

Supplementary Information

Binding kinetics drive G protein subtype selectivity at the β_1 -adrenergic receptor

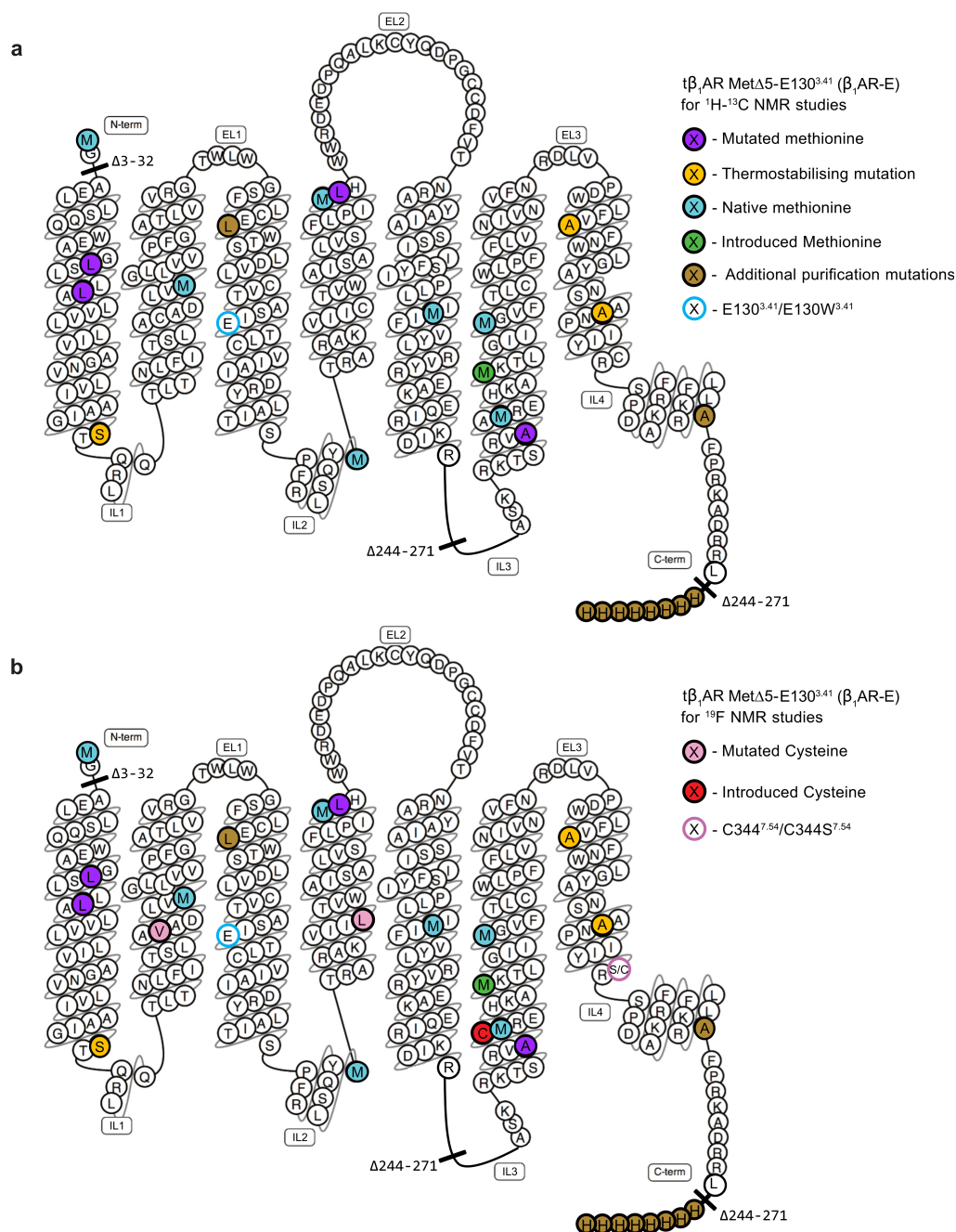
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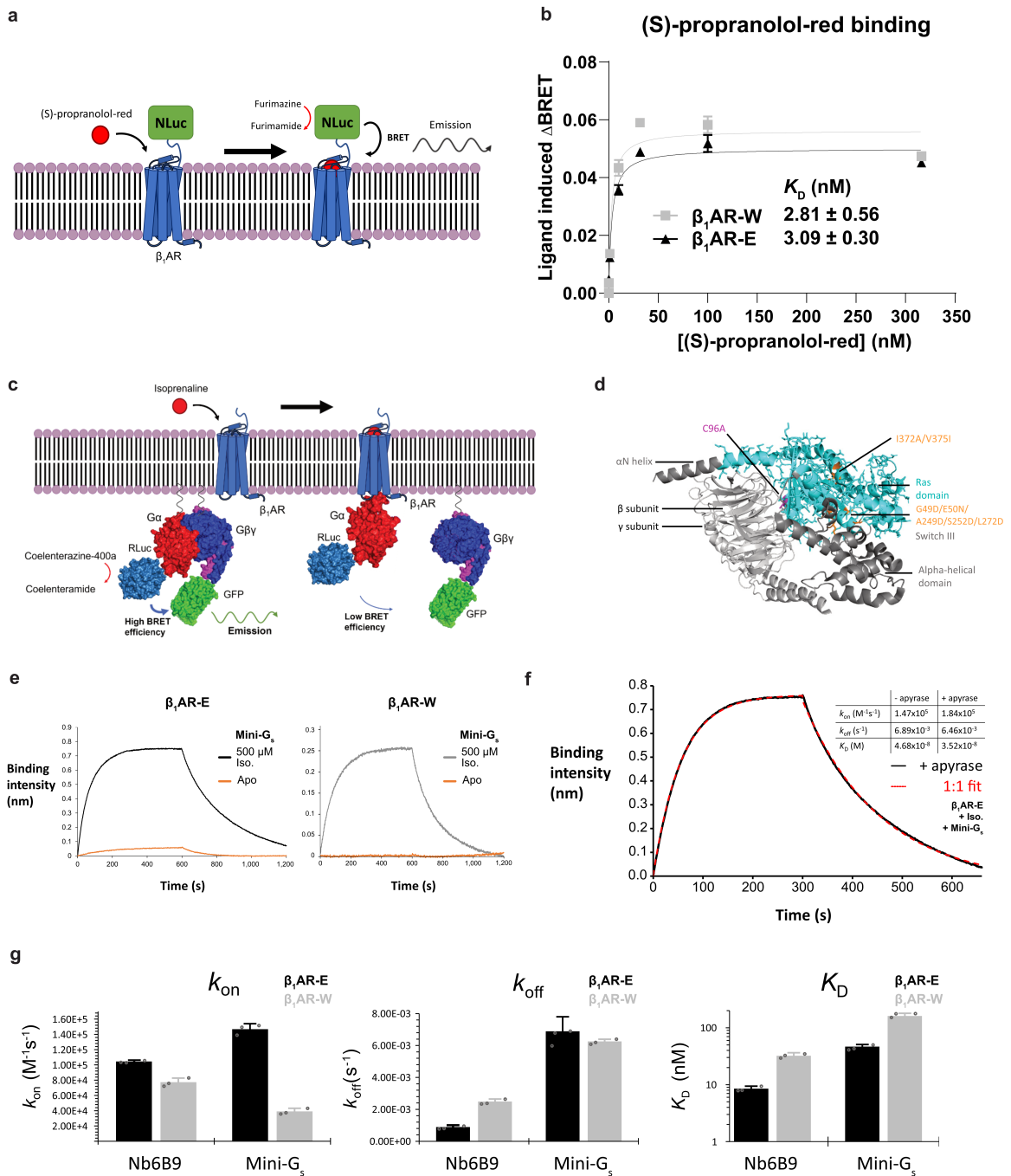
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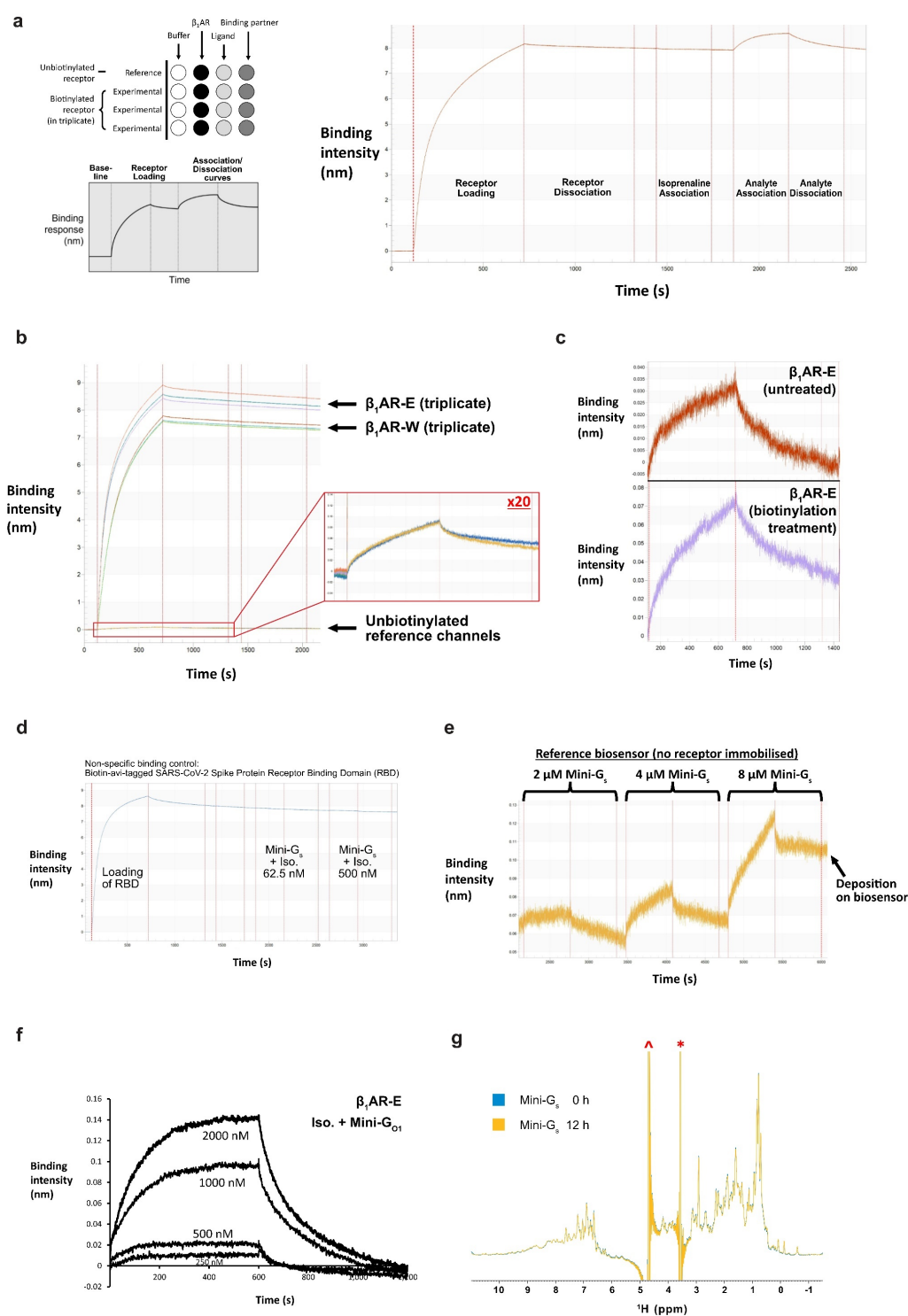


Supplementary Figure 1. Snake diagrams of the β_1AR constructs. a) The β_1AR construct used in 1H - ^{13}C HMQC NMR experiments and BLI assays. Intracellular loop (IL) and extracellular loop (EL) regions are indicated. All mutations are relative to the native turkey β_1AR sequence. β_1AR -E and β_1AR -W differed by mutation of position 130^{3.41}, shown with a light blue ring in TM3. 1H - ^{13}C NMR studies of β_1AR -E and β_1AR -W used constructs with the mutated methionine residues (purple), thermostabilising mutations (orange), and purification mutations (brown). Native methionine probes are shown in blue. NMR experiments using L289M^{6.34} had the introduced methionine mutation (green) present. BLI studies of β_1AR -E and β_1AR -W utilised the same constructs as 1H - ^{13}C NMR studies with an additional N-terminal avi-tag (GLNDIFEAQKIEWHE) between residues M1 and G2. **b)** The β_1AR construct used in ^{19}F NMR experiments. The constructs were the same as the β_1AR -E and β_1AR -W constructs used in 1H - ^{13}C NMR studies with additional mutations for ^{19}F labelling. TM7 labelling ($^{TET}C344^{7.54}$) used the mutated cysteines (pink) and native C344^{7.54} (pink ring). TM6 labelling ($A282C^{BTFA.6.27}$) used the mutated cysteines, introduced cysteine (red), and mutated C344S^{7.54} (pink ring). See methods for details of residue positions and explanations for mutations shown in **a)** and **b)**.



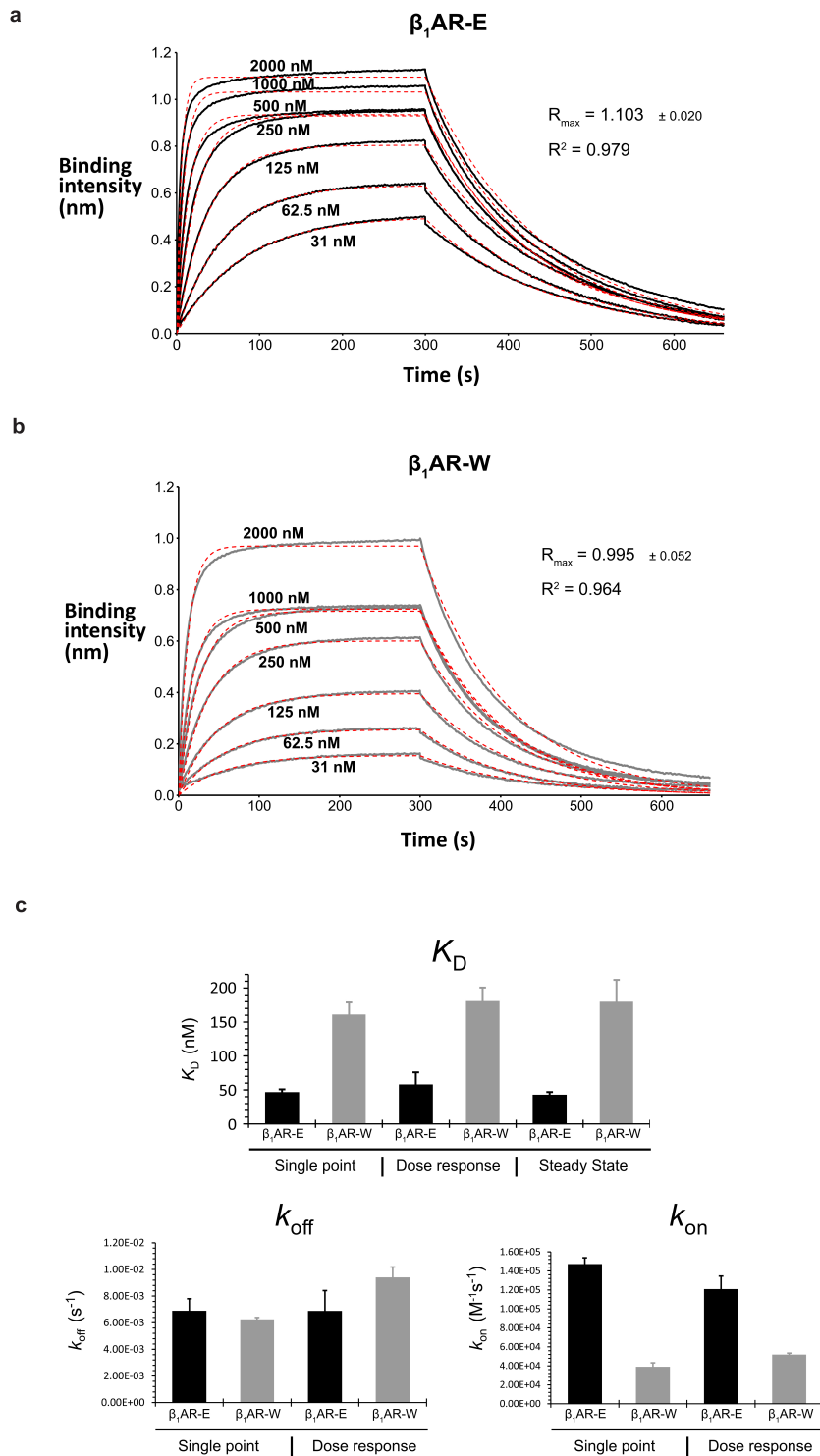
Supplementary Figure 2. Schemes for *in cell* validation of β_1AR NMR constructs, and validation of *in vitro* mini-G protein binding. a) A scheme for the luminescence-based ligand binding assay for determination of ligand K_i to β_1AR . N-Luciferase (NLuc) tagged β_1AR binds to (S)-propranolol-red¹. Catalysis of furimazine to furimamide by NLuc generates luminescence which is transferred by BRET to (S)-propranolol-red and emitted. Titration with non-BRET active ligand displaces (S)-propranolol-red and reduces the BRET signal, facilitating binding curve generation and K_i calculations². **b)** (S)-propranolol binding to the β_1AR constructs. K_D values are shown in the inset. **c)** A scheme of the TRUPATH assay³. $G\alpha$ and $G\beta\gamma$ proteins are labelled with R-Luc8 and GFP2, respectively. Binding of ligand to receptor induces receptor activation and G protein binding. Dissociation of the $G\alpha$ and $G\beta\gamma$ subunits reduces the BRET efficiency, therefore reducing emission intensity. Titration of ligand facilitates generation of EC_{50} curves. **d)** A structural comparison of $G\alpha_s$ (PDB: 6EG8)⁴ with mini-Gs protein⁵ showing conserved regions, deletions, and mutations. Cyan, conserved sequence, grey, deleted sequence, orange, point mutations, magenta, C96A mutation, white, $G\beta\gamma$ subunit. **e)** BLI binding curves showing the ligand dependence

of mini-G_s binding to β_1 AR constructs. Left, β_1 AR-E (black, 500 μ M isoprenaline (Iso.), orange, apo (no ligand)), right, β_1 AR-W (grey, 500 μ M isoprenaline, orange, apo (no ligand)). The mini-G_s concentration used was 2 μ M. **f)** BLI binding curves showing the effect of apyrase incubation on β_1 AR-E binding to mini-G_s in the presence of isoprenaline. Black solid line, mini-G_s binding following incubation with apyrase to remove bound GDP (overnight), red dotted line, 1:1 interaction stoichiometry model curve. Inset, equivalent kinetic data for binding curve compared to conditions without apyrase. **g)** BLI kinetic data for binding of isoprenaline-bound β_1 AR to Nb6B9, compared with binding to mini-G_s. Black, β_1 AR-E, grey, β_1 AR-W. Values were the average of triplicates (n=3 individual repeats). Error bars represent $\pm$ one standard deviation. Note the logarithmic axis for K_D data.



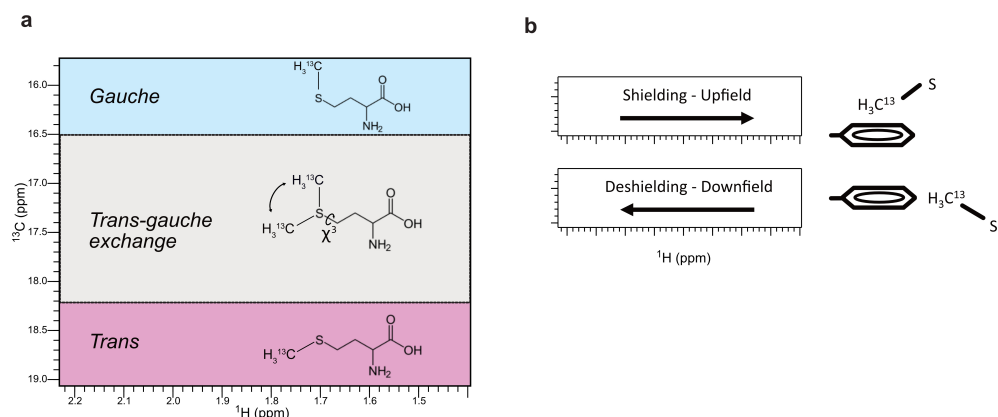
Supplementary Figure 3. Controls for β_1 AR-E BLI assays. **a)** A scheme and data to show BLI protocols. Left, a scheme showing the BLI experiment and wells. See methods for details. Right, data showing an example of loading of β_1 AR-E and generation of binding curves with isoprenaline and mini- G_s . The solid red line shows the binding response. Dotted vertical red lines separate each step. Note the dissociation step after receptor loading indicates biotinylated receptor is specifically immobilised onto the streptavidin biosensors. **b)** An example of biotinylated avi- β_1 AR-E and avi- β_1 AR-W loading onto streptavidin (SA) biosensors in triplicate. The inset boxed in red (y-axis enlarged 20x) demonstrates association of the unbiotinylated receptor controls is minimal, indicating biotin-specific loading onto the biosensors. **c)** Loading controls and biotinylation specificity controls for β_1 AR-E shows avi-tag specific biotinylation and minimal non-specific loading onto SA biosensors. Top, β_1 AR-E without the avi-tag and without biotinylation treatment. Bottom, β_1 AR-E without the avi-tag and with biotinylation treatment. **d)** Mini- G_s shows no binding to unrelated protein SARS-CoV2 Spike protein Receptor Binding

Domain (RBD). Blue, BLI trace showing loading of an avi-tagged and biotinylated RBD protein (300 nM) onto SA tips in β_1 AR BLI buffer, and association and dissociation steps assessing mini- G_s (62.5 and 500 nM) association, with isoprenaline (500 μ M). There is negligible interaction between the ligand and analyte, supporting a specific interaction between β_1 AR and mini- G_s . **e**) Concentration range determination. The reference biosensor (unbiotinylated avi- β_1 AR-E in the loading step) was dipped into wells containing mini-G analytes. Deposition of analyte on the biosensor is clearly visible at 8 μ M, limiting the concentration range for BLI experiments. Shown is an example for mini- G_s . **f**) BLI dose-response trial for mini- G_{o1} binding to β_1 AR-E in the presence of 500 μ M isoprenaline. The binding response is too small to reliably calculate binding affinities below 1000 nM. **g**) ^1H NMR assessment of analyte stability. Shown is a representative experiment with mini- G_s . Protein was incubated at 298 K for 12 h, and 1D ^1H NMR experiments were recorded at 0 h (blue) and 12 h (orange) time points. ^ indicates suppressed water signal, * indicates TrisHCl buffer signal.



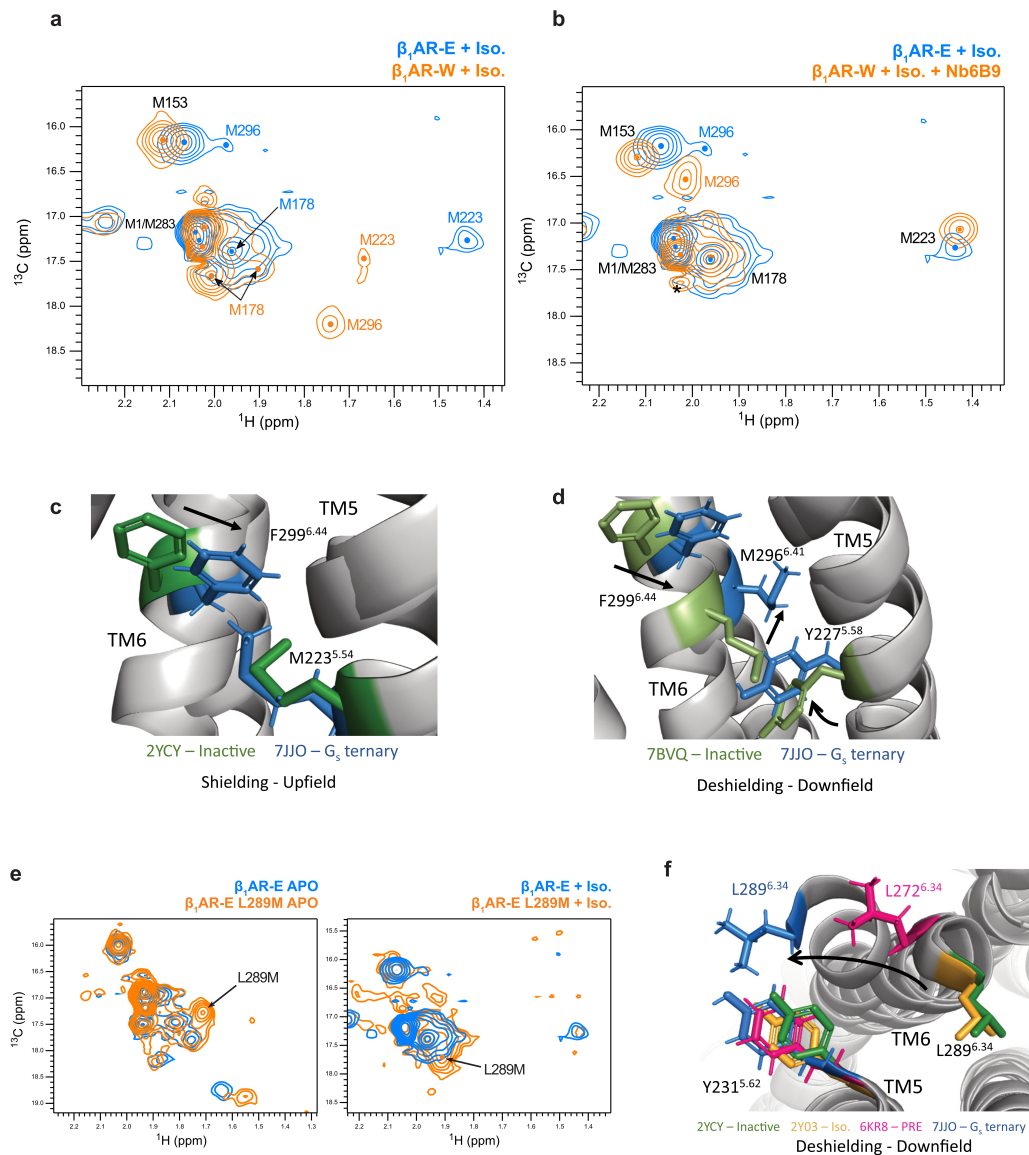
Supplementary Figure 4. Dose-response BLI experiments for $\beta_1\text{AR}$ binding to mini- G_s . **a)** A BLI dose-response experiment for $\beta_1\text{AR-E}$ binding to mini- G_s in the presence of isoprenaline. Raw data is shown as a solid black line, and 1:1 global fitting with $R_{\max}$ unlinked by sensors are shown in dashed red lines. Concentrations of mini- G_s are shown on the isotherms. The isoprenaline concentration was 500 μM . The curves were used to calculate K_D values using the 1:1 global fitting or by steady state response analysis. Steady state response curves were used to calculate $R_{\max}$ values, shown on the right with corresponding R^2 value. **b)** A BLI dose-response experiment for $\beta_1\text{AR-W}$ binding to mini- G_s in the presence of isoprenaline. Details are as described for **a)**. For **a)** and **b)**, note the differences in $R_{\max}$ between the two constructs may relate to the slightly smaller loading of $\beta_1\text{AR-W}$ relative to $\beta_1\text{AR-E}$ (Supplementary Fig. 3b). **c)** A comparison of K_D , k_{on} , and

k_{off} values calculated from optimised single concentration isotherms, dose-response curves with global fitting, and steady state response curves for $\beta_1\text{AR-E}$ and $\beta_1\text{AR-W}$ binding to mini- G_s in the presence of 500 μM isoprenaline. Values for the single point measurements are the mean of triplicates ($n=3$ individual repeats), and error bars show SD for these replicates. Values for the dose response and steady state measurements were calculated using the data shown in **a** and **b**, and error bars show calculated errors from Data Analysis V 11.0.0.4 (Pall ForteBio LLC) software. See Methods and Supplementary Methods for details on the fitting parameters.



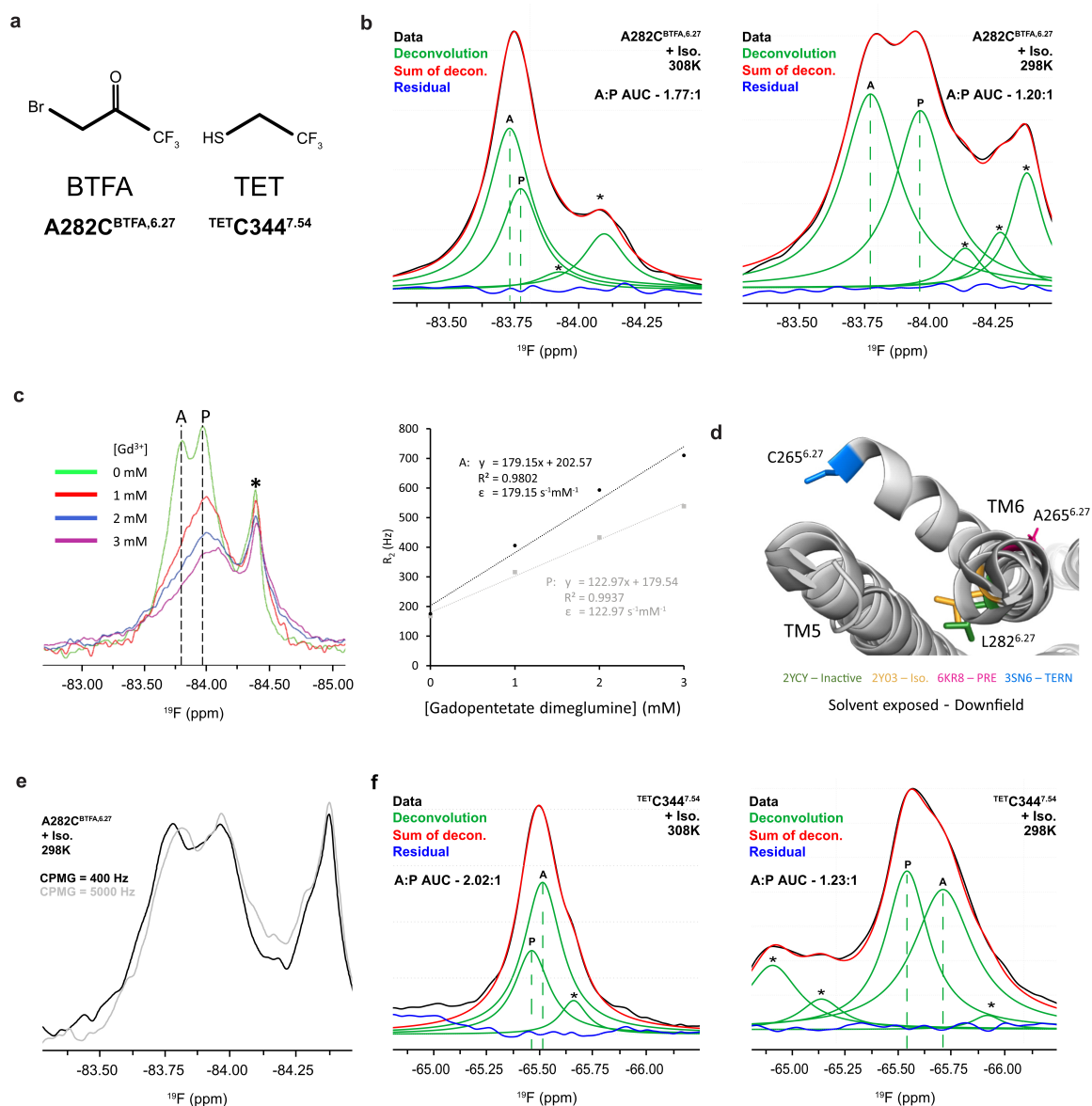
Supplementary Figure 5. The effect of ^{13}C -methyl methionine χ^3 conformers on methyl ^{13}C and ^1H chemical shifts.

a) The effect of χ^3 conformers on ^{13}C shifts. Trans and gauche χ^3 conformers have defined ^{13}C shift regions (red and blue, respectively), and ^{13}C chemical shift values between these regions represent χ^3 sampling both trans and gauche conformers in conformational exchange (grey). Methyl ^{13}C chemical shifts have been shown to be a suitable reporter of conformational changes related to receptor activation^{6,7}. **b)** The effect of ring currents of aromatic groups on ^1H chemical shifts of methyl group probes. Top, a face-on orientation of the aromatic system to the methyl probe affects ring current contributions to ^1H shifts through shielding of the protons, causing an upfield shift. Bottom, an edge-on orientation has the converse effect, deshielding protons and causing a downfield shift⁸.



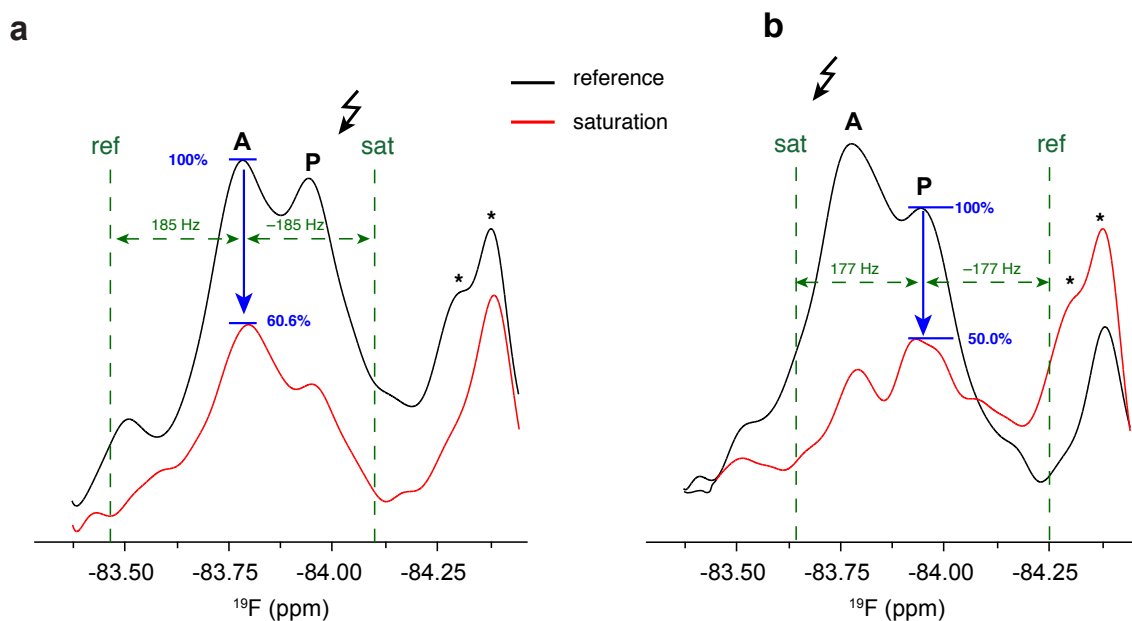
Supplementary Figure 6. Isoprenaline addition to $\beta_1\text{AR-E}$ results in population of an active state conformation similar to ternary complexes. **a)** An overlay of the ^1H - ^{13}C HMQC NMR spectra of isoprenaline-bound (Iso.) $\beta_1\text{AR-E}$ and isoprenaline-bound $\beta_1\text{AR-W}$, recorded at 308 K. **b)** An overlay of the ^1H - ^{13}C HMQC NMR spectra of isoprenaline-bound $\beta_1\text{AR-E}$ and isoprenaline-bound $\beta_1\text{AR-W}$ with 2 molar equivalents of Nb6B9, recorded at 308 K. * indicates an artifact related to ^1H 180° pulse imperfection. **c)** A comparison of structures of the inactive cyanopindolol-bound $\beta_1\text{AR}$ crystal structure (green - PDB: 2YCY)⁹ with the isoprenaline-bound $\beta_1\text{AR-G}_s$ ternary cryo-EM structure (blue - PDB: 7JJO)¹⁰ showing the conformation of aromatic residue F299^{6.44} and methyl probe M223^{5.54}. The benzyl ring of F299^{6.44} in the active receptor (blue) adopts a face-on position above M223^{5.54} that would exert a strongly shielding ring current shift. **d)** A comparison of the inactive carazolol-bound human $\beta_1\text{AR}$ crystal structure (light green - PDB: 6BVQ)¹¹ with the isoprenaline-bound $\beta_1\text{AR-G}_s$ ternary cryo-EM structure (PDB: 7JJO)¹⁰ showing the conformations of aromatic residues around methyl probe M296^{6.41}. Y227^{5.58} is absent from the inactive cyanopindolol-bound $\beta_1\text{AR}$ structure⁹ hence the use of an alternative inactive structure. Both Y227^{5.58} and F299^{6.44} adopt side-on orientations with respect to M296^{6.41} which would result in a strongly deshielding effect from the altered ring current contributions. **e)** An overlay of the ^1H - ^{13}C HMQC NMR spectra of apo $\beta_1\text{AR-E}$ L289M^{6.34} and apo $\beta_1\text{AR-E}$ L289M^{6.34} (left), and of isoprenaline-bound (Iso.) $\beta_1\text{AR-E}$ L289M^{6.34} and isoprenaline-bound $\beta_1\text{AR-E}$ L289M^{6.34} (right), recorded at 308 K. Experimental conditions were identical between the two experiments to facilitate assignment of L289M^{6.34}. **f)** A comparison of the inactive and active βAR structures showing the distance from aromatic residue Y231^{5.62} to L^{6.34} (L289M^{6.34} in our data). Structures: inactive cyanopindolol-bound $\beta_1\text{AR}$ crystal structure (green - PDB: 2YCY)⁹, isoprenaline-bound $\beta_1\text{AR}$ crystal structure (orange - PDB: 2Y03)¹², isoprenaline-bound $\beta_2\text{AR}$ PRE-based

structure (Pink - PDB: [6KR8](#))¹³, and isoprenaline-bound β_1 AR-G_s ternary cryo-EM structure (blue - PDB: [7JJO](#))¹⁰. L^{6.34} retains an edge-on orientation with Y231^{5.62}, thus the ring current from this orientation would result in a downfield ¹H shift which increases in magnitude with proximity.

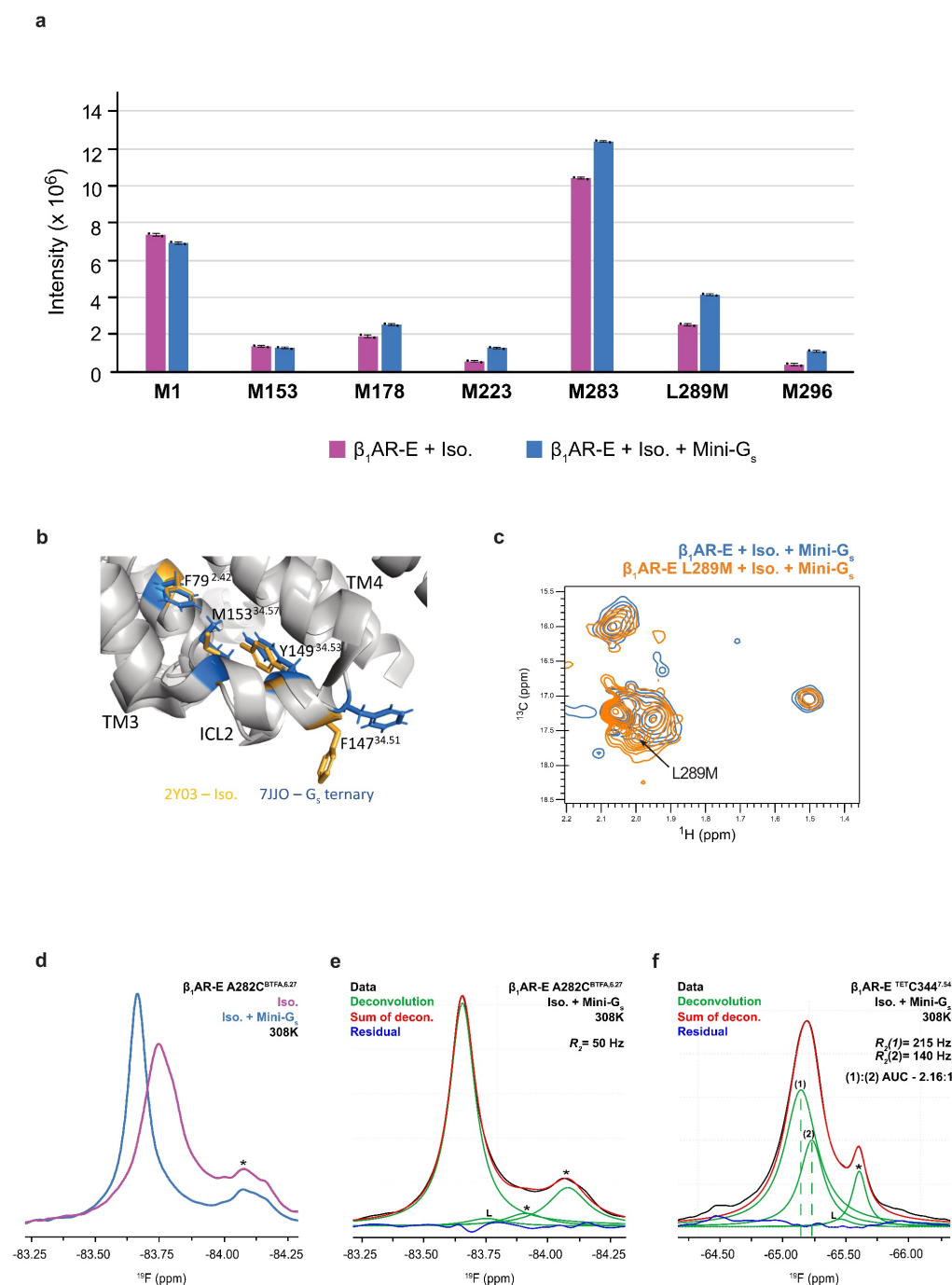


Supplementary Figure 7. ^{19}F NMR spectra show isoprenaline-bound $\beta_1\text{AR-E}$ occupies the active and pre-active states. **a)** The ^{19}F probes used in this study. BTFA forms stable C-S bonds. TET forms S-S disulphide bonds, which are more susceptible to hydrolysis. **b)** Deconvolution of A282C^{BTFA,6.27} ^{19}F NMR spectra recorded in the presence of 1 mM isoprenaline (Iso.) at 308 K (left) or 298 K (right). The chemical shifts of active state (A) and pre-active state (P) are indicated with dashed green lines. The residual (blue) is the subtraction of the sum of the simulated peaks (Sum of decon., red) from the raw data (black). Individual simulated peaks are shown in green. * indicates known degradation products. The ratio of the area under the curve (AUC) for the simulated active and pre-active states is shown. **c)** Solvent PRE experiments examining A282C^{BTFA,6.27} solvent accessibility. Left, reference spectra for a titration series of Gd^{3+} into samples with isoprenaline-bound A282C^{BTFA,6.27} at 298 K. The active (A) and pre-active (P) states are indicated. * indicates a degradation product. Right, a plot of R_2 relaxation rate against Gd^{3+} concentration. R_2 rates were calculated using a two-point relaxation measurement (see Methods). Linear fits and associated R^2 values are shown for the active (A, black) and pre-active (P, grey) states. The relaxation enhancement values (ϵ) due to Gd^{3+} addition are the gradient of the linear fits. **d)** A comparison of structures showing changes around the A282C^{BTFA,6.27} probe. Structures: inactive cyanopindolol-bound $\beta_1\text{AR}$ crystal structure (green - PDB: 2YCY)⁹, isoprenaline-bound $\beta_1\text{AR}$ crystal structure (orange - PDB: 2Y03)¹², isoprenaline-bound $\beta_2\text{AR}$ PRE-based structure (Pink - PDB: 6KR8)¹³, isoprenaline-bound $\beta_2\text{AR-G}_s$ complex crystal structure (Blue - PDB: 3SN6)¹⁴. L282^{6.27} moves from a less solvent exposed position behind TM5 to a solvent exposed position (A265^{6.27} in $\beta_2\text{AR}$) through TM6 rotation. A282C^{6.27} is unresolved in the $\beta_1\text{AR-G}_s$ ternary complex structure (PDB: 7JJO)¹⁰. **e)** Relaxation

dispersion (CPMG) experiments of isoprenaline-bound A282C^{BTFA,6.27} at 298 K with fast pulsing (5000 Hz, grey) and slow pulsing (400 Hz, black). See methods. **f**) Deconvolution of isoprenaline-bound ^{TET}C344^{7.54} ¹⁹F NMR spectra recorded at 308 K (left) or 298 K (right). Symbols are as in **b**).



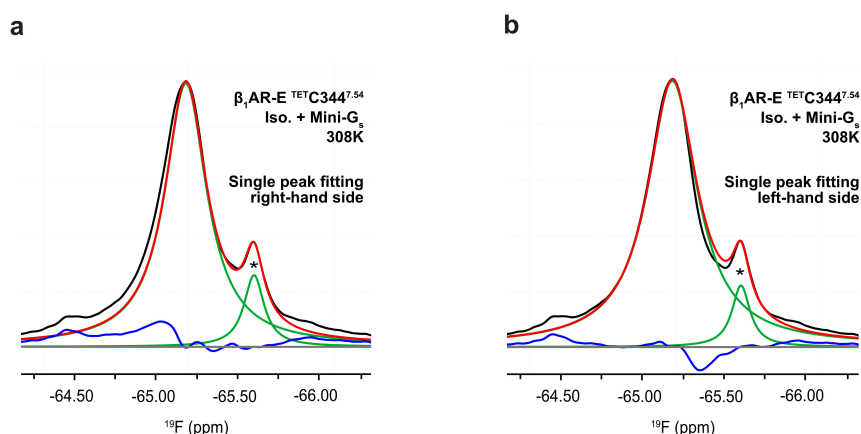
Supplementary Figure 8. Sub-second conformational exchange of $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ explored by ^{19}F NMR saturation transfer. Pairwise 1D ^{19}F NMR experiments were recorded at 298 K following irradiation with a weak RF field (irradiation time of 1 s, saturation field strength 25 Hz) applied at either of the two offset positions located symmetrically (dashed green lines with the corresponding offset positions of the saturation field indicated) around the peak of interest, which resulted in a saturation spectrum (red, irradiation at position 'sat') or a reference spectrum (black, irradiation at position 'ref'), respectively. At the corresponding position of the peak of interest, the reduction in signal intensity due to saturation transfer is indicated by a blue arrow, with the signal intensity in the saturation spectrum given as a percentage of the intensity in the reference spectrum. **a)** $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ bound to isoprenaline. The signal of the active state (A) shows attenuation upon irradiation of the pre-active state (P), indicative of conformational interchange between the A and P state. **b)** Similar to a) but here irradiation of the active state (A) leads to attenuation of the pre-active state (P) signal, again indicative of conformational exchange between the two states. The strongly overlapped signals of the A and P states required scaling of the saturation spectrum to enable a direct comparison of the signal intensities at the position of the peak of interest. The latter compensates for the loss in signal intensity at the peak of interest due to the reduced contribution from the attenuated saturated peak. Scaling factors were determined from the deconvolution shown in Supplementary Fig. 7b (right) whilst taking into account the residual intensity of the saturated peak in the saturated vs reference spectrum.



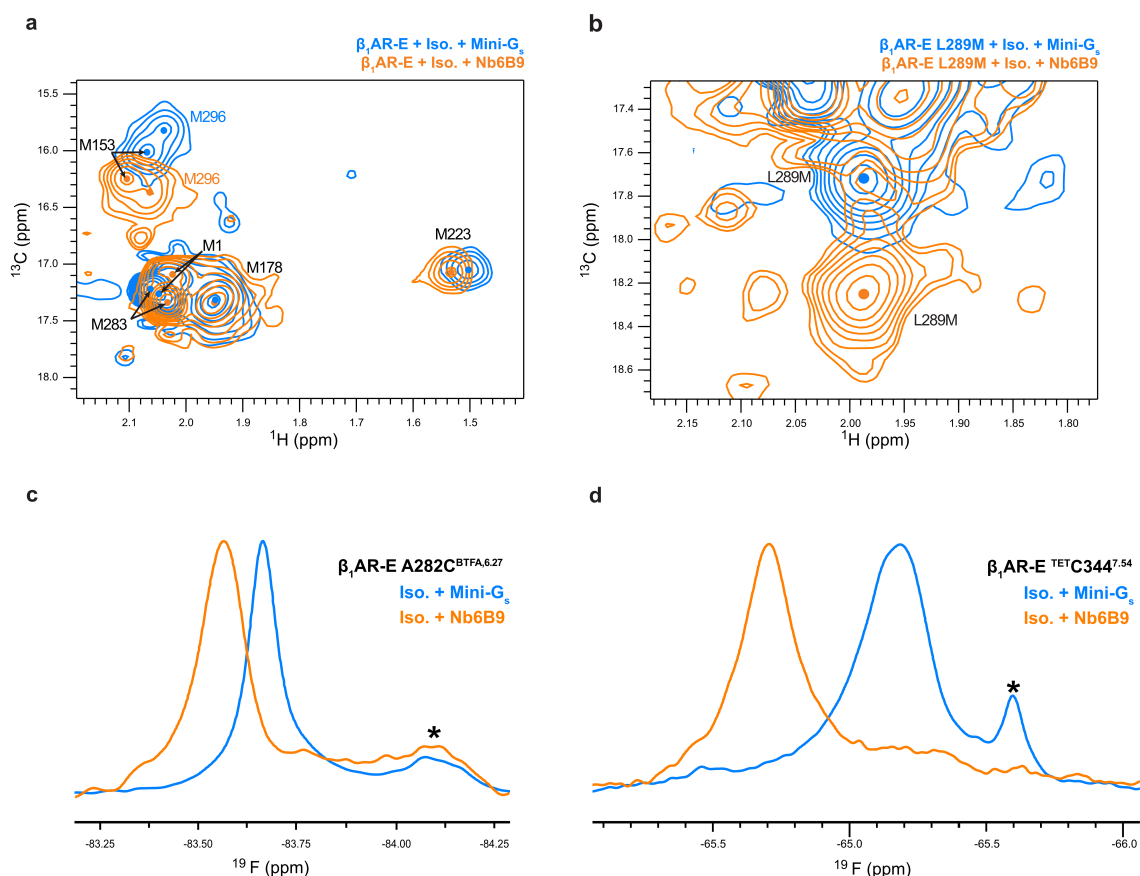
Supplementary Figure 9. $\beta_1\text{AR-E}$ undergoes additional conformational changes upon ternary complex formation.

a) A comparison of peak intensities from ^1H - ^{13}C HMQC spectra of isoprenaline-bound (Iso.) $\beta_1\text{AR-E}$ (purple) versus isoprenaline-bound $\beta_1\text{AR-E}$ with 2 molar equivalents of mini- G_s (blue). Note the largest relative difference between the two conditions are for M223^{5,54} and M296^{6,41}. Error bars indicate SD between two repeats. **b)** A comparison of $\beta_1\text{AR}$ structures showing the minor changes around the IL2 probe M153^{34,57} between the isoprenaline-bound $\beta_1\text{AR}$ crystal structure (orange - PDB: 2Y03)¹² and the isoprenaline-bound $\beta_1\text{AR-G}_s$ ternary cryo-EM structure (blue - PDB: 7JJO)¹⁰. **c)** An overlay of the ^1H - ^{13}C HMQC NMR spectra of isoprenaline-bound (Iso.) $\beta_1\text{AR-E}$ L289^{6,34} with 2 molar equivalents of mini- G_s , and isoprenaline-bound $\beta_1\text{AR-E}$ L289M^{6,34} with 2 molar equivalents of mini- G_s , both recorded at 308 K. **d)** ^{19}F NMR spectra of $\beta_1\text{AR-E}$ A282C^{BTFA,6,27} in the isoprenaline-bound (Iso.) state (purple) and mini- G_s (2 molar equivalents) ternary complex (blue), recorded at 308 K. * indicates a degradation product. **e)** Deconvolution of the $\beta_1\text{AR-E}$ A282C^{BTFA,6,27} ^{19}F NMR spectrum in the isoprenaline and mini- G_s (2 molar equivalents) ternary complex, recorded at 308 K. Addition of mini- G_s

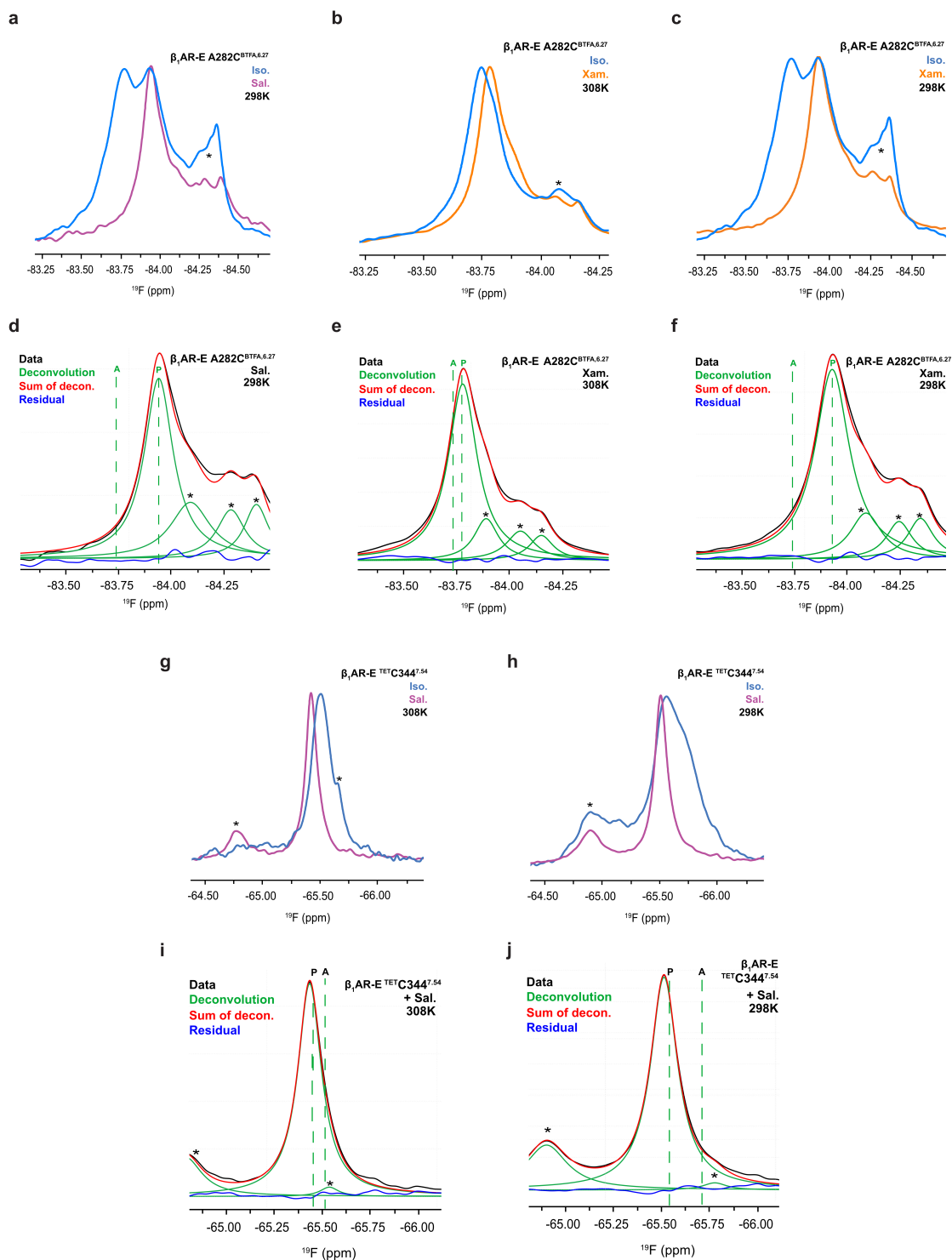
results in a single sharp ternary complex peak. The residual (blue) is the subtraction of the sum of the simulated peaks (Sum of decon., red) from the raw data (black). Individual simulated peaks are shown in green. * indicates known degradation products. L indicates residual ligand-bound population. R_2 transverse relaxation rate constant used to generate the simulated peak is indicated. **f)** Two-component deconvolution of the β_1 AR-E^{TET}C344^{7.54} ^{19}F NMR spectrum in the isoprenaline and mini-G_s (2 molar equivalents) ternary complex, recorded at 308 K. The chemical shifts of two ternary complex states (1) and (2) are indicated with dashed green lines. Colours and symbols are as in **e)**. The deconvolution consisting of two components results in a fit with a residual error that is clearly below the noise in the spectrum. The ratio of the area under the curve (AUC) for the simulated (1) and (2) states is shown. The R_2 relaxation rate constants used to generate the simulated peaks are indicated.



Supplementary Figure 10. Single-peak deconvolution of the TM7 ^{19}F NMR spectrum of $\beta_1\text{AR-E}$ in ternary complex with isoprenaline and mini- G_s indicate that the raw data for $\text{TET C344}^{7.54}$ cannot be fit by a single signal. **a) Single-peak deconvolution of the spectrum shown in Supplementary Figure 9f ($\beta_1\text{AR-E}^{\text{TET C344}^{7.54}}$ in the presence of isoprenaline and mini- G_s). The residual (blue) is the subtraction of the sum of the simulated peaks (Sum of deconv., red) from the raw data (black). Individual simulated peaks are shown in green. * indicates known degradation products. The single major peak is fit to minimise the residual on the right-hand side of the peak, with the centre of the simulated peak matching the centre of the raw data. **b)** Alternative single-peak deconvolution with the single major peak fit to minimise the residual on the left-hand side of the peak, with the centre of the simulated peak matching the centre of the raw data. Details are as in **a)**. Both **a)** and **b)** are single major peak deconvolutions of the data shown in Supplementary Fig. 9f (two-component deconvolution) and produce fits with residual errors that are well above the noise in the spectrum.**

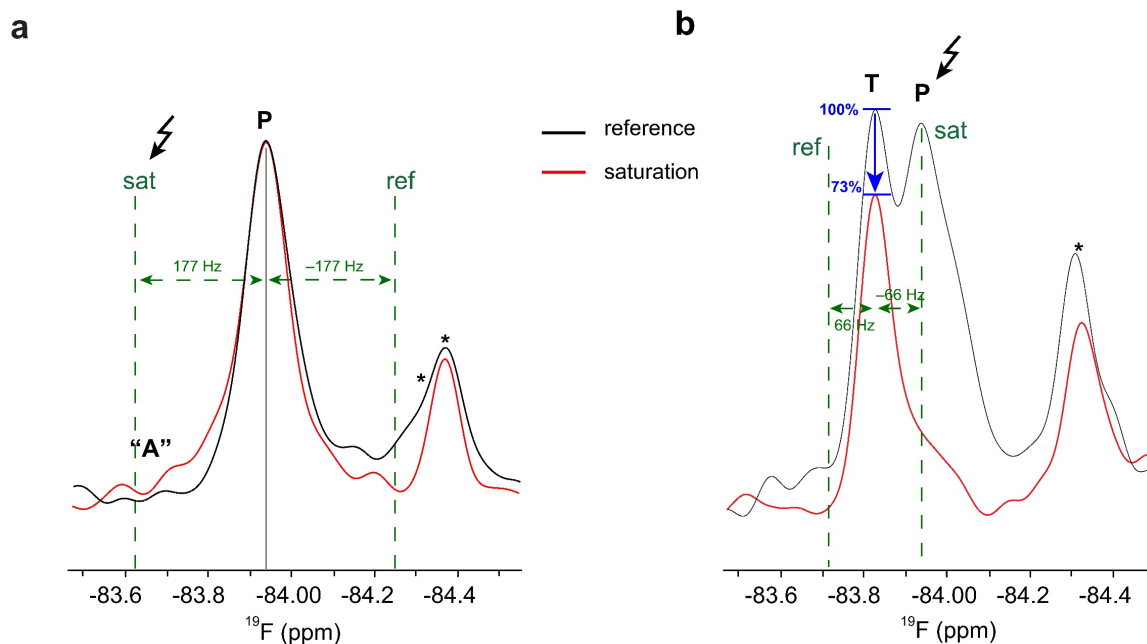


Supplementary Figure 11. Comparisons of isoprenaline-bound β_1 AR-E ternary complex spectra with mini-G_s or Nb6B9. **a)** An overlay of the ^1H - ^{13}C HMQC NMR spectra of isoprenaline-bound (Iso.) β_1 AR-E in complex with 2 molar equivalents mini-G_s or Nb6B9 (used in our previous work^{15,16}), recorded at 308 K. M178^{4,62} showed negligible chemical shift differences and M223^{5,54} small proton changes, suggesting similar arrangements around the orthosteric ligand pocket and conserved PIF region, respectively. M153^{34,57} and M296^{6,41} adopted downfield positions in the ^{13}C dimension for the mini-G_s complex relative to the Nb6B9 complex, indicating trans χ^3 rotamers and thus reduced conformational exchange and a more rigid conformation for the mini-G_s complex in IL2 and the core TM5-TM6 interface, respectively. The M283^{6,28} proton shift in the Nb6B9 complex was shifted upfield relative to the mini-G_s complex, further from the random coil value of 2.13 ppm¹⁷ indicating the TM6 tip was less flexible and less solvent exposed. **b)** An overlay of the ^1H - ^{13}C HMQC NMR spectra of isoprenaline-bound β_1 AR-E L289M^{6,34} in complex with 2 molar equivalents mini-G_s or Nb6B9. Experimental conditions were identical to **a)** aside from the L289M^{6,34} mutation. Similar proton shifts indicated a similar proximity of L289M^{6,34} to Y231^{5,62} thus similar rotations of TM6 between mini-G_s and Nb6B9 ternary complexes. The downfield shift in ^{13}C for Nb6B9 indicated a χ^3 trans conformation and a lower degree of flexibility in TM6. **c)** ^{19}F NMR spectra of A282C^{βTFA,6.27} in isoprenaline-bound β_1 AR-E in complex with 2 molar equivalents mini-G_s or Nb6B9, recorded at 308 K. The Nb6B9 complex is downfield shifted relative to mini-G_s ternary complex and broader lines suggest differing conformational adjustments at the tip of TM6 and conformational broadening on a slower μs -to- ms timescale. **d)** ^{19}F NMR spectra of TETC344^{7,54} in isoprenaline-bound β_1 AR-E in complex with 2 molar equivalents mini-G_s or Nb6B9, recorded at 308 K. The Nb6B9 complex broad peak was shifted downfield by 0.49 ppm relative to mini-G_s likely due to differences in solvent accessibility, possibly indicating sub-optimal contacts between receptor and nanobody in the TM7/IL4 region relative to mini-G_s interactions. Peaks are scaled to the same peak intensity in **c** and **d**.

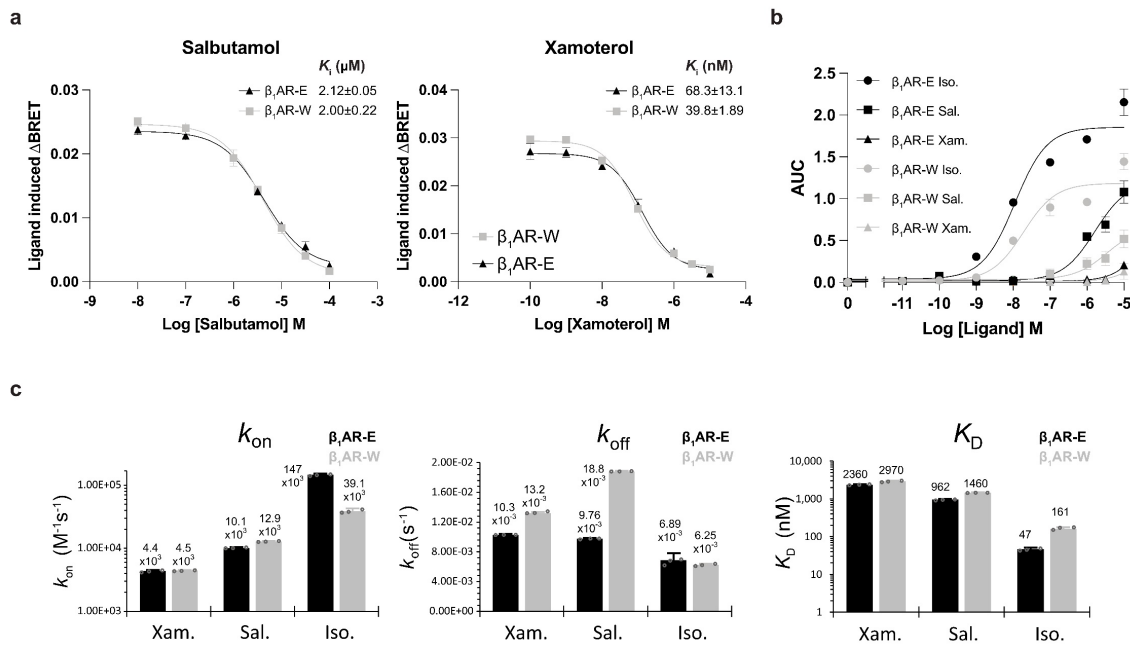


Supplementary Figure 12. ^{19}F NMR spectra show partial agonist bound $\beta_1\text{AR-E}$ only populates the inactive/pre-active state equilibrium. **a)** ^{19}F NMR spectra of $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ in the isoprenaline-bound (Iso.) state (blue) or salbutamol-bound (Sal.) state (purple), recorded at 298 K. * indicates degradation products. **b)** ^{19}F NMR spectra of $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ in the isoprenaline-bound (Iso.) state (blue) or xamoterol-bound (Xam.) state (orange), recorded at 308 K. * indicates degradation products. **c)** ^{19}F NMR spectra of $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ in the isoprenaline-bound (Iso.) state (blue) or xamoterol-bound (Xam.) state (orange), recorded at 298 K. * indicates degradation products. **d)** Deconvolution of the $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ ^{19}F NMR spectrum in the presence of 1 mM salbutamol, recorded at 298 K. The green dashed lines indicate the chemical shifts of the active (A) and pre-active (P) states observed in deconvolutions of isoprenaline spectra.

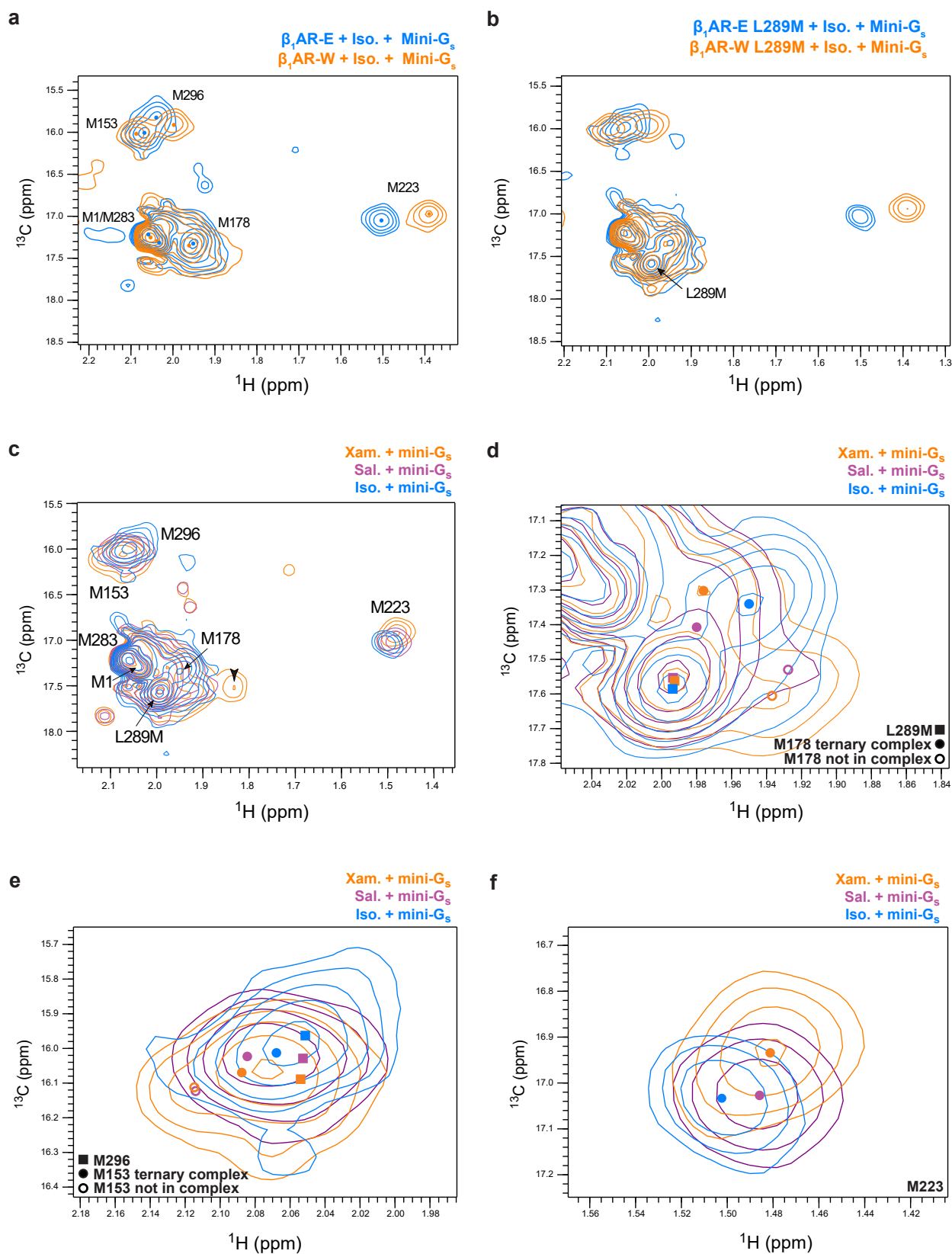
The residual (blue) is the subtraction of the sum of the simulated peaks (Sum of decon., red) from the raw data (black). Individual simulated peaks are shown in green. * indicates degradation products. **e)** Deconvolution of the β_1 AR-E A282C^{BTFA,6.27} ^{19}F NMR spectrum in the presence of 1 mM xamoterol, recorded at 308 K. See **d)** for symbols and colours. **f)** Deconvolution of the β_1 AR-E A282C^{BTFA,6.27} ^{19}F NMR spectrum in the presence of 1 mM xamoterol, recorded at 298 K. See **d)** for symbols and colours. **g)** ^{19}F NMR spectra of β_1 AR-E ^{TET}C344^{7.54} in the isoprenaline-bound state (blue) or salbutamol-bound state (purple), recorded at 308 K. * indicates degradation products. **h)** ^{19}F NMR spectra of β_1 AR-E ^{TET}C344^{7.54} in the isoprenaline-bound state (blue) or salbutamol-bound state (purple), recorded at 298 K. * indicates degradation products. **i)** Deconvolution of the β_1 AR-E ^{TET}C344^{7.54} ^{19}F NMR spectrum in the presence of 1 mM salbutamol, recorded at 308 K. See **d)** for symbols and colours. **j)** Deconvolution of the β_1 AR-E ^{TET}C344^{7.54} ^{19}F NMR spectrum in the presence of 1 mM salbutamol, recorded at 298 K. See **d)** for symbols and colours. Note for **a-c**, and **g-h**, peaks were scaled to the same intensity maxima.



Supplementary Figure 13. ^{19}F NMR saturation transfer experiments on $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ indicate partial agonists induce ternary complex formation via the pre-active state for $\beta_1\text{AR}$. Pairwise 1D ^{19}F NMR experiments were recorded following irradiation with a weak RF field (irradiation time of 1 s, saturation field strength 25 Hz) applied at either of the two offset positions located symmetrically (dashed green lines with the corresponding offset positions of the saturation field indicated) around the peak of interest, which resulted in a saturation spectrum (red, irradiation at position 'sat') or a reference spectrum (black, irradiation at position 'ref'), respectively. At the corresponding position of the peak of interest, the reduction in signal intensity due to saturation transfer is indicated by a blue arrow, with the signal intensity in the saturation spectrum given as a percentage of the intensity in the reference spectrum. **a)** $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ bound to xamoterol (500 μM) recorded at 298 K. Saturation of the active state position (using the same offset from the pre-active state signal as in Supplementary Fig. 8, right, 177 Hz) had a negligible effect on the pre-active state signal, indicating partial agonist-bound $\beta_1\text{AR-E}$ does not occupy the active state under these conditions. **b)** $\beta_1\text{AR-E A282C}^{\text{BTFA},6.27}$ in ternary complex with xamoterol (500 μM) and mini- G_s (0.75 molar equivalents). Experiments were recorded at 308 K to increase the separation of signals. Saturation of the pre-active state signal resulted in a decrease in the ternary complex signal, indicating chemical exchange between the pre-active state (P) and ternary complex (T). The saturation spectrum is shown scaled relative to its reference spectrum, in order to correct for the intensity loss at the signal position of the ternary complex (T) due to overlap with the saturated pre-active state (P) peak (see Supplementary Fig. 8).

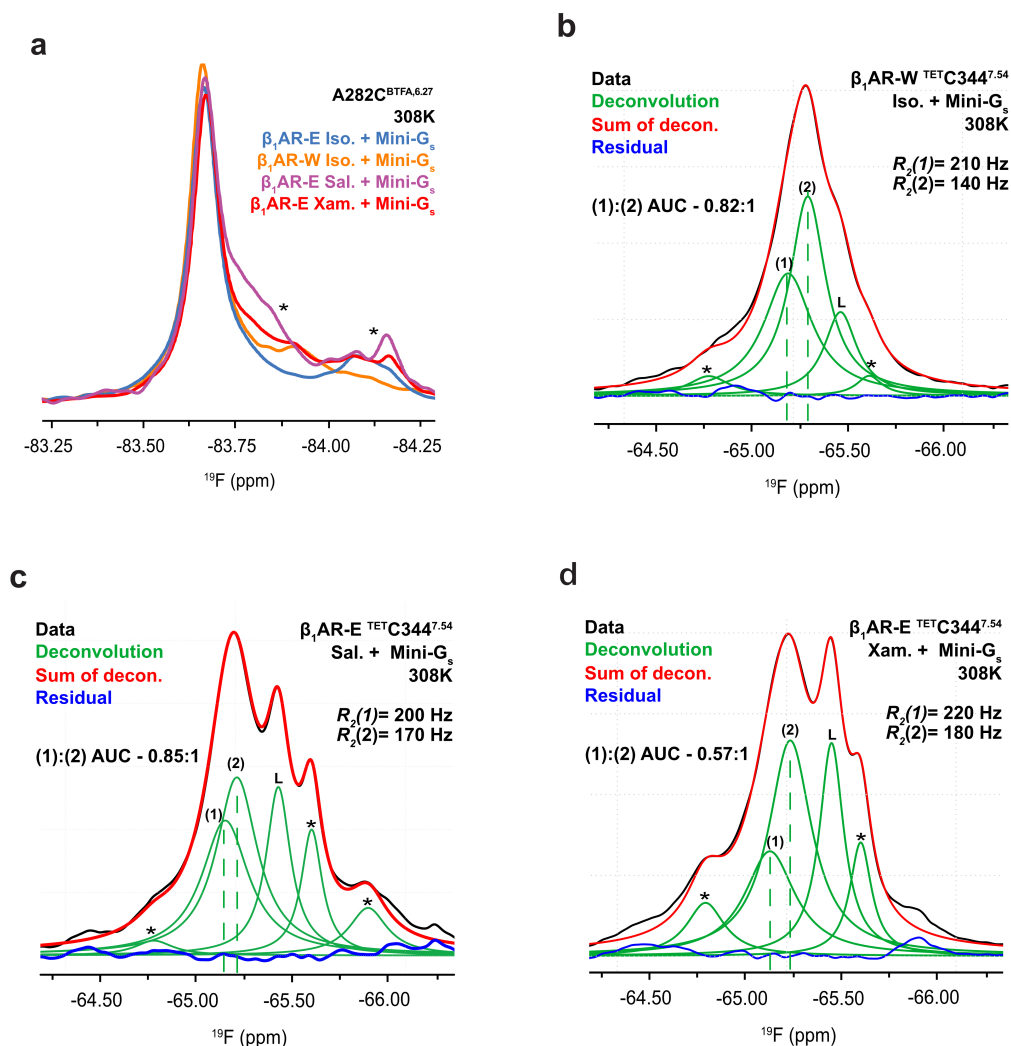


Supplementary Figure 14. Partial agonists reduce G protein coupling and mini-G_s binding affinities to β_1AR constructs relative to full agonist. **a)** Ligand binding affinities of the β_1AR constructs for salbutamol (left) and xamoterol (right) expressed in HEK293T cells, measured using the luminescence-based ligand binding assay shown in Supplementary Fig. 2a. K_i values are shown in inset. **b)** *In cell* measurements of the dissociation of the G protein G $\beta\gamma$ subunit from G α_s using the TRUPATH assay system, shown in Supplementary Fig. 2c, for partial agonists salbutamol (Sal., squares) and xamoterol (Xam., triangles) relative to full agonist isoprenaline (Iso., circles). The responses were measured for varying ligand concentrations to obtain EC₅₀ curves for β_1AR-E (black) and β_1AR-W (grey). Data are mean $\pm$ SEM of $n=3$ independent biological experiments, each performed in duplicate. **c)** BLI kinetic data for binding of β_1AR to mini-G_s in the presence of different ligands, xamoterol (Xam.), salbutamol (Sal.) and isoprenaline (Iso.). Black, β_1AR-E , grey, β_1AR-W . Values were the average of triplicates ($n=3$ individual repeats), and error bars indicate SD of these triplicates. Note for the logarithmic axes for k_{on} and K_D data. See Methods and Supplementary Methods for details.

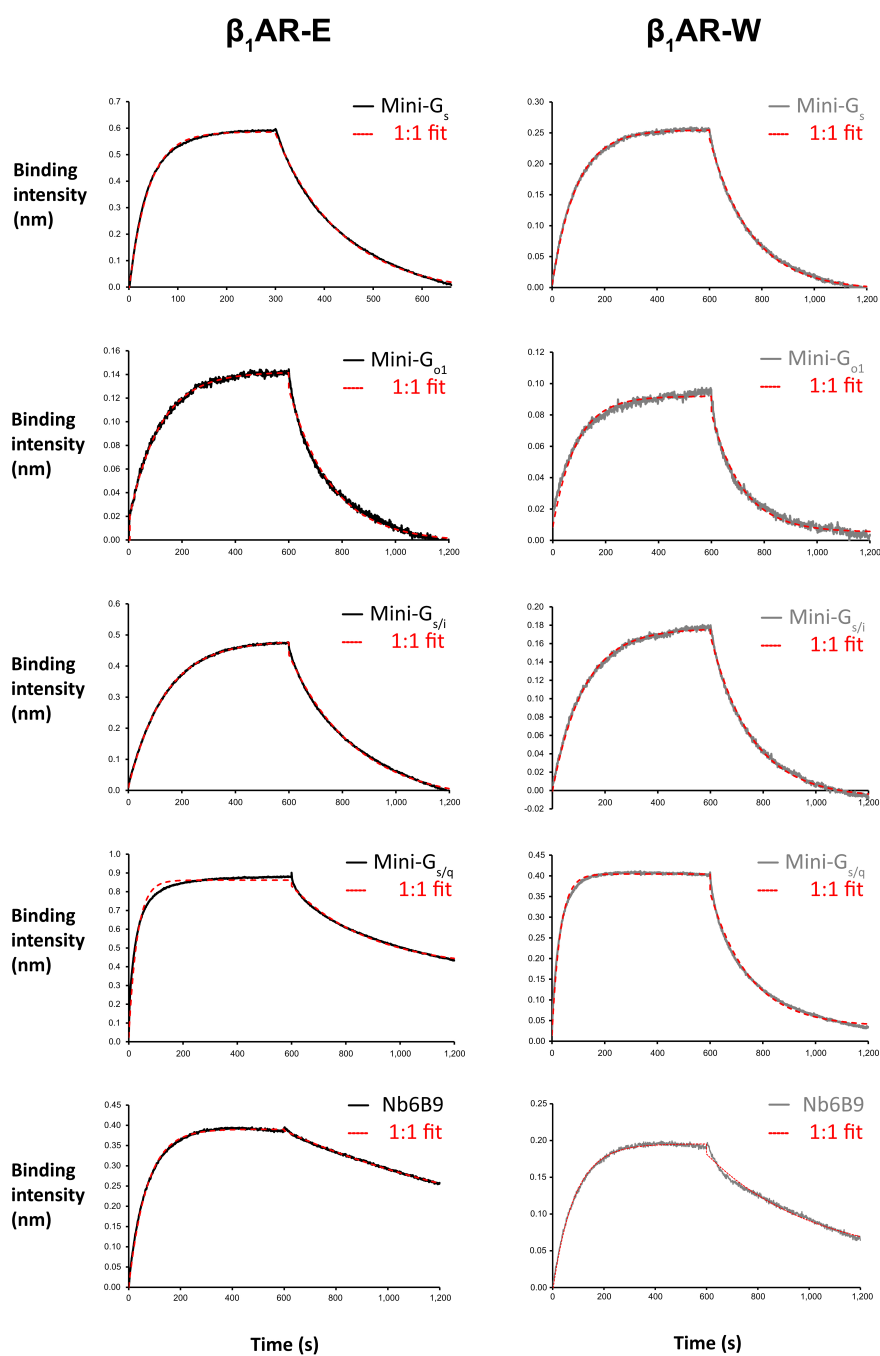


Supplementary Figure 15. ^1H - ^{13}C HMQC NMR spectra show E130W^{3,41} thermostabilising mutation and partial agonists affect ternary complex conformations. **a)** An overlay of the ^1H - ^{13}C HMQC NMR spectra of isoprenaline-bound (Iso.) $\beta_1\text{AR-E}$ with 2 molar equivalents of mini-G_s (blue) and isoprenaline-bound $\beta_1\text{AR-W}$ with 2 molar equivalents of mini-

G_s (orange), both recorded at 308 K. **b)** An overlay of the ¹H-¹³C HMQC NMR spectra of isoprenaline-bound β₁AR-E L289M^{6.34} with 2 molar equivalents of mini-G_s (blue) and isoprenaline-bound β₁AR-W L289M^{6.34} with 2 molar equivalents of mini-G_s (orange), both recorded at 308 K. **c)** An overlay of the ¹H-¹³C HMQC NMR spectra of mini-G_s bound β₁AR-E L289M^{6.34} recorded at 308 K in the presence of the ligands xamoterol (Xam., orange), salbutamol (Sal., purple), or isoprenaline (Iso., blue). The arrowhead indicates a residual ligand-bound population. **d)** Enlargement of **c)** in the M178^{4.62}/L289M^{6.34} region. M178^{4.62} peak positions are shown with filled circles and L289M^{6.34} peak positions are shown with squares. Residual M178^{4.62} ligand-bound peaks are shown with unfilled circles. **e)** Enlargement of **c)** in the M153^{34.57}/M296^{6.41} region. M153^{34.57} peak positions are shown with filled circles and M296^{6.41} peak positions are shown with squares. Residual M153^{34.57} ligand-bound peaks are shown with unfilled circles. **f)** Enlargement of **c)** in the M223^{5.54} region.



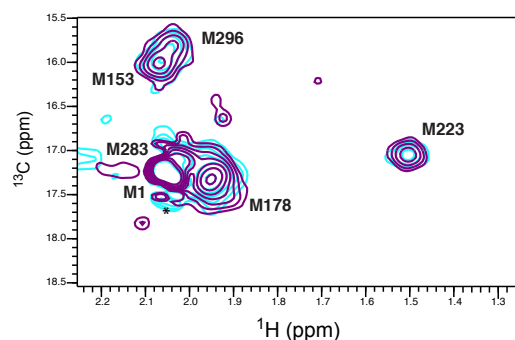
Supplementary Figure 16. ¹⁹F NMR spectra show E130W^{3.41} thermostabilising mutation and partial agonists affect ternary complex conformations. **a)** ¹⁹F NMR spectra of A282C^{BTFA,6.27} in the isoprenaline (Iso.) and mini-G_s ternary (2 molar equivalents) complex for β_1 AR-E (blue) or β_1 AR-W (orange), and A282C^{BTFA,6.27} in the salbutamol (Sal., high efficacy partial agonist, purple) or xamoterol (Xam., intermediate efficacy partial agonist, red) ternary complexes with mini-G_s (8 molar equivalents) for β_1 AR-E, recorded at 308 K. * indicates degradation products. **b)** Deconvolution of the β_1 AR-W^{TETC344^{7.54}} ¹⁹F NMR spectrum in the isoprenaline and mini-G_s (2 equivalents) ternary complex, recorded at 308 K. The chemical shifts of two ternary complex states (1) and (2) are indicated with dashed green lines. The residual (blue) is the subtraction of the sum of the simulated peaks (Sum of decon., red) from the raw data (black). Individual simulated peaks are shown in green. * indicates known degradation products. L indicates residual ligand-bound population. The ratio of the area under the curve (AUC) for the simulated (1) and (2) states is shown. The R_2 transverse relaxation rate constants used to generate the simulated peaks are indicated. **c)** Deconvolution of the β_1 AR-E^{TETC344^{7.54}} ¹⁹F NMR spectrum in the salbutamol and mini-G_s (8 molar equivalents) ternary complex, recorded at 308 K. Symbols (1), (2), *, L, AUC, and R_2 are as in **b)**. See **b)** for line colours. **d)** Deconvolution of the β_1 AR-E^{TETC344^{7.54}} ¹⁹F NMR spectrum in the xamoterol and mini-G_s (8 molar equivalents) ternary complex, recorded at 308 K. Symbols (1), (2), *, L, AUC, and R_2 are as in **b)**. See **b)** for line colours.



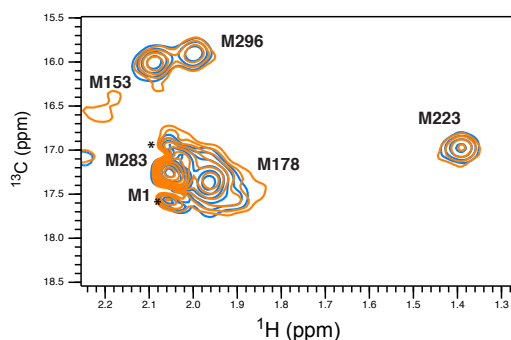
Supplementary Figure 17. Examples of isotherms for BLI experiments assessing β_1 AR binding kinetics to mini-G proteins and Nb6B9. Left, β_1 AR-E, right, β_1 AR-W. All binding curves were recorded in the presence of 500 μ M isoprenaline. Raw BLI data is shown as solid black lines for β_1 AR-E and solid grey lines for β_1 AR-W, and 1:1 stoichiometry model fits are shown as red dashed lines. Isotherms are representative data of $n=3$ individual replicates. See Methods and Supplementary Methods for experimental details. See Supplementary Table 4. for the concentrations of analyte used.

gaps are indicated with -. **b)** Sequence alignments of the mini-G_s protein with the mini-G chimeras mini-G_{s/i} and mini-G_{s/q} from the TCAT motif to the C-terminus¹⁸. Differences between the chimeras and the mini-G_s protein are shown underlined and in bold. **c)** Representative ¹H-¹³C HMQC NMR spectra of isoprenaline-bound β₁AR-E ternary complexes with 2 or 8 equivalents of mini-G proteins. Shown is an example for mini-G_{s/i}. The minimal differences between spectra indicate negligible binding kinetics-induced changes. **d)** A comparison of peak intensities from ¹H-¹³C HMQC spectra of isoprenaline-bound (Iso.) β₁AR-E in complex with 2 molar equivalents of different mini-G binding partners. **e)** An overlay of the ¹H-¹³C HMQC NMR spectra of isoprenaline-bound (Iso.) β₁AR-E L289M^{6,34} in complex with different mini-G chimeras recorded at 308 K. Left, mini-G_s, middle, mini-G_{s/i}, right, mini-G_{s/q}. All experiments used 2 molar equivalents of mini-G protein. **f)** An overlay of the ¹⁹F NMR spectra of isoprenaline-bound β₁AR-E A282C^{BTF_A,6,27} in complex with different mini-G proteins recorded at 308 K. The spectrum with mini-G_s used 2 molar equivalents of mini-G protein, all other spectra used 8 molar equivalents.

$\beta_1\text{AR-E} + \text{Iso.} + \text{Mini-G}_s$
 $\beta_1\text{AR-E} + \text{Iso.} + \text{Mini-G}_s$ (apyrase preincubation)



$\beta_1\text{AR-W} + \text{Iso.} + \text{Mini-G}_s$
 $\beta_1\text{AR-W} + \text{Iso.} + \text{Mini-G}_s$ (apyrase preincubation)



Supplementary Figure 19. Ternary complex spectra appearance does not depend on the presence of GDP.

Superposition of 2D ^1H - ^{13}C HMQC correlation spectra of $\beta_1\text{AR-E}$ (left) and $\beta_1\text{AR-W}$ (right) in ternary complex bound to isoprenaline and mini- G_s . The spectra were recorded either following preincubation with apyrase (5 units) for two hours (cyan for $\beta_1\text{AR-E}$, orange for $\beta_1\text{AR-W}$) or directly without preincubation (purple for $\beta_1\text{AR-E}$, blue for $\beta_1\text{AR-W}$). The signals of the ternary complexes are independent of the presence of GDP in solution, in agreement with our BLI experiments (Supplementary Fig. 2f) and consistent with a mutation in the mini-G proteins that makes them insensitive to GDP nucleotide binding when coupled to GPCRs⁵. Based on this comparison, all NMR and BLI experiments in this work were recorded without prior apyrase incubation. * indicates an artifact related to ^1H pulse imperfections.

Supplementary Table 1. Comparison of BLI binding kinetics for β_1 AR association with mini-G_s in the presence of 500 μ M isoprenaline using different modelling parameters. Single point measurements (single optimised concentrations), dose-response measurements (with global fitting), and steady state response measurements (using response curves following the equation $\text{Response} = (R_{\text{max}} * \text{Concentration}_{\text{analyte}})/(K_D + \text{Concentration}_{\text{analyte}})$). Values shown are triplicate averages (n=3 individual repeats) with $\pm$ one standard deviation.

Measurement method	Receptor Construct	K_D (M)	k_{on} ($\text{M}^{-1}\text{s}^{-1}$)	k_{off} (s^{-1})
Single point	β_1 AR-E	$4.68 \times 10^{-8} \pm 4.18 \times 10^{-9}$	$1.47 \times 10^5 \pm 6.86 \times 10^3$	$6.89 \times 10^{-3} \pm 9.10 \times 10^{-4}$
Dose-response	β_1 AR-E	$5.81 \times 10^{-8} \pm 1.79 \times 10^{-8}$	$1.21 \times 10^5 \pm 1.37 \times 10^4$	$6.89 \times 10^{-3} \pm 1.53 \times 10^{-3}$
Steady state	β_1 AR-E	$4.30 \times 10^{-8} \pm 3.90 \times 10^{-9}$	N/A	N/A
Single point	β_1 AR-W	$1.61 \times 10^{-7} \pm 1.79 \times 10^{-8}$	$3.91 \times 10^4 \pm 4.04 \times 10^3$	$6.25 \times 10^{-3} \pm 1.37 \times 10^{-4}$
Dose-response	β_1 AR-W	$1.81 \times 10^{-7} \pm 1.98 \times 10^{-8}$	$5.18 \times 10^4 \pm 1.54 \times 10^3$	$9.40 \times 10^{-3} \pm 7.78 \times 10^{-4}$
Steady state	β_1 AR-W	$1.80 \times 10^{-7} \pm 3.20 \times 10^{-8}$	N/A	N/A

Supplementary Table 2. BLI kinetic data for Nb6B9 binding to β_1 AR constructs. Kinetic values for binding of β_1 AR-E or β_1 AR-W to active state stabilising nanobody Nb6B9 in the presence of 500 μ M full agonist isoprenaline. Values shown are triplicate averages (n=3 individual repeats) with $\pm$ one standard deviation. See Methods and Supplementary Methods for details.

	β_1 AR-E	β_1 AR-W
k_{on} ($M^{-1} s^{-1}$)	$1.05 \times 10^5 \pm 1.44 \times 10^3$	$7.74 \times 10^4 \pm 5.32 \times 10^3$
k_{off} (s^{-1})	$8.93 \times 10^{-4} \pm 1.13 \times 10^{-4}$	$2.49 \times 10^{-3} \pm 1.62 \times 10^{-4}$
K_D (M)	$8.53 \times 10^{-9} \pm 9.60 \times 10^{-10}$	$3.23 \times 10^{-8} \pm 3.99 \times 10^{-9}$

Supplementary Table 3. BLI kinetic data for mini-G_s binding to β_1 AR constructs in the presence of different agonists.

Kinetic values for mini-G_s binding to β_1 AR-E or β_1 AR-W in the presence of 500 μ M of different ligands (isoprenaline – Iso., full agonist; salbutamol – Sal., partial agonist (high efficacy); xamoterol – Xam., partial agonist (intermediate efficacy))¹⁹. Top, k_{on} values, middle, k_{off} values, bottom, K_D values. Values shown are triplicate averages (n=3 individual repeats) with $\pm$ one standard deviation. See Methods and Supplementary Methods for details.

$$k_{on} (M^{-1}s^{-1})$$

	β_1 AR-E	β_1 AR-W
Iso.	$1.47 \times 10^5 \pm 6.86 \times 10^3$	$3.91 \times 10^4 \pm 4.04 \times 10^3$
Sal.	$1.01 \times 10^4 \pm 1.83 \times 10^2$	$1.29 \times 10^4 \pm 1.93 \times 10^2$
Xam.	$4.36 \times 10^3 \pm 1.12 \times 10^2$	$4.46 \times 10^3 \pm 1.55 \times 10^2$

$$k_{off} (s^{-1})$$

	β_1 AR-E	β_1 AR-W
Iso.	$6.89 \times 10^{-3} \pm 9.10 \times 10^{-4}$	$6.25 \times 10^{-3} \pm 1.37 \times 10^{-4}$
Sal.	$9.76 \times 10^{-3} \pm 5.99 \times 10^{-5}$	$1.88 \times 10^{-2} \pm 6.96 \times 10^{-5}$
Xam.	$1.03 \times 10^{-2} \pm 8.46 \times 10^{-5}$	$1.32 \times 10^{-2} \pm 1.40 \times 10^{-4}$

$$K_D (M)$$

	β_1 AR-E	β_1 AR-W
Iso.	$4.68 \times 10^{-8} \pm 4.18 \times 10^{-9}$	$1.61 \times 10^{-7} \pm 1.79 \times 10^{-8}$
Sal.	$9.62 \times 10^{-7} \pm 1.59 \times 10^{-8}$	$1.46 \times 10^{-6} \pm 1.66 \times 10^{-8}$
Xam.	$2.36 \times 10^{-6} \pm 4.38 \times 10^{-8}$	$2.97 \times 10^{-6} \pm 8.30 \times 10^{-8}$

Supplementary Table 4. BLI binding partner concentrations used for k_{on} , k_{off} and K_D determination based on single-concentration measurements. The binding partner concentrations correspond to conditions as established by pre-inspection of a concentration series that identified a BLI trace with a clear 1:1 monophasic behaviour (see Supplementary Methods).

Receptor Construct	β_1 AR ligand (500 μ M)	Binding partner	Binding partner concentration (nM)
β_1 AR-E	Isoprenaline	Mini-G _s	62.5
β_1 AR-E	Isoprenaline	Mini-G _{o1}	2000
β_1 AR-E	Isoprenaline	Mini-G _{s/i}	1000
β_1 AR-E	Isoprenaline	Mini-G _{s/q}	2000
β_1 AR-E	Salbutamol	Mini-G _s	2000
β_1 AR-E	Xamoterol	Mini-G _s	4000
β_1 AR-E	Isoprenaline	Nb6B9	125
β_1 AR-W	Isoprenaline	Mini-G _s	125
β_1 AR-W	Isoprenaline	Mini-G _{o1}	4000
β_1 AR-W	Isoprenaline	Mini-G _{s/i}	1000
β_1 AR-W	Isoprenaline	Mini-G _{s/q}	2000
β_1 AR-W	Salbutamol	Mini-G _s	1000
β_1 AR-W	Xamoterol	Mini-G _s	4000
β_1 AR-W	Isoprenaline	Nb6B9	125

Supplementary Table 5. BLI kinetic data for binding of mini-G proteins to β_1 AR constructs. Kinetic values for binding of β_1 AR-E or β_1 AR-W to different mini-G proteins in the presence of 500 μ M full agonist isoprenaline. Top, k_{on} values, middle, k_{off} values, bottom, K_D values. Values shown are triplicate averages (n=3 individual repeats) with $\pm$ one standard deviation. See Methods and Supplementary Methods for details.

$$k_{on} (M^{-1}s^{-1})$$

	β_1 AR-E	β_1 AR-W
Mini-G _s	1.47x10 ⁵ $\pm$ 6.86x10 ³	3.91x10 ⁴ $\pm$ 4.04x10 ³
Mini-G _{o1}	2.96x10 ³ $\pm$ 2.31x10 ²	1.32x10 ³ $\pm$ 3.22x10 ²
Mini-G _{s/i}	3.64x10 ³ $\pm$ 2.45x10 ²	2.51x10 ³ $\pm$ 4.42x10 ²
Mini-G _{s/q}	1.41x10 ⁴ $\pm$ 1.58x10 ²	1.25x10 ⁴ $\pm$ 1.84x10 ²

$$k_{off}(s^{-1})$$

	β_1 AR-E	β_1 AR-W
Mini-G _s	6.89x10 ⁻³ $\pm$ 9.10x10 ⁻⁴	6.25x10 ⁻³ $\pm$ 1.37x10 ⁻⁴
Mini-G _{o1}	3.93x10 ⁻³ $\pm$ 3.17x10 ⁻⁴	9.41x10 ⁻³ $\pm$ 6.54x10 ⁻⁴
Mini-G _{s/i}	3.97x10 ⁻³ $\pm$ 3.97x10 ⁻⁵	6.23x10 ⁻³ $\pm$ 1.55x10 ⁻⁴
Mini-G _{s/q}	3.43x10 ⁻³ $\pm$ 2.23x10 ⁻⁵	6.70x10 ⁻³ $\pm$ 1.06x10 ⁻⁴

$$K_D (M)$$

	β_1 AR-E	β_1 AR-W
Mini-G _s	4.68x10 ⁻⁸ $\pm$ 4.18x10 ⁻⁹	1.61x10 ⁻⁷ $\pm$ 1.79x10 ⁻⁸
Mini-G _{o1}	1.34x10 ⁻⁶ $\pm$ 1.88x10 ⁻⁷	7.41x10 ⁻⁶ $\pm$ 1.75x10 ⁻⁶
Mini-G _{s/i}	1.09x10 ⁻⁶ $\pm$ 7.82x10 ⁻⁸	2.53x10 ⁻⁶ $\pm$ 4.64x10 ⁻⁷
Mini-G _{s/q}	2.42x10 ⁻⁷ $\pm$ 1.19x10 ⁻⁹	5.34x10 ⁻⁷ $\pm$ 6.22x10 ⁻⁹

Supplementary Table 6. Exact p values for comparison of binding kinetics parameters between mini-G proteins for BLI experiments with β_1 AR-E in Figure 4c. The p values were calculated using unpaired two-tailed t-tests using the means and standard deviations in Supplementary Table 5, with a Bonferroni correction to the significance level for multiple comparisons. Significance level: $p = 0.05$, corrected to $p = 0.00833$.

Parameter	Comparison 1	Comparison 2	p value	Significant
k_{on}	Mini-G _s	Mini-G _{s/i}	0.0007535	Yes
k_{on}	Mini-G _s	Mini-G _{o1}	0.0007476	Yes
k_{on}	Mini-G _s	Mini-G _{s/q}	0.0008846	Yes
k_{on}	Mini-G _{s/i}	Mini-G _{o1}	0.02603	No
k_{on}	Mini-G _{s/i}	Mini-G _{s/q}	2.44e-06	Yes
k_{on}	Mini-G _{o1}	Mini-G _{s/q}	1.168e-06	Yes
k_{off}	Mini-G _s	Mini-G _{s/i}	0.03059	No
k_{off}	Mini-G _s	Mini-G _{o1}	0.02074	No
k_{off}	Mini-G _s	Mini-G _{s/q}	0.02221	No
k_{off}	Mini-G _{s/i}	Mini-G _{o1}	0.8716	No
k_{off}	Mini-G _{s/i}	Mini-G _{s/q}	0.0001875	Yes
k_{off}	Mini-G _{o1}	Mini-G _{s/q}	0.1098	No
K_D	Mini-G _s	Mini-G _{s/i}	0.001808	Yes
K_D	Mini-G _s	Mini-G _{o1}	0.007003	Yes
K_D	Mini-G _s	Mini-G _{s/q}	5.01e-05	Yes
K_D	Mini-G _{s/i}	Mini-G _{o1}	0.1431	No
K_D	Mini-G _{s/i}	Mini-G _{s/q}	0.002796	Yes
K_D	Mini-G _{o1}	Mini-G _{s/q}	0.009721	No

Supplementary Table 7. Exact p values for comparisons of binding kinetics parameters between β_1 AR-E and β_1 AR-W for each mini-G protein in BLI experiments in Figure 4c. The p values were calculated using unpaired two-tailed t-tests using the means and standard deviations in Supplementary Table 5. Significance level: $p = 0.05$.

Parameter	Mini-G protein	p value	Significant
k_{on}	Mini-G _s	9.992e-05	Yes
k_{on}	Mini-G _{o1}	0.002878	Yes
k_{on}	Mini-G _{s/i}	0.02884	Yes
k_{on}	Mini-G _{s/q}	0.0003839	Yes
k_{off}	Mini-G _s	0.3504	No
k_{off}	Mini-G _{o1}	0.001374	Yes
k_{off}	Mini-G _{s/i}	0.000861	Yes
k_{off}	Mini-G _{s/q}	0.0002065	Yes
K_D	Mini-G _s	0.005893	Yes
K_D	Mini-G _{o1}	0.02549	Yes
K_D	Mini-G _{s/i}	0.03005	Yes
K_D	Mini-G _{s/q}	9.11e-05	Yes

Supplementary Table 8. List of primers used in this study.

Primer Name	Primer Sequence
Mini-G _s C96A fw	GCCTTCAACGATGTGACTGCCATCATCTTCG
Mini-G _s C96A rv	CTGGATCCACTTGCGGCGTTCATCG
β ₁ AR W130E fw	GAGACCTTGTGCGTCATCG
β ₁ AR W130E rv	GATGCTTGCCGTCACG
β ₁ AR L289M fw	ATGAAGACATTGGGTATCATCATGG
β ₁ AR L289M rv	AGCTTTGTGTTCCCTCATGG
β ₁ AR N-Avi fw	GCAGAAAATTGAATGGCATGAAGGGGCCGAGCTGC
β ₁ AR N-Avi rv	GCTTCAAAAATATCGTTCAGGCCCATTTTGGGGGATCCGTATTTATAG

Supplementary Methods

BLI experimental procedure BLI experiments assessing β_1 AR binding kinetics to binding partners were performed in triplicate, with $n=3$ separate tips and corresponding wells for immobilisation of receptor and mini-G protein binding, and an additional reference channel. The reference channel wells were prepared with the exact conditions of each equivalent experimental well, with the exception of the receptor loading well which contained unbiotinylated avi- β_1 AR. The unbiotinylated avi- β_1 AR was prepared using material separated from the biotinylated avi- β_1 AR material prior to the biotinylation treatment step described in Methods.

Biosensor tips were blocked using 0.1% BSA by incubating in buffer for 10 minutes prior to experiments. BLI protocols for different experiments followed the same loading steps for receptor immobilisation. A baseline step (120 s) was recorded prior to receptor loading. Receptor loading and dissociation of any non-specifically bound material was performed as described in Methods. A baseline step was performed and an association step (300-600 s) into buffer containing agonist was performed to assess effects of ligand on BLI responses and as an additional blocking step prior to analyte association. Association and dissociation steps to assess analyte binding kinetics were performed as described in Methods, and baseline steps were performed between each new association/dissociation step. Reference channels followed the same protocols and were used to subtract any non-specific interactions between tips and analyte from the experimental data during processing. The $n=3$ individual binding isotherms, each generated from a different biosensor, were used to calculate binding kinetics k_{obs} and k_{off} , which then were used to obtain k_{on} and K_D values as detailed in the Methods section. Means and SDs of the $n=3$ individual repeats were then calculated for each kinetic parameter. A scheme and example of BLI procedures is shown in Supplementary Fig. 3a.

BLI controls Non-specific association of receptor to biosensors was assessed using reference channels with loading of unbiotinylated avi- β_1 AR constructs, and the β_1 AR-E construct used for NMR (no avi-tag) untreated and with biotinylation treatment. Each control showed minimal non-specific association of receptor to biosensors, and no significant loading of biotinylation treated β_1 AR-E indicated minimal off-target biotinylation of receptor at Lys residues outside the avi-tag. Loading controls are shown in Supplementary Fig. 3b-c. Specific association of mini-G_s with receptor was confirmed by loading of an unrelated biotinylated protein (biotinylated avi-tagged SARS-CoV2 Spike protein Receptor Binding Domain (RBD) (kindly provided by the Hyvönen group, Dept. of Biochemistry, University of Cambridge) onto biosensors in place of receptor and recording association and dissociation curves with mini-G_s and isoprenaline (Supplementary Fig. 3d).

Concentration ranges Loading of receptor to biosensors used 300 nM receptor. Agonist concentrations used were 500 μ M to ensure >99% bound receptor. Analyte concentrations ranged from 31 nM to 4000 nM. Above 4000 nM, deposition of analyte was observable on the reference channel biosensors, and thus no experimental conditions were used above this value. Concentration ranges used in BLI experiments are shown in Supplementary Table 4 and concentration trials on the reference channel are shown in Supplementary Fig. 3e.

BLI measurements and dose-responses Due to the substantially different affinities between the differing ternary complexes, different concentrations of analyte were utilised for each ternary complex, shown in Supplementary Table 4. Dose-response experiments and/or single point measurements were recorded to obtain kinetic values. Dose-response experiments were recorded with a dilution series and used to calculate K_D and R_{max} values as described in methods. Single point measurements were recorded through pre-inspection of isotherms recorded at 2-3 different concentrations and selection of the concentration which gave high-quality monophasic data with the lowest residuals. For the high affinity interactions (full agonist and canonical mini-G_s protein), both dose-response and optimised single point measurements were utilised, which gave similar results (Supplementary Fig. 4, Supplementary Table 1). Response curves were also plotted following the equation $\text{Response} = (R_{\text{max}} * \text{Concentration}_{\text{analyte}}) / (K_D + \text{Concentration}_{\text{analyte}})$.

Due to the concentration limit from deposition on biosensors described above, dose-response experiments were not performed for lower affinity interactions (non-canonical mini-G proteins, partial agonists, example of unused dose response for mini-G_{o1} shown in Supplementary Fig. 3f). Instead, single point measurements were recorded with optimised concentrations between 1 - 4 μ M to obtain sufficient signal to obtain kinetic parameters and give the lowest residuals.

Assessment of analyte stability The functionality and stability of analytes was assessed by ¹H 1D NMR experiments and comparing the amide fingerprint regions and well separated peaks in the upfield (0 to -1 ppm) region. Samples were measured at two time points separated at least 2-fold longer than BLI experiments and incubated at 298 K between these time points. An example of mini-G_s stability assessment is shown in Supplementary Fig. 3g.

Supplementary References

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