Supporting Information

Mehta et al. 10.1073/pnas.1217750110

SI Materials and Methods

RNA Extraction and Microarray Procedures. Whole blood was collected between 8:00 and 9:00 AM under fasting conditions in Tempus RNA tubes (Applied Biosystems). RNA was isolated using the Versagene kit (Gentra Systems), quantified using the Nanophotometer, and quality checks were performed on the Agilent Bioanalyzer. Only samples with RNA integrity numbers of ≥6 with clear 18S and 28S peaks on the Bioanalyzer were used for amplification, the average RNA integrity number was 7.87 (SD of 1.1). Next, 250 ng total RNA was reverse-transcribed and biotin-labeled using the Ambion kit (AMIL1791; Applied Biosystems), 750 ng of cRNA were hybridized to Illumina HT-12 v3.0 arrays (Illumina) and incubated overnight for 16 h at 55 °C. Arrays were washed, stained with Cy3 labeled streptavidin, dried and scanned on the Illumina BeadScan confocal laser scanner.

The 450 k Methylation Array Procedures. Genomic DNA was isolated from whole blood stored in EDTA tubes using the Gentra Puregene Kit (Qiagen). The DNA was quantified using the PicoGreen (Invitrogen) and the quality was checked on an agarose gel. A total of 1,000 ng DNA was treated with sodium bisulfite using the Zymo EZ-96 DNA Methylation Kit (Zymo Research) according to the manufacturer's protocol. The methylation assay was performed on 4 μL bisulfite-converted genomic DNA at 50 ng/μL in accordance with the Infinium HD Methylation Assay protocol. The DNA was amplified, fragmented and hybridized on the HumanMethylation 450k BeadChip (Illumina).

Separate Cohort Controls for Lymphocyte and Monocyte Cell Fractions. Data from 54 individuals [36 men and 18 women, mean (SD) age of 30 (1)] was used to interrogate potential correlations between the relative lymphocyte and monocyte fraction in whole blood and mRNA expression levels, while correcting for age and sex (2). The amount of $CD15–CD16^+$, $CD14^+$, $CD4^+$, and $CD8^+$ white blood cells was measured using FACS analysis from whole blood drawn at the same time-point as a Tempus RNA tube.

qPCR Procedures. cDNA was synthesized from 250 ng total RNA using SuperScript II Reverse Transcriptase (Invitrogen). qPCR was performed using the Universal Probe Library on the Roche LightCycler 480 (Roche Applied Science). Assays were designed using the Probe Finder Software (Roche Applied Science) and run in duplicates according to the manufacturer protocol, except for a total reaction volume of 10 μL. TBP was used as the endogenous control gene. Primer sequences are provided in Dataset S8.

DNA Methylation Validation Using Sequenom's EpiTYPER MassARRAY System. A total of 11 CpGs spanning two amplicon regions (SPON1 and TSPAN32) with a high density of CpGs were chosen for validation. Sequencing was performed at varionostic. Amplicons were designed using the Sequenom EpiDESIGNER software. All experimental procedures were performed under routine conditions as outlined in the Sequenom Methylation Analysis Application Note (www.sequenom.com/files/genetic-analysis-files/dna-methylation-pdfs/8876-007-r02_epityper-app-note) using the MassARRAY system from Sequenom. Robustness of the assay was tested using a pair of technical replicates (correlations: $r = 0.998$ for SPON1 and $r = 0.983$ for TSPAN32) for each and three controls (positive, negative methylation, and negative template control). Data analysis was performed by regressing the methylation percentage against the PTSD group using generalized linear models and adjusting for age, sex, ethnicity and substance abuse in R.

Pathway Analyses. The Ariadne Pathway Studio 8.0 (Ariadne Genomics/Elsevier) was used to deduce relationships between differentially expressed candidates using the Ariadne ResNet database based on cellular processes criteria using subnetwork enrichment analysis. Only categories with ≥five transcripts within a cellular process and relationships with ≥two references were considered were the analysis. Subnetwork enrichment analysis applies the Mann–Whitney version of gene-set enrichment analysis algorithm to each sub network to calculate P values. Results were corrected for multiple testing using the Bonferroni correction.

1. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 336(14):973–979.

2. Pace TW, et al. (2011) Increased peripheral NF-kappaB pathway activity in women with childhood abuse-related posttraumatic stress disorder. *Brain Behav Immun* 25(1):6–13.

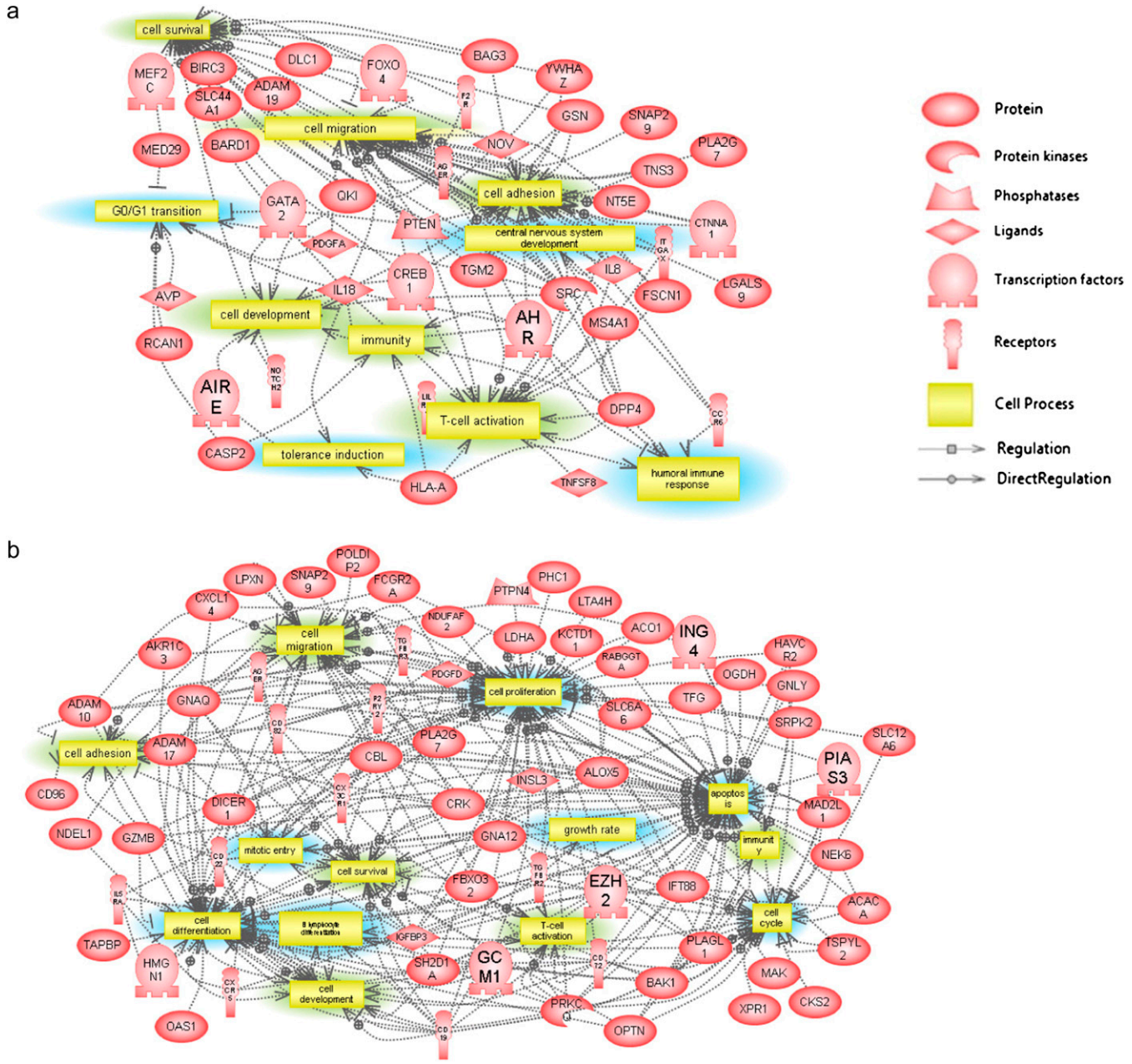

Fig. S1. Distinct biological networks enriched in child abuse vs. no child abuse groups. Significantly overrepresented cellular processes among transcripts in PTSD (*A*) with or (*B*) without child abuse using Pathway Studio after Bonferroni corrections for multiple testing. Details of the subnetwork cellular processes enrichment *P* values are shown in Dataset S3.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLS)
Dataset S5 (XLS)
Dataset S6 (XLS)
Dataset S7 (XLS)
Dataset S8 (XLS)