

1 **SUPPLEMENTARY INFORMATION**

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3 **Enhanced AMPAR-dependent synaptic transmission by**
4 **S-nitrosylation in the vmPFC contributes to chronic inflammatory**
5 **pain-induced persistent anxiety in mice**

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8 **Chen *et al.***

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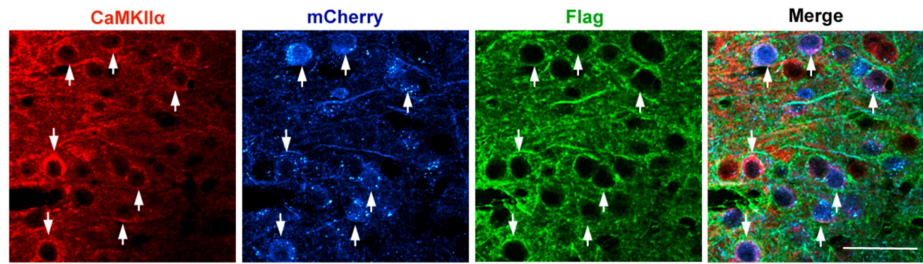
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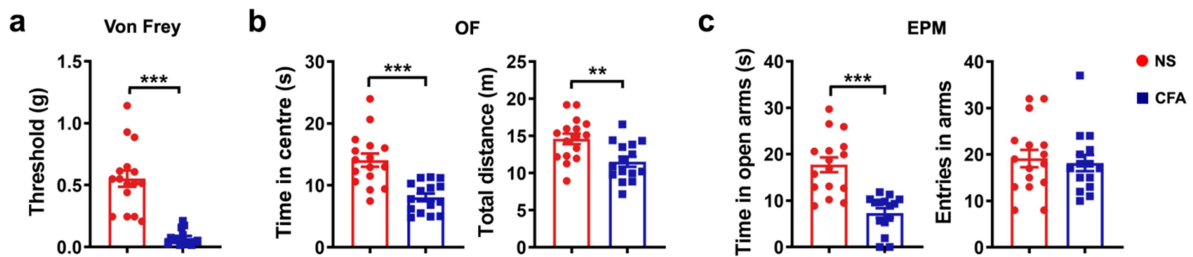
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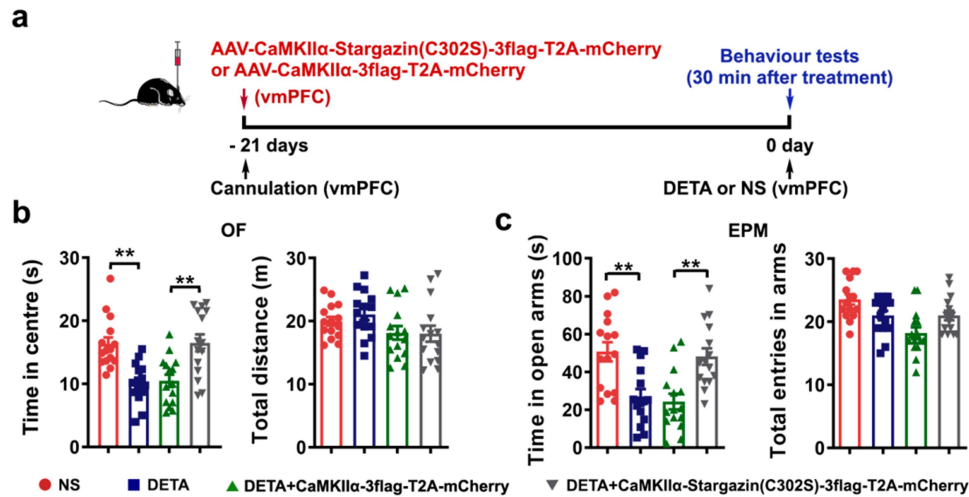
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Fig. S1 Expression of stargazin(C302S)-3flag in CaMKII α ⁺ neurons of vmPFC. Immunofluorescent images were captured from AAV-CaMKII α -stargazin(C302S)-3flag-T2A-mCherry-infected vmPFC at Day 21 after microinjection. Arrows marked flag⁺&mCherry⁺ cells co-labeled with CaMKII α ⁺ neurons. Similar results were observed with 4 mice. Scale bar, 50 μ m.



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Fig. S2 Behaviours of NS- or CFA-injected mice without ibuprofen-treatment. a-b Withdrawal threshold of the hind paw in von Frey tests (a), time in centre (b, left) and total distance (b, right) in OF tests, and time in open arms (c, left) and total entries in arms (c, right) in EPM tests at day 20 after CFA or NS injection. $n = 16$ for NS and $n = 15$ for CFA. Data are the mean \pm SEM; ** $P < 0.01$ and *** $P < 0.001$ (unpaired two-tailed Student's t-test for b (right), unpaired two-tailed Welch's t-test for b (left) or unpaired two-tailed Mann-Whitney U test for a and c).

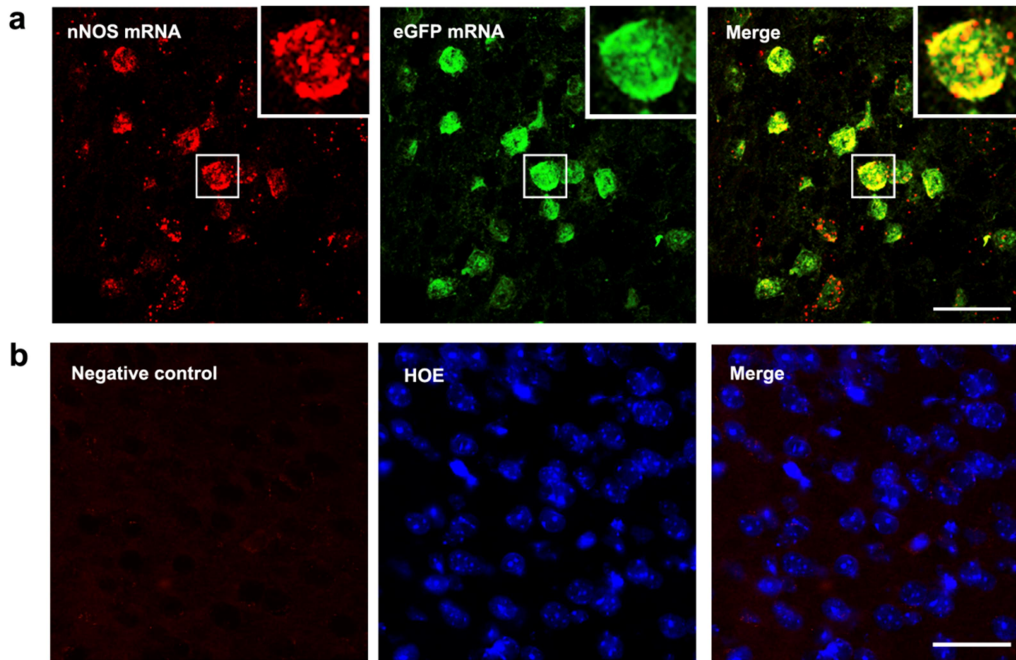


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2 **Fig. S3 Stargazin(C302S) mutant attenuates NO donor-induced anxiety.** **a** Scheme of
 3 experimental design. **b-c** Time in centre (b, left) and total distance (b, right) in OF tests, time
 4 in open arms (c, left), and total entries in arms (c, right) in EPM tests 30 min after
 5 DETA/NONOate (100 μ M, 1 μ l) or NS delivery through pre-implanted cannula. $n = 15$ for
 6 each group. Data are the mean \pm SEM; ** $P < 0.01$ and *** $P < 0.001$ (Tukey's test following
 7 ordinary one-way ANOVA for b (right), Tamhane's T2 test following Brown-Forsythe and
 8 Welch ANOVA for c (left) and Dunn's test following Kruskal-Wallis ANOVA for b (left) and
 9 c (right)).

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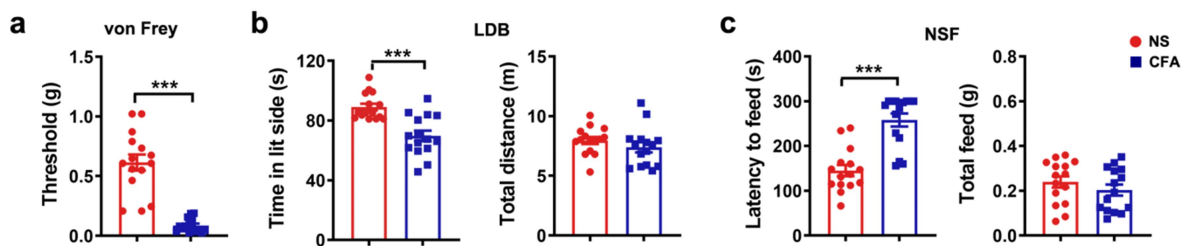
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3 **Fig. S4 Cre-dependent mRNA expression was specific to nNOS-expressing neurons. a**
4 Images of fluorescence in situ hybridization (FISH) showing colocalization of nNOS mRNA
5 and Cre-dependent eGFP mRNA in the vmPFC. Similar results were observed with 3 mice.
6 Scale bar, 50 μ m. **b** FISH images labeled with negative control probe. Similar results were
7 observed with 3 mice. Scale bar, 50 μ m.

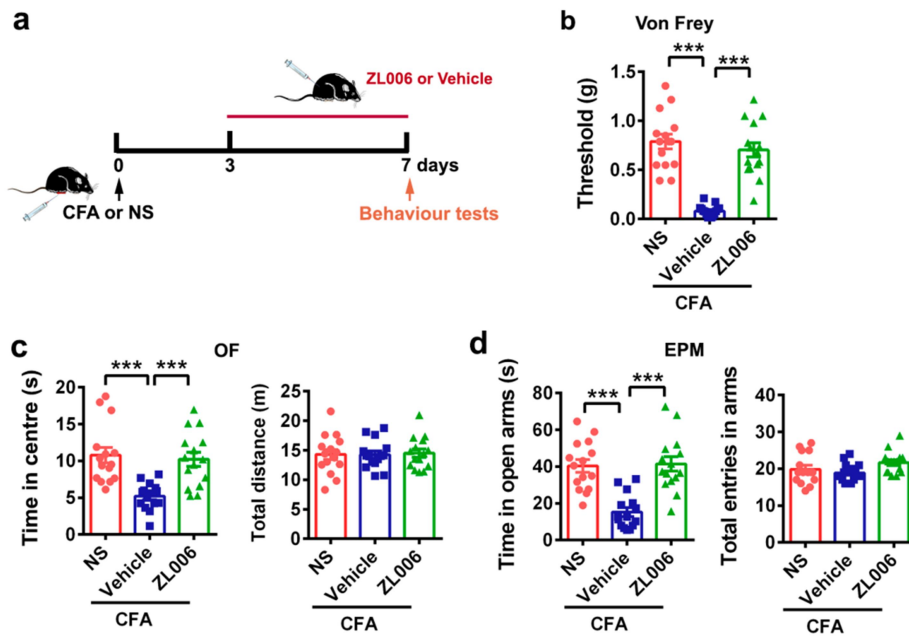
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10 **Fig. S5 Behaviors of NS- or CFA-injected mice without Ibuprofen-treatment. a-c**
11 Withdrawal threshold of hind paw in von Frey tests (a), time in side with light (b, left) and
12 total distance (b, right) in LDB tests, and latency to feed (c, left) and total feed (c, right) in
13 NSF tests at day 10 after CFA or NS injection. $n = 15$ for each group. Data are the mean \pm
14 SEM; *** $P < 0.001$ (unpaired two-tailed Student's t-test for a, b (right) and c (right), and
15 unpaired two-tailed Mann-Whitney test for b (left) and c (left)).

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3 **Fig. S6 ZL006 alleviated chronic pain-induced anxiety when it relieved chronic pain. a**

4 Scheme of experimental design. **b-d** Withdrawal threshold of the hind paw in von Frey tests

5 (b), time in centre (c, left) and total distance (c, right) in OF tests, and time in open arms (c,

6 left) and total entries in arms (c, right) in EPM tests at day 7 after CFA or NS injection. $n = 15$

7 for each group. Data are the mean \pm SEM; *** $P < 0.001$ (Tamhane's T2 test following

8 Brown-Forsythe and Welch ANOVA for b, c (left) and d (left), Tukey's test following ordinary

9 one-way ANOVA for c (right), Dunn's test following Kruskal-Wallis ANOVA for d (right)).

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