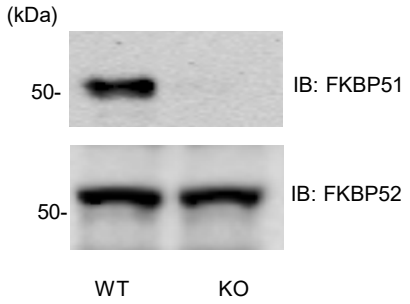
A



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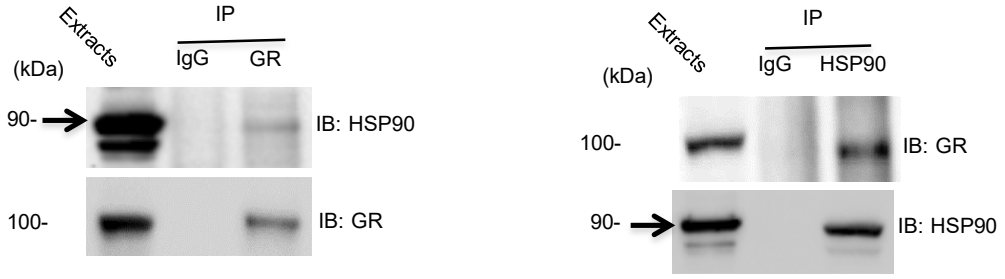
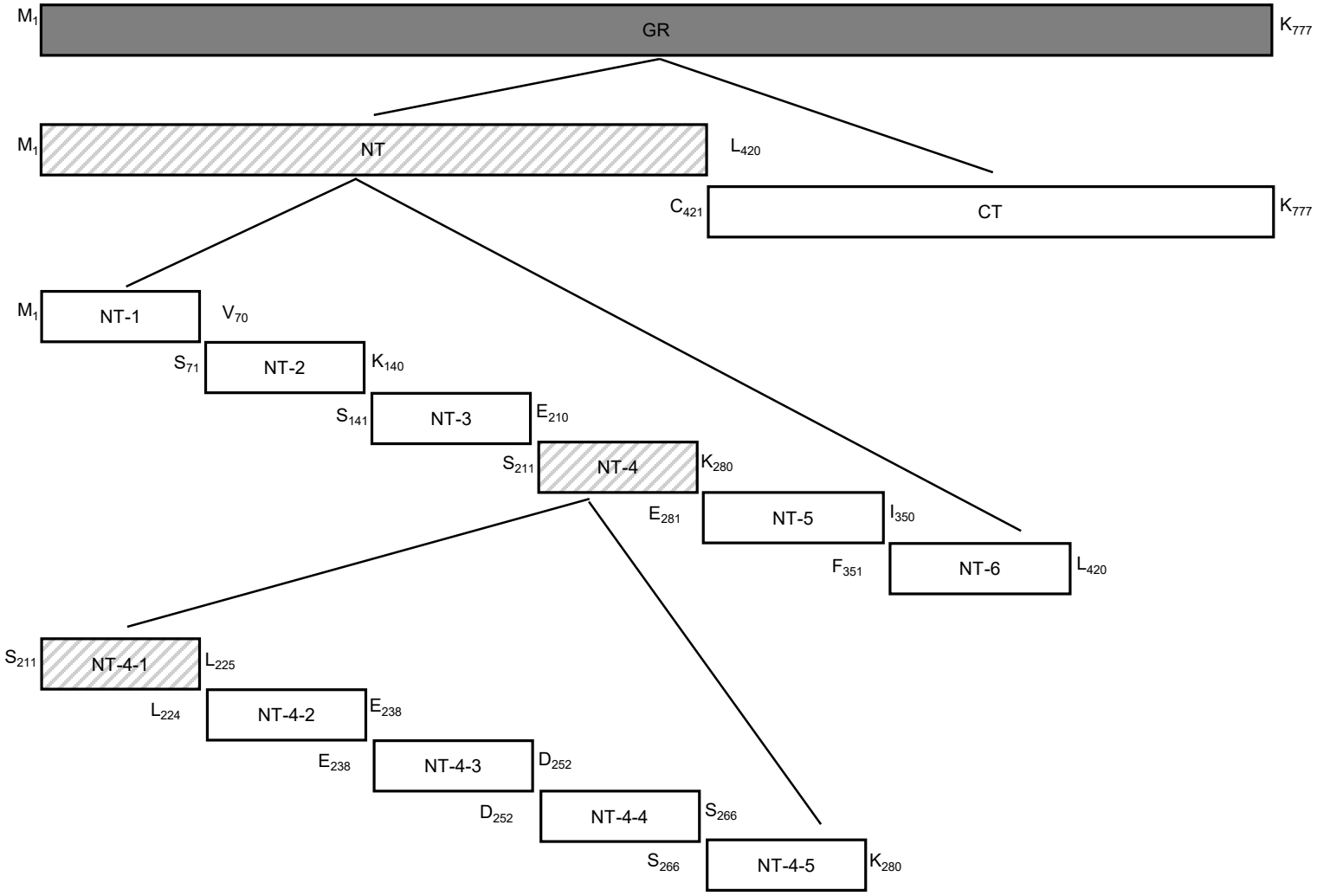
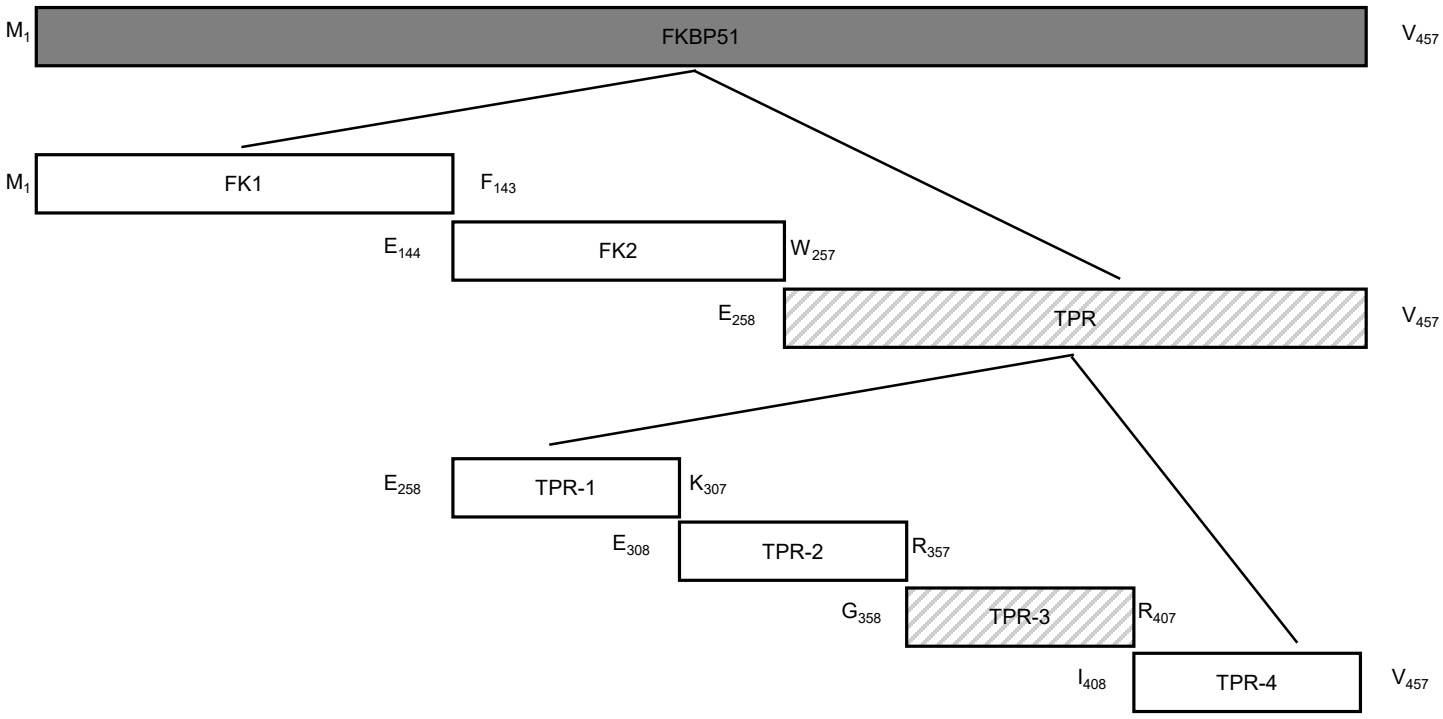


Supplemental Figure 1. The specificity of FKBP51 and FKBP52 antibodies and the interaction between GR and HSP90.

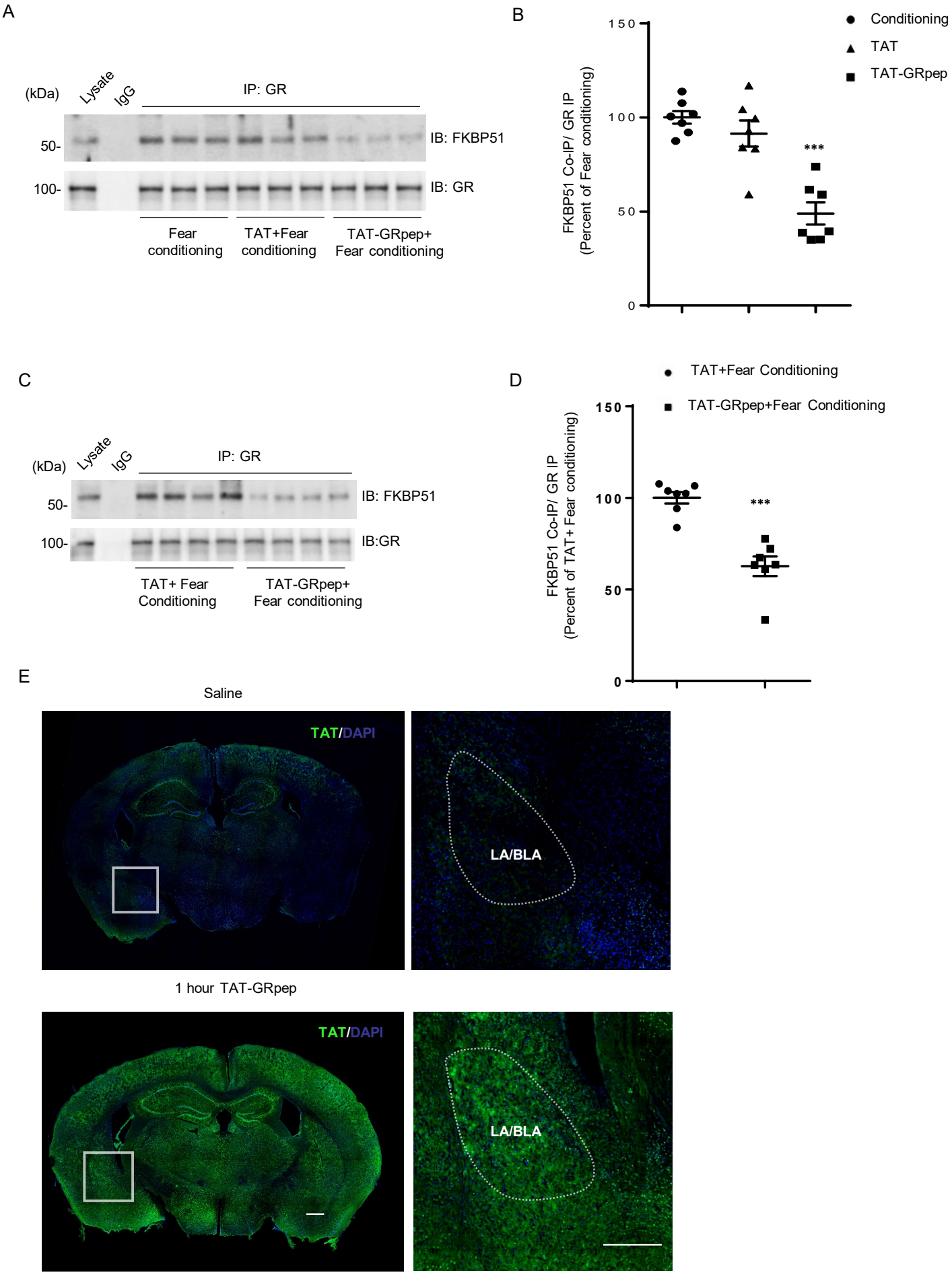
A. Western blot showing that anti-FKBP51 antibody fails to detect the FKBP51 band in brain lysate from FKBP51 knockout (KO) mice, but anti-FKBP52 antibody is able to detect the FKBP52 band in brain lysate from FKBP51 KO mice. Blots represent three independent experiments performed. **B.** In mouse brain lysate, GR antibody, but not IgG (negative control), co-immunoprecipitated with HSP90 (Left). HSP90 antibody, but not IgG (negative control), co-immunoprecipitated with GR (Right). Blots represent three independent experiments performed.



Supplemental Figure 2: Schematic representation of generated GST-fusion proteins encoding truncated GR segments.



Supplemental Figure 3: Schematic representation of generated GST-fusion proteins encoding truncated FKBP51 segments.


A
(kDa)
Lysate
IgG
IP: GR
50-
IB: FKBP51
100-
IB: GR
Fear conditioning
TAT+Fear conditioning
TAT-GRpep+ Fear conditioning
B
Conditioning
TAT
TAT-GRpep
FKBP51 Co-IP/ GR IP (Percent of Fear conditioning)
150
100
50
0
***
C
(kDa)
Lysate
IgG
IP: GR
50-
IB: FKBP51
100-
IB:GR
TAT+ Fear Conditioning
TAT-GRpep+ Fear conditioning
D
TAT+Fear Conditioning
TAT-GRpep+Fear Conditioning
FKBP51 Co-IP/ GR IP (Percent of TAT+ Fear conditioning)
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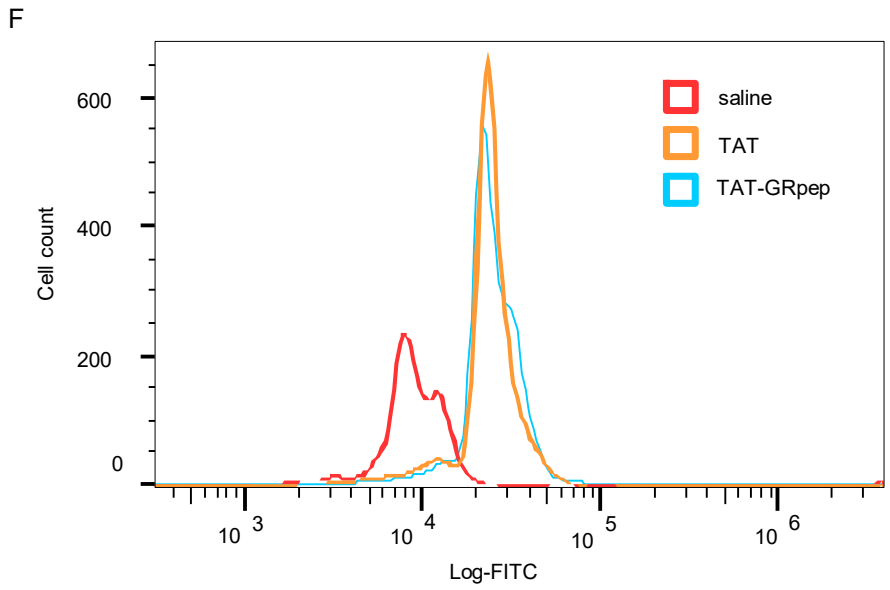
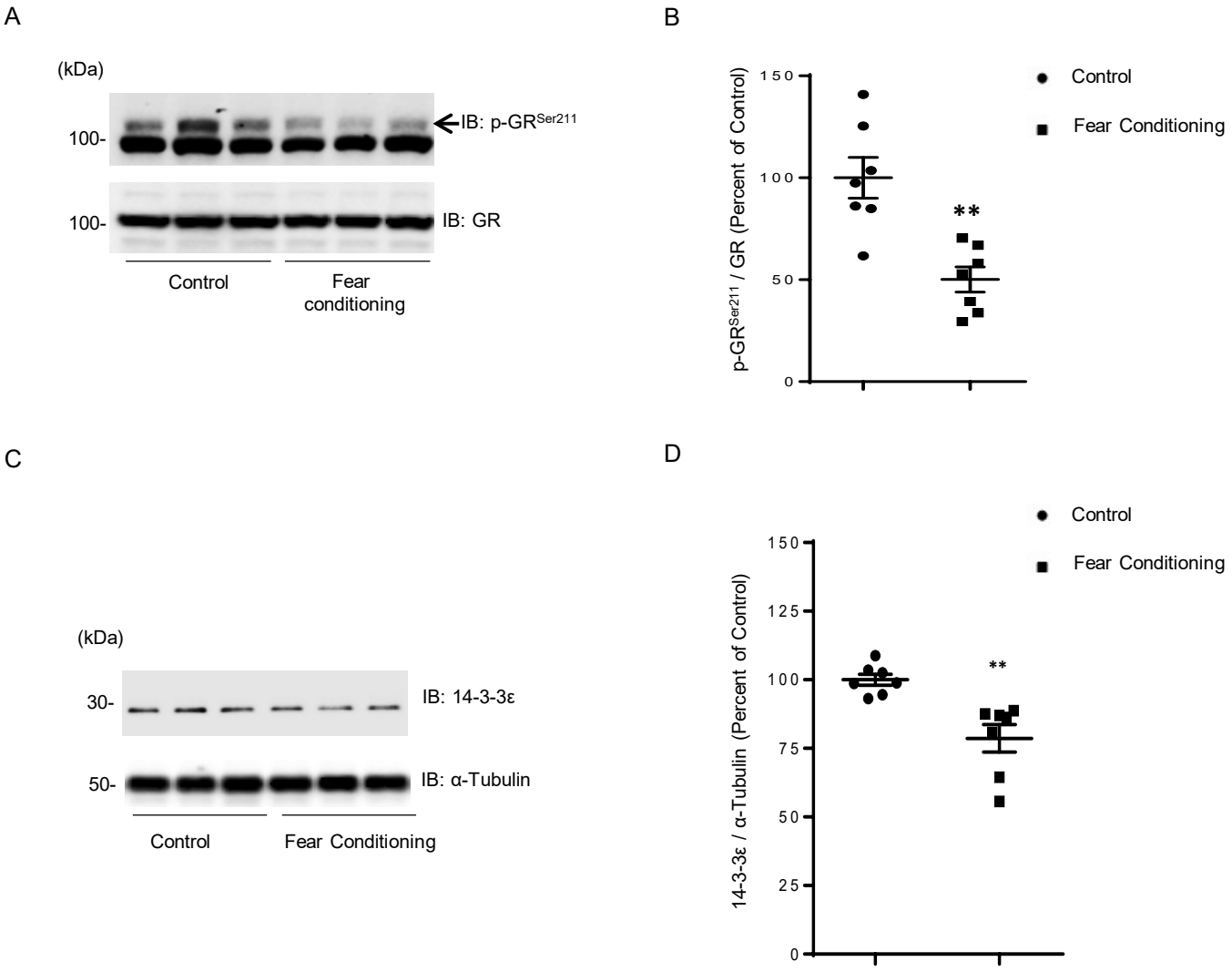
100
50
0
***
E
Saline
TAT/DAPI
LA/BLA
1 hour TAT-GRpep
TAT/DAPI
LA/BLA



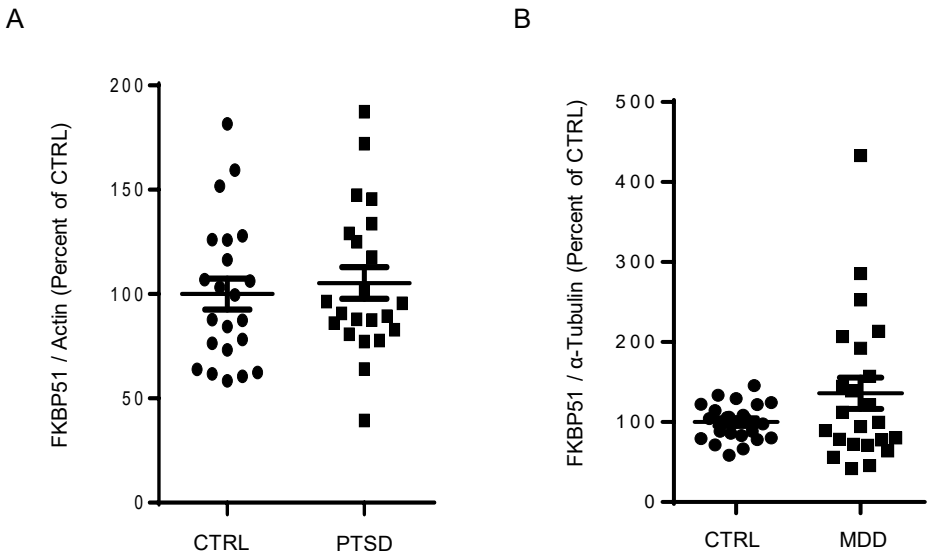
Supplemental Figure 4. TAT-GR peptide blocks the GR-FKBP51 complex

A-B. Co-immunoprecipitation shows that TAT-GRpep, but not TAT, is able to disrupt GR-FKBP51 complex in fear-conditioned mice. **A.** Representative western blot of FKBP51 and GR precipitated by GR antibody. **B.** Densitometric analysis of the level of FKBP51 co-immunoprecipitated by GR antibody in brain lysate of fear-conditioned mice injected with saline, TAT, or TAT-GRpep. The level of co-immunoprecipitated FKBP51 (FKBP51 Co-IP) was normalized after being divided by the level of precipitated GR (GR IP). Results for each sample are presented as the percentage of the fear conditioning group. ***$p < 0.001$ as compared to fear conditioning (Conditioning) samples, $n = 7$, $F_{2,18} = 23.61$, one-way ANOVA test followed by Dunnett's *post hoc* test. Data are shown as mean ± S.E.M. **C-D.** Co-immunoprecipitation shows that TAT-GRpep is able to disrupt the GR-FKBP51 complex in lymphocyte lysate from peripheral blood of fear-conditioned mice. **C.** Representative Western blot of GR and FKBP51 precipitated by GR antibody. **D.** Densitometric analysis of the level of FKBP51 co-immunoprecipitated by GR antibody in lymphocyte lysate of fear-conditioned mice injected with TAT, or TAT-GRpep. The level of co-immunoprecipitated FKBP51 (FKBP51 Co-IP) was normalized after being divided by the level of precipitated GR (GR IP). Results for each sample are presented as the percentage of TAT+fear conditioned group. ***$p < 0.001$ as compared to TAT+fear conditioned samples, $n = 7$, t-test. Data are shown as mean ± S.E.M. **E.** The presence of TAT-GRpep peptide in the mouse brain 1 hour after injection. Immunohistochemistry was performed with anti-TAT antibody on mouse brains after 1 hour injection of TAT-GRpep peptide or saline. Higher magnification images of the lateral and basolateral amygdalar nucleus (LA/BLA) are shown in the right. There was significantly more fluorescence observed with 1 hour TAT-GRpep peptide injection when compared to saline control. Scale bar: 500 µm. **F.** Flow cytometry analysis of lymphocytes from mice treated with saline, TAT or TAT-GRpep**.** Cells were stained by FITC conjugated anti-TAT antibody. Cells from both TAT and TAT-GRpep groups have similar but much higher FITC intensity than that from the saline group, which indicates that our peptides have entered the cells effectively. The graph represents three independent experiments performed.



Supplemental Figure 5. Lower levels of GR S_{211} phosphorylation and expression of 14-3-3ε in fear conditioned mice.

A-B. Western blot shows lower levels of GR phosphorylation at S_{211} in mouse brain lysate of fear-conditioned mice as compared to control mice. **A.** Representative Western blot of p-GR (Ser211) and total GR. GR was used as a loading control. **B.** Densitometric analysis of the levels of GR phosphorylation at S_{211} in brain lysate of fear-conditioned mice or control mice. The level of phosphorylated GR (S_{211}) was normalized after being divided by the level of GR. Results for each sample are presented as the percentage of the control sample. $^{**}p < 0.01$, n = 7, t-test. Data are shown as mean ± S.E.M. **C-D.** Western blot analysis shows lower levels of 14-3-3ε in brain lysate from fear-conditioned mice as compared to those of control mice. **C.** Representative Western blot of 14-3-3ε and α-Tubulin in the protein extract from mouse brain tissue. α-Tubulin was used as loading control. **D.** Densitometric analysis of expression levels of 14-3-3ε in fear-conditioned mice as compared to those of control mice. The levels of 14-3-3ε was normalized after being divided by the level of α-Tubulin. Results for each sample are presented as the percentage of the control samples on the same blot. $^{**}p < 0.01$ as compared to the control group, n = 7, t-test.



Supplemental Figure 6. Expression of FKBP51 in peripheral blood samples of PTSD patients and MDD patients.

A. Densitometric analysis of expression levels of FKBP51 in PTSD patients compared to control subjects. The levels of FKBP51was normalized after being divided by the level of Actin. Results for each sample are presented as the percentage of the control samples on the same blot. P=0.62 as compared to the control group, n = 22, t-test. **B.** Densitometric analysis of expression levels of FKBP51 in MDD patients compare to control subjects. The levels of FKBP51was normalized after being divided by the level of α-Tubulin. Results for each sample are presented as the percentage of the control samples on the same blot. P= 0.0841 as compared to the control group, n = 23, t-test.

Supplemental Table 1. Demographic and samples' clinical information of subjects recruited from CAMH

Variables	PTSD (N = 22)	CTRL (N = 22)	P
Age (years)	38.95 ± 11.62	29.68 ± 10.78	0.0089
Sex (female/male)	17 / 5	17 / 5	
Education (completed post-secondary)	11	13	
PCL-C score	62.32 ± 10.65	21.77 ± 4.96	$1.15 * 10^{-19}$

* Mean ± SD

Supplemental Table 2. Demographic and clinical information of subjects from Grady Trauma Project

Variables	PTSD (N = 21)	Trauma exposed control (N = 21)	P
Age (years)	35.90 ± 12.91	38.43 ± 12.58	0.52
Sex	Female	Female	
Education*	1.86 ± 1.46	2.38 ± 1.99	0.34
PSS_total*	27.71 ± 8.19	3.95 ± 3.20	$2.85 *10^{-15}$
PSS_intrusive*	7.29 ± 2.92	0.95 ± 1.43	$4.54 *10^{-11}$
PSS_avoidnumb*	11.62 ± 4.34	1.43 ± 1.94	$3.23 *10^{-12}$
PSS_hyperarousal*	8.81 ± 3.33	1.57 ± 1.66	$4.65 *10^{-11}$
Beck Depression Inventory*	17.67 ± 9.09	10.05 ± 7.48	0.0051
Childhood Trauma Questionnaire*	44.14 ± 10.79	38.33 ± 8.45	0.059
Race-ethnicity	African American	African American	
Employment*	0.29 ± 0.46	0.38 ± 0.50	0.52
Income*	1.76 ± 1.37	1.67 ± 1.46	0.83

* Mean ± SD
* Education: highest grade completed, 0 =<12, 1=12, 2 = GED, 3=some college, 4=completed tech/AB, 5= completed college, 6=graduate school
* PTSD Symptom Scale (PSS) total: continuous score 0-51 pt scale, higher = more ptsd symptoms
* PSS_intrusive: intrusive subscale of PSS
* PSS_avoidnumb: avoidance/numbing subscale of PSS
* PSS_hyperarousal: hyperarousal subscale of PSS
* Beck Depression Inventory: depression continuous variable, 0-63 pt scale, higher = more depression symptoms
* Childhood Trauma Questionnaire: 25-125 pt scale, higher number = more trauma
* Employment: 0 = not employed, 1= employed
* Income: monthly income household (0=<$250, 1=<$500, 2=<$1000, 3=<$2000, 4=>$2000)

Supplemental Table 3. Demographic and clinical information of MDD and control subjects

Variables	Control (N = 23)	Depression (N = 23)	P
Age (years)	44.21 ± 12.39	45.26 ± 12.93	0.78
Sex	14 Female + 9 Male	15 Female + 8 Male	
Education*	1.48 ± 1.68	1.96 ± 1.66	0.34
PTSD_diagnosis*	No	No	
BDI total score*	4.67 ± 3.49	29.11 ± 6.69	< 0.0001
Race-ethnicity	22 African American 1 Caucasian	21 African American 1 Caucasian 1 Other	
Employment*	0.17 ± 0.38	0.13 ± 0.34	0.69
Income*	1.63 ± 1.22	1.36 ± 1.43	0.50

* Mean ± SD
* Education: highest grade completed, 0 =<12, 1=12, 2 = GED, 3=some college, 4=completed tech/AB, 5= completed college, 6=graduate school
* PTSD_diagnosis: 0-3=no, 4=yes
* BDI total score: depression continuous variable, 0-63 pt scale, higher = more depression symptoms
* Employment: 0 = not employed, 1= employed
* Income: monthly income household (0=$0-249, 1=$250-499, 2=$500-999, 3=$1000-1999, 4=>$2000)