SUPPLEMENTAL FIGURES for

Modeling gene x environment interactions in PTSD
using glucocorticoid-induced transcriptomics in human neurons

AUTHORS: Michael S. Breen[1,2,3]*, Tom Rusielewicz[4]*, Heather N. Bader[1,5]*, Carina Seah[3,6,7,8]**, Changxin Xu[1,5]**, Christopher J. Hunter[4], Barry McCarthy[4], Mitali Chattopadhyay[1,5], Frank Desarnaud[1,5], Iouri Makotkine[1,5], Janine D. Flory[1,5], Linda M. Bierer[1,5], Migle Staniskyte[1,5], NYSCF Global Array Team[4], Scott A. Noggle[4], Daniel Paull[4#], Kristen J. Brennand[1,2,3,6,7,8,9#], Rachel Yehuda[1,5#]

AFFILIATIONS: [1]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, [2]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, [3]Pamela Sklar Division of Psychiatric Genomics, Icahn School of Medicine at Mount Sinai, New York, New York, 10029 USA; [4]The New York Stem Cell Foundation Research Institute, New York, New York, 10019 USA; [5]James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA; [6]Nash Family Department of Neuroscience, Friedman Brain Institute, [7] Black Family Stem Cell Institute, [8]Graduate School of Biomedical Science, Icahn School of Medicine at Mount Sinai, New York, NY 10029; [9]Department of Psychiatry, Yale University School of Medicine, New Haven CT 06511.

* Authors share first authorship
** Authors share second authorship
# Authors share last authorship

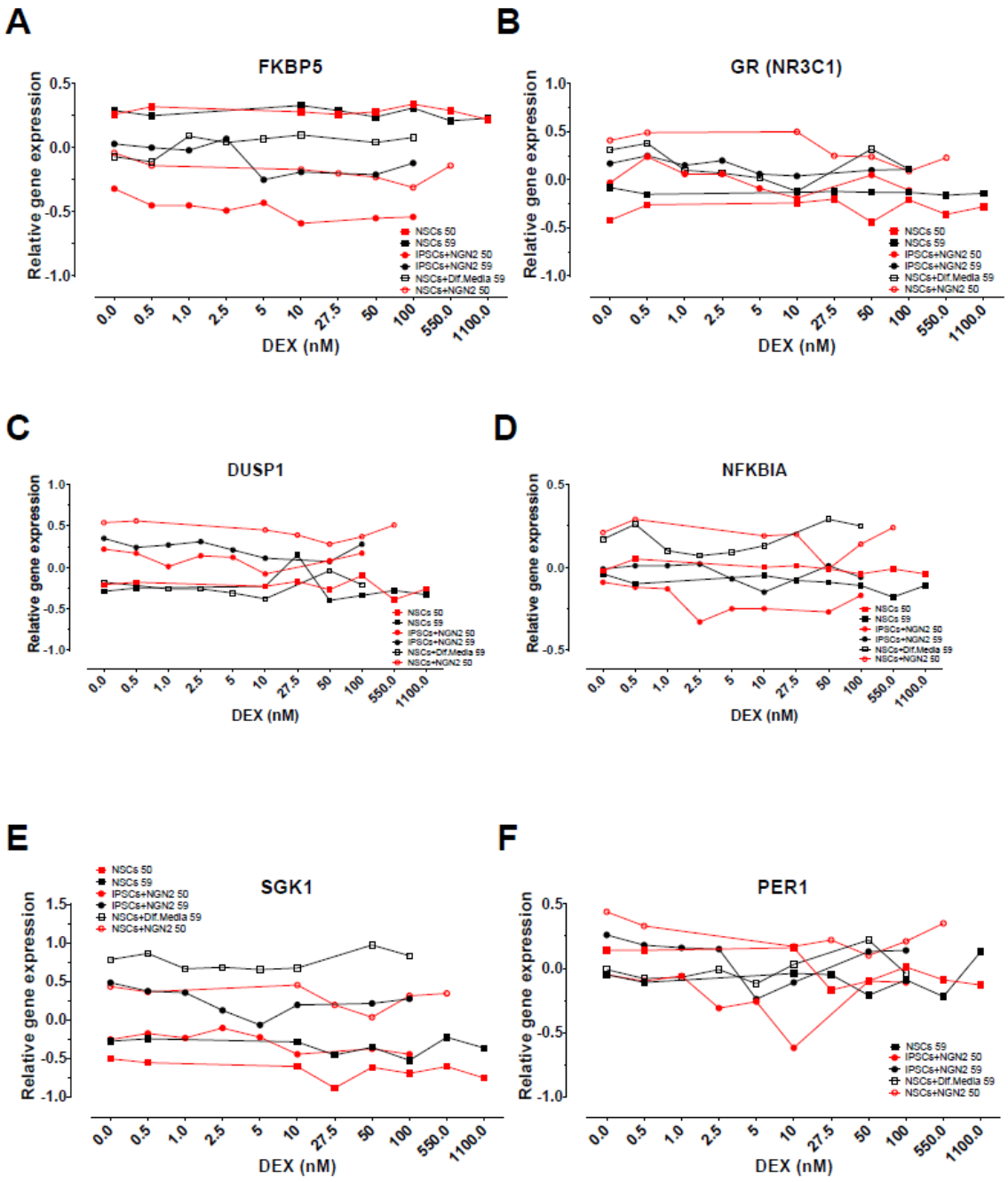

Figure S1. qPCR assays of DEX-stimulation in neuronal cells. (A-F) The expression of six glucocorticoid regulatory genes (y-axis) were examined across 11 distinct doses of DEX (x-axis). Four different cells were evaluated: i) Neural stem cells (NSCs), created using a commercial neural stem cell induction kit (A1647801, Life Technologies); ii) NSCs (as described in (1)) were treated with NGN2 lentivirus for conversion into neuronal populations; iii) hiPSC NGN2-derived glutamatergic neurons; iv) and a control neuronal cell line derived from the NSCs with a basic neuron conversion media composed of Neurobasal + B27 + DAPT (NSCs + alternative media).

Figure S2

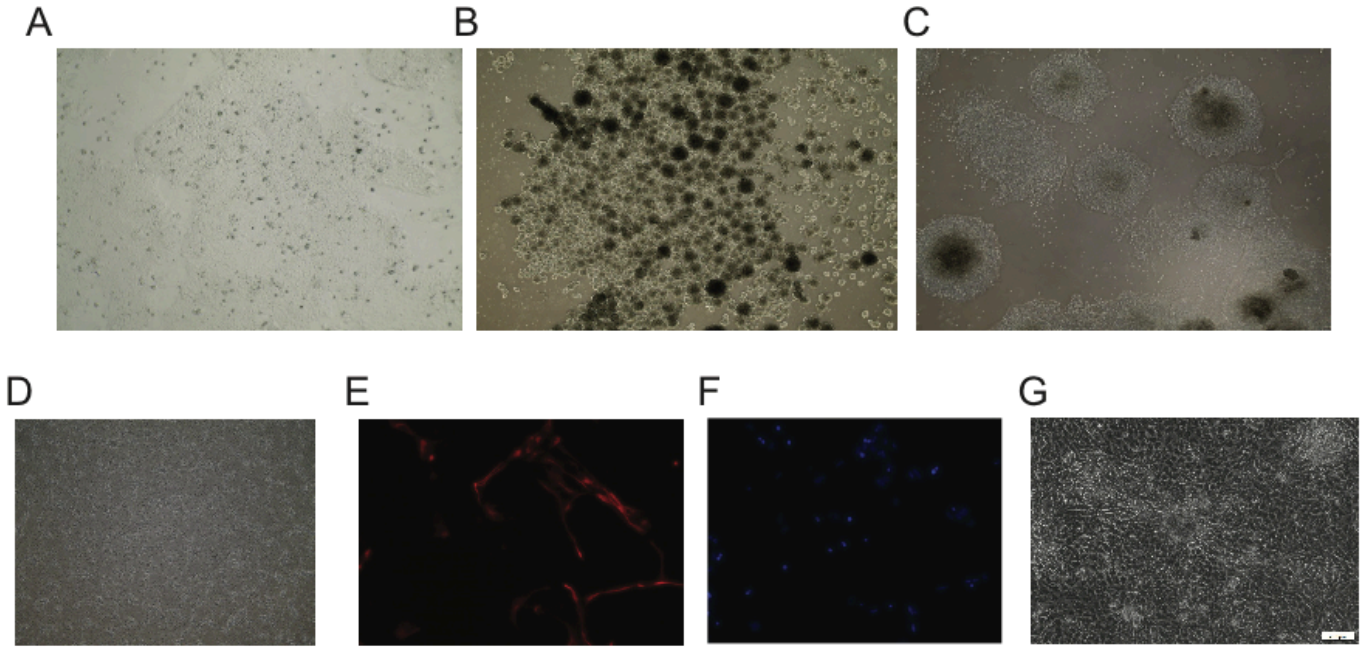

Figure S2. Immunostaining of hiPSC-derived mixed FB neurons. (**A**) Expansion of PTSD hIPSCs. (**B**) EBs in Stemflex Medium in N2/b27 DS medium. (**C**) Neural Rostte formation in Porn-Laminin coated plate. (**D**) NPC P0 after MACs sorting. (**E**) NPC NESTIN expression (red), indicating a forebrain-type identity. (**F**) Nuclei are labeled with DAPI (blue). (**G**) Six weeks matured Mixed forebrain before Hydrocortisone treatment. Scale bar = 200 μm.

Figure S3

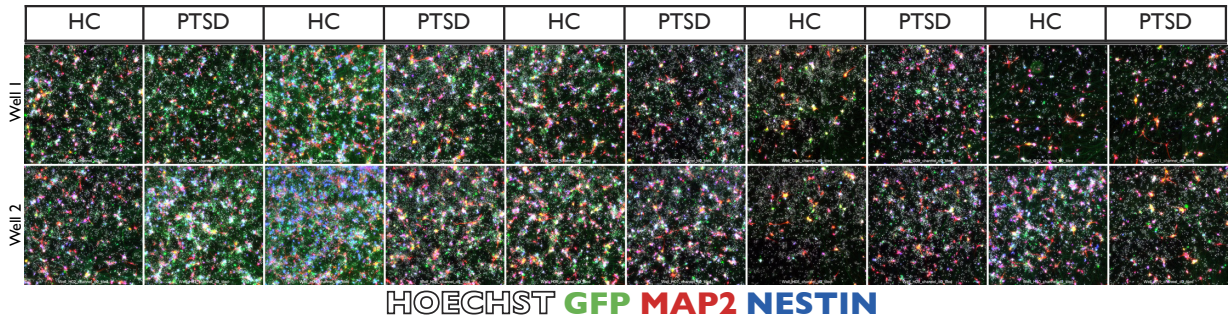

Figure S3. Immunostaining of hiPSC-derived *NGN2*-neurons. Immunostaining of HOECHST (white), GFP (green), MAP2 (red) and NESTIN (blue) across two wells for all 10 participants.

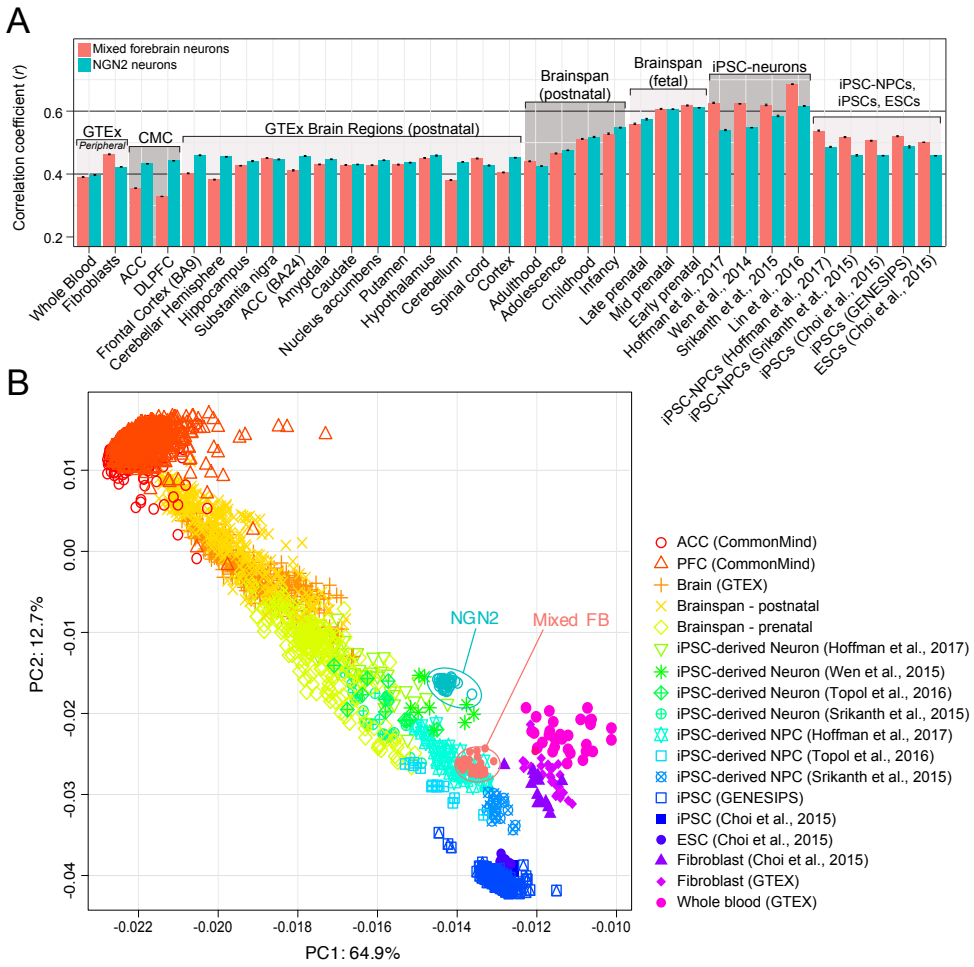

Figure S4. Developmental specificity of hiPSC-derived *NGN2*-and mixed forebrain neuron transcriptomes. We sought to confirm the developmental origin of our samples by integrating several RNA-seq data sets from postmortem brain tissue andhiPSC models. A total of 16 independent studies were collected covering 2716 independent samples and 12,140 genes. (**A**) We correlated full transcriptome profiles of our hiPSC-derived *NGN2*-(blue) and mixed forebrain neurons (red) to each of these independent samples and plotted the correlation coefficients across each cell type, brain region and developmental period. (**B**) Following standardized data pre-processing procedures, principal component analysis (PCA) stratified all gene expression samples into a distinct developmental axis starting with early embryonic stem cells (lower right) and subsequently moving into hiPSC-NPCs and hiPSC-neurons, and into prenatal and postnatal postmortem brain samples. Embryonic stem cells (ESCs) and hiPSCs clustered separately from hiPSC-NPCs and hiPSC-neurons, which in turn co-clustered with early prenatal brain samples. Notably, our hiPSC-neurons co-cluster with other hiPSC-neurons generated from previous reports, confirming their early developmental gene expression profiles.

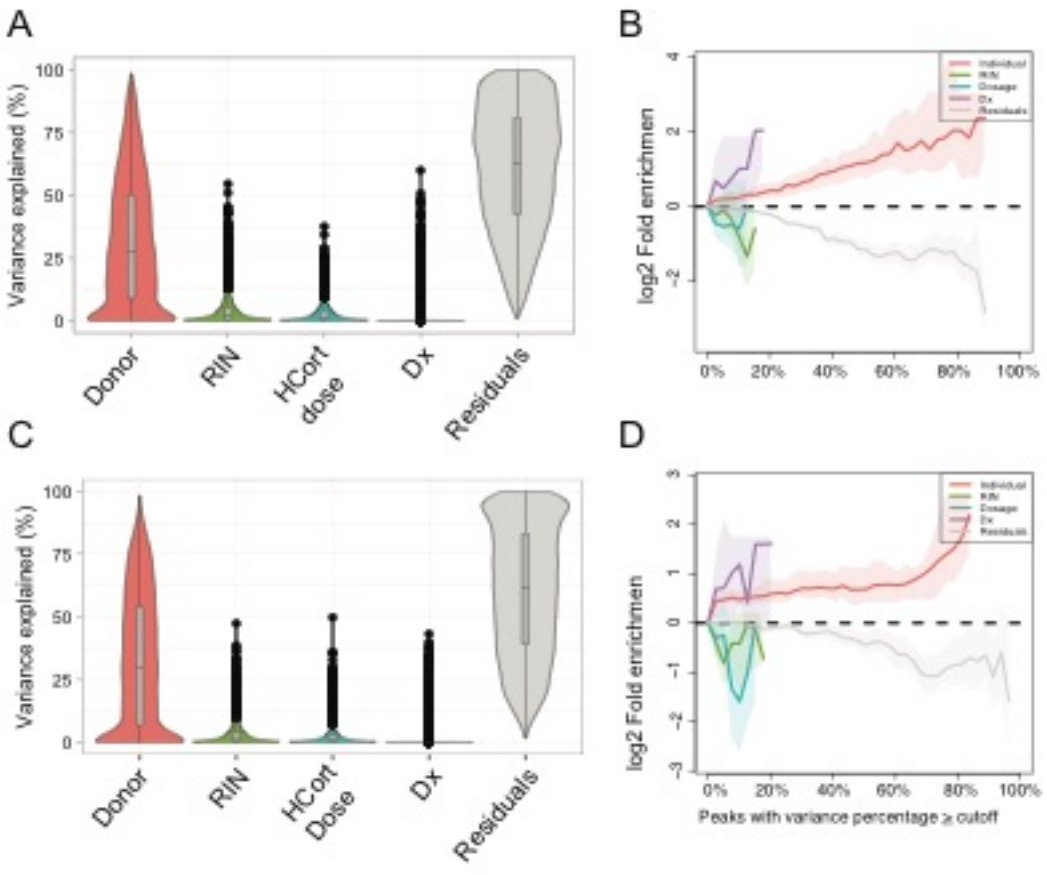

Figure S5. Transcriptome variance explained within each cell type. Violin plots display median transcriptome variance explained by donor as a repeated measure, RIN, PTSD diagnosis (Dx) and residuals in (A) *NGN2*-neurons and (C) mixed FB neurons. Genes that vary most across donors are enriched for brain cis-eQTLs in (B) *NGN2*-neurons and (D) mixed FB neurons. Fold enrichment (log_2) for the 2000 top cis-eQTLs discovered in post mortem dorsolateral prefrontal cortex data generated by the large brain meta-eQTL analysis (Sieberts *et al.,* 2020) shown for six sources of variation, plus residuals. Each line indicates the fold enrichment for genes with the fraction of variance explained exceeding the cutoff indicated on the *x*-axis. Enrichments are shown on the *x*-axis until less than 100 genes pass the cutoff.

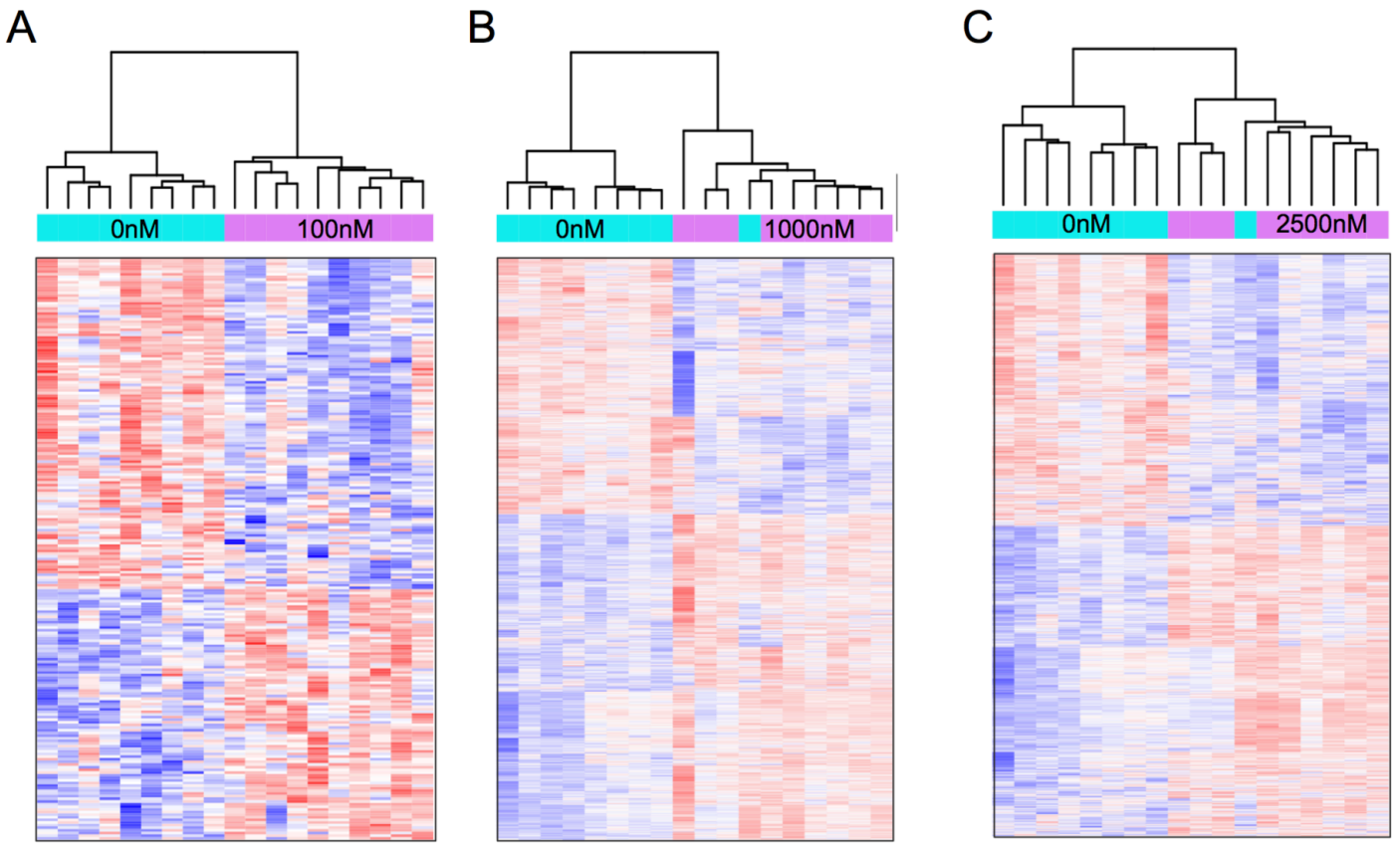

Figure S6. Preservation of HCort response across all donors. Heatmaps of differentially expressed genes (rows) that are independent of PTSD diagnosis across (**A**) 100nM of HCort, (**B**) 1000nM of HCort and (**C**) 2500nM of HCort. Blue indicate 0nM HCort and purple indicates HCort treated samples. Normalized expression is z-scale: red is high and blue is low (relative).

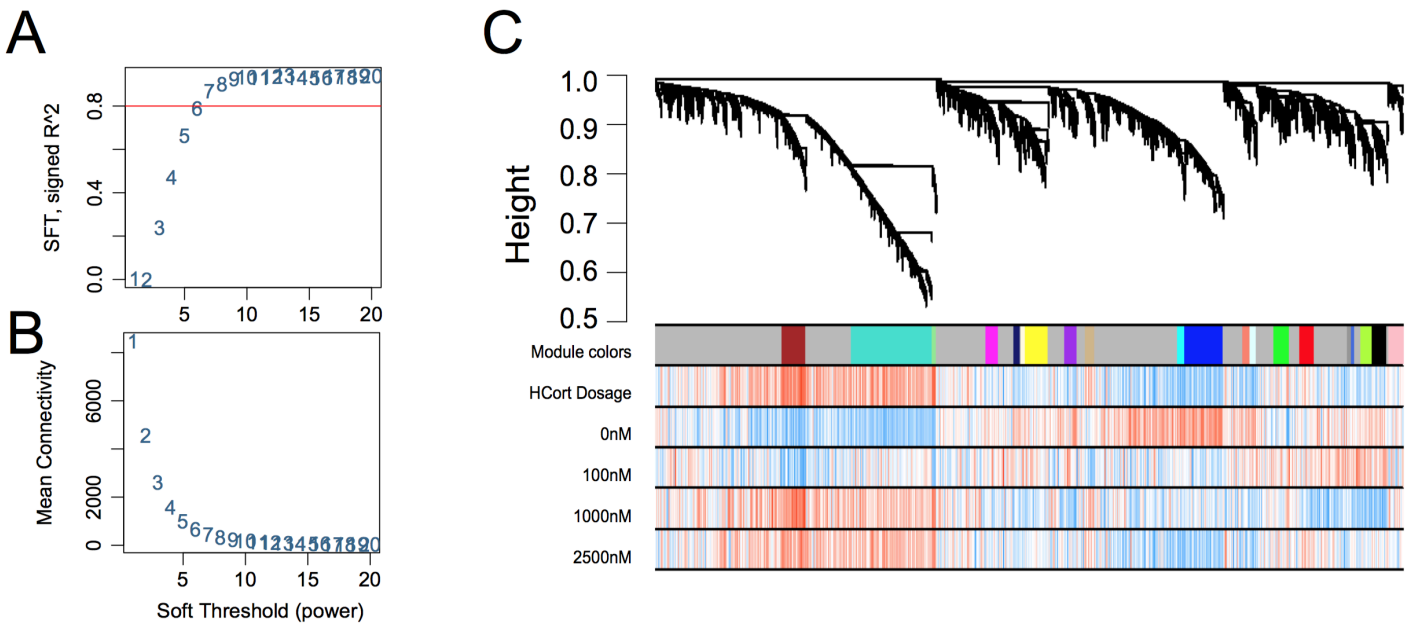

Figure S7. Weighted gene co-expression network analysis of *NGN2*-neurons. The β-power required to (**A**) satisfy scale free topology (SFT) and (**B**) corresponding mean connectivity in hiPSC-*NGN2*-neurons for gene co-expression network construction. As a rule of thumb, β-power's > 0.8 achieve scale free network topology, and a final β-power of 7 was used hiPSC-*NGN2*-neurons. **(C)** Hierarchical gene cluster tree and module structure and gene-HCort treatment color bands. The first color band underneath the tree indicates the 21 detected modules and subsequent bands indicate gene-HCort treatment correlation, when red indicates a strong relationship and blue indicates a strong negative relationship.

Figure S8

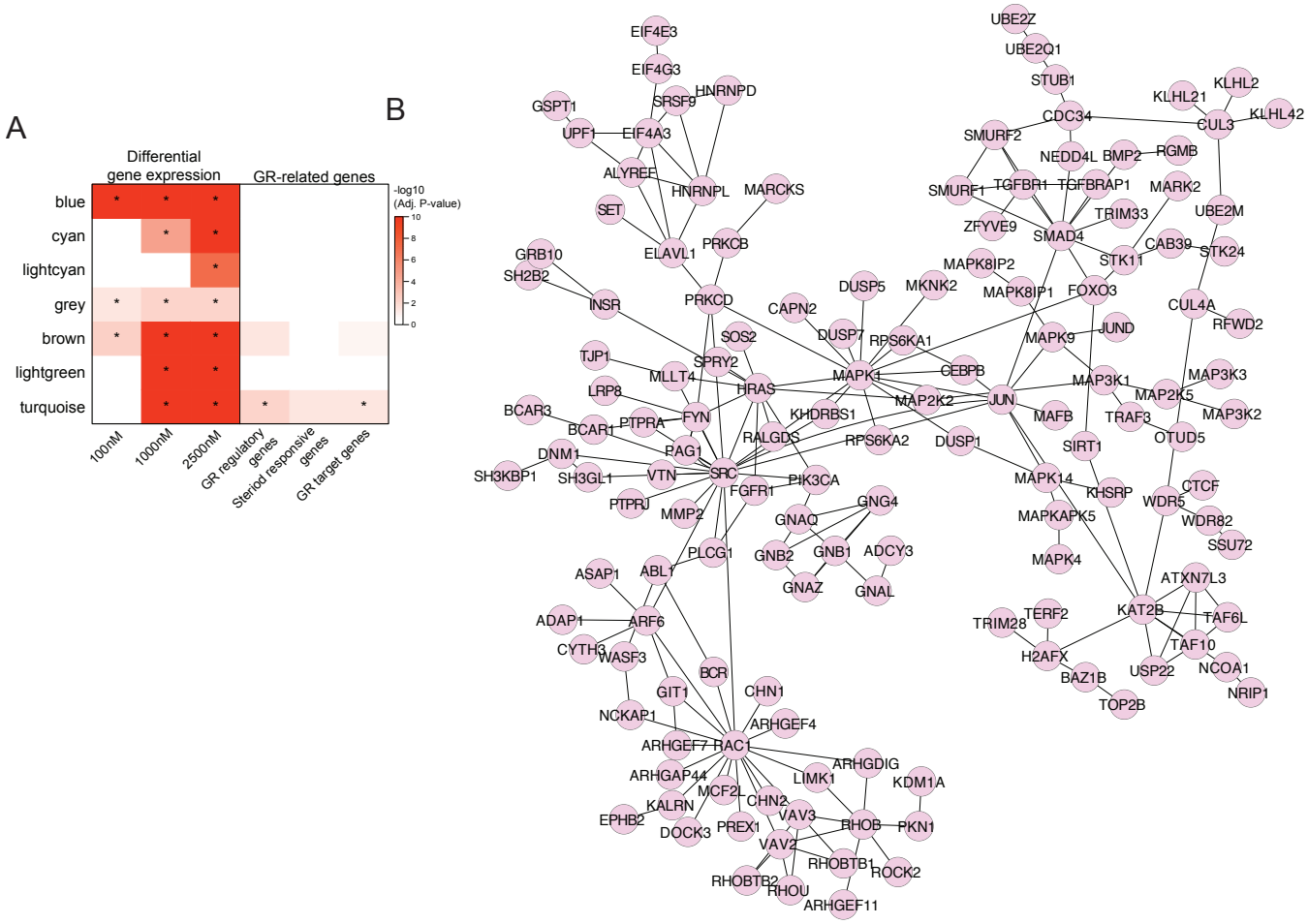

Figure S8. Characterization of *NGN2*-modules. (**A**) Enrichment of *NGN2*-co-expression modules for differentially expressed genes following HCort treatment (relative to 0nM) and a series of glucocorticoid receptor (GR) regulatory genes and ChiP-Seq binding sites. Enrichment was assessed using a Fisher's exact test and Bonferroni multiple test adjusted for the total number of modules. (**B**) Hub gene network for the turquoise module that is enriched for differentially expressed genes and GR-binding sites in neurons.

Figure S9

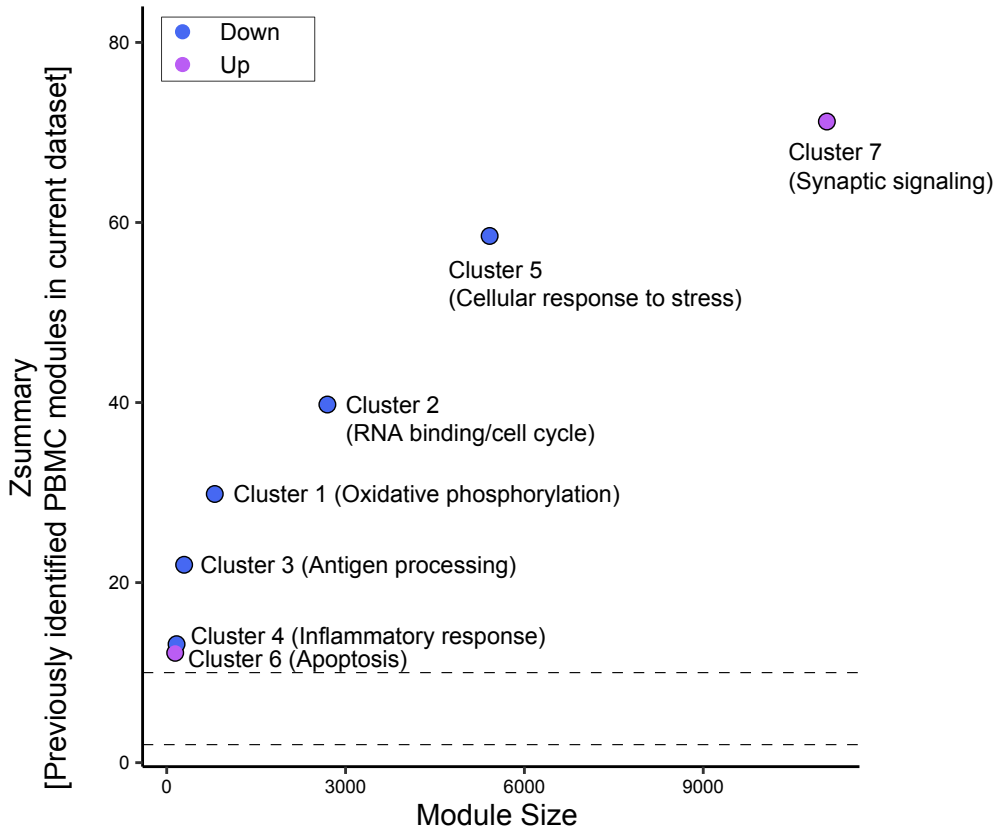

Figure S9. Gene set preservation analysis of PBMC responsive modules. Gene-set preservation analyses tested 7 previously identified co-expression modules that are significantly and dynamically regulated by dexamethasone (DEX) (Breen et al., 2019) for preservation in the current sample of 5 PTSD+ and 5 PTSD- donors. $Z_{summary}$ (y-axis) greater than 10 indicates strong preservation of all co-expression modules in these independent data. Module size is displayed on x-axis.

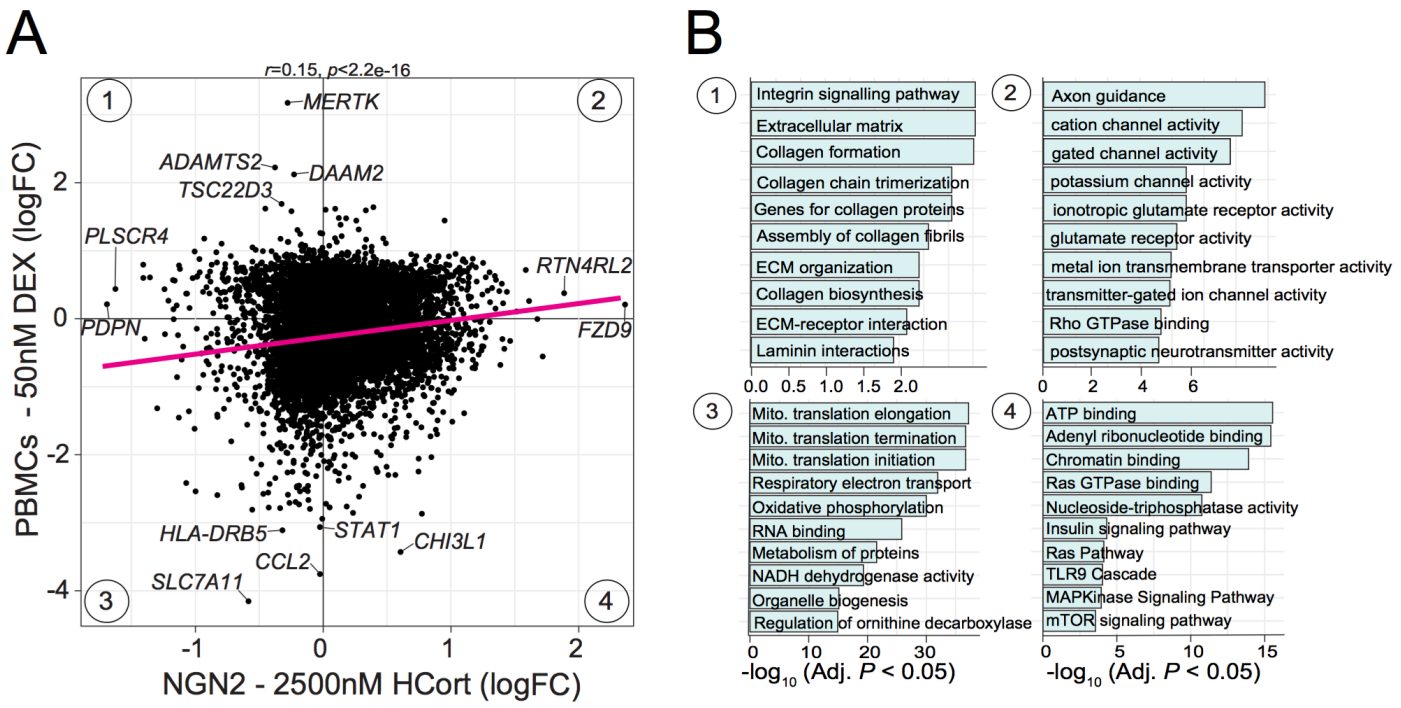

Figure S10. Concordance of glucocorticoid-stimulated gene expression between neurons and PBMCs. (**A**) A scatterplot showing the positive direction of effect of changes in expression observed in PBMCs to 50nM DEX (y-axis) to changes in expression in *NGN2*-neurons following 2500nM HCort. Four quadrants of genes were curated and subjected to functional annotation. (**B**) Genes that were up-regulated in PBMCs but down-regulated in neurons were implicated in extracellular matrix terms and processes. Genes that were up-regulated in PBMCs and neurons were implicated in axon guidance, potassium channel activity, Rho GTPase binding and postsynaptic neurotransmitter activity. Genes that were down-regulated in PBMCs and neurons were implicated in mitochondrial translation elongation, termination and initiation, as well as NADH dehydrogenase activity. Genes that were down-regulated in PBMCs but up-regulated in neurons were implicated ATP binding, chromatin binding and several inflammatory pathways, including Ras pathway, TRL9 cascade, MAPKinase signaling and mTOR signaling pathway.

Figure S11

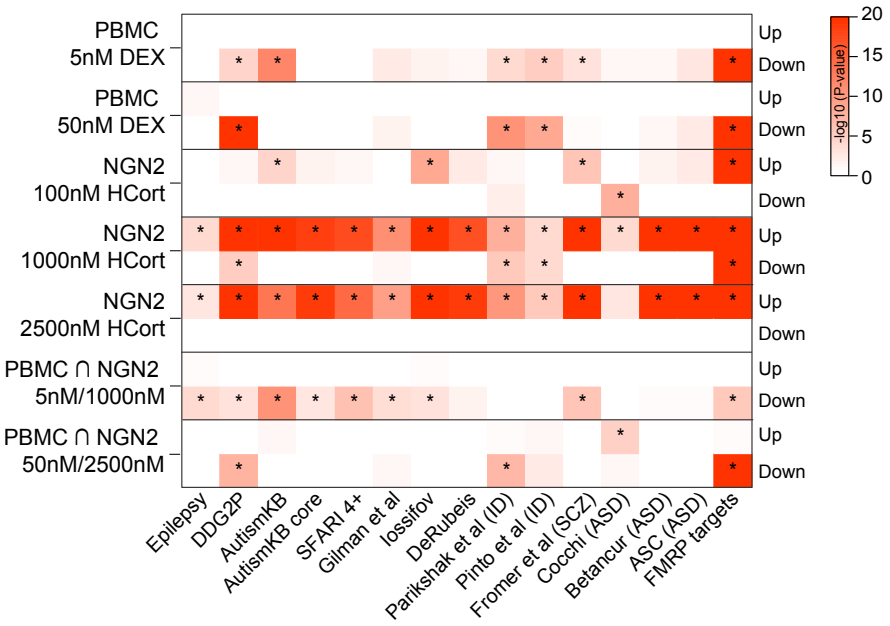

Figure S11. Enrichment of neurodevelopmental risk loci. Differentially expressed genes in both PBMCs and *NGN2*-neurons as a result of DEX and HCort treatment, respectively. Genes are parsed into those that are up- and down-regulated and tested for enrichment for 15 lists of neurodevelopmental risk loci. Enrichment was tested using a Fisher's exact test and adjusted for multiple comparisons using Bonferroni correction. Asterisks (*) indicate a significant enrichment (Adj. P<0.05)