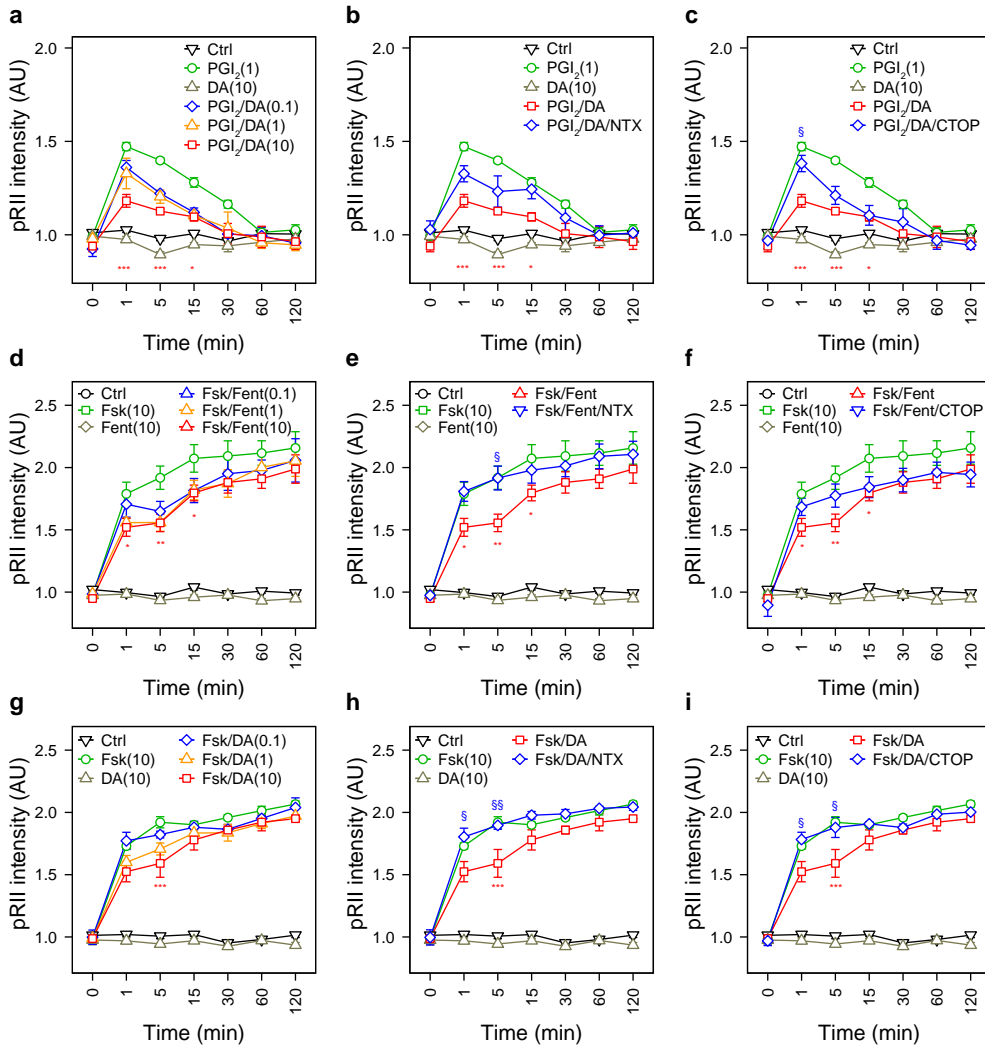


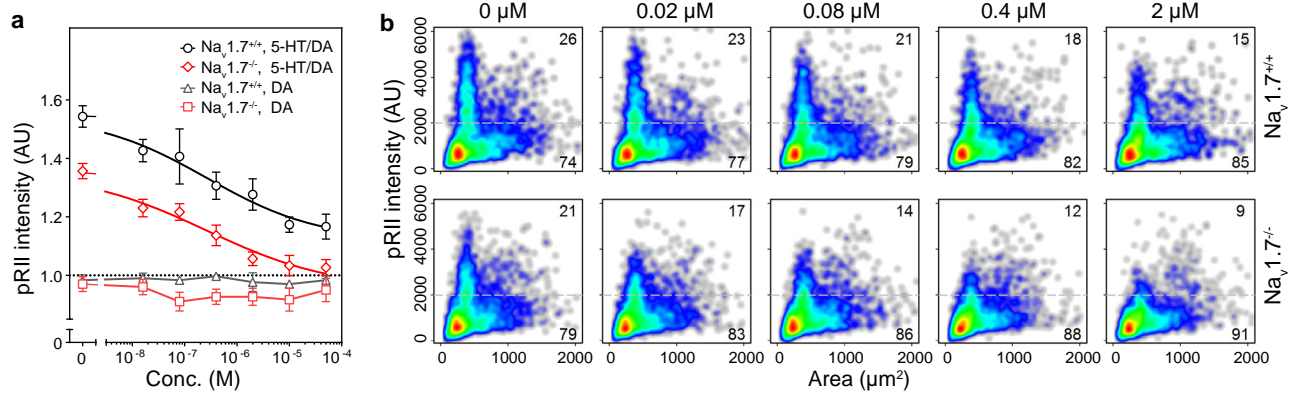
Supplemental Fig. S1. Experimental outline for analyzing Gs- and Gi-coupled GPCR signaling in sensory neurons by HCS microscopy. Dorsal root ganglia (DRGs) are collected, enzymatically digested, and cultured overnight. After stimulation with inflammatory mediators in the absence or presence of opioids, cells are fixed, and stained with antibodies to measure UCHL1 (pan-neuronal marker), RII β (nociceptors), and phospho-RII (PKA-II activity) signal intensities. Images were acquired with an automated Cellomics ArrayScan XTI microscope and analyzed using respective software resulting in single cell data. Green/red encircled neurons indicate automatically selected or rejected objects.

Supplemental Fig. S2. Opioids inhibit PGI₂- and Fsk-induced pRII increase in rat sensory neurons. **(A)** Time-course of pRII intensity after stimulation with PGI₂ (1 μ M) alone or in the presence of DAMGO (DA, 0.1-10 μ M). **(B, C)** Effect of the opioid receptor antagonist naltrexone (NTX, 10 μ M, B) and the MOR antagonist CTOP (10 μ M, C) on the inhibition of PGI₂-induced pRII by DAMGO. **(D)** Time-course showing the dose-dependent inhibition of Fsk (10 μ M) induced pRII response by fentanyl (Fent). **(E, F)** Time-course showing the effect of NTX (E) and CTOP (F) on the inhibition of Fsk-induced pRII increase by fentanyl. **(G)** The MOR-specific agonist DAMGO also inhibited Fsk-induced pRII increase, which was reverted by NTX **(H)** and CTOP **(I)**. Values are means \pm SEM; n = 3-4 independent experiments; total of >2000 neurons/condition; two-way ANOVA with Bonferroni's test; *P<0.05; **P<0.01; ***P<0.001 indicate significance levels between baseline and stimulated conditions; §P<0.05; §§P<0.01; §§§P<0.001 indicate significance levels between stimulated and inhibited conditions.

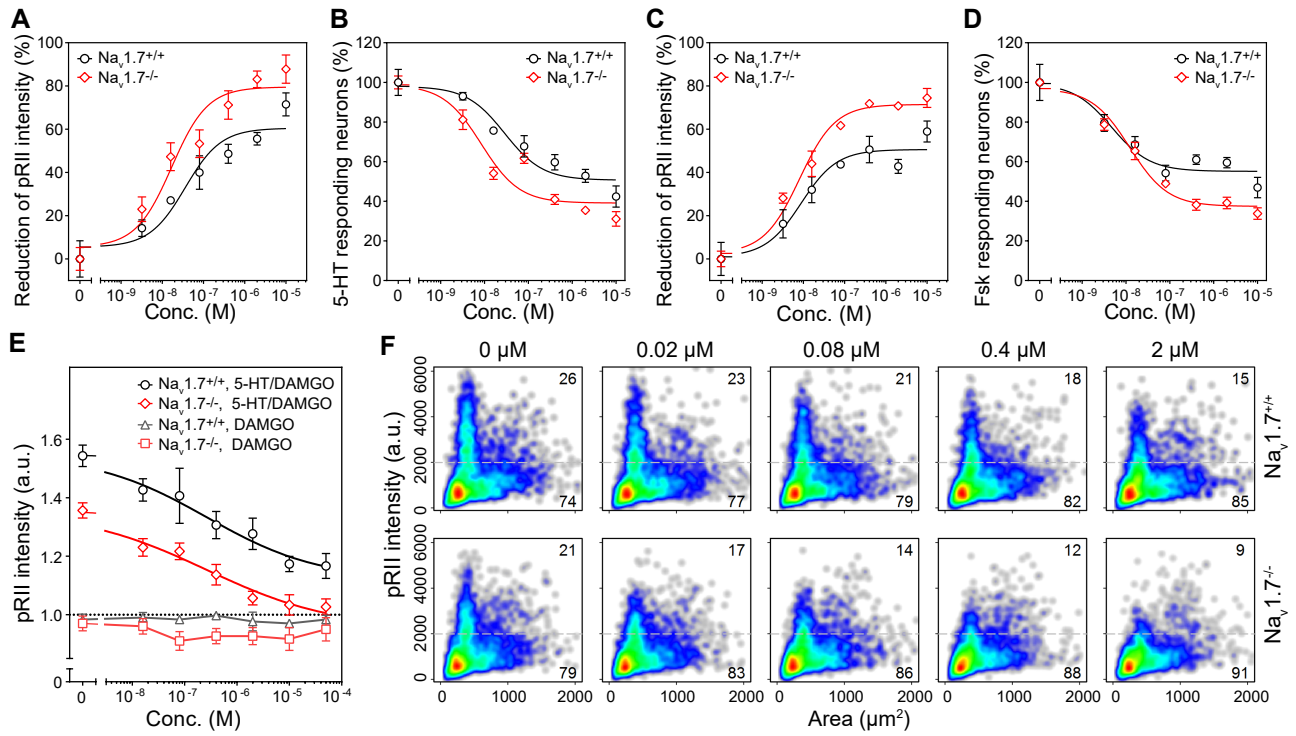
Supplemental Fig. S3. Enhanced efficacy of opioids to inhibit the induction of pRII by 5-HT or Fsk in Nav1.7-deficient sensory neurons. (A) Relative reduction of 5-HT-induced pRII intensity by increasing doses of fentanyl (Fent, 0-10 μ M) after 3 min stimulation of dorsal root ganglion neurons from Nav1.7-deficient (Nav1.7^{-/-}) and wild-type mice (Nav1.7^{+/+}). Data are means \pm SEM; Genotype effect: $F_{3,55} = 8.5$, $n = 4$ females per genotype; $P < 0.0001$ for whole curve; extra-sum-of-squares F test. **(B)** Inhibitory effect of fentanyl (0-10 μ M, 3 min) on the relative number of 5-HT (0.2 μ M, 3 min) responding neurons after 3 min stimulation. Genotype effect: $F_{3,55} = 9.6$, $n = 4$ females per genotype; $P < 0.0001$ for whole curve; extra-sum-of-squares F test. **(C)** Relative reduction of Fsk-induced pRII intensity by increasing doses of fentanyl (Fent, 0-10 μ M, 3 min). Genotype effect: $F_{3,50} = 15.1$, $n = 4$ females per genotype; $P < 0.0001$ for whole curve; extra-sum-of-squares F test. **(D)** Inhibitory effect of fentanyl (0-10 μ M, 3 min) on the relative number of Fsk (10 μ M, 3 min) responding neurons. Genotype effect: $F_{3,50} = 9.5$, $n = 4$ females per genotype; $P < 0.0001$ for whole curve; extra-sum-of-squares F test. **(E)** Dose-response curve showing the effect of increasing doses of DAMGO (0-50 μ M, 3 min) on the 5-HT (0.2 μ M, 3 min) induced pRII response. Data are means \pm SEM; $F_{2,36} = 7.7$; $n = 3$ males per genotype; $P < 0.01$ for whole curve; extra-sum-of-squares F test; $IC_{50} = 155$ nM vs. 250 nM in Nav1.7^{-/-} mice and wild-type litters. **(F)** Combined single cell data of pRII intensity vs. cell size of all sensory neurons shown in (A) representing >2500 neurons/condition.



Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3