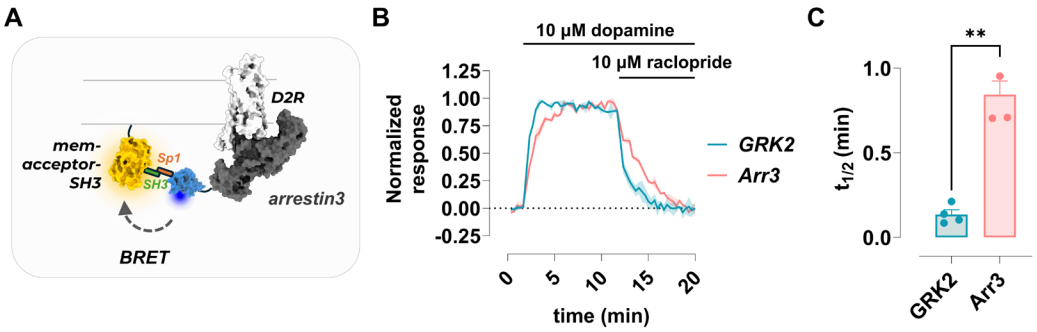
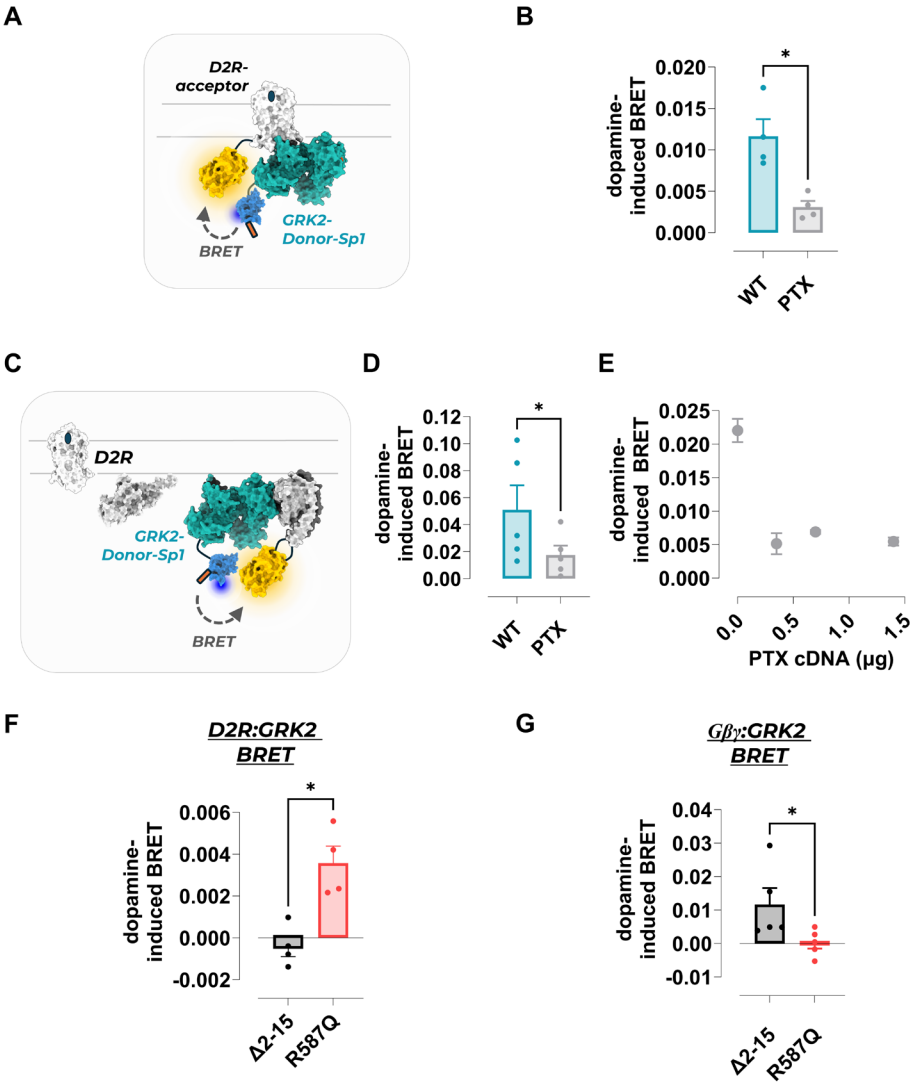
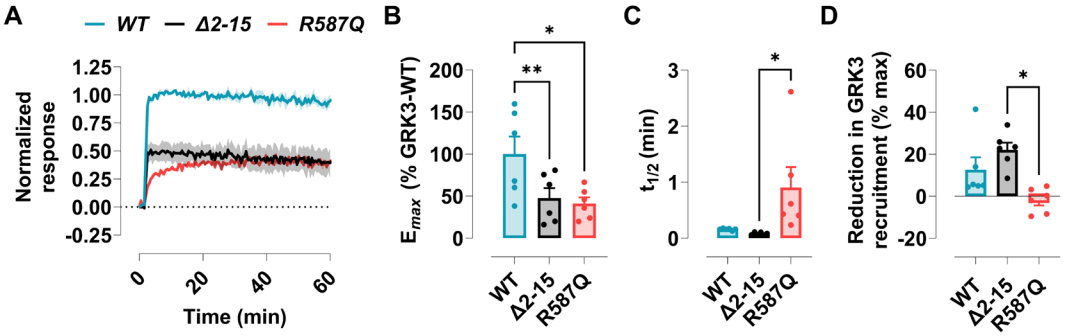
Supplementary Figures



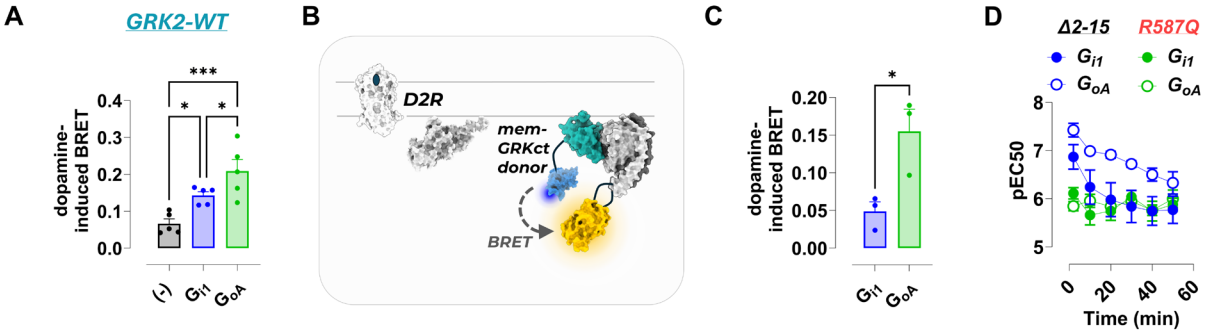
Supplementary Figure 1. A comparison of the kinetics of GRK2 and Arr3 recruitment to the plasma membrane in response to D2R activation according to bystander hiBRET, related to Figure 1. (**A**) A bystander hiBRET-based D2R-mediated Arr3 recruitment assay wherein Arr3 is fused to Sp1-Nanoluciferase donor (Sp1-donor-Arr3). Upon receptor activation, Sp1-donor-Arr3 is recruited to the plasma membrane where a membrane-anchored SH3-citrine acceptor is localized, resulting in an increase in BRET. (**B**) Representative traces of D2R-mediated GRK2 recruitment and Arr3 recruitment. (**D**) Summary of the on-kinetics of GRK2 recruitment and Arr3 recruitment. unpaired t-test, **p = 0.0016.



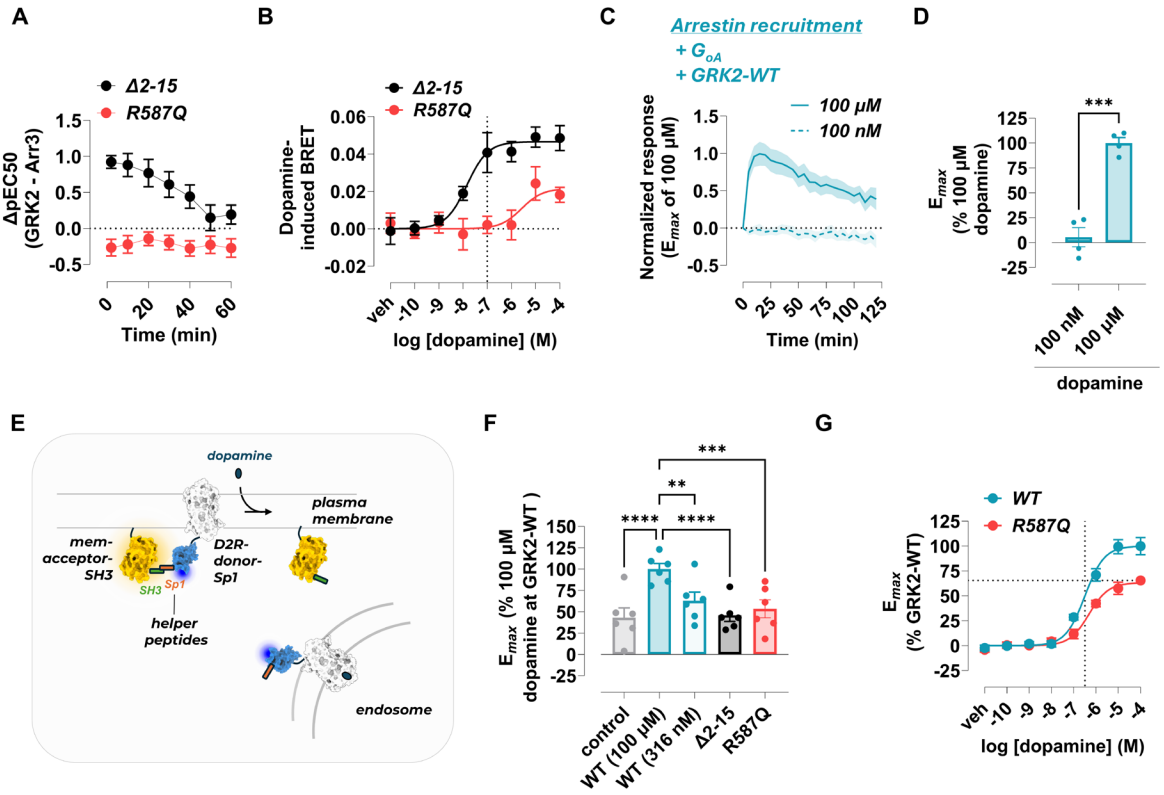
Supplementary Figure 2. Direct BRET between GRK2 and D2R or Gβγ, related to Figure 2. (**A**) Dopamine-induced D2R activation enhances BRET between GRK2-donor-Sp1 and mVenus acceptor-tagged D2R (D2R-acceptor). (**B**) PTX reduces but does not abolish dopamine-induced BRET between D2R and GRK2. paired t-test, *p < 0.05. (**C**) Dopamine-induced D2R activation enhances BRET between GRK2-donor-Sp1 and split mVenus acceptor-tagged $G\beta_1\gamma_2$. (**D**) PTX reduces but does not abolish dopamine-induced BRET between D2R and $G\beta_1\gamma_2$. paired t-test, *p < 0.05. (**E**) Recruitment of GRK2-donor-Sp1 to split acceptor-tagged $G\beta_1\gamma_2$ in the presence of increasing amount of PTX cDNA. n = 3 independent experiments each performed in triplicate. (**F**) Dopamine-induced D2R activation promotes the interaction between D2R-acceptor and GRK2(R587Q)-donor-Sp1 but not GRK2(Δ2-15)-donor-Sp1. paired t-test, *p < 0.05. (**G**) Dopamine-induced D2R activation promotes the interaction between split acceptor-tagged $G\beta_1\gamma_2$ and GRK2(Δ2-15)-donor-Sp1 but not GRK2(R587Q)-donor-Sp1. paired t-test, *p < 0.05.



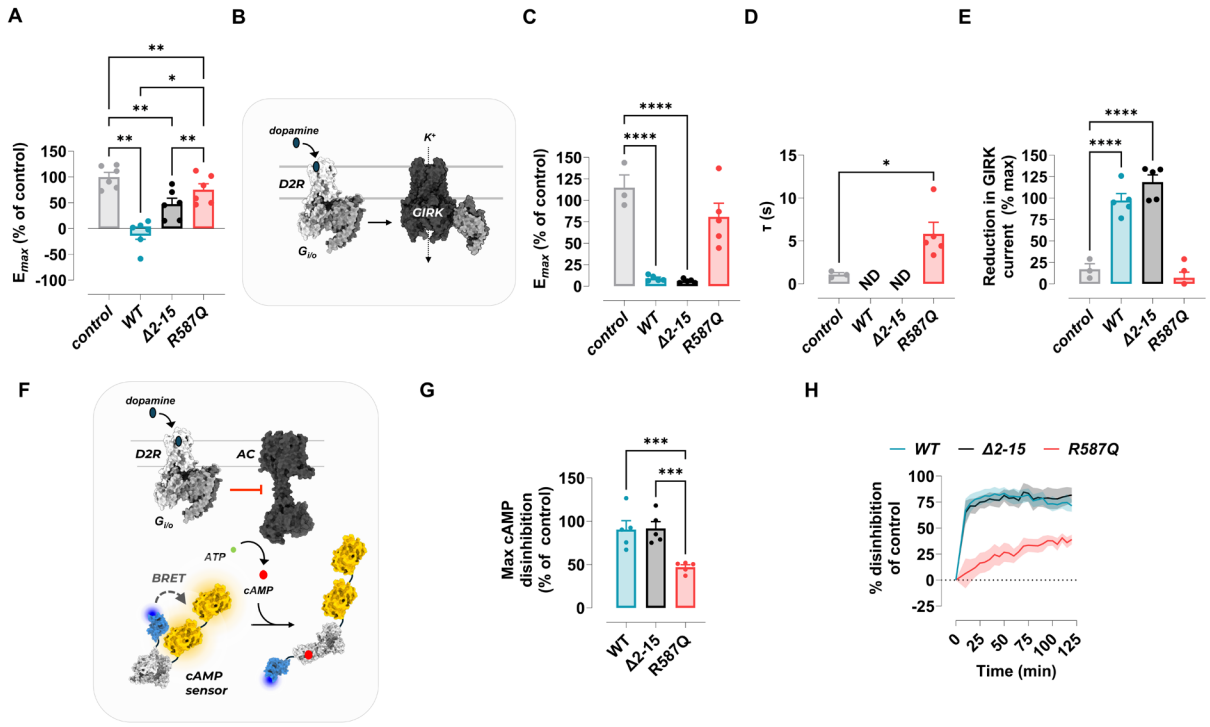
Supplementary Figure 3. Dynamics of Gβγ-dependent and -independent GRK3 recruitment, related to Figure 2. (**A**) Representative traces of the recruitment of GRK3-WT, GRK3(Δ2-15), or GRK3(R587Q) to the plasma membrane in response to 10 μM dopamine-induced D2R activation. (**C**) Maximal recruitment of GRK3-WT or GRK3 mutants to the plasma membrane. one-way ANOVA, $F = 20.1$, *$p < 0.05$, **$p < 0.01$. (**D**) Summary of the on-kinetics of GRK3-WT, GRK3(Δ2-15), or GRK3(R587Q) to the plasma membrane in response to 10 μM dopamine. RM one-way ANOVA, $F = 4.8$, *$p < 0.05$. (**E**) Reduction in maximal GRK3 recruitment to the plasma membrane after 60 minutes. RM one-way ANOVA, $F = 9.9$, **$p < 0.05$.



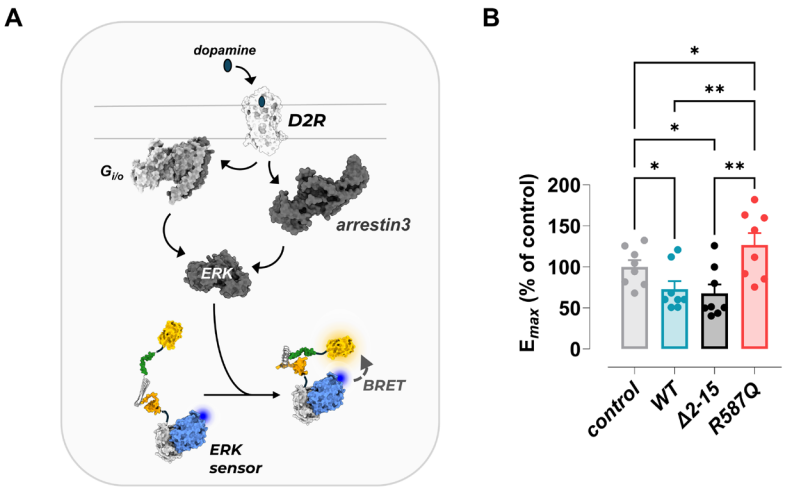
Supplementary Figure 4. GRK2 recruitment in the presence of G_{i1} or G_{oA}, related to Figure 3. (**A**) Summary of the effect of PTX resistant-G_{i1} or -G_{oA} overexpression on GRK2-WT recruitment compared to PTX alone (-) in response to 10 μM dopamine. RM one-way ANOVA, F = 24.0, Tukey post-hoc test, *p < 0.05, ***p < 0.001. (**B**) A BRET-based assay wherein D2R activation promotes the dissociation of a heterotrimeric G protein and the release of mVenus split acceptor-tagged $G\beta_1\gamma_2$, which then binds a Renilla luciferase donor tagged-C-terminal fragment of GRK3 (GRKct-donor). (**C**) Dopamine-induced D2R activation promotes greater $G\beta_1\gamma_2$ release from G_{oA} than G_{i1}. paired t-test, *p < 0.05. (**D**) Summary of the reduction in the potency of dopamine for GRK2(Δ2-15) or GRK2(R587Q) recruitment in the presence of PTX and either PTX-resistant $G\alpha_{i1}$ or $G\alpha_{oA}$.



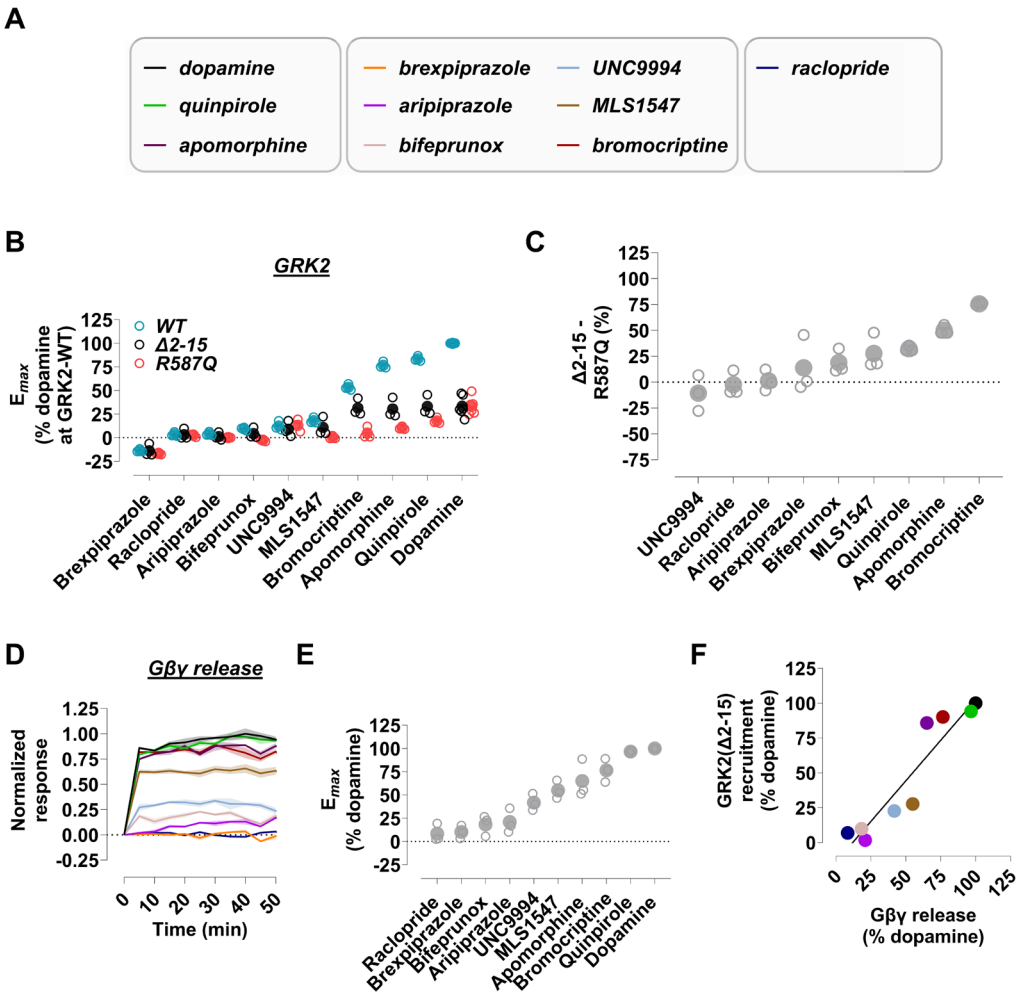
Supplementary Figure 5. Extended analysis of the effects of Gβγ-dependent and -independent GRK2 recruitment on arrestin, related to Figure 4. (**A**) Difference between the potency of dopamine of GRK2(Δ2-15) or GRK2(R587Q) and that for Arr3 in the presence of GRK2-WT. (**B**) 100 nM dopamine (*dotted line*) fully activates GRK2(Δ2-15) but has no effect on GRK2(R587Q) in cells co-expressing G_{oA}. (**C**) Representative trace of Arr3 recruitment in the presence of G_{oA} and GRK2-WT in response to 100 nM or 100 μM dopamine. (**D**) Summary of D2R-mediated Arr3 recruitment in the presence of G_{oA} and GRK2-WT in response to varying concentrations of dopamine. paired t-test, ***p < 0.001. (**E**) Schematic of a novel hiBRET-based D2R internalization assay. D2R was tagged with a donor and helper peptide Sp1, followed by co-expression with mem-acceptor-SH3 in ΔQ:Arr2/3 cells, resulting in BRET at the plasma membrane. Upon activation, the receptor undergoes GRK and arrestin dependent-internalization, reducing the BRET signal. (**F**) Summary of D2R-dependent internalization in ΔQ:Arr2/3 cells transfected with GRK2-WT, GRK2(Δ2-15), or GRK2(R587Q). Arr3 was co-expressed in all conditions. 100 μM dopamine was applied to all conditions, with an additional condition in which 316 nM dopamine was applied to GRK2-WT. RM one-way ANOVA, F = 13.1, **p < 0.01, ***p < 0.001, ****p < 0.0001. (**G**) Dose-response curves showing the similarity in the effect of (i) 316 nM dopamine on GRK2-WT-dependent Arr3 recruitment and (ii) 100 μM dopamine on GRK2(R587Q)-dependent Arr3 recruitment.



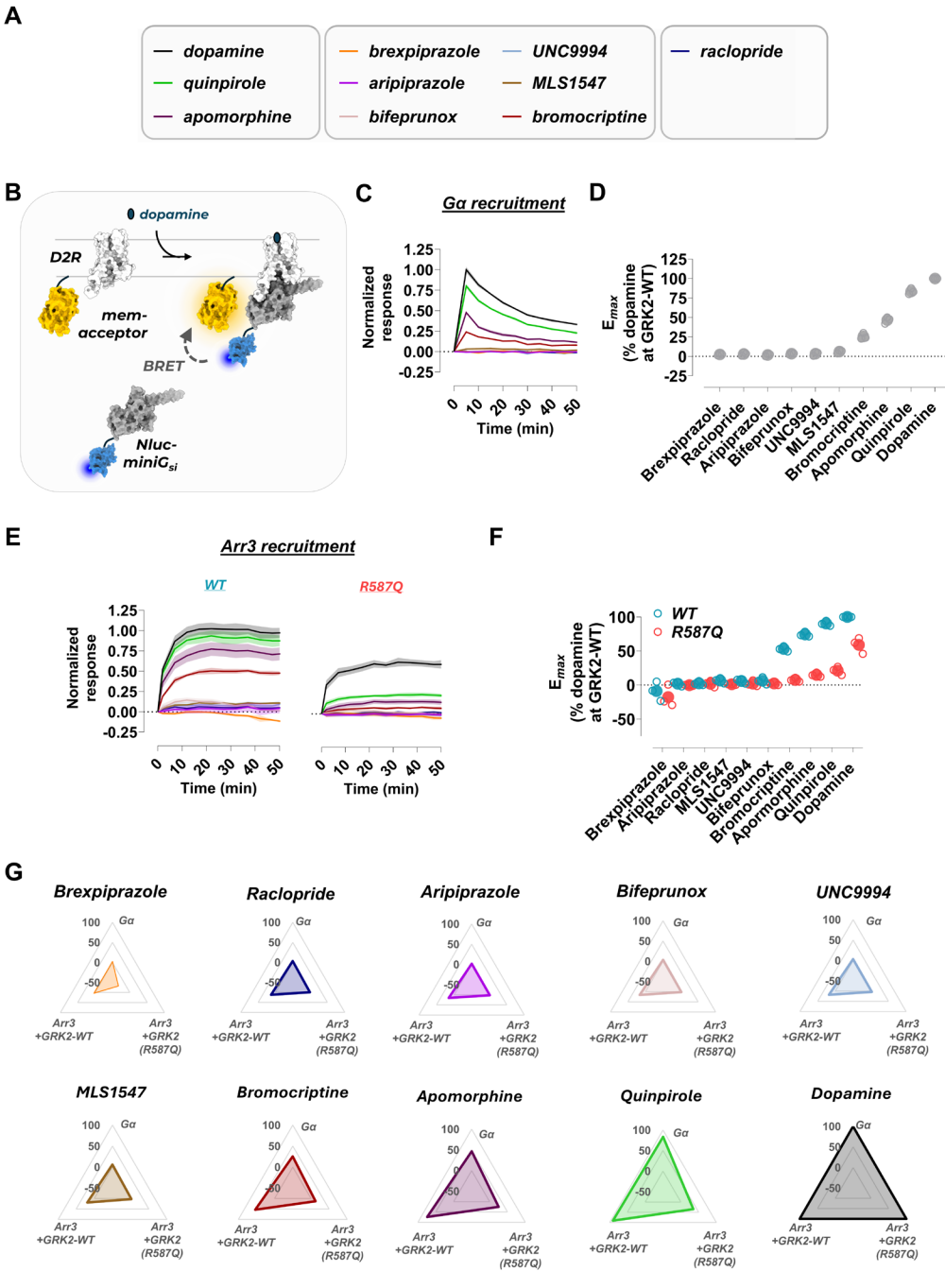
Supplementary Figure 6. Extended analysis of the effects of Gβγ-dependent and -independent GRK2 recruitment on G protein, related to Figure 4. (**A**) Summary of maximal D2R-depedent G protein activation in ΔQ:Arr2/3 cells transfected with GRK2-WT, GRK2(Δ2-15), or GRK2(R587Q). 100 μM dopamine was applied to all conditions. one-way ANOVA, F = 24.0, *p < 0.05, **p < 0.01. (**B**) Schematic of an electrophysiology-based D2R-mediated GIRK activation assay. (**C**) Summary of maximal D2R-mediated GIRK current in ΔQ:Arr2/3 cells transfected with GRK2-WT, GRK2(Δ2-15), or GRK2(R587Q). Arr3 was co-expressed in all conditions. 10 μM dopamine was applied to all conditions. one-way ANOVA, F = 25.3, ****p < 0.0001. (**D**) On-kinetics of dopamine-induced GIRK activation. unpaired t-test, *p < 0.05. (**E**) Desensitization of dopamine-induced GIRK current. one-way ANOVA, F = 51.9, ****p < 0.0001. (**F**) Schematic of BRET-based D2R-mediated cylic AMP (cAMP) inhibition assay using the cAMP sensor Nluc-EPAC-VV. (**G**) Summary of GRK2-dependent disinhibition of the inhibitory effect of D2R on cAMP. RM one-way ANOVA, F = 25.5, ***p < 0.001. (**H**) Summary of GRK2-dependent disinhibition of the inhibitory effect of D2R on cAMP over time.



Supplementary Figure 7. Extended analysis of the effects of Gβγ-dependent and -independent GRK2 recruitment on ERK signaling, related to Figure 4. (**A**) Schematic of D2R-mediated enhancement of ERK activity using an established BRET-based biosensor. (**B**) Summary of D2R-dependent effects on ERK activity in ΔQ:Arr2/3 cells transfected with GRK2-WT, GRK2(Δ2-15), or GRK2(R587Q) compared to control. Arr3 was co-expressed in all conditions. 100 μM dopamine was applied to all conditions. RM one-way ANOVA, F = 18.4, **p < 0.01, **p < 0.05.

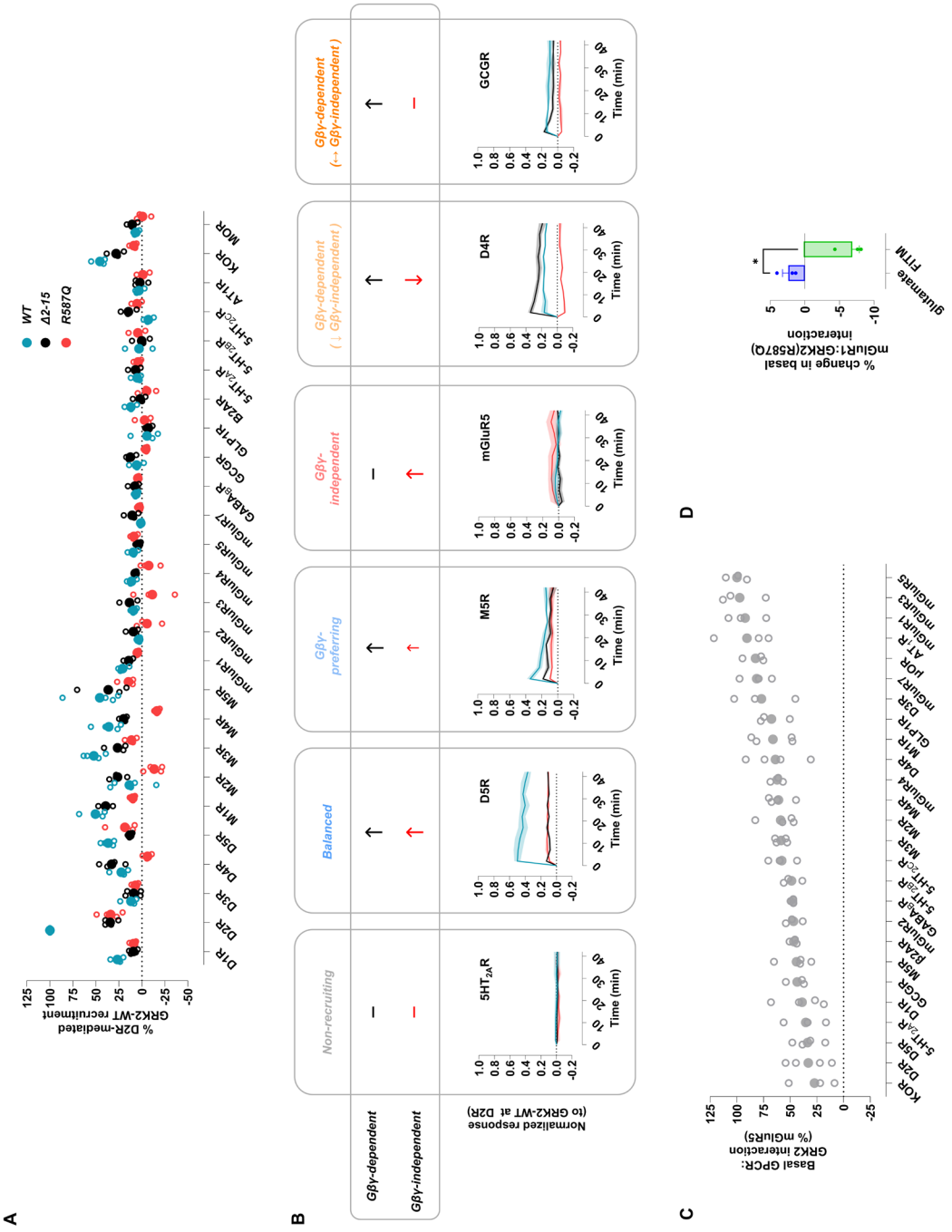


Supplementary Figure 8. Extended analysis of D2R ligand-induced effects on Gβγ-dependent and -independent GRK2 recruitment, related to Figure 5. (**A**) D2R ligands tested in this study. A saturating concentration of each compound (10 μM) was compared to dopamine (100 μM). (**B**) Summary of GRK2-WT, GRK2(Δ2-15), and GRK2(R587Q) recruitment in response to a panel of D2R ligands. Responses normalized to dopamine at GRK2-WT. (**C**) Preference of D2R ligands for GRK2(Δ2-15) over GRK2(R587Q). (**D**) Representative traces of $G\beta_1\gamma_2$ release in response to a panel of D2R ligands. (**E**) Summary of $G\beta_1\gamma_2$ release in response to a panel of D2R ligands. (**F**) Relationship between GRK2(Δ2-15) recruitment and $G\beta_1\gamma_2$ release in response to a panel of D2R ligands. Linear regression, Slope = 1.16, R^2 = 0.86.



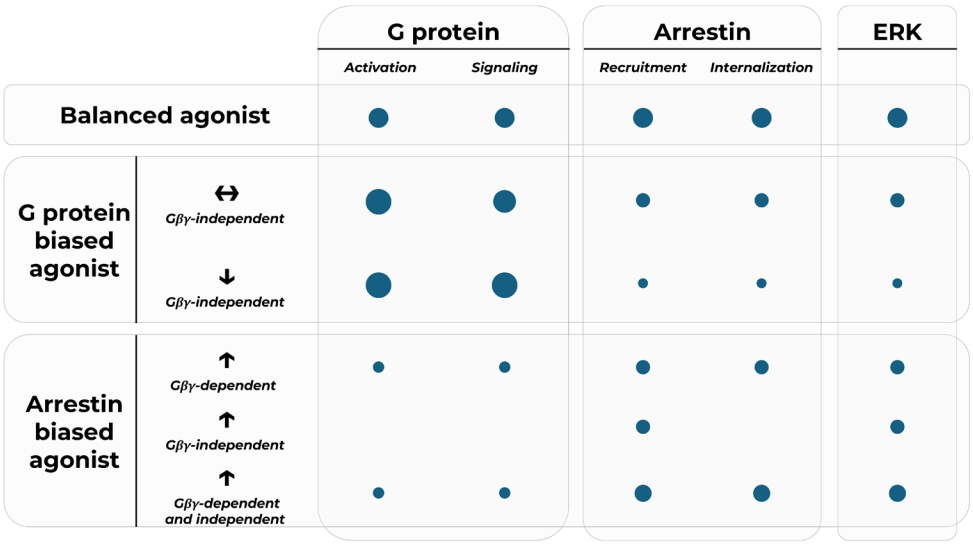
Supplementary Figure 9. Extended analysis of the effect of Gβγ-dependent and -independent GRK2 recruitment on biased signaling, related to Figure 5. (**A**) D2R ligands tested in this study. (**B**) Schematic of a bystander BRET-based G protein recruitment assay. The Gα subunit was engineered to operate independently of Gβγ, localize to the cytosol rather than the membrane, and is fused to an Nluc donor (Nluc–miniG$_{si}$). Receptor activation results in the recruitment of Nluc-miniG$_{si}$ to D2R, resulting in bystander BRET with a membrane-anchored acceptor (mem-acceptor). (**C**) Representative traces of Gα recruitment in response to a panel of D2R ligands. A saturating concentration of each compound (10 μM) was compared to dopamine (100 μM). (**D**) Summary of Gα recruitment in response to a panel of D2R ligands. (**E**) Representative traces of Arr3 recruitment in ΔQ:Arr2/3 cells overexpressing GRK2-WT (*left*) or

GRK2(R587Q) (*right*) recruitment in response to a panel of D2R ligands. A saturating concentration of each compound (10 μM) was compared to dopamine (100 μM). (**F**) Summary of GRK2-WT- or GRK2(R587Q)-dependent Arr3 recruitment in response to a panel of D2R ligands. (**G**) Bias plots for a panel of D2R ligands.



Supplementary Figure 10. Extended analysis of Gβγ-dependent and -independent GRK2 recruitment to diverse GPCRs, related to Figure 6. (**A**) Summary of GRK2-WT, GRK2(Δ2-15), and GRK2(R587Q) recruitment to a panel of GPCRs. The following agonists were applied at saturating concentrations: dopamine receptors D1R-D5R = 100 μM dopamine; muscarinic acetylcholine receptors

M1R-M5R = 10 μM acetylcholine; metabotropic glutamate receptors mGluR1-7 = 1 mM glutamate; GABA receptor $GABA_BR$ = 10 μM GABA; glucagon receptor GCGR = 100 μM glucagon; glucagon-like receptor GLP1R = 1 μM GLP-1; beta adrenergic receptor β2AR = 10 μM isoproterenol; serotonin receptors $5HT_{2A-C}$ = 10 μM serotonin; angiotensin receptor AT_1R = 10 μM angiotensin II; kappa opioid receptor κOR = 10 μM dynorphin A; mu opioid receptor μOR = 10 μM DAMGO. (**B**) Representative traces for GPCRs categorized based on their GRK2 recruitment profiles. This includes receptors that: (1) do not recruit GRK2 (*non-recruiting*), (2) recruit similar levels of GRK2(Δ2-15) and GRK2(R587Q) (*balanced*), (3) recruit more GRK2(Δ2-15) than GRK2(R587Q) (*Gβγ-preferring*), (4) recruit GRK2(R587Q) but not GRK2(Δ2-15) (*Gβγ-independent*) , (5) recruit GRK2(Δ2-15) but decrease in their basal association with GRK2(R587Q) (*Gβγ-dependent, ↓ Gβγ-independent*), and (6) recruit GRK2(Δ2-15) but have no effect on GRK2(R587Q) (*Gβγ-dependent, ↔ Gβγ-independent*). Receptors were classified as being capable of promoting Gβγ-dependent or -independent GRK2 recruitment if the magnitude of their effects on these mechanisms was >5% of GRK2-WT recruitment to D2R. (**C**) Summary of the basal association between GPCRs and GRK2(R587Q) ranked from lowest to highest. (**D**) Summary of the effect of glutamate (1 mM) or FITM (10 μM) on the basal association between mGluR1 and GRK2(R587Q). paired t-test, *$p < 0.05$.



Supplementary Figure 11. Predicted biased agonist profiles based on this study, related to Discussion. Endogenous ligands are, by definition, balanced agonists that fully engage both G proteins and arrestins. In principle, G protein biased agonists stabilize conformational states that preferentially couple to G proteins. However, Gβγ-dependent GRK2 recruitment counteracts this bias by suppressing G protein signaling and promoting receptor phosphorylation and subsequent arrestin recruitment. To maintain bias, G protein biased agonists should avoid non-canonical Gβγ-independent GRK2 recruitment, either by not activating this pathway or by suppressing basal receptor:GRK2 interactions. Conversely, arrestin biased agonists stabilize receptor states that favor arrestin binding. However, many GPCRs cannot efficiently recruit GRK2 in the absence of G protein activation and Gβγ release, limiting opportunities for arrestin-biased drug discovery. Agonists that preferentially stabilize receptor conformations that favor Gβγ-independent GRK2 recruitment — and thereby arrestin — independently of G protein represents a path towards purely arrestin biased agonists. However, such ligands would support ERK activation while only partially promoting arrestin recruitment and failing to drive arrestin-dependent internalization. Finally, arrestin biased agonists that promote both Gβγ-dependent and -independent would still require some degree of G protein activation but offer greater potential for arrestin recruitment.