Expression of the *PPM1F* Gene Is Regulated by Stress and Associated With Anxiety and Depression

Supplemental Information

Supplemental Methods

Animals

All experiments were performed on adult (2–3 months old) wild-type C57BL/6J mice obtained from Jackson Labs. Male and female mice were group-housed in a temperature-controlled vivarium, with ad libitum access to food and water. They were maintained on a 12-h light/dark cycle, with all behavioral procedures being performed during the light cycle. All procedures conducted at Emory were approved by the Institutional Animal Care and Use Committee of Emory University and in compliance with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All procedures conducted at Universitat Autònoma de Barcelona were approved by the Committee of Ethics of the Universitat Autònoma de Barcelona and the Generalitat de Catalunya. They were also carried out in accordance with the European Communities Council Directive (2010-63-UE) and Spanish legislation (RD 53/2013).

Immobilization Stress

Immobilization Stress (IMO) procedures, as previously described (1-3), were conducted in a room separate from housing and behavioral paradigms. Each animal was immobilized by gently restraining their four limbs in a prone position to metal arms attached to a wooden board for 2 h in a brightly lit room, previously shown to lead to a very large and robust behavioral and physiological stress response (4). All cage-mate animals received the same treatment of IMO or handling. Handling lasted ~ 1 min per mouse and consisted of letting the mouse walk on top of their home cage and in the hands of the experimenter

wearing latex gloves. After treatment, mice where returned to their home cage where they remained undisturbed until fear-learning procedures or sacrifice for gene expression analysis (2).

Drugs

Corticosterone Fluka (WA11926) was used at a 40 mg/kg dose. Vehicle was ethanol 5% in 0.9% NaCl. Corticosterone or vehicle were given 1 hour after IMO.

Microarray Hybridization and Analysis

Mice were sacrificed under basal conditions (Home Cage *Control Group*) and 6 or 8 days after a 2 hour immobilization (IMO *Stress Group*). The two amygdala microarrays and the mPFC microarray were performed in the same lab at the same time but originally designed for two different studies (amygdala microarrays for one study and mPFC microarray for another study). The reason to have two amygdala microarrays was to replicate the findings on day 6 with those on day 8 and get more robust results. When we analyzed the amygdala microarrays and the human data replicating those results we thought that it would be interesting to consider the mPFC microarray we already had (6 days). This is why there are two amygdala microarrays and one mPFC microarray. Brains were immediately fresh frozen on dry ice and stored at -80°C. Amygdala and medial prefrontal cortex (mPFC) tissue from both hemispheres was extracted by 1mm micropunch and each structure from each mouse was individually stored. Total RNA was isolated and purified from the tissue with the RNeasy Mini Kit catalog # 74106 (Qiagen) following the manufacturer's instructions. We obtained ~2 ug RNA per side for a total of ~4 ug per brain. For the microarray, only amygdala tissue was used with 4 animals per condition. Electrophoresis assays and electropherograms were performed to ensure RNA quality with an Agilent 2100 BioAnalyzer PicoChip (Agilent Technologies) before the microarray. Illumina Mouse WG-6 v2 Expression BeadChip microarray (Illumina, Inc.) was assayed for 45,281 transcripts. RNA quality control, hybridizations and preliminary

data analysis were conducted at the Cancer Genomics shared resource, Winship Cancer Institute (Emory University). Data retuned were Pair-wise two-condition comparisons including relative levels of RNA, fold change, and p-value. Heat maps with hierarchical clustering were performed with GENE-E (Broad Institute, Inc.) version 3.0.204. We first performed the amygdala RNA microarrays, and the top genes regulated after stress were selected using these criteria: a) Probe present at least one time in both lists (6 and 8 days after stress), b) >1.5 fold change compared to control, c) significant difference at p<.05 (nominal p-value), d) clearly present at moderate to high levels in amygdala (search in the Allen Brain Atlas). These top genes are shown in Supplemental Table S1. After analyzing this amygdala microarray, the mPFC microarray was performed following similar methodology. Given the small number of biological replicates, there was low statistical power to detect genes differentially expressed at 1.5-fold, and hence we adopted a more liberal threshold of 1.30 for the mPFC genes, in the interest of increasing the likelihood of discovery genes in common with those in the amygdala. Hence, the criteria for highly regulated probes on this microarray were: a) >1.30 fold change (There are only 6 genes which survive a 1.5 cut-off criteria), b) significant at nominal p<.05 (5), c) clearly present at moderate to high levels in amygdala (based on *Allen Brain Atlas*). Our analysis performed without adjusting for correct for multiple testing increases the risk of false positives. This is why we performed different replications of the gene expression studies in different cohorts of mice.

Bioinformatics of the Microarray Analysis

The top genes regulated after stress in different microarrays in amygdala and mPFC were further analyzed with bioinformatics to understand their functions in the brain. Note that *Ppm1f* was the only top gene present in all the microarrays (Figure 1E); The list of the genes studied are highlighted in yellow or red in Supplemental Tables S1, S2, S4 and S5. The functional profiles of genes identified in the microarray analysis were analyzed with DAVID Bioinformatics resources 6.8. This DAVID analysis was performed with

these parameters in Functional Annotation Chart (Thresholds: Count 2, EASE 0.1; Display: Benjamini, #Records 10, see Supplemental Figure S10). The top three functional pathways altered after stress in the amygdala and mPFC are depicted in Figure 2A. The detailed functions of the gene lists (Figure 2B) and network analysis (Supplementary Figure S1) were generated through the use of QIAGEN's Ingenuity Pathway Analysis (IPA® QIAGEN Redwood City, www.qiagen.com/ingenuity). The IPA analysis was performed using these parameters: A) Direct and indirect relationships; B) All node types; C) Confidence, experimentally observed; D) All species; and E) All tissues and cell lines.

Reverse Transcription and PCR Quantification

Total RNA was isolated with RNeasy kit Catalog # 74106 (Qiagen) or Maxwell RSC simplyRNA Tissue Kit catalog # AS1390 (Promega). Gene expression changes in the amygdala tissue was detected by relative quantitative reverse transcription PCR (FAST 7500; Applied Biosystems, Foster City, CA). Bilateral tissue punches were performed using a 1mm micropunch. At the time the mice were killed, brains were placed on ice and rapidly blocked, and tissue punches were obtained for isolation of RNA. cDNA was obtained by reverse transcription using the RT2 First Strand Kit catalog # 330401 (Qiagen) or High-Capacity cDNA Reverse Transcription Kit catalog # 4368814 (ThermoFisher Scientific) according to the manufacturer's instructions. TaqMan assays (Applied Biosystems, Carlsbad, CA) were used to quantify the expression of *Ppm1f* Mm01344949_m1 (#4331182) and *Camk2g* Mm00618054_m1 (#4331182) normalized to mouse *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase)(1). Graphics are represented by fold change obtained with the 2^-ddCt method.

RT-PCR Arrays

RNA was isolated and reverse transcribed as outlined in the section above. See Supplemental Table S3 for details on the SYBR green primers used. We used a custom plate format 24 x 4 catalog #CAPM10412 (Applied Biosystems, Carlsbad, CA). Results were normalized with GAPDH values as a reference. Half of the brains were collected 6 days after stress and the other half 8 days after stress in the "6 and 8 days after IMO" group.

Immunohistochemistry

Immunohistochemistry was performed by first removing cryoprotectant solution with 3 KPBS washes. Unspecific binding was next achieved with blocking buffer (2% albumin and 2% fetal calf serum) incubated for one hour at room temperature. The primary antibodies used were PPM1F polyclonal antibody PA5-15572 (Invitrogen, 1:50) and Camk2g monoclonal IgG1 Antibody 8G10C1 (Santa Cruz, 1:50); antibodies were diluted in blocking buffer and incubated overnight at 4°C. After primary antibody incubation, slices were washed 3 times with KPBS. Secondary antibodies used were goat anti-rabbit IgG conjugated with Alexa Fluor 488 (Jackson Laboratories,1:500) and goat anti-mouse IgG1 A21124 (Invitrogen, 1:500); antibodies were diluted in blocking buffer and incubated in agitation for 2h at room temperature. After incubation, slices were washed three times with KPBS and finally nuclei were stained with DAPI at 1:1000 dilution.

Immunofluorescence images were visualized and captured using Aixo Examiner D1 Confocal microscope (Carl Zeiss) and Zen2010 software (Zeiss). Fluorescence signal was captured under a 40x magnification lens with immersion oil. Z-stacks of 5 slices each were obtained. Three lasers were used with varying intensities, 488nm (7.5%) for channel 1, 568nm (8.0%) for channel 2 and 405nm (2.0%) for channel 3. Images were obtained at a 1024x 1024 resolution and in 16 bit color format. Master gain was set at 850 units and digital offset at -3500 units for both channels.

Images were processed using IMARIS 8.0 software (Bitplane). To define PPM1F cells, we created a surface mask on channel 1 using the background subtraction function for a 0.500 um diameter area, intensity threshold value was set at 1750 and filtering discarded values below 1500. The surface mask was manually corrected for artifacts. After creating this mask, we made an overlay mask to delimitate channel 2 signals inside PPM1F cells. To define CaMK2g positive cells we created a spots mask on the previously masked channel 2, XY diameter of the spots was set to 1um, Z diameter to 2um and threshold manually set at 4500. To extract the data we created a cell mask that was merged with the previously created masks. Mean fluorescence values for Ppm1f and Camk2g were averaged by photograph for each brain area and compared depending on the group (IMO, Control). To calculate the colocalization ratio, a cutoff criterion of 2 vesicles (detected by IMARIS software) of Camk2g per cell was used to identify positive cells, ratios were compared between groups.

Mouse Behavioral Assays:

Elevated plus maze. The elevated plus maze consisted of two open arms (50 × 6.5 cm) and two closed arms with a wall (50 × 6.5 × 15 cm) attached to a common central platform (6.5 × 6.5 cm) to form a cross. The maze was elevated 65 cm above the floor. Mice received an 8 µl/g systemic saline intraperitoneal (i.p.) injection 1 hour after IMO (5) only for experiments in Figure 1. Test sessions lasted 5 minutes and behaviors were continuously recorded using a video camera placed over the apparatus. Activity was analyzed with a stopwatch by a researcher blind to the each mouse treatment. Arm entry was considered complete if all four paws entered a closed or open arm from the central platform.

*Tail suspension test***.** Mice were suspended with duct tape ~2 cm at the end of the tail. The session lasted for 6 minutes. Behavior was recorded by a video camera and a researcher blind to the experimental groups analyzed the immobility time. Mice that climbed on their tail were removed from the analysis.

Mouse fear conditioning. Mice were fear conditioned in standard rodent modular test chambers (ENV-008-VP; Med Associates Inc, St. Albans, VT) with an inside area of 30.5cm (L) x 24.1cm (W) x 21.0cm (H). The tone conditioned stimulus was generated by a Tektronix function generator audio oscillator delivered through a high-frequency speaker (Motorola, Model 948) attached to side of each chamber. The illumination was provided by white light. Mice received 5 trials of a conditioned stimulus (CS; 30 second tone, 6 kHz, 70 dB) co-terminating with a footshock (500ms, 0.6mA) unconditioned stimulus (US). The PreCS and intertrial interval (ITI) were 180 seconds. The apparatus was cleaned with Quatricide® after each mouse.

Human Study Participants

Participants were recruited from general medical care waiting rooms at Grady Memorial Hospital by the Grady Trauma Project (GTP), which investigates genetic and environmental factors contributing to PTSD, from a population of inner city, low income, and high stress and trauma exposure (6-8). Inclusion criteria consisted of being 18 or older, understanding English, and being able to give informed consent. Exclusion criteria included being acutely suicidal, psychotic, or having acute medical problems. Participants gave informed consent, completed a battery of psychological measures, and provided blood for genetic materials. This study was approved by the Institutional Review Board of Emory University School of Medicine and Grady Memorial Hospital.

This is a cross-sectional study. Trauma was ascertained by participants' retrospective self-report using the Traumatic Event Inventory (TEI). The TEI is a 14-item instrument to assess for lifetime history of traumatic events. Traumas include categories such as natural disaster, serious accident or injury, life-threatening illness, combat-related or military-related trauma, witnessing a family member or close friend being attacked or murdered, being attacked with a weapon, witnessing violence between parents/caregivers as a child, excessive physical punishment during childhood, emotional abuse during

childhood, and sexual abuse during childhood, and any other traumatic event that the participant experienced but not listed on the TEI.

PTSD symptoms were assessed with the modified PTSD Symptom Scale (PSS) (9). A PSS cutoff score of 14 yielded a 91% sensitivity and 62% specificity for identifying PTSD compared to the diagnosis of PTSD made by the clinician-administered PTSD scale (10). Hence, we defined PSS scores ³ 14 to indicate significant current PTSD symptoms. Depressive symptoms were measured with the Beck Depression Inventory (BDI) (11). Among African American (AA) participants, a BDI cutoff score of 14 yielded a 88% sensitivity and 84% specificity compared to diagnosis of major depressive disorder made by a focused interview using DSM-IV criteria (12). Given these data, we used a BDI score ³ 14 to indicate significant current depression. Cases with current symptoms of comorbid PTSD and depression (PTSD&Dep) were defined as having PSS³ 14 and BDI³ 14. Controls were defined as not having either PTSD or depressive symptoms, as reflected by a PSS £7 and BDI £7, despite have been exposed to trauma.

Statistics

For the mouse studies, GraphPad v5.0 or SPSS v20 were used for statistics. The results are presented as means ± or + SEM, statistical significance was set at $P \leq 0.05$. Analysis of the distribution of the data and detection of outliers were performed and removed when necessary. T-test (two tailed, type 2), one-way ANOVA or repeated measures ANOVA were used were appropriate. For Figure 5, Mann-Whitney U test was used for comparisons when one of the distributions did not reach the normality and/or homoscedasticity criteria. Post-hoc analysis used in Figure 7 were DMS.

Figure S1: Principal variance component analysis and volcano plots for mouse amygdala and mPFC microarrays at 6, 7, or 8 days post IMO stress. A) Amygdala expression 6 days after IMO; red circles denote stressed mice and blue circles control mice. B) Amygdala expression 8 days after IMO; red circles denote stressed mice, blue circles control mice. C) mPFC expression 6 days after IMO; red circles denote stressed mice, blue circles control mice. D) Volcano plot for amygdala microarray 6 days after IMO. E) Volcano plot for amygdala microarray 8 days after IMO. F) Volcano plot for mPFC microarray 6 days after IMO.

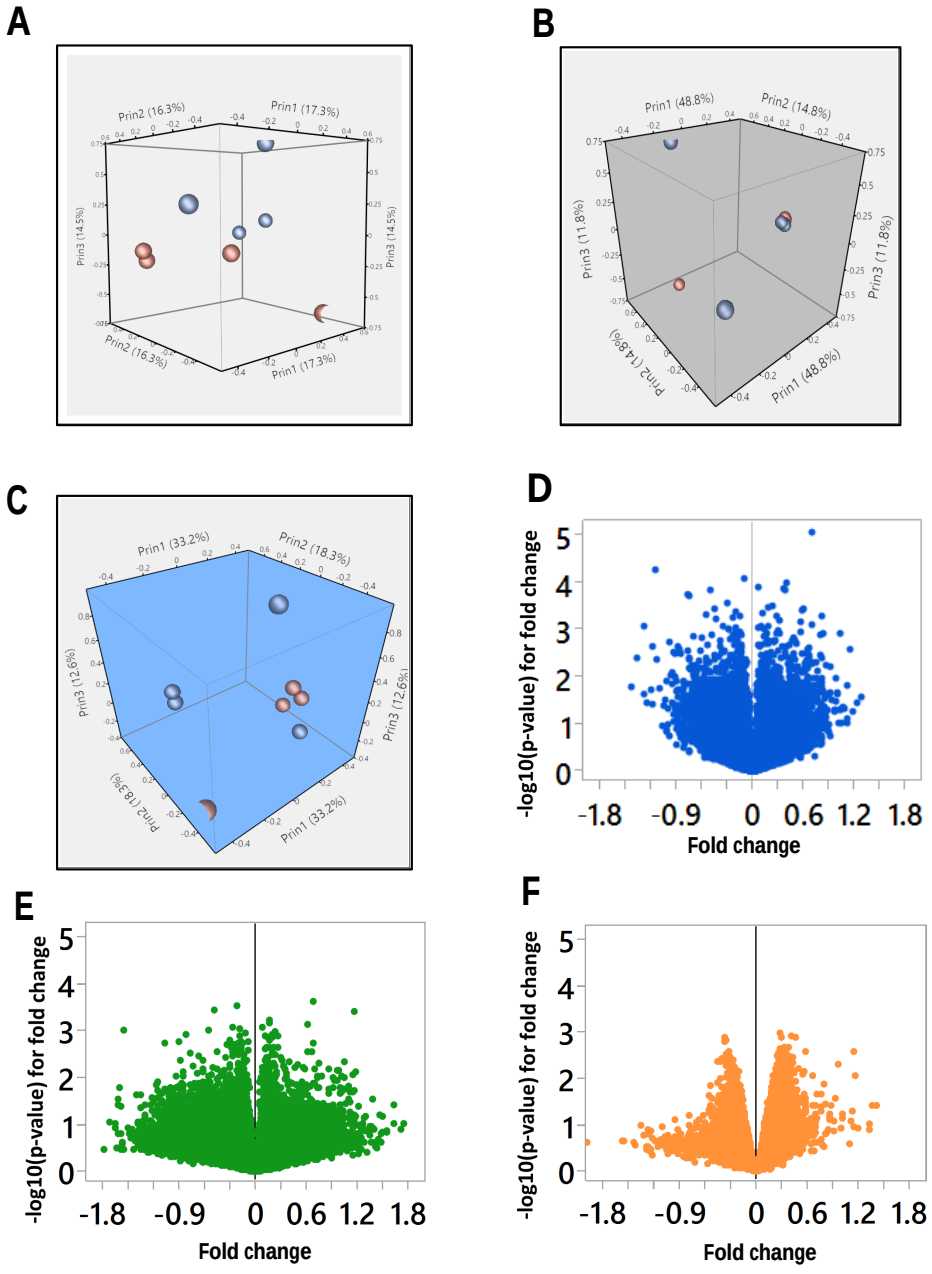
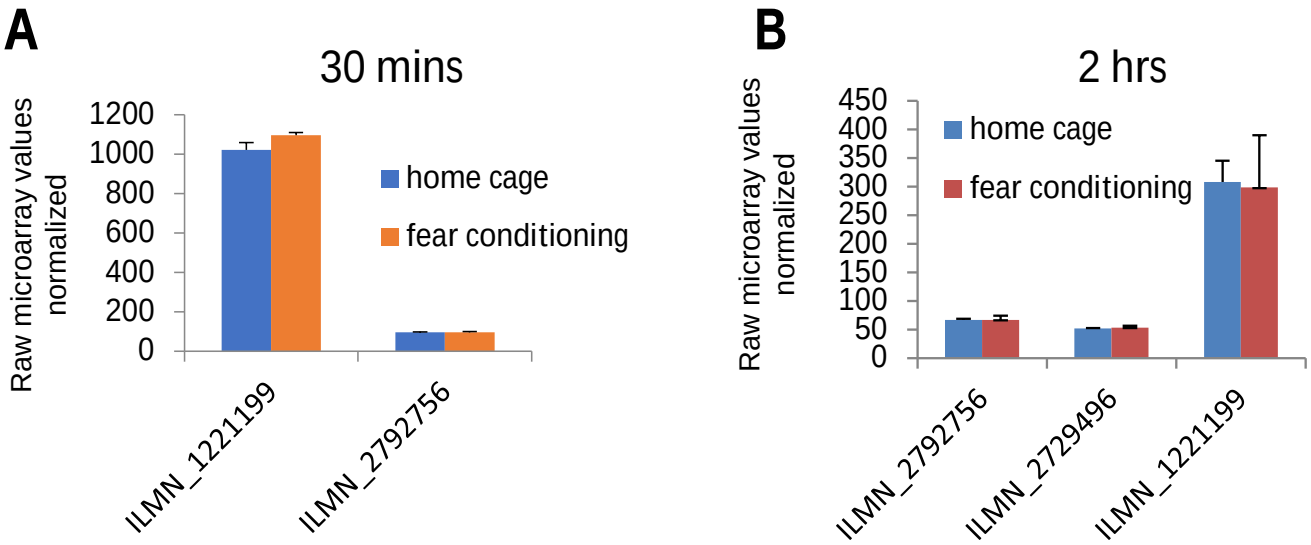


Figure S2. *Ppm1f* levels are not altered by fear conditioning.
Mice were exposed to cued-fear conditioning (FC) and sacrificed at the time points shown below for amygdala (A and B) and medial prefrontal cortext (mPFC, C) microarray studies. The control group was home cage which was not exposed to FC. Results showed that a mild stress such as FC did not alter *Ppm1f* mRNA levels in the amygdala or mPFC. N=4 per group.

Amygdala *Ppm1f* mRNA levels after fear conditioning



mPFC *Ppm1f* mRNA levels after after fear conditioning

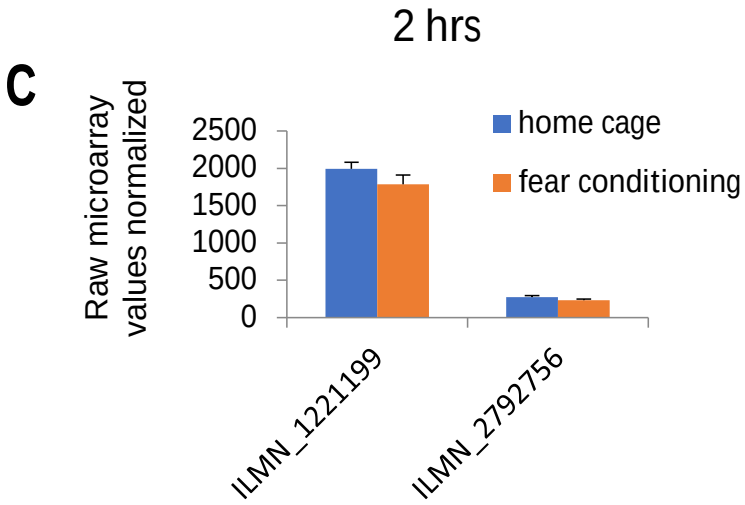


Figure S3. DAVID analysis. Functional analysis of the top genes regulated 6 and 8 days after stress in both the amygdala and mPFC. Results show that the Phosphoprotein, Cytoplasm and Alternative splicing are the three top categories involved in acute stress in mice.

Functional Annotation Chart

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	UP_KEYWORDS	Phosphoprotein	RT		80	32,0	1,9E-10	3,3E-8
	GOTERM_CC_DIRECT	cytoplasm	RT		68	27,2	5,2E-6	1,2E-3
	UP_KEYWORDS	Alternative splicing	RT		54	21,6	2,7E-7	1,5E-5
	GOTERM_CC_DIRECT	membrane	RT		54	21,6	7,8E-2	8,2E-1
	GOTERM_CC_DIRECT	nucleus	RT		53	21,2	7,1E-3	3,4E-1
	UP_SEQ_FEATURE	splice variant	RT		51	20,4	8,1E-5	3,5E-2
	GOTERM_MF_DIRECT	protein binding	RT		43	17,2	2,4E-4	3,2E-2
	UP_KEYWORDS	Nucleus	RT		38	15,2	1,3E-2	2,0E-1
	UP_KEYWORDS	Cytoplasm	RT		37	14,8	1,4E-2	1,9E-1
	UP_KEYWORDS	Coiled coil	RT		35	14,0	9,6E-5	3,3E-3

Figure S4. Network analysis of the top gene candidates regulated after stress in amygdala and the mPFC. PPM1F has direct relationships with the CaMKII family (blue arrow) and indirect relationship with BDNF, Creb, ERK1/2 and MAP2K1/2.

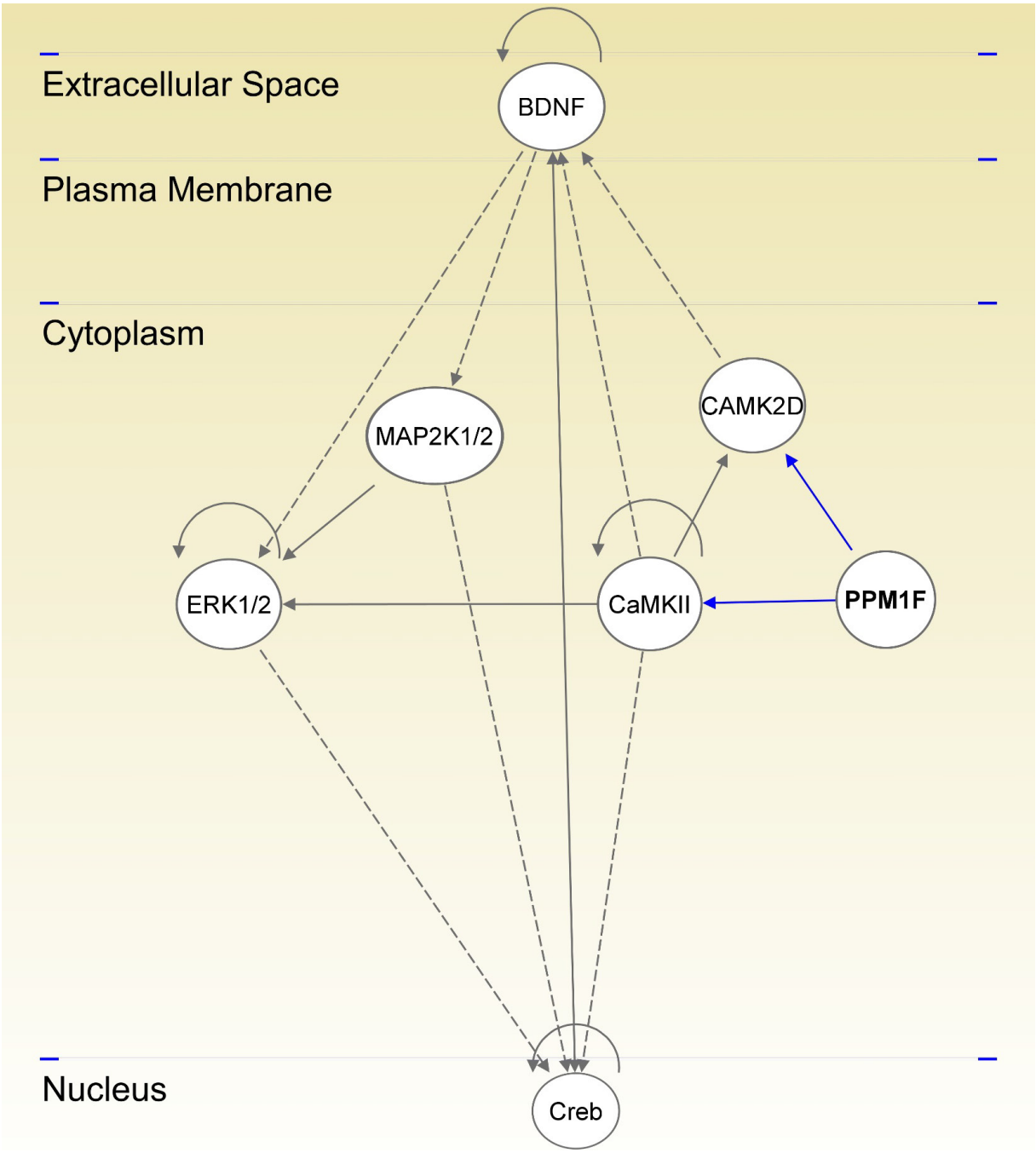
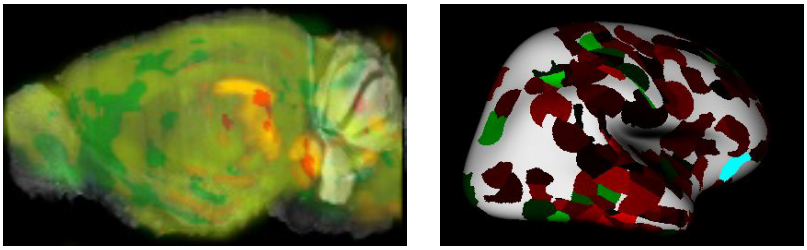


Figure S5. PPM1F expression in the brain. These images modified from the Allen Brain Atlas show that *PPM1F* mRNA expression in both mice and humans at basal levels is ubiquitous including the amygdala and the medial prefrontal cortex (mPFC). Legend of the images: Green = low expression; Yellow = moderate expression; Red = high expression. White arrow = amygdala; blue arrow = mPFC.

Expression of *PPM1F* in the Human and Mouse Brain



***PPM1F* expression in the Human and Mouse Brain mPFC and Amygdala**

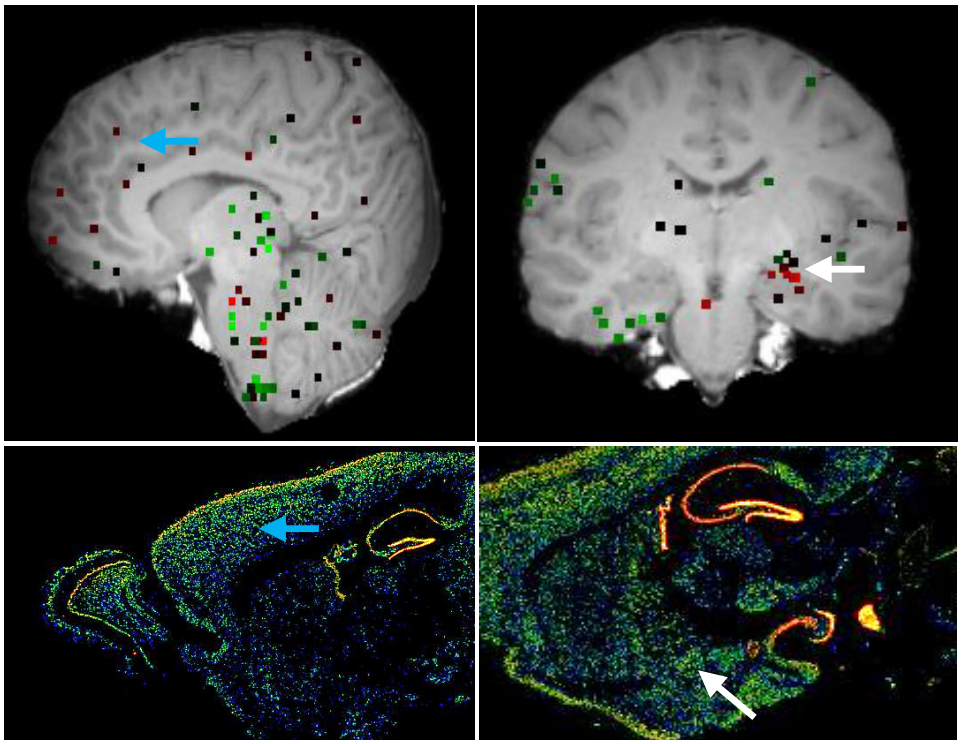
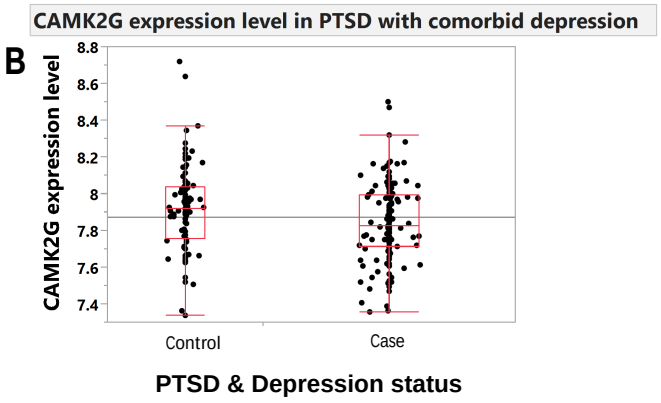


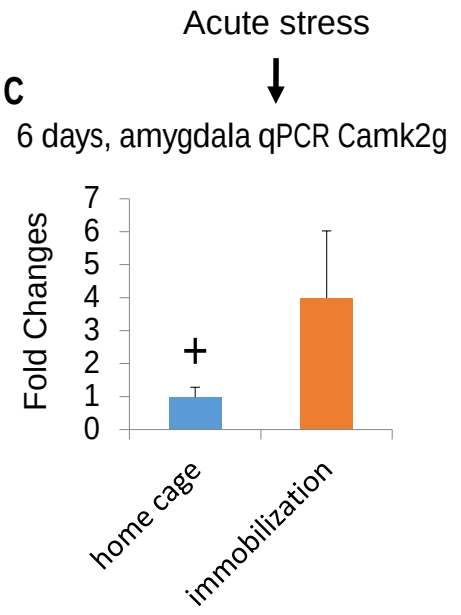
Figure S6. Camk2g mRNA levels are altered after stress in mouse amygdala and its blood mRNA levels are associated with PTSD&Depression.

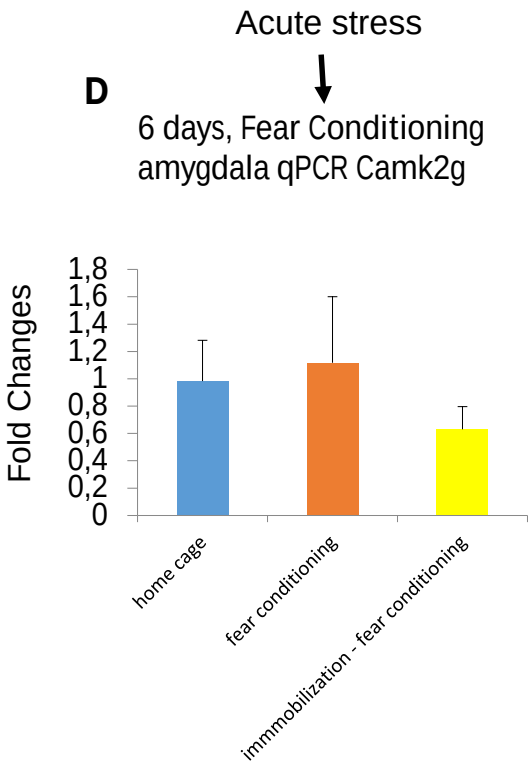
A) Top associations between CAMK2 blood mRNA level and PTSD&Dep, adjusting for gender, age, and population substructure (n=230); B) CAMK2G has significantly decreased expression level in blood in PTSD&Dep cases versus controls after adjusting for gender, age, and population substructure. C) There was an increase, marginally significant, in amygdala CamK2G mRNA basal levels 6 days after stress exposure t=2,213 df=8, p=0.0578, n=8 control group, n=3 stress group. D) Fear conditioning does not change amygdala CamK2g mRNA levels two hours later, home cage n=8, fear conditioning, n=7, immobilization-fear conditioning n=4.

A

Gene	Probe_ID	t-Statistic (controls-cases)	p-value
CAMK2D	ILMN_1683415	0.099	0.921
CAMK2G	ILMN_1809695	2.431	**0.016***
CAMK2G	ILMN_2359601	1.353	0.177







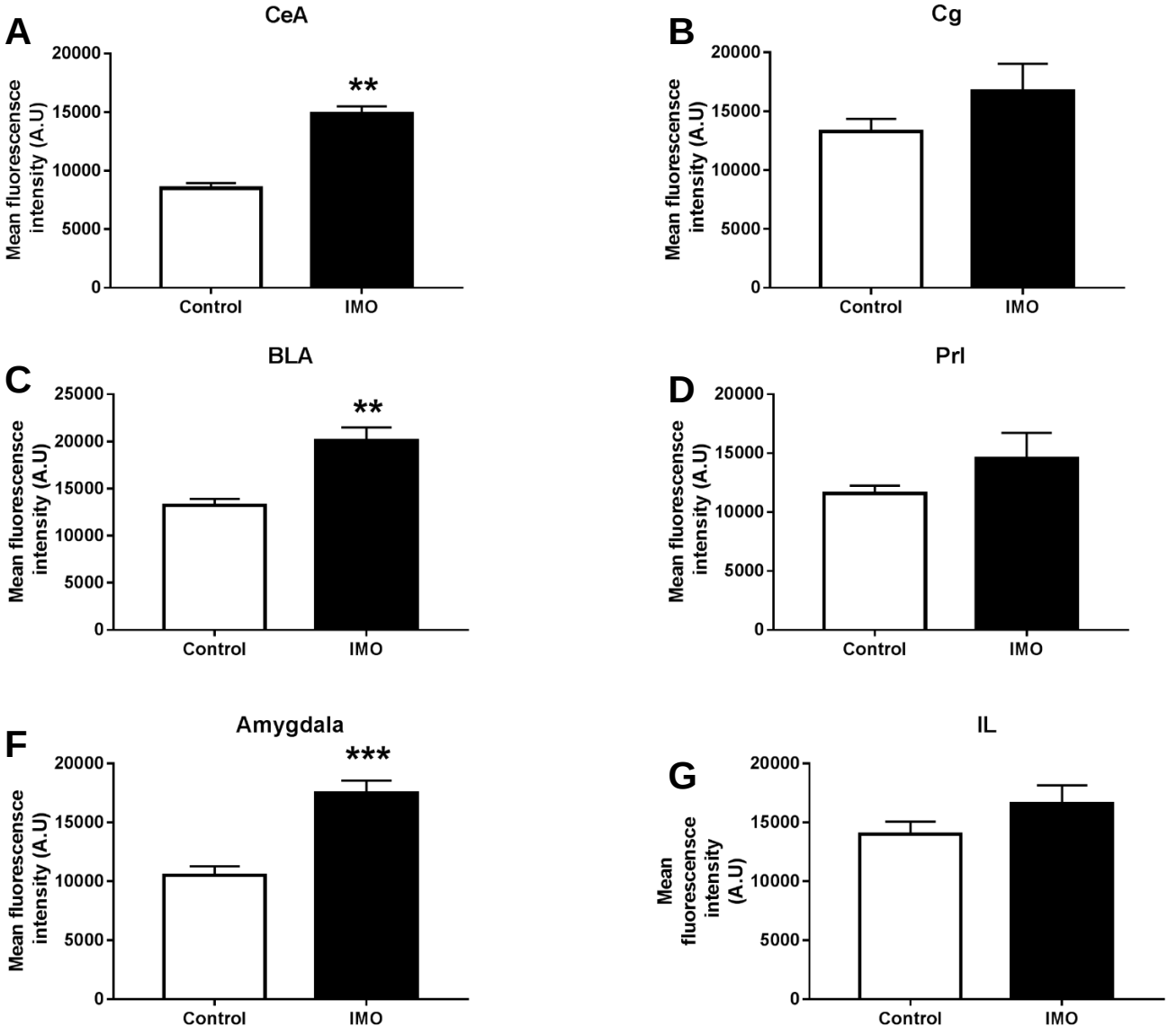


Figure S7.

Ppm1f is upregulated in the central amygdala (CeA) (A), Basolateral amygdala (BLA) and in the amygdala (CeA+BLA) 6 days after stress immobilization (IMO) in male mice. Moreover, IMO also enhanced Ppm1f levels in the medial prefrontal cortex (mPFC: Cg+Prl+IL). P*p≤0.05, **p≤0.01, ***p≤0.001. A.U.= Arbitrary Units. N=7-8 per group.

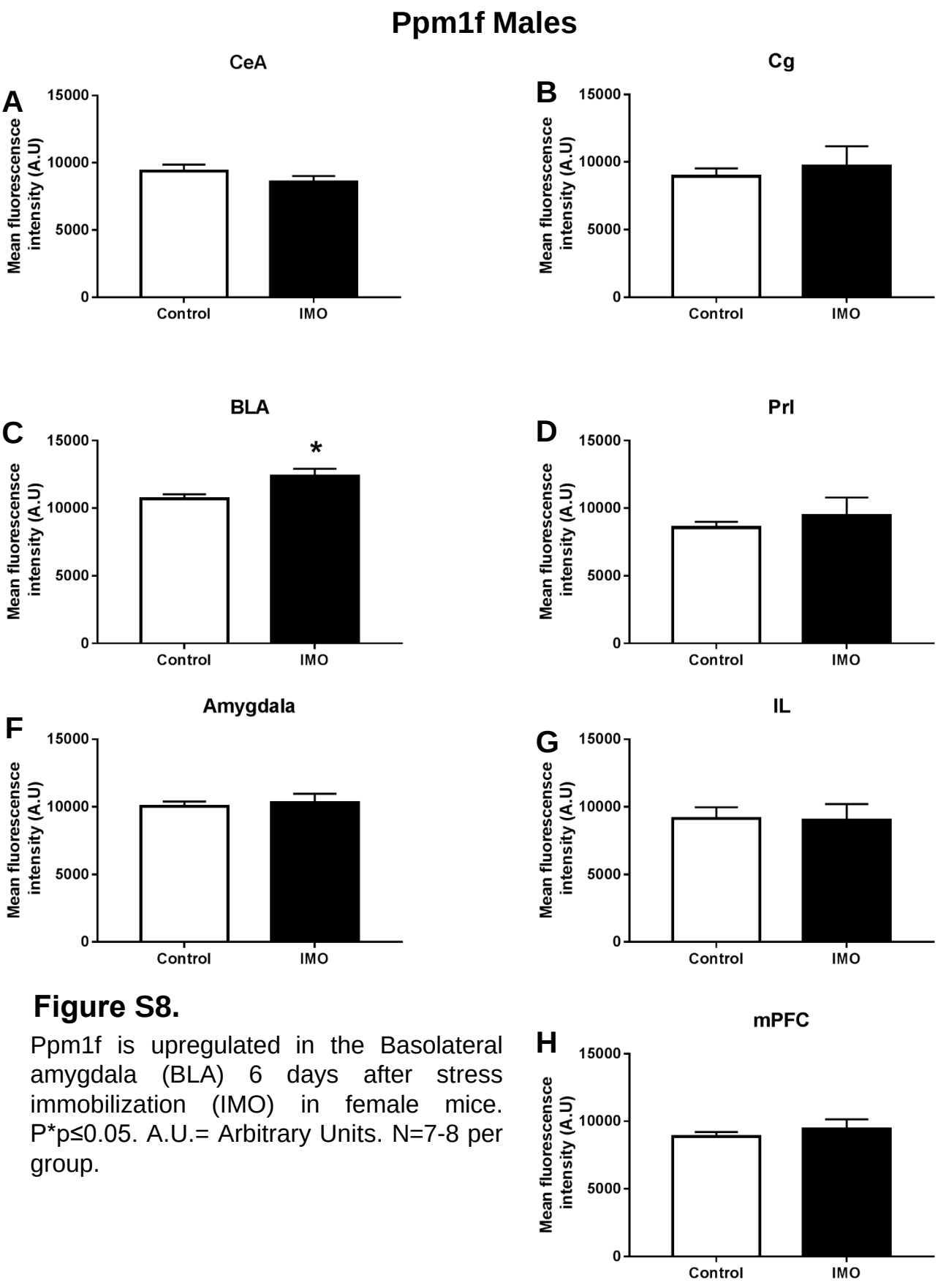


Figure S8.

Ppm1f is upregulated in the Basolateral amygdala (BLA) 6 days after stress immobilization (IMO) in female mice. P*p≤0.05. A.U.= Arbitrary Units. N=7-8 per group.

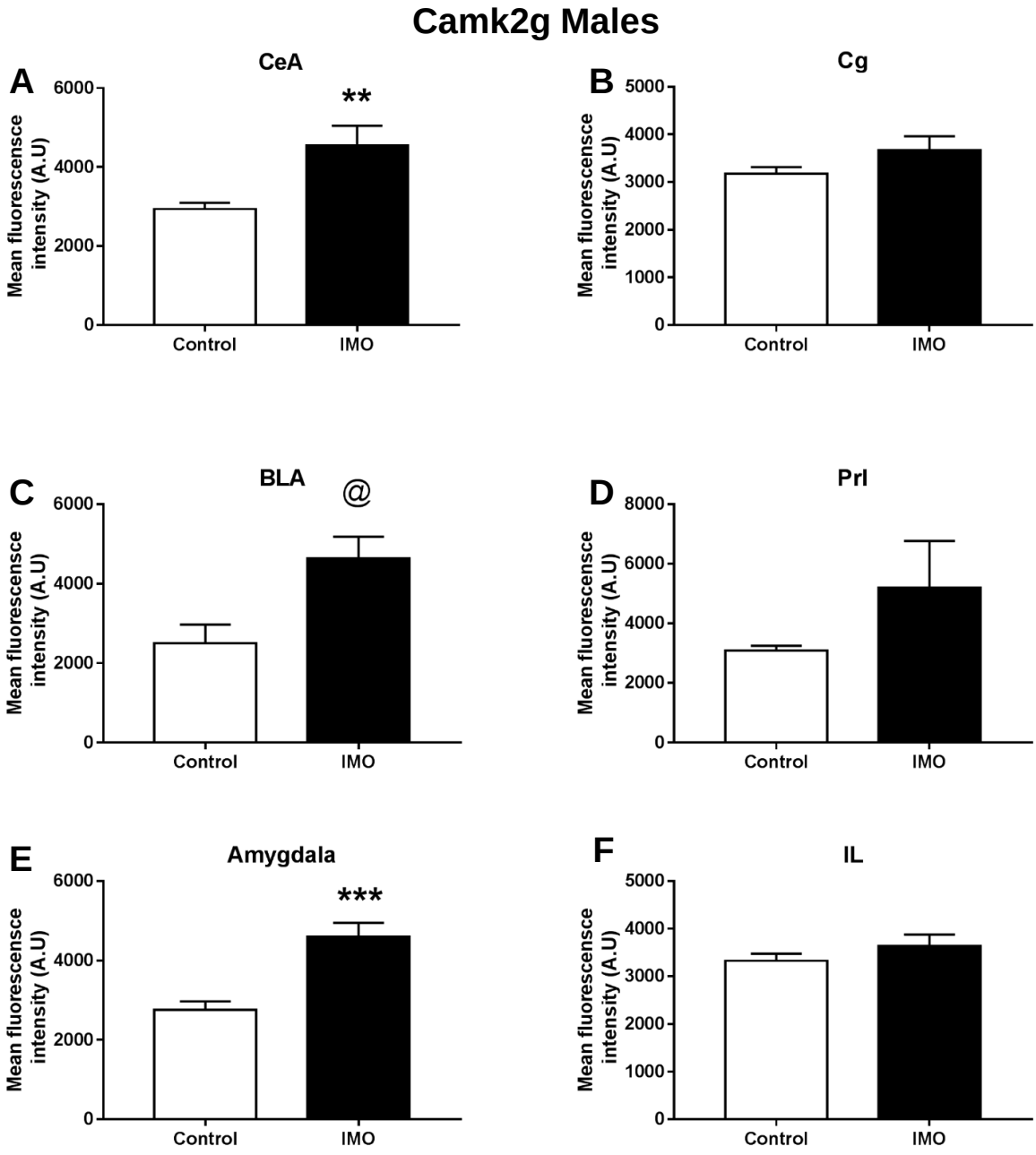


Figure S9.

Camk2g is upregulated in the central amygdala (CeA) (A), Basolateral amygdala (BLA) and in the amygdala (CeA+BLA) 6 days after stress immobilization (IMO) in male mice. Moreover, IMO also enhanced Ppm1f levels in the medial prefrontal cortex (mPFC: Cg+Prl+IL). P*p≤0.05, **p≤0.01, ***p≤0.001. A.U.= Arbitrary Units. N=7-8 per group.

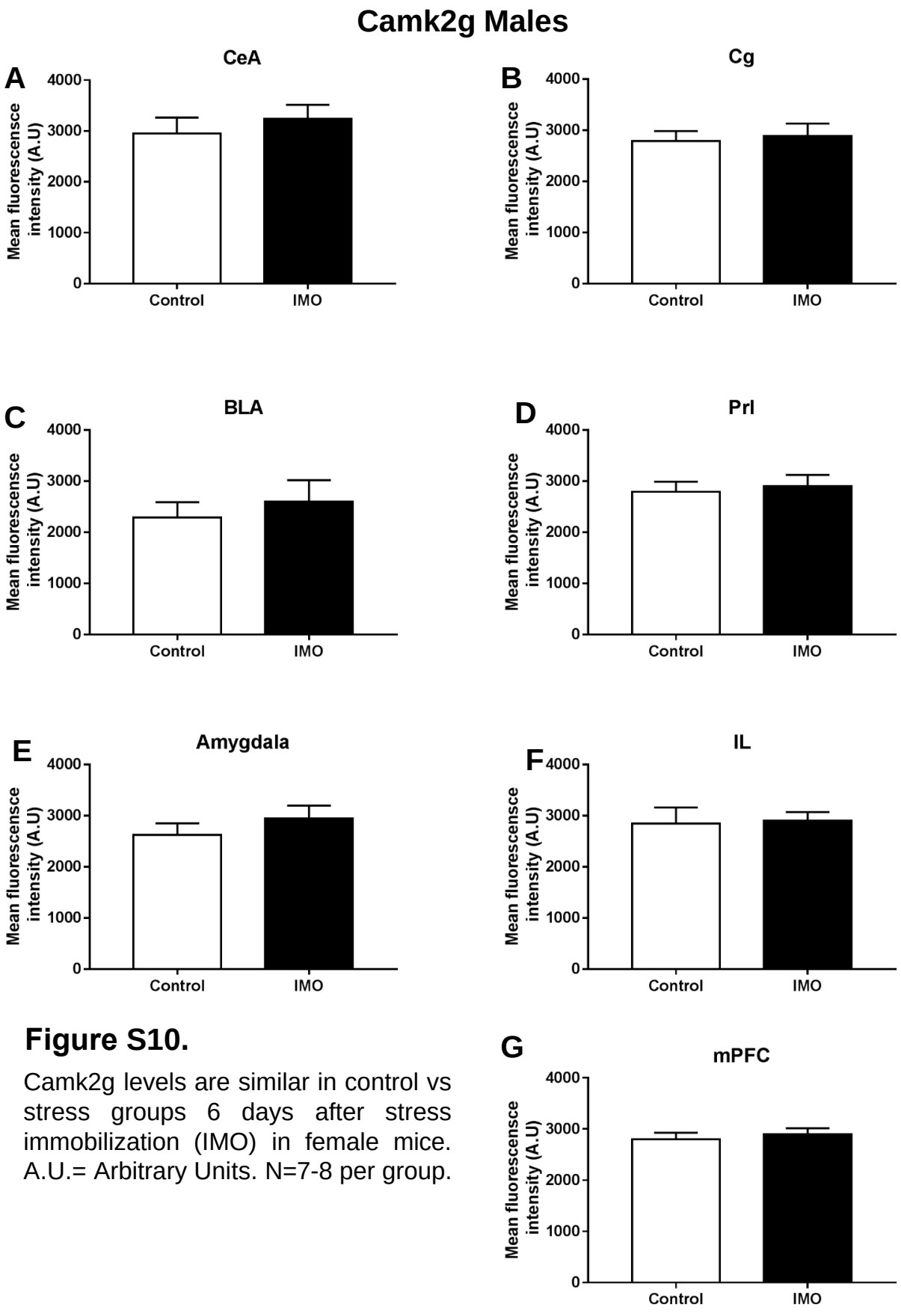


Figure S10.

Camk2g levels are similar in control vs stress groups 6 days after stress immobilization (IMO) in female mice. A.U.= Arbitrary Units. N=7-8 per group.

Supplemental Table 1. Gene regulation in the amygdala microarrays.

List of the genes that survived these criteria: a) Probe present at least one time in both lists (6 and 8 days after stress), b) >1.5 fold change compared to control, c) significant difference at p<.05 (nominal p-value), d) clearly present at moderate to high levels in amygdala (search in the Allen Brain Atlas).

Red color = High expression in the brain
Yellow color = Moderate expression in the brain

	UPREGULATED BY IMO			
Probe	gene name	P value	Fold Change	Amygdala Expression
ILMN_2900827	Ssbp4	0,002	4,59	High in CeA
ILMN_2751935	Sytl4	0,018	3,75	Moderate in CeA
ILMN_2485900	Ash2l	0,042	2,65	Moderate
ILMN_1245389	LOC236604	0,002	2,65	It is a predicted gene
ILMN_2644415	Pacsin1	0,032	2,34	Very high in BLA
ILMN_1215847	Gfap	0,002	2,28	Moderate
ILMN_2773012	Hbp1	0,002	2,18	Moderate
ILMN_2721748	C85492	0,036	2,14	Moderate
ILMN_2773012	Hbp1	0,002	1,93	High
ILMN_1220230	Hbp1	0,002	1,89	High
ILMN_2797374	Fbxl5	0,002	1,88	Moderate
ILMN_2729496	Ppm1f	0,026	1,88	Moderate
ILMN_2650392	Rbm28	0,002	1,69	Moderate in MeA
ILMN_2982890	Clpp	0,002	1,68	Moderate
ILMN_1254705	Atp5d	0,002	1,62	Moderate

	DOWNREGULATED BY IMO			
Probe	Gene name	P value	Fold Change	Amygdala Expression
ILMN_2751345	Arhgef6	0,026	-1,63	Moderate in CeA
ILMN_2651660	Ppp1r1b	0,026	-2,75	High in CeA
ILMN_2781689	Kif2a	0,026	-1,89	Moderate
ILMN_2485900	Ash2l	0,026	-1,88	Moderate
ILMN_1237360	Aebp2	0,026	-1,76	Moderate
ILMN_2425376	Cttnbp2	0,050	-1,74	Moderate
ILMN_2971567	Ddx24	0,026	-1,64	Moderate
ILMN_3052685	Tro	0,026	-1,58	High in CeA
ILMN_2437254	Fhl1	0,026	-1,57	High in CeA

Supplemental Table 2. RT-PCR arrays plates. This list shows the top hits of the microarray replicated by RT-PCR arrays including the controls used for these experiments (mGDC, RTC, PPC).

Gene Symbol	RefSeqID	Description	RT2 Catalog Number
Hbp1	NM_153198	High mobility group box transcription factor 1	PPM30281A
Ncapg	NM_019438	Non-SMC condensin I complex, subunit G	PPM58723A
Ssbp4	NM_133772	Single stranded DNA binding protein 4	PPM25373A
Sytl4	NM_013757	Synaptotagmin-like 4	PPM28561A
Ash2l	NM_011791	Ash2 (absent, small, or homeotic)-like (Drosophila)	PPM27101A
Pacsin1	NM_178365	Protein kinase C and casein kinase substrate in neurons 1	PPM25209A
Gfap	NM_001131020	Glial fibrillary acidic protein	PPM04716A
C85492	NM_153540	Expressed sequence C85492	PPM41418A
Fbxl5	NM_178729	F-box and leucine-rich repeat protein 5	PPM26815A
Ppm1f	NM_176833	Protein phosphatase 1F (PP2C domain containing)	PPM34145A
Rbm28	NM_133925	RNA binding motif protein 28	PPM28730A
Arhgef6	NM_152801	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	PPM35862A
Ppp1r1b	NM_144828	Protein phosphatase 1, regulatory (inhibitor) subunit 1B	PPM29180A
Kif2a	NM_001145779	Kinesin family member 2A	PPM40465A
Aebp2	NM_178803	AE binding protein 2	PPM30162A
Cttnbp2	NM_080285	Cortactin binding protein 2	PPM33999A
Ddx24	NM_001159502	DEAD (Asp-Glu-Ala-Asp) box polypeptide 24	PPM25034A
Tro	NM_001002272	Trophinin	PPM04339E
Fhl1	NM_001077362	Four and a half LIM domains 1	PPM05773A
GAPDH	NM_008084	Glyceraldehyde-3-phosphate dehydrogenase	PPM02946E
Rpl13a	NM_009438	Ribosomal protein L13a	PPM03694F
mGDC	Controls		
RTC			
PPC			

Supplemental Table 3. Amygdala microarray replications. From the initial gene list (Supplementary Figure 1) probes highlighted in red here are those replicated in two independent cohorts of mice - including *ppm1f*. Genes highlighted in yellow are genes replicated only in one cohort.

Amygdala			1 day after IMO		6 and 8 days after IMO		6 and 8 days after IMO		7 days after IMO	
			RT PCR ARRAYS		MICROARRAY		RT PCR ARRAYS		RT PCR ARRAYS	
Probe name	Cytoband	Gene	fold change	p value	fold change	p value	fold change	p value	fold change	p value
ILMN_1221199	16qA3	Ppm1f	3,12	0,159	1,88	0,026	1,49	0,011	1,30	0,007
ILMN_3020240	12qA3	Hbp1	-1,10	0,667	2,18	0,002	1,26	0,040	1,19	0,019
ILMN_2900827	8qB3.3	Ssbp4	1,37	0,444	4,59	0,002	1,41	0,004	1,18	0,012
ILMN_2662020	5qB3	Fbxl5	1,35	0,547	1,88	0,002	1,16	0,031	1,32	0,001
ILMN_2437254	XqA5	Fhl1	-1,74	0,308	-1,57	0,026	-1,42	0,019	-1,25	0,007
ILMN_2428596	unknown	Rbm28	1,24	0,675	1,69	0,002	1,05	0,450	1,14	0,011
ILMN_2764587	13qD2.1	Kif2a	1,14	0,774	-1,89	0,026	-1,43	0,004	-1,17	0,268
ILMN_1237360	6qG2	Aebp2	1,37	0,512	-1,76	0,026	1,00	0,987	1,12	0,041
ILMN_2747569	unknown	Ncapg	1,01	0,989	2,95	0,024	-2,02	0,011	1,16	0,257
ILMN_2751935	XqE3	Sytl4	1,24	0,648	3,75	0,018	1,04	0,913	-1,60	0,083
ILMN_2485900	unknown	Ash2l	-1,29	0,498	2,65	0,042	1,12	0,219	-1,03	0,532
ILMN_3074180	17qA3.3	Pacsin1	-1,25	0,756	2,34	0,032	1,10	0,125	1,04	0,587
ILMN_1214715	11qE1	Gfap	1,64	0,399	2,28	0,002	-1,11	0,489	-1,11	0,662
ILMN_3074279	9qF4	C85492	1,34	0,341	2,14	0,036	-1,16	0,080	-1,11	0,302
ILMN_2751345	XqA5	Arhgef6	1,25	0,523	-1,63	0,026	1,16	0,163	1,05	0,331
ILMN_2651660	11qD	Ppp1r1b	2,30	0,355	-2,75	0,026	-1,08	0,840	1,59	0,116
ILMN_1242097	6qA2	Cttnbp2	-1,23	0,648	-1,74	0,050	-1,21	0,154	1,06	0,401
ILMN_2747381	12qE	Ddx24	1,66	0,484	-1,64	0,026	-1,03	0,811	-1,01	0,851
ILMN_3052685	XqF3	Tro	1,50	0,614	-1,58	0,026	1,11	0,294	1,01	0,899

Supplementary Table 4. Associations between human blood mRNA levels of the five stress-regulated genes in mouse amygdala in PTSD&Dep, covarying for gender, age, and population substructure.

Gene	Probe_ID	t-Statistic (control-case)	p-value
PPM1F	ILMN_2059535	2.806	0.005
FBXL5	ILMN_2378100	1.573	0.117
	ILMN_1673370	1.090	0.277
FHL1	ILMN_1805842	-0.822	0.412
HBP1	ILMN_2160764	1.535	0.126
	ILMN_1685415	1.407	0.161
SSBP4	ILMN_1720137	-0.441	0.660
	ILMN_2327346	-1.224	0.222

Supplemental Table 5. Gene regulation of the mPFC microarray. List of the genes that survived these criteria: a) >1.30 fold change (There are only 6 genes which survive a 1.5 cut-off criteria), b) significant at nominal p<.05, c) clearly present at moderate to high levels in amygdala (based on Allen Brain Atlas).

Red color = High expression in the brain
Yellow color = Moderate expression in the brain

	UPREGULATED BY IMO			
Probe	Gene name	P value	Fold Change	mPFC Expression
ILMN_2620406	Nptx2	0,005	2,02	Moderate
ILMN_1232381	Slit2	0,004	1,69	Moderate
ILMN_2704570	Nell2	0,002	1,69	High
ILMN_2592486	Pglyrp1	0,003	1,67	Moderate
ILMN_1214326	Dnm	0,034	1,64	High
ILMN_1229830	Zfhx1b	0,003	1,59	Moderate
ILMN_2957555	Lmtk3	0,022	1,58	High
ILMN_2711366	Nell2	0,034	1,57	High
ILMN_1241551	Nell2	0,007	1,53	High
ILMN_2717716	Rapgef5	0,014	1,51	Moderate

	DOWNREGULATED BY IMO			
Probe	Gene name	P value	Fold Change	mPFC Expression
ILMN_1255462	Hbb-b1	0,033	-1,76	Moderate
ILMN_2626294	Pam	0,001	-1,59	Moderate
ILMN_2772998	Enpp2	0,025	-1,54	Moderate
ILMN_2813487	Per1	0,005	-1,54	Moderate
ILMN_1249378	Bhlhb2	0,007	-1,51	High
ILMN_2920736	Bbc3	0,046	-1,48	High
ILMN_2592779	Klhl9	0,029	-1,45	Moderate
ILMN_2950422	Tppp	0,014	-1,42	High
ILMN_2813484	Per1	0,025	-1,42	Moderate
ILMN_2681670	Vps37b	0,006	-1,41	Moderate
ILMN_2716838	Hspa4	0,004	-1,40	Moderate
ILMN_2676052	Tef	0,039	-1,39	Moderate
ILMN_2595359	Slc3a2	0,004	-1,37	Moderate
ILMN_2775885	Calm2	0,010	-1,36	Moderate
ILMN_1247787	ORF19	0,017	-1,36	Moderate
ILMN_1258688	Cbx6	0,020	-1,36	Moderate
ILMN_2616509	Arnt2	0,033	-1,36	Moderate
ILMN_1246340	9330185J12Rik	0,001	-1,36	Moderate
ILMN_2482572	Flnb	0,027	-1,36	Moderate
ILMN_1214470	Ids	0,046	-1,35	High
ILMN_2720813	Epb4.1	0,001	-1,35	High
ILMN_2598318	Lin7b	0,016	-1,35	Moderate
ILMN_2835686	Ywhaq	0,004	-1,35	Moderate
ILMN_2647864	Ogt	0,032	-1,34	High
ILMN_1226366	Uck1	0,001	-1,34	Moderate
ILMN_2670713	Cyfip2	0,003	-1,34	High
ILMN_2792756	Ppm1f	0,027	-1,34	Moderate

Supplemental Table 6. Sociodemographic characteristics of the 230 African American GTP participants.

Characteristics	Cases (n=142)	Controls (n=88)	*p*-value[a]
Age	41.7 ± 11.4	43.3 ± 14.5	0.222
Gender (% female)	76.1%	73.9%	0.754
Education			0.492
Highschool or below	66.7%	68.2%	
Some college or technical school	27.7%	22.7%	
College graduate or beyond	5.6%	9.1%	
Employment status (% currently employed)	18.4%	29.5%	0.054
PSS[b] score	27.2 ± 9.1	2.3 ± 2.4	<0.0001
BDI[c] score	27.7 ± 9.5	2.9 ± 2.2	<0.0001
Number of traumatic experiences	7.1 ± 3.6	3.6 ± 2.5	<0.0001

[a] *p*-values were from Wilcoxon Rank Sums or Fisher-Exact test

[b] PSS: PTSD symptom severity score from the PTSD Symptom Scale

[c] BDI: depression severity score from the Beck Depression Inventory

Supplemental Table 7. Sociodemographic characteristics of the CHDWB cohort.

Characteristics	Anxiety cases (n=151)	Controls (n=165)	*p*-value[a]
Age	48.1 ± 10.7	49.5 ± 11.1	0.323
Gender (% female)	73.5%	63.0%	0.053
Race			0.003
Caucasian	79.5%	64.2%	
African American	20.5%	35.8%	
Employment	100%	100	1.00
GAD-7 score	7.9 ± 3.6	0.4 ± 0.5	<0.0001

[a] *p*-values were from Wilcoxon Rank Sums or Fisher-Exact test

Supplemental Table 8: Comparing the GTP genetic dataset to GTP gene expression subset

	PSS score	BDI score	Sex
Genetic sample	***Mean ± SD; median***	***Mean ± SD; median***	***% female***
Cases with PTSD&Dep symptoms	27.8 ± 8.9; 27	27.5 ± 9.9; 26	75%
Controls	2.0 ± 2.2; 1	2.8 ± 2.3; 3	68%
Gene expression sample	***Mean ± SD; median***	***Mean ± SD; median***	***% female***
Cases with PTSD&Dep symptoms	26.8 ± 8.8; 26	28.0 ± 9.8; 26	79%
Controls	2.4 ± 2.4; 2	3.0 ± 2.3; 3	67%

PSS: PTSD symptom severity score from the PTSD Symptom Scale
BDI: Depression severity score from the Beck Depression Inventory

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