Supplemental Material

Tetrapeptide endomorphin analogs require both full length and truncated splice variants of the mu opioid receptor gene *Oprm1* for analgesia

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Supplemental Table 1: Summary of knockout models and endomorphin analog analgesia

	Knockout model			
Expression	**E1[a]**	**E11[b]**	**E1/E11[c]**	**Triple[d]**
MOR-1 Variants				
7TM	Lost	Retained	Lost	Lost
6TM	Retained	Lost	Lost	Retained
1TM	Lost	Retained	Lost	Lost
DOR-1	Retained	Retained	Retained	Lost
KOR-1	Retained	Retained	Retained	Lost
Analgesia				
Morphine	No	Yes	No	No
DAMGO	No	Yes	No	
IBNtxA	Yes	No	No	Yes
DAPP		No	No	
IDAPP		No	No	
Endomorphin 1	No	No	No	
Endomorphin 2	No	No	No	
6TM rescue				
IBNtxA		Yes	Yes	
DAMGO			No	
DAPP		Yes	No	
IDAPP		Yes	No	

[a] (*1*); [b] (*2*); [c] (*3*); [d](*4*)

Knockout models of Oprm1 have been described targeting exon 1 (E1), exon 11 (E11), both exon 1 and exon 11 (E1/E11) that selectively eliminate selected classes of splice variants, as indicated. Analgesia is affected differently for various compounds in these models. Morphine and IBNtxA are from the literature (*1, 2, 5*). The peptides are summarized from this report.

Supplemental Table 2: Oligodeoxynucleotide antisense targeting sequences

Target	Antisense Sequence	Mismatch Control Sequence
E1	CGCCCCAGCCTCTTCCTCT	CGCCCCGACCTCTTCCCTT
E1/2	CATTTTGGTATATCTTACAATCAC	**A)** TTGATTCTAATTTGCTTACAATCAC **B)** CATTTTGGTATATATCCTTACAAC
E11	GACAGTCACTGGTGCCTATGCAATG	GACGATCACGTGTGCTCATGACATG

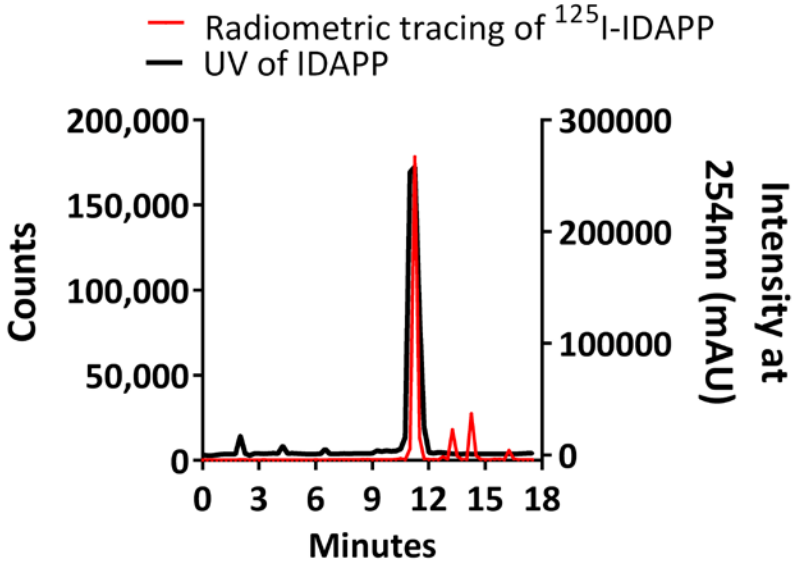
Antisense (AS) and mismatch (MIS) oligodeoxynucleotides were designed based upon the published sequence of the mouse mu opioid receptor gene, *Oprm1.* Mismatch for the E1 probe switched the order of two sets of two bases, maintaining a common base composition. Mismatch for the E11 probe switched the order of four sets of two bases, maintaining a common base composition. E1/2 AS was designed to target the splice site between exons 1 and 2, with half the probe annealing to exon 1 and the other to exon 2. Annealing to only exon 1 or 2 would not be of sufficient T_m to be active. Two mismatch probes were designed for the E1/2 AS. One probe (A) scrambled the 5' end, which targeted exon 1, while the other MS (B) scrambled the 3' end, which targeted exon 2. They were designed to anneal to only exon 2 (A) or exon 1 (B).

Supplemental Table 3: Summary of Antisense mapping on analgesia

	Antisense		
	E1	**E1/2 junction**	**E11**
MOR-1 Variants			
7TM	Lowered	Lowered	Unchanged
6TM	Unchanged	Unchanged	Lowered
1TM	Lowered	Unchanged	Unchanged
Analgesia			
Morphine	Lowered	Lowered	Unchanged
DAMGO	Lowered	Lowered	Unchanged
IDAPP	Lowered	Lowered	Lowered

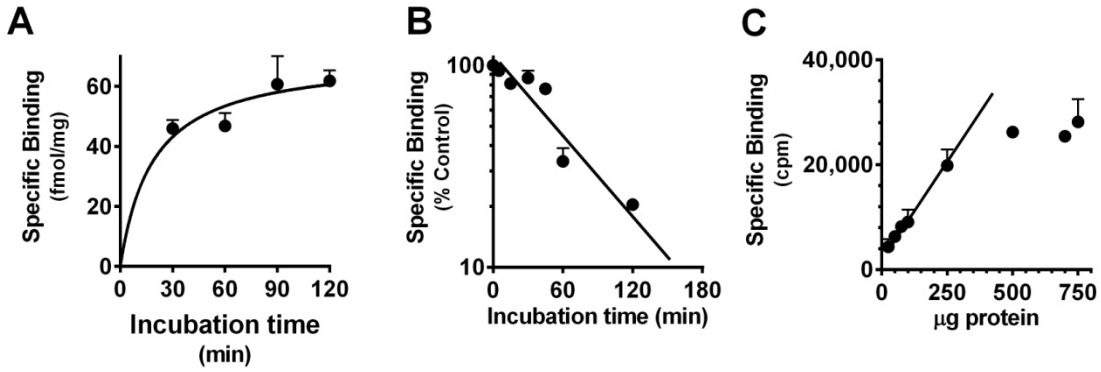
Antisense mapping is able to selectively downregulate selected sets of splice variants of the *Oprm1* receptor by targeting individual exons within the gene (*6-10*). The E1/2 junction antisense targets the junction between exon 1 and 2, which is unique to the 7TM receptors and selectively downregulates them (*10*). The analgesia results for morphine are from the literature and the peptides are summarized from this report.

Supplemental Figure 1: Reverse phase HPLC purification of ^{125}I-IDAPP



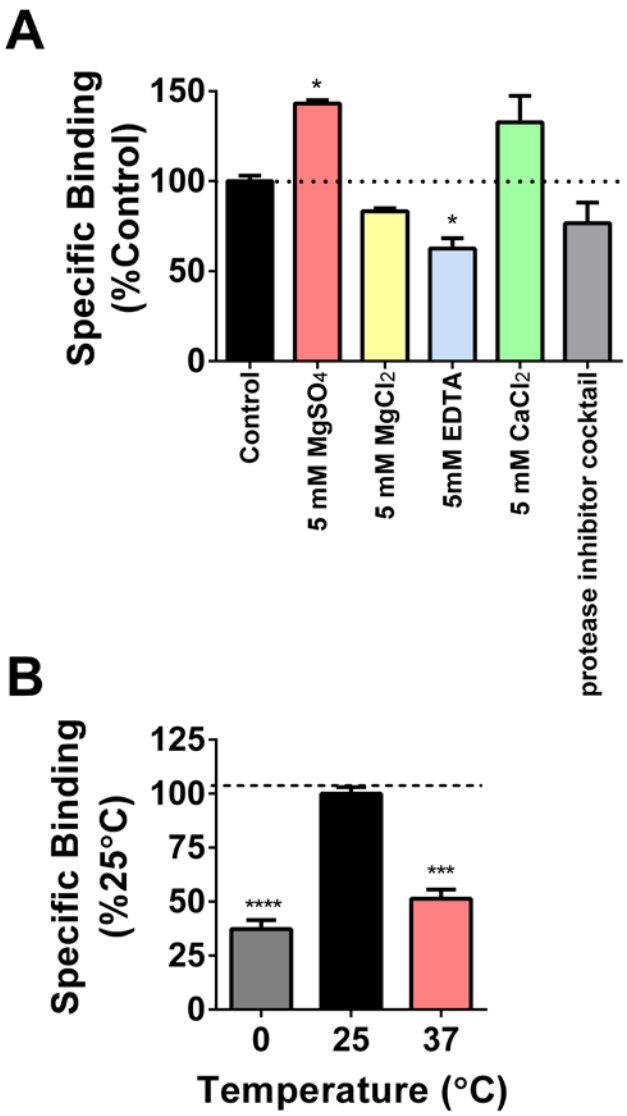
^{125}I-IDAPP was purified following iodination by HPLC as described in Methods. Comparisons of the radiometric tracing following radioiodination of ^{125}I-IDAPP (red) and the UV tracing of IDAPP run as a standard (black) indicates that the major radioactive product isolated during the radioiodination has the same retention time as the cold peptide.

Supplemental Figure 2: ^{125}I-IDAPP binding parameters



^{125}I-IDAPP binding (0.1 nM) was carried out in mouse brain and specific binding (the difference between total and 8 µM levallorphan) shown **(a)** Association of ^{125}I-IDAPP. The radioligand quickly reaches a saturated binding level, plateauing after 90 min. Association data was fit with a one site saturation curve (GraphPad Prism). **(b)** Dissociation of ^{125}I-IDAPP. The radioligand was incubated with mouse brain tissue for 90 minutes (time 0) at which time levallorphan (8 µM) was added. Linear regression of the log(binding) vs time yields a $t_{1/2}$ of 56 min. (**c)** Tissue linearity of ^{125}I-IDAPP. Tissue was incubated for 90 minutes. Binding was linear with tissue concentrations up to 250 µg.

Supplemental Figure 3: ^{125}I-IDAPP binding assay with different conditions



Binding was performed at 0.1 nM in mouse brain. **(a)** Different conditions were examined to determine their influence on specific binding. A one-way ANOVA ($F_{5,12}$=15.64, p<0.0001) with Bonferroni's post hoc analysis indicated that magnesium sulfate significantly increased binding whereas EDTA significantly decreased binding, *p<0.05. **(b)** Binding following incubation at different temperatures. A one-way ANOVA ($F_{2,6}$=73.88, p<0.0001) with Bonferroni's post hoc analysis indicated binding at 25 degrees differed from the other temperatures. ***p<0.001, ****p<0.0001

Supplemental Figure 4: Statistical analysis of ^{35}S-GTPγS stimulation in brain

	$F_{(2,6)}$	P Value	Bonferroni post hoc analysis		
			WT vs E11	E11 vs E1/E11	WT vs E1/E11
DAMGO	13.2	0.006	0.999	0.009	0.021
IDAPP	28.4	0.0009	0.047	0.015	0.0009
EM1	106.3	0.0001	0.006	0.0001	0.0003
DM2	150.5	0.0001	0.005	0.0001	0.0001

Anova with Bonferroni posthoc analysis of ^{35}S-GTPγS stimulation from Table 4.

References

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