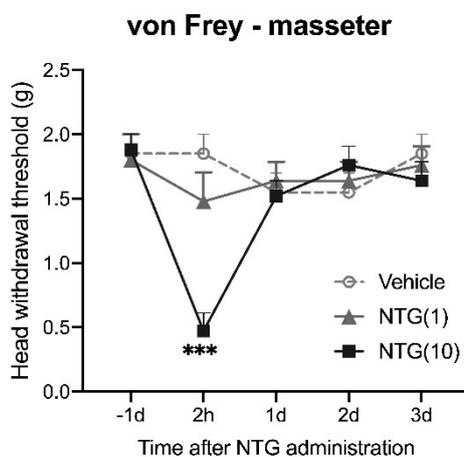
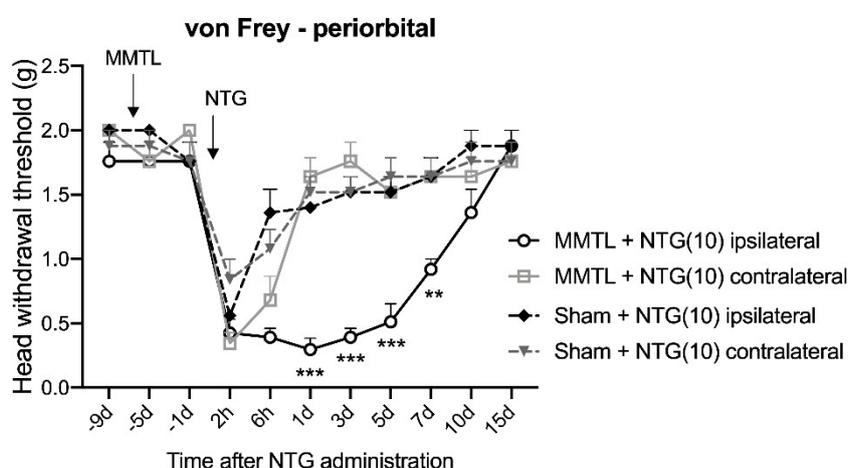




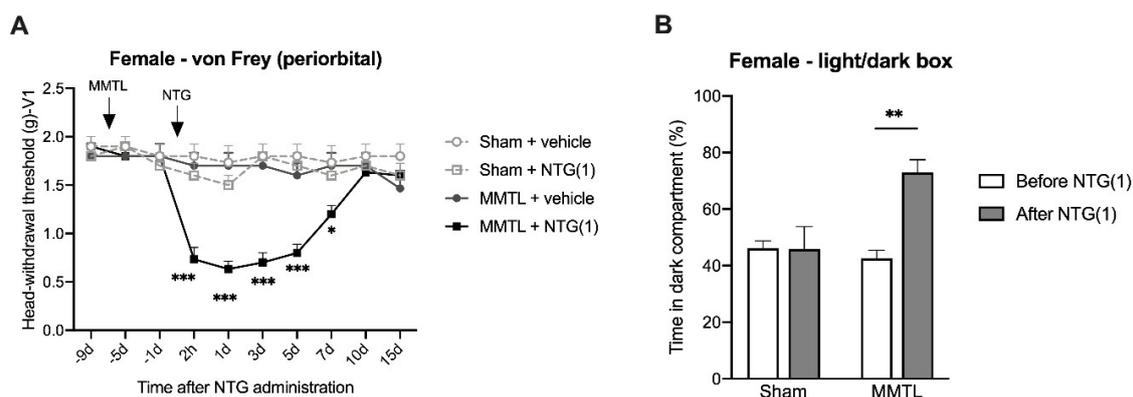
## Supplementary Materials



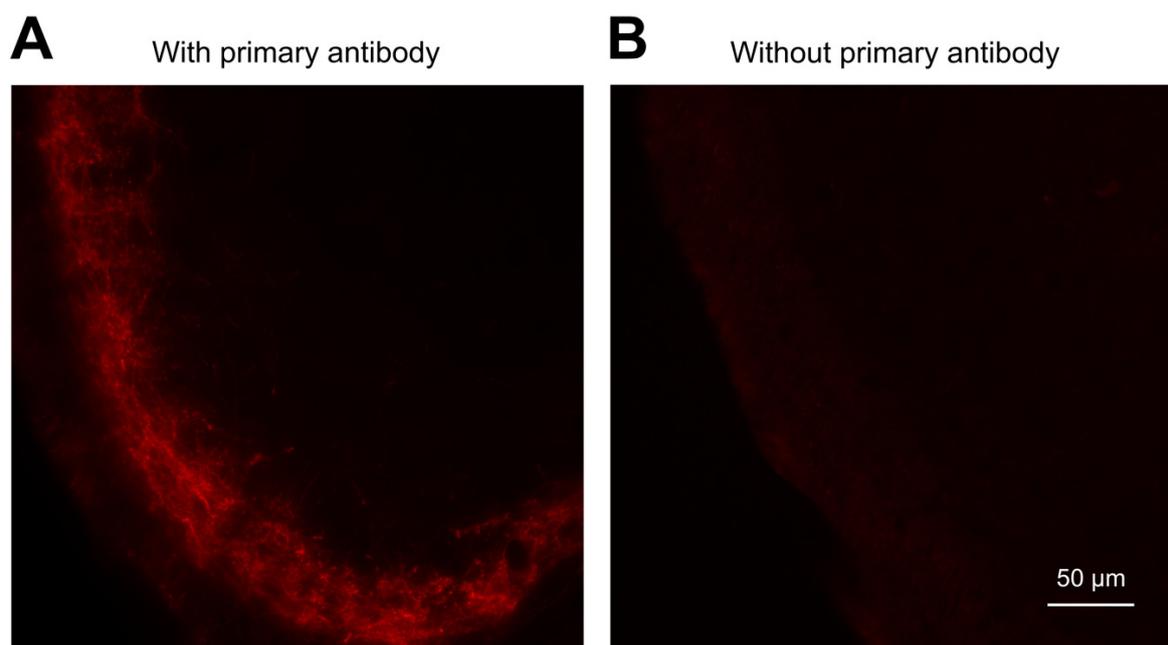
**Supplemental Figure 1.** Systemic injection of NTG induces mechanical hypersensitivity in trigeminal nerve V3-innervated masseter area of mice. Intraperitoneal injection (i.p.) of NTG at 10 mg/kg, but not 1 mg/kg, decreased the head withdrawal threshold in the skin area over masseter muscle ( $n = 5$  per group). \*\*\* $P < 0.001$  vs the vehicle-treated group. NTG, nitroglycerin; NTG(1): 1 mg/kg of nitroglycerin; NTG(10): 10 mg/kg of nitroglycerin.



**Supplemental Figure 2.** MMTL differentially affects NTG-induced mechanical hypersensitivity in ipsilateral and contralateral periorbital area. We observed that the MMTL significantly extended the NTG-decreased head withdrawal threshold in the ipsilateral periorbital area, but only slightly prolonged the NTG-decreased head withdrawal threshold on the contralateral side ( $n = 5$  per group). \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs the respective “MMTL + NTG(10) contralateral” group.  $F_{\text{treatment}}(3, 16) = 29.950$ ,  $P < 0.001$ ;  $F_{\text{time}}(10, 160) = 51.510$ ,  $P < 0.001$ ;  $F_{\text{interaction}}(30, 160) = 4.914$ ,  $P < 0.001$ . NTG(10), 10 mg/kg of nitroglycerin.



**Supplemental Figure 3.** MMTL enhances NTG-induced migraine-like hypersensitivity in female mice. **(A)** MMTL pretreatment enabled the lower dose (1 mg/kg) of NTG to decrease the head withdrawal threshold in the periorbital region ( $n = 6$  per group).  $F_{\text{treatment}}(3, 20) = 8.700$ ,  $P < 0.001$ ;  $F_{\text{time}}(9, 180) = 9.98$ ,  $P < 0.001$ ;  $F_{\text{interaction}}(27, 180) = 5.46$ ,  $P < 0.001$ .  $***P < 0.001$ ,  $*P < 0.05$  vs the corresponding time points in the “sham + vehicle” group. **(B)** MMTL pretreatment enabled the lower dose (1 mg/kg) of NTG to produce the light-aversive behavior ( $n = 6$  per group).  $F_{\text{treatment}}(1, 10) = 6.879$ ,  $P < 0.05$ ;  $F_{\text{time}}(1, 10) = 7.520$ ,  $P < 0.05$ ;  $F_{\text{interaction}}(1, 10) = 7.798$ ,  $P < 0.05$ .  $**P < 0.01$  as indicated in the figure. MMTL: masseter muscle tendon ligation; NTG: nitroglycerin; NTG(1): 1 mg/kg of nitroglycerin.



**Supplemental Figure 4.** Specificity of the CGRP antibody. Sp5C-containing brainstem tissues from wild-type mice were harvested for immunofluorescence staining. **(A)** The staining with primary CGRP antibody showed CGRP-positive signals in the superficial laminae of the Sp5C. **(B)** The staining with the primary CGRP antibody omitted showed no positive signals in the area.