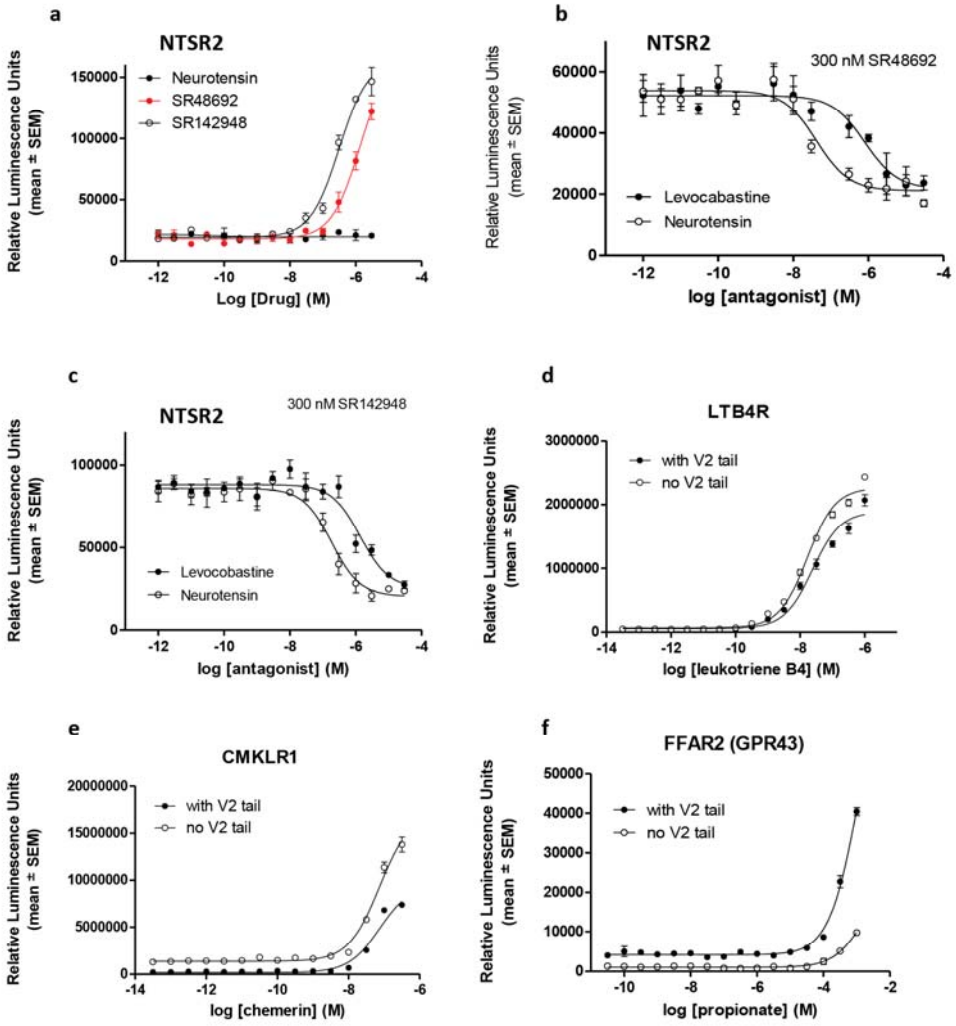
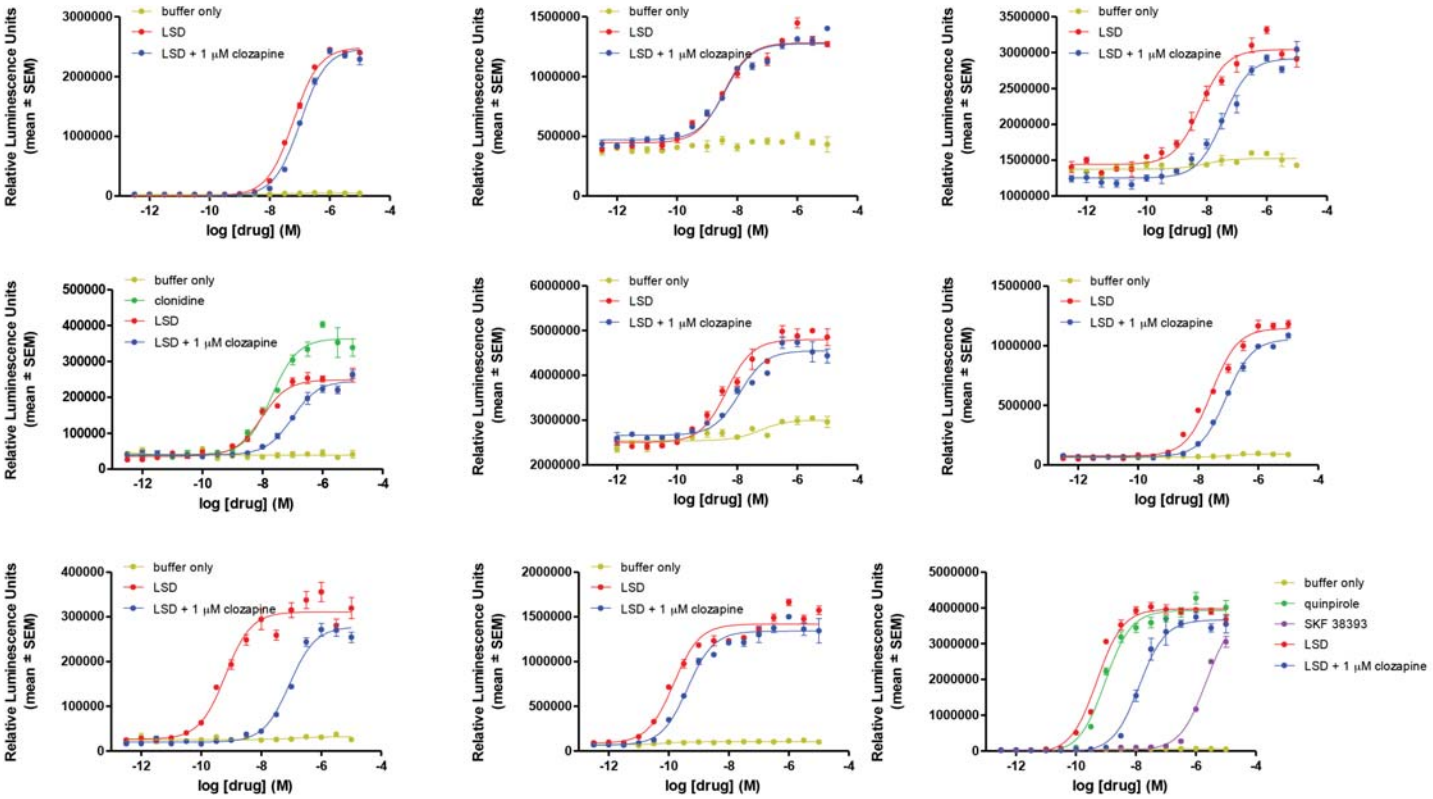



**Supplementary Figure 1**

Demonstration of the feasibility of testing for antagonist activity with the Tango β-arrestin recruitment assay and demonstration of the variability of the effects of removal of the V2 tail on activity in the Tango β-arrestin recruitment assay.

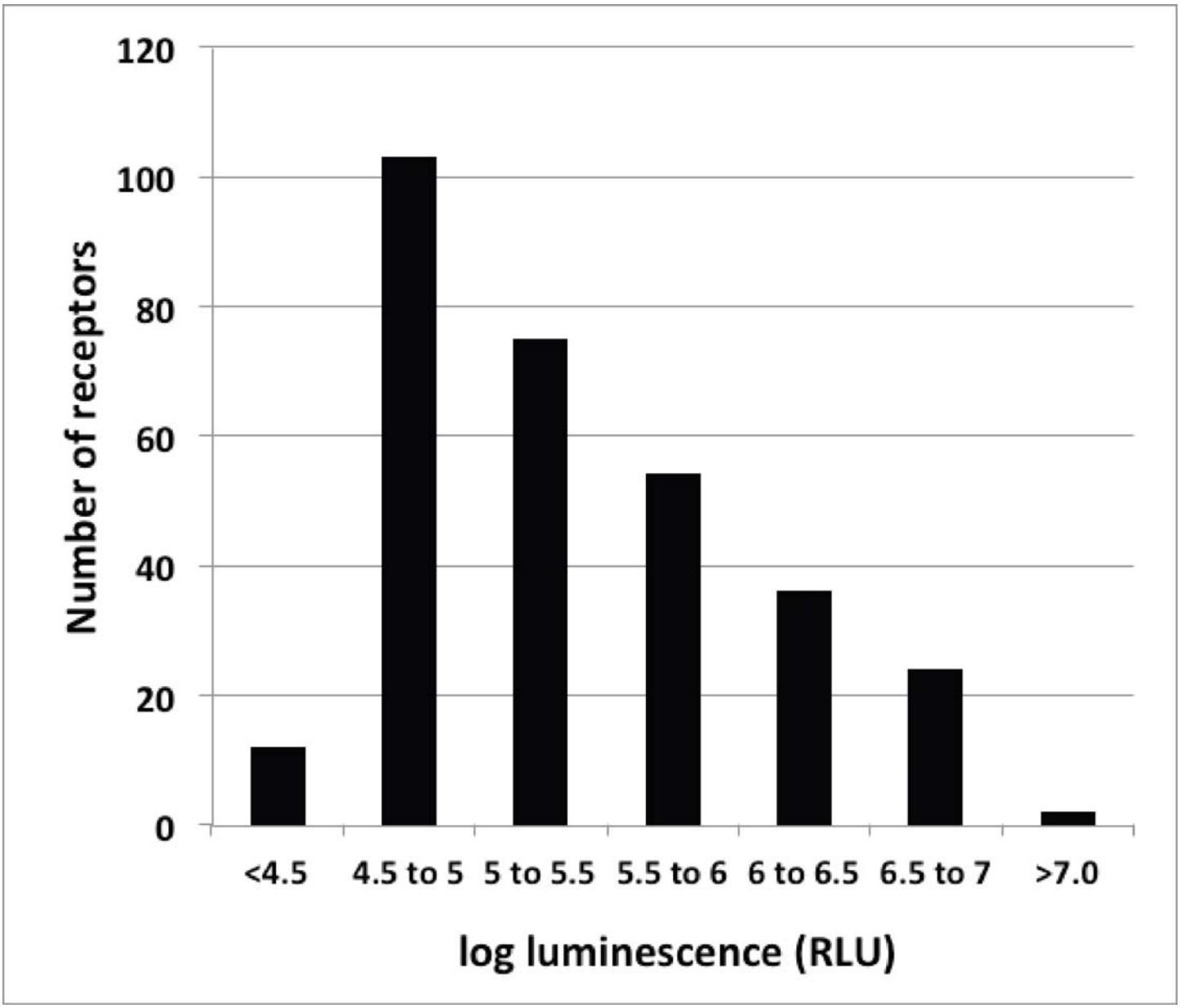
**(a-c)** Demonstration of the feasibility of testing for antagonist activity with the Tango β-arrestin–recruitment assay **(a)** Stimulation of neurotensin receptor NTSR2 activity by SR48692 or SR142948, but not neurotensin. **(b)** Inhibition of activity of SR48692 at the NTSR2 receptor by levocabastine or neurotensin. **(c)** Inhibition of activity of SR142948 at the NTSR2 receptor by levocabastine or neurotensin. **(d-f)** Demonstration of the variability of the effects of removal of the V2 tail on activity in the Tango β-arrestin recruitment assay. **(d)** Lack of effect of V2 tail removal using the LTB4R receptor. **(e)** Increased activity after V2 tail removal using the CMKLR1 receptor. **(f)** Decreased activity after V2 tail removal using the FFAR2 (GPR43) receptor.




**Supplementary Figure 2**

The effect of clozapine on concentration-response curves to LSD at various GPCR targets in the Tango β-arrestin recruitment assay.

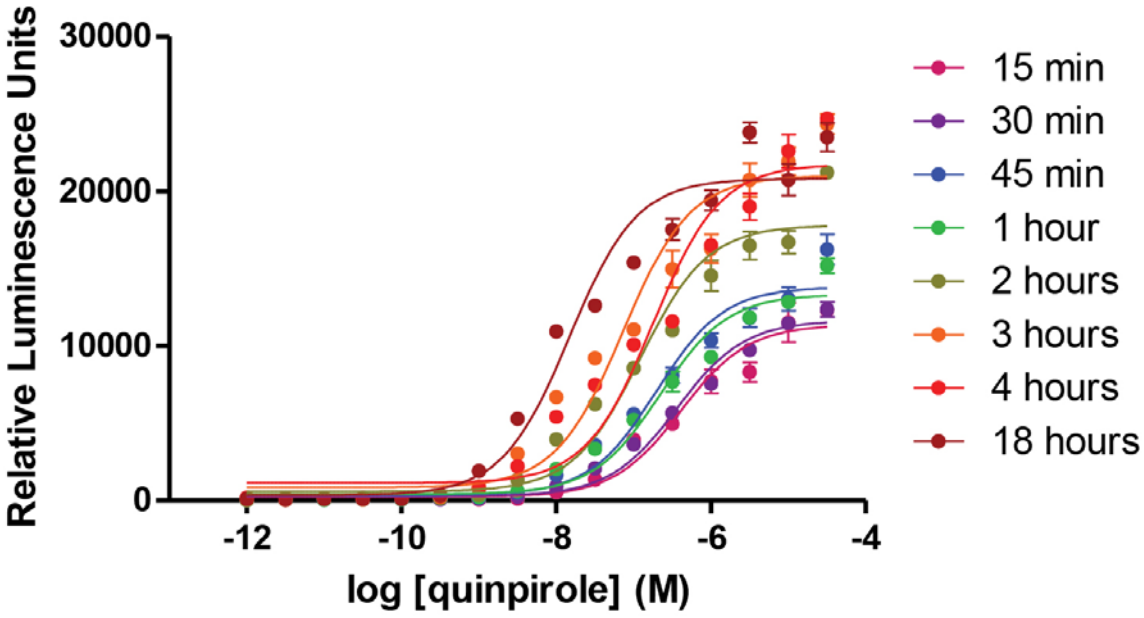
Data are shown as mean ± SEM of quadruplicate values, and curves were fitted using GraphPad Prism. **(a)** HTR1A, **(b)** HTR1D, **(c)** HTR1B, **(d)** ADRA2B, **(e)** HTR1E, **(f)** HTR1F, **(g)** HTR2A, **(h)** HTR5, **(i)** DRD2.




**Supplementary Figure 3**

Summary of constitutive activity of GPCR-Tango constructs used in this study.

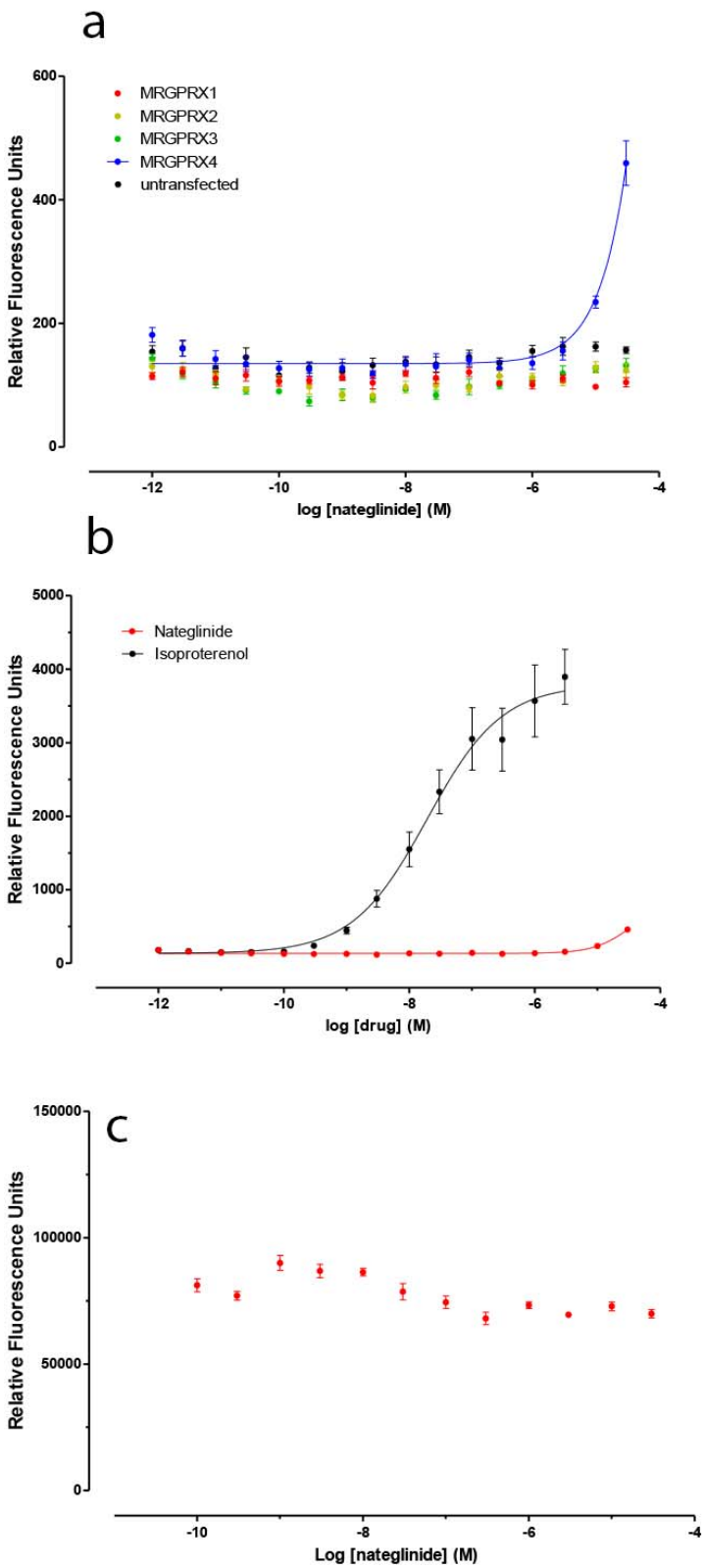
HTLA cells were transfected with various Tango constructs, plated into 384-well assay plates, and luminescence in relative luminescence units (RLU) was measured after overnight incubation in the absence of ligand. The constitutive activity of these constructs, *i.e.*, the ratio of the maximum to the minimum luminescence of the constructs, varied over a range of up to 551-fold in individual experiments.




**Supplementary Figure 4**

The effect of various lengths of exposure to agonist on the luminescence response in the Tango β-arrestin recruitment assay.

Cells transfected with the DRD2-Tango construct were plated into 384-well plates and incubated in medium containing 1% dialysed fetal bovine serum (dFBS) overnight. Then, medium was switched to serum-free, and cells were incubated for a further 4 hours. Various concentrations of the agonist quinpirole were added, and at different times were washed out and replaced with serum-free medium, with further incubation overnight. Plates were read the following day. Data are expressed as mean ± SEM of quadruplicate determinations, and curves were fitted using GraphPad Prism.

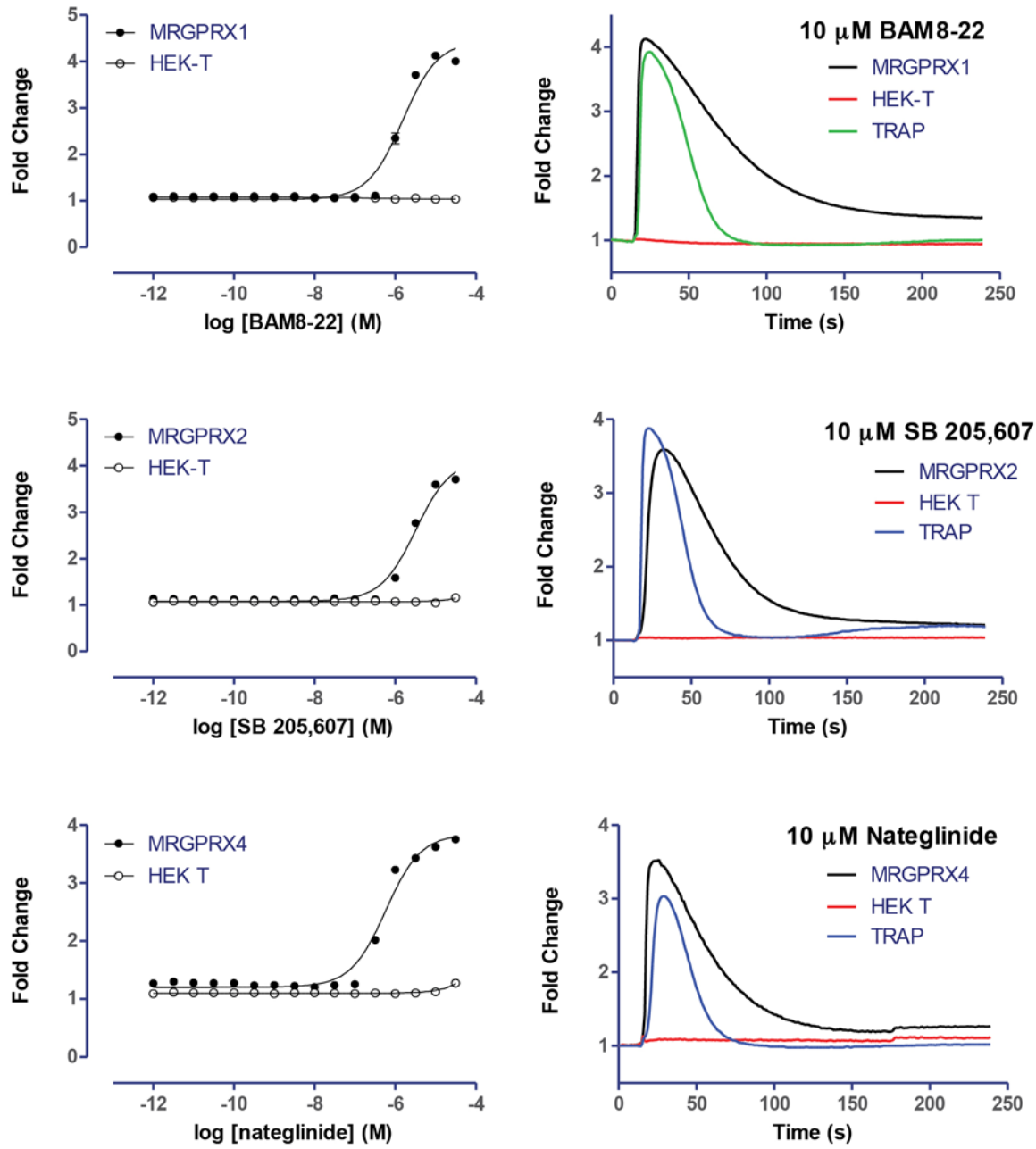



Nature Structural and Molecular Biology: doi:10.1038/nsmb.3014

**Supplementary Figure 5**

Concentration-response curves of nateglinide in $G_s$ and $G_i$ assays in MRGPRX receptor–expressing cells.

**a)** $G_s$ response to nateglinide in HEK293T cells expressing MRGPRX receptors, showing a $G_s$ response only in cells expressing MRGPRX4 receptors at high concentrations of nateglinide. **(b)** Comparison of the concentration-response curves in $G_s$ assays of nateglinide and isoproterenol in MRGPRX4-expressing HEK293T cells. **(c)** Concentration-response curve in $G_i$ assay of nateglinide in MRGPRX4-expressing HEK293T cells. Data are expressed as mean ± SEM of triplicate or quadruplicate determinations, and curves were fitted using Graphpad Prism.

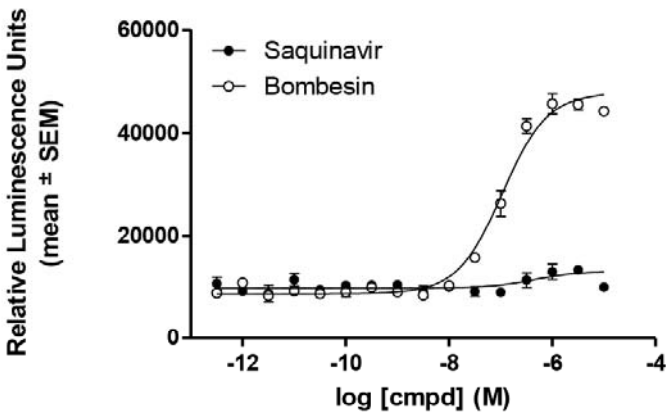



**Supplementary Figure 6**

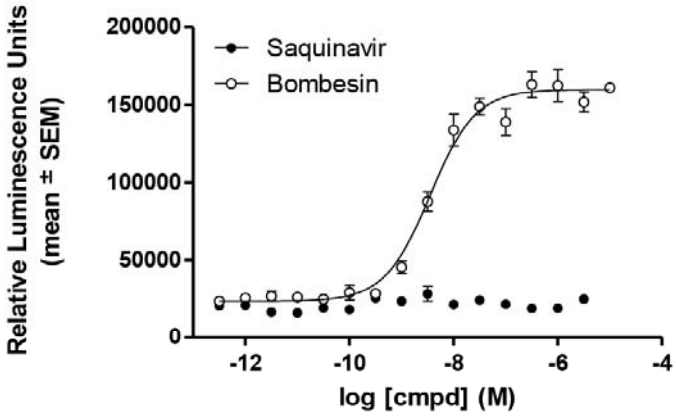
Calcium-mobilization responses in stable cell lines expressing MRGPRX receptors.

**(a,c,e)** Concentration-response curves; data are expressed as mean ± SEM, and curves were fitted using Graphpad Prism. **(b,d,f)** Time course of responses, showing representative curves of experiments done in triplicate. **(a, b)** Responses of MRGPRX1-expressing cells to BAM8-22. **(c,d)** Responses of MRGPRX2-expressing cells to SB 205,607. **(e,f)** Responses of MRGPRX4-expressing cells to nateglinide. TRAP is an agonist for endogenous PAR1 and serves as an internal control for the calcium mobilization assay.

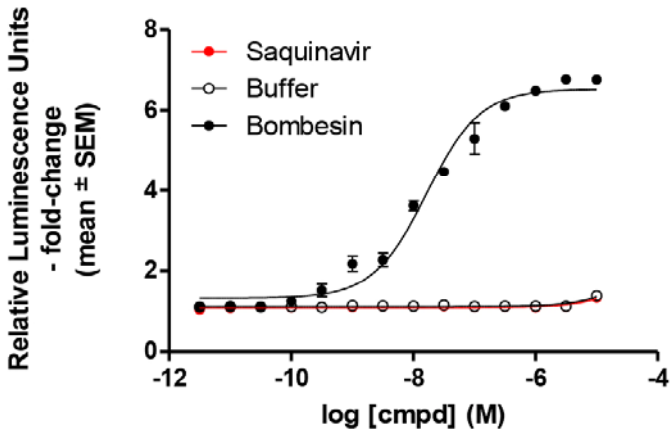
a


Relative Luminescence Units
(mean ± SEM)
60000
40000
20000
0
Saquinavir
Bombesin
-12
-10
-8
-6
-4
log [cmpd] (M)

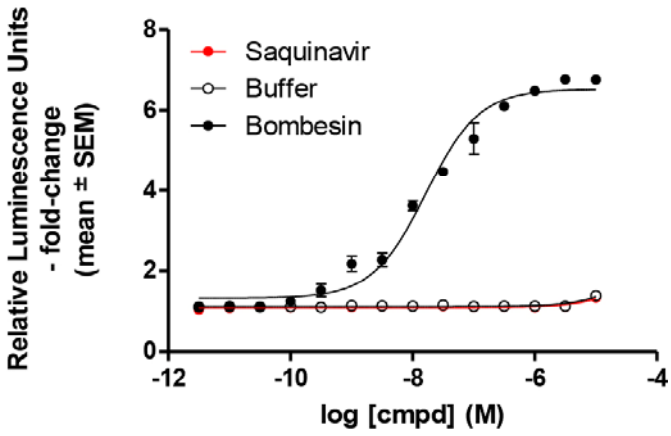
b


Relative Luminescence Units
(mean ± SEM)
200000
150000
100000
50000
0
Saquinavir
Bombesin
-12
-10
-8
-6
-4
log [cmpd] (M)

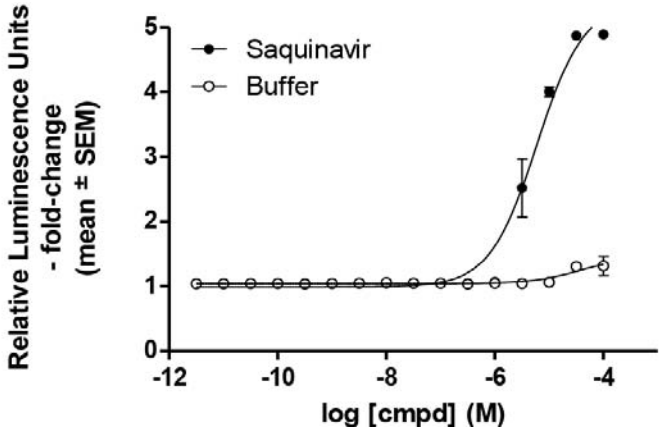
c


Relative Luminescence Units
- fold-change
(mean ± SEM)
8
6
4
2
0
Saquinavir
Buffer
Bombesin
-12
-10
-8
-6
-4
log [cmpd] (M)

d


Relative Luminescence Units
- fold-change
(mean ± SEM)
8
6
4
2
0
Saquinavir
Buffer
Bombesin
-12
-10
-8
-6
-4
log [cmpd] (M)

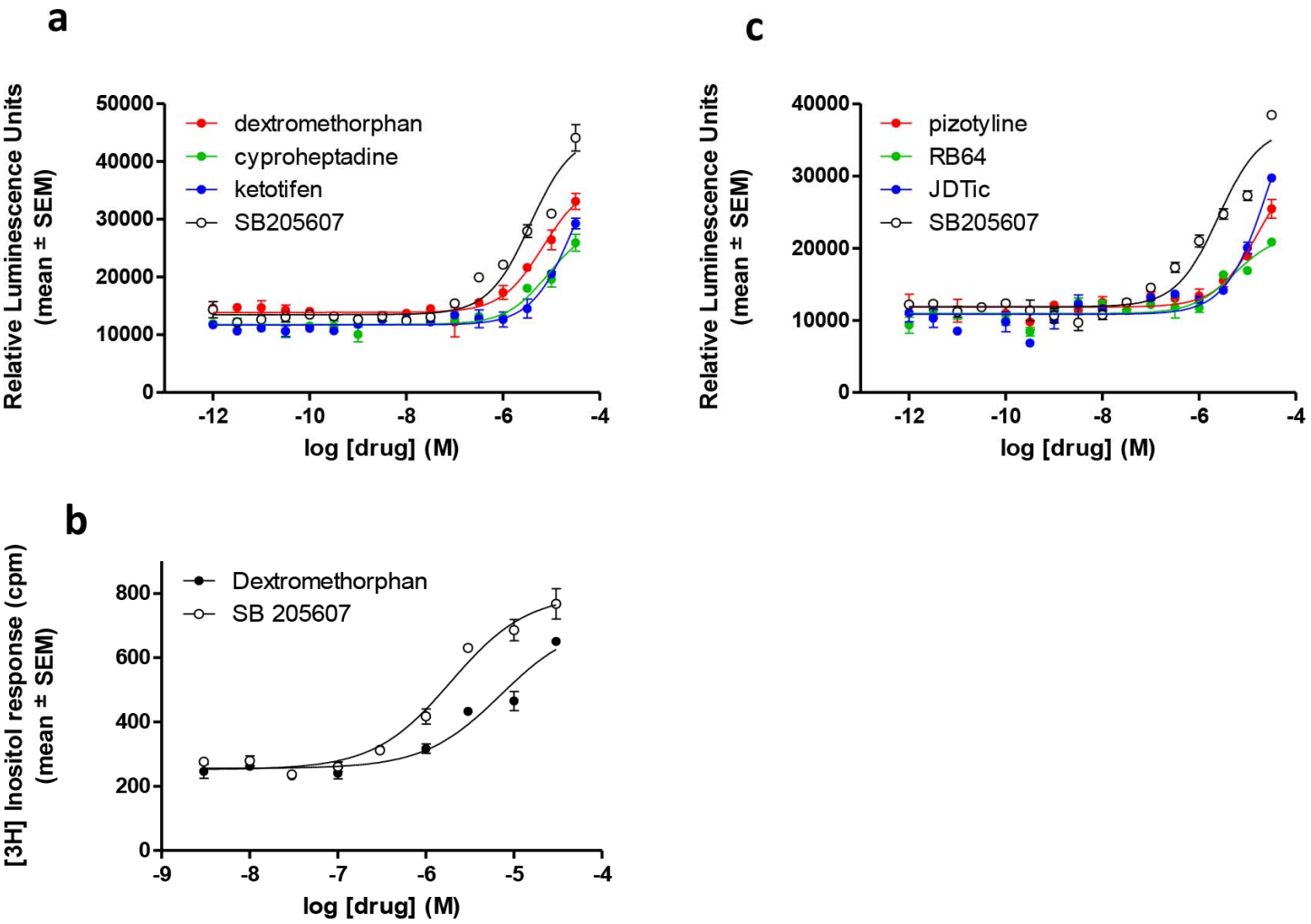
e


Relative Luminescence Units
- fold-change
(mean ± SEM)
5
4
3
2
1
0
Saquinavir
Buffer
-12
-10
-8
-6
-4
log [cmpd] (M)

**Supplementary Figure 7**

Concentration-response curves showing responses of bombesin receptors to the cognate ligand bombesin and saquinavir.

(a) BB1 receptor, Tango assay. (b) BB2 receptor, Tango assay. (c) BB1 receptor, calcium mobilization assay. (d) BB2 receptor, calcium mobilization assay. (e) BB3 receptor, PI hydrolysis assay. Data are expressed as mean ± SEM of triplicate determinations, and curves were fitted using Graphpad Prism.




**Supplementary Figure 8**

Concentration-dependent agonist activity of various compounds at MRGPRX2 receptors in the Tango assay compared with the activity of the known ligand SB 205607.

**(a)** and **(c)** Tango arrestin recruitment assays, **(b)** PI hydrolysis. Data are expressed as mean ± SEM of triplicate determinations, and curves were fitted using Graphpad Prism.

**Supplementary Table 1.** List of receptors used in this study, with references to earliest literature showing interactions of receptors with arrestins.

| Receptor | Validated in this study? | Previously known to interact with arrestin? | Earliest reference |
|---|---|---|---|
| ADCYAP1R1 (PACAP) | Y | Y | [1] |
| ADORA1 | Y | Y | [2] |
| ADORA2A | | Y | [3] |
| ADORA2B | | Y | [4] |
| ADORA3 | | Y | [5] |
| ADRA1A | | N | [6] |
| ADRA1B | Y | Y | [7] |
| ADRA1D | Y | Y | [8] |
| ADRA2A | Y | Y | [9] |
| ADRA2B | Y | Y | [10] |
| ADRA2C | Y | Y | [10] |
| ADRB1 | Y | Y | [11] |
| ADRB2 | Y | Y | [12] |
| ADRB3 | | N | [13] |
| AGTR1 | Y | Y | [14] |
| AGTR2 | | N | [15] |
| APJ | Y | Y | [16] |
| AVPR1A | Y | Y | [17] |
| AVPR1B | Y | N | this study |
| AVRP2 | Y | Y | [18] |
| BB3 (BRS3) | Y | Y | [19] |
| BDKRB1 | Y | Y | [20] |
| BDKRB2 | Y | Y | [21] |
| C3AR1 | Y | Y | [22] |
| C5A | | Y | [23] |
| CALCRb | | N | |
| CALCRL | | Y | [24] |
| CASR | | Y | [25] |
| CC10 (CCBP2) | | N | |
| CC2 | | Y | [26] |
| CC3 | | Y | [27] |
| CC4 | Y | Y | [27] |
| CC5 | | Y | [28] |
| CC6 | Y | Y | [27] |
| CC7 | | Y | [29] |
| CC8 | | Y | [27] |
| CCKAR | Y | N | |
| CCKBR | | Y | [30] |
| CCRL2 | | | |

| Receptor | Validated in this study? | Previously known to interact with arrestin? | Reference |
|---|---|---|---|
| CD97 | | | |
| CHRM1 | Y | Y | [31] |
| CHRM2 | Y | Y | [32] |
| CHRM3 | Y | Y | [33] |
| CHRM4 | Y | Y | [34] |
| CHRM5 | Y | N | this study |
| CMKLR1 | Y | Y | [35] |
| CMKOR1 | Y | N | this study |
| CNR1 | Y | Y | [36] |
| CNR2 | Y | Y | [37] |
| CRHR1 | | Y | [38] |
| CRHR2 | | Y | [39] |
| CX3C1 | Y | N | this study |
| CXC1 | Y | Y | [40] |
| CXC2 | Y | Y | [41] |
| CXC3 | | Y | [42] |
| CXC4 | Y | Y | [43] |
| CXC5 | | | |
| CXC6 | Y | Y | [27] |
| CXC7 | | Y | [44] |
| CYSLTR1 | Y | Y | [45] |
| CYSLTR2 | | | |
| DRD1 | Y | Y | [46] |
| DRD2 | Y | Y | [47] |
| DRD3 | Y | Y | [47] |
| DRD4 | Y | N | [48] |
| DRD5 | Y | Y | [48] |
| EBI2 (GPR183) | | Y | [49] |
| EDNRA | Y | Y | [50] |
| EDNRB | | Y | [51] |
| ELTD1 | | | |
| F2R | | Y | [52] |
| F2RL1 | | Y | [53] |
| F2RL2 | | | |
| F2RL3 | | | |
| FFAR1 (GPR40) | | | |
| FFAR2 (GPR43) | Y | Y | [54] |
| FFAR3 (GPR41) | | | |
| FPR1 | Y | Y | [55] |
| FPR2 (FPRL1) | Y | Y | [56] |
| FPR3 | | | |
| FSHR | | Y | [57] |
| GABBR1 | | | |

| Receptor | Validated in this study? | Previously known to interact with arrestin? | Reference |
|---|---|---|---|
| GAL1 | Y | Y | [27] |
| GAL2 | Y | Y | [27] |
| GAL3 | Y | N | this study |
| GCGR | Y | Y | [58] |
| GHRHR | | | |
| GHSR | Y | Y | [59] |
| GIPR | | | |
| GLP1R | Y | Y | [60] |
| GLP2R | | Y | [61] |
| GNRHR | Y | Y | [62] |
| GPBA | Y | N | this study |
| GPER (GPR30) | | Y | [63] |
| GPR1 | | Y | [35] |
| GPR101 | | | |
| GPR109A (HCA2) | | | |
| GPR109B (HCA3) | | | |
| GPR110 | | | |
| GPR111 | | | |
| GPR113 | | | |
| GPR114 | | | |
| GPR115 | | | |
| GPR116 | | | |
| GPR119 | | Y | [27] |
| GPR12 | | | |
| GPR120 (FFAR4) | | Y | [27] |
| GPR123 | | | |
| GPR124 | | | |
| GPR125 | | | |
| GPR126 | | | |
| GPR132 (G2A) | | Y | [64] |
| GPR133 | | | |
| GPR135 | | | |
| GPR139 | | | |
| GPR141 | | | |
| GPR142 | | | |
| GPR143 | | Y | [65] |
| GPR144 | | | |
| GPR146 | | | |
| GPR148 | | | |
| GPR149 | | | |
| GPR15 | | | |

| Receptor | Validated in this study? | Previously known to interact with arrestin? | Reference |
|---|---|---|---|
| GPR150 | | | |
| GPR151 | | | |
| GPR152 | | | |
| GPR153 | | | |
| GPR156 | | | |
| GPR157 | | | |
| GPR158 | | | |
| GPR160 | | | |
| GPR161 | | Y | 66 |
| GPR162 | | | |
| GPR17 | | Y | 67 |
| GPR171 | | | |
| GPR173 | | | |
| GPR174 | | | |
| GPR18 | | Y | 68 |
| GPR182 | | | |
| GPR19 | | | |
| GPR20 | | | |
| GPR21 | | Y | 27 |
| GPR22 | | | |
| GPR23 | | Y | 64 |
| GPR25 | | | |
| GPR26 | | | |
| GPR27 | | | |
| GPR3 | | Y | 69 |
| GPR31 | | | |
| GPR32 | | Y | 70 |
| GPR34 | | Y | 27 |
| GPR35 | Y | Y | 71 |
| GPR37 | | Y | 27 |
| GPR37L1 | | | |
| GPR39 | | Y | 72 |
| GPR4 | | | |
| GPR44 (CRTH2, PTGDR2) | Y | Y | 27 |
| GPR45 | | | |
| GPR50 | | | |
| GPR52 | | Y | |
| GPR55 | | Y | 64 |
| GPR56 | | Y | 73 |
| GPR6 | | | |
| GPR61 | | | |
| GPR62 | | | |
| GPR63 | | | |

| Receptor | Validated in this study? | Previously known to interact with arrestin? | Reference |
|---|---|---|---|
| GPR64 | | | |
| GPR65 | | | |
| GPR68 (OGR1) | | | |
| GPR75 | | | |
| GPR77 | | | |
| GPR78 | | | |
| GPR81 (HCA1) | | Y | 27 |
| GPR82 | | | |
| GPR83 | | | |
| GPR84 | | Y | 74 |
| GPR85 | | | |
| GPR87 | | Y | 75 |
| GPR88 | | Y | 27 |
| GPR92 | | Y | 64 |
| GPR97 | | Y | 74 |
| GPRC5A | | | |
| GPRC5B | | | |
| GPRCR5C | | | |
| GPRC5D | | | |
| GPRC6A | | | |
| GRM1 | | Y | 76 |
| GRM2 | | | |
| GRM3 | | | |
| GRM4 | | | |
| GRM5 | | | |
| GRM6 | | | |
| GRM7 | | Y | 77 |
| GRM8 | | | |
| GRPR (BB2) | Y | Y | 78 |
| HCRTR1 | Y | Y | 16 |
| HCRTR2 | Y | Y | 16 |
| HRH1 | Y | Y | 79 |
| HRH2 | Y | Y | 80 |
| HRH3 | Y | Y | 27 |
| HRH4 | Y | Y | 64 |
| HTR1A | Y | Y | 27 |
| HTR1B | Y | Y | 27 |
| HTR1D | Y | Y | 27 |
| HTR1E | Y | Y | 81 |
| HTR1F | Y | Y | 81 |
| HTR2A | Y | Y | 82, 83 |
| HTR2B | Y | Y | 84 |
| HTR2C (INI) | Y | Y | 85 |

| Receptor | Validated in this study? | Previously known to interact with arrestin? | Reference |
|---|---|---|---|
| HTR4 | Y | Y | 86 |
| HTR5 | Y | Y | 81 |
| HTR6 | Y | Y | 81 |
| HTR7 | Y | N | this study |
| KISS (GPR54) | | Y | 87 |
| LHCGR | | Y | 88 |
| LPAR1 (EDG2) | Y | Y | 89 |
| LPAR2 (EDG4) | Y | Y | 27 |
| LPAR3 (EDG7) | | | |
| LPAR5 (GPR92) | Y | N | this study |
| LPAR6 (P2RY5) | | | |
| LTB4R | Y | Y | 90 |
| LTB4R2b | | | |
| MAS1 | | | |
| MAS1L | | | |
| MC1R | Y | Y | 58 |
| MC2R | | Y | 58 |
| MC3R | Y | Y | 58 |
| MC4R | Y | Y | 91 |
| MC5R | Y | Y | 92 |
| MCHR1 | Y | Y | 16 |
| MCHR2 | Y | Y | 27 |
| MLNR | Y | Y | 93 |
| MRGPRD | | Y | 74 |
| MRGPRE | | | |
| MRGPRF | | | |
| MRGPRG | | | |
| MRGPRX1 | Y | Y | 74 |
| MRGPRX2 | Y | Y | 74 |
| MRGPRX3 | | | |
| MRGPRX4 | Y | N | this study |
| MTNR1A | Y | Y | 94 |
| MTNR1B | Y | Y | 95 |
| NMBR (BB1) | Y | N | this study |
| NMUR1 | Y | N | this study |
| NMUR2 | Y | N | this study |
| NPBW1 | | | |
| NPBW2 (GPR8) | | Y | 27 |
| NPFF1 | | | |
| NPFF2 | | | |
| NPS (GPR154) | Y | Y | 64 |
| NPY1R | Y | Y | 96 |
| NPY2R | Y | Y | 96 |

| Receptor | Validated in this study? | Previously known to interact with arrestin? | Reference |
|---|---|---|---|
| NPY4R | Y | Y | [96] |
| NPY5R | | Y | [96] |
| NTSR1 | | Y | [50] |
| NTSR2 | Y | N | this study |
| OPN3 | | | |
| OPN5 | | | |
| OPRD1 | Y | Y | [97] |
| OPRK1 | Y | Y | [98] |
| OPRL1 | Y | Y | [99] |
| OPRM1 | | Y | [97] |
| OXER1 | | | |
| OXGR1 | | Y | [74] |
| OXTR | Y | Y | [100] |
| P2RY1 | Y | Y | [31] |
| P2RY10 | | | |
| P2RY11 | | Y | [101] |
| P2RY12 | Y | Y | [102] |
| P2RY13 | Y | N | this study |
| P2RY14 | Y | N | this study |
| P2RY2 | Y | Y | [31] |
| P2RY4 | Y | Y | [101] |
| P2RY6 | Y | Y | [101] |
| PK1 | | | |
| PK2 | | Y | [103] |
| PRP | | | |
| PTAFR | Y | Y | [27] |
| PTGDR | | | |
| PTGER1 | Y | N | this study |
| PTGER2 | Y | Y | [104] |
| PTGER3 | Y | Y | [105] |
| PTGER4 | Y | Y | [106] |
| PTGFR | Y | N | [107], this study |
| PTGIR | Y | Y | [108] |
| PTH1R | Y | Y | [109] |
| PTH2R | | Y | [110] |
| QRFP (GPR103) | | Y | [74] |
| RXFP1 | | Y | [111] |
| RXFP2 | | N | [112] |
| RXFP3 | Y | Y | [27] |
| RXFP4 | Y | N | this study |
| S1PR1 (EDG1) | Y | Y | [113] |
| S1PR2 (EDG5) | Y | N | this study |
| S1PR3 (EDG3) | Y | Y | [27] |

| Receptor | this study? | Previously known to arrestin? | Reference |
|---|---|---|---|
| S1PR4 (EDG6) | | Y | [27] |
| S1PR5 (EDG8) | Y | Y | [27] |
| SCTR | Y | Y | [114] |
| SSTR1 | Y | Y | [31] |
| SSTR2 | Y | Y | [115] |
| SSTR3 | Y | Y | [116] |
| SSTR4 | Y | N | [117], this study |
| SSTR5 | Y | Y | [117] |
| SUCNR1 (GPR91) | | Y | [74] |
| TA1 | | Y | [118] |
| TAAR2 | | | |
| TAAR5 | | | |
| TAAR6 | | | |
| TAAR8 | | | |
| TAAR9 | | | |
| TACR1 | Y | Y | [119] |
| TACR2 | Y | N | this study |
| TACR3 | Y | Y | [120] |
| TBXA2R | | Y | [121] |
| TSHR | | Y | [122] |
| UTS2R | Y | Y | [123] |
| VIPR1 | | Y | [124] |
| VIPR2 | | Y | [125] |

**Supplementary Note 1.** The sequence of a typical Tango construct for the APJ apelin receptor in pcDNA3.1(+).

**MODULES:**

Not I – Kozak – Start – Signal/FLAG – Cla I – Start – receptor – Cla I – Age I – V2 tail – Age I – TEV cleavage site – Tta transcription factor – stop - Xho I

Note: everything must be in a single reading frame.

GCGGCCGCGCCACCATGAAGACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTATTCGCCGACTACAA
GGACGATGATGACGCCAGCatcgatATGGAGGAGGGCGGAGACTTTGACAATTATTACGGTGCCGATAAT
CAGTCCGAGTGCGAGTACACTGACTGGAAAAGCTCCGGTGCACTCATCCCTGCTATATATATGCTGGTGT
TTCTGTTGGGAACCACTGGAAACGGCCTCGTACTGTGGACTGTGTTTAGGAGTTCCCGGGAAAAACGGAG
GAGCGCCGATATTTTTATTGCAAGCCTGGCCGTGGCTGACCTTACGTTTGTGGTGACCCTGCCACTGTGG
GCAACCTACACATATCGCGATTACGATTGGCCTTTCGGAACATTCTTCTGCAAACTGAGTTCTTATCTCA
TCTTCGTTAATATGTATGCATCTGTCTTCTGTCTGACGGGCCTCAGTTTTGATCGGTACCTCGCGATTGT
TCGGCCCGTAGCTAATGCCAGACTGAGGCTGAGAGTCAGTGGGGCCGTCGCCACAGCAGTGCTCTGGGTG
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AGCAGATGTTTACCGATGCCCTTGGAATTGACGAGTACGGTGGGTAGctcgag

**Supplementary Note 1.** The sequence of a typical Tango construct for the APJ apelin receptor in pcDNA3.1(+).

**MODULES:**

Not I – Kozak – Start – Signal/FLAG – Cla I – Start – receptor – Cla I – Age I – V2 tail – Age I – TEV cleavage site – Tta transcription factor – stop - Xho I

Note: everything must be in a single reading frame.

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## Supplementary Note 2

## Parallel receptorome screening using β-arrestin recruitment assay

**Main equipment**: Liquid handling workstation for 96- and 384-well plates, luminescence counter
**Main reagent**: BrightGlo® from Promega
**Assay buffer**: 20 mM HEPES, 1x HBSS, pH 7.40

**Cell culture**. HTLA cells (a gift from Dr. Richard Axel), stably expressing a tTA-dependent luciferase reporter and a β-arrestin-TEV protease fusion gene, are maintained in DMEM supplemented with 10% FBS and 2 μg/ml Puromycin and 100 μg/ml Hygromycin. To set up the cells for transfection, HTLA cells are plated in DMEM supplemented with 10% dialyzed FBS in Poly-L-Lys (PLL) coated 384-well white clear bottom cell culture plates at a density of 15,000 to 20,000 cells in 50 μl per well and incubated overnight.

**DNA plate**. Each single DNA plasmid is plated using the liquid handling workstation into one well of a 96-well plate at 0.5 μg/well (enough for eight 384-well plates) in at least 10μl of assay buffer. Each plate includes 80 receptor DNA samples, positive controls in wells A12 and B12 (DNA plasmids for receptors with cognate ligands), transfection controls in wells A1, B1, G12, and H12 (DNA plasmid for YFP), and negative controls with buffer only (wells C1 to H1, and C12 to F12). We use $D_2$-Tango (quinpirole as agonist) or $V_2$-Tango (Vasopressin as agonist) constructs as positive assay controls. These plates are kept at -20C until ready for assay. Immediately before transfection (see below), two DNA plates are combined in a cell culture hood into one 384-well plate, with duplicate wells for every DNA, (250 μg/well and 50 μl) (see **Figures 1 and 2** for DNA maps in 96-and 384-well plate formats, respectively); immediately followed by calcium phosphate transfection (see below).

**Transfection using calcium phosphate precipitation protocol**. HTLA cells are plated as indicated above and incubated overnight before transfection. Plated DNA in 384-well plates (**Figure 2**) is first diluted to a final volume of 100 μl with 0.25 M $CaCl_2$ in TE buffer (1 mM Tris–HCl, 0.1 mM EDTA, pH 7.6). The diluted DNA is then dispensed onto the plated cells in duplicate (adjacent wells, 50μl/well) as shown in **Figure 2** (*i.e.,* A1 and A2, A3 and A4, …, P23 and P24). Thus, the DNA samples from two 96-well plates (p1 and p2) are transfected in one 384-well plate (P1), and therefore each DNA plasmid is transfected in duplicate. The DNA from the first 96-well plate (p1) is transfected in rows A to H and the DNA from the second plate (p2) is transfected in rows I to P of the 384-well assay plate (Figure 2). An equal volume (50μl) of 2x HBS solution (50 mM HEPES, 280 mM NaCl, 10 mM KCl, 1.5 mM $Na_2HPO_4$, pH 7.00) is added to the DNA/$CaCl_2$ solution to transfect cells. Plates are incubated overnight at 37°C.

**Assay procedure**. Each 384-well plate is designed to test one drug at multiple (up to 160) target receptors simultaneously. Odd-numbered columns contain Tango assay buffer to serve as a basal control and even-numbered columns contain drug stimulation solution prepared in sterile filtered Tango assay buffer at 6 μM (final concentration is 1 μM). The drug plate design is shown in **Figure 3**. On the day of the assay, growth medium is replaced by 50 μl of serum-free medium supplemented with penicillin/streptomycin 4 hours before stimulation. Cells are then stimulated by addition of drugs (10 μl per well) and incubated overnight at 37°C. The following day, medium and drug

solutions are removed and 20 µl per well of BrightGlo reagent (diluted 20-fold with Tango assay buffer) are added. The plate is incubated for at least 20 minutes at room temperature in the dark before luminescence is measured.

**Data processing and analysis.** The luminescence counter records relative luminescence units (RLU) and saves files in Excel spreadsheets for easy processing. For receptors that have positive controls (non-orphan receptors), activation relative to positive control (%) is calculated according to the following formula:

$$Activation\ (relative\ to\ positive\ control, \%) = \frac{(\text{test compound RLU}) - (\text{ave. negative control RLU})}{(\text{ave. positive control RLU}) - (\text{ave. negative control RLU})} * 100$$

For receptors that lack positive controls (orphan receptors), activation relative to baseline (%) is calculated according to the formula below:

$$Activation\ (relative\ to\ baseline, \%) = \frac{(\text{test compound RLU})}{(\text{ave.baseline RLU})} * 100$$

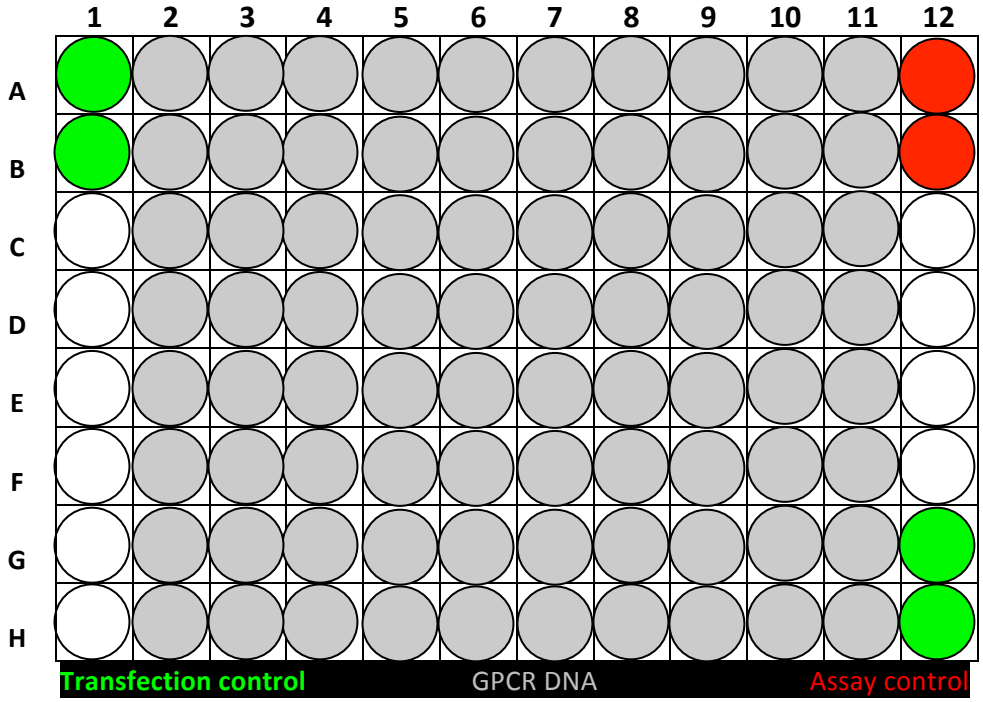



**Figure 1.** 96-well DNA map**.** Green wells contain a YFP plasmid for transfection control. Red wells contain non-orphan receptors for use as positive assay controls (D2-Tango and/or V2-tango constructs). Grey wells have DNA plasmids, each well has a different DNA plasmid. White wells contain transfection reagents only for use as a negative control.

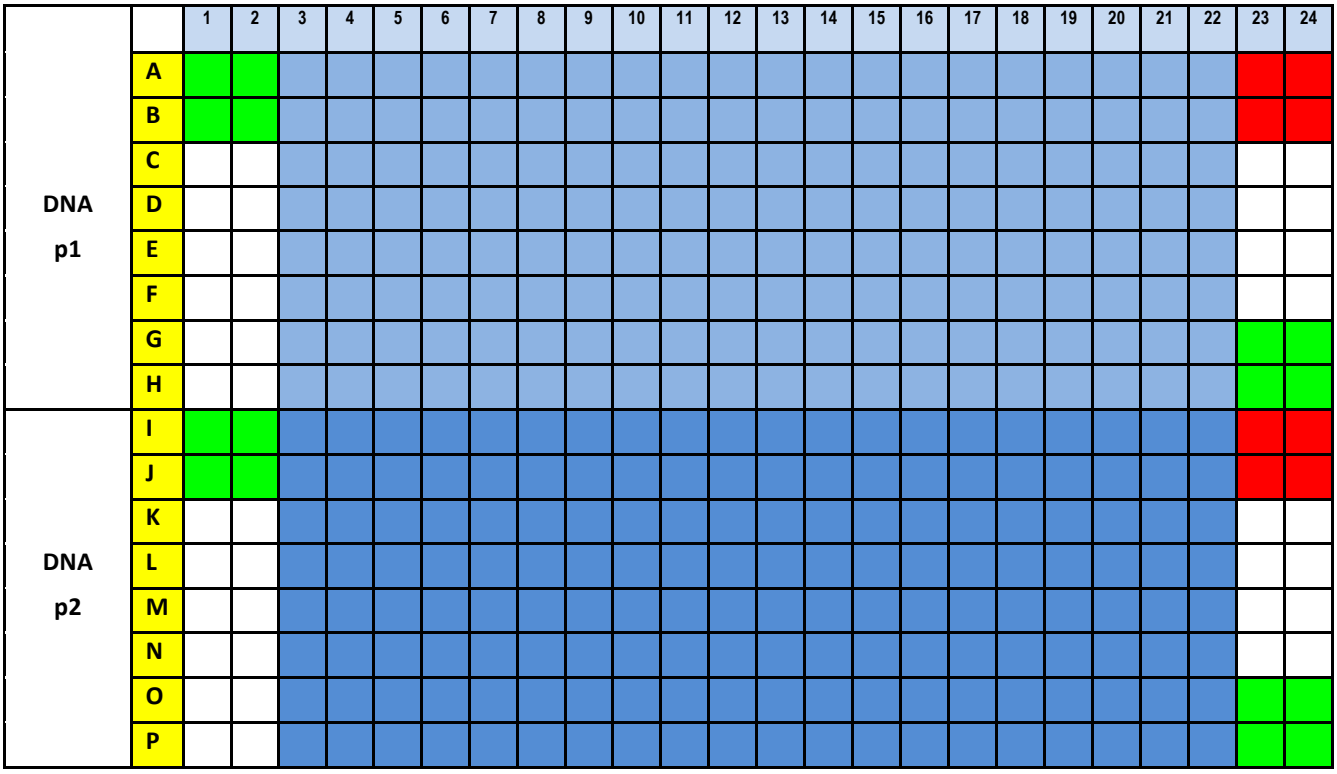



**Figure 2.** 384-well DNA plate for calcium precipitation and transfection (P1). DNA constructs are transfected in neighboring wells. Green wells are YFP-transfected wells for transfection controls. Red wells are assay controls with cells transfected with non-orphan receptors and stimulated with their cognate ligand. DNA from the 96-well plate (p1) is used to transfect rows A to H. DNA from a second plate (p2) is used to transfect rows I to P.

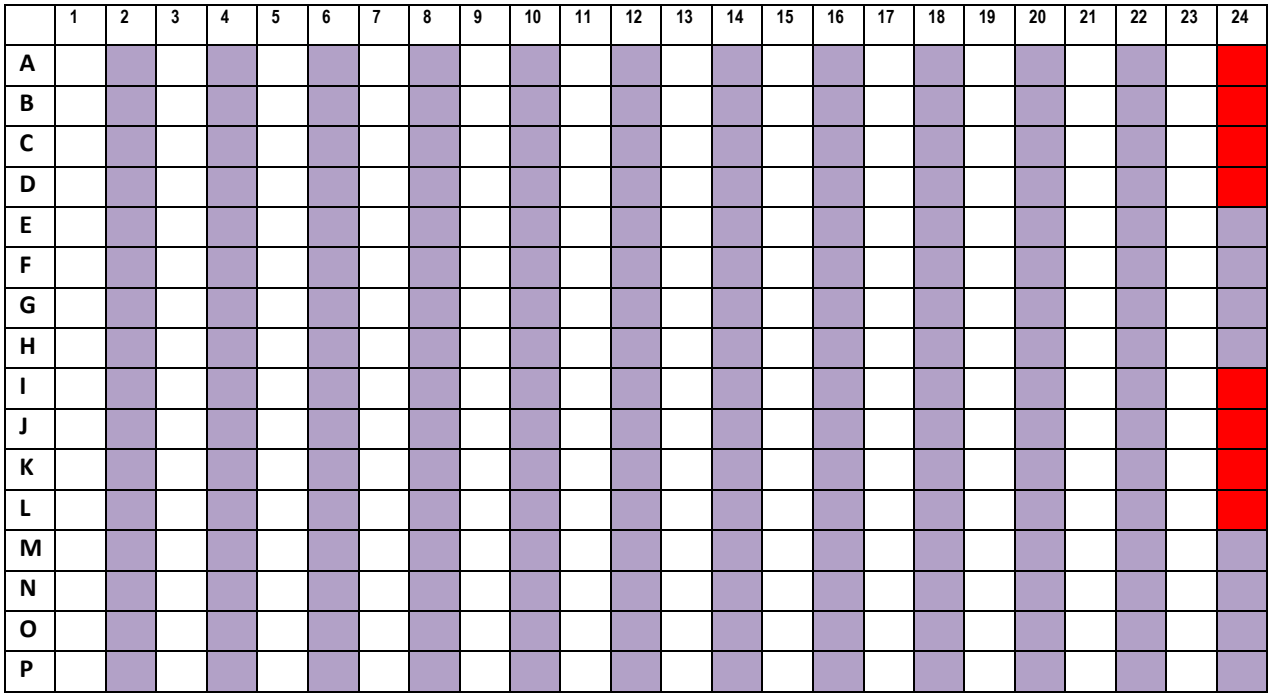
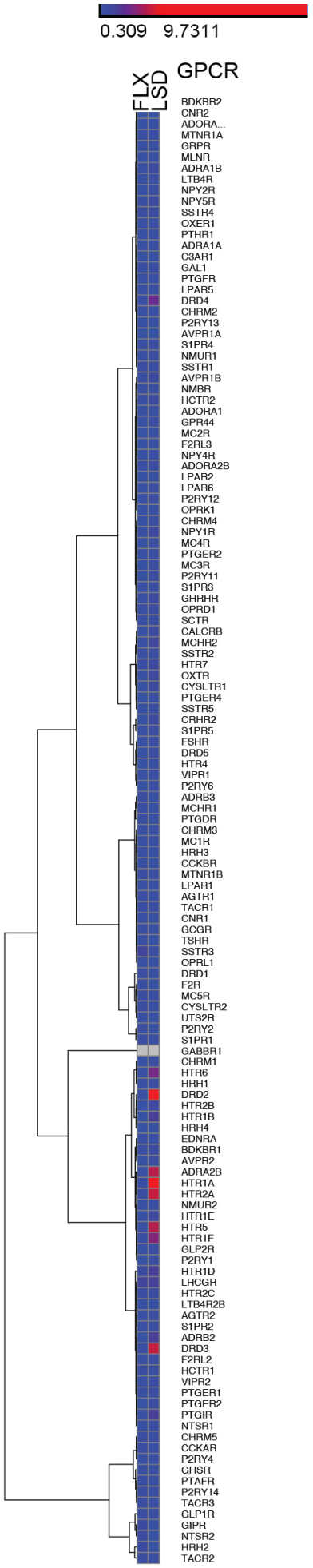



**Figure 3** 384-well drug plate design for stimulation. White wells are baseline controls with assay buffer; purple wells contain drug solution (6 μM). Columns 1 and 2 serve as negative controls to determine drug effects on non-transfected cells. Red wells are assay controls and are thus stimulated with cognate ligands (Quinpirole for $D_2$ and Vasopressin for $V_2$).




**SUPPLEMENTARY NOTE 3.**

Heat map of responses of 133 targets to 1 **μ**M concentrations of fluoxetine or LSD, with agonist activity shown in red.

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