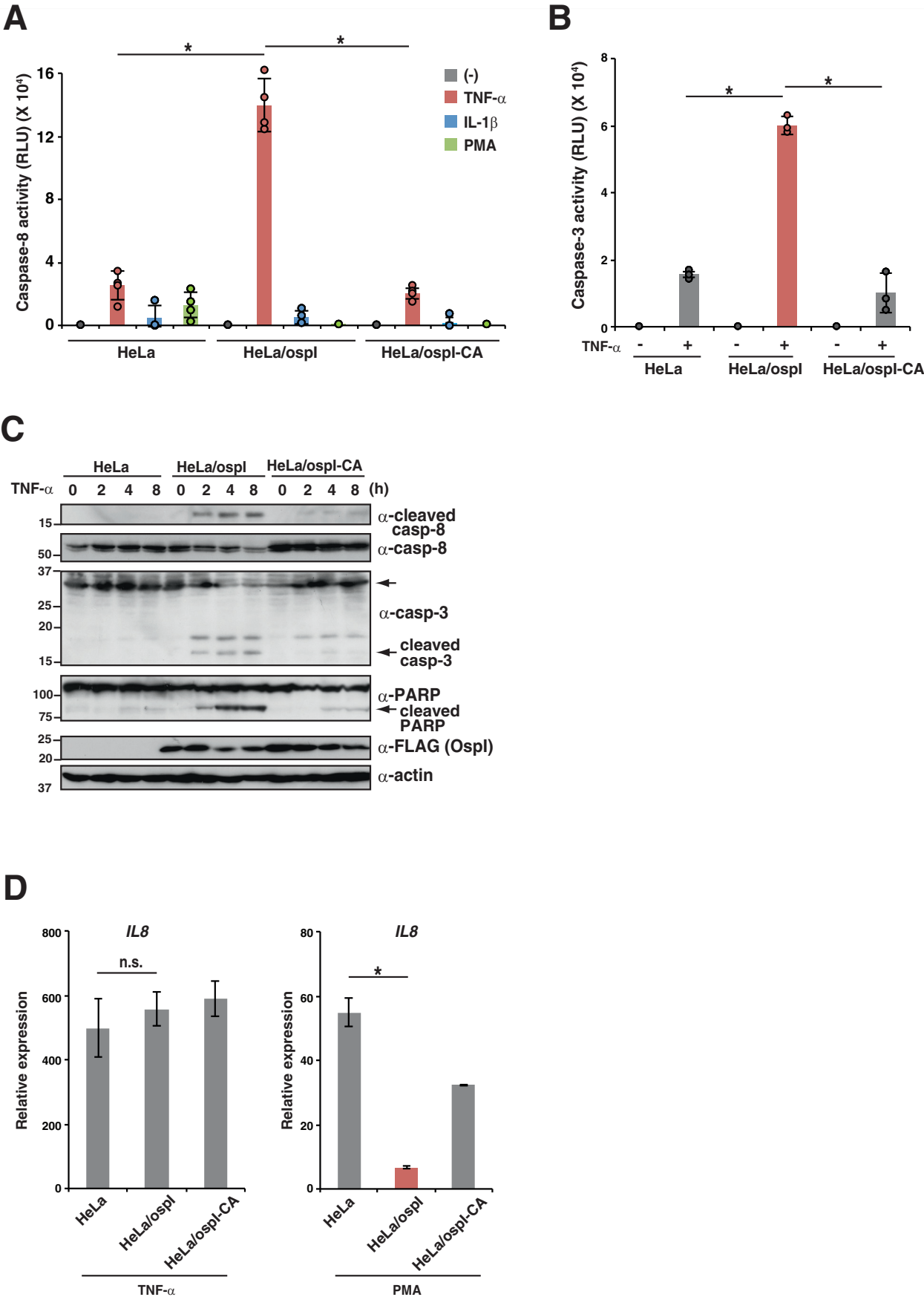
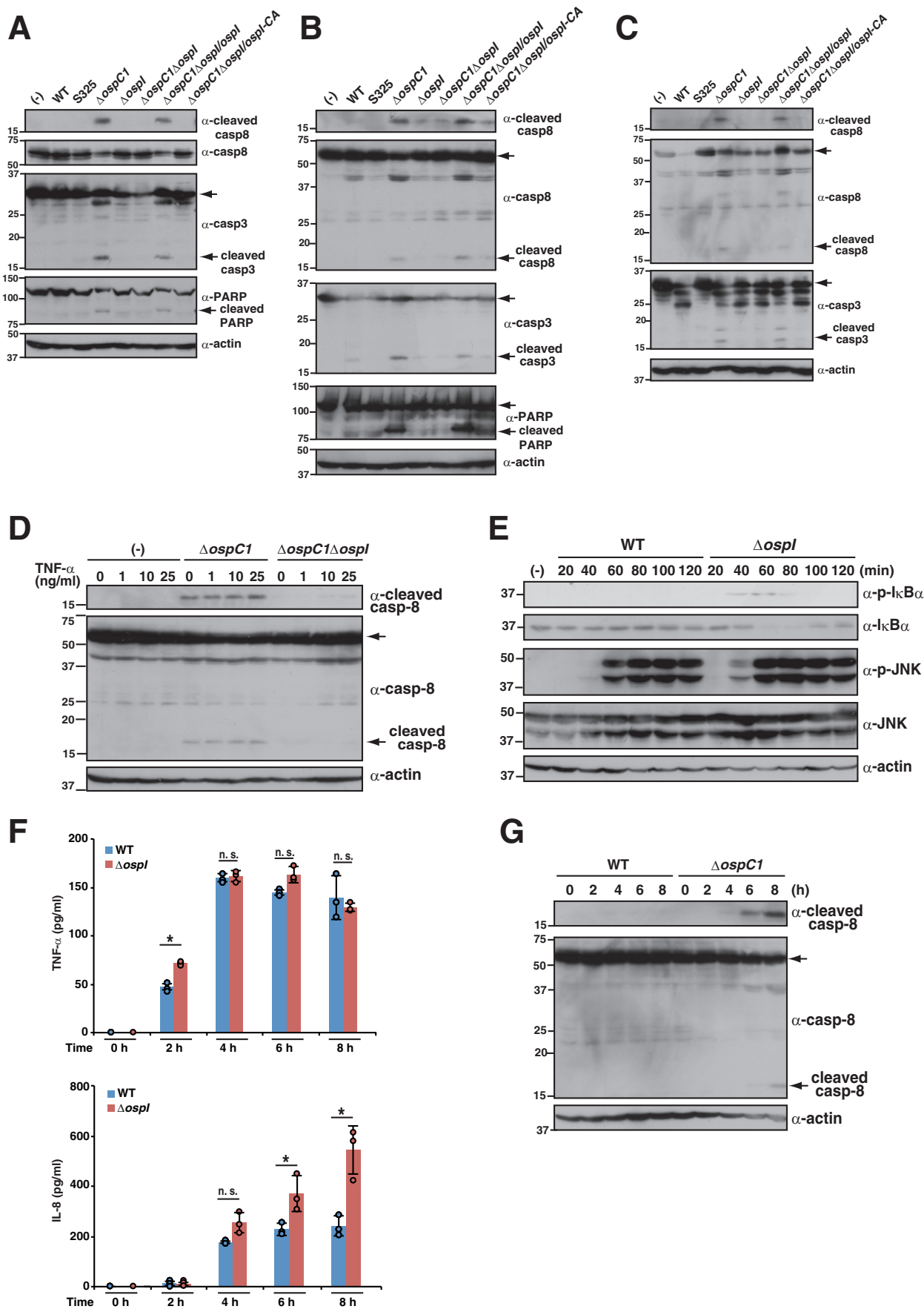


Expanded View Figures

Figure EV1. The *Shigella* effector Ospl triggers caspase-8 activation.

(A, B) HeLa cells stably expressing Ospl or Ospl-C62A were stimulated with TNF- α (25 ng/mL), IL-1 β (50 ng/mL), or PMA (50 μ g/mL) and incubated for 8 h. Caspase-8 activity (A) or caspase-3 activity (B) was measured and is reported as relative light units (RLU) of stimulated samples normalized to the value in unstimulated samples. (A) Data are expressed as the mean $\pm$ SD from quadruplicate and representative of three independent experiments (P values: $P < 0.0001$ (left) and $P < 0.0001$ (right); two-way ANOVA). (B) Data are expressed as the mean $\pm$ SD from triplicate and representative of three independent experiments (P values: $P < 0.0001$ (left) and $P < 0.0001$ (right); two-way ANOVA). (C) HeLa cells stably expressing Ospl or Ospl-C62A were stimulated with TNF- α (25 ng/mL). At the indicated time points, cells were harvested and cell lysates were subjected to immunoblotting. Data are representative of three independent experiments. (D) HeLa cells stably expressing Ospl or Ospl-C62A were stimulated with TNF- α (10 ng/mL) or PMA (50 μ g/mL) and incubated for 1 h. The expression level of the *IL8* gene was determined by real-time PCR. Relative expression change in gene expression was calculated using unstimulated cells as the control. Data are expressed as the mean $\pm$ SD from three independent experiments ($P = 0.8172$ for TNF- α and $P < 0.0001$ for PMA; two-way ANOVA). Data are considered significant when $P < 0.05$, with * $P < 0.05$ or n.s., not significant (A, B, D).





◀ **Figure EV2. The *Shigella* effector Ospl triggers caspase-8 activation.**

(A–C) HeLa (A), HCT116 (B), or T84 (C) cells were infected with the indicated *Shigella* strains and incubated for 8 h (A), 6 h (B), or 12 h (C). Cell lysates were subjected to immunoblotting. (D) HT-29 cells were infected with the indicated *Shigella* strains in the presence or absence of TNF- α and incubated for 8 h. Cell lysates were then subjected to immunoblotting. (E–G) HT-29 cells were infected with the indicated *Shigella* strains. Cell lysates (E, G) or culture supernatants (F) obtained at the indicated time points were subjected to immunoblotting (E, G) or ELISA (F). (F) Data are expressed as the mean $\pm$ SD from triplicate and representative of three independent experiments (TNF- α : $P = 0.0384$ for 2 h, $P > 0.9999$ for 4 h, $P = 0.2229$ for 6 h, and $P = 0.8763$ for 8 h. IL-8: $P = 0.4407$ for 4 h, $P = 0.0129$ for 6 h, and $P < 0.0001$ for 8 h; two-way ANOVA). Data are representative of three independent experiments (A–E, G). Molecular weights in immunoblots are in kDa. Data are considered significant when $P < 0.05$, with * $P < 0.05$ or n.s., not significant (F).

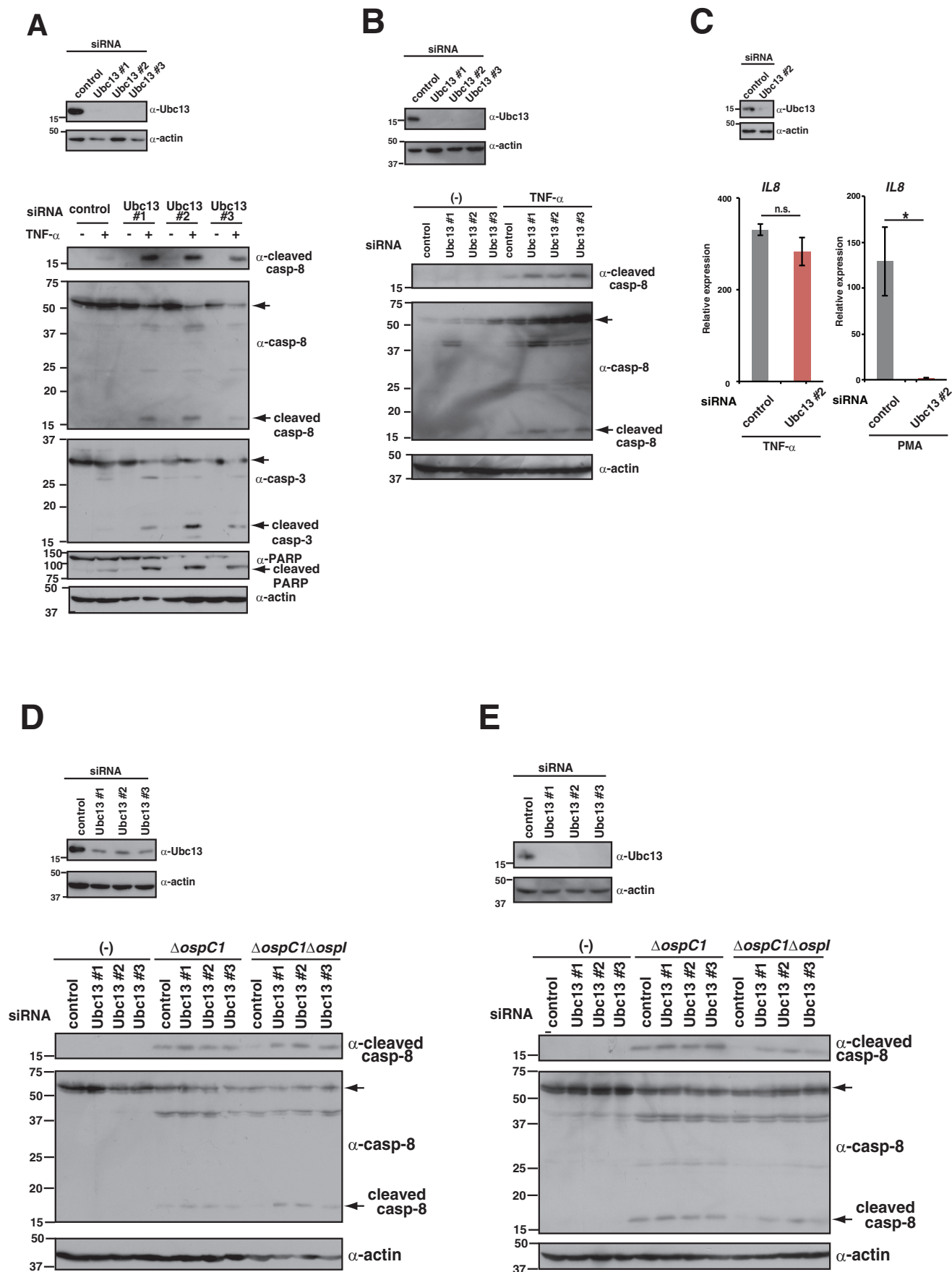
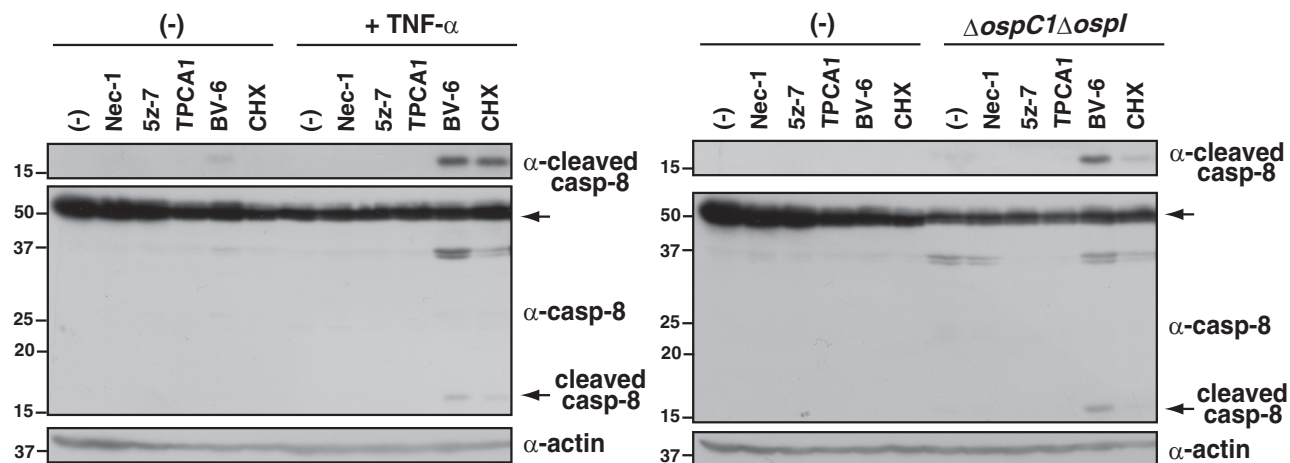
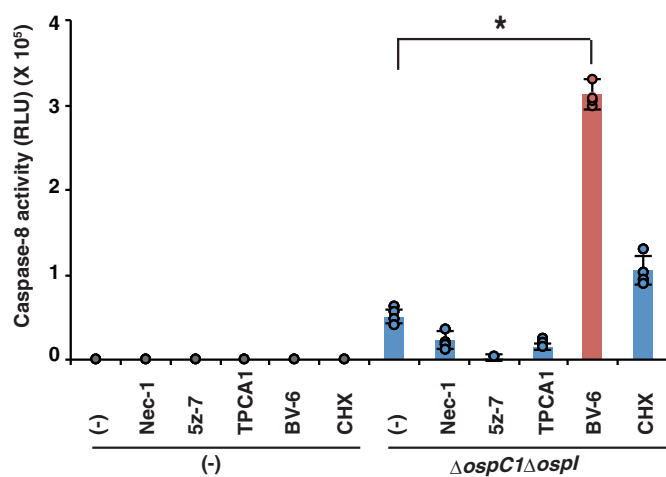
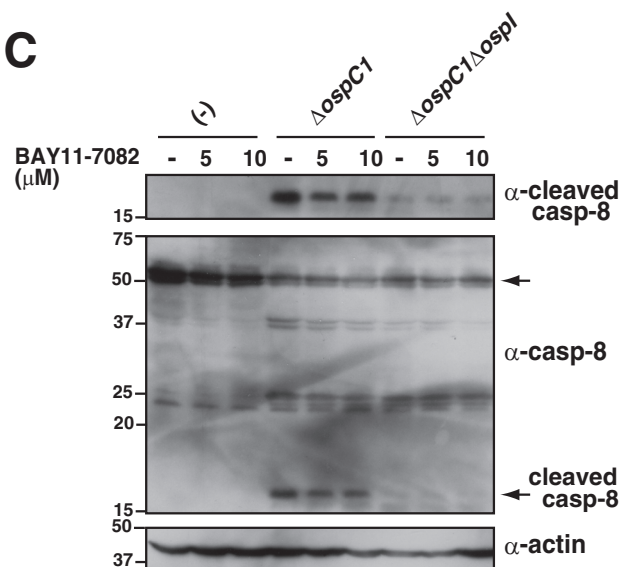
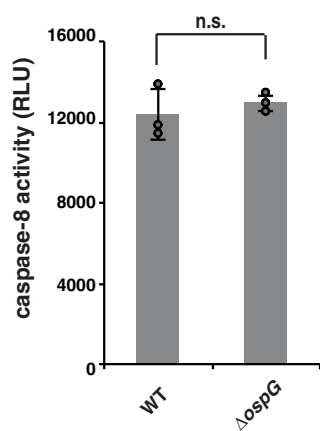
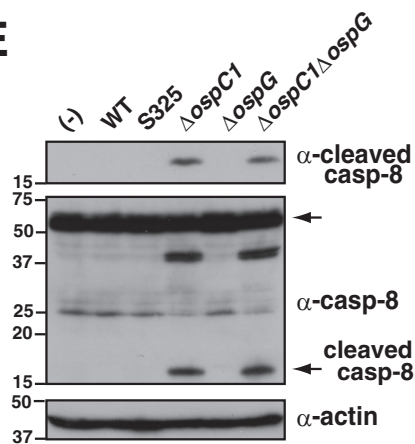


Figure EV3. Ubc13 inactivation triggers caspase-8 activation under TNF- α stimulation.

(A, B) HeLa (A) or HCT116 (B) cells treated with control or Ubc13 siRNAs were stimulated with TNF- α (25 ng/mL) and incubated for 8 h. Cell lysates were subsequently subjected to immunoblotting. The knockdown efficiency of the indicated siRNAs was assessed by immunoblotting (inset). (C) HeLa cells treated with control or Ubc13 siRNAs were stimulated with TNF- α (10 ng/mL) or PMA (50 μ g/mL) and incubated for 1 h. The expression level of the *IL8* gene was determined by real-time PCR. Relative expression change in gene expression was calculated using unstimulated cells as the control. Data are expressed as the mean $\pm$ SD from three independent experiments ($P = 0.2176$ for TNF- α and $P = 0.0278$ for PMA; two-tailed Student's *t* test). The knockdown efficiency of the indicated siRNAs was assessed by immunoblotting (inset). (D, E) HeLa (D) or HCT116 (E) cells treated with control or Ubc13 siRNAs were infected with the indicated *Shigella* strains and incubated for 8 h. Cell lysates were subjected to immunoblotting. The knockdown efficiency of the indicated siRNAs was assessed by immunoblotting (inset). Data are representative of three independent experiments (A, B, D, E). Molecular weights in immunoblots are in kDa. Data are considered significant when $P < 0.05$, with * $P < 0.05$ or n.s., not significant (C).

A**B****C****D****E**

◀ **Figure EV4. *Shigella* effector OspI-mediated IAP inactivation triggers caspase-8 activation.**

(A, B) HT-29 cells were stimulated with TNF- α (25 ng/mL) or infected with *Shigella* Δ ospC1 Δ ospI in the presence or absence of Nec-1 (50 μ M), 5z-7 (0.5 μ M), TPCA1 (5 μ M), BV-6 (1 μ M), or CHX (25 μ g/mL) and incubated for 8 h. Cell lysates were subjected to immunoblotting (A) or measurement of caspase-8 activity (B). Caspase-8 activity is reported in terms of relative light units (RLUs) of infected samples normalized to the values in uninfected samples. Data are expressed as the mean $\pm$ SD from quadruplicate and representative of three independent experiments (P value: $P < 0.0001$; two-way ANOVA). (C) HT-29 cells were infected with the indicated *Shigella* strains in the presence or absence of a BAY11-7082 inhibitor (5 or 10 μ M) and incubated for 8 h. Cell lysates were subjected to immunoblotting. (D) HT-29 cells were infected with the indicated *Shigella* strains and incubated for 8 h. Cells were then harvested and caspase-8 activity was measured. Caspase-8 activity is reported as RLUs of infected samples normalized to the values in uninfected samples. Data are expressed as the mean $\pm$ SD from triplicate and representative of three independent experiments ($P = 0.52$; two-tailed Student's t test). (E) HT-29 cells were infected with indicated *Shigella* strains and incubated for 8 h. Cell lysates were subjected to immunoblotting. Data are representative of three independent experiments (A, C, E). Molecular weights in immunoblots are in kDa. Data are considered significant when $P < 0.05$, with * $P < 0.05$ or n.s., not significant (B, D).

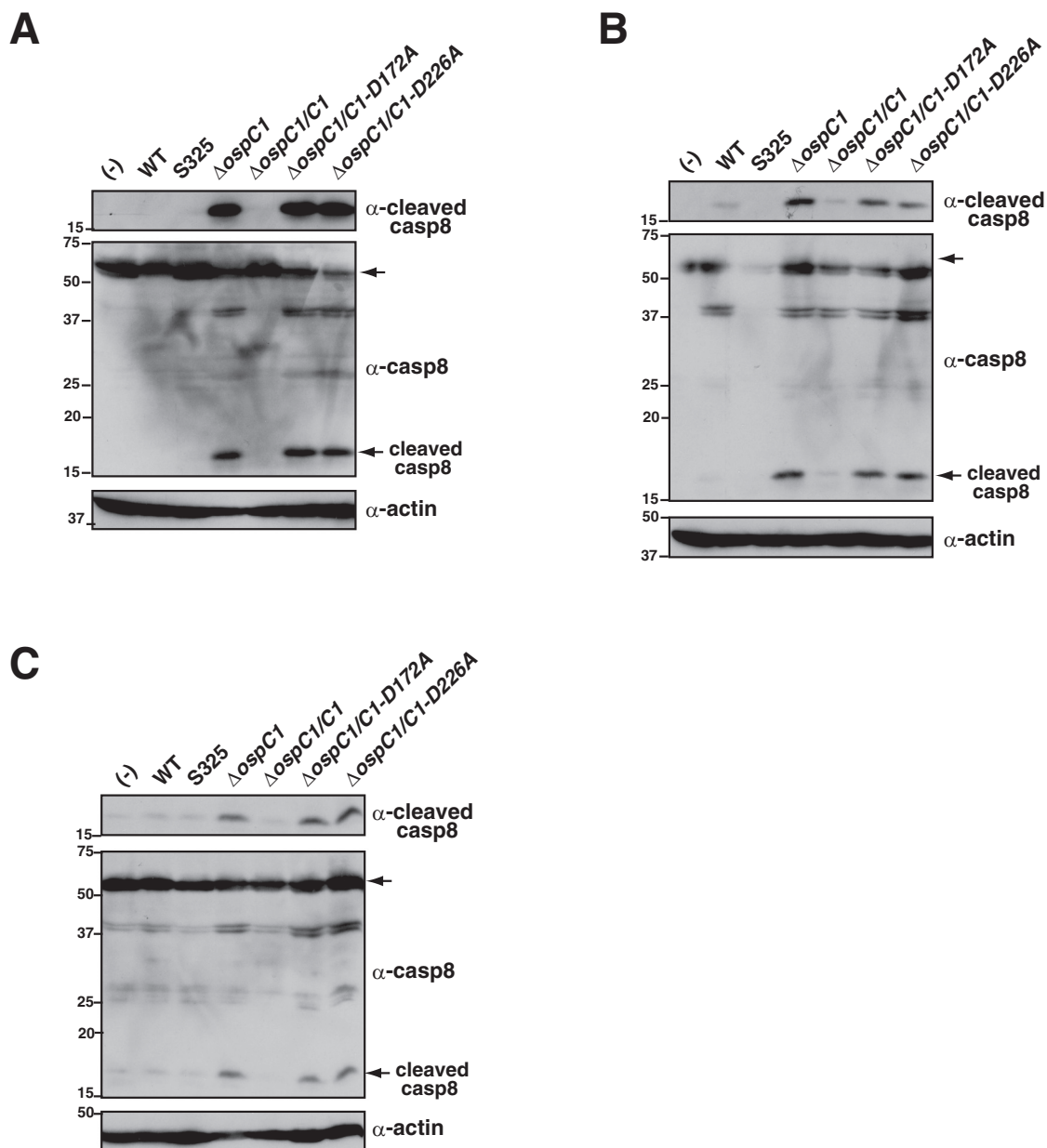


Figure EV5. The ADP-ribosylation activity of OspC1 is required for caspase-8 inhibition.

(A–C) HeLa (A), HCT116 (B) or T84 (C) cells were infected with *Shigella* WT, S325, Δ ospC1, Δ ospC1/ospC1 (Δ ospC1 complemented with ospC1), Δ ospC1/ospC1-D172A (Δ ospC1 complemented with ospC1-D172A), or Δ ospC1/ospC1-D226A (Δ ospC1 complemented with ospC1-D226A) strains and incubated for 6 h (A, B) or 12 h (C). Cell lysates were subjected to immunoblotting. All data are representative of three independent experiments. Molecular weights in immunoblots are in kDa.

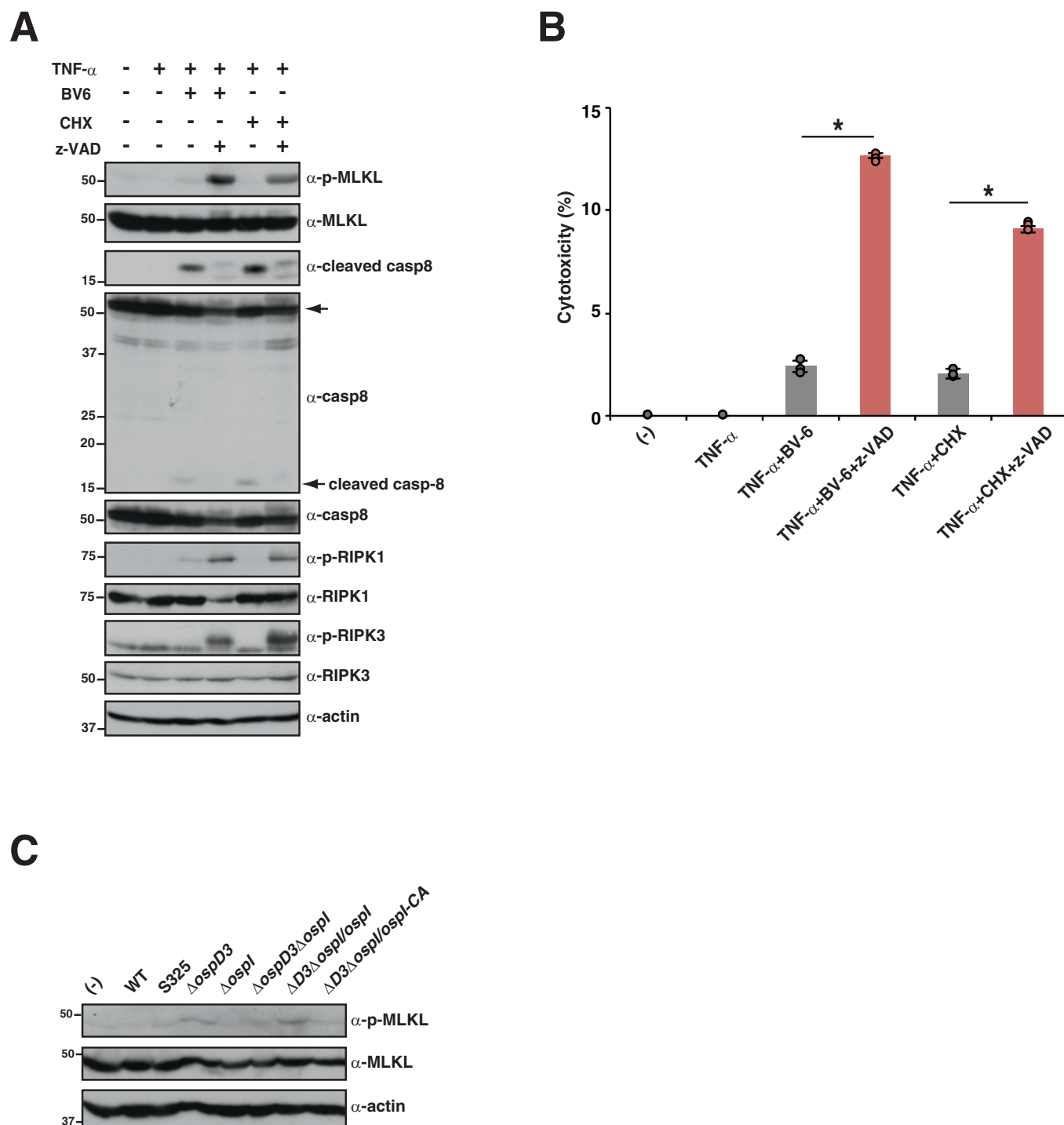


Figure EV6. Caspase-8 is the molecular switch for apoptosis and necroptosis.

(A, B) HT-29 cells were treated with TNF- α (25 ng/mL) plus the indicated inhibitors (BV-6, 1 μ M; CHX, 25 μ g/mL; Z-VAD-fmk, 10 μ M) and incubated for 8 h. Cell lysates and aliquots of cellular supernatants were subjected to immunoblotting (A) or cytotoxicity assays (B), respectively. (B) Data are expressed as the mean $\pm$ SD from triplicate and representative of three independent experiments (P values: $P < 0.0001$ (left) and $P < 0.0001$ (right); two-way ANOVA). (C) HT-55 cells were infected with the indicated *Shigella* strains and incubated for 12 h before cell lysates were subjected to immunoblotting. Data are representative of three independent experiments (A, C). Molecular weights in immunoblots are in kDa. Data are considered significant when $P < 0.05$, with * $P < 0.05$ (B).