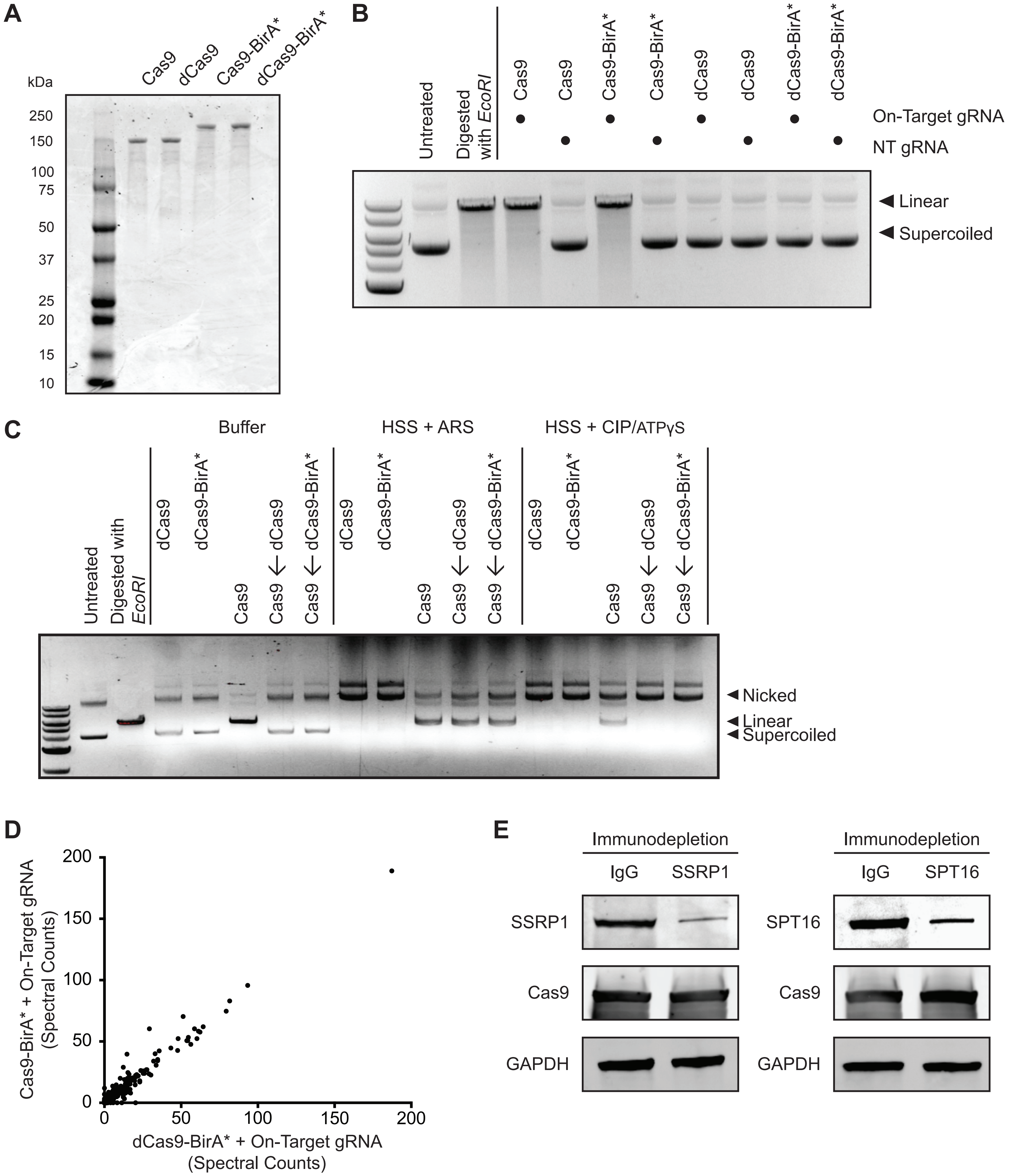

A
kDa
Cas9
dCas9
Cas9-BirA*
dCas9-BirA*
250
150
100
75
50
37
25
20
15
10
B
Untreated
Digested with EcoRI
Cas9
Cas9
Cas9-BirA*
Cas9-BirA*
dCas9
dCas9
dCas9-BirA*
dCas9-BirA*
On-Target gRNA
NT gRNA
● ● ● ●
● ● ● ●
Linear
Supercoiled
C
Buffer
HSS + ARS
HSS + CIP/ATPγS
Untreated
Digested with EcoRI
dCas9
dCas9-BirA*
Cas9
Cas9 ← dCas9
Cas9 ← dCas9-BirA*
dCas9
dCas9-BirA*
Cas9
Cas9 ← dCas9
Cas9 ← dCas9-BirA*
dCas9
dCas9-BirA*
Cas9
Cas9 ← dCas9
Cas9 ← dCas9-BirA*
Nicked
Linear
Supercoiled
D
Cas9-BirA* + On-Target gRNA (Spectral Counts)
200
150
100
50
0
0
50
100
150
200
dCas9-BirA* + On-Target gRNA (Spectral Counts)
E
Immunodepletion
IgG
SSRP1
SSRP1
Cas9
GAPDH
Immunodepletion
IgG
SPT16
SPT16
Cas9
GAPDH

Figure S1. Deployment of Recombinant Cas9-BirA* and dCas9-BirA* in HSS, Related to Figures 1 and 2

(A) Coomassie of purified Cas9, dCas9, Cas9-BirA*, and dCas9-BirA*.

(B) BirA* fusion does not compromise gRNA-targeting of Cas9 or Cas9's cleavage ability in vitro.

(C) BirA* fusion does not impede energy-dependent removal of Cas9 from plasmid substrates in HSS.

(D) Proteins identified by mass spectrometry plotted according to average number of spectral counts in Cas9-BirA*–on-target gRNA samples (N = 3 biological replicates) versus dCas9-BirA*–on-target gRNA samples (N = 3 biological replicates).

(E) Immunodepletion of SSRP1 or SPT16 from HSS.

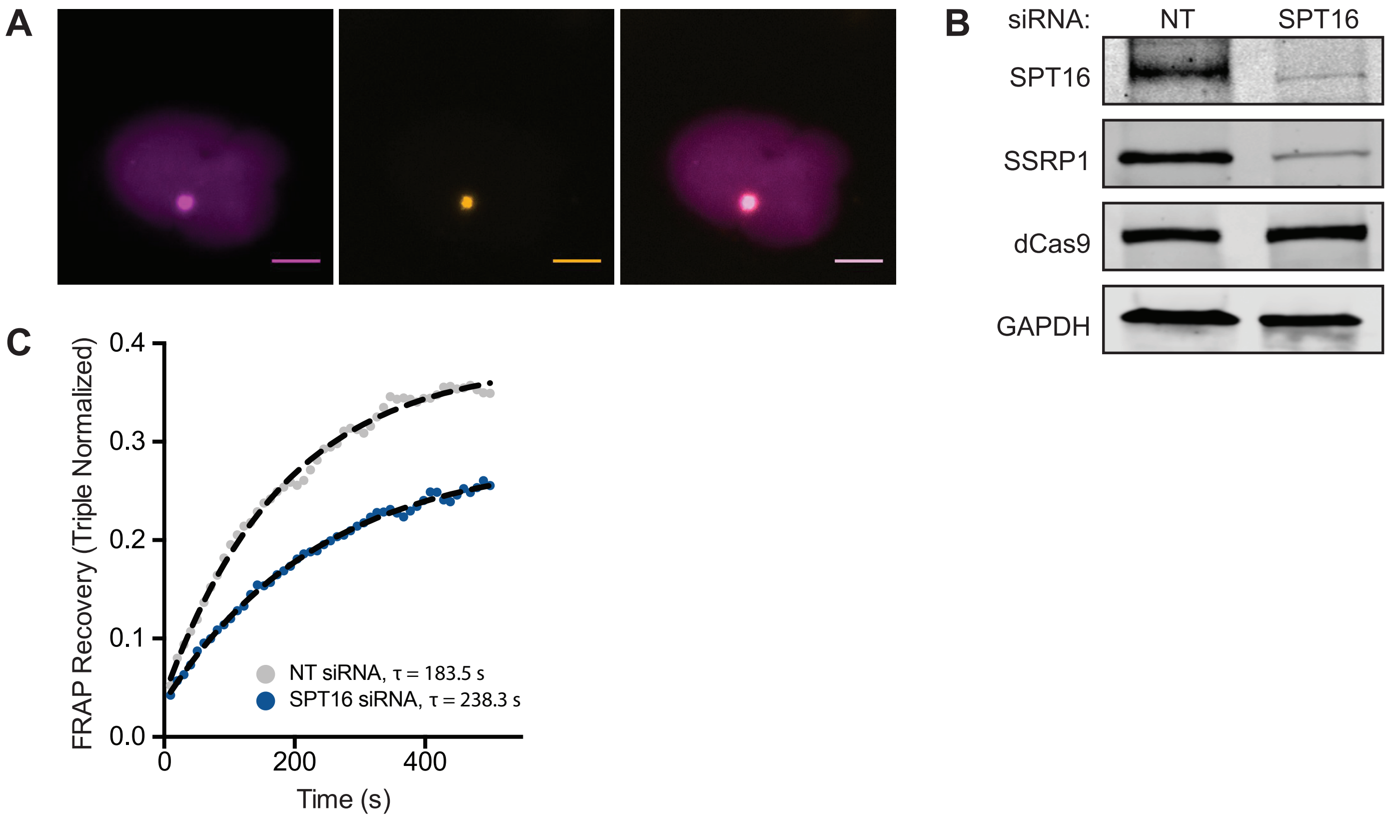


Figure S2. FRAP Imaging of dCas9-HaloTag after FACT Depletion, Related to Figure 3

(A) Colocalization of dCas9-HaloTag labeled with JF_{646} and programmed with a tetO gRNA (left panel) and mOrange-LacI-NLS (middle panel) at synthetic arrays. The right panel is an overlay of the two channels. Scale bars = 10 µm.

(B) Western blot of SPT16, SSRP1, dCas9, and GAPDH in U2OS cells transfected with either NT or SPT16 siRNAs.

(C) Mean FRAP recoveries with single component exponential fits.

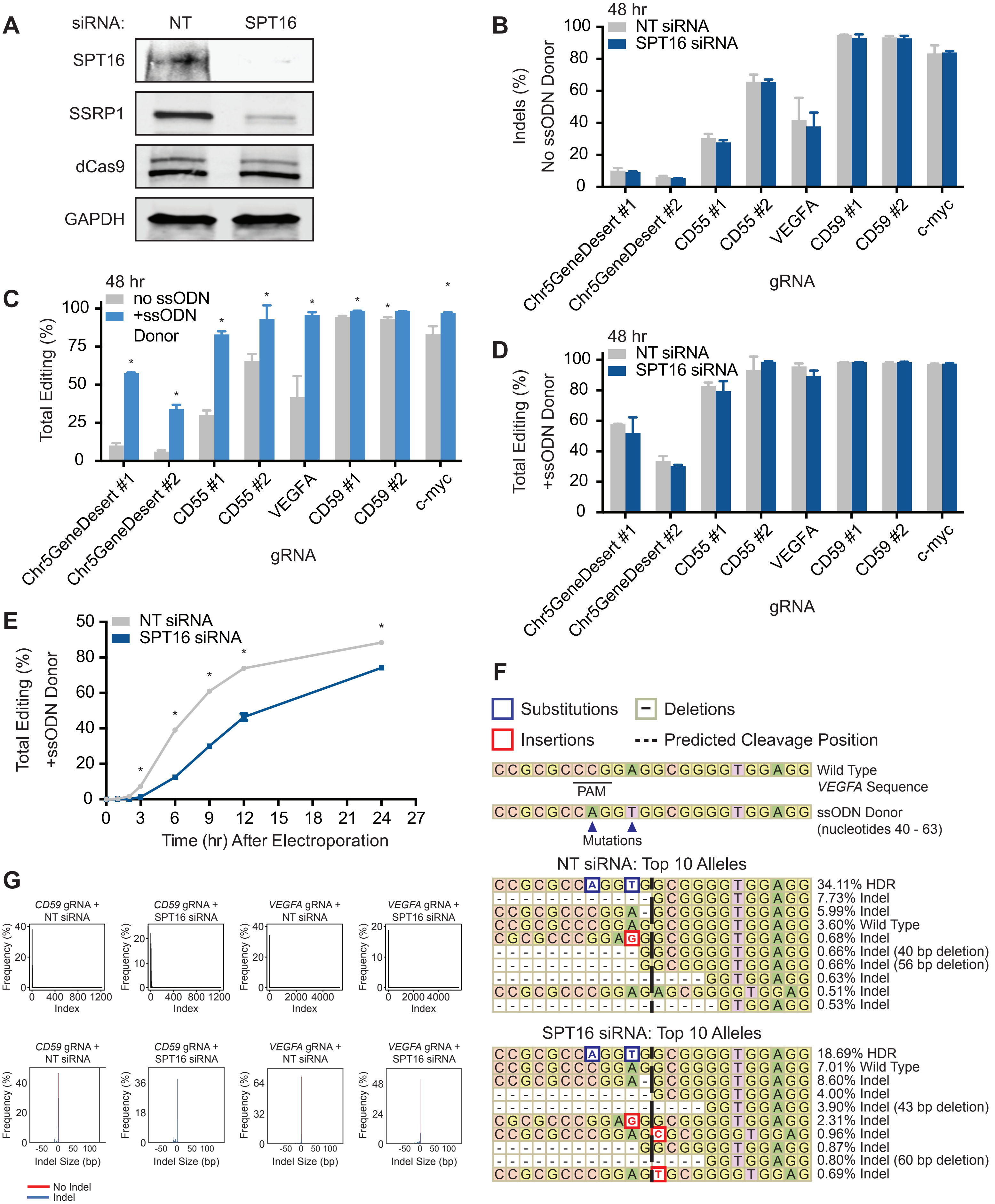

A
siRNA:
NT
SPT16
SPT16
SSRP1
dCas9
GAPDH
B
48 hr
NT siRNA
SPT16 siRNA
Indels (%)
No ssODN Donor
0
20
40
60
80
100
Chr5GeneDesert #1
Chr5GeneDesert #2
CD55 #1
CD55 #2
VEGFA
CD59 #1
CD59 #2
c-myc
gRNA
C
48 hr
no ssODN
+ssODN
Donor
Total Editing (%)
0
25
50
75
100
*
Chr5GeneDesert #1
Chr5GeneDesert #2
CD55 #1
CD55 #2
VEGFA
CD59 #1
CD59 #2
c-myc
gRNA
D
48 hr
NT siRNA
SPT16 siRNA
Total Editing (%)
+ssODN Donor
0
20
40
60
80
100
Chr5GeneDesert #1
Chr5GeneDesert #2
CD55 #1
CD55 #2
VEGFA
CD59 #1
CD59 #2
c-myc
gRNA
E
NT siRNA
SPT16 siRNA
Total Editing (%)
+ssODN Donor
0
20
40
60
80
50
*
0
3
6
9
12
15
18
21
24
27
Time (hr) After Electroporation
F
Substitutions
Deletions
Insertions
--- Predicted Cleavage Position
CCGCGCCCGGAGGCGGGGTGGAGG Wild Type
PAM
VEGFA Sequence
CCGCGCCAGGTGGCGGGGTGGAGG ssODN Donor
(nucleotides 40 - 63)
Mutations
NT siRNA: Top 10 Alleles
CCGCGCCAGGTGGCGGGGTGGAGG 34.11% HDR
-----------GCGGGGTGGAGG 7.73% Indel
CCGCGCCCGGA-GCGGGGTGGAGG 5.99% Indel
CCGCGCCCGGAGGCGGGGTGGAGG 3.60% Wild Type
CCGCGCCCGGAGGGCGGGGTGGAGG 0.68% Indel
----------GGCGGGGTGGAGG 0.66% Indel (40 bp deletion)
----------GGCGGGGTGGAGG 0.66% Indel (56 bp deletion)
---------------GGTGGAGG 0.63% Indel
CCGCGCCCGGAGAGCGGGGTGGAG 0.51% Indel
----------------GTGGAGG 0.53% Indel
SPT16 siRNA: Top 10 Alleles
CCGCGCCAGGTGGCGGGGTGGAGG 18.69% HDR
CCGCGCCCGGAGGCGGGGTGGAGG 7.01% Wild Type
CCGCGCCCGGA-GCGGGGTGGAGG 8.60% Indel
-----------GCGGGGTGGAGG 4.00% Indel
---------------GGTGGAGG 3.90% Indel (43 bp deletion)
CGCGCCCGGAGGGCGGGGTGGAGG 2.31% Indel
CCGCGCCCGGAGCGCGGGGTGGAG 0.96% Indel
----------GGCGGGGTGGAGG 0.87% Indel
---------------GGTGGAGG 0.80% Indel (60 bp deletion)
CCGCGCCCGGAGTGCGGGGTGGAG 0.69% Indel
G
CD59 gRNA + NT siRNA
CD59 gRNA + SPT16 siRNA
VEGFA gRNA + NT siRNA
VEGFA gRNA + SPT16 siRNA
Frequency (%)
Index
0 400 800 1200
0 2000 4000
Indel Size (bp)
-50 0 50 100
No Indel
Indel

Figure S3. Effects of FACT Depletion and ssODN Inclusion on Cas9 Editing Outcomes, Related to Figure 4

(A) Western blot of SPT16, SSRP1, dCas9, and GAPDH in K562 cells transfected with either NT or SPT16 siRNAs.

(B) Indel rates from amplicon-NGS sequencing of eight different loci after electroporation of Cas9 RNPs in the absence of an HDR donor (N = 3 biological replicates). Data are represented as mean ± standard deviation.

(C) Total editing rates from amplicon-NGS sequencing of eight different loci after electroporation of Cas9 RNPs in the absence or presence of an HDR donor (N = 3 biological replicates). Data are represented as mean ± standard deviation.

(D) Total editing rates from amplicon-NGS sequencing of eight different loci after electroporation of Cas9 RNPs in the presence of an HDR donor (N = 3 biological replicates). Data are represented as mean ± standard deviation.

(E) Total editing rates from amplicon-NGS sequencing of *VEGFA* 0, 1, 2, 3, 6, 9, 12, and 24 hours after electroporation of Cas9 RNPs in the presence of an HDR donor (N = 3 biological replicates). Data are represented as mean ± standard deviation.

(F) Representative alleles from cells edited with Cas9 programmed with the *VEGFA* gRNA and a PAM-out ssODN HDR donor.

(G) Histograms of indel distributions for *CD59* and *VEGFA* plotted by indel abundance (top) or indel size (bottom).

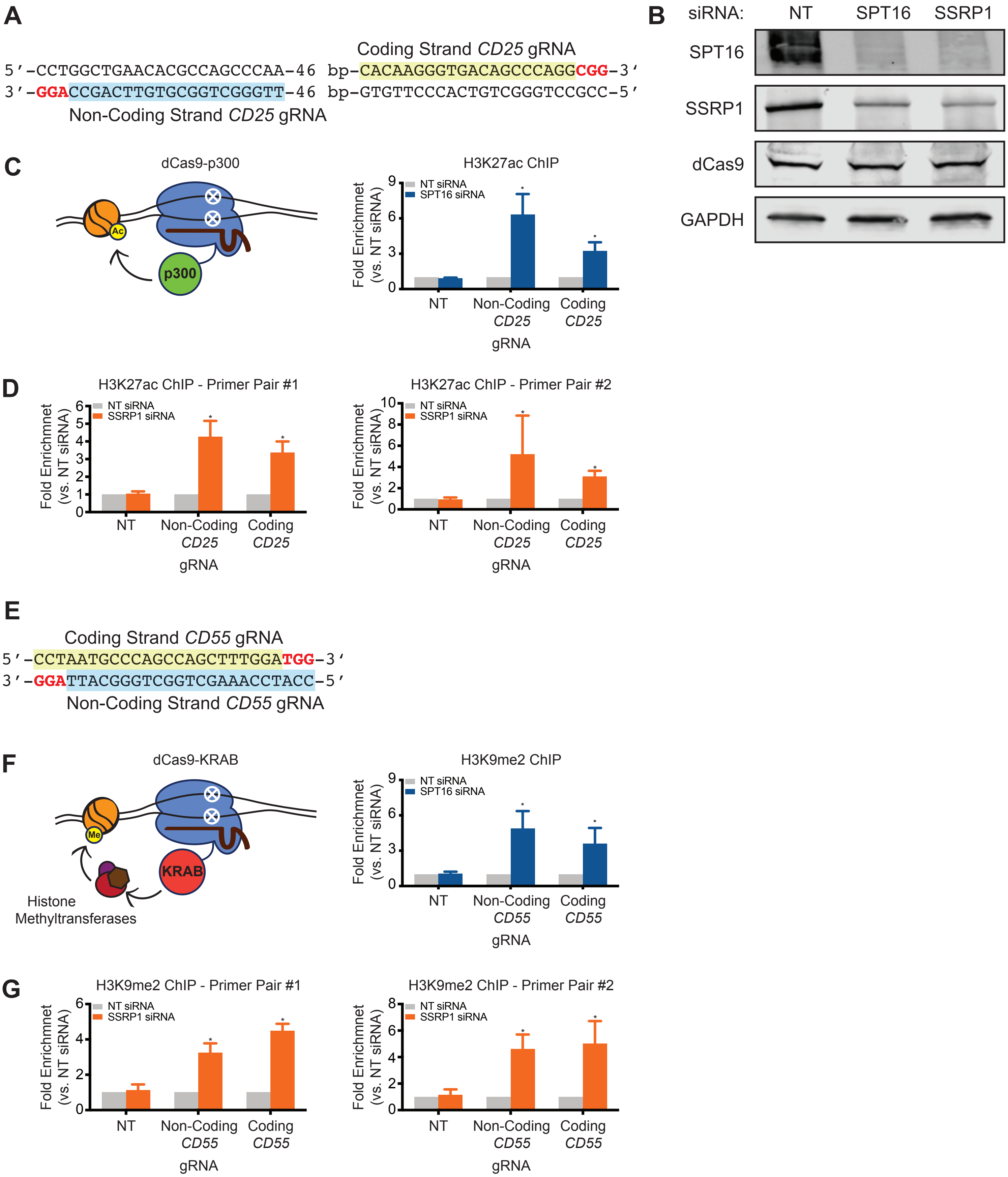

A
Coding Strand *CD25* gRNA
5′-CCTGGCTGAACACGCCAGCCCAA-46 bp-CACAAGGGTGACAGCCCAGGCGG-3′
3′-GGACCGACTTGTGCGGTCGGGTT-46 bp-GTGTTCCCACTGTCGGGTCCGCC-5′
Non-Coding Strand *CD25* gRNA
B
siRNA:
NT
SPT16
SSRP1
SPT16
SSRP1
dCas9
GAPDH
C
dCas9-p300
Ac
p300
H3K27ac ChIP
NT siRNA
SPT16 siRNA
Fold Enrichmnet (vs. NT siRNA)
0
3
6
9
NT
Non-Coding *CD25*
Coding *CD25*
gRNA
D
H3K27ac ChIP - Primer Pair #1
NT siRNA
SSRP1 siRNA
Fold Enrichmnet (vs. NT siRNA)
0
1
2
3
4
5
6
NT
Non-Coding *CD25*
Coding *CD25*
gRNA
H3K27ac ChIP - Primer Pair #2
NT siRNA
SSRP1 siRNA
Fold Enrichmnet (vs. NT siRNA)
0
2
4
6
8
10
NT
Non-Coding *CD25*
Coding *CD25*
gRNA
E
Coding Strand *CD55* gRNA
5′-CCTAATGCCCAGCCAGCTTTGGATGG-3′
3′-GGATTACGGGTCGGTCGAAACCTACC-5′
Non-Coding Strand *CD55* gRNA
F
dCas9-KRAB
Me
KRAB
Histone Methyltransferases
H3K9me2 ChIP
NT siRNA
SPT16 siRNA
Fold Enrichmnet (vs. NT siRNA)
0
3
6
9
NT
Non-Coding *CD55*
Coding *CD55*
gRNA
G
H3K9me2 ChIP - Primer Pair #1
NT siRNA
SSRP1 siRNA
Fold Enrichmnet (vs. NT siRNA)
0
2
4
6
NT
Non-Coding *CD55*
Coding *CD55*
gRNA
H3K9me2 ChIP - Primer Pair #2
NT siRNA
SSRP1 siRNA
Fold Enrichmnet (vs. NT siRNA)
0
2
4
6
8
NT
Non-Coding *CD55*
Coding *CD55*
gRNA

Figure S4. FACT Depletion Enhances Chromatin Marking by dCas9-p300 and dCas9-KRAB, Related to Figure 5

(A) Schematic of coding and non-coding strand CD25 gRNAs.

(B) Western blot of SPT16, SSRP1, dCas9, and GAPDH in HEK293T cells transfected with either NT, SPT16, or SSRP1 siRNAs.

(C) Knockdown of SPT16 increases H3K27 acetylation in HEK293T dCas9-p300 cells (N = 3 biological replicates). Fold enrichment is the amount of H3K27ac after SPT16 depletion normalized to the amount of H3K27ac after treatment with a NT siRNA. qPCR primers amplified regions that include the corresponding protospacer. Data are represented as mean ± standard deviation.

(D) Knockdown of SSRP1 increases H3K27 acetylation in HEK293T dCas9-p300 cells (N = 3 biological replicates). Fold enrichment is the amount of H3K27ac after SSRP1 depletion normalized to the amount of H3K27ac after treatment with a NT siRNA. qPCR primers amplified regions 9 base pairs upstream of the non-coding-strand gRNA protospacer and 46 base pairs upstream of the coding strand gRNA protospacer (Primer Pair #1) or regions that include the corresponding protospacers (Primer Pair #2). Data are represented as mean ± standard deviation.

(E) Schematic of coding and non-coding strand CD55 gRNAs.

(F) Knockdown of SPT16 increases H3K9 methylation in K562 dCas9-KRAB cells (N = 3 biological replicates). Fold enrichment is the amount of H3K9me2 after SPT16 depletion normalized to the amount of H3K9me2 after treatment with a NT siRNA. qPCR primers amplified a region that include the protospacers. Data are represented as mean ± standard deviation.

(G) Knockdown of SSRP1 increases H3K9 methylation in K562 dCas9-KRAB cells (N = 3 biological replicates). Fold enrichment is the amount of H3K9me2 after SSRP1 depletion normalized to the amount of H3K9me2 after treatment with a NT siRNA. qPCR primers amplified a region 66 base pairs upstream of both the coding and non-coding strand gRNA protospacers (Primer Pair #1) or a region that includes the protospacers (Primer Pair #2). Data are represented as mean ± standard deviation.

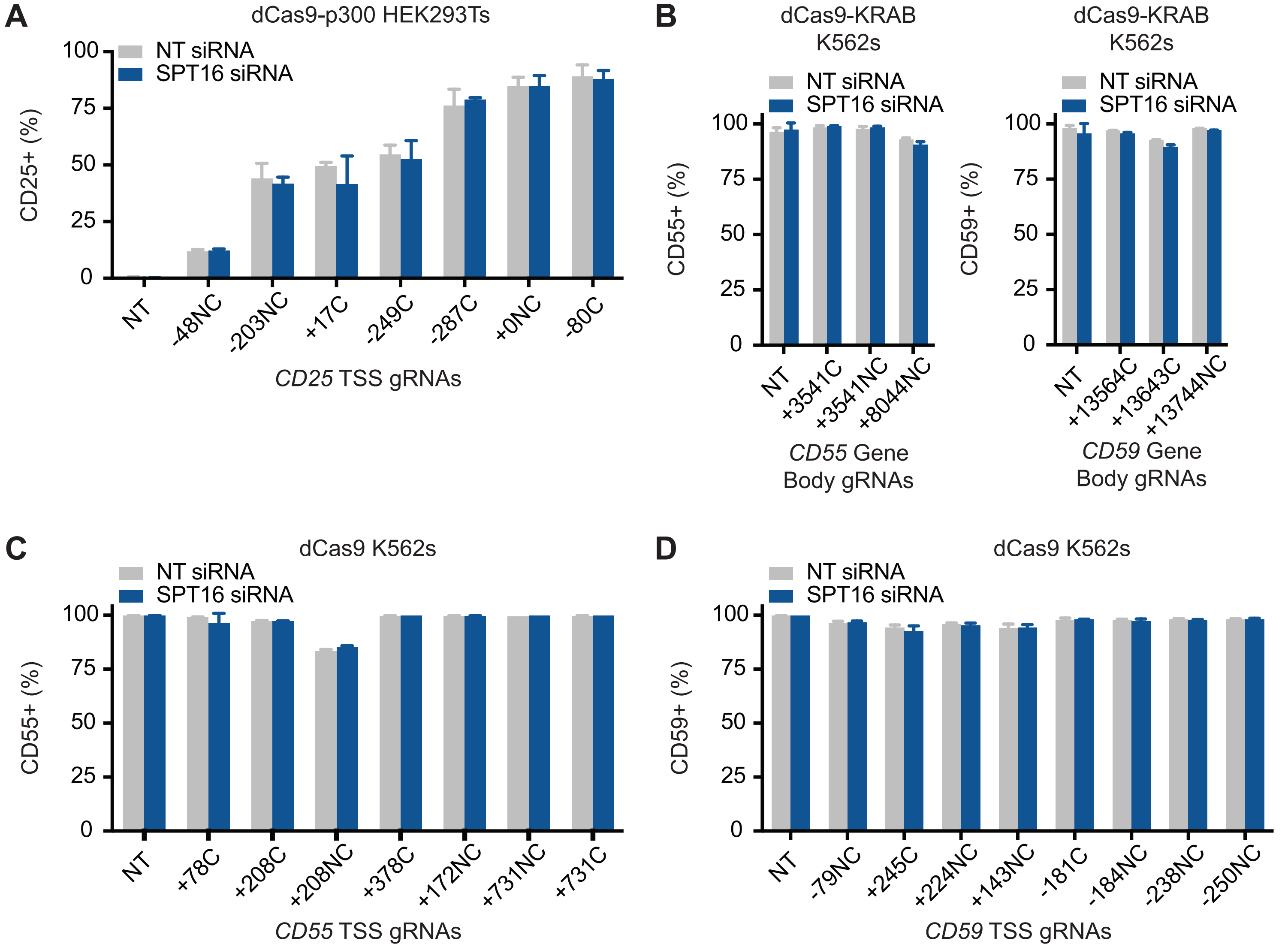


Figure S5. Enhancement of Transcriptional Engineering After FACT Depletion Requires Localizing KRAB Domain to TSSs, Related to Figure 5

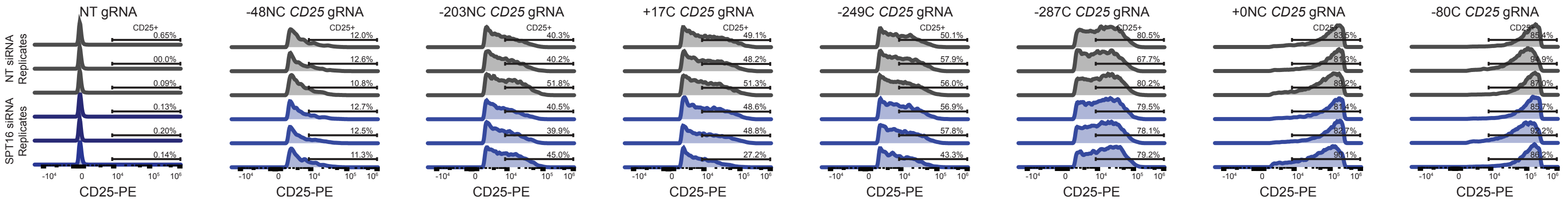
(A) FACT depletion does not affect *CD25* expression in HEK293T dCas9-p300 cells expressing *CD25* TSS gRNAs (N = 3 biological replicates). CRISPRa cells were stained with PE anti-CD25 after transfection of either NT or SPT16 siRNAs. gRNAs are labeled as in Figure 5D. Data are represented as mean ± standard deviation.

(B) Knockdown of FACT does not affect *CD55* (left) or *CD59* (right) expression in K562 dCas9-KRAB cells expressing *CD55* or *CD59* gene body gRNAs (N = 3 biological replicates). gRNAs are labeled as in Figure 5D. Data are represented as mean ± standard deviation.

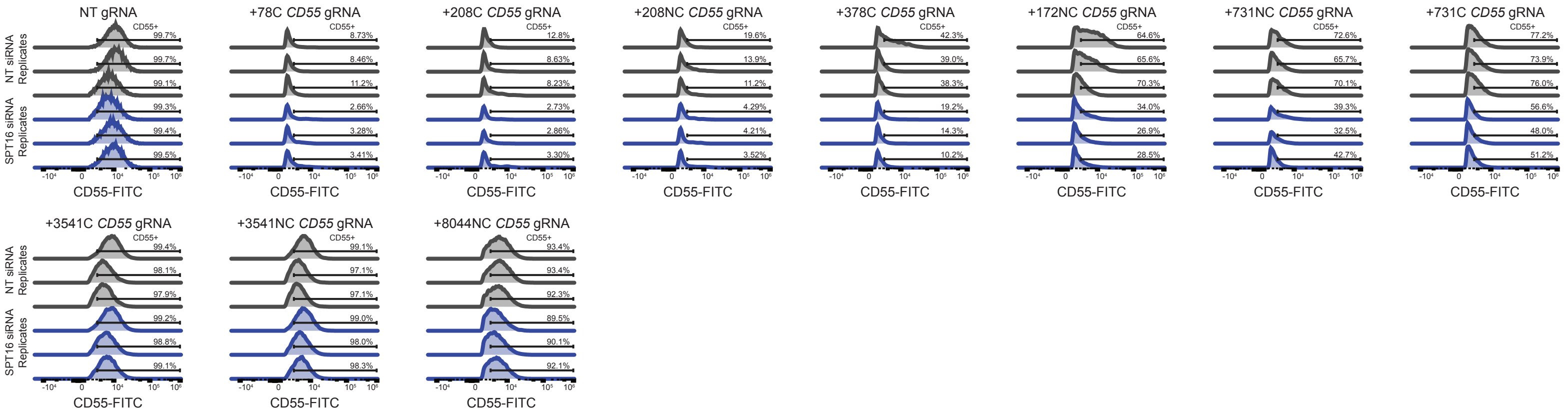
(C) Knockdown of FACT does not affect *CD55* expression in K562 dCas9 cells expressing *CD55* TSS gRNAs (N = 3 biological replicates). gRNAs are labeled as in Figure 5D. Data are represented as mean ± standard deviation.

(D) Knockdown of FACT does not affect *CD59* expression in K562 dCas9 cells expressing *CD59* TSS gRNAs (N = 3 biological replicates). gRNAs are labeled as in Figure 5D. Data are represented as mean ± standard deviation.

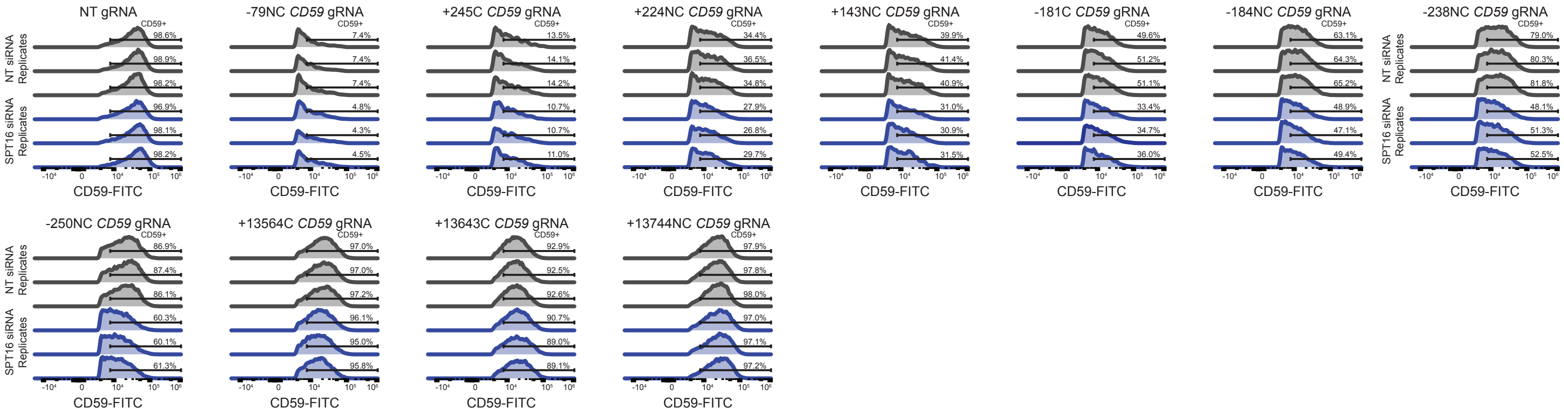
dCas9-p300 HEK293Ts - CD25 Levels



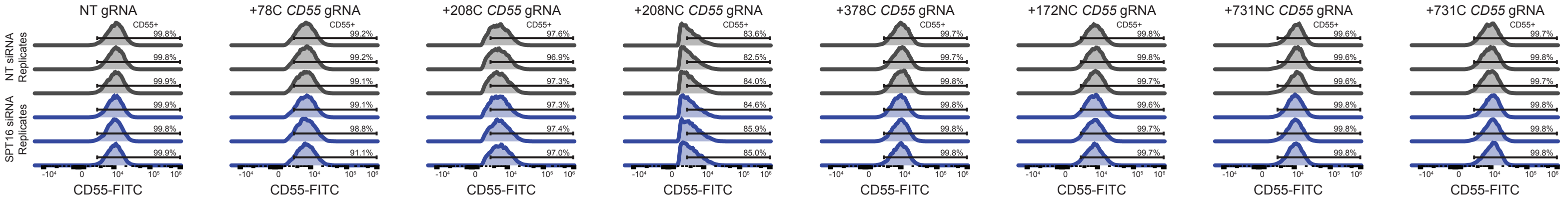
dCas9-KRAB K562s - CD55 Levels



dCas9-KRAB K562s - CD59 Levels



dCas9 K562s - CD55 Levels



dCas9 K562s - CD59 Levels

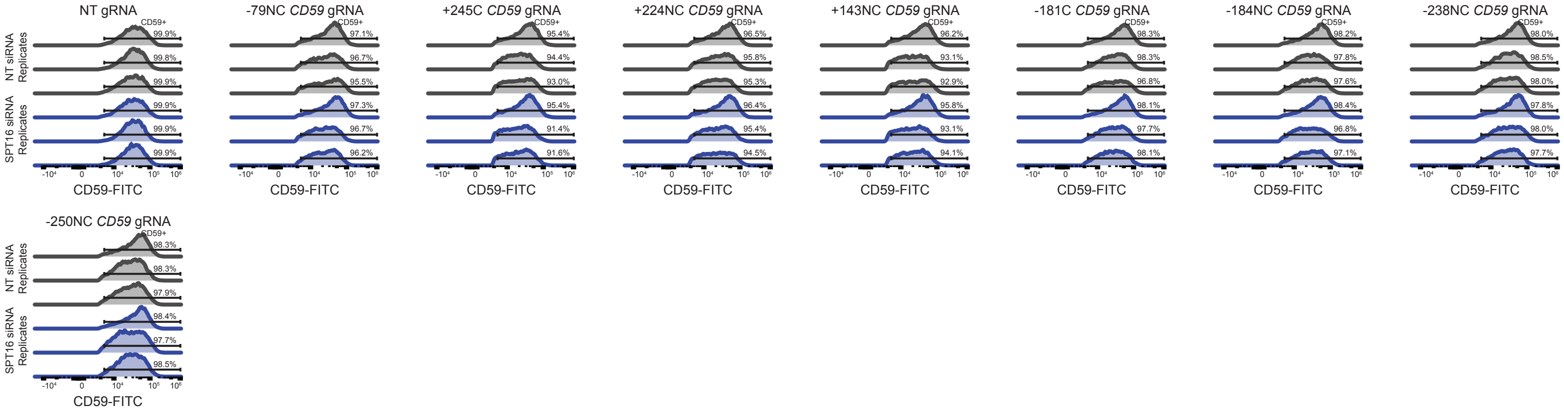


Figure S6. Histograms of CD25, CD55, and CD59 Levels After SPT16 Knockdown, Related to Figure 5

Cas9-BirA*:

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAADKKYSIGLDIGTNSVGWAVITDE
YKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLI
YLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSR
RLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQ
YADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG
ELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVV
DKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQK
KAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEE
NEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG
KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVK
VVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQ
ILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIK
KYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIET
NGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDP
KKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKD
LIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF
VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFK
YFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKGGGSG
GGSGGGSKDNTVPLKLIALLANGEFHSGEQLGETLGMSRAAINKHIQTLRDWGVDVFTVPGKG
YSLPEPIQLLNAKQILGQLDGGSVAVLPVIDSTNQYLLDRIGELKSGDACIAEYQQAGRGGRGR
KWFSPFGANLYLSMFWRLEQGPAAAIGLSLVIGIVMAEVLRKLGADKVRVKWPNDLYLQDRKL
AGILVELTGKTGDAAQIVIGAGINMAMRRVEESVVNQGWITLQEAGINLDRNTLAAMLIRELRAA
LELFEQEGLAPYLSRWEKLDNFINRPVKLIIGDKEIFGISRGIDKQGALLLEQDGIIKPWMGGEISL
RSAEKAYPYDVPDYA*

dCas9-BIrA*:

MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDE
YKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLI
YLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSR
RLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQ
YADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG
ELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVV
DKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQK
KAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEE
NEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG
KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVK
VVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQL
QNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDN
VPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQ
ILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIK
KYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIET
NGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDP

KKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKD
LIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF
VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFK
YFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKGGGSG
GGSGGGSKDNTVPLKLIALLANGEFHSGEQLGETLGMSRAAINKHIQTLRDWGVDVFTVPGKG
YSLPEPIQLLNAKQILGQLDGGSVAVLPVIDSTNQYLLDRIGELKSGDACIAEYQQAGRGGRGR
KWFSPFGANLYLSMFWRLEQGPAAAIGLSLVIGIVMAEVLRKLGADKVRVKWPNDLYLQDRKL
AGILVELTGKTGDAAQIVIGAGINMAMRRVEESVVNQGWITLQEAGINLDRNTLAAMLIRELRAA
LELFEQEGLAPYLSRWEKLDNFINRPVKLIIGDKEIFGISRGIDKQGALLLEQDGIIKPWMGGEISL
RSAEKAYPYDVPDYA*

Data S1. Amino Acid Sequences of Cas9-BirA* and dCas9-BirA*, Related to Figure 2

Supplemental Tables

gRNA	Sequence
TetO	TCTCTATCACTGATAGGGAG
Chr5 Gene Desert #1	CCAGAAATTAACTGTACCTG
Chr5 Gene Desert #2	GAAGGTCAGACATTAATGTG
CD55 #1	GCTGACTTGGCTTTAGGGGT
CD55 #2	AATGCCCAGCCAGCTTTGGA
VEGFA	GACCCCCTCCACCCCGCCTC
CD59 #1	TTCTCAGAACCTGGGCCAGG
CD59 #2	GGCCCAGGTTCTGAGAAGGC
c-myc	GCGTCCTGGGAAGGGAGATC
Non-coding CD25	TTGGGCTGGCGTGTTCAGCC
Coding CD25	CACAAGGGTGACAGCCCAGG
Non-coding CD55	TCCAAAGCTGGCTGGGCATT
Coding CD55	AATGCCCAGCCAGCTTTGGA
Non-targeting	ACGGAGGCTAAGCGTCGCAA
CD25 -48NC	TTGGGCTGGCGTGTTCAGCC
CD25 -203NC	GTGGGCTGGGGTTGATGAGA
CD25 +17C	CACAAGGGTGACAGCCCAGG
CD25 -249C	TTATGGGCGTAGCTGAAGAA
CD25 -287C	CACCCTACCTTCAACGGCAG
CD25 +0NC	TGGGTCCATCCAGTCTCTAT
CD25 -80C	AGATGAGAGAAGAGAGTGCT
CD55 +78C	GGCGCGCCATGACCGTCGCG
CD55 +208C	GGGAAGCCCCTGGGCTGGGT
CD55 +208NC	GGGAAGCCCCTGGGCTGGGT
CD55 +378C	GCTGACTTGGCTTTAGGGGT
CD55 +172NC	ACTCACCCCACACGGCCGGC
CD55 +731NC	TCCAAAGCTGGCTGGGCATT
CD55 +731C	AATGCCCAGCCAGCTTTGGA
CD55 +3541C	GCTTCTTGGAAGGCTGAGGC
CD55 +3541NC	GCCTCAGCCTTCCAAGAAGC
CD55 +8044NC	GCTACAAGGGAGGCTGAGGT
CD59 -79NC	CAGGATGCCCTTGCCCTCCC
CD59 +245C	TATCTACGAGGAAGGAGAAA
CD59 +224NC	AATGAAGCCAGCGTTCGGCT
CD59 +143NC	GGTCGAGTGGAAAGCGAGGA
CD59 -181C	TTCTCAGAACCTGGGCCAGG

CD59 -184NC	GGCCCAGGTTCTGAGAAGGC
CD59 -238NC	CCCAGGGAACTGAAAGTTTG
CD59 -250NC	AAAGTTTGGGGCGTCCTCCT
CD59 +13564C	TCAGACCAAAGGGGTGACTC
CD59 +13643C	GTTCCCACTCTACTGGCCCC
CD59 +13744NC	CAGTGTGGTAGTACACACTG

Table S2. Sequences of gRNAs, Related to Figures 3, 4, and 5

ssODN	Sequence
Chr5 Gene Desert #1	CATTTAAATCTCTCTACATGAAAAGATAATTGCTCCAGAAATTAACTGTACCTGGAATTCATTCATTTGGTCATGGCTAGTTTCTTATGTAGTGATGATTTGATATCAGAGCTAA
Chr5 Gene Desert #2	TATCAGAGCTAATTAAGATCGGGTCAGAGTTGGTGAAGGTCAGACATTAATGTGTAAGATCATTGAAATGATAATTATAAACAGATTAGGAAGCCACGGTCCTTTATAAGGGGTT
CD55 #1	CCAGCATTTGGGGCTCCTGCTGTGTCGGCCCCCAGCTGACTTGGCTTTAGGGGTAGACGTGGAGGGTTAAAGAGGCCCCGGCTGGGTTTGCGGAGCAGCCAAGCCTGGCAAAATC
CD55 #2	CCTAGGTGACTGTGGCCTTCCCCCAGATGTACCTAATGCCCAGCCAGCTTTGGAAAGCCGTACAAGTTTTCCCGAGGATACTGTAATAACGTACAAATGTGAAGAAAGCTTTGTG
VEGFA	GGACGAAAAGTTTCAGTGCGACGCCGCGAGCCCCGACCCCCTCCACCCCGCCACCTGGCGCGGGCTCCGGCCCCTGCCCGCGGCTCGCCGCCGCGTCCACTGTCCGCCGCCGGCC
CD59 #1	TTGAAGGTGCTCATTGGGTCCTGGCCACCCGGCCTTCTCAGAACCTGGGCCAGGATTCTGAGCTCCGCGCGGGGGTGGAGGGAGAGGAGGAGGTTCCTGCCGAGGTGCGGCTGCG
CD59 #2	TCTCCCTCCACCCCCGCGCGGAGCTCAGCCTCCTGGCCCAGGTTCTGAGAAGGCCTTGTGGCCAGGACCCAATGAGCACCTTCAAAACCCCAGGGAACTGAAAGTTTGGGGCGTC
c-myc	GTGGAAGAGCCGGGCGAGCAGAGCTGCGCTGCGGGCGTCCTGGGAAGGGAGATCCAAAGCGAATAGGGGGCTTCGCCTCTGGCCCAGCCCTCCCGCTGATCCCCCAGCCAGCGGT

Table S3. Sequences of ssODN HDR Donors, Related to Figure 4

Chr5 Gene Desert gRNA #1	
PCR #1 - Forward Primer	GACTACCTGCCCACATCGTTAC
PCR #1 - Reverse Primer	GAGGAAGGAATACACTCTCACC
PCR #2 - Forward Primer	gctcttccgatctGACTGTGGAGCCCTGCCTTTG
PCR #2 - Reverse Primer	gctcttccgatctCCCCTTATAAAGGACCGTGGC
Chr5 Gene Desert gRNA #2	
PCR #1 - Forward Primer	GACTGTGGAGCCCTGCCTTTG
PCR #1 - Reverse Primer	CCATCATTGTCCACAGGACAGC
PCR #2 - Forward Primer	gctcttccgatctGGGTTCATTCATTTGGTCATGGC
PCR #2 - Reverse Primer	gctcttccgatctGGGCTGGAGCTACCATTCTAC
CD55 gRNA #1	
PCR #1 - Forward Primer	AGGTCCAAGTCGGTCTCTGAG
PCR #1 - Reverse Primer	GGAGACAAAAGCAGAACTGAAGG
PCR #2 - Forward Primer	gctcttccgatctCCGCCGTCCTGTGCCTTTAAG
PCR #2 - Reverse Primer	gctcttccgatctCCACACGGCTGGACTCTGTC
CD55 gRNA #2	
PCR #1 - Forward Primer	CCACTCTCGACAGAGTCCAGC
PCR #1 - Reverse Primer	GTGACGTGCCAACAGGGTATAC
PCR #2 - Forward Primer	gctcttccgatctGCAACTGTGAGGACACTTGATAG
PCR #2 - Reverse Primer	gctcttccgatctCAATATCTGACCATTGACTGCCC
VEGFA	
PCR #1 - Forward Primer	GAGGTAGCAAGAGCTCCAGAGAG
PCR #1 - Reverse Primer	CTGGAGCACTGTCTGCGCACA
PCR #2 - Forward Primer	gctcttccgatctTGACGGACAGACAGACAGACAC
PCR #2 - Reverse Primer	gctcttccgatctGGCCCGAGCTAGCACTTCTC
CD59 gRNA #1	
PCR #1 - Forward Primer	GTAGGAAGCAGCTTCAGACTGC
PCR #1 - Reverse Primer	CTCGGCTCGGCTCACCCAAAC
PCR #2 - Forward Primer	gctcttccgatctGCAAATCCGAGGAGGACGCC
PCR #2 - Reverse Primer	gctcttccgatctCATTCTTTCGCTCCAGCCCGCA
CD59 gRNA #2	
PCR #1 - Forward Primer	GAAGCAGCTTCAGACTGCAGC
PCR #1 - Reverse Primer	GCCTTCGGGCCTTCTTACCTG
PCR #2 - Forward Primer	gctcttccgatctATGTCCCATAGCAAATCCGAGG
PCR #2 - Reverse Primer	gctcttccgatctCCCGCATTCTTTCGCTCCAGC
c-myc gRNA	
PCR #1 - Forward Primer	CCCTTTATAATGCGAGGGTCTG
PCR #1 - Reverse Primer	AATCCAGCGTCTAAGCAGCTGC
PCR #2 - Forward Primer	gctcttccgatctGGGCTTTATCTAACTCGCTGTAG

PCR #2 - Reverse Primer	gctcttccgatctTGCTATGGGCAAAGTTTCGTGG

Table S4. Sequences of Amplicon-NGS PCR Primers, Related to Figure 4

Template CD25 gRNA	
Upstream of Protospacer - Forward Primer	CTTCTCATCAACCCCAGCCC
Upstream of Protospacer - Reverse Primer	CCTAGCACTCTCTTCTCTCATCTC
Inclusive of Protospacer - Forward Primer	GAGATGAGAGAAGAGAGTGCTAG
Inclusive of Protospacer - Reverse Primer	ACCCTTGTGGGTCCATCCAG
Non-Template CD25 gRNA	
Upstream of Protospacer - Forward Primer	CTTCCCATCCCACATCCTCC
Upstream of Protospacer - Reverse Primer	CCCACATCAGCAGGTATGAATCC
Inclusive of Protospacer - Forward Primer	GAGAGCAACTCCTGACTCCG
Inclusive of Protospacer - Reverse Primer	CTTTCTCTGCAGAAGGCCCA
CD55 gRNAs	
Upstream of Protospacer - Forward Primer	CCACTCTCGACAGAGTCCAG
Upstream of Protospacer - Reverse Primer	CAAGTGTCCTCACAGTTGCTG
Inclusive of Protospacer - Forward Primer	CCTAGGTGACTGTGGCCTTC
Inclusive of Protospacer - Reverse Primer	GGCAGATCACTGAGTCCTTCTC
Reference (c-myc)	
Forward Primer	GCCGCATCCACGAAACTTTG
Reverse Primer	GCAAGGAGAGCCTTTCAGAGAAG

Table S5. Sequences of qPCR Primers for Chromatin Immunoprecipitation, Related to Figure 5