Cocaine-induced Chromatin Modifications Associate With Increased Expression and 3-Dimensional Looping of *Auts2*

Supplementary Information

Supplementary Methods

Animals

Eight week old male Sprague-Dawley rats (200-300 g) and 7- to 12-week-old C57BL/6J male mice (20–30 g) were purchased from the Jackson Laboratory, Bar Harbor, Maine. 2- to 3-month-old *Drd1* (D1)-tomato and *Drd2* (D2)-green fluorescent protein (GFP) bacterial artificial chromosome (BAC) transgenic mice of both sexes as well as male D1-Cre and D2-Cre BAC transgenic mice (25–32 g, C57BL/6J background; Gene Expression Nervous System Atlas [GENSAT] Project; www.gensat.org) were obtained from N. Heintz (Rockefeller University) and C. Gerfen (National Institute of Mental Health). Despite the fact that both lines are fully backcrossed to C57BL/6J, small differences have been seen in several baseline behaviors, such as cocaine conditioned place preference as is evidenced in Fig. 5.

We used rats and mice in this study to take advantage of strengths unique to each species. Rats were used for procedures that require a relatively large amount of tissue (e.g., 3C and 4C), which also enabled separate analysis of NAc core and shell subregions. Such separate subregion analyses are not feasible in mouse based on our extensive experience. Conversely, transgenic mouse lines enable the separate biochemical and behavioral analysis of cell type-specific regulation, which is not feasible in rat.

Mice had ad libitum access to food and were group-housed (2-5 mice per cage) at 22–25°C on a 12-hour light/dark cycle. They were habituated for at least 1 week before experimentation and assigned randomly to experimental groups. All behavioral experiments except for self-administration were performed during the light cycle. Behavioral experiments were centered around midday. Experiments were carried out by experimenters blind to animal treatment conditions and were conducted in accordance with the guidelines

of the Institutional Animal Care and Use Committees at Mount Sinai. Female mice were balanced by estrous cycle during experiments.

Chromosome conformation capture (3C) and circularized chromosome-conformation capture (4C)

Protocols were modified from (1). An overview of the protocol is shown in Fig. S14. Approximately 50 mg wet weight tissue—12-gauge punches from 5 males rats (4 punches of each NAc subregion/rat) or 14-gauge punches from 8 male mice (2 punches of whole NAc/mouse)—were Dounce-homogenized in lysis buffer (10 mM Tris-HCl, pH 8.0; 10 mM NaCl, 0.2% NP-40, protease inhibitor cocktail (Sigma, #P2714-1BTL)) and then cross-linked for 10 min at room temperature in 1% formaldehyde. Crosslinking was stopped by adding glycine to a final concentration of 0.125 mM, lysed and centrifuged for 5 min at 240 x g. The pellet was washed 4 times in the 1X New England Biolabs (NEB)-buffer applicable for the restriction enzyme of interest by centrifugation for 5 min at 2,650 x g and removing the supernatant. 0.1% SDS was added and samples were incubated at 65°C for 10 min to make chromatin accessible for digestion. SDS was quenched by adding Triton X-100 to a final concentration of 1%. Samples were digested with NcoI (for 4C, 6 bp-cutter) or Nla3 (for 3C, 4 bp-cutter) (both NEB, #R0193L and #R0125L, respectively) at 37°C overnight. The digestion enzyme was inactivated by adding SDS to a final concentration of 1.4% for 30 min at 65°C. Next, ligation in a large volume (7.55 ml) was performed at 16°C for 5 h after ligation buffer was added to the samples (500 mM Tris-HCl, pH 7.5, 100 mM $MgCl_2$, 100 mM DTT dithiothreitol, 0.9% Triton TX-100, 0.1 mg/ml bovine serum albumin (Sigma, # B42087), 1 mM ATP (Sigma, #A1852-1VL)). T4 DNA ligase (5 U/µl, Life Technologies, #15224-041) was added to 3 samples, while the 4th sample was used as a no ligase control (final ligase concentration: 6.6 U/ml). After ligation, samples were reverse cross-linked at 65°C overnight and again for 2 h with proteinase K (final concentration: 0.066 mg/ml, Life Technologies, # AM2546). DNA was extracted with 1:1 volume of phenol (pH 8.0, Fisher), and phenol-chloroform (1:1) (pH 8, Fisher). DNA was precipitated using 1/10 the volume of 3M sodium acetate (pH 5.4) and 2.5x the volume of ice-cold ethanol overnight at -80°C. The samples were centrifuged at 3,220 x g for 45 min at 4°C. The pellet was re-suspended in TE-buffer (pH 8.0) and transferred to a 2.0 ml tube. To increase the purity of the DNA, another cleanup with phenol and phenol-chloroform was conducted. Ethanol precipitation was performed for at least 1 h at -80°C and DNA was

pelleted by centrifugation at 18,000 x g at 4°C. The pellet was washed five times with 1 ml of 70% ethanol and re-dissolved in 50 µl of TE, pH 8.0 and incubated for 30 min at 37°C after adding 1 µl of RNAse A (20mg/ml, Life technologies, #12091-021). An additional DNA-purification was conducted by using the DNeasy Blood & Tissue-kit (Qiagen, #69504).

For 4C, the samples were re-suspended in 200 µl NEB-buffer and the entire 3C-procedure was repeated, using the 4 bp-cutter Dpn2 as second restriction enzyme (NEB, #R0543L). 4C-primers were designed for *Auts2* in an intronic, CpG-rich, promoter-proximal region. This region fulfilled the criteria of ideal length of the 4C-fragment (291 bp). The primers were designed with the UCSC genome browser (Rat: RN6; mouse: mm10), NCBI primer Blast, and IDT tools. 4C libraries were amplified by outwards-PCR with the 4C primers and purified with a Minelute PCR Purification kit (Qiagen, #28004) (PCR conditions: 2 min at 94°C; 30 cycles of: 15 sec at 94°C; 1 min at 55°C; 3 min at 68°C; 1x: 7 min at 68°C; hold at 4°C). Quality and amount of libraries and PCR products were assessed with an Agilent High Sensitivity DNA kit (Agilent Technologies, #5067-4626). For next-generation sequencing, the samples were processed with a TruSeq Nano DNA Library Prep Kit (Illumina, #FC-121-4001). The library was submitted for next-generation paired-end sequencing to the Mount Sinai sequencing core.

The 4C-sequencing data were first checked for quality using the various metrics generated by FastQC (v0.11.2) (2), which indicated a substantial presence of primer sequences in the reads. Cutadapt (v1.8.1) (3) was thus used to trim off the presence of either of the two primers used, and the trimmed reads were then aligned to the rat RN6 genome using default settings of Bowtie (v2.2.0) (4). The Bioconductor package FourCSeq (v1.2.0) (5) was used to generate the list of valid 4C fragments on the rat genome, after which a custom-written R script was used to count the reads aligning precisely at the edges of the fragments. Differential analysis of fragment read-counts between cocaine treatment and saline samples were then performed using the DESeq2 Bioconductor package (v1.6.3) (6).

For 3C, physical looping interactions were quantified by PCR. Primers were designed less than 120 bp from the Nla3 restriction site, using the UCSD genome browser, NCBI primer Blast, and IDT tools. Mouse

genome regions of interest were determined by using the USCS genome browser BLAT-tool to identify regions that are homologous to Rat 4C-areas. For PCR, GoTag Green Master Mix (Promega, #M7122), Primers A and B (0.5 µM each), 5% dimethyl sulfoxide (NEB, #12611), as well as a dilution series of library template, starting at 4 µl. PCR conditions were: 5 min at 95°C; 40 cycles of: 30 sec at 95°C; 30 sec at 58-61°C; 30 sec at 72°C; 1 x 30 sec at 95°C; 30 sec at 60°C' and 8 min at 72°C. The melting temperature was optimized for each primer pair separately. Primer specificity was confirmed with Sanger sequencing. The PCR products were resolved on a 2% agarose gel. Interaction levels between two primers were measured semi-quantitatively using band intensities with ImageJ after subtracting the background. Primers are listed in Table S5.

Cocaine administration

Cocaine was given by repeated injections or by self-administration. For the former, male and female mice received daily intraperitoneal (I.P.) injections of cocaine hydrochloride (Sigma, #C5776) for 7 consecutive days at 20 mg/kg body weight. Mice were analyzed 24 h after the final injection. Control mice received saline injections. Bilateral 14-gauge NAc punches were taken from each animal. Please note that cell type-specific analyses are not readily feasible in mouse self-administration models due to the relatively low throughput of this behavioral procedure and the requirement for relatively large amounts of tissue for cell isolation.

Cocaine self-administration

Male rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), implanted with chronic indwelling jugular catheters, and trained for i.v. self-administration as previously described (7; 8). The catheter tubing was passed subcutaneously from the back to the jugular vein and 1.2 cm of tubing was inserted into the vein and secured with silk suture. Following surgery, animals were singly housed, and allowed to recover for 48-72 h. Each animal was maintained on a reversed light-dark cycle (7:00 am lights off; 7:00 pm lights on) and all self-administration procedures occurred during the active/dark cycle. Sessions were 6 h in length and animals self-administered cocaine (0.8 mg/kg/inj over 5 sec) on a fixed-ratio 1 schedule of administration. At the beginning of each session a house light was illuminated

signifying the availability of drug. Concurrent with the start of each injection, the lever retracted, the house light was turned off, and a stimulus light was activated for 20 sec to signal a time-out period. Under these conditions, animals acquired a stable pattern of intake within 1 to 5 days. For self-administering animals, acquisition (day 1) was counted when the animal reached 70% responding on the active lever and 20 or more responses. Following acquisition, the animals were given unlimited access to a cocaine-paired lever for six hours per day for a period of 10 days. An inactive lever was present and responding was recorded, but had no programmed consequence. Control animals underwent the same experimental procedures but had access to a saline-paired active lever.

Human brain tissue

NAc samples from humans addicted to cocaine or matched controls, or a separate cohort of depressed patients and their matched controls, were acquired from the brain bank at McGill University. The cocaine cohort was composed of 30 male subjects, ranging in age between 15 and 53 years, the depression cohort consisted of 19 male subjects between 25 and 81 years. In all subjects, the death was sudden without protracted medical illness or a prolonged agonal state. Subjects met the Structured Clinical Interview for DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV) criteria for cocaine dependence or depression. Groups were matched for mean subject age, postmortem delay, and pH. For all subjects, psychological autopsies were performed as described previously (9). A sufficient number of female subjects was not available for study of possible sex differences in humans.

Quantitative PCR

Brain samples were homogenized in Trizol (Invitrogen) and processed according to the manufacturer's instructions. RNA was purified with an RNeasy Micro-kit (Qiagen, #74004). RNA was then reverse transcribed using a Bio-Rad iScript Kit (Biorad, #170-8890). cDNA was quantified by quantitative PCR (qPCR) using PerfeCTa SYBR Green Fast mix (Quanta Bioscience, #95073-012). Each reaction was run in triplicate and analyzed following the ΔΔCt method using up to 3 of the following control genes as normalization controls: glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), actin (*Actb*), 18S rRNA

(*RNA18S5*), and ß2-microglobulin (B2m). Primers were designed using the NCBI Primer design tool and are shown in Table S5.

FACS isolation of MSNs from D1-Tomato and D2-GFP mice

NAc punches from D1-Tomato and D2-GFP mice of both sexes were collected in ice-cold digestion buffer (88.5% Hibernate A (Brainbits, #HA-CaMg500), 5% w/v D-(+)-Trehalose dihydrate (Sigma, #T9531-25G), 0.05 mM APV (Sigma, #A5282), 0.025 U/ml RNA-se free DNAse I (NEB, #M0303L)) and digested in papain solution (Worthington Biochemical corporation, #LS3119 in FACS-buffer, see below) at 37°C on a rotor (225 rpm) for 45 min. Tissue was incubated for 5 min in 2 ml FACS-buffer (83% Hibernate A, 0.084 mM APV, 5.38% Ovomucoid Albumin Inhibitor solution (see below), 16.7 U/ml RNAse-free DNAse, 8% trehalose). Next, the tissue was titrated to allow for cells to gently disperse. The cell suspension was gently passed through a 70 µm filter mesh (BD, #352350) and kept on ice. To select for intact, dissociated cells, the samples were passed over a gradient: 32 ml of FACS buffer were added to one vial of Ovomucoid Albumin Inhibitor (Worthington Biochemical Corporation, #LK003182) and mixed well. 2 ml of gradient were added to a 15 ml tube and the cell suspension was slowly transferred on top of the gradient before being spun at 100 x g for 6 min at 4°C. After removing the gradient solution, 1 ml of FACS buffer was added to the pellet and centrifuged twice at 200 x g for 6 min at 4°C. Cells were re-suspended in 400 µl of FACS buffer and passed through a 40 µm filter mesh tube (Fisher, #08-771-23). 2.5 µl of DAPI (200 mg/µl, Thermo Fisher Scientific, # D3571) were added to the cell suspension. Cells were sorted on a BD FACS Aria II and collected into Trizol LS-reagent (Ambion, #10296-028) and RNA was extracted using the Direct-zol RNA miniprep kit with DNAse treatment (Zymo, #R2050), following manufacturer's recommendations. RNA quality was assessed with an Agilent RNA 6000 PICO kit (Agilent Technologies, #5067-1513).

Quantitative chromatin immunoprecipitation (qChIP)

qChIP was performed as previously described (10). DNA was purified with a PCR Purification Kit (Qiagen, #28104). Concentration and shearing efficiency were assessed by use of the dsDNA HS Assay and Agilent High-Sensitivity DNA kits (Thermo Fisher Scientific, #Q32851; Agilent Technologies, #5067-4626).

Amounts of chromatin and antibodies were optimized prior to the experiments. ChIP grade antibodies to H3K4me3 (Abcam, #ab8580, 5 µg per sample), H3K27ac (Abcam, # ab4729, 5 µg per sample) or the ChIPAb+ CTCF - ChIP Validated Antibody (5 µg per sample, EMD Millipore, #17-10044) were used per ChIP reaction. Controls were the PP64 Rabbit IgG (EMD Millipore, #PP64) and the mouse IgG provided in the CTCF-ChIP kit (EMD Millipore, #17-10044). Data are represented as ChIP/input. Primers are shown in Table S5.

Bisulfite sequencing

14 gauge NAc punches from 3 male mice were pooled and DNA was purified with a QIAamp DNA Mini-kit (Qiagen, #51304) followed by a Qiaquick PCR Purification kit (Qiagen, #28104). Bisulfite conversation was conducted with the EZ DNA methylation kit (Zymo, #D5001), starting with a DNA amount of 500 ng per sample. Primers were designed with the Zymo Research Primer Seeker-tool and validated by Sanger-sequencing. For PCR, Platinum™ Taq Green Hot Start DNA Polymerase and dNTP Mix (Thermo Fisher Scientific, #11966-018 and 18427-013) were used according to manufacturers' instructions. PCR conditions were: 2 min at 94°C; 40 cycles of: 30 sec at 94°C, 30 sec at 55-62°C, 1 min at 72°C; hold at 4°C. Products were purified with the Qiaquick PCR Purification kit. Quality and concentration of samples were assessed with dsDNA HS Assay and Agilent High-Sensitivity DNA kits (Thermo Fisher Scientific, #Q32851; Agilent Technologies, #5067-4626). Libraries were prepared with a TruSeq Nano DNA Library Prep Kit (Illumina, #FC-121-4003) according to manufacturers' instructions. Libraries were pooled and sequenced on an Illumina MiSeq sequencer, using a 600 cycle, V3-chemistry sequencing kit (Illumina, #MS-102-3003). Approximately 400,000 molecules were sequenced per library. Sequencing data were then analyzed using Bismark software (Reference: PMID: 21493656, http://www.bioinformatics.babraham.ac.uk/projects/bismark) to determine the level of methylation at individual CpG dinucleotide resolution. Briefly, sequences were aligned to both bisulfite converted and reference genomes using the Bowtie2 aligner, and the methylation status was determined for each individual CpG in each aligned molecule. For each CpG, we then calculated the level of methylation (fraction of molecules methylated at that CpG). Primers are shown in Table S5.

CRISPR epigenome-editing

A vector coding the dCas9-DNMT3ACD-DNMT3LCD-3xFLAG fusion gene was constructed by Gibson assembly. The human codon optimized nuclease-null Cas9 from *S. pyogenes* coding plasmid was a gift from George Church, Harvard University (Addgene, #48674). The sequence of the catalytic domain of *Dnmt3a* fused with the C-terminal domain of *Dnmt3L* was amplified from a published plasmid (11). The 3xFLAG tag was assembled by annealing the complementary partially overlapping oligonucleotides and using extension PCR. As a control construct, a FLAG-dCas9 was synthesized and cloned into an entry vector in conjunction with GENEWIZ. It was then cloned into a hSyn-GW-IRES-mCherry destination vector and validated by Sanger sequencing.

For designing guide RNAs, we followed the protocol from the Church laboratory provided as online source by Addgene (https://www.addgene.org/crispr/church/). Guide RNA sequences were de-novo synthesized using G-blocks from IDT. They were then cloned into a TOPO-vector using the Zero Blunt® TOPO® PCR Cloning Kit, with One Shot® TOP10 Chemically Competent *E. coli* cells (Thermo Fisher Scientific, #K280020) according to manufacturers' instructions. Constructs were amplified and harvested with a QIAGEN Plasmid Midi Kit (Qiagen, #12143). The sequence of guide RNAs was confirmed by Sanger sequencing. A non-targeting guide RNA was utilized as a control (12). The sequence GCG AGG TAT TCG GCT CCG CG was de-novo synthesized and cloned as described above. Guide RNA sequences are shown in Table S5.

miRNA vectors

miR constructs were designed with the Thermo Fisher Scientific BLOCK-iT™ RNAi Designer tool. The sequences were: *Auts2*-miR top: TGC TGT AAA GTG GAA GGT CGT GCC AGG TTT TGG CCA CTG ACT GAC CTG GCA CGC TTC CAC TTT A, *Auts2*-miR bottom: CCT GTA AAG TGG AAG CGT GCC AGG TCA GTC AGT GGC CAA AAC CTG GCA CGA CCT TCC ACT TTA C. The constructs were purchased from IDT and cloned into a pcDNA 6.2 GW/miR vector with the BLOCK-iT™ Pol II miR RNAi Expression Vector Kit (Thermo Fisher Scientific, #K493500) according to the manufacturers' instructions. The provided *LacZ* construct served as a control. They were then transformed into One Shot TOP10

chemically competent *E. coli* (Thermo Fisher Scientific, #C404003C404003) according to the manufacturers' instructions, amplified, and harvested with a QIAGEN Plasmid Midi Kit (Qiagen, #12143). The correctness of cloning was validated by Sanger sequencing.

Transfection and RNA purification from Neuro2A cells

Neuro2A cells (ATCC, #CCL131) were transfected with lipofectamine 2000 transfection reagent (Thermo Fisher Scientific, #11668-019) as described (13). Cells were rinsed with PBS, lysed and harvested 24 h later using the RNeasy Minikit (Qiagen, #74104) and Qiashredder columns (Qiagen, #79656) according to manufacturers' instructions. RNA was converted to cDNA and examined by qPCR as described above.

Herpes simplex virus (HSV) vectors

Overexpression vectors for *Auts2* and *Caln1* were purchased from Origene (# MR225787 and MR202484). They were cloned into a pENTR1A Dual Selection Vector (Life Technologies, #A10462). Products were purified from a 1% agarose gel with the Qiaquick Gel extraction kit (Qiagen, #28706), and the PCR purify kit (Qiagen, #28104). Ligation into the open pENTRA vectors was done with T4 ligase (1 U/μl, Life Technologies, #15224-041) at a vector/insert ratio of 1:3 (total DNA < 100 ng; PCR conditions: 15 h at 14°C; hold at 4°C). Constructs were transformed into One Shot TOP10 chemically competent *E. coli* (Thermo Fisher Scientific, #C404003C404003), amplified and harvested with a QIAprep spin miniprep kit (Qiagen, #27104), and validated by Sanger sequencing. The insert was then subcloned into a p1005-LS1L-vector by using the Gateway® LR Clonase® Enzyme mix (Thermo Fisher Scientific, #11791019) according to manufacturers' instructions. Correct clones were amplified, harvested with a Qiagen Plasmid Midi kit (Qiagen, #12143), and sent for HSV packaging to the viral core facility at the McGovern Institute for Brain Research at Massachusetts Institute of Technology. Proper expression of the viruses was confirmed under a fluorescent microscope as well as by qPCR from NAc punches of stereotaxically injected Cre driver mice vs. a GFP-control vector.

Conditioned place preference

Conditioned place preference was carried out in a 3-chambered box containing 2 end-chambers and a small middle chamber as described previously using Med Associates software (14). Prior to the experiment, mice were allowed to habituate to the test environment for 1 h.

Data analysis

Data were analyzed with Prism 6.0 (GraphPad Software, San Diego, California). Differences between 2 groups were assessed using Student's t-tests. One-way ANOVAs were used when 3 or more ungrouped comparisons were made, while 2-way ANOVAs were used in instances in which 4 groups were analyzed across 2 separate parameters. Following 2-way ANOVAs, Bonferroni post hoc testing was used to assess statistical differences for all specific comparisons, while Tukey post-hoc tests were used for One-way ANOVAs. For the analysis of changes in bisulfite sequencing over CG positions, One-way ANOVA was performed for each CG individually as baseline methylation varied considerably between CGs. Furthermore, methylation at various CGs may not be independent measures. All summary data are expressed as means ± SD. Significance was set at $p < 0.05$. See figure legends for statistical details of individual experiments, including statistical tests used, t, P, and F values, and number of subjects or samples tested.

Supplementary Tables

Table S1. Genes with most cocaine-induced chromatin modifications in NAc

Gene	No. modifications
Celf2	50
Csmd1	45
Myt1l	42
Dlgap2	40
Macrod2	39
Caln1	35
Auts2	34
Anks1b	32
Kirrel3	32

Based on analysis of data in Feng et al. (14).

Table S2. Cocaine-induced changes in *Auts2*-DNA interacting fragments (NAc shell)

NAc shell	chr	log2 FC	pvalue	padj	location	gene
12:26099150-26111925	chr12	11.61	0.000	0.000	intergenic	
12:27213674-27214837	chr12	11.82	0.000	0.000	intergenic	
12:27284010-27294234	chr12	-9.33	0.000	0.000	intergenic	
12:26156256-26157848	chr12	10.77	0.000	0.000	intergenic	
12:26141041-26143417	chr12	11.82	0.000	0.000	intergenic	
12:27582093-27583052	chr12	-9.37	0.000	0.000	intergenic	
12:26625862-26627898	chr12	10.27	0.000	0.000	intergenic	
12:26353729-26361053	chr12	-9.70	0.000	0.000	intergenic	
12:26541760-26542376	chr12	-9.22	0.000	0.000	intergenic	
12:26588563-26591425	chr12	-9.27	0.000	0.000	intergenic	
12:27119147-27123135	chr12	-7.50	0.000	0.000	intergenic	
12:26126413-26130008	chr12	-8.36	0.000	0.000	intergenic	
12:26164477-26167050	chr12	-8.52	0.000	0.000	intergenic	
12:26939653-26940640	chr12	-8.60	0.000	0.000	intergenic	
12:26778788-26779396	chr12	10.73	0.000	0.001	intergenic	
12:25752890-25754378	chr12	9.20	0.000	0.001	intergenic	
12:27447303-27452932	chr12	6.53	0.000	0.002	intergenic	
12:26617690-26617933	chr12	9.68	0.000	0.002	intergenic	
12:26721883-26722368	chr12	8.37	0.000	0.003	intergenic	
12:26149819-26156250	chr12	8.50	0.001	0.009	intergenic	
12:27135935-27140886	chr12	-6.89	0.001	0.009	intergenic	
12:27114577-27118474	chr12	-7.89	0.002	0.012	intergenic	
12:27242295-27243882	chr12	-4.85	0.004	0.024	intergenic	
12:27234679-27237963	chr12	3.93	0.006	0.037	intergenic	
1:187351014-187352900	chr1	6.17	0.007	0.044	intergenic	

NAc shell	chr	log2 FC	pvalue	padj	location	gene
12:27108002-27108982	chr12	-7.39	0.010	0.056	intergenic	
12:29680069-29681962	chr12	-7.06	0.011	0.065	Genebody	*Caln1*
16:10456002-10457885	chr16	6.81	0.013	0.072	intergenic	
12:29129317-29131210	chr12	-6.87	0.017	0.083	Genebody	*Wbscr17*
2:77391522-77392571	chr2	5.42	0.017	0.083	intergenic	
12:26283113-26288674	chr12	-6.83	0.017	0.083	intergenic	
12:27194780-27195797	chr12	4.32	0.020	0.088	intergenic	
15:5910643-5917898	chr15	5.49	0.020	0.088	intergenic	
19:54300740-54302072	chr19	6.62	0.020	0.088	intergenic	
15:5923148-5923796	chr15	6.52	0.020	0.088	intergenic	
12:27140892-27147114	chr12	-5.26	0.021	0.089	intergenic	
3:92145843-92156350	chr3	6.44	0.022	0.089	Genebody	*Trim44*
7:110980774-110981634	chr7	6.49	0.022	0.089	intergenic	
12:25946321-25949354	chr12	-5.55	0.028	0.110	intergenic	
9:97367591-97368093	chr9	1.03	0.029	0.111	intergenic	
13:74692155-74693558	chr13	6.16	0.034	0.119	intergenic	
8:122228129-122229181	chr8	6.16	0.034	0.119	Genebody	*Ubp1*
12:27523986-27525798	chr12	-4.15	0.034	0.119	Genebody	*Auts2*
10:105080222-105086179	chr10	6.06	0.036	0.125	Genebody	*Ten1*
12:27183781-27184286	chr12	4.27	0.042	0.142	Genebody	*Auts2*
16:10450034-10452370	chr16	4.78	0.048	0.158	intergenic	

Table S3. Auts2-transcripts

Transcript id	Transcript name	chrom	start	end	log2FC	p value	q value
ENSMUST00000061007	Auts2-201	chr5	131915306	133017693	-0.4673	0.0001	0.0023
ENSMUST00000071591	Auts2-012	chr5	132525600	133018668	-0.5240	0.4751	1.1580
ENSMUST00000100564	Auts2-003	chr5	132501849	133017902	0.0632	0.8320	1.1213
ENSMUST00000159507	Auts2-006	chr5	131951198	132013840	0.7907	0.2898	1.0428
ENSMUST00000160071	Auts2-008	chr5	131913205	132010267	-0.0960	0.3574	1.1065
ENSMUST00000160135	Auts2-011	chr5	131952440	132925230	0.2363	0.4573	1.1547
ENSMUST00000161108	Auts2-004	chr5	132244281	133018274	0.3812	0.2640	1.0089
ENSMUST00000161226	Auts2-001	chr5	131913205	133019214	0.1333	0.1470	0.7974
ENSMUST00000161374	Auts2-007	chr5	131915168	132014557	-0.0607	0.5644	1.1651
ENSMUST00000161804	Auts2-005	chr5	131913658	132092475	0.0330	0.6776	1.1540
ENSMUST00000162101	Auts2-010	chr5	131916427	131936019	0.0798	0.7390	1.1417
ENSMUST00000162979	Auts2-013	chr5	131921294	131931732	-0.4613	0.3906	1.1291

Table S4. Patient data

Group ID	Cause of Death	Age	Gender	Weight (g)	pH	PMD (h)	Comorbidity
control	Accident	19	male	1428	6.55	1.50	
control	Suicide	26	male	1902	6.97	5.00	
control	Natural	47	male	1412	6.49	3.50	
control	Natural	30	male	1517	6.37	11.00	
control	Accident	28	male	1565	6.32	2.25	
control	Natural	41	male	1376	6.00	3.00	
control	Accident	32	male	1516	6.67	4.00	
control	Natural	46	male	1210	6.42	1.50	
control	Natural	27	male	1595	6.55	3.00	
control	Suicide	25	male	1600	6.73	11.25	
control	Accident	15	male	1420	6.72	16.75	
control	Accident	42	male	1470	6.75	2.50	
control	Natural	18	male	1470	6.87	2.00	
control	Suicide	17	male	1460	6.95	7.50	
control	Accident	20	male	1533	6.30	12.00	
Cocaine dep	Accident	51	male	1428	6.73	1.50	Major Depression
Cocaine dep	Natural	39	male	1140	6.86	27.50	Depressive NOS
Cocaine dep	Suicide	36	male	1548	6.54	12.00	Bipolar Disorder
Cocaine dep	Suicide	45	male	1430	6.57	2.75	Major Depression
Cocaine dep	Suicide	35	male	1425	6.81	3.50	
Cocaine dep	Suicide	26	male	1480	6.67	13.50	Major Depression
Cocaine dep	Suicide	24	male	1460	6.89	2.50	
Cocaine dep	Suicide	48	male	1460	6.56	1.75	Major Depression
Cocaine dep	Suicide	33	male	1580	6.75	9.00	
Cocaine dep	Suicide	43	male	1445	6.78	5.25	Psychotic Disorder NOS
Cocaine dep	Accident	24	male	1480	6.33	7.00	
Cocaine dep	Suicide	39	male	1552	6.70	4.25	Major Depression
Cocaine dep	Suicide	38	male	1511.4	6.50	16.00	Depressive NOS
Cocaine dep	Suicide	53	male	1366.1	6.50	28.00	
Cocaine dep	Suicide	43	male	1411.5	6.90	19.00	

	Cause of Death	Age	Gender	Weight (g)	pH	PMD (h)	Comorbidity
Control	Suicide	31	male	1745	6.27	32.5	
Control	Natural	47	male	1412	6.49	12	
Control	Natural	30	male	1517	6.37	30	
Control	Natural	41	male	1376	6	24	
Control	Accident	32	male	1516	6.67	29.5	
Control	Natural	46	male	1210	6.42	19.5	
Control	Suicide	25	male	1600	6.73	36	
Control	Suicide	46	male	1600	6.83	59	
Control	Accident	42	male	1470	6.75	63	
Depression	Suicide	52	male	1370.5	6.3	29	
Depression	Suicide	53	male	1507	6.64	14	
Depression	Suicide	39	male	1264	6.6	25.5	
Depression	Suicide	49	male	1280	6.57	32	
Depression	Suicide	40	male	no info	6.96	22	
Depression	Suicide	53	male	no info	6.91	33.5	
Depression	Suicide	68	male	1100	6.93	32	
Depression	Suicide	51	male	1200	6.74	54	
Depression	Suicide	63	male	1420	6.95	50	
Depression	Suicide	49	male	1432.8	6.7	2.5	

Table S5. Sequences of primers and guide-RNAs

Name	Sequence
3C / 4C	
4C Rat Fvd	CCC TTT CCT GTT CCC CCC A
4C Rat Rev	TCT CCC GGT CTC GCT GTG
Rat Auts2-Caln1 Fvd	CCT CAG CCG AAG AGG ACA TC
Rat Auts2-Caln1 Rev	CTG TGC CGA TAA CCC TGG TG
Rat Control Fvd	AAG TGT CCG GAG AAA GGT GC
Rat Control Rev	CCA GCC CCT CAG CCT TTT T
Mouse Auts2-Caln1 Fvd	GAC TCT CTG CGC TTC CTC C
Mouse Auts2-Caln1 Rev	CTG CTC TCC TCC AAG GTT C
Mouse Control Fvd	CCA AGG TTG ATG CCT GGG AA
Mouse Control Rev	CTG CTCT GAC CCC ATT GTC C
qPCR	
Actb Mouse Fvd	TGT TAC CAA CTG GGA CGA CA
Actb Mouse Rev	GGG GTG TTG AAG GTC TCA AA
Auts2 Mouse Fvd	GAT GCA GTC AGA CCC ACA CA
Auts2 Mouse Rev	CGG CCG GTT AAA AGG CTC TA
B2M Mouse Fvd	GGA GAA TGG GAA GCC GAA CA
B2M Mouse Rev	TCT CGA TCC CAG TAG ACG GT
Caln1 Mouse Fvd	AAC GGC TTC ATC TCC AAG CA
Caln1 Mouse Rev	TGC ATG TCG AAC TGC CAG AA
Prodynorphin Mouse Fvd	AGG TTG CTT TGG AAG AAG GCT
Prodynorphin Mouse Rev	GAC GCT GGT AAG GAG TCG G
Proenkephalin Mouse Fvd	GAG AGC ACC AAC AAT GAC GAA
Proenkephalin Mouse Rev	TCT TCT GGT AGT CCA TCC ACC
Gapdh Mouse Fvd	AAC TTT GGC ATT GTG GAA GG
Gapdh Mouse Rev	ACA CAT TGG GGG TAG GAA CA
RNA18S5 Mouse Fvd	AGG GGA GAG CGG GTA AGA GA
RNA18S5 Mouse Rev	GGA CAG GAC TAG GCG GAA CA
Wbscr17 Mouse Fvd	AGA CGC CTT CCA CGA GAT CC
Wbscr17 Mouse Rev	GAT AAG CCA TTA AGC TGC CTG T
Auts2 Rat Fvd	GAT GCA GTC AGA CCC ACA CA
Auts2 Rat Rev	CGG CCG GTT AAA AGG CTC TA
B2M Rat Fvd	GTT TGA GGC CAG TCT GGG AG
B2M Rat Rev	GCA CTG GCT CTT TGT TTC CC
Caln1 Rat Fvd	AAC GGC TTC ATC TCC AAG CA
Caln1 Rat Rev	TGC ATG TCG AAC TGC CAG AA
Wbscr17 Rat Fvd	GCC TCA GGC AAG CTT TGT TC
Wbscr17 Rat Rev	GGC AGT CCC AAC AGA ATG GA
Actin Human Fvd	GTT GCT ATC CAG GCT GTG CT
Actin Human Rev	GAG GGC ATA CCC CTC GTA GA
Auts2 Human Fvd	GAT GCA GTC AGA CCC ACA CA
Auts2 Human Rev	CGG CCG GTT AAA AGG CTC TA
B2M Human Fvd	TTA AGT GGG ATC GAG ACA TGT AAG
B2M Human Rev	AGC AAG CAA GCA GAA TTT GGA A

Caln1 Human Fvd	GCC CAG CAA GAG GGA AAA GA
Caln1 Human Rev	AGA TGA AGC CGT TCC CAT CC
Gapdh Human Fvd	TGG GCA GCC GTT AGG AAA G
Gapdh Human Rev	AGT TAA AAG CAG CCC TGG TGA
Wbscr17 Human Fvd	TGT CTG GTT GAC CAC AGA GC
Wbscr17 Human Rev	CCT TGC CAC CAC AGT GAA GA
ChIP (all mouse)	
Auts2 -2 Fvd	TCG CTA AGC TGT ATG CCT GG
Auts2 -2 Rev	GAA CCA TCC GTG GAG AAG GG
Auts2 -1 Fvd	TTG AAG CGC TGG AGG TAA GG
Auts2 -1 Rev	ACA ACC CCA CAC CCA ACA AT
Auts2 4C Fvd	GGC TCC GAC AAG GAA GAC AA
Auts2 4C Rev	AGC TGG TCA TGG CAA ATC CA
Auts2 +1 Fvd	GGC GCG GTT AAA AGA ACC AT
Auts2 +1 Rev	GTG ATC GAG ACC TCC GCT TT
Auts2 +2 Fvd	GGG AGG GAA AAG CCC GTG
Auts2 +2 Rev	GAT CTT CCC CGT TCA CCT ACC
Auts2 +3 Fvd	GCA AGG CGA TTT CAG TGG TG
Auts2 +3 Rev	GCC GAG CAA AGT AAT GG CTG
Auts2 +4 Fvd	CGT GGT TAT GCT CCC CTC TC
Auts2 +4 Rev	AAT GCA TCC AAG GGA AGC CA
Caln1 4C.1 Fvd	GTG AAC TCG TGT CTT GGG CT
Caln1 4C.1 Rev	AAG GCA CTG CTG GAT GTC TG
Caln1 4C.2 Fvd	AGA CAT CCA GCA GTG CCT TC
Caln1 4C.2 Rev	CAG GTG CAT GGG GAG ATG AG
Caln1 4C.3 Fvd	GCC AGG AGG CTC ACA AGA TT
Caln1 4C.3 Rev	GGT GTG TAC CAC AGC CTC TC
Caln1 4C.4 Fvd	TGG GAG AGC CTG TCT CAG AA
Caln1 4C.4 Rev	CAC CGT GTG CTG GTA GAC AT
Caln1 4C.5 Fvd	GTC ACA GGA TAG GAT GCA GGT
Caln1 4C.5 Rev	ACC CTC CCT CTA CAG TTG CT
Caln1 4C.6 Fvd	GGC TCA TTT GCA TGC TGT CA
Caln1 4C.6 Rev	GTC TGA AGG TGT CCT GTG GC
Bisulfite sequencing (mouse)	
Auts2 F2 Fvd	GGG GYG GAG GAT GGG GGT TTT TTA TTG TTT TTT TTG
Auts2 F2 Rev	TCT CTT TTC CCC CCT ACC CTC CCT TCT TAT TCT TCT TC
Auts2 Pi Fvd	TTT GAA TTT TTT TTT TTT TAT TTA TTT GTT GTG GAG GG
Auts2 Pi Rev	AAC TCC RAC AAA AAA AAC AAT AAA AAA CCC CCA TCC TC
Auts2 Rho Fvd	GAA TAA GAA GGG AGG GTA GGG GGG AAA AGA GAA AG
Auts2 Rho Rev	TCA ACT CRC TCT CCA ACC CTA ACT TCC CTT AAA TAC
Caln1 F1 Fvd	TTA TAA GAT TTA GGG GAG TTA AAA TTG GGG AGA TG
Caln1 F1 Rev	ATA TAT ACA AAA ACC AAA AAT CCC AAT CAA ATA TCT C
Caln1 No2 Fvd	TTA GGG TTG TAG ATA TAG TAA ATA ATG GTT GTG GGG
Caln1 No2 Rev	CTC TCT CTT TCA ATC TCT CTC TCT CTA TCT CTT TCT C
Caln1 No3 Fvd	AAT AAA GTA GGA AGG GAT AGA AGG AGA TAT TTG ATT G
Caln1 No3 Rev	AAA TAAA ACA ACA ACT CAT CAA AAC TAC ATA CC

Guide-RNAs (mouse)	
gcontrol	TGG GCT CCA CAA CCC ATG A
gAuts2.1	GGA GTC CAT GTC CCC GCG T
gAuts2.2	CCC CGC GTC GGG CCA TCC A
gAuts2.3	ATG GTT CTT TTA ACC GCG C
gAuts2.4	GGT TCT TTT AAC CGC GCC G
gCaln1.1	AGT AAA GTC CTT ACC GTA T
gCaln1.2	CTT ACC GTA TAG GCA AGC A
gCaln1.3	ACA CCC GCA GTC CTA GTG C
gCaln1.4	ACA AAT GTC TAC CAG CAC A
gCaln1.5	GGG AGG GAC CGT TAT TCA G

Figure S1. Locations of primers and guide RNAs

4C-fragment (rat)

Fvd

AGCGGAGGTCGCGA***TCACAGCGAGACCGGGAGA***GACGCTCCCGGGCCGGCTTAGGAACCGGTGCGGCGGG
TGGTATAGGAACCGGCCGGACCCGGGCTCCCTCGCTGGCTTCATCGTCGGGCTCCGACAAGGAAGACAAT
GGAAAGCCCCCATCCTCCGCCCCGTCCCGGCCCAGACCCCCGCGGAGGAAGCGCAGAGAGTCCACCTCAG
CCGAAGAGGACATCATTGATGGATTTGCCATGACCAGCTTTGTTACTTTTGAAGCGCTGGAGGTAAGGGG
GGGACCCCCTTTCCTGTTCCCCCCATGGTTTCCTTTTATGCATGACACCCC

Reverse complement

GGGGTGTCATGCATAAAAGGAAACCATGGGGGGAACAGGAAAGGGGGTCCCCCCCCTTACCTCCAGCGCTT
CAAAAGTAACAAAGCTGGTCATGGCAAATCCATCAATGATGTCCTCTTCGGCTGAGGTGGACTCTCTGCG
CTTCCTCCGCGGGGGTCTGGGCCGGGACGGGGCGGAGGATGGGGGCTTTCCATTGTCTTCCTTGTCGGAG
CCCGACGATGAAGCCAGCGAGGGAGCCCGGGTCCGGCCGGTTCCTATACCACCCGCCGCACCGGTTCCTA
AGCCGGCCCGGGAGCGTC***TCTCCCGGTCTCGCTGTGA***TCGCGACCTCCGCT

Nco1 Fvd CCCTTTCCTGTTCCCCCCA

Dpn2 Rev ***TCTCCCGGTCTCGCTGTGA***

Location 3C-primers (rat)

Auts2

chr12: 27153362-27155765 Fvd strand

AAGTGTCCGGAGAAAGGTGCCTGCTACGCGTGCGCGCGCGAGTCCTCCGTGCGTTTGGCGGGATGTTGGG
GCAGCCTCCACGCCCTCGAACTTCAACTCCACACGGGCCCGTGACCGACCCCTCCAACATACCCCACAGC
CTCTGGAGACCCCTTCCGCCCCCAAGTGCGCAGGCCCGGCCGCCCCAGCGCGCATGCCCTGGGCTCGTGG
AGCGGTGGCCAGCCCCTCAGCCTTTTTGCTTTCTACACACTCTACAACTAGGGGAGGGGGGCGGGTCACG
TGATCCAGCCGGCCCCGAGGCGCGCGGGAGGAGACGTGGGGGGGTAGGACGGGAACGCGCGTGGGAGAGA
GGGAGAGAGGCGCGAGGGGAGGGGCTCTTCTCGGGAGCGCGCAGCCAGTGGTGCATGAGTGGAAGGGGCG
GCCCTCGGGAGCGCGCCCCGTGGTGCCGGGGTGGTGGTGGGGGGATTGCGGGGGGGAGAGAACGAGGCGT
GAGAGATTCTCGGGAGCCCGCGAAGGGGGAAGGGGGCGGCCCTCGGGAGCGCGCCGAGTGAGGCGTGAGG
GGAGGGGGGAGCCCTCGGGAGCGCGAGGAGAGCCAGGAGGGCGGTCCTCGGGAGCGCGCGCAGAGCGAGG
CGCGAAGAGGGCGGTCTTCCAGAGCGCTCGCCGGCGAGCGAGCTCGAGCGCGCGTGCAAGAGAGAGAGAG
GGAGAGAGAGAGGGAGAGAGAGAGACGGCAAGCGGGCGGGCGTGGGGCTGGGCCGAGGCGGGCGGCGGCG
GTGGGCCGGCCAGAGGGGGAGGAGCGGGCGCTGTGTGAGCGAGGCGGGCGGAGGATGAGGCCGTGCCCGG
GCTCTGGCTGCAGCCGCCCGGGGACACGCCGTGCACCGTCGGGCTCGGGGCTCCGGCGGCGGCGGCTGAG
GCAGCGTTAGCTGCGAGCGAGCGAGCGAGTGAGCGAGCGGCATCTCCCAGCGCTTCTTCCTCAGGCGGGG
CGACGCGAAGAAGAAGAAGAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCACCACACCGGTGTA
TCTCGGGTTGGAGACTGCTCTTTGCTTTCCTCATTGACTGACTCGCCCCCCCTTTATAATTTTTTTTTTT
TTGAGGAAGGGTGACCTCTGGAACTGGGGTGGGGGGAGACAATTTGTGTGCGTGCGTGCATGTGTATGTG
AGGAGGGGGTCGGTGGCACGCGCCAGGCTTTGCAATTCTTCTCTTCTTTCATCTTTTTCTCCTCCTCCCC
CCCCATCGAAGACCGAAAAAAAGAAAGGGAGAAAAAAAAAAAATCCCACAACTGCTTCAGCGTCCTCCTC
CTTCCCTTCCTCCTTTCCTCGGGCAACCTCTGTCTCCGCTCGTGATTGCGTGGTTATGCTCCCCTCTCTG
GGGGGTCTCGCCCCTCTCAGCTCGCTCTCTAGCCCTGGCTTCCCTTGGATACATTTCTTAAAAAAAGACT
AGGAGCCCGGGAGTGAGTTTTTCTGCGAGACGAGTGTGTGAGAGAGGCGGAGGTGTGGGGTGTTTTCCTG
CACGAGGGGGCGGGTGAAGTTCATTGCCACCCACCCCTCACTTCACCGTGACCTTTTCGGACCTCGGGCT
TTTTTTTTTCTTTTAATTTTTTTTGGGGGGGGGGTCTCTAGGAAAAGCGAGAAGCCAAGCGATTTCAGTG
GTGGGGAGAGAGCATGGCCGCGGGGAGGGCTTCCCCAGTCTCTTGATCAAAGCATTCCGCTATTCTGATT
TATTGCCTGCTGGTGAGTTATTTTTTTCCCCCTTTCCTCTGTAAAGGAGACCTGTGTGTTCAGCCATTAC
TTTGCTCGGCGCTGTTCGCAGGCATCTCCGACCCTCGGTGCAGAGGGGAGCCCCACACTTCGCCCTCTCG
CTTCCCCGTCCCTCCCTCCCCGTCTCCCCGCCCCTTCCCCCTTTTTTCTTTCTCTTTTTCCCCCTGCCCT
CCCTTCTTGTTCTTCTTGGGCTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATTCCTCCC
AGCCGCCTTCTCCCCCGCGCCCTTCCTCGCGGCTGGAGGGAAAAGCCCGTGCAGGAGAAGCGGGGGGGCT
CGGTGTCAATTTTTTTTTTCTTCTTGGTGTGTGTGTGTGTGTGTGTGTGTGTGTGGCCGCGGCCCGAG
CCTGTGGCGCGCGGGTAGGCGAACGGGGAAGATCCCCGGCGCGGTTAAAAGAACCATGGATGGCCCGACG
CGGGGACATGGACTCCGGAAAAAGCGGAGGTCGCGATCACAGCGAGACCGGGAGAGACGCTCCCGGGCCG
GCTTAGGAACCGGTGCGGCGGGTGGTATAGGAACCGGCCGGACCCGGGCTCCCTCGCTGGCTTCATCGTC
GGGCTCCGACAAGGAAGACAATGGCTTCATCGTCGGGCTCCGACAAGGAAGACAATGGAAAGCCCCCATC
CTCCGCCCCGTCCCGGCCCAGACCCCCGCGGAGGAAGCGCAGAGAGTCCACCTCAGCCGAAGAGGACATC
ATTGATGGATTTGCCATGACCAGCTTTGTTACTTTTGAAGCGCTGGAGGTAAGGGGGGGACCCCCTTTCC
TGTTCCCCCCATG

3C Auts2; Nla3 restriction site; 4C-bait; 3C-control

Caln1 4C-fragment

chr12:29680069-29681962 Fvd strand

GTGTGCTGAGGTCCTCTATGGTGTCTGAAGGTGTCCTGTGGCTCATTATGATATTAAAGCAGAGGTGGGG
TCTTATGATGCATGAAATTGTATATACAACTATATGTAACAGCATGCAAATGAGCCTTGCTATGTAAATG
AGCCAAAAGCTGGTGCTGAATTAACACAAAAGCCCCAGCTCTTTATGTAGCAGGGCAGTTGAGTGTTGAA
ATTAATATCTGTCTGTCTGTCTCTCTCTCTCTTTTTCTCTCTCTCTCCCTCTCCCTCCCTCTCTCTCTCC
CTCTCCCTTTCCCCTTTCTCTCTCTGTCTCTTTGTCTCTGTCTGTCTCTGTCTCTCTCTGTGTCTGTCTC
TCTGTCTCTCTCCATGTTCCTGTGTGTCTCTTTCTCTCTGTGTGTCTGTCTCTCCATATCTCTCTCTGTG
TCTCTGTCTCTCTGTCTCTCTCTCTGTCTCTCTGTCTCTCTCTGTCTCTGTCTGTCTCTCTGTGTGTGTG
TCTGTCTCTCCATCTCTCTGTGTGTCTCTGTGTGTGTCTGTTTCTCTGTCTCTCTCTGTCTCTCTCTCTC
TGTCTCTCTCTCTCTGTCTCTCTCTCTCTCTCTCTCTCTCTGTCTCTCTCTCTCTCTCTCTCTCTCTG
AACTGTATCTTCTCCTAACCAACCCTGTTAGAAAGTCTATTCTTCCTTAATCAGTAAACCCTCCCCCTAC
ATTTGCTACCGTTTGCTGCTTTTGTCCTATTCTTTGTTCCTGGATACAAGTACCTGCATCCTGTCCTGTG
GCTATGAAATCTCTAGACATCAGCCCCACCAGCAGAAAGCCCACCCCAGGATGGGGCGACAACTCAGCAA
AGCTGCCTGTCTCCTGGAGCCTGCCCGGGACAGCTCCACTGAATAATGCCTCCTCCCCACAGCCTTTATT
TACTGTGCCGATAACCCTGGTGTGGGGCCTTGGGAATCCTAAAATTTCATTAGCTTCCTGAACCTCGGAG
GAGAGCAGGTTGTGGGGATGTGTTGCATGCACATTTTCGTGTGGGTGTGTGCACATACTGTGTGCTGGTA
CACATTTGTAAAAGTGTATGCAGAAGCCAGAGATCCAGTTCAGTTGTCTTCCTCTACCGGTTTCTGCTTT
GTTTTTTGAGACAGGCTCTCCCACTGAACCCAGAGCTTACCAGTTCTGCTAAGCCGGTTAGGGCTCCTAC
TTATCTGCCTCCTCAGCACTAGGACTGCAGGTGTGCACCACAGCCCCCTGGCTCCTTTATGTGGGTGCTG
GGGTCCTAAACTCAAGATTTCATACTTACCTGTATGGTAAACAATTTACTGATTGAGGCGCCTCCCCGAT
CTTGGCTCCCCTAAATCTTGTGAGCCTCCAGGCTCCCTCCAGTGAAGAATCTTTCCATTCAGAGGAAATA
GAAACACAATAACACATCCTTTAAAAGTTAGCCAGGATGCAAGTTCCGGAGACTTAAATAATGTCTGGTC
TGTGTAGATGCCTGAGAATAACACAAGGTCAGACAGACGGCCCCCACCACACCCCCAGCAGCCACCTGGC
TGAGCCTTTGGTCCAGGTGCATGGGGAGATGAGGCAGGCTAGACAGGCCCTGCCAACAGCAGCCAGAATG
TCACAATTGGGCACAGAGTCATCCCAACAGTCTGGCACAGGTCAGTGAATGCAGGAAGGCACTGCTGGAT
GTCTGCTAGTGAGAGACATGAGCTGATGGGGTGTGCTGACCGCAGAAGCAAAAATGAGCTTAACCTAAGG
ACCTGAGTTCACCTGAAGGATGTATGGGGAAAGACTGTCCCTCACTACCTAACCTTCACAGAGACATCAG
GTCGATGGGGTCAGAATCTGCTGCGGGGGAGGAGGGACTTTTCTTGGTTCTGAATGGAGACTTGAAGGAT
GACT

3C Auts2; Nla3 restriction site

Location 3C-primers (mouse)

Auts2

chr5:132541150-132542774 Fvd strand

GGGGTAGAACCATCCGTGGAGAAGGGGGGCGCATTGTTGTGATGCCTGAACCCCTTTCTTTCCACCCATC
TGCTGTGGAGGGAGGTGGGGGTGCAGGAAGGGGGCGCAAGGGAAAGGCAAGCTGGACAAGAACGAAACCA
GTAGAAGACCAGACCAGGCATACAGCTTAGCGATTCCCTCCTTGCTGCCAATCTGCCCCCAGCTCCCCCT
ACATACAACCCCACACCCAACAATAAGACCTCGGCCGAGACTGAGAGCTTCGAGTGCGGCGGGGGAGGCA
AGAAAACCGGGGTGCAGCCGAGTGTGGGGGGTGTCATGCATAAAAGGAAACCATGGGGGGAACAGAAAAG
GGGGTCCCCCCTTACCTCCAGCGCTTCAAAAGTAACAAAGCTGGTCATGGCAAATCCATCAATGATGTCC
TCTTCGGCTGAGGTGGACTCTCTGCGCTTCCTCCGCGGGGGTCTGGGCCGGGACGGGGCGGAGGATGGGG
GCTTTCCATTGTCTTCCTTGTCGGAGCCCGACGATGAAGCCAGCGAGGGAGCCCGGGTCCGGCCGGCTCC
TATACCACCCGCCGCACCGGTTCCTAACCCGGCCCGGGAGCGTCTCTCCCGGTCTCGCTGTGATCGAGAC
CTCCGCTTTTTCCGGAGTCCATGTCCCCGCGTCGGGCCATCCATGGTTCTTTTAACCGCGCCGGGGATCT
TCCCCGTTCACCTACCCGCGCGCCACAGGCTCGGGCCGCGGCCACACACACACACACACACCCCAAGAAG
AAAAAAAAAATTGACACCGAGCCCCCCTTCCTCCTGCACGGGCTTTTCCCTCCCGCCGCGAGGAAGGG*CG*
*CGGGGG*AGAAAGCGGCTGGGAGAAATAAAAAAAAAAAAAAAAAAAAGAATCCGAAGAAGAACAAGAAGGG
AGGGCAGGGGGGAAAAGAGAAAGAAAAAAGGGGGAAGGGGCGGGGAGACGGGGAGGGAGGGACGGGGAAG
CGAGAGGGCGAAGTGTGGGGCTCCCCTCTGCACCGAGGGTCGGAGATGCCTGCGAACAGCGCCGAGCAAA
GTAATGGCTGAACACACAGGTCTCCTTTACAGAGGAAAGGGGGAAAAAAATAACTCACCAAGCAGGCAAT
AAATCAGAATAGCGGAATGCTTTGATCAAGAGACTGGGGAAGCCCTCCCCGCGGCCATGCTCTCTCCCCA
CCACTGAAATCGCCTTGCTTCTCCCTTTCTCTAGAGACCCCCCAAAAAATTTTAAAAAAAAAAAAGCCCG
AGGTCCGAAAAGGTCACGGTGAAGTGAGGGGTGGGTGGCAATGAACTTCACCCGCCCCCTCGTGCAGGAA
AACACCCCACACCTCCGCCTCTCTCACACACTCGCCTCGCAGAAAAACTCACTCCCGGGCTCCCAGTCTT
TTTTTAAGAAATGCATCCAAGGGAAGCCAGGGCTGGAGAGCGAGCTGAGAGGGGCGAGACCCCCCAGAGA
GGGGAGCATAACCACGCAATCACAAGCGGAGACAGAGGTTGCCCGAGGAAAGGAGGAAGGGAAGGAGGAG
GACGCTGAAGCAGTTGTGGGATTTTTTTTTTTCGGTCTTCAAGGGGGGGAGGAGGAGAAAAAGATGAAAG
AAGAGAAGAATTGCA

Caln1 – 4C-predicted fragment (mouse)

converted from rat to mouse via UCSC genome browser BLAT-tool

>mm10_dna range=chr5:130474541-130476365 Forward strand

CCAAGGTTGATGCCTGGGAACATCTTCCATCTCTCTTCCCCATTATTTACTGAGGCAGAATATGTCACTC
AAACCCAGAACTTACCCCTATGGCTACTGTAGCTAGCTAGCTTGCTCTGGGGGGAACCCCATGTCTCTGC
TTCTTTCCACATCAGGAACAACAGGCTGGCTCTCATAGTTCTGGGGATTTGAACTTTCGTCCTTATGCTT
GTCCAGCAAGTCCTTTAGCCACTGAGCCATTCTACCAGTCTCCATTCAGAACCAAGAAAAGCCCCTCCTC
CCCTACAGCAGATTCTGCTCTGACCCCATTGTCCCTATGCCTCTGTGAAAGTTAGGTAGTGAGGGACAGT
CTTTCCCGATACATCCTTCAGGTGAACTCGTGTCTTGGGCTAAAGCTCATTTTTGCTTCTGCGGTCATCA
CACCCCATCAGCTCATGCCTCTCACTAGCAGACATCCAGCAGTGCCTTCCTGCATTCACTGACCTGTGCC
AGACTGTTGGGATGACTCTGTGCCCAATTGTGACATTCCGGCTGCTGTTGGCAGGGCCTGTCTAGCCTGC
CTCATCTCCCCATGCACCTGGACCAGAGGCTCAGCCAGGTGGTTGCTGGGGGATGGTGGGGGTGGCAGTC
CATCTGACCTTATGTTATTCTCGGGCATCTACATAAACCAGACATTATTTCAGTCTCTGGAACTCTTGTC
CCGACTGTTCTGTAACTTTTTAAGGACATGCTACTGAGTTTCTATTTCCTCTGAATGGAAAAGATTCTTT
ACTGGCGGGAGCCAGGAGGCTCACAAGATTTAGGGGAGCTAAAATTGGGGAGATGCCTCAATCAGTAAAG
TCCTTACCGTATAGGCAAGCATGGGATCCTAAGTGTAGGTCCCCAGCACCCACATAAAAGAGCAGGGGGT
AGGGGGAGAGGCTGTGGTACACACCCGCAGTCCTAGTGCTGGAGAAGCAGAGCTAAGTAGGCTCCTTAGC
TAGCTTAGCTGCATTGGTAAGCTCTGAGTTCAGTGGGAGAGCCTGTCTCAGAAAAACAAAGCAGGAAGGG
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CAGGAAGCTAATGAAATTTTAGGATTCCCAAGGCCCCACCCCAGGGTTGTAGACACAGTAAATAATGGCT
GTGGGGAGGGACCGTTATTCAGTGGAGCTGTCTGGGGCAGGCTCCAGGAGGCATGCAGCTTTGATGAGTT
GTTGCCCCATCTTGGGGTGGGTTTTCTGTAGATGGCACTGATGTCTAGAAATTTTGTAGTCACAGGATAG
GATGCAGGTACTTGTATCCAGGAACAAAGAATAGGTCAAAGGCAGCAAACTGTAGCAACTGTAGAGGGAG
GGTTTACTGATTGAAGAAGAATAGACTTTCAAATAGTGTTGGTTAGGAAAAGATACAGTTGAGAGAGAGA
AAGAGACAGAGAGAGAGAGATTGAAAGAGAGAGACAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
AGATTTCAACAGCTCAAGTGCCCTGTTACATAAAGAGCTGGGGCTTTTGTGTTAATTCAACATCAGCTTT
TGGGCTTATTTACATAGCAAGGCTCATTTGCATGCTGTCACATATAGTTGTATATACAACTTCATGCATT
GAGTATCTCATTTGCATCATAAGACCCCACCTCTGTTTTAATATCATAATTAGCCACAGGACACCTTCAG
ACACC

green to green: 4C-predicted fragment; 3C Caln1; Nla3 restriction site; 3C control

Location ChIP-primers (mouse)

Auts2

chr5:132541150-132542774 >mm10_dna Reverse strand

TGCAATTCTTCTCTTCTTTCATCTTTTTCTCCTCCTCCCCCCCTTGAAGACCGAAAAAAAAAAATCCCAC
AACTGCTTCAGCGTCCTCCTCCTTCCCTTCCTCCTTTCCTCGGGCAACCTCTGTCTCCGCTTGTGATTGC
GTGGTTATGCTCCCCTCTCTGGGGGGTCTCGCCCCTCTCAGCTCGCTCTCCAGCCCTGGCTTCCCTTGGA
TGCATTTCTTAAAAAAAGACTGGGAGCCCGGGAGTGAGTTTTTCTGCGAGGCGAGTGTGTGAGAGAGGCG
GAGGTGTGGGGTGTTTTCCTGCACGAGGGGGCGGGTGAAGTTCATTGCCACCCACCCCTCACTTCACCGT
GACCTTTTCGGACCTCGGGCTTTTTTTTTTTTAAAATTTTTTGGGGGGTCTCTAGAGAAAGGGAGAAGCA
AGGCGATTTCAGTGGTGGGGAGAGAGCATGGCCGCGGGGAGGGCTTCCCCAGTCTCTTGATCAAAGCATT
CCGCTATTCTGATTTATTGCCTGCTTGGTGAGTTATTTTTTTCCCCCTTTCCTCTGTAAAGGAGACCTGT
GTGTTCAGCCATTACTTTGCTCGGCGCTGTTCGCAGGCATCTCCGACCCTCGGTGCAGAGGGGAGCCCCA
CACTTCGCCCTCTCGCTTCCCCGTCCCTCCCTCCCCGTCTCCCCGCCCCTTCCCCCTTTTTTCTTTCTCT
TTTCCCCCCTGCCCTCCCTTCTTGTTCTTCTTCGGATTCTTTTTTTTTTTTTTTTTTTTATTTCTCCCAG
CCGCTTTCTCCCCCCGCGCCCTTCCTCGCGGCGGGAGGGAAAAGCCCGTGCAGGAGGAAGGGGGGCTCGGT
GTCAATTTTTTTTTTCTTCTTGGGGTGTGTGTGTGTGTGTGTGGCCGCGGCCCGAGCCTGTGGCGCGCGG
GTAGGTGAACGGGGAAGATCCCCGGCGCGGTTAAAAGAACCATGGATGGCCCGACGCGGGGACATGGACT
CCGGAAAAAGCGGAGGTCTCGATCAGCAGCGAGACCGGGAGAGACGCTCCCGGGCCGGGTTAGGAACCGGT
GCGGCGGGTGGTATAGGAGCCGGCCGGACCCGGGCTCCCTCGCTGGCTTCATCGTCGGGCTCCGACAAGG
AAGACAATGGAAAGCCCCCATCCTCCGCCCCGTCCCGGCCCAGACCCCCGCGGAGGAAGCGCAGAGAGTC
CACCTCAGCCGAAGAGGACATCATTGATGGATTTGCCATGACCAGCTTTGTTACTTTTGAAGCGCTGGAG
GTAAGGGGGGACCCCCTTTTCTGTTCCCCCCATGGTTTCCTTTTATGCATGACACCCCCCACACTCGGCT
GCACCCCGGTTTTCTTGCCTCCCCCGCCGCACTCGAAGCTCTCAGTCTCGGCCGAGGTCTTATTGTTGGG
TGTGGGGTTGTATGTAGGGGGAGCTGGGGGCAGATTGGCAGCAAGGAGGGAATCGCTAAGCTGTATGCCT
GGTCTGGTCTTCTACTGGTTTCGTTCTTGTCCAGCTTGCCTTTCCCTTGCGCCCCCTTCCTGCACCCCCA
CCTCCCTCCACAGCAGATGGGTGGAAAGAAAGGGGTTCAGGCATCACAACAATGCGCCCCCCTTCTCCAC
GGATGGTTCTACCCC

Primer location color code:

Auts2 +4, Auts2 +3, Auts2 +2, Auts2 +1, Auts2 4C, Auts2 -1, Auts2 -2

4C-bait: green

Caln1 – 4C-predicted fragment (mouse)

chr5:130474787-130476365 Forward strand

AGTCTCCATTCAGAACCAAGAAAAGCCCCTCCTCCCCTACAGCAGATTCTGCTCTGACCCCATTG
TCCCTATGCCTCTGTGAAAGTTAGGTAGTGAGGGACAGTCTTTCCCGATACATCCTTCAGGTGAA
CTCGTGTCTTGGGCTAAAGCTCATTTTTGCTTCTGCGGTCATCACACCCCATCAGCTCATGCCTC
TCACTAGCAGACATCCAGCAGTGCCTTCCTGCATTCACTGACCTGTGCCAGACTGTTGGGATGAC
TCTGTGCCCAATTGTGACATTCCGGCTGCTGTTGGCAGGGCCTGTCTAGCCTGCCTCATCTCCCC
ATGCACCTGGACCAGAGGCTCAGCCAGGTGGTTGCTGGGGGATGGTGGGGGTGGCAGTCCATCTG
ACCTTATGTTATTCTCGGGCATCTACATAAACCAGACATTATTTCAGTCTCTGGAACTCTTGTCC
CGACTGTTCTGTAACTTTTTAAGGACATGCTACTGAGTTTCTATTTCCTCTGAATGGAAAAGATT
CTTTACTGGCGGGAGCCAGGAGGCTCACAAGATTTAGGGGAGCTAAAATTGGGGAGATGCCTCAA
TCAGTAAAGTCCTTACCGTATAGGCAAGCATGGGATCCTAAGTGTAGGTCCCCAGCACCCACATA
AAAGAGCAGGGGGTAGGGGGAGAGGCTGTGGTACACACCCGCAGTCCTAGTGCTGGAGAAGCAGA
GCTAAGTAGGCTCCTTAGCTAGCTTAGCTGCATTGGTAAGCTCTGAGTTCAGTGGGAGAGCCTGT
CTCAGAAAAACAAAGCAGGAAGGGATAGAAGGAGACACTTGACTGGGACCTCTGGCTTCTGCACA
CACTCATAACAAATGTCTACCAGCACACGGTGCGTGCACACACCTATACAAAAAAATGTGCATAC
AACCCATACCCACAACCTGCTCTCCTCCAAGGTTCAGGAAGCTAATGAAATTTTAGGATTCCCAA
GGCCCCACCCCAGGGTTGTAGACACAGTAAATAATGGCTGTGGGGAGGGACCGTTATTCAGTGGA
GCTGTCTGGGGCAGGCTCCAGGAGGCATGCAGCTTTGATGAGTTGTTGCCCCATCTTGGGGTGGG
TTTTCTGTAGATGGCACTGATGTCTAGAAATTTTGTAGTCACAGGATAGGATGCAGGTACTTGTA
TCCAGGAACAAAGAATAGGTCAAAGGCAGCAAACTGTAGCAACTGTAGAGGGAGGGTTTACTGAT
TGAAGAAGAATAGACTTTCAAATAGTGTTGGTTAGGAAAAGATACAGTTGAGAGAGAGAAAGAGA
CAGAGAGAGAGAGATTGAAAGAGAGAGACAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
GATTTCAACAGCTCAAGTGCCCTGTTACATAAAGAGCTGGGGCTTTTGTGTTAATTCAACATCAG
CTTTTGGGCTTATTTACATAGCAAGGCTCATTTGCATGCTGTCACATATAGTTGTATATACAACT
TCATGCATTGAGTATCTCATTTGCATCATAAGACCCCACCTCTGTTTTAATATCATAATTAGCCA
CAGGACACCTTCAGACACC

Primer location color code:

Caln1 -4, Caln1 -3 F, Caln1 -2 F, Caln1 -1 F, Caln1 +1 F, Caln1 +2 F

Location Bisulfite-primers

Auts2 (mouse)

chr5:132541150-132542774 Reverse strand

TGCAATTCTTCTCTTCTTTCATCTTTTTCTCCTCCTCCCCCCCTTGAAGACCGAAAAAAAAAAAT
CCCACAACTGCTTCAGCGTCCTCCTCCTTCCCTTCCTCCTTTCCTCGGGCAACCTCTGTCTCCGC
TTGTGATTGCGTGGTTATGCTCCCCTCTCTGGGGGGTCTCGCCCCTC*TCAGCTCGCTCTCCAGCC*
*CTGGCTTCCCTTGGATGC*ATTTCTTAAAAAAAGACTGGGAGCCCGGGAGTGAGTTTTTCTGCGAG
GCGAGTGTGTGAGAGAGGCGGAGGTGTGGGGTGTTTTCCTGCACGAGGGGGCGGGTGAAGTTCAT
TGCCACCCACCCCTCACTTCACCGTGACCTTTTCGGACCTCGGGCTTTTTTTTTTTTAAAATTTT
TTGGGGGGTCTCTAGAGAAAGGGAGAAGCAAGGCGATTTCAGTGGTGGGGAGAGAGCATGGCCGC
GGGGAGGGCTTCCCCAGTCTCTTGATCAAAGCATTCCGCTATTCTGATTTATTGCCTGCTTGGTG
AGTTATTTTTTTCCCCCTTTCCTCTGTAAAGGAGACCTGTGTGTTCAGCCATTACTTTGCTCGGC
GCTGTTCGCAGGCATCTCCGACCCTCGGTGCAGAGGGGAGCCCCACACTTCGCCCTCTCGCTTCC
CCGTCCCTCCCTCCCCGTCTCCCCGCCCCTTCCCCCTTTTTT**CTT*TCTCTTTTCCCCCCTGCCCT***
CCCTTCTTGTTC*TTCTTC*GGATTCTTTTTTTTTTTTTTTTTTTTTATTTCTCCCAGCCGCTTTCTC
CCCCGCGCCCTTCCTCGCGGCGGGAGGGAAAAGCCCGTGCAGGAGGAAGGGGGGCTCGGTGTCAA
TTTTTTTTTTCTTCTTGGGGTGTGTGTGTGTGTGTGTGGCCGCGGCCCGAGCCTGTGGCGCGCGG
GTAGGTGAACGGGGAAGATCCCCGGCGCGGTTAAAAGAACCATGGATGGCCCGACGCGGGGACAT
GGACTCCGGAAAAAGCGGAGGTCTCGATCACAGCGAGACCGGGAGAGACGCTCCCGGGCCGGGTT
AGGAACCGGTGCGGCGGGTGGTATAGGAGCCGGCCGGACCCGGGCTCCCTCGCTGGCTTCATCGT
CG*GGCTCCGA****CAAGGAAGACAATGGAAAGCCCCCATCCTC***CGCCCCGTCCCGGCCCAGACCCCCG
CGGAGGAAGCGCAGAGAGTCCACCTCAGCCGAAGAGGACATCATTGATGGATTTGCCATGACCAG
CTTTGTTACTTTTGAAGCGCTGGAGGTAAGGGGGGACCCCCTTTTCTGTTCCCCCCATGGTTTCC
TTTTATGCATGACACCCCCCACACTCGGCTGCACCCCGGTTTTCTTGCCTCCCCCGCCGCACTCG
AAGCTCTCAGTCTCGGCCGAGGTCTTTTGTATGTAGGGGGAGCTGGGGGCAGATTGGCAGCAAGG
AGGGAATCGCTAAGCTGTATGCCTGGTCTGGTCTTCTACTGGTTTCGTTCTTGTCCAGCTTGCCT
TTCCCTTGCGCCCCCTTCCTGCACCCCCACCT**CCCTCCACAGCAGATGGGTGGAAAGAAAGGGGT**
TCAGGCATCACAACAATGCGCCCCCCTTCTCCACGGATGGTTCTACCCC

Primer location color code (non-BS-converted analogue, reverse complements shown):

Auts2 F2 F, *Auts2 F2 R*, **Auts2 Pi F**, *Auts2 Pi R*, **Auts2 Rho F**, *Auts2 Rho R*

4C-bait: green; ChIP-changes: yellow

Caln1 (mouse)

chr5:130474787-130476365 Forward strand

AGTCTCCATTCAGAACCAAGAAAAGCCCCTCCTCCCCTACAGCAGATTCTGCTCTGACCCCATTGTCCCT
ATGCCTCTGTGAAAGTTAGGTAGTGAGGGACAGTCTTTCCCGATACATCCTTCAGGTGAACTCGTGTCTT
GGGCTAAAGCTCATTTTTGCTTCTGCGGTCATCACACCCCATCAGCTCATGCCTCTCACTAGCAGACATC
CAGCAGTGCCTTCCTGCATTCACTGACCTGTGCCAGACTGTTGGGATGACTCTGTGCCCAATTGTGACAT
TCCGGCTGCTGTTGGCAGGGCCTGTCTAGCCTGCCTCATCTCCCCATGCACCTGGACCAGAGGCTCAGCC
AGGTGGTTGCTGGGGGATGGTGGGGGTGGCAGTCCATCTGACCTTATGTTATTCTCGGGCATCTACATAA
ACCAGACATTATTTCAGTCTCTGGAACTCTTGTCCCGACTGTTCTGTAACTTTTTAAGGACATGCTACTG
AGTTTCTATTTCCTCTGAATGGAAAAGATTCTTTACTGGCGGGAGCCAGGAGGC**TCACAAGATTTAGGGG**
AGCTAAAATTGGGGAGATGCCTCAATCAGTAAAGTCCTTACCGTATAGGCAAGCATGGGATCCTAAGTGT
AGGTCCCCAGCACCCACATAAAAGAGCAGGGGGTAGGGGGAGAGGCTGTGGTACACACCCGCAGTCCTAG
TGCTGGAGAAGCAGAGCTAAGTAGGCTCCTTAGCTAGCTTAGCTGCATTGGTAAGCTCTGAGTTCAGTGG
GAGAGCCTGTCTCAGAAA**AACAAAGCAGGAAGGGATAGAAG*GAGACACTTGACTG***GGACCTCTGGCTTCT
*GCACACAC*TCATAACAAATGTCTACCAGCACACGGTGCGTGCACACACCTATACAAAAAAATGTGCATAC
AACCCATACCCACAACCTGCTCTCCTCCAAGGTTCAGGAAGCTAATGAAATTTTAGGATTCCCAAGGCCC
CACC**CCAGGGTTGTAGACACAGTAAATAATGGCTGTGGGG**AGGGACCGTTATTCAGTGGAGCTGTCTGGG
GCAGGCTCCAGGA*GGCATGCAGCTTTGATGAGTTGTTGCCCCATCT*TGGGGTGGGTTTTCTGTAGATGGC
ACTGATGTCTAGAAATTTTGTAGTCACAGGATAGGATGCAGGTACTTGTATCCAGGAACAAAGAATAGGT
CAAAGGCAGCAAACTGTAGCAACTGTAGAGGGAGGGTTTACTGATTGAAGAAGAATAGACTTTCAAATAG
TGTTGGTTAGGAAAAGATACAGTTGAGAGA*GAGAAAGAGACAGAGAGAGAGAGATTGAAAGAGAGAG*ACA
GGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGATTTCAACAGCTCAAGTGCCCTGTTACATAAAGA
GCTGGGGCTTTTGTGTTAATTCAACATCAGCTTTTGGGCTTATTTACATAGCAAGGCTCATTTGCATGCT
GTCACATATAGTTGTATATACAACTTCATGCATTGAGTATCTCATTTGCATCATAAGACCCCACCTCTGT
TTTAATATCATAATTAGCCACAGGACACCTTCAGACACC

ChIP-changes

Primer location color code (non-BS-converted analogue):

Caln1 F1 F, *Caln1 F1 R*, **Caln1 No2 F**, *Caln1 No2 R*, **Caln1 No3 F**, *Caln1 No3 R*

Location guide-RNAs

Auts2 (mouse)

chr5:132541357-132542348 Fvd strand

CCTACATACAACCCCACACCCAACAATAAGACCTCGGCCGAGACTGAGAGCTTCGAGTGCGGCGGGGAG
GCAAGAAAACCGGGGTGCAGCCGAGTGTGGGGGGTGTCATGCATAAAAGGAAACCATGGGGGGAACAGAA
AAGGGGGTCCCCCCTTACCTCCAGCGCTTCAAAAGTAACAAAGCTGGTCATGGCAAATCCATCAATGATG
TCCTCTTCGGCTGAGGTGGACTCTCTGCGCTTCCTCCGCGGGGGTCTGGGCCGGGACGGGGCGGAGGATG
GGGGCTTTCCATTGTCTTCCTTGTCGGAGCCCGACGATGAAGCCAGCGAGGGAGCCCGGGTCCGGCCGGC
TCCTATACCACCCGCCGCACCGGTTCCTAACCCGGCCCGGGAGCGTCTCTCCCGGTCTCGCTGTGATCGA
GACCTCCGCTTTTTTCCGGAGTCCATGT***CCCCGCGCTCGGGCCATCCATGGTTCTTTTAACCGCGCCG***GGGA
TCTTCCCCGTTCACCTACCCGCGCGCCACAGGCTCGGGCCGCGGCCACACACACACACACACACCCCAAG
AAGAAAAAAAAAATTGACACCGAGCCCCCCTTCCTCCTGCACGGGCTTTTCCCTCCCGCCGCGAGGAAGG
GCGCGGGGGAGAAAGCGGCTGGGAGAAATAAAAAAAAAAAAAAAAAAAAGAATCCGAAGAAGAACAAGAA
GGGAGGGCAGGGGGGAAAAGAGAAAGAAAAAAGGGGGAAGGGGCGGGGAGACGGGGAGGGAGGGACGGGG
AAGCGAGAGGGCGAAGTGTGGGGCTCCCCTCTGCACCGAGGGTCGGAGATGCCTGCGAACAGCGCCGAGC
AAAGTAATGGCTGAACACACAGGTCTCCTTTACAGAGGAAAGGGGGAAAAAAATAACTCACCAAGCAGGC
AATAAATCAGAATAGCGGAATGCTTTGATCAAGAGACTGGGGAAGCCCTCCCCGCGGCCATGCTCTCTCC
CCACCACTGAAA

4c-bait: green; ChIP-changes: yellow; CGs: purple; guideRNAs: blue; yellow CGs: strong bisulfite changes

Caln1 4C-bait (mouse)

chr5:130474787-130476365 Forward strand

AGTCTCCATTCAGAACCAAGAAAAGCCCCTCCTCCCCTACAGCAGATTCTGCTCTGACCCCATTGTCCCT
ATGCCTCTGTGAAAGTTAGGTAGTGAGGGACAGTCTTTCCCGATACATCCTTCAGGTGAACTCGTGTCTT
GGGCTAAAGCTCATTTTTGCTTCTGCGGTCATCACACCCCATCAGCTCATGCCTCTCACTAGCAGACATC
CAGCAGTGCCTTCCTGCATTCACTGACCTGTGCCAGACTGTTGGGATGACTCTGTGCCCAATTGTGACAT
TCCGGCTGCTGTTGGCAGGGCCTGTCTAGCCTGCCTCATCTCCCCATGCACCTGGACCAGAGGCTCAGCC
AGGTGGTTGCTGGGGGATGGTGGGGGTGGCAGTCCATCTGACCTTATGTTATTCTCGGGCATCTACATAA
ACCAGACATTATTTCAGTCTCTGGAACTCTTGTCCCGACTGTTCTGTAACTTTTTAAGGACATGCTACTG
AGTTTCTATTTCCTCTGAATGGAAAAGATTCTTTACTGGCGGGAGCCAGGAGGCTCACAAGATTTAGGGG
AGCTAAAATTGGGGAGATGCCTCAATC**AGTAAAGTCCTTACCGTAT**AGGCAAGCATGGGATCCTAAGTGT
AGGTCCCCAGCACCCACATAAAAGAGCAGGGGGTAGGGGGAGAGGCTGTGGTACACACCCGCAGTCCTAG
TGCTGGAGAAGCAGAGCTAAGTAGGCTCCTTAGCTAGCTTAGCTGCATTGGTAAGCTCTGAGTTCAGTGG
GAGAGCCTGTCTCAGAAAAACAAAGCAGGAAGGGATAGAAGGAGACACTTGACTGGGACCTCTGGCTTCT
GCACACACTCATAACAAATGTCTACCAGCACACGGTGCGTGCACACACCTATACAAAAAAATGTGCATAC
AACCCATACCCACAACCTGCTCTCCTCCAAGGTTCAGGAAGCTAATGAAATTTTAGGATTCCCAAGGCCC
CACCCCAGGGTTGTAGACACAGTAAATAATGGCTGTGGGGAGGGACCGTTATTCAGTGGAGCTGTCTGGG
GCAGGCTCCAGGAGGCATGCAGCTTTGATGAGTTGTTGCCCCATCTTGGGGTGGGTTTTCTGTAGATGGC
ACTGATGTCTAGAAATTTTGTAGTCACAGGATAGGATGCAGGTACTTGTATCCAGGAACAAAGAATAGGT
CAAAGGCAGCAAACTGTAGCAACTGTAGAGGGAGGGTTTACTGATTGAAGAAGAATAGACTTTCAAATAG
TGTTGGTTAGGAAAAGATACAGTTGAGAGAGAGAAAGAGACAGAGAGAGAGAGATTGAAAGAGAGAGACA
GGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGATTTCAACAGCTCAAGTGCCCTGTTACATAAAGA
GCTGGGGCTTTTGTGTTAATTCAACATCAGCTTTTGGGCTTATTTACATAGCAAGGCTCATTTGCATGCT
GTCACATATAGTTGTATATACAACTTCATGCATTGAGTATCTCATTTGCATCATAAGACCCCACCTCTGT
TTTAATATCATAATTAGCCACAGGACACCTTCAGACACC

ChIP-changes: yellow; CGs: purple; guideRNAs: blue

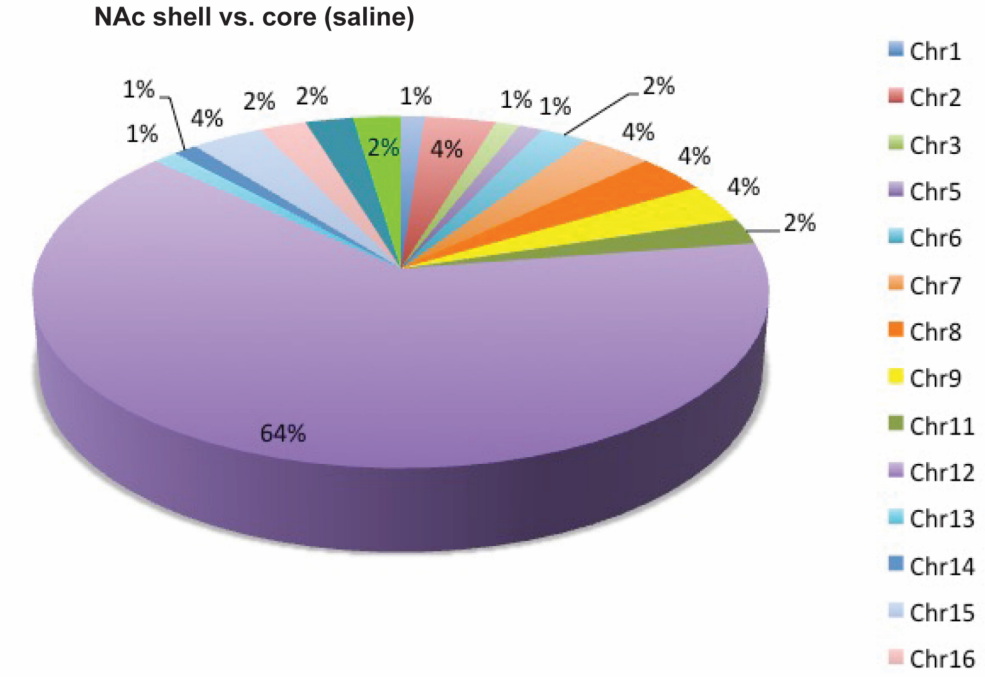

Figure S2. Auts2-interacting fragments differ substantially in NAc shell versus core. Pie chart of chromosomes with significantly different 4C-fragments in the saline condition. Total changes: 85 of 2159 fragments (3.93%).

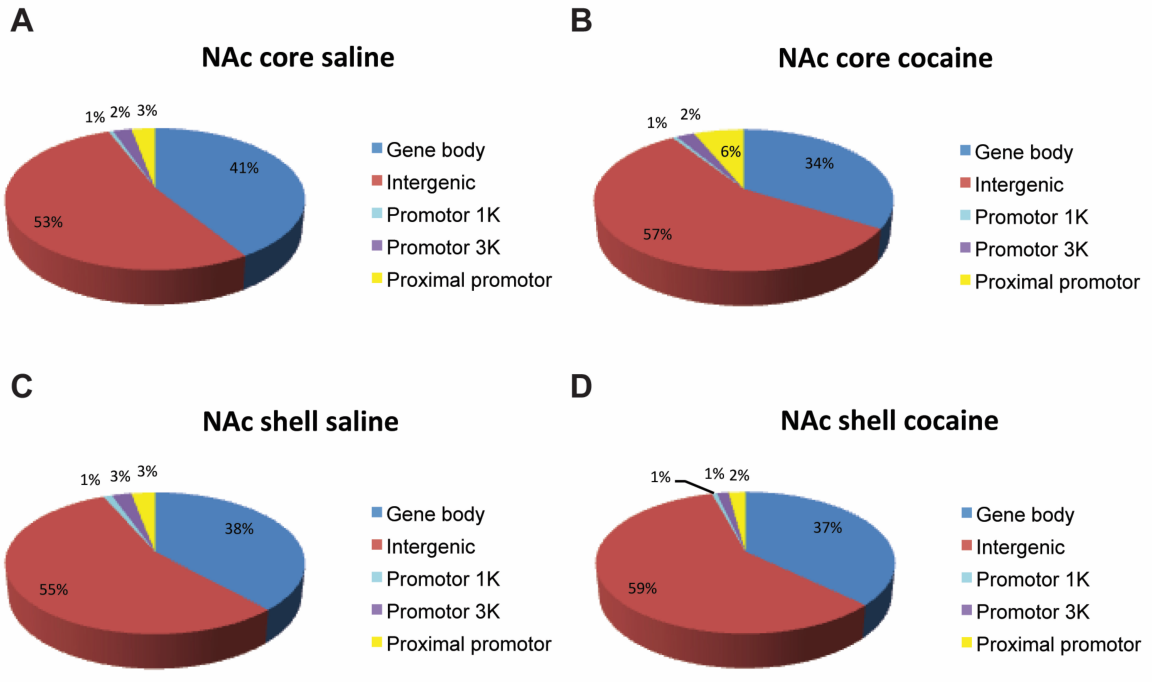

Figure S3. Composition of *Auts2*-interacting fragments in individual experimental groups. Pie chart of genomic regions with 4C-fragments that are bound to the Auts2 bait. (A) NAc core saline. (B) NAc core cocaine. (C) NAc shell saline. (D) NAc shell cocaine. Compositions did not differ significantly between groups.

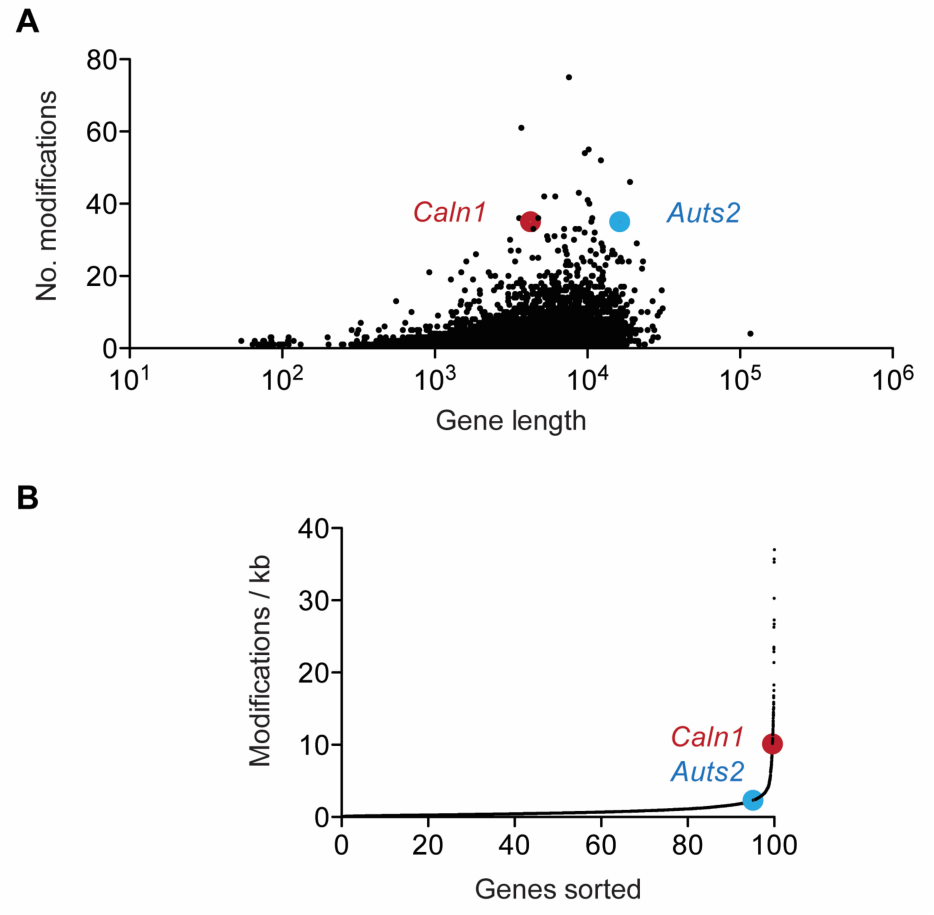

Figure S4. Plots of gene length and number of cocaine-induced modifications. (A) Number of modifications versus gene length. (B) Genes were sorted by the number of modifications per kb of length. *Caln1* and *Auts2* have above average numbers of chromatin modifications per kb. Those genes with the highest numbers of modifications per kb consisted in the majority of micro-RNA genes with only one chromatin-modification and may therefore be false positives.

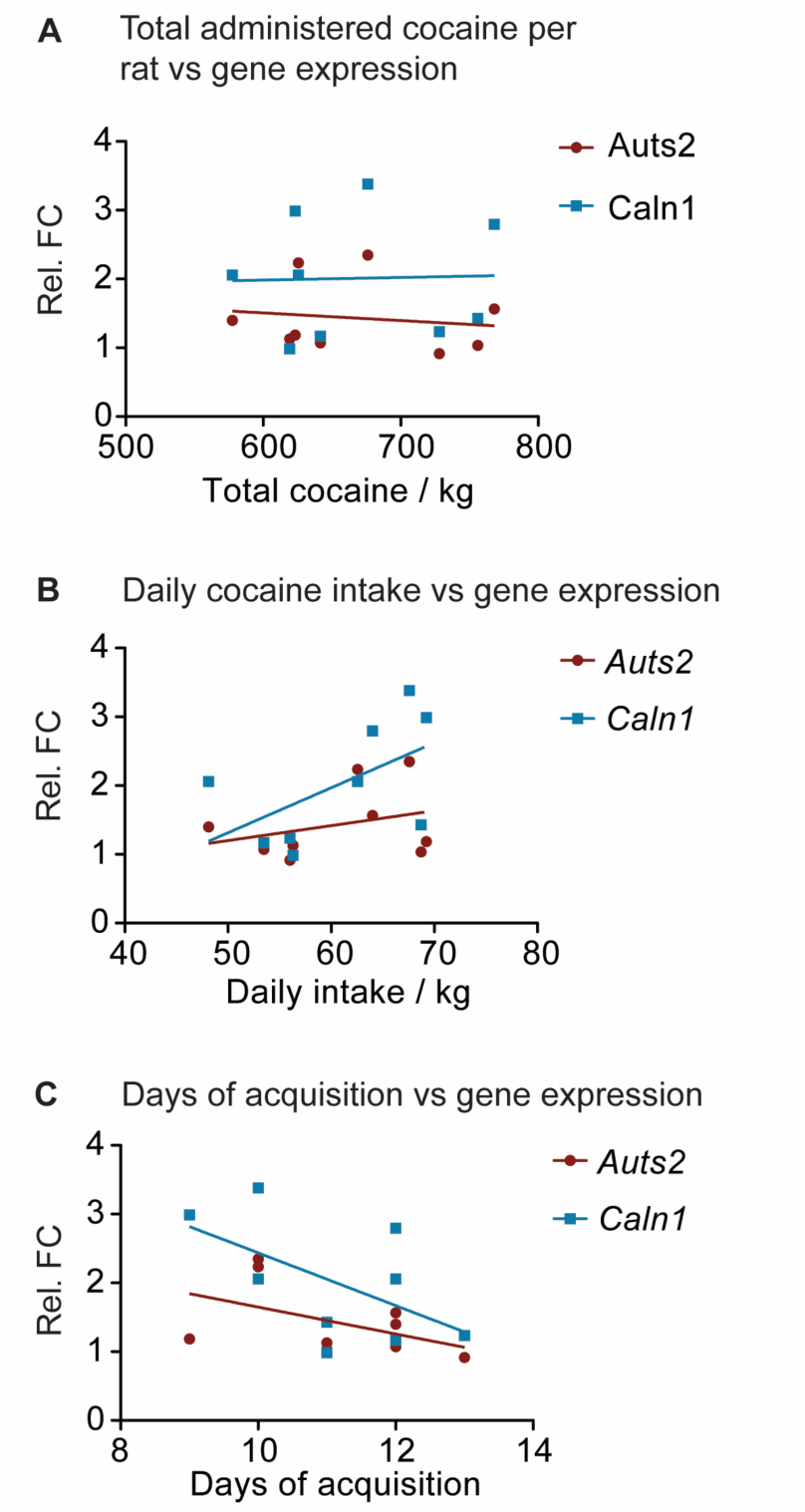

Figure S5. Cocaine self-administration in rats. There is no correlation between gene expression of *Auts2* and *Caln1*, and (A) total administered cocaine, (B) daily cocaine intake, or (C) days of acquisition. (A) Linear regression for Auts2: F(1,7)=0.146, P>0.05; Caln1: F(1,7)=0.006; P>0.05;

n=9. (B) Linear regression for Auts2: F(1,7)=0.744, P>0.05; Caln1: F(1,7)=3.184; P>0.05; n=9. (C) Linear regression for Auts2: F(1,7)=2.010, P>0.05; Caln1: F(1,7)=3.102; P>0.05; n=9.

Figure S6. Potential CTCF-binding sites

We defined a sequence as a potential CTCF-binding site if at least 10 of 14 nucleotides coincided with the consensus sequence CCGCGNGGNGGCAG determined by Bell and Felsenfeld (15).

Auts2 forward strand

consensus sequence: CCGCGNGGNGGCAG

CGGCGGGGGAGGCAA

CCGCGCCGGGGATC

GCGCGGGGGAGAAA

underlined: CGs detected by bisulfite sequencing

pink: changed CGs (bisulfite sequencing)

CCTGAACCCCTTTCTTTCCACCCATCTGCTGTGGAGGGAGGTGGGGGTGCAGGAAGGGGGCGCAAGGGAAAGGCAAGC
TGGACAAGAACGAAACCAGTAGAAGACCAGACCAGGCATACAGCTTAGCGATTCCCTCCTTGCTGCCAATCTGCCCCC
AGCTCCCCCTACATACAACCCCACACCCAACAATAAGACCTCGGCCGAGACTGAGAGCTTCGAGTGCGGCGGGGGAGG
CAAGAAAACCGGGGTGCAGCCGAGTGTGGGGGGTGTCATGCATAAAAGGAAACCATGGGGGGAACAGAAAAGGGGGTC
CCCCCTTACCTCCAGCGCTTCAAAAGTAACAAAGCTGGTCATGGCAAATCCATCAATGATGTCCTCTTCGGCTGAGGT
GGACTCTCTGCGCTTCCTCCGCCGGGGGTCTGGGCCGGGACGGGGCGGAGGATGGGGGCTTTCCATTGTCTTCCTTGTC
GGAGCCCGACGATGAAGCCAGCGAGGGAGCCCGGGTCCGGCCGGCTCCTATACCACCCGCCGCACCGGTTCCTAACCC
GGCCCGGGAGCGTCTCTCCCCGGTCTCGCTGTGATCGAGACCTCCGCTTTTTCCGGAGTCCATGTCCCCGCGTCGGGCC
ATCCATGGTTCTTTTAACCGCGCCGCGGGATCTTCCCCGTTCACCTACCCGCGCGCCACAGGCTCGGGCCGCGGCCACA
CACACACACACACACCCCAAGAAGAAAAAAAAAAATTGACACCGAGCCCCCCTTCCTCCTGCACGGGCTTTTCCCTCCC
GCCGCGAGGAAGGGCGCGGGGGAGAAAGCGGCTGGGAGAAATAAAAAAAAAAAAAAAAAAAAGAATCCGAAGAAGAAC
AAGAAGGGAGGGCAGGGGGGAAAAGAGAAAGAAAAAAGGGGGAAGGGGCGGGGAGACGGGGAGGGAGGGACGGGGAAG
CGAGAGGGCGAAGTGTGGGGCTCCCCTCTGCACCGAGGGTCGGAGATGCCTGCGAACAGCGCCGAGCAAAGTAATGGC
TGAACACACAGGTCTCCTTTACAGAGGAAAGGGGGAAAAAAATAACTCACCAAGCAGGCAATAAATCAGAATAGCGGA
ATGCTTTGATCAAGAGACTGGGGAAGCCCTCCCCGCGGCCATGCTCTCTCCCCACCACTGAAATCGCCTTGCTTCTCC
CTTTCTCTAGAGACCCCCCAAAAAATTTTAAAAAAAAAAAAGCCCGAGGTCCGAAAAGGTCACGGTGAAGTGAGGGGT
GGGTGGCAATGAACTTCACCCGCCCCCTCGTGCAGGAAAACACCCCACACCTCCGCCTCTCTCACACACTCGCCTCGC
AGAAAAACTCACTCCCGGGCTCCCAGTCTTTTTTTAAGAAATGCATCCAAGGGAAGCCAGGGCTGGAGAGCGAGCTGA

Caln1 reverse strand

As CTCF has a directionality of binding, any potential CTCF-binding site which interacts with the *Auts2* forward strand needs to be localized on the *Caln1* reverse strand.

consensus sequence: CCGCGNGGNGGCAG

GGGAGATGAGGCAG

underlined: CGs detected by bisulfite sequencing

GGTGTCTGAAGGTGTCCTGTGGCTAATTATGATATTAAAACAGAGGTGGGGTCTTATGATGCAAATGAGATACTCAAT
GCATGAAGTTGTATATACAACTATATGTGACAGCATGCAAATGAGCCTTGCTATGTAAATAAGCCCAAAAGCTGATGT
TGAATTAACACAAAAGCCCCAGCTCTTTATGTAACAGGGCACTTGAGCTGTTGAAATCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCCTGTCTCTCTCTTTCAATCTCTCTCTCTCTGTCTCTTTCTCTCTCTCAACTGTATCTTTTCCTA
ACCAACACTATTTGAAAGTCTATTCTTCTTCAATCAGTAAACCCTCCCTCTACAGTTGCTACAGTTTGCTGCCTTTGA
CCTATTCTTTGTTCCTGGATACAAGTACCTGCATCCTATCCTGTGACTACAAAATTTCTAGACATCAGTGCCATCTAC
AGAAAACCCACCCCAAGATGGGGCAACAACTCATCAAAGCTGCATGCCTCCTGGAGCCTGCCCCAGACAGCTCCACTG
AATAACGGTCCCTCCCCACAGCCATTATTTACTGTGTCTACAACCCTGGGGTGGGGCCTTGGGAATCCTAAAATTTCA
TTAGCTTCCTGAACCTTGGAGGAGAGCAGGTTGTGGGTATGGGTTGTATGCACATTTTTTTGTATAGGTGTGTGCACG
CACCGTGTGCTGGTAGACATTTGTTATGAGTGTGTGCAGAAGCCAGAGGTCCCAGTCAAGTGTCTCCTTCTATCCCTT
CCTGCTTTGTTTTTCTGAGACAGGCTCTCCCACTGAACTCAGAGCTTACCAATGCAGCTAAGCTAGCTAAGGAGCCTA
CTTAGCTCTGCTTCTCCAGCACTAGGACTGCGGGTGTGTACCACAGCCTCTCCCCCTACCCCCTGCTCTTTTATGTGG
GTGCTGGGGACCTACACTTAGGATCCCATGCTTGCCTATACGGTAAGGACTTTACTGATTGAGGCATCTCCCCAATTT
TAGCTCCCCTAAATCTTGTGAGCCTCCTGGCTCCCGCCAGTAAAGAATCTTTTCCATTCAGAGGAAATAGAAACTCAG
TAGCATGTCCTTAAAAAGTTACAGAACAGTCGGGACAAGAGTTCCAGAGACTGAAATAATGTCTGGTTTATGTAGATG
CCCGAGAATAACATAAGGTCAGATGGACTGCCACCCCCACCATCCCCCAGCAACCACCTGGCTGAGCCTCTGGTCCAG
GTGCATGGGGAGATGAGGCAGGCTAGACAGGCCCTGCCAACAGCAGCCGGAATGTCACAATTGGGCACAGAGTCATCC
CAACAGTCTGGCACAGGTCAGTGAATGCAGGAAGGCACTGCTGGATGTCTGCTAGTGAGAGGCATGAGCTGATGGGGT
GTGATGACCGCAGAAGCAAAAATGAGCTTTAGCCCAAGACACGAGTTCACCTGAAGGATGTATCGGGAAAGACTGTCC
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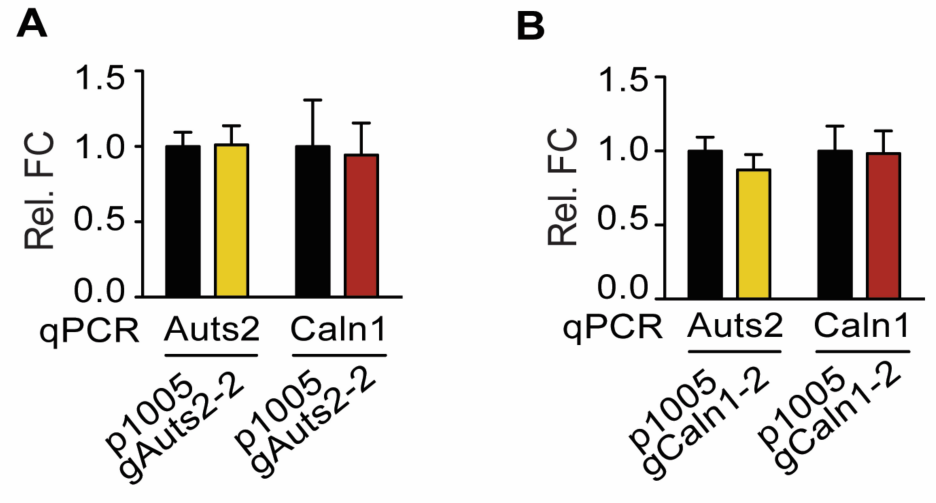

Figure S7. Gene expression of *Auts2* and *Caln1* is not affected by a FLAG-tag dCas9 CRISPR construct. (A) Neuro2A cells were co-transfected with the CRISPR-dCas9 and guide-RNA Auts2.2, which induced the highest gene expression change in *Caln1* when combined with a Dnmt3A/3L-dCas9. qPCR was conducted for *Auts2* and *Caln1*. No significant changes were detected. Two-way ANOVA: effect of CRISPR: $F(1,20)=0.013, P>0.05$. Effect of tested gene: $F(1,20)=0.027$, $P>0.05$. Interaction: $F(1,20)=0.027$, $P>0.05$, $n=6$. (B) Neuro2A cells were co-transfected with the CRISPR-dCas9 and guide-RNA Caln1.2, which induced the highest gene expression change in *Caln1* when combined with a Dnmt3a/3L-dCas9. qPCR was conducted for *Auts2* and *Caln1*. No significant changes were detected. Two-way ANOVA: effect of CRISPR: $F(1,20)=0.294$, $P>0.05$. Effect of tested gene: $F(1,20)=0.170$, $P>0.05$. Interaction: $F(1,20)=0.170$, $P>0.05, n=6$. Graphs show average +/- SEM.

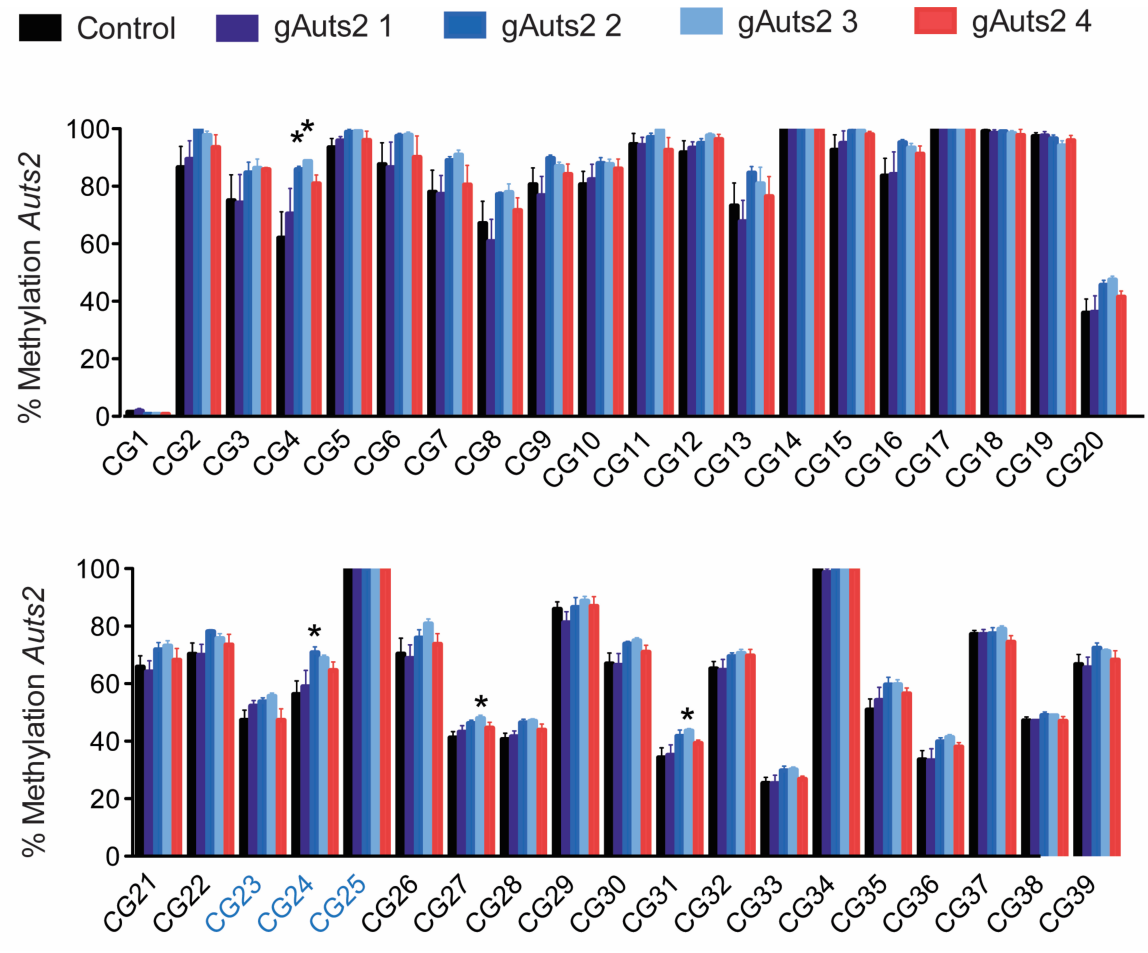

Figure S8. CRISPR-dCas9 induced DNA methylation on *Auts2*. Guide-RNAs were designed for *Auts2* and pyrosequencing was conducted on the *Auts2* region, which had been affected by cocaine (blue: putative CTCF binding site). Guide-RNAs induced a general increase in DNA-methylation. Two-way ANOVA: effect of guide-RNAs over all CGs: Ctrl vs. Auts2 1: $F(1,390)=0.011$, $P>0.05$; Ctrl vs. Auts2 2: $F(1,351)=52.810$, $P<0.001$***; Ctrl vs. Auts2 3: $F(1,390)=70.650$, $P<0.0001$***; Ctrl vs. Auts2 4: $F(1,390)=13.35$, $P<0.0001$***. One-way ANOVA and Dunnett posthoc test: effect of guideRNAs within one CG: CG4: Ctrl vs. Auts2 2: $P<0.05$*, Ctrl vs. Auts2 3: $P<0.05$*; CG24: Ctrl vs. Auts2 2: $P<0.05$*; CG27: Ctrl vs. Auts2 3: $P<0.05$*; CG 31: Ctrl vs. Auts2 3: $P<0.05$*, n=5-6. Graphs show average +/- SEM.

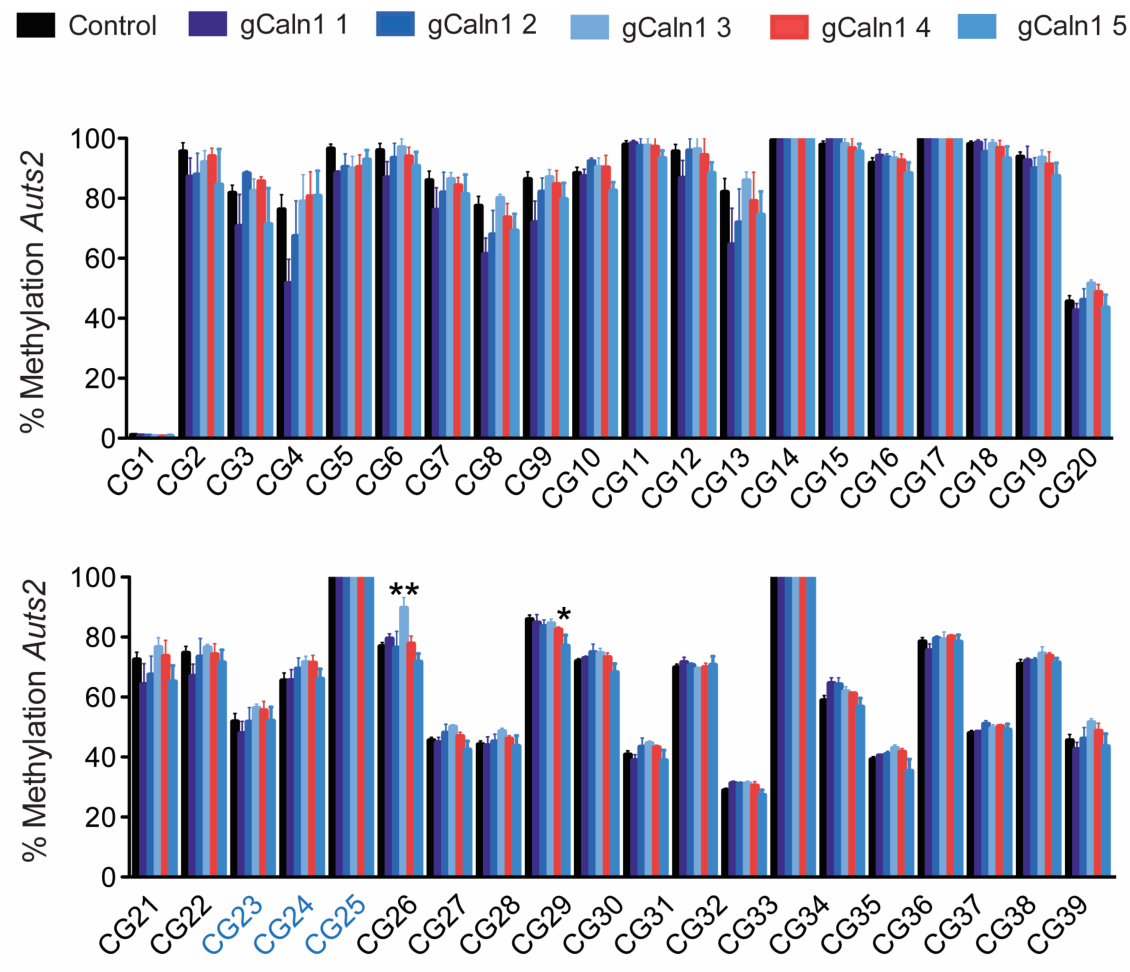

Figure S9. CRISPR-dCas9 induced DNA methylation changes on *Auts2*. Guide-RNAs were designed for *Caln1* and pyrosequencing was conducted on the *Auts2* region, which had been affected by cocaine (blue: putative CTCF binding site). Two-way ANOVA: effect of guide-RNAs over all CGs. Ctrl vs. Caln1 1: $F(1,480)=27.50$, $P<0.0001$***, interaction between CG and guide-RNA: $F(1,480)=2.33$, $P<0.0001$***; Ctrl vs. Caln1 2: $F(1,480)=0.8548$, $P>0.05$;Ctrl vs. Caln1 3: $F(1,480)=10.29$, $P<0.01$**; Ctrl vs. Caln1 4: $F(1,480)=0.605$, $P>0.05$; Ctrl vs. Caln1 5: $F(1,480)=21.72$, $P<0.0001$***. One-way ANOVA and Dunnett posthoc test: effect of guide-RNAs within one CG: CG26: Ctrl vs. Caln1 3: $P<0.01$**; CG29: Ctrl vs. Caln 1 5: $P<0.05$*; all others: n.s., n=4-10. Graphs show average +/- SEM.

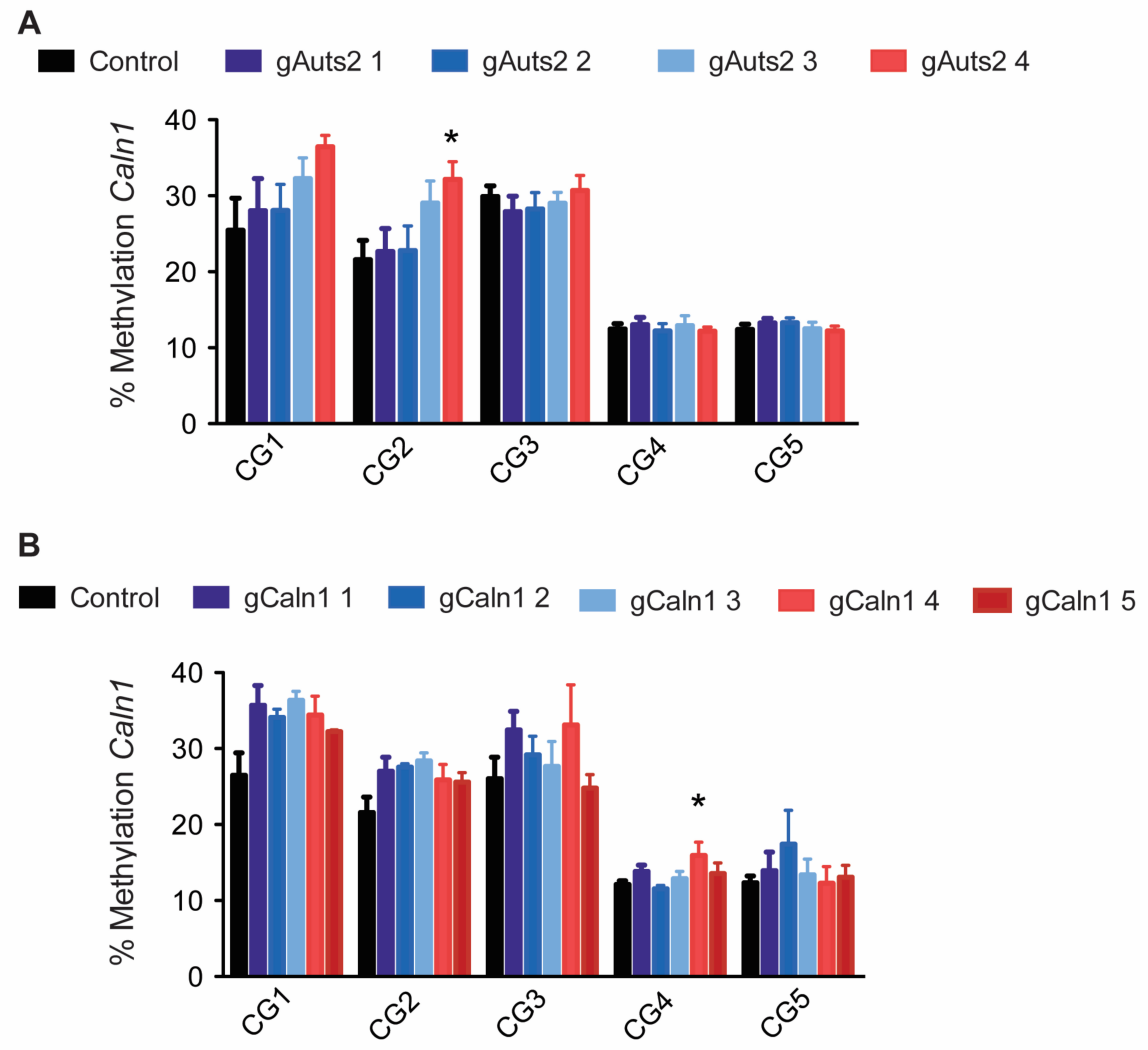

Figure S10. CRISPR-dCas9 induced DNA methylation on *Caln1*. Pyrosequencing was conducted on the *Caln1* region, which had been affected by cocaine. (A) Guide-RNAs were designed for *Auts2*. Two-way ANOVA: effect of guide-RNAs over all CGs: Ctrl vs. Auts2 1: $F(1,70)=0.186$, $P>0.05$; Ctrl vs. Auts2 2: $F(1,73)=0.153$, $P>0.05$; Ctrl vs. Auts2 3: $F(1,73)=4.336$, $P<0.05$*; Ctrl vs. Auts2 4: $F(1,73)=13.08$, $P<0.01$**, interaction between CG and guideRNA: $F(1,73)=4.702$, $P<0.05$*.One-way ANOVA and Dunnett posthoc test: effect of guideRNAs within one CG: CG2: Ctrl vs. Auts2 4: $P<0.05$*; all others: n.s., n=7-9. (B) Guide-RNAs were designed for Caln1. Two-way ANOVA: effect of guideRNAs over all CGs: Ctrl vs. Caln1 1: $F(1,193)=2.335$, $P<0.05$*; Ctrl vs. Caln1 2: $F(1,97)=6544$, $P<0.05$*; Ctrl vs. Caln1 3: $F(1,99)=10.450$, $P<0.01$**; Ctrl vs. Caln1 4: $F(1,94)=6.042$, $P<0.05$*; Ctrl vs. Caln1 5: $F(1,92)=1.718$, $P>0.05$. One-way ANOVA and Dunnett posthoc test: effect of guideRNAs within one CG CG4: Ctrl vs. Caln1 4: $P<0.05$*; all others: n.s., n=3-19. Graphs show average +/- SEM.

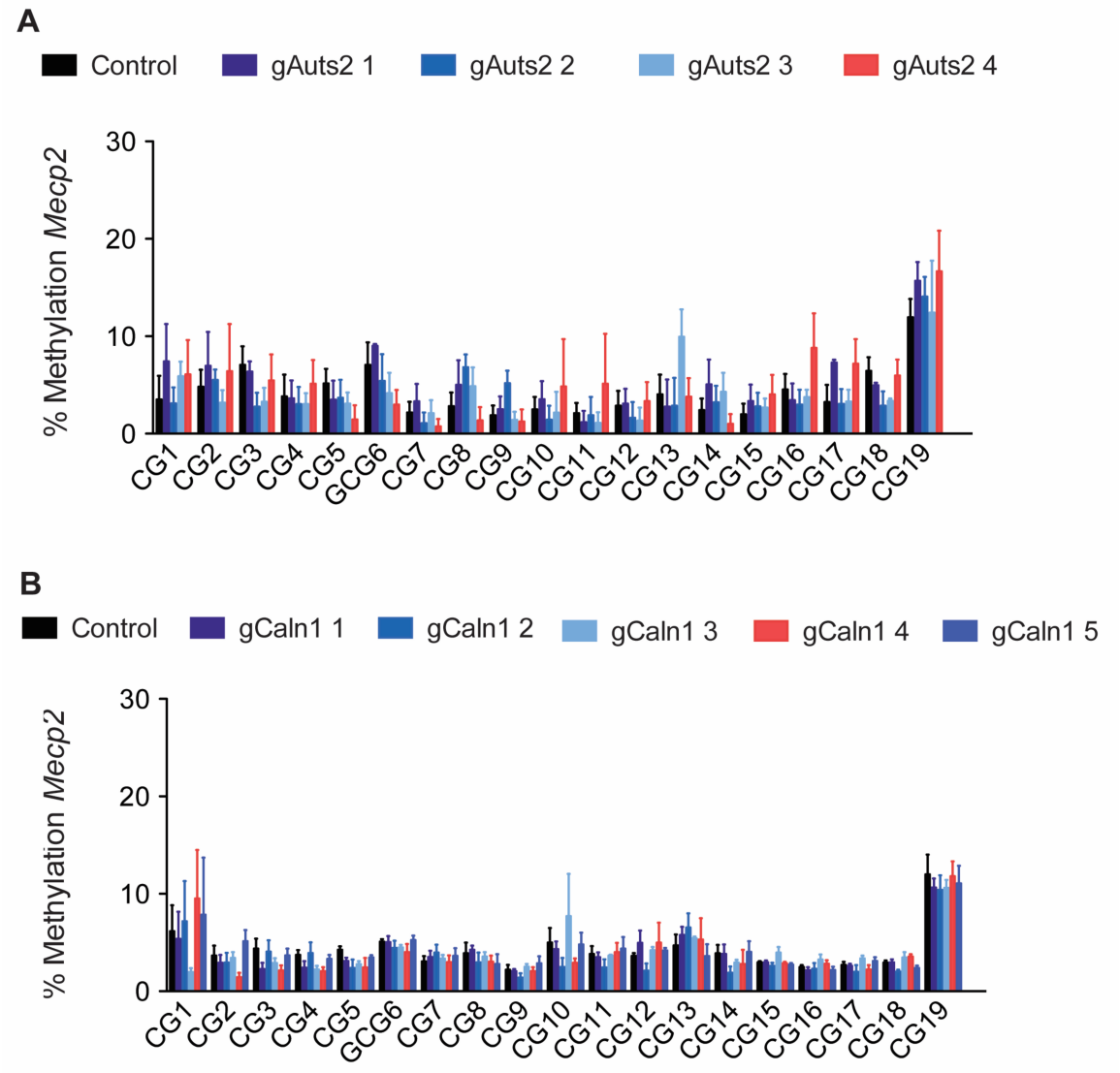

Figure S11. CRISPR-dCas9 did not induce DNA methylation on *Mecp2*. (A) Guide-RNAs were designed for *Auts2*. Two-way ANOVA: effect of guide-RNAs over all CGs: Ctrl vs. Auts2 1: P>0.05; F(1,76)=2.400; Ctrl vs. Auts2 2: F(1,76)=1.363, P>0.05; Ctrl vs. Auts2 3: F(1,94)=0.1908, P>0.05; Ctrl vs. Auts2 4: F(1,76)=0.600, P>0.05, n=3-4. (B) Guide-RNAs were designed for *Caln1*. Two-way ANOVA: effect of guide-RNAs over all CGs: Ctrl vs. Caln1 1: F(1,171)=0.928, P>0.05; Ctrl vs. Caln1 2: F(1,152)=3.020, P>0.05;Ctrl vs. Caln1 3: F(1,155)=0.516, P>0.05; Ctrl vs. Caln1 4: F(1,190)=0.900, P>0.05; Ctrl vs. Caln1 5: F(1,152)<0.001, P>0.05, n=4-6. Graphs show average +/- SEM.

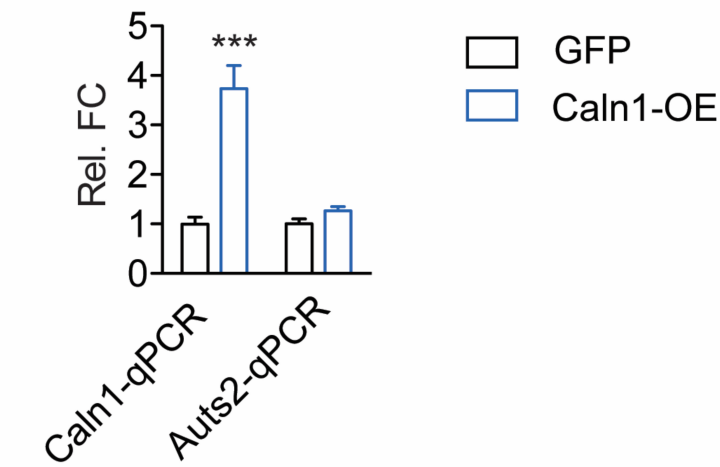

Figure S12. The HSV overexpressing Caln1 increased mRNA levels of *Caln1* but not *Auts2*. Caln1-HSV and GFP control were stereotaxically injected into mouse NAc and animals were analyzed 4 days later. mRNA levels were assessed by qPCR. Two-way ANOVA: effect of HSV: $F(1,46)=28.180$, $P<0.001$***; Effect of tested gene: $F(1,46)=19.090$, $P<0.001$***; Interaction: $F(1,46)=19.090$, $P<0.001$***, n=11-14. Bonferroni posthoc test: effect of HSV within Caln1-qPCR: $P<0.001$***; effect of HSV within Auts2-qPCR: $P>0.05$. Graphs show average +/- SEM.

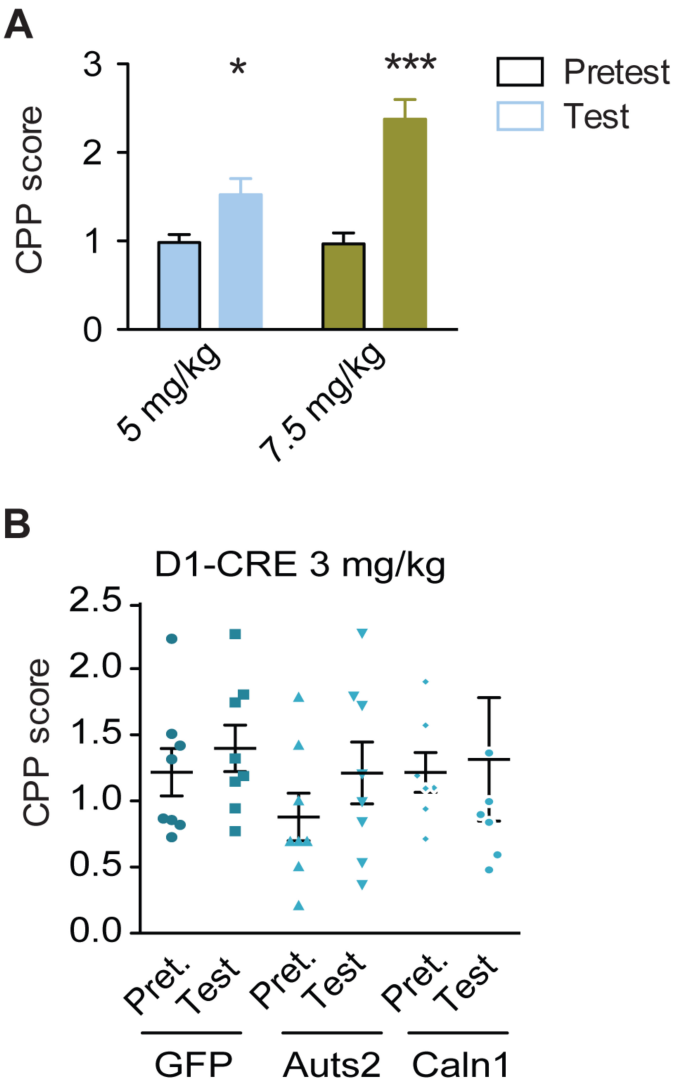

Figure S13. Optimization of conditioned cocaine place preference (CPP) in wild-type C57Bl/6J mice. (A) CPP was performed with 5 mg/kg and 7.5 mg/kg cocaine. 5 mg/kg induced CPP without having reached a ceiling effect and was therefore selected as the experimental dose in Fig 5. Two-way ANOVA: effect of dose: $F(1,36)=6.616$, $P<0.05$*; Effect of cocaine: $F(1,36)=36.180$, $P<0.0001$***; Interaction: $F(1,36)=67.150$, $P<0.05$*, n=10 (B) D1-Cre mice did not show an Auts2- or Caln1-induced difference in CPP Score at a lower 3 mg/kg dose. One-way ANOVA: $F(5,40)=0.534$, $P>0.05$, n=9-11. Graphs show average +/- SEM.

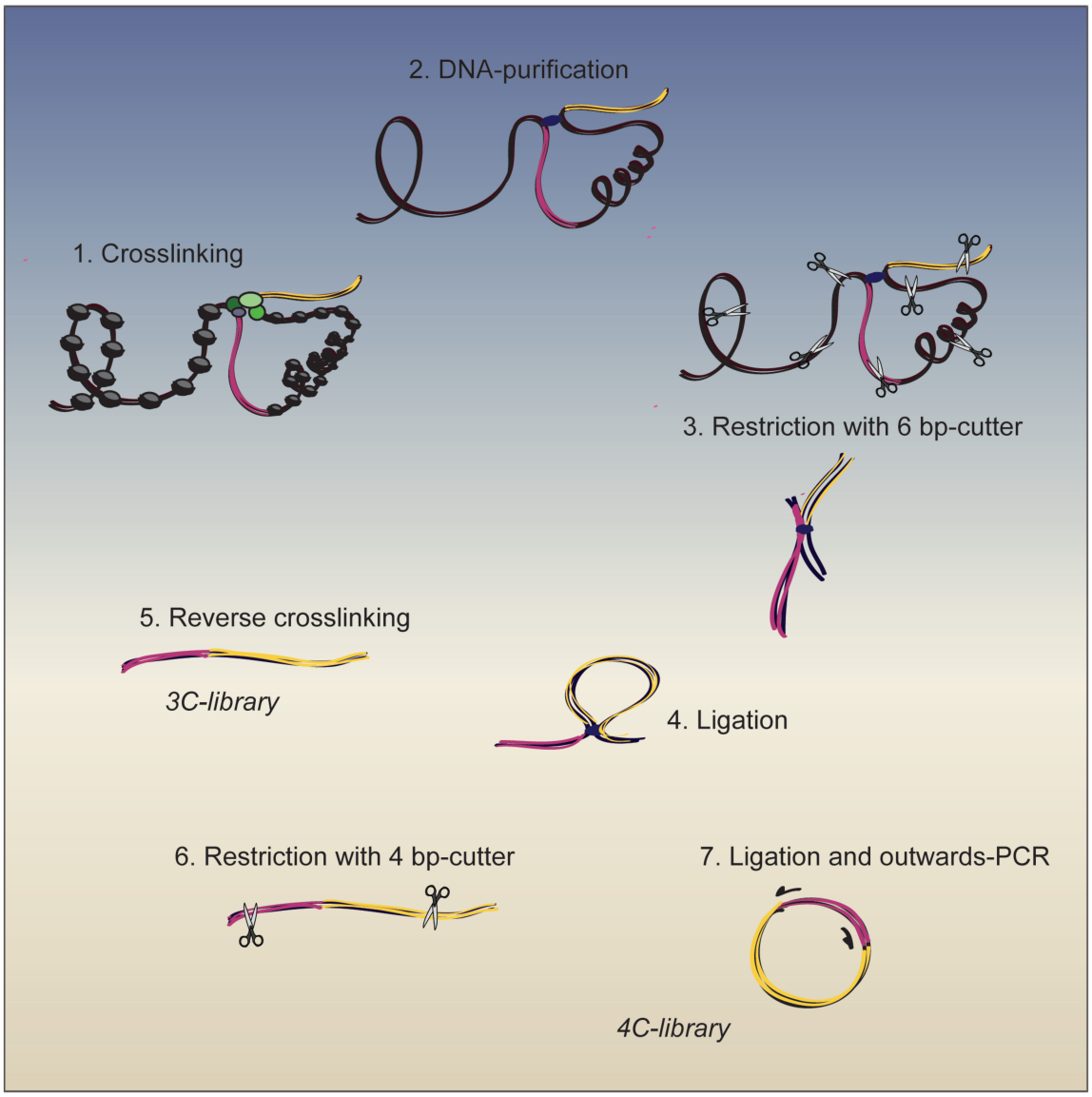

Figure S14. Generation of 3C- and 4C-libraries.

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