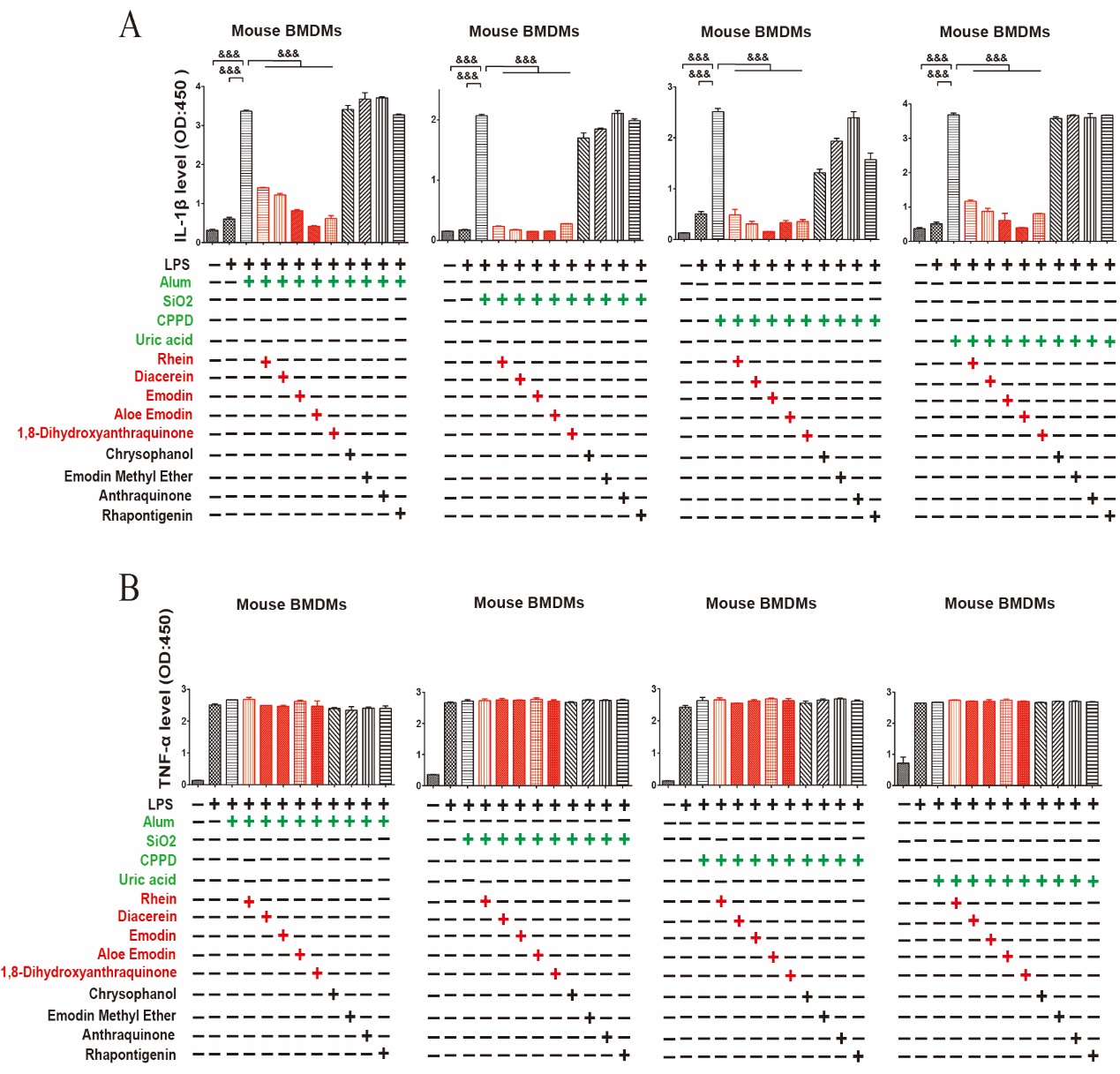
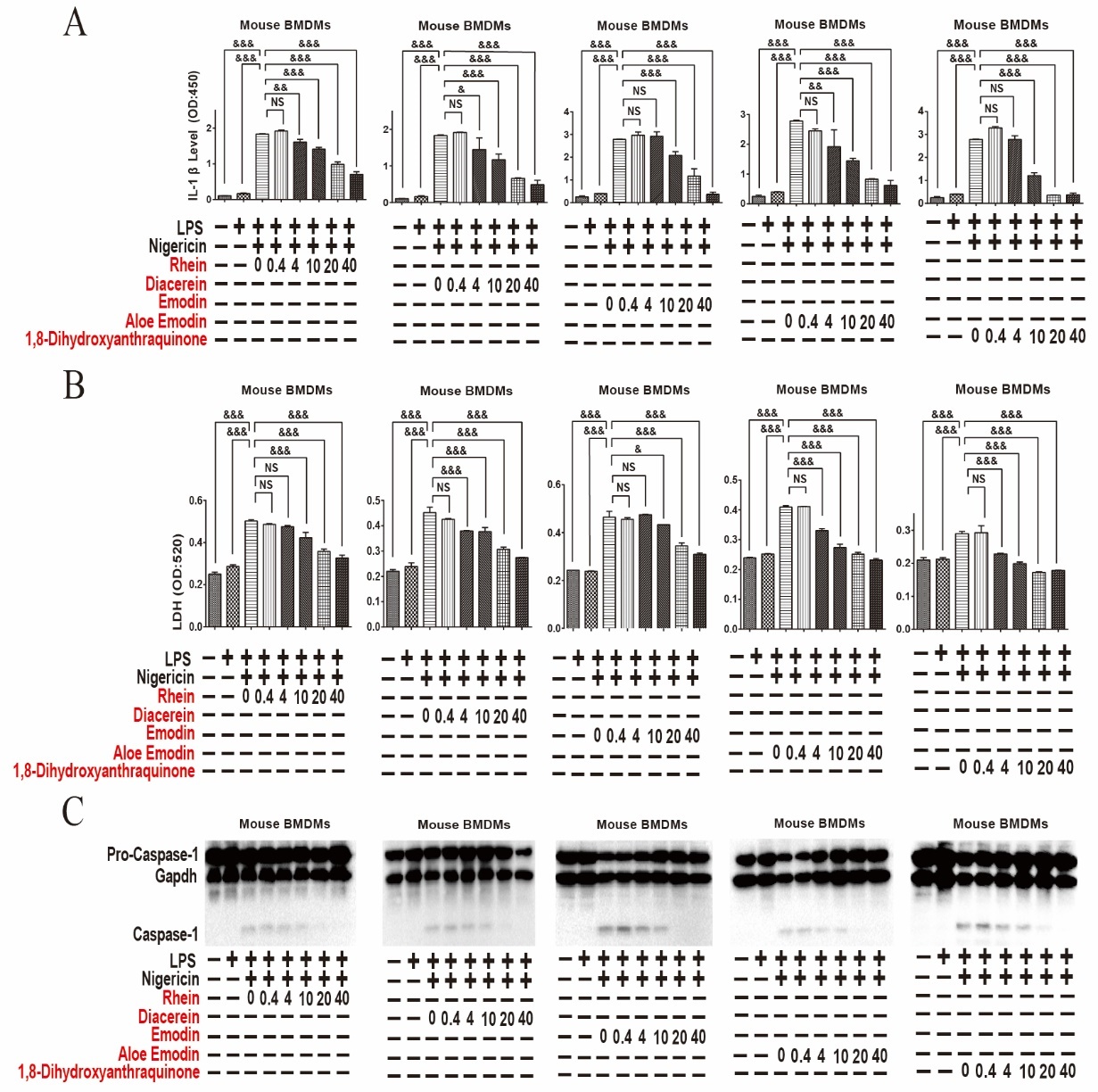
# Supplementary Materials

## Supplementary figures



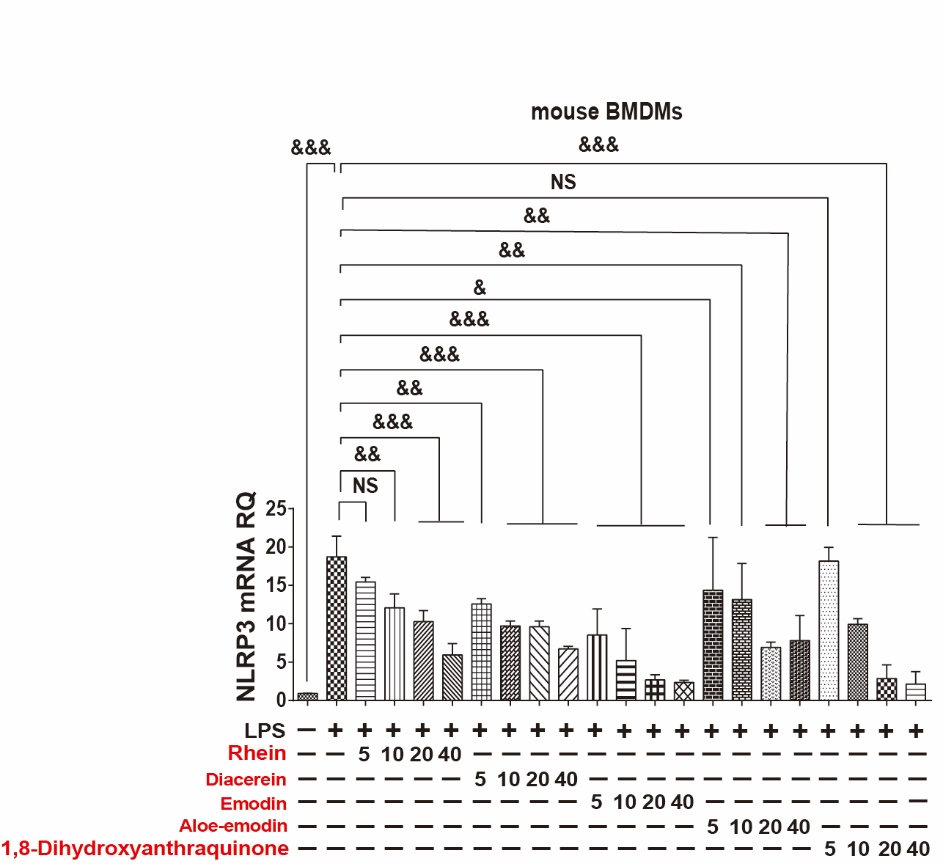
### Additional file 1: Fig S 1. RFAs inhibited NLRP3 inflammasome induced by uric acid, alum, SiO2 and CPPD.

Moue BMDMs were pretreated with RFAs (40μM rhein / 40μM diacerein, 40μM emodin, 40μM aloe emodin, 40μM 1,8-dihydroxyanthraquinone, 20μM chrysophanol, 4μM emodin methyl ether, 4μM anthraquinone) for 30 minutes and then stimulated with LPS (100ng / ml) for 4 hours. uric acid (100μg / ml), alum (100μg / ml), SiO2 (100μg / ml) and CPPD (100μg / ml) were added for 12 hours. IL-1β (**A**) and TNF-α (**B**) in cell culture supernatant were detected by ELISA (n=3 / group). Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ANOVA, analysis of variance; BMDMs, bone marrow-derived macrophages; CPPD, calcium pyrophosphate; ELISA, enzyme linked immunosorbent assay; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; SiO2, silicon dioxide; TNF-α, tumor necrosis factor-alpha.



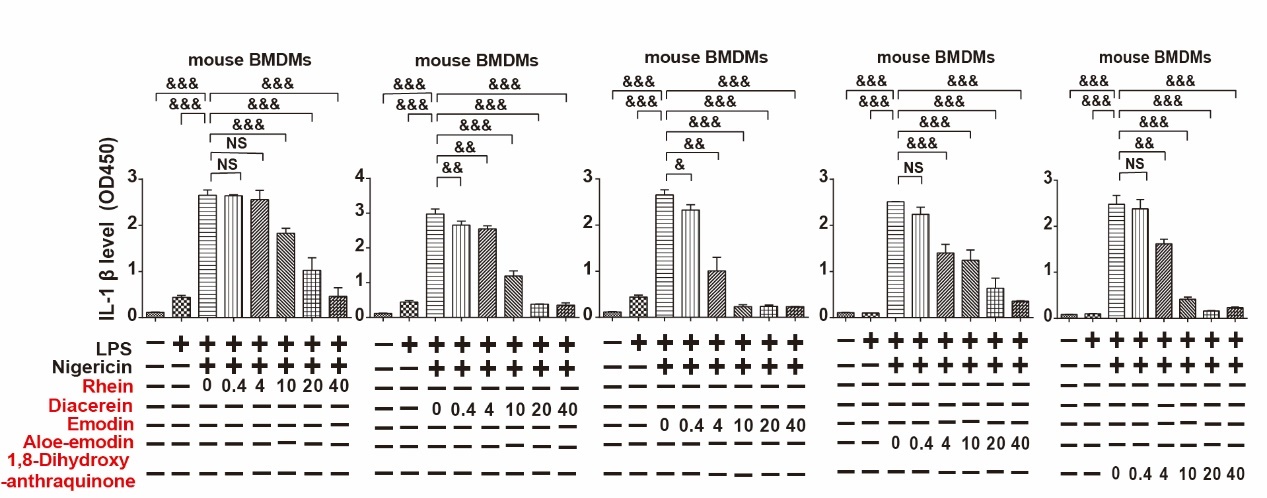
### Additional file 1: Fig S 2. RFAs inhibited NLRP3 inflammasome in a dose dependent manner.

Mouse BMDMs were pretreated by rhein, diacerein, emodin, aloe emodin and 1,8-dihydroxyanthraquinone with the concentration of 40μM, 20μM, 10μM, 4μM and 0.4μM for 30 minutes, and then stimulated with LPS (100ng / ml) for 4 hours. Nigericin (2.5μM) was added for 2 hours. IL-1β (**A**) in cell culture supernatant were detected by ELISA (n=3 / group), LDH (**B**) in cell culture supernatant were detected by biochemical kit (n=3 / group), The cleavage of pro-caspase-1 (**C**) was detected by western blot. Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ANOVA, analysis of variance; BMDMs, bone marrow-derived macrophages; ELISA, enzyme linked immunosorbent assay; IL-1β, interleukin-1 beta; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NS, no significance; TNF-α, tumor necrosis factor-alpha.



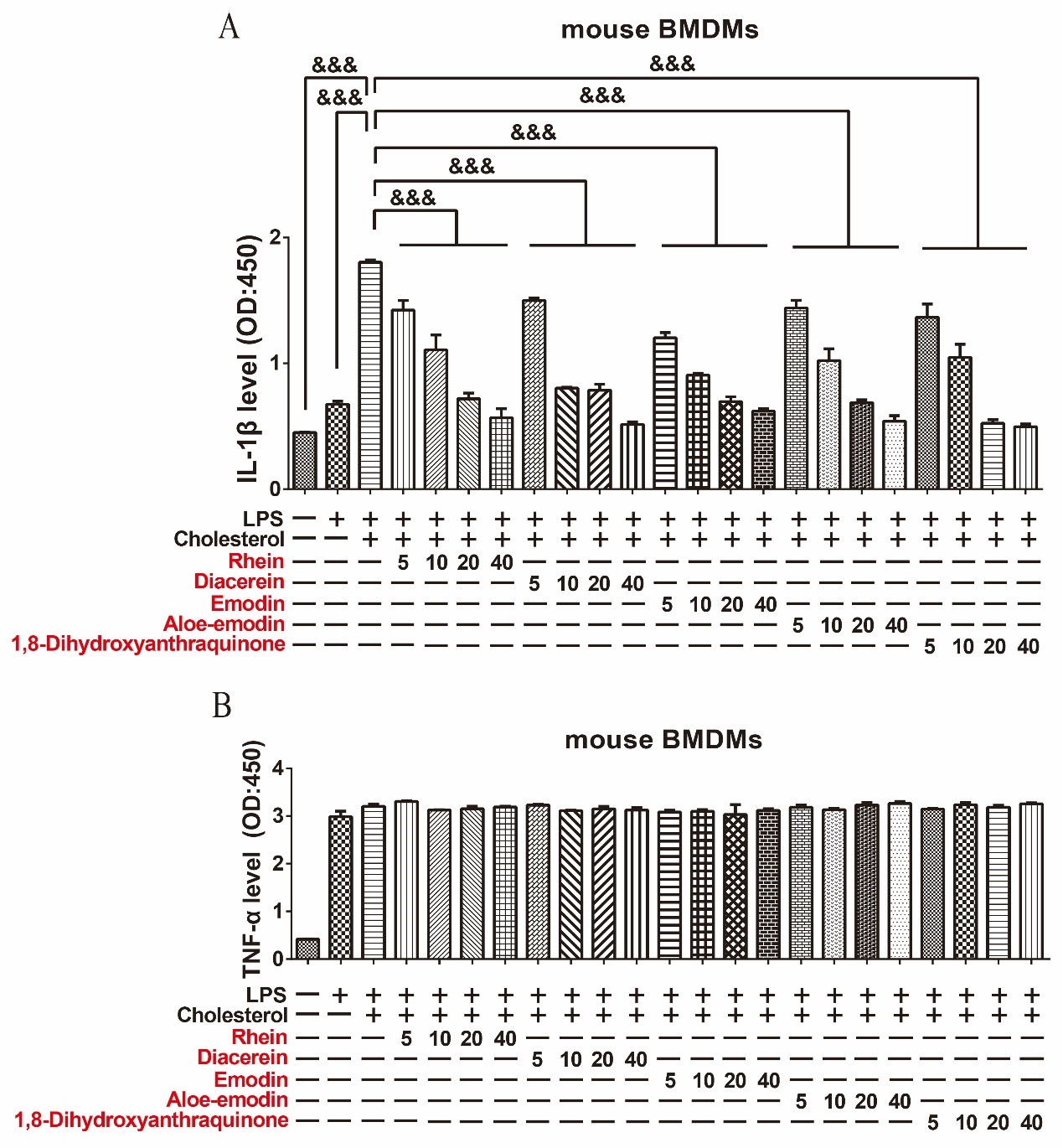
### Additional file 1: Fig S 3. RFAs inhibited the transcription of NLRP3 in a dose dependent manner.

Mouse BMDMs were pretreated by rhein, diacerein, emodin, aloe emodin and 1,8-dihydroxyanthraquinone with the concentration of 40μM, 20μM, 10μM and 5μM for 30min and then stimulated with 100ng / ml LPS for 1 hours. NLRP3 mRNA were detected by RT-qPCR (n=3 / group). Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ANOVA, analysis of variance; BMDMs, bone marrow-derived macrophages; LPS, lipopolysaccharide; NS, no significance; RT-qPCR, quantitative reverse transcription polymerase chain reaction.



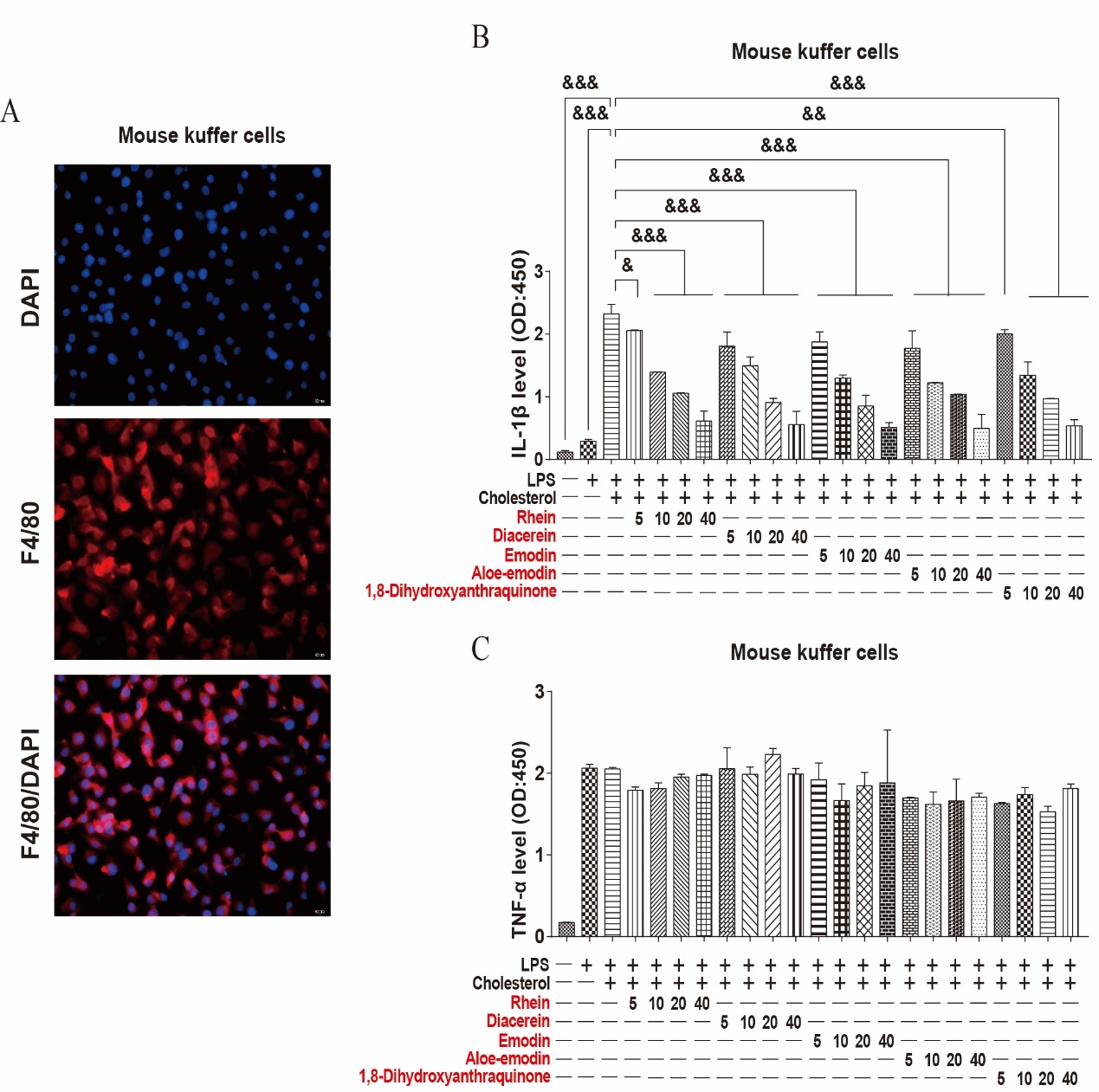
### Additional file 1: Fig S 4. RFAs inhibited the assembly of NLRP3 inflammasome in a dose dependent manner.

Mouse BMDMs were stimulated by LPS (100ng / ml) for 4 hours before the media was changed. Rhein, diacerein, emodin, aloe emodin and 1,8-dihydroxyanthraquinone with the concentrations of 40μM, 20μM, 10μM, 4μM and 0.4μM were added for 30 minutes and then stimulated with nigericin (2.5μM) for 2 hours. IL-1β in cell culture supernatant was detected by ELISA (n=3 / group). Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ANOVA, analysis of variance; BMDMs, bone marrow-derived macrophages; ELISA, enzyme linked immunosorbent assay; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide, NS, no significance.



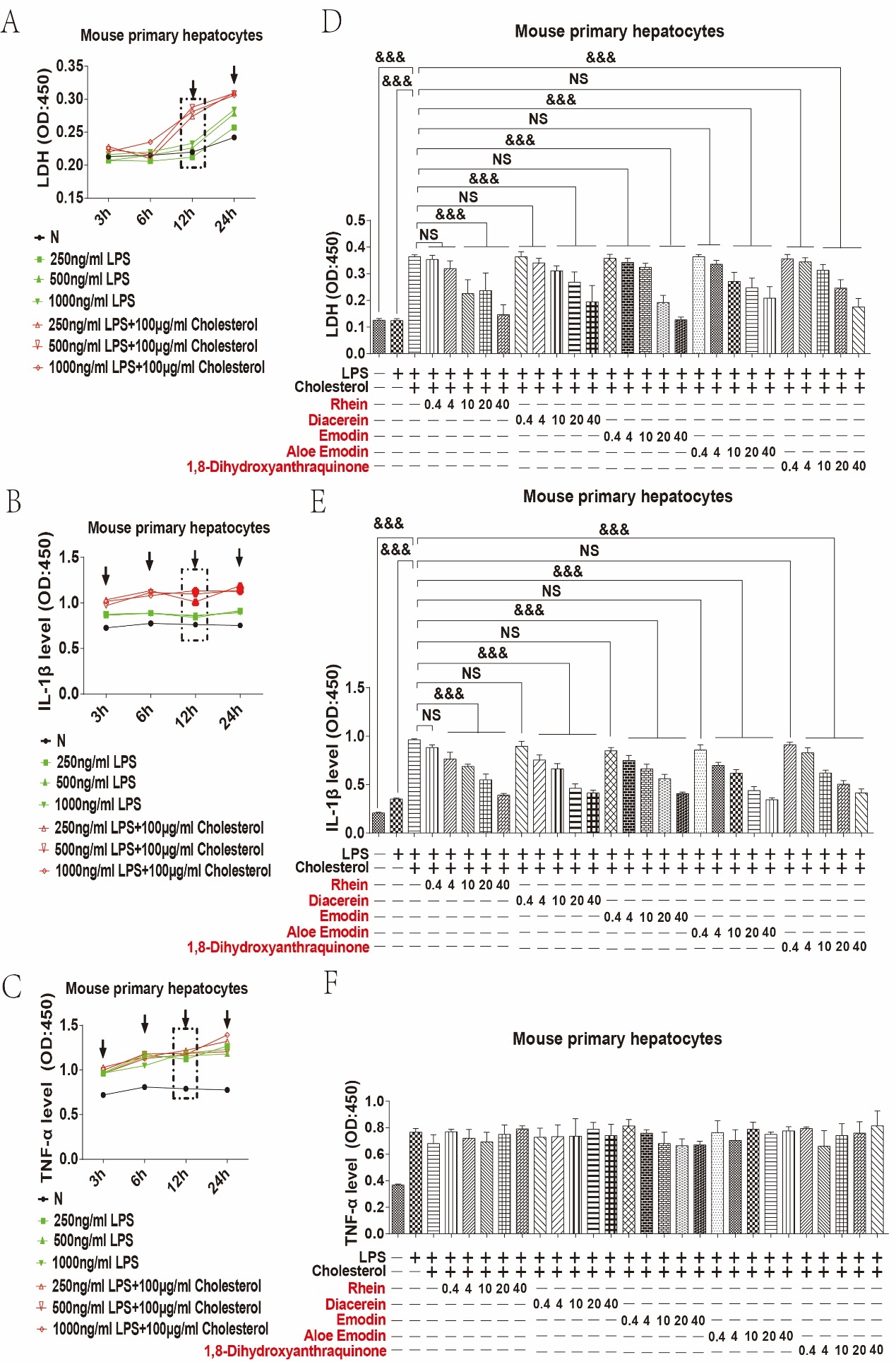
### Additional file 1: Fig S 5. RFAs inhibited cholesterol induced NLRP3 inflammasome in mouse BMDMs in a dose dependent manner.

Mouse BMDMs were pretreated by RFAs (Rhein, diacerein, emodin, aloe emodin and 1,8-dihydroxyanthraquinone with the concentrations of 40μM, 20μM, 10μM and 5μM) for 30 minutes and then stimulated with LPS (100ng/ml) for 4 hours and cholesterol (100μg / ml) for 12 hours. IL-1β (**A**) and TNF-α (**B**) in cell culture supernatant were detected by ELISA (n=3 / group). Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ANOVA, analysis of variance; BMDMs, bone marrow-derived macrophages; ELISA, enzyme linked immunosorbent assay; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha.



### Additional file 1: Fig S 6. RFAs inhibited cholesterol induced NLRP3 inflammasome in mouse Kuffer cells.

Mouse Kuffer cells were identified by immunofluorescence through detecting F4/80 (**A**). Mouse Kuffer cells were pretreated by RFAs (Rhein, diacerein, emodin, aloe emodin and 1,8-dihydroxyanthraquinone with the concentrations of 40μM, 20μM, 10μM and 5μM) for 30 minutes and then stimulated with LPS (100ng / ml) for 4 hours and cholesterol (100μg / ml) for 12 hours. IL-1β (**B**) and TNF-α (**C**) in cell culture supernatant were detected by ELISA (n=3 / group). Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ANOVA, analysis of variance; DAPI, 4',6-diamidino-2-phenylindole; ELISA, enzyme linked immunosorbent assay; IL-1β, interleukin-1 beta; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha.



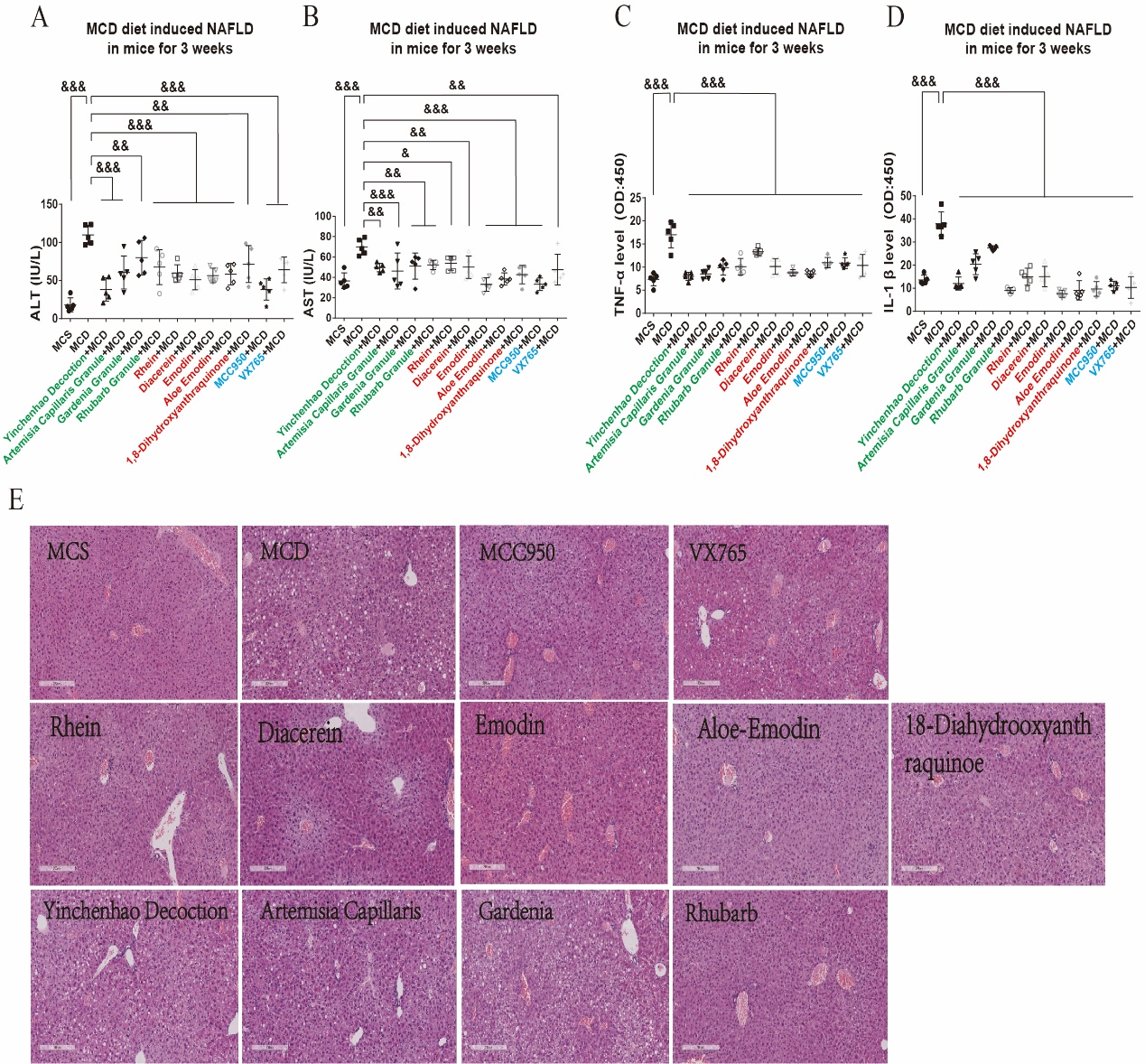
### Additional file 1: Fig S 7. RFAs inhibited cholesterol induced NLRP3 inflammasome in mouse primary hepatocytes.

Mouse primary hepatocytes were stimulated by LPS (250ng / ml, 500ng / ml and 1000ng / ml) for 4 hours and then stimulated by cholesterol (100μg / ml) for 12 hours, LDH (**A**) were detected by LDH kits (n=1 / group); IL-1β (**B**) and TNF-α (**C**) were detected by ELISA (n=1 / group). Mouse primary hepatocytes were pretreated by RFAs (Rhein, diacerein, emodin, aloe emodin and 1,8-dihydroxyanthraquinone with the concentrations of 40μM, 20μM, 10μM and 5μM) for 30 minutes and then stimulated with LPS (1000ng / ml) for 4 hours and cholesterol (100μg / ml) for 12 hours. LDH were detected by LDH kits (**D**) (n=3 / group); IL-1β (**E**) and TNF-α (**F**) were detected by ELISA (n=3 / group). Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ANOVA, analysis of variance; ELISA, enzyme linked immunosorbent assay; IL-1β, interleukin-1 beta; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NS, no significance; TNF-α, tumor necrosis factor-alpha.



### Additional file 1: Fig S 8. RFAs inhibited ALT and AST in mouse primary hepatocytes stimulated by LPS + cholesterol.

Mouse primary hepatocytes were stimulated by LPS (250ng / ml, 500ng / ml and 1000ng / ml) for 4 hours and then stimulated by cholesterol (100μg / ml) for 12 hours, ALT (**A**) and AST (**B**) were detected by ALT / AST kits (n=1 / group). Mouse primary hepatocytes were pretreated by RFAs (Rhein, diacerein, emodin, aloe emodin and 1,8-dihydroxyanthraquinone with the concentrations of 40μM, 20μM, 10μM and 5μM) for 30 minutes and then stimulated with LPS (1000ng / ml) for 4 hours and cholesterol (100μg / ml) for 12 hours. ALT (**C**) and AST (**D**) were detected by ALT / AST kits (n=3 / group). Data are presented as mean ± SEM. For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; ELISA, enzyme linked immunosorbent assay; IL-1β, interleukin-1 beta; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NS, no significance; TNF-α, tumor necrosis factor-alpha.



### Additional file 1: Fig S 9. RFAs improved MCD diet induced mice NAFLD by inhibiting NLRP3 inflammasome in the third week.

C57 BL/6 mice were fed by MCD diet for 3 weeks. RFAs (rhein / diacerein, emodin, aloe emodin, 1,8-dihydroxyanthraquinone), rhubarb and yinchenhao decoction as well as inflammasome inhibitors MCC950 and VX765 were given by gavage every 2 days when MCD diet treatment started. Serum ALT (**A**) and AST (**B**) were detected by ALT / AST kit, serum IL-1β (**C**) and TNF-α (**D**) were detected by ELISA. H&E stain and sirius red staining were also detected (**E**). Control group was fed by MCS diet (n=5 / group); Model group was fed by MCD diet (n=5 / group); Intervention groups were fed by MCD diet and different drugs (n=5 / group). For multiple comparisons, one-way ANOVA coupled with LSD’s post hoc testing was performed. &: P<0.05; &&: p<0.01; &&&: p<0.001. ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; ELISA, enzyme linked immunosorbent assay; H&E, hematoxylin and eosin; IL-1β, interleukin-1 beta; MCD, methionine and choline deficiency; MCS, methionine-choline-supplemented; TNF-α, tumor necrosis factor-alpha.

## Additional table

### Table S1 Main materials

|  |  |  |
| --- | --- | --- |
| **REAGENT** | **SOURCE** | **IDENTIFIER** |
| **Biochemical reagents & solutions** | | |
| Alum | Invivogen | 21645-51-2 |
| BSA | Sengon biotech | ST025 |
| CPPD | Sengon biotech | 7790-76-3 |
| DAPI | Beyotime | C1002 |
| LPS | Sigma | L2630 |
| Nigericin | Sigma | 481990 |
| SiO2 | Sengon biotech | 238-878-4 |
| 4% paraformaldehyde fix solution | Sengon biotech | E672002 |
| Lipofectamine 2000 | Invitrogen | 2030864 |
| Opti-MEM | Gibico | 2048098 |
| Protein A+G agrarose | Beyotime | P2055 |
| **Durgs** | | |
| Rhubarb | Tianjiang Pharmaceutical Co., Ltd | 18037004 |
| Rhein (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 171019/171109 |
| Aloe Emodin (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190307/171216 |
| Anthraquinone (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 170820 |
| Chrysophanol (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 180309 |
| Diacerin (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 171107/171224 |
| Emodin (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190120/180106 |
| Emodin methyl ether (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 180905 |
| 1,8-dihydroxyanthraquinone (Rhubarb) | Ronghe Pharmaceutical Technology Development Co., Ltd | 170716/180325 |
| Artemisia Capillaris | Tianjiang Pharmaceutical Co., Ltd | 18086954 |
| Artemisinin (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190420 |
| Caffeic acid (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190425 |
| Chlorogenic acid (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190327 |
| Crocin I (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190124 |
| Crocin II (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190305 |
| Ferulic acid (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 181109 |
| Hyperoside (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190402 |
| Quercetin (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190331 |
| Scopolactone (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190316 |
| 6-hydroxy-7-methoxycoumarin (Artemisia Capillaris) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190103 |
| Gardenia | Tianjiang Pharmaceutical Co., Ltd | 1807664 |
| Genipin (Gardenia) | Ronghe Pharmaceutical Technology Development Co., Ltd | 181229 |
| Geniposide (Gardenia) | Ronghe Pharmaceutical Technology Development Co., Ltd | 181205 |
| Geniposidic acid (Gardenia) | Ronghe Pharmaceutical Technology Development Co., Ltd | 181227 |
| Genipin-1-β-gentibioside (Gardenia) | Ronghe Pharmaceutical Technology Development Co., Ltd | 190223 |
| **Inhibitors:** | | |
| MCC950 | Selleck Biotechnology Co., Ltd | S8930 |
| VX765 | Selleck Biotechnology Co., Ltd | S2228 |
| **RNA isolation, reverse and amplification kits** | | |
| RNAfast200 kit | Fastagen | 220011 |
| Reverse transcriptionand kit | TAKARA | RR036 |
| Amplification kit | Toyobo | 857100 |
| **Primers** | | |
| Mouse β-actin primers | Genewiz | N/S |
| Mouse IL-1β primers | Genewiz | N/S |
| Mouse TNF-α primers | Genewiz | N/S |
| Mouse NLRP3 primers | Genewiz | N/S |
| **Antibody** | | |
| p-P65/P65 | CST | 3033/6956 |
| p-ERK/ERK | CST | 4730/4695 |
| p-JNK/JNK | CST | 4668/9252 |
| p-P38/P38 | CST | 4511/9212 |
| NLRP3 | Adipogen | AG-20B-0014 |
| Caspase-1 | Adipogen | AG-20B-0048 |
| ASC | CST | 67824 |
| GAPDH | Proteinteck | 60004-l-lg |
| β-actin | Proteinteck | 66009-l-lg |
| β-actin | CST | 4970 |
| α-tubulin | Proteinteck | 66031-l-lg |
| Anti-rabbit IgG-Cy3 | Abcam | ab6939 |
| **Critical commercial Assays** | | |
| ALT kit | Nanjing Jiancheng bioengineering institute | C009-2-1 |
| AST kit | Nanjing Jiancheng bioengineering institute | C010-2-1 |
| LDH kit | Nanjing Jiancheng bioengineering institute | A020-2-2 |
| IL-1β ELISA kits | Thermo Fisher | 88-7013 |
| TNF-α ELISA kits | Thermo Fisher | 88-7324 |