



Yupingfeng San Inhibits NLRP3 Inflammasome to Attenuate the Inflammatory Response in Asthma Mice

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Yupingfeng San (YPFS) is a representative Traditional Chinese Medicine (TCM) formula with accepted therapeutic effect on Asthma. However, its action mechanism is still obscure. In this study, we used network pharmacology to explore potential mechanism of YPFS on asthma. Nucleotide-binding oligomerization domain (NOD)-like receptor pathway was shown to be the top one shared signaling pathway associated with both YPFS and asthma. In addition, NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome was treated as target protein in the process of YPFS regulating asthma. Further, experimental validation was done by using LPS-stimulated U937 cells and ovalbumin (OVA)-sensitized BALB/c mice model. *In vitro* experiments showed that YPFS significantly decreased the production of TNF- α and IL-6, as well as both mRNA and protein levels of IL-1 β , NLRP3, Caspase-1 and ASC in LPS-stimulated U937 cells. *In vivo* experiment indicated that YPFS treatment not only attenuated the clinical symptoms, but also reduced inflammatory cell infiltration, mucus secretion and MUC5AC production in lung tissue of asthmatic mice. Moreover, YPFS treatment remarkably decreased the mRNA and protein levels of IL-1 β , NLRP3, Caspase-1 and ASC in lung tissue of asthmatic mice. In conclusion, these results demonstrated that YPFS could inhibit NLRP3 inflammasome components to attenuate the inflammatory response in asthma.

Keywords: Yupingfeng San, asthma, network pharmacology, NLRP3 inflammasome, inflammation

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many inflammatory cells play a role. This disease is characterized by recurrent episodes of wheezing, breathlessness, chest tightness and coughing (Braman, 2006; Mims, 2015). The cause of asthma is unknown, but it is generally thought that gene-environment interactions have played important roles in risk factors (Ober and Yao, 2011; Bonnelykke and Ober, 2016). Previous study revealed that genetic predisposition and

environmental exposures were most likely to cause inflammation in the lower airway (Bunyavanich and Schadt, 2015). Current research showed that Nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain-containing 3 (NLRP3), one of nucleotide-binding domain and leucine-rich repeat containing (NLR) family membranes, had a substantial impact on tissue inflammation including respiratory diseases like asthma and could aggravate the progress of asthma (Kufer and Sansonetti, 2011; Im and Ammit, 2014; Hosseini et al., 2015). For this reason, NLRP3 inflammasome has been suggested as an important target for inflammatory disease control (Peng et al., 2016). In addition, it has been reported that NLRP3 inflammasome activation could lead to the increased release of interleukin (IL)-1 β (Haneklaus and O'Neill, 2015). There's studies proved that NLRP3 mediated IL-1 β could lead to a vicious cycle between previous and future exacerbations in asthma (Kim et al., 2017). For this reason, NLRP3 inflammasome and IL-1 β have been suggested as important targets for inflammatory disease control (Fu et al., 2015; Peng et al., 2016).

Commonly, presently available drugs such as corticosteroids, biologic therapies have been found beneficial in controlling the asthma, but it had been proved with frequent side effects (Cooper et al., 2015). *Yupingfeng San* (YPFS), originally recorded in the book *Danxi Xinfu*, is a Chinese medicine formula consisting of *Astragalus membranaceus*, *Atractylodes macrocephala*, and *Saposhnikovia radix*. YPFS has been found to have therapeutic effect on asthma and with less adverse reactions (Wang et al., 2013; Zhong et al., 2014; Chan et al., 2016). Modern pharmacological research indicated that YPFS could regulate the releases of cytokines, like IL-1 β , necrosis factor alpha (TNF)- α and IL-6 (Du et al., 2013). However, the underlying mechanism of YPFS on asthma is still unknown and further inquiry is needed.

Traditional Chinese Medicine (TCM) is well-known for its multi-component, multi-target and multi-pathway. And most diseases are not simply caused by one single factor. The TCM treatments can be visualized as a complexity against complexity paradigm between multi-target therapy such as TCM and the complex biological networks of human diseases (Li et al., 2014). As a major method, network pharmacology provide a method to unravel the complex and holistic mechanisms of TCM in treating complex diseases (Goh et al., 2007). Moreover, successful attempts on TCM study have been achieved in our group by network pharmacology analysis (Niu et al., 2015; Zhao et al., 2015; Fan et al., 2016). Therefore, in this study, we explored the underlying mechanism of YPFS on asthma via network pharmacology analysis and experimental validation.

MATERIALS AND METHODS

Network Pharmacology Analysis

Chemical compounds with OB $\geq$ 30% or DL $\geq$ 0.18 in *Astragalus membranaceus*, *Atractylodes macrocephala*, and *Saposhnikovia radix* were obtained in Traditional Chinese Medicine Systems Pharmacology Database (TCMSP)¹. Those compounds of the

three drugs were combined and repeated compounds were deleted. The corresponding target of each component was acquired in TCMSP database. The corresponding gene name of each target protein was obtained in UniProt database² and non-human target proteins were excluded. Finally, the target protein regulating by YPFS was acquired. Asthma-related human genes were searched on National Center for Biotechnology Information's (NCBI) Gene database³. Then, the target genes of YPFS and asthma were mapped. The interaction protein of mapped gene was obtained through the String interaction database⁴ and the protein interaction network was constructed. The pathway enrichment of the interaction protein was used the Database for Annotation, Visualization and Integrated Discovery (DAVID)⁵, and broad signaling pathways were eliminated. The top five pathways were extracted and the matching genes of the NOD-like receptor pathway which was ranked first were constructed the interaction network.

Preparation of YPFS

All crude drugs were purchased from Beijing Tongrentang. 60 g *Astragalus membranaceus*, 20 g *Atractylodes macrocephala*, and 20 g *Saposhnikovia radix* were extracted twice with boiling water, each time 40 min (1:6 and 1:4, w/v) (Song et al., 2013). Concentrating the two filtrates liquid into extract with electric thermostatic water bath and then transferred to the vacuum oven for drying. After drying, the solid extract was crushed into powder and stored at 4°C.

Cell Culture

Human leukemic U937 cells were purchased from American Type Culture Collection (Manassa, VA, United States), and were cultured at 37°C in a humidified atmosphere with 5% CO₂ in RPMI 1640 medium (GIBCO, Gaithersburg, MD, United States) supplemented with 10% fetal bovine serum (FBS) (GIBCO, Gaithersburg, MD, United States) and 1% penicillin-streptomycin (PS) (GIBCO, Gaithersburg, MD, United States).

Cell Viability Assay

U937 cells were seeded at a density of 1.0×10^4 cells/well into a 96-well with RPMI 1640 containing 5% FBS and 1% PS at 37°C in a 5% CO₂ incubator. U937 were differentiated into macrophages by using phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO, United States) (0.01 μ g/ml). After 48 h, the cells were washed with phosphate-buffered saline solution (PBS) twice and incubated with YPFS (0–100 μ g/ml) for 45 h at 37°C in a 5% CO₂ incubator. Cells were added with 10 μ l CCK-8 reagent (Dojindo, Tokyo, Japan) into each well and further cultured for 3 h. The absorbance of each well was measured with a microplate reader (Bio-Tek Instruments, Winooski, VT, United States) at 450 nm.

²<http://www.uniprot.org/>

³<https://www.ncbi.nlm.nih.gov/genbank/>

⁴<https://string-db.org/>

⁵<https://david.ncicrf.gov/>

¹<http://ibts.hkbu.edu.hk/LSP/tcmsp.php>

YPFS Treatment of U937 Cells

U937 cells were plated in 6 well plates (1×10^6 cells/well) and incubated with PMA for 48 h at 37°C, 5% CO₂. Then cells were washed for three times in PBS and induced with lipopolysaccharides (LPS) (Sigma, St. Louis, MO, United States) (0.1 µg/ml, 2 h). U937 cells were incubated with YPFS (25 µg/ml) for another 48 h. INF39 (Selleck, Shanghai, China), a non-toxic, irreversible NLRP3 inhibitor able to decrease interleukin-1β release from macrophages, was used as the positive control at a concentration of 10 µM (Cocco et al., 2017). The supernatant was collected to detect levels of IL-1β, TNF-α and IL-6 by ELISA and the cells were harvested to detect levels of NLRP3, ASC, Caspase-1 and IL-1β with Real-Time PCR and western blotting.

Animals

Male BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd., (Beijing, China), weighted 18–20 g with 7 weeks of age. The mice were bred in the China Academy of Chinese Medical Sciences [certification NO. SCXK (JING) 2016-0021] and housed in a room with a temperature-, humidity- and light-controlled environment. They were fed food and water ad libitum, and allowed to acclimatize themselves for 1 week before the initiation of experiment. The study was approved by the Research Ethics Committee of Institute of Basic Theory of Chinese Medicine, China Academy of Chinese Medical Sciences. All animals were treated in accordance with the guidelines and regulations for the use and care of animals of the Center for Laboratory Animal Care, China Academy of Chinese Medical Sciences.

Induction of Asthma

To establish an asthma model, 0.5 ml mixture [10 µg ovalbumin (OVA), 2 mg sodium hydroxide and 0.5 ml saline] were injected on day 1, day 7, and day 14 for model group and administration group. The normal group was injected with the same amount of saline. From day 21, mice were sprayed with 1% OVA solution once a day for 30 min and sprayed for 12 days. The normal group was sprayed with saline.

Drug Treatments

All the mice were divided into 4 groups: normal group, model group, dexamethasone (Dex) group (1 mg/kg/d) and YPFS group (13 g/kg/d), each group contained 10 mice. The dose of Dex and YPFS were reference to related literature (Dong et al., 2012; Wang et al., 2013), and the administration start from the atomization period (day 21). In the 30 min before the atomization, the mice of the positive drug group were injected with 0.2 ml of Dex. The mice in the administration group were given 0.5 ml of YPFS solution, and the mice in the model group and the normal group were given 0.5 ml of saline.

Observation of Ethology

The ethology of the mice was evaluated by observation of the following parameters: Fur luster, touching of nose and scratching of ears, irritability, sneezing, rapid breathing and incontinence (Jia et al., 2017).

Histological Analysis

Left lungs were removed from mice of each group, and distended with 10% buffered formalin. The tissues were sliced and embedded in paraffin, and 4 µm sections were prepared for morphological evaluation with hematoxylin-eosin staining (HE) and for mucopolysaccharide staining with alcian blue-periodic acid-schiff (AB-PAS). Sections from all of the left lobes were examined blindly by three individuals. The scoring system for cell infiltration was as follows: 0, no cells; 1, a few cells; 2, a ring of cells 1 cell layer deep; 3, a ring of cells 2–4 cells deep; and 4, a ring of cells > 4 cells deep. Goblet cell hyperplasia in the airway epithelium was quantified based on a five-point system: 0, no goblet cells; 1, <25% of the epithelium; 2, 25–50% of the epithelium; 3, 50–75% of the epithelium; and 4, >75% of the epithelium. Mean scores were calculated from ten mice (Underwood et al., 1995; Dong et al., 2012).

Immunohistochemistry

Paraffin sections were deparaffinized in 100% xylene and rehydrated (100, 95, 90, 85, 80% ethanol). Endogenous peroxidase activity was blocked by treating with 3% hydrogen peroxide (10 min) followed by a PBS wash twice (5 min each). The sections were incubated with a MUC5AC antibody (Abcam, Cambridge, United Kingdom) at a 1:500 dilution in primary antibody dilution buffer (Beyotime, Shanghai, China) for 12 h at 4°C. The sections were washed three times (3 min each) in PBS, incubated with SignalStain Boost® IHC Detection Reagent (HRP, Mouse) (Cell Signaling Technology, Beverly, MA, United States) for 30 min at room temperature, and washed in PBS for three times (5 min each). Secondary antibody was detected with SignalStain® DAB Substrate Kit (Cell Signaling Technology, Beverly, MA, United States). Slides were counterstained with hematoxylin (Leagene, Beijing, China) and mounted. To evaluate the protein expression, semi-quantitative image analysis was employed to measure the mean optical density (MOD) using Image-Pro Plus 6.0 software.

Bronchoalveolar Lavage Fluid (BALF)

Cell Count

Bronchoalveolar lavage fluid was collected from the mice by endotracheal intubation using a catheter followed by lavage with 0.4 ml of PBS for three times as previously described (Mi Chun et al., 2017). After lavage, approximately 1 ml of BALF was recovered. Total BALF cells count was determined in a hemocytometer using trypan blue exclusion. Eosinophils, neutrophils, lymphocytes, and monocytes cell counts were determined in 300 BALF cells on cytospin smears of BAL samples from individual mice stained with Giemsa stain (Sigma, St. Louis, MO, United States).

ELISA

Cell supernatants were collected as well as serum in mice were prepared. The levels of IL-1β, TNF-α and IL-6 were detected by using commercially available ELISA kits (eBioscience, San Diego, CA, United States) according to the manufacturer's instructions.

Real Time-PCR

The mRNA levels of IL-1 β , NLRP3, cysteinyl aspartate specific proteinase (Caspase)-1 and advanced synthesis and catalysis (ASC) were analyzed by RT-PCR. Total RNA of lung tissue was isolated using TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa, Kusatsu, Japan) according to the manufacturer's instructions. This procedure was done under RNase-free conditions. The total RNA (1 μ g) was reverse transcribed to cDNA using PrimeScriptTM RT reagent Kit with gDNA Eraser (TaKaRa, Kusatsu, Japan) according to the instruction manual. The specific transcripts were quantified by quantitative RT-PCR using SYBR[®] Premix Ex TaqTM II (TliRNaseH Plus), ROX plus (TaKaRa, Kusatsu, Japan) and analyzed with ABI 7500 RT-PCR system (Applied Biosystems, Foster, CA, United States). Gene-specific primers were synthesized by Sangon Biotech (Shanghai, China) and the following primer sequences were used: CATGAGTGCTGCTTCGACAT (forward) and GCTTCAGTCCCACACACAGA (reverse) for H-NLRP3, CTGACGGATGAGCAGTACCA (forward) and AGTCCTTGCAGGTCCAGTTC (reverse) for H-ASC, GCTTTCTGCTCTTCCACACC (forward) and TCCTCCACATCACA GGAACA (reverse) for H-caspase-1, CCATGGACAAGCTGAG GAAG (forward) and GTGATCGTACAGGTGCATCG (reverse) for H-IL-1 β , TGGAGTCTACTGGCGTCTT (forward) and TGTCATATTTCTCGTGGTTCA (reverse) for H-GAPDH, GGAGGAAGAGGAGGAGGAAA (forward) and ACTGGAAGTGAGGTGGCTGT (reverse) for M-NLRP3, GGCTGCTGGATGCTCTGTA (forward) and AGGCTGGTGTGAAACTGAAGA (reverse) for M-ASC, CAGACAAGGGTGCTGAACAA (forward) and TCGGAATAACGGAGTCAATCA (reverse) for M-Caspase-1, TGGCAATGAGGATGACTTGT (forward) and TGGTGGTCGGAGATTCTGTA (reverse) for M-IL-1 β , AGGC CGGTGCTGAGTATGTC (forward) and TGCCTGCTT CACCACCTTCT (reverse) for M-GAPDH. The mRNA levels were normalized to GAPDH mRNA level. PCR conditions were shown as below: at 95°C for 30 s, 40 cycles at 95°C for 5 s and at 60°C for 30 s. Relative mRNA expression was calculated by comparative CT method.

Western Blotting

The levels of NLRP3, ASC, Caspase-1 and IL-1 β in mice were detected by western blotting. Tissue homogenates were prepared in lysis buffer (Beyotime, Shanghai, China), consisting of 1 nM phenylmethanesulfonyl fluoride (PMSF) (Beyotime, Shanghai, China). Proteins were denatured and equal amounts of proteins were electrophoresed in 12% bis-Tris/polyacrylamide gels (Beyotime, Shanghai, China) and transferred to Polyvinylidene fluoride (PVDF) (Amresco, Houston, TX, United States). Membranes with 0.45 μ m pore-size Millipore filter. The membranes were blocked for 2 h in blocking solution (TBST containing 5% skim milk powder and 0.1% Tween 20) and incubated overnight at 4°C with anti-NLRP3, anti-Caspase-1, anti-IL-1 β antibody (Abcam, Cambridge, United Kingdom) at 1:1000 and anti-ASC antibody (Abcam, Cambridge, United Kingdom) at 1:500 in TBST containing 5% bovine sera albumin. After that, incubation with horseradish

peroxidase-conjugated secondary antibody (1:10000 dilution in TBST containing 5% skim milk powder) was performed at room temperature for 2 h, and immunoreactivity was detected by using enhanced chemiluminescence (APPLYGEN, Beijing, China). Blots were scanned and analyzed for measurement of the band intensities with UN-SCAN-IT version 5.1 software. Band intensity was calculated as follows: band intensity = sum of all pixel values in the segment selected-background pixel value in that segment.

Statistical Analysis

Data were expressed as the mean $\pm$ SD of triplicate experiments and analyzed with SPSS version 17.0 software (SPSS Inc., Chicago, IL, United States). Statistically significant values were determined using ANOVA and Dunnett's *post hoc* test, and *p*-values of less than 0.05 were considered statistically significant.

RESULTS

YPFS Might Treat Asthma by Regulating the Expression of NLRP3 Inflammasome

Two hundred and thirty chemical compounds (Supplementary Tables 1–3), 372 human target proteins (Supplementary Table 4) and 793 asthma-related human genes (Supplementary Table 5) of YPFS were obtained in this study. The reaction network that YPFS antagonized asthma was built through String database (Supplementary Figure 1). DAVID database pathway enrichment analysis showed that the top five pathways of YPFS regulating asthma were NOD-like receptor signaling pathway, tumor necrosis factor (TNF) signal pathway, phosphatidylinositol-3-kinase (PI3K)-AKT signal pathway, Hypoxia-inducible factor (HIF)-1 signal pathway and nuclear factor-kappaB (NF- κ B) signal pathway (Table 1). Using the String database to visualize the matching gene of the NOD-like receptor pathway, we found that the NLRP3 inflammasome might play an important role in the process that YPFS regulating the asthma (Figure 1).

YPFS Inhibited the Increase of Inflammatory Cytokines in LPS-Stimulated U937 Cells

Our results showed that YPFS didn't exhibit obvious cytotoxicity at the concentration of 0, 3.125, 6.25 12.5 and 25 μ g/ml ($P > 0.05$) (Figure 2A). Subsequently, 25 μ g/ml YPFS was used for the

TABLE 1 | Top 5 shared signaling pathways of YPFS and asthma.

Pathway ID	Pathway description	False discovery rate	Observed gene count
4621	NOD-like receptor signaling pathway	1.69E-19	16
4668	TNF signaling pathway	2.00E-16	17
4151	PI3K-Akt signaling pathway	3.85E-15	24
4066	HIF-1 signaling pathway	9.63E-13	14
4064	NF- κ B signaling pathway	2.32E-12	13

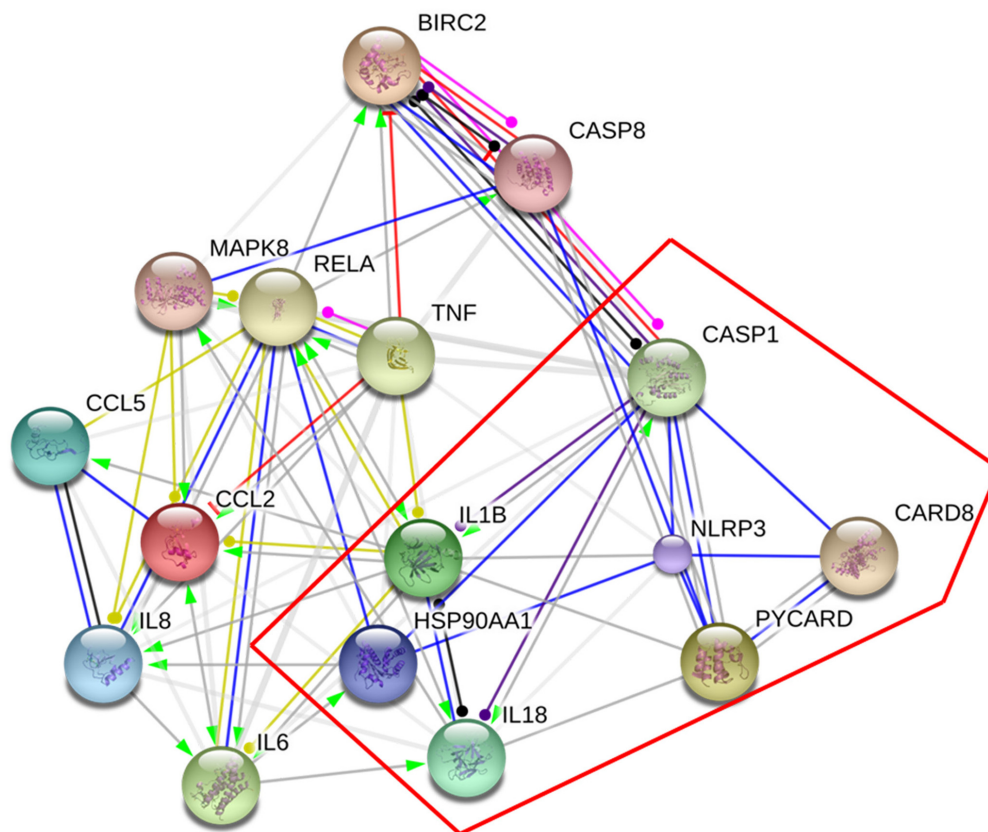


FIGURE 1 | Target genes of YPFS treating asthma. The genes in the red box were the key genes of NLRP3 inflammasome.

further experiments. As shown in **Figures 2B,C**, We found that cytokine production in supernatant were all significantly increased in the LPS stimulation group compared to the control group ($P < 0.01$). YPFS treatment was able to interfere TNF- α and IL-6 production, which was shown by lower levels of TNF- α and IL-6 than the LPS stimulation group ($P < 0.01$).

YPFS Reduced the Levels of NLRP3 Inflammasome Relative Genes and Proteins in LPS-Stimulated U937 Cells

To investigate whether YPFS regulate the expression of NLRP3 inflammasome, we detected the levels of NLRP3 inflammasome relative components. As shown in **Figure 3**, compared to the normal group, LPS stimulation significantly increased NLRP3, caspase-1 and ASC levels both at mRNA and protein levels ($P < 0.01$). Both YPFS and INF39 could significantly inhibit the mRNA and protein levels of NLRP3 inflammasome components compared to LPS stimulation group ($P < 0.05$ or $P < 0.01$).

YPFS Inhibited the Production of IL-1 β in LPS-Stimulated U937 Cells

Compared to the normal group, LPS stimulation significantly increased IL-1 β production (**Figure 4**). Both YPFS and

INF39 could significantly inhibit the IL-1 β production in the cell supernatants (**Figure 4A**). The mRNA (**Figure 4B**) and protein levels (**Figures 4C,D**) of IL-1 β in LPS-stimulated U937 cells were also remarkably decreased ($P < 0.01$).

YPFS Ameliorated Lung Inflammation, Mucus Secretion and MUC5AC Production in Asthma Mice

In order to verify the therapeutic effect of YPFS on asthma, OVA-sensitized asthma model was constructed. After sensitization, mice in model group showed obvious symptoms of nose touching and ear scratching, irritability, sneezing, rapid breathing and incontinence, while these symptoms were all improved after using YPFS. As shown in **Figures 5A,D**, OVA induction caused the infiltration of inflammatory cells which were mainly eosinophils into the perivascular connective tissues and peribronchiolar compared with the normal group. YPFS significantly attenuated the eosinophil-rich infiltration compared with the model group. To further determine the effect of YPFS on mucus secretion, adjacent lung sections were stained with AB-PAS (**Figures 5B,E**). There was marked goblet cell hyperplasia and mucus hypersecretion within the bronchi in the mice lungs from the model group

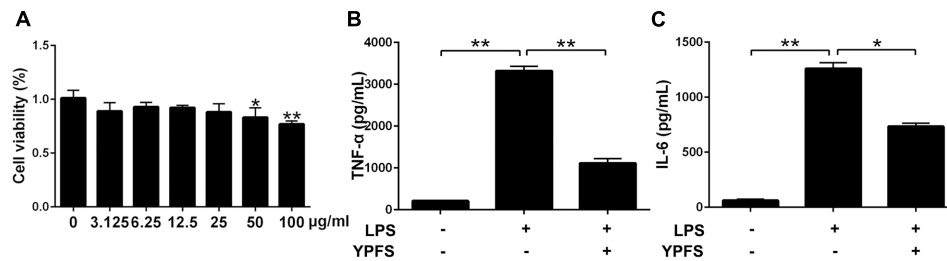


FIGURE 2 | *Yupingfeng San* inhibited the increase of inflammatory cytokines in LPS-stimulated U937 cells. **(A)** Effect of YPFS on cell viability of U937 was detected by CCK-8 reagent. The OD values were measured at 450 nm. Cell supernatants were collected for ELISA detection. The levels of TNF- α **(B)** and IL-6 **(C)** were shown as above. * $P < 0.05$, ** $P < 0.01$, compared with the model group.

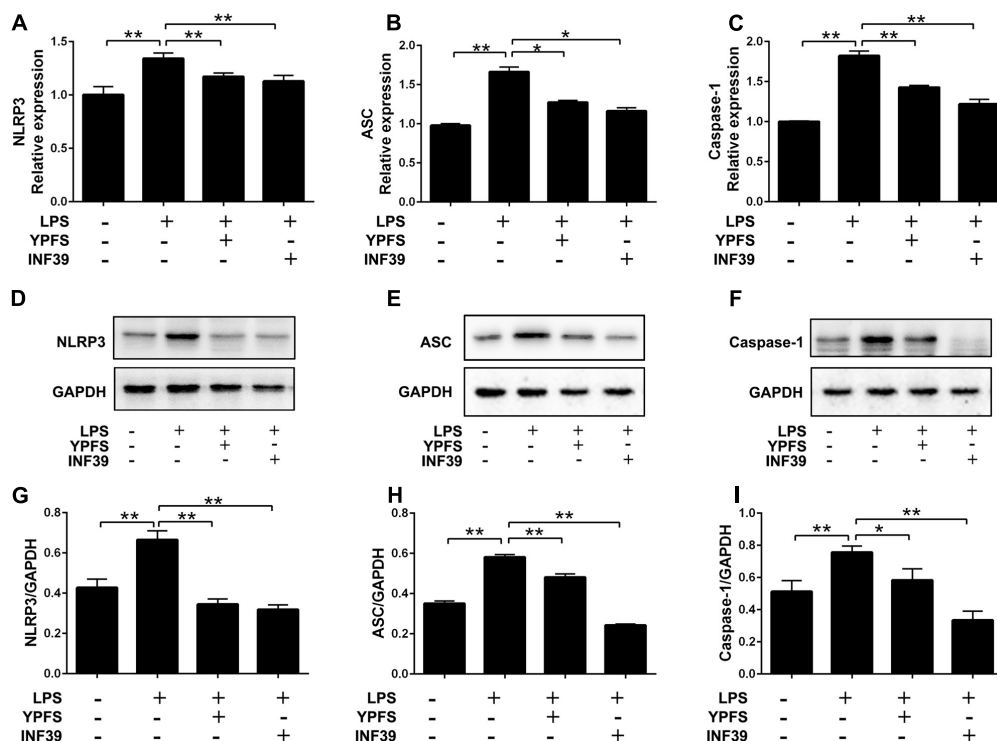


FIGURE 3 | *Yupingfeng San* reduced the expression of NLRP3 Inflammasome in LPS-stimulated U937 cells. Cells were pre-treated with PMA for 48 h and then incubated with LPS for 2 h. Subsequently, cells were stimulated with YPFS. INF39 was used as the positive control. After 48 h, cells were collected. **(A–C)** The mRNA levels of NLRP3, ASC and Caspase-1 were detected by real time-PCR. **(D–I)** The protein levels of NLRP3, ASC and Caspase-1 were detected by western blotting, and the ratio of NLRP3/GAPDH, ASC/GAPDH and Caspase-1/GAPDH were shown. * $P < 0.05$, ** $P < 0.01$, compared with model group.

but not in the lungs from the normal group. Treatment with YPFS markedly attenuated the OVA-induced mucus secretion. MUC5AC is a major mucin protein secreted from the airway surface epithelium and played important role in asthma (Tan et al., 2011). To further confirm the effect of YPFS on asthma, histological sections of lung tissue were stained immunohistochemically to detect MUC5AC level. Our studies have proved that MUC5AC expression was markedly upregulated in lung tissue from OVA-induced asthmatic mice. Compared with normal group, MUC5AC were significantly increased in the stained sections of model groups. When compared with the model group, MUC5AC were significantly

less in the stained sections of Dex group and YPFS group (Figures 5C,F).

YPFS Decreased Inflammatory Cell Numbers in BALF

To determine the effect of YPFS on inflammatory cell infiltration in the lungs of asthmatic mice, the total numbers of inflammatory cells in the BALF of the YPFS-treated and untreated asthmatic mice were determined. Compared with the normal mice, mice in model group had significantly increased numbers of total inflammatory cells. By comparison, the YPFS- and Dex-treated

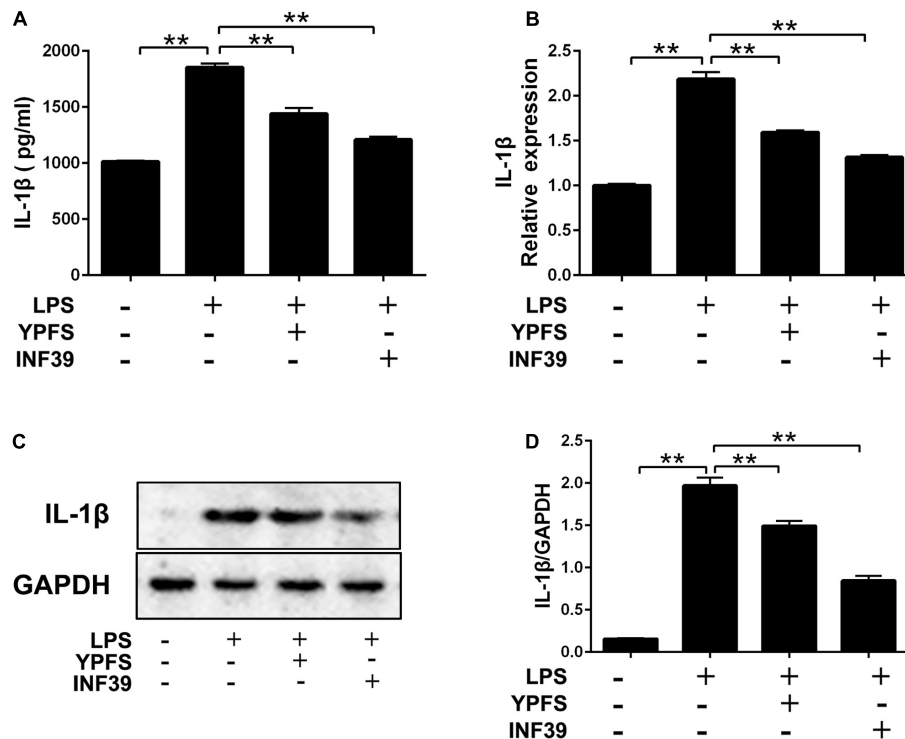


FIGURE 4 | *Yupingfeng San* inhibited the production of IL-1 β in LPS-stimulated U937 cells. **(A)** The level of IL-1 β in cell supernatant was evaluated by ELISA. **(B)** The mRNA level of IL-1 β in cells was assessed by real time-PCR. **(C,D)** The protein level of IL-1 β in cells was detected by Western blotting. ** $P < 0.01$, compared with model group.

mice had significantly fewer inflammatory cells in their BALF (Table 2). Analysis of the numbers of different inflammatory cells (eosinophils, neutrophils, macrophages, and lymphocytes) in the BALF showed that model group had very high numbers of eosinophils. Compared with model group, the YPFS-treated asthmatic mice had significantly fewer inflammatory cells, particularly eosinophils (Table 2).

YPFS Reduced Pro-inflammatory Cytokines Expression in Serum of Asthma Mice

To observe the effects of YPFS on production of pro-inflammatory cytokines in OVA-sensitized asthma mice, the expression of TNF- α and IL-6 in serum of OVA-sensitized asthma mice were examined by ELISA. Our results showed that levels of TNF- α and IL-6 in model group were all elevated. However, these effects were attenuated by YPFS treatment (Figure 6).

YPFS Inhibited the Expression of NLRP3 Inflammasome in OVA-Sensitized Asthma Mice

To further examine the mechanism of YPFS on OVA-sensitized asthma model, NLRP3 inflammasome level in lung tissues of mice in each group was detected. The results of RT-PCR showed that the mRNA levels of NLRP3, ASC and Caspase-1

in lung tissue was dramatically decreased after YPFS treatment compared to the model group (Figures 7A–C). Meanwhile, the protein levels of NLRP3, ASC, Caspase-1 and Pro-Caspase-1 in lung tissue of YPFS treated OVA-sensitized asthma mice also decreased significantly in comparison with those in model group (Figures 7D–J).

YPFS Inhibited the Expression of IL-1 β in OVA-Sensitized Asthma Mice

NLRP3 inflammasome activation could cause excessive IL-1 β activation (Menu and Vince, 2011). To further verify the effects of YPFS on production of IL-1 β , the level of IL-1 β in serum was detected. As shown in Figure 8A, the level of IL-1 β in the serum of model group was significantly higher than those in normal group. In contrast, higher level of IL-1 β was significantly inhibited by YPFS treatment. As the level of IL-1 β in serum was suppressed by YPFS, we wondered whether it could also be inhibited in the injured lung. As shown in Figures 8B–D, the mRNA and protein levels of IL-1 β in the lung from model group were apparently raised compared to normal group, which was significantly decreased by YPFS treatment.

DISCUSSION

Yupingfeng San is widely used to treat the diseases of respiratory systems and immune systems, especially asthma

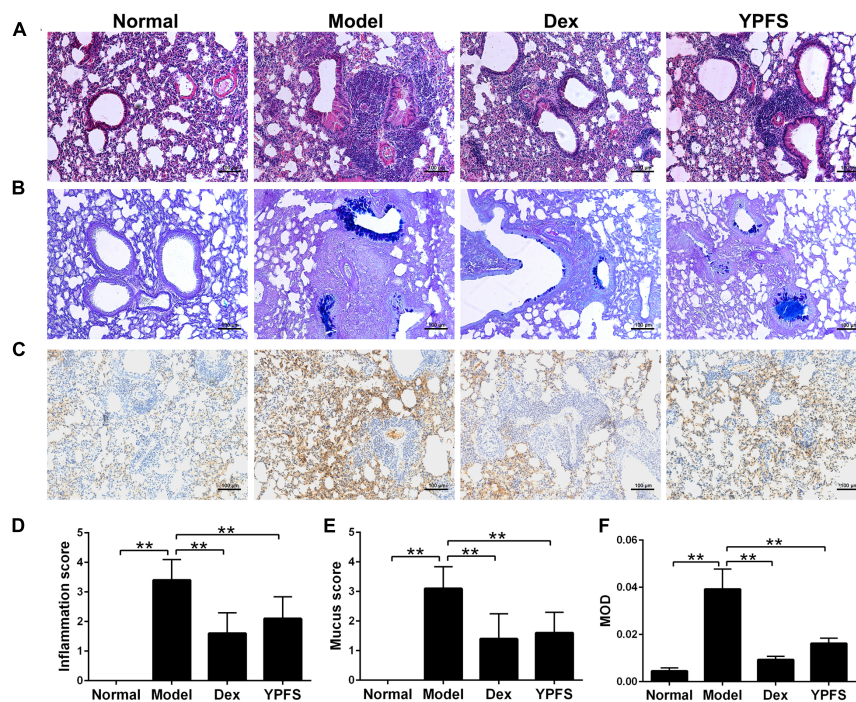


FIGURE 5 | *Yupingfeng San* ameliorated lung inflammation, mucus secretion and reduced MUC5AC in asthma mice. Asthma model was induced by OVA-sensitized BALB/c mice. From the start of the atomization period, YPFS intragastric administration or dexamethasone (Dex) injection was carried out. **(A)** The upper lobe of left lungs was collected and the histopathological changes of the lung tissue were observed by HE staining. **(B)** The mucus secretion in the bronchus was observed by AB-PAS staining. **(C)** Immunohistochemical staining for MUC5AC. Tissue sections from lungs in each group were stained with monoclonal MUC5AC antibody. **(D-F)** The inflammation score, mucus secretion score and MOD in each group were shown. MOD: mean optical density. All the images are shown at 100× magnification. ** $P < 0.01$, compared with the model group.

TABLE 2 | Effect of YPFS on bronchoalveolar lavage differential cell counts.

	Total cells ($\times 10^3$)	Eosinophils ($\times 10^3$)	Neutrophils ($\times 10^3$)	Lymphocytes ($\times 10^3$)	Macrophage ($\times 10^3$)
Normal	50.71 $\pm$ 9.65	0.43 $\pm$ 0.17	0.53 $\pm$ 0.09	0.29 $\pm$ 0.06	48.46 $\pm$ 9.43
Model	371.54 $\pm$ 60.98 ^{##}	117.91 $\pm$ 54.79 ^{##}	5.48 $\pm$ 0.95 ^{##}	5.97 $\pm$ 1.09 ^{##}	241.32 $\pm$ 21.75 ^{##}
Dex	200.31 $\pm$ 56.91 ^{**}	59.36 $\pm$ 27.78 [*]	2.56 $\pm$ 1.08 ^{**}	2.97 $\pm$ 1.51 ^{**}	110.27 $\pm$ 49.31 ^{**}
YPFS	263.66 $\pm$ 63.93 ^{**}	71.89 $\pm$ 26.51 [*]	3.29 $\pm$ 1.85 ^{**}	3.96 $\pm$ 1.46 ^{**}	183.51 $\pm$ 38.67 ^{**}

^{##} $P < 0.01$, compared with the normal group. ^{*} $P < 0.05$, ^{**} $P < 0.01$, compared with the model group.

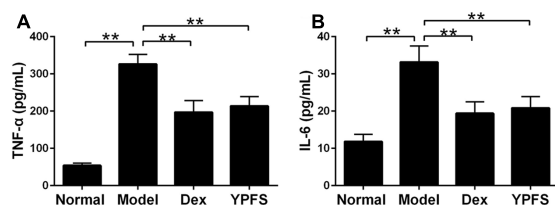


FIGURE 6 | *Yupingfeng San* reduced pro-inflammatory cytokines expression in serum of asthma mice. Mice were sacrificed and the serum was collected for ELISA detection. The levels of TNF- α **(A)** and IL-6 **(B)** were shown as above. ** $P < 0.01$, compared with the model group.

(Chen et al., 2014). Previous studies also found YPFS had anti-inflammatory effect and this effect was achieved through suppressing the transcript and protein expressions of pro-inflammatory cytokine (Du et al., 2013; Sun et al., 2017). Consistent with previous researches, our study confirmed the therapeutic effect of YPFS on asthma. It could improve the symptoms of asthma mice like, nose touching, ear scratching, irritability, sneezing, rapid breathing and incontinence. After treating with YPFS, inflammatory cell infiltration, mucus secretion and MUC5AC expression in lungs of asthmatic mice were significantly inhibited. It also decreased inflammatory cell numbers in BALF and TNF- α , IL-6 levels in asthmatic mice plasma.

Although lots of researches had confirmed the efficacy of YPFS, more mechanisms by which YPFS inhibit inflammatory response should be fully elucidated (Chen et al., 2014; Song et al., 2016). In this study, we used network pharmacological

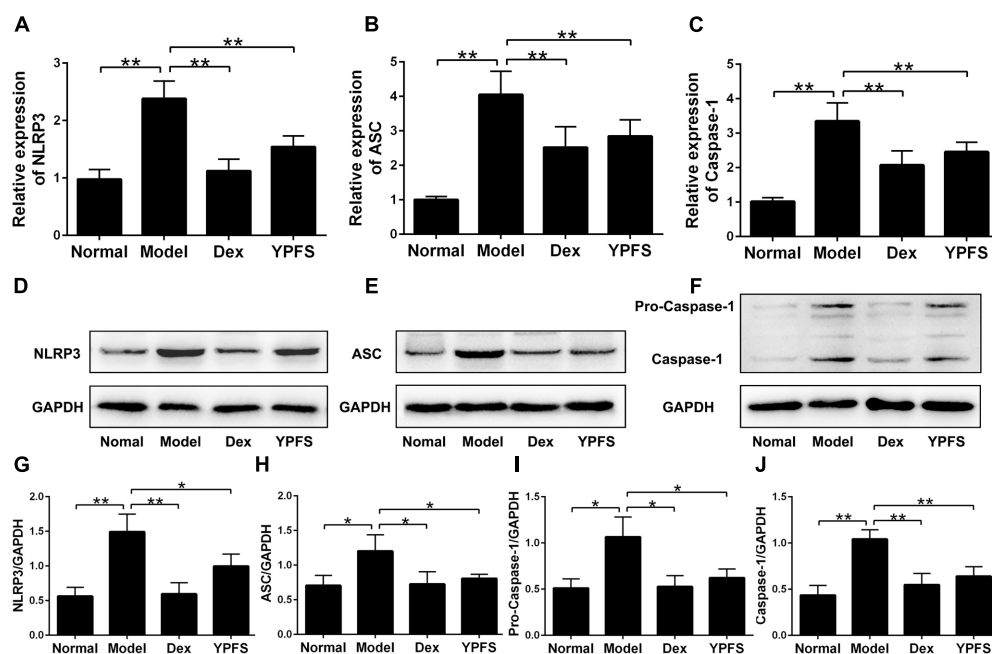


FIGURE 7 | *Yupingfeng San* inhibited the expression of NLRP3 inflammasome in OVA-sensitized asthma mice. After mice were sacrificed, the upper lobe of the right lung was collected for RT-PCR and western blotting detection. (A–C) The mRNA levels of NLRP3, ASC and Caspase-1 on the upper lobe of the right lung were detected by RT-PCR. (D–J) The protein levels of NLRP3, ASC, Caspase-1 and Pro-Caspase-1 were detected by western blotting, and the ratio of NLRP3/GAPDH, ASC/GAPDH, Caspase-1/GAPDH and Pro-Caspase-1/GAPDH were shown. * $P < 0.05$, ** $P < 0.01$, compared with the model group.

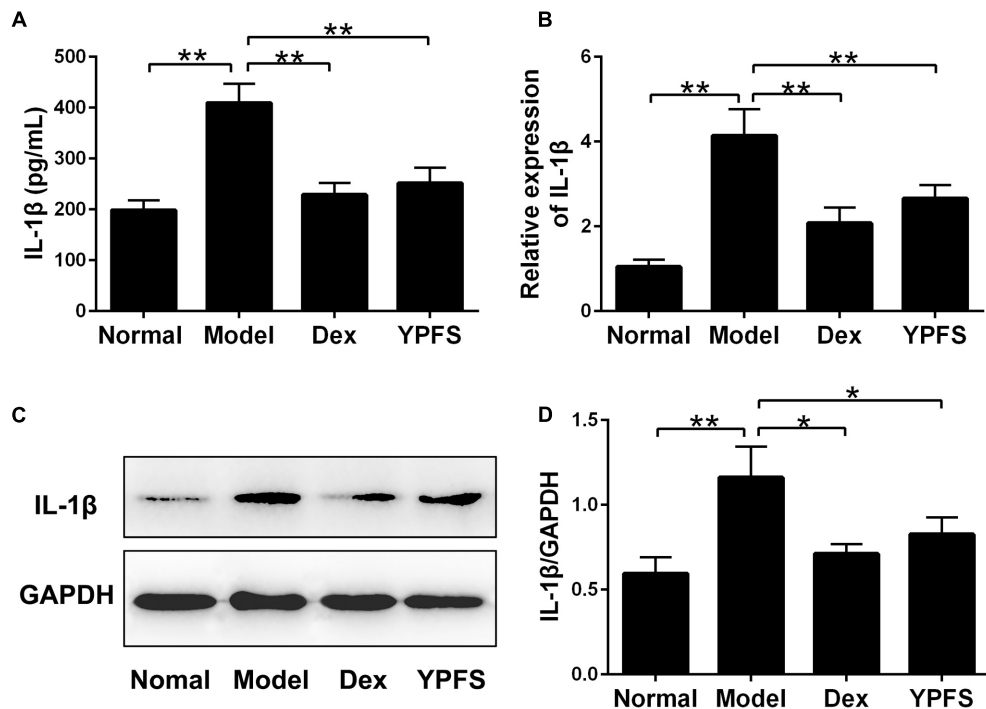


FIGURE 8 | *Yupingfeng San* inhibited the expression of IL-1β in OVA-sensitized asthma mice. (A) The level of IL-1β in the serum was detected by ELISA. (B) The mRNA level of IL-1β on the upper lobe of the right lung was detected by RT-PCR. (C,D) The protein level of IL-1β was detected by western blotting and the ratio of IL-1β/GAPDH was shown. * $P < 0.05$, ** $P < 0.01$, compared with the model group.

analysis to investigate the mechanisms of YPFS acting on asthma. Our analysis indicated that multiple pathways were closely associated with asthma, such as TNF signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, and NF- κ B signaling pathway. And these findings were consistent with previous studies (Crotty Alexander et al., 2013; Li et al., 2015, 2016; Schuliga, 2015; Peng et al., 2016). In addition, we found that NOD-like receptor signaling pathway was the top one shared signaling pathway of YPFS and asthma, which was not explored before. Studies on NOD-like receptor signaling pathways revealed that NLRP3, composed by NLRP3, ASC and Caspase-1, could be a very important immune receptor responsible for auto-inflammatory response (Martinon et al., 2009), and when excessive activated, NLRP3 inflammasome could lead to serious inflammatory conditions (Latz et al., 2013). Moreover, previous clinical and experimental studies demonstrated that the expression of NLRP3 inflammasome was up-regulated in patients with asthma and OVA-induced asthma model (Kim et al., 2017; Rossios et al., 2017), which indicated NLRP3 inflammasome played an important role in the pathology of asthma (Simpson et al., 2014). Our results showed that mRNA and protein levels of NLRP3, ASC and Caspase-1 in LPS-stimulated U937 cells as well as lung tissues of OVA-induced asthma mice were dramatically decreased after YPFS treatment, which suggested YPFS could inhibit the inflammatory response in OVA-induced asthma through suppressing the NLRP3 inflammasome.

In addition, more evidences implicated NLRP3 inflammasome activation could lead to the increased release of IL-1 β (Negash et al., 2013; Kim et al., 2017). And this conclusion was also confirmed in patients with asthma (Simpson et al., 2014). Further research revealed that NLRP3 inflammasome-mediated IL-1 β might be one of the key factors which could induce inflammation and result in asthma, and increasing IL-1 β could also be seen as a marker of asthma (Kim et al., 2015; Giuffrida and Valero, 2017). Therefore, targeting elevated IL-1 β instead of using corticosteroids might be an effective treatment for asthma. In this study, the expression of IL-1 β in LPS-stimulated U937 Cells, in serum and in the lung tissue of asthma mice was all detected. We found that YPFS not only significantly inhibited the IL-1 β production *in vitro*, but also decreased its levels in the periphery and lung tissue of OVA-induced asthma mice. These data indicated YPFS could inhibit inflammatory response in asthma through modulating NLRP3 inflammasome and subsequently down-regulating IL-1 β .

In this study, we provided an integrative analysis by combining network pharmacology prediction with experiment validation to understand the pharmacological mechanism of YPFS acting on asthma. Furthermore, our finding also

demonstrated that network pharmacology was a fairly reliable way to find TCM's target and possible mechanisms.

CONCLUSION

Our data in this research demonstrated that YPFS could attenuate inflammatory response in asthma through suppressing the NLRP3 inflammasome.

ETHICS STATEMENT

Full name of the ethics committee that approved the study. The study was approved by the Research Ethics Committee of Institute of Basic Theory of Chinese Medicine, China Academy of Chinese Medical Sciences. Consent procedure used for human participants or for animal owners. Male BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd., weighted 18–20 g with 7 weeks of age. The mice were bred and housed in a room with a temperature-, humidity- and light-controlled environment. They were fed food and water *ad libitum*, and allowed to acclimatize themselves for 1 week before the initiation of experiment. Any additional considerations of the study in cases where vulnerable populations were involved, for example minors, persons with disabilities or endangered animal species.

AUTHOR CONTRIBUTIONS

XL and JS performed the major research in equal contribution. DF, XQ, QG, KZ, HL, JS, CL, GZ, and AL provided the technical support. CM contributed to final approval of the version to be published. XH designed the study and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2017.00944/full#supplementary-material>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer YZ and handling editor declared their shared affiliation.

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Supplementary Material

Yupingfeng San inhibits NLRP3 inflammasome to attenuate the inflammatory response in asthma mice

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1 Supplementary Tables and Figures

1.1 Supplementary Tables

Supplementary Table 1. Chemical compounds of *Astragalus Membranaceus*

Mol ID	Chemical Compound	OB (%)	DL
MOL000114	vanillic acid	35.47	0.04
MOL000131	EIC	41.9	0.14
MOL000211	Mairin	55.38	0.78
MOL000239	Jaranol	50.83	0.29
MOL000296	hederagenin	36.91	0.75
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl-octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
MOL000354	isorhamnetin	49.6	0.31
MOL000371	3,9-di-O-methylnissolin	53.74	0.48
MOL000372	3-Hydroxy-2-picoline	62.47	0.02
MOL000374	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69
MOL000378	7-O-methylisomucronulatol	74.69	0.3
MOL000379	9,10-dimethoxypterocarpan-3-O- β -D-glucoside	36.74	0.92
MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	64.26	0.42
MOL000381	13-hydroxy-9,11-octadecadienoic acid	35.6	0.17
MOL005928	isoferulic acid	50.83	0.06
MOL000386	Fucopyranose, L-	42.51	0.03
MOL000387	Bifendate	31.1	0.67
MOL000389	FERULIC ACID (CIS)	54.97	0.06
MOL000392	formononetin	69.67	0.21
MOL000395	GGB	54.95	0.03
MOL000397	cis-p-Coumarate	45.98	0.04
MOL000398	isoflavanone	109.99	0.3
MOL000401	astragalosideI	46.79	0.11
MOL000403	astragalosideII	46.06	0.13
MOL000405	astragalosideIII	31.83	0.1
MOL000414	Caffeate	54.97	0.05
MOL000417	Calycosin	47.75	0.24
MOL000420	XLS	51.08	0.02
MOL000421	nicotinic acid	47.65	0.02
MOL000422	kaempferol	41.88	0.24
MOL000424	RAM	50.5	0.04

MOL000429	Crystal VI	83.96	0.02
MOL000430	betaine	40.92	0.01
MOL000432	linolenic acid	45.01	0.15
MOL000433	FA	68.96	0.71
MOL000434	acetylastragaloside I	43.54	0.09
MOL000435	acetylastragaloside I Qt	30.75	0.17
MOL000436	(Z)-1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one	87.51	0.15
MOL000438	(3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol	67.67	0.26
MOL000439	isomucronulatol-7,2'-di-O-glucoside	49.28	0.62
MOL000442	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48
MOL000054	L-	47.64	0.03
MOL000061	Prolinum	77.57	0.01
MOL000098	quercetin	46.43	0.28
MOL000437	Hirsutrin	1.86	0.77
MOL000423	rhamnocitrin-3-O-glucoside	2.87	0.76
MOL000415	rutin	3.2	0.68
MOL000396	(+)-Syringaresinol	3.29	0.72
MOL000375	5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside Qt	3.65	0.8
MOL000412	Mucronulatol	4.22	0.26
MOL000406	astragaloside III Qt	5.35	0.32
MOL000373	(2S)-4-methoxy-7-methyl-2-[1-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-methylol-tetrahydropyran-2-yl]oxyethyl]-2,3-dihydrofuro[3,2-g]chromen-5-one	5.38	0.81
MOL000377	7-hydroxy-3-(2-hydroxy-3,4-dimethoxyphenyl)chromone	5.45	0.3
MOL000416	Lariciresinol	5.53	0.38
MOL000410	Astragaloside IV Qt	7.07	0.32
MOL000408	astragaloside IV Qt	7.07	0.32
MOL000418	3'-Hydroxy-4'-methoxyisoflavone-7-O-beta-D-glucoside	10.05	0.81
MOL000391	Ononin	11.52	0.78
MOL000404	astragaloside II Qt	11.55	0.25
MOL000441	LUPENONE	11.66	0.78
MOL001955	Heriguard	11.93	0.33
MOL000356	lupeol	12.12	0.78
MOL000402	astragaloside I Qt	12.34	0.2
MOL000251	Rhamnocitrin	12.9	0.27
MOL000399	Docosanoate	15.69	0.26
MOL000376	7,2'-dihydroxy-3',4'-dimethoxyisoflavone-7-O-β-D-glucoside	16.16	0.86
MOL000400	Flavaxin	18.18	0.5

MOL000411	Astraisoflavanin	18.37	0.86
MOL000390	daidzein	19.44	0.19
MOL000295	alexandrin	20.63	0.63
MOL000440	isomucronulatol-7,2'-di-O-glucosiole_qt	23.42	0.79

Supplementary Table 2. Chemical compounds of *Atractylodes Macrocephala*

Mol ID	Chemical Compound	OB (%)	DL
MOL000018	(+/-)-Isoborneol	86.98	0.05
MOL000019	D-Camphene	34.98	0.04
MOL000020	12-senecieryl-2E,8E,10E-atractylentriol	62.4	0.22
MOL000021	14-acetyl-12-senecieryl-2E,8E,10E-atractylentriol	60.31	0.31
MOL000022	14-acetyl-12-senecieryl-2E,8Z,10E-atractylentriol	63.37	0.3
MOL000023	Hemo-sol	39.84	0.02
MOL000025	α -Longipinene	53.26	0.12
MOL000028	α -Amyrin	39.51	0.76
MOL000030	(1R)-2-methyl-1-phenylprop-2-en-1-ol	75.1	0.03
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yl]octan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78
MOL000038	Akridin	33.71	0.1
MOL000039	(1S,2R,4R)-Neoiso-dihydrocarveol	52.4	0.03
MOL000041	PHA	41.62	0.04
MOL000042	LPG	87.69	0.01
MOL000043	atractylenolide i	37.37	0.15
MOL000044	atractylenolideII	47.5	0.15
MOL000045	atractylenolide iii	68.11	0.17
MOL000046	atractylone	41.1	0.13
MOL000047	juniper camphor	33.3	0.1
MOL000048	(5E,9Z)-3,6,10-trimethyl-4,7,8,11-tetrahydrocyclohexa[b]furan	43.17	0.1
MOL000049	3 β -acetoxylatractylone	54.07	0.22
MOL000050	GLY	48.74	0
MOL000054	L-	47.64	0.03
MOL000056	DTY	57.55	0.05
MOL000057	DIBP	49.63	0.13
MOL000058	2-[(2R,5S,6S)-6,10-dimethylspiro[4.5]dec-9-en-2-yl]propan-2-ol	38.59	0.09
MOL000060	selina-4(14),7(11)-dien-8-one	32.31	0.1
MOL000061	Prolinum	77.57	0.01
MOL000064	D-Serin	83.59	0.01
MOL000065	ASI	79.74	0.02
MOL000066	alloaromadendrene	53.46	0.1
MOL000067	L-Valin	53.33	0.01
MOL000068	L-Ile	59.05	0.02
MOL000070	Ethyl pivaloylacetate	40.52	0.03

MOL000071	Istidina	53.18	0.03
MOL000072	8 β -ethoxy atractylenolide III	35.95	0.21
MOL000026	stigmast-22E-en-3beta-ol	10.39	0.75
MOL000062	biatractylolide	17.45	0.81
MOL000063	ATRACTYLODES MACROCEPHALA	14.6	0.81

Supplementary Table 3. Chemical compounds of *Saposhnikovia Radix*

Mol ID	Chemical Compound	OB (%)	DL
MOL000011	(2R,3R)-3-(4-hydroxy-3-methoxy-phenyl)-5-methoxy-2-methylol-2,3-dihydropyrano[5,6-h][1,4]benzodioxin-9-one	68.83	0.66
MOL000018	(+/-)-Isoborneol	86.98	0.05
MOL000023	Hemo-sol	39.84	0.02
MOL000114	vanillic acid	35.47	0.04
MOL000115	Undecenal	39.35	0.03
MOL000116	Nonanal	40.28	0.02
MOL000118	(L)-alpha-Terpineol	48.8	0.03
MOL000122	1,8-cineole	39.73	0.05
MOL000125	(-)-alpha-Pinene	46.25	0.05
MOL000131	EIC	41.9	0.14
MOL000162	beta-Chamigrene	31.99	0.08
MOL000172	Furol	34.35	0.01
MOL000173	wogonin	30.68	0.23
MOL000196	L-Bornyl acetate	65.52	0.08
MOL000198	(R)-linalool	39.8	0.02
MOL000202	Moslene	33.02	0.02
MOL000234	L-Limonen	38.09	0.02
MOL000266	beta-Cubebene	32.81	0.11
MOL000268	(1S,5S)-1-isopropyl-4-methylenebicyclo[3.1.0]hexane	46.21	0.04
MOL000271	l-carvone	49.47	0.03
MOL000302	Undekansaeure	30.14	0.03
MOL000358	beta-sitosterol	36.91	0.75
MOL000359	sitosterol	36.91	0.75
MOL000474	(-)-Epoxycaryophyllene	35.94	0.13
MOL000597	Neryl acetate	57.47	0.04
MOL000614	osthol	38.75	0.13
MOL000666	hexanal	55.71	0.01
MOL000668	PENTYLFURAN	54.59	0.02
MOL000675	oleic acid	33.13	0.14
MOL000676	DBP	64.54	0.13
MOL000699	m-Cymol	48.85	0.02
MOL000708	WLN: VHR	32.63	0.01
MOL000709	(S)-Matsutake alcohol	40.11	0.01
MOL000723	trans-2,4-decadial	51.03	0.02
MOL000905	()-beta-Pinene	44.77	0.05
MOL000914	(5S)-1-isopropyl-4-methylbicyclo[3.1.0]hex-3-ene	47.13	0.04
MOL000922	(R)-p-Menth-1-en-4-ol	32.16	0.03
MOL000974	cuminal	38.29	0.03
MOL001114	2-[(1R)-2,2,3-trimethyl-1-cyclopent-3-enyl]ethanal	45.71	0.03

MOL001121	19894-97-4	49.98	0.06
MOL001129	l-Verbenone	50.66	0.06
MOL001245	(1S,5R)-7,7-dimethyl-4-bicyclo[3.1.1]hept-3-enecarboxaldehyde	41.25	0.06
MOL001390	49070_FLUKA	85.51	0.12
MOL001494	Mandenol	42	0.19
MOL001578	Hypnon	48.19	0.02
MOL001641	METHYL LINOLEATE	41.93	0.17
MOL001889	Methyl linolelaidate	41.93	0.17
MOL001940	Falcarindiol	39.3	0.11
MOL001941	Ammidin	34.55	0.22
MOL001942	isoimperatorin	45.46	0.23
MOL001944	Marmesin	50.28	0.18
MOL001945	Majudin	42.21	0.13
MOL001949	panaxynol	42.44	0.1
MOL001950	psoralen	33.06	0.1
MOL001953	Uvadex	35.3	0.13
MOL002024	(1S,4R)-1,7,7-trimethylbicyclo[2.2.1]hept-2-ene	39.62	0.04
MOL002029	()-Cuparene	38.26	0.07
MOL002033	cis-Thujopsene	56.43	0.12
MOL002138	p-Cymen-8-ol	32.26	0.03
MOL002153	1H-Cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, (1aR-(1aalpha,4aalpha,7beta,7abeta,7balpha))-	82.33	0.12
MOL002198	Heptan	41.8	0
MOL002207	1(3H)-Isobenzofuranone, 3-butyl-3a,4,5,6-tetrahydro-, cis-(-)-	65.03	0.07
MOL002379	PTL	59.53	0
MOL002453	(-)-Comphene	34.98	0.04
MOL002480	Methylbutenol	54.58	0.01
MOL002520	.beta.-Fenchyl acetate, exo-	108.68	0.07
MOL002644	Phellopterin	40.19	0.28
MOL003040	Amylol	76.16	0
MOL003050	nonanoic acid	40.51	0.02
MOL003453	ZINC02571348	39.15	0.02
MOL003547	Azaron	38.39	0.06
MOL003588	Prangenidin	36.31	0.22
MOL003951	Benzyl isovalerate	58.44	0.05
MOL004480	acetic acid	47.87	0
MOL004775	Isobergapten	34.75	0.13
MOL004793	Marmesine	84.77	0.18
MOL005274	Neohexane	37.81	0.01
MOL005723	Phytodolor	52.32	0.1
MOL007502	NSC692928	43.31	0.1
MOL007514	methyl icoso-11,14-dienoate	39.67	0.23

MOL007545	lanceol	37.54	0.07
MOL010120	(1S,4R,4aR,8aR)-1-isopropyl-4,7-dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-4a-ol	62.54	0.09
MOL010244	(2S)-Flavanone	64.04	0.13
MOL011387	heptadeca-1,8-dien-4,6-diyn-3,10-diol	53.86	0.11
MOL011648	METHYL 10-OCTADECENOATE	31.9	0.17
MOL011730	11-hydroxy-sec-o-beta-d-glucosylhamaudol_qt	50.24	0.27
MOL011732	anomalin	59.65	0.66
MOL011737	divaricatacid	87	0.32
MOL011738	divaricataester,a	64.02	0.13
MOL011740	divaricatol	31.65	0.38
MOL011742	fraxidin	42.22	0.1
MOL011745	isofraxidin	39.75	0.1
MOL011746	isopimpinellin	43.14	0.17
MOL011747	ledebouriellol	32.05	0.51
MOL011748	nodakenetin	68.62	0.15
MOL011749	phelloptorin	43.39	0.28
MOL011753	5-O-Methylvisamminol	37.99	0.25
MOL011754	4-hydroxy-9-methoxyfuro[3,2-g]chromen-7-one	31.78	0.15
MOL011755	5-methoxy-8-hydroxypsoralen	48.4	0.15
MOL011756	7-octen-4-ol	33.99	0.01
MOL011860	d-Dihydrocarvone	62.94	0.03
MOL013077	Decursin	39.27	0.38
MOL013176	2-[(2S)-7-oxo-2,3-dihydrofuro[4,5-g]chromen-2-yl]propan-2-yl methylbut-2-enoate (E)-2-	17.32	0.36
MOL011752	sec-o-beta-d-glucosylhamaudol	15.08	0.75
MOL011750	prim-o-beta-d-glucosylcimifugin	27	0.79
MOL011743	hamaudol	21.46	0.24
MOL011741	fangfengalpyrimidine	15.37	0.24
MOL011739	divaricataester,b	26.1	0.39
MOL011736	3'-O-angeloylhamaudol	12.01	0.45
MOL011735	deltoin	14.25	0.36
MOL011734	Cimifugin	13.49	0.29
MOL011731	3'-O-Acetylhamaudol	26.21	0.34
MOL011729	11-hydroxy-sec-o-beta-d-glucosylhamaudol	16.62	0.77
MOL009356	Tectochrysin	9.57	0.2
MOL005786	Byakangelicin	27.67	0.35
MOL001946	Ammijin	15.48	0.69
MOL000663	lignoceric acid	14.9	0.33
MOL000661	PENTACOSANOIC ACID	14.57	0.37
MOL000373	(2S)-4-methoxy-7-methyl-2-[1-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-	5.38	0.81

	methylo[4,5-b]tetrahydropyran-2-yl]oxy-ethyl]-2,3-dihydrofuro[3,2-g]chromen-5-one		
MOL000357	Sitogluside	20.63	0.62

Supplementary Table 4. Human target proteins of YPFS

NO.	Target Proteins	NO.	Target Proteins	NO.	Target Proteins
1	AARS	2	ESR1	3	NCOA2
4	ABAT	5	ESR2	6	NDUFS1
7	ABI1	8	F13A1	9	NDUFS3
10	ACADM	11	F7	12	NDUFS8
13	ACADSB	14	FABP1	15	NDUFV1
16	ACAT2	17	FASN	18	NFS1
19	ACE	20	FKBP1A	21	NLRP3
22	ACHE	23	FN1	24	NOS1
25	ACY1	26	FOLH1	27	NOS2
28	ADH1A	29	FOS	30	NOS3
31	ADH1B	32	G6PD	33	NPL
34	ADH1C	35	GABRA1	36	NQO2
37	ADH4	38	GABRA2	39	NR3C1
40	ADH7	41	GABRA3	42	NR3C2
43	ADRA1A	44	GABRA4	45	NTRK2
46	ADRA1B	47	GABRA5	48	OAT
49	ADRA1D	50	GABRA6	51	OAZ3
52	ADRA2A	53	GABRB1	54	OPLAH
55	ADRA2B	56	GABRB3	57	OPRM1
58	ADRA2C	59	GALE	60	PAFAH1B3
61	ADRB1	62	GALT	63	PAM
64	ADRB2	65	GAMT	66	PC
67	ADSSL1	68	GAP43	69	PCCA
70	AGXT	71	GAPDHS	72	PDE3A
73	AGXT2	74	GATM	75	PDHB
76	AHCY	77	GBA2	78	PDX1
79	AHSA1	80	GCAT	81	PDXP
82	AKR1B1	83	GCG	84	PFKFB1
85	AKT1	86	GCLM	87	PFKFB4
88	ALAD	89	GGT5	90	PFKM
91	ALAS1	92	GIG18	93	PGD
94	ALAS2	95	GLB1	96	PGLS
97	ALDH18A1	98	GLDC	99	PGR
100	ALDH1A1	101	GLRA1	102	PHOSPHO1
103	ALDH1B1	104	GLRA2	105	PHYKPL
106	ALDH2	107	GLRA3	108	PIK3CG
109	ALDH4A1	110	GLUD1	111	PIM1
112	ALDH5A1	113	GLUD2	114	PIPOX
115	ALDH9A1	116	GLUL	117	PKIA
118	ALDOA	119	GLYAT	120	PLA2G1B
121	ALOX5	122	GLYATL1	123	PLA2G2A
124	AMD1	125	GM2A	126	PLA2G2E
127	AMT	128	GNMT	129	PLAU
130	AMY2A	131	GNPDA1	132	PLG
133	ANXA3	134	GNRH1	135	PNMT

136	APRT	137	GOT2	138	PNP
139	AR	140	GPHN	141	PON1
142	ARF1	143	GPI	144	PPARA
145	ARF4	146	GPT	147	PPARD
148	ARG1	149	GPT2	150	PPARG
151	ARG2	152	GRIA2	153	PPIA
154	ASL	155	GRIN1	156	PPIH
157	ASNA1	158	GRIN2A	159	PPOX
160	ASRGL1	161	GRIN2B	162	PRDX2
163	ATP5A1	164	GRIN2C	165	PRKACA
166	BAAT	167	GSK3B	168	PRKCD
169	BAX	170	GSR	171	PROSC
172	BBC3	173	GSTA1	174	PRSS1
175	BCHE	176	GSTA2	177	PRSS3
178	BCL2	179	GSTA5	180	PSMA1
181	BDNF	182	GSTM1	183	PTGER3
184	BHMT	185	GSTM4	186	PTGS1
187	BHMT2	188	GSTP1	189	PTGS2
190	C8G	191	GSTZ1	192	PTPN1
193	CA2	194	GUSB	195	PYCR1
196	CA4	197	HAGH	198	PYCR2
199	CAD	200	HAO1	201	PYGL
202	CALM1	203	HARS	204	PYGM
205	CASP3	206	HDAC8	207	PYY
208	CASP8	209	HDC	210	RB1
211	CASP9	212	HMGCR	213	RBKS
214	CAT	215	HMOX1	216	RBP2
217	CBR1	218	HS3ST3A1	219	RDH11
220	CBS	221	HSD17B1	222	RDH13
223	CCK	224	HSD17B8	225	RDH5
226	CCL2	227	HTR2A	228	RELA
229	CCNA2	230	HTR3A	231	REN
232	CCND1	233	IGHG1	234	RNASE1
235	CD36	236	IKBKB	237	RRM1
238	CDC25B	239	IL10	240	RTCA
241	CDK2	242	IL1B	243	RXRA
244	CDK4	245	IL6	246	RXRG
247	CDKN1A	248	IMPA1	249	SCD
250	CELA1	251	INS	252	SCN5A
253	CES2	254	ISYNA1	255	SDHA
256	CETP	257	JUN	258	SDS
259	CHDH	260	KCNH2	261	SELP
262	CHEK1	263	KCNMA1	264	SERPINA1
265	CHRM1	266	KDR	267	SERPINE1
268	CHRM2	269	KYAT1	270	SHMT1
271	CHRM3	272	KYNU	273	SHMT2

274	CHRM4	275	LARS	276	SI
277	CHRNA2	278	LCMT2	279	SIRT5
280	CHRNA7	281	LCT	282	SLC25A10
283	CITED1	284	LDHA	285	SLC25A12
286	CKM	287	LDHB	288	SLC25A13
289	COX5A	290	LPL	291	SLC25A15
292	CPA1	293	LTA4H	294	SLC2A2
295	CPB1	296	MAOA	297	SLC36A1
298	CPT1A	299	MAOB	300	SLC6A2
301	CRH	302	MAP1B	303	SLC6A3
304	CRP	305	MAP2	306	SLC6A4
307	CTH	308	MAPK10	309	SOAT1
310	CTNNB1	311	MAPK14	312	SOD1
313	CTRB1	314	MARS	315	SPR
316	CTSB	317	MAT1A	318	SRC
319	CTSD	320	MAT2A	321	SULT2B1
322	CXCL8	323	MCAT	324	TEP1
325	DAO	326	MCL1	327	TGFB1
328	DCXR	329	ME1	330	TGM7
331	DDAH2	332	ME2	333	TNF
334	DHODH	335	ME3	336	TNFSF13B
337	DHRX	338	METAP2	339	TP53
340	DMPK	341	MGAM	342	TPI1
343	DNPEP	344	MGMT	345	TRAPPC4
346	DPP4	347	MMP1	348	TREH
349	DRD1	350	MMP12	351	TRPV1
352	DUOX2	353	MMP3	354	TXNRD1
355	EDN1	356	MMP8	357	TYRP1
358	EGLN1	359	MPO	360	UCP2
361	EIF6	362	MTR	363	UCP3
364	ENPEP	365	NAGS	366	UROD
367	EPHX1	368	NCF1	369	WARS2
370	ERBB2	371	NCOA1	372	XDH

Supplementary Table 5. Asthma-related human genes

NO.	Genes	NO.	Genes	NO.	Genes	NO.	Genes
1	IL13	2	HNMT	3	JAK1	4	ITK
5	NPSR1	6	T	7	ATP2A2	8	TNFRSF14
9	IL33	10	CRB1	11	CYSLTR1	12	CRHR2
13	TSLP	14	P2RX7	15	STAT5A	16	RNASE2
17	HLA-DQB1	18	HAVCR1	19	IFNA1	20	PTGER3
21	TNF	22	IL15	23	ITGA4	24	GRK5
25	IL1RL1	26	IL12A	27	CRHR1	28	LTB4R
29	RAD50	30	VCAM1	31	TBXA2R	32	MIR15A
33	CHI3L1	34	CD274	35	PROS1	36	PMCH
37	SMAD3	38	CYP1B1	39	MYB	40	SLC25A46
41	ORMDL3	42	AHR	43	NR3C2	44	DENND1B
45	IL18R1	46	ASOBS	47	IL7	48	TLR10
49	ADRB2	50	ASRT8	51	EMSY	52	BMPR1B
53	GSDMB	54	ASRT6	55	GSTA1	56	ABI3BP
57	HLA-G	58	ASRT4	59	IL27	60	CCL3L1
61	VEGFA	62	ASRT3	63	STARD13	64	GSTO2
65	TGFB1	66	AASTH27	67	ALOX5AP	68	SVEP1
69	HLA-DRA	70	AASTH54	71	BMPR2	72	SERPINE2
73	ACE	74	AASTH55	75	PEBP1	76	TAGLN
77	FLG	78	AASTH38	79	IL9	80	LTBP1
81	IL10	82	AASTH17	83	SCGB3A2	84	IL31
85	IL6	86	AASTH31	87	SLC11A1	88	CCR4
89	ADIPOQ	90	AASTH7	91	ADCY3	92	IL20
93	MMP9	94	AASTH42	95	TAF7	96	NEIL1
97	HLA-DRB1	98	AASTH41	99	PLA2G2A	100	HAS2
101	BDNF	102	AASTH15	103	KITLG	104	PCDH9
105	VDR	106	AASTH16	107	HAVCR2	108	CCL7
109	HLA-DQA1	110	AASTH39	111	DEFB1	112	CRLF2
113	TLR4	114	AASTH2	115	CTTN	116	SPATS2L
117	RORA	118	AASTH3	119	IKZF2	120	IL19
121	IL1B	122	AASTH4	123	SFTPA1	124	GLRX
125	ALOX5	126	AASTH5	127	NAT1	128	MIR19A
129	GSTT1	130	AASTH6	131	STAT4	132	FBXL7
133	CRP	134	AASTH1	135	CHRM3	136	PCDH20
137	GSTM1	138	AASTH28	139	MMP12	140	FLT3LG
141	IFNG	142	AASTH32	143	ANXA5	144	MKLN1
145	MTHFR	146	AASTH56	147	ADAM12	148	PTPRD
149	IL6R	150	AASTH29	151	USP15	152	IL17RA
153	HLA-DPB1	154	AASTH37	155	PLA2G4A	156	KCNMB1
157	CXCL8	158	AASTH33	159	IL6ST	160	TFF2
161	APOE	162	AASTH36	163	CCL3	164	FABP5
165	EGFR	166	AASTH35	167	RYR2	168	EFNA1
169	SERPINE1	170	AASTH34	171	ITGB4	172	BPI
173	PTGS2	174	AASTH10	175	CD86	176	MYH11

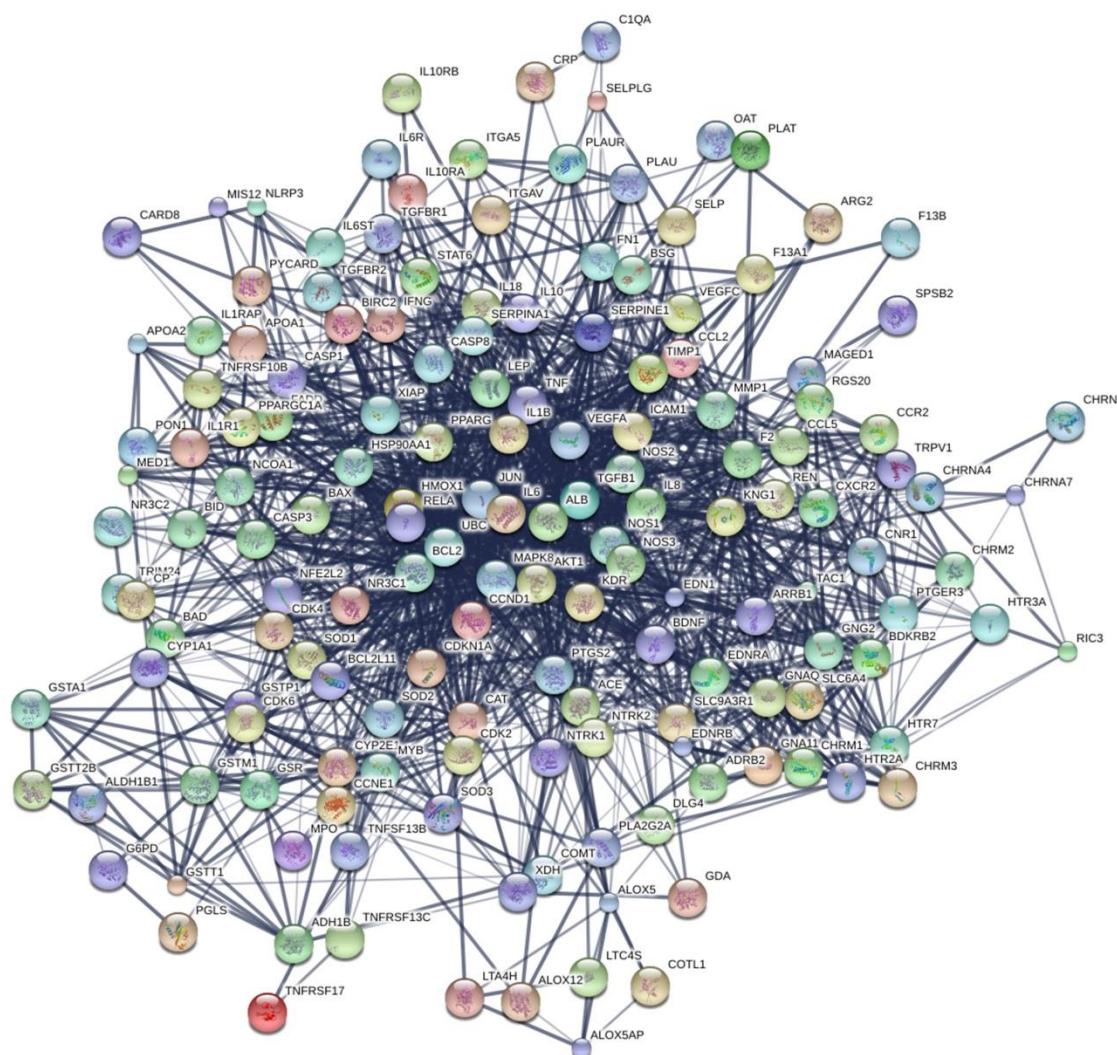
177	CFTR	178	AASTH8	179	SYNE2	180	CNTLN
181	IL2RA	182	AASTH11	183	EDNRB	184	TET1
185	GSTP1	186	AASTH13	187	MPL	188	TAC3
189	CDK2	190	AASTH12	191	RACK1	192	IKZF4
193	LEP	194	AASTH9	195	AREG	196	C10orf54
197	IL5	198	AASTH22	199	TNFSF4	200	LGALS7
201	STAT3	202	AASTH24	203	CSF3	204	PDCD1LG2
205	TBX21	206	AASTH18	207	P2RY12	208	AOC1
209	CCL2	210	AASTH19	211	NOD1	212	ADH5
213	SLC6A4	214	AASTH23	215	TUSC3	216	LTB4R2
217	NR3C1	218	AASTH26	219	PDCD4	220	GZMA
221	IL17A	222	AASTH25	223	CSF1R	224	CLCA1
225	GC	226	AASTH21	227	DGKH	228	DOCK10
229	IL18	230	AASTH20	231	TERC	232	CDHR3
233	S100B	234	AASTH46	235	CFL1	236	HLX
237	NOS3	238	AASTH45	239	TJP1	240	XCL1
241	CCL11	242	AASTH44	243	C5	244	PLA2G10
245	IL4	246	AASTH53	247	VTN	248	ICOSLG
249	CCR5	250	AASTH43	251	CCL20	252	SLURP1
253	BTNL2	254	AASTH51	255	CHRNA5	256	C3AR1
257	ICAM1	258	AASTH50	259	SLC24A2	260	IL13RA1
261	TNXB	262	AASTH49	263	MS4A2	264	SERPINB4
265	PPARG	266	AASTH47	267	RAB11FIP2	268	ZBTB10
269	IRF1	270	AASTH48	271	CCL18	272	TRPV2
273	GSDMA	274	AASTH52	275	DUSP1	276	EBI3
277	MYCN	278	AASTH14	279	MCAM	280	HHIP
281	CTLA4	282	AASTH40	283	CHRNA3	284	DLEU1
285	IL1R1	286	AASTH30	287	MYLK	288	MAP2K3
289	TLR2	290	CASR	291	TLR5	292	RAP1GAP2
293	CDH1	294	F2RL1	295	HSP90B1	296	PDE11A
297	HIF1A	298	MIR155	299	IL3	300	TIMD4
301	PTEN	302	IL5RA	303	ENPP2	304	KLHL5
305	FOXP3	306	ANGPT2	307	TLR8	308	IL9R
309	NFKB1	310	SCGB1A1	311	TREM1	312	CCL15
313	CD14	314	CREB1	315	CCR3	316	CCL1
317	ABCB1	318	ANK1	319	FTL	320	CCL24
321	PLA2G7	322	TGM2	323	TLR6	324	BST1
325	SOX9	326	ACO1	327	INHA	328	ZNF71
329	CTNNB1	330	ADAM33	331	KANK1	332	NTF4
333	PDE4D	334	IDO1	335	CXCR1	336	TRG
337	ERBB4	338	SELE	339	TSPO	340	EFR3B
341	MBL2	342	PROC	343	ITLN1	344	IGSF3
345	COMT	346	KLK3	347	ACKR1	348	RGS5
349	PRKCE	350	MIR146A	351	FCER2	352	COMMD10
353	HLA-E	354	NOS1	355	CCL17	356	RAMP1
357	CCL5	358	CTNNA3	359	S1PR1	360	PYHIN1
361	CD4	362	CCR2	363	CMA1	364	KIF3A

365	PON1	366	CALCA	367	C4A	368	NAGA
369	PTHLH	370	XIAP	371	ICOS	372	MIR192
373	SPP1	374	ADORA2A	375	IL16	376	DPP10
377	BCL2	378	IRAK3	379	CHRM2	380	ADAMTS9
381	HMGB1	382	FCGR2A	383	HERC5	384	DAP3
385	ITGB3	386	RNASE3	387	HTR3A	388	SEMA7A
389	MMP2	390	CAMP	391	SYNPO2	392	DLEU7
393	WDR36	394	AGL	395	SLPI	396	GLCCI1
397	NFE2L2	398	POSTN	399	IFNL1	400	CD53
401	CXCR4	402	NFKBIA	403	C5AR1	404	DEUP1
405	HMOX1	406	TGFBR1	407	PTGER4	408	SLC24A3
409	IL1RN	410	SLC26A4	411	CYP11A1	412	MINA
413	SOD1	414	ITGB2	415	LPIN2	416	MMP28
417	IL12B	418	TGFBR2	419	TRPM8	420	SSH1
421	MAPK1	422	IKZF3	423	IRF4	424	LEPQTL1
425	CYP2C19	426	S100A9	427	PBX2	428	IL18RAP
429	SOD2	430	FCGR3A	431	RGS2	432	FAM19A2
433	CCND1	434	ANXA2	435	KIR2DS2	436	SERBP1
437	TLR1	438	G6PD	439	CRIM1	440	SPTBN2
441	PTGDR	442	FGB	443	PTGDR2	444	ACSL3
445	CDH13	446	CYBA	447	XDH	448	HPGDS
449	CXCL12	450	TNFSF13B	451	AKR1C3	452	PTPRE
453	APOA1	454	CDH23	455	TNS1	456	ZNF365
457	GATA2	458	ADM	459	OSM	460	IL17RB
461	PRKCQ	462	FYN	463	PDE9A	464	NFIA
465	MMP1	466	MUSK	467	CHRM1	468	SIGLEC8
469	PARP1	470	MMP7	471	LAMC2	472	MMP25
473	SLC30A8	474	SH2B3	475	DENND1A	476	XKR6
477	EDN1	478	EGR1	479	ATG3	480	ENPP3
481	SLC22A5	482	F13A1	483	KIR2DL2	484	RETNLB
485	XRCC1	486	LGALS1	487	TPSAB1	488	ACAA1
489	KIT	490	PTX3	491	FADS2	492	SIGIRR
493	AGER	494	CSMD1	495	HRH1	496	CCL28
497	CXCL10	498	SELP	499	TRPA1	500	CPA3
501	IL4R	502	NPY	503	SCG3	504	KIFC1
505	CAV1	506	FGA	507	EDIL3	508	RANBP6
509	CD44	510	RBP4	511	PLD2	512	NPS
513	MIR21	514	F2R	515	RGS4	516	SLC26A9
517	ITGB1	518	EPHX1	519	HEXB	520	MMP21
521	NOTCH4	522	NPSR1-AS1	523	SFTPC	524	SOCS5
525	MAVS	526	SOCS3	527	CD276	528	MUC19
529	NAT2	530	IL23R	531	FPR2	532	PCDH1
533	PLAUR	534	ADRB3	535	IGHE	536	TNFAIP8L2
537	TLR9	538	VAV3	539	C6orf10	540	UNC119
541	LEPR	542	TXN	543	SPINK5	544	UBE3C
545	GHRL	546	DCLK1	547	RBM17	548	C5orf56

549	NOS2	550	LRP1	551	LTA4H	552	SLC30A4
553	KDR	554	PARK7	555	LGALS9	556	SEMA4A
557	NOD2	558	CEBPB	559	SPRY2	560	DCBLD2
561	BAX	562	LTF	563	GNGT1	564	LYNX1
565	CASP3	566	CLEC16A	567	CCR1	568	PPP1R12B
569	CYP1A1	570	SFTPD	571	TLE4	572	PSORS1C1
573	PTGER2	574	IL22	575	LY96	576	KCNIP4
577	PSAP	578	FGG	579	TGFBR3	580	BATF
581	LRRC32	582	IL21	583	IL25	584	C1orf100
585	PRKG1	586	HSPD1	587	MIR16-1	588	FOXA3
589	MAPK3	590	NGF	591	PAFAH1B1	592	LY86
593	IL2	594	HLA-DQA2	595	CYP2J2	596	INPP4A
597	HLA-DPA1	598	SMAD2	599	UTS2	600	TPSB2
601	FMR1	602	ANGPT1	603	STAT2	604	CHML
605	F3	606	HDAC2	607	ZBTB38	608	TBCD
609	SLC22A4	610	NPPA	611	ADAM8	612	C6orf118
613	HTR2A	614	DDX1	615	CCL22	616	CA10
617	PRKAA2	618	ADH1B	619	ACVR1	620	C11orf71
621	P2RY2	622	HLA-DOA	623	SDC4	624	TAC4
625	TNFSF10	626	MYD88	627	BDKRB1	628	CRTAM
629	STAT6	630	CTNND2	631	CTNNA1	632	STK10
633	CAT	634	CX3CR1	635	ADORA1	636	PGAP3
637	CFH	638	CD28	639	ATF6B	640	OR6X1
641	CHD7	642	F10	643	KIR2DL3	644	COL6A5
645	LTA	646	ETS1	647	TNFRSF13B	648	ZNF665
649	C3	650	MUC5AC	651	GCLC	652	LOC727896
653	FGF2	654	LTC4S	655	KIR2DL4	656	DTD1
657	CYP3A4	658	SOCS1	659	NDFIP1	660	OPN3
661	IL2RB	662	CD38	663	PDHA1	664	TPSD1
665	PRKCA	666	PTPN6	667	MAP3K1	668	FGFBP2
669	NQO1	670	TLR7	671	HTR4	672	SEMA3D
673	CASP8	674	IL17F	675	XPR1	676	LOC101060400
677	IL1A	678	PCDH15	679	SERPINB3	680	CRCT1
681	LGALS3	682	ADA	683	RIPK2	684	COL26A1
685	GAB1	686	CXCR3	687	MRC1	688	RASGRP4
689	RETN	690	CHRNA7	691	IL37	692	ZBP2
693	MPO	694	SIM2	695	TPT1	696	KLK9
697	ABCA1	698	MUC7	699	UGT1A6	700	GNG5P5
701	TLN1	702	S100A8	703	CD27	704	HCG23
705	RHOA	706	PECAM1	707	CCL26	708	RPS28P1
709	COL1A1	710	ARG1	711	FCRL3	712	LOC101928284
713	ABL1	714	APOH	715	IRAK4	716	ATPAF1
717	MIF	718	COL1A2	719	LAMA3	720	IGES
721	PLAU	722	GZMB	723	INSIG2	724	HLA-DRB2
725	SERPINA1	726	ALOX15	727	TNFRSF4	728	FSIP1
729	IGFBP3	730	PTGS1	731	CASP10	732	RGS7BP
733	EGF	734	NKX2-1	735	ATG5	736	ASB3

737	OPRK1	738	BST2	739	ARG2	740	PKDCC
741	NLRP3	742	TNC	743	CLDN5	744	LOC101929231
745	FN1	746	TAC1	747	HRH4	748	LOC101928940
749	CD40	750	PHF11	751	CD69	752	SPRR2B
753	TIMP1	754	ELN	755	PLXNA4	756	CNKSR3
757	FASLG	758	CHIT1	759	LAMB3	760	LOC102724802
761	CYP3A5	762	ELANE	763	IL1R2	764	TPSG1
765	CTGF	766	INHBA	767	NTF3	768	MRPL42
769	PMEL	770	TNFRSF10B	771	SLC6A11	772	SLC6A7
773	BCL2L1	774	NOX4	775	FBLN1	776	SCGB1C1
777	GATA3	778	TGFB2	779	ADCYAP1R1	780	CACNG6
781	CLU	782	HPSE2	783	CYSLTR2	784	ZNF432
785	CPS1	786	CXCR2	787	FLRT2	788	LYRM9
789	TRPV1	790	FCER1A	791	CHIA	792	MIR3162
793	NEWENTRY						

1.2 Supplementary Figures



Supplementary Figure 1. Reaction network of YPFS that antagonizing asthma. The reaction network of YPFS antagonizing asthma was built through String database. This network contains 158 nodes and 1730 interactive relationships. The thickness of the lines has positive correlation with interactive relationships between YPFS and asthma.