Table S4. G_i-mediated cAMP accumulation assay results of D3R.

Potency (pEC50) were extracted from a minimum of 3 independent assays in at least triplicate. pEC50 displayed values are mean ± SEM. Delta FOB for difference in either Fold of Basal (FOB) or Delta pEC50 when compared to wild-type receptor value. Average Emax and basal values were determined from "log(agonist) vs. response – Variable slope (four parameters) or log(inhibition) vs. response – Variable slope (four parameters)" function in Graphpad Prism 8.4 software (Graphpad Software Inc., San Diego, CA) and were divided by 10^3 for display, basal values are enclosed with parentheses in the table column. Color scheme is based on the effects of mutations on relative pEC50 and Fold of Basal (FOB) values with red for reduced potency/efficacy and blue for increased potency/efficacy when compared to wild-type values for each ligand. ND - not determined.

		D3-Dopamine					D3R-Rotigotine				
BW		**Emax (Basal)**	**FOB**	**ΔFOB**	**pEC50**	**ΔpEC50**	**Emax (Basal)**	**FOB**	**ΔFOB**	**pEC50**	**ΔpEC50**
	WT	2.0 ± 0.1 (8.4 ± 0.2)	4.2 ± 0.1	0	9.94 ± 0.12	0	1.8 ± 0.2 (6.2 ± 0.5)	3.5 ± 0.1	0	9.56 ± 0.02	0.00
2.61	V86A	2.1 ± 0.2 (7.3 ± 0.6)	3.4 ± 0.1	-0.8	9.83 ± 0.05	-0.11	2.4 ± 0.1 (6.6 ± 0.1)	2.8 ± 0.1	-1.4	9.38 ± 0.06	-0.18
2.64	L89A	2.0 ± 0.3 (3.2 ± 0.6)	1.6 ± 0.1	-2.6	9.55 ± 0.46	-0.39	2.7 ± 0.5 (3.5 ± 0.7)	1.3 ± 0.1	-2.9	9.61 ± 0.13	0.05
3.28	F106A	4.5 ± 0.8 (8.5 ± 1.5)	1.9 ± 0	-2.3	9.03 ± 0.41	-0.91	3.7 ± 0.7 (5.6 ± 0.9)	1.6 ± 0.1	-2.6	9.21 ± 0.13	-0.35
3.32	D110A	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3.33	V111A	2.3 ± 0.1 (8.3 ± 0.1)	3.6 ± 0.2	-0.6	9.57 ± 0.06	-0.37	2.2 ± 0.1 (6.7 ± 0.3)	3.1 ± 0.2	-1.1	9.67 ± 0.07	0.11
3.36	C114A	1.4 ± 0.2 (5.8 ± 0.7)	4.1 ± 0.2	-0.1	7.54 ± 0.07	-2.4	1.6 ± 0.2 (3.8 ± 0.5)	2.3 ± 0.1	-1.9	8.63 ± 0.02	-0.93
3.37	T115A	3.9 ± 0.3 (6.9 ± 0.6)	1.8 ± 0.1	-2.4	6.93 ± 0.12	-3.01	2.9 ± 0.4 (6.6 ± 0.8)	2.3 ± 0.1	-1.9	9.62 ± 0.06	0.06
4.56	V164A	1.7 ± 0.6 (4.6 ± 1.5)	2.6 ± 0.1	-1.6	9.57 ± 0.15	-0.37	2.4 ± 0.6 (5.5 ± 1.5)	2.3 ± 0.1	-1.9	9.81 ± 0.02	0.25
ECL2	C181A	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ECL2	S182A	1.7 ± 0.5 (6.1 ± 1.7)	3.6 ± 0.1	-0.6	9.91 ± 0.09	-0.03	2.0 ± 0.5 (6.6 ± 1.6)	3.3 ± 0.2	-0.9	9.85 ± 0.03	0.29
ECL2	I183A	2.2 ± 0.1 (8.1 ± 1.0)	3.6 ± 0.2	-0.6	9 ± 0.12	-0.94	2.2 ± 0.3 (8.0 ± 1.2)	3.5 ± 0.2	-0.7	10.09 ± 0.04	0.53
5.38	F188A	2.3 ± 0.1 (8.3 ± 0.3)	3.6 ± 0.1	-0.6	6.34 ± 0.03	-3.6	2.5 ± 0.1 (5.6 ± 0.2)	2.2 ± 0.1	-2.0	8.62 ± 0.06	-0.94
5.39	V189A	3.2 ± 0.5 (8.7 ± 1.4)	2.7 ± 0.1	-1.5	8.64 ± 0.05	-1.3	3.0 ± 0.5 (8.2 ± 1.7)	2.6 ± 0.2	-1.6	9.53 ± 0.04	-0.03
5.42	S192A	2.7 ± 0.5 (7.5 ± 1.8)	2.8 ± 0.2	-1.4	7.7 ± 0.09	-2.24	3.3 ± 0.6 (7.4 ± 1.7)	2.2 ± 0.2	-2.0	8.82 ± 0.07	-0.74
5.43	S193A	1.6 ± 0.2 (3.9 ± 0.8)	2.4 ± 0.3	-1.8	9.11 ± 0.46	-0.83	1.5 ± 0.2 (3.8 ± 0.8)	2.4 ± 0.2	-1.8	9.53 ± 0.10	-0.03
5.46	S196A	11.4 ± 1.5 (14.1 ± 1.8)	1.2 ± 0	-3	7.37 ± 0.19	-2.57	4.0 ± 0.5 (12.9 ± 2.5)	3.2 ± 0.3	-1.0	9.69 ± 0.04	0.13
6.48	W342A	5.5 ± 0.5 (6.8 ± 0.8)	1.2 ± 0	-3	9.38 ± 0.47	-0.56	4.6 ± 0.7 (5.6 ± 0.8)	1.2 ± 0.1	-3.0	10.47 ± 0.35	0.91
6.51	F345A	2.0 ± 0.3 (8.1 ± 0.8)	4.2 ± 0.5	0	5.49 ± 0.02	-4.45	2.6 ± 0.3 (6.4 ± 0.8)	2.5 ± 0.1	-1.7	6.91 ± 0.04	-2.65
6.52	F346A	2.5 ± 0.2 (7.6 ± 0.4)	3.1 ± 0.2	-1.1	10.2 ± 0.39	0.26	2.1 ± 0.2 (5.8 ± 0.6)	2.8 ± 0.1	-1.4	9.42 ± 0.05	-0.14
6.55	H349A	3.8 ± 0.2 (10 ± 0.1)	2.7 ± 0.1	-1.5	8.93 ± 0.4	-1.01	N.D.	N.D.	N.D.	N.D.	N.D.
7.35	Y365A	2.4 ± 0.3 (7.4 ± 1)	3 ± 0.1	-1.2	9.57 ± 0.61	-0.37	2.2 ± 0.2 (6.1 ± 0.8)	2.7 ± 0.1	-1.5	9.36 ± 0.1	-0.20
7.38	T368A	1.9 ± 0.2 (6.7 ± 0.9)	3.4 ± 0.1	-0.8	10.29 ± 0.42	0.35	1.7 ± 0.1 (5.2 ± 0.8)	3.0 ± 0.2	-1.2	9.73 ± 0.03	0.17
7.39	T369A	2.7 ± 0.4 (8.1 ± 1.5)	3 ± 0.1	-1.2	10.15 ± 0.41	0.21	2.5 ± 0.3 (7.4 ± 1.5)	2.9 ± 0.2	-1.3	9.83 ± 0.09	0.27
7.43	Y373A	3.1 ± 0.1 (4.4 ± 0.1)	1.4 ± 0	-2.8	5.85 ± 0.13	-4.09	N.D.	N.D.	N.D.	N.D.	N.D.