Determinants of ligand subtype-selectivity at α_{1A}-adrenoceptor revealed using Saturation Transfer Difference (STD) NMR.

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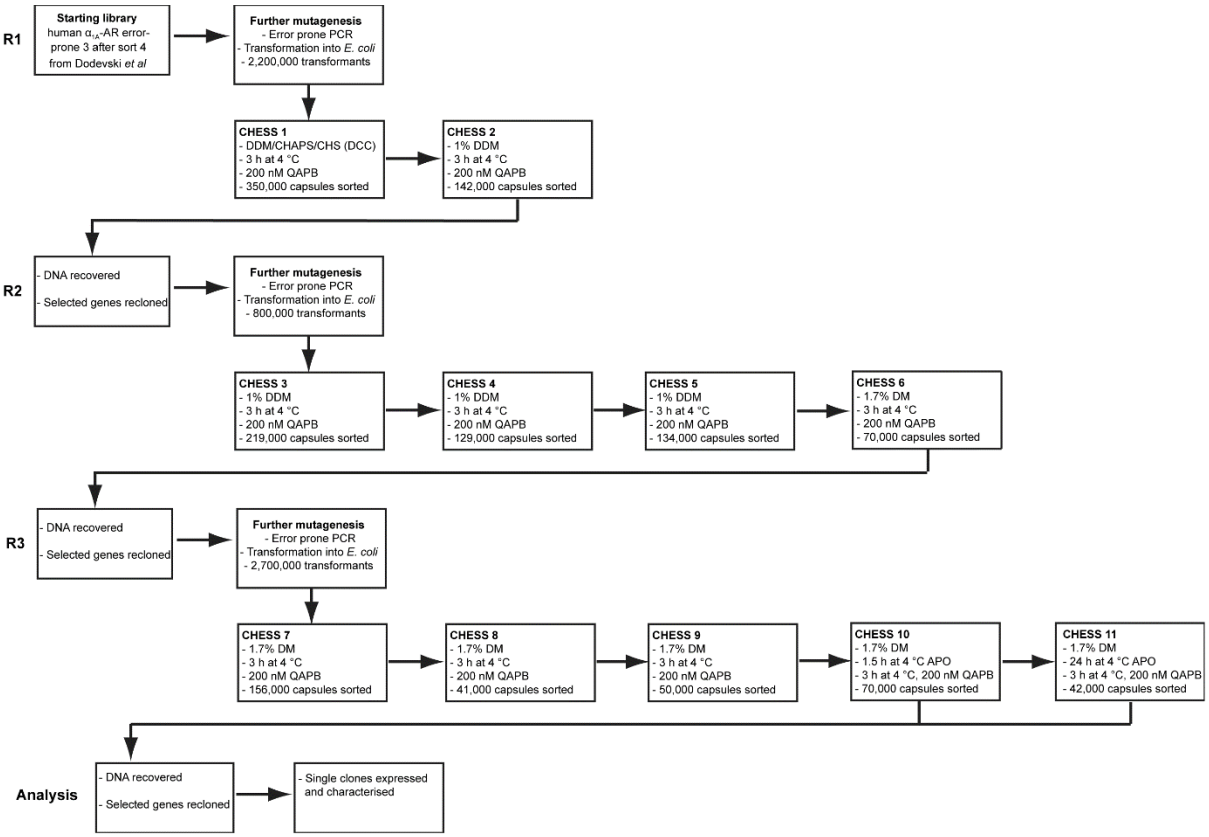
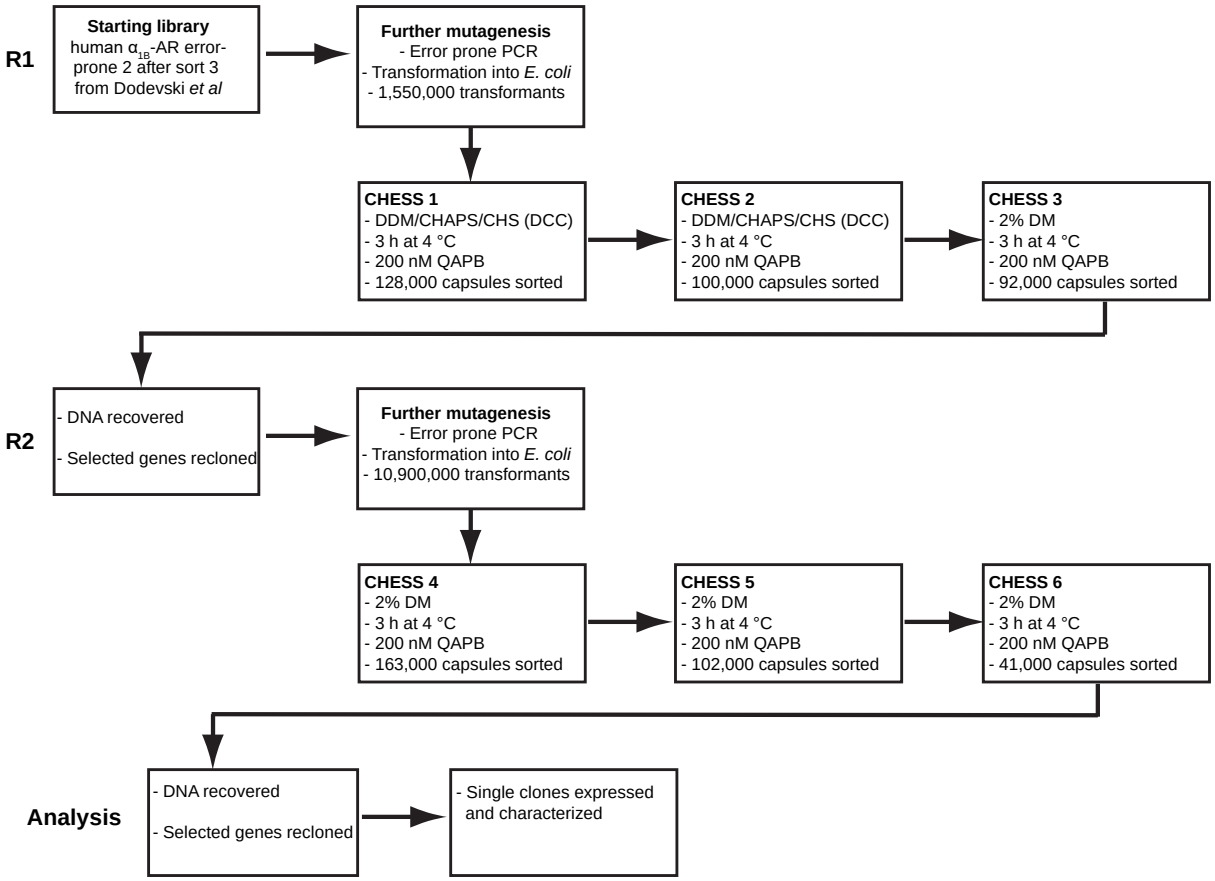
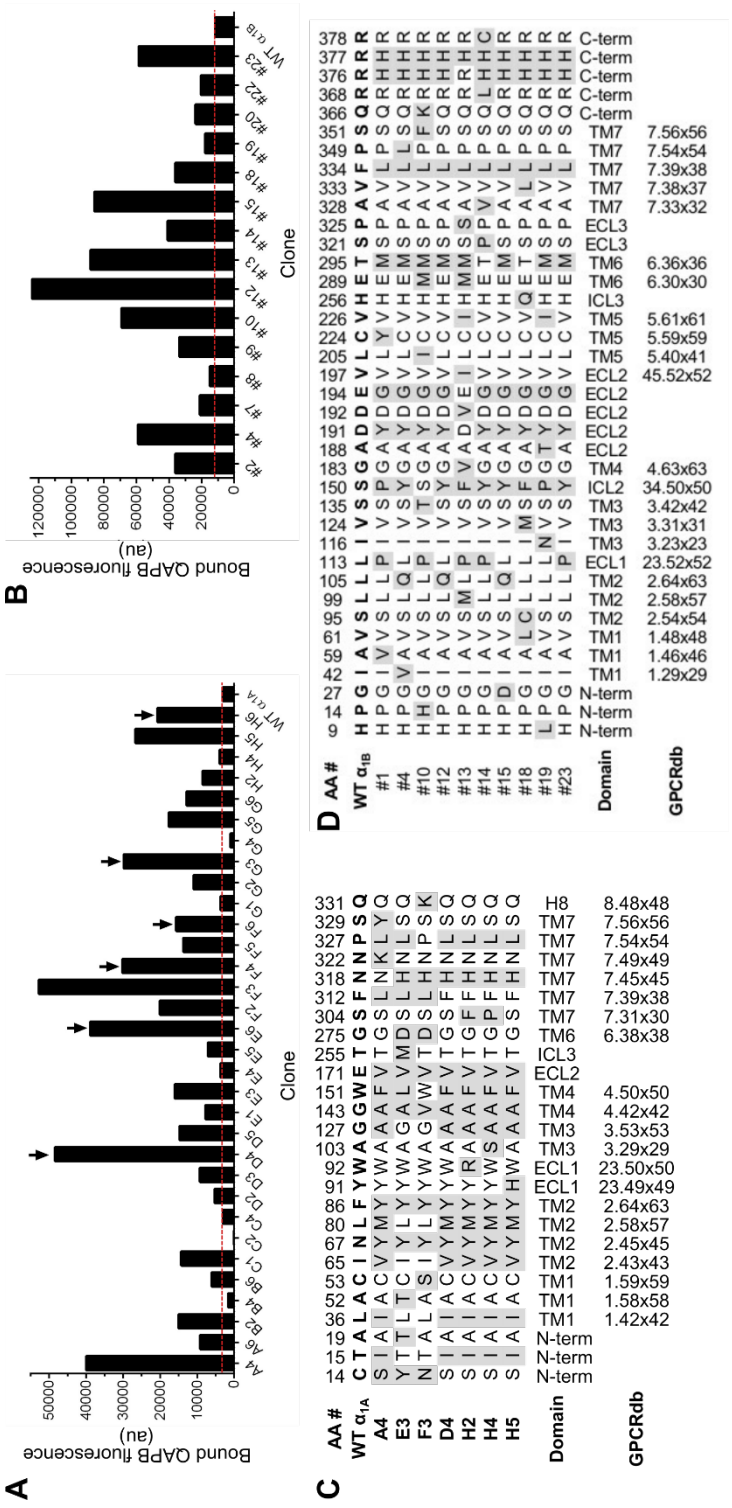
Supplementary Figures



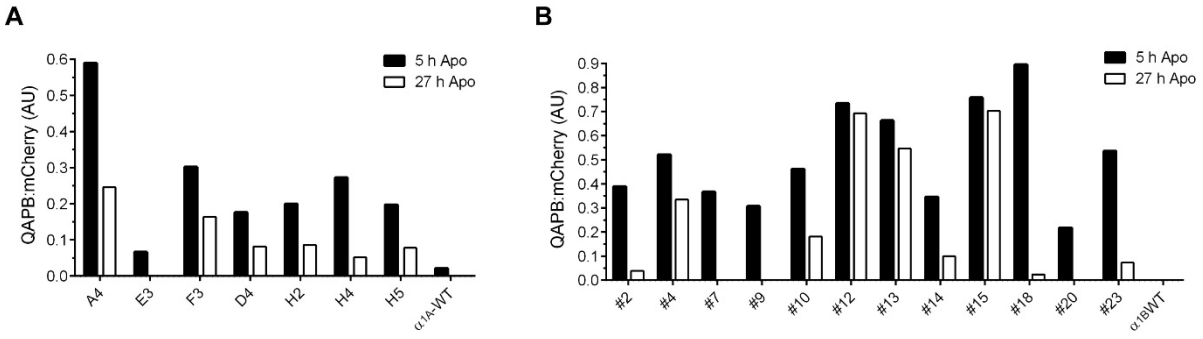
Supplementary Figure 1. Schematic diagram of the CHESS stabilization of α_{1A}-AR. A total of three mutagenesis rounds and 11 CHESS rounds were performed on α_{1A}-AR to generate a mutant library stable in n-Decyl-β-D-Maltopyranoside (DM) for 24 h apo-state at 4 °C.



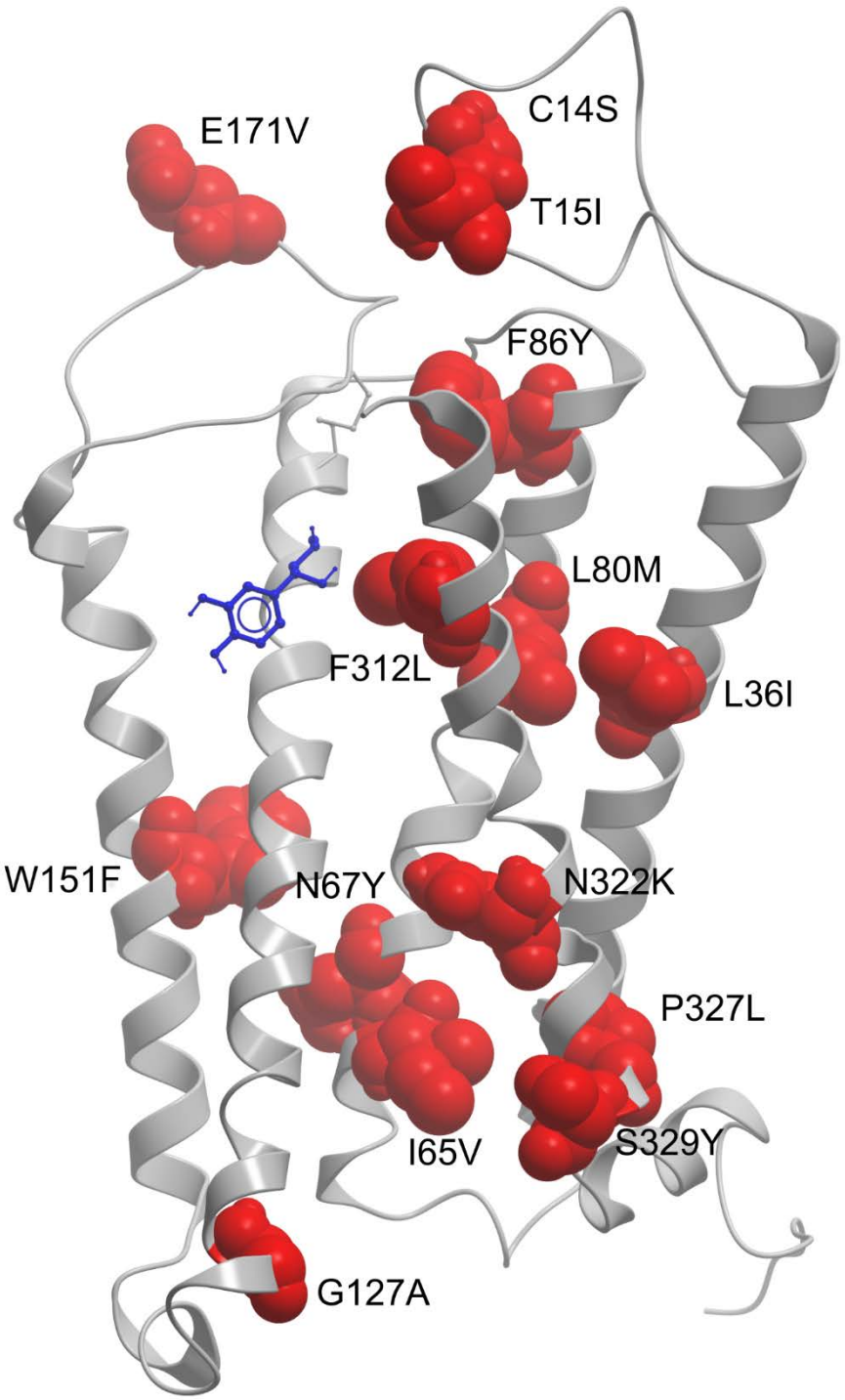
Supplementary Figure 2. Schematic diagram of the CHESS stabilization of α_{1B}-AR. A total two mutagenesis rounds and six CHESS rounds were performed on α_{1B}-AR to generate a mutant library stable in n-Decyl-β-D-Maltopyranoside (DM) at 4 °C.



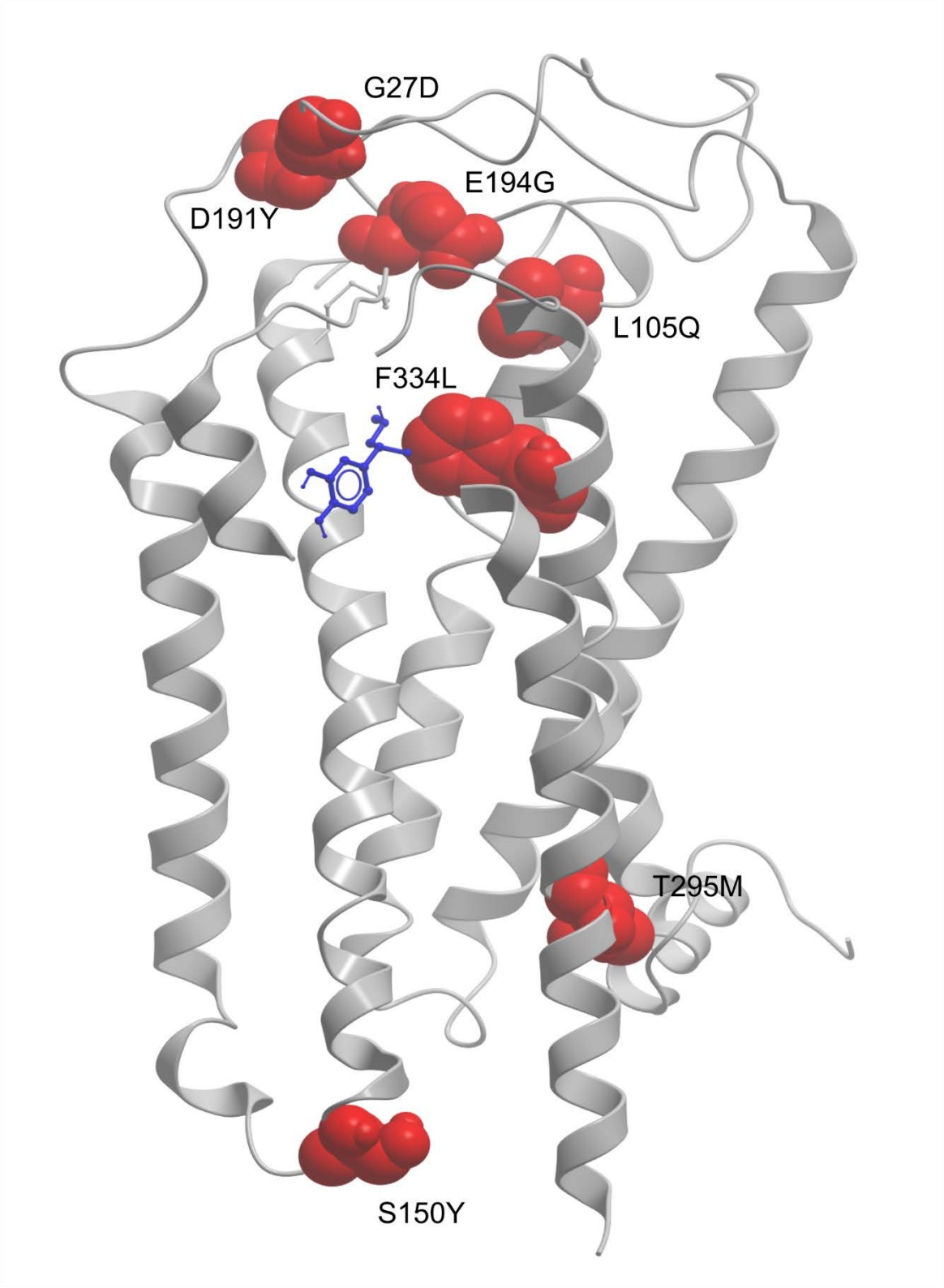
Supplementary Figure 3. Primary screening of CHESS stabilized clones. The specific binding of QAPB to (A) several α_{1A}-AR or (B) α_{1B}-AR selected clones after solubilization in DM for 3 h at 4 °C. Clones that did not express have been removed. Arrows indicate identical clones and the red dashed lines indicate the binding of unmodified receptors. Amino acid substitutions in selected (C) α_{1A}-AR or (D) α_{1B}-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}.



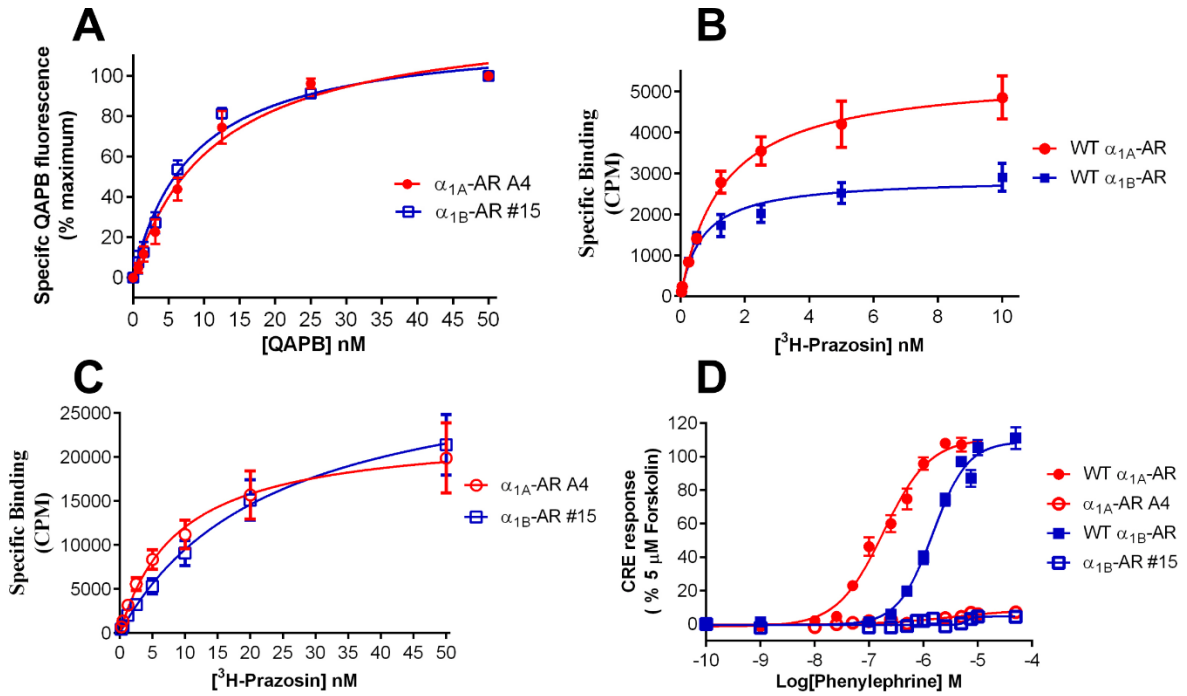
Supplementary Figure 4. Identification of stabilized α_1-ARs. (A) Secondary screening of seven unique α_{1A}-AR mutants evolved using CHESS for stability after DM solubilization for 5 h (black bars) and 27 h (white bars) in apo-state. (B) Secondary screening of 12 unique α_{1B}-AR mutants evolved using CHESS for stability after DDM solubilization for 5 h (black bars) and 27 h (white bars) in apo-state.



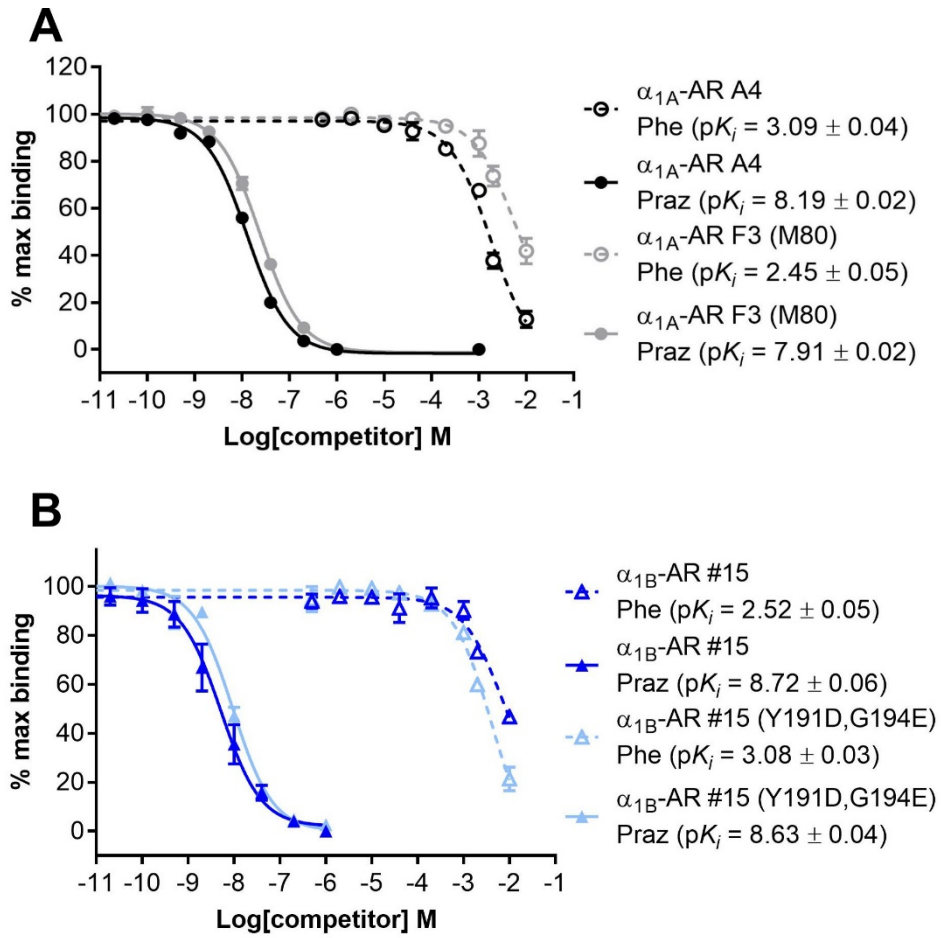
Supplementary Figure 5. Location of mutations mapped onto a homology model of α_{1A}-AR A4. TM5 and TM6, which do not contain any mutations, are not shown.



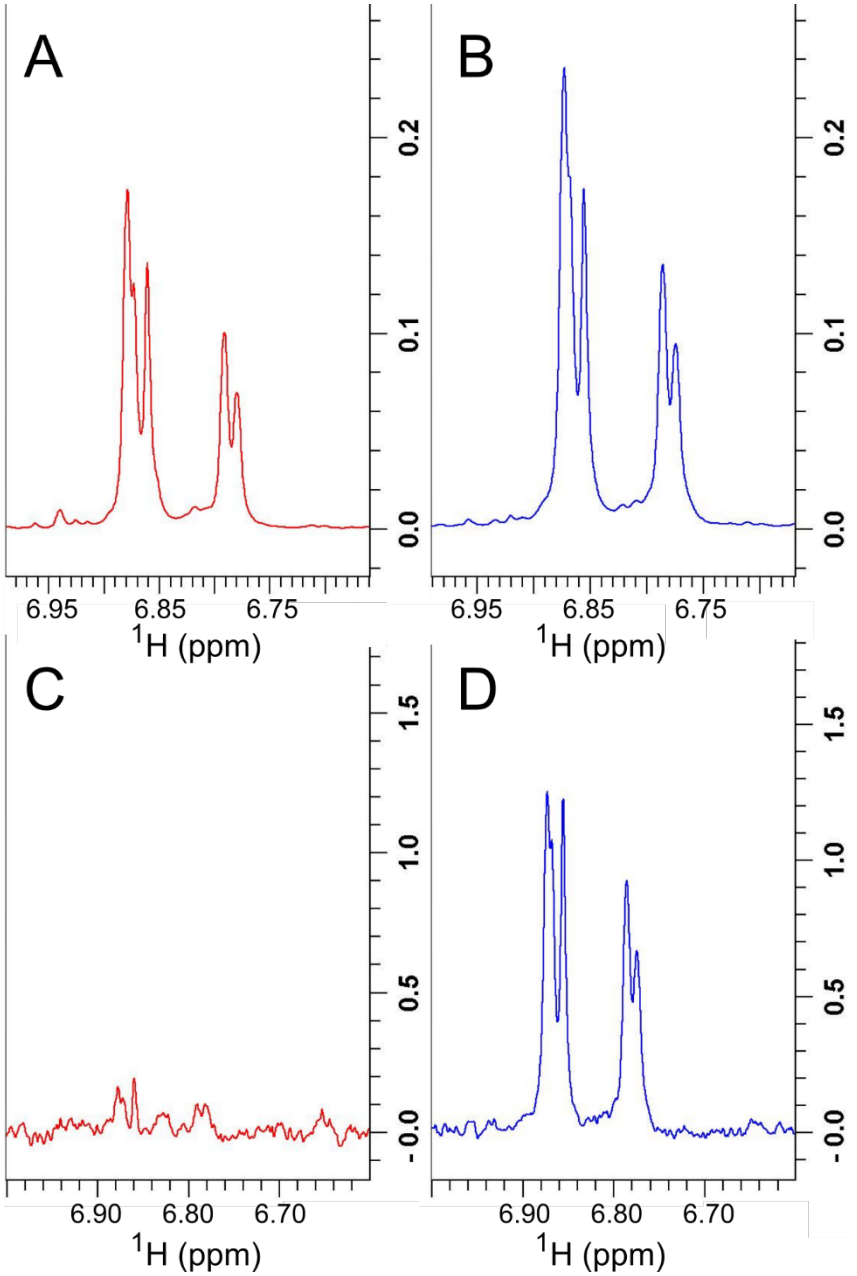
Supplementary Figure 6. Location of mutations mapped onto a homology model of α_{1B}-AR #15. Most of TM5 and the top of TM6, which do not contain any mutations, are not shown.



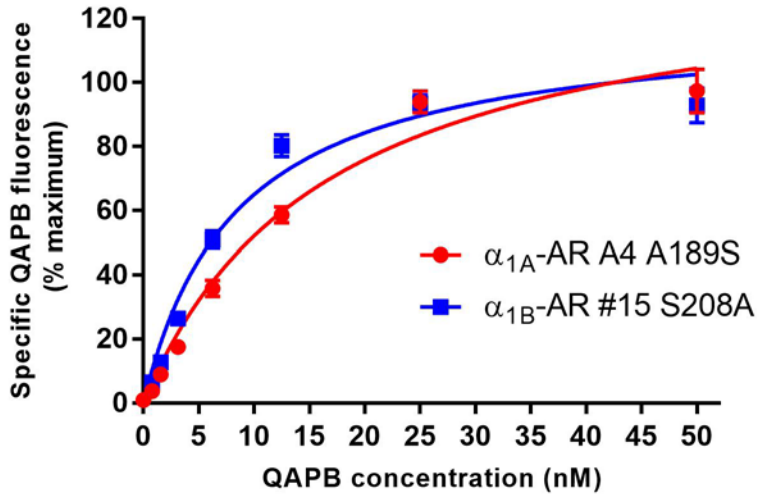
Supplementary Figure 7. Characterization of stabilized α1-ARs. (A) Saturation binding of QAPB to purified α_{1A}-AR A4 (red solid circles) and α_{1B}-AR #15 (open blue squares). (B) Saturation binding of ^{3}H-prazosin to COS-7 cells overexpressing: WT α_{1A}-AR (red solid circles); WT α_{1B}-AR (solid blue squares) or (C); α_{1A}-AR A4 (red open circles); and α_{1B}-AR #15 (blue open squares). (D) Phenylephrine induced activation of a cAMP response element (CRE) reporter gene in COS-7 cells transfected with WT α_{1A}-AR (red solid circles) and WT α_{1B}-AR (solid blue squares) but not in cells expressing α_{1A}-AR A4 (red open circles) or α_{1B}-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 α_{1A}-AR A4 (black circles and lines) and α_{1A}-AR F3 (grey circles and lines). (B) QAPB competition binding against purified α_{1B}-AR #15 (dark blue triangles and lines) and α_{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 α_{1A}-AR A4 (A189S) (red solid circles) and α_{1B}-AR #15 (S208A) (solid blue squares).