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Abstract

15-Lipoxygenase-2(15-LOX-2) is thought to regulate inflammation and immunological function; however, its mechanisms of action are still unclear. Furthermore, it has been reported that salidroside has anti-inflammatory properties, but its role in macrophage function has not been understood yet. In this study, we aimed to determine how 15-LOX-2expression levels affect the function of macrophages and the effect of salidroside on 15-LOX-2-deficient macrophages. We used multiple functional genetic strategies to determine 15-LOX-2 function in macrophages.15-LOX-2 deficiency promotes phagocytosis and proliferation of macrophages and impairs their apoptosis. Mechanistically, the expression levels of cyclophilinB (CypB) were upregulated in 15-LOX-2-deficient Ana-1 macrophages, whereas those ofcaspase-3were downregulated. Furthermore, RNA-seq analysis showed that inflammation, complement, and TNF-α signaling pathways were all activated in 15-LOX-2-deficient Ana-1 macrophages. Treatment of 15-LOX-2-deficient macrophages with salidroside, a natural product derived from *Rhodiola* species, effectively reversed the effects of 15-LOX-2 deficiency on caspase-3 and CypB levels, as well as on apoptosis and proliferation. In conclusion, our study shows that there is a newly identified link between 15-LOX-2 deficiency and salidroside in regulating macrophage survival, proliferation, and function. Salidroside may be a promising therapeutic strategy for treating inflammation-related diseases resulting from 15-LOX-2 deficiency.

Keywords:15-Lipoxygenase-2, macrophages, Rhodiola, salidroside, cyclophilin B

1. Introduction

Mononuclear phagocytes such as dendritic cells, monocytes, and macrophages play an essential role in inflammation by eliminating pathogens and producing inflammatory mediators [1,2]. Under inflammatory conditions, monocytes can differentiate into inflammatory macrophages, which can be further polarized to become either M1 or M2 macrophages[3].

Most studies defining the roles of macrophages in immunology have been based on their chemokine and cytokine profiles [4]. Arachidonic acid (AA), a lipid mediator, has been shown to be produced by a subset of macrophages. AA serves as a substrate for the biosynthesis of eicosanoids. More specifically, AA can be metabolized by arachidonic acid lipoxygenases (ALOXs), cyclooxygenases, and cytochromeP450 [5,6]. The metabolism of AA by 15-LOX-2 predominantly produces 15(S)-hydroxy-eicosatetraenoic acid [15(S)-HETE] that can bind to and activate peroxisome proliferator-activated receptor γ (PPARγ), which is known to be involved in inflammatory responses and inflammation-based diseases. 15(S)-HETE has been shown to have different effects on different diseases and cell types. For example, it can stimulate the proliferation of primary human pulmonary artery smooth muscle cells [7-9]. However, we and others have shown that it acts as a suppressor gene in tumorigenesis and suppresses tumor cell growth [10,11]. In macrophages,15-LOX-2 function and the regulatory mechanism of its expression along with strategies for therapeutic interventions are poorly understood.

Salidroside is an active component extracted from plants of the genus *Rhodiola*, which

are used intraditional Chinese medicine [12]. Previous studies have shown that salidroside exerts extensive pharmacological activities, such as antioxidant, anti-cancer, and anti-cardiovascular effects, by repressing inflammation and oxidative stress [13-17]. Notably, salidroside also has diverse roles in different cells under different conditions. For example, salidroside may protect quiescent hematopoietic stem cells (HSCs) from damage caused by oxidative stress and can cause proliferating HSCs to become quiescent *in vivo* [16]. Based on recent literature reports on the functions of salidroside, in this study, we used two strategies (shRNA and CRISPRa) to regulate the expression of 15-LOX-2 in macrophages and used salidroside to treat 15-LOX-2-deficient macrophages. We aimed to investigate whether and how lipoxygenase 15-LOX-2 induced macrophage dysfunction and find an effective treatment for diseases associated with this condition.

2. Materials and Methods

2.1 Cell lines

The Ana-1 cell line was purchased from Boster Biological Technology Co., Ltd. (Wuhan, China). RAW264.7 macrophages were obtained from the Chinese Academy of Sciences Shanghai Branch. Both cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). Human umbilical vein endothelial cells(HUVECs) were obtained from CCLY LAB, State Key Laboratory of Biotherapy, and cultured with Dulbecco's modified Eagle's medium containing 10%FBS. All cells were incubated in an incubator at 37°C with an atmosphere of 5% CO_2.

2.2 Cloning 15-LOX-2-underexpressing and overexpressing cell lines

To create a 15-LOX-2 shRNA, the appropriate15-LOX-2 primers were cloned into the pMSCV-mir30-SV40-GFP retroviral construct. Virus packaging and infection were performed as reported previously [10]. Cells stably expressing 15-LOX-2 shRNA were selected using G418. sgRNAs (Table 1) were designed using Broad Institute's web portal (https://www.genscript.com/gRNA-design-tool) and cloned into TRE-SV40-GFP/dCas9-VP64-IRES-AtTA. The CRISPR/dCas9(CRISPRa)system consisted of these two vectors, which were used to package the virus and coinfect cells, respectively. Cells expressing GFP were sorted using flow cytometry to identify and isolate cell lines with 15-LOX-2 upregulation.

2.3 RNA sequencing

Total RNA was sequenced using BGISEQ500, and the results of the sequencing were analyzed using 50-bp single-end reads. The reads were aligned to the reference genome (GRCm38) using STAR_2.6.0. Transcript abundance was normalized and measured in reads/fragments per kilo base per million mapped reads (RPKM/FPKM). DESeq2 was used to analyze differential gene expression. Genes with absolute fold changes in expression levels greater than 1 and a false discovery rate≤0.05 were considered differentially expressed genes. The characteristic differences between samples were assessed using principal component analysis(PCA). Based on the designated clusters, gene set enrichment analysis (GSEA) was performed to statistically analyze similarities and differences between two types of samples. The top100 genes in our RNAsequencing

data were ranked according to the degree of differential expression between the two groups. Genes with high and low expression and having an inflammatory signature were analyzed by GSEA.

2.4 Proliferation assay

The cells were incubated with the medium containing 10% FBS and supplemented with or without 100μM salidroside (cat: #CSN17210, CSNpharm, Shanghai, China). After 48 h, cell proliferation was assessed using a Cell-Light^{TM} Edu Apollo643 In Vitro Kit (cat: #C10310-2, Ribobio, Guangzhou, China) according to the manufacturer's protocol. The proportion of proliferating cells was determined using flow cytometry(ACEA/Agilent, NovoCyte,San Diego, CA, USA).

2.5 Apoptosis assay

Ana-1-sh15-LOX-2 cells were seeded at 3×10^5 cells/mL in 6-well plates. The cells were treated with or without 100μM salidroside dissolved in H_2O. After 48 h, the cells were assayed for apoptosis using an Annexin V PE Apoptosis Dtec Kit (cat: #559763,BD Biosciences, Franklin Lakes,NJ, USA) according to the manufacturer's protocol. For each sample, more than 20,000 cells were collected. The number of Annexin V- and 7ADD-positive cells was analyzed and expressed as a percentage of the total number of cells in four separate fields. Samples were analyzed using flow cytometry (BD Biosciences), and the data were analyzed using FlowJo v10.

2.6 Western blotting

The cells were lysed by the addition of RIPA (cat: #CW2333,CWBio,Beijing, China) containing protease inhibitors (cat: #A32961,ThermoFisher Scientific, Waltham, MA, USA). Proteins were separated by electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels and then transferred to polyvinylidene fluoride membranes (Immobilon-P,ThermoFisher Scientific). The membrane was blocked at 25℃with 5% fat-free milk in phosphate-buffered saline (PBS) and 0.1% Tween-20 (0.1% PBS-T) for 1 h and then incubated with the appropriate primary antibody [15-LOX-2, 1:200; caspase-3, 1:1000; cyclophilinB (CypB), 1:1000; β-tubulin, 1:1000] (Table2) overnight at 4°C, followed by incubation with the appropriate secondary antibody (anti-rabbit or anti-mouse IgG HRP-linked, 1:10000) for 1 h at room temperature. Immunoreactive proteins were detected using the VilberLourmat imaging system (Fusion Fx7,VilberLourmat,Marne-la-ValléeCedex 3, France).

2.7 Quantitative reverse transcription PCR

Total RNA was isolated from cells using RNAiso Plus reagent (cat: #9109, Takara, Dalian, China) and quantified using a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific). RNA was transcribed using RevertAid First Strand cDNA Synthesis Kit (cat: #K1622, Thermo Fisher Scientific).Reverse transcription PCR (Table 3) primers were designed usinghttps://pga.mgh.harvard.edu/primerbank/.Quantitative reverse transcription PCR(qRT-PCR) was performed using a PowerUp SYBR Green Master Mix (cat: #A25742,Thermo Fisher Scientific) and the LightCycler96 system (Roche,Basel,

Switzerland).

2.8 Oil Red O staining

To assess the effect of 15-LOX-2 on the phagocytosis of macrophages, sh15-LOX-2Ana-1 macrophages were seeded at a density of 2.5 × 10^5 cells/mL in 12-well plates and treated with 0.033mM oleicacid (cat: #YZ-2760S,Extrasynthese, Genay Cedex, France) and 0.066mM palmiticacid (cat: #SP9880, Solarbio, Beijing, China). After 48 h, the cells were fixed with 4% paraformaldehyde for 10minand stained with Oil Red O (cat: #O8010, Solarbio) at 37°C for 15min. Microscopy was performed using OLYMPUSDP73(OLYMPUS, Japan).

2.9 Lipid droplet staining and detection using a laser confocal microscope

Ana-1-sh15-LOX-2 cells were seeded at a density of 2.5 × 10^5 cells/mL in 12-well plates and treated with 0.033mM oleicacid and 0.066mM palmiticacid. After 48 h, the cells were fixed with 4% paraformaldehyde for 10min and stained with the cell membrane red fluorescent probe DiI(cat: #C1036,Beyotime,Shanghai, China) at 37°C for 15 min, followed by staining with DAPI (cat: #AR1176,BOSTER, Wuhan, China) for 5 min. Optical density (OD) was determined at 550nm for DiI and 360nm for DAPI using a microplate reader (FluoroskanAscent FL, Thermo Fisher Scientific). Lipid droplet density in sh15-LOX-2 cells was calculated as relative absorbance compared with that in control-shRen. Furthermore, the cells were analyzed using a laser confocal microscope (FV3000, OLYMPUS).

2.10Statistical analysis

All experiments were performed three times independently. Data are shown as mean ± SD. Comparisons between groups were analyzed using one-way ANOVA. Differences were expressed as p-values; $p < 0.05$ was considered statistically significant.

3. Results

3.1 15-LOX-2 deficiency promotes phagocytosis of macrophages

To validate the role of 15-LOX-2 in macrophages, two independent 15-LOX-2 shRNAs (sh15-LOX-2.1252 and sh15-LOX-2.2865) or control shRen were introduced into GFP and Neo vectors (Fig. 1a). Then, the 15-LOX-2 shRNAs were introduced into macrophages to construct stable cell lines witha 15-LOX-2 deficiency (Supplementary figure1a,b). Then, western blotting (WB) was performed to verify the efficiency of shRNA targeting of 15-LOX-2. As shown in Figure 1b, the expression of 15-LOX-2 in 15-LOX-2 shRNA Ana-1 macrophages was lower than that in shRen Ana-1 macrophages.

As is known, the formation of foam cells via increased phagocytosis of lipid droplets by macrophages is an important step in atherosclerosis (AS) [18]. Therefore, to further study the function of 15-LOX-2-deficientAna-1 macrophages, the phagocytosis of lipid droplets was analyzed. Notably, as shown in Figure 1c and Supplementary Fig.2a, the cytoplasm of 15-LOX-2-deficient Ana-1 macrophages were filled with lipid droplets, whereas few lipid droplets were observed in the cytoplasm of shRenAna-1 macrophages. The fluorescence OD also showed that lipid droplets were markedly higher in 15-LOX-2-deficient Ana-1

195 macrophages than in shRenAna-1 macrophages (Fig. 1d).These data suggest that the
196 phagocytosis of lipids by macrophages was enhanced following15-LOX-2 deficiency.
197
198 **3.2 15-LOX-2 deficiency promotes macrophage proliferation and inhibits its**
199 **apoptosis**
200 Based on the above data, we further assessed both proliferation and apoptosis in
201 15-LOX-2-deficient Ana-1 macrophages using Edu staining and Annexin V staining,
202 respectively. Increased proliferation was observed 15-LOX-2-deficient Ana-1
203 macrophages when compared with that in control Ana-1 macrophages (Fig. 2a); the
204 percentage of cells in S-phase in Ana-1 cells expressing shREN, sh15-LOX-2.1252, and
205 sh15-LOX-2.2865 macrophages were 16.5, 30.7, and 47.3% (n=3, $p<0.01$), respectively
206 (Fig. 2b). As shown in Figure2c, the percentage of early apoptotic cells was lower in
207 15-LOX-2 -deficient Ana-1 macrophages than in control Ana-1 macrophages. More
208 specifically, the percentage of early apoptotic Ana-1 macrophages expressing shREN,
209 sh15-LOX-2.1252, and sh15-LOX-2.2865 was 8.24, 2.61, and 1.71% (n=3, $p<0.05$),
210 respectively (Fig. 2d). All these data suggest that 15-LOX-2 deficiency may promote
211 macrophage activity.
212
213 **3.3 15-LOX-2 deficiency changes the expression of protein related to cell activity**
214 **and apoptosis**
215 It is known that the proliferation and apoptosis of macrophages with increased ability to
216 phagocytize lipids play an important role in the development of AS [19]. It has also been

reported that AS is a disease closely related to hypoxia, and CypB may inhibit cell death induced by hypoxia [20,21]. Therefore, WB was performed to assess the expression levels of CypB in 15-LOX-2-deficient Ana-1 macrophages. As shown in Figure 3a and b, compared with those in control Ana-1 macrophages, the expression levels of CypB in 15-LOX-2-deficient Ana-1 macrophages increased significantly($p<0.05$). Caspase-3 encodes a cysteine protease that has been linked to the promotion of cell apoptosis. WB was performed to examine the expression levels of caspase-3 protein in 15-LOX-2-deficient Ana-1 macrophages. As shown in Figure3c and d, compared with those in control, caspase-3 expression levels were significantly reduced in15-LOX-2-deficient Ana-1 macrophages.

To further confirm its function on apoptotic proteins, we designed sgRNAs targeting the untranslated regions of 15-LOX-2 and constructed the CRISPR/dCas9(CRISPRa) system (Supplementary Figure. 3a). 15-LOX-2 CRISPRa was used to construct RAW264.7macrophages and stable HUVECs, which have a relative function to Ana-1macrophages in AS development (Supplementary Figure. 1c-f). In contrast to 15-LOX-2-deficient cells, CypB expression levels were significantly decreased in RAW264.7 macrophages and HUVECs overexpressing 15-LOX-2 compared with those in control RAW264.7 macrophages and HUVECs, (Supplementary Figure. 4a–c). In addition, the expression levels of caspase-3 were upregulated in both cell types overexpressing 15-LOX-2 (Supplementary Figure. 5a–d).

3.4 15-LOX-2 deficiency is associated with enhancement of inflammation-related

pathways

To further explore the molecular mechanisms of 15-LOX-2 on macrophage function, RNA-seq was performed to analyze the transcriptomes of Ana-1 macrophages expressing sh15-LOX-2 or shRen. Both unsupervised clustering and PCA plots showed that Ana-1 macrophages expressing sh15-LOX-2.1252 or sh15-LOX-2.2865 were grouped together and clearly separated from shRen cells, indicating that the off-target effects of these two shRNAs are minimal (Fig. 4a and b). Notably, compared with those in the control shRen-associated transcriptome, multiple gene sets related to inflammation, complement pathway, and TNF-αsignaling pathway were activated in sh15-LOX-2-expressing cells (Fig. 4c–e). The results of qPCR revealed that compared with those in shREN control macrophages, *Cx3cl1*, *Il4*, and *Il10,*all related to the pathways identified, were upregulated in sh15-LOX-2-expressing Ana-1 macrophages. However, the expression of TNF-αwas downregulated, indicating that 15-LOX-2deficiency regulates the inflammatory response in Ana-1 macrophages (Fig. 4f).

3.5 Salidroside can reverse the abnormal function of macrophages caused by 15-LOX-2 deficiency

We investigated the effect of the natural product salidroside, which we hypothesized might reverse these changes in 15-LOX-2-deficient macrophages. Salidroside has been shown to exert various pharmacological effects, including antioxidative stress and anti-inflammatory properties [22,23]. In this study, after treatment of 15-LOX-2-deficient Ana-1 macrophages with salidroside, the upregulation of CypB expression caused by

15-LOX-2 deficiency was significantly reversed, returning to levels close to those of control Ana-1 macrophages (Fig. 5b and c). In addition, caspase-3 levels in 15-LOX-2-deficient cells were restored to normal levels by salidroside (Fig. 5d and e). Similar results were obtained from the analysis of cell proliferation and apoptosis in 15-LOX-2-deficient Ana-1 macrophages treated with salidroside. As shown in Figure 5f, the increased proliferation observed in 15-LOX-2-deficient macrophages was attenuated by salidroside. Similarly, as shown in Figure 5g, the reduced apoptosis observed in 15-LOX-2-deficient Ana-1 cells were dramatically restored to normal levels by salidroside. These data indicate that salidroside can ameliorate the changes in macrophages caused by 15-LOX-2 deficiency.

4. Discussion

In this study, we aimed to determine how 15-LOX-2 expression levels affect the function of macrophages. We also determined the effect of salidroside on15-LOX-2 deficiency-induced dysfunction in macrophages. We found that salidroside restored the changes in macrophages caused by 15-LOX-2 deficiency. Macrophages are now known to have diverse and context-dependent functions in a variety of pathophysiological settings [24]. There is a rapidly growing interest in understanding how metabolic process-related genes, including lipoxygenases, can affect the appropriate activation of macrophages to enable host defense mechanisms. Multiple studies have proven that 15-LOX-2 plays a role in cancer and diseases of lipid metabolism [10,25-27]. Recent studies have also suggested that lipids regulate the inflammatory responses and

283 phagocytosis of macrophages [28,29]. However, little is known about the importance of
284 15-LOX-2 and its relationship to physiological events in macrophages. In this study, using
285 both loss-of-function and gain-of-function models and transcriptomics approaches, we
286 highlight the fact that abnormal expression of 15-LOX-2 (either under- or overexpression)
287 regulates the phagocytosis of lipids by macrophages.

288 15-LOX-2 has been found to affect the development of tumors through an impact on
289 tumor cell apoptosis and proliferation in previous studies [30,31]. In this study, we found
290 that15-LOX-2 deficiency inhibited apoptosis and promoted the proliferation of
291 macrophages. It has also been reported that 15-LOX-2 products[15(S)-HETE] might
292 promotecaspase-3 activation and markedly inhibit the growth of tumor cells [10,32]. Here,
293 we found that changes in 15-LOX-2 levels may result in the abnormal expression of
294 caspase-3 protein, thus affecting cell survival. Notably, we found for the first time that
295 15-LOX-2 may regulate CypB expression in cells, which has been reported to prevent
296 hypoxia-induced cell death in other studies [33]. In our system, there was a negative
297 correlation between 15-LOX-2 and CypB levels. For example, when 15-LOX-2levels were
298 downregulated, CypB levels were accordingly increased. These results indicate
299 that15-LOX-2 deficiency may regulate genes related to the Hif1α pathway to impact cell
300 growth and functions.

301 In addition, we showed that 15-LOX-2 is a crucial anti-inflammatory and anti-oxidative
302 stress regulator. The downregulation of 15-LOX-2 expression serves as a positive
303 feedback mechanism to activate inflammatory, complement, and TNF-α signaling
304 pathways. Conversely, the upregulation of its expression inhibited the expression of

pro-inflammatory genes.

Salidroside has antioxidant and anti-inflammatory actions, and it may be used to treat diseases associated with these conditions [14,23]. It has been reported that salidroside may promote autophagy, inhibit oxidative stress, and prevent mitochondrial dysfunction by activating the AMPK pathway [34]. Notably, in our study, salidroside was shown to restore abnormal apoptosis and proliferation in macrophages caused by 15-LOX-2 deficiency. Furthermore, it restored the abnormal expression levels of caspase-3, CypB, and other inflammatory genes, which were induced by 15-LOX-2 deficiency, to normal levels.

The main limitation of our study is that our experiments were only carried out in vitro; in vivo experiments need to be performed to further verify our results. Furthermore, the activation of multiple pathways by15-LOX-2 deficiency shown by RNAseq results needs further functional investigation. Nevertheless, we demonstrated that 15-LOX-2 deficiency enhanced lipid phagocytosis by macrophages and several cytokines associated with those inflammatory pathways were regulated by 15-LOX-2.

In conclusion, our findings suggest that 15-LOX-2 deficiency could promote the development of inflammation-related diseases, and these diseases could be alleviated by salidroside. Further research is needed to explore the relationship between 15-LOX-2 and inflammatory diseases, such as AS and cancer, and elucidate the underlying mechanisms. Salidroside may be a promising therapeutic strategy to treat inflammation-related diseases resulting from 15-LOX-2 deficiency.

Data availability statement

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors Contribution

R.H designed the experiments. T.L, X.Y., H.W., X.Z., J.Y., C.Y., P.X., Y.W., D.W, T.X., H.Y.,

Y.C., L.X., X.Z., X.L. performed the experiments; Y.L. contributed to the RNAseq analysis; Z.X., C.Z. and R.H organized data and wrote the manuscript.

Competing Interests Statement

All authors declare no conflict of interest.

Figure legends

Figure 1

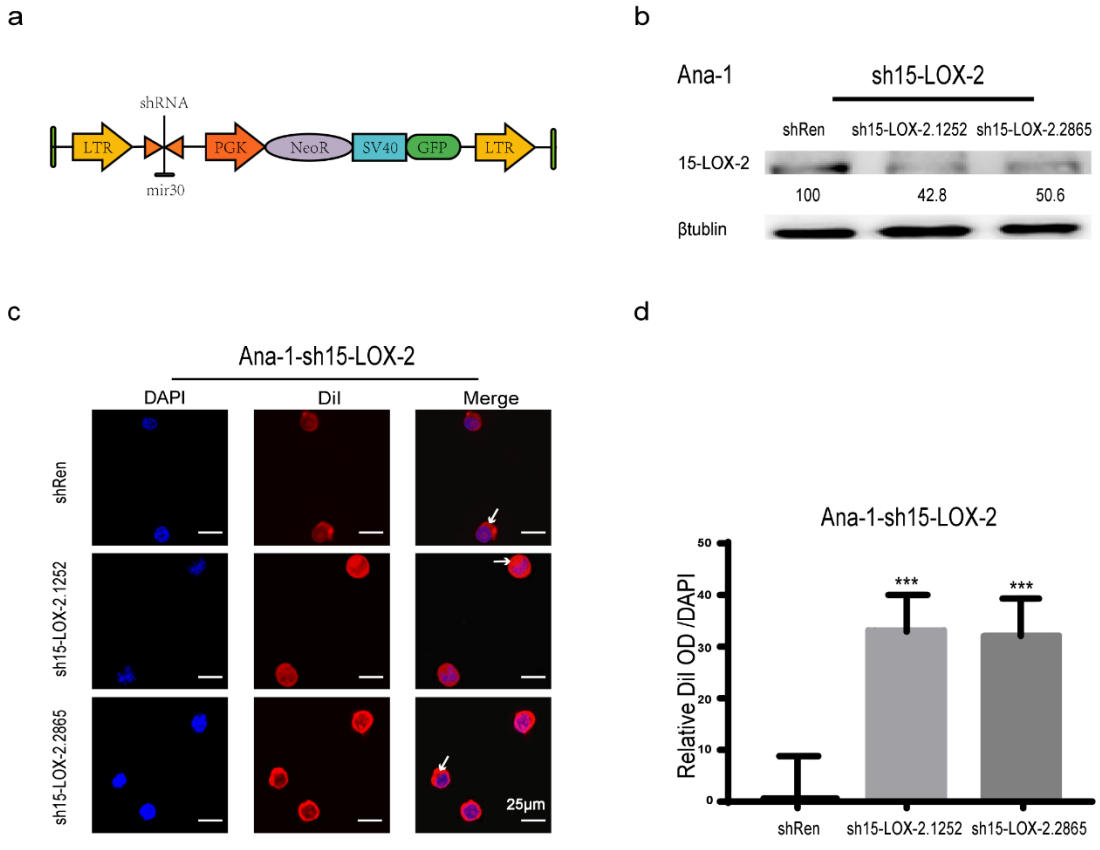


Figure 1. 15-LOX-2 deficiency promotes phagocytosis of macrophages.(a) Vector schematic of MLS, which was constructed in the self-activation retroviral backbone NeoR,

G418 (Geneticin) screening sequence. (b) The knockdown efficiency of 15-LOX-2 by shRNA in Ana-1 was detected by WB and quantitated using ImageJ, compared with shRen.(c) Subcellular localization of lipid droplets, Scale bar 25μm. White arrows show lipid droplets located in the cytoplasm; Red arrow shows cytoplasm without lipid droplets. (d)Lipid droplets per cell was determined using relative DiI Fluorescence OD compared to DAPI OD.(n=3), ***: P<0.001, vs shRen.

Figure 2

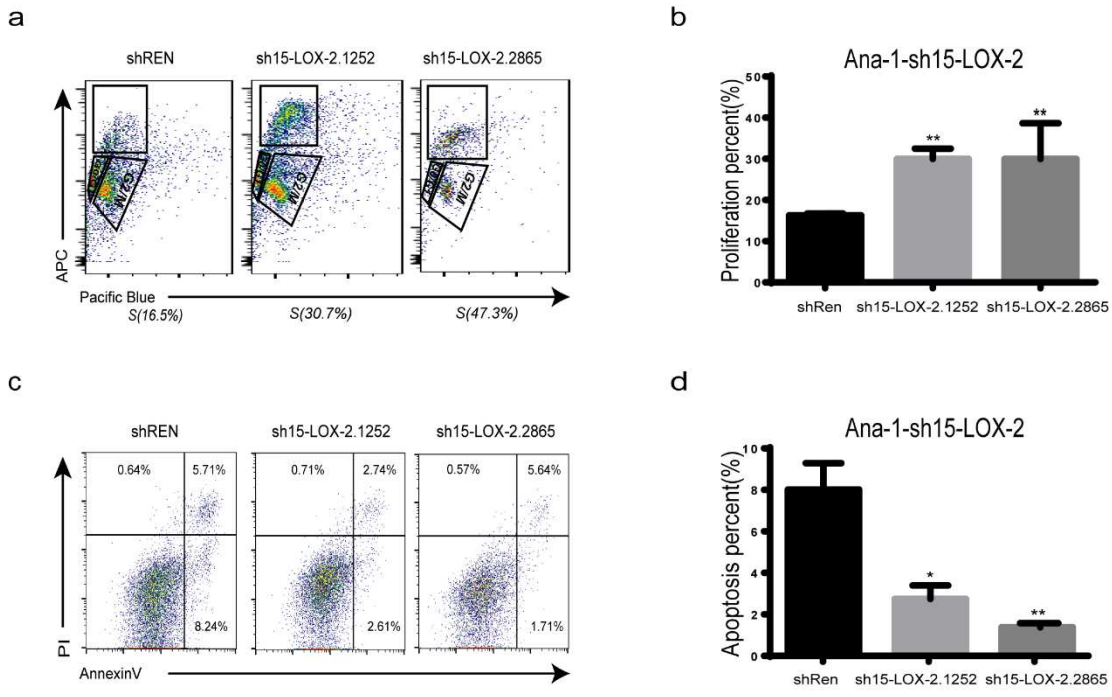
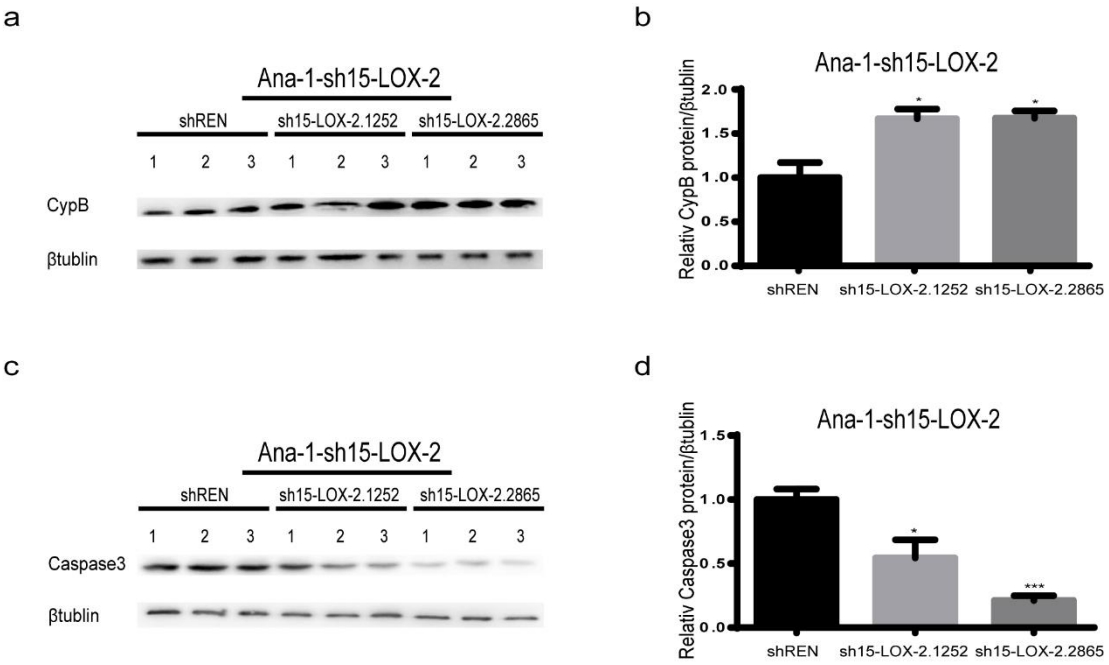


Figure 2.Effects of 15-LOX-2 deficiency on proliferation and apoptosis of macrophages.(a) Representative flow cytometry plot of proliferation of sh15-LOX-2 and shRen macrophages.(b) Proliferation percentage of sh15-LOX-2 and shRen (n=3), **: P<0.01, vs shRen.(c) Representative flow cytometry plot of apoptosis of sh15-LOX-2 and shRen macrophages.(d) Apoptosis percentage of sh15-LOX-2 and shRen (n=3), *: P<0.05, **:P<0.01, vs shRen.

Figure 3



458

459 **Figure 3. 15-Lox-2 deficiency attenuated the expression of proteins related to cell**

460 **activity and apoptosis.**(a, b) The expression of CypB in Ana-1- sh15-LOX-2 and shRen

461 macrophages was detected using WB and quantitated with ImageJ. Results are

462 presented as the mean ±SD (n=3), *:P<0.05, vs shRen.(c) The expression of caspase-3 in

463 Ana-1-sh15-LOX-2 and shRen macrophages was detected using WB. (d) Quantification

464 to (c) is presented as the mean ±SD (n=3), *: P<0.05, ***: P<0.001, vs shRen.

465

Figure 4

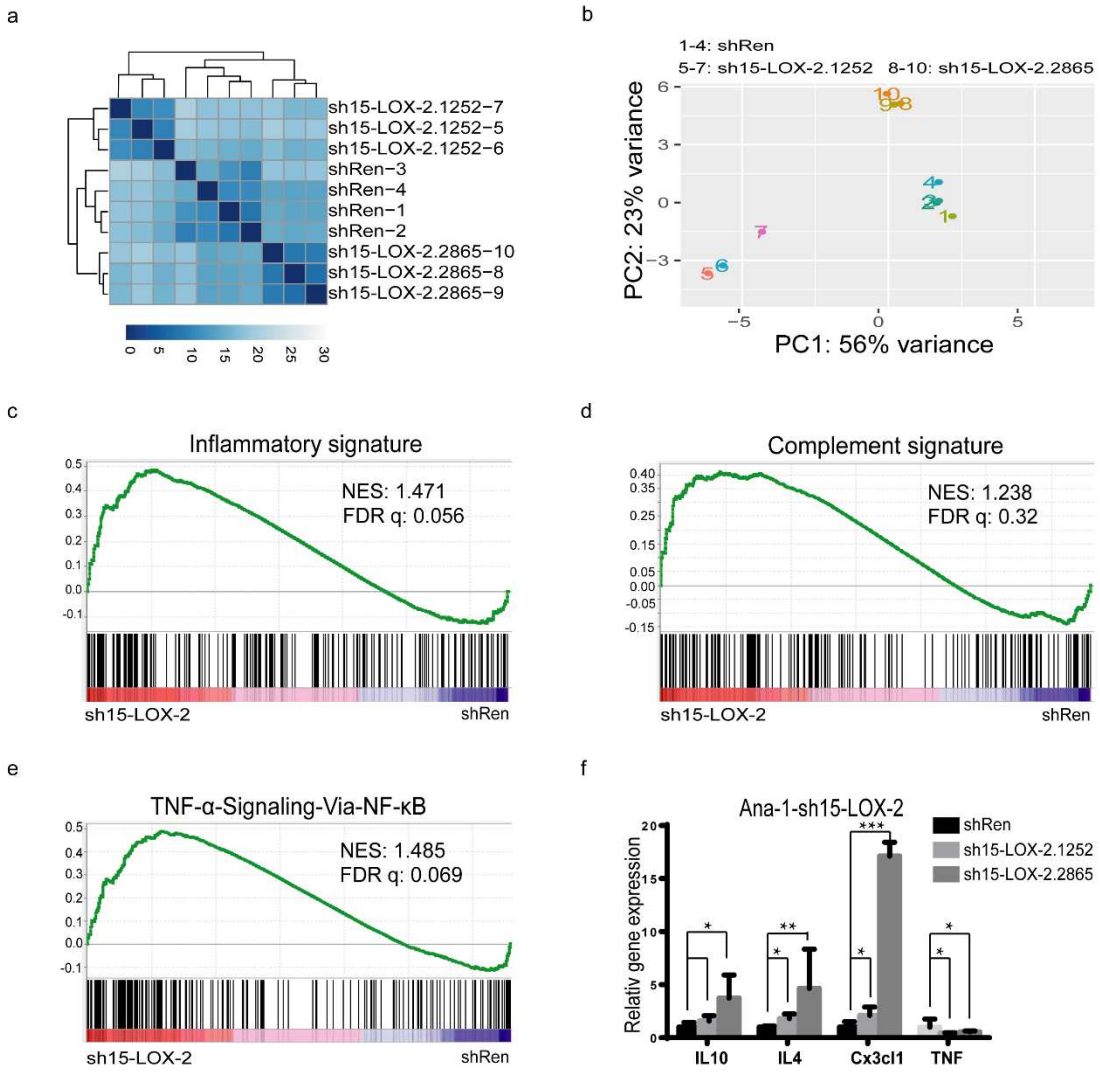


Figure 4. Deficiency of ALOX15B associated with activation of inflammation-related signaling pathway.(a) Unsupervised clustering of RNA-seq data sh15-LOX-2.1252, sh15-LOX-2.2865 or shRen macrophages.(b) PCA analysis of transcriptome ofsh15-LOX-2 and shRen macrophages.(c) GSEA shows positive enrichment of the hallmark-inflammatory-response gene sets in sh15-LOX-2 macrophages compared with shRen macrophages. NES, normalized enrichment score; FDR, false discovery rate. (d) GSEA shows positive enrichment of the hallmark-complement gene set in sh15-LOX-2 macrophages compared with shRen macrophages. (e) GSEA shows positive enrichment

of the hallmark-TNF-α-signaling-via-NF-κB gene set in sh15-LOX-2 macrophages compared with shRen macrophages. (f) Gene *(Il10, Il4, Cx3cl1 or TNF)* expression quantification of 15-LOX-2-deficient macrophages performed by qRT-PCR. Results are presented as the mean ±SD (n=3), *:P<0.05, vs shRen.

Figure 5

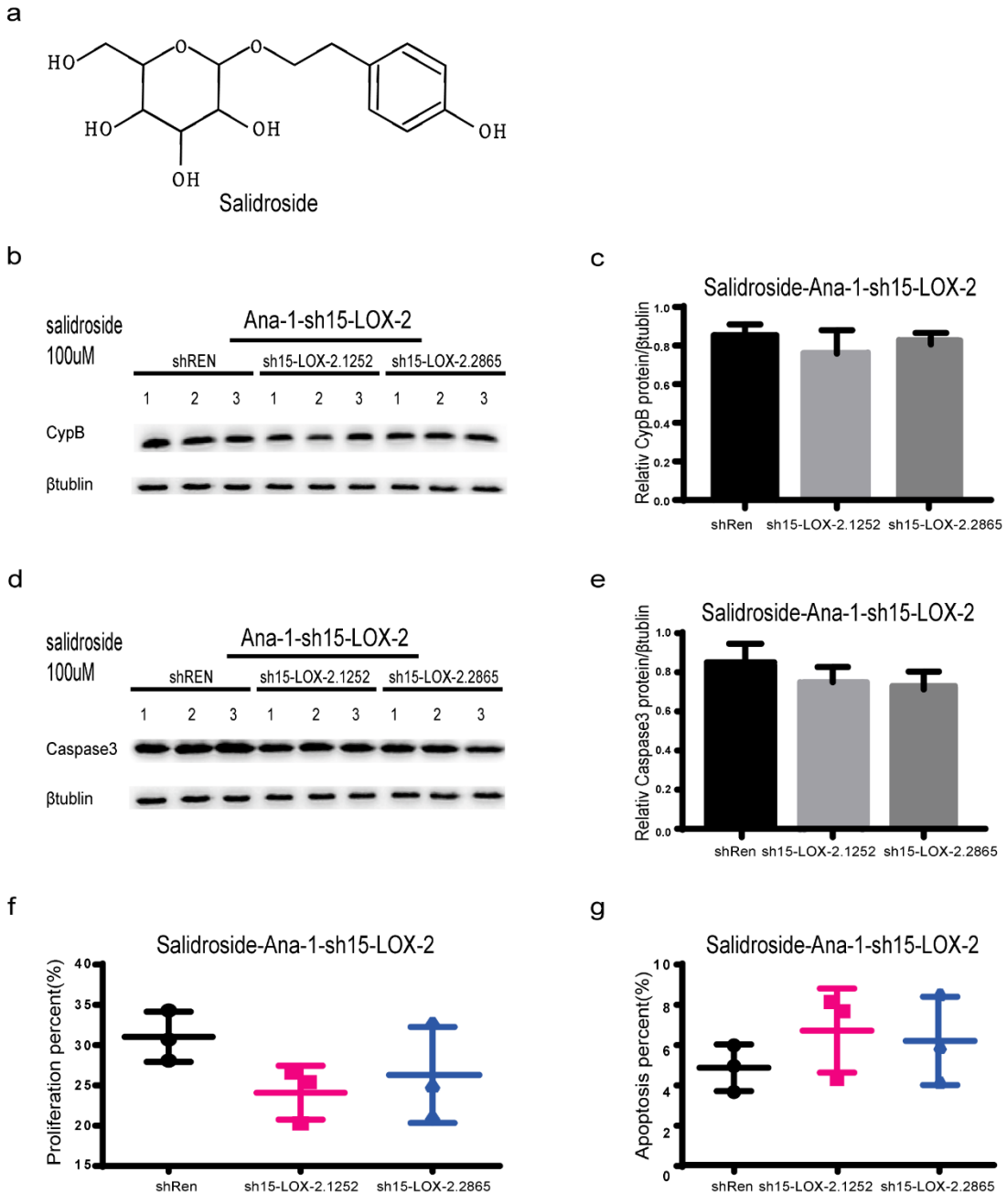


Figure 5. Salidroside can reverse the abnormal function of macrophages caused by 15-LOX-2 deficiency. (a) The chemical structure formula of salidroside. (b, c) Representative WB result shows CypB levels in sh15-LOX-2 and shRen macrophages treated with salidroside and quantitated with ImageJ. Results are presented as the mean ±SD (n=3), *:P<0.05, vs shRen. (d, e) Representative WB result shows the caspase-3 levels in sh15-LOX-2 and shRen macrophages treated with salidroside and quantitated using ImageJ. Results are presented as the mean ±SD (n=3), *: P<0.05, vs shRen. (f)Proliferation percentage of sh15-LOX-2 and shRen treated with salidroside (n=3). (G) Apoptosis percentage of sh15-LOX-2 and shRen treated with salidroside (n=3).

Tables

Table 1. sgRNA used in CRISPRa system

Primers	Primer sequence
15-LOX-2-sgRNA1 forward	5′-GGATGGGCGGGGCATCGCTG-3′
15-LOX-2-sgRNA1 reverse	5′-CAGCGATGCCCCGCCCATCC-3′
15-LOX-2-sgRNA2 forward	5′-CTCAAAGCAGCCTTGTGGCG-3′
15-LOX-2-sgRNA2 reverse	5′-CGCCACAAGGCTGCTTTGAG-3′
15-LOX-2-sgRNA3 forward	5′-CAAAACAAACAGACGTGGT-3′
15-LOX-2-sgRNA3 reverse	5′-ACCACGTCTGTTTGTTTTG-3′
15-LOX-2-sgRNA4 forward	5′-CATCATCCTGGCCTACATGG-3′
15-LOX-2-sgRNA4 reverse	5′-CCATGTAGGCCAGGATGATG-3′

Table 2. Antibodies used in the experiment

Antibodies	Source	Identification
15-LOX-2	Abcam	Cat: #ab23691
Caspase-3	CST	Cat: #9662S
CypB	CST	Cat: #43603
β-Tubulin	Thermo Fisher Scientific	Cat: #MA5-16308

Table 3. Primers used in qRT-PCR

Primers	Primer sequence
β-Actin (human)forward	5′-GTTGTCGACGACGAGCG-3′
β-Actin (human)reverse	5′-GCACAGAGCCTCGCCTT-3′
β-Actin (mouse) forward	5′-ATGGAGGGGAATACAGCCC-3′
β-Actin (mouse) reverse	5′-TTCTTTGCAGCTCCTTCGTT-3′
Cx3cl1 (mouse) forward	5′-ACGAAATGCGAAATCATGTGC-3′
Cx3cl1 (mouse) reverse	5′-CTGTGTCGTCTCCAGGACAA-3′
IL10 (mouse) forward	5′-GTGAGTGAGATGGGCATGTTT-3′
IL10 (mouse) reverse	5′-GAGTGGCAAGAAGGCTGGAT-3′
IL4 (mouse) forward	5′-GGTCTCAACCCCCAGCTAGT-3′
IL4 (mouse) reverse	5′-GCCGATGATCTCTCTCAAGTGAT-3′
TNF (mouse) forward	5′-CCCTCACACTCAGATCATCTTCT-3′
TNF (mouse) reverse	5′-GCTACGACGTGGGCTACAG-3″

Figures

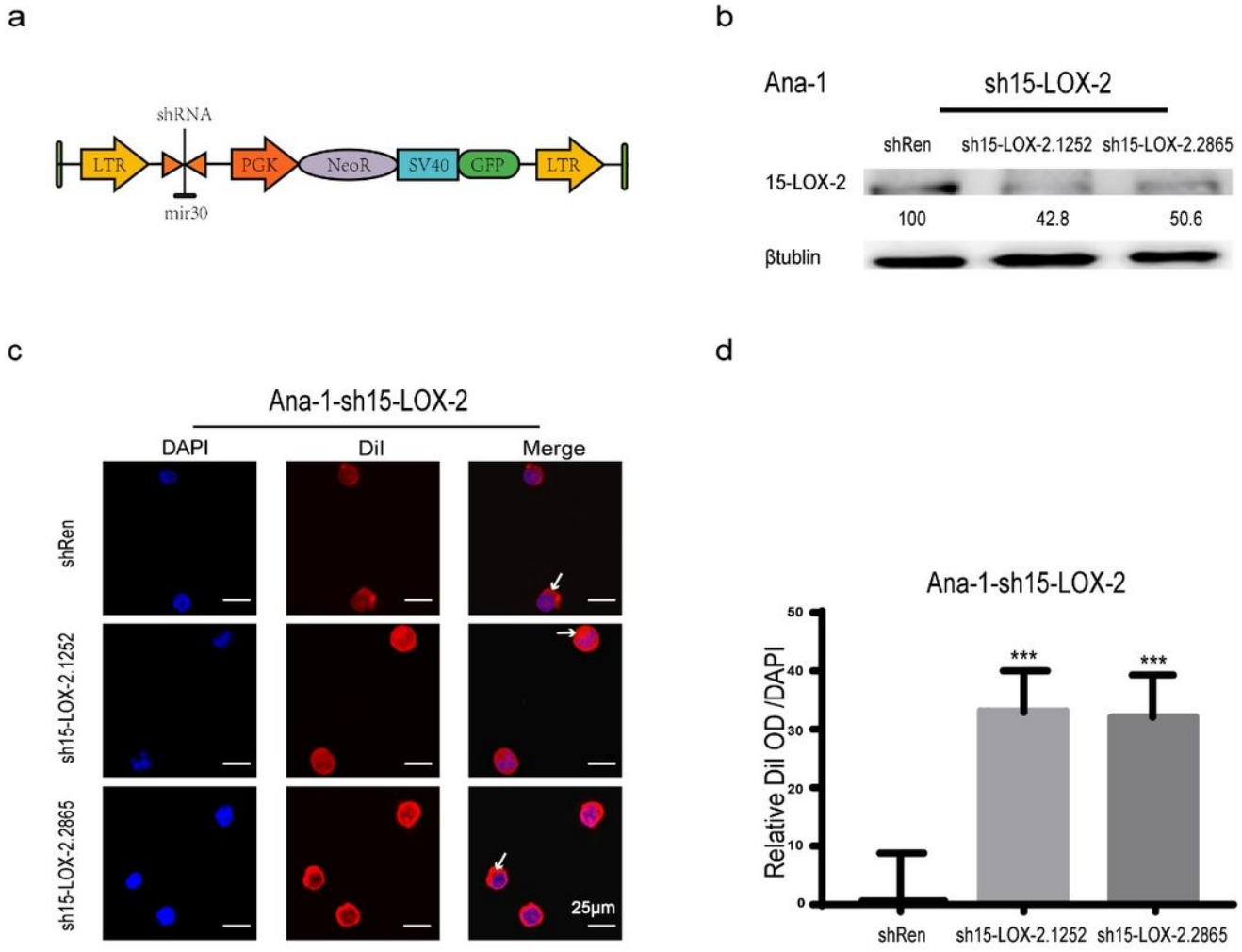


Figure 1

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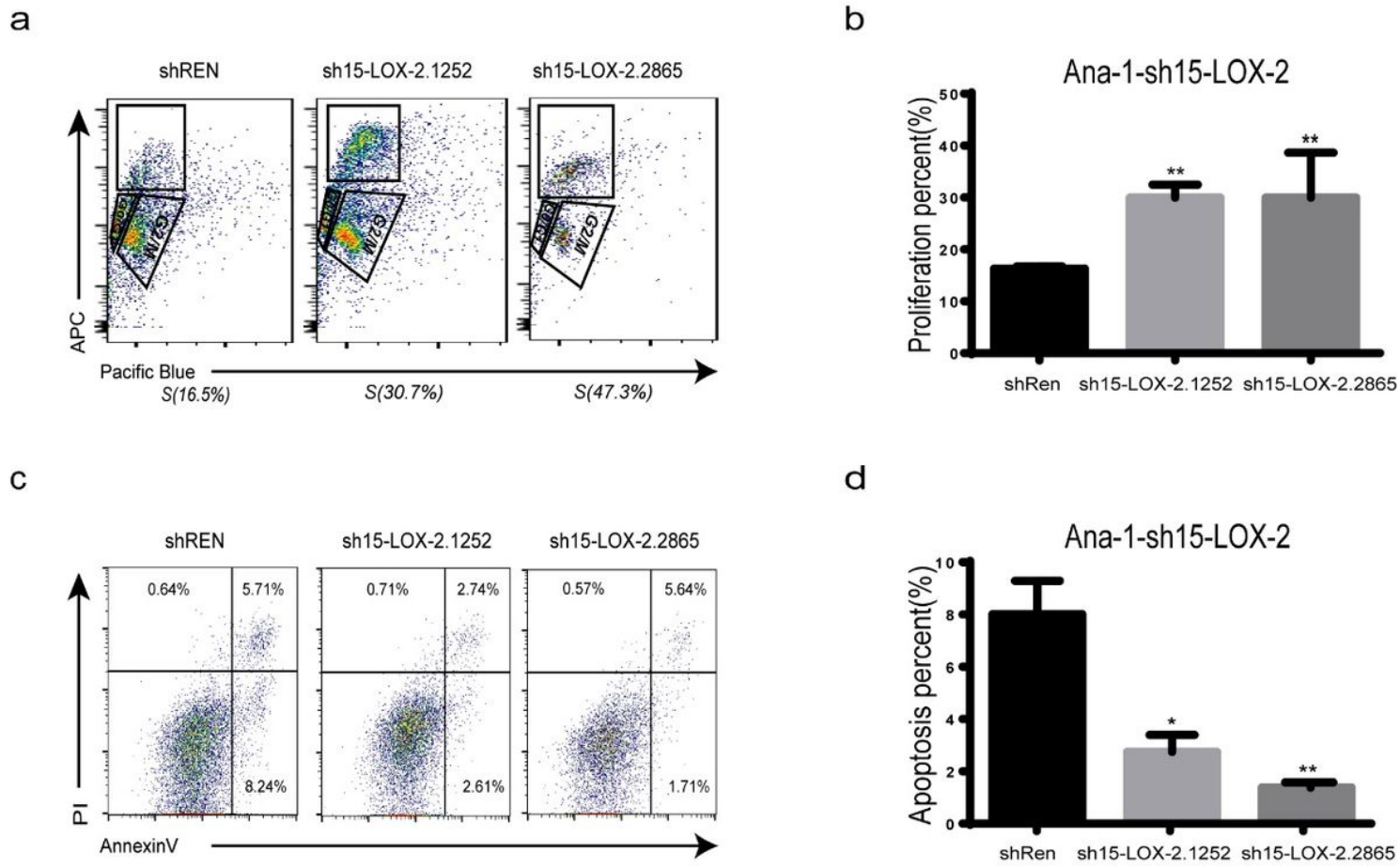

Figure 2

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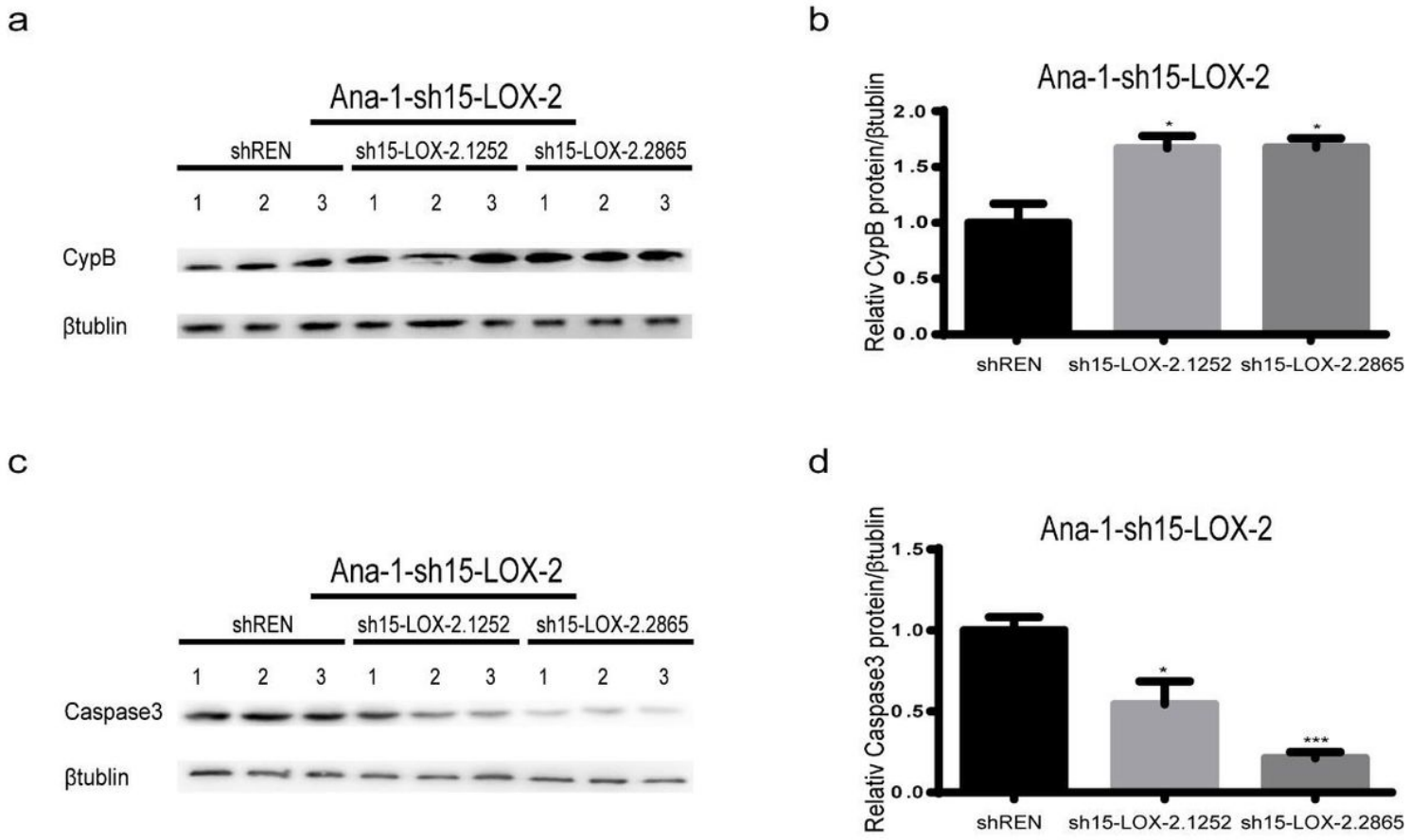

Figure 3

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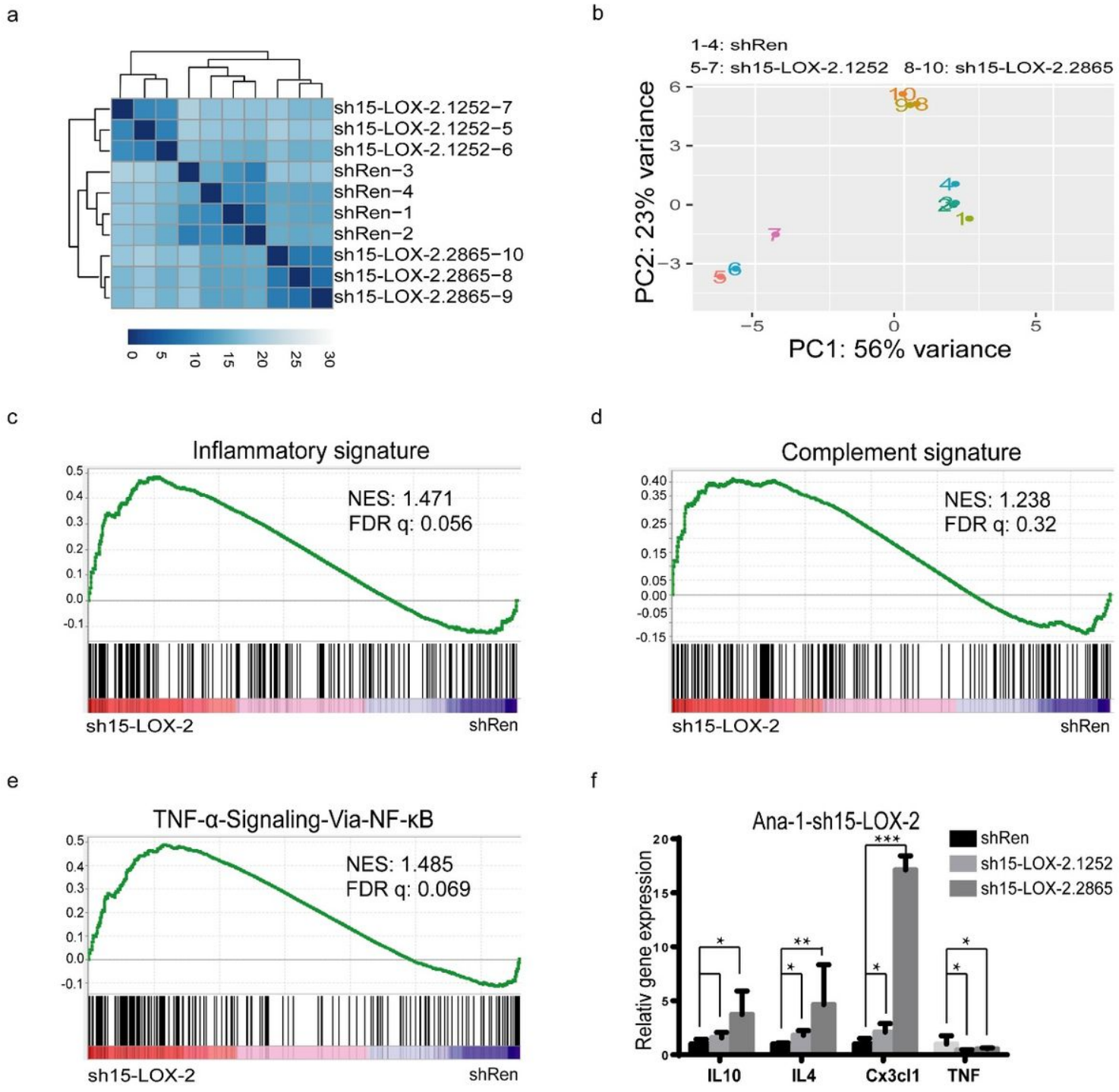

Figure 4

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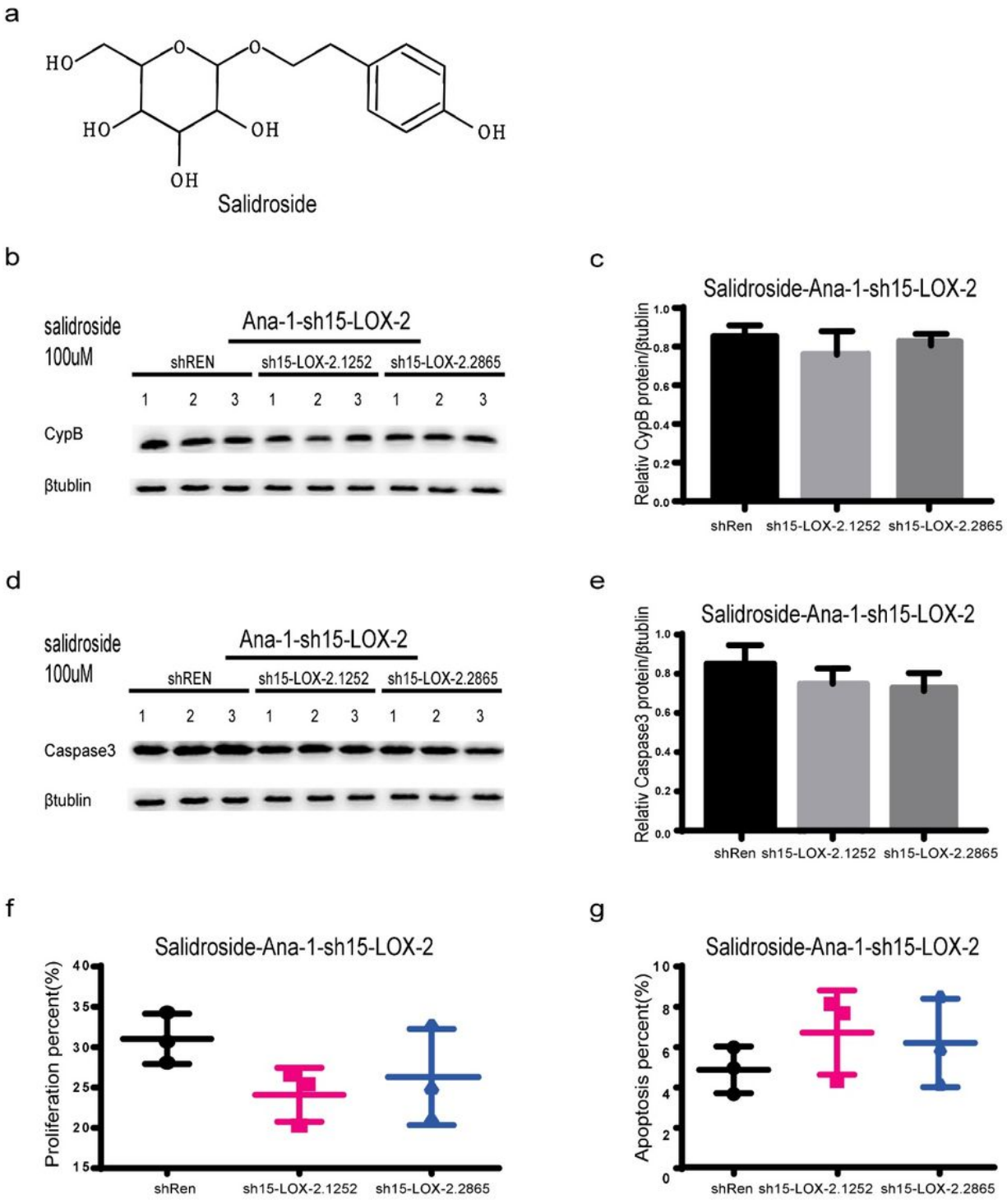

Figure 5

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- supplementaryinformation.pdf