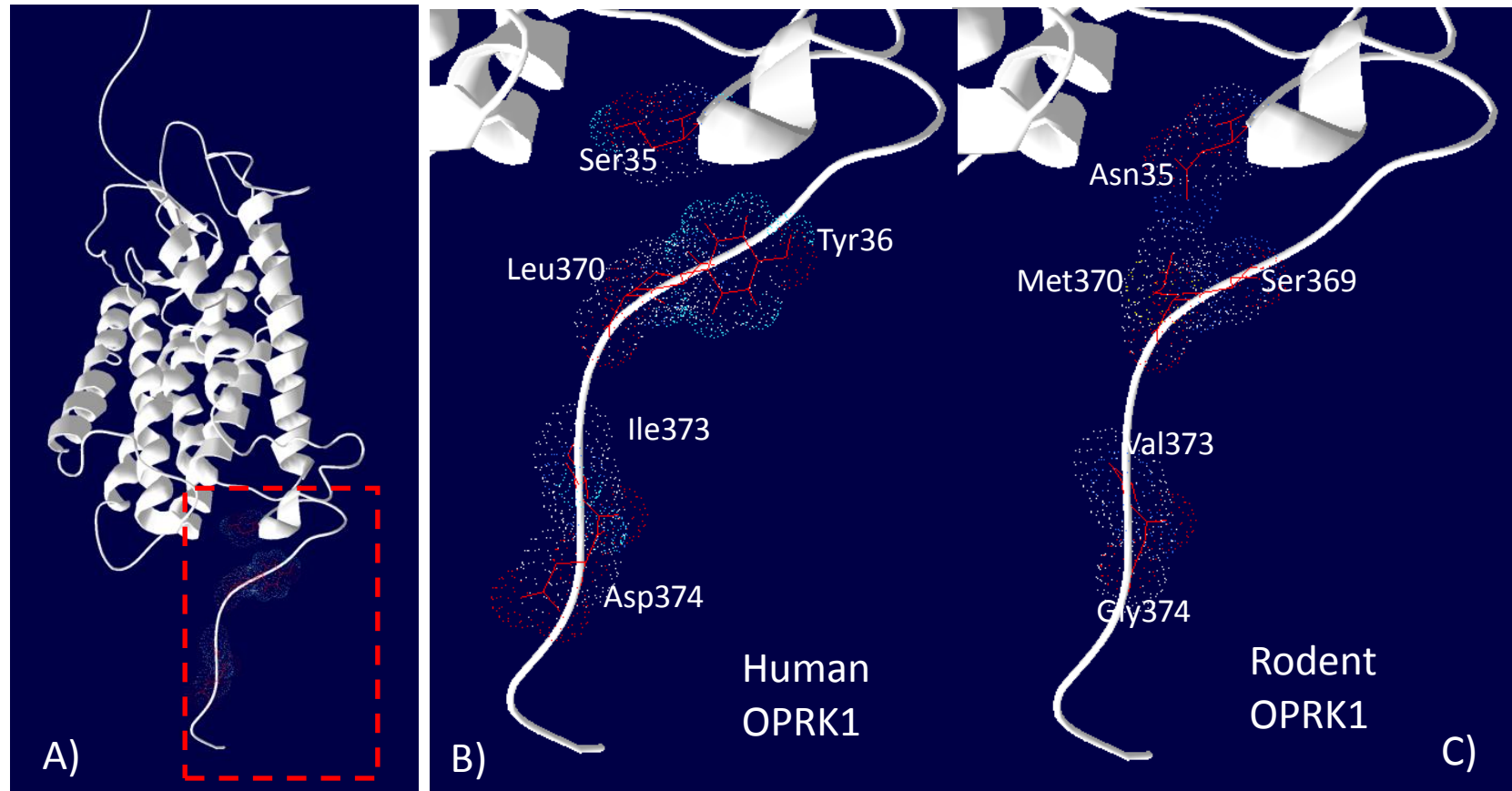


Human native kappa opioid receptor functions not predicted by recombinant receptors: Implications for drug design

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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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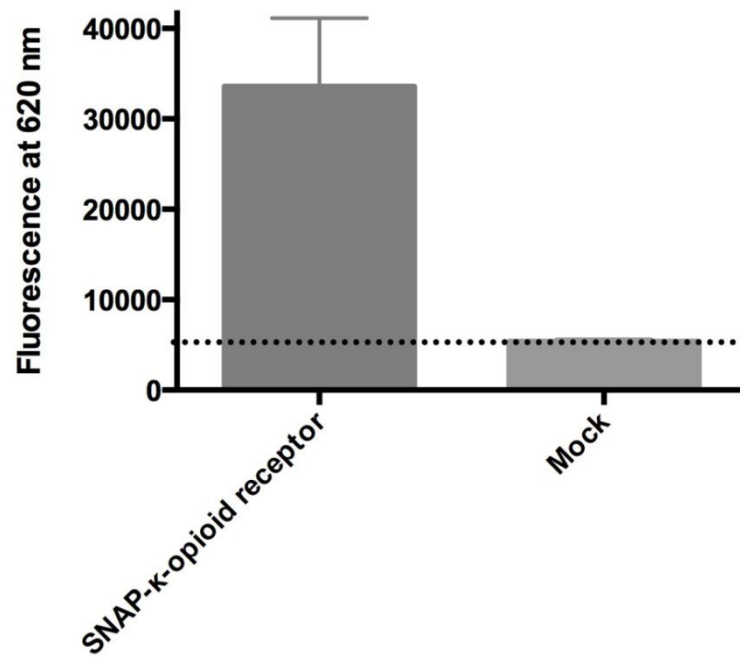
Supplementary Figure 2. Pilot study to examine the abilities of single concentrations of different κ receptor agonists to inhibit cholinergically-mediated contractions of human descending colon evoked by electrical field stimulation. All EC_{50} s are for the human receptor and except where stated are 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 μ M, 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 μ M: (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 \pm 0.07 | 7.01 \pm 0.07 | 1 μ M | 2 | -30, -35 | 17, 11 |
| (-) U50488 | 9.06 \pm 0.07 | 9.09 \pm 0.09 | 1 μ M | 2 | -44, -74 | 31, 27 |
| U62066 | 9.00 \pm 0.05 | 8.21 \pm 0.10 | 1 μ M | 2 | -39, -50 | 24, 24 |
| ICI204448 | 8.38 \pm 0.09 9.6 \pm 0.1* | 8.48 \pm 0.06 7.7 \pm 0.1* | 1 μ M | 6 | -57 \pm 10 | 13 \pm 3 |
| Asimadoline | 9.2 \pm 0.1* | 7.4 \pm 0.1* | 1 μ M | 6 | -29 \pm 4 | 40 \pm 4 |

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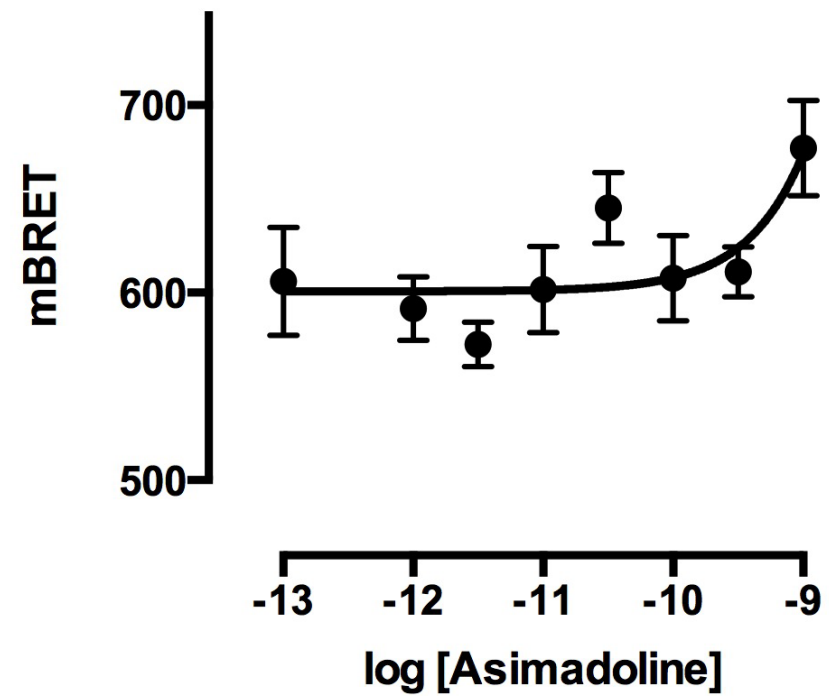
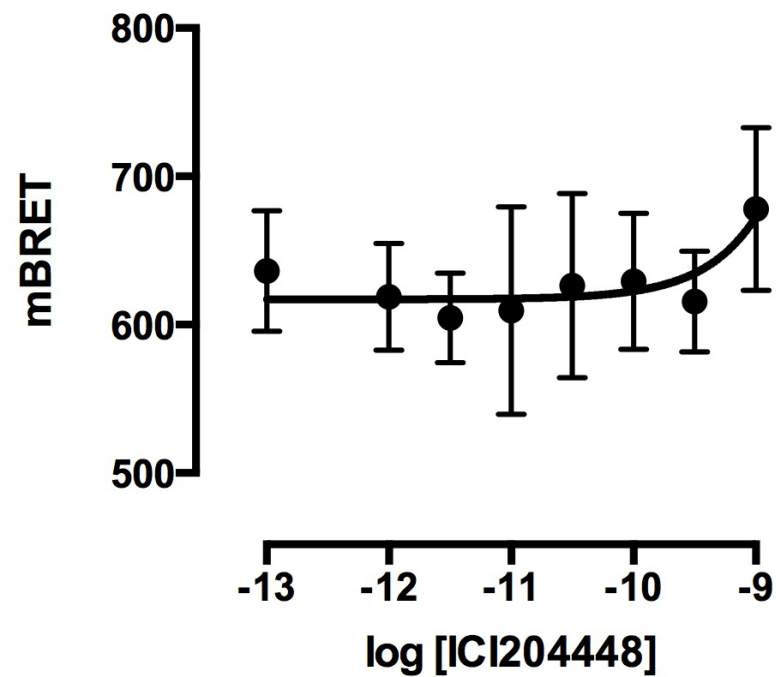
Supplementary Figure 3. Detection of SNAP-kappa-opioid receptor at the surface of HEK293 cells. As BG-Tb is not cell permeant, only receptors expressed at the cell surface and displaying the SNAP-tag outside the cell were labelled. Data are means \pm SD of a triplicate.



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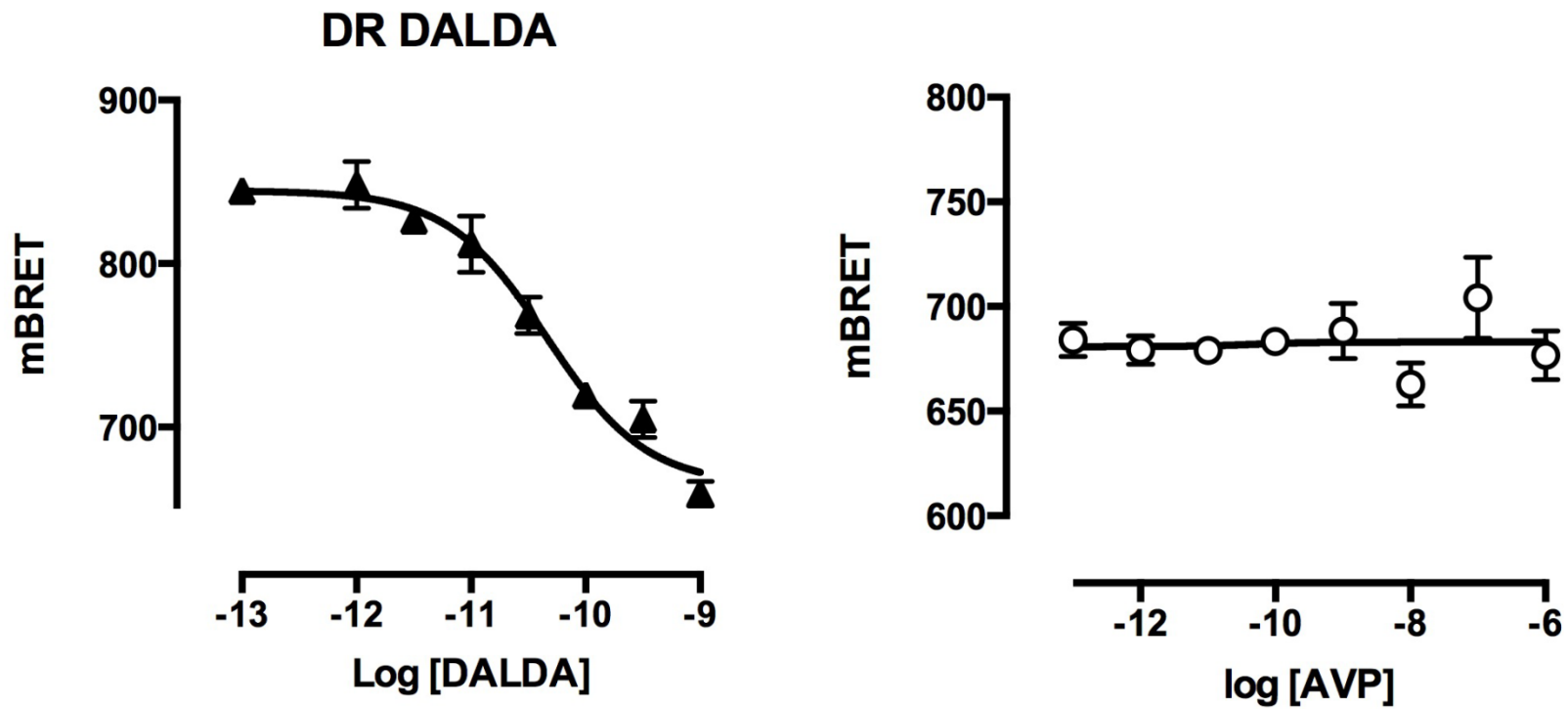
Supplementary Figure 4. BRET was measured in HEK293 cells co-expressing Gai1-Rluc8, $\beta 2$ and Venus- $\gamma 2$ (no receptor). Cells were stimulated with increasing concentrations of ICI204448 and asimadoline. Data are means \pm SD of a representative experiment.



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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 \pm SD of a representative experiment.



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

