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FAP expression in adipose tissue macrophages promotes obesity and metabolic inflammation

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Adipose tissue macrophages (ATM) are key players in the development of obesity and associated metabolic inflammation which contributes to systemic metabolic dysfunction. We here found that fibroblast activation protein α (FAP), a well-known marker of cancer-associated fibroblast, is selectively expressed in murine and human ATM among adipose tissue-infiltrating leukocytes. Macrophage FAP deficiency protects mice against diet-induced obesity and proinflammatory macrophage infiltration in obese adipose tissues, thereby alleviating hepatic steatosis and insulin resistance. Mechanistically, FAP specifically mediates monocyte chemokine protein CCL8 expression by ATM, which is further upregulated upon high-fat-diet (HFD) feeding, contributing to the recruitment of monocyte-derived proinflammatory macrophages with no effect on their classical inflammatory activation. CCL8 overexpression restores HFD-induced metabolic phenotypes in the absence of FAP. Moreover, macrophage FAP deficiency enhances energy expenditure and oxygen consumption preceding differential body weight after HFD feeding. Such enhanced energy expenditure is associated with increased levels of norepinephrine (NE) and lipolysis in white adipose tissues, likely due to decreased expression of monoamine oxidase, a NE degradation enzyme, by *Fap*$^{-/-}$ ATM. Collectively, our study identifies FAP as a previously unrecognized regulator of ATM function contributing to diet-induced obesity and metabolic inflammation and suggests FAP as a potential immunotherapeutic target against metabolic disorders.

macrophage | fibroblast activation protein (FAP) | CCL8 | obesity | inflammation

Significance

Macrophages play critical roles in the obesity-associated inflammation and energy storage. Identification of the factors that regulate the function of macrophages is helpful to develop new therapeutic target against obesity-associated metabolic disorders. We herein demonstrate that fibroblast activation protein (FAP), an extensively studied protein in cancer research, is preferably expressed in adipose tissue–resident macrophages and reveal a role of macrophage FAP in promoting diet-induced obesity and metabolic inflammation/dysfunction. We further uncover the underlying mechanism that macrophage FAP expression mediates inflammatory monocyte recruitment via upregulation of CCL8 and inhibits lipolysis and energy expenditure via controlling local norepinephrine metabolism. Thus, our study identifies FAP as a previously unrecognized molecular link between macrophage function and metabolic dysfunction.

The worldwide epidemic of obesity has contributed to the prevalence of many metabolic disorders including insulin resistance and type 2 diabetes as well as fatty liver/cirrhosis and cardiovascular disease (1). White adipose tissue (WAT) is an essential regulator of energy storage and systemic metabolic homeostasis. Accumulating evidence demonstrates that localized inflammation in adipose tissue is a predominant contributor to systemic low-grade inflammation and insulin resistance associated with obesity (2). Adipose tissue inflammation is characterized by increased accumulation and inflammatory polarization of immune cells as well as the release of pro-inflammatory cytokines (3, 4). Adipose tissue macrophages (ATM) represent the most abundant immune cells that infiltrate obese adipose tissues and are considered to be the major driver of obesity-associated chronic inflammation via the secretion of various inflammatory mediators (5). ATMs have been classified into two major subtypes: tissue-resident and monocyte-derived "recruited" macrophages (6). In the lean fat, resident ATM display the alternatively activated phenotype and function to maintain tissue homeostasis by resolving inflammation, while obesity-associated ATM adopted an inflammatory phenotype in the setting of metabolic stress, leading to unresolved local and systemic inflammation (7, 8). In addition to the classical inflammatory properties, recent observations in several models suggest that macrophages may also control adiposity and thermogenesis by modulation of lipid metabolism, tissue innervation, adrenergic signalling, and energy metabolism (9–12).

Recruited monocyte-derived macrophages contribute to dramatic increase in the number of ATM in obese adipose tissues, which is tightly linked to the degree of insulin resistance and metabolic dysregulation (5, 7). Blockade of recruited monocyte-derived macrophages by targeting critical monocyte chemoattractant has been shown to alleviate obesity-associated inflammation and associated metabolic disorders in mice (13–15). There are five members of monocyte chemotactic protein (MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MCP-5/CCL12), in which MCP1/CCL2 and its receptor CCR2 are well-known to mediate monocyte recruitment during obesity, contributing to metabolic inflammation and insulin resistance (15, 16). Notably, although genetic or pharmacologic interruption of CCR2 consistently reduced macrophage infiltration in the obese adipose tissue and

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improved insulin resistance (16, 17), the contrasting data were reported about the effect of CCL2 deficiency or blockade on macrophage infiltration in obese adipose tissue (18, 19), suggesting that other MCP members may be involved in the recruitment of monocyte-derived macrophages.

Fibroblast activation protein α (FAP), as a well-known marker for cancer-associated fibroblasts (CAFs), has been extensively studied in its tumor-promoting role (20). Our previous studies revealed a functional role of FAP in mediating secretion of inflammatory chemokines/cytokines by CAFs and consequent recruitment and activation of tumor-promoting myeloid cells (21–23). Interestingly, FAP was also reported to be expressed in several normal tissues of adult mice, including skin, adipose tissues, pancreas, skeletal muscle, and bone marrow, at lower levels than those in CAFs (24, 25). Moreover, ablation of the FAP-expressing cell causes loss of muscle mass and a reduction of B-lymphopoiesis and erythropoiesis in naive mice (25). However, it remains unclear that such effects were mediated by FAP itself or cell depletion. Recently, emerging evidence indicates a possible role of FAP in regulation of obesity and metabolism. FAP was first found to inactivate human FGF21 via proteolysis in vitro (26, 27). However, another study reported that lack of FAP had no effect on FGF21 levels in blood, adipose tissue, and liver in mice following fasting or HFD (high-fat-diet) feeding (28). Furthermore, conflicting phenotypes were reported in the mouse model of diet-induced obesity by using *Fap*$^{-/-}$ mice on FVB or C57BL/6 background with different gene-deletion strategies (28, 29). Therefore, the exact role of FAP in the regulation of obesity and associated metabolic dysfunction needs further exploration. We and others recently found that administration of the FAP inhibitor could reduce body weight and improve glucose metabolism, which is partly dependent on macrophages (30, 31). Given that FAP was previously reported to be expressed in tumor-associated macrophages in human breast cancer and murine lung cancer (32), we aimed to investigate whether ATM express FAP and its potential role in diet-induced obesity and metabolic dysfunction via regulation of ATM function.

In this study, we identify a selective expression of FAP in ATM and reveal a previously unrecognized function of macrophage FAP in promoting diet-induced obesity and metabolic inflammation, thereby contributing to systemic metabolic dysfunction. We further uncover the underlying mechanism that macrophage FAP mediates inflammatory monocyte recruitment via upregulation of CCL8 and inhibits energy expenditure via controlling local norepinephrine (NE) concentrations and lipolysis. These findings may have important implications for the development of FAP-targeted immunotherapies for obesity and its associated metabolic dysfunction.

Results

FAP Is Selectively Expressed in ATM. We first examined FAP expression in different adipose depots of naive male mice on C57BL/6 background and detected its expression in epididymal WAT (eWAT) and subcutaneous inguinal WAT (iWAT) at high levels and in brown adipose tissue (BAT) at much lower levels as determined by western blotting (Fig. 1*A*). To further determine cellular distribution of FAP in adipose tissues, flow cytometry analysis was performed and revealed FAP expression in both CD45$^+$ leukocytes and CD45$^-$ non-immune cells in eWAT and iWAT (Fig. 1*B*). Much lower percentages of FAP$^+$ cells were detected in BAT (Fig. 1*B*), which was consistent with the result of western blotting (Fig. 1*A*). Notably, almost all CD45$^+$ FAP$^+$ cells in WAT were F4/80$^+$CD11b$^+$ macrophages (Fig. 1*B*), which was confirmed by PCR analysis showing FAP was expressed in macrophages but not non-macrophage CD45$^+$ cells isolated from eWAT (*SI Appendix*, Fig. S1*A*). Moreover, FAP$^+$ macrophages comprised more than half of total macrophages in WAT (Fig. 1*C*). The expression of FAP in ATM was further confirmed by confocal immunofluorescence staining (Fig. 1*D*). We then examined whether FAP is expressed in macrophages derived from other types of normal tissues. Very little, if any, FAP expression was detected in bone marrow-derived macrophages (BMDM), peritoneal macrophages (PM), and macrophages from the lung, liver, and spleen except tumor-associated macrophages that were previously reported to express FAP (Fig. 1*E*) (32). Similar to murine ATM, CD14$^+$CD163$^+$ macrophages from human subcutaneous WAT also expressed FAP (Fig. 1*F*). Interestingly, we found that BMDM could acquire FAP expression after incubating with supernatants from eWAT explants, isolated stromal vascular fraction (SVF) or adipocytes (*SI Appendix*, Fig. S1*B*). However, TGF-β or PDGFα, both of which were reported to induce FAP expression in fibroblasts (33), failed to induce FAP expression in BMDM (*SI Appendix*, Fig. S1*C*). These data suggest that the WAT microenvironment favors FAP expression in macrophages. Collectively, these results demonstrate that FAP is selectively expressed in ATM, which may be involved in the regulation of ATM function.

Specific Deletion of FAP in Macrophages Protects Mice against Diet-Induced Obesity and Metabolic Dysfunction. To explore whether FAP expression in ATM influences obesity and associated metabolic dysfunction, we generated macrophage-selective *Fap*-deficient mice by crossing *Fap*-floxed mice with LysM-Cre transgenic mice on a C57BL/6 background (*Fap*Δ^{LysM}) (*SI Appendix*, Fig. S2) and exposed these mice and their littermate controls to HFD feeding. There were no differences in body weight and fat mass between *Fap*Δ^{LysM} mice and littermate controls after ND feeding (Fig. 2 *A* and *B*). *Fap*Δ^{LysM} mice exhibited obvious decreases in weight gain and adiposity compared with littermate controls after 12 wk of HFD feeding (Fig. 2 *A* and *B*). Consistently, histological analysis showed smaller adipocytes in both eWAT and iWAT of HFD-fed *Fap*Δ^{LysM} mice (Fig. 2*C*). Moreover, macrophage-specific deletion of *Fap* resulted in additional metabolic benefits, including alleviated hepatic steatosis with fewer accumulation of lipid droplets (Fig. 2 *D* and *E*), lower serum levels of triglycerides and total cholesterol (Fig. 2*F*), as well as improved glucose intolerance (GTT) and insulin resistance (ITT) after 12 wk of HFD feeding (Fig. 2*G*). Given that FAP was detected at very low, if any, levels in macrophages from other normal tissues investigated (Fig. 1*E*) as well as other leukocytes in adipose tissues (Fig. 1*B*), the phenotypes observed in *Fap*Δ^{LysM} mice could be largely attributable to FAP expression in ATM. Previous studies reported paradoxical results from global knockout of FAP in the HFD-induced obesity model (28, 29). We therefore examined the phenotype of HFD-fed *Fap*$^{-/-}$ mice which were obtained from JAX lab and widely used for research. We found that HFD feeding induced similar phenotypes in *Fap*$^{-/-}$ mice, including reduced body weight and adiposity as well as ameliorated metabolic syndromes such as hypertriglyceridemia, hypercholesterolemia, hepatic steatosis, and glucose intolerance/insulin resistance (*SI Appendix*, Fig. S3 *A*–*F*). Collectively, these results demonstrate that macrophages FAP expression contributes to HFD-induced obesity and associated metabolic dysfunction.

Specific Deletion of FAP in Macrophages Attenuates Obesity-Induced Systemic and Adipose Inflammation. We next examined whether the improvements in glucose metabolism and insulin resistance in *Fap*Δ^{LysM} mice are associated with alleviation of obesity-induced systemic and adipose inflammation. *Fap*Δ^{LysM} mice had significantly decreased serum levels of proinflammatory

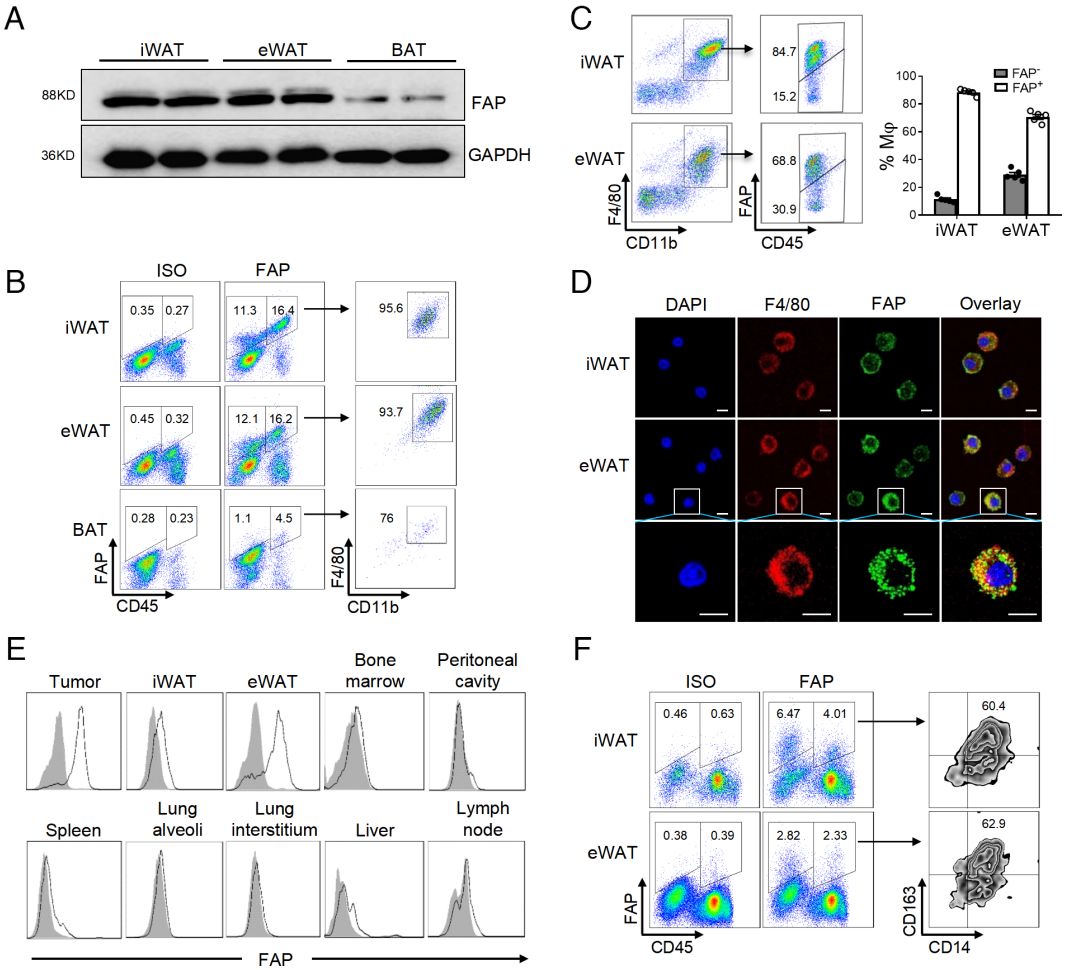

Fig. 1. FAP is selectively expressed in ATM. (*A* and *B*) Immunoblot analysis of FAP expression (*A*) and representative flow cytometry dot plots showing percentages of FAP$^+$ cells in SVF and FAP$^+$ CD45$^+$ cells in total macrophages (CD11b$^+$F4/80$^+$) (*B*) in inguinal subcutaneous WATs (iWAT), eWAT and interscapular BAT. (*C*) Representative flow cytometry dot plots and average percentages of FAP$^+$ macrophages and FAP$^-$ macrophages in total ATM. n = 5. (*D*) Immunofluorescent confocal images showing positive staining of F4/80 and FAP in sorted macrophages of iWAT and eWAT. DAPI (blue), F4/80 (red), FAP (green). (Scale bars, 5 μm.) (*E*) Representative histogram of FAP expression in macrophages derived from different types of tissue. (*F*) Representative flow cytometry dot and zebra plots showing FAP expression in SVF and CD45$^+$ FAP$^+$ cells in macrophages (CD14$^+$CD163$^+$) within human subcutaneous WAT. Data are represented as mean ± SEM. Results are representative of three independent experiments.

cytokines including tumor necrosis factor-α (TNF-α), IL-1β and IL-6 compared with littermate controls after 12-wk HFD feeding (Fig. 3*A*). Immunohistochemistry staining revealed lower numbers of F4/80$^+$ macrophage crown-like structures (CLSs), which is usually used to evaluate adipose inflammation (34), in eWAT of *Fap*Δ^{LysM} mice than those in littermate controls after 12-wk HFD feeding (Fig. 3*B*). Consistently, HFD-fed *Fap*Δ^{LysM} mice had significantly decreased numbers of total macrophages in eWAT as determined by flow cytometry (Fig. 3*C*). In obesity, the majority of recruited ATMs are positive for CD11c which has been considered as a useful marker to determine pro-inflammatory ATMs in both mouse and human studies (35, 36). We found significantly decreased percentages of proinflammatory CD11c$^+$ macrophages while comparable percentages of M2-like CD11c$^-$CD206$^+$ macrophages in eWAT *of Fap*Δ^{LysM} mice (Fig. 3*D*). Furthermore, significantly reduced percentages of T cells (both CD4$^+$ and CD8$^+$ cells) and natural killer (NK) cells while increased type 2 innate lymphoid cells (ILC2) and eosinophils were observed in eWAT of HFD-fed *Fap*Δ^{LysM} mice, suggesting that macrophage FAP expression deficiency causes less shift from anti-inflammatory toward inflammatory immune phenotype following HFD feeding (Fig. 3*E*, see gating scheme, *SI Appendix*, Fig. S4*A*). Similar alterations in infiltrating macrophages were observed in iWAT of *Fap*Δ^{LysM} mice after HFD feeding (*SI Appendix*, Fig. S4 *B–D*). Collectively, these results demonstrate that macrophage FAP expression promotes accumulation of proinflammatory macrophages in obese adipose tissues and consequent systemic inflammation.

FAP$^+$ATM Exhibit a Distinct Gene Expression Profile Characterized by Increased MCP Family Members, Particularly CCL8. We attempted to determine the downstream effector of FAP expression in ATM that mediates HFD-induced obesity and metabolic inflammation. To avoid the potential influence of differential adipose tissue microenvironment between WT and *Fap*$^{-/-}$ mice due to different adiposity after HFD feeding on the transcriptional profile of ATM, we isolated FAP$^+$ and FAP$^-$ macrophages by FACS from eWAT of WT mice fed with ND or HFD to perform unbiased RNA sequencing (RNA-seq). Differentially expressed genes were detected between FAP$^+$ATM and FAP$^-$ATM from mice fed with ND, in which *Ccl8* that encodes MCP2 was the most dramatically upregulated gene ($Log_2FC > 1$; adjusted $P < 0.05$) in FAP$^+$ATM (Fig. 4*A* and *SI Appendix*, Table S1). Moreover, *Ccl8* and *Ccl12*, both of which are located in close proximity to each other on chromosome, were among the most upregulated genes detected in FAP$^+$ATM from HFD-fed obese mice ($Log_2FC > 1$; adjusted $P < 0.05$) (Fig. 4*A*). In addition to *Ccl8*, *C6* that encodes

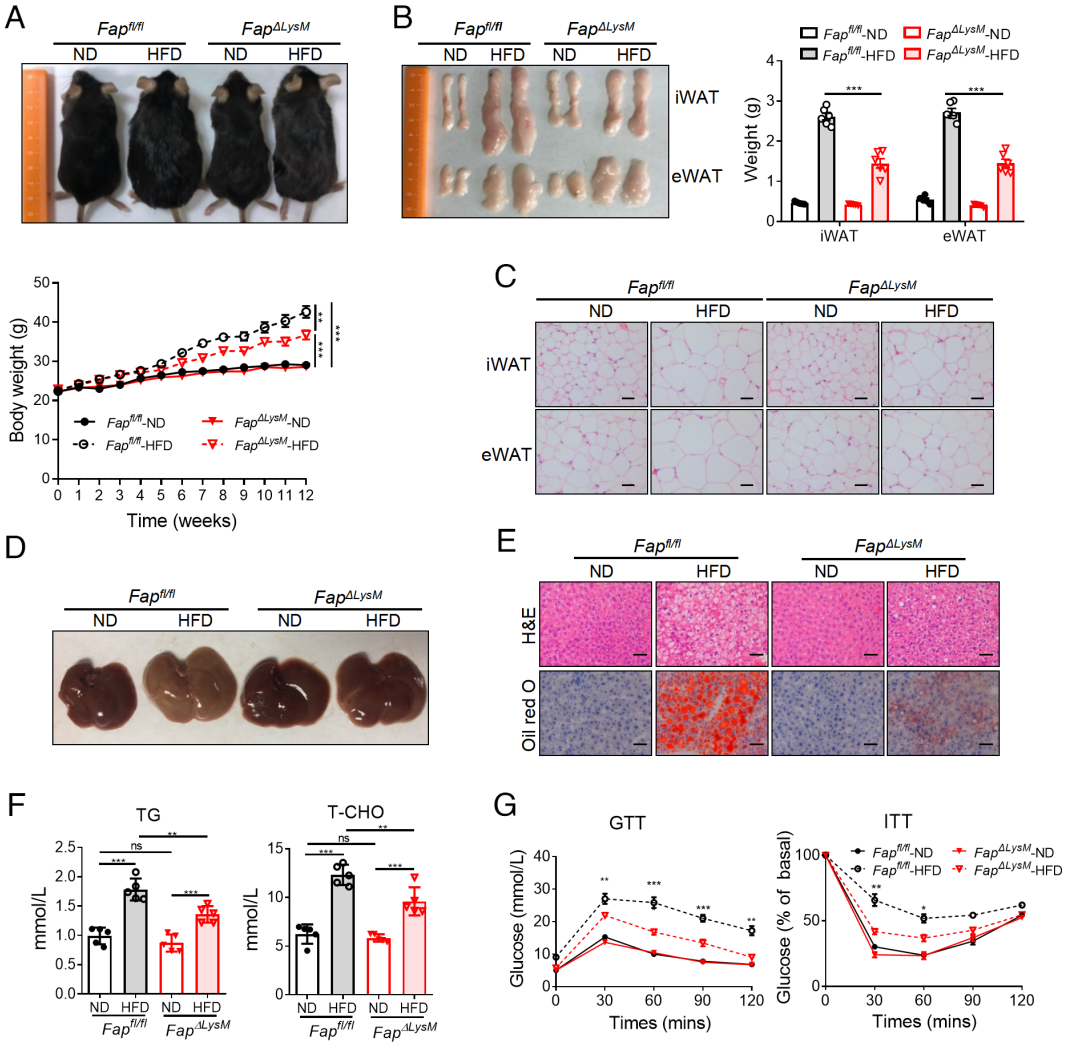

Fig. 2. Specific deletion of FAP in macrophages protects mice against diet-induced obesity and metabolic dysfunction. Macrophage-specific *Fap* deficient mice ($Fap^{\Delta LysM}$) and $Fap^{fl/fl}$ littermate controls were fed with ND or HFD for 12 wk. (*A*) Representative mouse photos (*Up*) and body weight curves (*Down*). (*B* and *C*) Representative photos and average weights (*B*) as well as representative images of hematoxylin and eosin (H&E) staining of iWAT and eWAT (*C*). n = 6 for *A* and *B*. (*D*) Representative photos of whole livers. (*E*) Images of H&E staining and Oil red O staining of liver sections. (Scale bars, 50 μm in *C* and *E*.) (*F*) Serum levels of triglycerides (TG) and total cholesterol (T-CHO). (*G*) Glucose tolerance test (GTT) and insulin tolerance test (ITT). n = 5 for *F* and *G*. Statistical comparisons were made using two-way ANOVA followed by Bonferroni post-tests. Data are represented as means ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and ns = no significance. Results are representative of two independent experiments.

complement 6 was another gene greatly affected by FAP expression under both steady and obese state, which was consistent with KEGG analysis showing that the majority of enriched pathways in FAP^+ATM were associated with infectious diseases (Fig. 4*B* and *SI Appendix*, Table S2). Additionally, FAP^+ATM exhibited enriched pathway associated with cytokine/chemokine signaling (Fig. 4*B*), primarily characterized by upregulated gene expression of C-C motif chemokine ligand, particularly MCP family members that are important for monocyte migration (Fig. 4*C* and *SI Appendix*, Table S3). In contrast, there were no obvious differences in gene expression of C-X-C motif chemokine ligands (*SI Appendix*, Fig. S5*A*).

FAP Is Predominantly Expressed in Resident ATM with No Effect on Proinflammatory Phenotype Induction. Interestingly, we noted that FAP^+ATM expressed much higher levels of several genes which encode markers for tissue-resident macrophages such as *Mrc1* (CD206), *Clec10a* (CD301), and *Timd4* (Tim4) (Fig. 4*C*). We then performed flow cytometry analysis to further investigate whether FAP is predominantly expressed in tissue-resident macrophages in adipose tissues. We found that FAP^+ATM consisted predominantly of $CD11c^-CD206^+$ resident macrophages while FAP^-ATM contained much less $CD11c^-CD206^+$ resident macrophages but more $CD11c^+$ proinflammatory macrophages in ND-fed mice, confirming FAP expression is enriched in resident ATM. Following HFD feeding, both FAP^-ATM and FAP^+ATM had obviously increased percentages of $CD11c^+$ proinflammatory macrophages, while FAP^+ATM still had more $CD206^+CD11c^-$ resident macrophages (*SI Appendix*, Fig. S5*B*). This is also confirmed by using TIM4 as a marker for tissue-resident macrophages that were reported to be independent of CCR2 and non-monocyte derived in adipose tissues (37), as FAP^+ATM were primarily made up of $TIM4^+CD11c^-$ macrophages especially in ND-fed mice, while FAP^-ATM contained mostly $TIM4^-CD11c^+$ macrophages (Fig. 4*D*). We then examined the origin of FAP^+ATM and FAP^-ATM by comparing WT and $Ccr2^{-/-}$ mice. HFD feeding increased $TIM4^-CD11c^+$ macrophages in FAP^-ATM from WT mice, which was dramatically reduced in those from $Ccr2^{-/-}$ mice (Fig. 4*D*). CCR2 deficiency led to even higher frequencies of $TIM4^+CD11c^-$ macrophages in FAP^+ATM (Fig. 4*D*), which could be due to less CCR2-dependent $TIM4^-CD11c^+$ macrophages. Interestingly, FAP^+ATM had obviously increased

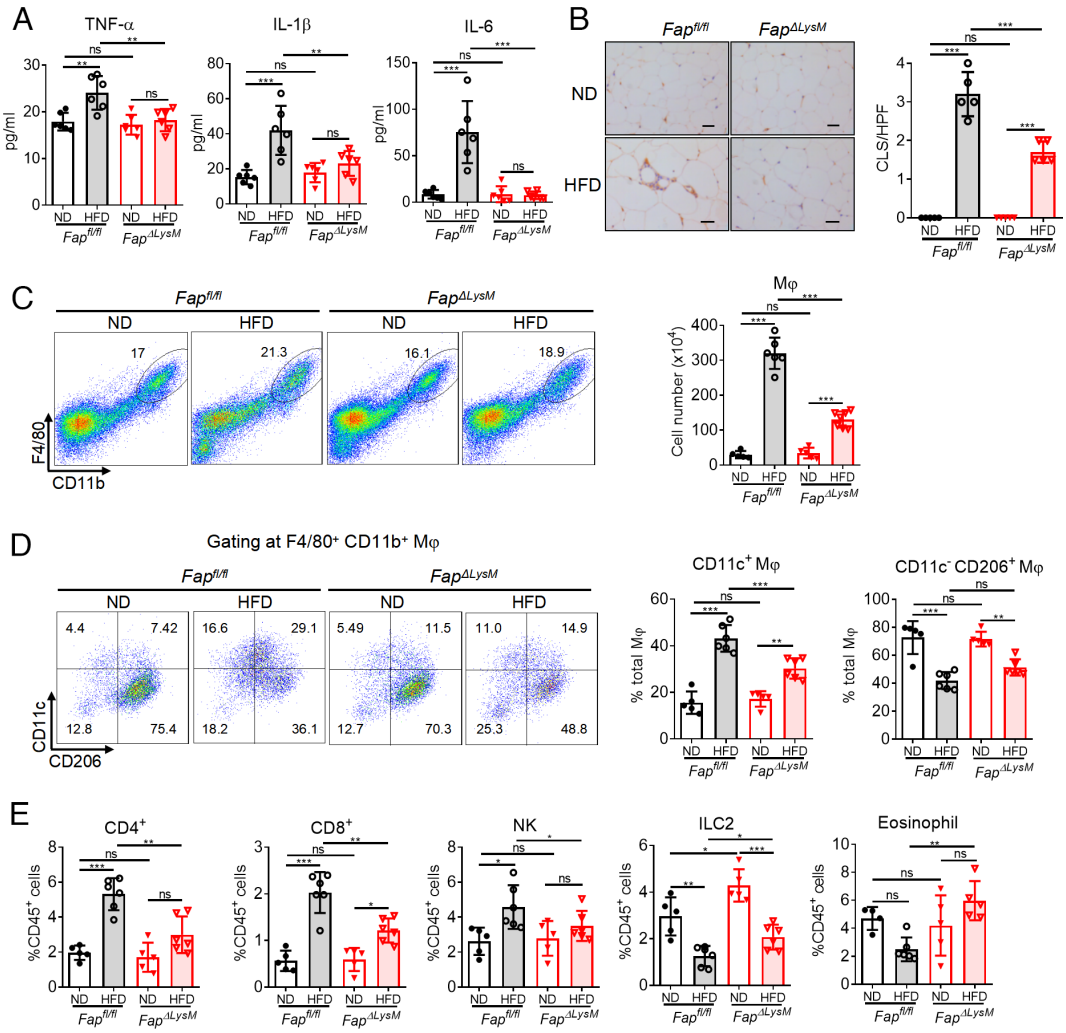

Fig. 3. Specific deletion of FAP in macrophages attenuates obesity-induced systemic and adipose inflammation. *Fap*$^{\Delta LysM}$ mice and *Fap*$^{fl/fl}$ littermate controls were fed with ND or HFD for 12 wk. (*A*) Serum levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β. n = 6. (*B*) Representative images of immunohistochemical staining of F4/80 for showing macrophage CLS and average numbers of CLS per high power field (HPF) in eWAT. (*C*) Representative flow cytometry dot plots and the absolute numbers of macrophages in the whole eWAT. (*D*) Representative flow cytometry dot plots as well as percentages of CD11c$^+$ proinflammatory macrophage and CD11c$^-$ CD206$^+$ M2-like macrophage in total macrophages of eWAT SVF. (*E*) Percentages of CD4$^+$ T cells, CD8$^+$ T cells, NK cells, innate lymphoid cells (ILC2), and eosinophils in CD45$^+$ cells of eWAT SVF. n = 5 to 6 for *B*, *C*, *D*, and *E*. Statistical comparisons were made using two-way ANOVA followed by Bonferroni post-tests. Data are represented as means ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and ns = no significance. Results are representative of two independent experiments.

CCR2-dependent TIM4$^-$CD11c$^+$ macrophages following HFD challenge (Fig. 4*D*). These results together suggest that FAP$^+$ATM are primarily composed of CCR2-independent non-monocyte-derived macrophages under steady-state condition, but CCR2-dependent monocyte-derived macrophages could acquire FAP expression when recruited into adipose tissues, partly contributing to the FAP$^+$ATM population.

In addition, there were no distinct differences in the expression of marker genes associated with M1- or M2-macrophages between FAP$^-$ATM and FAP$^+$ATM (Fig. 4*C*), suggesting FAP expression may not be related to a classical M1 or M2 activation phenotype. To directly investigate whether macrophage FAP expression affects the activation phenotypes of macrophages, PM from *Fap*$^{-/-}$ mice were transduced with adenovirus (Adv) to enforce FAP expression or Adv-eGFP as controls and subjected to a well-established in vitro polarization assay. As shown in *SI Appendix*, Fig. S5*C*, forced FAP expression had no obvious effect on expression of marker genes associated with classical M1- or M2-macrophages except increased expression of *Tnfα* and *Retnla*. Similarly, comparable expression levels of marker genes associated with classical M1- or M2- macrophages were observed in ATM isolated from WT and *Fap*$^{-/-}$ mice after 5-wk HFD feeding when there was no difference in adiposity between two genotypes (*SI Appendix*, Fig. S5*D*). A recent study identified Trem2$^+$ lipid-associated macrophage (LAM) subset prominently arising under obesity conditions from circulating monocytes as a key population responsible for regulating lipid catabolism and energy metabolism (11). We did not observe any differences in the expression of marker genes associated with LAM between FAP$^+$ATM and FAP$^-$ATM (*SI Appendix*, Fig. S5*E*). Collectively, our results suggest that macrophages FAP is dispensable for the regulation of classical M1/M2 polarization and FAP can not be used to distinguish M1- and M2- activation phenotype of ATM. Thus, it is highly likely that decreased infiltrating proinflammatory CD11c$^+$ ATM in HFD-fed *FapΔ*^{LysM} mice is due to less recruited macrophages.

Macrophages FAP Is Responsible for CCL8 Expression via Activating STAT3. Given that CCL8 is the highly affected genes in ATM by FAP expression under both steady state and obesity progression and functions as a monocyte chemokine, we speculated

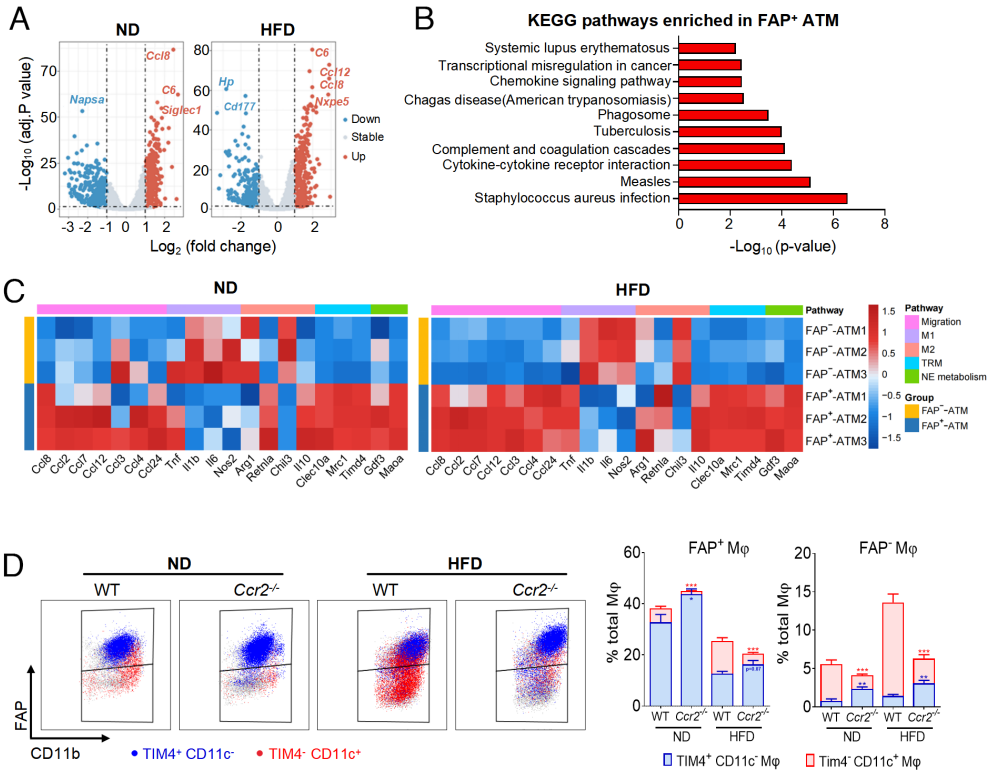

Fig. 4. Phenotypic characterization of FAP$^+$ATM. FAP$^+$ and FAP$^-$ ATM were sorted by FACS from eWAT of WT mice after 12-wk ND or HFD feeding and RNA-seq was performed. (*A*) Volcano plot showing differential gene expression in FAP$^+$ vs. FAP$^-$ ATM. (*B*) GSEA analysis of enriched KEGG pathways in FAP$^+$ATM from HFD-fed mice. (*C*) Heatmap showing differential gene expression in FAP$^+$ vs. FAP$^-$ ATM from ND- and HFD-fed mice. n = 3 for *A*, *B*, and *C*. (*D*) Representative flow cytometry dot plots (*Left*) and average frequencies (*Right*) of TIM4$^+$CD11c$^-$ and TIM4$^-$CD11c$^+$ macrophages of FAP$^+$ and FAP$^-$ ATM in total macrophages from eWAT of WT and *Ccr2*$^{-/-}$ mice. n = 5. Statistical comparisons were made using unpaired Student's *t* test (*D*). Data are represented as means ± SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Results are representative of two independent experiments in *D*.

that CCL8 may be involved in FAP$^+$ATM-mediated obesity-associated adipose inflammation via recruiting inflammatory monocytes. Since immune cells and adipocytes/preadipocytes can produce various chemokines to mediate the recruitment of immune cells and inflammation in obese adipose tissue, we first examined whether ATM are the major source for CCL8. To this end, different types of cells were isolated from eWAT of HFD-fed WT mice by FACS. qPCR analysis revealed that *Ccl8* was predominantly expressed by ATM at much higher levels than *Ccl2* and *Ccl7*, while *Ccl2* was abundantly expressed by ATM and macrophage-depleted SVF (Fig. 5*A*). Moreover, specific deletion of macrophage FAP reduced baseline gene expression of *Ccl8* in eWAT of ND-fed mice and dramatically reduced *Ccl8* after 12-wk HFD feeding (Fig. 5*B*). In contrast, mildly decreased *Ccl2* and unaltered *Ccl7* gene expression was observed in eWAT of HFD-fed *Fap*Δ^{LysM} mice (Fig. 5*B*). Dramatically decreased gene expression of *Ccl8* was also detected in ATM isolated from *Fap*$^{-/-}$ mice following 5-wk HFD feeding (Fig. 5*C*). A previous study shows that CD169$^+$ intestinal macrophages are the major source for CCL8 (38). Predominant CD169 expression were detected in FAP$^+$ATM but not FAP$^-$ATM by flow cytometric analysis (Fig. 5*D*), which is consistent with increased CD169 encoding gene *Siglec-1* mRNA levels in FAP$^+$ATM according to RNA-seq analysis (Fig. 5*E*). However, HFD feeding or macrophage FAP deletion did not affect CD169 expression levels by macrophages in adipose tissues (Fig. 5*F*). To investigate whether FAP expression is responsible for directly inducing *Ccl8* expression in macrophages, we examined CCL8 production in *Fap*$^{-/-}$ mice-derived PM that were transduced with Adv-FAP or control Adv-eGFP. As shown in Fig. 5*G*, forced FAP expression in resting PM significantly increased CCL8 expression, which was significantly upregulated by LPS/IFN-γ stimulation (382.3 ± 19.82 pg/mg vs. 558.0 ± 50.85 pg/mg, n = 8, *P* value < 0.01). In contrast, LPS/IFN-γ stimulation only mildly induced CCL8 production by control PM (46.99 ± 8.17 pg/mg vs. 75.00 ± 13.59 pg/mg, n = 8, *P* value = 0.09) (Fig. 5*G*), suggesting that CCL8 is not associated with classical M1 phenotype. In agreement with our previous finding that FAP activates STAT3 in CAFs to mediate their inflammatory phenotype (21), FAP overexpression increased phosphorylation of STAT3 in PM, which was further enhanced by LPS/IFN-γ stimulation (Fig. 5*H*). Importantly, adding Stattic, a STAT3 inhibitor, completely abrogated increases in CCL8 production by FAP-overexpressing PM (Fig. 5*G*). Collectively, these results demonstrate a critical role of FAP in promoting CCL8 expression in ATM which serve as the major source of CCL8 within obese adipose tissues. Collectively, these data suggest that macrophage FAP is required for the production of monocyte-recruiting chemokine CCL8.

FAP$^+$ Macrophage-Derived CCL8 Mediates the Recruitment of Inflammatory Monocytes. We then attempted to examine whether FAP$^+$ macrophages contribute to obesity-associated adipose inflammation by mediating monocyte recruitment via CCL8. To this end, we first investigated the impact of macrophage FAP deficiency on the recruitment of circulating monocytes into adipose tissues by tail vein injection of blood monocytes from CD45.1 mice into the HFD-fed *Fap*Δ^{LysM} mice or littermate controls. Flow cytometry revealed significantly lower percentages of transferred CD45.1$^+$ macrophages in total macrophages of iWAT and eWAT of *Fap*Δ^{LysM} mice compared with littermate controls (Fig. 6 *A* and *B*), indicating that lack of macrophage FAP impairs circulating monocyte recruitment. To seek the direct

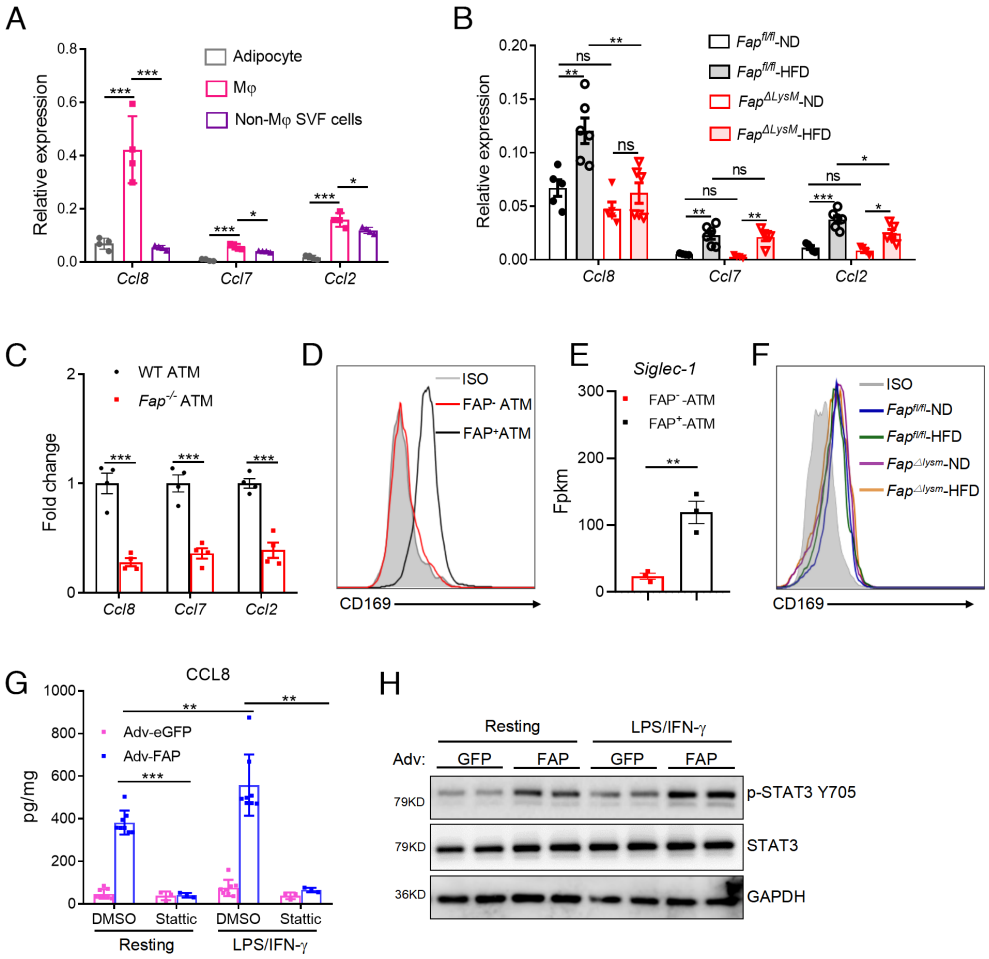

Fig. 5. Macrophages FAP is responsible for CCL8 expression via activating STAT3. (*A–C*) qPCR analysis of mRNA levels of Ccl8, Ccl7, and Ccl2 in indicated cell populations isolated from eWAT of naive WT mice (*A*), eWAT of *Fap*$^{\Delta LysM}$ mice and *Fap*$^{fl/fl}$ littermate controls after 12-wk ND or HFD feeding (*B*), and ATM sorted from eWAT of WT and *Fap*$^{-/-}$ mice after 5-wk HFD feeding (*C*). n = 4 for *A* and *C* and n = 5 to 6 for *B*. (*D*) Histogram of the fluorescence intensity of CD169 in FAP^{+} and FAP^{-} macrophages from eWAT of HFD-fed WT mice. (*E*) FPKM value of *Siglec-1* gene (encoding CD169) in isolated FAP^{+} and FAP^{-} macrophages from eWAT of HFD-fed WT mice. n = 3 for *E*. (*F*) Histogram of the fluorescence intensity of CD169 in ATM from eWAT of *Fap*$^{\Delta LysM}$ mice and *Fap*$^{fl/fl}$ littermate controls. (*G* and *H*) CCL8 concentrations (*G*) and immunoblot for total and phosphorylated STAT3 (pSTAT3) levels (*H*) of FAP-overexpressing PM or Adv-eGFP-transfected control PM under resting or LPS/IFN-γ stimulation with or without STAT3 inhibitor, Stattic. DMSO was used as vehicle control. n = 8 for *G*. Statistical comparisons were analyzed using unpaired Student's *t* test (*C* and *E*), one-way ANOVA (*A*) and two-way ANOVA followed by Bonferroni post-tests (*B* and *G*). Data are represented as means ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and ns = no significance. Results are pooled from 2 independent experiments in *G*. Results are representative of 2 independent experiments in *B*, *C*, *D*, and *H*.

evident that CCL8 is responsible for monocyte-attracting ability of FAP^{+} macrophages, an in vitro transwell chemotaxis assay was performed. As expected, isolated blood monocytes migrated in response to recombinant CCL8 (Fig. 6*C*). Similar numbers of migrated monocytes were detected in the lower chambers containing condition medium (CM) of FAP-overexpressing PM, which was significantly higher than those in CM of control PM or medium alone (Fig. 6*C*). Moreover, CM from LPS/IFN-γ-stimulated FAP-overexpressing PM attracted more monocytes (Fig. 6*C*), which was consistent with elevated CCL8 production (Fig. 5*G*). Importantly, knockdown *Ccl8* of FAP-overexpressing PM by small interfering RNA (siRNA) effectively reduced migrated monocyte numbers (Fig. 6*D*), emphasizing that CCL8 is required for the chemotactic activity of FAP-overexpressing PM toward monocyte. CCR2 is the receptor for all members of MCP including CCL8 and critical for monocyte recruitment. We further showed much less migrated *Ccr2*$^{-/-}$ monocytes than WT controls in CM of FAP-overexpressing PM (Fig. 6*E*). Collectively, these results demonstrate that macrophage FAP expression promotes recruitment of blood monocytes which differentiates into inflammatory macrophages during obesity via upregulating CCL8 production.

Adipose Tissue-Specific Overexpression of CCL8 Restores HFD-Induced Obesity and Metabolic Dysfunction in *Fap*$^{-/-}$ Mice. To directly address whether reduced CCL8 expression leads to the metabolic changes in *Fap*$^{-/-}$ mice, we generated CCL8-overexpressing adeno-associated virus (AAV) and locally administrated these viruses or control AAV into eWAT of *Fap*$^{-/-}$ mice followed by 16-wk HFD-feeding. Overexpression of CCL8 reversed the metabolic benefits in HFD-fed *Fap*$^{-/-}$ mice, as evidenced by restored weight gain, fat mass, hepatic steatosis, glucose intolerance, and insulin resistance to similar levels observed in HFD-fed WT controls (Fig. 7 *A–E*). Consistently, administration of CCL8-overexpressing AAV restored obesity-associated inflammation, as evidenced by restored serum levels of proinflammatory cytokines (Fig. 7*F*) and infiltration of inflammatory macrophages (Fig. 7 *G* and *H*) in obese eWAT. Considering the rescue effect of CCL8 overexpression on metabolic phenotypes in *Fap*$^{-/-}$ mice following HFD-feeding, we attempted to investigate whether further upregulation of CCL8 expression in WT HFD-fed mice could amplify such phenotypes. To this end, CCL8-overexpressing AAV or control AAV were locally administrated into eWAT of WT mice followed by 12-wk HFD feeding. CCL8 overexpression in WT mice caused mild increases in weight gain, fat mass, hepatic steatosis, and infiltrating inflammatory macrophages

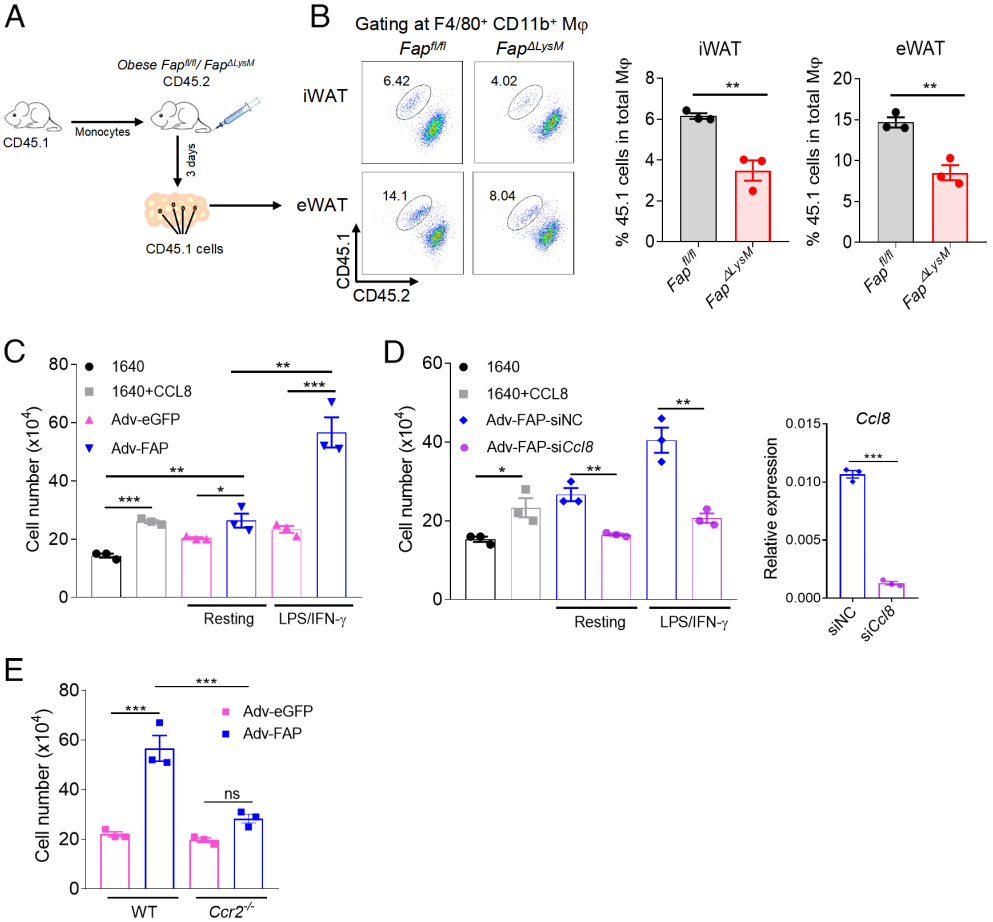

Fig. 6. FAP$^+$ macrophage-derived CCL8 mediates the recruitment of inflammatory monocytes. (*A*) Schematic protocol showing that blood CD11b$^+$Ly6C$^+$ monocytes were sorted from CD45.1 mice and intravenously (i.v.) transferred into 12-wk HFD-fed *Fap*$^{\Delta LysM}$ mice and *Fap*$^{fl/fl}$ littermate controls. (*B*) Representative flow cytometry dot plots and percentages of CD45.1$^+$ macrophages in total macrophages of iWAT and eWAT on day 3 after cell transfer. n = 3 for *B*. (*C* and *D*) In vitro transwell migration assay. Absolute numbers of migrated monocytes in response to conditioned medium (CM) of FAP-overexpressing PM and control PM with or without LPS/IFN-γ stimulation (*C*) and to CM of FAP-overexpressing PM transfected with scramble small interfering RNA (*siNC*) or *Ccl8* siRNA (*siCcl8*) (*D*). The lower chambers with 1640 media alone or with murine recombinant CCL8 (10 ng/mL) were used as negative or positive controls. (*E*) Absolute numbers of migrated WT or *Ccr2*$^{-/-}$ blood monocytes in response to CM of FAP-overexpressing PM and control PM with LPS/IFN-γ stimulation. Statistical comparisons were made using unpaired Student's *t* test (*B*) and two-way ANOVA followed by Bonferroni post tests (*C*–*E*). Data are represented as means ± SEM. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001. Results are representative of 2 independent experiments.

(*SI Appendix*, Fig. S6 *A*–*F*). However, CCL8 overexpression had no obvious effects on GTT and ITT (*SI Appendix*, Fig. S6*G*), suggesting that the extent of adipose tissue inflammation increased by CCL8 overexpression may be insufficient to further aggravate glucose intolerance and insulin resistance in WT HFD-fed mice which had normally increased endogenous CCL8 expression. Collectively, these results demonstrate that alleviated obesity and metabolic inflammation/dysfunction in *Fap*$^{-/-}$ mice could be secondary to reduced adipose CCL8 levels.

Macrophages FAP Deficiency Increases Energy Expenditure via Controlling Local NE Concentration and Lipolysis Following HFD-Feeding. Since *FapΔ*^{LysM} mice had obviously less adiposity in addition to reduced adipose tissue inflammation upon HFD feeding, we finally set out to investigate whether the propensity of *FapΔ*^{LysM} mice to gain less weight is due to increased energy expenditure. To this end, 5-wk HFD-fed *Fap*$^{fl/fl}$ and *FapΔ*^{LysM} mice were placed in metabolic cages for 24 h with 12-h light–dark cycles. There were no differences in food intake and body weight between *Fap*$^{fl/fl}$ and *FapΔ*^{LysM} mice after 5-wk HFD feeding (Fig. 8 *A* and *B*), which could avoid the influence of differential body weights on energy expenditure. EchoMRI scans revealed decreased fat mass but comparable lean mass in *FapΔ*^{LysM} mice (Fig. 8*C*). Despite equivalent physical activity (Fig. 8*D*), *FapΔ*^{LysM} mice had significantly higher energy expenditure than similarly treated littermate controls (Fig. 8*E*). Consistently, *FapΔ*^{LysM} mice had higher oxygen consumption (VO_2) (Fig. 8*F*), indicative of increased lipid oxidation. Lipolysis is essential for adipose tissue thermogenesis, as the fatty acids released from NE-induced lipolysis serve as both metabolic substrates fueling thermogenic respiration and obligatory activators for UCP1. Recent studies reported that monoamine oxidase-A (*Maoa*) was up-regulated in ATM of aging mice or sympathetic neuron–associated macrophages to facilitate NE degradation, thus inhibiting lipolysis (10, 12). Interestingly, our RNA-seq data showed obviously increased gene expression of *Maoa* in FAP$^+$ATM compared with FAP$^-$ATM (Fig. 4*C*). We further confirmed decreased *Maoa* expression in iWAT of *FapΔ*^{LysM} mice following 5-wk HFD feeding (Fig. 8*G*), suggesting a significant contribution of FAP$^+$ ATM to *Maoa* expression in WAT. Thus, we speculated that macrophage FAP is likely to inhibit NE concentration and lipolysis in WAT via increasing *Maoa* expression. To examine such possibility, we measured the levels of NE and phosphorylated hormone-sensitive lipase (pHSL) in iWAT of mice following 5-wk HFD feeding and found that *FapΔ*^{LysM} mice had significantly higher levels of NE levels (Fig. 8*H*) and pHSL (Fig. 8*I*) than littermate controls, suggesting enhanced NE-mediated lipolysis in the absence of macrophage FAP expression. However, there were no differences

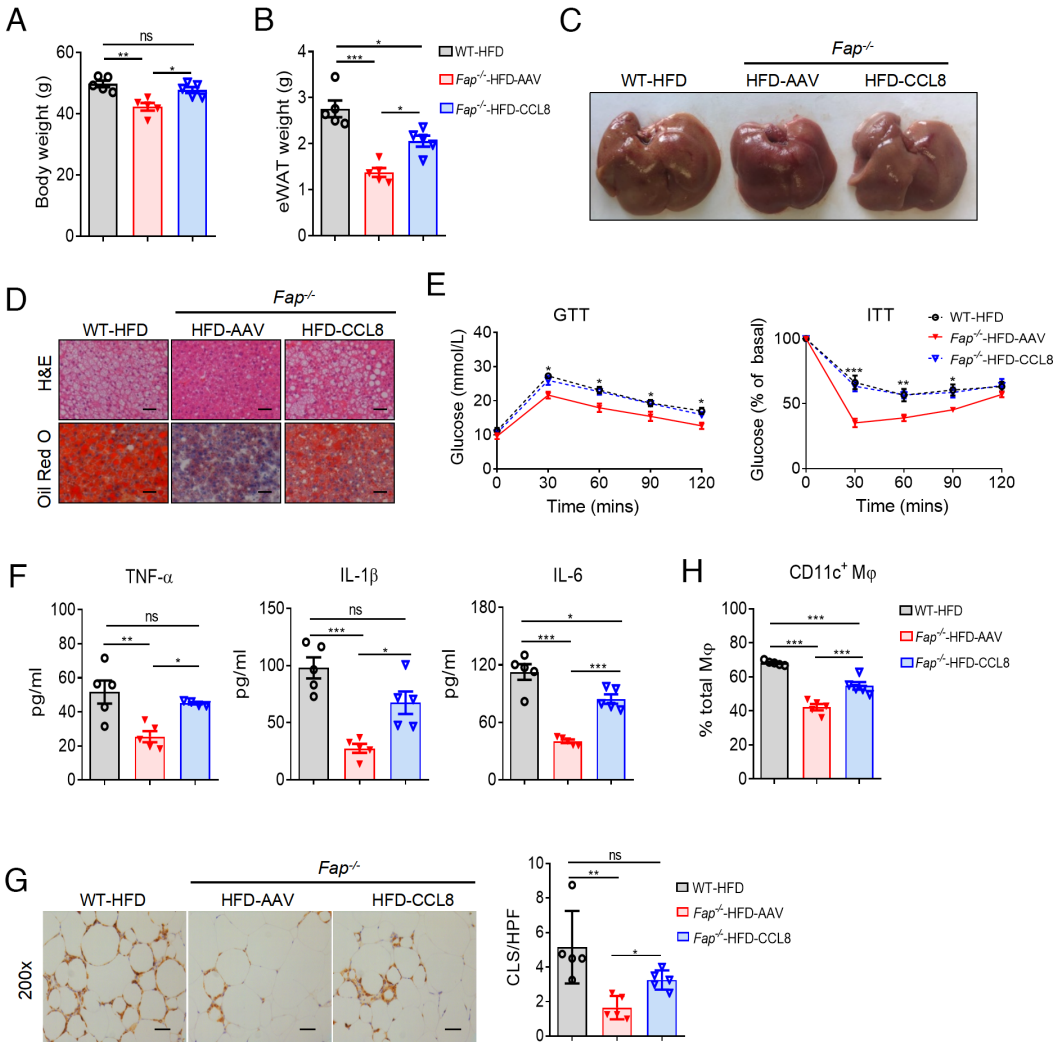

Fig. 7. Adipose tissue-specific overexpression of CCL8 restores HFD-induced obesity and metabolic dysfunction in *Fap*$^{-/-}$ mice. *Fap*$^{-/-}$ mice were locally injected with AAV expressing CCL8 or control AAV followed by 16-wk HFD feeding and WT mice were fed with HFD for 16 wk as controls. (*A*) Mouse body weights. (*B*) Average weights of eWAT. (*C*) Representative photos of whole livers. n = 5 for *A* and *B*. (*D*) Images of H&E staining and Oil red O staining of liver sections. (*E*) Glucose tolerance test (GTT) and insulin tolerance test (ITT). (*F*) Serum levels of pro-inflammatory cytokines TNF-α, IL-6, IL-1β. (*G*) Representative images of immunohistochemical staining of F4/80 for showing macrophage CLS and average numbers of CLS per high power field (HPF) in eWAT. (*H*) The percentages of pro-inflammatory CD11c^{+} macrophages in eWAT as determined by flow cytometry analysis. n = 5 for *E*, *F*, *G*, and *H*. (Scale bars, 50 μm in *D* and *G*.) Statistical comparisons were made using one-way ANOVA followed by Bonferroni post-tests. Data are represented as means ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

in UCP1 expression in iWAT and BAT between *FapΔ*^{LysM} mice and littermate controls (Fig. 8*J*). Consistently, macrophage FAP deficiency had no effect on brown fat marker gene UCP1 expression in both iWAT and BAT following injection of a β3-adrenergic agonist CL316,243 (*SI Appendix*, Fig. S7*A*) or cold exposure (*SI Appendix*, Fig. S7*B*). These data indicate that increased energy expenditure caused by macrophage FAP deficiency could not be due to beiging of iWAT. Collectively, these results suggest that macrophage FAP expression could suppress energy expenditure by controlling local concentration of NE and lipolysis following HFD feeding, thereby contributing to obesity.

Discussion

Despite being the well-known marker of CAF, FAP has long been shown to be expressed in several normal tissues including adipose tissue (24, 39). However, the knowledge about the physiological function of FAP in normal tissues is still limited. In this study, we identify the expression of FAP in ATM, which serves as an important regulator of macrophage function and contributes to diet-induced obesity and metabolic inflammation/dysfunction via promoting CCL8-mediated monocyte recruitment and restricting energy expenditure (Fig. 8*K*).

Although FAP expression was reported in tumor-associated macrophages and normal adipose tissue, there was no report about FAP expression in ATM to our knowledge. We first identified that FAP is selectively expressed in ATM but not other adipose tissue-infiltrating leukocytes as well as macrophages derived from other types of tissues in mice. FAP expression was also confirmed in human ATM. Furthermore, we found that, although FAP is preferentially expressed in CCR2-independent tissue-resident macrophages under steady-state condition, CCR2-dependent monocyte-derived macrophages also partly contribute to FAP^{+} ATM by acquiring FAP expression when they are recruited into adipose tissue. This is also supported by our findings that incubation with supernatants of adipose tissue or adipocytes could induce FAP expression in BMDM. Thus, FAP is not an ideal marker to determine the origin of tissue-resident macrophage in adipose tissues. Additionally, these results suggest that macrophage FAP may serve as sensor for WAT-derived substances; however, the identification of the factors that are responsible for FAP expression in ATM needs further study.

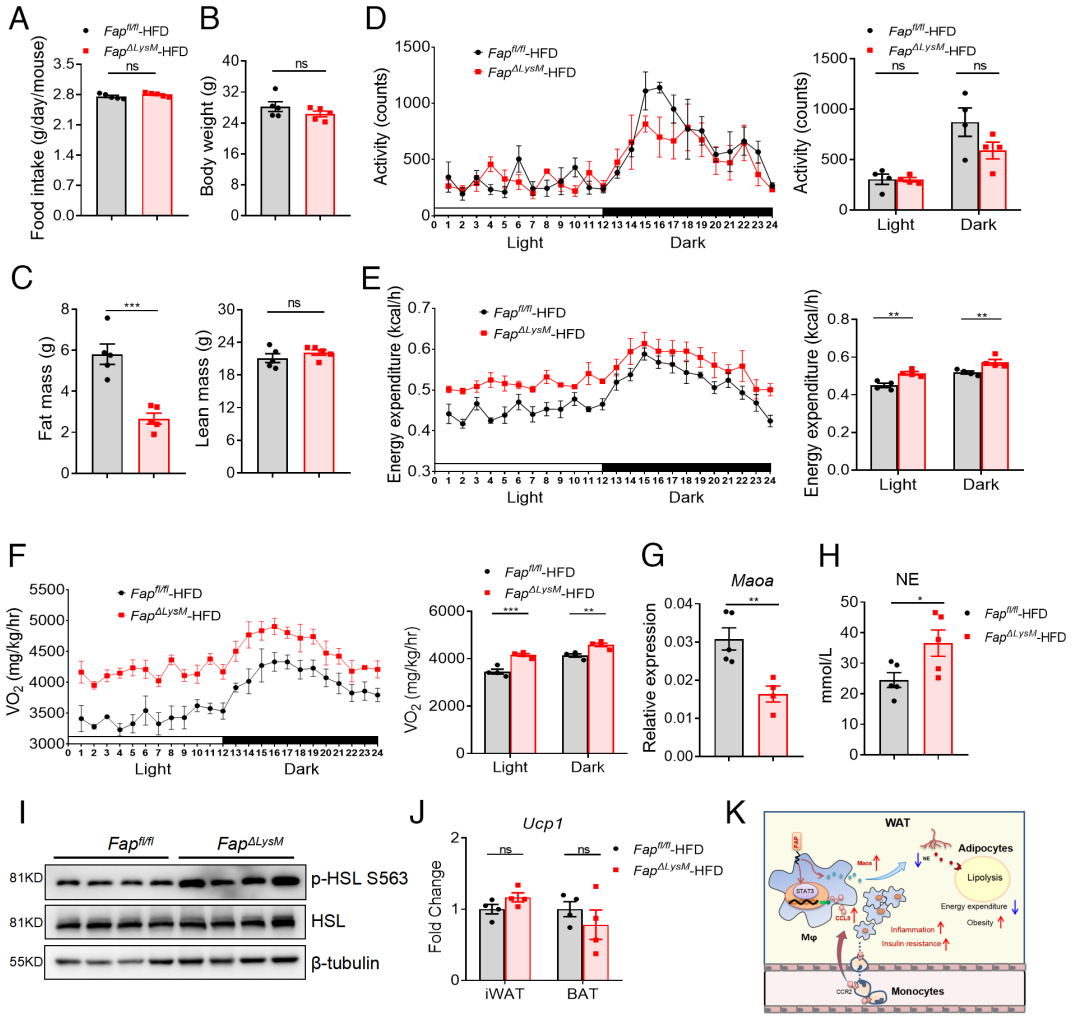

Fig. 8. Macrophages FAP deficiency increases energy expenditure in response to HFD feeding. $Fap^{\Delta LysM}$ mice and $Fap^{fl/fl}$ littermate controls were fed with HFD for 5 wk. (*A*) Food intake. (*B*) Body weight. (*C*) Fat mass and lean mass determined by EchoMRI. n = 5 for *A*, *B*, and *C*. (*D–F*) Locomotor activity (*D*), heat production (*E*), and oxygen consumption (VO_2) (*F*) monitored over a 24-h period (*Left*) and shown as averaged values (*Right*). n = 4 for *A*, *B*, and *C*. (*G–J*) *Maoa* mRNA levels (*G*), NE concentrations (*H*), representative immunoblot for total and phosphorylated HSL (pHSL) levels (*I*), and fold change of *Ucp1* mRNA levels in iWAT and BAT of different genotypes (*J*). n = 4 for *G*, *H*, and *J*. (*K*) A schematic diagram summarizing the mechanism by which FAP expression in ATM promotes diet-induced obesity and associated metabolic inflammation. Statistical comparisons were made using unpaired Student's *t* test. Data are represented as means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and ns = no significance. Results are representative of 2 independent experiments.

Importantly, we demonstrated that FAP expression in ATM is functional and involved in metabolic regulation, as macrophage FAP deficiency alleviated diet-induced obesity, metabolic inflammation, hepatic steatosis, and glucose intolerance. Such a phenotype is unlikely attributed to the ability of FAP to directly shift macrophages from proinflammatory to anti-inflammatory status, as we demonstrated that ATM isolated from WT and $Fap^{-/-}$ mice following HFD challenge exhibited no differences in classical marker gene expression profiles associated with M1 or M2-phenotypes and forced FAP expression in PM had no effect on the in vitro M1 or M2 macrophage polarization. In addition to inflammatory polarization of tissue-resident macrophages, increased recruitment of circulating monocytes into adipose tissue where they differentiate into proinflammatory macrophages makes a major contribution to obesity-associated local and systemic inflammation (5). We further showed that macrophage FAP deficiency led to decreases in accumulation of proinflammatory $CD11c^{+}$ ATM and CLS in obese adipose tissues after HFD feeding, which was further confirmed by adoptive transfer experiment. These data indicate that macrophage FAP promotes accumulation of proinflammatory ATM via monocyte recruitment, thereby contributing obesity-associated inflammation. Extensive studies have focused on the critical role of the CCL2–CCR2 axis in obesity-associated inflammation via monocyte recruitment (15, 16). Another important finding of our study is identification of CCL8 as the major downstream effector of FAP in ATM to mediate monocyte recruitment. Unbiased RNA-seq data revealed *Ccl8* as the most affected gene in ATM by FAP expression under both steady and obese states. In contrast, other monocyte chemokines including CCL2, CCL7, and CCL12 were only found upregulated at lesser extents in FAP^{+}ATM under obese state. Furthermore, forced FAP expression in macrophage markedly increased CCL8 expression via activation of STAT3, which was further enhanced by LPS/IFN-γ stimulation. This is consistent with the previous study showing that CCL8 does not belong to the "generic" pro-inflammatory cytokines associated with M1 macrophages (38) and our findings that LPS/IFN-γ stimulation had a very mild effect on CCL8 production in $Fap^{-/-}$macrophages (Fig. 5*G*). Collectively, these data demonstrate that FAP specifically mediates CCL8 expression in macrophages with no obvious effect on classical activation of proinflammatory M1 macrophage.

Several studies recently demonstrated that CCL8 regulates the trafficking of immune cells in barrier tissues to promote tissue inflammation. For example, CCL8 mediated the recruitment of a population of highly differentiated IL-5-producing Th2 cells and

$CD301b^+$ cDC2s into skin and skin-draining lymph nodes, respectively, to promote the development of skin allergic inflammation (40, 41). Another study further showed CCL8 as a chemotactic factor for ILC2 accumulation in lungs (42). Moreover, CCL8 was found to recruit monocytes in DSS-induced murine colitis as well as in human breast cancer (38, 43). However, the role of CCL8 in obesity-associated inflammation has yet been reported. Our study here demonstrates that CCL8 was predominantly expressed by macrophages in adipose tissue which is further upregulated during obesity under the control of FAP, thereby attracting more inflammatory monocytes to amplify obesity-associated inflammation. This is supported by the findings that CCL8 knockdown reduced the monocyte-recruiting ability of FAP^+ macrophages in vitro and CCL8 overexpression in $Fap^{-/-}$ mice restored diet-induced obesity, metabolic inflammation, and dysfunction. Thus, our study implies CCL8 as an important chemokine to alert nutrient stress in adipose tissue via recruiting inflammatory monocyte during obesity progression. The effect of CCL8 may also explain why some studies found unaltered monocyte recruitment in $Ccl2^{-/-}$ mice following HFD feeding (18, 19). Interestingly, we noted that CCL8 overexpression in WT HFD-fed mice aggravated obvious hepatic steatosis with more lipid deposition but had no obvious effect on glucose intolerance and insulin resistance. These results suggest a potential role of CCL8 in the progression of NAFLD associated with obesity. However, more detailed studies about how CCL8 regulates the development of NAFLD are warranted in the future.

Previous studies showed that the absence of proinflammatory macrophages does not influence diet-induced obesity, for example, impaired monocyte recruitment by CCR2 blockade does not prevent weight gain, but only metabolic inflammation and disorders (16, 37). It is worth noting that, in addition to alleviated metabolic inflammation, macrophage FAP deficiency led to less diet-induced weight gain. Moreover, enhanced whole-body energy expenditure was found in $Fap\Delta^{LysM}$ mice before they and littermate controls had divergent body weight. We further revealed that macrophage FAP deficiency was associated with decreased expression of *Maoa*, a NE-degrading enzyme, in ATM and adipose tissues following HFD feeding, which was accompanied by increases in local levels of NE and lipolysis and whole-body energy expenditure. These results support a recently proposed regulatory function of macrophages in adrenergic homeostasis (44). Previous studies showed that induction of the NLRP3 inflammasome and IL-1β expression enhances *Maoa* abundance in aging ATM to inhibit fasting-induced lipolysis. However, we found that FAP had no effect on IL-1β expression by macrophages, suggesting that FAP induced *Maoa* expression in a NLRP3 inflammasome-independent way. The exact mechanism how FAP regulates *Maoa* expression needs further study. Interestingly, despite increased energy expenditure, HFD challenge did not cause any differences in UCP1 expression between $Fap\Delta^{LysM}$ mice and littermate controls. Consistently, macrophage FAP deficiency had no effect on UCP1 expression in adipose tissue following cold exposure or CL316,243 stimulation. Thus, our study suggests that FAP is involved in macrophage function to control local NE concentrations and lipolysis in response to HFD challenge, thereby restricting energy expenditure to promote adiposity.

In summary, our study reveals a previously undescribed function of FAP as a previously unrecognized molecular link between macrophage function and metabolic dysfunction within adipose tissues and suggests that targeting macrophage FAP may represent a potential therapeutic strategy against obesity and its associated metabolic dysfunction.

Materials and Methods

Mice. WT mice on C57BL/6J and C57BL/6N were purchased from the Chinese Academy of Sciences (Shanghai, China) or Charles River (Beijing, China), respectively. $Fap^{fl/fl}$ mice were generated by Biocytogen Company (Shanghai, China) using CRISPR-Cas9 knockout strategy in C57BL/6N zygotes as depicted in *SI Appendix*, Fig. S2. $Fap^{-/-}$ mice (Strain # 024288), LysM-cre mice (Strain# 031674), and $Ccr2^{-/-}$ mice (Strain # 035229) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). The primer sequences for Genotyping are listed in *SI Appendix*, Table S4. All transgenic mice are on the C57BL/6 genetic backgrounds, and experiments were conducted with littermate controls. All mice used in experiments were age-matched males between 6- and 8-wk-old and housed under specific pathogen-free conditions. All animal experiments were conducted in accordance with protocols approved by the Animal Care and Use Committee at Shanghai Medical College, Fudan University.

In Vivo Studies. For HFD-induced obesity, 6-wk-old male mice were fed with either normal diet or HFD diet (60% kcal fat, #D12492, Research Diet). For in vivo migration, monocytes were isolated from 6~8-wk-old male CD45.1 mice on C57BL/6 background by density gradient centrifugation followed by FACS. In brief, density centrifugation was performed using Ficoll separation to isolate peripheral blood mononuclear cells. Subsequently, monocytes ($CD11b^+Ly6C^+$) were extracted from the mononuclear fraction using FACS (Moflo; Beckman Coulter, Carlsbad, CA). The monocytes were counted, and ~2×10^6 viable cells were suspended in 0.2 mL PBS and injected into the tail vein of $Fap^{fl/fl}$ or $Fap^{\Delta LysM}$ mice which have been fed with HFD for 12 wk. Three days after the injection, SVF of iWAT and eWAT was isolated and subjected to flow cytometry analysis. For CCL8 overexpression, 4-wk-old mice were intraperitoneally injected with AAV-CCL8 (5×10^{11} viral particles per mouse) following by HFD feeding for 16 wk. To induce the browning of WAT in vivo, mice were housed at 4 °C for 3 d, or intraperitoneally injected with 0.5 mg/kg of CL316,243 (Cat# C5976; Sigma-Aldrich) for 5 d. Mice were subsequently killed and adipose tissues were dissected for further analysis.

Human Samples. Human subcutaneous fat was obtained from underaged subjects that received plastic surgery in Ruijin Hospital, Shanghai JiaoTong University. Human samples were de-identified prior to use for the study.

Detailed methods used in this study are listed in *SI Appendix, SI Materials and Methods*.

Data, Materials, and Software Availability. The RNA-seq data reported in this paper have been deposited in the OMIX, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix: accession no. OMIX005260) (45). All study data are included in the article and/or supporting information.

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