Riz *et al.*, Supplementary Information

Supplementary Results

Derivation of iEBHX1S-4 erythroid progenitor cells from an ESC line with inducible TLX1 expression

We previously reported that enforced expression of *TLX1* in murine ESCs subjected to *in vitro* differentiation frequently results in the immortalization of various myeloerythroid precursors (Keller *et al.*, 1998). Therefore, to further investigate the mechanism of the TLX1-induced differentiation block, we were interested in extending this latter approach using an ESC line in which *TLX1* expression could be reversibly controlled. To obviate the possibility of interfering oncogenes being inadvertently activated by retroviral insertional mutagenesis (Du *et al.*, 2005), which could complicate interpretation of the results, we sought a less genotoxic strategy to achieve this goal. Toward this end, a TLX1 loxP-targeting vector (ploxTLX1) was generated by subcloning a TLX1-IRES-EGFP cassette into the plox plasmid (Kyba *et al.*, 2002) (Supplementary Figure 1a). The "Tet-on" target ESC line Ainv15 was electroporated with ploxTLX1 and the pSalk-Cre Cre recombinase expression plasmid, resulting in site-specific integration of the TLX1-IRES-EGFP cassette downstream of a promoter integrated into the *hprt* locus that is responsive to the tetracycline derivative doxycycline (Supplementary Figure 1b). The ESC lines with doxycycline-inducible *TLX1* expression that were established have been denoted iTLX1, following the nomenclature of Daley and colleagues (Kyba *et al.*, 2002). To determine whether *TLX1*(GFP) expression was reversibly regulated in the iTLX1 ESC line chosen for further study (TG7), the cells were cultured in the presence or absence of 1 μg/ml doxycycline. After 3 days in doxycycline-supplemented medium, GFP expression was detected by fluorescence microscopy (Supplementary Figure 1c) and flow cytometric analysis (Supplementary Figure 1d), and TLX1 expression was detected by Western blot analysis

(Supplementary Figure 1e). More importantly, coordinate down-regulation of TLX1 and GFP expression was observed when iTLX1 cells were subsequently cultured in the absence of doxycycline for 3 days (Supplementary Figure 1d,e). Thus, *TLX1*(GFP) expression is tightly regulated in the iTLX1 ESC line TG7 by the addition and removal of doxycycline.

Embryoid bodies were generated from iTLX1 ESCs essentially as described previously (Keller *et al.*, 1998) except that doxycycline was added at 1 μg/ml on day 4 of differentiation (Supplementary Figure 1f). After an additional 2 days of culture, iTLX1 embryoid bodies were dissociated and assayed for hematopoietic colony formation in methylcellulose-containing cultures supplemented with doxycycline. Colonies that developed were picked, transferred to liquid cultures, and propagated in the presence of interleukin-3 (IL-3), stem cell factor (SCF) and erythropoietin (Epo) (Keller *et al.*, 1998) plus or minus doxycycline. The iEBHX1S-4 clonal cell line was established from an embryoid body-derived hematopoietic cell culture that continued to proliferate (>6 months) in the presence of doxycycline.

Although the iEBHX1S-4 cell line was generated in the presence of IL-3, SCF and Epo, the cells showed no significant growth response to Epo, either alone or in pairwise combinations with IL-3 or SCF when maintained in the presence of doxycycline, but required IL-3 plus SCF for survival and proliferation (Supplementary Figure 1g). However, because the cells express Epo receptor mRNA (EpoR; see below), they are routinely maintained in IL-3, SCF and Epo (Supplementary Figure 2a). Notably, 3 days after removal of doxycycline from iEBHX1S-4 cultures, the majority of the cells became positive after benzidine staining, indicative of hemoglobin synthesis (Supplementary Figure 2b). Addition of bovine serum albumin, insulin and transferrin (Iscove *et al.*, 1980; Kurtz *et al.*, 1983) to doxycycline-minus cultures resulted in a greater percentage of cells exhibiting the features of terminal erythroid maturation, including enucleation (Supplementary Figure 2c). To further investigate the factor requirements of the

iEBHX1S-4 erythroid differentiative response, doxycycline was removed and the cells were cultured in the presence of various combinations of IL-3, SCF and Epo. The results shown in Supplementary Figure 2d demonstrated that Epo was necessary and sufficient for erythroid differentiation of iEBHX1S-4 cells following release of the TLX1-mediated block.

Flow cytometric analysis indicated that a subset of iEBHX1S-4 cells expressed the glycophorin A-associated surface antigen TER119 first detectable around the early proerythroblast stage of differentiation (Kina *et al.*, 2000), the percentage of which increased significantly 3 days after doxycycline withdrawal (Supplementary Figure 2e). Examination of genes known to be involved in erythropoiesis (Cantor and Orkin, 2002) by semi-quantitative RT-PCR showed that iEBHX1S-4 cells expressed mRNAs coding for the SCF receptor (c-Kit), EpoR, c-Myb and GATA-1 (Supplementary Figure 2f). In addition, embryonic (hbb-bH1/βH1) as well as adult (hbb-b1/βmajor) β-globin mRNA could be detected (Supplementary Figure 2f), indicating that the iEBHX1S-4 cell line was of embryonic erythroid origin (Keller *et al.*, 1998). Three days after doxycycline withdrawal, when the cells exhibited a decreased rate of proliferation (data not shown), down-regulation of c-kit and c-myb mRNAs was observed concomitant with up-regulation of Gata-1 transcripts (Supplementary Figure 2f); β-globin mRNA levels were also moderately increased at the 3-day time point (Supplementary Figure 2f). Previous studies have implicated the cyclin-dependent kinase inhibitor $p27^{Kip1}$ but not $p21^{Waf1/Cip1}$ in the growth arrest that accompanies terminal erythroid maturation (Hsieh *et al.*, 2000; Rylski *et al.*, 2003). Steady-state levels of the $p27^{Kip1}$ had significantly increased by 24 hours after doxycycline withdrawal whereas there was no significant increase in the levels of $p21^{Waf1/Cip1}$ at the 24-hour time point (Supplementary Figure 2g). In aggregate, the data suggested that iEBHX1S-4 cells were conditionally-arrested by *TLX1* just prior to or at an early proerythroblast-like stage of erythropoiesis (Zon *et al.*, 1991; Kapur and Zhang, 2001; Koury *et*

al., 2002). iEBHX1S-4 cells thus share properties with GATA-1-null G1E erythroid cells, which proliferate in an SCF-dependent manner and, upon restoration of GATA-1 function, undergo Epo-dependent terminal maturation (Weiss *et al.*, 1997; Hung *et al.*, 1999).

Bioinformatic promoter analysis of conditionally-regulated iEBHX1S-4 gene sets

Over-represented motifs within promoter regions were compiled by three different methods based on transcription factor motif matrices or *ab initio* motif discovery algorithms (Matys *et al.*, 2003; Frith *et al.*, 2004; Mariño-Ramírez *et al.*, 2004; Tharakaraman *et al.*, 2005) (see Materials and Methods). Two motifs, the CCAAT box and the motif recognized by "CAC-binding protein", were specifically over-represented in the promoters of the genes belonging to the up-regulated "Figure 1c subtrees", Ccne1-like, Hba-x-like and Hemgn-like gene sets (Supplementary Tables 8-11) but were not present above random expectation in the promoter regions of the genes belonging to the transiently up-regulated Apobec2-like gene set (Supplementary Table 12) or in the promoters of any of the genes from the two down-regulated gene sets (Supplementary Tables 13 and 14). Both elements have been shown to be important for developmental regulation of various globin genes (Miller and Bieker, 1993; Sabath *et al.*, 1998; Tewari *et al.*, 1998; Asano *et al.*, 1999; Basu *et al.*, 2005). In particular, they were demonstrated to be of functional relevance for *Hba-x*/ζ-globin expression (Sabath *et al.*, 1998). There are a number of transcription factors that recognize a CCAAT core sequence as part of their binding site, including NF-Y (Cohen *et al.*, 1986; Barnhart *et al.*, 1988) and C/EBPγ (Zafarana *et al.*, 2000), which are expressed in iEBHX1S-4 cells. The "CAC-binding protein" motif is recognized by the Krüppel-like transcription factors (KLFs) (Bieker, 2001). EKLF/KLF1 – the first of 16 KLFs to be identified, which is expressed in iEBHX1S-4 cells – recognizes the CAC motif in the β-globin promoter (Miller and Bieker, 1993) and functionally and physically interacts with

GATA-1 (Merika and Orkin, 1995). In addition, KLF1 has been implicated as a regulator of *Hemgn*/hemogen expression (Drissen *et al.*, 2005) and other genes required for the terminal maturation and hemoglobinization of erythroid cells (Coghill *et al.*, 2001; Drissen *et al.*, 2005).

In addition to the CCAAT and CAC-binding protein motifs, a bipartite "E-box-GATA" motif was found to be selectively over-represented in the promoter regions of the up-regulated Ccne1-like and Hba-x-like gene sets (Supplementary Tables 9 and 10). The motif, which consists of an E-box (CAGGTG) followed ~9 bp downstream by a GATA site, is recognized by an erythroid DNA-binding complex containing SCL and GATA-1 linked by LMO2 (Wadman *et al.*, 1997). The SCL-LMO2-GATA-1 complex activates expression of erythroid-lineage genes such as the red cell membrane proteins glycophorin A (recognized by the TER119 antibody) and protein 4.2, during terminal maturation (Xu *et al.*, 2003; Lahlil *et al.*, 2004). Additionally, when the up-regulated "Figure 1c subtrees" gene set was searched for positionally-clustered "word" combinations, the TGATTG sequence was found to cluster ~50 bp upstream of the transcriptional start sites ($P < 0.002$) (Supplementary Table 15). This motif corresponded to an atypical GATA-1 site which is present in the β-globin promoter at -266 (Barnhart *et al.*, 1989).

By comparison, bioinformatic analysis of the promoter regions of the transiently up-regulated Apobec2-like gene set revealed the exclusive over-representation of a Gfi-1b motif (Tong *et al.*, 1998) (Supplementary Table 12). Gfi (growth factor-independence)-1b is a zinc-finger transcriptional repressor that is essential for erythropoiesis and governs the proliferation of erythroid progenitors *in vitro* (Saleque *et al.*, 2002; Garcon *et al.*, 2005; Rodriguez *et al.*, 2005).

A common feature of all of the transcription factors implicated through the bioinformatic analysis is that their transcriptional activity is regulated by CBP (Blobel *et al.*, 1998; Boyes *et al.*, 1998; Huang *et al.*, 1999; Hung *et al.*, 1999; Zhang and Bieker, 1998; Zhang *et al.*, 2001). Specifically, acetylation of GATA-1 (Blobel *et al.*, 1998; Boyes *et al.*, 1998; Hung *et al.*, 1999),

KLF1 (Zhang and Bieker, 1998; Zhang *et al.*, 2001), NF-Y (Faniello *et al.*, 1999; Duan *et al.*, 2001), C/EBP family members (Mink *et al.*, 1997; Kovacs *et al.*, 2003) and SCL (Huang *et al.*, 1999) by CBP has been demonstrated to potentiate their transcriptional activity. Moreover, CBP acetylation of SCL correlates with disruption of SCL-ETO-2-Gfi-1b complexes – implicated in the transcription of the transiently up-regulated Apobec2-like gene set – during erythroid differentiation (Huang *et al.*, 1999; Schuh *et al.*, 2005).

Supplementary Materials and Methods

Cell lines and culture conditions

Mouse ESCs were co-cultured with G418-resistant primary mouse embryonic fibroblasts (Specialty Media, Phillipsburg, NJ) on gelatinized 35 mm tissue culture dishes. ESC maintenance medium consisted of Dulbecco's modified Eagle medium supplemented with 4.5 g/l glucose (Invitrogen Corp., Carlsbad, CA), 15% fetal bovine serum (FBS; BioWhittaker, Walkersville, MD), 10% leukemia inhibitory factor-conditioned medium from Chinese hamster ovary cells transfected with a leukemia inhibitory factor expression vector (Keller *et al.*, 1998), 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM monothioglycerol (Sigma-Aldrich Corp., St. Louis, MO). Cells were passaged every 3 days and maintained (8×10^4 to 1.5×10^6 cells per dish) at 37°C in a humidified atmosphere containing 5% CO_2 (Kyba *et al.*, 2002).

Human 293T embryonic kidney cells (293T/17, CRL-11268; American Type Culture Collection, Manassas, VA) were cultured in DMEM supplemented with 4.5 g/l glucose plus 10% FBS.

Construction of the ploxTLX1 plasmid and generation of the TLX1-inducible ESC line

The ploxTLX1 targeting plasmid was generated by cloning a TLX1-IRES-EGFP *Eco*RI-*Sal*I fragment into corresponding *Eco*RI and *Sal*I sites of the plasmid plox. The doxycycline-inducible targeting ESC line Ainv15 was electroporated (270 V, 960 μF; Gene Pulser II, Bio-Rad Laboratories, Hercules, CA) in the presence of 100 μg ploxTLX1 targeting plasmid and 20 μg pSalk-Cre Cre recombinase expression plasmid in electroporation buffer (Specialty Media). The Ainv15 cell line and the Cre-lox plasmids were gifts from Dr. George Daley (Children's Hospital Boston, Boston, MA) (Kyba *et al.*, 2002). Electroporated ESCs were co-cultured with

G418-resistant primary mouse embryonic fibroblasts in ESC maintenance medium supplemented with 500 μg/ml G418 (Invitrogen Corp.). Individual G418-resistant ESC colonies were picked and expanded, and examined by polymerase chain reaction (PCR) for confirmation of successful integration of the ploxTLX1 targeting plasmid. The primers used for PCR consisted of sequences within the ploxTLX1 targeting plasmid (*pgk* promoter region) and within the *neo* gene in the parental Ainv15 ESC line that would generate a 450 bp product. Primers: LoxinF 5'-CTAGATCTCGAAGGATCTGGAG-3', LoxinR 5'-ATACTTTCTCGGCAGGAGCA-3'. PCR conditions: 10 min at 95^{o}C; 40 cycles of 15 sec at 95^{o}C, 40 sec at 58^{o}C and 1 min at 72^{o}C; 5 min at 72^{o}C.

Embryoid body formation, *hematopoietic cell differentiation and iEBHX1S-4 erythroid progenitor cell line derivation*

Two days prior to embryoid body formation, ESCs were placed in ESC maintenance medium containing Iscove's modified Dulbecco's medium (IMDM; Invitrogen Corp.) instead of Dulbecco's modified Eagle medium. Embryoid body formation was induced as described (Keller *et al.*, 1998), with slight modifications according to a protocol provided by StemCell Technologies (Vancouver, BC, Canada). ESCs were mixed with ES-Cult^{TM} methylcellulose (StemCell Technologies) supplemented with 40 ng/ml SCF (PeproTech, Inc., Rocky Hill, NJ) and 150 μM monothioglycerol, seeded in 35 mm suspension dishes (StemCell Technologies) at a density of 5×10^3 cells/ml, and maintained at 37^{o}C in a humidified atmosphere containing 5% CO_2. Doxycycline (Sigma-Aldrich Corp.) in 0.5 ml ES-CULT medium was added to the dishes at 1 μg/ml on day 4 of differentiation. Embryoid bodies were recovered on day 6 of differentiation in IMDM/2% FBS, centrifuged at 300 *g* for 10 min, and then disaggregated for 3

min in the presence of 0.05% trypsin/EDTA (Invitrogen Corp.). Disaggregated embryoid bodies were passed through a 21G 38 mm needle to produce a single-cell suspension.

Individual disaggregated day-6 embryoid bodies (4×10^4 cells) were plated in methylcellulose-based MethoCult™ GF M3434 medium (StemCell Technologies) containing 50 ng/ml SCF, 10 ng/ml IL-3 (PeproTech, Inc.), 10 ng/ml IL-6 (PeproTech, Inc.), 3 U/ml Epo (Amgen, Thousand Oaks, CA) and 1 μg/ml doxycycline. After 6 days, individual colonies were picked by pipette, washed free of methylcellulose and placed in liquid cultures containing IMDM/10% FBS, 55 μM β-mercaptoethanol (Invitrogen Corp.), 2.5% SCF-conditioned medium from Chinese hamster ovary cells transfected with a SCF expression vector (Hawley *et al.*, 1996), 5% IL-3-conditioned medium from X630-rIL3 cells, 3 U/ml Epo and 1 μg/ml doxycycline.

iEBHX1 was established as a continuously growing cell line from a single colony and single-cell sorted (from 2×10^6 cells) based on GFP expression into 96-well plates containing the above described medium, from which the iEBHX1S-4 clone was obtained (the suffix S denotes "sorted"). Cell sorting was performed on a FACSVantage SE/FACSDiVa (BD Biosciences, San Jose, CA) instrument equipped with a CloneCyt Plus option that provides automated single-cell deposition capability. In order to examine the growth and differentiation potential of the iEBHX1S-4 cell line, its responsiveness to a variety of factors, including IL-3, SCF, Epo, thrombopoietin, IL-5, IL-6, IL-11, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage colony-stimulating factor, vascular endothelial growth factor and basic fibroblast growth factor, was evaluated in suspension and methylcellulose cultures. iEBHX1S-4 cultures were supplemented with 5% BIT (bovine serum albumin, insulin and human transferrin; StemCell Technologies), 1 mM sodium butyrate, 0.5 mM valproic acid or 50 nM trichostatin A (Sigma-Aldrich Corp.) where noted.

Flow cytometric and in situ analyses

Immunofluorescence flow cytometric analysis of iTLX1 TG7 ESCs and iEBHX1S-4 cells was performed using a BD FACSAria flow cytometer (BD Biosciences). Anti-SSEA-1 antibody was obtained from the Developmental Studies Hybridoma Bank, Department of Biological Sciences, University of Iowa (Iowa City, IA). Antibodies to Flk-1, Gr-1 (Ly-6G), Mac-1 (CD11b), CD18, CD41, CD49d (VLA-4), CD62L (L-selectin) and TER119 (Ly-76) were purchased from BD Biosciences Pharmingen (San Diego, CA). 2.4G2 rat anti-mouse CD16/CD32 monoclonal antibody (Mouse BD Fc Block™; BD Biosciences Pharmingen) was used to block FcγIII/II receptors. A rat monoclonal antibody to mouse neutrophils (clone 7/4) was purchased from Caltag Laboratories (Burlingame, CA). iTLX1 TG7 ESCs are SSEA-1^+; iEBHX1S-4 cells express the CD41 surface antigen (Ferkowicz *et al.*, 2003). Benzidine staining was performed by adding 20 µl of benzidine staining solution (500 µl 0.2% benzidine in 3% acetic acid added to 50 µl H_2O_2 immediately prior to staining) to 2×10^4 cells in 200 µl for 8 min.

Microarray profiling and RT-PCR

Microarray profiling was performed in The George Washington University Medical Center Genomics Core Facility essentially as described previously (Krasnoselskaya-Riz *et al.*, 2002; Riz and Hawley, 2005). In brief, total RNA was isolated by TRIzol Reagent (Invitrogen Corp.) and further purified using RNeasy mini spin columns (QIAGEN Inc., Valencia, CA). cDNA synthesis was performed with an HPLC purified T7-(dT)24 primer (Genset Oligos/Proligo LLC, Boulder, CO). Biotin-labeled cRNA synthesis, hybridization, washing, staining and scanning were carried out according to protocols provided by Affymetrix Inc. (Santa Clara, CA). Target cRNA prepared from two independent cultures of each cell line and from two independent cultures of each iEBHX1S-4 differentiation time point was hybridized to Affymetrix MG-

U74Av2 GeneChip oligonucleotide arrays. For all relative expression value comparisons, experimental and control hybridizations were processed simultaneously.

Quality control and low-level analysis of expression data were performed using Microarray Suite 5.0 software (MAS 5.0; Affymetrix). Subsequent data analysis was performed with GeneSpring software (Silicon Genetics/Agilent Technologies, Inc., Palo Alto, CA). Unsupervised hierarchical condition tree, gene tree and K-means clustering were based on standard correlations for log-transformed data averaged for two replicates. Gene annotation queries were conducted through the Affymetrix NetAffx Analysis Center (http://www.affymetrix.com/analysis/index.affx) and gene sets categorized based on Gene Ontology (GO) (Ashburner *et al.*, 2000) information from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). To compare iEBHX1S-4 differentiation expression profiles with published microarray expression data obtained for G1E cells (Welch *et al.*, 2004), profile neighbors were extracted from the NCBI GEO (Gene Expression Omnibus) DataSet (Record Accession Number GDS568) and analyzed in GeneSpring as a list of Affymetrix Probe Set IDs. Gene lists were deposited in the George Washington University List of Lists-Annotated (LOLA) database (http://www.lola.gwu.edu).

The expression profiles of selected genes obtained by microarray analysis were validated by real-time qRT-PCR using an ABI Prism 7000 Sequence Detection System (Krasnoselskaya-Riz *et al.*, 2002). Complementary DNA was synthesized using TaqMan reverse transcriptase and random Hexamers (Applied Biosciences, Branchburg, NJ) and amplified with the following TaqMan probe sets (Applied Biosciences) according to the manufacturer's protocol: Apobec2 (Mm00477588_m1), Ccne1 (Mm00432367_m1), Hba-x (Mm00439255_m1), Hemgn (Mm00519058_m1), and Egr1 (Mm00656724_m1).

For semi-quantitative RT-PCR, RNA isolation was performed using the High Pure RNA Isolation Kit as per the manufacturer's protocol (Roche Diagnostics Corp., Indianapolis, IN). cDNA synthesis was performed by reverse transcription of RNA templates using the TaqMan RT Kit and random Hexamers (Applied Biosciences). PCR was performed using PCR MasterMix (Promega Corp., Madison, WI), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control. Primers specific for c-kit, c-myb, EpoR, GATA-1, βH1-globin, β1-globin and GAPDH were as previously published (Keller *et al.*, 1993). PCR conditions: 10 min at 95^{o}C; 40 cycles of 15 sec at 95^{o}C, 40 sec at 56^{o}C (58 ^{o}C for c-kit and c-myb) and 30 sec at 72^{o}C; 5 min at 72^{o}C.

Statistical identification of putative transcription factor binding sites

Proximal promoter regions of gene sets consisted of nucleotides from -2,000 to +1000 or -2,000 to +300 relative to the corresponding transcriptional start sites, which were identified using information from the Database of Transcriptional Start Sites (DBTSS) (Yamashita *et al.*, 2006) mapped to NCBI mouse genome build 35.1. Over-represented motifs within promoter regions were identified by three different methods: (i) Using Clover (Cis-eLement OVERrepresentation) software (Frith *et al.*, 2004) and position frequency matrices derived from transcription factor binding sites from the TRANSFAC Professional database (Release 9.3) (Matys *et al.*, 2003); (ii) Using an enumerative motif finding algorithm (Mariño-Ramírez *et al.*, 2004), which applies *z*-scores to evaluate the over-representation of "words" with respect to transcriptional start sites; and (iii) Using an enumerative motif finding algorithm that identifies clusters of "words" unusually placed relative to transcriptional start sites (Tharakaraman *et al.*, 2005).

Immunoprecipitations, GST pull-downs and Western blotting

Immunoprecipitations and Western blotting were performed essentially as described previously (Owens *et al.*, 2003; Akimov *et al.*, 2005; Riz and Hawley, 2005). To prepare whole-cell lysates, cells were lysed on ice in RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl , 0.1% SDS, 0.5% Na-deoxycholate, 1 mM PMSF, 1 mM benzamidine, 10 μg/ml leupeptin, 10 μg/ml aprotinin). Insoluble materials were removed by centrifugation at 18,000*g* at 4°C for 20 min. To prepare nuclear lysates, cells were washed once in PBS and once in hypotonic buffer (10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM PMSF, 1 mM DTT), incubated in hypotonic buffer on ice for 15 min and centrifuged at 10,000*g* at 4°C for 10 min. Pelleted nuclei were extracted with NE buffer (20 mM HEPES pH 7.9, 25% glycerol, 0.25 M NaCl, 0.1% NP-40, 5 mM EDTA, 1 mM PMSF, 5 mM DTT) for 30 min on ice and insoluble materials were removed by centrifugation at 14,000 rpm at 4°C for 20 min. Whole-cell or nuclear lysates (500 μg) were precleared with protein A or recombinant protein G agarose beads (Invitrogen Corp.) for rabbit or mouse antibodies, respectively, for 1 hour, incubated at 4°C with 1-2 μg of antibody for 1-2 hours, and then incubated with protein A or protein G beads for an additional hour. Immunoprecipitates from whole-cell lysates were washed three times with RIPA buffer and once with TBS buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl). Immunoprecipitates from nuclear extracts were washed once with NE buffer, once with 0.3% Triton X-100, 75 mM NaCl, 15 mM Tris-HCl pH 7.5, and once with 300 mM NaCl, 15 mM Tris-HCl pH 7.5. Immunoprecipitates were resolved by 4%-20% or 4%-12% gradient SDS-PAGE and transferred to PVDF membranes for Western blotting. Antibodies used: rat anti-GATA-1 (N-1) monoclonal, mouse anti-p27 (F-8) monoclonal, rabbit anti-p21 (c-19) polyclonal (all from Santa Cruz Biotechnology Inc., Santa Cruz, CA), rabbit anti-CBP-NT polyclonal (Upstate USA, Inc., Charlottesville, VA), mouse monoclonal anti-CBP (R&D Systems, Minneapolis, MN), and mouse anti-FLAG-M5

monoclonal antibody (Sigma-Aldrich Co., St Louis, MO). Membrane-bound GATA-1 immunoprecipitates were probed with mouse anti-Ac-K-103 Acetylated Lysine monoclonal antibody (Cell Signaling Technology Inc., Danvers, MA) and rat anti-GATA-1 (N-6) monoclonal antibody (Santa Cruz Biotechnology Inc.). Membranes with $p27^{Kip1}$ and $p21^{Waf1/Cip1}$ immunoprecipitated proteins were probed with mouse anti-p27 (F-8) and anti-p21 (F-5) monoclonal antibodies (Santa Cruz Biotechnology Inc.). To identify GATA-1 and CBP in the CBP-precipitated samples, membranes were probed with rat anti-GATA-1 (N-1) and mouse anti-CBP (C-1) monoclonal antibodies, respectively (Santa Cruz Biotechnology Inc.). TLX1 and α-tubulin were identified with rabbit anti-TLX1 (HOX11 C-18) polyclonal and mouse anti-α-Tubulin (B-7) monoclonal antibodies (Santa Cruz Biotechnology Inc.) after direct immunoblotting of cell lysates. Where noted, control immunoprecipitations were performed using an irrelevant mouse monoclonal antibody recognizing the *Saccharomyces cerevisiae* GAL4 protein (GAL4 DBD RK5C1) (Santa Cruz Biotechnology Inc.).

The glutathione *S*-transferase (GST)-FLAG-TLX1 (full-length TLX1), GST-FLAG-TLX1 D2 (consisting of amino acids 98-330), GST-FLAG-TLX1 D6 (consisting of amino acids 2-260) and GST-FLAG-TLX1 HD (containing an internal deletion from amino acid 201 to amino acid 260) expression constructs were generated by subcloning PCR-amplified FLAG-tagged TLX1 cDNAs (Owens *et al.*, 2003) into the *Eco*RI site of pGEX-4T-1 (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). GST-FLAG-TLX1 fusion proteins expressed in T7 Express bacteria (New England Biolabs, Inc., Ipswich, MA) were purified by adsorption to glutathione-sepharose 4B beads (GE Healthcare Bio-Sciences Corp.) as described (Heidari *et al.*, 2002). After dialysis against PBS (1:500 ratio twice), 0.5 to 1 μg of GST-FLAG-TLX1 and control GST proteins were reapplied to glutathione-sepharose 4B columns and used for pull-down experiments (Berger and Hawley, 1997). Briefly, nuclear lysates (1 mg) from iEBHX1S-4

or 293T cells were incubated with immobilized GST-FLAG-TLX1 and GST proteins for 10 min at 4^{o}C and the columns were washed with 5-10 column volumes of 0.1% Triton X-100, 75 mM NaCl, 15 mM Tris-HCl pH 7.5, then with 0.3% Triton X-100, 75 mM NaCl, 15 mM Tris-HCl pH 7.5, and finally with PBS. Protein complexes were eluted with 2 column volumes of 10 mM glutathione, 50 mM Tris-HCl pH 8.0, and analyzed by Western blotting with mouse monoclonal anti-CBP (R&D Systems) and rabbit anti-GST (Z-5) (Santa Cruz Biotechnology Inc.) antibodies as described above. In pull-down experiments of endogenous CBP in iEBHX1S-4 cells, the eluates were concentrated 10x using Amicon Centricon YM-30 filters (Millipore Corp., Billerica, MA) prior to analysis by Western blotting.

Signal detection and analysis were carried out with alkaline phosphatase-conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) and the ECF Western Blotting Kit (GE Healthcare Bio-Sciences Corp.) on a Storm 860 PhosphorImager equipped with ImageQuant software (GE Healthcare Bio-Sciences Corp.).

Transient transfections

293T cells were seeded at a density of 5.0×10^5 cells/well in six-well plates. Six to twenty-four hours after seeding, the cells were cotransfected with the pRc/RSV-mCBP-HA mouse CBP expression vector (a gift from Dr. Richard Goodman, Oregon Health and Science University, Portland, OR) (Chrivia *et al.*, 1993; Kwok *et al.*, 1994), the pCMV2N3T-CBP human CBP expression vector (a gift from Dr. Annick Harel-Bellan, Institut Andre Lwoff, Villejuif, France) (Ramirez *et al.,* 1997) and/or the pcDNA-FLAG-TLX1 expression vector as described previously (Owens *et al.*, 2003; Riz and Hawley, 2005).

Confocal laser scanning microscopy and image analysis

iEBHX1S-4 cells (2.5 x 10^5) cultured in the presence or absence of 1 μg/ml doxycycline were centrifuged onto a microscope slide at 1000 rpm for 5 minutes using a Shandon Cytospin 4 instrument. The cells were then immediately fixed in 3.7% formaldehyde for 5 minutes at room temperature, rinsed with PBS, and permeabilized with 0.5% Triton X-100 in PBS for 15 minutes at room temperature. Following permeabilization, the cells were rinsed with PBS and blocked in PBS containing 10% FBS and 0.1% Triton X-100 for 1 hour at room temperature. The cells were then incubated with of rabbit polyclonal anti-TLX1 (HOX11 C-18) (Santa Cruz Biotechnology Inc.) and mouse monoclonal anti-CBP (R&D Systems) antibodies diluted to final concentrations of 0.4 μg/ml and 1.0 μg/ml, respectively, in PBS containing 1% FBS and 0.01% Triton X-100 for 1 hour at room temperature. The other antibodies used for staining and the concentration they were used at are as follows: rabbit anti-dimethyl-histone H3 (Lys9) diluted 1:250, rabbit anti-trimethyl-histone H3 (Lys9) diluted 1:250, mouse anti-HP1α at a final concentration of 2.3 μg/ml, and rabbit anti-CBP-NT diluted 1:500 (all from Upstate USA, Inc.). All antibodies were diluted in 1% FBS and 0.01% Triton X-100. The cells were rinsed with PBS and then incubated with Alexa Fluor 568-conjugated goat anti-rabbit and Alexa Fluor 488-conjugated goat anti-mouse secondary antibodies (Invitrogen Corp.) diluted 1:500 in PBS containing 1% FBS and 0.01% Triton X-100 for 1 hour at room temperature. The cells were rinsed with PBS and mounted with Fluoromount G (Electron Microscopy Science, Hatfield PA). 293T cells cultured in six-well plates containing fibronectin-coated coverslips (BD Biosciences) (Owens *et al.*, 2003) were transiently transfected with pcDNA-FLAG-TLX1, pRc/RSV-mCBP-HA or both expression vectors as described above. The total amount of DNA in each transfection was kept constant by the addition of an appropriate amount of the pcDNA3 plasmid. After 36 hours, the cells were processed as above for iEBHX1S-4 cells, except that the rabbit polyclonal anti-TLX1 antibody

was diluted to a final concentration of 0.133 µg/ml and the secondary antibodies were diluted to a final concentration of 1:1000. The slides were mounted with Prolong Antifade mounting medium (Invitrogen Corp.). The slides were sealed and images were acquired using the 60× oil immersion objective of a Bio-Rad MRC-1024 confocal laser scanning microscope equipped with an argon-krypton ion laser and LaserSharp 2000 software (Carl Zeiss MicroImaging, Inc., Thornwood, NY).

Confocal images were analyzed using Image-Pro Plus (Media Cybernetics, Silver Spring, MD). Quantification of TLX1 and CBP nuclear distribution in transfected 293T cells was obtained by drawing a line scan extending through the longest axis of the nucleus in the central slice of the image stack. The intensity of the pixels along the line was recorded and used to generate a histogram of signal intensity across the nucleus (Popratiloff *et al.*, 2003). Images were collected under identical conditions and signal intensity maps of the portions of the line scans outside the nuclei were compared to confirm that the level of background staining was consistent among images. Based on the number of pixels, the nucleus was divided into five sections of equal length. The average signal intensity of each section was calculated (n = 5 images per experimental group) and the unpaired Student's t-test was used to determine if there was a statistically significant difference between the TLX1 and/or CBP signal intensities in the peripheral sections versus the central sections of the nuclei (Ragoczy *et al.*, 2006).

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Supplementary Table 1

Genes analyzed from the Figure 1c subtrees of induced transcripts based on Gene Ontology (GO) "Transcription" group categorization

Affymetrix Probe Set ID	*Name*	*Gene*	*Entrez Gene*
100023_at	myeloblastosis oncogene-like 2	Mybl2	17865 Entrez gene
100050_at	inhibitor of DNA binding 1	Id1	15901 Entrez gene
100062_at	minichromosome maintenance deficient 3 (S. cerevisiae)	Mcm3	17215 Entrez gene
100148_at	CCCTC-binding factor	Ctcf	13018 Entrez gene
100156_at	minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)	Mcm5	17218 Entrez gene
100405_at	chromobox homolog 3 (Drosophila HP1 gamma)	Cbx3	12417 Entrez gene
100441_s_at	ankyrin 1, erythroid	Ank1	11733 Entrez gene
100451_at	heat shock factor 1	Hsf1	15499 Entrez gene
100461_at	polymerase (RNA) II (DNA directed) polypeptide J	Polr2j	20022 Entrez gene
100553_at	tripartite motif protein 27	Trim27	19720 Entrez gene
100616_at	centromere autoantigen A	Cenpa	12615 Entrez gene
100941_at	protein inhibitor of activated STAT 2	Pias2	17344 Entrez gene
100984_at	activating transcription factor 1	Atf1	11908 Entrez gene
101382_at	pre B-cell leukemia transcription factor 2	Pbx2	18515 Entrez gene
101445_at	DNA methyltransferase (cytosine-5) 1	Dnmt1	13433 Entrez gene
101465_at	signal transducer and activator of transcription 1	Stat1	20846 Entrez gene
101475_at	polycomb group ring finger 4	Pcgf4	12151 Entrez gene

101589_at	high mobility group nucleosomal binding domain 2	Hmgn2	15331 Entrez gene
101920_at	polymerase (DNA directed), epsilon 2 (p59 subunit)	Pole2	18974 Entrez gene
101930_at	nuclear factor I/X	Nfix	18032 Entrez gene
101958_f_at	transcription factor Dp 1	Tfdp1	21781 Entrez gene
101959_r_at	transcription factor Dp 1	Tfdp1	21781 Entrez gene
102054_at	ankyrin repeat and FYVE domain containing 1	Ankfy1	11736 Entrez gene
102242_at	period homolog 3 (Drosophila)	Per3	18628 Entrez gene
102382_at	aryl hydrocarbon receptor nuclear translocator-like	Arntl	11865 Entrez gene
102631_at	Bloom syndrome homolog (human)	Blm	12144 Entrez gene
102654_at	GATA binding protein 1	Gata1	14460 Entrez gene
102893_at	POU domain, class 2, transcription factor 1	Pou2f1	18986 Entrez gene
102963_at	E2F transcription factor 1	E2f1	13555 Entrez gene
102976_at	breast cancer 1	Brca1	12189 Entrez gene
103057_at	polymerase (DNA directed), delta 1, catalytic subunit	Pold1	18971 Entrez gene
103207_at	polymerase (DNA directed), alpha 1	Pola1	18968 Entrez gene
103285_at	methyl-CpG binding domain protein 4	Mbd4	17193 Entrez gene
103546_at	fos-like antigen 2	Fosl2	14284 Entrez gene
103598_at	DEAH (Asp-Glu-Ala-His) box polypeptide 9	Dhx9	13211 Entrez gene
104220_at	MAD homolog 6 (Drosophila)	Smad6	17130 Entrez gene
104476_at	retinoblastoma-like 1 (p107)	Rbl1	19650 Entrez gene
104644_at	kinesin family member 4	Kif4	16571

			Entrez gene
92199_at	signal transducer and activator of transcription 5B	Stat5b	20851 Entrez gene
92219_s_at	midline 1	Mid1	17318 Entrez gene
92234_at	retinoid X receptor alpha	Rxra	20181 Entrez gene
92235_g_at	retinoid X receptor alpha	Rxra	20181 Entrez gene
92244_at	exonuclease 1	Exo1	26909 Entrez gene
92257_at	circadian locomoter output cycles kaput	Clock	12753 Entrez gene
92468_at	ankyrin repeat domain 49	Ankrd49	56503 Entrez gene
92882_at	RAB1, member RAS oncogene family	Rab1	19324 Entrez gene
92933_at	POU domain, class 2, transcription factor 3	Pou2f3	18988 Entrez gene
93023_f_at	histone 1, H3g histone 1, H3f histone1, H3c histone1, H3d histone 1, H3b histone 1, H3e histone 1, H3h histone 1, H3i histone 1, H3a	Hist1h3g Hist1h3f Hist1h3c Hist1h3d Hist1h3b Hist1h3e Hist1h3h Hist1h3i Hist1h3a	260423 Entrez gene 319148 Entrez gene 319149 Entrez gene 319150 Entrez gene 319151 Entrez gene 319152 Entrez gene 319153 Entrez gene 360198 Entrez gene 97908 Entrez gene
93041_at	minichromosome maintenance deficient 4 homolog (S. cerevisiae)	Mcm4	17217 Entrez gene
93095_at	high mobility group box 1	Hmgb1	15289 Entrez gene
93112_at	minichromosome maintenance deficient 2 mitotin (S. cerevisiae)	Mcm2	17216 Entrez gene

93250_r_at	high mobility group box 2 similar to high mobility group protein B2 similar to high mobility group protein B2 similar to high mobility group protein B2 similar to high mobility group protein B2	Hmgb2 LOC433785 LOC433788 LOC433799 LOC545710	433785 Entrez gene 433788 Entrez gene 433799 Entrez gene 545710 Entrez gene 97165 Entrez gene
93356_at	minichromosome maintenance deficient 7 (S. cerevisiae)	Mcm7	17220 Entrez gene
93406_at	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein G (avian)	Mafg	17134 Entrez gene
93413_at	telomeric repeat binding factor 2	Terf2	21750 Entrez gene
93699_at	polymerase (DNA directed), gamma 2, accessory subunit	Polg2	50776 Entrez gene
93708_at	protein inhibitor of activated STAT 3	Pias3	229615 Entrez gene
94031_at	RAB2, member RAS oncogene family	Rab2	59021 Entrez gene
94467_at	CCAAT/enhancer binding protein zeta	Cebpz	12607 Entrez gene
94521_at	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	Cdkn2d	12581 Entrez gene
94709_at	glial cells missing homolog 2 (Drosophila)	Gcm2	107889 Entrez gene
94969_at	nuclear transcription factor-Y gamma	Nfyc	18046 Entrez gene
95479_at	nuclear DNA binding protein	MGI:1927354	57316 Entrez gene
95755_at	cold shock domain protein A	Csda	56449 Entrez gene
95795_at	suppressor of Ty 4 homolog 2 (S. cerevisiae)	Supt4h2	20923 Entrez gene
96084_at	heterogeneous nuclear ribonucleoprotein D-like	Hnrpdl	50926 Entrez gene
96192_at	trans-acting transcription factor 3	Sp3	20687 Entrez gene
96651_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	Smarce1	57376 Entrez gene

97327_at	flap structure specific endonuclease 1	Fen1	14156 Entrez gene
97789_at	forkhead box H1	Foxh1	14106 Entrez gene
97965_at	phospholipase A2, group VI	Pla2g6	53357 Entrez gene
98024_at	nuclear transcription factor-Y beta	Nfyb	18045 Entrez gene
98032_at	zinc finger protein 35	Zfp35	22694 Entrez gene
98289_at	E74-like factor 4 (ets domain transcription factor)	Elf4	56501 Entrez gene
98469_at	aurora kinase B	Aurkb	20877 Entrez gene
98770_at	centromere autoantigen C1	Cenpc1	12617 Entrez gene
99095_at	Max protein	Max	17187 Entrez gene
99486_at	centromere autoantigen B	Cenpb	12616 Entrez gene
99558_at	cyclin C	Ccnc	51813 Entrez gene
99564_at	ubiquitin-like, containing PHD and RING finger domains, 1	Uhrf1	18140 Entrez gene
99578_at	topoisomerase (DNA) II alpha	Top2a	21973 Entrez gene
99587_at	RAB7, member RAS oncogene family	Rab7	19349 Entrez gene
99908_at	paired box gene 4	Pax4	18506 Entrez gene
99917_at	enhancer of zeste homolog 2 (Drosophila)	Ezh2	14056 Entrez gene
99932_at	zinc finger and BTB domain containing 17	Zbtb17	22642 Entrez gene

Supplementary Table 2

Genes analyzed from the Figure 1c set of Ccne1-like profile neighbors of induced transcripts based on GO "Transcription" group categorization

Affymetrix Probe Set ID	*Name*	*Gene*	*Entrez Gene*
100050_at	inhibitor of DNA binding 1	Id1	15901 Entrez gene
100452_at	Kruppel-like factor 1 (erythroid)	Klf1	16596 Entrez gene
100461_at	polymerase (RNA) II (DNA directed) polypeptide J	Polr2j	20022 Entrez gene
100616_at	centromere autoantigen A	Cenpa	12615 Entrez gene
100941_at	protein inhibitor of activated STAT 2	Pias2	17344 Entrez gene
100984_at	activating transcription factor 1	Atf1	11908 Entrez gene
101382_at	pre B-cell leukemia transcription factor 2	Pbx2	18515 Entrez gene
101475_at	polycomb group ring finger 4	Pcgf4	12151 Entrez gene
101589_at	high mobility group nucleosomal binding domain 2	Hmgn2	15331 Entrez gene
101888_at	RAR-related orphan receptor alpha	Rora	19883 Entrez gene
101930_at	nuclear factor I/X	Nfix	18032 Entrez gene
101958_f_at	transcription factor Dp 1	Tfdp1	21781 Entrez gene
101959_r_at	transcription factor Dp 1	Tfdp1	21781 Entrez gene
102242_at	period homolog 3 (Drosophila)	Per3	18628 Entrez gene
102654_at	GATA binding protein 1	Gata1	14460 Entrez gene
102819_at	nucleosome assembly protein 1-like 2	Nap1l2	17954 Entrez gene
102963_at	E2F transcription factor 1	E2f1	13555 Entrez gene

103285_at	methyl-CpG binding domain protein 4	Mbd4	17193 Entrez gene
103546_at	fos-like antigen 2	Fosl2	14284 Entrez gene
103598_at	DEAH (Asp-Glu-Ala-His) box polypeptide 9	Dhx9	13211 Entrez gene
104220_at	MAD homolog 6 (Drosophila)	Smad6	17130 Entrez gene
92235_g_at	retinoid X receptor alpha	Rxra	20181 Entrez gene
92244_at	exonuclease 1	Exo1	26909 Entrez gene
92468_at	ankyrin repeat domain 49	Ankrd49	56503 Entrez gene
93089_at	eukaryotic translation initiation factor 4A2	Eif4a2	13682 Entrez gene
93129_at	cut-like 2 (Drosophila)	Cutl2	13048 Entrez gene
93250_r_at	high mobility group box 2 similar to high mobility group protein B2 similar to high mobility group protein B2 similar to high mobility group protein B2 similar to high mobility group protein B2	Hmgb2 LOC433785 LOC433788 LOC433799 LOC545710	433785 Entrez gene 433788 Entrez gene 433799 Entrez gene 545710 Entrez gene 97165 Entrez gene
93708_at	protein inhibitor of activated STAT 3	Pias3	229615 Entrez gene
94031_at	RAB2, member RAS oncogene family	Rab2	59021 Entrez gene
94521_at	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	Cdkn2d	12581 Entrez gene
95791_s_at	splicing factor, arginine/serine-rich 2 (SC-35)	Sfrs2	20382 Entrez gene
95795_at	suppressor of Ty 4 homolog 2 (S. cerevisiae)	Supt4h2	20923 Entrez gene
96050_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	Smarcb1	20587 Entrez gene
96084_at	heterogeneous nuclear ribonucleoprotein D-like	Hnrpdl	50926 Entrez gene

96192_at	trans-acting transcription factor 3	Sp3	20687 Entrez gene
97327_at	flap structure specific endonuclease 1	Fen1	14156 Entrez gene
97789_at	forkhead box H1	Foxh1	14106 Entrez gene
97965_at	phospholipase A2, group VI	Pla2g6	53357 Entrez gene
98002_at	interferon regulatory factor 8	Irf8	15900 Entrez gene
98032_at	zinc finger protein 35	Zfp35	22694 Entrez gene
98038_at	high mobility group box 3	Hmgb3	15354 Entrez gene
98469_at	aurora kinase B	Aurkb	20877 Entrez gene
98981_s_at	transcription factor 12	Tcf12	21406 Entrez gene
99486_at	centromere autoantigen B	Cenpb	12616 Entrez gene
99587_at	RAB7, member RAS oncogene family	Rab7	19349 Entrez gene
99932_at	zinc finger and BTB domain containing 17	Zbtb17	22642 Entrez gene

Supplementary Table 3

Genes analyzed from the Figure 1c set of Hba-x-like profile neighbors of induced transcripts based on GO "Transcription" group categorization

Affymetrix Probe Set ID	*Name*	*Gene*	*Entrez Gene*
100156_at	minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)	Mcm5	17218 Entrez gene
100405_at	chromobox homolog 3 (Drosophila HP1 gamma)	Cbx3	12417 Entrez gene
100701_r_at	nuclear receptor subfamily 5, group A, member 1	Nr5a1	26423 Entrez gene
101305_at	POU domain, class 3, transcription factor 3	Pou3f3	18993 Entrez gene
101310_at	T-cell acute lymphocytic leukemia 2	Tal2	21350 Entrez gene
102614_at	prospero-related homeobox 1	Prox1	19130 Entrez gene
102715_at	nuclear receptor subfamily 2, group F, member 1	Nr2f1	13865 Entrez gene
102882_at	zinc finger protein 46	Zfp46	22704 Entrez gene
103204_r_at	E2F transcription factor 8	E2f8	108961 Entrez gene
103990_at	FBJ osteosarcoma oncogene B	Fosb	14282 Entrez gene
104292_at	eyes absent 2 homolog (Drosophila)	Eya2	14049 Entrez gene
104408_s_at	SRY-box containing gene 18	Sox18	20672 Entrez gene
160109_at	SRY-box containing gene 4	Sox4	20677 Entrez gene
161279_f_at	Ring finger protein 12, mRNA (cDNA clone MGC:13712 IMAGE:4193003)	Rnf12	19820 Entrez gene
161340_r_at	paired-like homeodomain transcription factor 2	Pitx2	18741 Entrez gene
161858_f_at	retinoblastoma 1	Rb1	19645 Entrez gene
161965_r_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	Smarcd2	83796 Entrez gene

92249_g_at	nuclear receptor subfamily 4, group A, member 2	Nr4a2	18227 Entrez gene
92275_at	transcription factor AP-2, gamma	Tcfap2c	21420 Entrez gene
92299_at	jumonji, AT rich interactive domain 1A (Rbp2 like)	Jarid1a	214899 Entrez gene
92342_at	SRY-box containing gene 1	Sox1	20664 Entrez gene
92705_at	T-box 2	Tbx2	21385 Entrez gene
92781_at	thymopoietin	Tmpo	21917 Entrez gene
92926_at	myeloproliferative leukemia virus oncogene	Mpl	17480 Entrez gene
92933_at	POU domain, class 2, transcription factor 3	Pou2f3	18988 Entrez gene
93041_at	minichromosome maintenance deficient 4 homolog (S. cerevisiae)	Mcm4	17217 Entrez gene
93095_at	high mobility group box 1	Hmgb1	15289 Entrez gene
93356_at	minichromosome maintenance deficient 7 (S. cerevisiae)	Mcm7	17220 Entrez gene
93468_at	AF4/FMR2 family, member 1	Aff1	17355 Entrez gene
94128_at	BRCA1 associated RING domain 1	Bard1	12021 Entrez gene
94212_at	TSC22 domain family 3	Tsc22d3	14605 Entrez gene
94467_at	CCAAT/enhancer binding protein zeta	Cebpz	12607 Entrez gene
94709_at	glial cells missing homolog 2 (Drosophila)	Gcm2	107889 Entrez gene
95673_s_at	Brain abundant, membrane attached signal protein 1 (Basp1), mRNA	Basp1	70350 Entrez gene
96500_at	GATA binding protein 5	Gata5	14464 Entrez gene
97555_at	chromobox homolog 7	Cbx7	52609 Entrez gene
97777_at	NK2 transcription factor related, locus 5 (Drosophila)	Nkx2-5	18091 Entrez gene
97996_at	poly (A) polymerase alpha	Papola	18789 Entrez

			gene
98073_at	cut-like 1 (Drosophila)	Cutl1	13047 Entrez gene
98839_at	sine oculis-related homeobox 2 homolog (Drosophila)	Six2	20472 Entrez gene
99385_at	POU domain, class 3, transcription factor 2	Pou3f2	18992 Entrez gene
99808_at	homeo box B13	Hoxb13	15408 Entrez gene

Supplementary Table 4

Genes analyzed from the Figure 1c set of Hemgn-like profile neighbors of induced transcripts based on GO "Transcription" group categorization

Affymetrix Probe Set ID	*Name*	*Gene*	*Entrez Gene*
101305_at	POU domain, class 3, transcription factor 3	Pou3f3	18993 Entrez gene
101310_at	T-cell acute lymphocytic leukemia 2	Tal2	21350 Entrez gene
102643_at	homeo box A2	Hoxa2	15399 Entrez gene
102715_at	nuclear receptor subfamily 2, group F, member 1	Nr2f1	13865 Entrez gene
102882_at	zinc finger protein 46	Zfp46	22704 Entrez gene
103204_r_at	E2F transcription factor 8	E2f8	108961 Entrez gene
103229_at	nuclear receptor coactivator 1	Ncoa1	17977 Entrez gene
103990_at	FBJ osteosarcoma oncogene B	Fosb	14282 Entrez gene
104292_at	eyes absent 2 homolog (Drosophila)	Eya2	14049 Entrez gene
104408_s_at	SRY-box containing gene 18	Sox18	20672 Entrez gene
160109_at	SRY-box containing gene 4	Sox4	20677 Entrez gene
161340_r_at	paired-like homeodomain transcription factor 2	Pitx2	18741 Entrez gene
161521_at	protein phosphatase 1, regulatory (inhibitor) subunit 12C	Ppp1r12c	232807 Entrez gene
161858_f_at	retinoblastoma 1	Rb1	19645 Entrez gene
161965_r_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	Smarcd2	83796 Entrez gene
162016_f_at	forkhead box C2	Foxc2	14234 Entrez gene
92249_g_at	nuclear receptor subfamily 4, group A, member 2	Nr4a2	18227 Entrez gene

92342_at	SRY-box containing gene 1	Sox1	20664 Entrez gene
92697_at	forkhead box A1	Foxa1	15375 Entrez gene
92705_at	T-box 2	Tbx2	21385 Entrez gene
92781_at	thymopoietin	Tmpo	21917 Entrez gene
92926_at	myeloproliferative leukemia virus oncogene	Mpl	17480 Entrez gene
92933_at	POU domain, class 2, transcription factor 3	Pou2f3	18988 Entrez gene
92935_at	CBFA2T1 identified gene homolog (human)	Cbfa2t1h	12395 Entrez gene
93616_g_at	pre B-cell leukemia transcription factor 3	Pbx3	18516 Entrez gene
97123_at	nuclear receptor subfamily 0, group B, member 2	Nr0b2	23957 Entrez gene
97704_at	elongation factor RNA polymerase II 2	Ell2	192657 Entrez gene
97777_at	NK2 transcription factor related, locus 5 (Drosophila)	Nkx2-5	18091 Entrez gene
97937_at	Kruppel-like factor 5	Klf5	12224 Entrez gene
98073_at	cut-like 1 (Drosophila)	Cutl1	13047 Entrez gene
99527_at	nuclear factor, erythroid derived 2, like 3	Nfe2l3	18025 Entrez gene

Supplementary Table 5

Genes analyzed from the Figure 1c set of Apobec2-like profile neighbors of induced transcripts based on GO "Transcription" group categorization

Affymetrix Probe Set ID	*Name*	*Gene*	*Entrez Gene*
100301_at	estrogen related receptor, beta	Esrrb	26380 Entrez gene
101059_at	necdin	Ndn	17984 Entrez gene
101704_at	hepatocyte nuclear factor 4, gamma	Hnf4g	30942 Entrez gene
102235_at	lung carcinoma myc related oncogene 1	Lmyc1	16918 Entrez gene
102580_r_at	homeo box A6	Hoxa6	15403 Entrez gene
102716_at	eyes absent 3 homolog (Drosophila)	Eya3	14050 Entrez gene
103050_at	transcription factor 21	Tcf21	21412 Entrez gene
103761_at	transcription factor CP2-like 1	Tcfcp2l1	81879 Entrez gene
104494_at	RIKEN cDNA 4921515A04 gene	4921515A04Rik	268301 Entrez gene
104506_at	nuclear receptor subfamily 1, group I, member 3	Nr1i3	12355 Entrez gene
104592_i_at	myocyte enhancer factor 2C	Mef2c	17260 Entrez gene
160615_at	protein inhibitor of activated STAT 3	Pias3	229615 Entrez gene
161798_r_at	general transcription factor IIIC, polypeptide 4	Gtf3c4	269252 Entrez gene
92334_at	transcriptional regulator protein	MGI:1927479	57230 Entrez gene
92445_at	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	Cacna1a	12286 Entrez gene
92469_at	secreted frizzled-related sequence protein 4	Sfrp4	20379 Entrez gene
92726_at	SRY-box containing gene 6	Sox6	20679 Entrez gene

92753_at	myeloid ecotropic viral integration site-related gene 2	Mrg2	17537 Entrez gene
92990_at	zinc finger protein 93	Zfp93	22755 Entrez gene
93075_r_at	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	Nfatc2	18019 Entrez gene
94203_at	RIKEN cDNA A630025C20 gene	A630025C20Rik	212391 Entrez gene
96507_at	even skipped homeotic gene 2 homolog	Evx2	14029 Entrez gene
96994_at	hypermethylated in cancer 1	Hic1	15248 Entrez gene
97159_at	autoimmune regulator (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy)	Aire	11634 Entrez gene
97745_at	homeo box A4	Hoxa4	15401 Entrez gene
98821_at	insulin promoter factor 1, homeodomain transcription factor	Ipf1	18609 Entrez gene
99900_at	cone-rod homeobox containing gene	Crx	12951 Entrez gene

Supplementary Table 6

Genes analyzed from the set of 6-hour down-regulated transcripts obtained by K-means clustering of the expression profiling data based on GO "Transcription" group categorization

Affymetrix Probe Set ID	*Name*	*Gene*	*Entrez Gene*
101162_at	myogenic factor 5	Myf5	17877 Entrez gene
101396_at	transcription factor 2	Tcf2	21410 Entrez gene
101904_at	SET and MYND domain containing 1	Smyd1	12180 Entrez gene
102048_at	ankyrin repeat domain 1 (cardiac muscle)	Ankrd1	107765 Entrez gene
102087_at	homeo box A3	Hoxa3	15400 Entrez gene
102579_f_at	homeo box A6	Hoxa6	15403 Entrez gene
102668_at	peroxisome proliferator activated receptor alpha	Ppara	19013 Entrez gene
102955_at	nuclear factor, interleukin 3, regulated	Nfil3	18030 Entrez gene
103283_at	E74-like factor 5	Elf5	13711 Entrez gene
104240_at	cut-like 1 (Drosophila)	Cutl1	13047 Entrez gene
104590_at	myocyte enhancer factor 2C	Mef2c	17260 Entrez gene
160604_at	forkhead box C1	Foxc1	17300 Entrez gene
160731_at	RAB25, member RAS oncogene family	Rab25	53868 Entrez gene
161418_r_at	nuclear receptor subfamily 5, group A, member 1	Nr5a1	26423 Entrez gene
161584_r_at	E74-like factor 1	Elf1	13709 Entrez gene
162314_at	CCR4-NOT transcription complex, subunit 7	Cnot7	18983 Entrez gene
92248_at	nuclear receptor subfamily 4, group A, member 2	Nr4a2	18227 Entrez gene

92255_at	homeo box B4	Hoxb4	15412 Entrez gene
92344_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3	Smarca3	20585 Entrez gene
92535_at	early B-cell factor 1	Ebf1	13591 Entrez gene
92614_at	inhibitor of DNA binding 3	Id3	15903 Entrez gene
92717_at	neurogenic differentiation 1	Neurod1	18012 Entrez gene
92883_at	RAB1, member RAS oncogene family	Rab1	19324 Entrez gene
92891_f_at	homeo box C9	Hoxc9	15427 Entrez gene
92894_s_at	nuclear factor I/A	Nfia	18027 Entrez gene
92896_s_at	single-minded homolog 2 (Drosophila) similar to single-minded 2 protein similar to single-minded 2 protein	Sim2 LOC547289 LOC547335	20465 Entrez gene 547289 Entrez gene 547335 Entrez gene
92902_at	myeloblastosis oncogene-like 1	Mybl1	17864 Entrez gene
92999_at	sine oculis-related homeobox 4 homolog (Drosophila)	Six4	20474 Entrez gene
93230_at	neurogenin 1	Neurog1	18014 Entrez gene
93371_at	basonuclin 1	Bnc1	12173 Entrez gene
93418_g_at	myocyte enhancer factor 2B	Mef2b	17259 Entrez gene
93613_at	MAD homolog 3 (Drosophila)	Smad3	17127 Entrez gene
94100_s_at	transient receptor potential cation channel, subfamily C, member 4	Trpc4	22066 Entrez gene
94636_at	homeo box A13	Hoxa13	15398 Entrez gene
94710_g_at	glial cells missing homolog 2 (Drosophila)	Gcm2	107889 Entrez gene
95391_at	ventral anterior homeobox containing gene 1	Vax1	22326 Entrez

			gene
95584_at	developmental pluripotency associated 2	Dppa2	73703 Entrez gene
95968_at	expressed sequence C77691	C77691	97222 Entrez gene
97357_at	myocyte enhancer factor 2C	Mef2c	17260 Entrez gene
98780_at	homeo box B3	Hoxb3	15410 Entrez gene
99058_at	high mobility group AT-hook 2	Hmga2	15364 Entrez gene
99427_at	homeo box D12	Hoxd12	15432 Entrez gene
99432_at	cytoplasmic linker 2	Cyln2	269713 Entrez gene
99965_at	vitamin D receptor	Vdr	22337 Entrez gene

Supplementary Table 7

Genes analyzed from the set of 12-hour down-regulated transcripts obtained by K-means clustering of the expression profiling data based on GO "Transcription" group categorization

Affymetrix Probe Set ID	*Name*	*Gene*	*Entrez Gene*
100130_at	Jun oncogene	Jun	16476 Entrez gene
100962_at	Ngfi-A binding protein 2	Nab2	17937 Entrez gene
101797_at	sine oculis-related homeobox 6 homolog (Drosophila)	Six6	20476 Entrez gene
102984_g_at	MAD homolog 1 (Drosophila)	Smad1	17125 Entrez gene
103925_at	myeloid/lymphoid or mixed lineage-leukemia translocation to 3 homolog (Drosophila)	Mllt3	70122 Entrez gene
104233_at	TEA domain family member 1	Tead1	21676 Entrez gene
104376_at	histone deacetylase 5	Hdac5	15184 Entrez gene
104381_at	nuclear receptor subfamily 1, group H, member 3	Nr1h3	22259 Entrez gene
104462_at	hypermethylated in cancer 1	Hic1	15248 Entrez gene
104698_at	GATA binding protein 6	Gata6	14465 Entrez gene
160901_at	FBJ osteosarcoma oncogene	Fos	14281 Entrez gene
161113_at	estrogen receptor 1 (alpha)	Esr1	13982 Entrez gene
161383_r_at	TEA domain family member 1	Tead1	21676 Entrez gene
161390_r_at	POU domain, class 6, transcription factor 1	Pou6f1	19009 Entrez gene
161468_f_at	Heart and neural crest derivatives expressed transcript 1 (Hand1), mRNA	Hand1	15110 Entrez gene
161699_i_at	interferon regulatory factor 6	Irf6	54139 Entrez gene
161994_f_at	protein inhibitor of activated STAT 3	Pias3	229615 Entrez gene
92186_at	aristaless related homeobox gene (Drosophila)	Arx	11878 Entrez

			gene
92667_at	androgen receptor	Ar	11835 Entrez gene
92910_at	aryl hydrocarbon receptor nuclear translocator 2	Arnt2	11864 Entrez gene
92930_at	distal-less homeobox 5	Dlx5	13395 Entrez gene
92974_at	zinc finger protein 37	Zfp37	22696 Entrez gene
93142_at	BTB and CNC homology 1	Bach1	12013 Entrez gene
93350_f_at	zinc finger protein 422	Zfp422	67255 Entrez gene
93528_s_at	Kruppel-like factor 9	Klf9	16601 Entrez gene
93601_at	kelch-like ECH-associated protein 1	Keap1	50868 Entrez gene
93657_at	Spi-B transcription factor (Spi-1/PU.1 related)	Spib	272382 Entrez gene
93728_at	TSC22 domain family, member 1	Tsc22d1	21807 Entrez gene
94687_at	forkhead box B1	Foxb1	64290 Entrez gene
94811_s_at	general transcription factor II H, polypeptide 1 necdin	Gtf2h1 Ndn	14884 Entrez gene 17984 Entrez gene
95315_at	RIKEN cDNA 4732429I09 gene	4732429I09Rik	243906 Entrez gene
96580_at	pre B-cell leukemia transcription factor 3	Pbx3	18516 Entrez gene
96940_at	TEA domain family member 2	Tead2	21677 Entrez gene
96993_at	paired box gene 5	Pax5	18507 Entrez gene
97193_at	Transcription factor CP2-like 1 (Tcfcp2l1), mRNA	Tcfcp2l1	81879 Entrez gene
97497_at	Notch gene homolog 1 (Drosophila)	Notch1	18128 Entrez gene
97747_r_at	homeo box A4	Hoxa4	15401 Entrez gene

98083_at	Kruppel-like factor 6 (Klf6), mRNA	Copeb	23849 Entrez gene
98340_at	expressed sequence AA407331	AA407331	106839 Entrez gene
98579_at	early growth response 1	Egr1	13653 Entrez gene
98838_at	paired box gene 9	Pax9	18511 Entrez gene
99665_at	special AT-rich sequence binding protein 1	Satb1	20230 Entrez gene
99875_at	hairless	Hr	15460 Entrez gene
99901_at	polymerase I and transcript release factor	Ptrf	19285 Entrez gene
99914_at	heart and neural crest derivatives expressed transcript 2	Hand2	15111 Entrez gene

Supplementary Table 8

Motifs identified in the promoter regions of the genes analyzed from the Figure 1c subtrees of induced transcripts based on Gene Ontology (GO) "Transcription" group categorization (see Supplementary Table 1)

Motif	*Raw Score*	*P-value (from randomizing matrix)*
M00791|HNF-3	140	0.002
M00422|FOXJ2	139	0.007
M00933|Sp1	130	0.002
M00932|Sp1	122	0
M00196|Sp1	114	0
M00931|Sp1	111	0
M00720|CAC-binding protein (KLF1)	92.5	0.001
M00289|HFH-3	87.4	0.003
M00255|GC box	87.1	0
M01068|UF1H3BETA	84.8	0
M00302|NF-AT	66.6	0.006
M00403|aMEF-2	66.1	0.002
M00405|MEF-2	63.1	0.001
M00500|STAT6	62.5	0.008
M00238|Barbie box	51.5	0.003
M00808|Pax	41.9	0
M00141|Lyf-1	39.6	0.003
M00804|E2A	36.7	0.002
M00793|YY1	36	0
M00648|MAF	35.9	0.004
M00287|NF-Y (CCAAT)	33.5	0
M00340|c-Ets-2	33	0.006
M00979|PAX6	32.6	0
M00929|MyoD	31.9	0.009
M00175|AP-4	31.8	0.001
M00777|STAT	31.4	0.006

M00794|TTF-1	31	0.01
M00006|MEF-2	29.4	0.003
M00644|LBP-1	28.8	0.009
M00291|Freac-3	27.4	0.007
M00927|AP-4	25.7	0.009
M00176|AP-4	25	0.01
M00531|NERF1a	23.7	0.001
M00254|CCAAT	22.3	0.001
M00983|MAF	22.2	0.003
M00775|NF-Y (CCAAT)	20.3	0
M00209|NF-Y (CCAAT)	19.6	0
M00069|YY1	19.2	0
M00687|α-CP1 ≡ NF-Y (CCAAT)	15	0.002
M00185|NF-Y (CCAAT)	14.2	0.006
M00264|Staf	13.7	0.001
M00005|AP-4	12.8	0.003
M00526|GCNF	10	0
M00261|Olf-1	5.18	0.007

Listed are over-represented motifs within promoter regions identified using Clover (Cis-eLement OVERrepresentation) software (Frith *et al*., 2004) and position frequency matrices derived from transcription factor binding sites from the TRANSFAC® Professional database (Release 9.3) (Matys *et al*., 2003). The raw score quantifies the degree of the presence of the motif in the gene set and the *P*-value indicates the probability of obtaining a raw score of this size or greater by chance. If the *P*-value is very low (e.g., ≤ 0.01), the motif is considered to be significantly over-represented in the gene set. Motifs highlighted in blue, which are specifically present in this and the Ccne1-like, Hba-x-like and Hemgn-like gene sets, are discussed in the text.

Supplementary Table 9

Motifs identified in the promoter regions of the genes analyzed from the Figure 1c set of Ccne1-like profile neighbors of induced transcripts based on GO "Transcription" group categorization (see Supplementary Table 2)

Motif	*Raw Score*	*P-value (from randomizing matrix)*
M00933|Sp1	89.7	0.004
M00932|Sp1	83.9	0
M00196|Sp1	79.3	0
M00422|FOXJ2	78.9	0.003
M01068|UF1H3BETA	72.4	0
M00931|Sp1	71.9	0.001
M00720|CAC-binding protein (KLF1)	57.4	0.002
M00255|GC box	56.9	0.001
M00302|NF-AT	50.6	0.001
M00238|Barbie box	37.8	0.004
M00971|Ets	36.9	0.009
M00771|ETS	33.8	0.003
M00216|TATA	31.2	0.01
M00935|NF-AT	29.1	0.002
M00793|YY1	28.2	0
M00648|MAF	27.8	0.003
M00712|myogenin	24.7	0.009
M00929|MyoD	24.4	0.002
M00340|c-Ets-2	24.2	0.003
M00141|Lyf-1	23.5	0.005
M00777|STAT	23.4	0.003
M00339|c-Ets-1	22.9	0.007
M00804|E2A	22.1	0.006
M00644|LBP-1	22.1	0.001
M00175|AP-4	21.6	0.001
M01035|YY1	21.4	0.007

M00808|Pax	20.4	0
M00927|AP-4	19.8	0.002
M00176|AP-4	18.1	0.005
M00979|PAX6	17.7	0
M00531|NERF1a	16.8	0.001
M00277|Lmo2 complex	15	0.007
M00005|AP-4	14.2	0
M00974|SMAD	12.5	0.01
M00002|E47	9.81	0.008
M00638|HNF-4alpha	9.75	0.003
M01031|HNF4	8.38	0.008
M00733|SMAD-4	8.16	0.007
M00212|Retroviral poly A	7.53	0.009
M00069|YY1	7.39	0.006
M00287|NF-Y (CCAAT)	6.59	0.004
M00135|Oct-1	5.7	0.006

The motif highlighted in red, which is specifically present in this and the Hba-x-like gene set, is discussed in the text.

Supplementary Table 10

Motifs identified in the promoter regions of the genes analyzed from the Figure 1c set of Hba-x-like profile neighbors of induced transcripts based on GO "Transcription" group categorization (see Supplementary Table 3)

Motif	*Raw Score*	*P-value (from randomizing matrix)*
M00933|Sp-1	72.8	0.005
M00979|PAX6	67.3	0
M00932|Sp-1	63.3	0.002
M00196|Sp1	61.6	0.004
M00931|Sp-1	59.1	0.001
M00255|GC box	57.9	0.001
M00720|CAC-binding protein (KLF1)	50.7	0.01
M00808|Pax	48.6	0
M00302|NF-AT	37.7	0.001
M00238|Barbie box	30.8	0.006
M00141|Lyf-1	23.8	0.001
M00482|PITX2	22.2	0
M00804|E2A	21.3	0.001
M00622|C/EBPγ (CCAAT)	20.6	0.01
M00929|MyoD	19.8	0.008
M00777|STAT	18.8	0.003
M00277|Lmo2 complex	15	0.004
M00794|TTF-1	15	0.005
M00526|GCNF	14	0
M00175|AP-4	13.6	0.009
M00531|NERF1a	10.6	0.008
M00287|NF-Y (CCAAT)	10.3	0.001
M00193|NF-1	9.48	0.006
M00005|AP-4	9.41	0.006
M00687|α-CP1 ≡ NF-Y (CCAAT)	6.28	0.009

Supplementary Table 11

Motifs identified in the promoter regions of the genes analyzed from the Figure 1c set of Hemgn-like profile neighbors of induced transcripts based on GO "Transcription" group categorization (see Supplementary Table 4)

Motif	*Raw Score*	*P-value (from randomizing matrix)*
M00720|CAC-binding protein (KLF1)	32.9	0.001
M00238|Barbie box	30	0.002
M00302|NF-AT	26.7	0.003
M00808|Pax	21	0
M00979|PAX6	19.5	0
M00141|Lyf-1	17.4	0.002
M00777|STAT	16.3	0.001
M00804|E2A	15.5	0.002
M01036|COUPTF	13.7	0.008
M00531|NERF1a	11	0.002
M00794|TTF-1	9.7	0.003
M00193|NF-1	6.28	0.008
M00687|α-CP1 ≡ NF-Y (CCAAT)	5.98	0.001
M00158|COUP-TF, HNF-4	5.59	0.006
M00733|SMAD-4	3.89	0.01
M00287|NF-Y (CCAAT)	3.37	0.006

Supplementary Table 12

Motifs identified in the promoter regions of the genes analyzed from the Figure 1c set of Apobec2-like profile neighbors of induced transcripts based on GO "Transcription" group categorization (see Supplementary Table 5)

Motif	*Raw Score*	*P-value (from randomizing matrix)*
M00933|Sp-1	52.4	0
M00931|Sp-1	50	0.001
M00932|Sp-1	49.2	0
M00196|Sp1	49.1	0
M00255|GC box	37.9	0
M00979|PAX6	35	0
M00808|Pax	26.9	0
M00302|NF-AT	22.2	0
M00929|MyoD	17	0.01
M00804|E2A	13.7	0.008
M00935|NF-AT	13	0.004
M00977|EBF	12.9	0.01
M00777|STAT	12.3	0.004
M00158|COUP-TF, hnf-4	12.3	0
M01028|NRSF	12.1	0.001
M01031|HNF4	11.4	0.005
M00256|NRSF	8.55	0
M00250|Gfi-1	8.31	0.01
M00325|neural-restr.-silencer-element	6.45	0.003

The motif highlighted in green, which is specifically present in this gene set, is discussed in the text.

Supplementary Table 13

Motifs identified in the promoter regions of the genes analyzed from the set of 6-hour down-regulated transcripts obtained by K-means clustering of the expression profiling data based on GO "Transcription" group categorization (see Supplementary Table 6)

Motif	*Raw Score*	*P-value (from randomizing matrix)*
M00932|Sp-1	77.7	0.002
M00196|Sp1	73.2	0.003
M00931|Sp-1	68.4	0.006
M01010|HMGIY	60.4	0.004
M00255|GC box	53.8	0.009
M00979|PAX6	39.7	0
M00302|NF-AT	34.9	0.01
M00808|Pax	34.8	0
M00804|E2A	31.5	0
M01036|COUPTF	26.4	0
M00141|Lyf-1	25.9	0.002
M00929|MyoD	22.8	0.005
M00777|STAT	20.5	0.008
M00974|SMAD	18.1	0.004
M00087|Ik-2	16.2	0.006
M00482|PITX2	15.3	0.002
M00175|AP-4	12.9	0.01
M01031|HNF4	12.9	0.008
M00531|NERF1a	11.5	0.01
M00005|AP-4	11.1	0.004
M00158|COUP-TF, HNF-4	9.89	0.002
M00457|STAT5A (homodimer)	5.84	0.007
M00459|STAT5B (homodimer)	4.87	0.002

Supplementary Table 14

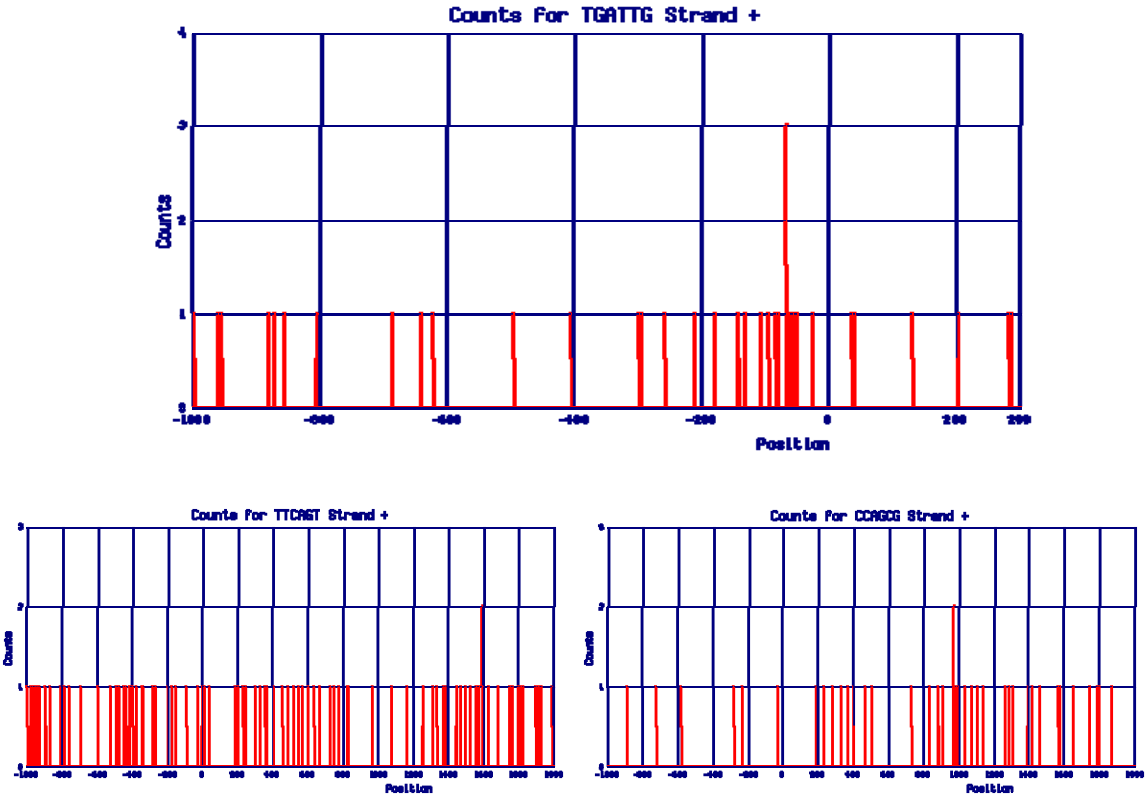
Motifs identified in the promoter regions of the genes analyzed from the set of 12-hour down-regulated transcripts obtained by K-means clustering of the expression profiling data based on GO "Transcription" group categorization (see Supplementary Table 7)

Motif	*Raw Score*	*P-value (from randomizing matrix)*
M00982|KROX	100	0.009
M00933|Sp-1	93.4	0.006
M00196|Sp1	88.7	0.002
M00932|Sp-1	88.4	0.001
M00931|Sp-1	79.4	0.001
M00255|GC box	71.5	0
M00791|HNF-3	66.6	0.009
M00979|PAX6	38.6	0
M00941|MEF-2	32.1	0.009
M00808|Pax	26.8	0
M00141|Lyf-1	25.4	0.008
M00470|AP-2gamma	23.5	0.003
M00777|STAT	20	0.003
M00087|Ik-2	14.4	0.008
M00794|TTF-1	14	0.006
M00006|MEF-2	10.9	0.007
M00531|NERF1a	10.4	0.009
M00231|MEF-2	6.33	0.01

Supplementary Table 15

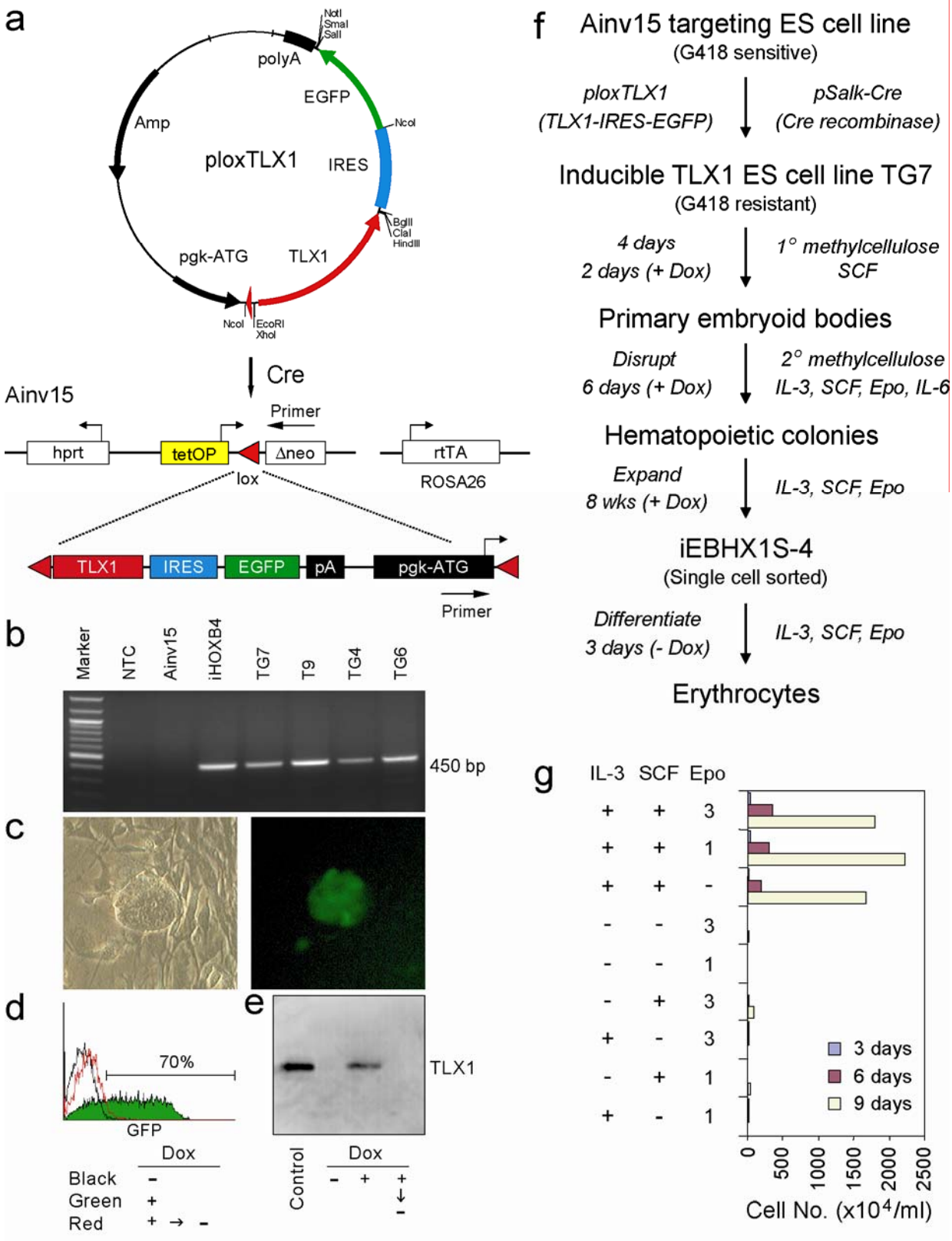
Positionally clustered six-letter "word", TGATTG, identified in the promoter regions of the genes analyzed from the Figure 1c subtrees of induced transcripts based on Gene Ontology (GO) "Transcription" group categorization (see Supplementary Table 1).

Rank	*Word*	*Count*	*Pnlty per Pos*	*Local Max*	*P-value*	*Mtc P-value*
1	TTCAGT	26	1.003861e-01	5.237365e+00	1.057322e-04	4.330793e-01
2	CCAGCG	22	8.494208e-02	4.376475e+00	8.833988e-04	3.618401e+00
3	GCGCCG	41	1.583012e-01	4.411765e+00	1.498340e-03	6.137201e+00
4	**TGATTG**	**29**	**1.119691e-01**	**4.258229e+00**	**1.594406e-03**	**6.530688e+00**
5	CTTAGA	27	1.042471e-01	4.221771e+00	1.635609e-03	6.699453e+00
6	GGTGCG	22	8.494208e-02	4.137344e+00	1.668312e-03	6.833405e+00
7	GGGGCG	59	2.277992e-01	4.501486e+00	1.698136e-03	6.955564e+00
8	TGGCGG	48	1.853282e-01	4.416162e+00	1.733549e-03	7.100615e+00
9	CACAAG	28	1.081081e-01	4.189658e+00	1.847273e-03	7.566429e+00
10	GGGCTC	47	1.814672e-01	4.369449e+00	1.921873e-03	7.871991e+00



Over-represented six letter "words" were evaluated for cluster significance (representative block alignments are illustrated; transcription start site = position 0) using an enumerative motif finding algorithm (Tharakaraman *et al.*, 2005) and queried for functional relevance through the TRANSFAC public database website (Release 7.0, Biobase GmbH) http://www.gene-regulation.com/cgi-bin/pub/databases/transfac/search.cgi . The only sequence that satisfied these criteria, TGATTG, is discussed in the text.

Supplementary Figure 1

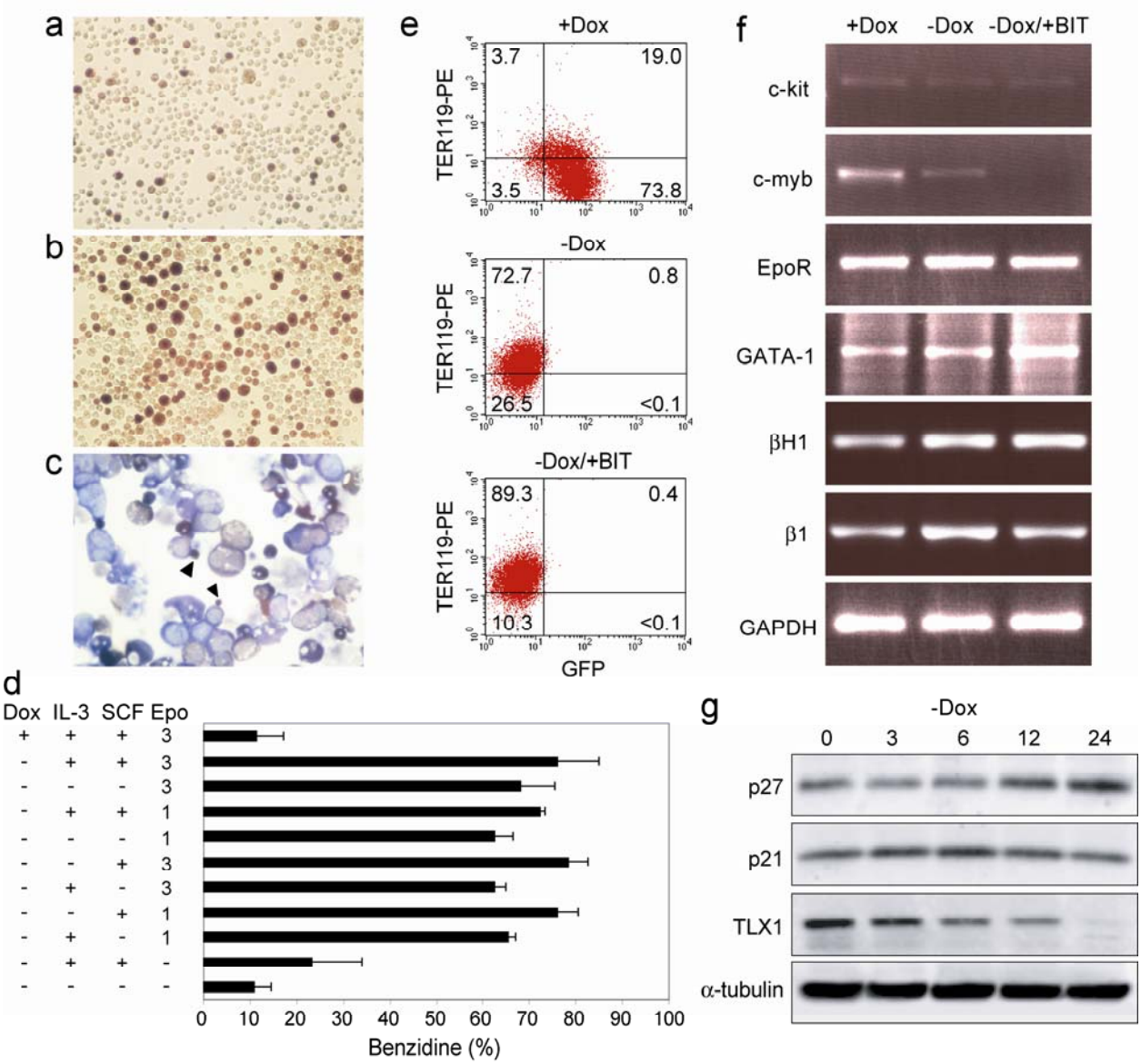


Supplementary Figure 1 Legend

Generation of a *TLX1*-inducible ESC line and conditionally-arrested iEBHX1S-4 erythroid progenitor cells. (**a**) Schematic representation of the integrated TLX1-IRES-EGFP expression cassette in iTLX1 ES cells. Successful Cre-mediated recombination between the loxP site in the ploxTLX1 targeting plasmid and the loxP site upstream of the *hprt* locus in the Ainv15 ES cell line places the TLX1-IRES-EGFP cassette under the control of a doxycycline-responsive promoter and restores G418 resistance by reconstituting a functional neomycin (*neo*) drug resistance gene. A reverse tetracycline-controlled transactivator (rtTA) responsive to doxycycline is integrated into the constitutive ROSA26 locus. tetOP, rtTA-dependent promoter consisting of tetracycline operator sequences fused to a minimal promoter. (**b**) Individual G418-resistant colonies were picked and expanded. Successful targeting was confirmed by the presence of a diagnostic 450-bp fragment generated by PCR analysis of genomic DNA using primers specific for *pgk* promoter sequences in the ploxTLX1 plasmid and neo sequences in the *hprt* homing site. NTC = no template control; Ainv15 = negative control parental ESC line; iHOXB4 = positive control ESC line containing a doxycycline-inducible HoxB4 cDNA in the *hprt* locus; TG4, TG6 and TG7 = iTLX1 clones containing correctly targeted TLX1-IRES-EGFP cassettes; T9 = iTLX1 clone containing a correctly targeted TLX1 cDNA minus a linked EGFP gene. (**c**) *Left* Bright-field image of TG7 iTLX1 ESCs cultured in the presence of 1 μg/ml doxycycline. *Right* Fluorescence image of the same cells demonstrating GFP expression. (**d**) Flow cytometric analysis of GFP expression in TG7 iTLX1 ESCs grown in the absence of doxycycline (-), grown in the presence of 1 μg/ml doxycycline for 3 days (+), and grown in the presence of 1 μg/ml doxycycline for 3 days and then in the absence of doxycycline for 3 days (+ → -). (**e**) Western blot analysis of TLX1 expression in: NIH3T3 cells constitutively expressing *TLX1* (Control); TG7 iTLX1 ESCs grown in the absence of doxycycline (-); TG7 iTLX1 ESCs grown in the

presence of 1 μg/ml doxycycline for 3 days (+); and TG7 iTLX1 ESCs grown in the presence of 1 μg/ml doxycycline for 3 days and then in the absence of doxycycline for 3 days (+ → -). (**f**) Protocol used to generate the iEBHX1S-4 cell line. Abbreviation: Dox, doxycycline. (**g**) Growth factor responsiveness of iEBHX1S-4 cells cultured in the presence of 1 μg/ml doxycycline. Abbreviations: + IL-3, 5% IL-3-conditioned medium; + SCF, 5% SCF-conditioned medium; 1 Epo, 1 U/ml Epo; 3 Epo, 3 U/ml Epo.

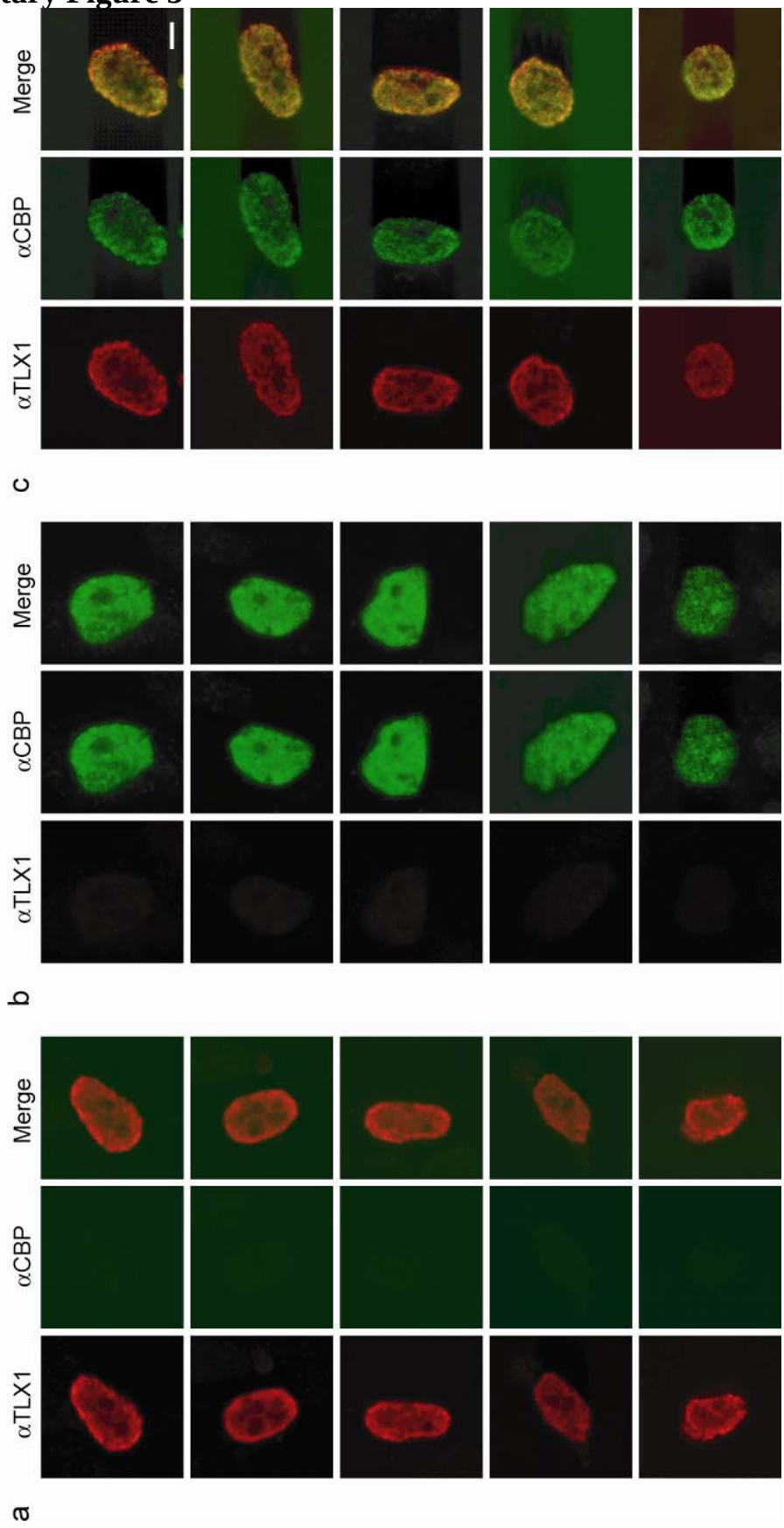
Supplementary Figure 2

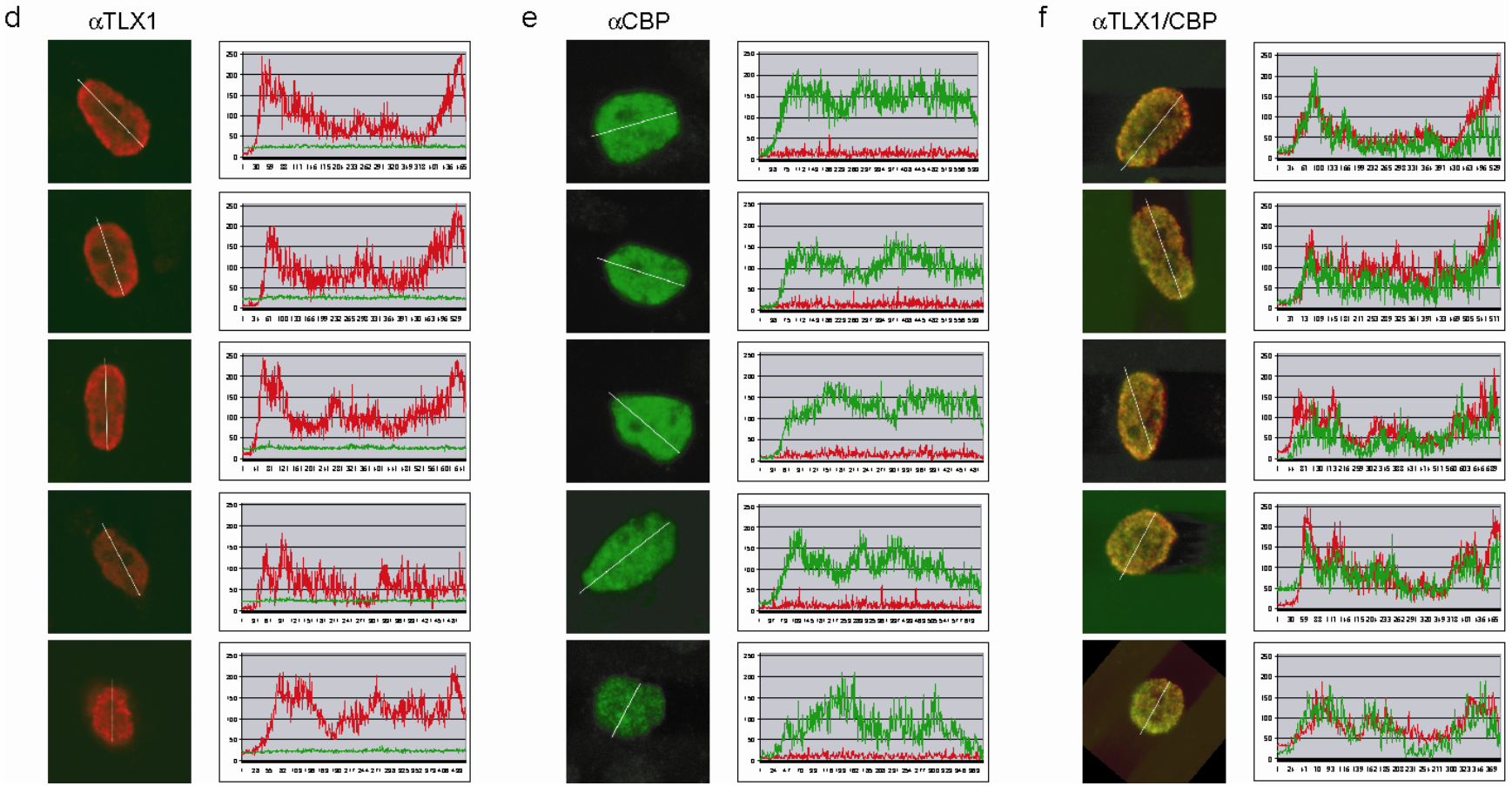


Supplementary Figure 2 Legend

Erythroid differentiation potential of iEBHX1S-4 cells. Photomicrographs of cultures (**a,b**) and cytospin preparations (c) of benzidine-stained iEBHX1S-4 cells cultured in the presence (**a**) or absence (**b,c**) of 1 μg/ml doxycycline for 3 days. In (**c**), differentiation was carried out in the presence of 5% BIT (bovine serum albumin, insulin and human transferrin). Arrowheads represent enucleated benzidine-positive cells. (**d**) Differentiation of iEBHX1S-4 cells cultured in the absence of doxycycline (Dox) for 3 days in various combinations of IL-3, SCF and Epo. Differentiation potential was assayed by the percentage of cells positive for benzidine staining. Epo was added at either 3 U/ml or 1 U/ml. (**e**) Flow cytometric analysis of glycophorin A/TER119 expression by iEBHX1S-4 cells cultured in the presence (+Dox) or absence (-Dox) of 1 μg/ml doxycycline for 3 days in IL-3, SCF and Epo. +BIT, differentiation was carried out in the presence of 5% BIT. Iso, isotype control (black line). (**f**) RT-PCR gene expression analysis of iEBHX1S-4 cells cultured in the presence or absence of 1 μg/ml doxycycline (Dox) as described above. EpoR, Epo receptor; c-kit, SCF receptor; βH1, embryonic βH1-globin; β1, adult βmajor-globin; GAPDH (glyceraldehyde-3-phosphate dehydrogenase), RT-PCR amplification and loading control. (**g**) Western blot analysis showing $p27^{Kip1}$ and $p21^{Waf1/Cip1}$ cyclin-dependent kinase inhibitor expression levels in iEBHX1S-4 cells cultured in the absence of doxycycline (Dox) for the indicated times (hours).

Supplementary Figure 3




d
αTLX1
e
αCBP
f
αTLX1/CBP

Supplementary Figure 3 Legend

TLX1 interaction with CBP and colocalization in 293T cells. 293T cells transiently transfected with TLX1 (**a**), CBP (**b**), or TLX1 and CBP (**c**) expression vectors were labeled with anti-TLX1 (αTLX1; Alexa Fluor 568, red) and anti-CBP (αCBP; Alexa Fluor 488, green) antibodies, and immunofluorescence staining was analyzed by confocal laser scanning microscopy. The right panels show the merged green and red images at the same focal plane with overlapping regions of protein distribution appearing yellow. Size bar, 10 μm. (**d**) Quantitative analysis of the images in (**a**) revealed that there was a statistically significant difference between the distribution of TLX1 in the peripheral versus the central region of the nucleus ($P = 0.024$). (**e**) Quantitative analysis of the images in (**b**) revealed that there was no statistically significant difference between the distribution of CBP in the peripheral versus the central region of the nucleus ($P = 0.328$). (**f**) When TLX1 was coexpressed with CBP (**c**), a substantial fraction of CBP colocalized with TLX1 (Pearson correlation coefficient, $r = 0.672$) preferentially relocalizing at the nuclear periphery ($P = 0.001$, peripheral versus central localization).