

## Supplemental information

### Generation of SGIP1<sup>-/-</sup> mice

SGIP1<sup>-/-</sup> mice were generated in-house starting with embryonic stem cells obtained from the European Conditional Mouse Mutagenesis Program (EUCOMM). The cells (Sgip1<sup>tm1a(EUCOMM)Hmgu</sup>) carried a SGIP1 gene (GeneBank Accession: NM\_001285852) modified by homologous recombination. The ES cells were of C57Bl/NCrl background.

In these cells, the FRT sites flanked exon 2 of the SGIP1 gene and the LoxP sites bordered additional sequences (Suppl. Fig. 1A). Using a laser-assisted technique, ES cells were injected into 8-cell stage embryos to generate chimeric mice. Sgip1<sup>tm1a+/-</sup> mice were crossed. Selected offspring were mated with Flp-expressing Gt(ROSA)26Sor<sup>tm2(CAG-Flpo,-EYFP)lcs</sup>, to delete aberrant sequences, and their offspring were further crossed with a Cre-recombinase expressing strain Gt(ROSA)26Sor<sup>tm1(ACTB-cre,-EGFP)lcs</sup> to excise the Exon 2. Both mice were from the same source (Bircling, Dierich et al., 2012). This resulted in a shift of the frame in the SGIP1 allele, causing global loss of SGIP1 protein in homozygous SGIP1<sup>-/-</sup> mice. The mice underwent more than 10 backcrossing to obtain constant C57Bl/NCrl background. This also led to the exclusion of the FLP and Cre recombinase coding sequences and to homogenization to C57Bl/NCrl background.

The ablation of SGIP1 was confirmed by PCR analysis and immunoblotting (Suppl. Fig. 1B and 1C).

### Genotyping

Mice were genotyped using tail snip samples collected at the time of weaning. Tails were digested, genomic DNA isolated, and purified using the QIAGEN DNAeasy kit (QIAGEN) according to the manufacturer's instructions. After the extractions, 1.0 µg of DNA was amplified by Polymerase chain reaction (PCR), using combination of three primers designed to identify both the wild-type and targeted alleles annealing inside and outside the deleted region.

Primer A: AGGCACAGCATCCTTAGGCACAGC,

Primer B: GAATGTATCAGGGAAGGTTTCAGCC,

Primer C: TGAAGTATGGCGAGCTCAGACC

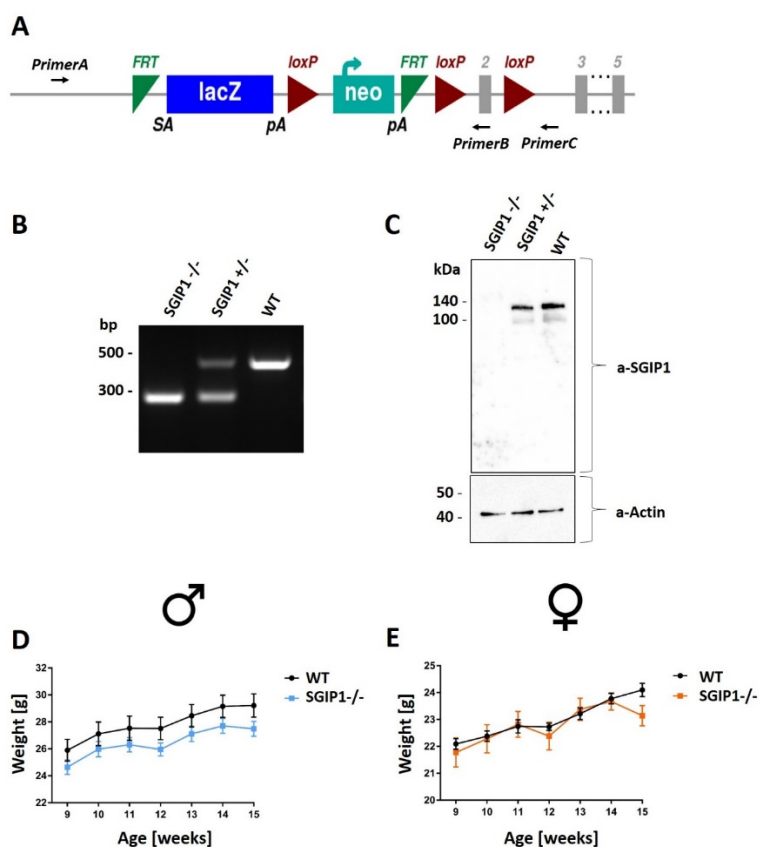
The A-B primer pair allowed amplification of a 456-bp band, that corresponds to the wild-type allele. The A-C primer pair allowed detection of a 245-bp band corresponding to the sequence of the targeted allele. For the PCR reaction we used a polymerase without proofreading activity (Promega GoTaq<sup>®</sup> Green Master Mix). Denaturing, annealing, and extension steps were performed; initial step 95°C lasted 5 minutes, followed by 30 cycles of 95°C for 30 s, annealing at 60°C for 30 s, and extensions at 72°C for 2 minutes. The reaction was terminated at 72°C for 10 minutes, followed by 4°C till separation and visualization of the PCR products on 1.5% agarose gel.

### Immunoblot analysis

Protein samples (50 µg of brain homogenate) were separated on SDS-PAGE Tris-glycine gel (10%) and transferred onto nitrocellulose membranes (Pall Corporation, USA). Membranes were blocked in 5% powdered milk, labeled with home-made guinea pig anti-SGIP1 antibody (H09/h23) 1:1000 characterized in our previous study (Hajkova et al., 2016) and secondary goat anti-guinea pig IgG-HRP antibody (Santa Cruz Biotechnology, USA) 1:5000.

Samples were visualized using the SuperSignal West FEMTO chemiluminescent substrate system (Thermo Fisher Scientific, USA) and detected on the LAS-300 system (Fujifilm, USA).

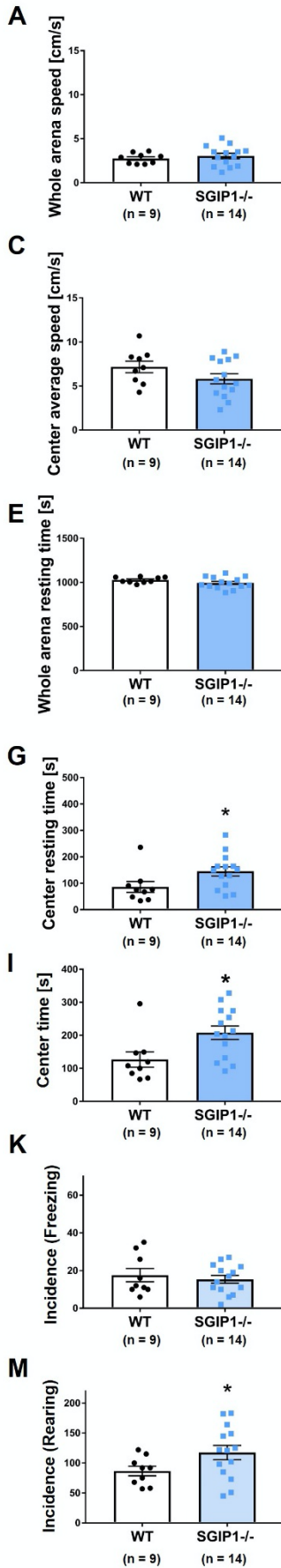
## Supplemental Figures



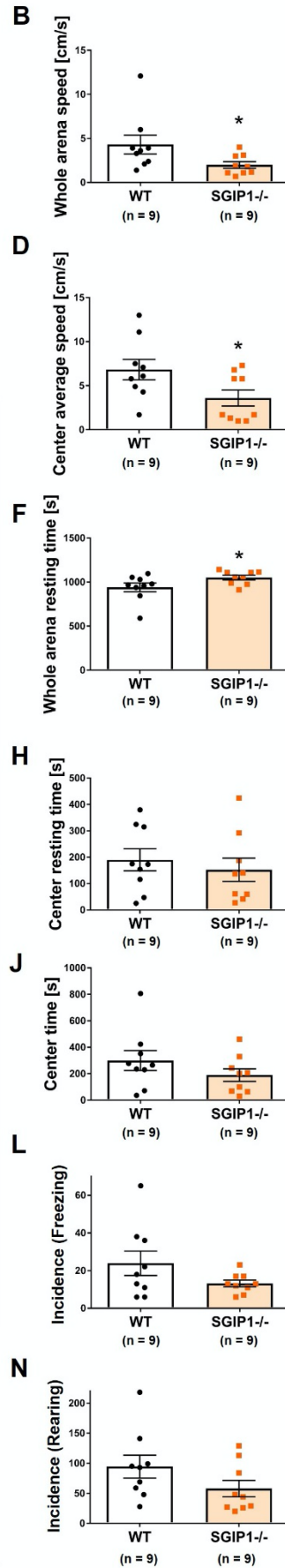
### Supplemental figure 1. SGIP1<sup>-/-</sup> mice generation and characterization

Schematic depiction of the targeting construct used for homologous recombination of the SGIP1 gene. FRT sites allow enzymatic removal of the sequences used for selection, while two loxP sites flank exon 2. Removal of the critical exon 2 leads to loss of SGIP1 protein expression from the targeted allele. Arrows indicate the PCR probe annealing sites. Primer A: aggcacagcatccttaggcacagc, Primer B: gaatgtatcaggaaggttcagcc, Primer C: tgaactgatggcgagctcagacc. (A). The enzymatic excision of exon 2 was confirmed by PCR. The PCR products of the analysis of DNA, extracted from tail biopsy of the parental heterozygous (SGIP1<sup>+/-</sup>) generation, and selected siblings used in the experiment; knock-out (SGIP1<sup>-/-</sup>) and wild type (WT) mice (B). The absence of SGIP1 protein in SGIP1<sup>-/-</sup> mice was confirmed by immunoblotting from the brain homogenates, separated on SDS-PAGE, and detected with the anti-SGIP1 antibody (C). The body weights of the animals were measured during the behavioral testing and no difference between SGIP1<sup>-/-</sup> and WT animals was found (males: (D), females: (E)). The data in panels D, E were analyzed by two-way ANOVA with repeated measures and are presented as means ± SEM

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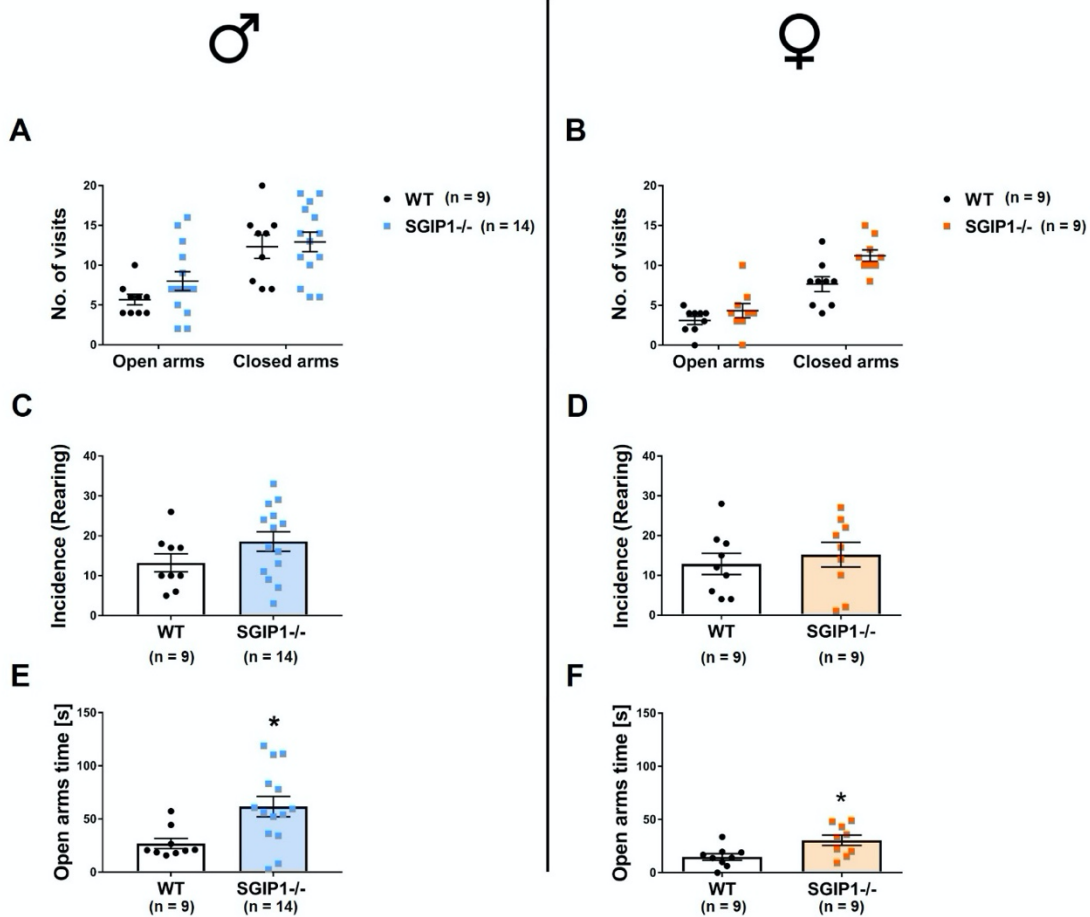


### **Supplemental figure 2. Additional parameters of the open field (OF) test.**

The entire arena (A) and center (C) velocity and resting time (E) were not altered in SGIP1<sup>-/-</sup> males. The SGIP1<sup>-/-</sup> females moved throughout the open field arena and in its center more slowly than WT controls (B, D) and rested more frequently over the entire arena (F). When in the center, SGIP1<sup>-/-</sup> male mice rested more than WT mice (G), while there was no difference in female cohorts (H).

For clarity, we show the raw data for times spent in the center of the open field arena. Male SGIP1<sup>-/-</sup> mice spent more time in the center of the open field (I), we did not detect any significant differences between the female SGIP1<sup>-/-</sup> and WT mice (J). Both male and female SGIP1<sup>-/-</sup> mice exhibited similar numbers of freezing episodes in the open field test as the WT controls (males: K, females: L). SGIP1<sup>-/-</sup> male mice showed higher incidence of rearing (M), and there was no difference in female cohorts (N)

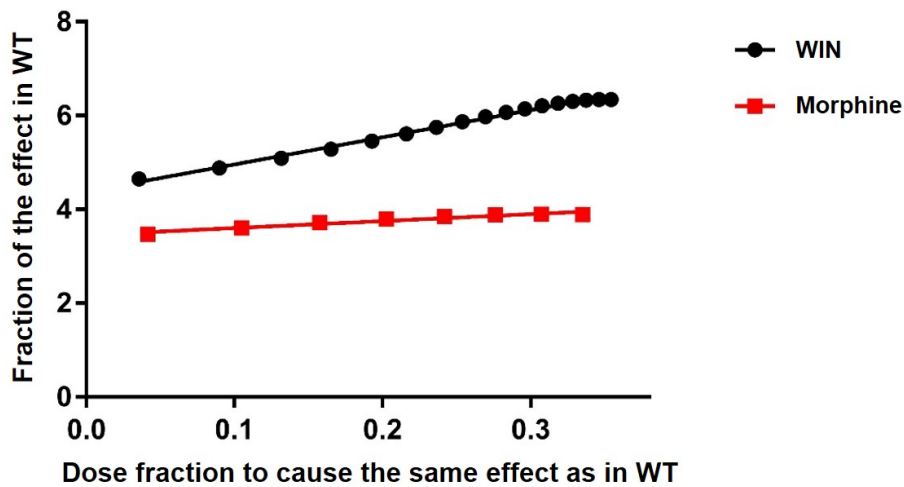
The data were analyzed by Mann-Whitney U test (B, F, G, I, J, ) or by parametric test when the data were normally distributed (A, C-E, H, K-N) and are presented as means ± SEM. \* p<0.05



### Supplemental figure 3. Additional parameters of the elevated plus maze (EPM) tests.

The number of visits into open and closed arms of the EPM is not altered by the deletion of the SGIP1 gene (males: A, females: B). Similarly, the incidence of rearing behavior is comparable in SGIP1<sup>-/-</sup> and WT mice (males: C, females: D). Both male and female SGIP1<sup>-/-</sup> mice spent more time in the open arms of the EPM (males: E, females: F).

The data were analyzed by Mann-Whitney U test (E) or by t-test where the data were normally distributed (C, D, F). The numbers of visits in the EPM were analyzed by two-way ANOVA (A, B) and are presented as means  $\pm$  SEM. \* p < 0.05.



**Supplemental figure 4. Comparison of WIN and morphine effects on antinociception in SGIP1<sup>-/-</sup> and WT males.**

Latency to dose analyses. Ligand concentration required for a specific MPE in the SGIP1 KO mice divided by the ligand concentration needed to reach the same MPE in WT mice was plotted on the X-axis, while the ratio of the MPEs for that dose of ligand in the WT mice is plotted on the Y axis. The lines for the two ligands were then fit by linear regression. The results of the linear regressions are  $y=5.789x+4.38$  and  $y=1.487x+3.453$  for WIN and morphine, respectively. The steeper slope of the WIN line is consistent with a synergistic-like effect with SGIP1 deletion, morphine-induced antinociception is additive-like with SGIP1 absence.

**Supplemental video. A representative behaviors of the mice following THC withdrawal.**

SGIP1<sup>-/-</sup> and WT males were treated with intraperitoneal injections of THC (10 mg/kg) for 9 consecutive days. The mice were injected with a vehicle 30 min after the last drug deliveries, and after another 30 minutes CB1R inverse agonist rimonabant (10 mg/kg) was intraperitoneally injected, as illustrated in (Fig. 3E).

The sample video starts 25 minutes after the rimonabant injections.

*Left chamber:* SGIP1<sup>-/-</sup> male treated with THC.

*Center chamber:* SGIP1<sup>-/-</sup> male chronically treated with vehicle.

*Right chamber:* WT male chronically treated with THC.

Parametric tests						
	Sex	Mean, n		P value		
		WT	SGIP1 <sup>-/-</sup>			
Y-maze, % of SA	M	62.03, n=9	59.11, n=14	0.4533		
	F	70.34, n=9	56.36, n=9	0.1195		
Multiple t-tests						
	Prepulse intensity [db]	Mean, n		t ratio	df	P value
		WT	SGIP1 <sup>-/-</sup>			
PPI MALES, % of PPI	70	29.85	38.11	1.14	21	0.4754
	77	40.32	58.08	2.645	21	0.0592
	82	49.31	59.44	1.343	21	0.4754
	85	53.91	62.56	1.101	21	0.4754
PPI FEMALES, % of PPI	70	30.78	32.98	0.3213	16	0.9526
	77	47.34	45.74	0.2497	16	0.9526
	82	51.42	61.47	2.683	16	0.0637
	85	54.33	56.68	0.4796	16	0.9526

**Supplemental table 1. Statistical analysis of behavior tests results presented in Fig. 1.**

Results of parametric t-tests (SA) and multiple t-tests (PPI). SA, spontaneous alteration; PPI, pre-pulse inhibition

Parametric tests and Mann–Whitney U tests

	Sex	Median, n/ mean, n		U value	P value
		WT	SGIP1 <sup>-/-</sup>		
OF, center time [%]	M	9.025, n=9	17.41, n=14	26	0.0191
	F	18.39, n=9	15.79, n=9		
OF, whole arena distance [cm]	M	3286, n=9	3666, n=14		0.3982
	F	3854, n=9	2379, n=9		
OF, whole arena speed [cm/s]	M	2.756, n=9	3.05, n=14	15	0.0228
	F	3.6, n=9	1.8, n=9		
OF, center speed [cm/s]	M	7.178, n=9	5.821, n=14		0.1362
	F	6.833, n=9	3.6, n=9		
OF, whole arena resting time [s]	M	1028, n=9	994.5, n=14	16.5	0.0334
	F	968.4, n=9	1060, n=9		
OF, center resting time [s]	M	75.7, n=9	151.8, n=14	28	0.0275
	F	190.1, n=9	152.4, n=9		
OF, center permane nce time [s]	M	108.3, n=9	208.9, n=14	26	0.0191
	F	265.3, n=9	201.8, n=9		
OF, freezing episodes	M	17.56, n=9	15.36, n=14		0.6023

OF,	F	23.89, n=9	13.22, n=9		0.1446
	M	86.56, n=9	117.5, n=14		0.0421
rearing	F	94.44, n=9	57.78, n=9		0.1378
incidence					
EPM,	M	6.89, n=9	19.18, n=14	25	0.0158
open					
arms					
time					
[%]					
	F	4.944, n=9	10.16, n=9		0.0173
EPM,	M	1275, n=9	1526, n=14		0.0322
total					
distance					
[cm]					
	F	1437, n=9	1792, n=9		0.0146
EPM,	M	13.22, n=9	18.57, n=14		0.1217
rearing					
	F	12.89, n=9	15.22, n=9		0.5764
EPM,	M	20.65, n=9	57.49, n=14	25	0.0158
open					
arms					
time [s]					
	F	14.83, n=9	30.49, n=9		0.0173
LDB,	M	6.567, n=9	11.63, n=14	36	0.0956
light					
side					
time					
[%]					
	F	1.467, n=9	4.167, n=9	29	0.3284
LDB,	M	980.8, n=9	1109, n=14		0.5433
total					
distance					
[cm]					
	F	745, n=9	1087, n=9		0.1014
TST,	M	84.92, n=9	51.78, n=14		0.0334
immobil					
ity [s]					
	F	150.3, n=9	92.94, n=9		0.0013

Two-way ANOVA						Bonferroni's multiple comparisons test				
	Sex	Source of Variation	F (DFn, DFd)	P value	X axis factor	Mean 1	Mean 2	95.00% CI of diff.	P value	
Weights [g]	M	Interaction	F (6, 126) = 1.17	0.3277						
		Weeks	F (6, 126) = 141.30	<0.0001						
		Genotype	F (1, 21) = 2.16	0.1564						
	F	Interaction	F (6, 114) = 2.87	0.0121						
		Weeks	F (6, 114) = 37.73	<0.0001						
		Genotype	F (1, 19) = 0.26	0.6167						
EPM, visits	M	Interaction	F (1, 21) = 2.40	0.1364						
		Arms	F (1, 21) = 106.70	<0.0001						
		Genotype	F (1, 21) = 0.77	0.3901						
	F	Interaction	F (1, 16) = 3.40	0.0836						
		Arms	F (1, 16) = 81.92	<0.0001						
		Genotype	F (1, 16) = 6.91	0.0182						
FC, freezing time [%]	M	Interaction	F (2, 50) = 0.35	0.7099						
		Context, Cue	F (2, 50) = 39.89	<0.0001						
		Genotype	F (1, 25) = 0.49	0.4926						
	F	Interaction	F (2, 50) = 0.41	0.6656						
		Context, Cue	F (2, 50) = 36.64	<0.0001						

FE, freezing time [log(s)]		Genotype	F (1, 25) = 0.2823 1.21						
	M	Interaction	F (7, 154) = 0.9760 = 0.24						
		Days	F (7, 154) = 7.77 <0.0001						
FE, freezing time [%]		Genotype	F (1, 22) = 0.3586 0.88						
	F	Interaction	F (4, 88) = 4.05 0.0047	Day 1	30.39	30.05	-19.76	to	>0.9999 20.44
		Days	F (4, 88) = 8.40 <0.0001	Day 2	57.42	22.68	14.64	to	<0.0001 54.85
		Genotype	F (1, 22) = 12.77 0.0017	Day 3	35.17	14.83	0.24	to	0.0459 40.44
				Day 4	30.90	9.58	1.22	to	0.0319 41.43
			Day 5	27.61	8.15	-0.65	to	0.0630 39.56	

**Supplemental table 2. Statistical analysis of behavior test results presented in Fig. 2 and Suppl. Fig 1-3.** Results of parametric, nonparametric (Mann-Whitney) t-tests and two-way ANOVA. For the results of the Mann-Whitney test we show median values and U-values, for the results of parametric tests for the data with normal distribution we show mean values.

Cannabinoid tetrad test

Three-way ANOVA				Bonferroni's multiple comparisons test				
	Source of Variation	F (DFn, DFd)	P value	X axis factor	Mean 1	Mean 2	95.00% CI of diff.	P value
Ring test %MPE	Days	F (2, 64) = 43.56	<0.0001	Day 1				
	Genotype	F (1, 32) = 14.52	0.0006	WT_VEH vs. WT_THC	-1.40	72.95	-88.94 to -59.76	<0.0001
	THC or VEH	F (1, 32) = 649.10	<0.0001	WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	-1.40	1.88	-17.88 to 11.31	>0.9999
	Days x Genotype	F (2, 64) = 4.71	0.0123	WT_THC vs. SGIP1 <sup>-/-</sup> _THC	72.95	75.22	-16.86 to 12.32	>0.9999
	Days x THC or VEH	F (2, 64) = 42.32	<0.0001	SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	1.88	75.22	-87.93 to -58.74	<0.0001
	Genotype x THC or VEH	F (1, 32) = 10.21	0.0031	Day 4				
	Days x Genotype x THC or VEH	F (2, 64) = 7.75	0.0010	WT_VEH vs. WT_THC	-0.29	42.32	-57.20 to -28.02	<0.0001
				WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	-0.29	0.55	-14.60 to 14.58	>0.9999
				WT_THC vs. SGIP1 <sup>-/-</sup> _THC	42.32	61.05	-33.32 to -4.137	0.0014
				SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	-0.28	61.05	-75.92 to -46.74	<0.0001
				Day 8				
				WT_VEH vs. WT_THC	-0.24	30.71	-45.55 to -16.37	<0.0001
				WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	-0.24	0.55	-15.38 to 13.80	>0.9999
				WT_THC vs. SGIP1 <sup>-/-</sup> _THC	30.71	56.22	-40.10 to -10.92	<0.0001

				SGIP1 <sup>-/-</sup> _VEH	0.55	56.22	-70.27 to	<0.0001
				vs. SGIP1 <sup>-/-</sup> _THC			-41.08	
Tail flick	Days	F (2, 64) =	<0.0001	Day 1				
			13.29					
%MPE	Genotype	F (1, 32) =	0.0278	WT_VEH vs.	-2.43	14.36	-30.77 to	0.0042
			5.31	WT_THC			-2.82	
	THC or VEH	F (1, 32) =	<0.0001	WT_VEH vs.	-2.43	-1.70	-14.70 to	>0.9999
			71.56	SGIP1 <sup>-/-</sup> _VEH			13.24	
	Days x Genotype	F (2, 64) =	0.1994	WT_THC vs.	14.36	29.91	-29.52 to	0.0130
			1.65	SGIP1 <sup>-/-</sup> _THC			-1.57	
	Days x THC or VEH	F (2, 64) =	0.0026	SGIP1 <sup>-/-</sup> _VEH	-1.70	29.91	-45.58 to	<0.0001
			6.56	vs. SGIP1 <sup>-/-</sup> _THC			-17.64	
	Genotype x THC or VEH	F (1, 32) =	0.0406	Day 4				
			4.56					
	Days x Genotype x THC or VEH	F (2, 64) =	0.1740	WT_VEH vs.	-4.20	8.62	-26.80 to	0.1256
			1.80	WT_THC			1.15	
				WT_VEH vs.	-4.20	-3.03	-15.15 to	>0.9999
				SGIP1 <sup>-/-</sup> _VEH			12.80	
				WT_THC vs.	8.62	11.41	-16.76 to	>0.9999
				SGIP1 <sup>-/-</sup> _THC			11.19	
				SGIP1 <sup>-/-</sup> _VEH	-3.03	11.41	-28.41 to	0.0340
				vs. SGIP1 <sup>-/-</sup> _THC			-0.46	
				Day 8				
				WT_VEH vs.	-4.47	3.84	-22.28 to	>0.9999
				WT_THC			5.67	
				WT_VEH vs.	-4.47	-5.35	-13.09 to	>0.9999
				SGIP1 <sup>-/-</sup> _VEH			14.85	
				WT_THC vs.	3.84	12.12	-22.26 to	>0.9999
				SGIP1 <sup>-/-</sup> _THC			5.69	
				SGIP1 <sup>-/-</sup> _VEH	-5.35	12.12	-31.44 to	0.0022
				vs. SGIP1 <sup>-/-</sup> _THC			-3.50	

Temperature % change	Days	F (2, 64) = <0.0001 15.39	Day 1					
	Genotype	F (1, 32) = 0.0415 4.51	WT_VEH vs. WT_THC	1.51	-4.89	2.81	to <0.0001	
	THC or VEH	F (1, 32) = <0.0001 45.72	WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	1.51	0.74	-2.83	to >0.9999	
	Days x Genotype	F (2, 64) = 0.1194 2.20	WT_THC vs. SGIP1 <sup>-/-</sup> _THC	-4.89	-0.95	-0.16	to 0.0837	
	Days x THC or VEH	F (2, 64) = <0.0001 33.53	SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	0.74	-0.95	5.48	to <0.0001	
	Genotype x THC or VEH	F (1, 32) = 0.324 1.00	Day 4					
	Days x Genotype x THC or VEH	F (2, 64) = 0.2986 1.23	WT_VEH vs. WT_THC	0.04	-0.43	-3.13	to >0.9999	
			WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	0.04	-0.83	-2.73	to >0.9999	
			WT_THC vs. SGIP1 <sup>-/-</sup> _THC	-0.43	-0.95	-3.07	to >0.9999	
			SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	-0.83	-0.95	-3.48	to >0.9999	
			Day 8					
			WT_VEH vs. WT_THC	0.28	-0.56	-2.76	to >0.9999	
			WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	0.28	0.41	-3.73	to >0.9999	
			WT_THC vs. SGIP1 <sup>-/-</sup> _THC	-0.56	-0.78	-3.37	to >0.9999	
		SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	0.41	-0.78	-2.40	to >0.9999		
Rotarod [s]	Days	F (3, 96) = 0.0009 6.01						
	Genotype	F (1, 32) = 0.1440 2.24						

THC or VEH	F (1, 32) = 0.0460 4.31
Days x Genotype	F (3, 96) = 0.3287 1.16
Days x THC or VEH	F (3, 96) = 0.0058 4.43
Genotype x THC or VEH	F (1, 32) = 0.0827 3.21
Days x Genotype x THC or VEH	F (3, 96) = 0.3632 1.08

**Supplemental table 3. Statistical analysis of the cannabinoid tetrad test results presented in Fig. 3.**  
Results of three-way ANOVA analysis.

THC withdrawal test

Three-way ANOVA				Bonferroni's multiple comparisons test				
Source of Variation	F (DFn, DFd)	P value	X axis factor	Mean 1	Mean 2	95.00% CI of diff.	P value	
Headshakes (log transformation)	Withdrawal treatment	F (2, 64) = 17,80	<0.0001	THC				
	Genotype	F (1, 32) = 0,9613	0.3342	WT_VEH vs. WT_THC	4.20	3.74	-0.98 to 1.89	>0.9999
	THC or VEH	F (1, 32) = 13,47	0.0009	SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	4.58	3.63	-0.48 to 2.39	>0.9999
	Withdrawal treatment x Genotype	F (2, 64) = 0,2308	0.7946	VEH				
	Withdrawal treatment x THC or VEH	F (2, 64) = 27,40	<0.0001	WT_VEH vs. WT_THC	4.00	2.08	0.48 to 3.36	0.0007
	Genotype x THC or VEH	F (1, 32) = 0,0006585	0.9797	SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	4.49	2.27	0.79 to 3.67	<0.0001
	Withdrawal treatment x Genotype x THC or VEH	F (2, 64) = 2,606	0.0817	Rimonabant				
				WT_VEH vs. WT_THC	4.01	3.87	-1.30 to 1.58	>0.9999
				SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	3.77	4.48	-2.15 to 0.73	>0.9999
				WT_VEH				

				THC	vs.	4.20	4.00	-0.93	to	>0.9999
				VEH				1.33		
				THC	vs.	4.20	4.01	-0.95	to	>0.9999
				rimonabant				1.32		
				VEH	vs.	4.00	4.01	-1.15	to	>0.9999
				rimonabant				1.12		
				WT_THC						
				THC	vs.	3.74	2.08	0.53	to	0.0001
				VEH				2.80		
				THC	vs.	3.74	3.87	-1.26	to	>0.9999
				rimonabant				1.00		
				VEH	vs.	2.08	3.87	-2.93	to -	<0.0001
				rimonabant				0.66		
				SGIP1 <sup>-/-</sup> _VEH						
				THC	vs.	4.58	4.49	-1.04	to	>0.9999
				VEH				1.22		
				THC	vs.	4.58	3.77	-0.32	to	0.8897
				rimonabant				1.94		
				VEH	vs.	4.49	3.77	-0.41	to	>0.9999
				rimonabant				1.90		
				SGIP1 <sup>-/-</sup> _THC						
				THC	vs.	3.63	2.27	0.23	to	0.0047
				VEH				2.50		
				THC	vs.	3.63	4.48	-1.99	to	0.6334
				rimonabant				0.28		
				VEH	vs.	2.27	4.48	-3.34	to -	<0.0001
				rimonabant				1.08		
Paw shakes (log transformat ion)	Withdrawal treatment	F (2, 64) = 69.02	<0.0001	THC						
	Genotype	F (1, 32) = 5.56	0.0247	WT_VEH		3.64	2.74	-0.59	to	>0.9999
				vs.				2.39		
				WT_THC						
	THC or VEH	F (1, 32) = 18.92	0.0001	WT_VEH		3.64	3.35	-1.20	to	>0.9999
				vs. SGIP1 <sup>-/-</sup> _VEH				1.79		

Withdrawal treatment x Genotype	F (2, 64) = 0.0258 3.88	WT_THC vs. SGIP1 <sup>-/-</sup> _THC	2.74	1.56	-0.31 to 2.67	0.4730
Withdrawal treatment x THC or VEH	F (2, 64) = <0.0001 39.72	SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	3.35	1.56	0.29 to 3.28	0.0045
Genotype x THC or VEH	F (1, 32) = 0.2224 1.55	VEH				
Withdrawal treatment x Genotype x THC or VEH	F (2, 64) = 0.0781 2.65	WT_VEH vs. WT_THC	3.52	1.85	0.18 to 3.16	0.0122
		WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	3.52	3.17	-1.14 to 1.84	>0.9999
		WT_THC vs. SGIP1 <sup>-/-</sup> _THC	1.85	0.38	-0.02 to 2.97	0.0577
		SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	3.17	0.38	1.30 to 4.29	<0.0001
		Rimonabant				
		WT_VEH vs. WT_THC	3.84	4.01	-1.66 to 1.32	>0.9999
		WT_VEH vs. SGIP1 <sup>-/-</sup> _VEH	3.84	3.63	-1.28 to 1.71	>0.9999
		WT_THC vs. SGIP1 <sup>-/-</sup> _THC	4.01	3.88	-1.36 to 1.62	>0.9999
		SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	3.63	3.88	-1.75 to 1.24	>0.9999



Withdrawal treatment x Genotype	F (2, 64) = 0.9341	0.07	VEH					
Withdrawal treatment x THC or VEH	F (2, 64) = 0.3079	1.20	WT_VEH vs. WT_THC	49.56	13.78	-1.43 to 73.00	0.0774	
Genotype x THC or VEH	F (1, 32) = 0.4416	0.61	SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	45.44	3.00	5.24 to 79.65	0.0092	
Withdrawal treatment x Genotype x THC or VEH	F (2, 64) = 0.3127	1.18	Rimonabant					
			WT_VEH vs. WT_THC	81.22	36.56	7.46 to 81.87	0.0043	
			SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	72.89	31.44	4.24 to 78.65	0.0128	
			THC VEH	49.89	49.56	-30.25 to 30.91	>0.9999	
			THC rimonabant	49.89	81.22	-61.91 to -0.75	0.0379	
			VEH rimonabant	49.56	81.22	-62.25 to -1.09	0.0335	
			WT_THC					
			THC VEH	31.44	13.78	-12.91 to 48.25	>0.9999	
			THC rimonabant	31.44	36.56	-35.69 to 25.47	>0.9999	
			VEH rimonabant	13.78	36.56	-26.43 to 47.98	>0.9999	
			SGIP1 <sup>-/-</sup> _VEH					

THC vs. VEH	51.67	45.44	-24.36 to 36.80	>0.9999
THC vs. rimonabant	51.67	72.89	-51.80 to 9.36	>0.9999
VEH vs. rimonabant	45.44	72.89	-58.02 to 3.136	0.1522
SGIP1 <sup>-/-</sup> _THC				
THC vs. VEH	10.11	3.00	-23.47 to 37.69	>0.9999
THC vs. rimonabant	10.11	31.44	-51.91 to 9.25	>0.9999
VEH vs. rimonabant	3.00	31.44	-59.02 to 2.14	0.1075

R based linear model

Jumps

contrast	null.value	estimate	std.error	statistic	adj.p.value
THC					
WT_VEH vs. WT_THC	0	-1.44E-10	2858.766	-5.04E-14	>0.9999
SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	0	16.79851	1905.844	0.008814	>0.9999
WT_THC vs. SGIP1 <sup>-/-</sup> _THC	0	16.7985077	2021.452996	0.008310115	>0.9999
VEH					
WT_VEH vs. WT_THC	0	4.39E-13	2858.766	1.54E-16	>0.9999
SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	0	19.36346	1905.844	0.01016	>0.9999
WT_THC vs. SGIP1 <sup>-/-</sup> _THC	0	19.36345705	2021.452882	0.00957898	>0.9999
Rimonabant					
WT_VEH vs. WT_THC	0	20.62986	2021.453	0.010205	>0.9999
SGIP1 <sup>-/-</sup> _VEH vs. SGIP1 <sup>-/-</sup> _THC	0	5.46806	1.002107	5.456561	<0.0001
WT_THC vs. SGIP1 <sup>-/-</sup> _THC	0	0.943557858	0.128119212	7.364686723	<0.0001

WT_VEH					
THC vs. rimonabant	0	- 1.45E- 10	2858.7 66	-5.06E- 14	>0.9999
THC vs. VEH	0	- 1.45E- 10	2858.7 66	-5.08E- 14	>0.9999
WT_THC					
THC vs. rimonabant	0	20.629 86	2021.4 53	0.01020 5	>0.9999
THC vs. VEH	0	- 6.24E- 13	2858.7 66	-2.18E- 16	>0.9999
SGIP1-/-_VEH					
THC vs. rimonabant	0	16.105 36	1905.8 44	0.00845 1	>0.9999
THC vs. VEH	0	- 1.45E- 13	2695.2 7	-5.39E- 17	>0.9999
SGIP1-/-_THC					
THC vs. rimonabant	0	4.7749 13	0.7100 84	6.72443 3	<0.0001
THC vs. VEH	0	2.5649 49	0.7337 99	3.49543 7	0.0054

**Supplemental table 4. Statistical analysis of the withdrawal experiment results presented in Fig. 3.**

Results of three-way ANOVA (headshakes, paw shakes, scratching/grooming) and R based general linear model (jumps). In order to analyze data with equal variances the headshakes and paw shakes data were log transformed.

Parametric test and Mann–Whitney U test

	Sex	Median, n/ mean, n	U value	P value	
		WT	SGIP1 <sup>-/-</sup>		
Tail flick, average latency	M	1.983, n=9	2.872, n=14	8	0.0002
	F	1.336, n=11	2.122, n=11		0.0032
Two-way ANOVA					
	Sex	Source of Variation	F (DFn, DFd)	P value	
Tail flick, single trial latency	M	Interaction	F (2, 42) = 0.0718	0.9309	
		Trials	F (2, 42) = 0.0401	0.9608	
		Genotype	F (1, 21) = 9.933	0.0048	
	F	Interaction	F (2, 40) = 0.9584	0.3921	
		Trials	F (2, 40) = 3.117	0.0552	
		Genotype	F (1, 20) = 12.1	0.0024	

**Supplemental table 5. Statistical analysis of tail flick tests results presented in Fig. 4.**

The results of parametric t-test, Mann-Whitney U test and two-way ANOVA.

R based linear model

	contrast	null.value	estimate	std.error	statistic	adj.p.value
Day	Baseline					
1	WT_RIM vs. WT_VEH	0	0.132593	0.357999	0.370372	>0.9999
	SGIP1-/-_RIM vs. SGIP1-/-_VEH	0	0.254815	0.357999	0.711776	>0.9999
	SGIP1-/-_RIM vs. WT_RIM	0	1.744074	0.357999	4.871733	0.0002
	SGIP1-/-_VEH vs. WT_VEH	0	1.621852	0.357999	4.530329	0.0002
	SGIP1-/- vs. WT	0	3.862963	0.253143	15.25998	<0.0001
	RIM vs. VEH	0	3.995556	0.253143	15.78377	<0.0001
	30 min					
	WT_RIM vs. WT_VEH	0	-0.30444	0.357999	-0.85041	>0.9999
	SGIP1-/-_RIM vs. SGIP1-/-_VEH	0	-1.37444	0.357999	-3.83924	0.0046
	SGIP1-/-_RIM vs. WT_RIM	0	1.254444	0.357999	3.504047	0.0170
	SGIP1-/-_VEH vs. WT_VEH	0	2.324444	0.357999	6.492885	<0.0001
	SGIP1-/- vs. WT	0	3.969815	0.253143	15.68208	<0.0001
	RIM vs. VEH	0	1.559444	0.253143	6.160322	<0.0001
	60 min					
	WT_RIM vs. WT_VEH	0	0.057778	0.357999	0.161391	>0.9999
	SGIP1-/-_RIM vs. SGIP1-/-_VEH	0	-0.56556	0.357999	-1.57977	0.9290
	SGIP1-/-_RIM vs. WT_RIM	0	1.365556	0.357999	3.814414	0.0045
	SGIP1-/-_VEH vs. WT_VEH	0	1.988889	0.357999	5.555575	<0.0001
	SGIP1-/- vs. WT	0	4.081481	0.253143	16.1232	<0.0001
	RIM vs. VEH	0	4.056111	0.253143	16.02298	<0.0001
	WT					
	60 min vs Baseline	0	0.103704	0.170846	0.607001	>0.9999
	30 min vs Baseline	0	0.00037	0.170846	0.002168	>0.9999
	SGIP1-/-					
	60 min vs Baseline	0	-0.86759	0.215951	-4.01754	0.0023
	30 min vs Baseline	0	0.106852	0.170846	0.625427	>0.9999
Day	Baseline					
3	WT_RIM vs. WT_VEH	0	0.417407	0.357999	1.165947	0.9970
	SGIP1-/-_RIM vs. SGIP1-/-_VEH	0	-0.15222	0.357999	-0.4252	>0.9999
	SGIP1-/-_RIM vs. WT_RIM	0	1.205926	0.357999	3.368519	0.0250
	SGIP1-/-_VEH vs. WT_VEH	0	1.775555	0.357999	4.95967	<0.0001
	SGIP1-/- vs. WT	0	3.462592	0.253143	13.67839	<0.0001
	RIM vs. VEH	0	2.707407	0.310036	8.732558	<0.0001
	30 min					
	WT_RIM vs. WT_VEH	0	0.145556	0.357999	0.406581	>0.9999

SGIP1-/-_RIM vs. SGIP1-/-_VEH	0	-0.61333	0.357999	-1.71323	0.8681
SGIP1-/-_RIM vs. WT_RIM	0	1.005556	0.357999	2.808824	0.1395
SGIP1-/-_VEH vs. WT_VEH	0	1.764444	0.357999	4.928633	<0.0001
SGIP1-/- vs. WT	0	1.909444	0.253143	7.542938	<0.0001
RIM vs. VEH	0	1.909444	0.253143	7.542938	<0.0001
60 min					
WT_RIM vs. WT_VEH	0	0.63	0.357999	1.759783	0.8424
SGIP1-/-_RIM vs. SGIP1-/-_VEH	0	0.142222	0.357999	0.39727	>0.9999
SGIP1-/-_RIM vs. WT_RIM	0	1.214444	0.357999	3.392315	0.0244
SGIP1-/-_VEH vs. WT_VEH	0	1.702222	0.357999	4.754827	<0.0001
SGIP1-/- vs. WT	0	2.339444	0.253143	9.24158	<0.0001
RIM vs. VEH	0	2.339444	0.253143	9.24158	<0.0001
WT					
60 min vs Baseline	0	0.087778	0.170846	0.513782	>0.9999
30 min vs Baseline	0	0.035556	0.170846	0.208114	>0.9999
SGIP1-/-					
60 min vs Baseline	0	0.05537	0.170846	0.324096	>0.9999
30 min vs Baseline	0	-0.07019	0.170846	-0.41081	>0.9999

**Supplemental table 6. Statistical analysis of CB1R antagonist experiment results presented in Fig. 5.**

The results of R based analysis by general linear model with a set of contrasts between defined groups.

Test	Data in the figures
Parametric t-test	1A-B, 2B-D, 2F-H, 2J, 2L-N, 4B, Suppl.2A, Suppl.2C-E, Suppl.2H, Suppl.2K-N, Suppl.3C-D, Suppl.3F
Mann-Whitney U-test	2A, 2E, 2I, 2K, 4A, Suppl.2B, Suppl.2F-G, Suppl.2I-J, Suppl.3E
Multiple t-tests	1C-D
Two-way ANOVA with repeated measures	2O-R, 4C-D, Suppl.1D-E, Suppl.3A-B
Three-way ANOVA	3A-D, 3F-H
Nonlinear regression, ED <sub>50</sub>	4E-J
General linear model	5A-B
General linear model, Poisson distribution	3I

**Supplemental table 7. Overview of used statistical tests.**