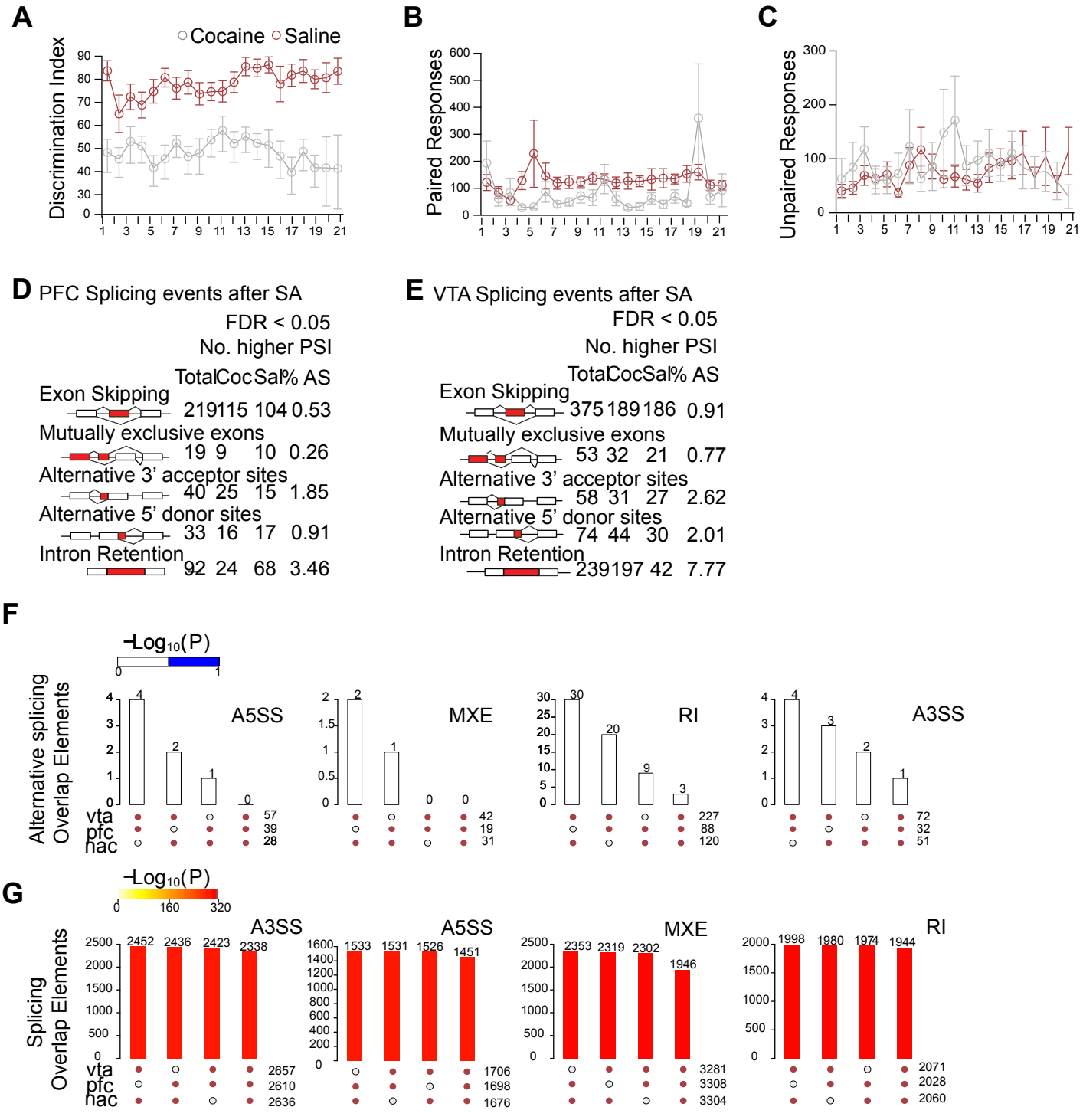
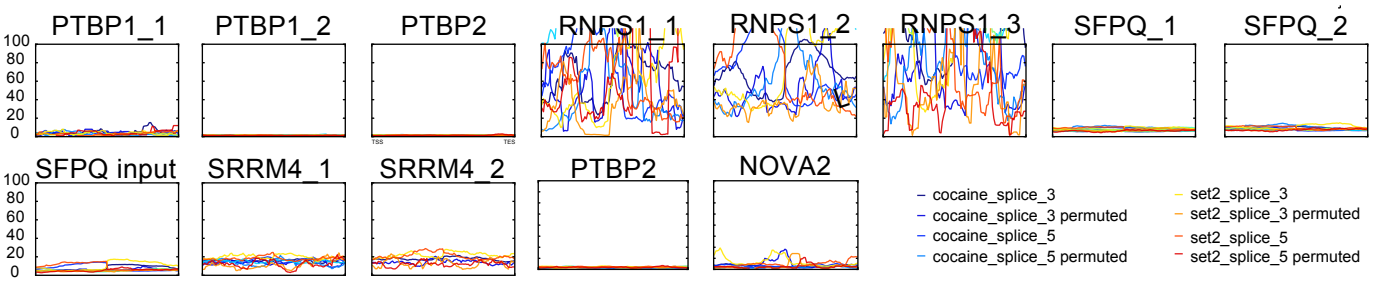
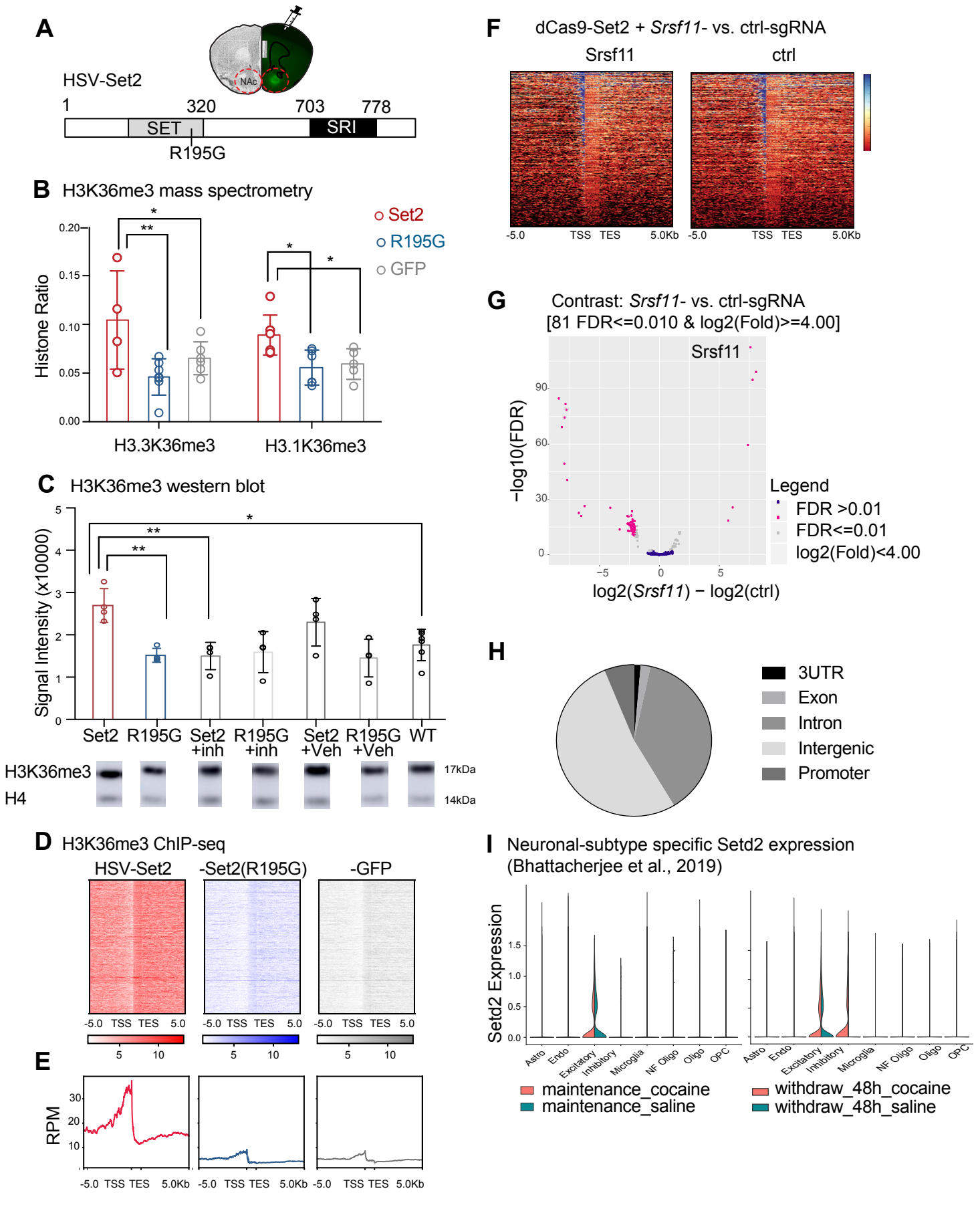


Supplement Figure 1, Related to Figure 1. Cocaine SA behavior and alternative splicing profiles in multiple brain regions. (A) Cocaine SA mice show greater discrimination between the active (cocaine-paired) and inactive (no response-paired) wheels compared to saline SA mice across sessions (Two-way repeated measures ANOVA: interaction effect $F(20,440) = 1.002$, $P=0.4584$; drug effect $F(1,22)=36$, $P<0.0001$; session effect $F(24,440) =1.411$, $P=0.1116$). (B) Cocaine SA mice spin the active wheel more than saline SA mice across sessions. (Two-way repeated measures ANOVA: interaction effect $F(20,440) = 1.487$, $P =0.0810$; drug effect $F(1,22)=9.406$, $P=0.0056$; session effect $F(24,440) =0.9203$, $P=0.5612$). (C) Cocaine SA mice spin the inactive wheel less than saline SA mice across sessions (Two-way repeated measures ANOVA: Interaction effect $F(20,440)=1.407$, $P=0.1136$; drug effect $F(1,22)=0.07903$, $P=0.7812$; session effect $F(24,440) =0.9474$, $P=0.5266$). (D-E) Alternative splicing summary comparing cocaine and saline SA (F) PFC and (G) VTA identified by rMATS. Only alternative splicing events that are FDR < 0.05 are shown in the table. (F-G) Comparisons of alternatively spliced transcripts (top panel; FDR < 0.05, Δ Psi > 0.1) and total spliced transcripts (bottom) among three different tissue types (NAc, PFC and VTA) and splicing types (MXE, A3SS, A5SS, IR). Significance of each overlap was calculated by hypergeometric tests. Red suggests highly significant overlap.



Supplement Figure 2, Related to Figure 3. Profile plots of splice factor motifs identified at cocaine SA alternative splicing junctions (top, “bell-curve” like, gray) and permuted control (bottom, “flat-line” like, red).


A
HSV-Set2
NAc
1
320
703
778
SET
SRI
R195G
B
H3K36me3 mass spectrometry
Set2
R195G
GFP
Histone Ratio
0.20
0.15
0.10
0.05
0.00
H3.3K36me3
H3.1K36me3
C
H3K36me3 western blot
Signal Intensity (x10000)
Set2
R195G
Set2 +inh
R195G +inh
Set2 +Veh
R195G +Veh
WT
H3K36me3
H4
17kDa
14kDa
D
H3K36me3 ChIP-seq
HSV-Set2
-Set2(R195G)
-GFP
-5.0 TSS TES 5.0
5
10
E
RPM
30
20
10
-5.0 TSS TES 5.0Kb
F
dCas9-Set2 + Srsf11- vs. ctrl-sgRNA
Srsf11
ctrl
-5.0 TSS TES 5.0Kb
G
Contrast: Srsf11- vs. ctrl-sgRNA
[81 FDR<=0.010 & log2(Fold)>=4.00]
Srsf11
−log10(FDR)
log2(Srsf11) − log2(ctrl)
Legend
FDR >0.01
FDR<=0.01
log2(Fold)<4.00
H
3UTR
Exon
Intron
Intergenic
Promoter
I
Neuronal-subtype specific Setd2 expression
(Bhattacherjee et al., 2019)
Setd2 Expression
Astro
Endo
Excitatory
Inhibitory
Microglia
NF Oligo
Oligo
OPC
maintenance_cocaine
maintenance_saline
withdraw_48h_cocaine
withdraw_48h_saline

Supplement Figure 3, Related to Figure 4. Set2 overexpression globally enriched H3K36me3 and dCas9-Set2 epigenetic editing was specific to target, Srsf11.

(A) Representative image of HSV-Set2-IRES-GFP expression in NAc (top) and schematic of Set2 histone methyltransferase, showing location of point mutation in catalytically dead Set2(R195G) control. SRI: Set2 Rpb1 interacting domain.

(B) Quantitative mass spectrometry of H3.1 and H3.3 K36me3 in HSV-Set2, HSV-Set2(R195G) and HSV-GFP injected NAc (Two-way ANOVA: interaction effect $F(2,29) = 1.0521$, $P=0.362$; Histone variant effect $F(1,29)=0.411$, $P=0.526$, virus effect $F(1,456) = 9.7348$, $P<0.001$)).

(C) Histone western blot of H3K36me3 and Histone H4 on NAc tissue treated with HSV-Set2, HSV-Set2(R195G), HSV-Set2 with inhibitor, HSV-Set2(R195G) with inhibitor, HSV-Set2 with vehicle, HSV-Set2(R195G) with vehicle, and wildtype (WT) NAc. H3K36me3 is enriched in Set2 vs Set2(R195G) or WT controls, but H3K36me3 does not differ between controls. Set2 inhibitor Bay598 restored Set2-mediated increased H3K36me3 to WT level (One-way ANOVA with Tukey's multiple comparisons: Set2 vs R195G: **P = 0.0071, Set2 vs WT: *P = 0.0248, R195G vs WT, P = 0.9626; Set2 vs Set2+Inhibitor: **P = 0.0062, Set2 vs Set2+Vehicle: P = 0.8099, WT vs Set2+Inhibitor: P = 0.9504).

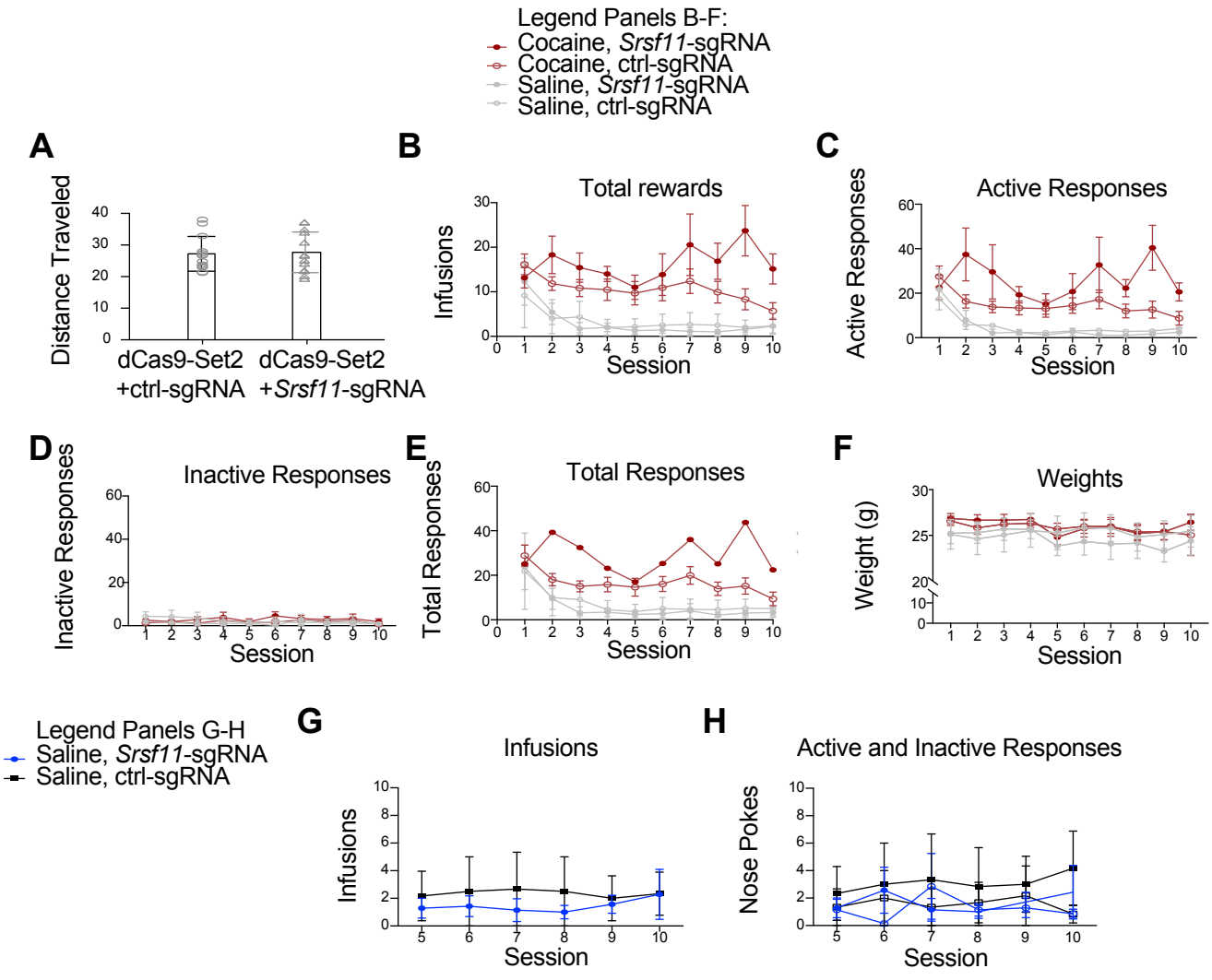
(D) Heatmaps and (E) profile plots of H3K36me3 distribution 5kb upstream and downstream of gene bodies in NAc following injection of HSV-Set2 (red), HSV-Set2(R195G) (blue), or HSV-GFP (gray).

(F) Heatmaps of genome-wide H3K36me3 enrichment in N2a cells transfected with dCas9-Set2 and Srsf11-sgRNA or ctrl-sgRNA identified by CUT&RUN-seq. No difference in enrichment distribution was observed between the two treatments.

(G) Volcano plot from DiffBind analysis between dCas9-Set2 with Srsf11-sgRNA and ctrl-sgRNA. Srsf11 was the highest differentially enriched gene (Fold change = 152.2, FDR = 5e-75). Pink dots indicate other significantly differential H3K36me3 enriched peaks (log2(Fold-change)>=4).

(H) Genomic locations of 122 identified H3K36me3 differential enrichment in dCas9-Set2 with Srsf11-sgRNA or ctrl-sgRNA. The majority of off-target enrichment are at intergenic regions.

(I) Setd2 expression in different PFC cell types of mice from cocaine SA and cocaine SA followed by 48-hour withdrawal.



Supplement Figure 4, Related to Figure 5. Epigenetic editing of Srsf11 was sufficient to increase cocaine reward behavior.

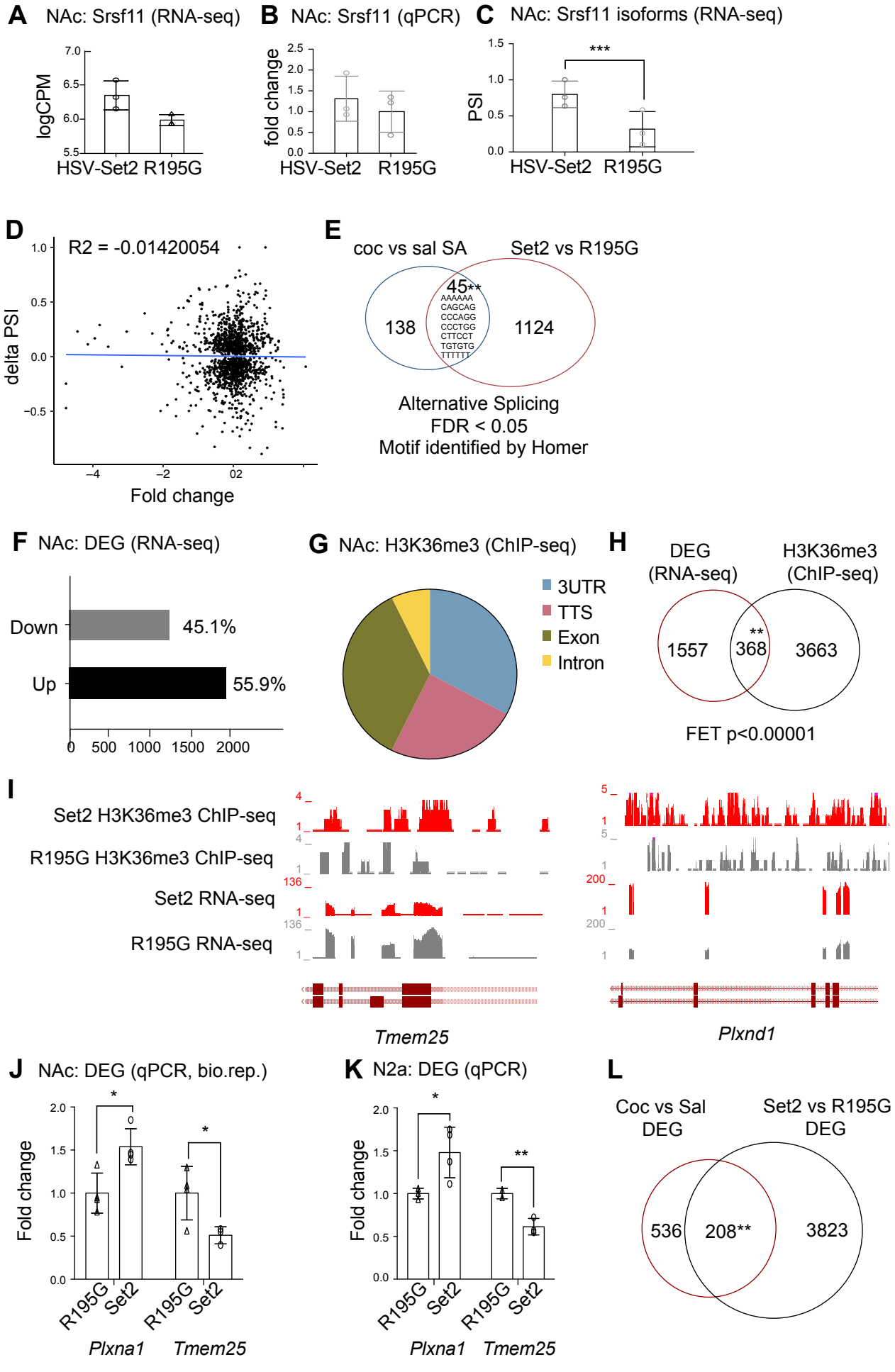
(A) Distance traveled in CPP pre-tests of dCas9-Set2 with Srsf11-sgRNA or ctrl-sgRNA injected animals. (Student's t-test, non-significant).

(B-E) Average rewards (infusions) (B), active responses (C), inactive responses (D) and total responses (E) for 10-day cocaine (red) and saline (grey) SA for both dCas9-Set2 with Srsf11-sgRNA or ctrl-sgRNA treatments.

(F) Weights (g) for 10-day cocaine (red) and saline (grey) SA (n=12) for both dCas9-Set2 with Srsf11-sgRNA or ctrl-sgRNA (One-way ANOVA, non-significant).

(G) Average saline infusions (n=12 per group) after viral delivery per session. No difference in infusion rate between dCas9-Set2 with Srsf11-sgRNA or ctrl-sgRNA treated mice (Two-way ANOVA, non-significant; Session x Plasmid $F(4,44) = 0.325$, $P=0.8597$; Plasmid $F(1,11)=0.2678$, $P=0.6151$; Subject $F(11.44)=33.87$, $P<0.001$).

(H) Average active and inactive responses (n=12 per group) after viral infusion (Day 4) per session for saline SA. Mice showed the same active and inactive rate for saline between dCas9-Set2 with with Srsf11-sgRNA or ctrl-sgRNA (Three-way ANOVA, non-significant; Session x Lever x Plasmid $F(4,44) = 1.527$, $P=0.2110$; Level x Plasmid $F(1,11)=0.8892$, $P=0.0866$; Session x Plasmid $F(4,44) =0.1570$, $P=0.9588$; Session x Lever $F(2.746,30.21)=0.6203$, $P=0.5937$; Plasmid $F(1,11)=0.1794$, $P=0.68$; Lever $F(0.6353,6.988)=1.923$, $P=0.1942$; Session $F(4.0, 44)=0.3154$, $P=0.8661$).


A
NAc: Srsf11 (RNA-seq)
logCPM
HSV-Set2 R195G
B
NAc: Srsf11 (qPCR)
fold change
HSV-Set2 R195G
C
NAc: Srsf11 isoforms (RNA-seq)
PSI
***
HSV-Set2 R195G
D
R2 = -0.01420054
delta PSI
Fold change
E
coc vs sal SA
Set2 vs R195G
138
45**
AAAAAA
CAGCAG
CCCAGG
CCCTGG
CTTCCT
TGTGTG
TTTTTT
1124
Alternative Splicing
FDR < 0.05
Motif identified by Homer
F
NAc: DEG (RNA-seq)
Down
45.1%
Up
55.9%
G
NAc: H3K36me3 (ChIP-seq)
3UTR
TTS
Exon
Intron
H
DEG
(RNA-seq)
H3K36me3
(ChIP-seq)
1557
**
368
3663
FET p<0.00001
I
Set2 H3K36me3 ChIP-seq
R195G H3K36me3 ChIP-seq
Set2 RNA-seq
R195G RNA-seq
Tmem25
Plxnd1
J
NAc: DEG (qPCR, bio.rep.)
Fold change
R195G Set2 R195G Set2
Plxna1 Tmem25
K
N2a: DEG (qPCR)
Fold change
R195G Set2 R195G Set2
Plxna1 Tmem25
L
Coc vs Sal
DEG
Set2 vs R195G
DEG
536
208**
3823

Supplement Figure 5, Related to Figure 6. Set2 overexpression in NAc regulated alternative splicing and gene expression.

(A) Quantification of Srsf11 expression from RNA-seq of HSV-R195G and HSV-Set2 injected NAc (FDR > 0.1, non-significant).

(B) Quantification from Srsf11 expression levels by qPCR HSV-R195G and -Set2 injected NAc from a biological replicate cohort (Student t-test, non-significant).

(C) Srsf11 PSI values quantified by rMATS in HSV-R195G and -Set2 NAc (FDR < 0.001).

(D) Scatter plot of expression fold change and ΔPSI of the same transcript. Correlation (blue line) R2 = 0.0002017 suggest little relationship the two variables.

(E) Venn diagram showing overlap alternatively spliced transcripts (FDR < 0.05, ΔPSI > 0.1) by HSV-Set2 vs HSV-Set2(R195G) and cocaine vs saline SA (FET, P < 0.0001).

(F) HSV-Set2 up- and down-regulates gene expression. Among the regulated genes, 45.1% were down-regulated and 55.9% were upregulated relative to HSV-Set2(R195G).

(G) Genomic distribution of H3K36me3 enrichment in HSV-Set2 overexpression NAc.

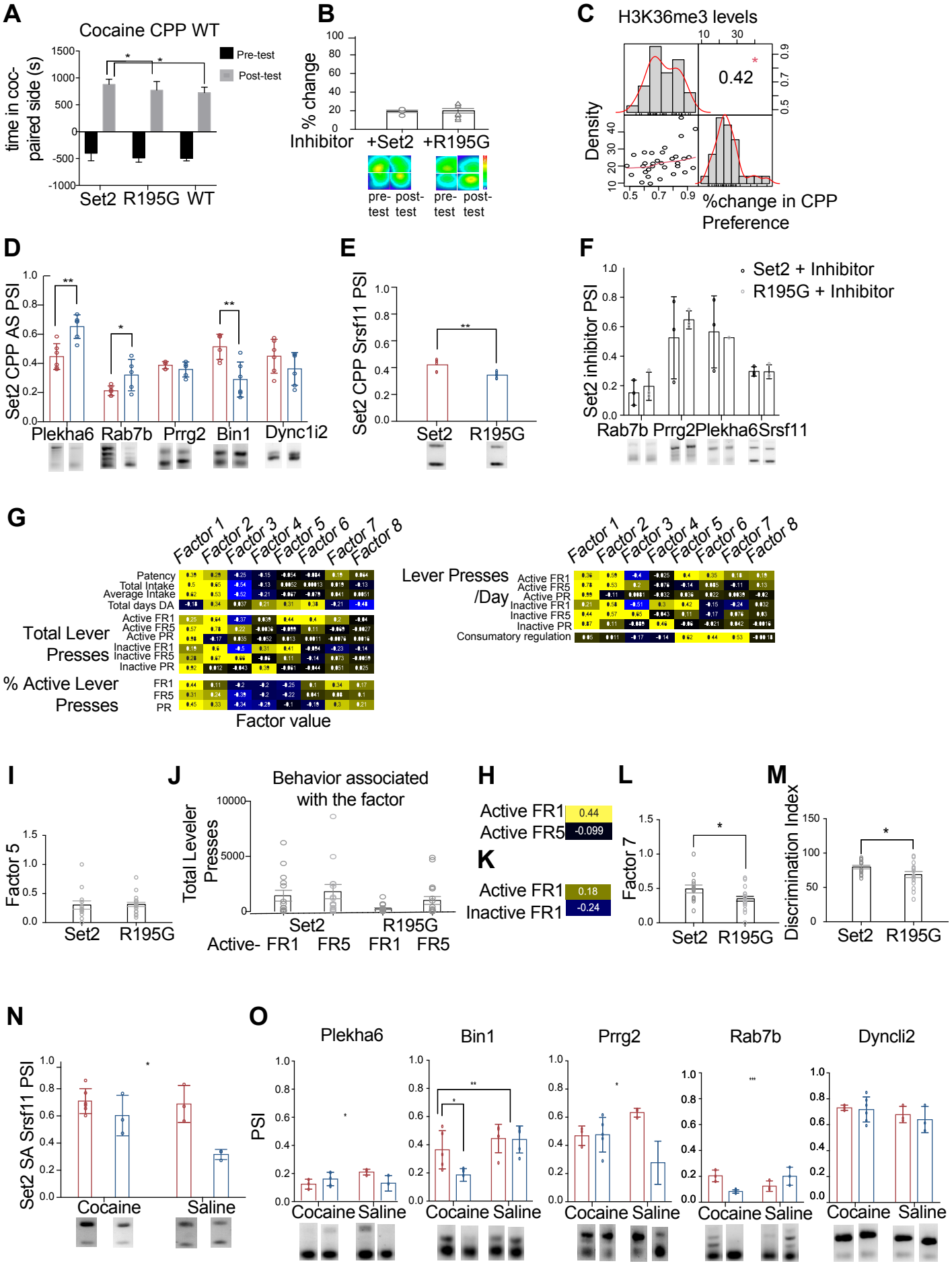
(H) Venn diagram showing overlap between DEG identified by RNA-seq and H3K36me3 enriched genes following HSV-Set2 injection in NAc, relative to HSV-Set2(R195G) (FET P < 0.00001).

(I) Visualization of H3K36me3 ChIP- and RNA-seq from HSV-Set2 and -Set2(R195G) for representative up-regulated gene Tmem25 and down-regulated gene Plxnd1.

(J) qPCR validation of Set2-mediated DEGs (Student's t-test, * P<0.05, n=3 biological replicates).

(K) qPCR validation of Set2-mediated DEGs from transfected N2a cells (n=3 biological replicates) (Student's t-test, *P<0.05, ** P<0.01).

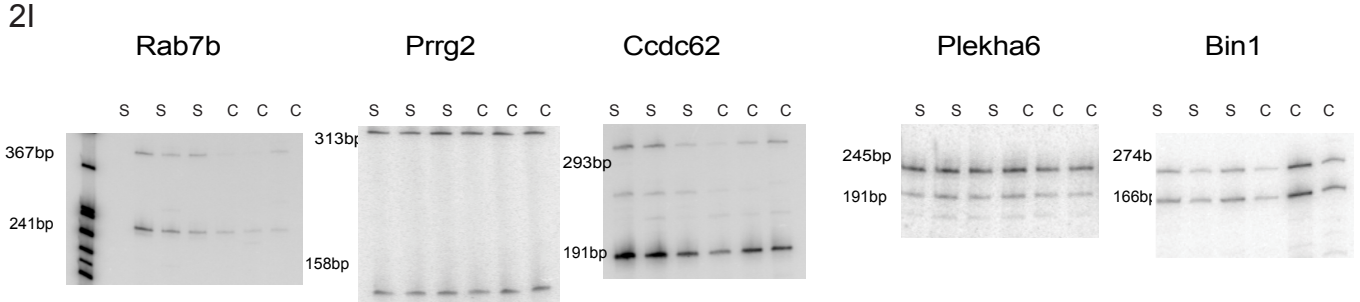
(L) Venn diagram showing overlap between DEGs mediated by cocaine vs saline SA and HSV-Set2 vs HSV-Set2(R195G) (FET P< 0.00001).


A
Cocaine CPP WT
time in coc-paired side (s)
Pre-test
Post-test
Set2 R195G WT
B
% change
Inhibitor +Set2 +R195G
pre-test post-test pre-test post-test
C
H3K36me3 levels
Density
0.42
%change in CPP Preference
D
Set2 CPP AS PSI
Plekha6 Rab7b Prrg2 Bin1 Dync1i2
E
Set2 CPP Srsf11 PSI
Set2 R195G
F
Set2 + Inhibitor
R195G + Inhibitor
Set2 inhibitor PSI
Rab7b Prrg2 Plekha6 Srsf11
G
Factor 1 Factor 2 Factor 3 Factor 4 Factor 5 Factor 6 Factor 7 Factor 8
Patency
Total Intake
Average Intake
Total days DA
Total Lever Presses
Active FR1
Active FR5
Active PR
Inactive FR1
Inactive FR5
Inactive PR
% Active Lever Presses
FR1
FR5
PR
Factor value
Lever Presses /Day
Consumatory regulation
I
Factor 5
Set2 R195G
J
Behavior associated with the factor
Total Leveler Presses
Active- FR1 FR5 FR1 FR5
H
Active FR1 0.44
Active FR5 -0.099
K
Active FR1 0.18
Inactive FR1 -0.24
L
Factor 7
Set2 R195G
M
Discrimination Index
Set2 R195G
N
Set2 SA Srsf11 PSI
Cocaine Saline
O
PSI
Plekha6 Bin1 Prrg2 Rab7b Dyncli2
Cocaine Saline

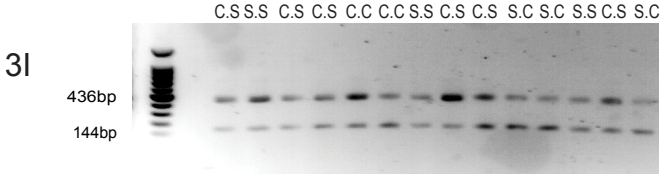
Supplement Figure 6, Related to Figure 7. Set2 overexpression increased cocaine reward behavior.
(A) HSV-Set2 injected mice showed greater CPP score than HSV-Set2(R195G) injected and wildtype (WT) in CPP behavioral test (2way RM ANOVA, for HSV-Set2 vs HSV-Set2(R195G), interaction P=0.04, virus effect P = 0.762, drug effect P < 0.001; for HSV-Set2 vs WT, interaction P=0.0386, virus effect P = 0.2673, drug effect P < 0.001; **P<0.01, * P<0.05). No difference between HSV-Set2(R195G) injected and WT animals in CPP test (2way repeated-measures ANOVA, interaction P=0.5203, virus effect P = 0.6913, drug effect P < 0.001).
(B) No difference in percentage time change in cocaine CPP between Set2+inhibitor and R195G+inhibitor.
(C) Correlation plot (R=0.42, P<0.05*) of H3K36me3 levels and time change in cocaine CPP within each animal.
(D) Quantification of Plehha6, Rab7b, Prrg2, Bin1, Dyncli2 alternative isoform expression of NAc injected with HSV-Set2 or HSV-Set2(R195G) in NAc following cocaine CPP. (Student's t-test, Plehha6 P=0.002, Rab7b P=0.044, Prrg2 P=0.29, Bin1 P=0.0042, Dyncli2 P=0.22; n=6 per treatment, **P<0.01, * P<0.05).
(E) Quantification of Srsf11 alternative isoform expression in HSV-Set2, HSV- Set2(R195G) animals underwent cocaine CPP. Set2 showed higher Srsf11 inclusion levels than R195G (Student's t-test, P=0.003, n = 6 **P<0.01)
(F) Quantification of Srsf11, Plehha6, Rab7b, Prrg2, alternative isoform expression of NAc injected with HSV-Set2 or HSV-Set2(R195G) with Set2 inhibitor Bay598 (Two-way ANOVA with Tukey's multiple comparison, non-significant).
(G) Factor analysis was used to reduce multidimensional behavioral data to factors. The association of each factor with each behavioral endpoint included in the analysis is displayed. Factors were positively (yellow), negatively (blue), or not associated (black) with each endpoint. These particular associations allowed for the interpretation of the how each factor related to Set2 affected cocaine SA behaviors.
(H-M) Data for individual animals for each behavior and each factor are presented. (H, K) Factor loading of factors 5 and 7 with self- administration behaviors (yellow = positive; blue = negative) are presented. (I, (L) Individual data presented for the behaviors associated with each factor. (I) Factor 5 is associated with consummatory regulation. It positively associated with paired lever under a fixed-ratio 1 and negatively associated with unpaired lever under a fixed-ratio 5, and (L) Factor 7 is positively associated with paired lever and negatively associated with unpaired lever under a fixed-ratio 1. (J, M) Individually transformed data for factor 5 (J), which is associated with total levels presses (Student's t-test, * P<0.05), and (M) factor 7, which is associated with discrimination index (Student's t-test, * P<0.05).
(N) Quantification of Srsf11 alternative isoform expression of NAc injected with HSV-Set2 or HSV-Set2(R195G) in animals that underwent cocaine and saline SA (Two-way ANOVA with Tukey's multiple comparison, interaction F(1, 11) = 5.252 P=0.0427, drug effect F(1, 11) = 7.097, P=0.0220, virus effect F(1, 11) = 17.14, P=0.0016).
(O) Quantification of Plehha6, Rab7b, Prrg2, Bin1, Dyncli2 alternative isoform expression of NAc injected with HSV-Set2 or HSV-Set2(R195G) in animals that underwent cocaine and saline SA. (Two-way ANOVA, Plehha6 interaction F(1, 10) = 6.195 P=0.032, drug effect F (1, 10) = 1.41, P=0.26, virus effect F (1, 10) = 0.82, P=0.39 ; Rab7b interaction F(1, 11) = 20.36 P=0.0009, drug effect F (1, 11) = 0.76, P=0.40, virus effect F (1, 11) = 1.048, P=0.33; Prrg2 interaction F(1, 10) = 9.264 P=0.012, drug effect F (1, 10) = 0.082, P=0.78, virus effect F (1, 10) = 8.476, P=0.0155; Bin1 interaction F(1, 11) = 4.089 P=0.0592, drug effect F (1, 11) = 15.18, P=0.0012, virus effect F (1, 11) = 4.881, P=0.0412; Dyncli2 interaction F(1, 11) = 0.085 P=0.7758, drug effect F (1, 11) = 2.131, P=0.17, virus effect F (1, 11) = 0.34, P=0.57; **P<0.01, * P<0.05).

Data S1, Related to Figures 2, 3, 4, 6.Full gels used for quantification of splicing PCR

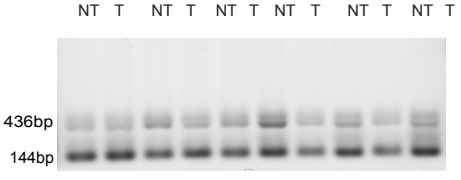
2I



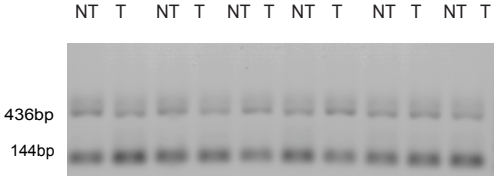
3I



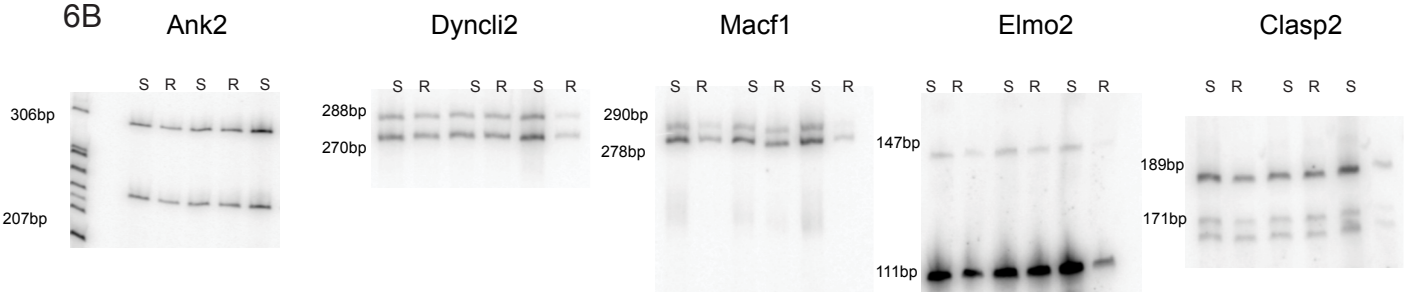
4G



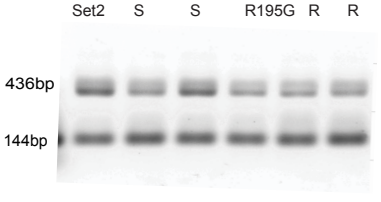
4H



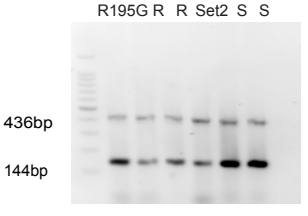
6B



6H

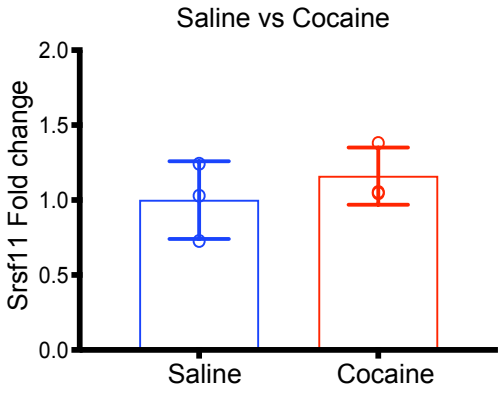


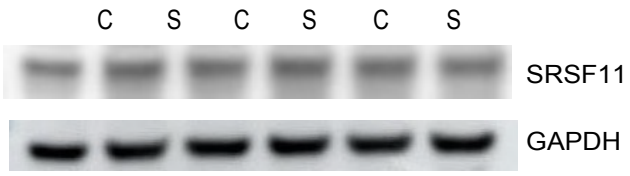
6I



Data S2, Related to Figures 3, 4, 6. Full western blots for protein quantification.

FIGURE 3G





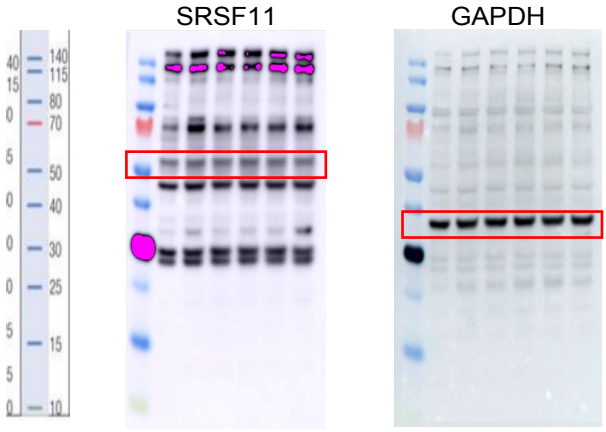
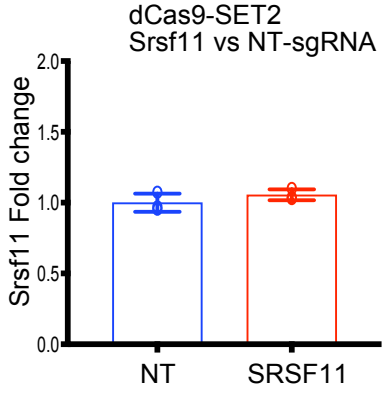
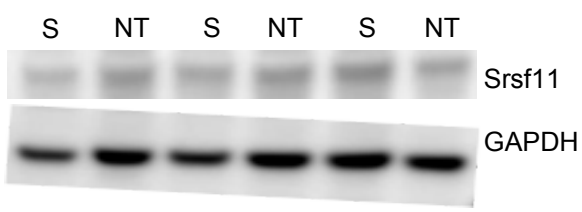


FIGURE 4I





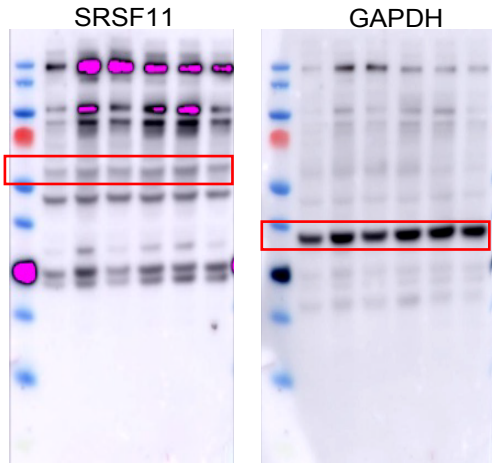


FIGURE 6J

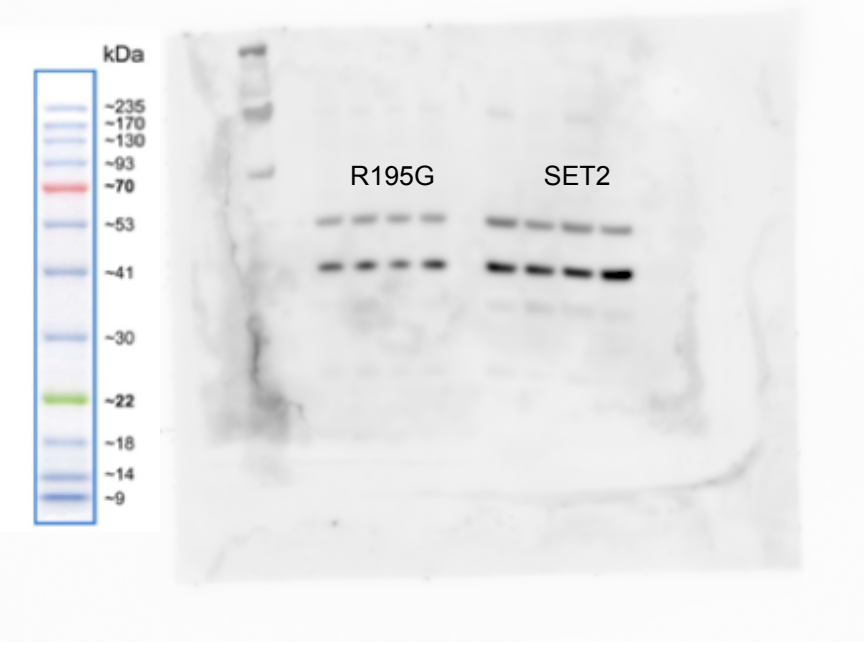


Table S1: Oligonucleotides	
Srsf11 mRNA qPCR Primers	F:ACTGGAGTCTACCCCCTTGT
	R:CCACCCAACACTAGCCATCT
Srsf11 splicing PCR primers	F:GCTTTTGAAAGTTGAGAGCACACCCC
	R:ACCTGCCACTGCATTAGCTGGTGCC
Rab7b splicing PCR primers	F: GACACAGGTGGTCAGGAGCGGTTCC
	R: CTATGAGGTGGTTCTCTGCGGTGCCC
Prrg2 splicing PCR primers	F: ACACAGTATGAGGGGCCGTCCTTCCC
	R: TCCCTTGCCATTGTACGTATAGCTTTCCC
Ccdc62 splicing PCR primers	F: TCTTCAGAAGACGCAGCAGCAGCTCC
	R: CTCTCACCACACACGTCTCCGCCATC
Plekha6 splicing PCR primers	F: TCCTACAGCCGTGCCCGCATCTACTC
	R: TCGTTTAGCTTGAAGGTGTGGAGGCC
Bin1 splicing PCR primers	F: TCCCCTGCTGCTACCCCTGAGATCAG
	R: AGGTTGCTTCACTGGCTGCTGTCTCC
Srsf11-T1-sgRNA	GATCAAGATCTCGCTCGAGG
Srsf11-N2-sgRNA	TGATTAGATCTACTTCACCGAGG
Srsf11-N3-sgRNA	CAGCTGCTTGATCACGCAAATGG