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Modulation of Fatty Acid Synthase Degradation by Concerted Action of p38 MAP Kinase, E3 Ligase COP1, and SH2-Tyrosine Phosphatase Shp2*

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Background: FASN is a key enzyme in lipid metabolism, and its overexpression is associated with a variety of human malignancies.
Results: Shp2 promotes FASN ubiquitination by acting as an adapter linking FASN with COP1 and p38.
Conclusion: Shp2 may act as an adaptor in targeting FASN for its degradation.
Significance: This study identifies a new function for Shp2 in lipid metabolism and tumor suppression.

The Src-homology 2 (SH2) domain-containing tyrosine phosphatase Shp2 has been known to regulate various signaling pathways triggered by receptor and cytoplasmic tyrosine kinases. Here we describe a novel function of Shp2 in control of lipid metabolism by mediating degradation of fatty acid synthase (FASN). p38-phosphorylated COP1 accumulates in the cytoplasm and subsequently binds FASN through Shp2 here as an adapter, leading to FASN-Shp2-COP1 complex formation and FASN degradation mediated by ubiquitination pathway. By fasting p38 is activated and stimulates FASN protein degradation in mice. Consistently, the FASN protein levels are dramatically elevated in mouse liver and pancreas in which Shp2/Ptpn11 is selectively deleted. Thus, this study identifies a new activity for Shp2 in lipid metabolism.

Protein-tyrosine phosphatase Shp2, encoded by the human gene *Ptpn11,* is a widely expressed cytoplasmic enzyme containing two Src-homology 2 (SH2)[3] domains at its amino-terminal end and a single catalytic (PTPase) domain (1, 2). Shp2 has been implicated in several signaling pathways, such as Ras-ERK, PI3K-AKT, NF-κB, Stat3, and NFAT, in regulation of multiple cellular activities. Most functions of Shp2 require its catalytic activity, and mutations that lead to constitutive activation of the phosphatase have been implicated in leukemogenesis, thus identifying *PTPN11/Shp2* as the first human proto-oncogene encoding a PTPase (3). In contrast to the leukemogenic effect, more recently Ptpn11/Shp2 has been found a tumor-suppressor function in liver (4). Neuronal Shp2 acts to promote hypothalamic leptin signaling in energy balance (5). However, it is unclear whether this molecule has a direct role in regulation of lipid metabolism.

Fatty acid synthase (FASN) is a key enzyme in fatty acid biosynthesis, which plays an important role in energy homeostasis (6). The expression of FASN is relatively low in normal tissue since fatty acid is generally supplied by diet, but aberrantly elevated in various types of cancer including breast, prostate, colon, ovarian, and esophageal carcinomas (7–10). Interestingly, *de novo* synthesized fatty acids regulate glucose, lipid, and cholesterol metabolism through activation of PPARα in adult liver (11). Prolonged fasting induced fatty liver in liver-specific *FASN* knock-out mice, which is associated with obesity, type 2 diabetes, and insulin resistance (11, 12). It has been suggested a role of isopeptidase USP2a in modulation of FASN stability in prostate cancer cells (13), although the molecular mechanism for control of FASN degradation remains to be elucidated.

Herein we report a new function for Shp2 acting as an adaptor molecule connecting ubiquitin E3 ligase COP1 (also known as RFWD2) with FASN, thereby regulating FASN ubiquitination and degradation. This adapter function of Shp2 does not require its phosphatase activity but engages in the two SH2 domains. Of note, the transcription factor sterol regulatory element-binding protein 1c (SREBP1c), which directs *FASN* gene expression, is activated by PI3K-AKT and ERK signaling pathways (14–16), which are coordinately regulated by Shp2 (1, 2). Therefore, Shp2 acts to modulate FASN expression and degradation via two distinct mechanisms, thus revealing its multifaceted roles in regulation of cellular activities.

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[3] The abbreviations used are: SH2, Src-homology 2; FASN, fatty acid synthase; MEF, mouse embryonic fibroblast.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—All cell lines including human embryonic kidney 293T cells, HeLa cells, and mouse embryonic fibroblasts (MEFs) $p38^{+/+}$ and $p38^{-/-}$, primary MEFs $JNK1^{+/+}$, and $JNK1^{-/-}$, 3T3 $JNK1/2^{+/+}$, and $JNK1/2^{-/-}$ were cultured in DMEM containing 10% FBS. Cell transfections were performed with Lipofectamine reagent (Invitrogen) or FuGENE® HD Transfection Reagent (Roche) according to the manufacturer's instruction.

Plasmids, Antibodies, and Reagents—The different Shp2 plasmids including the wild-type, catalytically inactive C459S, constitutively active D61A and Shp2-ΔSH2 (deletion of amino acids 1–209 containing N-SH2 and C-SH2 domain regions) were subcloned in the vector pEF-HA (17). Similarly, N-SH2 (1–106 aa) or C-SH2 (107–220 aa) region was also cloned into the pEF-HA vector. pCDNA3-HA-COP1 was described previously (18). Antibodies used were Shp2 (B-1 and C-18), FASN (H-300), COP1 (K-16), p38 (C-20), Ub (A-5), and HA (rabbit IgG) from Santa Cruz Biotechnology, p-p38 (28B10, Cell Signaling), β-actin (AC-15, Sigma), anti-HA (mouse IgG, Roche). Anti-phosphothreonine-proline/phosphoserine-proline antibody (anti-pTP/pSP, ab9344) was from Abcam (Hong Kong) Ltd. Alex Flour 488, 568 (goat anti-rabbit or mouse), and Vectashield DAPI were from Invitrogen. The proteasome inhibitors MG132 and LAC, PI3K inhibitor LY294002, ERK inhibitor PD98059, p38 kinase inhibitor SB203580, JNK kinase inhibitor SP600125, and MEK inhibitor U0126 were from Calbiochem. Okadaic acid (an inhibitor of the serine/threonine phosphatase, #5934) was from Cell Signaling. Human Shp2 siRNA (sc-36488), COP1 siRNA (sc-45541), and Scrambled/Control siRNA-A (sc-37007) were from Santa Cruz Biotechnology. We also designed siRNA to target the Shp2 cDNA sequence: Shp2 siRNA, 5′-gcaguuaaauugugcgcugua-3′ and Mismatch siRNA, 5′-gcaguuaaacggugcgcugua-3′, were custom-synthesized commercially. The siRNA transfections were performed according to the siRNA Transfection Protocol from the instructions for Lipofectamine 2000.

Immunoprecipitation and Western Blot—For Western blotting analysis, anti-β-actin was used at a 1:5000 dilution, other primary antibodies were used at a 1:1000 dilution. For determination of protein ubiquitination, cells were lysed in the cell lysis buffer A (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, and protease inhibitors) on ice. Lysates from cell cultures or tissues (500–1000 μg) were pre-cleared by incubation with protein A/G plus-agarose (Santa Cruz Biotechnology) and then incubated with 1–2 μg of anti-Shp2, or anti-FASN or anti-HA(m) and normal rabbit IgG (from Santa Cruz Biotechnology) for 2 h at 4 °C. Protein A/G plus-agarose (30–40 μl) were added into the mixture and incubated with agitation for an additional 4 h at 4 °C. The immunoprecipitated samples were extensively washed with the cell lysis buffer and subjected to the Western blotting analysis.

Immunofluorescence Staining—$p38^{+/+}$ and $p38^{-/-}$ MEFs were directly seeded into the uncoated 35-mm dishes at a density of 1.5×10^5 cells. After 24 h of transfection, cells were washed in PBS once, fixed with 4% freshly prepared formaldehyde in PBS for 8–10 min, and then washed three times with PBS. Cells were permeabilized with 0.1% saponin/PBS for 15 min, blocked in 2.5% NGS (normal goat serum) in 0.1% saponin/PBS for 30 min-24 h, incubated in the primary antibody (FASN, dilution 1:200; Shp2, dilution 1:200; COP1, dilution 1:100) diluted in blocking solution for 1 h, washed three times with blocking solution, and then incubated in the second antibody (Alexa 568 anti-rabbit, or Alexa 488 anti-mouse, dilution 1:500) in blocking solution for 30 min. Then these were washed twice with blocking solution and once with PBS. DAPI was added for DNA staining. Pictures were taken with a Fluoview 1000 Olympus Laser Point Scanning Confocal Microscope.

Mass Spectrometry—To purify the Shp2 complex, lysates generated from 1×10^8 cells were incubated with anti-SYP (rabbit polyclonal Shp2 homemade antibody) or anti-Shp2(C-18) and protein A/G-agarose resins in buffer A. After three washes with the same buffer, immunoprecipitates were resolved by SDS-PAGE. After in-gel digestion with highest grade trypsin, the digest was injected into a high-pressure liquid chromatography instrument (LC Packings Inc.), which first separates the peptides on a reverse-phase column, from which they elute directly into a quadrupole time-of-flight mass spectrometer (Q-TOF API-US) equipped with a nanoelectrospray ionization source (Waters-Micromass), at the core facility of the Sanford/Burnham Medical Research Institute.

RESULTS AND DISCUSSION

Shp2 Interacts with FASN—Shp2 has been implicated in a number of cytoplasmic signaling pathways, although the molecular mechanisms remain to be elucidated. Toward this goal, we resorted to search for Shp2-interacting partners by mass spectrometry analysis of proteins that are coimmunoprecipitated with Shp2. Surprisingly, one of the molecules identified in Shp2-coimmunoprecipitates is FASN in MEF cells (Fig. 1*A*), and human 293T and HeLa cells (data not shown). To verify the Shp2/FASN association, we immunoprecipitated 293T cell lysates with an anti-Shp2 antibody and analyzed precipitated proteins by immunoblotting with a specific antibody against FASN (Fig. 1*B*), which confirmed Shp2/FASN interaction between the two endogenous proteins in cells. We then examined the expression levels of FASN in various mouse tissues, and found that FASN contents were most abundant in liver and adipose tissues, with low levels detected in the heart (Fig. 1*C*). Coimmunoprecipitation assay detected a complex containing Shp2 and FASN in mouse liver and pancreas lysates (Fig. 1*D*). Thus, Shp2 binds FASN *in vitro* and *in vivo*.

Shp2 Negatively Regulates FASN Protein Levels—To explore the biological significance for Shp2 interaction with FASN, we transfected 293T cells with a Shp2 expression construct and examined its effect on FASN protein content. As shown in Fig. 2*A*, with progressively increasing amounts of Shp2, FASN protein levels decreased correspondingly, indicating a negative correlation between Shp2 and FASN expression. To assess whether Shp2 affects FASN stability, we measured the protein amounts at different time points following cycloheximide treatment. The half-life of FASN shown was shortened from 15.4 to 9.7 h in cells transfected with HA-Shp2 (Fig. 2*B*). In contrast, Shp2 knockdown mediated by Shp2-specific siRNA resulted in

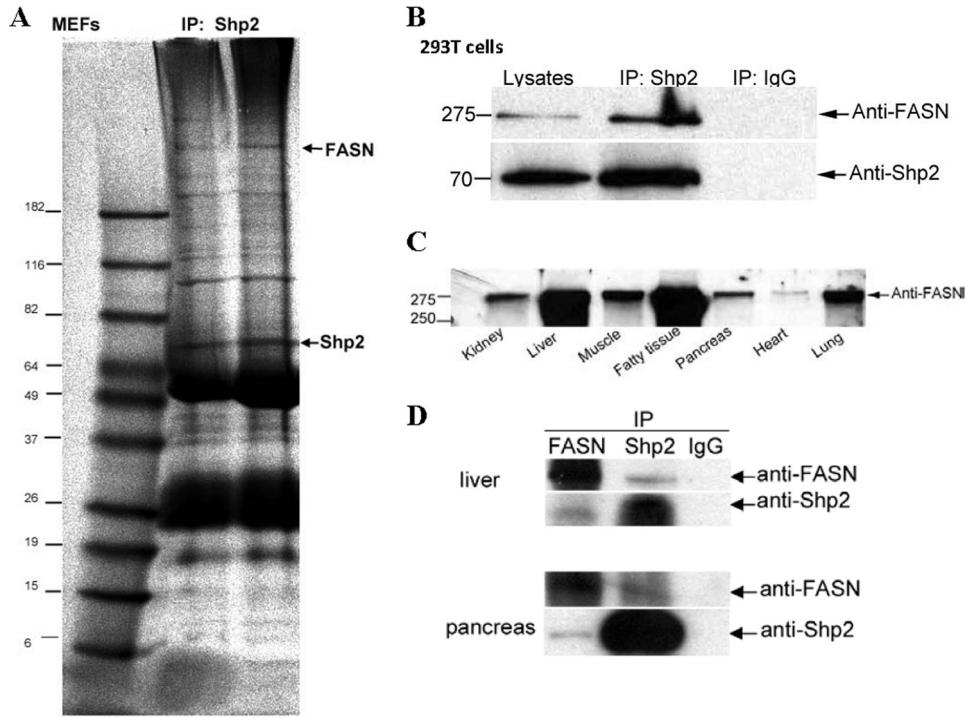

FIGURE 1. **Shp2 binds to FASN.** *A*, MEF cell lysates were immmunoprecipitated with an anti-Shp2 antibody, and co-precipitated proteins were analyzed and identified by mass spectrometry, showing that FASN is one of the Shp2-binding proteins. *B*, 293T lysates were immmunoprecipitated with Shp2 (C-18) antibody and coprecipitated proteins were immunoblotted with FASN or Shp2 (B1) antibodies. *C*, expression levels of FASN protein in different mouse tissues were detected by immunoblotting. *D*, lysates from wild-type mouse liver or pancreas were immunoprecipitated with antibodies to FASN or Shp2, and then detected by immunoblotting.

increased amounts of FASN protein (Fig. 2*C*), indicating a role of Shp2 in facilitating FASN degradation in cells.

In previous experiments, we generated liver-specific and pancreas-specific *Shp2/Ptpn11* knock-out mice, using the cre-loxP system (19, 20). We measured FASN protein levels in these Shp2-deficient mouse organs. Results shown here demonstrate that Shp2 ablation in the liver led to elevated FASN protein levels, as compared with wild-type mouse liver (Fig. 2*D*). Similar results were obtained with conditional knock-out of Shp2 in the pancreas (Fig. 2*E*). H&E staining of pancreatic sections revealed increased fat tissue when Shp2 was deleted (Fig. 2*F*).

Shp2-mediated Degradation of FASN Is Independent of Its Phosphatase Activity—We asked if degradation of FASN was indeed controlled by the ubiquitination pathway. As shown in Fig. 3*A*, the ubiquitination of FASN in coimmunoprecipitates was dramatically increased following treatment with proteasome inhibitor MG132 or lactacystin, compared with the controls. This result is consistent with a previous report on regulation of FASN by ubiquitin-mediated degradation in prostate cancer (13). To determine the molecular mechanism for Shp2 regulation of FASN protein levels, we transiently expressed wild-type (WT), catalytically inactive mutant (C459S), constitutively active mutant (D61A), and a N+C SH2 domain deletion mutant (Shp2-ΔSH2) in 293T cells. As shown in Fig. 3*B*, expression of the WT and two mutants (C459S or D61A) of Shp2 significantly decreased the protein level of FASN, compared with the vector control. However, the deletion mutant Shp2-ΔSH2 did not have any effect on FASN expression. These results suggest a mechanism for Shp2 control of FASN protein level through its SH2 domain in a phosphatase-independent fashion.

We next determined whether Shp2 expression affected the FASN level through protein degradation via the ubiquitin-proteasome system. Wild-type and mutant Shp2 expression constructs were co-transfected with His_6-tagged ubiquitin into 293T cells. Cell lysates were prepared in a denaturing 6 M guanidinium-HCl buffer, and His_6-tagged ubiquitin was precipitated with Ni^{2+}-NTA-agarose beads. Immunoblotting of precipitates showed markedly increased levels of FASN protein ubiquitination in 293T cells transfected with WT, CS, or DA mutants of Shp2, compared with the empty vector or the mutant Shp2-ΔSH2 (Fig. 3*C*). Taken together, these results suggest a role of Shp2 in control of FASN degradation by ubiquitination, which is independent of the catalytic activity but requires its SH2 domains.

FASN Degradation Is Mediated by an E3 Ligase COP1—COP1 is an E3 ubiquitin ligase that is involved in the ubiquitination of various protein substrates to trigger their proteasomal degradation (21). The first identified direct mammalian substrate of COP1 is p53 tumor suppressor (22). COP1 also targets JUN and shows that COP1 acts as a bridge between JUN, DET1, and the large E3 ligase complex that contains CUL4A and DDB1 (23). It has also been reported putative roles for COP1 in cell metabolism including glucose (24) and lipid metabolism (18). Importantly, COP1 can promote the degradation of acetyl-CoA carboxylase (ACC), a rate-limiting enzyme in fatty acid synthesis (18, 21). To test if COP1 as an ubiquitin E3 ligase also mediates FASN degradation, we transiently transfected the

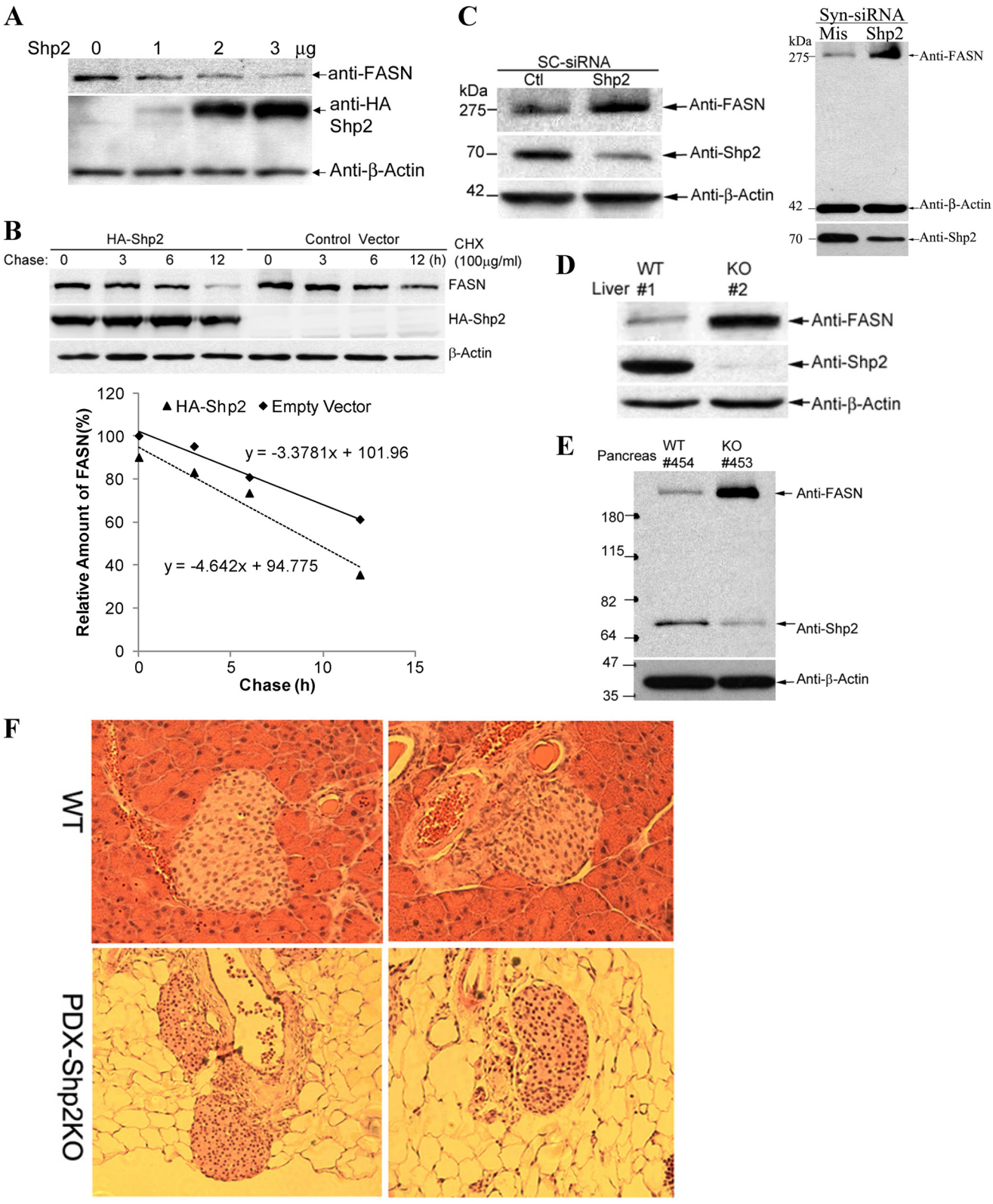

FIGURE 2. **Shp2 facilitates FASN degradation.** *A*, overexpression of Shp2 decreases FASN protein levels. 293T cells were transfected with 1, 2, or 3 μg of HA-Shp2 plasmids, and 48 h later, transfected cells were lysed for immunoblot with antibodies to FASN, HA, or β-actin. *B*, half-life of FASN in 293T cells transfected with or without HA-Shp2. 24 h after transfection, the cells was treated with cycloheximide (100 μg/ml) and harvested in SDS lysis buffer at the indicated time points. Relative amounts of FASN were quantified and normalized to β-actin, and plotted (the experiment repeated three times). *C*, Shp2 knockdown by siRNA leads to increased level of FASN protein. 293T cells were transfected with Shp2-specific siRNA, and transfected cells were lysed 48 h later for immunoblot with antibodies to FASN, Shp2, or β-actin. *Left panel*, SC-siRNA (from Santa Cruz Biotechnology); *Right panel*, Syn-siRNA (designed sequences, custom-synthesized commercially). *D* and *E*, Western blotting analysis showing high levels of FASN protein in Shp2 conditional knock-out mouse tissues, liver (*D*) and pancreas (*E*). *F*, H&E staining of pancreas sections from control or pancreas-specific Shp2 knock-out mouse (Pdx-Shp2KO) (19).

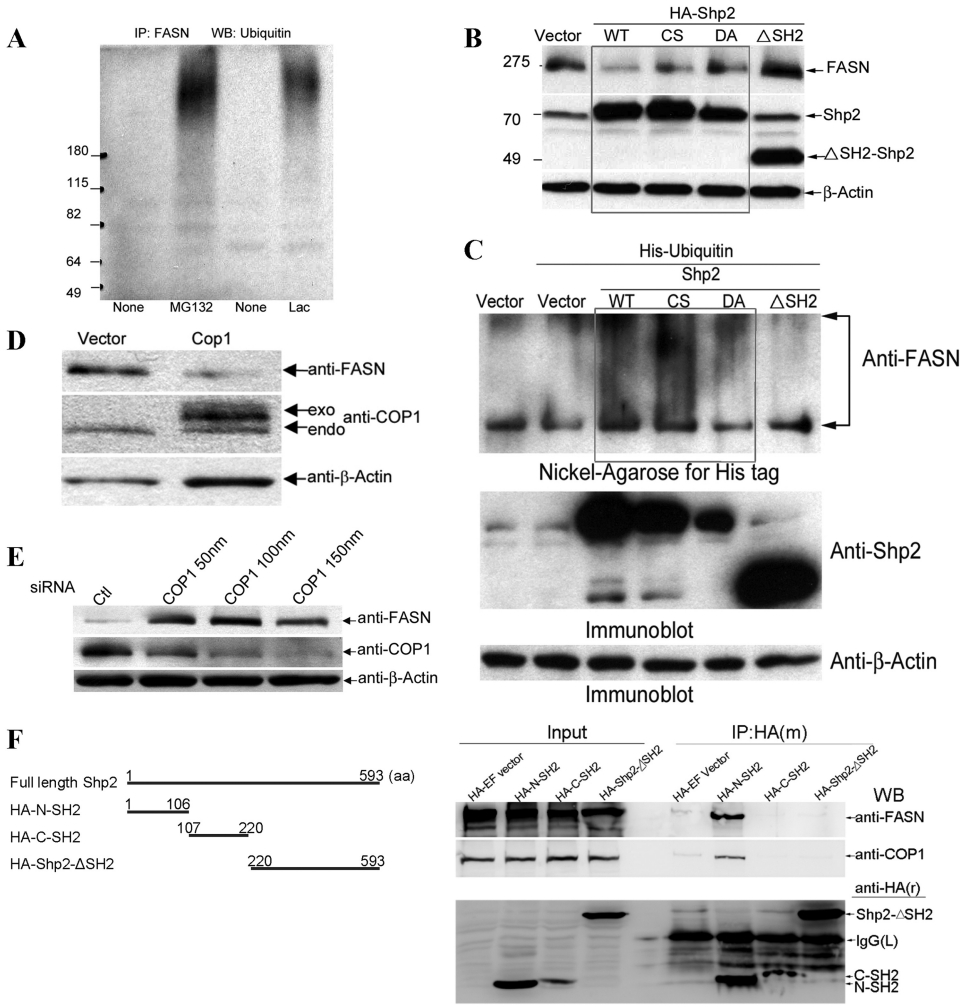

FIGURE 3. **FASN degradation is controlled by ubiquitin E3 ligase COP1.** *A*, FASN protein was degraded by ubiquitinylation. 293T cells treated with proteasome inhibitors Lactacystin (20 μM) or MG132 (20 μM) for 6 h were lysed for coimmunoprecipitation with FASN antibody, and followed by immunoblot with ubiquitin antibody. *B*, lysates from 293T cells transfected with empty vector, three mutants CS, DA, and ΔSH2, or wild-type Shp2, were analyzed by immunoblotting. *C*, the same Shp2 constructs were co-transfected with His-ubiquitin into 293T cells. The assay was performed for precipitation of the tagged ubiquitin with Ni^{2+}-NTA-agarose beads, as previously described (31). *D*, overexpression of COP1 decreased protein level of FASN. 293T cells transfected with COP1 expression vector were lysed for immunoblot with antibodies to FASN, COP1, or β-actin. *E*, COP1 knockdown by siRNA leads to increase in FASN protein amounts. 293T cells were transfected with siRNA-COP1, and at 48 h after transfection, cells were lysed for immunoblot with antibodies to FASN, COP1, or β-actin. *F*, 293T cells were transfected with pEF-HA, or pEF-HA-N-SH2, or pEF-HA-C-SH2 or pEF-HA-Shp2-ΔSH2 plasmid. Lysates from the cells at 48 h after transfection were used for immunoprecipitation with HA(m) antibody, then detected by immunoblotting with antibodies to HA(r), FASN, and COP1.

COP1 expression plasmid into 293T cells and showed that COP1 was able to significantly decrease the protein level of FASN (Fig. 3*D*). In contrast, down-regulation of endogenous COP1 by siRNA caused accumulation of FASN protein (Fig. 3*E*). Thus, overexpression or knockdown of COP1 had reciprocal effects on the protein level of FASN (Fig. 3, *D* and *E*), indicating that COP1 directly affects FASN degradation.

Shp2 protein contains one PTPase domain and two SH2 domains (N-SH2 and C-SH2). Since the SH2 domains are required for promoting degradation of FASN (Fig. 3, *B* and *C*), next we want to investigate whether one or both of SH2 domain(s) are able to directly interact with FASN and/or COP1. We generated and transfected three constructs HA-N-SH2, HA-C-SH2, and HA-Shp2-ΔSH2 into 293T cells. Coimmunoprecipitation/Western blot assays showed that N-SH2 domain of Shp2 complexed with both FASN and COP1, whereas either C-SH2 domain or Shp2-ΔSH2 (mainly PTPase domain) could not bind to FASN and COP1 (Fig. 3*F*, *IP*), suggesting that FASN degradation mediated by COP1 is involved in the direct interaction of Shp2 N-SH2 domain with FASN and COP1. However, three deletion mutants including N-SH2 domain did not lead to FASN protein degradation (Fig. 3*F*, *Input*), indicating that full-length Shp2 is required for this pathway.

p38 MAP Kinase Is Involved in FASN Degradation—To probe the molecular mechanism underlying FASN degradation, we treated cells with small molecule inhibitors LY294002 for PI3K, PD98059 for ERK, SB203580 for p38 MAP kinase, SP600125 for JNK, and U0126 for MEK, respectively. p38 MAP kinase inhibitor SB203580 significantly increased FASN protein level, SP600125 and U0126 caused a decrease, and LY294002 and PD98058 did not have a significant effect on FASN protein levels (Fig. 4*A*). To establish a role of p38, we examined FASN expression in $p38^{+/+}$ and $p38^{-/-}$ MEF cells. FASN protein level was much higher in $p38^{-/-}$ MEF than in $p38^{+/+}$ MEF cells (Fig. 4*B*), consistent with the experimental results using the chemical inhibitors shown in Fig. 4*A*. Notably,

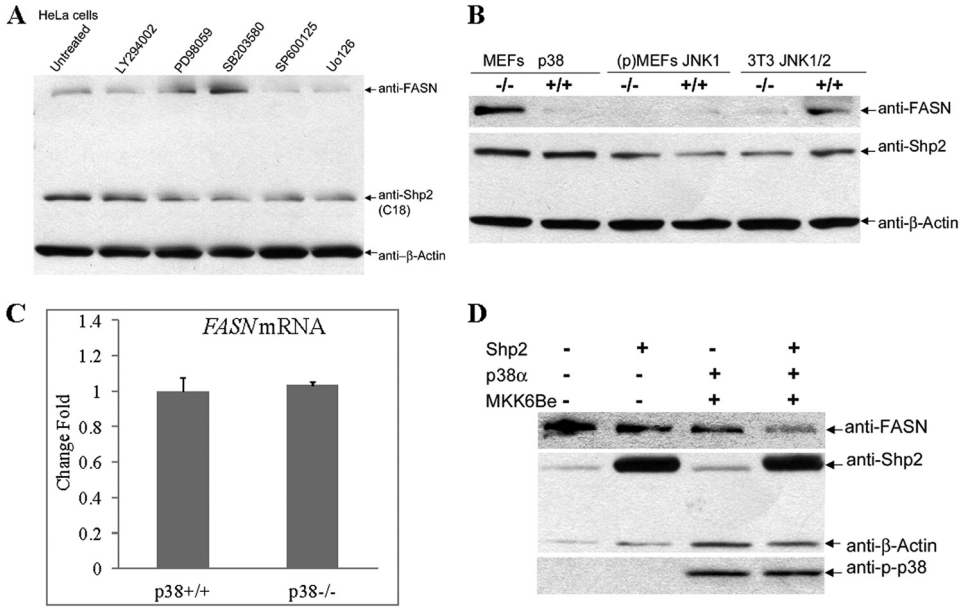

FIGURE 4. **p38 MAP kinase is involved in FASN degradation.** *A*, FASN protein was affected by kinase inhibitors. Hela cells were treated with 10 μm of different inhibitors LY294002, PD98059, SB203580, SP600125, or U0126 for 24 h, and then lysed for immunoblotting. *B*, comparison of FASN protein levels in $p38^{-/-}$ and $p38^{+/+}$ MEFs, $JNK1^{-/-}$ and $JNK1^{+/+}$ MEFs, $JNK1/2^{-/-}$ and $JNK1/2^{+/+}$ MEF cells. *C*, *FASN* mRNA levels in $p38^{+/+}$ and $p38^{-/-}$ MEFs were determined by qRT-PCR. Each value represents the mean ± S.E. of three independent experiments with triplicates each. Differences between two groups were analyzed using the *t* test (two-tailed and unpaired), *p* value = 0.4317. *D*, activated/phosphorylated p38 increased FASN degradation mediated by Shp2. 293T cells were transfected with Shp2 with or without p38α+MKK6Be. After 48 h, transfected cells were lysed for immunoblotting with antibodies to FASN, Shp2, β-actin, or p-P38.

the FASN protein levels were lower in $JNK1^{-/-}$ MEFs and JNK1/2 double knock-out ($JNK1/2^{-/-}$) cells than in wild-type cells (Fig. 4*B*).

We tested whether p38-mediated regulation of FASN occurs at the transcription and/or mRNA stability level. qRT-PCR analysis detected similar levels of FASN mRNA levels in $p38^{+/+}$ and $p38^{-/-}$ MEF cells (Fig. 4*C*), thus excluding the possibility that p38 regulates FASN gene transcription or mRNA stability. We also investigated possible concerted functions of p38 and Shp2 in controlling degradation of FASN protein. As shown in Fig. 4*D*, the decrease in FASN protein levels was more severe in 293T cells transfected with p38/MKK6Be and Shp2 (*lane 4*), than in cells transfected with either p38/MKK6Be or Shp2 alone.

p38 MAP Kinase Engages FASN-Shp2-COP1 Complex Formation in the Cytoplasm—It has been shown that ionizing radiation (IR) triggered an ATM-mediated COP1 serine 387 (S387) phosphorylation leading to IR-induced COP1 nuclear exclusion (25). Also, COP1 S387 phosphorylation is essential for 14-3-3σ induced COP1 nuclear export (26). Therefore, we wanted to test whether p38 MAP kinase can also phosphorylate COP1. We used a very useful tool, okadaic acid, an inhibitor of the serine/threonine protein phosphatases PP1 and PP2A, to treat $p38^{+/+}$ and $p38^{-/-}$ MEFs. After treatment of okadaic acid (2 μm) for 60 min, cells were lysed for immunoprecipitation with anti-pTP/pSP antibody, and then detected by immunoblotting with antibody to COP1. The results showed that COP1 phosphorylation was easily detected in $p38^{+/+}$ MEFs, and strongly increased by okadaic acid treatment when compared with DMSO treatment. In contrast, COP1 phosphorylation was almost not detected under treatment of either okadaic acid or DMSO in $p38^{-/-}$ MEFs (Fig. 5*A*). Moreover, we transiently transfected plasmids MKK6Be with or without p38α-WT and p38α-KR (a dominant-negative mutant) into 293T cells. Lysates were used for immunprecipitation with anti-pTP/pSP antibody and then detected with anti-COP1 antibody. Compared with control, COP1 phosphorylation was significantly increased by p38α-WT but not by p38α-KR (Fig. 5*B*). These suggest that p38 can phosphorylate COP1 in cells.

The data shown above suggest participation of three proteins Shp2, COP1, and p38 in mediating FASN protein degradation. We then explored the biochemical mechanisms linking these proteins to examine whether endogenous COP1 binds FASN and/or Shp2 in $p38^{+/+}$ and $p38^{-/-}$ MEF cells. COP1 bound both FASN and Shp2 only in $p38^{+/+}$ MEFs but not in $p38^{-/-}$ MEFs, suggesting that p38 is requested for COP1 binding to its target protein FASN and in interaction with Shp2 (Fig. 5*C*).

We further examined localization and association of Shp2, FASN, and COP1 in $p38^{+/+}$ and $p38^{-/-}$ MEF cells by immunofluorescence staining and confocal microscopy. Both Shp2 and FASN were mainly cytoplasmic, and their colocalization showed no difference between $p38^{+/+}$ and $p38^{-/-}$ MEFs (Fig. 5*D*, *upper two panels*), indicating that p38 does not affect binding of FASN with Shp2. However, COP1 was largely detected in the cytoplasm in $p38^{+/+}$ MEFs, whereas increased nuclear distribution of COP1 was observed in $p38^{-/-}$ MEFs, likely due to p38-dependent movement of COP1 from the nucleus to the cytoplasm, thereby increasing COP1 binding to FASN and Shp2 in the cytoplasm, as revealed by COP1 colocalization with both FASN and Shp2 (Fig. 5*D*, *4th* and *6th panels*), consistent with the coimmunoprecipitation data (Fig. 5*C*). These results support a model that p38-phosphorylated COP1 accumulates in the cytoplasm and subsequently binds FASN through Shp2 here as a bridge molecule, leading to FASN-Shp2-COP1 com-

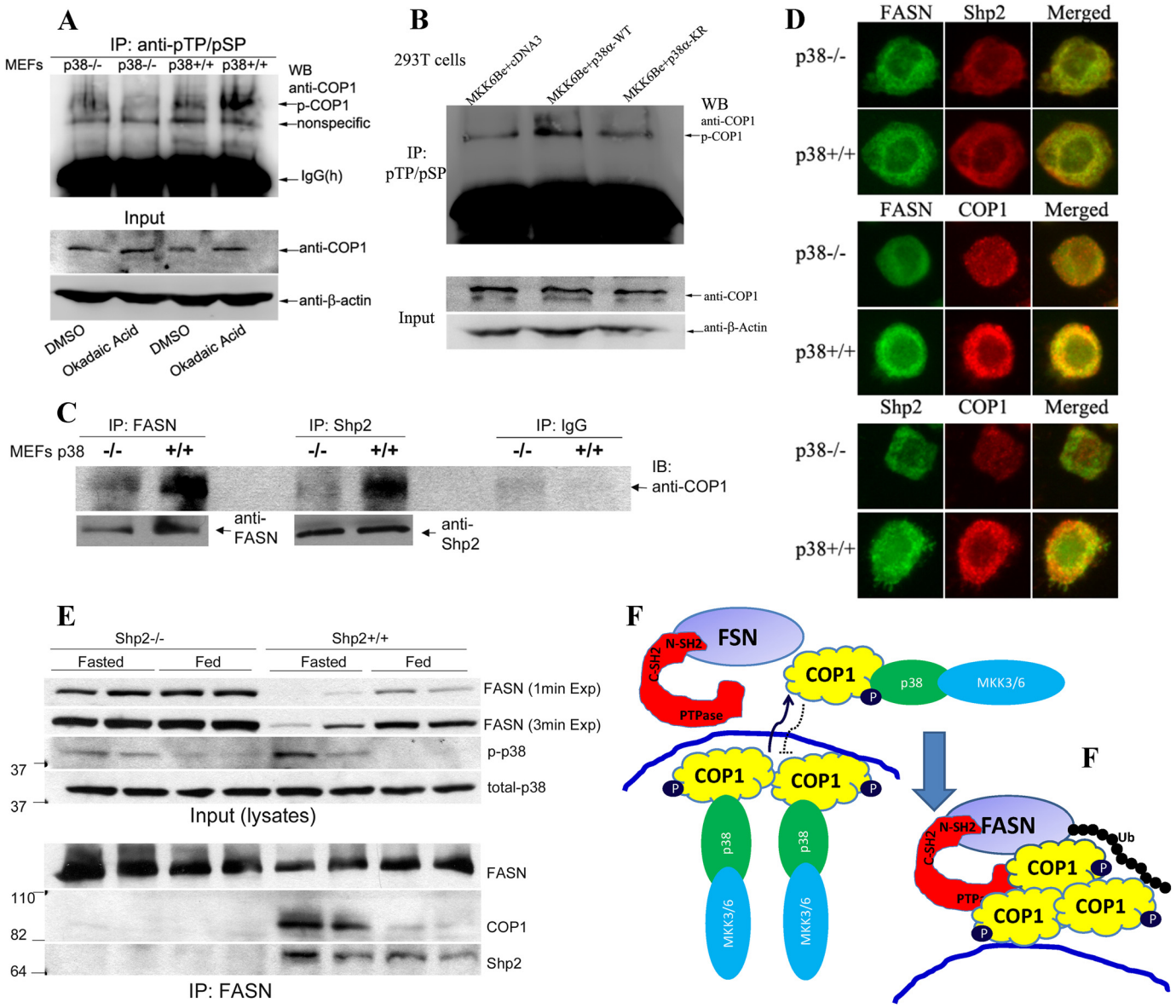

FIGURE 5. **FASN regulation by the p38-COP1-Shp2 pathway.** *A*, lysates from p38$^{+/+}$ and p38$^{-/-}$ MEFs treated with okadaic acid (2 μm for 60 min) were coimmunoprecipitated with anti-pTP/pSP antibody and then detected by immunoblotting with antibody to COP1. *B*, 293T cells were transfected with plasmids MKK6Be with or without p38α-WT and p38α-KR (mutant). After 48 h, transfected cells were lysed for immunprecipitation with anti-pTP/pSP antibody and then detected with anti-COP1 antibody. *C*, endogenous COP1 binds FASN and Shp2 in p38$^{+/+}$ but not in p38$^{-/-}$ MEFs. Lysates from p38$^{+/+}$ and p38$^{-/-}$ MEFs were coimmunoprecipitated with antibodies to FASN, Shp2, or control IgG, and then detected by immunoblotting with antibody to COP1. *D*, p38$^{+/+}$ and p38$^{-/-}$ MEF cells were processed for immunostaining and confocal microscopy to visualize the localization of Shp2, FASN, and COP1. *E*, wild-type and liver-specific Shp2 knock-out C57BL/6 mice were either fasted for 24 h, or fed regular chow food. Liver lysates from sacrificed mice were subjected to immunoblot and Co-IP experiments with indicated antibodies. *F*, a model for FASN regulation by the p38-COP1-Shp2 pathway.

plex formation in the cytoplasm and FASN degradation mediated by ubiquitination pathway.

FASN Regulation by a New Pathway of p38-COP1-Shp2 in Vivo—To further evaluate the p38-COP1-Shp2 pathway in regulation of FASN degradation, wild-type and liver-specific Shp2 knock-out (LSKO) mice (8 weeks old) were either fasted for 24 h or fed with regular food. Liver lysates of these mice were analyzed by immunoblotting (Fig. 5*E*, *upper 4 panels*). FASN protein levels were much higher in livers of Shp2$^{-/-}$ mice than wild-type control. Furthermore, FASN protein levels were decreased by fasting in wild-type but not in LSKO mice, although increased p-p38 signals were detected in livers of both wild-type and Shp2$^{-/-}$ mice upon fasting. Consistently, coimmunoprecipitation assay of liver lysates (Fig. 5*E*, *bottom 3 panels*) showed that binding of FASN and COP1 in fasted mice was higher than in fed wild-type mice, while this binding was not observed in either fasted or fed Shp2-deficient liver. These results suggest that Shp2 is required for control of FASN protein levels in the liver, and that p38 plays a central role in orchestrating molecular interaction of FASN, Shp2, and COP1 for FASN degradation.

In summary, we identify a novel and unexpected function for Shp2 acting as an adaptor protein likely through its N-SH2 domain in targeting FASN for its ubiquitination and degradation, and propose a model for FASN regulation by the p38-COP1-Shp2 pathway (Fig. 5*F*). This action does not depend on

its catalytic activity and is evidently distinct from its previously revealed roles in modulating various cytoplasmic signaling pathways. Shp2 is an abundantly expressed enzyme in mammalian cells, which makes it possible to have multiple activities in cell regulation. COP1 is a key regulator of lipid metabolism to maintain energy homeostasis through association with acetyl-coenzyme A (18), and also plays an important role in maintenance of glucose homeostasis through association with phosphorylated TORC2 (24). Therefore, COP1 may contribute to insulin resistance and diabetes in humans. p38, a member of MAP kinase family, plays key roles in cytoplasmic-nuclear signaling elicited by inflammatory cytokines and environmental stress such as ultraviolet, heat shock, and ischemia (27). p38 is activated in mouse liver and hypothalamus during fasting as well in the livers of both type 1 and type 2 diabetic mice (28–30). By acting as an adapter linking FASN with COP1, Shp2 executes a new function of promoting degradation of FASN, which is a key enzyme in lipid metabolism, and its overexpression is associated with a variety of human malignancies. This study identifies a new function for Shp2 in both lipid metabolism and tumor suppression.

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