TAL1 cooperates with PI3K/AKT pathway activation in T-cell acute lymphoblastic leukemia

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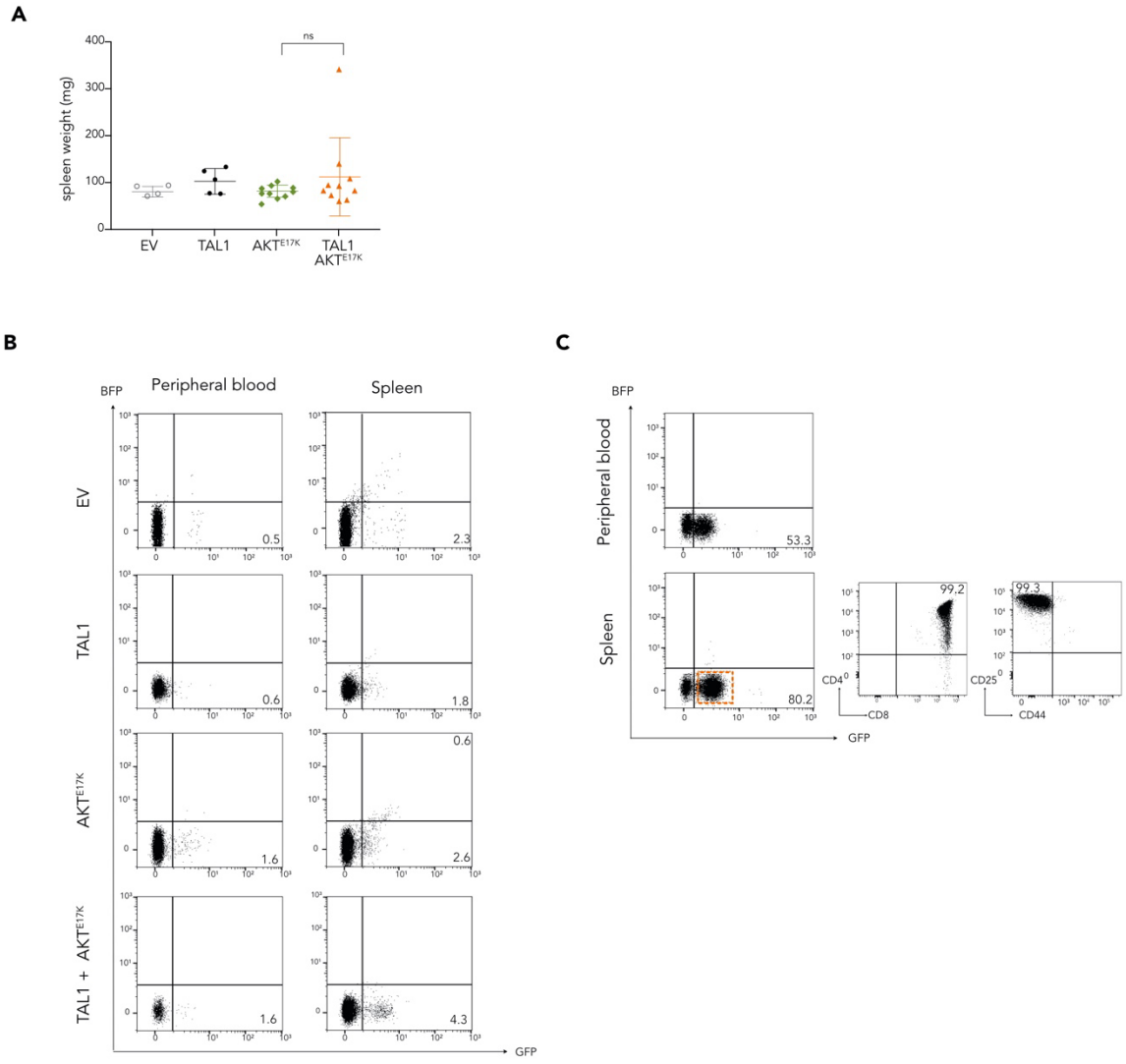
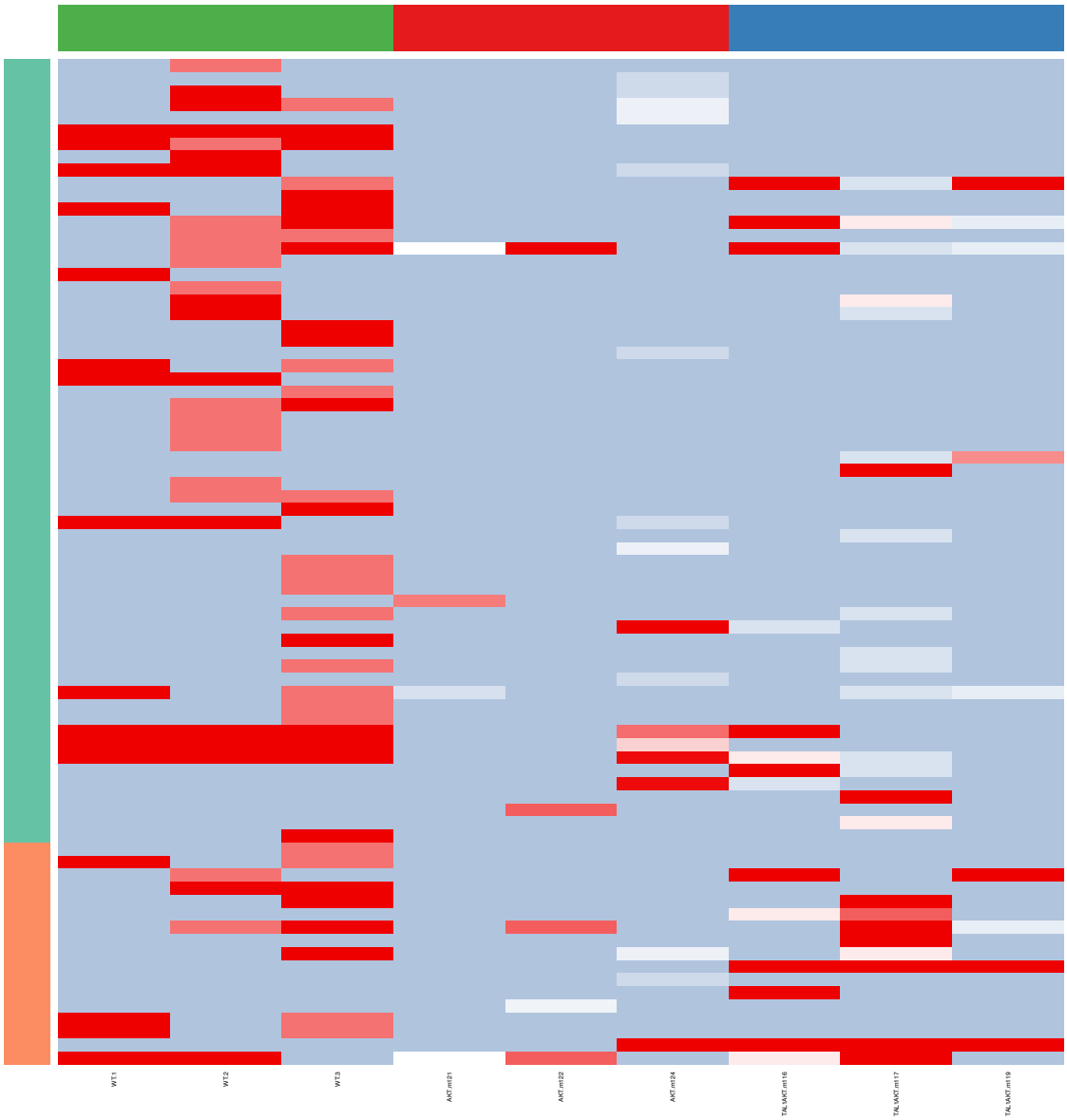
Supplementary Figures



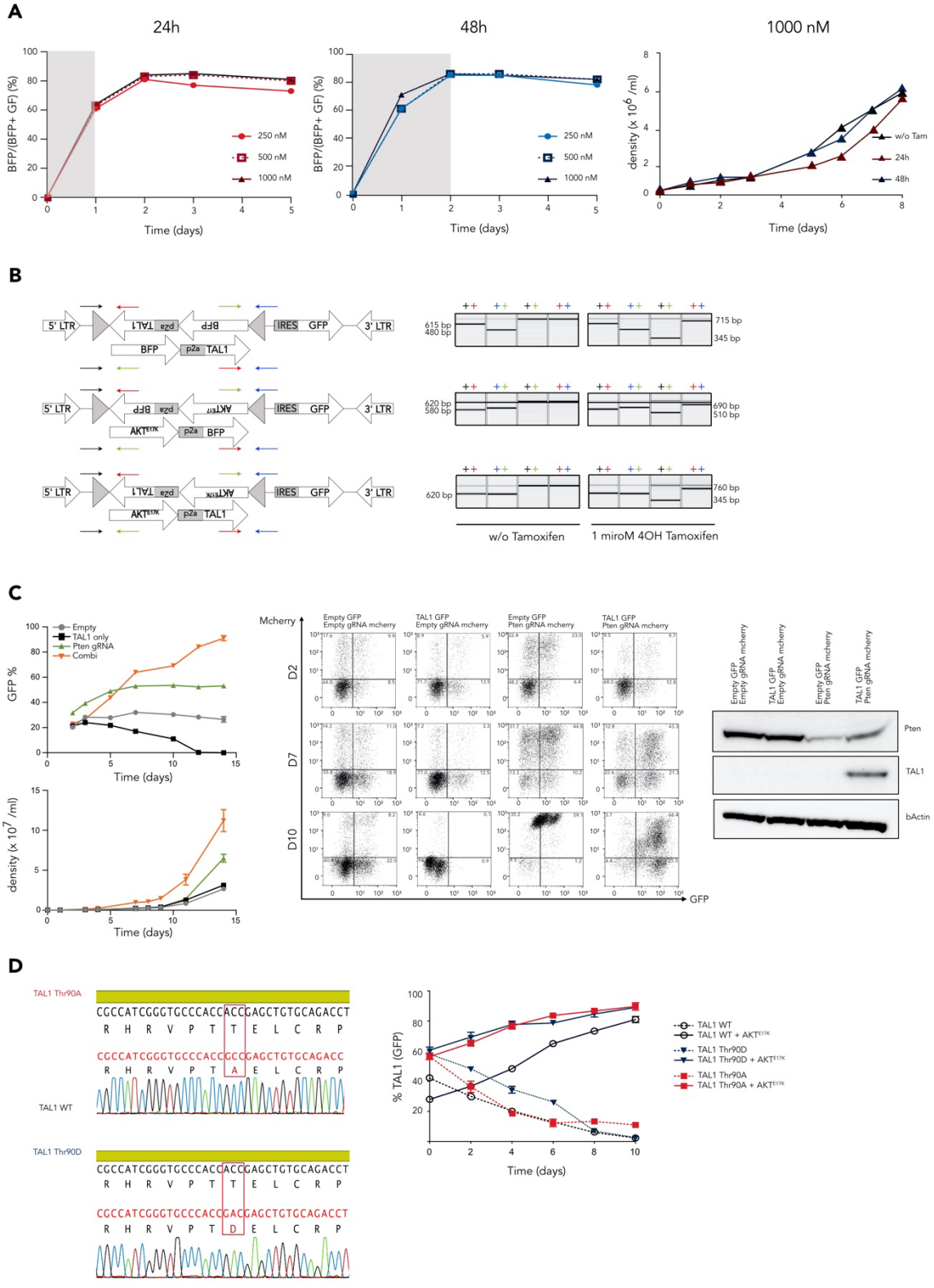
Supplementary Figure 1.

A, Plot showing spleen weight at time of sacrifice. P-values were calculated using the one-way ANOVA with Tukey correction to account for multiple comparisons. **B**, Flow cytometry analysis of peripheral blood and spleen at time of sacrifice- BFP y-axis; GFP x-axis. **C**, Flow cytometry analysis of peripheral blood and spleen of secondary transplant mouse. Spleen cells were) were stained for CD8 (APC-eFluor 780, X-axis) and CD4 (PE-Cy7, Y axis) and stained for CD25 (APC, X-axis) and CD44 (PerCP-Cy5.,5 Y-axis).



Supplementary Figure 2. Clonality of mouse lymphoma/leukemia.

RNA-seq data from RNA extracted from thymus cells in wild type mice (n=3, left), AKT(E17K) (n=3, middle) or TAL1+AKT(E17K) (n=3, right) lymphoma/leukemia cells (primary disease) was analyzed for the expression of the various variable regions for T-cell receptor alpha or beta. Upon rearrangement of the T-cell receptors, only one variable region is selected and expressed and therefore expression data provides an estimate of the various clones present in thymus. The pattern observed in AKT(E17K) or TAL1+AKT(E17K) mice corresponds to an oligoclonal origin of the disease.


A
24h
48h
1000 nM
BFP/(BFP+ GF) (%)
Time (days)
250 nM
500 nM
1000 nM
density (x 10^6 /ml)
w/o Tam
24h
48h
B
5' LTR
TAL1
BFP
IRES
GFP
3' LTR
AKT^E17K
615 bp
480 bp
715 bp
345 bp
620 bp
580 bp
690 bp
510 bp
760 bp
w/o Tamoxifen
1 miroM 4OH Tamoxifen
C
Empty
TAL1 only
Pten gRNA
Combi
GFP %
density (x 10^7 /ml)
Mcherry
D2
D7
D10
GFP
Empty GFP Empty gRNA mcherry
TAL1 GFP Empty gRNA mcherry
Empty GFP Pten gRNA mcherry
TAL1 GFP Pten gRNA mcherry
Pten
TAL1
bActin
D
TAL1 Thr90A
CGCCATCGGGTGCCCACCACCGAGCTGTGCAGACCT
R H R V P T T E L C R P
CGCCATCGGGTGCCCACCGCCGAGCTGTGCAGACC
R H R V P T A E L C R P
TAL1 WT
TAL1 Thr90D
CGCCATCGGGTGCCCACCACCGAGCTGTGCAGACCT
R H R V P T T E L C R P
CGCCATCGGGTGCCCACCGACGAGCTGTGCAGACCT
R H R V P T D E L C R P
% TAL1 (GFP)
TAL1 WT
TAL1 WT + AKT^E17K
TAL1 Thr90D
TAL1 Thr90D + AKT^E17K
TAL1 Thr90A
TAL1 Thr90A + AKT^E17K

Supplementary Figure 3.

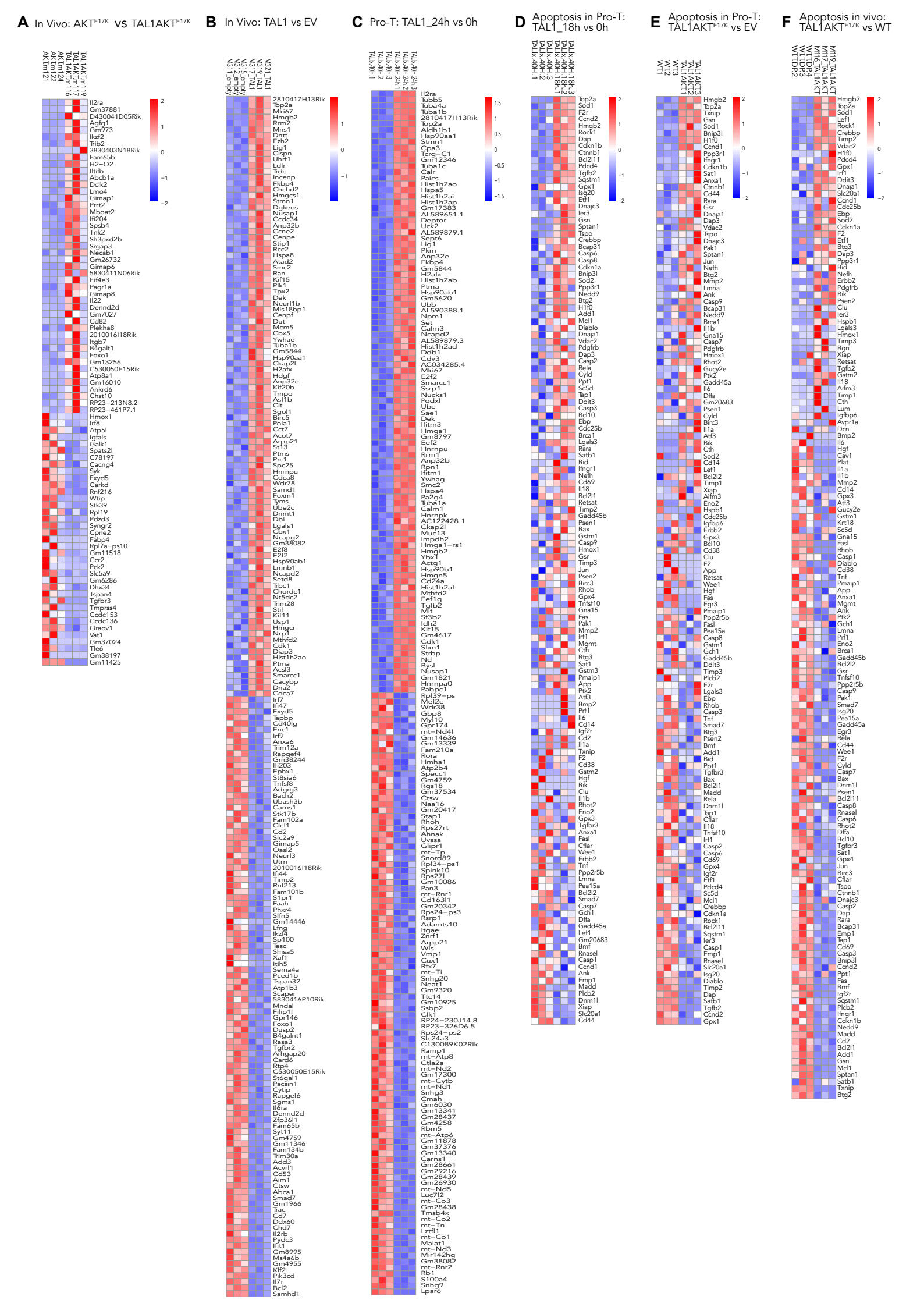
A, Treatment of Cre-ER cells with empty vector: percentage of floxed cells (= BFP/(BFP+GFP)) over time. Cells were treated with different doses of 4-OH-Tamoxifen (250-500-100 nM). Cells were treated during 24h (left) or during 48h (middle). Right: effect on cell viability after treatment with 1000 nM 4OH-Tamoxifen for different hours. **B**, Schematic overview of different inducible constructs. Colored arrows indicate different primers (left). Results of PCR with indicated primers after DNA extraction of pro-T cells showing successful flipping of the construct on DNA level. (right). **C**, Pro-T cells derived from a CAS9 donor mice and transduced with different constructs. Left: growth curves showing GFP% (= TAL1 or empty vector) over time and cell density (mean with SD) over time, both showing that TAL1-Pten^{del} combination rescues TAL1 cell growth. Middle: Flow cytometry analysis of pro-T cells at different time points. Right: Western blotting, confirming expression of TAL1 in TAL1-Pten^{del} transformed pro-T cells and confirming deletion of Pten in Pten^{del} and TAL1-Pten^{del} cells. **D**, Results of sanger sequencing after PCR-mutagenesis of TAL1 showing point mutations at Thr90. Threonine was either replaced by an alanine (above) or an aspartic acid (below). Right: % of GFP = (TAL1) over time of different TAL1 variants (WT, Thr90A, Thr90D).

(next page)

Supplementary Figure 4. Heatmaps of deregulated genes in pro-T cells or mouse models.

Figure shows selected heatmaps of comparisons between various conditions in pro-T cells or mouse lymphoma/leukemia models. Genes are indicated in rows, samples in columns.

Additional data on deregulated gene expression based on RNA-seq data is provided in supplementary excel tables.


A In Vivo: AKT^{E17K} vs TAL1AKT^{E17K}
AKTm121
AKTm122
AKTm124
TAL1AKTm116
TAL1AKTm117
TAL1AKTm119
Il2ra
Gm37881
D430041D05Rik
Agfg1
Gm973
Ikzf2
Trib2
3830403N18Rik
Fam65b
H2−Q2
Itifb
Abcb1a
Dclk2
Lmo4
Gimap1
Prrt2
Mboat2
Ifi204
Spsb4
Tnk2
Sh3pxd2b
Srgap3
Necab1
Gm26732
Gimap6
5830411N06Rik
Eif4e3
Pagr1a
Gimap8
Il22
Dennd2d
Gm7027
Cd82
Plekha8
2010016I18Rik
Itgb7
B4galt1
Foxo1
Gm13256
C530050E15Rik
Atp8a1
Gm16010
Ankrd6
Chst10
RP23−213N8.2
RP23−461P7.1
Hmox1
Irf8
Atp5l
Igfals
Galk1
Spats2l
C78197
Cacng4
Syk
Fxyd5
Carkd
Rnf216
Wtip
Stk39
Rpl19
Pdzd3
Syngr2
Cpne2
Fabp4
Rpl7a−ps10
Gm11518
Ccr2
Pck2
Slc5a9
Gm6286
Dhx34
Tspan4
Tgfbr3
Tmprss4
Ccdc153
Ccdc136
Oraov1
Vat1
Gm37024
Tle6
Gm38197
Gm11425
2
1
0
−1
−2
B In Vivo: TAL1 vs EV
M321_TAL1
M317_TAL1
M319_TAL1
M312_empty
M313_empty
M311_empty
2810417H13Rik
Top2a
Mki67
Hmgb2
Rrm2
Mns1
Dntt
Ezh2
Lig1
Clspn
Uhrf1
Ldlr
Trdc
Incenp
Fkbp4
Chchd2
Hmgcs1
Stmn1
Dgkeos
Nusap1
Ccdc34
Anp32b
Ccne2
Cenpe
Stip1
Rcc2
Hspa8
Atad2
Smc2
Ran
Kif15
Plk1
Tpx2
Dek
Neurl1b
Mis18bp1
Cenpf
Dut
Mcm5
Cbx5
Ywhae
Tuba1b
Gm5844
Hsp90aa1
Ckap2l
H2afx
Hdgf
Anp32e
Kif20b
Tmpo
Asf1b
Cit
Sgol1
Birc5
Pola1
Cct7
Acot7
Arpp21
St13
Ptms
Prc1
Spc25
Hnrnpu
Cdca8
Wdr78
Samd1
Foxm1
Tyms
Ube2c
Dnmt1
Dbi
Lgals1
Cbx1
Ncapg2
Gm38082
E2f8
E2f2
Hsp90ab1
Lmnb1
Ncapd2
Setd8
Trbc1
Chordc1
Nt5dc2
Trim28
Stil
Kif11
Usp1
Hmgcr
Nrp1
Mthfd2
Cdk1
Diap3
Hist1h2ao
Ptma
Acsl3
Smarcc1
Cacybp
Dna2
Cdca7
Irf7
Ifi47
Fxyd5
Tapbp
Cd40lg
Enc1
Irf9
Anxa6
Trim12a
Rapgef4
Gm38244
Ifi203
Ephx1
St8sia6
Tnfsf8
Adgrg3
Bach2
Ubash3b
Carns1
Stk17b
Fam102a
Clcf1
Cd2
Slc2a9
Gimap5
Oasl2
Neurl3
Utrn
2010016I18Rik
Ifi44
Timp2
Rnf213
Fam101b
S1pr1
Faah
Phxr4
Slfn5
Gm14446
Lfng
Ikzf4
Sp100
Tesc
Shisa5
Xaf1
Itih5
Sema4a
Pced1b
Tspan32
Atp1b3
Scaper
5830416P10Rik
Mndal
Filip1l
Gpr146
Foxo1
Dusp2
B4galnt1
Rasa3
Tgfbr2
Arhgap20
Card6
Rtp4
C530050E15Rik
St6gal1
Pacsin1
Cytip
Rapgef6
Sgms1
Il6ra
Dennd2d
Zfp36l1
Fam65b
Syt11
Gm4759
Gm11346
Fam134b
Trim30a
Add3
Acvrl1
Cd53
Aim1
Ctsw
Abca1
Smad7
Gm1966
Trac
Cd7
Ddx60
Chd7
Il2rb
Pydc3
Ifit1
Gm8995
Ms4a6b
Gm4955
Klf2
Pik3cd
Il7r
Bcl2
Samhd1
2
1
0
−1
C Pro-T: TAL1_24h vs 0h
TALk 40h.1
TALk 40h.2
TALk 40h.3
TALk 40h 24h.1
TALk 40h 24h.2
TALk 40h 24h.3
Il2ra
Tubb5
Tuba4a
Tuba1b
2810417H13Rik
Top2a
Aldh1b1
Hsp90aa1
Stmn1
Cpa3
Tcrg−C1
Gm12346
Tuba1c
Calr
Paics
Hist1h2ao
Hspa5
Hist1h2ai
Hist1h2ap
Gm17383
AL589651.1
Deptor
Ube2c
AL589879.1
Sept6
Lig1
Pkm
Anp32e
Fkbp4
Gm5844
H2afx
Hist1h2ab
Ptma
Hsp90ab1
Gm5620
Ubb
AL590388.1
Npm1
Set
Calm3
Ncapd2
AL589879.3
Hist1h2ad
Ddb1
Cdv3
AC034285.4
Mki67
E2f2
Smarcc1
Ssrp1
Nucks1
Podxl
Ubc
Sae1
Dek
Ifitm3
Hmga1
Gm8797
Eef2
Hnrnpu
Rrm1
Anp32b
Rpn1
Ifitm1
Ywhag
Smc2
Hspa4
Pa2g4
Tuba1a
Calm1
Hnrnpk
AC122428.1
Ckap2l
Muc13
Impdh2
Hmga1−rs1
Hmgb2
Ybx1
Actg1
Hsp90b1
Hnrnp5
Cd24a
Hist1h2af
Mthfd2
Eef1g
Tgfb2
Mif
Sf3b2
Idh2
Kif15
Gm4617
Cdk1
Sfxn1
Strbp
Ncl
Bysl
Nusap1
Gm1821
Hnrnpa0
Pabpc1
Rpl39−ps
Mef2c
Wdr38
Gbp8
Myl10
Gpr174
mt−Nd4l
Gm14636
Gm13339
Fam210a
Rora
Hmha1
Atp2b4
Specc1
Gm4759
Rgs18
Gm37534
Ctsw
Naa16
Gm20417
Stap1
Rhoh
Rps27rt
Ahnak
Uvssa
Cliprl
mt−Tp
Snord89
Rpl34−ps1
Spink10
Rps27l
Gm10086
Pan3
mt−Rnr1
Cd163l1
Gm20342
Rps24−ps3
Rsrp1
Adamts10
Itgae
Znrf1
Arpp21
Wls
Vmp1
Cux1
Rfx7
mt−Ti
Snhg20
Neat1
Gm9320
Ttc14
Gm10925
Sbpp2
Clk1
RP24−230J14.8
RP23−326D6.5
Rps24−ps2
Slc24a3
C130089K02Rik
Ramp1
mt−Atp8
Ctla2a
mt−Nd2
Gm17300
mt−Cytb
mt−Nd1
Snhg3
Cmah
Gm6030
Gm13341
Gm28437
Gm4258
Rbm5
mt−Atp6
Gm11878
Gm37376
Gm13340
Carns1
Gm28661
Gm29216
Gm28439
Gm26930
mt−Nd5
Luc7l2
mt−Co3
Gm28438
Tmsb4x
mt−Co2
mt−Tn
Lztfl1
mt−Co1
Malat1
mt−Nd3
Mir142hg
Gm38082
mt−Rnr2
Rb1
S100a4
Snhg9
Lpar6
1.5
1
0.5
0
−0.5
−1
−1.5
D Apoptosis in Pro-T: TAL1_18h vs 0h
TALk 40h.1
TALk 40h.2
TALk 40h.3
TALk 40h 18h.1
TALk 40h 18h.2
TALk 40h 18h.3
Top2a
Sod1
F2r
Ccnd2
Hmgb2
Rock1
Dap
Cdkn1b
Ctnnb1
Bcl2l11
Pdcd4
Tgfb2
Sqstm1
Gpx1
Isg20
Etf1
Dnajc3
Ier3
Gsn
Sptan1
Tspo
Crebbp
Bcap31
Casp6
Casp8
Cdkn1a
Bnip3l
Sod2
Ppp3r1
Nedd9
Btg2
H1f0
Add1
Mcl1
Diablo
Dnaja1
Vdac2
Pdgfrb
Dap3
Casp2
Rela
Cyld
Ppt1
Sc5d
Tap1
Ddit3
Casp3
Bcl10
Ebp
Cdc25b
Brca1
Lgals3
Rara
Satb1
Bid
Ifngr1
Nefh
Cd69
Il18
Bcl2l1
Retsat
Timp2
Gadd45b
Psen1
Bax
Gstm1
Casp9
Hmox1
Gsr
Timp3
Jun
Psen2
Birc3
Rhob
Gpx4
Tnfsf10
Gna15
Fas
Pak1
Mmp2
Irf1
Mgmt
Cth
Btg3
Sat1
Gstm3
Pmaip1
App
Ptk2
Atf3
Bmp2
Prf1
Il6
Cd14
Igf2r
Cd2
Il1a
Txnip
F2
Cd38
Gstm2
Hgf
Bik
Clu
Il1b
Rhot2
Eno2
Gpx3
Tgfbr3
Anxa1
Fasl
Cflar
Wee1
Erbb2
Tnf
Ppp2r5b
Lmna
Pea15a
Bcl2l2
Smad7
Casp7
Gch1
Dffa
Gadd45a
Lef1
Gm20683
Bmf
Rnasel
Casp1
Ccnd1
Ank
Emp1
Madd
Plcb2
Dnm1l
Xiap
Slc20a1
Cd44
2
1
0
−1
−2
E Apoptosis in Pro-T: TAL1AKT^{E17K} vs EV
WT1
WT2
WT3
TAL1AKT1
TAL1AKT2
TAL1AKT3
Hmgb2
Top2a
Txnip
Gsn
Sod1
Bnip3l
H1f0
Cd44
Rara
Gsr
Dnaja1
Dap3
Vdac2
Tspo
Dnajc3
Pak1
Sptan1
Jun
Nefh
Btg2
Mmp2
Lmna
Ank
Casp9
Bcap31
Nedd9
Brca1
Il1b
Gna15
Casp7
Pdgfrb
Hmox1
Rhot2
Gucy2e
Ptk2
Gadd45a
Il6
Dffa
Gm20683
Psen1
Cyld
Birc3
Il1a
Atf3
Bik
Cth
Sod2
Cd14
Lef1
Bcl2l2
Timp1
Xiap
Aifm3
Eno2
Hspb1
Cdc25b
Igfbp6
Erbb2
Gpx3
Bcl10
Cd38
Clu
F2
App
Retsat
Wee1
Hgf
Fas
Egr3
Pmaip1
Ppp2r5b
Fasl
Pea15a
Casp8
Gstm1
Gch1
Gadd45b
Ddit3
Timp3
Plcb2
F2r
Lgals3
Ebp
Rhob
Casp3
Tnf
Smad7
Cd14
Igf2r
Cd2
Il1a
Txnip
F2
Cd38
Gstm2
Hgf
Bik
Clu
Il1b
Rhot2
Eno2
Gpx3
Tgfbr3
Anxa1
Fasl
Cflar
Il18
Tnfsf10
Irf1
Casp2
Casp6
Cd69
Gpx4
Igf2r
Etf1
Pdcd4
Sc5d
Mcl1
Crebbp
Cdkn1a
Rock1
Bcl2l11
Sqstm1
Ier3
Casp1
Emp1
Rnasel
Slc20a1
Isg20
Diablo
Timp2
Dap
Satb1
Tgfb2
Ccnd2
Gpx1
2
1
0
−1
−2
F Apoptosis in vivo: TAL1AKT^{E17K} vs WT
WTDP.2
WTDP.4
WTDP.3
M116_TAL1AKT
M117_TAL1AKT
M119_TAL1AKT
Hmgb2
Top2a
Sod1
Lef1
Rock1
Crebbp
Timp2
Vdac2
H1f0
Pdcd4
Gpx1
Irf1
Ddit3
Dnaja1
Slc20a1
Ccnd1
Cdc25b
Ebp
Sod2
Cdkn1a
F2
Etf1
Btg3
Dap3
Ppp3r1
Bid
Nefh
Erbb2
Pdgfrb
Bik
Psen2
Clu
Ier3
Hspb1
Lgals3
Hmox1
Timp3
Bgn
Xiap
Retsat
Tgfb2
Gstm2
Il18
Aifm3
Timp1
Cth
Lum
Igfbp6
Avpr1a
Dcn
Bmp2
Il6
Hgf
Cav1
Plat
Il1a
Il1b
Mmp2
Cd14
Gpx3
Atf3
Gucy2e
Gstm1
Krt18
Sc5d
Gna15
Fasl
Rhob
Casp1
Diablo
Cd38
Tnf
Pmaip1
App
Anxa1
Mgmt
Ank
Ptk2
Gch1
Lmna
Prf1
Eno2
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Gadd45b
Bcl2l2
Gsr
Tnfsf10
Ppp2r5b
Casp9
Pak1
Smad7
Isg20
Pea15a
Gadd45a
Btg3
Rela
Cd44
Wee1
F2r
Cyld
Casp7
Bax
Dnm1l
Psen1
Bcl2l11
Casp8
Rnasel
Casp6
Rhot2
Dffa
Bcl10
Tgfbr3
Sat1
Gpx4
Jun
Birc3
Cflar
Tspo
Ctnnb1
Dnajc3
Casp2
Dap
Cdkn1a
Bcap31
Emp1
Tap1
Cd69
Casp3
Bnip3l
Ccnd2
Slc20a1
Ppt1
Fas
Bmf
Igf2r
Sqstm1
Plcb2
Ifngr1
Cdkn1b
Nedd9
Madd
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Sptan1
Satb1
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2
1
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