SUPPLEMENTARY METHODS

**Mice:** The constructs were derived from PAC54A19 following digestion with restriction enzymes capable of generating the 5’ and 3’ flanking regions indicated in Figures 1a, 2a, 2c, 6a, 4Sa and 5Sa-d. Pvul was utilized for constructs A, D, F and G; AscII and BsiWI for constructs B and E; Pvul and FspI for construct C. Homologous recombination was used to obtain constructs B, and D-G, that have been verified by sequencing. Digested DNA was separated with field inversion gel electrophoresis (FIGE, Bio-Rad) for 17-24h. After cutting out gels containing genomic DNAs, gels were electroeluted with dialysis tubing (SnakeSkin pleated dialysis tubing 10,000 MWCO, Pierce cat. # 68100). DNAs were precipitated and then suspended in 1mM Tris-HCl (pH7.6)/0.1 mM EDTA.

DNAs were injected into fertilized oocytes of FVB/N mice and implanted into uteri of pseudopregnant FVB/N according to standard procedure. Screening of transgenic mice was carried out by Southern blot and by PCR using probes located in exon 8 (see Genotyping section for details) of the *hCD34* gene, a region which is highly divergent from the murine sequence, allowing for specific detection of the human transgene in murine tissues. Subsequently additional PCR reactions were carried out with primers located in various region of the gene (see Genotyping section) to check for construct integrity.

*hCD34* transgenic mice were bred to conditional Runx1 knockout mice (Runx1<sup>F/F</sup>) (Growney et al., 2005) and to Mx1-Cre mice (Kuhn et al., 1995), to obtain interferon (IFN)-inducible Runx1 gene excision.

**Genotyping transgenic mice with Southern Blot Analysis and PCR:** For Southern Blot analysis fifteen micrograms of DNA were digested with BamHI and separated on 0.8% agarose gels, transferred to nylon membranes (Hybond N+, GE Healthcare Biscence) in 0.4 NaOH for 24 hours, and immobilized by UV cross-linking (Stratalinker, Stratagene, La Jolla, CA). An EcoRI-digested 2kb human *CD34* genomic DNA fragment encompassing exons 2-4 was used
as a probe and labeled with \( [\alpha-^32P] \) deoxycytidine triphosphate 110 TBq/mM (3,000 Ci/mM) by random priming; this detects a 6.7-kb BamHI fragment. Membranes were washed with 0.2X SSC/0.1% SDS at 65° C and exposed using a BioMax Intensifying Screen (Kodak, Rochester, NY).

Several sets of polymerase chain reaction (PCR) primers were generated to confirm transgene structures. Primers 5’-AGAAGAGATGAGGTGTGAGGAT- 3’ and 5’-GGATCC ACAAGAATGAGCATGTA-3’ were used to amplify a fragment 7 kb 3’ from exon 8, the most 3’ exon of human CD34; 5’-ATGGGTTGAGG ACCTGAAGTGGT- 3’ and 5’-TGGATTCAGTTTTGTTTCC-3’ were used to amplify a 500-bp genomic fragment located 38 kb 5’ of the transcription start site; 5’-GTGCTTTCATGGAGAG CGGTTTTA- 3’ and 5’-TAAGACCTCAAG GGGTGGACTC-3’ to amplify a genomic DNA fragment located 1 kb 5’ of the transcription start site; 5’-AAAGTTAAGTGAAGTGGAGCTGGACTGGAGAAG-3’ and 5’-TGGATAGGTAACACTGCCTCTCAGGAAAGCTGAA CGAGCAT- 3’ and 5’-GCTACTAACT TGGGCTCTCCAGGAAAGCTGAA CGAGCAT- 3’ to amplify a genomic DNA fragment encompassing the promoter-exon 2 region; 5’-TCTACTCCAACCTCTACGCTACT-3’ and 5’-GCCTGTCCCTGGCACTCGACAAC-3’ to amplify a genomic DNA fragment located 19 kb downstream from the end of exon 8; 5’- TGGGGCCTCTCCAGGAAAGCTGAA CGAGCAT- 3’ and 5’-GCTACTAACT TGGGCTCTCCAGGAAAGCTGAA CGAGCAT- 3’ to amplify a genomic DNA fragment encompassing exon 1; 5’-CAAGACACTGTTGGAACACTT- 3’ and 5’-GAAGCCATGGAGATCAG- 3’ to amplify a genomic DNA fragment encompassing exon 8; 5’-GACCCATCGGCACGAGCTATCTCT-3’ and 5’-ACAGGGGCACGTTTTGACCA-3’ to amplify a 413-bp genomic DNA fragment located 12 kb upstream of the transcription start site; 5’- TGG TGGGGAACCTCTTGGACTGTGA- 3’ and 5’-GGCGCCTGAGCTGGGATTACTG-3’ to amplify a 469-bp genomic DNA fragment located 13.6 kb upstream of the transcription start site; 5’-GCAACCCAGCCTCCCTCCTAAGC- 3’ and 5’-CACACCTCGGACTGACAACCTCAG-3’ to amplify a 551-bp genomic DNA fragment encompassing the transcriptional start site and exon 1; 5’-TGGAAAGTAGCAGGGGGA ACG-3’ and 5’-TCAAACAGGATGGGGTCAAAC-3’ to amplify a 594-bp genomic DNA fragment located 22 kb downstream of exon 8; 5’-TGCGCTCGAGT GCAACACAAGGACTG-3’ to amplify a genomic DNA
fragment encompassing exon 8; 5'-TATGGGAGTCTGA GGCAAGCAGA-3' and 5'-GTCACTG
TCACTGGGATGCAG-3' to amplify a genomic DNA fragment located 15.2 kb downstream of
exon 8; 5'-CATCATCTGCCCAGG GC7GCTT-3' and 5'-CCATCACCACATCCTCAAAGA-3' to
amplify a 521-bp genomic DNA fragment located 0.5 kb upstream of the transcriptional start site.
PCRs were performed with Taq Pro Red Complete (2 mM MgCl, Denville Scientific), with
cycles at 95C, 30 sec; 58C, 30 seconds; and 72C 50 seconds for 35 cycles after 5 minutes of
denaturation at 95C.

Oligonucleotides to genotype for Runx1 loxP/loxP sites were as follows: 5'-GAGTCC
CAGCTGTCAATTCC-3' and 5'-GGTGATGGTCAGAGTGAAGC-3'; and for the Mx-Cre allele:
5'-TGTTCATT TACTGACC-3' and 5'-CGCCGCATAACCAGTGAAC-3'. Complete excision
of the Runx1 floxed/floxed allele was demonstrated by Real Time PCR (Figure 2S) and at the
genomic level by PCR (data not shown), as previously described (Growney et al, 2005)

**Flow Cytometric Analysis and Cell Sorting:** Dead cells were excluded by using propidium
iodide staining. After lysis of erythrocyte with ACK buffer, staining was performed using
antibodies directed against lineage negative markers (CD45/B220, CD19, CD8, CD4, Gr1, all
Tricolor-conjugated from eBioscience clones RA36B2, eBio1D3, 53-6.7, GK1.5, RB6.8C5,
respectively), Sca1 (Pacific Blue-conjugated clone D7 Biolegend 108120), c-kit (APC-
conjugated clone 2B8, eBioscience 17117183), CD48 (FITC-conjugated clone HM48-1
Biolegend 103404), CD150 (PE-cy7-conjugated clone TC15-12F12.2 Biolegend 115914),
human CD34 (PE-conjugated clone 8G12, BD-Pharmingen 348057). For the analysis of the
congenic FVB/N transplantation, donor and recipient cells were distinguished by using Ly5.1
(APC-conjugated clone A20, eBioscience 170453) or Ly5.2 (PE-conjugated clone 104,
eBioscience 12045482). For mature cells analysis we used Gr-1 (FITC-conjugated clone
RB68C5, BD-Pharmingen 553127), Mac-1 (TC-conjugated clone RM2806 Caltag), c-kit (biotin-
conjugated clone 2B8, eBioscience 553353), Ter119 (biotin-conjugated clone TER-119,
eBioscience 13592185), CD41 (FITC-conjugated clone MWReg30, BD-Pharmingen 553848),
Levantini et al., Runx1 and regulation of CD34 in HSC

B220 (FITC-conjugated clone RA36B2 eBioscience 11045285), CD3 (TC-conjugated clone RM3406, Caltag).

Cells sorted for the 3C assay have been lineage depleted before FACS-sorting by utilizing the Streptavidin Microbeads kit from Macs Miltenyi Biotec per the manufacturer’s instructions.

**Nuclear extracts preparation and Electrophoretic Mobility Shift Assay:** After the cells were washed in 1 × phosphate-buffered saline, the volume of the cell pellet was determined, the cells were resuspended in equal volume of ice-cold buffer A (10 mmol/L HEPES pH 7.9, 1.5 mmol/L MgCl₂, 10 mmol/L KCl, 0.5 mmol/L dithiothreitol (DTT), and protease inhibitor cocktail [MiniComplete, Roche]), and allowed to swell on ice for 15 minutes. The cell membranes were disrupted by applying 5 to 10 rapid strokes in and out of a 1 mL syringe attached to a 25-gauge needle. The nuclei were pelleted in a microfuge at 12,000 g for 20 seconds, resuspended in two thirds of the original cell pellet volume of buffer C (20 mmol/L HEPES pH 7.9, 25% glycerol, 420 mmol/L NaCl, 1.5 mmol/L MgCl₂, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, and protease inhibitor cocktail [MiniComplete, Roche]), incubated at 4°C for 30 minutes with occasional flicking and centrifuged for 5 minutes at 4°C at 12,000g in a microfuge. The supernatant was aliquoted and stored at −80°C. Concentration of the nuclear extracts was determined by Bradford assay by using the Protein Assay reagent (Bio-Rad Laboratories).

Double-stranded oligonucleotides (sequences are shown in Figure S4 b) were end-labeled with ³²P-γATP and Polynucleotide Kinase to a specific activity of 10⁸ to 10⁹ cpm/µg. Nuclear extracts (about 20 µg protein) were incubated for 20 minutes on ice with 5 × 10⁴ cpm of labeled DNA. The reaction mixture also contained, 20 mM HEPES pH 7.6, 100 mM KCl, 2 mM EDTA, 2 mM DTT, 10% glycerol, 2.5 µg of poly dI-dC, and 5 micrograms bovine serum albumin (BSA) in a total volume of 25 µL. Where indicated, 1 µL of anti-FLAG M2 monoclonal antibody (Sigma, cat# F3165) or RUNX1 Ab (rabbit polyclonal, Calbiochem PC284) were added to binding reactions. Unlabeled competitor oligonucleotides were added to the binding reactions at
a 200-fold excess immediately before the addition of the radioactive probe. The competitors were: RUNX binding site from the human M-CSF receptor promoter (5'-CCAAACTCTGTGG TTGCCTTG-3') and c-myb binding consensus sequence (5'-TACAGGCATAACGGTTCCGT AGTGA-3'). Unbound probe and DNA-protein complexes were separated by electrophoresis through 4% (19:1 acrylamide:bis-acrylamide mix) polyacrylamide gels in 0.5 × TBE (45 mmol/L Tris-borate, 1 mmol/L EDTA, pH 8) at 4°C, at 150 V.

**Chromosome Conformation Capture:** 1 x 10⁷ KG1a and HL60 cells were utilized for the 3C assay, in which interaction of the promoter with 7 downstream fragments, including the DRE, was analyzed. SLAM⁺ LSKs pooled from 25 mice carrying construct A, and pooled LSKs from 18 mice carrying construct F were used to study the interaction between the promoter and the DRE element. Cord blood cells were expanded in culture and ~130K hCD34⁺ and ~150K hCD34⁻ cells were utilized to analyze the interaction between the promoter and the DRE fragment. The sequence for the hybridization probe utilized in the 3C assay was 5'-FAM-tcatgcacatcctcacctcattca-BHQ1-3'. The primers to detect promoter interaction with the DRE and fragments 1-6 (Figure 5a) were as follows: promoter/DRE interaction: sense 5'-ctggcaag aacatctagagatag-3'; antisense 5'-tccagtctccacatctgctg-3'; promoter/fragment 1: sense 5'-ctggca agaacatctagagatag-3'; antisense 5'-tcagacttttcaatggctctaca-3'; promoter/fragment 2: sense 5'-ctggcaagaacatctagagatag-3'; antisense 5'-tcttcagtccatgctccaca-3'; promoter/fragment 3: sense 5'-ctggcaagaacatctagagatag-3'; antisense 5'-cgcagtttcttagggtcagc-3'; promoter/fragment 4: sense 5'-ctggcaagaacatctagagatag-3'; antisense 5'-ctggcaagaacatctagagatag-3'; promoter/fragment 5: sense 5'-ctggcaagaacatctagagatag-3'; antisense 5'-ggggtgaa gaaaagaggaaa-3'; promoter/fragment 6: sense 5'-ctggcaagaacatctagagatag-3'; antisense 5'-tagtgccaaatgg gaacaca-3'. Efficiency of these primers was tested on limiting dilutions (1:10, 1:100, 1:1000, and 1:10000) of the control library (PAC), prepared as previously described (Dostie & Dekker, 2007). The primer pair utilized to detect the interaction between the Promoter-DRE fragments showed an efficiency of 98.4%; all the remaining primer pairs (utilized to detect the interaction of the
Promoter with the other 6 downstream fragments shown in Figure 5a) showed a comparable efficiency of 96.4 +/- 1.24 %.

The efficiency of restriction enzyme digestion in KG1a and HL60 cells was calculated using the formula described by Hagège et al (Nature Protocols, 2007), and was >90% at all the HindIII junctions (surrounding the Promoter fragment, the DRE, and the 6 downstream fragments preceding the DRE, and shown in Figure 5a). The primers used to test HindIII digestion efficiency at the upstream and downstream border of the analyzed fragments were as follows:

For the promoter HindIII upstream boundary: sense 5’-gatcacatgagccaggagt-3’; antisense 5’-ctggcaagaacatcaggtag-3’; for the promoter downstream boundary: sense 5’-gaagtgccccagag-3’; antisense 5’-tgtaggggtctttgtagagtgag-3’; for fragment 1 upstream boundary: sense 5’-cgtaggggtctttgtagagtgag-3’; antisense 5’-cgtaggggtctttgtagagtgag-3’; for fragment 1 downstream boundary/fragment 2 upstream boundary: sense 5’-gcttattcttttttaaggtaatgt-3’; antisense 5’-tcttcagctccaccttcac-3’; fragment 2 downstream boundary/fragment 3 upstream boundary: sense 5’-tgggggagatcacaga-3’; antisense 5’-gtgagaacatgcgggtttg-3’; fragment 3 downstream boundary/fragment 4 upstream boundary: sense 5’-gaaggggagatcacaga-3’; antisense 5’-cgtaggggtctttgtagagtgag-3’; fragment 4 downstream boundary/fragment 5 upstream boundary: sense 5’-cgtaggggtctttgtagagtgag-3’; antisense 5’-cgtaggggtctttgtagagtgag-3’; fragment 5 downstream boundary/fragment 6 upstream boundary: sense 5’-ttaaaaagcagtaggcctttctgtcgt-3’; antisense 5’-gcacttggtcctttctgtcgt-3’; fragment 2 downstream boundary/DRE upstream boundary: sense 5’-gccctgtctttctgtcgt-3’; antisense 5’-tgacattctggtctttctgtcgt-3’; DRE downstream boundary: sense 5’-tc tgtgagtttctgagaggg-3’; antisense 5’-aagggcatacagcctcactaa-3’.

Interaction frequencies for the cell lines were calculated by dividing the test 3C libraries (obtained on the hCD34⁺ cell line KG1a and the hCD34⁻ cell line HL60) signals by control library
(PAC) signals. Interaction frequency of the DRE and Pr fragment in SLAM⁺ LSKs were calculated by dividing the test 3C libraries (obtained on the hCD34⁺ SLAM⁺ LSKs and hCD34⁻ lin⁺ cells from mice carrying Construct A) signals by their internal control signal indicating the amount of DNA used per each amplification. Similarly, interaction frequencies in LSKs from transgenic mice carrying 4 mutant RUNX sites (construct F), and in hCD34⁺ and hCD34⁻ cord blood cells, were calculated as described above for mice carrying Construct A.


SUPPLEMENTARY LEGENDS

Figure S1: Runx1 is expressed in SLAM<sup>+</sup> LSKs (related to Fig. 3)
Real-time PCR analysis of SLAM<sup>+</sup> LSKs and total bone marrow (BM) cells was performed on FVB/n wt mice (n=7). Runx1 expression is indicated as percentage of 18S expression by the black columns.

Figure S2: Runx1 expression is abolished in SLAM<sup>+</sup> LSKs from hCD34<sup>+</sup> conditional Runx1 null mice (related to Fig. 4)
Real-time PCR analysis of SLAM<sup>+</sup> cells demonstrated complete abrogation of Runx1 expression in PIPC-treated hCD34+ Runx1 KO mice, as compared to PIPC-treated hCD34<sup>+</sup> Runx1 WT mice. Gene expression was assayed 1 month after last PIPC injection. These data are representative of an experiment with 6 WT and 6 KO mice.

Figure S3: Characterization of cells used for the 3C assay in Figure 5
a) Real-time PCR analysis was performed on the human CD34<sup>+</sup> cell line KG1a and the human CD34<sup>-</sup> cell line HL60. Runx1 expression is indicated as percentage of 18S, by the black columns. b) Nuclear extracts from KG1a and HL60 cells were analyzed by western blot analysis stained with anti-RUNX1 antibody (upper panel). Staining with anti-β-actin antibody (lower panel) served as a loading control. c) Real-time PCR analysis was performed on KG1a and HL60 cells to confirm levels of hCD34 expression. hCD34 expression is indicated as percentage of 18S, by the black columns. d) The upper panel indicates how the sorting gates were selected. LSK from mice carrying construct A have been subdivided into SLAM<sup>+</sup> cells, which stained
99.4% positive for hCD34. After sorting, ~ 200 purified cells were subjected to analysis to verify the purity of the collected samples. The lower panel demonstrates that 98.6% of the purified cells are indeed positive for LSK markers, 98.3 % positive for SLAM markers, and are all positive for expression of hCD34. e) Sorting gates applied to obtain hCD34⁺ and hCD34⁻ cells from human umbilical cord blood grown in culture for 10 days.

Figure S4:
-Mutation in the RUNX sites abolishes binding of RUNX1 (related to Fig. 6). a) Nuclear extracts from 293T cells transiently transfected with FLAG-tagged RUNX1 expression vector were analyzed by western blot stained with anti -FLAG antibody (upper panel). Staining with anti-β-actin antibody (lower panel) served as a loading control. b) The nuclear extracts shown in (a) were used in EMSA. The sources of protein are indicated above the gels: 293T (untransfected 293T cells), or RUNX1 (293T cells transfected with FLAG-RUNX1); the probes are marked below. “Probe” indicates binding reactions in the absence of nuclear extract (NE); the “+” symbol indicates presence of NE. Binding is indicated by the lateral arrow ( ); supershift by the lateral thick arrow ( ). For supershifts, anti-FLAG antibody (Ab) was included in the reactions. Competition experiments were performed with either self competitor (S), PU.1-gene derived oligonucleotide containing previously identified RUNX1 site (R) (Huang et al., 2008), oligonucleotide with mutated RUNX site (m), and a c-myb site (c). The left panel shows binding assays relative to RUNX site #3 (wt and mutated as indicated below); the right panel shows binding assays to RUNX site #4 (wt and mutated).

-Deletion of the E-Box/GATA motifs within the DRE does not affect human CD34 expression in SLAM⁺ LSKs (related to Fig. 6) c) Construct A was modified to specifically delete the E-Box/GATA motifs (construct G). d) Fluorescent cytometry performed on bone marrow cells from mice carrying construct G. Deletion of the E-Box/GATA motifs does not impact hCD34 expression in SLAM⁺ cells. Shown is a representative example from one of 11
founder lines. T-test (tails 2; type 3) showed that the percentages observed in eleven independent mice transgenic for construct G (n=3 per founder, a part from 2 lines in which germ line transmission was not observed) were not statistically different from the data obtained in mice carrying construct A (p=0.2).

Figure S5: Transgenic constructs design (related to Supplemental Methods)
PAC54A19 was modified by lambda red recombination system (Datsenko and Wanner, 2000) to obtain constructs B, and D-G, as follows. a) Construct B: A FRT(green triangle)-flanked chloramphenicol (Cm) resistant cassette with homologous sequences at both 5’ (HS, blue box) and 3’ (HS, red box) was amplified by PCR and inserted in PAC54A19. The “FLPing-out” of the inserted antibiotic gene (Cm) was achieved by Flp recombinase in bacteria. The final construct was verified by PCR screening and sequence analysis and contained 19.6 kb of 3’ region immediately after exon 8. b) Construct D: The FRT-flanked Zeocin resistant cassette with homologous sequences at both the 5’ (HS, blue box) and 3’ (HS, red box) was amplified by PCR and inserted in PAC54A19. The final construct was verified by PCR screening and sequencing analysis, and did not contain the DRE sequence. c) Construct E: loxP-flanked Zeocin resistant cassette with homolog sequences at both 5’ (HS, blue box) and 3’ (HS, red box) was amplified by PCR and inserted by lambda red recombination system. Antibiotic resistance gene (Zeocin) was removed by Cre recombinase in bacteria. The final construct was verified by sequencing analysis and PCR screening, and contained the DRE immediately after exon 8. d) Constructs F and G: The DRE has been modified by lambda red recombination system with Neo selection/counter-selection system, as following: the boxed sequences (RUNX sites 1-4) indicates the region that has been modified in construct F (RUNX site 1 TGTGGG => TGTAGG; RUNX site 2 TGTGGA => TGTA GA; RUNX site 3 TGTGGG => TGTAGG; RUNX site 4 TGTTGGTG GG => TGTGTAGGG); the underlined sequence indicates the region that has been deleted in construct G.
Figure S6: Alignment of the hCD34 RUNX sites 1-4, with homologous sequences in chimp, rhesus monkey, and mouse (related to Discussion).

The four RUNX consensus binding sites (1-4) are indicated in the figure. This RUNX cluster is highly conserved in primates, in which CD34 is a marker of LT-HSC, whereas in mice the cluster is not conserved, a part from site 2.
Table S1: hCD34+ SLAM+ LSKs provide long term reconstitution (related to Fig. 2)

The table lists percentage of donor-derived long-term reconstitution for each hematopoietic lineage, +/- standard deviation. Six Ly5.2 recipient mice were lethally irradiated and transplanted with hCD34+ SLAM+ LSK cells from mice transgenic for constructs A, B, and E. Mice were transplanted with fifty Ly5.1 SLAM+ LSK cells from each construct. Long-term reconstitution was scored five months after transplantation.

Lethally irradiated mice that did not receive any transplanted bone marrow cells succumbed a few weeks after irradiation. These results demonstrate that LT-HSCs are driving hCD34 expression in transgenic mice that contain the DRE.

<table>
<thead>
<tr>
<th>Transgenic donor constructs</th>
<th>Granulocytes</th>
<th>Monocytes</th>
<th>Erythroid cells</th>
<th>B cells</th>
<th>T cells</th>
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<tbody>
<tr>
<td>A: 25.6 kb 3’</td>
<td>19.3 +/- 11.8</td>
<td>22.1 +/- 8.8</td>
<td>10.5 +/- 4.3</td>
<td>26.6 +/- 13.2</td>
<td>17.6 +/- 7</td>
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<tr>
<td>B: 19.6 kb 3’</td>
<td>23.8 +/- 13.4</td>
<td>18.8 +/- 13.1</td>
<td>12.3 +/- 6.2</td>
<td>23.3 +/- 13.3</td>
<td>19.5 +/- 10.6</td>
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<tr>
<td>E: DRE 3’</td>
<td>21.5 +/- 9.6</td>
<td>18 +/- 10.6</td>
<td>19 +/- 11.5</td>
<td>19.8 +/- 10.4</td>
<td>19 +/- 11.1</td>
</tr>
</tbody>
</table>
Runx1 expression in SLAM+ LSKs

- hCD34+ Runx1 WT
- hCD34+ Runx1 KO

Levantini_Fig. S2
Levantini_Fig. S3

(a) Runx1 expression versus %18S

(b) NE

(c) hCD34 expression versus %18S

(d) Sorting gates

Post-sorting analysis

LSK → SLAM⁺ → hCD34 in SLAM⁺

CD150

CD48

FSC

hCD34

C-kit

sca-1

11.7

7.8

99.4

98.3

98.6

98.6

100

50
Cord Blood

e

unstained  hCD34-stained

46% 39%
Levantini_Fig. S4

(a) Western blot analysis using anti-FLAG and anti-actin antibodies.

(b) EMSA with probes for wt site #3 and mut site #3, and wt site #4 and mut site #4.

Sequences:
- wt site #3: TGGAAGCC TGGGTGAACCT
- wt site #4: GAAGGATGGTGGCATTAT

S 17
<table>
<thead>
<tr>
<th>Construct name</th>
<th>Founder lines</th>
<th>hCD34 in SLAM+ LSKs</th>
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<tbody>
<tr>
<td>G</td>
<td>11</td>
<td>yes</td>
</tr>
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</table>

**Diagram c**

- **PvuI** restriction sites:
  - 18.3 kb
  - 24.8 kb
  - 25.6 kb
- Genes of interest:
  - E-Box/GATA #1
  - E-Box/GATA #2
  - DRE

**Diagram d**

- **LSK**
  - **SLAM**
    - **G**
      - C-Kit
      - sca-1
      - 1.3
    - **CD48**
      - CD150
      - 9.3
    - **FSC**
      - hCD34
      - 98.8

Levantini Fig. S4 continued
GGAGGAGCAGGTATAGAAGGGCCAGCCAGGGGCCTGTTGTGCTCTTTGCAATTGTTGCGAGGGGCTGAACCTCCAGCCCCTTGGGTTTGGTTCCACCCCTGGCCTGTCCCTGGCACTCGACAACTGCTCCTGTGCACT
GATTTGGAGCAGGCCTGGGGCTAGGGGATGGTCAGGGAAGGGTGTACAGGGAAGTAGATCAGACAGCAAAGATAAACATGGTGTTCTTTTACTGTACTCTCCACTGGGCTGTCCCTGGTTACCGGGGCAAACAGAAGCAGT
ATGAAAATCATGCCTTTCGTTCAGTGGAAAAATTTGGCTCCTCCTCCCGGCT

Levantini_Fig. S5