Human native kappa opioid receptor functions not predicted by recombinant receptors: Implications for drug design.

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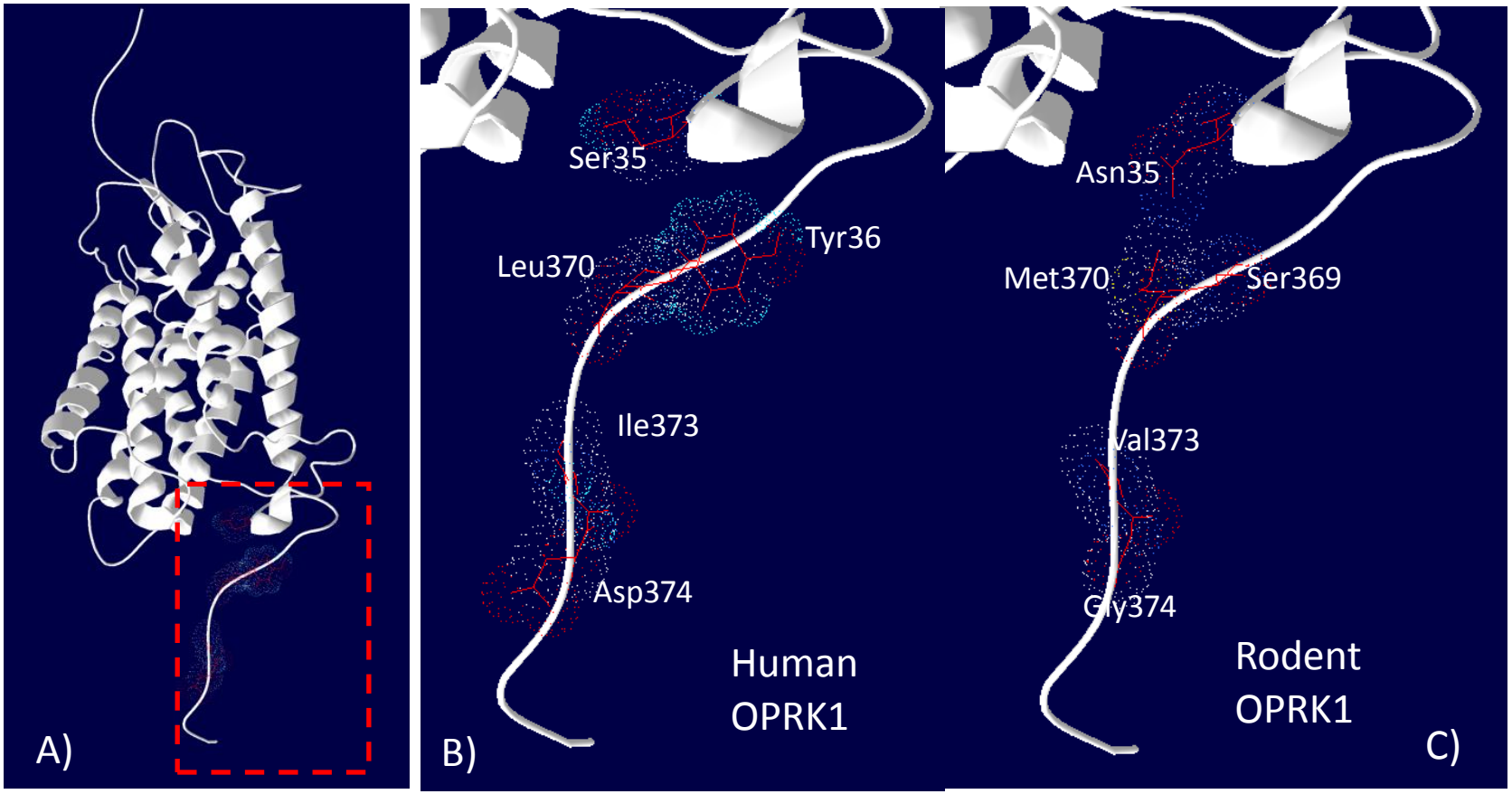
Supplementary Figure 1. A) Homology modelled structure of human OPRK1 (PDB id 2a0d), B) Highlighted human residues in the OPRK1 C-terminal region, C) Highlighted rodent residue substitutions in the OPRK1 C-terminal region



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Supplementary Figure 2. Pilot study to examine the abilities of single concentrations of different κ receptor agonists to inhibit cholinergically-mediated contractions of human descending colon evoked by electrical field stimulation. All EC_{50}s are for the human receptor and except where stated are taken from White, K.L. *et al*. (Identification of novel functionally selective κ-opioid receptor scaffolds. Mol. Pharmacol. 85, 83-90, 2014); the remainder (*) are data from the experiments documented in the body of the accompanying paper, measuring ability to recruit G Proteins (BRET) and internalise (TR-FRET) the receptor. Similarly, the data for ICI204448 and asimadoline, obtained using human colon in the presence of L-NAME 300 μM, are from the experiments found in the body of the accompanying paper. For the new experiments with human colon, dynorphin A 1-13, (-) U50488 and U62066 (each from Sigma, UK) were studied using tissue from two patients, again in the presence of L-NAME 300 μM: (1) Rectum, female, 33, cancer; (2) descending colon, male, 51, cancer. All strips displayed contractions during EFS (inhibited by κ agonism) and after-contractions (unaffected by each ligand).

Ligand	Recombinant human receptor		Human intestine			
	G protein pEC_{50}	Arrestin pEC_{50}	Concentration	N	% inhibition of contractions evoked during EFS	Time to max effect (minutes)
Dynorphin 1-13	8.68 ± 0.07	7.01 ± 0.07	1 μM	2	-30, -35	17, 11
(-) U50488	9.06 ± 0.07	9.09 ± 0.09	1 μM	2	-44, -74	31, 27
U62066	9.00 ± 0.05	8.21 ± 0.10	1 μM	2	-39, -50	24, 24
ICI204448	8.38 ± 0.09 9.6 ± 0.1*	8.48 ± 0.06 7.7 ± 0.1*	1 μM	6	-57 ± 10	13 ± 3
Asimadoline	9.2 ± 0.1*	7.4 ± 0.1*	1 μM	6	-29 ± 4	40 ± 4

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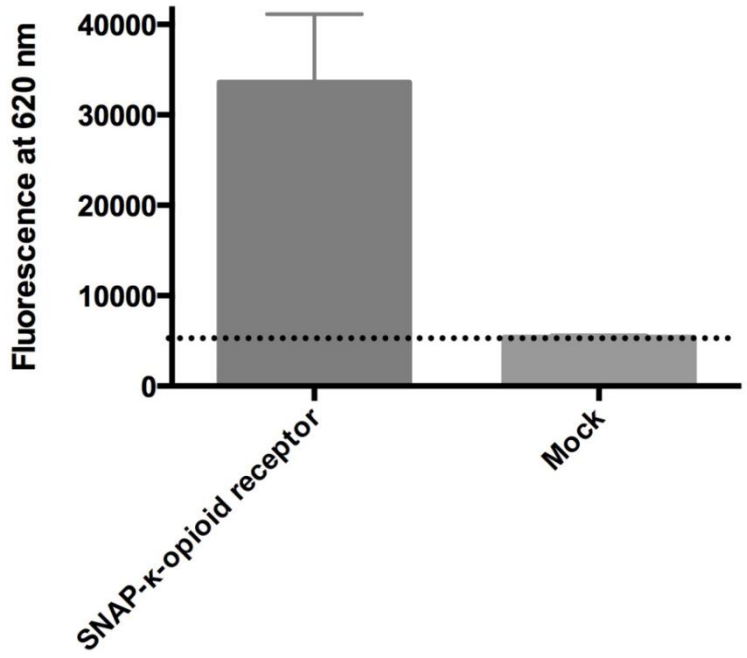
Supplementary Figure 3. Detection of SNAP-kappa-opioid receptor at the surface of HEK293 cells. As BG-Tb is not cell permeant, only receptors expressed at the cell surface and displaying the SNAP-tag outside the cell were labelled. Data are means ± SD of a triplicate.



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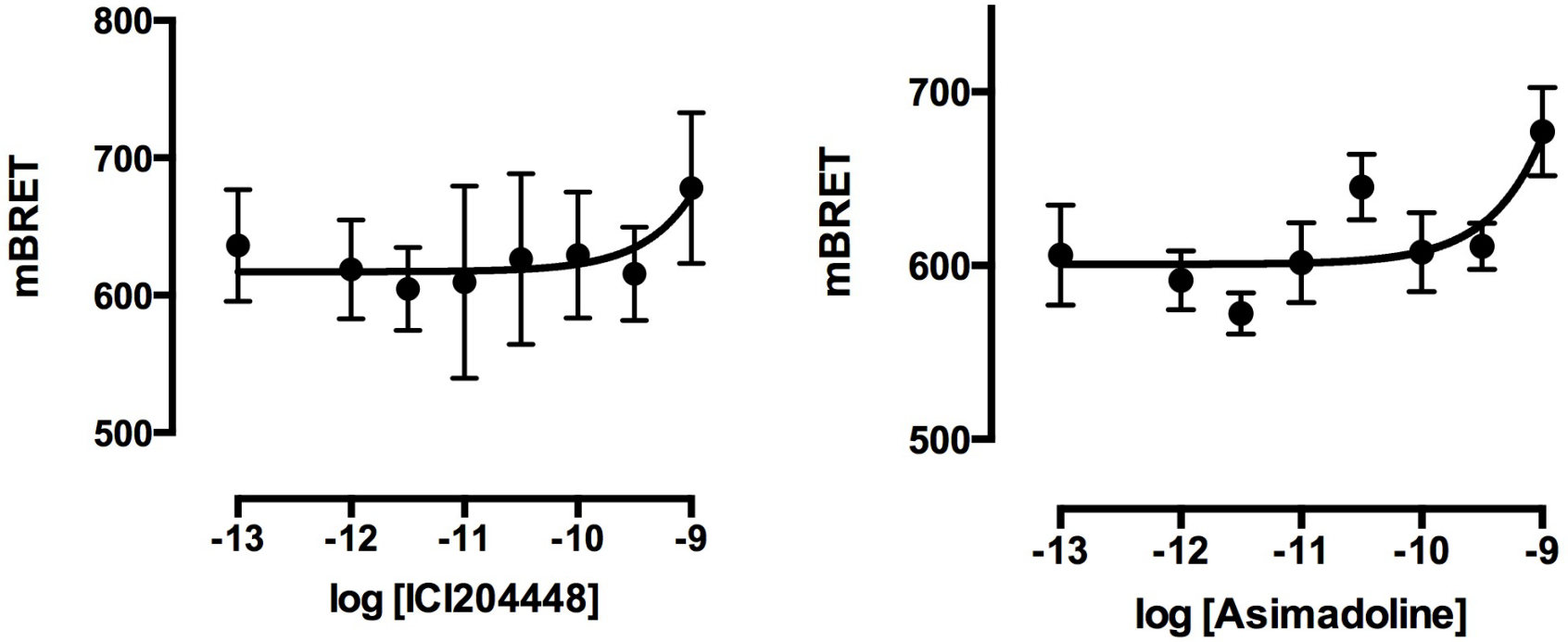
Supplementary Figure 4. BRET was measured in HEK293 cells co-expressing Gαi1-Rluc8, β2 and Venus-γ2 (no receptor). Cells were stimulated with increasing concentrations of ICI204448 and asimadoline. Data are means ± SD of a representative experiment.



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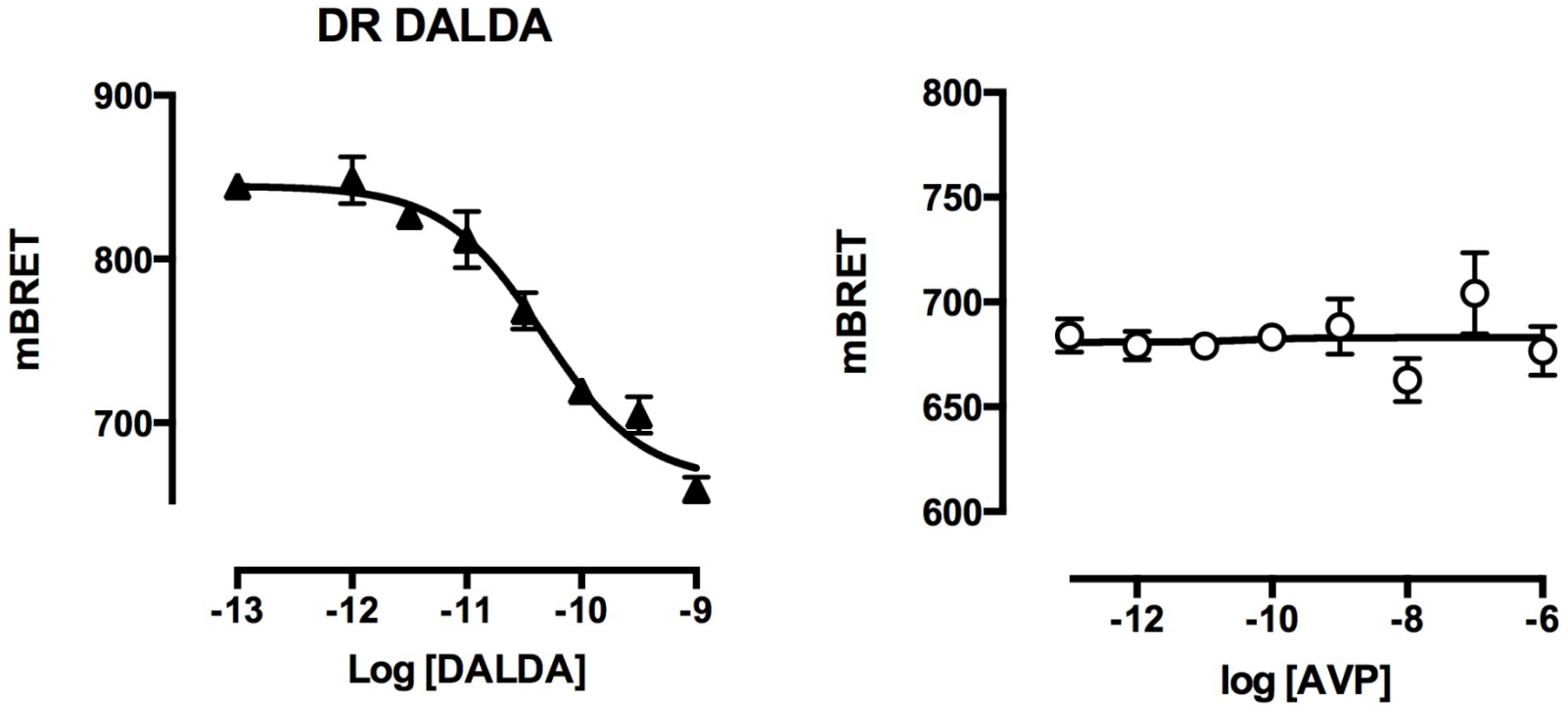
Supplementary Figure 5. (a) Dose response of DALDA on MOR (Gi-coupled receptor) and (b) AVP on V2 (Gs-coupled receptor). DALDA EC50 = 4.10-11M. Data are means ± SD of a representative experiment.



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Supplementary Figure 6. a) Time course of V2 internalization with 1µM AVP. b) Dose response of AVP on V2 at T = 40 min. AVP EC50 = 1.10-9M. Data are means ± SD of a representative experiment.