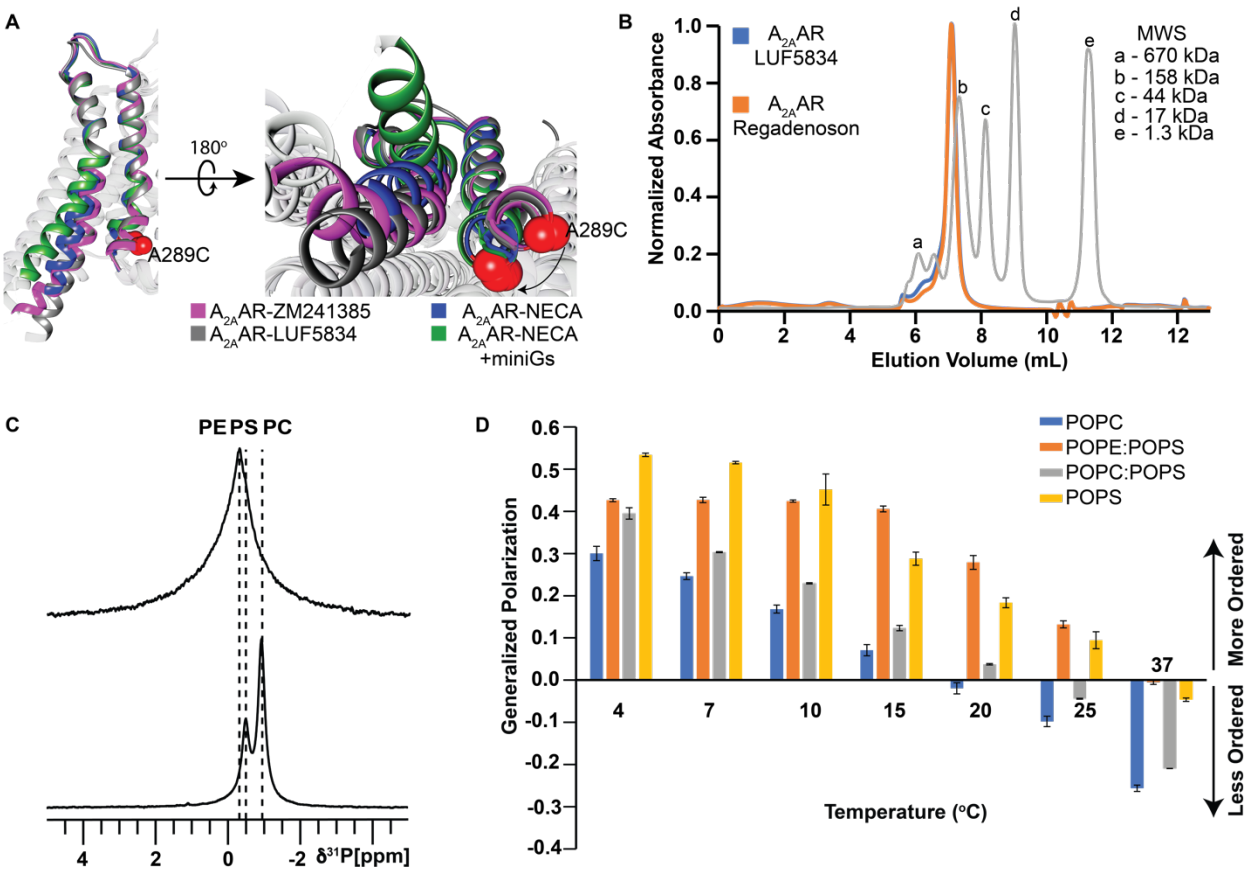


**Figure S1: Biophysical characterization and membrane fluidity measurements of nanodiscs of varied lipid compositions. Related to Figures 1, 2 and 3.**

(A) Structures of $A_{2A}AR$ in complex with antagonist ZM241385 (pink ribbon, PDB ID: 3EML), partial agonist LUF5834 (grey ribbon, PDB ID: 7ARO), agonist NECA (blue ribbon, PDB ID: 2YDV), and agonist NECA + mini-Gs (green ribbon, PDB ID: 5G53). For clarity, only helices 6 and 7 are shown here. (Right) Intracellular view of helices 6 and 7, highlighting structural changes on helix 6 and 7. Position 289 is used as a site for $^{19}F$-NMR labelling and shown as a red sphere.

(B) Analytical size exclusion chromatograms of $A_{2A}AR$ in complex with Regadenoson (orange) and LUF5834 (blue) in POPC:POPS (70:30) nanodiscs. The grey chromatogram represents molecular weight standards with the molecular weights indicated on the corresponding peaks.

(C) 1-dimensional $^{31}P$-NMR of nanodiscs containing $A_{2A}AR[A289C^{TET}]$ and POPE:POPS (70:30, molar ratio) or $A_{2A}AR[A289C^{TET}]$ and POPC:POPS (70:30, molar ratio). Signals assigned to POPE, POPS, and POPC are indicated by the vertical dashed lines with

chemical shifts consistent with the manufacturer's reported values. The relative intensities of the two observed signals were qualitatively consistent with the expected lipid ratios in the nanodisc samples.

(D) Laurdan-based fluidity measurements of membrane lipids in nanodiscs containing POPC, POPE:POPS (70:30), POPC:POPS(70:30), and POPS across a range of temperature from 4 $^{o}$C to 37 $^{o}$C. Generalized polarization values are plotted as the mean ± s.e.m. for three independent trials.

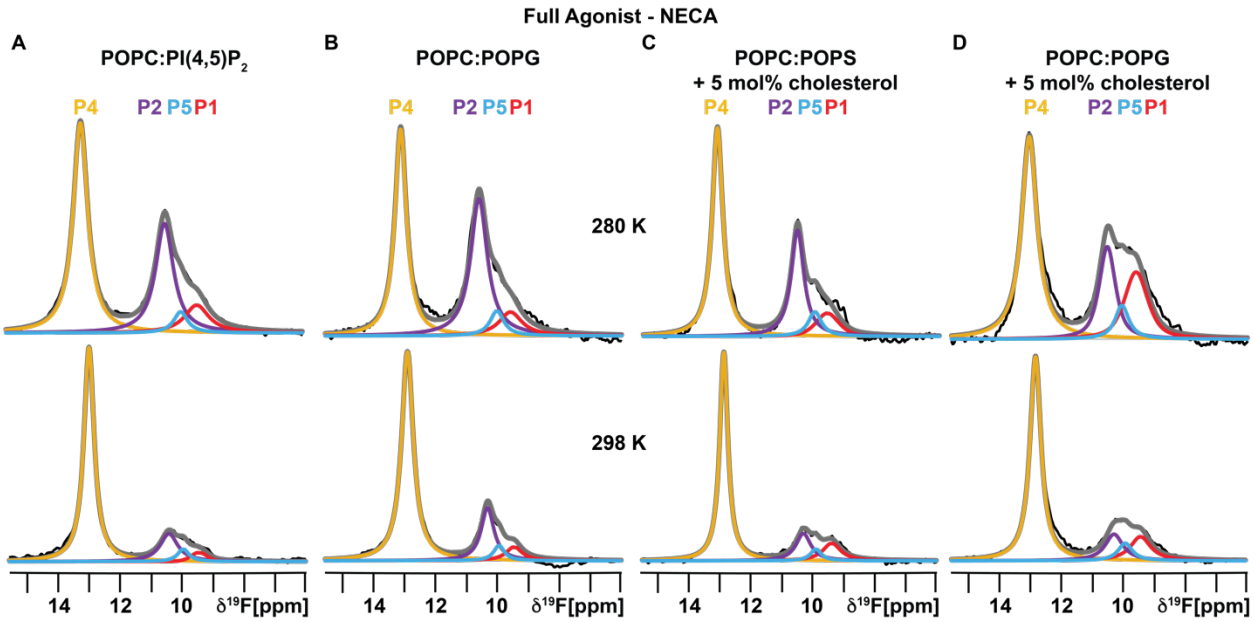


**Figure S2. Variable temperature $^{19}$F-NMR spectra of agonist-bound $A_{2A}$AR reconstituted in nanodiscs of varied lipid composition as a response to temperature. Related to Figure 1.**

(A – D) 1-dimensional $^{19}$F NMR spectra of $A_{2A}$AR-NECA reconstituted in (A) POPC:PI(4,5)$P_2$ (95:5, molar ratio), (B) POPC:POPG (70:30, molar ratio), (C) POPC:POPS:Cholesterol (70:30:5 mol%), and (D) POPC:POPG:Cholesterol (70:30:5 mol%) recorded at 280 K and 298 K.

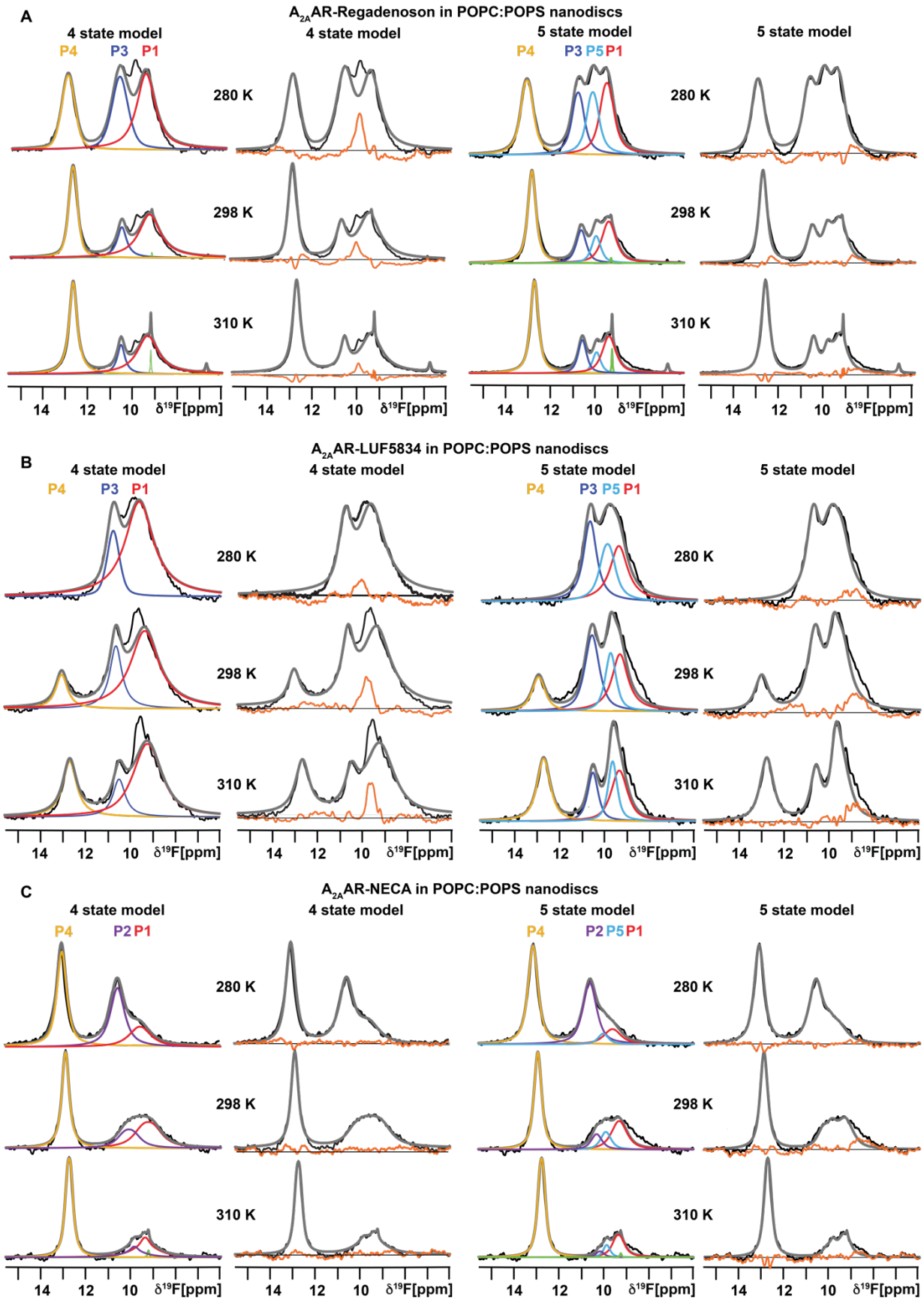

**Figure S3: $^{19}$F-NMR data fitting to different models. Related to Figure 1 and 2.**

(A – C) $^{19}$F NMR data of (A) $A_{2A}$AR-Regadenoson, (B) $A_{2A}$AR-LUF5834, and (C) $A_{2A}$AR-NECA in nanodiscs containing POPC:POPS (70:30, molar ratio) recorded at 280 K, 298 K, and 310 K. In each panel, the data on the left half are fit to a 4-state model and the date on the right half are fit to a 5-state model, as indicated. Other presentation details are same as Figure 1.

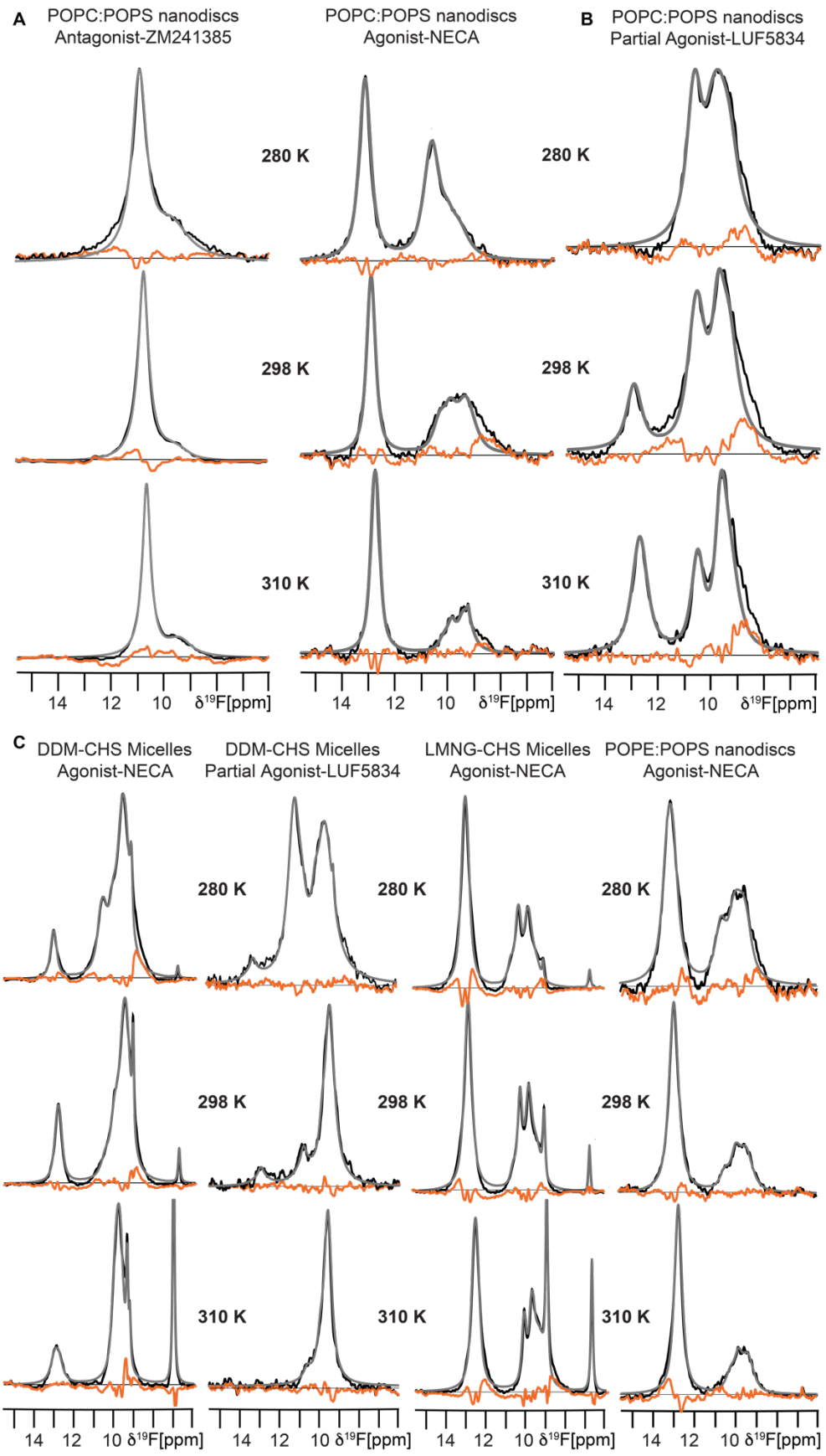

**Figure S4: Residual differences between the raw data and summation of the individually fit components from Figures 1, 2, and 3.**

(A – C) The experimental data are shown in black, the grey lines superimposed on the spectra are the total sums of the individual deconvolutions, and the orange line is the calculated difference between the grey and black lines.

**Table S1: Relative populations of the $A_{2A}$AR conformational states observed in $^{19}$F-NMR. Related to Figure 1, 2, and 3.**

The relative populations of the $A_{2A}$AR conformational states as observed in the $^{19}$F-NMR spectra are tabulated below. The value reported for each conformation is a ratio of the integrated area of the specific deconvoluted peak to the total integral of all signals from 6 ppm to 15.5 ppm, excluding any signals from free TET.

| Ligand | Ligand effiicacy | Membrane mimetic | Composition | Temperature | Relative populations | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | P4 | P3 | P2 | P1 | P5 |
| NECA | Agonist | Micelles | DDM/CHS | 280 K | 0.10 | 0.00 | 0.22 | 0.50 | 0.18 |
| NECA | Agonist | Micelles | DDM/CHS | 298 K | 0.12 | 0.00 | 0.07 | 0.63 | 0.18 |
| NECA | Agonist | Micelles | DDM/CHS | 310 K | 0.14 | 0.00 | 0.00 | 0.69 | 0.17 |
| | | | | | | | | | |
| LUF5834 | Partial Agonist | Micelles | DDM/CHS | 280 K | 0.04 | 0.40 | 0.11 | 0.38 | 0.08 |
| LUF5834 | Partial Agonist | Micelles | DDM/CHS | 298 K | 0.07 | 0.11 | 0.04 | 0.70 | 0.08 |
| LUF5834 | Partial Agonist | Micelles | DDM/CHS | 310 K | 0.00 | 0.00 | 0.11 | 0.62 | 0.27 |
| | | | | | | | | | |
| NECA | Agonist | Micelles | LMNG/CHS | 280 K | 0.36 | 0.04 | 0.23 | 0.11 | 0.26 |
| NECA | Agonist | Micelles | LMNG/CHS | 298 K | 0.51 | 0.03 | 0.13 | 0.15 | 0.18 |
| NECA | Agonist | Micelles | LMNG/CHS | 310 K | 0.48 | 0.00 | 0.13 | 0.22 | 0.17 |
| | | | | | | | | | |
| ZM241385 | Antagonist | Nanodiscs | POPC:POPS (70:30) | 280 K | 0.00 | 0.70 | 0.00 | 0.30 | |
| ZM241385 | Antagonist | Nanodiscs | POPC:POPS (70:30) | 298 K | 0.00 | 0.71 | 0.00 | 0.29 | |
| ZM241385 | Antagonist | Nanodiscs | POPC:POPS (70:30) | 298 K | 0.00 | 0.89 | 0.00 | 0.11 | |
| | | | | | | | | | |
| NECA | Agonist | Nanodiscs | POPC:POPS (70:30) | 280 K | 0.44 | 0.00 | 0.37 | 0.11 | 0.05 |
| NECA | Agonist | Nanodiscs | POPC:POPS (70:30) | 298 K | 0.47 | 0.00 | 0.10 | 0.27 | 0.11 |
| NECA | Agonist | Nanodiscs | POPC:POPS (70:30) | 310 K | 0.68 | 0.00 | 0.04 | 0.24 | 0.08 |
| | | | | | | | | | |
| NECA | Agonist | Nanodiscs | POPC:PI(4,5)$P_2$ (95:5) | 280 K | 0.51 | 0.00 | 0.32 | 0.11 | 0.05 |
| NECA | Agonist | Nanodiscs | POPC:PI(4,5)$P_2$ (95:5) | 298 K | 0.74 | 0.00 | 0.17 | 0.04 | 0.05 |
| | | | | | | | | | |
| NECA | Agonist | Nanodiscs | POPC:POPG (70:30) | 280 K | 0.43 | 0.00 | 0.41 | 0.10 | 0.06 |
| NECA | Agonist | Nanodiscs | POPC:POPG (70:30) | 298 K | 0.70 | 0.00 | 0.19 | 0.06 | 0.05 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| NECA | Agonist | Nanodiscs | POPC:POPS (70:30) w/ 5 mol% cholesterol | 280 K | 0.52 | 0.00 | 0.31 | 0.10 | 0.08 |
| NECA | Agonist | Nanodiscs | POPC:POPS (70:30) w/ 5 mol% cholesterol | 298 K | 0.67 | 0.00 | 0.16 | 0.12 | 0.05 |
| | | | | | | | | | |
| NECA | Agonist | Nanodiscs | POPC:POPG (70:30) w/ 5 mol% cholesterol | 280 K | 0.50 | 0.00 | 0.21 | 0.21 | 0.07 |
| NECA | Agonist | Nanodiscs | POPC:POPG (70:30) w/ 5 mol% cholesterol | 298 K | 0.65 | 0.00 | 0.14 | 0.14 | 0.07 |
| | | | | | | | | | |
| NECA | Agonist | Nanodiscs | POPE:POPS (70:30) | 280 K | 0.47 | 0.00 | 0.15 | 0.26 | 0.13 |
| NECA | Agonist | Nanodiscs | POPE:POPS (70:30) | 298 K | 0.66 | 0.00 | 0.05 | 0.19 | 0.11 |
| NECA | Agonist | Nanodiscs | POPE:POPS (70:30) | 310 K | 0.65 | 0.00 | 0.07 | 0.18 | 0.09 |
| | | | | | | | | | |
| Regadenoson | Partial Agonist | Nanodiscs | POPC:POPS (70:30) | 280 K | 0.24 | 0.27 | 0.00 | 0.30 | 0.19 |
| Regadenoson | Partial Agonist | Nanodiscs | POPC:POPS (70:30) | 298 K | 0.40 | 0.17 | 0.00 | 0.30 | 0.13 |
| Regadenoson | Partial Agonist | Nanodiscs | POPC:POPS (70:30) | 310 K | 0.45 | 0.16 | 0.00 | 0.29 | 0.10 |
| | | | | | | | | | |
| LUF5834 | Partial Agonist | Nanodiscs | POPC:POPS (70:30) | 280 K | 0.00 | 0.42 | 0.00 | 0.46 | 0.12 |
| LUF5834 | Partial Agonist | Nanodiscs | POPC:POPS (70:30) | 298 K | 0.15 | 0.34 | 0.00 | 0.32 | 0.20 |
| LUF5834 | Partial Agonist | Nanodiscs | POPC:POPS (70:30) | 310 K | 0.31 | 0.19 | 0.00 | 0.30 | 0.20 |