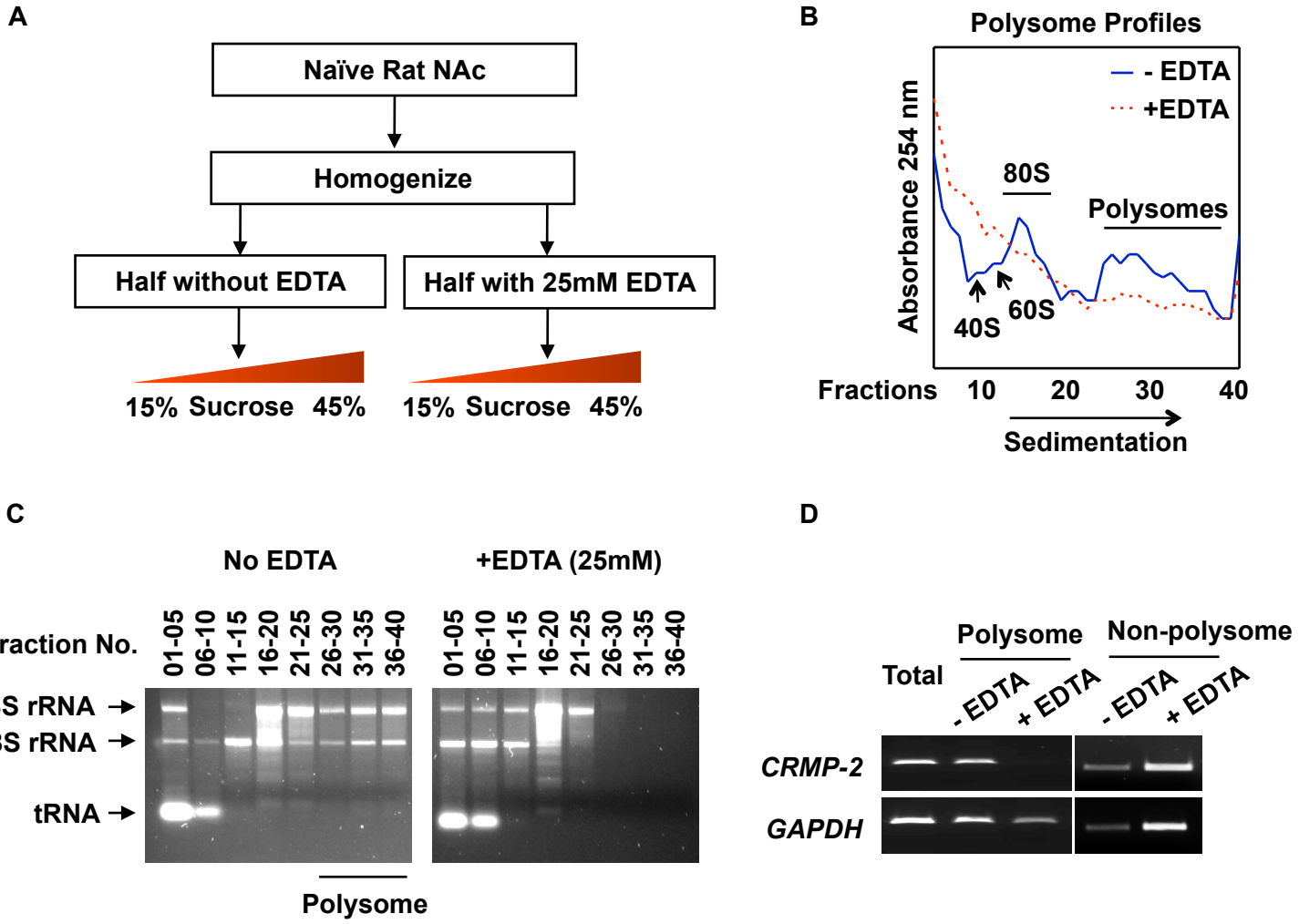
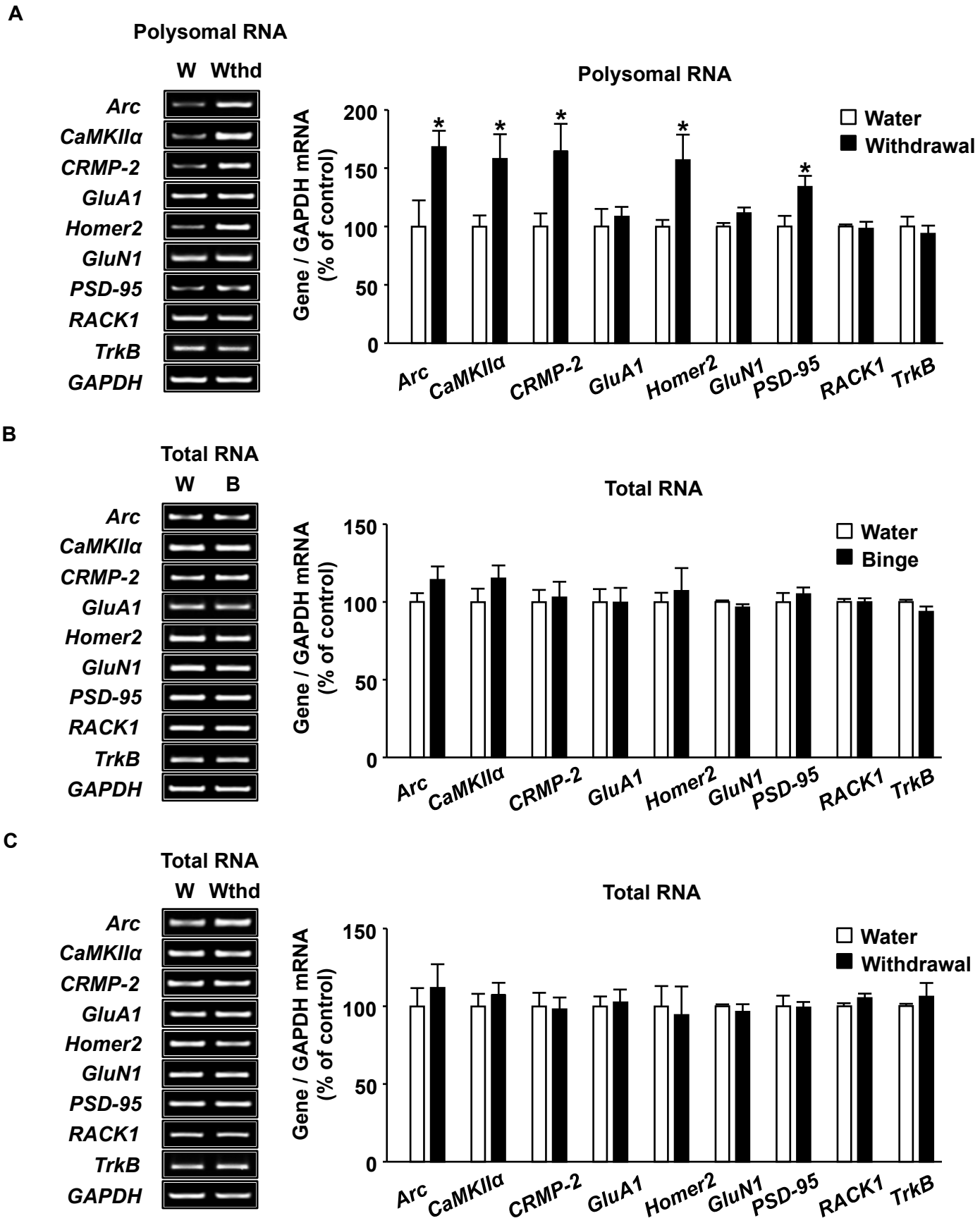
Supplementary Figure 1

Purification of polysomal RNA fraction from rat NAc



Supplementary Figure 2

Alcohol withdrawal promotes the mRNA translation, but does not change the transcription



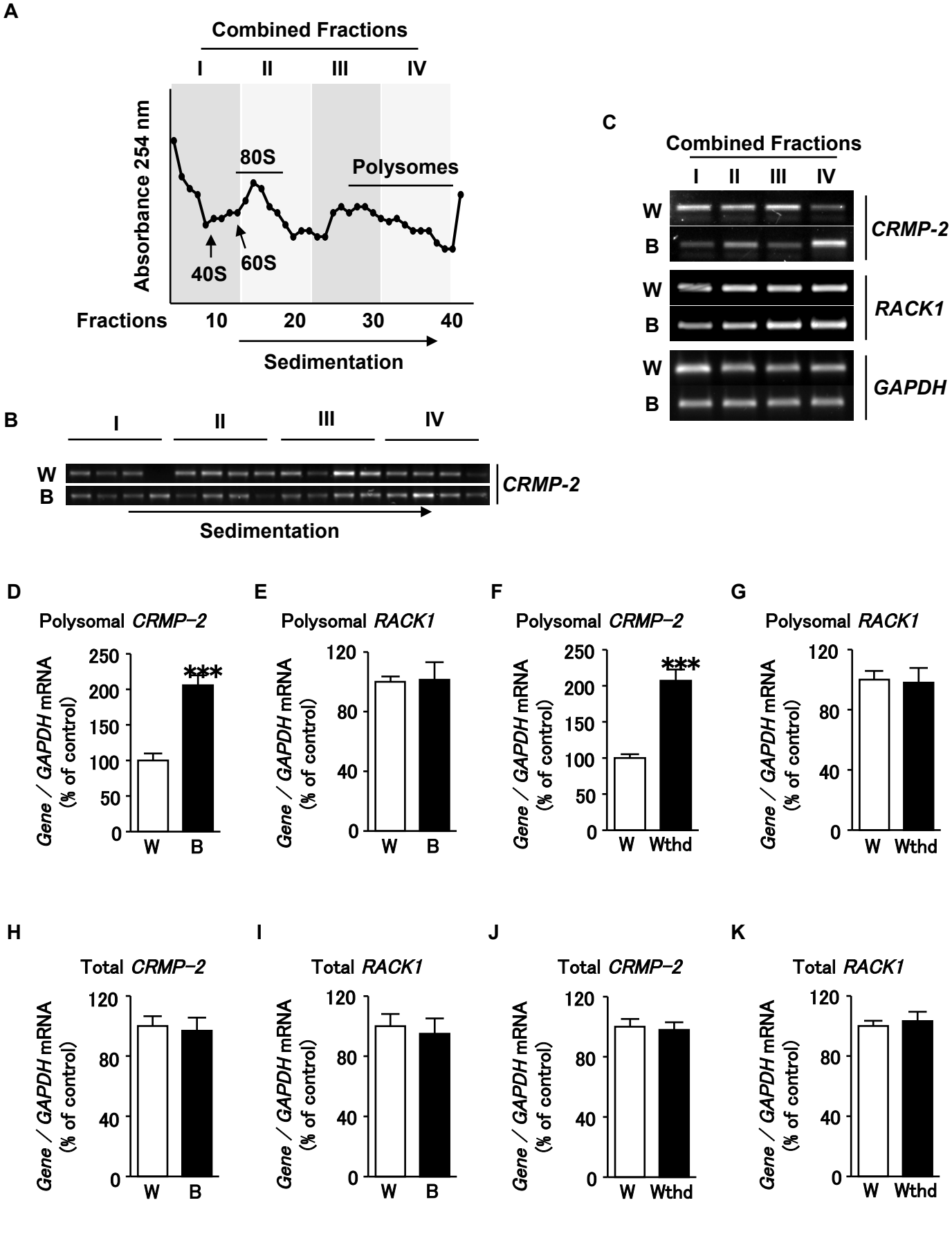
Supplementary Figure 3

TOP analysis of candidate mRNAs

	Transcription start site (TSS, +1 to +20, RefSeq)	
Rat *Arc* (NM_019361)	AGTGCTCTGGCGAGTAGTCC…	Non-TOP
Rat *CaMKIIα* (NM_012920)	AGTCCCGAGCCTAAAGCCTC…	Non-TOP
Rat *CRMP-2* (NM_001105717)	TTTTTCCGCCCTAGCTGGAT…	TOP-like
Rat *GluA1* (NM_031608)	AATTCGGCACGAGCTCGGCT…	Non-TOP
Rat *Homer2* (NM_053309)	GGCACGAGCGGGAGGGACCG…	Non-TOP
Rat *PSD-95* (NM_019621)	GCAAAACTCCAATGAAGTCA…	Non-TOP

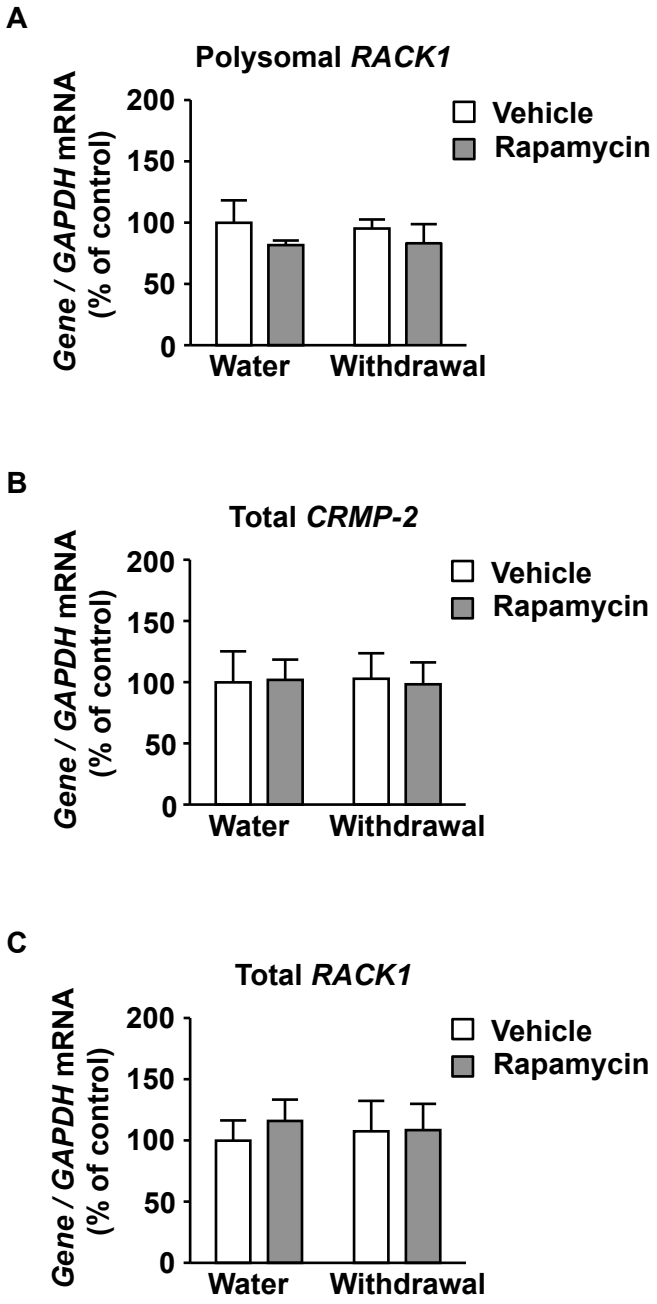
Supplementary Figure 4

Alcohol intake promotes the translation of *CRMP-2* mRNA



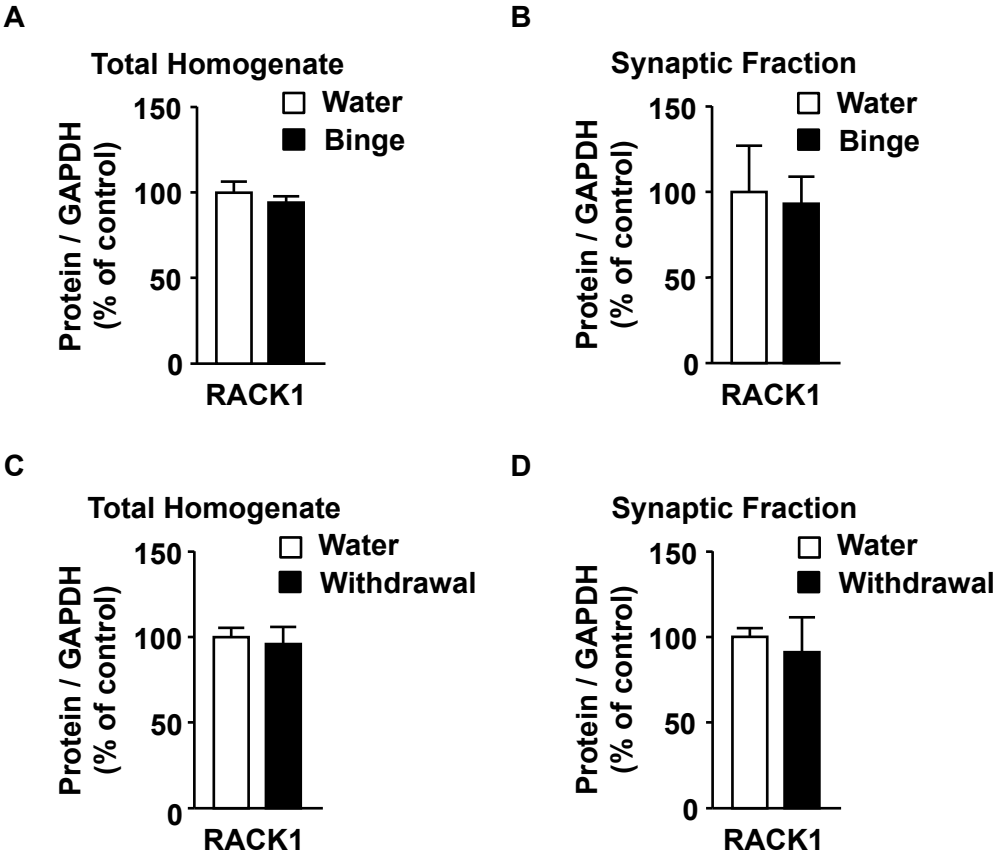
Supplementary Figure 5

Alcohol withdrawal does not alter the translation of *RACK1* mRNA and the transcription of *CRMP-2* and *RACK1* with or without rapamycin



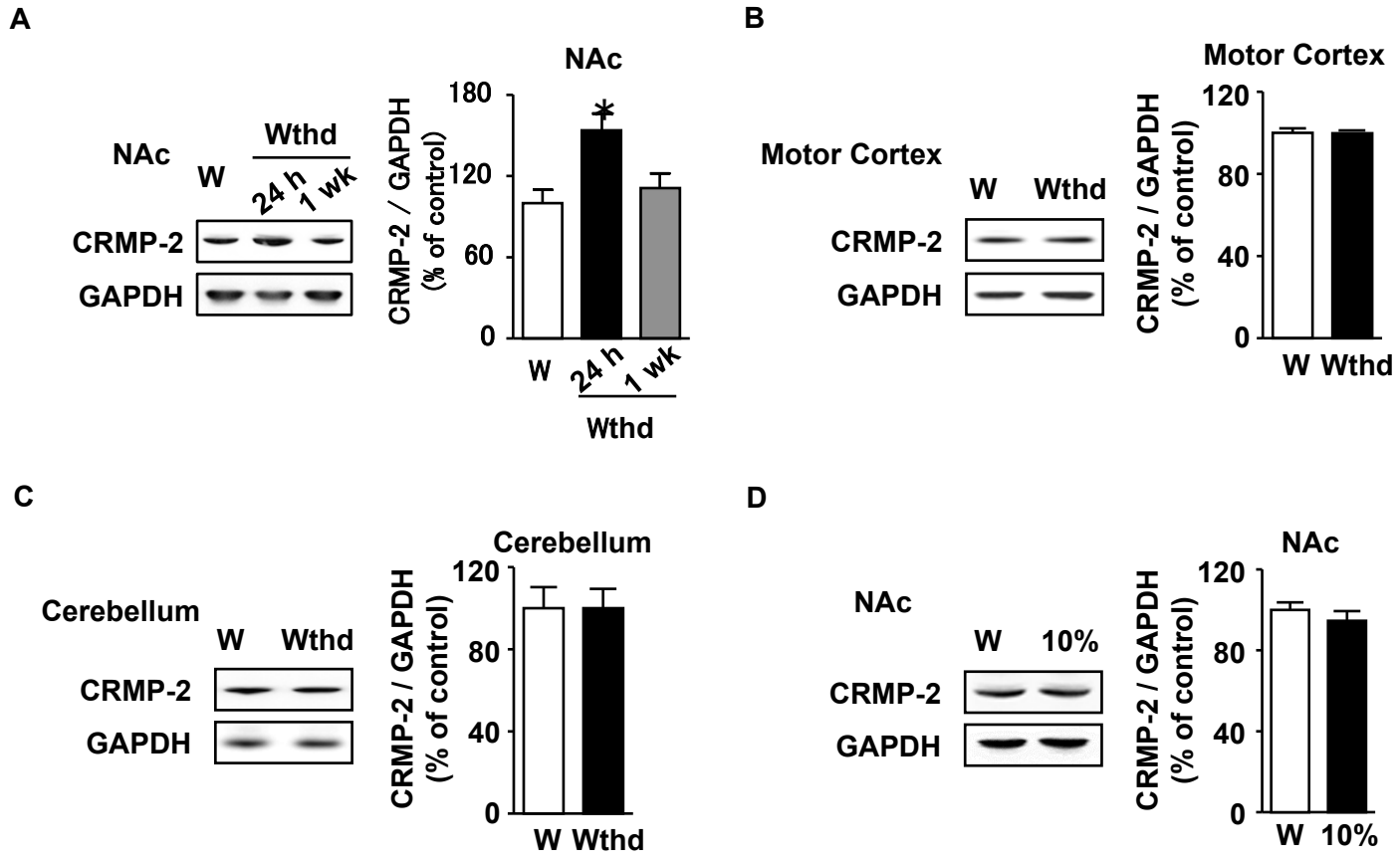
Supplementary Figure 6

Alcohol intake does not change the protein levels of RACK1



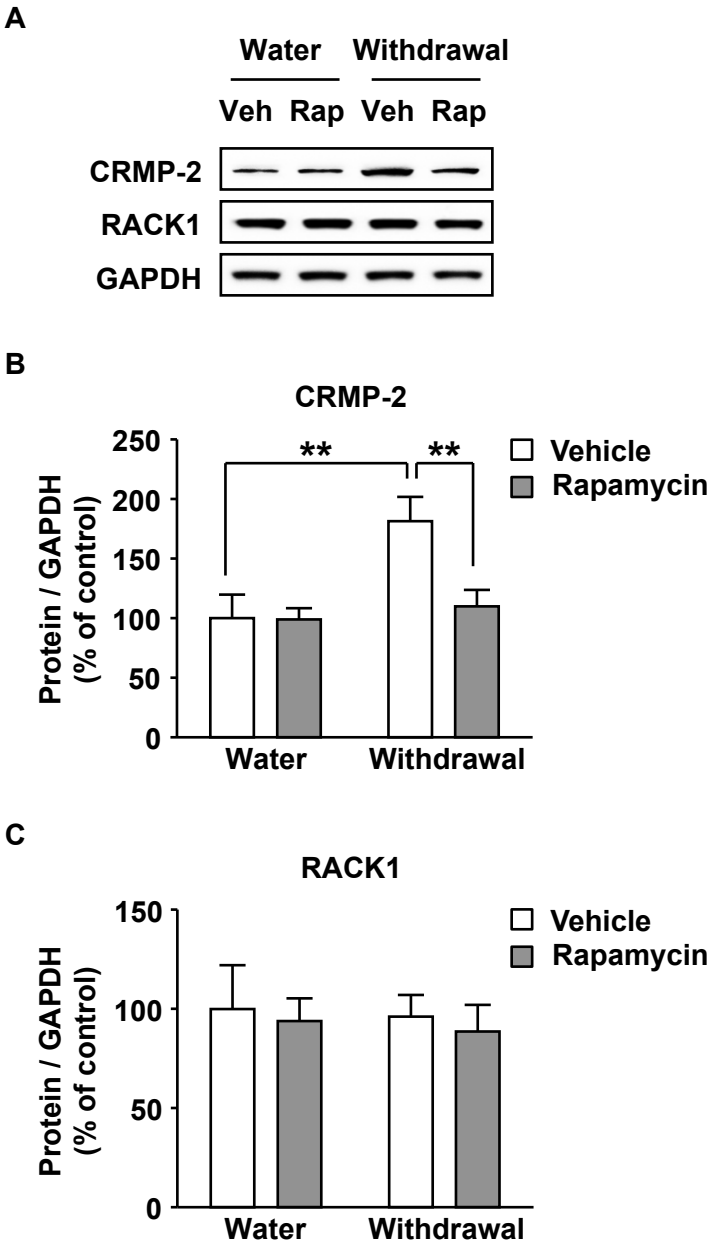
Supplementary Figure 7

Alcohol exposure does not alter CRMP-2 levels in the Nac, Cerebellum and the Motor Cortex



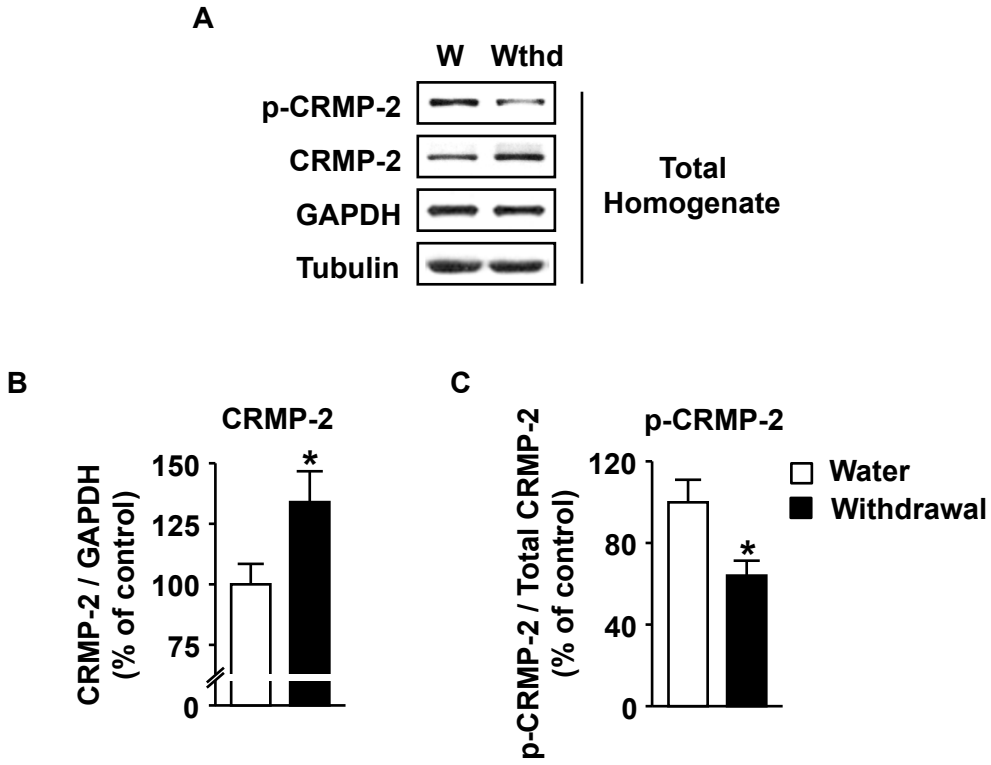
Supplementary Figure 8

Alcohol withdrawal increases CRMP-2 protein levels in an mTORC1-depepdent manner in the mouse NAc



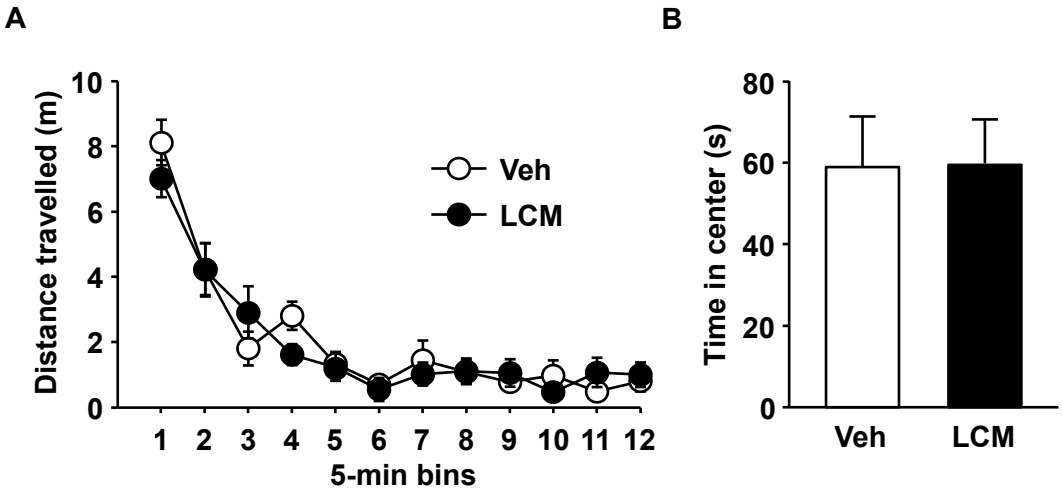
Supplementary Figure 9

Alcohol withdrawal increases CRMP-2 protein levels and blocks CRMP-2 phosphorylation in the rat NAc



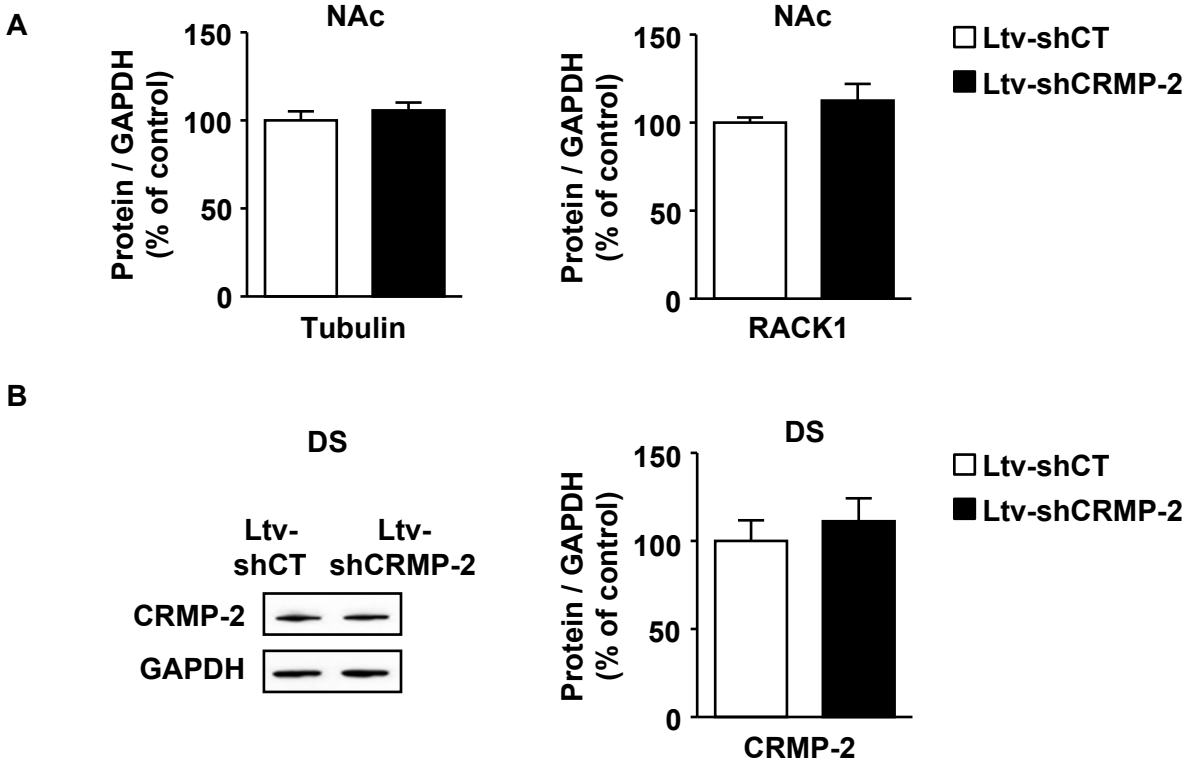
Supplementary Figure 10

Lacosamide does not alter locomotion or anxiety-like behavior



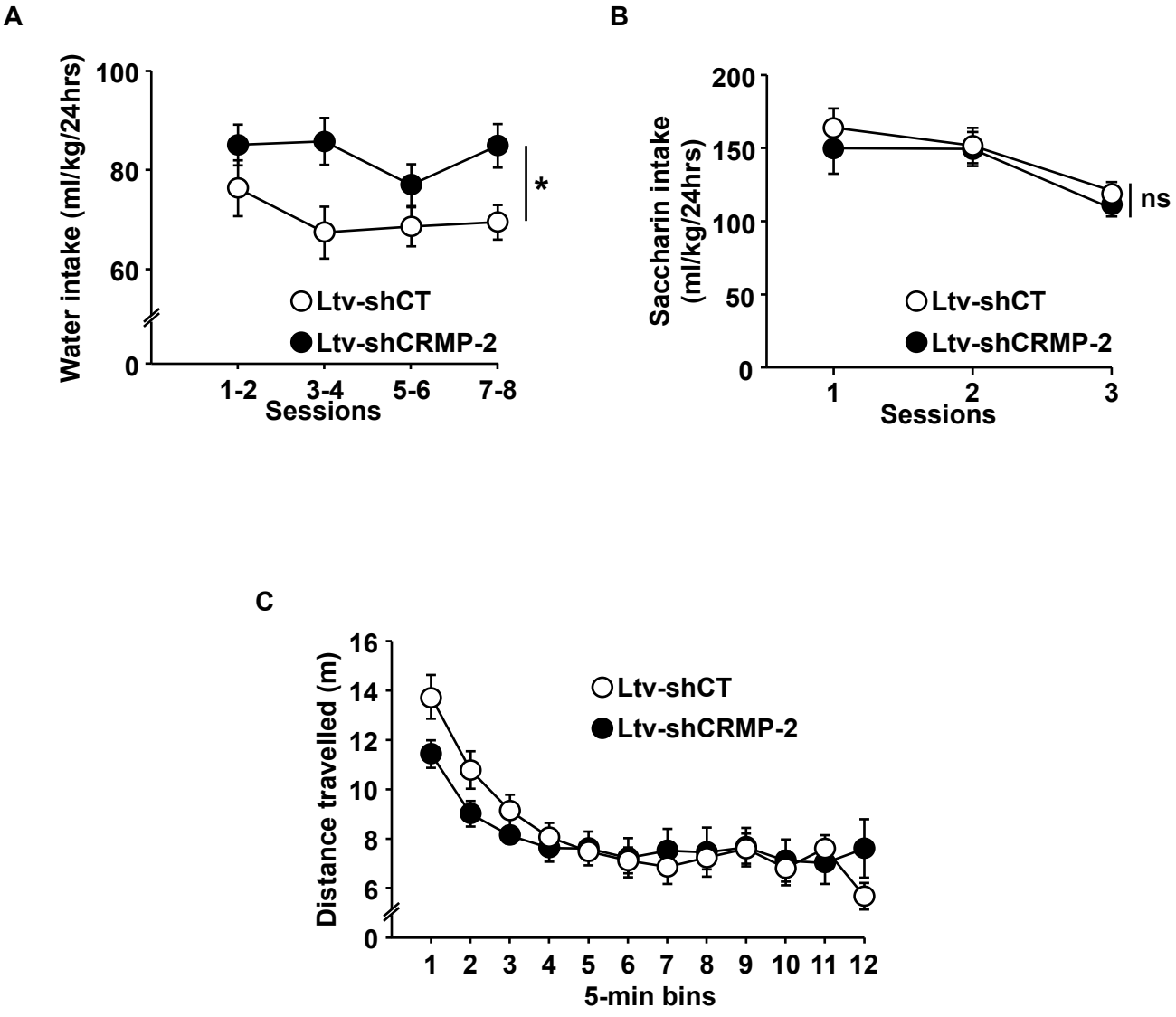
Supplementary Figure 11

Infusion of Ltv-shCRMP-2 into the NAc does not change tubulin and RACK1 protein levels in the NAc and CRMP-2 level in the dorsal striatum



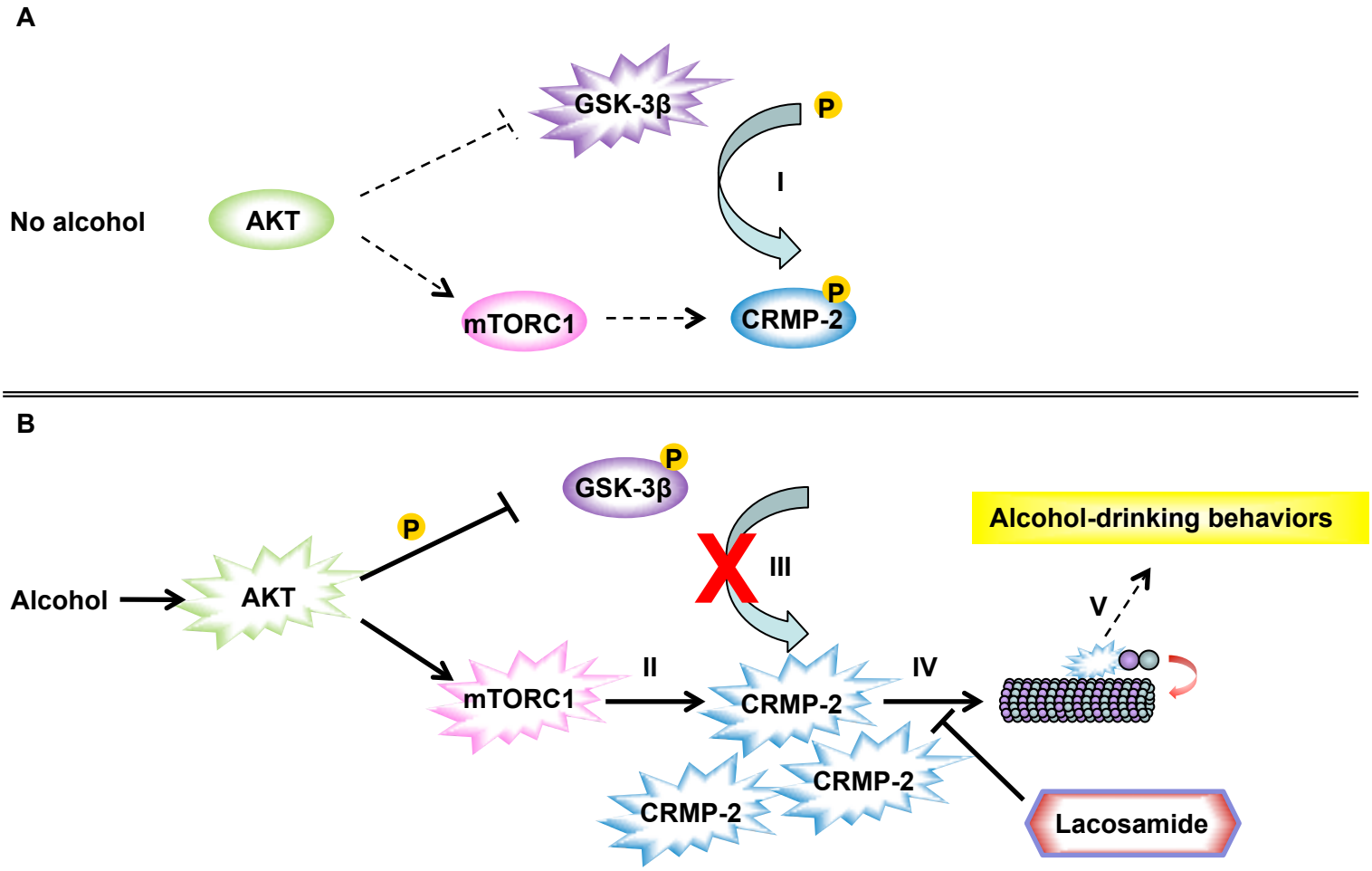
Supplementary Figure 12

Knockdown of CRMP-2 in the mouse NAc increases water intake, but does not alter saccharine intake and basal locomotor activity



Supplementary Figure 13

Dual regulation of CRMP-2 in the NAc promotes alcohol drinking



Supplementary Information

Supplementary Materials and Methods

Materials

Anti-phospho-CRMP-2 (Thr514) (#9397), anti-CRMP-2 (#9393), anti-phospho-GSK-3β (#9323) were obtained from Cell Signaling Technology (Danvers, MA). Anti-GAPDH (sc-25778), anti-α-tubulin (sc-8035), donkey anti-rabbit IgG-HRP (sc-2313) and donkey anti-mouse IgG-HRP (sc-2314) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-RACK1 (610178) was purchased from BD (Franklin Lakes, NJ). Rabbit anti-green fluorescence protein (GFP) (ab290) was purchased from Abcam (Cambridge, MA, USA). Anti-GSK-3β (#05-412) and nitrocellulose membranes were purchased from Millipore (Billerica, MA). Microtubule binding protein spin-down assay biochem kit and microtubules/tubulin *in vivo* assay biochem kit were from Cytoskeleton (Denver, CO). EDTA-free complete mini Protease Inhibitor Cocktails were from Roche (Indianapolis, IN). Phosphatase Inhibitor Cocktails 1 and 2, and Proteinase K were from Sigma Aldrich (St. Louis, MO). EDTA solution (0.5 M), TRIzol reagent and NuPAGE Bis-Tris precast gels were purchased from Life Technologies (Grand Island, NY). Enhance Chemiluminescence (ECL) was from GE Healthcare (Buckinghamshire, UK). Pierce BCA protein assay kit was obtained from Thermo Scientific (Rockford, IL). Terra™ qPCR Direct SYBR® Premix was from Clontech (Mountain View, CA). The HIV-1 p24 antigen ELISA kit for determining the titer of lentivirus was purchased from ZeptoMetrix Corporation (Buffalo, NY). Alcohol was purchased from Gold Shield Chemical (Hayward,

CA). Lacosamide was purchased from Selleck Chemicals (Houston, TX). Rapamycin was purchased from LC Laboratories (Woburn, MA). Recombinant RNasin ribonuclease inhibitor, reverse-transcription system and PCR master mix were from Promega (Madison, WI). Ribonucleoside vanadyl complex (RVC) was purchased from New England Biolabs (Ipswich, MA). Polyallomer ultracentrifuge tubes for sucrose gradient centrifugation and microtubule content assay were purchased from Beckman Coulter (Brea, CA). Other common reagents were from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Primer sequences for RT-PCR or quantitative PCR

The following primers were used: Rat Arc: upstream 5'-GGG AGG TCT TCT ACC GTC TG-3', downstream 5'-CTT CAC CGA GCC CTG TTT-3'; rat CaMKIIα: upstream 5'-AGC AGC AGG CAT GGT TTG-3', downstream 5'-AGT GGA GCG GTG CGA GAT-3'; rat CRMP-2: upstream 5'-ATG GAA GCT CTG GTG AAG GA-3', downstream 5'-CAT TCT CTG CGT GGA CTT GA -3'; rat GluA1: upstream 5'-AGG ACC TAC ATC GTC ACT ACT A-3', downstream 5'-CGT CCC TCT TCA AAC TCT T-3'; rat Homer2: upstream 5'-GTG AGA TCA ACA GGG AGA AGG-3', downstream 5'-ACG CAC CCG CAT TAC AGA-3'; rat GluN1: upstream 5'-GTT CGG TAT CAG GAA TGC G-3', downstream 5'-GGT GCT CGT GTC TTT GGA-3'; rat PSD-95: upstream 5'-GAC TGC GGT TTC TTG AGC-3', downstream 5'-GTT GGC ACG GTC TTT GGT-3'; rat RACK1: upstream 5'-CAC TTT GTT AGC GAT GTT GT-3', downstream 5'-TGC TTG CCT TCA

TTG AGA-3'; rat TrkB: upstream 5'-CAA TGC CTT GTT GTA TTC C-3', downstream 5'-GTC TCA CTC CTG CTG TGC-3'; rat GAPDH: upstream 5'-TCA ACG GCA CAG TCA AGG-3', downstream 5'-ACC AGT GGA TGC AGG GAT-3'.

Collection of brain samples for biochemical analyses

Rats and mice underwent an intermittent access to 20% alcohol two-bottle choice (2BC) drinking paradigm for 2 months. Rats were anesthetized by isoflurane and decapitated immediately after the end of the 30 minutes binge drinking session (binge) or at the end of the last 24 hours of alcohol deprivation (withdrawal) (Timeline, Figure 1A). Mice were killed by cervical dislocation and decapitated immediately after the end of the last 24 hours of alcohol withdrawal. For moderate drinking of alcohol, rats underwent continuous access to 10% alcohol two-bottle choice drinking paradigm for 21 days and were dissected at the end of last drinking session. Brains were then quickly removed and placed on an ice-cold platform prior to dissection except for the microtubule content assay experiment for which brains were immediately placed and dissected on a 37°C metal heating block.

Crude synaptosomal fraction and western blot analysis

The NAc was homogenized in ice-cold Krebs buffer (125 mM NaCl, 1.2 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $CaCl_2$, 22 mM Na_2CO_3, 1.2 mM NaH_2PO_4, 320 mM sucrose and 10 mM glucose, pH7.4) with protease and phosphatase inhibitors. One fifth of the

homogenate was saved for total homogenate analysis and the rest of the homogenate was centrifuged at 1,000 g, 4°C for 10 minutes to obtain a pellet containing heavy membranes and debris. The supernatant was further centrifuged at 16,000 g, 4°C for 20 minutes. The resulting pellet contained crude synaptic fraction. Both the total homogenate and the synaptic fraction were resolved in radioimmunoprecipitaion assay (RIPA) buffer (25 mM Tris pH7.6, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA). Protein concentration was determined by BCA protein assay kit according to the manufacturer's protocol.

Equal amounts of protein samples (40 μg) were denatured in Laemmli buffer, boiled for 10 minutes, resolved on NuPAGE 10% Bis-Tris gels and transferred to nitrocellulose membranes. Then, membranes were blocked in 5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) at room temperature for 1 hour and incubated with appropriate primary antibodies in the same blocking solution at 4°C overnight. After extensive washing with TBST, bound primary antibodies were detected with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized by ECL. After determining the level of phospho-proteins, membranes were stripped in stripping buffer (25 mM glycine, 1% SDS, pH3.0) at room temperature for 30 minutes, followed by reblocking and reprobing with the appropriate total antibodies.

Microtubule binding assay

The binding of CRMP-2 to microtubules in the rat NAc was detected by using microtubule binding protein spin-down assay biochem kit according to manufacturer's instructions. Specifically, 5 mg/ml tubulin protein in a general tubulin buffer (80 mM PIPES, 2 mM $MgCl_2$, 0.5 mM EGTA, pH7.0) plus 1 mM GTP was incubated at 35ºC for 20 minutes to generate microtubules. The NAc was homogenized in a tubulin buffer plus protease inhibitor and phosphatase inhibitor cocktails. One hundred µg of the NAc homogenate was incubated with 20 µg of pre-assembled microtubules at room temperature (total 125 µl) for 30 minutes. Twenty µl of the mixture was taken from each sample to be used as a total fraction that contains total CRMP-2 and total tubulin. The rest of the sample was loaded onto a 165 µl cushion buffer (80 mM PIPES, 1 mM $MgCl_2$, 1 mM EGTA, 60% glycerol, pH7.0, 1.25 µM taxol) and centrifuged at 28,700 rpm, room temperature for 40 minutes using a SW55 rotor (Beckman Coulter, Brea, CA). Thirty µl of the supernatant was used for testing unbound CRMP-2 and free tubulin. The pellet, which included microtubules-bound CRMP-2, was carefully collected and subjected to western blot analysis.

Microtubule content assay

Microtubule content was determined by microtubules/tubulin *in vivo* assay biochem kit according to manufacturer's instructions. Microtubules are very sensitive to temperature change; therefore, all the equipment and buffers were pre-warmed to 37ºC and the dissecting procedure was performed on a 37ºC metal heat block. Specifically, the rat

NAc was homogenized in 1 ml LMS2 buffer (100 mM PIPES, 5 mM $MgCl_2$, 1 mM EGTA, 30% (*v/v*) glycerol, 0.1% Tween 20, 100 μM GTP, 1 mM ATP, 0.1% beta-mercaptoethanol, 0.001% antifoam, pH6.9) plus protease inhibitors. The lysate was immediately centrifuged at 2,000 g, 37ºC for 5 minutes. The same amount of supernatant from each sample was taken out for protein concentration determination and used as the total fraction. The same amount of remaining supernatant was transferred into an ultracentrifuge tube and centrifuged at 28,700 rpm, 37ºC for 30 minutes using a SW55 rotor (Beckman Coulter, Brea, CA). After centrifugation, the supernatant was carefully transferred into a tube and placed on ice. The pellet was resuspended in the same volume of ice cold Ca-LMS2 (2 mM $CaCl_2$ in LMS2) and kept at room temperature for 15 minutes to depolymerize microtubules. The content of microtubules in each sample was determined by western blot analysis and expressed as the ratio of microtubules-associated tubulin to free tubulin.

Infusion of lentivirus

Mice were anesthetized using isoflurane. Bilateral microinfusions were made using stainless steel injectors (33 gauge, Hamilton) into the NAc (1 infusion site per hemisphere; the stereotaxic coordinates were anterioposterior +2.1 mm from bregma; mediolateral ±0.75 mm from bregma and dorsoventral -4.35 mm from the skull surface). Animals were infused with Ltv-shCT or Ltv-shCRMP-2 (1.2 μl/side) at a titer of 2×10^7 pg/ml and at an injection rate of 0.1 μl/min. After each infusion, the injectors were left in place for an additional 15 min to allow the virus to diffuse. Mice recovered for 3 weeks before

experiments were initiated. Mice were sacrificed at the end of behavior experiment and the virus infected area was visualized in 50 µm coronal sections using Zeiss LSM 510 META laser confocal microscope (Zeiss MicroImaging, Jena, Germany). Only data from mice with infection localized in the NAc were included in the analysis. Four out of 26 mice infused with either the Ltv-shCT or the Ltv-shCRMP-2 virus were excluded from the analysis: two had a unilateral infection, one had an off target infection and one had a very weak infection.

Immunohistochemistry

Animals were euthanized in chambers slowly filled with carbon dioxide and perfused with phosphate-buffered saline (PBS), followed by ice cold 4% paraformaldehyde (PFA) in PBS. Brains were removed, post-fixed in 4% PFA for 2 hours, and transferred to a PBS/30% sucrose solution and stored for 3 days at 4°C. 50 µm-thick frozen coronal sections were cut on a cryostat (Leica CM3050, Leica Biosystems, Buffalo Grove, IL, USA), collected in 12-well plates, and stored in PBS at 4°C. Sections containing the infusion site in the NAc were selected, blocked with 5% normal donkey serum in PBS/0.3% Triton X-100 for 1 hour and rinsed in PBS. Sections were then incubated for 24 hours at 4°C on an orbital shaker with rabbit anti-GFP antibodies (1:1000) diluted in PBS/0.1% Triton X-100 containing 1% Bovine Serum Albumin (BSA). Next, sections were washed in PBS, incubated for 4 hours with the secondary antibodies (Alexa Fluor 488-labeled donkey anti-rabbit; 1:500) diluted in PBS/0.1% Triton X-100 containing 1% BSA. After

staining, sections were rinsed in PBS and coverslipped using Vectashield mounting medium. Images were acquired using Zeiss LSM 510 META laser confocal microscope (Zeiss MicroImaging, Jena, Germany) using manufacturer recommended filter configurations.

Effect of Lacosamide on alcohol drinking, voluntary sucrose consumption, locomotion and anxiety-like behavior

After 2 months of intermittent-access to 20% alcohol, Lacosamide (20 mg/kg for rats and 20 or 50 mg/kg for mice) was systemically administered 90 minutes before the beginning of a drinking session. Water and alcohol bottles were weighted 30 minutes (rats) or 4 hours (mice) after the beginning of the session (binge) as well as at the end of the drinking session (24 hours intake).

For sucrose consumption, alcohol naïve rats were given a free choice between 0.32% sucrose and tap water under an intermittent-access drinking paradigm for 2 weeks. The placement (left or right) of each solution was alternated between each session to control for side preference. Lacosamide (20 mg/kg) was systemically administered 90 minutes before the beginning of a drinking session. Water and sucrose bottles were weighed 30 minutes after the beginning of the session.

The effect of Lacosamide on locomotor activity and anxiety-like behavior of rats was measured in the activity monitoring chambers described above. The open field (43 cm × 43 cm) was divided into central (28 cm × 28 cm) and peripheral areas. Lacosamide (20

mg/kg) was systemically administered 90 minutes before the beginning of a 60-minute locomotor activity session. Anxiety-like behavior was determined by measuring the time spent in the central area of the open field during the first 10 minutes of the session.

Supplementary Figure legends

Supplementary Figure 1. Purification of polysomal RNA fraction from rat NAc. Polysomal fractionation was conducted as described previously [1]. **(A)** Before loading onto sucrose gradient, the NAc homogenates were partitioned into two equal samples, one of which contained 25 mM EDTA. **(B)** Forty fractions from each sample were removed after sucrose gradient centrifugation and absorbance was measured at 254 nm. **(C)** RNA was visualized by migrating on a 1.5% agarose gel. Fraction 01-05 mainly contains tRNAs. Fractions 06-15 are enriched with 40S ribosomal subunit as well as 18S rRNA. Fractions 16-25 are enriched with 60S ribosomal subunit and 28S rRNA. Note that the ribosomal RNA redistributes towards the lower density factions in the presence of EDTA, which disrupts polysomes. **(D)** Polysomal RNA in fractions 26-40 and non-polysomal RNA in fractions 01-15 were purified and combined for RT-PCR analysis of *CRMP-2* and *GAPDH*.

Supplementary Figure 2. Alcohol withdrawal after excessive drinking of alcohol promotes mRNA translation, but does not change transcription of candidate genes. Rats were trained to drink alcohol as described in Figure 1. The NAc was removed after 30 minutes binge drinking of alcohol (**A**, binge) or 24 hours after the end of the last drinking session (**B**, withdrawal). Total polysomal RNA and total RNA were isolated and mRNA levels were determined by RT-PCR analysis. Optical density quantification is expressed as the ratio of each gene to *GAPDH*. Data are presented as mean ± SEM and expressed as percentage of control. Significance was determined using two-tailed unpaired *t*-test. **(A)**

Arc, $t_{(6)}$=2.582, p=0.042; *CaMKIIα*, $t_{(6)}$=2.495, p=0.047; *CRMP-2*, $t_{(6)}$=2.485, p=0.048; *GluA1*, $t_{(6)}$=0.497, p=0.637; *Homer2*, $t_{(6)}$=2.512, p=0.046; *GluN1*, $t_{(6)}$=1.971, p=0.096; *PSD-95*, $t_{(6)}$=2.596, p=0.041; *RACK1*, $t_{(6)}$=0.291, p=0.781; *TrkB*, $t_{(6)}$=0.563, p=0.593. n=4 for each group. **(B)** *Arc*, $t_{(6)}$=1.379, p=0.217; *CaMKIIα*, $t_{(6)}$=1.241, p=0.261; *CRMP-2*, $t_{(6)}$=0.229, p=0.827; *GluA1*, $t_{(6)}$=0.032, p=0.976; *Homer2*, $t_{(6)}$=0.448, p=0.670; *GluN1*, $t_{(6)}$=1.608, p=0.159; *PSD-95*, $t_{(6)}$=0.699, p=0.511; *RACK1*, $t_{(6)}$=0.038, p=0.971; *TrkB*, $t_{(6)}$=1.668, p=0.146. n=4 for each group. **(C)** *Arc*, $t_{(6)}$=0.615, p=0.561; *CaMKIIα*, $t_{(6)}$=0.650, p=0.540; *CRMP-2*, $t_{(6)}$=0.167, p=0.873; *GluA1*, $t_{(6)}$=0.240, p=0.818; *Homer2*, $t_{(6)}$=0.247, p=0.813; *GluN1*, $t_{(6)}$=0.718, p=0.500; *PSD-95*, $t_{(6)}$=0.107, p=0.918; *RACK1*, $t_{(6)}$=1.502, p=0.184; *TrkB*, $t_{(6)}$=0.690, p=0.516. n=4 for each group. *p<0.05.

Supplementary Figure 3. TOP analysis of candidate mRNAs. The annotations of TSS (+1 to +20) for the rat mRNAs whose translation was activated by alcohol in Figure 1, including *Arc, CaMKIIα, CRMP-2, GluA1, Homer2* and *PSD-95* mRNAs, were retrieved from RefSeq data base (http://www.ncbi.nlm.nih.gov/refseq/). Potential TOP-like signature sequences were analyzed according to published criteria [2]. The stretch of pyrimidines after the TSS of TOP-like mRNA is highlighted in red.

Supplementary Figure 4. Excessive alcohol intake promotes the translation of *CRMP-2* mRNA. Rats were trained to drink alcohol as described in Figure 1. The NAc was removed 30 minutes after the beginning (binge, B) or 24 hours after the end of the last

drinking session (withdrawal, Wthd). **(A)** A representative polysomal profile showing collected fractions for mRNA distribution analysis. **(B)** A representative RT-PCR result of *CRMP-2* mRNA levels in indicated fractions from one control and one binge drinking rat NAc samples. **(C)** RT-PCR analysis of mRNA levels in each of the combined 4 fractions after polysomal profiling. **(D-K)** Polysomal RNA and total RNA were isolated and mRNA levels were determined by RT-q-PCR analysis. Data are expressed as the average ratio of *CRMP-2* or *RACK1* to *GAPDH* ± SEM, and are expressed as percentage of water control. Significance was determined using two-tailed unpaired *t*-test. **(D)** Polysomal *CRMP-2*, binge, $t_{(10)}$=6.117, p=0.0001, n=6 for each group; **(E)** Polysomal *RACK1*, binge, $t_{(10)}$=0.118, p=0.908, n=6 for each group; **(F)** Polysomal *CRMP-2*, withdrawal, $t_{(10)}$=6.537, p<0.0001, n=6 for each group; **(G)** Polysomal *RACK1*, withdrawal, $t_{(10)}$=0.170, p=0.869, n=6 for each group; **(H)** Total *CRMP-2*, binge, $t_{(10)}$=0.286, p=0.781, **n=6 for each group**; **(I)** Total *RACK1*, binge, $t_{(10)}$=0.381, p=0.711, n=6 for each group; **(J)** Total *CRMP-2*, withdrawal, $t_{(10)}$=0.280, p=0.785, n=6 for each group; **(K)** Total *RACK1*, withdrawal, $t_{(10)}$=0.464, p=0.653, n=6 for each group. ***p<0.001.

Supplementary Figure 5. Alcohol withdrawal does not alter the translation of *RACK1* mRNA and the transcription of *CRMP-2* and *RACK1* with or without rapamycin. Rats were trained to drink alcohol as described in Figure 1. Three hours before the end of the last 24 hours of alcohol withdrawal, rats were systemically administered with 10 mg/kg of rapamycin or vehicle. The NAc was removed three hours after rapamycin or vehicle

treatment and subjected to polysomal RNA and total RNA fractionation. The mRNA levels were determined by RT-qPCR. Data are presented as the average ratio of *CRMP-2* or *RACK1* to *GAPDH* mRNA ± SEM and expressed as percentage of control (water plus vehicle group). Significance was determined using two-way ANOVA. **(A)** mRNA levels of *RACK1* in the polysomal fraction. Two-way ANOVA showed no main effect of alcohol ($F_{(1,16)}$=0.014, p=0.907) and rapamycin ($F_{(1,16)}$=1.422, p=0.251) and no interaction ($F_{(1,16)}$=0.0692, p=0.796). n=5 for each group. **(B)** The mRNA levels of *CRMP-2* in total RNA. Two-way ANOVA showed no main effect of alcohol ($F_{(1,16)}=2.75\times10^{-4}$, p=0.987) and rapamycin ($F_{(1,16)}=6.14\times10^{-3}$, p=0.938) and no interaction ($F_{(1,16)}$=0.0262, p=0.873). n=5 for each group. **(C)** The mRNA levels of *RACK1* in total RNA. Two-way ANOVA showed no main effect of alcohol ($F_{(1,16)}=1.21\times10^{-5}$, p=0.997) and rapamycin ($F_{(1,16)}$=0.168, p=0.687) and no interaction ($F_{(1,16)}$=0.138, p=0.715). n=5 for each group.

Supplementary Figure 6. Binge drinking of alcohol and withdrawal do not change the protein levels of RACK1. Data quantification of RACK1 immunoreactivity was shown in Figure 2A-D. Data are expressed as the average ratio ± SEM of RACK1 to GAPDH, and are expressed as percentage of water control. Significance was determined using two-tailed unpaired *t*-test. **(A)** Protein levels in the total homogenate of binge drinking rats. RACK1, $t_{(10)}$=0.759, p=0.465. n=6 for each group. **(B)** Protein levels in the synaptic fraction of binge drinking rats. RACK1, $t_{(10)}$=0.223, p=0.828. n=6 for each group. **(C)** Protein levels in the total homogenate 24 hours after withdrawal. RACK1, $t_{(10)}$=0.335, p=0.729. n=6 for each

group. **(D)** Protein levels in the synaptic fraction 24 hours after withdrawal. RACK1, $t_{(10)}$=0.419, p=0.683. n=6 for each group.

Supplementary Figure 7. CRMP-2 levels in the NAc are unaltered after one week of withdrawal or in response to moderate intake of alcohol. CRMP-2 levels are unaltered in the cerebellum and the motor cortex of rats consuming 20% alcohol. **(A)** One week withdrawal after excessive drinking of alcohol does not change CRMP-2 protein levels in the NAc. Rats experienced 2 months of intermittent access to 20% alcohol two-bottle choice drinking paradigm. Control animals (water, W) underwent the same paradigm but had access to water only. The NAc was removed 24 hours (Wthd 24 h) or one week after the end of the last drinking session (withdrawal 1 week, Wthd 1wk) and subjected to western blot analysis. CRMP-2 (Wthd 24 h), $t_{(6)}$=3.412, p=0.014; CRMP-2 (Wthd 1 wk), $t_{(6)}$=0.762, p=0.475. n=4 for each group. **(B-C)** Excessive drinking of alcohol does not change CRMP-2 protein levels in motor cortex and cerebellum. Rats experienced 2 months of intermittent access to 20% alcohol two-bottle choice drinking paradigm. Control animals (water, W) underwent the same paradigm but had access to water only. The motor cortex and cerebellum were removed 24 hours after the end of the last drinking session (withdrawal, Wthd) and subjected to western blot analysis. **(B)** Protein levels of CRMP-2 in the motor cortex. CRMP-2, $t_{(4)}$=0.06, p=0.95. n=3 for each group. **(C)** Protein levels of CRMP-2 in the cerebellum. CRMP-2, $t_{(4)}$=0.003, p=0.99. n=3 for each group. **(D)** Moderate drinking of alcohol does not change CRMP-2 protein levels in the NAc. Rats experienced

21 days of continuous access to 10% alcohol two-bottle choice drinking paradigm. Control animals (water, W) underwent the same paradigm but had access only to water. The NAc was removed at the end of the last drinking session and subjected to western blot analysis. CRMP-2, $t_{(6)}$=0.908, p=0.399. n=4 for each group.

Supplementary Figure 8. Alcohol withdrawal increases CRMP-2 protein levels in an mTORC1-depepdent manner in the mouse NAc. Mice experienced 2 months of intermittent access to 20% alcohol two-bottle choice drinking paradigm. Control animals (Water) underwent the same paradigm but had only access to water. Three hours before the end of the last 24 hours of alcohol withdrawal, mice were systemically administered with 20 mg/kg of rapamycin (Rap) or vehicle (Veh). The NAc was removed three hours after rapamycin or vehicle and the protein levels were determined by western blot analysis. Data are presented as the average ratio of CRMP-2 or RACK1 to GAPDH protein levels ± SEM and expressed as percentage of control (water plus vehicle group). Significance was determined using two-way ANOVA and the method of contrasts. **(A)** The protein levels of CRMP-2, RACK1 and GAPDH in total homogenate were determined by western blot analysis. **(B)** CRMP-2 protein level. Two-way ANOVA showed a significant main effect of alcohol ($F_{(1,16)}$=7.891, p=0.013) and rapamycin ($F_{(1,16)}$=4.81, p=0.043), and a significant interaction ($F_{(1,16)}$=4.54, p=0.049); and *post hoc* Student–Newman–Keuls test detected a significant difference between water and alcohol within the vehicle group (q=4.94, p=0.003) and a significant difference between vehicle and rapamycin within the alcohol group

(q=4.324, p=0.008). n=5 for each group. **(C)** RACK1 protein level. Two-way ANOVA showed no main effect of alcohol ($F_{(1,16)}$=0.091, p=0.767) and rapamycin ($F_{(1,16)}$=0.201, p=0.66) and no interaction ($F_{(1,16)}$=2.47×10^{-3}, p=0.961). n=5 for each group. **p<0.01.

Supplementary Figure 9. Alcohol withdrawal increases CRMP-2 protein levels and blocks CRMP-2 phosphorylation in the total homogenate of rat NAc. Rats were trained to drink alcohol as described in Figure 1. The NAc was removed 24 hours after the end of the last drinking session (withdrawal) and the protein levels were determined by western blot analysis. Optical density quantification is expressed as the ratio of CRMP-2 to GAPDH or phospho-CRMP-2 to total CRMP-2 levels. Data are presented as mean ± SEM and expressed as percentage of control. Significance was determined using two-tailed unpaired t-test. **(A)** The phosphorylation level and protein level of CRMP-2 in total homogenate of rat NAc that was used for microtubules binding assay (Figure 3E-F). The phosphorylation level of CRMP-2 and total protein level of CRMP-2, GAPDH and tubulin in total homogenate were determined by western blot. **(B)** CRMP-2 protein level, $t_{(11)}$=2.338, p=0.039. **n=7** for water control group and n=6 for alcohol withdrawal group. **(C)** [T^{514}]CRMP-2 phosphorylation level, $t_{(12)}$=2.693, p=0.019. n=7 for each group. *p<0.05.

Supplementary Figure 10. Lacosamide does not alter locomotion or anxiety-like behavior. Naïve rats were systemically administered with 20 mg/kg of Lacosamide (LCM) or vehicle (Veh) 90 minutes before the behavior test. Locomotion was determined by measuring the

distance travelled in bins and anxiety-like behavior was determined by measuring the time spent in the center of the chamber during a 60-minute locomotor activity session. Results are expressed as mean ± SEM. Significance was determined using two-way RM-ANOVA **(A)** or two-tailed paired *t*-test **(B)**. **(A)** Locomotion test of every 5 minutes**.** Two-way RM-ANOVA showed no main effect of Lacosamide ($F_{(1,11)}$=0.112, p=0.744), a significant effect of time ($F_{(11,121)}$=34.152, p<0.001) and no interaction between Lacosamide and time ($F_{(11,120)}$=1.636, p=0.097). n=12 for each group. **(B)** Anxiety-like behavior test at the first 10 minutes of the session, $t_{(10)}$=0.0469, p=0.963. n=12 for each group.

Supplementary Figure 11. Infusion of Ltv-shCRMP-2 into the NAc does not change tubulin and RACK1 protein levels in the NAc and CRMP-2 level in the dorsal striatum. Ltv-shCT (2×10^7 pg/ml) or Ltv-shCRMP-2 (2×10^7 pg/ml) was infused bilaterally into the mouse NAc (1.2 µl/side). The NAc and dorsal striatum (DS) were dissected at the end of behavioral experiment described in Figure 7 and used for western blot analysis. Data are presented as mean ± SEM. Significance was determined using two-tailed unpaired *t*-test. **(A)** Ltv-CRMP-2 infection does not change tubulin or RACK1 expression in the NAc. Histograms show the ratio of tubulin to GAPDH levels (left), $t_{(6)}$=0.8506, p=0.427; and the ratio of RACK1 to GAPDH levels (right), $t_{(6)}$=1.248, p=0.258. n=4 **for each group**. **(B)** Ltv-CRMP-2 infection in the NAc does not change CRMP-2 expression in the DS. Left, the protein levels of CRMP-2 and GAPDH were determined by western blot analysis. Right,

the histogram shows the ratio of CRMP-2 to GAPDH level, $t_{(6)}$=0.6438, p=0.543. n=4 **for each group.**

Supplementary Figure 12. Knockdown of CRMP-2 in the mouse NAc increases water intake, but does not alter saccharine intake and basal locomotor activity. Ltv-shCT (2×10^7 pg/ml) or Ltv-shCRMP-2 (2×10^7 pg/ml) was infused bilaterally into the mouse NAc (1.2 μl/side). After three weeks of recovery, mice underwent an intermittent-access to 20% alcohol two-bottle choice drinking procedure for 8 sessions. Water intake **(A)** and saccharin intake **(B)** were measured after each 24-hour drinking session and expressed as an average of every 2 drinking sessions for water and single session for saccharin. Data are expressed as mean ± SEM. Significance was determined using two-way RM-ANOVA. **(A)** Water intake. Two-way RM-ANOVA showed a significant main effect of virus infusion ($F_{(1,20)}$=6.155, p=0.022), no effect of session ($F_{(3,60)}$=2.175, p=0.100) and no interaction between virus infusion and session ($F_{(3,60)}$=1.265, p=0.294). n=11 for each group. **(B)** One week after the alcohol drinking test, the same mice were subjected to an intermittent-access to saccharin two-bottle choice drinking procedure for 3 sessions. Saccharin intake was measured after each session. Two-way RM-ANOVA found no main effect of virus infusion ($F_{(1,19)}$=0.730, p=0.403), a significant effect of session ($F_{(2,32)}$=9.461, p<0.001) and no interaction between virus infusion and session ($F_{(2,32)}$=0.262, p=0.771). n=10 for Ltv-shCT group and n=11 for Ltv-shCRMP-2 group. **(C)** 8 weeks after virus infusion, locomotor activity was determined by measuring the distance travelled in bins during a 60-minute

locomotor activity session. Data are expressed as mean ± SEM of distance travelled in bins of every 5 minutes. Two-way RM-ANOVA showed no main effect of virus infusion ($F_{(1,20)}$=0.0819, p=0.778), a significant effect of session ($F_{(11,220)}$=28.054, p<0.001) and a significant interaction between virus infusion and session ($F_{(11,220)}$=3.165, p<0.001). n=11 for each group. *p<0.05.

Supplementary Figure 13. Model: Dual regulation of CRMP-2 in the NAc promotes alcohol drinking. **(A)** In the absence of alcohol, AKT and mTRC1 activity are low. In contrast, GSK-3β is constitutively active, resulting in the hyperphosphorylation of CRMP-2 (I). **(B)** Excessive drinking of alcohol activates the AKT pathway in the NAc [3]. AKT activates mTORC1 in response to alcohol [4], leading to an increase of *CRMP-2* mRNA translation (II). Activated AKT phosphorylates and inhibits GSK-3β, resulting in the blockade of CRMP-2 phosphorylation (III). Together, these two events result in the accumulation of hypophosphorylated CRMP-2 in the NAc. Hypophosphorylated CRMP-2 binds with microtubules and promotes microtubule assembly (IV), which in turn contributes to excessive alcohol drinking (V). Lacosamide, which prevents CRMP-2 binding to microtubules, reduces alcohol drinking.

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