Supporting Information

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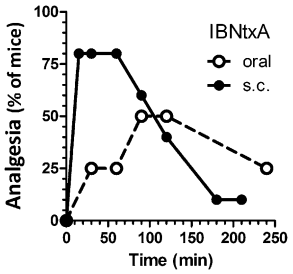

Fig. S1. Time course of oral and s.c. IBNtxA analgesia. Groups of mice received IBNtxA s.c. (0.75 mg/kg s.c.; $n = 20$) or orally (5 mg/kg s.c.; $n = 8$) by gavage and were tested for analgesia at the indicated times.

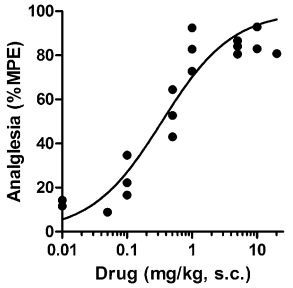

Fig. S2. IBNtxA analgesia in WT mice. Three independent determinations of the cumulative dose–response curves were performed on groups of mice ($n = 10$) for IBNtxA analgesia in the tail-flick assay. The means of each dose in each determination were determined as percentage maximal possible effect (%MPE) [(observed latency − baseline latency)/(maximal latency − baseline latency)]. Each value is shown for each of the three independent determinations.

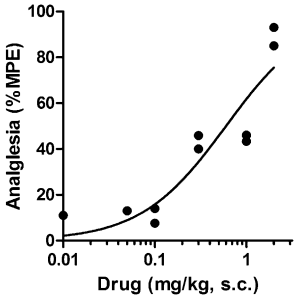

Fig. S3. IBNtxA hot plate assay analgesia in WT mice. Cumulative dose–response curves were determined in the 55 °C hot plate assay for two groups of mice ($n = 8$) tested independently. Results were evaluated as %MPE [(observed latency − baseline latency)/(maximal latency − baseline latency)] and shown as the average of each group ($n = 8$). $ED_{50} = 0.6$ mg/kg s.c.

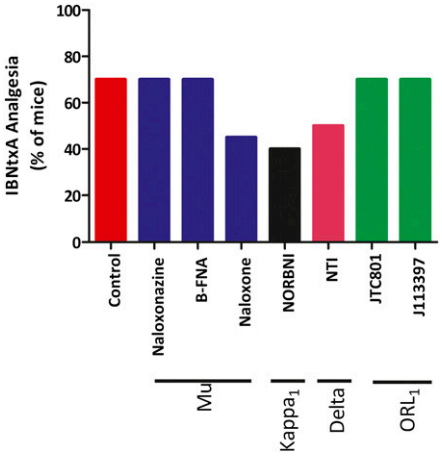

Fig. S4. Sensitivity of IBNtxA analgesia to opioid antagonists. Groups of mice ($n \geq 10$) received IBNtxA (0.75 mg/kg s.c.) and the indicated antagonist. Naloxonazine (35 mg/kg s.c.) and β-funaltrexamine (β-FNA; 40 mg/kg s.c.) were administered at 24 h before agonist testing. Naloxone (10 mg/kg s.c.), norbinaltorphimine (norBNI; 10 mg/kg s.c.), naltrindole (NTI; 20 mg/kg s.c.), JTC801 (30 mg/kg s.c.), and J113397 (30 mg/kg s.c.) were administered immediately before IBNtxA. All analgesia testing was performed 30 min after IBNtxA administration.

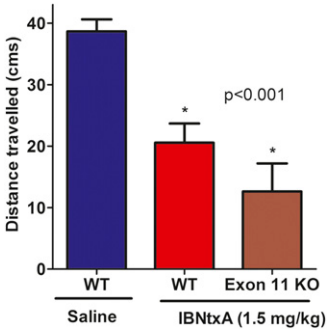

Fig. S5. IBNtxA inhibition of gastrointestinal transit in WT and exon 11 MOR-1 KO mice. Groups of mice ($n = 10$) received either saline (control) or IBNtxA (1.5 mg/kg s.c.) and followed by a charcoal meal by gavage. Animals were killed 30 min later, and the distance traveled by charcoal was measured. IBNtxA decreased transit in WT mice and in the exon 11 MOR-1 KO mouse compared with untreated control WT mice ($P < 0.001$). The decrease in transit for IBNtxA in the exon 11 MOR-1 KO mouse was not significantly different from the WT mice, contrasting with its loss of analgesic activity in the exon 11 MOR-1 KO mice.

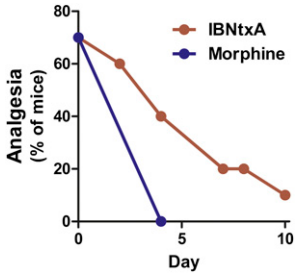

Fig. S6. Effect of chronic dosing on morphine and IBNtxA analgesia. Groups of mice ($n \geq 20$) were treated with either morphine (6 mg/kg s.c.) or IBNtxA (1 mg/kg s.c.) twice daily for 5 or 10 d, respectively. Tail-flick latencies were determined before and 30 min after each injection.

Table S1. ^{125}I-BNtxA binding in triple-KO and WT mice brain membranes with and without blockers

Mice	K_D, nM	B_{max}, fmol/mg of protein
WT C57	0.12 ± 0.03	284 ± 8.96
Triple-KO	0.16 ± 0.04	61 ± 1.6
WT C57 with blockers	0.12 ± 0.04	47 ± 8.4

Mouse brain membranes were prepared as described in *Materials and Methods*. Saturation studies were carried out with ^{125}I-BNtxA alone (WT C57 and triple-KO) or in WT C57 membranes in the presence of mu {[D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP), 1 μM}, kappa$_1$ (U50,488H, 1 μM), and delta {[D-Pen^2,D-Pen^5]enkephalin (DPDPE); 1 μM} blockers. Results are the means ± SEM of three independent determinations.