

Supplemental Legends:

Figure S1. YopE does not affect caspase-1 activation in response to *Yersinia* infection and Caspase-1 activation occurs in response *Yersinia* infection in NLRP3- or NLRC4-deficient cells. (A) C57BL/6 bone marrow macrophages were infected with isogenic WT, $\Delta yopE$, $\Delta yopJ$, and $\Delta yopEJ$ strains, and lysates harvested at indicated times post-infection. Cell lysates were probed with anti-caspase-1 antibodies to assay for presence of cleaved p10 subunit. *Salmonella typhimurium* (Stm) and isogenic Stm $\Delta sipB$ serve as positive and negative controls, respectively. The presence or absence of YopE does not impact the extent of caspase-1 activation in response to *Y. pseudotuberculosis* infection. (B) BMMs from *Nlrc4*^{-/-} (left panel) or *Nlrp3*^{-/-} (right panel) mice were infected with WT, $\Delta yopEHJ$, or T3SS *Yersinia* strains, or with *S. typhimurium* (Stm) or treated with LPS+Nigericin (Ng) as indicated. Cell lysates were harvested at indicated times post-infection and probed for caspase-1 activation (p10).

Figure S2. Bacterial flagellin is not responsible for caspase-1 activation in response to the *Yersinia* T3SS. C57BL/6 macrophages were infected with isogenic bacterial strains of the T3SS strain (A) or $\Delta yopJK$ strain (B) in which the *fliC* locus, which encodes bacterial flagellin was either ablated by in-frame deletion ($\Delta fliC$) or left intact, as indicated. Whole cell lysates were harvested at 0 and 120 minutes post-infection, as indicated. (C) BMMs from B6 or *Nlrc4*^{-/-} mice were also infected with $\Delta yopJK$ or $\Delta yopJK fliC$ strains, or $\Delta yopJ$ strain as negative control, and analyzed for caspase-1 cleavage.

Figure S3. YopK prevents macrophage production of caspase-1 dependent cytokines and *Yersinia* flagellin does not contribute to IL-1 β production (A) Macrophages from B6, *Asc*^{-/-} and *Nlrp3*^{-/-} mice were treated with 50 ng/ml LPS for 3 hours, infected with indicated bacterial strains, supernatants were harvested 2 hours post-infection and analyzed by ELISA for production of IL-1 β and IL-6 or (B) IL-18. (C) Macrophages from B6, *Nlrp3*^{-/-}, and *Nlrc4*^{-/-} mice were treated with 50 ng/ml LPS for 3 hours infected with indicated bacterial strains, supernatants were harvested 2 hours post-infection and analyzed by ELISA for production of IL-1 β and IL-6.

Figure S4. LPS pretreatment inhibits caspase-1 activation in response to wild-type *Yersinia* but not in response to the *Yersinia* T3SS. C57BL/6 BMMs were either pretreated with LPS for 2 hours prior to infection or left untreated, followed by infection with indicated bacterial strains. Whole cell lysates were harvested at 0, 60, and 120 minutes post-infection, as indicated, and analyzed by SDS-PAGE and western blotting for the caspase-1 p10 subunit.

Figure S5. YopK is translocated in a T3SS-dependent manner but does not prevent caspase-1 activation *in trans*. (A) BMMs from C57BL/6 mice were infected either singly at an MOI of 15 with T3SS strain containing vector control or a YopK expression plasmid, or co-infected at an MOI of 30 with both bacterial strains. At 60 and 120 minutes post-infection, whole-cell lysates were harvested and assayed for caspase-1 activation by western blotting. (B) BMMs were infected with T3SS or $\Delta yopB$ *Yersinia* strains expressing a YopK-GSK tagged protein (C) BMMs were infected with T3SS, T3SS harboring a YopK expression plasmid, and T3SS harboring an isogenic YopK-GSK tag expression plasmid. At indicated times post-infection, whole cell lysates were harvested and assayed for caspase-1 activation by western blotting for caspase-1 p10. (D) BMMs were infected with indicated *Yersinia* strains and harvested 120 minutes post-infection. Cell lysates were assayed for caspase-1 activation by western blotting.

Figure S6. Caspase-1 deficient mice are not generally more susceptible to bacterial infection and T3SS vector and T3SS pYopK initially colonize spleen at equal levels. (A) Age-matched WT (white circles) or *Casp1*^{-/-} (gray circles) mice were infected intraperitoneally with 1×10^5 cfu of IP2666c *phoP* bacteria and bacterial loads in the spleen determined four days post-infection. (B) Age-matched WT (C57BL/6) mice were infected intraperitoneally with either T3SS vector control (black squares) or T3SS pYopK (black triangles) *Yersinia*. Spleen homogenates were prepared 24 hours post-infection and assayed for bacterial CFU/g spleen.

Supplemental Table 1. Bacterial strains and plasmids used in this work

Strain Genotype	Description	Source or Reference
IP2666 (WT)	Wild-type <i>Y. pseudotuberculosis</i> O:3 strain	(Black and Bliska, 1997)
pYV-	IP2666 cured of virulence plasmid	(Grabenstein et al., 2004)
<i>phoP</i>	pYV- <i>phoP</i> mutant	(Grabenstein et al., 2004)
<i>yopJ</i>	IP2666 <i>yopJ</i> mutant	(Lilo et al., 2008)
<i>yopEJ</i>	IP2666 <i>yopEJ</i> double mutant	(Lilo et al., 2008)
<i>yopEHJ</i>	IP2666 <i>yopEHJ</i> triple mutant	This work, (Viboud and Bliska, 2001)
<i>yopK</i>	IP2666 <i>yopK</i> mutant	This work, (Ryndak et al., 2005)
<i>yopJK</i>	IP2666 <i>yopJK</i> double mutant	This work
<i>yopJKB</i>	IP2666 <i>yopJKB</i> triple mutant	This work
mCD1	Modified CD1 virulence plasmid from <i>Y. pestis</i> lacking all known effectors but expressing functional type III secretion system	(Bartra et al., 2006)
T3SS	pYV- reconstituted with mCD1	This work
pProH (Vector)	pMMB67EH plasmid containing <i>yopH</i> promoter	(Black and Bliska, 1997)
pPYopK	pProH driving <i>YopK</i> expression	This work
T3SS pYopK	T3SS containing pPYopK	This work
T3SS pYopK-GSK	T3SS containing pPYopK-GSK-3 β tag fusion	This work
T3SS pYopK-FLAG	T3SS containing pProH- <i>YopK</i> -3X FLAG fusion	This work

Supplemental References:

Bartra, S.S., Jackson, M.W., Ross, J.A., and Plano, G.V. (2006). Calcium-regulated type III secretion of Yop proteins by an *Escherichia coli* hha mutant carrying a *Yersinia pestis* pCD1 virulence plasmid. *Infect Immun* 74, 1381-1386.

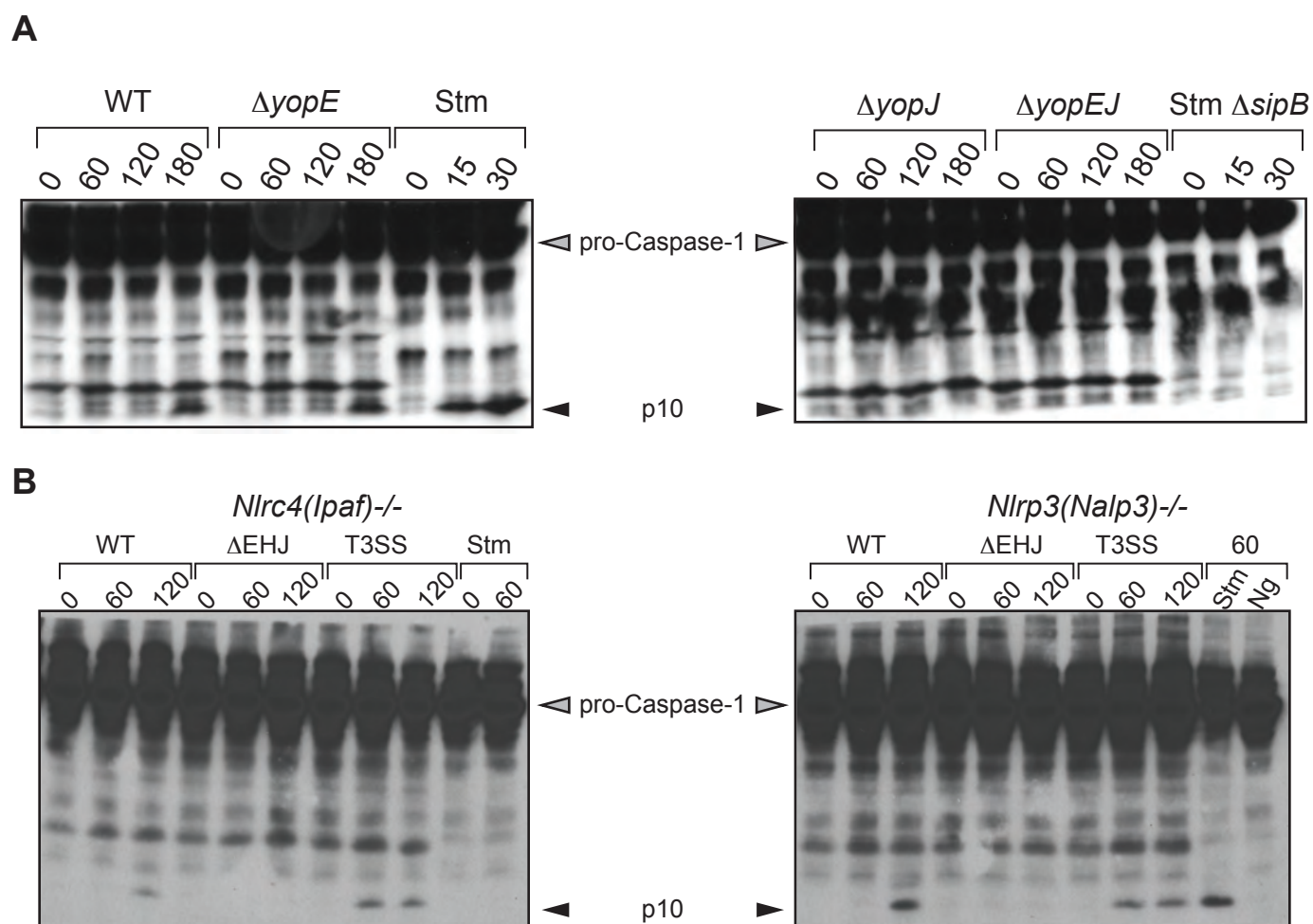
Black, D.S., and Bliska, J.B. (1997). Identification of p130Cas as a substrate of *Yersinia* YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J* 16, 2730-2744.

Grabenstein, J.P., Marceau, M., Pujol, C., Simonet, M., and Bliska, J.B. (2004). The response regulator PhoP of *Yersinia pseudotuberculosis* is important for replication in macrophages and for virulence. *Infect Immun* 72, 4973-4984.

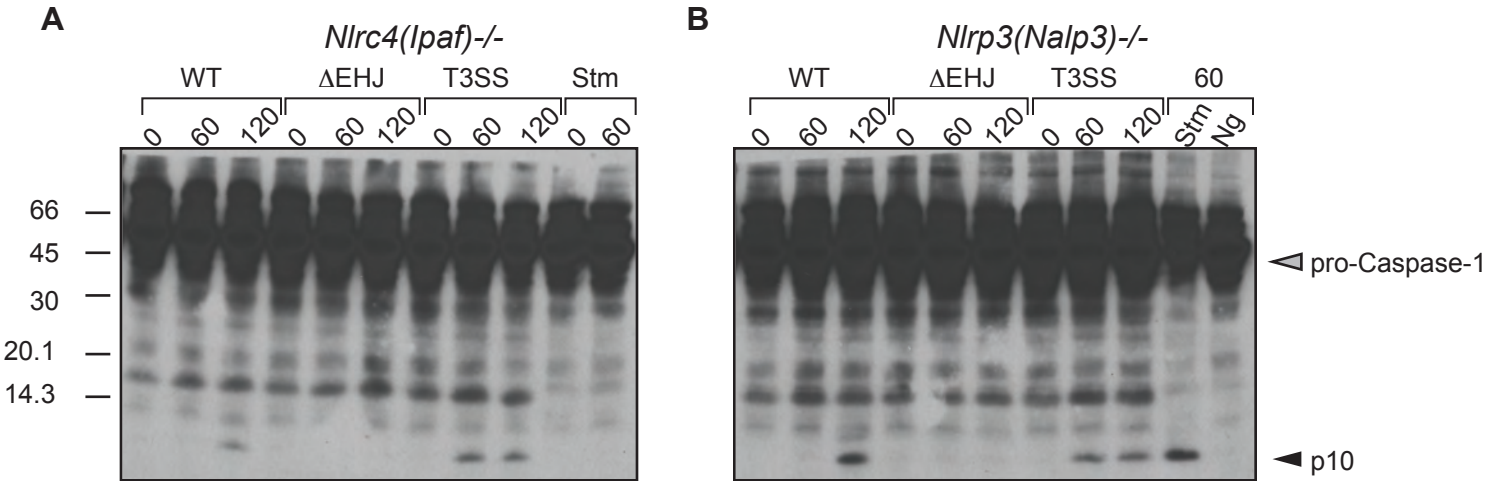
Lilo, S., Zheng, Y., and Bliska, J.B. (2008). Caspase-1 activation in macrophages infected with *Yersinia pestis* KIM requires the type III secretion system effector YopJ. *Infect Immun* 76, 3911-3923.

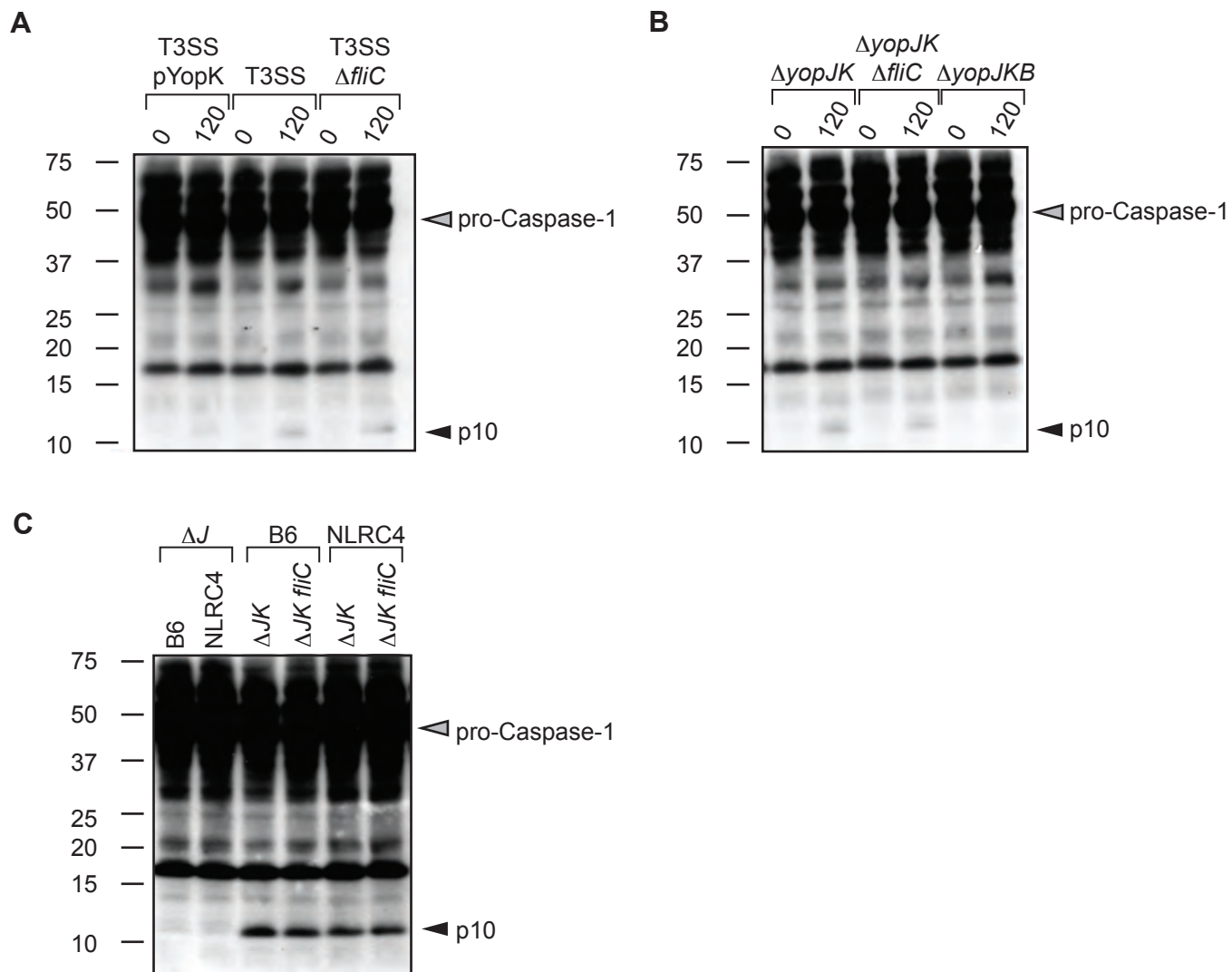
Ryndak, M.B., Chung, H., London, E., and Bliska, J.B. (2005). Role of predicted transmembrane domains for type III translocation, pore formation, and signaling by the *Yersinia pseudotuberculosis* YopB protein. *Infect Immun* 73, 2433-2443.

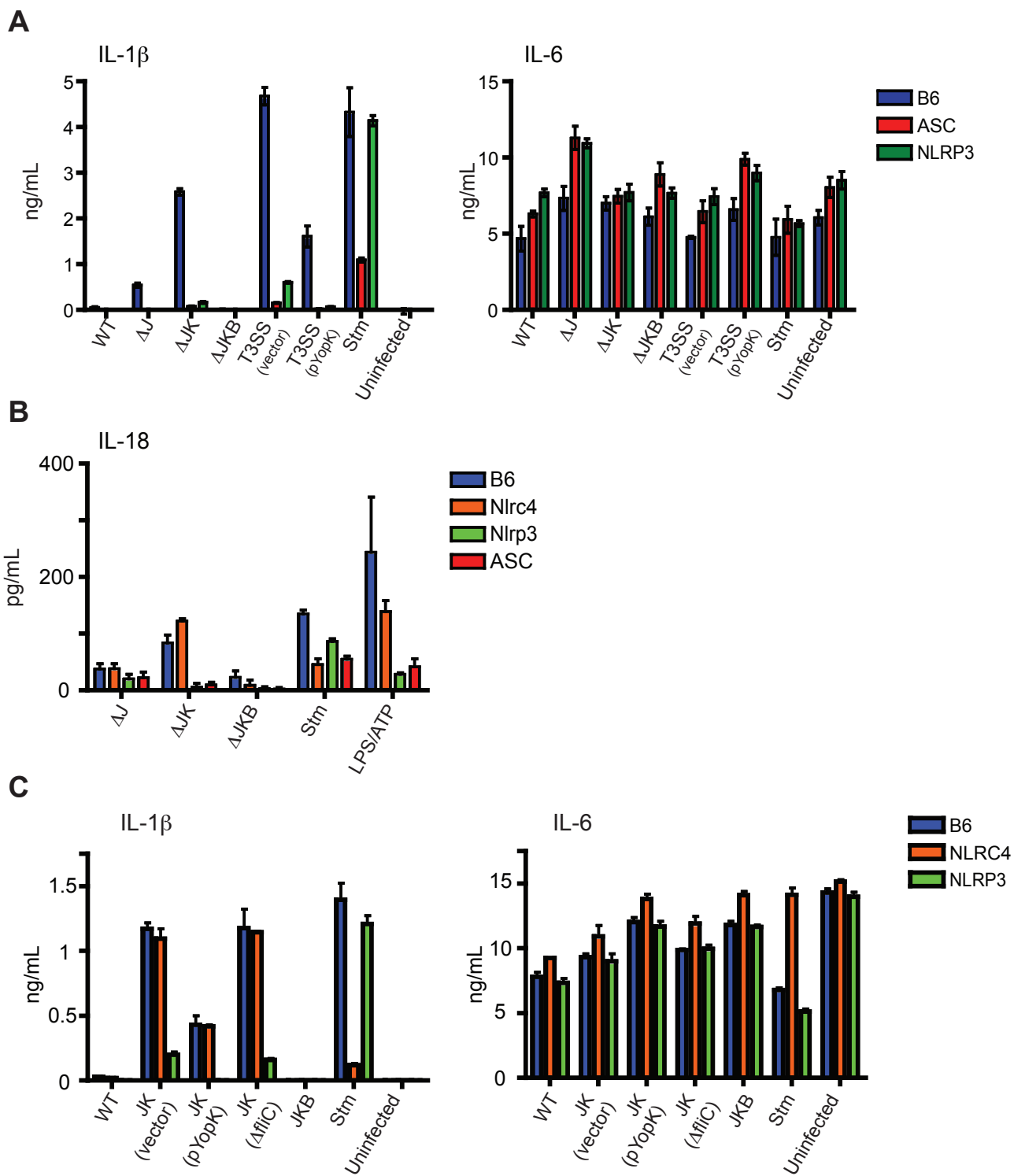
Viboud, G.I., and Bliska, J.B. (2001). A bacterial type III secretion system inhibits actin polymerization to prevent pore formation in host cell membranes. *Embo J* 20, 5373-5382.



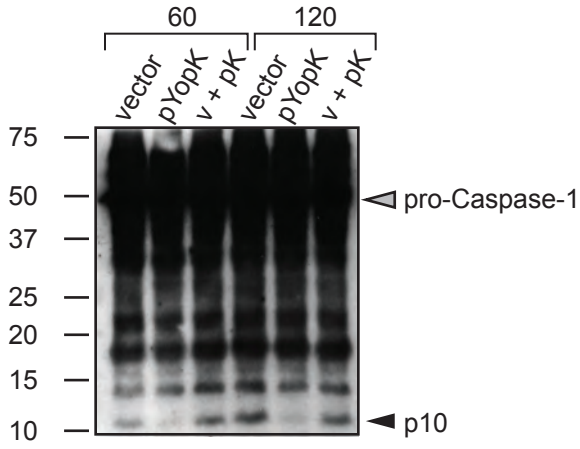
Brodsky et al. Supplemental Figure 2



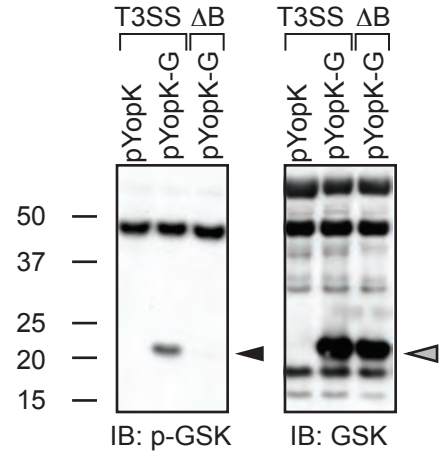




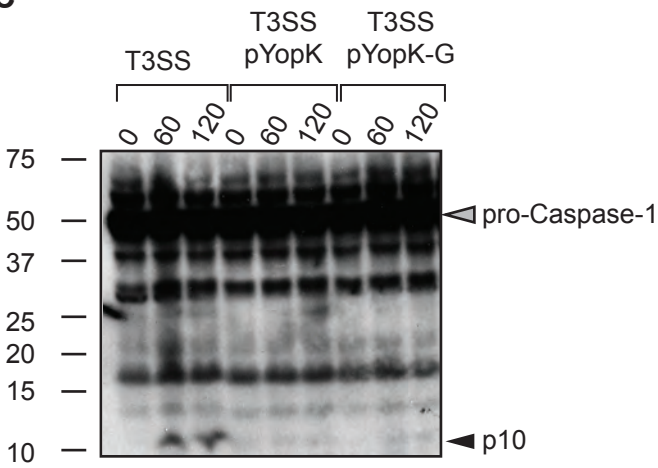
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B



C



D

