Supplementary Materials

A Split Luciferase Complementation Assay for the Quantification of β-Arrestin2 Recruitment to Dopamine D_2-like Receptors

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1. Radioligand Saturation Binding Data

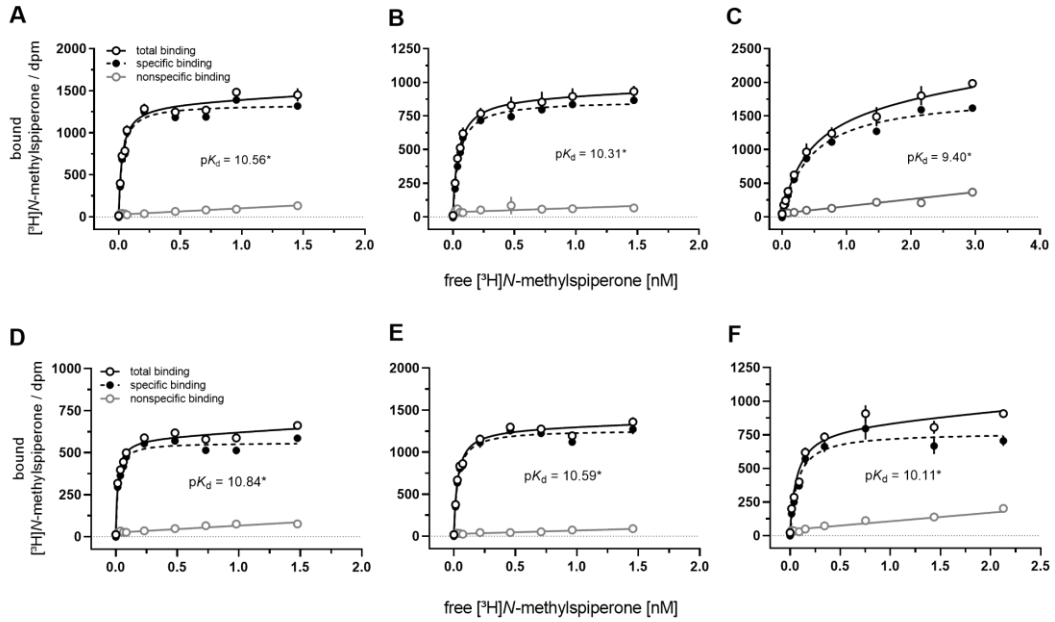

Figure S1. Radioligand saturation binding curves with whole HEK293T ELucN-βarr2 cells expressing the D_{2long}R-ELucC (A), D_3R-ELucC (B) or $D_{4.4}$R-ELucC (C) fusion proteins and homogenates from cells expressing the wild-type D_{2long}R (D), D_3R (E) or $D_{4.4}$R (F). Corresponding dissociation constants are provided in Table 1 in the manuscript. Graphs represent means ± SEM from one representative experiment performed in triplicate of three independent experiments. *pK_d values are given as mean from three independent experiments.

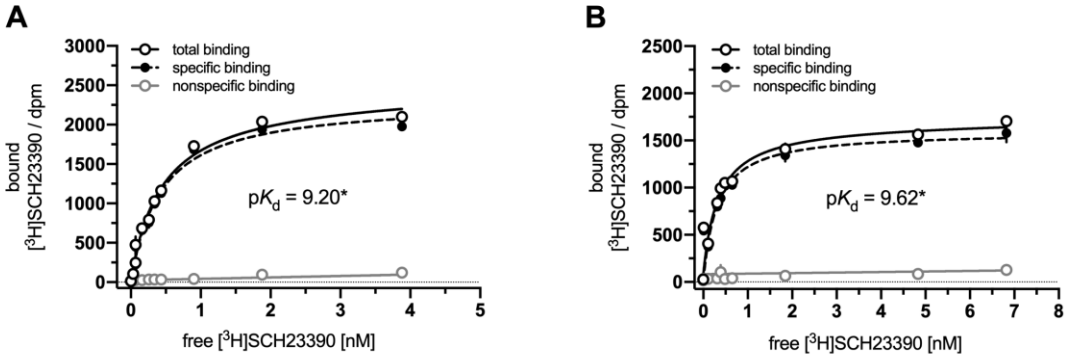

Figure S2. Radioligand saturation binding curves with whole HEK293T ELucN-βarr2 cells expressing the D_1R-ELucC (A) fusion proteins and homogenates from cells expressing the wild-type D_1R (B). Corresponding dissociation constants are provided in Table S1 in the Supplementary Materials. Graphs represent means ± SEM from one representative experiment performed in triplicate of three independent experiments. *pK_d values are given as mean from three independent experiments.

Table S1. Dissociation constants (pK_d values) of [^{3}H]SCH23390 determined in radioligand saturation binding experiments at receptors fused to the C-terminal fragment of the Emerald luciferase using whole cells and at wild-type receptors using homogenates. Data represent means ± SEM determined in three independent experiments, each performed in triplicate.

	D_1R	
	ELucC Fusion Protein	**wt**
pK_d	9.20 ± 0.09	9.62 ± 0.07

2. β-. Arrestin2 Recruitment Data

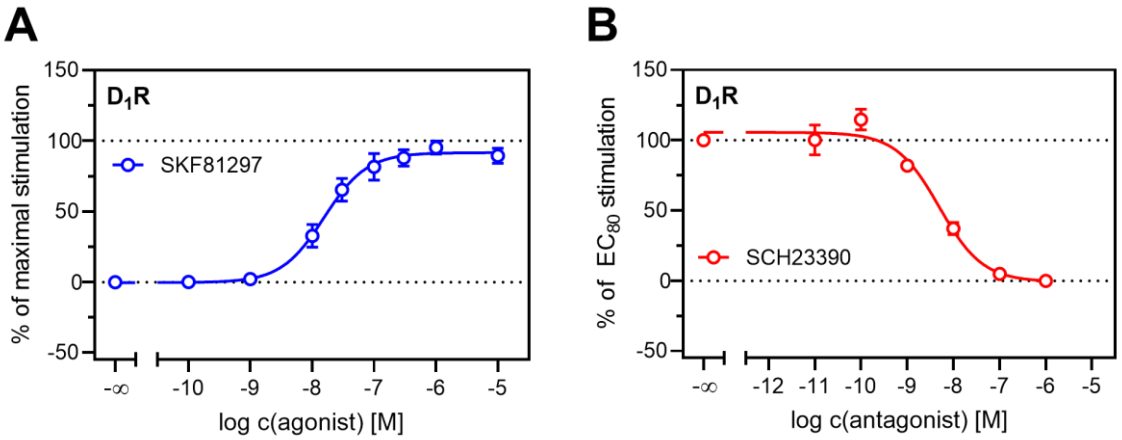

Figure S3. Characterization of the standard agonist SKF81297 and standard antagonist SCH23390 in the β-arrestin2 recruitment assay at the D_1R. Data of the agonist were normalized to the maximal stimulation (100%) and a solvent control (0%). Antagonist data were normalized to the signal elicited by SKF81297 at a concentration corresponding to the EC_{80} (100%) and a solvent control (0%). Obtained pEC_{50} and pK_b values are presented in Table S2. Data represent means ± SEM from at least three independent experiments, each performed in triplicate.

Table S2. pEC_{50}, E_{max} and pK_b values of SKF81297 and SCH23390 analysed in the newly developed β-arrestin2 recruitment assay at the D_1R. For comparison, pK_i values from previously published data are included. Data represent means ± SEM from N independent experiments, each performed in triplicate.

		β-Arrestin2 Recruitment			Ref.
Receptor	**cpd**	**pEC_{50}**	**pK_b**	**N**	**pK_i**
D_1R	SKF81297	7.75 ± 0.15		3	7.47[1]
	SCH23390		8.84 ± 0.07	3	9.33[2]

3. References

1. Andersen, P. H.; Jansen, J. A. Dopamine Receptor Agonists: Selectivity and Dopamine D_1 Receptor Efficacy. *Eur. J. Pharmacol. Mol. Pharmacol.* **1990**, *188* (6), 335–347.
2. Sunahara, R. K.; Guan, H.-C.; O'Dowd, B. F.; Laurier, L. G.; Ng, G.; George, S. R.; Torchia, J. Cloning of the Gene for a Human Dopamine D_5 Receptor with Higher Affinity for Dopamine than D_1. **1991**, *350*, 614-619.