Supplementary information

Seven supplement figures were included in the supplemental information.

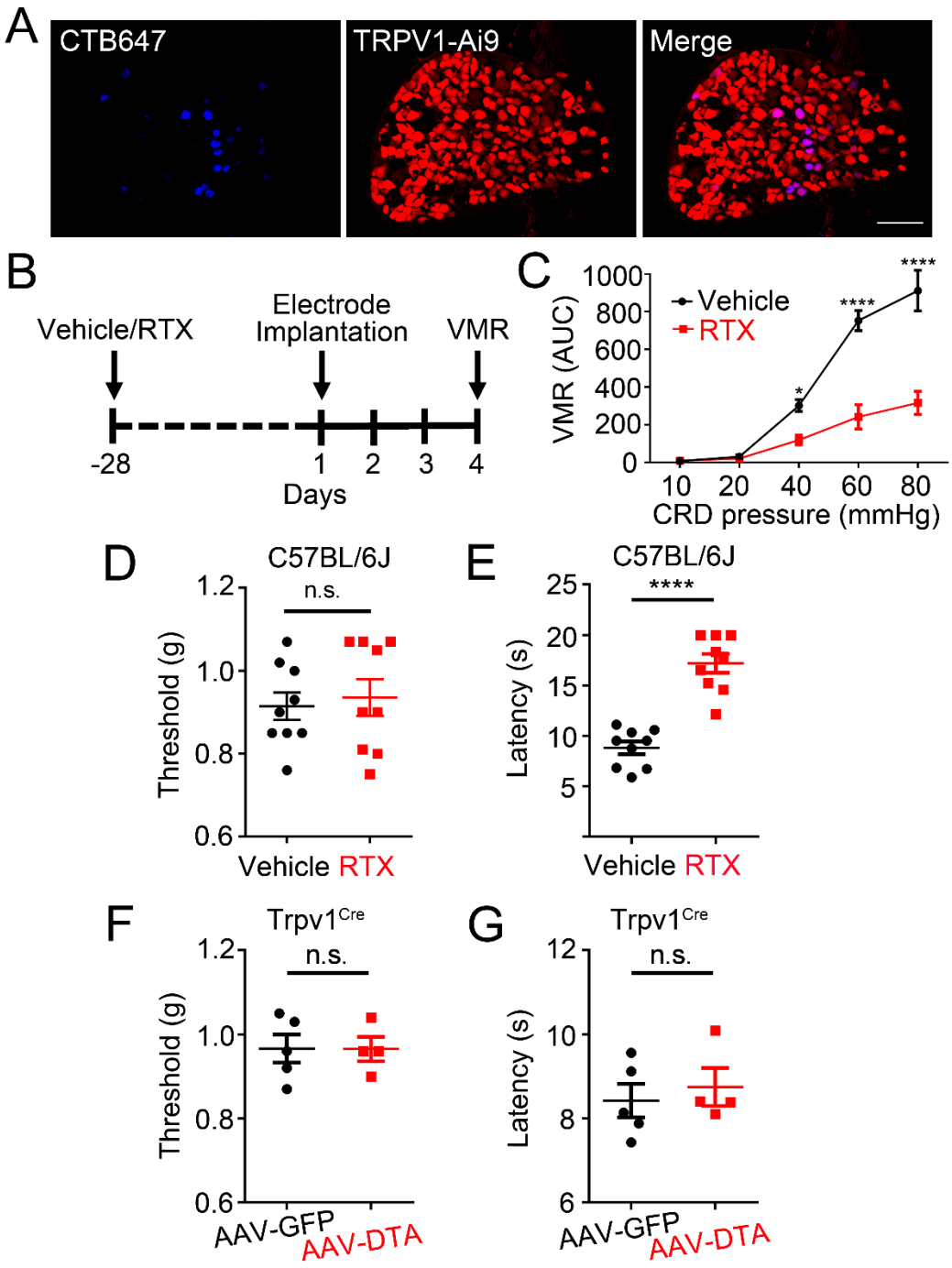

Figure S1 (Related to Figure 1). Effects of ablation of TRPV1-expressing nociceptors on visceral and somatic sensations. (A). Representative images of CTB-647 retrogradely labeled neurons in the L6 DRG sections from Trpv1^{Cre}::Ai9 mice. CTB 647-positive neurons appear blue,

TRPV1-tdTomato-positive neurons appear red. Images shown here were representative of three independent experiments using tissues from six different mice. Scale bar=100 μm. (**B**). Schematic representation of RTX treatment and VMR measurement. (**C**). Summary data showing that ablation of TRPV1-expressing nociceptors with RTX significantly reduces CRD-induced VMR. *P < 0.05, ****P < 0.0001, two-way ANOVA, n=5 mice per group. (**D**-**E**). Summary data showing that ablation of TRPV1-expressing nociceptors with RTX did not significantly change mechanical pain (**D**), but significantly reduced thermal pain in the paw (**E**). n.s., no significant difference, ****P<0.0001 unpaired *t* test, n=9 mice per group. (**F**-**G**). Viral-mediated ablation of colon-innervating TRPV1-expressing neurons did not significantly affect either mechanical pain (**F**) or thermal pain (**G**). n.s., no significant difference, unpaired *t* test, n=4 to 5 mice per group. All data are expressed as means ± S.E.

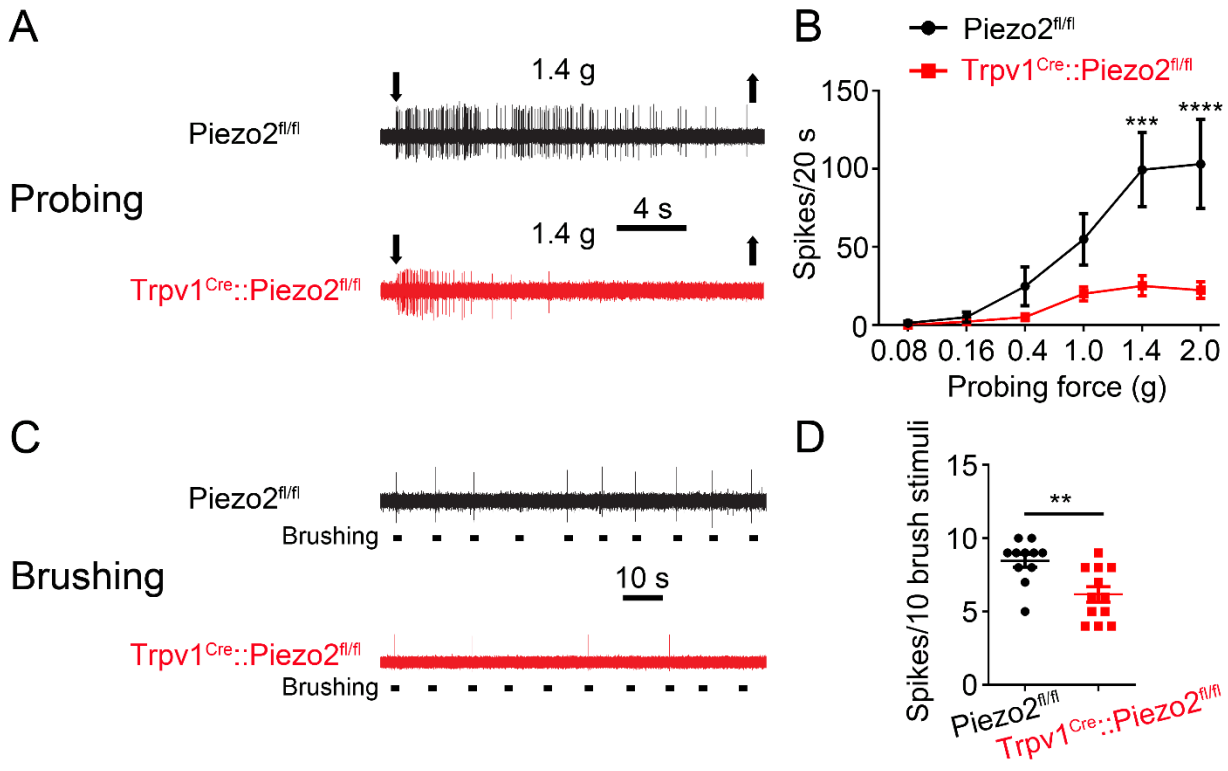

Figure S2 (Related to Figure 2). Conditional knockout of Piezo2 from TRPV1-lineage neurons attenuates colon afferent firing in response to mechanical probing and brushing. **(A)**. Representative traces of colon pelvic nerve recordings from Piezo2$^{fl/fl}$ and Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice in response to probing. **(B)**. Summary data of colon pelvic nerve recording of Piezo2$^{fl/fl}$ and Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice in response to probing. ***P <0.001, ****P < 0.0001, two-way ANOVA, n=9 units from 3 mice for Piezo2$^{fl/fl}$ group and n=9 units from 4 mice for Trpv1^{Cre}::Piezo2$^{fl/fl}$ group. **(C)**. Representative traces of colon pelvic nerve recordings from Piezo2$^{fl/fl}$ and Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice in response to brushing. **(D)**. Summary data of colon pelvic nerve recording of Piezo2$^{fl/fl}$ and Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice in response to brushing. **P <0.01, unpaired *t* test, n=11 units from 8 mice for Piezo2$^{fl/fl}$ group and n=12 units from 9 mice for Trpv1^{Cre}::Piezo2$^{fl/fl}$ group.

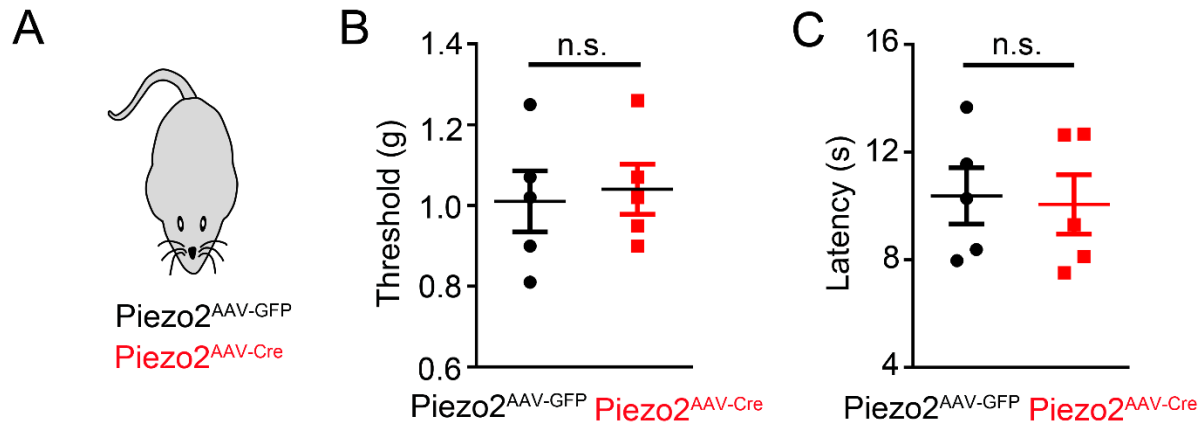

Figure S3 (Related to Figure 3). Virally-mediated knockdown of PIEZO2 function from colon-innervating DRG neurons does not affect somatic pain. (**A**). Schematic representation of Piezo2$^{fl/fl}$ mice. (**B**). PIEZO2$^{AAV\text{-}GFP\text{-}Cre}$ mice showed comparative somatic mechanical pain with PIEZO2$^{AAV\text{-}GFP}$ mice in the paw. (**C**). PIEZO2$^{AAV\text{-}GFP\text{-}Cre}$ mice showed comparative thermal pain with PIEZO2$^{AAV\text{-}GFP}$ mice in the paw. n.s., no significant difference, unpaired *t* test, n=5 mice per group. All data are expressed as means ± S.E.

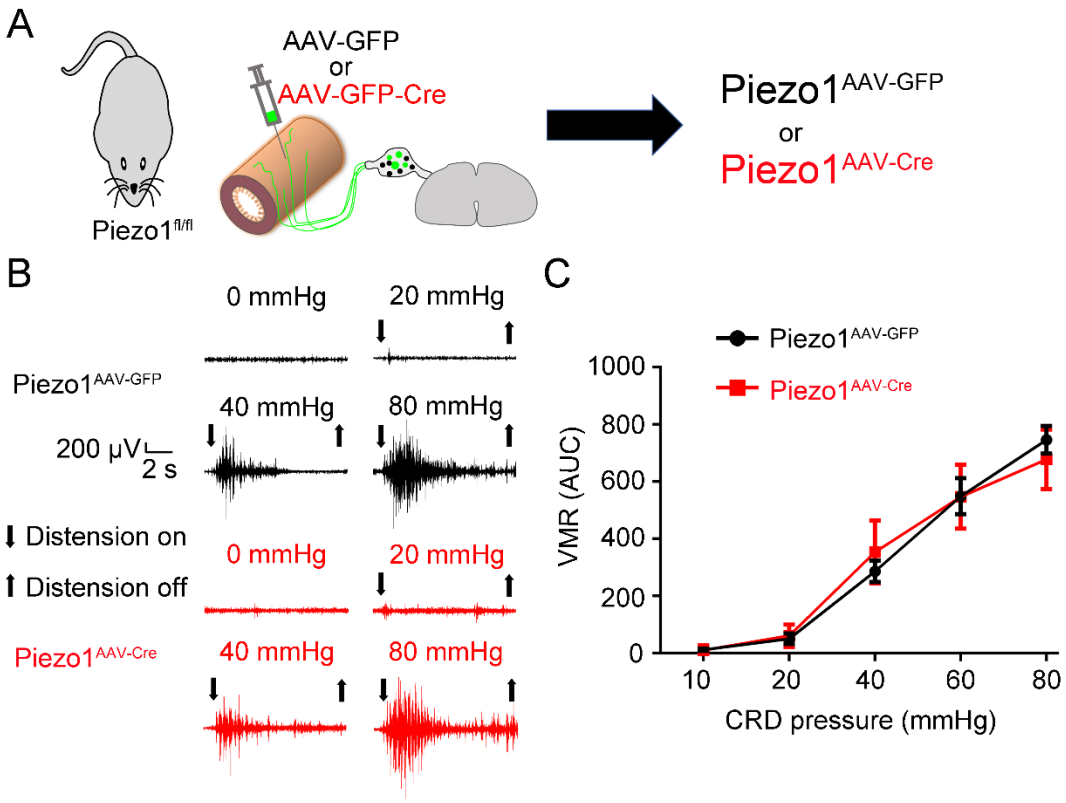

Figure S4 (Related to Figure 3). Virally-mediated knockdown of PIEZO1 function in colon-innervating DRG neurons does not affect visceral mechanical nociception. (**A**). Schematic representation of intracolonic injection of AAV encoding GFP or GFP-Cre into the Piezo1$^{fl/fl}$ mice. (**B**). Representative electromyogram recording elicited by graded CRD pressures in PIEZO1$^{AAV-GFP}$ mice and PIEZO1$^{AAV-GFP-Cre}$ mice. (**C**). Summary data showing that PIEZO1$^{AAV-GFP-Cre}$ mice display comparable CRD-induced VMRs with those in the PIEZO1$^{AAV-GFP}$ mice. n.s., no significant difference, two-way ANOVA, n=5 mice per group. All data are expressed as means ± S.E.

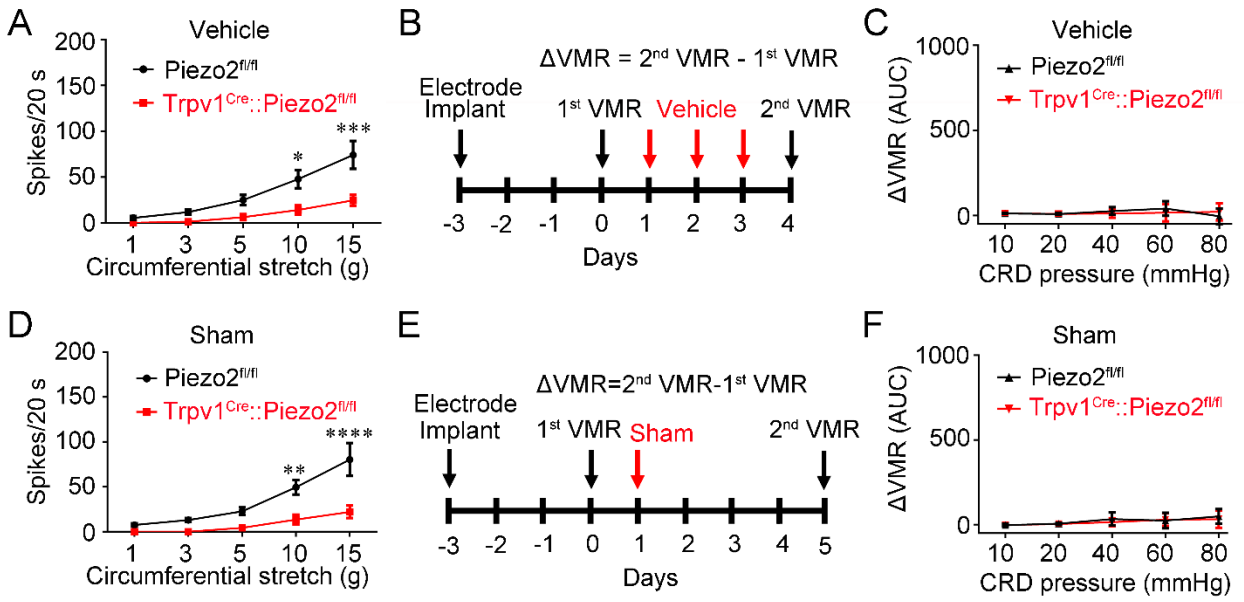

Figure S5 (Related to Figure 4 and Figure 5). Vehicle for zymosan treatment or sham for PCO surgery does not induce visceral hypersensitivity. (A). Summary data of stretch stimuli-induced nerve firings of colon nerve recording in Piezo2$^{fl/fl}$ and Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice subjected to vehicle treatment. *P < 0.05; ***P<0.001, two-way ANOVA. n=20 units from 6 mice for Piezo2$^{fl/fl}$ group and n=12 units from 4 mice for Trpv1^{Cre}::Piezo2$^{fl/fl}$ group. **(B).** Schematic diagram showing the timing of vehicle treatment and VMR recording. **(C).** Summary data showing that neither Piezo2$^{fl/fl}$ nor Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice displayed visceral hypersensitivity after vehicle treatment. **(D).** Summary data of stretch stimuli-induced nerve firings of colon nerve recording in Piezo2$^{fl/fl}$ and Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice subjected to sham surgery. **P < 0.01; ****P<0.0001, two-way ANOVA. n=18 units from 6 mice for Piezo2$^{fl/fl}$ group and n=14 units from 5 mice for Trpv1^{Cre}::Piezo2$^{fl/fl}$ group. **(E).** Schematic diagram showing the timing of sham surgery and VMR recording. **(F).** Summary data showing that neither Piezo2$^{fl/fl}$ nor Trpv1^{Cre}::Piezo2$^{fl/fl}$ mice displayed visceral hypersensitivity after sham surgery. Two-way ANOVA, n=5 mice per group.

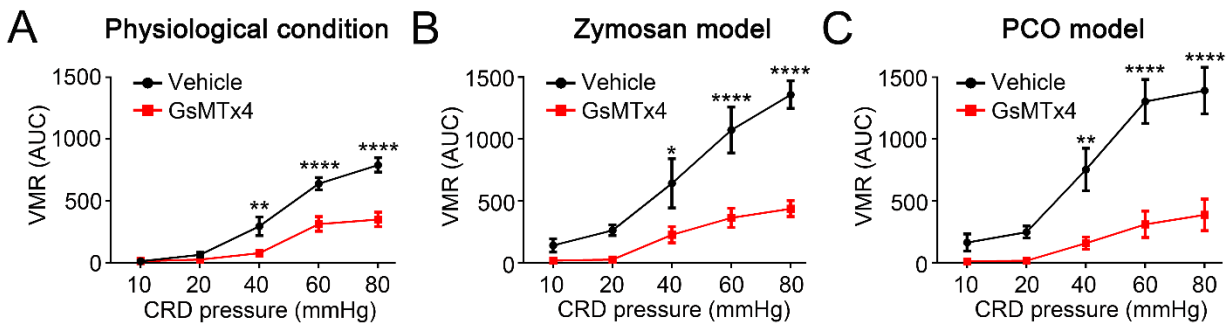

Figure S6 (Related to Figure 4 and Figure 5). Pretreatment with GsMTx4 inhibits CRD-induced visceral nociception under physiological condition and visceral hypersensitivity induced by zymosan or PCO. (A). CRD-induced VMR after application of vehicle or GsMTx4 in wild-type mice under normal conditions. **(B)**. CRD-induced VMR after application of vehicle or GsMTx4 in zymosan-treated mice. **(C)**. CRD-induced VMR after application of vehicle or GsMTx4 in mice subjected to PCO procedure. Two-way ANOVA, n=5 mice per group. *P<0.05, **P<0.01, ****P<0.0001. All data are expressed as means ± S.E.

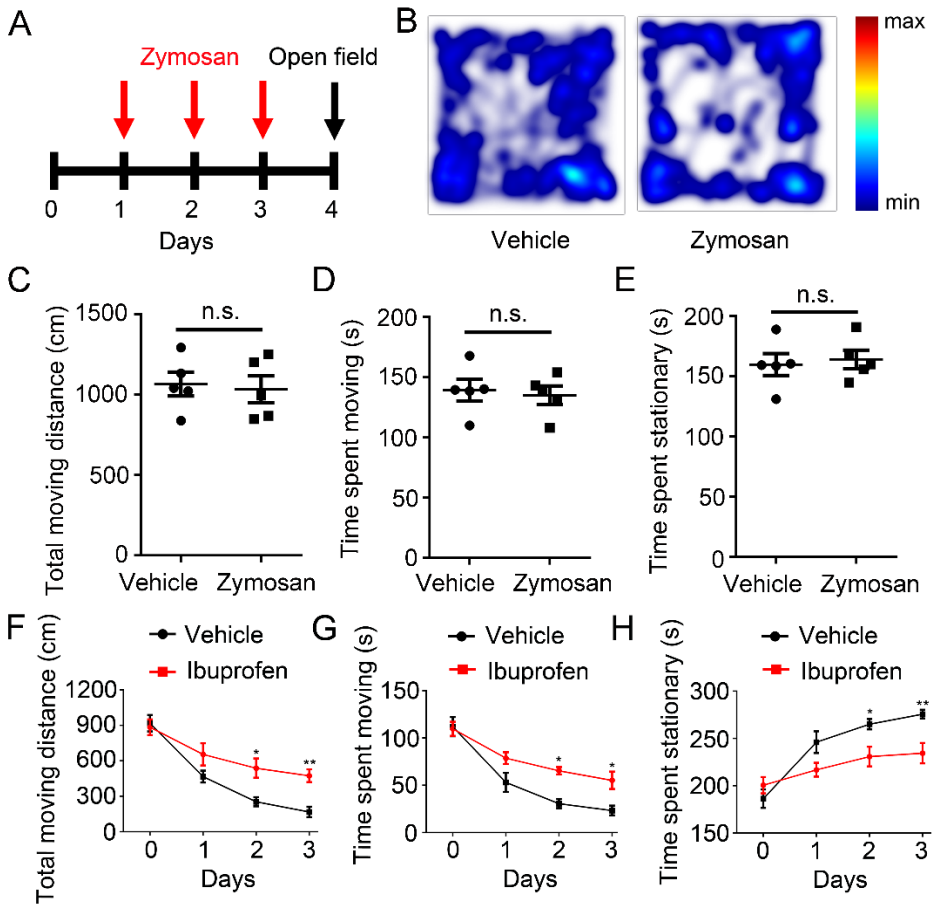

Figure S7 (Related to Figure 7). PCO but not Zymosan treatment affects pain-related voluntary movements. (**A**). Schematic representation of zymosan treatment and open field recording**.** (**B**). Representative images illustrating heat maps of voluntary movements of mice subjected to vehicle treatment (left) and zymosan treatment (right) in open field test using home cages. (**C-E**). Quantification of voluntary movements including total moving distance (**C**), total time spent on moving (**D**) and total time spent on stationary (**E**) in vehicle- and zymosan-treated mice. n.s., no significant difference, unpaired *t* test, n=5 mice per group. All data are expressed as means ± S.E. **(F-H).** Ibuprofen-treated PCO mice showed significantly increased moving distance (**F**), total time spent on moving (**G**), and decreased time spent on stationary (**H**) compared to vehicle-treated PCO mice. Intraperitoneal injection of ibuprofen at a dose of 50 mg/kg was administered 30 min before open field test. Two-way ANOVA, n=5 mice per group. *$p < 0.05$, **$p < 0.01$. All data are expressed as means ± S.E.