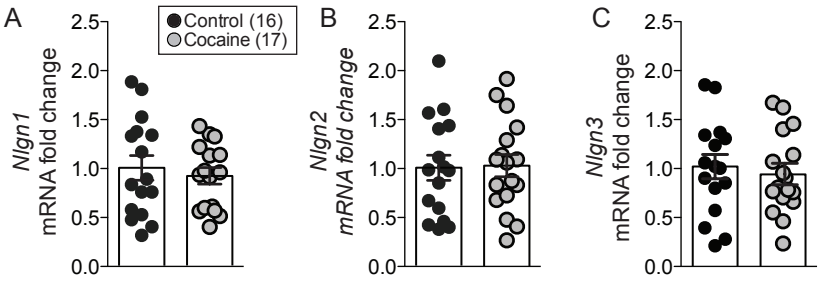
Figure S1. Postmortem NAc *NLGN2* in Cocaine Users



D ● Controls

Gender	Age	PMI	Cause of Death
Female	41	3.5	Suicide
Male	20	12	Accident
Male	17	7.5	Suicide
Male	18	2	Natural
Male	42	2.5	Accident
Male	15	16.75	Accident
Male	25	11.25	Suicide
Male	27	3	Natural
Male	46	1.5	Natural
Male	32	4	Accident
Male	41	3	Natural
Male	28	2.25	Accident
Male	30	11	Natural
Male	47	3.5	Natural
Male	26	5	Suicide
Male	19	1.5	Accident

E ○ Cocaine

Gender	Age	PMI	Cause of Death
Female	50	7	Suicide
Male	38	16	Suicide
Male	53	28	Suicide
Male	43	19	Suicide
Male	51	2	Natural
Male	51	1.5	Accident
Male	39	27.5	Natural
Male	36	12	Suicide
Male	45	2.75	Suicide
Male	35	3.5	Suicide
Male	26	13.5	Suicide
Male	24	2.5	Suicide
Male	48	1.75	Suicide
Male	33	9	Suicide
Male	43	5.25	Suicide
Male	24	7	Accident
Male	39	4.25	Suicide

Figure S2. Immunohistochemistry behavioral data.

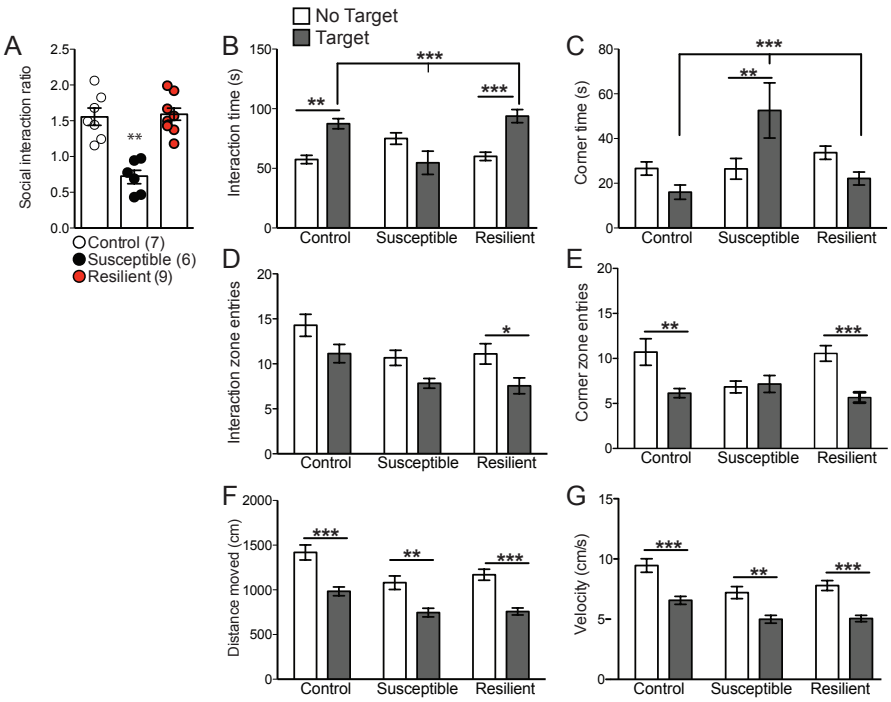


Figure S3. miRNA vector

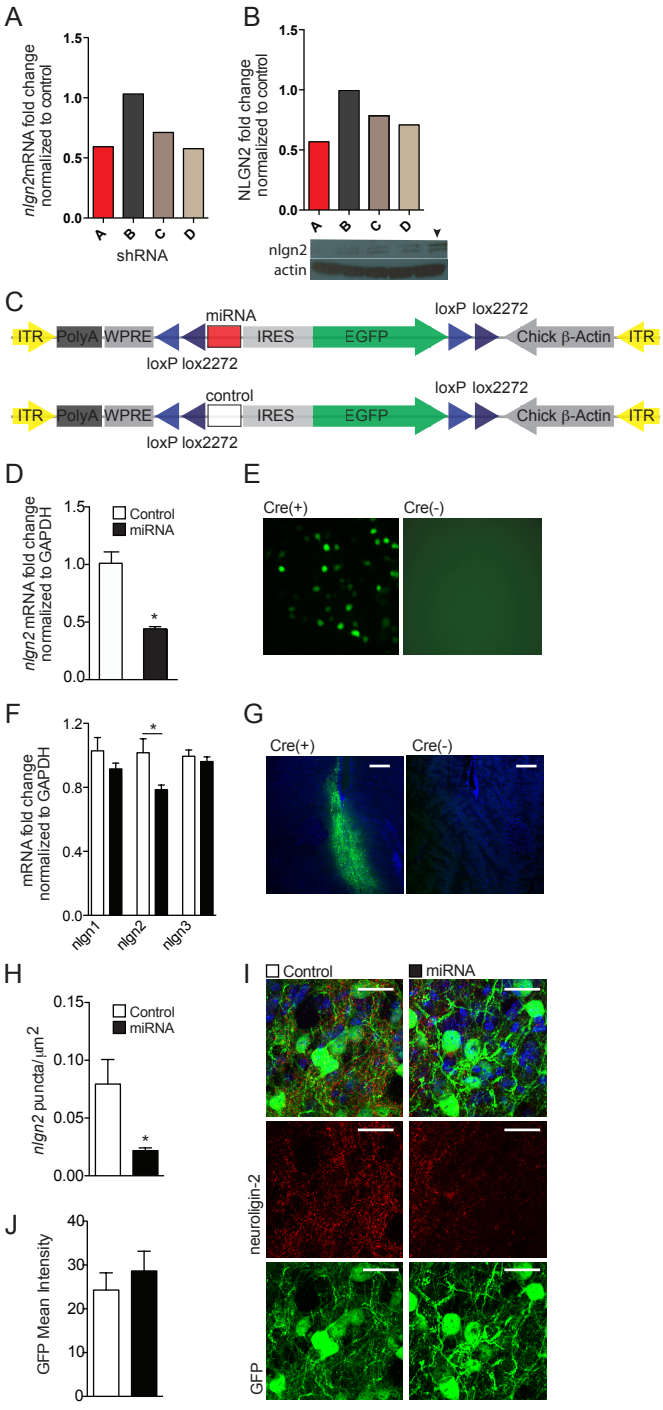


Figure S4. miRNA vector effects on mini inhibitory currents.

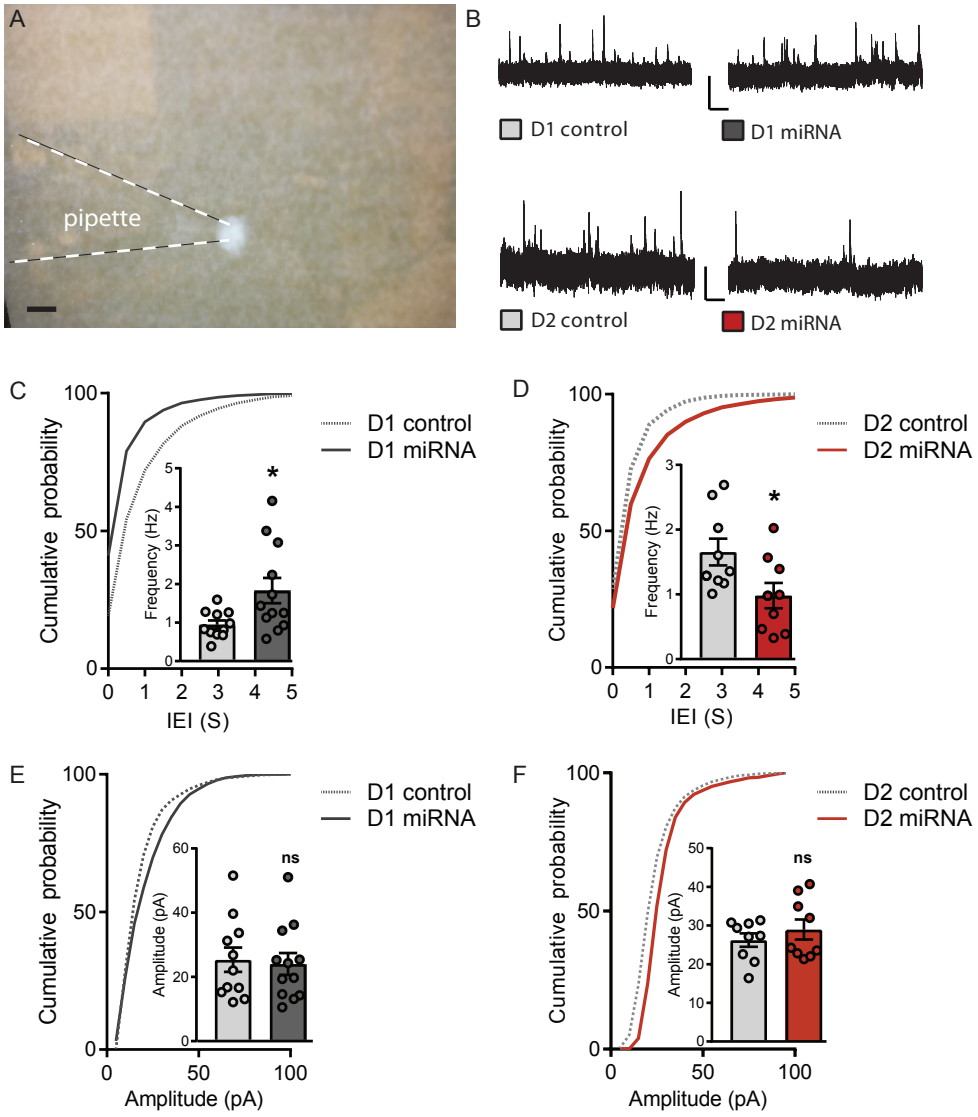


Figure S5. Subthreshold defeat timeline and data.

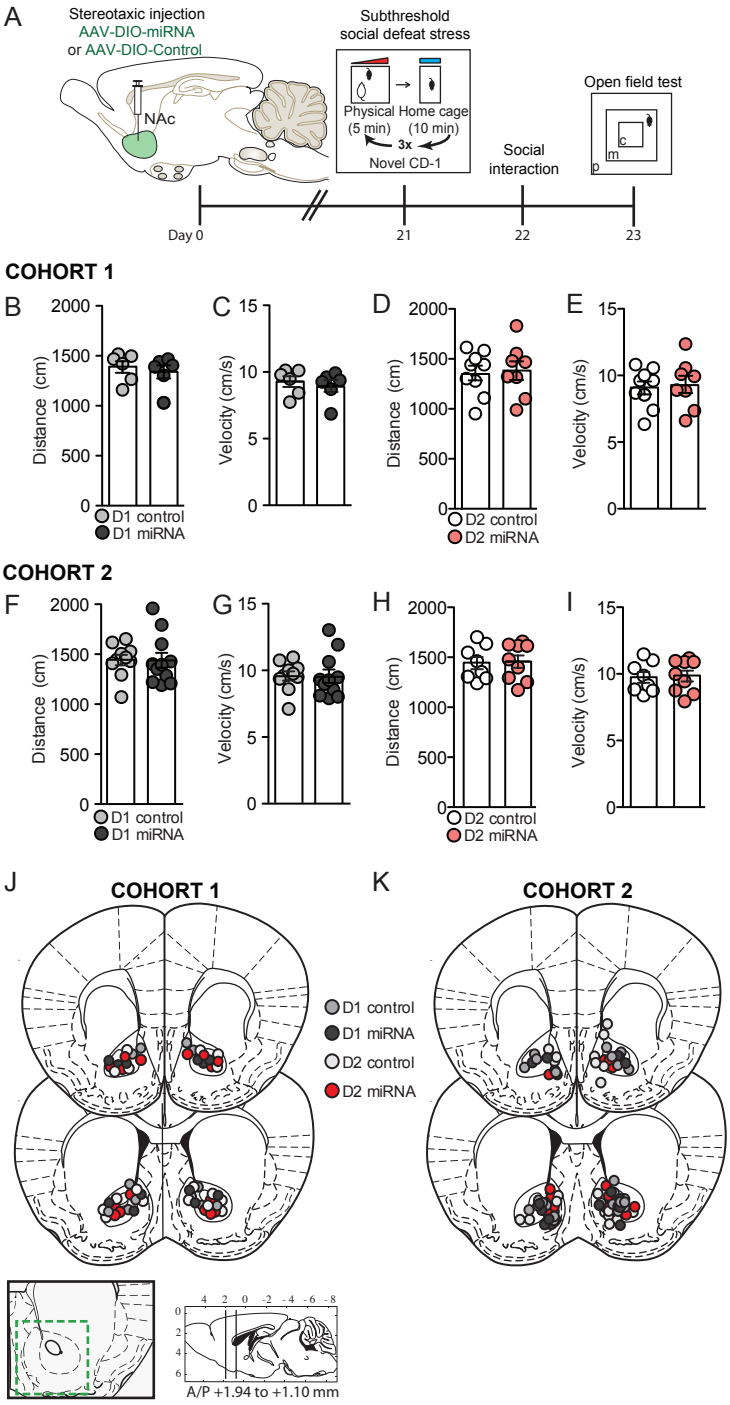


Figure S6. No stress controls nlgn2 miRNA knockdown.

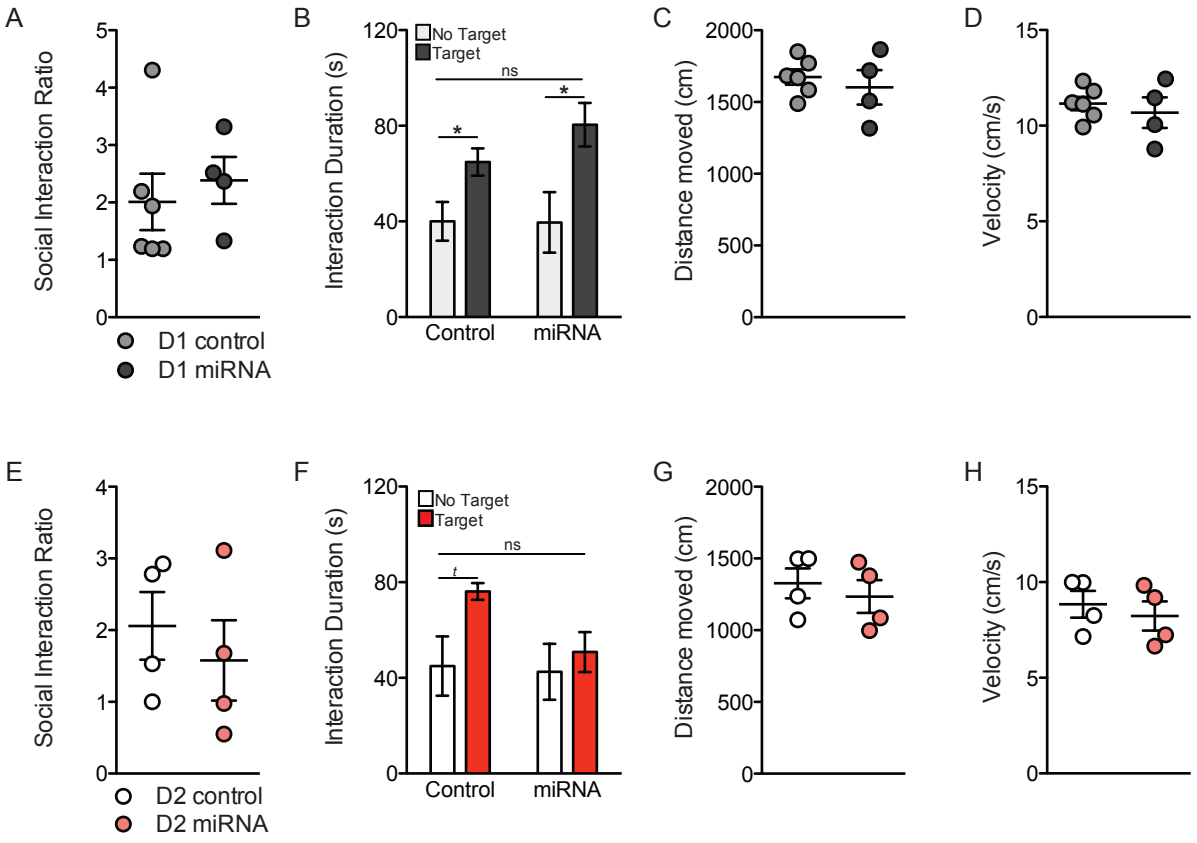


Figure S7. Open Field Test.

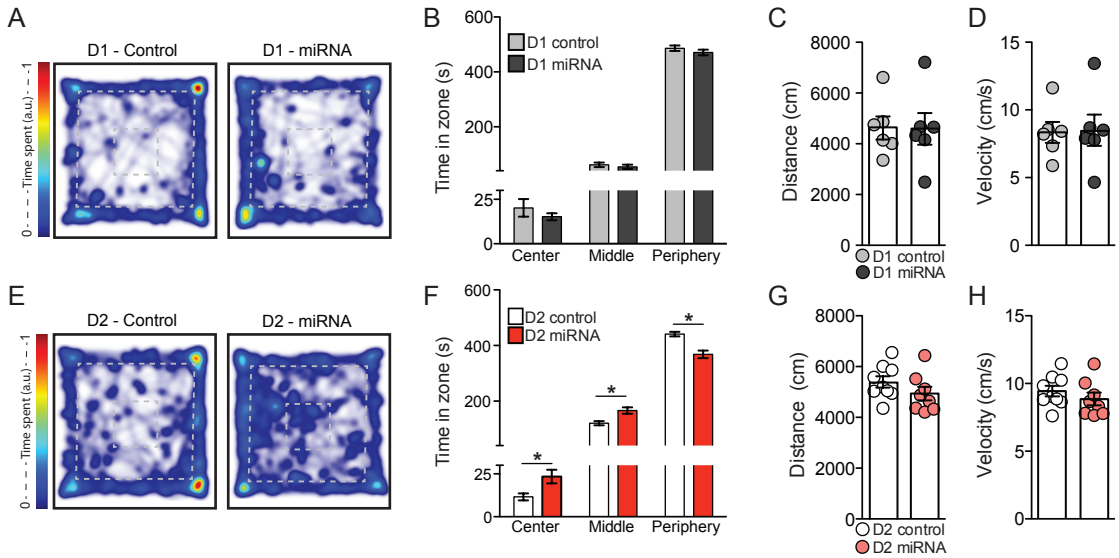


Table S1. Postmortem MDD Demographics

Control samples

Gender	Age	PMI	Cause of death
M	31	32.5	Suicide
M	47	12	Natural
M	30	30	Natural
M	41	24	Natural
M	32	29.5	Accident
M	46	19.5	Natural
M	25	36	Suicide
M	46	59	Suicide
M	42	63	Accident
F	66	61	Accident
F	81	13.5	Natural
F	78	7.5	Accident
F	41	3.5	Suicide
F	76	7	Accident

Major depressive disorder samples

Gender	Age	PMI	Antidepressants	Cause of death
M	42	21	On	Suicide
M	39	19	On	Suicide
M	48	21.5	On	Suicide
M	22	24	On	Suicide
F	46	15	On	Suicide
F	55	36	On	Suicide
M	53	41	On	Suicide
M	36	36.5	On	Suicide
M	39	18.5	On	Suicide
M	35	31	On	Suicide
F	40	49.5	On	Suicide
F	54	28.5	On	Suicide
M	47	2.5	On	Suicide
M	63	24	On	Suicide
F	49	14.5	On	Suicide
M	64	6.5	On	Suicide
M	52	29	Off	Suicide
M	39	25.5	Off	Suicide
M	49	32	Off	Suicide
M	40	22	Off	Suicide
M	53	33.5	Off	Suicide
F	25	20	Off	Suicide
M	68	32	Off	Suicide
M	51	54	Off	Suicide
M	63	50	Off	Suicide
F	44	60	Off	Suicide
F	32	41	Off	Suicide
F	55	2.5	Off	Suicide
M	49	2.5	Off	Suicide

PMI, post mortem interval (hours)

Table S2. CD-1 aggressor behavioral analysis from resident-intruder interaction.

CD-1 Attacks	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	12.14	3.77	7	0.738	26.19	9.74	7	0.998	91.85	32.19	7	0.722
D1-miRNA	10.71	1.80	7		26.21	5.53	7		105.96	21.45	7	
D2-control	12.33	2.93	6	0.314	26.23	3.94	6	0.150	55.20	44.06	6	0.596
D2-miRNA	18.50	5.03	6		58.65	20.41	6		30.15	12.34	6	

CD-1 Social Approach	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	10.86	1.52	7	0.170	33.99	6.66	7	0.284	20.37	8.72	7	0.669
D1-miRNA	8.00	1.23	7		23.78	6.22	7		25.70	8.51	7	
D2-control	13.33	2.51	6	0.129	56.52	12.99	6	0.074	34.20	16.42	6	0.225
D2-miRNA	7.83	2.18	6		26.40	7.65	6		79.87	31.31	6	

CD-1 Social Withdrawal	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	0.00	0.00	7	0.147	0.00	0.00	7	0.150	600.00	0.00	7	0.188
D1-miRNA	0.29	0.18	7		1.29	0.84	7		523.68	54.63	7	
D2-control	1.00	0.68	6	0.670	1.42	1.01	6	0.472	409.21	120.73	6	0.661
D2-miRNA	0.67	0.33	6		3.26	2.24	6		476.83	88.74	6	

CD-1 Social Chasing	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	7.14	1.40	7	0.738	16.47	3.78	7	0.576	24.30	8.13	7	0.414
D1-miRNA	8.00	2.07	7		20.18	5.24	7		63.52	45.66	7	
D2-control	6.67	1.36	6	0.598	22.43	9.18	6	0.798	58.37	47.84	6	0.484
D2-miRNA	8.33	2.74	6		19.26	7.89	6		22.32	13.21	6	

CD-1 Explores	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	26.14	3.16	7	0.147	166.51	20.74	7	0.214	21.42	6.11	7	0.190
D1-miRNA	18.86	3.49	7		124.45	24.45	7		42.25	13.71	7	
D2-control	30.17	3.77	6	0.225	198.23	21.57	6	0.496	8.01	3.07	6	0.069
D2-miRNA	23.17	3.89	6		177.40	20.07	6		18.21	3.95	6	

Table S3. D1-Cre and D2-Cre behavioral analysis from CD-1 resident-intruder interaction.

Cre Social Approach	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	2.29	1.06	7	0.018	3.63	1.63	7	0.006	275.39	97.40	7	0.282
D1-miRNA	8.00	1.80	7		19.76	4.56	7		155.08	43.93	7	
D2-control	2.50	0.56	6	0.477	5.10	1.42	6	0.433	218.95	89.55	6	0.921
D2-miRNA	3.50	1.23	6		7.79	2.98	6		233.98	117.15	6	

Cre Social Withdrawal	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	7.86	1.28	7	0.777	12.37	2.62	7	0.428	34.29	11.92	7	0.559
D1-miRNA	8.57	2.10	7		16.51	4.32	7		61.57	43.75	7	
D2-control	6.67	1.56	6	0.629	17.72	4.68	6	0.744	103.37	94.28	6	0.976
D2-miRNA	8.50	3.33	6		14.91	6.97	6		99.82	63.95	6	

Cre Social Chasing	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	0.00	0.00	7	0.175	0.00	0.00	7	0.147	600.00	0.00	7	0.161
D1-miRNA	0.43	0.30	7		1.06	0.68	7		461.05	92.91	7	
D2-control	0.33	0.21	6	0.687	0.36	0.23	6	0.344	409.22	120.73	6	0.997
D2-miRNA	0.50	0.34	6		1.06	0.67	6		408.53	121.24	6	

Cre Explores	Bouts			t-test	Duration			t-test	Latency			t-test
	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value	MEAN	SEM	n	p-value
D1-control	12.57	2.86	7	0.173	37.46	10.86	7	0.122	65.93	45.68	7	0.954
D1-miRNA	19.29	3.65	7		66.42	13.57	7		69.25	32.80	7	
D2-control	12.17	1.19	6	0.760	46.42	8.74	6	0.300	66.64	42.49	6	0.451
D2-miRNA	12.67	1.05	6		34.26	6.88	6		30.60	17.37	6	

Table S4. qRT-PCR primer sequences.

Human gene	Forward Primer	Reverse Primer
Gapdh	IDT PrimeTime	IDT PrimeTime
Nlgn2	GTGAAAACATCGCCCACTTT	TGGTGGGAGAGGATCAGAAG
Nlgn1	IDT PrimeTime	IDT PrimeTime
Nlgn3	IDT PrimeTime	IDT PrimeTime

Mouse gene	Forward Primer	Reverse Primer
nlgn1	ACAGGAGAACATCGTTTCCAGCCT	ATACAGGAGCAAACTGAGTGGCGT
nlgn2	TTCCCACCACTCAGAAGGAC	GTGCTGTCTTCTCGGTCACA
nlgn3	GTGAAATCCTGGGTCCTGTG	GTCTTCATCTTCATCCCCGTC
gapdh	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT

Cell-type specific role for nucleus accumbens neuroligin-2 in depression and stress susceptibility. Mitra Heshmati, Hossein Aleyasin, Caroline Menard, Daniel J. Christoffel, Meghan E. Flanigan, Madeline L. Pfau, Georgia E. Hodes, Ashley E. Lepack, Lucy K. Bicks, Aki Takahashi, Ramesh Chandra, Gustavo Turecki, Mary Kay Lobo, Ian Maze, Sam A. Golden, and Scott J. Russo*

SI Appendix Figure Legends

Figure S1. Postmortem NAc NLGN2 In Cocaine Users. Transcriptional profiling of human postmortem nucleus accumbens in control subjects and chronic cocaine users shows no significant gene expression differences in (A) neuroligin-1, (B) neuroligin-2, and (C) neuroligin-3 (Student's t-test, $p>0.05$, n = 16, 17). Detailed demographics shown in the table below for (D) control subjects and (E) chronic cocaine users. All data are represented as mean ± standard error of the mean.

Figure S2. Immunohistochemistry Behavioral Data. Detailed chronic social defeat stress behavioral data for immunohistochemistry study in Figure 2A-D. (A) Susceptible mice display significantly decreased social interaction ratio (one-way ANOVA: $F_{2,21} = 21.56$, ***$p<0.001$, n = 7, 6, 9). Analysis of (B) interaction duration (two-way ANOVA: $F_{2,38} = 15.71$, ***$p<0.001$), (C) corner duration (two-way ANOVA: $F_{2,38} = 8.625$, ***$p<0.001$, **$p<0.01$), (D) interaction zone entries, (E) corner zone entries, (F) distance moved, and (G) velocity. Analysis was performed by one-way or two-ANOVA followed by Bonferroni posttests. All data are represented as mean ± standard error of the mean.

Figure S3. Design, *in vitro* and *in vivo* validation of miRNA vector. Testing of 4 shRNA constructs lettered A-D showed (A) neuroligin-2 mRNA fold change and (B) changes in neuroligin-2 protein relative to scrambled control (arrowhead). shRNA constructs A,C,D were chosen for designing (C) *Cre*-conditional microRNA vector. (D) Neuroligin-2 knockdown after transfection in Neuro-2A cells (Student's t-test, *$p<0.01$, n = 3 replicates). (E) GFP expression was only observed in the presence of *Cre* recombinase. (F) Selective neuroligin-2 knockdown after *in vivo* infection in nucleus accumbens of D1-*Cre* mice (Student's t-test, *$p<0.05$, n = 5, 6). (G) *In vivo* virus expression in *Cre*-positive and *Cre*-negative mice. Scale bar: 200 μm. (H) Neuroligin-2 protein puncta are reduced in the NAc after *in vivo* virus expression (Student's t-test, *$p<0.05$, n = 4, 5), with no significant change in GFP mean intensity between groups (Student's t-test, $p>0.05$). (I) Representative images showing control and miRNA virus expression and neuroligin-2 immunohistochemistry in D1-*Cre* mice. Scale bar: 20 μm.

Figure S4. miRNA vector effects on mini inhibitory currents. (A) Representative image of virus-infected medium spiny neuron in slice preparation, scale bar 10 μm (B) Representative traces of mini inhibitory postsynaptic currents (mIPSC) recorded from D1- or D2- cells expressing AAV-Control or AAV-miRNA. (C) mIPSC frequency increases in D1-positive cells with miRNA expression. (Student's t-test, *$p<0.05$, n = 11-12 cells from 3-4 mice per group). (D) mIPSC frequency decreases in D2-positive cells with miRNA expression (Student's t-test, *$p<0.05$, n = 9 cells from 2-3 mice per group). There is no significant change in mIPSC amplitude in either (E) D1-positive (Student's t-test, $^{ns}p>0.05$)

or (F) D2-positive cells (Student's t-test, $^{ns}p>0.05$) with miRNA knockdown. Kolmogorov-Smirnov tests were used to analyze differences in cumulative probability between groups.

Figure S5. Subthreshold defeat timeline and data. (A) Experimental timeline for subthreshold social defeat stress and open field test experiments. There is no effect of (B, D) distance traveled or (C, E) velocity between D1-*Cre* groups in cohort 1 nor cohort 2 (Student's t-test, $p>0.05$, n = 6-11). No significant difference in (F, H) distance traveled and (G, I) velocity was observed between D2-*Cre* mice expressing the control or miRNA viruses in either cohorts (Student's t-test, $p>0.05$, n = 8-9). All data are represented as mean ± standard error of the mean. (J) Dot schematic indicating sites of NAc virus expression in cohort 1. (K) Dot schematic indicating sites of NAc virus expression in cohort 2. Images adapted from the Allen Brain Atlas.

Figure S6. No stress controls with neuroligin-2 miRNA knockdown. There is no effect of neuroligin-2 knockdown on baseline social interaction ratio (S6A and S6E, Student's t-test, $p>0.05$). While there is an effect of target ($^{*}p<0.05$, $^{t}p=0.05$), there is no effect of virus on interaction duration across groups and no significant interaction overall (S6B and S6F, two-way ANOVA, $p>0.05$). There is no significant difference in distance moved (S6C and S6G) or velocity (S6D and S6H, Student's t-test, $p>0.05$). All data are represented as mean ± standard error of the mean.

Figure S7. Open field test. (A) Representative heat maps of D1-*Cre* control and D1-*Cre* miRNA mice. (B) There was no significant difference between D1-*Cre* groups in time spent

in the center, middle, or periphery of the open field (two-way ANOVA, $p>0.05$). There was no effect of (C) distance traveled or (D) velocity between D1-*Cre* groups (Student's t-test, $p>0.05$). (E) Representative heat maps of D2-*Cre* control and D2-*Cre* miRNA mice in the open field. (F) D2 miRNA group spent significantly more time in the center and middle zones and less time in the periphery (two-way ANOVA: $F_{2,45} = 25.56$, ***$p<0.001$). There was no difference in (G) distance traveled or (H) velocity between D2-*Cre* groups (Student's t-test, $p>0.05$). All data are represented as mean ± standard error of the mean.

Table S1. Postmortem MDD Demographics.

Table S2. CD-1 aggressor behavioral analysis from resident-intruder interaction.

Table S3. D1-*Cre* and D2-*Cre* behavioral analysis from CD-1 resident-intruder interaction.

Table S4. qRT-PCR primer sequences.

SI Materials and Methods.

Experimental Animals

D1-*Cre* and D2-*Cre* BAC transgenic heterozygous mice (courtesy of Eric Nestler, Icahn School of Medicine at Mount Sinai) were bred to c57bl6/j wild-type females from Jackson Laboratories (Bar Harbor, ME), and the resulting 7 to 10 week-old heterozygous male

offspring were used for behavioral studies. Pups were weaned at 3-4 weeks of age and genotyped by Transnetyx (Cordova, MN). Transgenic mice were previously backcrossed onto a c57bl6/j background for at least 10 generations. 8 week-old c57bl6/j male mice from Jackson were used for immunohistochemistry after chronic social defeat stress experiments. 7-9 week-old D1-Cre-RiboTag and D2-Cre-RiboTag male mice were used for cell-type specific ribosome immunoprecipitation as previously described(1). Singly-housed male CD-1 mice (4-month old sexually experienced retired breeders) from Charles River Laboratories (Wilmington, MA) were used in social defeat experiments. All mice were group housed in a controlled environment (12 h light/dark cycle) with food and water available *ad libitum.* Mice were singly housed one-week prior to resident-intruder testing and 24 hours prior to social interaction testing. Behavioral assessments and tissue collections were performed during the animals' light phase. All experiments were performed in accordance with the Icahn School of Medicine and University of Maryland Institutional Animal Care and Use Committees.

Chronic Social Defeat Stress

Chronic social defeat stress was performed as previously described (2, 3). All aggressors were screened for aggressive behavior prior to use according to published protocols (4). Experimental mice encountered a novel CD1 aggressor for 10 minutes daily over 10 consecutive days. Mice were housed opposite a perforated plexiglass barrier between defeat sessions to enable continuous sensory contact with the aggressor. After the 10 days, experimental mice were singly housed overnight and underwent social interaction testing the following day.

Subthreshold Defeat Stress

Subthreshold social defeat was used to measure increased susceptibility to stress following a single day of social defeat stress. Based on previous studies, these conditions do not cause social avoidance in control mice, but manipulations that promote susceptibility will reveal a social avoidance phenotype after subthreshold defeat (5-7). Experimental mice were exposed to a novel CD-1 aggressor for five minutes followed by 10 minutes rest in the home cage, and this was repeated for a total of three aggressive interactions. Twenty four hours later, mice were assessed using the social interaction test.

Social Interaction Test

Social interaction testing was performed as previously described (4). Mice were placed over two 150-second trials into a novel, open field arena with an interaction zone delineated. In the first "No target" trial, an empty cage was placed in the interaction zone. In the second "Target" trial, a novel CD-1 mouse was placed inside the cage in the interaction zone. Time spent in the interaction zone, distance traveled, and velocity was measured using Ethovision video tracking software (Noldus Technology). The social interaction ratio was calculated as time spent in the interaction zone in the "Target" trial divided by time spent in the interaction zone in the "No target" trial. Susceptible mice were defined by having a social interaction ratio less than 1 (3, 4). Heat maps were generated using the "heat map" function on the Ethovision software and represent the mouse's movement in the arena during the "Target" trial. Heat map scale bar represents the percentage of time spent at each XY coordinate during the trial.

Home Cage Aggression

Experimental mice were singly housed and exposed for 10 minutes to a novel, D1-*Cre* or D2-*Cre* conspecific intruder of approximately 5 weeks age and 15 grams weight. Attack latency was manually scored by a blinded experimenter.

Defensive Behavior

Singly-housed CD-1 mice were screened for aggressive behavior according to social defeat protocols. Experimental mice were exposed for 10 minutes to a novel, CD-1 aggressor. Defensive behaviors by the experimental mice against the CD-1 aggressor were scored during post hoc video analysis by a blinded experimenter. Behaviors were identified as biting the aggressor, boxing the aggressor, or holding down the CD-1 aggressor when attacked. Aggressive behavior by CD-1 aggressors and social behaviors by CD-1, D1-*Cre,* and D2-*Cre* were scored in an automated manner using Cleversys software.

Tube Test

The tube test protocol was based on previous reports using a non-automated test (8-10). Following 2 days of habituation to the tube, mice were simultaneously placed at opposite ends of a clear plastic tube measuring 3cm wide and 30cm long. The tube was narrow enough for one mouse to walk through without reversing direction. Mice of the same strain and weight were tested pairwise using a round robin design for a total of 3 trials, placing one control virus-injeced mouse against one miRNA virus-injected mouse. The tube was cleaned between trials. Trials over 120s or in which the mice did not meet in the center of

the tube were discarded. The end of the trial was marked by the "loser" having retreated with all four paws outside of the tube. The "winner" was the mouse that forced its opponent to retreat. The percentage of trials won is suggested to be a measure of social dominance (11).

Open Field Test

The open field test was performed as previously described (5). Experimental mice were placed inside a novel box and movements inside the box were tracked over 10 minutes using Ethovision software as a measure of exploratory-based anxiety. Less time spent in the corners of the box and more time spent in the center of the box is interpreted as decreased anxiety-like behavior.

MicroRNA vector

Commercially available mouse short hairpin RNA (shRNA) against the neuroligin-2 gene were purchased (Origene, TG516880) and transfected in Neuro2A cells (ATCC) using Effectene (Qiagen) and common procedures. Cells were harvested for either RNA or protein followed by qPCR and Western blot for neuroligin-2 to validate knockdown. Of the four shRNA tested, three showed reduction in both messenger RNA (mRNA) and protein expression of neuroligin-2. These shRNA sequences were then used to create microRNA (miRNA) oligos using the instructions outlined in the BLOCK-iTTM Pol II miR RNAi Expression Vector Kit (Invitrogen, K4936-00). The following miR sequence was validated for best knockdown efficiency and later packaged in the AAV virus: TGCTGCAAGTTGTCGGTGAACCACACGTTTTGGCCACTGACTGACGTGTGGTTCCGACAACTTG

miR constructs were next sub-cloned into a bicistronic AAV-IRES-GFP vector (Cell Biolabs, Inc., VPK-418) using BamH1 and XhoI sites. The kit provided a negative control that does not target any sequence in the vertebrate genome, and this non-targeting miR sequence was similarly cloned to generate the control virus used in all studies. To construct a Cre-dependent version, we modified an AAV-FLEX-rev-ChR2-Tdtomato vector; a kind donation to Addgene from Dr. Sternson's laboratory (Addgene #18917) by insertion of an AvrII site on the 5' end of the gene of interest. The modified vector was linearized using AvrII and KpnI double digestion. The miR-IRES-GFP cassette was then reversely subcloned into the AAV-FLEX vector using Gibson assembly method (New England Biolabs, Cat# 2611) and online primer design tool. Resulting AAV-DIO-miR-IRES-GFP constructs were verified by sequencing and validated for GFP expression and mRNA knockdown in Neuro2A cell culture before being packaged into AAV viral vectors. AAV constructs were packaged into high titer viral particles (10^{12}, serotype AAV-2) by the University of North Carolina at Chapel Hill Gene Therapy Center (UNC Vector Core).

Western Blot

Neuro2A cells were collected and protein was isolated in RIPA buffer using common procedures. Equal amounts of protein were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in 5% milk and incubated overnight in milk with goat polyclonal antibody against neuroligin-2 (1:1000, Abcam ab77595).

Stereotaxic Surgery

Surgical procedures were performed according to previously published methods (5, 7, 12). Mice were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg), and positioned in a small-animal stereotax (David Kopf Instruments). The skull surface was exposed and thirty-three-guage syringe needles (Hamilton Co.) were used to bilaterally infuse 0.5 μL of virus (AAV-DIO-miRNA or AAV-DIO-control virus) into the NAc (bregma coordinates: anteroposterior, +1.5mm; mediolateral, +1.6mm; dorsoventral, -4.4mm, 10 degree angle) at a rate of 0.1 mL/min. All behavioral tests were performed 3 weeks after AAV virus infection.

Perfusion

Following behavioral studies, mice were anesthetized with 15% chloral hydrate. Transcardial perfusion was performed with cold phosphate-buffered saline (pH 7.4) followed by 4% paraformaldehyde in phosphate-buffered saline. Brains were dissected and post fixed overnight in 4% paraformaldehyde. Brains were cut into 50 μm coronal slices on a Leica vibratome to evaluate virus expression in the nucleus accumbens.

Immunohistochemistry

Brains were perfused as described above, flash frozen after cyroprotection with sucrose, and 40 μm slices containing the nucleus accumbens were cut on a cryostat (Leica CM1850). The following primary antibody dilution was used in blocking solution (3% normal donkey serum and 0.3% Triton X in PBS) with free-floating incubation overnight: 1:500 neuroligin-2 (rabbit, Abcam ab36602). Slices were then incubated in secondary fluorescent antibody (Donkey Cy2 or Cy3, Jackson ImmunoResearch) for 2 hours. Images were acquired on a

Zeiss LSM 780 confocal microscope using a 100X oil immersion objective (pixel size of 0.17 microns). Images were deconvoluted with AutoQuant and protein puncta were quantified using Image J 3D Object Counter.

RiboTag Protocol

The RiboTag procedure to immunoprecipitate ribosomes from NAc of D1-Cre-RiboTag (D1-Cre-RT) and D2-Cre-RiboTag (D2-Cre-RT) mice was performed as previously described(1). In brief, NAc tissue from 3 mice was homogenized in 800 µl homogenization buffer and the clear supernatant was collected after centrifugation 10,000 × *g* for 10. The supernatant was added directly to the HA-coupled beads (Invitrogen: 100.03D; Covance: MMS-101R) for constant rotation overnight at 4°C. The following day, magnetic beads were added to the lysate for constant rotation overnight at 4°C. The beads were washed three times in high-salt buffer. Finally, RNA was extracted by adding TRK lysis buffer to the pellet provided in MicroElute Total RNA Kit (Omega) according to the manufacturer's instructions. 300–400 ng of cDNA was synthesized using the reverse transcriptase iScript cDNA synthesis kit (Bio-Rad). qRT-PCR was performed with PerfeCTa SYBR Green FastMix (Quanta). Quantification of mRNA changes was performed using the $-\Delta\Delta C_T$ method described previously(1) using GAPDH as a housekeeping gene.

Quantative Polymerase Chain Reaction (qPCR)

For qPCR of whole brain, bilateral 14-gauge tissue punches containing the NAc were collected on ice after rapid decapitation and immediately placed on dry ice or stored at -80°C until further processing. For qPCR of Neuro2A cell culture, cells were collected using

standard procedures. RNA isolation was performed using TRIzol (Invitrogen) homogenization and chloforom layer separation, followed by processing of the RNA layer (RNAeasy MicroKit, Qiagen). RNA was analyzed with NanoDrop (Thermo Scientific) and reverse transcribed to cDNA (qScript Kit, VWR). For qPCR, cDNA was diluted to 1 ng/μL and 3μL was used in each reaction. The reaction mixture consisted of 5μL Perfecta SYBR Green (VWR), 0.5μL each of forward and reverse primers, 1μL of water and the cDNA template. Samples were heated to 95°C for 2 minutes followed by 40 cycles of 95°C for 15 seconds (s), 60°C for 33 s and 72°C for 33 s. Analysis was performed using the $-\Delta\Delta C_T$ method, with sample normalization to GAPDH. Please see Table S10 for a list of primer pairs. IDT PrimeTime primers were purchased from Integrated DNA Technologies.

Acquisition of Postmortem Human Tissue

Nucleus accumbens whole tissue resections were collected at the local medical examiners offices, after obtaning next-of-kin permission, by the Quebec Suicide Brain Bank at the Douglas Hospital Research Center under an approval of the Douglas Hospital Research Center's Research Ethics Committee. The NAc was dissected and immediately placed in a mixture of dry ice and isopentane (1:1 (vol:vol)) and stored at -80°C until further processed for total RNA using TRIzol (Invitrogen). Groups were matched for race, gender, age, pH, postmortem interval (PMI) and RNA integrity number (RIN). Acceptable RIN values ranged from 6.1 - 9.5 (Agilent 2100 Bioanalyzer). Subjects were excluded if they had a known history of neurological disease or head injury. For each subject, a review of clinical records, telephone interviews with primary caregivers, toxicology screen, and independent diagnosis by 3 to 4 mental health care professionals using the Diagnostic and Statistical

Manual of Mental Disorders IV was confirmed. Individual demographic characteristics are listed in Supplementary Tables 1 and 5.

Electrophysiology. D1-Cre and A2A-Cre BAC transgenic mouse were bred in house and 5 week old mice were injected with AAV-FLEX- control or miR-1,2. Whole-cell recordings were obtained 10-14 days post-injection from MSNs expressing GFP which was excited using a mercury arc lamp with a bandpass filter (HQ545/303) in the NAc (identified by the presence of the anterior commissure) using IR-DIC optics. To minimize stress and to obtain healthy slices, mice were anaesthetized and perfused immediately for 40–60 s with ice-cold aCSF (artificial cerebrospinal fluid), which contained in mM: 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4, 11 D-glucose, 18 $NaHCO_3$, 2.5 $CaCl_2$ and 1.2 $MgCl_2$ (oxygenated with 95% O_2 and 5% CO_2, pH 7.4, 295–305 mOsm). Acute brain slices were cut using a microslicer (Leica 1200S) in cold sucrose aCSF which contained in mM: 254 mM sucrose, 23 KCl, 1.25 NaH_2PO_4, 10 D-glucose, 24 $NaHCO_3$, 2 $CaCl_2$ and 2 $MgCl_2$ saturated by 95% O_2 and 5% CO_2. Slices were maintained in holding chambers with aCSF which contained: 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4, 11 D-glucose, 18 $NaHCO_3$, 2.5 $CaCl_2$ and 1.2 $MgCl_2$ (oxygenated with 95% O_2 and 5% CO_2, pH 7.4, 295–305 mOsm) for 1 h at 32 °C aCSF. Patch pipettes (3–5 MΩ) for voltage-clamp recordings were filled with internal solution containing the following (mM): 117 cesium methanesulfonate, 2.8 NaCl, 0.4 EGTA, 5 tetraethylammonium chloride, 20 HEPES, 24magnesium ATP and 0.4 GTP (pH 7.2, 285 mOsm). Whole-cell recordings were carried out using aCSF at 32 °C (flow rate = 2.5 ml min^{-1}). MSNs were voltage-clamped at 0 mV. Recordings were performed using a MultiClamp 700B (Molecular Devices), filtered at 2 kHz and digitized at 10 kHz. Data acquisition and analysis were performed on-line using

Axograph X. Input and series resistance were monitored continuously and experiments were discarded if either parameter changed by > 20%. Miniature inhibitory postsynaptic currents were recorded in the presence of tetrodotoxin (500nM), D-APV (100μM) and NBQX (100μM). At least 200 events per cell were acquired in 15 s blocks and detected using a threshold of 7pA and all events included in the analysis were verified by eye.

Statistics

All data are expressed as the mean ± SEM. Mean differences between groups were determined using two-tailed Student's t-test, one-way analysis of variance (ANOVA) or two-way ANOVA, followed by Bonferroni post-tests if the main effect was significant at $p<0.05$. Kolmogorov-Smirnov tests were used to analyze population shifts in electrophysiology cumulative probability data. Statistical analyses were performed using Prism 5.0 (GraphPad Software).

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