

# Curcumin Inhibits the Proteolytic Process of SREBP-2 by First Inhibiting the Expression of S1P rather than Directly Inhibiting SREBP-2 Expression.

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## Research

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# Abstract

**Objective:** Many studies have demonstrated that curcumin can downregulate mRNA levels of sterol regulatory element-binding proteins (SREBP-2), however, our study did not find similar results. This study was designed to demonstrate that curcumin inhibits the proteolytic process of SREBP-2 by first inhibiting the expression of membrane-bound transcription factor site-1 protease (S1P) rather than directly inhibiting SREBP-2 expression.

**Methods:** After curcumin treatment, Caco-2 cells were collected to observe the dose- and time-dependent dynamics of precursor and mature SREBP-2, transcription factor specific protein 1 (SP-1) and SREBP cleavage-activating protein (SCAP). After curcumin treatment, SREBP-2 distribution was detected in the cells and S1P protein expression were examined.

**Results:** Curcumin could downregulate mRNA levels of SREBP2, SP-1 and SCAP, but it did not simultaneously downregulate the expression of precursor SREBP-2 (pSREBP-2) and SCAP. Curcumin can inhibit the proteolytic process of SREBP-2, reduce the production of mature SREBP-2 (mSREBP-2) and change the cellular distribution of SREBP-2. The inhibitory effect of curcumin on SP-1 protein expression is short-acting.

**Conclusion:** Curcumin can inhibit the SREBP-2 proteolytic process to reduce mSREBP-2 which functions as a transcription factor, affecting the regulation of cholesterol metabolism-related genes. This process may be achieved by regulating the expression of S1P. Curcumin does not directly inhibit the expression of mSREBP-2 protein, and it has no such inhibitory effect for at least a short period of time, although curcumin does reduce the amount of mSREBP-2 protein.

## 1. Introduction

Many studies have indicated that the formation of cholesterol gallstones is closely related with the absorption of cholesterol in the small intestine. A high-fat diet can promote the formation of cholesterol stones and reducing cholesterol absorption in the small intestine can inhibit the formation of calculus.<sup>[1]</sup> The Niemann-Pick C1-like 1 ( ) protein of the intestinal epithelial brush-border membrane is a cholesterol transporter <sup>[2, 3]</sup>. Kumar et al <sup>[4]</sup> found that curcumin can inhibit NPC1L1 expression to reduce cholesterol absorption in Caco-2 cells, which is mediated by SREBP-2. We used a high-fat diet mouse model to identify that curcumin can reduce the formation of cholesterol gallstones in a process regulated by SREBP-2.<sup>[5]</sup>

SREBP-2 is a member of the SREBP family which includes three proteins: SREBP-1a, SREBP-1c and SREBP-2. SREBP-2 is mainly involved in the activation of low-density lipoprotein receptors and the expression regulation of cholesterol metabolism pathways related proteins. The SREBP-2 precursor protein (precursor SREBP-2, pSREBP-2) is a membrane protein with a molecular weight of approximately 125 kDa which is localized on the endoplasmic reticulum as a hairpin structure and contains three domains: the N-terminal (about 480 amino acids), C-terminal (about 590 amino acids) and middle

regions (about 80 amino acids)<sup>[6, 7]</sup>. The C-terminus of the pSREBP-2 connects with the C-terminus of the SREBP cleavage-activating protein (SCAP) to form the SREBP/SCAP complex. The sterol-sensing domain (SSD) consists of 2–6 transmembrane helices near the N-terminus of SCAP. The SREBP/SCAP complex anchors to the endoplasmic reticulum by linking to the endoplasmic reticulum intrinsic structural protein insulin induced gene (INSIG) <sup>[7]</sup>. As sterol concentration decreases, the linkage between SCAP and INSIG disappears and the SCAP conformation changes, leading to the transport of the SREBP/SCAP complex from the endoplasmic reticulum to the Golgi apparatus <sup>[7]</sup>. In the Golgi body, site-1 protease (S1P) cleaves SREBP-2 protein in the hydrophilic ring of SREBP-2. The site-2 protease (S2P) cleaves the N-terminal domains of SREBP-2 across the Golgi membrane. The N-terminal SREBP-2 (mSREBP-2, approximately 62 kDa) is then released into the nucleus to act as a transcription factor via its NH<sub>2</sub>-terminal bHLH transcription factor domains<sup>[6, 7]</sup> (Sketch Map). Only mSREBP-2 has transcription factor functions, not its precursor, and the two forms have different molecular weights.

Many studies have confirmed that curcumin can inhibit SREBP-2 mRNA expression<sup>[4, 5, 8–12]</sup>. This may depend on the inhibition of specificity protein-1 (SP-1) protein expression, which can bind to the GC-box in the SREBP-2 promoter sequence<sup>[8]</sup>. Curcumin is reported to inhibit both the transcription and translation of SREBP-2<sup>[9, 11, 12]</sup>. Curcumin likely directly inhibits SREBP-2 protein expression through inhibiting its transcription. In this study, we identified that although curcumin down-regulated SREBP-2 transcription, the amount of pSREBP-2 did not decrease, but mSREBP-2 did decrease. Therefore, we speculate that the direct effect of curcumin on SREBP-2 in Caco-2 cells may depend on inhibiting its proteolysis process and reducing SREBP-2 protein activity rather than inhibiting SREBP-2 translation.

## 2. Materials And Methods

### 2.1 Cell culture:

Human colorectal adenocarcinoma cell line (Caco-2) was purchased from the Cell Bank of the Chinese Academy of Sciences and cells were grown routinely in T-75-cm<sup>2</sup> plastic flasks at 37°C in a 5% CO<sub>2</sub>-95% air environment. The culture medium consisted of MEM (Invitrogen 11090081), 20% FBS (Gibco), 1% Glutamax (Invitrogen 35050061), 1% Non-essential Amino Acid (Invitrogen 11140050), 1% Sodium Pyruvate 100mM Solution (Invitrogen 11360070), 100 IU/ml penicillin, and 100 µg/ml streptomycin (Life Technologies, Inc.). Cells from passages between 20 and 40 were plated in six-well Falcon plates at a density of 2×10<sup>4</sup> cells/cm<sup>2</sup> and were fed with fresh incubation media every alternate day. Curcumin was obtained from Sigma (St. Louis, MO).

### 2.2 RNA extraction and qPCR:

Total RNA was prepared from Caco-2 cells using an RNAiso Plus (TAKARA, 9108) according to the manufacturer's instructions. Equal amounts of RNA from both treated and control samples were reverse

transcribed and amplified in one step reaction using 5×PrimeScript RT Master Mix (TAKARA, RR036A). Real-time PCR was performed using 2 × ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co.,Ltd) with gene-specific primers as follows:

human SREBP-2: Forward Primer CTCCATTGACTCTGAGCCAGGA; ReversePrimer  
GAATCCGTGAGCGGTCTACCAT

human SCAP: Forward Primer TCACGTTGCAGCCGTCTTCCTT; Reverse Primer  
CAGGATGCCAATCCAGACAACG

human SP-1: Forward Primer ACGCTTCACACGTTCCGGATGAG; Reverse Primer  
TGACAGGTGGTCACTCCTCATG

human S1P: Forward Primer CTACTIONATGGAGGAATGCCGACAG; Reverse Primer  
CTCCGTTCTGTGGCAAATAGGG

human S2P: Forward Primer ACGGCGGAAAGCAAGGATGCTT; Reverse Primer  
GTGCCAAAGTCTGCATCAGCGT

human GAPDH: Forward Primer GTCTCCTCTGACTTCAACAGCG; Reverse Primer  
ACCACCCTGTTGCTGTAGCCAA

## 2.3 Western Blot

Total protein was extracted by suspending the cell pellet in a cell lysis buffer containing 50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 1 mM EDTA supplemented with protease inhibitor cocktail from Roche (Sigma, St. Louis, MO) [4]. The BCA kit (Abcam, ab102536) was used to determine the protein concentration. Protein (100µg) from both control and treated samples was subjected to 4-20% SDS-PAGE. The resolved proteins in the gel were transferred to a PVDF membrane electrophoretically. The membrane was separately incubated first with Anti-SREBP2 antibody (ab30682), Anti-SCAP antibody (ab190103), Anti-SP1 antibody (ab227383), Anti-S1P antibody (ab140592), Anti-S2P antibody (ab140594), Anti-GAPDH antibody (ab181602) from Abcam (1:1000) and then with goat anti-rabbit secondary antibody (1:10000) conjugated with horseradish peroxidase followed by ECL detection from Bio-Rad (Hercules, CA).

## 2.4 Immunofluorescence

The cells were 4% paraformaldehyde fixed (15min) at room temperature and then incubated with 0.1% TritonX-100 in PBS for 15min to permeabilize the cells and block non-specific protein-protein interaction with 5% BSA for 30min. The cells were then incubated with the antibody SREBP2 1:100 overnight at 4°C. The secondary antibody (green) Alexa Fluor 488 goat anti-rabbit IgG (H+L) 1:200 was incubated 2h at

RT, then ER-tracker Red Beyotime C1041 was used to label ER membranes (red) at a 1:200 dilution for 30min. DAPI Beyotime C1005 1:1000 was used to stain the cell nuclei (blue) for 5min.

## 2.5 Statistics

Results are expressed as means $\pm$ SE. Student's t-test was used for statistical analysis. A P value of 0.05 or less was considered statistically significant.

## 3. Results

### 3.1 Curcumin inhibits the expression of SREBP-2, SCAP and SP-1

Many studies report inhibited expression of SREBP-2 mRNA upon curcumin treatment [4, 5, 8–12]. Our study showed consistent results, with mRNA levels of SREBP-2, SCAP and SP-1 mRNA decreasing as the curcumin concentration increased. With 50  $\mu$ M curcumin, the mRNA levels of SREBP-2, SCAP and SP-1 mRNA were not significantly different at 48 h compared to 24 h. There was also no significant change in levels of SREBP-2 and SP-1 mRNA when the curcumin concentration was 25  $\mu$ M or 50  $\mu$ M (Fig. 1a). Thus, we assayed the time course of curcumin at a 25  $\mu$ M concentration. As shown in Fig. 1b, mRNA levels for SREBP-2, SCAP and SP-1 were initially inhibited, yet this inhibition lessened with prolonged treatment. Levels of SP-1 were seen to completely recover by 48 h, while SREBP-2 and SCAP mRNA levels were slightly lower in treated cells even at 96 h. We also found that the expression of SREBP-2, SCAP, and SP-1 mRNA were down-regulated by curcumin treatment at 12 h, with SP-1 levels decreasing faster than SREBP-2 or SCAP (Fig. 1c). This strongly supports the study by Kang et al identifying that SP-1 acts as a transcription factor binding to the GC-box on the SREBP-2 promoter to regulate SREBP-2 gene expression<sup>[8]</sup>. To diminish the effects of cell proliferation, we set up control groups at each time point. We found that the expression of SREBP-2 and SCAP was lower with curcumin than in the control group. SP-1 expression was inhibited at 24 h under curcumin treatment and the effect was eliminated after 48 hours, consistent with the time and effect of curcumin in Kang et al<sup>[8]</sup> (Fig. 1d).

### 3.2. Curcumin did not simultaneously down-regulate the expression of SREBP-2 and SCAP proteins.

After treatment with curcumin in Caco-2 cells, although SREBP-2, SCAP, and SP-1 mRNA expression were down-regulated in both dose-effect and time-effect tests, there was no simultaneous down-regulation of protein expression. As shown in Fig. 2a, at 24 h treatment with an increased concentration of curcumin, the levels of pSREBP-2 (130 kDa) and SCAP showed no significant difference compared to the control group, yet mSREBP-2 protein (55-70kDa) was decreased. SP-1 expression gradually decreased with increasing curcumin concentration, but no change in SP-1 expression was seen after 48 h treatment. The

time course results showed that the protein levels of pSREBP-2 and SCAP did not decrease although their mRNAs were downregulated following curcumin treatment. The pSREBP-2 and SCAP protein levels showed no significant change compared to the control group at 72 h treatment. However, at 96 h treatment, the levels of pSREBP-2 and SCAP protein were decreased. The level of mSREBP-2 mature protein was also decreased upon curcumin treatment compared to the control group (Fig. 2b). The observation that curcumin treatment did not decrease the level of pSREBP-2 while mSREBP-2 protein levels were downregulated suggest that curcumin may inhibit proteolysis of the pSREBP-2.

### **3.3. Curcumin inhibits the proteolysis of the SREBP-2 precursor and decreases the level of SREBP-2 mature protein**

To test this hypothesis, we examined the distribution of SREBP-2 in the cytoplasm and nucleus early in curcumin treatment. As shown in Fig. 3a, mature SREBP (55–70 kDa) decreased in curcumin treatment, but the pSREBP-2 (130 kDa) did not decrease with prolonged treatment. After 24 h curcumin treatment, western blotting showed that the amount of pSREBP-2 in the cytoplasm was not significantly different from that of the control group, while the amount of mSREBP-2 protein in the nucleus was less than in the control group (Fig. 3b). Tracking co-localization of SREBP-2 protein with the endoplasmic reticulum showed that the amount of mSREBP-2 in the nucleus was reduced and more pSREBP-2 stayed in the cytosol and endoplasmic reticulum after curcumin intervention (Fig. 3c).

### **3.4. Curcumin may down-regulate the expression of S1P.**

To explore the mechanism of curcumin inhibiting proteolysis of the pSREBP-2, we examined the expression of S1P and S2P relative to SREBP-2 proteolysis after curcumin intervention. We found that after 24 h treatment, 25  $\mu$ M curcumin could down-regulate the expression of S1P but not S2P in CaCo-2 cells (Fig. 4). Therefore, we speculate that curcumin may affect the proteolysis process of SREBP-2 by inhibiting the expression of S1P.

## **4. Discussion**

Curcumin is a chemical component extracted from the rhizome of Zingiberaceae and Araceae. Studies have described various effects of curcumin including reducing blood fat, choleric actions, and anti-tumor, anti-inflammatory, and anti-oxidation effects [13–16]. Several studies have indicated that the effect of curcumin on SREBPs is related to the mechanism of curcumin regulating blood lipid metabolism [4, 5, 8–12]. Kumar et al [4] found that curcumin can regulate the expression of NPC1L1 through SREBP-2 to inhibit cholesterol absorption in Caco-2 cells. We used a mouse model of cholesterol gallstone formation induced by a high-fat diet to show these results. Our preliminary study further confirmed that curcumin can reduce cholesterol gallstone formation and inhibit NPC1L1[5]. As we investigated how curcumin

affects NPC1L1 expression through SREBP-2, we found different results from previous studies. Although curcumin lowered SREBP-2 mRNA levels, as in other studies, we did not see simultaneously down-regulated SREBP-2 protein levels. In the Caco-2 cell line, SREBP-2 mRNA expression decreased with higher doses (Fig. 1a) and times (Fig. 1b) of curcumin treatment. However, we did not find pSREBP-2 precursor levels to be inhibited (Fig. 2). Western blotting showed that while pSREBP-2 (almost 130 kDa) wasn't significantly down-regulated, the amount of mSREBP-2 protein (55–70 kDa) decreased (Fig. 2). The SREBP-2 mRNA levels were inconsistent with pSREBP-2 levels, which was particularly evident through the curcumin time course. Regardless of whether curcumin was administered long-term (0 ~ 72 h) or short-term (within 24 h), the pSREBP-2 did not decrease following with the mRNA inhibition. Kang et al <sup>[8]</sup> found that the curcumin-mediated reduction of SREBP-2 promoter activity was dependent on the inhibition of SP-1. Consistent with Kang's study, we also found downregulated transcriptional and translational levels of SP-1 at 24 h treatment, but this down-regulation was relieved after 48 h treatment (Fig. 1b, Fig. 2b). This suggests that curcumin's effect on SREBP-2 may not involve SP-1, or that there may be another pathway. Considering the amount that mSREBP-2 protein decreased after curcumin treatment and that SREBP-2 is activated as a transcription factor through the proteolytic process <sup>[6, 7]</sup>, we hypothesized that the effect of curcumin on SREBP-2 may depend on its proteolytic process. Endoplasmic reticulum co-localization showed that curcumin modulated SREBP-2 distribution within the cell, decreased mSREBP-2 in the nucleus, and inhibited the proteolysis process (Fig. 3c). In Brown and Goldstein's research <sup>[6, 7]</sup>, they suggest that the SREBP-2/SCAP complex is the real substrate of S1P and key for the proteolytic process of SREBP-2. Therefore, we also detected SCAP in our study. SCAP and SREBP-2 showed consistent changes in transcription and translation. These results suggest that SCAP may not be a target of curcumin. As S1P protein levels decreased after curcumin treatment, curcumin may inhibit the expression of SREBP-2 by inhibiting the expression of S1P.

Several other studies have demonstrated inconsistency between SREBP-2 transcription and translation levels. Field et al <sup>[17]</sup> showed in rat liver cells that increased cholesterol in Caco-2 cells inhibited proteolysis of SREBP-2. The mSREBP-2 was decreased but pSREBP-2 levels were not significantly changed, and this process was accompanied by inhibition of SREBP-2 gene expression. Liu et al <sup>[10]</sup> reported that the change of SREBP-2 protein was not statistically significant after curcumin treatment compared with controls. Sato et al <sup>[18]</sup> found that the SREBP-2 gene includes a Sterol Regulatory Element (SRE) identical to the one on the promoter sequence of the human LDL receptor, thus SREBP-2 may regulate its own expression through modulating sterol levels. When the proteolytic process of SREBP-2 was inhibited, mSREBP-2 transcription factor levels decreased, which affected the expression of cholesterol metabolism related proteins and also the expression of SREBP-2. These results may explain why the expression of SREBP-2 mRNA does not follow the protein level.

Some studies have reported downregulated SREBP-2 upon curcumin treatment<sup>[9, 11, 12]</sup>. However, these studies do not show the molecular weight of the protein markers in their figures, making it hard to determine the molecular weight of the SREBP-2 detected in their western blots. Thus, the detected protein may be mSREBP-2 rather than pSREBP-2. Liu et al <sup>[10]</sup> studied the function of curcumin using antibodies

against SREBP-2 purchased from Santa Cruz which have only been used to detect pSREBP-2. Our results indicate that curcumin first affects the SREBP-2 proteolysis process rather than inhibiting its protein expression. Protein expression inhibition may be a long-term effect. The inhibitory effect of curcumin on the transcription of SREBP-2 mRNA persisted long-term, with decreased mRNA of the pSREBP-2 detected after 96 hours treatment (Fig. 2b). When SREBP-2 proteolysis is inhibited, pSREBP-2 may be degraded by other pathways, and its gene expression may remain continuously inhibited, eventually leading to down-regulation of the pSREBP-2 protein expression. This mechanism still requires further experimentation to fully understand.

The exact mechanism for how curcumin reduces the hydrolysis of SREBP-2 and decreases mSREBP-2 protein levels through inhibiting S1P expression remains unknown. However, we conclude here that the mechanism of curcumin treatment on SREBP-2 is not through short-term inhibition of protein expression, although curcumin can indeed inhibit the transcription of SREBP-2 mRNA long-term. Thus, it appears that curcumin plays an important role via inhibiting the activity of SREBP-2 rather than directly affecting its expression of gene and protein.

## Abbreviations

INSIG: Intrinsic Structural Protein Insulin Induced Gene, NPC1L1: Niemann-Pick C1-like 1, SREBP-2: Sterol Regulatory Element-Binding Proteins, pSREBP-2: precursor SREBP-2, mSREBP-2: mature SREBP-2, SCAP: SREBP Cleavage-Activating Protein, SP-1: Specific Protein 1, S1P: Site-1 Protease, S2P: Site-2 Protease, SSD: Sterol-Sensing Domain, SRE: Sterol Regulatory Element

## Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Yongnan Li: performed the research, wrote and approved the manuscript.

Shuodong Wu: wrote and approved the manuscript

Acknowledgements: Not applicable

## References

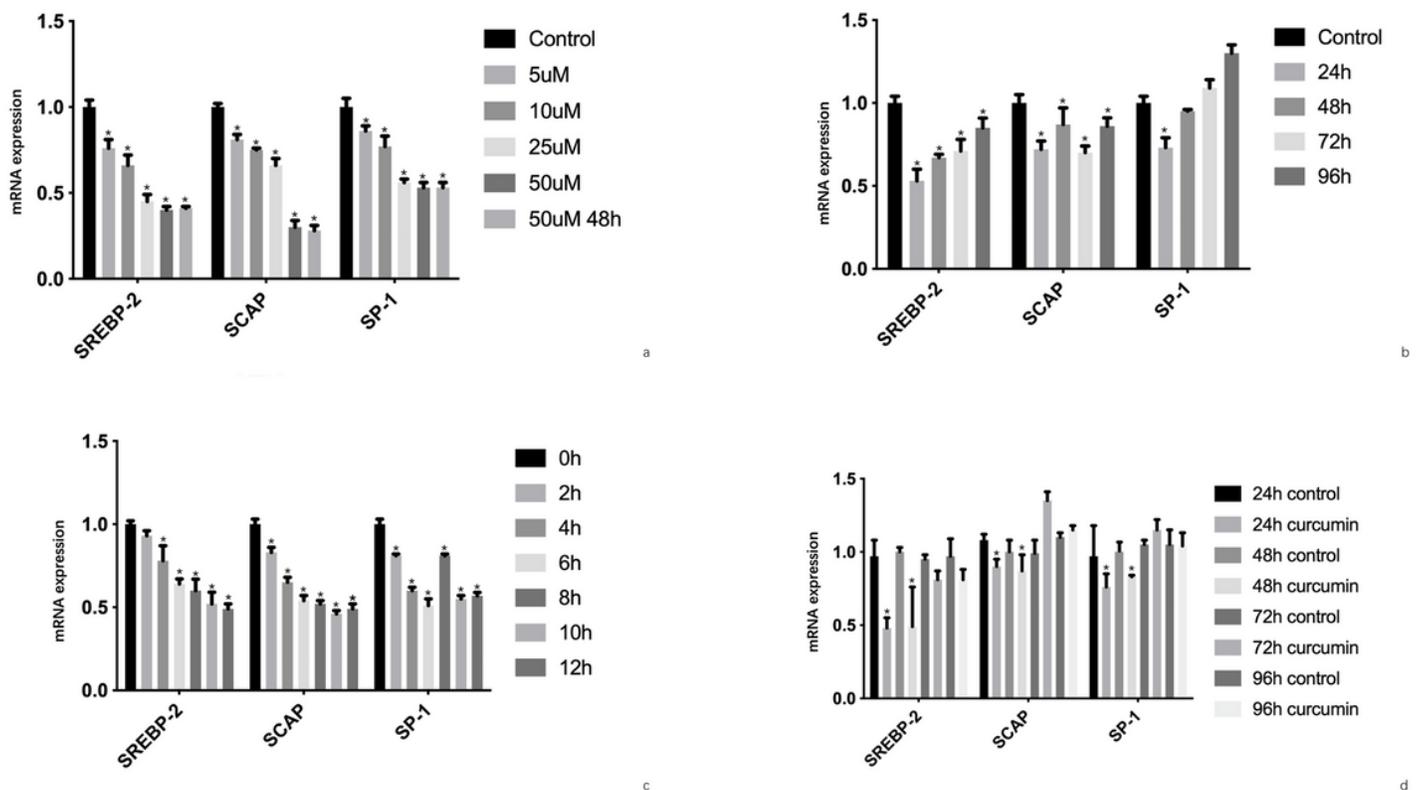
1. Wang H H, Portincasa P, Mendez–Sanchez N, et al. Effect of ezetimibe on the prevention and dissolution of cholesterol gallstones. *Gastroenterology*, 2008, 134(7): 2101-2110.
2. Altmann S W, Davis H R, Zhu L, et al. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science*, 2004, 303(5661): 1201-1204.
3. Alrefai W, Annaba F, Sarwar Z, et al. Modulation of human Niemann-Pick C1 like 1 gene expression by sterol: role of sterol regulatory element binding protein-2. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 2007.
4. Kumar P, Malhotra P, Ma K, et al. SREBP2 mediates the modulation of intestinal NPC1L1 expression by curcumin. *American Journal of Physiology-Heart and Circulatory Physiology*, 2011.
5. Li Y, Li M, Wu S, et al. Combination of curcumin and piperine prevents formation of gallstones in C57BL6 mice fed on lithogenic diet: whether NPC1L1/SREBP2 participates in this process? *Lipids in health and disease*, 2015, 14(1): 100.
6. Brown M S, Goldstein J L. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proceedings of the National Academy of Sciences*, 1999, 96(20): 11041-11048.
7. Brown M S, Radhakrishnan A, Goldstein J L. Retrospective on cholesterol homeostasis: the central role of scap. *Annual review of biochemistry*, 2018, 87: 783-807.
8. Kang Q, Chen A. Curcumin inhibits srebp-2 expression in activated hepatic stellate cells in vitro by reducing the activity of specificity protein-1. *Endocrinology*, 2009, 150(12): 5384-5394.
9. Ding L, Li J, Song B, et al. Curcumin rescues high fat diet-induced obesity and insulin sensitivity in mice through regulating SREBP pathway. *Toxicology and applied pharmacology*, 2016, 304: 99-109.
10. Liu Z, Cui C, Xu P, et al. Curcumin Activates AMPK Pathway and Regulates Lipid Metabolism in Rats Following Prolonged Clozapine Exposure. *Frontiers in neuroscience*, 2017, 11: 558.
11. Feng D, Zou J, Zhang S, et al. Hypocholesterolemic activity of curcumin is mediated by down-regulating the expression of Niemann-Pick C1-like 1 in hamsters. *Journal of agricultural and food chemistry*, 2017, 65(2): 276-280.
12. Kang Q, Chen A. Curcumin suppresses expression of low-density lipoprotein (LDL) receptor, leading to the inhibition of LDL-induced activation of hepatic stellate cells. *British journal of pharmacology*, 2009, 157(8): 1354-1367.
13. Panahi Y, Ahmadi Y, Teymouri M, et al. Curcumin as a potential candidate for treating hyperlipidemia: a review of cellular and metabolic mechanisms. *Journal of cellular physiology*, 2018, 233(1): 141-152.
14. Moghaddam N S A, Oskouie M N, Butler A E, et al. Hormetic effects of curcumin: What is the evidence? *Journal of cellular physiology*, 2019, 234(7): 10060-10071.
15. Zingg J M, Hasan S T, Meydani M. Molecular mechanisms of hypolipidemic effects of curcumin. *Biofactors*, 2013, 39(1): 101-121.
16. Nelson K M, Dahlin J L, Bisson J, et al. The essential medicinal chemistry of curcumin: miniperspective. *Journal of medicinal chemistry*, 2017, 60(5): 1620-1637.

17. Field F J, Born E, Murthy S, et al. Regulation of sterol regulatory element-binding proteins by cholesterol flux in CaCo-2 cells. *Journal of lipid research*, 2001, 42(10): 1687-1698.
18. Sato R, Inoue J, Kawabe Y, et al. Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein-2. *Journal of Biological Chemistry*, 1996, 271(43): 26461-26464.

## Supplemental Material

Sketch Map-Normally, INSIG binds to the sterol-sensing domain (SSD) of SCAP (blue transmembrane region in SCAP). As the sterols concentration decreases, the connection between SCAP and INSIG disappears, the complex of SREBP/SCAP is transported from the endoplasmic reticulum to the Golgi apparatus. S1P and S2P were sequentially involved in cleaving SREBP-2 and releasing the mSREBP-2 into the cell nucleus.

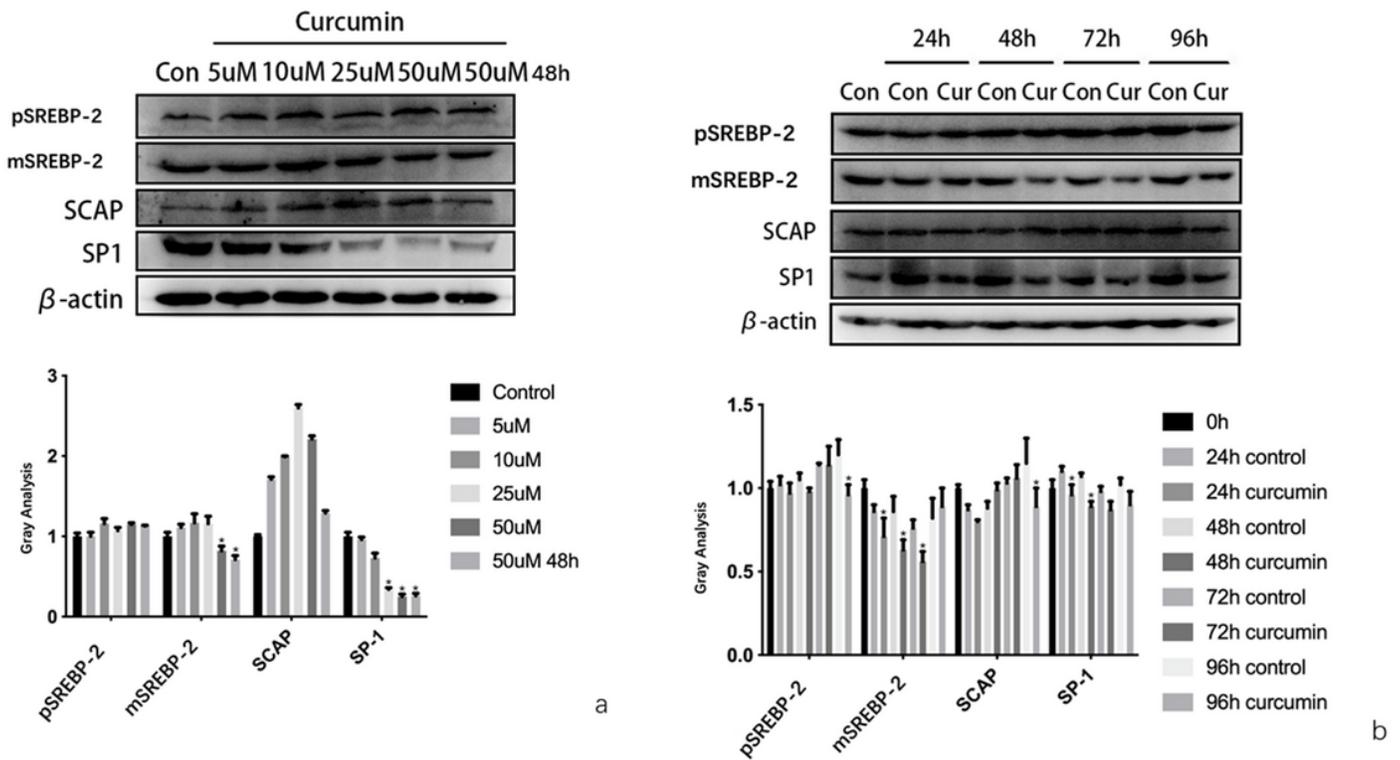
## Figures



**Figure 1**

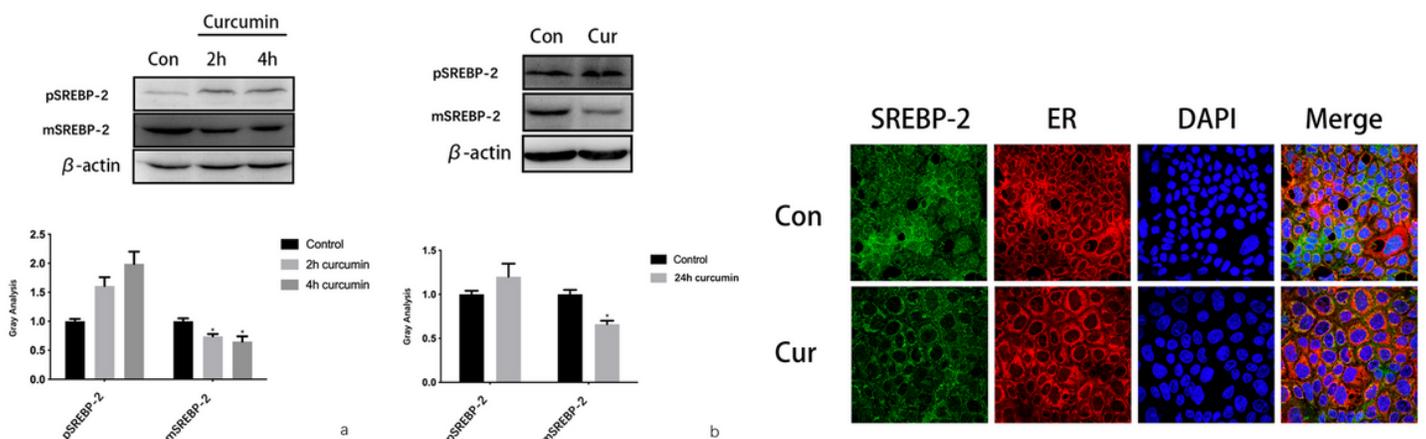
Dose response and time effects of curcumin on the mRNA expression of SREBP-2, SP-1 and SCAP. a. mRNA expression at 24 h and 48h cultivation in different concentrations of curcumin \*,  $P < 0.05$ , vs. the untreated control. b, mRNA expression under 25µM curcumin treatment from 0 ~ 96h. \*,  $P < 0.05$ , vs. the untreated control. c, mRNA expression under 25µM curcumin treatment from 0 ~ 12h. \*,  $P < 0.05$ , vs. the

untreated control. d, The comparison of mRNA expression in control group and curcumin group at each time point. \*,  $P < 0.05$ , vs. the untreated control in each point-in-time.



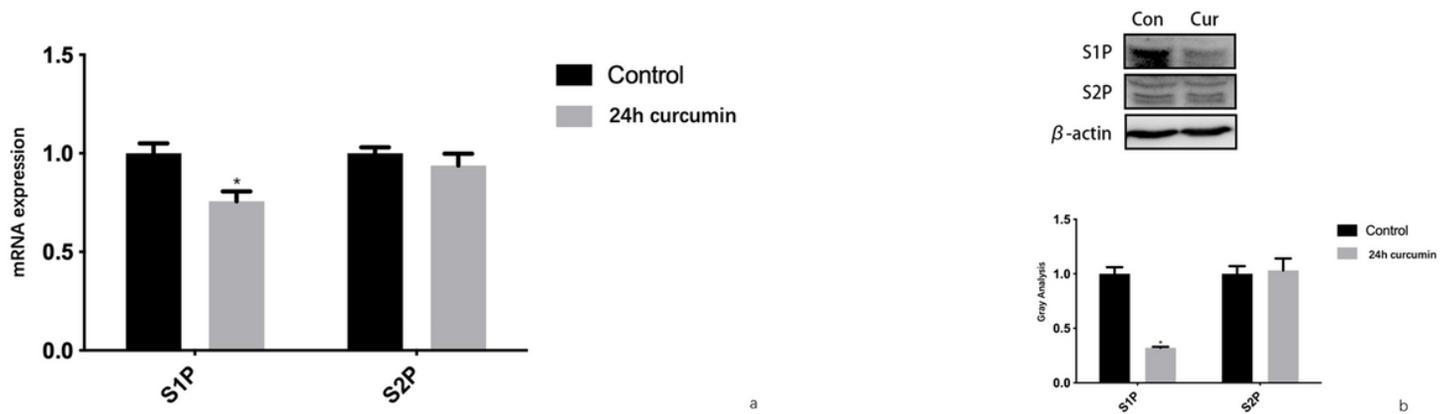
**Figure 2**

In western blotting analysis, the precursor and mature SREBP-2, SP-1, SCAP in cells were treated with curcumin at different concentrations.  $\beta$ -actin was used as an invariant control for equal loading. Representative was shown from three independent experiments. a, The detection of protein expression and the gray value analysis under different concentrations of curcumin treatment at 24h and under 50 $\mu$ M curcumin treatment at 48h. \*,  $P < 0.05$ , vs. the untreated control; #,  $P > 0.05$ , vs. the untreated control. b, The expression of protein and the gray value analysis under 25 $\mu$ M curcumin treatment from 0 to 96h cultivation. \*,  $P < 0.05$ , vs. the untreated control; #,  $P > 0.05$ , vs. the untreated control.



### Figure 3

The intracellular distribution changes of precursor and mature SREBP-2 incubated for different time (0-24h) under curcumin intervention. a. The expression changes of precursor and mature SREBP-2 in the cytoplasm and nucleus of precursor and mature SREBP-2 for different time(0-4h) under 25 $\mu$ M curcumin intervention was detected by western blot and the gray intensity was analyzed. \*,  $P < 0.05$ , vs. the untreated control; #,  $P > 0.05$ , vs. the untreated control. b. Western Blot was used to detect the protein expression in the cytoplasm and nucleus of precursor and mature SREBP-2 after 24h cultivation under 25 $\mu$ M curcumin intervention. \*,  $P < 0.05$ , vs. the untreated control; #,  $P > 0.05$ , vs. the untreated control. c. The images of co-localization of SREBP-2 in endoplasmic reticulum after 24h cultivation under 25 $\mu$ M curcumin intervention. Green fluorescence indicates the SREBP-2, red fluorescence indicates the endoplasmic reticulum and blue fluorescence indicates the cell nucleus.



### Figure 4

The expression of S1P/S2P mRNA and protein under 25 $\mu$ M curcumin treatment. a, The change of mRNA expression. \*,  $P < 0.05$ , vs. the untreated control (first column). b. The western blot and the gray value analysis results.  $\beta$ -actin was used as an invariant control for equal loading. \*,  $P < 0.05$ , vs. the untreated control (first column).

## Supplementary Files

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- [SketchMap.TIF](#)