First cohort description

Subject recruitment. One hundred ninety six (196) healthy European American pain-free females with an age range of 18 to 34 years were genotyped and phenotyped. Demographic characteristics of the cohort at the time of recruitment have been previously described (1-3). This study was nested within a larger prospective study conducted at the University of North Carolina at Chapel Hill that was designed to examine whether pain sensitivity is a risk factor for the development of TMD (2). Subjects were recruited to participate in a 3-year prospective study designed to identify risk factors that contribute to the onset of myogenous TMD via advertisements placed in local newspapers. A comprehensive medical history was obtained from each subject to rule out previous or current history of pain-related disorders and other medically-related conditions that might alter their pain perception. The study was conducted with both written and verbal informed consent using protocols reviewed and approved by the UNC's Committee on Investigations Involving Human Subjects.

Subjects were pain phenotyped with respect to their sensitivity to pressure pain, heat pain, and ischemic pain. Indices of the temporal summation of heat evoked pain were also examined. To control for the effects of menstrual cycle on pain sensitivity all pain measurements, except pressure pain threshold, were performed during the follicular phase (between days 3 and 10) of the subject's menstrual cycle. All subjects were asked to refrain from consuming over-the-counter pain relieving medications for at least 48 hours before visiting the laboratory and all subjects were free of prescription pain medications for at least two weeks prior to testing. During each session, pain measurements were performed in the following order: pressure pain, thermal pain, temporal summation of heat pain, and ischemic pain. The sequence of procedures was not randomized between subjects because of the possible long lasting effects of the more prolonged noxious stimuli (i.e. ischemic pain & repeated application of high intensity heat pulses) on neural and hormonal systems.

Pain Phenotyping Procedures

A detailed description of the phenotyping procedures used to assess this cohort has been published previously (1,3). Measures of pressure pain, heat pain, and responses to repetitive noxious heat stimuli were obtained.

Pressure Pain Threshold. Pressure pain threshold (PPT) was assessed over the right and left temporalis muscle, masseter muscle, temporomandibular joint, and ventral surface of the wrist with a hand-held pressure algometer (Pain Diagnosis and Treatment, Great Neck, NY). The PPT was defined as the amount of pressure (kg) at which the subjects first perceived the stimulus to be painful. One pre-trial assessment was performed at each site followed by additional assessments until two consecutive measures were obtained that differed by less than 0.2 kg. The values from the right and left sides were averaged to obtain one pressure pain threshold value per anatomical site. This procedure yielded four measures of pressure pain threshold at four anatomical sites.

Heat Pain Threshold and Tolerance. Measures of thermal pain threshold and tolerance were obtained with a 10 mm diameter computer controlled contact thermal stimulator. Thermal stimuli were applied to the skin overlaying the right masseter muscle, right forearm, and dorsal surface of the right foot. Thermal pain threshold was defined as the temperature at which the subjects first perceived heat pain, whereas thermal pain tolerance was defined as the temperature at which the subjects would no longer tolerate the pain and requested the removal of the stimulus. Six heat ramps were applied to each site for each measure from a neutral adapting temperature of 32ºC at a rate of 0.5ºC/sec, which produces a relatively selective activation of heat C-fibers (4,5). This procedure yielded six measures: heat pain threshold and heat pain tolerance at three anatomical sites.

Responses to Repeated Heat Stimuli. Responses to sequential presentations of heat pulses were assessed. A total of fifteen 53ºC heat pulses were applied to the skin overlying the thenar region of the right hand. Each heat pulse was 1.5 sec in duration and was delivered at a rate of 10ºC/sec from a 40ºC base temperature with an inter-trial interval of 1.5 sec. Subjects were instructed to verbally rate the intensity of each thermal pulse using a 0 to 100 numerical scale with '0' representing 'no sensation', '20' representing 'just painful', and '100' representing 'the most intense pain imaginable'. Each subject's ability to summate responses to repeated heat stimuli was quantified to derive four measures:

First Pulse Response (FPR): The numeric response to the first pulse was used as a marker of initial resting state sensitivity to thermal pain; Rate of Rise (RR): The average rate of change across the first 6 pulses was used as one measure of temporal summation of nociception, a phenomenon that is thought to be mediated by the central integration of C-fiber input. This average rate of change was computed for each individual from the parameter estimate of the least-squares regression line that was fitted for the subject's first 6 verbal responses; Area Under the Curve (AUC): The integrated effects of initial sensitivity and subsequent temporal summation were quantified by computing the total AUC plotted for each individual's responses across all 15 pulses; Delta: The total amount of change was computed by subtracting the rating for the first pulse from the highest rated response. This was used as a second measure of temporal summation, capturing the maximum amount of summation among all 15 pulses. Derived parameters RR and Delta are measures of the temporal summation of heat pain, whereas parameters FPR and AUC are indices of the overall sensitivity to heat pain.

Ischemic Pain Threshold and Tolerance. A modified submaximal effort tourniquet procedure was used to evoke ischemic pain. The subject's right arm was elevated for 30 sec followed by the inflation of a blood pressure cuff to 220 mmHg. A stopwatch was started and the subject squeezed a handgrip dynamometer at 30% of maximum force of grip for 20 repetitions. The times to ischemic pain onset and tolerance were determined. The tourniquet remained in place for 25 min or until pain tolerance had appeared. This procedure yielded two measures: ischemic pain threshold and ischemic pain tolerance.

Generation of a summary pain z-score. Each enrollee in the analyzed cohort was quantified for responsiveness to a set of noxious stimuli applied to various anatomical sites (1-3). We calculated a derived, unitary measure of pain sensitivity for both cutaneous and deep muscle pain, which are modified by μ-receptor agonists (6-9). A single measure of pain sensitivity was computed for each subject from the sixteen experimental pain procedures. We first reversed the direction of measurement (by subtracting from zero) twelve of the measures that quantified threshold or tolerance to experimental pain: four measures of pressure pain threshold, six measures of thermal threshold/tolerance, and two measures of ischemic threshold/tolerance. The four measures of responses to repeated heat stimuli was retained as its original value. All sixteen measures were standardized to unit normal deviates (z-scores) by subtracting the sample mean, then dividing by the sample standard deviation. Given that all tested individual pain measures are influenced by μ-receptor agonists, we hypothesized that functional genetic polymorphisms in *OPRM1* would be associated with population variations in the aggregate measure of experimental pain sensitivity. For consistency with our previous work (2-3,10-11) a sum of all 16 z-scores has been used for characterization of association of genotypes with the aggregate measure of pain. Alternatively, an average of z-scores, or a centered and scaled sum could be taken instead. However, such normalizations amount to a shift or rescaling of the overall measure, and thus would not affect the outcome of the statistical analysis.

Second cohort description

Subjects. Second cohort has been recruited at the University of Florida at Gainesville. Subjects included 133 healthy Caucasians volunteers (80 female, 53 male) recruited via posted advertisements from the local community. All participants were healthy nonsmokers and were free of clinical pain, psychiatric disturbance, substance abuse, or use of centrally acting medications. Subjects refrained from any over-the-counter medication use for at least 24 h prior to testing. Forty-seven (47%) of the women were taking oral contraceptives.

General Experimental Procedures. All subjects participated in two experimental sessions separated by at least 2 days. The data presented herein represent baseline pain responses obtained as part of an ongoing investigation of opioid analgesia (12). All subjects participated in 2 experimental sessions, one involving administration of morphine and the other involving saline placebo, in counterbalanced order. In order to reduce error variability the baseline values from the two sessions were averaged for each subject. For women, all sessions were conducted during the follicular phase of the menstrual cycle, between days 4 and 10 after the onset of menses. For 36 of the women the two sessions occurred within 2-7 days in the same menstrual cycle, and for 59 of the women the two sessions occurred in separate menstrual cycles and were separated by approximately 4 weeks. For men, sessions were separated by

equal intervals, with 34 men being tested within one week and 38 being tested approximately 4 weeks apart. At a separate introductory session, approximately one week before the experimental sessions were conducted, all subjects provided verbal and written informed consent and completed a series of health and psychological questionnaires. All sessions were conducted by two experimenters, either two females or one female and one male. Each experimental session started with insertion of an intravenous cannula from which 5 ml of blood was drawn, followed by a 15-min rest period, during which blood pressure and heart rate were monitored. Next, baseline experimental pain testing was performed, including assessment of thermal pain, pressure pain, and ischemic pain (described in detail below). After the pre-drug pain testing, a 15-minute rest period was observed, followed by double-blind intravenous bolus administration of either morphine (0.08 mg/kg) or saline, in randomized order. Fifteen minutes after drug administration, resting cardiovascular measures were again obtained, after which pain testing was repeated in a manner identical to the pre-drug testing. All procedures were approved by the University of Florida's Institutional Review Board.

Pain Testing Procedures. The following experimental pain procedures were conducted. Pressure and thermal pain were delivered first in counterbalanced order, separated by a 5-min rest period. Ischemic pain always occurred last in order to reduce the possibility of carry-over effects. Prior to each pain procedure, digitally recorded instructions were played for the subject.

Pressure Pain Threshold. A handheld algometer (Pain Diagnostics and Therapeutics, Great Neck, NY) was used to assess pressure pain threshold. Mechanical pressure was applied using a 1 cm^2 probe. An application rate of 1 kg/s was utilized, as this rather slow application rate reduces artifact associated with reaction time (13). Subjects were instructed to report when the pressure first became painful. Pressure pain thresholds (PPT) were assessed at 3 sites: the center of the right upper trapezius (posterior to the clavicle), the right masseter (approximately midway between the ear opening and the corner of the mouth), and the right ulna (on the dorsal forearm, approximately 8 cm distal to the elbow) with the order of site presentation counterbalanced. Pressure pain thresholds were assessed 3 times at each site, and the average of the three assessments was computed and used in subsequent analyses.

Thermal Pain Threshold and Tolerance. The first thermal procedure involved assessment of heat pain threshold and tolerance. Contact heat stimuli were delivered using a computer-controlled Medoc Thermal Sensory Analyzer (TSA-2001, Ramat Yishai, Israel), which is a Peltier-based stimulator. Temperature levels were monitored by a contactor-contained thermistor, and returned to a preset baseline of 32ºC by active cooling at a rate of 10ºC/s. The 3 x 3-cm contact probe was applied to the right ventral forearm. In separate series of trials, warmth thresholds, heat pain thresholds, and heat pain tolerances were assessed using an ascending method of limits. From a baseline of 32ºC, probe temperature increased at a rate of 0.5ºC/s until the subject responded by pressing a button to indicate when they first felt pain and when they no longer felt able to tolerate the pain. This slow rise-time was selected as a test of pain evoked mainly by stimulation of C-nociceptive afferents, as has been previously demonstrated (4,5). Four trials of heat pain threshold (HPTh), and heat pain tolerance (HPTo) were presented to each subject. The position of the thermode was altered slightly between trials (though it remained on the ventral forearm) in order to avoid either sensitization or response suppression of cutaneous heat nociceptors. For each measure, the average of all four trials was computed for use in subsequent analyses.

Temporal Summation of Thermal Pain. After a 5-min rest period, the temporal summation procedure was conducted. This procedure involved administration of brief, repetitive, suprathreshold heat pulses to assess first and second pain and temporal summation of the latter (14). Subjects rated thermal pain intensity of 10 repetitive heat pulses applied to the right dorsal forearm. The target temperatures were delivered for less than 1 s, with a 2.5-s interpulse interval during which the temperature of the contactor returned to a baseline of 40ºC. Subjects were asked to rate the peak pain for each of the 10 heat pulses. Because subjects vary in their responses to heat pain, we examined temporal summation at 2 different stimulus intensities. This increased the likelihood that at least one set of stimuli would be at least moderately painful yet tolerable for the vast majority of subjects. Therefore, 2 sets of target temperatures, 49ºC and 52ºC, were used. Subjects were instructed to verbally rate the intensity of each thermal pulse using a numerical rating scale as previously described (15), on which 0

represented no sensation, 20 represented a barely painful sensation and 100 represented the most intense pain imaginable. Subjects were told that the procedure would be terminated when they reported a rating of 100, when 10 trials had elapsed, or when they wished to stop. The average of the 10 ratings was determined for each subject and used in subsequent analyses.

Modified Submaximal Tourniquet Procedure. Following the first two pain procedures, a 5-min rest period was observed, after which subjects underwent the modified submaximal tourniquet procedure (16,17). The right arm was exsanguinated by elevating it above heart level for 30 s, after which the arm was occluded with a standard blood pressure cuff positioned proximal to the elbow and inflated to 240 mm Hg using a Hokanson E20 Rapid Cuff Inflator. Subjects then performed 20 handgrip exercises of 2-s duration at 4-s intervals at 50% of their maximum grip strength. Subjects were instructed to report when they first felt pain (ischemic pain threshold, IPTh) then to continue until the pain became intolerable (ischemic pain tolerance, IPTo), and these time points were recorded. Every 30 s, subjects were prompted to alternately rate either the intensity or unpleasantness of their pain using joint numerical (0-20) and verbal descriptor box scales. An uninformed 15-min time limit was observed. Changes in ischemic pain tolerance were not used as analgesic measures due to a ceiling effect, because more than 30% of patients achieved a maximum tolerance time during baseline assessment. In addition to IPTh and IPTo, two total pain scores were created, one for pain intensity and one for pain unpleasantness, by summing all ratings obtained during the procedure. To replace missing values created by subjects terminating the procedure before the time limit, the last rating provided was carried forward.

Selection of the new candidate SNPs

Having established the areas of exonic conservation within the *OPRM1* gene locus, we selected a set of candidate SNPs that potentially cover all of the functional allelic diversity of the gene including the newly identified exonic and promoter regions. SNPs were selected based on the following three criteria. First, we restricted our choice based on the frequency of the SNP because abundant SNPs with a minor allele frequency in population >0.15 rather than rare mutations are more likely to contribute to complex traits like pain responsiveness (18). Second, we chose SNPs that are most likely to impact gene function, which are SNPs in the coding region, exon-intron junctions, 5' promoter regions, putative transcription factor binding sites (TFBS) and 3' and 5’ untranslated regions (UTRs). Third, we chose equally spaced SNPs to represent the haplotype structure of the *OPRM1* gene (19). Table 1 presents a summary of the characteristics and potential functional significance of the selected SNPs. For the predicted exons, regions flanking the ~ 300 nt of the conservation zone were also considered. We also chose several abundant SNPs in the intronic regions at an interval of ~10 kb to obtain a surrogate marker for functional alleles, which are in the same haploblock, moderately abundant and effective but unknown; or to be a candidate for a functional SNP situated within an unidentified exon. We evaluated SNPs within the *OPRM1* gene locus with the emphasis on the newly identified exons and promoter sites.

Supplementary Figures

Figure S1. **Alignment of conserved mouse and human exon 11 region.** Exon 11 nucleotides are shown in upper case; nucleotides in intronic sequences are shown in lower case, putative transcription factor binding sites in conserved promoter region are underlined.

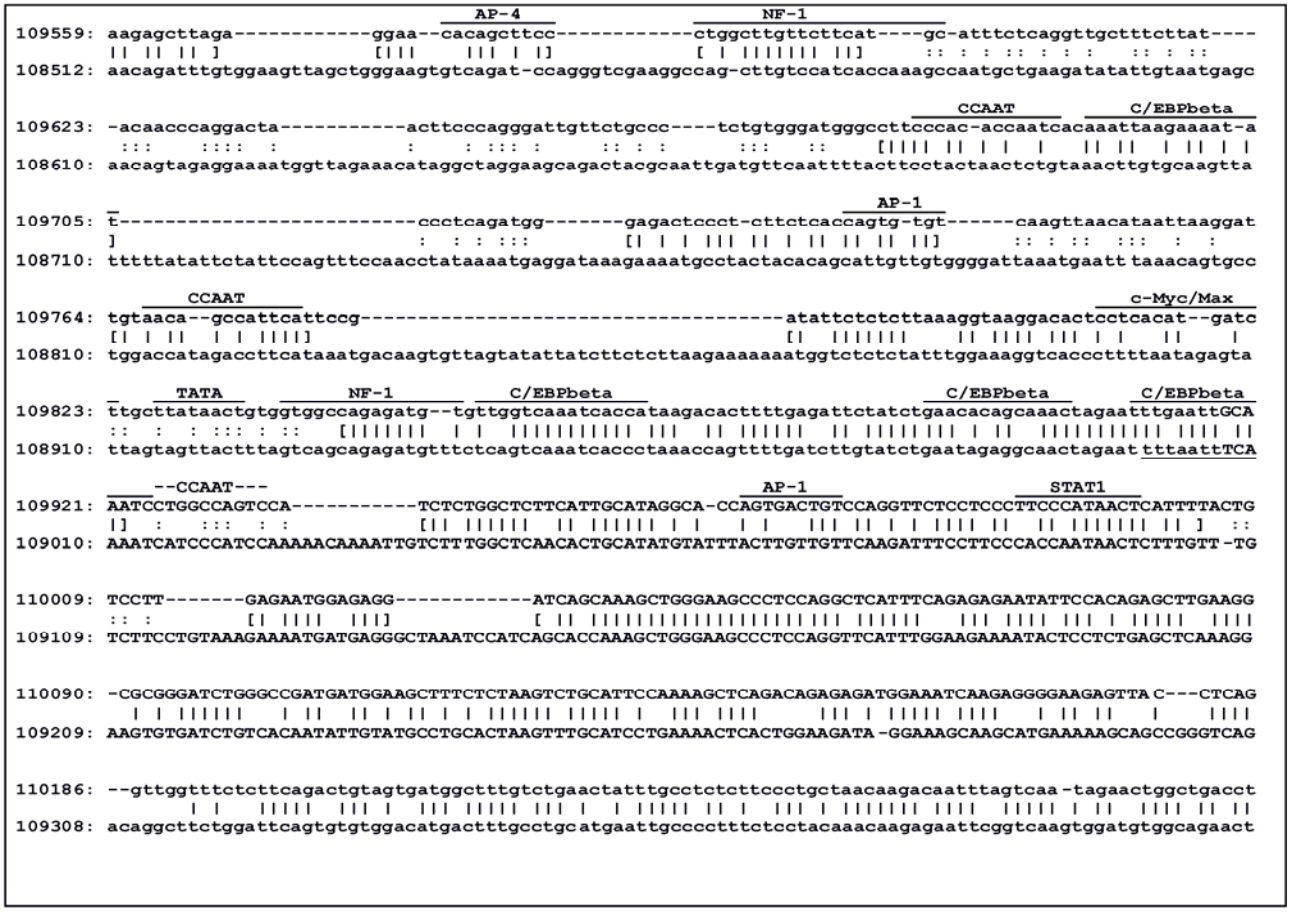

Figure S2. **Sliding window –ln(p-value) plots for the overall haplotype association tests with increasing window size.** P-values correspond to a haplotype that includes indicated SNP as the first one. The horizontal line indicates a level of significance at $\alpha = 0.05$, corrected for the number of independent tests in each SW. Significantly associated SNPs are listed in each figure and the SNP that tags the haplotype with maximum effect is specified under each plot.

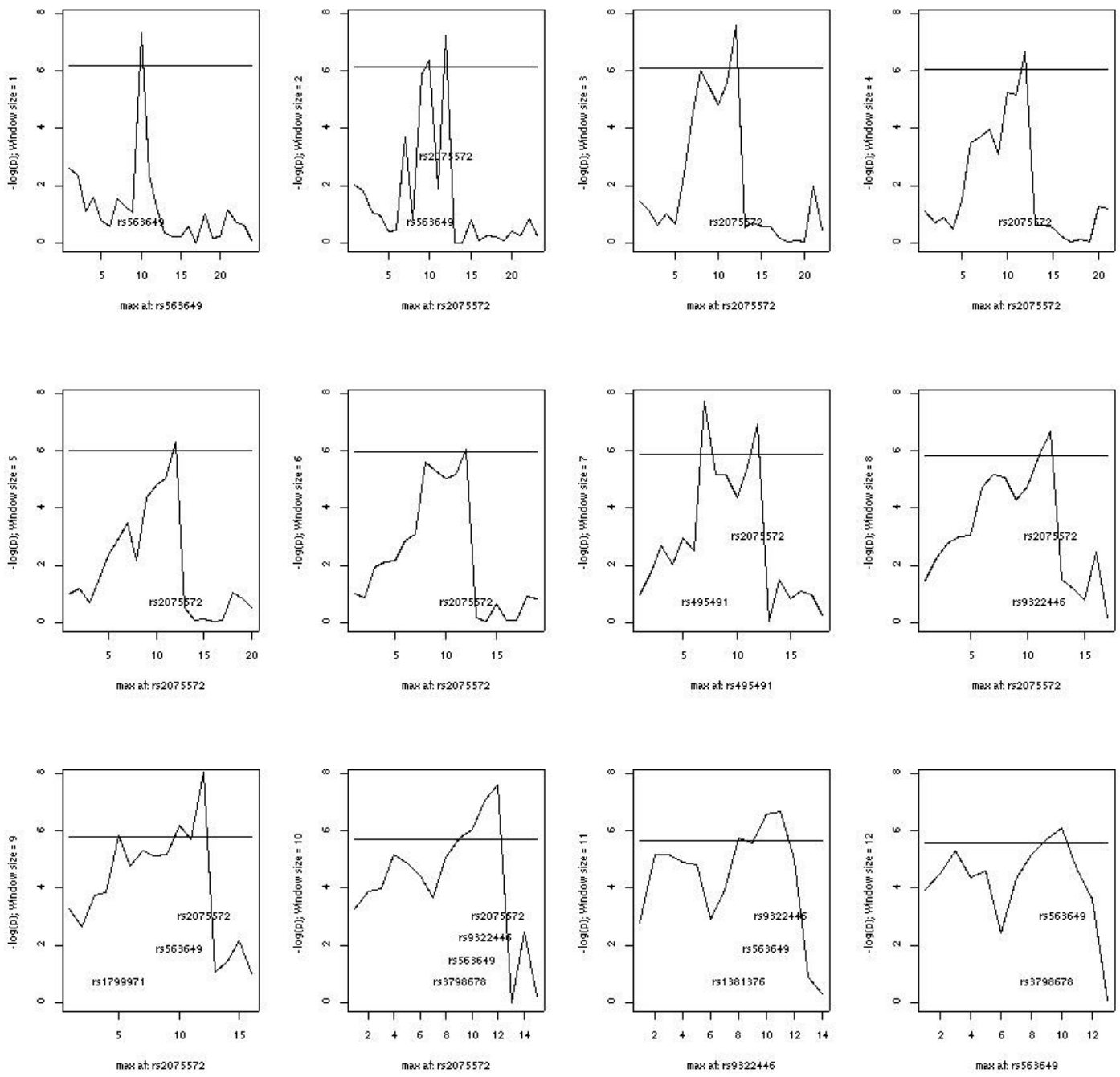

Figure S3. *OPRM1* LD Matrix in Caucasian women. Each box represents % LD [D'] between pairs of markers, as generated by Haploview (Whitehead Institute for Biomedical Research, USA). D' is color coded, red box indicating complete [1.00] D' between locus pairs.

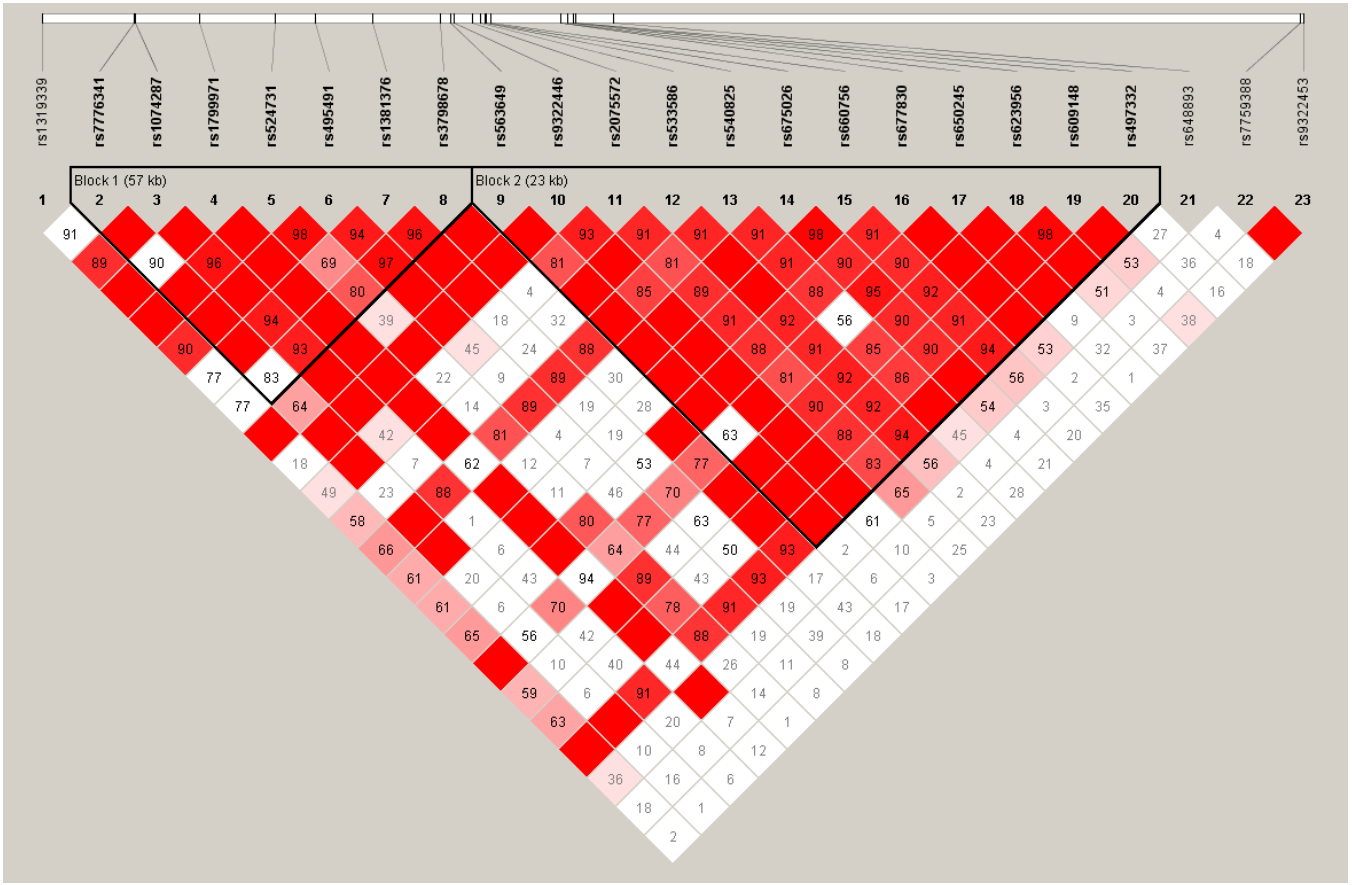

Figure S4. Cloning of exons 13-2 junction. To identify exact borders of exon 13-exon 2 junction, total RNA samples from human medulla oblongata (MO), frontal lobe (FL), nucleus accumbens (NA) and spinal cord (Table S6) were amplified with primers hU2-4L3 followed by secondary PCR with primers hU2-hL1 (Table S5) using Taq DNA polymerase (Invitrogen, Carlsbad, CA). Samples were purified using QIAquick PCR purification kit (Qiagen, Valencia, CA), followed by TOPO-TA cloning reaction (Invitrogen, Carlsbad, CA). Twenty four random clones of each tissue were selected and submitted for sequencing (MWG Biotech USA, High Point, NC). Clones were sequenced using both hU2 and hL1 primers. The tissue-specific distribution of MOR-1K1 and MOR-1K2 isoforms in presented in Table S5.

Sequences of the exon 13-exon 2 junctions in MOR-1K1 and MOR-1K2 isoforms

OPRM1 genome	GAGTGAAACT GAGGTACTAT TACTAAATTA AGGTA::intron:: TAGATACA CCAAGATGAA
MOR-1K1	GAGTGAAACT GAGGTACTAT TACTAAATTA AG:::::::::::::::::::::::::::: ATACA CCAAGATGAA
MOR-1K2	GAGTGAAACT GAG:: ATACA CCAAGATGAA

Figure S5. Human exon 13 promoter region for MOR-1K1 and MOR-1K2 isoforms. Transcription start is shown in red; putative transcription factor binding sites in the vicinity of promoter region are underlined.

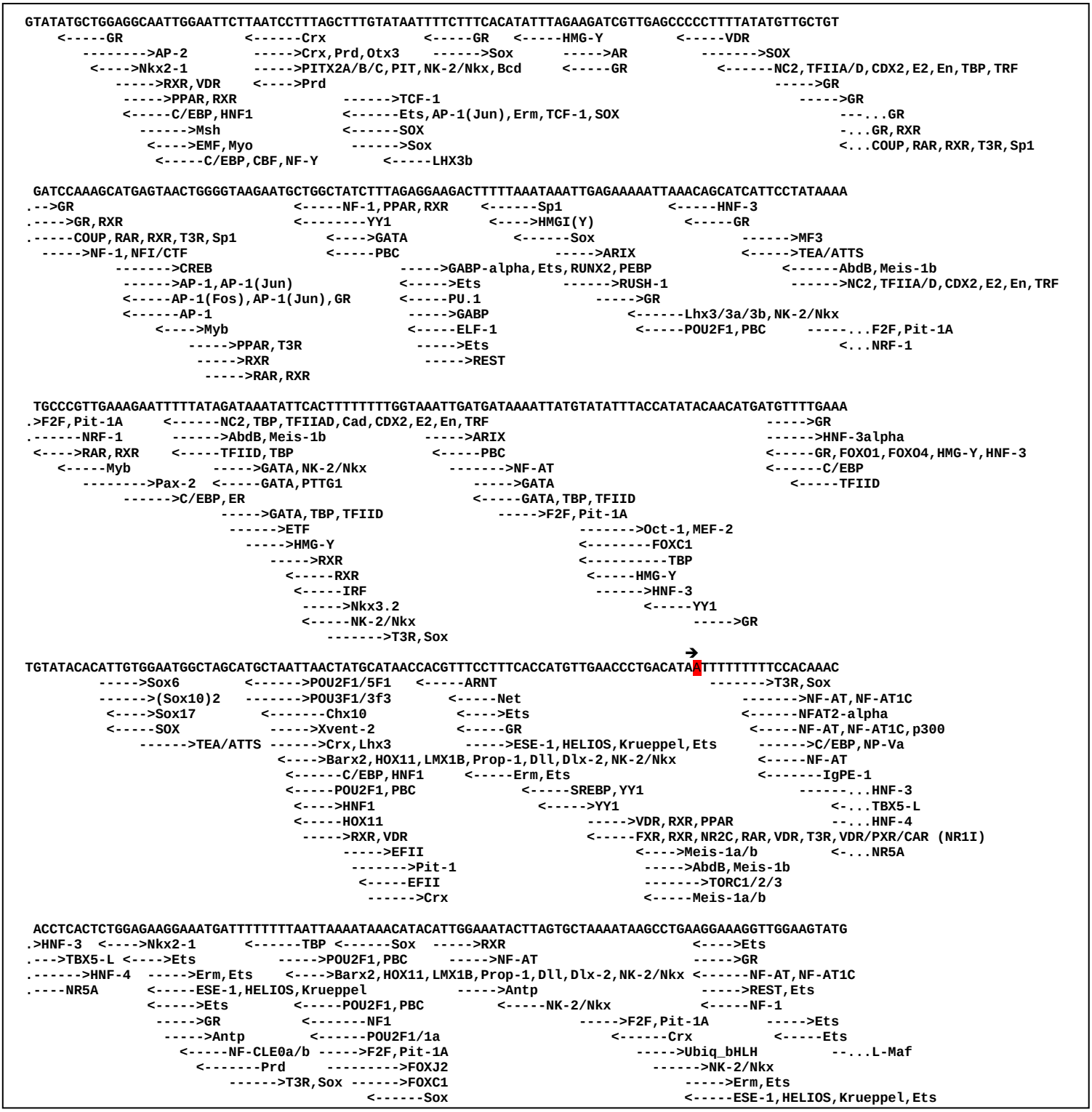

Figure S6. Oligonucleotides used for cloning of putative IRES element

The *C* and *T* allelic variants of putative IRES were cloned into a NF-kB-SEAP reporter vector (Takara Bio) between transcription and translation start sites of SEAP reporter. The IRES sequence oligonucleotides (direct and reverse complement strands) were ordered from ITD. The oligonucleotides were re-constituted in 150mM NaCl solution to yield a finial concentration of 200ng/μL. The direct and reverse complement strands were mixed and allowed to anneal in a thermocycler at a decreasing temperature gradient with 10° C decrements from 90° C to 30° C, the length of each decrement was 2 min. The annealed ds-oligos were then diluted to 10ng/ μL with 10mM Tris buffer. Then 6ng of the double stranded oligonucleotide were mixed with 100 ng of linearized pNF-kB-SEAP vector and ligated with T4 DNA Ligase (New England Biolabs, Ipswich, MA) into Hind III and Nru I restriction sites at 16° C overnight. The ligated constructs were transformed into Mach-10 cells (Invitrogen) following the manufacturer specifications. The resulted clones were extracted using Qiagen Maxi Kit and confirmed by sequencing in UNC sequencing facility. The sequences of the cloned oligonucleodies were as follows:

1 IRS-SEAP-C Direct:
AGCTTTCTAGATGTCTTTAGATCATGCAGGTCTATAACCAACGGTGAATCTAGCAAAAGTTATTTTCT
CTTTTGGGGAGAAACTTCTTCTTATTAAAAAAATAAAAAATGTTTCAACTTCTTGAGCACCAAGGTC
AATTATTCTAATCG

2 IRS-SEAP-C REV/C:
CGATTAGAATAATTGACCTTGGTGCTCAAGAAGTTGAAACATTTTTTATTTTTTTAATAAGAAGAAG
TTTCTCCCCAAAAGAGAAAATAACTTTTGCTAGATTCACCGTTGGTTATAGACCTGCATGATCTAAA
GACATCTAGAA

3 IRS-SEAP-T Direct
AGCTTTCTAGATGTCTTTAGATCATGCAGGTCTATAACCAATGGTGAATCTAGCAAAAGTTATTTTCT
CTTTTGGGGAGAAACTTCTTCTTATTAAAAAAATAAAAAATGTTTCAACTTCTTGAGCACCAAGGTC
AATTATTCTAATCG

4 IRS-SEAP-T Rev/C
CGATTAGAATAATTGACCTTGGTGCTCAAGAAGTTGAAACATTTTTTATTTTTTTAATAAGAAGAAG
TTTCTCCCCAAAAGAGAAAATAACTTTTGCTAGATTCACCATTGGTTATAGACCTGCATGATCTAAA
GACATCTAGAA

Supplementary tables:

Table S1. Primer and probe sequences for 5' nuclease genotyping of *OPRM1* SNPs*

hCV number	rs number		primer sequences
809932	650245	Forward primer	GGCCACTGAGCTACAATGCA
		Reverse primer	TCATATGAAATAGTTACAAGCCTTTGCAAAC
		Allele 1 probe (FAM)	TAGAGCGTTAATTTT
		Allele 2 probe (VIC)	TCTAGAGCATTAATTTT
809931	497332	Forward primer	GGGTTTTCACTTTTGTTTTACTTTGTTTTCTG
		Reverse primer	TGTACCAATTATAATGCTTATTCTTAGCAATGTATATT
		Allele 1 probe (FAM)	ATACCCCAAAGGTACATAG
		Allele 2 probe (VIC)	TTTATACCCCAAAGCTACATAG
8950109	1319339	Forward primer	AGAAACATAGGCTAGGAAGCAGACT
		Reverse primer	GCACAAGTTTACAGAGTTAGTAGGAAGT
		Allele 1 probe (FAM)	AATTGAACATCAGTTGCG
		Allele 2 probe (VIC)	TGAACATCAATTGCG
27335972	677830	Forward primer	CCTTGGCCACTGAGCTACAAT
		Reverse primer	GAAATAGTTACAAGCCTTTGCAAACTCA
		Allele 1 probe (FAM)	TGGAGACTGCCCTGC
		Allele 2 probe (VIC)	AAATGGAGACTACCCTGC
3073593	623956	Forward primer	TGCTACCTCTCACATTCTATGTTTCTATAGT
		Reverse primer	AAAAACCAAGCATACTAGAAGTGTTCTCT
		Allele 1 probe (FAM)	AATACAGTAGTTACTAGAGAAA
		Allele 2 probe (VIC)	CAGTAGTTGCTAGAGAAA
3073616	1294094	Forward primer	CCAAACTTGCAGGATATTTTCAGCAAA
		Reverse primer	CTTCCCATTCCCTCATCTAAGCT
		Allele 1 probe (FAM)	CTGTCCTTTGATTATCT
		Allele 2 probe (VIC)	TCTGTCCTTTGTTTATCT
N/A	7776341	Forward primer	ACAGCACTGGGTCTTACTCAAG
		Reverse primer	CCCCAAGTCCTGTGTGAACATAG
		Allele 1 probe (FAM)	CAGGGAATGGTTATAG
		Allele 2 probe (VIC)	CCAGGGAATTGTTATAG
809981	1074287	Forward primer	GCCACGTTCTAGAGTCAAAAACCTTAG
		Reverse primer	AAGGACTGCATTTTGTGGTTTGAG
		Allele 1 probe (FAM)	TGTGGCTGAGGTAGTA
		Allele 2 probe (VIC)	CTGTGGCTGAAGTAGTA
8950105	1323040	Forward primer	CCTTGTTCTTGTGGTTGCTACATG
		Reverse primer	CAGGAAACATTTTGATGGGATGTAAGG
		Allele 1 probe (FAM)	TTCTCACAGAATTAC
		Allele 2 probe (VIC)	CCTTCTCATAGAATTAC
N/A	7775848	Forward primer	GTCCCTGGTGGCTTATTTAGTTCAT
		Reverse primer	ACTCTTCAATGCTCAAACACAGACA
		Allele 1 probe (FAM)	ATCCAAGAAAATACG
		Allele 2 probe (VIC)	ACAATCCAAGAATATACG
8950070	1042753	Forward primer	GGGTCAACTTGTCCCACTTAGATG
		Reverse primer	GGGCACAGGCTGTCTCT
		Allele 1 probe (FAM)	CCGCACCGACCTG
		Allele 2 probe (VIC)	CCGCACCAACCTG
8950075	1799972	Forward primer	GGCCGTCAGTACCATGGA
		Reverse primer	CAAGGCATCAGTGCAATTGCT
		Allele 1 probe (FAM)	AGCGCTGCCCCCA
		Allele 2 probe (VIC)	CAGCGCTGTCCCCA
32237197	12205732	Forward primer	GAGAAGACTGTCATCCTGTAGGGTA
		Reverse primer	CCTTGGAGTCAATCATTTTATAAATCAGCTTT
		Allele 1 probe (FAM)	ACATGTCCAAACTC
		Allele 2 probe (VIC)	AAGTAACATATCCAAACTC
32404373	3798678	Forward primer	GTGAACTTATGAAAAAGGTAGTTGAGCAA
		Reverse primer	TGTGTGTGTGTGTGTGTGTGT
		Allele 1 probe (FAM)	ATGTGGAATTGAACACACA
		Allele 2 probe (VIC)	ATGATGTGGAATTAAACACACA

* Primes and probes information for other markers (SNPs rs ## 540825, 533586, 6912029, 9322453, 9322446, 7759388, 660756, 1799971, 524731, 495491, 675026, 1381376, 563649, 2075572, 609148, 648893) is not available; assays were purchased as ABI Assays-On-Demand (ABI, Foster City, CA).

Table S2. Morphine-Induced Changes in Pain Ratings as a Function of *OPRM1* rs563649 Genotype

	2 Major Alleles	1 or 2 Minor Alleles	p-value	Effect Size
Summed Z-score	-0.95 (0.52) ***	0.63 (1.19) ***	0.22	0.32
Ischemic Pain Threshold Change Score*	72.6 (167.9)***	-21.2 (97.5)****	0.056	0.61
Pain Intensity Change Score**	35.45 (47.25) ***	9.24 (47.95) ***	0.08	0.55
Pain Unpleasantness Change Score**	36.05 (47.91) ***	10.54 (48.49) ***	0.09	0.53

* ischemic threshold is presented as change from pre-drug values (sec) and higher values indicate greater analgesia. **These change scores were computed as follows. Each participant underwent the submaximal effort tourniquet procedure twice, once before and once after administration of morphine. During each tourniquet procedure, ratings of pain intensity and unpleasantness were obtained at one-minute intervals. These ratings were summed across the entire tourniquet procedure, and change scores were derived by subtracting post-drug summed scores from pre-drug summed scores, such that larger values indicate greater analgesia. ***Presented as mean (standard deviation)

Table S3. Human RNA samples (Takara Bio)
Except for the total brain and frontal lobe samples, each batch consists of pooled RNA samples from a number of individuals.

Clontech #	total RNA
636530	human total brain
636562	human medulla oblongata (MO)
636563	human frontal lobe (FL)
636569	human nucleus accumbens (NA)
636572	human pons (P)
636580	human blood peripheral leucocytes
636554	human spinal cord (SC)
636616	mouse spinal cord

Table S4. RT-PCR amplification primers

exon	Primers*	Tm	primer sequence
13	mU2	56	CCTGGCGGTGAGCTGATAAAGACTGAG
13	mU3	56	GGTATGCCTTTGAACACTGGTC
2	mL1	65	ACCGGGTGGCAGACGGCAATGTA
2	mL3	62	ATTACGGGCAGACCAATGGCAGAAGA
1	h1U-1	69	GTGCCCGCCCGGCCGTCAGTA
13	hU-5	64	ATGGCCCCGGTCACTAAACGTCTTCAC
4	hL-3	63	CCACTGGGTGTCTTGCACAAACTGGAC
13	hU2	58	TAAGCCTGAAGGAAAGGTTGGAAGTAT
2	hL5	63	AAGGGCAGGGTACTGGTGGCTAAG
13	h13U-1	61	CCATGGCCCCGGTCACTAAAC
13	h13U-2	60	CTAAACAAAGGGCCTCCAACCAACAG
13	h13U-3	61	AAAGCCCCCAAGTCTGAAAATGTGGAG
5	h5L-1	61	CTGGCATGCTGGCAAAAGGGTAAC
5	h5L-2	57	TTATCACCGAGGTTATCAAGCATTCC
5	h5L-3	56	AATCCCCATGACTTATTTCTTATGTGC
5	h5L-4	63	CTTGGCTGGAGGTCCCTTGATAACTGC
0/7	h0/7L-1	63	TGTAAAGATCTGGGCCATGCTGACTGC
0/7	h0/7L-2	57	AAGTTGCCTTTCACTCTGTCACAT

* m - primers for mouse mRNAs, h - primers for human mRNAs, U-upstream PCR primer, L-downstream PCR primer

PCR amplification conditions were:

1). mouse exons 13 to 2: mU2-mL3, mU2-mL1 and mU3-mL3: 94ºC 5 min (94ºC 30 sec, 63ºC 30 sec, 72ºC 3 min) 35 cycles, 72ºC 15 min.

2). human exons 1 to 4 and 13 to 4: h1U1-hL3 and hU5-hL3: 94ºC 5 min (94ºC 30 sec, 63ºC 30 sec, 72ºC 3 min) 35 and 38 cycles respectively, 72ºC 15 min.

3). human exons 13 to 2: hU2-hL5: 94ºC 5 min (94ºC 30 sec, 57ºC 30 sec, 72ºC 30 sec) 30 cycles, 72ºC 15 min.

4) human exons 13 to 5: h13U1-h5L1 or h5L2 or h5L3: 94ºC 5 min (94ºC 30 sec, 61 or 57 or 56ºC 30 sec, 72ºC 2 min) 30 cycles, 72ºC 15 min. h13U2-h5L1: 94ºC 5 min (94ºC 30 sec, 60ºC 30 sec, 72ºC 2 min) 30 cycles, 72ºC 15 min. h13U3-h5L1 or h5L4: 94ºC 5 min (94ºC 30 sec, 61ºC 30 sec, 72ºC 2 min) 30 cycles, 72ºC 15 min. h13U5-h5L1: 94ºC 5 min (94ºC 30 sec, 61ºC 30 sec, 72ºC 2 min) 30 cycles, 72ºC 15 min. Secondary PCR was performed using same primers and conditions for an additional 20 cycles.

5) human exons 13 to O/7: h13U1-hO/7L2 and h13U5-O/7L2: 94ºC 5 min (94ºC 30 sec, 57ºC 30 sec, 72ºC 2 min) 30 cycles, 72ºC 15 min. h13U1-hO/7L1 and h13U3-hO/7L1: 94ºC 5 min (94ºC 30 sec, 61ºC 30 sec, 72ºC 2 min) 30 cycles, 72ºC 15 min. h13U2-hO/7L1: 94ºC 5 min (94ºC 30 sec, 60ºC 30 sec, 72ºC 2 min) 30 cycles, 72ºC 15 min. Secondary PCR was performed using same primers and conditions for an additional 20 cycles.

Table S5. Tissue-specific distribution of MOR-1K1 and MOR-1K2 isoforms

Tissue	Clones sequenced	Corresponded to *MOR-1K1*	Corresponded to *MOR-1K2*
medulla oblongata	21	21	0
spinal cord	22	0	22
frontal lobe	23	7	23
nucleus accumbens	21	4	17

Expression of putative IRES element.

Human neuroblastoma BE2C cells (ATCC, Manassas, VA) were grown to 90% confluency in 12-well plates and transiently transfected using Lipofectamine-2000 (Invitrogen) with mixture of either 0.3µg of pNF-kB-SEAP-IRES(C/T) constructs or original pNF-kB-SEAP reporter vector and 0.03µg of pCMC-Luc (CLONTECH) vector per well. The levels of SEAP mRNA and enzymatic activity were determined at 8, 24 and 48 hours after transfection, by real-time PCR using SYBRGreen PCR kit (ABI) and by luminometry using a Great Escape kit (Takara Bio). Data were normalized for transfection efficiency by measuring the mRNA levels of Luc for each experimental point, measured by Real-Time PCR using SYBRGreen PCR kit (ABI) . Total RNA was isolated using the RNeasy Mini kit (QIAGEN). The isolated RNA was treated with TURBO DNA-free kit (Ambion, Austin, TX) and reverse transcribed by SuperScript III reverse transcriptase (Invitrogen). The cDNA for SEAP and Luc was amplified using forward and reverse PCR primers (AGAGATACGCCCTGGTTCCT and CCAACAC-CGGCATAAAGAAT, respectively, for Luc and GCCGACCACTCCCACGTCTT and CCCGCTCTCGCTCTCGGTAA, respectively, for SEAP). ABI 7900 Real Time Fluorescence Detection System (ABI) was used for measuring fluorescence.

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