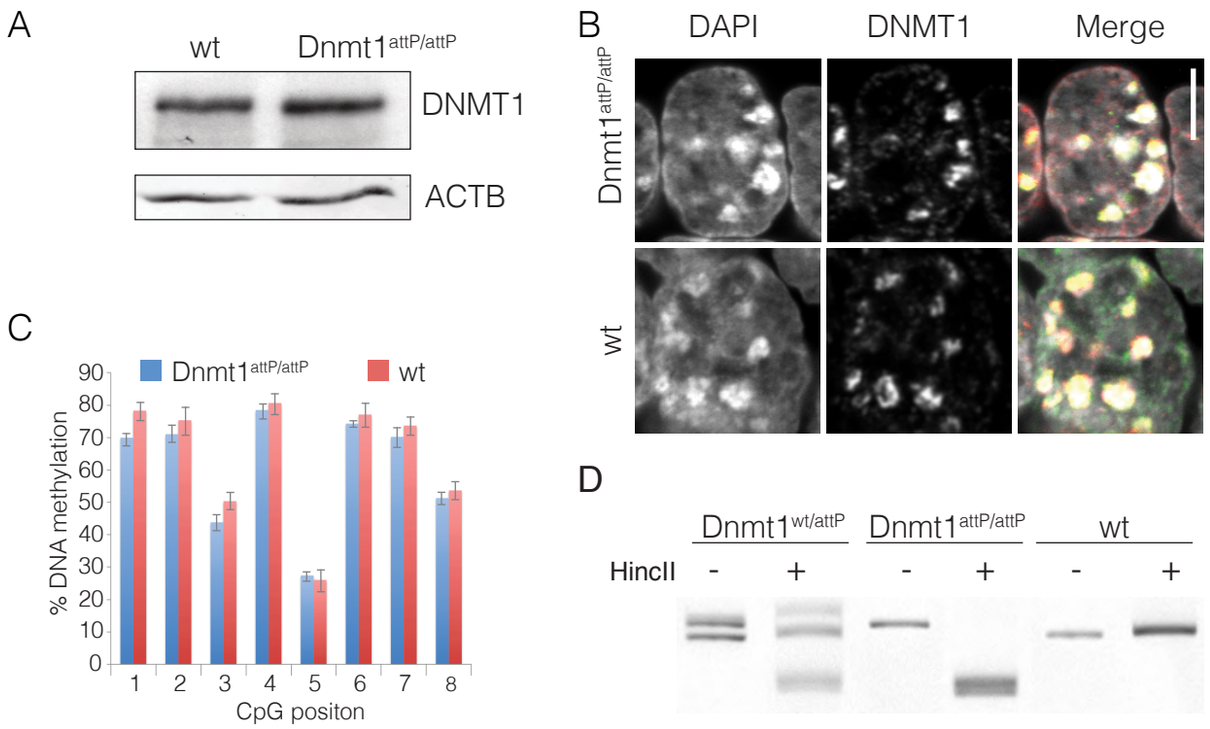
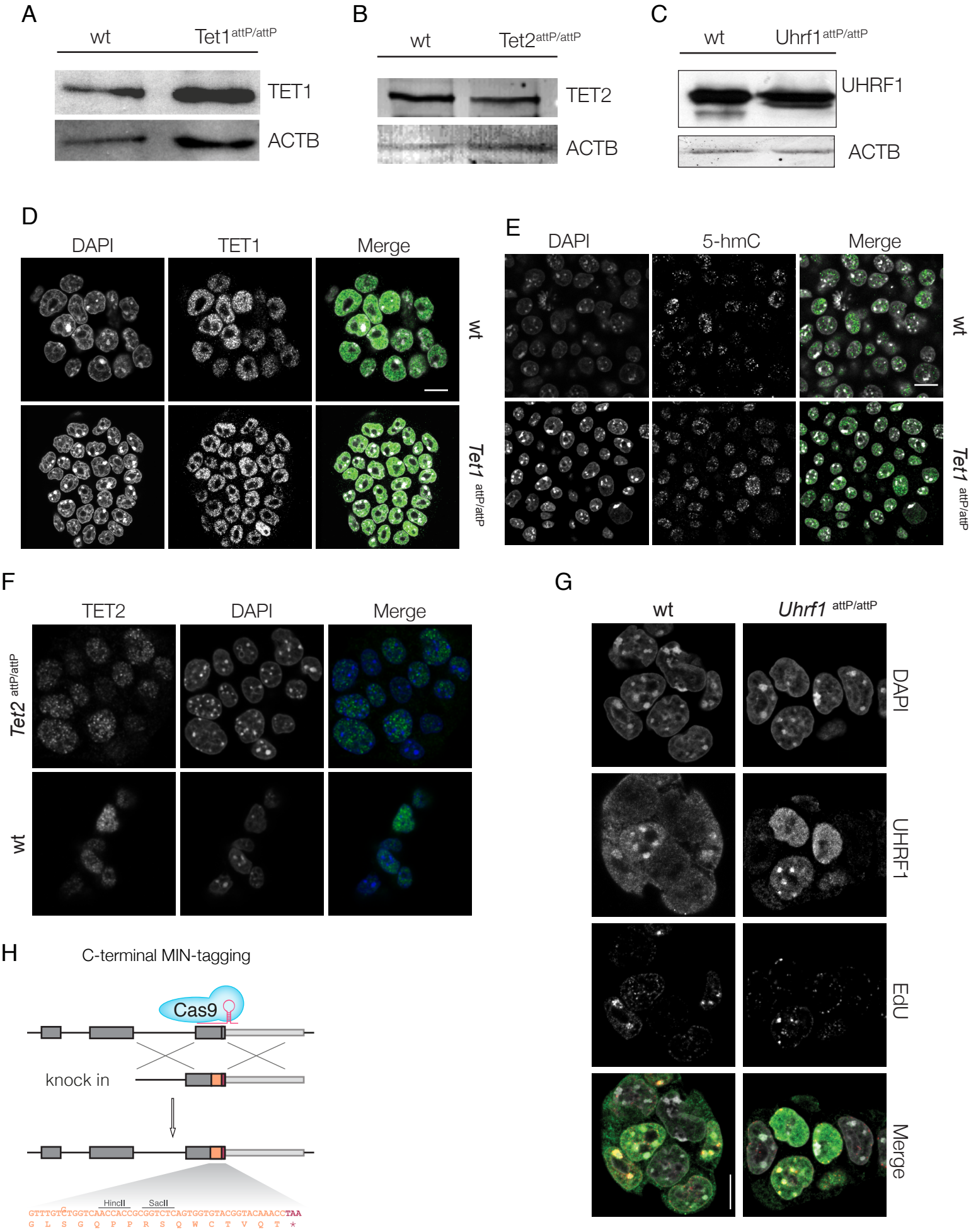
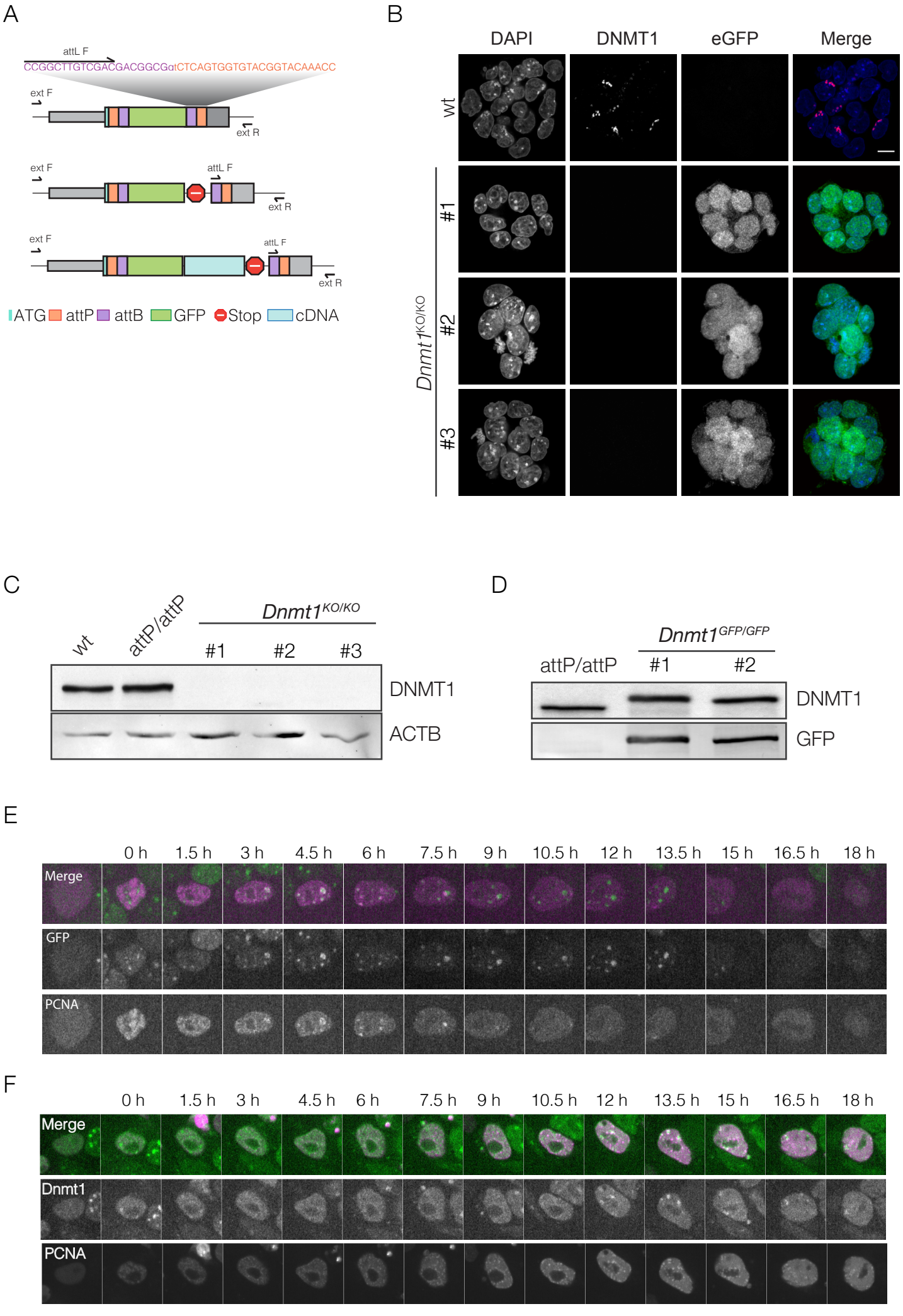
Supplementary Figure 1



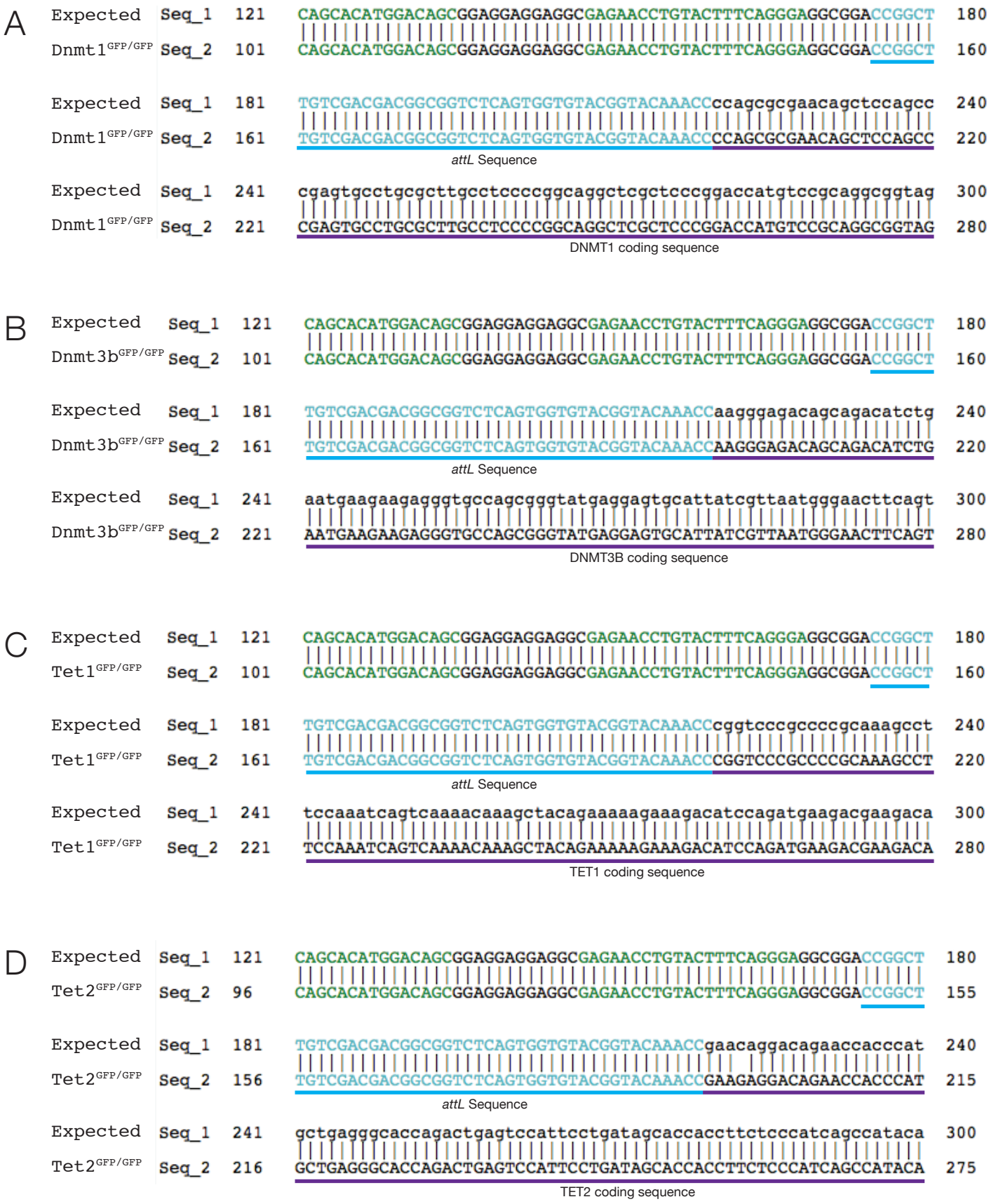
Supplementary Figure 2



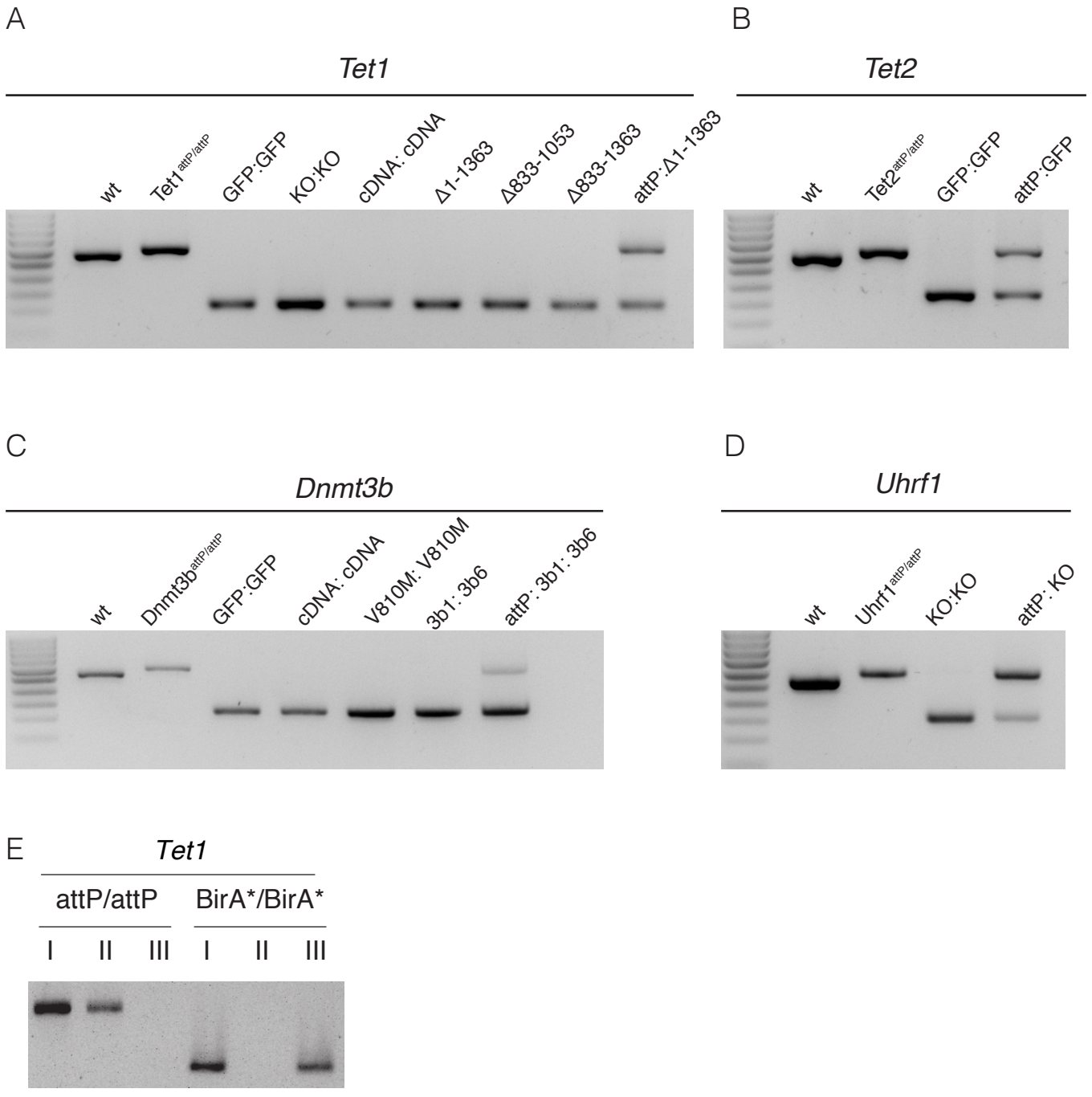
Supplementary Figure 3



Supplementary Figure 4

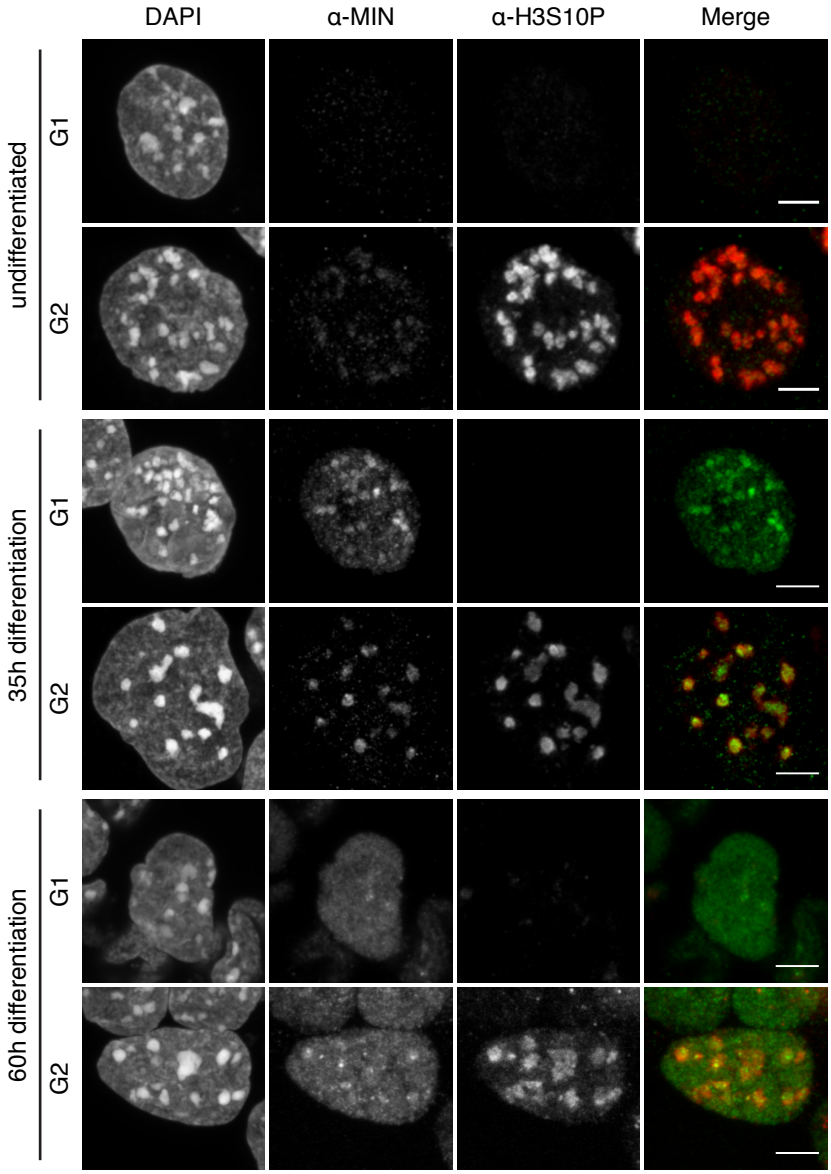


Supplementary Figure 5



Supplementary figure 6

A



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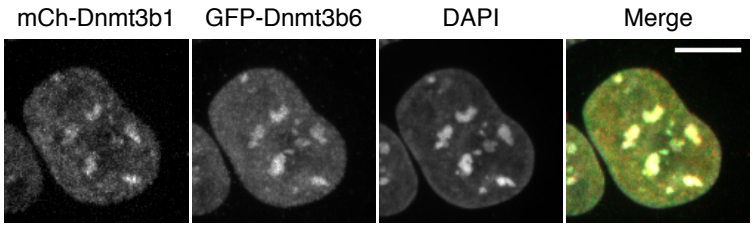

Supplemental Figure and Video Legends

Supplemental Figure S1. Characterization of MIN-tagged DNA methyltransferase cell lines.

(A) Western blot analysis of DNMT1 expression levels in the homozygous *Dnmt1*$^{attP/attP}$ and wild type J1 cells. Beta-actin is used as a loading control. **(B)** Immunofluorescence stainings of Dnmt1 in wt and Dnmt1$^{attP/attP}$ cells. Scale bar represent 5 μm. **(C)** DNA methylation analysis of the major satellite repeats in *Dnmt1*$^{attP/attP}$ and wild type cells. **(D)** Example of the screening PCRs, with and without HincII treatment, of clones found to be heterozygous and homozygous for the MIN-tag at the *Dnmt1* locus. Monoallelic and biallelic insertions of the MIN-tag can be distinguished by complete and incomplete digests, respectively. **(E)** Western blot analysis of DNMT3A expression levels in a heterozygous (#2) and homozygous (#1, #3-4) *Dnmt3a*$^{attP/attP}$ cell lines compared to wild type cells. Beta-actin is used as a loading control. **(F)** DNA methylation analysis of major satellite repeats in *Dnmt3a*$^{attP/attP}$ compared to wt cells. **(G)** Immunofluorescence stainings of DNMT3A together with the replication marker EdU in wt cells and the the homozygous *Dnmt3a*$^{attP/attP}$ clone #1. Scale bar represents 10 μm. **(H)** Immunofluorescence stainings of DNMT3B in *Dnmt3b*$^{attP/attp}$ and wt cells after 35 hours of EpiLC differentiation. Scale bar represents 5 μm. Error bar represent standard deviation (n=2).

Supplemental Figure S2. Characterization of MIN-tagged Tet1, Tet2 and Uhrf1 cell lines and C-terminal MIN-tag integration.

(A-C) Western blot analysis of TET1, TET2, and UHRF1 expression levels in the homozygous *Tet1*$^{attP/attP}$, *Tet2*$^{attP/attP}$, and N-terminal *Uhrf1*$^{attP/attP}$ cell lines, respectively, compared to the wt J1 control. β-Actin (ACTB) was used as loading control. **(D)** Immunofluorescence stainings of TET1 in wt and *Tet1*$^{attP/attP}$ cells. **(E)** Immunofluorescence stainings of 5-hydroxymethylcytosine (5-hmC) in wt and *Tet1*$^{attP/attP}$ cells. **(F)** Immunofluorescence stainings of TET2 in wt and *Tet2*$^{attP/attP}$ cells. **(G)**

Immunofluorescence stainings of UHRF1 in wt and *Uhrf1*$^{attP/attP}$ cells. DAPI is used for DNA counterstaining; scale bars represent 15 *μ*m. **(H)** Schematic overview of CRISPR/Cas-assisted C-terminal integration of the MIN-tag. MIN-tag donors contain the attP site (depicted in orange) flanked by sequences (200-300 for PCR fragments or 76 for ssDNA oligos) homologous to 5' and 3' of the target gene stop codon (depicted in red). Restriction enzyme sites available for restriction fragment analysis based screening are shown above the attP sequence.

Supplemental Figure S3. Evaluating functionality of Bxb1 mediated recombination in *Dnmt1*$^{attP/attP}$ cells.

(A) Schematic outline of the multiplex PCR strategy to identify positive recombination events and their zygosity. **(B)** Immunofluorescence stainings of DNMT1 and GFP in wt cells and three *Dnmt1*$^{KO/KO}$ clones. Diffuse GFP indicates a successful integration of the KO cassette into the locus. **(C)** Western blot analysis of DNMT1 expression levels in three *Dnmt1*$^{KO/KO}$ clonal cell lines generated by Bxb1-mediated insertion of a knock-out cassette, compared to wt and *Dnmt1*$^{attP/attP}$ cells. **(D)** Western blot analysis of DNMT1 and GFP expression in *Dnmt1*$^{attP/attP}$ cells and two homozygous GFP-knock in cell lines (*Dnmt1*$^{GFP/GFP}$ #1-2) generated by Bxb1 mediated insertion. **(E-F)** Live cell imaging of *Dnmt1*$^{GFP/GFP}$ and *Dnmt1*$^{cDNA/cDNA}$ cells transiently expressing RFP-labeled PCNA, a DNA replication marker, during cell-cycle progression. Scale bars represent 5 μm

Supplemental Figure S4. Alignments of the expected sequence flanking the attL site after recombination

Alignments of the expected sequence flanking the attL site after recombination of the attB-GFP KI at the *Dnmt1*, *Dnmt3b*, *Tet1*, and *Tet2* locus (A-D) with the sequencing results from the *Dnmt1*$^{GFP/GFP}$, *Dnmt3b*$^{GFP/GFP}$, *Tet1*$^{GFP/GFP}$, and *Tet2*$^{GFP/GFP}$ cell lines.

Supplemental Figure S5. Demonstration of Bxb1 mediated recombination in multiple MIN-tagged genes.

(A-D) Gel electrophoresis of the multiplex PCR (using the attL primer and locus specific

external primers, see also Table S1) performed on cell lines generated by Bxb1-mediated integration of various MIN-tag toolbox components (Table S5) into the loci of: (A) Tet1, (B) Tet2, (C) Dnmt3b, and (D) Uhrf1. Equal mixtures of genomic DNA from non-recombined cell lines and recombined cell lines are used to control for possible amplification biases arising from the use of different locus specific external primers. **(E)** PCR to confirm insertion of the BirA* cassette into the Tet1 genomic locus. I: multiplex PCR, II: wt specific PCR, III: attL (recombination) specific PCR

Supplemental Figure S6. Cell cycle analysis of DNMT3b localization during differentiation.

(A) Immunofluorescence stainings of MIN-tagged DNMT3B and Histone 3 Serine 10 phosphorylation (H3S10P), a marker of G2/M phase (Ref Hendzel:1997wo) during differentiation of naive pluripotent *Dnmt3b*$^{attP/attP}$ stem cells into epiblast-like cells. Cells were fixed directly after (0 h)35 h, or 60 hafter induction of differentiation. The H3S10P mark was used to determine if cells were in G2 or G1 phase in order to assess whether changes in DNMT3B localization during differentiation are cell-cycle dependent. Scale bar represents 5 μm. **(B)** Fluorescence microscopy images of *Dnmt3b*$^{mCh\text{-}3b1/GFP\text{-}3b6}$ cells fixed after 35 h of differentiation. Both DNMT3B isoforms (GFP-DNMT3B1in green and mCh-DNMT3B6 in red) localize at chromocenters (visible as bright DAPI spots). Scale bar represents 5 μm

Supplemental Video 1. Live cell imaging of *Dnmt3b*$^{GFP/GFP}$ cells during differentiation.

Long-term (60 h), live cell imaging tracking the transition of *Dnmt3b*$^{GFP/GFP}$ cells from the naive pluripotency ground state into the primed, epiblast-like state. Images were acquired once per hour and entailing at least 10 μm z-stacks. The left panel depicts the projection of GFP signal, while the right panel shows that projection superimposed onto the acquired brightfield images.

Supplemental Tables (S1-S5)

Table S1: CRISPR/Cas9-mediated MIN-tag insertion efficiencies

Gene	Position	MIN-tag Donor	Heterozygotes	Homozygotes	TOTAL
Dnmt1	N-terminal	PCR Product	1/67 (1.5%)	1/67 (1.5%)	2/67 (2.9%)
Dnmt3a	N-terminal	PCR Product	0/86 (0%)	3/86 (3.5%)	3/86 (3.5%)
Dnmt3b	N-terminal	ssDNA oligo	0/65(0%)	1/65(1.5%)	1/65 (1.5%)
Uhrf1	N-terminal	PCR Product	0/6 (0%)	1/6(16.7%)	1/6 (16.7%)
Uhrf1	C-terminal	ssDNA oligo	2/36 (5.5%)	2/36 (5.6 %)	4/36 (11.1%)
Tet1	N-terminal	PCR Product	0/70(0%)	1/70 (1.4%)	1/70 (1.4%)
Tet2	N-terminal	PCR Product	1/24 (4.2%)	2/24 (8.3%)	3/24 (12.5%)
Tet3	N-terminal	PCR Product	0/38 (0%)	2/38 (5.3%)	2/38 (5.3%)

Table S2: Oligonucleotide sequences used for CRISPR/Cas assisted targeting and screening

Name	Sequence
Dnmt1	
gRNA_F	TGTTCGCGCTGGCATCTTGCGTTTTAGAGCTAGAAATAGCAAG
gRNA_R	GCAAGATGCCAGCGCGAACACGGTGTTTCGTCCTTTCCAC
surrogate_F	CTAGCTGTTCGCGCTGGCATCTTGCAGGGGATTCC
surrogate_R	CCGGAGGAATCCCCTGCAAGATGCCAGCGCGAACAG
internal_R	CACTATAGCCAGGAGGTGTGGG
internal_F	TGTACCGTACACCACTGAGACCGCGGTGGTTGACCAGACAAACCCATCTTGCAGGTTGCA GACGACAG
external_R	GTCTGGTCAACCACCGCGGTCTCAGTGGTGTACGGTACAAACCCCAGCGCGAACAGCTCC AGC
external_F	GCGCGACAGGAAGCACAGCC
screening_F	GTCGCAGCACGGACGAG
Uhrf1 (N)	
gRNA_F	CATCGGCATCATGTGGATCCGTTTTAGAGCTAGAAATAGCAAG
gRNA_R	GGATCCACATGATGCCGATGCGGTGTTTCGTCCTTTCCAC
surrogate_F	CTAGCCATCGGCATCATGTGGATCCAGGGGATTCCT
surrogate_R	GGCCAGGAATCCCCTGGATCCACATGATGCCGATGG
internal_R	CATCGGCATCATGTGGATCCGTTTTAGAGCTAGAAATAGCAAG
internal_F	GGATCCACATGATGCCGATGCGGTGTTTCGTCCTTTCCAC
external_R	ACCACCGCGGTCTCAGTGGTGTACGGTACAAACCTGGATCCAGGTTCGAACTATG
external_F	CTATTGCTTGGTGGCTTTGAG
screening_F	GGCAATTCACATTCAAGTGTCCC
Uhrf1 (C)	
gRNA_F	TGCCTGGGTCTCAGCATCACGTTTTAGAGCTAGAAATAGCAAG
gRNA_R	GTGATGCTGAGACCCAGGCACGGTGTTTCGTCCTTTCCAC
surrogate_F	CTAGCTGCCTGGGTCTCAGCATCACCGGGGATTCCT
surrogate_R	CCGGAGGAATCCCCGGTGATGCTGAGACCCAGGCAG
ssDNA oligo	CAGCTCCCCAACCCGGGTGAACCAGCCCTTGCAGACCATTCTCAACCAGCTCTTCCCTGG CTATGGCAGCGGCCGGGGTTTGTCTGGTCAACCACCGCGGTCTCAGTGGTGTACGGTACA AACCTGATGCTGAGACCCAGGCAGAGGGCTCATGGTTCCAACTTCATAGTGTGTTTAGCT TGAAGGTGTTGTCCTTCACG
external_R	TTTCTAGGCAGCTGGTGTGG
external_F	TGTACGTGAGAGGACGGAGT
screening_F	TGTTGCCAGGAGCTACCAAG
Dnmt3a	
gRNA_F	GGGCCGCTGGAGGGCATTGCGTTTTAGAGCTAGAAATAGCAAG
gRNA_R	GCAATGCCCTCCAGCGGCCCCGGTGTTTCGTCCTTTCCAC
surrogate_F	CTAGCGGGCCGCTGGAGGGCATTGCTGGGGATTCCT
surrogate_R	CCGGAGGAATCCCCAGCAATGCCCTCCAGCGGCCCG
internal_R	CTTCTCTTCCCCACAGGCAG
internal_F	ACCACTGAGACCGCGGTGGTTGACCAGACAAACCCATTGCTGGGCAGTAGGCG
external_R	ACCACCGCGGTCTCAGTGGTGTACGGTACAAACCCCCTCCAGCGGCCCCG
external_F	GTTCCCAGCCAAGCACCTAT
screening_F	ATGGTCCTGCAACCAGAGTG
Dnmt3b	
gRNA_F	TTCCCCACAGGAAACAATGAGTTTTAGAGCTAGAAATAGCAAG
gRNA_R	TCATTGTTTCCTGTGGGGAACGGTGTTTCGTCCTTTCCAC

surrogate_F	CTAGCTTCCCCACAGGAAACAATGAAGGGGATTCCT
surrogate_R	CCGGAGGAATCCCCTTCATTGTTTCCTGTGGGGAAG
ssDNA oligo	GAACTGGTGGTGTAAACCTTGCAGTGTGCCCTGTCTGCCTCTTACATATCCTGATCTTTC CCCACAGGAAACAATGGGTTTGTCTGGTCAACCACCGCGGTCTCAGTGGTGTACGGTACA AACCAAGGGAGACAGCAGACATCTGAATGAAGAAGAGGGTGCCAGCGGGTATGAGGAGTG CATTATCGTTAATGGGAACT
external_R	ACCACCGCGGTCTCAGTGGTGTACGGTACAAACCGGAGACAGCAGACATCTGAATG
external_F	ATCTGTCATGGAACCTGCCG
screening_F	GAGCTGGCCAATTGCAGAAC
Tet1	
gRNA_F	AGACATGGCTGCAGAGTAAGCGGTGTTTCGTCCTTTCCAC
gRNA_R	CTTACTCTGCAGCCATGTCTAGCTTTCTTGTACAAAGTTGGCAT
surrogate_F	CTAGCCTTACTCTGCAGCCATGTCTCGGGGATCCCT
surrogate_R	CCGGAGGGATCCCCGAGACATGGCTGCAGAGTAAGG
internal_R	ACTCAGTCTCCCAAATGCTGG
internal_F	ACCACTGAGACCGCGGTGGTTGACCAGACAAACCAGACATGGCTGCAGAGTAAGTAAAG
external_R	ACCACCGCGGTCTCAGTGGTGTACGGTACAAACCCGGTCCCGCCCCGCAAAG
external_F	TCGGGGTTTTGTCTTCCGTT
screening_F	GGGCAATGTTGTGACTCATGC
Tet2	
gRNA_F	CGAAGCAAGCCTGATGGAACGTTTTAGAGCTAGAAATAGCAAG
gRNA_R	GTTCCATCAGGCTTGCTTCGCGGTGTTTCGTCCTTTCCAC
surrogate_F	CTAGCCGAAGCAAGCCTGATGGAACAGGGGATTCCT
surrogate_R	CCGGAGGAATCCCCTGTTCCATCAGGCTTGCTTCGG
internal_R	ACCACTGAGACCGCGGTGGTTGACCAGACAAACCCATCAGGCTTGCTTCGGGG
internal_F	ACCACCGCGGTCTCAGTGGTGTACGGTACAAACCGAACAGGACAGAACCACCCAT
external_R	TGGTTCACTGACTGTGCGTT
external_F	CCAGGATCACACAGGAAGCA
screening_F	GGATGGAGCCCAGAGAGAGA
Tet3	
gRNA_F	GTTCCAGGTCAGATGGACTCGTTTTAGAGCTAGAAATAGCAAG
gRNA_R	GAGTCCATCTGACCTGGAACCGGTGTTTCGTCCTTTCCAC
surrogate_F	CTAGCGTTCCAGGTCAGATGGACTCAGGGGATTCCT
surrogate_R	CCGGAGGAATCCCCTGAGTCCATCTGACCTGGAACG
internal_R	ACCACTGAGACCGCGGTGGTTGACCAGACAAACCCATCTGACCTGGAACAGGTC
internal_F	ACCACCGCGGTCTCAGTGGTGTACGGTACAAACCGACTCAGGGCCAGTGTACC
external_R	CAGTCGGGCTTCTGGTCTAC
external_F	GATCTGAGCTCTCACAGGGC
screening_F	AGTAGACAGGGCCTTGGGAT
attL_F	CCGGCTTGTCGACGACG

Table S3: Bxb1-mediated recombination efficiencies

Gene	Integration Construct	Heterozygotes	Homozygotes	TOTAL
Dnmt1	attB-GFP	N/A	13/31 (41.9%)	13/31 (41.9%)
Dnmt3b	attB-GFP	0/3 (0%)	1/3 (33.3%)	1/3 (33.3%)
Tet1	attB-GFP	14/45 (31.1%)	13/45 (28.9%)	27/45 (60%)
Tet2	attB-GFP	28/81 (34.6%)	15/81(18.5%)	43/81 (53%)
Dnmt1	attB-GFP-STOP-Poly(A)	2/23 (8.7%)	13/23 (56.5%)	15/23 (65.2%)
Uhrf1	attB-GFP-STOP-Poly(A)	5/32 (15.6%)	14/32 (43.8%)	19/32 (59.4%)
Dnmt1	attB-GFP-cDNA-STOP-Poly(A)	1/15 (6.6%)	9/15 (60%)	10/15 (66.6%)
Dnmt3b	attB-GFP-cDNA-STOP-Poly(A)	28/84 (33.3%)	26/84 (31%)	54/84 (64.3%)
Tet1	attB-GFP-cDNA-STOP-Poly(A)	12/58 (20.7%)	7/58 (12.1%)	19/58 (32.8%)
Dnmt3b	attB-GFP/mCh-cDNA-STOP-Poly(A) PuroR/neoR	29/102 (28.4%)	64/102 (62.7%)	93/102 (91.2%)

Table S4: Evaluation of FRAP protein kinetics

	GFP-DNMT3B	mCh-DNMT3B1	GFP-DNMT3B6
Mobile fraction [A]	87	81	100
Diffusion coef. [µm²/s]	4.2E-03	1.2E-03	4.1E-02
Half-time recovery [s]	42.2	94.8	5.1

Table S5: The MIN-tag toolbox

Name	Fluorescent protein	Application
Universal constructs		
attB-GFP	GFP	GFP KI
attB-mCh	mCherry	mCherry KI
attB-GFP-T2A-BirA*	GFP	Protein interaction
attB-GFP-Poly(A)	GFP	KO
attB-mCh-Poly(A)	mCherry	KO
attB-GFP-Poly(A)-NeoR	GFP	KO /w selection
attB-GFP-Poly(A)-PuroR	GFP	KO /w selection
attB-mCh-Poly(A)-NeoR	mCherry	KO /w selection
attB-mCh-Poly(A)-PuroR	mCherry	KO /w selection
Gene specific cDNA KI constructs		
attB-GFP-Dnmt1-Poly(A)	GFP	cDNA KI
attB-GFP-Dnmt3b1-Poly(A)	GFP	cDNA KI
attB-GFP-Dnmt3b6-Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b_C656A_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b_D809G_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b_dX_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b_G655S_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b_L656T_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b_V718G_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b_V810M_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b6_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b1_dPWWP_Poly(A)	GFP	cDNA KI
attB_eGFP_Dnmt3b1_dPHD_Poly(A)	GFP	cDNA KI
attB_mCh_Dnmt3b_C656A_Poly(A)	mCherry	cDNA KI
attB_ mCh _Dnmt3b_D809G_Poly(A)	mCherry	cDNA KI
attB_ mCh _Dnmt3b_dX_Poly(A)	mCherry	cDNA KI
attB_ mCh _Dnmt3b_G655S_Poly(A)	mCherry	cDNA KI
attB_ mCh _Dnmt3b_L656T_Poly(A)	mCherry	cDNA KI
attB_ mCh _Dnmt3b_V718G_Poly(A)	mCherry	cDNA KI
attB_ mCh _Dnmt3b_V810M_Poly(A)	mCherry	cDNA KI
attB_ mCh _Dnmt3b6_Poly(A)	mCherry	cDNA KI
attB-GFP-Dnmt3b1-Poly(A) -NeoR	GFP	cDNA KI /w selection
attB-GFP-Dnmt3b6-Poly(A) -NeoR	GFP	cDNA KI /w selection
attB_eGFP_Dnmt3b_C656A_Poly(A)-NeoR	GFP	cDNA KI /w selection
attB_eGFP_Dnmt3b_D809G_Poly(A)-NeoR	GFP	cDNA KI /w selection
attB_eGFP_Dnmt3b_dX_Poly(A)-NeoR	GFP	cDNA KI /w selection
attB_eGFP_Dnmt3b_G655S_Poly(A)-NeoR	GFP	cDNA KI /w selection
attB_eGFP_Dnmt3b_L656T_Poly(A)-NeoR	GFP	cDNA KI /w selection
attB_eGFP_Dnmt3b_V718G_Poly(A)-NeoR	GFP	cDNA KI /w selection

attB_eGFP_Dnmt3b_V810M_Poly(A)-NeoR	GFP	cDNA KI /w selection
attB_eGFP_Dnmt3b6_Poly(A)-NeoR	GFP	cDNA KI /w selection
attB- mCh -Dnmt3b1-Poly(A) -NeoR	mCherry	cDNA KI /w selection
attB- mCh -Dnmt3b6-Poly(A) -NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_C656A_Poly(A)-NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_D809G_Poly(A)-NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_dX_Poly(A)-NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_G655S_Poly(A)-NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_L656T_Poly(A)-NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_V718G_Poly(A)-NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_V810M_Poly(A)-NeoR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b6_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB- mCh -Dnmt3b1-Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB- mCh -Dnmt3b6-Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_C656A_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_D809G_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_dX_Poly(A)- PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_G655S_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_L656T_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_V718G_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b_V810M_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB_ mCh _Dnmt3b6_Poly(A)-PuroR	mCherry	cDNA KI /w selection
attB-GFP-Tet1-Poly(A)	GFP	cDNA KI
attB-GFP-Tet1d1-389-Poly(A)	GFP	cDNA KI
attB-GFP-Tet1d390-565-Poly(A)	GFP	cDNA KI
attB-GFP-Tet1d566-833-Poly(A)	GFP	cDNA KI
attB-GFP-Tet1d834-1053-Poly(A)	GFP	cDNA KI
attB-GFP-Tet1d1054-1363-Poly(A)	GFP	cDNA KI
attB-GFP-Tet1d1-833-Poly(A)	GFP	cDNA KI
attB-GFP-Tet1d834-1363-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2d1-225-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2d226-398-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2d399-650-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2d651-848-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2d849-1038-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2d1-650-Poly(A)	GFP	cDNA KI
attB-GFP-Tet2d651-1038-Poly(A)	GFP	cDNA KI
attB-GFP-Uhrf1-Poly(A)	GFP	cDNA KI
attB-GFP-Uhrf1dSRA-Poly(A)	GFP	cDNA KI

Supplemental Table Legends

Table S1: CRISPR/Cas9-mediated MIN-tag insertion efficiencies

For MIN-tag Insertion, J1 mESCs transfected with the appropriate MIN-tag donor oligonucleotides or PCR products along with the Cas9, gRNA, and CRISPR surrogate reporter vector were single cell sorted after enriching for cells with CRISPR/Cas activity. The number of clones with either a monoallelic or biallelic insertion of the MIN-Tag is shown in relation to the number of clones screened.

Table S2: Oligonucleotide sequences used for CRISPR/Cas assisted targeting and screening

DNA oligonucleotides used for the generation of target specific gRNA expression vectors, surrogate reporters, and homology donors for MIN-tag integration.

Table S3: Bxb1-mediated recombination efficiencies

For Bxb1-mediated recombination, J1 mESCs transfected with NLS-Bxb1, the Bxb1 surrogate reporter, and the respective attB-site containing integration construct were single-cell sorted after enrichment for cells with Bxb1 activity. The number of clones with either a monoallelic or biallelic integration of the listed construct is shown in relation to the total number of clones screened.

Table S4: Evaluation of FRAP protein kinetics

Evaluation of FRAP kinetics (w/o 5-azadC treatment) performed in Dnmt3bGFP/GFP and Dnmt3bmCh-3b1/GFP-3b6 cells

Table S5: The MIN-tag toolbox

Vectors generated for Bxb1 mediated recombination into MIN-tagged cell lines. KO: knockout, KI: knockin