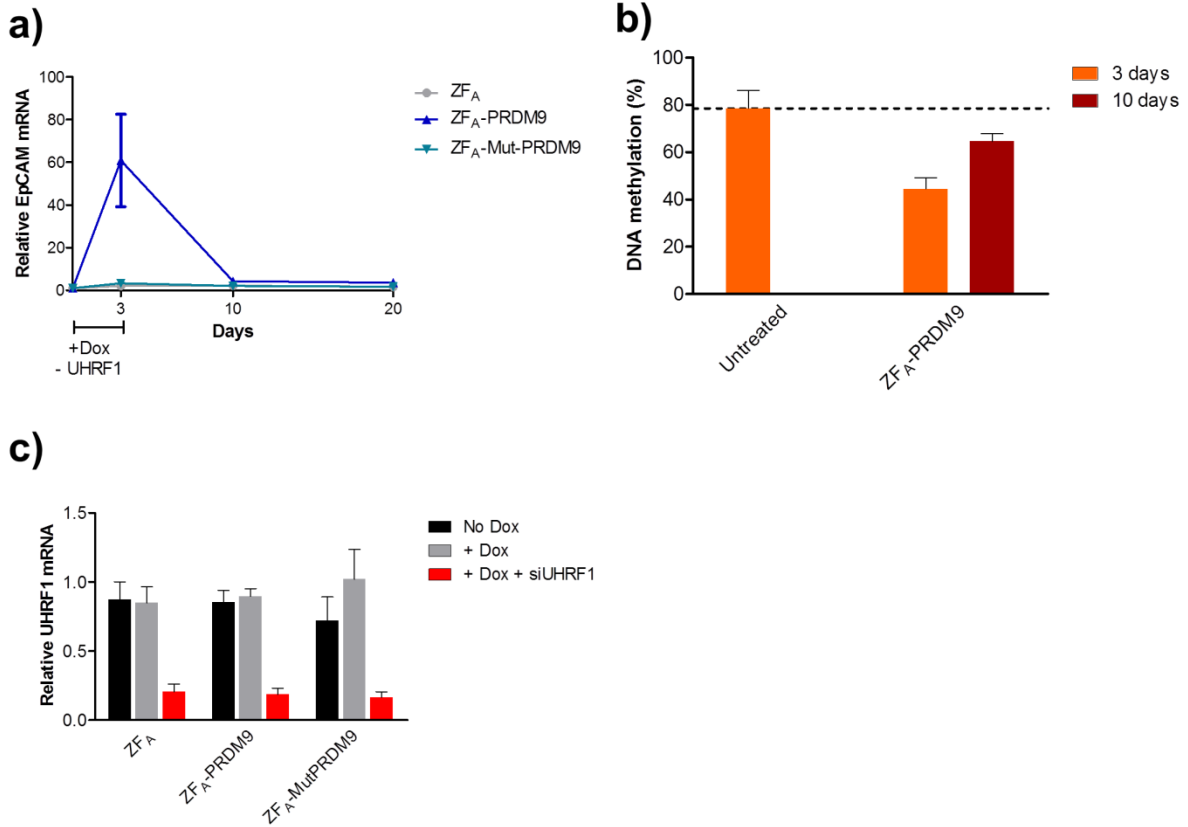**Supplementary Figure 1** Epigenetic landscape of target genes in the studied cell lines (a) ENCODE data depicting the promoter and first exon of the three genes in question. Dark green bars represent the CpG island; light green (unmehtylated), yellow (partially methylated or red (hypermethylated) bars represent DNA methylation profiles; black represent the H3K4me3 peaks of HEK293 and A549; in orange the region targeted with gRNAs (b) DNA methylation levels determined by pyrosequencing of the promoter area. Bars represent the mean methylation of 7-10 CpGs (c) FLAG ChIP-qPCR enrichment at the promoter region of 4 genes compared to levels of DNA methylation in three different cell lines (d) DNA methylation levels determined by pyrosequencing of the promoter area of A2780 (all genes) and HeLA (EpCAM). Bars represent the mean methylation of 7-10 CpGs (e) Protein expression of ZF-fusions in A2780 cells. (n = 3 independent experiments; error bars ± s.d.)

Supplementary Figure 2 Downregulation of key regulator of epigenetic memory through maintenance of DNA methylation after replication (a) Relative *EpCAM* mRNA expression, at each specific time point after Dox and downregulation of UHRF1 for 3 days (b) DNA methylation levels determined by pyrosequencing of the promoter area. Bars represent the mean methylation of 7-10 CpGs (c) Relative *UHRF1* expression after siRNA treatment in all the stable cell lines and treatments. ($n = 3$ independent experiments; error bars ± s.d.)

a)
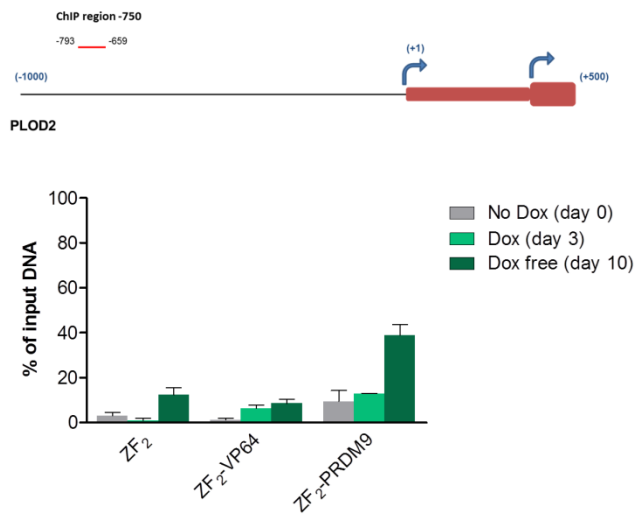
Supplementary Figure 3 Spreading of H3K4me3 750 bps upstream of targeting region (a) H3K4me3 ChIP-qPCR enrichment at the region 750 bps upstream of the PLOD2 promoter. (n = 3 independent experiments; error bars ± s.d.)
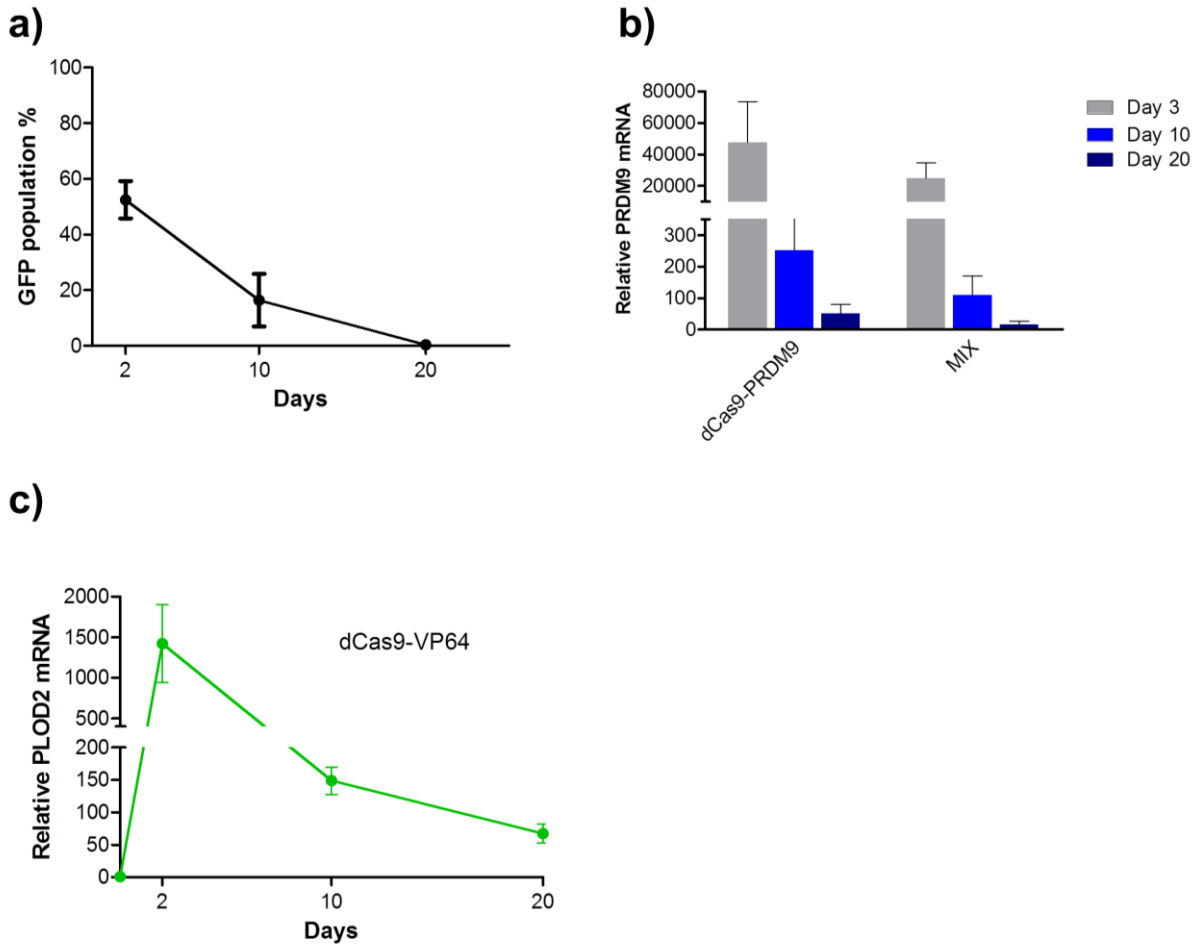
Supplementary Figure 4 (a) Transfection efficiency and transition after subculturing for C33a cells measured as GFP using a CMV-GFP plasmid. (b) Relative expression of PRMD9 in Hela cells treated with 5aza and transfected with the dCas9-effector domains shows the loss of PRDM9 expression (c) Relative PLOD2 mRNA, after co-transfection in C33a cells of dCas9 –VP64 and a combination of gRNAs. (*n* = 3 independent experiments; error bars ± s.d.)

Supplementary Table 1. Primers for effector domain amplification.

Effector domain primers		
Target	Forward Primer (5'-3')	Reverse Primer (5'-3')
PRDM9	CTGACGCGTATGGCCTTAAGAGTGGAACA	GGTTAATTAAAGAGGAGTGATTGCGTTCTA
DOT1L	TTAACGCGTGGGGAGAAGCTGGAGCTG	CGTTAATTAATTGGTTCTTCTTGGGCTT
UBE2A	TTAACGCGTATGTCCACCCCGGCTCGG	CGTTAATTAAACAATCACGCCAGCTTTG
MutPRDM9	CTTTGGCCCTTATGAGGCCCGAATTACAGAAGACG	CGTCTTCTGTAATTCGGGCCTCATAAGGGCCAAAAG
MutDOT1L	CACCTCAGGACCAAAGGCAAAATCAGCCACAAATATA	CCAACACGAGTGTTATATTTGTGGCTGATTTTGCCTTTGGTCCTGAGGTG

Supplementary Table 2. gRNA information (in bold the regions shared between gRNA and zinc finger proteins).

Sp-dCas9		
Target Location	Protospacer Sequence (5'- 3')	Genomic Location (GRCh38/hg19 Assembly)
EPCAM A+	CAGC**GCCGGGGCTGGGGGAG**	Chr2: 47,369,152-47,369,172
EPCAM A-	**CCAGCCCCGGC**GCTGCAGTT	Chr2: 47,369,147-47,369,167
EPCAM B+	CT**ACTCACTCCCCCAACTCC**	Chr2: 47,369,179-47,369,198
EPCAM B-	CG**GGAGTTGGGGGAGTGAGT**	Chr2: 47,369,181-47,369,200
ICAM C+	**CGGAGCTGAAGCGGCC**AGCG	Chr19: 10,270,941-10,270,961
ICAM C-	CTCGCT**GGCCGCTTCAGCTC**	Chr19: 10,270,944-10,270,963
RASSF1a X+	TGTGA**GGAGGGGACGAAGGA**	Chr3: 50,341,033-50,341,052
RASSF1a X-	**TCCT**CACACCCCACCCCGGA	Chr3: 50,341,045-50,341,064
RASSF1a Z+	TGGGGGA**GGCGCTGAAGTCG**	Chr3: 50,340,881-50,340,900
RASSF1a Z-	**CCGACTTCAGCGCC**TCCCCC	Chr3: 50,340,880-50,340,899
RASSF1a Y+	AAGGAAGGGCAAGGCGGGGG	Chr3: 50,341,010-50,341,029
PLOD2 g2	**GCTGTGGAAGCT**ACCGGGGC	Chr3: 146,161,525-146,161,544
PLOD2 g1	CCACTCCCAAAGCTAAGTGC	Chr3: 146,161,426-146,161,445
PLOD2 g3	GAGCCTCCACACGTAGCCGC	Chr3: 146,161,374-146,161,393
PLOD2 g3	TGAGCAAACAGTCCAGACGT	Chr3: 146,161,327-146,161,346

Supplementary Table 3. Zinc finger information.

ZFP		
Target Location	Target site (5'- 3')	Genomic Location (GRCh38/hg19 Assembly)
EPCAM A	**GCCGGGGCTGGGGGAG**GG	Chr2: 47,369,157-47,369,174
EPCAM B	**ACTCACTCCCCCAACTCC**	Chr2: 47,369,181-47,369,198
ICAM CD54	TC**CGGAGCTGAAGCGGCC**	Chr19: 10,270,940-10,270,957
RASSF1a X	**GGAGGGGACGAAGGA**GGG	Chr3: 50,341,030-50,341,047
RASSF1a Z	**GGCGCTGAAGTCG**GGGCC	Chr3: 50,340,876-50,340,893
PLOD2 ZF2	GAGGCT**GCTGTGGAAGCT**	Chr3: 146,161,533-146,161,550
PLOD2 ZF8	GGGGCCGTGGGCGCCTGA	Chr3: 146,161,016-146,161,033

Supplementary Table 4. Quantitative reverse transcription PCR

qRT-PCR primers			
Target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe
GAPDH	CCACATCGCTCAGACACCAT	GCGCCCAATACGACCAAAT	CGTTGACTCCGACCTTCACCTTCCC
RASSF1a	AAGTTCACCTGCCACTACCG	AAGGTCAGGTGTCTCCCACT	
PLOD2	GGGAGTTCATTGCACCAGTT	GAGGACGAAGAGAACGC	

Supplementary Table 5. ChIP-qPCR primers

ChIP primers			
Target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Probe
EPCAM ChIP	CCCAACTCCCGGGCGGTGACT	TGTCCTCCCGACGCGGACCC	
RASSF1a ChIP	TGGGGTGTGAGGAGGGGACGA	AGAGCCGCGCAATGGA	
PLOD2 ChIP region 2	GAGGCTGCTGTGGAAGCTA	GGTTTGGGAGAGGGAGGAG	AGACGGGAACACCGCCCTCC
PLOD2 ChIP region 8	CACCGACGACCTCACTCA	TCGCGAGAACGCAGAGAC	CTGCGTTACGCGCCGCTC
PLOD2 ChIP region -750	ACAAAACGTGATCATAATGGAA	ATTTAGGCAGGAGATTCCAGAA	

Supplementary Table 6. Pyrosequencing primers

Pyrosequencing primers			
Target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Pyrosequencing
ICAM1	GGGGAAGTTGGTAGTATTTAAAAGT	[Btn] CCTTCCCCTCCCAAACAAATACTACAATTA	GTTAGATTGTTTTAGT
EPCAM	TGGGGGAGGGGAGTTTATT	[Btn] ACCCAACTCCACAACTCT	AGGGGAGTTTATTTATTTTTTTA
RASSF1a	AAGGAGGGAAGGAAGGGTAAG	gggacaccgctgatcgtttaCCCCCAACTCAATAAACTCAAACTCCCC	GAAGGAAGGGTAAGG
PLOD2	AAGTAGTGGGGGTTAAGG	[Btn] CCAAAAAAACCTTAAACTCTTTCCAATAAT	GGGTTAAGGTTTAGAGA
Universal	[Btn] gggacaccgctgatcgttta		

Supplementary Note 1. CCDS Sequences of amplified epigenetic enzymes (in-between green is amplified region, in red the nucleotides changed for site directed mutagenesis)

PRDM9

ATGAGCCCTGAAAAGTCCCAAGAGGAGAGCCCAGAAGAAGACACAGAGAGAACAGAGCGGAAGCCCATGGT
CAAAGATGCCTTCAAAGACATTTCCATATACTTCACCAAGGAAGAATGGGCAGAGATGGGAGACTGGGAGAA
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CCTAGGCAGCAAGTCAAACCTCCTTGGATGGCCTTAAGAGTGGAACAGCGTAAACACCAGAAGGGAATGCC
CAAGGCGTCATTCAGTAATGAATCTAGTTTGAAAGAATTGTCAAGAACAGCAAATTTACTGAATGCAAGTGGC
TCAGAGCAGGCTCAGAAACCAGTGTCCCCTTCTGGAGAAGCAAGTACCTCTGGACAGCACTCAAGACTAAAA
CTGGAACTCAGGAAGAAGGAGACTGAAAGAAAGATGTATAGCCTGCGAGAAAGAAAGGGTCATGCATACAAA
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CAGTCTGCCCCCAGGGCTGAGAATTGGGCCATCAGGCATCCCTCAGGCTGGGCTTGGAGTATGGAATGAGG
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DOT1L

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UBE2A

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