Supplemental Materials and Methods

***β3AR* mRNA expression and β3-AR signaling assessment.**

Expression levels of *β3AR* were determined by quantitative qRT-PCR. Briefly, RNA was isolated from frozen adipose tissue using Qiagen RNeasy tissue kit following the manufacturer's protocol (Qiagen). cDNA was synthesized from the isolated RNA using SuperScript III and random hexamers (Invitrogen). Quantitative PCR was performed with the Light Cycler-fast start master SYBR green mix (Roche Diagnostics) with the following primer sets: b3ar-for: 5'-cag cca gcc ctg ttg aag-3' and b3ar-rev: 5'-cct tca tag cca tca aac ctg-3'. To evaluate the *in vivo* β3-adrenergic signaling, CL316, 243 was injected intraperitoneally into BALB/c and B6 *ob/ob* mice. Before injection, 15 and 30 min latter blood was collected from tail vain and analyzed for serum glycerol levels.

Supplemental figure legends

Supplemental figure 1. *Agrp* deletion improve glucose homeostasis in *ob/ob* mice. (**A**) Representation of fasting glucose and fasting insulin levels measured in serum from C57BL6/J (B6) *ob/ob* and *ob/ob Agrp*$^{-/-}$ males. (**B**) HOMA index was calculated in B6 *ob/ob* and *ob/ob Agrp*$^{-/-}$ mice (n=5). Date are expressed as average±SEM. Unpaired t-tests were performed *, # $P<0.05$ for glucose and insulin, respectively.

Supplemental figure 2. BALB/c mice are resistant to obesity and glucose intolerance induced by high fat diet. (**A**) Body composition (fat mass and fat-free mass) were analyzed in BALB/c and C57BL6/J (B6) males and females fed a high fat diet (HFD) for 3 months (n=5-11). (**B**) Fasting glycemia and insulin level were measured in 3 months HFD mice after a 12h fast. *, # $P<0.05$ for glucose and insulin, respectively. (**C**) Glucose tolerance test (GTT) was performed by measuring glycemia after an overnight fast at the indicated times after intraperitoneal injection of glucose, and area under the curve (AUC) was calculated in HFD BALB/c and B6 females (n=4). Date are expressed as average±SEM. Unpaired t-tests were performed ***$P<0.0005$.

Supplemental figure 3. **Energy expenditure and adipose tissue lipases expression in BALB/c and C57BL6/J mice on high fat diet (HFD**) (**A**) Oxygen consumption normalized to fat-free body mass was determined during light and dark cycle (n=4). (**B**) adipose triglyceride lipase (ATGL) and (**C**) comparative gene identification-58 (CGI-58) protein, (**D**) total hormone sensitive lipase (HSL), (**E**) phosphorylated HSL on S660expression in inguinal adipose tissue

from BALB/c and B6 fed a HFD for 3 months (n=5). Date are expressed as average±SEM. Two-way ANOVA (A) and unpaired t-tests (B, C, D, E) were performed *P<0.05, **P<0.005.

Supplemental figure 4. Unchanged β3-adrenergic signaling between BALB/c and B6 *ob/ob* mice. (**A**) Quantification by qRT-PCR of mRNA expression of the beta3 adrenergic receptor (*β3-AR*) normalized to *Actin*. (**B**) Glycerol concentrations measured in fed mice before CL 316,243 administration, 15 min and 30 min following intraperitoneal injection in BALB/c and B6 *ob/ob* mice. (n=5). Date are expressed as average±SEM. Unpaired t-tests were performed *P<0.05

Supplemental figure 5. HSL and Perilipin A protein expression in BALB and B6 *ob/ob* adipose tissue. Immunoblots and densitometry analysis of (**A**) hormone-sensitive lipase (HSL), phosphorylated HSL on (**B**) S563 and (**C**) S660, (**D**) Perilipin A in inguinal fat from BALB/c, C57BL6/J (B6) and F1 *ob/ob* (n=6). Data are expressed as average±SEM. One-way ANOVA (A) and unpaired t-tests (B, C, D) tests were performed *P<0.05, **P<0.005,

Supplemental figure 6. CAY10499 inhibits β3AR agonist induced lipolysis. FFA release from fat explants incubated with or without CL 316,243 and CAY10499. (n=6). Data are expressed as average±SEM. One-way ANOVA test was performed *P<0.05.

Supplemental Table 1. Genotyping of SNPs located on mouse chromosome 2.

The underlined markers were not found polymorph between C57BL6/J and BALB/c parental strains

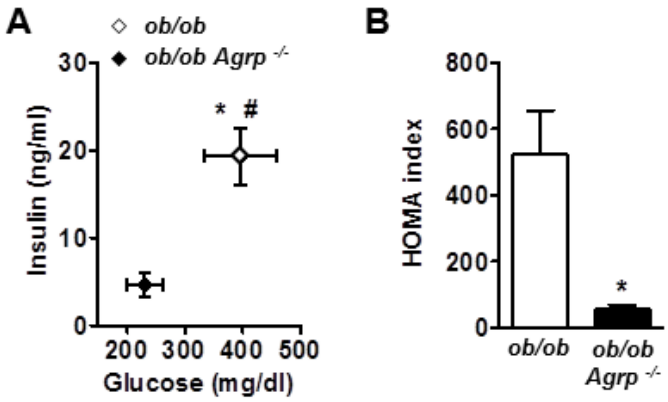
[1] *from the Mouse Genome Database (www.phenome.jax.org)*

[2] *Digestion product size obtained after amplification and digestion, with the indicated enzyme, of genomic DNA from C57BL6/J and BALB/c mice*.

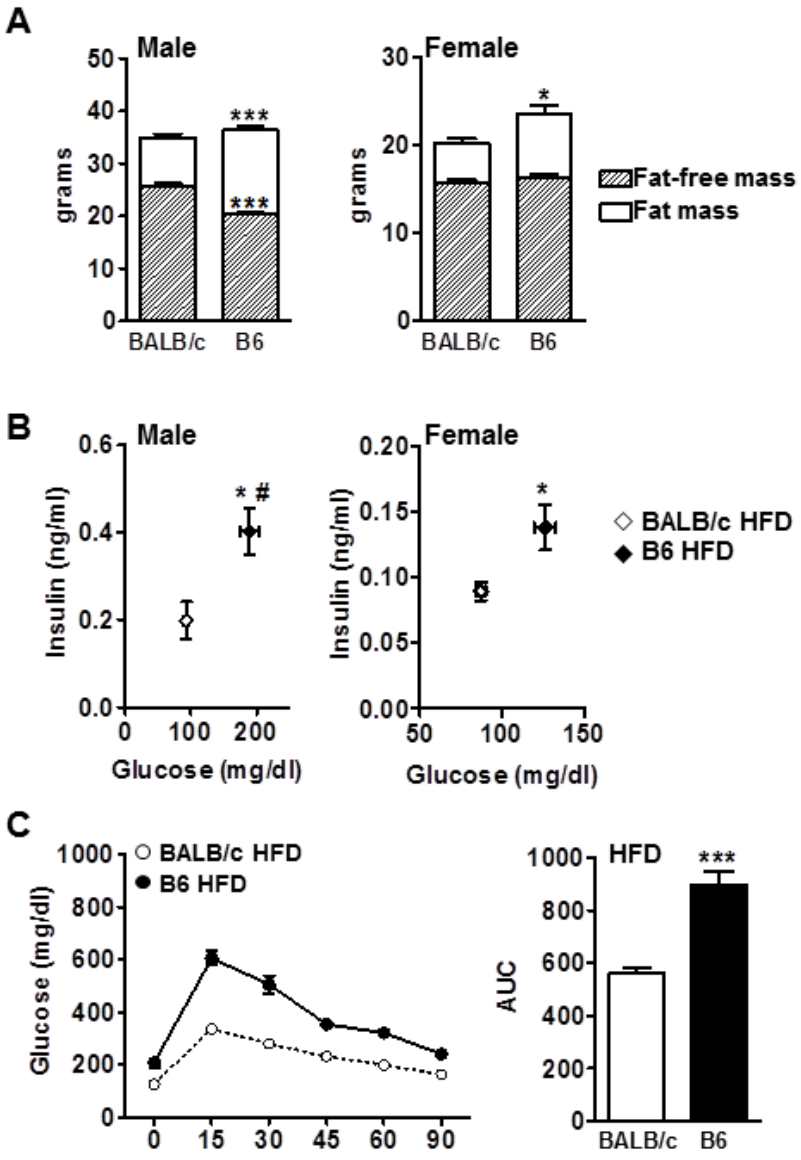
Marker ID	Chr.2 location (Mb)	Primer sequence 5'-3'	Alleles[1]	Enzyme	Expected restriction fragment length[1]	Results	
						C57BL6/J DNA	BALB/c DNA
rs3678168	5.57	For: cattcaagagccggcaaat Rev: taagagggccagcattttg	B6: G BALB: T	Alu1	B6: 96+55bp BALB: 151bp	96+55bp	151bp
rs27233322	24.7	For: tcaccgtcctctgttttaat Rev: ttgaatggtgtttcttttcc	B6: A BALB: T	Pst1	B6: 112+89bp BALB: 201bpp	112+89bp	112+89bp
rs3722052	27	For: atcaaaaggggcaaagtc Rev: tctttggaggtgtattcaaaa	B6: C BALB: T	Rsa1	B6: 203bp BALB: 136+67bp	203bp	203bp
rs27909730	46.8	For: aaaactgaaaagtcaacagacttga Rev: tttgtttgtttgtttgttttgttg	B6: T BALB: G	Alu1	B6: 95+79bp BALB: 174bp	95+79bp	95+79bp
rs27923765	47.7	For: tttggttataggcctgggaat Rev: ttatcaaatcaaattcaaaaggataac	B6: T BALB: C	Alu1	B6: 72+31bp BALB: 103bp	72+31bp	72+31bp
rs27927249	51.7	For: attctttctggcaccattgc Rev: ccagttttggggaattgtct	B6: C BALB: A	Bsaj1	B6: 72+64bp BALB: 136bp	72+64bp	72+64bp
rs27908937	56.9	For: gtcattggcctcagatgat Rev: tgagtcaaaaccacaaactct	B6: C BALB: G	Pst1	B6: 111+90bp BALB: 201bp	111+90bp	111+90bp
rs28057095	60.3	For: gtggtttagatcccgtttgc Rev: ttctgtgctgcaagcatttc	B6: T BALB:C	Alu1	B6: 105+65bp BALB: 170bp	105+65bp	105+65bp
rs27991516	64.7	For: gaccaggaggtagccatcaa Rev: acattggcaacctggagaag	B6: T BALB: G	EcoR1	B6: 102+96bp BALB: 198bp	102+96bp	102+96bp

rs27987286	66.9	For: acactcggacaaccagatcc Rev: tgacgtcatctgggctgata	B6: G BALB: C	BsaJ1	B6: 91+59bp BALB: 150bp	150bp	150bp
rs13476554	67.09	For: tctgtctgtgtggagtgcttg Rev: gcatgcgaaggcagttacta	B6: A BALB: T	Tru91	B6: 127+23bp BALB: 150bp	150bp	150bp
rs4136610	70	For: tggctcggttgtactgtctg Rev: ttttccactctggaccaaca	B6: G BALB: A	Hinf1	B6: 124+26bp BALB: 150bp	124+26bp	150bp
rs3667007	81	For: ctggtgcaaacactttggtc Rev: tttggcatagggcactaaaga	B6: C BALB: T	BsaJ1	B6: 122+55bp BALB: 177bp	122+55bp	177bp

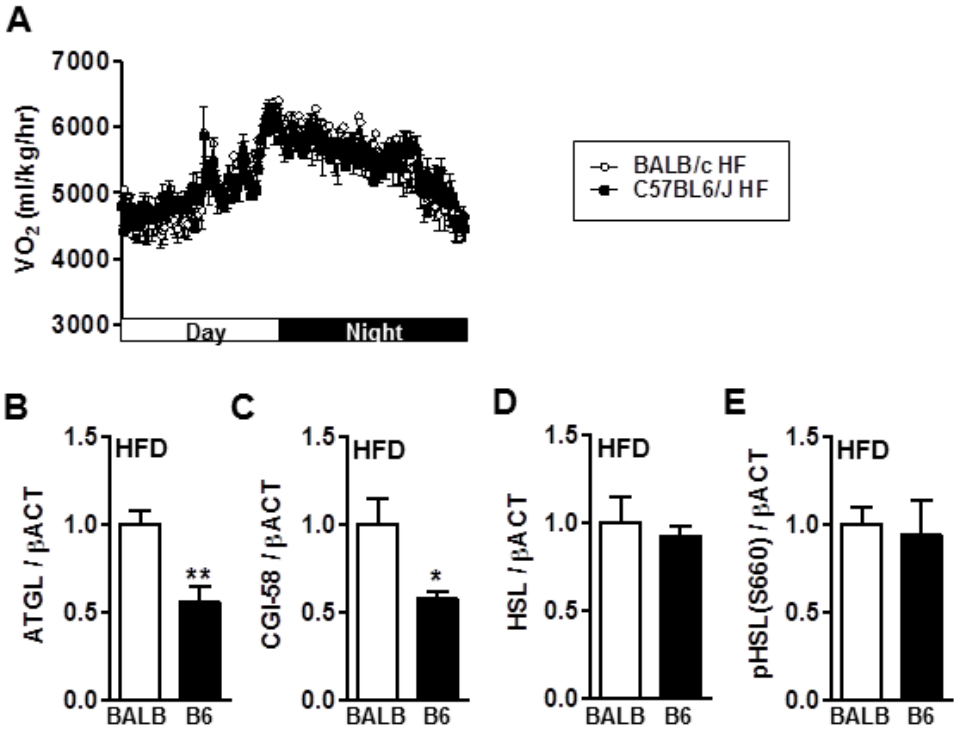
Supplemental Figure 1



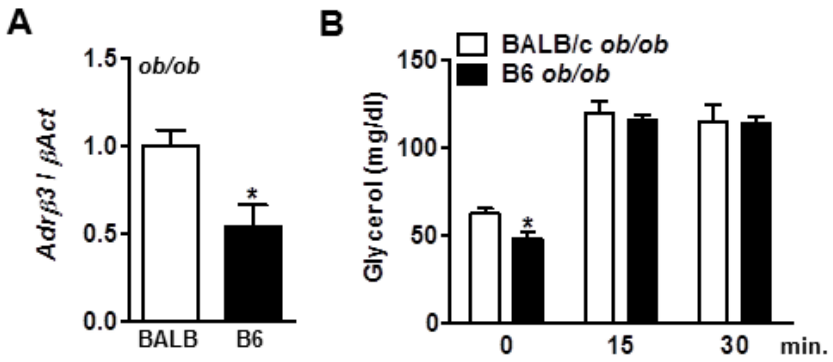
Supplemental Figure 2



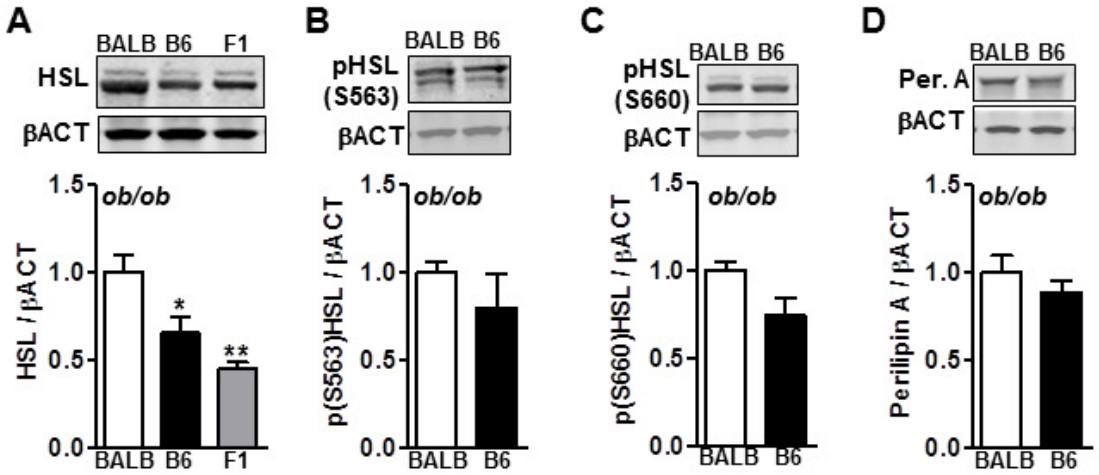
Supplemental Figure 3



Supplemental Figure 4



Supplemental figure 5



Supplemental figure 6