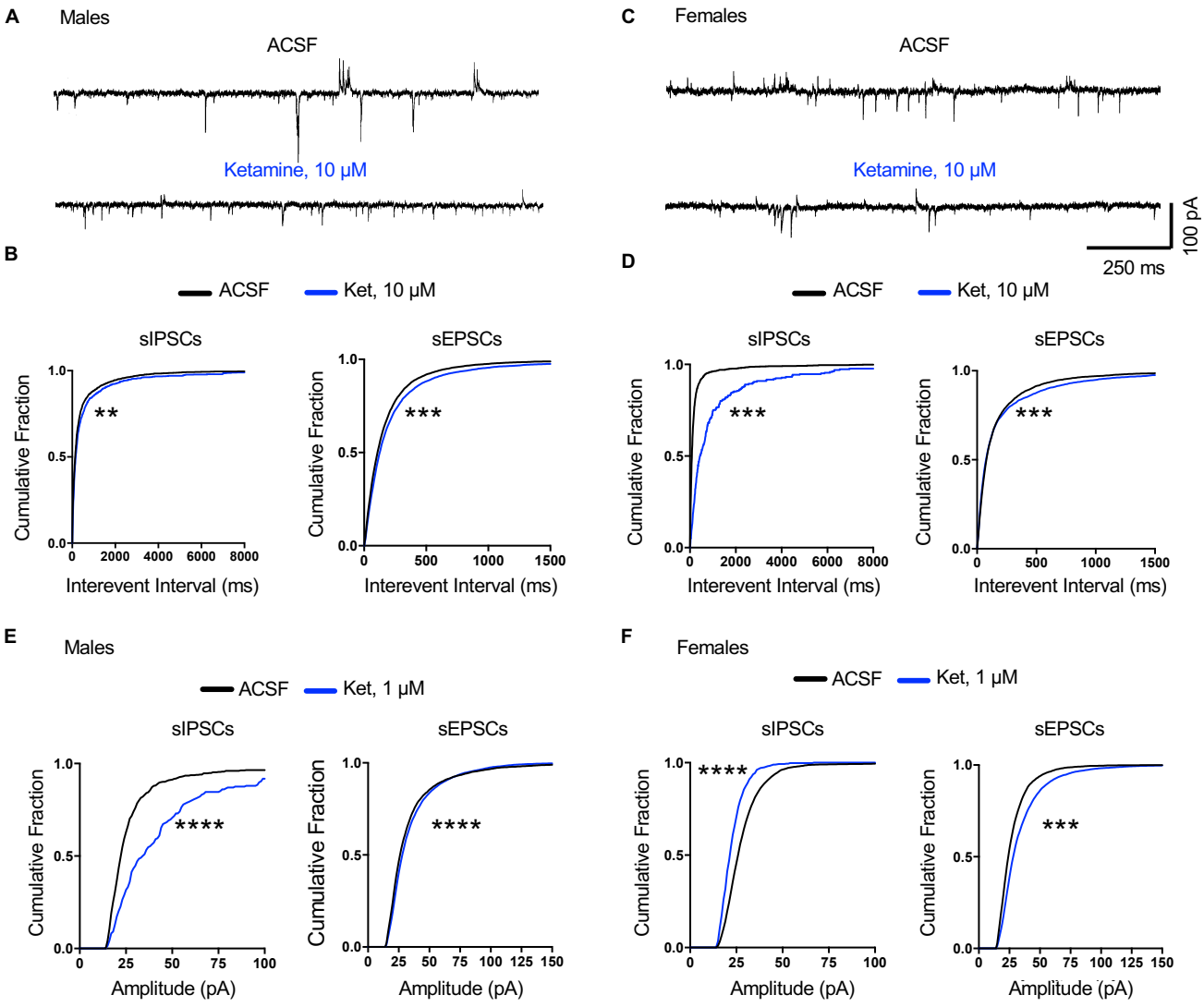
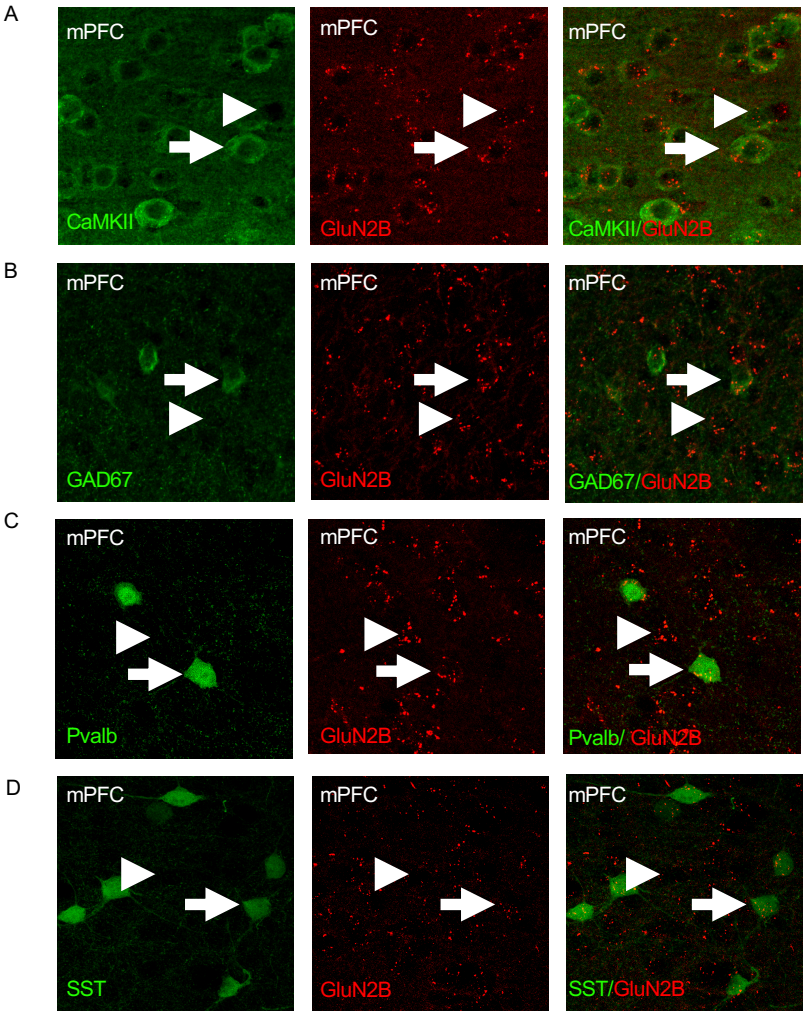
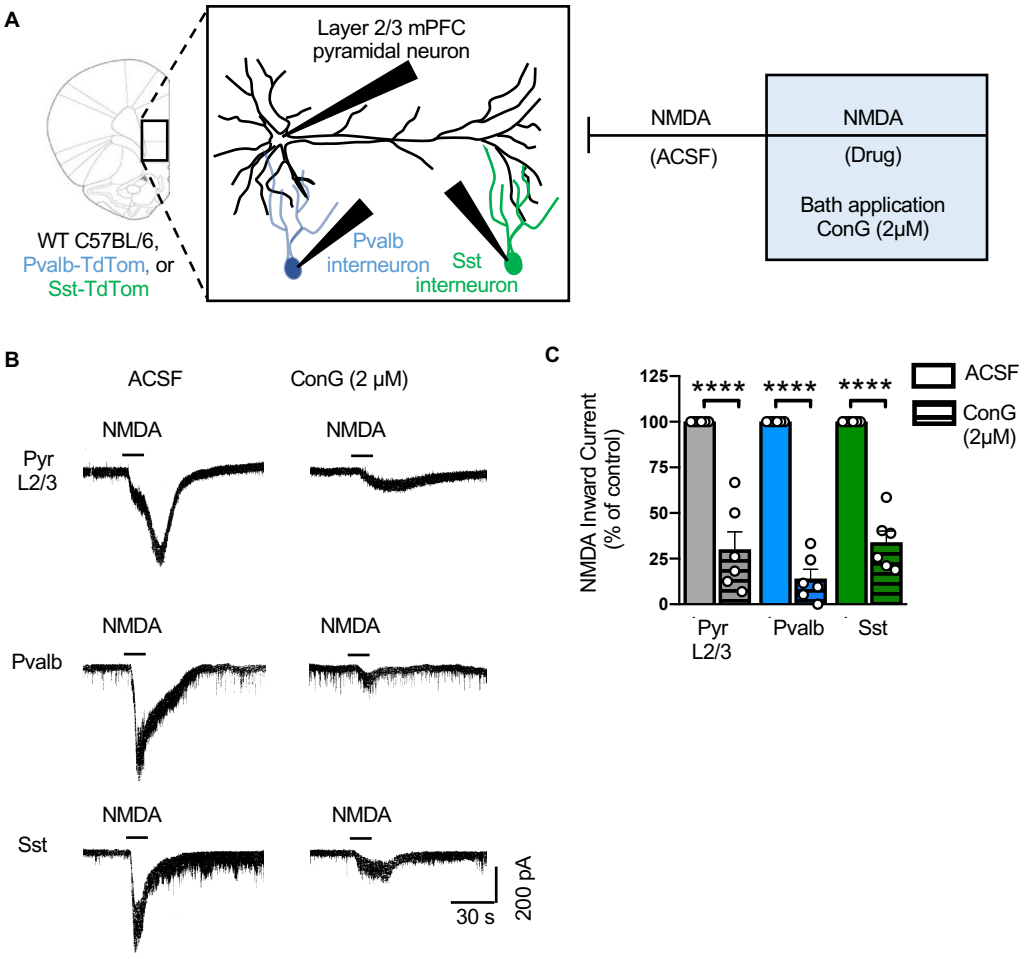
Supplemental Figure 1



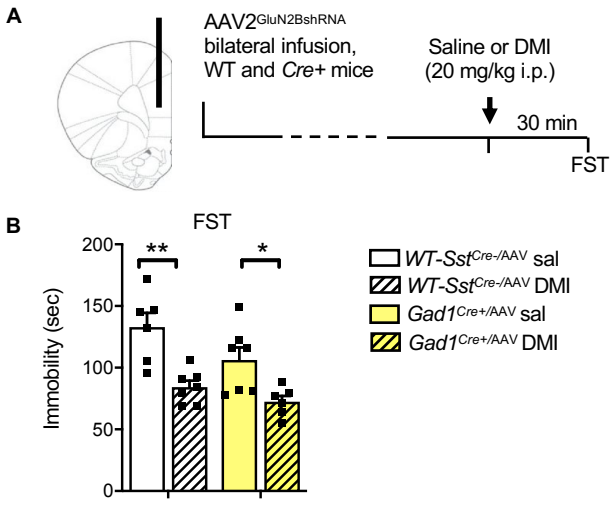
Supplemental Figure 1. 10 μM ketamine reduces spontaneous inhibitory postsynaptic currents in mPFC slices. (A,C) Representative traces of spontaneous inhibitory postsynaptic currents (sIPSCs) and spontaneous excitatory postsynaptic currents (sEPSCs) from layer V pyramidal neurons of (A) male and (C) female mice before and during application of 10 μM ketamine. (B) In male mice, 10 μM ketamine significantly decreases both sIPSCs and sEPSCs (n=9-10 cells from 15 mice). (d) In female mice, 10 μM ketamine significantly decreases both sIPSCs and sEPSCs (n=7-8 cells from 10 mice). (E) In male mice, 1 μM ketamine significantly increases both sIPSC and EPSC amplitude (n=9-10 cells, 10 mice). (F) In female mice, 1 μM ketamine significantly decreases sIPSC amplitude and increases sEPSC amplitude (n=7-8 cells, 5 mice). Data represented as the cumulative probability of (B,D) the interevent interval or (E-F) amplitude. Kolmogorov–Smirnov two-sample test was used. **, $P<0.01$, ***, $P<0.001$, ****, $P<0.0001$. Abbreviations: ACSF = artificial cerebrospinal fluid, Ket = ketamine.



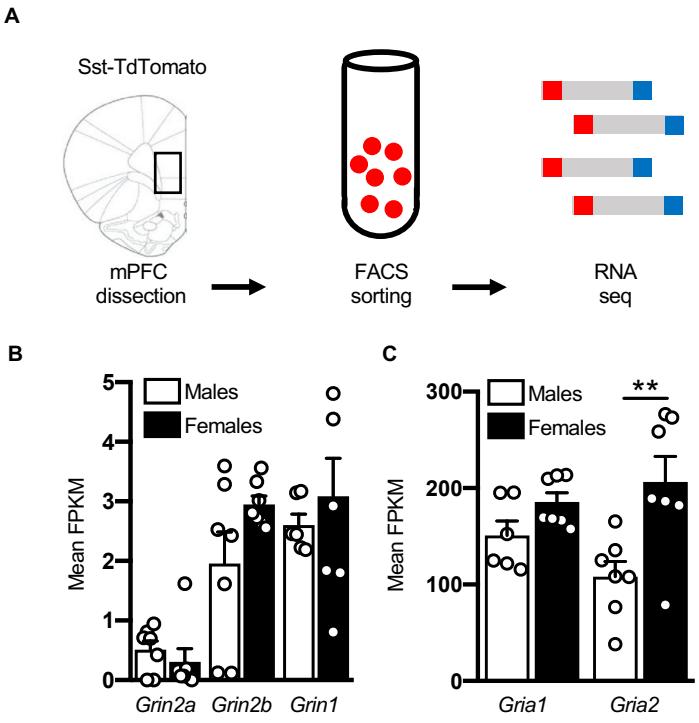
Supplemental Figure 2. GluN2B subunits colocalize with excitatory and inhibitory neurons in the mPFC. (A-D) Representative confocal images of immunofluorescent labeling in the mPFC are shown. GluN2B (red) co-localizes with markers of pyramidal neurons (CaMKII, A), GABAergic interneurons (Gad67, B), and two subtypes of interneurons, parvalbumin (Pvalb, C), and somatostatin (Sst, D). Magnified images (zoom 3), original magnification 40x. Arrows show neurons co-labeled for specific cell marker and GluN2B.



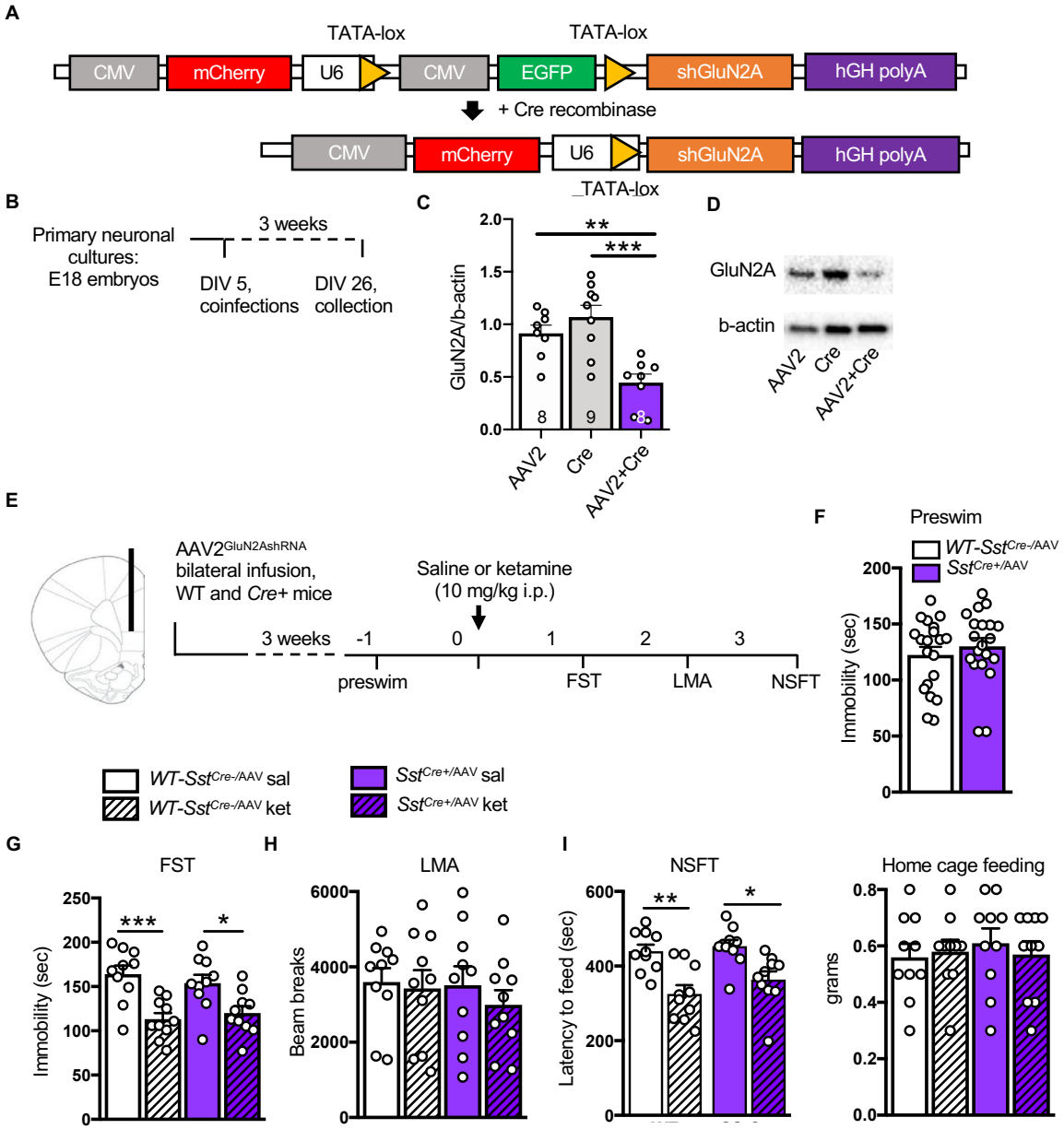
Supplemental Figure 3. GluN2B-selective antagonist blocks NMDA-induced inward currents in excitatory and inhibitory neurons in the mPFC. (A) Brain slice electrophysiology schematic. (B) Representative traces of NMDA-induced inward currents before and after 2 μM conantokin G (Con G). (C) Application of 2 μM Con G significantly reduces NMDA-induced inward currents in layer II/III pyramidal neurons (Pyr) and parvalbumin (*Pvalb*) and somatostatin (*Sst*) interneurons compared to basal ACSF recordings (n=6 cells per cell type, paired t tests (before and after ketamine) by cell type, Pyr: t_{10}=7.319, P<0.0001, Pvalb: t_{10}=16.88, P<0.0001, Sst: t_{10}=10.74, P<0.0001,****, P<0.0001). Data are represented as mean % of control ± SEM.



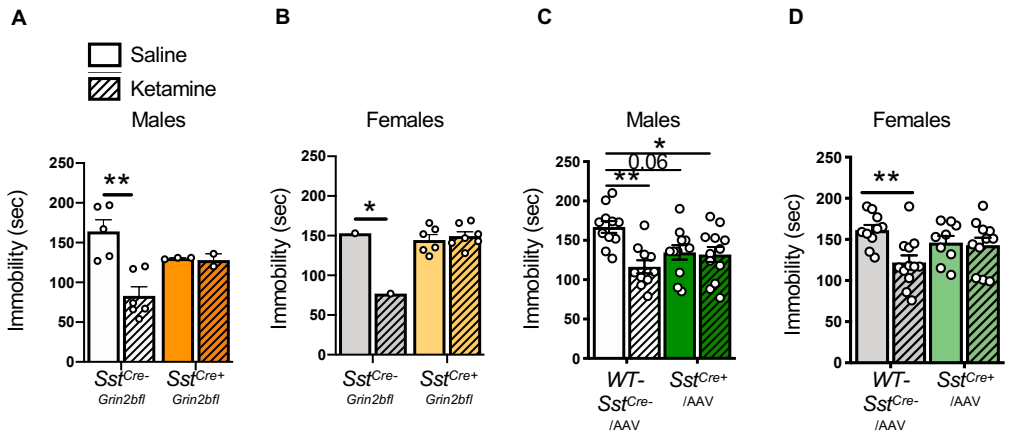
Supplemental Figure 4. Infusion of AAV2^{GluN2BshRNA} into *Gad1*$^{Cre+}$does not block the antidepressant-like effects of desipramine. (A) Procedure schedule. (B) Both *WT-Gad1*$^{Cre-}$ and *Gad1*$^{Cre+}$ male mice receiving desipramine (DMI) showed significantly reduced immobility in the forced swim test (FST) compared to saline controls (n=6-7 per group, two-way ANOVA with Tukey's multiple comparisons, genotype: : $F_{1,22}$=5.24, P=0.03, treatment: $F_{1,22}$=24.0, P<0.001, *, P<0.05,**, P<0.01).



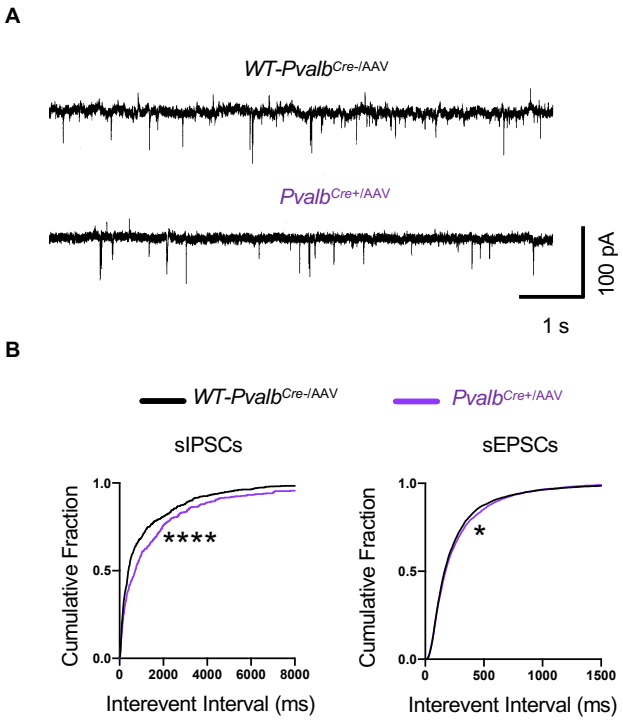
Supplemental Figure 5. *Sst*-interneurons in mPFC of both male and female mice express more *Grin2b* than *Grin2a* mRNA. (A) Procedure schematic. (B,C) RNA-Seq data obtained from FACS sorted Sst+ interneurons from mPFC of male or female Sst-tdTomato reporter mice revealed no significant sex differences in mean FPKM values for *Grin2a, Grin2b or Grin1* (B; n=6-7 per sex, gene: $F_{2,35}$=24.51, P<0.0001) but female mice showed significantly higher *Gria2* expression than males (C; n=6-7 per sex, sex: $F_{1,23}$=13.56, P= 0.0012). Data are represented as mean ± SEM. Two-way ANOVA with Tukey's multiple comparisons. **, P<0.01


A
TATA-lox
TATA-lox
CMV
mCherry
U6
CMV
EGFP
shGluN2A
hGH polyA
+ Cre recombinase
CMV
mCherry
U6
shGluN2A
hGH polyA
TATA-lox
B
Primary neuronal cultures: E18 embryos
3 weeks
DIV 5, coinfections
DIV 26, collection
C
GluN2A/b-actin
AAV2
Cre
AAV2+Cre
D
GluN2A
b-actin
AAV2
Cre
AAV2+Cre
E
AAV2GluN2AshRNA bilateral infusion, WT and Cre+ mice
Saline or ketamine (10 mg/kg i.p.)
3 weeks
preswim
FST
LMA
NSFT
WT-SstCre-/AAV sal
WT-SstCre-/AAV ket
SstCre+/AAV sal
SstCre+/AAV ket
F
Preswim
WT-SstCre-/AAV
SstCre+/AAV
Immobility (sec)
G
FST
Immobility (sec)
H
LMA
Beam breaks
I
NSFT
Latency to feed (sec)
Home cage feeding
grams

Supplemental Figure 6. Infusion of AAV2^{GluN2AshRNA} into *Sst*$^{Cre+}$ male mice does not block the antidepressant-like effects of ketamine. (A) Schematic of the pGluN2AshRNA sMACK-down construct before and after introduction of Cre-recombinase to generate the active construct. (B) Procedure schedule. (B) There was a significant reduction in GluN2A protein in the AAV2^{GluN2AshRNA}+AAV1-Cre group (AAV2+Cre) compared to AAV2^{GluN2AshRNA} (AAV2) or AAV2-Cre alone (Cre) (n=8-9 per group, $F_{2,22}$=11.88, P=0.0003). (D) Representative images of Western blots. Lanes were run on the same gel and were contiguous. (E) Schematic of surgical and behavioral schedule. (F) GluN2A knockdown in *Sst*+ neurons in male mice had no effect on baseline immobility (preswim; n=20 per group). (G) Both *WT-Sst*$^{Cre-}$-ket and *Sst*$^{Cre+}$-ket mice showed significantly reduced immobility in the forced swim test (FST) compared to controls (n=10 per group, treatment: $F_{1,36}$=24.7, P<0.001). (H) No significant differences were found in number of beam breaks in the locomotor activity assay (n=10 per group,). (I) Both *WT-Sst*$^{Cre-}$ and *Sst*$^{Cre+}$ mice receiving ketamine showed significantly reduced latency to feed in the novelty-suppressed feeding test (NSFT) compared to controls (n=10 per group, treatment: $F_{1,36}$=26.2, P<0.001). No significant differences were observed in home cage feeding (n=10 per group). Data are represented as mean ± SEM. Western: one-way ANOVA with Tukey's multiple comparisons. Preswim : unpaired two-tailed t test. FST, NSFT and LMA: two-way ANOVA with or without Tukey's multiple comparisons. *, P<0.05, **, P<0.01***, P<0.001. Abbreviations: sal = saline, ket = ketamine.



Supplemental Figure 7. Genetic deletion of *Grin2b* from *Sst*+ interneurons: behavioral data by sex. (A) In males, only control $Sst^{Cre\text{-}Grin2bfl}$-ketamine mice showed a significant reduction in time spent immobile compared to control (n=2-6 per group, genotype: $F_{1,12}$=0.156, P=0.70, treatment: $F_{1,12}$=8.12, P<0.01, genotype x treatment: $F_{1,12}$=7.23, P<0.01). (B) In females, only control $Sst^{Cre\text{-}Grin2bfl}$-ketamine mice showed a significant reduction in time spent immobile when compared to their control (n=1-7 per group, genotype: $F_{1,11}$=7.46 P<0.05, treatment: $F_{1,11}$=9.22, P<0.05, genotype x treatment: $F_{1,11}$=12.0, P<0.01). (C,D) For comparison, $Sst^{Cre/AAV}$ (C) male and (D) female FST data is reshown. Only male and female *WT- $Sst^{Cre\text{-}/AAV}$*-ketamine mice showed significantly reduced immobility in the forced swim test (FST) compared to their controls (n=10-12 (C) and 9-12 (D) per group, males: treatment: $F_{1,40}$=9.248, P=0.0041, genotype x treatment: $F_{1,40}$=7.453, P=0.0094, females: treatment: $F_{1,38}$=6.567, P=0.0145, genotype x treatment: $F_{1,38}$=4.744, P=0.0357). Data are represented as mean ± SEM. Two-way ANOVAs with Tukey's multiple comparisons. *, P<0.05, **, P<0.01.



Supplemental Figure 8. Infusion of AAV2^{GluN2BshRNA} into mPFC of *Pvalb*$^{Cre+}$ male mice decreases mPFC layer V spontaneous postsynaptic currents. (A) Representative traces of spontaneous inhibitory postsynaptic currents (sIPSCs) and spontaneous excitatory postsynaptic currents (sEPSCs) from layer V pyramidal neurons of WT-*Pvalb*$^{Cre-}$ and *Pvalb*$^{Cre+}$ male mice. (B) *Pvalb*$^{Cre+}$ mice show a significant decrease in sIPSCs and a smaller, but significant, decrease in sEPSCs (n=17-22 cells from 3 mice, Kolmogorov–Smirnov two-sample test ,*, P<0.05, ****, P<0.0001). Data represented as the cumulative probability of the interevent interval.