A Signaling-Biased and Constitutively Active Dopamine D2 Receptor Variant

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Supporting Information for Publication:

Table S1. D2 receptor density in HEK293 cells

Receptor	Arrestin Recruitment (No GRK)		Gαo Activation		Gαi Activation	
	B_{max}	N	B_{max}	N	B_{max}	N
$D2_L$-WT	2.6 ± 0.5	7	2.3 ± 0.5	7	2.3 ± 0.8	2
$D2_L$-$I^{212}F$	0.9 ± 0.1*	6	1.1 ± 0.2**	7	1.0 ± 0.2	2
$D2_S$-WT	2.5 ± 0.3	3	4.6 ± 1.0	7	3.2 ± 0.6[a]	3
$D2_S$-$I^{212}F$	1.0 ± 0.01	2	2.3 ± 0.5**	7	1.1 ± 0.2[a],***	3

For most experiments included in Tables 1 and 2, replicate plates were prepared for analysis of receptor density. B_{max} values (Mean ± S.E.M., pmol/mg of membrane protein) were determined by saturation analysis of the binding of [^{3}H]spiperone to a crude membrane fraction. In some BRET experiments, the number of cells was not sufficient to start replicate plates for binding. In some experiments, the number of replications (N) for radioligand binding was greater than the number of replications of the G protein activation and arrestin recruitment assays because results from replicate plates of both donor-only and donor+acceptor conditions were included as separate measurements. [a]from van der Weijden et al. (8). Student's *t*-test: *p<0.05; **p<0.01; ***p<0.001 compared to D2-WT. No statistical comparison of means was carried out for groups with N = 2.

Table S2. Arrestin recruitment: Cmpd101 pretreatment

Receptor $D2_L$	**Arrestin Recruitment** (n=3)			
	$-LogEC_{50}$		E_{max} (% of WT+GRK)	
	+GRK2	No GRK2	+GRK2	No GRK2
WT Vehicle	7.8 ± 0.02	6.8 ± 0.02†††	100 ± 2	61 ± 3†††
I212F Vehicle	8.2 ± 0.03*	7.2 ± 0.04**,†††	55 ± 2*** (-45%)	19 ± 1***,††† (-69%)
WT Cmpd101	6.9 ± 0.03	6.7 ± 0.04	28 ± 2	17 ± 1††
I212F Cmpd101	7.5 ± 0.04***	7.3 ± 0.2***	12 ± 1*** (-57%)	6 ± 1*** (-65%)

HEK293 cells were pretreated with vehicle or the GRK2/3 inhibitor Cmpd101 (30 μM), 30 min before adding quinpirole and coelenterazine *h*. Data are presented as mean ± SEM. E_{max} was calculated as described in Table 1, and is expressed as a percentage of E_{max} for $D2_L$-WT with added GRK2. For D2-$I^{212}F$, the percent reduction compared to the corresponding D2-WT E_{max} is included in parentheses. N = 3 independent experiments for each condition. B_{max} values (pmol/mg protein) were 1.68 ± 0.08 ($D2_L$-WT, no GRK2), 0.57 ± 0.03 ($D2_L$-$I^{212}F$, no GRK2), 2.27 ± 0.16 ($D2_L$-WT, + GRK2), and 0.93 ±0.25 ($D2_L$-$I^{212}F$, + GRK2). Statistical differences were calculated by 2-way ANOVA followed by Turkey's post-hoc test (*p<0.05, **p<0.01, ***p<0.001 compared to the corresponding D2-WT condition; ††p<0.01, †††p<0.001 compared to the corresponding + GRK2 condition).

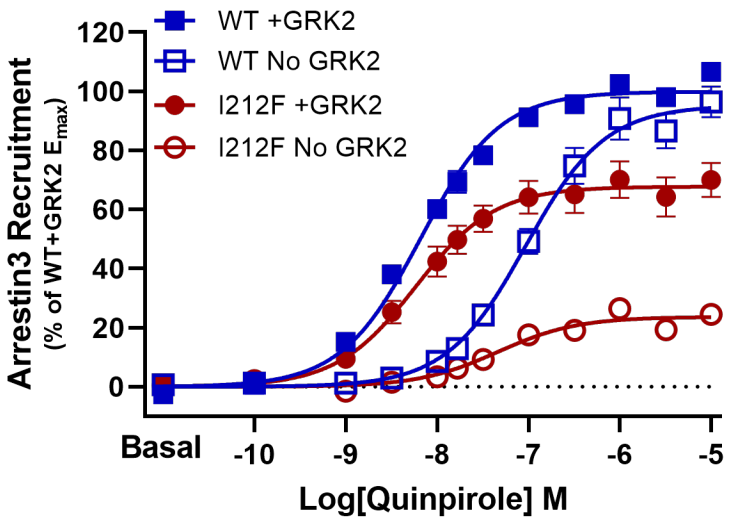

Figure S1.**Arrestin recruitment with matched receptor expression levels.** Arrestin3 recruitment was measured in HEK293 cells co-transfected with $D2_L$-WT (50 ng plasmid DNA) or $D2_L$-$I^{212}F$ (250 ng plasmid DNA) and with GRK2 (+GRK2) or nonspecific plasmid DNA (No GRK2). Values plotted are the means ± SD of 3-4 independent experiments performed in quadruplicate. Data from each independent experiment were normalized by subtracting the baseline and expressed as a percentage of maximum arrestin3 recruitment by D2-WT+GRK2. D2 receptor B_{max} values were 0.46 ± 0.05 pmol/mg protein (D2-WT, No GRK2), 0.48 ± 0.04 pmol/mg (D2-WT, +GRK2), 0.55 ± 0.01 pmol/mg (D2-$I^{212}F$, No GRK2), and 0.54 ± 0.07 pmol/mg (D2-$I^{212}F$, +GRK2). Omitting overexpressed GRK2 decreased arrestin recruitment for D2-$I^{212}F$ by 65%, whereas there was no significant effect of omitting GRK2 on maximal response for D2-WT at this lower level of expression. On the other hand, the potency of quinpirole at D2-WT decreased from 6 nM in the presence of GRK2 to 100 nM in the absence of GRK2, and at D2-$I^{212}F$ from 6 nM to 50 nM.

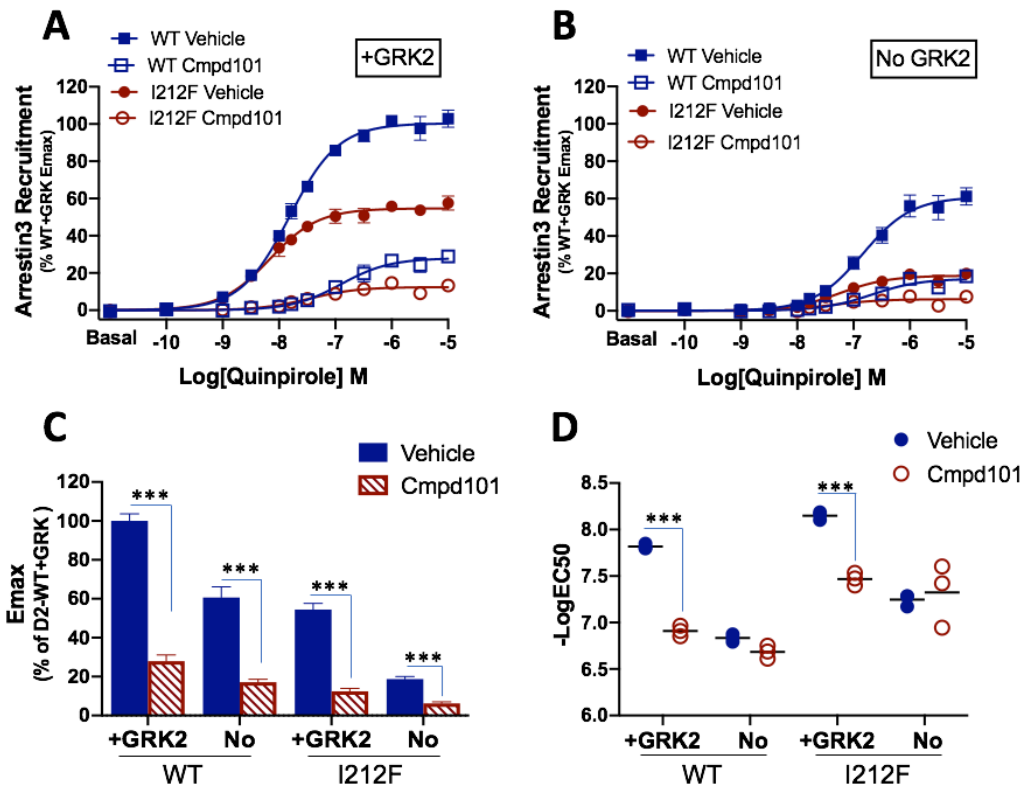

Figure S2. **Effect of Cmpd101 on Arrestin3 recruitment by D2L.** Arrestin3 recruitment was measured in HEK293 cells co-transfected with GRK2 (+GRK2) or nonspecific plasmid DNA (No GRK2) and pretreated with vehicle or the GRK2/3 inhibitor Cmpd101 (30 μM, 30 min). **A and B**, quinpirole concentration-response curves for $D2_L$-WT (WT) or $D2_L$-$I^{212}F$ (I212F) with GRK2 (**A**) or in the absence of overexpressed GRK2 (**B**). Data from each independent experiment were normalized by subtracting the baseline and expressed as a percentage of maximum arrestin3 recruitment by D2-WT +GRK2. Values plotted are the means ± SD of 3 independent experiments performed in quadruplicate. **C and D**, values from Table S2 for $D2_L$-WT (WT) or $D2_L$-$I^{212}F$ (I212F) with (+GRK2) or without (No) GRK2, in the presence (Cmpd101) or absence (vehicle) of Compound 101. **C,** E_{max}, expressed as the percentage of E_{max} for D2-WT with GRK2, and **D,** quinpirole potency, expressed as the $-LogEC_{50}$. Statistical differences determined as described in Table S2 (***$p < 0.001$).

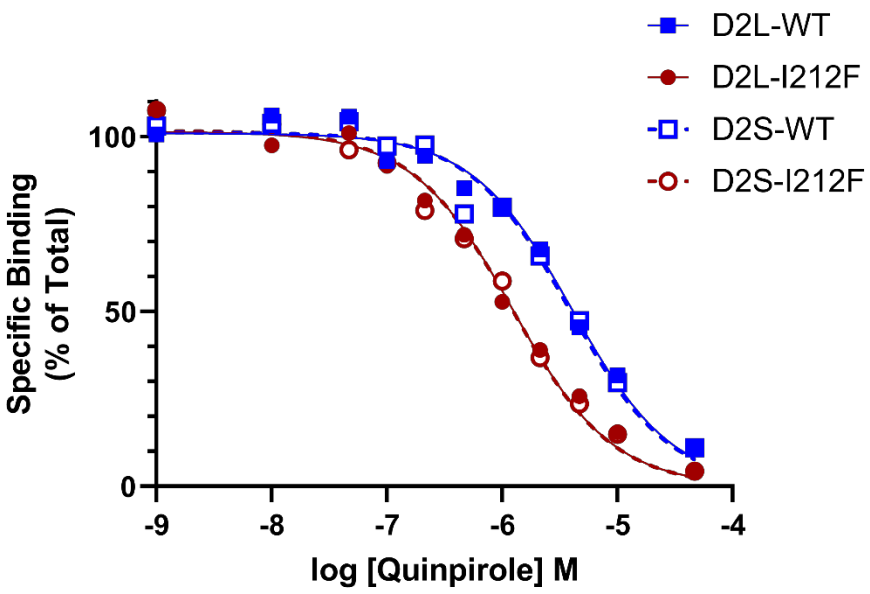

Figure S3. Quinpirole competition binding curves. Representative curves are shown for inhibition of the binding of [^{3}H]spiperone (87 pM) by various concentrations of quinpirole in membranes prepared from HEK293 cells stably expressing each of the variants. K_i values in this experiment were 1.24 μM ($D2_L$-WT), 0.35 μM ($D2_L$-$I^{212}F$), 1.19 μM ($D2_S$-WT), and 0.39 μM ($D2_S$-$I^{212}F$). The leftward shift in the quinpirole competition curves for $D2_{L/S}$-$I^{212}F$ relative to $D2_{L/S}$-WT indicates that the mutation increased the affinity of the D2 receptor for that agonist, consistent with many studies of constitutively active GPCRs.