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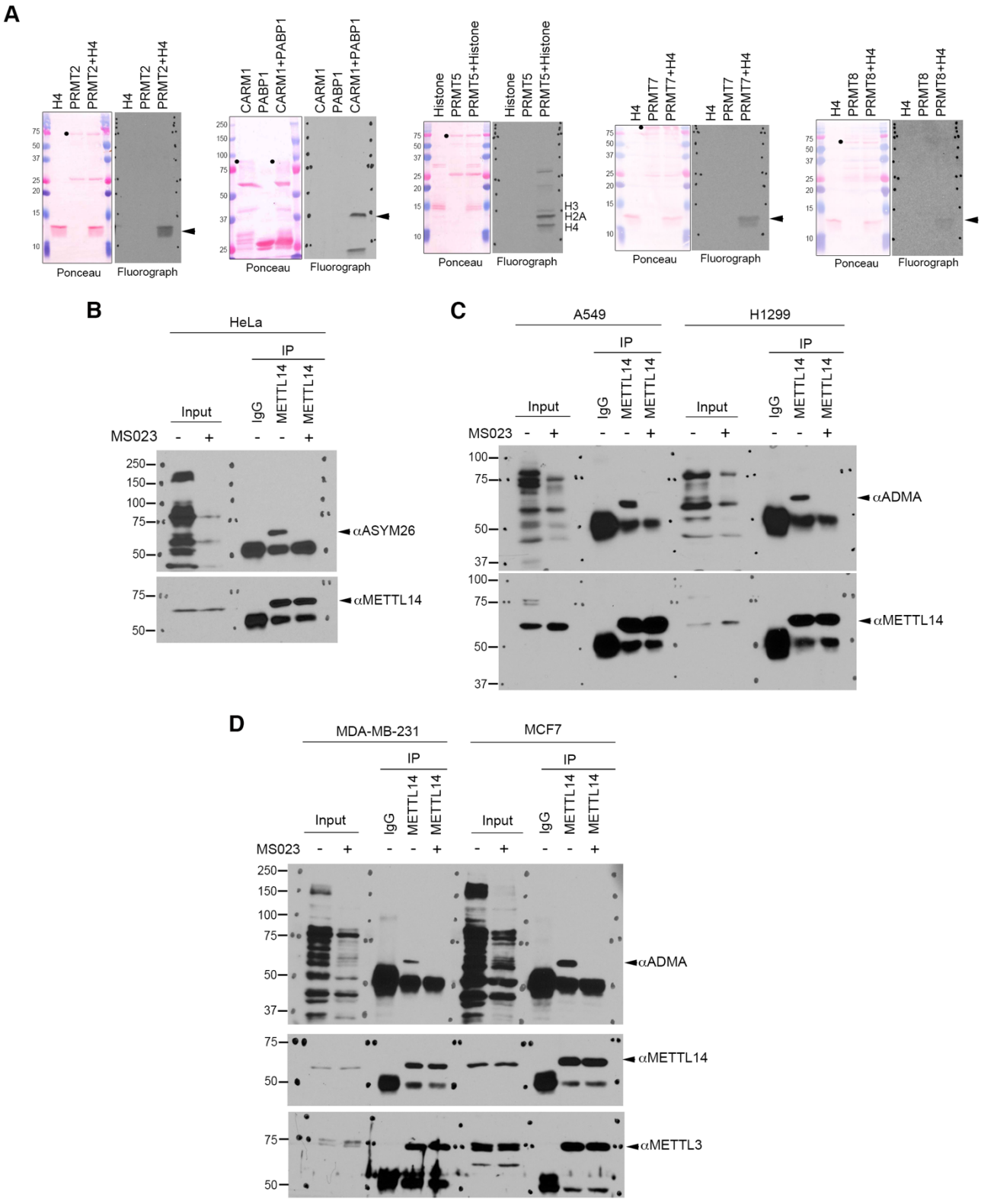
Appendix Figure S1

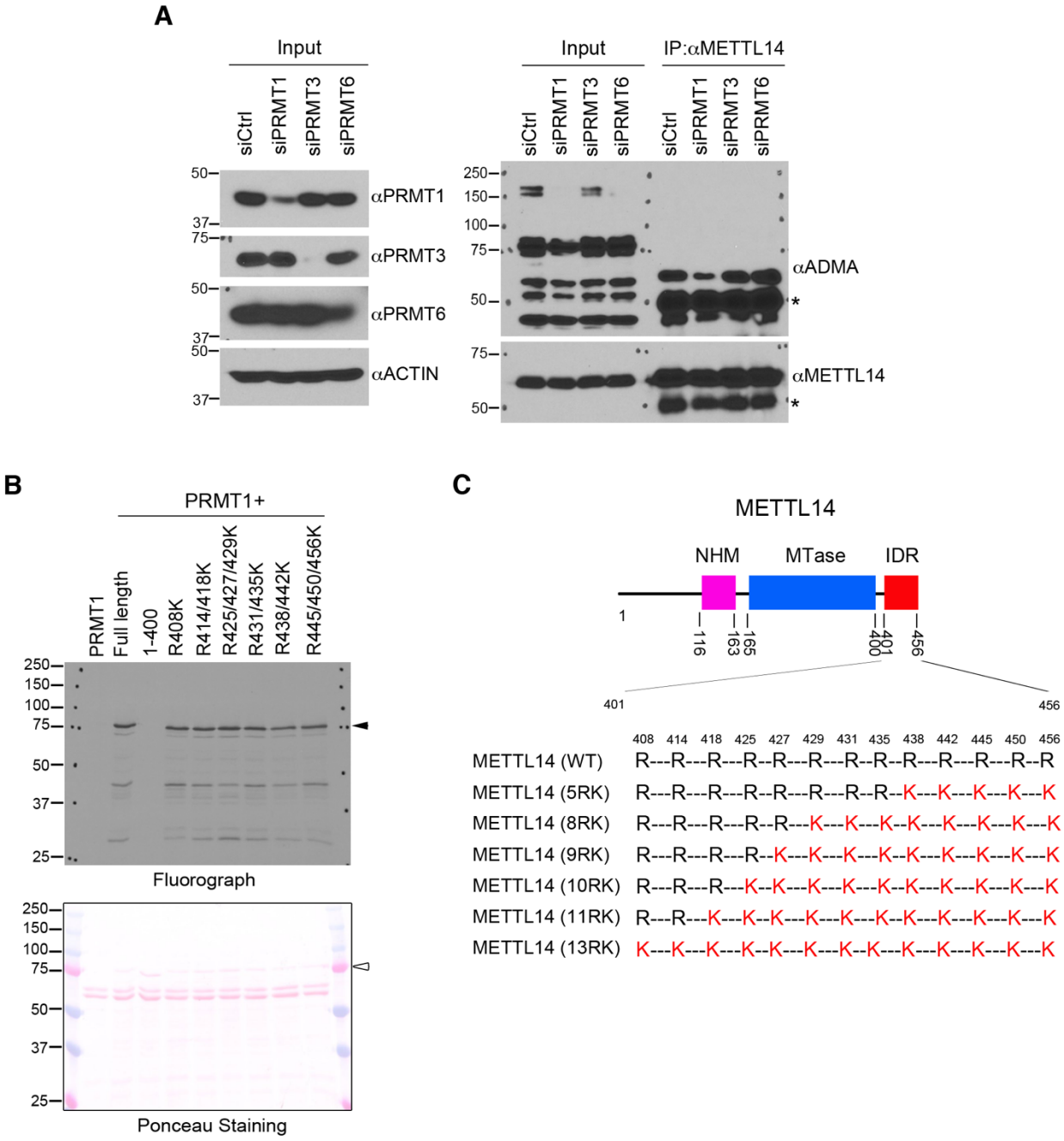
Appendix Figure S1. METTL14 harbors a conserved arginine/glycine (RGG/RG)-rich C-terminus. The C-terminal intrinsically disordered region (IDR) of METTL14 is conserved among species. METTL14 protein sequences from *Drosophila melanogaster*, *Danio rerio*, *Xenopus tropicalis*, *Gallus gallus*, *Mus musculus*, and *Homo sapiens* were aligned using ClustalW software. Colored strips indicate the conserved protein domains/motifs. The C-terminal IDR, which contains multiple RGG motifs, is conserved in vertebrates, whereas the same region in Drosophila harbors a much shorter RGG motif.

Appendix Figure S2



Appendix Figure S2. Characterization of METTL14 arginine methylation *in vitro* and *in vivo*.
(A) *In vitro* methylation assays were performed to confirm the activities of PRMTs used in Figure 1B. Recombinant proteins of PRMTs were incubated with their respective substrates, including histone H4 (H4), Polyadenylate-binding protein 1 (PABP1), and core histones. The Ponceau staining shows the loading of the recombinant proteins. Black dots indicate PRMT enzymes; triangles indicate fluorograph signals from substrate methylation. Human cervical cancer cell line HeLa (B), Lung cancer cell line A549 and H1299 (C), and breast cancer cell line MDA-MB231 and MCF7 (D) were either left untreated or treated with Type I PRMT inhibitor MS023 (1 μM, 48 h). The level of METTL14 arginine methylation was detected by IP/Western blot analysis using indicated antibodies.

Appendix Figure S3



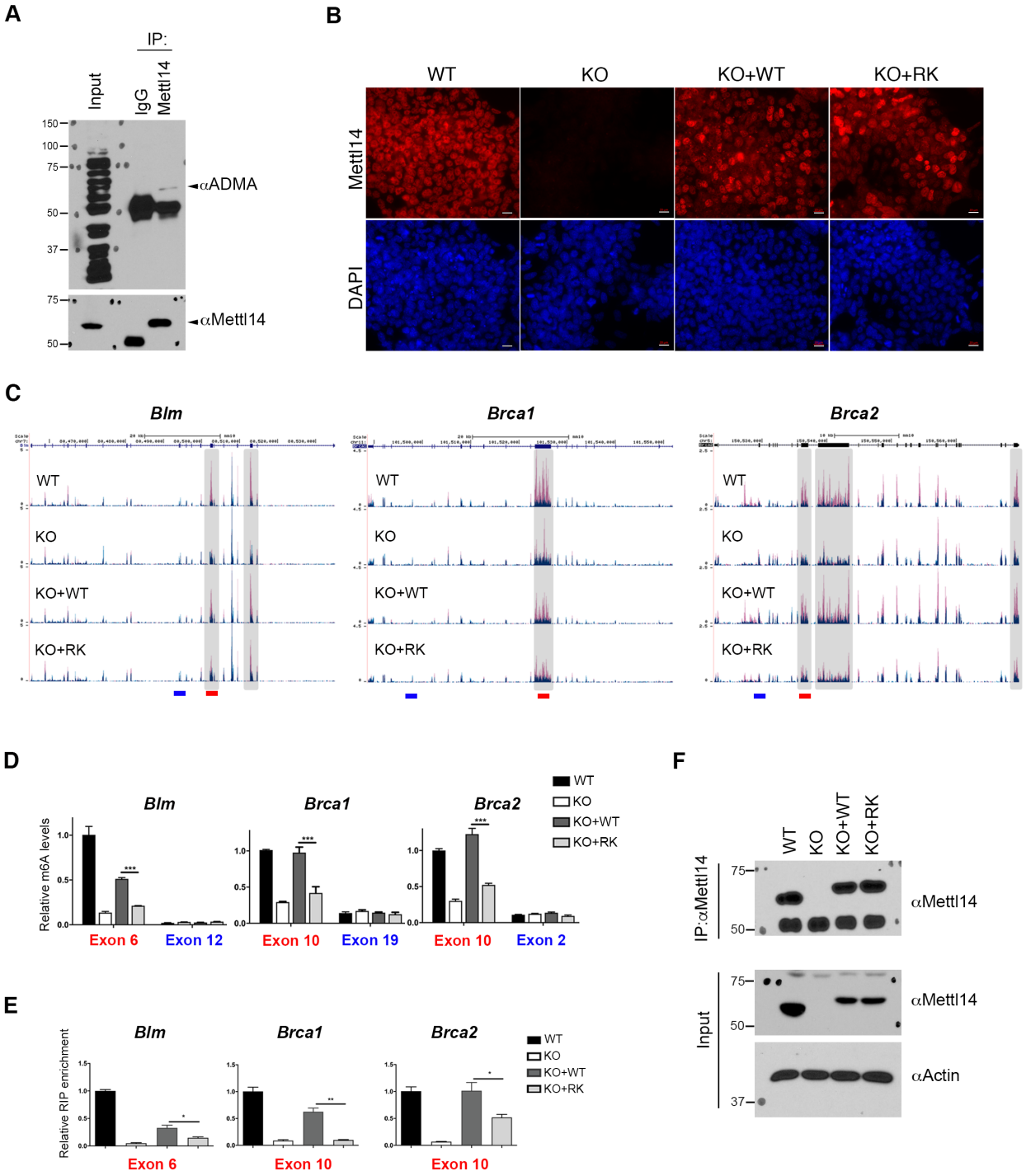
Appendix Figure S3. Identification of PRMT1-catalyzed methylation sites on METTL14.

(A) PRMT1, but not PRMT3 and PRMT6, is responsible for METTL14 arginine methylation *in vivo*. The levels of METTL14 arginine methylation were compared in cells transfected with control siRNA (siCtrl), PRMT1-specific siRNA (siPRMT1), PRMT3-specific siRNA (siPRMT3), and PRMT6-specific siRNA (siPRMT6). The knockdown efficiency was confirmed by Western blot analysis of total cell lysates using indicated antibodies. The levels of METTL14 arginine methylation were detected by IP/WB analysis.

(B) Selective mutation analysis of single, double, or triple arginine sites does not impair METTL14 methylation *in vitro*. The *in vitro* methylation assays were performed by incubating recombinant PRMT1 with purified GST-tagged WT, 1-400 truncation, and various arginine to lysine (R-to-K) METTL14 mutants. The Ponceau S staining shows the loading of the recombinant proteins used in the exact methylation assay.

(C) Schematic representation of the mutated arginine residues in each METTL14 mutant constructs used in Figure 3B and Figure 3C.

Appendix Figure S4



Appendix Figure S4. Characterization of METTL14 arginine methylation-dependent m^{6}A sites in mESCs.

(A) Detection of METTL14 arginine methylation in mESCs. METTL14 was immunoprecipitated from mESCs, and Western blot analysis was performed to detect its methylation using anti-ADMA and anti-METTL14 antibodies.

(B) Detection of METT1L4 expression in WT, *Mettl14* KO, KO+WT, KO+RK mESCs by immunofluorescence using an anti-METTL14 antibody. DAPI staining indicates the cell nucleus. Scale bar: 20 μM

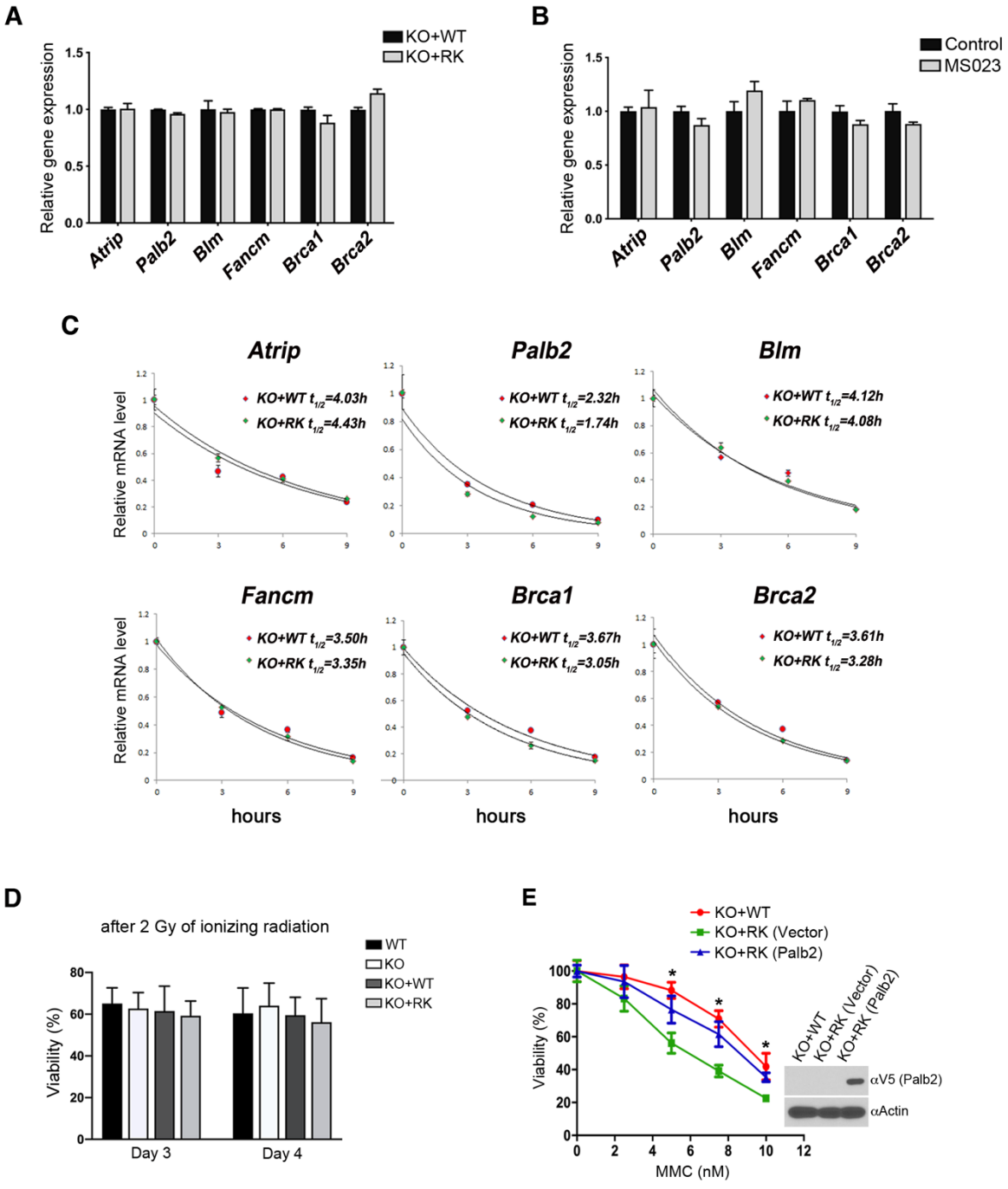
(C) UCSC Genome Browser custom tracks of m^{6}A-seq reads along the indicated mRNAs in WT, *Mettl14* KO, KO+WT, and KO+RK mESCs. The y-axis represents the normalized number of reads. Blue reads are from non-immunoprecipitated input libraries, and red reads are from m^{6}A-IP libraries. Above the custom tracks, the thick blue boxes represent the protein coding regions (CDSs), the thin blue boxes represent the untranslated regions (UTRs), and the blue lines represent introns. The bars at the bottom of the custom tracks indicate the amplicon locations for MeRIP (m^{6}A-IP)-qPCR assays (D) and METTL14 RIP-qPCR assays (E) to detect m^{6}A-positive (red) and negative (blue) regions.

(D) MeRIP (m^{6}A-IP)-qPCR assays were performed for WT, *Mettl14* KO, KO+WT, and KO+RK mESCs to validate the MeRIP-seq results. Four target mRNAs encoded by genes in the Fanconi anemia pathway were analyzed. m^{6}A-negative regions of the transcripts (blue) were included as negative controls. Data are shown as mean ± SD from three biological replicates. ***, $p < 0.001$.

(E) METTL14 RIP-qPCR assays were performed for WT, *Mettl14* KO, KO+WT, and KO+RK mESCs to compare the binding of WT and RK mutant METTL14 to the indicated mRNA targets. Primers (red color) that amplify m^{6}A positive regions of the transcripts were used. Data are shown as mean ± SD from three biological replicates. *, $p < 0.05$; **, $p < 0.01$.

(F) The amount of METTL14 protein immunoprecipitated in the RIP experiments described in Figure 6C and Appendix Figure S4E was detected by Western blot analysis.

Appendix Figure S5



Appendix Figure S5. Examine the impact of METTL14 arginine methylation loss on mRNA expression, stability, and cellular response to DNA damage.

(A) The mRNA levels of Fanconi anemia pathway genes were analyzed by RT-qPCR for mESCs expressing WT and RK mutant METTL14. Data are shown as mean ± SD from three biological replicates.

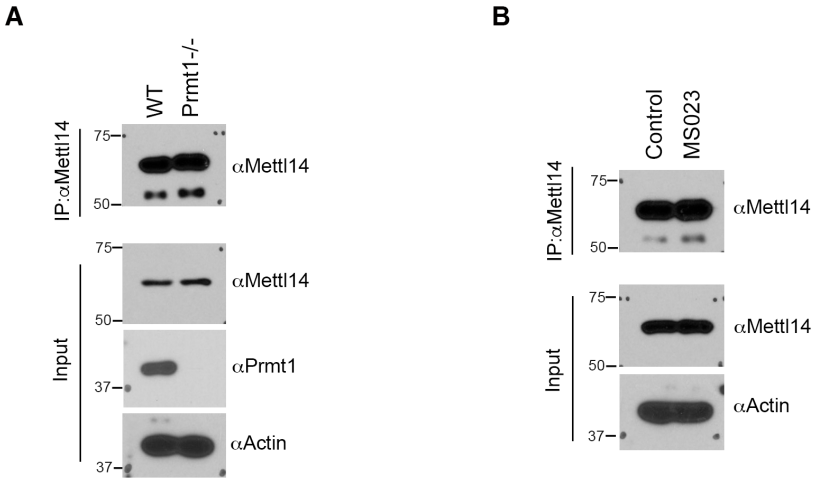
(B) The mRNA levels of Fanconi anemia pathway genes were analyzed by RT-qPCR for mESCs treated with DMSO (control) or type I PRMT inhibitor (MS023). Data are shown as mean ± SD from three biological replicates.

(C) mRNA half-life assays were performed to compare the mRNA stability of genes involved in the Fanconi anemia pathway for mESCs expressing WT and RK mutant METTL14.

(D) The viability of WT, *Mettl14* KO, KO+WT, and KO+RK mESCs was measured on days 3 and 4 after ionizing radiation (2 Gy).

(E) The KO+RK mESCs transfected with V5-tagged Palb2, as well as KO+WT and KO+RK mESCs, were treated with various amounts of MMC. Cell viability was measured on day 4. The expression of transfected Palb2 was confirmed by Western blot analysis using an anti-V5 antibody. Data are shown as mean ± SD from three biological replicates. *, $p < 0.05$.

Appendix Figure S6



Appendix Figure S6. The amount of METTL14 protein immunoprecipitated in the RIP experiments performed in *Prmt1* KO (A) and MS023-treated (B) mESCs, as described in Figure EV5B, was detected by Western blot analysis.

Appendix Table S1. Primers used in this study

Primer Name	Primer sequence (5'-3')
Cloning primers	
GST-METTL14 Forward	CGGGATCCATGGATAGCCGCTTGC
GST-METTL14 Reverse	CCGCTCGAGTTATCGAGGTGGAAAG
GST-METTL14 (1-400) Forward	CGGGATCCATGGATAGCCGCTTGC
GST-METTL14 (1-400) Reverse	CCGCTCGAGTTAAGGCGATTTTGGTCG
3xFlag-METTL14 Forward	CCCAAGCTTATGGATAGCCGCTTGC
3xFlag-METTL14 Reverse	GGGGTACCTTATCGAGGTGGAAAG
3xFlag-METTL14 (1-400) Forward	CCCAAGCTTATGGATAGCCGCTTGC
3Flag-METTL14 (1-400) Reverse	GGGGTACCTTAAGGCGATTTTGGTCG
GFP-WTAP Forward	CCGCTCGAGCTATGACCAACGAAGAAC
GFP-WTAP Reverse	CGGGATCCTTACAAAACTGAACC
pLV-EF1a-IRES-Blast METTL14 Forward	CGGGATCCATGGACTACAAAGACCATGA
pLV-EF1a-IRES-Blast METTL14 Reverse	CGGAATTCTTATCGAGGTGGAAAG
HA-METTL14 Forward	CGGAATTCGGATGGATAGCCGCTTGC
HA-METTL14 Reverse	CCGCTCGAGTTATCGAGGTGGAAAG
METTL14 site mutagenesis primers	
METTL14 R408K-Forwrad	CAAATCTAAATCTGAC**AAA**GGAGGTGGAGCTCCC
METTL14 R408K-Reverse	GGGAGCTCCACCTCC**TTT**GTCAGATTTAGATTTG
METTL14 R414K/R418K-Forward	GGAGGTGGAGCTCCC**AAA**GGTGGAGGA**AAA**GGTGG AACTTCTGC
METTL14 R414K/R418K-Reverse	GCAGAAGTTCCACC**TTT**TCCTCCACC**TTT**GGGAGCTC CACCTCC
METTL14 R425K/R427K/R429K-Forward	GGAACTTCTGCTGGC**AAA**GGA**AAA**GAA**AAA**AATAGAT CTAACTTC
METTL14 R425K/R427K/R429K-Reverse	GAAGTTAGATCTATT**TTT**TTC**TTT**TCC**TTT**GCCAGCAGA AGTTCC
METTL14 R431K/R435K-Forward	GGACGAGAAAGAAAT**AAA**TCTAACTTC**AAA**GGAGAAA GAGGTGGC
METTL14 R431K/R435K-Reverse	GCCACCTCTTTCTCC**TTT**GAAGTTAGA**TTT**ATTTCTTTC TCGTCC
METTL14 R438K/R442K-Forward	CTAACTTCCGAGGAGAA**AAA**GGTGGCTTT**AAA**GGGG GCCGTGGAGGAG
METTL14 R438K/R442K-Reverse	CTCCTCCACGGCCCCC**TTT**AAAGCCACC**TTT**TTCTCCT CGGAAGTTAG
METTL14 R445K-Forward	GGCTTTAGAGGGGGC**AAA**GGAGGAGCACACAG
METTL14 R445K-Reverse	CTGTGTGCTCCTCC**TTT**GCCCCCTCTAAAGCC
METTL14 R450K-Forward	GTGGAGGAGCACAC**AAA**GGTGGCTTTCCACCTC
METTL14 R450K-Reverse	GAGGTGGAAAGCCACC**TTT**GTGTGCTCCTCCAC
METTL14 R456K-Forward	GGTGGCTTTCCACCT**AAA**TAAGGTACCAGTCG
METTL14 R456K-Reverse	CGACTGGTACCTTA**TTT**AGGTGGAAAGCCACC
RT-qPCR primers	
Atrip-Forward	CTCATAAGGTCCGCCGATTAG
Atrip-Reverse	CTGCTCAGAAGGTGACAAAGA
Blm-Forward	TGTGATTCATGCATCTCTTCCTAAA
Blm-Reverse	CAGCTCGGCCGGATTCT

Brca1-Forward	GGAGATGTTGTGACTGGAAGAA
Brca1-Reverse	GTGAAGGGCTCACAACAATAGA
Brca2-Forward	TCCCCCCTACCATCAGTTTG
Brca2-Reverse	CAGTGGTAGAGTTTGACTTCGTTCTT
Fancm-Forward	GGCAGAACGTGTCCAAGATTG
Fancm-Reverse	GCGGAGCCTTTTCTGATGTT
Palb2-Forward	CTGGTGATGACAGTGAAAAGCAA
Palb2-Reverse	CAGGCCAAGCATAGCTTTTATATCT
RIP-qPCR primers	
Atrip-Forward	ATCTTTAGCAGTGGGTGCTG
Atrip-Reverse	GGTCCAGACTTGTGCAGATAC
Blm-Forward	GGAAGATTTGCTGGCTGGAA
Blm-Reverse	ACGGCCAGGCTTCCTAT
Brca1-Forward	GCTAACTGTGTGCACTGTACT
Brca1-Reverse	GAGGGACGATTTGAGAGACATAC
Brca2-Forward	CAGTGAAACAAGAACTGATGAA
Brca2-Reverse	GATCACTCTCTCTTAGTTCCATTT
Fancm-Forward	TGTGTCTGGAAGGCATTCTG
Fancm-Reverse	GGGATTGGTGATATGGCTCTAC
Palb2-Forward	GAGGTGCGGGCTGATTT
Palb2-Reverse	CCAGGACCTGCTGGAAAG

Appendix Table S2. Key reagents used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-METTL14 antibody	Sigma	HPA038002
Rabbit anti-METTL3 antibody	Bethyl Laboratories	A301-567A
Mouse anti-RNA Pol II antibody	Active Motif	102660
Rat anti-RNA Pol II (pSer2) antibody	Active Motif	91115
Mouse anti-RNA Pol II (pSer5) antibody	Santa Cruz Biotechnology	sc-47701
Rabbit Asymmetric Di-Methyl Arginine antibody	Cell Signaling Technology	13522
Rabbit anti-ADMA (ASYM26) antibody	A gift from Dr. Stéphane Richard	
Rabbit anti-m6A antibody	Synaptic Systems	202003
Mouse anti-Flag antibody	Sigma	F3165
Mouse anti-GFP antibody	Santa Cruz Biotechnology	sc-9996
Rabbit anti-GFP antibody	Invitrogen	A6455
Mouse anti-HA antibody	Biolgend	901513
Rabbit anti-HA antibody	Cell Signaling Technology	3724S
Rabbit anti-PRMT1 antibody	Bethyl Laboratories	A300-722A
Rabbit anti-PRMT3 antibody	A gift from Dr. Mark T.Bedford	
Rabbit anti-PRMT6 antibody	IMGENEX	IMG-506
Rabbit anti-Atrip antibody	ABClonal	A7139
Rabbit anti-Fancm antibody	Proteintech	12954-1-AP
Rabbit anti-Palb2 antibody	Proteintech	14340-1-AP
Mouse anti- β-Actin antibody	Sigma	A2228
Normal Rabbit IgG	Cell Signaling Technology	2729
Normal Mouse IgG	Santa Cruz Biotechnology	sc-2025
Goat anti-Mouse Alexa Fluor 555 Secondary Antibody	Invitrogen	A-21422
Goat Anti-Mouse HRP Secondary Antibody	Invitrogen	62-6520
Donkey Anti-Rabbit HRP Secondary Antibody	GE Healthcare	NA934V
Bacterial and Virus Strains		
E. coli DH5α	New England Biolabs	C2987H
E. coli BL21	New England Biolabs	C2530H
NEB® Stable Competent E. coli	New England Biolabs	C3040H
Chemicals, Peptides, and Recombinant Proteins		
S-adenosyl-l-[methyl-^{3}H] methionine, (SAM[^{3}H])	PerkinElmer	NET155V250UC
MS023	Selleck Chemicals	S8112
Adox	APExBIO	B6120
Cisplatin	APExBIO	A8321
MMC	Cayman Chemical	11435
Blasticidin	Selleck Chemicals	S7419
RNase A	Thermo Scientific	EN0531
Recombinant Mouse LIF	Gemini Bio	400-495
3x Flag Peptide	APExBIO	A6001
DAPI	Sigma	D9542
Recombinant 3xFlag-METTL14	This study	N/A
Recombinant 3xFlag-METTL3	This study	N/A
Recombinant GST-METTL14 (WT)	This study	N/A
Recombinant GST-METTL14 (1-400)	This study	N/A
Recombinant GST-METTL14 (R408K)	This study	N/A

Recombinant GST-METTL14 (R414K/R418K)	This study	N/A
Recombinant GST-METTL14 (R425K/R427K/R429K)	This study	N/A
Recombinant GST-METTL14 (R431K/R435K)	This study	N/A
Recombinant GST-METTL14 (R438K/R442K)	This study	N/A
Recombinant GST-METTL14 (R445K/R450K/R456K)	This study	N/A
Recombinant GST-PRMT1	This study	N/A
Recombinant GST-PRMT2	This study	N/A
Recombinant GST-PRMT3	This study	N/A
Recombinant GST-CARM1	This study	N/A
Recombinant Myc-PRMT5	This study	N/A
Recombinant GST-PRMT6	This study	N/A
Recombinant GST-PRMT7	This study	N/A
Recombinant GST-PRMT8	This study	N/A
Critical Commercial Assays		
Cell Counting Kit-8	Dojindo Molecular Technologies	CK04-20
Alkaline Phosphatase Staining Kit	BioPioneer	SC-003
Streptavidin 96-well scintillant coated microplate	PerkinElmer	SMP103001PK
High-Capacity cDNA Reverse Transcription Kit	Applied Biosystems	4368813
Power SYBR™ Green PCR Master Mix	Applied Biosystems	4367659
Streptavidin agarose beads	Millipore	16-126
Anti-FLAG® M2 Magnetic Beads	Sigma	M8823
Experimental Models: Cell Lines		
HEK-293	ATCC	CRL-1573
Hela	ATCC	CCL-2
MCF7	ATCC	HTB-22
MDA-MB-231	ATCC	HTB-26
A549	ATCC	CCL-185
H1299	ATCC	CRL-5803D
Mettl14 WT Mouse embryonic stem cells	A gift from Dr. Jacob Hanna	
Mettl14 KO Mouse embryonic stem cells	A gift from Dr. Jacob Hanna	
Mettl14 KO+WT Mouse embryonic stem cells	This study	N/A
Mettl14 KO+5RK Mouse embryonic stem cells	This study	N/A
Mettl14 KO+13RK Mouse embryonic stem cells	This study	N/A
Oligonucleotides		
Biotin labeled GGACU RNA: 5'UACACUCGAUCU**GGACU**AAAGCUGCUC3'	IDT	N/A
FAM labeled GGACU RNA: 5'UACACUCGAUCU**GGACU**AAAGCUGCUC3'	IDT	N/A
PRMT1 siRNA	Qiagen	SI02663493
PRMT3 siRNA	Dharmacon	J-026786-09
PRMT6 siRNA	Dharmacon	J-007773-05
Other Oligonucleotides used in this study, please see Supplementary Table 4		
Recombinant DNA		
pcDNA3/Flag-METTL14	Addgene	53740
pcDNA3/Flag-METTL3	Addgene	53739
p3xFlag-CMV7.1	Sigma	E7533
p3xFlag-CMV7.1 METTL14 (WT)	This study	N/A

p3xFlag-CMV7.1 METTL14 (1-400)	This study	N/A
p3xFlag-CMV7.1 METTL14 (R408K)	This study	N/A
p3xFlag-CMV7.1 METTL14 (R414K/R418K)	This study	N/A
p3xFlag-CMV7.1 METTL14 (R425K/R427K/R429K)	This study	N/A
p3xFlag-CMV7.1 METTL14 (R431K/R435K)	This study	N/A
p3xFlag-CMV7.1 METTL14 (R438K/R442K)	This study	N/A
p3xFlag-CMV7.1 METTL14 (R445K/R450K/R456K)	This study	N/A
p3xFlag-CMV7.1 METTL14 (5RK)	This study	N/A
p3xFlag-CMV7.1 METTL14 (13RK)	This study	N/A
pGEX-4T-1	GE Healthcare	28954549
pGEX-4T-1-METTL14 (WT)	This study	N/A
pGEX-4T-1-METTL14 (1-400)	This study	N/A
pGEX-4T-1-METTL14 (R408K)	This study	N/A
pGEX-4T-1-METTL14 (R414K/R418K)	This study	N/A
pGEX-4T-1-METTL14 (R425K/R427K/R429K)	This study	N/A
pGEX-4T-1-METTL14 (R431K/R435K)	This study	N/A
pGEX-4T-1-METTL14 (R438K/R442K)	This study	N/A
pGEX-4T-1-METTL14 (R445K/R450K/R456K)	This study	N/A
pGEX-4T-1-METTL14 (5RK)	This study	N/A
pGEX-4T-1-METTL14 (13RK)	This study	N/A
pCMV-HA	Takara	635690
HA-METTL14	This study	N/A
HA-METTL14 13RK	This study	N/A
pEGFP-C1	Clontech	6084-1
GFP-WTAP	This study	N/A
GFP-PRMT1	This study	N/A
GFP-PRMT3	This study	N/A
GFP-PRMT6	This study	N/A
pLV-EF1a-IRES-Blast	Addgene	85133
pLV-EF1a-IRES-Blast METTL14 WT	This study	N/A
pLV-EF1a-IRES-Blast METTL14 5RK	This study	N/A
pLV-EF1a-IRES-Blast METTL14 13RK	This study	N/A