

Supplemental Material

Supplementary Table 1

Liver		
Gene	F/R	Sequence (5'-3')
Serine palmitoyltransferase, long chain base subunit (<i>Sptlc3</i>)	F	AGG TTCAGCATGTCCTCTGCA
	R	ACCGAGCTCATCAACGACTTC
insulin receptor substrate 1 (<i>Irs1</i>)	F	GTCCACGCTGTCCCTTTCTC
	R	TTTCCTGGGTAGTGAATTTGTTCT
Housekeeping genes		
Glyceraldehyde-3- phosphate dehydrogenase (<i>Gapdh</i>)	F	TCAAGAAGGTGGTGAAGCAG
	R	TGGGAGTTGCTGTTGAAGTC
Succinate dehydrogenase complex subunit (<i>Sdha</i>)	F	TACAAAGTGCGGGTTCGATGA
	R	TGTTCCCAAACGGCTTCT

White adipose tissue		
Gene	F/R	Sequence (5'-3')
<i>Leptin</i>	F	GGTGAGCGGGATCAGGTTTT
	R	TCACCCCTCAGATCCTCCAA
Housekeeping genes		

Tyrosine 3 mono oxygenase tryptophan 5 mono oxygenase (<i>Ywhaz</i>)	F	CTTGTGAGGCTGTGACACAAACA
	R	CAAGAGTGTGCACGCAGACA
Non-POU-domain- containing, octamer binding protein (<i>Nono</i>)	F	CTGTCTGGTGCATTCTGA ACTAT
	R	AGCTCTGAGTTCATTTTCCCATG

Brown adipose tissue

Gene	F/R	Sequence (5'-3')
Leptin	F	GGTGAGCGGGATCAGGTTTT
	R	TCACCCCTCAGATCCTCCA
Housekeeping genes		
Ubiquitin C (<i>Ubc</i>)	F	AGACAGACGTACCTTCCTCACCA
	R	CCCATCACACCCAAGAACAAG
valosin containing protein (<i>Vcp</i>)	F	TGGCCGTCTAGATCAGCTCAT
	R	TTTCGCAGATTGGCTTTTAGG

Supplementary Figures

Figure S1. Related to Fig.1

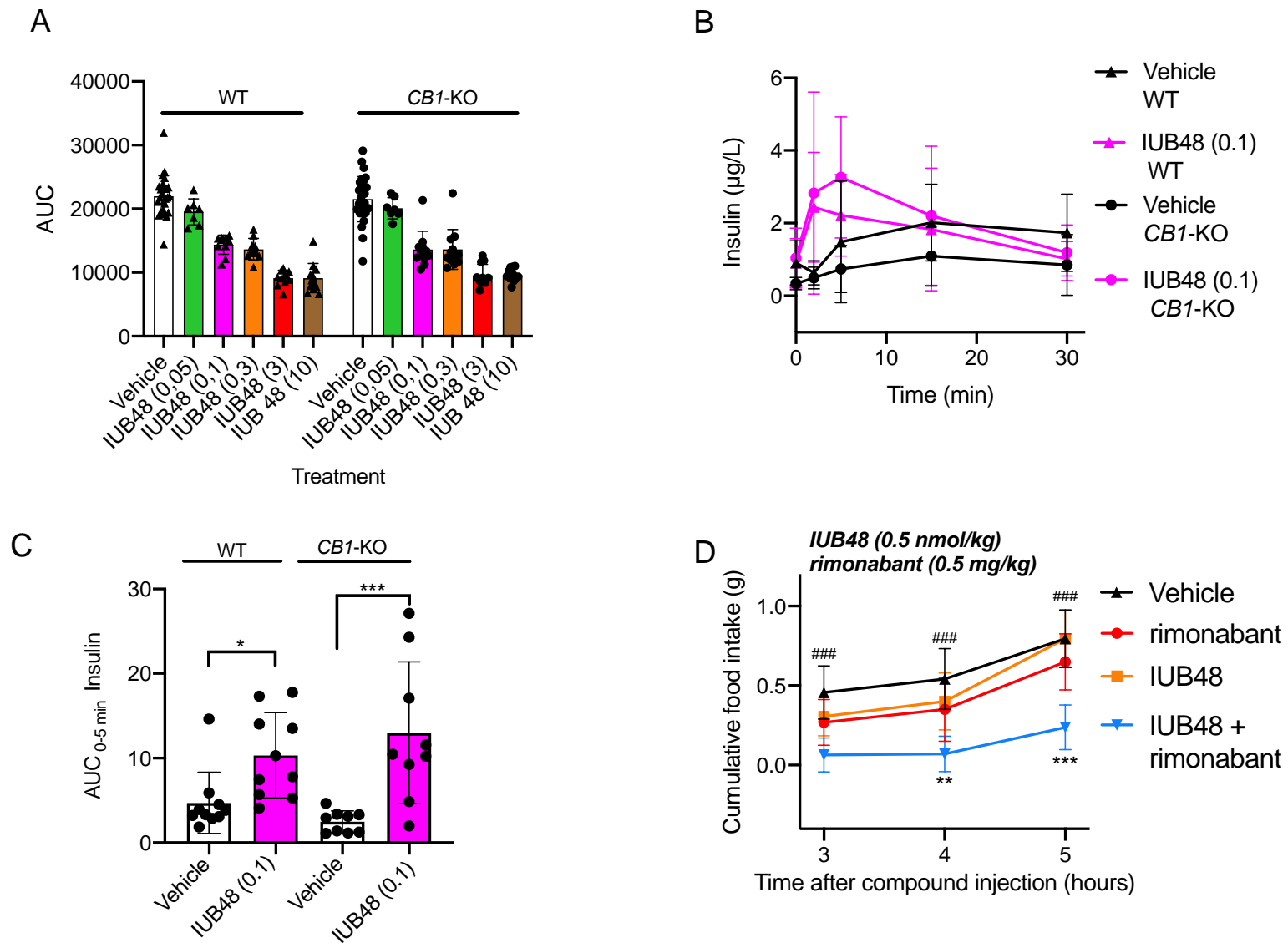


Figure S1: (A) Area under the curve (AUC) related to the glucose tolerance test described in Fig 1C. Data were analyzed by 2-way ANOVA, and no significant interaction between genotype and treatment was detected. Genotype effect was: $F(1, 146)=0.007798$, $P=0.9298$; drug effect was: $F(5, 146)=122.2$, $P<0.0001$; drug x genotype effect was: $F(5, 146)=0.2756$, $P=0.9259$. (B) Glucose-stimulated plasma insulin secretion in 15 hrs-fasted WT or *CBI*-KO mice pre-treated with IUB48 at the dose of 0.1 nmol/kg, 1 hour before the glucose challenge. $N=9/10$ per treatment or genotype. (C) The acute insulin response related to the experiment in (B) was calculated as the area under the curve (AUC) of insulin concentrations during the first 5 min after glucose injection. Data were analyzed by 2-way ANOVA followed by Sidak's multiple comparison test. Genotype x treatment effect was not significant [$F(1, 34)=2.104$, $P=0.1561$]. Treatment effect was: $F(1, 34)=22.67$, $P<0.0001$], genotype effect was: $F(1, 34)=0.01583$, $P=0.9006$]. Post-test: * $P<0.05$ and *** $P<0.001$ as indicated in the graph. (D) Cumulative food intake in a cohort of normal-weight C57BL/6J mice analyzed under non-fasting conditions (light-phase) after treatment with vehicle, IUB48 (0.5 nmol/kg), rimonabant (0.5 mg/kg), or the combination of the two drugs. All mice were singly-housed for at least 4 weeks before the start of the study and during the actual study. $N=8/9$ per group. Data were analyzed by two-way ANOVA repeated measurements with Tukey's multiple comparison test for individual time points. Treatment effect was $F(3, 30)=18.13$, $P<0.0001$; time x treatment effect was $F(6, 60)=3.623$, $P=0.0039$. Post-test: ### $P<0.001$ comparing vehicle vs IUB48 + rimonabant. ** $P<0.01$ and *** $P<0.001$ comparing IUB48 + rimonabant vs monotherapies.

All data are mean \pm SD.

Figure S2. Related to Fig.2

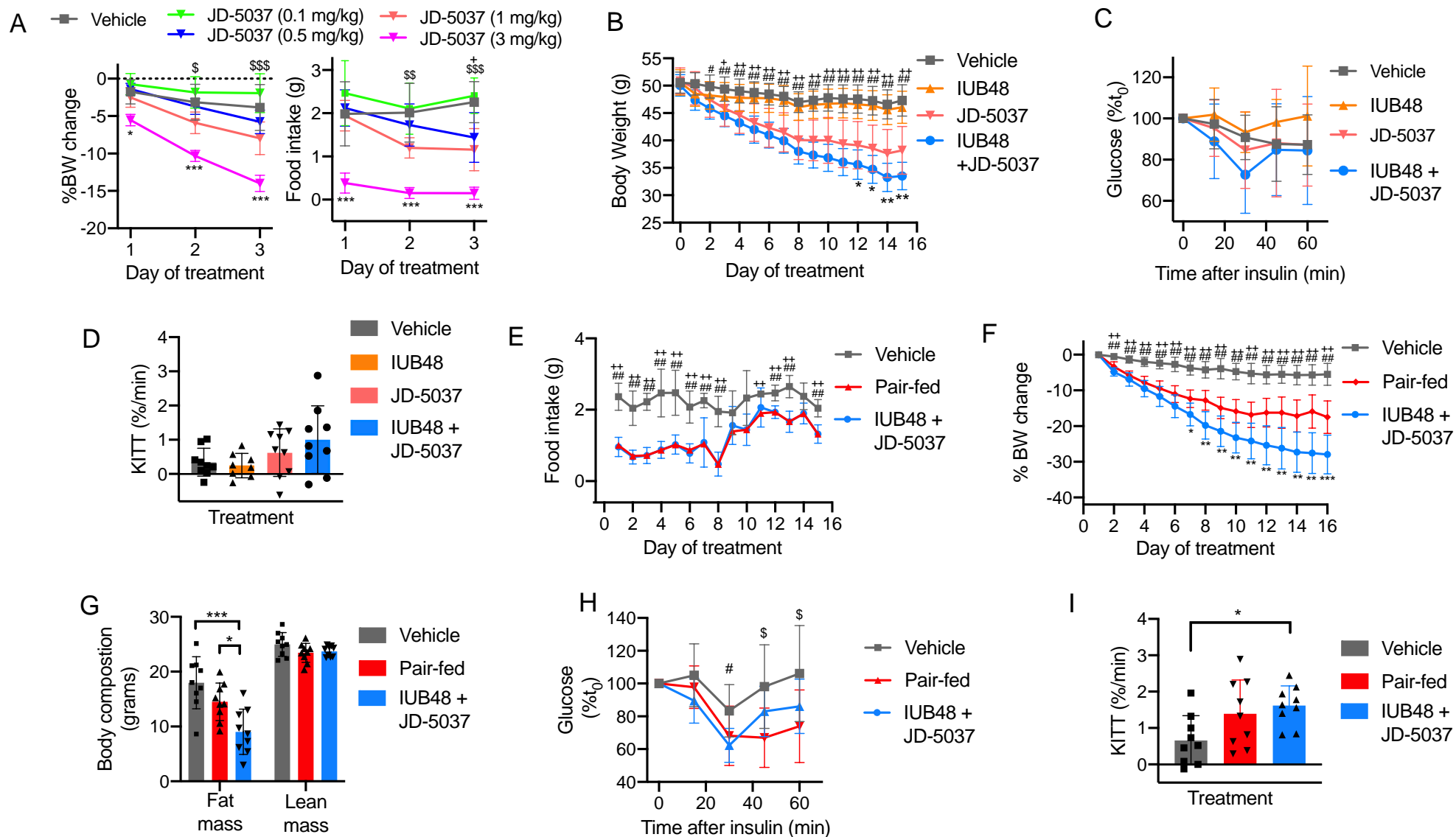


Figure S2.

(A) One cohort of C57BL6/J DIO mice was treated for 3 days with vehicle (Saline in 1% Tween, 4% DMSO) or JD-5037 at different doses, as indicated in the graphs. N=7/14 per treatment or dose. All mice were single-housed for at least 4 weeks before the start of the study and during the actual study. Data were analyzed by two-way ANOVA repeated measurements with Tukey's multiple comparison test for individual time points. For the measurement of body weight (BW) changes, treatment effects was: $F(4, 38) = 28.72$, $P < 0.0001$; time x treatment effect was: $F(8, 76) = 22.42$; $P < 0.0001$. For food intake, treatment effects was: $F(4, 38) = 32.46$, $P < 0.0001$; time x treatment effects was: $F(8, 76) = 3.611$; $P = 0.0013$. Post-test: $\$P < 0.05$, $\$$$$P < 0.001$ comparing vehicle vs JD-5037 1mg/kg; + $P < 0.05$ comparing vehicle vs JD-5037 0.5mg/kg; * $P < 0.05$, *** $P < 0.001$ comparing JD-5037 3 mg/kg vs all other groups. (B) Raw data (body weight in grams) relative to Fig. 2A. Data were analyzed by two-way ANOVA repeated measurements with Tukey's multiple comparison test for individual time points. Treatment effect is $F(3, 31) = 21.84$, $P < 0.0001$; time x treatment effect is $F(45, 465) = 23.99$, $P < 0.0001$. Post-test: # $P < 0.05$, ## $P < 0.01$ comparing vehicle vs JD-5037; + $P < 0.05$, ++ $P < 0.01$ comparing vehicle vs IUB48 + JD-5037; * $P < 0.05$ and ** $P < 0.01$ comparing IUB48 + JD-5037 vs monotherapies. (C) Effects on insulin tolerance test (percentage of change from basal glucose levels) at day 8 of treatment in the animals described in Figure 2. The test was performed using 0.75 U/kg lean mass of insulin (Humulin, Eli Lilly) after 5 hours of fasting. Data were analyzed by two-way ANOVA repeated measurements, and no statistical changes between groups were detected. Treatment effect $F(3, 30) = 2.622$, $P = 0.0688$; time x treatment effect $F(12, 120) = 1.327$, $P = 0.2120$. (D) Effects on glucose disappearance rate (KITT) from the analysis of insulin tolerance described in (C). Data were analyzed by one-way ANOVA, and no statistical changes between groups were detected [$F(3, 31) = 2.234$, $P = 0.1039$].

(E-F) Effects on daily food intake (grams) (E) and body weight (BW) change (% change from day 0) (F) in one cohort of C57BL6/J DIO mice treated with daily injections of vehicle (saline with 1% Tween and 4% DMSO i.p. + saline subq.) or the combination JD-5037 + IUB48 (1 mg/kg of JD-5037 i.p. + 100 nmol/kg of IUB48 subq.). A third group of animals was treated with vehicle (saline with 1% Tween and 4% DMSO i.p. + saline subq.) and pair-fed to mice treated with JD-5037 + IUB48. N=9 mice per group. The animals were housed as n=1/2 per cage throughout the study, using an equal number of cages (N=6) per treatment group. Such a housing paradigm was setup at least 4 weeks before the start of the study. N=6 independent food intake measures were obtained by the average amount of food daily consumed divided by the number of animals contained in each cage. Data were analyzed by two-way ANOVA repeated measurements with Tukey's multiple comparison test for individual time points. In E, treatment effect was: $F(2, 15)=90.54$, $P<0.0001$; time x treatment effect was: $F(28, 210)=3.982$, $P<0.0001$. In F, treatment effect was: $F(2, 24) = 67.56$, $P<0.0001$; time x treatment effect was: $F(30, 360)=28.40$, $P<0.0001$. Post-test: ++ $P<0.01$ comparing vehicle vs JD-5037 + IUB48; ## $P<0.01$ comparing vehicle vs pair-fed; * $P<0.05$, ** $P<0.01$, *** $P<0.001$ comparing JD-5037 + IUB48 vs pair-fed. **(G)** Effects on body composition at day 16 of treatment, as described in E-F. Data were analyzed by 1-way ANOVA [fat mass: $F(2, 24)=10.74$, $P=0.0005$; lean mass $F(2, 24)=2.123$, $P=0.1416$] followed by Tukey's post-test. Post-test: * $P<0.05$ and *** $P<0.001$ as indicated in the graphs. **(H)** Effects on insulin tolerance (% change from basal glucose levels) at D15 of treatment in the animals described in (F-G). The animals were treated with 0.75 U/kg of Insulin (Actarapid, Novo Nordisk) after 5 hours of fasting. Data were analyzed by two-way ANOVA repeated measurements with Tukey's multiple comparison test for individual time points. Treatment effects: $F(2, 24)=4.320$, $P=0.0250$; time x treatment effect: $F(8, 96)=4.358$, $P=0.0002$. Post-test: # $P<0.05$ comparing vehicle vs IUB48 + JD-5037, \$ $P<0.05$ comparing vehicle vs pair-fed. **(I)** Effects on glucose

disappearance rate (KITT) from the analysis of insulin tolerance described in (**H**). Data were analyzed by one-way ANOVA [F(2, 24)=4.152, P=0.0283] with Tukey's multiple comparison test. Post-test: *P<0.05 as indicated in the graph.

All data are mean \pm SD.

Figure S3. Related to Fig.4

● Vehicle ● Semaglutide ● JD-5037 ● Semaglutide + JD-5037

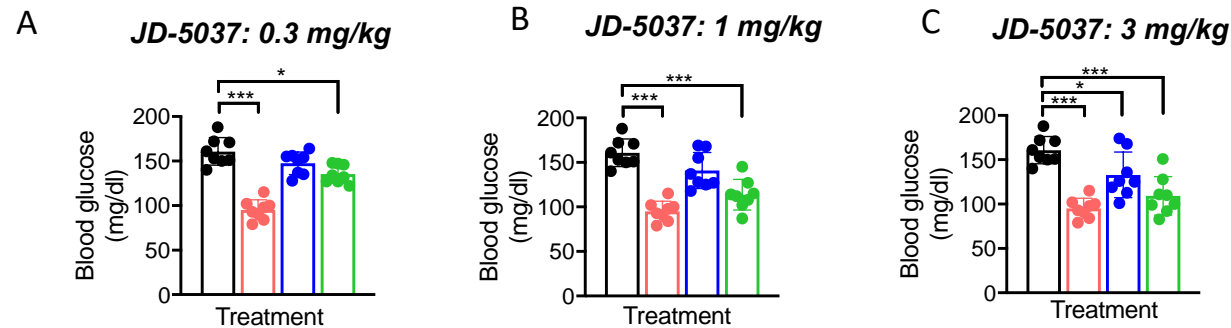


Figure S3

Effects on basal glucose levels (T0) related to the insulin tolerance test described in Fig. 4. The measurements were performed at the end of the chronic study described in Fig 4. Data were analyzed by one-way ANOVA [$F(7, 56) = 12.27, P < 0.0001$] followed by Tukey's post-test. The visual and statistical representation of the different groups have been separated in different panels (A,B, C) corresponding to different doses of JD-5037 (0.3 mg/kg, 1 mg/kg, 3 mg/kg) to increase the readability of the results. Post-test results were: * $P < 0.05$ and *** $P < 0.001$ as indicated.

All data are mean \pm SD.