

## Supplementary Information

### **P2X2 receptors in pyramidal neurons are critical for regulating vulnerability to chronic stress**

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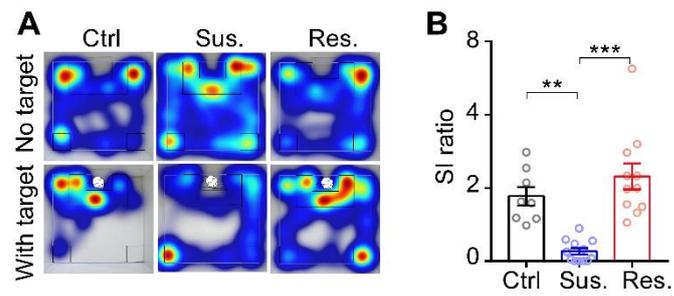
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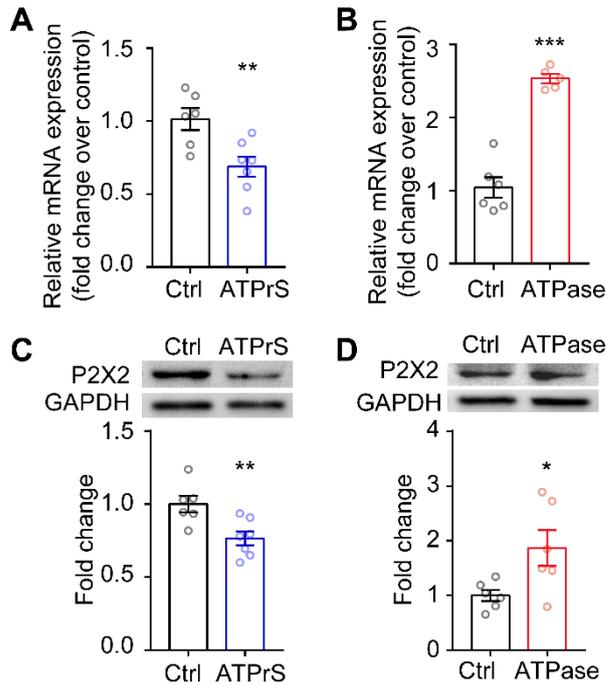
**Figure. S1**



**Figure. S1. Social interaction test.** (A) Representative heatmaps of normalized time spent during SI test of C57BL/6J mice. (B) Statistical comparison of SI ratio after CSDS (n = 8 -12, n = 8 -12, \*p = 0.042, one-way ANOVA). The data are shown as mean ± SEM. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

Related to Figure 1.

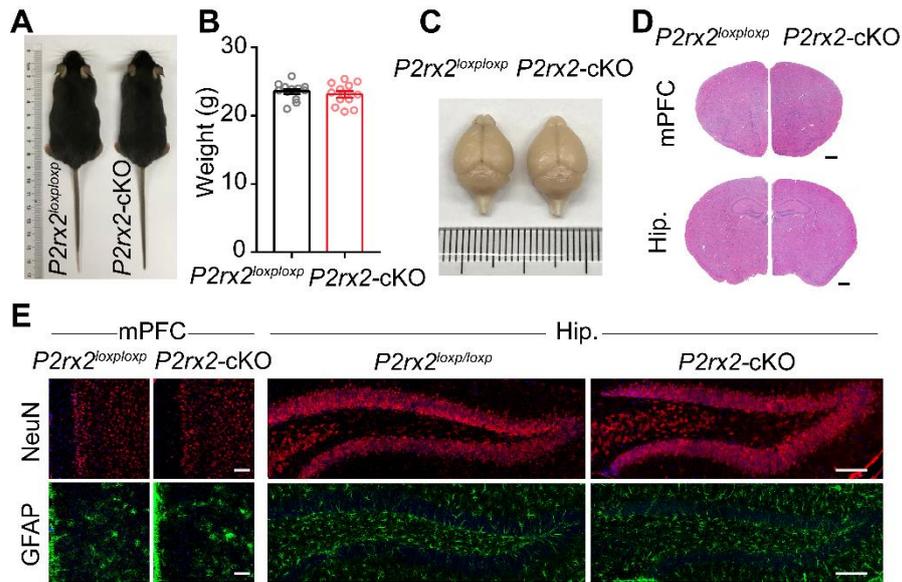
**Figure. S2.**



**Figure. S2. P2X2 expression of cultured PFC neurons treated with ATP $\gamma$ S or ATPase**

**treatment.** (A-B) Statistic analysis of P2X2 mRNA expression in the PFC cultured neurons after ATP $\gamma$ S (A) ( $n = 6-7$ ,  $t_{(11)} = 3.203$ ,  $p = 0.008$ , unpaired t test) or ATPase treatment (B) ( $n = 5-6$ ,  $t_{(9)} = 8.968$ ,  $p < 0.001$ , unpaired t test). (C-D) Western blots representation (top) and quantification (bottom) of P2X2 protein level in the PFC cultured neurons after ATP $\gamma$ S (C) ( $n = 6-7$ ,  $t_{(11)} = 3.734$ ,  $p = 0.003$ , unpaired t test) or ATPase treatment (D) ( $n = 6$ ,  $t_{(10)} = 2.520$ ,  $p = 0.030$ , unpaired t test). The data are shown as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

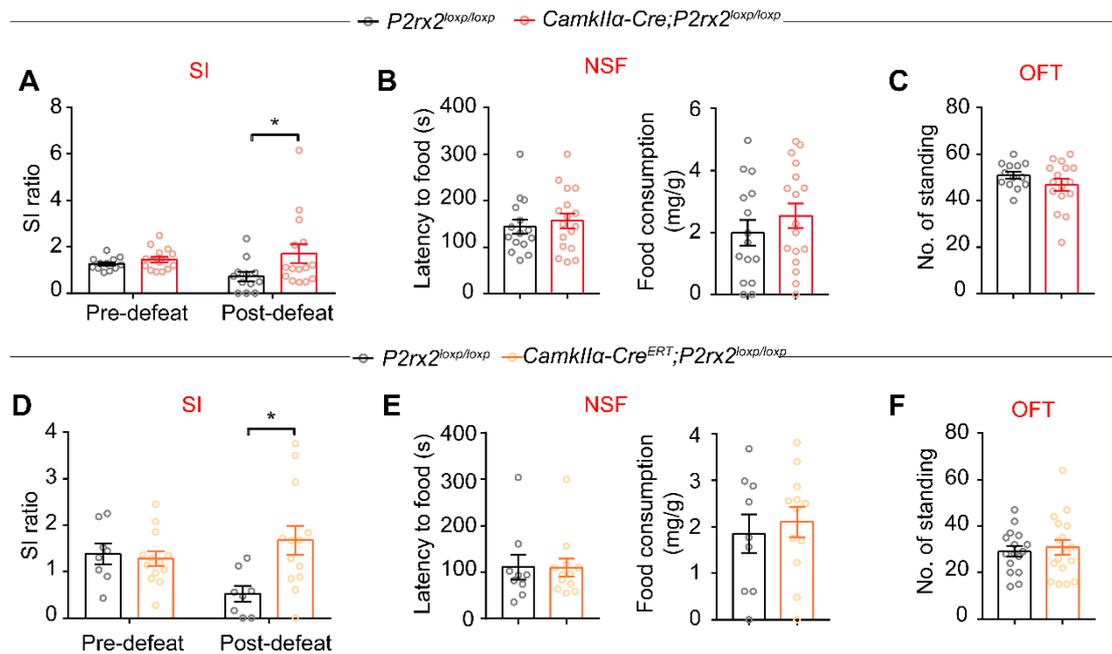
**Figure. S3**



**Figure. S3 Generation of *P2rx2-cKO* mice.** (A-B) Representative images (A) and body weight (B) ( $n = 12$ ,  $t_{22} = 0.209$ ,  $p = 0.840$ ; unpaired t test) of adult *P2rx2-cKO* and control mice. (C-D) Gross appearance (C) and H&E-stained coronal sections (mPFC or hippocampus, Scale bar, 500  $\mu$ m) of the brain (D) of *P2rx2-cKO* or littermate control mice. (E) Immunofluorescence for NeuN (red) and GFAP (green) in the mPFC or hippocampus of *P2rx2-cKO* and littermate control mice. Scale bar, 50  $\mu$ m. The data are shown as mean  $\pm$  SEM.

Related to Figure. 2.

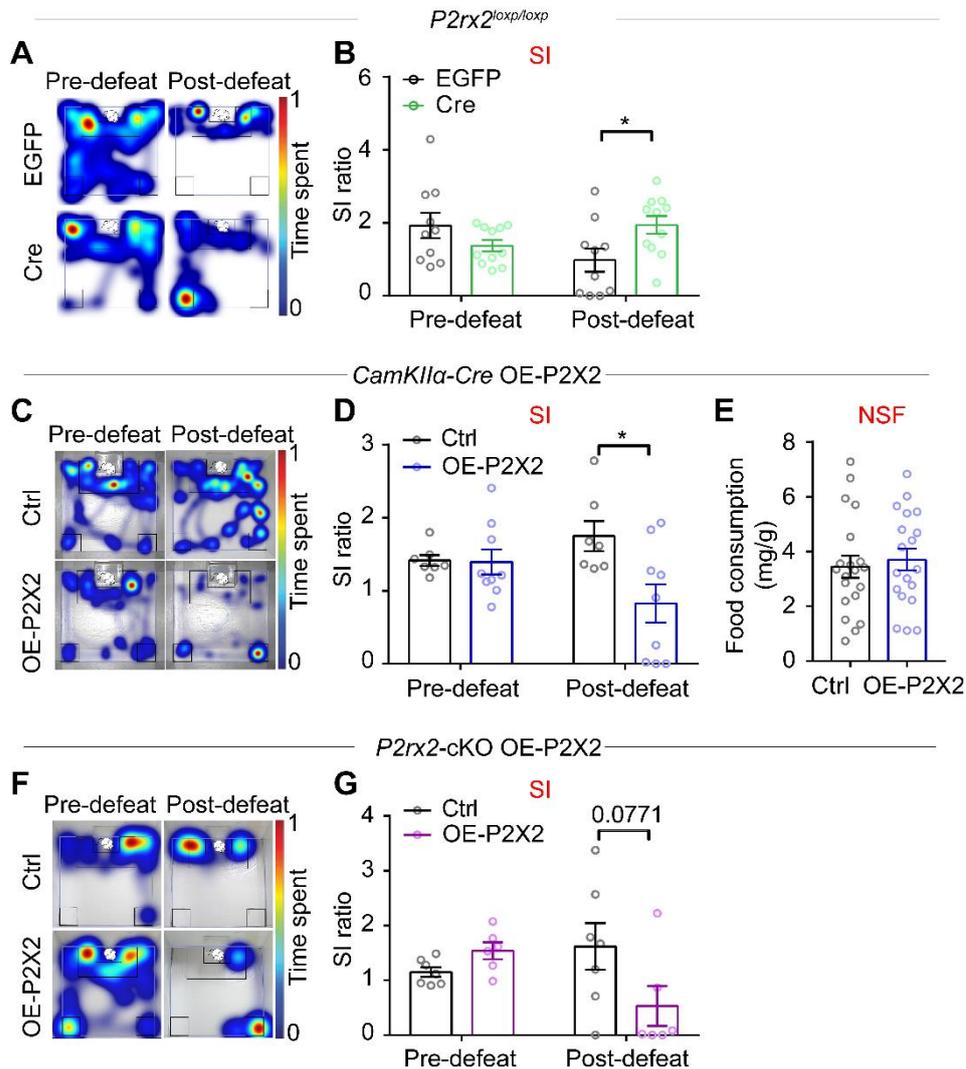
**Figure. S4**



**Figure. S4. Relative behaviors of *P2rx2* conditional knockout mice.** (A) SI ratio before and after CSDS of *P2rx2*-cKO and control mice (n = 12-14, p = 0.042, interaction effect, matching two-way ANOVA). (B-C) Statistics analysis of *P2rx2*-cKO and control mice in NSF (B, latency to food:  $t_{(30)} = 0.579$ , p = 0.570; food consumption:  $t_{(30)} = 0.960$ , p = 0.340; n = 15-17, unpaired t test) and OFT (C, number of standing: n = 13-16,  $t_{(27)} = 1.288$ , p = 0.210, unpaired t test). (D) Social interaction ratio before and after CSDS of *CamkIIa-Cre<sup>ERT</sup>; P2rx2<sup>loxp/loxp</sup>* and control mice (n = 8-13, p = 0.030, interaction effect, matching two-way ANOVA). (E-F) Statistics analysis of *CamkIIa-Cre<sup>ERT</sup>; P2rx2<sup>loxp/loxp</sup>* and control mice in NSF (E) (latency to food:  $t_{(19)} = 0.018$ , p = 0.986; food consumption:  $t_{(19)} = 0.491$ , p = 0.629; n = 9-12, unpaired t test) and OFT (F) (number of standing: n = 14-16,  $t_{(31)} = 0.421$ , p = 0.680, unpaired t test). The data are shown as mean  $\pm$  SEM. \*p < 0.05.

Related to Figure 2.

**Figure. S5**



**Figure. S5. Relative behaviors of P2X2 local knockdown or overexpression mice. (A)**

Representative heatmaps of normalized time spent during SI test before (left) and after (right)

CSDS of *P2rx2<sup>loxp/loxp</sup>* mice injected with AAV-CamkII $\alpha$ -EGFP-Cre or control virus. **(B)** SI ratio

(n = 10-11, p = 0.010, interaction effect, matching two-way ANOVA) of *P2rx2<sup>loxp/loxp</sup>* mice

injected with AAV-CamkII $\alpha$ -EGFP-Cre or control virus. **(C)** Representative heatmaps of

normalized time spent during SI test before (left) and after (right) CSDS of *CamkIIa-Cre* mice

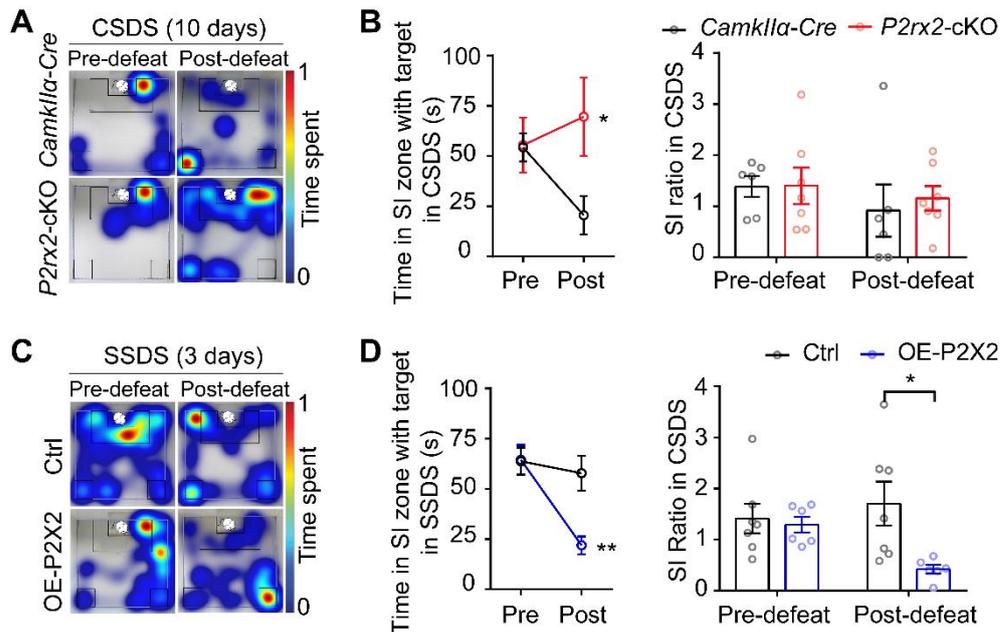
injected with AAV-DIO-P2X2 or control virus. **(D-E)** SI ratio **(D)** (n = 7-9, p = 0.035, interaction

effect, matching two-way ANOVA) and NSF **(E)** (n = 11-12,  $t_{(21)} = 0.384$ , p = 0.700; unpaired t

test) of *CamkII $\alpha$ -Cre* mice injected with AAV-DIO-P2X2 or control virus. **(F)** Representative heatmaps of normalized time spent during SI test before (left) and after (right) CSDS of *P2rx2-cKO* mice injected with AAV- DIO-P2X2 or control virus. **(G)** SI ratio (n = 6-7, p = 0.025, interaction effect, matching two-way ANOVA) of *P2rx2-cKO* mice injected with AAV- DIO-P2X2 or control virus. The data are shown as mean  $\pm$  SEM. \*p < 0.05.

Related to Fig. 3.

**Figure. S6**



**Figure. S6. CSDS results of mice applying AAV-DIO-GCaMP6s virus. (A, B)** Representative

heatmaps (A) and analysis of social interaction (B) ( $n = 6-7$ , time in the interaction zone with

target:  $p = 0.017$ ; SI ratio:  $p = 0.747$ ; interaction effect, matching two-way ANOVA) of *P2rx2-*

*cKO* and *Camk1la-cre* control mice injected with AAV-DIO-GCaMP6s virus in CSDS paradigm.

(C, D) Representative heatmaps (C) and analysis of social interaction (D) ( $n = 6-7$ , time in the

interaction zone with target:  $*p = 0.035$ ; SI ratio:  $p = 0.060$ ; interaction effect, matching two-way

ANOVA) of P2X2 overexpression and control mice injected with AAV-DIO-GCaMP6s virus. The

data are shown as mean  $\pm$  SEM.  $*p < 0.05$ ,  $**p < 0.01$ .

Related to Figure 4.

**Supplementary Table 1**

Gene	Former primer	Reverse primer
<i>P2rx1</i>	CCG AAG CCT TGC TGA GAA	GGT TTG CAG TGC CGT ACA T
<i>P2rx2</i>	CAG AAC TGG CAC ACA AGG G	CAG TCA CAC AGA AAG GAG CC
<i>P2rx3</i>	GGT GGC TGC CTT CAC TTC	TCA GCC CCT TTG AGG AAA
<i>P2rx4</i>	CCA ACA CTT CTC AGC TTG GAT	TGG TCA TGA TGA AGA GGG AGT
<i>P2rx5</i>	CAC AGT CAT CAA CAT TGG TTC C	AGG TAG ATA AGT ACC AGG TCA CAG AAG
<i>P2rx6</i>	TGT CCC CAG TAC TCC TTC CA	CAC CAG TGA TTG GCT GTC C
<i>P2rx7</i>	GGG GGT TTA CCC CTA CTG TAA	GCT CGT CGA CAA AGG ACA C
<i>Synaptophysin</i>	TCT TTG TCA CCG TGG CTG TGT T	TCC CTC AGT TCC TTG CAT GTG T
<i>SNAP 25</i>	CTG GCA TCA GGA CTT TGG TT	ATT ATT GCC CCA GGC TTT TT
<i>Synasin 1</i>	CCA GCT CAA CAA ATC CCA GT	GGT GTC AGT CGG AGA AGA GG
<i>PSD 95</i>	TCT GTG CGA GAG GTA GCA GA	AAG CAC TCC GTG AAC TCC TG
<i>Rab4b</i>	ACT ATT GGC GTG GAG TTT GG	CAC AGA GGA TGA CCA CGA TG
<i>Tubb4</i>	GGG GAC CTC AAC CAC CTA GT	ATC CTG GCA TGA AGA AGT GG
<i>MAP 2</i>	TCA GGA GAC AGG GAG GAG AA	GTG TGG AGG TGC CAC TTT TT
<i>VGLUT2</i>	GCT CAC CTC TAC CCT CAA TAT	CCA CTT GCT CCA TAT CCC ATG

	G	
<i>VGAT</i>	ACG ACA AAC CCA AGA TCA CG	AAG ATG ATG AGG AAC AAC CCC
<i><math>\beta</math>-actin</i>	CCA CCA TGT ACC CAG GCA TT	CGG ACT CAT CGT ACT CCT GC

**Supplementary Table 1. qPCR primers.**