Supplemental Materials

Dynamic interaction between TAL1 oncoprotein and LSD1 regulates TAL1 function in hematopoiesis and leukemogenesis

LSD1 siRNA sequences:

LSD1 siRNA target sequence 1: CUAUAAAGCUCCAAUACUG; target sequence 2: GUAAAGCCACCCAGAGAUA; target sequence 3: GACAAGCUGUUCCUAAAGA; target sequence 4: UGAAUUAGCUGAAACACAA.

Supplemental Figure legends:

Supplement Figure S1. Pre-TCRα is a TAL1 target in T cell acute lymphoblastic leukemia. **(A)** The effect of Ser172 phosphorylation on the LSD1 recruitment. The purified GST and GST-TAL1 fusion proteins were incubated with ATP in the presence or absence of the catalytic subunit of PKA. The reaction was further incubated with purified LSD1. The bound LSD1 was resolved in SDS-PAGE and visualized by Western blotting analysis (Top). Loading of GST fusion proteins were shown (Bottom). **(B)** Western blotting analysis of TAL1 mutant expression in control or TAL1 mutant overexpressed Jurkat cells. **(C)** Alignment of the mouse and human pre-TCRα enhancers.

Supplemental Figure S2. PKA mediated phosphorylation controls LSD1 recruiment and transcription of the TAL1 target genes in T-ALL cells. (A) Jurkat cells were treated with PKA inhibitor H89 or PKA activator forskolin for 2 hrs. The nuclear extracts were prepared and precipitated TAL1 antibody. The immunoprecipitates were analyzed by WB with LSD1 (Top), HDAC1 (Middle), or phosphor-PKA (Bottom) antibodies. **(B)** Treatment of H89 or Forskolin doesn't affect the level of LSD1. Jurkat cells were treated with PKA inhibitor H89 or PKA

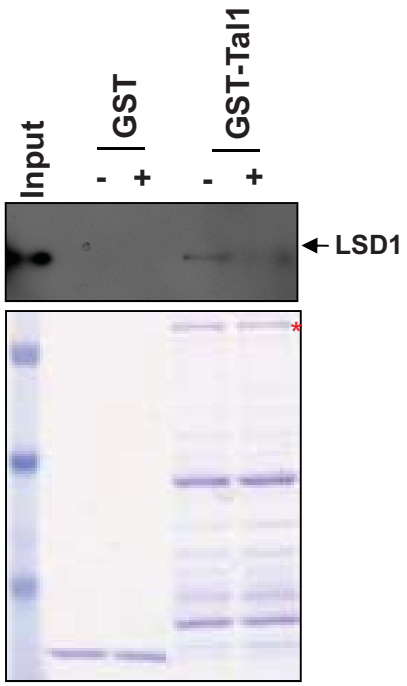
activator forskolin for 2 hrs. The nuclear extracts were prepared and analyzed by Western blotting using LSD1 antibody. **(C)** ChIP analysis of H3K4me2 enrichment at the *p21* enhancer from Jurkat cells transduced with wide type Flag-TAL1, Flag-TAL1$^{\Delta 142\text{-}185}$, and Flag-TAL1^{S172A}. **(D)** ChIP analysis of H3K4me2 enrichment at the *p21* enhancer using cross-linked chromatin from Jurkat cells treated with PKA inhibitor H89 and activator forskolin. **(E)** ChIP analysis of H3K4me2 enrichment at the *Runx1* promoter from Jurkat cells transduced with wide type Flag-TAL1, Flag-TAL1$^{\Delta 142\text{-}185}$, and Flag-TAL1^{S172A}. **(F)** ChIP analysis of enrichment of H3K4 methylation at the p21 enhancer comparing the control and LSD1 KD Jurkat cells.

Supplemental Figure S3. Knock down of TAL1 inhibits leukemic T cell colony formation and induces T cell apoptosis. (A) A soft agar colony formation assay was carried out in Jurkat cells stably expressed Dox-inducible TAL1 shRNA in the absence or presence of Doxycycline**.** **(B)** The FITC annexin V staining of apoptosis cells was analyzed from WT control and Jurkat cells stably expressed Dox-inducible TAL1 shRNA in the absence or presence Doxycycline for 72 or 96 hours. Pink: Total cells; Green: live cells; and Blue: Apoptotic cells. **(C)** Total RNA from Dox treated Jurkat cells for 0, 72, and 96 hrs was isolated and analyzed for the expression levels of *STIL and actin* by RT-PCR. **(D and E)** ChIP analysis of H3K4me1 **(D)** and H3K4me2 **(E)** in the inducible TAL1 KD Jurkat cells treated with or without doxycycline.

Supplemental Figure S4. PKA inhibitor H89 blocks EPO-induced erythroid differentiation. **(A)** CD34+ cells were treated with 5 μM of H89 and differentiated with erythroid differentiation medium (including EPO) for 7 days. The cells were analyzed for CD34 or CD36 positive population by FACS analysis. (B) Total RNA from CD34+ cells and CD36+ cells treated with or

without 5 μM of H89 was isolated and analyzed for the expression levels of *p4.2 and actin* by RT-PCR.

A



Commassie stain

B

Alignment of mouse_pTa (upper line) and human_pTa (lower line)
Identity=70.43%

```
                                    E1
mouse_pTa.txt   CCCCTGTCCCATCTGCACTGTGGTCGCAGAGCCTGGGCCA
human_pTa.txt   CTCTTTCCCCATCTGTAATGTGGTAACCGAGCCCGAGCCA
Consensus       c c t  cccatctg a tgtggt  c gagcc g gcca

mouse_pTa.txt   GCACTTCCCCAGACTGGAGAATGACAGGCAGAGTCGTTAG
human_pTa.txt   GCTCCTCCCTGGGCTGGAGGGT....GGGAGAGTCGTTAG
Consensus       gc c tccc  g ctggag  t    gg agagtcgttag
                    E2
mouse_pTa.txt   GGACACCTGCCTCCCCTCCTCCCACTCCAGGGCGGGAAGC
human_pTa.txt   GGACACCTGCCCCGCTCCCTCCCCCTCCCGGGGGGGAACC
Consensus       ggacacctgcc c c  cctccc ctcc ggg gggaa c
                                 E3
mouse_pTa.txt   CACCATGCTG.....CACCTGGGCCTG..CAGGGCCTGGG
human_pTa.txt   CACCGCGACGGCGCGCACCTGGGCGCGGGCTGTGCCTGGG
Consensus       cacc  g  g     cacctgggc  g  c g gcctggg
                    E4
mouse_pTa.txt   AACACGTGGAGCCCACAGTACCCAAAGCCCCTCCCCCGC
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Consensus       aac cg gg g cc c g   cca     cc
```
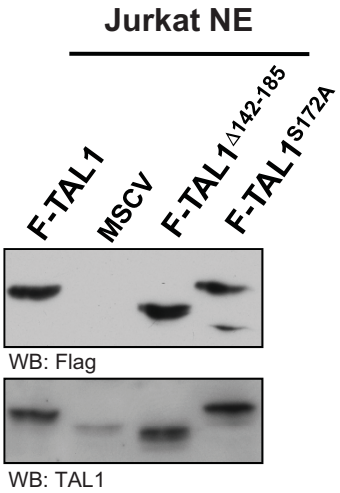
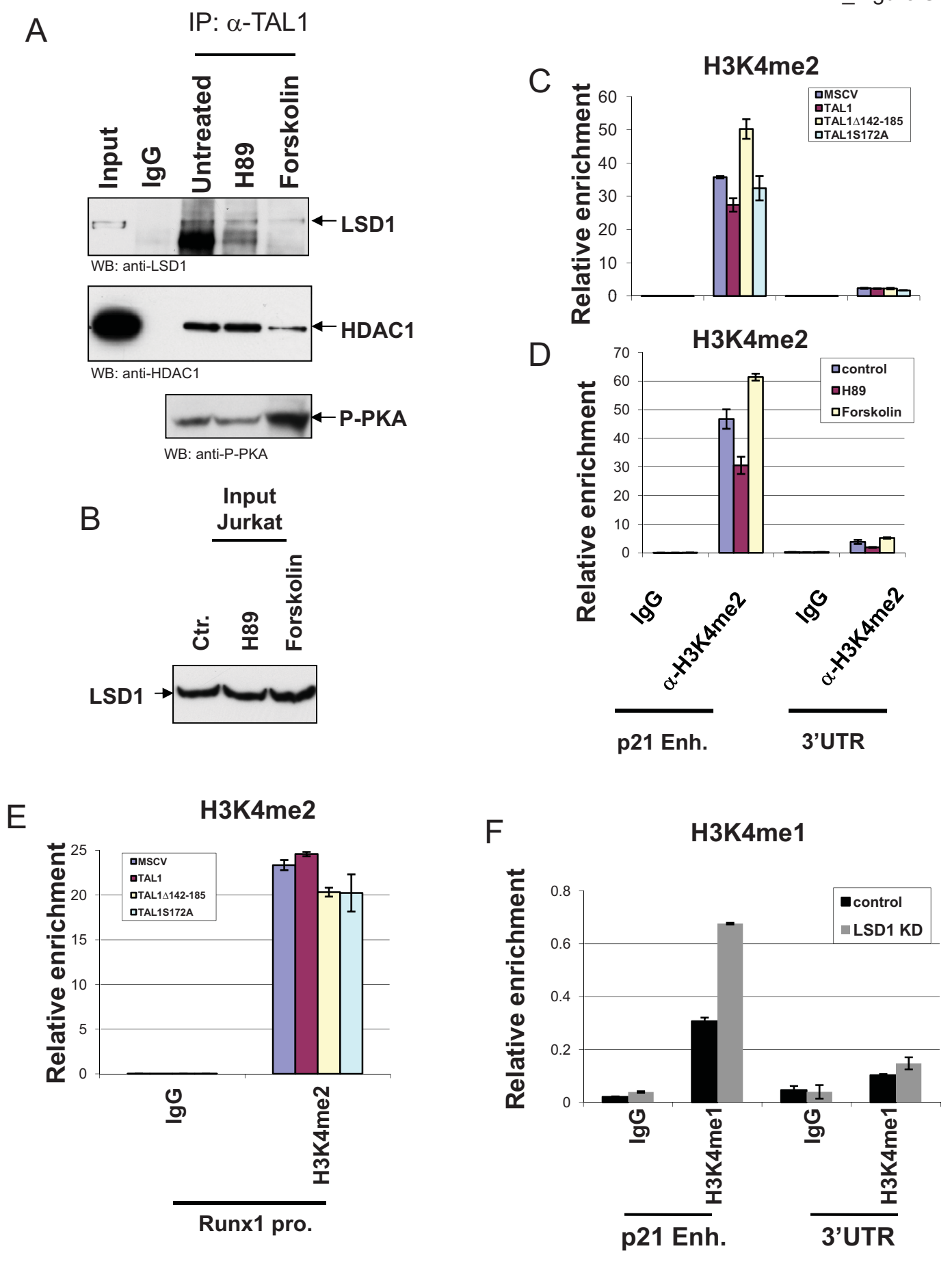
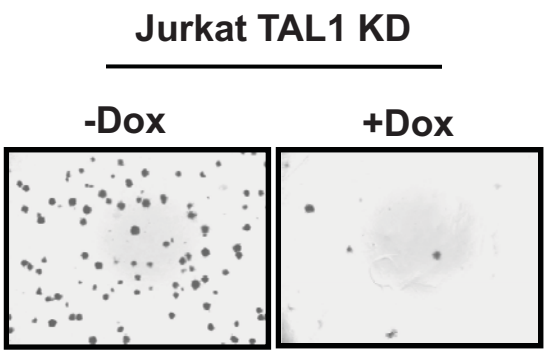

C

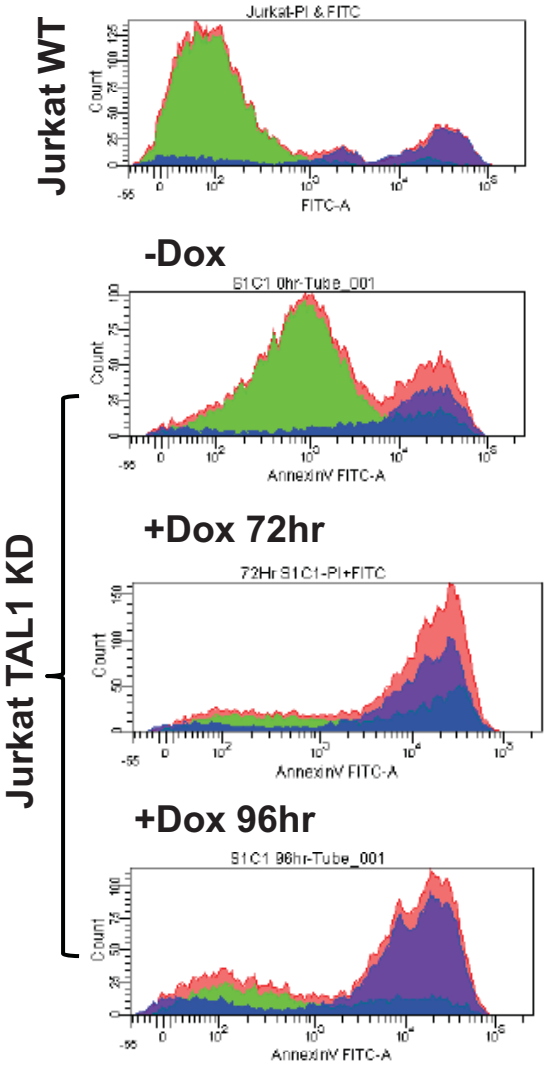



A
IP: α-TAL1
Input
IgG
Untreated
H89
Forskolin
LSD1
WB: anti-LSD1
HDAC1
WB: anti-HDAC1
P-PKA
WB: anti-P-PKA
B
Input
Jurkat
Ctr.
H89
Forskolin
LSD1
C
H3K4me2
Relative enrichment
MSCV
TAL1
TAL1Δ142-185
TAL1S172A
D
H3K4me2
Relative enrichment
control
H89
Forskolin
IgG
α-H3K4me2
IgG
α-H3K4me2
p21 Enh.
3'UTR
E
H3K4me2
Relative enrichment
MSCV
TAL1
TAL1Δ142-185
TAL1S172A
IgG
H3K4me2
Runx1 pro.
F
H3K4me1
Relative enrichment
control
LSD1 KD
IgG
H3K4me1
IgG
H3K4me1
p21 Enh.
3'UTR

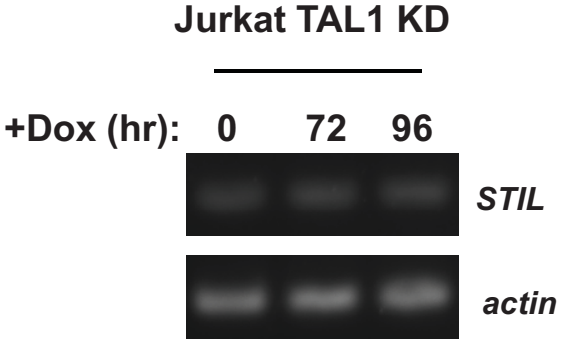
A


Jurkat TAL1 KD
-Dox
+Dox

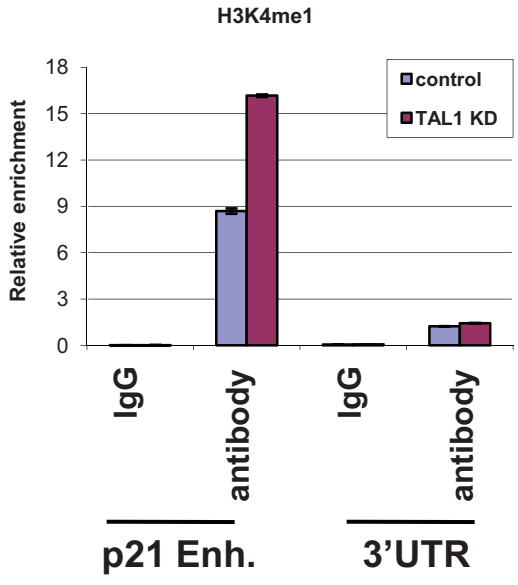
B


Jurkat WT
Jurkat-PI & FITC
Count
FITC-A
Jurkat TAL1 KD
-Dox
S1C1 0hr-Tube_001
AnnexinV FITC-A
+Dox 72hr
72Hr S1C1-PI+FITC
AnnexinV FITC-A
+Dox 96hr
S1C1 96hr-Tube_001
AnnexinV FITC-A

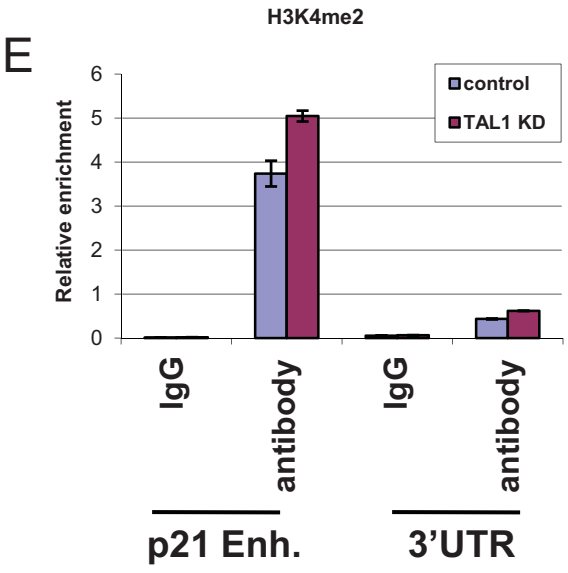
C


Jurkat TAL1 KD
+Dox (hr): 0 72 96
STIL
actin

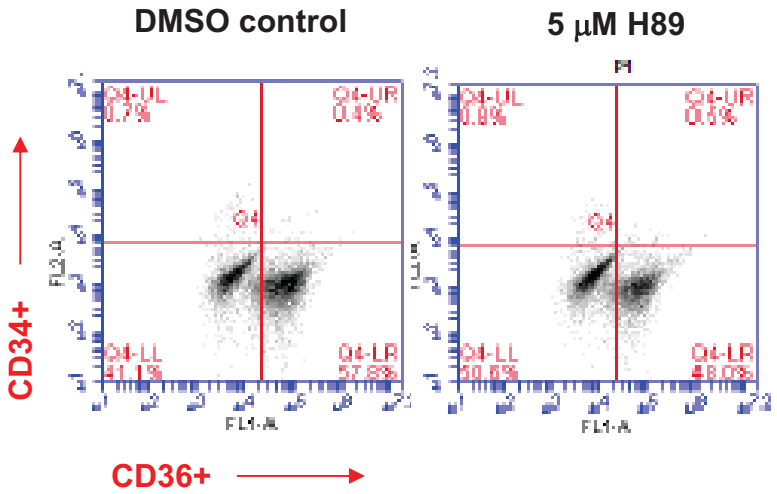
D


H3K4me1
Relative enrichment
control
TAL1 KD
IgG
antibody
IgG
antibody
p21 Enh.
3'UTR

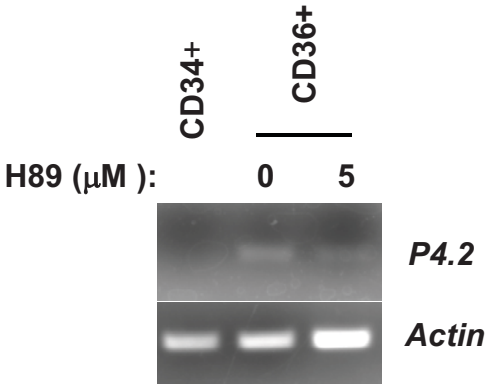
E


H3K4me2
Relative enrichment
control
TAL1 KD
IgG
antibody
IgG
antibody
p21 Enh.
3'UTR

A


DMSO control
5 μM H89
Q4-UL
0.7%
Q4-UR
0.4%
Q4
Q4-LL
41.1%
Q4-LR
57.8%
Q4-UL
0.9%
Q4-UR
0.5%
Q4
Q4-LL
50.6%
Q4-LR
48.0%
FL2-A
FL1-A
CD34+
CD36+

B


CD34+
CD36+
H89 (μM):
0
5
P4.2
Actin