Supplemental Material

The G protein-coupled estrogen receptor of the trigeminal ganglion regulates acute and chronic itch in mice

Jun Li [1, 2, 3†], Po Gao [2, 3†, *], Siyu Zhang [2, 3, 4], Xiaoqi Lin [2, 3], Junhui Chen [2, 3], Song Zhang [2, 3], Yingfu Jiao [2, 3], Weifeng Yu [2, 3], Xiaoqiong Xia [1*], Liqun Yang [2, 3*].

[1]Department of Anesthesiology, Chaohu Hospital Affiliated to Anhui Medical University, Chaohu, Anhui, 238000, China

[2]Department of Anesthesiology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China

[3]Key Laboratory of Anesthesiology (Shanghai Jiao Tong University), Ministry of Education, China

[4]Department of Anesthesiology, The Second Affiliated Hospital of Jiaxing University, Jiaxing, 314000, Zhejiang, China

† These authors have contributed equally to this work and share first authorship.

*** Correspondence:**

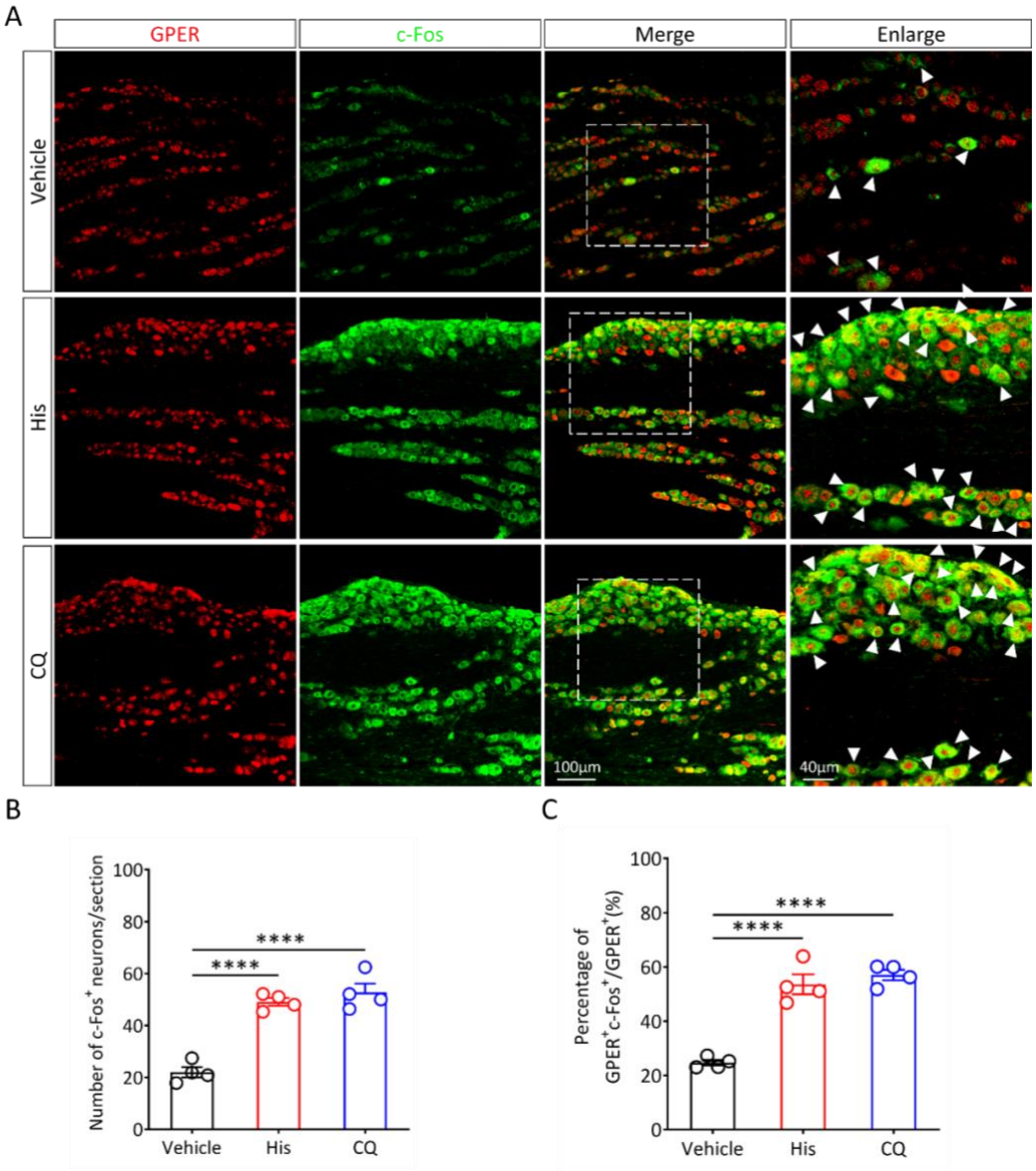
Po Gao and Liqun Yang, Department of Anesthesiology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Email: gaopo0908@163.com and lqyang72721@126.com

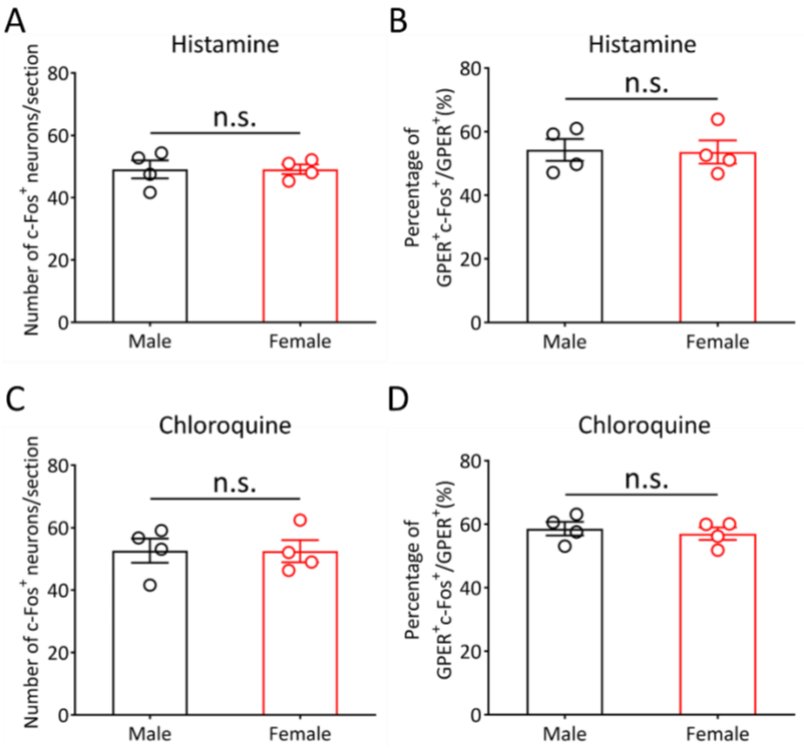
Xiaoqiong Xia, Department of Anesthesiology, Chaohu Hospital Affiliated to Anhui Medical University, Chaohu, Anhui, 238000, China.

Email: xxq2366833@sina.com

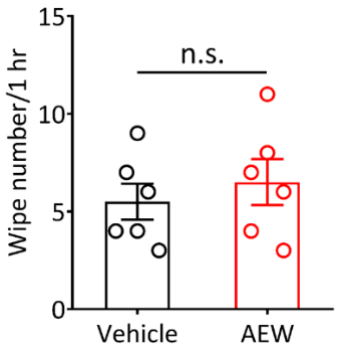
Supplemental Figures



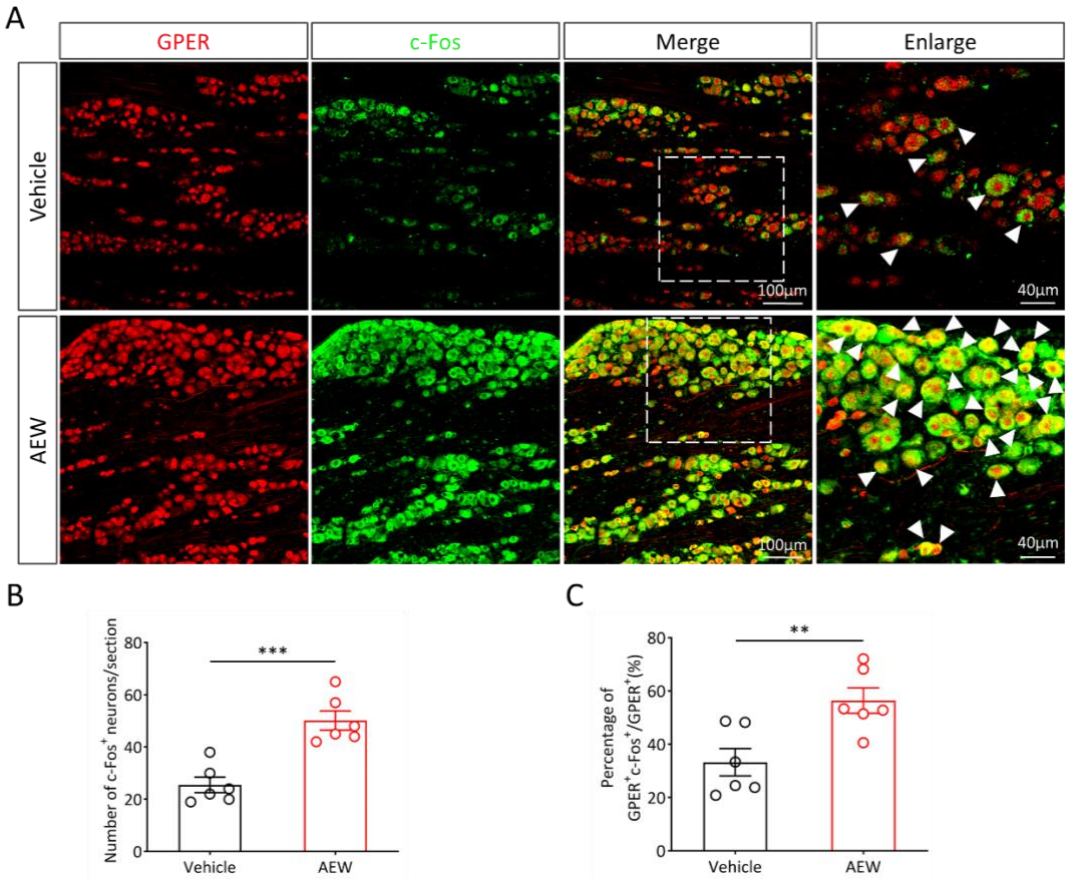
Supplemental Figure 1. GPER$^+$ neurons in the TG of female mice are significantly activated by acute itch stimuli. **(A)** Representative immunofluorescence images showing the colocalization of GPER (red) and c-Fos (green) in TG after administration of vehicle (saline), histamine, or chloroquine (scale bar: 100 μm or 40 μm). **(B)** Quantitative analysis of the number of c-Fos$^+$ neurons in TG after acute itch stimuli (n = 4 per group, ****p < 0.0001, one-way ANOVA with Tukey's *post hoc* test). **(C)** Quantitative analysis of the percentage of activated GPER$^+$ neurons in total GPER$^+$ neurons in the TG subjected to acute itch stimuli (n = 4 mice per group, ****p < 0.0001, one-way ANOVA with Tukey's *post hoc* test)



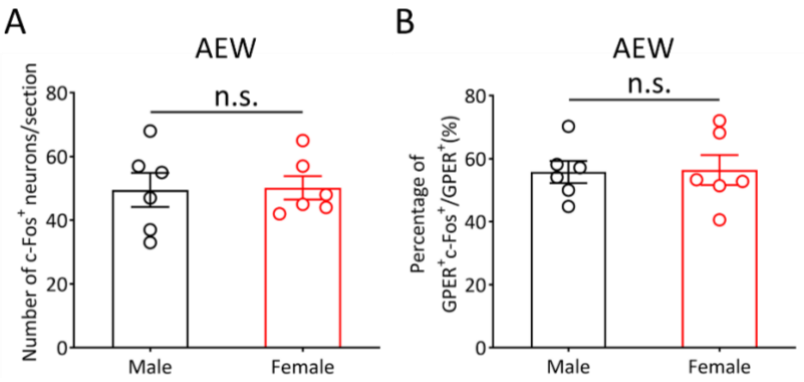
Supplemental Figure 2. There is no significant difference in the number of activated GPER$^+$ neurons in the TG between male and female mice during acute itch processing. **(A, B)** Quantitative analysis of the number of c-Fos$^+$ neurons and the percentage of activated GPER$^+$ neurons in total GPER$^+$ in the TG of male and female mice after histamine stimuli (n = 4 per group, n.s.: no statistical difference, unpaired Student's *t*-test). **(C, D)** Quantitative analysis of the number of c-Fos$^+$ neurons and the percentage of activated GPER$^+$ neurons in total GPER$^+$ in the TG of male and female mice after chloroquine stimuli (n = 4 per group, n.s.: no statistical difference, unpaired Student's *t*-test).



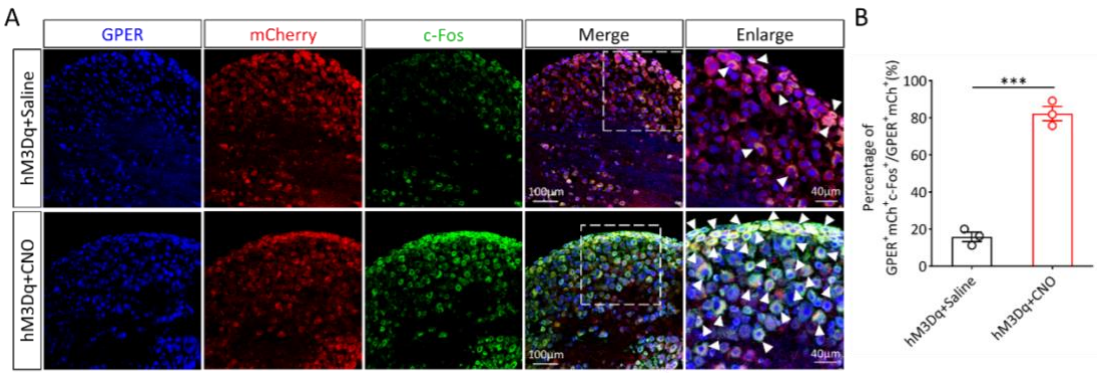
Supplemental Figure 3. The wiping behavior (related to pain) was not significantly changed in male mice treated with AEW compared to the vehicle group (n = 6 per group, n.s.: no statistical difference, unpaired Student's *t*-test).



Supplemental Figure 4. GPER$^+$ neurons in the TG of female mice are significantly activated under AEW-induced chronic itch condition. **(A)** Representative immunofluorescence images showing the colocalization of GPER (red) and c-Fos (green) in TG of vehicle- and AEW-treated mice (scale bar: 100 μm or 40 μm). **(B)** Quantitative analysis of the number of c-Fos$^+$ neurons in TG under AEW-induced chronic itch conditions (n = 6 per group, ***$p < 0.001$, unpaired Student's *t*-test). **(C)** Quantitative analysis of the percentage of activated GPER$^+$ neurons in total GPER$^+$ neurons of the TG under AEW-induced chronic itch conditions (n = 6 per group, **$p < 0.01$ unpaired Student's *t*-test).



Supplemental Figure 5. There is no significant difference in the number of activated GPER$^+$ neurons in the TG between male and female mice under chronic itch conditions. **(A)** Quantitative analysis of the number of c-Fos$^+$ neurons in TG of male and female mice under AEW-induced chronic itch conditions (n = 6 per group, n.s.: no statistical difference, unpaired Student's *t*-test). **(B)** Quantitative analysis of the percentage of activated GPER$^+$ neurons in total GPER$^+$ neurons in TG of male and female mice under AEW-induced chronic itch conditions (n = 6 per group, n.s.: no statistical difference, unpaired Student's *t*-test).



Supplemental Figure 6. The GPER neurons in the TG were successfully activated by chemogenetic regulation. **(A)** Representative immunofluorescence images showing the colocalization of GPER (blue), mCherry (red) and c-Fos (green) in TG after administration of CNO or saline (scale bar: 100 μm or 40 μm). (B) Quantitative analysis of the percentage of $GPER^+/mCh^+/c\text{-}Fos^+$ neurons in total $GPER^+/mCh^+$ neurons after the administration of CNO or saline (n = 3 per group, ***$p < 0.001$, unpaired Student's *t*-test).