**Supplemental Figure Legends**

**Supplemental Figure 1: Role of the MyD88 and TRIF signaling pathways in NLRP3 activation in response to TLR ligands or Listeria infection.**

A-E, graphs showing the densitometric values (arbitrary units) of caspase-1 p20 bands in Figure 1A-E.

*F*, *G***,** immunoblots of caspase-1 in the culture supernatants (*upper panels*) or cell lysates (*lower panels*) after infection of NLRP3-KO or N1-8 macrophages with Listeria (MOI 100) for different periods of time (*F*), or infection of the indicated mouse knockout macrophage with Listeria (MOI 100) for 60 min (*G*).

*H, I,* confocal images of stable NLRP3-KO cell lines expressing WT NLRP3-GFP (*H*) or Walker A/B mutant NLRP3-GFP (*I*), left untreated (Un) or infected with Listeria (Listeria) for 45 min as indicated. The green and blue signals represent NLRP3 and nuclear fluorescence, respectively. Bar: 10 $\mu$m.

*J*, immunoblots of caspase-1 in culture supernatants (Sup) or cell lysates (Lys) after stimulation of WT or Walker A/B mutant NLRP3-GFP-expressing macrophages with LPS plus ATP or infection with Listeria (MOI 100) for 45 min as indicated.

**Supplemental Figure 2: TLR signaling through the MyD88-IRAK4-IRAK1 pathway is required for rapid activation and oligomerization of NLRP3.**

*A*, immunoblots of caspase-1 and HMGB1 in the culture supernatants (Sup) or cell lysates (Lys) of mouse macrophages derived from (WT), MyD88-KO, TRIF-KO, IRAK4-KO, IRAK1-KO or TBK1-KO mice, stimulated simultaneously with LPS, Pam3CSK4 (Pam), or Poly I:C plus ATP for 45 min as indicated.

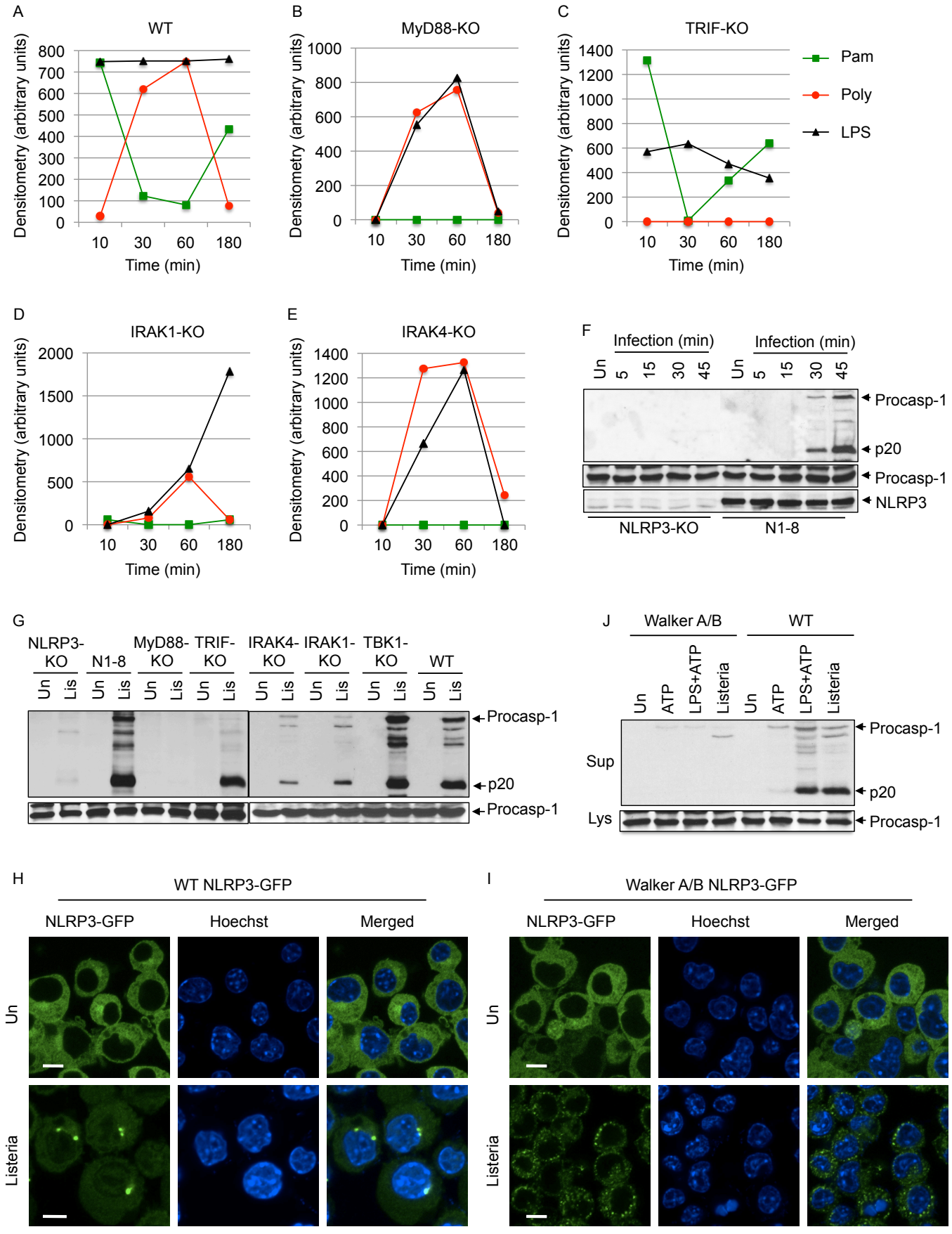
*B***,** confocal images of stable NLRP3-KO cell lines expressing WT NLRP3-GFP left untreated (Un) or treated with PamCSK4 (Pam, 45 min), Poly I:C (Poly, 90 min), PamCSK4 plus nigericin (Pam + Nig, 45 min), Poly I:C for 45 min followed by nigericin for an additional 45 min (Poly45 + Nig) or nigericin alone (Nig, 45 min) as indicated. The green and blue signals represent NLRP3 and nuclear fluorescence, respectively. Bar: 10 $\mu$m.

*C*, a graph showing the percentages of cells containing NLRP3 specks after treatments with PamCSK4 (Pam, 45 min), Poly I:C (Poly, 90 min), PamCSK4 plus nigericin (Pam + Nig, 45 min), PamCSK4 for 45 min followed by nigericin for an additional 45 min (Pam45 + Nig), Poly I:C plus nigericin (Poly + Nig, 45 min), Poly I:C for 45 min followed by nigericin for an additional 45 min (Poly45 + Nig) or nigericin alone (Nig, 45 min) as indicated.

*D*, a graph showing the densitometric values (arbitrary units) of caspase-1 p20 bands in Figure 3A.

E, immunoblots of NLRP3, pro-IL-1β and procaspase-1 in cell lysates of WT macrophages treated with LPS for the indicated periods of time in the absence or presence of actinomycin D (Act D, 0.5 μg/ml) as indicated. Note inhibition of NLRP3 and pro-IL-1β upregulation in cells treated with actinomycin D ($4^{th}$ and $5^{th}$ lanes).

## Supplemental Figure 1



## Supplemental Figure 2