Expanded View Figures

Figure EV1. GLMN colocalizes with cIAPs in macrophages.
Upper panel: Immunohistochemical analysis reveals the colocalization of endogenous GLMN (red) and cIAP2 (green) in *Shigella* WT-infected BMDMs (see also Fig 1D). The intensities of the fluorescence signals along with the white arrows were measured and are shown in the graph. Scale bars, 10 μm. Data represent one of three similar independent experiments. Bottom panel: Intracellular localization of endogenous cIAP2 is not affected by LPS priming. Scale bar, 10 μm.

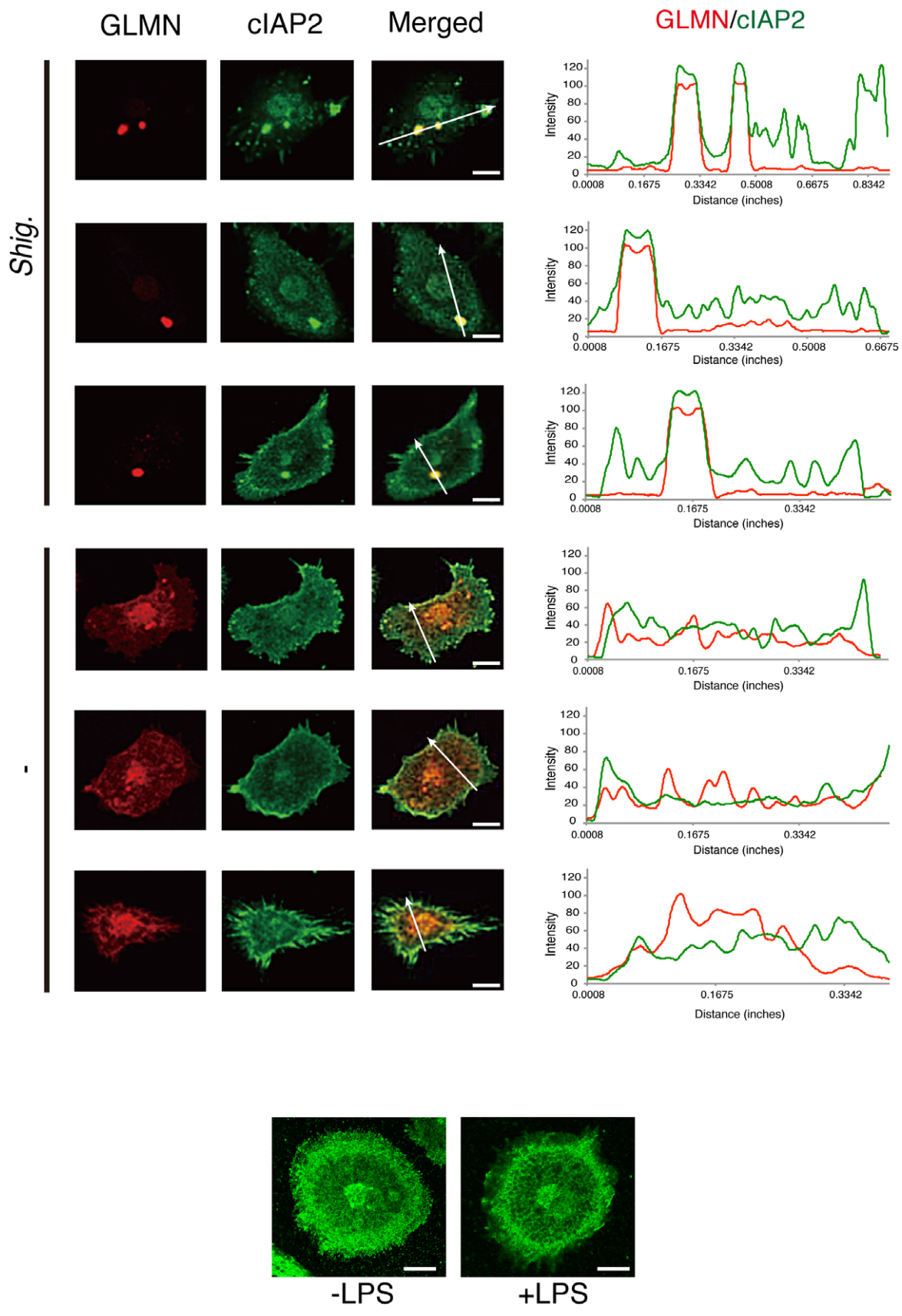

Figure EV1.

Figure EV2. cIAP1, cIAP2, XIAP, and GLMN influence inflammasome activation.

A Knockdown efficiencies of cIAP1, cIAP2, and XIAP in the cells used in this study were assessed by RT–PCR (see also Fig 2A–D). Actin mRNA levels were used for normalization. $*P < 0.01$.

B Expression levels of cIAP1, cIAP2, and XIAP in the cells used in this study (see also Fig 2F and G). $*P < 0.01$.

C Influence of cIAP1, cIAP2, XIAP, and GLMN on inflammasome activation caused by infection with *Shigella*, *Salmonella*, or *Pseudomonas aeruginosa*. WT BMDMs were subjected to siRNA-mediated knockdown of cIAP1/cIAP2, XIAP, or GLMN. Non-targeting siRNA (NT-CTL) was used as a negative control. Two days after siRNA nucleofection, cells were infected with each type of bacteria at an MOI of 10 (*Shigella*) or 1 (*Salmonella* and *P. aeruginosa*). IL-1β levels were measured at the indicated times post-infection. IL-18, CXCL2, and IL-6 levels were measured at 2 h post-infection (see also Figs 2A and B, and 3A and D). Knockdown of cIAP1/cIAP2 decreased IL-1β and IL-18 levels, indicating downregulation of inflammasomes. On the contrary, knockdown of XIAP or GLMN increased IL-1β and IL-18 levels. CXCL2 and IL-6 cytokine levels were not meaningfully affected by the presence or absence of cIAP1/2, XIAP, and GLMN. $*P < 0.01$.

D CXCL2 and IL-6 cytokine levels in cIAP1/2-, XIAP-, and GLMN-knockdown cells treated with stimulators of AIM2 or NLRP3 inflammasomes. cIAP1/2-, XIAP-, or GLMN-knockdown cells were stimulated with stimulators of AIM2 or NLRP3 inflammasomes, and supernatants were collected and subjected to CXCL2 and IL-6 ELISA as described for IL-1β (see also Figs 2C and D, and 3B and C).

Data information: The error bars represent the SD of the measurements. Statistical analyses were performed using the Mann–Whitney *U*-test. $n = 3$. Data are representative of three independent experiments.

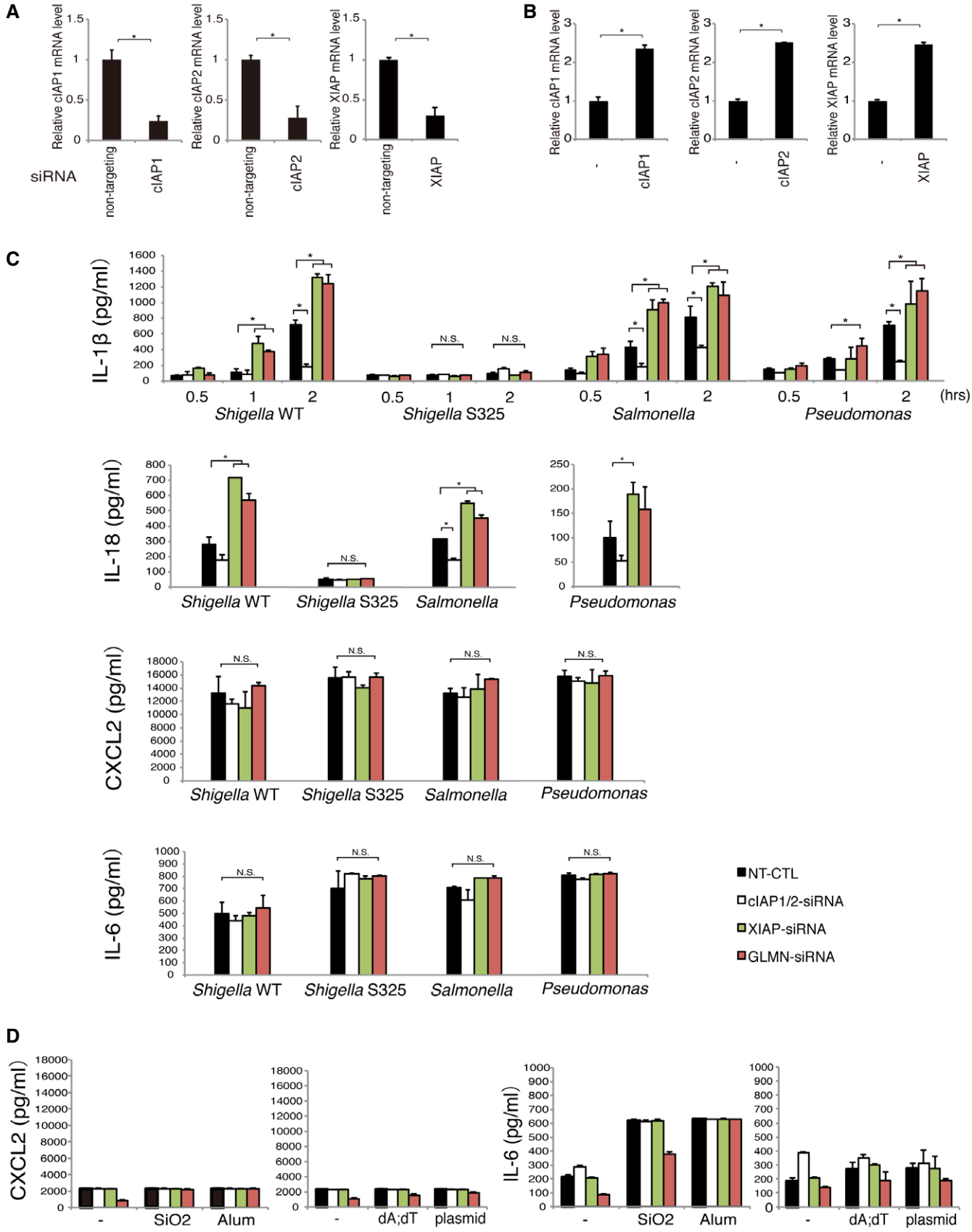
A
Relative cIAP1 mRNA level
Relative cIAP2 mRNA level
Relative XIAP mRNA level
siRNA
non-targeting
cIAP1
cIAP2
XIAP
B
Relative cIAP1 mRNA level
Relative cIAP2 mRNA level
Relative XIAP mRNA level
-
cIAP1
cIAP2
XIAP
C
IL-1β (pg/ml)
IL-18 (pg/ml)
CXCL2 (pg/ml)
IL-6 (pg/ml)
N.S.
0.5
1
2
(hrs)
Shigella WT
Shigella S325
Salmonella
Pseudomonas
NT-CTL
cIAP1/2-siRNA
XIAP-siRNA
GLMN-siRNA
D
CXCL2 (pg/ml)
IL-6 (pg/ml)
-
SiO2
Alum
dA;dT
plasmid

Figure EV2.

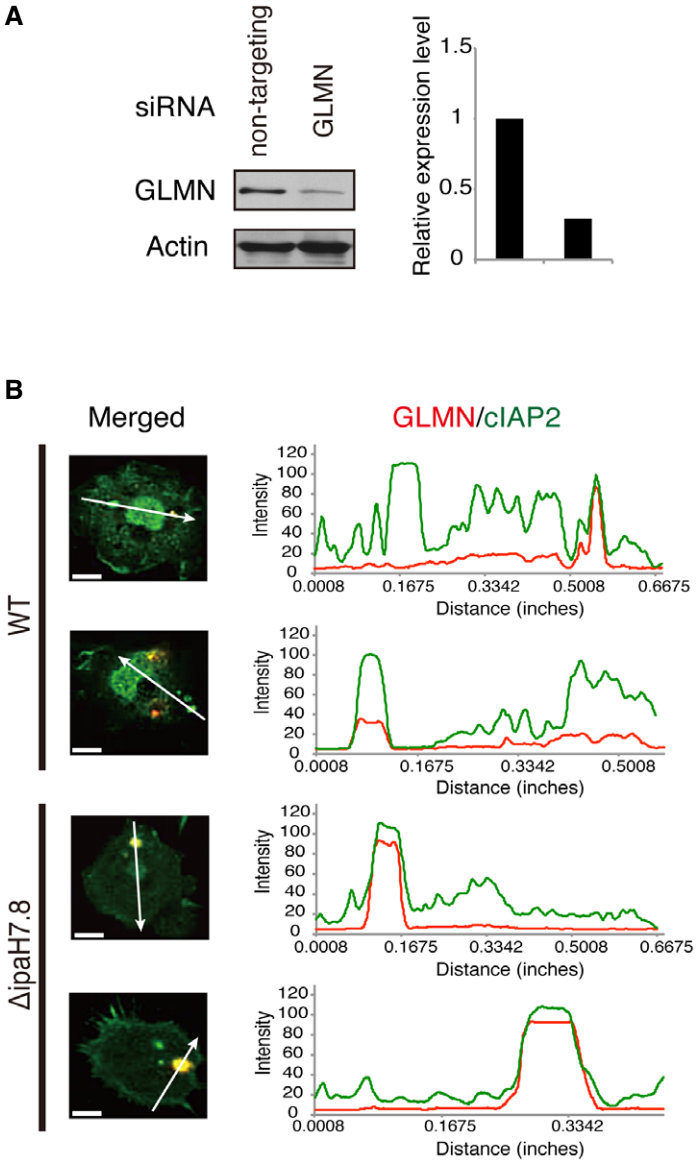

Figure EV3. Efficiency of siRNA-mediated knockdown of GLMN.

A Macrophages were subjected to siRNA-mediated knockdown of GLMN (or non-targeting siRNA as the control), and then, GLMN expression levels were assessed by immunoblotting with anti-GLMN antibody (see also Fig 3).

B Colocalization analysis. Graph shows the intensities of the fluorescence signals along the white arrows. Scale bars: 10 μm.

C BMDMs were infected with indicated bacterial strains. Cells were harvested 1 h post-infection, followed by immunoblotting to measure the level of endogenous GLMN. Data represent one of three similar experiments.

Source data are available online for this figure.

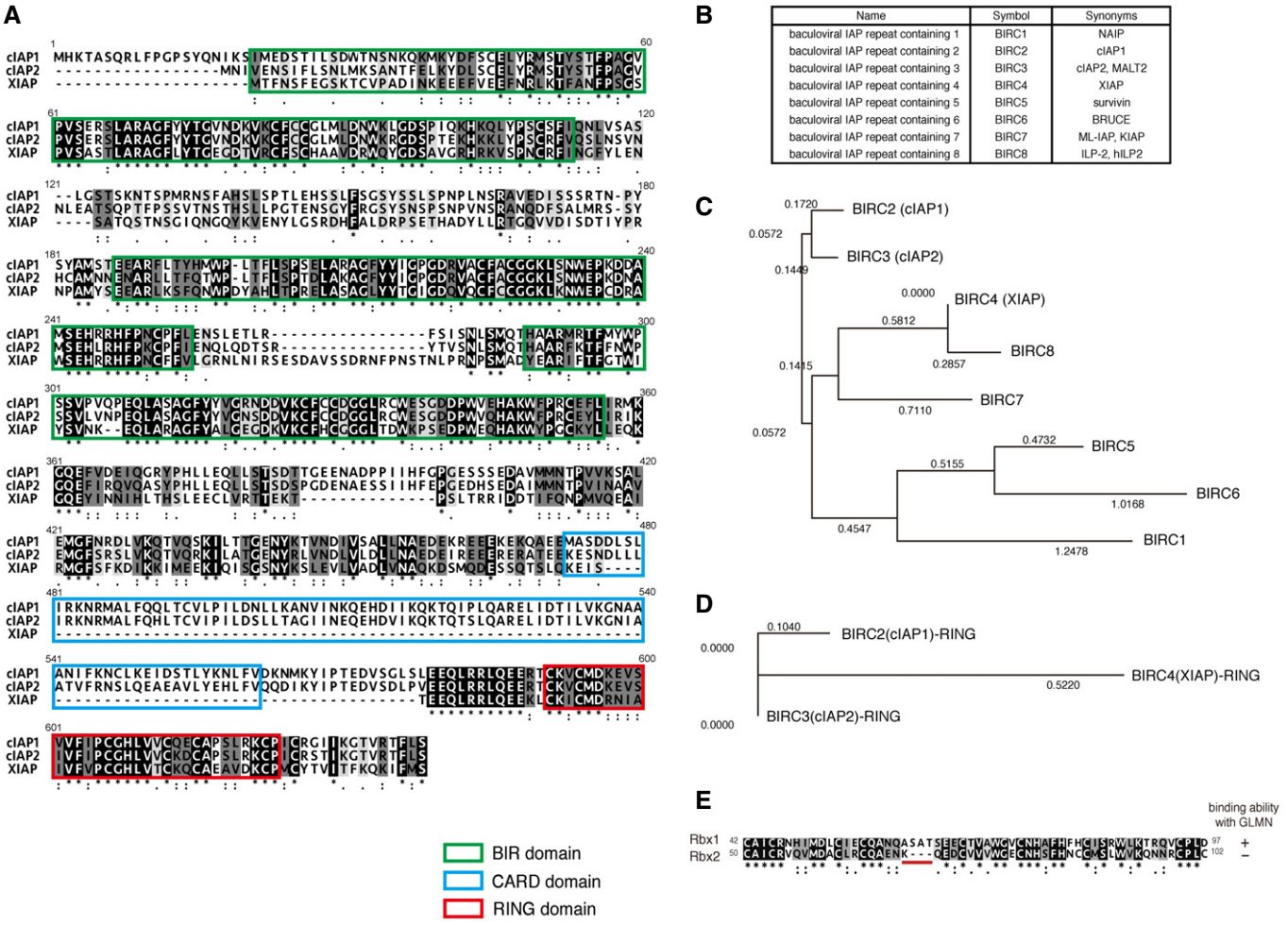

Figure EV4. Sequence alignment and phylogenetic tree building for cIAP, cIAP2, and XIAP.

A Comparison of amino acid sequences of cIAP, cIAP2, and XIAP, aligned using ClustalW. Green, blue, and red boxes indicate the BIRs, CARD, and RING, respectively. The three BIRs and RING are highly conserved among all three proteins. XIAP lacks the CARD, which is well conserved between cIAP1 and cIAP2.

B A list of the BIRC family members. The human BIRC family consists of eight members. A common feature of all BIRCs is the presence of BIR domain(s) in one to three copies. BIRC2 (cIAP1), BIRC3 (cIAP2), BIRC4 (XIAP), BIRC7, and BIRC8 possess RING zinc-finger domains that can act as E3 ligases.

C, D Phylogenetic analysis of BIRC family members. The neighbor-joining (NJ) method was used to create a phylogenetic tree using the Genetyx-Mac software. The amino acid sequences of full-length of BIRC family members (C) or RING domains (D) were analyzed. The phylogenetic tree shows that cIAP1 and cIAP2 are closely related to each other, whereas XIAP is located on another branch.

E Amino acid sequence alignment of the RING domains of Rbx1 and Rbx2. Identical amino acids (black) and similar amino acids (gray) are shaded. The RING domain is highly conserved between Rbx1 and Rbx2, except for the motif underlined in red.