

**Supplementary figure 1.** Directionalities of Gs protein activation BRET between Gs and  $\gamma 2$  or  $\gamma 7$  probes. Dopamine dose response of D1R activation is detected by BRET change between (A)  $\gamma 2$ -Rluc8 and Gs-Venus, (C) Gs-Rluc8 and  $\gamma 7$ -Venus, (E)  $\gamma 7$ -Rluc8 and Gs-Venus. Isoproterenol dose response of  $\beta 2AR$  activation is detected by BRET change between (B)  $\gamma 2$ -Rluc8 and Gs-Venus, (D) Gs-Rluc8 and  $\gamma 7$ -Venus, (F)  $\gamma 7$ -Rluc8 and Gs-Venus. Insertion positions are: orange = 69-70 aa, blue = 72-73 aa, red = 100-101 aa, and green = 155-156 aa.

**Supplementary figure 2. A-B.** Dose-response curves of Gs protein activation BRET for  $\beta 2AR$  with isoproterenol (A) and for D1R with dopamine (B). Different colors represent insertion positions for Rluc (orange = 67-68 aa, red = 99-100 aa, and green = 154-155 aa.) Dose response curves represent the means  $\pm$  S.E.M. of more than 5 experiments performed in triplicate.

**Supplementary figure 3. A-B.** Dose-response curves of Gs protein engagement BRET for  $\beta 2AR$  with isoproterenol (A) and for D1R with dopamine (B). Different colors represent insertion positions for Venus (black = 7-8 aa, orange = 67-68 aa, blue = 71-72 aa, red = 99-100 aa, green = 154-155 aa, and yellow = 175-176 aa.) Dose response curves represent the means  $\pm$  S.E.M. of more than 5 experiments performed in triplicate.

**Supplementary figure 4. A.** Scheme for the engagement BRET between D1R-Rluc and Golf-Venus. **B.** Dose-response curves of dopamine induced BRET between D1R-Rluc and Golf-Venus with and without co-expression of Ric8B (orange or black curve respectively). Dose response curves represent the means  $\pm$  S.E.M. of more than 3 experiments performed in triplicate.

**Supplementary figure 5. A-B.** Dose-response curves of dopamine induced BRET between D1R-Rluc and Golf-Venus. Different combinations of  $\beta \gamma$  subunits were tried in  $\gamma 2$  (A) or  $\gamma 7$  subunit (B) co-expression with  $\beta 1$  or  $\beta 2$  (black or orange). Dose response curves represent the means  $\pm$  S.E.M. of more than 3 experiments performed in triplicate.

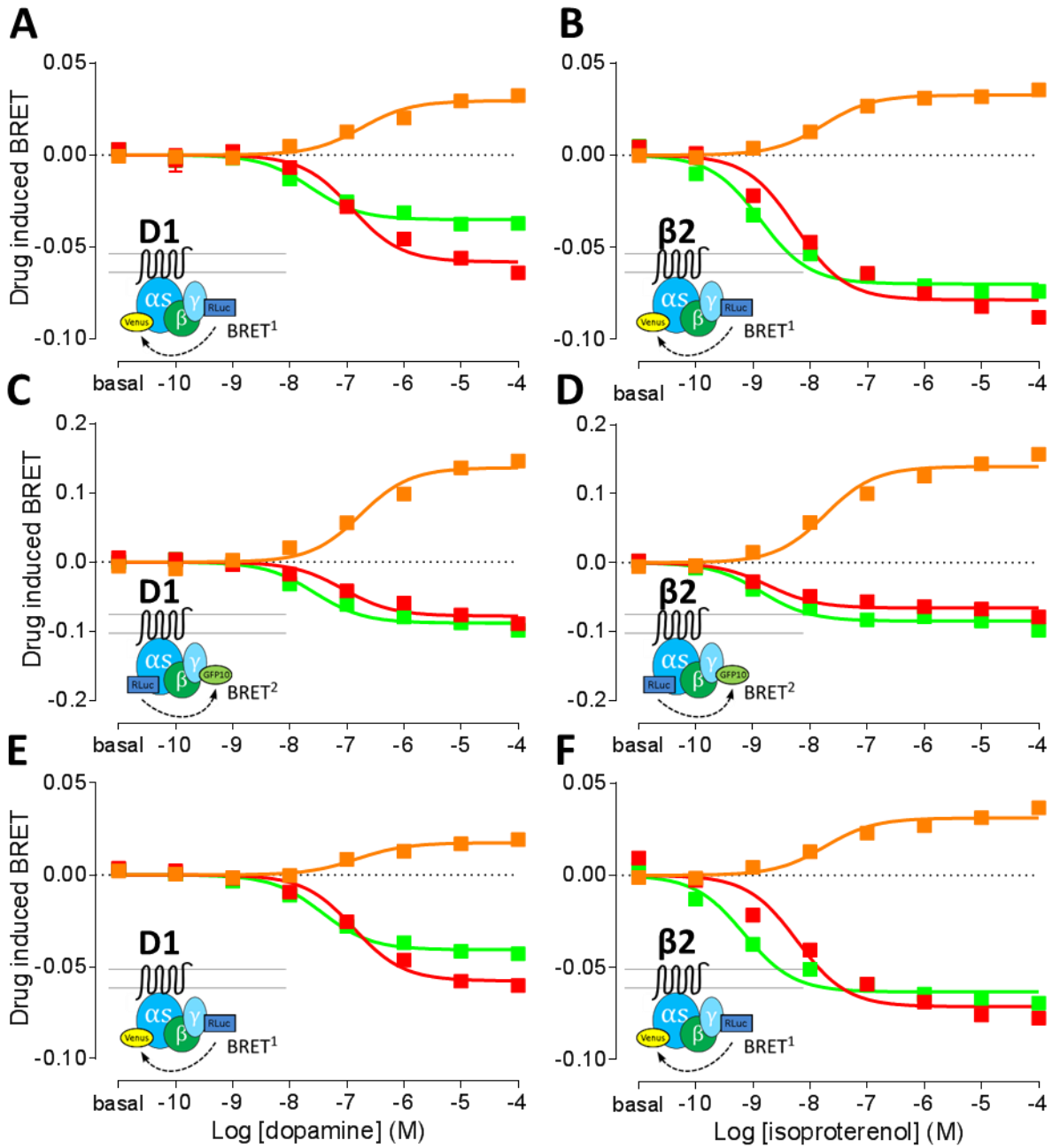
**Supplementary figure 6. A-B.** Dose-response curves of G protein engagement BRET between D2R and Gi1 with dopamine (A) and between M1R and Gq with carbachol (B). Different colors represent insertion positions for Venus (orange = 60-61 [Gi1] or 66-67 [Gq] aa, red = 91-92 [Gi1] or 97-98 [Gq] aa, and green = 145-146 [Gi1] or 150-151 [Gq] aa.) Dose response curves represent the means  $\pm$  S.E.M. of more than 3 experiments performed in triplicate.

**Supplementary table 1.** Distance between the C $\alpha$  atoms of experimental insertion points for Gs and  $\gamma 2$  subunits coupled to  $\beta 2AR$ . The distance between each numbered residue in Gs and N-terminus of  $\gamma 2$  subunit monitored during molecular dynamics is listed for closed and opened conformations (and the difference of the two) of Gs- $\beta 1$ - $\gamma 2$  heterotrimeric G protein. Values for positions 67 and 71 were based on

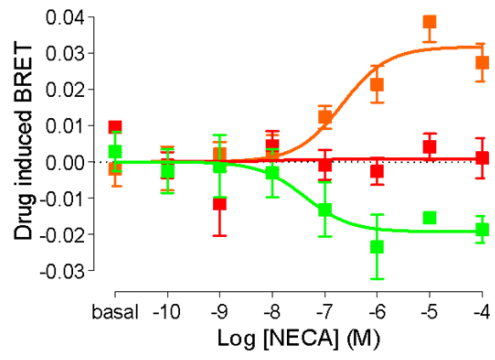
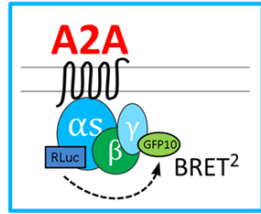
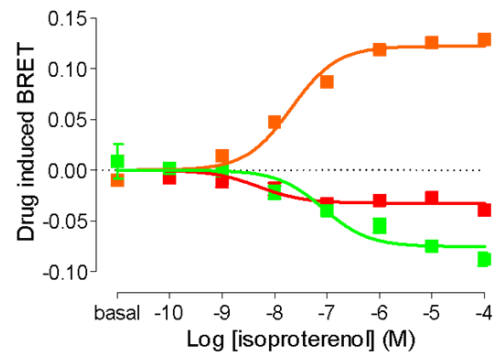
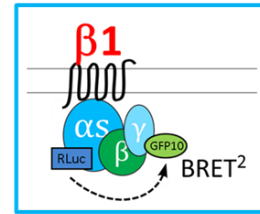
simulation model as they were not well-resolved in the crystals. Directionalities of BRET results are also listed in the last column.

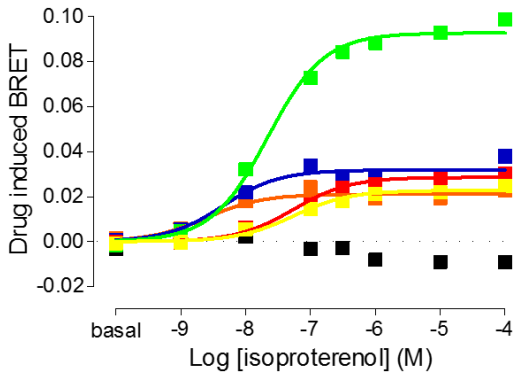
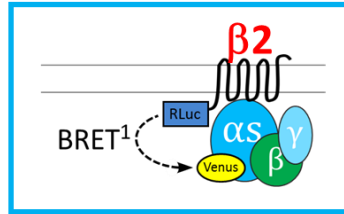
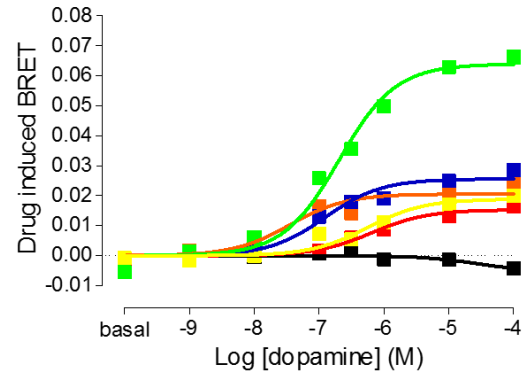
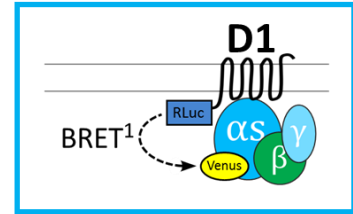
**Supplementary table 2.** Directionalities of Gs protein activation BRET between Golf and  $\gamma 7$  probes. First two rows and next two rows show agonist-induced BRET for D1R and  $\beta 2$ AR respectively. First row shows the directionality for BRET change between Golf-Rluc and  $\gamma 7$ -GFP10. Second row shows the directionality for BRET change in reciprocal configuration between  $\gamma 7$ -Rluc and Golf-Venus. Third and fourth rows are the same for  $\beta 2$ AR. The probe insertion positions for Golf are labeled across.

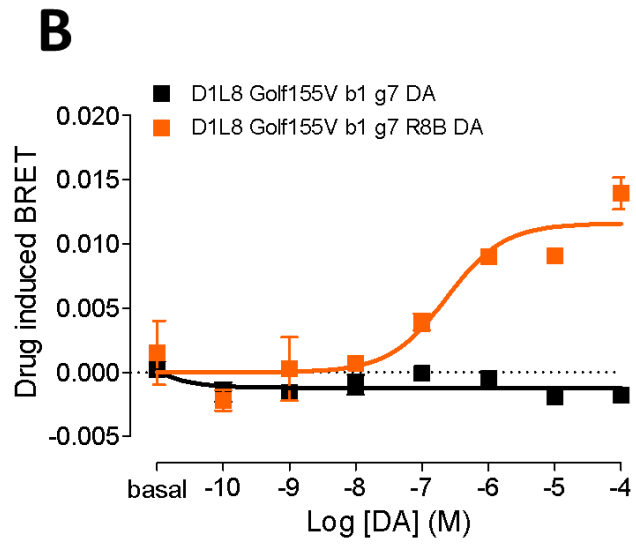
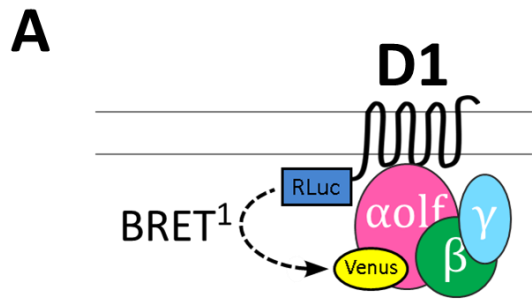
**Supplementary table 3.** Agonist induced engagement and activation of Gs and Golf in D1R. Data were fit by non-linear regression to a sigmoidal dose-response relationship against the agonist concentration.  $EC_{50}$  and  $E_{max}$  values are means  $\pm$  S.E.M. of more than 5 experiments performed in triplicate.  $E_{max}$  values are expressed in % normalized to dopamine results.



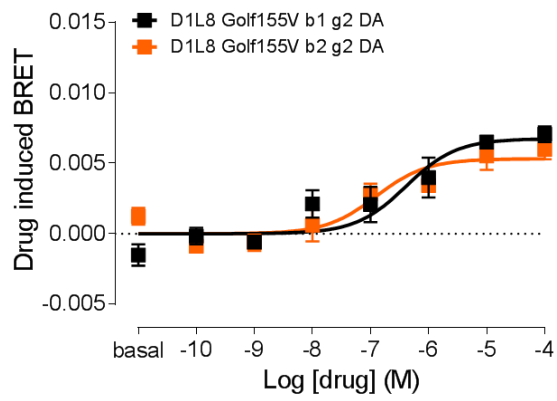
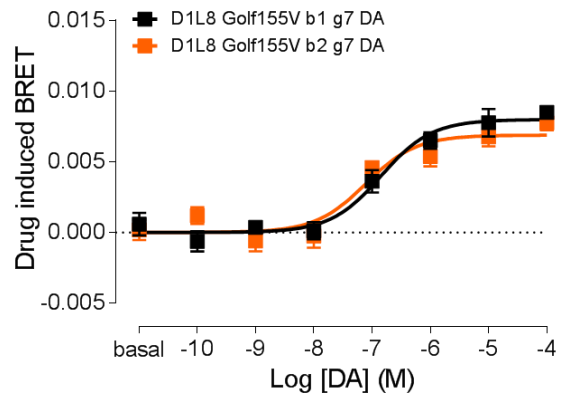
**Supplementary Figure 1**

**A****B****Supplementary Figure 2**

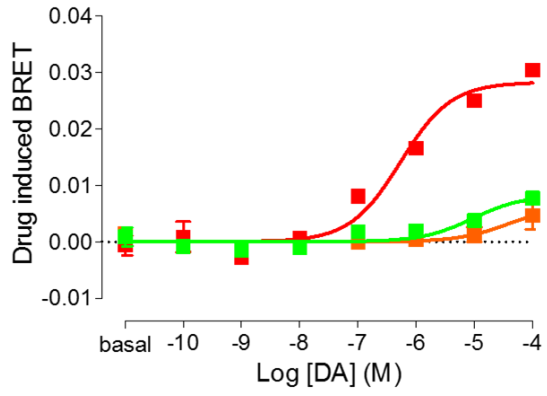
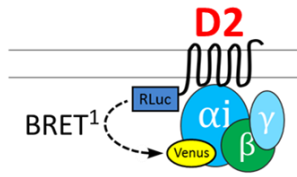
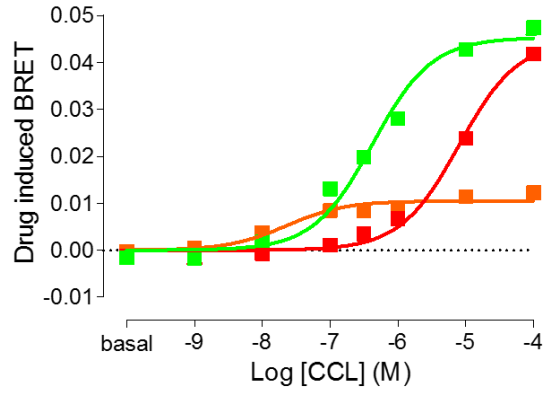
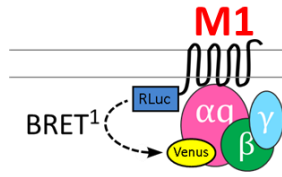
**A****B****Supplementary Figure 3**



**Supplementary Figure 4**

**A****B**

**Supplementary Figure 5**

**A****B****Supplementary Figure 6**



	Rluc location (# amino acid)	Inactive (Å)	Active (Å)	$\Delta$ distance (Å)	BRET ISO (efficacy)
Hinge Loop	67	40	33	-7	++
	71	40	35	-5	++
$\alpha$ -helical	99	16	45	29	--
	154	12	37	25	--
	175	36	45	9	0
Ras-like	305	37	42	5	0
	338	34	34	0	0
	349	31	31	0	0

**Supplementary Table 1**

		69 (ΔBRET)	100 (ΔBRET)	155 (ΔBRET)
β2AR	GolfL_γ7G	+	=	-
	GolfV_γ7L	+	-	-
D1R	GolfL_γ7G	+	=	-
	GolfV_γ7L	+	-	-

**Supplementary Table 2**

		Dopamine		Norepinephrine	
		EC <sub>50</sub> (nM)	E <sub>max</sub> (%)	EC <sub>50</sub> (nM)	E <sub>max</sub> (%)
Gs	GsL_γ7G	75.3 ± 9.6	100.0 ± 1.9	693.4 ± 131.1	97.5 ± 3.5
	D1L_GsV	49.0 ± 4.3	100.0 ± 1.3	1002.3 ± 116.3	99.2 ± 2.2
Golf	GolfL_γ7G	140.0 ± 64.5	100.0 ± 9.2	1336.6 ± 746.7	68.7 ± 10.4
	D1L_GolfV	111.9 ± 25.4	100.0 ± 3.9	2280.3 ± 784.5	97.8 ± 7.9

**Supplementary Table 3**