Expanded View Figures

Figure EV1. Licochalcone B inhibits NLRP3 inflammasome activation.

A BMDMs were primed with LPS for 4 h and then treated with LicoB 1 h prior to stimulation of nigericin for 45 min. Activity of caspase-1 in culture supernatants of BMDMs were shown.

B, C BMDMs were primed with LPS for 4 h and then treated with LicoB for 1 h, prior to stimulation with nigericin for 45 min or ATP for 1 h. Western blot analyses of GSDMD, pro-caspase-1 (p45), pro-IL-1β, NLRP3 and ASC in the whole cell lysate (WCL); activated caspase-1 (p20) and cleaved IL-1β (p17) in the culture SN of BMDMs. Coomassie Blue staining was used as the SN loading control, while lamin B was used as the lysate loading control.

D–F PMA-primed THP-1 cells were treated with LicoB 1 h prior to stimulation with nigericin for 45 min. Western blot analyses of pro-caspase-1 (p45), pro-IL-1β, NLRP3 and ASC in the WCL; and activated caspase-1 (p20) and cleaved IL-1β (p17) (D) in the culture supernatants (SN) of THP-1 cells. Caspase-1 activity (E) and IL-1β secretion (F) in the SN were measured.

G, H Human PBMCs were primed with LPS for 4 h and then treated with LicoB 1 h prior to stimulation with nigericin for 45 min (G) or with ATP for 1 h (H). Caspase-1 activity in supernatant was measured.

Data information: Error bars, mean ± SEM from three biological replicates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and n.s.: not significant (one-way ANOVA with Dunnett's *post hoc* test).
Source data are available online for this figure.

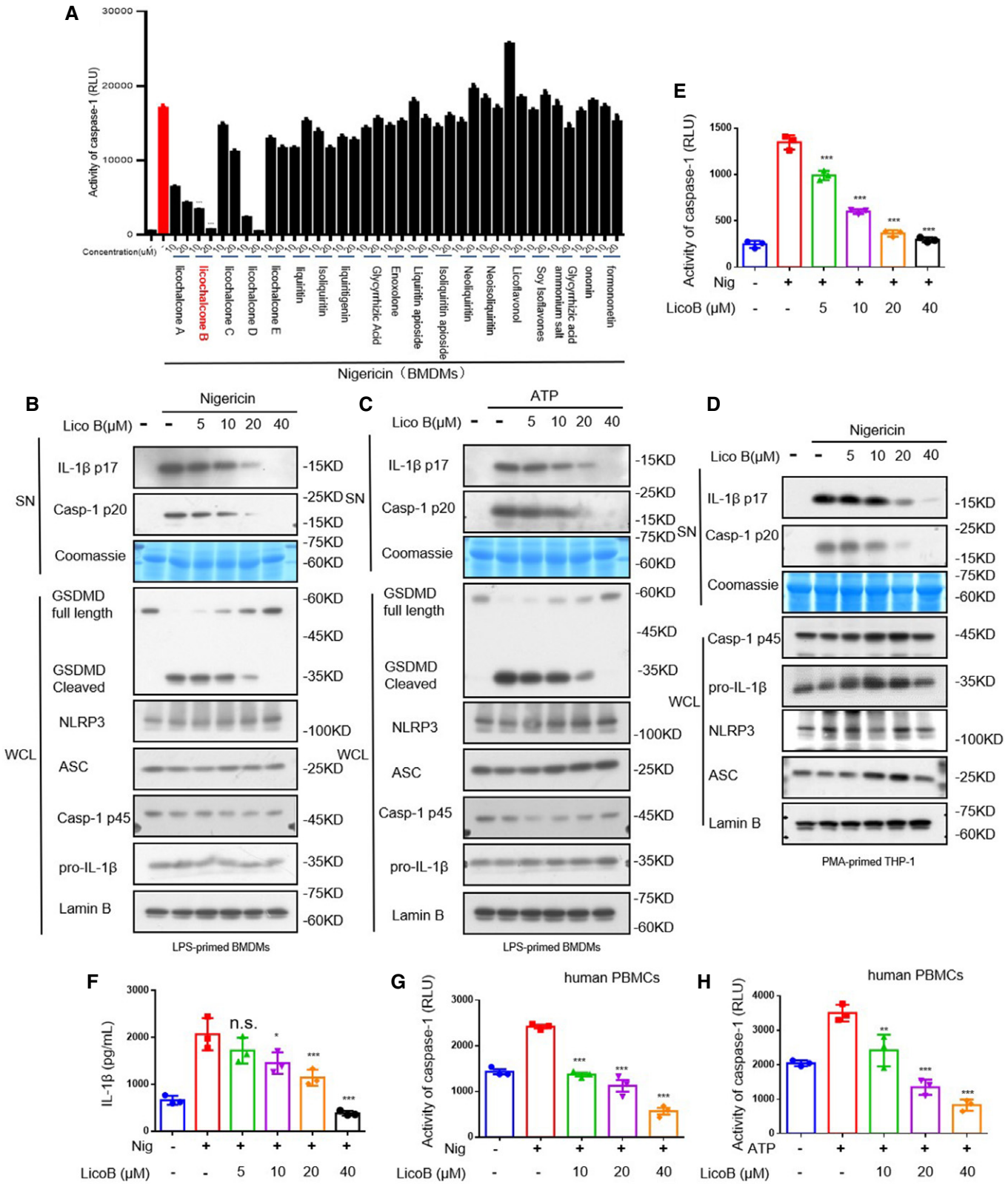

Figure EV1.

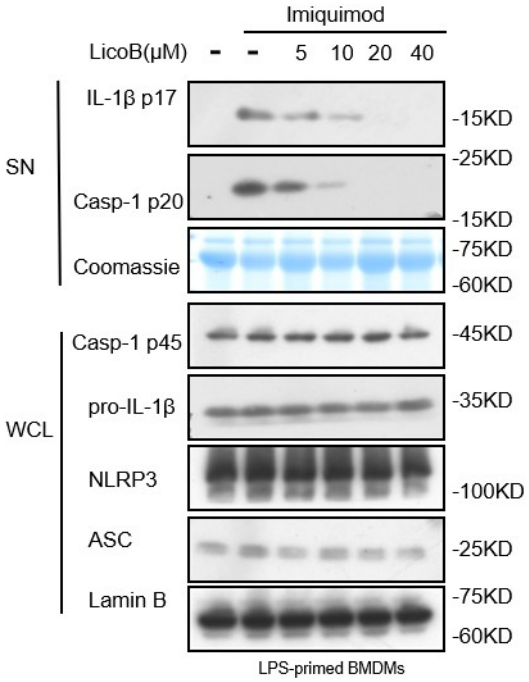

Figure EV2. Licochalcone B inhibits K^+ Efflux-Independent NLRP3 Inflammasome Activation in BMDMs.

BMDMs were primed with LPS for 4 h and then treated with LicoB for 1 h prior to stimulation with imiquimod (70 μM) for 1 h. Western blot analyses of pro-caspase-1 (p45), pro-IL-1β, NLRP3 and ASC in the whole cell lysate (WCL); activated caspase-1 (p20) and cleaved IL-1β (p17) in the culture SN of BMDMs were shown. Coomassie Blue staining was used as the SN loading control, while lamin B was used as the lysate loading control.

Source data are available online for this figure.

Figure EV3. Licochalcone B directly binds to NEK7 but does not affect the kinase activity of NEK7.

A Cell lysates of PMA-primed THP-1 treated with nigericin or not were incubated with sepharose or LicoB-sepharose. The pull-down samples and input were analysed by Western blot.

B Cell lysates of PMA-primed THP-1 were incubated with sepharose or LicoB-sepharose in the presence of different concentrations of free LicoB (0.5 and 1 mM). The pull-down samples and input were analysed by Western blot.

C Cell lysates of LPS-primed hPBMCs treated with nigericin or not were incubated with sepharose or LicoB-sepharose. The pull-down samples and input were analysed by Western blot.

D Cell lysates of LPS-primed hPBMCs were incubated with sepharose or LicoB-sepharose in the presence of different concentrations of free LicoB (0.5 and 1 mM). The pull-down samples and input were analysed by Western blot.

E NEK7 was incubated with β-casein and ATP in the presence of different concentrations of LicoB. NEK7 kinase activity was measured using an ADP-based phosphatase coupled kinase assay.

F Cell lysates of LPS-primed BMDMs treated with nigericin or not were incubated with Sepharose, Sepharose-LicoA or Sepharose-LicoB. The pull-down samples and input were analysed by Western blot.

G, H Human monocytes were treated with LicoB for 1 h, prior to stimulation with LPS (200 ng/ml) for 14 h. (G) Western blot analyses of pro-caspase-1 (p45), pro-IL-1β, NLRP3 and ASC in the whole cell lysate (WCL); cleaved IL-1β (p17) in the culture SN of BMDMs were shown. Coomassie Blue staining was used as the SN loading control, while lamin B was used as the lysate loading control. IL-1β secretion (H) in the supernatant were measured by ELISA.

Data information: Error bars, mean ± SEM from three biological replicates. $^{**}P < 0.01$, $^{***}P < 0.001$ and n.s.: not significant (one-way ANOVA with Dunnett's *post hoc* test).
Source data are available online for this figure.

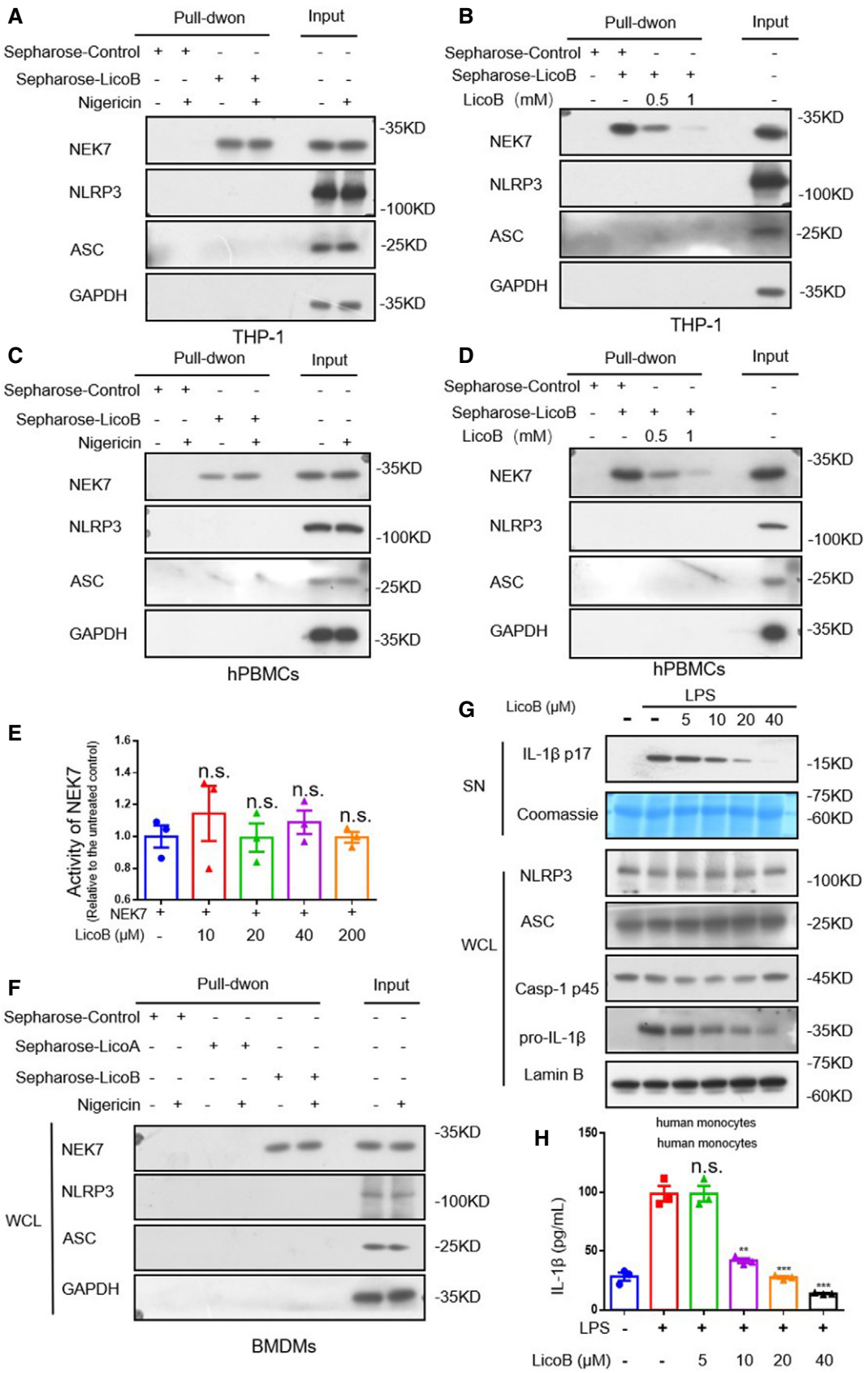

Figure EV3.