Rousseau^{4,6}, Fabien Guidez^{3,4}, Laurence Petit^{1,2}, Marika Pla^{3,4}, Denis Clay^{7,8}, Fabien Guimiot⁹, Sandra Sanfilippo^{1,2,10}, Sebastien Jacques¹¹, Pierre de la Grange¹², Noémie Robil¹², Jean Soulier^{4,6} and Michèle Souyri^{*1,2,3,4}

Inventory of Supplemental Informations

- **Table S1, related to Figure 6 and transcriptome analysis section in the Results**, reports all the GO processes and genes associated (DAVID, KEGG and reactome analysis) in genes in *Fancg*^{-/-} E12.5 Pl and E14.5 FL and human FA FL HSCs.
- **Table S2, related to Figure 6 and transcriptome analysis section in the Results**, shows expression of few cell cycle regulators in E12.5 FL and E14.5 FL *Fancg*^{-/-} HSCs and in human FA FL HSCs.
- **Table S3, related to Figure 6 and transcriptome analysis section in the Results**, shows transcriptional regulators and markers important for HSC development and maintenance in E12.5 FL and E14.5 FL *Fancg*^{-/-} HSCs and in human FA FL HSCs.
- **Figure S1, related to Figure 2**, shows that the percentage of HSC-enriched cells in *Fancg*^{-/-} E12.5 Pl or FL, and E14.5 FL is identical to that detected in WT Pl or FL.
- **Figure S2, related to Figure 3**, reports long-term primary and secondary hematopoietic reconstitution ability of E14.5 FL LSA HSCs.
- **Figure S3**, reports analysis of apoptosis in *Fancg*^{-/-} and WT E12.5 FL LSA HSC population (**related to Figure 4**), and qPCR verification of the level of expression of few cell cycle and HSC-related genes (**related to Figure 6 and Tables S2 and S3**), and analysis of phospho-P53 in HCs and HSCs populations from *Fancg*^{-/-} and WT E12.5 FL (**related to Table S2**).

Supplemental Figures and Legends

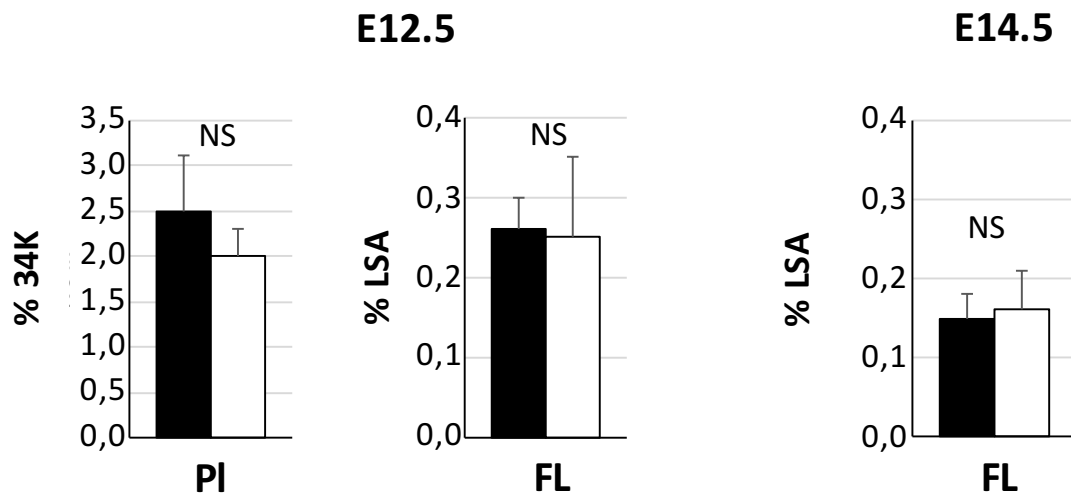


Figure S1. Related to Figure 2. Percentage of HSC-enriched populations.

Percentage of HSC-enriched cells in the PI (34K, n=9 - distinct experiments) and FL (LSA, n=7 - distinct experiments) at E12.5 and in the FL (LSA, n=6 - distinct experiments) at E14.5.

Black bars: WT; White bars: *Fancg*^{-/-}.

NS: not significant.

Error bars correspond to standard deviation (SD).

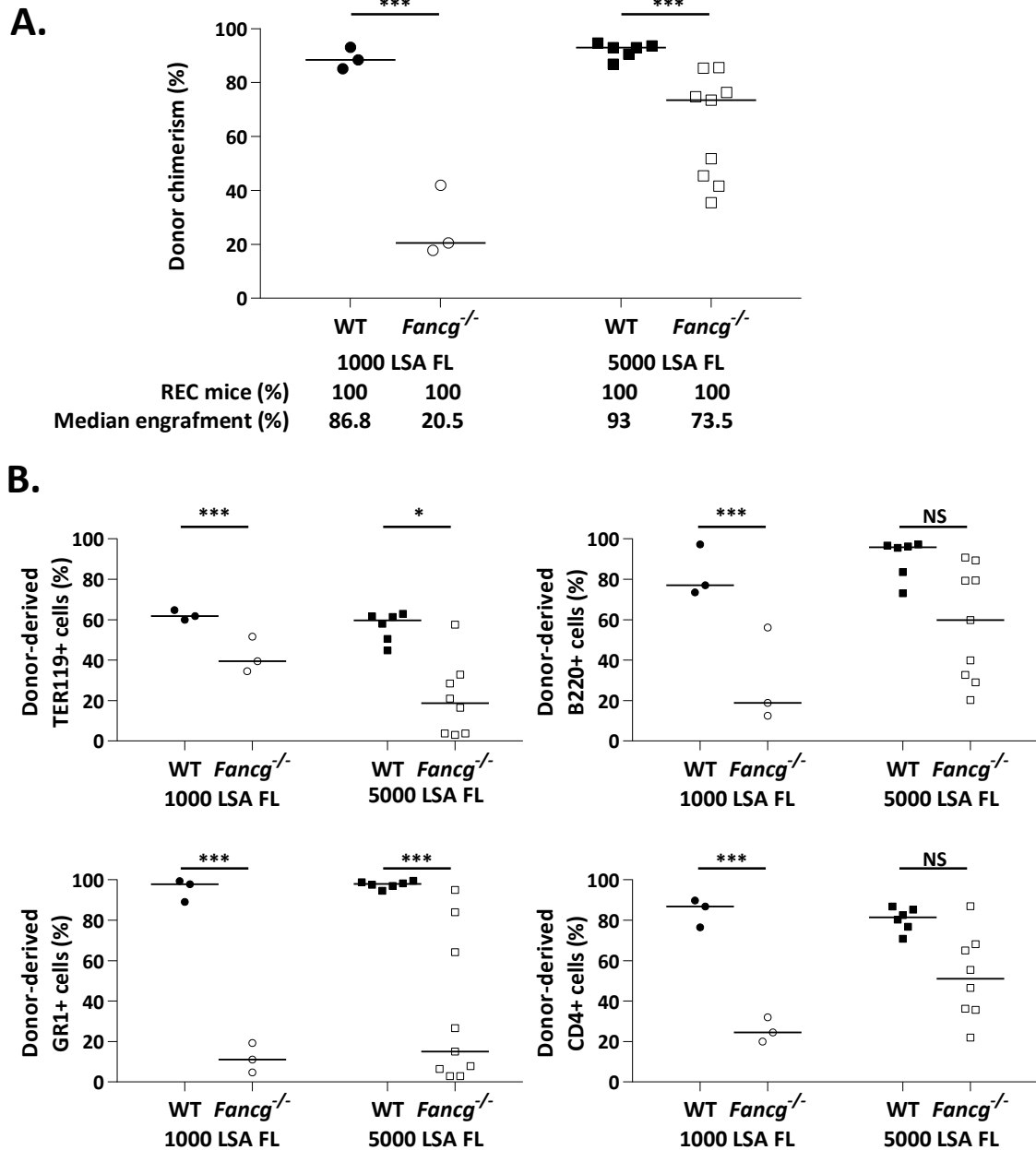


Figure S2. Related to Figure 3. High LTR ability defect and impaired myeloid potential of E14.5 *Fancg*^{-/-} FL LSA HSCs. (A) CD45.2 chimerism in the blood of primary recipients 16- to 20-week post injection of 1.10^3 (circles) or 5.10^3 (squares) WT (black) or *Fancg*^{-/-} (white) LSA cells. (B) Analysis of the BM hematopoietic content of primary recipients transplanted with LSA cells, expressed as a percentage of CD45.2⁺ erythroid (TER119), myeloid (GR1), B lymphoid (B220) and T-lymphoid (CD4) cells. Circles: 1.10^3 LSA injected; squares: 5.10^3 LSA injected; black: WT FL or PI cells; White: *Fancg*^{-/-} FL or PI cells. (C) Secondary transplantations. Engraftment was considered as positive when CD45.2 chimerism in the blood was >5%. *** p<0.001; * p<0.05; NS: not significant.

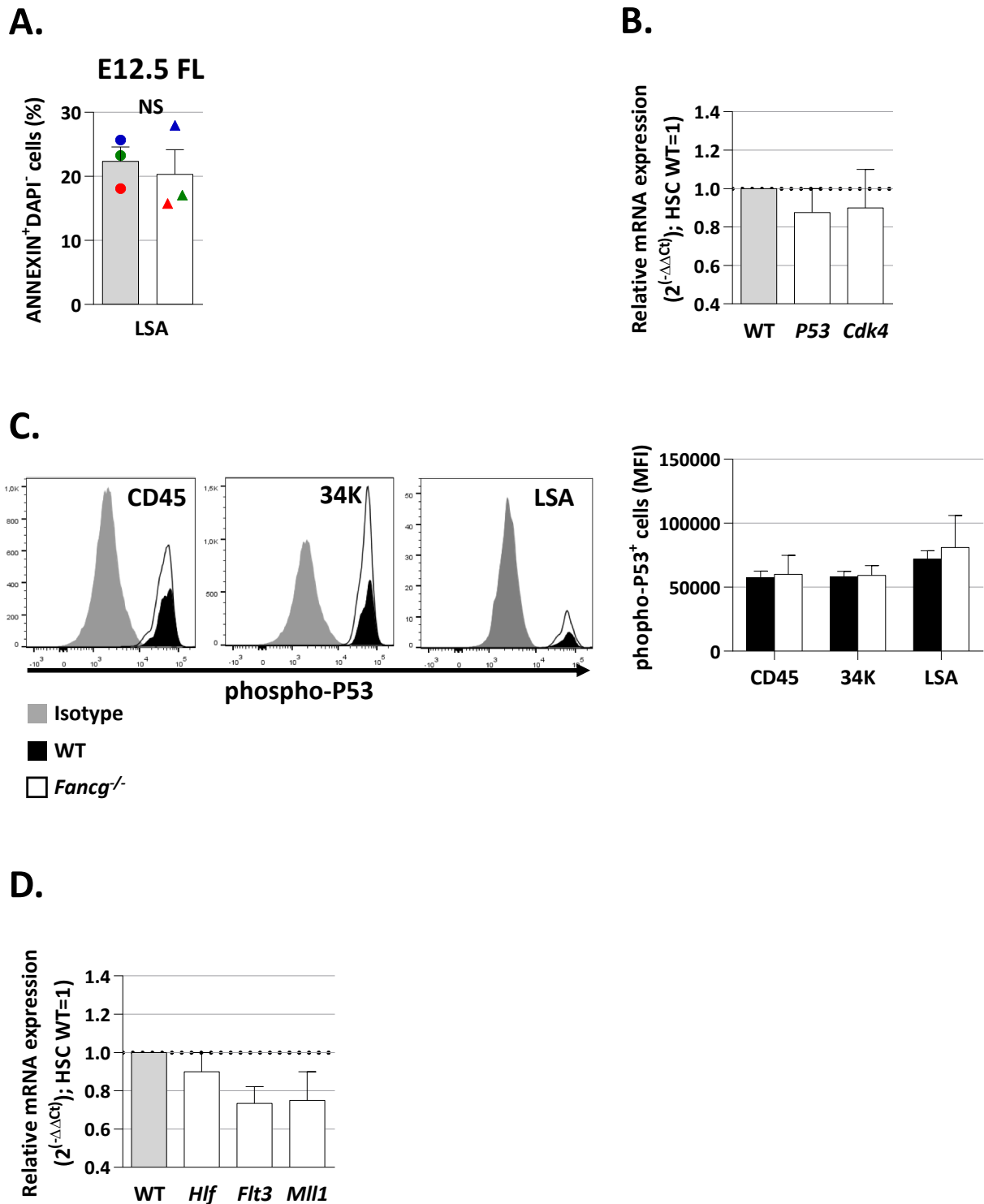


Figure S3. Related to Figure 4 and Figure 6. Annexin, p53, and qPCR. (A) ANNEXIN⁺DAPI⁻ LSA cells in E12.5 FL (n=3, dots of same colors are related to the same experiment), measured by flow-cytometry. Grey bars: WT; White bars: *Fancg*^{-/-}. NS: not significant. (B, D) qPCR of relative mRNA expression of (B) cell cycle related genes (*P53*, *Cdk4*) and (D) HSC related genes (*Hlf*, *Flt3*, *Mll1*) in E12.5 FL HSCs. Grey bars: WT; White bars: *Fancg*^{-/-}. (C) Left panel, histograms of phospho-P53 expression in CD45⁺, 34K and LSA in E12.5 FL. Right panel, MFI of phospho-P53 in CD45⁺, 34K and LSA in E12.5 FL. Grey: Isotype; Black: WT; White: *Fancg*^{-/-}, n=3. Error bars correspond to standard deviation (SD).

Supplemental Tables

Table S1. Related to Figure 6. GO processes for up- and down-regulated genes in *Fancg*^{-/-} E12.5 Pl and E14.5 FL and human FA FL HSCs determined by DAVID, KEGG and Reactome pathways (excel file).

Table S2. Related to Figure 6. Expression of cell cycle regulators in E12.5 FL and E14.5 FL *Fancg*^{-/-} HSCs and in human FA FL HSCs

	MOUSE		Type(s)	E12.5 FL HSC <i>Fancg</i> ^{-/-} vs WT E14.5 FL HSC <i>Fancg</i> ^{-/-} vs WT			HUMAN	Hu FL CSH FA vs N			
	EntrezGeneID	Symbol		p-value	Fold-Change	p-value		Fold-Change	EntrezGene ID	Symbol	p-value
positive regulators cell cycle	12534	<i>Cdk1</i>	kinase (G2/M)	0.0265882	-1.95099	0.523601	1.19537	983	<i>CDK1</i>	0.049633	-2.34181
	12566	<i>Cdk2</i>	kinase (G1/S and S)	0.244252	-1.47903	0.861626	1.05902	1017	<i>CDK2</i>	0.0286387	-1.50515
	12567	<i>Cdk4</i>	kinase (G1)	0.061724	-2.40922	0.638159	1.23345	1019	<i>CDK4</i>	0.137186	1.44103
	12571	<i>Cdk6</i>	kinase (G1)	0.068171	-2.01864	0.624994	1.196	1021	<i>CDK6</i>	0.413603	-2.13399
	12530	<i>Cdc25a</i>	phosphatase	0.070752	-2.0569	0.256828	1.54979	993	<i>CDC25A</i>	0.899374	-1.11772
	12545	<i>Cdc7</i>	kinase	0.00571329	-1.52194	0.278122	1.15937	8317	<i>CDC7</i>	0.0184651	-10.022
positive regulators G1/S	72391	<i>Cdkn3</i>	phosphatase	0.0123337	1.80254	0.163852	-1.35654	1033	<i>CDKN3</i>	0.163052	-4.89689
	26965	<i>Cull1</i>	enzyme	0.940323	1.02756	0.865013	1.06371	8454	<i>CUL1</i>	0.0227364	-2.8474
	26554	<i>Cul3</i>	enzyme	0.0762675	-1.38092	0.81476	-1.04139	8452	<i>CUL3</i>	0.00796146	-8.41682
	27214	<i>Dbp4</i>	kinase	0.100316	-1.58151	0.48082	-1.20889	10926	<i>DBF4</i>	0.422796	-1.40464
	27401	<i>Skp2</i>	enzyme	0.29602	-1.32127	0.505338	1.19212	6502	<i>SKP2</i>	0.0341479	-1.60341
	12575	<i>Cdkn1a (p21)</i>	kinase	0.19388	-1.29206	0.00169713	2.03639	1026	<i>CDKN1A</i>	0.627505	1.69843
negative regulators G1 and G1/S	12576	<i>Cdkn1b (p27)</i>	kinase	0.242822	-1.33249	0.935519	-1.01964	1027	<i>CDKN1B</i>	0.269765	-1.56777
	12577	<i>Cdkn1c (p57)</i>	other	0.976823	-1.0031	0.0818228	-1.21492	1028	<i>CDKN1C</i>	0.0528907	1.40915
	12578	<i>Cdkn2a (p16)</i>	transcription regulator	0.0138273	1.51116	0.481858	-1.11356	1029	<i>CDKN2A</i>	0.0579896	1.67837
	12580	<i>Cdkn2c (p18)</i>	transcription regulator	0.169685	1.64242	0.78489	1.10051	1031	<i>CDKN2C</i>	0.993546	-1.00365
	12581	<i>Cdkn2d (p19)</i>	transcription regulator	0.328384	-1.68009	0.901609	1.06678	1032	<i>CDKN2D</i>	0.163026	1.95938
	22059	<i>Tp53</i>	transcription regulator	0.020398	-2.28048	0.329335	1.38025	7157	<i>TP53</i>	0.0357746	-4.79119
DNA Damage Response	12649	<i>Chek1</i>	kinase	0.101596	-1.71194	0.62796	1.16524	1111	<i>CHEK1</i>	0.476714	-1.3565
	11920	<i>Atm</i>	kinase	0.0196423	-1.36019	0.235506	1.15751	472	<i>ATM</i>	0.0146579	-4.59635
	245000	<i>Atr</i>	kinase	0.295867	-1.155	0.155405	1.21996	545	<i>ATR</i>	0.0882164	-2.72483
	26416	<i>Mapk14</i>	kinase	0.100101	-1.78115	0.870933	-1.05612	1432	<i>MAPK14</i>	0.155265	-1.71515

italics : p-value > 0.05

Table S3. Related to Figure 6. Expression of transcriptional regulators and markers important for HSC development and maintenance in E12.5 FL and E14.5 FL *Fancg*^{-/-} HSCs and in human FA FL HSCs

MOUSE EntrezGeneID	Symbol	Type(s)	E12.5 FL HSC <i>Fancg</i> ^{-/-} vs WT		E14.5 FL HSC <i>Fancg</i> ^{-/-} vs WT		HUMAN Entrez Gene ID		Hu FL HSC FA vs N	
			p-value	Fold-Change	p-value	Fold-Change	Symbol	p-value	Fold-Change	
17863	<i>Myb</i>	transcription regulator	0.551/93	-1.26695	0.764629	1.12565	4602	<i>MYB</i>	0.72529	-1.82171
14461	<i>Gata2</i>	transcription regulator	0.000671183	-1.63055	0.139091	1.19841	2624	<i>GATA2</i>	0.839851	1.55964
15405	<i>Hoxa9</i>	transcription regulator	0.791312	1.04128	0.132993	-1.2687	3205	<i>HOXA9</i>	0.0187017	3.59795
217082	<i>Hlf</i>	transcription regulator	0.00182585	-1.94873	0.394645	-1.16941	3131	<i>HLF</i>	0.400577	-1.89707
17268	<i>Mets1</i>	transcription regulator	0.00111461	-1.83682	0.253189	1.19943	4211	<i>MEIS1</i>	0.209917	-2.5383
16909	<i>Lmo2</i>	transcription regulator	0.041514	-1.67244	0.74554	1.07963	4005	<i>LMO2</i>	0.0156993	-1.60739
12394	<i>Runx1</i>	transcription regulator	0.0226876	-2.04944	0.196683	1.46733	861	<i>RUNX1</i>	0.77931	-1.62861
21349	<i>Tal1</i>	transcription regulator	0.876997	-1.03302	0.826557	-1.04709	6886	<i>TALI</i>	0.013999	-1.40094
14056	<i>Ezh2</i>	transcription regulator	0.242662	-1.2982	0.851762	1.0417	2146	<i>EZH2</i>	0.0680242	-1.86875
12393	<i>Runx2</i>	transcription regulator	0.0705636	-1.26931	0.69666	1.05007	860	<i>RUNX2</i>	0.427646	-1.71222
14011	<i>Etv6</i>	transcription regulator	0.0481506	-2.1165	0.703837	1.14525	2120	<i>ETV6</i>	0.149461	-3.75014
13876	<i>Erg</i>	transcription regulator	0.00811629	-2.00213	0.651597	-1.11153	2078	<i>ERG</i>	0.951723	1.02782
17128	<i>Smad4</i>	transcription regulator	0.00303934	-2.15952	0.6264	1.11578	4089	<i>SMAD4</i>	0.0758063	-3.40223
21423	<i>Tcf3 (E2a)</i>	transcription regulator	0.0101073	-1.85672	0.409928	1.19673	6929	<i>TCF3 (E2A)</i>	0.976242	-1.00542
20230	<i>Satb1</i>	transcription regulator	0.00000373983	-1.9032	0.188524	-1.13722	6304	<i>SATB1</i>	0.0455969	-2.32733
14247	<i>Fli1</i>	transcription regulator	0.0210975	-2.09813	0.600603	1.16741	2313	<i>FLI1</i>	0.305429	-1.83813
17480	<i>Mpl</i>	transmembrane receptor	0.00652311	-2.59841	0.934073	-1.02601	4352	<i>MPL</i>	0.772362	-1.70948
16590	<i>Kit</i>	transmembrane receptor	0.00876411	-2.15246	0.996552	1.00113	3815	<i>KIT</i>	0.0279414	-5.30959
14255	<i>Fh3</i>	kinase	0.0000856308	-3.26137	0.678667	1.10044	2322	<i>FLT3</i>	0.0150671	-11.4272
12490	<i>CD34</i>	other	0.0296424	-1.77092	0.578961	1.1452	947	<i>CD34</i>	0.291241	-2.26558
17064	<i>CD93</i>	other	0.0492916	-1.5755	0.732869	1.07704	22918	<i>CD93</i>	0.743419	-1.30972
12767	<i>Cxcr4</i>	G-protein coupled recep	0.0453386	-2.00912	0.883063	1.04921	7852	<i>CXCR4</i>	0.00546894	-2.60175
110454	<i>Ly6a (Sca1)</i>	other	0.525021	-1.17961	0.869235	-1.04344				
13435	<i>Dnmt3A</i>	enzyme	0.0000019344	-1.81074	0.0054889	1.30033	1788	<i>DNMT3A</i>	0.194286	-2.16926
13436	<i>Dnmt3B</i>	enzyme	0.00615053	-3.07848	0.313937	1.44877	1789	<i>DNMT3B</i>	0.545676	-1.53146
269966	<i>NUP98</i>	transporter	0.163985	-1.71656	0.819614	1.08966	4928	<i>NUP98</i>	0.237552	-2.46241
380669	<i>Lin28b</i>	other	0.0117365	-2.11293	0.377657	1.26962	389421	<i>LIN28B</i>	0.414911	-2.09804
15364	<i>Hmga2</i>	enzyme	0.0219313	-1.54098	0.191152	1.26183	8091	<i>HMG42</i>	0.0138053	-3.28699

italics : *p-value* > 0.05

Supplemental Experimental Procedures

RT-qPCR

For RT-qPCR assay, RNA were reverse-transcribed 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 for RT-qPCR assay are listed below.

Target	Sens	Primers 5'-3'
Cell cycle markers		
<i>P53</i>	Forward	GAGGCCGGCTCTGAGTATACCA
	Reverse	GGCAGGCACAAACACGAACC
<i>Cdk4</i>	Forward	CGGCCTGTGTCTATGGTCTG
	Reverse	GAAGCAGGGGATCTTACGCT
HSCs markers		
<i>Hlf</i>	Forward	GCAGCCGTCTACGTTTTCAA
	Reverse	CAGGATGCTTTCTCACCTGC
<i>Flt3</i>	Forward	TGTCAGTAATGATTCTTGAGACCGT
	Reverse	GCACACTGGAGGTCTTCTGG
<i>Mll1</i>	Forward	GGCCCTGTTGAATTCTCGGA
	Reverse	GGGAGCTTCGGGAAGGTATG
Housekeeping genes		
<i>Gapdh</i>	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	TGTAGACCATGTAGTTGAGGTCA