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Cidea controls lipid droplet fusion and lipid storage in brown and white adipose tissue

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Excess lipid storage in adipose tissue results in the development of obesity and other metabolic disorders including diabetes, fatty liver and cardiovascular diseases. The lipid droplet (LD) is an important subcellular organelle responsible for lipid storage. We previously observed that Fsp27, a member of the CIDE family proteins, is localized to LD-contact sites and promotes atypical LD fusion and growth. Cidea, a close homolog of Fsp27, is expressed at high levels in brown adipose tissue. However, the exact role of Cidea in promoting LD fusion and lipid storage in adipose tissue remains unknown. Here, we expressed Cidea in Fsp27-knockdown adipocytes and observed that Cidea has similar activity to Fsp27 in promoting lipid storage and LD fusion and growth. Next, we generated *Cidea* and *Fsp27* double-deficient mice and observed that these animals had drastically reduced adipose tissue mass and a strong lean phenotype. In addition, *Cidea/Fsp27* double-deficient mice had improved insulin sensitivity and were intolerant to cold. Furthermore, we observed that the brown and white adipose tissues of *Cidea/Fsp27* double-deficient mice had significantly reduced lipid storage and contained smaller LDs compared to those of *Cidea* or *Fsp27* single deficient mice. Overall, these data reveal an important role of Cidea in controlling lipid droplet fusion, lipid storage in brown and white adipose tissue, and the development of obesity.

Cidea, Fsp27, brown adipose tissue, white adipose tissue, lipid droplet fusion

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The amount of lipid storage in white adipose tissue (WAT) is controlled by multiple metabolic pathways, and excessive lipid storage has been linked to the development of various diseases including obesity, insulin resistance, fatty liver formation and cardiovascular disease [1,2]. The lipid droplet (LD), a subcellular organelle containing a phospholipid monolayer structure and neutral lipid core, is the primary site for lipid storage and plays a crucial role in controlling lipid homeostasis [3−6]. LD growth from a small to a large LD in the WAT is the most important step in the course of obesity development.

Fsp27 is a member of the CIDE family that is expressed at high levels in WAT and at moderate levels in brown adipose tissue (BAT) [7]. Fsp27 is enriched at the LD contact site (LDCS), it promotes lipid exchange among contacted

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LDs and the fusion of smaller LDs to form a large LD [8]. Fsp27 also cooperates with Perilipin1, another adipocyte-specific LD-associated protein to promote LD growth and unilocular LD formation in white adipocytes [9]. In addition, animals with an *Fsp27* deficiency have a markedly reduced adiposity index and a lean phenotype in addition to "browning" WAT that accumulates multiple small LDs and possesses increased mitochondrial activity [7,10].

Cidea, a close homolog of Fsp27 [11], is highly expressed in the BAT of animals fed a normal diet [12]. Specific and high levels of Cidea expression are also detected in mammary gland during pregnancy and lactation where the protein controls lipid secretion and newborn survival [13]. The higher expression of both Fsp27 and Cidea is also observed in the livers of mice that are fed a high fat diet (HFD) and in *leptin*-deficient (*ob/ob*) obese animals [14,15]. We have previously observed that *Cidea*-deficient mice have mildly reduced fat accumulation under HFD feeding conditions and slightly increased whole body energy expenditure [12]. Brown adipocytes from *Cidea*-deficient animals exposed to cold or at old age (9 months old) have decreased lipid storage, increased lipolysis and the accumulation of smaller LDs [12]. However, the reduction of LD sizes and lipid storage in the BAT of *Cidea*-deficient mice is not observed in animals fed a normal diet at a young age. In addition, the BAT of *Fsp27*-deficient mice contains larger LDs and has mildly increased cellular TAG levels [7]. These data raise the question of whether Cidea has similar functions as Fsp27 in controlling LD fusion and lipid storage in adipose tissues.

Here, we introduced Cidea into Fsp27-knockdown adipocytes and observed that Cidea promotes LD fusion and restores LD growth in Fsp27-knockdown adipocytes. Furthermore, we generated *Cidea/Fsp27* double-deficient mice and systematically evaluated the role of Cidea in controlling animal adiposity, lipid storage in BAT and WAT in comparison to the single deficiency animals. Our data reveal that Cidea is a crucial regulator in controlling the development of obesity by modulating LD fusion and lipid storage in BAT and WAT.

1 Materials and methods

1.1 Plasmid and siRNA

Full length cDNAs encoding Cidea and Fsp27 were cloned into the pEGFP-N1 vector as previously described [8]. siRNA-resistant Fsp27-GFP was generated using the PCR-based site-directed mutagenesis method (Transgen, China). The accuracy of each plasmid DNA was verified by sequencing analysis. The siRNA sequence for Fsp27 is aatcgtggagacagaagaata.

1.2 Cell culture

Preadipocytes (3T3-L1) were cultured in DMEM (Invitrogen, USA) containing 10% FBS (Invitrogen, USA) and induced to differentiate into mature adipocytes as previously described [7]. At day 6 after differentiation, adipocytes were digested with trypsin and electroporated with siRNA and plasmid using the Amaxa Nucleofector II electroporation device (Lonza, Germany) with the A-033 program according to the manufacturer's instructions.

1.3 Lipid droplets

The LD imaging methods, and methods used to measure the diameter of the largest lipid droplet and the lipid exchange rate between contacted LDs were similar to previously described ones [8,9].

1.4 Mouse handling

All of the mice used in the studies were male. The methods utilized in adiposity index, hematoxylin and eosin staining (H&E), electron microscopy (EM), food intake measurement and core body temperature experiments were essentially the same as previously described [7,12]. The TAG content of the BAT and WAT were measured as previously described [15]. Mouse experiments were performed in the animal facility of the Center of Biomedical Analysis at Tsinghua University (Beijing, China). Mouse handling procedures were in accordance with the Responsible Care and Use of Laboratory Animals guideline set by Tsinghua University.

1.5 Western blot

Tissues or cultured cells for Western blot analysis were lysed as previously described [7]. Antibodies against Cidea and Fsp27 were generated as previously described [7,16]. Antibodies against GFP (Santa Cruz Biotechnology, USA), actin (Sigma, USA), cytochrome c (Pharmingen, USA), Cox4 (Molecular Probes, USA), CPT1 (Alpha Diagnostic, USA) and CPT2 (Alpha Diagostic, USA) were used for Western blot analysis.

1.6 Measurement of the levels of TAG, NEFA, glucose and insulin serum

TAG and NEFA serum levels were measured using the triglyceride (Sigma, USA) and FFA kits (Wako, Japan), respectively. The serum insulin level was measured with the insulin ELISA kit (Millipore, USA). The blood glucose level was measured using a Roche blood glucose monitoring system (ACCU-CHEK Advantage II, Roche, Switzerland).

1.7 GTT and ITT

For the glucose tolerance tests, the mice were deprived of food for 16 h and were then injected with glucose (2 g glu-

cose kg^{-1} body weight) through intraperitoneal injection. For the insulin tolerance tests, the mice were injected with recombinant human insulin (0.5 U kg^{-1} body weight, Sigma, USA) after 4 h of fasting. Blood from the eye area was collected at the indicated time and the glucose level was measured using a Roche blood glucose monitoring system (ACCU-CHEK Advantage II, Roche, Switzerland).

1.8 Statistical analysis

All statistical analyses were performed using GraphPad Prism v 4.0 software. The data are expressed as mean±SEM or the mean±SD, as indicated in the figure legends, and the *P* value was calculated using the non-parametric Student's *t*-test.

2 Results

2.1 Cidea promotes LD fusion and restores Fsp27 activity in white adipocytes

To evaluate the role of Cidea in controlling LD fusion and growth in adipocytes, we introduced plasmid expressing Cidea or Fsp27 (siRNA-resistant) together with siRNA against Fsp27 into 3T3-L1 adipocytes by electroporation. Fsp27 depletion in 3T3-L1 adipocytes resulted in the accumulation of large amount of smaller LDs and drastically reduced the lipid exchange rate among contacted LDs (Figure 1A–D). The reintroduction of Fsp27-GFP into the Fsp27-knockdown adipocytes restored the ability of these cells to store lipids because the LD sizes were markedly increased and the lipid exchange activity among contacted LDs was similar to that of wild-type cells (Figure 1A–D).

When Cidea-GFP was introduced into the Fsp27-knockdown adipocytes, the GFP fluorescent signal was clearly detected and enriched at the LDCS (Figure 1B). Fsp27-knockdown adipocytes expressing Cidea also accumulated significantly larger LDs, with the size and distribution of LDs similar to those in wild-type adipocytes and Fsp27-knockdown adipocytes expressing Fsp27 (Figure 1B and C). In addition, the lipid exchange rate was significantly increased in Fsp27-knockdown adipocytes expressing Cidea (Figure 1D). These data indicate that Cidea acts similarly to Fsp27 in promoting LD fusion and growth in mature adipocytes.

2.2 Markedly reduced adiposity and the strong lean phenotype in *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice

To evaluate the physiological role of Cidea in controlling adipose tissue mass and the development of obesity, we generated *Cidea/Fsp27* double-deficient (*Cidea*$^{-/-}$/*Fsp27*$^{-/-}$) mice, and the fat tissues from various anatomical locations were dissected. As shown in Figure 2A, the abdominal region of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice contained a very low amount of fat tissue compared to that of wild-type mice. Further

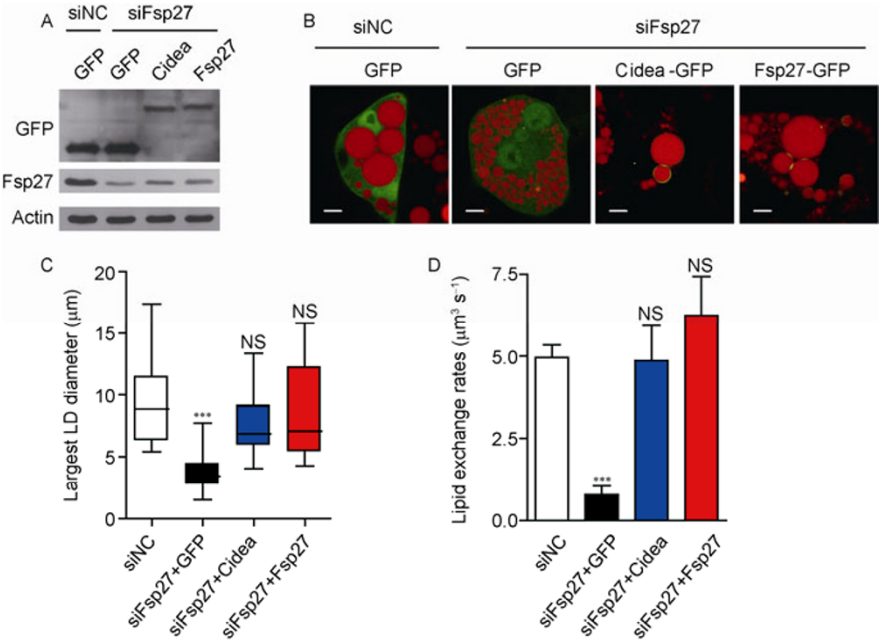

Figure 1 Cidea promotes lipid droplet fusion in Fsp27-knockdown adipocytes. A, Western blot showing the expression level of Fsp27-GFP and Cidea-GFP in Fsp27-knockdown adipocytes. B, Representative images showing that Cidea and Fsp27 promote the formation of larger LDs in Fsp27-knockdown adipocytes. GFP, Cidea-GFP and Fsp27-GFP (siRNA-resistant) together with siRNA against Fsp27 were transfected into adipocytes by electroporation. Scale bar, 10 μm. The LDs were labeled with Bodipy 558/568 C12 fatty acid dye (BODIPY-FA, red). C, Quantitative analysis of the diameter of the largest LDs in B (mean±SD; $n \geqslant 30$ for each group). D, Cidea and Fsp27 restore the lipid exchange rate in Fsp27-knockdown adipocytes (mean±SEM; $n \geqslant 8$ for each group). ***, $P<0.001$; NS, no significant difference.

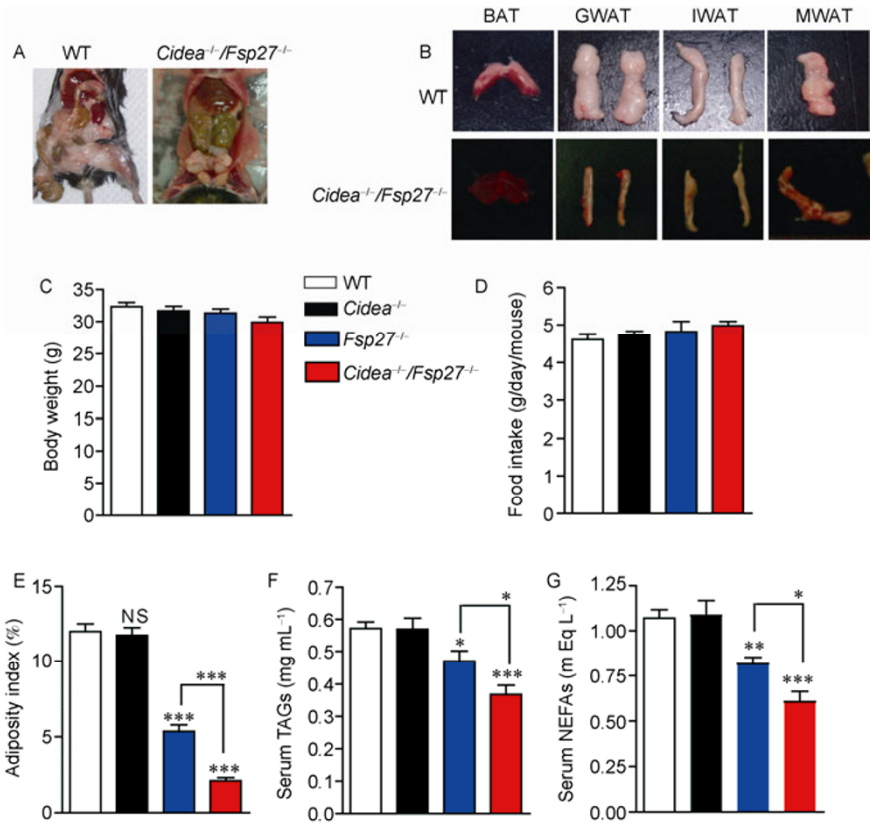

Figure 2 Lean phenotype of the *Cidea* and *Fsp27* double-deficient mice. A, A representative photograph of the abdominal region of dissected 3-month-old wild-type (WT) and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. B, The broad loss of adipose tissue mass in the interscapular brown adipose tissue (BAT), the gonadal white adipose tissue (GWAT), the inguinal white adipose tissue (IWAT) and the mesenteric white adipose tissue (MWAT) in 3-month-old *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. Body weight (C), Food intake (D), Adiposity index (E), Serum TAGs (F) and NEFAs levels (G) of 3-month-old WT, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. In total, 5 mice were used in this experiment. The data are the mean±SEM.*, P<0.05; ***, P<0.001.

analysis indicated that the fat pads from all anatomical locations (BAT, gonadal fat, inguinal fat, and mesenteric fat) were markedly smaller compared to those in wild-type mice (Figure 2B). In contrast to the reduced body fat, the total body weight and food intake were similar between wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ animals (Figure 2C and D).

Quantitative analysis of the adiposity index showed that the *Cidea* deficiency did not affect the adiposity index of 3-month-old mice (Figure 2E). Consistently, plasma levels of TAG and NFFA were similar between wild-type and *Cidea*$^{-/-}$ mice (Figure 2F and G). In contrast, the animals with an *Fsp27* deficiency had an approximately 55% reduction in adiposity, however, *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ animals had a further reduction of adiposity (2.1% for *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ vs 5.4% for *Fsp27*$^{-/-}$ mice, 12% for wild-type and 11.8% for *Cidea*$^{-/-}$ mice). Plasma levels of TAG and NEFA in *Fsp27*$^{-/-}$ mice were lower compared to wild-type and *Cidea*$^{-/-}$ mice (Figure 2F and G). A further reduction in TAG and NFFA plasma levels was observed in *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice (Figure 2F and G). Overall, these data clearly indicate that animals with a deficiency in both *Cidea* and *Fsp27* have a more severe phenotype compared to animals with a single deficiency as shown by their drastically reduced fat accumulation and strong lean phenotype.

2.3 *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice have improved insulin sensitivity and are intolerant to cold

As reduced plasma levels of TAG and NFFA and the lean phenotype often result in an improved insulin sensitivity; we next measured the plasma levels of glucose and insulin in wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice under fed and fasting conditions. The *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice had reduced plasma levels of insulin under fed condition (Figure 3A). The insulin plasma levels under fasting conditions, and the glucose plasma levels under both fed and fasting conditions were similar among these four types of mice (Figure 3A and B). Next, we systematically measured the rate of glucose disposal and insulin sensitivity in wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice using the glucose tolerance test (GTT) and insulin tolerance test (ITT). Consistent with previous observations [7], the *Fsp27*$^{-/-}$ mice had significantly reduced blood glucose lev-

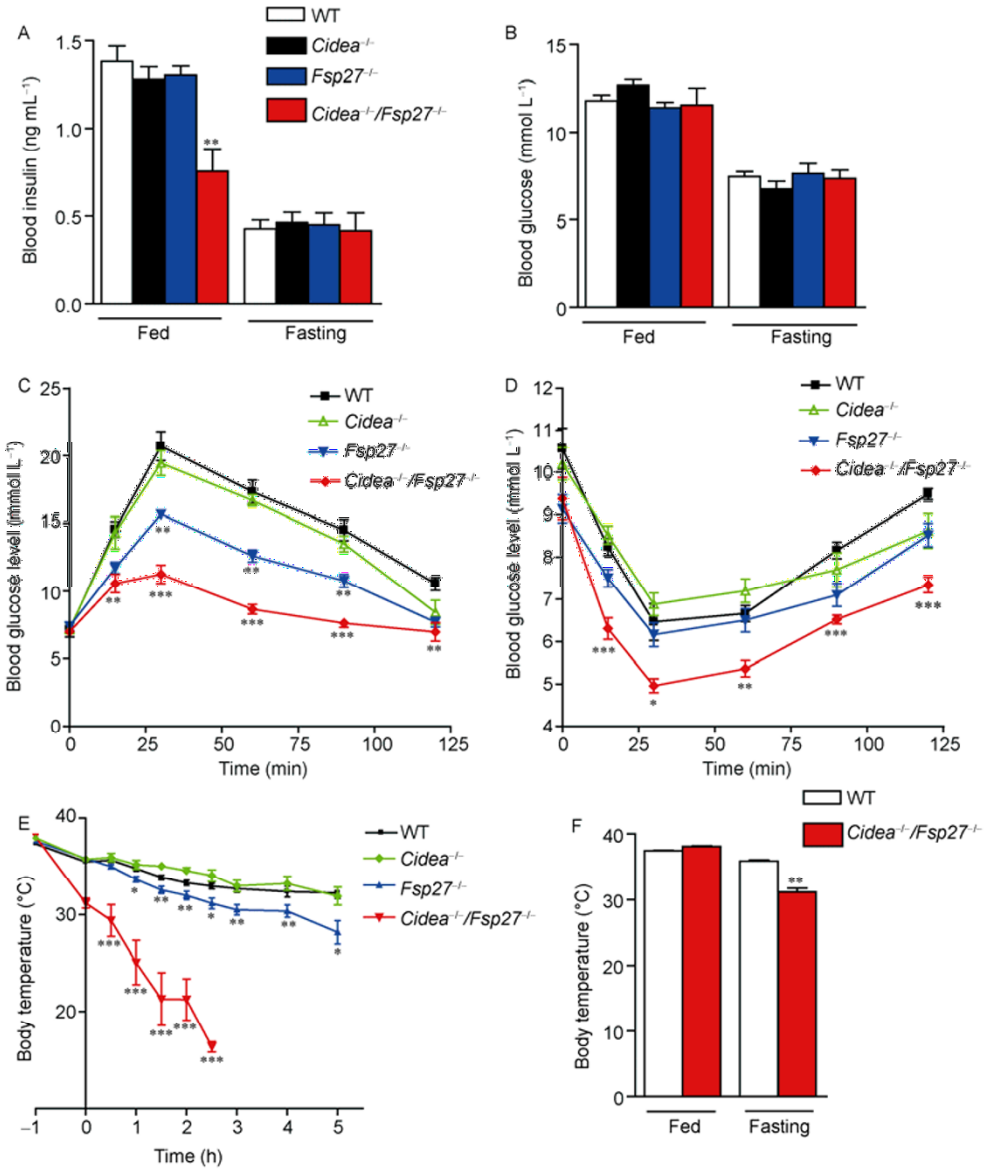

Figure 3 Increased insulin sensitivity in *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. Blood insulin (A) and glucose levels (B) of 3-month-old wild-type (WT), *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice under fed and fasting conditions. In total, 7 mice from each group were used. GTT (C) and ITT (D) experiments using 3-month-old WT, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. In total, 5 mice were used in this experiment. E, Core body temperature of WT, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice after the animals were exposed to cold (4°C). In total, 10 WT, 7 *Cidea*$^{-/-}$, 10 *Fsp27*$^{-/-}$ and 9 *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice were used for this experiment. F, Body temperature of WT and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice under fed or fasting (16 hr) conditions. In total, 14 WT and 9 *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice were used for this experiment. The data are the mean±SEM. *, P<0.05; **, P<0.01; ***, P<0.001.

els after the administration of the exogenous load of glucose compared to the wild-type mice (Figure 3C). The rate of glucose disposal was further increased in the *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice compared to that in the *Fsp27*$^{-/-}$ mice using the GTT tests (Figure 3C). When excessive amounts of insulin was administered in the ITT, the wild-type, *Cidea*$^{-/-}$ and *Fsp27*$^{-/-}$ mice had similar levels of blood glucose; however, the blood glucose levels were significantly reduced in *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice (Figure 3D). These data suggest that the *Cidea/Fsp27* double-deficient mice have improved insulin sensitivity compared to wild-type and *Cidea* or *Fsp27* single-deficient mice.

To evaluate whether reduced fat accumulation in animals affects their ability to adapt to the cold environment, we exposed wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice to a cold (4°C) environment and measured their core body temperature. Upon exposure to the cold, the *Cidea*$^{-/-}$ mice had a slightly increased core body temperature compared to the wild-type mice (Figure 3E). Consistent with our previous observations [7], the *Fsp27*$^{-/-}$ mice had significantly lower body temperatures compared to wild-type and *Cidea*$^{-/-}$ mice when exposed to cold because their body temperatures dropped to lower than 30°C 5 h after cold exposure (Figure 3E). The *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice showed little tolerance for cold as shown by their core body temperature dropping from 37°C to 30°C within 1 h after cold ex-

posure. Two and a half hours after cold exposure, the core body temperature in *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice was approximately 10°C and was not able to survive further (Figure 3E). Under fasting conditions, the *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice also had a reduced core body temperature compared to wild-type mice (Figure 3F). Therefore, mice deficient in both *Cidea* and *Fsp27* are severely intolerant to cold.

2.4 Dramatically reduced lipid storage in the BAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice

BAT is the only tissue that expresses both Cidea and Fsp27 in adult mice fed a normal diet (ND); therefore, we systematically analyzed the BAT phenotype in animals with a single deficiency in *Cidea* or *Fsp27* or double deficiencies in both genes. The Cidea expression levels in the BAT of *Fsp27*$^{-/-}$ mice and the Fsp27 levels in the BAT of *Cidea*$^{-/-}$ mice were similar to those of wild-type mice, indicating no compensatory increase in the expression levels of these proteins (Figure 4A). The BAT weight and its total amount of TAG in wild-type and *Cidea*$^{-/-}$ mice were similar (Figure 4B and C). However, the weight and total amount of cellular TAG in the BAT of the *Fsp27*$^{-/-}$ mice were significantly higher compared to those of wild-type and *Cidea*$^{-/-}$ mice

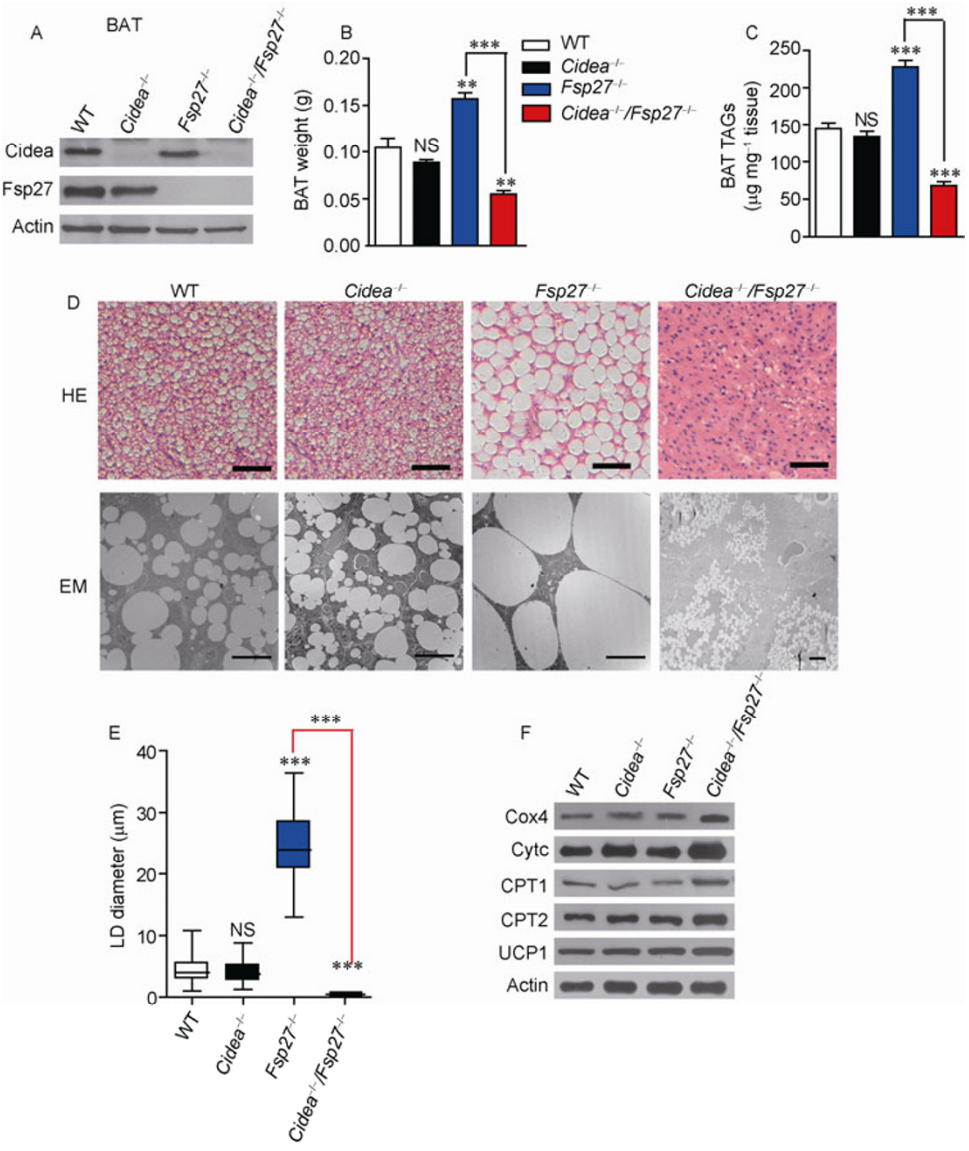

Figure 4 Reduced lipid storage in the BAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. A, Representative Western blot of Cidea and Fsp27 in the BAT of wild-type (WT), *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. Actin was used as the loading control. BAT weight (B) and TAG content (C) of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. In total, 5 mice were used in this experiment. D, Morphology of BAT of WT, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. HE, hematoxylin and eosin staining. EM, electron microscope image. 3-month-old mice were used for the experiment. Scale bar=50 μm for HE and 10 μm for EM. E, The average LD diameter in the brown adipocytes of WT, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$*Fsp27*$^{-/-}$ mice. The diameter of LDs in 5 brown adipocytes of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice was measured. F, Western blot analysis of various mitochondrial proteins in the BAT of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. Cytc, cytochrome c. Actin was used as the loading control. The data are the mean±SEM.*, P<0.05; **, P<0.01; ***P, <0.001.

(Figure 4B and C), which is consistent with our previous observations [7]. In contrast, the BAT weight and the total amount of TAG in the BAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice were dramatically decreased compared to those of the other three types of mice (Figure 4B and C).

BAT morphology was further analyzed by hematoxylin and eosin staining (H&E) and electron microscopy (EM). As shown in Figure 4D and E, the BAT in the wild-type and *Cidea*$^{-/-}$ mice contained similar sized LDs. Consistent with the higher tissue weight and cellular TAG content in the BAT of *Fsp27*$^{-/-}$ mice, the *Fsp27*$^{-/-}$ brown adipocytes contained larger LDs compared to the wild-type and *Cidea*$^{-/-}$ mice (Figure 4D). The average diameter of the LDs in *Fsp27*$^{-/-}$ brown adipocytes was 24.37±1.952 μm, which was dramatically higher than that in the wild-type brown adipocytes (4.52±0.338 μm, P<0.0001, Figure 4E). The LD sizes of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ brown adipocytes were smaller compared to those of the other three types of mice (Figure 4D), with an average diameter of 0.59±0.028 μm (Figure 4E). The expression levels of mitochondrial proteins, including UCP1, Cox4, cytochrome c, CPT1 and CPT2 in the BAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice were significantly higher than those in the BAT of the other three genotypes (Figure 4F). These data suggest that the BAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice accumulates drastically smaller LDs, has reduced lipid storage and increased mitochondrial activity.

2.5 Reduced lipid storage and the accumulation of markedly smaller LDs in the WAT of the *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice

Although Cidea is not detected in the WAT of the wild-type mice fed a normal diet, its protein was detected in the gonadal WAT (GWAT) of *Fsp27*$^{-/-}$ mice [7] (Figure 5A). However, the Fsp27 protein levels were similar in the GWAT of the wild-type and *Cidea*$^{-/-}$ mice (Figure 5A). To evaluate the role of Cidea in regulating the LD sizes and lipid storage in the WAT, we measured the tissue weight and cellular TAG content in the GWAT of all four genotypes. The tissue weight and cellular TAG levels in *Cidea*$^{-/-}$ mice were similar to those in wild-type mice (Figure 5B and C). *Cidea*$^{-/-}$ white adipocytes also contained a unilocular large LD that was similar in size to that in wild-type cells (Figure 5D and E). In contrast, the tissue weight, the cellular TAG content and the LD sizes in the GWAT of *Fsp27*$^{-/-}$ mice were significantly reduced (Figure 5B–E). Further reductions in tissue weight and the TAG levels were observed in the GWAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice (Figure 5B and C). In addition, the GWAT of *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice contained multilocular LDs (Figure 5D). The average diameter of the LDs in the white adipocytes of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice was 0.609±0.0154 μm, much smaller than that in *Fsp27*$^{-/-}$ white adipocytes (2.399±0.211 μm, P<0.0001, Figure 5E). The expression levels of Perilipin1 and Perilipin 2 were increased in the GWAT of *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice when compared to those of wild-type and *Cidea*$^{-/-}$ mice (Figure 5F). The expression level of mitochondrial proteins UCP1, Cox4, cytochrome c, CPT1 and CPT2 were higher in the GWAT of *Fsp27*$^{-/-}$ mice when compared to that in wild-type and *Cidea*$^{-/-}$ mice (Figure 5F). A further increase in the protein levels of Cox4, cytochrome c, CPT1 and CPT2 were observed in the GWAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice (Figure 5F). Overall, these data suggest that Cidea contributes to lipid storage in the WAT because *Cidea/Fsp27* double deficiencies resulted in a further reduction of lipid storage and the accumulation of markedly smaller LDs.

3 Discussion

Excess lipid storage in adipose tissue has been linked to the development of obesity and other metabolic disorders. LD fusion and growth play crucial roles in controlling lipid storage and obesity development. Here, we showed that Cidea promotes LD fusion and growth in adipocytes. Our results using *Fsp27* and *Cidea* double-deficient mice indicate that these two proteins act in concert to control lipid storage, insulin sensitivity and energy homeostasis by regulating LD fusion and growth in the BAT and WAT.

By introducing Cidea into differentiated 3T3-L1 adipocytes in which Fsp27 is depleted, we observed almost a complete restoration of LD fusion activity evaluated by the lipid exchange activity and the sizes of the LDs (Figure 1). Therefore, Cidea acts similarly to Fsp27 in promoting LD fusion and growth in adipocytes. Its role in controlling lipid storage and the development of obesity *in vivo* is further confirmed in *Cidea/Fsp27* double-deficient mice because these animals have a broad and severe loss of adipose tissue mass from all anatomical locations due to their inability to store lipids. In many animal models, the loss of the ability to store lipids in adipose tissue (a condition of lipodystrophy) often results in ectopic lipid storage and the development of insulin resistance [17]. However, we did not observe increased ectopic lipid storage in the liver and in the blood of *Cidea/Fsp27* double-deficient mice. In addition, *Cidea/Fsp27* double-deficient mice have significantly improved insulin sensitivity. Lack of ectopic lipid storage and increased insulin sensitivity are likely contributed by the increased energy expenditure and mitochondrial activity in both the BAT and WAT because we observed broadly increased expression of mitochondrial proteins in uncoupling, the electron transport chain, oxidative phosphorylation and fatty acid oxidation pathways. Therefore, the depletion of Cidea and Fsp27 converts both the BAT and WAT into more metabolically active organs compared to the single depletion, and results in increased local consumption of

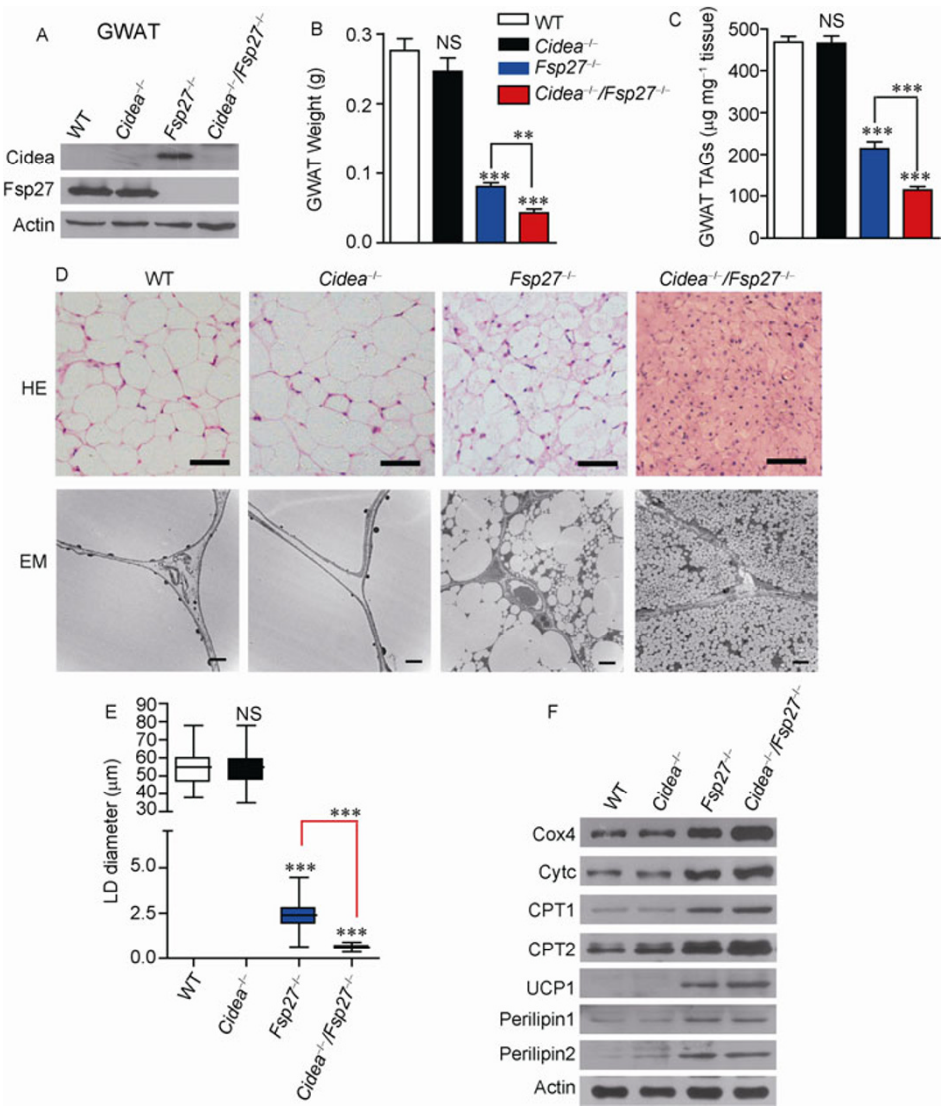

Figure 5 Reduced lipid storage and smaller LDs in the GWAT of *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. A, Western blots showing the levels of Cidea and Fsp27 in the GWAT of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. Actin was used as the loading control. GWAT weight (B) and TAG content (C) of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. In total, 5 mice were used in this experiment. D, Morphology of the GWAT of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. HE, hematoxylin and eosin staining. EM, electron microscope image. Three- month-old mice were used for the experiment. Scale bar=50 μm for HE and 2 μm for EM. E, The LD diameter in the white adipocytes of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. The diameter of the LDs in 5 white adipocytes of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice was measured. F, Western blot analysis of various proteins in the GWAT of wild-type, *Cidea*$^{-/-}$, *Fsp27*$^{-/-}$ and *Cidea*$^{-/-}$/*Fsp27*$^{-/-}$ mice. Cytc, cytochrome c. Actin was used as the loading control. The data are the mean±SEM.*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

lipids and improved insulin sensitivity.

Interestingly, *Cidea*/*Fsp27* double-deficient mice are severely intolerant to cold. Cold intolerance is also observed in the *Fsp27*-deficient mice, albeit with less severity. Reduced lipid storage in the WAT of *Fsp27*-deficient or *Cidea/Fsp27* double-deficient mice may result in lower FFA output and limited substrate supply to the BAT (non-shivering) and the muscles (shivering) that are important for heat production and temperature control upon cold exposure.

BAT is the only tissue that expresses both Cidea and Fsp27. Under normal diet conditions, Fsp27 compensates for Cidea function and no obvious phenotype was observed in the BAT of *Cidea*-deficient mice. Upon cold exposure or due to old age, Cidea plays a dominant and crucial role in controlling LD sizes and lipid storage in the BAT [12]. Intriguingly, brown adipocytes with a single deficiency in *Fsp27* have slightly increased lipid storage and larger LDs without altered Cidea expression; however, the underlying mechanism remains unclear. In several genetically modified lean animal models such as aP2-SREBP1c transgenic mice and aP2-FOXC2 transgenic mice [18–19], decreased lipid storage in the WAT has been coupled with increased lipid storage in the BAT. Potentially, the WAT with reduced li-

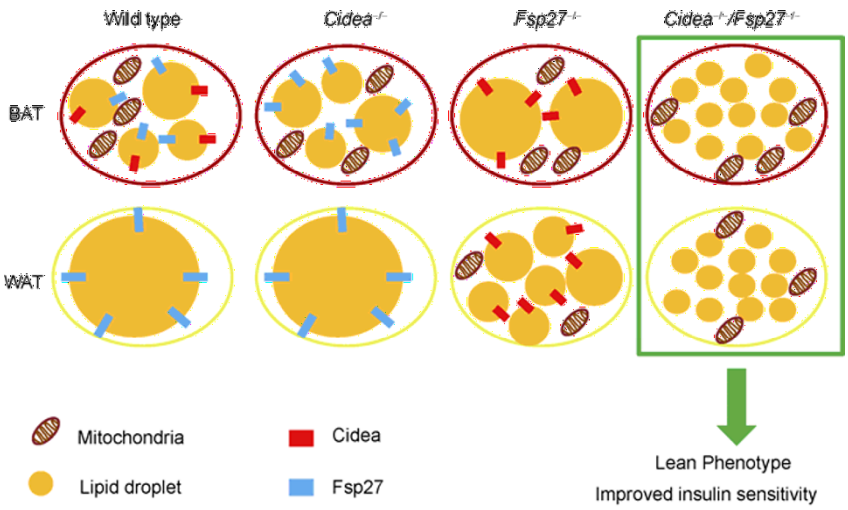

Figure 6 Summary of the role of Cidea and Fsp27 in controlling lipid metabolism in brown adipose tissue (BAT), white adipose tissue (WAT) and whole body insulin sensitivity.

pid storage (in the case of *Fsp27* deficiency) may secrete several unknown factors to inhibit BAT function. The loss of Fsp27 may also result in the upregulation of an activator of Cidea, leading to increased Cidea activity and the formation of larger LDs.

Cidea expression was not detected in the mouse WAT under normal feeding conditions; however, Cidea protein levels in the WAT are significantly elevated in *Fsp27*-deficient mice, most likely by compensating for the loss of Fsp27. The compensatory effect of Cidea in the WAT is validated by *Cidea/Fsp27* double-deficient mice because their white adipocytes contained reduced cellular TAG and accumulated drastically smaller LDs (Figure 6). Therefore, in addition to Fsp27, Cidea also plays a role in promoting lipid storage and LD fusion in the WAT. Interestingly, we observed that the diameters of LDs from brown and white adipocytes of *Cidea/Fsp27* double-deficient mice that lack LD fusion and growth activity are approximately 0.5–0.6 μm, possibly reflecting the general LD sizes before CIDE-mediated LD fusion (Figure 6). Interestingly, both Cidea and Fsp27 are expressed in human fat tissues [20,21]. Cidea and Fsp27 may play important roles in lipid storage in human adipose tissue, thereby contributing to the development of human obesity.

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