The truncated RUNX1/ETO activates VLA-4-dependent adhesion and migration of hematopoietic progenitor cells

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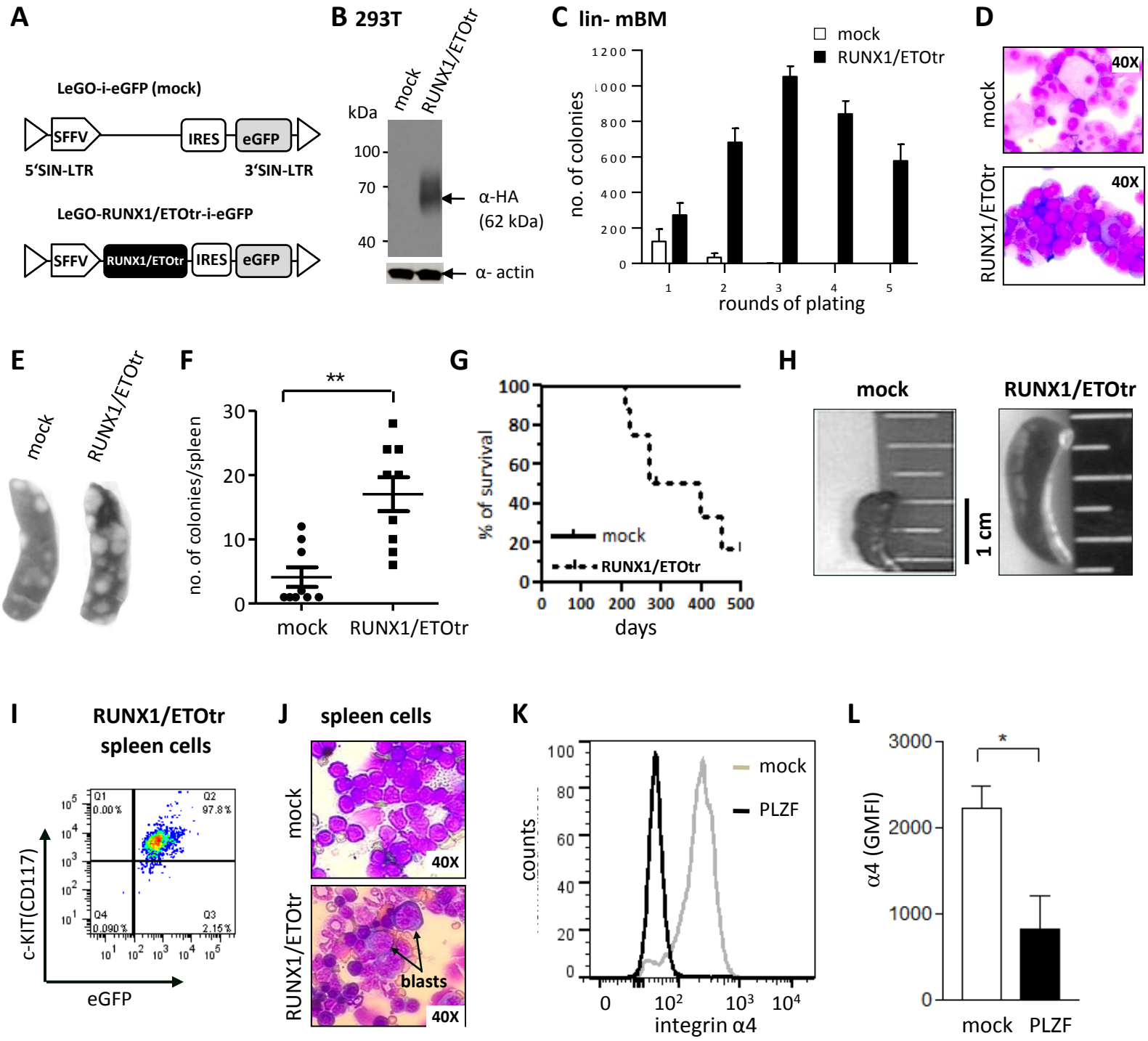
doi:10.3324/haematol.2014.106088

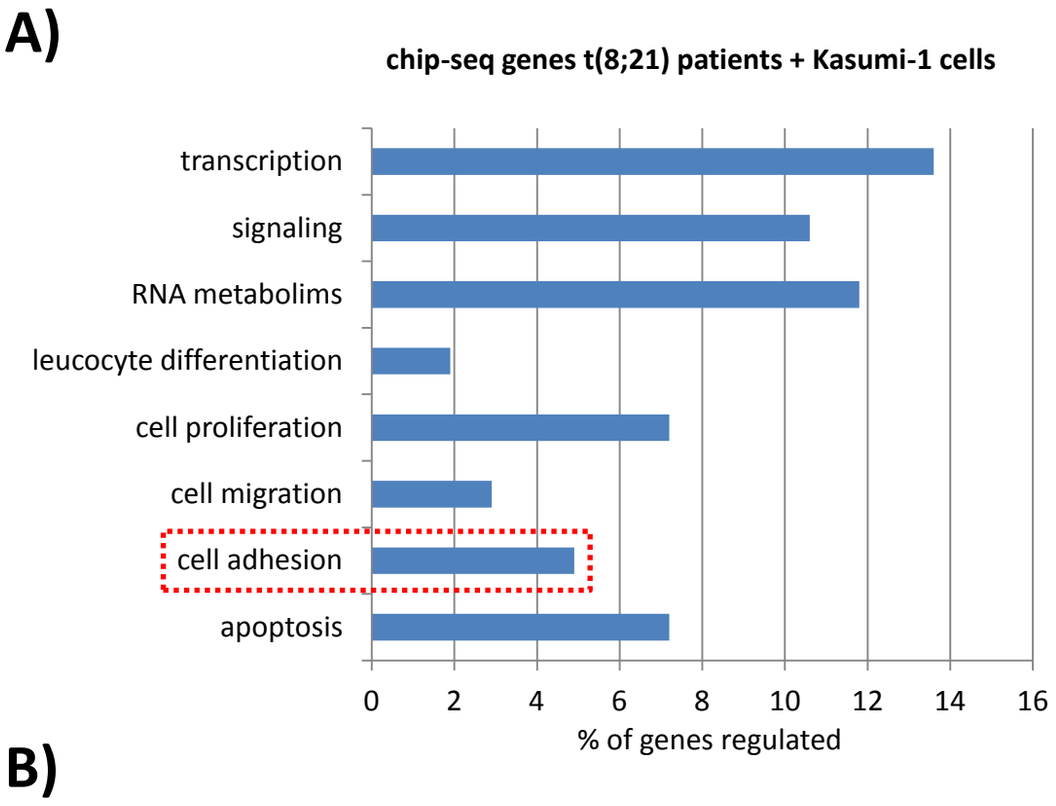
Suppl. Fig. 1



Supplementary Figure 1. Transplantation of RUNX1/ETOtr lin- mBM cells induced leukemia phenotype in mice. (A) Structure of LeGO-iG2 (mock) and LeGO-RUNX1/ETOtr vectors. (B) Expression of HA-tagged RUNX1/ETOtr in transfected 293T cells assessed using western blot. A total of 10 µg of each cell lysate was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. The protein was then transferred onto a nitrocellulose membrane. The membrane was blocked with 5% skimmed milk in 1x TBS-T buffer (50 mM Tris (tris (hydroxymethyl) aminomethane)–HCl (pH 7.6), 150 mM NaCl, and 0.1% Tween 20) and incubated with HA-tagged primary antibody overnight. Next, the membrane was washed with 1x TBS-T and incubated with an HRP-labeled secondary antibody. Protein detection was performed using a standard chemiluminescence method. (C) Transduced lin- mBM cells were seeded in methylcellulose supplemented with 10 ng/mL mIL3, 50 ng/mL mSCF, 100 ng/mL hFLT3L and 100 ng/mL hIL11 (R&D Systems). After 7 days, colonies containing more than 50 cells were counted as single colony. The depicted data represent five replating cycles. (D) Simultaneously, cells from the second replating cycle were stained for cytomorphological analysis using cytospin method. A total of $1x10^5$ cells were loaded into a cytofunnel (Thermo Scientific, Schwerte, Germany) and centrifuged for 7 minutes at 800 rpm. The cells were then stained with May-Grünwald stain (Sigma-Aldrich) for 3 minutes followed by Giemsa stain (Sigma-Aldrich) for 5 minutes. Cytomorphology of the cells was documented using light microscopy at 40x magnification. (E) Transduced lin- mBM cells were transplanted into lethally γ-irradiated (11 Gy) mice. At day 10, the spleens of transplanted mice were isolated and fixed in Tellesniczky's fixative solution. Representative images of spleen colonies and (F) respective colony numbers are shown. (G) Two days after isolation and prestimulation with 10 ng/ml IL-3, 50 ng/ml IL-6 and 50 ng/ml SCF, C57BL/6 lin- mBM cells were transduced with the lentiviral vectors leGO-empty-IRES-eGFP (mock) and leGO-RUNX1/ETOtr-IRES-eGFP. Two days after transduction 5x10E5/animal progenitor cells were transplanted into lethally γ-irradiated (11 Gy) C57BL/6 mice by tail-vein injection. Subsequent to transplantation, mice were given neomycin for 2 weeks. Transplanted mice were observed daily for sings of disease. Survival curves of lethally irradiated mice transplanted with transduced lin- mBM cells are shown. mock, n = 5; RUNX1/ETOtr, n = 10. (H) Typical spleen size of the transplanted group. (I) Expression of eGFP/c-KIT in the spleen cells of RUNX1/ETOtr-transduced lin- mBM cell-transplanted mice. (J) Cytomorphology of spleen cells of transplanted mice. (K) Typical histogram for expression levels of integrin α4 subunit on the surface of Kasumi-1 cells transduced with mock or PLZF and (L) quantitative value thereof.

Suppl. Fig. 2

A)



B)

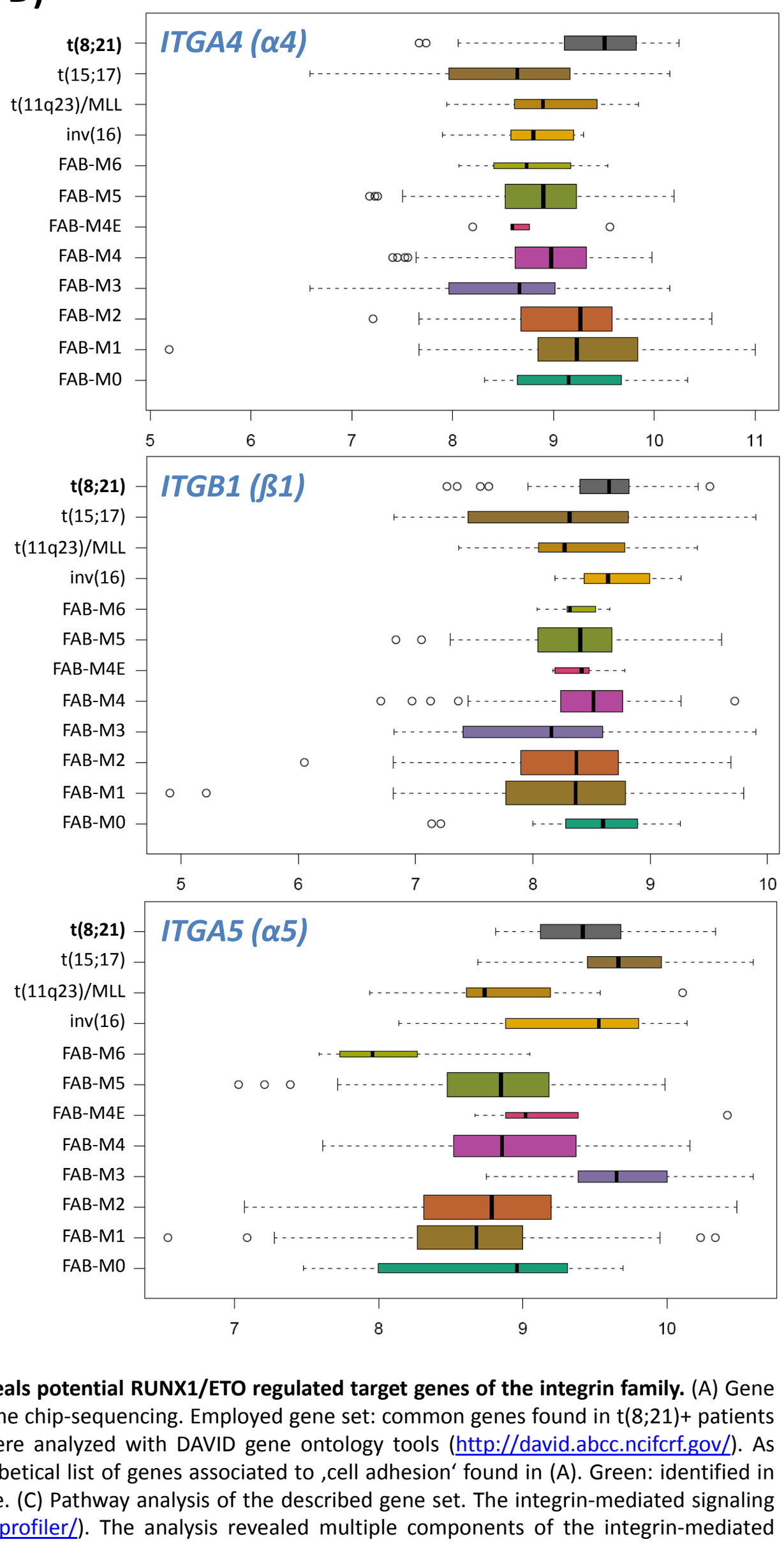
‚cell adhesion' associated candidates, chip-seq common genes t(8;21) patients + Kasumi-1	
ADAM metallopeptidase domain 8	integrin, beta 1 (ß1)
B-cell CLL/lymphoma 2	integrin, beta 2
CD164 molecule, sialomucin	intercellular adhesion molecule 3
CD300a molecule	metastasis suppressor 1
CD34 molecule	mucin 4, cell surface associated
CD44 molecule	myelin associated glycoprotein
CD47 molecule	myelin protein zero-like 3
CD58 molecule	myosin binding protein C, cardiac
CD9 molecule	myosin, heavy chain 9, non-muscle
CD97 molecule	neurexin 2
F11 receptor	neuronal growth regulator 1
PTK2B protein tyrosine kinase 2 beta	neuropilin 1
Beta-1,4-Galactosyltransferase 2	ninjurin 1
cadherin-like 23	ninjurin 2
carboxypeptidase X (M14 family), member 1	parvin, gamma
catenin, delta 1	paxillin
chemokine (C-C motif) ligand 5	PPARD
claudin 14	plexin C1
coagulation factor V	CD2BP1
G-CSF-R	PTP1B
CDK5R1	ras homolog gene family, member B
elastin microfibril interfacer 1	RAC1
endoglin	selectin P ligand
endomucin	sialic acid binding Ig-like lectin 8
FEZ1	sorbin and SH3 domain containing 1
flotillin 2	stabilin 1
glycoprotein Ib (platelet), alpha polypeptide	sushi, nidogen and EGF-like domains 1
hairy and enhancer of split 1	syndecan 3
heparan sulfate proteoglycan 2	SSX2IP
insulin-like growth factor binding protein 7	thrombospondin 2
integrin, alpha 4 (α4)	SRC
integrin, alpha M	

described identified

C)

gPROFILER pathway analysis: integrin-mediated signaling pathway GO:0007229 ; (p-value 4.46e-03)	
membrane	**intracellular signaling**
ADAM11	MYH9
CD47	PRAM1
FGR	PTK2,PTK2B
ITGA4 (α4)	PXN
ITGAM	RCC2, RAC1
ITGB1 (ß1)	TEC
ITGB2	TSPAN32
	TYROBP
	VAV1, VAV3

D)



Supplementary Figure 2. *In silico* analyses of chip-sequencing data reveals potential RUNX1/ETO regulated target genes of the integrin family. (A) Gene ontology analysis of RUNX1/ETO target genes identified by whole-genome chip-sequencing. Employed gene set: common genes found in t(8;21)+ patients and Kasumi-1 cells (1336 genes; Ptasinska et al., 2012). Gene lists were analyzed with DAVID gene ontology tools (http://david.abcc.ncifcrf.gov/). As depicted, about 5% of genes are associated to 'cell adhesion'. (B) Alphabetical list of genes associated to ‚cell adhesion' found in (A). Green: identified in our study; yellow: described in the literature as RUNX1/ETO target gene. (C) Pathway analysis of the described gene set. The integrin-mediated signaling pathway was identified with gPROFILER analysis (http://biit.cs.ut.ee/gprofiler/). The analysis revealed multiple components of the integrin-mediated signaling pathway as potential RUNX1/ETO targets, suggesting that RUNX1/ETO disturbs the integrin signaling pathways at multiple levels. (D) Analysis of AML gene array data sets obtained from patients with t(8;21), t(15;17), t(11q23)/MLL, inv(16) and AML FAB subgroups (Verhaak et al., 2009) through leukemia gene atlas (http://www.leukemia-gene-atlas.org/LGAtlas/; Hebestreit et al., 2012). Depicted are results for expression levels of ITGA4 (α4), ITGB1 (ß1) and ITGA5 (α5) in the respective AML subgroups.

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Verhaak RG, Wouters BJ, Erpelinck CA, Abbas S, Beverloo HB, Lugthart S, et al. Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling. Haematologica. 2009 Jan;94(1):131-4.

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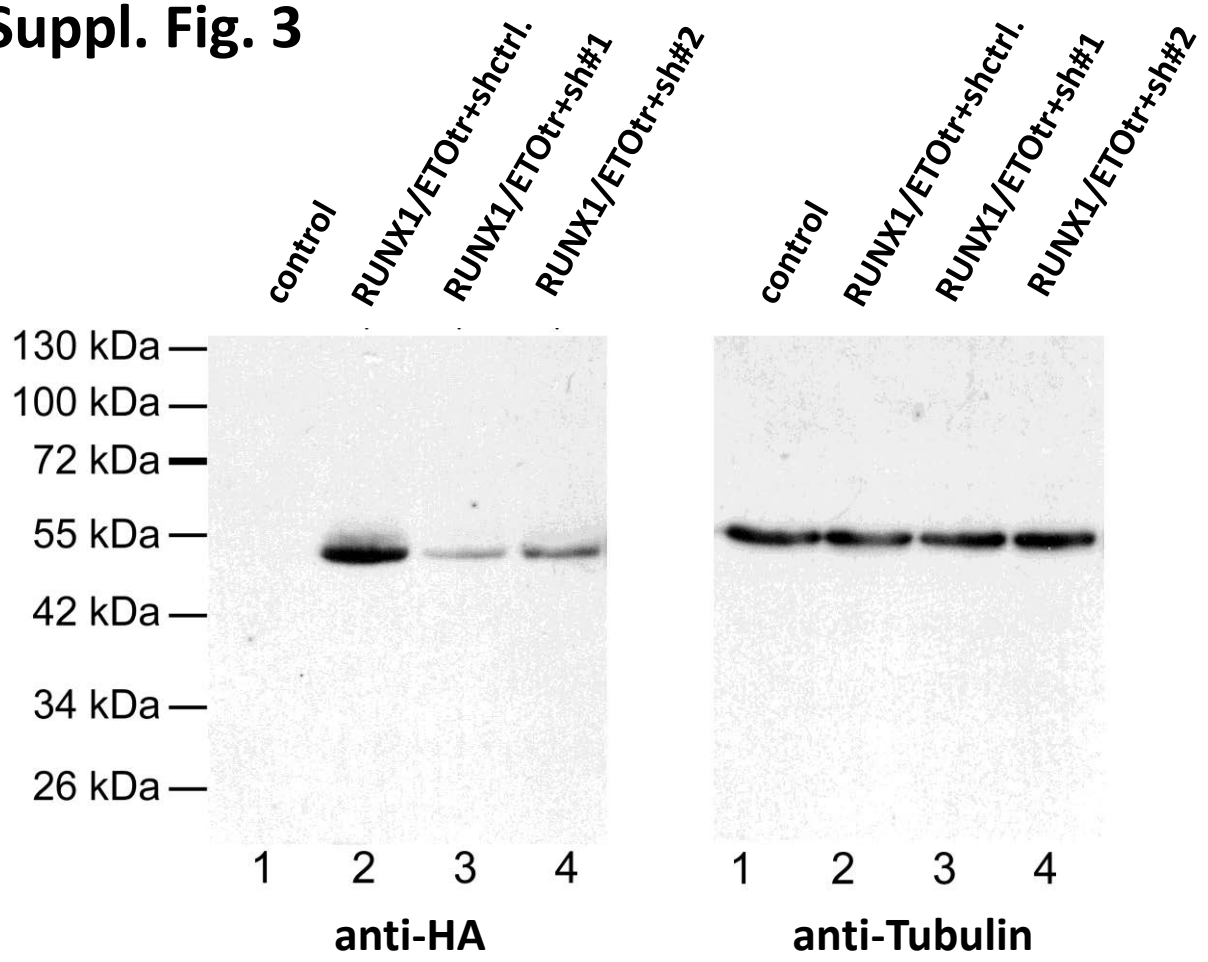
Suppl. Fig. 3



Supplementary Figure 3. Validation of shRNAs targeting the breakpoint of RUNX1/ETO. Two different shRNA sequences were cloned into the lentiviral expression vector SiEW and tested for their efficiency to downregulate RUNX1/ETOtr. 293T cells were transfected with expression vectors for RUNX1/ETOtr and the indicated shRNA. Cellular lysates of transfected 293T cells were analyzed for HA-RUNX1/ETOtr and Tubulin as loading control by western blotting analyses. Sh#1 was used for further experiments (shRE). shRNA#1: 5'-ACCTC ACCTCGAAATCGTACTGAGAA TCAAGAG TTCTCAGTACGA TTTCGAGGT TT-3'; shRNA#2: 5'-ACCTCC CTCGAAATCGTACTGAGAAG TCAA GAG CTTCTCAGTACGATTTCGAGG TT-3' (underlined: target sequences).