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Supplementary Information for

Biased signaling by endogenous opioid peptides

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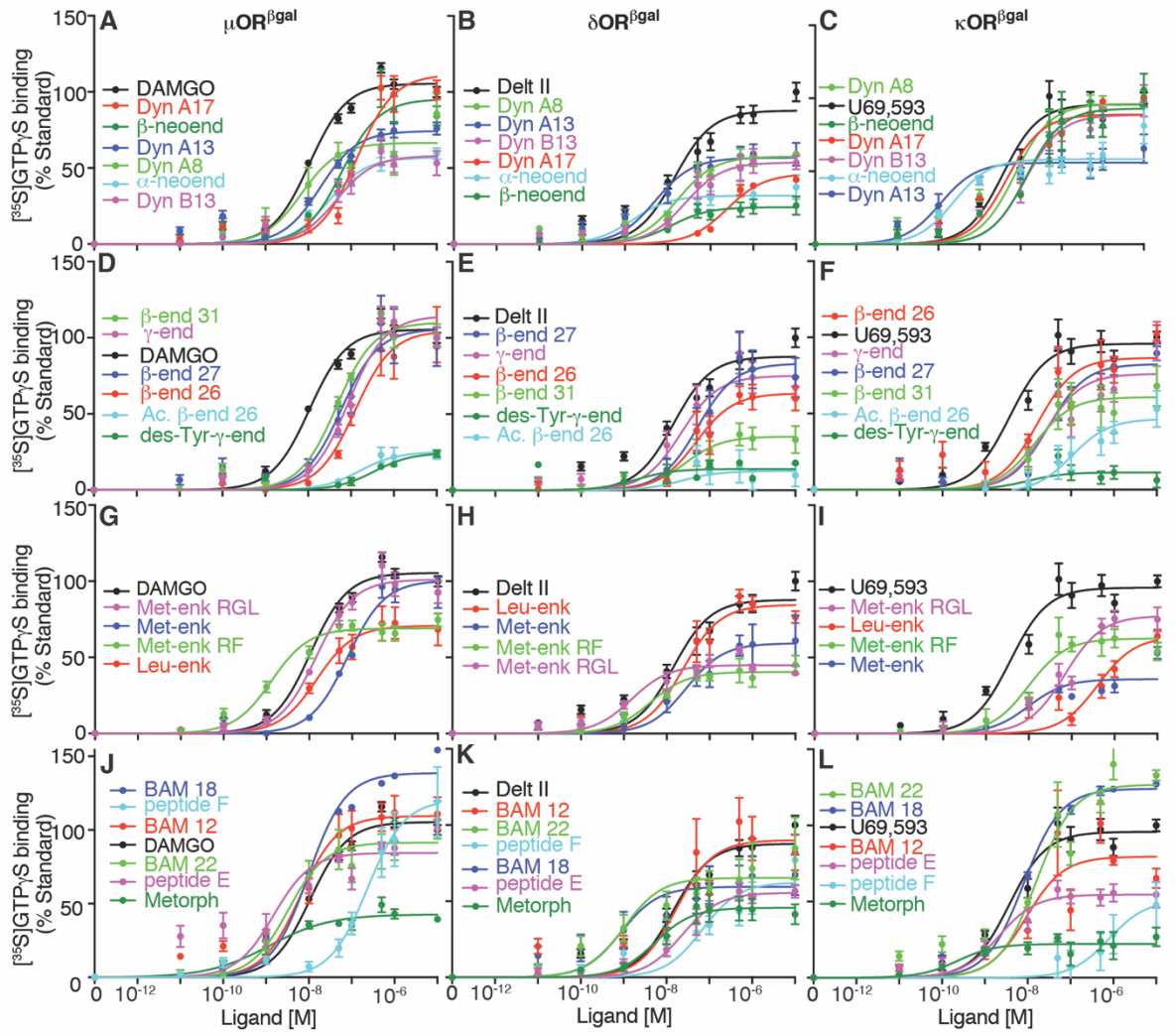

Fig.S1 [^{35}S]GTPγS binding at μOR, δOR and κOR by endogenous opioid peptides.
[^{35}S]GTPγS binding by opioid peptides generated from pro-dynorphin (**A-C**), POMC (**D-F**) or pro-enkephalin (**G-L**) processing. Membranes (20 μg) from cells expressing either $\mu^{\beta gal}$OR, $\delta^{\beta gal}$OR or $\kappa^{\beta gal}$OR were treated without or with different concentrations of peptides (10^{-12} -10^{-5} M) as described in Methods. DAMGO was used as standard for μOR, deltorphin II for δOR and U69,593 for κOR. Data are mean ± SE from 3-6 independent experiments.

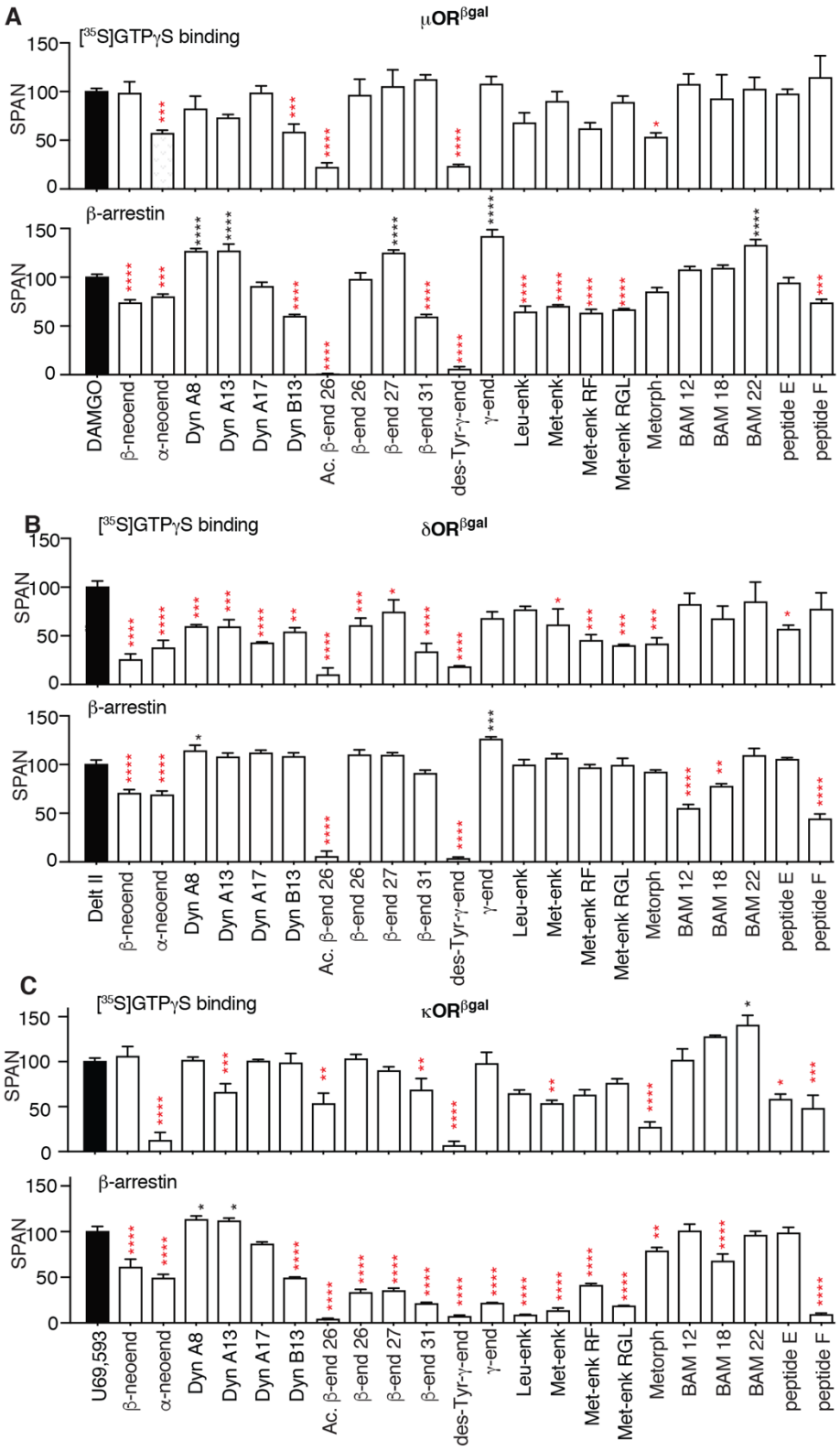

Fig. S2. Identification of opioid peptides that induce a maximal response. SPAN values (i.e. E_{max}) obtained from fitting dose response curves in Figs S1 and S3 to the four parameter logistic equation in Prism 7.0 were analyzed using one-Way ANOVA followed by Dunnett's posthoc test to identify opioid peptides that induced maximal response at [^{35}S]GTPγS binding or β-arrestin recruitment at μOR (**A**), δOR (**B**) or κOR (**C**). *p>0.05; **p>0.01; ***p>0.001;****p>0.0001. * in black indicates better than that of standard. * in red indicates lower than that of standard.

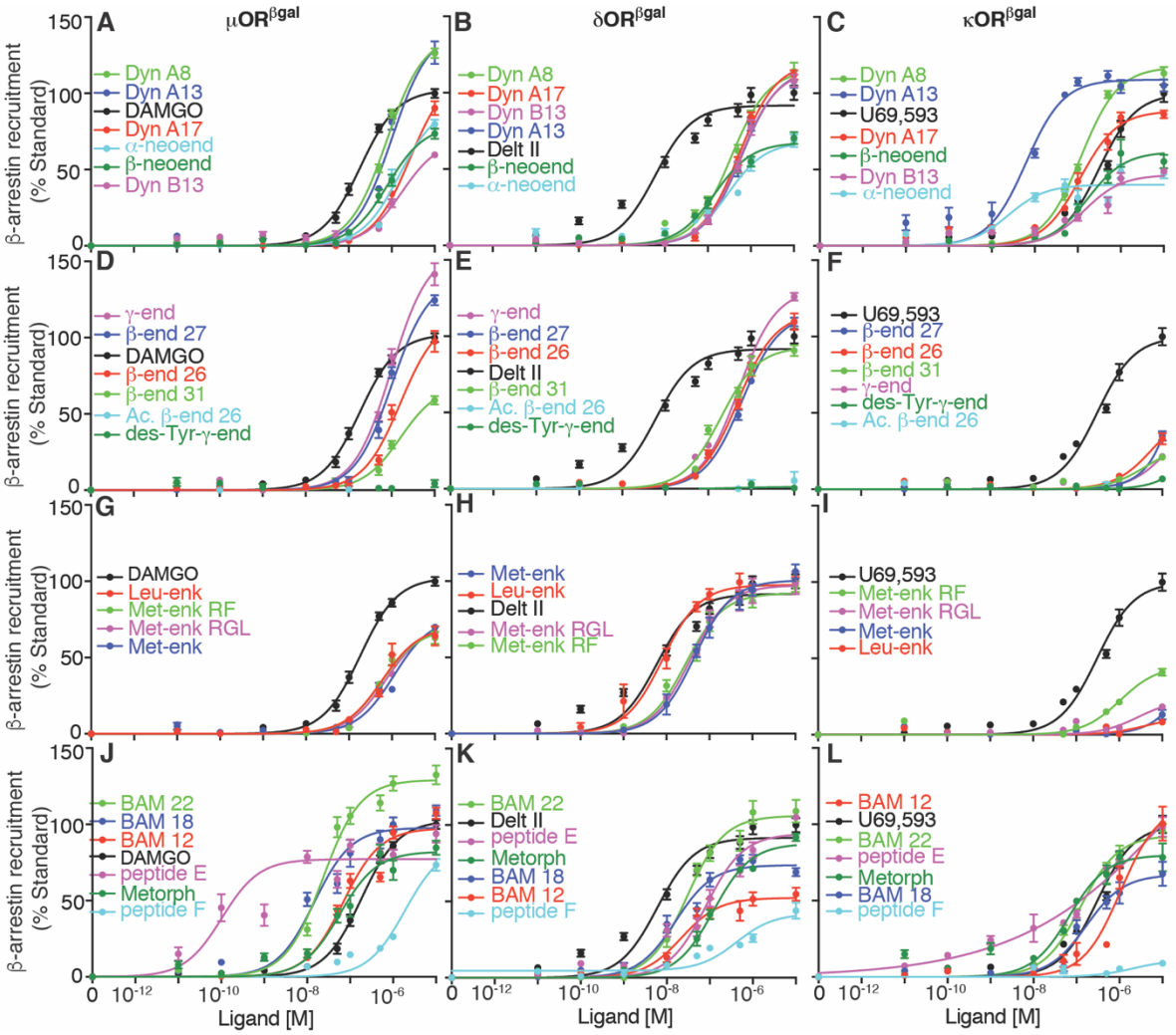

Fig.S3 β-arrestin recruitment at μOR, δOR and κOR by endogenous opioid peptides. β-arrestin recruitment by opioid peptides generated from pro-dynorphin (**A-C**), POMC (**D-F**) or pro-enkephalin (**G-L**) processing. Cells (5,000 cells/well) expressing either $\mu^{\beta gal}$OR, $\delta^{\beta gal}$OR or $\kappa^{\beta gal}$OR were treated without or with different concentrations of peptides (10^{-12} - 10^{-5} M) as described in Methods. DAMGO was used as standard for μOR, deltorphin II for δOR and U69,593 for κOR. Data are mean ± SE from 3 independent experiments.

Supplemental Table 1. Displacement binding parameters by endogenous opioid peptides at μOR, δOR or κOR

	μOR			δOR			κOR		
	IC_{50}[M]	n_H	% displaced at 10 μM	IC_{50}[M]	n_H	% displaced at 10 μM	IC_{50}[M]	n_H	% displaced at 10 μM
Standards									
DAMGO	8.4±1.4E-11; 1.4±1.2E-7	36.8%	100±3	3.4±2.0E-5	n.a.	28±1	3.5±1.6E-7	n.a.	36±6
DeltII	1.3±1.5E-6	n.a.	27±6	4.4±1.4E-10; 5.7±1.4E-7	50.4%	100±2	4.6±1.7E-8	n.a.	35±4
U69,593	7.9±1.6E-7	n.a.	19±4	3.5±3.0E-7	n.a.	22±8	8.9±2.1E-11; 5.7±1.5E-8	34.1%	100±2
Pro-dynorphin derived peptides									
β-neoend	3.0±2.1E-10; 6.6±1.2E-7	19.3%	89±4	1.0±1.5E-9; 3.3±1.4E-7	38.1%	77±3	1.8±1.3E-9; 1.3±1.5E-6	52.1%	100±2
α-neoend	2.2±1.7E-11; 1.6±1.2E-7	28.8%	73±4	5.0±2.1E-11; 2.4±1.3E-8	27.6%	91±1	2.2±1.6E-10; 5.5±1.3E-8	35.7%	95±2
Dyn A8	1.1±1.0E-11; 1.3±1.2E-7	24.4%	56±4	1.5±1.6E-10; 3.1±1.3E-7	31.4%	82±2	7.0±1.5E-11; 3.6±1.3E-7	38.7%	95±1
Dyn A13	8.0±1.4E-11; 9.6±1.4E-8	49.2%	69±4	7.1±1.4E-10; 1.2±1.4E-6	45.8%	87±2	3.6±2.8E-11; 5.4±1.0E-8	14.6%	84±2
Dyn A17	6.2±2.1E-10; 4.0±1.4E-7	28.2%	51±3	7.9±1.4E-11; 3.7±1.2E-7	30.4%	77±2	5.9±1.6E-11; 2.7±1.5E-7	42.5%	98±2
Dyn B13	3.4±1.9E-10; 2.0±1.4E-7	32.3%	58±3	4.8±1.4E-9; 8.3±1.4E-7	47.2%	86±2	1.9±1.4E-11; 3.1±1.0E-7	31.7%	96±1
Pro-opiomelanocortin derived peptides									
Ac.β-end 26	2.1±1.3E-7	n.a.	31±3	4.4±1.5E-11; 5.8±1.3E-7	37.3%	64±1	1.1±2.3E-10; 4.7±1.0E-7	19.1%	51±2
β-end 26	4.7±1.6E-10; 1.7±1.2E-6	23.9%	100±1	7.2±2.0E-11; 3.8±1.2E-8	23.5%	83±2	1.2±1.4E-8; 5.3±2.4E-6	39.3%	91±3
β-end 27	1.1±2.0E-10; 7.0±1.2E-7	19.4%	100±1	1.0±1.8E-10; 1.3±1.3E-7	29.3%	87±1	7.0±3.8E-11; 2.0±1.3E-7	17.1%.	38±2
β-end 31	2.8±1.7E-11; 3.6±1.1E-7	16.4%	100±1	9.2±1.6E-11; 7.4±1.2E-8	29.5%	88±2	9.6±2.0E-11; 1.5±1.0E-7	23.0%	70±2
Des-Tyr-γ-end	4.5±1.3E-7	n.a.	30±2	3.0±1.5E-8	n.a.	59±2	3.0±1.6E-11; 7.6±1.4E-7	38.3%	58±2
γ-end	4.9±1.2E-10; 1.6±1.2E-6	48.4%	82±1	4.3±1.5E-11; 1.3±1.4E-7	42.1%	77±2	1.9±1.5E-10; 6.2±1.3E-8	44.2%	86±2
Pro-enkephalin derived peptides									
Leu-enk	3.1±1.8E-10; 1.3±1.4E-7	36.4%	77±4	1.3±1.9E-9; 4.7±1.4E-7	30.8%	57±2	1.0±2.3E-10; 7.1±1.5E-7	30.3%	53±3
Met-enk	1.6±3.0E-10; 8.8±1.3E-8	21.8%	77±3	2.1±1.5E-10; 9.8±1.2E-8	31.6%	56±2	2.2±1.5E-10; 6.7±1.3E-7	37.7%	65±2
Met-enk RF	1.3±1.4E-9; 7.3±1.8E-7	58.6%	95±1	2.1±2.3E-10; 6.9±1.2E-8	21.8%	74±1	4.5±1.9E-11; 1.1±1.4E-7	38.3%	62±1
Met-enk RGL	2.4±1.9E-9; 2.1±2.9E-6	52.8%	84±5	5.7±1.5E-10; 3.1±1.3E-8	39.3%	72±1	1.7±1.9E-11; 2.1±1.5E-7	28.0%	61±4
Metorphamide	2.5±1.5E-10; 4.6±2.0E-7	59.3%	85±3	8.9±1.1E-10; 6.8±1.3E-7	37.3%	81±2	1.1±1.3E-10; 3.0±1.2E-7	34.6%	78±1
BAM 12	6.3±2.3E-11; 6.1±2.0E-8	48.1%	83±8	6.8±1.0E-11; 8.9±1.0E-8	28.9%	81±3	8.1±1.6E-10; 4.8±1.4E-7	37.9%	85±2
BAM 18	9.2±1.7E-11; 2.1±1.4E-7	37.2%	79±5	9.1±1.3E-11; 2.4±1.4E-7	49.1%	71±2	6.2±1.4E-10; 7.7±1.5E-7	45.9%	100±2
BAM 22	2.1±1.6E-10; 1.4±1.8E-7	55.9%	93±4	1.2±1.3E-9; 3.0±1.4E-6	43.4%	94±1	4.1±1.4E-11; 9.4±1.2E-8	40.4%	100±1
Peptide E	9.3±1.3E-10; 8.8±1.4E-7	53.0%	97±2	3.6±1.4E-10; 1.9±1.3E-7	41.8%	53±1	5.6±2.1E-11; 5.4±1.2E-8	21.0%	97±2
Peptide F	8.0±1.8E-10; 6.6±1.6E-7	43.1%	92±2	2.2±1.9E-10; 9.0±1.6E-7	38.1%	54±4	8.8±1.5E-10; 3.2±1.3E-7	32.2%	96±2

Displacement binding assays were carried out with [^{3}H] diprenorphine (3 nM) using membranes (20 μg) expressing either $\mu^{\beta gal}$OR, $\delta^{\beta gal}$OR or $\kappa^{\beta gal}$OR and without or with different concentrations (10^{-12} - 10^{-5} M) of opioid peptides in 50 mM Tris-Cl buffer containing 100 mM NaCl, 10 mM $MgCl_2$, 0.2 mM EGTA and protease inhibitor cocktail as described in Methods. Data are mean ± SE (n=3). β-neoend, β-neoendorphin; α-neoend, α-neoendorphin; Ac., Acetyl; BAM, Bovine adrenal medulla; Dyn, Dynorphin; end, endorphin; enk, enkephalin; n_H, % receptors in high affinity state; n.a., not applicable.

Supplemental Table 2. Four parameter logistic analysis for [^{35}S]GTPγS binding and β-arrestin recruitment by endogenous opioid peptides at μOR

Ligand	Sequence	[^{35}S]GTPγS binding			β-arrestin recruitment		
		EC_{50} [M]	Emax at 10 μM	Hill Slope	EC_{50} [M]	Emax at 10 μM	Hill Slope
Standard							
DAMGO		9.0±1.1E-9	100±3	0.87±0.08	1.7±1.1E-7	100±3	1.08±0.06
Pro-dynorphin derived peptides							
β-neoend	YGGFLRKYP	5.1±1.2E-8	81±6	1.35±0.33	6.6±1.1E-7	74±3	1.14±0.12
α-neoend	YGGFLRKYPK	3.1±1.2E-8	57±3	0.69±0.08	9.1±1.0E-7	80±3	2.60±0.32
Dyn A8	YGGFLRRI	2.5±1.7E-8	82±13	0.40±0.09	6.2±1.0E-7	126±3	1.58±0.10
Dyn A13	YGGFLRRIRPKLK	1.7±1.2E-8	73±4	0.68±0.08	7.6±1.0E-7	127±7	1.93±0.21
Dyn A17	YGGFLRRIRPKLKWDNQ	1.1±1.1E-7	97±6	2.06±0.50	1.3±1.1E-6	90±5	1.82±0.18
Dyn B13	YGGFLRRQFKVVT	4.2±1.3E-8	51±8	2.03±0.93	1.1±1.1E-6	58±2	1.42±0.26
Pro-opiomelanocortin derived peptides							
Ac.β-end 26	Ac-YGGFMTSEKSQTPLVTLFKNAIIKNA	1.3±1.3E-7	22±2	1.77±0.76	n.d.	n.d.	n.d.
β-end 26	YGGFMTSEKSQTPLVTLFKNAIIKNA	1.1±1.2E-7	96±17	1.28±0.32	9.6±1.1E-7	98±7	2.08±0.32
β-end 27	YGGFMTSEKSQTPLVTLFKNAIIKNAY	5.0±1.3E-8	90±12	1.38±0.51	7.6±1.0E-7	125±3	1.68±0.16
β-end 31	YGGFMTSEKSQTPLVTLFKNAIIKNAYKKGE	4.2±1.1E-8	99±7	1.43±0.22	9.9±1.1E-7	59±3	1.86±0.35
Des-Tyr-γ-end	GGFMTSEKSQTPLVTL	3.3±1.3E-7	23±2	1.01±0.23	n.d.	4±2	n.d.
γ-end	YGGFMTSEKSQTPLVTL	8.5±1.1E-8	96±6	1.37±0.23	7.4±1.1E-7	142±7	1.60±0.15
Pro-enkephalin derived peptides							
Leu-enk	YGGFL	1.2±1.4E-8	68±11	0.95±0.27	5.2±1.1E-7	64±6	1.69±0.33
Met-enk	YGGFM	6.8±1.1E-8	90±10	1.36±0.27	9.1±1.1E-7	70±2	0.98±0.08
Met-enk RF	YGGFMRF	1.6±1.3E-9	62±6	0.95±0.22	4.9±1.1E-7	63±4	1.76±0.28
Met-enk RGL	YGGFMRGL	1.2±1.2E-8	89±7	1.24±0.23	6.2±1.1E-7	66±1	1.13±0.08
Metorphamide	YGGFMRRV-NH_2	1.7±1.5E-9	43±2	0.38±0.05	5.9±1.2E-8	85±5	0.76±0.10
BAM 12	YGGFMRRVGRPF	4.5±1.4E-9	106±11	0.59±0.10	1.0±1.1E-7	107±4	0.70±0.05
BAM 18	YGGFMRRVGRPFWWMDYQ	1.1±1.7E-8	92±25	0.74±0.24	2.0±1.2E-8	109±3	0.55±0.05
BAM 22	YGGFMRRVGRPFWWMDYQKRYG	7.4±1.5E-9	102±12	0.51±0.09	2.5±1.1E-8	132±6	1.11±0.10
Peptide E	YGGFMRRVGRPFWWMDYQKRYGGFL	2.1±1.5E-9	97±5	0.29±0.03	5.9±1.5E-10	94±6	0.32±0.04
Peptide F	YGGFMKKMDELYPLEVEEEANGGEVLGK RYGGFM	2.3±1.3E-7	114±23	0.98±0.21	1.3±1.2E-6	74±4	0.99±0.17

Analysis of data for [^{35}S]GTPγS binding and β-arrestin recruitment by endogenous opioid peptides at μOR (Supl. Fig.1. and 3) fitted to the four parameter logistic equation in Prism 7.0.

β-neoend, β-neoendorphin; α-neoend, α-neoendorphin; Ac., Acetyl; BAM, Bovine adrenal medulla; Dyn, Dynorphin; end, endorphin; enk, enkephalin. n.d., not detectable.

Supplemental Table 3. Four parameter logistic analysis for [^{35}S]GTPγS binding and β-arrestin recruitment by endogenous opioid peptides at δOR

Ligand	Sequence	[^{35}S]GTPγS binding			β-arrestin recruitment		
		EC_{50} [M]	Emax at 10 μM	Hill Slope	EC_{50} [M]	Emax at 10 μM	Hill Slope
Standard							
Delt II		1.7±1.3E-8	100±6	0.44±0.04	6.2±1.2E-9	100±5	0.49±0.03
Pro-dynorphin derived peptides							
β-neoend	YGGFLRKYP	1.1±1.7E-8	25±6	0.63±0.19	1.6±1.1E-7	70±4	0.78±0.06
α-neoend	YGGFLRKYPK	3.3±1.9E-9	37±8	0.38±0.08	2.9±1.1E-7	69±4	0.71±0.07
Dyn A8	YGGFLRRI	1.4±1.3E-8	59±2	0.65±0.09	3.3±1.1E-7	114±6	0.82±0.05
Dyn A13	YGGFLRRIRPKLK	4.2±1.5E-9	59±8	0.55±0.18	5.1±1.1E-7	108±4	1.30±0.09
Dyn A17	YGGFLRRIRPKLKWDNQ	1.9±1.1E-7	42±1	1.58±0.22	4.6±1.0E-7	112±3	1.29±0.07
Dyn B13	YGGFLRRQFKVVT	2.4±1.4E-8	54±5	0.75±0.18	4.8±1.1E-7	107±4	1.14±0.08
Pro-opiomelanocortin derived peptides							
Ac.β-end 26	Ac-YGGFMTSEKSQTPLVTLFKNAIIKNA	n.d.	10±7	n.d.	n.d.	5±6	n.d.
β-end 26	YGGFMTSEKSQTPLVTLFKNAIIKNA	4.8±1.2E-8	60±8	1.15±0.26	4.4±1.1E-7	110±5	1.05±0.07
β-end 27	YGGFMTSEKSQTPLVTLFKNAIIKNAY	6.1±1.1E-8	74±2	1.08±0.16	5.4±1.1E-7	109±3	0.96±0.05
β-end 31	YGGFMTSEKSQTPLVTLFKNAIIKNAYKKGE	2.6±1.7E-8	33±9	0.77±0.29	1.8±1.1E-7	91±4	1.10±0.08
Des-Tyr-γ-end	GGFMTSEKSQTPLVTL	1.3±1.3E-9	18±1	0.18±0.07	n.d.	n.d.	n.d.
γ-end	YGGFMTSEKSQTPLVTL	4.0±1.3E-8	68±7	0.60±0.10	4.6±1.0E-7	126±3	0.90±0.04
Pro-enkephalin derived peptides							
Leu-enk	YGGFL	1.8±1.3E-8	76±4	1.21±0.29	7.6±1.2E-9	98±5	0.79±0.10
Met-enk	YGGFM	2.8±1.4E-8	61±6	0.72±0.17	5.3±1.1E-8	106±5	0.79±0.08
Met-enk RF	YGGFMRF	5.9±1.5E-9	45±6	0.58±0.11	3.1±1.1E-8	96±3	0.76±0.07
Met-enk RGL	YGGFMRGL	8.7±1.4E-10	40±1	0.86±0.24	3.7±1.1E-8	99±7	0.93±0.11
Metorphamide	YGGFMRRV-NH_2	6.3±1.5E-9	41±7	1.14±0.40	1.4±1.1E-7	92±2	0.81±0.05
BAM 12	YGGFMRRVGRPF	2.3±1.3E-8	82±2	0.59±0.10	2.5±1.2E-8	55±4	1.02±0.21
BAM 18	YGGFMRRVGRPFWWMDYQ	1.3±1.5E-9	67±3	0.37±0.05	1.7±1.2E-8	69±3	0.74±0.08
BAM 22	YGGFMRRVGRPFWWMDYQKRYG	6.0±1.6E-9	85±10	0.33±0.05	3.2±1.1E-8	109±7	0.93±0.11
Peptide E	YGGFMRRVGRPFWWMDYQKRYGGFL	2.1±1.3E-8	57±4	0.76±0.15	1.1±1.2E-7	105±2	0.54±0.04
Peptide F	YGGFMKKMDELYPLEVEEEANGGEVLGK RYGGFM	1.4±1.4E-7	77±6	0.41±0.07	2.6±1.3E-7	44±5	0.49±0.06

Analysis of data for [^{35}S]GTPγS binding binding and β-arrestin recruitment by endogenous opioid peptides at δOR (Supl.Fig.1 and 3) fitted to the four parameter logistic equation in Prism 7.0.

β-neoend, β-neoendorphin; α-neoend, α-neoendorphin; Ac., Acetyl; BAM, Bovine adrenal medulla; Dyn, Dynorphin; end, endorphin; enk, enkephalin. n.d., not detectable.

Supplemental Table 4. Four parameter logistic analysis for [^{35}S]GTPγS binding and β-arrestin recruitment by endogenous opioid peptides at κOR

Ligand	Sequence	[^{35}S]GTPγS binding			β-arrestin recruitment		
		EC_{50} [M]	Emax at 10 μM	Hill Slope	EC_{50} [M]	Emax at 10 μM	Hill Slope
Standard							
U69,593		3.8±1.3E-9	100±4	0.73±0.10	3.0±1.1E-7	100±6	0.80±0.06
Pro-dynorphin derived peptides							
β-neoend	YGGFLRKYP	1.8±1.5E-8	106±11	0.51±0.10	1.6±1.2E-7	55±4	1.27±0.20
α-neoend	YGGFLRKYPK	5.0±1.7E-9	81±12	0.27±0.04	5.8±1.5E-9	48±3	0.36±0.05
Dyn A8	YGGFLRRI	4.1±1.3E-9	101±4	0.45±0.05	1.1±1.1E-7	113±4	1.14±0.07
Dyn A13	YGGFLRRIRPKLK	3.3±1.7E-10	66±10	0.34±0.06	5.7±1.2E-9	105±4	0.77±0.08
Dyn A17	YGGFLRRIRPKLKWDNQ	4.6±1.3E-9	100±2	0.93±0.71	1.0±1.1E-7	86±3	0.97±0.10
Dyn B13	YGGFLRRQFKVVT	1.4±1.3E-8	98±11	0.50±0.06	1.4±1.2E-7	49±1	0.56±0.07
Pro-opiomelanocortin derived peptides							
Ac.β-end 26	Ac-YGGFMTSEKSQTPLVTLFKNAIIKNA	2.0±1.7E-7	53±11	0.65±0.23	n.d.	n.d.	n.d.
β-end 26	YGGFMTSEKSQTPLVTLFKNAIIKNA	2.6±1.6E-8	103±5	0.41±0.08	1.6±1.3E-6	33±4	2.13±0.95
β-end 27	YGGFMTSEKSQTPLVTLFKNAIIKNAY	3.9±1.4E-8	90±5	0.78±0.21	4.6±1.5E-6	35±3	2.46±1.14
β-end 31	YGGFMTSEKSQTPLVTLFKNAIIKNAYKKGE	2.0±1.6E-8	68±13	0.98±0.40	1.3±1.4E-6	21±2	2.64±1.96
Des-Tyr-γ-end	GGFMTSEKSQTPLVTL	n.d.	6±5	n.d.	n.d.	7±2	n.d.
γ-end	YGGFMTSEKSQTPLVTL	8.2±1.5E-8	98±13	0.48±0.10	2.0±1.9E-6	21±1	2.41±2.02
Pro-enkephalin derived peptides							
Leu-enk	YGGFL	3.6±1.4E-7	64±4	0.60±0.12	n.d.	8±1	n.d.
Met-enk	YGGFM	1.0±1.3E-7	53±4	0.38±0.05	2.2±3.4E-6	13±3	2.90±4.31
Met-enk RF	YGGFMRF	1.4±1.3E-8	54±6	1.47±0.46	8.3±1.1E-7	41±2	1.18±0.21
Met-enk RGL	YGGFMRGL	6.0±1.2E-8	75±8	0.85±0.15	1.5±1.3E-6	18±1	0.72±0.15
Metorphamide	YGGFMRRV-NH_2	9.3±1.5E-10	27±6	0.35±0.12	6.3±1.2E-8	78±9	0.89±0.14
BAM 12	YGGFMRRVGRPF	2.3±1.7E-8	65±6	0.49±0.12	8.0±1.1E-7	100±8	2.62±0.39
BAM 18	YGGFMRRVGRPFWWMDYQ	7.9±1.1E-9	127±2	0.76±0.04	1.7±1.2E-7	68±8	0.97±0.14
BAM 22	YGGFMRRVGRPFWWMDYQKRYG	1.7±1.3E-8	133±3	0.50±0.07	1.4±1.1E-7	96±5	0.84±0.07
Peptide E	YGGFMRRVGRPFWWMDYQKRYGGFL	2.3±1.5E-9	55±9	0.62±0.12	7.7±1.2E-8	98±6	0.40±0.04
Peptide F	YGGFMKKMDELYPLEVEEEANGGEVLGK RYGGFM	6.7±1.7E-7	48±15	0.89±0.45	8.4±1.4E-7	9±2	2.83±3.04

Analysis of data for [^{35}S]GTPγS binding binding and β-arrestin recruitment by endogenous opioid peptides at κOR (Supl Fig.1 and 3) fitted to the four parameter logistic equation in Prism 7.0.

β-neoend, β-neoendorphin; α-neoend, α-neoendorphin; Ac., Acetyl; BAM, Bovine adrenal medulla; Dyn, Dynorphin; end, endorphin; enk, enkephalin. n.d., not detectable.

Supplemental Table 5. cAMP inhibition by endogenous opioid peptides in CHO cells expressing Flag-tagged opioid receptors

Ligand	cAMP inhibition					
	CHO-Flag μOR		CHO-Flag δOR		CHO-Flag κOR	
	EC_{50} [M]	% inhibition at 10 μM	EC_{50} [M]	% inhibition at 10 μM	EC_{50} [M]	% inhibition at 10 μM
Standard						
DAMGO	6.8±1.3E-9	100±2				
Delt II			1.9±1.6E-8	100±6		
U69,593					4.9±1.3E-10	100±8
Pro-dynorphin derived peptides						
Dyn A8	4.8±1.2E-9	76±1**	1.4±1.6E-10	67±3****	5.7±1.2E-9	144±3***
Dyn A13	6.9±1.3E-9	88±1	2.0±1.4E-10	84±6*	8.8±1.2E-10	147±3****
Dyn A17	1.3±1.2E-9	95±11	9.2±1.6E-10	96±8	1.0±1.2E-9	145±10***
Dyn B13	7.5±1.5E-10	116±10	5.5±2.0E-10	54±7****	2.1±1.3E-9	99±4
Pro-opiomelanocortin derived peptides						
β-end 26	2.7±1.4E-8	75±3**	1.9±1.3E-8	66±4****	3.1±1.6E-9	73±10*
β-end 27	6.3±1.5E-9	70±1**	2.8±1.3E-8	59±3****	3.7±2.1E-9	69±6 **
β-end 31	7.8±1.4E-9	107±9	6.0±1.3E-9	70±3***	5.9±1.5E-9	92±12

Analysis of data for inhibition of cAMP levels in Fig.1, 2 and 3. Data is normalized to that of standards: DAMGO, Delt II, or U69,593. end, endorphin. *p<0.05; **p<0.01;***p<0.001; ****p<0.0001; One-way ANOVA

Supplemental Table 6. Bias Analysis for endogenous opioid peptides at μOR

`	GTP Log RA	β-arrestin Log RA	Δ log RA for GTP (DAMGO)	Δ log RA for β-arrestin (DAMGO)	Δ Δ log RA (GTP –barr)	Bias Factor (GTP – βarr)	Bias Factor (βarr-GTP)
Standard							
DAMGO	7.878±0.09942	6.566±0.05345	0.000±0.141	0.000±0.076	0.000±0.160	1	
Pro-dynorphin derived peptides							
β-neoend	7.147±0.1008	5.700±0.06622	-0.731±0.142	-0.866±0.085	0.135±0.165	1.365	
α-neoend	7.447±0.1760	5.674±0.07533	-0.431±0.202	-0.892±0.092	0.461±0.222	2.891	
Dyn A8	8.072±0.1706	6.281±0.05310	0.194±0.197	-0.285±0.075	0.479±0.211	3.013	
Dyn A13	7.562±0.1490	6.193±0.05301	-0.316±0.179	-0.373±0.075	0.057±0.194	1.140	
Dyn A17	6.838±0.09630	5.535±0.06391	-1.040±0.193	-1.031±0.083	-0.009±0.162		1.021
Dyn B13	7.314±0.1655	5.041±0.07307	-0.564±0.193	-1.525±0.091	0.962±0.213	9.141	
Pro-opiomelanocortin derived peptides							
β-end 26	6.938±0.09504	5.752±0.06070	-0.94±0.138	-0.814±0.081	-0.126±0.160		1.337
β-end 27	7.155±0.09187	6.180±0.05338	-0.723±0.135	-0.386±0.076	-0.337±0.155		2.173
β-end 31	7.306±0.08552	5.356±0.09037	-0.572±0.131	-1.210±0.105	0.638±0.168	4.345	
γ-end	7.066±0.08313	6.377±0.05131	-0.812±0.130	-0.189±0.074	-0.623±0.149		4.198
Pro-enkephalin derived peptides							
Leu-enk	7.827±0.1445	5.517±0.06727	-0.051±0.175	-1.049±0.086	0.998±0.195	9.954	
Met-enk	7.098±0.09254	5.339±0.07151	-0.780±0.136	-1.227±0.089	0.447±0.163	2.799	
Met-enk RF	8.851±0.1888	5.587±0.06224	0.973±0.213	-0.979±0.062	1.952±0.229	89.537	
Met-enk RGL	7.812±0.1040	5.412±0.06845	-0.066±0.144	-1.154±0.087	1.088±0.168	12.246	
Metorphamide	9.068±0.2590	6.551±0.05676	1.190±0.277	-0.015±0.078	1.205±0.288	16.032	
BAM 12	7.994±0.1082	6.896±0.05303	0.116±0.147	0.330±0.075	-0.214±0.165		1.637
BAM 18	7.927±0.1416	7.403±0.05919	0.049±0.173	0.837±0.080	-0.788±0.191		6.138
BAM 22	8.479±0.1400	7.848±0.05875	0.601±0.172	1.282±0.079	-0.681±0.189		4.797
Peptide E	8.128±0.1646	7.193±0.07454	0.250±0.192	0.627±0.092	-0.377±0.213		2.382
Peptide F	6.591±0.1147	5.579±0.07094	-1.287±0.152	-0.987±0.089	-0.300±0.176		1.995

Bias analysis was carried out as described in Methods. RA= τ/K_A

Supplemental Table 7. Bias Analysis for endogenous opioid peptides at δOR

	GTP Log RA*	β-arrestin Log RA*	Δ log RA for GTP (DAMGO)	Δ log RA for β-arrestin (DAMGO)	Δ Δ log RA (GTP –barr)	Bias Factor (GTP – βarr)	Bias Factor (βarr-GTP)
Standard							
Delt II	8.056±0.2085	7.890±0.05763	0.000±0.295	0.000±0.082	0.000±.306	1.00	
Pro-dynorphin derived peptides							
β-neoend	7.147±0.1008	5.700±0.06622	-0.731±0.142	-0.866±0.085	0.135±0.165	1.365	
α-neoend	7.447±0.1760	5.674±0.07533	-0.431±0.202	-0.892±0.092	0.461±0.222	2.891	
Dyn A8	8.072±0.1706	6.281±0.05310	0.194±0.197	-0.285±0.075	0.479±0.211	3.013	
Dyn A13	7.562±0.1490	6.193±0.05301	-0.316±0.179	-0.373±0.075	0.057±0.194	1.140	
Dyn A17	6.838±0.09630	5.535±0.06391	-1.040±0.193	-1.031±0.083	-0.009±0.162		1.021
Dyn B13	7.314±0.1655	5.041±0.07307	-0.564±0.193	-1.525±0.091	0.962±0.213	9.141	
Pro-opiomelanocortin derived peptides							
β-end 26	6.476±0.1871	6.387±0.04864	-1.580±0.280	-1.503±0.075	-0.077±0.290		1.194
β-end 27	7.106±0.1894	6.296±0.05637	-0.949±0.282	-1.594±0.081	0.645±0.293	4.416	
β-end 31	5.773±0.2159	6.699±0.04868	-2.283±0.300	-1.191±0.075	-1.092±0.309		12.359
γ-end	7.475±0.1836	6.782±0.04557	-0.581±0.278	-1.108±0.073	0.527±0.287	3.365	
Pro-enkephalin derived peptides							
Leu-enk	7.588±0.1972	7.970±0.05895	-0.468±0.287	0.80±0.082	-0.548±0.299		3.532
Met-enk	6.564±0.1869	7.279±0.04663	-1.492±0.280	-0.611±0.074	-0.881±0.290		7.603
Met-enk RF	5.984±0.1921	7.337±0.04690	-2.072±0.284	-0.553±0.074	-1.519±0.293		33.037
Met-enk RGL	6.501±0.1871	7.329±0.04759	-1.555±0.280	-0.561±0.075	-0.994±0.290		9.863
Metorphamide	6.584±0.1851	6.779±0.04514	-1.472±0.279	-1.111±0.073	-0.361±0.288		2.296
BAM 12	8.115±0.2004	6.386±0.04887	0.059±0.289	-1.504±0.076	1.563±0.299	36.559	
BAM 18	7.679±0.1877	7.115±0.04400	-0.377±0.281	-0.775±0.073	0.398±0.290	2.500	
BAM 22	7.943±0.1949	7.589±0.04843	-0.113±0.285	-0.301±0.075	0.188±0.295	1.542	
Peptide E	6.734±0.1825	7.020±0.04438	-1.322±0.277	-0.870±0.073	-0.452±0.286		2.831
Peptide F	6.698±0.1830	5.812±0.06696	-1.358±0.277	-2.078±0.088	0.720±0.291	5.248	

Bias analysis was carried out as described in Methods. RA= τ/K_A

Supplemental Table 8. Bias Analysis for endogenous opioid peptides at κOR

	GTP Log RA	β-arrestin Log RA	Δ log RA for GTP (DAMGO)	Δ log RA for β-arrestin (DAMGO)	Δ Δ log RA (GTP –barr)	Bias Factor (GTP – βarr)	Bias Factor (βarr-GTP)
Standard							
U69,593	7.36±0.574	6.302±0.06124	0.000±0.812	0.000±.087	0±.816	1.000	
Pro-dynorphin derived peptides							
β-neoend	7.169±0.5155	5.900±0.07049	-0.191±0.772	-0.402±0.093	0.211±0.777	1.626	
α-neoend	6.461±0.5048	5.792±0.07036	-0.899±0.764	-0.510±0.093	-0.389±0.770		2.483
Dyn A8	7.513±0.5547	6.964±0.05749	0.153±0.798	0.662±0.084	-0.509±0.803		3.177
Dyn A13	6.237±0.5215	8.243±0.07183	-1.123±0.776	1.941±0.094	-3.064±0.781		1158.777
Dyn A17	7.283±0.6806	6.536±0.06153	-0.077±0.890	0.234±0.087	-0.311±0.895		124.165
Dyn B13	6.935±0.5313	5.266±0.07009	-0.425±0.782	-1.076±0.093	0.651±0.788	4.477	
Pro-opiomelanocortin derived peptides							
β-end 26	6.784±0.4554	4.289±0.1126	-0.576±0.780	-2.013±0.128	1.437±0.790	27.353	
β-end 27	6.314±0.5215	4.214±0.1225	-1.046±0.776	-2.088±0.137	1.042±0.788	11.015	
β-end 31	5.872±0.5194	4.318±0.1753	-1.488±0.774	-1.984±0.186	0.496±0.796	3.133	
γ-end	6.325±0.5062	4.269±0.1816	-1.035±0.765	-2.033±0.192	0.998±0.789	9.954	
Pro-enkephalin derived peptides							
Leu-enk	4.635±0.5869	3.091±0.4059	-2.797±0.821	-3.211±0.410	0.414±0.918	2.594	
Met-enk	4.537±0.5956	3.462±0.2521	-2.823±0.827	-2.840±0.259	0.017±0.867	1.040	
Met-enk RF	5.888±0.5247	4.676±0.1088	-1.472±0.778	-1.626±0.125	0.154±0.788	1.426	
Met-enk RGL	5.802±0.5264	3.816±0.1726	-1.558±0.779	-2.486±0.183	0.928±0.800	8.472	
Metorphamide	4.195±0.6843	6.594±0.05774	-3.165±0.893	0.292±0.084	-3.457±0.897		3250.87
BAM 12	6.722±0.5058	6.029±0.05245	-0.588±0.765	-0.273±0.081	-0.315±0.769		2.099
BAM 18	8.310±0.719	6.011±0.06417	0.950±0.920	-0.291±0.089	1.263±0.991	17.418	
BAM 22	8.195±0.5777	6.606±0.05867	0.835±0.814	0.304±0.085	0.551±0.819	3.396	
Peptide E	6.022±0.5136	6.725±0.06223	-1.338±0.770	0.423±0.087	-1.761±0.775		59.979
Peptide F	4.075±0.7043	3.643±0.3512	-3.285±0.909	-2.659±0.356	-0.626±0.976		4.977

Bias analysis was carried out as described in Methods. RA= τ/K_A