Science Advances
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Supplementary Materials for

CTNNB1/β-*catenin* dysfunction contributes to adiposity by regulating the cross-talk of mature adipocytes and preadipocytes

Maopei Chen, Peng Lu, Qinyun Ma, Yanan Cao, Na Chen, Wen Li, Shaoqian Zhao, Banru Chen, Juan Shi, Yingkai Sun, Hongbin Shen, Liangdan Sun, Juan Shen, Qijun Liao, Yifei Zhang, Jie Hong, Weiqiong Gu, Ruixin Liu*, Guang Ning*, Weiqing Wang*, Jiqiu Wang*

*Corresponding author. Email: xiner198287@163.com (R.L.); gning@sibs.ac.cn (G.N.); wqingw61@163.com (W.W.); wangjq@shsmu.edu (J.W.)

Published 8 January 2020, *Sci. Adv.* **6**, eaax9605 (2020)
DOI: 10.1126/sciadv.aax9605

This PDF file includes:

Supplementary Materials

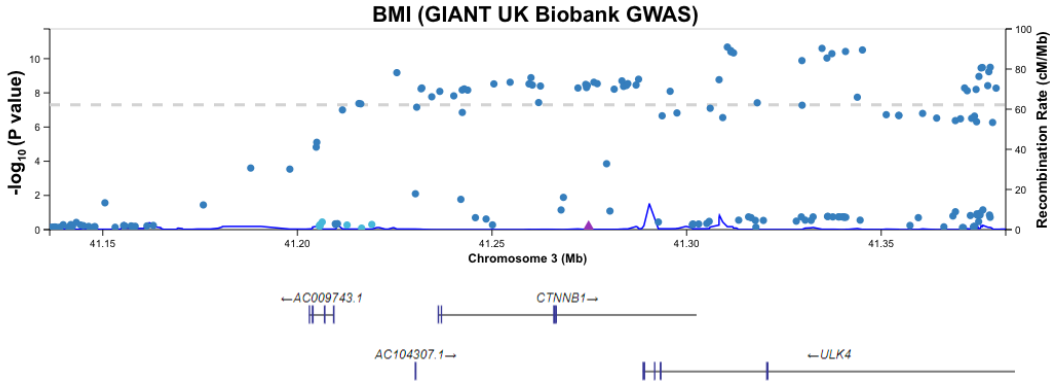

Fig. S1. Common noncoding variants in and around *CTNNB1* show significant association signals with BMI in GIANT UK Biobank study (*25*). This region plot of BMI around *CTNNB1* gene came from Type 2 Diabetes Knowledge Portal (T2D Portal) (CTNNB1. type2diabetesgenetics.org. 2019 Aug 15; http://www.type2diabetesgenetics.org/gene/geneInfo/CTNNB1).http://www.type2diabetesgenetics.org/).

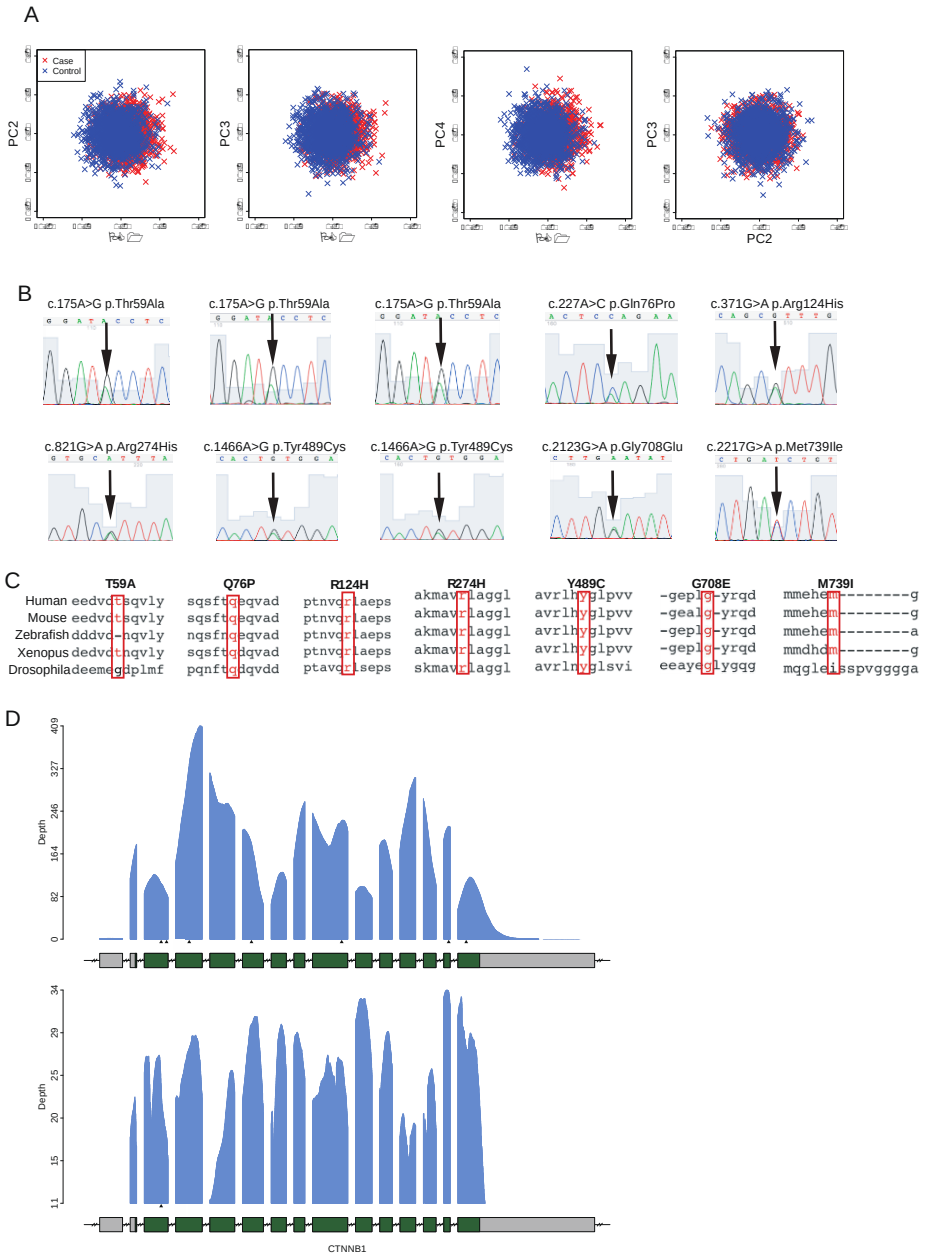

Fig. S2. Genetic mutations in the *CTNNB1* gene in young obese and control subjects. (**A**), Principal component analysis for 1,408 young, severely obese cases and 1,455 non-obese controls. The principal components were calculated based on 18,631 SNPs retained after linkage disequilibrium pruning. Plots of the first and the second to forth principal components were drawn. (**B**)**,** Sanger sequencing of the indicated mutations. Arrows indicated mutation sites. (**C**), Sequence conservation of the indicated mutation sites in *CTNNB1* among different species. (**D**), Schematic representation of CTNNB1 protein with mutations in obese group (the upper) and control group (the lower). Black triangle indicated the location of mutation sites.

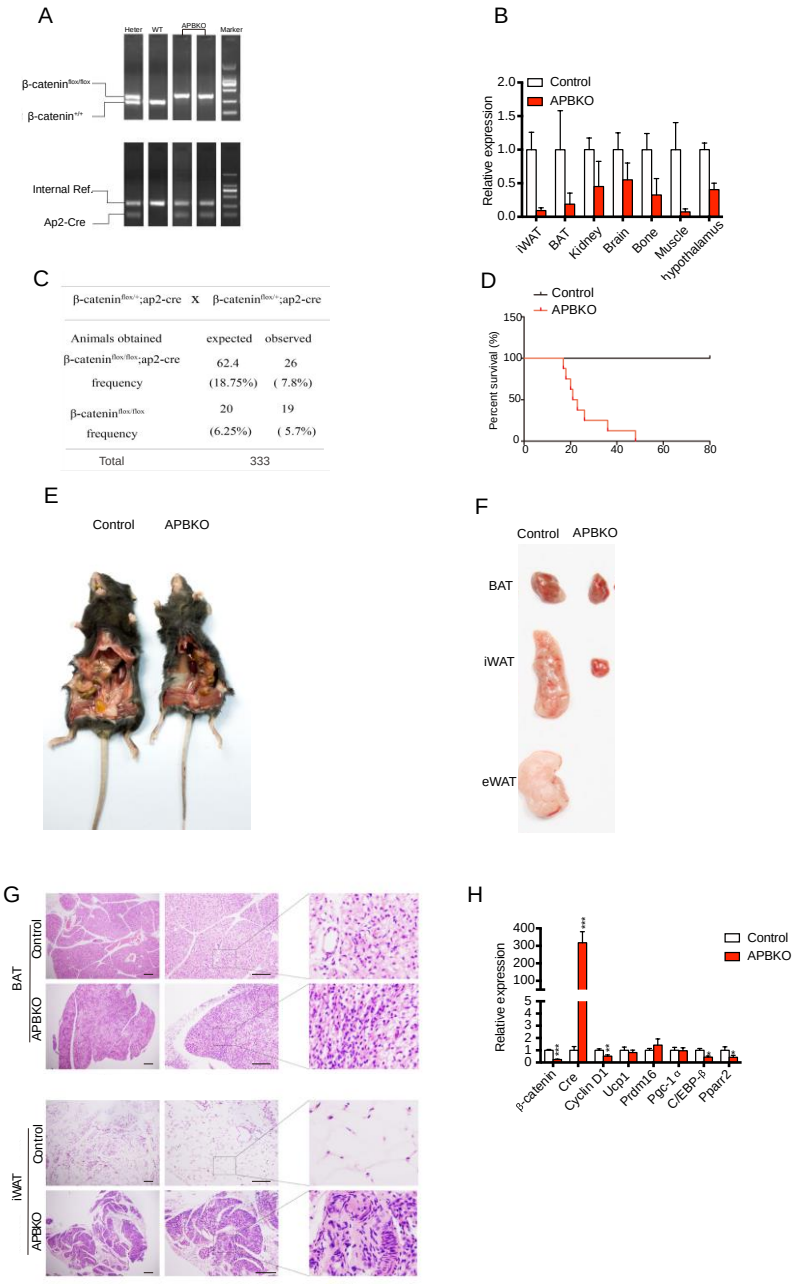

Fig. S3. Phenotypes of APBKO mice. (**A**), Genotyping of wild-type, heterozygous *β-catenin*$^{flox/flox}$ (control) mice and *β-catenin*$^{flox/flox}$;aP2-Cre (APBKO) mice. (**B**), *β-catenin* expression in the indicated tissues. (**C-D**), Birth rate (C) and survival curve (D) for control and APBKO mice. (**E-G**), Representative gross images of mice (E), dissected BAT, iWAT and eWAT (F), and H&E staining of BAT and iWAT (G) from male control and APBKO mice aged 8 weeks. Left panel, 100× magnification; middle panel, 200× magnification. Scale bar, 100 μm. (**H**), Gene expression involved in thermogenesis in BAT from male control and APBKO mice. Control, *β-catenin*$^{flox/flox}$ mice; APBKO, *β-catenin*$^{flox/flox}$;aP2-Cre mice. iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue; BAT, brown adipose tissue. Data are shown

as mean ± SEM. * P<0.05, ** P<0.01, ***P<0.001.

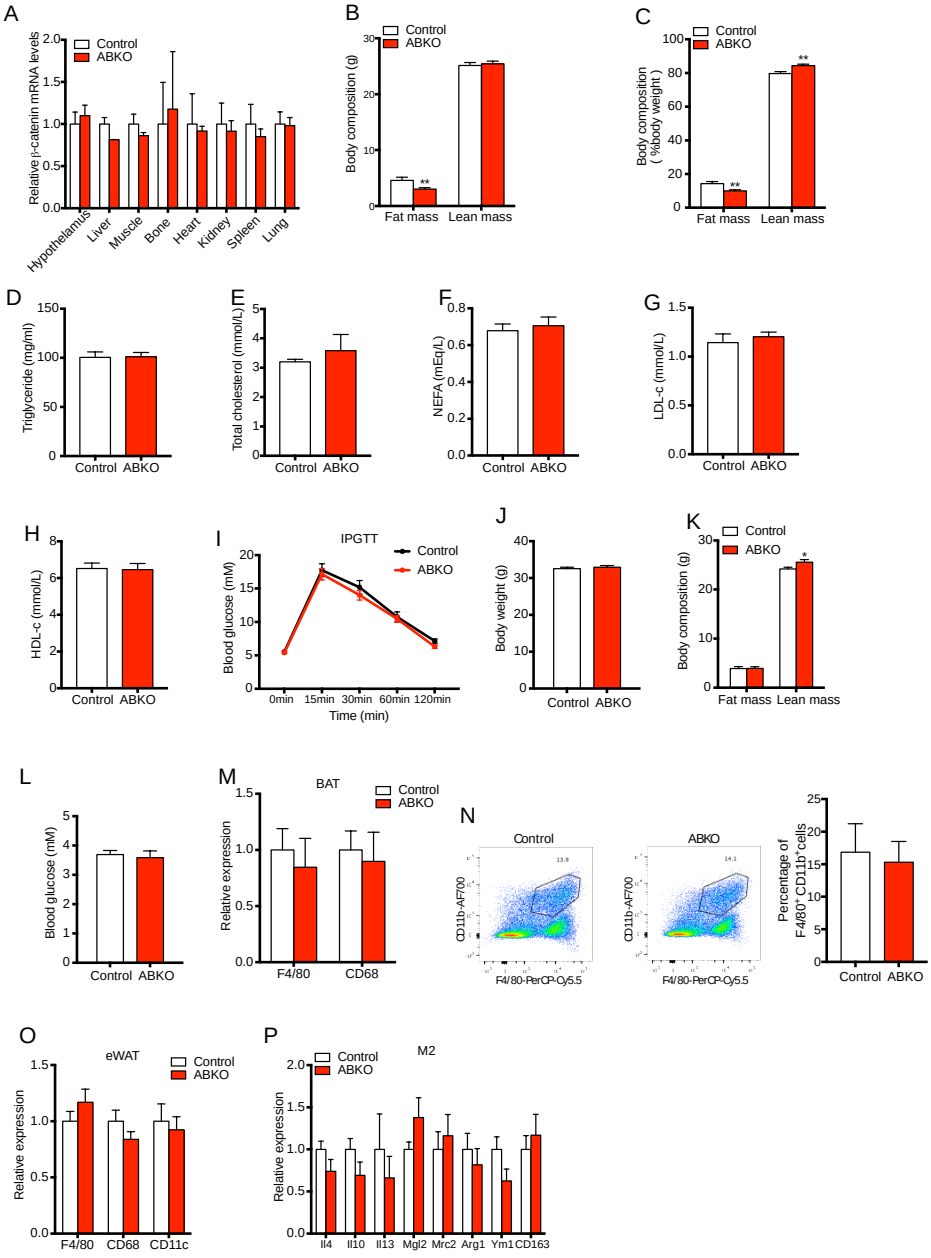

Fig. S4. Metabolic parameters in ABKO mice fed normal chow diet at young and old ages. (**A**), Relative *β-catenin* mRNA levels in non-fat tissues between control and ABKO mice fed chow diet (n=3 for each group). (**B-C**), Body composition (B) and the percentage of body composition to body weight (C) in control and ABKO mice fed chow diet at 26 weeks old (n=7 for control mice, n=13 for ABKO mice). (**D-H**), Serum triglyceride (D), total cholesterol (E), NEFA (F), LDL-c (G) and HDL-c (H) levels in control and ABKO mice at 26 weeks chow diet (n=7 for control mice, n=13 for ABKO mice). (**I**), Blood glucose levels during IPGTT in control and ABKO mice at 24 weeks chow diet (n=15 for control mice, n=19 for ABKO mice). (**J-K**), Body weight (J) and body composition (K) of control and ABKO mice at 18 months old in chow diet (n=8 for each group). L, Blood glucose levels of control and ABKO mice

after 16 hours fasting at 18 months old in chow diet (n=8 for each group). (**M**), Relative mRNA levels of macrophage markers in BAT in 26 weeks HFD-fed control and ABKO mice (n=13 for each group). (**N**), Left, representative images of cell sorting of $F4/80^{+}CD11b^{+}$ cells from eWAT SVF of 26 weeks HFD-fed control and ABKO mice by FACS; Right, quantification of the percentage of $F4/80^{+}CD11b^{+}$ cells to $CD45^{+}$ cells in SVF of eWAT (n=4 for each group). (**O**), Relative mRNA levels of macrophage markers in eWAT in 26 weeks HFD-fed control and ABKO mice (n=13 for each group). (**P**), Relative mRNA levels of type II macrophage (M2) markers in iWAT of 26 weeks HFD-fed control and ABKO mice (n=6-13 for control mice, n=6-12 for ABKO mice). Control, *β-catenin*$^{flox/flox}$ mice; ABKO, *β-catenin*$^{flox/flox}$; Adiponectin-Cre mice. eWAT, epididymal white adipose tissue; BAT, brown adipose tissue. HFD, high-fat diet. Data are shown as mean ± SEM. * $P<0.05$.

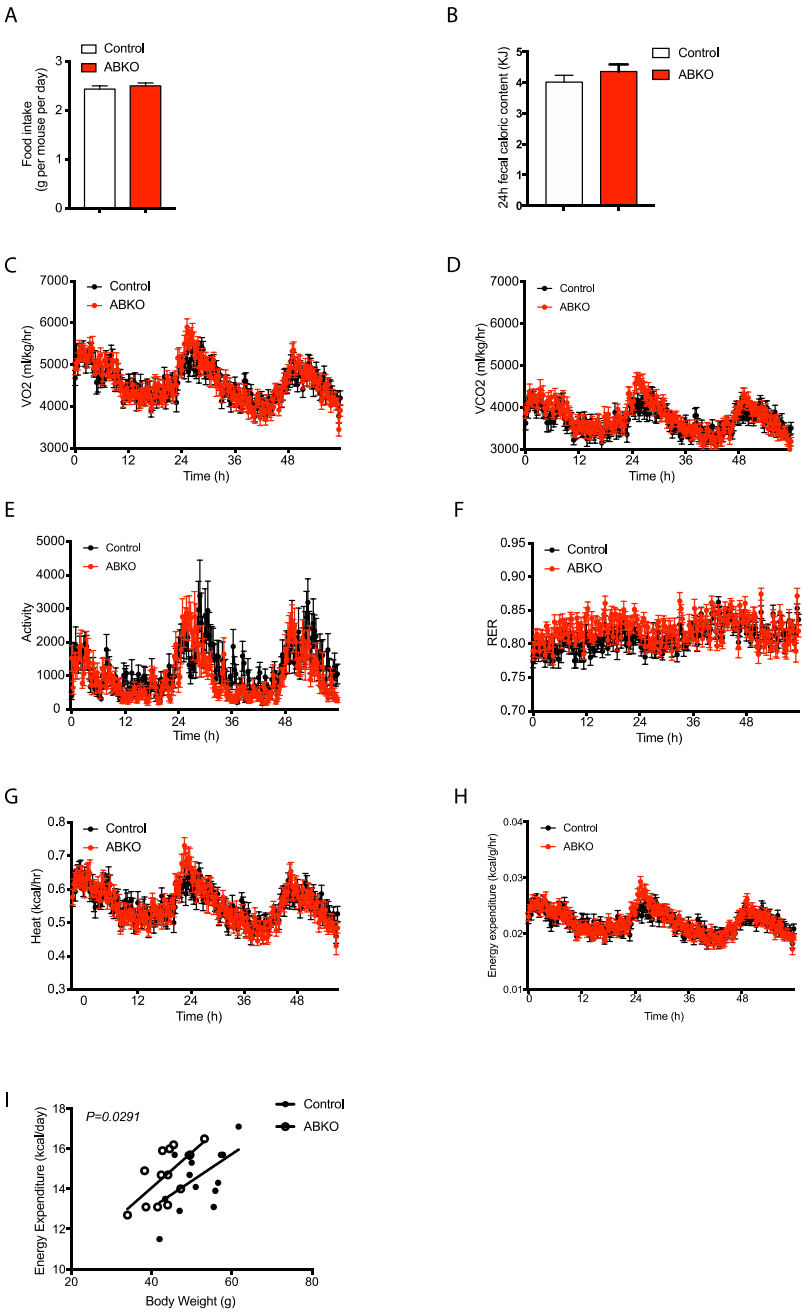

Fig. S5. Food intake, fecal calorie excretion, and energy expenditure in ABKO mice fed HFD. (**A**), 24h food intake in control and ABKO mice (n=20 for control mice, n=15 for ABKO mice). (**B**), 24h fecal caloric content of 30 weeks HFD-fed control and ABKO mice (n=10 for each group). (**C-E**), whole-body oxygen (O_2) consumption (C), carbon dioxide (CO_2) production (D), physical activity (E) from 26 weeks HFD-fed male control and ABKO mice during a 24-hour period (n=15 for control mice, n=13 for ABKO mice). (**F-H**), Respiratory exchange ratio (RER) (F), heat (G) and energy expenditure (H) is calculated for 26 weeks HFD-fed male control and ABKO mice (n=15 for control mice, n=13 for ABKO mice). (**I**), Energy expenditure was plotted in relation to body weight using ANCOVA. Control, *β-catenin*$^{flox/flox}$ mice; ABKO, *β-catenin*$^{flox/flox}$;Adiponectin-Cre mice. HFD, high-fat diet. Data are shown as mean ± SEM.

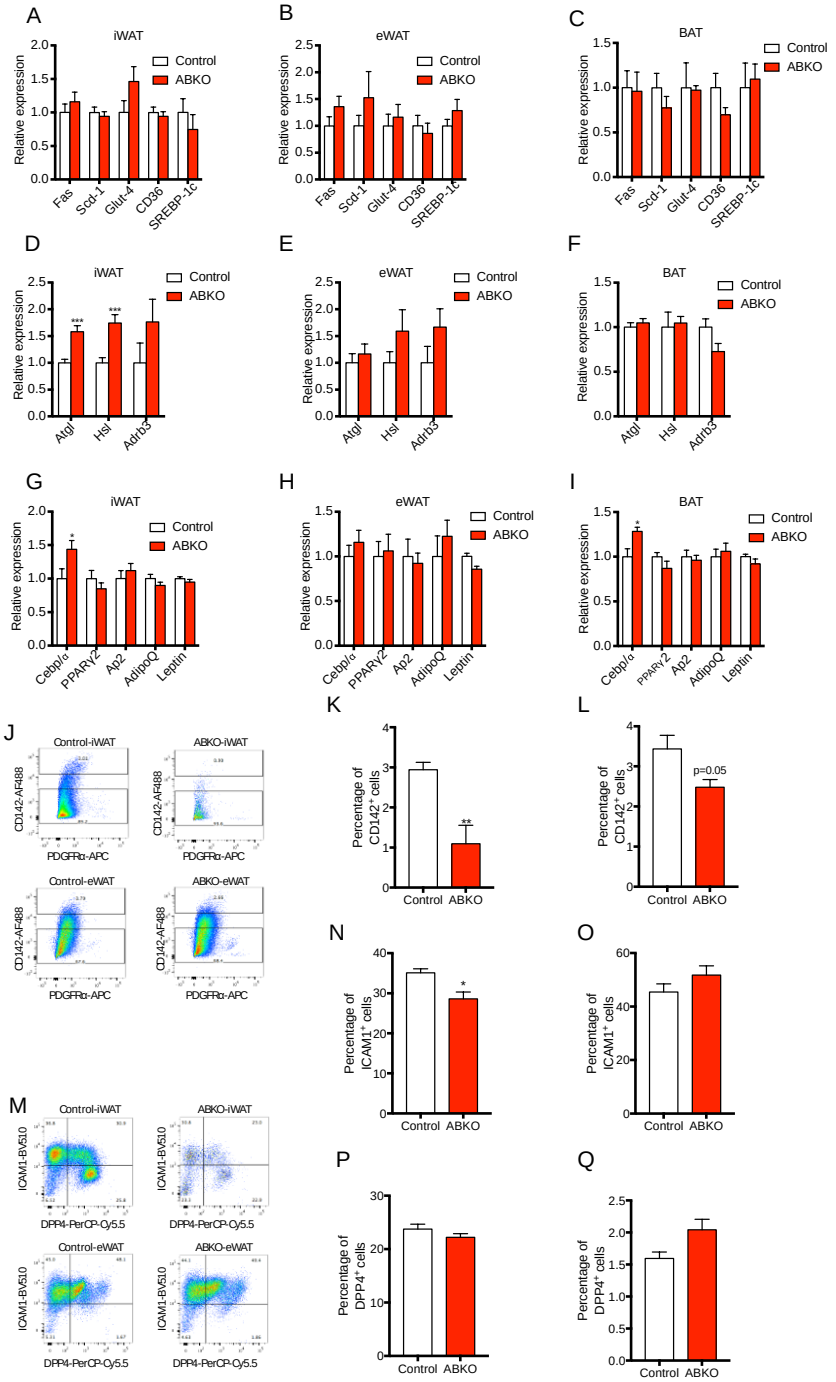

Fig. S6. The expression changes of genes involved in adipogenesis and the percentage changes of progenitors in adipose tissues of ABKO mice fed HFD. (**A-C**), Relative mRNA levels of lipogenesis genes in iWATs (A), eWATs (B) and BATs (C) (n=6-13 for each group). (**D-F**), Relative mRNA levels of lipolysis genes in iWATs (D), eWATs (E) and BATs (F) (n=6-13 for each group). (**G-I**), Relative mRNA levels of adipogenesis genes in iWATs (G), eWATs (H) and BATs (I) (n=6-13 for each

group). (**J**), Representative images of cell sorting of $Cd142^+$ cells from iWAT and eWAT SVF of 26 weeks HFD-fed control and ABKO mice by FACS. (**K-L**), quantification of the percentage of $Cd142^+$ cells in $Cd45^-Cd31^-$ cells from SVF of iWAT and eWAT, respectively (n=4 for each group). (**M**), Representative images of cell sorting of adipose precursor subpopulations from iWAT and eWAT SVF of 26 weeks HFD-fed control and ABKO mice by FACS. (**N-O**), quantification of the percentage of $Icam1^+$ $Dpp4^-$ cells in $Cd45^-Cd31^-$ $Cd142^-$cells from SVF of iWAT (N) and eWAT (O), respectively (n=4 for each group). (**P-Q**), quantification of the percentage of $Dpp4^+Icam1^-$ cells in $Cd45^-Cd31^-$ $Cd142^-$cells from SVF of iWAT (P) and eWAT (Q), respectively (n=4 for each group). Control, *β-catenin*$^{flox/flox}$ mice; ABKO, *β-catenin*$^{flox/flox}$;Adiponectin-Cre mice. iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue; BAT, brown adipose tissue. Data are shown as mean ± SEM. * $P<0.05$.

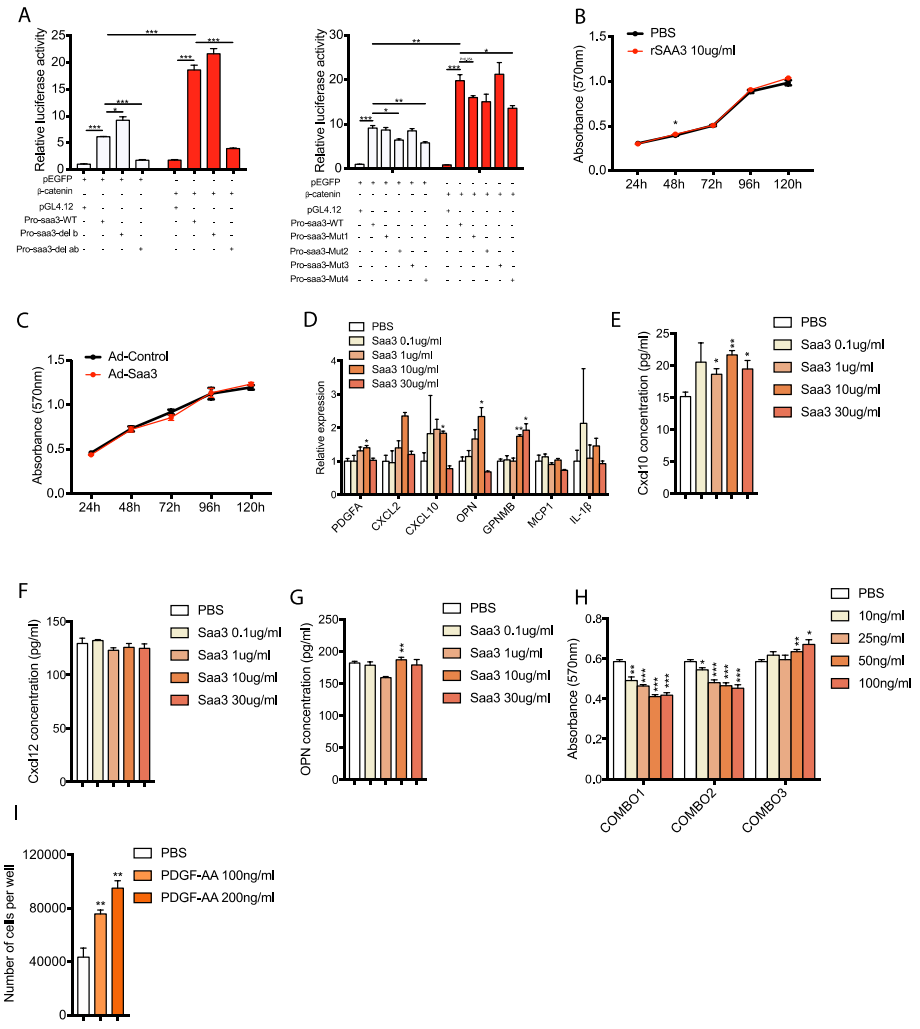

Fig. S7. Cell viability of Saa3-treated preadipocytes and the expression of secreted factors in Saa3-treated macrophages. (**A**), Luciferase reporter assay performed in 3T3-L1 cell line 48 hours after transfection with the indicated plasmids. Left, relative luciferase activity of *Saa3* deletion mutants with or without β-catenin; right, relative luciferase activity of *Saa3* point mutants with or without β-catenin. pRL-TK (expressing Renilla luciferase) was used as the normalized control. (**B**), Cell viability of 3T3-L1 cells treated with PBS or recombinant Saa3 (10 μg/ml) during 120 hours. (**C**), Cell viability of SVFs infected with control adenovirus or *Saa3* overexpressing adenovirus during 120 hours. (**D**), Relative mRNA levels of inflammatory and growth factors expressed by Raw264.7 treated with the increasing doses of recombinant Saa3 protein. (**E-G**), Concentrations of Cxcl10 (E), Cxcl12 (F) and OPN (G) in the conditioned medium of Raw264.7 cells treated with the increasing doses of recombinant Saa3 protein. (**H**), Cell viability of 3T3-L1 preadipocytes treated with different combination formula consisting of the recombinant proteins for 120 hours. COMBO1 contained Il-1β, Il-6 and Mcp-1;

COMBO2 contained Ccl12, Cxcl2, Cxcl10 and Cxcl12; COMBO3 contained OPN, Gpnmb and PDGF-AA. (**I**), Cell number of 3T3-L1 preadipocytes treated with different concentrations of PDGF-AA recombinant protein for 120 hours. Data are shown as mean ± SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Table S1. The BMI association signals of common variants in and around *CTNNB1* from GIANT UK Biobank GWAS.

Variants	dbSNP	Function	Major allele	Minor allele	P-value	Effect	Gene
3_41310470_G_A	*rs9814633*	intron	G	A	2.10E-11	0.0122	ULK4
3_41334787_T_G	*rs17267020*	intron	T	G	2.50E-11	0.012	ULK4
3_41345172_C_T	*rs4118041*	intron	C	T	3.10E-11	0.012	ULK4
3_41311362_G_C	*rs9816029*	intron	G	C	3.40E-11	0.012	ULK4
3_41340824_A_G	*rs9852315*	intron	A	G	3.80E-11	0.0119	ULK4
3_41311387_G_A	*rs6793667*	intron	G	A	4.00E-11	0.012	ULK4
3_41312055_G_A	*rs9820765*	intron	G	A	4.60E-11	0.0119	ULK4
3_41337338_C_A	*rs11718282*	intron	C	A	5.10E-11	0.0119	ULK4
3_41336053_C_A	*rs11717079*	intron	C	A	9.20E-11	0.0117	ULK4
3_41329633_T_C	*rs9879363*	intron	T	C	1.30E-10	0.0117	ULK4
3_41378012_A_C	*rs9835228*	intron	A	C	3.20E-10	0.0117	ULK4
3_41375994_A_T	*rs1352272*	intron	A	T	3.30E-10	0.0115	ULK4
3_41375667_G_A	*rs6790569*	intron	G	A	3.50E-10	0.0115	ULK4
3_41377659_A_G	*rs6786286*	intron	A	G	5.80E-10	0.0115	ULK4
3_41225545_T_C	*rs4973925*	intergenic variant	T	C	6.60E-10	0.0107	
3_41375107_T_A	*rs9877071*	intron	T	A	1.10E-09	0.0112	ULK4
3_41260017_G_A	*rs13075993*	intron	G	A	1.30E-09	0.0104	CTNNB1
3_41287657_A_G	*rs11716602*	downstream variant	A	G	1.60E-09	0.0105	ULK4
3_41308348_T_C	*rs11707955*	intron	T	C	1.70E-09	0.0105	ULK4
3_41283382_G_A	*rs11129895*	downstream variant	G	A	2.00E-09	0.0104	ULK4
3_41254646_G_T	*rs2140090*	intron	G	T	2.40E-09	0.0102	CTNNB1
3_41276166_A_T	*rs3774371*	intron	A	T	2.40E-09	0.0102	CTNNB1
3_41277040_C_T	*rs11564465*	intron	C	T	2.90E-09	0.0102	CTNNB1
3_41284891_G_A	*rs9883839*	downstream variant	G	A	2.90E-09	0.0102	ULK4
3_41250481_G_A	*rs6776881*	intron	G	A	3.00E-09	0.0102	CTNNB1
3_41259511_G_A	*rs4973927*	intron	G	A	3.00E-09	0.0102	CTNNB1
3_41274072_C_G	*rs11564450*	intron	C	G	3.20E-09	0.0101	CTNNB1
3_41260369_C_T	*rs111353008*	intron	C	T	3.50E-09	0.0101	CTNNB1
3_41287018_T_C	*rs11711946*	downstream variant	T	C	3.50E-09	0.0102	ULK4
3_41274477_A_G	*rs11564454*	intron	A	G	3.70E-09	0.0101	CTNNB1
3_41284536_C_A	*rs9883073*	downstream variant	C	A	3.70E-09	0.0101	ULK4
3_41377373_A_G	*rs901688*	intron	A	G	3.80E-09	0.0104	ULK4

3_41262444_T_C	*rs13072632*	intron	T	C	4.00E-09	0.0101	CTNNB1
3_41283793_G_C	*rs9311265*	downstream variant	G	C	4.20E-09	0.0101	ULK4
3_41274270_A_T	*rs11564452*	intron	A	T	4.90E-09	0.01	CTNNB1
3_41231968_C_T	*rs9872276*	upstream gene variant	C	T	5.20E-09	0.0101	CTNNB1
3_41272081_C_A	*rs1880481*	intron	C	A	5.20E-09	0.01	CTNNB1
3_41371414_C_T	*rs6801474*	intron	C	T	5.20E-09	0.0124	ULK4
3_41379517_T_C	*rs880603*	intron	T	C	5.30E-09	0.0103	ULK4
3_41231784_T_A	*rs4974074*	upstream gene variant	T	A	5.80E-09	0.0101	CTNNB1
3_41242863_A_G	*rs2371452*	intron	A	G	5.90E-09	0.01	CTNNB1
3_41281388_T_G	*rs2953*	3' UTR	T	G	6.10E-09	0.01	CTNNB1
3_41374373_C_T	*rs7610589*	intron	C	T	6.30E-09	0.0124	ULK4
3_41242338_C_A	*rs4533622*	intron	C	A	6.80E-09	0.0099	CTNNB1
3_41243742_T_G	*rs3915129*	intron	T	G	6.90E-09	0.0099	CTNNB1
3_41372090_C_T	*rs9820239*	intron	C	T	7.50E-09	0.0123	ULK4
3_41295767_T_C	*rs11129896*	intron	T	C	8.10E-09	0.0099	ULK4
3_41236581_G_C	*rs9870255*	intron	G	C	8.20E-09	0.0099	CTNNB1
3_41240177_G_A	*rs3864004*	5' UTR	G	A	1.50E-08	0.01	CTNNB1
3_41234516_C_G	*rs9859392*	upstream gene variant	C	G	1.70E-08	0.0097	CTNNB1
3_41343854_T_G	*rs7637185*	intron	T	G	1.80E-08	0.0097	ULK4
3_41261979_A_G	*rs1798802*	intron	A	G	3.70E-08	-0.0094	CTNNB1
3_41318158_C_T	*rs1949836*	intron	C	T	3.80E-08	0.0095	ULK4
3_41215796_C_T	*rs9814976*	regulatory region variant	C	T	4.20E-08	0.0094	
3_41216266_C_A	*rs9815735*	regulatory region variant	C	A	4.40E-08	0.0094	

The BMI association signals of common variants with P-value<5.0×10^{-8} in *CTNNB1* and its adjacent loci from GIANT and UK Biobank GWAS (25) were shown. All results came from Type 2 Diabetes Knowledge Portal (T2D Portal) (http://www.type2diabetesgenetics.org/) and only variants which located on chromosome 3 between *41,136,351* and *41,381,986* were included.

Table S2. Rare missense variants in the *CTNNB1* gene in young, severely obese cases and controls.

Position*	Nucleotide Change†	Amino Acid Change†	State	Cases (1408)	Controls (1455)	BMI of cases (kg/m^2)	BMI of controls (kg/m^2)	SIFT	PolyPhen2	gnomAD (8624 EAS)	Transcriptional activity
3:41266178	c.175A>G	p.Thr59Ala	Het	3	2	30.11, 39, 36.41	22.04, 18.29	Tolerated	Benign	0	active
3:41266230	c.227A>C	p.Gln76Pro	Het	1	0	32.3	-	Tolerated	Benign	0	-
3:41266447	c.244A>G	p.Ile82Val	Het	0	1	-	19.69	Tolerated	Benign	0	
3:41266574	c.371G>A	p.Arg124His	Het	1	0	30.85	-	Damaging	Possibly Damaging	0	active
3:41267237	c.821G>A	p.Arg274His	Het	1	0	33.61	-	Damaging	Possibly Damaging	0	active
3:41275300	c.1466A>G	p.Tyr489Cys	Het	2	0	36.4, 45.94	-	Tolerated	Benign	6	-
3:41279553	c.2123G>A	p.Gly708Glu	Het	1	0	40.3	-	Tolerated	Benign	0	active
3:41280704	c.2217G>A	p.Met739Ile	Het	1	0	30.2	-	Tolerated	Benign	8	-

* NCBI Build 37. † Variations are based on RefSeq records NM_001904.3 and NP_001895.1. Homo, homozygous; Het, heterozygous. Functional prediction was conducted by SIFT (https://sift.bii.a-star.edu.sg/) (*47*) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) (*48*). EAS, East Asians

Table S3. The clinical parameters related to obesity in gain-of-function *CTNNB1* carriers.

	Non-carrier Controls Mean (95% CI)	**Ob_1**	**Ob_2**	**Ob_3**	**Ob_4**
CTNNB1 Mutation	Wild type	p.T59A	p.T59A	p.R124H	p.R274H
Age (years)	17.5 (17.4, 17.7)	19.0	17.0	18.0	17.0
Obesity phenotypes					
Birth weight (g)	3398.4 (3322.5, 3474.3)	3150.0	2700.0	4000.0	/
BMI (kg/m^2)	34.6 (34.1, 35.2)	36.4	30.1	30.9	33.6
VAT (cm^2)	103.4 (92.6, 114.2)	**75.60***	**85.90***	**59.40***	**73.50***
SAT (cm^2)	371.4 (344.3, 398.6)	373.6	240.9	310.7	402.9
TAT (cm^2)	474.8 (442.5, 507.2)	449.2	326.8	370.1	476.4
Waist (cm)	104.6 (103.2, 106.0)	107.5	**93.2***	**93.5***	**104.0***
Hip (cm)	114.2 (113.1, 115.2)	121.0	107.2	101.5	123.0
WHR	0.92 (0.91, 0.93)	**0.89***	**0.87***	0.92	**0.85***
Liver enzymes					
ALT (IU/L)	51.8 (44.5, 59.2)	**21.0***	**20.0***	**11.0***	**16.0***
AST (μIU/mL)	33.5 (29.6, 37.5)	**14.0***	**19.0***	**13.0***	**20.0***
GGT (μIU/mL)	28.7 (25.5, 31.9)	**19.0***	**16.0***	**12.0***	**16.0***
Glucose parameters					
OGTT-BG0 (mmol/L)	5.00 (4.89, 5.89)	4.75	4.70	4.80	5.10
OGTT-BG120 (mmol/L)	7.72 (7.36, 8.09)	6.30	9.30	6.80	6.10
OGTT-INS0 (μIU/mL)	26.03 (24.21, 27.85)	32.57	39.20	14.31	7.10
OGTT-INS120 (μIU/mL)	174.90 (159.25, 190.57)	337.30	291.20	117.50	67.30
HbA1c (%)	5.72 (5.57, 5.87)	5.40	5.10	5.80	/
Matsuda Index	33.37 (30.67, 36.07)	18.73	14.49	45.18	75.97
Lipids profile					
TG (mmol/L)	1.34 (1.33, 1.72)	1.06	1.61	0.95	1.04
TC (mmol/L)	4.34 (4.32, 4.53)	4.29	5.56	3.22	4.24
HDL-c (mmol/L)	1.10 (1.09, 1.22)	1.11	1.14	0.96	1.00
LDL-c (mmol/L)	2.75 (2.67, 2.84)	2.54	3.82	2.16	2.95

224 age-, geography, nationality-matched young female subjects without *CTNNB1* mutations were recruited as the controls, in which 68 subjects received abdominal Computed Tomography (CT) scanning for the quantification of subcutaneous and visceral fat areas. Data for controls are means (95% confidential interval, 95% CI). * Actual values of more than 3/4 mutant carriers are below 95% CI. BMI, body mass index. VAT, visceral adipose tissue areas. SAT, subcutaneous adipose tissue areas. TAT, total adipose tissues areas, equal to the sum of VAT plus SAT. WHR, waist-to-hip ratio. ALT, alanine aminotransferase. AST, aspartate aminotransferase. GGT, gamma glutamyl transpeptidase. OGTT, oral glucose tolerance test. BG0 and BG120, blood glucose levels at 0 and 120 minutes of OGTT. INS0 and INS120, plasma insulin levels at 0 and 120 minutes. Matsuda Index, a surrogate index of insulin sensitivity, was calculated as follows: $\{10{,}000/[\text{fasting glucose} \times \text{fasting insulin} \times (\text{glucose}_{mean} \times \text{insulin}_{mean})]^{1/2}\}$.