Epigenetic regulation of synaptic remodeling in stress disorders

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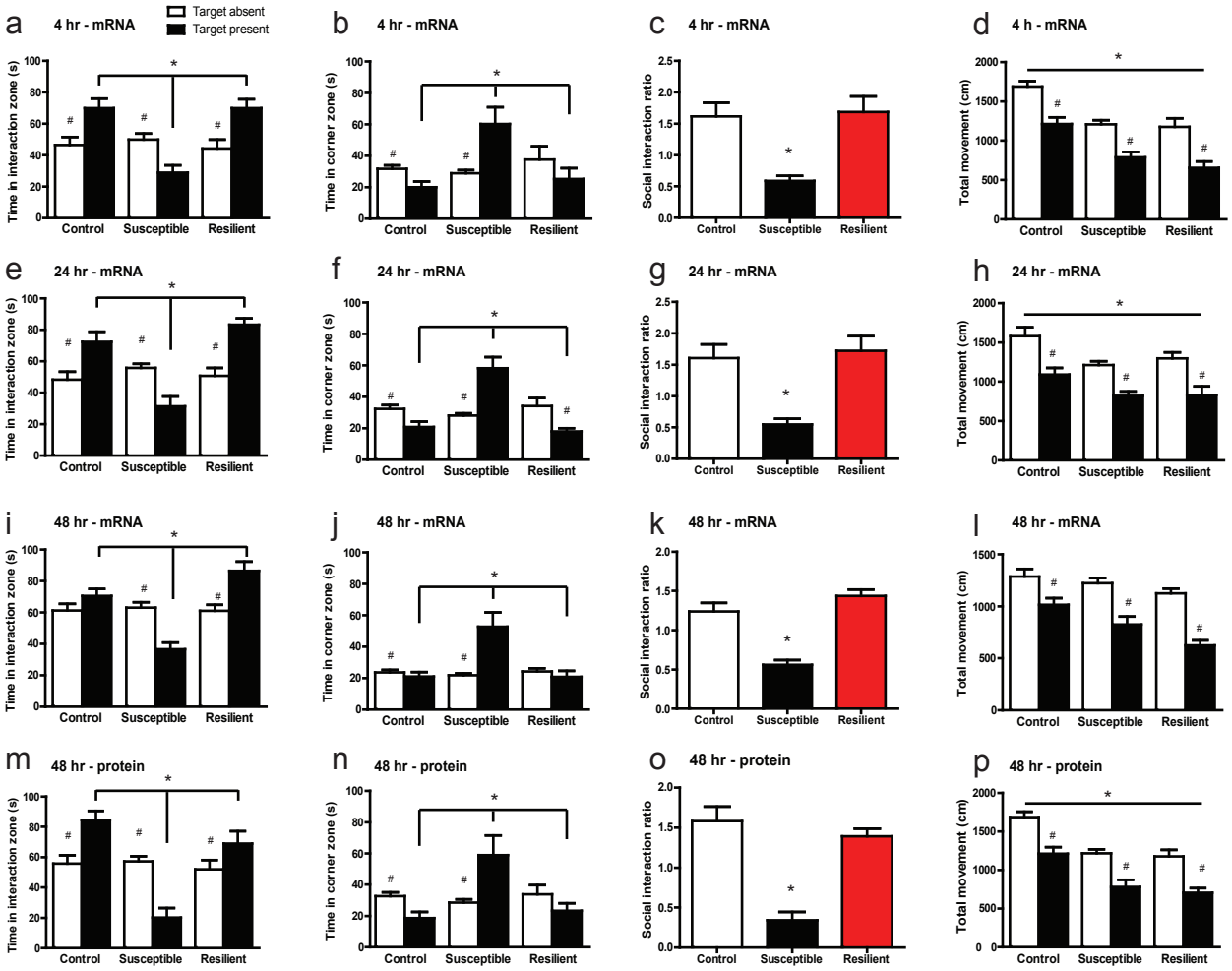
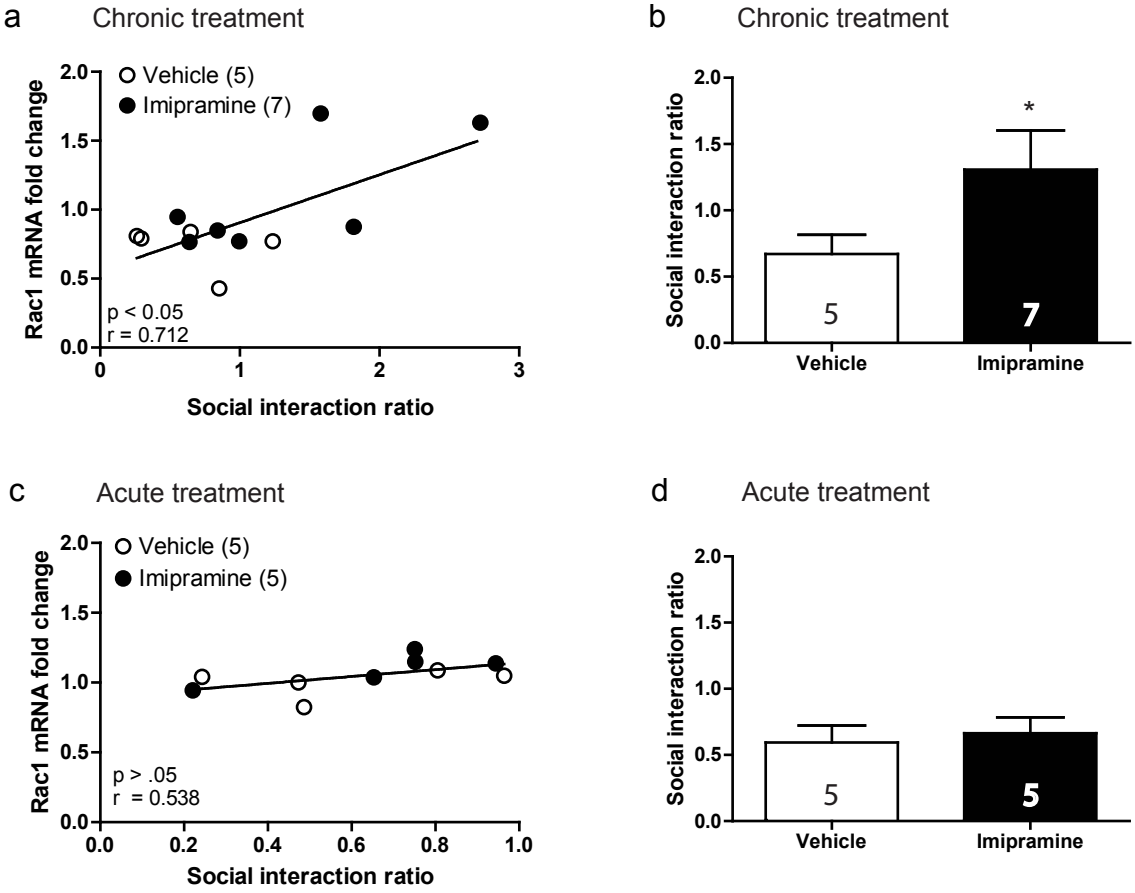
Supplemental Figure 1



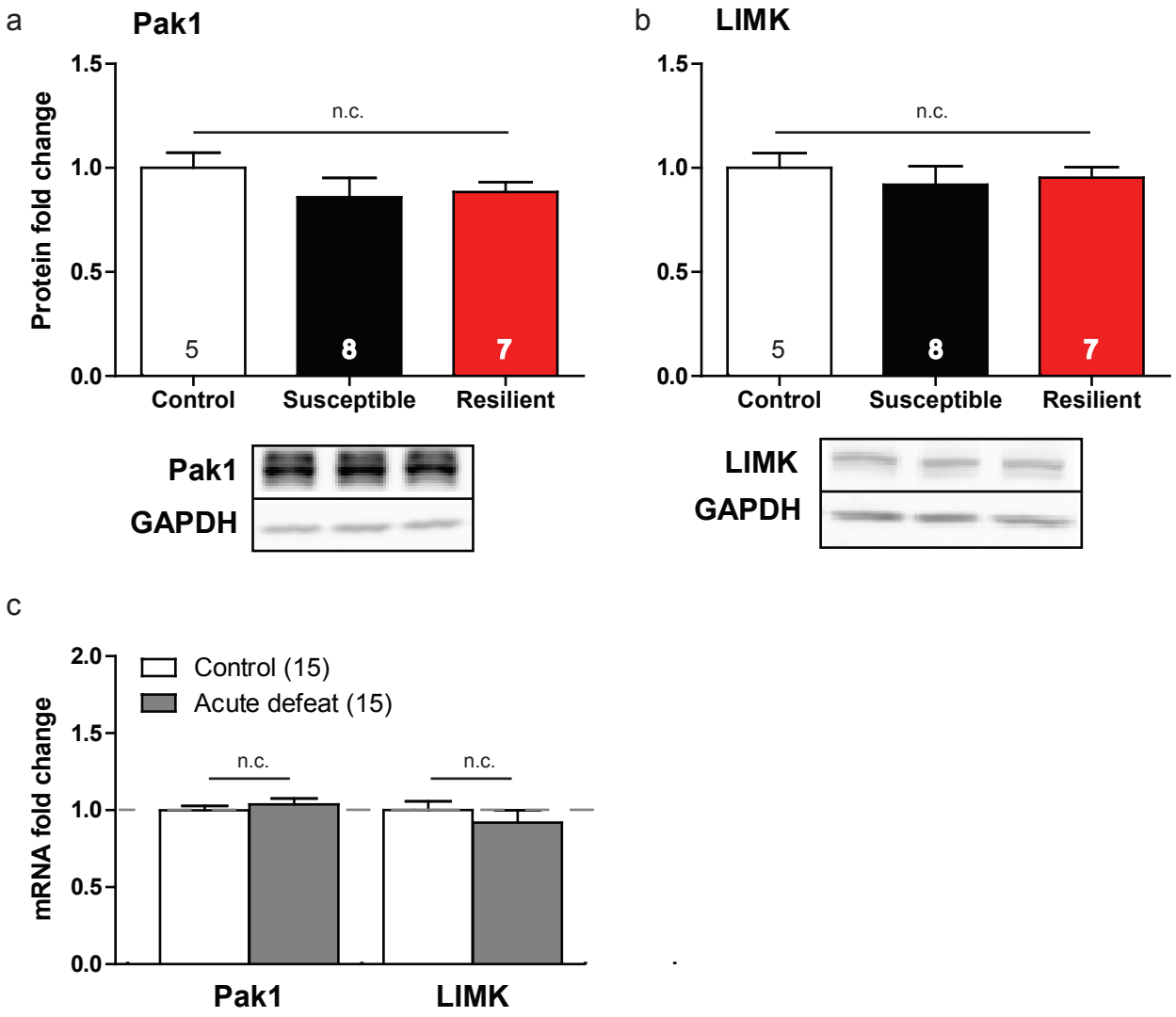
Supplemental Figure 1. Detailed behavioral data from small RhoGTPase mRNA time course and protein expression experiments. During social interaction testing, several parameters are automatically recorded for analysis, including total duration of time spent in the interaction zone **(a,e,i,j),** total time spent in the corner zones (**b,f,j,n**), total distance travelled (**d,h,l,p**), and social interaction ratio (**c,g,k,o**). Social avoidance behavioral characteristics are highly consistent across all experiments, such that susceptible mice spend less time in the interaction zone and more time in the corner zone compared to their control and resilient littermates, while still maintaining similar levels of overall locomotion. * or # is P <0.05 by one-way (**c,g,k,o**) and two-way (**a-b,d-f, h-j,l-m,p**) ANOVA. * designate between group statistics (ie, control vs. susceptible), and # designates within group statistics (ie, target absent vs. target present). All data presented as mean ± SEM.

Supplemental Figure 2



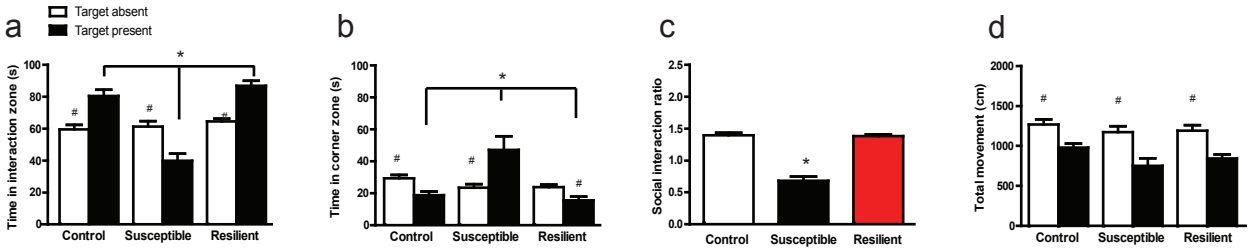
Supplemental Figure 2. Behavioral response of previously susceptible mice to acute and chronic imipramine treatments. (**b**) Chronic, but not (**d**) acute, impramine (20 mg kg^{-1}, i.p.) reverses social avoidance behavior in susceptible mice. This social avoidance behavior is correlated to Rac1 mRNA expression in mice that have received (**a**) chronic, but not (**c**) acute, impramine. All data presented as mean ± SEM, group size is indicated in legends and within graph bars. **P* <0.05 by Pearsons r (**a,c**) or students t–test (**b,d**).

Supplemental Figure 3



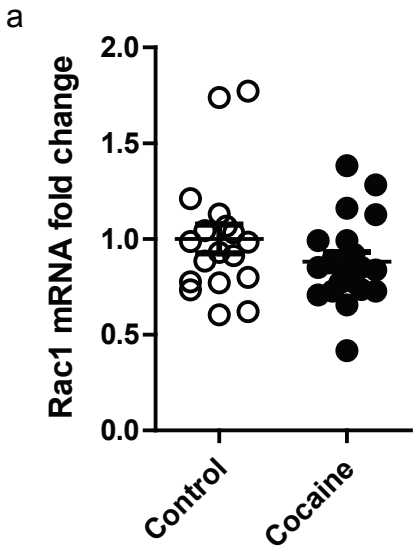
Supplemental Figure 3. Total Pak1 and LIMK protein levels in the mouse NAc 48–hr after chronic social defeat stress. Neither Pak1 (**a**) or LIMK (**b**) show stress–induced regulation of protein consistent with mRNA expression profile (see **Fig. 1a**). All data are normalized to GAPDH protein levels, group size is indicated within bars. Similarly, a single acute 10 minute social defeat (**c**) bout does not lead to alterations in either Pak1 or LIMK transcriptional activity in the NAc, group size indicated in legend. **P* <0.05 by one–way ANOVA (**a–b**) or Student's *t*–test (**c**).

Supplemental Figure 4



Supplemental Figure 4. Detailed behavioral data acquired from mice using site–directed quantitative chromatin–immunoprecipitation experiments. Susceptible mice spend less time in the interaction (**a**) and corner (**b**) zones than control and resilient mice. This results in susceptible mice having a decreased social interaction ratio (**c**) compared to control and resilient mice, although all groups maintain similar overall levels of locomotion (**d**). * or # is P <0.05 by one–way (**c**) and two–way (**a–b,d**) ANOVA. * designate between group statistics (ie, control vs. susceptible), and # designates within group statistics (ie, target absent vs. target present). All data presented as mean ± SEM, 10 mice per group.

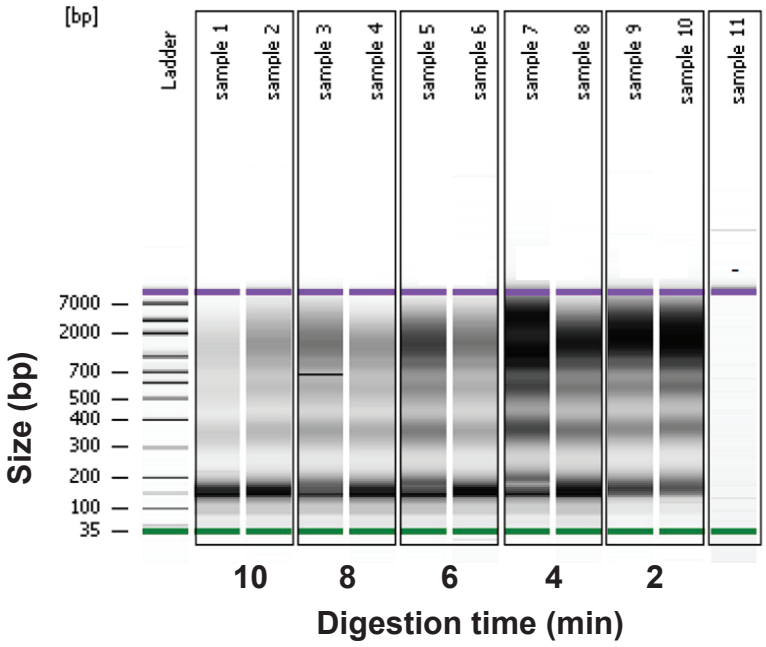
Supplemental Figure 5



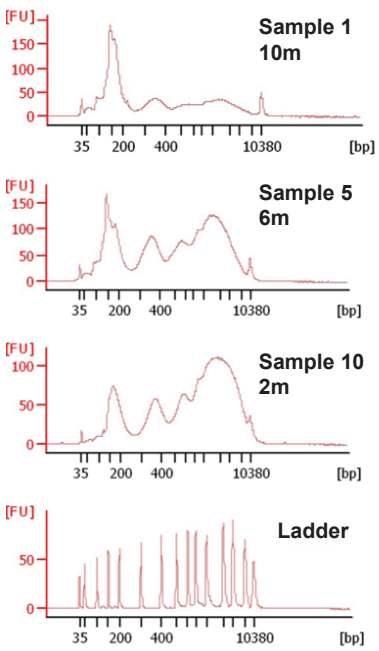
Supplemental Figure 5. Rac1 mRNA expression in postmortem human NAc from cocaine addicts. Data shows no change in Rac1 mRNA in cocaine addicts compared to controls. All data presented as mean ± SEM, group size of 15 per condition. *$P < 0.05$ by students t–test.

Supplemental Figure 6

a

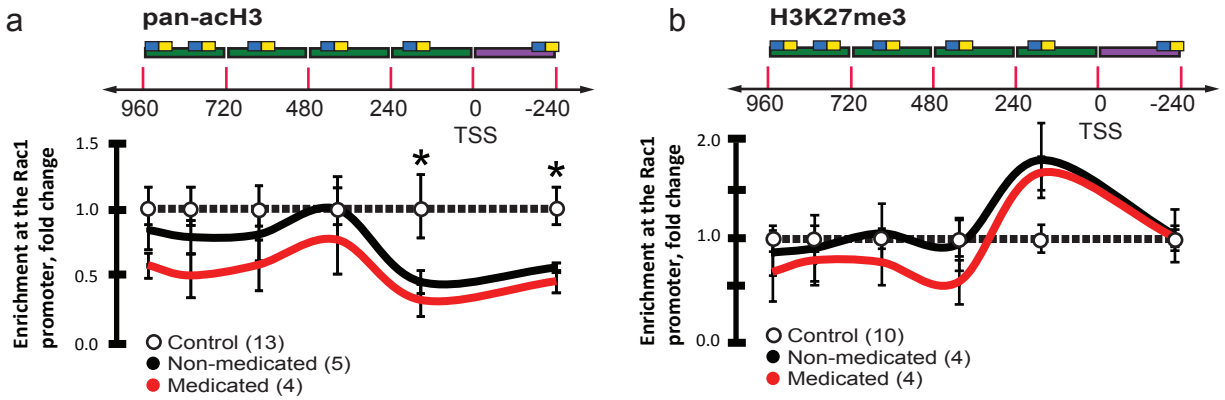


b



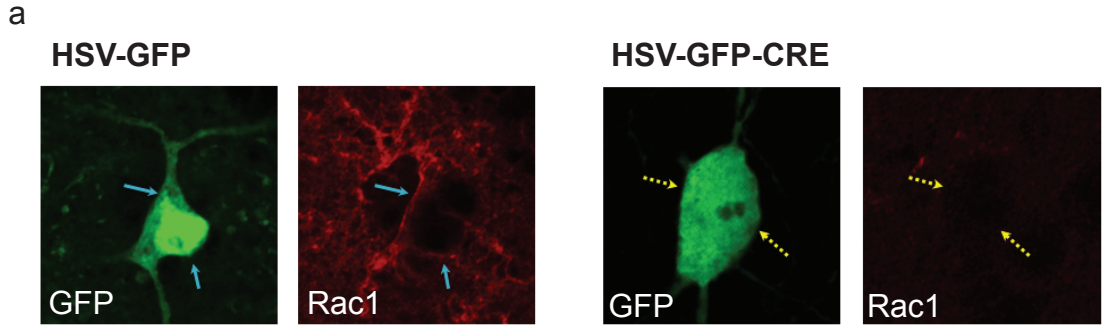
Supplemental Figure 6. Micrococcal nuclease (MNase) digestion of postmortem human NAc tissue results in finely delineated DNA fragment sizes. (**a**) We optimized the MNase digestion assay to ensure that DNA fragment sizes were ~150 to 170 basepairs in size, or a single mononucleosome. This digital gel shows how altering the duration of MNase digestion results in a range of DNA fragment lengths. (**b**) The optimal duration of MNase digestion was found to be 10 minutes (2 units/ml), shown as a plot comparing basepair fragment size and frequency within a sample.

Supplemental Figure 7



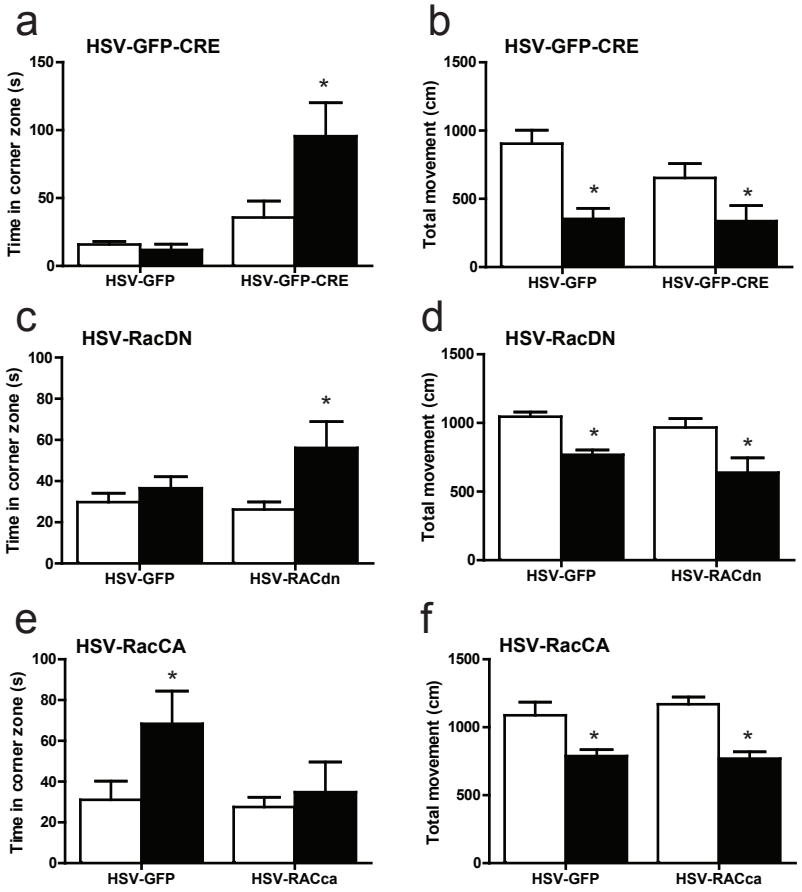
Supplemental Figure 7. Epigenetic regulation of Rac1 mRNA expression in MDD patients separated by antidepressant medication status. Although there is a significant effect of depression on Rac1 mRNA expression levels, there is no effect of having anti–depressant medication in the patients system at time of death, as determined by toxicology. In both cases there is no effect of medication status on permissive H3 acetylation (**a**) or repressive H3K27me3 (**b**) along the human *Rac1* promoter and upstream regions (group size indicated in legend). *$P < 0.05$, **$P < 0.01$ by one–way ANOVA (**a**,**b**).

Supplemental Figure 8



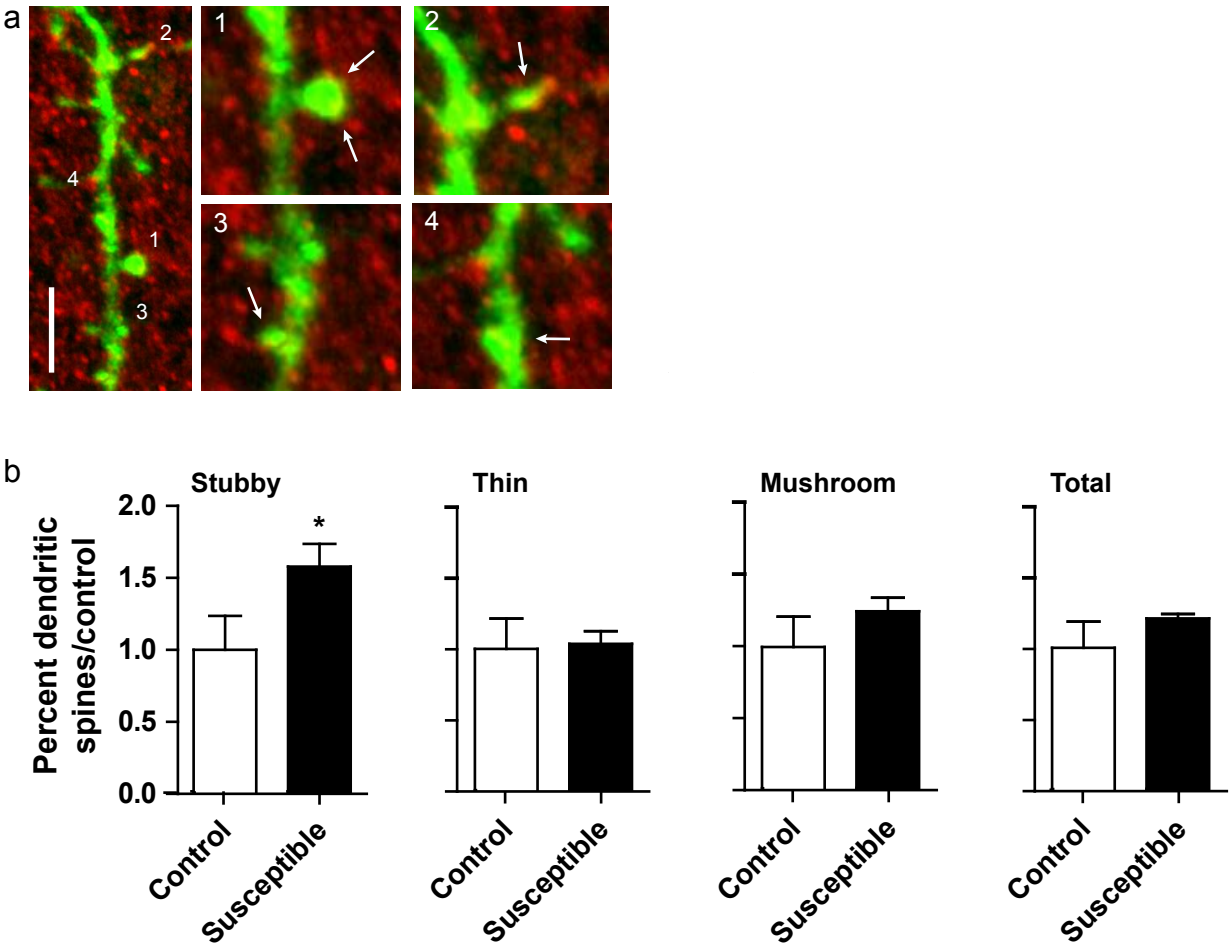
Supplemental Figure 8. Validation of a herpes–simplex virus (HSV) containing Cre in NAc of *fl/fl Rac1* mice. (**a**) Overexpression of a control HSV–GFP virus in the NAc of *f/f/ Rac1* mice does not alter somal Rac1 expression, while (**b**) overexpression of a HSV–GFP–CRE expressing virus lowers Rac1 levels as determined by immunohistochemistry. The blue arrows indicate Rac1 staining in the somatic plasma membrane of a GFP–infected MSN. Yellow arrows indicate the absence of Rac 1 staining of a HSV–GFP–Cre infected MSN.

Supplemental Figure 9



Supplemental Figure 9. Regulation of Rac1 by viral–mediated gene transfer and gene knockout modulates stress–related behavior in mice. (**a,b**) Removal of *Rac1* via infection of a Cre expressing HSV in the NAc of fl/fl *Rac1* mice promotes susceptibility to depression–like behavior as measured by increased corner time, and decreased total movement when a target is present. (**c,d**). (**e,f**) Viral–mediated gene transfer of RacCA into the NAc of susceptible mice is sufficient to reverse social avoidance behavior after chronic social defeat as measured by decreased time spent in the corners. All data presented as mean ± SEM. $^{*}P < 0.05$ by two–way ANOVA (**a–f**).

Supplemental Figure 10



Supplemental Figure 10. Chronic social defeat stress modulates the spatial dynamics of cofilin localization in the synapse. (**a**) Representative 63x confocal z–stack of cofilin puncta co–localizing with GFP–infected MSN dendritic segments. Numbers draw attention to different spine types (1, mushroom; 2, thin; 3, stubby; 4, dendritic shaft). White arrows in insets represent cofilin and GFP co–localized puncta. Measurement bar is 5 μm. Within the IHC tissue used in **Figure 5**, we also performed dendritic spine counts using NeuronStudio to determine the frequency of dendritic spines by type on MSNs following chronic social defeat stress. As has been previously reported, (**b**) stress resulted in an induction in stubby dendritic spines relative to control animals. However, as this was performed in 40 μm sections, we could not trace all dendrites back to their respective soma. *P <0.05 by Student's t–test.

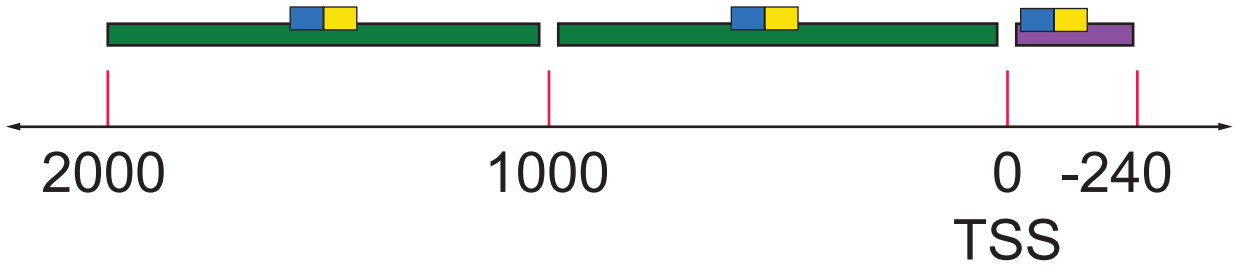
Supplemental Table 1. Mouse qPCR primer pairs

Gene	Forward Primer	Reverse Primer
Kal7	GCGAAGACCTTATCCAGCAG	GGGGTGTCTTGTTGTTGGAC
FARP2	GAACTGGAAAAGGCCACAAA	CTCAGCCAGACGGTAGTG
Tiam1	CAACTCCCTGGGTGACTTGT	ATTCCGGCAGTAGCTTGAGA
αPIX	ACGGAAAATGGAAGTCACCA	GCCTCCTTCTTCAACTCGTG
βPIX	CTCACTCCATCCAGCAAACA	CCAGCTGATGGTGGTGTGT
Vav1	AGCCCGCTACGACTTCTGT	AGCCTTGCTGTCCCTTCTTA
Vav3	GCCGAAGATGCAGGTCATTA	GGATATGCAATGGTGGTCCT
Rac1	GGTAGGTGATGGGAGTCAGC	CTGAAGTGCGACACCACTGT
Rac2	ACCATCGAGAAGCTGAAGGA	CAATATCCTTGGCCAGTGC
Rac3	CACACACACCCATCCTTCTG	TAGGTTATGGGTGCCAGCTT
Pak1	TTACTCCAACTCGGGAEGTG	GTTCCGGGTCAAAGCATCT
LIMK	ACCAGACCGTGGTAACTCCA	AGCAGAGGCTGGGATAGCAC
Cofilin	GTCAAGATGCTGCCAGACAA	TGAACACCAGGTCCTCCTTC
GAPDH	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG

Supplemental Table 2.
Rac1 mRNA expression and behavior in other brain regions

Brain Region		Rac1 mRNA fold change			fl/fl Rac1 Microdefeat Behavior					
					Time in interaction zone			Social interaction ratio		
		Susceptible	Resilient	p value	GFP	CRE	p value	GFP	CRE	p value
Nucleus accumbens	MEAN	0.65	0.91	**0.007**	100.50	17.33	**0.005**	1.17	0.22	**0.001**
	SEM	0.08	0.05		16.68	9.93		0.17	0.12	
		↓	↔			↓			↓	
Dorsal Striatum	MEAN	0.75	0.71	**0.003**	38.51	51.52	**0.836**	0.99	0.95	**0.951**
	SEM	0.06	0.03		21.54	13.49		0.73	0.23	
		↓	↓			↔			↔	
Prefrontal Cortex	MEAN	0.93	1.06	**0.691**	80.96	112.55	**0.376**	1.25	1.83	**0.216**
	SEM	0.14	0.08		25.77	12.89		0.36	0.22	
		↔	↔			↔			↔	

Supplemental Table 3. Mouse qChIP *Rac1* primer pairs and locations



Primer location (relative to TSS)	Forward	Reverse
~ -50 bp	GGAGGCCGGATGTGAGTG	AGCAGCCACCACCCAAAG
~ 500 bp	TCATACCGTCGTGAGGTTCA	GCGGAAGCAGTAGAATCCTG
~ 1500 bp	GTCTGGGAAATTCTCGTGGA	CCAGGAATTCCAGCCATCCTA

TSS, Transcription start site.

Supplemental Table 4. Complete demographics for Texas MDD cohort

Texas Demographics

Gender	Age	PMI	Cause of death
M	31	16	HTCVD
M	63	14	acute myocardial infarction
M	19	20	gunshot wound
M	48	15	mitral valve regurgitation
M	20	21	blunt force injury
M	60	20	surgical complication
M	43	15	HTCVD
M	60	11	HTCVD
M	63	12	acute myocardial infarction
M	34	23	ASCVD
M	48	20	ASCVD
M	54	11.4	HTCVD
M	77	13.4	pancreatic cancer
M	60	27	acute myocardial infarction

Gender	Age	PMI	Antidepressants	Cause of death
M	33	18	On	suicide
M	40	18	On	suicide
M	35	9	On	suicide
M	61	20	On	suicide
M	50	23	On	suicide
M	65	14	On	HTCVD
M	25	21	Off	suicide
M	42	17	Off	suicide
M	24	18	Off	suicide
M	18	22	Off	suicide
M	61	19	Off	suicide

HTCVD, hypertensive cardiovascular disease; ASCVD, atherosclerotic cardiovascular disease; PMI, post mortem interval

Supplemental Table 5. Complete demographics for Montreal MDD chart

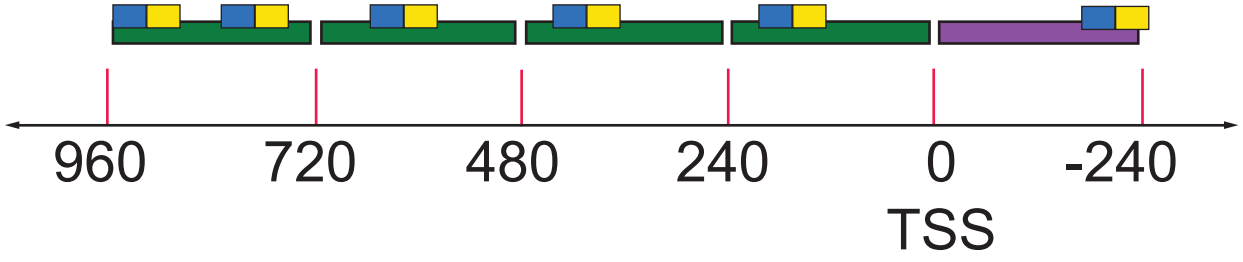
Montreal Demographics

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	52	24	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

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	53	14	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

Supplemental Table 6. Human qChIP *Rac1* primers and locations



Primer location (relative to TSS)	Forward	Reverse
~ -200 bp	CGCCGCTTCCTATCTCAG	CACCACCACACACTTGATGG
~ 170 bp	CATTCCCGAAGTCCAGAGAA	GCCGCTCCACTCACATCC
~ 400 bp	CTGCCTCAGCCTCCTGAGTA	GCCAACATGGTGAAACACTG
~ 615 bp	TCCCAAAGTGCTGGGTTTAC	AGCGAAACTCCGTCTCAAAA
~ 800 bp	AACCAAAGTGCTGGGATGAC	GGTTTCAGTGAGCCCAGATG
~ 915 bp	GCCTCCCAGGTTCAAGAGAT	TAGATTAGCAGGGCGTGGAG

TSS, transcription start site