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Supplemental Information

An Adversarial DNA N^6-Methyladenine-Sensor Network Preserves Polycomb Silencing

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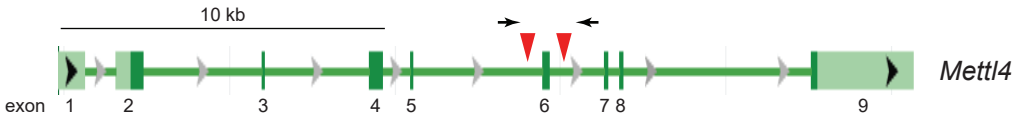
A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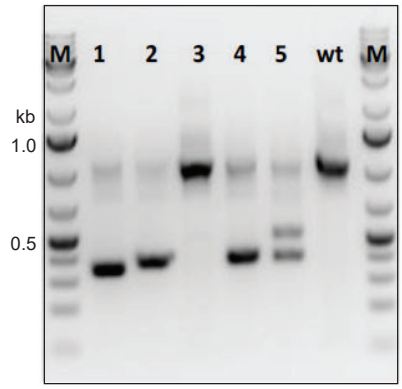
exon 1 2 3 4 5 6 7 8 9
Mettl4

B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kb
1.0
0.5

C

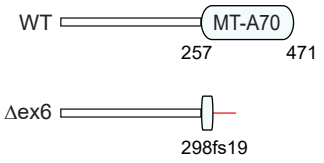

WT
MT-A70
257
471
Δex6
298fs19

Figure S1. Generation of *Mettl4* deficient mice, Related to Figures 1 and 2

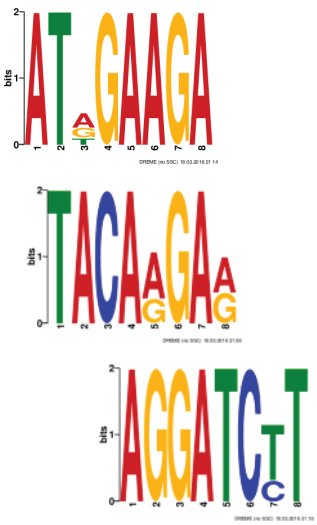
(A) Exonic structure of murine *Mettl4.* Colored boxes show numbered exons, dark green indicates protein coding regions. Red triangles denote sites targeted by each CRISPR sgRNA. Black arrows flanking exon 6 indicate the positions of primers used for genotyping analysis.

(B) Identification of *Mettl4* deletion founder mice. Pups were screened by PCR using primers adjacent to the sgRNA/Cas9-targeted sites flanking exon 6 of *Mettl4*. WT, wild-type control sample. M, DNA size marker.

(C) Diagram of proteins encoded by wild-type *Mettl4* and the ∆exon6 allele, which introduces a translational frameshift that ablates the MT-A70 methyltransferase domain.

mouse ESCs
6mA MeDIP

4922 peaks



mouse E9.5
6mA MeDIP

2510 peaks

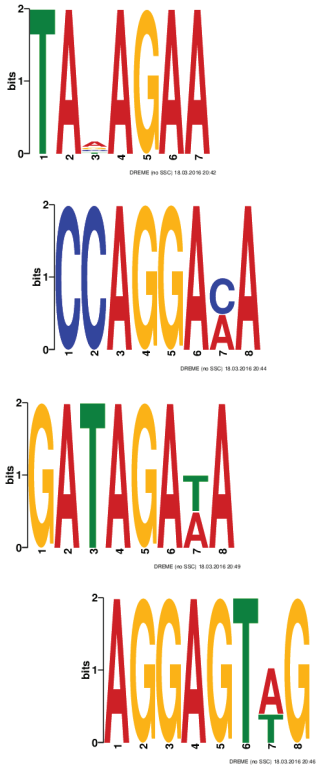
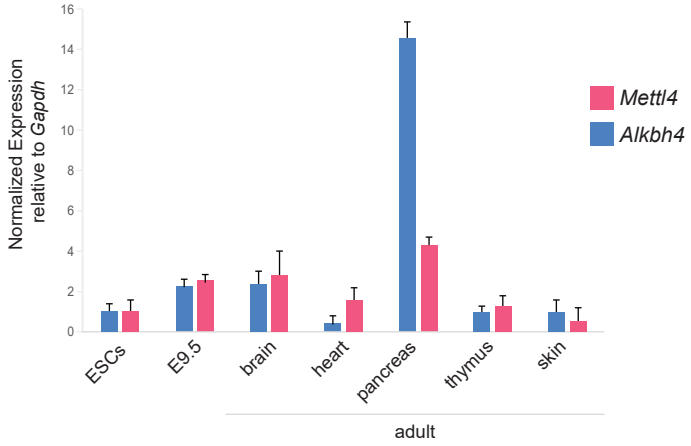


Figure S2. Sequence motifs associated with 6mA, Related to Figure 1

Top-scoring DNA sequence motifs associated with 6mA identified by MeDIP-seq analysis of genomic DNA prepared from murine ESCs (top) and E9.5 embryos (bottom). E-values for all motifs are $<10^{-7}$.

A


Normalized Expression
relative to Gapdh
16
14
12
10
8
6
4
2
0
Mettl4
Alkbh4
ESCs
E9.5
brain
heart
pancreas
thymus
skin
adult

B

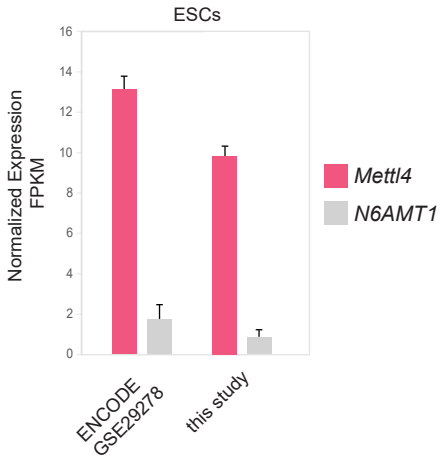
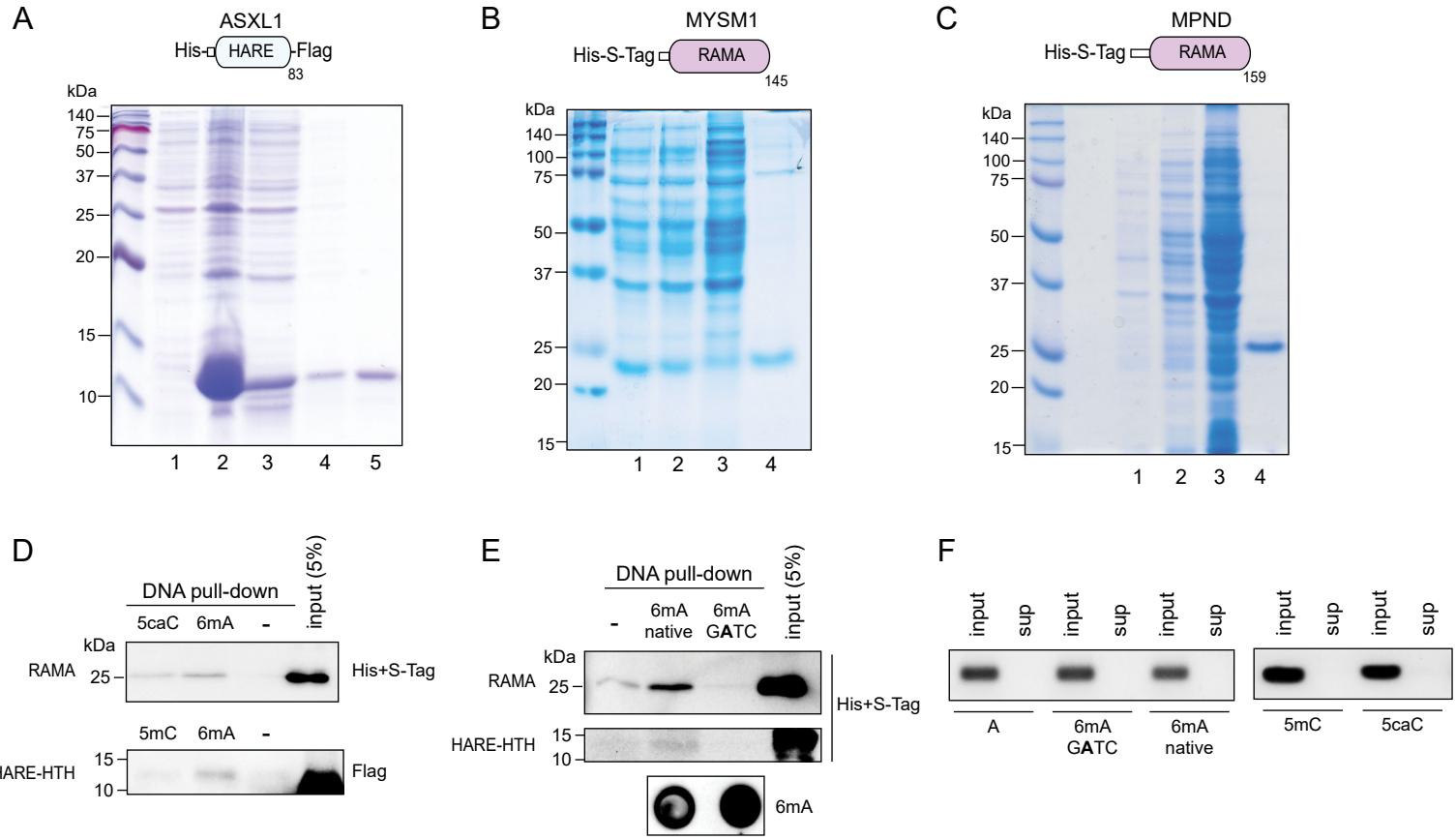

ESCs
Normalized Expression
FPKM
16
14
12
10
8
6
4
2
0
Mettl4
N6AMT1
ENCODE
GSE29278
this study

Figure S3. Expression of *Alkbh4* and *Mettl4* in mouse embryos and adult tissues, Related to Figure 2

(A) RT-qPCR analysis of *Mettl4* and *Alkbh4* expression in ESCs (strain C57Bl/6x129Sv), E9.5 embryos and in the indicated tissues obtained from 12-week-old adult mice. Expression levels are normalized to *Gapdh.* The mean expression level for each gene in ESCs is set as 1. Error bars indicate s.e.m. (n=2 experiments).

(B) Transcript levels of *Mettl4* and *N6AMT1* in mouse ESCs as determined by RNA-seq analysis. Error bars indicate s.e.m. from n=2 experiments from each study.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ASXL1
His-
HARE
Flag
83
kDa
140
75
50
37
25
20
15
10
1 2 3 4 5
B
MYSM1
His-S-Tag
RAMA
145
kDa
140
100
75
50
37
25
20
15
1 2 3 4
C
MPND
His-S-Tag
RAMA
159
kDa
140
100
75
50
37
25
20
15
1 2 3 4
D
DNA pull-down
5caC
6mA
-
input (5%)
kDa
RAMA
25
His+S-Tag
5mC
6mA
-
HARE-HTH
15
10
Flag
E
DNA pull-down
-
6mA native
6mA GATC
input (5%)
kDa
RAMA
25
His+S-Tag
HARE-HTH
15
10
6mA
F
input
sup
input
sup
input
sup
input
sup
input
sup
A
6mA GATC
6mA native
5mC
5caC

Figure S4. Selective interaction of HARE-HTH and RAMA domains with 6mA, Related to Figure 3

(A) Total protein was recovered from uninduced bacteria (lane 1) or postinduction cultures of bacteria expressing His- and Flag-tagged HARE-HTH domain (lane 2). Bacterial lysates containing recombinant HARE-HTH were incubated with nickel resin, and proteins washed (lane 3) or eluted (lanes 4 and 5) from the resin were resolved by SDS-PAGE and stained with Coomassie Blue.

(B) Protein lysates prepared from postinduction cultures of bacteria expressing His-tagged MYSM1 RAMA domain (lanes 1 and 2) were incubated with nickel resin, and proteins washed (lane 3) or eluted (lane 4) from the resin were resolved by SDS-PAGE and stained with Coomassie Blue.

(C) Total protein was recovered from uninduced bacteria (lane 1) or postinduction cultures of bacteria expressing His- and S-tagged MPND RAMA domain (lane 2). Bacterial lysates containing His-RAMA were incubated with nickel resin, and proteins washed (lane 3) or eluted (lane 4) from the resin were resolved by SDS-PAGE and stained with Coomassie Blue.

(D) In vitro DNA pull-down assay. Purified HARE-HTH or MPND RAMA domains were incubated with untreated resin or resin coated with duplex DNA oligonucleotide containing 6mA in a native sequence context (5'-AAC**A**GAAG**A**GG-3'), or 5mC or 5-carboxylcytosine (5caC) in a CpG sequence context. After extensive washing, bound proteins or input samples were resolved by SDS-PAGE and detected by immunoblotting (n=2 experiments).

(E) Purified HARE-HTH and MPND RAMA domains were incubated with untreated resin or resin coated with duplex DNA oligonucleotides containing 6mA in a native sequence context or Dam-specified context (5'-G**A**TC-3'). After extensive washing, bound proteins or inputs were resolved by SDS-PAGE and immunoblotting using His and S-Tag antisera (upper panels). 6mA in each pull-down was detected by dot blot using 6mA antisera (bottom panel) (n=3 experiments).

(F) Biotinylated duplex DNA containing the indicated standard or modified bases was incubated in the presence of streptavidin agarose resin. Coupling to the resin was verified by agarose gel electrophoresis of 5% of each input and unbound supernatant (sup). Resin containing immobilized DNA was used for pull-down assays.

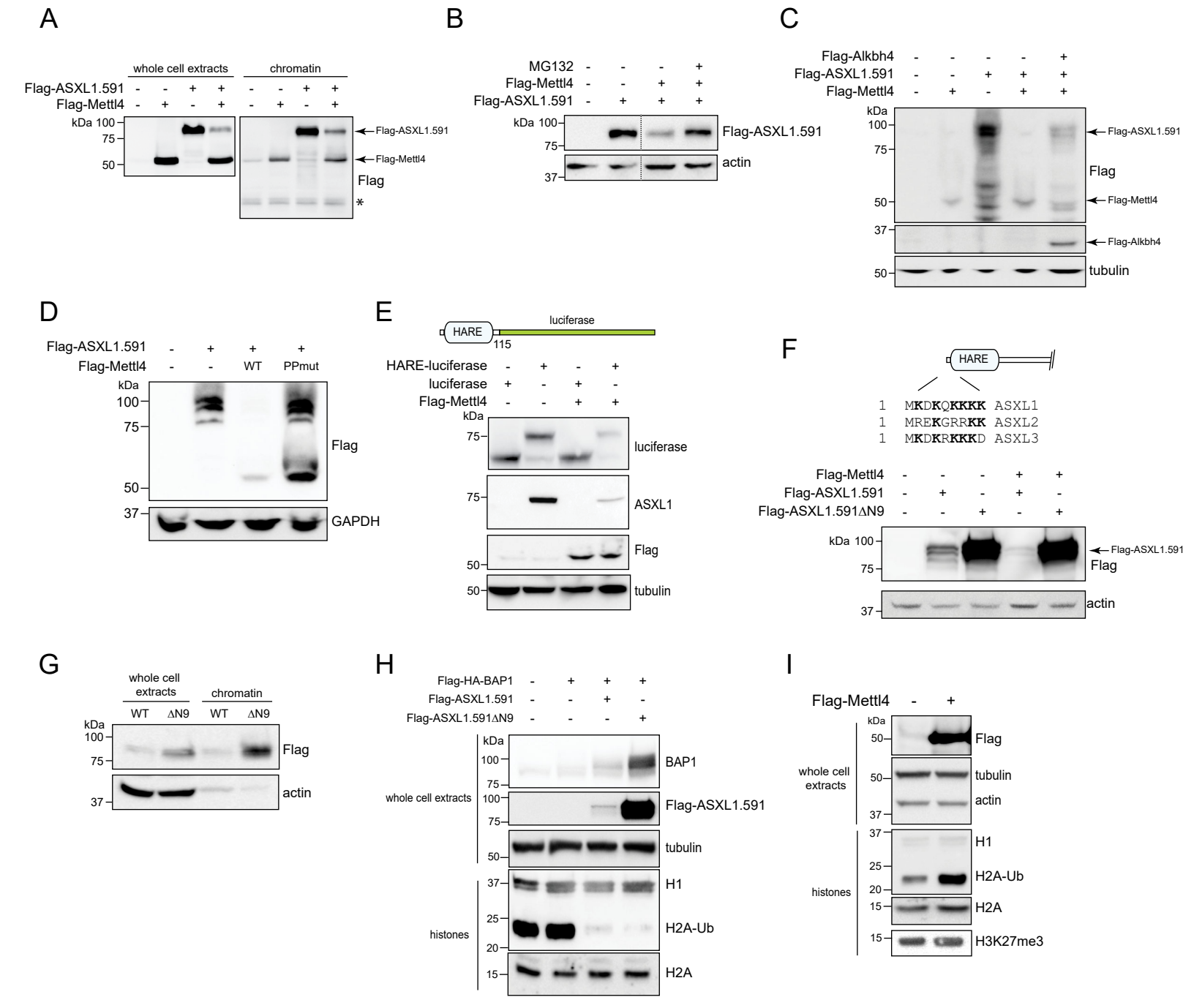
A
whole cell extracts
chromatin
Flag-ASXL1.591
Flag-Mettl4
kDa 100
75
50
Flag-ASXL1.591
Flag-Mettl4
Flag
*
B
MG132
Flag-Mettl4
Flag-ASXL1.591
kDa 100
75
37
Flag-ASXL1.591
actin
C
Flag-Alkbh4
Flag-ASXL1.591
Flag-Mettl4
kDa
100
75
50
37
50
Flag-ASXL1.591
Flag
Flag-Mettl4
Flag-Alkbh4
tubulin
D
Flag-ASXL1.591
Flag-Mettl4
WT
PPmut
kDa
100
75
50
37
Flag
GAPDH
E
HARE
luciferase
115
HARE-luciferase
luciferase
Flag-Mettl4
kDa
75
75
50
50
luciferase
ASXL1
Flag
tubulin
F
HARE
1 MKDKQKKKK ASXL1
1 MREKGRRKK ASXL2
1 MKDKRKKKD ASXL3
Flag-Mettl4
Flag-ASXL1.591
Flag-ASXL1.591ΔN9
kDa 100
75
37
Flag-ASXL1.591
Flag
actin
G
whole cell extracts
chromatin
WT
ΔN9
WT
ΔN9
kDa
100
75
37
Flag
actin
H
Flag-HA-BAP1
Flag-ASXL1.591
Flag-ASXL1.591ΔN9
kDa
whole cell extracts
100
75
100
75
50
37
25
20
15
histones
BAP1
Flag-ASXL1.591
tubulin
H1
H2A-Ub
H2A
I
Flag-Mettl4
kDa
whole cell extracts
50
50
37
histones
37
25
20
15
15
Flag
tubulin
actin
H1
H2A-Ub
H2A
H3K27me3

Figure S5. 6mA deposition triggers proteolysis of ASXL1 and MPND, Related to Figure 3

(A) HEK293T cells were transfected with vector control or plasmid expressing Flag-ASXL1.591, in the absence or presence of Flag-Mettl4. Proteins in whole cell extracts or chromatin were resolved by SDS-PAGE and immunoblotting with Flag antisera. Asterix shows nonspecific band in chromatin fraction (n=4 experiments).

(B) HEK293T cells expressing Flag-ASXL1.591 or Flag-Mettl4 were incubated in the absence or presence of proteasome inhibitor MG132 (2.5 μM). Proteins in whole cell extracts were resolved by SDS-PAGE and immunoblotting with the indicated antisera. An irrelevant lane was removed from both images (n=2 experiments).

(C) Alkbh4 rescues ASXL1.591 from proteolysis. HEK293T cells expressing the indicated combinations of Flag-Alkbh4, Flag-ASXL1.591 and Flag-Mettl4 were harvested and lysed, and proteins in whole cell extracts resolved by SDS-PAGE and immunoblotting with the indicated antisera. Tubulin, loading control (n=3 experiments).

(D) HEK293T cells expressing Flag-ASXL1.591 and wild-type or catalytically inactive (PPmut) variants of Flag-Mettl4 were harvested and proteins in whole cell extracts were resolved by SDS-PAGE and immunoblotting. GAPDH, loading control (n=2 experiments).

(E) The N-terminal of ASXL1 functions as a 6mA-activated destruction module. HEK293T cells expressing luciferase or HARE-HTH-luciferase fusion protein, each in the absence or presence of Flag-Mettl4, were lysed and proteins in whole cell extracts were resolved by SDS-PAGE and immunoblotting with the indicated antisera (n=4 experiments).

(F) Sequence alignment of the N-terminal regions of human ASXL1-3 with lysines shown in bold (top). Lower panels, HEK293T cells were transfected with plasmids expressing Flag-ASXL1.591 or Flag-ASXL1.591ΔN9, which contains a deletion of amino acids 2-9, in the absence or presence of Flag-Mettl4 (n=3 experiments).

(G) HEK293T cells were transfected with plasmids expressing Flag-ASXL1.591 or Flag-ASXL1.591ΔN9. Proteins in whole cell extracts or a chromatin-enriched subcellular fraction were resolved by SDS-PAGE and immunoblotting (n=2 experiments).

(H) HEK293T cells expressing the indicated combinations of Flag-HA-BAP1, Flag-ASXL1.591 or Flag-ASXL1.591ΔN9 were lysed and proteins in whole cell extracts (upper panels) or purified histones (lower panels) were resolved by SDS-PAGE and immunoblotting with the indicated antisera (n=2 experiments).

(I) HEK293T cells were transfected with empty vector or plasmid expressing Flag-Mettl4. Proteins in whole cell extracts (upper panels) or purified histones (lower panels) were resolved by SDS-PAGE and immunoblotting (n=2 experiments).