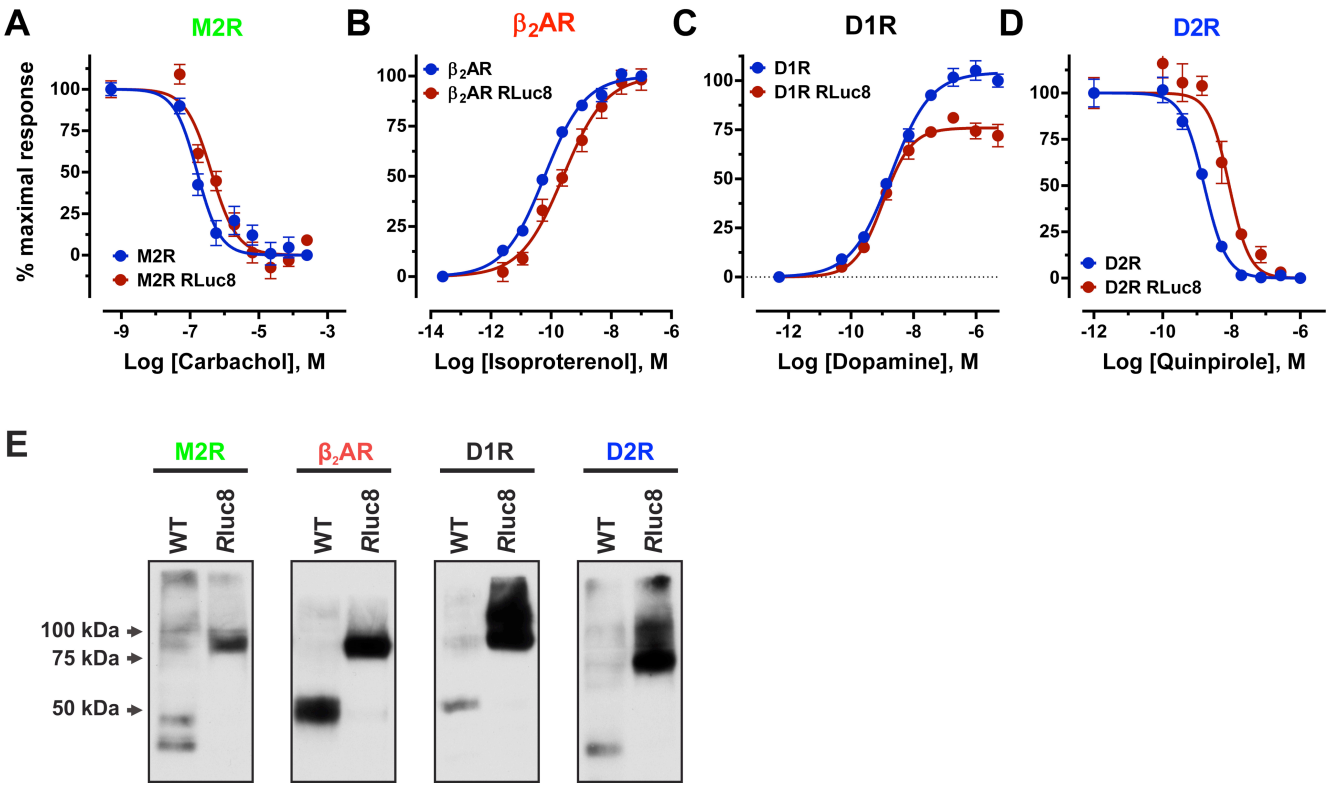
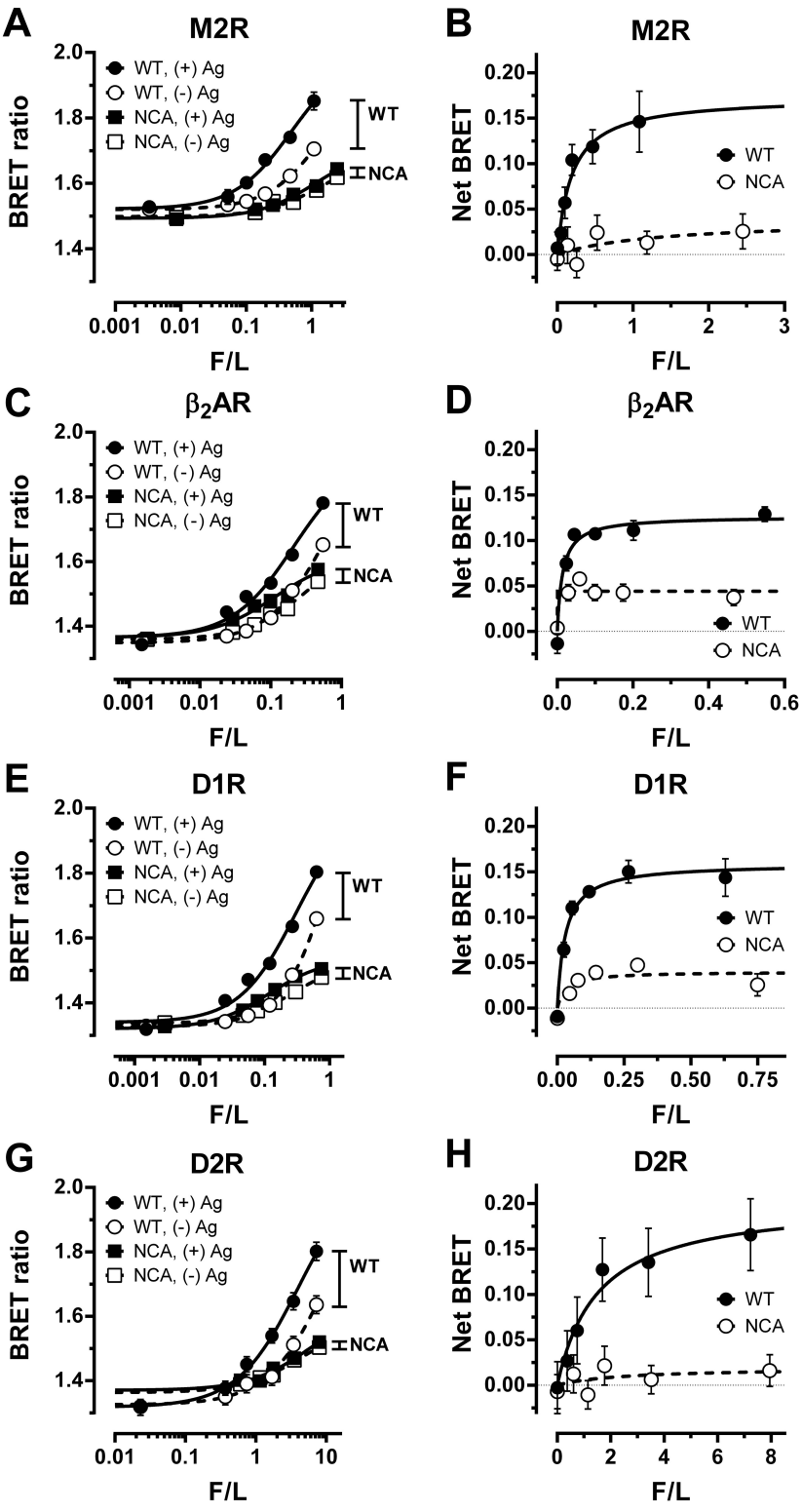
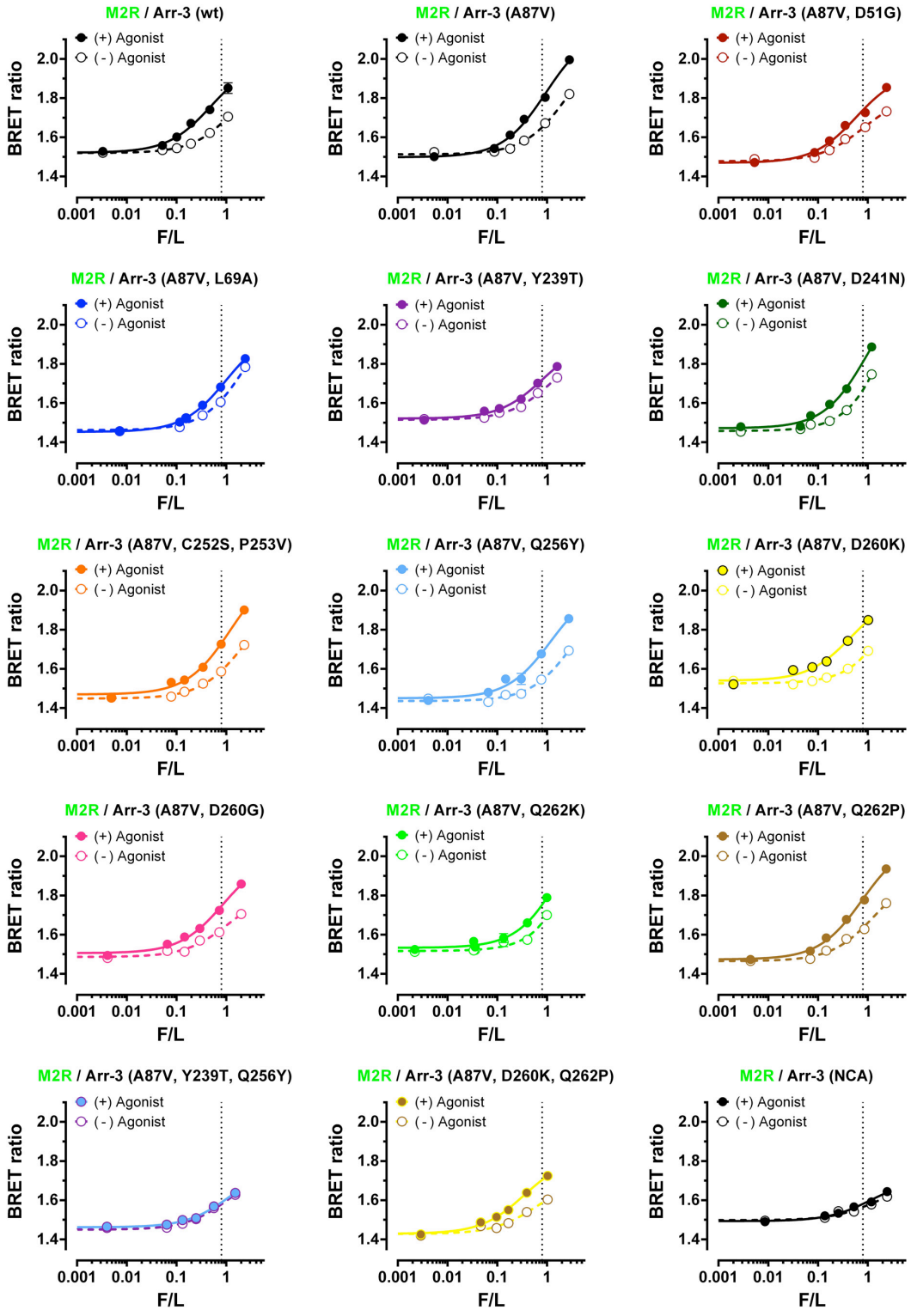


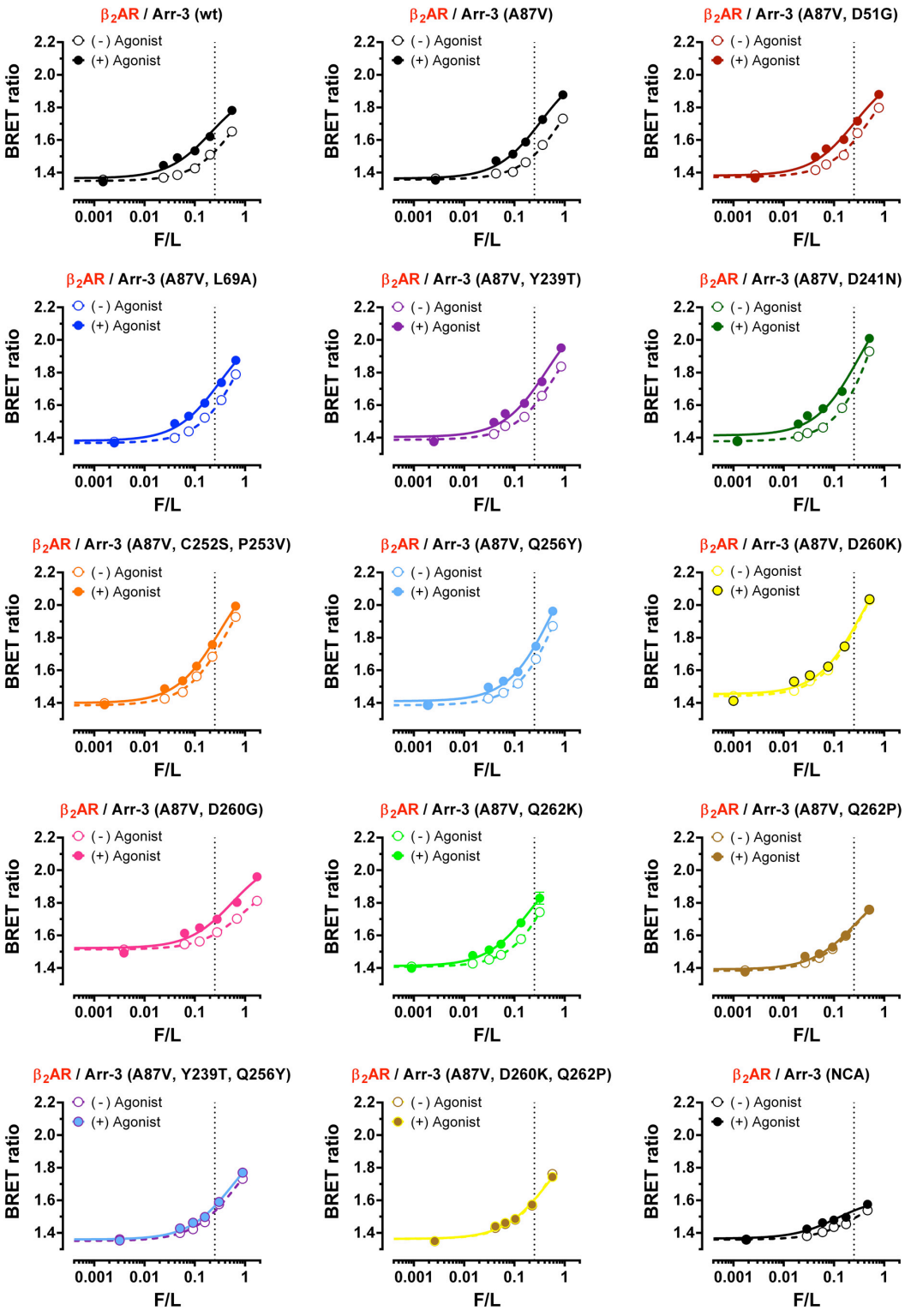
Suppl. Fig. S1. Functional activity of receptor-luciferase fusions. Indicated WT and *Renilla* luciferase-tagged M2R (**A**), β_2AR (**B**), D1R (**C**), or D2R (**D**) were co-expressed with GloSensor (clone 22f) in COS7 cells. Transfections were performed using Lipofectamine 2000 (Life Technologies, Grand Island, NY) for β_2AR and D1R, and FuGENE HD (Promega, Madison, WI) for the M2R or D2R. Cells expressing G_s-coupled β_2AR (**B**) and D1R (**C**) were challenged with indicated concentrations of isoproterenol or dopamine, respectively, and the luminescence of GloSensor (reflecting cAMP levels) was measured after five minutes with Wallac 1420 VICTOR^2 plate reader (PerkinElmer) and normalized to the signal obtained in the presence of 10 μM of the direct adenylyl cyclase activator forskolin. Maximum effect of both β_2AR and D1R was close to that of 5 μM forskolin (**B,C**). For the Gi-coupled D2R (**D**), cells were primed with indicated concentrations of quinpirole for 15 minutes followed with 2.5 μM forskolin. Luminescence was measured 30 minutes after agonist addition and 15 minutes after forskolin challenge. For the M2R transfected cells, (**A**) indicated concentrations of carbamylcholine were added after a 15 minute pretreatment with 2.5 μM forskolin and luminescence measured 5 minutes afterwards. Maximum reduction of forskolin-stimulated cAMP production by fully activated M2R and D2R was 88% and 52%, respectively. All receptors contained N-terminal triple-HA-tag. Comparative expression of WT and luciferase-tagged receptors was determined using Western blot with anti-HA antibody (**E**). The results indicated that all four *Renilla* luciferase-tagged receptors are functional, although their potency in all cases was lower than that of corresponding untagged WT GPCRs, in line with an earlier report that β_2AR-GFP (which is similar in size to luciferase) is active, but less potent than untagged β_2AR (1).



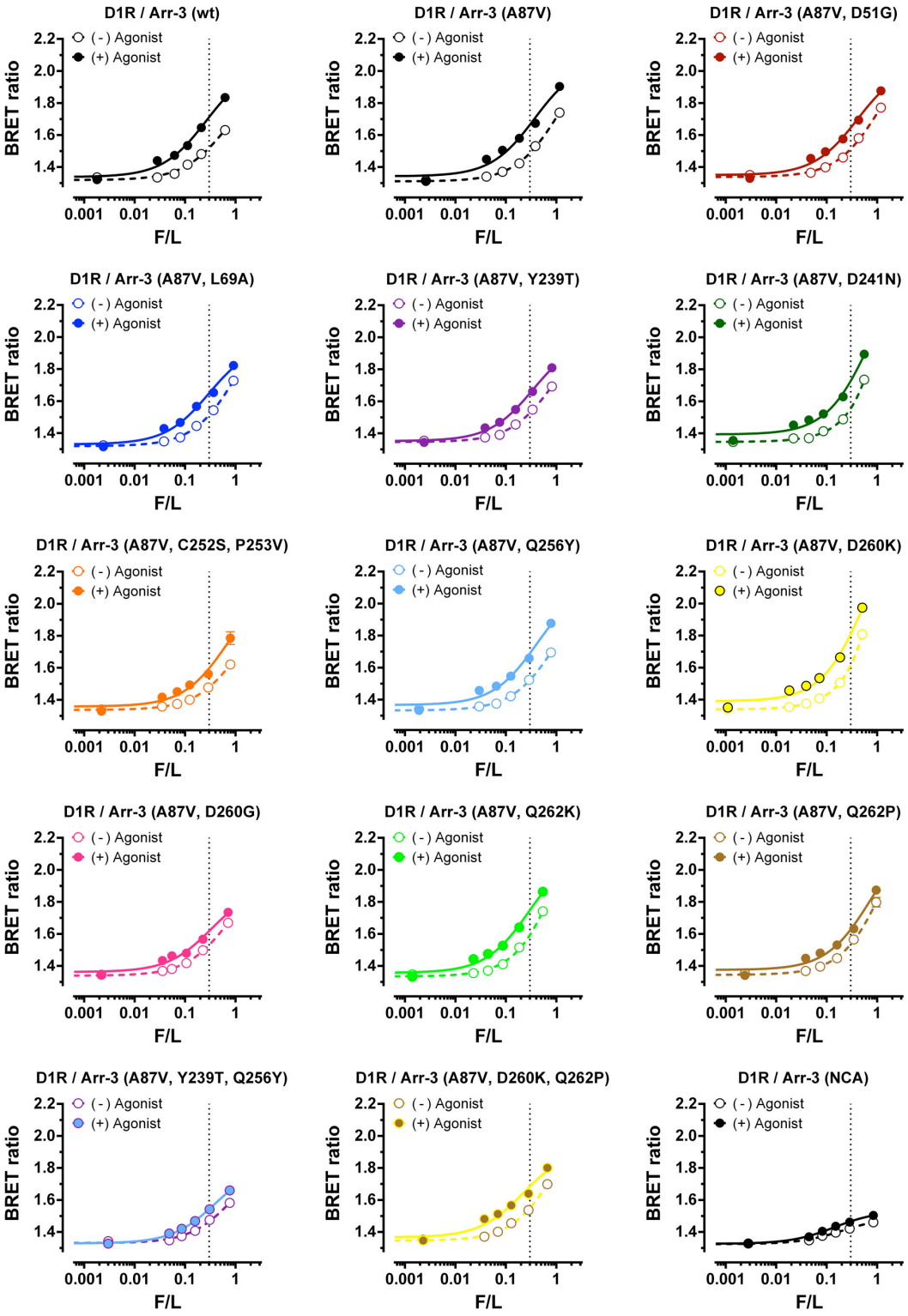
Suppl. Fig. S2. The comparison of WT arrestin-3 and NCA mutant. A-H. BRET in intact COS-7 cell was used to determine the interactions of increasing amounts of Venus-tagged WT arrestin-3 and NCA mutant with luciferase-tagged human M2R (**A,B**), β_2AR (**C,D**), D1R (**E,F**), or D2R (**G,H**). **A,C,E,G.** Raw BRET ratios in the presence (filled symbols) or absence (open symbols) of the respective agonist (25 μM carbamylcholine for M2R, 10 μM isoproterenol for β_2AR, 10 μM dopamine for D1R, or 10 μM quinpirole for D2R; 15 min at 37ºC) are plotted as a function of Venus-Arr-3 expression (fluorescence) normalized by receptor expression (luciferase luminescence) (F/L). Data were fitted to a single site sigmoid binding model. Bracketed lines on the right represent the difference between BRET in the presence and absence of the respective agonist. BRET ratios for NCA (described in (2)) were used to determine non-specific "bystander" BRET. **B,D,F,H.** Net BRET (increase in BRET signal induced by agonist stimulation) for the indicated arrestin-receptor combinations was calculated as the difference in BRET ratios in the presence and absence of agonists. Receptor stimulation induces saturable WT arrestin-3 binding (filled circles), whereas the NCA mutant (open circles) demonstrates very low to non-existent binding. A single site hyperbola-based model (GraphPad Prism 6.00) was used to fit the data. Means ± SE of six repeats in a representative experiment (out of three to fifteen performed) are shown.



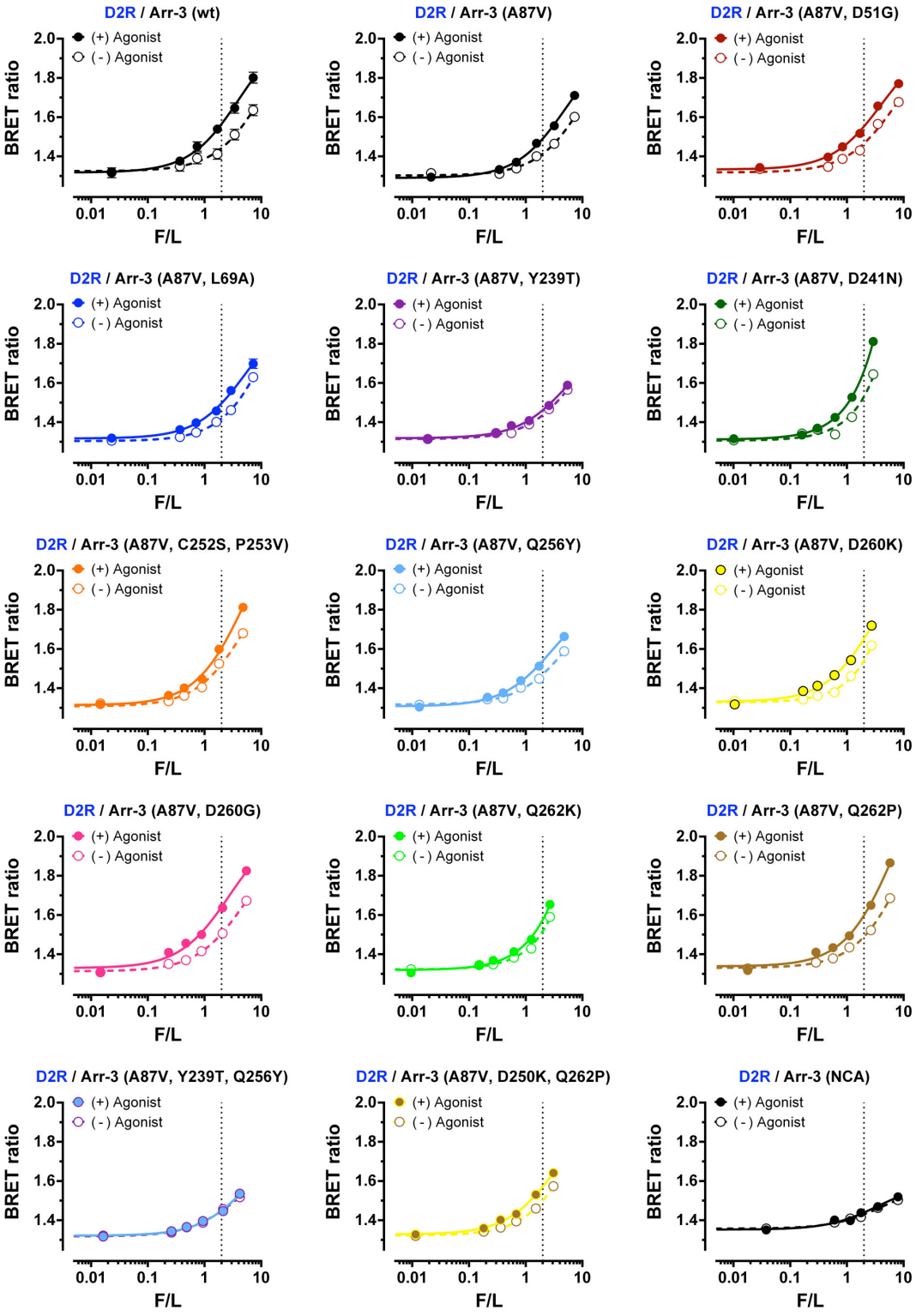
Suppl. Fig. S3. The interactions of WT and mutant forms of arrestin-3 with M2R. BRET ratios obtained with indicated arrestin-3 variants in the presence (filled circles) or absence (open circles) of agonist (25 μM carbamylcholine) are plotted as a function of Venus-Arr-3 fluorescence over M2R-*R*luc8 luminescence (F/L). Dotted vertical lines represent the F/L value at which the BRET ratios were used to determine the ΔBRET ratios in Suppl. Fig. S7 and Fig.5. Means ± SE of six repeats in a representative experiment (from a total of three to fifteen experiments performed). Data were fitted using a one site sigmoid model (GraphPad Prism 6.00).



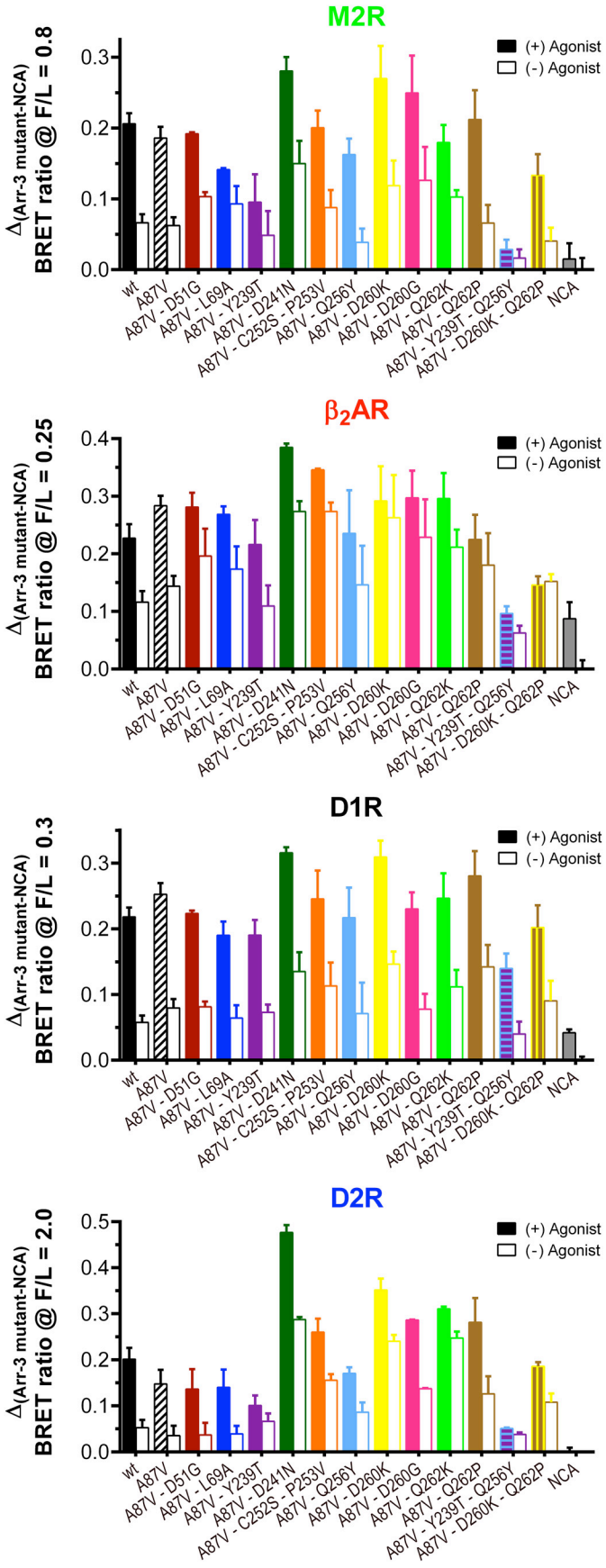
Suppl. Fig. S4. The interactions of WT and mutant forms of arrestin-3 with β2AR. BRET ratios obtained with indicated arrestin-3 variants in the presence (filled circles) or absence (open circles) of agonist (10 μM isoproterenol) are plotted as a function of Venus-Arr-3 fluorescence over β2AR-*R*luc8 luminescence (F/L). Dotted vertical lines represent the F/L value at which the BRET ratios were used to determine the ΔBRET ratios in Suppl. Fig. S7 and Fig.5. Means ± SE of six repeats in a representative experiment (from a total of three to fifteen experiments performed). Data were fitted using a one site sigmoid model (GraphPad Prism 6.00).



Suppl. Fig. S5. The interactions of WT and mutant forms of arrestin-3 with D1R. BRET ratios obtained with indicated arrestin-3 variants in the presence (filled circles) or absence (open circles) of agonist (10 μM dopamine) are plotted as a function of Venus-Arr-3 fluorescence over D1R-*R*luc8 luminescence (F/L). Dotted vertical lines represent the F/L value at which the BRET ratios were used to determine the ΔBRET ratios in Suppl. Fig. S7 and Fig.5. Means ± SE of six repeats in a representative experiment (from a total of three to fifteen experiments performed). Data were fitted using a one site sigmoid model (GraphPad Prism 6.00).



Suppl. Fig. S6. The interactions of WT and mutant forms of arrestin-3 with D2R. BRET ratios obtained with indicated arrestin-3 variants in the presence (filled circles) or absence (open circles) of agonist (10 μM quinpirole) are plotted as a function of Venus-Arr-3 fluorescence over D2R-*R*luc8 luminescence (F/L). Dotted vertical lines represent the F/L value at which the BRET ratios were used to determine the ΔBRET ratios in Suppl. Fig. S7 and Fig.5. Means ± SE of six repeats in a representative experiment (from a total of three to fifteen experiments performed). Data were fitted using a one site sigmoid model (GraphPad Prism 6.00).



Suppl. Fig. S7. The same residues in arrestin-3 mediate basal and agonist-promoted interactions with different GPCRs. Based on the data shown in suppl. Figs S3-S6, ΔBRET ratios were calculated by subtracting BRET ratio obtained with arrestin-3-NCA in the absence of agonist for each receptor (considered as baseline or "bystander" BRET) from the BRET ratios determined for different mutants with the corresponding receptor combination in the presence (filled bars) or absence (empty bars) of the respective agonist at F/L values indicated in Suppl. Figs. S3-S6. Selected F/L values are different for M2R, β2AR, D1R, and D2R, but the same for the binding of different mutants with each receptor. These ΔBRET ratios in the presence or absence of agonist were plotted in Fig. 5. Means ± SE of three to fifteen independent experiments performed in six repeats are shown.

1. Kallal, L., Gagnon, A. W., Penn, R. B., and Benovic, J. L. (1998) *J Biol Chem* **273**, 322-328
2. Gimenez, L. E., Kook, S., Vishnivetskiy, S. A., Ahmed, M. R., Gurevich, E. V., and Gurevich, V. V. (2012) *J Biol Chem* **287**, 9028-9040