SUPPLEMENTARY DATA

Online Supplementary Material

Cell Culture and Adipocyte Differentiation

HEK 293T cells were purchased from the American Type Culture Collection (ATCC). MEFs were prepared by culturing small tissue explants from mice at E13.5. Cells were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% FBS. 3T3-L1 preadipocytes were maintained in DMEM supplemented with 10% new born calf serum. 3T3-L1 cells were induced to differentiate 2 days after confluence (day 0) with DMEM containing 10% FBS and the full differentiation cocktail of 0.5 mM 1-methyl-3-isobutylxanthine, 1 μM dexamethasone, and 1 μg/ml of insulin (MDI) for 2 days. After 2 days, the medium was replaced with DMEM containing 10% FBS and 1 μg/ml insulin, and then the cells were subsequently fed every other day with DMEM containing 10% FBS. Fully differentiated adipocytes were used for experiments after 7 days. The two cell lines were checked for short tandem repeats DNA profiles. When searched the short tandem repeat database published on ATCC website, we found that all the two cell lines are the same with those in ATCC. The absence of mycoplasma was confirmed.

Oil Red-O staining

After days of differentiation, cells were washed with PBS, fixed with freshly prepared 4% paraformaldehyde for 1 h, incubated with Oil red-O working solution for 30 min at room temperature, and then washed with PBS.

Cytoplasmic and Nuclear Extract Preparation.

Cells were resuspended in buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.15% NP-40, 0.1 mM EDTA, pH 8.0, 0.1 mM EGTA, pH 8.0, 1 mM DTT and protease inhibitors) and incubated on ice for 15 min. The supernatant containing the cytoplasmic extract and the precipitate containing the nuclei were separated by centrifugation. The precipitated nuclei were resuspended in buffer B (20 mM HEPES, pH 7.9, 400 mM NaCl, 0.5% NP-40, 1 mM EDTA, pH 8.0, 1 mM EGTA, pH 8.0, 1 mM DTT, and protease inhibitors), rotated at 4 °C for 1 h, and cleared by centrifugation.

Energy Expenditure

Metabolic cage studies were conducted using an Automated Home Cage Phenotyping System (PhenoMaster, TSE system). Mice were acclimated in the metabolic cage for 5 days before the experiments. Food intake, movement, O_2, CO_2 and heat production were measured every 30 min for each mouse over a period of 5 days.

Co-immunoprecipitation and GST-pull down

Cells or cells transfected with the indicated plasmids were lysed for subsequent co-immunoprecipitation (Co-IP). The cell lysates were incubated with GSK3β antibody (Cell Signaling Technology, cat. no.9832) and protein A/G agarose (Thermo Scientific, Waltham, MA) or EZview™ Red Anti-HA Affinity Gel (Sigma-Aldrich, cat. no. E6779). The immune complexes were analyzed by western blotting. For the pull-down assay, GST fusion proteins and His fusion proteins were expressed in the E. coli strain BL21. Lysates were incubated with glutathione–Sepharose beads to purify the GST fusion proteins. The beads were centrifugated and then incubated with His fusion proteins extracts. The immune complexes were detected by western blotting.

Primary Oligodendrocyte and neuron cell culture

Mouse oligodendrocyte precusor cells (OPCs) were isolated from cortices of mouse brains at postnatal day 6. Briefly, dissociated mouse cortices were resuspended in panning buffer. The cells were incubated 45 min on panning plates coated with anti-O4 antibody to harvest OPCs. The adherent cells were trypsinized and plated onto poly-D-lysine-coated plates. Cells were grown in SATO media with addition of PDGF-AA (20 ng/ml) and NT3 (1 ng/ml). For primary neuron culture, cerebral cortex was dissected from 14-day mice and dissociated by trypsin digestion and trituration. Cells were plated in poly-L-lysine coated 6-well plates, cultured in Neurobasal medium supplemented with B27, 200 mM L-glutamine, and 1% penicillin and streptomycin. Antimitotic reagents, Uridine 5'-Triphosphate and 5-Fluoro-2'-deoxy Uridine (10 mg/ml each) were added to limit glia proliferation.

Immunofluorescence

Mice at postnatal day 14 were anesthetized with pentobarbital and perfused with PBS followed by 4% paraformaldehyde (PFA). Brains were dissected, fixed in 4% PFA overnight, dehydrated in 25% sucrose at 4°C, embedded in tissue freezing medium and cryosectioned at 12 μm. Cryosections were permeabilized and blocked in blocking buffer (0.4% Triton X-100 and 5% normal donkey serum in PBS) for 30min at room temperature and

incubated with primary antibodies (NeuN, Millipore, mab377; olig2, R&D systems, BAF2418; Pygo2, Abcam, ab109001) overnight at 4 °C. The next day, sections were washed 3 times with PBS and incubated with Alexa Fluor-conjugated secondary antibodies for 1 h at room temperature (with the addition of DAPI). Images were acquired with a Leica SP8 confocal microscope.

Flow cytometry and cell sorting

Isolated SVF cells were stained with indicated antibodies and sorted with a MoFlo Astrios EQS instrument. The following antibody were used: PDGFRα PE (BioLegend, 135905), CD45 APC–eFluor 780 (eBioscience; 47-0451-80), CD31 PE–Cy7 (eBioscience, 25-0311-82), Ter119 PE (eBioscience, 12-5921-82), CD29 Alexa Fluor 700 (BioLegend, 102218), CD34 Alexa Fluor 647 (BioLegend, 119314), Sca-1 Pacific Blue (BD Biosciences, 560653), CD24 FITC (eBioscience, 11-0242-82). Data analysis was performed using Kaluza Analysis software.

Supplementary Table 1. Target Sequences of shRNA.

Gene		Target Sequence 5'→3'
Mouse Pygo2	shRNA1	ATACTCAGGGTCCTGCATATT
	shRNA2	GGGATTTGGTCCCATGATCTC
Mouse Axin2	shRNA	GAGCTGGTTGTCACCTACTTT
Control	shRNA	TTCTCCGAACGTGTCACGT

Supplementary Table 2. Antibodies for western blotting.

Antibody	Company	Catalogue no
Pygo1	Abcam	ab75216
FABP4	Proteintech	12802-1-AP
C/EBPα	Santa Cruz Biotechnology	sc-61
PPARγ	Abcam	ab41928
Axin2	Abcam	ab109307
β-Catenin	Upstate	05-613
TCF4	Millipore	05-511
GSK3β	Santa Cruz Biotechnology	sc-9166
C/EBPβ	Santa Cruz Biotechnology	sc-150
Snail	Proteintech	13099-1-AP
GAPDH	Sigma-Aldrich	G9295
LaminB	Santa Cruz Biotechnology	sc-6216
ERK1/2	Proteintech	66192-1-Ig
Axin1	Affinity Biosciences	DF9264
HA	Sigma-Aldrich	H6908
Myc	Sigma-Aldrich	C3956
His	Sigma-Aldrich	SAB1306085

SUPPLEMENTARY DATA

Supplementary Table 3. Primers for real-time qPCR.

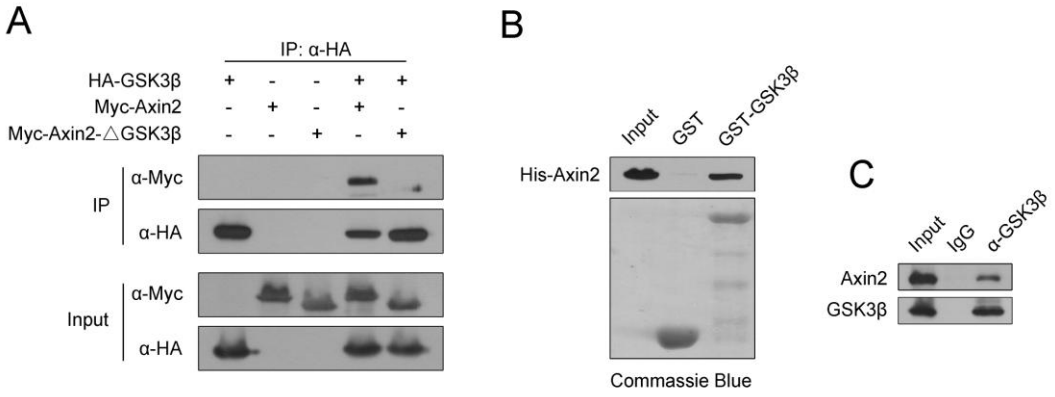
Gene		Primer sequence 5' →3'
Mouse Pygo1	F	CAACTCGGAAGCCCAGATAAG
	R	CAGCCACTAGATGGTCGGAG
Mouse Pygo2	F	GAGCCCTGAAAAGAAGCGAAG
	R	GAGACTGGTTGAAGGGTTGACTG
Human Pygo2	F	GCCGGTCTGCAAATGAAG
	R	GGACTGCCAAGGAATGGAG
Mouse Axin2	F	TGACTCTCCTTCCAGATCCCA
	R	TGCCCACACTAGGCTGACA
Mouse Snail	F	TGCCTTGTGTCTGCACGAC
	R	CTTGGTGCTTGTGGAGCAAG
Mouse C/EBPα	F	AGGGACTGGAGTTATGACAAG
	R	CCAGCCGTTAGTGAAGAGTC
Mouse PPARγ1	F	TGAAAGAAGCGGTGAACCACTG
	R	TGGCATCTCTGTGTCAACCATG
Mouse PPARγ2	F	GTTTTATGCTGTTATGGGTG
	R	GTAATTTCTTGTGAAGTGCTCATAG
Mouse FABP4	F	GAAAACGAGATGGTGACAAGC
	R	GCCCTTTCATAAACTCTTGTGG
Mouse Fasn	F	GGCTGAGGCCTTATGCTTCT
	R	CGATCTTCCAGGCTCTTCAG
Mouse Fatp1	F	CGCTTTCTGCGTATCGTCTG
	R	GATGCACGGGATCGTGTCT
Mouse Adiponectin	F	GATGGCACTCCTGGAGAGAA
	R	CAGCTCCTGTCATTCCAACA
Mouse Resistin	F	AAGAACCTTTCATTTCCCCTCCT
	R	GTCCAGCAATTTAAGCCAATGTT
Mouse Leptin	F	CAAGACCATTGTCACCAGGA
	R	TGAAGCCCAGGAATGAAGTC
Mouse CoupTFII	F	CTCAAAGTGGGCATGAGAC
	R	AGGTACGAGTGGCAGTTG
Mouse TNFα	F	CCATTCCTGAGTTCTGCAAAGG
	R	AAGTAGGAAGGCCTGAGATCTTATC
Mouse IL-6	F	GAAATGCCACCTTTTGACAGTG
	R	TGGATGCTCTCATCAGGACAG
Mouse Lef1	F	CGTCACACATCCCGTCAGATG
	R	ATGGGTAGGGTTGCCTGAATC
Mouse MCAD	F	AGGGTTTAGTTTTGAGTTGACGG
	R	CCCCGCTTTTGTCATATTCCG
Mouse Cpt1b	F	GCACACCAGGCAGTAGCTTT
	R	CAGGAGTTGATTCCAGACAGGTA
18s	F	GTCTGTGATGCCCTTAGATG
	R	AGCTTATGACCCGCACTTAC

SUPPLEMENTARY DATA

Supplementary Table 4. Primers for ChIP Assay.

Gene		Primer sequence 5' →3'
Mouse C/EBPα	F	CAGGAGTCAGTGGGCGTTGC
	R	AGGATGGTGCCTGCTGGGTC
Mouse PPARγ	F	GTGATTAGGAGTTTCAACCAAAGA
	R	CTGTGAGGGGCGTGAACTGT

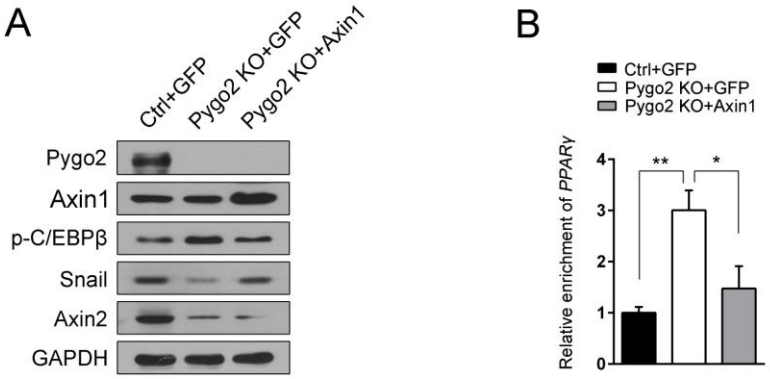
Supplementary Figure 1. Axin2 directly associates with GSK3β in preadipocytes.
(**A**) GSK3β was shown to interact with Axin2, but not Axin2-ΔGSK3β. Shown are the results of Co-IP experiments using 293T cells co-transfected with HA-GSK3β and Myc-Axin2 or Myc-Axin2-ΔGSK3β. (**B**) An in vitro pull-down assay demonstrated direct binding between GSK3β and Axin2. (**C**) Endogenous Co-IP experiments between GSK3β and Axin2 from extracts of non-transfected 3T3-L1 preadipocytes revealed that GSK3β pulled down Axin2.



SUPPLEMENTARY DATA

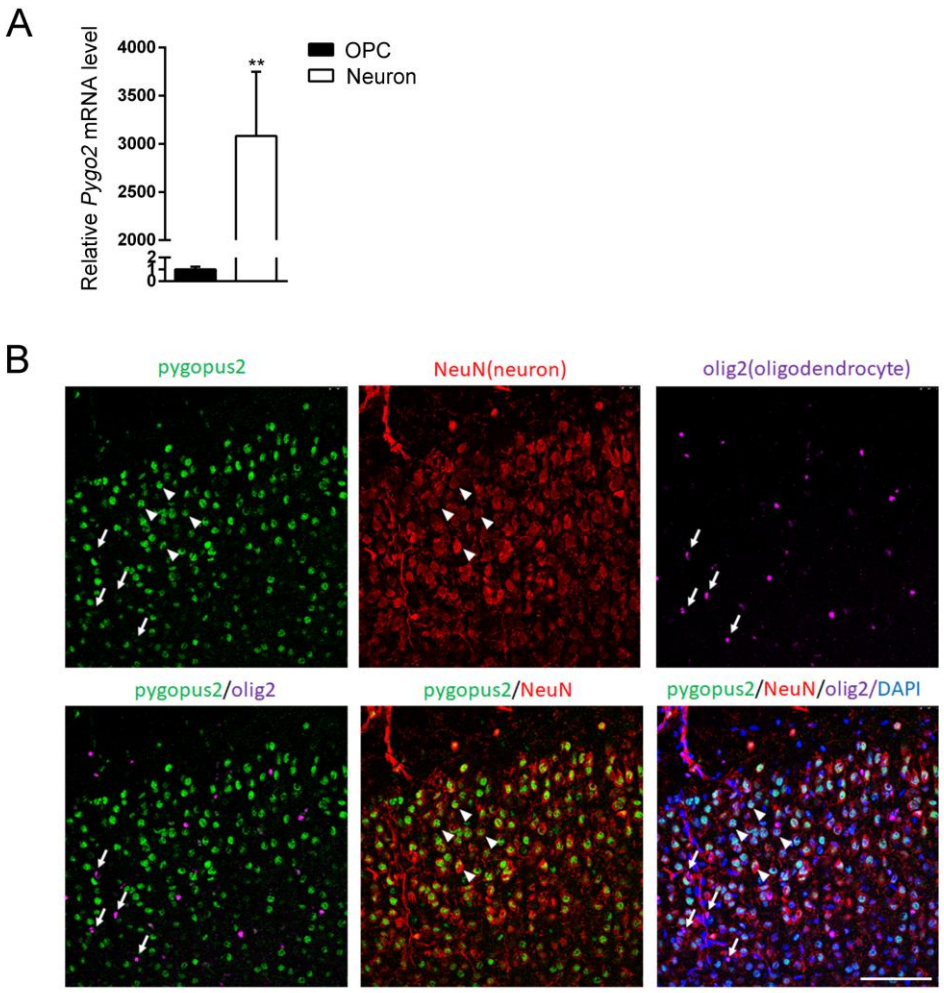
Supplementary Figure 2. Simultaneous overexpression of Axin1 also significantly compromised the Pygo2 knockout-induced C/EBPβ and Snail function changes.

The expression of Pygo2 was knocked out by CRISPR/CAS9 technique with or without lentivirus overexpression of Axin1 to the level that could compensate the reduction of Axin2. (**A**) The phosphorylated C/EBPβ and Snail protein expression were detected by western blotting. (**B**) The C/EBPβ occupancy on the PPARγ promoter was assessed by ChIP assay. For **B**, each bar represents the average value of three independent experiments and error bars represent SD. **P* <0.05, ***P* < 0.01.



SUPPLEMENTARY DATA

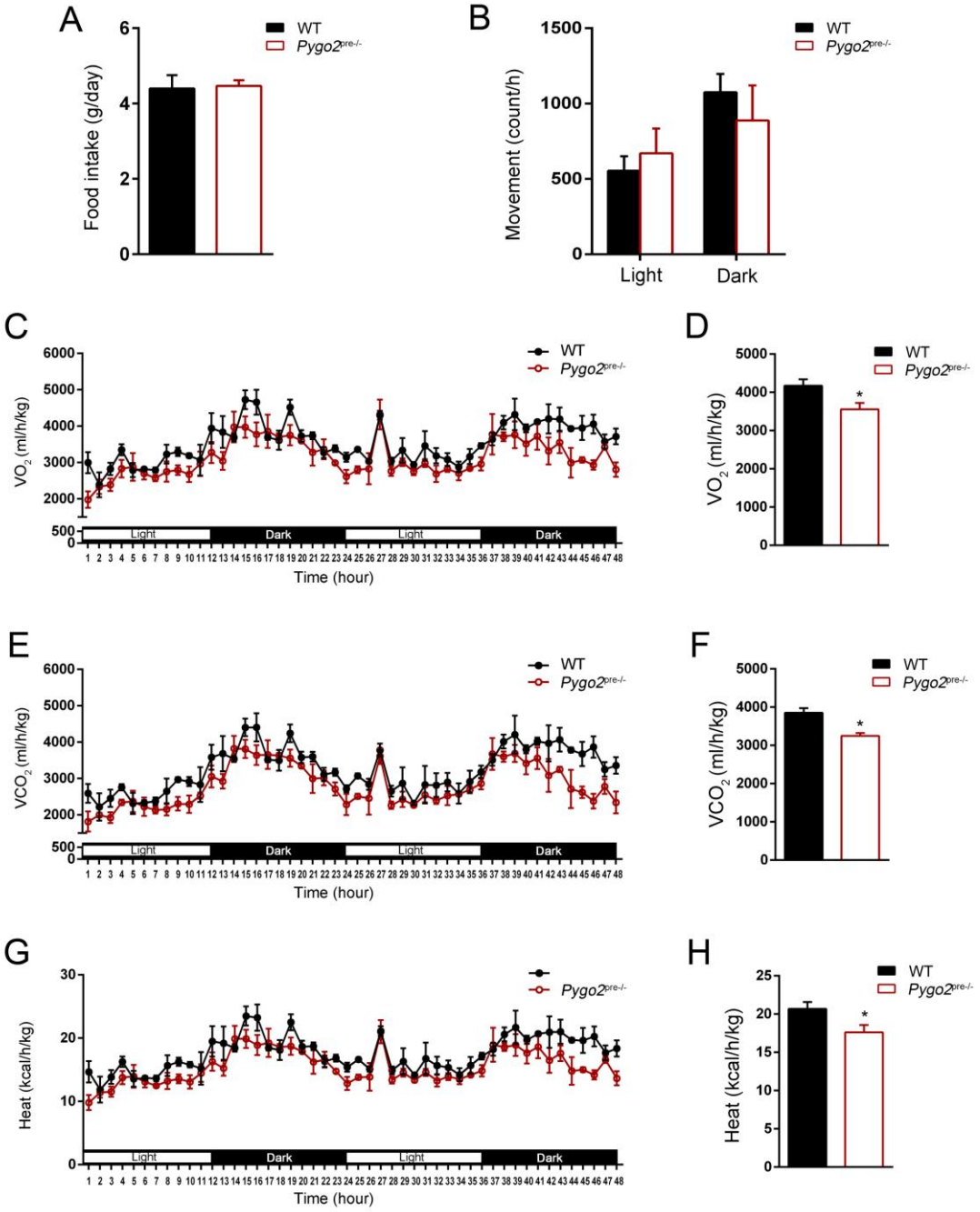
Supplementary Figure 3. Pygo2 does not express in OPC cells.
(**A**) Neuron and OPC cells were isolated from mouse brain (n=3); Pygo2 mRNA expression was assessed by real-time qPCR. (**B**) Pygo2 protein expression and localization in mouse brain were assessed by confocal immunofluorescence. Arrows indicated oligo2 positive cells and arrowheads indicated NeuN positive cells, scale bar, 100μm. For **A**, Error bars represent SEM. $^{**}P < 0.01$.



SUPPLEMENTARY DATA

Supplementary Figure 4. *Pygo2*$^{pre-/-}$ mutation does not affect food intake and movement, but affect energy expenditure.

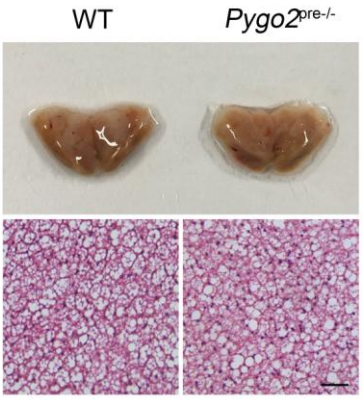
Food intake (**A**) and movement (**B**) was measured over 5 days in 10-week-old *Pygo2*$^{pre-/-}$ and WT mice (n=6) maintained on a normal chow diet. (**C**, **E** and **G**) O_2 consumption (**C**), CO_2 production (**E**) and heat production (**G**) in *Pygo2*$^{pre-/-}$ and WT mice (n=6) during two complete 12 h light-dark cycles were assessed. (**D**, **F** and **H**) Average O_2 consumption (**D**), average CO_2 consumption (**F**) and average heat production (**H**) of *Pygo2*$^{pre-/-}$ and WT mice during the 5 days measurement. Error bars represent SEM. **P* <0.05.



SUPPLEMENTARY DATA

Supplementary Figure 5. No significant difference about brown adipose tissue phenotype between *Pygo2*$^{pre-/-}$ mice and wild-type controls.

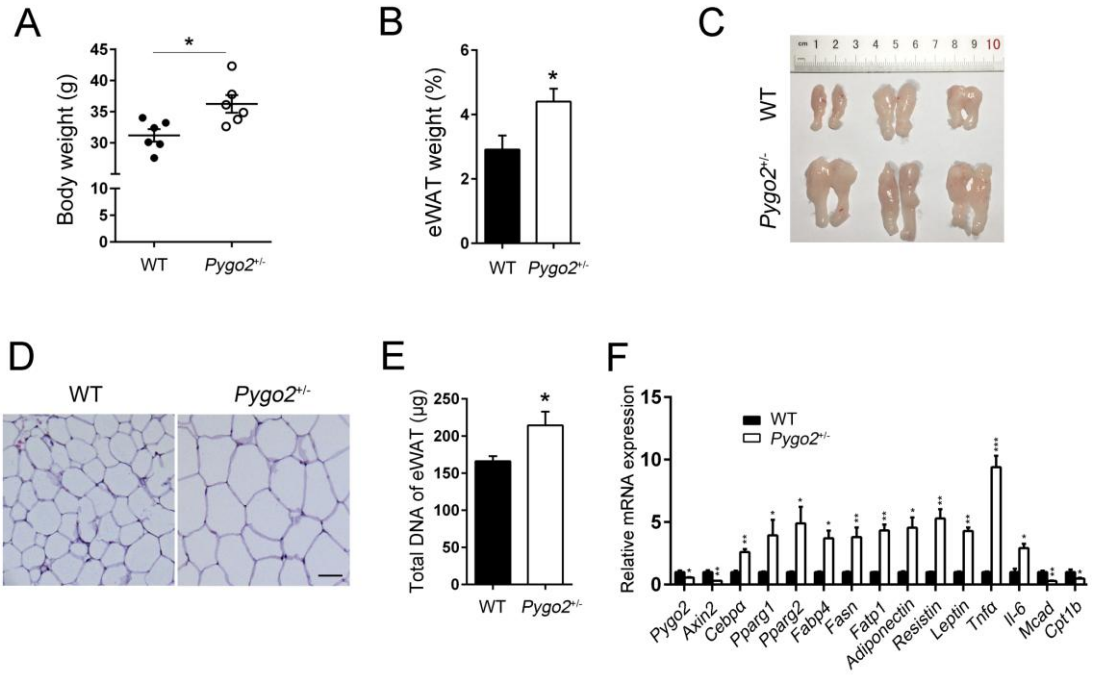
Macroscopic view and hematoxylin/eosin staining of the interscapular BAT of 14-week-old *Pygo2*$^{pre-/-}$ and WT mice. Scale bar, 50 μm.



SUPPLEMENTARY DATA

Supplementary Figure 6. *Pygo2*$^{+/-}$ mice present increased adiposity and elevated gene expression related to adipogenesis and glucose metabolism under high fat diet condition.

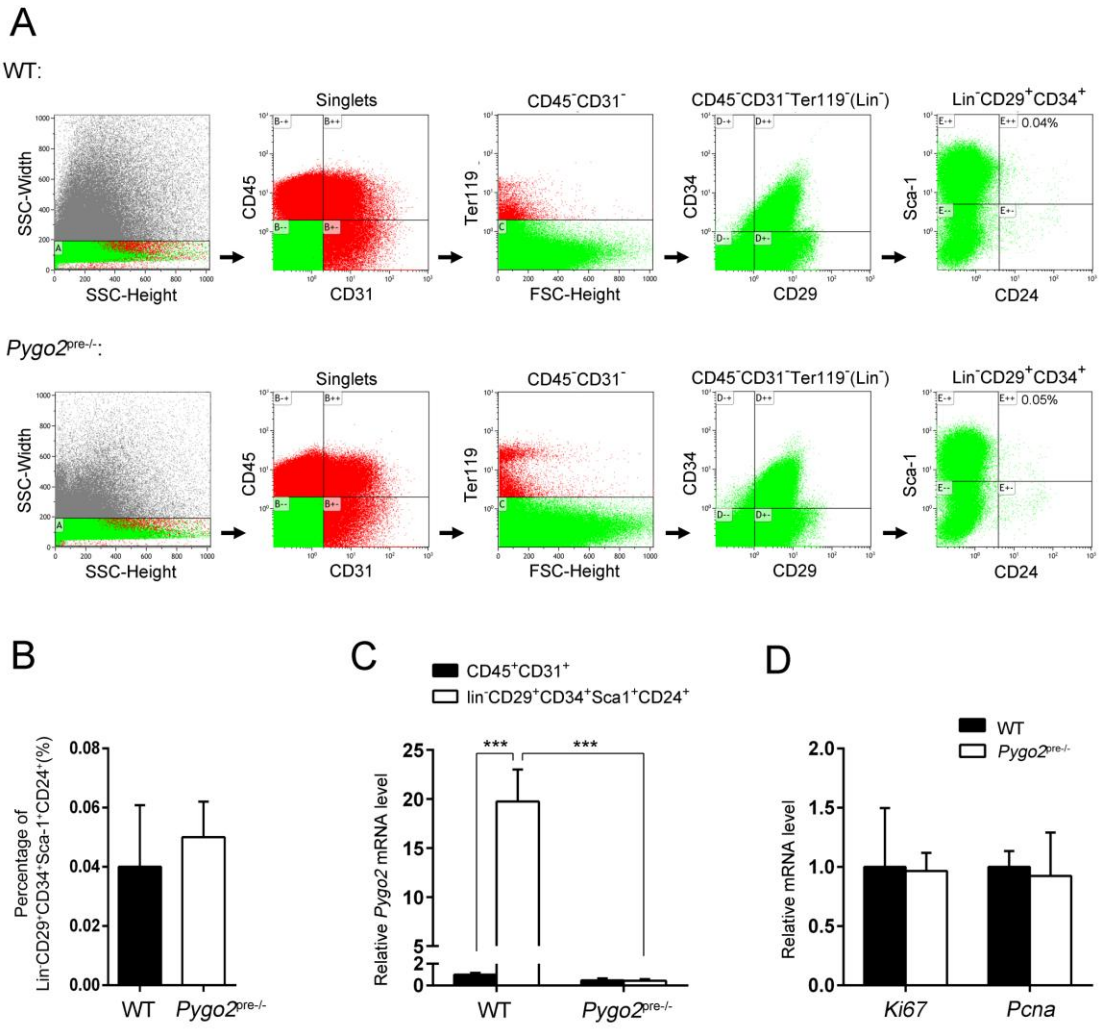
The male *Pygo2*$^{+/-}$ and WT mice fed at 8-week-old with HFD for 6 weeks. (**A**) Body weight of the male *Pygo2*$^{+/-}$ and WT (n=6) mice was evaluated. (**B** and **C**) The ratio of epididymal WAT to body weight in the *Pygo2*$^{+/-}$ and WT (n=6) mice (**B**), and macroscopic view of the epididymal WAT (**C**). (**D**) Hematoxylin/eosin staining of the epididymal WAT of the *Pygo2*$^{+/-}$ and WT mice. Scale bar, 50 μm. (**E**) Total DNA content of epididymal WAT in *Pygo2*$^{+/-}$ and WT (n=4) mice. (**F**) Real-time qPCR analysis of the indicated mRNAs in the epididymal WAT of *Pygo2*$^{+/-}$ and WT mice (n=3). Error bars represent SEM. **P* <0.05, ***P* < 0.01, ****P* < 0.001.



SUPPLEMENTARY DATA

Supplementary Figure 7. Pygo2 expresses in Lin$^-$CD29$^+$CD34$^+$Sca-1$^+$CD24$^+$ cells but not CD45$^+$CD31$^+$ cells, and Pygo2 deletion does not affect preadipocyte commitment and/or proliferation.

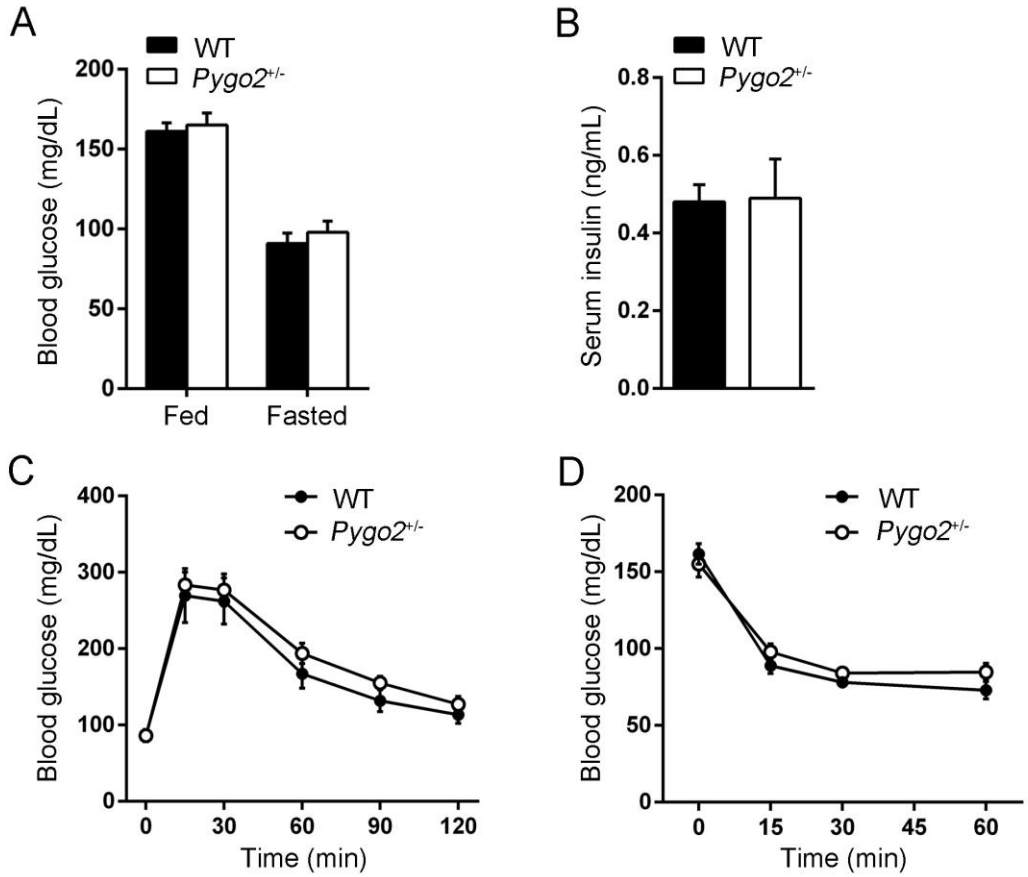
SVF cells were isolated from mixed white adipose tissue of five WT or *Pygo2*$^{pre-/-}$ mice. Cell populations are identified via flow cytometry by staining of SVF cells with fluorescently labeled antibodies against the indicated cell surface proteins. (**A**) CD45$^+$CD31$^+$ cell population and Lin$^-$CD29$^+$CD34$^+$Sca-1$^+$CD24$^+$ cell population were sorted by this strategy. (**B**) Shown is the comparison of Lin$^-$CD29$^+$CD34$^+$Sca-1$^+$CD24$^+$ cell population between the WT and *Pygo2*$^{pre-/-}$ mice. (**C**) *Pygo2* mRNA expression was assessed by real-time qPCR. (**D**) The mRNA expression levels of *Ki67* and *Pcna* in the above cell population were compared between the WT and *Pygo2*$^{pre-/-}$ mice. Each bar represents the average value from cells of three independent sorting experiments. Error bars represent SEM. ***$P < 0.001$.



SUPPLEMENTARY DATA

Supplementary Figure 8. No difference of glucose metabolism in *Pygo2*$^{+/-}$ and WT mice fed a normal chow diet.

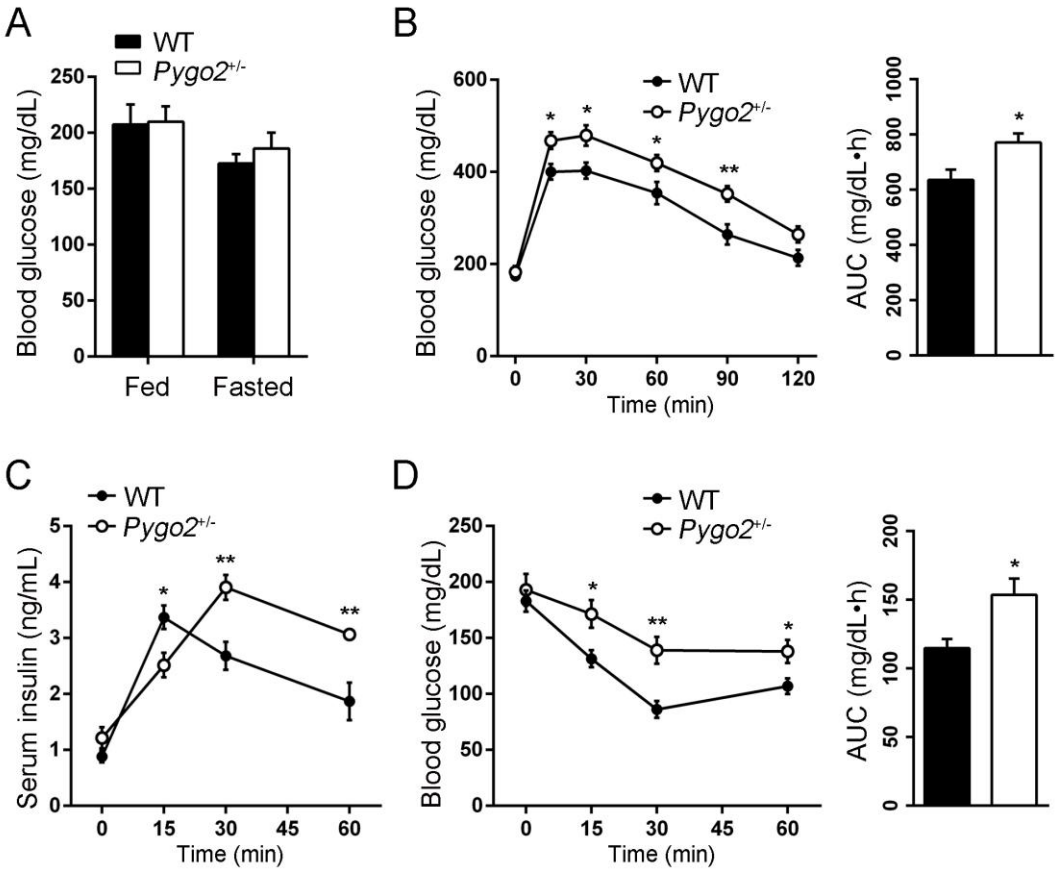
(**A**) Feeding and fasting blood glucose levels in 15-week-old *Pygo2*$^{+/-}$ and WT mice fed a normal chow diet (n=8). (**B**) Fasting serum insulin concentration in 15-week-old *Pygo2*$^{+/-}$ and WT mice fed a normal chow diet (n=6). (**C**) Glucose tolerance test. The blood glucose were determined at the indicated times in *Pygo2*$^{+/-}$ and WT mice described as A (n=8). (**D**) Insulin tolerance test. Blood glucose were measured at the indicated times in *Pygo2*$^{+/-}$ and WT mice described as A (n=8). Error bars represent SEM.



SUPPLEMENTARY DATA

Supplementary Figure 9. HFD feeding impaired glucose tolerance and decreased systemic insulin sensitivity in *Pygo2*$^{+/-}$ mice.

(**A**) Feeding and fasting blood glucose levels in *Pygo2*$^{+/-}$ and WT mice fed at 8-week-old with HFD for 6 weeks (n=6).(**B** and **C**) Glucose tolerance test. The blood glucose (**B**) and serum insulin (**C**) were determined at the indicated times in *Pygo2*$^{+/-}$ and WT mice with HFD described as A (n=9). The graphs represent the AUC (**B**, right). (**D**) Insulin tolerance test. Blood glucose were measured at the indicated times in *Pygo2*$^{+/-}$ and WT mice with HFD described as A (n=8). The graphs represent the AUC (**D**, right). Error bars represent SEM.*P <0.05, **P < 0.01.



SUPPLEMENTARY DATA

Supplementary Figure 10. No activation of COUP-TFII by either β-Catenin or Pygo2.
(**A** and **B**) *COUP-TFII* mRNA levels were measured by real-time qPCR and β-Catenin or Pygo2 overexpression level in 3T3-L1 cells were detected by western blotting. Each bar represents the average value of three independent experiments and error bars represent SD.