Supplementary Online Methods

Animals

Male C57BL/6J mice (20–30g, The Jackson Laboratory) were obtained at 7–8 weeks of age and allowed 1 week acclimation to housing facilities prior to the start of experiments. Male fl/fl Rac1 mice (20–30g) were bred at the Mount Sinai School of Medicine and used at 7–8 weeks old. Male CD–1 mice (35–45g, Charles River Laboratories) utilized as aggressors were sexually–experienced retired breeders at least 4 months of age. Outside of use in chronic social defeat and chronic variable stress experiments, all mice were single housed and maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. Behavioral assessments and tissue collection were performed 1 h into the animals' dark phase. Mouse procedures were performed in accordance with the Institutional Animal Care and Use Committee guidelines of the Mount Sinai School of Medicine.

Chronic social defeat stress

Chronic social defeat stress was performed as previously described [19,26,54,55]. CD–1 mice were screened for aggressive characteristics prior to the start of social defeat experiments based on previously described criteria [26], and housed within the social defeat cage (26.7w x 48.3d x 15.2h cm; Allentown Inc) 24 hours prior to the start of defeats on one side of a clear, perforated Plexiglass divider (0.6 × 45.7 × 15.2 cm; Nationwide Plastics). Briefly, experimental C57BL/6J mice were subjected to a novel CD–1 aggressor mouse for 10 minutes once per day, over 10 consecutive days. Following the 10 minutes of agonistic interaction, the experimental mice were removed to the opposite side of the social defeat cage and allowed sensory contact during the following 24 hour period. Control mice were housed 2 mice per cage, on opposite sides of the perforated divider, rotated daily in a manner similar to the defeat group, but never exposed to aggressive CD–1 mice. Twenty–four hours following the final social defeat stress experimental C57BL/6J mice were singly housed. In a variation on this protocol, for tissue collected at more immediate time points (4 and 24 hours) following social defeat, we exposed mice to 9 consecutive defeats, performed behavioral testing for social avoidance, and then performed the final 10th defeat and sacked at the necessary time point.

Microdefeat stress

To measure increased susceptibility to stress we adapted a subthreshold variation on the chronic social defeat protocol, as previously described [19,26]. Using this protocol, experimental C57BL/6J mice were subjected to a novel CD–1 aggressor for 3 consecutive 5 minute defeat bouts, each separated by 15 minutes. Twenty–four hours later mice were assessed for social avoidance behavior. Under control conditions, this protocol does not result in social avoidance behavior, but is sensitive to pro–susceptibility factors.

Social avoidance testing (Social interaction test)

Social interaction testing was performed as previously described [26,55]. All social interaction testing was performed under red–light conditions. Mice were placed in a novel interaction

open–field arena custom–crafted from opaque Plexiglas (42 × 42 × 42 cm; Nationwide Plastics) with a small animal cage placed at one end. Their movements were then automatically monitored and recorded (Ethovision 3.0; Noldus Information Technology) for 2.5 minutes in the absence (target absent phase) of a novel CD–1 mouse. This phase is used to determine baseline exploratory behavior. We then immediately measure 2.5 minutes of exploratory behavior in the presence of a caged CD–1 mouse (target present phase), again recording total distance travelled and duration of time spent in the interaction and corner zones. Social interaction behavior is than calculated as total time spent in each zone, or as a ratio of the time spent in the interaction with target present divided by the time spent in the interaction zone with the target absent. All mice with a ratio above 1.0 were classified as resilient, and all mice below 1.0 were classified as susceptible.

Sucrose preference testing

To determine if mice acquired anhedonic responses to any experimental manipulations, we performed a standard sucrose preference assay. Immediately following the final chronic social defeat stress session, or the final microdefeat bout, mice had their standard water bottle removed and replaced with two 50ml conical tubes with sipper tops filled with water. Following a 24 hour habituation period, water from one 50ml conical tube was replaced with 1% sucrose. All tubes were weighed and mice were allowed 24 hours to drink. Tubes were then re–weighed and their location in the wire top was switched prior to a second 24 hour period of drinking. At the end of the second day of sucrose testing, sucrose preference was calculated as the total amount of sucrose consumption divided by the total amount of fluid consumed over the 2 days of sucrose availability.

Imipramine treatment

Following social interaction testing, mice are sorted into either susceptible or resilient phenotypes based on interaction scores as described above. Mice were then divided into treatment groups. For each group (control, susceptible), animals received either daily intraperitoneal injections of imipramine (20 mg/kg) for 35 d or vehicle. Previous work has shown that this imipramine treatment paradigm completely reverses the social interaction deficit seen in susceptible animals, which otherwise persists unabated in vehicle–treated susceptible animals [27]. All mice were killed 24 h after the last imipramine or vehicle injection, and NAc dissections were obtained by punch dissection as described previously [27] .

Transcriptional profiling

NAc samples were collected and processed as described previously [27]. Briefly, bilateral 14 gauge NAc, mPFC or dorsal striatum punches were collected on ice following rapid decapitation, and then immediately placed on dry ice and stored at –80˚C until use. We then isolated RNA using TriZol (Invitrogen) homogenization and chloroform layer separation. The clear RNA layer was then processed (RNAeasy MicroKit, Qiagen) and analyzed with NanoDrop. 500ng of RNA was then reverse transcribed to cDNA (qScript Kit, VWR). For quantitative PCR (qPCR), cDNA was diluted to 500µl and 3µl was used for each reaction. The reaction mixture consisted of Perfecta SYBR Green (5 µl), forward and reverse primers (0.5 µl each), water (1 µl), and cDNA template. Samples were then heated to 95°C for 2 min followed by 40 cycles of

95°C for 15 s, 60°C for 33 s, and 72°C for 33 s. Analysis was done via the ΔΔC(t) method [54]. Samples were normalized to GAPDH. Primer pairs are shown in **Supplemental Table 1** (Integrated DNA Technologies).

Western blotting

Bilateral NAc punches, taken as described above, were sonicated in a detergent–based lysis buffer [50mM Tris–HCl, pH 7.5, 2 mM MgCl2, 150 mM NaCl, 1% Triton X–100, protease inhibitor cocktail (Roche), 1 μM sodium vanadate, 30 mM sodium pyrophosphate, and 30 mM sodium fluoride] containing phosphatase and protease inhibitors (Sigma). Samples were then centrifuged for 30 min at 14,000 RPM at 4°C and the supernatant was collected and quantified with the Microplate BCA Protein Assay Kit (Thermo Scientific). Equal amounts of protein sample were separated by SDS–PAGE (BioRad, 4 –20% polyacrylamide gels) and transferred to nitrocellulose membranes (BioRad). The membranes were blocked in 5% milk and incubated overnight with primary antibodies in 5% BSA or milk. Primary antibodies used were as follows: anti–Rac1, 1:500 (Cytoskeleton, Inc.); anti–Pak1, 1:1000; anti–LIMK, 1:1000 (Cell Signaling Technology). Antibody binding was detected using the Oddessey Fluorescence Detection System (LiCOR), with proprietary LiCOR secondary antibodies (1:50,000). All samples were normalized to GAPDH (1:50,000; Cell Signaling Technology).

Mouse chromatin–immunoprecipitation

Site–directed qChIP was performed for pan–acH3 and H3K27me3 as described previously [28]. Briefly, brains were rapidly removed and bilateral 14 gauge NAc punches taken from two adjacent 1mm coronal slices containing the NAc. Punches were then immediately cross–linked in 1% formaldehyde for 15 minutes at room temperature, and quenched by addition of glycine at a final concentration of 0.125 M. Punches were then washed 5 times in cold PBS containing proteinase inhibitors (1 mM PMSF, 1 μg/ml apoprotin, and 1 μg/ml pepstatin A) and frozen on dry ice. For each sample, the total NAc punches of 2 mice were combined, balanced by social interaction ratio behavior, resulting in 8 NAc punches per sample.

Chromatin was solubilized and extracted by SDS–detergent lysis buffer, followed by a series of sonications. First, samples were sonicated twice for 7 seconds with a desktop sonicator (40% power, Ultrasonic Processor; Cole Parmer) at low power in 500μl SDS Lysis buffer [1% SDS, 50mM Tris–HCl (pH 8.1), 10mM EDTA] and then diluted with 1100μl ChIP dilution buffer [1.10% Triton X–100, 167mM NaCl, 16.7mM Tris–HCl (pH 8.1), 1.2mM EDTA, 0.01% SDS] and sonicated (setting 4, Sonic Dismembranator 550; Fisher Scientific) at high power 5x times for 15 seconds per sonication. 400μl of sheared chromatin was used for each immunoprecipitation, brought to a final volume of 600μl with ChIP dilution buffer. 5% of each sample was put aside to be used as input for data normalization. This chromatin shearing protocol reliably produces fragments 350–700bp in length [28]. Chromatin immunopreciptation was then performed with 7 μg of anti–acetyl H3 and anti–H3K27me3 antibodies (EMD Millipore) per sample, conjugated to magnetic Dynabeads (M–280 Sheep anti–Rabbit IgG; Invitrogen). IgG control antibody–conjucated beads were also used, and failed to show enrichment. Beaded antibodies were incubated with the immunoprecipitated chromatin overnight (~16 hours) at 4˚C and then washed 8x times in ChIP RIPA buffer [0.7%% Na–Deoxycholate, 500mM LiCl, 50mM Hepes–KOH (pH

7.6), 1% NP–40, 1mM EDTA]. Beads were removed by heating to 65˚C, shaken at 1000rpm for 30 minutes on a Thermomixer and spun down before the supernatant collected. Chromatin in the supernatant and input samples was then reverse cross–linked by heating to 65˚C overnight. DNA was then purified for RT–PCR analysis with a QIAquick PCR Purification Kit (Qiagen). Levels of specific histone modifications at each gene promoter of interest were determined by measuring the amount of acetylated or methylated histone–associated DNA by quantitative real–time PCR, as detailed previously. For the purposes of mapping out the chromatin landscape within, and upstream of, the *Rac1* promoter, we used the primers listed in **Supplemental Table 3** (Integrated DNA Technologies).

Human post–mortem tissue

For the Texas cohort, whole tissue NAc resections were collected and provided by the Dallas Brain Collection, where tissue is collected from the Dallas Medical Examiner's Office and University of Texas (UT) Southwestern's Tissue Transplant Program under review of The UT Southwestern Institutional Review Board [56]. For the Montreal cohort, whole tissue NAc resections were collected 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. Briefly, after obtaining next of kin permission, brain tissue is collected from cases at the local Medical Examiners Offices. Subjects with known history of neurological disorders or head injury are excluded and blood toxicology screens out any subject using illicit drugs and psychotropic medications. Clinical records and collateral information from telephone interviews with a primary caregiver is obtained for each case. Three to four mental health professionals carry out an extensive review of the clinical information and make independent diagnoses followed by a consensus diagnosis using DSM IV criteria. Demographic characteristics associated with the tissue are presented in Supplemental Table 4 and 5. . The two groups were matched as closely as possible for race, gender, age, pH, postmortem interval (PMI) and RIN (RNA integrity number). Acceptable RIN values were within the range of 6.1 to 9.5.

In each case, cerebral hemispheres were cut coronally into 1–2cm blocks. The nucleus accumbens was dissected from the appropriate coronal section (**Fig. 3a**) and immediately placed in a mixture of dry ice and isopentane (1:1, v:v). The frozen tissue was then pulverized on dry ice and stored at –80°C.

pH and RIN determination was done as previously described [56]. Briefly, tissue weighing approximately 150 mg was punched from the cerebellum, homogenized in 5 ml of ddH_2O (pH adjusted to 7.00) and centrifuged for 3 min at 8000g at 4 °C. pH of the supernatant was measured in duplicate (Thermo–Electron Corporation). RIN determination was performed by isolating total RNA using Trizol (Invitrogen) followed by analysis with an Agilent 2100 Bioanalyzer.

Human chromatin–immunoprecipitation

Site–directed qChIP was performed as previously described [30], using a micrococcal nuclease (MNase) based assay allowing for high–resolution mapping of the human gene promoter and upstream regions. Briefly, 50mg of human NAc tissue was lightly homogenized with 550µl

Douncing buffer [10mM Tris–HCl (pH 8.0), 4mM $MgCl^2$, 1mM $CaCl^2$] in a 1mL loose–fit glass homogenizer. The homogenate was then digested with miccrococal nuclease enzyme (2 units/mL) for 10 minutes in a 37˚C water bath. We have found that this reaction yields the optimal DNA basepair fragment size of ~150–160bp (**Supplemental Fig. 7**). The digestion reaction was terminated by addition of 10mM EDTA, pH 8.0. The digested chromatin was then further incubated in SDS Lysis buffer [1% SDS, 50mM Tris–HCl (pH 8.1), 10mM EDTA] for 60 minutes on wet ice, and lightly vortexed every 10 minutes. The lysed chromatin was then centrifuged at 3000g for 20 minutes at 4˚C, and the supernatant collected. 400µl of the digested chromatin supernatant was then used for each chromatin immunoprecipitation, brought to 500µl final volume with Incubation buffer [500mM NaCl, 200mM Tris–HCl (pH 8.0), 50mM EDTA (pH 8.0)].

Chromatin immunopreciptation was then performed with 7 µg of anti–acetyl H3 and anti–H3K27me3 antibodies (EMD Millipore) per sample, conjugated to magnetic Dynabeads (M–280 Sheep anti–Rabbit IgG; Invitrogen). IgG control antibody–conjucated beads were also used, and failed to show enrichment. Beaded antibodies were incubated with the immunoprecipitated chromatin overnight (~16 hours) at 4˚C and then washed 8x times in ChIP RIPA buffer [0.7%% Na–Deoxycholate, 500mM LiCl, 50mM Hepes–KOH (pH 7.6), 1% NP–40, 1mM EDTA]. Beads were removed by heating to 65˚C and shaken at 1000rpm for 30 minutes on a Thermomixer, and then spun down to remove the supernatant. Chromatin in the supernantent and input samples was reverse cross–linked by heating to 65˚C overnight. DNA was then purified for RT–PCR analysis with a QIAquick PCR Purification Kit (Qiagen). Levels of specific histone modifications at each gene promoter of interest were determined by measuring the amount of acetylated or methylated histone–associated DNA by quantitative real–time PCR, as detailed previously. For the purposes of mapping out the chromatin landscape within, and upstream of, the *Rac1* promoter the primers listed in **Supplemental Table 6** were used (Integrated DNA Technologies).

Imaging and NeuronStudio

For spine analysis, dendritic segments 50–150 µm away from the soma were randomly chosen from herpes simplex viral (HSV)–infected cells that express XFP in a 4% PFA fixed 200 µm coronal slice cover–slipped in VectaShield setting medium. Images were acquired on a confocal LSM 710 (Carl Zeiss) for morphological analysis as described previously [57]. Neurons were selected from the NAc shell. To qualify for spine analysis, dendritic segments had to satisfy the following requirements: (1) the segment had to be completely filled (all endings were excluded), (2) the segment must be at least 50 µm from the soma, and (3) the segment could not be overlapping with other dendritic branches. Dendritic segments were imaged using a 100× lens (numerical aperture 1.4; Carl Zeiss) and a zoom of 2.5. Pixel size was 0.03 µm in the *x*–*y* plane and 0.01 µm in the *z* plane. Images were taken with a resolution of 1024 × ~300–350 (the *y* dimension was adjusted to the particular dendritic segment to expedite imaging), pixel dwell time was 1.58 µm/s, and the line average was set to 4. An average of two dendrites per neuron on five neurons per animal totaling ~2500 dendritic spines per experimental group were analyzed. For quantitative analysis of spine size and shape, NeuronStudio was used with the rayburst algorithm described previously [58]. NeuronStudio classifies spines as thin, mushroom, or

stubby based on the following values: (1) aspect ratio, (2) head to neck ratio, and (3) head diameter. Spines with a neck can be classified as either thin or mushroom, and those without a significant neck are classified as stubby. Spines with a neck are labeled as thin or mushroom based on head diameter. These parameters have been verified by comparison with trained human operators.

Immunohistochemistry

For cofilin immunohistochemisty, mice were perfused with 4% PFA as indicated in the main methods section. Forty µm coronal slices were washed in PBS–T (0.4% Triton X–100 in PBS) and transferred to blocking solution (0.2% PBS–T in 5% normal donkey serum) for 2 hours before overnight incubation (0.2% PBS–T in 5% normal donkey serum) in primary antibody solution (anti–Cofilin, 1:2000; Abcam). Slices were then washed in 1x PBS, and incubated for 2 hours with secondary antibody for fluorescent detection. Slices were cover–slipped with DPX mounting media. For floxed Rac1 knockout validation, a monoclonal anti–Rac1 antibody (Millipore; 1:500) was used with the same procedure.

Immunohistochemistry quantitative analysis

We acquired z–stack images of NAc dendritic segments and colocalized cofilin puncta on a LSM–710 (Carl Zeiss) confocal microscope, using identical paramaters for those used during spine count experiments. This allowed us to determine dendritic spine morphology within the 40 µm tissue sections on each dendritic segment. To correct the photon point–spread function and improve resolution we independently deconvolved the GFP and cofilin channels (Autodeblurr; AutoQuant), and remerged them into a single z–stack. With the previously created NeuronStudio dendritic spine maps, we identified colocalized cofilin puncta manually on merged RGB images (Image J Software), and used orthogonal verification to ensure puncta were located within discrete spine heads. All immunohistological images were taken with identical acquisition parameters using Zen software.

Stereotaxic surgery and viral gene transfer

We performed all surgeries under aseptic conditions using anesthetic as described previously [17,59]. Briefly, Mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and positioned in a small–animal stereotaxic instrument (David Kopf Instruments), and the skull surface was exposed. Thirty–three–gauge syringe needles (Hamilton Co.) were used to bilaterally infuse 0.5µl of virus ($1.5x10^8$ infectious units/ml) expressing a control green fluorescent protein (GFP) or either Cre, a dominant–negative (Rac DN) or constitutively active (Rac CA) mutant to activate or inhibit Rac1 signaling into the NAc (bregma coordinates: anteroposterior, +1.5 mm; mediolateral, +1.6 mm; dorsoventral, – 4.4 mm) at a rate of 0.1 ml/min. All behavioral analyses were conducted between days 3–4 post infusion during maximal HSV expression.

Osmotic minipump surgery and infusion

Mice were anesthetized as detailed above. Mice were surgically implanted with two subcutaneous Alzet minipumps (model 1002; Durect) and bilateral guide cannulae (Plastics One) targeting the NAc. One day before surgery, two cannulae (28 gauge stainless steel) were

filled with MS–275 (100 μm; provided by the Broad Institute) or 5% hydroxypropyl β–cyclodextrin vehicle (Trappsol; CTD), and each pedestal within the assembly was separately affixed via vinyl tubing to a minipump, each loaded with drug or vehicle. The minipumps were activated on the evening before surgery (by incubating them at 40°C) to initiate a continuous delivery at 0.25 μl/h over 10 d. Briefly, the surgical procedure began with an incision over the skull, and the skin was spread apart under the scapulae to create an area for positioning the minipumps on the back. Bilateral cannulae were delivered into the NAc according to bregma: anteroposterior, +1.5; mediolateral, +1.0; dorsoventral, −4.5. Cannulae were permanently fixed to the skull with Loctite skull adhesive (Henkel). Cannulae, tubing, and minipumps were all secured under the skin using Vetbond tissue adhesive (3M) and two staples.