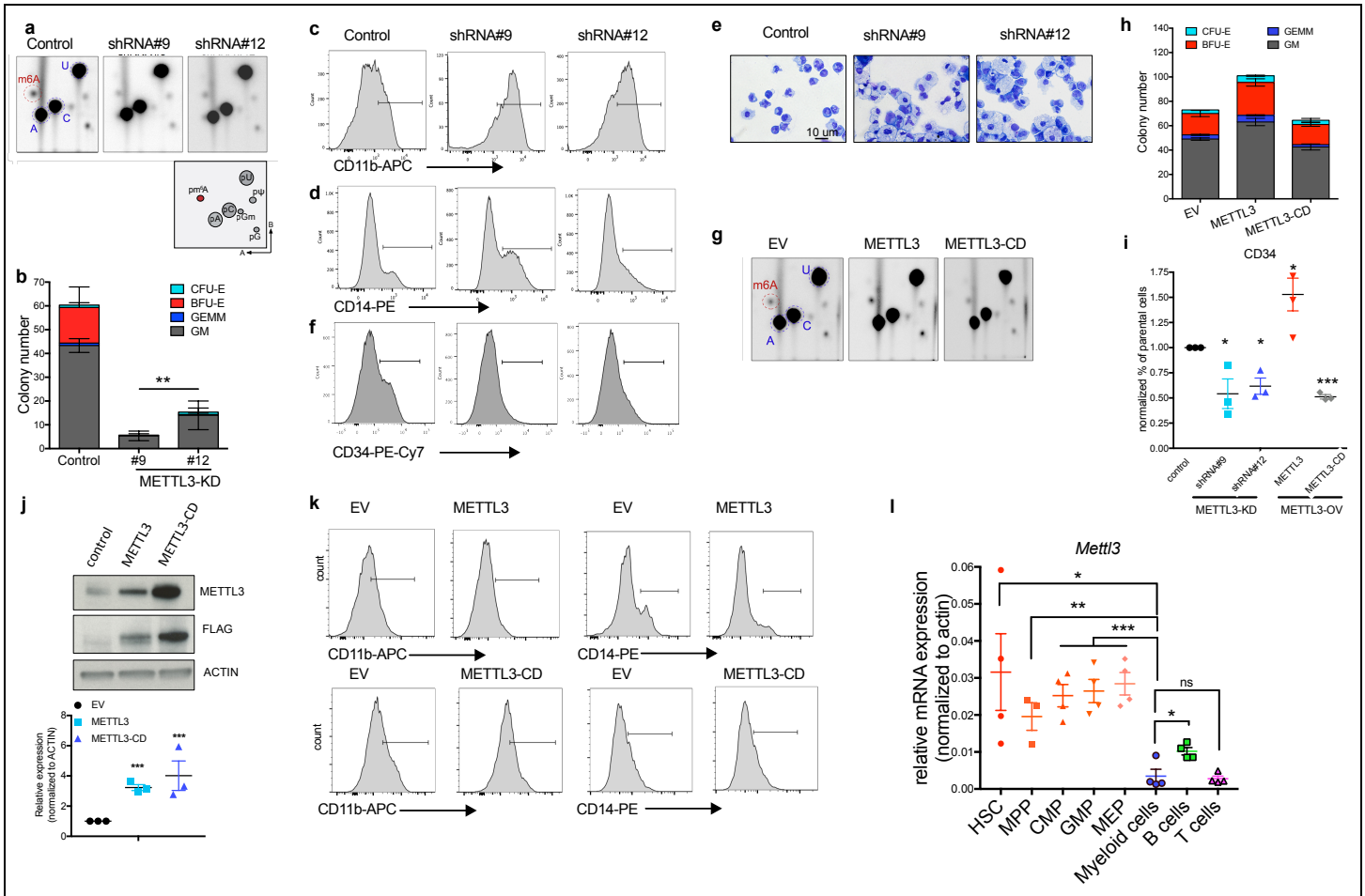
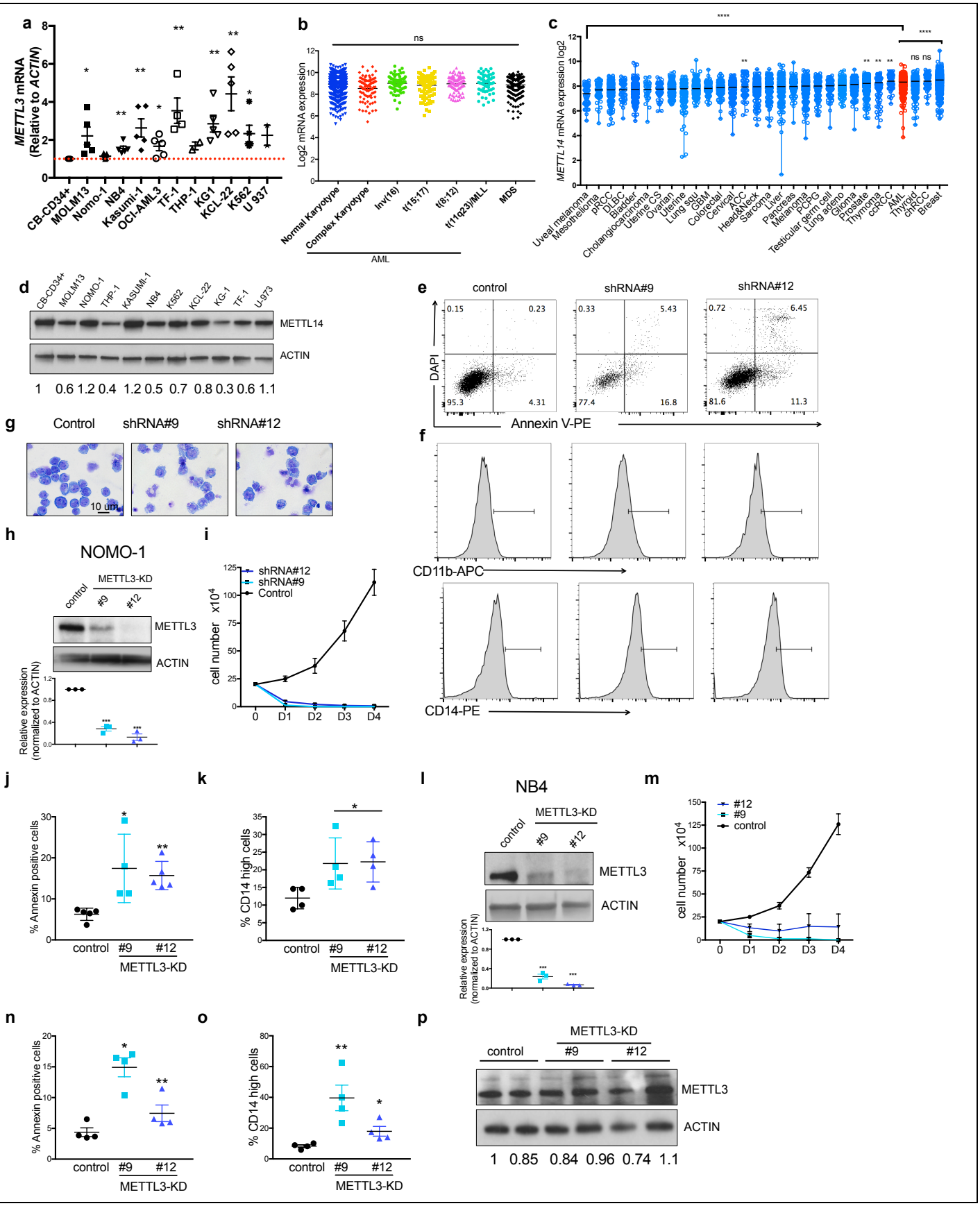


Supplementary Figure 1

m^{6}A is required to maintain hematopoietic stem/progenitor cells in an undifferentiated state.

(**a**) Representative TLC imagines of **Figure 1b**. (**b**) Colony forming assay of HSPCs in **Figure 1(a-e)**. 10^4 CD34+ cells were plated from control or *METTL3* knockdown cells four days post-transduction. The total number of colony forming units (CFUs) was scored two weeks after plating. Macrophage (light blue), Erythroid (red), GEMM =Granulocyte/Erythrocyte/Macrophage/Megakaryocyte (dark blue), and GM = Granulocyte/Macrophage (grey). n=3 independent experiments. ** p <0.01 two tailed *t* -test. (**c-d**) Myeloid differentiation of HSPCs in **Figure 1(a-e)**. Representative FACS analysis used to quantify differentiation of HSPCs in **Figure 1e**. (**e**) Morphological analysis of HSPCs in **Figure 1(a-e)**. Cytospins of control and *METTL3* knockdown normal hematopoietic cells were prepared and stained with GIEMSA. (**f**) Expression of stem/progenitor cells CD34+ surface marker. Representative FACS analysis used to quantify differentiation of HSPCs in **Supplemental figure 1i**. (**g**) Representative TLC imagines of **Figure 1f**.(**h**) Colony forming assay of HSPCs in **Figure 1 (f-h)**. n=2 independent experiments. (**i**) Quantitative summary of FACS analysis of CD34+ expression in control and METTL3-knockdown and in HSPCs transduced with EV and wild type METTL3 and METTL-CD. *p < 0.05, **p < 0.01, two-tailed *t* test. (**j**) Immunoblot analysis of METTL3 expression in HSPCs in **figure 1 (f-h)**. Top: representative immunoblot images. Bottom:

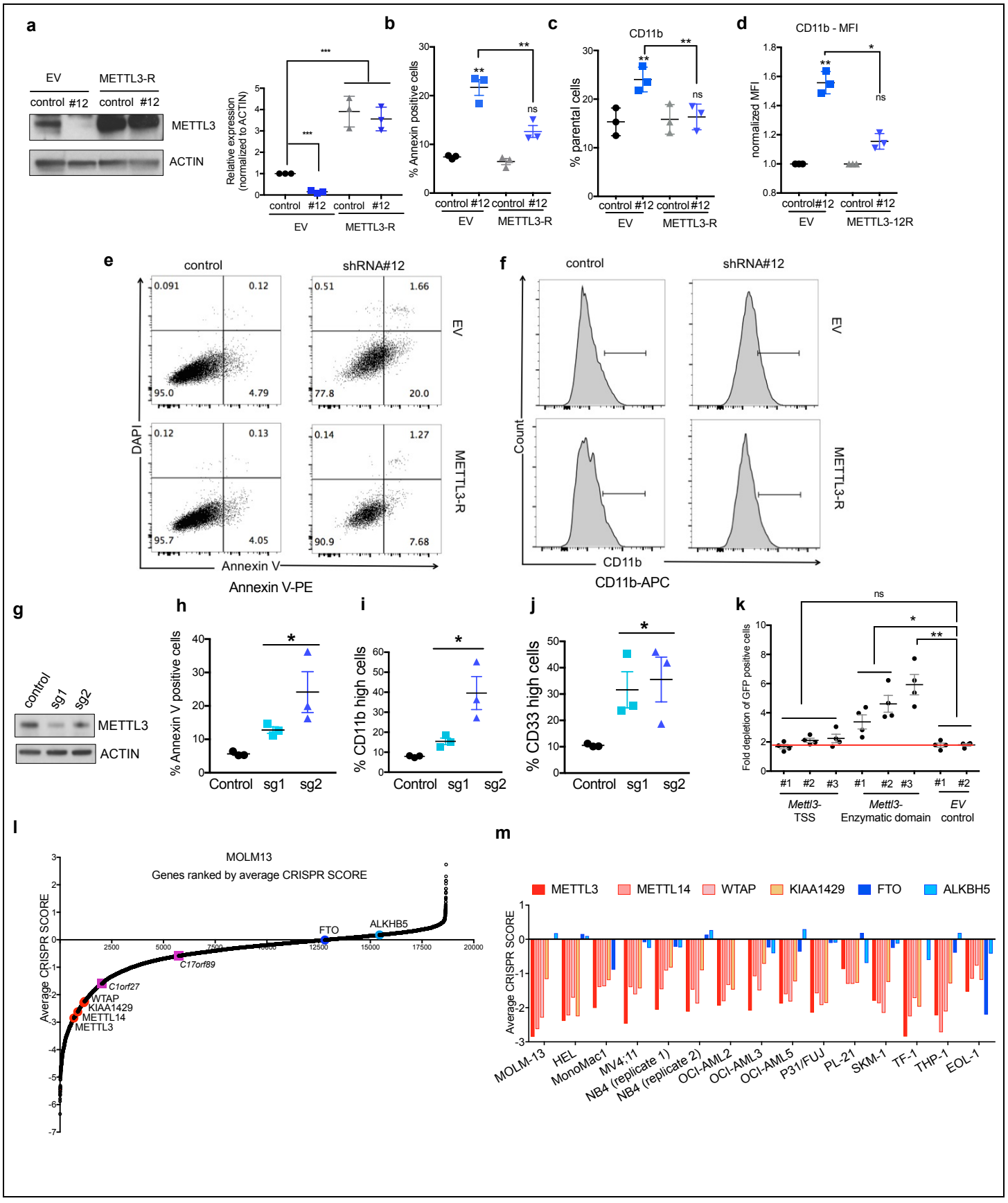
quantitative summary of the immunoblots. n=3 independent experiments; error bars, s.e.m. * p<0.05, **p<0.01,***p<0.001 two-tailed *t* test(**k**) Representative FACS analysis used to quantify differentiation of HSPCs in **Figure 1h**. (**l**) *Mettl3* mRNA expression in mouse hematopoietic cells. Bone marrow cells from n=4 wild type C57BL/6J mice were harvest. Cells from different population were sorted based on surface markers previously reported[1]. HSC: Hematopoietic stem cells; MPP: Multi-potent progenitor; GMP: Granulocyte Macrophage progenitor; CMP: Common Myeloid progenitor; MEP: Megakaryocyte-Erythrocyte progenitor; Myeloid cells (Gr1+Mac1+); B cells (B220+) and T cells (IgM+). n=4 independent experiments; error bars, s.e.m. * p<0.05, **p<0.01,***p<0.001 two-tailed *t* test


a
METTL3 mRNA (Relative to ACTIN)
CB-CD34+
MOLM13
Nomo-1
NB4
Kasumi-1
OCI-AML3
TF-1
THP-1
KG1
KCL-22
K562
U 937
b
Log2 mRNA expression
ns
Normal Karyotype
Complex Karyotype
Inv(16)
t(15;17)
t(8;12)
t(11q23)/MLL
MDS
AML
c
METTL14 mRNA expression log2
Uveal melanoma
Mesothelioma
pRCC
DLBC
Bladder
Cholangiocarcinoma
Uterine CS
Ovarian
Uterine
Lung squ
GBM
Colorectal
Cervical
ACC
Head&Neck
Sarcoma
Liver
Pancreas
Melanoma
PCPG
Testicular germ cell
Lung adeno
Glioma
Prostate
Thymoma
ccRCC
AML
Thyroid
chRCC
Breast
d
CB-CD34+
MOLM13
NOMO-1
THP-1
KASUMI-1
NB4
K562
KCL-22
KG-1
TF-1
U-973
METTL14
ACTIN
1 0.6 1.2 0.4 1.2 0.5 0.7 0.8 0.3 0.6 1.1
e
control
shRNA#9
shRNA#12
DAPI
Annexin V-PE
0.15
0.23
95.3
4.31
0.33
5.43
77.4
16.8
0.72
6.45
81.6
11.3
f
CD11b-APC
CD14-PE
g
Control
shRNA#9
shRNA#12
10 um
h
NOMO-1
METTL3-KD
control
#9
#12
METTL3
ACTIN
Relative expression (normalized to ACTIN)
i
shRNA#12
shRNA#9
Control
cell number x10^4
0
D1
D2
D3
D4
j
% Annexin positive cells
control
#9
#12
METTL3-KD
k
% CD14 high cells
control
#9
#12
METTL3-KD
l
NB4
METTL3-KD
control
#9
#12
METTL3
ACTIN
Relative expression (normalized to ACTIN)
m
#12
#9
control
cell number x10^4
0
D1
D2
D3
D4
n
% Annexin positive cells
control
#9
#12
METTL3-KD
o
% CD14 high cells
control
#9
#12
METTL3-KD
p
METTL3-KD
control
#9
#12
METTL3
ACTIN
1 0.85 0.84 0.96 0.74 1.1

Supplementary Figure 2

METTL3 is essential for acute myeloid leukemia cells

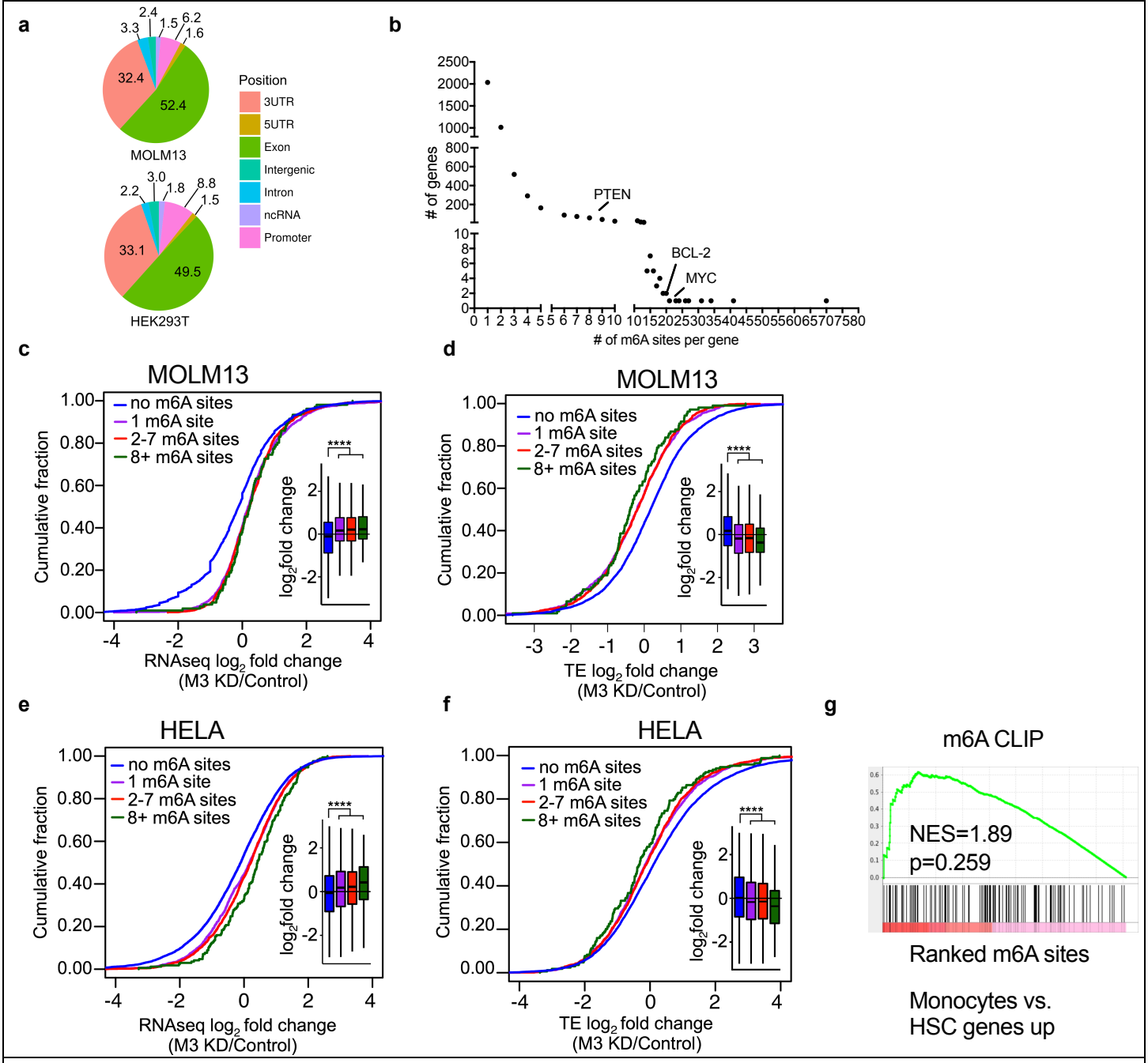
(**a**) mRNA expression of *METTL3* in 11 AML cell lines versus control HSPCs. RNA was extracted from cells and *METTL3* mRNA was quantified by qPCR. *ACTIN* serves as control. (**b**) *METTL3* mRNA expression AML patients. The graph shows the log_2 expression of *METTL3* from patients with various subtypes of AML and MDS. Normal karyotype, n=989; Complex karyotype, n=87; AML Inv(16), n=77; AML t(15;17), n=87; AML t(8;12), n=98, AML t(11q23)/MLL, n=58; MDS, n=228. (BloodPool data of *METTL3* probe 209265_s_at from U133 Plus 2.0 array)[2]. (**c**) *METTL14* mRNA expression in AML compared to other cancers. **** p<0.00001, ** p<0.01 ns not significant, ANOVA with multiple comparisons. (**d**) METTL14 protein levels in normal HSPCs and AML cell lines. Quantitative summary is shown. (**e**) Representative FACS analysis used to quantify apoptosis of MOLM13 cells in **Figure 2f**. (**f**) Representative FACS analysis used to quantify differentiation of MOLM13 cells in **Figure 2g**. (**g**) Morphological analysis of HSPCs in **Figure 2(g)**. Cytospins of control and *METTL3* knockdown MOLM13 cells were prepared and stained with GIEMSA. (**h-o**) METTL3 depletion multiple AML cell lines induces differentiation and apoptosis. Immunoblot of METTL3 expression four days post-transduction with shRNA lentivirus in NOMO-1 cells (**h**) and NB4 cells (**l**). Quantitative summary is shown. Knockdown of *METTL3* reduced proliferation in NOMO-1 cells (**i**) and NB4 cells (**m**). NOMO-1 and NB4 cells had increased apoptosis (**j** and **n**, respectively) and increased myeloid differentiation (**k** and **o**, respectively) following METTL3 depletion. n=3 independent experiments; error bars, s.e.m. * p<0.05, **p<0.01,***p<0.001 two-tailed *t* test. (**p**) Immunoblot analysis of MOLM13 *METTL3* knockdown cells that outgrow in moribund leukemic mice. Leukemia cells were sorted for human CD45 positive (a marker for hematopoietic cells) from leukemic mice in **Figure 2h**. ACTIN servers as loading control. Quantitative summary is shown.


a
EV
METTL3-R
control #12 control #12
METTL3
ACTIN
Relative expression (normalized to ACTIN)
control #12 control #12
EV
METTL3-R
b
% Annexin positive cells
control #12 control #12
EV
METTL3-R
c
CD11b
% parental cells
control #12 control #12
EV
METTL3-R
d
CD11b - MFI
normalized MFI
control#12 control #12
EV
METTL3-12R
e
control
shRNA#12
EV
METTL3-R
DAPI
Annexin V
Annexin V-PE
f
control
shRNA#12
EV
METTL3-R
Count
CD11b
CD11b-APC
g
control
sg1
sg2
METTL3
ACTIN
h
% Annexin V positive cells
Control sg1 sg2
i
% CD11b high cells
Control sg1 sg2
j
% CD33 high cells
Control sg1 sg2
k
Fold depletion of GFP positive cells
#1 #2 #3 #1 #2 #3 #1 #2
Mettl3-TSS
Mettl3-Enzymatic domain
EV control
l
MOLM13
Genes ranked by average CRISPR SCORE
Average CRISPR SCORE
FTO
ALKHB5
C17orf89
C1orf27
WTAP
KIAA1429
METTL14
METTL3
m
METTL3 METTL14 WTAP KIAA1429 FTO ALKBH5
Average CRISPR SCORE
MOLM-13
HEL
MonoMac1
MV4;11
NB4 (replicate 1)
NB4 (replicate 2)
OCI-AML2
OCI-AML3
OCI-AML5
P31/FUJ
PL-21
SKM-1
TF-1
THP-1
EOL-1

Supplementary Figure 3

METTL3 is required for survival of acute myeloid leukemia cells

(**a**) Immunoblot of control or knockdown MOLM13 cells previously transduced with empty vector (EV) or METTL3-12R resistant to targeting by shRNA#12. Top: representative immunoblot images. Bottom: quantitative summary of the immunoblots. n=3 independent experiments; error bars, s.e.m. * p<0.05, **p<0.01,***p<0.001 two-tailed *t* test. (**b-d**) Overexpression of the shRNA-resistant METTL3-12R rescues survival and differentiation defects of MOLM13 cells depleted of endogenous METTL3. Quantitative summary of Annexin V staining (**b**), percentage of CD11b cells (**c**) and MFI of CD11b expression (**d**). n=3 independent experiments, error bars, s.e.m. * p<0.05, **p<0.001 two-tailed t test. (**e-f**) Representative FACS analysis used to quantify apoptosis of MOLM13 cells in **Supplemental Figure 2b** and differentiation in **Supplemental Figure 2c-d**. (**g-j**) CRISPR/Cas9 mediated- deletion of METTL3 in MOLM13 cells. (**g**) Immunoblot showing METTL3 expression in MOLM13 cells upon CRISPR/Cas9-mediated deletion with two independent sgRNAs (sg1 and sg2) three days post transduction. ACTIN serves as loading control. Cells were assayed for Annexin V positivity and (**h**) and differentiation (**i-j**) as previously described with *METTL3* shRNAs. n=3 independent experiments; error bars, s.e.m. * p<0.05, **p<0.001, *** p <0.0001 two-tailed *t* test. (**k**) The enzymatic domain of METTL3 is essential for AML cell viability. Mouse MLL-AF9 $NRAS^{G12D}$ constitutively expressing Cas9 (RN2-Cas9) cells were transduced with guide RNAs (gRNAs) that specifically target regions of METTL3. Targeted regions include the transcription start site (TSS, black bars), METTL3 enzymatic domain (blue bars), or an empty virus control (grey bars). Viability was measured as the fold depletion of GFP positive cells (gRNAs transduced cells) beginning two days post-transduction. n=4 independent experiments, error bars, s.e.m. * p<0.05, **p<0.001 two-tailed t test. (**l**) CRISPR score rank of members of the RNA methylation "writers" complex and "erasers" in MOLM13 cells. CRISPR score (CS) is the average $\log_2$ fold-change in the abundance of all single guide (sg)RNAs targeting the gene after 14 population doublings[3]. Members of the "writer" complex METTL3, METTL14, WTAP, and KIAA1429 were highlighted in red. The "erasers" were highlighted in blue. C1orf27 and C17orf89, highlighted in purple, were genes previously reported to be essential for survival of leukemia cells. (**m**) CRISPR score of members of the RNA methylation "writers" complex and "erasers" across all 14 tested leukemia cell lines[3].

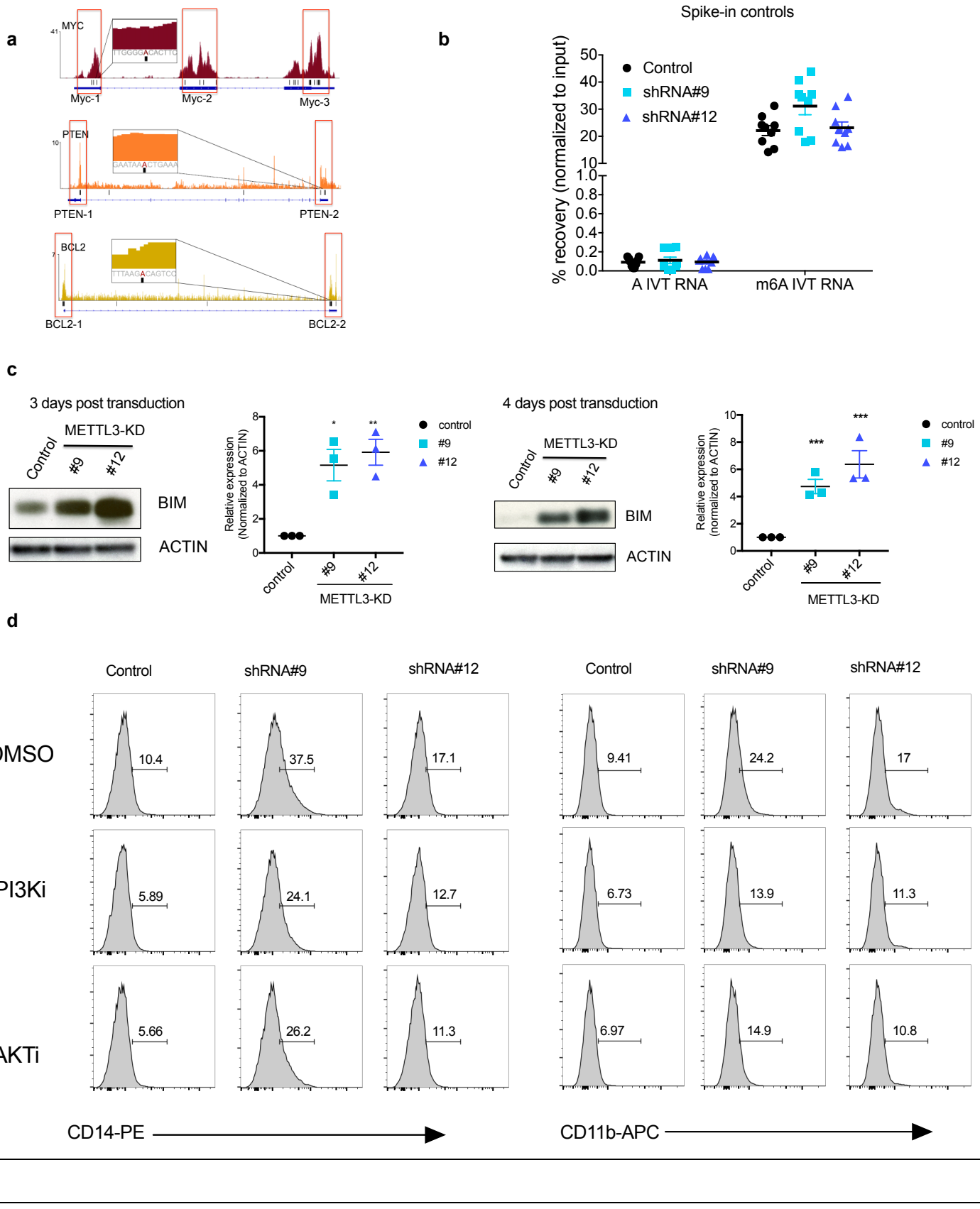


Supplementary Figure 4

Mapping m^{6}A in MOLM13 at single-nucleotide resolution

(**a**) The distribution of m^{6}A in MOLM13 cells compared to the distribution of HEK293T cells (**b**) Plot showing the number of m^{6}A sites per gene (x-axis) versus the number of genes (y-axis). (**c**) Correlation between the number of m^{6}A sites and mRNA expression. Transcripts were binned based on the number of m^{6}A sites: no sites, 1 site, 2-7 sites, or 8+ sites and analyzed their expression. n=3 independent replicates per condition, **** $p<2.2e^{-16}$, one-way ANOVA with Tukey's post-hoc test. (**d**) Translational efficiency is correlated with the number of m^{6}A sites per transcript. Transcripts were binned based on the number of m^{6}A sites: no sites, 1 site, 2-7

sites, or 8+ sites and analyzed their translational efficiency. n=3 independent replicates per condition, **** $p<2.2e^{-16}$, one-way ANOVA with Tukey's post-hoc test. (**e**) Correlation between m^6A sites and mRNA expression after *METTL3* knockdown in HeLa cells. n=1 independent replicate per condition, **** $p<2.2e^{-16}$, one-way ANOVA with Tukey's post-hoc test. (**f**) Correlation between m^6A sites and translational efficiency (TE) after *METTL3* knockdown in HeLa cells. n=1 independent replicate per condition, **** $p<2.2e^{-16}$, one-way ANOVA with Tukey's post-hoc test. (g) The Gene set promoting monocyte differentiation enrichment analysis for m6A ranked lists.


a
MYC
Myc-1
Myc-2
Myc-3
PTEN
PTEN-1
PTEN-2
BCL2
BCL2-1
BCL2-2
b
Spike-in controls
% recovery (normalized to input)
Control
shRNA#9
shRNA#12
A IVT RNA
m6A IVT RNA
c
3 days post transduction
METTL3-KD
Control
#9
#12
BIM
ACTIN
Relative expression (Normalized to ACTIN)
control
#9
#12
METTL3-KD
4 days post transduction
d
Control
shRNA#9
shRNA#12
DMSO
PI3Ki
AKTi
10.4
37.5
17.1
9.41
24.2
17
5.89
24.1
12.7
6.73
13.9
11.3
5.66
26.2
11.3
6.97
14.9
10.8
CD14-PE
CD11b-APC

Supplementary Figure 5

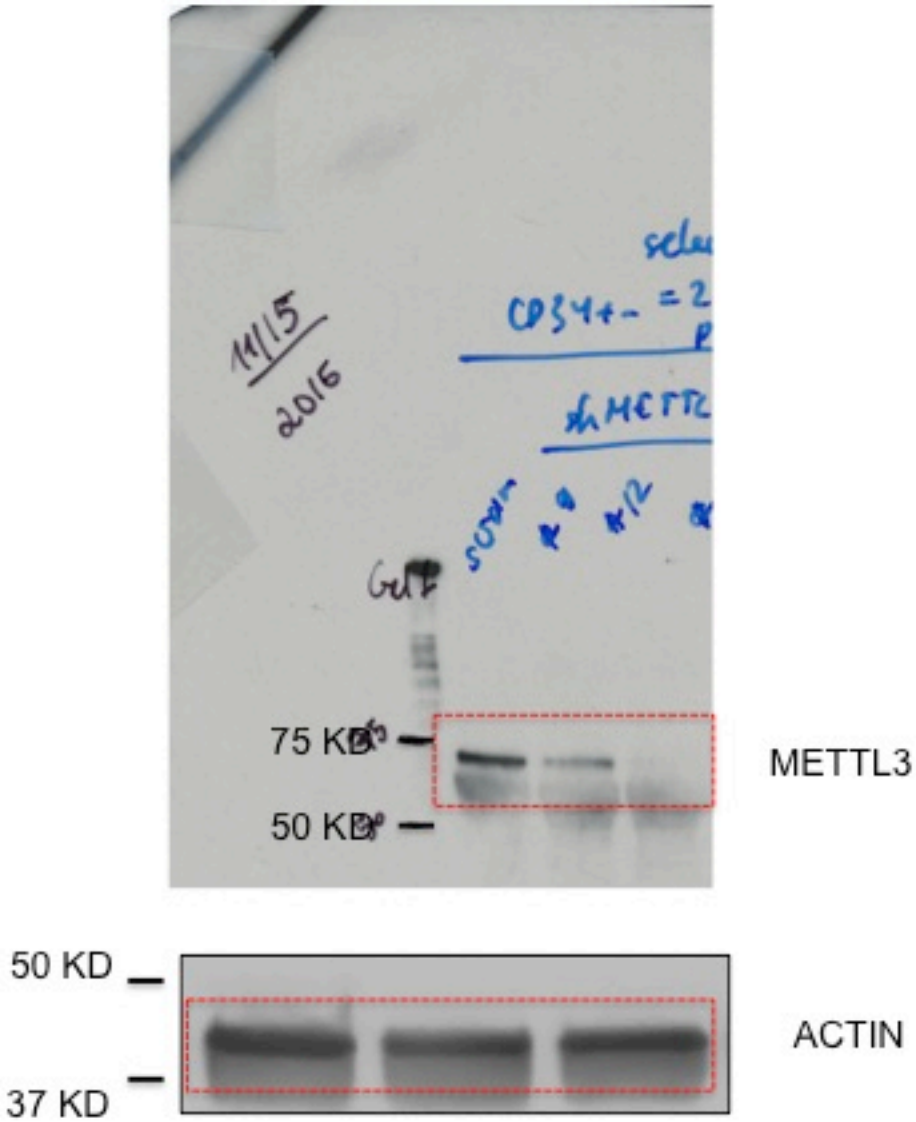
m^{6}A modulates expression of targets c-MYC, PTEN and BCL2 and attenuation of the AKT pathway.

(**a**) m^{6}A sites in *MYC*, *PTEN*, and *BCL2*. Reads (unique tags per millions) from miCLIP in MOLM13 with called m^{6}A sites indicated with black vertical bars. Zoomed-in areas show individual m^{6}A sites called based on C→T transitions. (**b**) Validation of meRIP-qPCR with spike-in RNA controls. Negative and positive control RNAs were in vitro transcribed with either ATP or m^{6}ATP in the reaction. 100 ng of each RNA were added to poly-A+ purified RNA from MOLM-13 cells immediately before immunoprecipitation with an anti-m^{6}A antibody. Plots represent the recovery of the control RNAs after immunoprecipitation as a percentage of the total input. (**c**) Immunoblot analysis for the protein level of BIM upon METTL3 depletion in MOLM13 cells. The panels are representative blots from three days or four days post-transduction of MOLM13 cells with 2 shRNAs targeting METTL3. ACTIN serves as loading control. Left: representative immunoblot images. Right: quantitative summary of the immunoblots. n=3 independent experiments; error bars, s.e.m. * $p<0.05$, **$p<0.01$,***$p<0.001$ two-tailed *t* test. (**d**) Inhibition of the AKT pathway inhibits myeloid differentiation of MOLM13 METTL3 knocked down cells. Representative FACS analysis used to quantify differentiation of MOLM13 cells in **Figure 4g-h.**

Reference:

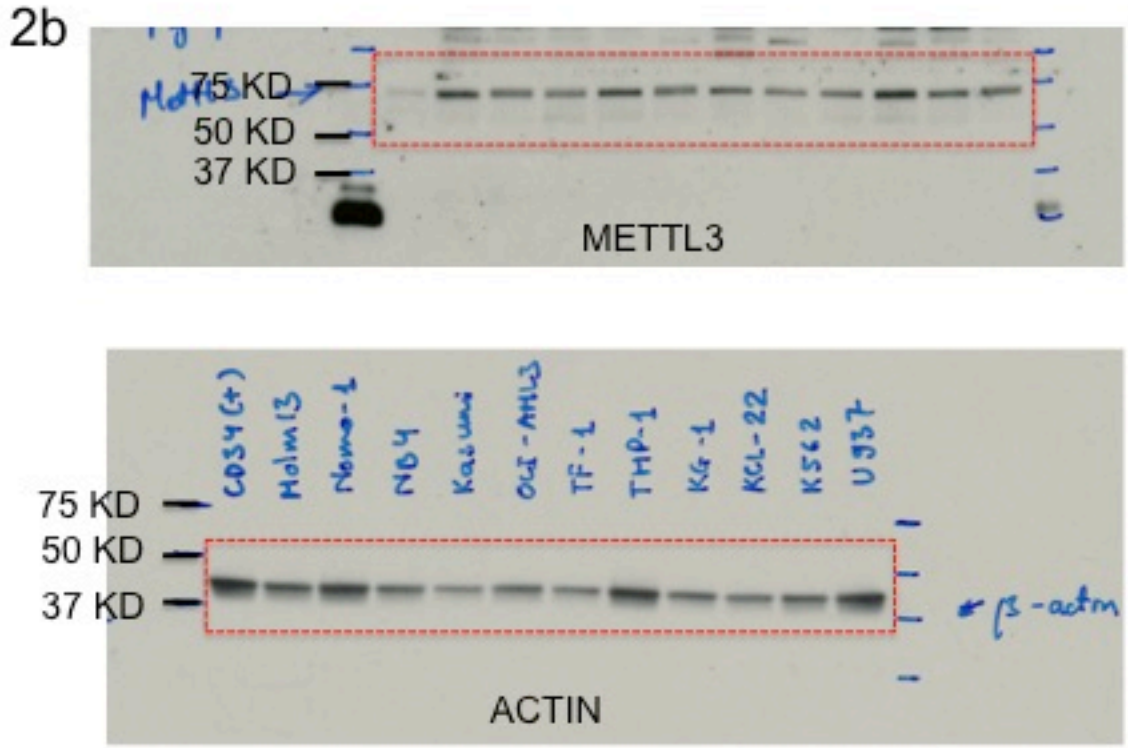
1. Kharas, M.G.*, et al.* Musashi-2 regulates normal hematopoiesis and promotes aggressive myeloid leukemia. *Nat Med* **16**, 903-908 (2010).
2. Bagger, F.O.*, et al.* BloodSpot: a database of gene expression profiles and transcriptional programs for healthy and malignant haematopoiesis. *Nucleic acids research* **44**, D917-924 (2016).
3. Wang, T.*, et al.* Gene Essentiality Profiling Reveals Gene Networks and Synthetic Lethal Interactions with Oncogenic Ras. *Cell* **168**, 890-903 e815 (2017).

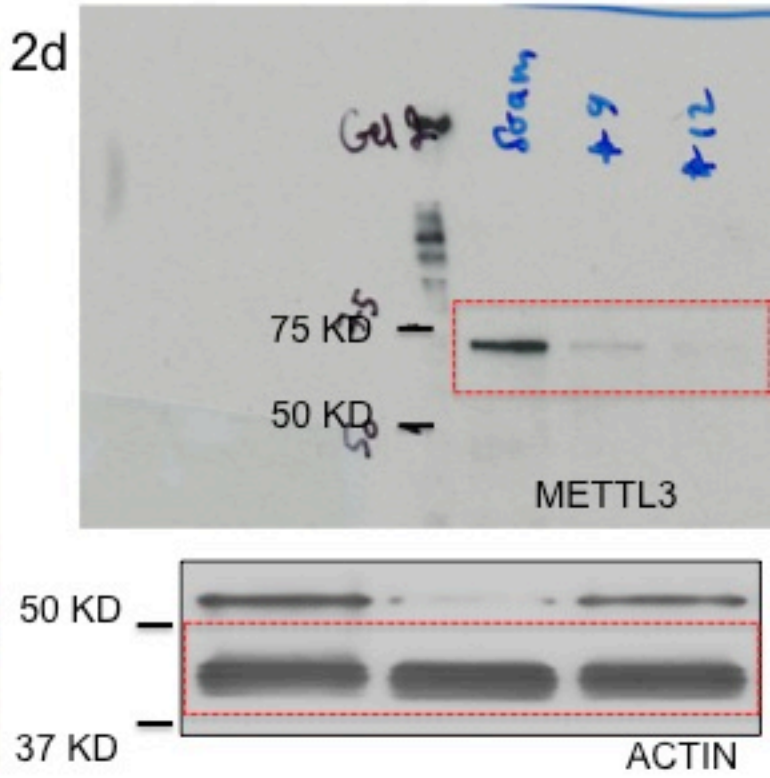
1a



METTL3 KD in CD34+ cells

Figure 1


2b
75 KD
50 KD
37 KD
METTL3
COS4 (+)
Molm13
Nomo-1
NB4
Kasumi
Oci-AML3
TF-1
THP-1
KG-1
KCL-22
K562
U937
75 KD
50 KD
37 KD
β-actin
ACTIN
Cell panel


2d
75 KD
50 KD
METTL3
50 KD
37 KD
ACTIN
METTL3 KD in MOLM13 cells

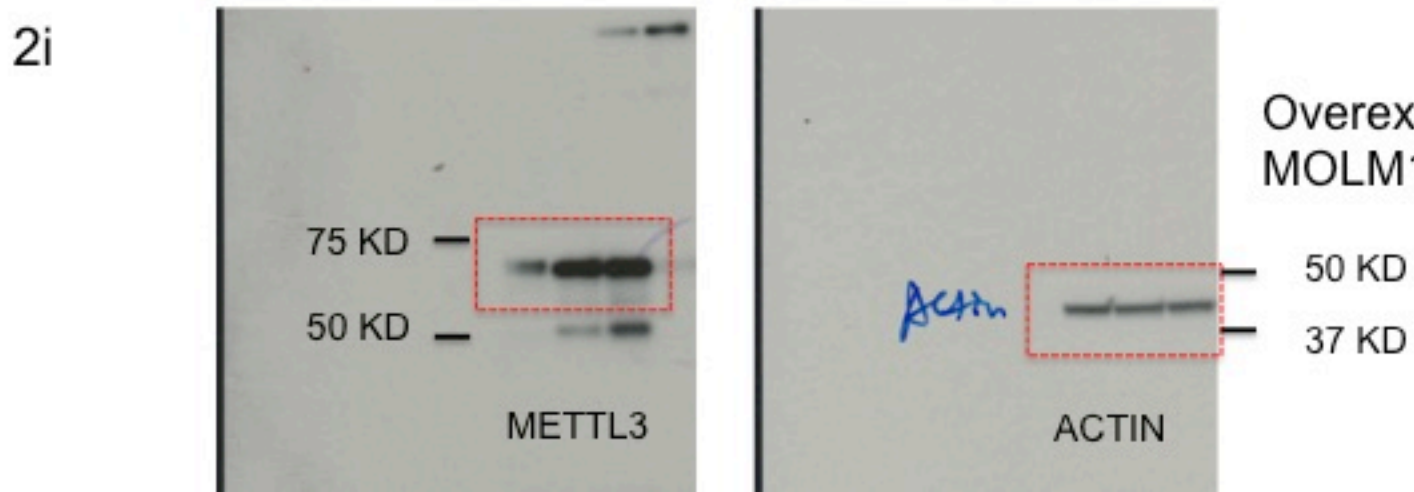
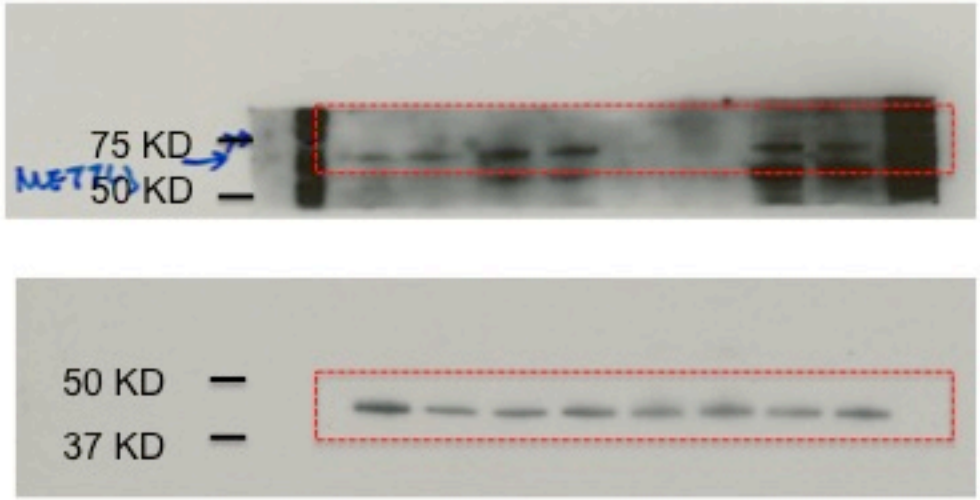

2i
75 KD
50 KD
METTL3
Actin
ACTIN
50 KD
37 KD
Overexpression in
MOLM13 cells

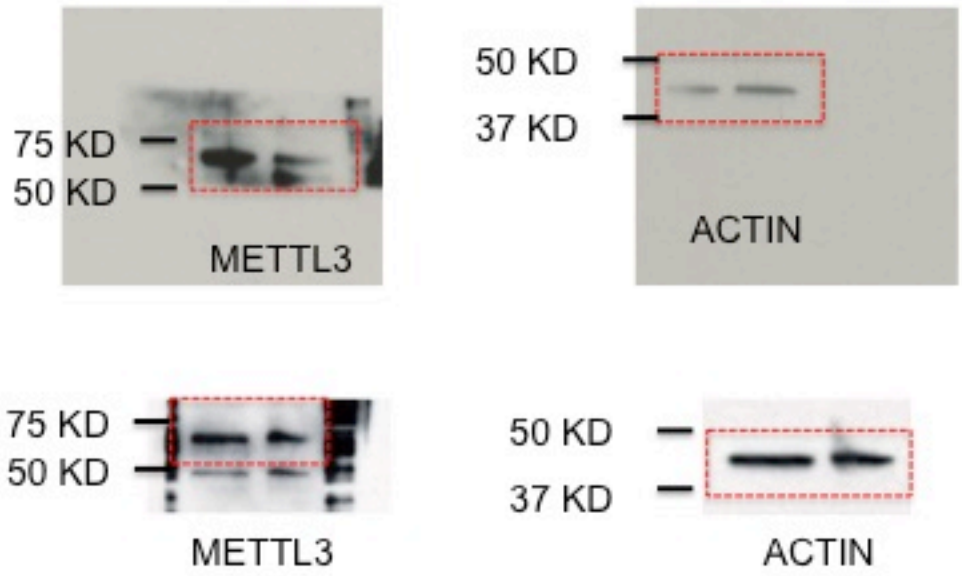
Figure 2

2k



human patients panel

2n



METTL3 KD in human AML patient

Figure 2

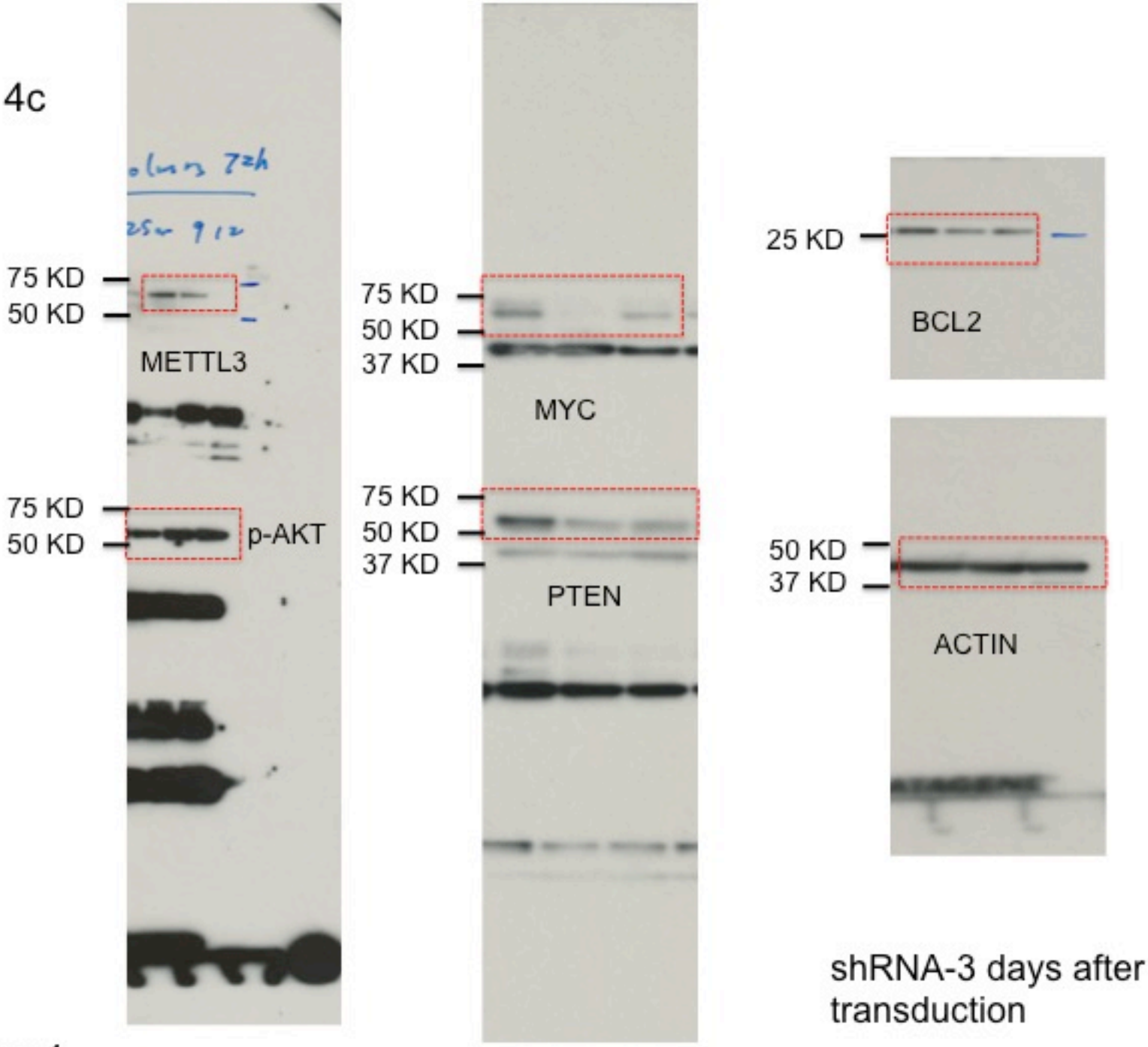

4c
75 KD
50 KD
METTL3
75 KD
50 KD
p-AKT
75 KD
50 KD
37 KD
MYC
75 KD
50 KD
37 KD
PTEN
25 KD
BCL2
50 KD
37 KD
ACTIN
shRNA-3 days after transduction

Figure 4

4d

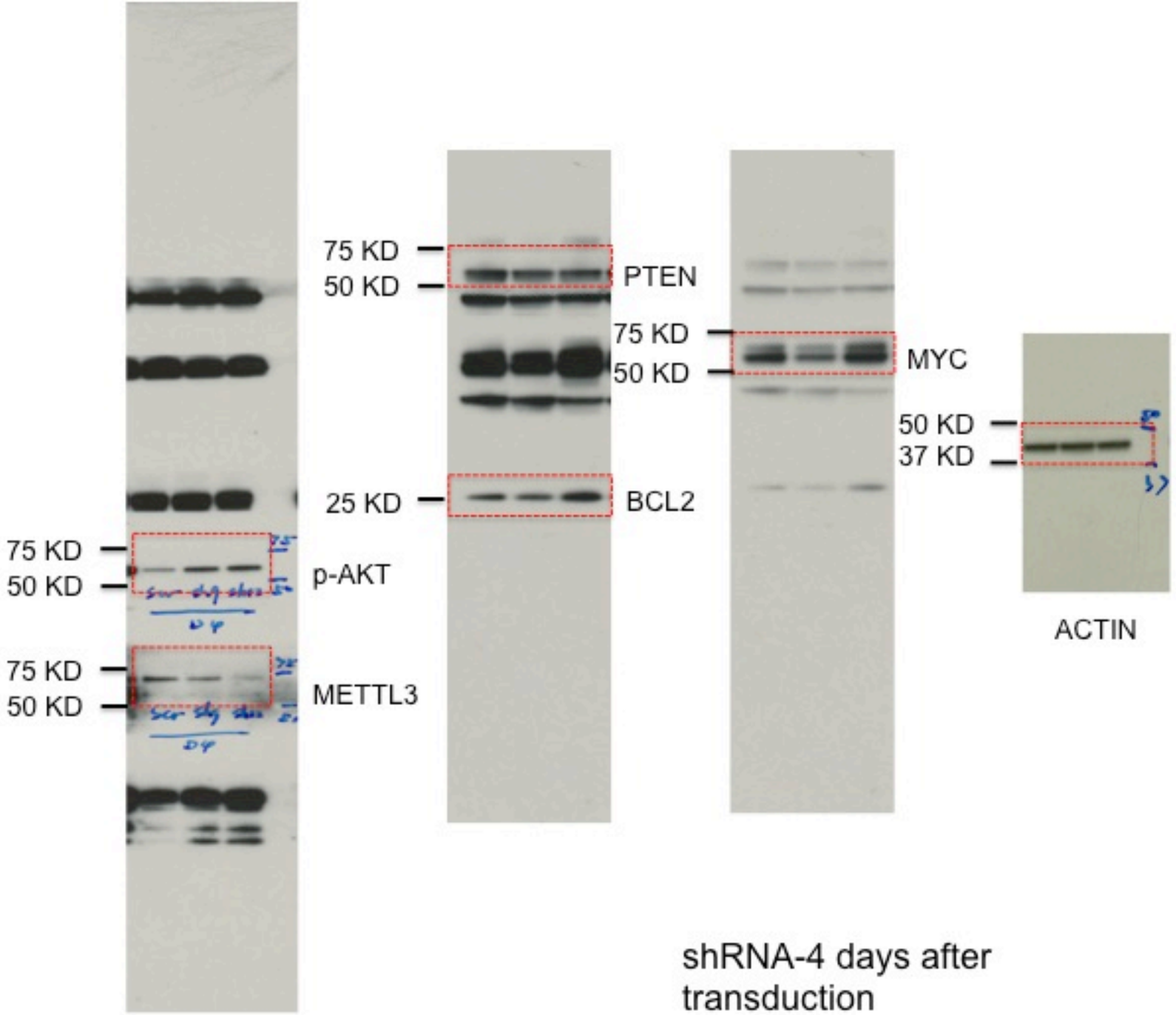
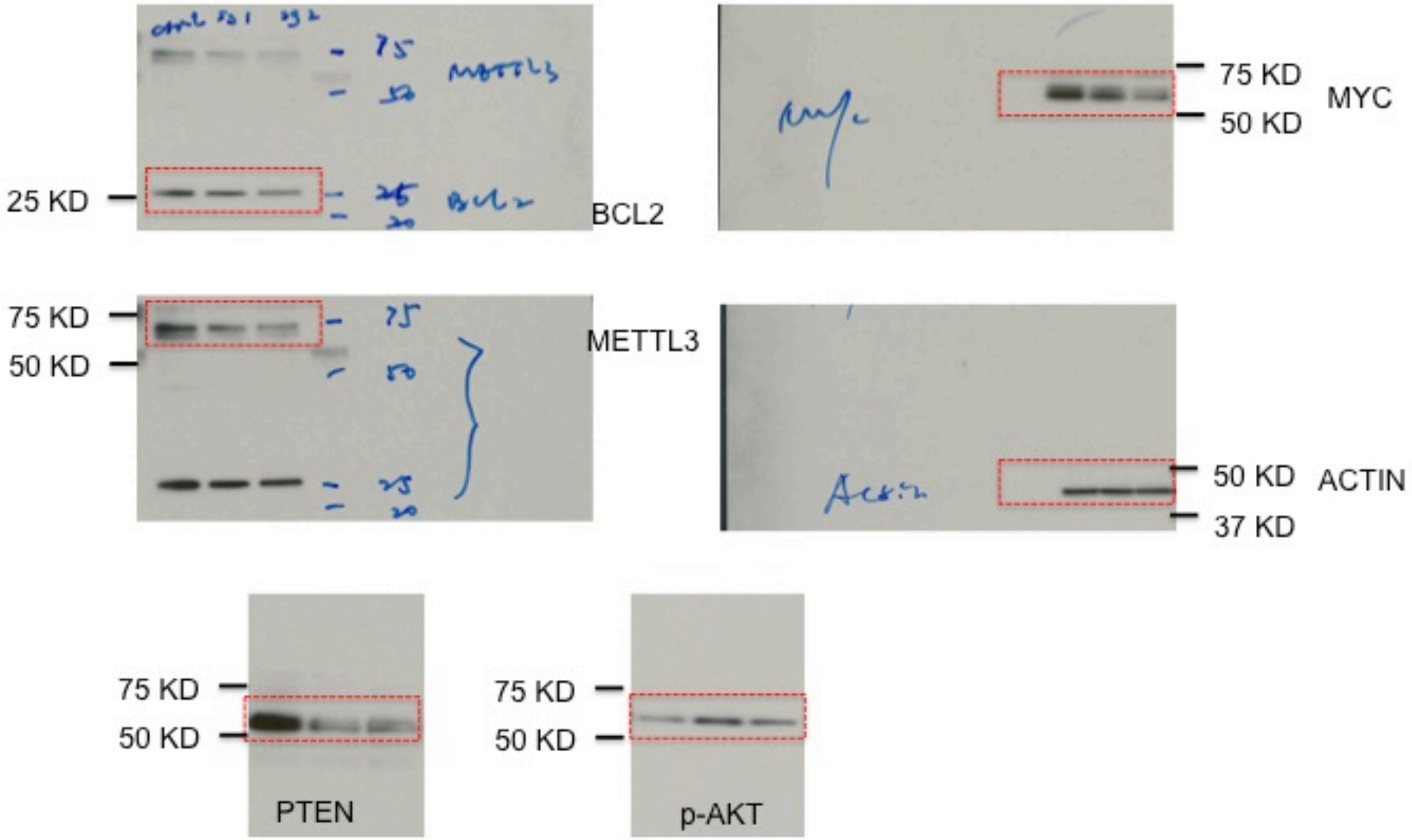

75 KD
50 KD
p-AKT
75 KD
50 KD
METTL3
75 KD
50 KD
PTEN
25 KD
BCL2
75 KD
50 KD
MYC
50 KD
37 KD
ACTIN
shRNA-4 days after transduction

Figure 4

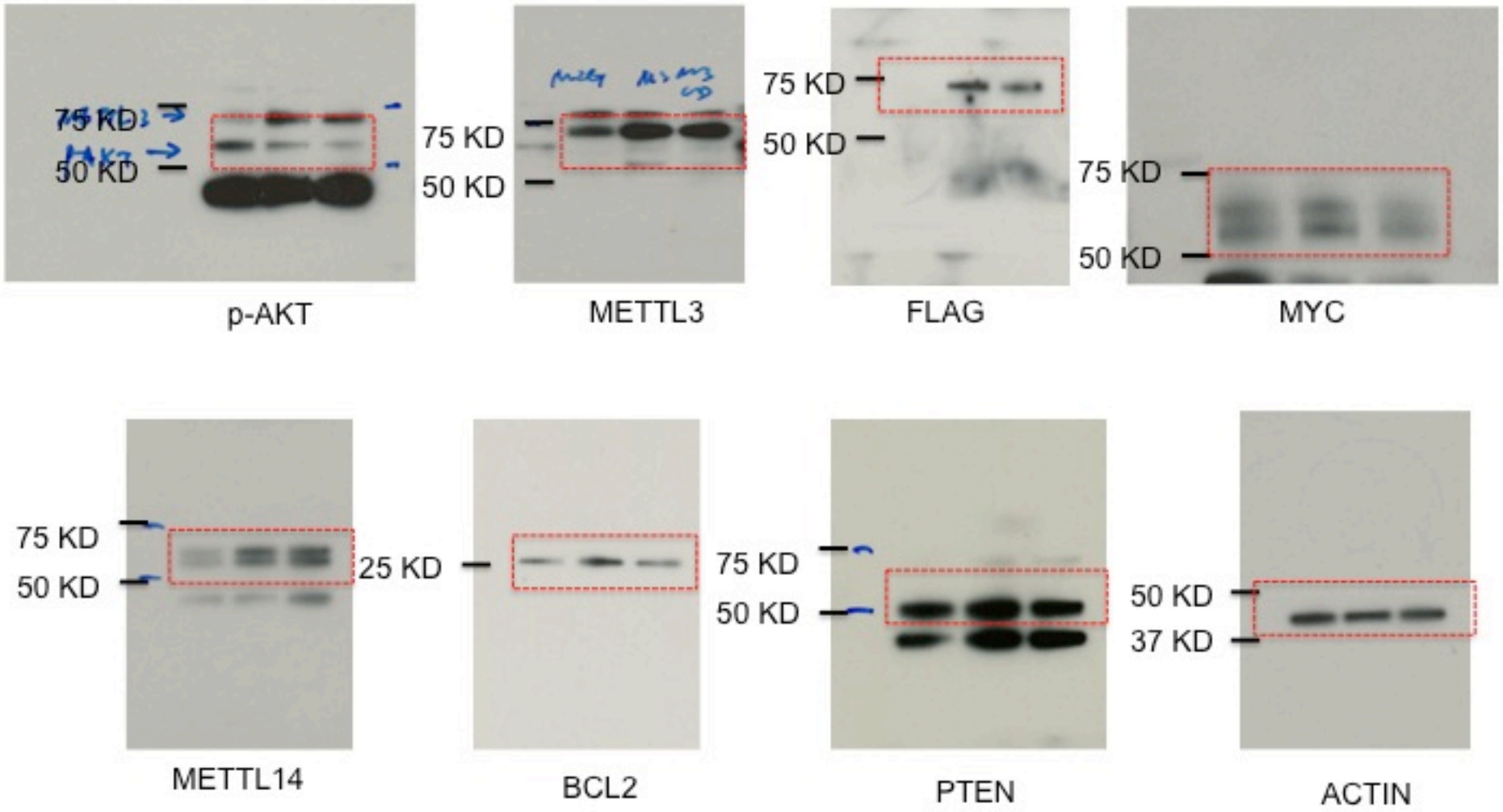
4e



sgRNA-3days after transduction

Figure 4

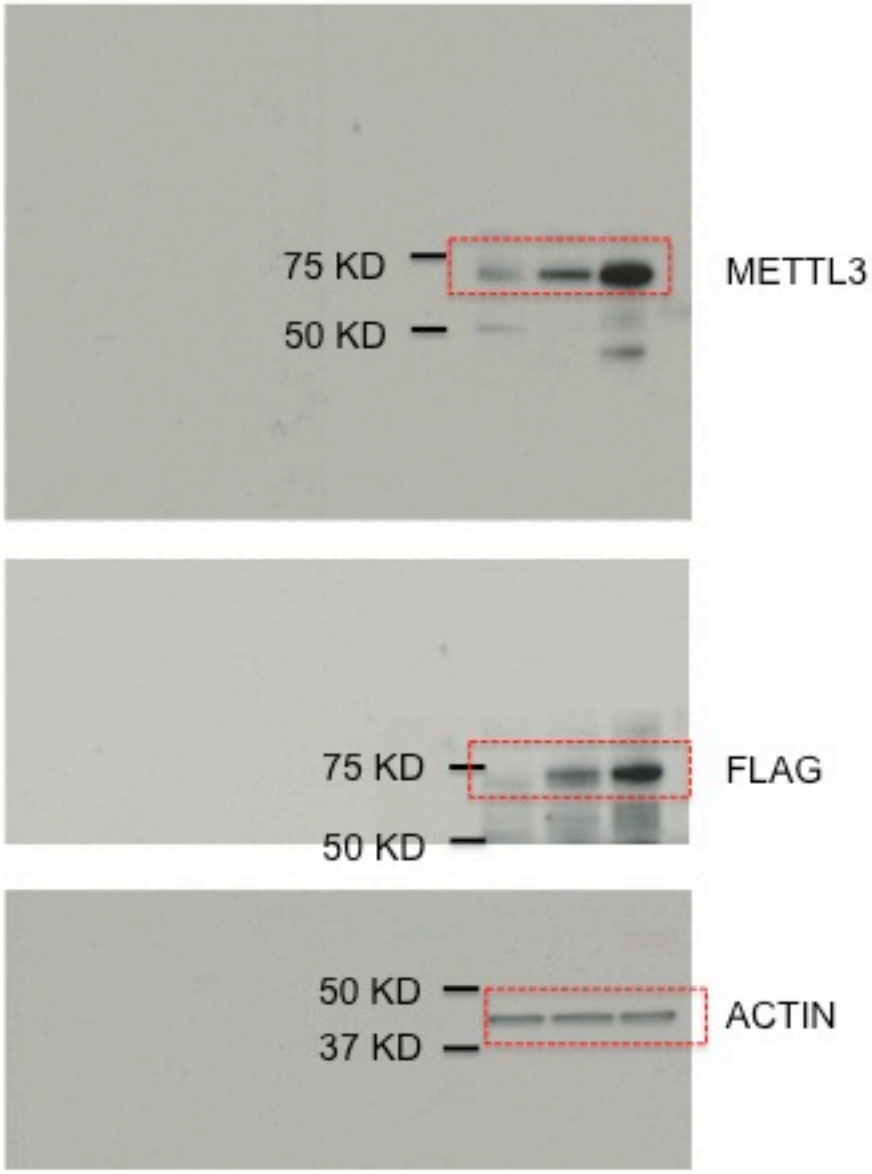
4f



Overexpression of METTL3 and METTL3-CD

Figure 4

S1j



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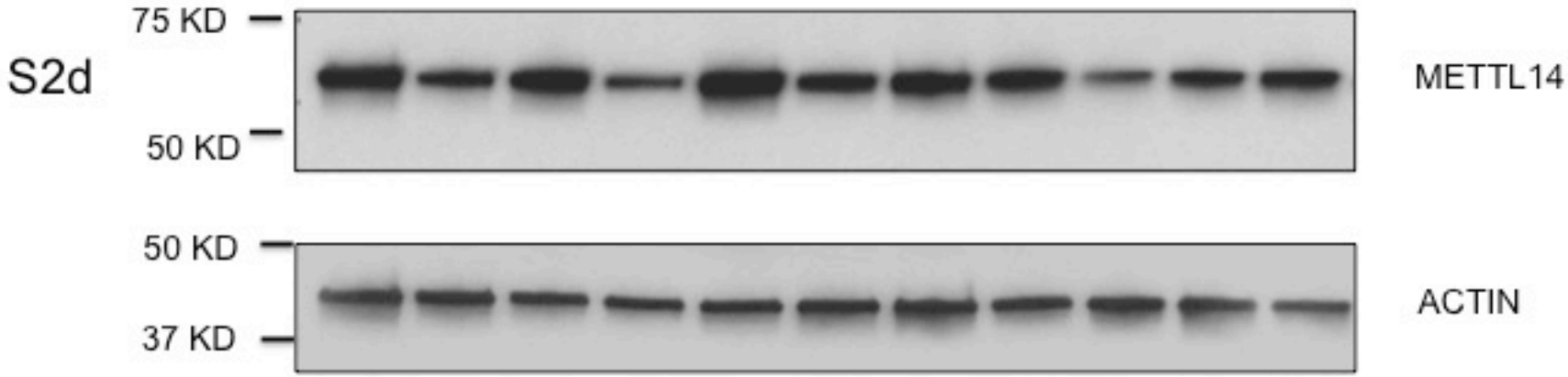
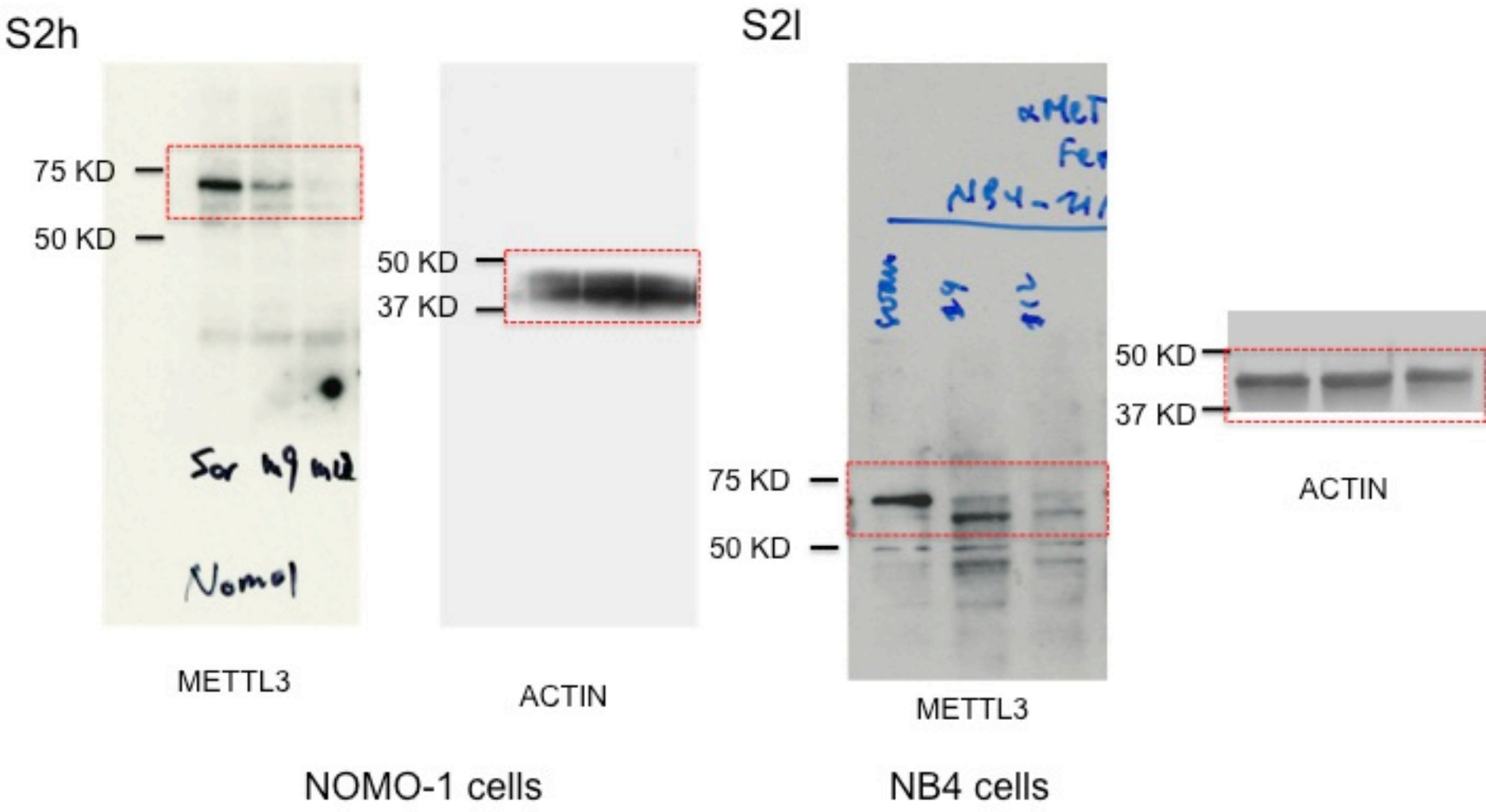
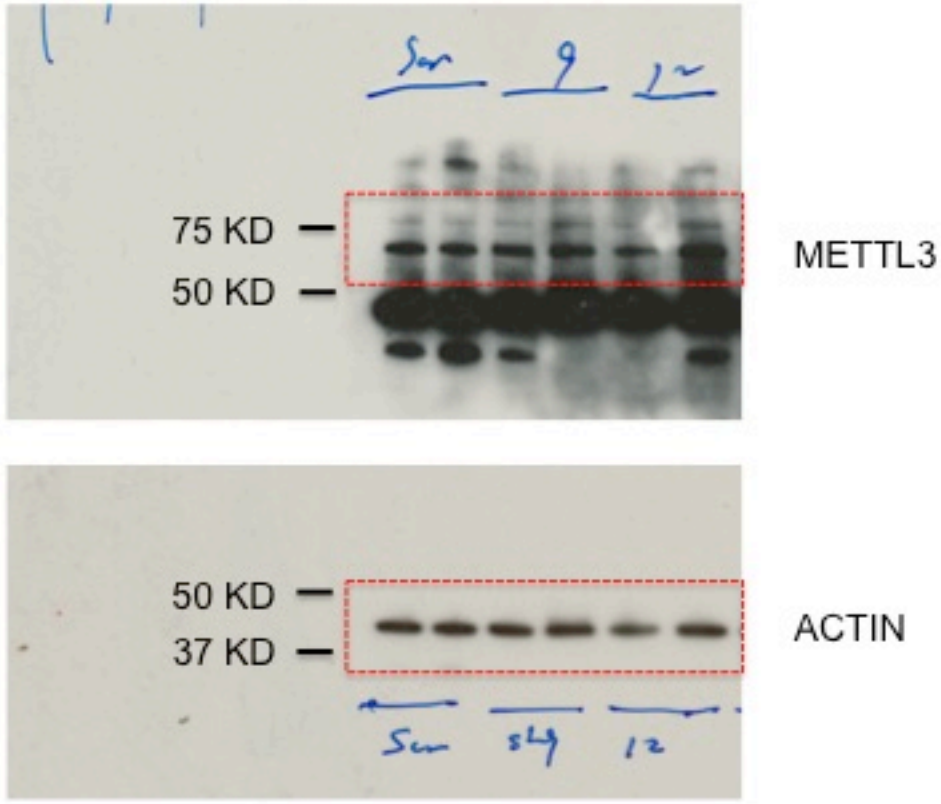


Supplementary Fig. 2



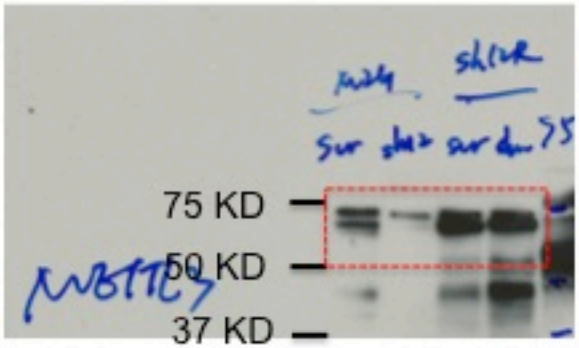
Supplementary Fig. 2

S2p

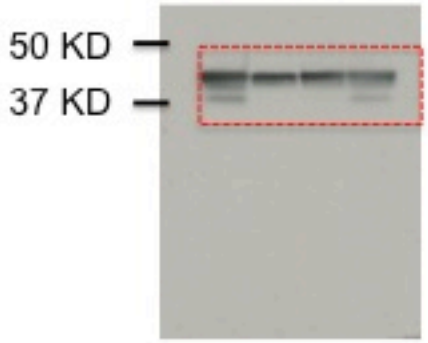


Supplementary Fig. 2

S3a

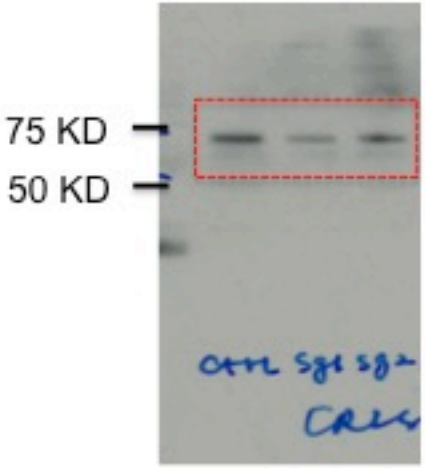


METTL3

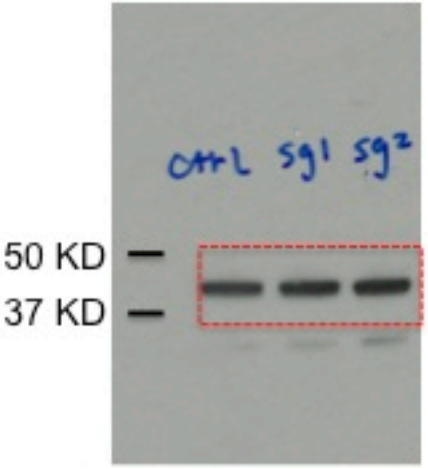


ACTIN

S3g



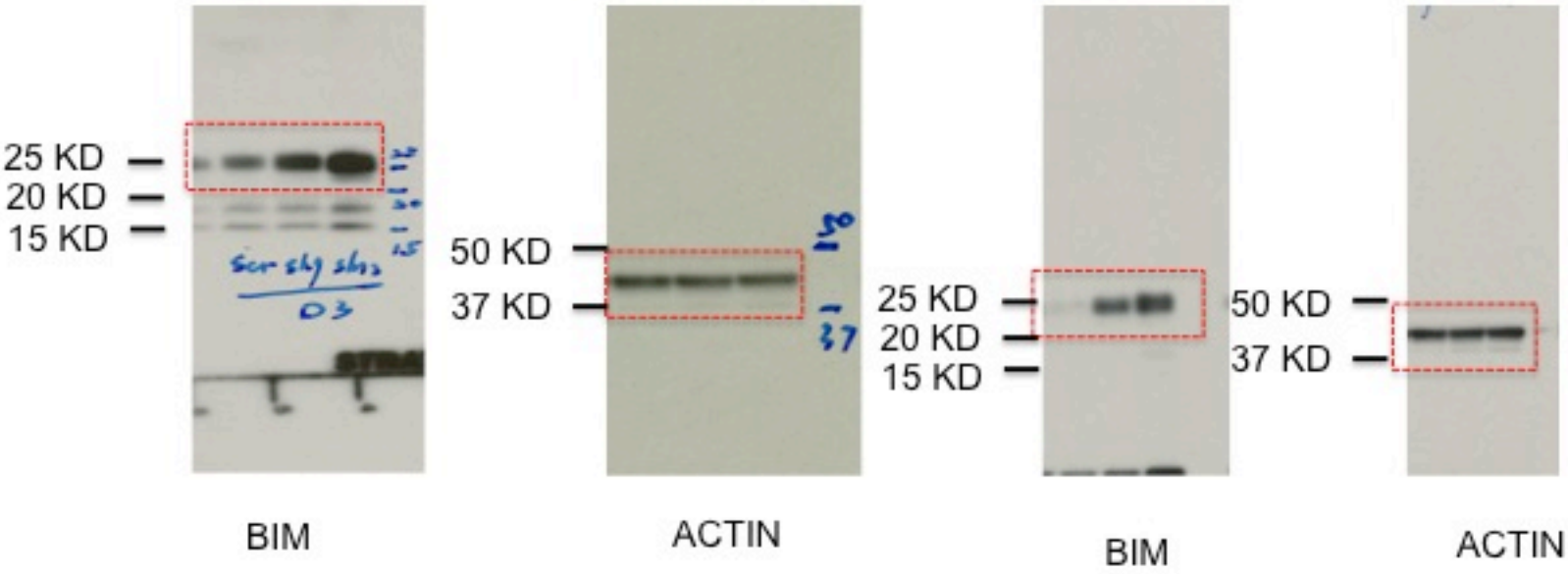
METTL3



ACTIN

Supplementary Fig. 3

S5c



Supplementary Fig. 5