Determinants of ligand subtype-selectivity at α₁A-adrenoceptor revealed using Saturation Transfer Difference (STD) NMR.

Kelvin J. Yong, Tasneem Vaid, Patrick J. Shilling, Feng-Jie Wu, Lisa M. Williams, Mattia Deluigi, Andreas Plückthun, Ross A.D. Bathgate, Paul R. Gooley*, and Daniel J. Scott*

Supplementary Figures

Supplementary Figure 1. Schematic diagram of the CHESS stabilization of α₁A-AR. A total of three mutagenesis rounds and 11 CHESS rounds were performed on α₁A-AR to generate a mutant library stable in n-Decyl-β-D-Maltopyranoside (DM) for 24 h apo-state at 4 °C.
Supplementary Figure 2. Schematic diagram of the CHESS stabilization of α₁B-AR. A total two mutagenesis rounds and six CHESS rounds were performed on α₁B-AR to generate a mutant library stable in n-Decyl-β-D-Maltopyranoside (DM) at 4 °C.
**Supplementary Figure 3. Primary screening of CHESS stabilized clones.** The specific binding of QAPB to (A) several α₁A-AR or (B) α₁B-AR selected clones after solubilization in DM for 3 h at 4 °C. Clones that did not express have been removed. Arrows indicate identical clones and the red dashed lines indicate the binding of unmodified receptors. Amino acid substitutions in selected (C) α₁A-AR or (D) α₁B-AR mutants. The sequence position is indicated by AA#, and the domain location of mutations listed (N-terminus (N-Term), transmembrane domains (TM), extracellular loops (ECL), intracellular loops (ICL), helix 8 (H8) and C-terminus (C-Term)). GPCRdb numbering of each position is also listed {Isberg, 2015 #1477}. 
Supplementary Figure 4. Identification of stabilized $\alpha_1$-ARs. (A) Secondary screening of seven unique $\alpha_{1A}$-AR mutants evolved using CHESS for stability after DM solubilization for 5 h (black bars) and 27 h (white bars) in apo-state. (B) Secondary screening of 12 unique $\alpha_{1B}$-AR mutants evolved using CHESS for stability after DDM solubilization for 5 h (black bars) and 27 h (white bars) in apo-state.
Supplementary Figure 5. Location of mutations mapped onto a homology model of α1A-AR A4. TM5 and TM6, which do not contain any mutations, are not shown.
Supplementary Figure 6. Location of mutations mapped onto a homology model of $\alpha_{1B}$-AR #15. Most of TM5 and the top of TM6, which do not contain any mutations, are not shown.
**Supplementary Figure 7. Characterization of stabilized α1-ARs.** (A) Saturation binding of QAPB to purified α1A-AR A4 (red solid circles) and α1B-AR #15 (open blue squares). (B) Saturation binding of 3H-prazosin to COS-7 cells overexpressing: WT α1A-AR (red solid circles); WT α1B-AR (solid blue squares) or (C); α1A-AR A4 (red open circles); and α1B-AR #15 (blue open squares). (D) Phenylephrine induced activation of a cAMP response element (CRE) reporter gene in COS-7 cells transfected with WT α1A-AR (red solid circles) and WT α1B-AR (solid blue squares) but not in cells expressing α1A-AR A4 (red open circles) or α1B-AR #15 (blue open squares). Data are mean ± SEM from three independent experiments. Data are mean ± SEM from three independent experiments.
Supplementary Figure 8. (A) QAPB competition binding against purified $\alpha_{1A}$-AR A4 (black circles and lines) and $\alpha_{1A}$-AR F3 (grey circles and lines). (B) QAPB competition binding against purified $\alpha_{1B}$-AR #15 (dark blue triangles and lines) and $\alpha_{1B}$-AR #15 (Y191D, G194E) (pale blue triangles and lines). Open symbols and dashed lines in (A) and (B) indicate competition with phenylephrine (Phe), whereas solid symbols and lines are from competition with prazosin (Praz).
Supplementary Figure 9. No receptor STD NMR control. 1D proton spectra of the aromatic protons of epinephrine in DDM buffer with (A) no receptor and (B) with receptor. STD NMR spectra of the aromatic protons of epinephrine in DDM buffer with (C) no receptor and (D) with receptor.
Supplementary Figure 10. (A) Saturation binding of QAPB to purified $\alpha_{1A}$-AR A4 (A189S) (red solid circles) and $\alpha_{1B}$-AR #15 (S208A) (solid blue squares).