## **Supplementary Information**

# MeCP2 binds to methylated DNA independently of phase separation and heterochromatin organisation

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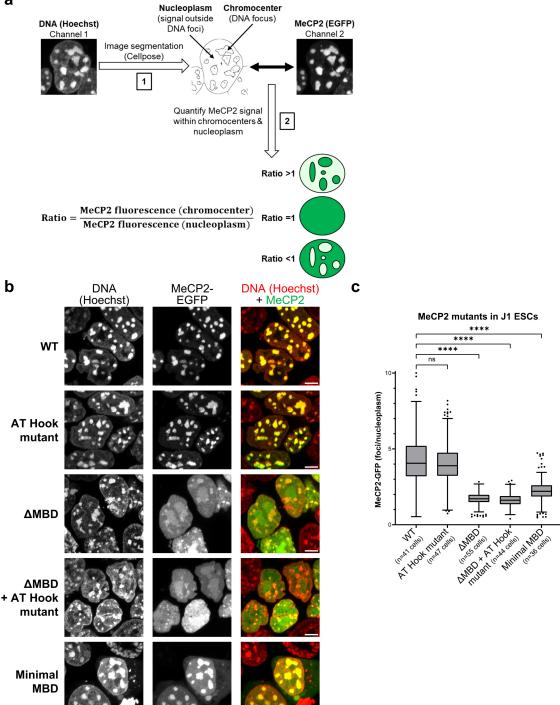
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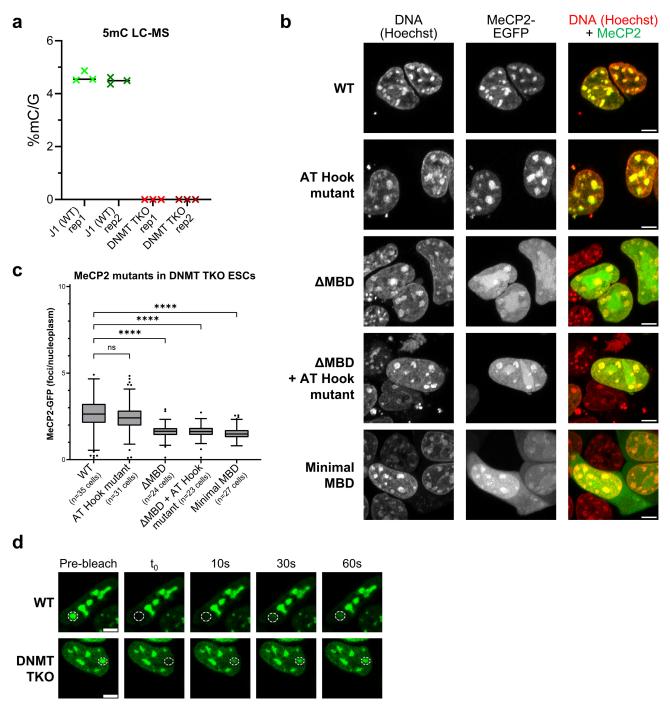
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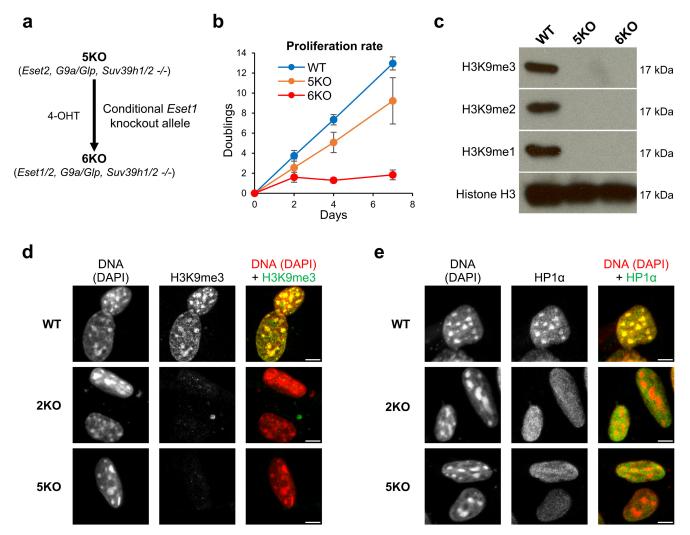
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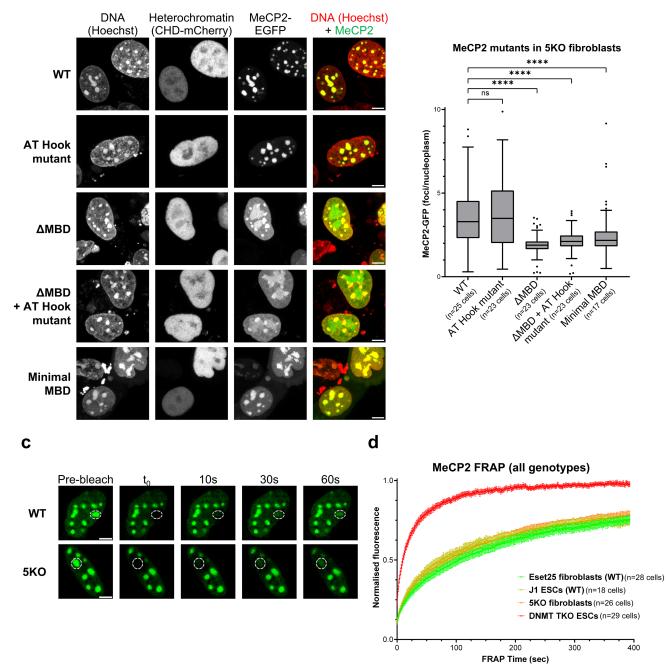
**Supplementary Fig. 1 (related to Fig. 1)** | **a.** Diagram showing the strategy for quantifying the relative enrichment of MeCP2 within chromocenters (DNA-dense foci) in mouse cells. In each cell, the fluorescence ratio was calculated for each individual chromocenter. **b.** Live-cell imaging of J1 ESCs transfected with EGFP-MeCP2 wild-type and mutant constructs (see Fig. 1a). Hoechst staining was used to visualise DNA. Scale bars:  $5\mu$ m. **c.** Box plot showing the quantification of wild-type and mutant MeCP2 fluorescence at DNA-dense foci (relative to nucleoplasm) in J1 ESCs, as described in panel b. The box lower and upper limits correspond to the 25th and 75th percentiles, respectively, with the centre line corresponding to the median. Whiskers extend up to 1.5 times the inter-quartile distance according to Tukey's method, and individual points are outliers. The number of analysed cells from two independent experiments are: WT n= 41 cells, AT Hook mutant n= 47 cells,  $\Delta$ MBD n= 55 cells,  $\Delta$ MBD + AT Hook mutant n= 44 cells, Minimal MBD n= 36 cells. Stars indicate statistical significance compared to wild-type MeCP2 (Brown-Forsythe and Welch ANOVA test). The Source data for this Supplementary Figure is associated with the Source data for Figure 1.



Supplementary Fig. 2 (related to Fig. 1) | a. Mass spectrometric quantification of 5-methylcytosine (relative to total guanosine pool) from genomic DNA in the indicated ESC lines (two independent replicates). Individual data points are technical replicates and lines correspond to the median. **b.** Live-cell imaging of DNMT TKO ESCs transfected with EGFP-MeCP2 wild-type and mutant constructs (see Fig. 1a). Hoechst staining was used to visualise DNA. Scale bars: 5µm. c. Box plot showing the quantification of wild-type and mutant MeCP2 fluorescence at DNA-dense foci (relative to nucleoplasm) in DNMT TKO ESCs, as described in panel b. The box lower and upper limits correspond to the 25th and 75th percentiles, respectively, with the centre line corresponding to the median. Whiskers extend up to 1.5 times the inter-quartile distance according to Tukey's method, and individual points are outliers. The number of analysed cells from two independent experiments are: WT n= 35 cells, AT Hook mutant n= 31 cells, ΔMBD n= 24 cells, ΔMBD + AT Hook mutant n= 23 cells, Minimal MBD n= 27 cells. Stars indicate statistical significance compared to wild-type MeCP2 (Brown-Forsythe and Welch ANOVA test). d. Live-cell imaging showing the fluorescence recovery after photobleaching (FRAP) of wild-type EGFP-MeCP2 in wild-type (J1) and DNMT TKO ESCs. Scale bars: 5µm. The Source data for this Supplementary Figure is associated with the Source data for Figure 1.

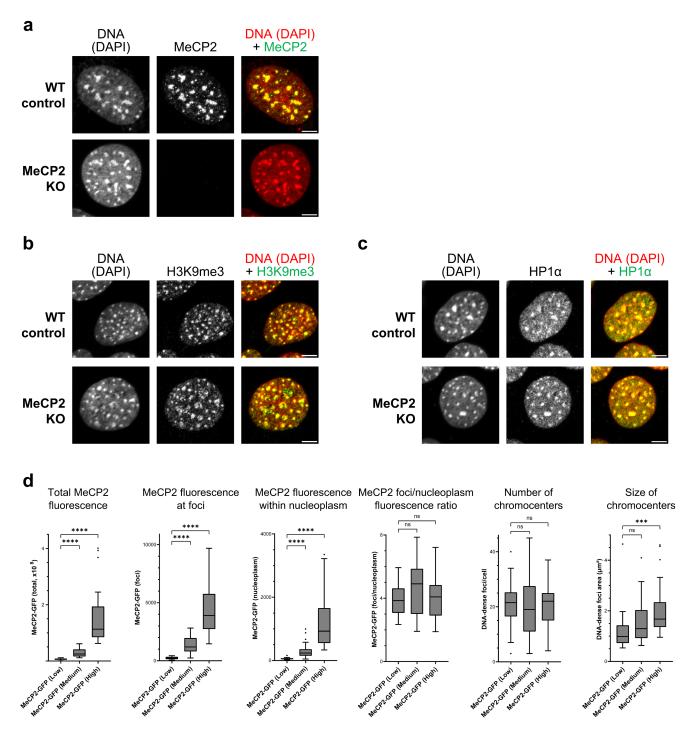


**Supplementary Fig. 3 (related to Fig. 2)** | **a.** Diagram showing the genotype of H3K9 lysine methyltransferase knockout cell lines. *5KO* fibroblasts lack five out of six methyltransferase genes and can be converted to a *6KO* state upon tamoxifen induction. **b.** Growth curves showing that Eset1 knockout (*6KO*) leads to a lethal phenotype within 2 to 4 days. Error bars: SD. **c.** Western blot analysis of canonical heterochromatin marks H3K9me1/2/3 in the indicated cell lines. Total histone H3 was probed as a positive control. **d, e.** Immunofluorescence of H3K9me3 (d) and HP1 $\alpha$  (e) in wild-type (Eset25) and H3K9 lysine methyltransferases knockout fibroblasts (*2KO/5KO*). DAPI staining was used to visualise DNA. Scale bars: 5µm. The Source data for this Supplementary Figure is associated with the Source data for Figure 2.

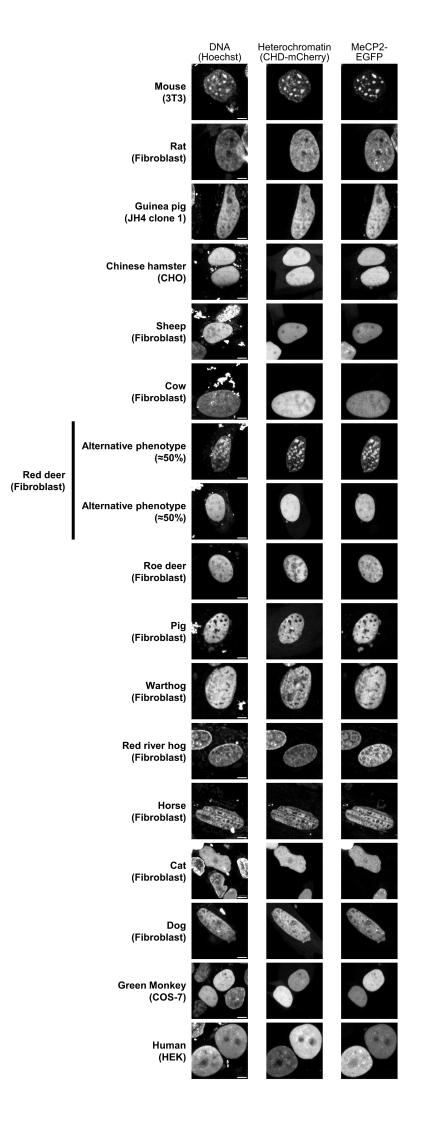


Supplementary Fig. 4 (related to Fig. 2) | a. Live-cell imaging of 5KO fibroblasts transfected with EGFP-MeCP2 wild-type and mutant constructs (see Fig. 1a). Hoechst staining and CHD-mCherry reporter were used to visualise DNA and heterochromatin, respectively. Scale bars: 5µm. b. Box plot showing the quantification of MeCP2 wild-type and mutant fluorescence at DNA-dense foci (relative to nucleoplasm) in 5KO fibroblasts, as described in panel a. The box lower and upper limits correspond to the 25th and 75th percentiles, respectively, with the centre line corresponding to the median. Whiskers extend up to 1.5 times the inter-guartile distance according to Tukey's method. and individual points are outliers. The number of analysed cells from two independent experiments are: WT n= 25 cells, AT Hook mutant n= 23 cells, ΔMBD n= 23 cells, ΔMBD + AT Hook mutant n= 23 cells, Minimal MBD n= 17 cells. Stars indicate statistical significance compared to wild-type MeCP2 (Brown-Forsythe and Welch ANOVA test). c. Live-cell imaging showing the fluorescence recovery after photobleaching (FRAP) of wild-type EGFP-MeCP2 in wild-type (Eset25) and 5KO fibroblasts. Scale bars: 5um. d. Graph showing the FRAP quantification of wild-type EGFP-MeCP2 in all mutant lines used in this study (see Fig. 1 and 2). The number of analysed cells from two independent experiments are: Eset25 (WT) n= 28 cells, J1 (WT) n= 18 cells, 5KO n= 26 cells, DNMT TKO n= 29 cells. Error bars: SEM. The Source data for this Supplementary Figure is associated with the Source data for Figure 2.

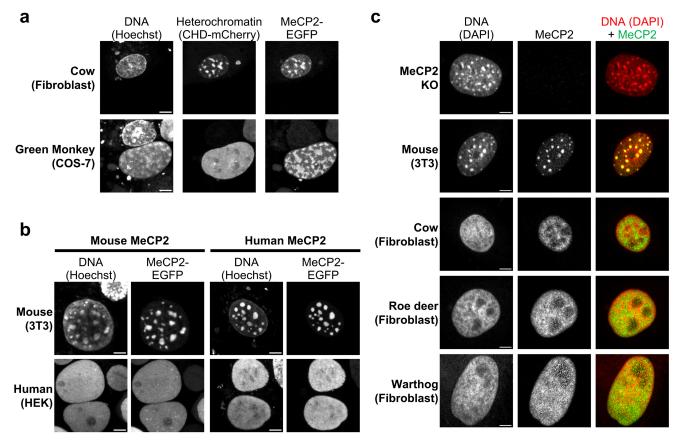
b



**Supplementary Fig. 5 (related to Fig. 2) | a, b, c.** Immunofluorescence of MeCP2 (a), H3K9me3 (b) and HP1 $\alpha$  (c) in wild-type (2-17) and *MeCP2* knockout fibroblasts. DAPI staining was used to visualise DNA. Scale bars: 5µm. **d.** Box plots showing the quantification of different parameters in transfected *Mecp2* knockout fibroblasts (see Fig. 2f) divided into three categories depending on their MeCP2 expression levels (low: n=34 cells; medium: n=33 cells; high: n=34 cells). The box lower and upper limits correspond to the 25th and 75th percentiles, respectively, with the centre line corresponding to the median. Whiskers extend up to 1.5 times the inter-quartile distance according to Tukey's method, and individual points are outliers. Stars indicate statistical significance between groups of MeCP2 expression level (Brown-Forsythe and Welch ANOVA test). The Source data for this Supplementary Figure is associated with the Source data for Figure 2.



**Supplementary Fig. 6 (related to Fig. 3)** | Live-cell imaging of all studied mammalian cell lines transfected with wild-type EGFP-MeCP2. Hoechst staining and CHD-mCherry reporter were used to visualise DNA and heterochromatin, respectively. Scale bars: 5µm. Of note, red deer cells presented a mixed population with two distinct phenotypes characterised by diffuse or spotty MeCP2/Heterochromatin, respectively. The Source data for this Supplementary Figure is associated with the Source data for Figure 3.

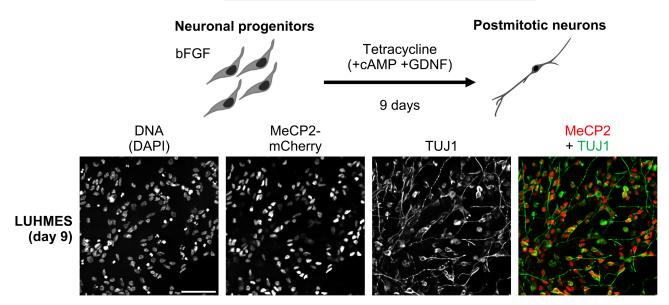


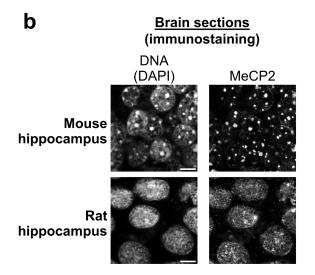
**Supplementary Fig. 7 (related to Fig. 3) | a.** Live-cell imaging showing a rare sub-population of cow and monkey cells with spotty signal when transfected with wild-type EGFP-MeCP2 (see Fig. 3c). Hoechst staining and CHD-mCherry reporter were used to visualise DNA and heterochromatin, respectively. Scale bars: 5µm. **b.** Live-cell imaging of mouse (3T3) and human (HEK) cell lines transfected with mouse (left) or human (right) wild-type EGFP-MeCP2. Hoechst staining was used to visualise DNA. Scale bars: 5µm. **c.** Immunofluorescence of endogenous MeCP2 in the indicated mammalian cell lines and *MeCP2* knockout fibroblasts (negative control). DAPI staining was used to visualise DNA. Scale bars: 5µm. The Source data for this Supplementary Figure is associated with the Source data for Figure 3.

	1 10	20	30	40	50	60	70	80
MOUSE	MVAGMLGLR <mark>E</mark> EKSE		· .	KKEDKEGKHE		EPAEAGKAET		· ·
RAT CHINESE_HAMSTER	MVAGMLGLR <mark>K</mark> EKSE MVAGMLGLR <mark>E</mark> EKSE			KK <mark>EDK</mark> EGKHE KKEDKEGKHE		EPAEAGKAET EPAEAGKAET		
SHEEP	MVAGMLGLR <mark>e</mark> ekse	E <mark>QDLQGL</mark> KD <mark>B</mark>	CPLKFKK <mark>V</mark> KK <mark>D</mark>	<mark>kk</mark> edk <mark>egkhe</mark>	P L Q P A A H H S A	EPAEAGKAET	SE <mark>g</mark> sg <mark>s</mark> apavpe	ASAS
COW RED_DEER	MVAGMLGLR <mark>E</mark> EKSE MVAGMLGLR <mark>E</mark> EKSE			KKEDKEGKHE KKEDKEGKHE		EPAEAGKAET EPAEAGKAET		
PIG HORSE	MVAGMLGLR <mark>E</mark> EKSE MVAGMLGLR <mark>E</mark> EKSE			KK <mark>DDR</mark> EGKHE KKEDKEGKHE		EPAEAGKAET EPAEAGKAET		
CAT	MVAGMLGLREEKSE MVAGMLGLR <mark>E</mark> EKSE					EPAEAGKAET		
DOG AFRICAN_GREEN_MONKEY	MVAGMLGLR <mark>E</mark> EKSE MVAGMLGLR <mark>E</mark> EKSE					EPAEAGKAET EPAEAGKAET		
HUMAN	MVAGMLGLR <mark>E</mark> EKSE							
MOUSE	90 PKORRSIIRDRGPM				130 INBOCKAERS	140 KVELTAVEEK		160
RAT	PKQRRSIIRDRGPM							
CHINESE_HAMSTER SHEEP	PKQRRSIIRDRGPM PKQRRSIIRDRGPM							
COW	PKQRRSIIRDRGPM	YDDPTLPEGV	TRKLKQRKSG	RSAGKYDVYL	INPQGKAFRS	KVELIAYFEK	VGDTSLDPNDFD	FTVT
RED_DEER PIG	PKQRRSIIRDRGPM PKQRRSIIRDRGPM							
HORSE CAT	PKQRRSIIRDRGPM PKQRRSIIRDRGPM							
DOG	PKQRRSIIRDRGPM	YDDPTLPEGW	TRKLKQRKSG	RSAGKYDVYL	INPQGKAFRS	KVELIAYFEK	VGDTSLDPNDFD	FTVT
AFRICAN_GREEN_MONKEY HUMAN	PKQRRSIIRDRGPM PKQRRSIIRDRGPM							
	170	180	190	200	210	220	230	240
MOUSE RAT	GR JSP SRREQKPPK. GR JSP SRREQKPPK.						PFQASPGGKGEG PFQASPGGKGEG	
CHINESE_HAMSTER	GR SPSRREQKPPK	KPKSPKAPGI	GRGRGRPKGS	GTGRPKAASS	EGVQVKRVLE	KSPGKL <mark>L</mark> VKM	P F Q A S P G <mark>G K</mark> N E G	GGAT
SHEEP COW	GRJSPSRREQKPPK GRJSPSRREQKPPK						PFQAAPGSKAEG PFQAAPGSKAEG	
RED_DEER PIG	GRJSPSRREQKPPK GRJSPSRREQKPPK						P F Q A A P G S K A E G P F Q A S P G S K A E G	GGAT GGAT
HORSE	GR JSP SRREQKPPK	KPKSPKAPG1	GRGRGRPKGS	<b>GTARPKAAA</b> S	EGVQVKRVLE	K S P G K L <mark>L</mark> V K M	P F Q A S P G <mark>S K</mark> A E G	GGAT
CAT DOG	GR 3SP SRREQKPPK GR 3SP SRREQKPPK	KPKSPKAPG KPKSPKAPG	GRGRGRPKGS GRGRGRPKGS	GTARPKAAAS GTARPKAATS	EGVQVKRVLE EGVOVKRVLE	KSPGKL <mark>L</mark> VKM KSPGKLLVKM	P F Q A S P G <mark>S K</mark> A E G P F O A S P G S K A E G	
AFRICAN_GREEN_MONKEY	GR SPSRREQKPPK	KPKSPKAPG'	GRGRGRPKGS	GTTRPKAATS	EGVQVKRVLE	KSPGKL <mark>L</mark> VKM	P F Q T S P G <mark>G K</mark> A E G	GGAT
HUMAN	GRESPERREQKPPK	KPKSPKAPG'	GRGRGRPKGS	GTTRPKAATS	EGVQVKRVLE	KSPGKLUVKM	PFQTSPG <mark>GK</mark> AEG	GGAT
	250	260	270	280	290	300	310	320
MOUSE							KRKTRETVSIEV	
RAT CHINESE HAMSTER	TSAQVMVIKRPGRK TSAQVMVIKRPGRK						KRKTRETVSIEV KRKTRETVSIEV	
SHEEP COW	TS <mark>AQVMVIKRPGRK</mark> TS <mark>AQVMVIKRPGRK</mark>						KRKTRETVSIEV KRKTRETVSIEV	
RED_DEER	TS <mark>A</mark> QVMVIKRPGRK	RKAEADPQAI	PKKRGRKPGS	VVAAA <mark>a</mark> aeak	KKAVKESSIR	SV <mark>Q</mark> ETVLPIK	KRKTRETVSIEV	KEVV
PIG HORSE	TSAQVMVIKRPGRK. TSAQVMVIKRPGRK.						KRKTRETVSIEV KRKTRETVSIEV	
CAT	TS <mark>A</mark> QVMVIKRPGRK	RKAEADPQAI	PKKRGRKPGS	VVAAA <mark>a</mark> aeak	KKAVKESSIR	SV <mark>Q</mark> ETVLPIK	KRKTRETVSIEV	KEVV
DOG AFRICAN_GREEN_MONKEY	TS <mark>AQVMVIKRPGRK</mark> TS <mark>T</mark> QVMVIKRPGRK						KRKTRETVSIEV KRKTRETVSIEV	
HUMAN	TS <mark>T</mark> QVMVIKRPGRK	RKAEADPQAI	PKKRGRKPGS	VVAAAAAAEAK	KKAVKESSIR	SVQETVLPIK	KRKTRETVSIEV	KEVV
	330	340	350	360	370	380	390	
MOUSE	KPLLVSTLGEKSGK	· ·	KSKE <mark>S</mark> PKGR	SSSA <mark>s.</mark> SPP	ккенннннн	SESTKAPMPL	L <mark>PSP<mark>P</mark> PPEP</mark> E	
RAT CHINESE_HAMSTER	KPLLVSTLGEKSGK KPLLVSTLGEKSGK			SSSAS.SPP. SSSS <mark>S</mark> AS <mark>SPP</mark>			LPPPP. PPEPC LPPPPPPPEPE	
SHEEP	KPLLVSTLGEKSGK	GLKTCKSPGI	R <b>KSKE</b> S <mark>S</mark> PKGR	SGSA <mark>S</mark> SPP	ккенннннн	V <mark>E P P K A P</mark> A P L	LLPPPPPPPEPC	SSED
COW RED_DEER	KPLLVSTLGEKSGK KPLLVSTLGEKSGK			SGSAS . SPP SGSA <mark>S . SPP</mark>	ккенннннн	A <mark>E</mark> P P K <mark>A P</mark> A <mark>P L</mark>	LLPPPPPP <mark>PPEP</mark> C LLPP <mark>PPP<mark>PPEP</mark>C</mark>	
PIG HORSE	KPLLVSTLGEKSGK KPLLVSTLGEKSGK			SSSTS SPP	ККЕНННННН	AEPPKAPAPL AEPPRAPAPL	LPPPPPPP <mark>PPEP</mark> C LPPP <b>P</b> PP <b>PPEP</b> C	
CAT	KPLLVSTLGEKSGK	GLKTCKSPGI	<b>KSKE</b> S <mark>S</mark> PKGR	GGSAS. SPP	ККЕНННННН	SEPPKAPAPL	L P P P <mark>P</mark> P P <b>P P E P</b> C	SSED
DOG AFRICAN GREEN MONKEY	KPLLVSTLGEKSGK KPLLVSTLGEKSGK	GLKTCKSPGI	<b>KSKE</b> S <b>S</b> PKGR	SSSAS SPP	ККЕНННННН	SESPKAPVPL	LPPLPPPPPEPE	SSED
HUMAN	KPLLVSTLGEKSGK	GLKTCKSPGE	RKSKE <mark>s</mark> pkgr	SSSA <mark>S</mark> SPP	ккенннннн	SESPKAPVPL	LPPL <mark>P</mark> PP <mark>PPEP</mark> E	SSED
	400 410	420	) 430	440		450	460	
MOUSE	PISPPEPQDLSSSI	CKEEKMPRG	SLESDGCPKE	PAKTOPMVAT	TT	TVAEKYKHRG	EGERKDIVSSSM	IPRPN
RAT CHINESE HAMSTER	PISPPEPÕDLSS <mark>SI</mark> PISPPEPÕDLSSSI	CKEEKMPRAC	SLESDGCPKE	PAKTQPMVAA	AATTTTTT	TVAEKYKHRG	EGERKDIVSSSM	IPRPN
SHEEP	L TOLL PL OD TOOOT						EGERKDIVSSSM	IPRPN
	PASPPEPQDLSSSV							
COW RED DEER	PASPPEPQDLSSSV	C K E E K M P R A (	SLESDGCPKE	PAKTQPALAT	A A	PATEKYKHRG		IPRPN
RED_DEER PIG	PASPPEPQDLSSSV PASPPEPQDLSSSI PASPPEPQDLSSNV	CKEEKMPRAC CKEEKMPRAC CREEKMPRAC	SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTOPALAT PAKTOPTLAT PAKTOPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM	IPRPN
RED_DEER PIG HORSE CAT	PASPPEPQDLSSSV PASPPEPQDLSSSI PASPPEPQDLSSSV PTSPPEPQDLSSV PASPPEPQDLSSGV	CKEEKMPRAC CKEEKMPRAC CREEKMPRAC CKEEKMPRGC CKEEKMPRGC	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTOPALAT PAKTOPTLAT PAKTOPAVAT PAKTOPAVAT PAKTOPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN
RED_DEER PIG Horse	PASPPEPQDLSSSV PASPPEPQDLSSSI PASPPEPQDLSSSV PTSPPEPQDLSSSV PASPPEPQDLSSGV PASPPEPQDLSSGV	CKEEKMPRA CKEEKMPRA CREEKMPRA CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMARG	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTOPALAT PAKTOPTLAT PAKTOPAVAT PAKTOPAVAT PAKTOPAVAT PAKTOPTVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAADKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG	PASPPEPQDLSSSV PASPPEPQDLSSSI PASPPEPQDLSSSV PTSPPEPQDLSSV PASPPEPQDLSSGV	CKEEKMPRAC CKEEKMPRAC CREEKMPRAC CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMARG CKEEKMPRG	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG AFRICAN_GREEN_MONKEY	PASPPEPOLSSSV PASPPEPOLSSSI PASPPEPOLSSSV PTSPPEPOLSSSV PASPPEPOLSSGV PASPPEPOLSSGV PTSPPEPOLSSSV PTSPPEPOLSSSV	CKEEKMPRAC CKEEKMPRAC CREEKMPRAC CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMARG CKEEKMPRG	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER FIG HORSE CAT DOG AFRICAN_GREEN_MONKEY HUMAN	PASPPEPOLSSV   PTSPPEPOLSSV   PTSPPEPOLSSV   PTO	CKEEKMPRA CKEEKMPRA CREEKMPRA CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG AFRICAN_GREEN_MONKEY HUMAN MOUSE RAT	PASPPEPOLSSSV PASPPEPOLSSSI PASPPEPOLSSSV PTSPPEPOLSSSV PASPPEPOLSSGV PTSPPEPOLSSGV PTSPPEPOLSSGV PTSPPEPOLSSSV 470 480 REEPVDSRTPVTER	CKEEKMPRA CKEEKMPRA CREEKMPRA CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG AFRICAN_GREEN_MONKEY HUMAN MOUSE	PASPPEPODLSSSV PASPPEPODLSSSV PASPPEPODLSSNV PASPPEPODLSSVV PASPPEPODLSSGV PTSPPEPODLSSGV PTSPPEPODLSSSV 470 480 REEPVDSRTPVTER	CKEEKMPRA CKEEKMPRA CREEKMPRA CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG VS VS	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG AFRICAN_GREEN_MONKEY HUMAN MOUSE RAT CHINESE_HAMSTER SHEEP COW	PASPPEPOLSSSV PASPPEPOLSSSI PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSGV PASPPEPOLSSGV PTSPPEPOLSSGV PTSPPEPOLSSSV 470 480 REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER	CKEEKMPRA CKEEKMPRA CREEKMPRA CKEEKMPRG CKEEKMPRG CKEEKMARG CKEEKMPRG CKEEKMPRG CKEKMPRG VS VS	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG AFRICAN_GREEN_MONKEY HUMAN MOUSE RAT CHINESE_HAMSTER SHEEP COW RED_DEER FIG	PASPPEPOLSSSV PASPPEPOLSSSI PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSGV PTSPPEPOLSSGV PTSPPEPOLSSGV PTSPPEPOLSSSV 470 480 REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER	CKEEKMPRA CKEEKMPRA CREEKMPRA CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG VS VS VS VS VS VS	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG AFRICAN_GREEN_MONKEY HUMAN MOUSE RAT CHINESE_HAMSTER SHEEP COW RED_DEER	PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSSV PTSPPEPOLSSGV PTSPPEPOLSSGV 470 480 REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER	CKEEKMPRA CKEEKMPRA CREEKMPRA CKEEKMPRG CKEEKMARG CKEEKMARG CKEEKMARG CKEEKMPRG CKEEKMPRG CKEEKMPRG VS VS VS VS VS VS VS VS	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
RED_DEER PIG HORSE CAT DOG AFRICAN_GREEN_MONKEY HUMAN MOUSE RAT CHINESE_HAMSTER SHEEP COW RED_DEER PIG HORSE CAT DOG	PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSSV PASPPEPOLSSGV PASPPEPOLSSGV PTSPPEPOLSSGV PTSPPEPOLSSSV 470 480 REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER REEPVDSRTPVTER	CKEEKMPRA CKEEKMPRA CKEEKMPRG CKEEKMPRG CKEEKMARG CKEEKMARG CKEEKMPRG CKEEKMPRG CKEEKMPRG CKEEKMPRG VS VS VS VS VS VS VS VS VS	SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE SLESDGCPKE	PAKTQPALAT PAKTQPTLAT PAKTQPAVAT PAKTQPAVAT PAKTQPAVAT PAKTQPTVAT PAKTQPAVAT	A A A A A	PATEKYKHRG PATEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG TAAEKYKHRG	EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM EGERKDIVSSSM	IPRPN IPRPN IPRPN IPRPN IPRPN
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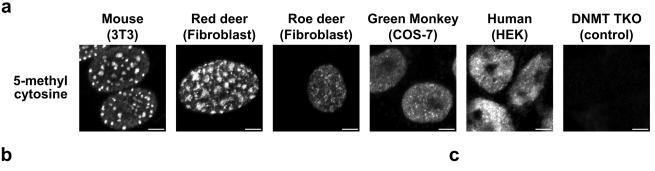
**Supplementary Fig. 8 (related to Fig. 3)** | Protein alignment of MeCP2 across mammalian species. Identical residues are white on a red background; conservative substitutions found in several mammalian species are in red text with white background. Brackets indicate the MBD domain (residues 78-162) which is strictly conserved. The Source data for this Supplementary Figure is associated with the Source data for Figure 3.

#### Lund Human Mesencephalic (LUHMES) cell line





**Supplementary Fig. 9 (related to Fig. 3) | a.** Immunofluorescence of TUJ1 in human postmitotic neurons (LUHMES) expressing endogenously tagged MeCP2-mCherry. DAPI staining was used to visualise DNA. Scale bar: 50µm. Diagram adapted from Shah et al, Wellcome Open Res, 2016 under the terms of a Creative Commons Attribution License (<u>https://creativecommons.org/licenses/by/4.0</u>). **b.** Immunofluorescence of endogenous MeCP2 in mouse and rat brain sections (hippocampus). DAPI staining was used to visualise DNA. Scale bars: 5µm. The Source data for this Supplementary Figure is associated with the Source data for Figure 3.



5' CAAGACGAAAGGATGTCTGAATCCCCTGTGGAGACCACAGAGAAAGACCTAGTTCCCCACCTCATC 3' GTTCTGCTTTCCTACAGACTTAGGGGACACCTCTGGTGTCTCTTTCTGGATCAAGGGGTGGAGTAG <u>Hpall/Mspl</u>

GCGACCGGAGGCCTCACATCCTTTGAAAACTCCAGAGGTACGCGGAGATCAGTGCCTCCAAAGGAG CGCTGGCCTCCGGAGTGTAGGAAACTTTTGAGGTCTCCATGCGCCTCTAGTCACGGAGGTTTCCTC

CCTTGGTCTCCCGCCTCAGCTGGAGAGGCGTCCCAATTGCCCTGCCAAGCCTCGAGGAGAATCCCG GGAACCAGAGGGCGGAGTCGACCTCTCCCGCAGGGTTAACGGGACGGTTCGGAGCTCCTCTTAGGGC

AGTTGTCCCTCGCAACTAGGCAGGAGTCCTGACGTCGCTGAAGAAACACGTGTGGGAAGGGCCCAT TCAACAGGGAGCGTTGATCCGTCCTCAGGACTGCAGCGACTTCTTTGTGCACACACCTTCCCGGTA

CTAGAGGGATCCTGAGGTCACTGTAGCAACACGAAAGAGCTCCGTGGACCAAAAATCAACTCGAGA GATCTCCCTAGGACTCCAGTGACATCGTTGTGCTTTCTCGAGGCACCTGGTTTTTAGTTGAGCTCT

#### d

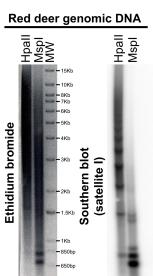
#### HpyCH4IV

5' GGACCTGGAATATGGCGAGAAAACTGAAAAATCACGGAAAATGAGAAATACACACTTTAGG<mark>ACGT</mark>

3' CCTGGACCTTATACCGCTCTTTTGACTTTTAGTGCCTTTTACTCTTTATGTGTGAAATCCTGCA Apol GAAATATGGCGAGGAAAACTGAAAAAGGTGGAAAATTTAGAAATGTCCACTGTAGGACGTGGAA CTTTATACCGCTCCTTTTGACTTTTTCCACCTTTTAAATCTTTACAGGTGACATCCTGCACCTT

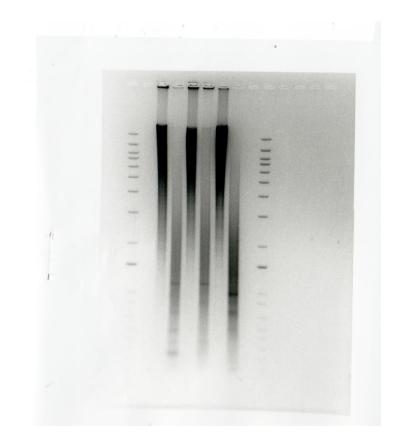
 $\begin{array}{l} {\tt TATGGCAAGAAAACTGAAAATCATGGAAAATGAGAAACATCCACTTGACGACTTGAAAAATGAC\\ {\tt ATACCGTTCTTTTGACTTTTAGTACCTTTTACTCTTTGTAGGTGAACTGCTGAACTTTTTACTG\\ {\tt HpyCH4IV}\\ {\tt GAAATCACTAAAAAACGTGAAAATGAGAAATGCACACTGAA} \begin{array}{l} 3'\\ {\tt CTTTAGTGATTTTTTGCA}{\tt CTTTTACTCTTTACGTGTGACTT} \begin{array}{l} 5' \end{array}$ 

**Supplementary Fig. 10 (related to Fig. 4) | a.** Immunofluorescence of 5-methylcytosine in the indicated mammalian cell lines. Scale bars: 5µm. **b.** Consensus sequence of red deer satellite I. The restriction site for Hpall/Mspl enzymes used for Southern blot is highlighted in orange. **c.** Ethidium bromide staining (left) and Southern blot (right) using a probe for satellite I DNA repeats with red deer genomic DNA digested with a methylation-sensitive (HpalI) or -insensitive (Mspl) restriction enzyme. Discrete bands at the bottom of the gel with Mspl, but not HpalI, digested DNA indicates abundant and highly methylated DNA repeats in red deer cells. MW: Molecular weight marker. **d.** Consensus sequence of mouse major satellite DNA. The restriction sites for HpyCH4IV and Apol enzymes used for Southern blot are highlighted in red and blue, respectively. The Source data for this Supplementary Figure is associated with the Source data for Figure 4.

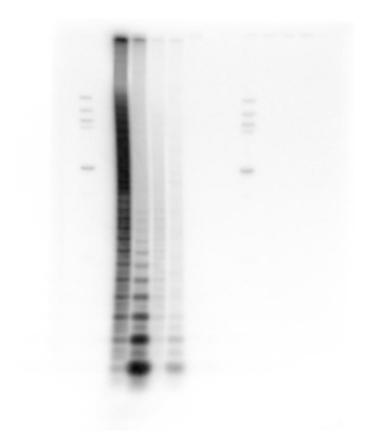


## Figure 4C

## Left panel (Gel, ethidium bromide staining)

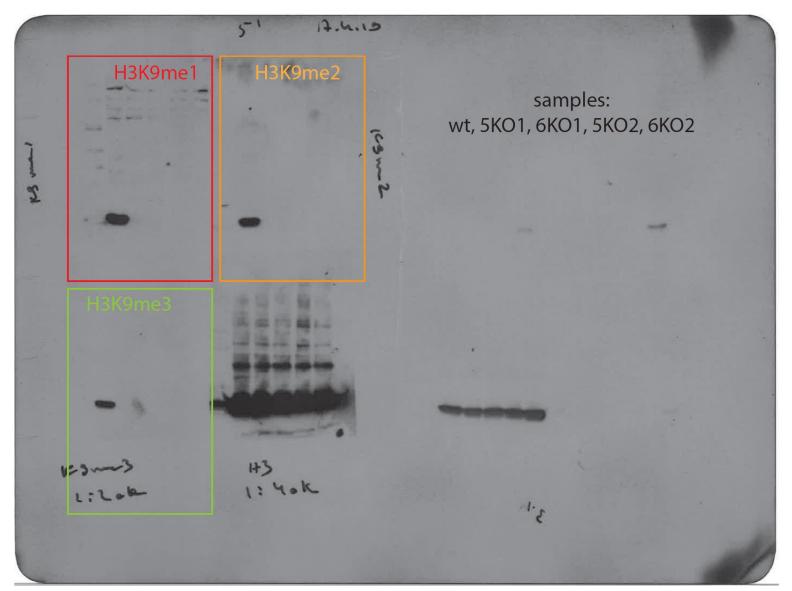


# Right panel (Southern blot, satellite DNA probe)



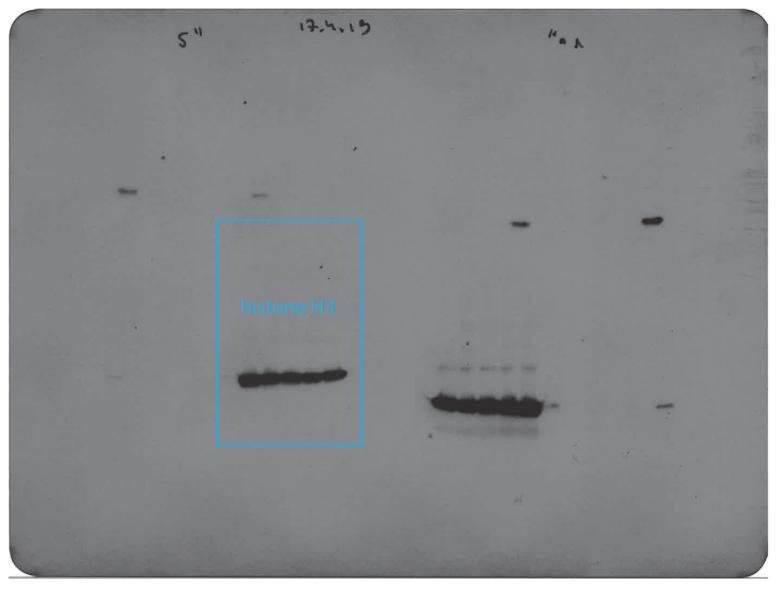
# Supplementary Figure 3C (related to Figure 2)

Western blot



# Supplementary Figure 3C (related to Figure 2)

### Western blot



# Supplementary Figure 10C (related to Figure 4)

Left panel (Gel, ethidium bromide staining)



Right panel (Southern blot, satellite DNA probe)

