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Supplementary Information for

Exploring the signaling space of a GPCR using bivalent ligands with a rigid oligoproline backbone

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This PDF file includes:

Supplementary Figure 1: Synthesis and characterization of ligands

Agonists

Monovalent agonists were published in:

Dobitz, S., Wilhelm, P., Romantini, N., De Foresta, M., Walther, C., Ritler, A., Schibli, R., Berger, P., Deupi, X., Béhé, M. and Wennemers, H., **2020**. Distance-Dependent Cellular Uptake of Oligoproline-Based Homobivalent Ligands Targeting GPCRs—An Experimental and Computational Analysis. *Bioconjugate Chemistry*, *31*(10), pp.2431-2438.

Divalent agonists

AG-2-BBN-1 agonist/ agonist 10 Å

AG-2-BBN-2 agonist/ agonist 20 Å

AG-2-BBN-3 agonist/ agonist 30 Å

published in:

Dobitz, S., Wilhelm, P., Romantini, N., De Foresta, M., Walther, C., Ritler, A., Schibli, R., Berger, P., Deupi, X., Béhé, M. and Wennemers, H., **2020**. Distance-Dependent Cellular Uptake of Oligoproline-Based Homobivalent Ligands Targeting GPCRs—An Experimental and Computational Analysis. *Bioconjugate Chemistry*, *31*(10), pp.2431-2438.

Antagonists

Monovalent antagonists are unpublished (characterization is in the following pages)

Divalent ligands:

AN-2-BBN-1 antagonist/ antagonist 10 Å

AN-2-BBN-2 antagonist/ antagonist 20 Å

AN-2-BBN-3 antagonist/ antagonist 30 Å

Of these 3 compounds, only the second one **AN-2-BBN-2** antagonist/ antagonist 20 Å was published under the name ligand **8** in:

Kroll, C., Mansi, R., Braun, F., Dobitz, S., Maecke, H.R. and Wennemers, H., 2013. Hybrid bombesin analogues: combining an agonist and an antagonist in defined distances for optimized tumor targeting. *Journal of the American Chemical Society*, *135*(45), pp.16793-16796.

The 10 Å and the 30 Å are unpublished (characterization is in the following pages).

Hybrids

H-2-BBN-1 agonist/ antagonist 10 Å

H-2-BBN-2 agonist/ antagonist 20 Å

H-2-BBN-3 agonist/ antagonist 30 Å

Hybrid ligands were all published in:

Kroll, C., Mansi, R., Braun, F., Dobitz, S., Maecke, H.R. and Wennemers, H., 2013. Hybrid bombesin analogues: combining an agonist and an antagonist in defined distances for optimized tumor targeting. *Journal of the American Chemical Society*, *135*(45), pp.16793-16796.

Synthesis and characterization of the unpublished ligands

All novel molecules were synthesized by manual solid phase peptide synthesis (SPPS).

Protocol A: Resin Swelling

The resin was agitated in CH_2Cl_2 for 15 min at rt. Afterwards, the resin was washed with DMF (5x) and CH_2Cl_2 (5x).

Protocol B: Fmoc Deprotection

20% *v/v* piperidine in DMF were added to the resin and the mixture was agitated for 5 min at rt, drained, and the procedure was repeated for another 15 min at rt. Then, the resin was washed with DMF (5x) and CH_2Cl_2 (5x).

Protocol C: Peptide Coupling

A mixture of Fmoc-AA-OH (3 equiv.), HATU (3 equiv.), and Hünig's base (6 equiv.) in DMF was agitated for 2 min at rt. Then, the mixture was added to the deprotected resin and agitated for 1.5 h at rt. Afterwards, the resin was washed with DMF (5x) and CH_2Cl_2 (5x). Reaction completion was monitored *via* Kaiser or Chloranil test. In case of incomplete reactions, double couplings followed by acylation were performed.

Protocol D: Coupling of Propiolic Acid

The resin was thoroughly washed with CH_2Cl_2 (5x). Then, a mixture of propiolic acid (3 equiv.) and EEDQ (3 equiv.) in CH_2Cl_2 was added to the deprotected resin and agitated for 1.5 h at rt. Afterwards, the resin was washed with CH_2Cl_2 (10x). The procedure was repeated for another 1.5 h at rt.

Protocol E: Coupling of Proline-Based Trimers

A mixture of the Fmoc-protected trimer (3 equiv.), HATU (3 equiv.), and Hünig's base (6 equiv.) in DMF was agitated for 2 min at rt. Then, the mixture was added to the deprotected resin and agitated for 25 min at 75 °C in the microwave. Afterwards, the resin was washed with DMF (5x) and CH_2Cl_2 (5x). Reaction completion was monitored *via* Kaiser or Chloranil test. In case of incomplete reactions, double couplings followed by acylation were performed.

Protocol F: Click Chemistry on Solid Support

Prior to the reaction, the resin was dried under high vacuum for 1 h. Under Ar atmosphere, a mixture of [Cu(MeCN)4]PF6 (1 equiv.), TBTA (1 equiv.), and the corresponding alkyne (2 equiv.) in degassed NMP/DMSO (10:1) was added to the dried resin. The resulting suspension was agitated in the microwave for 2 h at 80 °C. Afterwards, the resin was washed with a mixture of sodium diethyldithiocarbamate (0.5% *w/v*) and Hünig's base (0.5% *v/v*) in DMF until it became almost colourless again (agitation for 2 min). Subsequently, the resin was washed with DMF (5x) and CH_2Cl_2 (5x). Reaction completion was monitored *via Protocol M*.

Protocol G: Cleavage from Resin and Global Deprotection

The resin was thoroughly washed with CH_2Cl_2 (5x). Then, the resin was agitated with a mixture of TFA/phenol/anisole/H_2O (85:5:5:5) for 4 h at rt. Afterwards, the mixture was filtered and two-thirds of the liquid were removed *in vacuo*. The reduced mixture was poured over ice-cold ether. The resulting suspension was centrifuged, decanted, solubilized in MeCN/milliQ-H_2O (1:1), and lyophilized. After lyophilization, the peptides were subsequently purified *via* RP-HPLC with different gradients.

Protocol H: Cleavage of Side Chain Protected Peptide Fragments from Resin

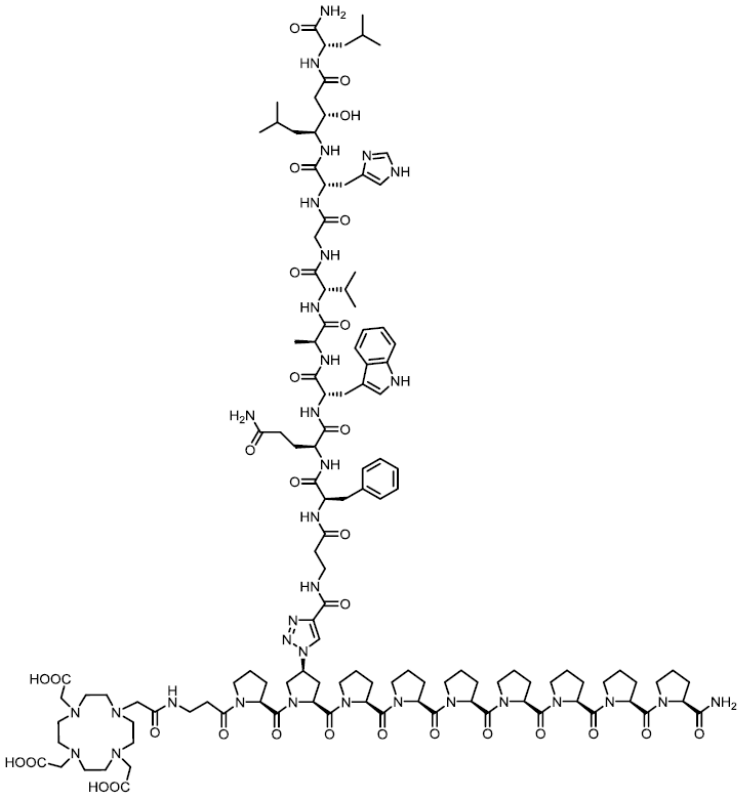
This protocol was used to obtain side chain protected peptide fragments through mild cleavage from Sieber amide. The resin was thoroughly washed with CH_2Cl_2 (5x). Then, two solutions were prepared: (i) the neutralization solution contained 10% *v/v* pyridine in MeOH (5 equiv., about 2.2 mL per 10 mL of cleavage solution) and (ii) the cleavage solution contained 1% *v/v* TFA in CH_2Cl_2 (1.5 mL/100 mg resin). The cleavage solution was added to the resin and agitated for 1.5 min at rt. Then, the cleavage solution was drained into the neutralization solution. This procedure was repeated 9x. Afterwards, two-thirds of the mixture were removed *in vacuo*. The reduced mixture was poured over ice-cold milliQ-H_2O. The resulting suspension was centrifuged, decanted, and re-suspended in ice-cold milliQ-H_2O. This procedure was repeated 2x. Then, the peptide was suspended in MeCN/milliQ-H_2O (1:1) and lyophilized. After lyophilization, the crude peptides were used for click chemistry on solid support without further purification.

Protocol I: Test Cleavage

A few resin beads were thoroughly washed with CH_2Cl_2 (5x). Then, the resin beads were agitated with a mixture of TFA/TIS/H_2O (90:5:5) for 45 min at rt. Afterwards, the mixture was filtered and poured over ice-cold ether. The resulting suspension was centrifuged, decanted, solubilized in MeCN/milliQ-H_2O (1:1), and subsequently subjected to LC-ESI-MS analysis.

All ligands were purified by RP-HPLC (Dionex UHPLC, Ultimate 3000) using a Reprosil Gold 120 C18, 10 µm, 150 × 16 mm column with a flow of 6 mL/min at 50 °C. Appropriate gradients of MeCN (solvent A) and a mixture of Milli-Q-H_2O/ MeCN/TFA (1000:10:1, solvent B) were used. High resolution mass spectrometry on Bruker solariX ESI/MALDI-FTICRMS instrument was used to verify the synthesized peptides. For HR-MALDI-MS, α-cyano-4-hydroxycinnamic acid was used as the matrix. The purity was determined by reversed phase UHPLC on an Ultimate 3000 from Dionex equipped with either ReproSil Gold 120 C18 (5 µm, 150 × 4 mm column with a flow of 1 mL/min at 50 °C using an appropriate gradient of MeCN (solvent A) and a mixture of Milli-Q-H_2O/MeCN/TFA (1000:10:1, solvent B). Peptide purities are listed in the SI as purity determined by analytical RP-HPLC (%) using UV detection corresponding to the percentage of desired peptide in relation to the total amount of material absorbing at λ = 214 nm.

Monovalent Antagonist **AN-1-BBN-1**

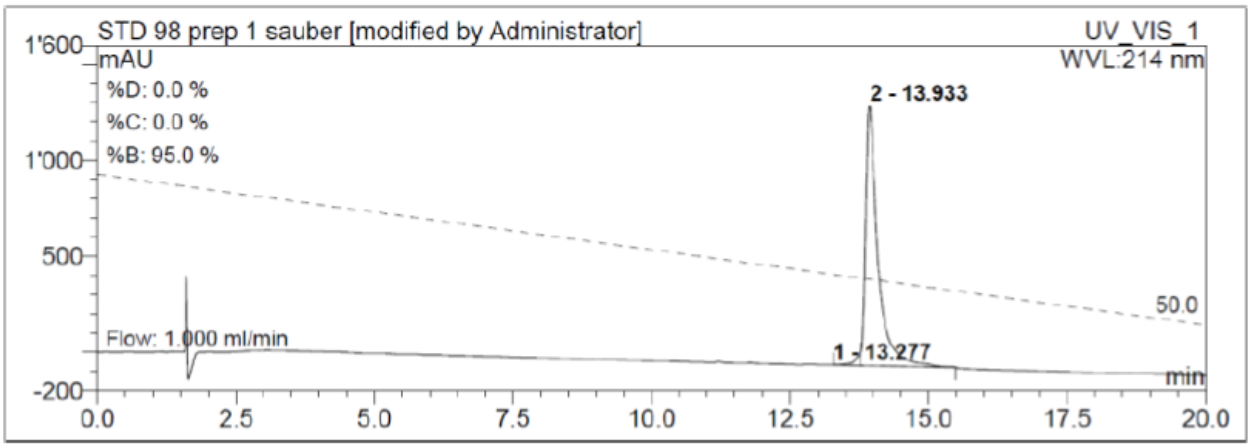


The peptide was synthesized on 200 mg Rink amide ChemMatrix® resin according to *Protocol A*, *B*, *C*, *D*, *E*, *F*, *G, H* and *I* affording 13.9 mg of white solid.

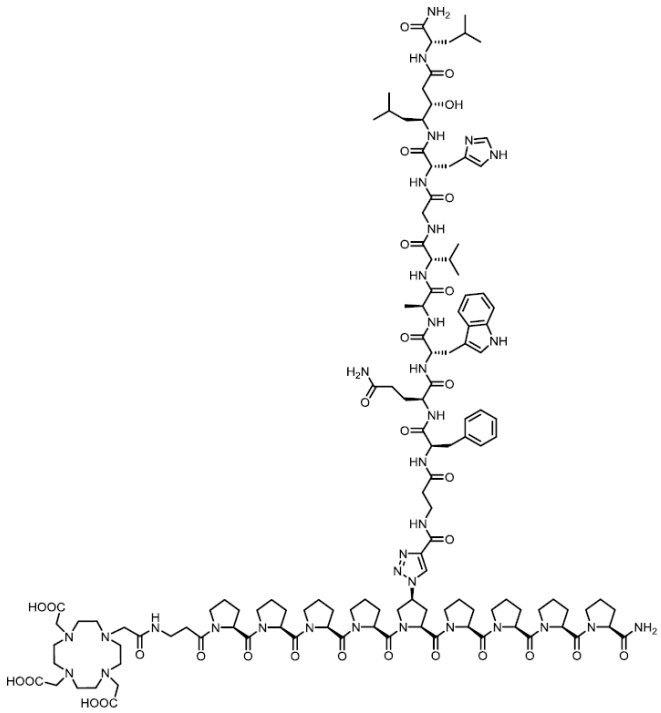
HR-MS (MALDI): m/z: calcd for $C_{125}H_{182}N_{33}O_{30}^{+}$ $[M+H]^{+}$ 2625.3725, found 2625.3743; calcd for $C_{125}H_{181}N_{33}NaO_{30}^{+}$ [M+Na]+ 2647.3544, found 2647.3564.

Analytical HPLC: Reprosil Gold C18, 5 µm, (150x4) mm, (95-50)% B in 20 min, 1.0 mL/min, 50 °C

Rt = 13.933 min, purity: 98%



Monovalent Antagonist **AN-1-BBN-2**

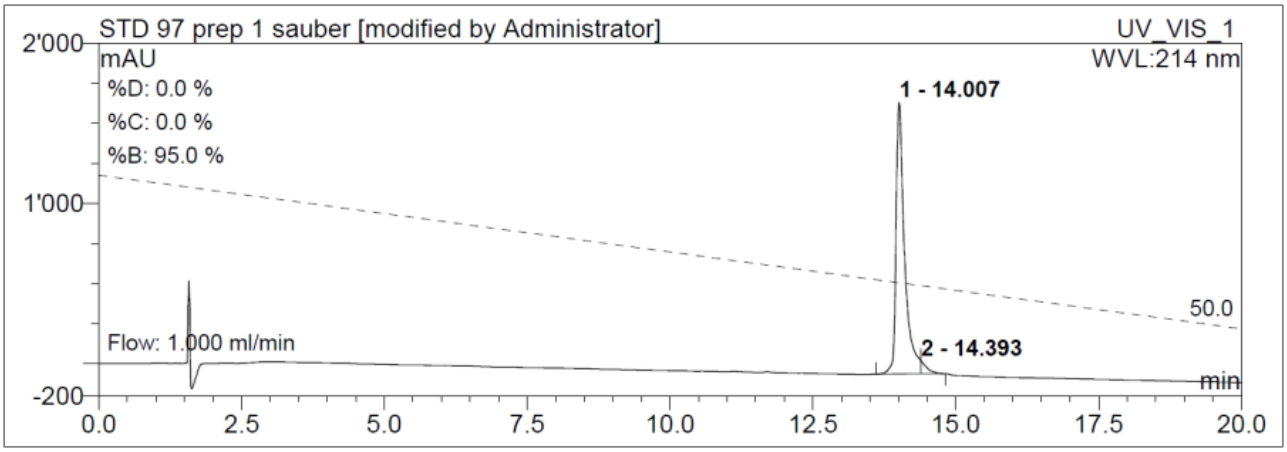


The peptide was synthesized on 200 mg Rink amide ChemMatrix® resin according to *Protocol A*, *B*, *C*, *D*, *E*, *F*, *G, H* and *I* affording 11.5 mg of white solid.

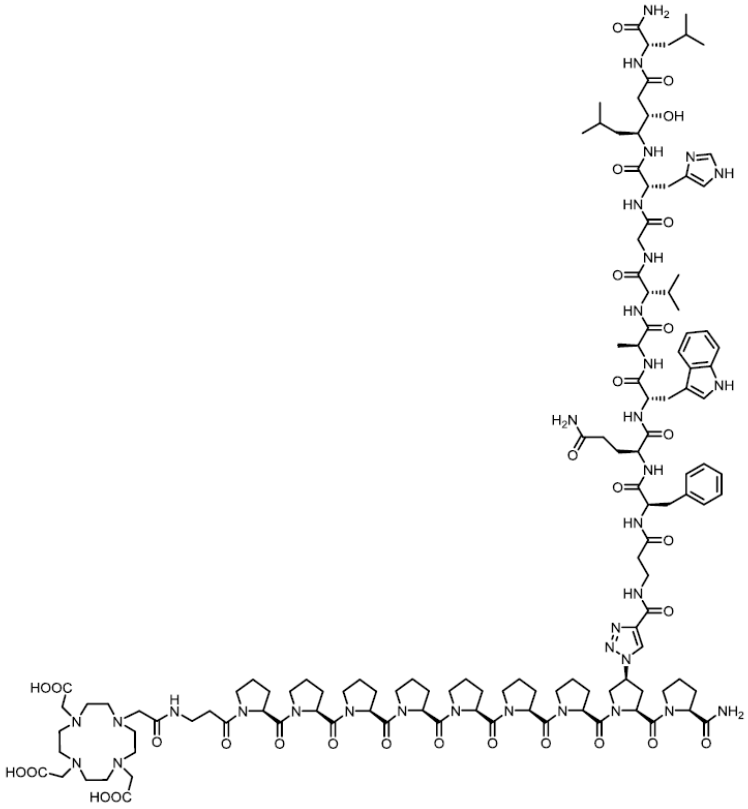
HR-MS (MALDI): m/z: calcd for $C_{125}H_{182}N_{33}O_{30}^{+}$ $[M+H]^{+}$ 2625.3725, found 2625.3725; calcd for $C_{125}H_{181}N_{33}NaO_{30}^{+}$ $[M+Na]^{+}$ 2647.3544, found 2647.3551.

Analytical HPLC: Reprosil Gold C18, 5 µm, (150x4) mm, (95-50)% B in 20 min, 1.0 mL/min, 50 °C

Rt = 14.007 min, purity: 97%



Monovalent Antagonist **AN-1-BBN-3**

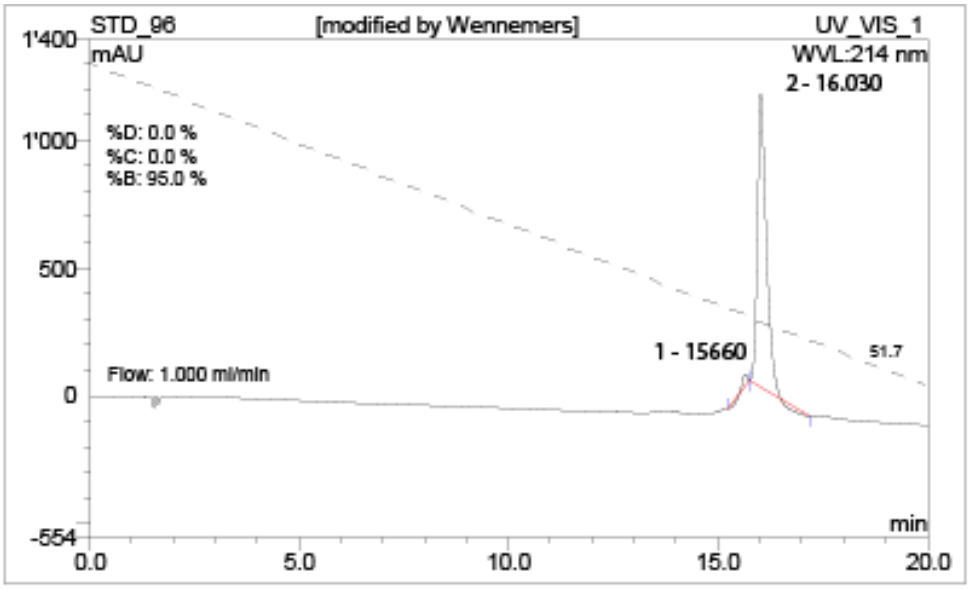


The peptide was synthesized on 200 mg Rink amide ChemMatrix® resin according to *Protocol A*, *B*, *C*, *D*, *E*, *F*, *G, H* and *I* affording 16.0 mg of white solid.

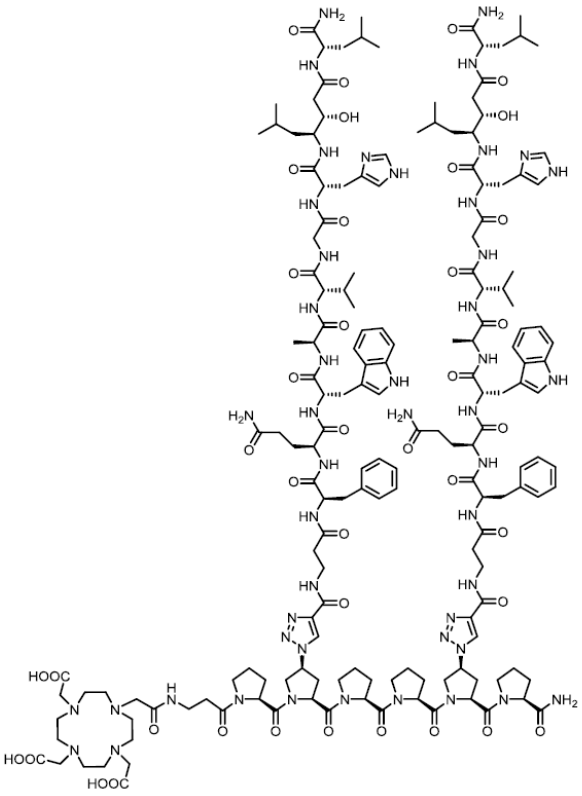
HR-MS (MALDI): m/z: calcd for $C_{125}H_{182}N_{33}O_{30}^{+}$ $[M+H]^{+}$ 2625.3725, found 2625.3725; calcd for $C_{125}H_{181}N_{33}NaO_{30}^{+}$ $[M+Na]^{+}$ 2647.3544, found 2647.3553.

Analytical HPLC: Reprosil Gold C18, 5 µm, (150x4) mm, (95-50)% B in 20 min, 1.0 mL/min, 50 °C

Rt = 16.030 min, purity: 97%



10 Å Homodivalent Antagonist **AN-2-BBN-1**

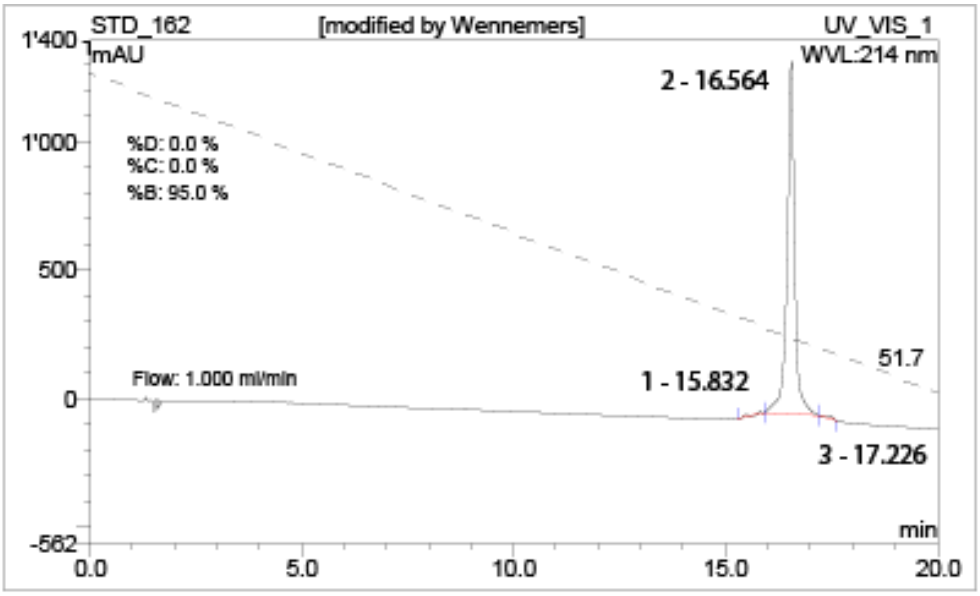


The peptide was synthesized on 200 mg Rink amide ChemMatrix® resin according to *Protocol A*, *B*, *C*, *D*, *E*, *F*, *G, H* and *I* affording 5.9 mg of white solid. The analytical data of the ligand were in line with the literature.213

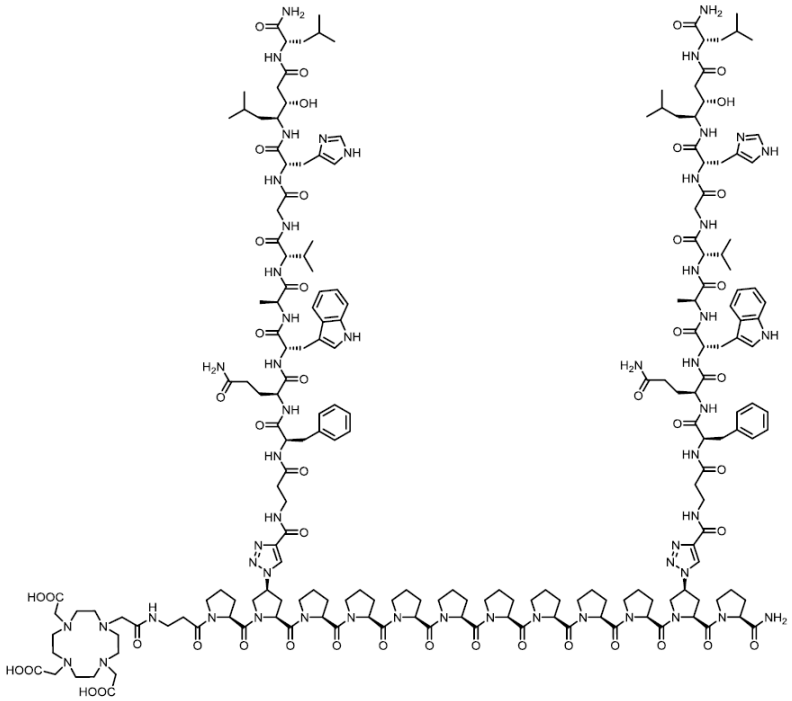
HR-MS (MALDI): m/z: calcd for $C_{171}H_{245}N_{48}O_{40}^{+}$ $[M+H]^{+}$ 3610.8607, found 3610.8469; calcd for $C_{171}H_{244}N_{48}NaO_{40}^{+}$ $[M+Na]^{+}$ 3632.8427, found 3632.8307.

Analytical HPLC: Reprosil Gold C18, 5 µm, (150x4) mm, (95-40)% B in 20 min, 1.0 mL/min, 50 °C

Rt = 16.564 min, purity: 98%



30 Å Homodivalent Antagonist **AN-2-BBN-3**

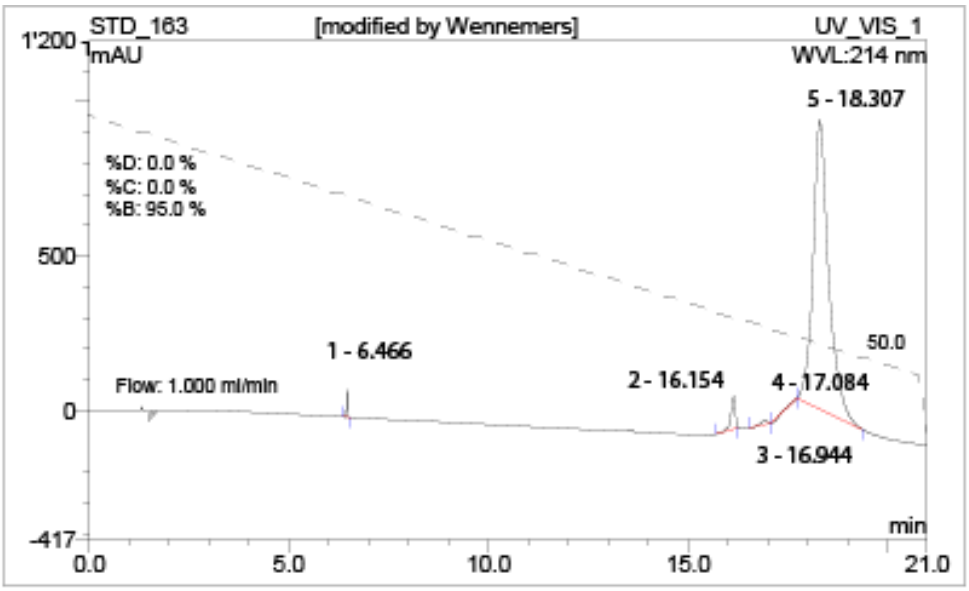


The peptide was synthesized on 200 mg Rink amide ChemMatrix® resin according to *Protocol A*, *B*, *C*, *D*, *E*, *F*, *G, H* and *I* affording 6.7 mg of white solid.

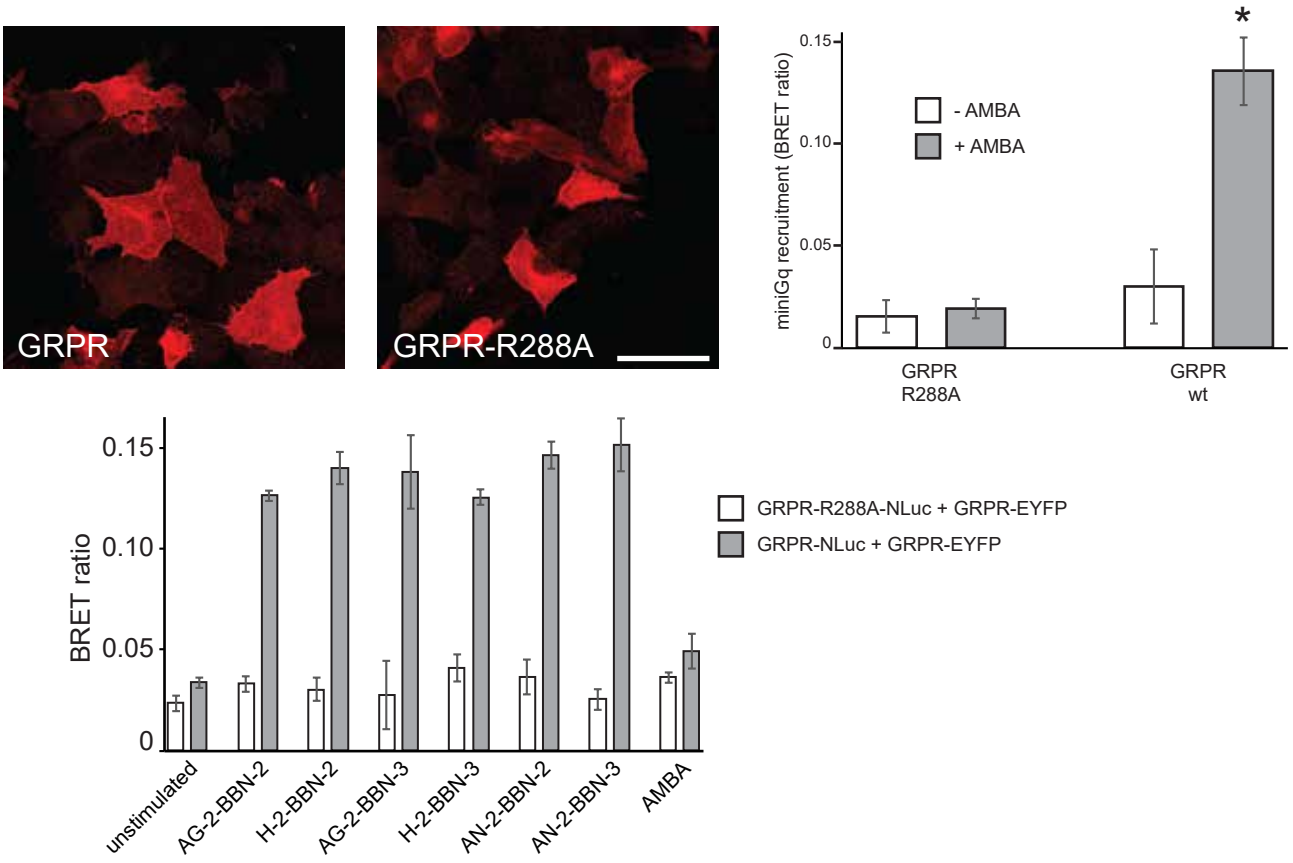
HR-MS (MALDI): m/z: calcd for $C_{201}H_{287}N_{54}O_{46}^{+}$ $[M+H]^{+}$ 4193.1773, found 4193.1912; calcd for $C_{201}H_{286}KN_{54}O_{46}^{+}$ $[M+K]^{+}$ 4231.1332, found 4231.1301.

Analytical HPLC: Reprosil Gold C18, 5 µm, (150x4) mm, (95-50)% B in 20 min, 1.0 mL/min, 50 °C

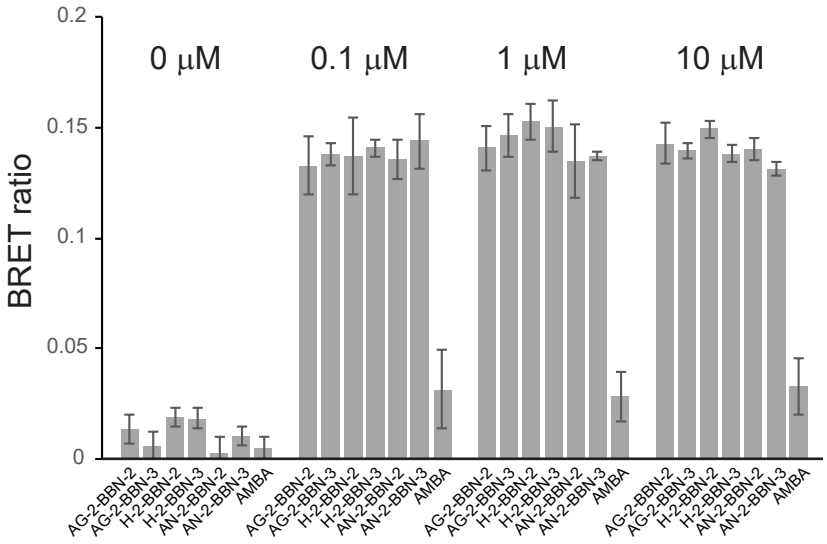
Rt = 18.307 min, purity: 92%



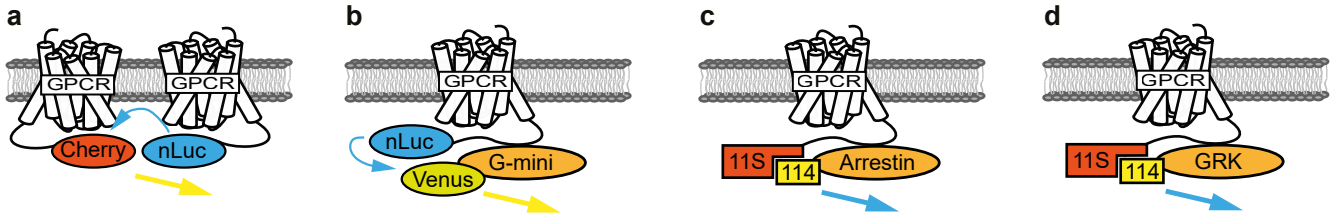
Supplementary Figure 2a: Dimerization depends on ligand binding. (Top left) GRPR and GRPR-R288A, a mutant that was previously shown to have reduced receptor binding, were transiently expressed in HEK293. Forty hours after transfection, cells were fixed with formaldehyd and immunostained a with polyclonal rabbit anti GRPR antibody (Atlas antibodies, HPA069604). Mutant as well as wildtype receptor appear at similar levels on the plasma membrane. Scale bar: 50 μm. (Top right) Mutant GRPR-R288A does not recruit miniGq after activation with AMBA suggesting that AMBA does not bind this mutant receptor (* P< 0.005).
(bottom) GRPR-NLuc (wt and R288A) was coexpressed with wildtype GRPR-EYFP. No dimerization was observed when mutant GRPR-R288A was used suggesting that ligand binding is necessary for dimerization. Results of a representative experiment (mean ± SD) are shown.



Supplementary Figure 2b: Dimerization at different ligand concentrations. Dimerization was measured using HEK293 cells co-expressing a NLuc- and EYFP-tagged GRPR. Cells were stimulated with bivalent compounds and AMBA as control at indicated concentrations. BRET ratio was measured 20 minutes after stimulation. Dimers were also observed at higher concentration suggesting that no high dose inhibition of dimerization occurs. Results of a representative experiment (mean ± SD) are shown.



Supplementary Figure 3: Recruitment assays. (a) Dimerization assay based on BRET. Light generated by NanoLuciferase is transfered to Cherry when two receptor are in close proximity and then emited with a longer wavelength. (b) BRET assay for measurement of mini-Gq recruitment. (c,d) Arrestin and GRK recruitment were mesured with a split NanoLuc complementation assay. The large subunit of NanoLuc (11S) was linked to GRPR, whereas the small unit (114) was linked to arrestin and GRK. Enzymatic activity is restored when GRPR and arrestin/ GRK are in close proximity. (e) Construction details of GRK2-114, GRK3-114, and GRK5-114.

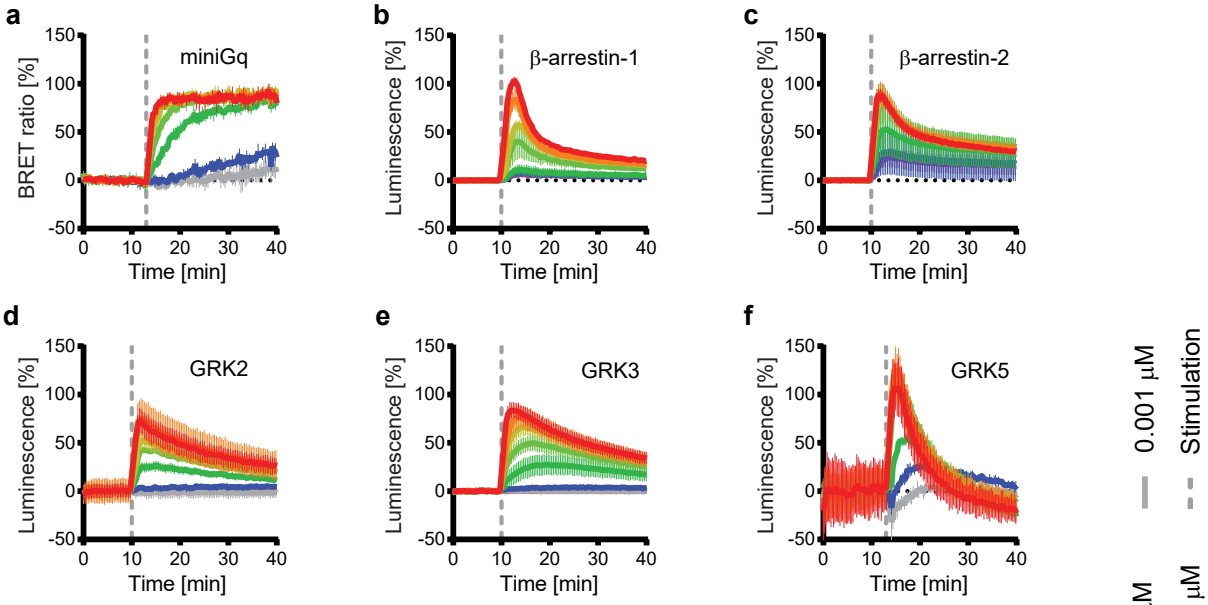


e

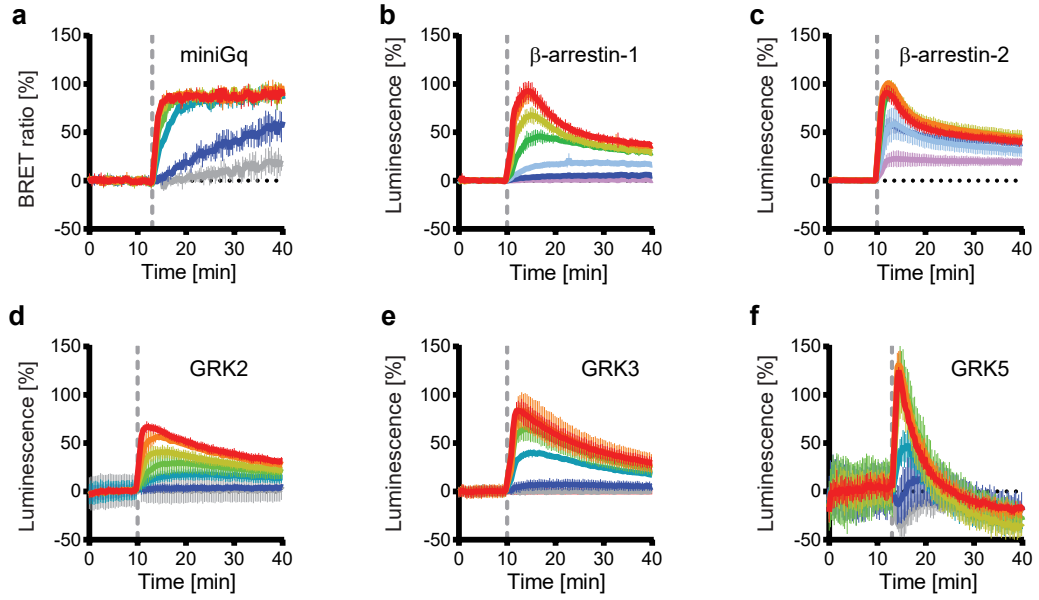
	BamHI	Linker	Subunit 114
GRK2 (accession NM_001619.5, 689 aa)		GSGGSGGGGSGGSSSG	VTGYRLFEEIL*
GRK3 (accession NM_005160.4, 688 aa)		GSGGSGGGGSGGSSSG	VTGYRLFEEIL*
GRK5 (accession NM_005308.3, 590 aa)		GSGGSGGGGSGGSSSG	VTGYRLFEEIL*

Supplementary Figure 4: Time-resolved measurement of adaptor protein recruitment to GRPR. Cells were stimulated with different concentrations of the indicated compound at the indicated time point (dashed line). (a) BRET-based assay for miniGq recruitment. (b) Nanoluciferase complementation assay for β-arrestin-1, (c) β-arrestin-2, (d) GRK2, (e) GRK3, (f) GRK5.

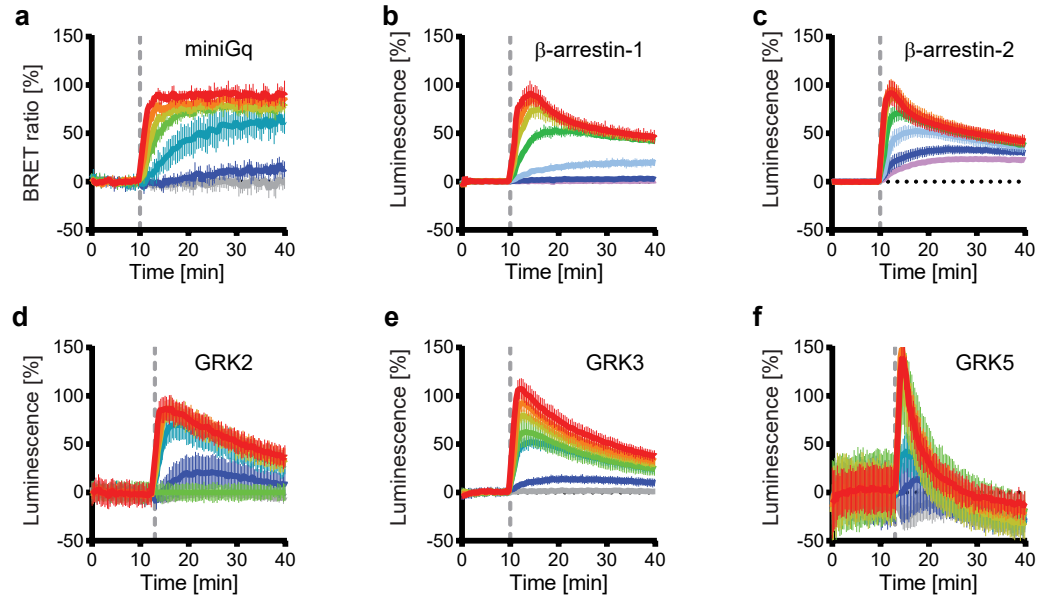
Supplementary Figure 4a: AG-1-BBN-1



Supplementary Figure 4b: AG-1-BBN-2

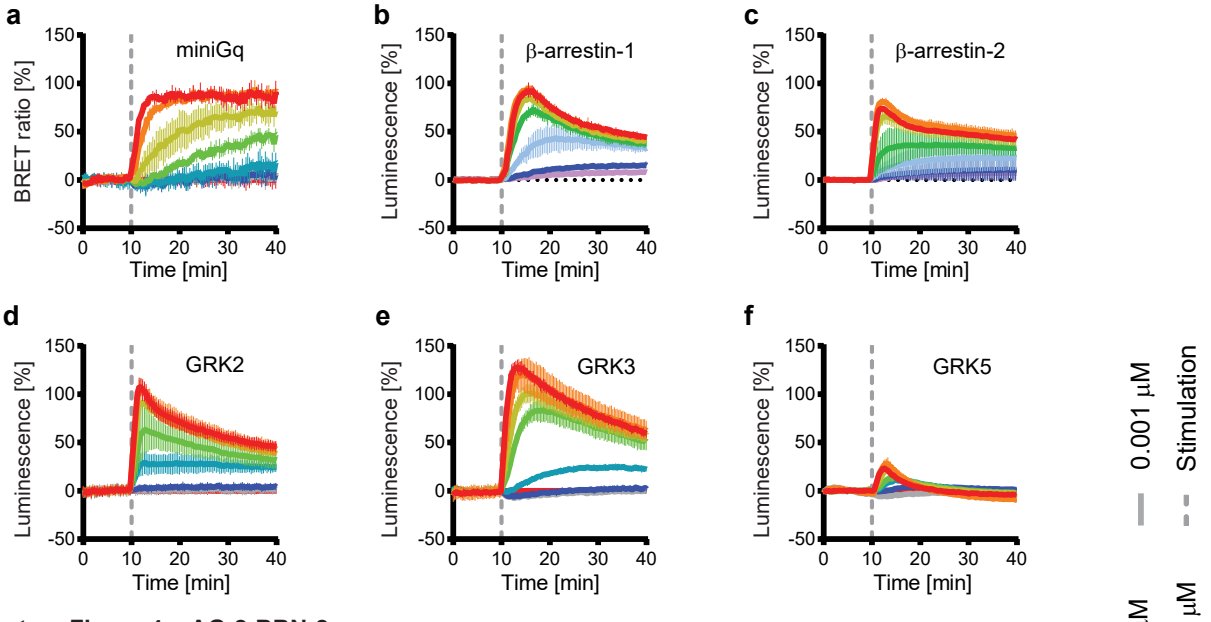


Supplementary Figure 4c: AG-1-BBN-3

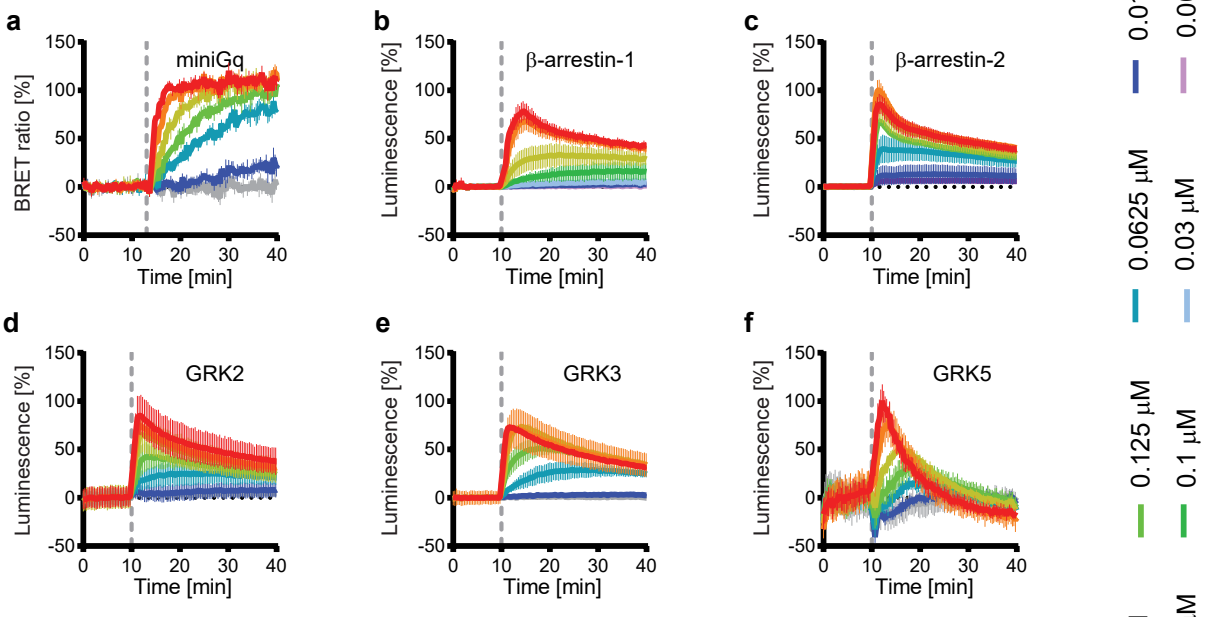


Supplementary Figure 4: Time-resolved measurement of adaptor protein recruitment to GRPR. Cells were stimulated with different concentrations of the indicated compound at the indicated time point (dashed line). (a) BRET-based assay for miniGq recruitment. (b) Nanoluciferase complementation assay for β-arrestin-1, (c) β-arrestin-2, (d) GRK2, (e) GRK3, (f) GRK5.

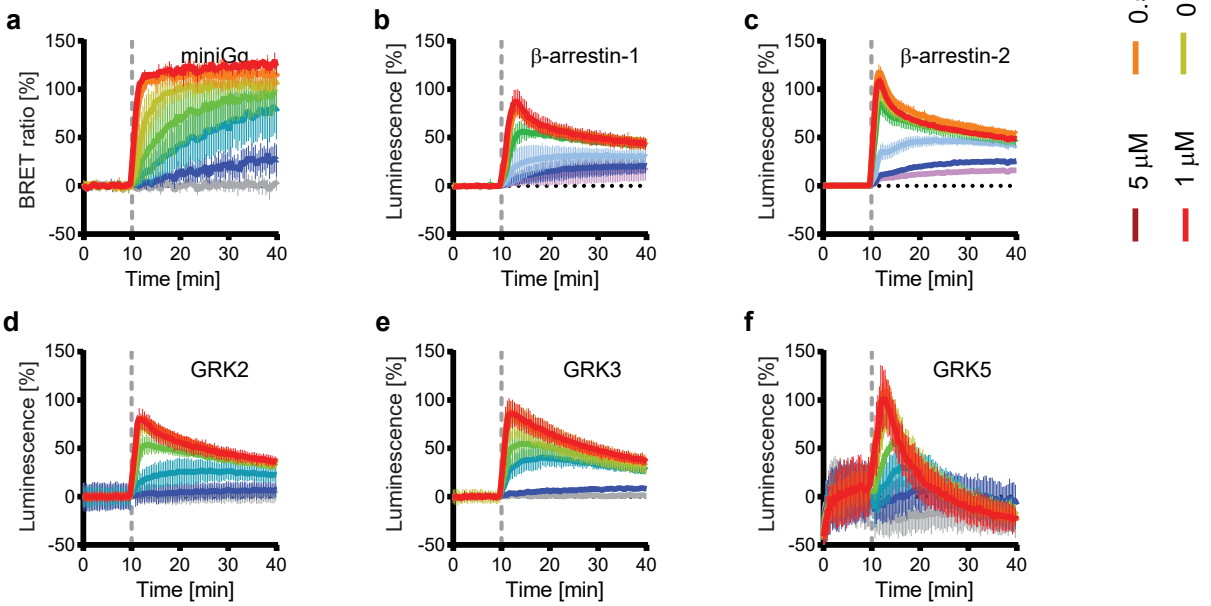
Supplementary Figure 4d: AG-2-BBN-1



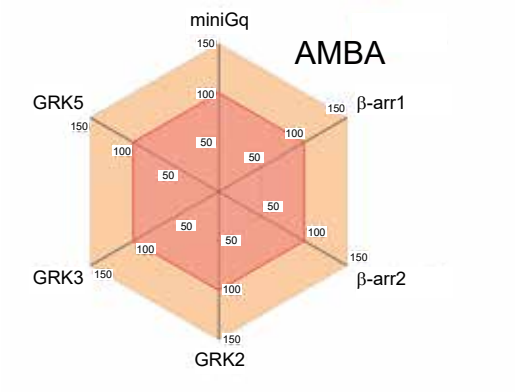
Supplementary Figure 4e: AG-2-BBN-2

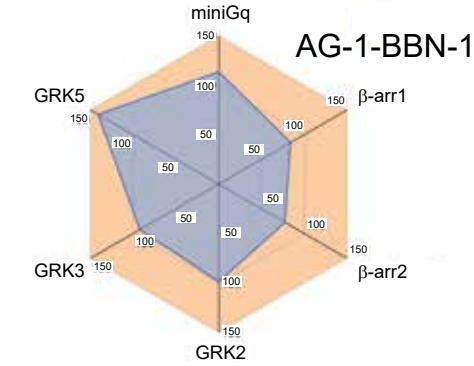


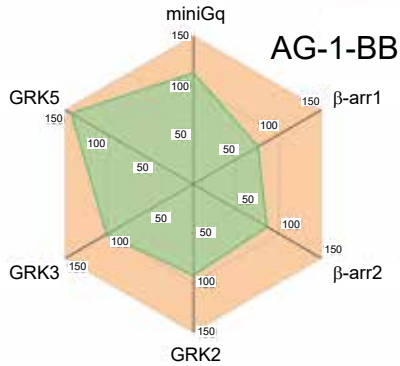
Supplementary Figure 4f: AG-2-BBN-3

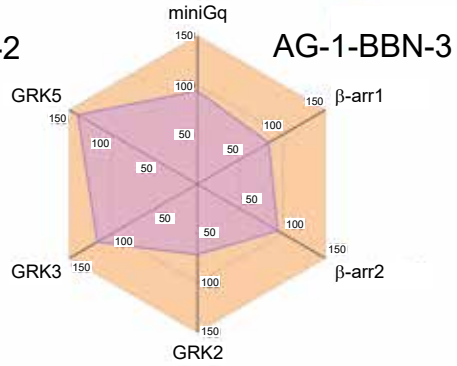


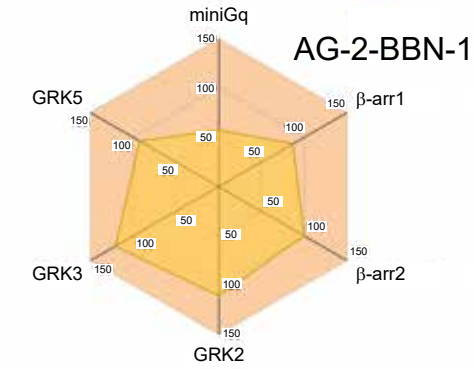
Supplementary Figure 5a: Spiderwebs of efficacy values. Values of dimerizing compunds are shown on a green background. Non-dimerizing compound on orange background.

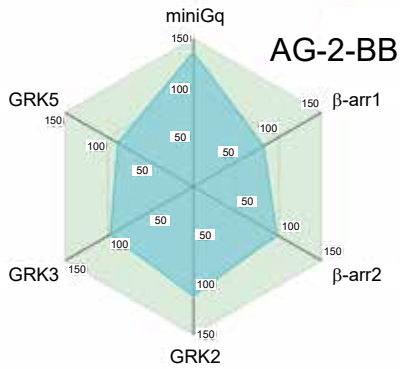


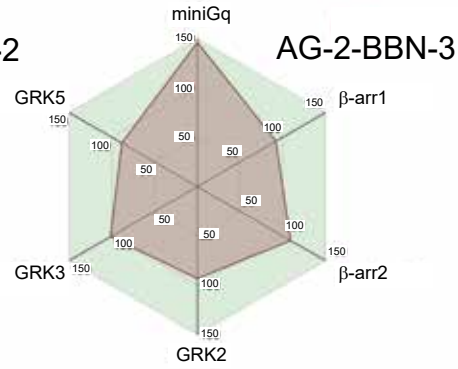


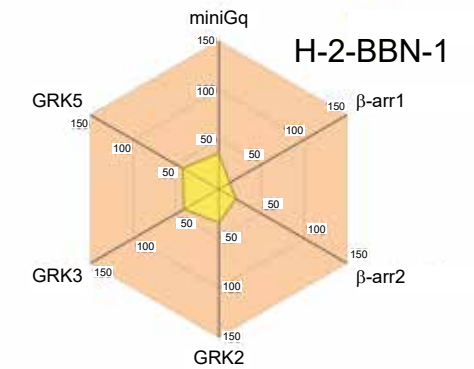


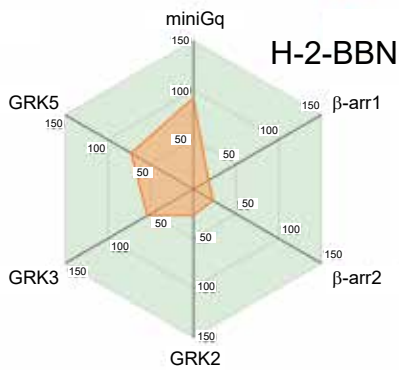


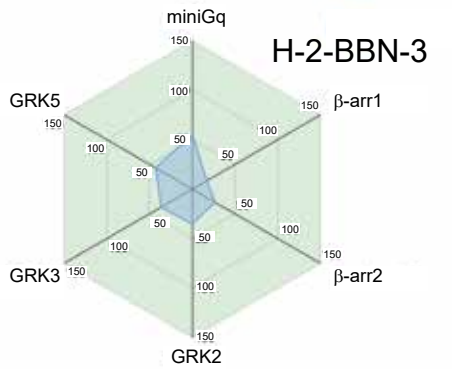




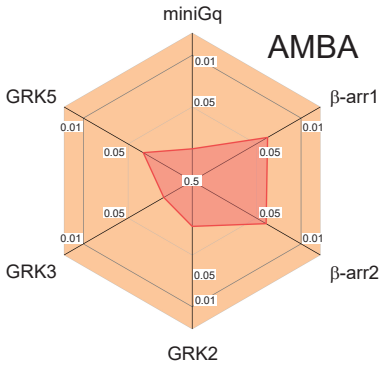


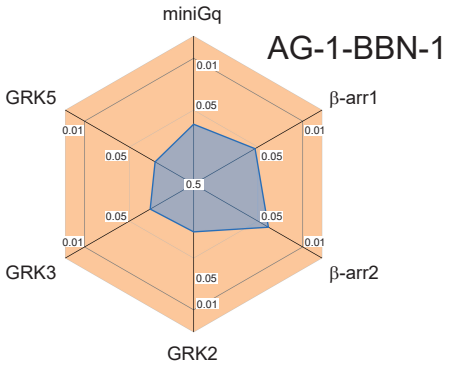


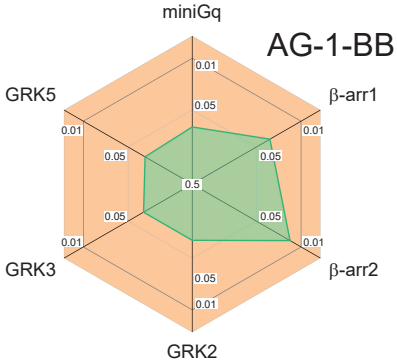


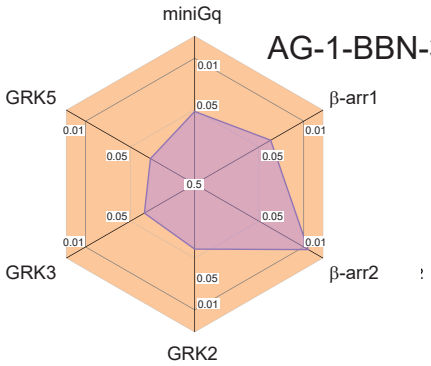


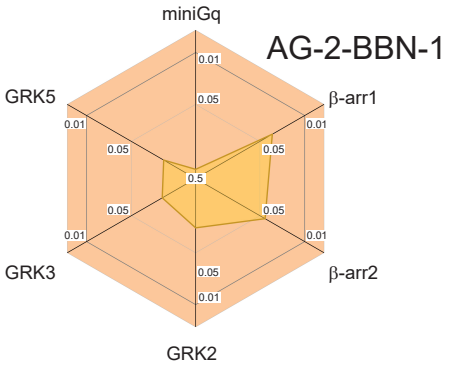
Supplementary Figure 5b: Spiderwebs of potency values. Values of dimerizing compounds are shown on a green background. Non-dimerizing compound on orange back-ground. Concentrations are in micromolar.

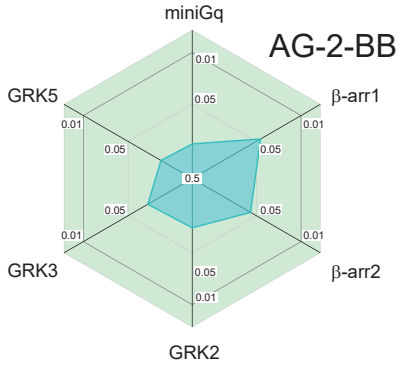


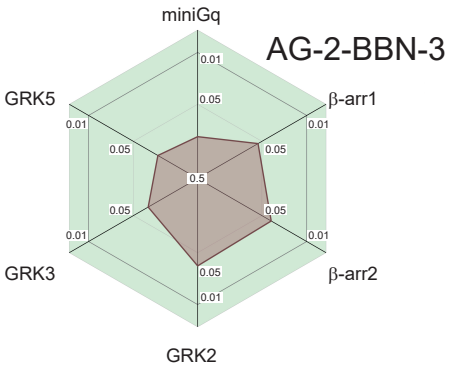




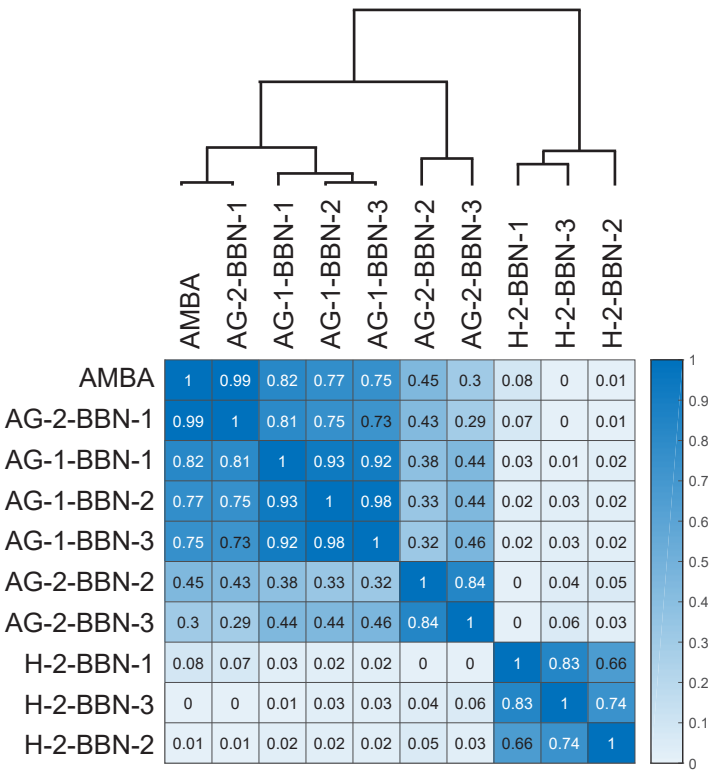




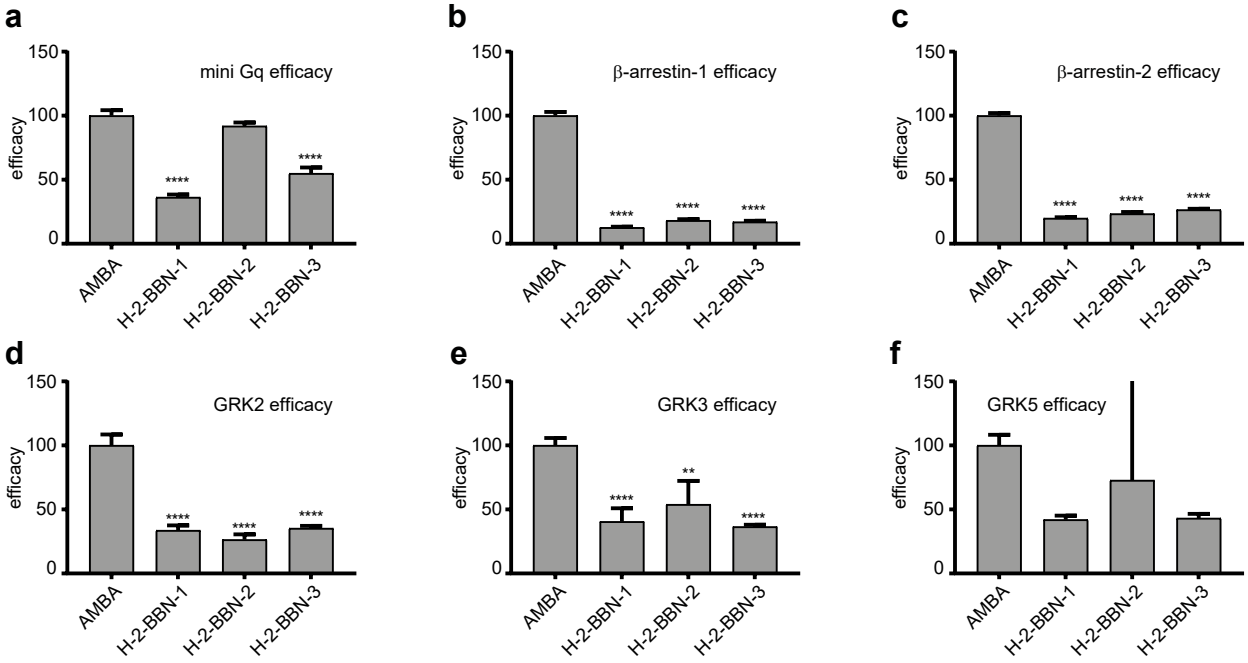




Supplementary Figure 6: Ligand classification according to signaling profiles.
Heatmap and dendrogram were calculated as follows:
1. Each adaptor was represented by two column vectors (one for the efficacy and one for the potency) with 10 values (one for each compound). Dimerization was represented by an additional column vector (0: not dimeric, 1: dimeric). In order to correct the 10x13-matrix, composed of those vectors, for scale differences between parameters, we standardized each column to range between 0 and 1.
2. k-means clustering was performed 1000 times with k=3 and the squared Euclidean distance to quantify the fraction of times each compound clustered together resulting in a 10x10 frequency matrix ranging from 1 (always clustered together) to 0 (never clustered together).
3. The frequency matrix was visualized by a heatmap and a dendrogram using the functions from MATLAB.
4. Compounds were reordered in the heatmap according to the dendrogram.



Supplementary Figure 7. Efficacy of hybrid compounds. Efficacy of tested ligands for recruitment of (a) miniGq, (b) β-arrestin-1, (c) β-arrestin-2, (d) GRK2, (e) GRK3, and (f) GRK5. The asterisks' indicate statistically significant difference to the reference compound AMBA. * significantly different with P ≤ 0.05, ** significantly different with P ≤ 0.01, *** significantly different with P ≤ 0.001, **** significantly different with P ≤ 0.0001.



Supplementary Figure 8a: Pairwise comparison of statistical significance. * significantly different with P ≤ 0.05, ** significantly different with P ≤ 0.01, *** significantly different with P ≤ 0.001, **** significantly different with P ≤ 0.0001.

Gq potency

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		**	**	***	****	ns	ns
AG-1-BBN-1	**		ns	ns	****	*	ns
AG-1-BBN-2	**	ns		ns	****	ns	ns
AG-1-BBN-3	***	ns	ns		****	**	ns
AG-2-BBN-1	****	****	****	****		****	****
AG-2-BBN-2	ns	*	ns	**	****		ns
AG-2-BBN-3	ns	ns	ns	ns	****	ns	

Gq efficacy

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	ns	ns	****	***	****
AG-1-BBN-1	ns		ns	ns	****	ns	**
AG-1-BBN-2	ns	ns		ns	****	ns	**
AG-1-BBN-3	ns	ns	ns		***	***	****
AG-2-BBN-1	****	****	****	***		****	****
AG-2-BBN-2	***	ns	ns	***	****		ns
AG-2-BBN-3	****	**	**	****	****	ns	

β-arrestin-1 potency

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	ns	ns	ns	ns	ns
AG-1-BBN-1	ns		ns	ns	ns	ns	ns
AG-1-BBN-2	ns	ns		ns	ns	ns	ns
AG-1-BBN-3	ns	ns	ns		ns	ns	ns
AG-2-BBN-1	ns	ns	ns	ns		ns	ns
AG-2-BBN-2	ns	ns	ns	ns	ns		ns
AG-2-BBN-3	ns	ns	ns	ns	ns	ns	

β-arrestin-1 efficacy

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	***	ns	ns	*	ns
AG-1-BBN-1	ns		ns	ns	ns	ns	ns
AG-1-BBN-2	***	ns		ns	ns	ns	ns
AG-1-BBN-3	ns	ns	ns		ns	ns	ns
AG-2-BBN-1	ns	ns	ns	ns		ns	ns
AG-2-BBN-2	*	ns	ns	ns	ns		ns
AG-2-BBN-3	ns	ns	ns	ns	ns	ns	

β-arrestin-2 potency

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	*	***	ns	***	ns
AG-1-BBN-1	ns		ns	*	ns	**	ns
AG-1-BBN-2	*	ns		ns	**	****	ns
AG-1-BBN-3	***	*	ns		***	****	**
AG-2-BBN-1	ns	ns	**	***		ns	ns
AG-2-BBN-2	***	**	****	****	ns		*
AG-2-BBN-3	ns	ns	ns	**	ns	*	

β-arrestin-2 efficacy

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		****	*	ns	ns	ns	ns
AG-1-BBN-1	****		ns	**	***	***	****
AG-1-BBN-2	*	ns		ns	ns	ns	****
AG-1-BBN-3	ns	**	ns		ns	ns	*
AG-2-BBN-1	ns	***	ns	ns		ns	ns
AG-2-BBN-2	ns	***	ns	ns	ns		ns
AG-2-BBN-3	ns	****	****	*	ns	ns	

Supplementary Figure 8b: Pairwise comparison of statistical significance. * significantly different with P ≤ 0.05, ** significantly different with P ≤ 0.01, *** significantly different with P ≤ 0.001, **** significantly different with P ≤ 0.0001.

GRK2 potency

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	ns	ns	ns	ns	*
AG-1-BBN-1	ns		ns	ns	ns	ns	ns
AG-1-BBN-2	ns	ns		ns	ns	ns	ns
AG-1-BBN-3	ns	ns	ns		ns	ns	ns
AG-2-BBN-1	ns	ns	ns	ns		ns	ns
AG-2-BBN-2	ns	ns	ns	ns	ns		ns
AG-2-BBN-3	*	ns	ns	ns	ns	ns	

GRK2 efficacy

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	ns	ns	ns	ns	ns
AG-1-BBN-1	ns		ns	ns	ns	ns	ns
AG-1-BBN-2	ns	ns		ns	ns	ns	ns
AG-1-BBN-3	ns	ns	ns		*	*	ns
AG-2-BBN-1	ns	ns	ns	*		ns	ns
AG-2-BBN-2	ns	ns	ns	*	ns		ns
AG-2-BBN-3	ns	ns	ns	ns	ns	ns	

GRK3 potency

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	*	*	ns	ns	*
AG-1-BBN-1	ns		ns	ns	ns	ns	ns
AG-1-BBN-2	*	ns		ns	ns	ns	ns
AG-1-BBN-3	*	ns	ns		ns	ns	ns
AG-2-BBN-1	ns	ns	ns	ns		ns	ns
AG-2-BBN-2	ns	ns	ns	ns	ns		ns
AG-2-BBN-3	*	ns	ns	ns	ns	ns	

GRK3 efficacy

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	ns	ns	ns	ns	ns
AG-1-BBN-1	ns		ns	ns	ns	ns	ns
AG-1-BBN-2	ns	ns		ns	ns	ns	ns
AG-1-BBN-3	ns	ns	ns		ns	ns	ns
AG-2-BBN-1	ns	ns	ns	ns		ns	ns
AG-2-BBN-2	ns	ns	ns	ns	ns		ns
AG-2-BBN-3	ns	ns	ns	ns	ns	ns	

GRK5 potency

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		ns	ns	ns	ns	ns	ns
AG-1-BBN-1	ns		ns	ns	ns	ns	ns
AG-1-BBN-2	ns	ns		ns	ns	ns	ns
AG-1-BBN-3	ns	ns	ns		ns	ns	ns
AG-2-BBN-1	ns	ns	ns	ns		ns	ns
AG-2-BBN-2	ns	ns	ns	ns	ns		ns
AG-2-BBN-3	ns	ns	ns	ns	ns	ns	

GRK5 efficacy

	AMBA	AG-1-BBN-1	AG-1-BBN-2	AG-1-BBN-3	AG-2-BBN-1	AG-2-BBN-2	AG-2-BBN-3
AMBA		**	**	**	ns	ns	ns
AG-1-BBN-1	**		ns	ns	***	***	***
AG-1-BBN-2	**	ns		ns	***	***	***
AG-1-BBN-3	**	ns	ns		***	***	***
AG-2-BBN-1	ns	***	***	***		ns	ns
AG-2-BBN-2	ns	***	***	***	ns		ns
AG-2-BBN-3	ns	***	***	***	ns	ns	