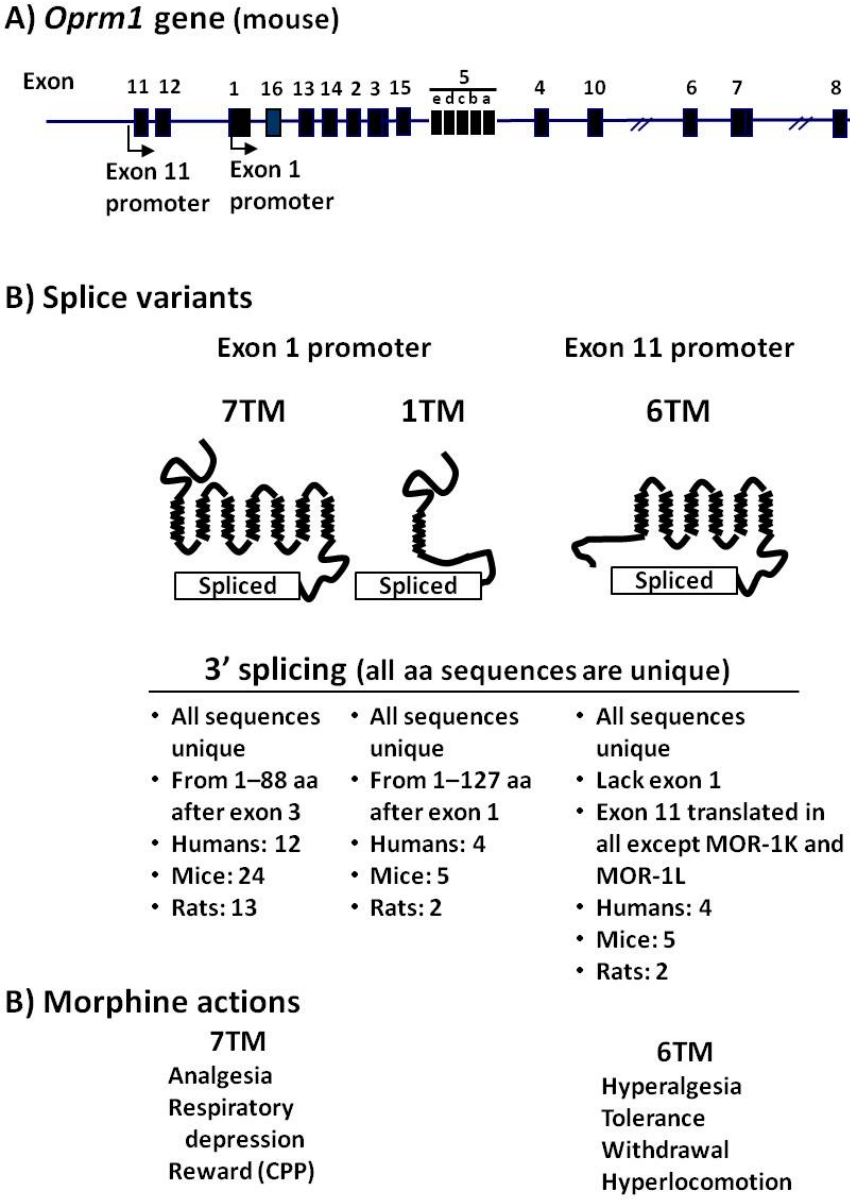
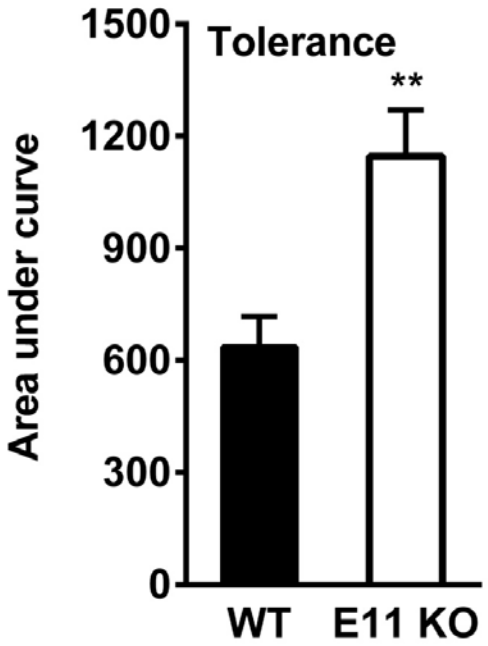
Supplemental Figure 1: Splice variants generated from *Oprm1*.



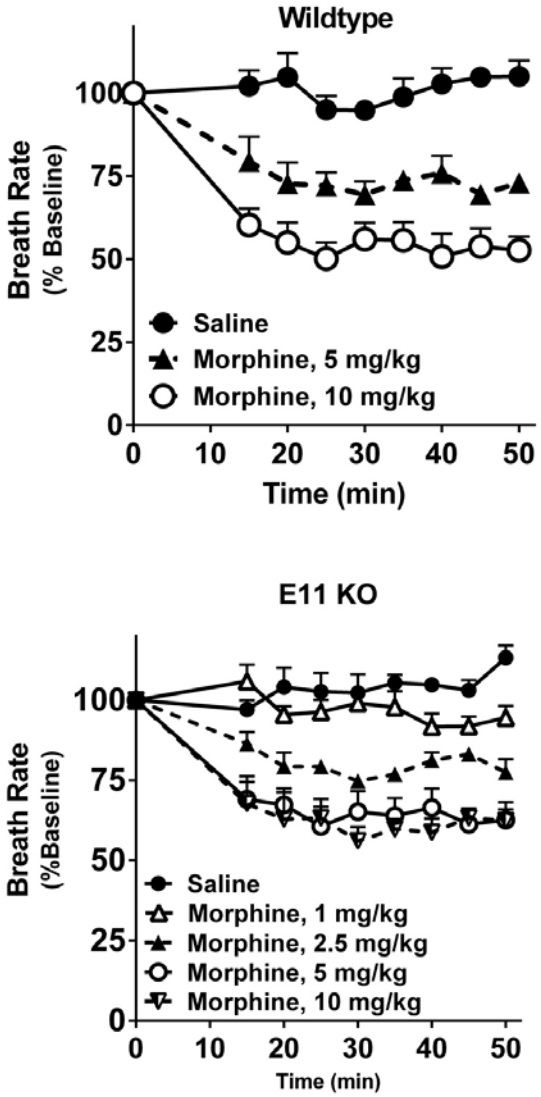
A) The mu opioid receptor gene *Oprm1* contains two independent promoters associated with exons 1 and 11. **B)** Variants associated with the exon 1 promoter include 7TM and 1TM variants. 7TM variants are composed of exons 1, 2 and 3, as well as at least one additional downstream exon resulting from 3' splicing. The 1TM variants contain a single transmembrane domain encoded by exon 1 followed by alternatively spliced exons generating the C-terminus. 6TM variants are associated with exon 11. They lack the first TM domain encoded by exon 1 due to exon skipping. Translation starts from exon 11 (27 aa in mice) and proceeds through exons 2 and 3 followed by downstream alternative splicing. Exceptions to this are MOR-1K and MOR-1L, in which exon 11 is not translated and translation of these variants is initiated within exon 2. 3' splicing results in unique amino acid (aa) sequences for each variant.

Supplemental Figure 2: Area under the curve of morphine tolerance



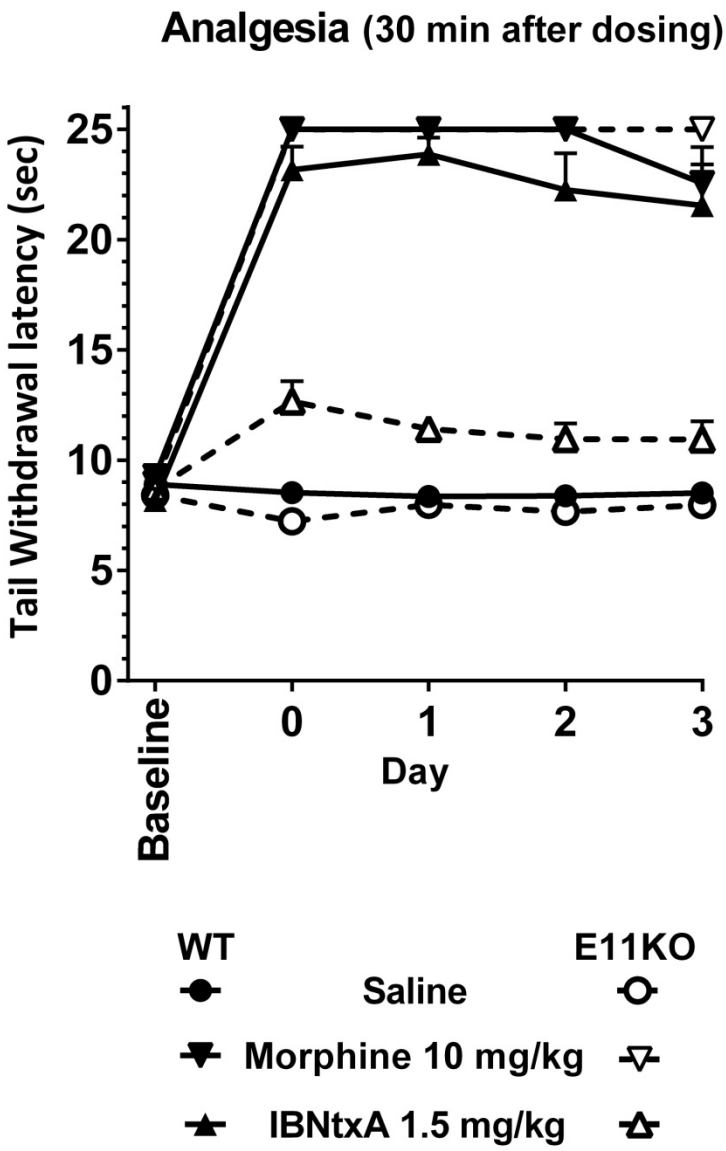
Groups of mice received morphine for 21 days as described in the legend of Figure 1C. The area under the curve (AUC) of Figure 1C was calculated using GraphPad Prism. Data represent mean ± s.e.m. for each genotype. Comparison of the two groups with Student's t-test indicated a significantly greater area for the E11 KO mice compared to WT mice, consistent with a slower development of tolerance in the E11 KO mice (t_{35}=3.54, p=0.0012).

Supplemental Figure 3: Time action of morphine effects on respiratory rate



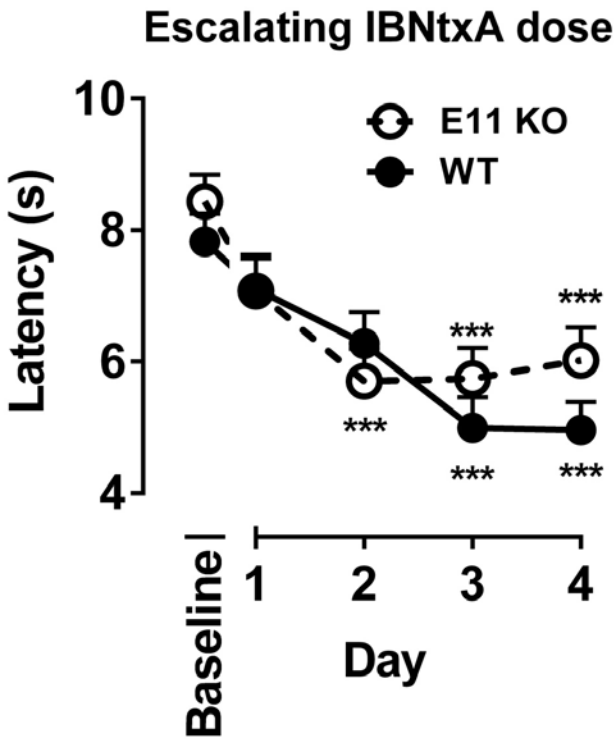
Groups of WT and E11 KO mice (n=5-8) received saline or morphine (1, 2.5, 5, 10 mg/kg, s.c.) and breath rate was monitored for 50 min. **A)** Breath rate in WT mice following morphine. A repeated measures ANOVA with Bonferroni post hoc comparisons test indicated WT mice showed a dose dependent response to morphine (repeated measures ANOVA, time: $F_{8,160}$=, p<0.0001; dose: $F_{2,20}$=90.5, p<0.0001; interaction: $F_{16,160}$=5.9, p<0.0001). **B)** Breath rate in E11 KO mice following morphine. E11 KO mice also showed significant decreases in breath rate following morphine (repeated measures ANOVA, time: $F_{8,160}$=14.62, p<0.0001; dose: $F_{4,20}$=44.39, p<0.0001; interaction: $F_{32,160}$=3.73, p<0.0001).

Supplemental Figure 4: Morphine and IBNtxA analgesia in the tail immersion assay



Groups of WT and E11 KO mice (n=10-20 per group) were tested in the warm water tail immersion assay 3 times to obtain a baseline. Then, mice were administered saline, morphine (10 mg/kg, s.c.) or IBNtxA (1.6 mg/kg, s.c.) on the indicated days and latencies were determined 30 min later. Data are reported as the mean ± s.e.m. latency. Morphine values reached cutoff for all animals in both the WT and the E11 KO groups, so some of the values cannot be seen on the figure due to overlap of symbols.

Supplemental Figure 5: Effects of escalating IBNtxA Dosing on tail withdrawal latencies



Groups of mice (n=10) were tested in the warm water tail immersion assay 3 times to obtain a baseline. Then, mice were administered saline or IBNtxA and latencies were determined again 24 later. Following testing, drug was administered again, and tail withdrawal latency was assessed again 24 hours later. This was repeated two more times. The dose of IBNtxA was 2 mg/kg, s.c. on Days 1-3 and 4 mg/kg on Day 4).