Supplemental Materials

Transcritional Regulatory Networks Downstream of TAL1/SCL in T-cell Acute Lymphoblastic Leukemia

Palomero et al.

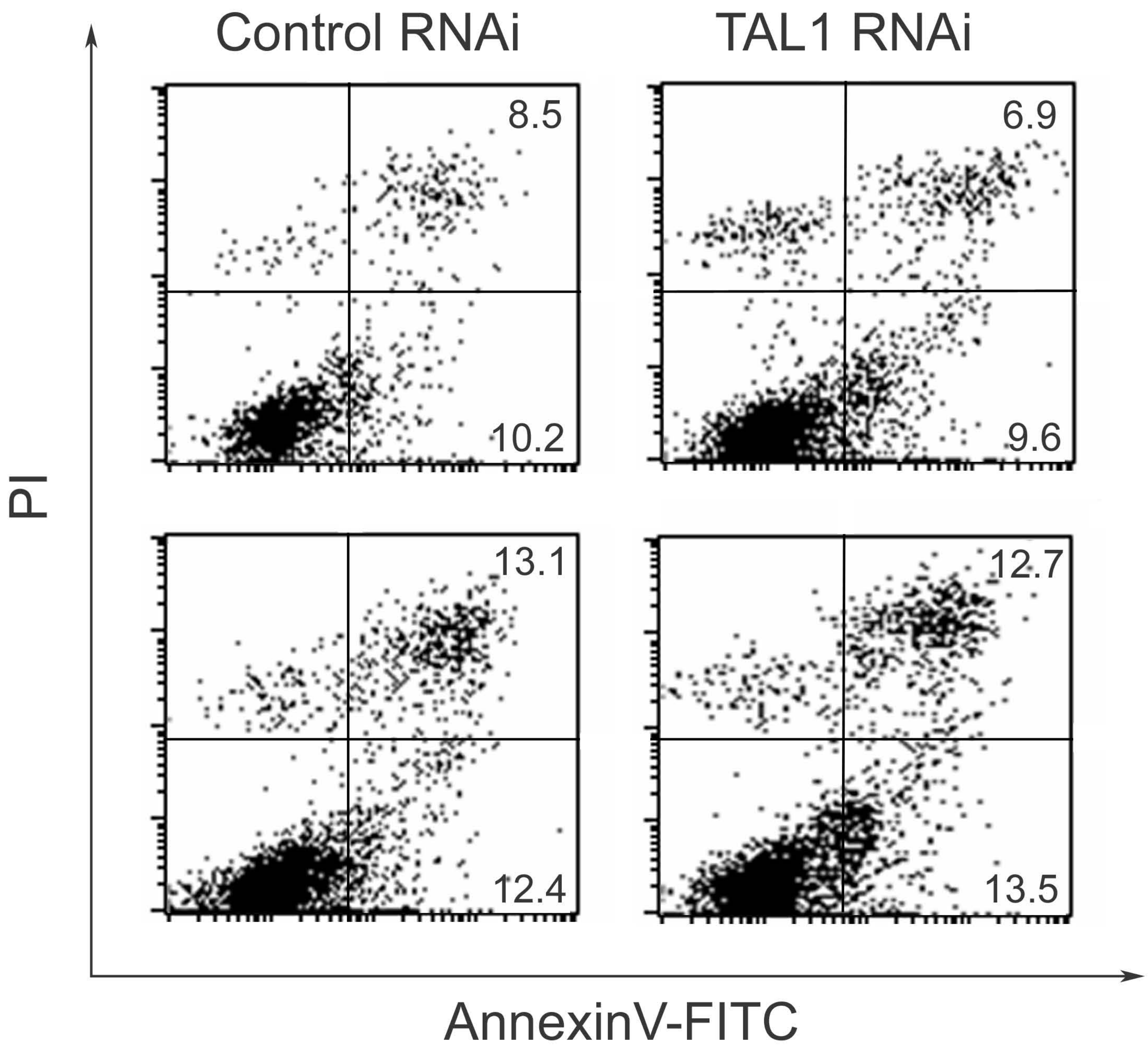
Control RNAi
TAL1 RNAi
8.5
10.2
6.9
9.6
13.1
12.4
12.7
13.5
PI
AnnexinV-FITC

Figure S1. Downregulation of TAL1 by RNA interference does not affect apoptosis in Jurkat cells. Annexin V staining was used to quantify apoptosis rates in Jurkat cell clones expressing TAL1 shRNA (right panels) or a control shRNA (left panels). The percentage of apoptotic cells is indicated in the bottom right corner of each graph, while the dead cell percentage is indicated in the top right corner. PI: propidium iodide.

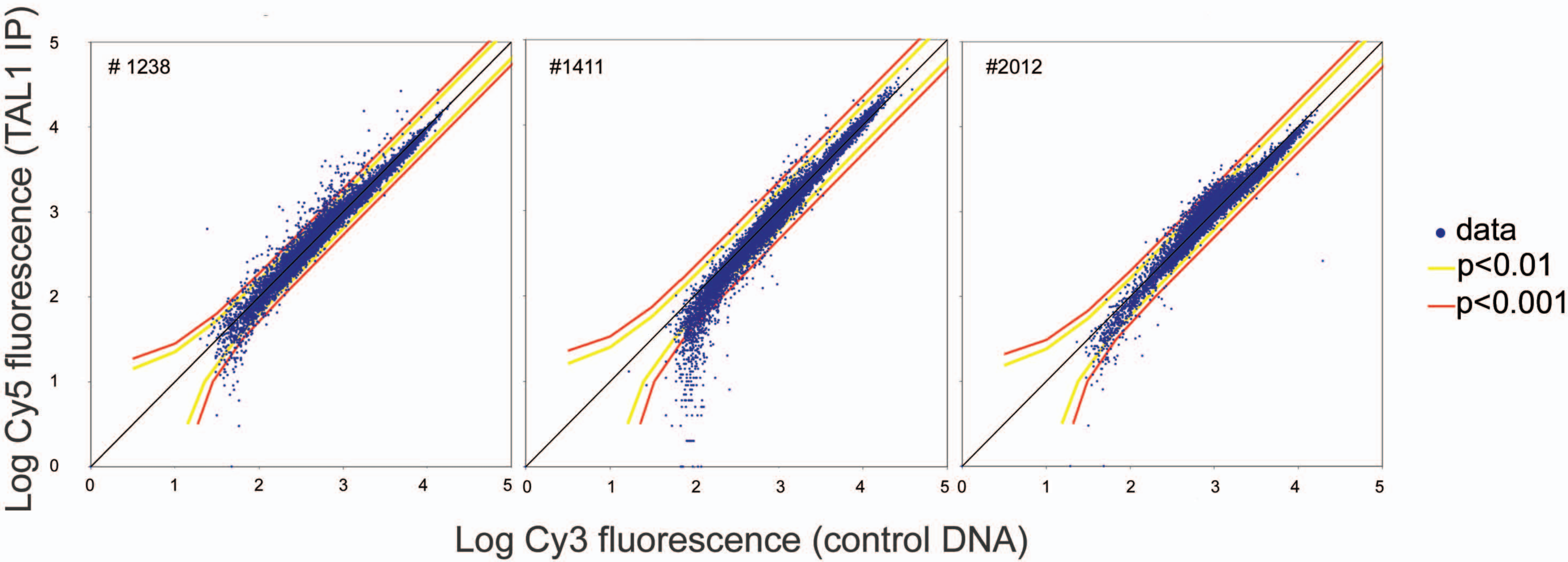

Figure S2. Scatter plots of chromatin immunoprecipitations performed with TAL1 #370 antibody in Jurkat cells. The array number is indicated in the top corner of each graph. The confidence levels calculated by the error model are represented by the red and yellow lines and the corresponding p-values are indicated in the graph legend.

A

B

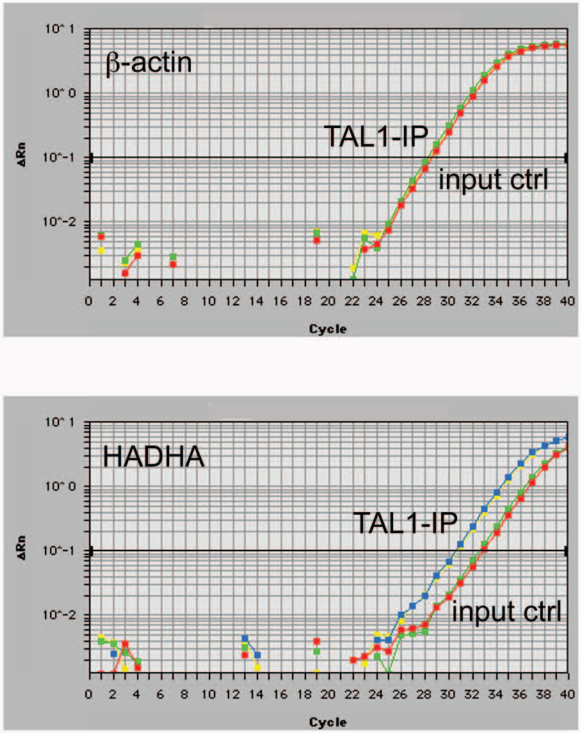

Figure S3. Target validation by quantitative real-time PCR (qRT-PCR). A. Amplification of β-actin in the control DNA (input control, green and red lines) and in the TAL1 immunoprecipitate (TAL1-IP yellow and blue lines) B. Amplification of a representative TAL1 target, HADHA promoter region, in the control genomic DNA and the TAL1 immunoprecipitate. Note the 2-cycle difference (4 fold enrichment) between the amount of HADHA in the control and the TAL1 immunoprecipitate. Differences over 1-cycle (2-fold) in the amplification of promoter regions bound by TAL1 in control DNA and TAL1 immunoprecipitates were considered to verify the binding. In the graphs, the number of amplification cycles is in the X axis, while the intensity of the fluorescence of the amplified product is represented in the Y axis (see also Estimation of False Positive Rates in the Supplemental Materials).

Name	Description	p-value
IFRD1	interferon-related developmental regulator 1	0.009927703
PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	0.00445631
ATRX	alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)	0.010847575
CDK6	cyclin-dependent kinase 6	0.044093956

Table S1. Genes regulated by TAL1 knockdown are identified as direct target of this transcription factor using ChIP on chip. The p-value determined by the error model applied to the ChIP on chip fluorescence data is indicated in the right column.

Name	Description	TAL1	E2A	HEB
Signal transduction-Receptor				
MUC16	mucin 16	red	red	blue
PTPRU	protein tyrosine phosphatase, receptor type, U	red	red	red
IL10RA	interleukin 10 receptor, alpha	red	blue	red
EPHB1	EphB1	blue	blue	blue
IFNAR1	interferon (alpha, beta and omega) receptor 1	red	red	blue
MUC1	mucin 1, transmembrane	red	blue	blue
CALCYON	calcyon; D1 dopamine receptor-interac. protein	red	blue	blue
PTPRCAP	protein tyrosine phosphatase, receptor type	red	red	red
OR2W1	olfactory receptor, fam. 2, subfam. W, member 1	blue	blue	blue
TMEFF1	transmembrane protein with EGF-like domains	red	red	blue
Signal transduction-Other				
TRAF3	TNF receptor-associated factor 3	blue	blue	blue
TNFAIP1	tumor necrosis factor, alpha-induced protein 1	red	blue	red
MLLT4	MLL leukemia translocated to, 4 (AF4)	red	red	blue
MADHIP	MAD interacting protein	red	blue	blue
CGR11	cell growth regulatory with EF-hand domain	blue	red	blue
ARAF1	v-raf murine viral oncogene homolog 1	red	red	red
RQCD1	RCD1 required for cell differentiation1 homolog	blue	red	red
TTC3	tetratricopeptide repeat domain 3	blue	blue	blue
Transcription Regulation				
NR4A3	nuclear receptor subfam. 4, group A, 3	red	red	red
DED	apoptosis antagonizing transcription factor	red	red	red
NFYA	nuclear transcription factor Y, alpha	red	blue	blue
ZNF74	zinc finger protein 74 (Cos52)	red	blue	blue
GS2NA	nuclear autoantigen	blue	red	red
LANP-L	leucine-rich acidic protein-like protein	blue	blue	blue
TCF7	transcription factor 7 (T-cell specific, HMG-box)	red	blue	blue
DNA repair				
G22P1	thyroid autoantigen 70kD (Ku antigen)	red	red	red
PMS2	PMS2 postmeiotic segregation increased 2	red	red	red
Ligands				
AZGP1	alpha-2-glycoprotein 1, zinc	red	red	red
MSLN	mesothelin	red	blue	blue
GDF5	growth differentiation factor 5	red	blue	blue
LOC56920	semaphorin sem2	blue	red	blue
Transporters-channel/pore				
CHRNA5	cholinergic receptor, nicotinic, alpha polypeptide 5	red	red	red
ACCN2	amiloride-sensitive cation channel 2, neuronal	blue	blue	blue
CACNG4	calcium channel, voltage-dep., γ sub. 4	red	blue	red
KCNJ9	potassium inwardly-rect. channel, subfam. J, 9	red	blue	blue
SLC4A11	solute carrier family 4	red	blue	blue
OKB1	organic cation transporter OKB1	red	red	red
Transporters-lipids/small molecules				
ABCC12	ATP-binding cassette, sub-fam. C (CFTR/MRP)	blue	red	blue
Protein degradation and processing				
PRSS8	protease, serine, 8 (prostasin)	red	blue	blue
SERPINB2	serine (or cysteine) proteinase inhibitor	red	blue	blue
CUL4A	cullin 4A	red	blue	blue
Vesicle trafficking, storage and cytoskeleton				
RAB33A	RAB33A, member RAS oncogene family	red	red	red
MAP2	microtubule-associated protein 2	red	blue	blue
RPS3A	ribosomal protein S3A	red	red	red
LRBA	vesicle trafficking, beach and anchor cont.	red	red	red
RAB40B	RAB40B, member RAS oncogene family	grey	grey	grey
DOCK1	dedicator of cyto-kinesis 1	red	red	blue
AP4B1	adaptor-related protein complex 4, beta 1 subunit	red	red	red
Enzymes				
NCF1	neutrophil cytosolic factor 1	red	blue	blue
DDC	dopa decarboxylase	blue	blue	blue
HADHA	hydroxyacyl-Coenzyme A dehydrog. (...) alpha	red	red	red
BHMT2	betaine-homocysteine methyltransferase 2	red	blue	blue
MVD	mevalonate (diphospho) decarboxylase	red	blue	red
MGAT3	mannosyl -acetylglucosaminyltransferase	blue	blue	red
LOC51171	retinal short-chain dehydrog./red. retSDR3	red	red	red
SULT1A3	sulfotransferase family, cytosolic, 1A, member 3	red	red	red
ARSA	arylsulfatase A	red	red	red
PTE1	peroxisomal acyl-CoA thioesterase	red	blue	red
Immune-system related proteins				
PLA2G2A	phospholipase A2, group IIA	red	blue	blue
C3	complement component 3	red	blue	blue
Unknown function				
LOC51184	protein x 0004	red	red	red
FRG1	FSHD region gene 1	red	blue	blue
FLJ12150	hypothetical protein FLJ12150	red	blue	blue
FLJ22529	hypothetical protein FLJ22529	red	red	red
FLJ14981	hypothetical protein FLJ14981	red	blue	blue
FLJ10661	hypothetical protein FLJ10661	red	red	red
MGC14136	hypothetical protein MGC14136	red	red	red
FLJ10637	hypothetical protein FLJ10637	red	red	red
PRO2859	hypothetical protein PRO2859	red	blue	blue
LOC51035	ORF	red	blue	blue
FLJ23231	hypothetical protein FLJ23231	red	red	red

Table S2. Analysis of TAL1, E2A and HEB binding to TAL1 direct targets identified by ChIP on chip by gene-specific quantitative PCR. Binding is confirmed when a two-fold enrichment is detected in the immunoprecipitate versus the control whole cell extract. Verified binding is represented in red, while blue indicates binding was not detected. Grey symbol in RAB40B promoter indicates no amplification.

Symbol	Name	Genomic location	Cell line/ Tissue	Reference
-	otogelin-like gene	intron	erythoid lineage (erythroleukemia cell lines and embryonic differenciation towards the erythroid/megakaryocityc lineage	Cohen-Kaminsky et al., 1998
RALDH2	retinaldehyde dehydrogenase2	intron	T-ALL cell line	Ono et al., 1998
TCRA	T-cell receptor alpha precursor	enhancer	T-ALL cell line	Bernard et al., 1998
GATA1	GATA binding protein 1 (globin transcription factor 1)	enhancer	in vitro experiments	Vyas et al., 1999
PTCRA	pre-T-cell receptor alpha precursor	enhancer	primitive and preleukemic mouse thymocytes	Herblot at al., 2000; Tremblay et al. 2003; Hansson et al, 2003
CD4	CD4 antigen	enhancer	thymocytes and T-ALL cell lines and mouse T-ALL	Herblot at el., 2000; O'Neil et al., 2004
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	proximal promoter	hematopoietic progenitors and erythroleukemia cell lines	Lecuyer et al., 2002
p4.2	protein 4.2	proximal promoter	erythroleukemia cell line	Xu et al., 2003
p16INK4A	cyclin-dependent kinase inhibitor 2A; CDKN2A	proximal promoter	in vitro experiments, reporter assays in non hematopoietic cell lines	Hansson et al., 2003
GPA	GlycophorinA	proximal promoter	erythroleukemia cell line	Lahlil et al., 2004

Table S3. Previously described TAL1 targets

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