

GPCR kinase knockout cells reveal the impact of individual GRKs on arrestin binding and GPCR regulation

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Supplementary Information

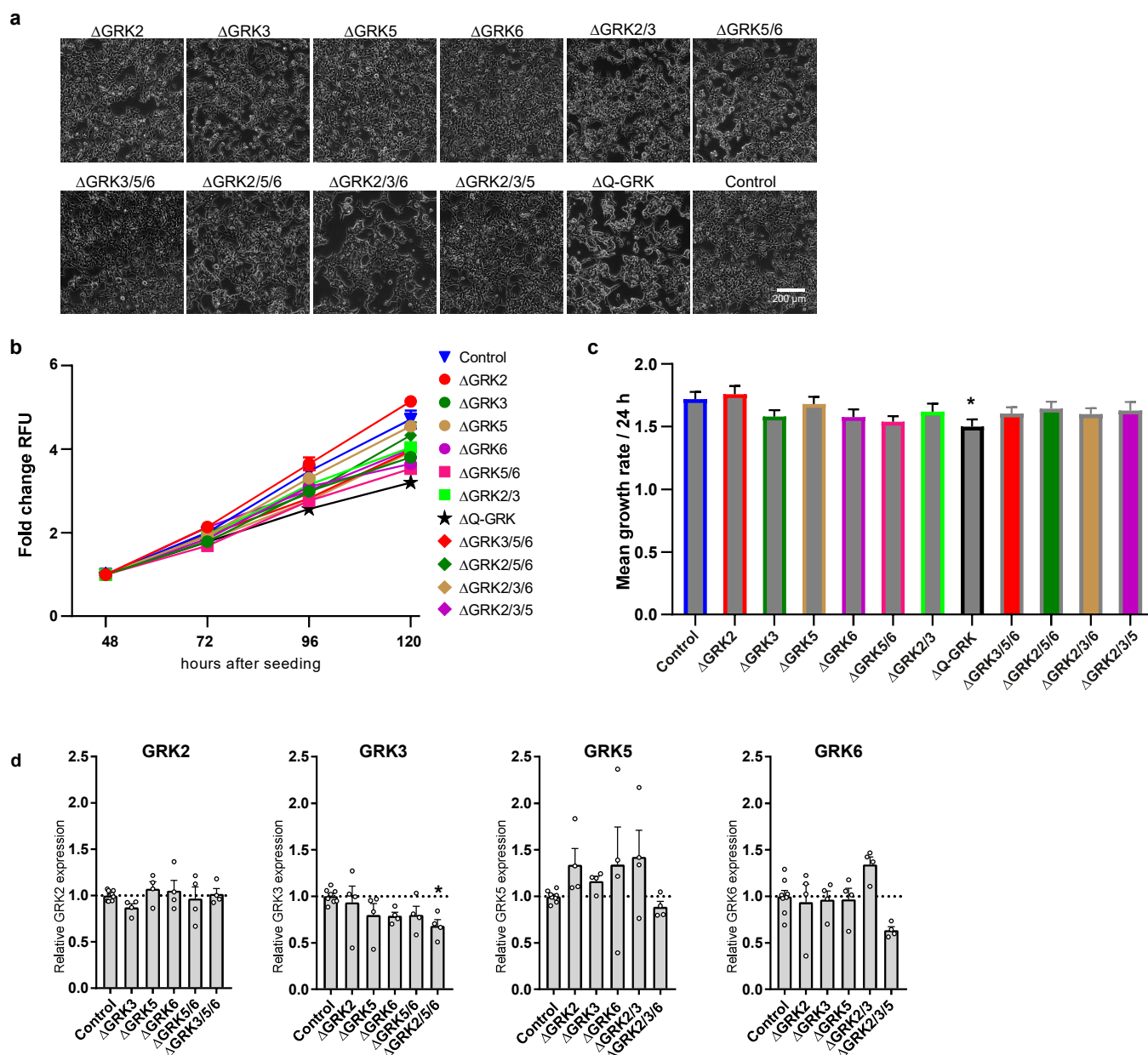
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Supplementary Figures 1-15

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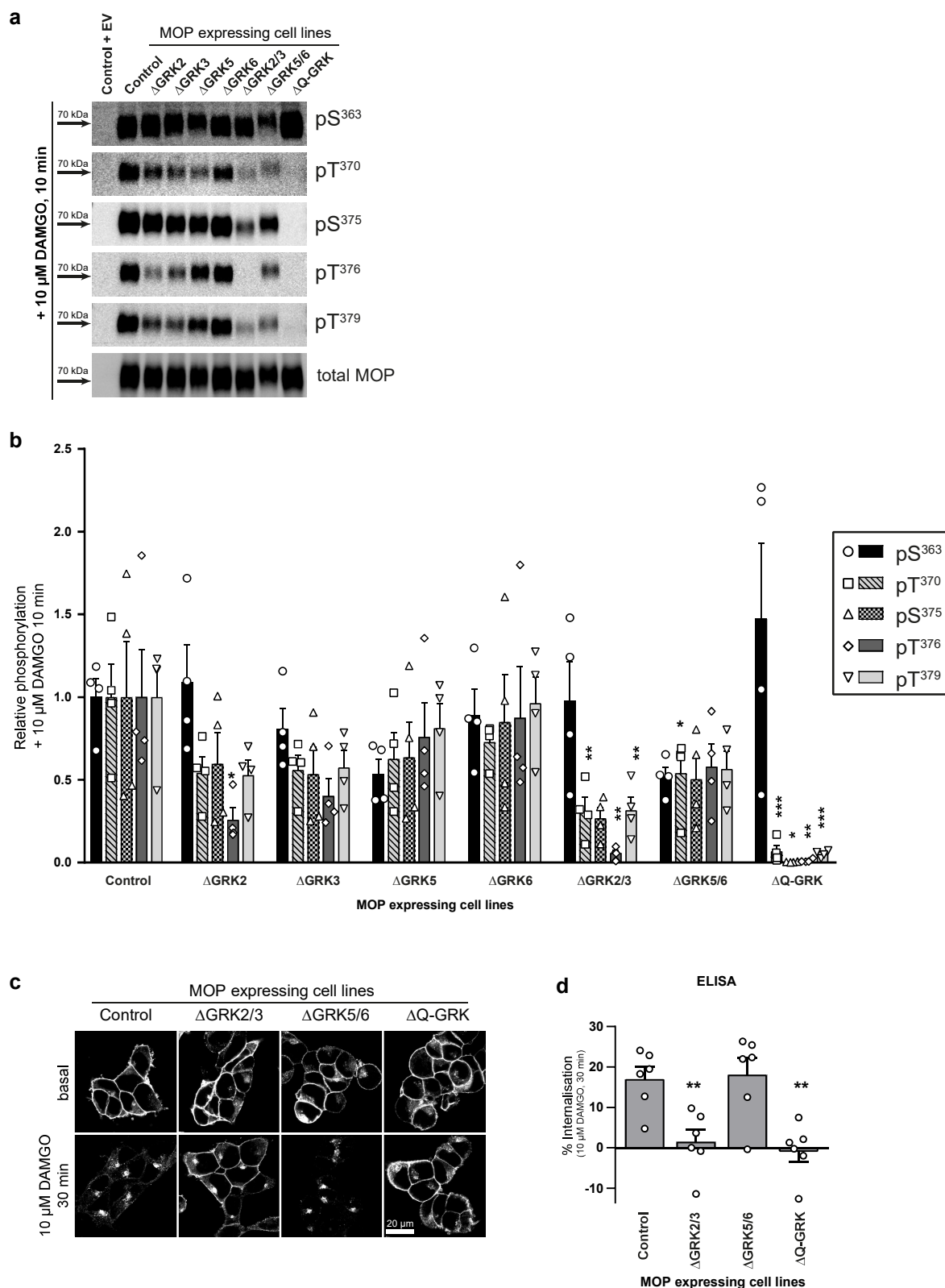
Supplementary References

Supplementary Figure 1



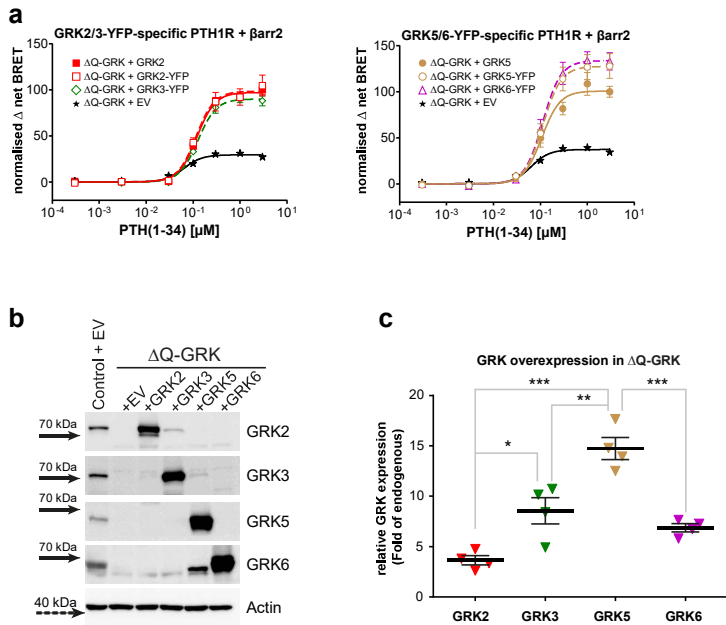
Supplementary Figure 1: Characterisation of GRK knockout cell properties. **a** Single (Δ GRK2, 3, 5, 6), double (Δ GRK2/3; 5/6), triple (Δ GRK3/5/6, 2/5/6, 2/3/6, 2/3/5), and quadruple (Δ Q-GRK) GRK knockout cells were generated using the CRISPR/Cas9 technology and established as single cell clones. Cell morphology was examined regularly. Representative images acquired by phase contrast microscopy are shown. **b** Cell viability was determined by adding CellTiter-Blue reagent (Promega) at indicated timepoints and measuring relative fluorescence units (RFU) after 90 minutes of incubation. Shown are the respective growth curves as mean of $n = 3$ independent experiments $\pm$ SEM, expressed as fold change compared to 48 h after seeding. **c** Growth rates per 24 h were calculated from data shown in **b** for each cell clone and are presented in bar graphs as mean $\pm$ SEM. Growth rates of generated cell clones were compared to the growth rate of Control cells using ANOVA and two-sided Dunnett's test (* $p < 0.05$). **d** Quantification of the untargeted GRKs was performed by analysing four independent Western blots (representative blot in Figure 1a) made from $n = 4$ independent protein lysates of each cell clone. Data are depicted as mean $\pm$ SEM, normalised to the respective remaining expression in Control cells. GRK expression levels were compared to expression in Control cells using ANOVA and two-sided Dunnett's test (* $p < 0.05$). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

Supplementary Figure 2



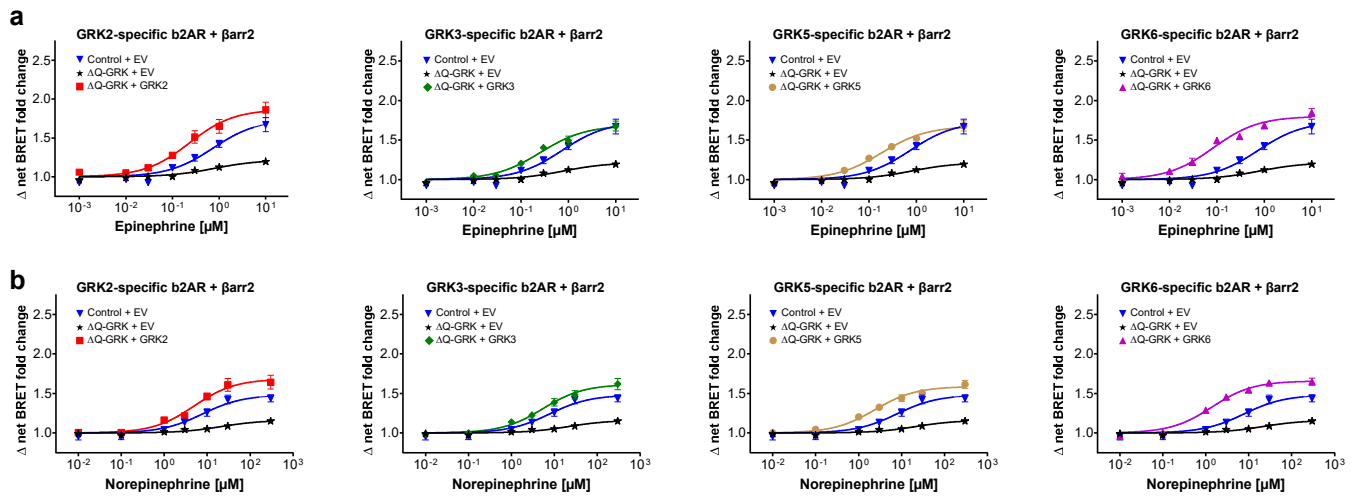
Supplementary Figure 2: GRK knockout cells confirm GRK-specific μ -opioid receptor (MOP) phosphorylation. **a-d** Δ GRK clones and Control cells stably expressing an N-terminally HA-tagged MOP were used for analysis of site-specific phosphorylation and receptor internalisation. **a** Prior to immunoprecipitation, cells were treated with the MOP agonist [D-Ala², N-MePhe⁴; Gly-oI]-enkephalin (DAMGO) as indicated and subsequently analysed for site-specific phosphorylation by Western blot. Shown are representative blots of $n = 4$ independent experiments. **b** Blots were quantified and relative phosphorylation compared to the respective Control signal of the indicated GRK target sites is shown as mean $\pm$ SEM. Statistical significance compared to signal obtained in Control cells was determined by ANOVA and two-sided Dunnett's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). **c** Δ GRK MOP cells were stimulated with DAMGO as indicated or left untreated (basal). After fixation, the cells were stained with anti-HA antibody followed by Alexa488-conjugated secondary antibody, and examined by confocal microscopy. Shown are representative images of $n = 5$ independent experiments. **d** Receptor internalisation was measured by ELISA. Data represent per cent loss of cell-surface receptors in DAMGO treated cells as compared to vehicle stimulated sister cultures. Data are presented as mean of $n = 6$ independent experiments $\pm$ SEM. Differences in internalisation between indicated cell clones and Control cells were statistically analysed using ANOVA and two-sided Dunnett's test (** $p < 0.01$). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

Supplementary Figure 3



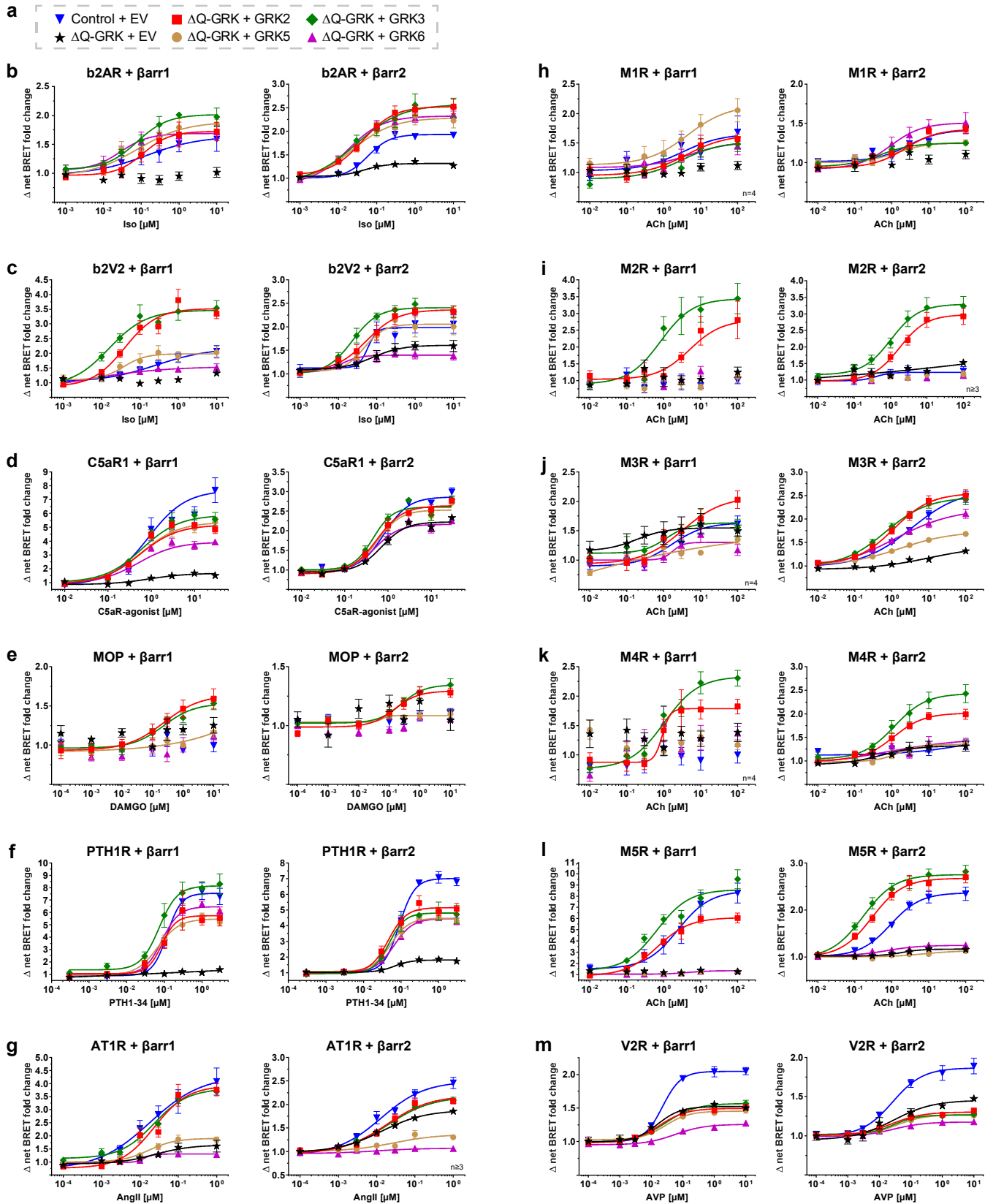
Supplementary Figure 3: Verification of GRK-YFP-mediated β -arrestin recruitment and quantification of GRK overexpression levels. **a** Δ Q-GRK cells were transfected with either YFP-tagged or untagged GRKs as indicated and β -arrestin2 (β arr2) recruitment to the parathyroid hormone 1 receptor (PTH1R) was measured using our NanoBRET β arr recruitment assay. Data points are presented as the mean $\pm$ SEM of the calculated Δ net BRET fold change from $n = 3$ independent experiments. The utilisation of the GRK-YFP constructs resulted in comparable β arr2 recruitment as in presence of the untagged GRKs (corresponds to Figure 1f). **b, c** Representative Western blot (**b**) and quantification (**c**) of $n = 4$ independent experiments showing the overexpression of GRK 2, 3, 5, or 6 in Δ Q-GRK in comparison to endogenous levels (Control + empty vector (EV)). The actin-normalised values are represented as fold expression of respective signal from endogenously expressed GRKs detected on the same membrane. GRK fold overexpressions were compared using ANOVA and two-sided Tukey's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files

Supplementary Figure 4



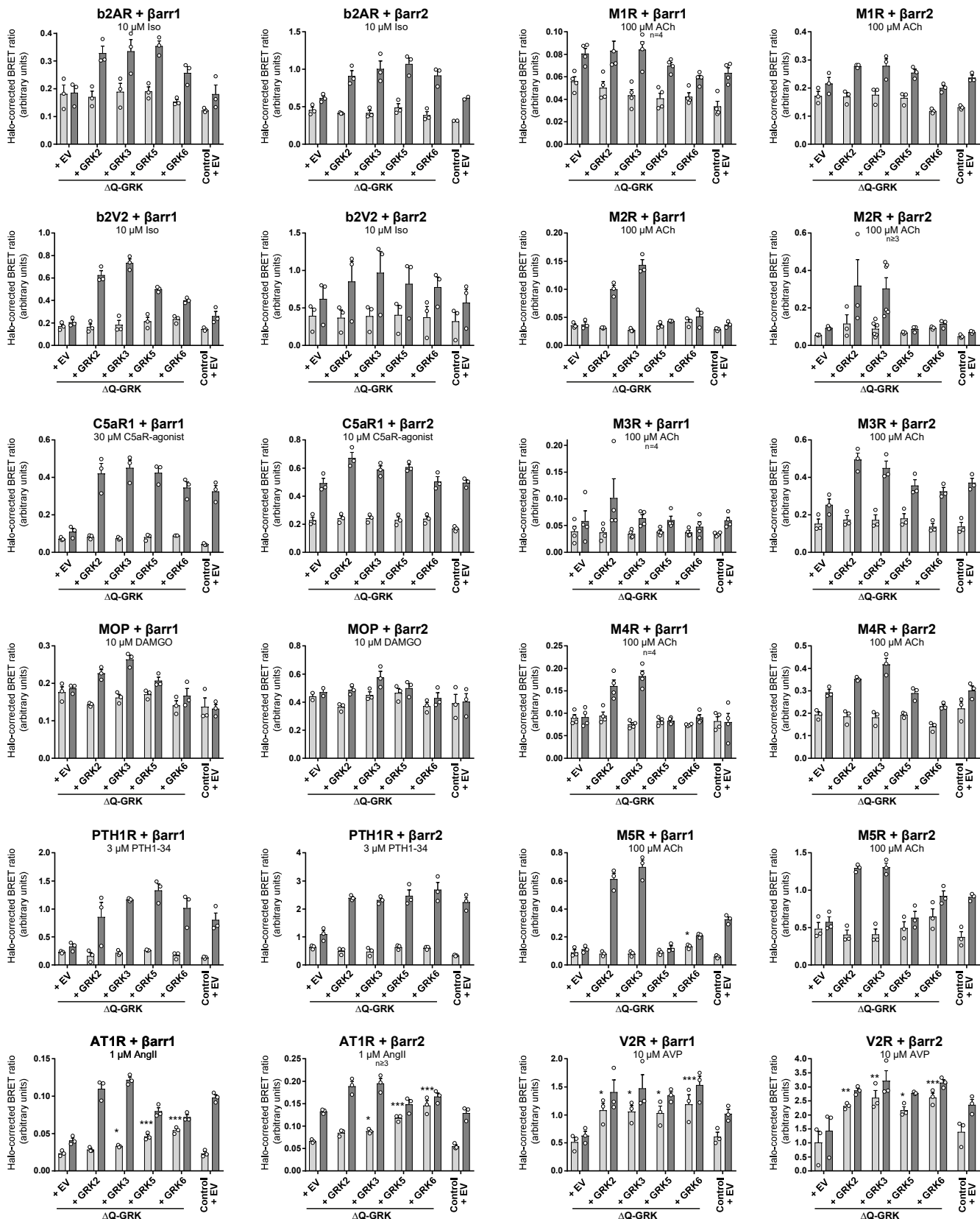
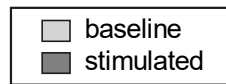
Supplementary Figure 4: GRK-specific β -arrestin 2 (β arr2) recruitment to the β 2 adrenergic receptor (b2AR) induced by endogenous ligands. **a, b** GRK-specific β arr2 recruitment to the b2AR was measured in presence of the endogenous ligands epinephrine (**a**) or norepinephrine (**b**) (correspond to Figure 2c and d, respectively). Individual GRK isoforms were overexpressed in Δ Q-GRK as indicated. For better comparability, the data for the Control + empty vector (EV) and Δ Q-GRK + EV conditions are depicted in each panel. The data are shown as Δ net BRET fold change as mean of $n = 3$ independent experiments $\pm$ SEM.

Supplementary Figure 5



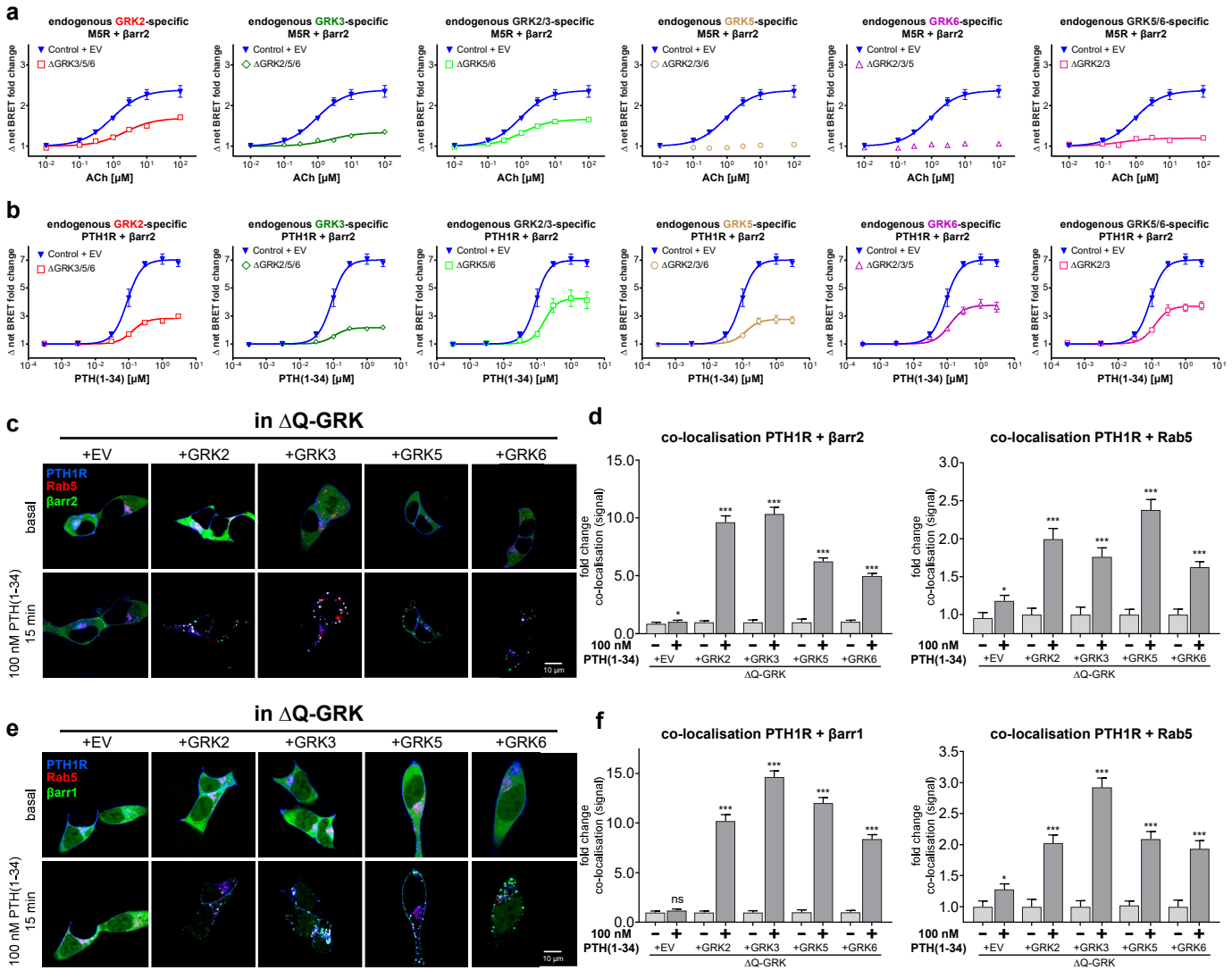
Supplementary Figure 5: Evaluation of GRK-specific β -arrestin (β arr) recruitment for twelve GPCRs. **a** Legend for concentration-response curves shown in (b-m). **b-m** The Halo-Tag- β arr fusion protein is recruited to a NanoLuciferase (NanoLuc)-tagged GPCR upon agonist activation and subsequent receptor phosphorylation. For the parathyroid hormone 1 receptor (PTH1R) and the vasopressin 2 receptor (V2R), the BRET pair was swapped. Overexpression of single GRKs in Δ Q-GRK cells allows the assessment of the impact of individual GRKs on this process. Δ Q-GRK or Control cells were transfected with the respective tagged GPCR and β arr1 or 2 fusion constructs. Additionally, either GRK2, 3, 5, 6, or empty vector (EV) were co-transfected as indicated. The dynamic BRET changes are shown as ligand concentration-response curves normalised to baseline values and vehicle control. All data points are calculated as Δ net BRET fold change as the mean of $n = 3$ independent experiments (exceptions are indicated in the respective panels) $\pm$ SEM. Results of the statistical analysis are listed in Supplementary Table 3 and plotted in Figure 3i.

Supplementary Figure 6



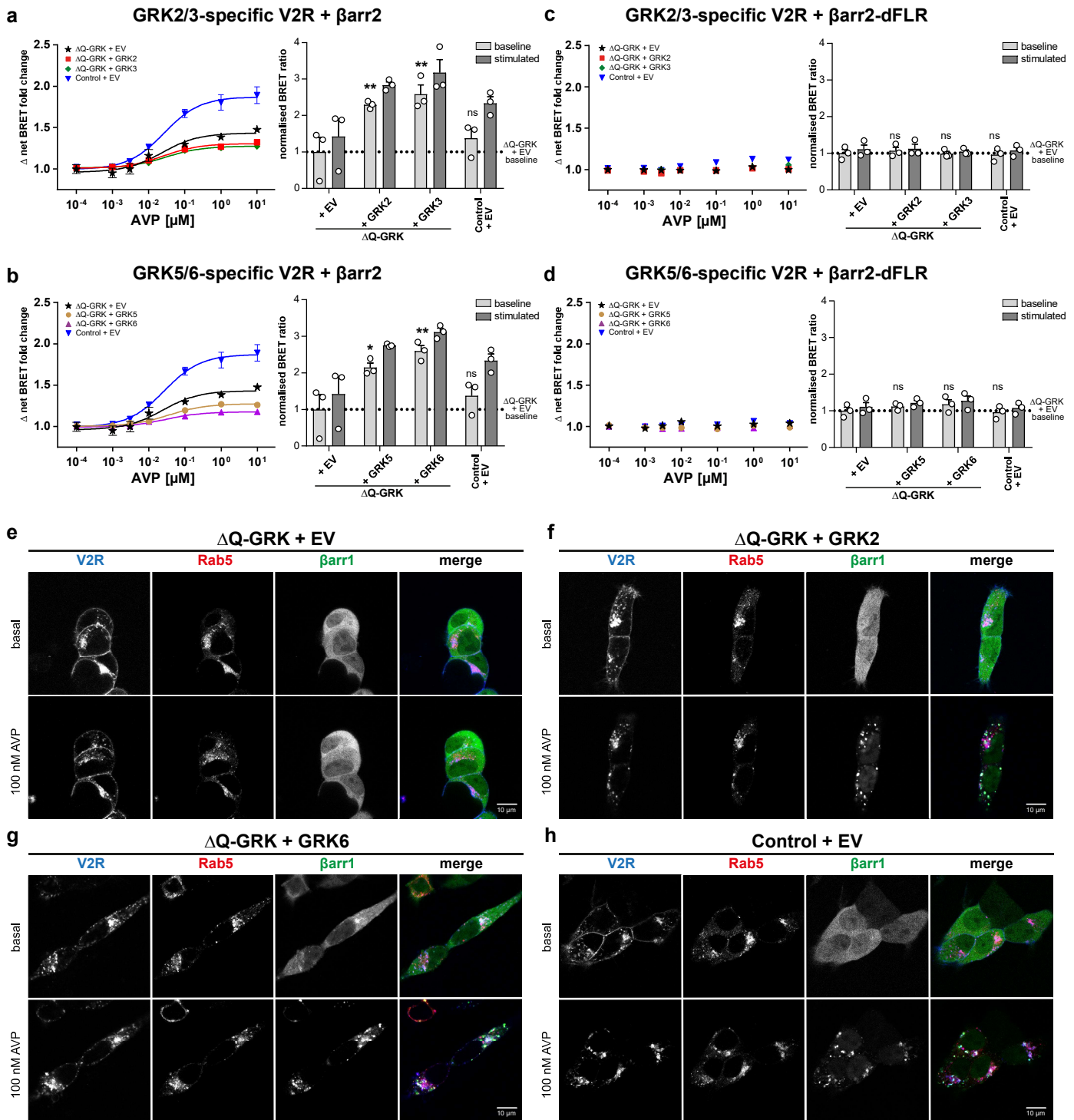
Supplementary Figure 6: Detailed analysis of basal and stimulated BRET ratios of GRK-specific β -arrestin (β arr) recruitment to twelve GPCRs. The data shown are derived from concentration-response curves in Supplementary Figure 5. Δ Q-GRK or Control cells were transfected with the NanoLuciferase (NanoLuc)-tagged GPCR and Halo-Tag- β arr1 or 2 fusion constructs. For the parathyroid hormone 1 receptor (PTH1R) and the vasopressin 2 receptor (V2R), the BRET pair was swapped. Additionally, either GRK2, 3, 5, 6, or the empty vector (EV) were co-transfected. The non-normalised, Halo-corrected BRET ratio is presented before (baseline) and after stimulation with saturating agonist concentrations (stimulated) as indicated. The bar graphs show the mean of $n = 3$ independent measurements (exceptions are indicated in the respective panels) $\pm$ SEM. To test whether the baseline BRET ratios were significantly elevated compared to the respective Δ Q-GRK + EV baseline, an ANOVA and one-sided Dunnett's test was performed (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Baselines without indication of significance were found to be not significantly different from Δ Q-GRK + EV. All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

Supplementary Figure 7



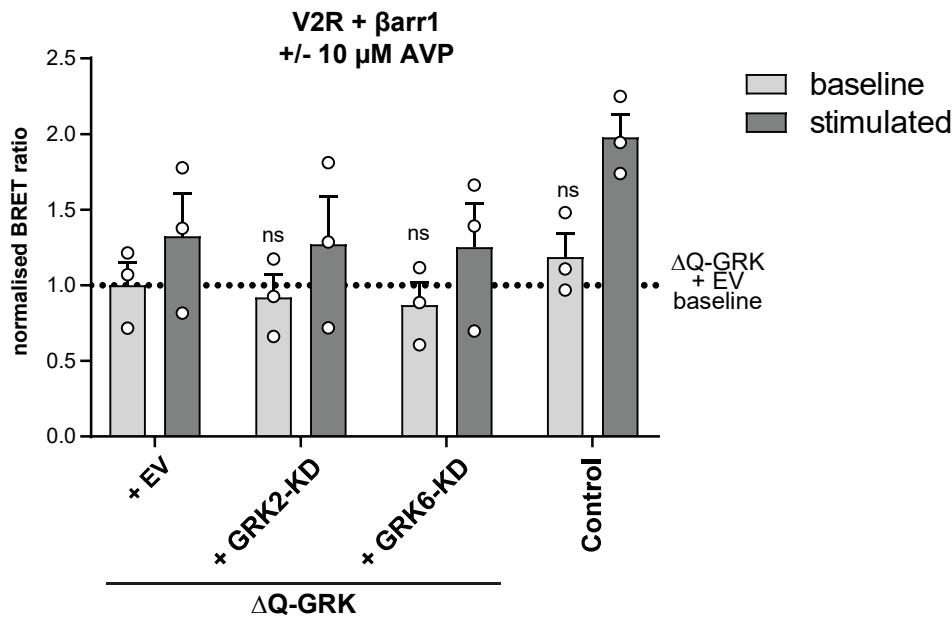
Supplementary Figure 7: GRK-specific β -arrestin2 (β arr2) recruitment to muscarinic M5 acetylcholine receptor (M5R) and parathyroid hormone 1 receptor (PTH1R) under endogenous GRK expression levels and β arr1 and 2 translocation to PTH1R with individual GRK overexpression. **a, b** β arr2 recruitment to the M5R (**a**) or PTH1R (**b**) in cells expressing all endogenous GRKs (Control + EV), only one remaining endogenous GRK (Δ GRK3/5/6, 2/5/6, 2/3/6, 2/3/5), or two remaining endogenous GRKs (Δ GRK5/6, 2/3) as indicated is shown as Δ net BRET fold change (mean of $n = 3$ independent experiments $\pm$ SEM). For easier comparison, Control curves are depicted in every panel. **c-f** Confocal live-cell microscopy was performed using Δ Q-GRK cells transfected with PTH1R-CFP (blue), Rab5-mCherry (red), β arr2-YFP (**c**) or β arr1-YFP (**e**) expression constructs (green) and individual untagged GRK2, 3, 5, or 6. Shown are representative images, taken before (basal) and after 15 minutes of stimulation with 100 nM parathyroid hormone (1-34) (PTH(1-34)). The normalised co-localisation of PTH1R and β arr2 or Rab5 (**d**) and β arr1 or Rab5 (**f**) was quantified using Squash and SquashAnalyst (number of images per respective condition; for β arr2 Δ Q-GRK + EV (52), Δ Q-GRK + GRK2 (44), Δ Q-GRK + GRK3 (41), Δ Q-GRK + GRK5 (44), Δ Q-GRK + GRK6 (51); for β arr1 Δ Q-GRK + EV (38), Δ Q-GRK + GRK2 (44), Δ Q-GRK + GRK3 (44), Δ Q-GRK + GRK5 (51), Δ Q-GRK + GRK6 (40)). Data are presented as mean fold change in co-localisation signal $\pm$ SEM. Statistical analysis was performed using a two-way mixed-model ANOVA followed by a two-sided paired t-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns (not significant)). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

Supplementary Figure 8



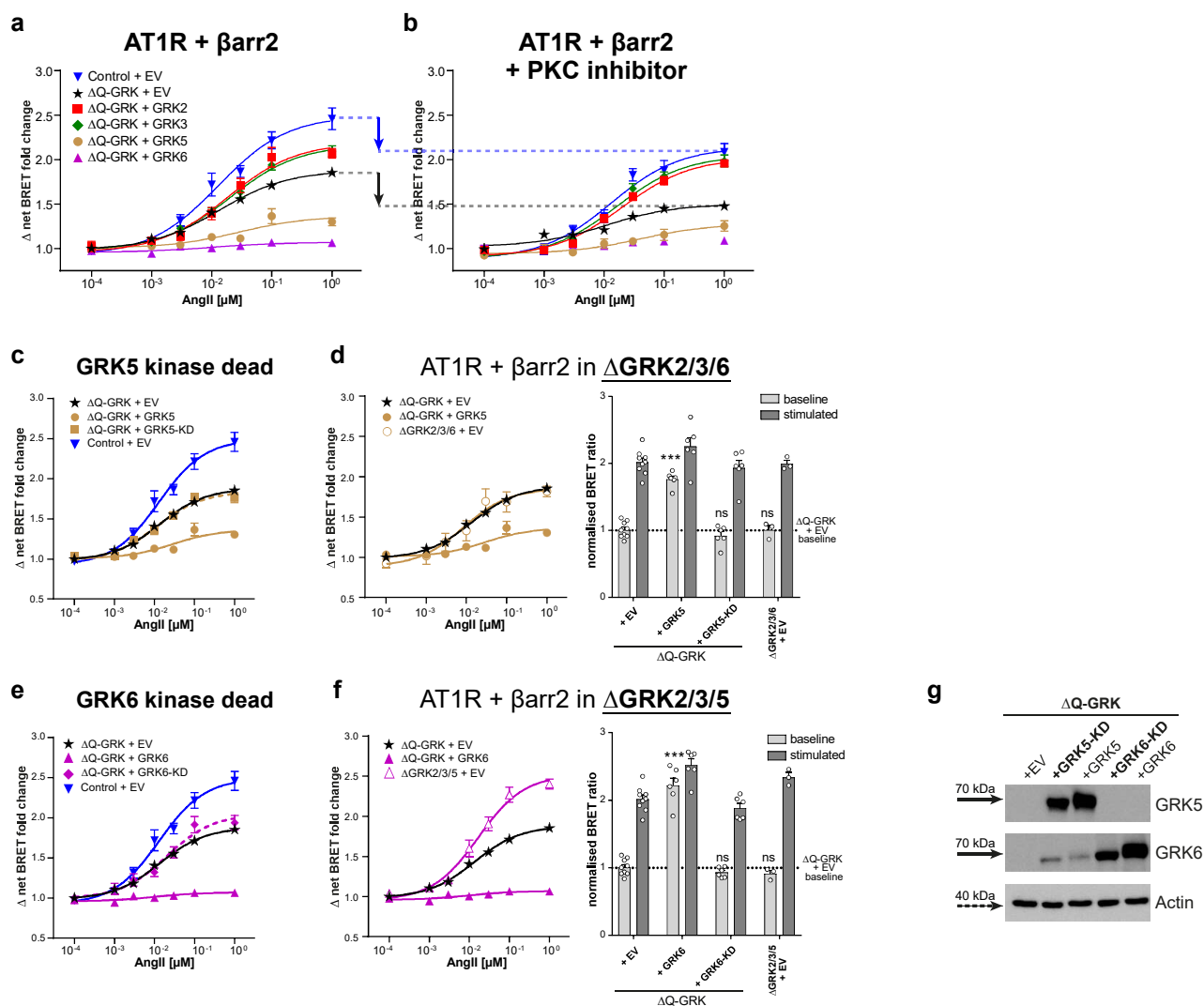
Supplementary Figure 8: GRK-specific β -arrestin 2 (β arr2) recruitment to vasopressin 2 receptor (V2R) and single channel images of β arr1 translocation to V2R. a-d Δ Q-GRK or Control cells were transfected with V2R-Halo-Tag and one of the following β arr2-NanoLuciferase (NanoLuc) fusion constructs: wild type (a, b) or β arr2 lacking the finger loop region (dFLR; c, d). Additionally, either GRK2, 3, 5, 6 or the empty vector (EV) were transfected as indicated. The dynamic BRET changes are shown as ligand concentration-response curves normalised to baseline values and vehicle control. All data points are calculated as Δ net BRET fold change as the mean of $n = 3$ independent experiments $\pm$ SEM. The same dataset is presented as bar graphs, displaying the mean BRET-values $\pm$ SEM before (baseline) and after stimulation with 10 μ M [Arg8]-vasopressin (AVP; stimulated), normalised to the basal BRET ratio derived from the Δ Q-GRK + EV condition (dashed line). To test whether the baseline BRET ratios were significantly elevated compared to the respective Δ Q-GRK + EV baseline, an ANOVA and one-sided Dunnett's test was performed (* $p < 0.05$; ** $p < 0.01$; ns (not significant)). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files. e-h (Corresponds to Figure 4e) Δ Q-GRK or Control cells were transfected with V2R-CFP (blue), Rab5-mCherry (red), β arr1-YFP (green), and either EV, GRK2, or GRK6 as indicated. Images were taken before (basal) and after 15 minutes of 100 nM AVP stimulation (number of images per respective condition; Δ Q-GRK + EV (35), Δ Q-GRK + GRK2 (35), Δ Q-GRK + GRK6 (33), Control + EV (30)). Single channels and overlay of all three channels from the corresponding images shown in Figure 4e are depicted here.

Supplementary Figure 9



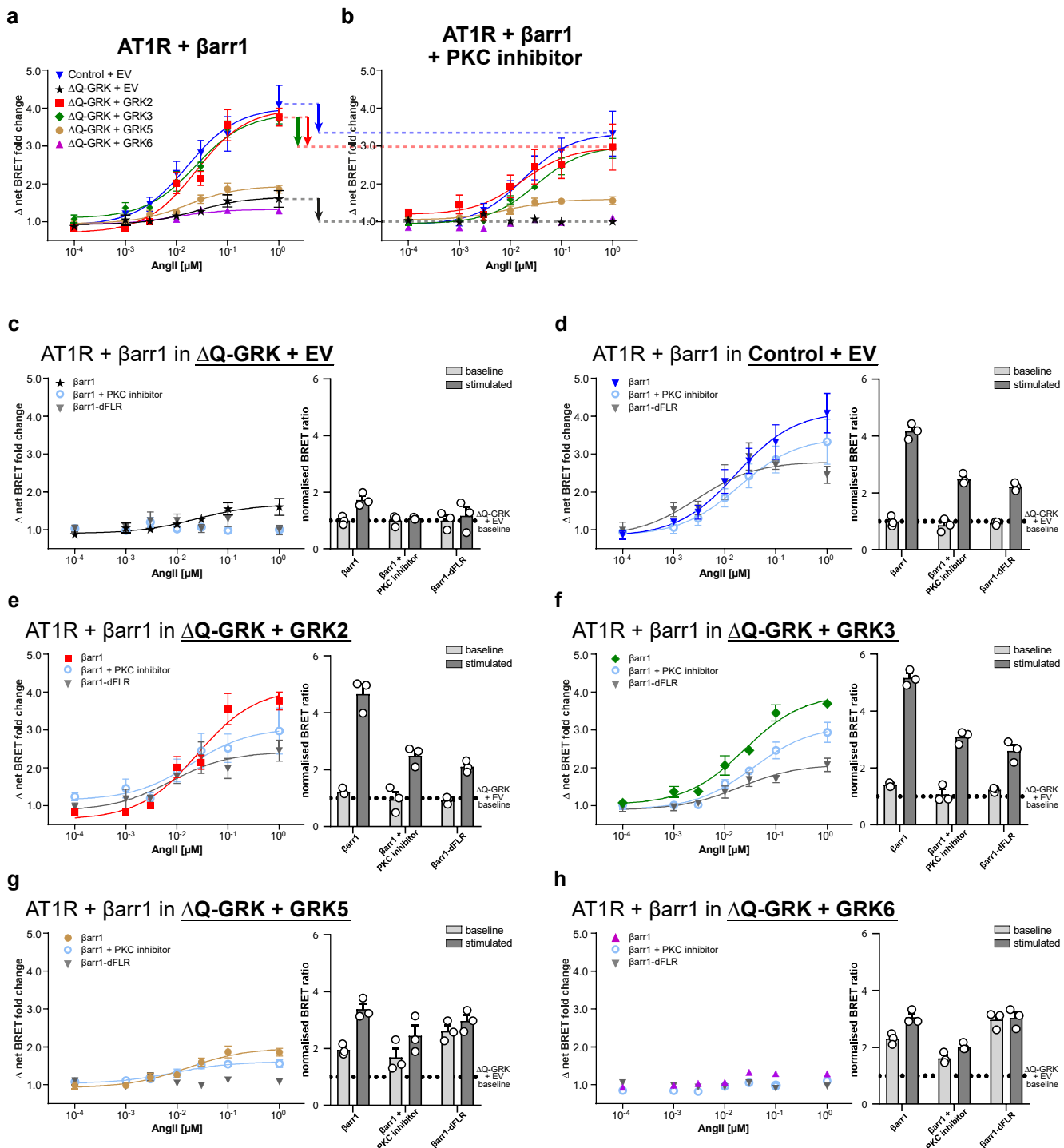
Supplementary Figure 9: β -arrestin 1 (β arr1) recruitment to vasopressin 2 receptor (V2R) with kinase-dead GRK constructs. Δ Q-GRK or Control cells were transfected with V2R-Halo-Tag and β arr1-NanoLuciferase (NanoLuc) fusion construct. Additionally, kinase-dead (KD) mutants of either GRK2 (K220R) or 6 (K215R), or the empty vector (EV) were transfected as indicated. The dataset is presented as mean of $n = 3$ independent experiments $\pm$ SEM in bar graphs, displaying the measured BRET-values before (baseline) and after stimulation with 10 μ M [Arg8]-vasopressin (AVP; stimulated), normalised to the basal BRET ratio derived from the Δ Q-GRK + EV condition (dashed line). To test whether the baseline BRET ratios were significantly elevated compared to the respective Δ Q-GRK + EV baseline, an ANOVA and one-sided Dunnett's test was performed (ns (not significant)). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files. Recruitment data for Control + EV and Δ Q-GRK + EV were measured again in the same experiment as the GRK-KD mutants and are not identical to data presented in Figure 4a, b for these conditions.

Supplementary Figure 10



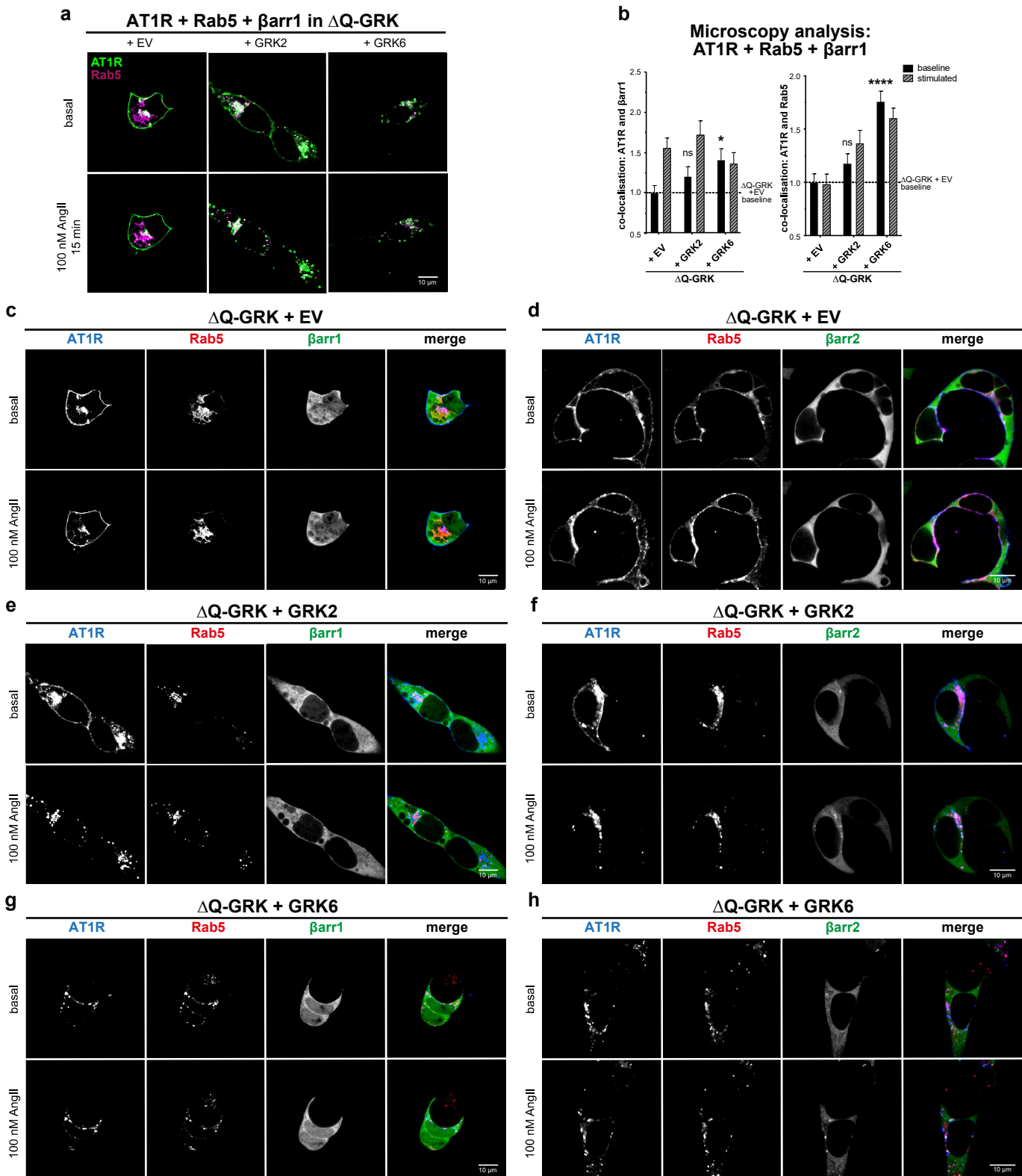
Supplementary Figure 10: β -arrestin2 (β arr2) recruitment to angiotensin 1 receptor (AT1R) with PKC inhibition, kinase dead GRK5 and 6 constructs, and under endogenous GRK5 and 6 expression. **a, b** Δ Q-GRK cells were transfected with AT1R-NanoLuciferase (NanoLuc), Halo-Tag- β arr2, and either one of the GRKs or the empty vector (EV) as indicated. β arr2 recruitment to the AT1R was measured in absence or presence of PKC inhibitor Gö6983 (500 nM) for one hour. Angiotensin II (AngII)-induced dynamic BRET changes are shown as concentration-response curves. To emphasise the impact of PKC on β arr2 recruitment, dynamic BRET changes of Figure 5a-f in absence (**a**) or presence of the PKC inhibitor Gö6983 (500 nM) (**b**) are shown as concentration-response curves. Downward arrows and dashed lines indicate the loss in dynamic recruitment by PKC inhibition. **c-f** Corresponding to the data shown in Figure 5e and f, Δ Q-GRK cells were transfected with AT1R-NanoLuc and Halo-Tag- β arr2. The concentration-dependent recruitment of β arr2 was measured in the presence of co-transfected GRK5 and 6 kinase-dead (KD, K215R) mutants (**c, e**) or in Δ GRK2/3/6 or Δ GRK2/3/5 (**d, f**). Recruitment data generated for Control + EV, Δ Q-GRK + EV, and Δ Q-GRK + GRK5 or 6 overexpression (Figure 5a, b, e, f) are depicted again to allow comparability. The data are also presented in respective bar graphs, displaying the mean BRET ratios $\pm$ SEM before (baseline) and after stimulation with 1 μ M AngII (stimulated), normalised to the basal BRET ratio derived from Δ Q-GRK + EV baseline (**d, f**). To test whether the baseline BRET ratios were significantly elevated compared to Δ Q-GRK + EV baseline, an ANOVA and one-sided Dunnett's test was performed ($*** p < 0.001$; ns (not significant)). All concentration-response curve data points are calculated as Δ net BRET fold change normalised to baseline values and vehicle control, represented as the mean of $n = 3$ independent experiments $\pm$ SEM. All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files. **g** Representative Western blot showing the overexpression of GRK5 or 6 and GRK5- or 6-KD mutants in Δ Q-GRK.

Supplementary Figure 11



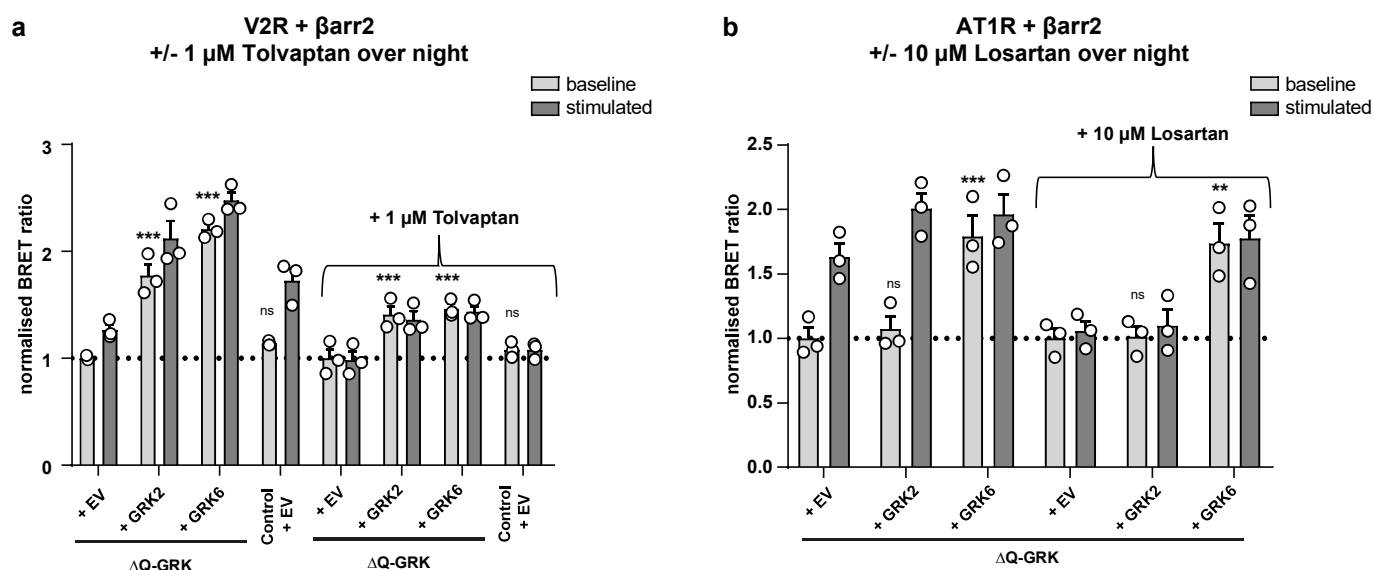
Supplementary Figure 11: GRK- and PKC-specific β -arrestin1 (β arr1) recruitment to angiotensin 1 receptor (AT1R). **a, b** Δ Q-GRK or Control cells were transfected with AT1R-NanoLuciferase (NanoLuc), Halo-Tag- β arr1 and either GRK2, 3, 5, 6, or the empty vector (EV) as indicated. Upon stimulation with Angiotensin II (AngII), dynamic BRET changes are shown as concentration-response curves recorded in absence (**a**) or presence of the PKC inhibitor G6983 (500 nM) for one hour (**b**). Downward arrows and dashed lines indicate the loss in dynamic recruitment by PKC inhibition. **c-h** To emphasise the contribution of the individual GRKs to the complex formation of β arr1 with AT1R, data shown in (**a, b**) and Supplementary Figure 5g (left panel), are now divided into smaller panels according to the individual GRK condition. Additionally, an analogous measurement using a Halo-Tag- β arr1 construct lacking the finger loop region (dFLR) was performed for each condition to test for the formation of a “hanging” complex. All data points are calculated as Δ net BRET fold change normalised to baseline values and vehicle control, represented as the mean of $n = 3$ independent experiments $\pm$ SEM. Data of these panels are also presented in respective bar graphs, displaying the mean BRET ratios $\pm$ SEM before (baseline) and after stimulation with 1 μ M AngII (stimulated), normalised to the basal BRET ratio derived from the corresponding Δ Q-GRK + EV condition. The results of the statistical analysis of displayed data are listed in Supplementary Table 4. All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

Supplementary Figure 12



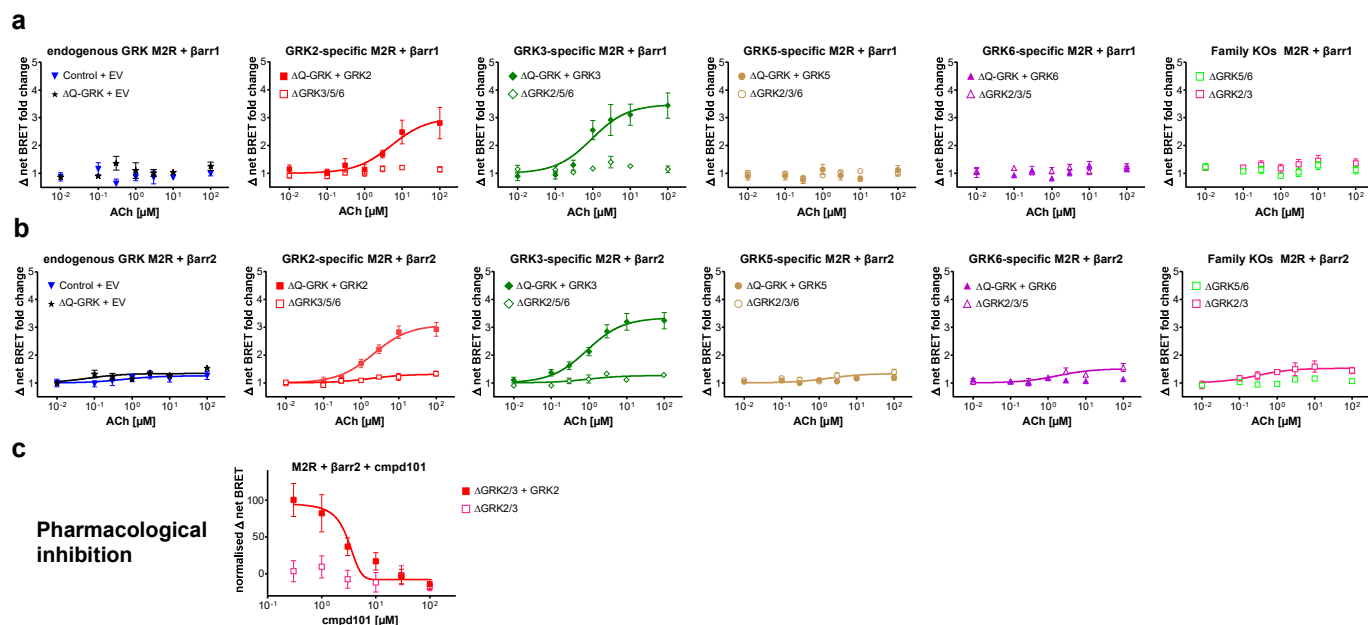
Supplementary Figure 12: β -arrestin (β arr) 1 and 2 translocation to angiotensin 1 receptor (AT1R). Corresponding to Figure 6a, b, GRK2- and 6-dependent AT1R internalisation in Δ Q-GRK is shown. **a, b** Analogous experiments were performed as depicted in Figure 6a, b, but using β arr1. In short: Δ Q-GRK cells were transfected with hsp-AT1R-CFP (green), Rab5-mCherry (magenta), β arr1-YFP, and either empty vector (EV), GRK2, or GRK6. Images were taken before (basal) and after 15 minutes of 100 nM Angiotensin II (AngII) stimulation (number of images per respective condition for β arr1; Δ Q-GRK + EV (32), Δ Q-GRK + GRK2 (31), Δ Q-GRK + GRK6 (33)). Representative images are shown in **(a)**. The co-localisation of AT1R and β arr1 or Rab5 was quantified using Squash and Squash Analyst. Data are presented as mean fold change in co-localisation signal $\pm$ SEM, normalised to unstimulated Δ Q-GRK + EV condition **(b)**, dashed line). Co-localisation under unstimulated conditions was compared using ANOVA and two-sided Dunnett's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns (not significant)). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files. **c-h** Depiction of single channels and overlay (merge) of hsp-AT1R-CFP (blue), Rab5-mCherry (red), and β arr1 **(c, e, g)** and 2 **(d, f, h)**-YFP (green). Additionally, cells were transfected with EV **(c, d)**, GRK2 **(e, f)**, or GRK6 **(g, h)**. Representative images before (basal) and after 15 minutes of 100 nM AngII stimulation are shown (number of images per respective condition for β arr2; Δ Q-GRK + EV (31), Δ Q-GRK + GRK2 (31); Δ Q-GRK + GRK6 (33)).

Supplementary Figure 13



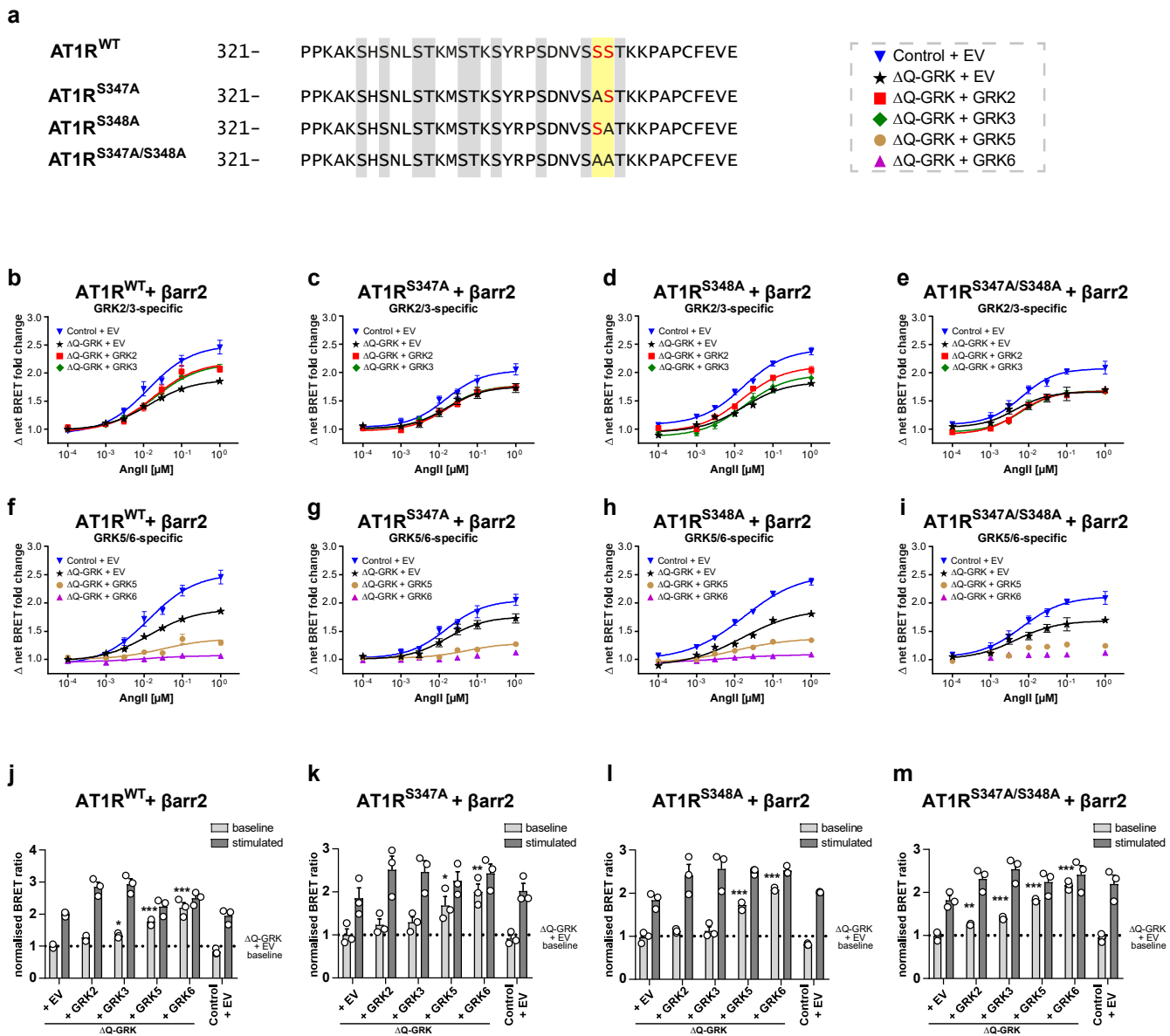
Supplementary Figure 13: Pharmacological inhibition of receptor activity does not suffice to abolish pre-coupled β -arrestin2 (β arr2) complexes with vasopressin 2 receptor (V2R) and angiotensin 1 receptor (AT1R). Δ Q-GRK or Control cells were transfected with (a) V2R-Halo-Tag and β arr2-NanoLuciferase (NanoLuc) fusion constructs or (b) AT1R-Nanoluc and β arr2-Halo-Tag plasmids. Additionally, GRK2 or 6, or the empty vector (EV) were co-transfected as indicated. 4 h after transfer into 96-well plates, the cells were pre-treated with 1 μ M Tolvaptan, 10 μ M Losartan, or vehicle, respectively, overnight. All washing steps prior to the measurement were conducted in presence of unchanged Tolvaptan or Losartan concentrations. The dataset is presented as mean of $n = 3$ independent experiments $\pm$ SEM in bar graphs, displaying the measured BRET-values before (baseline) and after stimulation with 1 μ M AVP, or 1 μ M AngII, respectively, normalised to the basal BRET ratio derived from the respective Δ Q-GRK + EV condition (dashed line). To test whether the baseline BRET ratios were significantly elevated compared to the respective Δ Q-GRK + EV baseline, an ANOVA and one-sided Dunnett's test was performed (** $p < 0.01$; *** $p < 0.001$; ns (not significant)). All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

Supplementary Figure 14



Supplementary Figure 14: GRK-specific β -arrestin (β arr)1 and 2 recruitment to muscarinic M2 acetylcholine receptor (M2R) with endogenous expression of or individually overexpressed GRKs. a, b The recruitment of β arr1 (a) or β arr2 (b) to the M2R was measured in presence of no GRK (Δ Q-GRK + empty vector (EV)), all endogenous GRKs (Control + EV), single overexpressed GRKs (Δ Q-GRK + GRK, data identical to Supplementary Figure 5i), with only one endogenous GRK left (Δ GRK3/5/6, Δ GRK2/5/6, Δ GRK2/3/6, Δ GRK2/3/5) or with a knockout of the GRK families (Δ GRK2/3 or Δ GRK5/6). Data are presented as Δ net BRET fold change (means of three independent experiments $\pm$ SEM). **c** In addition, the experiment using β arr2 was carried out in Δ GRK2/3 cells with or without overexpression of GRK2 that had been pre-incubated with different concentrations of cmpd101 for 10 minutes prior to stimulation with 100 μ M of acetylcholine (ACh). Data are presented as normalised Δ net BRET as means of $n = 3$ independent experiments $\pm$ SEM.

Supplementary Figure 15



Supplementary Figure 15: GRK-specific β -arrestin 2 (β arr2) recruitment phosphorylation-deficient angiotensin 1 receptor (AT1R) mutants. **a** According to the proposed inhibitory phosphorylation sites by Mayer *et al.*¹, three AT1R constructs were created carrying alanine substitutions in the C-terminus using site-directed mutagenesis. Positions of proposed inhibitory sites are highlighted in yellow, other potential phosphorylation sites in grey. **b-i** ΔQ-GRK or Control cells were transfected with indicated AT1R variants carrying C-terminal Nano luciferase (NanoLuc), Halo-Tag- β arr2 and either GRK2, 3, 5, 6, or the empty vector (EV) as indicated. Upon stimulation with Angiotensin II (AngII), dynamic BRET changes are shown as concentration-response curves. All data points are calculated as Δ net BRET fold change normalised to baseline values and vehicle control, represented as the mean of $n = 3$ independent experiments $\pm$ SEM. Data of these panels are also presented in respective bar graphs (**j-m**), displaying the mean BRET ratios $\pm$ SEM before (baseline) and after stimulation with 1 μ M AngII (stimulated), normalised to the basal BRET ratio derived from the corresponding ΔQ-GRK + EV condition. To test whether the baseline BRET ratios were significantly elevated compared to ΔQ-GRK + EV baseline, an ANOVA and one-sided Dunnett's test was performed (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Data of β arr2 recruitment to AT1R wildtype (**b, f, j**) are also presented in Supplementary Figure 5g and 6. All exact p values, test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

Supplementary Table 1: Results of the statistical analysis of each concentration-response curve presented. For each indicated condition the BRET ratio after ligand application in the highest concentration was compared to the recorded BRET ratio after vehicle stimulation using two-sided Student's t-test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Significant differences were considered as functional β -arrestin recruitment. All test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

b2AR		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	3.06E-01
	ΔQ-GRK + GRK2	8.57E-07 ***
	ΔQ-GRK + GRK3	3.10E-04 ***
	ΔQ-GRK + GRK5	7.47E-06 ***
	ΔQ-GRK + GRK6	1.45E-05 ***
	Control + EV	8.80E-03 **
βarr2	ΔQ-GRK + EV	1.46E-05 ***
	ΔQ-GRK + GRK2	3.13E-05 ***
	ΔQ-GRK + GRK3	3.28E-04 ***
	ΔQ-GRK + GRK5	3.20E-04 ***
	ΔQ-GRK + GRK6	7.87E-07 ***
	Control + EV	3.20E-09 ***
b2AR + Epinephrine		
βarr	condition	p value
βarr2	ΔQ-GRK + EV	4.04E-04 ***
	ΔQ-GRK + GRK2	8.51E-06 ***
	ΔQ-GRK + GRK3	6.08E-07 ***
	ΔQ-GRK + GRK5	6.03E-06 ***
	ΔQ-GRK + GRK6	3.38E-07 ***
	Control + EV	4.90E-05 ***
b2AR + Norpinephrine		
βarr	condition	p value
βarr2	ΔQ-GRK + EV	6.09E-04 ***
	ΔQ-GRK + GRK2	1.81E-04 ***
	ΔQ-GRK + GRK3	2.42E-05 ***
	ΔQ-GRK + GRK5	7.89E-09 ***
	ΔQ-GRK + GRK6	7.77E-09 ***
	Control + EV	9.02E-05 ***
β2V2		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	1.45E-01
	ΔQ-GRK + GRK2	3.14E-07 ***
	ΔQ-GRK + GRK3	2.96E-05 ***
	ΔQ-GRK + GRK5	5.63E-05 ***
	ΔQ-GRK + GRK6	3.78E-03 **
	Control + EV	1.66E-04 ***
βarr2	ΔQ-GRK + EV	1.13E-03 **
	ΔQ-GRK + GRK2	2.96E-06 ***
	ΔQ-GRK + GRK3	1.46E-06 ***
	ΔQ-GRK + GRK5	1.84E-05 **
	ΔQ-GRK + GRK6	4.54E-03 **
	Control + EV	1.96E-04 ***
V2R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	1.76E-04 ***
	ΔQ-GRK + GRK2	3.45E-08 ***
	ΔQ-GRK + GRK3	5.51E-07 ***
	ΔQ-GRK + GRK5	5.40E-06 ***
	ΔQ-GRK + GRK6	1.11E-04 ***
	Control + EV	2.39E-11 ***
βarr2	ΔQ-GRK + EV	1.14E-03 **
	ΔQ-GRK + GRK2	1.54E-07 **
	ΔQ-GRK + GRK3	3.87E-07 ***
	ΔQ-GRK + GRK5	1.20E-06 **
	ΔQ-GRK + GRK6	4.90E-05 ***
	Control + EV	4.38E-07 ***
βarr1 - dFLR	ΔQ-GRK + EV	9.93E-01
	ΔQ-GRK + GRK2	8.81E-01
	ΔQ-GRK + GRK3	1.25E-02 *
	ΔQ-GRK + GRK5	1.63E-01
	ΔQ-GRK + GRK6	8.26E-01
	Control + EV	3.34E-03 **
βarr2 - dFLR	ΔQ-GRK + EV	8.86E-02
	ΔQ-GRK + GRK2	3.52E-01
	ΔQ-GRK + GRK3	9.03E-01
	ΔQ-GRK + GRK5	7.29E-01
	ΔQ-GRK + GRK6	7.64E-01
	Control + EV	5.39E-02
βarr1	ΔQ-GRK + GRK2-KD	7.45E-05 ***
	ΔQ-GRK + GRK6-KD	1.39E-05 ***
MOP		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	4.40E-02 *
	ΔQ-GRK + GRK2	1.48E-04 ***
	ΔQ-GRK + GRK3	6.73E-05 ***
	ΔQ-GRK + GRK5	4.99E-02 *
	ΔQ-GRK + GRK6	3.86E-01
	Control + EV	9.38E-01
βarr2	ΔQ-GRK + EV	9.50E-01
	ΔQ-GRK + GRK2	2.11E-05 ***
	ΔQ-GRK + GRK3	1.17E-05 ***
	ΔQ-GRK + GRK5	4.76E-02 *
	ΔQ-GRK + GRK6	1.80E-01
	Control + EV	7.20E-02
M1R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	2.28E-01
	ΔQ-GRK + GRK2	1.61E-05 ***
	ΔQ-GRK + GRK3	6.74E-05 ***
	ΔQ-GRK + GRK5	7.13E-05 ***
	ΔQ-GRK + GRK6	3.12E-02 *
	Control + EV	7.58E-03 **

M1R		
βarr	condition	p value
βarr2	ΔQ-GRK + EV	1.26E-01
	ΔQ-GRK + GRK2	1.49E-04 ***
	ΔQ-GRK + GRK3	4.19E-05 ***
	ΔQ-GRK + GRK5	5.44E-05 ***
	ΔQ-GRK + GRK6	1.02E-03 **
	Control + EV	5.53E-05 ***
M2R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	3.06E-01
	ΔQ-GRK + GRK2	7.08E-03 **
	ΔQ-GRK + GRK3	6.21E-07 **
	ΔQ-GRK + GRK5	6.08E-01
	ΔQ-GRK + GRK6	3.32E-01
	Control + EV	4.60E-01
βarr2	ΔQ-GRK + EV	2.99E-05 ***
	ΔQ-GRK + GRK2	3.24E-05 ***
	ΔQ-GRK + GRK3	9.80E-08 ***
	ΔQ-GRK + GRK5	1.61E-01
	ΔQ-GRK + GRK6	3.37E-02 *
	Control + EV	1.06E-02 *
βarr1	ΔGRK3/5/6	2.53E-01
	ΔGRK2/5/6	3.29E-01
	ΔGRK2/3/6	7.45E-01
	ΔGRK2/3/5	4.41E-01
	ΔGRK5/6	5.70E-01
	ΔGRK2/3	2.90E-01
βarr2	ΔGRK3/5/6	3.76E-03 **
	ΔGRK2/5/6	8.87E-03 **
	ΔGRK2/3/6	6.28E-03 **
	ΔGRK2/3/5	6.00E-04 ***
	ΔGRK5/6	4.02E-01
	ΔGRK2/3	3.01E-03 **
M3R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	1.43E-02 *
	ΔQ-GRK + GRK2	1.71E-04 **
	ΔQ-GRK + GRK3	3.54E-03 **
	ΔQ-GRK + GRK5	1.42E-03 **
	ΔQ-GRK + GRK6	2.45E-02 *
	Control + EV	9.20E-04 ***
βarr2	ΔQ-GRK + EV	8.03E-05 ***
	ΔQ-GRK + GRK2	1.99E-07 ***
	ΔQ-GRK + GRK3	1.47E-06 ***
	ΔQ-GRK + GRK5	1.04E-07 ***
	ΔQ-GRK + GRK6	1.29E-08 ***
	Control + EV	1.34E-07 ***
M4R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	7.82E-02
	ΔQ-GRK + GRK2	1.51E-05 ***
	ΔQ-GRK + GRK3	1.49E-08 ***
	ΔQ-GRK + GRK5	2.66E-01
	ΔQ-GRK + GRK6	5.22E-02
	Control + EV	6.88E-01
βarr2	ΔQ-GRK + EV	6.80E-03 **
	ΔQ-GRK + GRK2	1.68E-05 ***
	ΔQ-GRK + GRK3	4.57E-06 ***
	ΔQ-GRK + GRK5	4.44E-03 **
	ΔQ-GRK + GRK6	4.29E-03 **
	Control + EV	1.60E-02 *
M5R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	1.32E-01
	ΔQ-GRK + GRK2	4.84E-05 ***
	ΔQ-GRK + GRK3	6.51E-06 ***
	ΔQ-GRK + GRK5	4.60E-02 *
	ΔQ-GRK + GRK6	7.27E-03 **
	Control + EV	1.33E-04 **
βarr2	ΔQ-GRK + EV	5.00E-03 **
	ΔQ-GRK + GRK2	2.34E-07 ***
	ΔQ-GRK + GRK3	8.03E-06 **
	ΔQ-GRK + GRK5	3.88E-02 *
	ΔQ-GRK + GRK6	2.94E-03 **
	Control + EV	2.61E-04 ***
βarr2	ΔGRK3/5/6	1.98E-09 ***
	ΔGRK2/5/6	2.99E-05 ***
	ΔGRK2/3/6	3.20E-01
	ΔGRK2/3/5	2.29E-01
	ΔGRK5/6	1.61E-12 ***
	ΔGRK2/3	1.92E-04 ***
C5aR1		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	9.31E-03 **
	ΔQ-GRK + GRK2	1.27E-05 ***
	ΔQ-GRK + GRK3	4.46E-05 ***
	ΔQ-GRK + GRK5	1.55E-05 ***
	ΔQ-GRK + GRK6	3.87E-09 ***
	Control + EV	6.52E-05 ***
βarr2	ΔQ-GRK + EV	6.91E-08 ***
	ΔQ-GRK + GRK2	1.32E-07 ***
	ΔQ-GRK + GRK3	2.11E-07 ***
	ΔQ-GRK + GRK5	4.09E-07 ***
	ΔQ-GRK + GRK6	1.38E-06 ***
	Control + EV	5.30E-09 ***

PTH1R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	8.94E-03 **
	ΔQ-GRK + GRK2	1.58E-05 ***
	ΔQ-GRK + GRK3	1.08E-07 ***
	ΔQ-GRK + GRK5	4.39E-07 ***
	ΔQ-GRK + GRK6	2.14E-07 ***
	Control + EV	1.28E-04 ***
βarr2	ΔQ-GRK + EV	5.53E-08 ***
	ΔQ-GRK + GRK2	1.40E-05 ***
	ΔQ-GRK + GRK3	3.58E-05 ***
	ΔQ-GRK + GRK5	4.45E-08 ***
	ΔQ-GRK + GRK6	2.53E-07 ***
	Control + EV	5.35E-09 ***
βarr2	ΔQ-GRK + GRK2-YFP	2.20E-07 ***
	ΔQ-GRK + GRK3-YFP	1.04E-06 ***
	ΔQ-GRK + GRK5-YFP	3.90E-06 ***
	ΔQ-GRK + GRK5-YFP	1.76E-07 ***
βarr2	ΔGRK3/5/6	3.74E-07 ***
	ΔGRK2/5/6	2.34E-08 ***
	ΔGRK2/3/6	1.44E-04 ***
	ΔGRK2/3/5	5.82E-05 ***
	ΔGRK5/6	1.02E-03 **
	ΔGRK2/3	1.11E-03 **
AT1R		
βarr	condition	p value
βarr1	ΔQ-GRK + EV	1.21E-03 **
	ΔQ-GRK + GRK2	1.56E-05 ***
	ΔQ-GRK + GRK3	1.15E-13 ***
	ΔQ-GRK + GRK5	9.57E-06 ***
	ΔQ-GRK + GRK6	4.48E-04 ***
	Control + EV	8.71E-05 ***
βarr2	ΔQ-GRK + EV	4.55E-21 ***
	ΔQ-GRK + GRK2	9.64E-14 ***
	ΔQ-GRK + GRK3	3.76E-08 ***
	ΔQ-GRK + GRK5	7.13E-07 ***
	ΔQ-GRK + GRK6	6.81E-03 **
	Control + EV	5.56E-16 ***
βarr1 + PKC inhibitor	ΔQ-GRK + EV	9.40E-01
	ΔQ-GRK + GRK2	9.89E-03 **
	ΔQ-GRK + GRK3	1.72E-05 ***
	ΔQ-GRK + GRK5	1.58E-04 ***
	ΔQ-GRK + GRK6	1.13E-01
	Control + EV	1.88E-05 ***
βarr2 + PKC inhibitor	ΔQ-GRK + EV	2.58E-05 ***
	ΔQ-GRK + GRK2	5.15E-12 ***
	ΔQ-GRK + GRK3	6.82E-08 ***
	ΔQ-GRK + GRK5	2.61E-04 ***
	ΔQ-GRK + GRK6	1.44E-01
	Control + EV	1.53E-14 ***
βarr1 - dFLR	ΔQ-GRK + EV	9.93E-01
	ΔQ-GRK + GRK2	1.15E-03 **
	ΔQ-GRK + GRK3	2.77E-05 ***
	ΔQ-GRK + GRK5	2.59E-01
	ΔQ-GRK + GRK6	4.32E-01
	Control + EV	5.41E-04 ***
βarr2 - dFLR	ΔQ-GRK + EV	2.66E-01
	ΔQ-GRK + GRK2	2.99E-05 ***
	ΔQ-GRK + GRK3	1.45E-05 ***
	ΔQ-GRK + GRK5	5.19E-01
	ΔQ-GRK + GRK6	6.08E-01
	Control + EV	1.42E-04 ***
βarr2	ΔQ-GRK + GRK5-KD	9.73E-12 ***
	ΔQ-GRK + GRK6-KD	2.26E-10 ***
βarr2	ΔGRK2/3/6	3.52E-05 ***
	ΔGRK2/3/5	1.40E-11 ***
AT1R-S347A		
βarr	condition	p value
βarr2	ΔQ-GRK + EV	7.30E-03 **
	ΔQ-GRK + GRK2	2.91E-02 *
	ΔQ-GRK + GRK3	1.08E-02 *
	ΔQ-GRK + GRK5	4.99E-03 **
	ΔQ-GRK + GRK6	1.53E-01
	Control + EV	3.90E-05 ***
AT1R-S348A		
βarr	condition	p value
βarr2	ΔQ-GRK + EV	1.39E-03 **
	ΔQ-GRK + GRK2	2.88E-02 *
	ΔQ-GRK + GRK3	2.30E-02 *
	ΔQ-GRK + GRK5	9.31E-03 **
	ΔQ-GRK + GRK6	2.62E-02 *
	Control + EV	5.84E-07 ***
AT1R-S347A/S348A		
βarr	condition	p value
βarr2	ΔQ-GRK + EV	8.12E-03 **
	ΔQ-GRK + GRK2	7.90E-03 **
	ΔQ-GRK + GRK3	1.54E-02 *
	ΔQ-GRK + GRK5	6.10E-02
	ΔQ-GRK + GRK6	2.46E-01
	Control + EV	4.10E-02 *

Supplementary Table 2: Overview of the length (number of amino acids) of intracellular loop 3 (IL3), C-terminus (C-term), pre-dominantly coupled G α isoform^{2,3}, and class (according to Oakley *et al.*⁴) for each analysed GPCR (information from GPCRdb.org) with respective numbers of putative serine and threonine (S/T) phosphorylation sites, clusters (PPP, PXPP) and patterns (PXXPPXP, PXPXXP). While X represents any amino acid, P may be a serine, threonine, or a negatively charged amino acid (glutamic acid or aspartic acid). Phosphorylation sites and motifs were detected using Python 3.8.7. Custom code can be accessed via doi: 10.5281/zenodo.5764249.

GRK-specific β arr recruitment	class	G α	GPCR	β -arrestin	IL3							C-term						
					length	P count	P density	PPP count	PXPP count	PXPXXP count	PXXPPXP count	length	P count	P density	PPP count	PXPP count	PXPXXP count	PXXPPXP count
GRK2/3	B	G α_s	b2V2	β arr1	24	3	13%	0	0	1	0	26	11	42%	4	2	1	1
	A	G α_i	M2R	β arr1 + β arr2	152	31	20%	8	8	6	1	10	1	10%	0	0	0	0
	A	G α_i	M4R	β arr1 + β arr2	156	24	15%	5	4	3	4	8	1	13%	0	0	0	0
	A	G α_q	M5R	β arr1 + β arr2	213	40	19%	14	5	4	8	21	1	5%	0	0	0	0
	A	G α_i	MOP	β arr1 + β arr2	5	1	20%	0	0	0	0	46	11	24%	1	0	2	1
GRK2/3/5/6	A	G α_s	b2AR	β arr1 + β arr2	24	3	13%	0	0	1	0	72	13	18%	1	1	0	0
	B	G α_i	C5aR1	β arr1	5	2	40%	0	0	0	0	37	11	30%	2	2	1	1
	A	G α_q	M3R	β arr2	211	51	24%	13	5	9	4	29	1	3%	0	0	0	0
	B	G α_s	PTH1R	β arr1 + β arr2	8	1	13%	0	0	0	0	105	21	20%	3	5	2	1
not significant	B	G α_s	b2V2	β arr2	24	3	13%	0	0	1	0	26	11	42%	4	2	1	1
	B	G α_i	C5aR1	β arr2	5	2	40%	0	0	0	0	37	11	30%	2	2	1	1
	A	G α_q	M1R	β arr1 + β arr2	128	22	17%	12	4	3	3	25	3	12%	0	0	0	0
	A	G α_q	M3R	β arr1	211	51	24%	13	5	9	4	29	1	3%	0	0	0	0
pre-coupling	B	G α_q	AT1R	β arr1 + β arr2	3	0	0%	0	0	0	0	39	12	31%	2	1	1	2
	B	G α_s	V2R	β arr1 + β arr2	16	2	13%	0	0	0	0	26	11	42%	4	2	1	1

min  max

Supplementary Table 3: Multiple comparisons of GRK-specific β -arrestin recruitment. The p values presented in this table were used to generate the heatmap in Figure 3 i. BRET fold changes at saturating ligand concentrations of at least $n = 3$ independent experiments were compared using ANOVA and two-sided Bonferroni's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). All test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

GPCR	β arr	ΔQ -GRK vs. GRK2		ΔQ -GRK vs. GRK3		ΔQ -GRK vs. GRK5		ΔQ -GRK vs. GRK6		ΔQ -GRK vs. Control	
		non adj.	adj.	non adj.	adj.	non adj.	adj.	non adj.	adj.	non adj.	adj.
AT1R	β arr1	0,004	0,040 *	0,006	0,050	0,687	1,000	0,618	1,000	0,001	0,008 **
AT1R	β arr2	0,155	1,000	0,117	1,000	0,000	0,000 ***	0,000	0,000 ***	0,002	0,015 *
b2AR	β arr1	0,024	0,220	0,005	0,042 *	0,011	0,098	0,026	0,230	0,010	0,088
b2AR	β arr2	0,003	0,031 *	0,004	0,034 *	0,017	0,155	0,009	0,079	0,086	0,770
b2V2	β arr1	0,000	0,002 **	0,000	0,001 ***	0,089	0,801	0,619	1,000	0,078	0,704
b2V2	β arr2	0,064	0,573	0,064	0,572	0,271	1,000	0,570	1,000	0,216	1,000
C5aR1	β arr1	0,000	0,001 **	0,000	0,000 ***	0,000	0,002 **	0,007	0,064	0,000	0,000 ***
C5aR1	β arr2	0,180	1,000	0,318	1,000	0,367	1,000	0,870	1,000	0,063	0,566
M1R	β arr1	0,079	0,713	0,149	1,000	0,001	0,013 *	0,175	1,000	0,360	1,000
M1R	β arr2	0,073	0,658	0,399	1,000	0,368	1,000	0,030	0,273	0,081	0,731
M2R	β arr1	0,030	0,274	0,005	0,043 *	0,827	1,000	0,882	1,000	0,700	1,000
M2R	β arr2	0,018	0,158	0,003	0,029 *	0,553	1,000	0,492	1,000	0,649	1,000
M3R	β arr1	0,050	0,447	0,825	1,000	0,561	1,000	0,209	1,000	0,641	1,000
M3R	β arr2	0,000	0,004 **	0,001	0,007 **	0,167	1,000	0,008	0,068	0,001	0,005 **
M4R	β arr1	0,092	0,828	0,002	0,014 *	0,653	1,000	0,958	1,000	0,138	1,000
M4R	β arr2	0,007	0,066	0,000	0,002 **	0,818	1,000	0,650	1,000	0,873	1,000
M5R	β arr1	0,001	0,009 **	0,000	0,000 ***	0,977	1,000	0,929	1,000	0,000	0,000 ***
M5R	β arr2	0,000	0,000 ***	0,000	0,000 ***	0,903	1,000	0,710	1,000	0,000	0,004 **
MOP	β arr1	0,079	0,707	0,141	1,000	0,633	1,000	0,456	1,000	0,169	1,000
MOP	β arr2	0,017	0,152	0,004	0,035 *	0,540	1,000	0,922	1,000	0,510	1,000
PTHR	β arr1	0,009	0,082	0,000	0,002 **	0,015	0,134	0,004	0,039 *	0,001	0,008 **
PTHR	β arr2	0,001	0,007 **	0,002	0,016 *	0,005	0,046 *	0,005	0,048 *	0,000	0,000 ***
V2R	β arr1	0,894	1,000	0,480	1,000	0,740	1,000	0,029	0,260	0,000	0,001 ***
V2R	β arr2	0,236	1,000	0,125	1,000	0,105	0,946	0,031	0,278	0,005	0,044 *

GPCR	β arr	Control vs. GRK2		Control vs. GRK3		Control vs. GRK5		Control vs. GRK6	
		non adj.	adj.	non adj.	adj.	non adj.	adj.	non adj.	adj.
AT1R	β arr1	0,400	1,000	0,340	1,000	0,002	0,017 *	0,000	0,003 **
AT1R	β arr2	0,326	1,000	0,406	1,000	0,000	0,000 ***	0,000	0,000 ***
b2AR	β arr1	0,448	1,000	0,966	1,000	0,709	1,000	0,435	1,000
b2AR	β arr2	0,105	0,948	0,115	1,000	0,392	1,000	0,235	1,000
b2V2	β arr1	0,006	0,053	0,002	0,022 *	0,941	1,000	0,183	1,000
b2V2	β arr2	0,476	1,000	0,475	1,000	0,881	1,000	0,083	0,747
C5aR1	β arr1	0,243	1,000	0,963	1,000	0,145	1,000	0,004	0,035 *
C5aR1	β arr2	0,543	1,000	0,333	1,000	0,288	1,000	0,047	0,420
M1R	β arr1	0,442	1,000	0,652	1,000	0,020	0,179	0,717	1,000
M1R	β arr2	0,953	1,000	0,324	1,000	0,352	1,000	0,591	1,000
M2R	β arr1	0,015	0,132	0,002	0,021 *	0,867	1,000	0,812	1,000
M2R	β arr2	0,007	0,062	0,001	0,010 **	0,889	1,000	0,813	1,000
M3R	β arr1	0,120	1,000	0,806	1,000	0,301	1,000	0,092	0,832
M3R	β arr2	0,899	1,000	0,829	1,000	0,008	0,071	0,172	1,000
M4R	β arr1	0,004	0,035 *	0,000	0,001 ***	0,338	1,000	0,151	1,000
M4R	β arr2	0,010	0,089	0,000	0,002 **	0,943	1,000	0,768	1,000
M5R	β arr1	0,041	0,366	0,349	1,000	0,000	0,000 ***	0,000	0,000 ***
M5R	β arr2	0,187	1,000	0,086	0,772	0,000	0,004 **	0,001	0,009 **
MOP	β arr1	0,005	0,049 *	0,010	0,093	0,350	1,000	0,502	1,000
MOP	β arr2	0,058	0,525	0,014	0,122	0,962	1,000	0,573	1,000
PTHR	β arr1	0,222	1,000	0,472	1,000	0,147	1,000	0,396	1,000
PTHR	β arr2	0,039	0,351	0,017	0,149	0,006	0,052	0,006	0,050 *
V2R	β arr1	0,000	0,001 ***	0,000	0,002 **	0,000	0,000 ***	0,000	0,000 ***
V2R	β arr2	0,001	0,005 **	0,000	0,002 **	0,000	0,002 **	0,000	0,001 ***

Supplementary Table 4: Complete results of statistical analysis of BRET ratios shown in Figure 5 and Supplementary Figure 11 c-h. Statistical significance was determined by two-way mixed model ANOVA followed by two-sided paired t-test comparing stimulated BRET ratios to their corresponding baseline as well as two-sided Tukey's test comparing baselines or stimulated BRET ratios between each other (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). All test statistics, effect sizes, confidence intervals, and degrees of freedom are provided in the Source Data files.

comparing baseline and stimulated				
condition	β -arrestin	+ β arr	+ β arr-dFLR	+ β arr + PKC inhibitor
Control + EV	β arr1	0.00444 **	0.00829 **	0.00300 **
Δ Q-GRK + EV	β arr1	0.00469 **	0.36109	0.57302
Δ Q-GRK + GRK2	β arr1	0.00791 **	0.00185 **	0.00744 **
Δ Q-GRK + GRK3	β arr1	0.00171 **	0.01415 *	0.00712 **
Δ Q-GRK + GRK5	β arr1	0.00395 **	0.00407 **	0.01342 *
Δ Q-GRK + GRK6	β arr1	0.01012 *	0.45936	0.00920 **
Control + EV	β arr2	0.00859 **	0.09450	0.00030 ***
Δ Q-GRK + EV	β arr2	0.00033 ***	0.86111	0.00981 **
Δ Q-GRK + GRK2	β arr2	0.00469 **	0.05126	0.00141 **
Δ Q-GRK + GRK3	β arr2	0.00601 **	0.05231	0.00347 **
Δ Q-GRK + GRK5	β arr2	0.04058 *	0.01303 *	0.02003 *
Δ Q-GRK + GRK6	β arr2	0.01930 *	0.34421	0.10205

comparing baseline and baseline				
condition	β -arrestin	β arr vs. β arr-dFLR	β arr vs. β arr + PKC inhibitor	β arr-dFLR vs. β arr + PKC inhibitor
Control + EV	β arr1	0.93184	0.62262	0.82231
Δ Q-GRK + EV	β arr1	1.00000	1.00000	1.00000
Δ Q-GRK + GRK2	β arr1	0.45148	0.51739	0.99077
Δ Q-GRK + GRK3	β arr1	0.50462	0.14492	0.57972
Δ Q-GRK + GRK5	β arr1	0.18168	0.69820	0.06282
Δ Q-GRK + GRK6	β arr1	0.03082 *	0.02745 *	0.00092 ***
Control + EV	β arr2	0.23070	0.50580	0.78438
Δ Q-GRK + EV	β arr2	1.00000	1.00000	1.00000
Δ Q-GRK + GRK2	β arr2	0.01233 *	0.00609 **	0.76853
Δ Q-GRK + GRK3	β arr2	0.00600 **	0.00945 **	0.92324
Δ Q-GRK + GRK5	β arr2	0.00269 **	0.00002 ****	0.00000 ****
Δ Q-GRK + GRK6	β arr2	0.11392	0.05662	0.84639

comparing stimulated and stimulated				
condition	β -arrestin	β arr vs. β arr-dFLR	β arr vs. β arr + PKC inhibitor	β arr-dFLR vs. β arr + PKC inhibitor
Control + EV	β arr1	0.00003 ****	0.00002 ***	0.32708
Δ Q-GRK + EV	β arr1	0.17696	0.11616	0.94195
Δ Q-GRK + GRK2	β arr1	0.00051 ***	0.00014 **	0.50894
Δ Q-GRK + GRK3	β arr1	0.00006 ****	0.00004 ***	0.18578
Δ Q-GRK + GRK5	β arr1	0.56456	0.01106	0.41309
Δ Q-GRK + GRK6	β arr1	0.99380	0.00593 **	0.00694 **
Control + EV	β arr2	0.03493 *	0.12697	0.58481
Δ Q-GRK + EV	β arr2	0.10554	0.33143	0.65260
Δ Q-GRK + GRK2	β arr2	0.00629 **	0.00779 **	0.97985
Δ Q-GRK + GRK3	β arr2	0.01525 *	0.01205 *	0.98205
Δ Q-GRK + GRK5	β arr2	0.09398	0.00124 **	0.01643 *
Δ Q-GRK + GRK6	β arr2	0.00648 **	0.01708 *	0.67218

Supplementary Table 5: gRNA sequences for targeting different GRKs. Complementary forward and reverse oligos were annealed and ligated into the BsmBI-restricted lentiCRISPR v2 vector⁵ (Addgene #52961). gRNA sequence is shown in capital letters, overhangs for ligation in lowercase letters. Sequences of gRNAs were validated in Sanjana *et al.*⁵

		Oligo forward	Oligo reverse
GRK2	1	caccg CTTGACTCATACATCATGA	aaac TCATGATGTATGAGTCGAAGc
	2	caccgCTCAGTGGCACTCTTCGAGA	aaac TCTCGAAGAGTGCCACTGAGc
	3	caccgCGCCGCCAAGATGGCGGACC	aaacGGTCCGCCATCTTGGCGGCGc
	4	caccgGCACACACCTGGAAGAGATC	aaacGATCTCTTCCAGGTGTGTGCc
GRK3	1	caccgTTATGATGCCTACATCATGA	aaacTCATGATGTAGGCATCATAAc
	2	caccgCTGATTGAAAATCTTGTCAA	aaacTTGACAAGATTTTCAATCAGc
	3	caccgGCTCGCCAACATGGCGGACC	aaacGGTCCGCCATGTTGGCGAGCc
	4	caccgTTATTTTATTTCTCTCTGCA	aaacTGCAGAGAGAAATGAAATAAc
GRK5	1	caccgCGGAAAAGCAGCCTCCCGAT	aaacATCGGGAGGCTGCTTTTCCGc
	2	caccgGTTCTCCCCTCAGGTACTCG	aaacCGAGTACCTGAGGGGAGAACc
	3	caccgAGAACCATTCCACGAATATC	aaacGATATTCGTGGAATGGTTCTc
	4	caccgGCCAGTGTGAAGACCTCCGA	aaacTCGGAGGTCTTCACACTGGCc
GRK6	1	caccgCGGCAAAAGGGGCCACGCTC	aaacGAGCGTGGCCCCCTTTTGCCGc
	2	caccgCATGCCTTCGCTTGTCATC	aaacGATGACAAGCGGAAGGCATGc
	3	caccgATGACAAGCGGAAGGCATGT	aaacACATGCCTTCGCTTGTCATc
	4	caccgCAATACCGAGTCCTGGGCAA	aaacTTGCCCAGGACTCGGTATTGc

Supplementary References

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