Supplementary Information

$G_{o/z}$-biased coupling profile of the dopamine D3 receptor

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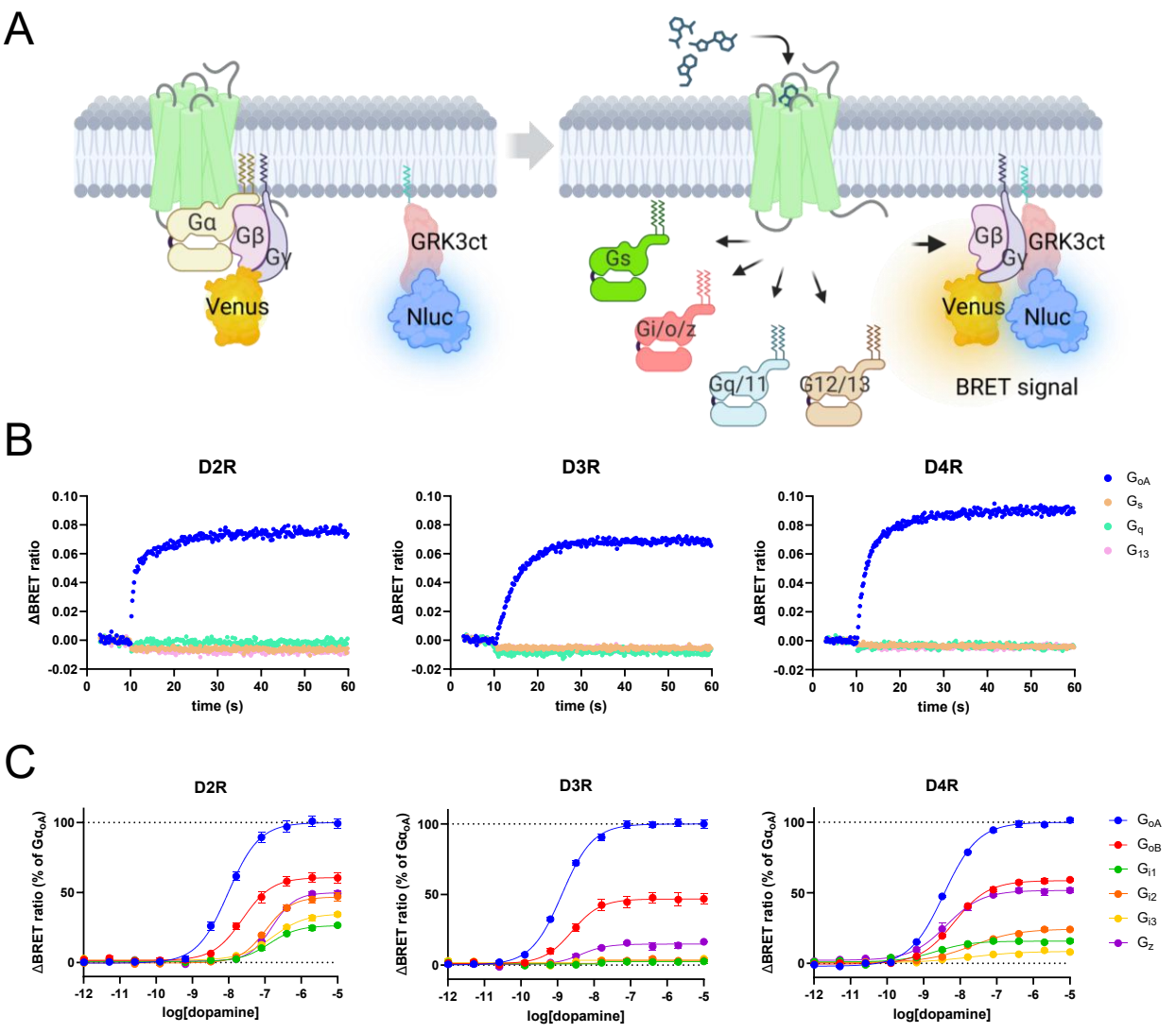
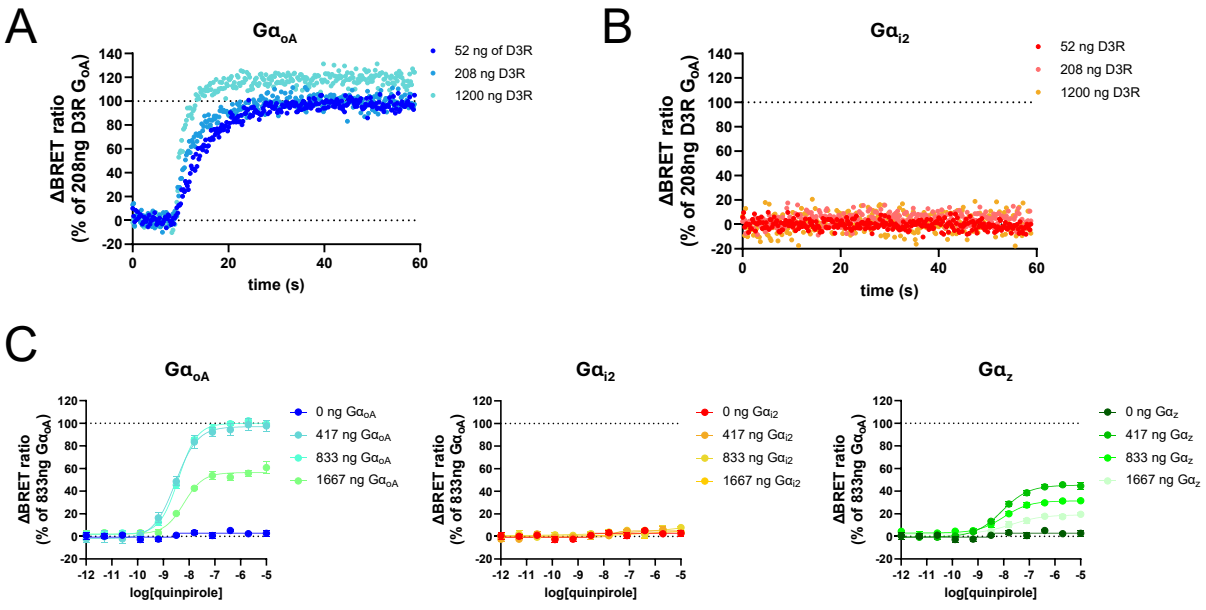
Supplementary Figure 1



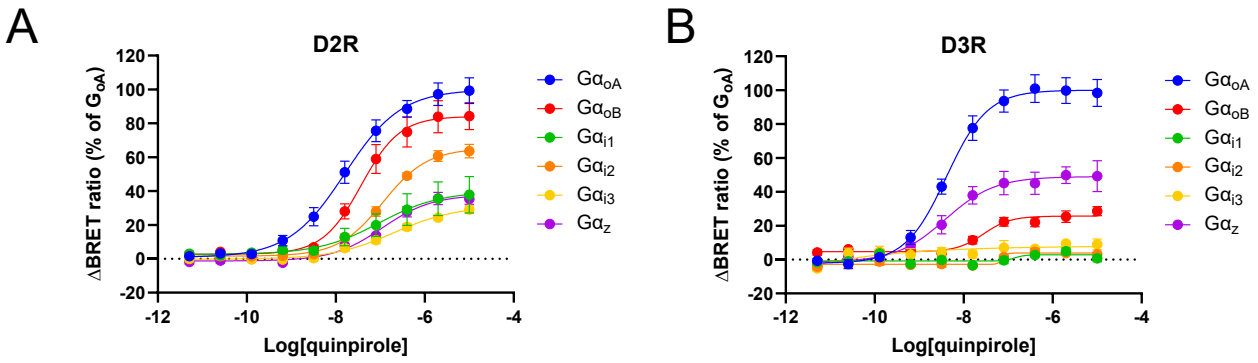
Supplementary Figure 1. Dopamine-mediated activation of $G\alpha_{i/o/z}$ family members by D2R, D3R, and D4R. (**A**) Schematics of the G protein nanoBRET assay. Cells are transfected with 5 components: a GPCR, a wild-type Gα protein, Gβ1-venus$^{156-239}$ and Gγ2-venus$^{1-155}$, that by heterodimerizing reconstitute a complete fluorescent venus protein, and masGRK3CT-Nluc. Ligand stimulation induces dissociation of the heterotrimeric G protein complex and association of the Gβγ heterodimer with GRK3ct, containing a Gβγ binding site. This interaction brings the donor and acceptor in close proximity, generating an increase in BRET signal. Created in BioRender. (**B**) Kinetics curves of dopamine receptor activation of a representative heterotrimeric G protein for each family. 10 μM dopamine application at 10 seconds stimulates the activation of $G\alpha_{oA}$ but not $G\alpha_{s}$, $G\alpha_{q}$, or $G\alpha_{13}$ by D2R, D3R and D4R. (**C**) Concentration-response curves showing D2R, D3R, and D4R activation of each $G\alpha_{i/o/z}$ family member by dopamine. Data are shown as mean ± SEM of values normalized to $G\alpha_{oA}$ signal. N=5 independent replicates.

Supplementary Figure 2



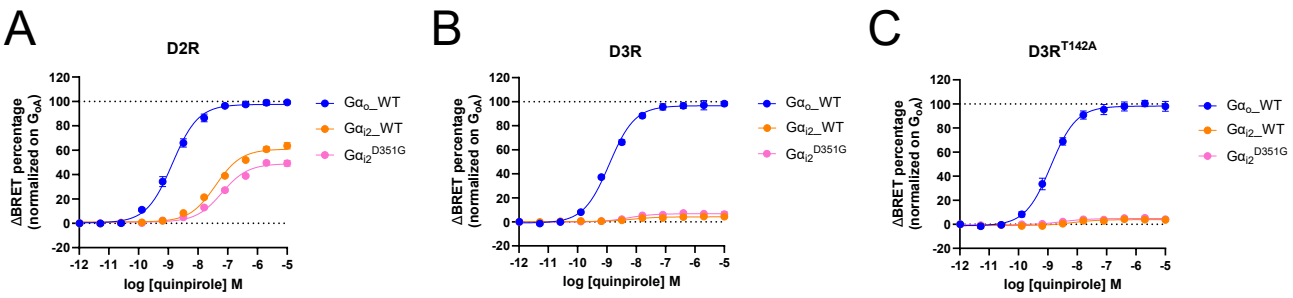
Supplementary Figure 2. Lack of $G\alpha_{i2}$ activation by D3R is independent of receptor or G protein expression levels. (**A-B**) To assess the impact of receptor expression levels, increasing amounts of DNA plasmid encoding D3R were transfected over 2500 ng of total DNA. Empty vector pcDNA3.1 was co-transfected to maintain a constant amount of total DNA transfected. Cells were stimulated with 100 µM dopamine at 10 seconds. Representative kinetic activation profiles measured using the G protein nanoBRET assay for D3R co-transfected with either $G\alpha_{oA}$ (**A**, positive control) or $G\alpha_{i2}$ (**B**). (**C**) Concentration–response curves measured using the G protein nanoBRET assay for a fixed amount of D3R co-transfected with increasing amounts of DNA plasmids encoding either $G\alpha_{oA}$, $G\alpha_{i2}$, or $G\alpha_{z}$. Empty vector pcDNA3.1 was co-transfected to maintain a constant amount of total DNA transfected. Cells were stimulated with increasing concentrations of quinpirole. Data were normalized to the signal obtained by transfecting 833 ng of $G\alpha_{oA}$ and shown as mean ± SEM. N=5 independent replicates.

Supplementary Figure 3



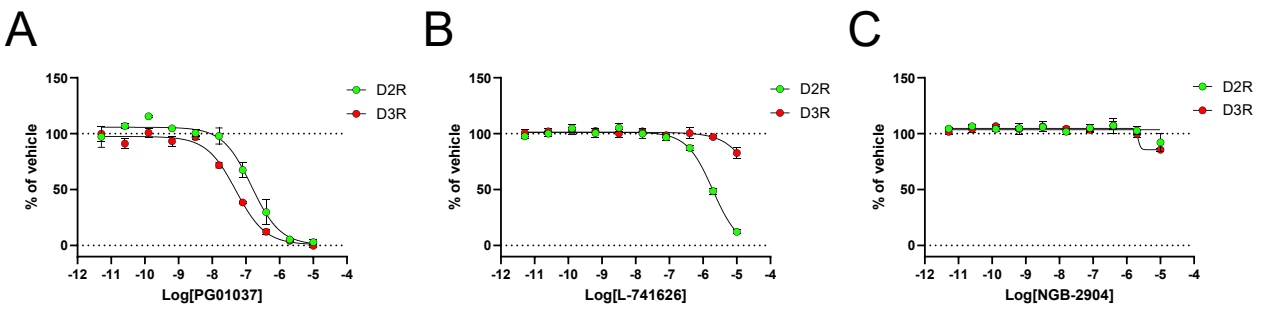
Supplementary Figure 3. D2R and D3R response to quinpirole in CHO cells. Concentration–response curves were obtained using the G protein nanoBRET assay in CHO cells transfected with D2R (**A**) or D3R (**B**) and indicated Gα proteins. Cells were stimulated with increasing concentrations of quinpirole. Data are shown as mean ± SEM of values normalized to the $G\alpha_{oA}$ signal. N=5 independent replicates.

Supplementary Figure 4



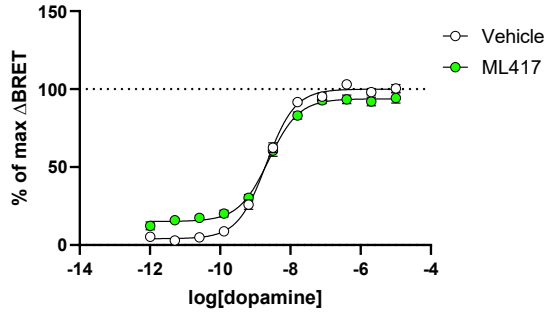
Supplementary Figure 4. Mutating D3R^{T142A} and Gα_{i2}^{D351G} has no effect on G-protein coupling. (**A-C**) Concentration-response curves measured using the G protein nanoBRET assay for D2R (**A**), D3R (**B**) and D3R^{T142A} (**C**) co-transfected with Gα_{oA}, Gα_{i2}, and Gα_{i2}^{D351G}. All experiments were conducted in wild-type HEK293T cells stimulated with increasing concentrations of quinpirole. Data were normalized to the signal obtained with Gα_{oA} and shown as mean ± SEM. N=4 independent replicates.

Supplementary Figure 5



Supplementary Figure 5. D2R and D3R antagonist selectivity. Concentration-responses obtained by stimulating D2R (green) or D3R (red) with 3.2 nM dopamine (EC_{80}) in the presence of PG01037 (**A**), L-741626 (**B**), and NGB-2904 (**C**).

Supplementary Figure 6



Supplementary Figure 6. Weak partial agonism of ML417 at the D2R does not inhibit dopamine responses. Concentration-response curves measured using the G protein nanoBRET assay for D2R in wild-type HEK293T cells co-transfected with $G\alpha_{oA}$. Increasing concentrations of dopamine were applied in the presence of vehicle (white) or 100 nM ML417 (green). Data shown as mean ± SEM. N=5 independent replicates.