Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Chemoproteomics-based target profiling of sinomenine reveals multiple protein regulators of inflammation

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Supplementary Figures and Tables

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Materials

Sinomenine, SIN-Y1 and SIN-CW were extracted and synthesized in our lab. They were dissolved in DMSO (Sigma-Aldrich, MO, USA) to a final concentration of 300 μM and stocked in -20 °C for subsequent research. Tris [(1-benzyl-1H-1, 2, 3-triazol-4-yl) methyl] amine (TBTA, 510758-28-8), and Tris (2-carboxyethyl) phosphine (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The TAMRA-azide and biotin-PEG3-azide (CAS: 875770-34-6) were purchased from Click Chemistry Tools, LLC (Scottsdale, AZ, USA). $CuSO_4$ was purchased from Beijing Chemical Factory (Beijing, China). Pierce Streptavidin Agarose Resins (20353) were purchased from Thermo Scientific. LPS was purchased from Sigma-Aldrich (USA) and lipofectamine RNAiMAX reagent (13778150) was purchased from Invitrogen (USA). Ripk3 Polyclonal antibody (17563-1-AP) was purchased from Proteintech. Mouse TNF-α Elisa kits (Liv, CK-E20852), IL-6 Elisa kits (Liv, CK-E20188) and IL-1β Elisa kits (Liv, CK-E20174) were purchased from Beijing Bizi Biotechnology Co. LTD. The other Mouse TNF-α Elisa kits and IL-6 Elisa kits were purchased from R&D Systems Inc. and Novus Biologicals, LLC., respectively. The siRNAs were designed and synthesized by Genepharma Company (Suzhou, China).

Cell culture

RAW 264.7 cell was purchased from China Infrastructure of Cell Line Resource (Beijing, China). These cells were grown in the Dulbecco's Modified Eagle's Medium-F12 basic medium (Invitrogen, CA, USA) supplemented with 10% inactivated fetal bovine serum (FBS, Invitrogen) and cultured at 5% CO_2 and 37 °C.

The chemical synthesis of SIN derivative (SIN-Y1)

The synthetic route of SIN-Y1 is shown in **Figure 1a** according to our previous report [1]. Sinomenine hydrochlorid 2 g (5.46 mmol), 10% Pd-C 400 mg, and five drops of hydrochloric acid were dissolved in methanol (65 mL) under the pressure of 1.8 kg/cm^{-2}, and the reaction was finished after 6 h. The solution was then filtered through Celite and evaporated to dryness. The sample was dissolved with chloroform, adjusting to pH=7 by saturated sodium bicarbonate solution. The mixture was purified by column chromatography on silica gel eluted with dichloromethane: methanol (50:1-20:1, with 2‰ ammonia) to get SIN-Y1 as white solid with 71.2% yield. Analytical data for the purified probe in the spectra of ^{1}H NMR and ^{13}C NMR (**Figure S1 and Figure S2**) were shown as follows:

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (1H, d, *J* = 8.30 Hz, H-2), 6.56 (1H, d, *J* = 8.30 Hz, H-1), 6.25 (1H, br s, -OH), 4.27 (1H, d, *J* = 13.00 Hz, H-5a), 3.92 (1H, dd , *J* = 12.00, 6.80Hz, H-7), 3.81 (3H, s, 3-OCH_3), 3.41 (3H, s, 7-OCH_3), 3.16 (1H, br s, H-9), 2.96 (1H, d, *J* = 18.70 Hz, H-10a), 2.80 (1H, dd, *J* = 18.70, 6.30 Hz, H-10b), 2.68-2.53 (2H, m, H-16), 2.51 (3H, s, -NCH_3), 2.28 (1H, d, *J* = 12.90 Hz, H-5b), 2.22-2.15 (2H, m, H-8), 1.96-1.94 (2H, m, H-15), 1.65-1.55 (1H, m, H-14). ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.11, 145.45, 144.96, 128.71, 121.56, 118.79, 109.65, 83.32, 58.18, 57.59, 56.28, 48.49, 46.90, 43.17, 42.19, 41.45, 37.24, 34.35, 23.75. MS-ESI (m/z): 332.20 $[M + H]^+$.

The anti-inflammatory activity test of SIN derivative (SIN-Y1)

To determine the anti-inflammatory activity of SIN-Y1, RAW 264.7 cells were seeded at 5×10^5 cells/well in 24-well plates for 24 h and pretreated with SIN (10 μM or 100 μM), SIN-Y1 (10 μM or 100 μM) and isopyknic DMSO for 1 h, respectively. Treated cells were further incubatd with LPS (0.01 or 0.1 μg/mL) for 12 h. The cell supernatant were collected and kept at -20 °C before analysis. The levels of TNF-α (**Figure 1b**) and IL-1β (**Figure S4**) in the supernatant were diluted and measured by Elisa kit according to the manufacturer's instructions.

The chemical synthesis of SIN-based probe (SIN-CW)

The synthetic route of SIN-CW is shown in **Figure 1c**. To a stirred mixture in dry acetonitrile (6 mL) of SIN (131.6 mg, 0.4 mmol) and potassium tert-butyl alcohol (44.8 mg, 0.4 mmol) was added propargyl bromide (70.8 mg, 0.6 mmol) in dry acetonitrile (2.4 mL) dropwise over 45 min. The reaction mixture was stirred for 8 h at room temperature. The reaction was monitored by TLC. After filtration, the filtrate was evaporated under reduced pressure. Then the compound was separated and purified readily by HPLC. The mobile phase system for eluting was composed of water solution containing 0.1% trifluoroacetic acid (Solvent A) and acetonitrile containing 0.1% trifluoroacetic acid (Solvent B) with a flow rate of 3 mL/min. The protocol for the gradient eluting was listed as follows: 0-5 min, 4% B; 5-70 min, 4~30% B. The desired elution was combined, concentrated nad lypholized to give the SIN-based probe (20.5 mg). The structure of purified probe was verified by ^{1}H-NMR(500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) at room temperature. Analytical data for the purified probe in the spectra of ^{1}H NMR and ^{13}C NMR (**Figure S5 and Figure S6**) were shown as follows: ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 6.75 (1H, d, *J* = 8.3 Hz, H-2), 6.64 (1H, d, *J* = 8.3 Hz, H-1), 5.49 (1H, d, *J* = 2.1 Hz, H-8), 4.94 (2H, br s, H_2-1'), 4.39 (1H, d, *J* = 15.7 Hz,

H-5a), 3.83 (3H, s, 3-OCH_3), 3.71 (1H, d, *J* = 7.0 Hz, H-16a), 3.60 (1H, m, H-16b), 3.50 (3H, s, 7-OCH_3), 3.40-3.45 (1H, m, H-9), 2.94 (1H, s, H-3'), 2.78-2.84 (1H, m, H-10a), 2.63 (3H, s, -NCH_3), 2.63 (1H, d, *J* = 15.7 Hz, H-5b); 2.20-2.27 (1H, m, H-10b), 2.10-2.14 (1H, m, H-14), 1.24 (1H, d, *J* = 2.3 Hz, H-15b), 1.23 (1H, d, *J* = 7.0 Hz, H-15a). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 191.6, 152.5, 146.1, 144.5, 124.0, 119.2, 118.7, 110.2, 110.1, 82.2, 70.6, 66.4, 55.9, 55.2, 55.0, 51.6, 50.4, 46.5, 40.6, 39.1, 38.7, 27.3. HRMS (ESI) calculated for $C_{22}H_{25}NO_4$ $[M + H]^+$ 368.18564, found 368.18563.

Activity detection of SIN-CW

To determine the effect of SIN and SIN-CW on the viability of RAW 264.7 cell, cells were seeded at 1 × 10^5 cells/well in 96-well plates for 24 h, followed by incubation with different concentrations of SIN and SIN-CW (0.1 nM, 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM and 1000 μM) for 24 h. The viability of the cells was determined using the MTT assay and the absorbance was measured at 450 nm.

To determine the anti-inflammatory activity of SIN and SIN-CW on RAW 264.7 cells, cells were seeded at 1 × 10^5 cells/well in 96-well plates for 24 h and pretreated with SIN (0.1, 0.5, 1, 5, 10, 50, 100 or 300 μM), SIN-CW (0.1, 0.5, 1, 5, 10, 50, 100 or 300 μM) and isopyknic DMSO for 1 h, respectively. Treated cells were further incubated with LPS (0.01μg/mL) for 12 h. And the levels of TNF-α and IL-6 in the collected cell supernatant were measured by Elisa kits according to the manufacturer's instructions.

The in-gel fluorescence labeling of SIN-CW in RAW 264.7

Raw 264.7 cells were cultured in the 6-well plates and grown to 90% confluence before they were harvested with cell Scraper and collected by centrifuge at 1000 g for 3 min. The cell pellets were re-suspended in 300 μL lysis buffer and lyzed by sonication (25% amplification, 5 seconds on and 2 seconds off for 4 cycles). After that, the protein lysis was centrifuged (4°C, 14000 g) for 30 min and then divided into 5 equal portions of 40 μL. Each sample was added 50 μL PBS and incubation with 10 μL different concentrations of SIN-CW (0, 100, 500, 1000 and 3000 μM) for 1 h. Then each sample was added 90 μL PBS (containing 1% protease inhibitor) and 8 μL click buffer, including 4 μL TAMRA-azide (10 mM in DMSO), 2 μL TBTA (10 mM in DMSO) and 2 μL TCEP (100 mM in ddH_2O). A total 198 μL mixtures were gently vortexed before adding 2 μL $CuSO_4$ (100 mM in ddH_2O). The mixture was then shaked (400 rpm) in an incubation shaker at 37 °C for 1 h. The labeled proteins were precipitated by adding 800 μL prechilled acetone and washed the pellets with 500 μL ice-cold methanol twice. The

pellets were air-dried, followed by addition of 40 μL 0.4% SDS-PBS and 10 μL 5 × SDS loading buffer to dissolve the proteins. The samples were denatured by heating at 95 °C for 10 min and subsequently separated in 10% SDS-PAGE gel. The fluorescence gels were imaged using Typhoon FLA 9500 laser scanner (GE Healthcare Bio-Sciences, Piscataway, NJ) and stained with coomassie brilliant blue for visualization the total proteins.

For the competitive labeling assay, cell lysate samples were treated with high and different concentration of SIN (200, 400, 800, 1600, 3200 and 6400 μM) for 60 min before incubating with SIN-CW (200 μM), then treated as described above.

Pull-down assay

We identified the targets of SIN through a gel-free ABPP. Raw 264.7 cells were cultured in the 6-well plates and grown to 90% confluence before incubation with SIN-CW (100 μM) for 1 h. Then, these cells were harvested, lyzed as according to the procedure mentioned in the section of **The in-gel fluorescence labeling of SIN-CW in RAW 264.7**. After that, as much volume of protein lysis buffer was collected as possible, followed by addition of PBS (containing 1% protease inhibitor) to a total 190 μL reaction volume. Then, 4 μL biotin-PEG3-azide (10 mM in DMSO), 2 μL TBTA (10 mM in DMSO) and 2 μL TCEP (100 mM in ddH_2O) were added for every protein sample. Then, 2 μL $CuSO_4$ (100 mM in ddH_2O) was added in the pre-mixtures above to initiate the cycloaddition. The mixtures were incubated and shaked in an incubation shaker (400 rpm, 37 °C) for 1 h. The labeled proteins were precipitated by adding 800 μL prechilled acetone and washed the pellets with 500 μL ice-cold methanol twice. The pellets were air-dried, followed by addition of 800 μL 0.4% SDS-PBS and added 50 μL streptavidin-beads. After rotating incubation for 3 h at RT, these beads were collected by centrifuge at 2500 g for 5 min, and washed successively with 1 mL 0.4% SDS-PBS three times, 1 mL 6 M Urea for twice and 1 mL PBS three times, for subsequent experiment.

Trypsin digestion on beads

Following the pull down assay, the beads were re-suspended in 500 μL 6 M Urea/PBS solution and then added 25 μL dithiothreitol (DTT, 200 mM). The mixture in PE tube was incubated and shocked in 65 °C water bath for 15 min. Then, the mixture was incubated and shocked in 35 °C water bath for 30 min prior to the addition of iodoacetamide (IAA, 25 μL, 400 mM). After adding 950 μL ddH_2O, the mixture was

centrifuged (1400 g,4 °C) for 3 min. After discarding the supernatant, the beads were added 200 μL 2 M Urea/PBS solution, 2 μL 100 mM $CaCl_2$ and 4 μL 500 ng/μL trypsin (Promega, MADISON, WI, USA), followed by incubating and shaking overnight at 37 °C. The supernatant was collected after centrifugation (1400 g) for 3 min, the beads was washed by 50 μL ddH_2O twice. The three portions of supernatant were merged together, followed by addition of formic acid to a final concentration of 1% formic acid. Samples were desalted by using Pierce™ C18 Tips (100 μL, Thermo) according to the instructions and subsequently dried in a lyophilizer. Then, the peptide obtained from each sample was dissolved in 10 μL ddH_2O. The solution was then thoroughly vortexed and centrifuged (13200 rpm) for 10 min at 4 °C. The supernatant (6 μL) was injected into mass spectrometry machine for analysis

LC-MS/MS analysis

The peptides were analyzed by using Orbitrap Fusion Lumos mass spectrometer (Thermo fisher scientific, USA). After loading on a trap packed with Thermo Scientific™ Acclaim™ PepMap™ 100 C18 column (0.3 mm × 5 mm, 5 μm particle size), the chromatographic separation of peptides (6 μL) was carried out on an Acclaim PepMap RSLC C18 column (50 μm × 15 cm, 2 μm particle size) the column temperature was set at 40 °C. The mobile phase is composed of buffer A (water solution containing 0.1% formic acid) and buffer B (an aqueous solution containing 80% acetonitrile and 0.08% formic acid) with a flow rate of 0.3 μL/min. The protocol for the gradient eluting was listed as follows: 0-5 min, 4% B; 5-70 min, 4~30% B; 70-75 min, 30~80% B; 75-80 min, 80% B; 80-85 min, 80~4% B; 80-85 min, 4% B. MS parameters were listed as follows: resolution, 60000; AGC target, 400000; maximum IT (ms), 50; scan range (m/z), 350 to 1500; Target FDR (strict) for PSMs, 0.01; Min. Precursor mass, 350 Da; Max. Precursor mass, 5000 Da. MS^2 parameter were listed as follows: resolution, 15000; AGC target, 5.0e4; maximum IT (ms), 30; HCD Collision Energy (%): 30.

The obtained raw mass spectrometric data was inputted into the Thermo Proteome Discoverer 2.3 software for searching the specific protein information in the uniprot-mouse database. The parameters of “Sequest HT” were set as follows: Trypsin（Full）was selected as the digestion enzyme. Max. Missed Cleavage Sites was 2, Min. Peptide Length was 6 and Max. Peptide Length was 144. Precusor Mass Tolerance was 10 ppm and Fragment Mass Tolerance was 0.02 Da. The modification of Carbamidomethyl/+57.021 Da in cysteine was set in Static Modification section.

Westen blot analysis

To validate the protein targets identified by LC-MS/MS, we randomly selected Ripk3 for western blotting experiment. Raw 264.7 cells were cultured in the 10-cm dishes and grown to 90% confluence before they were harvested and lyzed. Then, 90 μL each protein lysis sample was incubated with 10 μL different concentrations of SIN-CW (0, 30, 300 and 3000 μM) for 1 h, respectively. After that, the click chemistry step and pull-down experiment were carried out as mentioned in the section of **Pull-down assay**. After eluting at 95℃ for 20 min, these bound proteins enriched with streptavidin beads were separated on a 10% SDS-PAGE gel and immunoblotted with Ripk3 Polyclonal antibody (**Figure S9**).

Enrichment analysis

KEGG pathway

The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used for the KEGG analysis. First of all, the 92 target genes were inputted in the upload section, and then “uniprot accession” was selected as identifier before submitting the gene list. Secondly, the species option was selected as “Mus musculus”. Then, above gene list was analyzed with functional annotation tool. In annotation summary results section, KEGG pathway was selected.

GO enrichment analysis

GO enrichment analysis results were also obtained in DAVID as mentioned above. 92 target genes list was analyzed with functional annotation tool. Three analysis items were selected in annotation summary results: cell components, molecular functions, and biological processes. Finally, we obtained the results of the GO analysis. There were 25 cellular components were identified when corrected by a *P* value ≤ 0.05 (**Figure S11a**). The top ten cellular components (ranked by *P* value) are nucleus, extracellular exosome, nucleolus, small-subunit processome, cytoplasm, Pwp2p-containing subcomplex of 90S preribosome, nuclear speck, spliceosomal complex, nucleoplasm and cell-cell adherens junction in turn. Meanwhile, 25 biological processes were identified when corrected by a *P* value ≤ 0.05 (**Figure S11b**) and the top ten including mRNA processing, RNA processing, rRNA processing, RNA splicing, cell-cell adhesion, maturation of SSU-rRNA from tricistronic rRNA transcript, cell redox homeostasis, oxidation-reduction process, cortical actin cytoskeleton organization and I-kappaB kinase/NF-kappaB signaling. Furthermore, 10 molecular functions were identified when corrected by a P value ≤ 0.05

(**Figure S11c**), including poly(A) RNA binding, RNA binding, nucleotide binding, mRNA binding, cadherin binding involved in cell-cell adhesion, oxidoreductase activity, 3'-5' exonuclease activity, snoRNA binding, ATP binding, NAD binding.

RNA interference for genes knockdown

Cells (5×10^5/mL) were seeded in the 12 well plates and then growed for 12 h. Then, cells were transfected with 80 nM small interfering RNA (siRNA) of negative control (NC) or siRNA of every relative gene in the presence of 6 μL lipofectamine^{TM} 2000 RNAiMAX transfection reagent for 24h in 1 mL of complete growth medium DMEM containing 10% FBS, according to the manufacturer protocol.

Sequences of effective 14 siRNAs (**Figure S12**) were as follows:

siRipk3_1: 5’ CCACAGAACAUGGAACCAUTT 3’; siRipk3_2: 5’ AUGGUUCCAUGUUCUGUGGTT 3’; siSnx5_1: 5’ GCAAAGGAUCUCUUGUAUATT 3’; siSnx5_2: 5’ UAUACAAGAGAUCCUUUGCTT 3’; siUtp18_1: 5’ GGAUCUCGUCAGUGCAGUUTT 3’; siUtp18_2: 5’ AACUGCACUGACGAGAUCCTT 3’; siTrmt2a_1: 5’ CCAUGGCCAUUGCCUACUUTT 3’; siTrmt2a_2: 5’ AAGUAGGCAAUGGCCAUGGTT 3’; siDdx27_1: 5’ GCGGGCAGCCACUACACUATT 3’; siDdx27_2: 5’ UAGUGUAGUGGCUGCCCGCTT 3’; siDId_1: 5’ GCUGCAAACAGCAGAGCUATT 3’; siDId_2: 5’ UAGCUCUGCUGUUUGCAGCTT 3’; siPtges3_1: 5’ CCAAAUGAUUCCAAGCAUATT 3’; siPtges3_2: 5’ UAUGCUUGGAAUCAUUUGGTT 3’; siPsmd6_1: 5’ GCUUAUAUUGUGUGGCUAUTT 3’; siPsmd6_2: 5’ AUAGCCACACAAUAUAAGCTT 3’; siPrdx4_1: 5’ CCGAAUCUCUGCAAGGCUUTT 3’; siPrdx4_2: 5’ AAGCCUUGCAGAGAUUCGGTT 3’; siNcaph_1: 5’ CCGGGUUCCAGUUUACUAATT 3’; siNcaph_2: 5’ UUAGUAAACUGGAACCCGGTT 3’; siAdh5_1: 5’ GGAUCAUUGGUAUCGACAUTT 3’; siAdh5_2: 5’ AUGUCGAUACCAAUGAUCCTT 3’; siMyh11_1: 5’ GGGCUUCAAUGAAGAGGAATT 3’; siMyh11_2: 5’ UUCCUCUUCAUUGAAGCCCTT 3’; siSyne3_1: 5’ GGGCUUGAAGGAGAAGCAATT 3’; siSyne3_2: 5’ UUGCUUCUCCUUCAAGCCCTT 3’; siDbnl_1: 5’ CCAACUACAGCUUCCAUAATT 3’; siDbnl_2: 5’ UUAUGGAAGCUGUAGUUGGTT 3’.

RNA extraction and quantification

Total RNA of RAW 264.7 cells were lysed and isolated with TransZol Up Plus RNA Kit (TransGen Biotech, Beijing, China). The concentration of total RNA samples was detected by NanoPhotometer N50 (Implen, Germany). One microgram total RNA was used for reverse transcription using TransScript One-Step

gDNA Removal and cDNA Synthesis SuperMix kit (TransGen Biotech). cDNAs of each gene were quantified by real-time quantitative polymerase chain reaction (RT-qPCR). RT-qPCR was performed in the 7900HT96 fast real-time PCR system (Applied Biosystems, USA) using the transStart Tip Green qPCR SuperMix kit (TransGen Biotech, Beijing, China). The following system conditions were used: 1 cycle at 95 °C for 30 s, 40 cycles at 95 °C for 15 s and at 60 °C for 60 s, 1 cycle at 95 °C for 15 s, at 60 °C for 15 s and at 95 °C for 15 s. Quantifications of transcripts of the target genes were normalized to GAPDH level, and the relative expression of each target gene was calculated using the $2^{-\Delta\Delta Ct}$ method. The following 15 primers were used: *Ripk3*: 5′tcagggagatggaagacacg and 5′ccgaactgtgcttggtcatac; *Syne3*: 5′tgcaggcagaaaactccaag and 5′gcagatcttccagctcgttg; *Ddx27*: 5′agaggtcccagtttgaaatcct and 5′gccttcttgcttgtgttggt; *Dbnl*: 5′gcagaaggaaagggcaatgt and 5′agaagacagagtgctaggcg; *Ptges3*: 5′gagaatccggccagtcatg and 5′ctacatcctcatcaccaccca; *Myh11*: 5′gctagatgaggccacagaga and 5′ttgaagtctgagtcccgagc; *Utp18*: 5′tggtgttacttccttggcct and 5′ccctgccctttttcattccct; *Snx5*: 5′caggaatgctgccagaagtt and 5′tcagagtagacaggtcagttgt; *Ncaph*: 5′aggctgctaccattctgacc and 5′agttggaggtgtcgttaggg; *Prdx4*: 5′ccacttctttctgacctgaacc and 5′aaggcttgaaccaaacgcag; *Prpf6*: 5′ggttccatcgcactgtgaag and 5′ttccccaatcttcctctgcc; *Trmt2a*: 5′caccccattccatccagtca and 5′tcctgtgctcctatacttccc; *Dld*: 5′ttggggcaggagtaattggt and 5′ccatctgacttcttggtggc; *Psmd6*: 5′ggggcagagatccttgaagt and 5′gacctatatgattccagcagctg; *Gapdh*: 5′gtgtttcctcgtcccgtaga and 5′gccgtgagtggagtcatact.

Levels of inflammatory cytokines (TNF-α, IL-1β, and IL-6) in the supernatant of each group were detected by ELISA

After the target protein in RAW 264.7 cell was specifically knocked down by corresponding siRNA (80 nM), the inflammatory cell model was induced by addition of LPS (0.1 μg/mL) to these transfected cells. The RAW 264.7 cells were divided into seven groups: A group, normal cells; B group, inflammatory cell model induced by LPS; C group, inflammatory cell model induced by LPS + SIN; D group, cells transfected by siRNA-NC; E group, cells transfected by siRNA (target protein); F group, cells transfected by siRNA (target protein) + LPS; G group, cells transfected by siRNA (target protein) + LPS + SIN. The levels of released inflammatory factors (TNF-α, IL-1β, and IL-6) in the supernatant of the cells in each group were detected by ELISA. The ELISA procedure was carried out according to the instructions.

Statistics

Data are represented as means ± SD, the unpaired student *t*-test was used for two-group comparison. When *p*-value was less than 0.05 recognized as statistically significant.

References

1. C. J. Wei, F. Xu, M. J. Shi, J. W. Hu, J. J. Wang, B. Zhen, X. Wang, T. F. Ji, J. H. Wang, G. H. Du, *J. Asian Nat. Prod. Res*., 2018, **20**, 277-291.

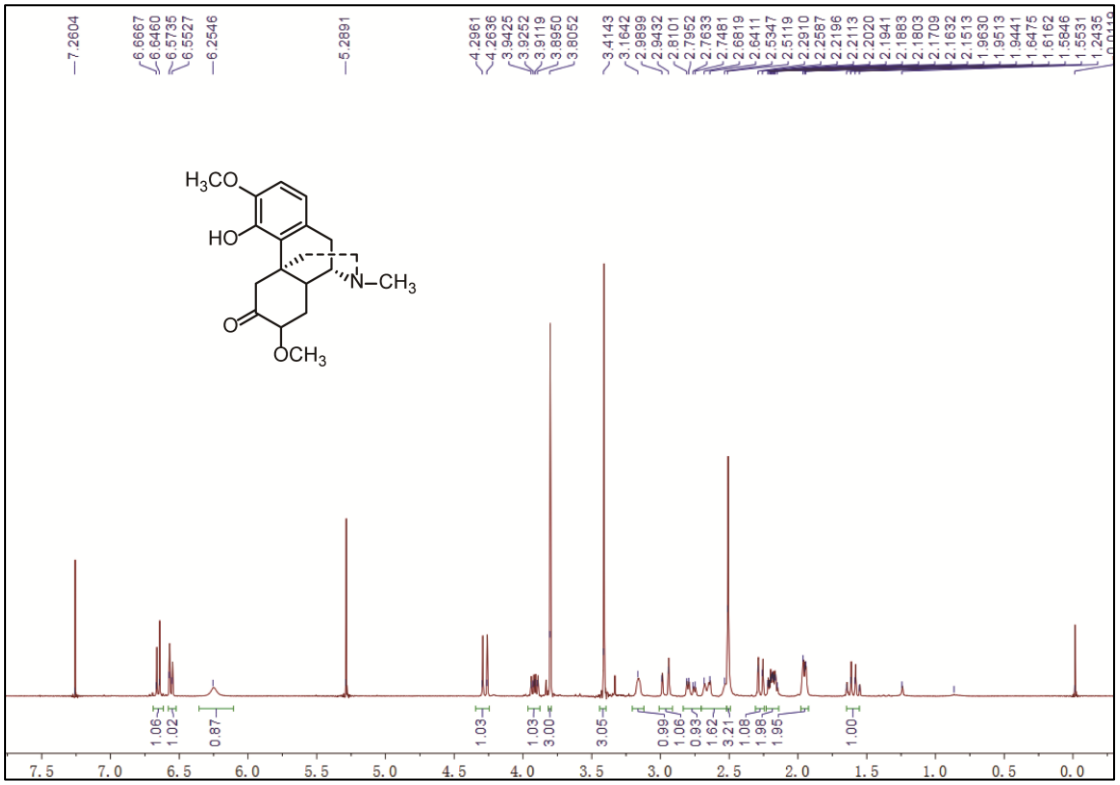

Figure S1. The ^{1}H NMR spectrum of SIN-Y1 in $CDCl_3$**.**

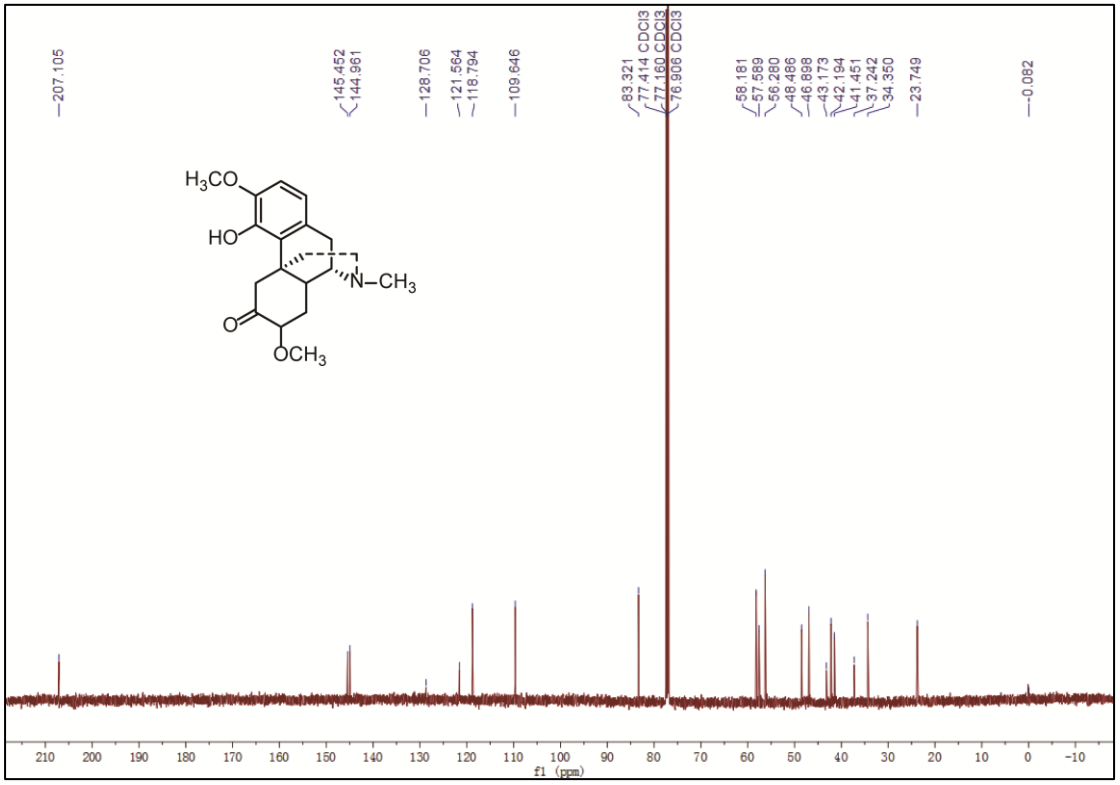

Figure S2. The ^{13}C NMR spectrum of SIN-Y1 in $CDCl_3$.

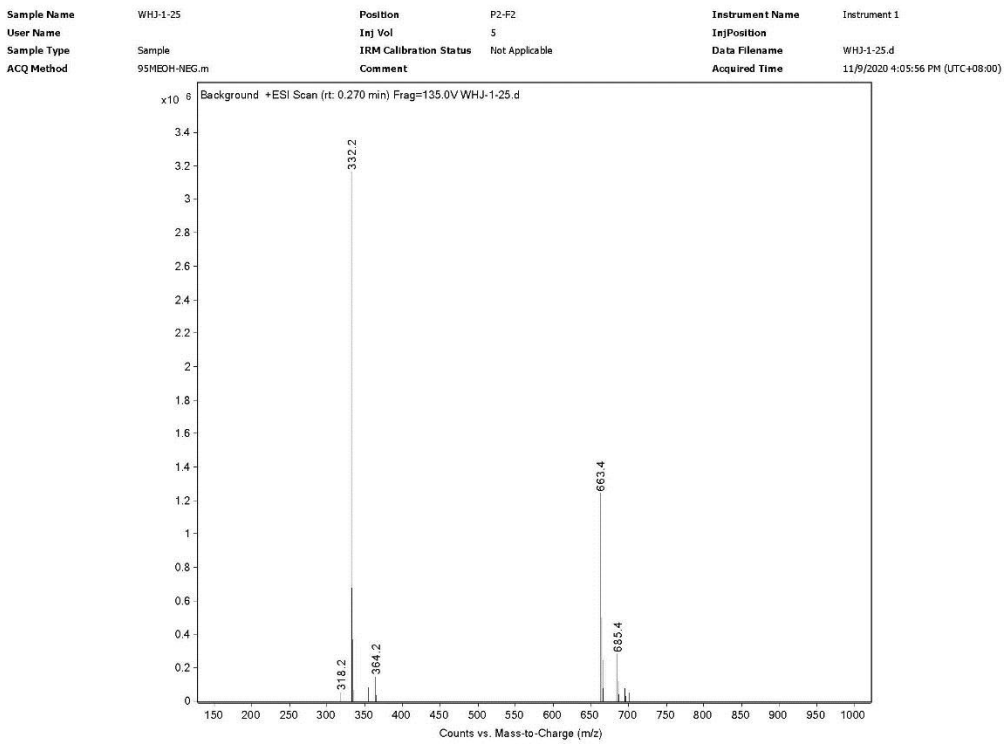

Figure S3. The mass spectrum of SIN-Y1.

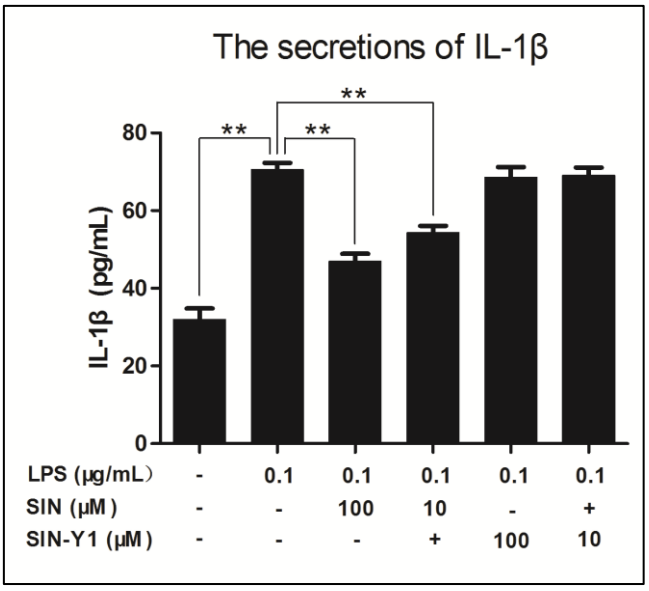

Figure S4. Effects of SIN and SIN-Y1 on the secretions of IL-1β in cells treated by two concentrations (10 and 100 μM).

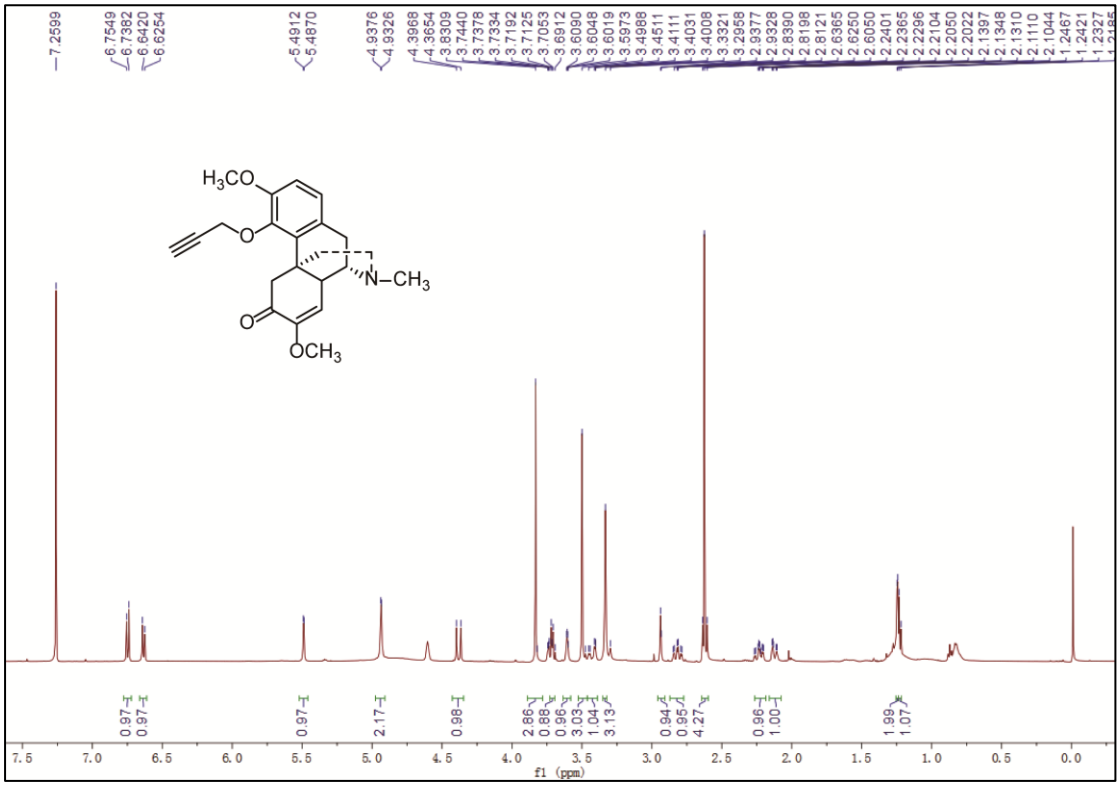

Figure S5. The ^{1}H NMR spectrum of SIN-CW in $CDCl_3$.

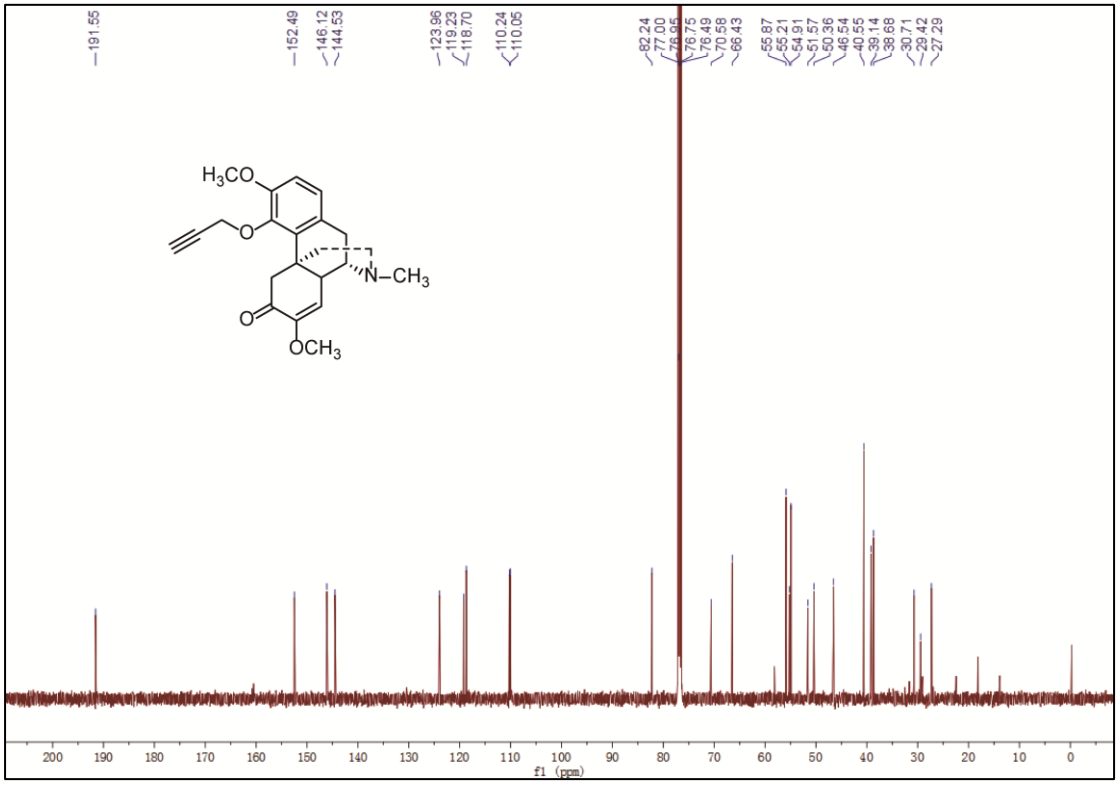

Figure S6. The ^{13}C NMR spectrum of SIN-CW in $CDCl_3$.

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
368.18564	368.18563	0.01	10.5	C22 H26 O4 N

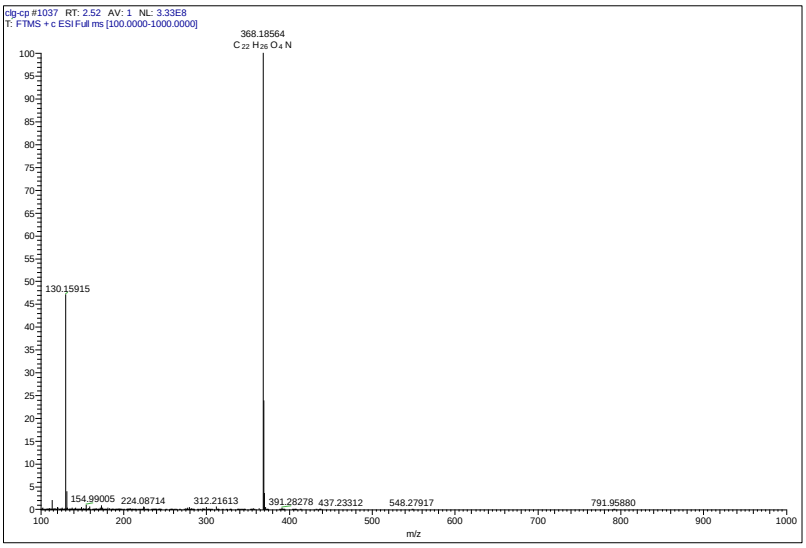

Figure S7. The high-resolution mass spectrum of SIN-CW.

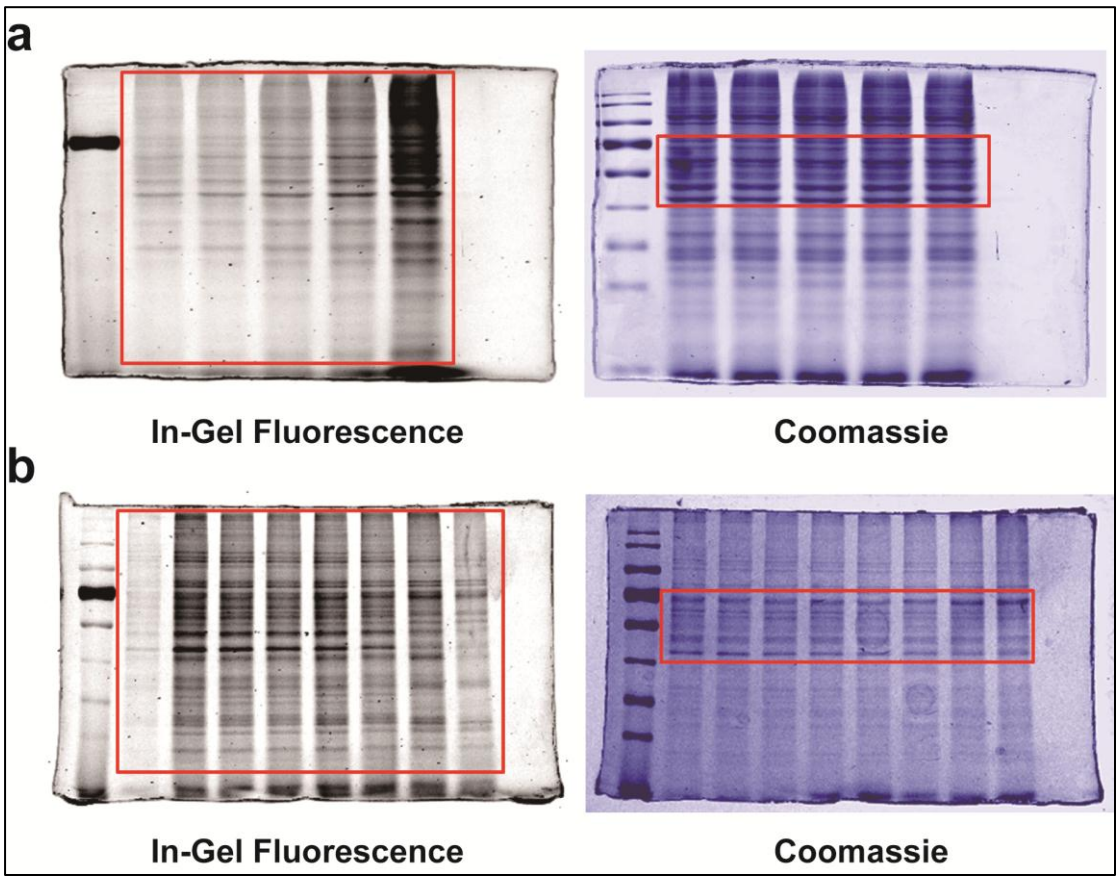

Figure S8. Uncropped gels/blots. (a) Concentration-dependent labeling of cell lysate by SIN-CW in SDS-PAGE gel *in vitro*. Left, the gel was scanned by Typhoon; Right, the gel was stained by coomassie

blue; (b) Comparative labeling of cell lysate by SIN and its probe in gel *in vitro*. Left, the gel was scanned by Typhoon; Right, the gel was stained by coomassie blue.

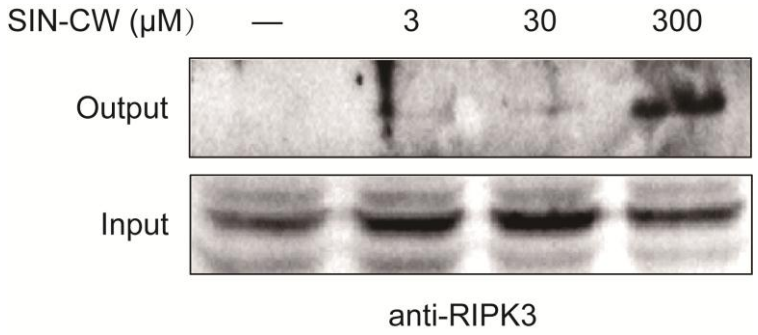

Figure S9. Validation of the covalent labeling of SIN-CW probe on Ripk3 in the cell lysate of RAW 264.7 cells. The labeled Ripk3 were conjugated with biotin through click chemistry ('input'), pulled down by streptavidin beads and eluted by boiling the beads at 95 °C ('output'). The input and output samples were analyzed by anti-Ripk3 immunoblotting.

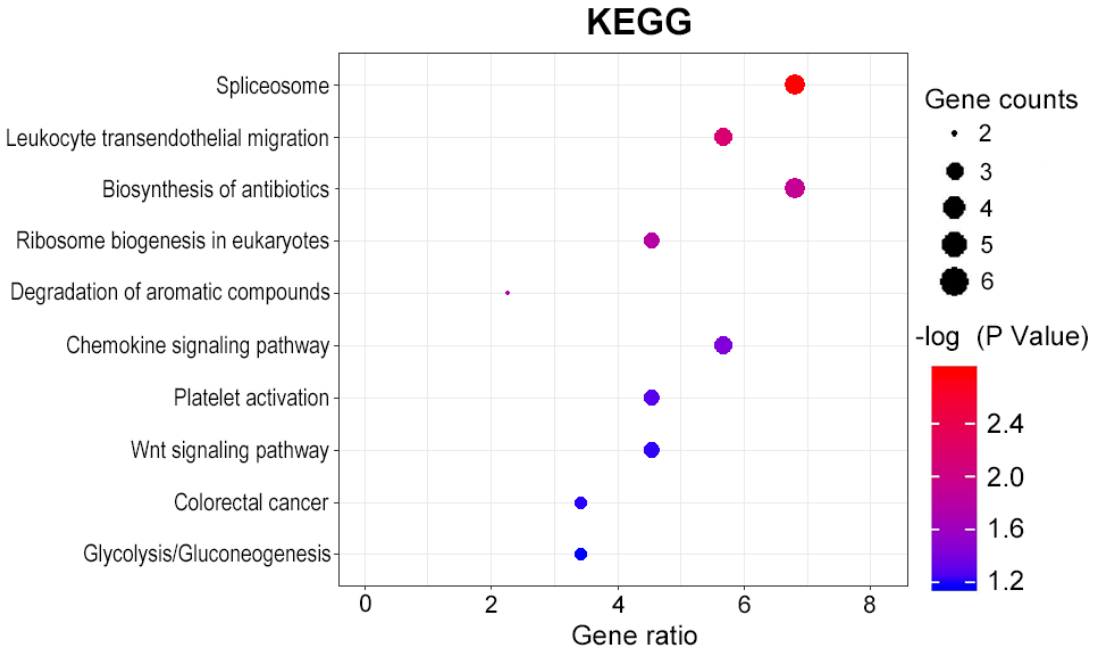

Figure S10. Bubble charts of KEGG pathway analysis. Note: pathway (Y axis), gene ratio (X axis), p value (colour) and gene counts (size).

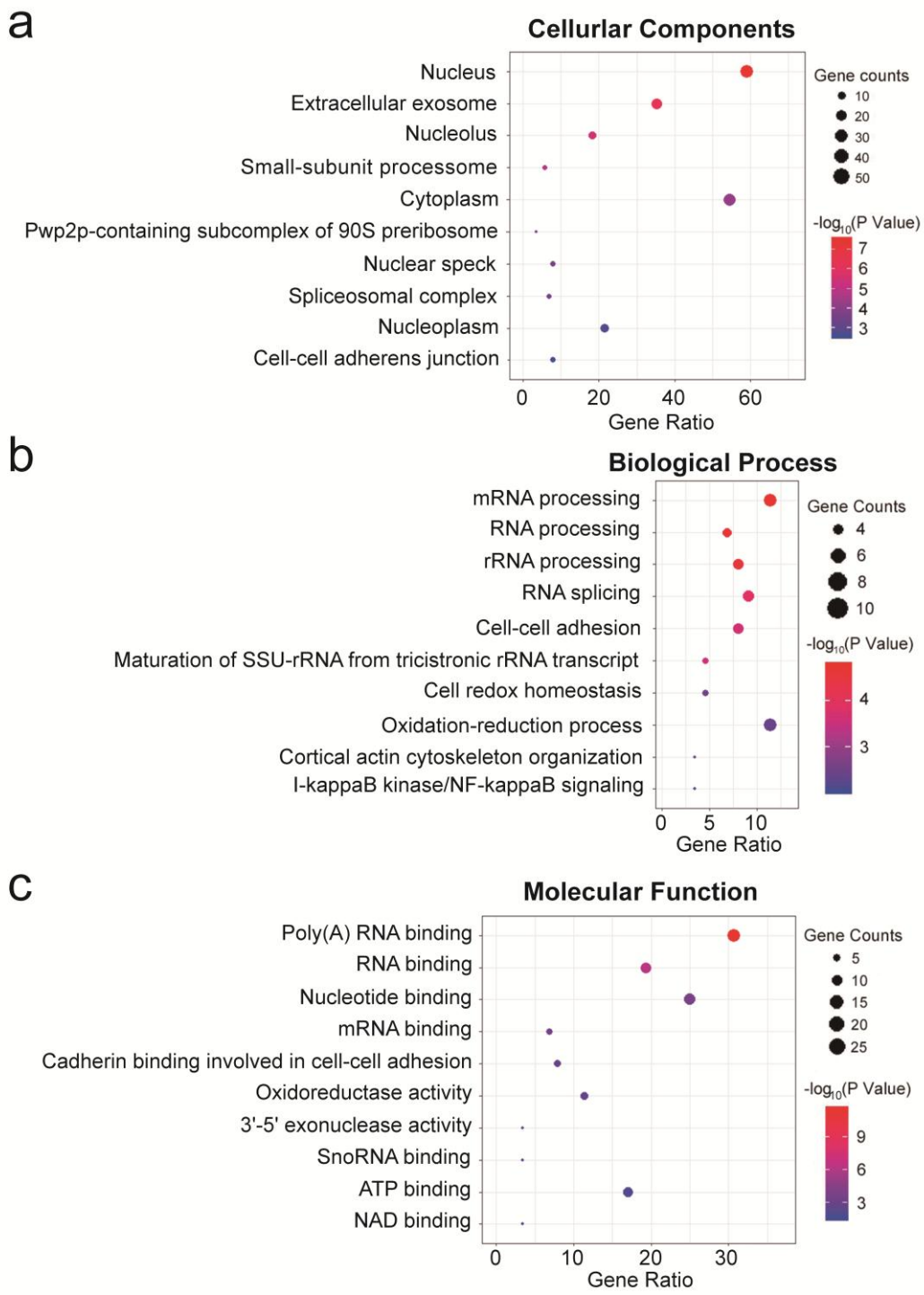

Figure S11. Bubble charts of GO analysis. (a) Cell components that the identified 92 proteins located. (b) Biological processes that the identified 92 proteins involved (c) Molecular functions that the identified 92 proteins involved. The cell components, biological processes or molecular functions served as Y axis. The gene ratio (the number of differentially expressed genes in relative channel/the number of differentially expressed genes in this channel) served as X axis. The color denoted $\log_{10}(p$ value) and the size represents gene counts.

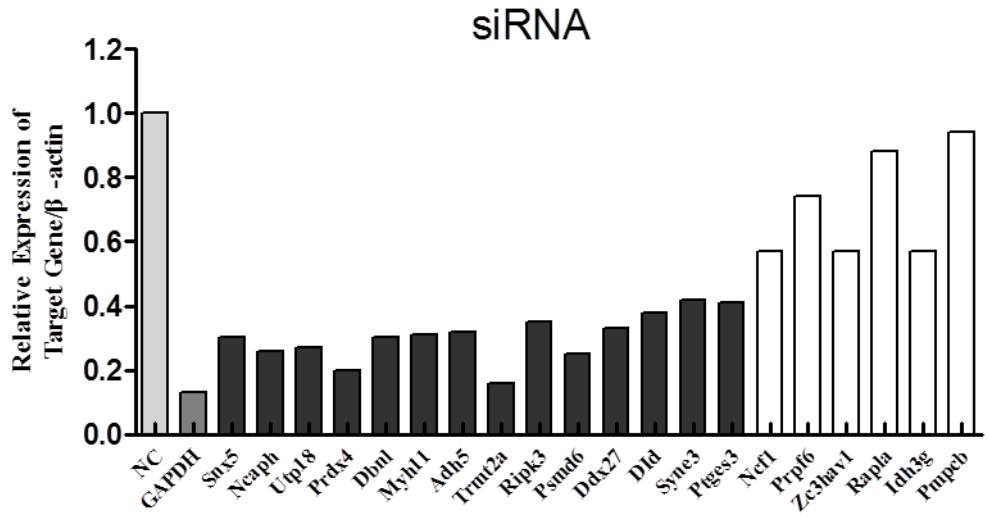

Figure S12. Selection of effective siRNA for corresponding gene. The relative mRNA level of 20 genes and *GAPDH* in RAW 264.7 cells was determined by RT-PCR after transfected with 80 nM effective siRNA (silencing efficacy＞50%) for corresponding gene,respectively.

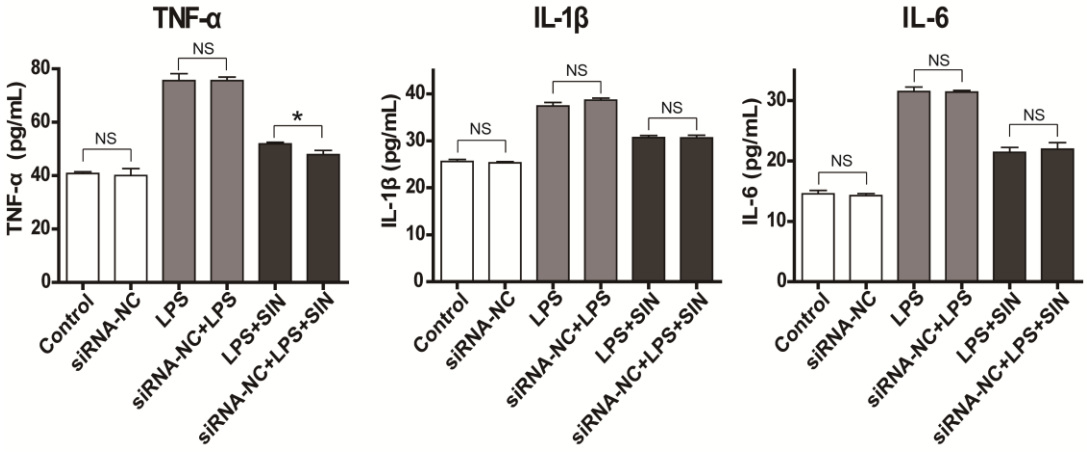

Figure S13. The secretion of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW 264.7 cells after transfection with siRNA-NC and SIN treatment. *NS Represents not statistically significant and *Represents P < 0.05 between two groups.*

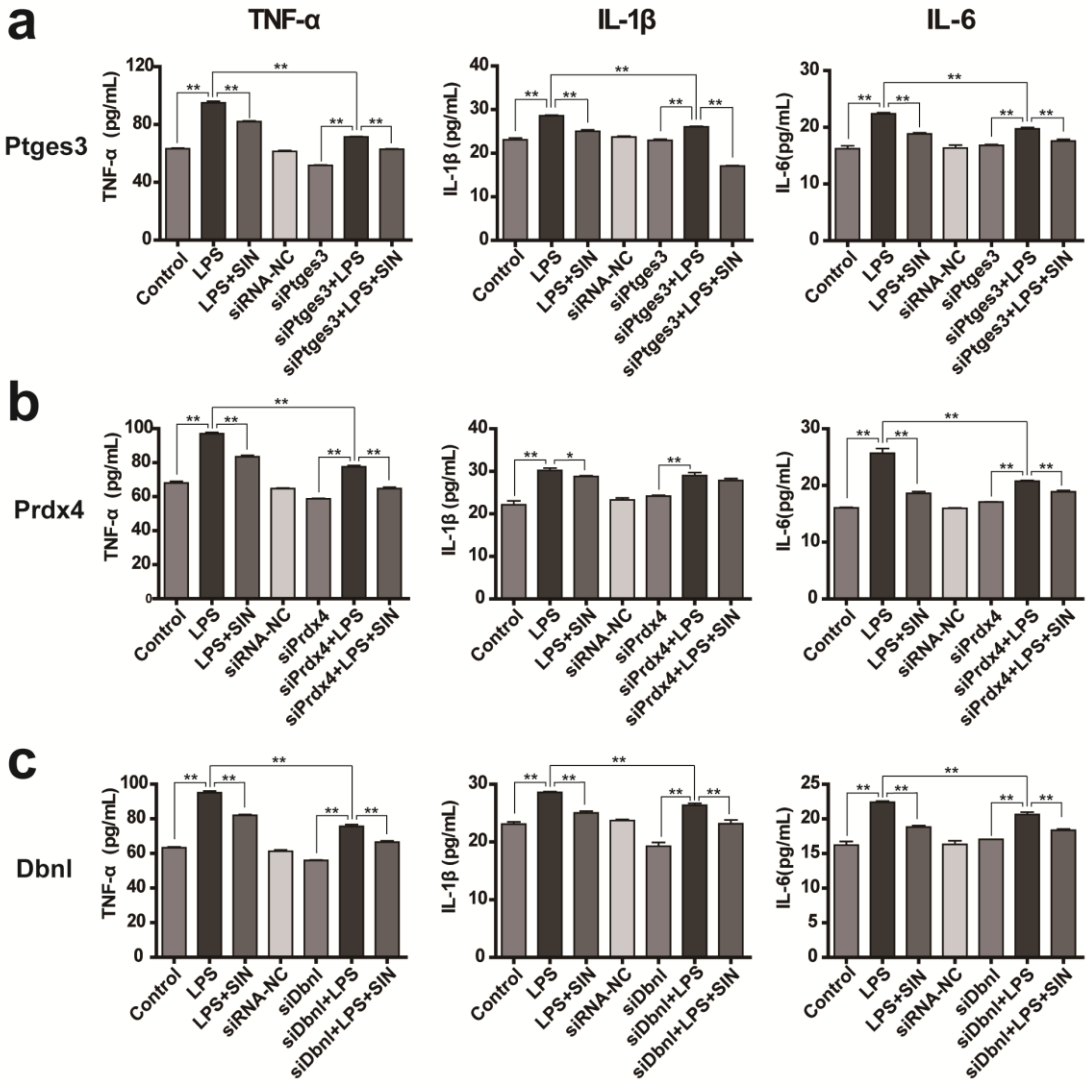

Figure S14. The secretion of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW 264.7 cells after transfection with designated siRNA (*Ptges3*, *Prdx4* and *Dbnl*)and SIN treatment. Knockdown anyone of Ptges3, Prdx4 and Dbnl in transcription level could decrease the overall expression of inflammatory cytokines in RAW 264.7 cells injured with LPS. **Represents P < 0.05 and **Represents P < 0.01 between two groups.*

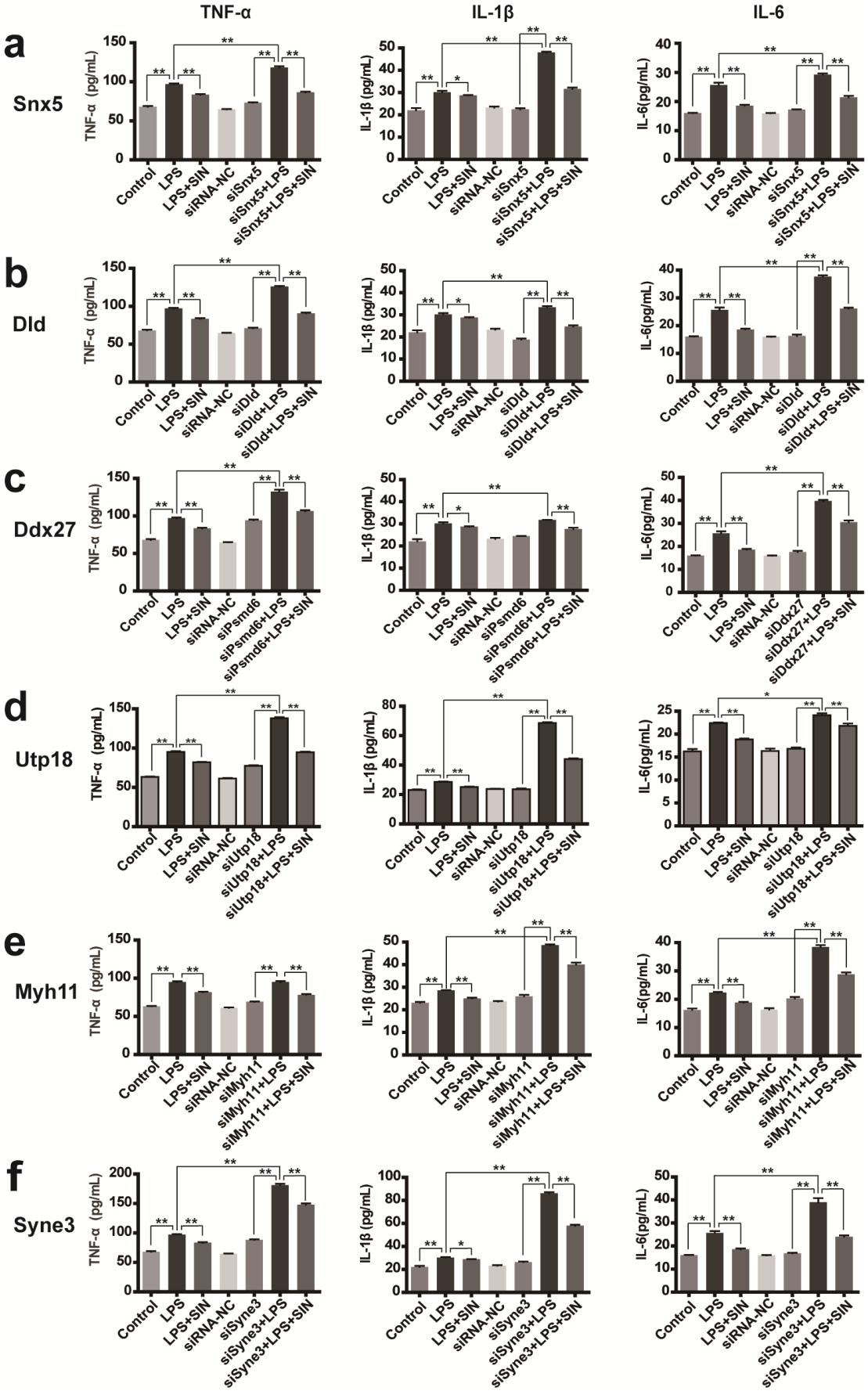
TNF-α
IL-1β
IL-6
a
Snx5
b
Dld
c
Ddx27
d
Utp18
e
Myh11
f
Syne3
TNF-α (pg/mL)
IL-1β (pg/mL)
IL-6(pg/mL)
Control
LPS
LPS+SIN
siRNA-NC
siSnx5
siSnx5+LPS
siSnx5+LPS+SIN
siDld
siDld+LPS
siDld+LPS+SIN
siPsmd6
siPsmd6+LPS
siPsmd6+LPS+SIN
siDdx27
siDdx27+LPS
siDdx27+LPS+SIN
siUtp18
siUtp18+LPS
siUtp18+LPS+SIN
siMyh11
siMyh11+LPS
siMyh11+LPS+SIN
siSyne3
siSyne3+LPS
siSyne3+LPS+SIN

Figure S15. The secretion of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW 264.7 cells after transfection with designated siRNA (*SNX5, Dld, Ddx27, Utp18, Myh11* and *Syne3*) and SIN treatment. Knockdown one of 6 genes in transcription level could increase the overall expression of inflammatory cytokines in RAW 264.7 cells injured with LPS. **Represents P < 0.05 and **Represents P < 0.01 between two groups.*

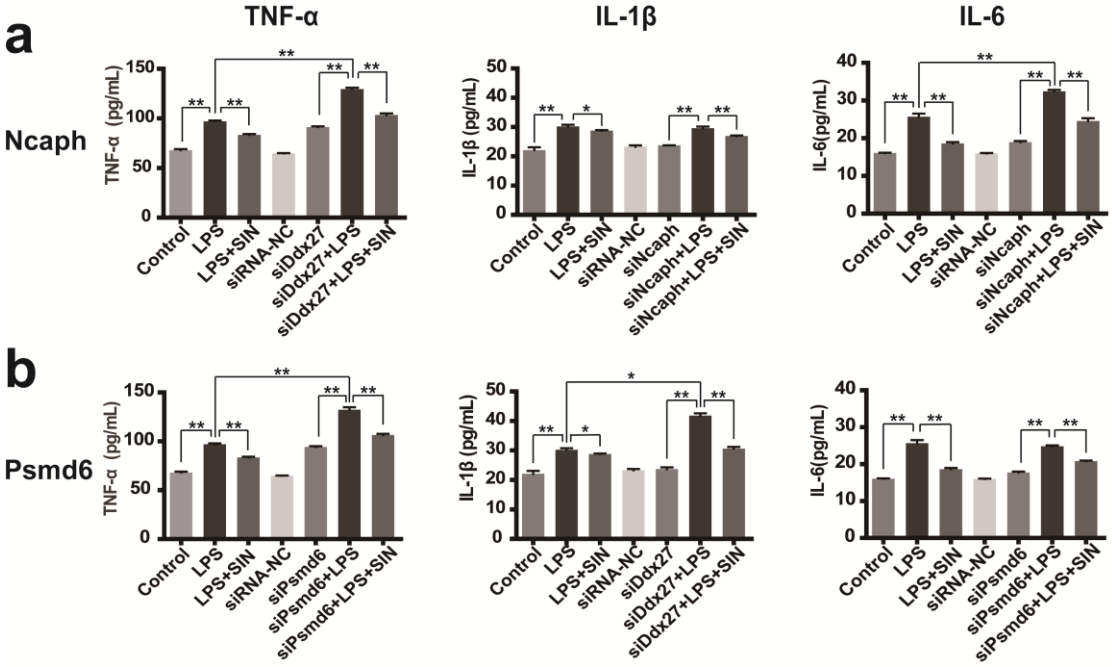

Figure S16. The secretion of TNF-α, IL-1β and IL-6 in LPS-stimulated RAW 264.7 cells after transfection with designated siRNA (*Ncaph* and *Psmd6*) and SIN treatment. Knockdown one of Ncaph and Psmd6 in transcription level could change the expression of inflammatory cytokines in different manners in RAW 264.7 cells injured with LPS. **Represents P < 0.05 and **Represents P < 0.01 between two groups.*

Table S1. Lists of 14 hits targeted only in the probe group and with effective siRNA for subsequent gene knockdown experiments

Protein	Gene name	Fold Change[a]	*P*-value
Receptor-interacting serine/threonine-protein kinase 3	*Ripk3*	999 (∞)	1.05×10^{-7}
26S proteasome non-ATPase regulatory subunit 6	*Psmd6*	999 (∞)	1.83×10^{-6}
Dihydrolipoyl dehydrogenase	*Dld*	999 (∞)	5.6×10^{-6}
Nesprin-3	*Syne3*	999 (∞)	2.28×10^{-5}
tRNA (uracil-5-)-methyltransferase homolog A	*Trmt2a*	999 (∞)	0.002386
Peroxiredoxin-4	*Prdx4*	999 (∞)	0.002776
Condensin complex subunit 2	*Ncaph*	999 (∞)	0.004247
Sorting nexin-5	*Snx5*	999 (∞)	0.004553
U3 small nucleolar RNA-associated protein 18 homolog	*Utp18*	999 (∞)	0.006165
Alcohol dehydrogenase class-3	*Adh5*	999 (∞)	0.020269
Myosin-11	*Myh11*	999 (∞)	0.022428
Prostaglandin E synthase 3	*Ptges3*	999 (∞)	0.026051
Drebrin-like protein	*Dbnl*	999 (∞)	0.026054
Probable ATP-dependent RNA helicase DDX27	*Ddx27*	999 (∞)	0.040732

[a] The fold change represents the mean abundances of targeted protein in probe group compared with that in control group. The fold change of those proteins appeared only in probe group was denoted 999 (∞).

Table S2. The level of TNF-α, IL-1β and IL-6 in cells after knockdown of the designated genes (*Ripk3*, *Ptges3*, *Prdx4* or *Dbnl*)

Gene	Group	TNF-α	IL-1β	IL-6
	control	63.294±0.861	23.085±0.696	16.210±0.907
	LPS	95.064±1.733	28.584±0.260	22.400±0.317
	LPS+SIN	81.995±0.829	25.031±0.533	18.826±0.372
Ripk3	siRNA-NC	62.307±0.783	23.702±0.360	16.331±0.902
	siRipk3	57.178±1.074	18.437±0.368	15.688±0.054
	siRipk3+LPS	74.041±1.795	21.709±0.148	19.100±0.078
	siRipk3+LPS+SIN	66.621±1.485	20.283±0.263	16.644±0.449
	control	63.294±0.861	23.085±0.696	16.210±0.907
	LPS	95.064±1.733	28.584±0.260	22.400±0.317
	LPS+SIN	81.995±0.829	25.031±0.533	18.826±0.372
Ptges3	siRNA-NC	62.307±0.783	23.702±0.360	16.331±0.902

	siPtges3	51.775±0.322	22.963±0.415	16.818±0.257
	siPtges3+LPS	71.467±0.265	26.043±0.234	19.719±0.438
	siPtges3+LPS+SIN	62.883±0.533	17.054±0.178	17.595±0.466
	control	68.070±1.692	22.124±1.613	16.055±0.151
	LPS	97.022±1.537	30.186±0.978	25.697±1.407
	LPS+SIN	83.486±1.431	28.782±0.264	18.621±0.554
Prdx4	siRNA-NC	65.752±1.898	23.284±0.761	15.978±0.157
	siPrdx4	58.766±0.477	24.160±0.306	17.083±0.031
	siPrdx4+LPS	77.573±1.293	28.970±1.299	20.762±0.278
	siPrdx4+LPS+SIN	64.784±1.301	27.851±0.745	18.893±0.403
	control	63.294±0.861	23.085±0.696	16.210±0.907
	LPS	95.064±1.733	28.584±0.260	22.400±0.317
	LPS+SIN	81.995±0.829	25.031±0.533	18.826±0.372
Dbnl	siRNA-NC	62.307±0.783	23.702±0.360	16.331±0.902
	siDbnl	55.983±0.414	19.268±1.118	17.036±0.009
	siDbnl+LPS	75.651±1.888	26.374±0.565	20.633±0.567
	siDbnl+LPS+SIN	66.550±1.117	23.168±1.115	18.350±0.317

Table S3. The level of TNF-α, IL-1β and IL-6 in cells after knockdown of the designated genes (*Snx5, Dld, Ddx27, Utp18, Myh11, ADH5* or Syne3)

Gene	Group	TNF-α	IL-1β	IL-6
	control	68.070±1.692	22.124±1.613	16.055±0.151
	LPS	97.022±1.537	30.186±0.978	25.697±1.407
	LPS+SIN	83.486±1.431	28.782±0.264	18.621±0.554
Snx5	siRNA-NC	65.752±1.898	23.284±0.761	15.978±0.157
	siSnx5	73.304±0.941	22.519±0.891	17.286±0.030
	siSnx5+LPS	118.372±2.763	48.016±0.580	29.267±0.701
	siSnx5+LPS+SIN	86.553±1.211	31.626±1.091	21.467±0.898

	control	68.070±1.692	22.124±1.613	16.055±0.151
	LPS	97.022±1.537	30.186±0.978	25.697±1.407
	LPS+SIN	83.486±1.431	28.782±0.264	18.621±0.554
Dld	siRNA-NC	65.752±1.898	23.284±0.761	15.978±0.157
	siDld	70.887±1.297	18.840±0.788	16.286±0.822
	siDld+LPS	125.962±1.411	33.375±0.964	37.674±0.779
	siDld+LPS+SIN	90.629±1.593	24.748±0.898	26.140±0.573
	control	68.070±1.692	22.124±1.613	16.055±0.151
	LPS	97.022±1.537	30.186±0.978	25.697±1.407
	LPS+SIN	83.486±1.431	28.782±0.264	18.621±0.554
Ddx27	siRNA-NC	65.752±1.898	23.284±0.761	15.978±0.157
	siDdx27	91.374±1.197	23.696±0.966	17.577±0.862
	siDdx27+LPS	129.621±2.192	41.810±1.363	39.741±0.747
	siDdx27+LPS+SIN	103.485±2.786	30.584±1.081	30.511±1.292
	control	63.294±0.861	23.085±0.696	16.210±0.907
	LPS	95.064±1.733	28.584±0.260	22.400±0.317
	LPS+SIN	81.995±0.829	25.031±0.533	18.826±0.372
Utp18	siRNA-NC	62.307±0.783	23.702±0.360	16.331±0.902
	siUtp18	77.376±1.284	23.583±0.852	16.823±0.477
	siUtp18+LPS	137.983±2.291	68.555±0.978	24.118±0.760
	siUtp18+LPS+SIN	94.700±1.350	44.010±1.062	21.811±0.885
	control	63.294±0.861	23.085±0.696	23.085±0.696
	LPS	95.064±1.733	28.584±0.260	28.584±0.260
	LPS+SIN	81.995±0.829	25.031±0.533	25.031±0.533
Myh11	siRNA-NC	62.307±0.783	23.702±0.360	23.702±0.360
	siMyh11	69.260±0.604	25.981±1.060	25.981±1.060
	siMyh11+LPS	95.180±2.125	48.606±0.682	48.606±0.682
	siMyh11+LPS+SIN	78.426±1.774	40.036±1.425	40.036±1.425
	control	63.294±0.861	23.085±0.696	16.210±0.907

	LPS	95.064±1.733	28.584±0.260	22.400±0.317
	LPS+SIN	81.995±0.829	25.031±0.533	18.826±0.372
ADH5	siRNA-NC	62.307±0.783	23.702±0.360	16.331±0.902
	siADH5	70.824±0.690	30.125±1.114	16.912±0.501
	siADH5+LPS	158.798±4.357	40.567±1.521	32.785±0.673
	siADH5+LPS+SIN	127.258±4.776	34.014±1.496	27.439±1.581
	control	68.070±1.692	22.124±1.613	16.055±0.151
	LPS	97.022±1.537	30.186±0.978	25.697±1.407
	LPS+SIN	83.486±1.431	28.782±0.264	18.621±0.554
Syne3	siRNA-NC	65.752±1.898	23.284±0.761	15.978±0.157
	siSyne3	88.215±1.372	26.258±1.108	16.864±0.314
	siSyne3+LPS	181.149±3.797	86.117±1.704	39.028±3.135
	siSyne3+LPS+SIN	147.960±3.590	57.925±1.431	24.076±0.923

Table S4. The level of TNF-α, IL-1β and IL-6 in cells after knockdown of the designated genes (*Ncaph, Trmt2a* or *Psmd6*)

Gene	Group	TNF-α	IL-1β	IL-6
	control	68.070±1.692	22.124±1.613	16.055±0.151
	LPS	97.022±1.537	30.186±0.978	25.697±1.407
	LPS+SIN	83.486±1.431	28.782±0.264	18.621±0.554
Ncaph	siRNA-NC	65.752±1.898	23.284±0.761	15.978±0.157
	siNcaph	78.746±2.621	23.715±0.094	18.958±0.517
	siNcaph+LPS	111.406±1.209	29.619±0.923	32.401±0.709
	siNcaph+LPS+SIN	86.361±0.791	26.950±0.272	24.558±1.229
	control	63.294±0.861	23.085±0.696	16.210±0.907
	LPS	95.064±1.733	28.584±0.260	22.400±0.317
	LPS+SIN	81.995±0.829	25.031±0.533	18.826±0.372
Trmt2a	siRNA-NC	62.307±0.783	23.702±0.360	16.331±0.902

	siTrmt2a	65.777±0.941v	20.667±0.284	15.853±0.376
	siTrmt2a+LPS	75.827±2.193	37.220±0.965	43.557±1.534
	siTrmt2a+LPS+SIN	69.488±2.338	30.972±0.665	29.282±0.943
	control	68.070±1.692	22.124±1.613	16.055±0.151
	LPS	97.022±1.537	30.186±0.978	25.697±1.407
	LPS+SIN	83.486±1.431	28.782±0.264	18.621±0.554
Psmd6	siRNA-NC	65.752±1.898	23.284±0.761	15.978±0.157
	siPsmd6	94.334±1.227	24.523±0.062	17.735±0.406
	siPsmd6+LPS	132.476±4.291	31.780±0.056	24.830±0.431
	siPsmd6+LPS+SIN	106.398±2.308	27.640±1.123	20.805±0.330