PNAS
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Supplementary Information for

Regulation of adipose tissue inflammation by interleukin 6

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Figures S1 to S7

www.pnas.org/cgi/doi/10.1073/pnas.1920004117

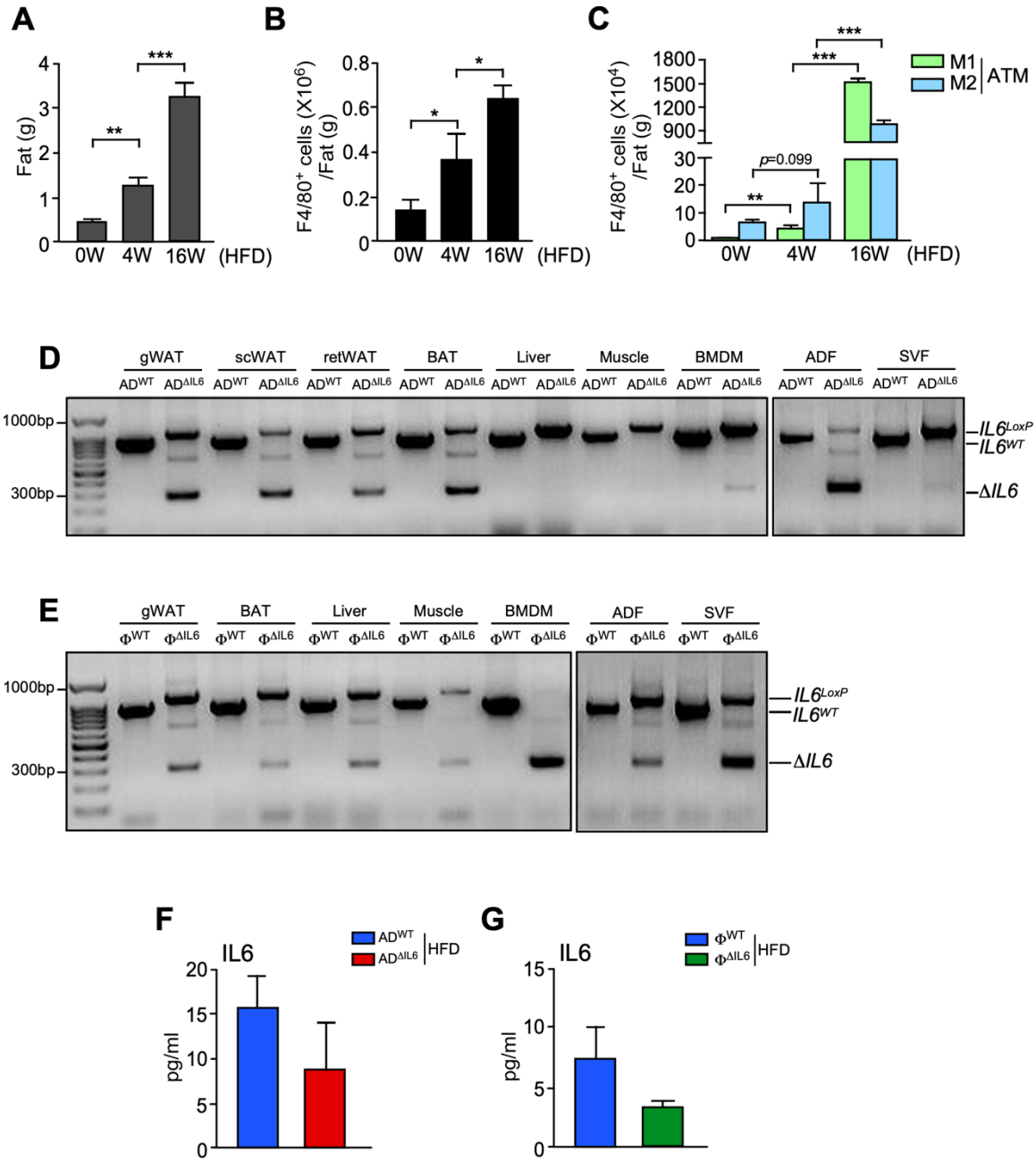

Fig. S1. (*A*) The gonadal fat mass from wild-type mice fed a HFD for 0, 4, and 16 wks was examined at age 24 wks (mean ± S.E.M.; $n = 2$; $^{**}p < 0.01$, $^{***}p < 0.001$) . (*B,C*) Immunophenotyping of adipose tissue macrophage (ATM) number was examined by flow cytometry (mean ± S.E.M.; $n = 2$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). (*D, E*) Genomic DNA isolated from gonadal (epididymal) white fat (gWAT), sub-cutaneous inguinal WAT (scWAT), retroperitoneal WAT (retWAT), brown adipose tissue (BAT), liver, muscle, bone marrow-derived macrophages (BMDM), adipocyte fraction of gWAT (ADF), and stromal vascular fraction of gWAT (SVF) from AD^{WT} and $AD^{\Delta IL6}$ mice and from Φ^{WT} and $\Phi^{\Delta IL6}$ mice was examined by PCR analysis. (*F,G*) The serum concentration of IL6 in HFD-fed Control and IL6-deficient mice was measured by ELISA (mean ± S.E.M.; $n = 5 \sim 7$).

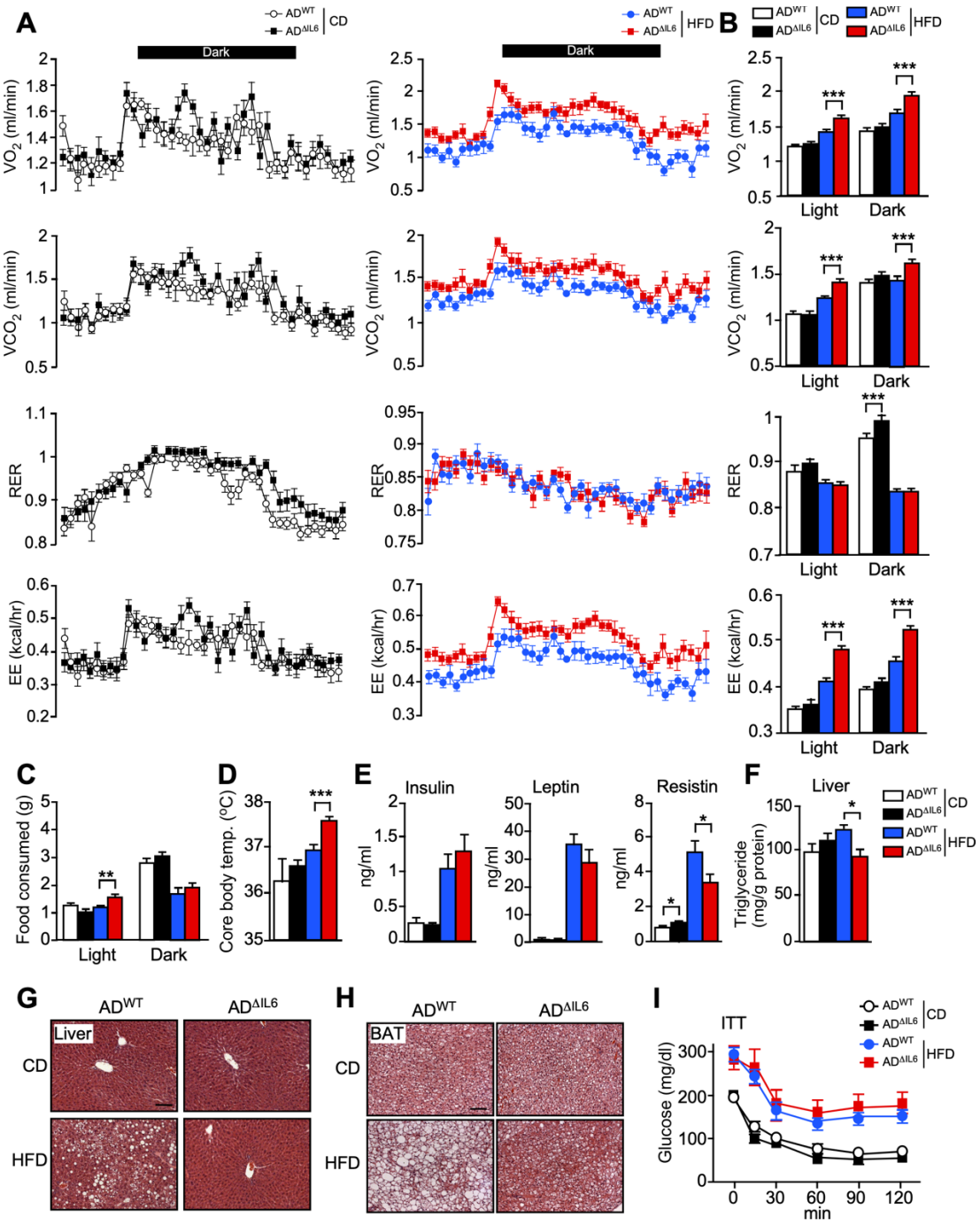

Fig. S2. (*A-C*) Metabolic cage analysis of chow diet (CD)-fed and high fat diet (HFD)-fed (6 wk.) mice was performed. VO_2, VCO_2, respiratory exchange ratio (RER), energy expenditure (EE), and food intake per mouse are presented for AD^{WT} and $AD^{\Delta IL6}$ mice (mean ± S.E.M.; n = 7 ~ 8; ***p < 0.001). (*D*) Core body temperature was measured by telemetry using an implanted probe (mean ± S.E.M.; n = 5 ~ 8; ***p < 0.001). (*E*) The blood concentration of insulin, leptin, and resistin was measured in AD^{WT} and $AD^{\Delta IL6}$ mice fed a CD or a HFD (16 wk.) (mean ± S.E.M.; n = 7 ~ 14; *p < 0.05). (*F*) The amount of hepatic triglyceride was measured (mean ± S.E.M.; n = 5 ~ 6; *p < 0.05). (*G*) Sections of liver of CD-fed and HFD-fed AD^{WT} and $AD^{\Delta IL6}$ mice (16 wk.) were stained with hematoxylin & eosin (scale bar = 100 μm). (*H*) Sections of BAT were stained with hematoxylin & eosin (H&E) (scale bar = 100 μm). (*I*) CD-fed and HFD-fed (16 wk.) mice were examined by insulin tolerance tests (ITT) (mean ± S.E.M.; n = 5 ~ 13).

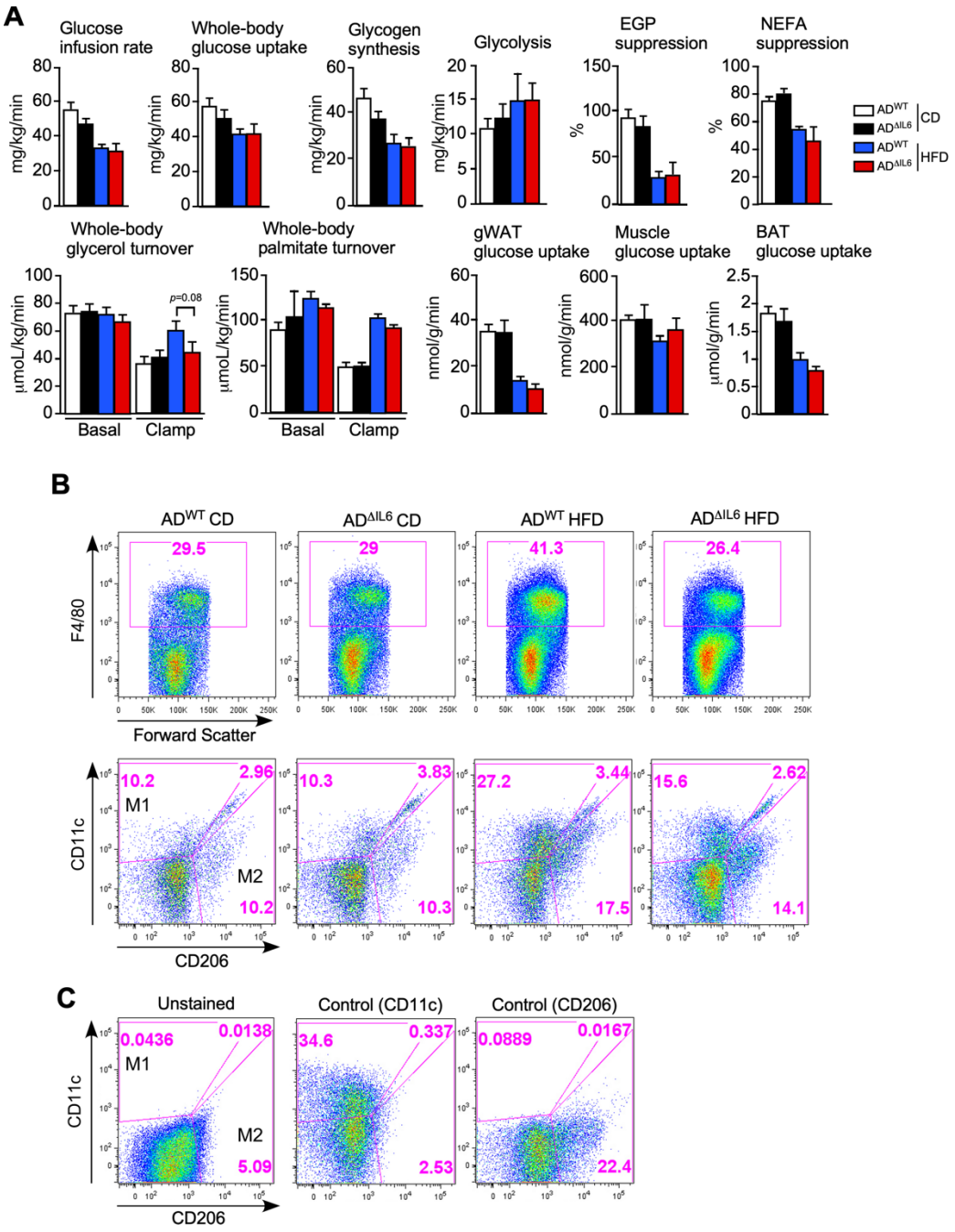

Fig. S3. (*A*) Hyperinsulinemic-euglycemic clamp study. The glucose infusion rate, whole-body glucose uptake, glycogen synthesis, glycolysis, endogenous glucose production (EGP), non-esterified fatty acid (NEFA) suppression, whole-body glycerol turnover, whole-body palmitate turnover, and glucose uptake in gWAT, muscle, and BAT were measured during clamps with CD-fed and HFD-fed (8 wk.) mice (mean ± S.E.M.; n=5~7). (*B*) The SVF of gWAT from CD-fed and HFD-fed AD^{WT} and $AD^{\Delta IL6}$ mice (16 wk.) was isolated and examined by flow cytometry. Representative data are presented. The $F4/80^{+}$ cells (*upper panels*) were examined by staining with CD11c and CD206 (*lower panels*). (*C*) The strategy employed for flow cytometry analysis is presented. $F4/80^{+}$ cells stained without (un-stained; *left panel*) or with CD206 plus an isotype control antibody for CD11c (*middle panel*) or CD11c plus an isotype control antibody for CD206 (*right panel*) were examined. Gates for M1-like ($CD11c^{+}$ $CD206^{-}$) and M2-like ($CD11c^{-}$ $CD206^{+}$) macrophages are indicated. Representative data are presented.

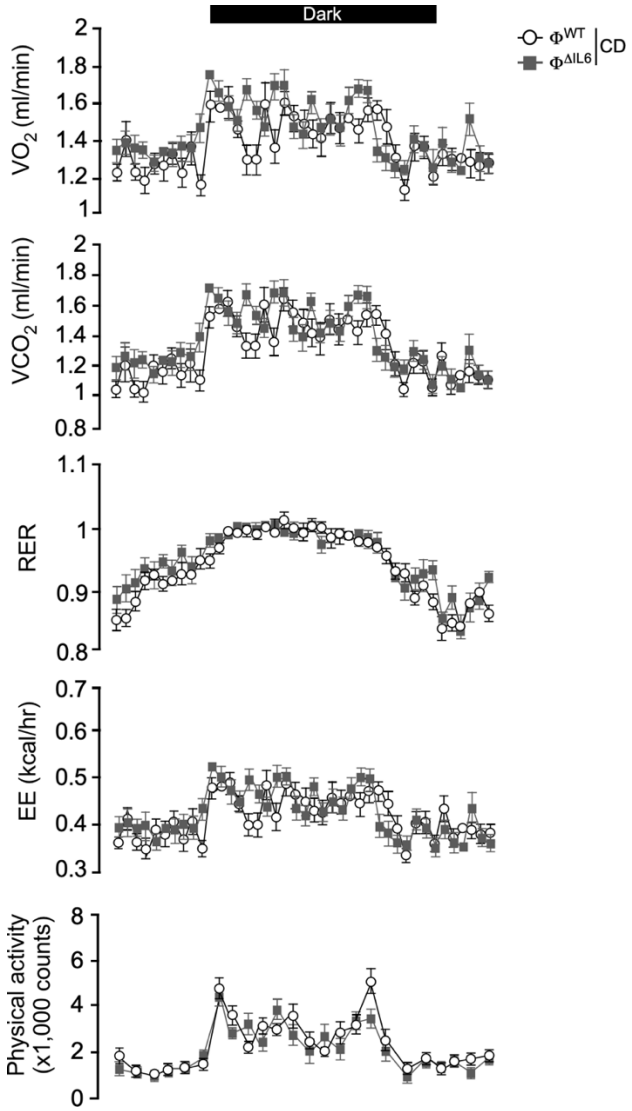

Fig. S4. Metabolic cage analysis of CD-fed Φ^{WT} and $\Phi^{\Delta IL6}$ mice (6 wk.) during a 24h period. The VO_2, VCO_2, RER, EE, and physical activity per mouse are presented (mean ± S.E.M.; *n* = 7 ~ 8).

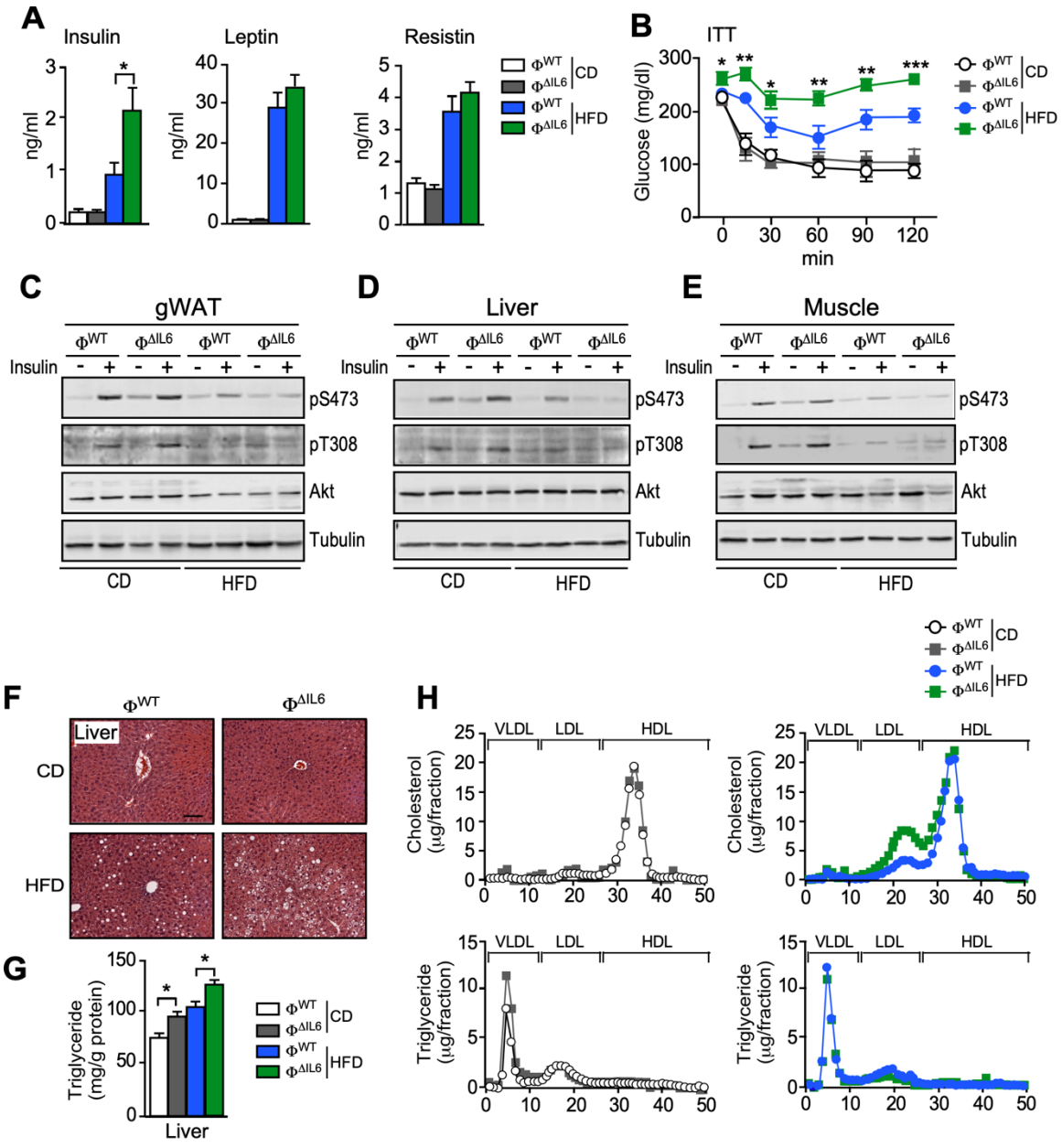

Fig S5. (*A*) The blood concentration of insulin, leptin, and resistin was measured in CD-fed and HFD-fed Φ^{WT} and $\Phi^{\Delta IL6}$ mice (16 wk.) (mean ± S.E.M.; $n = 6 \sim 8$; *$p < 0.05$). (*B*) CD-fed and HFD-fed (16 wk.) mice were examined by ITT (mean ± S.E.M.; $n = 5 \sim 8$; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (*C-E*) The CD and HFD-fed mice (16 wk.) were fasted overnight and then treated by intraperitoneal injection with 1 U/kg insulin (15 min). Immunoblot analysis of gWAT, liver, and gastrocnemius muscle was performed by probing with antibodies to pThr^{308}-Akt, pSer^{473}-Akt, Akt, and αTubulin. (*F*) Liver sections of CD-fed and HFD-fed Φ^{WT} and $\Phi^{\Delta IL6}$ mice (16 wk.) were stained with H&E (scale bar = 100 μm). (*G*) The amount of hepatic triglyceride was measured (mean ± S.E.M.; $n = 5 \sim 8$; *$p < 0.05$). (*H*) Blood lipoprotein analysis in Φ^{WT} and $\Phi^{\Delta IL6}$ mice on CD or HFD for 16 wk. was measured by fast performance liquid chromatography (FPLC) separation of pooled plasma ($n = 5$).

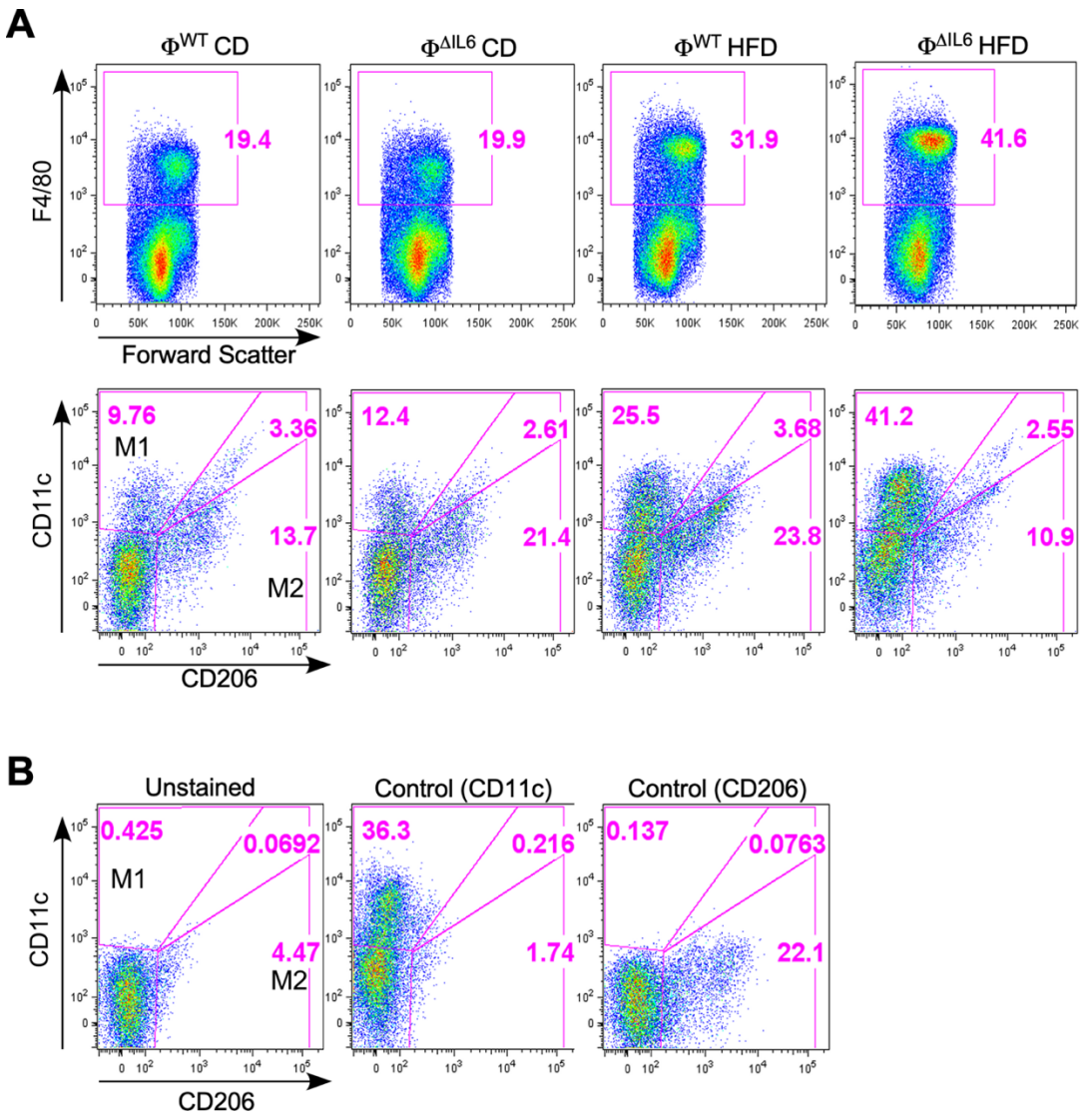

Fig S6. (A) The gWAT SVF from CD-fed and HFD-fed (16 wk) Φ^{WT} and $\Phi^{\Delta IL6}$ mice was examined by flow cytometry. Representative data are presented. (B) The strategy employed for flow cytometry analysis is presented. F4/80$^+$ cells stained without (un-stained; *left panel*) or with CD206 plus an isotype control antibody for CD11c (*middle panel*) or CD11c plus an isotype control antibody for CD206 (*right panel*) were examined. Gates for M1-like (CD11c$^+$ CD206$^-$) and M2-like (CD11c$^-$ CD206$^+$) macrophages are indicated. Representative data are presented.

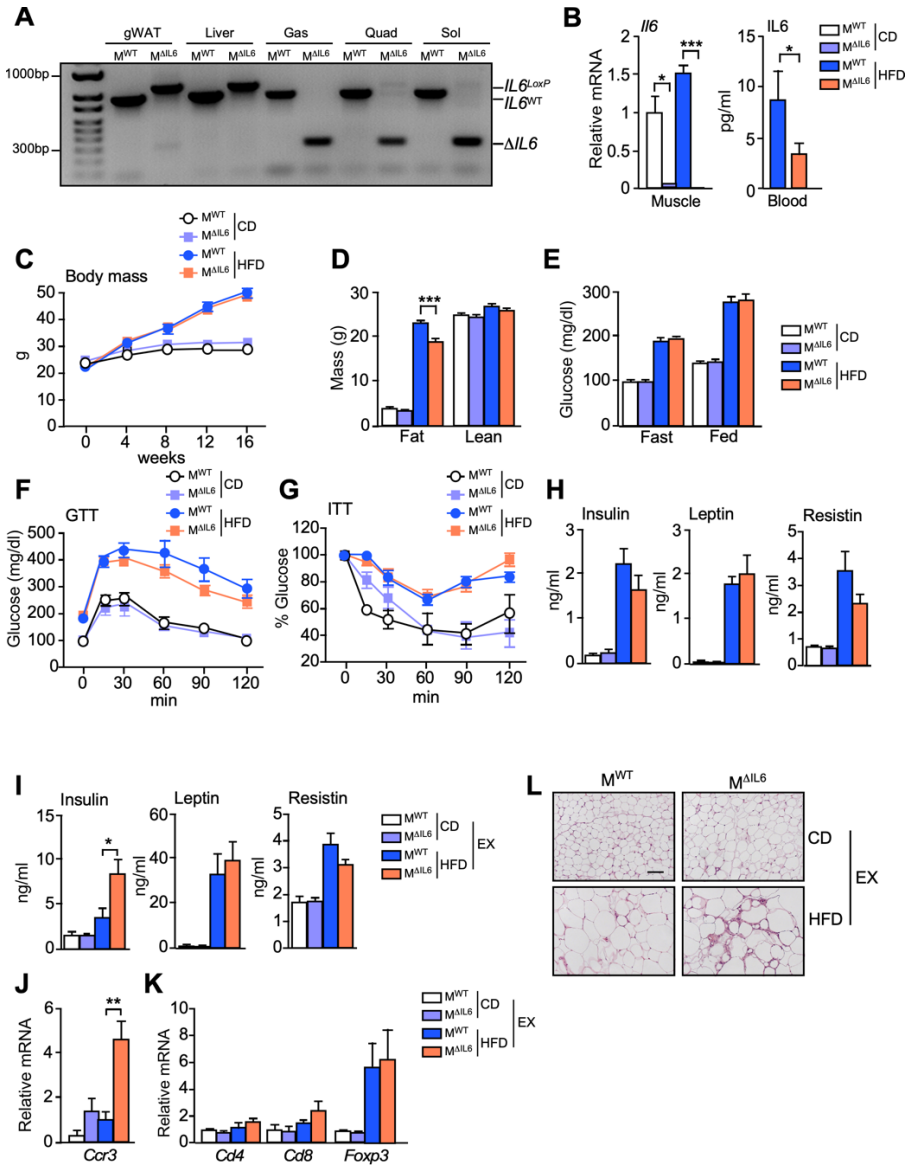

Fig S7. (*A*) Genomic DNA isolated from gWAT, liver, gastrocnemius muscle (Gas), quadriceps muscle (Quad), and soleus muscle (Sol) from M^{WT} and $M^{\Delta IL6}$ mice was examined by PCR analysis. (*B*) Total Gas muscle RNA isolated from CD-fed HFD-fed (16 wk.) M^{WT} and $M^{\Delta IL6}$ mice was examined using RT-qPCR analysis to detect *Il6* mRNA expression (*left panel*) and serum IL6 was measured by ELISA (*right panel*) (mean ± S.E.M.; n = 5 ~ 7; *p < 0.05, ***p < 0.001). (*C*) The change in body mass of CD-fed and HFD-fed M^{WT} and $M^{\Delta IL6}$ mice is presented (mean ± S.E.M.; n = 6 ~ 11). (*D*) Body composition was examined by ^{1}H-MRS (mean ± S.E.M.; n = 6 ~ 11; ***p < 0.001). (*E*) The blood concentration of glucose in overnight fasted mice and the blood glucose concentration in fed mice were measured (mean ± S.E.M.; n = 7 ~ 11). (*F*) The CD-fed and HFD-fed (16 wk.) mice were examined by GTT (mean ± S.E.M.; n =6 ~ 10). (*G*) The CD-fed and HFD-fed (16 wk.) mice was examined by ITT (mean ± S.E.M.; n = 6 ~ 10). (*H*) The blood concentration of insulin, leptin, and resistin of CD-fed and HFD-fed (16 wk.) M^{WT} and $M^{\Delta IL6}$ mice was measured (mean ± S.E.M.; n = 7 ~ 11). (*I*) The blood concentration of insulin, leptin, and resistin of exercised (EX) CD-fed and HFD-fed (16 wk.) M^{WT} and $M^{\Delta IL6}$ mice was measured (mean ± S.E.M.; n = 7 ~ 11; *p < 0.05). (*J, K*) The relative expression of the eosinophil marker (*Ccr3*) and T cell markers (*Cd4 and Cd8, and Foxp3)* was measured by RT-PCR assays of mRNA (mean ± S.E.M.; n = 5 ~ 7; **p < 0.01). (*L*) Sections of gWAT prepared from exercised CD-fed and HFD-fed (16 wk.) M^{WT} and $M^{\Delta IL6}$ mice were stained with H&E (scale bar = 100 μm).