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Targeting IκB Kinase β in Adipocyte Lineage Cells for Treatment of Obesity and Metabolic Dysfunctions

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ABSTRACT

IκB kinase β (IKKβ), a central coordinator of inflammation through activation of nuclear factor-κB, has been identified as a potential therapeutic target for the treatment of obesity-associated metabolic dysfunctions. In this study, we evaluated an antisense oligonucleotide (ASO) inhibitor of IKKβ and found that IKKβ ASO ameliorated diet-induced metabolic dysfunctions in mice. Interestingly, IKKβ ASO also inhibited adipocyte differentiation and reduced adiposity in high-fat (HF)-fed mice, indicating an important role of IKKβ signaling in the regulation of adipocyte differentiation. Indeed, CRISPR/Cas9-mediated genomic deletion of IKKβ in 3T3-L1 preadipocytes blocked these cells differentiating into adipocytes. To further elucidate the role of adipose progenitor IKKβ signaling in diet-induced obesity, we generated mice that selectively lack IKKβ in the white adipose lineage and confirmed the essential role of IKKβ in mediating adipocyte differentiation in vivo. Deficiency of IKKβ decreased HF-elicited adipogenesis in addition to reducing inflammation and protected mice from diet-induced obesity and insulin resistance. Further, pharmacological inhibition of IKKβ also blocked human adipose stem cell differentiation. Our findings establish IKKβ as a pivotal regulator of adipogenesis and suggest that overnutrition-mediated IKKβ activation serves as an initial signal that triggers adipose progenitor cell differentiation in response to HF feeding. Inhibition of IKKβ with antisense therapy may represent as a novel therapeutic approach to combat obesity and metabolic dysfunctions. STEM CELLS *2016;34:1883–1895*

SIGNIFICANCE STATEMENT

Obesity is associated with both increased adipocyte size and adipocyte number but the the mechanisms underlying nutritionally induced hyperplasia remain elusive. Our findings establish IκB kinase β (IKKβ) as an important regulator of adipogenesis and adipose tissue development. Overnutrition-mediated IKKβ activation may serve as an initial signal that triggers adipose progenitor cell differentiation in response to consumption of a high-fat diet. Our studies also demonstrate IKKβ as a potential target for future anti-obesity drugs and provide evidence for the use of appropriate IKKβ antisense oligonucleotides as a potential therapeutic strategy to treat obesity and metabolic disease.

INTRODUCTION

Obesity is a rapidly growing epidemic representing a growing serious health threat in an increasing number of countries as the number of overweight and obese individuals are expected to increase to over half of the world's population by 2030 [1]. There is an urgent need to understand the mechanisms underlying obesity and obesity-related metabolic diseases. Obesity is associated with both increased adipocyte size (hypertrophy) and adipocyte number (hyperplasia). Adipocyte number is a major determinant of fat mass in adults [2, 3] and approximately 10% of the body's adipocytes are re-generated annually at all adult ages [3]. Obese individuals also have a significantly greater number of adipocytes added per year than lean individuals [2, 3], suggesting that regulation of new adipocyte production is a potential therapeutic target to treat obesity. However, the mechanisms underlying nutritionally induced hyperplasia remain largely unknown.

It is generally accepted that obesity is associated with a state of chronic low-grade inflammation that is a major contributor to type 2 diabetes and atherosclerosis [4, 5]. Many inflammatory pathways that contribute to the pathogenesis of insulin resistance and atherosclerosis are regulated by the transcriptional factor nuclear

factor-κB (NF-κB), a master regulator of the innate and adaptive immune responses [6]. IκB kinase (IKK) β is the predominant catalytic subunit of the IKK complex and is required for activation of NF-κB by inflammatory mediators in the canonical or classical activation pathway [6–9]. It has been well established that overnutrition can lead to IKKβ activation in vitro and in vivo [10–12], and recent studies have implicated IKKβ as a key molecular link between obesity, inflammation and metabolic disorders [5, 13, 14]. For example, diet-induced insulin resistance has been associated with the activation of IKKβ/NF-κB in multiple tissues including liver, adipose tissue, and brain [10, 15–20]. Deletion of IKKβ in the liver improved diet-induced insulin resistance, and deficiency of IKKβ in myeloid cells rendered global insulin sensitivity upon high-fat (HF) feeding [17]. By contrast, constitutive activation of IKKβ in the liver caused systemic insulin resistance [18]. Activation of IKKβ in the hypothalamus has also been linked to obesity and metabolic disease [19, 21].

We have recently demonstrated that IKKβ functions in smooth muscle cells (SMCs) to regulate vascular inflammatory responses and atherosclerosis development [8]. Of particular interest is that many adipocyte precursor cells express SMC markers and ablation of IKKβ blocked adipocyte differentiation in vitro and in vivo, suggesting that IKKβ functions in adipocyte precursor cells to regulate adipose tissue development [8]. In the present study, we explored a novel and efficient pharmacological approach to inhibit IKKβ in vivo by using antisense oligonucleotides (ASOs) and found that ASO-mediated IKKβ knockdown ameliorated diet-induced obesity and metabolic disorders in mice. Interestingly, IKKβ ASO also inhibited high-fat diet (HFD)-elicited adipocyte differentiation and reduced adipose tissue growth. As the functions of IKKβ in regulating adipogenesis and adipose tissue development has not been fully understood, we then selectively deleted IKKβ in the white adipose lineage in mice to further elucidate the role of adipose progenitor cell IKKβ signaling in obesity and metabolic function. Deficiency of IKKβ decreased adipogenesis and systemic inflammation elicited by HF feeding, leading to reduction in diet-induced obesity and insulin resistance. Last, inhibition of IKKβ in human adipose stem cells also blocked adipogenesis in these cells. Our results establish IKKβ as an important regulator of adipogenesis and adipose tissue development. Overnutrition-mediated IKKβ activation may serve as an initial signal that triggers adipose progenitor cell differentiation in response to consumption of a HFD. Antisense therapy targeting IKKβ may present as a novel therapeutic approach to combat obesity and metabolic dysfunctions.

Materials and Methods

Animals

For the IKKβ ASO studies, 8-week-old male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME, http://www.jax.org/) were fed a normal chow diet (ND) or a Western-type HFD (21.2% fat, 0.2% cholesterol; Harlan Teklad, Hayward, CA, http://www.harlan.com/) and received biweekly intraperitoneal injections of either a nontargeting control ASO (5′-CCTTCCCTGAAGGTTCCTCC-3′) or an IKKβ targeted ASO (5′-GCAGACTCTCATCCTCCGTC-3′) for 8 weeks at a dose of 25 mg/kg body weight/week. The 20-mer phosphorothioate ASOs were designed to contain 2′-O-methoxyethyl groups at positions 1 to 5 and 15 to 20, and were generated and purified by Ionis Pharmaceuticals (Carlsbad, CA, http://www.ionispharma.com/). For cell lineage analysis, PDGFRβ-Cre mice [22] were crossed with Rosa26^{lacZ} reporter mice [23] to generate PDGFRβ-Cre/Rosa26^{lacZ} mice. To delete IKKβ in adipocyte lineage cells, mice containing loxP-flanked IKKβ alleles (IKKβ$^{F/F}$) [8, 24] was crossed with PDGFRβ-Cre transgenic mice [22] to generate PDGFRβ-Cre/IKKβ$^{F/F}$ mice (termed as IKKβ$^{\Delta PDGFR\beta}$). For obesity studies, 4-week-old male IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ littermates were fed a ND or a HFD for 16 weeks until euthanization at 20 weeks of age. For the Bromodeoxyuridine (BrdU) studies, 4-week-old male IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ littermates were intraperitoneal injected with 50 mg/kg body weight/day BrdU (TCI America, Portland, Oregon, http://www.tcichemicals.com/en/us/index.html) and mice were placed on a HFD for 7 days prior to euthanization. All animals were housed in a specific pathogen-free environment with a light-dark cycle, under a protocol approved by the University of Kentucky Institutional Animal Care and Use Committee.

Metabolic Analyses

Body weight was measured weekly and body composition was measured by nuclear magnetic resonance (NMR) spectroscopy (Echo MRI, Houston, TX, http://www.echomri.com/). Intraperitoneal glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed, as previously described [8]. Plasma insulin was measured using a Rat/Mouse Insulin ELISA kit (Millipore, Billerica, Massachusetts, http://www.emdmillipore.com/US/en). Plasma TNFα levels were measured by a mouse TNFα ELISA kit (BioLegend, San Diego, CA, http://www.biolegend.com/). Plasma cytokine levels were measured by a mouse cytokine multiplex assay kit and a BioPlex 200 system (Bio-Rad Laboratories, Hercules, California, http://www.bio-rad.com/). For insulin stimulation studies, 20-week-old male IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ littermates or ASO-treated C57BL/6 mice were injected with insulin (0.35 U/kg body weight) into the inferior vena cava [25]. After 5 minutes, mice were euthanized and tissues were collected for protein isolation and Western blot analysis.

Adipose Stromal Vascular (SV) Cell Isolation and Differentiation

Adipose SV cells and mature adipocytes were isolated as previously described [8]. The SV cells were used for RNA and protein isolation or cultured in 12-well plates for differentiation. For differentiation assays, SV cells were induced by high-glucose Dulbecco's modified Eagle's medium containing dexamethasone (1 μM), isobutylmethylxanthine (0.5 mM), insulin (10 μg/ml), rosiglitazone (1 μM), and 10% fetal bovine serum (FBS) until they were ready for analysis [8].

RNA Isolation and Quantitative Real Time PCR (QPCR) Analysis

Total RNA was isolated from mouse tissues or cells using TRIzol Reagent (Life Technologies, Grand Island, NY, https://www.thermofisher.com/) and QPCR was performed using gene-specific primers and the SYBR Green PCR kit (Life Technologies) as previously described [26, 27]. The sequences of primer sets used in this study are listed in Supporting Information Table 1.

Quantification of Mitochondrial DNA

Genomic DNA was extracted from subcutaneous white adipose tissue (WAT) by the DNAeasy Blood and Tissue kit (Qiagen,

Germantown, MD, http://www.qiagen.com). QPCR was performed on COX1 mitochondrial DNA and normalized to a nuclear 28S sequence [28, 29]. The sequences of primer sets used in this study are listed in Supporting Information Table 1.

Human Subjects and Adipose Stem Cell Isolation

Human adipocytes were derived from the differentiation of adult-derived human adipose stem cells (ADHASC) as described previously [30, 31]. The adipose tissue was from a collagenase digestion of the lipoaspirate of patients undergoing liposuction of subcutaneous fat. These patients were generally young, healthy women undergoing cosmetic procedures, and this method of collection was approved by the University of Kentucky Institutional Review Board. Differentiation was induced using differentiation media as previously described [30, 31]. At least 80%–90% of the cells developed lipid droplets in 7–10 days.

Statistical Analysis

All data are presented as the mean ± SEM. Individual pairwise comparisons were analyzed by two-sample, two-tailed Student's *t* test unless otherwise noted, with $p < .05$ was regarded as significant. Two-way analysis of variance (ANOVA) was used when multiple comparisons were made followed by a Bonferroni multiple comparisons test. *N* numbers are listed in figure legends.

Results

ASO-Mediated IKK*β* Knockdown Protects Mice from Diet-Induced Obesity

We recently demonstrated that IKKβ inhibitors can inhibit adipocyte differentiation in vitro and ameliorate diet-induced adiposity in mice [8]. To explore novel and more efficient pharmacological approaches for IKKβ inhibition, we utilized second-generation ASO targeting technology [32] and investigated the impact of IKKβ ASO on diet-induced obesity and metabolic disorders. Intraperitoneal delivery of ASO has been shown to reach a variety of tissues including liver and adipose tissue [33, 34]. Indeed, we found that IKKβ ASO can efficiently decrease IKKβ gene expression in multiple tissues including liver, skeletal muscle (Sk.M), and WAT at a dose of 25 mg/kg BW/week (Fig. 1A). Interestingly, IKKβ mRNA levels were not significantly altered in brown adipose tissue (BAT) by IKKβ ASO treatment at this dose. Western blot analysis also confirmed the specific and efficient IKKβ knockdown in tissues including liver and WAT as IKKα protein levels were not affected by IKKβ ASO treatment (Fig. 1B).

We next determined whether IKKβ ASO treatment can ameliorate diet-induced obesity. Groups of 8-week-old wild-type male mice were fed a ND or HFD and were treated with control ASO or IKKβ ASO for 8 weeks (Fig. 1C). HF-feeding increased body weight in mice treated with both control and IKKβ ASOs. However, IKKβ ASO treatment significantly decreased HFD-induced body weight gain and adiposity. While lean mass was slightly but significantly increased in IKKβ ASO-treated mice, fat mass was decreased by 45% in HFD-fed mice treated with IKKβ ASO (Fig. 1D). IKKβ ASO was able to decrease both subcutaneous (sub) WAT and visceral WAT including epididymal (epi) and retroperitoneal (retro) fat pads in HFD-fed mice as compared with littermate controls (Fig. 1E).

IKK*β* ASO Improves Insulin Sensitivity and Reverses Hepatic Steatosis in Obese Mice

Obesity is frequently associated with metabolic disorders such as insulin resistance and hepatic steatosis. We next investigated whether IKKβ ASO treatment can protect HFD-fed mice from these disorders. IKKβ ASO-treated mice had decreased fasting plasma glucose and insulin concentrations as compared with control mice (Fig. 1F), suggesting improved diabetic phenotype. Upon glucose and insulin tolerance test, IKKβ ASO-treated mice had improved glucose tolerance and showed an increased hypoglycemic response to the injected insulin (Fig. 1G). To further assess the impact of IKKβ ASO treatment on insulin signaling, HFD-fed mice were injected with insulin prior to euthanization and phosphorylation of Akt was analyzed in multiple tissues. IKKβ ASO was able to enhance phosphorylation of Akt in response to insulin in liver, Sk.M and WAT (Fig. 1H). While hepatic IKKβ signaling has been demonstrated to contribute to obesity-associated insulin resistance [17, 18], the role of adipose IKKβ signaling in the regulation of insulin sensitivity has not been well-defined. We then performed adipose glucose uptake assays. Interestingly, adipose tissue explants from IKKβ ASO-treated mice had elevated glucose uptake in the absence of insulin and insulin stimulation further enhanced glucose update by adipose tissues (Fig. 1I). Therefore, the enhanced Akt phosphorylation and increased glucose uptake in adipose tissue likely contribute to the improved insulin sensitivity in IKKβ ASO-treated mice. Further, HF feeding also caused lipid accumulation and hepatic steatosis in control mice. However, IKKβ ASO-treated mice were protected from these detrimental effects (Fig. 1J, 1K). Consistently, hepatic triglyceride and cholesterol contents were significantly reduced in IKKβ ASO-treated mice (Fig. 1L). Collectively, these results suggest that pharmacological inhibition of IKKβ by ASO ameliorates obesity-associated metabolic disorders in mice.

IKK*β* Regulates Murine Adipocyte Differentiation

We next investigated whether IKKβ ASO treatment can also affect IKKβ expression in adipocyte precursor cells and affect adipocyte differentiation. Indeed, we found that IKKβ expression was decreased in both adipose SV cells and mature adipocytes in IKKβ ASO-treated mice (Fig. 2A). Consistent with our previous study [8], ASO-mediated IKKβ knockdown diminished the ability of adipose SV cells to differentiate into adipocytes (Fig. 2B). As expected, the expression levels of adipogenic genes including PPARγ, Zfp423, and C/EBPs were significantly decreased in epiWAT from IKKβ ASO-treated mice (Fig. 2C). Further, IKKβ ASO treatment also decreased the expression of a known NF-κB target, Smad ubiquitination regulatory factor 2 (Smurf 2) (Fig. 2D). Smurf2 is an ubiquitin E3 ligase that regulates proteasome-mediated degradation of several proteins including β-catenin [8, 35, 36]. Consistently, ASO-mediated IKKβ knockdown increased nuclear β-catenin protein levels in epiWAT (Fig. 2E). Since Wnt/β-catenin signaling has been well defined to inhibit adipogenesis in vitro and in vivo [37, 38], the increased Wnt signaling likely contributes to the decreased adipogenesis in IKKβ ASO-treated mice.

To further define the role of IKKβ in adipogenesis, we used the CRISPR-Cas9 system to delete the IKKβ gene in murine 3T3-

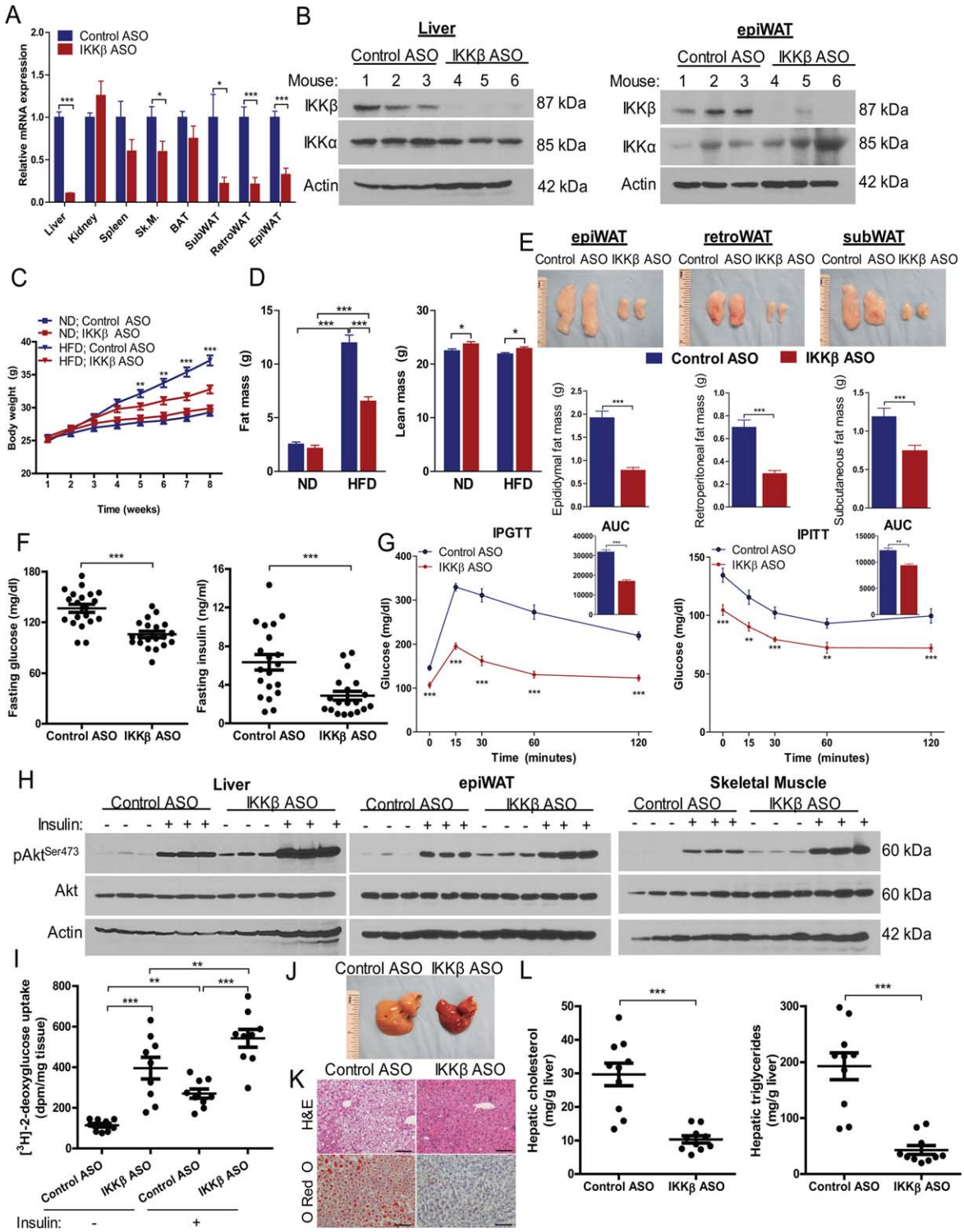

Figure 1. Pharmacological inhibition of IKKβ with ASOs protects mice from diet-induced obesity, improves insulin sensitivity, and reverses hepatic steatosis in obese mice. **(A)**: IKKβ mRNA expression in liver, kidney, spleen, Sk.M., BAT, subWAT, retroWAT, and epiWAT from mice treated with control ASO or IKKβ ASO for 8 weeks ($n = 6$-10; $^{*}p < .05$, $^{***}p < .001$, assessed by Student's *t* test). **(B)**: Western blot analysis of IKKβ and IKKα expression in liver and epiWAT from mice treated with control or IKKβ ASO for 4 weeks. **(C, D)**: Growth curves (C), and fat mass and lean mass (D) of ND and HFD-fed mice treated with control ASO or IKKβ ASO ($n = 10$ for ND and 30 for HFD; $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$, assessed by Student's *t*-test (C) or two-way analysis of variance (D)). **(E)**: Representative images of adipose depots (top) and weight of adipose depots (bottom) from mice treated with control or IKKβ ASO for 8 weeks ($n = 20$; $^{***}p < 0.001$, assessed by Student's *t* test). Results are presented as means ± SEM. **(F):** Fasting plasma glucose and insulin levels of HFD-fed mice treated with control or IKKβ ASO ($n = 29$-30; $^{***}p < .001$, assessed by Student's *t* test). **(G)**: IPGTT, IPITT, and area of curve (AUC) of IPGTT and IPITT of HFD-fed mice treated with control or IKKβ ASO ($n = 8$-10; $^{**}p < .01$, $^{***}p < .001$, assessed by Student's *t* test). **(H)**: Western blot analysis of phosphorylated Akt and total Akt levels in liver, epiWAT, and Sk.M of control or IKKβ ASO-treated mice injected with saline or 0.35 U/kg body weight. **(I)**: Glucose uptake was measured in primary adipose tissues from mice treated with control or IKKβ ASO ($n = 9$; $^{**}P < 0.01$, $^{***}P < 0.001$, assessed by two-way analysis of variance). **(J, K)**: Representative appearance (J) and hematoxylin and eosin (top) and Oil-red-O (bottom) stained sections (K) of livers from mice treated with control or IKKβ ASO (scale bar =100 μm). **(L)**: Hepatic cholesterol and triglyceride levels of mice treated with control or IKKβ ASO ($n = 10$; $^{***}p < .001$, assessed by Student's *t* test). Results are presented as means ± SEM. Abbreviations: IKKβ, IκB kinase β; HFD, high-fat diet; ASO, antisense oligonucleotide; ND, normal chow diet; Sk.M., skeletal muscle; BAT, brown adipose tissue; subWAT, subcutaneous white adipose tissue; retroWAT, retroperitoneal WAT; epiWAT, epididymal WAT; IPGTT, intraperitoneal glucose tolerance test; IPITT, intraperitoneal insulin tolerance tests.

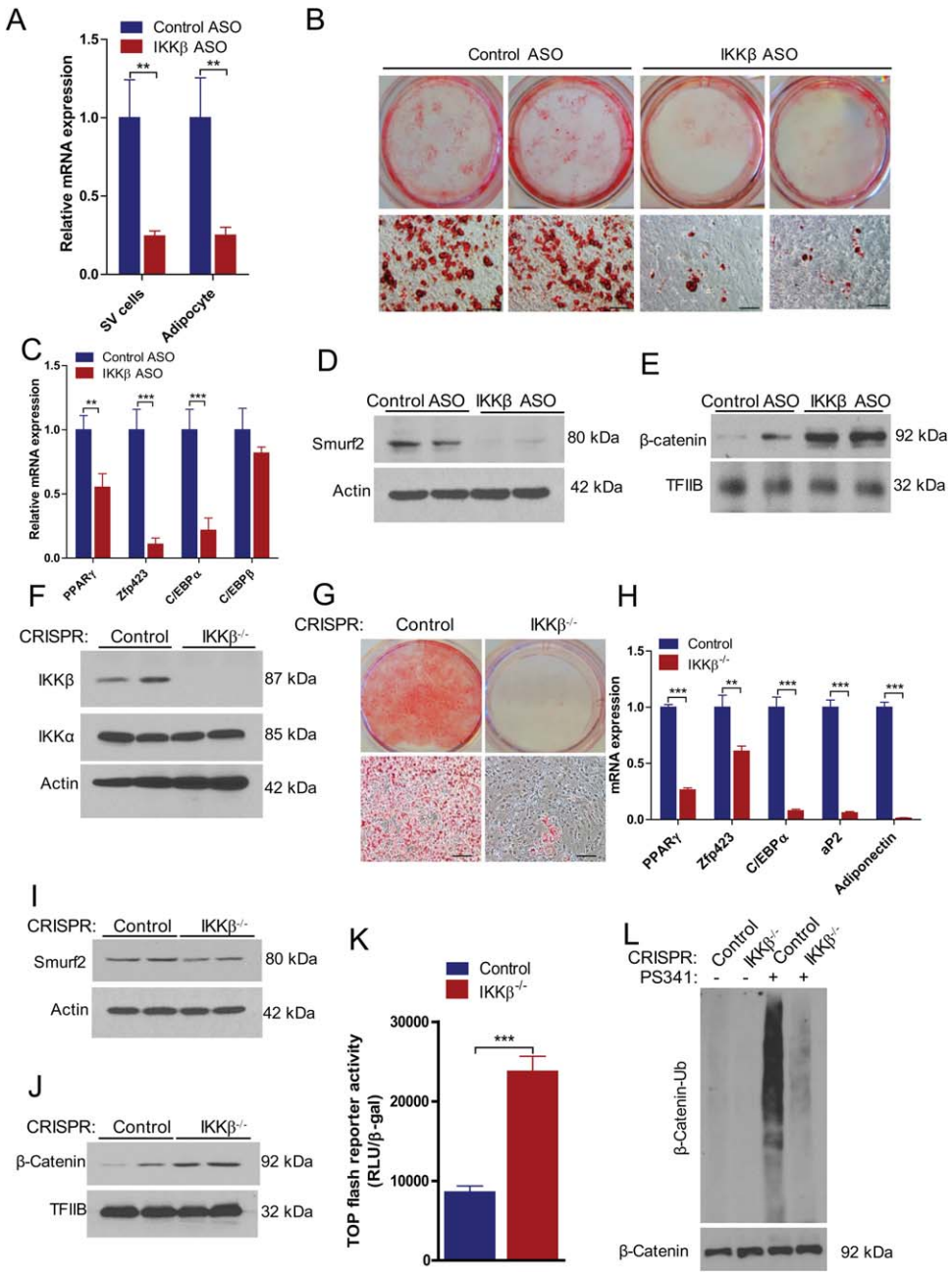

Figure 2. IKKβ regulates murine adipocyte differentiation. **(A)**: IKKβ mRNA expression in adipose stromal vascular (SV) cells and mature adipocytes isolated from epididymal WAT (epiWAT) of mice treated with control or IKKβ ASO ($n = 6$; $^{**}p < .01$, assessed by Student's *t* test). **(B)**: Oil-red-O staining of adipose SV cells isolated from epiWAT of mice treated with control and IKKβ ASO induced by differentiation media (scale bar = 100 μm, bottom panels). **(C)**: Expression of adipogenic genes in epiWAT of control and IKKβ ASO treated mice was measure by QPCR ($n = 6$; $^{**}p < .01$, $^{***}p < .001$, assessed by Student's *t*-test). **(D)**: Western blot analysis of Smurf2 protein levels in epiWAT. **(E)**: Western blot analysis of nuclear β-catenin levels in epiWAT. (**F**): Western blot analysis of IKKβ and IKKα protein levels in control 3T3-L1 cells or CRISPR-mediated IKKβ-deficient 3T3-L1 cells. **(G)**: Oil-red-O staining of control and IKKβ-deficient 3T3-L1 cells induced by differentiation media (scale bar = 100μm, bottom panels). **(H)**: Expression of adipogenic genes and adipocyte markers in control or IKKβ-deficient 3T3-L1 cells ($n = 6$; $^{**}p < .01$, $^{***}p < .001$, assessed by Student's *t* test). **(I)**: Western blot analysis of Smurf2 protein levels in control or IKKβ-deficient 3T3-L1 cells. **(J)**: Western blot analysis of nuclear β-catenin protein levels in control or IKKβ-deficient 3T3-L1 cells. **(K)**: β-catenin reporter (TOP-flash reporter) activity in control or IKKβ-deficient 3T3-L1 cells ($n = 6$; $^{***}p < .001$, assessed by Student's *t* test). **(L)**: Control or IKKβ-deficient 3T3-L1 cells were treated with vehicle control or 100 nM PS-341, as indicated, for 4 hours. β-catenin was immunoprecipitated with anti-β-catenin antibodies and then probed with anti-ubiquitin monoclonal antibodies. The whole cell lysates were probed with anti-β-catenin antibodies as an internal control. Results are presented as means ± SEM. Abbreviations: IKKβ, IκB kinase β; ASO, antisense oligonucleotide; Smurf2, Smad ubiquitination regulatory factor 2.

L1 preadipocytes (Fig. 2F). We found that deletion of IKKβ almost completely blocked 3T3-L1 cell differentiation (Fig. 2G). Gene expression analysis showed that mRNA levels of adipogenic genes and adipocyte markers including PPARγ and adiponectin were significantly decreased by IKKβ-deficiency (Fig. 2H). Consistently, deletion of IKKβ decreased the expression of Smurf2 (Fig. 2I) and increased nuclear β-catenin accumulation (Fig. 2J), leading to increased β-catenin activity in these cells

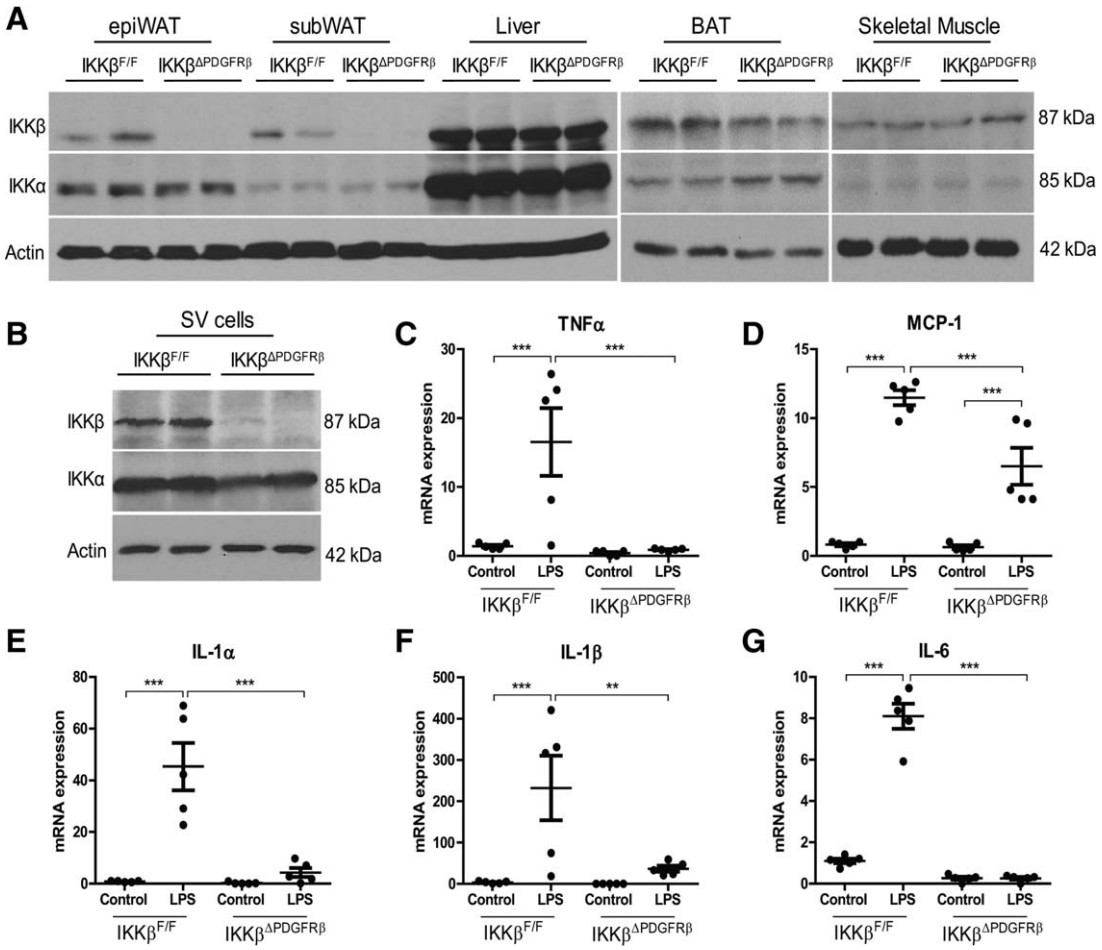

Figure 3. Generation of mice lacking IKKβ in the white adipose lineage. **(A)**: Western blot analysis of IKKβ and IKKα protein levels in epiWAT, subWAT, liver, BAT, and skeletal muscle (Sk.M.) of IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice. **(B)**: Western blot analysis of IKKβ and IKKα protein levels in adipose SV cells isolated from IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice. **(C–G)**: Adipose SV cells isolated from IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice were treated with LPS for 3 hours. The expression levels for proinflammatory cytokines including TNFα (C), MCP-1 (D), IL-1α (E), IL-1β (F), and IL-6 (G) were examined by QPCR ($n = 5$; $^{**}p < .01$, $^{***}p < .001$, assessed by two-way analysis of variance). Results are presented as means ± SEM. Abbreviations: IKKβ, IκB kinase β; epiWAT, epididymal WAT; BAT, brown adipose tissue; subWAT, subcutaneous white adipose tissue; retroWAT, retroperitoneal WAT.

(Fig. 2K). We further confirmed that β-catenin ubiquitination was inhibited by IKKβ deficiency in 3T3-L1 cells (Fig. 2L), suggesting the impact of reduced Smurf2 expression. Taken together, these results confirm the important role of IKKβ in adipogenesis and indicate that pharmacological inhibition of IKKβ by ASO can decrease adipogenesis and diet-induced adiposity.

Targeted Deletion of IKKβ in the White Adipose Lineage

Since ASO treatment affects many tissues and cell types, it is not clear how significantly the ASO-mediated IKKβ knockdown in adipose progenitors contributes to the beneficial effects on obesity and metabolic disorder in mice. Compared with other tissues or cell types, the role of IKKβ signaling in adipose progenitors has not been well-defined. To investigate the function of IKKβ signaling in adipose progenitors, we sought to generate a mouse model that selectively lacks IKKβ in white adipose lineage. Recent studies have identified a subset of perivascular cells as adipose progenitors which express multiple mural cell markers including platelet-derived growth factor receptor (PDGFR) β, α smooth muscle actin, and NG2 [39]. For example, both PDGFRβ, a human mesenchymal stem cell (MSC) marker [40], and PDGFRα, another isoform of PDGFR, have been confirmed to be adipocyte progenitor markers by independent groups [41–45]. Consistently, we also found that the high expression levels of PDGFRβ in adipose SV cells as compared with mature adipocytes (Supporting Information Fig. 1A). We then crossed PDGFRβ promoter-driven Cre mice [22] with Rosa26^{lacZ} reporter mice that express β-galactosidase (lacZ) in target tissues upon Cre-mediated excision of a "Stop" sequence to generate PDGFRβ-Cre/Rosa26^{lacZ} mice (Supporting Information Fig. 1B). While none of adipose SV cells from control Rosa26^{lacZ} mice was lacZ^{+} cells, many of adipose SV cells from PDGFRβ-Cre/Rosa26^{lacZ} mice were lacZ^{+} cells (Supporting Information Fig. 1C). Staining of the white adipose depots and tissue sections also indicated that PDGFRβ-Cre is activated in adipose progenitors that give rise to white adipocytes as PDGFRβ generated strong lacZ expression in adipocytes in addition to the vasculature (Supporting Information Fig. 1D, 1E). Taken together, our results are consistent with previous reports [39, 41] and confirm that PDGFRβ is a marker for adipocyte lineage cells.

We then deleted IKKβ in adipocyte lineage cells by intercrossing PDGFRβ-Cre mice with mice containing loxP-flanked

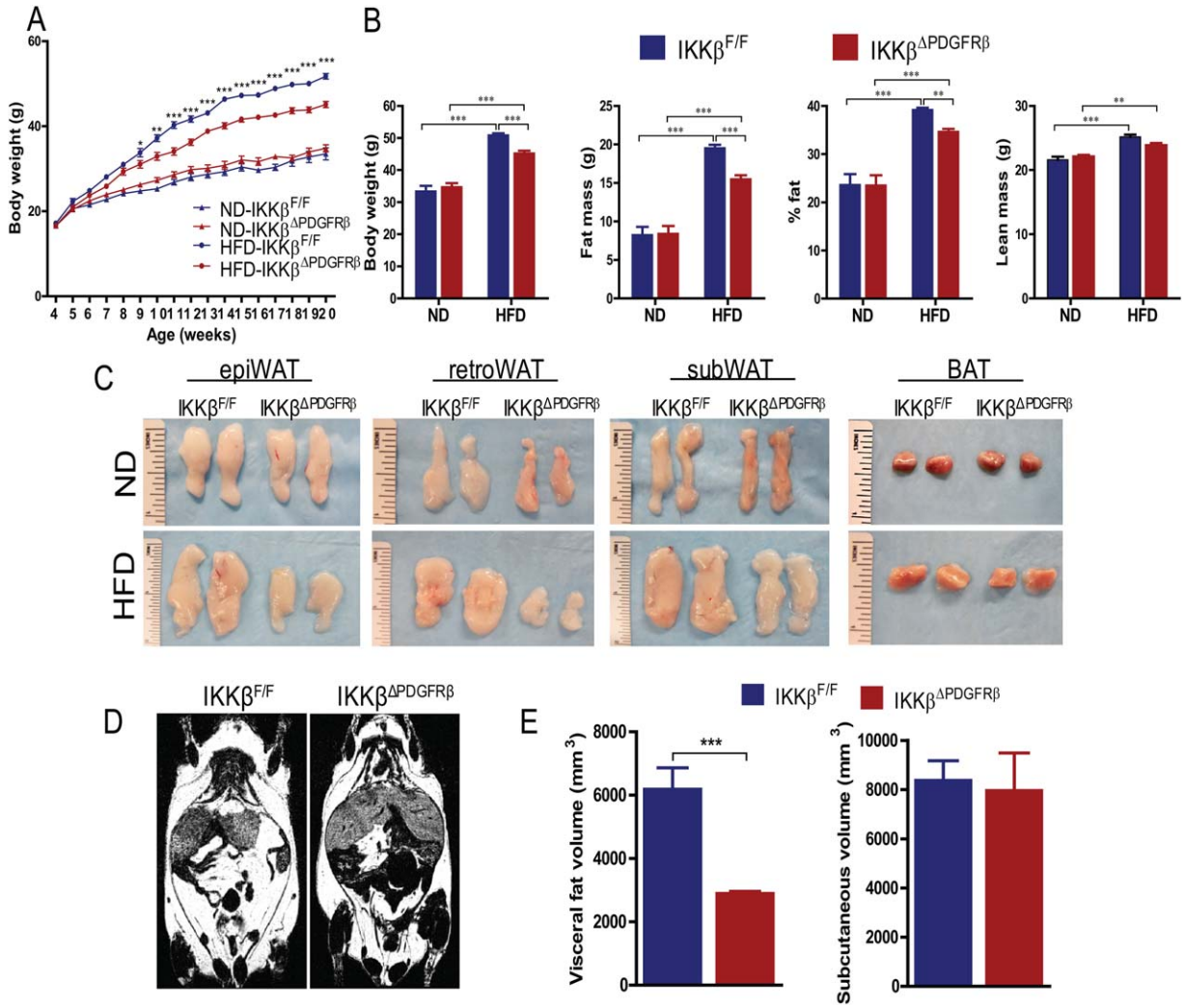

Figure 4. Deficiency of IKKβ in adipocyte lineage cells renders mice resistant to diet-induced obesity. **(A)**: Growth curves of ND or HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice ($n = 9$-18, ***$p < .001$ when comparing HFD-fed IKKβ$^{F/F}$ mice to HFD-fed IKKβ$^{\Delta PDGFR\beta}$ mice, assessed by Student's *t*-test). **(B)**: Body weight, fat mass, percentage of fat, and lean mass of 10-week-old IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice fed a ND or HFD for 16 weeks ($n = 7$-19; **$p < .01$, ***$p < .001$, assessed by two-way analysis of variance). **(C)**: Representative photographs of epiWAT, retroWAT, subWAT, and BAT from ND or HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice. **(D, E)**: Representative coronal section MRI images (D) and visceral and subcutaneous adipose tissue volume (E) of HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice ($n = 3$; ***$p < .001$, assessed by Student's *t* test). Results are presented as means ± SEM. Abbreviations: ND, normal chow diet; HFD, high-fat diet; BAT, brown adipose tissue; subWAT, subcutaneous white adipose tissue; retroWAT, retroperitoneal WAT; epiWAT, epididymal WAT.

IKKβ alleles (IKKβ$^{F/F}$) [8, 24]. The resulting PDGFRβ-Cre/IKKβ$^{F/F}$ (termed IKKβ$^{\Delta PDGFR\beta}$) mice were viable and appeared indistinguishable from their control IKKβ$^{F/F}$ littermates. Western blot analysis confirmed the deletion of IKKβ in WAT including epiWAT and subWAT but not in BAT, liver, and Sk.M (Fig. 3A). As expected, PDGFRβ-Cre-mediated IKKβ deletion also caused reduced IKKβ expression in adipose SV cells of IKKβ$^{\Delta PDGFR\beta}$ mice (Fig. 3B). Further, IKKβ deficiency reduces NF-κB-mediated pro-inflammatory gene expression in response to lipopolysaccharide (LPS) in adipose SV cells of IKKβ$^{\Delta PDGFR\beta}$ mice (Fig. 3C–3G), suggesting impaired NF-κB activation in these cells.

Deficiency of IKK*β* in Adipocyte Lineage Cells Renders Mice Resistant to Diet-Induced Obesity

To determine the role of adipose progenitor IKKβ signaling in obesity, IKKβ$^{F/F}$, and IKKβ$^{\Delta PDGFR\beta}$ littermates were fed a ND or a HFD for 16 weeks. There were no significant differences in body weight for ND-fed mice. HFD-feeding elicited increases in body weight in both IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice. However, IKKβ deficiency significantly decreased diet-induced bodyweight gain in IKKβ$^{\Delta PDGFR\beta}$ mice as compared with IKKβ$^{F/F}$ mice (Fig. 4A). While lean mass was not affected by IKKβ deficiency, HFD-fed IKKβ$^{\Delta PDGFR\beta}$ mice had significantly decreased fat mass as compared with control littermates (Fig. 4B).

HFD-fed IKKβ$^{\Delta PDGFR\beta}$ mice also had decreased visceral adipose pads including epididymal and retroperitoneal fat pads. However, deficiency of IKKβ did not affect the size or weight of subWAT and BAT (Fig. 4C, Supporting Information Fig. 2A, 2B). As subWAT often undergoes "browning" process which enhances oxidative metabolism and protects mice from metabolic dysfunctions [28, 46–48], we then examined whether deficiency of IKKβ affects browning of subWAT in these mice. Interestingly,

the expression levels of beige or brown adipocyte markers as well as mitochondrial function-related genes such as UCP-1 and PGC-1α [28, 47, 48] were not affected in subWAT of IKK$\beta^{\Delta PDGFR\beta}$ mice (Supporting Information Fig. 3A). Consistently, IKK$\beta^{\Delta PDGFR\beta}$ mice also had comparable mitochondrial content in subWAT as that of control littermates (Supporting Information Fig. 3B). MRI analyses also confirmed that IKK$\beta^{\Delta PDGFR\beta}$ mice had a significantly decreased volume of visceral adipose tissue but a similar subcutaneous adipose tissue volume as compared with IKK$\beta^{F/F}$ mice under HF feeding conditions (Fig. 4D, 4E). We next analyzed the expression levels of IKKβ and adipose progenitor markers in SV cells isolated from subWAT and epiWAT of control mice. Interestingly, IKKβ expression levels were significantly higher in visceral SV cells than subcutaneous SV cells. Further, the expression levels of adipose progenitor markers such as PDGFRβ were much higher in visceral SV cells as compared with subcutaneous SV cells (Supporting Information Fig. 4), indicating a more abundant adipose progenitor cell population in visceral WAT. Collectively, these results demonstrate that PDGFRβ-Cre-driven IKKβ deficiency in the white adipose lineage decreases diet-induced obesity and visceral adiposity.

IKKβ-Deficient Mice are Protected from Obesity-Associated Metabolic Disorders

We next investigated whether deficiency of IKKβ also protected mice from obesity-associated insulin resistance. IKK$\beta^{\Delta PDGFR\beta}$ mice had comparable glucose and insulin levels and similar glucose tolerance and insulin tolerance as that of IKK$\beta^{F/F}$ when fed a ND (Fig. 5A, 5B; Supporting Information Fig. 5). As expected, HF feeding increased plasma glucose and insulin levels in both IKK$\beta^{F/F}$ and IKK$\beta^{\Delta PDGFR\beta}$ mice (Fig. 5A). Although glucose concentrations were not affected by IKKβ deficiency, HFD-fed IKK$\beta^{\Delta PDGFR\beta}$ mice had significantly decreased insulin concentrations as compared with HFD-fed control littermates (Fig. 5A). Consistently, IKK$\beta^{\Delta PDGFR\beta}$ mice also had improved glucose tolerance and insulin tolerance as compared with IKK$\beta^{F/F}$ mice under HF feeding conditions (Fig. 5B). PDGFRβ-Cre-mediated IKKβ deletion also enhanced phosphorylation of Akt in response to insulin in WAT but not in liver and Sk.M (Fig. 5C). Further, adipose tissue explants from IKK$\beta^{\Delta PDGFR\beta}$ mice had significantly increased insulin-stimulated glucose uptake compared with that of IKK$\beta^{F/F}$ mice (Fig. 5D).

Obesity-associated macrophage infiltration contributes to the development of systemic insulin resistance [49]. Although IKKβ expression levels were not affected in macrophages of IKK$\beta^{\Delta PDGFR\beta}$ mice (Fig. 5E), macrophage infiltration was substantially decreased in WAT of IKK$\beta^{\Delta PDGFR\beta}$ mice (Fig. 5F, Supporting Information Fig. 6), suggesting that deficiency of IKKβ in adipose lineage blocks the increased inflammatory infiltrates in WAT under obese conditions. Consistently, the mRNA levels of macrophage markers and several key pro-inflammatory cytokines including TNFα, MCP-1, and IL-1β were significantly decreased in WAT of IKK$\beta^{\Delta PDGFR\beta}$ mice (Fig. 5G). We next measured plasma cytokine levels to determine whether IKKβ-deficient mice also had decreased systemic inflammation. Deficiency of IKKβ significantly decreased HFD-induced plasma pro-inflammatory cytokines including TNFα, MCP-1 and IL-6 in IKK$\beta^{\Delta PDGFR\beta}$ mice (Fig. 5H). Taken together, deficiency of IKKβ improved insulin signaling in adipose tissue and protected mice from obesity-associated metabolic disorders.

Deficiency of IKKβ Inhibits Adipogenesis in Mice

We next investigated whether deficiency of IKKβ can decrease HF feeding-elicited adipogenesis in vivo. For this experiment, IKK$\beta^{F/F}$ and IKK$\beta^{\Delta PDGFR\beta}$ mice were treated with BrdU during the first week of HF feeding. We then used BrdU as a marker to track newly differentiated adipocytes in these mice after 7 days of HFD. Immunostaining of adipose tissue sections with BrdU antibodies revealed that IKK$\beta^{\Delta PDGFR\beta}$ mice had significantly decreased BrdU positive adipocytes in WAT (Fig. 6A, 6B), indicating decreased adipogenesis. We also assessed the adipogenic potential of adipose SV cells isolated from visceral adipose tissue of IKK$\beta^{F/F}$ and IKK$\beta^{\Delta PDGFR\beta}$ mice and confirmed that IKKβ deficiency impaired the adipogenic potential of SV cells from IKK$\beta^{\Delta PDGFR\beta}$ mice (Fig. 6C). Further, the expression levels of adipocyte markers and key adipogenic genes including PPARγ, C/EBPα, and Zfp423, were significantly reduced by IKKβ deficiency (Fig. 6D). Consistent with results from IKKβ ASO treatment and 3T3-L1 experiments (Fig. 3), IKK$\beta^{\Delta PDGFR\beta}$ mice had decreased Smurf2 levels and increased nuclear β-catenin levels in epiWAT (Fig. 6E, 6F) as well as in primary adipose SV cells (Fig. 6G, 6H). Further, deficiency of IKKβ substantially decreased β-catenin ubiquitination in adipose SV cells (Fig. 6I), which likely contributes to the accumulation of nuclear β-catenin in IKK$\beta^{\Delta PDGFR\beta}$ mice.

Inhibition of IKKβ Decreases Adipogenesis in Human Adipose Stem Cells

In addition to murine 3T3-L1 preadipocytes and primary SV cells, we also determined whether IKKβ regulates the differentiation of human adipose stem cells. Adult-derived human adipose stem cells, also known as ADHASCs [30, 31, 50], were isolated from healthy subjects. Consistent with previous reports [30, 31, 50], human adipose stem cells were able to efficiently differentiate into mature adipocytes in vitro and at least 80%–90% of the cells developed lipid droplets in 7–10 days (Fig. 7A). Pharmacological inhibition of IKKβ by BMS-345541 strongly inhibited human adipose stem cell differentiation and repressed human adipogenic gene expression (Fig. 7B). BMS-345541 treatment also led to reduced Smurf2 expression and decreased β-catenin ubiquitination, leading to increased nuclear β-catenin levels (Fig. 7C–7E), which likely contribute to the decreased adipogenesis in human adipose stem cells. Collectively, these results demonstrated IKKβ as an important regulator of both human and murine adipocyte differentiation.

Discussion

As a central coordinator of inflammatory responses, IKKβ signaling in multiple tissues including liver, pancreas, and brain have been associated with obesity and obesity-related metabolic dysfunctions [15, 17–19, 21, 51]. High doses of IKKβ inhibitors such as salicylates have been used to treat inflammatory conditions including diabetes in humans for more than a century [52, 53]. Moreover, inhibition of IKKβ activity by salicylates also protected mice against insulin resistance triggered by HFD or obesity [15, 16]. Interestingly, long-term anti-inflammatory therapy has also been associated with

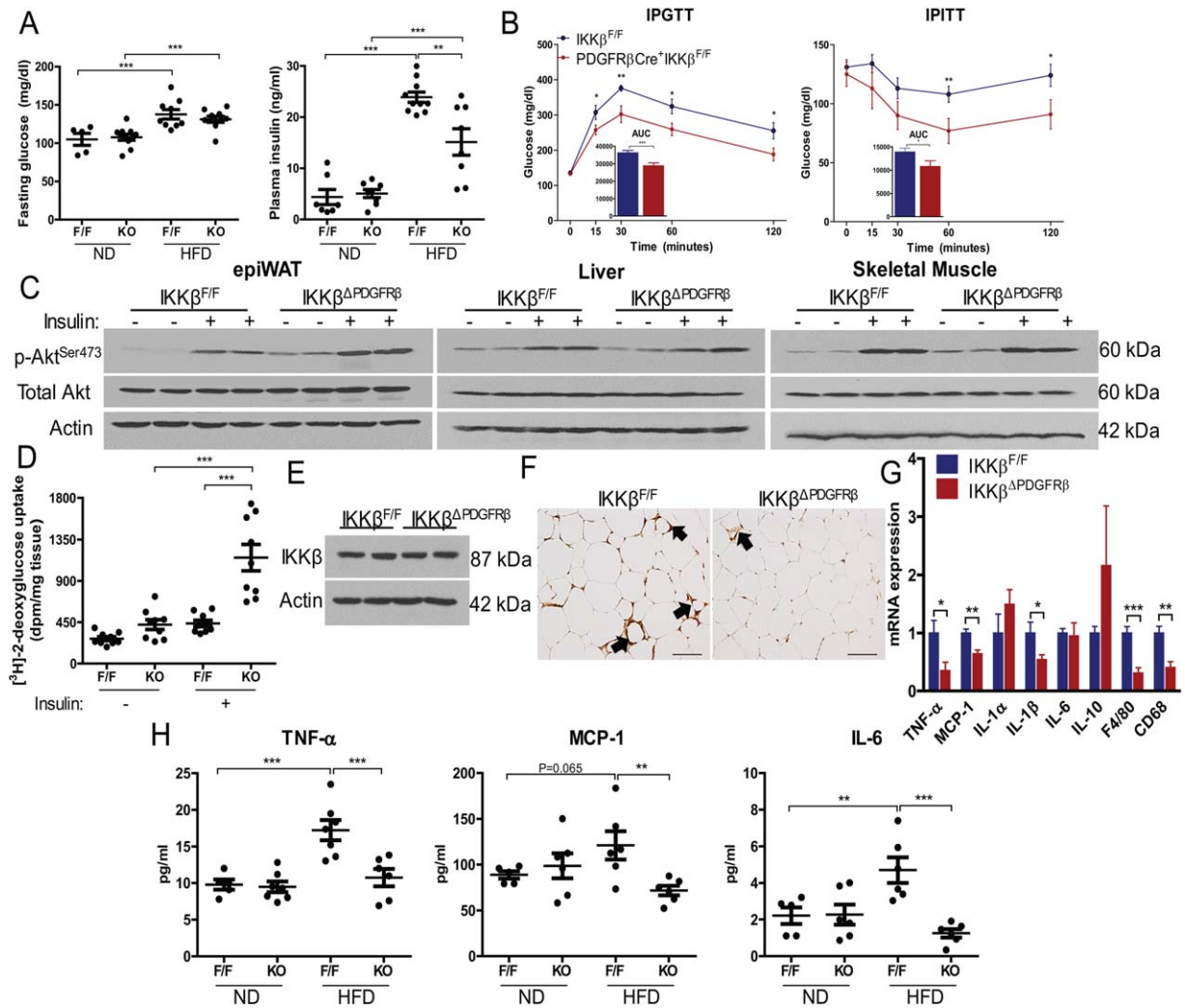

Figure 5. IKKβ-deficient mice are protected from obesity-associated metabolic disorders. **(A)**: Fasting plasma glucose and insulin levels in ND or HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice ($n = 5$-11; $^{**}p < .01$, $^{***}p < .001$, assessed by two-way analysis of variance). **(B)**: IPGTT, IPITT, and area under the curve (AUC) of IPGTT and IPITT of HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice ($n = 6$-11; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, assessed by Student's *t* test). **(C)**: Western blot analysis of phosphorylated Akt and total Akt levels in epiWAT, liver, and Sk.M of IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice injected with saline or 0.35U/kg body weight. **(D)**: Glucose uptake was measured in primary adipose tissues from HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice ($n = 9$; $^{***}p < .001$, assessed by two-way ANOVA). **(E)**: Western blot analysis of IKKβ protein levels in peritoneal macrophages of IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice. **(F)**: Representative immunohistochemistry for the macrophage marker, F4/80 in epiWAT from HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice (scale bar = 100μm). **(G)**: The expression levels of pro-inflammatory genes and macrophage markers in epiWAT of HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice were measured by QPCR ($n = 5$; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, assessed by Student's *t* test). **(H)**: Plasma cytokine levels of ND or HFD-fed IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice ($n = 5$-7; $^{**}p < .01$, $^{***}p < .001$, assessed by two-way ANOVA). Results are presented as means ± SEM. Abbreviations: epiWAT, epididymal WAT; ND, normal chow diet; HFD, high-fat diet; IKKβ, IκB kinase β.

weight loss in human studies [54]. We recently demonstrated that salicylates and the potent IKKβ inhibitor, BMS-345541, can also inhibit adipocyte differentiation in a dose-dependent manner [8]. In the current study, we demonstrated that IKKβ ASO can efficiently decrease IKKβ expression in various tissues including liver and WAT, and ameliorate diet-induced obesity and metabolic disorders in mice. To our knowledge, our study is the first to use ASOs targeting IKKβ in vivo and investigate its metabolic impact. Consistent with previous reports demonstrating that hepatic IKKβ signaling contribute to insulin resistance [17, 18], IKKβ ASO-treated mice had improved insulin signaling in the liver. Nevertheless, knockdown of IKKβ in WAT also resulted in enhanced Akt phosphorylation and increased glucose uptake. Therefore, the improved diabetic phenotype in IKKβ ASO-treated mice was likely due to the repressed IKKβ signaling in multiple tissues including liver and WAT. In addition to improved insulin signaling, IKKβ ASO treatment also repressed IKKβ expression in SV cells, leading to decreased adipogenesis in WAT. Collectively, our studies demonstrate IKKβ as a potential target for future anti-obesity drugs and provide evidence for the use of appropriate IKKβ ASOs as a potential therapeutic strategy to treat obesity and metabolic disease (Supporting Information Fig. 7).

Despite substantial progress in defining transcription factors such as PPARγ and C/EBPs in the regulation of committed preadipocyte differentiation, the initial signals that trigger adipose progenitor cells commitment to adipocyte lineage in response to overnutrition remain unknown. In addition to its well-established role as a central mediator of inflammation and immune responses, IKKβ/NF-κB signaling plays key roles

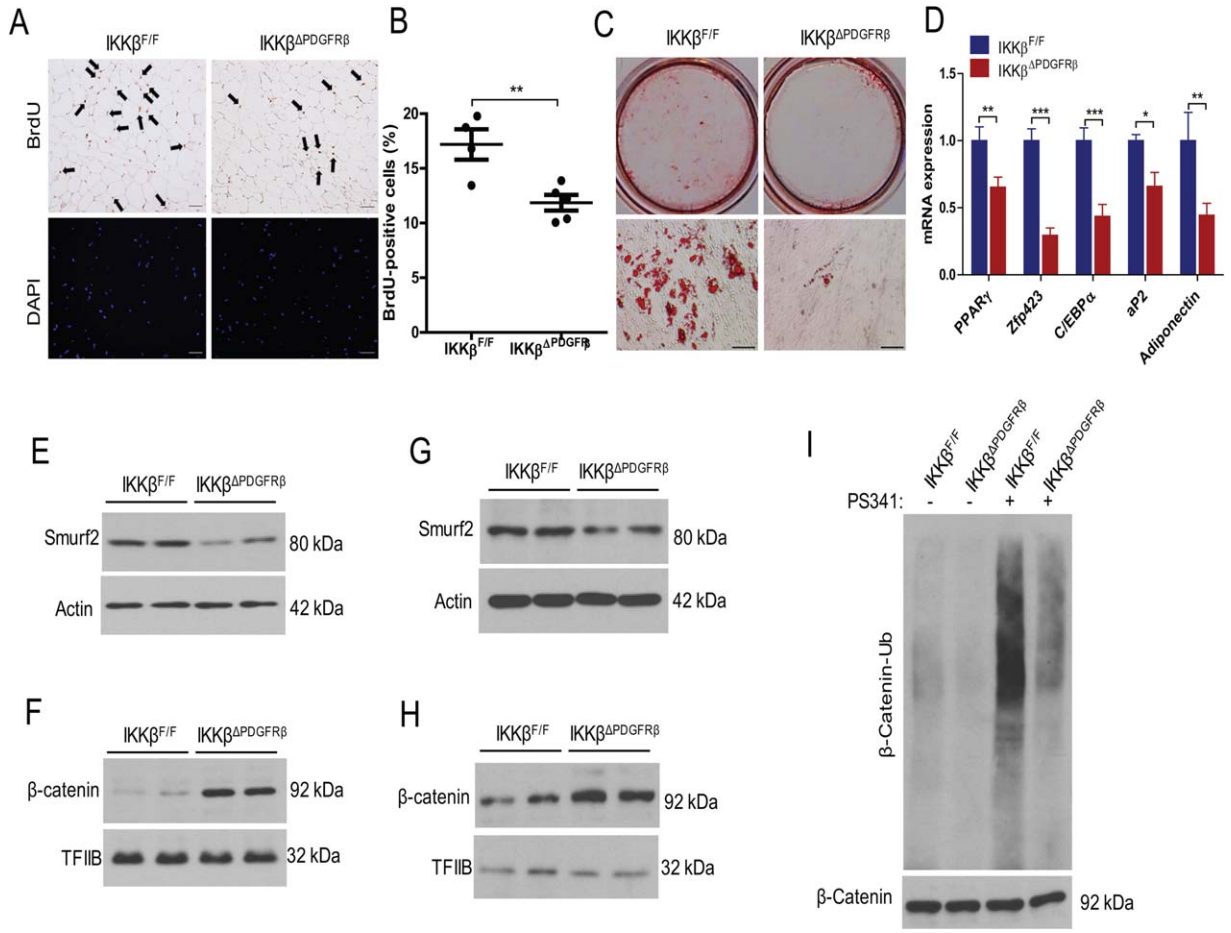

Figure 6. Deficiency of IKKβ inhibits adipogenesis in mice. **(A, B)**: Representative images (A) and quantification (B) of immunostaining for BrdU in epiWAT from BrdU-treated IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice fed a high-fat diet for 7 days ($n = 4$-5; $^{**}p < .01$, significance assessed by Student's *t* test). The nuclei were stained with DAPI (blue) and the BrdU-positive cells are indicated by arrows. **(C)**: Oil-red-O staining of adipose stromal vascular (SV) cells isolated from epiWAT of IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice induced by differentiation media. (scale bar = 100μm, bottom panels). **(D)**: The expression levels of adipogenic genes and adipocyte markers in adipose SV cells of IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice were measured by QPCR ($n = 5$-8; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, assessed by Student's *t* test). **(E, F)**: Western blot analysis of Smurf2 protein levels (E) and nuclear β-catenin protein levels (F) in epiWAT of IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice. **(G, H)**: Western blot analysis of Smurf2 protein levels (G) and nuclear β-catenin protein levels (H) of adipose SV cells isolated from IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice. **(I)**: Adipose SV cells isolated from IKKβ$^{F/F}$ and IKKβ$^{\Delta PDGFR\beta}$ mice were treated with vehicle control or 100 nM PS-341, as indicated, for 4 hours. β-catenin was immunoprecipitated with anti-β-catenin antibodies and then probed with anti-ubiquitin monoclonal antibodies. The whole cell lysates were probed with anti-β-catenin antibodies as an internal control. Results are presented as means ± SEM. Abbreviations: BrdU, Bromodeoxyuridine; IKKβ, IκB kinase β; Smurf2, Smad ubiquitination regulatory factor 2.

in the regulation of hematopoietic cell development and stem cell differentiation [36, 55–57]. It has also been reported that NF-κB activity increases during adipocyte differentiation [58] and IKKβ/NF-κB activation is associated with increased adipogenesis and insulin resistance in maternal obesity [59]. Further, pro-inflammatory signals have been demonstrated to be important for adipogenesis in vivo and many IKKβ/NF-κB activators including LPS, IL-1β, and MCP-1 can stimulate adipogenesis and promote adipocyte differentiation [60–62]. While the role of IKKβ signaling in regulating HFD-elicited tissue inflammation and insulin resistance is well recognized [10, 13, 14], it remained elusive if activation of IKKβ also mediates adipogenesis and adipose tissue growth in response to overnutrition. Since ASOs affects many cell types, we then selectively deleted IKKβ in white adipose lineage in mice and confirmed that deficiency of IKKβ in adipocyte lineage cells decreased adipogenesis and systemic inflammation elicited by HF feeding, leading to resistance to diet-induced obesity and insulin resistance.

Intriguingly, IKKβ$^{\Delta PDGFR\beta}$ mice displayed no significant phenotype under standard laboratory conditions but were resistant to obesity when challenged with a HFD, indicating that HFD-induced IKKβ activation in adipose progenitors is essential for HFD-induced adipose tissue growth. Consistent with our previous report [8], deficiency of IKKβ significantly decreased Smurf 2 expression and substantially inhibited β-catenin ubiquitination in adipose SV cells, leading to accumulation of nuclear β-catenin and increased β-catenin activity. Wnt/β-catenin signaling plays an important role in the regulation of MSC lineage and has been well defined to inhibit adipogenesis in vitro and in vivo [37, 38, 63]. Consistently, Cre-mediated IKKβ deletion or ASO-mediated IKKβ knockdown impaired the adipogenic potential of adipose SV cells in mice. We also demonstrated, for the first time, that inhibition of IKKβ activity can also block human adipose stem cells differentiation, suggesting that IKKβ plays an important role in regulation of both murine and human adipocyte differentiation.

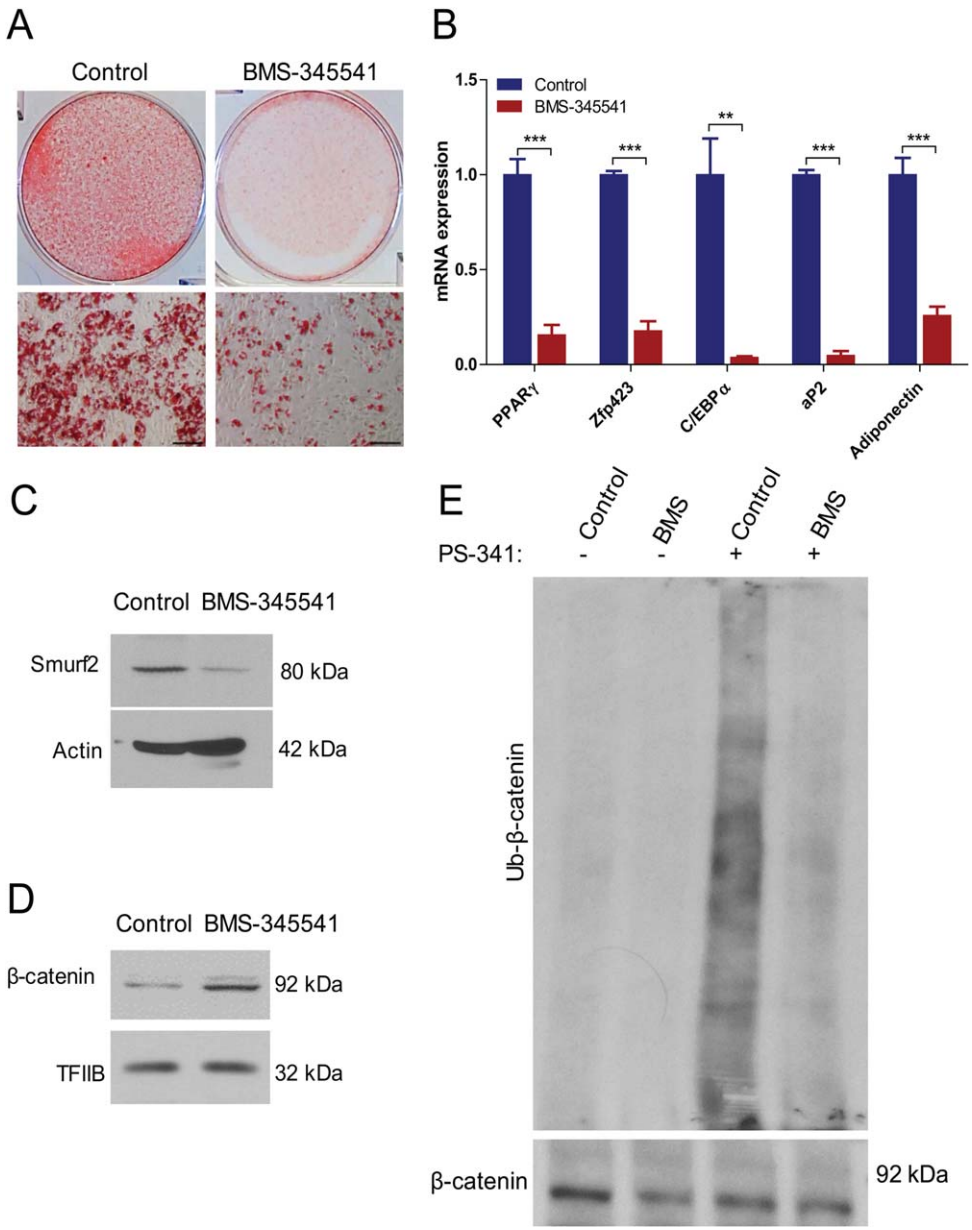

Figure 7. Inhibition of IKKβ decreases adipogenesis in human adipose stem cells. **(A)**: Oil-red-O staining of adult-derived human adipose stem cells induced by differentiation media or media containing 5 μM IKKβ inhibitor BMS-345541 (scale bar = 100μm, bottom panels). **(B)**: The expression levels of adipogenic genes and adipocyte markers of human adipose stem cells treated with vehicle control or 5 μM BMS-345541 were measured by QPCR ($n = 4$; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, assessed by Student's t test). **(C, D)**: Western blot analysis of Smurf2 protein levels (C) and nuclear β-catenin protein levels (D) in control or BMS-345541-treated human adipose stem cells. **(E)**: Control or BMS-345541-treated human adipose stem cells were treated with vehicle control or 100 nM PS-341, as indicated, for 4 hours. β-catenin was immunoprecipitated with anti-β-catenin antibodies and then probed with anti-ubiquitin monoclonal antibodies. The whole cell lysates were probed with anti-β-catenin antibodies as an internal control. $^{**}P < 0.01$, $^{***}P < 0.001$. Results are presented as means ± SEM. Abbreviations: IKKβ, IκB kinase β; Smurf2, Smad ubiquitination regulatory factor 2.

It is worth noting that IKKβ$^{\Delta PDGFR\beta}$ mice had decreased visceral adipose tissue but comparable subcutaneous adipose tissue as control mice when fed a HFD. There is a major ontogenetic difference between visceral and subcutaneous fat as they have different developmental origins [64]. Wang et al. [46] demonstrated that different fat depots have extensive differences in adipogenic potential. Visceral adipose tissue has a high capacity for adipogenesis in vivo but subcutaneous adipogenesis is limited [46]. A recent study also confirmed that HF feeding rapidly and specifically activates adipogenesis in visceral but not subcutaneous depots in mice [65]. Further, subcutaneous fat depot can also undergo extensive browning process after cold exposure and the appearance brown-like cells or beige cells are mainly found in subcutaneous fat but not in visceral fat [46]. It has been recently demonstrated that the PDGFRβ-positive adipocyte lineage cells contribute to beige adipogenesis after prolonged cold exposure [45]. While deficiency of IKKβ did not affect the beige or brown adipocyte marker expression or mitochondrial content in subWAT of IKKβ$^{\Delta PDGFR\beta}$ mice after HF feeding, it would be of interest to investigate whether IKKβ$^{\Delta PDGFR\beta}$ mice have affected beige

adipocyte formation or mitochondrial function after chronic cold exposure in the future. Consistent with previous studies demonstrating that human visceral adipose tissue expresses high levels of IKKβ as compared with subcutaneous adipose tissue [66], we also found that IKKβ expression levels were significantly higher in murine visceral SV cells than in murine subcutaneous SV cells. Moreover, the expression levels of adipose progenitor markers including PDGFRβ were much higher in visceral SV cells than in subcutaneous SV cells. It is plausible that visceral adipose tissue has more abundant adipose progenitor population and activation of IKKβ signaling is required for these cells differentiating into adipocytes in response to HF feeding. Deletion of IKKβ in adipocyte lineage cells therefore has more impact on visceral adipose tissue growth than that of subcutaneous adipose tissue when challenged with a HFD. Further studies will be required to determine the detailed mechanisms that account for the different effects of IKKβ deficiency on visceral versus subcutaneous fat. While both subcutaneous and visceral fat are increased in obese mice and human, accumulation of visceral but not subcutaneous adipose tissue contributes to the increased risk of obesity-associated metabolic dysfunctions [67]. Our data suggest that targeting IKKβ in adipocyte lineage cells may represent a novel therapeutic approach to reduce visceral adipose tissue mass in obesity.

Conclusion

Our study revealed a pivotal role of IKKβ in the regulation of adipocyte differentiation and adipose tissue growth in obesity. ASO-mediated IKKβ knockdown protected mice from diet-induced obesity and metabolic dysfunctions. Deficiency of IKKβ in adipocyte lineage cells also inhibited HF feeding-elicited adipocyte differentiation and adipose tissue growth, leading to resistance to obesity and insulin resistance. Our findings suggest that overnutrition-induced IKKβ activation in adipose progenitors is an important trigger for adipocyte differentiation and IKKβ antisense inhibition may represent as a novel therapeutic approach to combat obesity and related metabolic dysfunctions.

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Author Contributions

R.N.H.: Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript. Y.S., S.-H.P., and Z.L.: Collection and/or assembly of data, final approval of manuscript. R.G.L.: Provision of study material or patients, final approval of manuscript. B.Z.: Provision of study material or patients, final approval of manuscript. P.A.K.: Provision of study material or patients, final approval of manuscript. C.Z.: Conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

R.G.L. is an employee at Ionis Pharmaceuticals. P.A.K. received research funding from Kindex Pharmaceuticals. The other authors indicate no potential conflicts interest.

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