Viral transduction and reprogramming culture

The plasmids pMXs-hSOX2, pMXs-hC-MYC, pMXs-hOCT3/4 and pMXs-hKLF4 [1] were individually transfected together with packaging vectors in 293T cells. Virus was harvested 36 hours post-infection, concentrated by ultracentrifugation for 1.5 h at 25,000 rpm in a Beckman SW28 rotor and resuspended in phosphate-buffered saline (200 µl). The virus was used to infect KBM7 cells in three consecutive rounds of spin-infection with an interval of 12 hours. Conditions were used that resulted in an infection percentage of >95% of pLIB-EGFP (Clontech) that was taken along in a separate infection as a control. After three days of culture in regular medium (IMDM 10% FCS, L-glutamine, and penicillin–streptomycin), the cells were taken up in Knockout™ DMEM (Invitrogen) supplemented with L-glutamine, nonessential amino acids, knockout serum replacement (Invitrogen), and 4 ng/ml basic FGF. Subsequently the cells were plated in different densities on irradiated murine embryonic fibroblasts. Four independent infections of KBM7 cells with 1 million cells each were performed. Cells were typically plated with 50,000 cells per plate on MEFs. The large majority of colonies arising were granulated cell clusters not resembling hES cells. From a total of 20 plates 6 colonies were picked that resembled hES cells based on morphology. From these, 2 iPS cell clones colonies were expanded. For early passages iPS cells were propagated only manually, whereas for later passages occasional collagenase treatment (1 mg/ml) was used. Imatinib sensitivity was tested by adding imatinib (LC Laboratories, Woburn, MA) at a concentration of 1 µM.

iPS culture and *in vitro* differentiation

(**I.**) Spontaneous iPS cell differentiation was induced by embryoid body (EB) formation in low adherence dishes in DMEM supplemented with 15% defined fetal bovine serum, 1% (v/v) MEM non-essential amino acids (Invitrogen), 1 mM L-glutamine, 0.1 mM beta-mercaptoethanol (Sigma), and penicillin-streptomycin. Medium was replaced every 2 to 3 days. After 15 to 20 days, floating EBs were transferred to tissue culture dishes for adherent culture in DMEM with 10 to 30% fetal bovine serum and penicillin-streptomycin. (**II.**) Directed differentiation of KBM7-iPS toward neural lineages was conducted essentially as described previously for human ES cells,[2] using 300 ng/ ml Noggin for induction of neuroepithelial cells (21 days), followed by expansion of neural precursors with bFGF (9 days) and final neuronal differentiation without bFGF in N2-based medium (10 to 15 days). (**III.**) Hematopoietic differentiation was conducted according to embryoid body-based protocols with cytokine supplementation, in principle as previously published.[3] Embryoid bodies were generated as described above. After 4 days, EB medium was exchanged for hematopoietic differentiation medium: StemSpan serum-free medium (Stem Cell Technologies) supplemented with cytokines BMP4 (10 ng/ml), VEGF (10 ng/ml), SCF (25 ng/ml), TPO (20 ng/ml), Flt3-ligand (10 ng/ml), and bFGF (10 ng/ml). Medium was changed every 4 to 8 days, and cells from control versus imatinib-treated groups were subjected to flow cytometric surface antigen analysis (CD45, CD43) after 30 days.

RT-PCR

Total RNA was prepared as described in the RNeasy Mini Kit (Qiagen, Valencia, CA) with on-column DNase I digestion. One microgram total RNA from each sample was used for Oligo(dT)20-primed reverse transcription, which was carried out as described in the product protocol (SuperScriptTM III First-Strand Synthesis System for RT-PCR, Invitrogen). PCR was performed using AccuPrime™ Taq DNA Polymerase High Fidelity using 28 PCR cycles. PCR primer sequences are listed in table 1.

Immunostaining and alkaline phosphatase staining
Cells were fixed in 4% paraformaldehyde in PBS for 20 min at room temperature and rinsed with PBS. Cells were permeabilized in 0.1% Triton X-100 in PBS containing 10% normal donkey serum and 1% BSA for 45 min at room temperature. Cells were rinsed with PBS and then incubated overnight at 4°C with appropriate dilutions of primary antibodies in PBS containing 10% normal donkey serum and 1% BSA. Cells were then labeled with the appropriate fluorescently tagged secondary antibodies (Invitrogen). Primary antibodies used were: Tra-1–81 (MAB4381, Millipore), Oct-3/4 (R&D Systems), CD43 (MT1, Santa Cruz), CD9 (clone 209306, R&D Systems), Nestin (clone 196908, Neuromics). Alkaline phosphatase staining was performed with alkaline phosphatase staining kit (Vector Laboratories) according to product protocol.

Western blot analysis
Cells were lysed directly in Laemmli sample buffer, separated on a NuPAGE Novex 4-12% Bis-Tris gel (Invitrogen), and transferred to a polyvinylidene difluoride membrane (Millipore). Immunoblots were processed according to standard procedures, using primary antibodies for NANOG (R&D Systems), E-cadherin (clone 180215, R&D Systems), LIN28 (R&D Systems), CD43 (MT1, Santa Cruz).

Teratoma formation
Cells were harvested by collagenase IV treatment, centrifuged, and the pellets were suspended in DMEM/F12. The cells collected from a 50% confluent 10 cm dish were injected subcutaneously to dorsal flank of a NOD-SCID mouse. 26 weeks after injection, tumors were dissected and fixed with PBS containing 4% paraformaldehyde. Paraffin-embedded tissue was sectioned and stained with hematoxylin and eosin (H&E).

Methylation analysis
Genomic DNA was extracted using the Qiamp DNA mini kit (Qiagen) and subjected to bisulfite conversion using the Qiagen EpiTect Bisulfite Kit. Promoter regions of OCT4 and NANOG were amplified using previously described primers:[1,4]
OCT4 Forward: ATTTGTTTTTTGGGTAGTTAAAGGT
OCT4 Reverse: CCAACTATCTTCATCTTAATAACATCC
NANOG S: TGGTTAGGTTGGTTTTAAATTTTTG
NANOG AS: AACCCACCCTTATAAATTCTCAATTA
PCR products were cloned using the STRATACLONE vector (Stratagene) and sequenced using M13 forward primers.

Table S1. Primer sequences for RT-PCR

Gene		Nucleotide sequence
REX1	F	AACCATCGCTGAGCTGAAACAAATG
	R	CAGGATGGGTTGAGAAAACTCACCC
FGF4	F	GGACACCCGCGACAGCCTGCTGGAG
	R	AGGAAGTGGGTGACCTTCATGGTGG
TDGF1	F	ACGATGTGCGCAAAGAGAACTGTGG
	R	GGTCAATGTCGATTAATAGTAGCTT
OCT4	F	GCTTGGGCTCGAGAAGGATGTGGTC
	R	CTTCCCTCCAACCAGTTGCCCCAAAC
SOX2	F	GGGAAATGGGAGGGGTGCAAAAGAGG
	R	TTGCGTGAGTGTGGATGGGATTGGTG
NANOG	F	CAGCCCTGATTCTTCCACCAGTCCC
	R	TGGAAGGTTCCCAGTCGGGTTCACC
GDF3	F	CTTATGCTACGTAAAGGAGCTGGG
	R	GTGCCAACCCAGGTCCCGGAAGTT
LIN28	F	TGCGGGCATCTGTAAGTGG
	R	GGAACCCTTCCATGTGCAG
ZIC3	F	GCAAGTCTTTCAAGGCGAAG
	R	TAGGGCTTGTCCGAGGTATG
CD43	F	GTCCTTATCAGCCGAGCCGG
	R	GGATCCTAGAGAGTTTGCTGTTATG
CD45	F	CCACTAGAGGCTGAATTCCAGAGAC
	R	GTTTGCTGAGATCCATCCCTGCAG
ACTB	F	GCTCGTCGTCGACAACGGCTC
	R	CAAACATGATCTGGGTCATCTTCTC
TERT	F	CCTGCTCAAGCTGACTCGACACCGTG
	R	GGAAAAGCTGGCCCTGGGGTGGAGC
BCR-ABL	F	TGGAGCTGCAGATGCTGACCAACTCG
	R	ATCTCCACTGGCCACAAAATCATACA

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2. Sonntag K-C, Pruszak J, Yoshizaki T, van Arensbergen J, Sanchez-Pernaute R, Isacson O. Enhanced yield of neuroepithelial precursors and midbrain-like dopaminergic neurons from human embryonic stem cells using the bone morphogenic protein antagonist noggin. *Stem cells* (Dayton, Ohio). 2007;25 (2):411–418.
3. Raya A, Rodríguez-Pizà I, Guenechea G, et al. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature*. 2009;460 (7251):53–59.
4. Yu JY, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318 (5858):1917–1920.

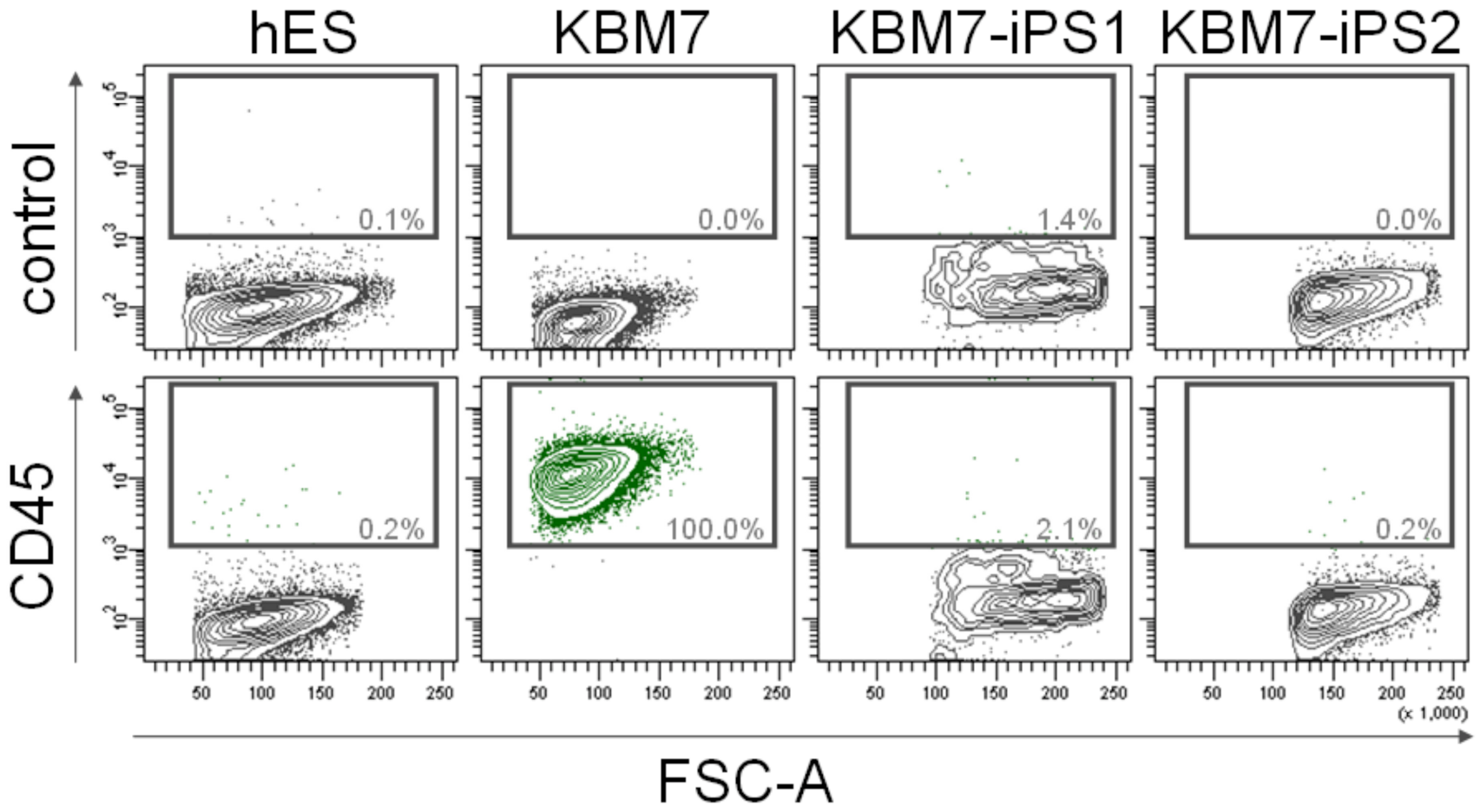

Figure S1. Flow cytometric analysis of CD45 surface expression
In contrast to the original KBM7 cells, reprogrammed KBM7-iPS cell lines do not express the pan-hematopoietic CD45 surface antigen. Upper panels: control without primary antibody; lower panels: cells stained with mouse anti-human CD45 primary antibody, followed by Alexa-488-conjugated goat anti-mouse secondary antibody.

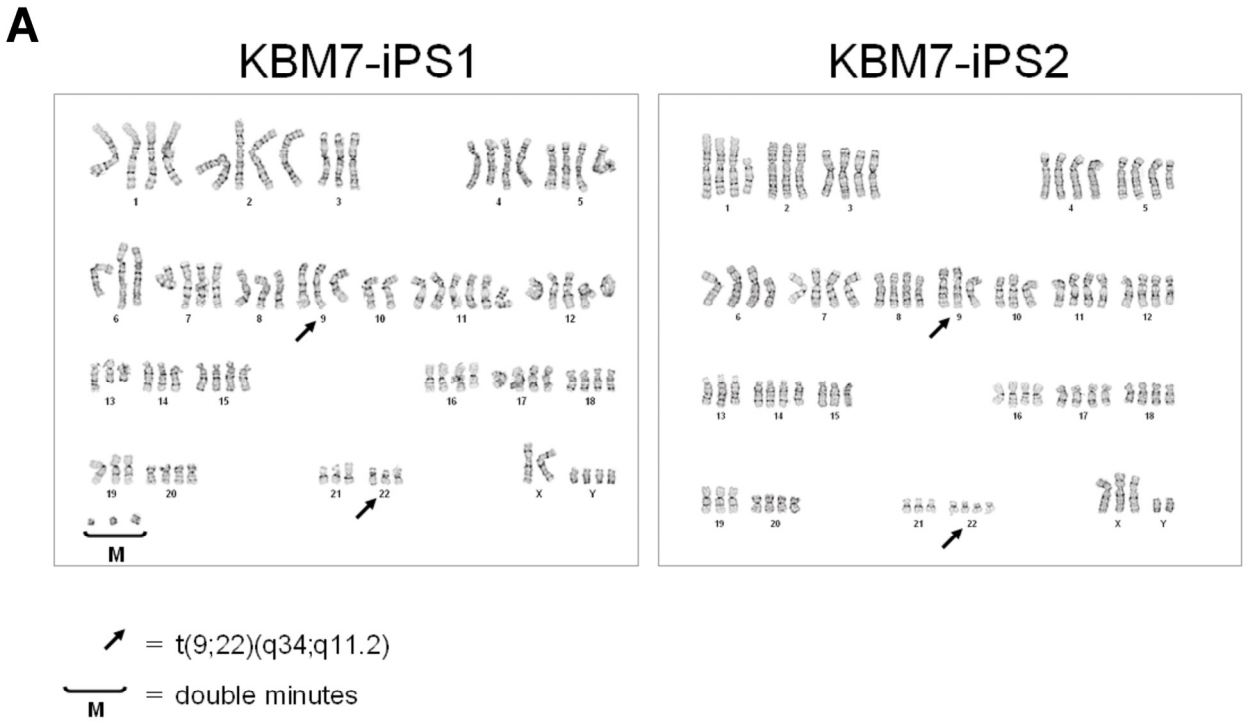

Figure S2. KBM7-iPS cells contain the Philadelphia chromosome and express BCR-ABL
(A) KBM7-derived iPS cell lines contain chromosomal structural aberrations observed in the original CML cancer cell line (KBM7), including the translocation (9;22)(q34;q11.2) (Philadelphia chromosome; indicated by arrows) underlying the BCR/ABL fusion transcript. Both lines show abnormal male, near tetraploid karyotype with complex numerical and structural changes, including double minute chromosomes in KBM7-iPS1 (indicated by "M"; left panel), and del(5)(q15q33), del(6)(q13q25), and del(9)(q13q22) in KBM7-iPS2 (right panel). (B) Western Blot analysis of cell lysates of parental KBM7, KBM7-iPS, and human embryonic stem cells.

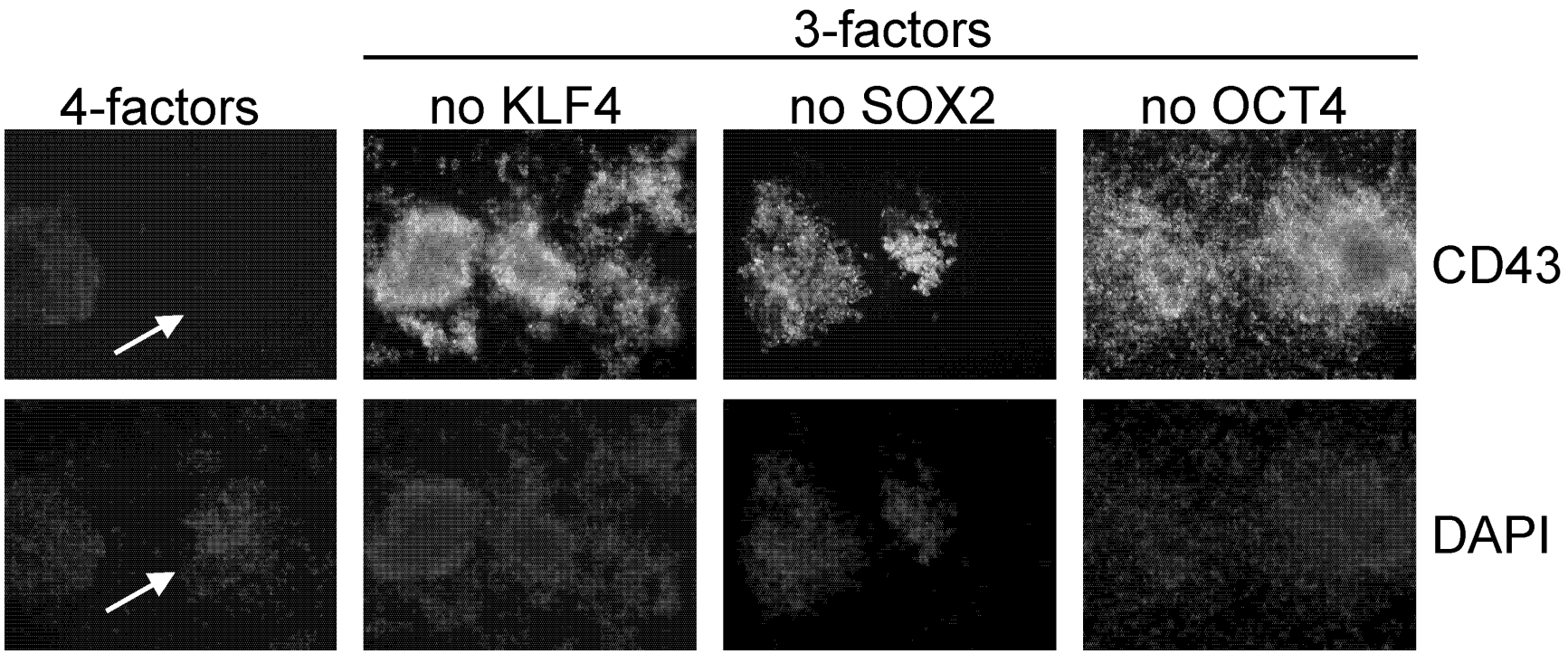

Figure S3. Immunofluoresence for hematopoietic marker CD43 (sialophorin)
Appearance of CD43-negative colonies after seven days requires combinatorial application of all four factors, c-MYC, OCT4, SOX2, and KLF4. Absence of c-MYC results in cell death and failure in the formation of adherent clones (not shown). CD43 immunofluorescence in green (upper row); nuclei in blue (DAPI, bottom row).

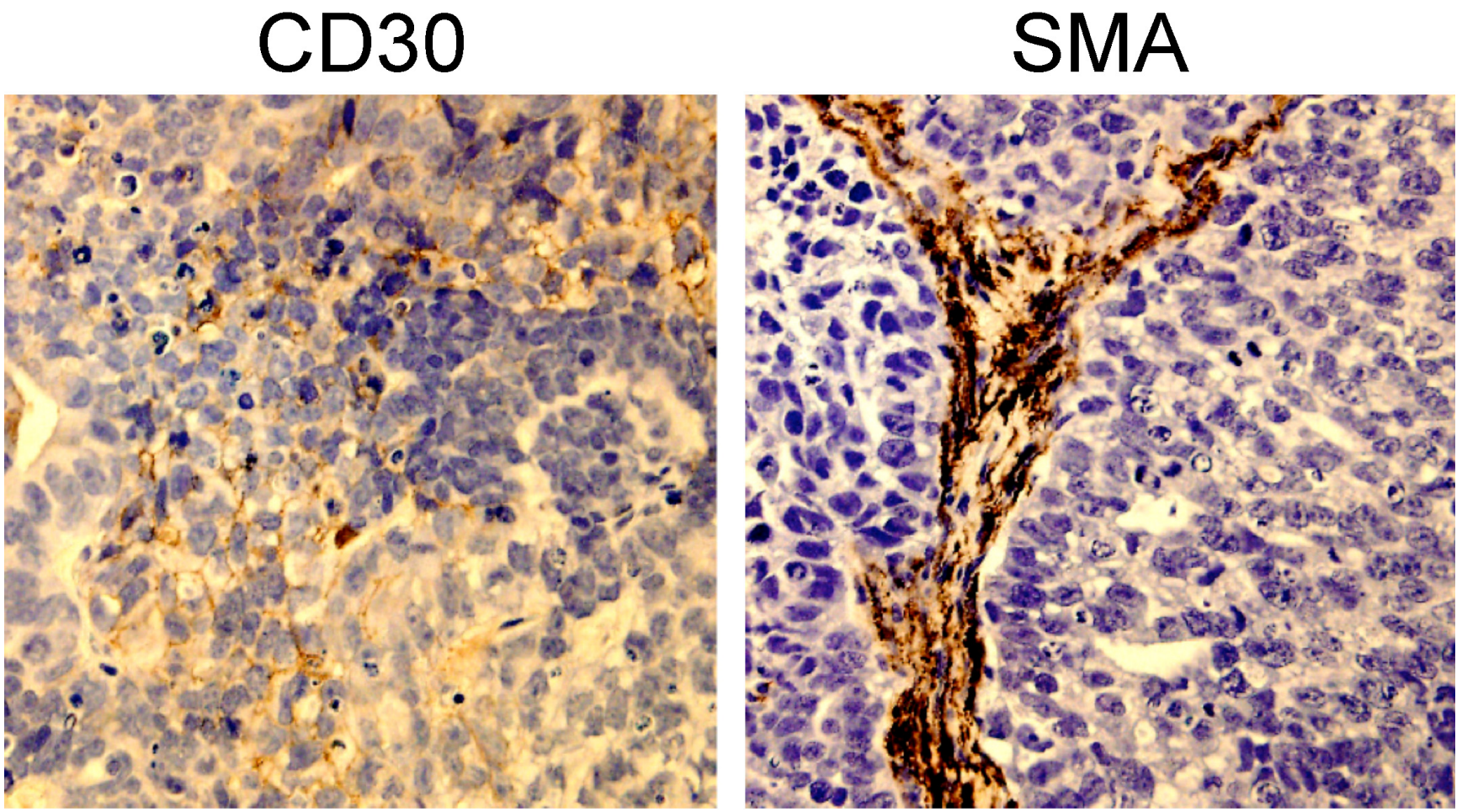

Figure S4. Immunohistochemical staining of KBM7-iPS teratoma
CD30 staining indicates areas of immature embryonal carcinoma and smooth muscle actin (SMA) indicates areas of mesodermal muscle tissue in the teratoma.

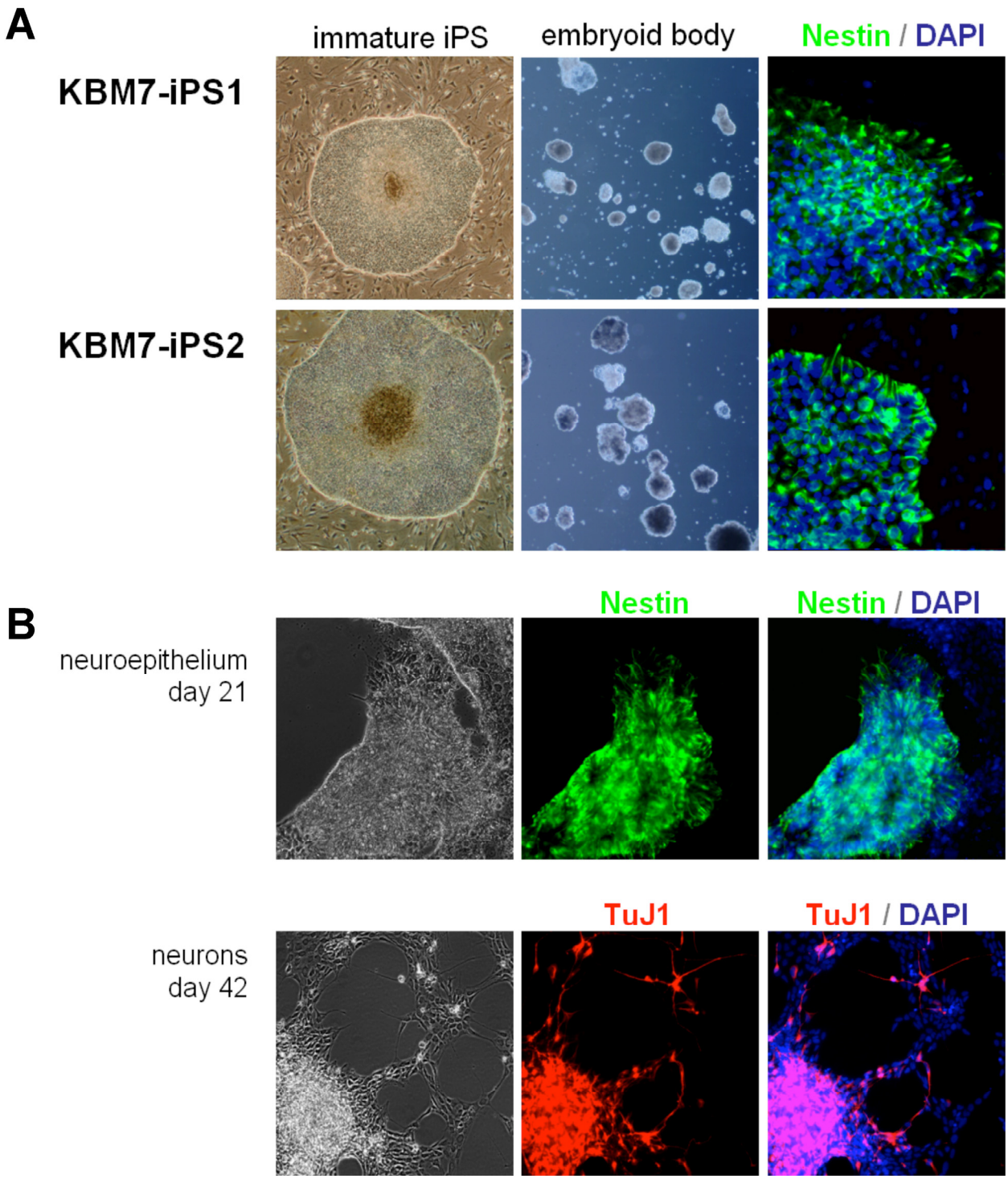

Figure S5. *In vitro* differentiation of KBM7-iPS cells
(A) Typical morphology of immature KBM7-iPS cells (7 days after manual passage; left column), of embryoid bodies (5-8 days after initiating EB formation; mid column) and Nestin-positive derivatives emerging in KBM7-iPS differentiation culture 10-15 days after plating of EBs on adherent substrates (upper panels: KBM7-iPS1; lower panels: KBM7-iPS2). (B) Directed differentiation of KBM7-iPS2 cells toward neural lineage utilizing the BMP-antagonist Noggin resulted in occurrence of characteristic Nestin-positive neuroepithelial rosette-like structures (upper row; 21 days). ß-III-tubulin (TuJ1)-positive cells of neuronal morphology were observed after continued maturation *in vitro* (lower row; 42 days).

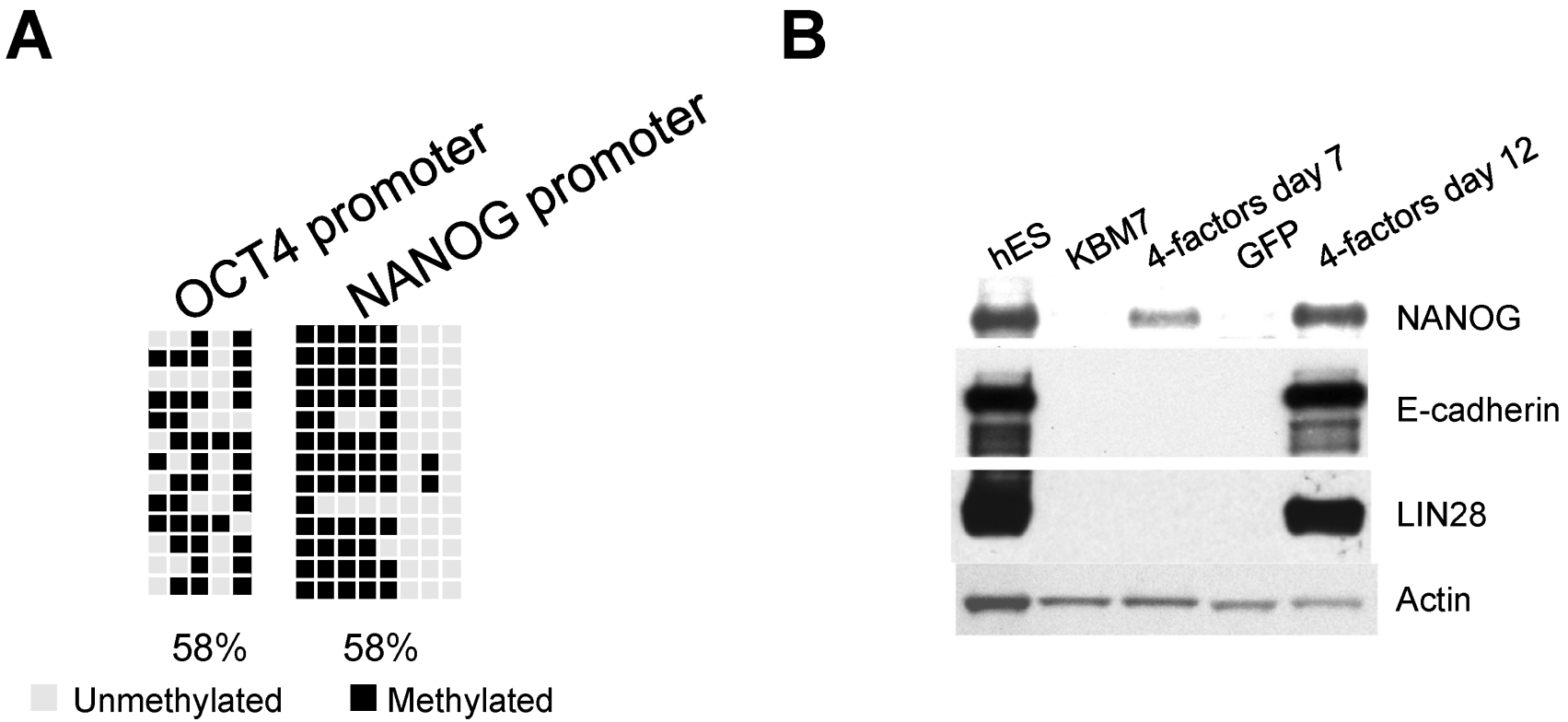

Figure S6. Promotor methylation and protein expression changes induced by reprogramming factors
(A) Methylation analysis of the OCT4 and NANOG promoter region in KBM7 cells 7 days after infection with retroviruses encoding the four reprogramming factors. Light gray squares indicate unmethylated, black squares methylated CpGs and numbers indicate the percentage methylated CpGs. (B) Western blot analysis of cell lysates of human ES cells (BG01), KBM7, KBM7 infected with retroviruses encoding the four reprogramming factors for 7 or 12 days and KBM7 cells infected with retroviruses encoding GFP.

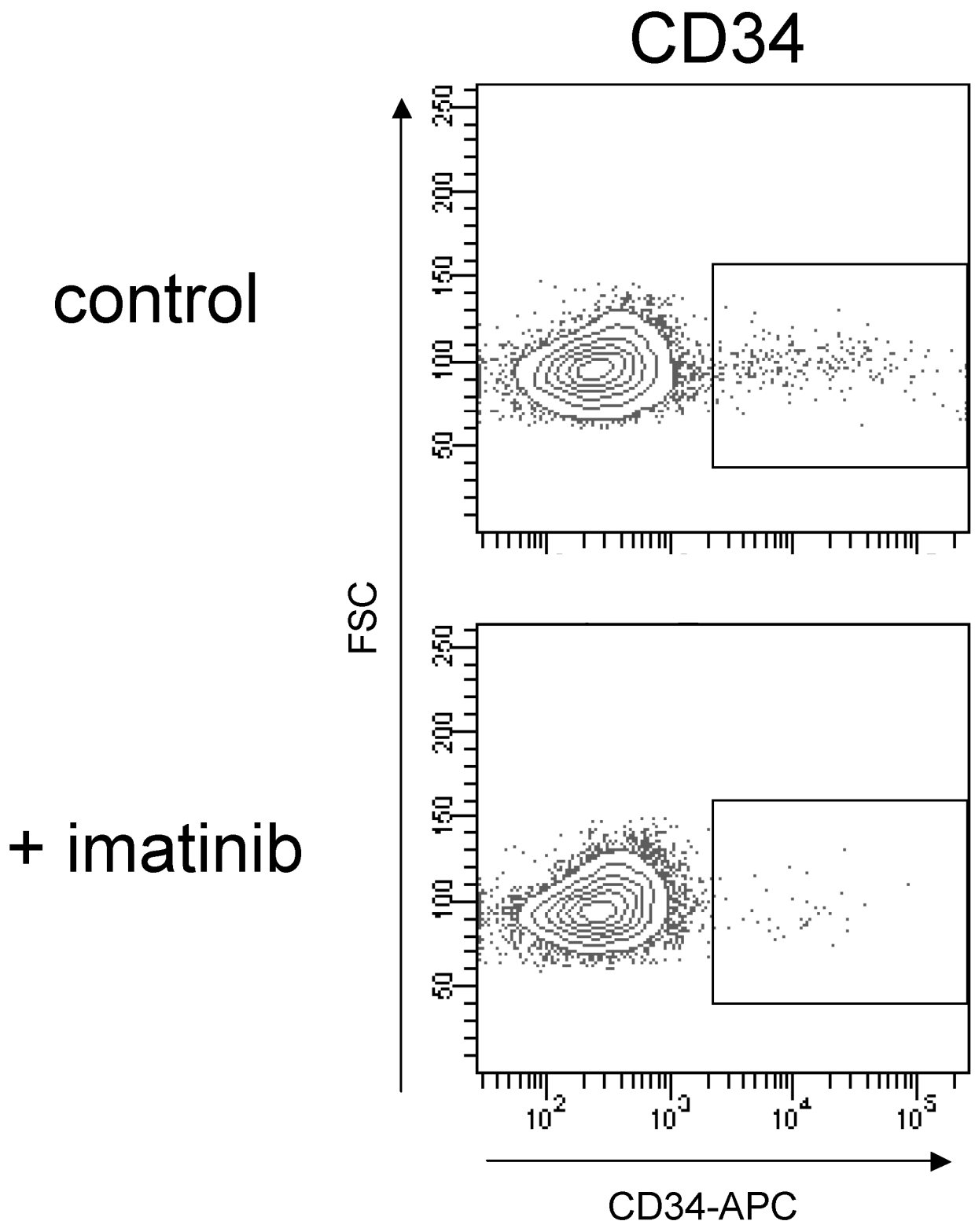

Figure S7. CD34 positive subpopulation is sensitive to imatinib
Flow cytometric analysis of KBM7-iPS cells differentiated into the hematopoietic lineage showed a distinct subpopulation stained by CD34 antibodies. Imatinib treatment resulted in a reduction of this subpopulation.