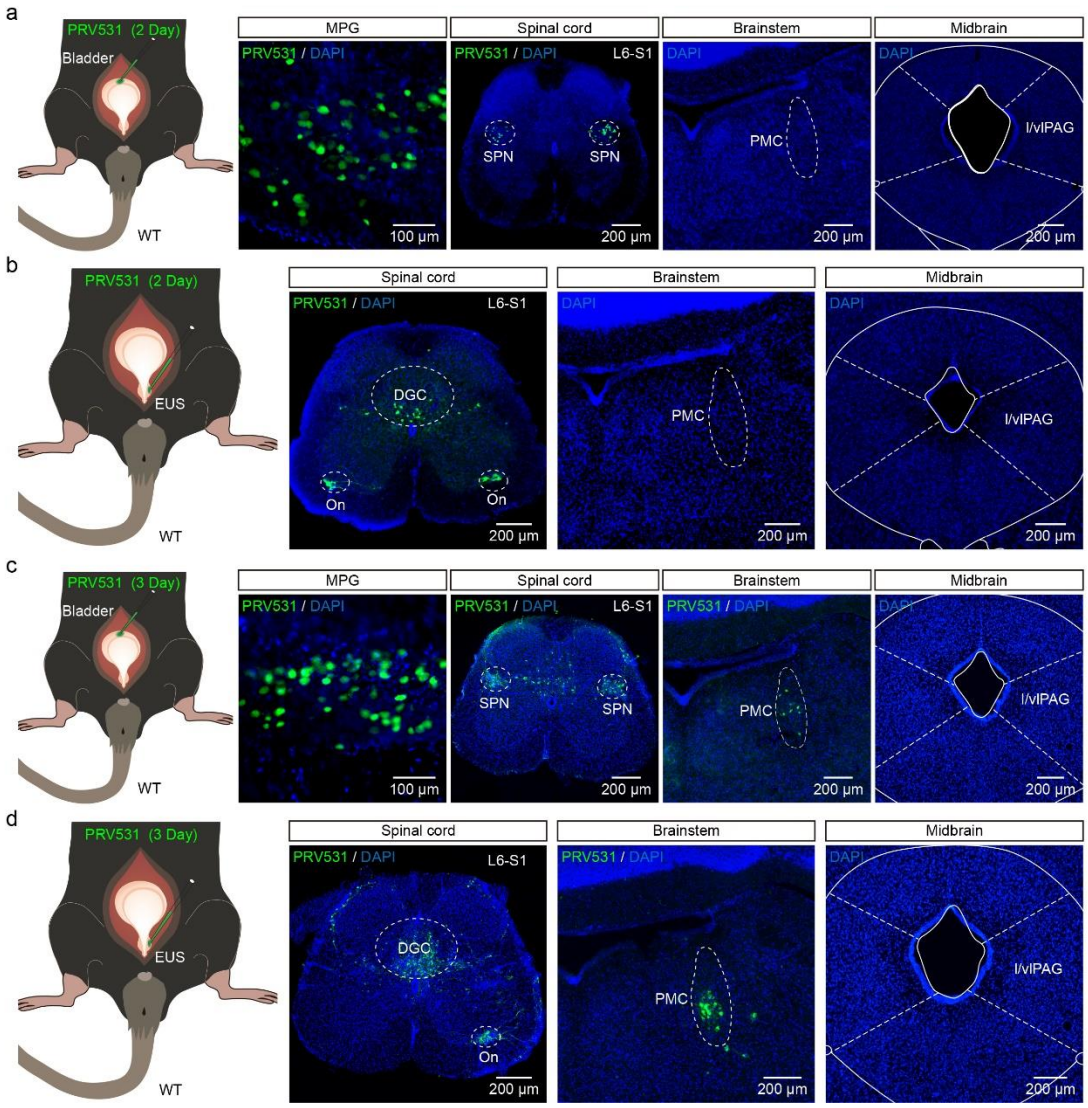
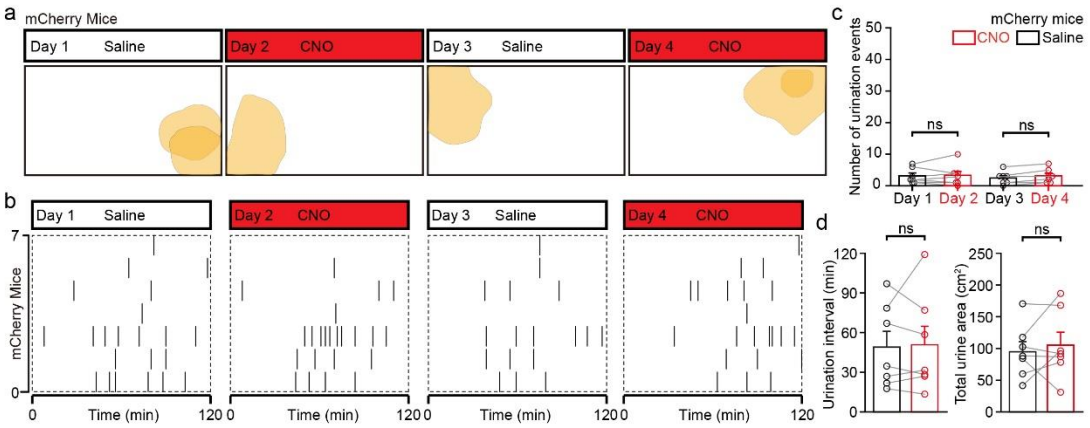
Supplementary Information



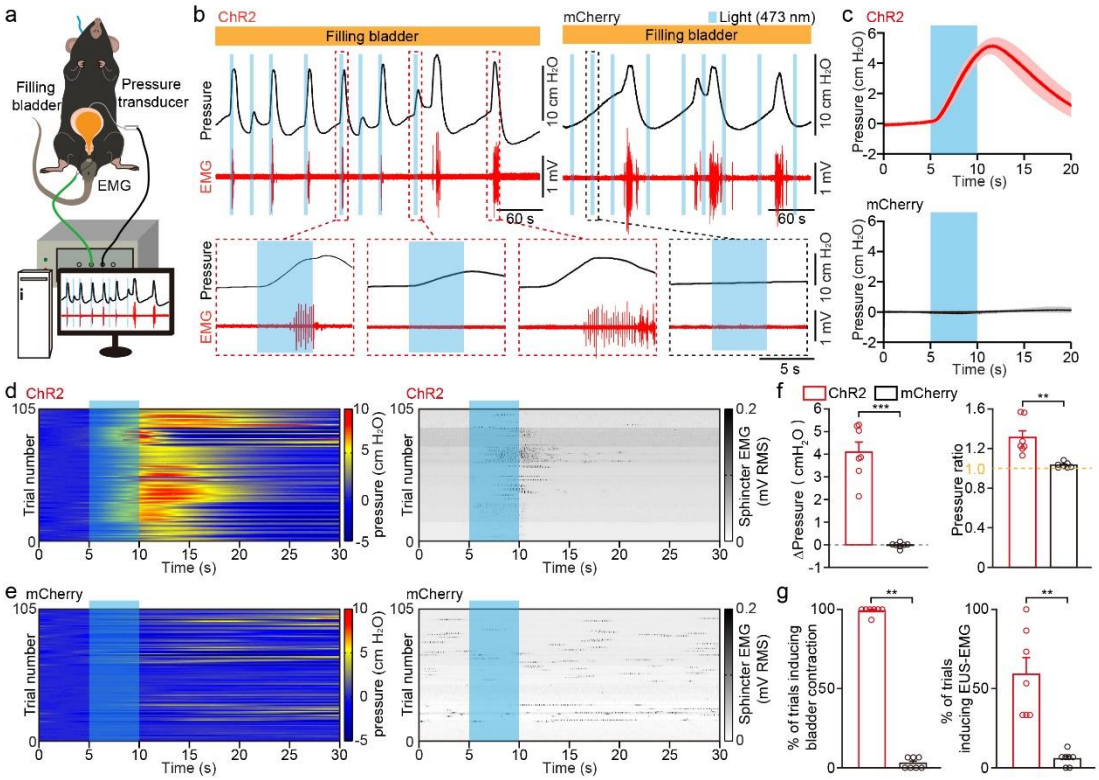
Supplementary Figure 1. Distribution of PRV-labeled neurons in major pelvic ganglion and central nervous system after PRV-EGFP injection into the bladder wall or the EUS at different time points

a Representative images showing PRV-labeled neurons in the major pelvic ganglion (MPG) and the sacral parasympathetic nucleus (SPN) at 48 hours after PRV-EGFP injection into the bladder wall. **b** Representative images of PRV-labeled neurons in the Onuf's nucleus (On) and dorsal gray commissure (DGC) at 48 hours after PRV-EGFP injection into the EUS. **c** Representative images showing PRV-labeled neurons in the MPG, SPN, and PMC at 72 hours after PRV-EGFP injection into the bladder wall. **d** Representative images of PRV-labeled neurons in the On, DGC, and PMC at 72 hours after PRV-EGFP injection into the EUS. For each time point, experiments were conducted using two male mice.



Supplementary Figure 2. Chemogenetic activation of mCherry-labeled PMC-projecting l/vlPAG SST⁺ neurons has no effects on urination patterns following CNO administration

a Representative urine spots deposited on filter papers from mCherry-labeled mice treated with saline or CNO. **b** Raster plots showing urination events during the 4-day behavioral test. **c** Daily quantification of urination events on filter paper from mCherry-labeled mice (n = 7 mice). *P* = 0.285 (Friedman' s test). **d** Quantification of the urination interval and total urine area following saline or CNO administration from mCherry-labeled mice (n = 7 mice). Left, *P* = 0.824 (saline versus CNO, paired *t* test); *P* = 0.524 (saline versus CNO, paired *t* test). All data are presented as mean ± SEM.



Supplementary Figure 3. Optogenetic activation of PMC-projecting l/vlPAG SST⁺ neurons elicits urination in anesthetized mice

a Schematic depicting optogenetic stimulation combined with cystometry (continuous saline infusion into the bladder) and EUS-EMG recording in anesthetized mice. **b** Representative traces of bladder pressure (black) and EUS-EMG activity (red) recorded from ChR2-expressing and mCherry-expressing PMC-projecting l/vlPAG SST⁺ neurons during photostimulation. Blue bars indicate periods of light stimulation. **c** Averaged bladder pressure traces from experimental mice expressing ChR2 (red) and control mice expressing mCherry (black), aligned to the onset of photostimulation. **d** Heatmaps of individual bladder pressure and EUS-EMG traces aligned with photostimulation from experimental mice expressing ChR2 (n = 105 trials from 7 mice). **e** Heatmaps of individual bladder pressure and EUS-EMG traces aligned with photostimulation from control mice expressing mCherry (n = 105 trials from 7 mice). **f** Quantification of the pressure change (ΔP) between the end pressure of the 5-s photostimulation and the pressure before stimulation (left), as well as the pressure ratio (calculated as the ratio of the end pressure of the 5-s photostimulation over the pressure before stimulation, right). n = 7 mice for each group. Left, ***P < 0.001 (unpaired t test); right, **P = 0.002 (Wilcoxon rank-sum test). **g** Quantification of the percentage of photostimulation trials inducing bladder contraction (left) and EUS bursting activity (right). n = 7 mice for each group. Left, **P = 0.001 (Wilcoxon rank-sum test); right, **P = 0.002 (unpaired t test). All data are presented as mean ± SEM.