PNAS

Supporting Information for

Decorating chromatin for enhanced genome editing using CRISPR-Cas9

Evelyn Chen[a,b,1], Enrique Lin-Shiao[a,b,1,2], Marena Trinidad[a,b], Mohammad Saffari Doost[a,b], David Colognori[a,b], and Jennifer A. Doudna[a-h,3]

[a]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA USA 94720
[b]Innovative Genomics Institute, University of California, Berkeley, Berkeley, CA USA 94720
[c]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA USA 94720
[d]Department of Chemistry, University of California, Berkeley, Berkeley, CA USA 94720
[e]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA USA 94720
[f]California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA USA 94720
[g]Gladstone Institutes, University of California, San Francisco, CA USA 94158
[h]Gladstone-University of California, San Francisco Institute of Genomic Immunology, San Francisco, CA USA 94158

[1]E.C. and E.L.-S. contributed equally to this work.
[2]Present address: Cystic Fibrosis Foundation, Bethesda MD USA 20814.
[3]Correspondence to: doudna@berkeley.edu.

This PDF file includes:

- Figures S1 to S4
- Tables S1 to S4
- SI References

A)

	PTM	Biological Process	Presumed Role in DNA Repair
PRDM9	H3K4me3 H3K36me3	Localization of meiotic recombination hotspots	H3K36me3 and H3K4me3 recruit ZCWPW1 to facilitate repair of SPO11-induced DSBs via HR
SETD2	H3K36me3	DNA repair by HR, mismatch repair, V(D)J recombination, transcription fidelity	H3K36me3 recruits CtIP to promote DSB resection and RPA and RAD51 foci formation
SETMAR	H3K4me3 H3K36me2	DNA repair by NHEJ, supression of chromosomal translocations	H3K36me2 stabilizes NSB1 and Ku70 at DSB sites to promote NHEJ repair.

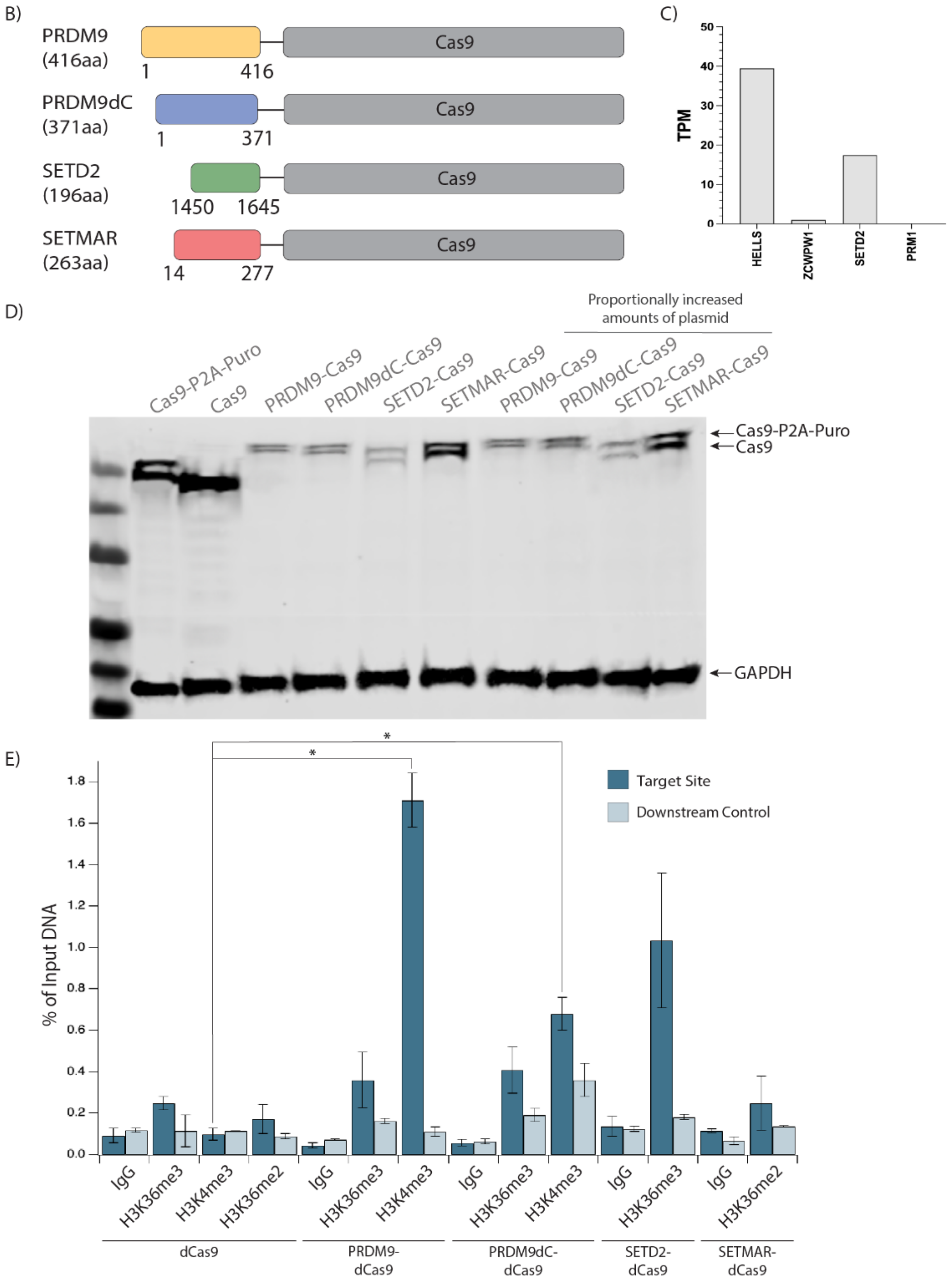


Fig. S1. Cas9-methyltransferase fusion protein design to modulate the choice of DNA repair pathway

A) Summary of histone methyltransferases used for Cas9 fusion protein engineering, histone posttranslational modifications (PTM) deposited, biological processes, and their presumed role in DNA repair.

B) Schematic of four Cas9-methyltransferase fusion proteins designed to decorate chromatin to modulate the choice of DNA repair pathway following Cas9-induced DSBs. The histone methyltransferases are fused to Cas9 at the N-terminus, and the amino acid positions of the fused proteins selected for cloning are indicated.

C) Expression levels of relevant genes (HELLS, ZCWPW1) based on RNA-seq experiments in HEK293T cells. Positive control (SETD2) and negative control (PRM1) genes were included.

D) Western blot analysis of fusion protein expression in HEK293T cells. GAPDH antibody was used as a loading control. Double bands were due to incomplete cleavage at the self-cleaving P2A peptide, leading to an uncleaved byproduct (1).

E) H3K36me3, H3K4me3, and H3K36me2 enrichment shown as a percentage of input DNA measured by ChIP-qPCR at site C9 in HEK293T cells at 3 dpt. Cells were transfected with plasmids expressing the dCas9 fusion proteins and sgRNA. Data represents mean ± s.d. (n = 2). $^*P < 0.05$, determined by student's two-tailed t-test.

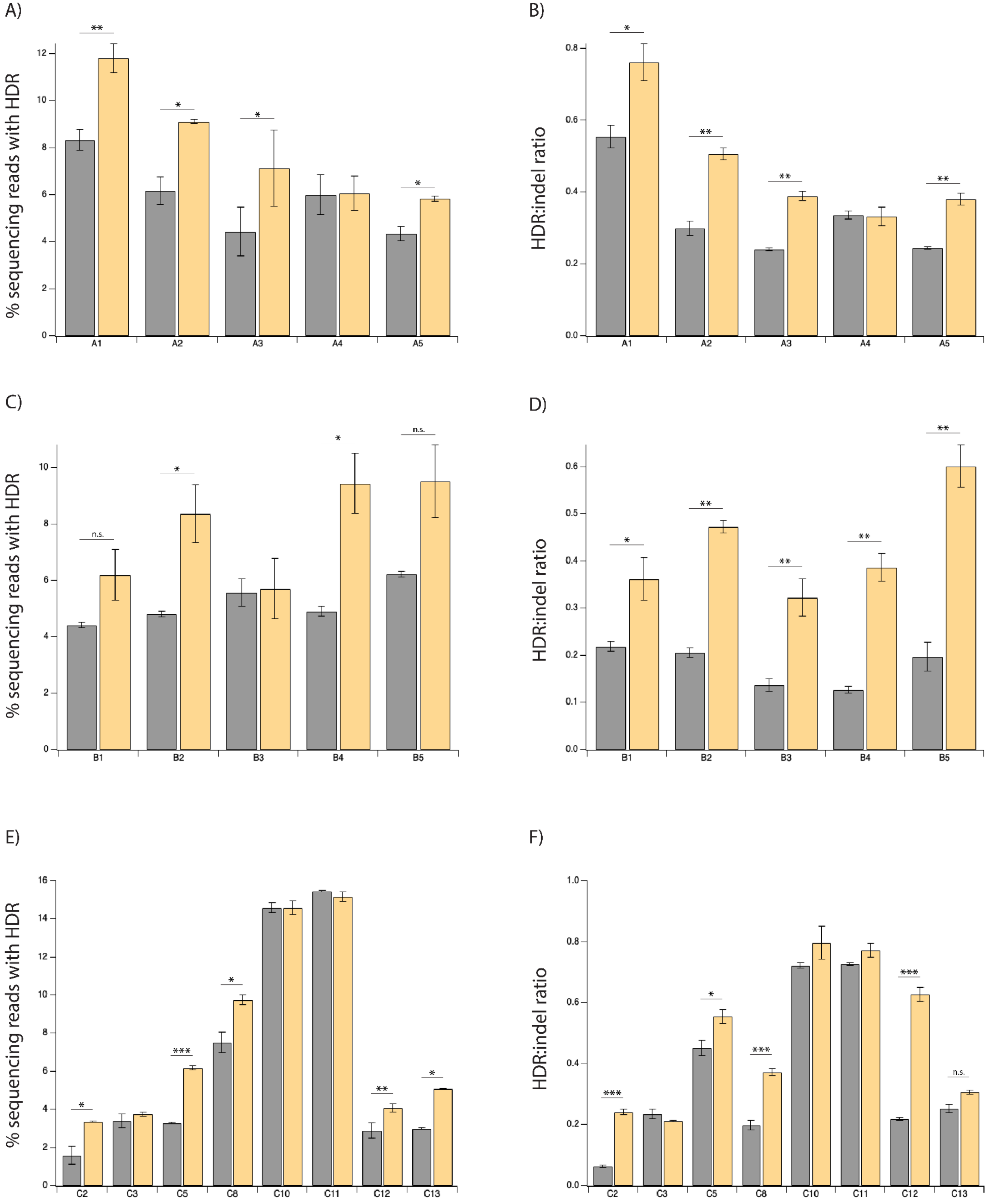

A)
% sequencing reads with HDR
0
2
4
6
8
10
12
A1
A2
A3
A4
A5
**
*
*
*
B)
HDR:indel ratio
0.0
0.2
0.4
0.6
0.8
A1
A2
A3
A4
A5
*
**
**
**
C)
% sequencing reads with HDR
0
2
4
6
8
10
B1
B2
B3
B4
B5
n.s.
*
*
n.s.
D)
HDR:indel ratio
0.0
0.1
0.2
0.3
0.4
0.5
0.6
B1
B2
B3
B4
B5
*
**
**
**
**
E)
% sequencing reads with HDR
0
2
4
6
8
10
12
14
16
C2
C3
C5
C8
C10
C11
C12
C13
*
***
*
**
*
F)
HDR:indel ratio
0.0
0.2
0.4
0.6
0.8
1.0
C2
C3
C5
C8
C10
C11
C12
C13
***
*
***
***
n.s.

Fig. S2. HDR efficiency and HDR:indel ratio improvement by PRDM9-Cas9 at multiple genomic loci

A) HDR frequency and **B)** HDR:indel ratio measured by NGS at five genomic loci enriched with H3K36me3 in HEK293T cells transfected with PRDM9-Cas9 and sgRNA with ssODN template.

C) HDR frequency and **D)** HDR:indel ratio measured by NGS at five genomic loci enriched with H3K36me3 and H3K4me3 in HEK293T cells transfected with PRDM9-Cas9 and sgRNA with ssODN template.

E) HDR frequency and **F)** HDR:indel ratio measured by NGS at eight genomic loci lacking H3K36me3 and H3K4me3 in HEK293T cells transfected with PRDM9-Cas9 and sgRNA with ssODN template.

For A-F, data represents mean ± s.d. ($n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, determined by student's two-tailed t-test.

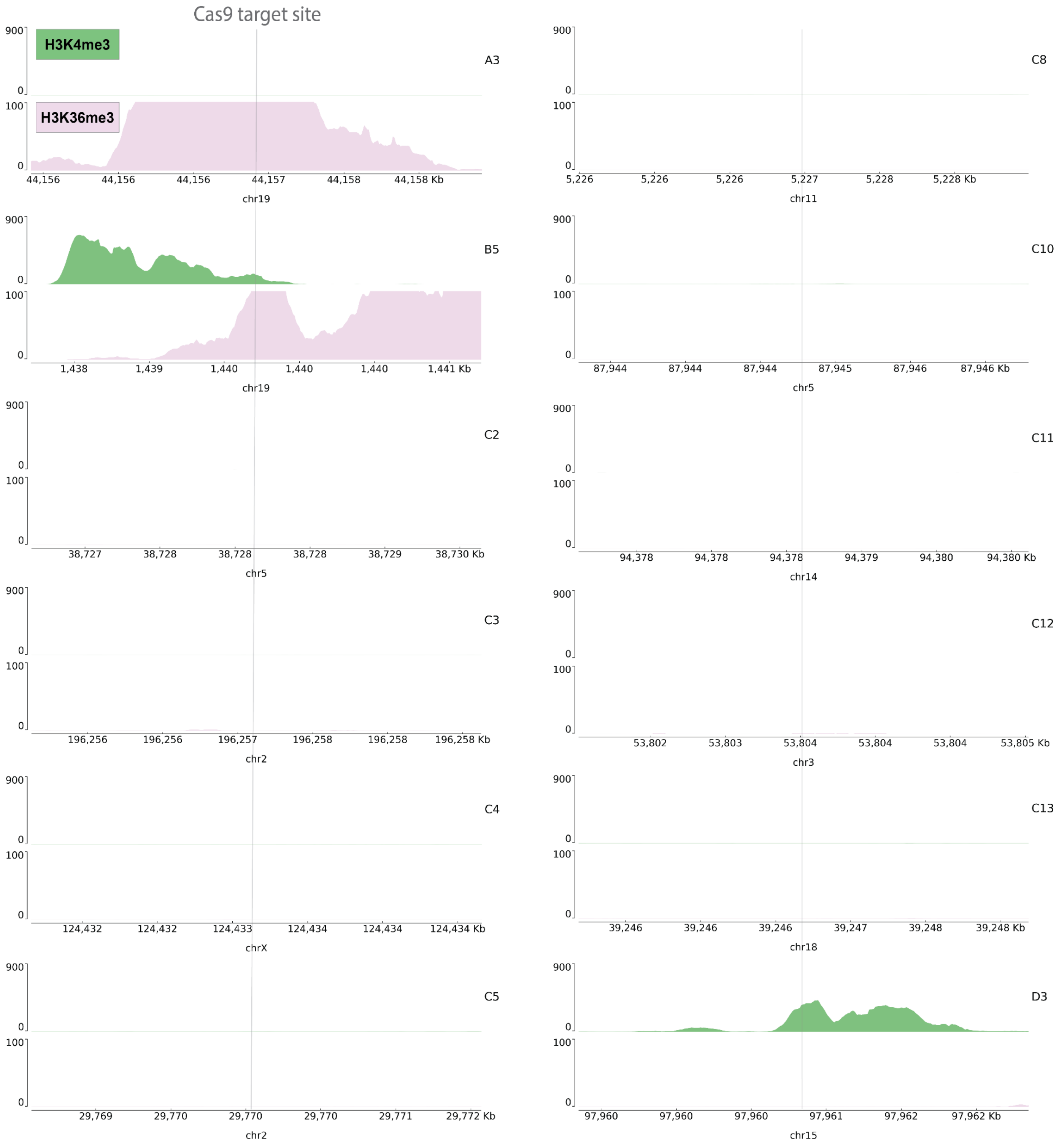
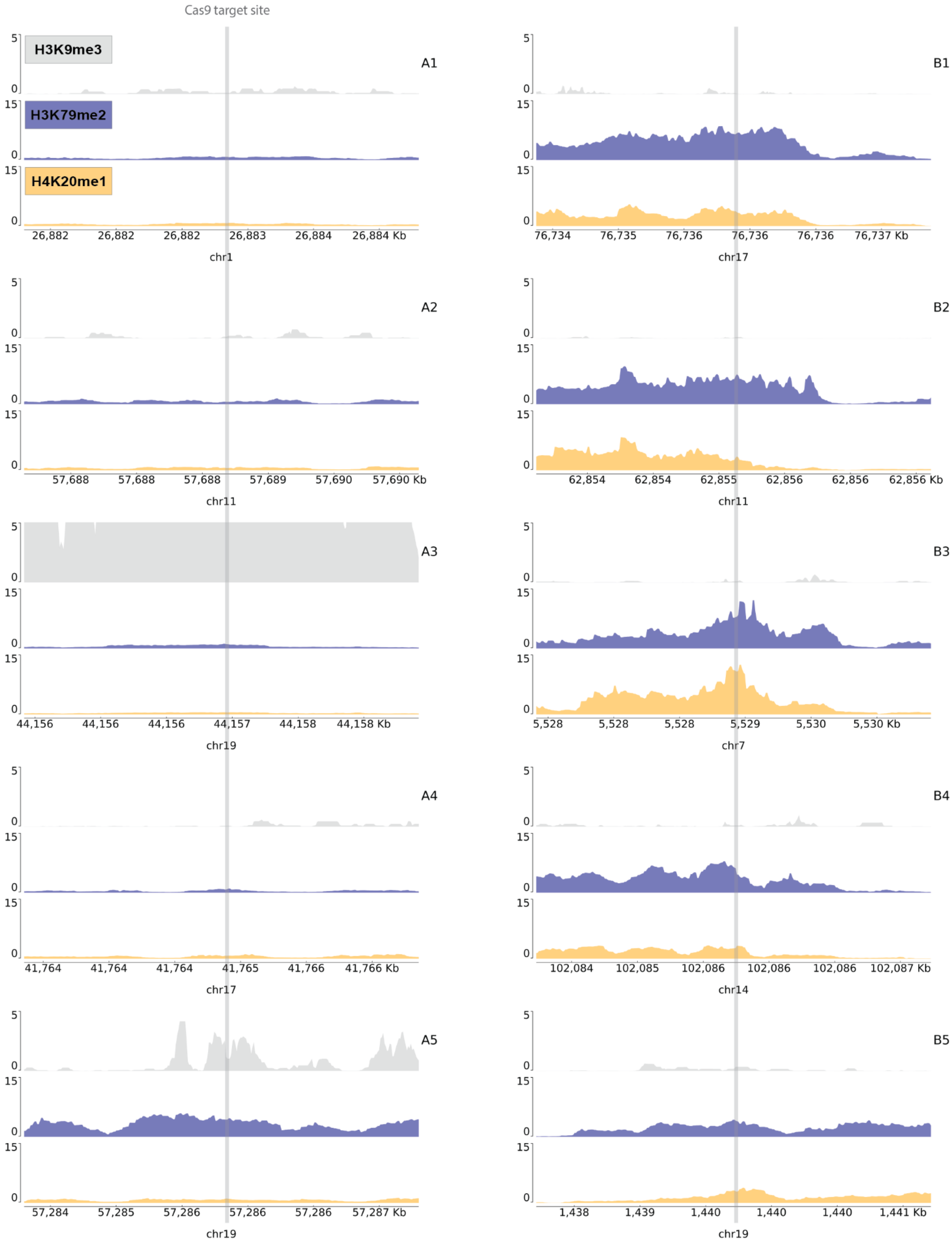


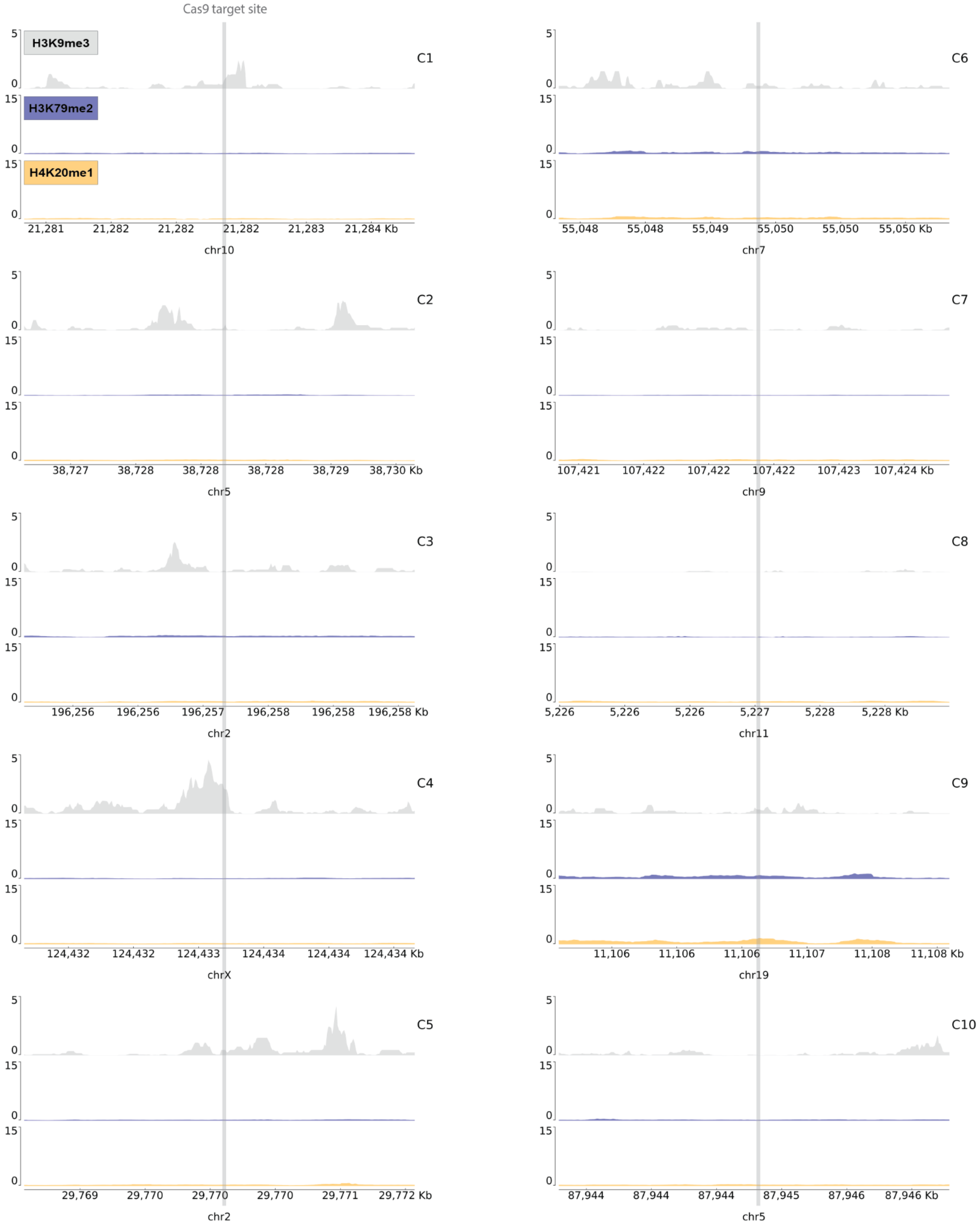
Fig. S3. UCSC genome browser tracks showing endogenous levels of H3K4me3 and H3K36me3 enrichment at additional genomic sites

Tracks represent target site ± 1.5 kilobases (kb) in HEK293 cells based on ENCODE ChIP-seq datasets for H3K4me3 (green) and H3K36me3 (pink). Each plot spans 3 kb and the y-axis reports the negative-log p-value for peak enrichment.

A)


Cas9 target site
H3K9me3
H3K79me2
H4K20me1
5
0
15
0
15
0
A1
26,882
26,882
26,882
26,883
26,884
26,884 Kb
chr1
B1
76,734
76,735
76,736
76,736
76,736
76,737 Kb
chr17
A2
57,688
57,688
57,688
57,689
57,690
57,690 Kb
chr11
B2
62,854
62,854
62,855
62,856
62,856
62,856 Kb
chr11
A3
44,156
44,156
44,156
44,157
44,158
44,158 Kb
chr19
B3
5,528
5,528
5,528
5,529
5,530
5,530 Kb
chr7
A4
41,764
41,764
41,764
41,765
41,766
41,766 Kb
chr17
B4
102,084
102,085
102,086
102,086
102,086
102,087 Kb
chr14
A5
57,284
57,285
57,286
57,286
57,286
57,287 Kb
chr19
B5
1,438
1,439
1,440
1,440
1,440
1,441 Kb
chr19

B)


Cas9 target site
H3K9me3
H3K79me2
H4K20me1
5
0
15
0
15
0
C1
21,281 21,282 21,282 21,282 21,283 21,284 Kb
chr10
C2
38,727 38,728 38,728 38,728 38,729 38,730 Kb
chr5
C3
196,256 196,256 196,257 196,258 196,258 196,258 Kb
chr2
C4
124,432 124,432 124,433 124,434 124,434 124,434 Kb
chrX
C5
29,769 29,770 29,770 29,770 29,771 29,772 Kb
chr2
C6
55,048 55,048 55,049 55,050 55,050 55,050 Kb
chr7
C7
107,421 107,422 107,422 107,422 107,423 107,424 Kb
chr9
C8
5,226 5,226 5,226 5,227 5,228 5,228 Kb
chr11
C9
11,106 11,106 11,106 11,107 11,108 11,108 Kb
chr19
C10
87,944 87,944 87,944 87,945 87,946 87,946 Kb
chr5

C)

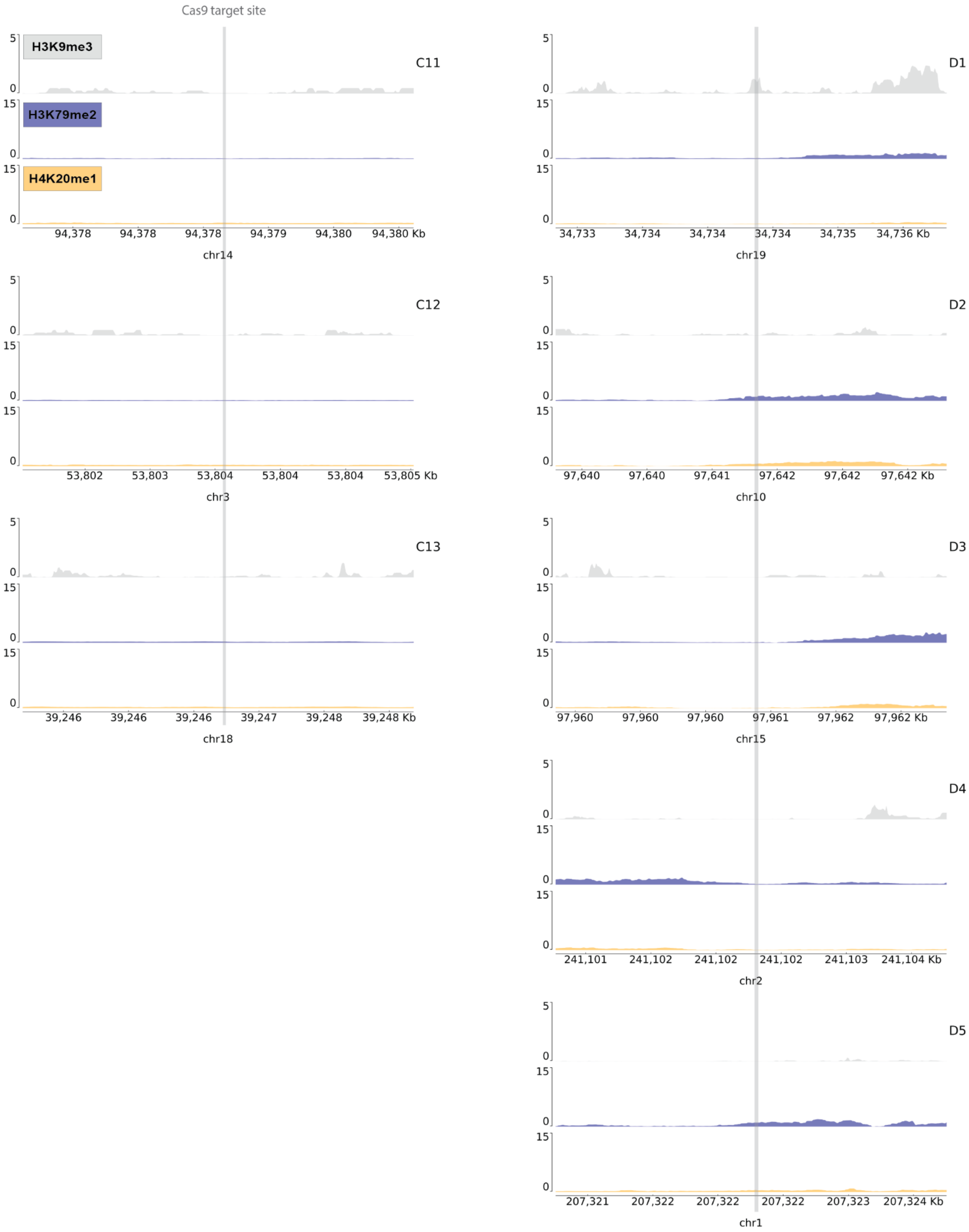

Cas9 target site
H3K9me3
H3K79me2
H4K20me1
5
0
15
0
15
0
C11
94,378 94,378 94,378 94,379 94,380 94,380 Kb
chr14
C12
53,802 53,803 53,804 53,804 53,804 53,805 Kb
chr3
C13
39,246 39,246 39,246 39,247 39,248 39,248 Kb
chr18
D1
34,733 34,734 34,734 34,734 34,735 34,736 Kb
chr19
D2
97,640 97,640 97,641 97,642 97,642 97,642 Kb
chr10
D3
97,960 97,960 97,960 97,961 97,962 97,962 Kb
chr15
D4
241,101 241,102 241,102 241,102 241,103 241,104 Kb
chr2
D5
207,321 207,322 207,322 207,322 207,323 207,324 Kb
chr1

Fig. S4. UCSC genome browser tracks showing normalized endogenous levels of H3K9me3, H3K79me2, and H4K20me1 at genomic sites
Tracks represent target site ± 1.5 kilobases (kb) in HEK293 cells based on ENCODE ChIP-seq datasets for H3K9me3 (gray), H3K79me2 (blue), and H4K20me1 (yellow) at **A)** sites A1-A5 and B1-B5, **B)** sites C1-C10, and **C)** sites C11-C13 and D1-D5. Each plot spans 3 kb and the y-axis reports the negative-log p-value for peak enrichment.

Table S1. Single guide RNA (sgRNA) sequences and HDR products

Site	Locus	sgRNA and PAM sequence	Change encoded by ssODN template
A1	GPN2	GGACAGCTCTGGATGTCCCAtgg	GGACAGCTCTGGATGTGCCAtgg
A2	ZDHHC5	GTTGTCACAGACACTGCAGTggg	GTTGTCACAGACACTGGAGTggg
A3	ZNF234	TATTAAGTGCTGATCTACGAcgg	TATTAAGTGCTGATCTTCGAcgg
A4	JUP	TCAGTCAGCCATGCTCCCCGtgg	TCAGTCAGCCATGCTCGCCGtgg
A5	ZNF460	GTGGAAGAACTCAATCCTAAggg	GTGGAAGAACTCAATCGTAAggg
B1	SRSF2	GGAGACCGCAGCTTTAAAGGggg	GGAGACCGCAGCTTTATAGGggg
B2	SNHG1	CATGGTTTATGCTCTTACAGagg	CATGGTTTATGCTCTTTCAGagg
B3	ACTB	GCTCGTGTGACAAGGCCATGagg	GCTCGTGTGACAAGGCGATGagg
B4	HSP90AA1	TTCACTGGTTAAGTGAGCGGtgg	TTCACTGGTTAAGTGACCGGtgg
B5	RPS15	AAAGGCCTCTAAAAACGCAGggg	AAAGGCCTCTAAAAACCCAGggg
C1	NEBL	ACAATGCAAGAGGCTCCGTAggg	ACAATGCAAGAGGCTCGGTAggg
C2	OSMR-DT	ACTGAATTCCTTGAAGACCGggg	ACTGAATTCCTTGAAGTCCGggg
C3	HECW2	GGATGAGCGTCCCAACCACTggg	GGATGAGCGTCCCAACGACTggg
C4	TENM1	CTTGGTTGAATCTAATCACGggg	CTTGGTTGAATCTAATGACGggg
C5	ALK	GTCTCTTGCTGGATATGGGAagg	GTCTCTTGCTGGATATCGGAagg
C6	EGFR	GTAATGAAATGAGTTGGGGCagg	GTAATGAAATGAGTTGCGGCagg
C7		GGCCCAGACTGAGCACGTGAtgg	GGCCCAGACTGAGCAAGTGAtgg
			GGCCCAGACTGAGCAAGTGATTT (PAM mutation)
C8	HBB	GTAACGGCAGACTTCTCCTCagg	GTAACGGCAGACTTCTCCACagg
C9	LDLR	CAGAGCACTGGAATTCGTCAggg	CAGAGCACTGGAATTAGTCAggg
			CAGAGCACTGGAATTAGTCAGTG (PAM mutation)
C10		GAACACAAAGCATAGACTGCggg	GAACACAAAGCAGAGACTGCggg
			GAACACAAAGCAGAGACTGCGAC (PAM mutation)
C11	SERPINA1	TGCTGACCATCGACGAGAAAggg	TGCTGACCATCGACAAGAAAggg
C12	CACNA1D	GGAGCAGGAGTATTTCAGTAGTGagg	GGAGCAGGAGTATTTCAGTTGTGagg
C13		AAGATGCAAGGTTTGTGTCTagg	AAGATGCAAGGTTTGTCTCTagg
D1	ZNF181	TTGCCCGCTCACCAGCGACGcgg	TTGCCCGCTCACCAGCCACGcgg
D2	PI4K2A	GCATCCCTAAATCCTGCCAGggg	GCATCCCTAAATCCTGGCAGggg
D3	ARRDC4	GGCAGGAAAGAGTCGCCCGGcgg	GGCAGGAAAGAGTCGCGCGGcgg
D4	MTERF4	GGCCGAACGCAGCCATAGCGcgg	GGCCGAACGCAGCCATTGCGcgg
D5	CD55	GCAGCATCGTGTGCTCCACAcgg	GCAGCATCGTGTGCTCGACAcgg
BFP-GFP		GCTGAAGCACTGCACGCCATggg	GCTGAAGCACTGCACGCCGTACG

Table S2. Single-stranded oligonucleotide (ssODN) donor templates

Site	Template Sequence
A1	CACTAACCAAGCTCCAAAAGTCTGGCTGCCTCTTGGACAGCTCTGGATGTGCCATGGACCCCTGAACACAGCACATCTGCCAGCTCTTGTCCCTTTCATCC
A2	GTCTTACCCCACCCAGGGTGCTTACCTCCACACAGTTGTCACAGACACTGGAGTGGGAACATCGAGGGGGACGGTAAAAGCGGCAGGTGGCACACCATTTC
A3	TATGGTTTCTCTCCTGTGTGGACCATGCAATGATTATTAAGTGCTGATCTTCGACGGAAGTTCTTACCACATGTATCACATTTGAATGGTTTCTCCCCAGT
A4	CGGCTGGGGTGGGAGCTCTGCCCAGCTGCCAGGCTCAGTCAGCCATGCTCGCCGTGGCTGATGGCACCCCTATCCCTGCCCCAGGTGGGATGCAGGCCCTG
A5	AAAACAAGGAGGCCATAAAGAATGGGAGACATGAGTGGAAGAACTCAATCGTAAGGGAACAGGGCAGCAGAGTGAGGGGTAGACGTGGTGACTCCACGCTT
B1	TCTGCAATACTGGCCAATATTCTTTTATCAAACAGGAGACCGCAGCTTTATAGGGGGAAAATGCAGACGTTGGATAAAAACAGCAAGAAATAGTCATTTTC
B2	AGCCAGCGTTACAGTAATGTTCCAGGTAGGTGTACATGGTTTATGCTCTTTCAGAGGAGACCTTGTAGATAACCACTCCATGATGAACACAAAATGACAAG
B3	TAAGACAGTGTTGTGGGTGTAGGTACTAACACTGGCTCGTGTGACAAGGCGATGAGGCTGGTGTAAAGCGGCCTTGGAGTGTGTATTAAGTAGGTGCACAG
B4	ACAGTGAGGACAGACACAGGTAGGCACCTGAACATTCACTGGTTAAGTGACCGGTGGAAGGGTGGGGTGCTGCAACCCTTGGACCCCTGGGGATGCGGTGA
B5	CTCAGACAAGGTGCTGCAGCGTACAGCTCGGGCCAAAGGCCTCTAAAAACCCAGGGGAAGGCAGGTAGGGCGCAGTGGCTCCCGCCTGTGATCCCAGCACT
C1	GGAATTCCTTCATTACAAATATTTATTAGTACCTACAATGCAAGAGGCTCGGTAGGGAGCACAAACCTAAGGAAAGGTGCAAACAAACAAAACCAAGCTTT
C2	GAGCTTATGGTAGAGTTAGAGAAAAGAATGCACAACTGAATTCCTTGAAGTCCGGGGCCTCGACTTAAAATGTGATTATAGGGAGTGGAGTGGAAGGGGTC
C3	TATGCTGTTCCTTTTAATTCTCTGGAGGTTACAGGGATGAGCGTCCCAACGACTGGGTAGAGCAAAGCATTATTTTACTATGGCTTGCCTACATTTGAGGG
C4	TTACATCACTGTCATGTTAGGGTTTTCTGTTATTCTTGGTTGAATCTAATGACGGGGAAGACAGTGCTCTGTTAGGCTTTGGTCTTGACTGGAGTTACTCA
C5	ATTTCTCCAATTCCAGATAGTAAAGAAGCCTTTGGTCTCTTGCTGGATATCGGAAGGGGATTTGTGGATCCAGCTGCTTCTTGAATGAACTTTCATCAAGG
C6	CTTCAGGAGAAATAATGAAGAAAGAGGCGGTTTGGTAATGAAATGAGTTGCGGCAGGAGATTACGGTCATTTCAAGTTATATTCAAATTCTCTTCTTTTTG
C7 (lacks PAM mutation)	GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCAAGTGATGGCAGAGGAAAGGAAGCCCTGCTTCCTCCAGAGGGCGTCGCAGGAC
C7 (contains PAM mutation)	GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCAAGTGATTTCAGAGGAAAGGAAGCCCTGCTTCCTCCAGAGGGCGTCGCAGGAC
C8	ACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTTCTCCACAGGAGTCAGATGCACCATGGTGTCTGTTTGAGGTTGCTAGTGAACAC
C9 (lacks PAM mutation)	ATCAACACACTCTGTCCTGTTTTCCAGCTGTGGCCACCTGTCGCCCTGACTAATTCCAGTGCTCTGATGGAAACTGCATCCATGGCAGCCGGCAGTGTGA
C9 (contains PAM mutation)	ATCAACACACTCTGTCCTGTTTTCCAGCTGTGGCCACCTGTCGCACTGACTAATTCCAGTGCTCTGATGGAAACTGCATCCATGGCAGCCGGCAGTGTGA

C10 (lacks PAM mutation)	TTTTCCAGCCCGCTGGCCCTGTAAAGGAAACTGGAACACAAAGCAGAGACTGCGGGGCGGGCCAGCCTGAATAGCTGCAAACAAGTGCAGAATATCTGAT
C10 (contains PAM mutation)	TTTTCCAGCCCGCTGGCCCTGTAAAGGAAACTGGAACACAAAGCAGAGACTGCGACGCGGGCCAGCCTGAATAGCTGCAAACAAGTGCAGAATATCTGAT
C11	CATGGGTATGGCCTCTAAAAACATGGCCCCAGCAGCTTCAGTCCCTTTCTTGTCGATGGTCAGCACAGCCTTATGCACGGCCTGGAGGGGAGAGAAGCAG
C12	ATGGCTATTTCAGGGACCCCCACTGCTTGGGGGAGCAGGAGTATTTCAGTTGTGAGGAATGCTACGAGGATGACAGCTCGCCCACCTGGAGCAGGTGAGCT
C13	AGAACTGGAATCACTATATGTTTATGTGAAAAAAATGAACCTAGAGACAAACCTTGCATCTTTCAAAAAATTAACCAAAAATAGATTGTAGACATAAATG
D1	TCTGGGCAGTGTCGTCCCCAGAGGTCGGCGGCCGTTGCCCGCTCACCAGCCACGCGGGGCGCCTGCGGGGACGGTGAGGCCCTGCTGAGGACTCCGGCCAG
D2	TTCCGCAGAGCGTCCCCAGCTCCAGTCCTCAGTGGCATCCCTAAATCCTGGCAGGGGCTGGTACTGTGAAGTTTCTCCCTTTTCCCTCTGGCTGTGCGAAG
D3	GCGACCCCGGCTCCGGGCCTCTGCCGACCTCAGGGGCAGGAAAGAGTCGCGCGGCGGGATGGGCGGGGAGGCTGGGTGCGCGGCGGCCGTGGGTGCCGAGG
D4	AGAAGCCCGCGCGCCCAGCTCGAGCTTACCTGACGGCCGAACGCAGCCATTGCGCGGAGAAGATGGCAGCAGTTACGGCGCCGGAAGCAGCGGTCCTCCCC
D5	AATGCCACACCTGAAAGAGATGACATGCAAGTTTGCAGCATCGTGTGCTCGACACGGCTGGACTCTGTCGAGAGTGGGGAACGGTCAGCGGTCTGGCCTCC
BFP-GFP	GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACGTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGA

Table S3. PCR primers for constructing next-generation sequencing libraries

Site	Primer
A1 (for)	TCCCTACACGACGCTCTTCCGATCTAGGAGGGAGCAGGTTTGTCA
A1 (rev)	GTTCAGACGTGTGCTCTTCCGATCTGGACACCTACCCATGACCTTG
A2 (for)	TCCCTACACGACGCTCTTCCGATCTGATGATTTCCGAGCTCCCCT
A2 (rev)	GTTCAGACGTGTGCTCTTCCGATCTGGATGAAGACAATGAAGGTTAAGCA
A3 (for)	TCCCTACACGACGCTCTTCCGATCTAGGGCCTTCATACATGCTTCC
A3 (rev)	GTTCAGACGTGTGCTCTTCCGATCTACACTTACCACAGTCCTCACAT
A4 (for)	TCCCTACACGACGCTCTTCCGATCTGCTGGTGAGTATGATGGCCTTG
A4 (rev)	GTTCAGACGTGTGCTCTTCCGATCTCTGTTGCTGGTCAGGTGCTT
A5 (for)	TCCCTACACGACGCTCTTCCGATCTGCTATTTGGAGTTCCCTCCC
A5 (rev)	GTTCAGACGTGTGCTCTTCCGATCTAGGAAATGAAGAACACCCCCG
B1 (for)	TCCCTACACGACGCTCTTCCGATCTAGCTTAGATAATAATGGCTGTTTCG
B1 (rev)	GTTCAGACGTGTGCTCTTCCGATCTAGAAACTGGTCCCTGGAGGA
B2 (for)	TCCCTACACGACGCTCTTCCGATCTGATGTTCAGCCCACAAGAGC
B2 (rev)	GTTCAGACGTGTGCTCTTCCGATCTACATCACTTGAAAGTTCAGCCA
B3 (for)	TCCCTACACGACGCTCTTCCGATCTTCTGGTGTTTGTCTCTCTGACTA
B3 (rev)	GTTCAGACGTGTGCTCTTCCGATCTGGACATGCAGAAAGTGCAAAG
B4 (for)	TCCCTACACGACGCTCTTCCGATCTAGTAACTGTGATCACCAAACATAAC
B4 (rev)	GTTCAGACGTGTGCTCTTCCGATCTGATCGTTGGGCAAACACAAA
B5 (for)	TCCCTACACGACGCTCTTCCGATCTTGAACTCCTGGGCTCAA
B5 (rev)	GTTCAGACGTGTGCTCTTCCGATCTCGTGTGACTGCAGAGATTC
C1 (for)	TCCCTACACGACGCTCTTCCGATCTAATGGATGAGCTCTCTGCGG
C1 (rev)	GTTCAGACGTGTGCTCTTCCGATCTACACTATGGTAGCCGTCCCT
C2 (for)	TCCCTACACGACGCTCTTCCGATCTGCTGAGAAGGACTTCAAGAGAT
C2 (rev)	GTTCAGACGTGTGCTCTTCCGATCTCCATATGTGAGAAATAAACAGTCCTAC
C3 (for)	TCCCTACACGACGCTCTTCCGATCTAGTAGTTTAGAAAGGAAACTGGCAA
C3 (rev)	GTTCAGACGTGTGCTCTTCCGATCTAAAACCTTGCCTGAAAGGCTCA
C4 (for)	TCCCTACACGACGCTCTTCCGATCTAGGTGATTCATCCAGAGGTGTA
C4 (rev)	GTTCAGACGTGTGCTCTTCCGATCTAAACCACACATCTAGCCTGG
C5 (for)	TCCCTACACGACGCTCTTCCGATCTGGTAGTTTACCCTCCTCCTCTA
C5 (rev)	GTTCAGACGTGTGCTCTTCCGATCTAGGCATTGATTGTGCTATCTCTA
C6 (for)	TCCCTACACGACGCTCTTCCGATCTGGTATCTGCCCAGAAAGCTCTA
C6 (rev)	GTTCAGACGTGTGCTCTTCCGATCTGCTGAGTGCAGTGGGAGAGA
C7 (for)	GCTCTTCCGATCTGGAAACGCCCATGCAATTAGTC
C7 (rev)	GCTCTTCCGATCTCTTGTCAACCAGTATCCCGGTG
C8 (for)	GCTCTTCCGATCTCTTGATACCAACCTGCC
C8 (rev)	GCTCTTCCGATCTTAAAAGTCAGGGCAGAGCCA

C9 (for)	GCTCTTCCGATCTGCCCTGCTTCTTTTTCTCTGGT
C9 (rev)	GCTCTTCCGATCTACCATTAACGCAGCCAACTTCA
C10 (for)	GCTCTTCCGATCTCCAGCCCCATCTGTCAAACT
C10 (rev)	GCTCTTCCGATCTGAATGGATTCCTTGGAAACAATG
C11 (for)	GCTCTTCCGATCTTTTGTTGAACTTGACCTCGGGG
C11 (rev)	GCTCTTCCGATCTTCAGGGAGGGAGAGGATGTG
C12 (for)	TCCCTACACGACGCTCTTCCGATCTAGGAGATGAACAGCTCCCAACTA
C12 (rev)	GTTCAGACGTGTGCTCTTCCGATCTGCTTTTTAGGGGAAGCCCAAGT
C13 (for)	GCTCTTCCGATCTTGACACACACACACATGAAAT
C13 (rev)	GCTCTTCCGATCTCCAAACGCAAGATCATGTAGA
D1 (for)	TCCCTACACGACGCTCTTCCGATCTGTCCGCCCCGCTGTAG
D1 (rev)	GTTCAGACGTGTGCTCTTCCGATCTACCCAGGGCCTGAGCC
D2 (for)	TCCCTACACGACGCTCTTCCGATCTGGTGAGTGCGGGGGTG
D2 (rev)	GTTCAGACGTGTGCTCTTCCGATCTTCCTCCCCCACCAAGTAGG
D3 (for)	TCCCTACACGACGCTCTTCCGATCTCCTTACCCTGCCGCGAG
D3 (rev)	GTTCAGACGTGTGCTCTTCCGATCTCTCGTCCTCGAACACCAGAC
D4 (for)	TCCCTACACGACGCTCTTCCGATCTGGGCCGGAAGTGAAGGAC
D4 (rev)	GTTCAGACGTGTGCTCTTCCGATCTACCTGGCCGTGAAACACTC
D5 (for)	TCCCTACACGACGCTCTTCCGATCTAGGGAGGGCTCAAAGAGACT
D5 (rev)	GTTCAGACGTGTGCTCTTCCGATCTTTCAAGACACAAGCCCCCTT

Table S4. ChIP-qPCR primers

Site	Primer
C7 (for)	CGCCTGTGATGGGCTAATTG
C7 (rev)	GGCCTCTCCAGCCTCATTTG
C7 control (for)	CCACATTGGCTCCACCCATC
C7 control (rev)	CTTGGTTGTGTCACGTGGTT
C9 (for)	GTTGGCTGCGTTAATGGTGA
C9 (rev)	TCATTTGCAAGCAGCAAGGC
C9 control (for)	AGGCTGAGACAGGAGAATCA
C9 control (rev)	GAATGCAATGGCACGATATTGG

SI References

1. J. H. Kim, *et al.*, High cleavage efficiency of a 2A peptide derived from porcine teschovirus-1 in human cell lines, zebrafish and mice. *PLOS ONE* **6**, e18556 (2011).