SUPPLEMENTARY DATA

Non-canonical Wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction

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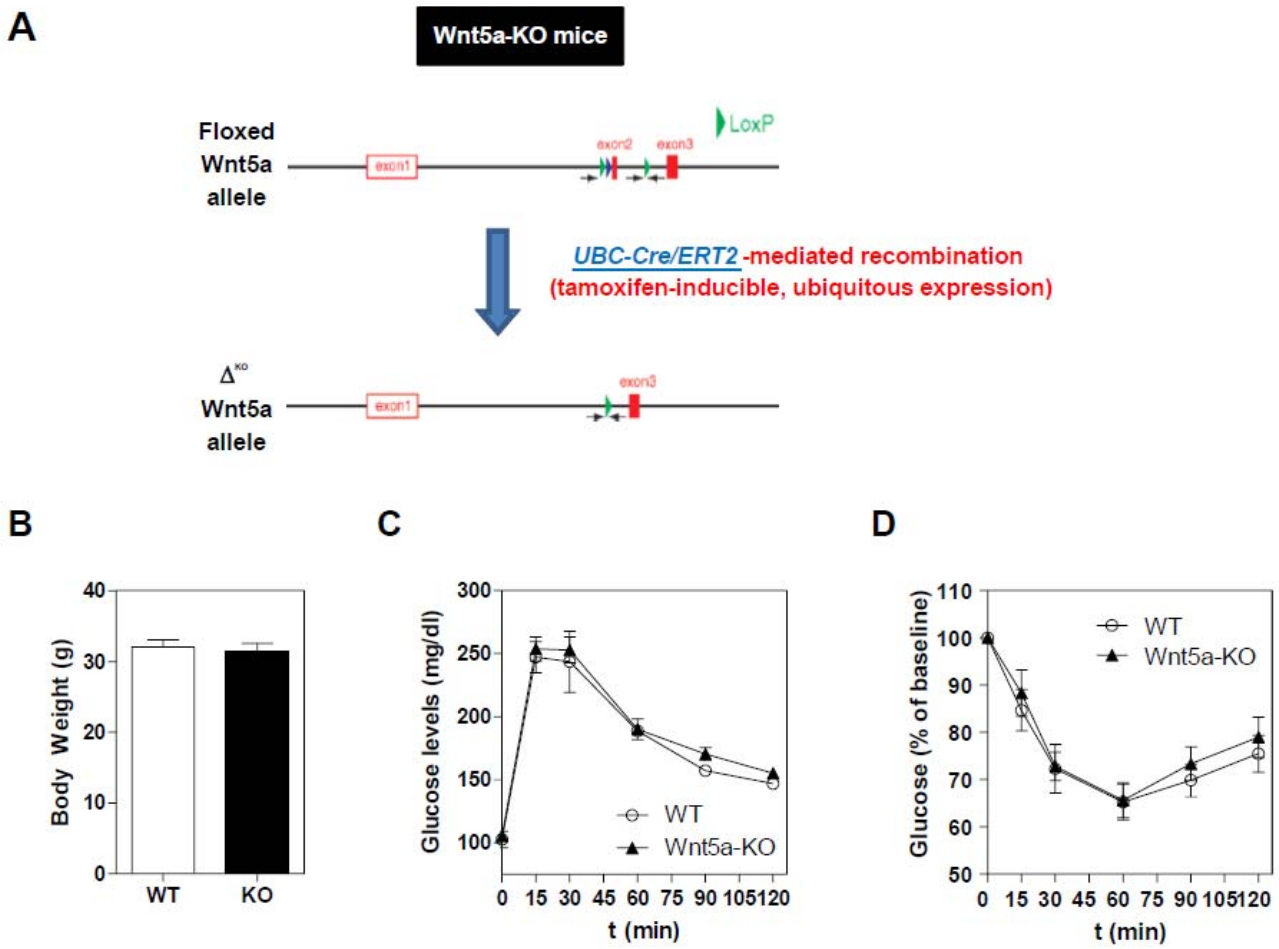
Supplementary Figure 1. Wnt5a ablation does not affect glucose homeostasis in lean mice. (**A**) Schematic representation of the Cre-LoxP strategy used to generate whole-body inducible Wnt5a-deficient mice. (**B**) Body weight, (**C**) GTT and (**D**) ITT of 20-week-old Wnt5a-KO mice and WT littermates fed standard chow diet (n=6-8).



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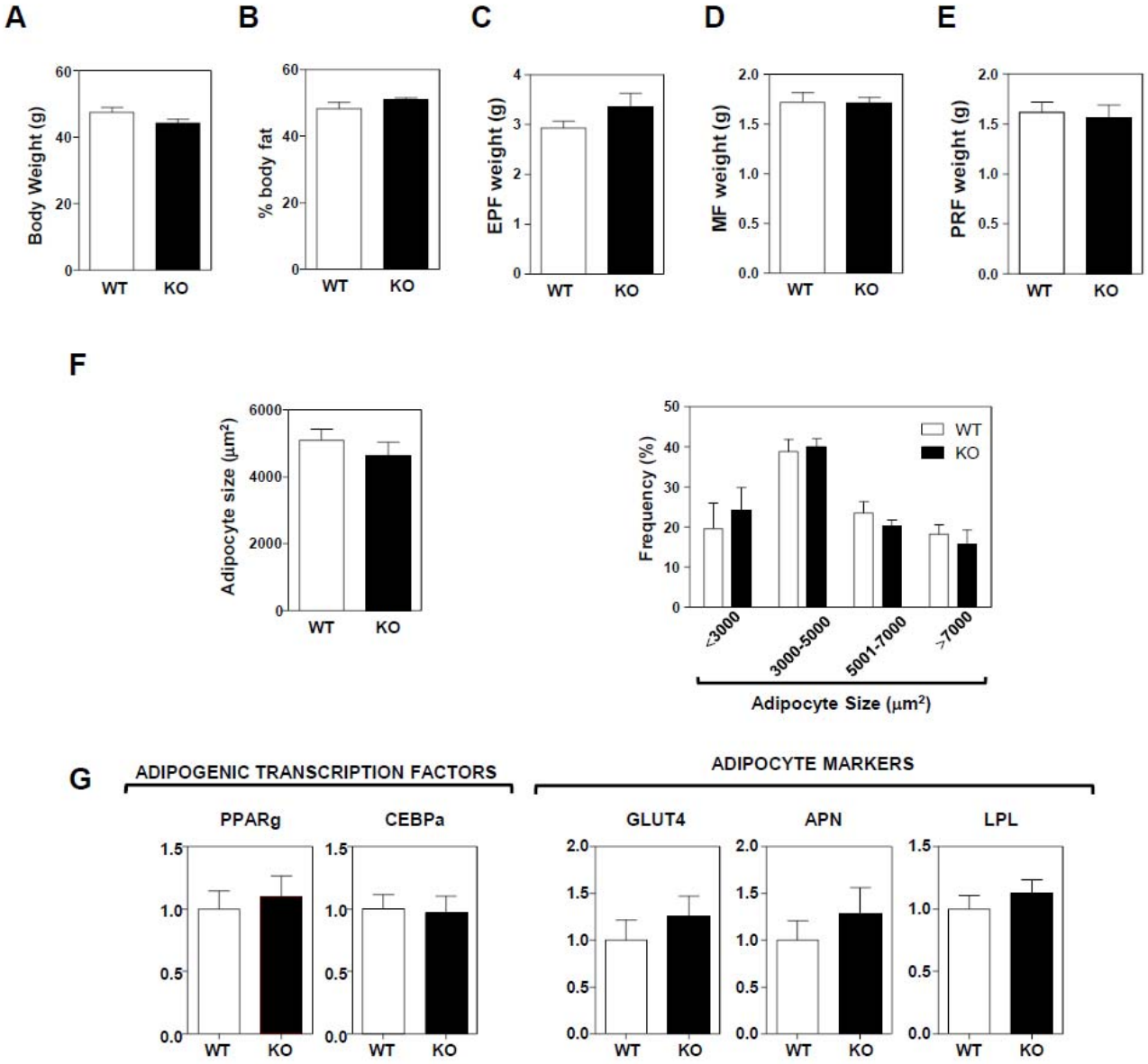
Supplementary Figure 2. Wnt5a ablation does not affect adipogenic expansion of white adipose tissue in HFHS-fed mice. Wnt5a-KO mice and WT littermates were fed a HFHS for 12 weeks (n=4-7). (**A**) Body weight. (**B**) % body fat mass assessed by magnetic resonance imaging (EchoMRI-700, Echo Medical System). (**C**) Epididymal fat weight. (**D**) Mesenteric fat weight. (**E**) Peri-renal fat weight. (**F**) Adipocyte size evaluated by computer-assisted morphometric analysis using Adiposoft (Galarraga et al, J Lipid Res. 2012 Dec;53(12):2791-6). (**G**) qRT-PCR analysis of transcript expression of several adipogenic transcription factors and adipocyte markers.



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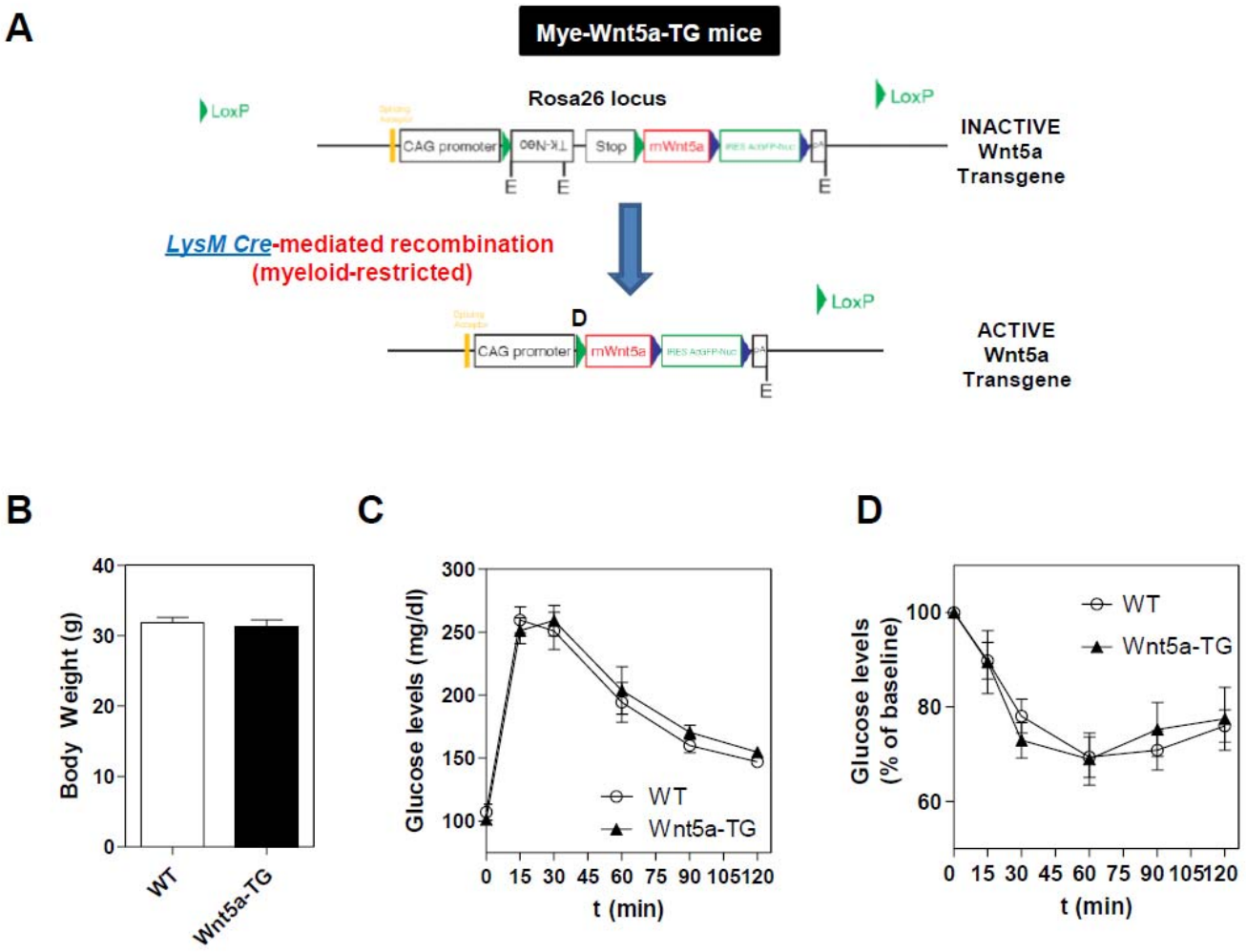
Supplementary Figure 3. Myeloid-specific Wnt5a overexpression does not affect glucose homeostasis in lean mice. (**A**) Schematic representation of the Cre-LoxP strategy used to generate myeloid-specific Wnt5a-overexpressing mice. (**B**) Body weight, (**C**) GTT and (**D**) ITT of 20-week-old Mye-Wnt5a-TG mice and WT littermates (n=6-8) fed standard chow diet.



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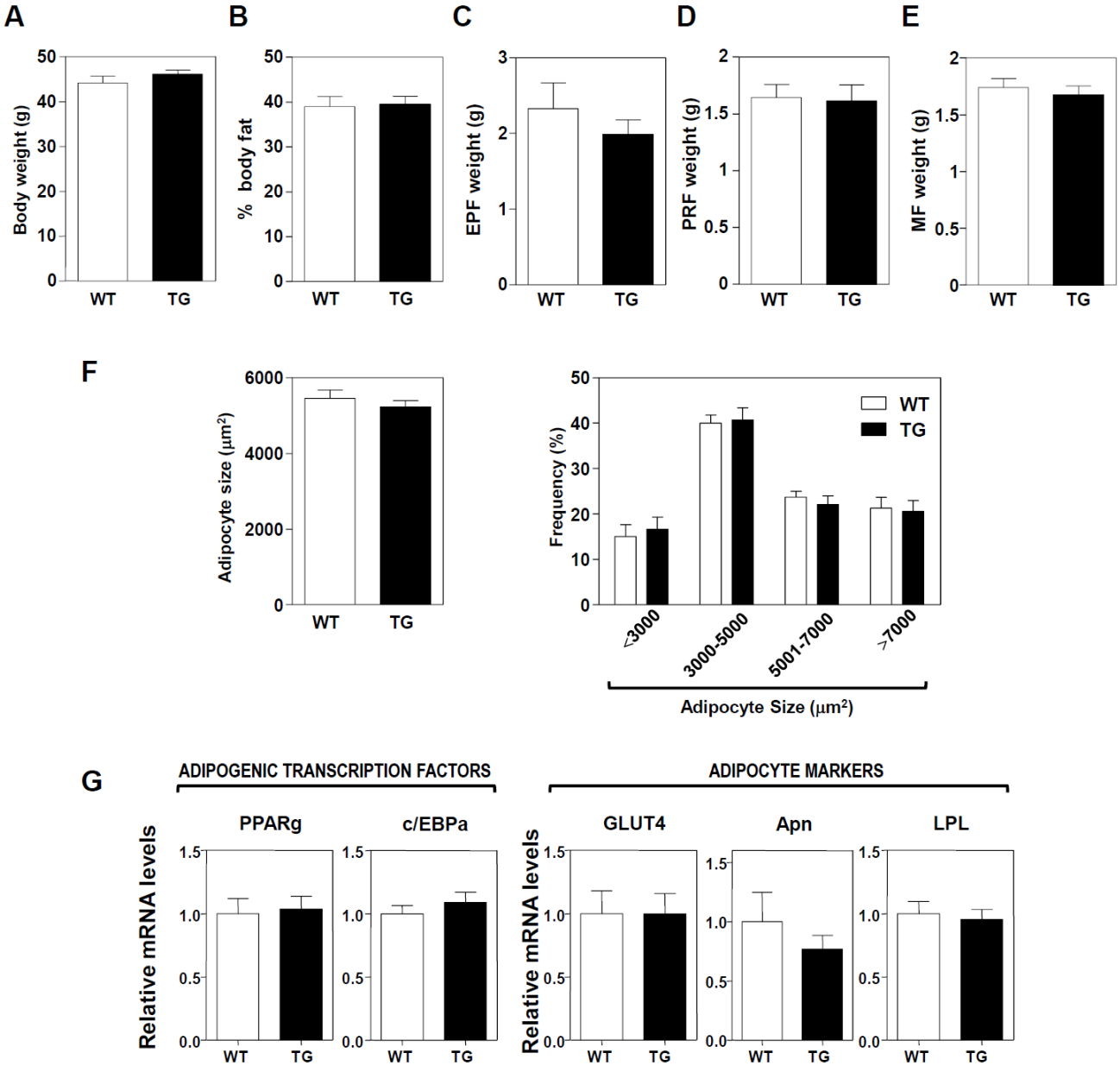
Supplementay Figure 4. Myeloid-specific Wnt5a overexpression does not affect adipogenic expansion of white adipose tissue in HFHS-fed mice. Mye-Wnt5a-TG mice and WT littermates were fed a HFHS for 12 weeks (n=4-9). (**A**) Body weight. (**B**) % body fat mass assessed by magnetic resonance imaging (EchoMRI-700, Echo Medical System). (**C**) Epididymal fat weight. (**D**) Mesenteric fat weight. (**E**) Peri-renal fat weight. (**F**) Adipocyte size evaluated by computer-assisted morphometric analysis. (**G**) qRT-PCR analysis of transcript expression of several adipogenic transcription factors and adipocyte markers in epididymal fat.



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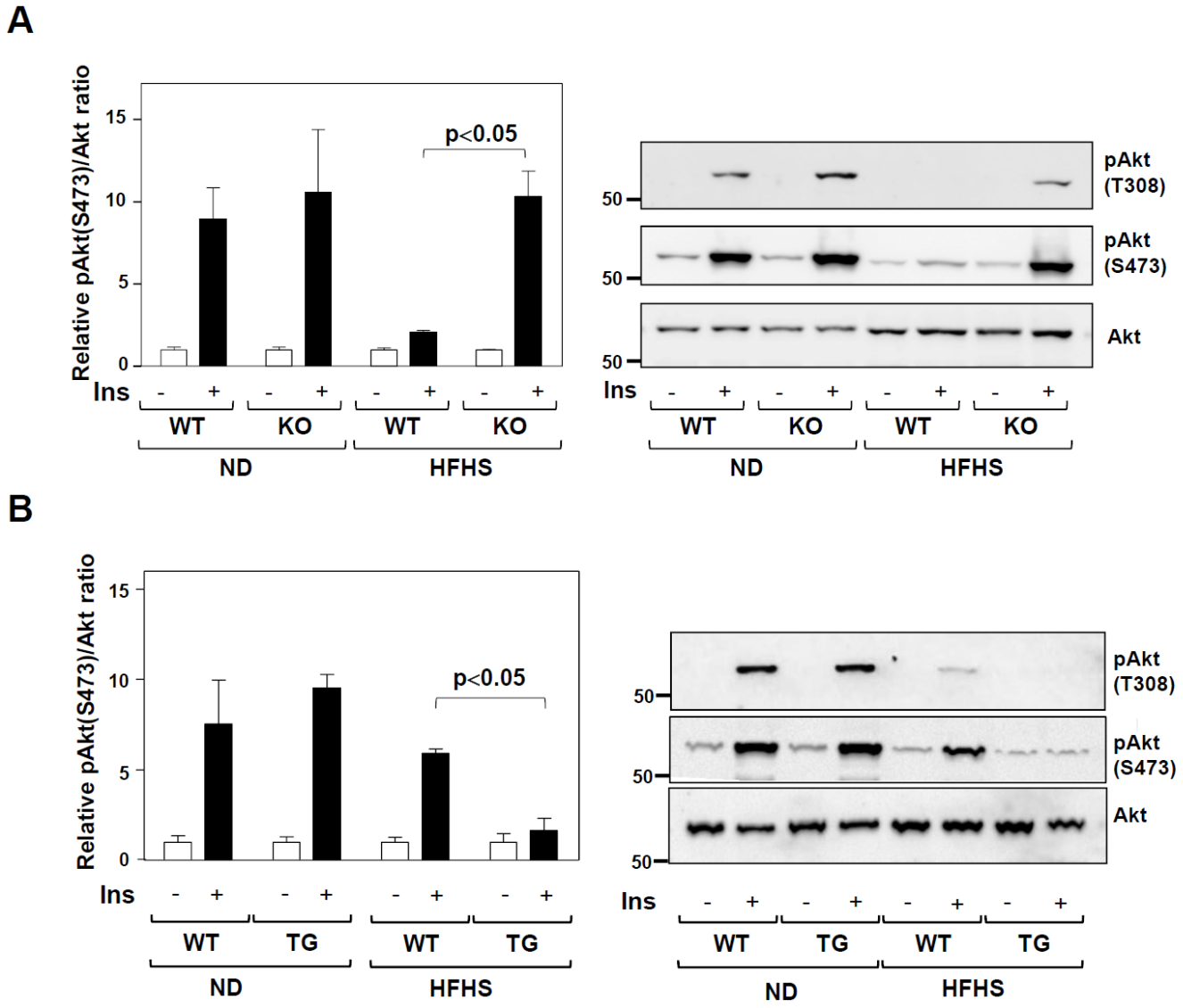
Supplementary Figure 5. Wnt5a-induced pro-inflammatory activation of macrophages promotes defective insulin signaling in adipocytes. (**A, B**) Epididymal WAT from normal diet (ND)- and HFHS-fed Wnt5a-KO and WT littermates (A) or Mye-Wnt5a-TG and WT littermates (B) was collected 10 minutes after intraperitoneal delivery of 0.75 U/kg of insulin, and Akt phosphorylation was evaluated by Western Blot. At least three independent pools of two mice per genotype and dietary regimen were analyzed. *Left*, densitometric quantification of the pAkt(S473)/Akt ratio. *Right*, representative immunoblot.



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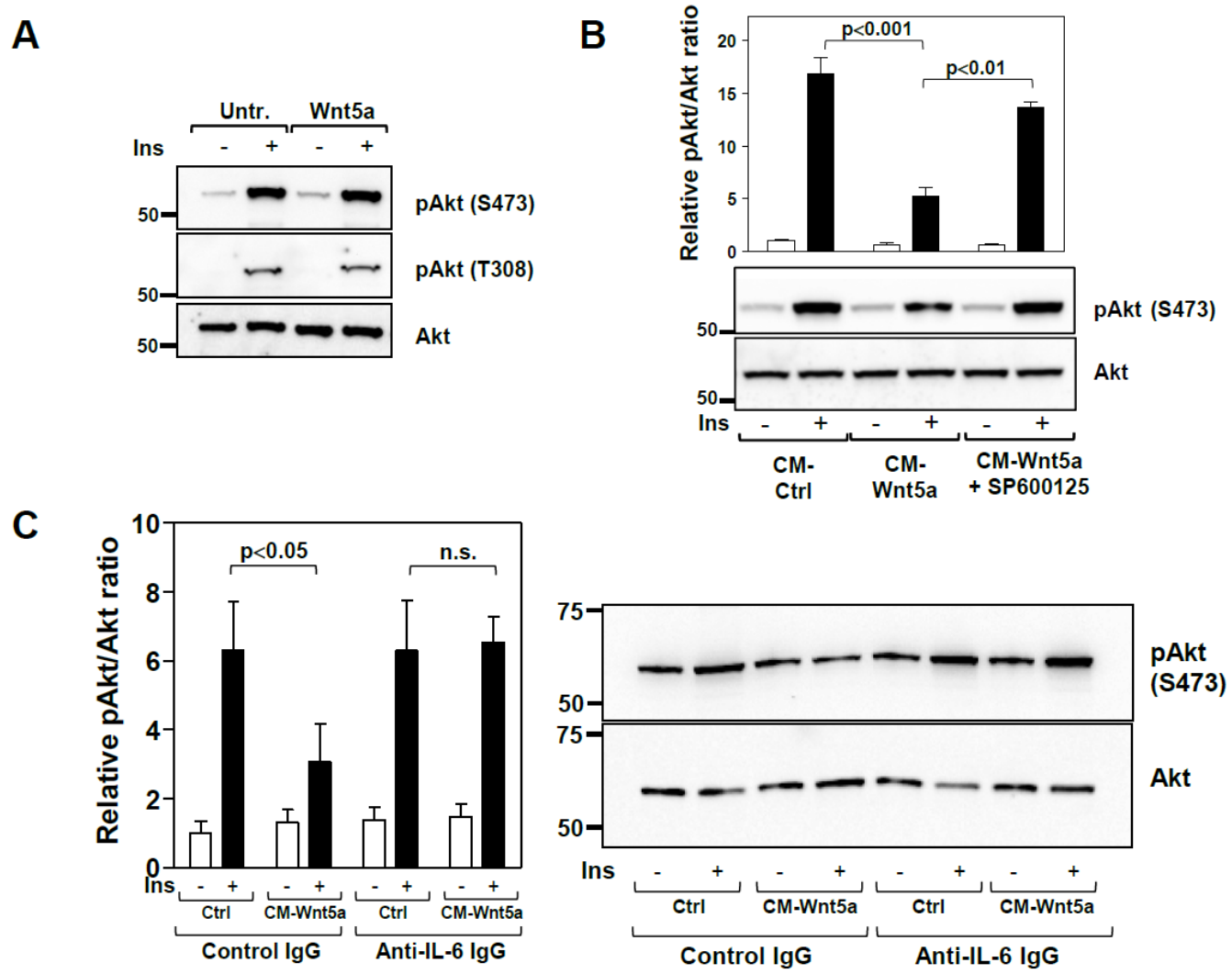
Supplementary Figure 6. JNK activation and IL-6 induction in macrophages mediate the effects of Wnt5a on adipocyte inulin signaling (**A**) Western Blot analysis of the effects of recombinant Wnt5a in insulin-induced Akt phosphorylation in cultured adipocytes. Mouse 3T3-L1 cells were maintained in DMEM with 10% calf serum and differentiated into adipocytes by treatment with DMEM supplemented with 5 µg/ml of insulin, 0.5 mM 1-methyl-3-isobutyl-xanthin, and 1 µM dexamethasone. Differentiated 3T3-L1 adipocytes were treated with recombinant Wnt5a (200 ng/ml) for 24 h and then stimulated with 100 nM insulin for 10 minutes. A representative experiment is shown. (**B**) Differentiated 3T3-L1 adipocytes were treated with 100 nM insulin for 10 min after a 24 h incubation with conditioned medium obtained from untreated, Wnt5a-treated or Wnt5a/SP600125-treated BM-derived macrophages. Insulin-induced Akt phosphorylation was analyzed by Western Blot. *Top*, densitometric quantification of the pAkt(S473)/Akt ratio. The graph shows the average of three independent experiments. *Bottom*, representative immunoblot. (**C**) Differentiated 3T3-L1 adipocytes were treated with 100 nM insulin for 10 min after a 24 h incubation with conditioned medium obtained from untreated or Wnt5a-treated macrophages containing 1 µg/ml IL-6-neutralizing antibody or control IgG (R&D Systems). Insulin-induced Akt phosphorylation was analyzed by Western Blot. *Left*, densitometric quantification of the pAkt(S473)/Akt ratio. The graph shows the average of three independent experiments. *Right*, representative immunoblot.



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Supplementary Table 1. Human study population characteristics.
Data expressed as Mean±SEM

Clinical parameter	
Age (years)	42±2
Female sex (%)	74%
BMI (kg/m^2)	45±1
Waist circumference (cm)	132±3
Weight (kg)	125±5
Systolic Blood Pressure	127±2
Diastolic Blood Pressure	73±1
Insulin (mU/ml)	15±3
Fasting glucose (mg/dl)	118±13
HOMA	4±1
HbA1C (%)	6.3±0.4
Triglycerides (mg/dl)	122±13
Total cholesterol (mg/dl)	175±7
HDL-cholesterol (mg/dl)	42±1
LDL-cholesterol (mg/dl)	109±5
Diabetes (%)	34%
Hypertension (%)	51%
Hypercholesterolemia (%)	20%

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Supplementary Table 2. Primers used for mouse gene expression analysis by qRT-PCR

Gene	FW primer	RV primer
36B4	GCTCCAAGCAGATGCAGCA	CCGGATGTGAGGCAGCAG
TNF-α	CGGAGTCCGGGCAGG	GCTGGGTAGAGAATGGATGAA
MCP-1	CAGCCAGATGCAGTTAACGC	GCCTACTCATTGGGATCATCTTG
IL-6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
Wnt5a	CTGGCTCCTGTAGCCTCAAG	GCCGCGCTATCATACTTCTC
Wnt5a (Exon 2-3)	GAGGTGCCATGTCTTCCAAG	TACTTCTGACATCTGAACAGGG
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
CD68	TTCTCCAGCTGTTCACCTTGACCT	GTTGCAAGAGAAACATGGCCCGAA
CD11c	ATGGAGCCTCAAGACAGGAC	GGATCTGGGATGCTGAAATC