Supplementary Information

Supplementary Table 1. **Alcohol consumption in g/kg during the first 4 hrs and over 24 hrs.** IA 20% - Mice underwent an intermittent access to 20% (IA 20%) 2-BC procedure alcohol for a total of 21 sessions of 24-hrs over a total of 49 days (e.g. 21 sessions) and alcohol and water intake were recorded immediately after the first 4 hrs of access (to assess binge-like drinking) and at the end of the 24 hrs of access. CA 10% - Mice had continuous access to one bottle of 10% alcohol (v/v) and one bottle of water for 21 consecutive days. Alcohol intake was recorded at the end of the final 24 hrs drinking session. n=10 per group.

Supplementary Figure 1. Timeline of experiments. (A) Timeline of the molecular experiments (Figures 1 and 2 and Supplementary Figures 2 and 3A,B). C57BL/6 mice underwent 21 sessions (7 weeks) of IA 20% using a two-bottle choice drinking paradigm; one group of animals was sacrificed after 4 hrs of alcohol intake (binge drinking) or after 24 hrs of abstinence. Another group of mice underwent 21 sessions of continuous access to 10% alcohol. Mice drinking water only were used as a control. (B) Overexpression of miR-30a-5p in the mPFC (Figure 4 and Supplementary Figure 5). AdV-miR-30a-5p or AdV-SCR was infused in the mPFC and five days later, the mPFC of one group of mice was dissected to analyze mRNA and miRNA expression, another group of mice was used to measure alcohol drinking (IA 20%), and a third group of mice was used to measure saccharin intake. (C). Timeline of the inhibition of the miR-30a-5p in the mPFC (Figure 5). LNA-miR-30a-5p or LNA-SCR was infused in the mPFC of mice with a history of 21 sessions (7 weeks) of IA 20%. One group was sacrificed after 2 hrs to analyze mRNA and miRNA expression and a second group was used to measure alcohol drinking during 3 intermittent sessions of 24 hrs. After one week of abstinence, saccharin

drinking was measured for 24 hrs on the same group for 6 sessions before testing infusion of LNA-miR-30a-5p or LNA-SCR.

Supplementary Figure 2. Excessive alcohol intake reduces *BDNF* mRNA expression in the mPFC. Mice underwent IA to 20% alcohol for a total of 21 drinking sessions or water only. Four hrs after the beginning of the last alcohol drinking session, the mPFC was removed and *BDNF* mRNAs were analyzed by qPCR. (**A**) Data are expressed as mean ratio of *BDNF*/*GAPDH* and plotted as percentage of control ± SEM, ***P<0.001, (two-tailed unpaired t-test; $t_{(16)}$=5.07, P<0.001). (**B**) Correlation plot between *BDNF* mRNA expression in the mPFC (*BDNF*/*GAPDH*) and alcohol intake during the 4 hrs binge session. Correlations were analyzed by linear regression and R^2 value was calculated (R^2=0.70, P=0.01). n=8-10.

Supplementary Figure 3. Alcohol drinking does not alter *BDNF* mRNA and miRNA expression in the hippocampus. (**A-D**) Mice underwent IA to 20% alcohol procedure for a total of 21 drinking sessions while a second group consumed water only. The hippocampus was collected 4 hrs after the beginning of the last alcohol drinking session (**A** and **C,** Binge) or 24 hrs after the end of the last alcohol drinking session (**B** and **D,** Abstinence). (**E-F**) The hippocampus was collected immediately at the end of 21 days of a CA to alcohol 10% or water only (6 hrs after the beginning of the dark cycle, i.e. at the same time of the day as dissection after binge). (**A, B** and **E**) *BDNF* mRNA was measured by RT-PCR. mRNA data are expressed as a mean ratio of *BDNF*/*GAPDH* and plotted as percentage of control ± SEM. (**C, D** and **F**) miRNAs expressions were measured by qPCR. miRNA data are expressed as mean ± SEM fold change ($2^{-\Delta\Delta Ct}$), normalized to control U6 small nuclear RNA (snRNA) levels. (**A**) *BDNF* ($t_{(6)}$=-0.15,

P=0.88) (**B**) *BDNF* ($t_{(10)}$=0.94, *P*=0.37) (**C**) miR-30a-5p ($t_{(6)}$=0.53, *P*=0.61), miR-195 ($t_{(6)}$=0.30, *P*=0.77), miR-1 ($t_{(6)}$=0.02, *P*=0.98), miR-124($t_{(6)}$=1.32, *P*=0.23), 5srRNA ($t_{(6)}$=1.94, *P*=0.08), Internal Control ($t_{(6)}$=0.71, *P*=0.50) (**D**) miR-30a-5p ($t_{(11)}$=1.12, *P*=0.28), miR-195 ($t_{(11)}$=1.28, *P*=0.23), miR-1 ($t_{(11)}$=1.37, *P*=0.19), miR-124 ($t_{(11)}$=1.59, *P*=0.14), 5srRNA ($t_{(11)}$=0.60, *P*=0.56) and Internal Control ($t_{(11)}$=0.71, *P*=0.50) (**E**) *BDNF* ($t_{(5)}$=-0.48, *P*=0.32) (**F**) miR-30a-5p ($t_{(5)}$=0.12, *P*=0.91), miR-195 ($t_{(5)}$=-0.36, *P*=0.73), miR-1 $t_{(5)}$=-0.52, *P*=0.63), miR-124 ($t_{(5)}$=-0.116, *P*=0.91), 5srRNA, ($t_{(5)}$=0.20, *P*=0.85) and Internal Control ($t_{(5)}$=0.006, *P*=0.49). (**A** and **C**) n=4, (**B** and **D**) n=5-7, (**E-F**) n=3-4.

Supplementary Figure 4. miR-30a-5p binding site is localized within the 3' UTR of the BDNF gene. HEK 293FT cells were transfected with pUSE-MBP-Flag-GFP-Wt-3'UTR(BDNF) and pRNAT-H1.1-SCR (1), pUSE-MBP-Flag-GFP-Wt-3'UTR(BDNF) and pRNAT-H1.1-miR-30a-5p (2), pUSE-MBP-Flag-GFP-Mut-3'UTR(BDNF) and pRNAT-H1.1-SCR (3), pUSE-MBP-Flag-GFP-Mut-3'UTR(BDNF) and pRNAT-H1.1-miR-30a-5p (4), pUSE-MBP-Flag-GFP-Wt-3'UTR(BDNF) (5) and pUSE-MBP-Flag-GFP-Mut-3'UTR(BDNF) (6). Cells were harvested 24 hrs after transfection and western blot analysis was used for detection. Anti-Flag antibodies were used to detect the levels of Wt-3'UTR(BDNF) and Mut-3'UTR(BDNF), and anti-actin antibodies were used as a loading control. Data are expressed as a mean ratio of Flag/Actin and plotted as percentage of control (Wt-3'UTR(BDNF) and SCR) ± SEM. Two-way RM-ANOVA revealed a significant interaction between Wt-3'UTR(BDNF) or Mut-3'UTR(BDNF) and SCR or miR-30a-5p ($F_{(1,20)}$=19.65, *P*<0.001). SNK *post hoc* test: ***P<0.001. n = 6.

Supplementary Figure 5. Adenoviral mediated overexpression of miR-30a-5p in the mPFC is specific to neurons and reduces *BDNF* expression. Mice were infused with AdV-miR-30a-5p or AdV-SCR ($1x10^8$ ifu/ml) and brain sections were harvested 5 days later. (**A**) Coronal sections containing the mPFC stained for GFP (green), the neuronal marker NeuN (red) or the glial marker GFAP (red). (**B**) Image is a representative of data shown in Figure 4B.

Supplementary Figure 6. Schematic representation of the cannulae placement in the mPFC. The location of the cannulae tips are represented by black circles in the coronal sections. Distance relative to Bregma is indicated in mm.

References

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Supplementary Table 1

	IA 20% (in g/kg)	Continuous 10 % (in g/kg)
4 hrs	6 ± 0.3	3.35 ± 0.2
24 hrs	20 ± 0.8	7 ± 0.2

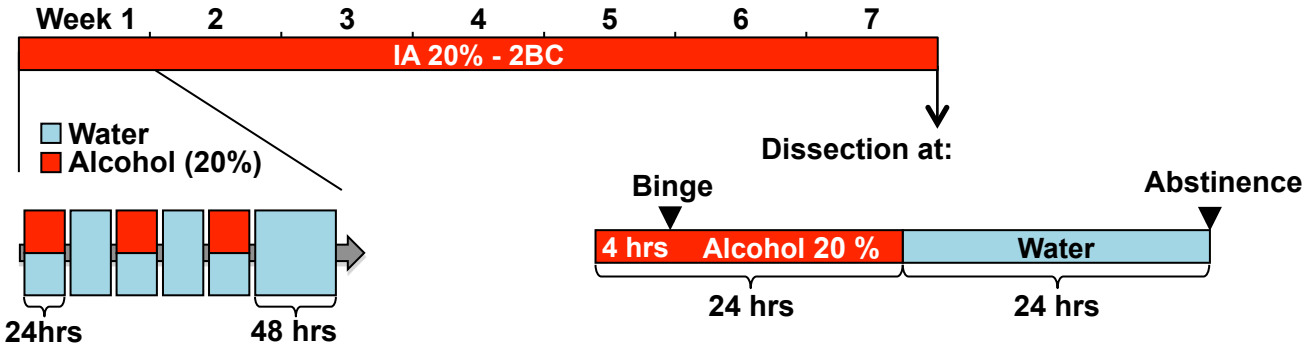
Supplementary Figure 1

Timeline of Experiments

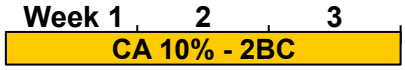
A

Timeline of molecular experiments

Intermittent access to 20% alcohol (IA 20%); 3 access sessions/week for 7 weeks = 21 sessions (Figures 1 and Supplementary Figures 2 and 3A-D)



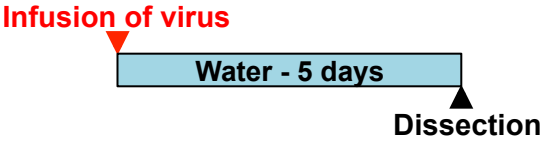
Continuous access to 10% alcohol; access is continuous for 3 weeks = 21 days of drinking (Figures 2 and Supplementary Figures 3E-F)



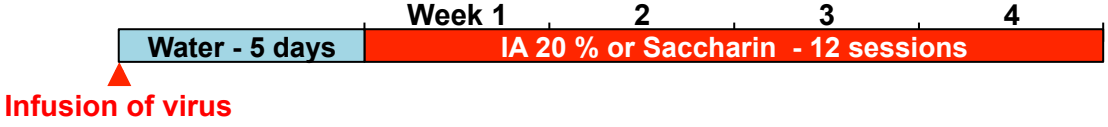
B

Overexpression of miR-30a-5p in the mPFC

Molecular (Figure 4A-B and Supplementary Figure 5)



Behavior(Figure 4C-H)



Supplementary Figure 1

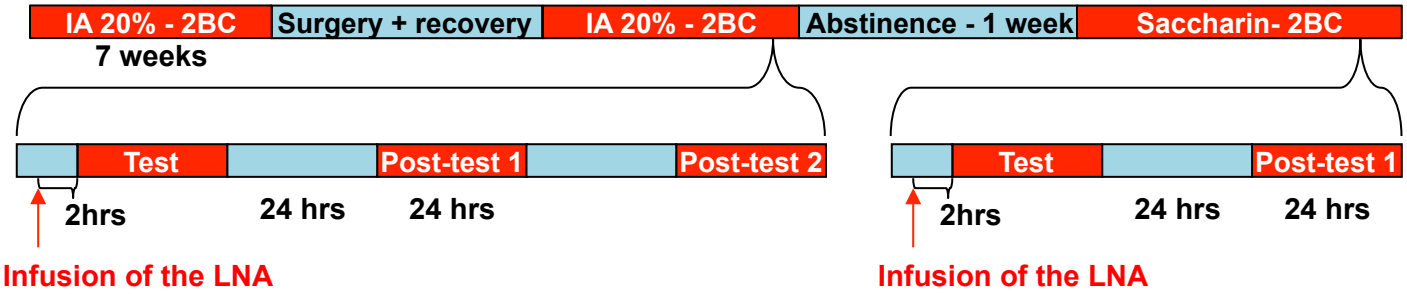
C

Inhibition of the miR-30a-5p in the mPFC

Intermittent access to 20% alcohol (IA 20%); 3 access sessions/week for 7 weeks = 21 sessions (Figure 5)

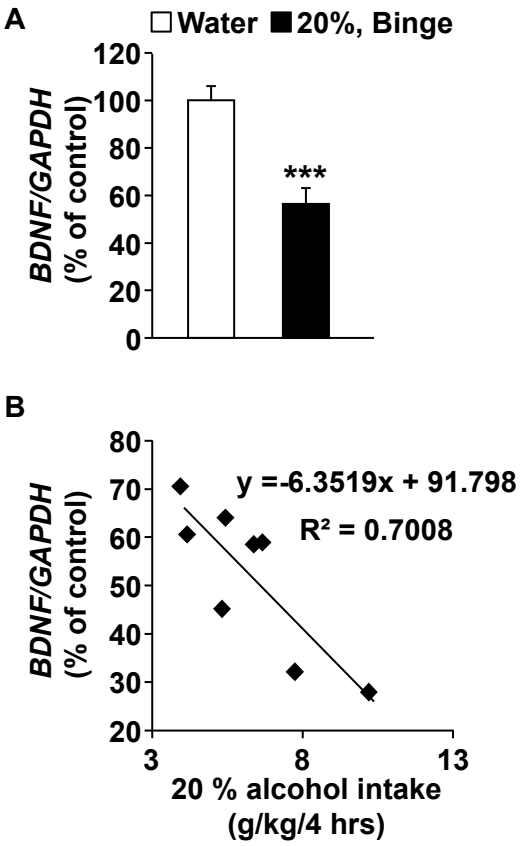
Molecular (Figure 5A-B)

Behavior (Figure 5C-H)



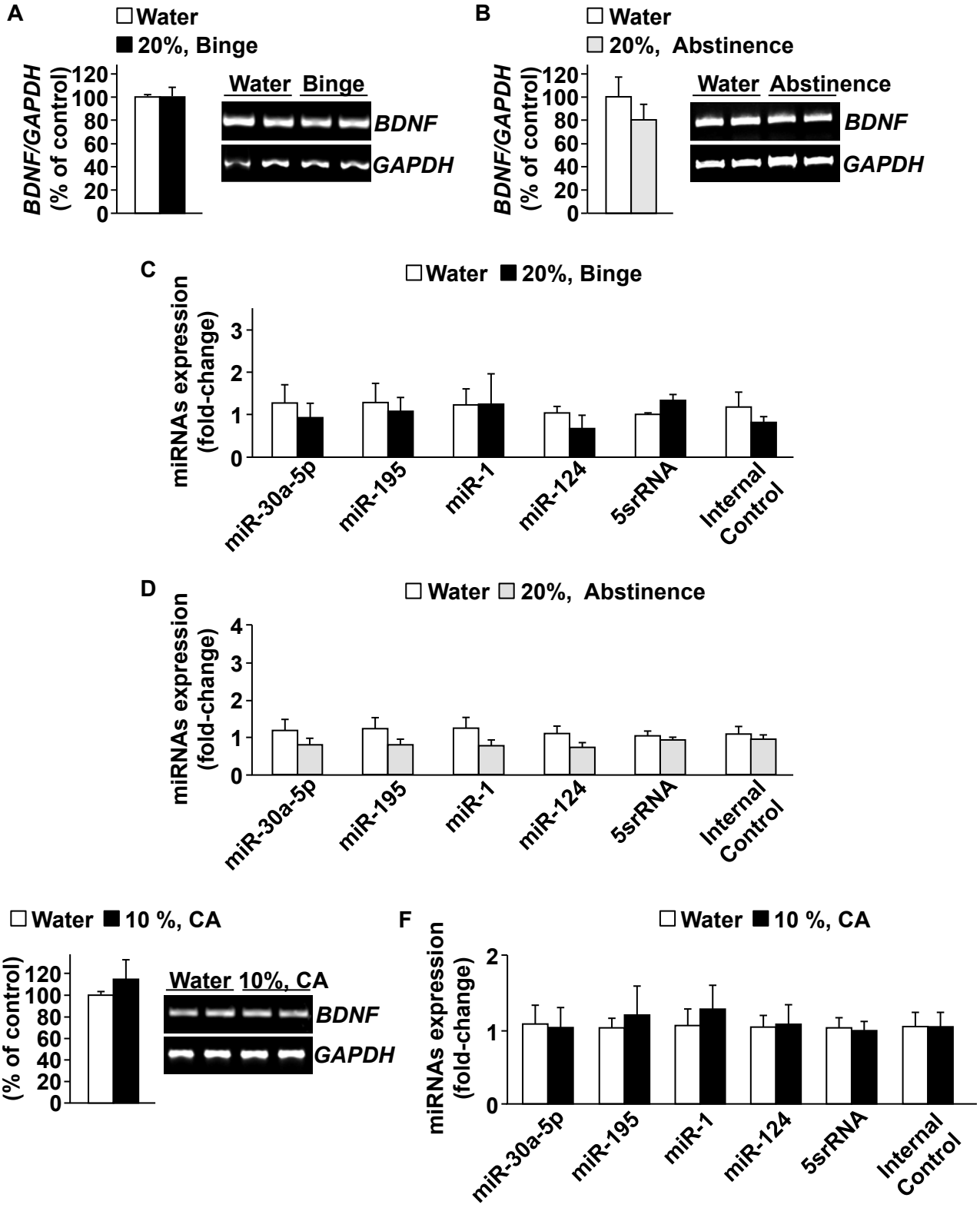
Supplementary Figure 2

Excessive alcohol intake reduces *BDNF* mRNA expression in the mPFC



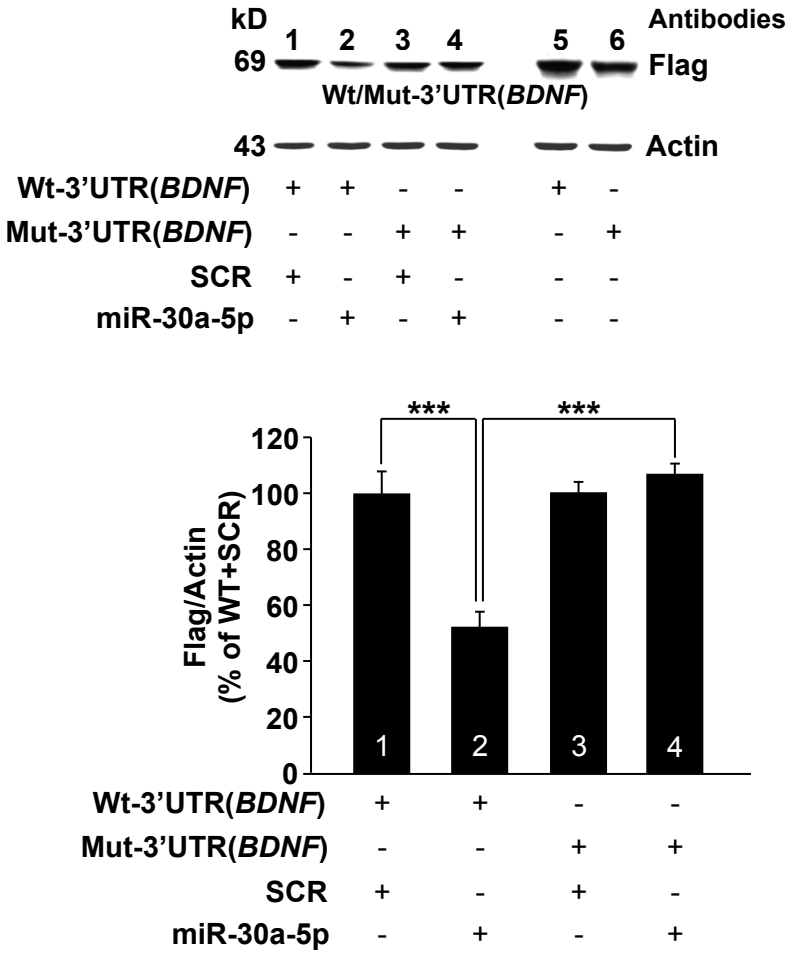
Supplementary Figure 3

Alcohol drinking does not alter *BDNF* expression and miRNA expression in the hippocampus



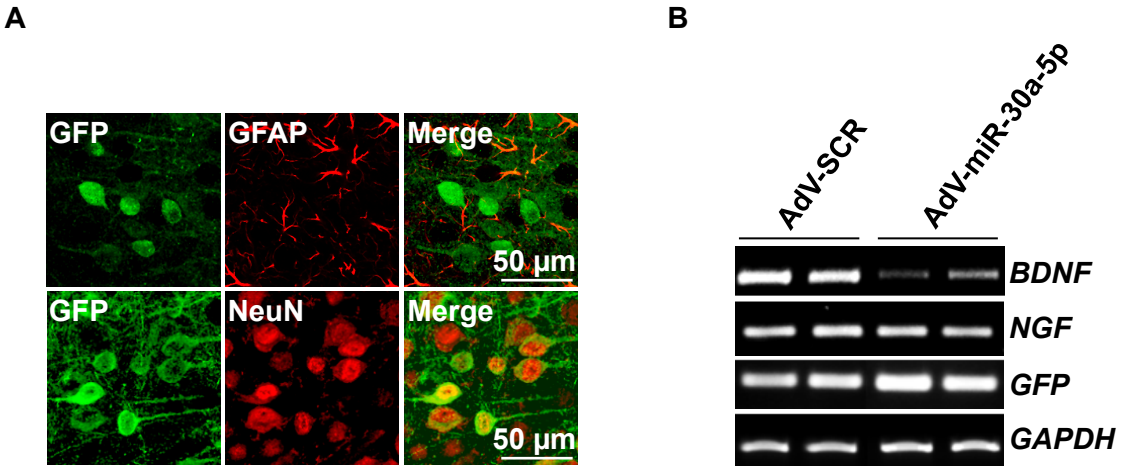
Supplementary Figure 4

miR30-5a binding site is localized within the 3' UTR of the *BDNF* gene



Supplementary Figure 5

Adenoviral mediated overexpression of miR30a-5p in the mPFC is specific to neurons and reduces *BDNF* expression.



Supplementary Figure 6

Schematic representation of cannulae placement in the mPFC