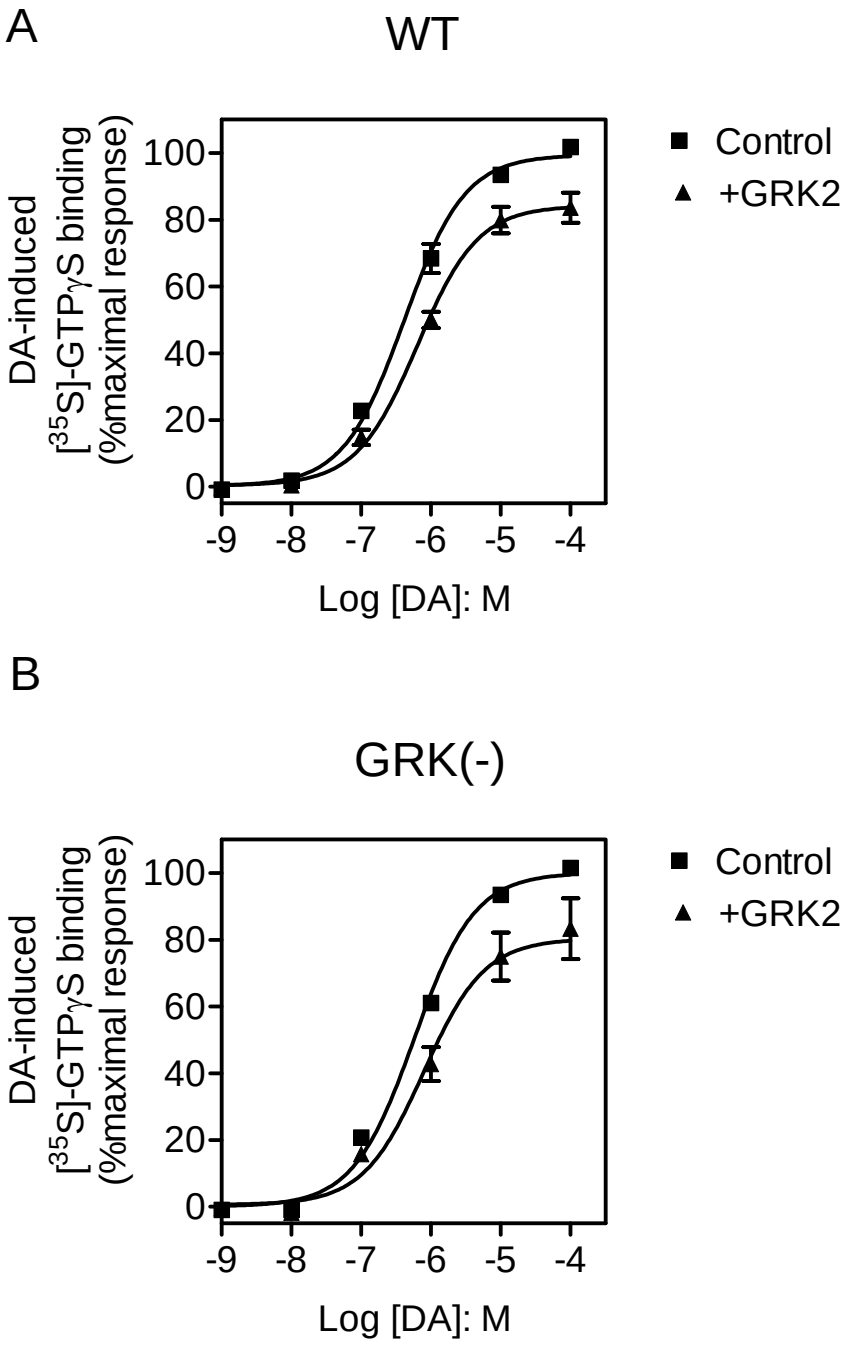
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Effect of GRK2 over-expression on D_2 DAR-mediated [^{35}S]-GTPγS binding. DA-stimulated [^{35}S]-GTPγS binding was determined in membranes prepared from HEK293T cells transiently expressing WT (**A**) or GRK(-) (**B**) D_2 DARs along with pcDNA (control) or GRK2. The amount of DA-stimulated [^{35}S]-GTPγS binding is calculated as described in the "Experimental Procedures" and expressed as the percent maximum of the control (pcDNA). The data shown represent the means ± S.E. values from 6-8 experiments. Average estimated EC_{50} parameters are as follows: WT D_2 DAR, 0.47 ± 0.08 μM for control and 0.63 ± 0.06 μM for GRK2 over-expression; GRK(-) D_2 DAR, 0.57 ± 0.04 μM for control and 0.86 ± 0.15 μM for GRK2 over-expression.

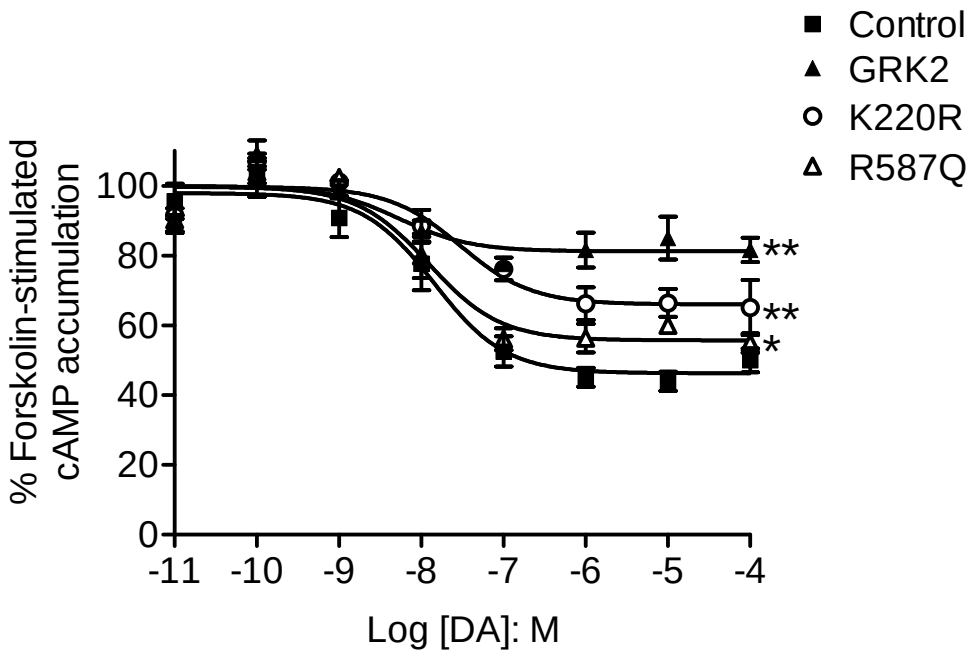
Supplemental Figure 2. Effect of GRK2, GRK2-K220R, and GRK2-R587Q expression on D_2 DAR-mediated inhibition of cAMP accumulation in HEK293 cells. DA inhibition of cAMP accumulation was measured using intact HEK293 cells transiently expressing D_2 DAR along with pcDNA (control), GRK2, GRK2-K220R, or GRK2-R587Q. Cells were incubated with various concentrations of DA for 10 min in the presence of 3 μM forskolin. cAMP accumulation was then assessed as described under "Experimental Procedures." The data shown represent the mean ± S.E. values from four to five experiments and are expressed as a percentage of forskolin-stimulated cAMP accumulation in the absence of DA. The average estimated IC_{50} parameters are as follows: Control, 23.0 ± 12.7 nM; GRK2, 7.5 ± 2.4 nM; GRK2-K220R, 61.9 ± 36.0 nM; GRK2-R587Q, 15.7 ± 4.6 nM. The maximal inhibitions were calculated from the best-fit curves of each experiment and compared. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with the values of the control group, Student's *t* test, unpaired.

Supplemental Figure 3. Co-immunoprecipitation of GRK2 and its mutants with the D_2 DAR. **A,** HEK293T cells were transfected with FLAG-tagged D_2 DAR plus WT GRK2 or various GRK2 mutants as indicated. Solubilized cell lysates were immunoprecipitated (IP) using anti-FLAG-agarose, separated by SDS-PAGE, and immunoblotted (IB) with an anti-GRK2 antibody as described in Fig 10. Then, the blot was stripped and re-probed with HRP-conjugated anti-FLAG antibody (M2). The gels were scanned and, using standard densitometry analysis, GRK2 co-immnuoprecipitation intensity was normalized to D_{2L} DAR immunoprecipitation and compared with that of WT GRK2 as percentages. Data are expressed as mean ± S.D. from two independent experiments. There was no significant difference between the GRK2 constructs in their ability to co-immunoprecipitate with the D_2 DAR. **B,** HEK293T cells were transfected with either FLAG-tagged WT D_2 DAR or FLAG-tagged GRK(-) D_2 DAR plus GRK2. Cells were incubated in the absence (C) or presence of 10 μM DA for 30 min before harvesting. Solubilized cell lysates were prepared and subjected to IP using anti FLAG-agarose as described in **A**. Normalized intensities of co-immunoprecipitated GRK2 were compared with that of WT D_{2L} DAR as described in **A**. Data are expressed as mean ± S.E. from three independent experiments. Preincubation with DA did not significantly affect the ability of GRK to co-immunoprecipitate with the D_2 DAR.

Supplemental Figure 1

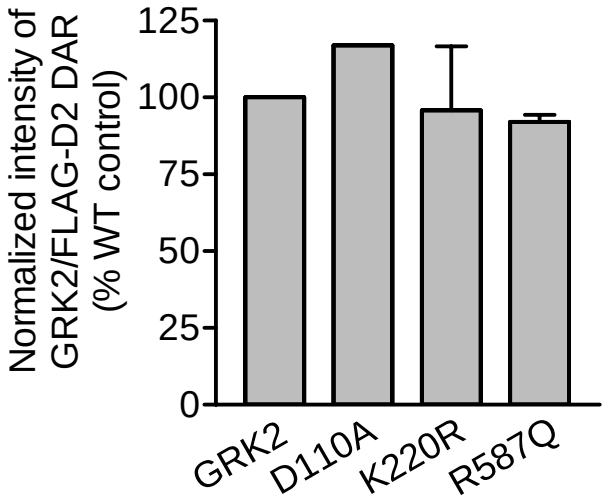


Supplemental Figure 2



Supplemental Figure 3

A



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