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Supplemental information

A non-canonical retina-ipRGCs-SCN-PVT visual pathway for mediating contagious itch behavior

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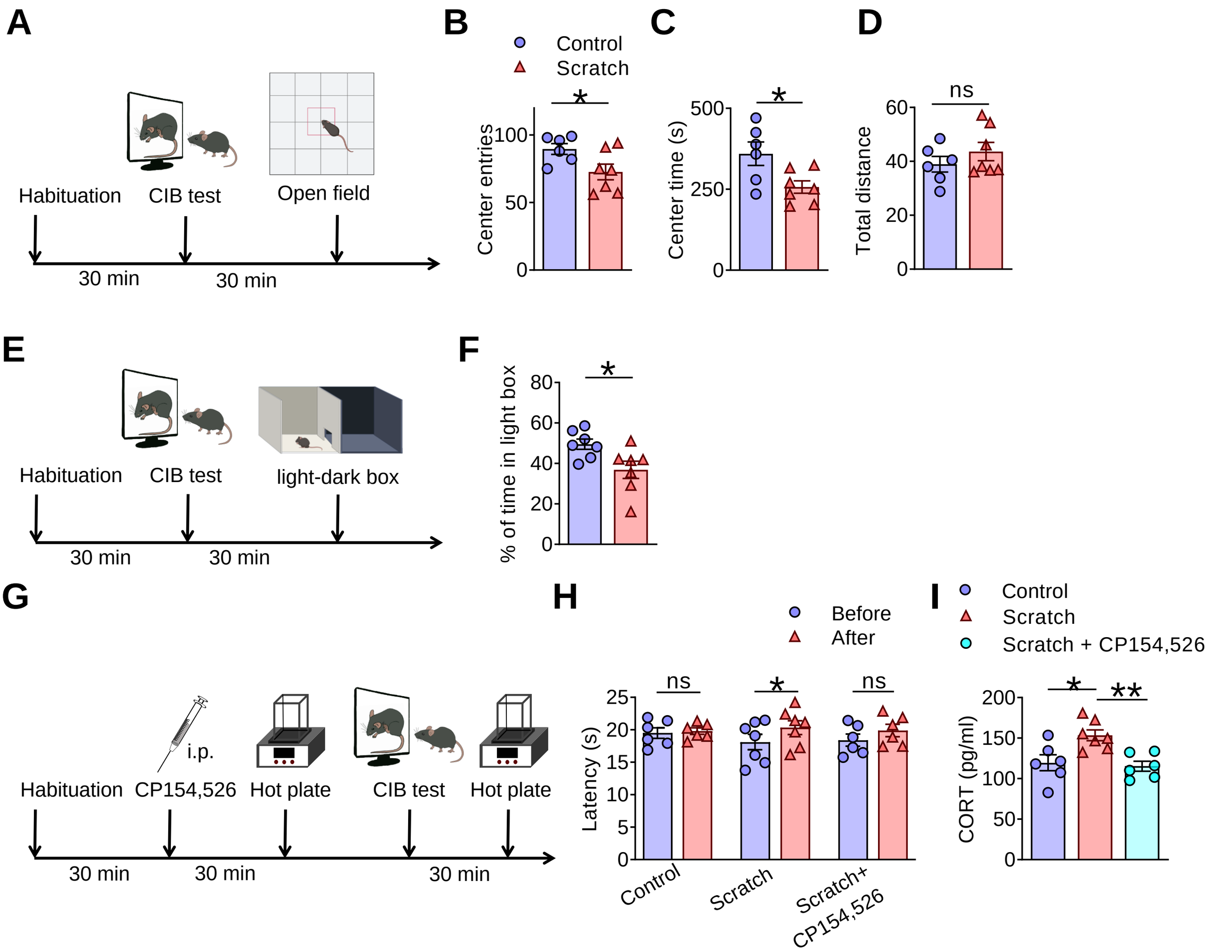

Figure S1. Scratching motion induces stress/anxiety-like behavior in mice. (**A**) Experimental schedule and cartoon illustrations for the open field behavior test. Mice were habituated in the behavior room for 30 min, followed by the CIB test and then tested in the open field box (**B, C, D**) The frequency for center entries (**B,** t = 2.313, df = 11, *P* = 0.0411), the time spent in the center (**C,** t = 2.633, df = 11, *P* = 0.0233) and the total travel distance (**D**, t = 1.043, df = 11, *P* = 0.3195) of mice in the open field test. (**E**) Experimental schedule and cartoon illustrations for the light-dark box behavior test. Mice were habituated in the behavior room for 30 min, followed by the CIB test and then tested light-dark box. (**F**) Percentage of time spent in the light box (t = 2.547, df = 12, *P* = 0.0256). (**G**) Schedule of the hot plate and stress hormone tests. (**H**) Hot plate latency at 50 ° of control mice or mice pretreated with the anti-stress antagonist (CRF1 receptor antagonist, CP154, 526, i.p.) before and after the CIB test (Scratch, t = 2.450, df = 6, *P* = 0.0498). (**I**) Corticosterone (CORT) level in the control mice or mice pretreated with CP154, 526 (i.p.) after the CIB test (F = 7.867, *P* = 0.0042). Unpaired *t* test in **B**, **C**, **D** and **F**. Paired *t* test in **H**. One-way ANOVA in **I**. Mice (n = 6 or 7/group). Data are presented as mean ± SEM. ns, not significant, * *P* < 0.05, * **P* < 0.01.

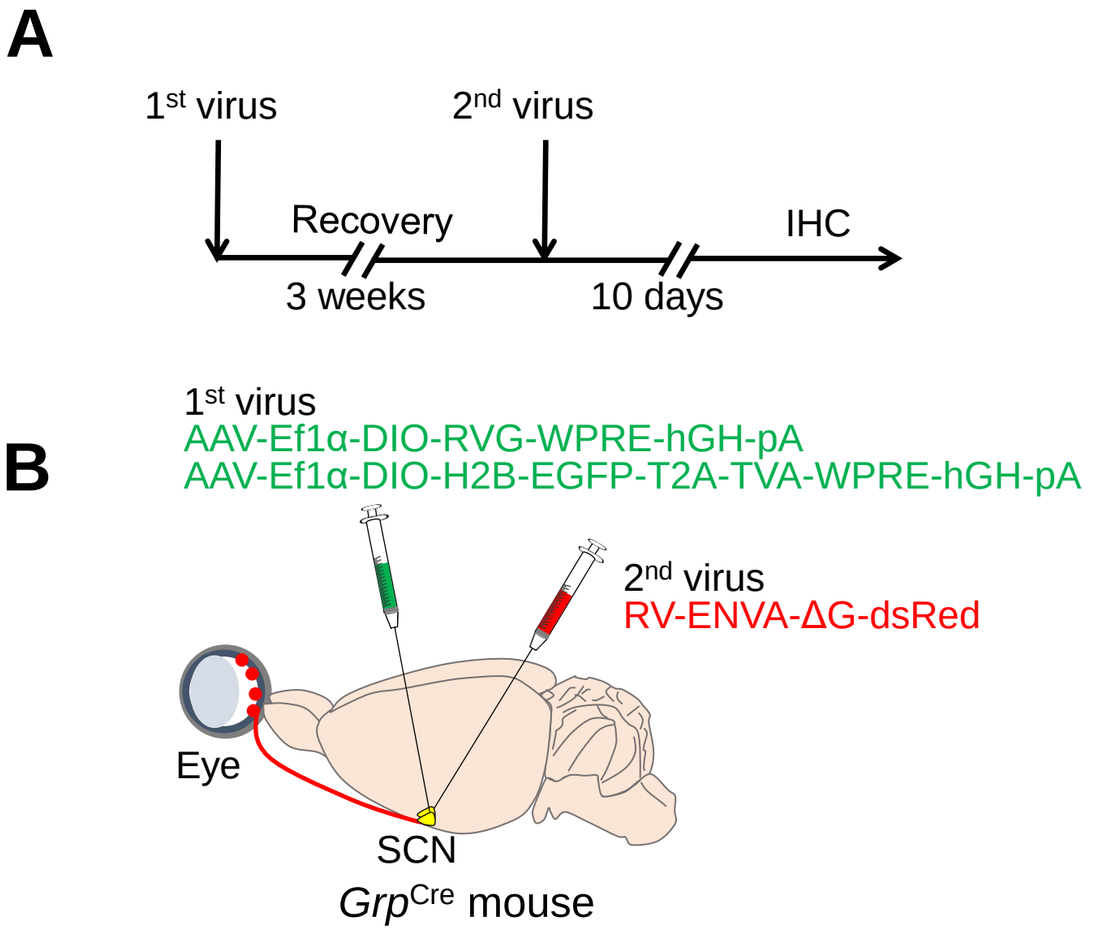

Figure S2. SCN GRP neurons receive direct monosynaptic input from ipRGCs. A-B, Schedule of virus monosynaptic tracing of SCN GRP neurons input. A mix of helper viruses: AAV-Ef1α-DIO-RVG-WPRE-hGH-pA and AAV-Ef1α-DIO-H2B-EGFP-T2A-TVA-WPRE-hGH-pA was injected into the SCN. 3 weeks later, the second virus RV-ENVA-ΔG-dsRed was injected into the SCN. **C**, IHC image of EGFP and dsRed in the SCN. EGFP in green indicate the expression of helper virus, yellow (white arrows) indicate the expression of RV in helper expression cells, which is the starter cells. Red only cells are the input cells. Scale bar in **C**, 50 μm. n = 3. **D**, IHC image of dsRed cells and melanopsin (in cyan) in the retina (whole-mounted), Scale bar in **D**, 100 μm. Arrows indicate the overlap of dsRed cells with melanopsin. White box indicate the zoomed areas in **E** and **F**. Scale bar in **E** and **F**, 20 μm. n=3.
Related to Figure 1.

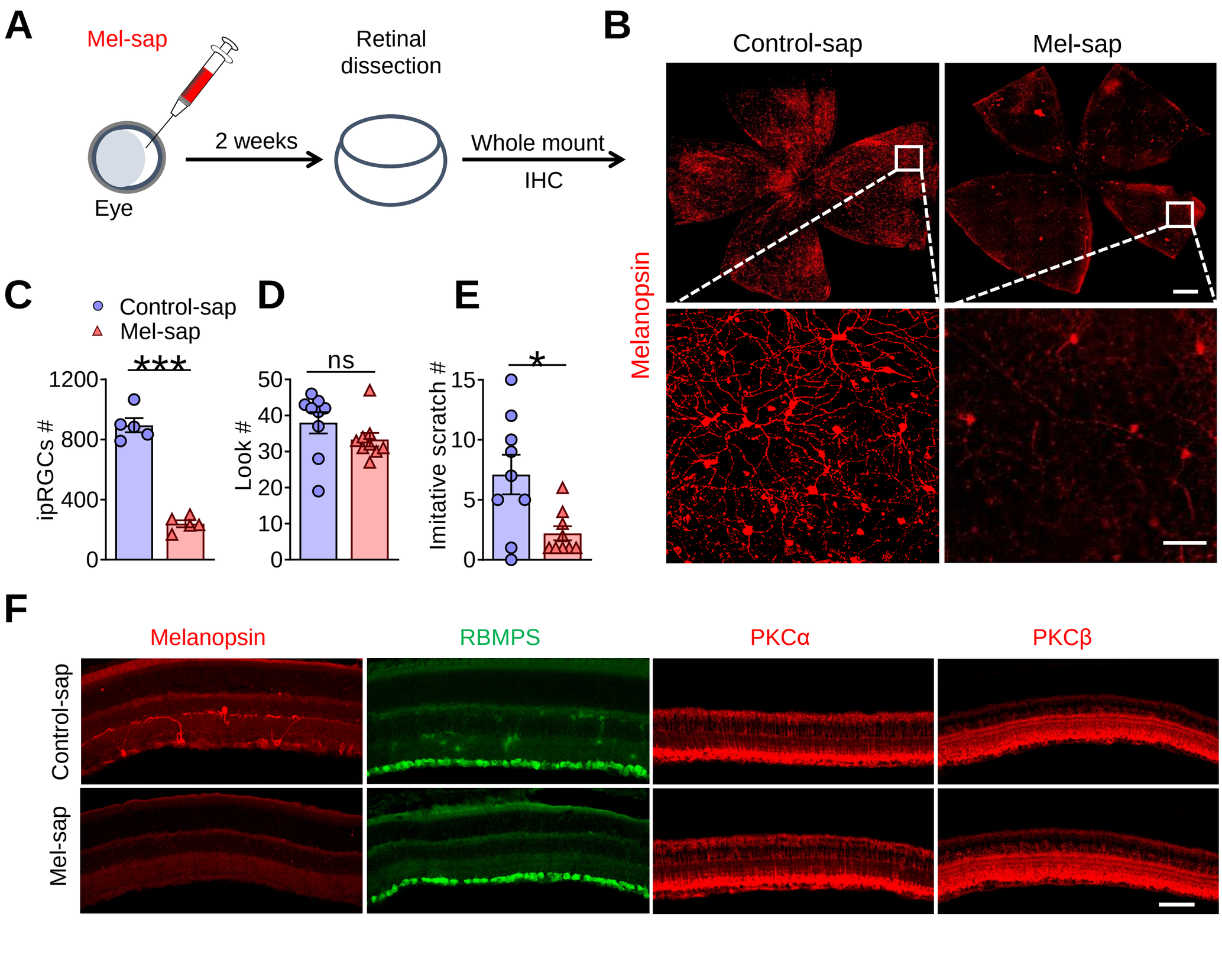

Figure S3. Ablation of ipRGCs by Mel-sap attenuates CIB. (**A**) Schematic of intravitreal injection of Melanopsin-saporin (Mel-sap) and dissection of the whole retina for whole-mount IHC. (**B**) ipRGCs labeled with Melanopsin in retina flat mounts treated with control-sap and Mel-sap, respectively. Scale bar, 500 μm (top panel), 100 μm (bottom panel). (**C**) Mel-sap significantly reduced the number of ipRGCs relative to the control. n = 5, t = 12.55, df = 8, $P < 0.0001$. n =5. (**D)** The mean number of look behaviors of control-sap and Mel-sap mice toward the scratching demonstrator. t = 1.938, df = 18, $P = 0.0685$. (**E**) The mean number of imitative scratches of control-sap and Mel-sap mice toward the scratching demonstrator. t = 3.182, df = 18, $P = 0.0052$. n = 9 for control-sap, n = 11 for Mel-sap. (**F**) IHC image of Melanopsin, RBMPS, PKCα and PKCβ in the coronal section of retina of control-sap or Mel-sap treated mice. Scale bar in H, 100 μm. n = 3. Data are presented as mean ± SEM. Unpaired *t* test in **C**-**E**. ns, not significant. *$P < 0.05$. ***$P < 0.001$.
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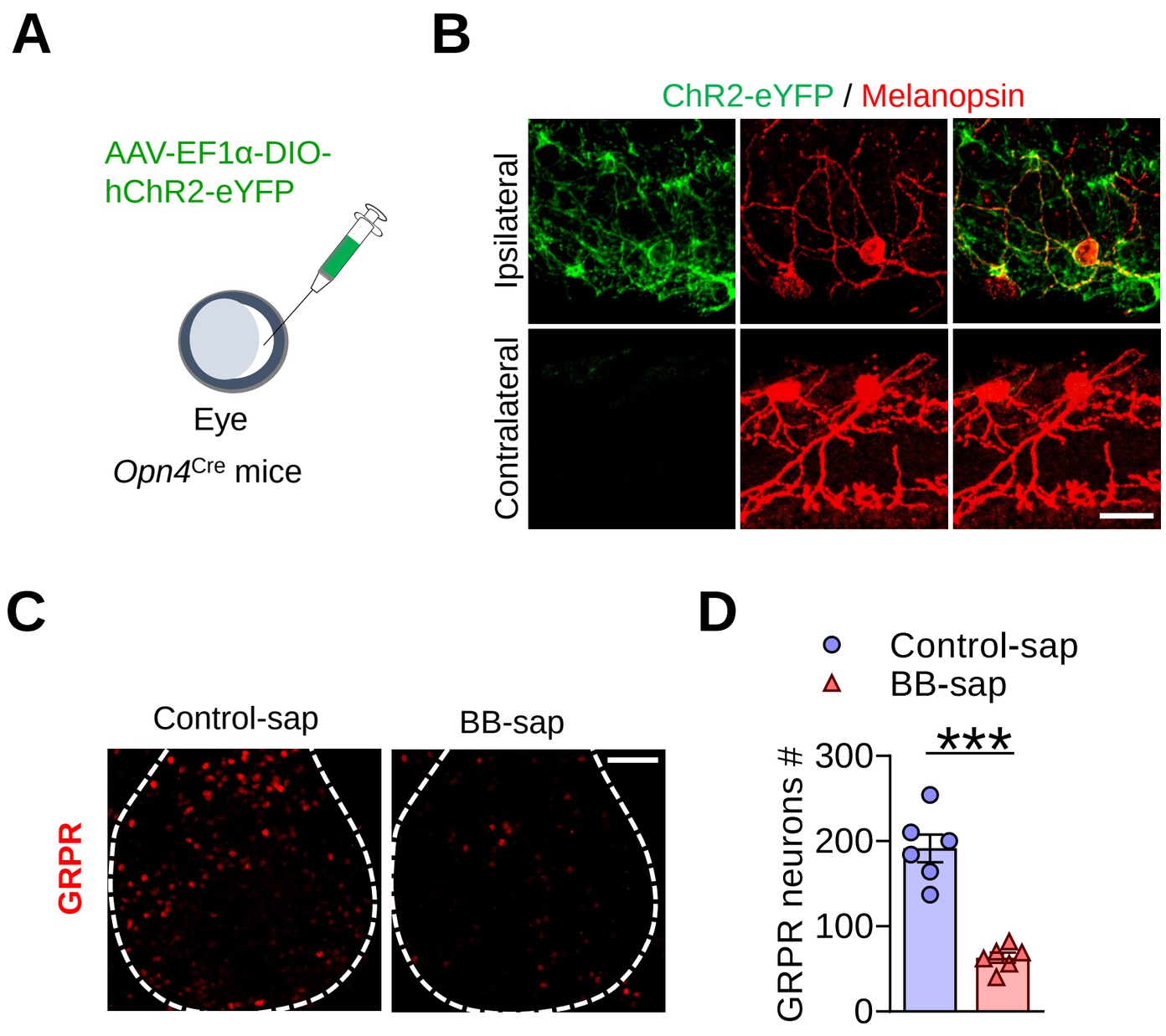

Figure S4. (**A**) Schematic illustration of intravitreal injection of AAV-EF1α-DIO-ChR2-eYFP in *Opn4*^{Cre} mice. (**B**) Overlapping expression of ChR2-eYFP and Melanopsin in the ipsilateral and contralateral retina of *Opn4*^{Cre} mice after ChR2 virus injection in the right eye. Scale bar, 50 μm. n = 3. (**C**) GRRP staining in the SCN of mice treated with control-sap and BB-sap. (**D**) Significantly reduced number of GRPR neurons in the SCN of mice treated with BB-sap relative to control-sap (**D**, t = 7.357, df = 10, $P < 0.0001$). n = 6 mice per group, Unpaired test in **D**. * $P < 0.05$, Scale bar in **B**, **C**, 50 μm. Related to Figure 2.

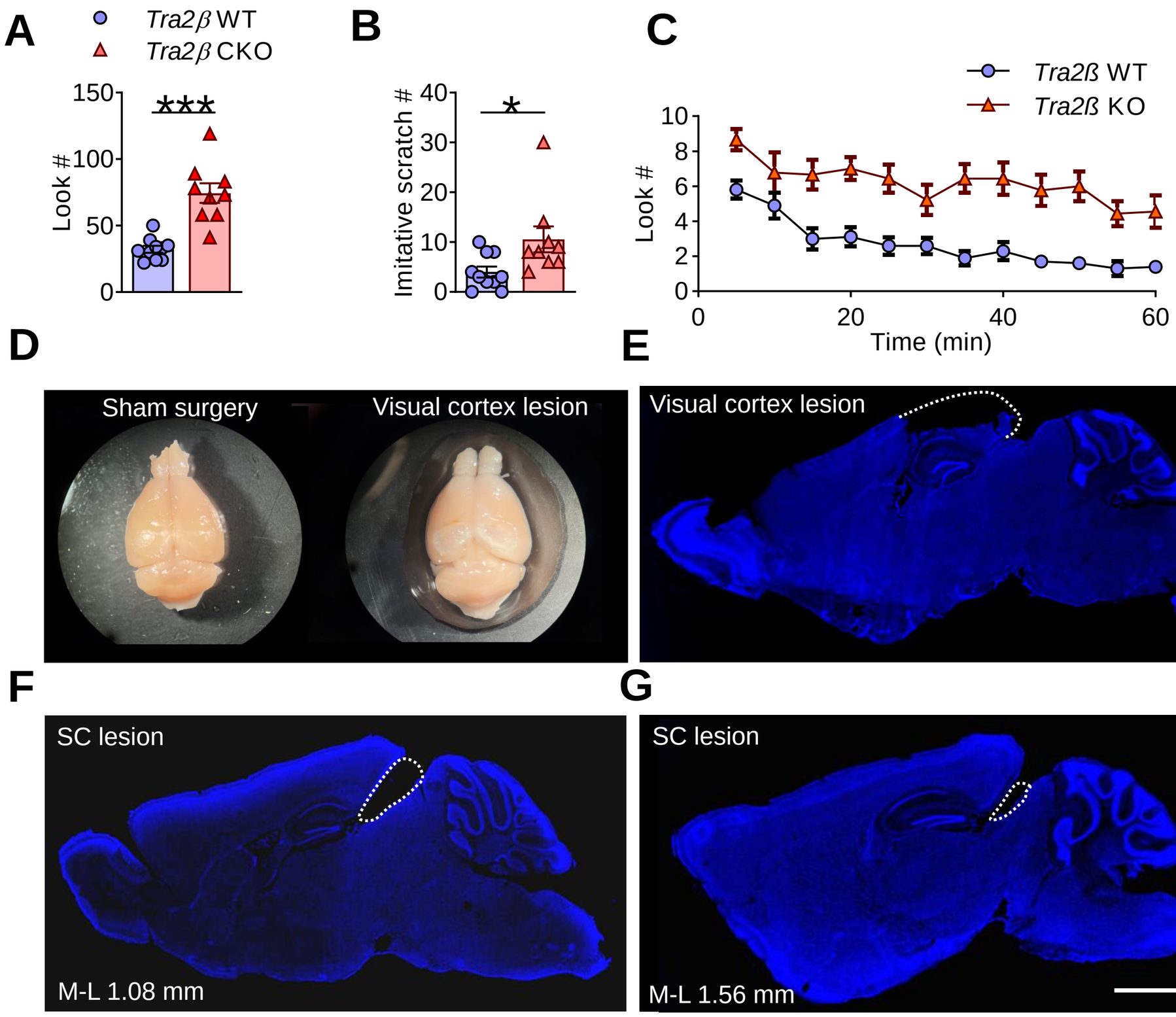

Figure S5. **CIB of cortexless mice.** (**A** and **B**) Significantly increased number of looks (**A**, t = 5.604, df = 17, $P < 0.0001$) and imitative scratches (**B**, t = 2.401, df = 17, $P = 0.0281$) of *Tra2β* CKO (*Tra2β*$^{f/f}$;*Emx1*^{Cre}) relative to that of *Tra2β* WT (*Tra2β*$^{f/f}$; *Emx1*^{Cre}). (**C**) Time course of mean number of looks of *Tra2β* CKO and WT mice. n = 9 ~ 10/group. (**D**) Images showing the brain tissue after sham surgery and visual cortex lesion surgery. (**E**) Sagittal sections of the brain (blue: DAPI) showing visual cortex lesion. (**F** and **G**) Sagittal section of the brain (blue: DAPI) showing SC lesion with different anatomic coordinates. Data are presented as mean ± SEM. Unpaired *t* test in **A**, **B**, * $P < 0.05$. *** $P < 0.001$. Scale bar, 2.5 mm.
Related to Figure 3.

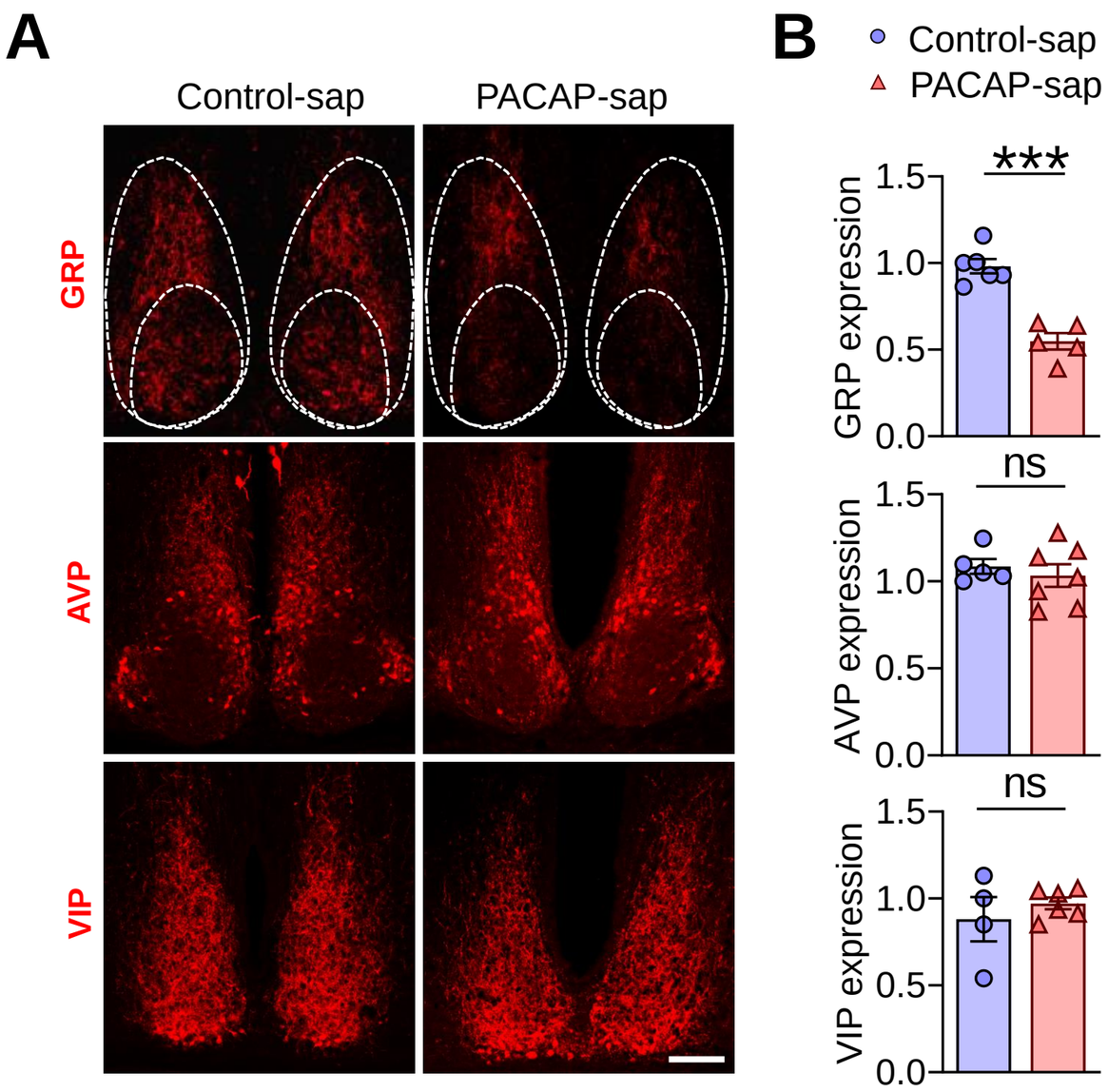

Figure S6. Ablation of PAC1R neurons reduces GRP expression. (**A**) Representative IHC images showing expression of GRP, AVP and VIP in the SCN after control-sap or PACAP-sap treatment. (**B)** Mean expression of GRP (top, t = 6.900, df = 9, $P < 0.0001$), AVP (middle, t = 0.6153, df = 10, $P = 0.5521$), and VIP (bottom, t = 0.8387, df = 8, $P = 0.4260$), in the SCN after Control-sap or PACAP-sap treatment. Data are presented as mean ± SEM. Unpaired *t* test in **B**. ns, not significant. *$P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.
Related to Figure 5.

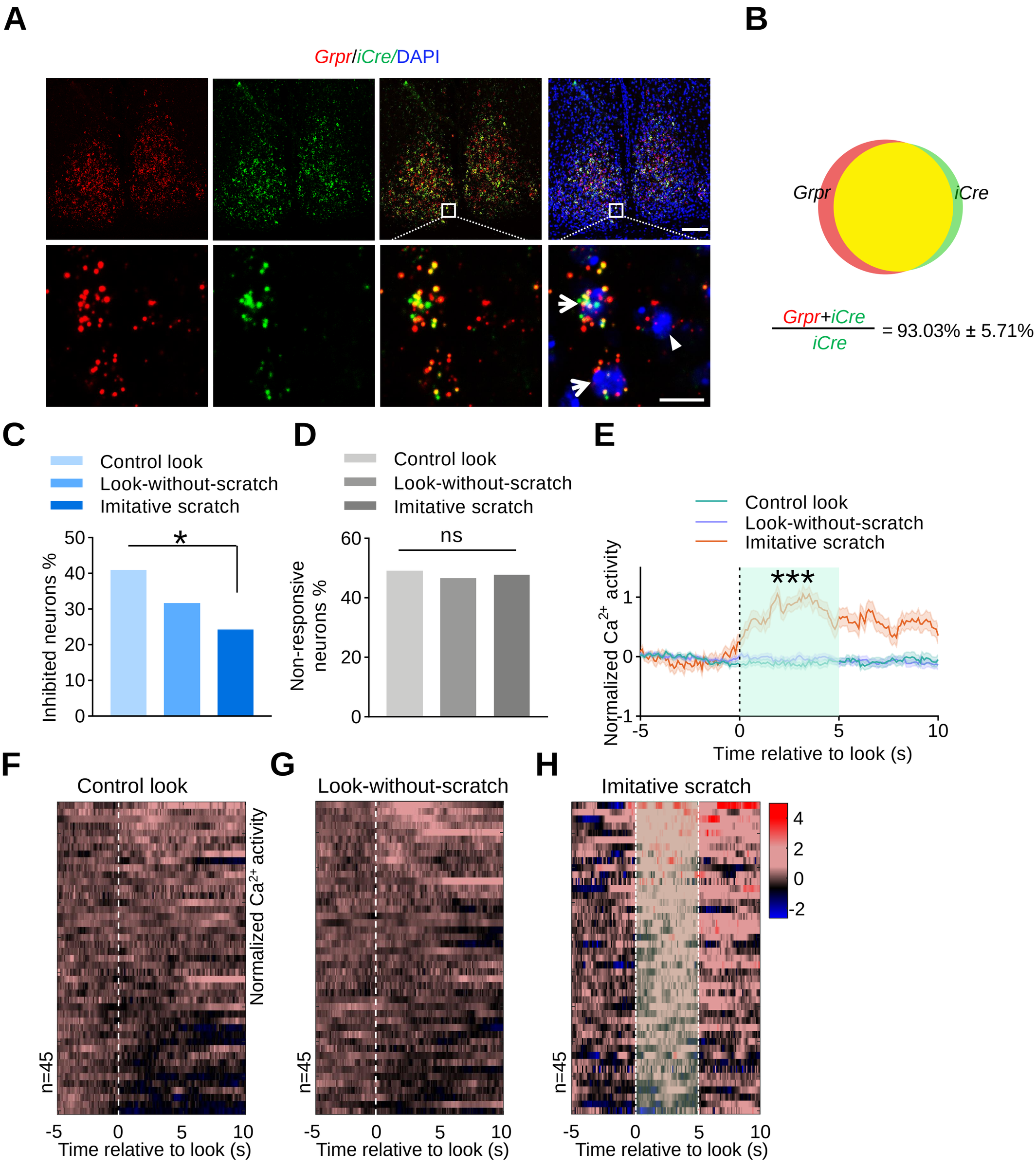

Figure S7. **Validation of SCN *iCre* expression and the Ca^{2+} activity of GRPR neurons during the CIB test.** (**A**) RNAscope ISH images showing the overlapping expression of *Grpr* (red) and *iCre* (green) in the SCN of $Grpr^{iCre}$ mice. (**B**) 93.0% of *iCre* cells express *Grpr.* Scale bar, upper panel 100 μm, bottom panel 10 μm. n = 3. (**C, D**) Mean percentage of GRPR cells showing inhibited Ca^{2+} response under three behavioral conditions (**C**), of non-responsive GRPR cells showing no change of Ca^{2+} response (**D**) under three behavioral conditions. (**E**) For the 45 neurons that were activated in the imitative scratch, we also compared their response to the other two look. Comparison of the mean Ca^{2+} traces of the 45 activated GRPR cells response corresponding to the look in the three groups (see F, G, H) F = 198.5, $P < 0.0001$. (**F, G, H**) Heatmap of normalized Ca^{2+} activity of the 45 activated individual GRPR cells in the control look (**F**), look-without-scratch (**G**) and imitative scratch (**H**) groups. Data are presented as mean ± SEM. Chi-square test in **C** and **D**. ns, not significant. *$P < 0.05$, **$P < 0.001$,
Related to Figure 6.

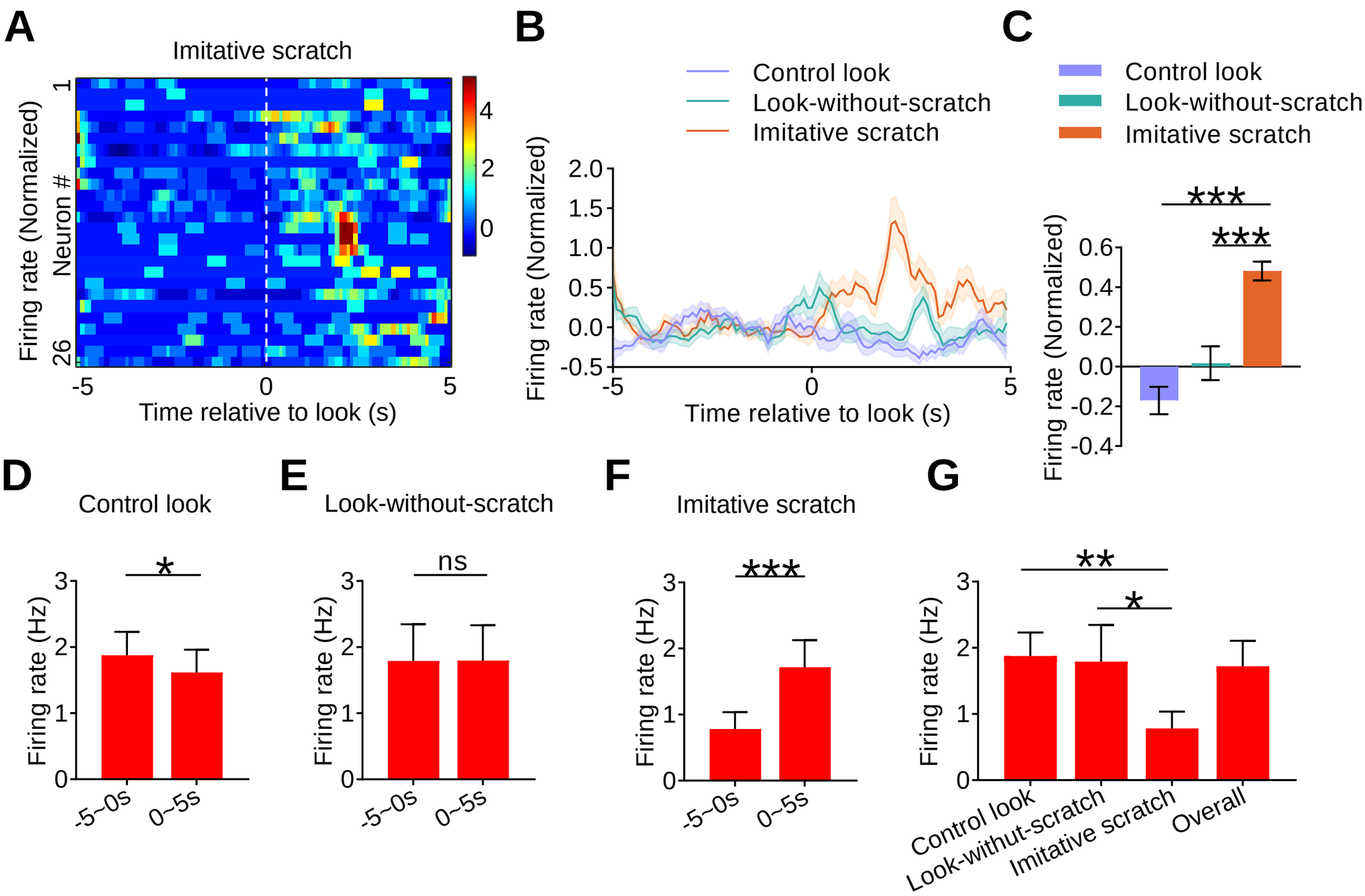

Figure S8. Distinct firing patterns of SCN GRPR itch neurons during the CIB test. (A) Heatmap of the normalized firing rate of GRPR itch neurons (n = 26) corresponding to the imitative scratch behavior. The time for the onset of look behaviors is 0 s. (B) The average traces of normalized firing rates of GRPR itch neurons (n = 26) during the CIB test. A significantly increased response after the onset of the look was seen in the imitative scratch group but not in the control look and look-without-scratch groups. (C) Comparison of the normalized firing rate of GRPR itch neurons 5s after look under three behavioral conditions. (D) Comparison of the mean firing rate of GRPR itch neurons (n = 26) before and after the control look (1.88 Hz vs. 1.62 Hz, $P = 0.0209$). (E) Comparison of the mean firing rate of GRPR itch neurons (n = 26) before and after the look-without-scratch (1.79 Hz vs. 1.79 Hz, $P = 0.98$). (F) Comparison of the mean firing rate of GRPR itch neurons 5 s before and after the imitative scratch (0.78 Hz vs. 1.72 Hz, $P < 0.001$). (G) Comparison of the mean baseline firing rate of GRPR itch neurons across three behavior groups (control look 1.88 Hz, look-without-scratch 1.79 Hz and imitative scratch 0.78 Hz). Paired *t* test in D, E, F, G. Unpaired *t* test in I. ns, not significant. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
Related to Figure 7.

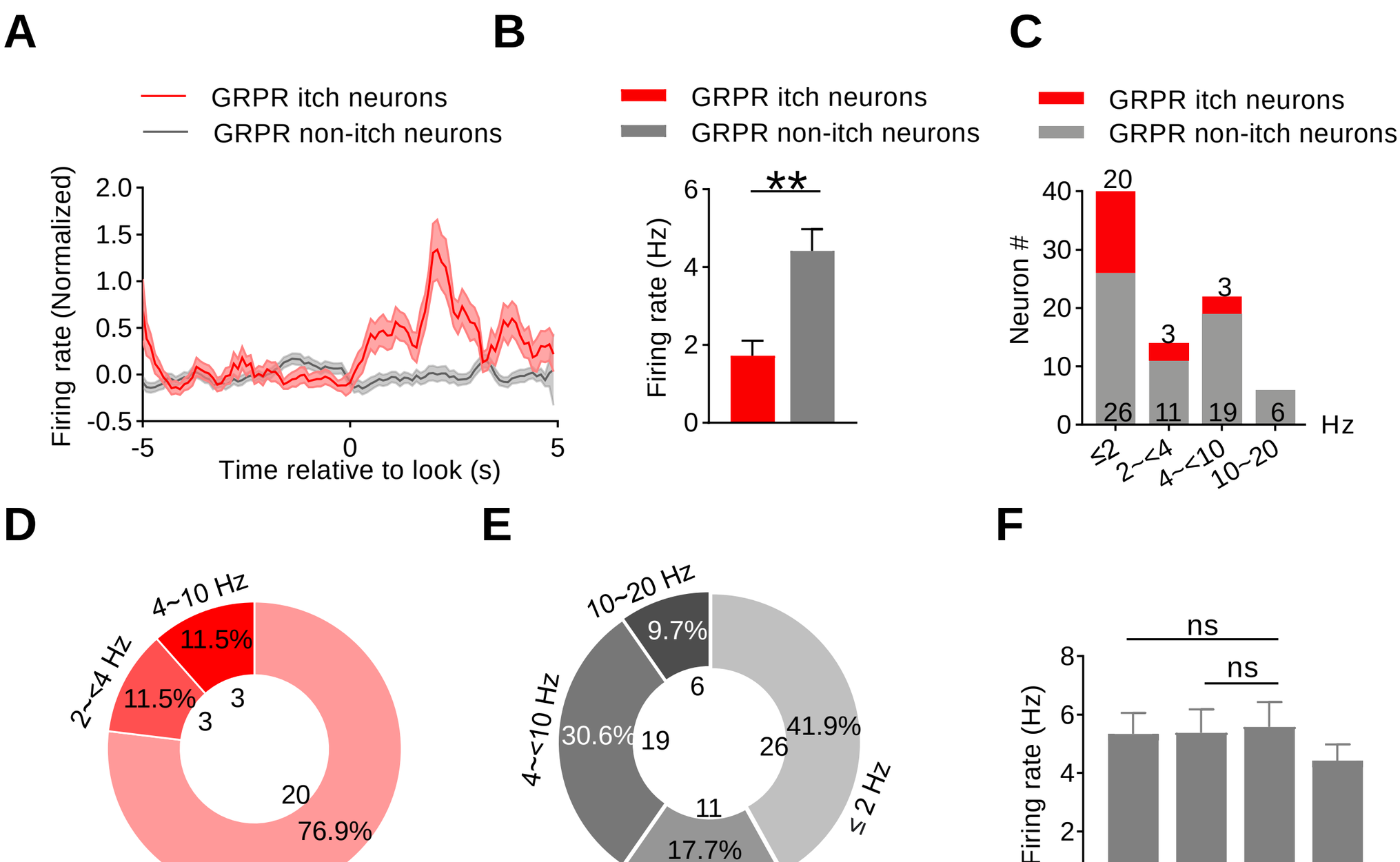

Figure S9. **Distinct firing patterns of GRPR itch and non-itch neurons.** (**A**) The average normalized trace of firing rate for GRPR itch neurons (n = 26) showed an increased firing rate in the imitative scratch group. No increase was observed for GRPR non-itch neurons (n = 62). (**B**) The mean firing rate of GRPR itch neurons and GRPR non-itch neurons (1.72 ± 0.39 Hz vs. 4.42 ± 0.55 Hz, *P* = 0.0009). (**C**) Distribution of the overall firing rate of GRPR neurons (n = 88) according to their response to scratching motion. (**D**) Distribution of GRPR itch neurons according to their overall firing rate. (**E**) Distribution of GRPR non-itch neurons according to their overall firing rate. (**F**) The mean baseline firing rate of GRPR non-itch neurons across three behavioral groups (the control look 5.33 ± 0.73 Hz, look-without-scratch 5.37 ± 0.81 Hz and imitative scratch 5.59 ± 0.84 Hz) and the mean firing rate across the 2 h recording session (Overall, 4.42 ± 0.55 Hz). (**G**) The mean firing rate of GRPR non-itch neurons (n = 62) before and after the control look (5.33 ± 0.73 Hz vs 5.05 ± 0.68 Hz, *P* = 0.0012). (**H**) The mean firing rate of GRPR non-itch neurons (n = 62) before and after the look-without-scratch (5.37 ± 0.81 Hz vs 5.3 ± 0.79 Hz, *P* = 0.5999). (**I**) The mean firing rate of GRPR non-itch neurons (n = 62) before and after the imitative scratch (5.59 ± 0.84 Hz vs 5.34 ± 0.8 Hz, *P* = 0.4793). Data are presented as mean ± SEM. Mann-Whitney test in (**B**). Friedman test with Dunn's multiple comparisons test in **F.** Wilcoxon signed-rank test in **G, H, I.** ns, not significant. ***P* < 0.01, ****P* < 0.001.
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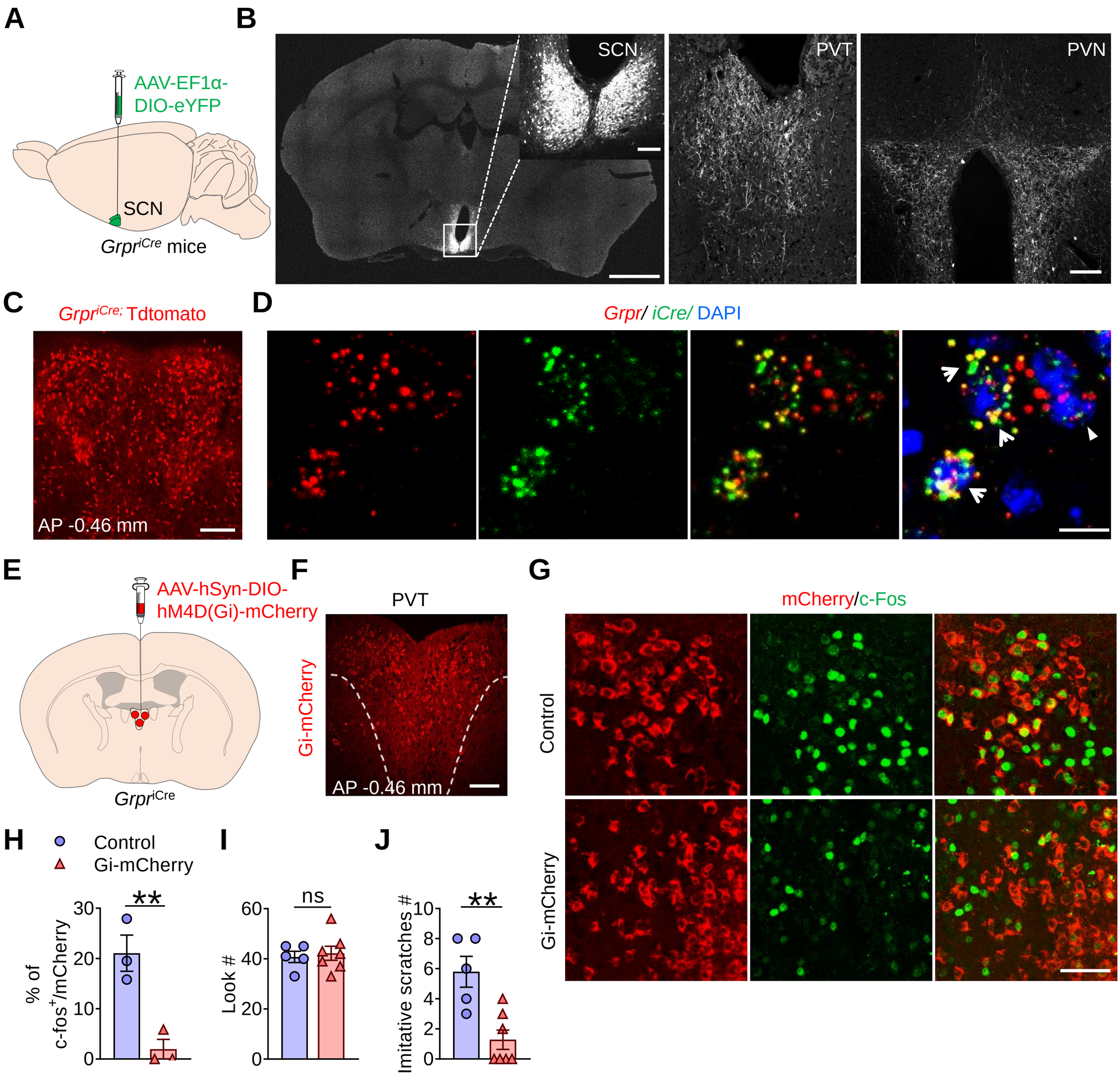

Figure S10. PVT GRPR neuron mediated CIB as output of SCN for CIB. (A) Schematic of injection of AAV-hSyn-DIO-eYFP in the SCN of *Grpr*^{iCre} mice. The terminals of SCN GRPR neurons were examined by IHC in the brain. **(B)** IHC images showing the GRPR neurons in the SCN and their terminals in the PVT and PVN, PVT, the paraventricular nucleus of the thalamus. PVN, the paraventricular nucleus of the hypothalamus. Scale bar in whole brain 1 mm, SCN/PVN, 100 μm. **(C)** Representative IHC image showing the expression of Tdtomato in the PVT of *Grpr*^{iCre}; Tdtomato mice. Scale bar, 100 μm. (**D**) RNAscope ISH images showing the overlapping expression of *Grpr* (red) and *iCre* (green) in the PVT of *Grpr*^{iCre} mice. White arrows indicate *Grpr / iCre* co-expression. Scale bar, 10 μm. n=3. (**E**) Schematic of injection of AAV-hSyn-DIO-hM4D(Gi)-mCherry injection in the PVT of *Grpr*^{iCre} mice. (**F**) Representative IHC image showing the expression of G_i-mCherry in the PVT. Scale bar, 100 μm. (**G**) Representative IHC image showing the expression of mCherry and c-Fos in the PVT after Clozapine injection in mice with control virus or G_i-mCherry. Scale bar, 20 μm. (**H**) Mean percentage of c-Fos and mCherry double stained cells out of mCherry expression cells ($t = 4.682$, $df = 4$, $P = 0.0094$). n = 3. (**I-J**) Mean number of look (**I,** $t = 0.3896$, $df = 10$, $P = 0.7050$) and imitative scratch (**J**, $t = 3.943$, $df = 10$, $P = 0.0028$) of mice with Control virus (n = 5) or Gi-mCherry (n = 7) after clozapine injection. Unpaired *t* test in **H**-**J**. ns, not significant. ****P* < 0.01.