Supporting Information for

Original article

Costunolide covalently targets NACHT domain of NLRP3 to inhibit inflammasome activation and alleviate NLRP3-driven inflammatory diseases

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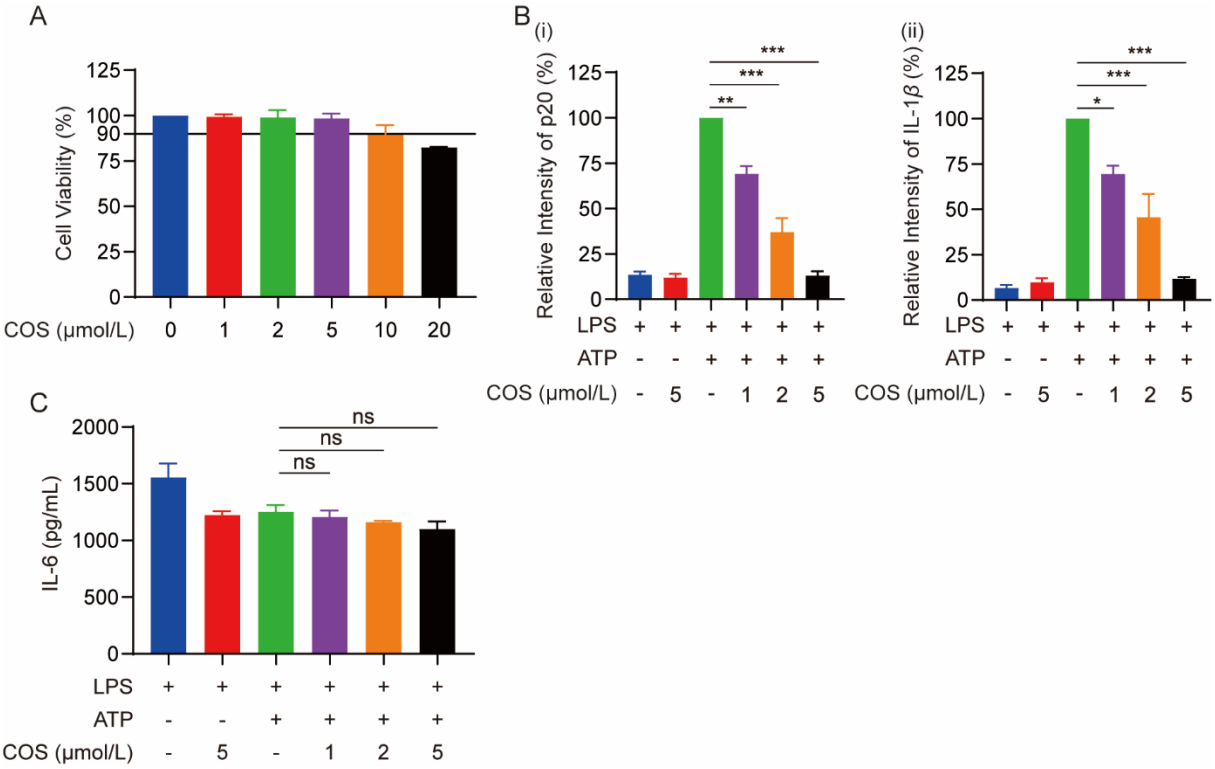
Contents in Supporting Information

Table S1

Figs. S1–S15

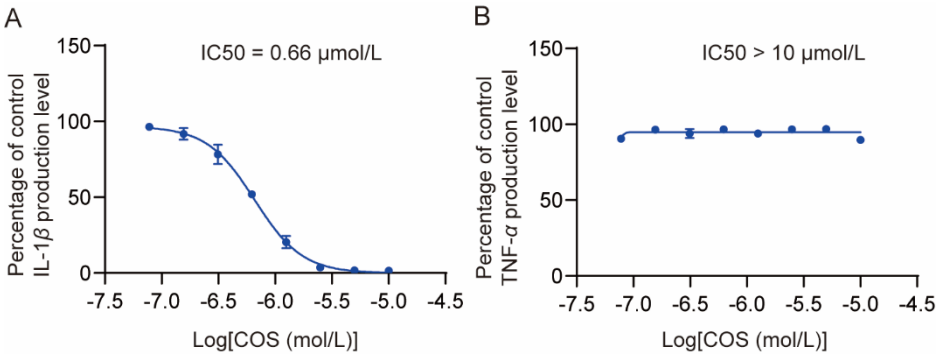
Table S1 List of 119 natural compounds.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Artemisinic acid	Engeletin	8-*O*-Acetylshanzhiside methyl ester	Rutin	Rapamycin	Praeruptorin A	4-Methylesculetin	coniferin	5-Methyl-7-methoxyisoflavone	7,8-Dihydroxy-4-phenylcoumarin	Jatrorrhizine hydrochloride	Chrysin	Pregnenolone	Reserpine	Methyl eugenol	Methyl cholate	Salidroside
2	Skimmin	Salvianolic acid A	Geniposide	Imperatorin	Artemisinin	Hydroxyprogesterone	Astrasieversianin VII	Tubeimoside II	Notoginsenoside Fa	8-*O*-Acetylharpagide	Caffeic Acid	Khellin	Casanthranol	Isorhamnetin	Coenzyme Q10	Aleuritic acid	Esculin
3	Acetylcimigenol 3-*O*-alpha-L-arabinopyran	Baicalein	(+)-Pteryxin	Alismoxide	Dehydroevodiamine	Notopterol	Hyoscyamine sulfate hydrate	Tuberstemonine	Thiocolchicoside	Plantagoside	Berbamine dihydrochloride	Aloin	Isoimperatorin	Spinosin	Quercetin	*Trans*-Zeatin Riboside	Aminophylline
4	Picfeltarraenin IA	Sucralose	Gossypol acetic acid	Ecliptasaponin A	Nonacosane	Mandelic acid	*N*-Benzylpalmitamide	5*β*-Pregnane-3*α*,20*α*-diol	Picroside II	Khasianine	Farrerol	Oxysophocarpine	2′-Deoxyinosine	Thymopentin	Bilobetin	Liensinine diperchlorate	Costunolide
5	Ganoderic acid G	Corydaline	Vinblastine sulfate	Azelaic acid	Harpagide	Arbutin	Chenodeoxycholic acid	Thymol	Cryptotanshinone	(−)-Epicatechin gallate	Corilagin	Penicillin G sodium salt	Resveratrol	Hydrocortisone	Toddalolactone	Sec-*O*-Glucosylhamaudol	Phellodendrine
6	Yangonin	Gigantol	5-Acetylsalicylic acid	Cyclocytidine hydrochloride	Rosmarinic acid	Epicatechin	Cinnamic aldehyde	Riboflavin	Aristolochic acid	Tubuloside A	Panaxadiol	(+)-Catechin hydrate	Oxymatrine	ADP	Secoxyloganin	Pseudolaric acid A	L-Thyroxine
7	SN38	Chlorogenic Acid	6-Hydroxyflavone	Schizandrin B	Ginsenoside Rb1	5-Hydroxy-1,7-diphenyl-6-hepten-3-one	Pterostilbene	Genistein	Apigenin	Schizandrin A	Isocorynoxeine	Hederasaponin B	Betulinic acid	Blinin	Notoginsenoside Fe	Isotretinoin	Laurocapram



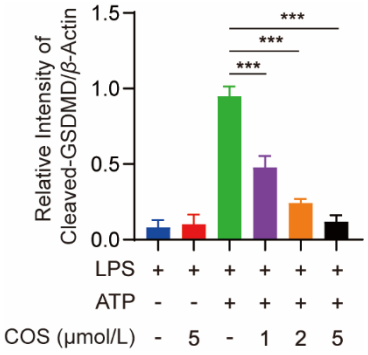
Supplementary Figure S1

(**A**) BMDMs were pretreated with different doses of COS for 24 h, cell counting kit-8 assay was evaluated for cellular viability. (**B**) Densitometric quantification of p20 (i) and IL-1*β* (ii) described in Fig. 1C. (**C**) IL-6 production was measured by ELISA in SN from LPS-primed BMDMs treated with or without COS for 0.5 h, and stimulated with ATP for 0.5 h. Data are presented as the mean ± SEM, $n = 3$; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.



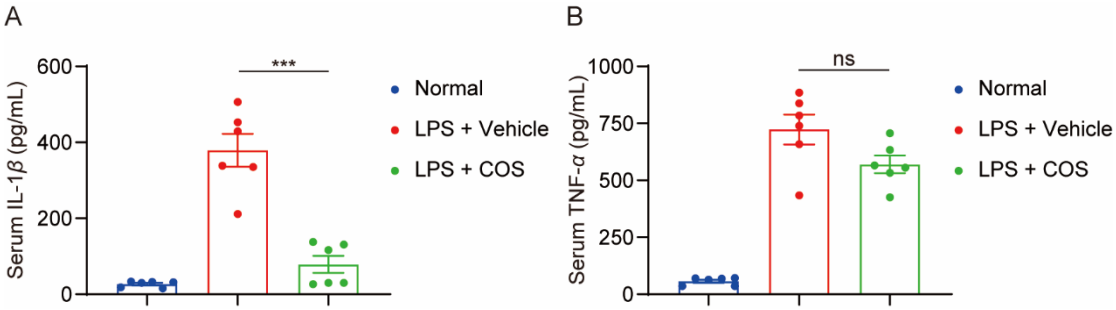
Supplementary Figure S2

BMDMs were primed with LPS for 3 h and then pretreated with different doses of COS for 30 min and stimulated with ATP for 30 min. Production of IL-1*β* (**A**) and TNF-*α* (**B**) was measured by ELISA and then the level is normalized to that of DMSO-treated control cells. Nonlinear regression analysis was performed, and the curve of Log[COS (μmol/L)] *versus* the normalized response is presented (n = 3).



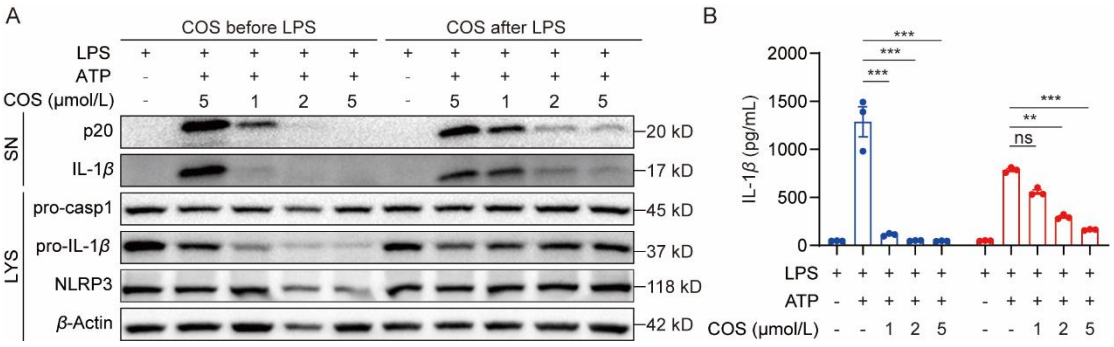
Supplementary Figure S3

Densitometric quantification of cleaved-GSDMD described in Fig. 1F. Data are presented as the mean ± SEM, $n = 3$; ***$P < 0.001$.



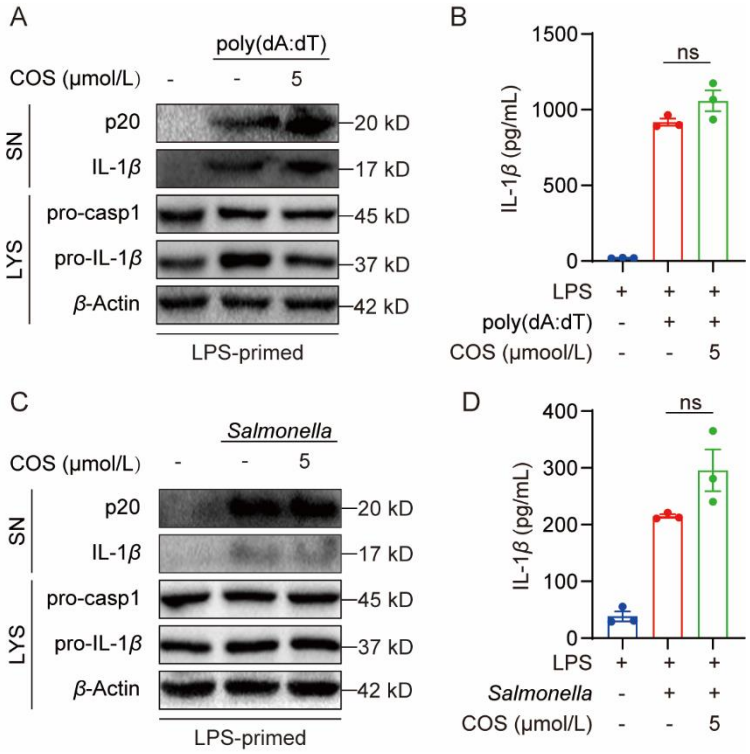
Supplementary Figure S4

ELISA of IL-1β (**A**) and TNF-α (**B**) in the serum of mice intraperitoneally injected with LPS (10 mg/kg) and treated with or without COS (40 mg/kg). Data are presented as the mean ± SEM, $n = 6$ per group; ***$P < 0.001$; ns, not significant.



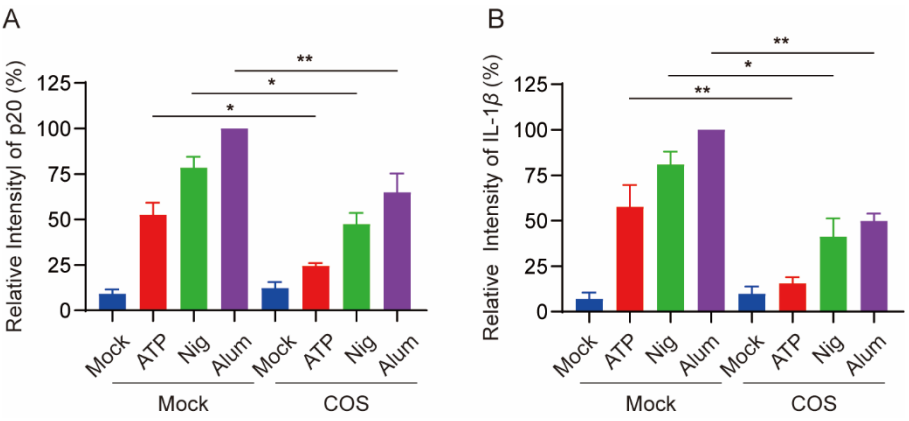
Supplementary Figure S5

Western blotting analysis of IL-1*β* and p20 levels (**A**) in SN or ELISA of IL-1*β* in culture SN (**B**) of BMDMs treated with COS (5 μmol/L) before or after LPS challenged and stimulated with ATP for 0.5 h. Data are presented as the mean ± SEM, $n = 3$; $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant.



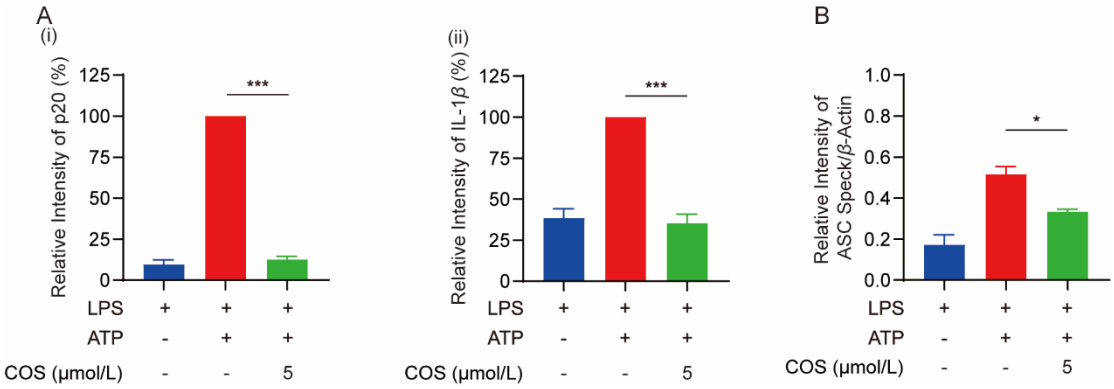
Supplementary Figure S6

(**A**, **B**) Western blotting analysis of IL-1*β* and p20 levels (**A**) in SN or ELISA of IL-1*β* in culture SN (**B**) of LPS-primed BMDMs treated with of COS (5 μmol/L) and stimulated with poly (dA:dT) for 4 h. (**C**, **D**) Western blotting analysis of IL-1*β* and p20 levels (**C**) or ELISA (**D**) of IL-1*β* in culture SN of LPS-primed BMDMs treated with of COS (5 μmol/L) and stimulated with *Salmonella* for 4 h. Data are presented as the mean ± SEM, $n = 3$. ns, not significant.



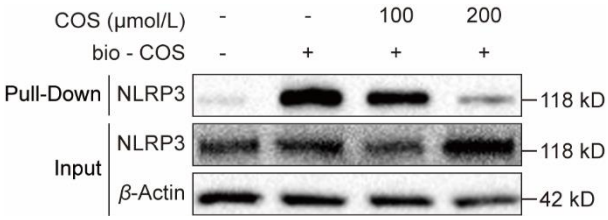
Supplementary Figure S7

Densitometric quantification of p20 (**A**) and IL-1β (**B**) described in Fig. 1J. Data are presented as the mean ± SEM, $n = 3$; *$P < 0.05$, **$P < 0.01$.



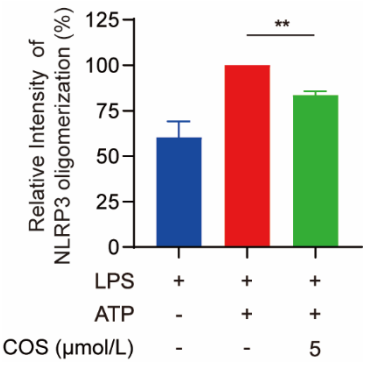
Supplementary Figure S8

(**A**) Densitometric quantification of p20 (i) and IL-1*β* (ii) described in Fig. 2D. (**B**) Densitometric quantification of ASC Speck described in Fig. 2D. Data are presented as the mean ± SEM, $n = 3$; *$P < 0.05$, ***$P < 0.001$.



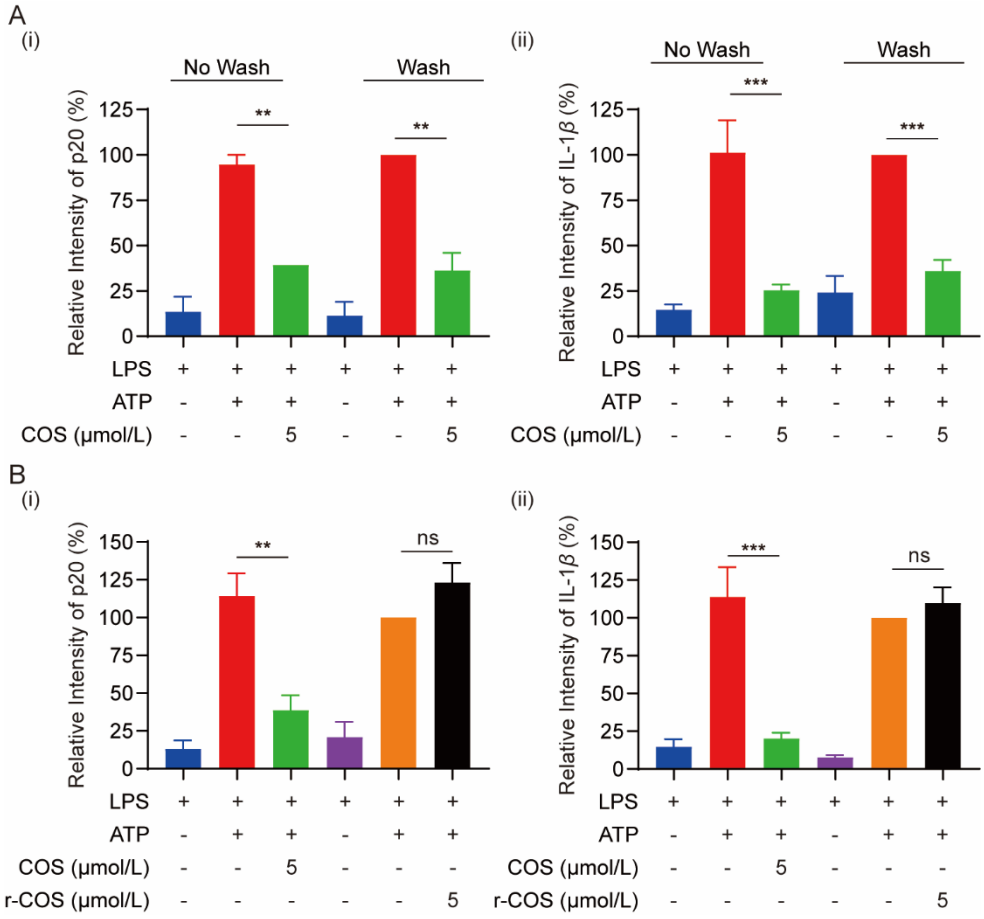
Supplementary Figure S9

Streptavidin-covered beads with bio-COS (100 μmol/L) and different concentrations of free COS were incubated with the cell lysates of HEK-293T cells transfected with high expression plasmid of Flag-NLRP3 for 6 h. As indicated, the levels of bound proteins (Pull-down) and total proteins (Input) were determined using western blotting (n = 3).



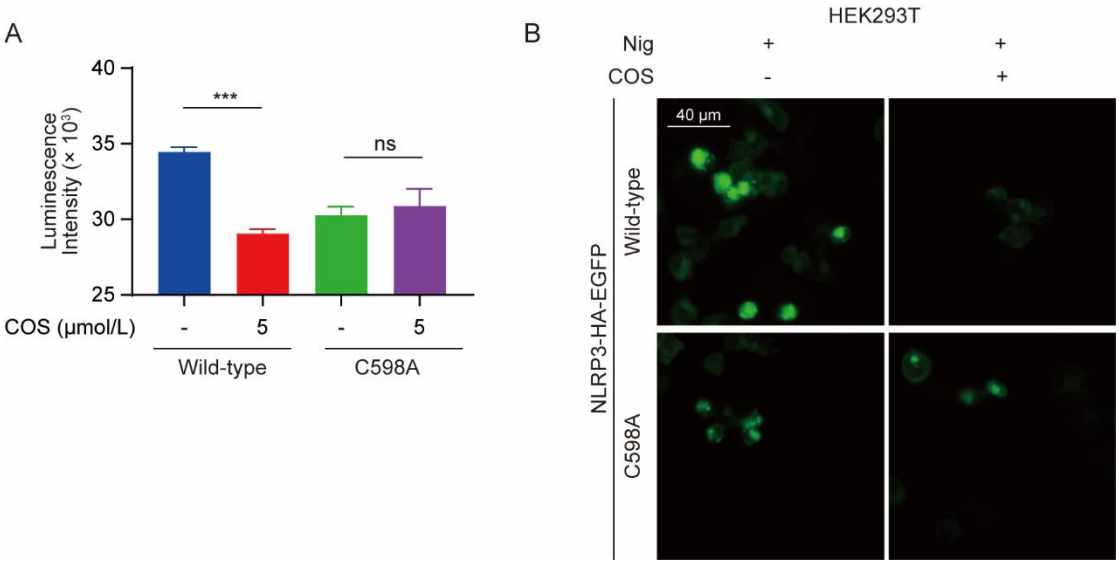
Supplementary Figure S10

Densitometric quantification of NLRP3 oligomerization described in Fig. 3F. Data are presented as the mean ± SEM, $n = 3$; $^{**}P < 0.01$.



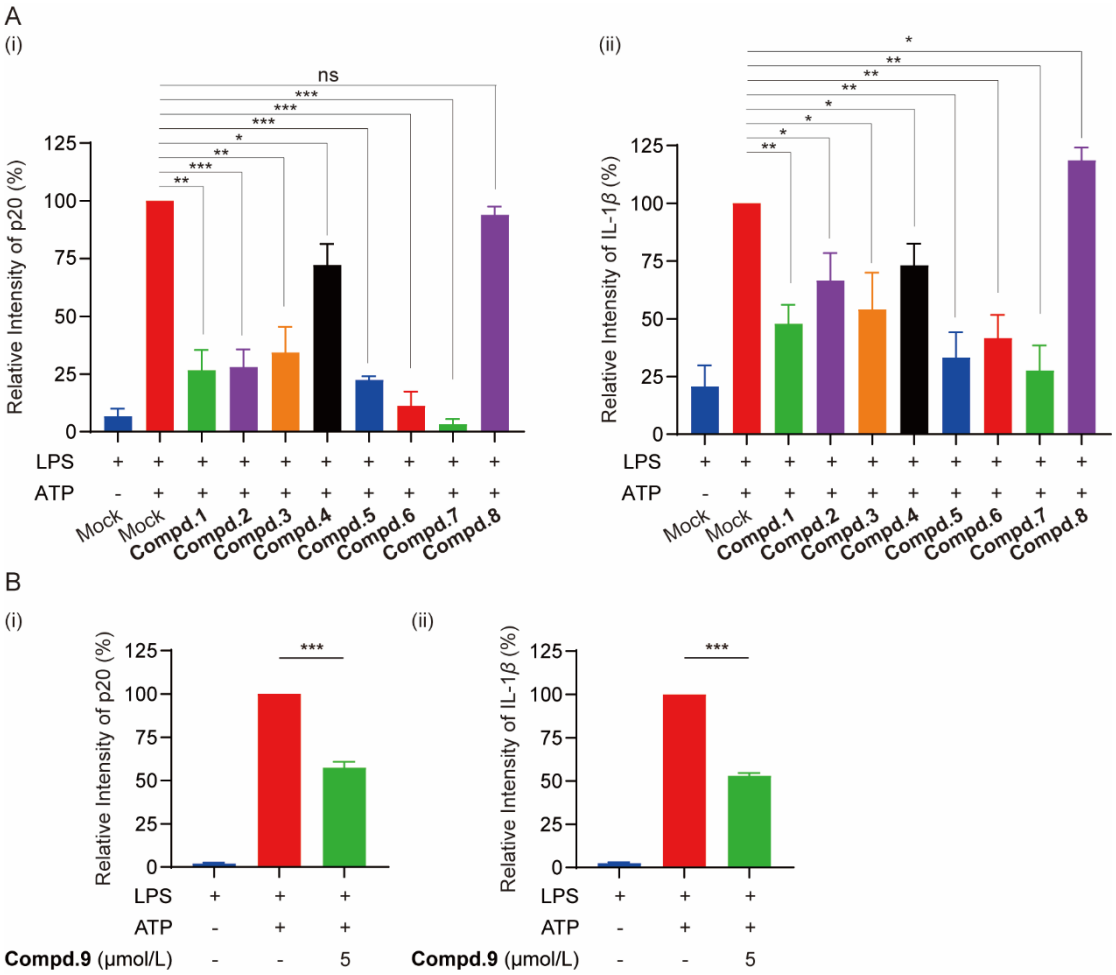
Supplementary Figure S11

(**A**) Densitometric quantification of p20 (i) and IL-1β (ii) described in Fig. 4A. (**B**) Densitometric quantification of ASC Speck described in Fig. 4D. Data are presented as the mean ± SEM, $n = 3$; $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant.



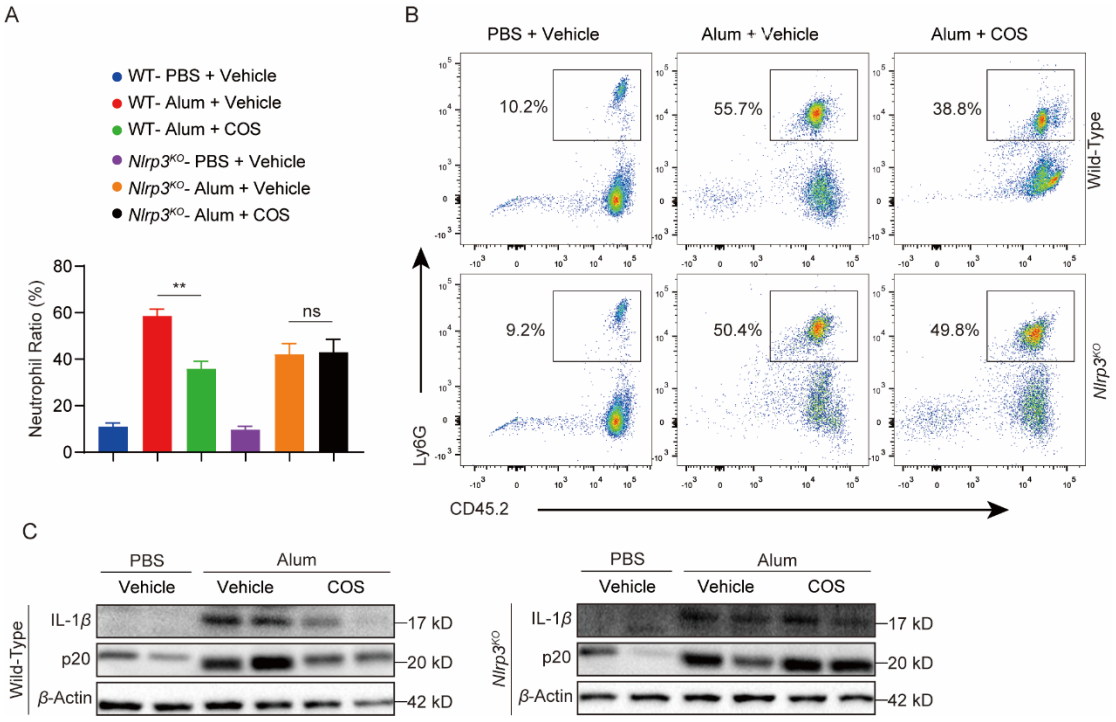
Supplementary Figure S12

(**A**) ATPase activity assay for WT or C598A NACHT protein treated with or without COS (5 μmol/L). (**B**) Fluorescence analysis of HEK-293T cells expressing HA-EGFP-tagged WT or C598A NLRP3 treated with or without COS (5μmol/L). Data are presented as the mean ± SEM of three separate experiments performed in duplicate (*n*=3 per group); ***$P < 0.001$; ns, not significant.



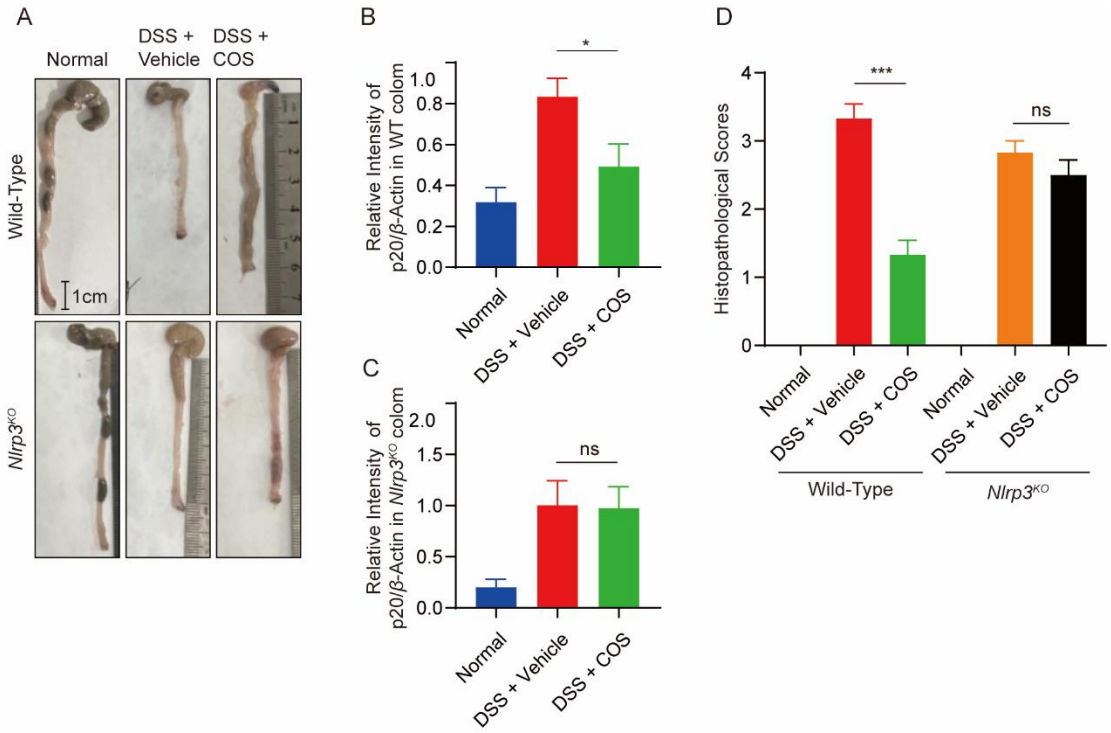
Supplementary Figure S13

(**A**) Densitometric quantification of p20 (i) and IL-1β (ii) described in Fig. 5C. (**B**) Densitometric quantification of p20 (i) and IL-1β (ii) described in Fig. 5F. Data are presented as the mean ± SEM, $n = 3$; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns, not significant.



Supplementary Figure S14

WT or *Nlrp3*^{KO} mice were intraperitoneally injected with Alum crystals (1 mg per mouse) in the presence or absence of COS (40 mg/kg). n = 6 per group. (**A**) FACS analysis of neutrophil ratios in the peritoneal cavity. (**B**) Representative figures in FACS analysis. (**C**) Western blotting analysis of mature IL-1β and p20 levels in peritoneal cells from WT or *Nlrp3*^{KO} mice. Data are presented as the mean ± SEM. $^{**}P < 0.01$; ns, not significant.



Supplementary Figure S15

(**A**) Representative photographs to show the colon length. (**B**) Densitometric quantification of p20 in WT mice colon described in Fig. 7E. (**C**) Densitometric quantification of p20 in *Nlrp3*^{KO} mice colon described in Fig. 7F. (**C**) Histopathological scores of paraffin-embedded colon tissues H&E staining. Data are presented as the mean ± SEM, (n = 6 per group); *P < 0.05, ***P < 0.001; ns, not significant.