Cell Reports, Volume *43*

Supplemental information

Plp1-expresssing perineuronal DRG cells facilitate colonic and somatic chronic mechanical pain involving Piezo2 upregulation in DRG neurons

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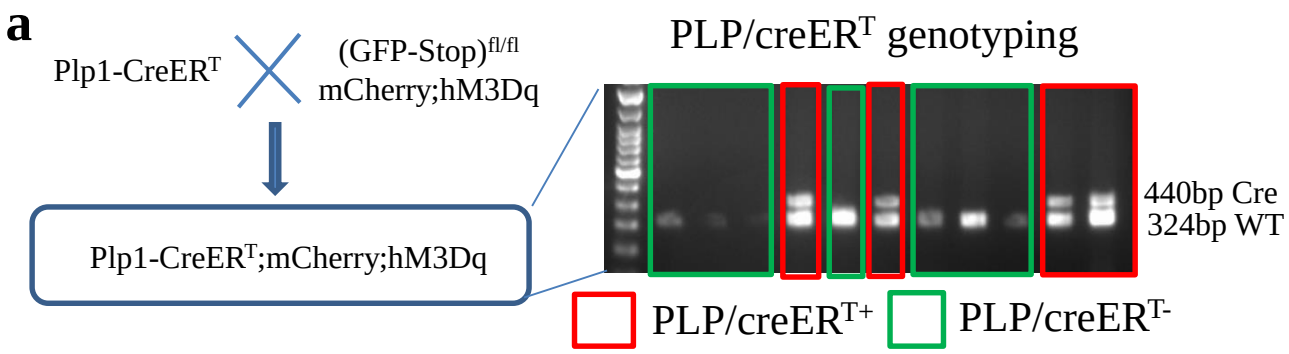

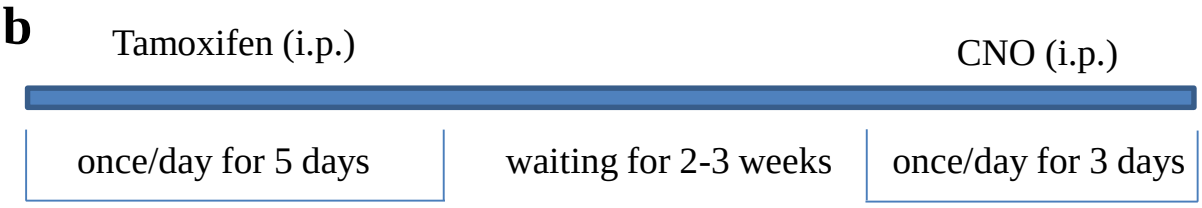

c

Experimental groups

CNO: CNO dissolved in DMSO and prepared in saline to treat PLP/creER^{T+};mCherry;hM3Dq mice (designated as Plp1;hM3Dq)

vehicle: Equal amount of DMSO in saline to treat PLP/creER^{T+};mCherry;hM3Dq mice (designated as Plp1;hM3Dq)

CNO-off: CNO-treated PLP/creER^{T-};mCherry;hM3Dq mice (designated as neg;hM3Dq)

Figure S1 (related to Figures 1-2): **Experimental design to characterize the role of SGCs in hypersensitivity and pain.** (a): Breeding strategies and genotyping. (b): Tamoxifen treatment schedules. (c): Experimental groups.

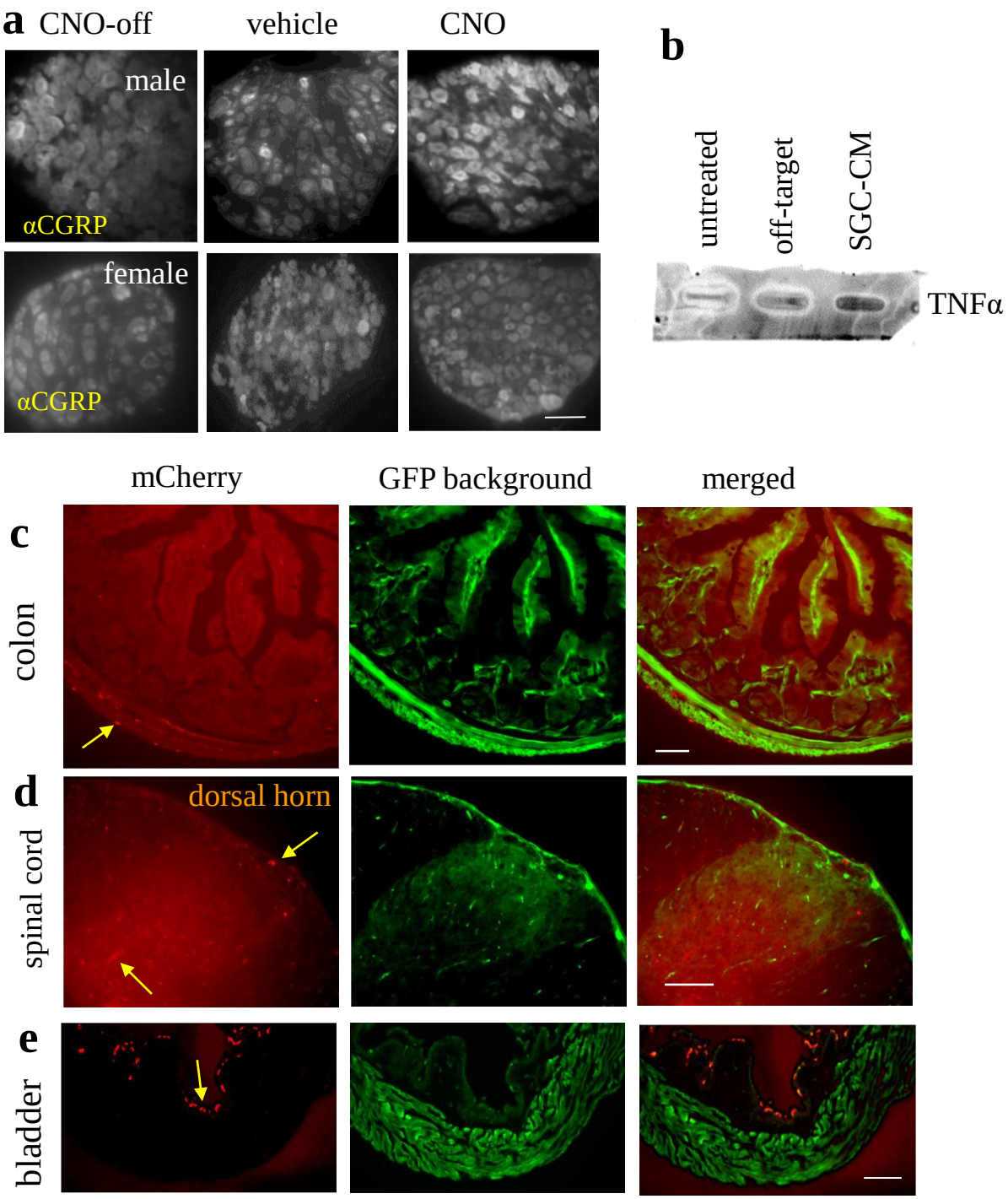

Figure S2 (related to Figures 2-3): **PLP/creERT-driven activation on CGRP expression in DRG, TNFα production in SGCs, and mCherry expression in the colon, spinal cord and urinary bladder.** (a): CNO treatment of Plp1;hM3Dq mice increases CGRP expression in DRG of male mice but not female mice. Bar=30 µm. (b): CNO-treated SGC culture from Plp1;hM3Dq mice contain a higher level of TNFα. (c): $Plp1^{CreERT}$- driven mCherry expression is visible in the muscular layer of the distal colon. Bar=100 µm. (d): $Plp1^{CreERT}$- driven mCherry expression is evident in the dorsal horn of the spinal cord and deep laminea. Bar = 100 µm. (e) $Plp1^{CreERT}$- driven mCherry expression is strongly present in the urinary bladder. Bar=600 µm.

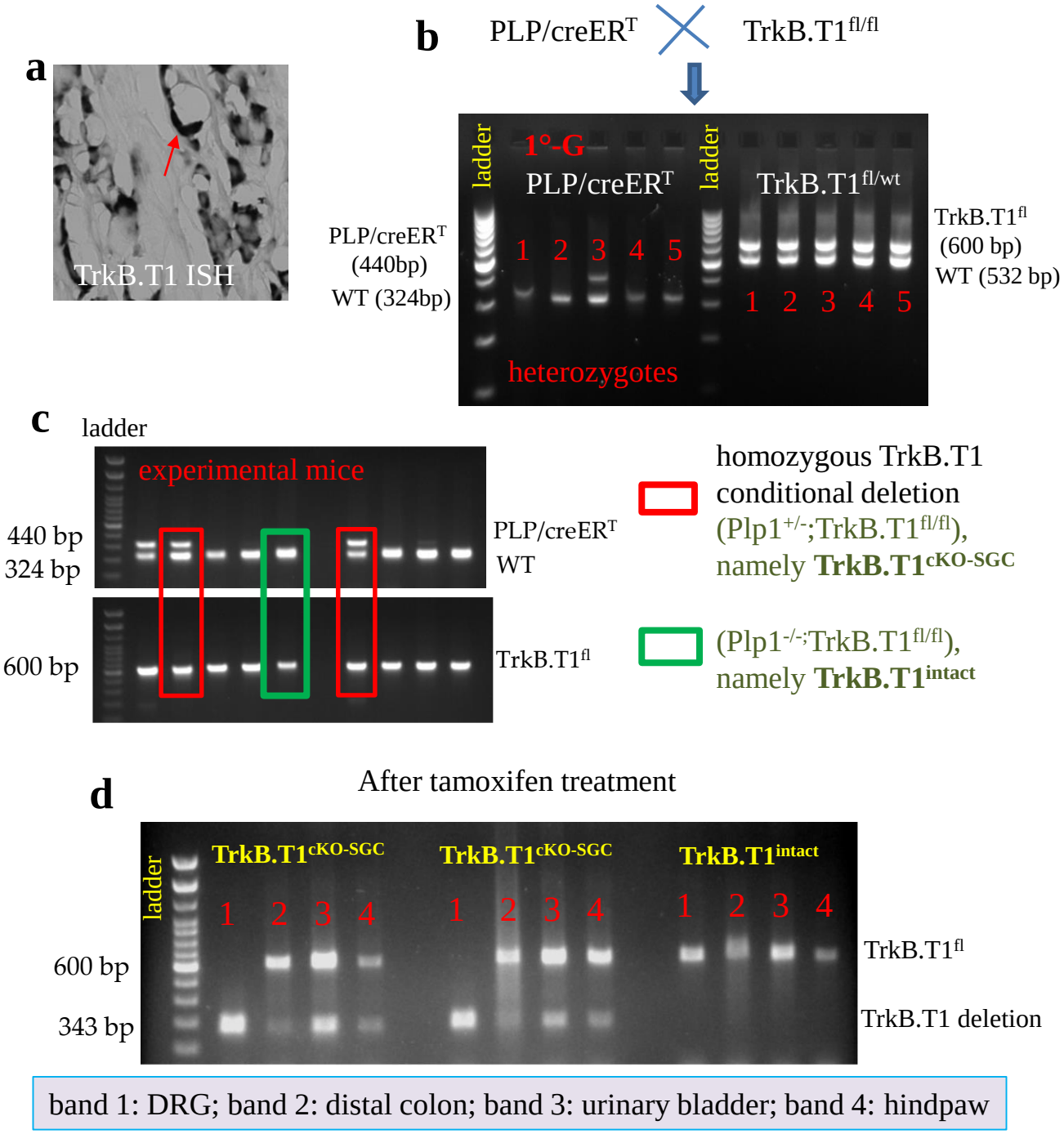

Figure S3 (related to Figure 3): **Generation of inducible TrkB.T1 conditional deletion from SGCs.** (a): TrkB.T1 in situ hybridization of DRG sections showing SGC expression (indicated by arrow). Bar = 40 µm. (b): First generation from breeders of PLP/creER^{T} and floxed TrkB.T1 mice are heterozygotes. (c): Experimental mice with homozygous TrkB.T1 deletion. (d): Tissue-specific genotyping after tamoxifen treatment showing TrkB.T1 is largely deleted from DRGs in TrkB.T1$^{cKO-SGC}$ mice (2 distinct animals) but not from TrkB.T1^{intact} mice.

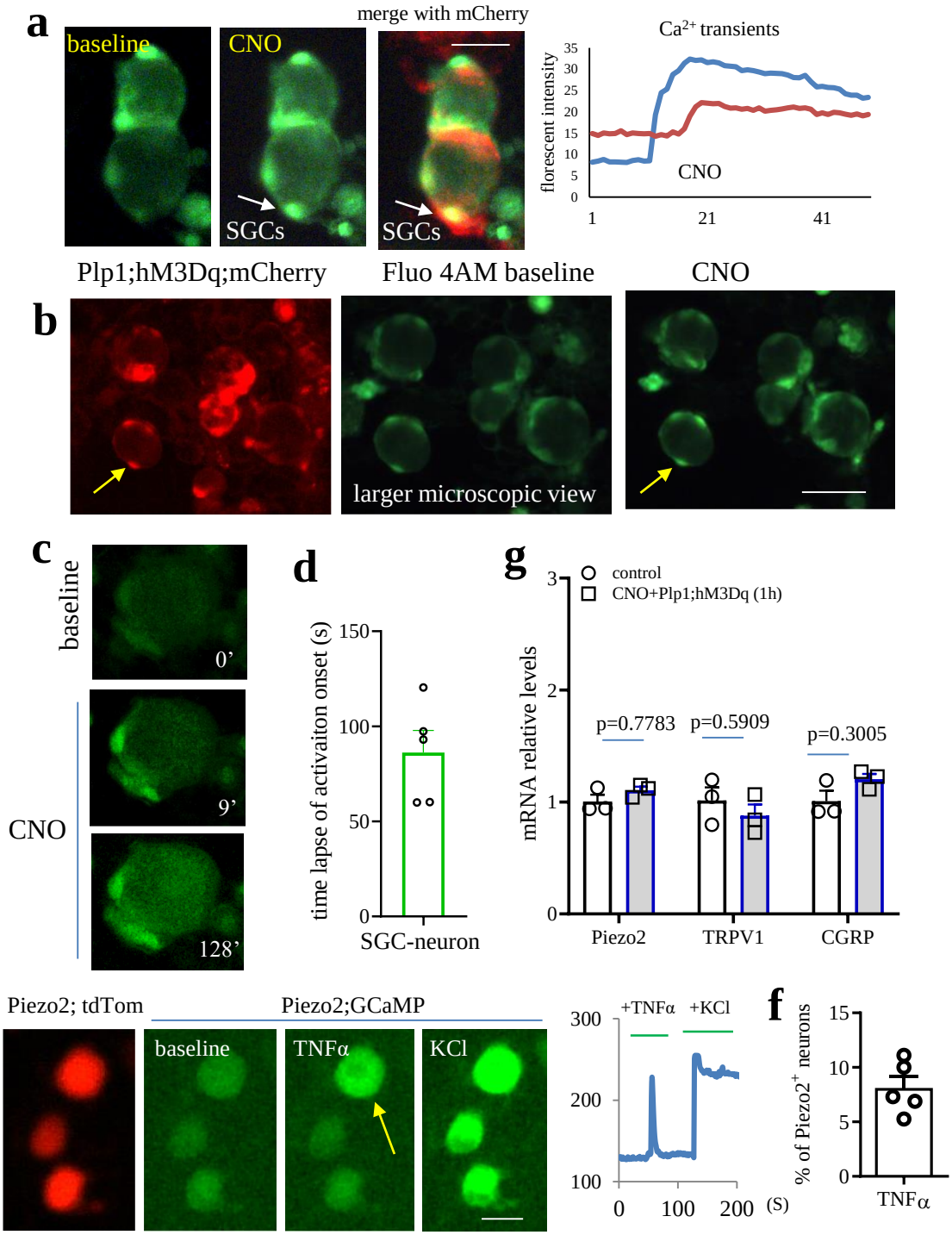

Figure S4 (related to Figures 4 and 5): **DRG SGCs-neuron crosstalk.** (a-b): CNO stimulation of DRG SGC-neuron unit culture from Plp1;hM3Dq mice increases Ca^{2+} transients exclusively in SGCs (1 min recording). Bar = 100 µm. (c): activation of SGCs by CNO leads to adjacent neuron activation (10 min recording). Bar = 50 µm. (d): Average time lapses between activation of SGCs and adjacent DRG neurons after CNO treatment. (e): TNFα (1 ng/mL) stimulation evokes the activity of $Piezo2^{+}$ DRG neurons examined by Piezo2 Cre-driven GCaMP intensity. Bar=100 µm. (f): percentage of $Piezo2^{+}$ neurons activated by TNFα. (g): No changes in the mRNA levels of Piezo2, TRPV1 and CGRP in DRG examined at 1 h after chemogenetic activation of SGCs. Two-way ANONA followed by Šídák's multiple comparisons test. Data are presented as mean ± SEM.

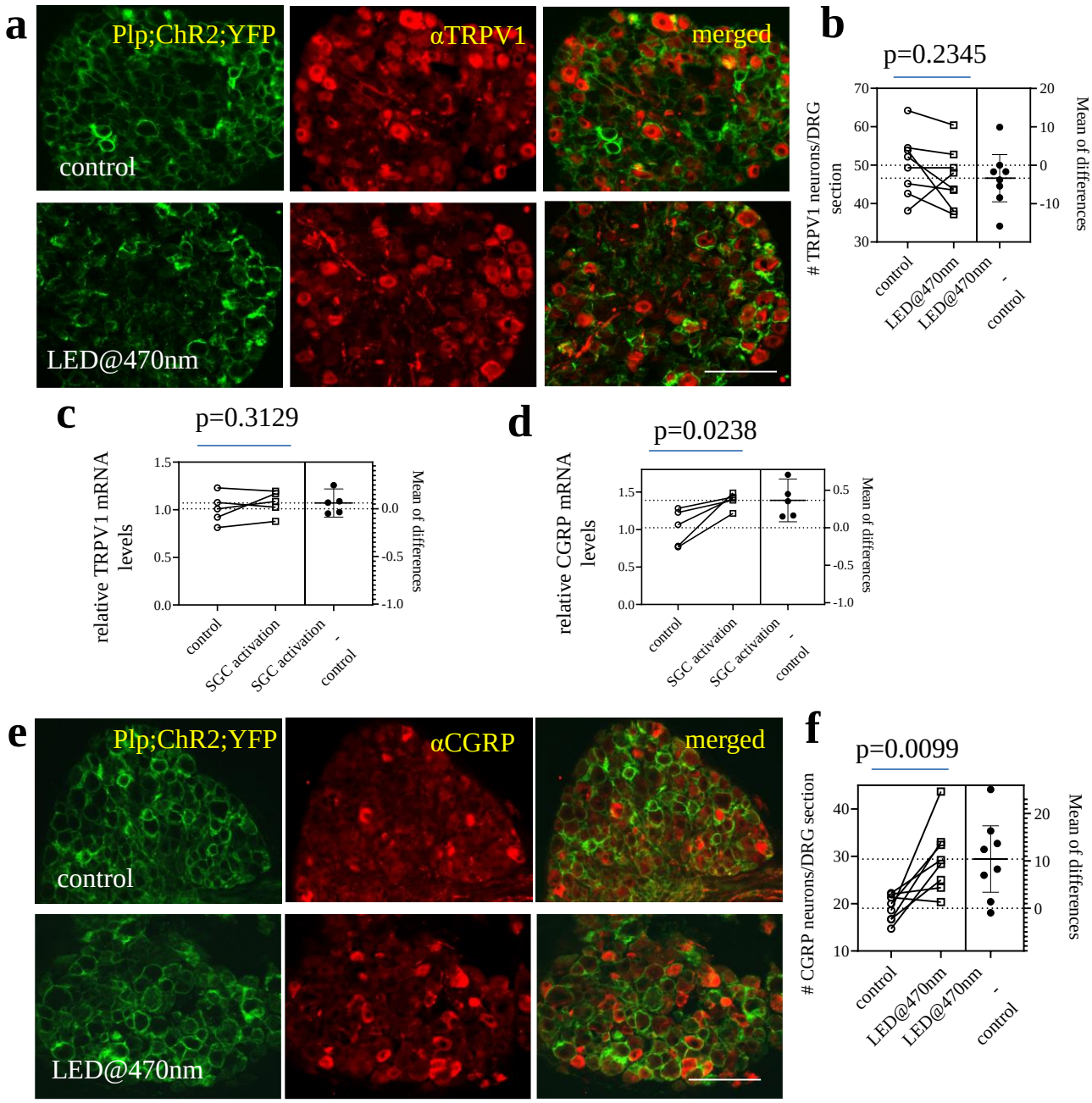

Figure S5 (related to Figure 4): **Alterations in the expression of neurochemical coding in DRG neurons after optogenetic activation of SGCs from DRG explant culture.** (a-b): TRPV1 expression in DRG neurons with or without LED stimulation of DRG explants of Plp1;ChR2-YFP mice. Bar = 100 µm. n=8 pairs of DRG explants. Paired two-tailed *t* test. Data are presented as mean ± SEM. (c): TRPV1 mRNA levels in DRG with or without LED stimulation of DRG explants of Plp1;ChR2-YFP mice. n=5, paired two-tailed *t* test. (d): CGRP mRNA levels in DRG with or without LED stimulation of DRG explants of Plp1;ChR2-YFP mice. n=5, paired two-tailed *t* test. (e-f): CGRP expression in DRG neurons with or without LED stimulation of DRG explants of Plp1;ChR2-YFP mice. Bar = 100 µm. n=8 pairs of DRG explants. Data are presented as mean ± SEM. Paired two-tailed *t* test.

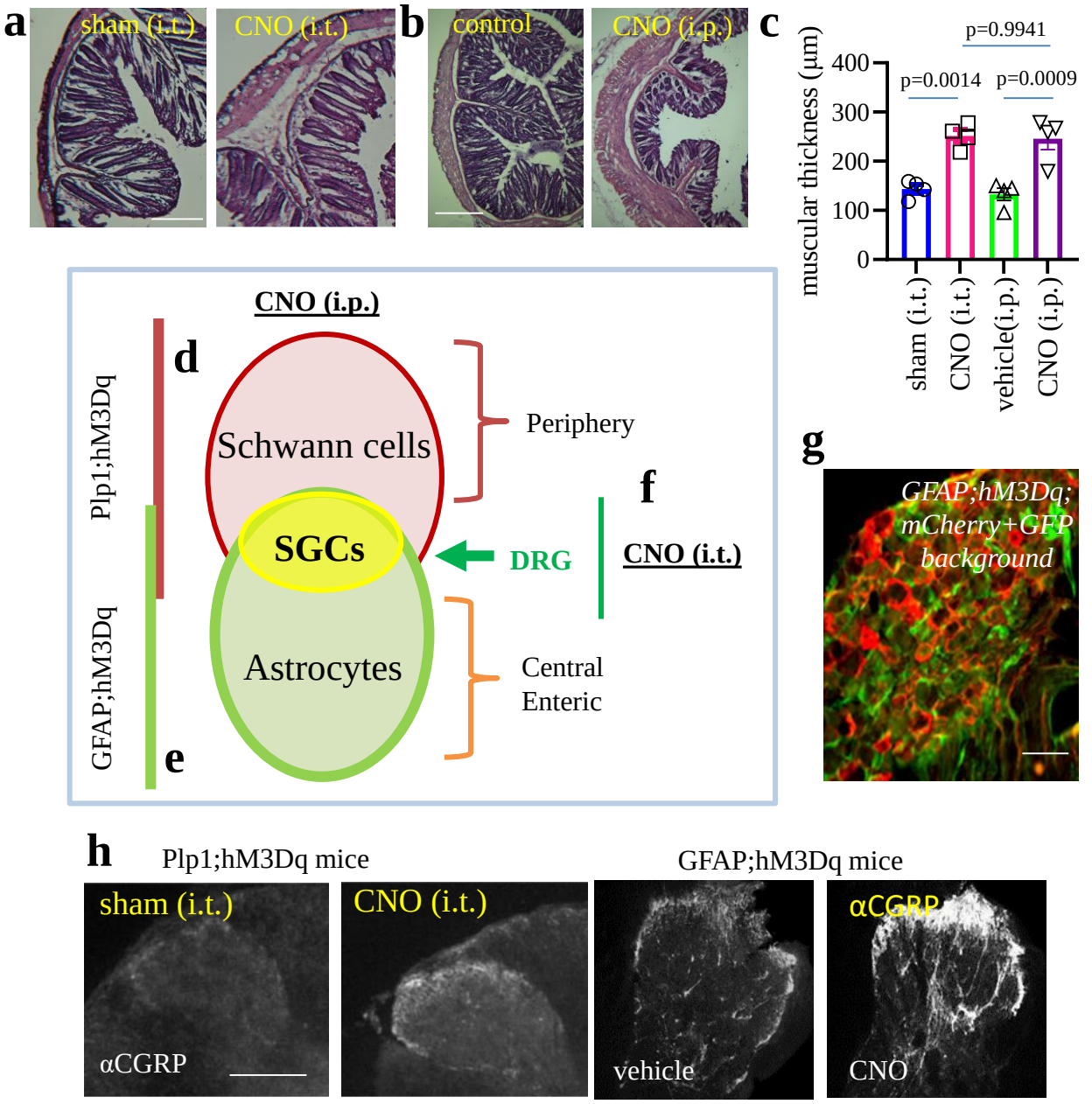

Figure S6 (related to Figures 6 and 7): **Effects of SGC activation on colonic inflammation and CGRP spinal cord release.** (a): Intrathecal (i.t., 1 dose) injection of CNO to tamoxifen-treated Plp1;hM3Dq mice changes colonic morphology. (b): Intraperitoneal (i.p. 1 dose) injection of CNO to tamoxifen-treated Plp1;hM3Dq mice changes colonic morphology. Bar = 1 mm. (c): The thickness of colonic muscular walls. n=4. Data are presented as mean ± SEM. One-way ANOVA with Tukey's multiple comparisons test. (d-f): Three unique strategies to activate SGCs. Intraperitoneal (i.p.) injection of clozapine N-oxide (CNO) to Plp1;hM3Dq mice to activate SGCs and Schwann cells (d); (2) injection of CNO (i.p.) to GFAP;hM3Dq mice to activate SGCs and astrocytes (e); and (3) intrathecal (i.t.) injection of CNO to Plp1;hM3Dq mice to more specifically target glial cells in DRG including SGCs (f). (g): GFAP;hM3Dq mice show mCherry expression in SGCs of DRG. Bar= 60 µm. (h): CNO (i.t.) treatment of Plp1;hM3Dq mice or CNO (i.p.) treatment of GFAP;hM3Dq mice induces CGRP spinal release. Bar = 200 µm.

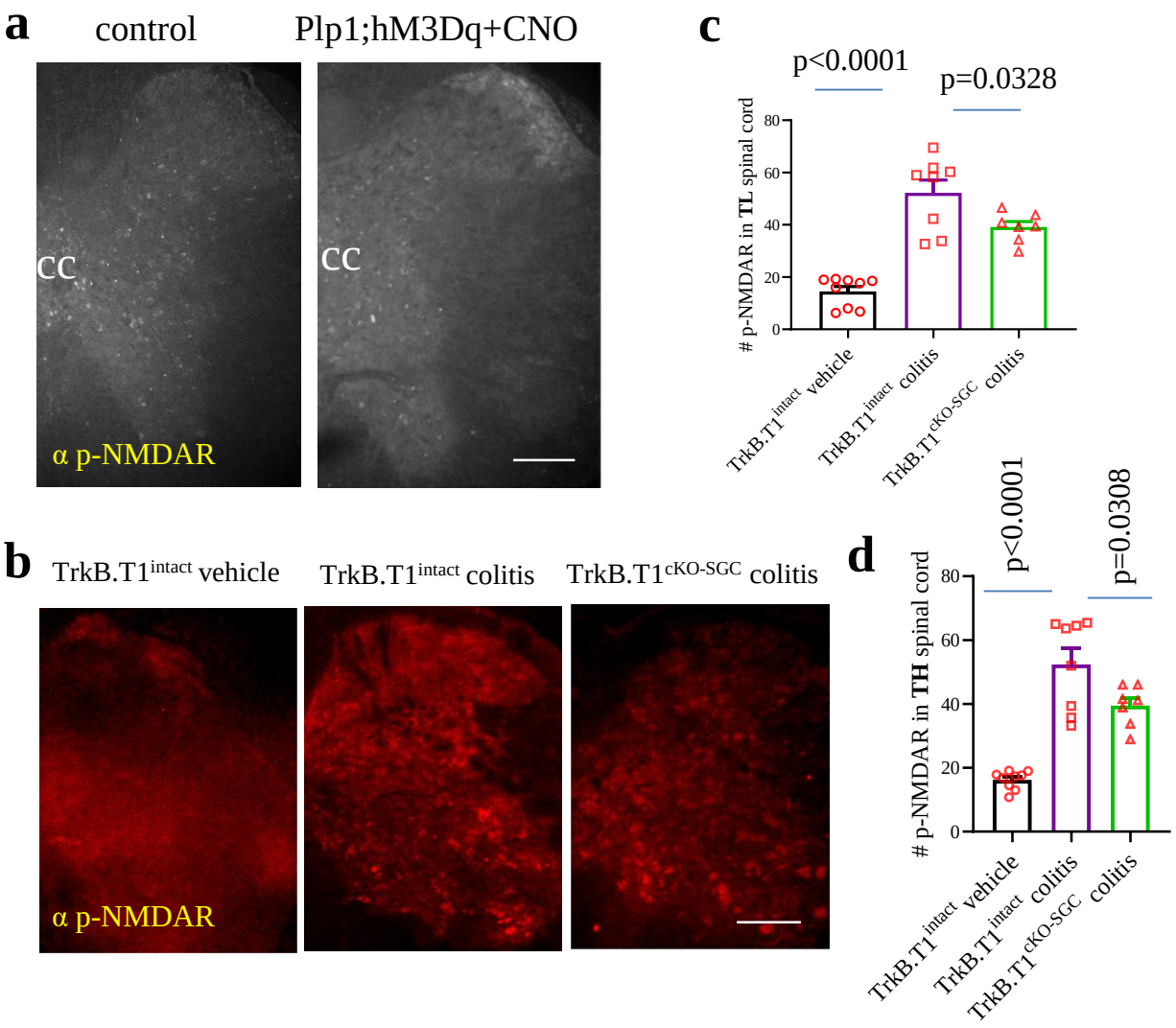

Figure S7 (related to 7): **Effects of SGC activation on p-NMDAR expression in the spinal cord.** (a): CNO treatment of Plp1;hM3Dq mice increases p-NMDAR expression in the dorsal horn of the spinal cord. (b-d): Increases in p-NMDAR expression in the spinal cord of TrkB.T1^{intact} mice after colonic inflammation (n=9 for control and n=8 for colitis) and the effects of TrkB.T1$^{cKO-SGC}$ (n=7 mice) on inflammation-induced p-NMDAR expression in thoracolumbar (c: TL) and thoracic (d: TH) segments. Data are presented as mean ± SEM. One-way ANOVA with Tukey's multiple comparisons test. Bar = 200 µm.