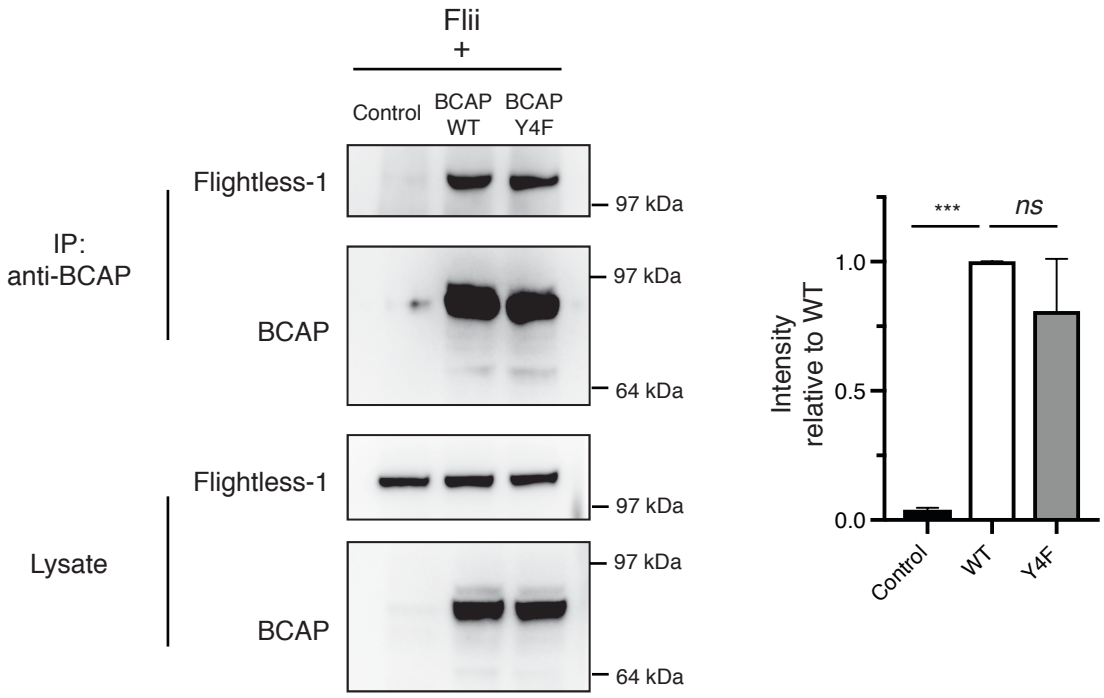
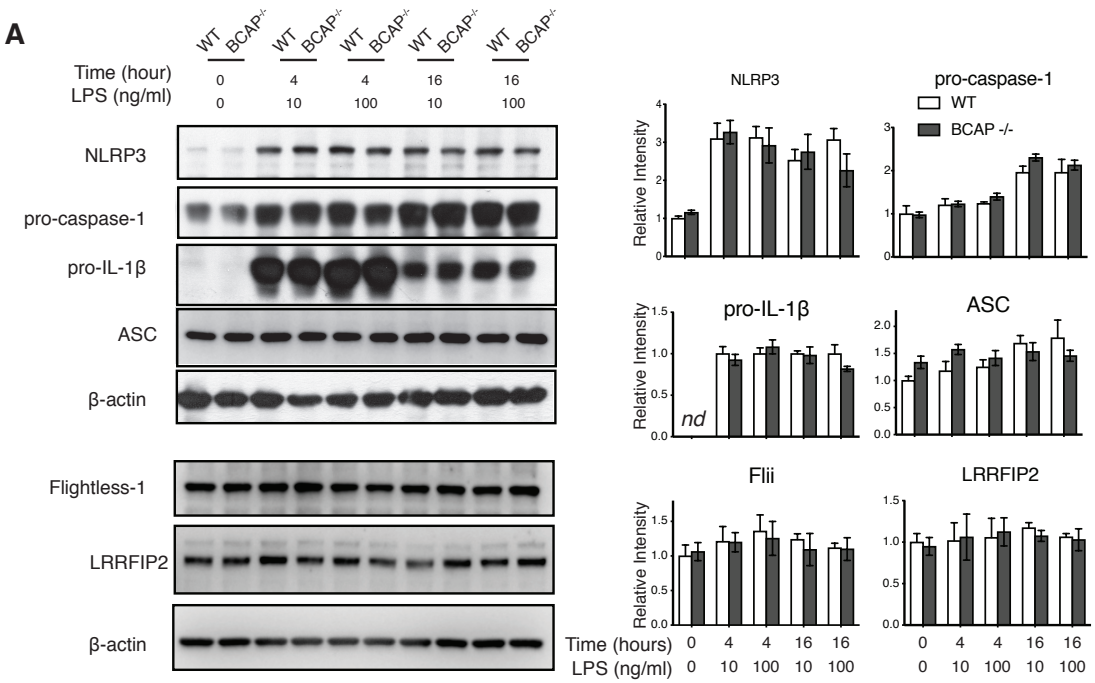
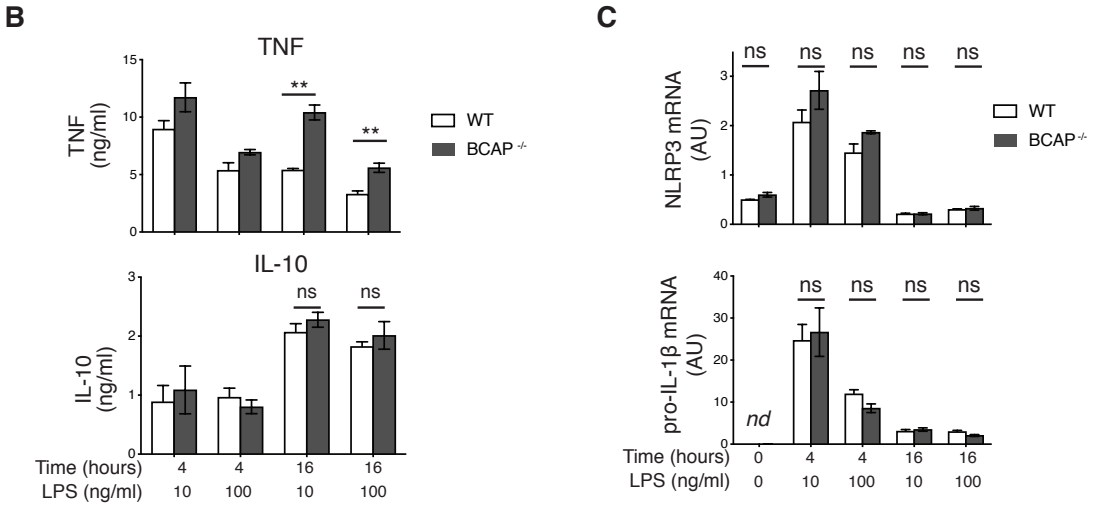


Figure S1. BCAP YxxM tyrosines are not required for association with Flightless-1. Western blot analysis of the indicated proteins in lysates from 293T cells co-transfected with plasmids encoding wild-type BCAP or Y4F BCAP mutant and Flightless-1 and then immunoprecipitated for BCAP. Blots (left) are representative of 3 independent experiments. Quantified band intensity values (right) of Flightless-1 in immunopreciptates are means + SEM pooled from all experiments. *ns,* not significant; ***P<0.001 by One way ANOVA with Tukey's post-test.


A
WT
BCAP-/-
Time (hour)
LPS (ng/ml)
NLRP3
pro-caspase-1
pro-IL-1β
ASC
β-actin
Flightless-1
LRRFIP2
β-actin
NLRP3
pro-caspase-1
WT
BCAP -/-
Relative Intensity
pro-IL-1β
ASC
nd
Flii
LRRFIP2
Time (hours)
LPS (ng/ml)


B
TNF
TNF (ng/ml)
WT
BCAP -/-
IL-10
IL-10 (ng/ml)
ns
Time (hours)
LPS (ng/ml)
C
NLRP3 mRNA (AU)
pro-IL-1β mRNA (AU)
nd

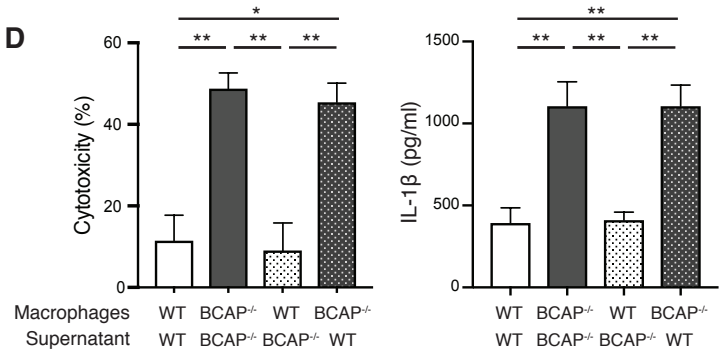

D
Cytotoxicity (%)
IL-1β (pg/ml)
Macrophages
Supernatant

Figure S2. No difference in expression of NLRP3 inflammasome components or soluble factors induced by LPS between WT and BCAP$^{-/-}$ macrophages. (**A**) Western blot analysis for indicated proteins in WT and BCAP$^{-/-}$ BMDMs primed with 10 or 100 ng/ml of LPS for 4 or 16 h. Blots (left) are representative of and quantified band intensity values (right) are pooled from 3 experiments. Band intensities are normalized to untreated WT BMDMs (NLRP3, pro-caspase-1, ASC, Flightless-1, LRRFIP2) or WT BMDMs + 10 ng/ml LPS for 4 h (pro-IL-1β). (**B**) ELISA analysis of TNF and IL-10 secretion by WT and BCAP$^{-/-}$ BMDMs in response to LPS stimulation. Data are means ± SEM of 3 experiments performed in triplicate. (**C**) Quantitative RT-PCR analysis of mRNA encoding NLRP3 and pro-IL-1β in BMDMs before and after LPS priming. Data are means ± SEM of 3 experiments performed in triplicate. (**D**) Cell death measured by LDH release and ELISA analysis of IL-1β secretion by BMDMs primed with LPS for 4 hours, and then cultured for an additional 2 hours in supernatants from either LPS-primed WT or BCAP$^{-/-}$ macrophages as indicated before treatment with nigericin for 30 min. Data are means + SEM of 2 independent experiments performed in triplicate. *nd,* not detected; *ns,* not significant; **P*<0.05 and ***P* < 0.01 by two-sided unpaired *t*-test.

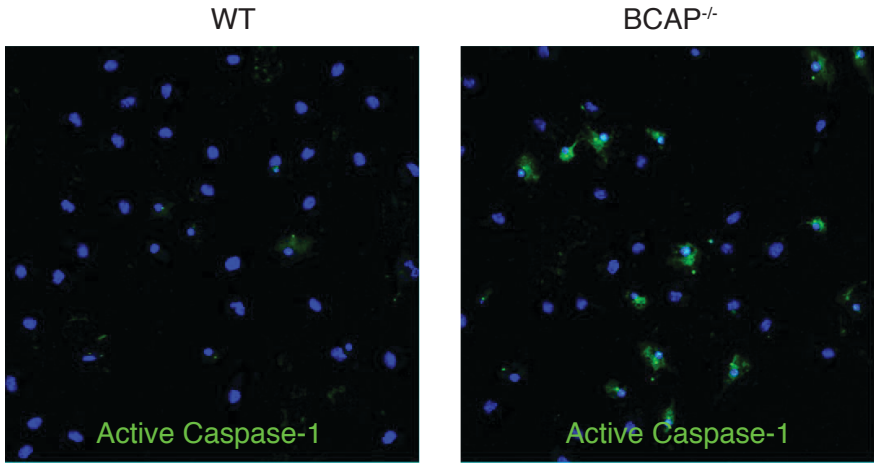


Figure S3. BCAP$^{-/-}$ macrophages have increased caspase-1 activation after *YpstbΔ infection*. Fluorescence analysis of BMDMs primed overnight with LPS (100 ng/ml) and then infected with a 10:1 MOI *YpstbΔ* for 2 hours. Cells were stained with the fluorescent active caspase-1 probe FAM-YVAD-FMK (green) and Hoescht 33342 (DNA, blue). Data are representative of 3 independent experiments, which are quantitated in Fig. 4A.

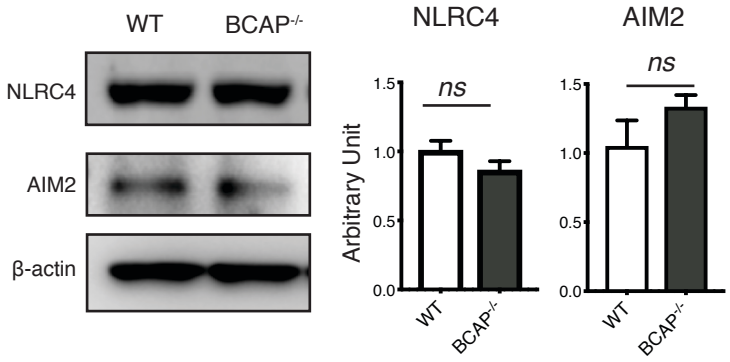


Figure S4. No difference in NLRC4 or AIM2 expression between WT and BCAP$^{-/-}$ macrophages. Western Blot analysis of NLRC4 and AIM2 in unprimed WT and BCAP$^{-/-}$ BMDMs. Blots (left) are representative of and quantified band intensity values are means ± SEM pooled from 3 biological replicates. *ns,* not significant by two-sided unpaired *t*-test.

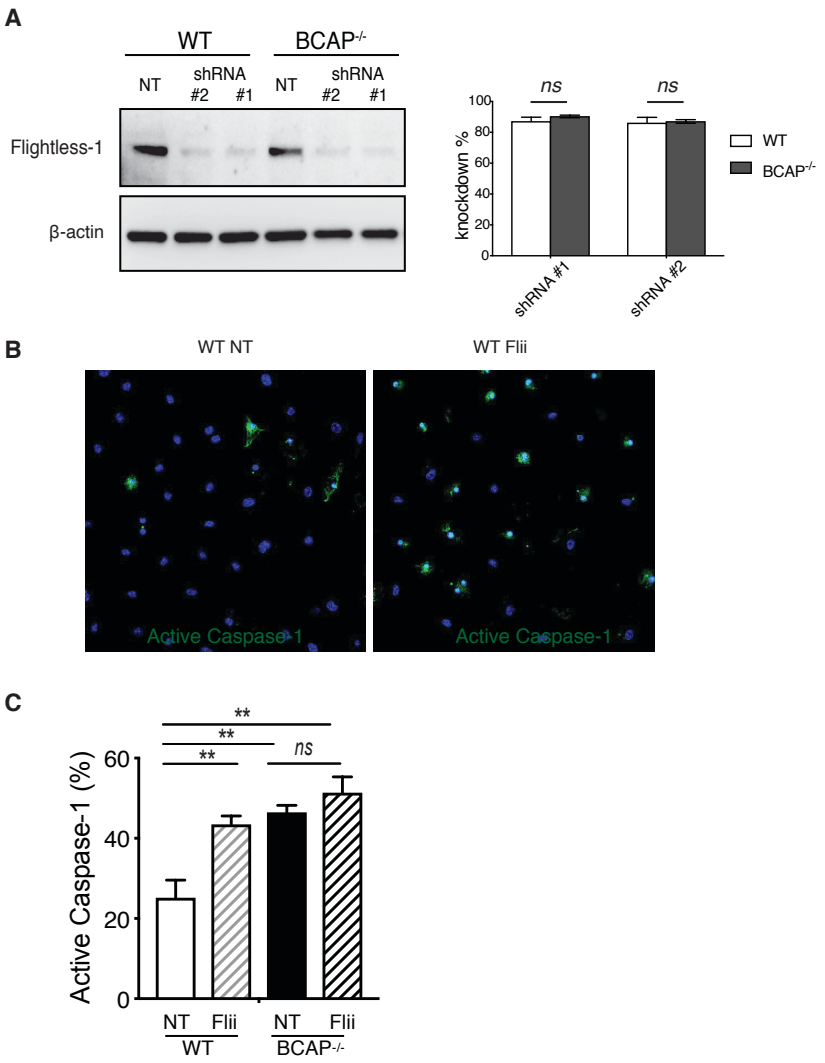


Figure S5. BCAP inhibition of inflammasome activation requires Flightless-1. (A) Western blot analysis of Flightless-1 knockdown in BMDMs infected with lentiviruses encoding for 2 different Flightless-1-targeting shRNAs. Blots (left) are representative of and quantified band intensity values (right) are means + SEM pooled from 3 independent experiments. (**B**) Immunofluorescence analysis of active caspase-1 (green) and DNA (blue) in BMDM transduced with non-targeting (NT) or Flii-specific shRNA#1 (Flii) and primed overnight with LPS, then infected with *YpstbΔ* for 1 hour. Images are representative of 3 independent experiments, which are quantitated in Fig. 7C. (**C**) Immunofluorescence analysis for active caspase-1 in WT BMDM transduced with NT or Flii-specific shRNA #2 that were LPS-primed for 4hrs, then stimulated with nigericin. Data are means + SEM from 3 independent experiments. *ns*, not significant; and $^{**}P<0.01$ by One way ANOVA with Tukey's post-test.

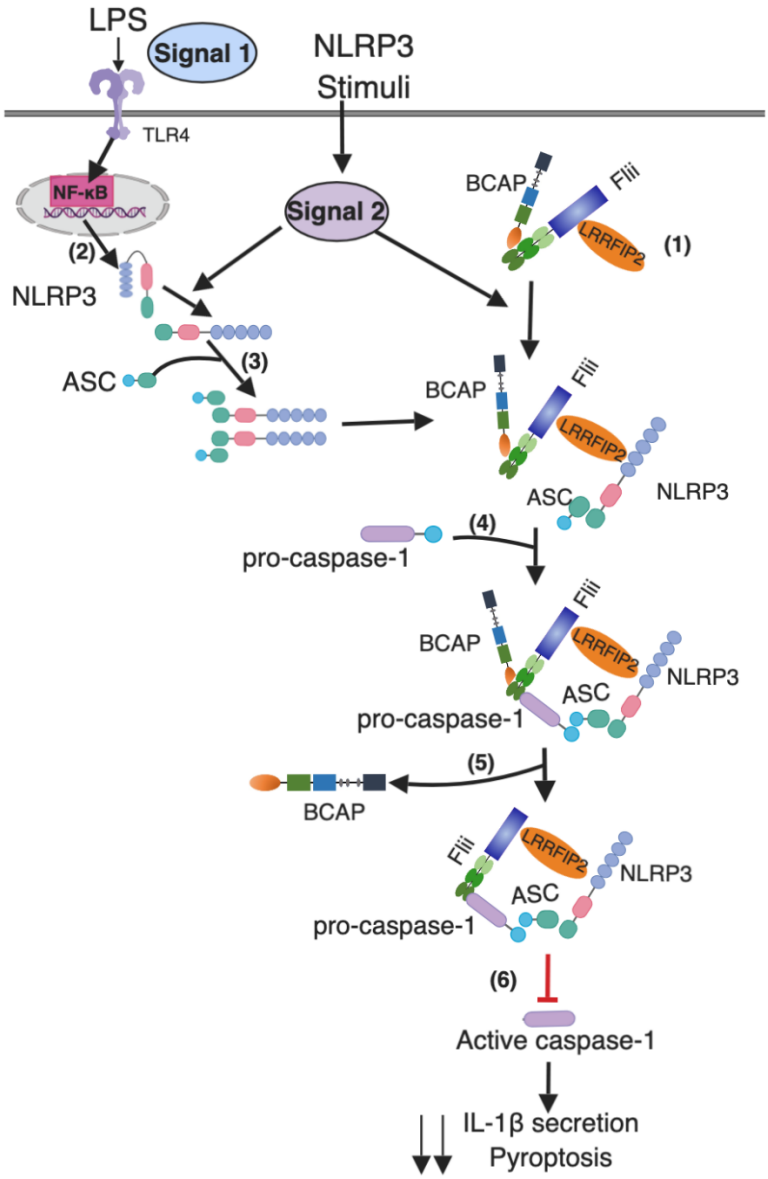


Figure S6. Model of NLRP3 inflammasome inhibition by BCAP and Flightless-1. (1) In resting BMDMs, BCAP is associated with the NLRP3 inflammasome regulators Flightless-1 and LRRFIP2. **(2)** During inflammasome priming (Signal 1), LPS signals through NF-κB and induces the transcription of NLRP3 and pro-IL-1β. **(3)** NLRP3 activation (Signal 2) causes the oligomerization of NLRP3 and subsequent ASC recruitment and oligomerization to the pre-inflammasome complex. **(4)** Upon signal 2, BCAP facilitates the association of the Flightless-1-LRRFIP2 complex with pro-caspase-1 and the NLRP3/ASC pre-inflammasome focus. **(5)** BCAP is then released from this complex. **(6)** The Flightless-1 mediated caspase-1 inhibition leads to reduced pyroptosis and IL-1β secretion. Figure was made with BioRender.