

Appendices

Results of statistical analysis of protein-protein interaction

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

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.

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

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.

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

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.

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

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.

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

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.

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

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 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

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.

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

Table 9. 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.