Expanded View Figures

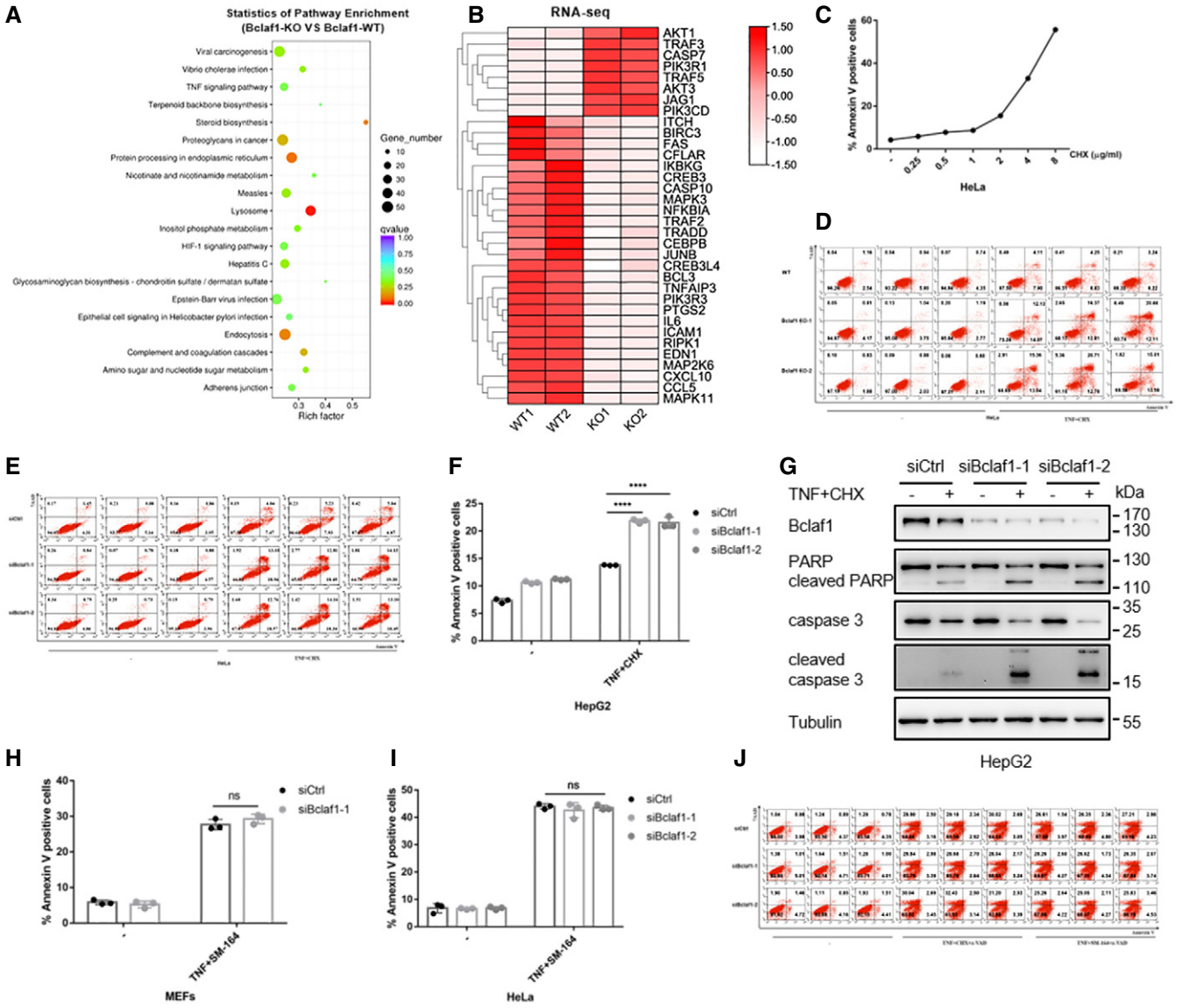

Figure EV1. Bclaf1 deficiency sensitizes cells to apoptosis in response to TNF^{+}CHX but not to necroptosis.

A, B Duplicates of WT and Bcalf1-KO HeLa cells were subject to RNA-seq analysis. (A) The top 20 enriched pathways obtained in the KEGG pathway analysis. (B) Heatmap of the different expression genes (DEGs) ($P < 0.05$, logFc>1) involved in TNF signaling pathway. The color in the heatmap represents the scaled FPKM of DEGs in the indicated samples.

C HeLa cells pretreated with increasing concentrations of CHX for 30min were treated with TNF (10 ng/ml) for 12 h in the presence of CHX. The cells were then subjected to Annexin V/7AAD staining followed by flow cytometry analysis. All Annexin V positive cells were counted for analysis.

D, E The FACS profile of Annexin V/7AAD staining of HeLa cells described in Fig 1A (D), and in Fig 1B (E).

F, G HepG2 cells were transfected with siCtrl or siBclaf1-1 and siBclaf1-2 and then treated and analyzed as described in Fig 1A.

H, I MEFs (H) and HeLa cells (I) transfected with siCtrl or siBclaf1 siRNAs were treated with SM-164 (100 nM) for 30 min prior to TNF (10 ng/ml) stimulation. After TNF treatment for 12 h, the cells were subjected to Annexin V staining followed by flow cytometry analysis.

J The FACS profile of Annexin V/7AAD staining of MEFs described in Fig 1D and E.

Data information: Data are shown as mean ± SD. $n = 3$ biological replicates. ns, not significant; ****$P < 0.0001$. One-way ANOVA test.

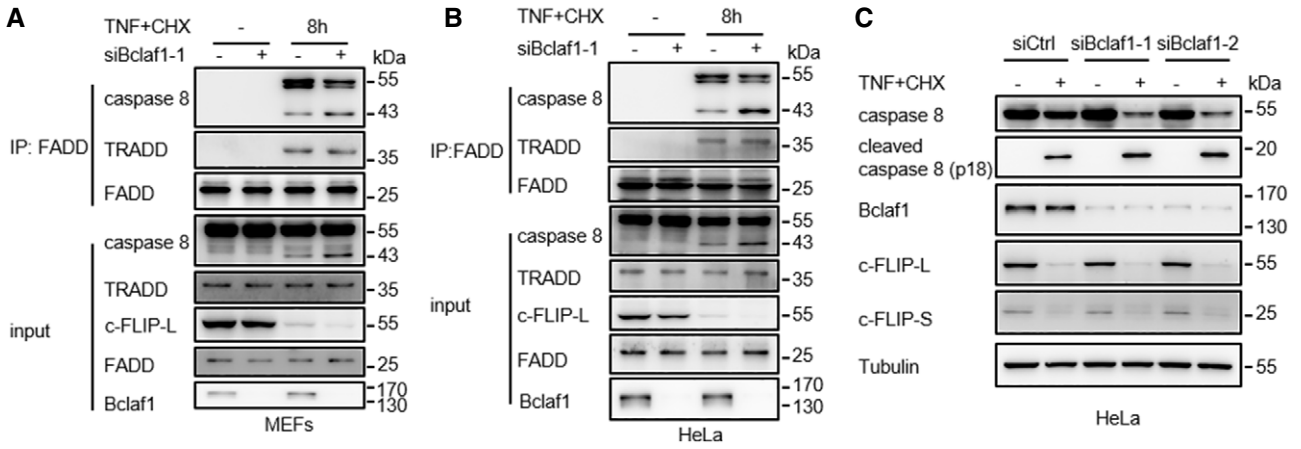

Figure EV2. Bclaf1 knockdown enhances the process and activation of caspase 8.

A, B MEFs and HeLa cells pretreated with CHX (1 μg/ml) for 30 min followed by stimulation with TNF (10 ng/ml) in the presence of CHX for indicated times were subject to FADD immunoprecipitation, and then analyzed by Western blotting.

C HeLa cells transfected with siCtrl or siBclaf1-1 and siBclaf1-2 were treated as described above followed by Western blotting analysis.

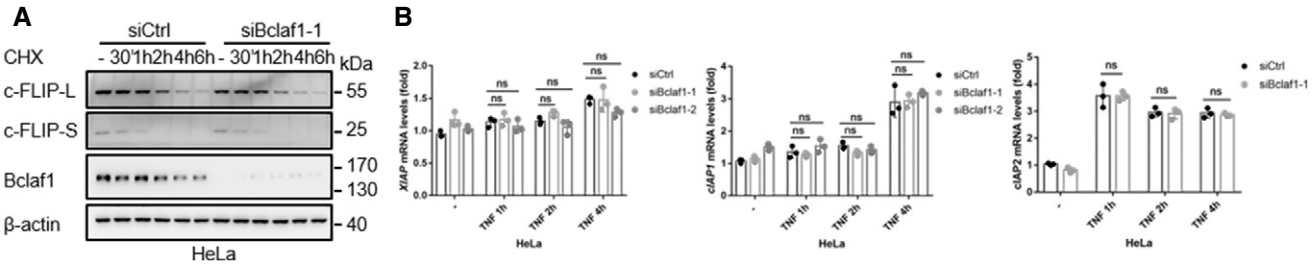

Figure EV3. siBclaf1 has no influence on the half-life of c-FLIP and transcriptions of other anti-apoptotic genes.

A HeLa cells were transfected with siCtrl or siBclaf1-1 were treated with 50 μg/ml CHX for indicated times followed by Western blotting analysis.

B HeLa cells were transfected with siCtrl or siBclaf1-1 and siBclaf1-2 were treated with TNF (10 ng/ml) for indicated hours followed by total RNA extraction and RT–PCR analysis. Data are shown as mean ± SD. $n = 3$ biological replicates. ns, not significant. Two-way ANOVA test.

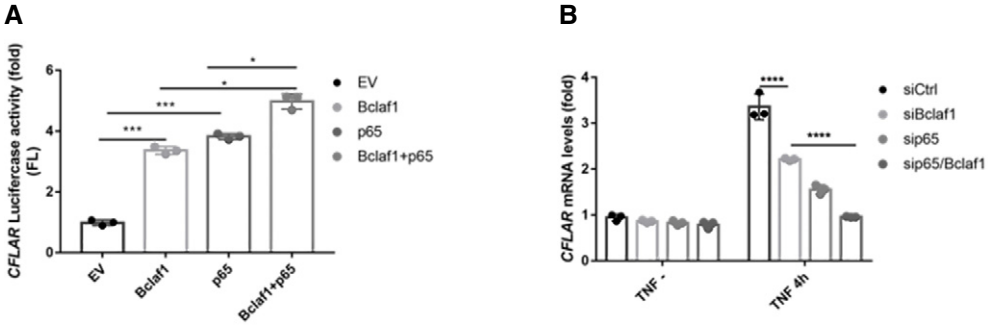

Figure EV4. Bclaf1-induced *CFLAR* upregulation is not through p65.

A *CFLAR* promoter was co-transfected with Bclaf1 and/or p65 into HeLa-Bclaf1-KO cells, and the luciferase activity was measured as described in Fig 4G.
B HeLa cells were transfected with siRNAs against Bclaf1 and/or p65. 24 h post-transfection, the cells were treated with TNF (10 ng/ml) for 4 h. The mRNA was extracted and subjected to real-time RT–PCR analysis.

Data information: Data are shown as mean ± SD. $n = 3$ biological replicates. *$P < 0.05$; ***$P < 0.001$; ****$P < 0.0001$. One-way ANOVA test.

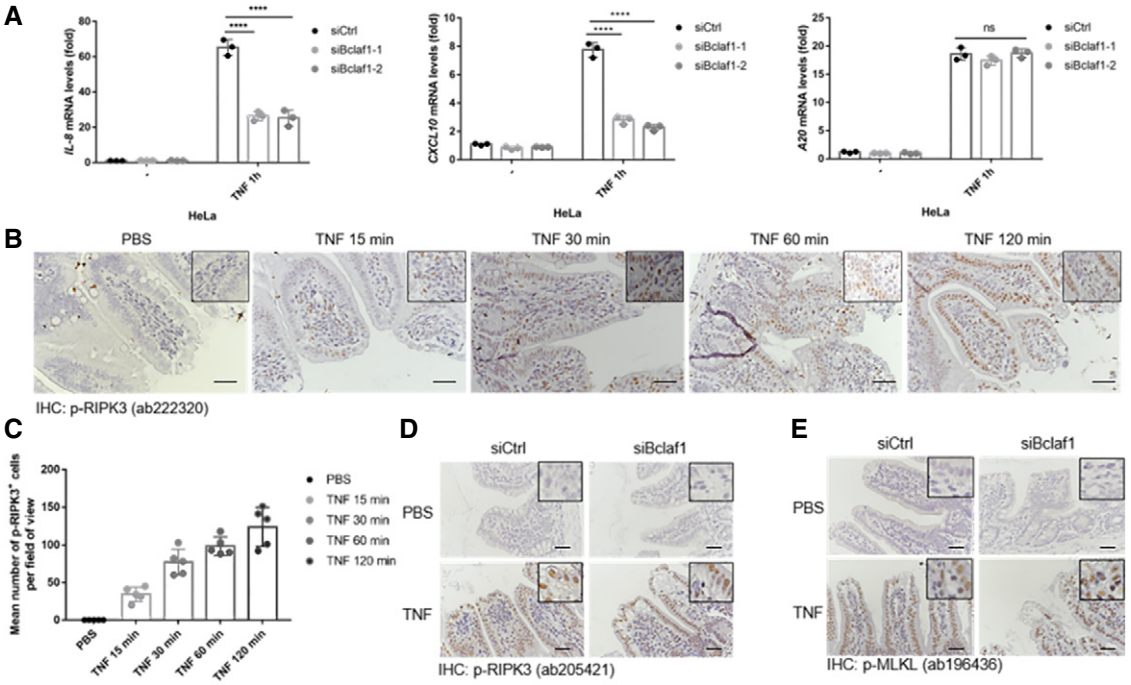

Figure EV5. siBclaf1 decreases IL-8 expression but has no influence on necroptotic pathway.

A HeLa cells transfected with siCtrl or siBclaf1-1 and siBclaf1-2 were treated with TNF (10 ng/ml) for indicated hours followed by total RNA extraction and RT–PCR analysis. Data are shown as mean ± SD. $n = 3$ biological replicates. ns, not significant; ****$P < 0.0001$. One-way ANOVA test.

B, C The mice were treated with mTNF for indicated time before sacrifice, and the small intestines were excised and processed for immunohistochemical staining by the anti-p-RIPK3 antibody ab222320 (B) and p-RIPK3$^+$ positive cells in five fields per intestine were quantified (C). Data are shown as mean ± SD. $n = 5$ mice. Scale bars: 50 μm.

D, E siRNAs against Bclaf1 and control siRNAs (siCtrl)-injected mice were treated with mTNF for 2 h before sacrifice, and immunohistochemically stained with another anti-p-RIPK3 antibody ab205421 (D) and an anti-p-MLKL (E) antibody. Scale bars: 50 μm.