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Supplementary Materials for

Identification of Therapeutic Targets for Quiescent, Chemotherapy-Resistant Human Leukemia Stem Cells

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Other Supplementary Material for this manuscript includes the following:
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ID	Gender	Age	FAB	Cytogenetics	U133	1.0ST	qPCR	25-gene hierarchical clustering	Flow cytometry					Positive LSC markers by flow cytometry
					Fig. 2A, Table S1	Fig. 2A, Table S1	Fig. S1	Fig. 2C	Fig. 4A,B	CD32		CD25 MFI	CD18 MFI	CD93 MFI	
										MFI	Pattern				
1	M	53	M0	complex including -7					Yes	2229	a	na	na	na	32
2	M	60	M0	complex including t(11;19)(q23;p13.1)				P(1)	Yes	206	c	215	141	91	none
3	M	79	M1	complex	P(1)R(2)	R(1)	R(1)		Pilot case	657	b	363	925	529	18
4	M	55	M1	+8, t(8;13)(q22;q12)				P(1)	Yes	343	c	884	993	122	25, 18
5	F	64	M1	complex involving t(15;17)				R(1)	Yes	158	c	1798	577	231	25
6	M	44	M1	inv(12)(p13q22)					Yes	1012	c	269	77	299	32
7	M	69	M1	complex					Yes	4516	a	165	967	360	32, 18
8	M	69	M1	complex				P(1)	Yes	1413	a	312	1549	576	32, 18
9	na	na	M1	46, XY, -7				R(1)	Yes	48	c	190	51	211	none
10	M	32	M1	add(3)(q21), add(4)(q31), -7, +mar				P(1)	Yes	1013	a	154	146	181	32
11	M	25	M2	t(1;16)(q32;q24), t(6;9)(p23;q34)	P(1)R(1)	R(1)	R(1)		Pilot case	1335	a	219	1842	333	32, 18
12	F	70	M2	complex	P(1)	R(1)	R(1)		Pilot case	118	b	322	635	1	none
13	M	61	M2	t(3;4)(q27;p12)	P(1)				Yes	1486	a	2870	1110	112	32, 25, 18
14	F	52	M2	add(5)(q?31), t(7;11)(p15;p15), +8, add(12)(p11.2)	P(1)				Yes	118	c	159	190	112	none
15	F	58	M2	46, XX		R(1)	R(1)		Pilot case	948	a	1810	688	923	32, 25, 93
16	M	48	M2	t(7;17)(q32;q21)					Yes	1516	a	292	79	136	32
17	M	35	M2	complex including t(8;21)(q22;q22)				P(1)	Yes	401	c	1504	270	325	25
18	M	59	M2	complex including -5, -7					Yes	222	c	125	196	1103	93
19	M	43	M2	complex					Yes	379	b	372	84	246	93
20	M	na	M2	na					Yes	78	b	749	121	120	none
21	M	45	M2	ins(1;?)(q21;?), +8					Yes	159	b	363	1141	285	18
22	F	56	M2	complex including -7				P(1)	Yes	122	c	148	950	224	18
23	M	62	M2	complex					Yes	1937	a	533	1859	1196	32, 18, 93
24	M	49	M2	+10, ?11, add(9)(q13)					Yes	292	b	120	504	111	none
25	F	54	M2	t(8;21)(q22;q22), t(9;12)(p22;p11.2)				P(1)	Yes	316	b	243	135	72	none
	M	na	M2	na	P(1)R(1)				No	na	na	na	na	na	na
26	M	na	M4	na	P(1)R(1)	R(1)	R(1)		Pilot case	1187	a	296	4116	2107	32. 18, 93
27	F	48	M4	46, XX	P(1)R(1)				Pilot case	1559	a	368	1192	326	32. 18
28	M	43	M4-Eo	inv(16)(p13q22), +22		P(1)			Yes	1281	a	214	71	70	32
29	M	56	M4	add(2)(q2?3)					Yes	340	b	855	260	127	25
30	M	61	M5a	complex					Yes	1737	a	171	1867	372	32.18
31	M	64	M7	complex including -7					Yes	3726	a	370	123	285	32
32	F	59	undetermined	t(6;9)(p23;q34)					Yes	4892	a	413	535	279	32
33	M	57	MDS/AML	complex	P(1)				Yes	399	b	3339	101	673	25
34	M	69	MDS/AML	+8	P(2)*				Yes	268	c	131	652	151	none
35	M	66	MDS/AML	del(11)(p11.2)	P(1)				Yes	144	c	152	38	48	none
36	M	87	MDS/AML	na	P(1)				Yes	241	c	421	128	168	none
37	F	67	MDS/AML	+8	P(1)				Yes	98	c	92	118	53	none
38	F	82	MDS/AML	t(6;9)(p23;q34)	P(1)				Yes	486	b	320	115	152	none
39	M	45	MDS/AML	t(3;19)(q21;p13)					Yes	2201	a	1155	nd	nd	32, 25
40	M	62	MDS/AML	+1, der(1;7)(q10;p10)					Yes	927	a	1033	53	195	32, 25
41	M	66	MDS/AML	na					Yes	487	b	2878	60	63	25
42	M	59	MDS/AML	del(5)(q22q33), del(11)(q13q23)					Yes	718	b	1312	471	163	25
43	F	63	MDS/AML	complex including 5q-, -7				R(1)	Yes	121	c	952	303	468	25
44	M	68	MDS/AML	50, XY, del(20)(q11.2), +del(20)(q11.2), +21, +21, +21				R(2)	Yes	309	c	4789	90	291	25
45	M	83	MDS/AML	complex					Yes	412	c	1651	451	666	25
46	M	69	MDS/AML	complex including -7					Yes	286	c	912	232	287	25
47	M	60	MDS/AML	t(3;3)(q21;q26)				R(2)	Pilot case	709	b	182	1588	279	18
48	M	71	MDS/AML	complex					Yes	162	c	431	764	535	none
49	M	71	MDS/AML	t(1;3)(p36;q21)				P(1)R(2)	Yes	59	b	281	84	105	none
50	M	63	MDS/AML	46, XY					Yes	171	c	316	324	135	none
51	F	62	MDS/AML	del(7)(q11.2q22)					Pilot case	598	b	281	3178	221	18
52	M	61	MDS/AML	46, XY					Yes	693	b	323	207	193	none
53	M	65	MDS/AML	-7, der(12)(1;12)(q21;p13)					Yes	1051	a	119	110	742	32
54	F	63	MDS/AML	complex					Yes	351	b	33	282	213	none
55	M	63	MDS/AML	46, XY					Yes	3118	a	244	1144	176	32, 18
56	F	64	MDS/AML	na					Yes	71	c	311	28	42	none
57	M	60	MDS/AML	del(13)(q12q14)					Yes	134	c	70	97	95	none
58	M	66	MDS/AML	t(4;21)(q31;q22)					Yes	126	b	183	140	147	none
59	M	60	MDS/AML	46, XY				P(1)	Yes	463	b	511	74	134	none
60	M	60	MDS/AML	47, XY, +8				P(1)	Yes	485	c	481	147	179	none
61	F	59	MDS/AML	46, XX				P(1)	Yes	2184	a	68	138	113	32
				% positive						21/61 (34.4%)		15/61 (24.6%)	15/61 (24.6%)	4/61 (6.6%)	39/61 (63.9%)

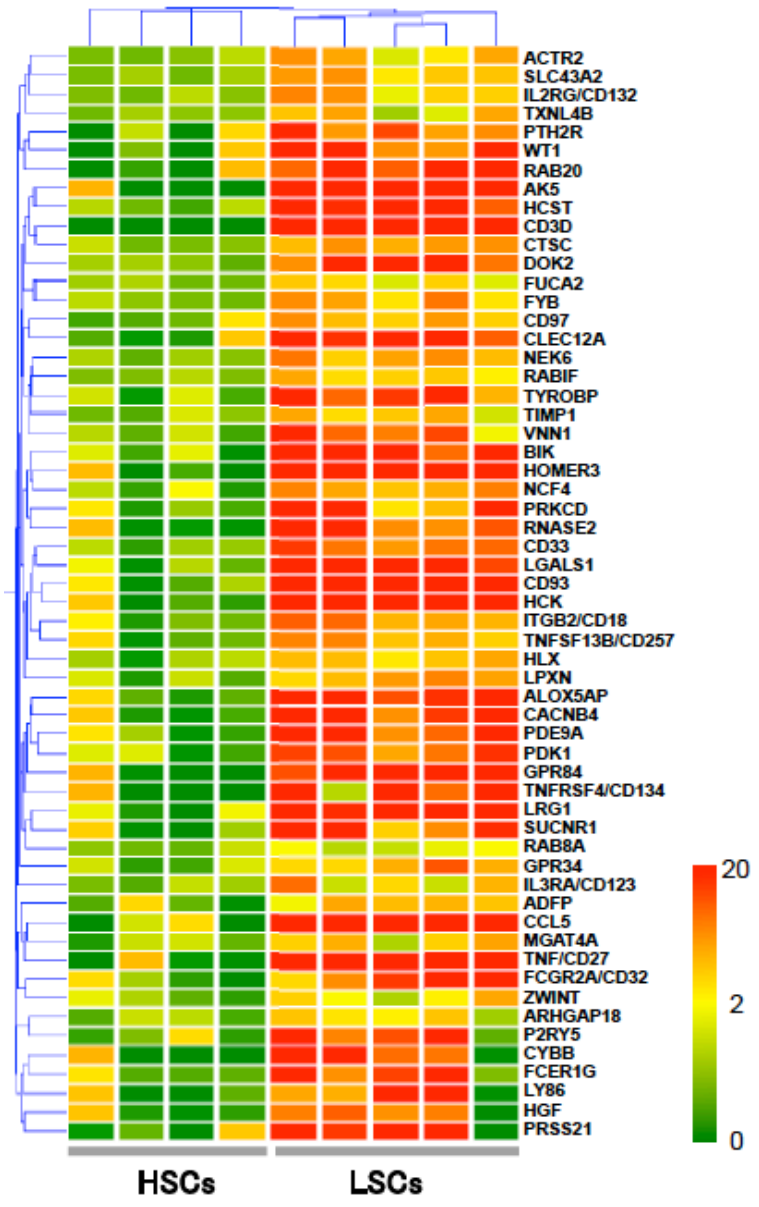
Supplementary Table 1. Summary of analyses performed on AML patient samples. P(x) and R(y) indicate that the analysis was performed on original patient LSCs at n=x and recipient BM LSCs at n=y, respectively. *For case #34, a pre-treatment sample and a sample obtained at the time of relapse were analyzed. Pilot cases are the group of eight representative samples in which initial flow cytometry analyses were performed to determine which molecules are most frequently expressed on LSCs. MFI refers to mean fluorescence intensity in LSCs.

Supplementary Table 2. List of LSC signature genes.

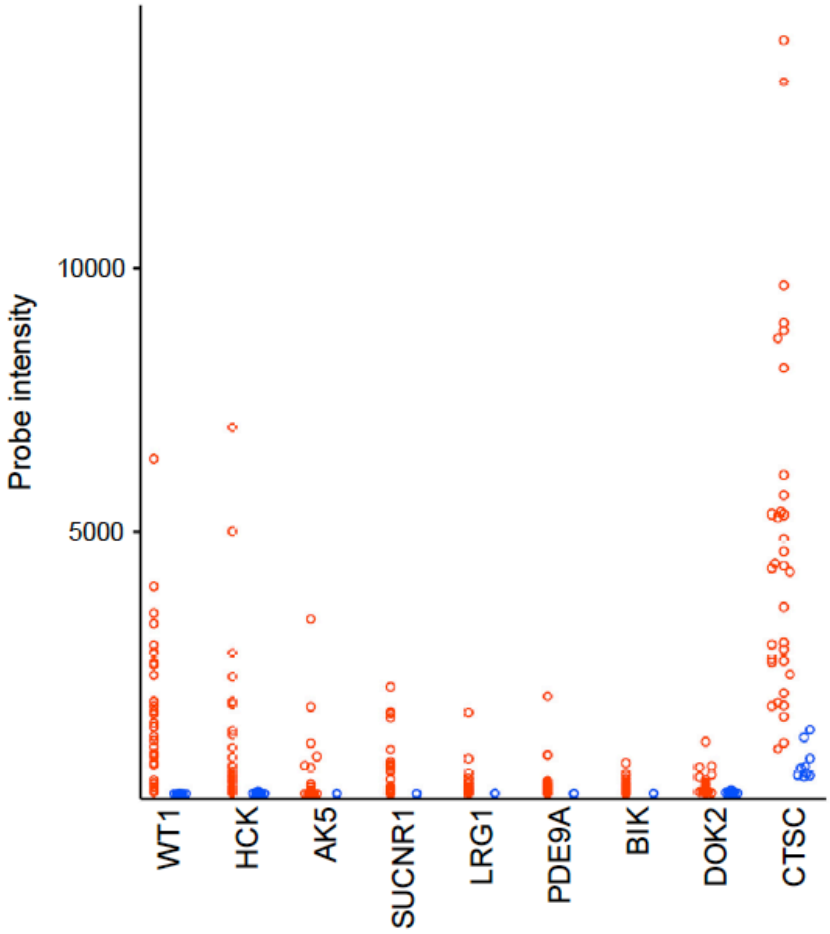
A total of 259 genes were identified through comprehensive expression analyses using the Human Genome U133 Plus 2.0 (U133) and Human Gene 1.0ST (1.0ST) GeneChips. Genes in Group 1 were found overrepresented in LSCs compared with HSCs in both array platforms. Genes in Group 2 were found overrepresented in LSCs and not expressed in HSCs in either platform. Entrez gene ID, HUGO gene symbol, probe set ID in U133 and 1.0ST platforms, fold difference of expression between LSC and HSC, chromosome number, gene name, and functional class of genes are shown.

Supplementary Table 3. List of primers and probes for qRT-PCR.

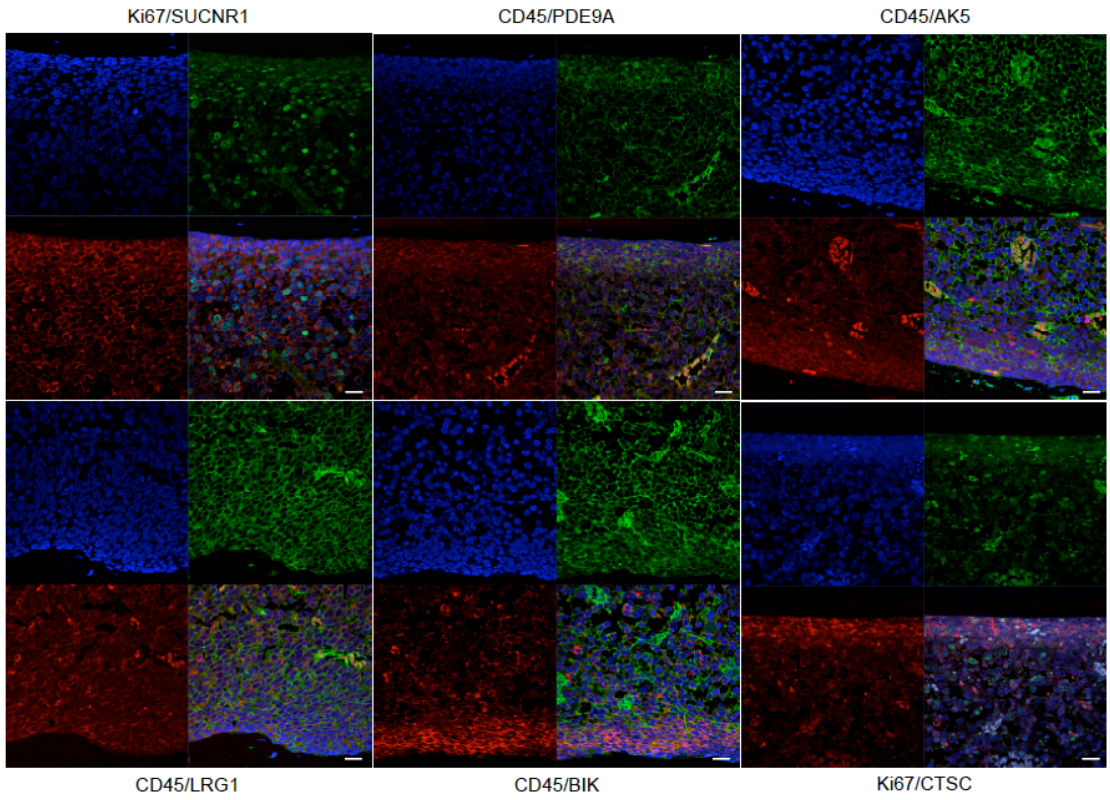
The first column contains the HUGO gene symbol with the name of dual fluorescence-labeled probes and primers corresponding to the each gene listed in the second column. Third, fourth and fifth columns represent DNA sequence, melting temperature, and length of the each probes and primers, respectively. Sixth and seventh columns provide DNA sequence and length of the amplified products, respectively.



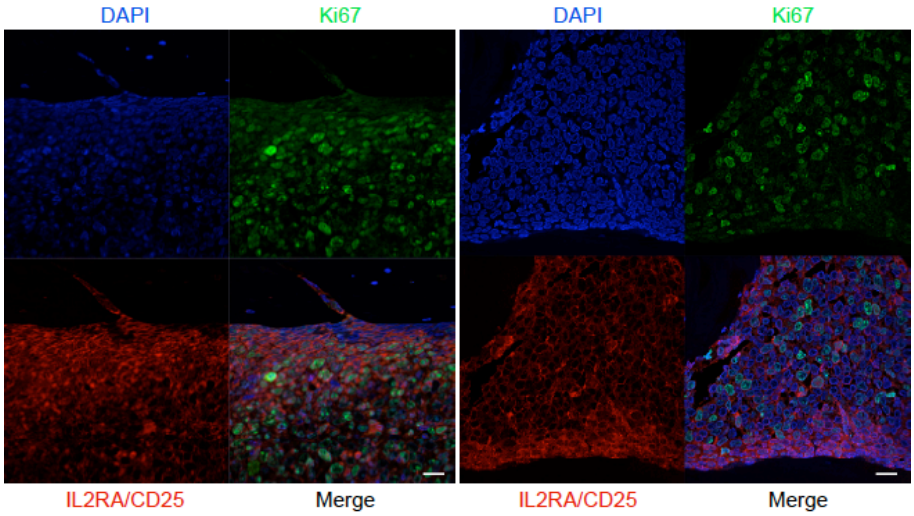
Supplementary Figure 1. LSC-specific expression of 58 genes was validated by qRT-PCR. Of the 136 candidate genes examined, 58 showed mRNA levels significantly higher in AML LSCs compared with those in normal human HSCs ($P < 0.05$ by either Kruskal-Wallis, Wilcoxon-Mann-Whitney, or student's t-test), as represented in a heat-map. In each panel, red, yellow and green represent high, intermediate, and low levels of expression, respectively.



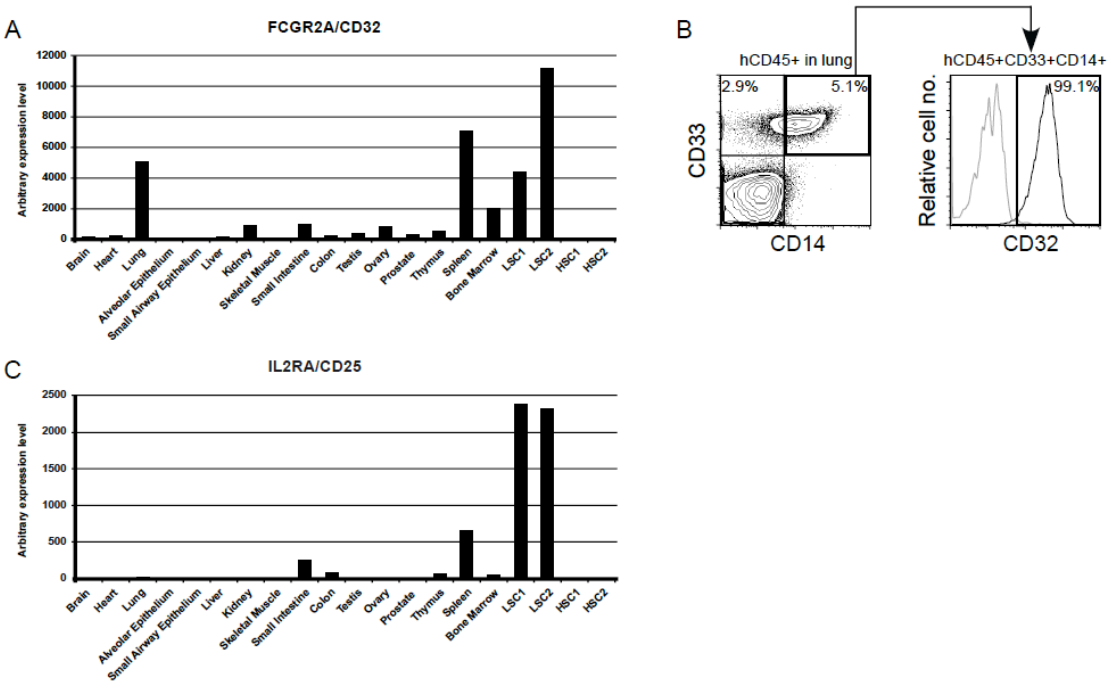
Supplementary Figure 2. WT1 and HCK are frequently overexpressed in LSCs. Expression levels of WT1, HCK, DOK2, AK5, SUCNR1, LRG1, PDE9A, BIK and CTSC genes in LSCs (red circles, N=30) and HSCs (blue circles, N=9) obtained using U133 plus array platform. Among the nine genes, WT1 and HCK were most frequently over-expressed in LSCs.



Supplementary Figure 3. Candidate LSC target molecules show expression in human AML cells lining the BM endosteum. LSC target molecules SUCNR1, PDE9A, AK5, DOK2, LRG1, BIK and CTSC are expressed in AML cells lining the BM endosteum. Target molecules (red) are co-labeled with hCD45 (green) for PDE9A, AK5, DOK2, LRG1 and BIK and with Ki67 (green) for SUCNR1 and CTSC. Nuclei are labeled with DAPI (blue). The BM %hCD45+CD33+ was greater than 98% in each recipient, indicating that nearly all the cells in the BM are human AML cells. Scale bar, 20μm.



Supplementary Figure 4. CD25 is expressed in cell cycle-quiescent AML cells in the BM endosteal region. CD25 (red) is expressed extensively in AML cells in the BM endosteal region and that are predominantly Ki67 negative (green), in femurs from two AML-engrafted recipients. The %hCD45+hCD33+ cells in the recipient BM were 98.4 and 99.6%, indicating that nearly all the cells in the BM are human AML cells. Nuclei are labeled with DAPI. Scale bar, 20μm.



Supplementary Figure 5. Expression of CD32 and CD25 in nonhematopoietic tissues. (A) CD32 expression was evaluated by qRT-PCR in various human tissues. **(B)** hCD45+CD33+CD14+ alveolar macrophages highly express CD32 at the protein-level. **(C)** CD25 expression was evaluated by qRT-PCR, showing little to no CD25 transcript in various non-hematopoietic organs.