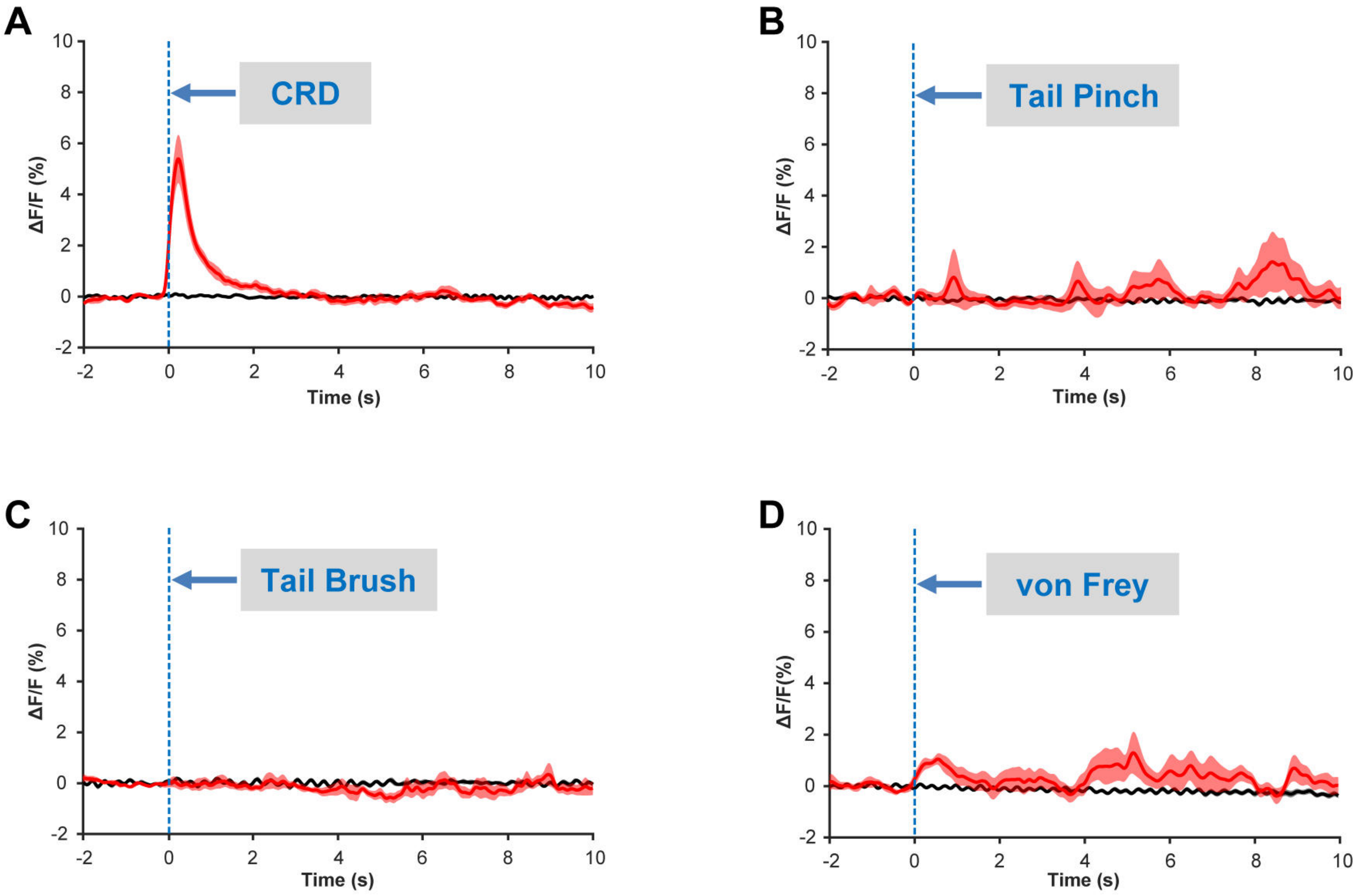

Figure S1. **Optical recording of the calcium activity of LSV neurons in awake mice following visceral and somatic stimulation. Related to Figure 2.** (A) Representative fluorescence traces of GCaMP6f (red) and GFP (black) were recorded from LSV neurons following CRD stimulation. (B) Representative fluorescence traces of GCaMP6f (red) and GFP (black) were recorded from LSV neurons under tail pinch stimulation. (C) Representative fluorescence traces of GCaMP6f (red) and GFP (black) were recorded from LSV neurons following the tail brush stimulation. (D) Representative fluorescence traces of GCaMP6f (red) and GFP (black) were recorded from LSV neurons following the von Frey filament stimulation.

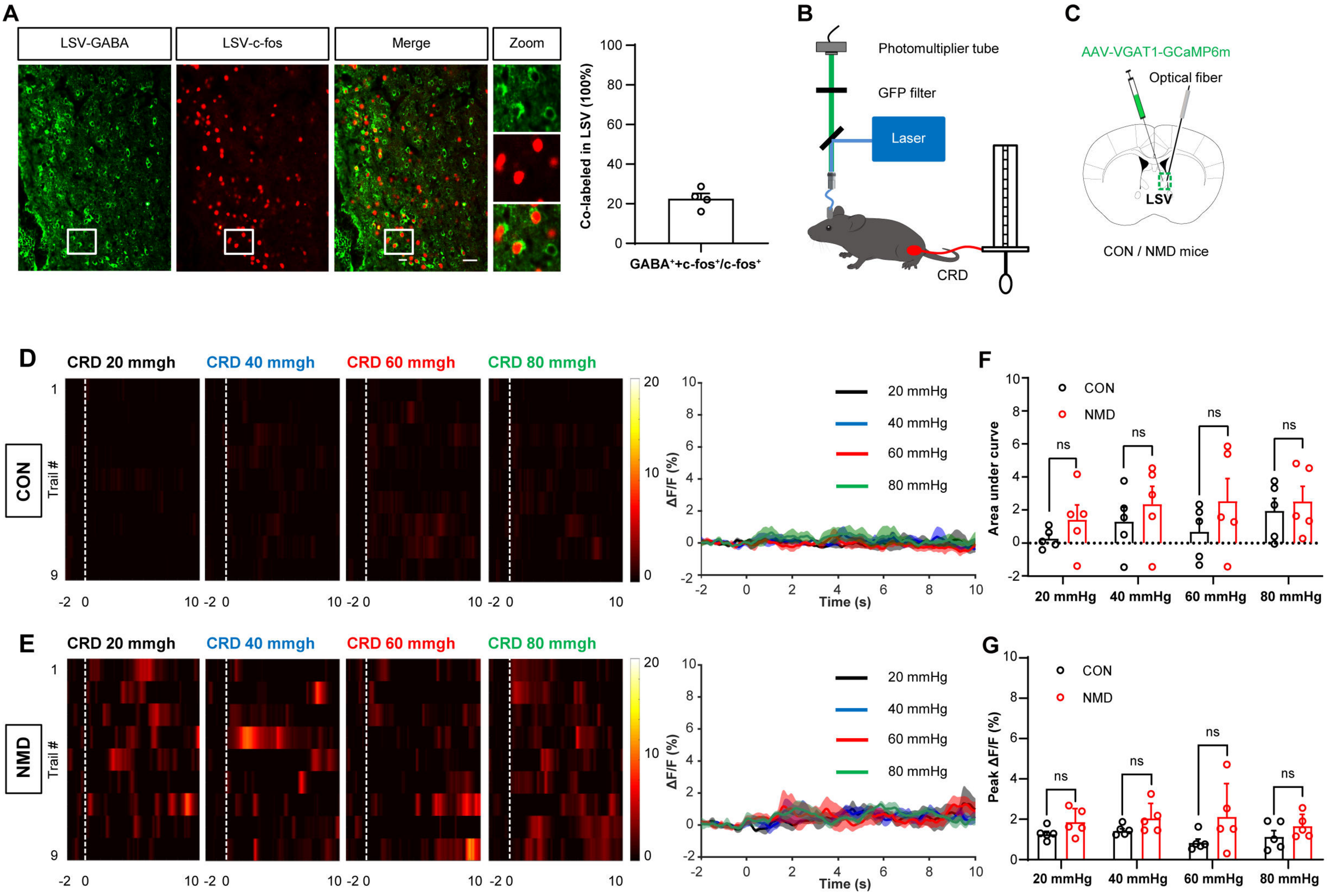

Figure S2. **LSV GABAergic neurons are not activated in visceral pain behavior. Related to Figure 3.** (A) Representative images of c-fos (red) and GABA (green) co-expression in LSV (left) and co-expression ratios (right). Scale bar, 50 μm. (B) Schematic of the recording system for evaluating calcium signals with fiber photometry and visceral pain behavior caused by CRD stimuli. (C) Schematic of stereotactic injection virus. (D and E) Heatmap and average Ca^{2+} transients of LSV GABAergic neurons in CON and NMD mice receiving CRD stimulation at 20, 40, 60 and 80 mmHg, respectively. (F) The area under the curve of calcium activity of LSV GABAergic neurons in CON and NMD mice receiving CRD stimulation at 20, 40, 60 and 80 mmHg, respectively (F (3, 12) = 0.6533, $P > 0.05$, two-way repeated measure ANOVA followed by Bonferroni's post hoc test, n = 5 mice per group).(G) Averaged peak ΔF/F of calcium activity of LSV GABAergic neurons in CON and NMD mice receiving CRD stimulation at 20, 40, 60 and 80 mmHg, respectively (F (3, 12) = 0.2937, $P > 0.05$, two-way repeated measure ANOVA followed by Bonferroni's post hoc test, n = 5 mice per group). n.s. indicates non-significant differences, $P > 0.05$.

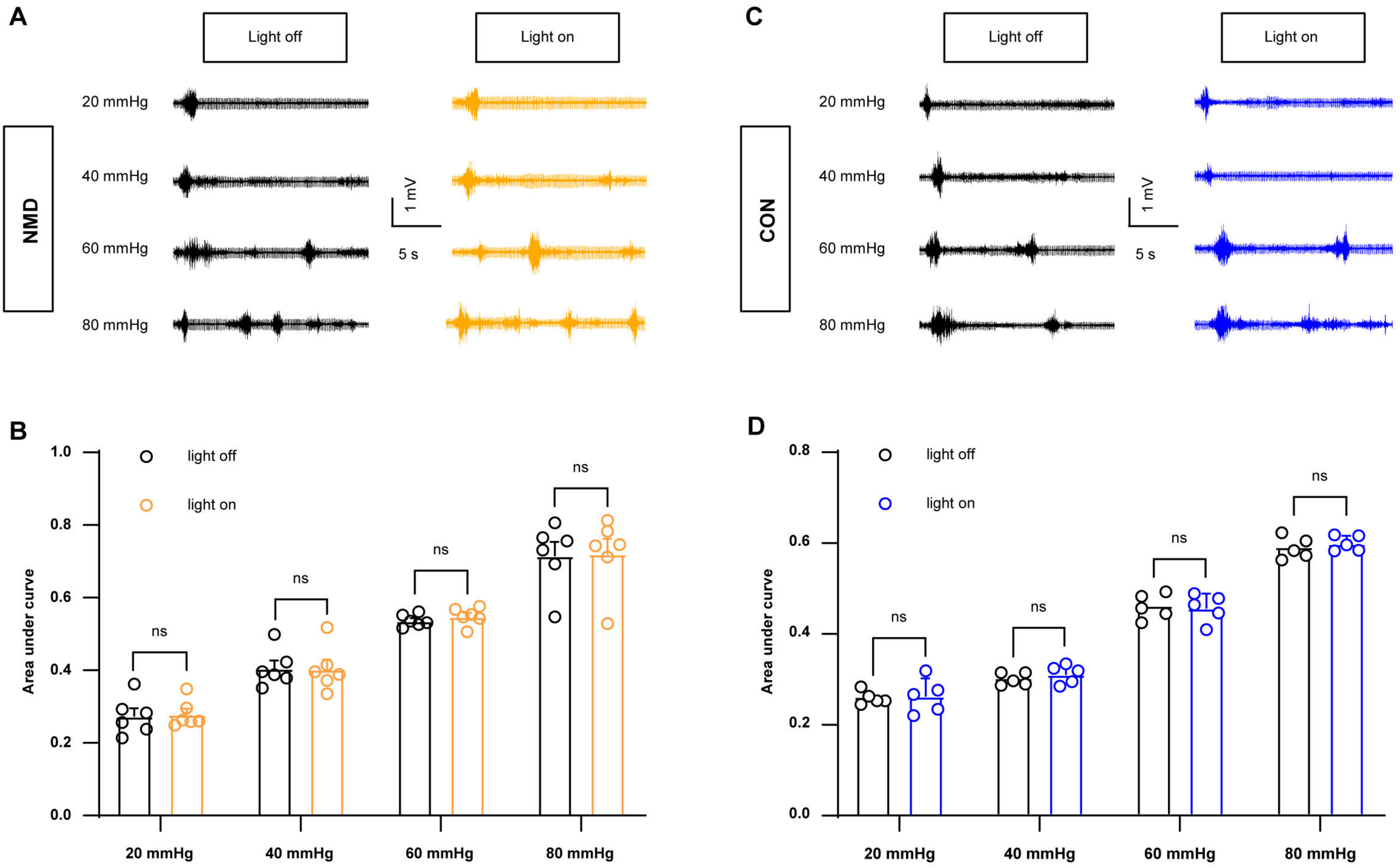

Figure S3. **Optogenetic manipulation does not change visceral pain following LSV injection of the control virus. Related to Figure 4.** (A) Representative EMG traces of NMD mice were recorded at 20, 40, 60 and 80 mmHg, respectively. (B) The area under the curve of EMG at 20, 40, 60 and 80 mmHg in NMD mice (F (3, 15) = 46.46, $P > 0.05$, two-way repeated measure ANOVA followed by Bonferroni's post hoc test, n = 6 mice per group). (C) Representative EMG traces of CON mice were recorded at 20, 40, 60 and 80 mmHg, respectively. (D) The area under the curve of EMG at 20, 40, 60 and 80 mmHg in CON mice (F (3, 12) = 230.4, $P > 0.05$, two-way repeated measure ANOVA followed by Bonferroni's post hoc test, n = 5 mice per group). n.s. indicates non-significant differences, $P > 0.05$.

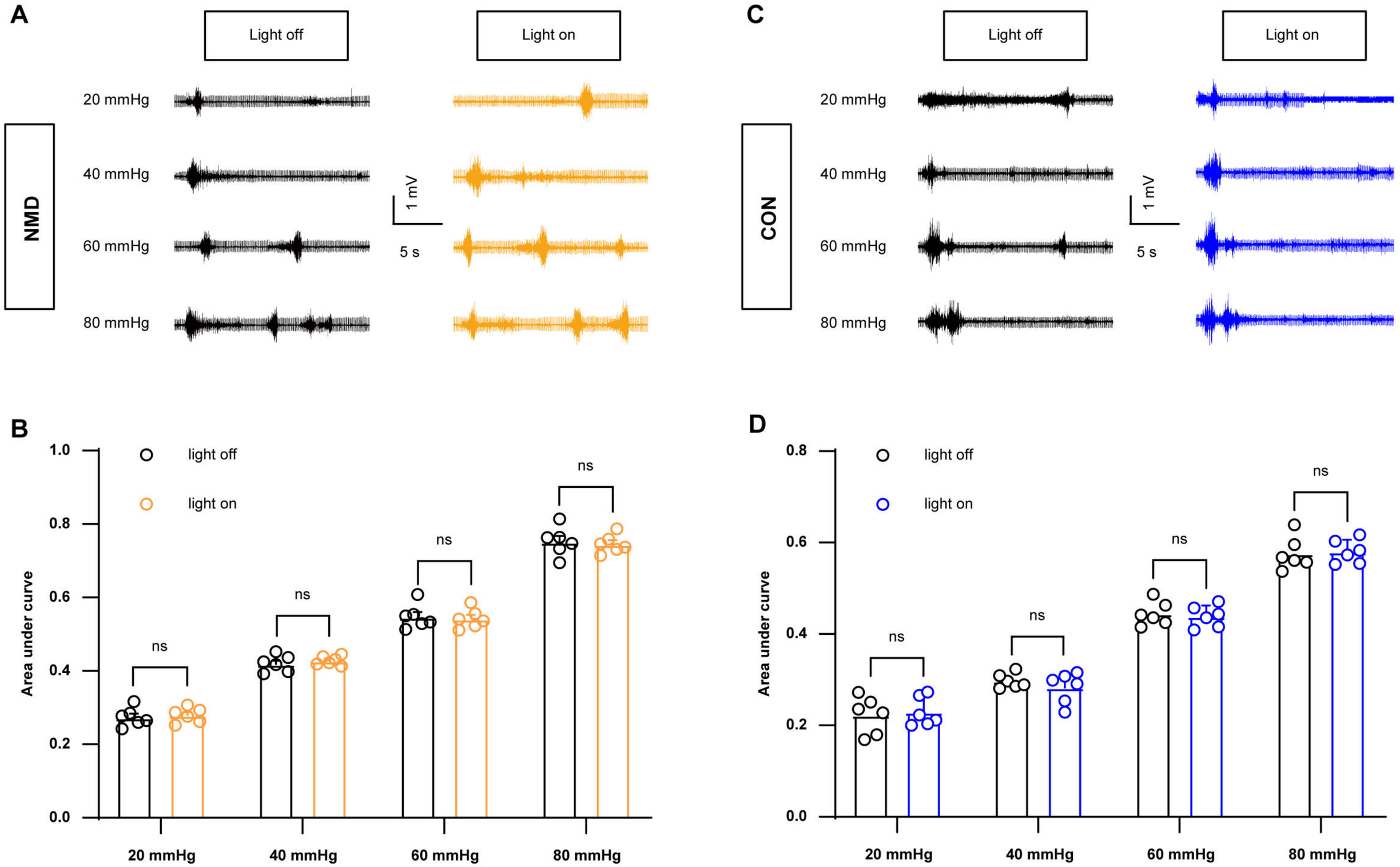

Figure S4. **Optogenetic manipulation does not change visceral pain following PVH injection of the control virus. Related to Figure 6.** (A) Representative EMG traces of NMD mice were recorded at 20, 40, 60 and 80 mmHg, respectively. (B) The area under the curve of EMG at 20, 40, 60 and 80 mmHg in NMD mice (F (3, 15) = 475.5, $P > 0.05$, two-way repeated measure ANOVA followed by Bonferroni's post hoc test, n = 6 mice per group). (C) Representative EMG traces of CON mice were recorded at 20, 40, 60 and 80 mmHg, respectively. (D) The area under the curve of EMG at 20, 40, 60 and 80 mmHg in CON mice (F (3, 15) = 220.1, $P > 0.05$, two-way repeated measure ANOVA followed by Bonferroni's post hoc test, n = 6 mice per group). n.s. indicates non-significant differences, $P > 0.05$.