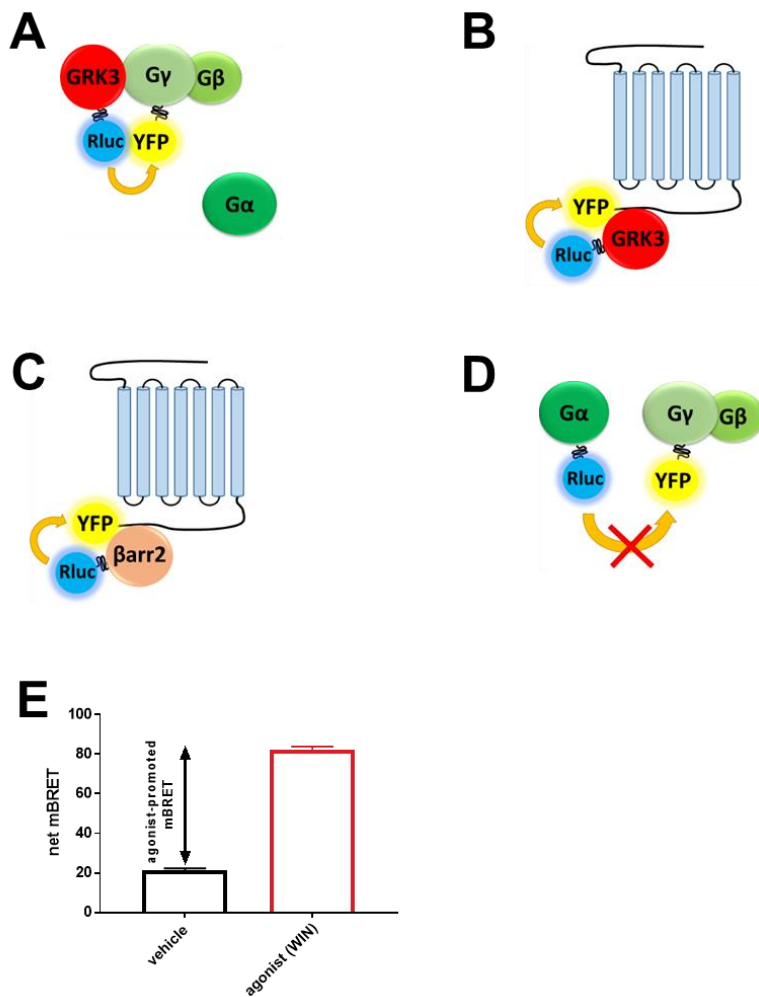


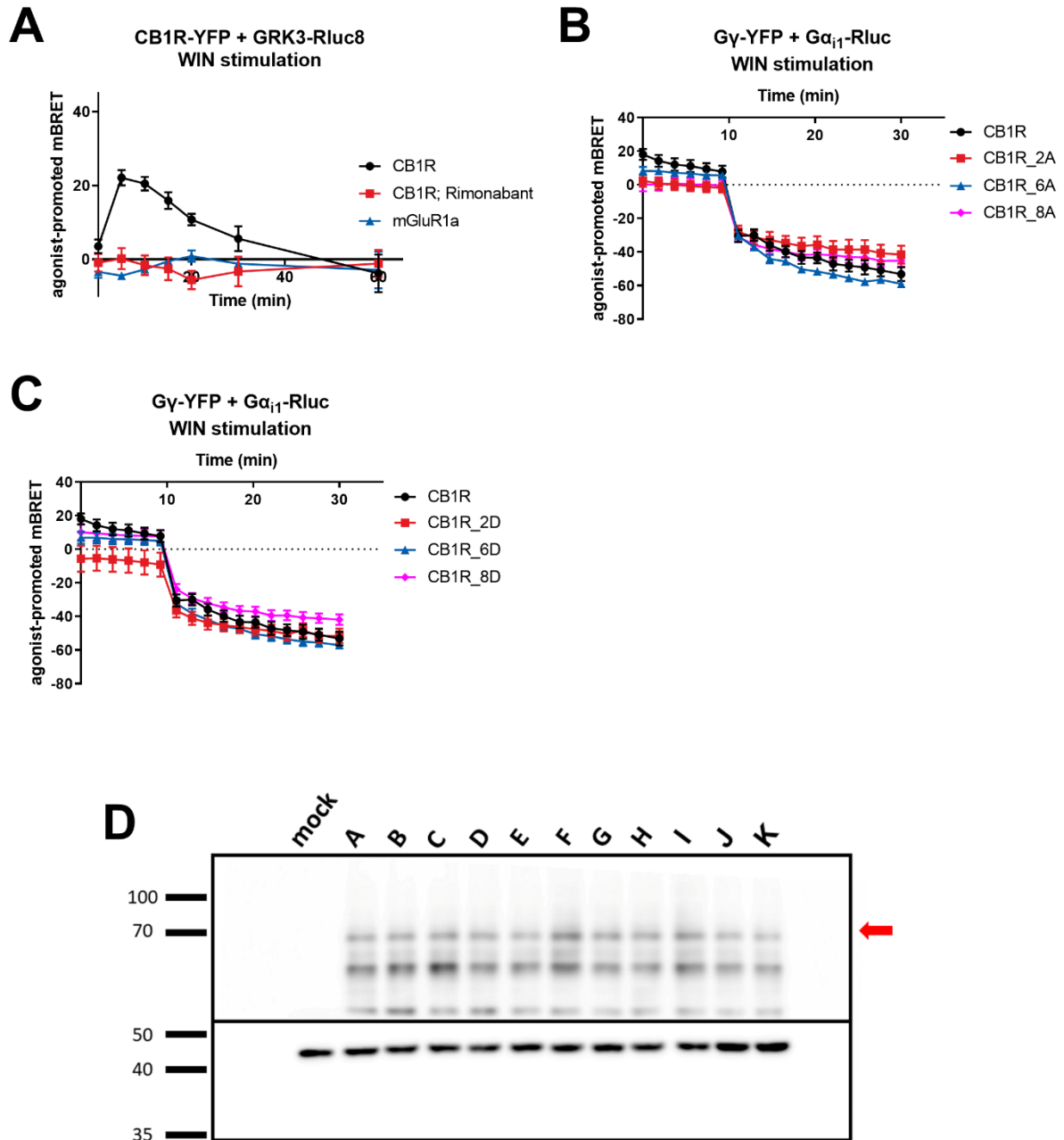
SGIP1 modulates kinetics and interactions of the cannabinoid receptor 1 and G protein-coupled receptor kinase 3 signalosome

Supplementary material

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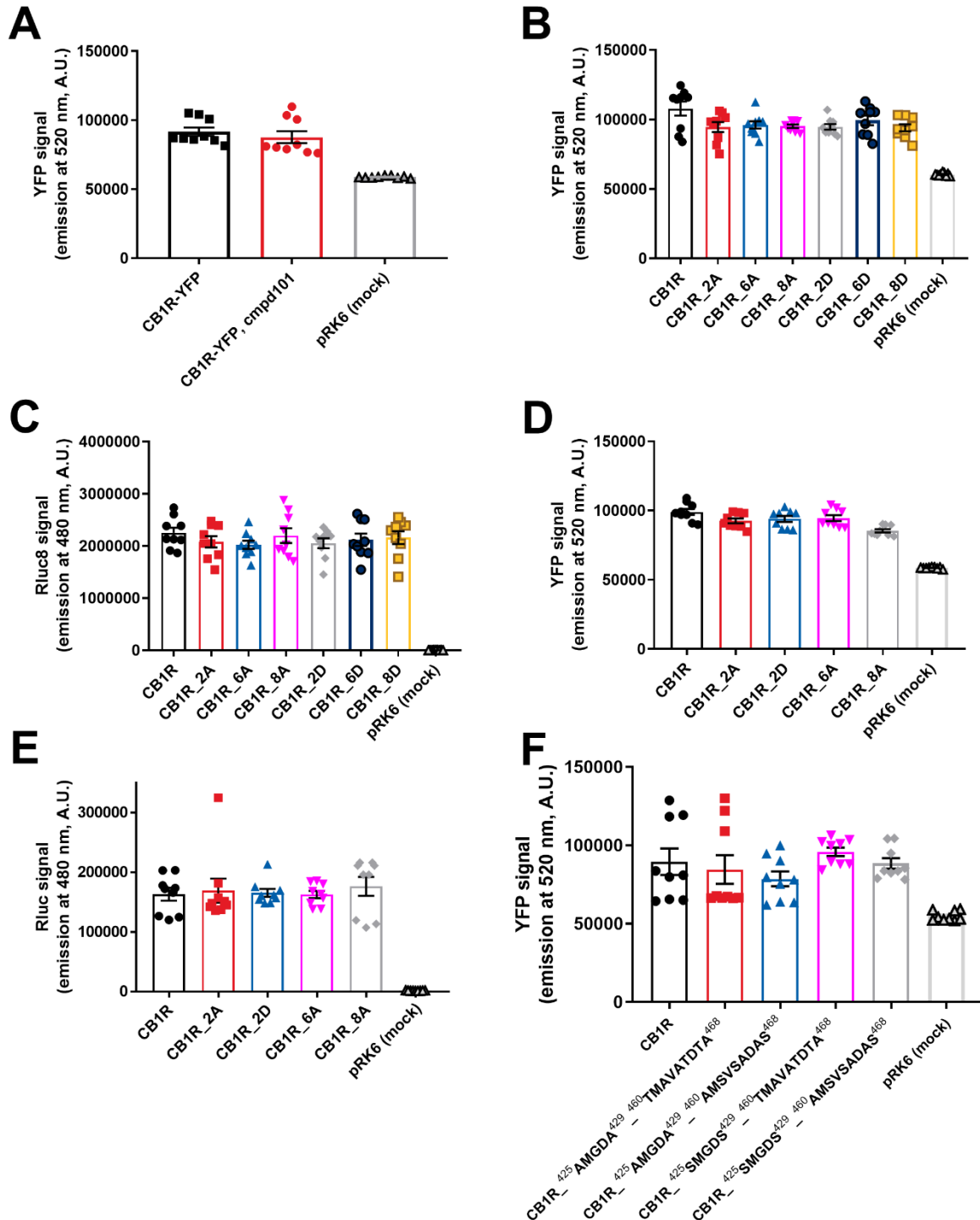


Suppl. Fig. 1. BRET-based sensors used in the study. **A)** Schematic representation of GRK3-Rluc8 with G γ -YFP complexes formation generating an increase in BRET signal. **B)** Schematic representation of GRK3-Rluc8 recruitment to CB1R-YFP upon CB1R activation. The formation of GRK3-CB1R complexes is observed as an increase in BRET signal efficiency. **C)** Schematic representation of the recruitment of β -arrestin2-Rluc by the activated CB1R-YFP generating an increase in BRET signal. **D)** Schematic representation of the activation of heterotrimeric G proteins which is observed as a decrease in BRET signal due to dissociation/conformational change of G α_i from G $\beta\gamma$ subunits. **E)** Agonist-promoted mBRET was calculated by subtracting the BRET ratio obtained in the absence of agonist from the one obtained following agonist application and multiplied by 1000.

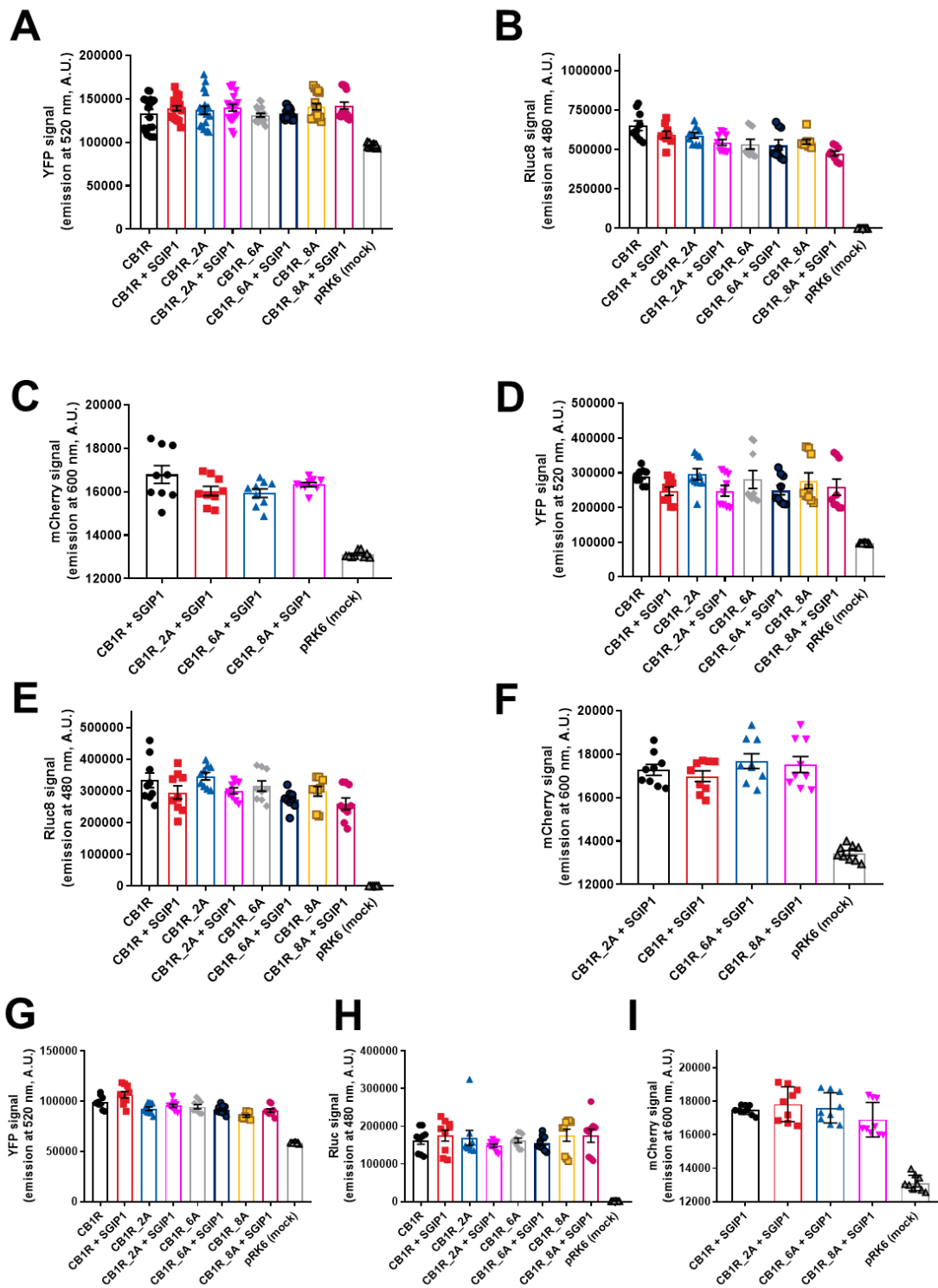


Suppl. Fig. 2. GRK3 recruitment by WIN-activated CB1R. CB1R phosphorylation mutants retain the capability to induce rapid dissociation of G protein subunits. Mutant CB1Rs variants have similar levels of expression to wild-type CB1R. A) Kinetic profiles of GRK3-RLuc8 recruitment by WIN-activated CB1R-YFP/mGluR1a in HEK293 cells pretreated or not with rimonabant. HEK293 cells were transiently cotransfected with the plasmid coding for CB1R-YFP or mGluR1a and GRK3-RLuc8. After sixteen hours, cells were pretreated or not for 30 minutes with rimonabant (45 μ M) prior to the stimulation with the CB1R agonist WIN (1 μ M). **B)** Alanine mutant CB1Rs preserve the ability to activate of G proteins. HEK293 cells were transiently cotransfected with CB1R-SNAP variant, G α_i -Rluc8, G β -Flag, G γ -YFP (2:1:1:1 ratio). Firstly, basal BRET was measured for 10 minutes. Afterward, cells were stimulated by 1 μ M WIN. **C)** Aspartic acid mutant CB1Rs preserve the ability to activate of G proteins. HEK293 cells were transiently cotransfected with CB1R-SNAP variant, G α_i -Rluc8, G β -Flag, G γ -YFP (2:1:1:1 ratio). Firstly, basal BRET was measured for 10 minutes. Afterward, cells were stimulated by 1 μ M WIN. **D)** Mutant CB1Rs variants have similar levels of expression to wild-type CB1R. HEK293

cells were transfected with the indicated CB1R variant or with empty plasmid pRK6 (mock). Cell lysates were separated by SDS-PAGE and subjected to Western blotting. Membranes were stained with either anti-GFP antibody for detection of CB1R-YFP variants (top blot) or anti-Actin antibody (actin) to normalize for loading and transfer of proteins (bottom blot). Legend: mock (pRK6 empty vector transfection), A) CB1R, B) CB1R_2A, C) CB1R_6A, D) CB1R_8A, E) CB1R_2D, F) CB1R_6D, G) CB1R_8D, H) CB1R⁴²⁵SMGDS⁴²⁹⁻⁴⁶⁰TMAVATDTA⁴⁶⁸, I) CB1R⁴²⁵AMGDA⁴²⁹⁻⁴⁶⁰TMAVATDTA⁴⁶⁸, J) CB1R⁴²⁵SMGDS⁴²⁹⁻⁴⁶⁰AMSVSADAS⁴⁶⁸, K) CB1R⁴²⁵AMGDA⁴²⁹⁻⁴⁶⁰AMSVSADAS⁴⁶⁸.



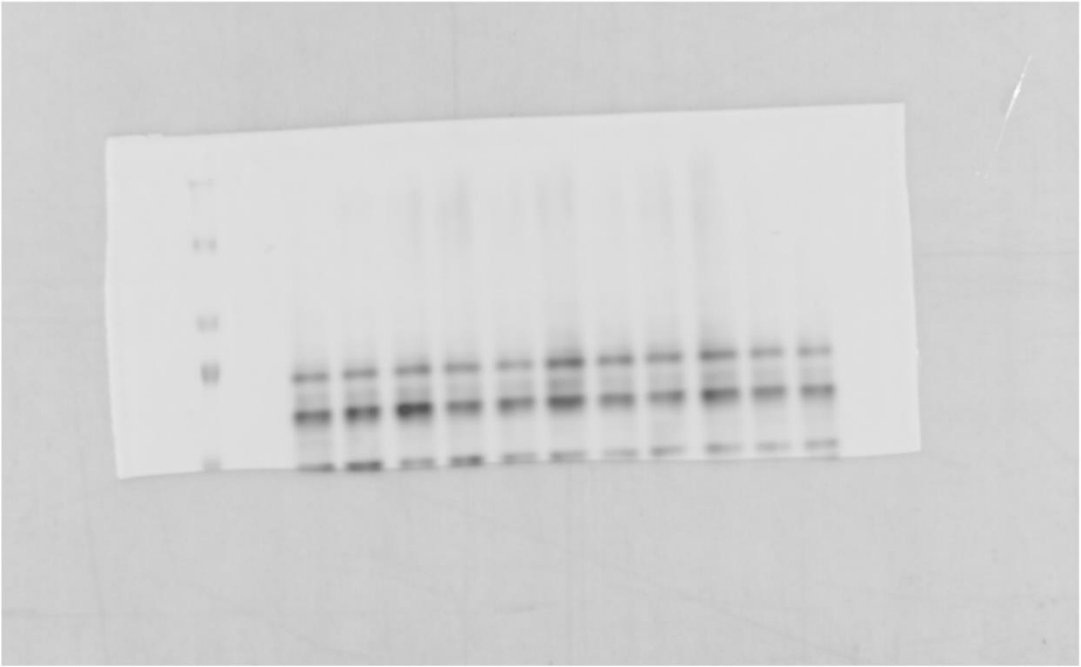
Suppl. Fig. 3. Expression levels of CB1R-YFP variants, GRK3-Rluc8 and β -arrestin2-Rluc in transiently transfected HEK293 cells. Emission of CB1R-YFP variants was measured at 520 nm on Mithras LB 940 microplate reader after excitation at 485 nm. Emission of Rluc constructs was measured at 480 nm on Mithras LB 940 microplate reader 5 minutes after adding coelenterazine h. **A)** Cmpd101 does not affect the expression of CB1R-YFP. Expression level determination of CB1R-YFP in presence/absence of compd101. **B)** Expression levels of CB1R-YFP variants. **C)** GRK3-Rluc8 in cells coexpressing mutant CB1Rs. **D)** Expression levels of CB1R-YFP variants. **E)** β -arrestin2-Rluc in cells coexpressing mutant CB1R. **F)** Expression level determination of CB1R-YFP variants.



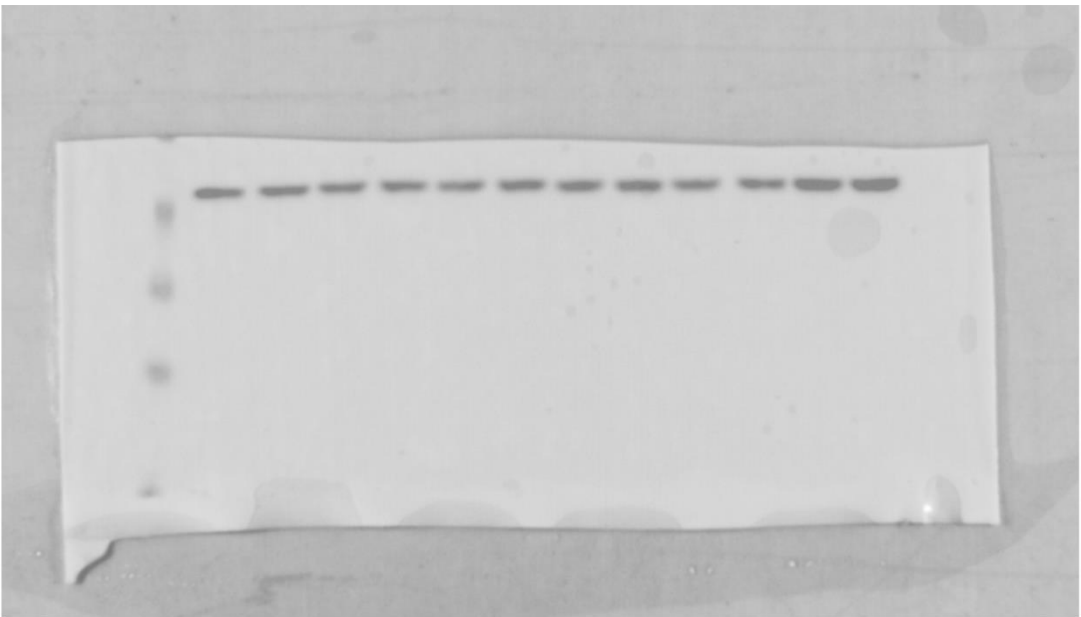
Suppl. Fig. 4. Expression level determination of CB1R-YFP variants, GRK3-Rluc8, β -arrestin2-Rluc and SGIP1-mCherry in transiently transfected HEK293 cells. Emission of YFP proteins was measured at 520 nm on Mithras LB 940 microplate reader after excitation at 485 nm. Emission of Rluc constructs was measured at 480 nm on Mithras LB 940 microplate reader 5 minutes after adding coelenterazine h. Emission of SGIP1-mCherry was measured at 600 nm on Mithras LB 940 microplate after excitation at 540 nm. **A)** Expression level determination of CB1R-YFP variants in cells coexpressing SGIP1-mCherry

and GRK3-Rluc8. **B)** GRK3-Rluc8 in cells coexpressing mutant CB1Rs. **C)** SGIP1-mCherry expression in cells coexpressing different CB1R receptors. **D)** Expression level determination of CB1R-YFP variants in cells coexpressing SGIP1-mCherry and β -arrestin2-Rluc. **E)** β -arrestin2-Rluc in cells coexpressing mutant CB1Rs. **F)** SGIP1-mCherry expression in cells coexpressing different CB1R receptors. **G)** Expression level of $G\gamma$ -YFP cells coexpressing distinct CB1R-SNAP variant, SGIP1-mCherry and GRK3-Rluc8. **H)** GRK3-Rluc8 in cells coexpressing mutant CB1R-SNAP. **I)** SGIP1-mCherry expression in cells coexpressing different CB1R receptors.

A



B



Suppl. Fig. 5. Full uncropped immunoblot image from Supp. Fig. 2D.

A Gy-YFP + GRK3-Rluc8 +- cmpd101

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x cmpd101 treatment	30198	4	7550	F (4, 40) = 58.33	P<0.0001
Time	154175	4	38544	F (4, 40) = 85.88	P<0.0001
cmpd101 treatment	145702	1	145702	F (1, 40) = 1126	P<0.0001

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + cmpd101					
0 min	9.834	-4.627 to 24.30	No	ns	0.3197
5 min after WIN stimulation	103.0	88.50 to 117.4	Yes	****	<0.0001
15 min after WIN stimulation	112.0	97.50 to 126.4	Yes	****	<0.0001
30 min after WIN stimulation	95.31	80.85 to 109.8	Yes	****	<0.0001
60 min after WIN stimulation	82.29	67.83 to 96.75	Yes	****	<0.0001

B

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
WIN concentration x cmpd101 treatment	1141	7	163.0	F (7, 112) = 0.6954	P=0.6758
WIN concentration	257479	7	36783	F (7, 112) = 156.9	P<0.0001
cmpd101 treatment	373.6	1	373.6	F (1, 16) = 1.178	P=0.2938

C

CB1R-YFP + GRK3-Rluc8 +- cmpd101

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x cmpd101 treatment	1213	5	242.5	F (5, 80) = 19.57	P<0.0001
Time	5161	5	1032	F (2.043, 32.69) = 83.29	P<0.0001
cmpd101 treatment	2480	1	2480	F (1, 16) = 23.54	P=0.0002

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + cmpd101					
0 min	0.5299	-6.814 to 7.874	No	ns	>0.9999
5 min after WIN stimulation	18.58	11.56 to 25.59	Yes	****	<0.0001
10 min after WIN stimulation	16.43	8.811 to 24.04	Yes	****	<0.0001
15 min after WIN stimulation	12.65	6.149 to 19.16	Yes	***	0.0002
30 min after WIN stimulation	3.689	-3.301 to 10.68	No	ns	0.5737
60 min after WIN stimulation	5.629	-3.573 to 14.83	No	ns	0.4104

D

CB1R-YFP + β -arrestin2-Rluc +- cmpd101

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x cmpd101 treatment	3681	5	736.1	F (5, 80) = 16.98	P<0.0001
Time	3436	5	687.2	F (5, 80) = 15.85	P<0.0001
cmpd101 treatment	4368	1	4368	F (1, 16) = 35.04	P<0.0001

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + cmpd101					
0 min	-1.184	-7.590 to 5.223	No	ns	0.9946
5 min after WIN stimulation	28.70	16.21 to 41.20	Yes	****	<0.0001
10 min after WIN stimulation	24.25	11.42 to 37.07	Yes	***	0.0003
15 min after WIN stimulation	18.10	5.596 to 30.61	Yes	**	0.0031
30 min after WIN stimulation	6.890	-2.177 to 15.96	No	ns	0.2007
60 min after WIN stimulation	-0.4430	-11.34 to 10.46	No	ns	>0.9999

Suppl. table 1. Statistical analysis of kinetics of presented protein-protein interactions. Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.

Gy-YFP + GRK3-Rluc8 – CB1R, CB1R_2A, CB1R_6A, CB1R_8A

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x CB1R variant	35899	12	2992	F (12, 128) = 7.938	P<0.0001
Time	319548	4	79887	F (1.627, 52.06) = 212.0	P<0.0001
CB1R variant	113790	3	37930	F (3, 32) = 13.53	P<0.0001

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0 min					
CB1R vs. CB1R 2A	-12.77	-65.76 to 40.23	No	ns	0.9993
CB1R vs. CB1R 6A	7.168	-47.44 to 61.78	No	ns	>0.9999
CB1R vs. CB1R 8A	-16.29	-69.39 to 36.81	No	ns	0.9925
5 min after WIN stimulation					
CB1R vs. CB1R 2A	-18.82	-74.00 to 36.36	No	ns	0.9878
CB1R vs. CB1R 6A	29.46	-31.48 to 90.40	No	ns	0.8438
CB1R vs. CB1R 8A	59.70	3.323 to 116.1	Yes	*	0.0330
15 min after WIN stimulation					
CB1R vs. CB1R 2A	-33.03	-85.00 to 18.95	No	ns	0.4629
CB1R vs. CB1R 6A	36.71	-22.02 to 95.44	No	ns	0.5177
CB1R vs. CB1R 8A	64.44	10.25 to 118.6	Yes	*	0.0135
30 min after WIN stimulation					
CB1R vs. CB1R 2A	-50.33	-93.09 to -7.561	Yes	*	0.0145
CB1R vs. CB1R 6A	21.47	-24.16 to 67.09	No	ns	0.8665
CB1R vs. CB1R 8A	43.76	1.341 to 86.17	Yes	*	0.0401
60 min after WIN stimulation					
CB1R vs. CB1R 2A	-32.50	-85.49 to 20.48	No	ns	0.4907
CB1R vs. CB1R 6A	18.94	-39.45 to 77.32	No	ns	0.9927
CB1R vs. CB1R 8A	33.50	-21.05 to 88.05	No	ns	0.5199

Suppl. table 2. Statistical analysis of kinetics of presented protein-protein interactions. *Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.*

A Gy-YFP + GRK3-Rluc8 – CB1R +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	37097	5	7419	F (5, 80) = 13.81	P<0.0001
Time	533999	5	106800	F (1.427, 22.83) = 198.8	P<0.0001
+/- SGIP1	21381	1	21381	F (1, 16) = 2.940	P=0.1057

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + SGIP1					
0 min	15.55	-40.96 to 72.05	No	ns	0.9625
5 min after WIN stimulation	-34.67	-98.43 to 29.08	No	ns	0.5364
15 min after WIN stimulation	-43.23	-108.7 to 22.22	No	ns	0.3258
30 min after WIN stimulation	-52.62	-104.4 to -0.8715	Yes	*	0.0452
60 min after WIN stimulation	-79.20	-130.1 to -28.31	Yes	**	0.0016

B Gy-YFP + GRK3-Rluc8 – CB1R_2A +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	37906	5	7581	F (5, 80) = 30.30	P<0.0001
Time	632583	5	126517	F (2.341, 37.46) = 505.7	P<0.0001
+/- SGIP1	6581	1	6581	F (1, 16) = 4.507	P=0.0497

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R_2A vs CB1R_2A + SGIP1					
0 min	20.60	-1.657 to 42.85	No	ns	0.0785
5 min after WIN stimulation	-14.09	-49.76 to 21.58	No	ns	0.8176
15 min after WIN stimulation	-17.28	-45.26 to 10.69	No	ns	0.4000
30 min after WIN stimulation	-29.85	-63.24 to 3.547	No	ns	0.0946
60 min after WIN stimulation	-84.26	-122.6 to -45.93	Yes	****	<0.0001

C Gy-YFP + GRK3-Rluc8 – CB1R_6A +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	32253	5	6451	F (5, 80) = 21.12	P<0.0001
Time	387015	5	77403	F (1.798, 28.77) = 253.5	P<0.0001
+/- SGIP1	72338	1	72338	F (1, 16) = 20.37	P=0.0004

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R_6A vs CB1R_6A + SGIP1					
0 min	-8.203	-36.64 to 20.24	No	ns	0.9439
5 min after WIN stimulation	-63.58	-112.6 to -14.60	Yes	**	0.0079
15 min after WIN stimulation	-71.15	-117.3 to -25.02	Yes	**	0.0017
30 min after WIN stimulation	-71.14	-115.6 to -26.69	Yes	**	0.0013
60 min after WIN stimulation	-94.90	-144.0 to -45.85	Yes	***	0.0002

D Gy-YFP + GRK3-Rluc8 – CB1R_8A +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	13219	5	2644	F (5, 80) = 10.65	P<0.0001
Time	124357	5	24871	F (2.275, 36.40) = 100.2	P<0.0001
+/- SGIP1	8.017	1	8.017	F (1, 16) = 0.004352	P=0.9482

Suppl. table 3. Statistical analysis of kinetics of presented protein-protein interactions. Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.

CB1R-YFP + GRK3-Rluc8 – CB1R, CB1R_2A, CB1R_6A, CB1R_8A

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x CB1R variant	19456	15	1297	F (15, 160) = 17.41	P<0.0001
Time	27135	5	5427	F (3.012, 96.40) = 72.84	P<0.0001
CB1R variant	58182	3	19394	F (3, 32) = 46.48	P<0.0001

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0 min					
CB1R vs. CB1R 2A	-6.820	-13.87 to 0.2316	No	ns	0.0588
CB1R vs. CB1R 6A	0.1555	-6.227 to 6.538	No	ns	0.9999
CB1R vs. CB1R 8A	0.7040	-10.93 to 12.34	No	ns	0.9975
5 min after WIN stimulation					
CB1R vs. CB1R 2A	-38.91	-54.16 to -23.66	Yes	****	<0.0001
CB1R vs. CB1R 6A	13.73	2.633 to 24.83	Yes	*	0.0148
CB1R vs. CB1R 8A	18.17	6.791 to 29.56	Yes	**	0.0022
10 min after WIN stimulation					
CB1R vs. CB1R 2A	-40.99	-60.87 to -21.12	Yes	***	0.0003
CB1R vs. CB1R 6A	15.66	4.394 to 26.93	Yes	**	0.0068
CB1R vs. CB1R 8A	21.06	9.619 to 32.50	Yes	***	0.0007
15 min after WIN stimulation					
CB1R vs. CB1R 2A	-51.33	-71.93 to -30.72	Yes	****	<0.0001
CB1R vs. CB1R 6A	12.71	1.939 to 23.48	Yes	*	0.0187
CB1R vs. CB1R 8A	12.62	0.5938 to 24.64	Yes	*	0.0384
30 min after WIN stimulation					
CB1R vs. CB1R 2A	-40.31	-55.68 to -24.95	Yes	****	<0.0001
CB1R vs. CB1R 6A	1.751	-8.621 to 12.12	No	ns	0.9576
CB1R vs. CB1R 8A	-1.273	-14.38 to 11.83	No	ns	0.9919
60 min after WIN stimulation					
CB1R vs. CB1R 2A	-18.20	-40.74 to 4.351	No	ns	0.1280
CB1R vs. CB1R 6A	-4.015	-16.58 to 8.551	No	ns	0.7916
CB1R vs. CB1R 8A	-8.775	-25.21 to 7.664	No	ns	0.4283

Suppl. table 4. Statistical analysis of kinetics of presented protein-protein interactions. Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.

A CB1R-YFP + GRK3-Rluc8 +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	2379	5	475.8	F (5, 80) = 7.613	P<0.0001
Time	28003	5	5601	F (2.339, 37.42) = 89.61	P<0.0001
+ - SGIP1	5295	1	5295	F (1, 16) = 28.23	P<0.0001

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + SGIP1					
0 min	3.686	-3.527 to 10.90	No	ns	0.5737
5 min after WIN stimulation	-16.55	-29.22 to -3.871	Yes	**	0.0085
10 min after WIN stimulation	-19.44	-32.26 to -6.621	Yes	**	0.0026
15 min after WIN stimulation	-24.81	-38.13 to -11.50	Yes	***	0.0003
30 min after WIN stimulation	-19.03	-32.69 to -5.373	Yes	**	0.0043
60 min after WIN stimulation	-7.876	-25.03 to 9.275	No	ns	0.7054

B CB1R_2A-YFP + GRK3-Rluc8 +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	4649	5	929.8	F (5, 80) = 6.885	P<0.0001
Time	78239	5	15648	F (2.298, 36.77) = 115.9	P<0.0001
+ - SGIP1	8754	1	8754	F (1, 16) = 12.67	P=0.0026

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R_2A vs CB1R_2A + SGIP1					
0 min	2.844	-7.480 to 13.17	No	ns	>0.9999
5 min after WIN stimulation	-4.964	-22.11 to 12.19	No	ns	>0.9999
10 min after WIN stimulation	-23.70	-46.61 to -0.7937	Yes	*	0.0406
15 min after WIN stimulation	-19.70	-44.26 to 4.860	No	ns	0.1634
30 min after WIN stimulation	-35.82	-54.95 to -16.69	Yes	***	0.0002
60 min after WIN stimulation	-26.70	-56.97 to 3.577	No	ns	0.1042

C CB1R_6A-YFP + GRK3-Rluc8 +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	752.9	5	150.6	F (5, 80) = 2.776	P=0.0231
Time	3873	5	774.6	F (2.474, 39.58) = 14.28	P<0.0001
+ - SGIP1	122.9	1	122.9	F (1, 16) = 1.095	P=0.3108

D CB1R_8A-YFP + GRK3-Rluc8 +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	371.8	5	74.36	F (5, 80) = 1.187	P=0.3231
Time	1134	5	226.8	F (3.327, 53.23) = 3.619	P=0.0157
+ - SGIP1	1.071	1	1.071	F (1, 16) = 0.005455	P=0.9420

Suppl. table 5. Statistical analysis of kinetics of presented protein-protein interactions. Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.

CB1R-YFP + β -arrestin2-Rluc – CB1R, CB1R_2A, CB1R_6A, CB1R_8A

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x CB1R variant	4037	15	269.1	F (15, 160) = 2.709	P=0.0010
Time	6623	5	1325	F (3.664, 117.3) = 13.33	P<0.0001
CB1R variant	6412	3	2137	F (3, 32) = 20.31	P<0.0001

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0 min					
CB1R vs. CB1R 2A	3.975	-3.738 to 11.69	No	ns	0.4657
CB1R vs. CB1R 6A	2.515	-5.670 to 10.70	No	ns	0.8090
CB1R vs. CB1R 8A	4.040	-2.256 to 10.34	No	ns	0.2753
5 min					
CB1R vs. CB1R 2A	13.41	3.295 to 23.52	Yes	**	0.0099
CB1R vs. CB1R 6A	25.44	16.73 to 34.15	Yes	****	<0.0001
CB1R vs. CB1R 8A	24.66	17.33 to 31.99	Yes	****	<0.0001
10 min					
CB1R vs. CB1R 2A	16.76	3.889 to 29.63	Yes	**	0.0095
CB1R vs. CB1R 6A	26.08	15.65 to 36.50	Yes	****	<0.0001
CB1R vs. CB1R 8A	25.48	13.11 to 37.85	Yes	***	0.0001
15 min					
CB1R vs. CB1R 2A	7.380	-8.523 to 23.28	No	ns	0.5235
CB1R vs. CB1R 6A	15.89	6.848 to 24.93	Yes	***	0.0008
CB1R vs. CB1R 8A	18.19	5.630 to 30.76	Yes	**	0.0049
30 min					
CB1R vs. CB1R 2A	4.511	-15.05 to 24.07	No	ns	0.9066
CB1R vs. CB1R 6A	6.602	-10.88 to 24.08	No	ns	0.6796
CB1R vs. CB1R 8A	10.87	-6.153 to 27.89	No	ns	0.2651
60 min					
CB1R vs. CB1R 2A	0.7437	-16.29 to 17.78	No	ns	0.9992
CB1R vs. CB1R 6A	2.150	-15.91 to 20.21	No	ns	0.9849
CB1R vs. CB1R 8A	-2.384	-20.23 to 15.46	No	ns	0.9788

Suppl. table 6. Statistical analysis of kinetics of presented protein-protein interactions. Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.

A CB1R-YFP + β -arrestin2-Rluc +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	4799	5	959.8	F (5, 95) = 7.702	P<0.0001
Time	32246	5	6449	F (2.222, 42.23) = 51.76	P<0.0001
+ - SGIP1	14155	1	14155	F (1, 19) = 90.51	P<0.0001

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + SGIP1					
0 min	3.561	-3.424 to 10.55	No	ns	0.5754
5 min after WIN stimulation	-20.24	-26.60 to -13.89	Yes	****	<0.0001
10 min after WIN stimulation	-33.77	-46.43 to -21.10	Yes	****	<0.0001
15 min after WIN stimulation	-29.90	-39.31 to -20.48	Yes	****	<0.0001
30 min after WIN stimulation	-29.97	-50.39 to -9.538	Yes	**	0.0030
60 min after WIN stimulation	-18.19	-42.16 to 5.768	No	ns	0.2082

B CB1R_2A-YFP + β -arrestin2-Rluc +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	1502	5	300.4	F (5, 80) = 2.566	P=0.0332
Time	10293	5	2059	F (3.343, 53.49) = 17.58	P<0.0001
+ - SGIP1	1533	1	1533	F (1, 16) = 7.890	P=0.0126

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + SGIP1					
0 min	-3.811	-12.49 to 4.866	No	ns	0.7501
5 min after WIN stimulation	-5.929	-18.19 to 6.332	No	ns	0.6554
10 min after WIN stimulation	-17.69	-30.32 to -5.060	Yes	**	0.0055
15 min after WIN stimulation	-14.55	-33.18 to 4.071	No	ns	0.1720
30 min after WIN stimulation	-8.504	-30.32 to 13.31	No	ns	0.8336
60 min after WIN stimulation	5.272	-15.68 to 26.22	No	ns	0.9702

C CB1R_6A-YFP + β -arrestin2-Rluc +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	1502	5	300.4	F (5, 80) = 2.566	P=0.0332
Time	10293	5	2059	F (5, 80) = 17.58	P<0.0001
+ - SGIP1	1533	1	1533	F (1, 16) = 7.890	P=0.0126

Šidák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
CB1R vs CB1R + SGIP1					
0 min	8.039	-0.6709 to 16.75	No	ns	0.0790
5 min after WIN stimulation	-1.879	-15.85 to 12.10	No	ns	0.9991
10 min after WIN stimulation	0.4922	-11.38 to 12.36	No	ns	>0.9999
15 min after WIN stimulation	3.491	-9.635 to 16.62	No	ns	0.9662
30 min after WIN stimulation	1.389	-8.755 to 11.53	No	ns	0.9988
60 min after WIN stimulation	10.08	-8.389 to 28.55	No	ns	0.5371

D CB1R_8A-YFP + β -arrestin2-Rluc +- SGIP1

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x +- SGIP1	161.3	5	32.27	F (5, 80) = 0.3685	P=0.8687
Time	478.0	5	95.61	F (3.074, 49.18) = 1.092	P=0.3622
+ - SGIP1	170.2	1	170.2	F (1, 16) = 1.030	P=0.3253

Suppl. table 7. Statistical analysis of kinetics of presented protein-protein interactions. Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.

A

Gy-YFP + GRK3-Rluc8 – CB1R, CB1R_2D, CB1R_6D, CB1R_8D

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x CB1R variant	12075	12	1006	F (12, 128) = 4,095	P<0,0001
Time	385956	4	96489	F (1,372, 43,90) = 392,7	P<0,0001
CB1R variant	24512	3	8171	F (3, 32) = 5,273	P=0,0045

Šídák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0 min					
CB1R vs. CB1R_6D	10,73	-28,60 to 50,05	No	ns	0,8251
CB1R vs. CB1R_2D	4,043	-35,39 to 43,48	No	ns	0,9881
CB1R vs. CB1R_8D	-1,474	-40,78 to 37,83	No	ns	0,9994
5 min after WIN stimulation					
CB1R vs. CB1R_6D	29,59	-9,139 to 68,31	No	ns	0,1560
CB1R vs. CB1R_2D	-10,31	-49,24 to 28,61	No	ns	0,8547
CB1R vs. CB1R_8D	27,77	-10,15 to 65,69	No	ns	0,1708
15 min after WIN stimulation					
CB1R vs. CB1R_6D	26,87	-11,66 to 65,39	No	ns	0,2099
CB1R vs. CB1R_2D	-10,70	-49,53 to 28,12	No	ns	0,8398
CB1R vs. CB1R_8D	29,50	-8,333 to 67,33	No	ns	0,1383
30 min after WIN stimulation					
CB1R vs. CB1R_6D	14,67	-16,96 to 46,29	No	ns	0,5418
CB1R vs. CB1R_2D	-11,23	-40,96 to 18,50	No	ns	0,6695
CB1R vs. CB1R_8D	19,54	-10,28 to 49,36	No	ns	0,2520
60 min after WIN stimulation					
CB1R vs. CB1R_6D	-11,81	-53,29 to 29,68	No	ns	0,8336
CB1R vs. CB1R_2D	-35,87	-75,60 to 3,856	No	ns	0,0800
CB1R vs. CB1R_8D	5,121	-35,72 to 45,96	No	ns	0,9814

B

CB1R-YFP + GRK3-Rluc8 – CB1R, CB1R_2D, CB1R_6D, CB1R_8D

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x CB1R variant	2412	15	160,8	F (15, 160) = 5,586	P<0,0001
Time	37038	5	7408	F (2,362, 75,57) = 257,4	P<0,0001
CB1R variant	878,1	3	292,7	F (3, 32) = 1,125	P=0,3534

Šídák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0 min					
CB1R vs. CB1R_2D	3,622	-2,664 to 9,908	No	ns	0,3721
CB1R vs. CB1R_6D	2,412	-3,927 to 8,752	No	ns	0,6920
CB1R vs. CB1R_8D	-2,758	-8,720 to 3,204	No	ns	0,5531
5 min after WIN stimulation					
CB1R vs. CB1R_2D	3,850	-8,120 to 15,82	No	ns	0,7788
CB1R vs. CB1R_6D	8,043	-1,332 to 17,42	No	ns	0,1045
CB1R vs. CB1R_8D	16,93	8,586 to 25,27	Yes	***	0,0002
10 min after WIN stimulation					
CB1R vs. CB1R_2D	-4,682	-16,07 to 6,709	No	ns	0,6204
CB1R vs. CB1R_6D	2,261	-5,535 to 10,06	No	ns	0,8328
CB1R vs. CB1R_8D	9,436	2,071 to 16,80	Yes	*	0,0107
15 min after WIN stimulation					
CB1R vs. CB1R_2D	-2,805	-14,03 to 8,416	No	ns	0,8750
CB1R vs. CB1R_6D	1,579	-4,991 to 8,149	No	ns	0,8967
CB1R vs. CB1R_8D	5,608	-2,182 to 13,40	No	ns	0,2017
30 min after WIN stimulation					
CB1R vs. CB1R_2D	-7,898	-20,18 to 4,386	No	ns	0,2846
CB1R vs. CB1R_6D	-3,031	-14,31 to 8,248	No	ns	0,8628
CB1R vs. CB1R_8D	-6,091	-16,65 to 4,464	No	ns	0,3705
60 min after WIN stimulation					
CB1R vs. CB1R_2D	-0,4438	-14,09 to 13,20	No	ns	0,9997
CB1R vs. CB1R_6D	2,642	-9,330 to 14,61	No	ns	0,9149
CB1R vs. CB1R_8D	-0,2189	-15,03 to 14,59	No	ns	>0,9999

C

CB1R-YFP + β -arrestin2-Rluc – CB1R, CB1R_2D, CB1R_6D, CB1R_8D

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Time x CB1R variant	3403	15	226,9	F (15, 160) = 2,492	P=0,0025
Time	9819	5	1964	F (3,235, 103,5) = 21,58	P<0,0001
CB1R variant	3527	3	1176	F (3, 32) = 18,21	P<0,0001

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
0 min					
CB1R vs. CB1R 2D	-2,787	-9,004 to 3,429	No	ns	0,5562
CB1R vs. CB1R 6D	-3,510	-11,11 to 4,087	No	ns	0,5542
CB1R vs. CB1R 8D	-1,158	-7,425 to 5,109	No	ns	0,9447
5 min after WIN stimulation					
CB1R vs. CB1R 2D	10,46	1,223 to 19,69	Yes	*	0,0255
CB1R vs. CB1R 6D	23,23	16,56 to 29,90	Yes	****	<0,0001
CB1R vs. CB1R 8D	21,76	15,12 to 28,40	Yes	****	<0,0001
10 min after WIN stimulation					
CB1R vs. CB1R 2D	14,13	4,347 to 23,90	Yes	**	0,0058
CB1R vs. CB1R 6D	19,73	8,405 to 31,06	Yes	***	0,0008
CB1R vs. CB1R 8D	19,12	8,490 to 29,75	Yes	***	0,0007
15 min after WIN stimulation					
CB1R vs. CB1R 2D	8,000	-0,8757 to 16,88	No	ns	0,0840
CB1R vs. CB1R 6D	10,54	-0,9145 to 21,99	No	ns	0,0754
CB1R vs. CB1R 8D	7,805	-2,337 to 17,95	No	ns	0,1601
30 min after WIN stimulation					
CB1R vs. CB1R 2D	-3,095	-21,14 to 14,95	No	ns	0,9563
CB1R vs. CB1R 6D	8,065	-10,38 to 26,51	No	ns	0,5892
CB1R vs. CB1R 8D	4,101	-12,88 to 21,08	No	ns	0,8773
60 min after WIN stimulation					
CB1R vs. CB1R 2D	2,852	-18,69 to 24,39	No	ns	0,9800
CB1R vs. CB1R 6D	1,108	-16,31 to 18,52	No	ns	0,9975
CB1R vs. CB1R 8D	6,264	-10,34 to 22,87	No	ns	0,6728

Suppl. table 8. Statistical analysis of kinetics of presented protein-protein interactions. Curves were compared by two-way ANOVA followed by Sidak's multiple comparison test. If the curves were significantly different, post hoc analysis of time points was performed.