**Supplemental Digital Content**

**Ciszek BP et al.** Persistent catechol-O-methyltransferase-dependent pain is initiated by peripheral beta-adrenergic receptors

**Supplementary Materials**

Figure 1. Sustained administration of the COMT inhibitor OR486 leads to increased mechanical and thermal pain sensitivity in male and female rats.

Figure 2. Male and female adrenalectomized rats fail to develop OR486-induced pain sensitivity.

Figure 3. Sustained peripheral, intrathecal or intracerebroventricular administration of βAR antagonists, in the absence of systemic administration of OR486, does not alter pain sensitivity.

Figure 4. Intrathecal administration of high dose propranolol does not alter OR486-induced pain sensitivity.

Figure 5. Intracerebroventricular administration of high dose propranolol does not alter OR486-induced pain sensitivity.

This supplementary material has been provided by the authors to give the readers additional information about their work.
Supplemental Fig 1. Sustained administration of the COMT inhibitor OR486 leads to increased mechanical and thermal pain sensitivity in male and female rats. Compared to vehicle, sustained OR486 administration produces (A) mechanical allodynia, (B) mechanical hyperalgesia and (C) thermal hyperalgesia in males and females. N=6 per group. Data are expressed as mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 different from Veh/M; †††P<0.001, ††P<0.01, †P<0.05 different from Veh/F. Abbreviations: Baseline (BL), catechol-o-methyltransferase (COMT), Female (F), Male (M), Vehicle (Veh).
Supplemental Fig 2. Male and female adrenalectomized rats fail to develop OR486-induced pain sensitivity. In Shm animals, sustained systemic OR486 administration produces (A) mechanical allodynia, (B) mechanical hyperalgesia and (C) thermal hyperalgesia. In contrast, Adx animals fail to develop OR486-induced (D) mechanical allodynia, (E) mechanical hyperalgesia or (F) thermal hyperalgesia. N=5-6 per group.

Data are expressed as mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 different from Veh/M; †††P<0.001, ††P<0.01, †P<0.05 different from Veh. Abbreviations: Adrenalectomized (Adx), Baseline (BL), Female (F), Male (M), Sham (Shm), Vehicle (Veh).
Supplemental Fig 3. Sustained peripheral, intrathecal or intracerebroventricular administration of βAR antagonists, in the absence of systemic administration of OR486, does not alter pain sensitivity. (A-C) Peripheral, (D-F) i.t. or (G-I) i.c.v. delivery of the non-selective βAR antagonist propranolol, β2AR antagonist ICI-118,551 or β3AR antagonist SR59230A does not alter (A,D,G) mechanical allodynia, (B,E,H) mechanical hyperalgesia or (C,F,I) thermal hyperalgesia. N=8-9 per group. **P<0.01, *P<0.05 different from Veh/Veh.

Abbreviations: Baseline (BL), Beta-Adrenergic Receptor (βAR), ICI-118,551 (ICI), intracerebroventricular (i.c.v.), intrathecal (i.t.), propranolol (prop), SR59230A (SR), Vehicle (Veh).
Supplemental Fig 4. Intrathecal administration of high dose propranolol does not alter OR486-induced pain sensitivity. Intrathecal delivery of a high dose of the non-selective βAR antagonist propranolol (100ug/day) alongside sustained OR486 administration does not alter (A) mechanical allodynia, (B) mechanical hyperalgesia or (C) thermal hyperalgesia. N=4 for Veh/Veh, OR486/Veh and OR486/Prop; and N=2 for OR486/Prop HD. Data are expressed as mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 different from Veh/Veh. Abbreviations: Baseline (BL), Beta-Adrenergic Receptor (βAR), High Dose (HD), Propranolol (Prop), Vehicle (Veh).
Supplemental Fig 5. Intracerebroventricular administration of high dose propranolol does not alter OR486-induced pain sensitivity. Intracerebroventricular delivery of a high dose of the non-selective βAR antagonist propranolol (100ug/day) alongside sustained OR486 administration does not alter (A) mechanical allodynia, (B) mechanical hyperalgesia or (C) thermal hyperalgesia. N=3-4 per group. Data are expressed as mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 different from Veh/Veh. Abbreviations: Baseline (BL), Beta-Adrenergic Receptor (βAR), High Dose (HD), Propranolol (prop), Vehicle (Veh).