**Abstinence from escalation of cocaine intake changes the microRNA landscape in the cortico-accumbal pathway**

**(Supplementary Materials)**

**Figure S1.** Dissection of rat brain nucleus accumbens and prefrontal cortex.

**Figure S2.** Cocaine withdrawal/abstinence-associated miRNA-mRNA-pathway network predicted by IPA.

**Figure S3.** IPA miRNA Target Filter prediction of transcription factors (TFs) & nuclear receptors (NRs) potentially targeted by differentially expressed miRNAs in the IL-PFC of rats with protracted abstinence.

**Figure S4.** IPA miRNA Target Filter prediction of miRNA-mRNA-pathway regulatory networks containing miRNAs differentially expressed in the IL-PFC of rats with protracted abstinence.

**Figure S5.** IPA miRNA Target Filter prediction of miRNA-mRNA-pathway regulatory networks containing miRNAs differentially expressed in the PL-PFC of rats with either withdrawal or protracted abstinence.

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**Table S3d.** Correlation of Z Shock with expression levels of top differentially expressed miRNAs ($|FC| \geq 2.0$ & $P < 0.05$) in three rat brain regions by partial correlation analysis
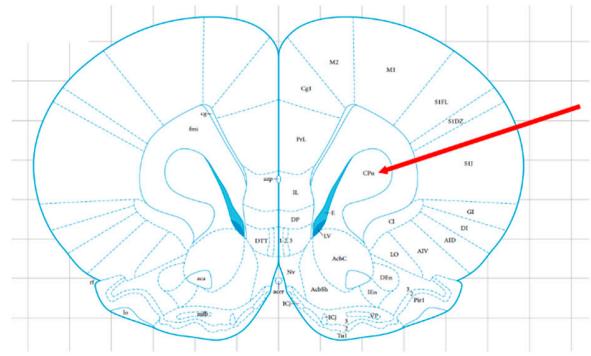
**Figure S1a:** Rat nucleus accumbens
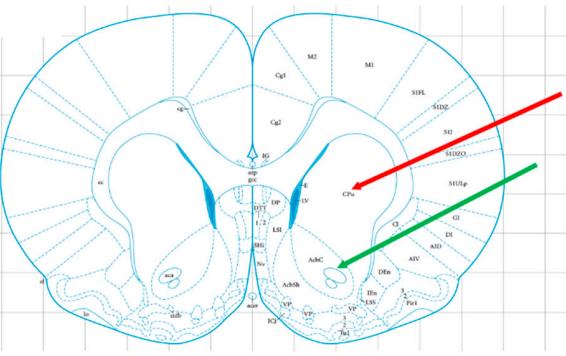


**Figure S1b:** Rat prefrontal cortex
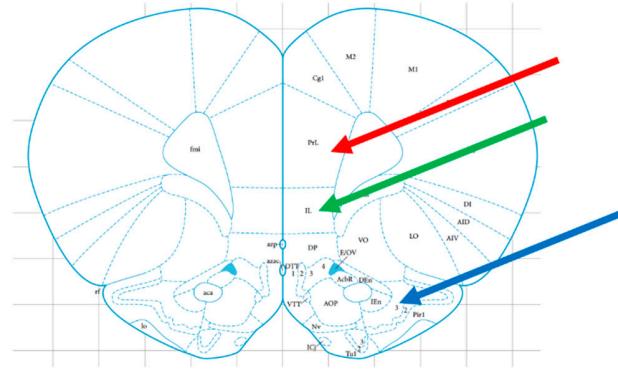
**Figure S1.** Dissection of rat brain nucleus accumbens and prefrontal cortex. Figure S1a: The red arrow indicates the striatum [or corpus striatum (CPu)] and the green arrow indicates the anterior commissure. Figure S1b: The red arrow indicates prelimbic prefrontal cortex; the green arrow indicates infralimbic prefrontal cortex; and the blue arrow indicates the recognizable ventral portion.
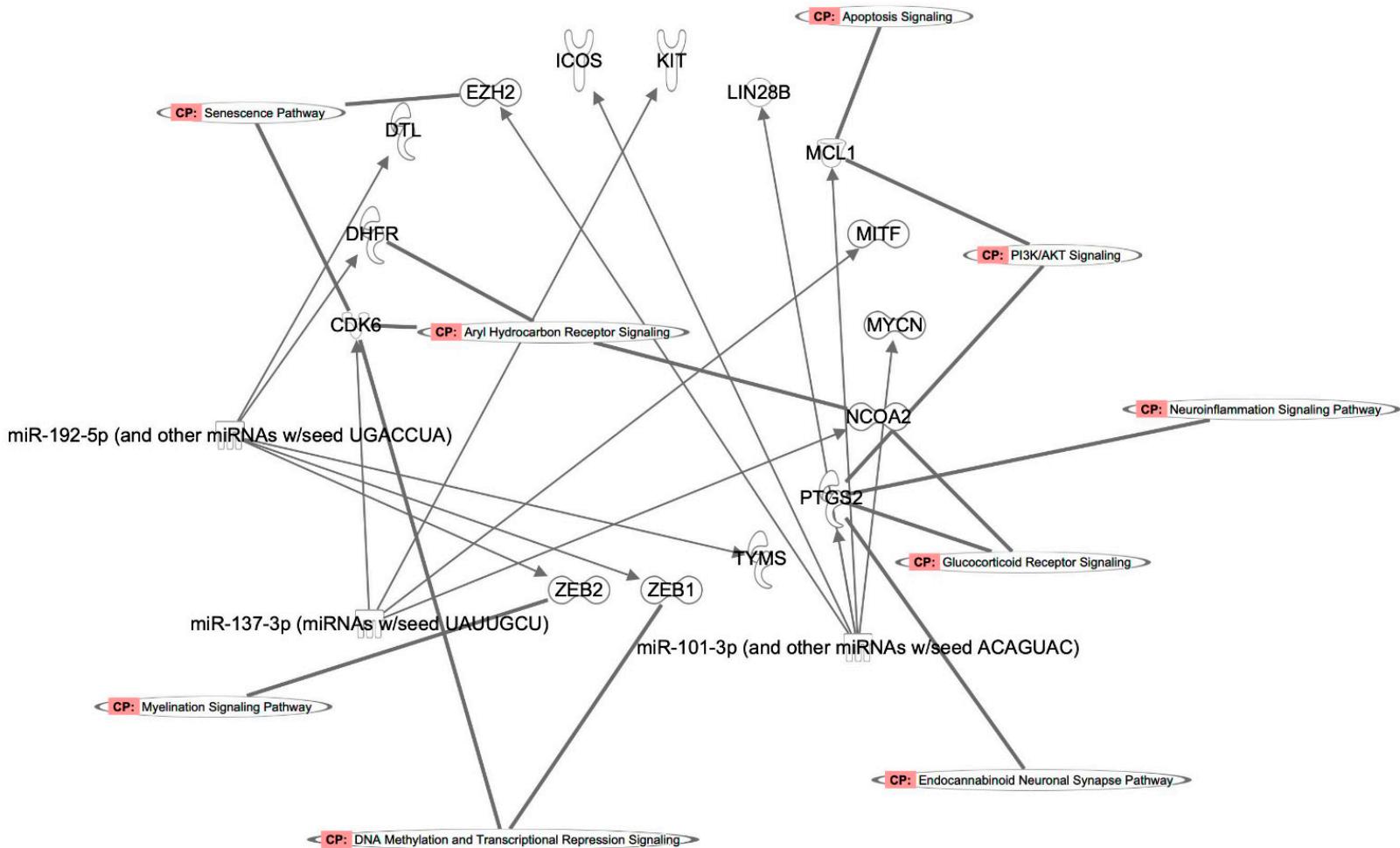
**Figure S2.** Cocaine withdrawal/abstinence-associated miRNA-mRNA-pathway network constructed by Ingenuity Pathway Analysis (IPA). The miRNA Target Filter function in IPA was applied to predict target genes (or mRNAs) of differentially expressed miRNAs. miRNAs were filtered to include those predicted to be target genes (or mRNAs) participating in canonical pathways associated with nervous system processes or signaling pathways. miRNAs and their target mRNAs (after filtering) along with relevant canonical pathways enriched in miRNA target mRNA sets (as determined by the IPA database) were visualized. Three such miRNAs (rno-miR101a-3p, rno-miR192-3p, and rno-miR137-3p) were identified. Pathways predicted to be regulated by these three miRNAs include: *Endocannabinoid Neuronal Synapse Pathway*, *Neuroinflammation signaling Pathway*, *PI3/Akt Signaling*, and *Apoptosis Signaling* for rno-miR101-3p; *DNA Methylation and Transcriptional Repression Signaling Pathway* and *Myelination Signaling Pathway* for rno-miR192-3p; and *Aryl Hydrocarbon Receptor Signaling and Senescence Pathway* for miR137-3p.
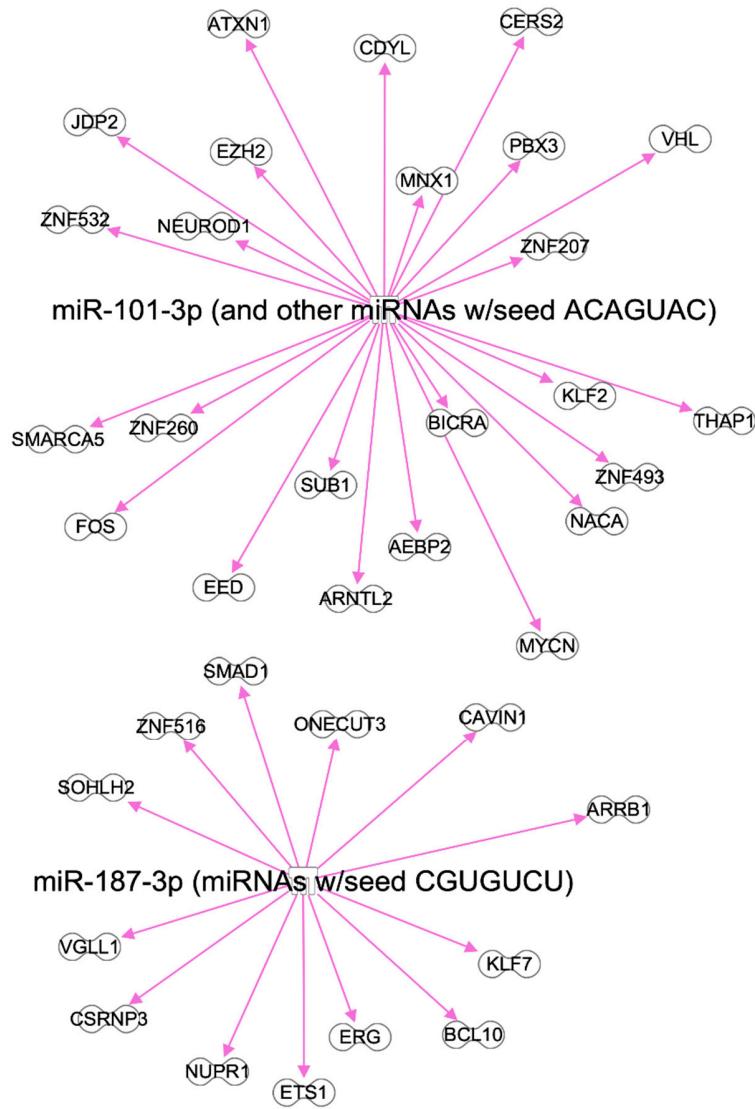
**Figure S3**. IPA miRNA Target Filter prediction of transcription factors (TFs) & nuclear receptors (NRs) potentially targeted by differentially expressed miRNAs in the IL-PFC of rats with protracted abstinence. miR-101a-3p and miR-187-3p are uniquely differentially expressed only during the protracted abstinence (**figure 7**). Target TFs & NRs of miR-101a-3p include FOS, NEUROD1, KLF2, and ZNFs. Target TFs & NF2 of miR-187-3p include KLF7, ZNF516, and BCL10. While mi-R101a-3p is upregulated, mi-R187-3p is downregulated (**Table 2**). It is expected that the expression of TFs & NFs targeted by mi-R101a-3p is decreased and the expression of TFs & NFs targeted by mi-R187-3p is increased.
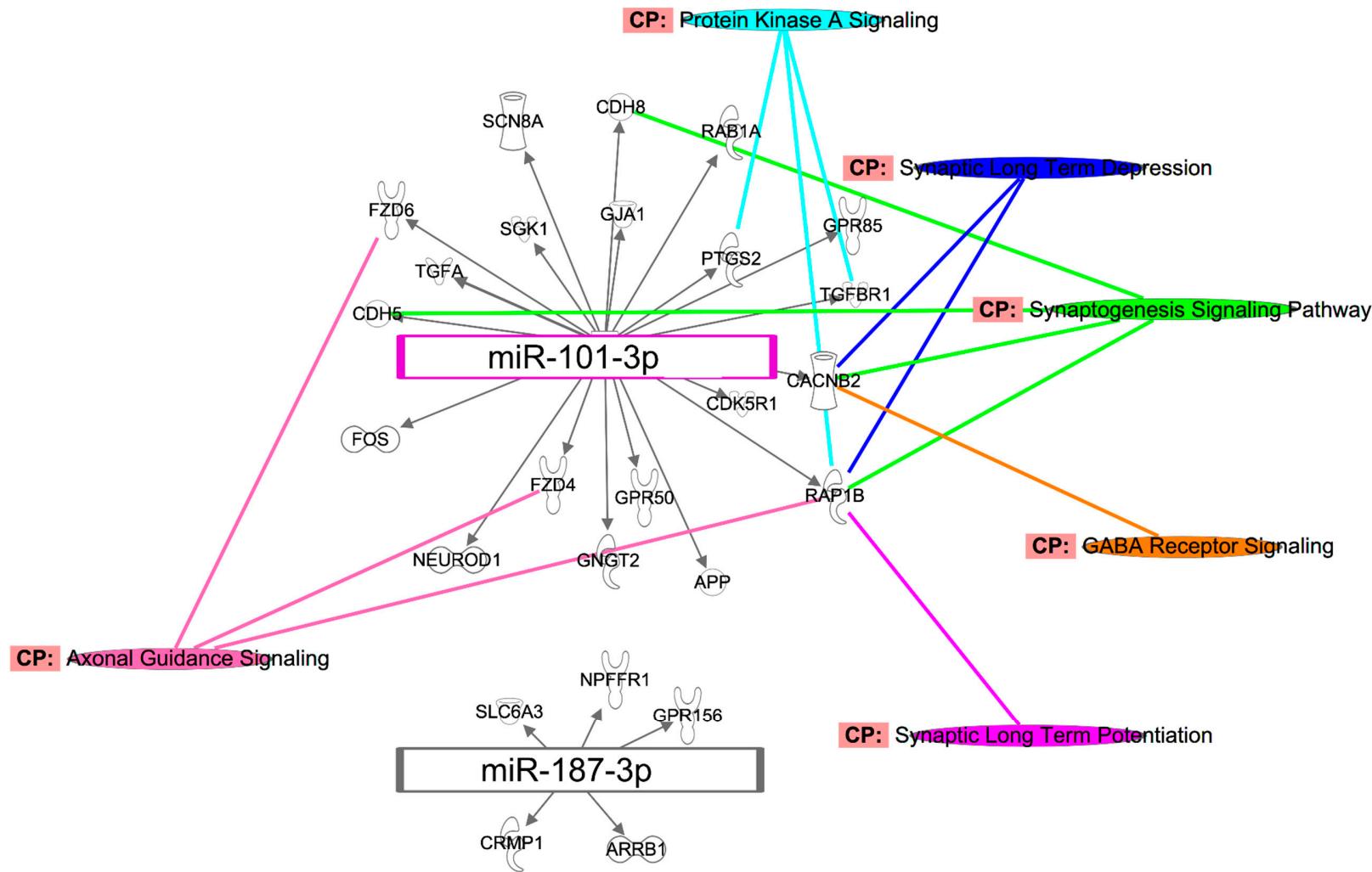
**Figure S4**. IPA miRNA Target Filter prediction of miRNA-mRNA-pathway regulatory networks containing miRNAs differentially expressed in the IL-PFC of rats with protracted abstinence (see **figure 7**). Putative targets of miR-101a-3p include those that are relevant to synaptogenesis signaling, synaptic long-term potentiation (LTP) and long-term depression (LTD), axonal guidance signaling, protein kinase A signaling, and GABA receptor signaling. Examples of miR-101a-3p targeted mRNAs include the calcium voltage-gated channel auxiliary subunit beta 2 gene (*CACNB2*), which is involved in synaptic LTD and GABA receptor signaling. miR-101a-3p is upregulated and hence, its targets are likely downregulated. The solute carrier family 6A member 3 gene (*SLC6A3*), which encodes the dopamine transporter, is a putative target of miR187-3p. miR187-3p is downregulated, hence the dopamine transporter gene is likely upregulated.
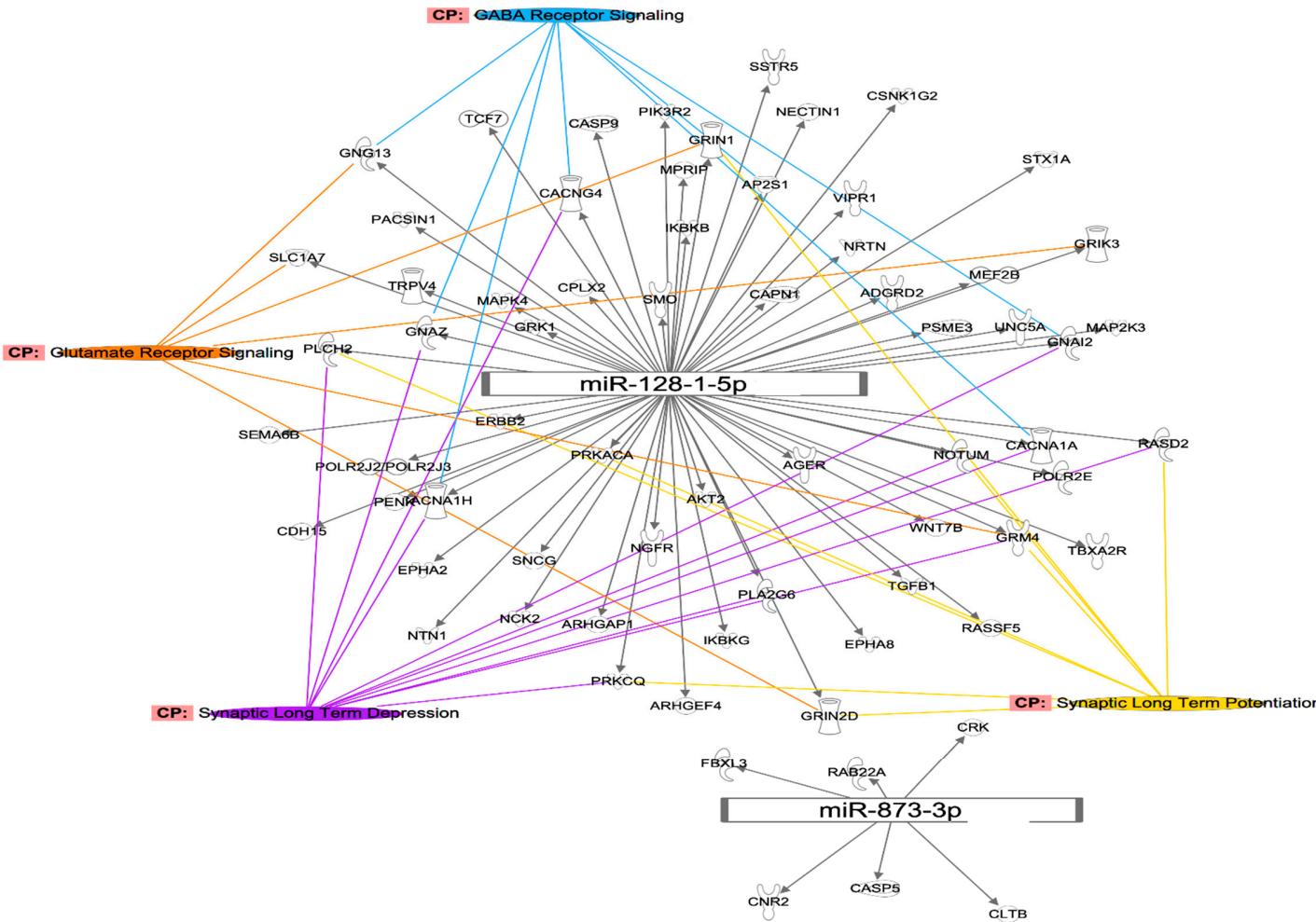
**Figure S5**. IPA miRNA Target Filter prediction of miRNA-mRNA-pathway regulatory networks containing miRNAs differentially expressed in the PL-PFC of rats with either withdrawal or protracted abstinence. miR-128-1-5p and miR-873-3p were differentially expressed in the PL-PFC due to either an 18-hr withdrawal or a 4-week abstinence (see **figure 7**). Examples of the predicted targets of miR-128-1-5p include the PI3 kinase receptor 2 gene (*PI3KR2*) and the phospholipase A2 group VI gene (*PLA2G6*). Predicted targets of miR-128-1-5p also include the glutamate ionotropic receptor NMDA type subunit 1 gene (*GRIN1*), the glutamate ionotropic receptor NMDA type subunit 2D gene (*GRIN2D*), the metabotropic glutamate receptor 4 gene (*GRM4*), the glutamate ionotropic receptor kainate type subunit 3 gene (*GRIK3*), and the solute carrier family 1 member 7 gene (*SLC1A7*; encoding the glutamate transporter EAAT5). These targets are involved in glutamate receptor signaling as well as synaptic LTD and LTP pathways. Additionally, miR-128-1-5p was predicted to target those genes encoding several subunits of the voltage gated calcium channels (CACNA1A and CACNA1H) and the calcium channel auxiliary subunit gamma 4 (CACNG4). miR-128-1-5p is downregulated and miR-873-3p is upregulated, and hence their targets are expected to be increased and decreased, respectively.

**Table S1.** The concentration and RNA integrity number (RIN) of 36 rat brain RNA samples

| RNA Samples | Rat ID | Alcohol treatment | Brain Region | Conc. (ng/ul) | RNA integrity number (RIN) |
|---|---|---|---|---|---|
| 1 | M170 | 4-week abstinence | Prelimbic Prefrontal Cortex | 51.4 | 8.9 |
| 2 | M170 | 4-week abstinence | Infralimbic Prefrontal Cortex | 47.6 | 8.7 |
| 3 | M170 | 4-week abstinence | Nucleus Accumbens | 23.3 | 9.1 |
| 4 | M363 | 4-week abstinence | Prelimbic Prefrontal Cortex | 48.0 | 7.4 |
| 5 | M363 | 4-week abstinence | Infralimbic Prefrontal Cortex | 30.1 | 7.9 |
| 6 | M363 | 4-week abstinence | Nucleus Accumbens | 38.4 | 8.1 |
| 7 | M472 | 4-week abstinence | Prelimbic Prefrontal Cortex | 37.4 | 8.5 |
| 8 | M472 | 4-week abstinence | Infralimbic Prefrontal Cortex | 42.7 | 9.1 |
| 9 | M472 | 4-week abstinence | Nucleus Accumbens | 41.6 | 9.2 |
| 10 | M456 | 4-week abstinence | Prelimbic Prefrontal Cortex | 46.8 | 9.1 |
| 11 | M456 | 4-week abstinence | Infralimbic Prefrontal Cortex | 21.6 | 8.7 |
| 12 | M456 | 4-week abstinence | Nucleus Accumbens | 50.7 | 8.2 |
| 13 | M153 | 18-hr withdrawal | Prelimbic Prefrontal Cortex | 33.5 | 9.0 |
| 14 | M153 | 18-hr withdrawal | Infralimbic Prefrontal Cortex | 55.0 | 9.0 |
| 15 | M153 | 18-hr withdrawal | Nucleus Accumbens | 30.9 | 9.0 |
| 16 | M366 | 18-hr withdrawal | Prelimbic Prefrontal Cortex | 50.0 | 8.8 |
| 17 | M366 | 18-hr withdrawal | Infralimbic Prefrontal Cortex | 49.6 | 8.2 |
| 18 | M366 | 18-hr withdrawal | Nucleus Accumbens | 52.6 | 8.9 |
| 19 | M172 | 18-hr withdrawal | Prelimbic Prefrontal Cortex | 48.5 | 9.3 |
| 20 | M172 | 18-hr withdrawal | Infralimbic Prefrontal Cortex | 60.7 | 8.9 |
| 21 | M172 | 18-hr withdrawal | Nucleus Accumbens | 47.5 | 9.1 |
| 22 | M154 | 18-hr withdrawal | Prelimbic Prefrontal Cortex | 42.2 | 8.8 |
| 23 | M154 | 18-hr withdrawal | Infralimbic Prefrontal Cortex | 45.8 | 8.9 |
| 24 | M154 | 18-hr withdrawal | Nucleus Accumbens | 53.3 | 9.2 |
| 25 | M157 | Naïve | Prelimbic Prefrontal Cortex | 38.0 | 9.0 |
| 26 | M157 | Naïve | Infralimbic Prefrontal Cortex | 45.7 | 9.2 |
| 27 | M157 | Naïve | Nucleus Accumbens | 51.4 | 9.2 |
| 28 | M378 | Naïve | Prelimbic Prefrontal Cortex | 41.8 | 8.5 |
| 29 | M378 | Naïve | Infralimbic Prefrontal Cortex | 49.1 | 8.9 |
| 30 | M378 | Naïve | Nucleus Accumbens | 53.0 | 9.1 |
| 31 | M476 | Naïve | Prelimbic Prefrontal Cortex | 43.3 | 9.1 |
| 32 | M476 | Naïve | Infralimbic Prefrontal Cortex | 42.9 | 9.2 |
| 33 | M476 | Naïve | Nucleus Accumbens | 41.0 | 8.1 |
| 34 | M480 | Naïve | Prelimbic Prefrontal Cortex | 44.9 | 8.6 |
| 35 | M480 | Naïve | Infralimbic Prefrontal Cortex | 63.5 | 9.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 36 | M480 | Naïve | Nucleus Accumbens | | | 39.7 | | 9.1 |

**Table S2.** Addiction behavior Z scores and addiction index of rats with an 18-hr withdrawal or a 4-week abstinence

| | Group A (4-week abstinence) | | | | Group B (18-hr withdrawal) | | | |
|---|---|---|---|---|---|---|---|---|
| | Rat 1 | Rat 2 | Rat 3 | Rat 4 | Rat 1 | Rat 2 | Rat 3 | Rat 4 |
| Z ESC | 1.133 | 0.367 | -0.2 | 0.69 | 0.675 | 0.382 | 0.934 | 0.874 |
| Z PR | 0.123 | 1.325 | 0.445 | -1.015 | -0.135 | 0.445 | -0.521 | -0.135 |
| Z shock | 1.116 | -0.556 | 0.087 | 2.016 | -0.299 | 1.759 | -0.17 | 0.988 |
| Addiction Index | 0.791 | 0.379 | 0.111 | 0.564 | 0.081 | 0.862 | 0.081 | 0.576 |

Mean±SD of addiction index in Group A: 0.461±0.288.
Mean±SD of addiction index in Group B: 0.400±0.386.
t-test of addiction index differences between Group A and Group B rats: t = 0.25, $P = 0.807$.

**Table S3a.** Correlation of addiction index with expression levels of top differentially expressed miRNAs ($|FC| \geq 2.0$ & $P < 0.05$) in three rat brain regions by partial correlation analysis

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 0.791 | IL-PFC | -0.032 | 0.512 | 0.201 | -0.643 | -0.970 | -1.086 | -0.460 | 0.705 | -0.246 | -0.834 | -0.262 | -0.983 | -0.841 | -1.349 | 0.781 | -0.487 |
| A_Rat 2 | 0.379 | IL-PFC | 0.432 | -0.772 | 0.577 | -0.203 | 1.417 | 0.601 | 0.034 | 1.503 | 1.173 | -1.074 | 0.361 | -0.249 | 0.368 | -0.219 | -0.913 | 0.314 |
| A_Rat 3 | 0.111 | IL-PFC | -0.357 | -0.072 | -0.306 | 0.250 | 0.038 | -0.545 | -0.346 | 0.112 | -0.660 | 0.309 | 0.832 | 0.551 | 1.044 | 0.673 | -0.837 | 0.414 |
| A_Rat 4 | 0.564 | IL-PFC | -0.001 | 0.450 | -0.421 | 0.452 | -0.512 | 1.076 | 0.645 | -2.253 | 0.068 | 1.584 | -1.029 | 0.541 | -0.693 | 0.886 | 1.107 | -0.301 |
| B_Rat 1 | 0.081 | IL-PFC | 0.157 | 0.975 | -0.675 | -0.842 | 3.567 | -0.205 | 1.695 | -2.181 | -1.589 | 2.368 | -1.697 | -0.276 | -3.313 | -0.420 | 3.015 | -0.844 |
| B_Rat 2 | 0.862 | IL-PFC | 0.103 | -0.756 | 0.680 | 1.252 | -0.107 | -0.681 | 1.466 | -0.435 | 2.263 | -0.828 | 1.578 | -2.191 | 1.392 | 1.256 | -0.597 | 0.909 |
| B_Rat 3 | 0.081 | IL-PFC | -0.735 | 0.266 | -0.787 | -1.393 | -1.939 | 0.943 | -1.943 | 1.023 | 0.139 | -2.081 | 0.744 | 0.609 | 0.423 | 0.364 | -2.330 | -0.406 |
| B_Rat 4 | 0.576 | IL-PFC | 0.366 | -0.423 | 0.555 | 1.224 | -1.498 | 0.037 | -1.284 | 1.526 | -0.782 | 0.580 | -0.473 | 1.906 | 1.656 | -1.251 | 0.101 | 0.468 |
| Partial correlation of addiction index with miRNA expression levels in the infralimbic prefrontal cortex (IL-PFC): | | | | | | | | | | | | | | | | | | |
| estimate | | | 0.444 | -0.367 | 0.732 | 0.601 | -0.341 | -0.371 | 0.202 | 0.082 | 0.539 | -0.154 | 0.184 | -0.453 | 0.312 | -0.090 | 0.074 | 0.407 |
| statistic | | | 1.110 | -0.882 | 2.400 | 1.680 | -0.812 | -0.894 | 0.461 | 0.184 | 1.431 | -0.349 | 0.418 | -1.135 | 0.734 | -0.203 | 0.165 | 0.996 |
| *p*-value | | | 0.318 | 0.418 | 0.062 | 0.154 | 0.454 | 0.412 | 0.664 | 0.862 | 0.212 | 0.742 | 0.693 | 0.308 | 0.496 | 0.847 | 0.875 | 0.365 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 0.791 | PL-PFC | -1.066 | 0.439 | -1.039 | -0.196 | 0.032 | -1.339 | 0.394 | 0.888 | 0.302 | 0.283 | 1.036 | 0.202 | 0.040 | 0.452 | -1.023 | 0.244 |
| A_Rat 2 | 0.379 | PL-PFC | 0.984 | -0.013 | 0.067 | 0.067 | -0.069 | 0.636 | -0.588 | 0.374 | 0.272 | 1.177 | 1.250 | 0.045 | 0.237 | -0.700 | -0.519 | 0.556 |
| A_Rat 3 | 0.111 | PL-PFC | -1.151 | -0.702 | -0.388 | 1.136 | 0.110 | 0.203 | -0.617 | -2.007 | 0.408 | 0.880 | -0.208 | -0.059 | 0.566 | -0.962 | 0.695 | -0.097 |
| A_Rat 4 | 0.564 | PL-PFC | 1.260 | 0.392 | 1.254 | -0.926 | -0.068 | 0.355 | 1.443 | 0.871 | -0.855 | -2.361 | -1.734 | -0.125 | -0.798 | 1.332 | 0.825 | -0.606 |
| B_Rat 1 | 0.081 | PL-PFC | -0.503 | 1.665 | -0.165 | -0.359 | -0.027 | -0.827 | 1.027 | 0.172 | -1.273 | 0.159 | -0.590 | 0.169 | -0.379 | 1.359 | 0.946 | -0.788 |
| B_Rat 2 | 0.862 | PL-PFC | 1.179 | 1.802 | 0.527 | 1.174 | 0.052 | 0.298 | 1.178 | -0.144 | 1.563 | -0.028 | 0.825 | 1.234 | 1.229 | -2.561 | -0.454 | 0.608 |
| B_Rat 3 | 0.081 | PL-PFC | 0.297 | -4.374 | -0.028 | -1.739 | 0.127 | -0.076 | -1.230 | 0.190 | -1.958 | -0.698 | -0.901 | -1.719 | -0.940 | 1.505 | -1.258 | -0.679 |
| B_Rat 4 | 0.576 | PL-PFC | -0.915 | 0.877 | -0.336 | 0.628 | -0.142 | 0.644 | -1.173 | -0.222 | 1.343 | 0.453 | 0.462 | -0.041 | -0.165 | -0.385 | 0.589 | 0.759 |
| Partial correlation of addiction index with miRNA expression levels in the prelimbic prefrontal cortex (PL-PFC): | | | | | | | | | | | | | | | | | | |
| estimate | | | 0.239 | 0.529 | 0.109 | 0.341 | -0.298 | 0.007 | 0.396 | 0.413 | 0.671 | -0.130 | 0.462 | 0.634 | 0.427 | -0.472 | -0.235 | 0.640 |
| statistic | | | 0.551 | 1.392 | 0.245 | 0.812 | -0.697 | 0.015 | 0.964 | 1.014 | 2.024 | -0.292 | 1.166 | 1.835 | 1.056 | -1.196 | -0.540 | 1.861 |
| *p*-value | | | 0.605 | 0.223 | 0.816 | 0.454 | 0.517 | 0.989 | 0.379 | 0.357 | 0.099 | 0.782 | 0.296 | 0.126 | 0.339 | 0.285 | 0.612 | 0.122 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 0.791 | NAc | -0.667 | 0.630 | -0.377 | -0.412 | 0.924 | -0.870 | 0.534 | -0.417 | 0.067 | -0.246 | 0.121 | 0.100 | 0.140 | 0.076 | -0.376 | -0.319 |
| A_Rat 2 | 0.379 | NAc | -2.222 | 0.577 | -1.308 | -2.016 | -0.381 | -1.787 | -0.032 | -1.168 | -1.892 | -0.910 | -2.265 | -1.073 | -1.660 | 1.199 | 0.564 | -2.278 |
| A_Rat 3 | 0.111 | NAc | 1.027 | 0.087 | 0.885 | 0.435 | -0.667 | 0.401 | -1.266 | 0.647 | 0.615 | 0.315 | 1.296 | 0.119 | 0.278 | -0.585 | -0.299 | 0.663 |
| A_Rat 4 | 0.564 | NAc | 1.833 | -1.245 | 0.704 | 1.957 | 0.135 | 2.251 | 0.588 | 0.832 | 1.005 | 0.808 | 0.540 | 0.887 | 1.237 | -0.618 | 0.075 | 1.940 |
| B_Rat 1 | 0.081 | NAc | 0.908 | 0.424 | 0.292 | -0.105 | 0.514 | -0.067 | 1.573 | 0.392 | -0.673 | 0.440 | 0.267 | 1.467 | -0.956 | 0.519 | 0.160 | -0.113 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B_Rat 2 | 0.862 | NAc | -1.595 | -0.175 | -1.284 | -1.174 | 0.405 | -1.204 | 1.699 | -0.768 | -0.633 | -0.724 | -1.133 | -1.216 | 0.062 | 0.577 | 0.106 | -1.021 |
| B_Rat 3 | 0.081 | NAc | 0.856 | -0.212 | 0.979 | 0.549 | -0.167 | 1.595 | -0.863 | 0.845 | 0.136 | -0.078 | 0.894 | 0.086 | 0.168 | -1.169 | -0.153 | 0.644 |
| B_Rat 4 | 0.576 | NAc | -0.159 | -0.062 | 0.209 | 0.808 | -0.756 | -0.298 | -2.444 | -0.399 | 1.224 | 0.398 | 0.093 | -0.169 | 0.786 | 0.001 | -0.132 | 0.529 |
| Partial correlation of addiction index with miRNA expression levels in the nucleus accumbens (NAc): | | | | | | | | | | | | | | | | | | |
| | | estimate | -0.493 | -0.119 | -0.602 | -0.170 | 0.400 | -0.376 | 0.257 | -0.581 | 0.071 | -0.312 | -0.413 | -0.463 | 0.310 | 0.319 | -0.052 | -0.178 |
| | | statistic | -1.268 | -0.267 | -1.686 | -0.387 | 0.976 | -0.909 | 0.594 | -1.598 | 0.159 | -0.734 | -1.015 | -1.168 | 0.730 | 0.753 | -0.116 | -0.405 |
| | | *p*-value | 0.261 | 0.800 | 0.153 | 0.715 | 0.374 | 0.405 | 0.578 | 0.171 | 0.880 | 0.496 | 0.357 | 0.296 | 0.498 | 0.485 | 0.912 | 0.702 |

IL-PFC: Infralimbic prefrontal cortex; PL-PFC: Prelimbic prefrontal cortex; NAc: Nucleus accumbens.

Normalized expression levels of miRNAs are listed under the name of each miRNA.

**Table S3b.** Correlation of Z ESC with expression levels of top differentially expressed miRNAs ($|FC| \geq 2.0$ & $P < 0.05$) in three rat brain regions by partial correlation analysis

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 1.133 | IL-PFC | -0.032 | 0.512 | 0.201 | -0.643 | -0.970 | -1.086 | -0.460 | 0.705 | -0.246 | -0.834 | -0.262 | -0.983 | -0.841 | -1.349 | 0.781 | -0.487 |
| A_Rat 2 | 0.367 | IL-PFC | 0.432 | -0.772 | 0.577 | -0.203 | 1.417 | 0.601 | 0.034 | 1.503 | 1.173 | -1.074 | 0.361 | -0.249 | 0.368 | -0.219 | -0.913 | 0.314 |
| A_Rat 3 | -0.200 | IL-PFC | -0.357 | -0.072 | -0.306 | 0.250 | 0.038 | -0.545 | -0.346 | 0.112 | -0.660 | 0.309 | 0.832 | 0.551 | 1.044 | 0.673 | -0.837 | 0.414 |
| A_Rat 4 | 0.690 | IL-PFC | -0.001 | 0.450 | -0.421 | 0.452 | -0.512 | 1.076 | 0.645 | -2.253 | 0.068 | 1.584 | -1.029 | 0.541 | -0.693 | 0.886 | 1.107 | -0.301 |
| B_Rat 1 | 0.675 | IL-PFC | 0.157 | 0.975 | -0.675 | -0.842 | 3.567 | -0.205 | 1.695 | -2.181 | -1.589 | 2.368 | -1.697 | -0.276 | -3.313 | -0.420 | 3.015 | -0.844 |
| B_Rat 2 | 0.382 | IL-PFC | 0.103 | -0.756 | 0.680 | 1.252 | -0.107 | -0.681 | 1.466 | -0.435 | 2.263 | -0.828 | 1.578 | -2.191 | 1.392 | 1.256 | -0.597 | 0.909 |
| B_Rat 3 | 0.934 | IL-PFC | -0.735 | 0.266 | -0.787 | -1.393 | -1.939 | 0.943 | -1.943 | 1.023 | 0.139 | -2.081 | 0.744 | 0.609 | 0.423 | 0.364 | -2.330 | -0.406 |
| B_Rat 4 | 0.874 | IL-PFC | 0.366 | -0.423 | 0.555 | 1.224 | -1.498 | 0.037 | -1.284 | 1.526 | -0.782 | 0.580 | -0.473 | 1.906 | 1.656 | -1.251 | 0.101 | 0.468 |
| | | Partial correlation of Z ESC with miRNA expression levels in the infralimbic prefrontal cortex (IL-PFC): | | | | | | | | | | | | | | | | |
| | | estimate | 0.027 | -0.116 | -0.059 | -0.622 | -0.271 | 0.120 | 0.048 | 0.829 | 0.027 | -0.116 | -0.059 | -0.622 | -0.271 | 0.120 | 0.048 | 0.829 |
| | | statistic | 0.060 | -0.261 | -0.132 | -1.776 | -0.629 | 0.269 | 0.108 | 3.310 | 0.060 | -0.261 | -0.132 | -1.776 | -0.629 | 0.269 | 0.108 | 3.310 |
| | | *p*-value | 0.954 | 0.804 | 0.900 | 0.136 | 0.557 | 0.798 | 0.918 | **0.021** | 0.542 | 0.427 | 0.961 | 0.526 | 0.165 | 0.150 | 0.386 | 0.842 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 1.133 | PL-PFC | -1.066 | 0.439 | -1.039 | -0.196 | 0.032 | -1.339 | 0.394 | 0.888 | 0.302 | 0.283 | 1.036 | 0.202 | 0.040 | 0.452 | -1.023 | 0.244 |
| A_Rat 2 | 0.367 | PL-PFC | 0.984 | -0.013 | 0.067 | 0.067 | -0.069 | 0.636 | -0.588 | 0.374 | 0.272 | 1.177 | 1.250 | 0.045 | 0.237 | -0.700 | -0.519 | 0.556 |
| A_Rat 3 | -0.200 | PL-PFC | -1.151 | -0.702 | -0.388 | 1.136 | 0.110 | 0.203 | -0.617 | -2.007 | 0.408 | 0.880 | -0.208 | -0.059 | 0.566 | -0.962 | 0.695 | -0.097 |
| A_Rat 4 | 0.690 | PL-PFC | 1.260 | 0.392 | 1.254 | -0.926 | -0.068 | 0.355 | 1.443 | 0.871 | -0.855 | -2.361 | -1.734 | -0.125 | -0.798 | 1.332 | 0.825 | -0.606 |
| B_Rat 1 | 0.675 | PL-PFC | -0.503 | 1.665 | -0.165 | -0.359 | -0.027 | -0.827 | 1.027 | 0.172 | -1.273 | 0.159 | -0.590 | 0.169 | -0.379 | 1.359 | 0.946 | -0.788 |
| B_Rat 2 | 0.382 | PL-PFC | 1.179 | 1.802 | 0.527 | 1.174 | 0.052 | 0.298 | 1.178 | -0.144 | 1.563 | -0.028 | 0.825 | 1.234 | 1.229 | -2.561 | -0.454 | 0.608 |
| B_Rat 3 | 0.934 | PL-PFC | 0.297 | -4.374 | -0.028 | -1.739 | 0.127 | -0.076 | -1.230 | 0.190 | -1.958 | -0.698 | -0.901 | -1.719 | -0.940 | 1.505 | -1.258 | -0.679 |
| B_Rat 4 | 0.874 | PL-PFC | -0.915 | 0.877 | -0.336 | 0.628 | -0.142 | 0.644 | -1.173 | -0.222 | 1.343 | 0.453 | 0.462 | -0.041 | -0.165 | -0.385 | 0.589 | 0.759 |
| | | Partial correlation of Z ESC with miRNA expression levels in the prelimbic prefrontal cortex (PL-PFC): | | | | | | | | | | | | | | | | |
| | | estimate | -0.073 | 0.429 | -0.202 | -0.358 | -0.340 | -0.493 | -0.314 | 0.102 | -0.183 | -0.115 | -0.451 | 0.089 | -0.313 | 0.592 | 0.220 | -0.582 |
| | | statistic | -0.164 | 1.061 | -0.460 | -0.857 | -0.810 | -1.266 | -0.739 | 0.230 | -0.417 | -0.260 | -1.130 | 0.199 | -0.736 | 1.644 | 0.504 | -1.600 |
| | | *p*-value | 0.876 | 0.337 | 0.665 | 0.431 | 0.455 | 0.261 | 0.493 | 0.827 | 0.694 | 0.806 | 0.310 | 0.850 | 0.495 | 0.161 | 0.636 | 0.170 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 1.133 | NAc | -0.667 | 0.630 | -0.377 | -0.412 | 0.924 | -0.870 | 0.534 | -0.417 | 0.067 | -0.246 | 0.121 | 0.100 | 0.140 | 0.076 | -0.376 | -0.319 |
| A_Rat 2 | 0.367 | NAc | -2.222 | 0.577 | -1.308 | -2.016 | -0.381 | -1.787 | -0.032 | -1.168 | -1.892 | -0.910 | -2.265 | -1.073 | -1.660 | 1.199 | 0.564 | -2.278 |
| A_Rat 3 | -0.200 | NAc | 1.027 | 0.087 | 0.885 | 0.435 | -0.667 | 0.401 | -1.266 | 0.647 | 0.615 | 0.315 | 1.296 | 0.119 | 0.278 | -0.585 | -0.299 | 0.663 |
| A_Rat 4 | 0.690 | NAc | 1.833 | -1.245 | 0.704 | 1.957 | 0.135 | 2.251 | 0.588 | 0.832 | 1.005 | 0.808 | 0.540 | 0.887 | 1.237 | -0.618 | 0.075 | 1.940 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B_Rat 1 | 0.675 | NAc | 0.908 | 0.424 | 0.292 | -0.105 | 0.514 | -0.067 | 1.573 | 0.392 | -0.673 | 0.440 | 0.267 | 1.467 | -0.956 | 0.519 | 0.160 | -0.113 |
| B_Rat 2 | 0.382 | NAc | -1.595 | -0.175 | -1.284 | -1.174 | 0.405 | -1.204 | 1.699 | -0.768 | -0.633 | -0.724 | -1.133 | -1.216 | 0.062 | 0.577 | 0.106 | -1.021 |
| B_Rat 3 | 0.934 | NAc | 0.856 | -0.212 | 0.979 | 0.549 | -0.167 | 1.595 | -0.863 | 0.845 | 0.136 | -0.078 | 0.894 | 0.086 | 0.168 | -1.169 | -0.153 | 0.644 |
| B_Rat 4 | 0.874 | NAc | -0.159 | -0.062 | 0.209 | 0.808 | -0.756 | -0.298 | -2.444 | -0.399 | 1.224 | 0.398 | 0.093 | -0.169 | 0.786 | 0.001 | -0.132 | 0.529 |
| Partial correlation of Z ESC with miRNA expression levels in the nucleus accumbens (NAc): | | | | | | | | | | | | | | | | | | |
| estimate | | | 0.040 | 0.005 | 0.044 | 0.208 | 0.486 | 0.140 | 0.033 | -0.019 | 0.179 | 0.100 | 0.046 | 0.250 | 0.204 | -0.133 | -0.220 | 0.162 |
| statistic | | | 0.090 | 0.012 | 0.099 | 0.475 | 1.245 | 0.315 | 0.074 | -0.042 | 0.408 | 0.225 | 0.103 | 0.577 | 0.467 | -0.301 | -0.504 | 0.366 |
| *p*-value | | | 0.932 | 0.991 | 0.925 | 0.655 | 0.268 | 0.765 | 0.944 | 0.968 | 0.700 | 0.831 | 0.922 | 0.589 | 0.660 | 0.776 | 0.635 | 0.729 |

IL-PFC: Infralimbic prefrontal cortex; PL-PFC: Prelimbic prefrontal cortex; NAc: Nucleus accumbens.

Normalized expression levels of miRNAs are listed under the name of each miRNA.

**Table S3c.** Correlation of Z PR with expression levels of top differentially expressed miRNAs ($|FC| \geq 2.0$ & $P < 0.05$) in three rat brain regions by partial correlation analysis

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 0.123 | IL-PFC | -0.032 | 0.512 | 0.201 | -0.643 | -0.970 | -1.086 | -0.460 | 0.705 | -0.246 | -0.834 | -0.262 | -0.983 | -0.841 | -1.349 | 0.781 | -0.487 |
| A_Rat 2 | 1.325 | IL-PFC | 0.432 | -0.772 | 0.577 | -0.203 | 1.417 | 0.601 | 0.034 | 1.503 | 1.173 | -1.074 | 0.361 | -0.249 | 0.368 | -0.219 | -0.913 | 0.314 |
| A_Rat 3 | 0.445 | IL-PFC | -0.357 | -0.072 | -0.306 | 0.250 | 0.038 | -0.545 | -0.346 | 0.112 | -0.660 | 0.309 | 0.832 | 0.551 | 1.044 | 0.673 | -0.837 | 0.414 |
| A_Rat 4 | -1.015 | IL-PFC | -0.001 | 0.450 | -0.421 | 0.452 | -0.512 | 1.076 | 0.645 | -2.253 | 0.068 | 1.584 | -1.029 | 0.541 | -0.693 | 0.886 | 1.107 | -0.301 |
| B_Rat 1 | -0.135 | IL-PFC | 0.157 | 0.975 | -0.675 | -0.842 | 3.567 | -0.205 | 1.695 | -2.181 | -1.589 | 2.368 | -1.697 | -0.276 | -3.313 | -0.420 | 3.015 | -0.844 |
| B_Rat 2 | 0.445 | IL-PFC | 0.103 | -0.756 | 0.680 | 1.252 | -0.107 | -0.681 | 1.466 | -0.435 | 2.263 | -0.828 | 1.578 | -2.191 | 1.392 | 1.256 | -0.597 | 0.909 |
| B_Rat 3 | -0.521 | IL-PFC | -0.735 | 0.266 | -0.787 | -1.393 | -1.939 | 0.943 | -1.943 | 1.023 | 0.139 | -2.081 | 0.744 | 0.609 | 0.423 | 0.364 | -2.330 | -0.406 |
| B_Rat 4 | -0.135 | IL-PFC | 0.366 | -0.423 | 0.555 | 1.224 | -1.498 | 0.037 | -1.284 | 1.526 | -0.782 | 0.580 | -0.473 | 1.906 | 1.656 | -1.251 | 0.101 | 0.468 |

Partial correlation of Z ESC with miRNA expression levels in the infralimbic prefrontal cortex (IL-PFC):

| | | | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| estimate | | | 0.410 | 0.217 | 0.624 | 0.592 | 0.022 | -0.381 | -0.278 | -0.310 | 0.522 | 0.878 | 0.838 | 0.406 | 0.719 | -0.100 | -0.260 | 0.643 |
| statistic | | | 1.004 | 0.498 | 1.786 | 1.644 | 0.048 | -0.921 | -0.647 | -0.728 | 1.367 | 4.104 | 3.431 | 0.994 | 2.313 | -0.225 | -0.602 | 1.876 |
| *p*-value | | | 0.361 | 0.640 | 0.134 | 0.161 | 0.963 | 0.399 | 0.546 | 0.499 | 0.230 | **0.009** | 0.019 | 0.366 | 0.069 | 0.831 | 0.573 | 0.119 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 0.123 | PL-PFC | -1.066 | 0.439 | -1.039 | -0.196 | 0.032 | -1.339 | 0.394 | 0.888 | 0.302 | 0.283 | 1.036 | 0.202 | 0.040 | 0.452 | -1.023 | 0.244 |
| A_Rat 2 | 1.325 | PL-PFC | 0.984 | -0.013 | 0.067 | 0.067 | -0.069 | 0.636 | -0.588 | 0.374 | 0.272 | 1.177 | 1.250 | 0.045 | 0.237 | -0.700 | -0.519 | 0.556 |
| A_Rat 3 | 0.445 | PL-PFC | -1.151 | -0.702 | -0.388 | 1.136 | 0.110 | 0.203 | -0.617 | -2.007 | 0.408 | 0.880 | -0.208 | -0.059 | 0.566 | -0.962 | 0.695 | -0.097 |
| A_Rat 4 | -1.015 | PL-PFC | 1.260 | 0.392 | 1.254 | -0.926 | -0.068 | 0.355 | 1.443 | 0.871 | -0.855 | -2.361 | -1.734 | -0.125 | -0.798 | 1.332 | 0.825 | -0.606 |
| B_Rat 1 | -0.135 | PL-PFC | -0.503 | 1.665 | -0.165 | -0.359 | -0.027 | -0.827 | 1.027 | 0.172 | -1.273 | 0.159 | -0.590 | 0.169 | -0.379 | 1.359 | 0.946 | -0.788 |
| B_Rat 2 | 0.445 | PL-PFC | 1.179 | 1.802 | 0.527 | 1.174 | 0.052 | 0.298 | 1.178 | -0.144 | 1.563 | -0.028 | 0.825 | 1.234 | 1.229 | -2.561 | -0.454 | 0.608 |
| B_Rat 3 | -0.521 | PL-PFC | 0.297 | -4.374 | -0.028 | -1.739 | 0.127 | -0.076 | -1.230 | 0.190 | -1.958 | -0.698 | -0.901 | -1.719 | -0.940 | 1.505 | -1.258 | -0.679 |
| B_Rat 4 | -0.135 | PL-PFC | -0.915 | 0.877 | -0.336 | 0.628 | -0.142 | 0.644 | -1.173 | -0.222 | 1.343 | 0.453 | 0.462 | -0.041 | -0.165 | -0.385 | 0.589 | 0.759 |

Partial correlation of Z ESC with miRNA expression levels in the prelimbic prefrontal cortex (PL-PFC):

| | | | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| estimate | | | -0.012 | -0.662 | -0.352 | 0.128 | 0.352 | 0.187 | 0.134 | 0.516 | 0.407 | -0.375 | 0.486 | -0.380 | 0.286 | -0.694 | -0.271 | 0.535 |
| statistic | | | -0.026 | -1.975 | -0.841 | 0.289 | 0.841 | 0.427 | 0.302 | 1.346 | 0.995 | -0.906 | 1.242 | -0.919 | 0.668 | -2.157 | -0.629 | 1.415 |
| *p*-value | | | 0.980 | 0.105 | 0.439 | 0.784 | 0.439 | 0.687 | 0.775 | 0.236 | 0.365 | 0.407 | 0.269 | 0.400 | 0.534 | 0.083 | 0.557 | 0.216 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 0.123 | NAc | -0.667 | 0.630 | -0.377 | -0.412 | 0.924 | -0.870 | 0.534 | -0.417 | 0.067 | -0.246 | 0.121 | 0.100 | 0.140 | 0.076 | -0.376 | -0.319 |
| A_Rat 2 | 1.325 | NAc | -2.222 | 0.577 | -1.308 | -2.016 | -0.381 | -1.787 | -0.032 | -1.168 | -1.892 | -0.910 | -2.265 | -1.073 | -1.660 | 1.199 | 0.564 | -2.278 |
| A_Rat 3 | 0.445 | NAc | 1.027 | 0.087 | 0.885 | 0.435 | -0.667 | 0.401 | -1.266 | 0.647 | 0.615 | 0.315 | 1.296 | 0.119 | 0.278 | -0.585 | -0.299 | 0.663 |
| A_Rat 4 | -1.015 | NAc | 1.833 | -1.245 | 0.704 | 1.957 | 0.135 | 2.251 | 0.588 | 0.832 | 1.005 | 0.808 | 0.540 | 0.887 | 1.237 | -0.618 | 0.075 | 1.940 |
| B_Rat 1 | -0.135 | NAc | 0.908 | 0.424 | 0.292 | -0.105 | 0.514 | -0.067 | 1.573 | 0.392 | -0.673 | 0.440 | 0.267 | 1.467 | -0.956 | 0.519 | 0.160 | -0.113 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B_Rat 2 | 0.445 | NAc | -1.595 | -0.175 | -1.284 | -1.174 | 0.405 | -1.204 | 1.699 | -0.768 | -0.633 | -0.724 | -1.133 | -1.216 | 0.062 | 0.577 | 0.106 | -1.021 |
| B_Rat 3 | -0.521 | NAc | 0.856 | -0.212 | 0.979 | 0.549 | -0.167 | 1.595 | -0.863 | 0.845 | 0.136 | -0.078 | 0.894 | 0.086 | 0.168 | -1.169 | -0.153 | 0.644 |
| B_Rat 4 | -0.135 | NAc | -0.159 | -0.062 | 0.209 | 0.808 | -0.756 | -0.298 | -2.444 | -0.399 | 1.224 | 0.398 | 0.093 | -0.169 | 0.786 | 0.001 | -0.132 | 0.529 |
| | | Partial correlation of Z ESC with miRNA expression levels in the nucleus accumbens (NAc): | | | | | | | | | | | | | | | | |
| | | estimate | -0.840 | 0.732 | -0.733 | -0.916 | -0.185 | -0.893 | 0.033 | -0.777 | -0.738 | -0.799 | -0.707 | -0.701 | -0.757 | 0.744 | 0.431 | -0.918 |
| | | statistic | -3.463 | 2.401 | -2.407 | -5.115 | -0.420 | -4.449 | 0.074 | -2.764 | -2.448 | -2.975 | -2.238 | -2.198 | -2.592 | 2.487 | 1.069 | -5.168 |
| | | *p*-value | **0.018** | 0.062 | 0.061 | **0.004** | 0.692 | **0.007** | 0.944 | **0.040** | 0.058 | **0.031** | 0.075 | 0.079 | **0.049** | 0.055 | 0.334 | **0.004** |

IL-PFC: Infralimbic prefrontal cortex; PL-PFC: Prelimbic prefrontal cortex; NAc: Nucleus accumbens.

Normalized expression levels of miRNAs are listed under the name of each miRNA.

**Table S3d.** Correlation of Z Shock with expression levels of top differentially expressed miRNAs ($|FC| \geq 2.0$ & $P < 0.05$) in three rat brain regions by partial correlation analysis

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 1.116 | IL-PFC | -0.032 | 0.512 | 0.201 | -0.643 | -0.970 | -1.086 | -0.460 | 0.705 | -0.246 | -0.834 | -0.262 | -0.983 | -0.841 | -1.349 | 0.781 | -0.487 |
| A_Rat 2 | -0.556 | IL-PFC | 0.432 | -0.772 | 0.577 | -0.203 | 1.417 | 0.601 | 0.034 | 1.503 | 1.173 | -1.074 | 0.361 | -0.249 | 0.368 | -0.219 | -0.913 | 0.314 |
| A_Rat 3 | 0.087 | IL-PFC | -0.357 | -0.072 | -0.306 | 0.250 | 0.038 | -0.545 | -0.346 | 0.112 | -0.660 | 0.309 | 0.832 | 0.551 | 1.044 | 0.673 | -0.837 | 0.414 |
| A_Rat 4 | 2.016 | IL-PFC | -0.001 | 0.450 | -0.421 | 0.452 | -0.512 | 1.076 | 0.645 | -2.253 | 0.068 | 1.584 | -1.029 | 0.541 | -0.693 | 0.886 | 1.107 | -0.301 |
| B_Rat 1 | -0.299 | IL-PFC | 0.157 | 0.975 | -0.675 | -0.842 | 3.567 | -0.205 | 1.695 | -2.181 | -1.589 | 2.368 | -1.697 | -0.276 | -3.313 | -0.420 | 3.015 | -0.844 |
| B_Rat 2 | 1.759 | IL-PFC | 0.103 | -0.756 | 0.680 | 1.252 | -0.107 | -0.681 | 1.466 | -0.435 | 2.263 | -0.828 | 1.578 | -2.191 | 1.392 | 1.256 | -0.597 | 0.909 |
| B_Rat 3 | -0.170 | IL-PFC | -0.735 | 0.266 | -0.787 | -1.393 | -1.939 | 0.943 | -1.943 | 1.023 | 0.139 | -2.081 | 0.744 | 0.609 | 0.423 | 0.364 | -2.330 | -0.406 |
| B_Rat 4 | 0.988 | IL-PFC | 0.366 | -0.423 | 0.555 | 1.224 | -1.498 | 0.037 | -1.284 | 1.526 | -0.782 | 0.580 | -0.473 | 1.906 | 1.656 | -1.251 | 0.101 | 0.468 |
| Partial correlation of Z ESC with miRNA expression levels in the infralimbic prefrontal cortex (IL-PFC): | | | | | | | | | | | | | | | | | | |
| estimate | | | 0.132 | 0.406 | 0.294 | 0.171 | -0.191 | -0.141 | 0.556 | 0.275 | 0.398 | -0.590 | -0.129 | 0.448 | 0.151 | 0.231 | 0.115 | 0.207 |
| statistic | | | 0.297 | 0.992 | 0.689 | 0.387 | -0.436 | -0.319 | 1.495 | 0.639 | 0.971 | -1.633 | 0.291 | 1.120 | 0.342 | 0.530 | 0.259 | 0.472 |
| *p*-value | | | 0.778 | 0.367 | 0.522 | 0.714 | 0.681 | 0.763 | 0.195 | 0.551 | 0.376 | 0.163 | 0.783 | 0.314 | 0.746 | 0.619 | 0.806 | 0.656 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 1.116 | PL-PFC | -1.066 | 0.439 | -1.039 | -0.196 | 0.032 | -1.339 | 0.394 | 0.888 | 0.302 | 0.283 | 1.036 | 0.202 | 0.040 | 0.452 | -1.023 | 0.244 |
| A_Rat 2 | -0.556 | PL-PFC | 0.984 | -0.013 | 0.067 | 0.067 | -0.069 | 0.636 | -0.588 | 0.374 | 0.272 | 1.177 | 1.250 | 0.045 | 0.237 | -0.700 | -0.519 | 0.556 |
| A_Rat 3 | 0.087 | PL-PFC | -1.151 | -0.702 | -0.388 | 1.136 | 0.110 | 0.203 | -0.617 | -2.007 | 0.408 | 0.880 | -0.208 | -0.059 | 0.566 | -0.962 | 0.695 | -0.097 |
| A_Rat 4 | 2.016 | PL-PFC | 1.260 | 0.392 | 1.254 | -0.926 | -0.068 | 0.355 | 1.443 | 0.871 | -0.855 | -2.361 | -1.734 | -0.125 | -0.798 | 1.332 | 0.825 | -0.606 |
| B_Rat 1 | -0.299 | PL-PFC | -0.503 | 1.665 | -0.165 | -0.359 | -0.027 | -0.827 | 1.027 | 0.172 | -1.273 | 0.159 | -0.590 | 0.169 | -0.379 | 1.359 | 0.946 | -0.788 |
| B_Rat 2 | 1.759 | PL-PFC | 1.179 | 1.802 | 0.527 | 1.174 | 0.052 | 0.298 | 1.178 | -0.144 | 1.563 | -0.028 | 0.825 | 1.234 | 1.229 | -2.561 | -0.454 | 0.608 |
| B_Rat 3 | -0.170 | PL-PFC | 0.297 | -4.374 | -0.028 | -1.739 | 0.127 | -0.076 | -1.230 | 0.190 | -1.958 | -0.698 | -0.901 | -1.719 | -0.940 | 1.505 | -1.258 | -0.679 |
| B_Rat 4 | 0.988 | PL-PFC | -0.915 | 0.877 | -0.336 | 0.628 | -0.142 | 0.644 | -1.173 | -0.222 | 1.343 | 0.453 | 0.462 | -0.041 | -0.165 | -0.385 | 0.589 | 0.759 |
| Partial correlation of Z ESC with miRNA expression levels in the prelimbic prefrontal cortex (PL-PFC): | | | | | | | | | | | | | | | | | | |
| estimate | | | 0.269 | -0.068 | 0.433 | 0.636 | -0.435 | 0.077 | 0.230 | -0.323 | 0.311 | 0.161 | 0.023 | -0.208 | 0.229 | -0.213 | 0.170 | 0.258 |
| statistic | | | 0.624 | -0.151 | 1.075 | 1.845 | -1.081 | 0.174 | 0.528 | -0.762 | 0.732 | 0.364 | 0.052 | -0.474 | 0.527 | -0.488 | 0.385 | 0.596 |
| *p*-value | | | 0.560 | 0.886 | 0.332 | 0.124 | 0.329 | 0.869 | 0.620 | 0.480 | 0.497 | 0.731 | 0.961 | 0.655 | 0.621 | 0.647 | 0.716 | 0.577 |

| Rats | Addiction Index | Brain Region | rno-miR-101a-3p | rno-miR-1188-3p | rno-miR-136-3p | rno-miR-137-3p | rno-miR-187-3p | rno-miR-192-5p | rno-miR-292-5p | rno-miR-381-3p | rno-miR-382-3p | rno-miR-448-3p | rno-miR-499-5p | rno-miR-551b-3p | rno-miR-582-3p | rno-miR-666-3p | rno-miR-764-5p | rno-miR-872-5p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A_Rat 1 | 1.116 | NAc | -0.667 | 0.630 | -0.377 | -0.412 | 0.924 | -0.870 | 0.534 | -0.417 | 0.067 | -0.246 | 0.121 | 0.100 | 0.140 | 0.076 | -0.376 | -0.319 |
| A_Rat 2 | -0.556 | NAc | -2.222 | 0.577 | -1.308 | -2.016 | -0.381 | -1.787 | -0.032 | -1.168 | -1.892 | -0.910 | -2.265 | -1.073 | -1.660 | 1.199 | 0.564 | -2.278 |
| A_Rat 3 | 0.087 | NAc | 1.027 | 0.087 | 0.885 | 0.435 | -0.667 | 0.401 | -1.266 | 0.647 | 0.615 | 0.315 | 1.296 | 0.119 | 0.278 | -0.585 | -0.299 | 0.663 |
| A_Rat 4 | 2.016 | NAc | 1.833 | -1.245 | 0.704 | 1.957 | 0.135 | 2.251 | 0.588 | 0.832 | 1.005 | 0.808 | 0.540 | 0.887 | 1.237 | -0.618 | 0.075 | 1.940 |
| B_Rat 1 | -0.299 | NAc | 0.908 | 0.424 | 0.292 | -0.105 | 0.514 | -0.067 | 1.573 | 0.392 | -0.673 | 0.440 | 0.267 | 1.467 | -0.956 | 0.519 | 0.160 | -0.113 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B_Rat 2 | 1.759 | NAc | -1.595 | -0.175 | -1.284 | -1.174 | 0.405 | -1.204 | 1.699 | -0.768 | -0.633 | -0.724 | -1.133 | -1.216 | 0.062 | 0.577 | 0.106 | -1.021 |
| B_Rat 3 | -0.170 | NAc | 0.856 | -0.212 | 0.979 | 0.549 | -0.167 | 1.595 | -0.863 | 0.845 | 0.136 | -0.078 | 0.894 | 0.086 | 0.168 | -1.169 | -0.153 | 0.644 |
| B_Rat 4 | 0.988 | NAc | -0.159 | -0.062 | 0.209 | 0.808 | -0.756 | -0.298 | -2.444 | -0.399 | 1.224 | 0.398 | 0.093 | -0.169 | 0.786 | 0.001 | -0.132 | 0.529 |
| | | Partial correlation of Z ESC with miRNA expression levels in the nucleus accumbens (NAc): | | | | | | | | | | | | | | | | |
| | | estimate | 0.094 | -0.627 | -0.087 | 0.390 | 0.315 | 0.203 | 0.212 | -0.011 | 0.510 | 0.216 | 0.076 | -0.060 | 0.744 | -0.156 | -0.261 | 0.402 |
| | | statistic | 0.211 | -1.801 | -0.196 | 0.946 | 0.743 | 0.464 | 0.484 | -0.024 | 1.325 | 0.494 | 0.170 | -0.134 | 2.487 | -0.354 | -0.604 | 0.983 |
| | | *p*-value | 0.841 | 0.132 | 0.852 | 0.387 | 0.491 | 0.662 | 0.649 | 0.982 | 0.242 | 0.642 | 0.872 | 0.899 | 0.055 | 0.738 | 0.572 | 0.371 |

IL-PFC: Infralimbic prefrontal cortex; PL-PFC: Prelimbic prefrontal cortex; NAc: Nucleus accumbens.

Normalized expression levels of miRNAs are listed under the name of each miRNA.