CODA-RET reveals functional selectivity as a result of GPCR heteromerization

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Supplementary Table 1. Characterization of D1R, D2R homo- and heteromers.

Receptor	G protein	Treatment	EC_{50} (Mean±SD) (M)	n
D1R	Gαs-RLuc+ mVenus-γ2	Dopamine	$7.66x10^{-8}$±$1.05x10^{-8}$	3
		SKF83822	$3.00x10^{-7}$±$6.48x10^{-8}$	2
D2R	Gαi-RLuc+ mVenus-γ2	Dopamine	$3.95x10^{-8}$±$2.48x10^{-9}$	3
		NPA	$7.94x10^{-9}$±$4.76x10^{-9}$	6
		NPA+Sulpiride	$1.07x10^{-6}$±$6.17x10^{-7}$	4
D1R-RLuc8	Gs-mVenus	Dopamine	$1.77x10^{-6}$±$1.09x10^{-6}$	4
		DAR100	$3.80x10^{-7}$±$2.14x10^{-7}$	3
D1R-RLuc8	Gs+V2β1 + V1γ2	Dopamine	$2.24x10^{-6}$±$1.23x10^{-6}$	2
	Gi+V2β1 + V1γ2	Dopamine	$2.61x10^{-6}$±$5.92x10^{-7}$	2
	no alpha+ V2β1 + V1γ2	Dopamine	$2.9x10^{-6}$	1
D2R-RLuc8	Gi91-mVenus	Dopamine	$1.89x10^{-6}$±$9.47x10^{-7}$	3
		NPA	$3.59x10^{-7}$±$7.71x10^{-8}$	4
	Gi91-mVenus no βγ	NPA	$8.48x10^{-7}$±$1.61x10^{-7}$	3
	Gi60-mVenus	NPA	$1.15x10^{-6}$±$9.88x10^{-6}$	2
	Gi122-mVenus	NPA	$5.34x10^{-7}$±$8.91x10^{-8}$	3
	Gi+V2β1 + V1γ2	Dopamine	$4.71x10^{-7}$±$1.78x10^{-6}$	3
	Gs+V2β1 + V1γ2	Dopamine	$4.97x10^{-7}$	1
	no alpha+ V2β1 + V1γ2	Dopamine	$3.34x10^{-7}$	1
D1R-L1 + D1R-L2	Gs-mVenus	DAR100+ 10^{-6} SCH23390	$1.06x10^{-7}$±$3.05x10^{-8}$	2
		SKF83822+SCH23390	$3.82x10^{-6}$±$3.57x10^{-6}$	2
		SCH23390+ 10^{-6} DAR100	$8.73x10^{-8}$±$3.20x10^{-8}$	3
D2R-L1 + D1R-L2	Gs-mVenus	DAR100+ 10^{-6} SCH23390	$3.69x10^{-6}$±$4.15x10^{-7}$	2
D2R-L1 + D2R-L2	Gi60-mVenus	NPA	$3.21x10^{-7}$	1
	Gi122-mVenus	NPA	$5.34x10^{-7}$	1
	Gi91-mVenus	Sulpiride+10^{-6} NPA	$1.63x10^{-8}$±$5.13x10^{-9}$	2
		Sulpiride+10^{-5} NPA	$5.38x10^{-6}$±$2.26x10^{-6}$	3
		Sulpiride+ 10^{-5} Quinpirole	$5.95x10^{-8}$	1
		Dopamine+Sulpiride	NF	2
		Quinpirole+Sulpiride	NF	2
		Quinpirole +SCH23390	$1.52x10^{-6}$±$2.02x10^{-7}$	2
		NPA+Sulpiride	$5.64x10^{-6}$±$1.73x10^{-6}$	5
		NPA+SCH23390	$1.91x10^{-7}$±$5.02x10^{-8}$	3

Supplementary Table 1 continuation.

Receptor	G protein	Treatment	EC_{50} (Mean±SD) (M)	n
D2R-L1 + D2R-L2	Gi+V2β1 + V1γ2	NPA	$4.87x10^{-8}$±$1.94x10^{-8}$	4
	no alpha+ V2β1 + V1γ2	NPA	$5.08x10^{-8}$±$3.56x10^{-8}$	4
	Gi+V2β1 + V1γ2	Dopamine	$1.15x10^{-6}$±$4.42x10^{-7}$	3
	Gi+V2β1 + V1γ2	Quinpirole	$7.64x10^{-7}$±$4.10x10^{-8}$	3
	Gi+V2β1 + V1γ2	NPA+10^{-7} Sulpiride	$4.97x10^{-7}$±$2.95x10^{-7}$	2
	Gi+V2β1 + V1γ2	NPA+10^{-5} Sulpiride	NF	2
	Gi+V2β1 + V1γ2	Sulpiride+10^{-6} NPA	$1.63x10^{-8}$±$5.63x10^{-9}$	2
	mVenus-γ2	Quinpirole	$2.07x10^{-6}$±$1.34x10^{-6}$	3
D1R-L1 + D2R-L2	Gs-mVenus	SKF83822	$1.49x10^{-7}$±$6.02x10^{-8}$	2
		NPA	$3.99x10^{-7}$±$1.78x10^{-7}$	2
	Gi-mVenus	Quinpirole	$2.79x10^{-6}$±$1.31x10^{-6}$	2
D2(D114A)R-L1 + D1R-L2	Gs-mVenus	SKF83822	$1.34x10^{-7}$±$4.29x10^{-8}$	2
		Dopamine	$5.41x10^{-7}$±$1.08x10^{-7}$	2
D2R-L1 + D2R-L2 + D1R	Gi-mVenus	Quinpirole	$3.25x10^{-6}$±$4.48x10^{-7}$	3
D1R-L1 + D1R-L2 + D2R	Gs-mVenus	SKF83822	$5.58x10^{-8}$±$2.46x10^{-8}$	2

n: number of independent experiments performed with triplicate samples

NF: not fit

Supplementary Table 2. BRET titration experiments.

BRET pair	$BRET_{50}$ (Mean±SD)	n
D2R-RLuc8 + D2R-mVenus	$3.75x10^{-3}$±$1.83x10^{-3}$	3
D1R-RLuc8 + D1R-mVenus	$1.32x10^{-3}$±$9.53x10^{-4}$	2
D1R-RLuc8 + D2R-mVenus	$1.43x10^{-3}$±$5.40x10^{-4}$	2

n: number of independent experiments performed with triplicate samples

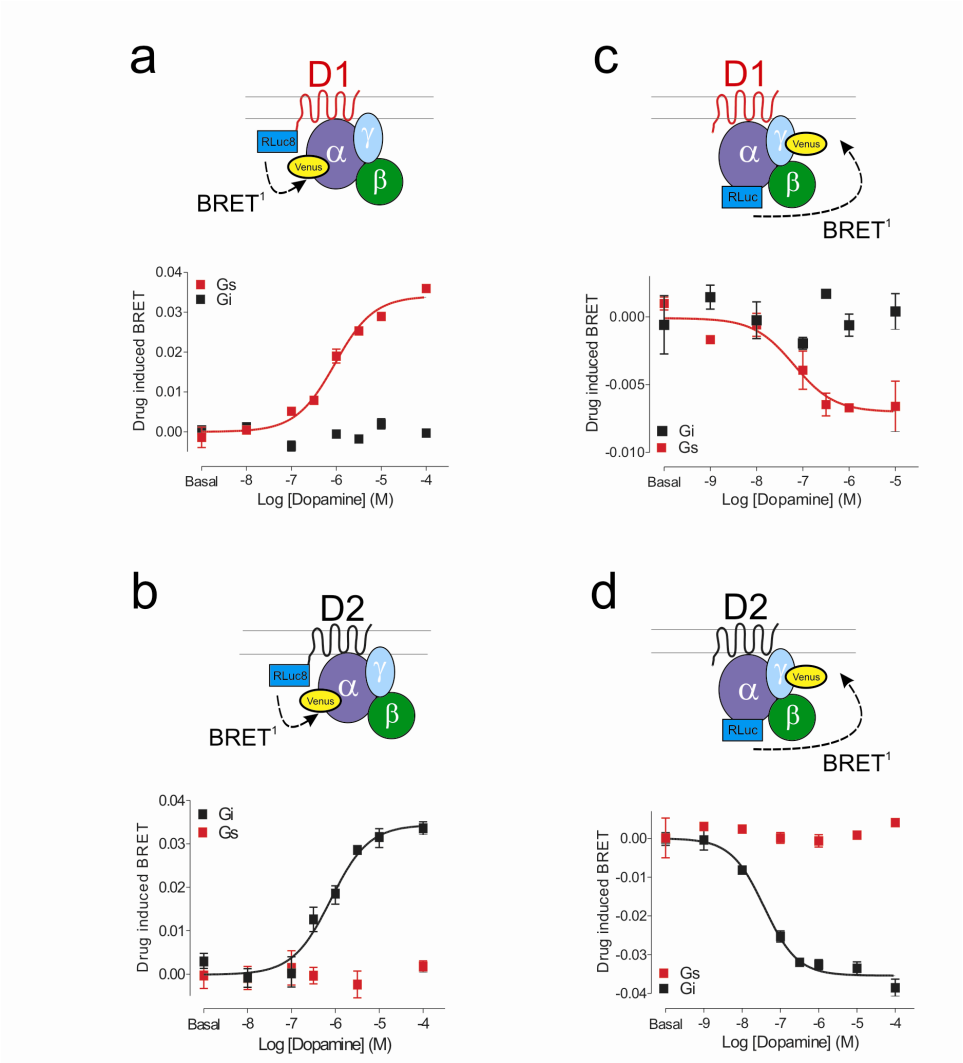
Supplementary Table 3. Ligand effects on BRET experiments performed in cells co-expressing constant amounts of D1R-RLuc8 and titrating amounts of D2R-mVenus.

BRET pair	Treatment (10^{-6}M)	$BRET_{50}$ (Mean±SD)	n
D1R-RLuc8 + D2R-mVenus	Dopamine	$1.16x10^{-3}$±$7.46x10^{-4}$	2
	Quinpirole	$1.09x10^{-3}$±$3.54x10^{-4}$	2
	NPA	$1.46x10^{-3}$±$5.29x10^{-4}$	2
	SKF83822	$1.32x10^{-3}$±$1.27x10^{-4}$	2
	Sulpiride	$1.33x10^{-3}$±$3.12x10^{-4}$	2
	SCH23390	$1.63x10^{-3}$±$4.80x10^{-4}$	2
	vehicle	$1.43x10^{-3}$±$5.40x10^{-4}$	2

n: number of independent experiments performed with triplicate samples

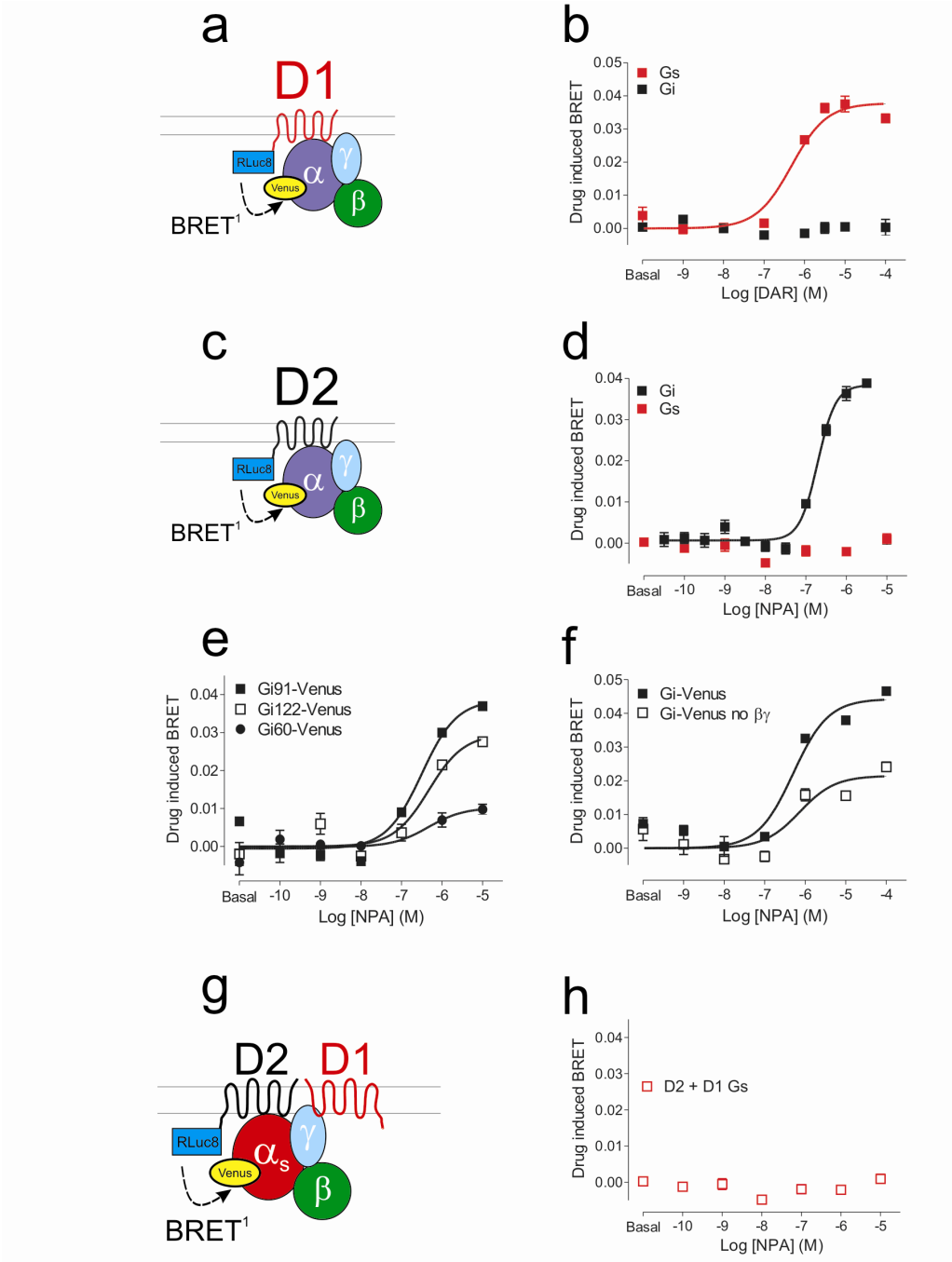
SUPPLEMENTARY FIGURES

Supplementary Figure 1



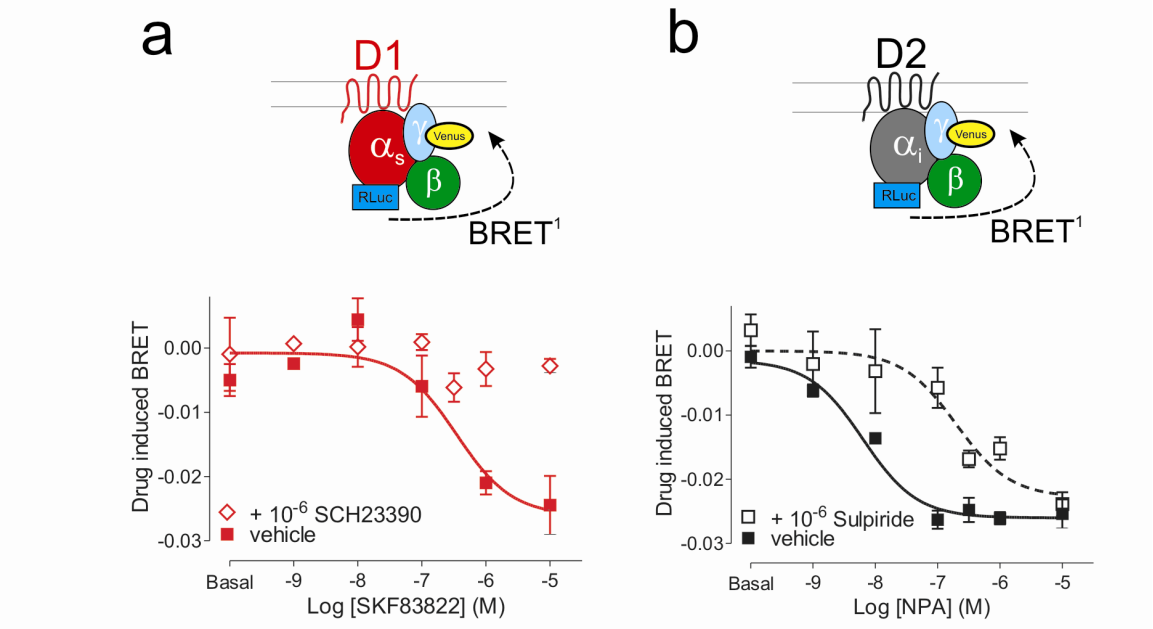
Supplementary Figure 1. *G protein BRET biosensors measure conformational changes associated with dopamine receptor activation.* HEK 293T cells transiently expressing D1R (**a**) or D2R (**b**) full length RLuc8 C-terminal fusions with Gαs-mVenus (red symbols and curves) or Gαi-mVenus (black symbols and curves) together with unlabeled β_1 and γ_2; or the unfused receptor (**c**-**d**) together with Gαs-RLuc (red symbols and curves) or Gαi-RLuc (black symbols and curves) and mVenus-γ_2 and unfused β_1 were harvested 48 h post-transfection, washed with PBS, centrifuged and resuspended in PBS. Cells were distributed in 96-well plates and preincubated with coelenterazine H for 1 min and then with increasing concentrations of dopamine for 1 min. $BRET^1$ was performed as described in Materials and Methods and results were fit by non-linear regression to a sigmoidal dose-response relationship against the dopamine concentration. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 2



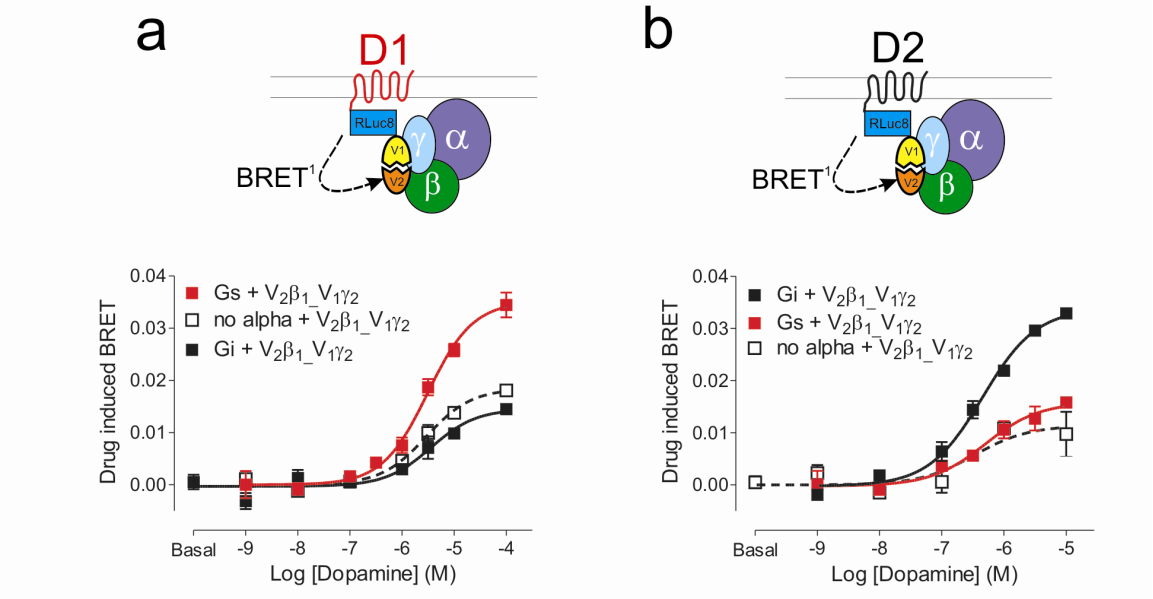
Supplementary Figure 2. *Optimization and characterization of the full length receptor–G protein BRET biosensors*. HEK 293T cells transiently expressing D1R (**a**-**b**) or D2R (**c**-**h**) full length RLuc8 C-terminal fusions, in the presence (**g**-**h**) or in the absence (**c**-**f**) of unfused D1R, together with Gαs-mVenus (red symbols and curves) or Gαi-mVenus (black symbols and curves) and unlabeled β_1 and γ_2 or not (**f,** open symbols) were prepared and analyzed as described in SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 3



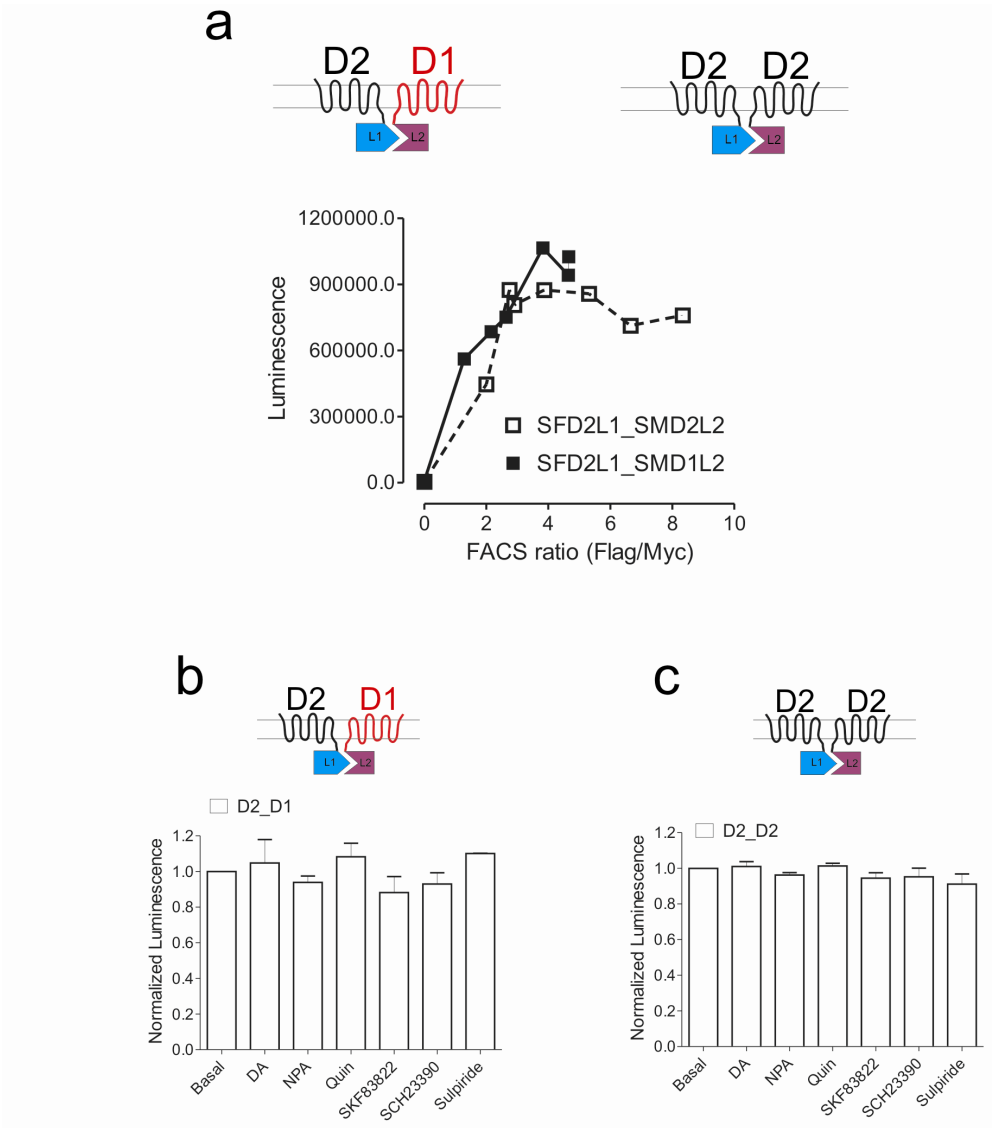
Supplementary Figure 3. *G protein biosensor detects an active conformation*. HEK 293T cells transiently expressing D1R and Gαs-mRLuc (**a**) or D2R and Gαi-mRLuc (**b**) together with mVenus-γ_2 unlabeled β_1 and γ_2 were prepared as explained for SF1. In experiments in which antagonists were used (open symbols and curves), the antagonists (1 μM) were preincubated for 15 mins at RT before the addition of the substrate and the tested agonist. BRET^1 was measured as explained in Material and Methods and analyzed as for SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 4



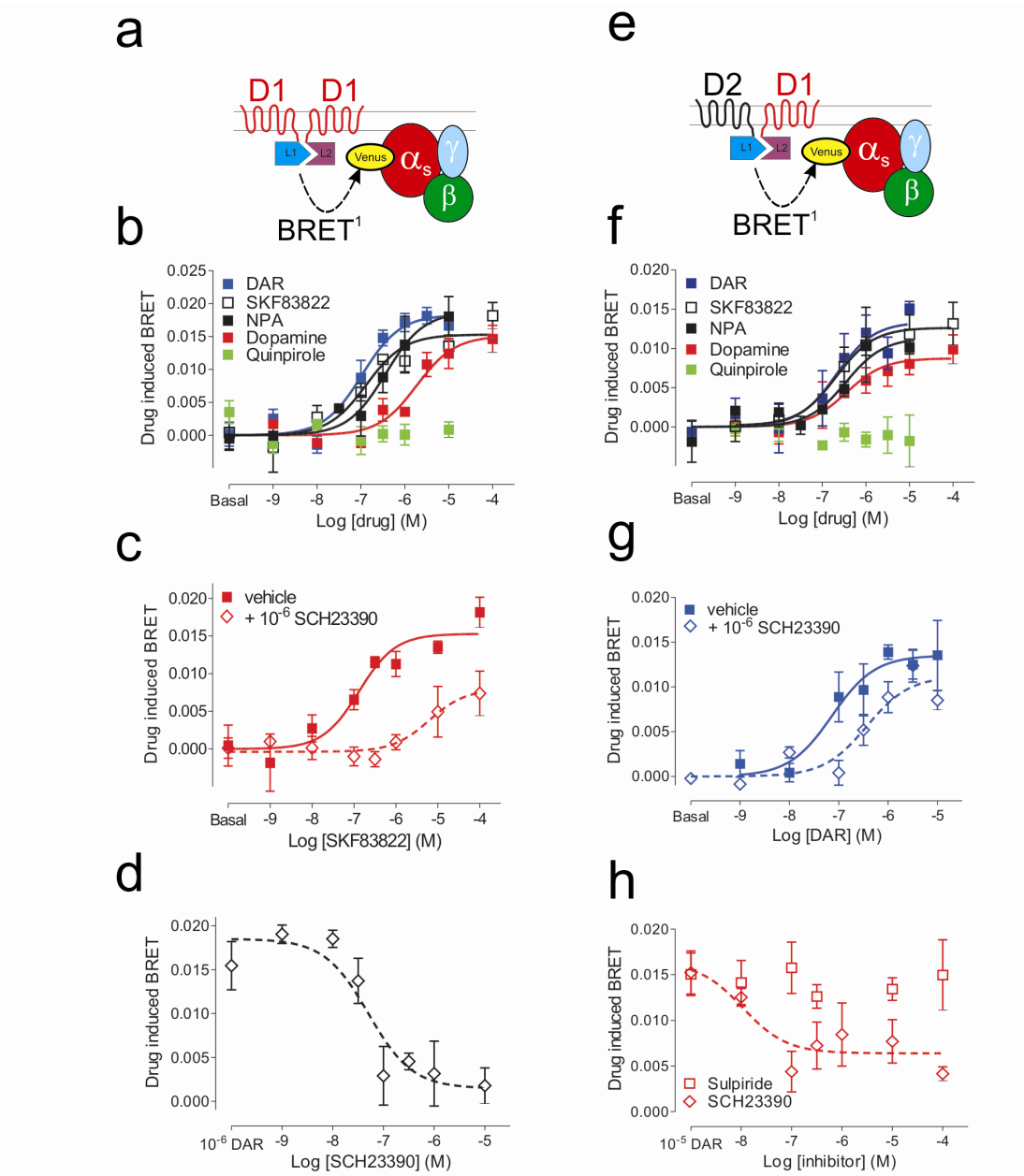
Supplementary Figure 4. *Characterization of a new biosensor to detect BRET changes at the level of the receptor and $\beta_1\gamma_2$.* HEK 293T cells coexpressing D1R (**a**) or D2R (**b**) full length RLuc8 C-terminal fusions together with V2 (C-terminal domain of mVenus, aa 156-end) at the N terminus of the β_1 subunit and V1 (N terminus of mVenus, aa 1-155) at the N terminus of the γ_2 with or without coexpression of the corresponding unlabeled Gα subunit were prepared and analyzed as described in SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 5



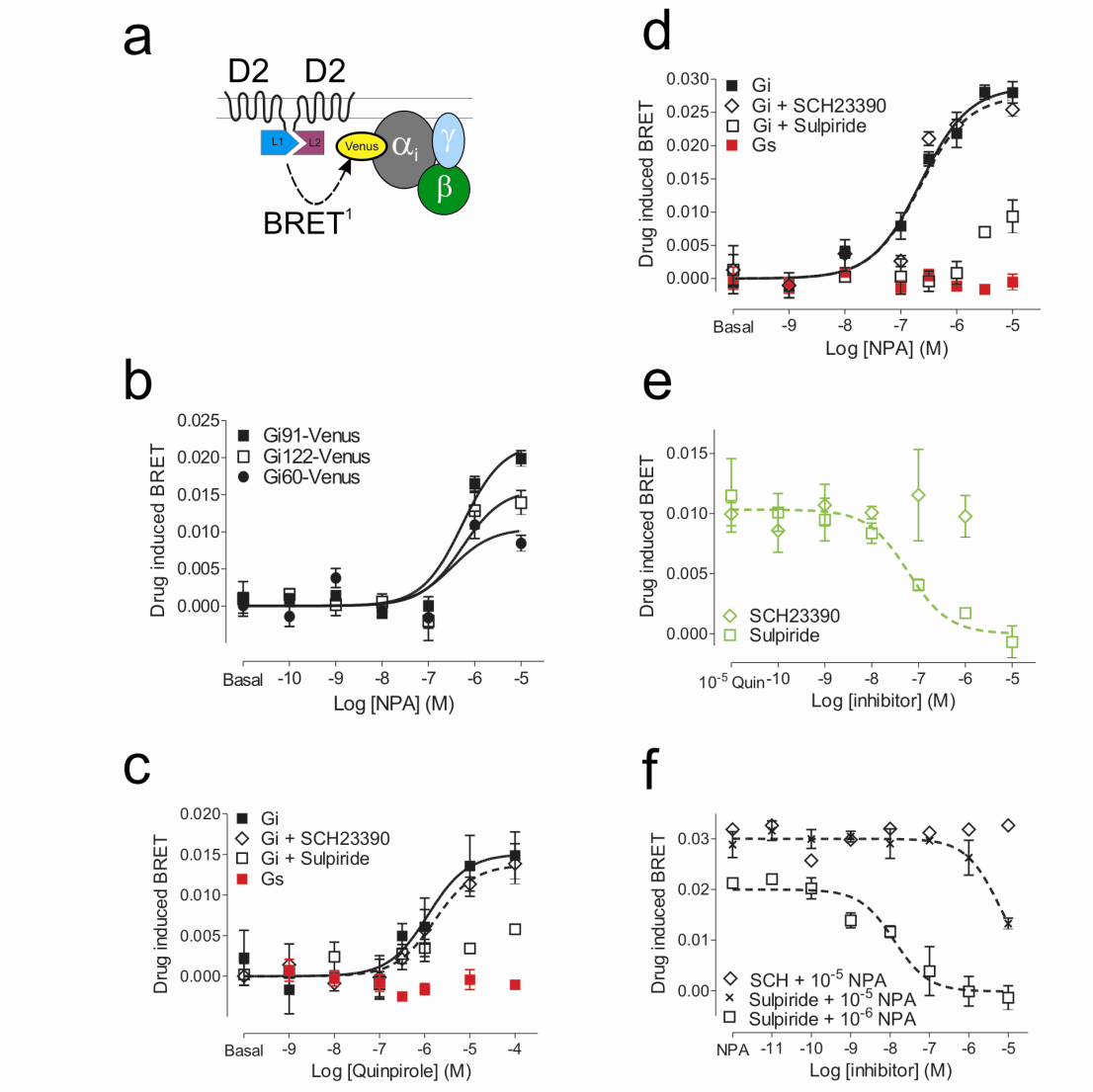
Supplementary Figure 5. *D2R interacts similarly with itself and with D1R and ligand binding to D2R and/or to D1R does not alter luminescence complementation.* HEK 293T cells transiently coexpressing D2R-L1 and D2R-L2 (**a**-open symbols and dashed line- and **c**) or D1R-L2 (**a**-closed symbols and continuous line- and **b**) were harvested 48 h post-transfection, washed with PBS, centrifuged and resuspended in PBS. Unfiltered luminescence was recorded for 1 s (Gain 3000) in the presence or in the absence of several selective and nonselective agonists and selective inverse agonists. Background was determined with cells expressing only one of the receptor probes and the signal to background ratio was plotted against the FACS ratio (**a**). For the FACS ratio, cells transfected in parallel were labeled with primary and secondary antibodies as described in the Material and Methods. Relative staining for each receptor was determined independently in the same cells with the same secondary anti-mouse antibody to determine the FACS ratio. Representative results from at least 2-3 independent experiments are shown.

Supplementary Figure 6



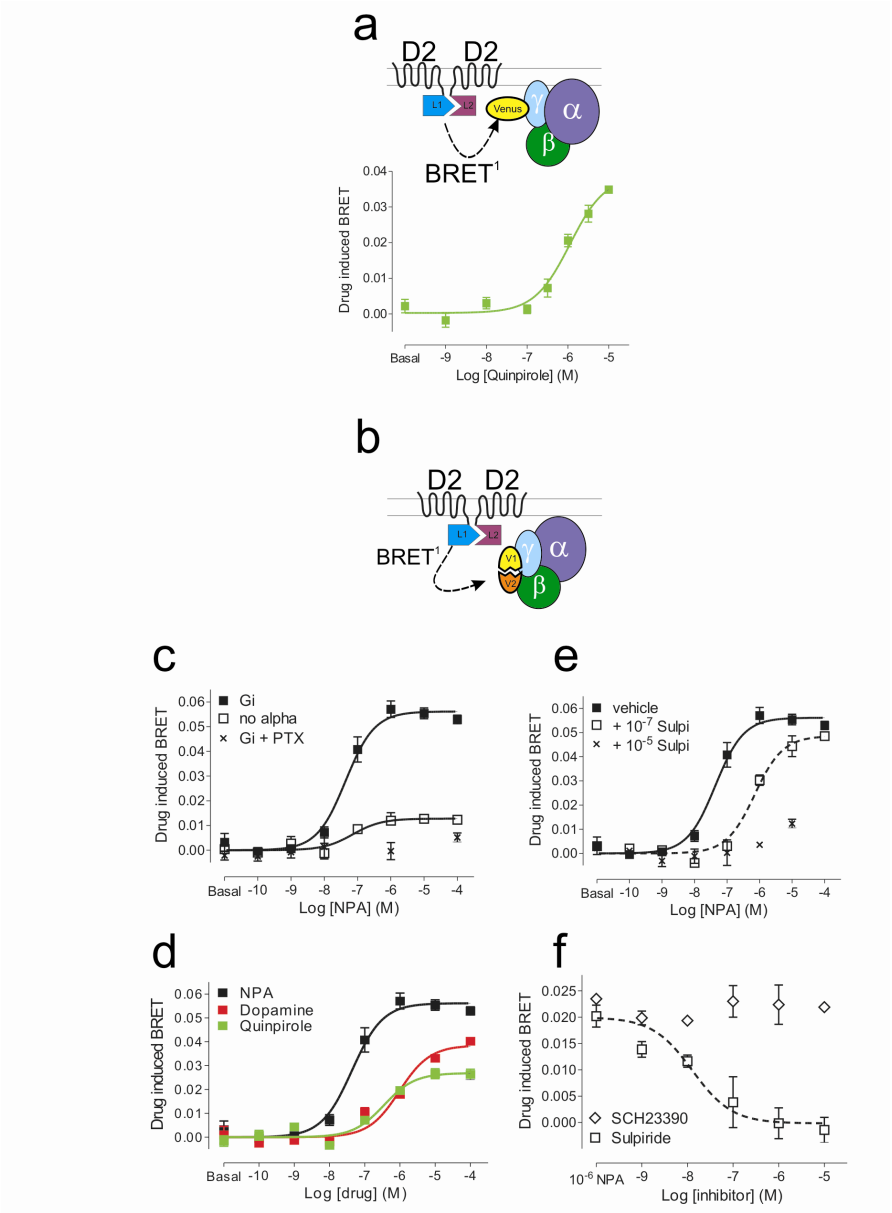
Supplementary Figure 6. *D1R agonism at D1R homo- and heteromers.* HEK 293T cells coexpressing D1R-L1, D1R-L2 (**a**-**d**); or D2R-L1, D1R-L2 together with Gαs-mVenus (**e**-**h**) and with unlabeled βγ were prepared as explained for SF1. In experiments in which antagonists were used (**c**-**d**,**g**-**h,** open symbols and curves), the antagonists (1 μM) were preincubated for 15 mins at RT before the addition of the substrate and the tested agonist. BRET[1] was measured as explained in Material and Methods and analyzed as for SF1. The graphs are representative of 1-5 independent experiments performed with triplicate samples (error bars represent S.E.M.), and summary data are presented in Supplementary Table 1.

Supplementary Figure 7



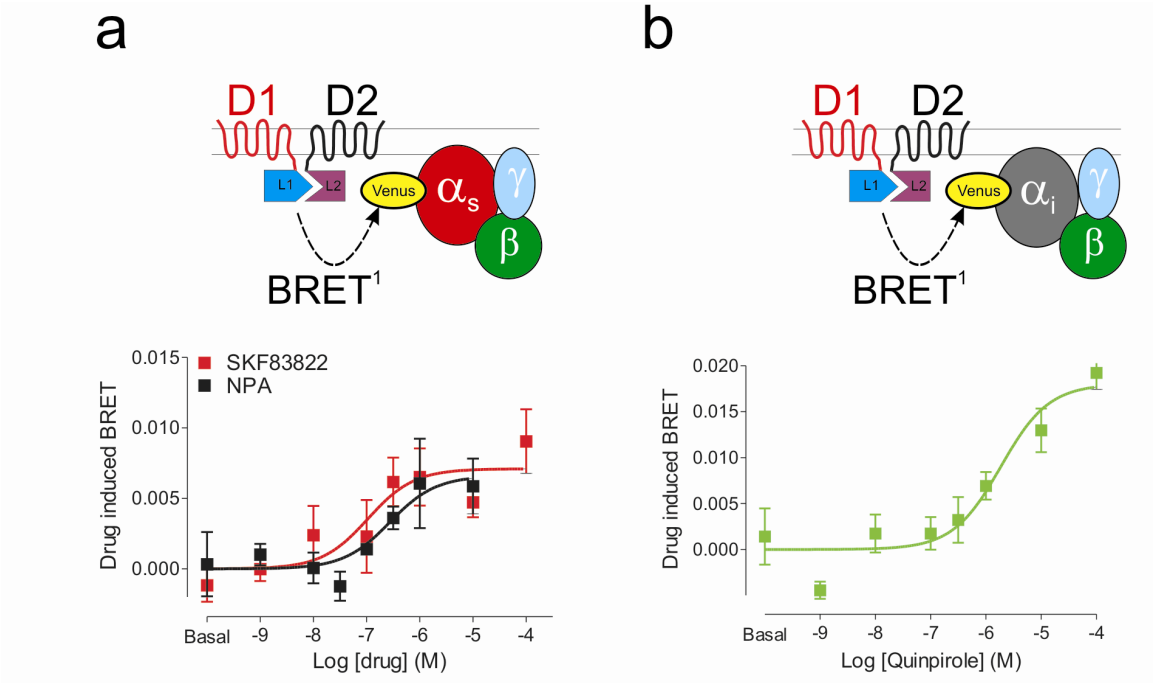
Supplementary Figure 7. *Characterization of the* Gα-based biosensors for *D2R homomers.* HEK 293T cells coexpressing D2R-L1, D2R-L2 and Gαs-mVenus (red in **c**-**d**) or Gαi-mVenus (black in **b**-**d**) together with unlabeled βγ were prepared as described in SF1. In experiments in which antagonists were used (**c**-**f,** open symbols), the antagonists (1 μM) were preincubated for 15 mins at RT before the addition of the substrate and the tested agonist. BRET[1] was measured as explained in Material and Methods and analyzed as for SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 8



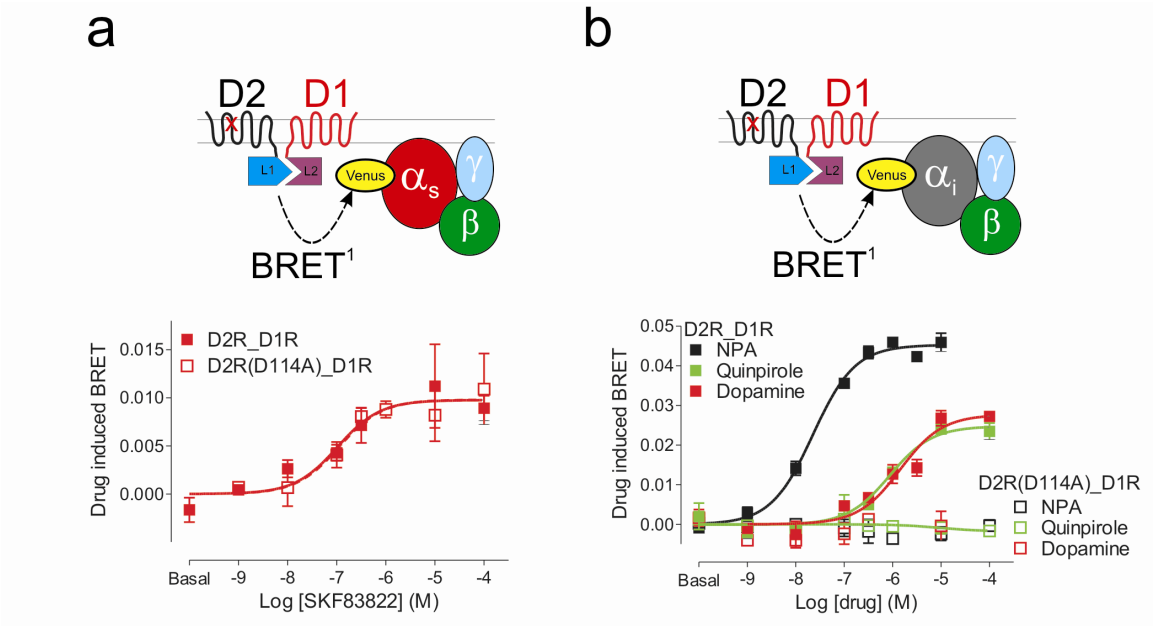
Supplementary Figure 8. *Characterization of the $\beta_1\gamma_2$ and γ_2-based biosensors for D2R homomers.* HEK 293T cells coexpressing D2R split RLuc8 C-terminal fusions together with mVenus-γ_2 (**a**) or V2 (C-terminal domain of mVenus, aa 156-end) at the N terminus of the β_1 subunit and V1 (N terminus of mVenus, aa 1-155) at the N terminus of the γ_2 and unlabeled Gαi (**b**-**f**) together with unlabeled Gαi or not (**c**, open squares) and unlabeled β_1 and were prepared as described in SF1. In experiments in which antagonists were used (**e**-**f,** open symbols), the antagonists (1 μM) were preincubated for 15 mins at RT before the addition of the substrate and the tested agonist. BRET^1 was measured as explained in Material and Methods and analyzed SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 9



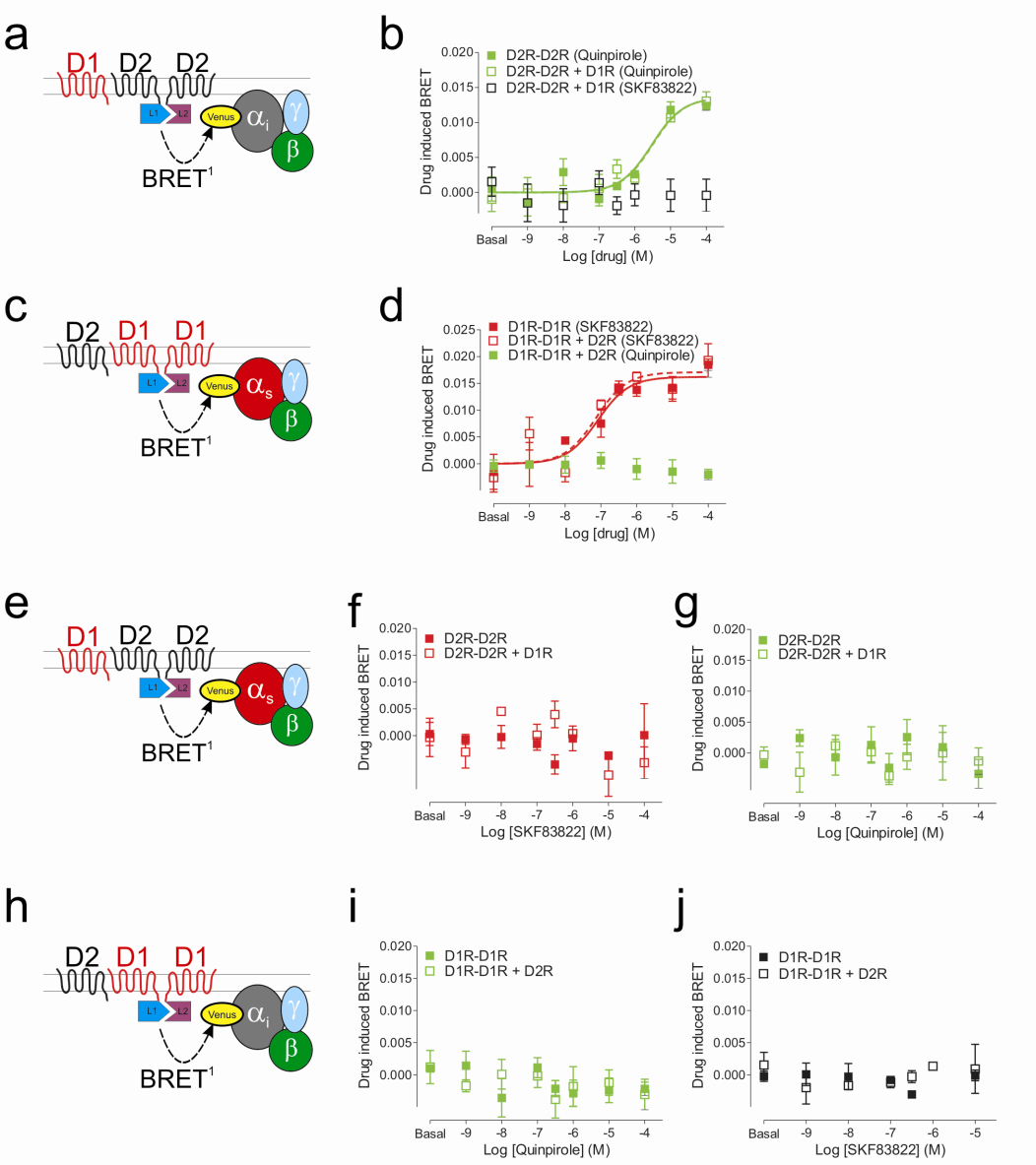
Supplementary Figure 9. *Similar BRET after swapping the protomers in the D1R-D2R heteromer*. HEK 293T cells coexpressing D1R-L1, D2R-L2 and Gαs-mVenus (**a**) or Gαi-mVenus (**b**) together with unlabeled βγ were prepared as described in SF1. $BRET^1$ was measured as explained in Material and Methods and analyzed as for SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 10



Supplementary Figure 10. *D1R agonists target D1R and D2R agonists target D2R to cause the G protein conformational changes: a mutagenesis approach*. HEK 293T cells coexpressing wild-type or mutant (D114A)D2R-L1, D1R-L2 and Gαs-mVenus (**a**) or Gαi-mVenus (**b**) together with unlabeled βγ were prepared as described SF1. BRET[1] was measured as explained in Material and Methods and analyzed as for SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.

Supplementary Figure 11



Supplementary Figure 11. *Untagged receptor does not promote BRET changes or alter the coupling of the complemented receptor biosensor.* HEK 293T cells coexpressing D2R-L1, D2R-L2, untagged D1R and Gαi-mVenus (**a**-**b**) or Gαs-mVenus (**e**-**g**) together with unlabeled βγ were prepared as described in SF1. Identical procedure was applied to cells coexpressing D1R-L1, D1R-L2, untagged D2R and Gαs-mVenus (**c**-**d**) or Gαi-mVenus (**h**-**j**) together with unlabeled βγ. BRET was measured as explained in Material and Methods and analyzed as for SF1. Representative experiments performed with triplicate samples (error bars represent S.E.M.) are shown, and summary data are presented in Supplementary Table 1.