Supplemental Information

Prophylactic ketamine alters nucleotide and neurotransmitter metabolism in brain and plasma following stress

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FIGURE LEGENDS

Figure S1. Positive mode metabolites changed in the PFC following prophylactic ketamine administration and CFC stress. (n = 9-10 male mice per group). Error bars represent ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S2. Negative mode metabolites changed in the PFC following prophylactic ketamine administration and CFC stress. (n = 9-10 male mice per group). Error bars represent ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S3. Positive and negative mode metabolites changed in the HPC following prophylactic ketamine administration and CFC stress. (n = 9-10 male mice per group). Error bars represent ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S4. Prophylactic ketamine does not impact freezing behavior, and does not significantly alter purine or pyrimidine metabolism in the PFC and HPC. **(A)** Experimental design. **(B-C)** Prophylactic ketamine does not alter context exposure or context re-exposure as measured by freezing when compared with prophylactic saline administration. Only 3 purine metabolites are significantly altered by ketamine administration in one hemisphere of the **(D-J)** PFC or **(K-O)** HPC. Only 2 pyrimidines are significantly altered by ketamine administration in one hemisphere of the **(P-T)** PFC, or **(U-W)** HPC. (n = 8-9 male mice per group). Error bars represent ± SEM. * $p < 0.05$, ** $p < 0.01$. Sal, saline; K, ketamine; RE, re-exposure; HPC, hippocampus; PFC, prefrontal cortex; Sac, sacrifice; QToF, quadrupole time-of-flight; LCMS, liquid chromatography mass spectrometry; min, minutes; dUDP, deoxyuridine-diphosphate.

Figure S5. Prophylactic ketamine without CFC stress does not significantly alter purine or pyrimidine metabolism in plasma. **(A-J)** Purine metabolites were not significantly changed following prophylactic ketamine administration. **(K-O)** Pyrimidine metabolites were not significantly changed following prophylactic ketamine administration. (n = 8-9 male mice per group). Error bars represent ± SEM.

Table S1. Statistical analysis summary for CFC data, weights of HPC and PFC samples used for analyses, and statistical analysis summary for metabolomics data.

Table S2. Prophylactic ketamine prior to CFC stress results in altered purine and pyrimidine metabolism**.** Of the 8 metabolites changed following prophylactic ketamine and CFC stress, 6 metabolites were changed in the same direction in both hemispheres of the PFC and HPC and these metabolites were primarily involved in purine and pyrimidine biosynthesis.

Table S3. Amino acid-derived neurotransmitters and precursors are significantly changed following prophylactic ketamine and CFC stress in the PFC, HPC, and plasma. Amino-acid derived neurotransmitters and their precursors were next analyzed for alterations following prophylactic ketamine administration and CFC stress. Interestingly, in the PFC and HPC almost all inhibitory neurotransmitter metabolites were increased (e.g. alanine, gamma-aminobutyric acid (GABA), taurine) and nearly all excitatory neurotransmitter metabolites were decreased (e.g. serine, tryosine, and phenylalanine). The main exception to this observation was glutamic acid, a precursor to GABA. Therefore, it is possible that the increase in glutamic acid is directly related to the increase in GABA. These data suggest that prophylactic ketamine may increase inhibitory tone in the brain following administration, resulting in long-lasting protection. As performed on brain tissue, metabolomic profiling was

performed on plasma samples. A number of amino acid-derived neurotransmitters and precursors were altered following prophylactic ketamine and CFC stress. Interestingly, as in the brain tissue, two excitatory neurotransmitters serine and glutamic acid were decreased. However, unlike in the brain tissue, GABA, an inhibitory neurotransmitter, was decreased. These data suggest that metabolite profiles of plasma samples may indicate effectiveness of prophylactic treatment.