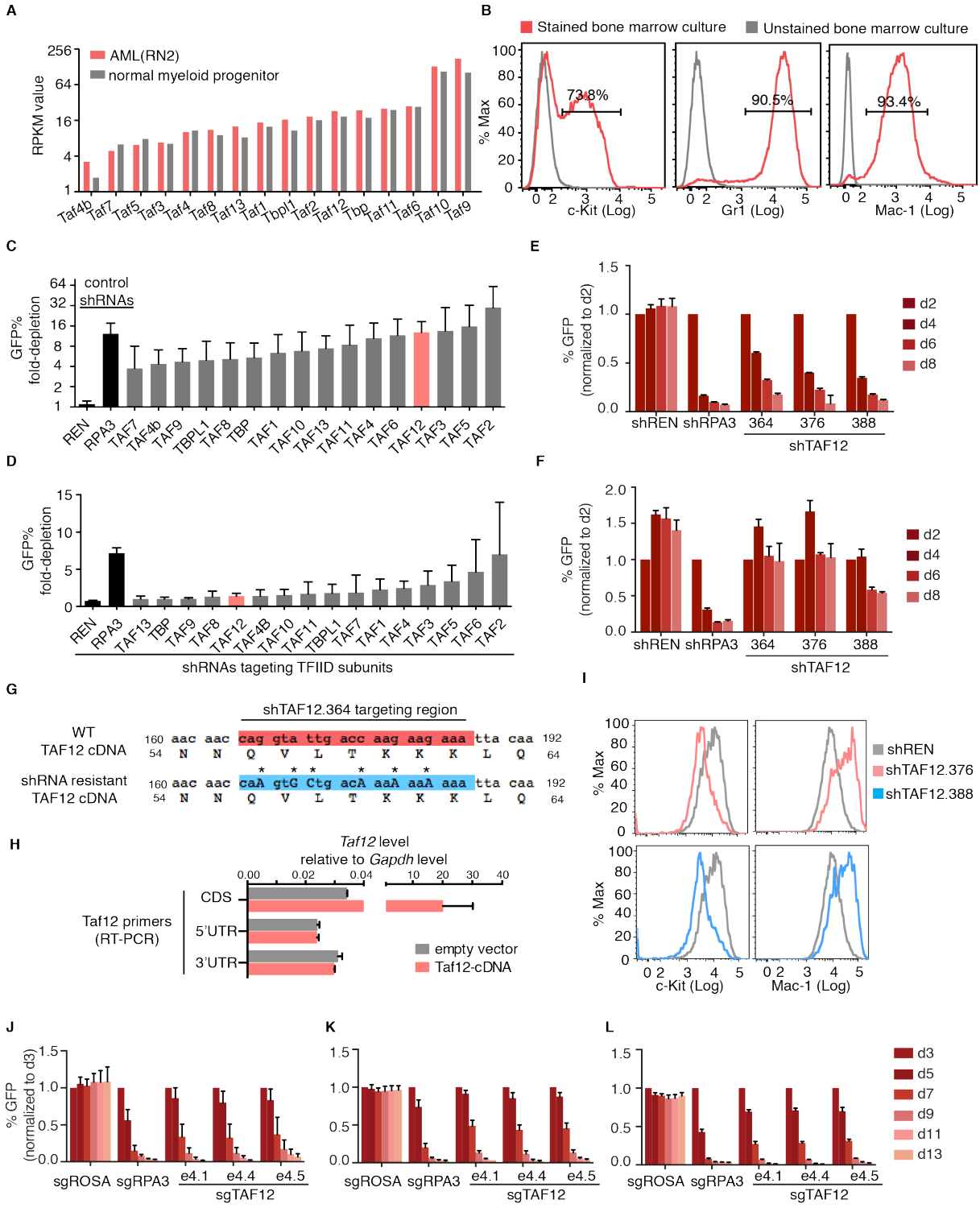

A
AML(RN2)
normal myeloid progenitor
RPKM value
Taf4b Taf7 Taf5 Taf3 Taf4 Taf8 Taf13 Taf1 Tbpl1 Taf2 Taf12 Tbp Taf11 Taf6 Taf10 Taf9
B
Stained bone marrow culture
Unstained bone marrow culture
% Max
73.8%
90.5%
93.4%
c-Kit (Log)
Gr1 (Log)
Mac-1 (Log)
C
control
shRNAs
GFP% fold-depletion
REN RPA3 TAF7 TAF4b TAF9 TBPL1 TAF8 TBP TAF1 TAF10 TAF13 TAF11 TAF4 TAF6 TAF12 TAF3 TAF5 TAF2
D
GFP% fold-depletion
REN RPA3 TAF13 TBP TAF9 TAF8 TAF12 TAF4B TAF10 TAF11 TBPL1 TAF7 TAF1 TAF4 TAF3 TAF5 TAF6 TAF2
shRNAs tageting TFIID subunits
E
% GFP (normalized to d2)
d2 d4 d6 d8
shREN shRPA3 364 376 388
shTAF12
F
% GFP (normalized to d2)
d2 d4 d6 d8
shREN shRPA3 364 376 388
shTAF12
G
shTAF12.364 targeting region
WT TAF12 cDNA
shRNA resistant TAF12 cDNA
H
Taf12 level relative to Gapdh level
Taf12 primers (RT-PCR)
CDS
5'UTR
3'UTR
empty vector
Taf12-cDNA
I
% Max
shREN
shTAF12.376
shTAF12.388
c-Kit (Log)
Mac-1 (Log)
J
% GFP (normalized to d3)
sgROSA sgRPA3 e4.1 e4.4 e4.5
sgTAF12
K
sgROSA sgRPA3 e4.1 e4.4 e4.5
sgTAF12
L
d3 d5 d7 d9 d11 d13
sgROSA sgRPA3 e4.1 e4.4 e4.5
sgTAF12

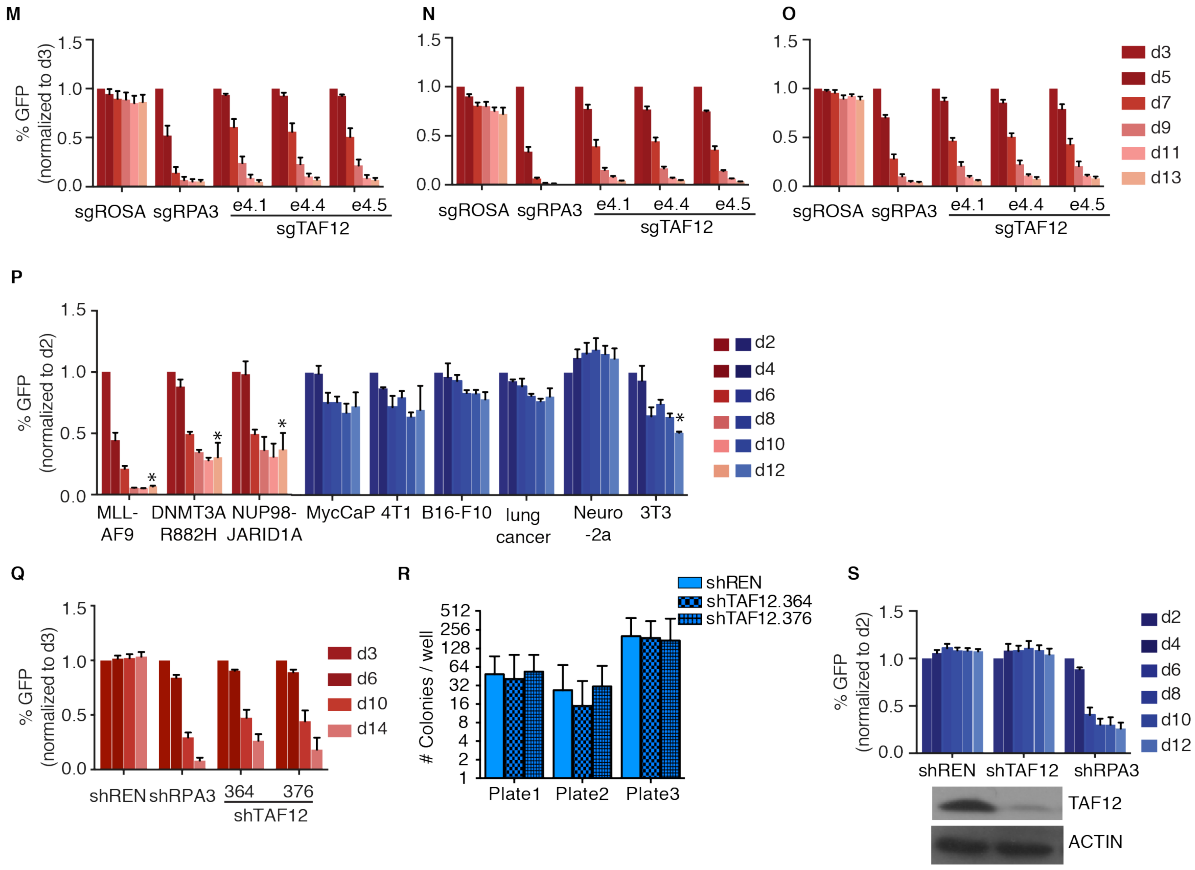


Figure S1. shRNA screen identifies TAF12 as an acquired dependency in AML. Related to Figure 1.

(A) Bar graph showing the reads per kilobase per million mapped reads (RPKM) of 16 genes encoding TFIID subunits from RNA-seq analysis in the RN2 cell line (murine MLL-AF9/Nras^{G12D} AML) and normal myeloid progenitor cells derived from the bone marrow of adult mice. For RN2 cells, data are presented as the mean of two biological repeats performed in RN2 cells on day 3 post viral transduction of shREN. For normal myeloid progenitor cells, data are presented as the value of a single RNA-seq experiment performed in normal progenitor cells on day 3 post viral transduction of shREN.

(B) Representative flow cytometry plots showing the relative abundance of myeloid-lineage markers in bone marrow cultures. Bone marrow cultures were stained with antibodies against the stem cell marker c-Kit and the myeloid lineage markers Mac-1 and Gr-1 followed by flow cytometry analysis. Unstained cultures were used as negative control.

(C-D) Bar graph summary of the negative-selection shRNA screen in RN2 cells (C) and normal myeloid progenitor cells (D). For shRNAs targeting TFIID subunits, data are presented as the mean ± SD fold-depletion of all the shRNAs targeting the same gene, n=5-6. For control shRNAs, data are presented as the mean ± SD fold-depletion of three technical repeats. Black bars highlight negative (shREN) and positive control shRNA (shRPA3). GFP fold-depletion is plotted as a d2 to d12 ratio of GFP% for RN2 cells and as a d2 to d8 ratio of GFP% for normal myeloid progenitors.

(E-F) Validation of three independent TAF12 shRNAs on the growth of RN2 cells (E) and normal myeloid progenitor cells (F) in liquid culture. Bar graphs represent mean ± SEM GFP% normalized to d2, n=3.

(G) Illustration of TAF12 cDNA rescue assay. Multiple silent mutations were cloned into the TAF12 cDNA to disrupt base-pairing with TAF12 shRNA (#364).

(H) RT-PCR analysis of endogenous TAF12 transcript level in RN2 cells stably expressing FLAG tagged TAF12 cDNA. Bar graph represents mean ± SEM, n=3.

(I) Flow cytometry analysis of RN2 cells on day 4 post infection with control or two additional TAF12 shRNAs (#376, #388), showing c-Kit downregulation and Mac-1 upregulation following TAF12 knockdown.

(J-O) GFP depletion assays in the human AML cell line MOLM-13 carrying MLL-AF9 (J), human AML cell line MV4-11 carrying MLL-AF4 (K), human AML cell line OCI-AML3 carrying $DNMT3A^{R882C}$ (L), human lymphoma/leukemia cell line U937 carrying CALM-AF10 (M), human

AML cell line HEL carrying $JAK2^{V617F}$ (N) and human CML cell line K562 carrying BCR-ABL (O) after lentiviral infection of CRISPR guide RNAs (sgRNAs) linked with GFP. Bar graphs represent mean ± SEM GFP% normalized to d3, n=3.

(P) GFP depletion assays in the indicated cell lines after retroviral infection with a different TAF12 shRNA (#376) linked with GFP. Bar graphs represent mean ± SEM GFP% normalized to d2, n=3. * indicates a >2-fold ratio of d2 to d12 GFP% and $p < 0.05$. p value was calculated using unpaired Student's t-test by comparing GFP% on day 2 and that on day 12.

(Q) GFP depletion assay in murine *Dnmt3a*$^{-/-}$ hematopoietic stem cells (HSC) in liquid culture. Bar graphs represent mean ± SEM GFP% normalized to d3, n= 4, including 2 biological and 2 technical repeats.

(R) Colony formation assay in murine *Dnmt3a*$^{-/-}$ HSCs. Data are presented as mean ± SEM, n=2.

(S) GFP depletion assay and western blot analysis in Neuro-2a cells infected with control or TAF12 (#364) shRNAs. Bar graph (top) represents mean ± SEM GFP% normalized to d2, n=3.

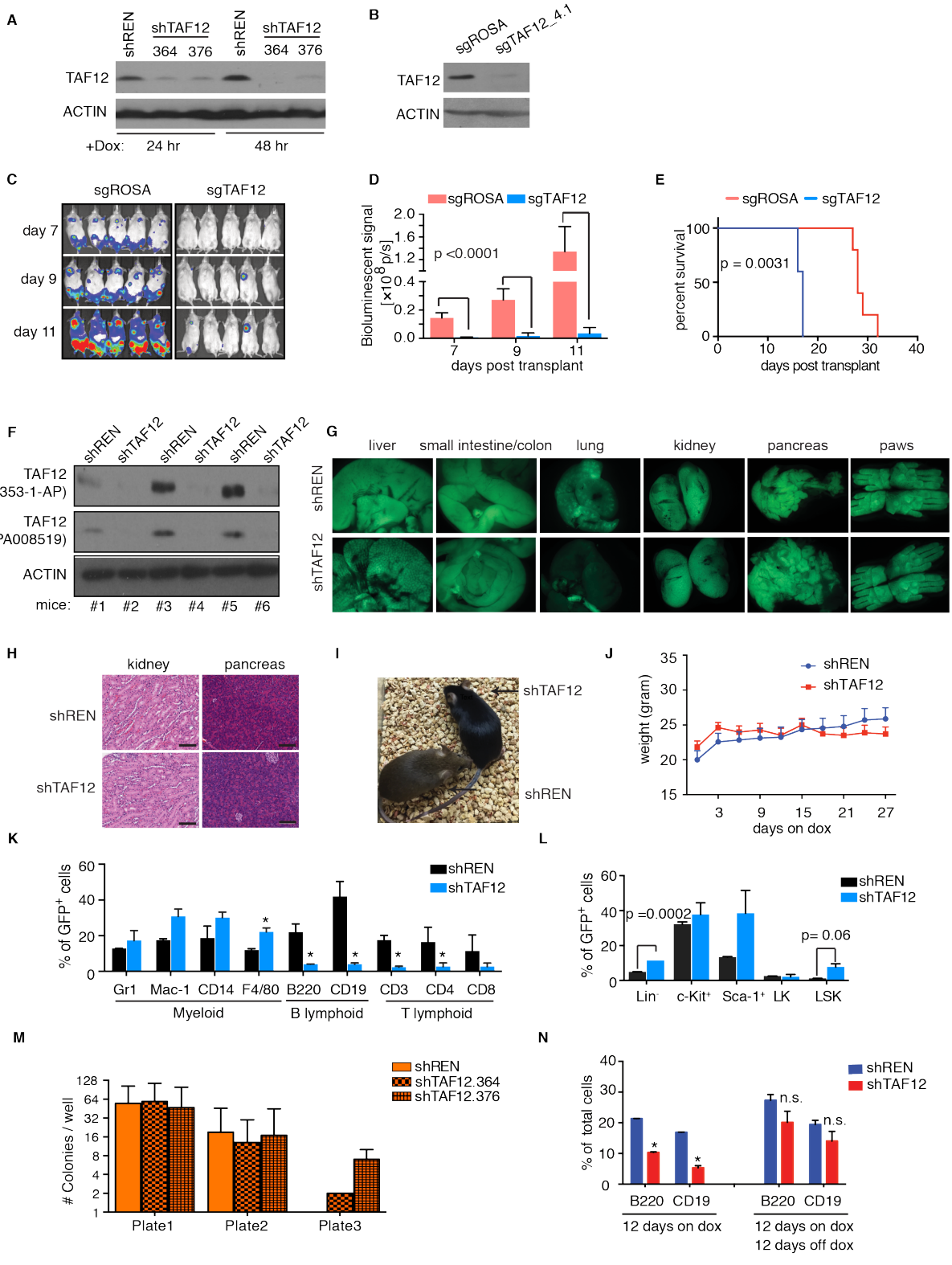

A
shREN
shTAF12
364 376
shREN
shTAF12
364 376
TAF12
ACTIN
+Dox: 24 hr 48 hr
B
sgROSA
sgTAF12_4.1
TAF12
ACTIN
C
sgROSA
sgTAF12
day 7
day 9
day 11
D
sgROSA sgTAF12
Bioluminescent signal [x10^8 p/s]
p <0.0001
days post transplant
E
sgROSA sgTAF12
percent survival
p = 0.0031
days post transplant
F
shREN shTAF12 shREN shTAF12 shREN shTAF12
TAF12 (12353-1-AP)
TAF12 (HPA008519)
ACTIN
mice: #1 #2 #3 #4 #5 #6
G
liver
small intestine/colon
lung
kidney
pancreas
paws
shREN
shTAF12
H
kidney
pancreas
shREN
shTAF12
I
shTAF12
shREN
J
shREN
shTAF12
weight (gram)
days on dox
K
shREN
shTAF12
% of GFP+ cells
Gr1 Mac-1 CD14 F4/80 B220 CD19 CD3 CD4 CD8
Myeloid
B lymphoid
T lymphoid
L
shREN
shTAF12
% of GFP+ cells
p =0.0002
p= 0.06
Lin- c-Kit+ Sca-1+ LK LSK
M
shREN
shTAF12.364
shTAF12.376
Colonies / well
Plate1 Plate2 Plate3
N
shREN
shTAF12
% of total cells
n.s.
n.s.
B220 CD19
12 days on dox
B220 CD19
12 days on dox
12 days off dox

Figure S2. Dox-inducible shRNA system and the consequences of TAF12 knock down in vivo. Related to Figure 2.

(A) Western blot analysis of TAF12 in RN2 cells transduced with dox-regulated control (shREN) or TAF12 shRNAs (#364, #376) following dox treatment in vitro for 24 or 48 hr.

(B) Western blot analysis of TAF12 in RN2-Cas9 cells transduced with TAF12 guide RNA (#4.1) on day 4 post viral transduction and G-418 selection.

(C) Bioluminescent imaging of mice transplanted with 100, 000 RN2-Cas9 cells transduced with CRISPR guide RNA targeting ROSA26 locus (sgROSA) or TAF12 (sgTAF12). Mice were imaged on day 7, 9 and 11 post transplantation.

(D) Quantification of bioluminescent imaging from (C). Values represent photons per second (p/s) of bioluminescent signal detection. Data are presented as mean ± SEM, n=4-5. p value was calculated using unpaired Student's t-test. n=4 or 5.

(E) Survival curves of mice transplanted with RN2-Cas9 cells transduced with CRISPR gRNAs. p value was calculated using Log-rank (Mantel-Cox) test. 4-5 mice were evaluated per condition.

(F) Western blot analysis of TAF12 protein level in whole bone marrow cells of three control (shREN) and three TAF12 shRNA (#364) transgenic mice following 4-week dox treatment. Two different TAF12 antibodies were assayed.

(G) Representative GFP fluorescent images of organs from transgenic mice treated with dox for 4 weeks.

(H) Hematoxylin and eosin staining of tissues from transgenic mice treated with dox for 4 weeks. Scale bars represent 100 μm.

(I) Representative pictures of control and TAF12 shRNA transgenic mice after 4 weeks of dox treatment.

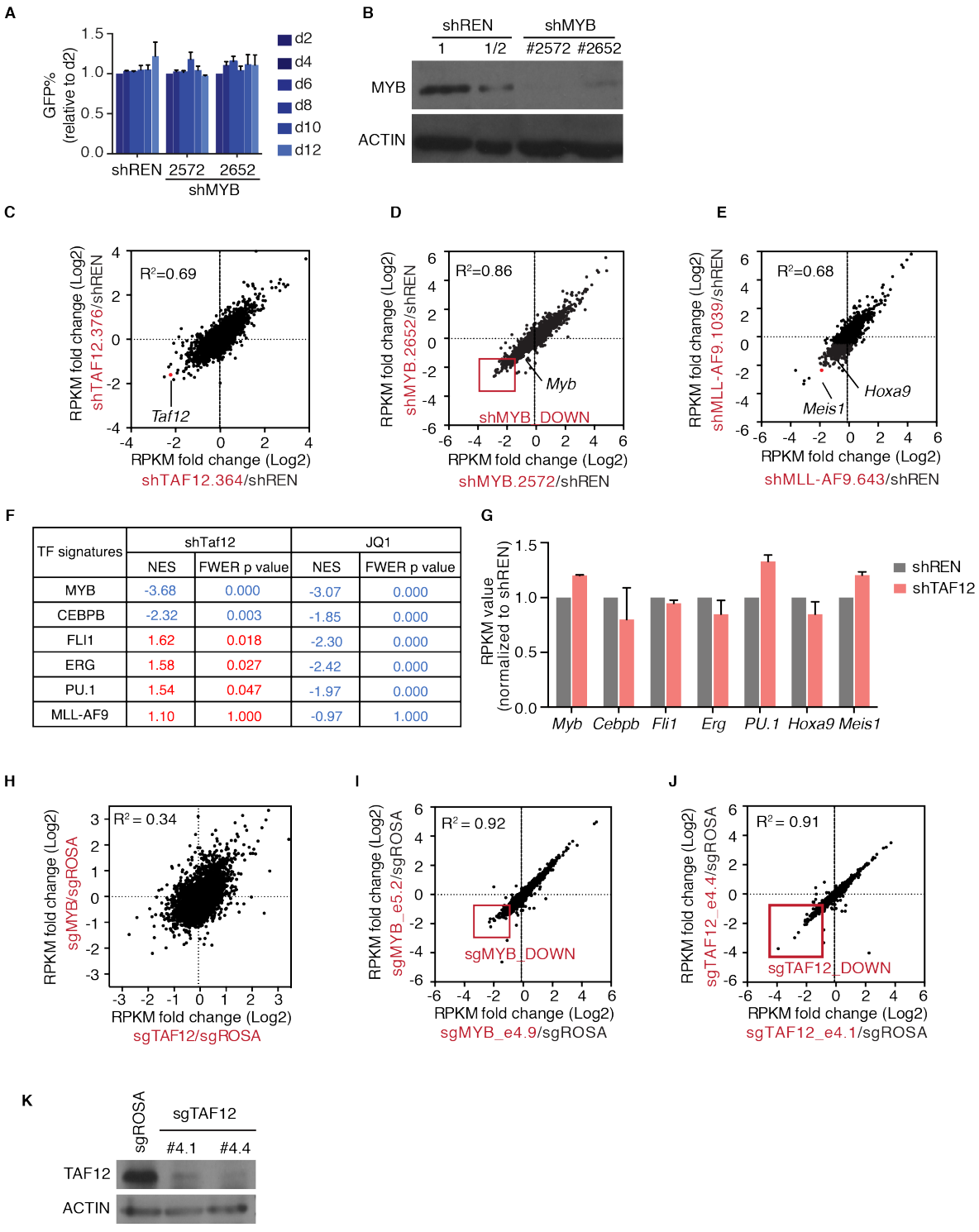
(J) Measurement of mice weight over 4 weeks of dox treatment. Data are presented as mean ± SEM, n= 6, including 3 males and 3 females.

(K) Flow cytometry analysis of peripheral blood stained with antibodies against the indicated cell surface markers from transgenic mice treated with dox for 4 weeks. Gating was first performed on $GFP^+/shRNA^+$ cells, prior to measurement of marker staining. p value was calculated using unpaired Student's t-test, *p <0.05. Data are presented as mean ± SEM, n=3.

(L) Flow cytometry analysis of $Lineage^-$ (Lin^-), c-Kit^+ and Sca-1^+ cell population within bone marrow cells from transgenic mice treated with dox for 4 weeks. Gating was first performed on $GFP^+/shRNA^+$ cells, prior to measurement of marker staining. p value was calculated using unpaired Student's t-test. Data are presented as mean ± SEM, n=2.

(M) Colony formation assay with serial replating in murine hematopoietic stem cells (HSCs). Sca-1^+ cells were transduced with control or TAF12 shRNA in MLS-E vector. On day2 post infection, 100 $GFP^+/Lineage^-/Sca\text{-}1^+/c\text{-}Kit^+/CD48^-/CD150^+$ HSCs were sorted for colony analysis. At the end of each round of plating, 5,000 GFP^+ cells were resorted for the next passage.

(N) Flow cytometry analysis showing the reversibility of B cell population upon removal of dox in transgenic shRNA mice. Bone marrow cells from mice treated with dox for 12 days (left panel) and mice with 12-day dox treatment followed by removal of dox for 12 days (right panel) were stained with antibodies against the B cell surface markers B220 and CD19, followed by flow cytometry analysis. Since GFP will not be expressed upon removal of dox, gating was performed only on live cells (SSC/FSC). p value was calculated using unpaired Student's t-test, *p <0.05. Data are presented as mean ± SEM, n=3.



TF signatures	shTaf12		JQ1	
	NES	FWER p value	NES	FWER p value
MYB	-3.68	0.000	-3.07	0.000
CEBPB	-2.32	0.003	-1.85	0.000
FLI1	1.62	0.018	-2.30	0.000
ERG	1.58	0.027	-2.42	0.000
PU.1	1.54	0.047	-1.97	0.000
MLL-AF9	1.10	1.000	-0.97	1.000

Figure S3. Overlapping transcriptional regulation by MYB and TAF12. Related to Figure 3.

(A) GFP depletion assay in Neuro-2a cells transduced with control or MYB shRNAs. Data are presented as mean ± SEM GFP% normalized to d2. n=3.

(B) Western blot analysis of MYB in RN2 cells transduced with dox-regulated control or MYB shRNAs following dox treatment for 24 hr. A 2-fold dilution of shREN sample (1/2 shREN) was performed to estimate MYB knockdown.

(C-E) Scatter plots showing the correlation of RNA-seq data using two independent shRNAs targeting TAF12 (C), MYB (D) and MLL-AF9 (E) in RN2 cells. 8,044 expressed genes with RPKM ≥5 in control sample (shREN) are plotted. The red dots highlight the shRNA targeted genes or known downstream target genes. The red box in (D) highlights the MYB target gene signatures defined as top 200 downregulated genes following shRNA mediated MYB knockdown.

(F) Gene set enrichment analysis (GSEA) of target gene signatures of different hematopoietic TFs upon TAF12 knockdown or JQ1 (BET bromodomain inhibitor) treatment. Normalized enrichment score (NES) and family-wise error rate (FWER) p value for indicated TF signatures are shown. TF signatures were defined as top 200 downregulated genes following TF knockdown. RNA-seq data for defining CEBPB, FLI1, ERG and PU.1 signatures as well as JQ1 treatment were obtained from a previous study (Roe et al., 2015).

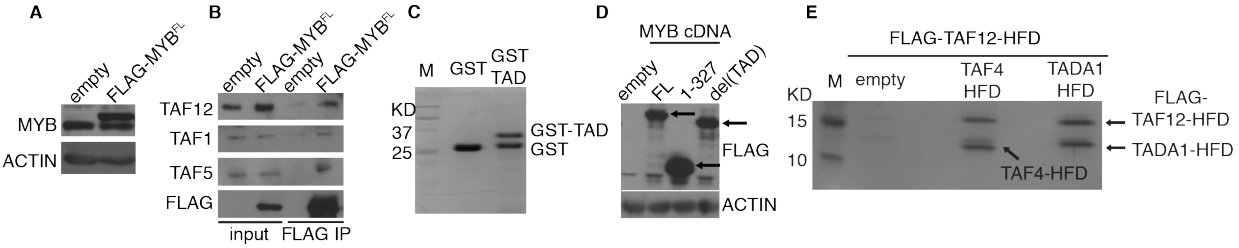
(G) Bar graph showing RPKM values of the indicated TFs from RNA-seq analysis in RN2 cells on day 3 post viral infection of control or TAF12 shRNA. Data are presented as mean ± SEM, normalized to shREN sample, n=2.

(H) Scatter plot showing the correlation of global transcriptional change upon sgRNA mediated targeting of TAF12 and MYB in the human AML cell line MOLM-13. For sgRNAs targeting MYB and TAF12, the RPKM value was calculated as the mean of two different gRNAs. For

control sgRNA, RPKM value was calculated as the mean of two biological repeats. 7,707 expressed genes with RPKM ≥5 in control sample (sgROSA) are plotted.

(I-J) Scatter plots showing the correlation of RNA-seq data using two different sgRNAs targeting MYB (I) and TAF12 (J) in MOLM-13 cells. 7,707 expressed genes with RPKM ≥5 in control sample (sgROSA) are plotted. The red boxes highlight the MYB (I) and TAF12 (J) target gene signatures defined as top 200 downregulated genes following sgRNA mediated MYB or TAF12 knockout in MOLM-13 cells.

(K) Western blot analysis of TAF12 protein level in MOLM-13-Cas9 cells transduced with control or TAF12 gRNAs (#4.1 and #4.4) on day 4 post viral transduction and G-418 selection.

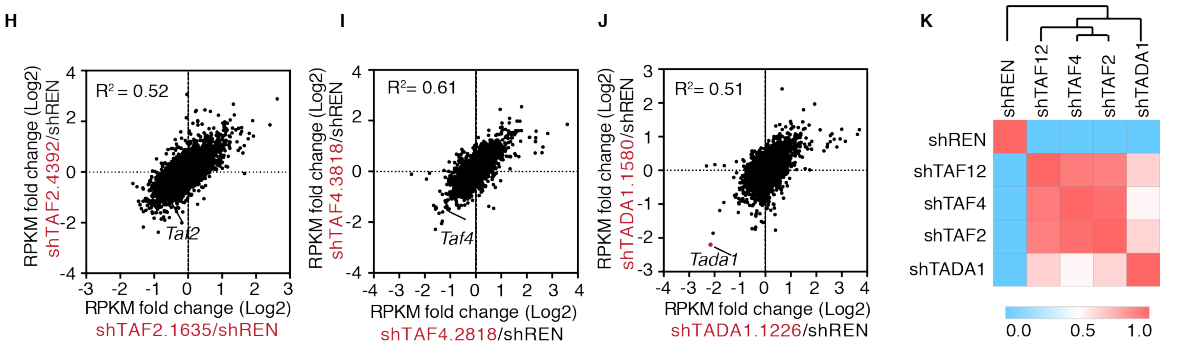


F

TFIID subunits	MWT (kDa)	# peptides_AML		# peptides_fibroblast	
		Mock IP	FLAG-TAF12 IP	Mock IP	FLAG-TAF12 IP
TAF12	17.9	0	36	0	22
TAF1	214.3	0	360	0	54
TAF2	126.9	0	182	0	38
TAF3	105.1	0	119	0	41
TAF4A	105.2	0	156	0	36
TAF5	87.0	0	215	0	47
TAF6	72.6	0	189	0	63
TAF7	39.1	0	39	0	20
TAF8	34.0	0	33	0	19
TAF9	29.0	0	246	0	21
TAF10	21.8	0	41	0	13
TAF11	23.3	0	13	0	14
TAF13	14.3	0	4	0	13
TBP	34.7	0	13	0	8

G

SAGA subunits	MWT (kDa)	# peptides_AML		# peptides_fibroblast	
		Mock IP	FLAG-TAF12 IP	Mock IP	FLAG-TAF12 IP
TAF12	17.9	0	36	0	22
TAF9	29.0	0	246	0	21
TAF10	21.8	0	41	0	13
TRRAP	436.9	0	443	0	134
KAT2A	93.3	0	41	0	22
ATXN7	92.6	0	36	0	6
ATXN7L1	78.0	0	46	0	8
ATXN7L2	73.9	0	21	0	8
TAF6L	67.2	0	90	0	38
TAF5L	65.9	0	68	0	29
SUPT20H	59.5	0	54	0	20
TADA3	48.9	0	26	0	22
TADA2B	48.5	0	26	0	16
SUPT7L	45.9	0	33	0	15
SUPT3	41.2	0	70	0	6
TADA1	37.4	0	31	0	11



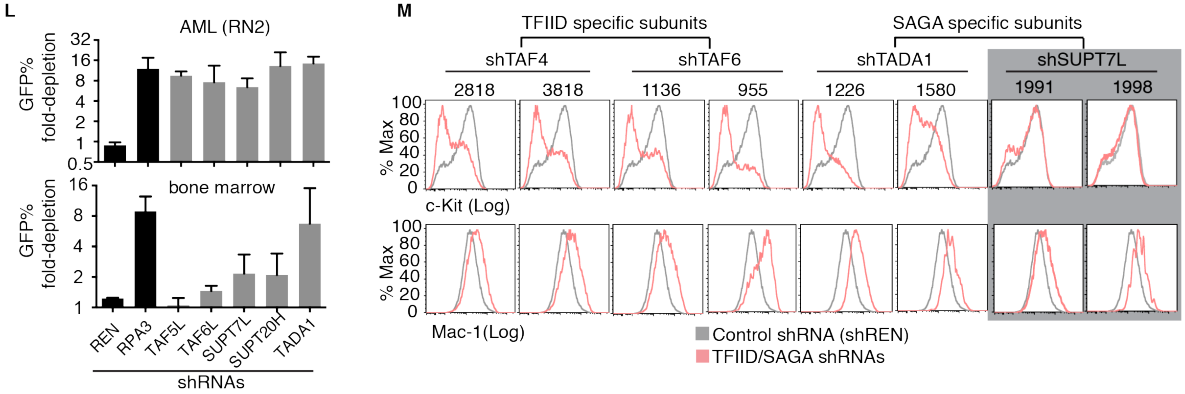


Figure S4. Additional data pertaining to SAGA and TAF12/TAF4 reconstitution experiments. Related to Figure 4.

(A) Western blot analysis of MYB protein level in RN2 cells stably expressing FLAG tagged MYB or empty vector.

(B) IP-western blot analysis evaluating the interaction between FLAG-MYB and endogenous TFIID subunits in HEK 293T cells. FLAG IP was performed in nuclear lysates prepared from HEK 293T cells 48 hr post transfection of FLAG-MYB or empty vector (PCDNA3).

(C) Coomassie staining of recombinant GST and GST tagged TAD protein purified from *E. coli*.

(D) Western blot analysis of FLAG tagged MYB fragments used in shRNA/cDNA rescue experiments.

(E) Silver staining analysis of the TAF12/TAF4 and TAF12/TADA1 HFD heterodimers. FLAG IP was performed in HEK 293T cells 48 hr post transfection of FLAG-TAF12-HFD together with untagged TAF4-HFD or TADA1-HFD or empty vector (PCDNA3). Elute from the FLAG IP was used for SDS-PAGE analysis followed by silver staining.

(F-G) Tables showing TFIID (F) and SAGA (G) subunits recovered from mass spectrometry analysis of FLAG IP performed in RN2 cells and immortalized fibroblast (iMEF) stably overexpressing FLAG-TAF12 or empty vector (mock IP).

(H-J) Scatter plots showing the correlation of RNA-seq data using two independent shRNAs targeting TAF2 (H), TAF4 (I) and TADA1 (J) in RN2 cells. All RNA-seq experiments were performed on day 3 post viral infection of the indicated shRNAs.

(K) Heat map of similarity matrix and unsupervised hierarchal clustering showing the correlation of gene expression change following TAF12, TAF2, TAF4 and TADA1 knockdown. The RPKM values of 2166 genes, which include all the genes with ≥1.5-fold-change in RPKM value in at least

one knockdown sample were used for the analysis. One minus Pearson correlation was used for the distance metric.

(L) Bar graph summary of the GFP fold-depletion of shRNAs targeting SAGA subunits in RN2 cells (upper panel) and normal myeloid progenitor cells (lower panel). For shRNAs targeting SAGA subunits, data are presented as the mean ± SD GFP% fold-depletion of all the shRNAs targeting the same gene, n=5-6. For control shRNAs, data are presented as the mean ± SD GFP% fold-depletion of three technical repeats. Black bars highlight negative (shREN) and positive (shRPA3) control shRNA. GFP% fold-depletion is plotted as a d2 to d12 ratio of GFP% for RN2 cells and a d2 to d8 ratio of GFP% for normal myeloid progenitors.

(M) Flow cytometry analysis of RN2 cells stained with antibodies against c-Kit (surface marker of leukemia stem cells) and Mac-1 (surface marker of macrophage cells) on day 4 post infection with shRNAs targeting TFIID specific or SAGA specific subunits. The box highlights the shRNAs which do not cause c-Kit downregulation and Mac-1 upregulation.

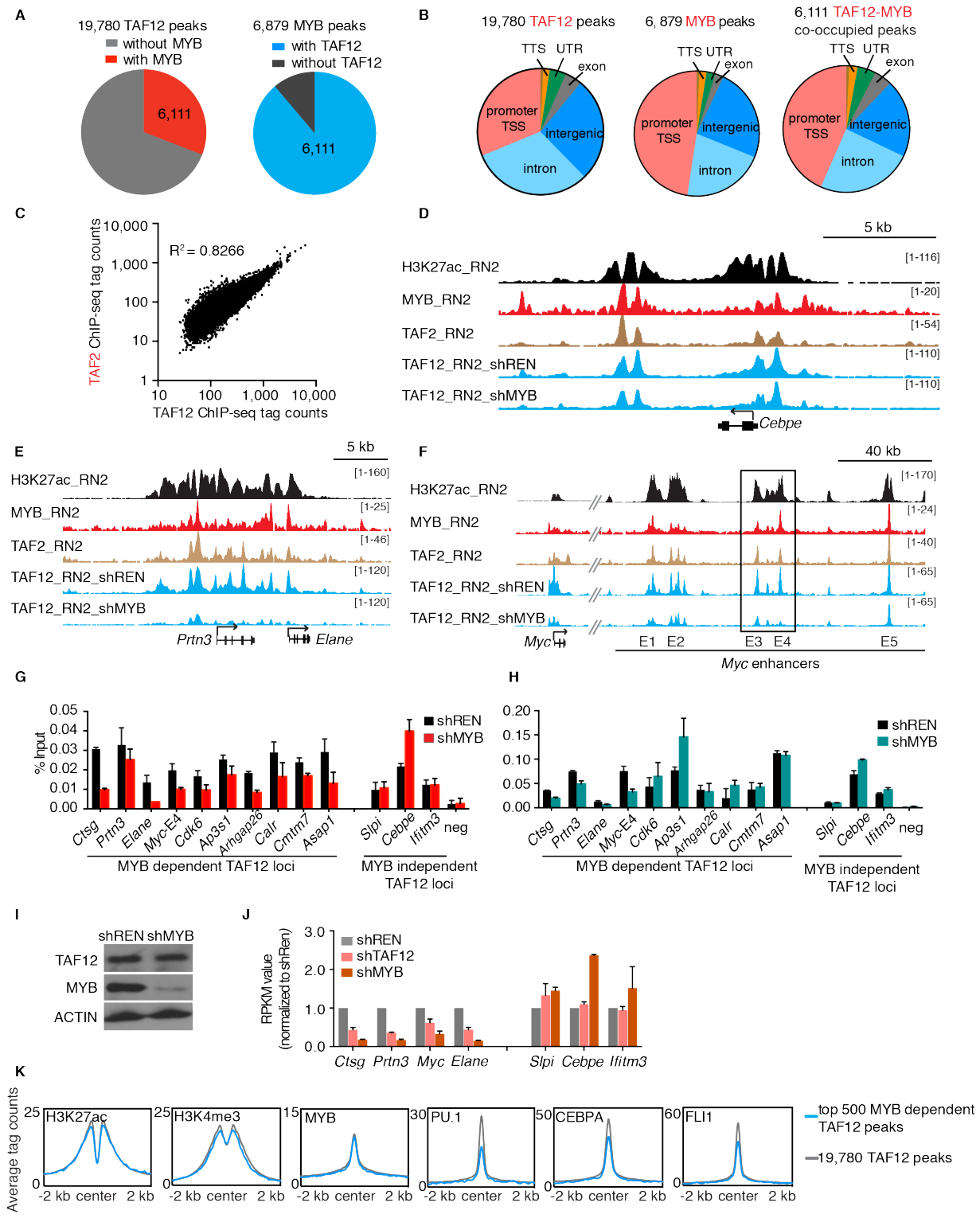


Figure S5. Additional analysis of TAF12 and MYB ChIP-seq datasets. Related to Figure 5.

(A) Pie charts showing the number of overlapped peaks between MYB and TAF12 ChIP-seq. FLAG-TAF12 ChIP-seq was performed in RN2 cells stably expressing 3* FLAG-TAF12. MYB ChIP-seq data was generated in a previous study (Roe et al., 2015). 19,780 high confident TAF12 peaks were defined as the overlapped peaks (more than 500 bp overlap) between two biological repeats of FLAG-TAF12 ChIP-seq.

(B) Pie charts showing genomic location of indicated sets of peaks. TTS: transcription termination site; UTR: untranslated region.

(C) Scatter plot showing the correlation of ChIP-seq tag counts between TAF2 and TAF12 ChIP-seq. Tag counts on 19,780 TAF12 peaks are plotted.

(D-F) Additional examples of ChIP-seq occupancy profiles of H3K27ac, MYB, TAF2 and TAF12 showing the insensitivity of TAF12 to MYB knockdown at the *Cebpe* locus (D) and the loss of TAF12 upon MYB knockdown at *Prtn3/Elane* (E) and *Myc* (F) loci.

(G-H) ChIP-qPCR analysis of TAF10 (G) and KAT2A (also called GCN5, H) at the indicated MYB dependent/independent TAF12 loci following MYB knockdown. ChIP-qPCR was performed in RN2 cells transduced with dox-regulated control or MYB shRNA (#2652) following 24-hr dox treatment.

(I) Western blot analysis showing TAF12 and MYB protein level upon MYB knockdown. Whole cell lysates from RN2 cells transduced with dox-regulated control or MYB shRNA (#2652) and treated with dox for 24 hr were used for analysis.

(J) Bar graph showing the RPKM values of genes near to indicated loci upon TAF12 or MYB knockdown from the previously described RNA-seq experiments. Data are presented as mean ± SEM, normalized to shREN sample, n=2.

(K) ChIP-seq meta profiles of indicated histone modifications and hematopoietic TF occupancy at the top 500 MYB dependent TAF12 peaks versus the 19,780 TAF12 peaks. Average tag counts from ChIP-seq experiments are plotted at the 2 kb interval around the summit of TAF12 peaks.

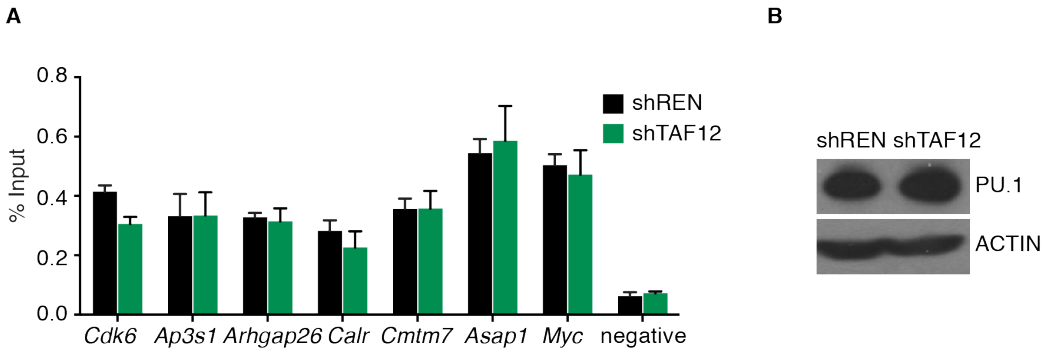


Figure S6. Additional data/controls. Related to Figure 6.

(A) ChIP-qPCR analysis of PU.1 at the indicated genomic loci, showing that PU.1 chromatin occupancy was not affected by TAF12 knockdown. ChIP was performed in RN2 cells on day 3 post viral infection of control or TAF12 shRNA (#364). Data are presented as mean ± SEM, n=3. (B) Western blot analysis showing PU.1 protein level following TAF12 knockdown. Whole cell lysates prepared form RN2 cells on day 3 post viral infection of control or TAF12 shRNA (#364) were used for analysis.

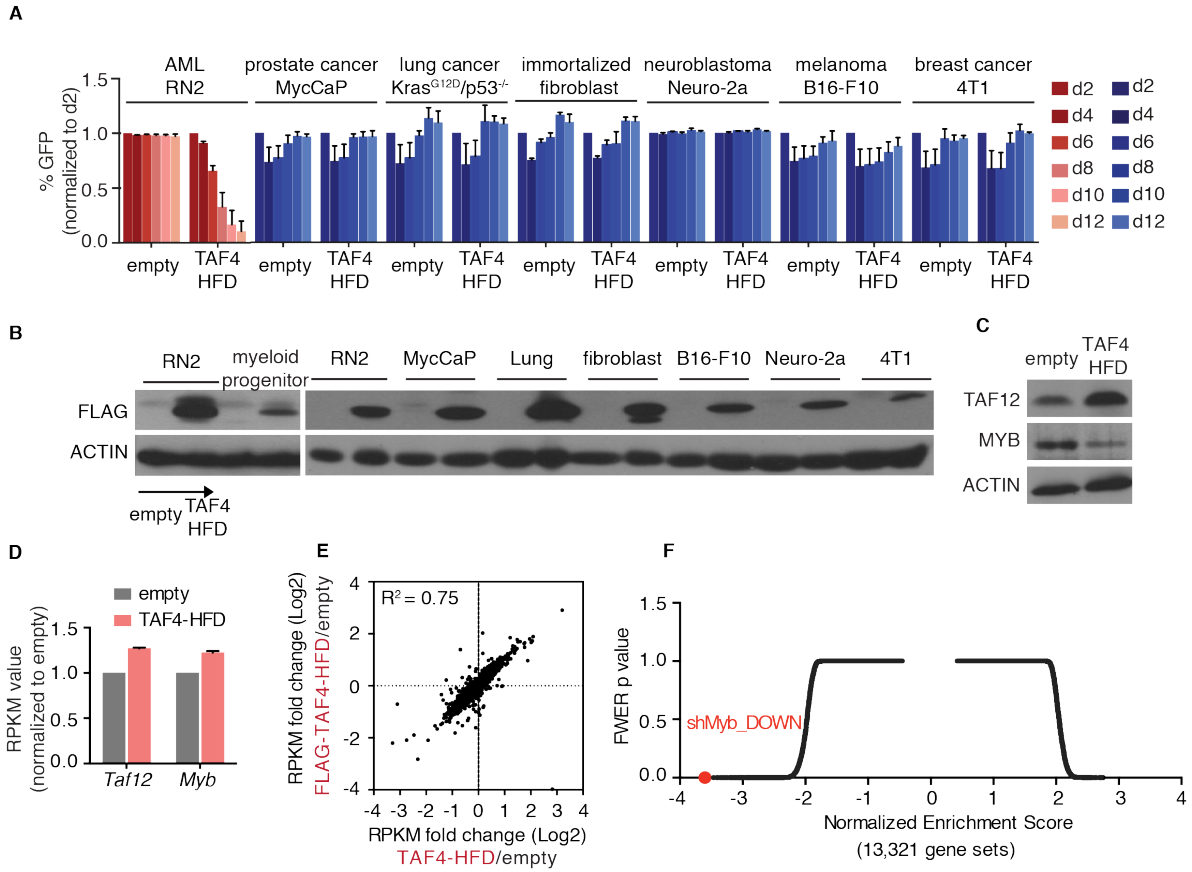


Figure S7. Additional analysis of TAF4-HFD experiments. Related to Figure 7.

(A) GFP depletion assays in the indicated cell lines overexpressing TAF4-HFD or empty vector (PIG vector). Data are presented as mean ± SEM GFP% normalized to d2, n=3.

(B) Western blot analysis of FLAG-TAF4-HFD level in the indicated cell lines on day 3 post viral infection of FLAG-TAF4-HFD or empty vector (PIG vector).

(C) Western blot analysis of TAF12 and MYB protein level in RN2 cells on day 3 post viral infection of TAF4-HFD or empty vector.

(D) Bar graph showing the mRNA level of *Taf12* and *Myb* upon TAF4-HFD overexpression, from the previously described RNA-seq experiments. Data are presented as mean ± SEM RPKM value, normalized to empty control sample, n=2.

(E) Scatter plot showing the correlation of RNA-seq data in RN2 cells overexpressing TAF4-HFD and FLAG-TAF4-HFD. 8,044 expressed genes with RPKM ≥5 in the empty vector are plotted.

(F) GSEA of TAF4-HFD overexpression over empty vector in RN2 cells. Normalized enrichment score (NES) and FWER p value were ranked and plotted for 13,321 gene sets which include all gene sets in the Molecular Signature Database v5.1. and a MYB signature, defined as top 200 downregulated genes following MYB knockdown in RN2 cells. Each gene signature is represented as a dot.