Supplemental Tables

Table S1. Comparison of KOP-Nb39 interaction in the presence of Gαi1 or β-arrestin2. Data represent mean EC_{50} (pEC_{50}± SEM) or E_{max} %±SEM and experiments were performed in triplicate. Related to Figure S1.

Table S2. Comparison of KOP agonists binding affinity and functional activity between KOP wt and D138$^{3.32}$A mutant. Data represent mean Ki (pKi± SEM), EC_{50} (pEC_{50}± SEM) or E_{max} %±SEM and experiments were performed in triplicate. Related to Figure 3.

Table S3. Mutagenesis studies of residues in the hydrophobic binding pocket. The compounds were tested at indicated mutants in cAMP inhibition assay. Data represent mean EC_{50} (pEC_{50}± SEM) or E_{max} %±SEM and experiments were performed in triplicate. Related to Figure 3.

Table S4. Mutagenesis studies of residues in the hydrophobic binding pocket. The compounds were tested at indicated mutants in Tango β-arrestin2 recruitment assay. Data represent mean EC50 (pEC50± SEM) or Emax %±SEM and experiments were performed in triplicate. Related to Figure 3.

Table S5. Comparison of functional activity of MP1104 and IBNtxA in GTPγ[^{35}S] and BRET assays. Data represent mean EC_{50} (pEC_{50}± SEM) or E_{max} %±SEM and experiments were performed in triplicate. Related to Figure S4.

Table S1

Receptor	BRET assay (KOP-Rluc + Nb39-YFP)					
	MP1104		SalA		Dyn A, 1-17	
	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM
KOP wt	0.17 (9.77±0.08)	100±2	13.4 (7.87±0.04)	100±2	57.6 (7.24±0.11)	100±5
+1 µg β-arrestin2	0.13 (9.88±0.07)	112±2	15.6 (7.81±0.04)	120±2	72.1 (7.14±0.15)	150±8
+5 µg β-arrestin2	0.22 (9.65±0.08)	124±1	9.32 (8.03±0.04)	130±2	60.9 (7.22±0.14)	161±3
+10 µg β-arrestin2	0.24 (9.62±0.09)	138±1	7.80 (8.11±0.04)	147±2	28.3 (7.55±0.16)	161±5

Receptor	BRET assay (KOP-Rluc + Nb39-YFP)					
	MP1104		SalA		Dyn A, 1-17	
	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM
KOP wt	0.19 (9.71±0.09)	100±2	12.5 (7.90±0.04)	100±2	42.8 (7.37±0.14)	100±5
+1 µg Gαi1	0.17 (9.77±0.07)	81±2	15.03 (7.82±0.06)	76±2	79.2 (7.10±0.13)	62±3
+5 µg Gαi1	0.14 (9.85±0.09)	67±1	10.9 (7.96±0.08)	60±1	117.2 (6.93±0.18)	42±3
+10 µg Gαi1	0.13 (9.88±0.11)	48±1	9.11 (8.04±0.08)	43±1	79.9 (7.09±0.20)	28±3

Table S2

			KOP wt	KOP D138$^{3.32}$A
Dynorphin A 1-17	Ki, nM (pKi ±SEM)		2.40 (8.58±0.03)	N.D
	Gi	EC_{50}, nM (pEC_{50}±SEM)	0.014 (10.86±0.05)	N.D
		E_{max}% ±SEM	100±2	
	Arrestin,	EC_{50}, nM (pEC_{50}±SEM)	20.38 (7.69±0.12)	N.D
		E_{max}% ±SEM	54±3	
MP1104	Ki, nM (pKi ±SEM)		0.22 (9.65±0.02)	0.34 (9.46±0.05)
	Gi	EC_{50}, nM (pEC_{50}±SEM)	0.003 (11.60±0.04)	0.041 (10.39±0.06)
		E_{max}% ±SEM	102±1	95±2
	Arrestin	EC_{50}, nM (pEC_{50}±SEM)	0.035 (10.45±0.03)	0.82 (9.08±0.09)
		E_{max}% ±SEM	109±5	78±2
SalA	Ki, nM (pKi ±SEM)		2.32 (8.63±0.05)	2.59 (8.58±0.05)
	Gi	EC_{50}, nM (pEC_{50}±SEM)	0.023 (10.65±0.07)	0.12 (9.93±0.05)
		E_{max}% ±SEM	104±4	100±2
	Arrestin	EC_{50}, nM (pEC_{50}±SEM)	3.17 (8.50±0.05)	88.7 (7.05±0.06)
		E_{max}% ±SEM	100±2	87±3

N.D, no activity detected.

Table S3

KOP mutation	cAMP inhibition assay					
	MP1104		IBNtxA		U69,593	
	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM
wt	0.003 (11.60±0.02)	100±1	0.002 (11.70±0.03)	102±1	0.32 (9.50±0.04)	102±2
$W287^{6.48}L$	0.003 (11.60±0.05)	100±1	0.005 (11.30±0.02)	102±1	310 (6.50±0.01)	89±4
$G319^{7.42}L$	1.21 (8.92±0.05)	100±2	12.2 (7.91±0.05)	99±2	318 (6.50±0.08)	91±5
$Y320^{7.43}L$	0.30 (9.50±0.05)	100±2	0.91 (9.04±0.03)	107±2	291 (6.54±0.08)	99±5

KOP mutation	cAMP inhibition assay					
	U50,488		Dyn A, 1-17		SalA	
	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM
wt	0.076 (10.12±0.05)	103±2	0.01 (10.90±0.04)	103±1	0.02 (10.70±0.03)	104±1
$W287^{6.48}L$	0.57 (9.24±0.04)	99±2	0.90 (9.05±0.04)	93±1	9.26 (8.03±0.06)	93±2
$G319^{7.42}L$	75.2 (7.12±0.08)	91±4	20.5 (7.69±0.06)	84±2	492 (6.3±0.07)	92±5
$Y320^{7.43}L$	30.4 (7.52±0.07)	100±3	8.74 (8.06±0.03)	99±2	N.D	N.D

N.D, no activity detected.

Table S4

KOP mutation	β-arrestin2 recruitment assay					
	MP1104		IBNtxA		U69,593	
	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM
wt	0.22 (9.64±0.04)	100±2	0.31 (9.52±0.04)	96±2	6.74 (8.17±0.06)	78±2
$W287^{6.48}L$	2.67 (8.57±0.06)	100±3	116 (6.90±0.09)	80±4	N.D	N.D
$G319^{7.42}L$	177.8 (6.75±0.10)	100±6	N.D	N.D	N.D	N.D
$Y320^{7.43}L$	58.1 (7.24±0.07)	100±4	676 (6.17±0.09)	140±10	N.D	N.D

KOP mutation	β-arrestin2 recruitment assay					
	U50,488		Dyn A, 1-17		SalA	
	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}±SEM)	Emax% ±SEM
wt	3.11 (8.50±0.08)	86±2	10.5 (8.00±0.12)	65±3	8.78 (8.06±0.08)	86±3
$W287^{6.48}L$	N.D	N.D	N.D	N.D	N.D	N.D
$G319^{7.42}L$	324 (6.49±0.10)	65±6	N.D	N.D	N.D	N.D
$Y320^{7.43}L$	N.D	N.D	N.D	N.D	N.D	N.D

N.D, no activity detected.

Table S5

Mutation	GTPγ[^{35}S] assay							
	MP1104		IBNtxA		SalA		DAMGO	
	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM
KOP wt	0.016 (10.78±0.07)	94±2	0.018 (10.73±0.06)	94±2	0.086 (10.07±0.05)	100±4	N.M	N.M
KOP $Y312^{7.35}W$	0.14 (9.86±0.05)	107±2	0.10 (10.00±0.06)	109±2	0.58 (9.23±0.06)	100±2	N.M	N.M
MOP wt	0.43 (9.36±0.08)	101±3	0.31 (9.51±0.05)	92±1	N.M	N.M	1.21 (8.92±0.05)	98±3

Mutation	BRET assay							
	MP1104		IBNtxA		SalA		DAMGO	
	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM	EC_{50}, nM (pEC_{50}± SEM)	Emax% ±SEM
KOP wt	0.60 (9.22±0.05)	112±2	0.75 (9.12±0.03)	116±2	9.13 (8.04±0.05)	100±2	N.M	N.M
KOP $Y312^{7.35}W$	0.28 (9.55±0.07)	111±2	9.02 (9.02±0.09)	70±2	154 (6.81±0.08)	100±4	N.M	N.M
MOP wt (+GRK2)	0.24 (9.63±0.06)	86±1	0.37 (9.43±0.06)	51±1	N.M	N.M	9.93 (8.03±0.07)	100±2

N.M, not measured.