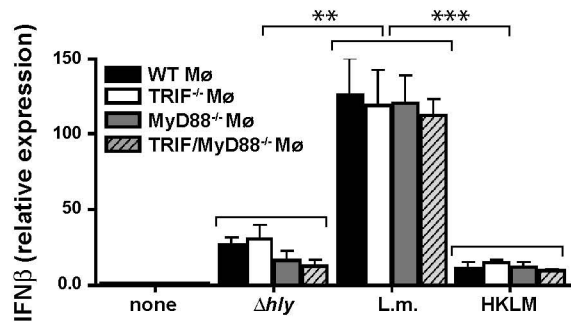
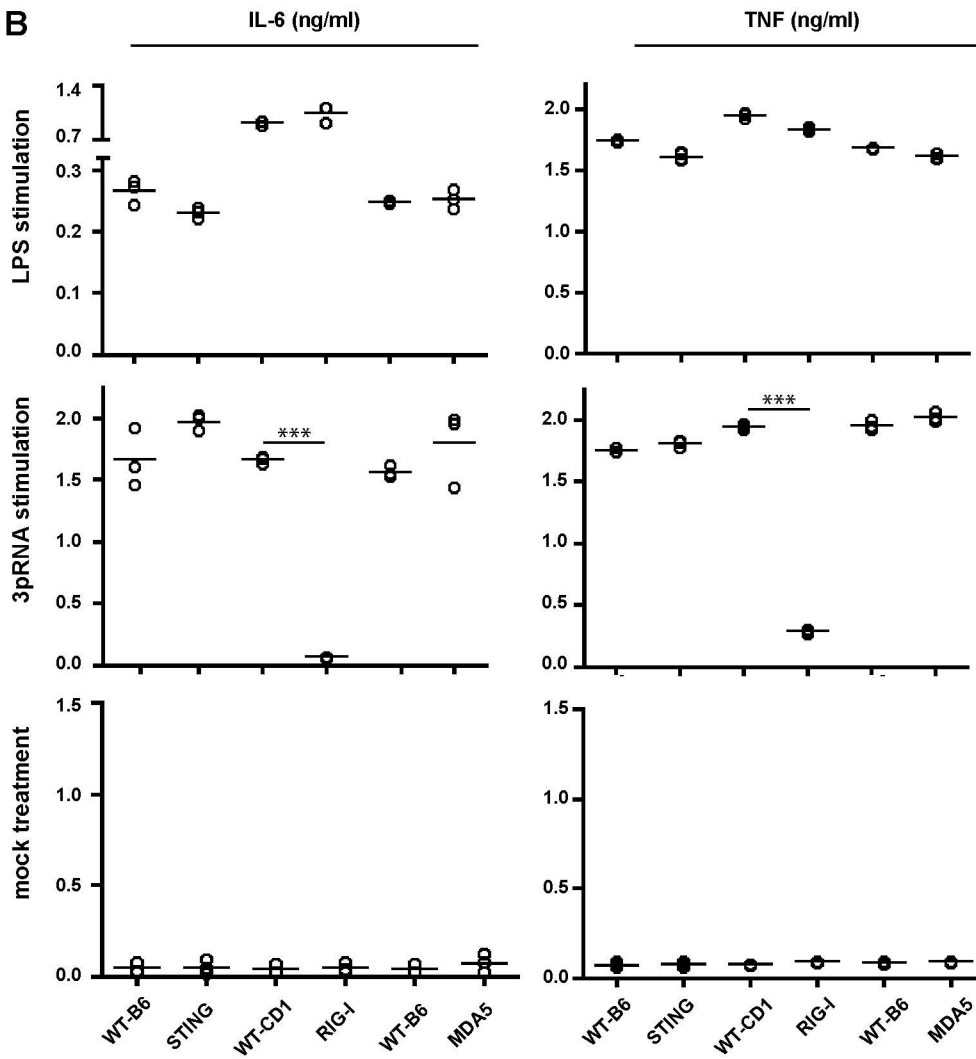


A



B



C

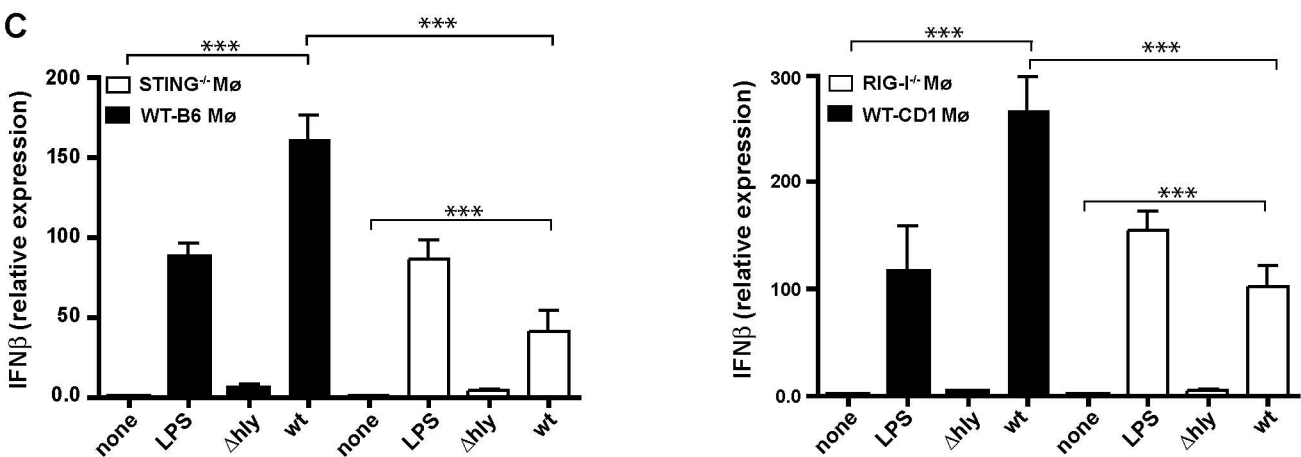
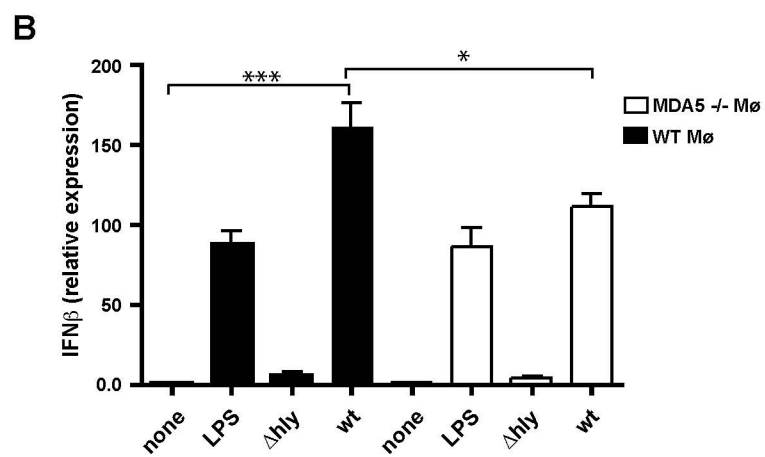
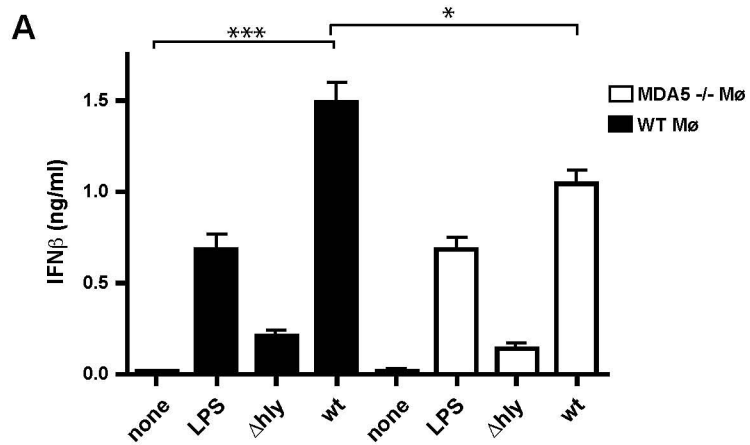
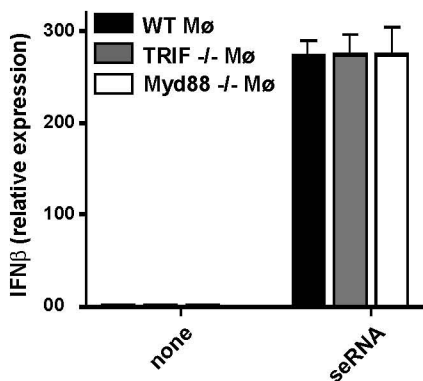


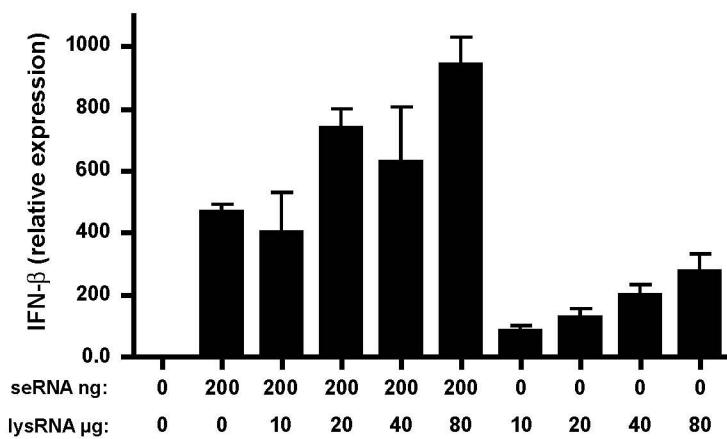
Figure S2



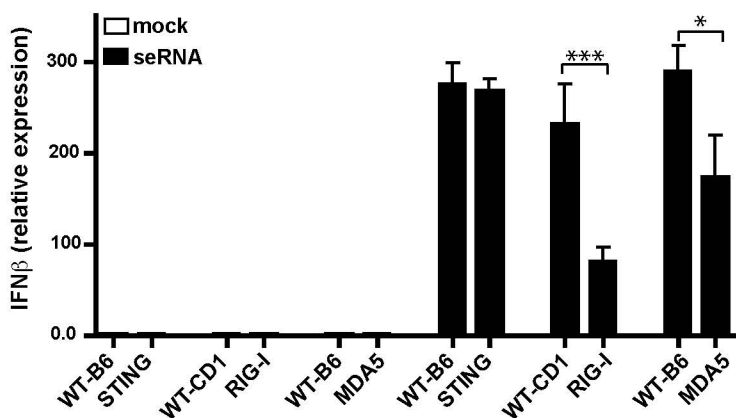
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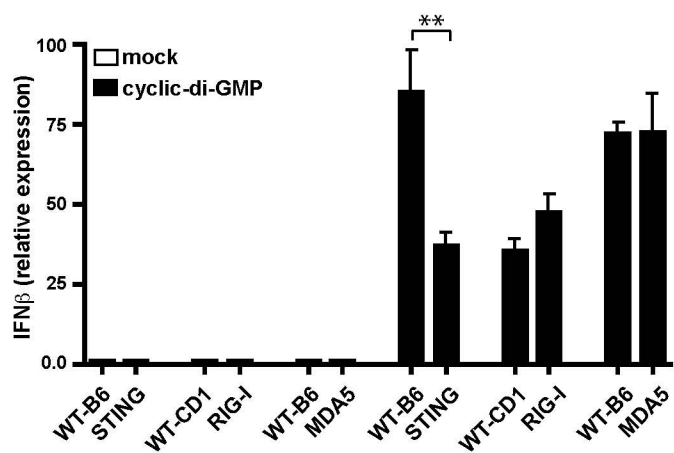
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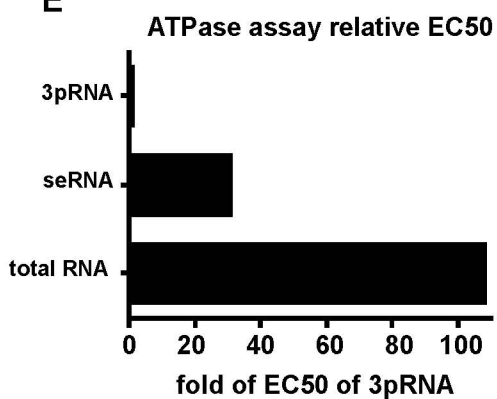
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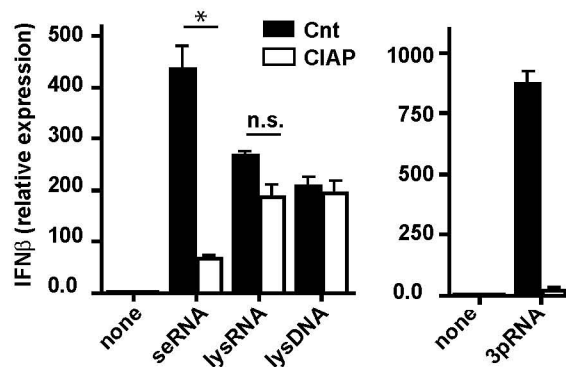
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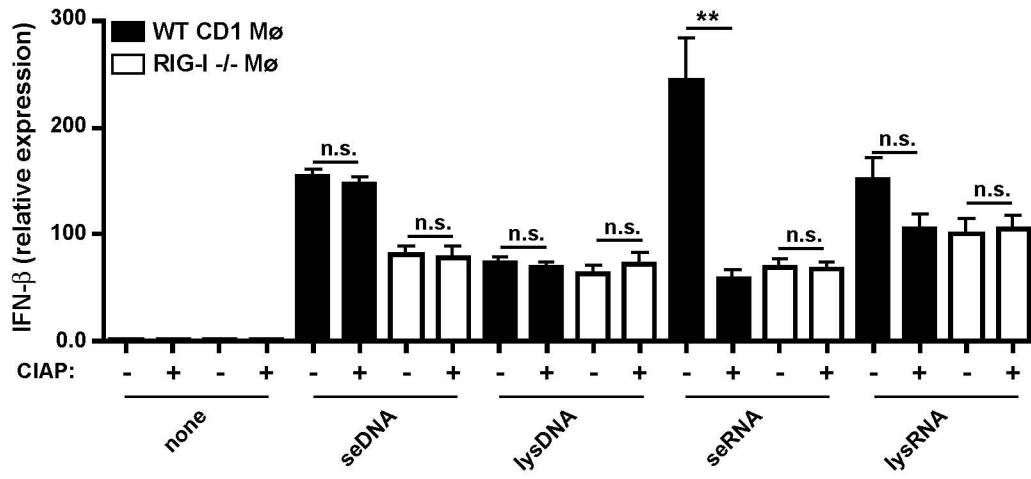
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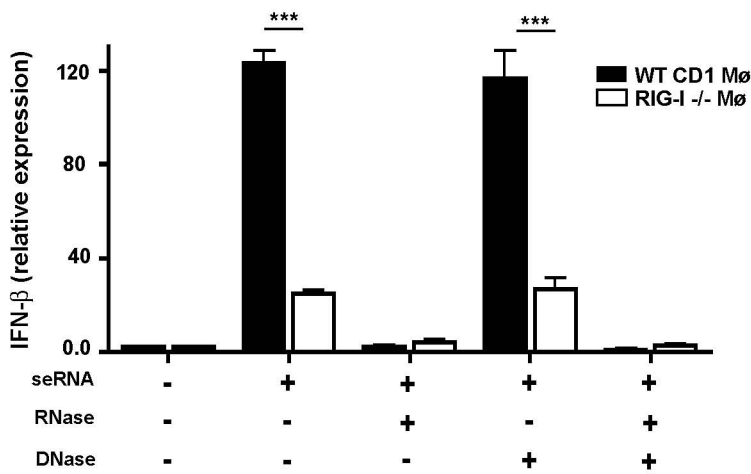
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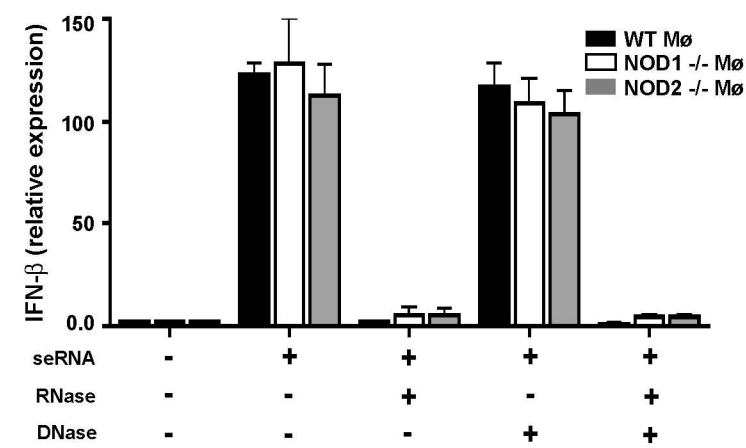
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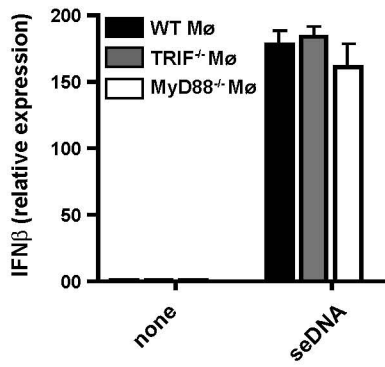
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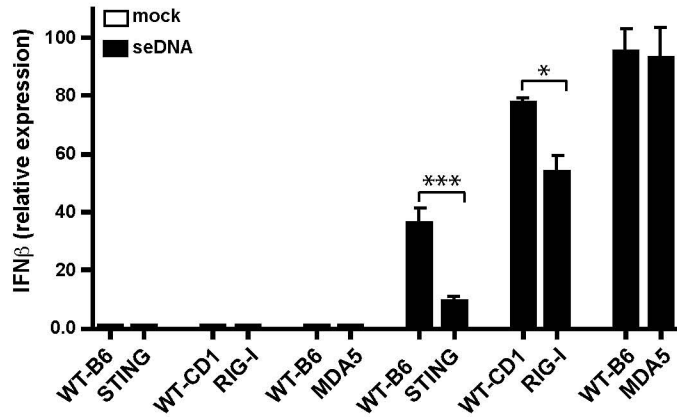
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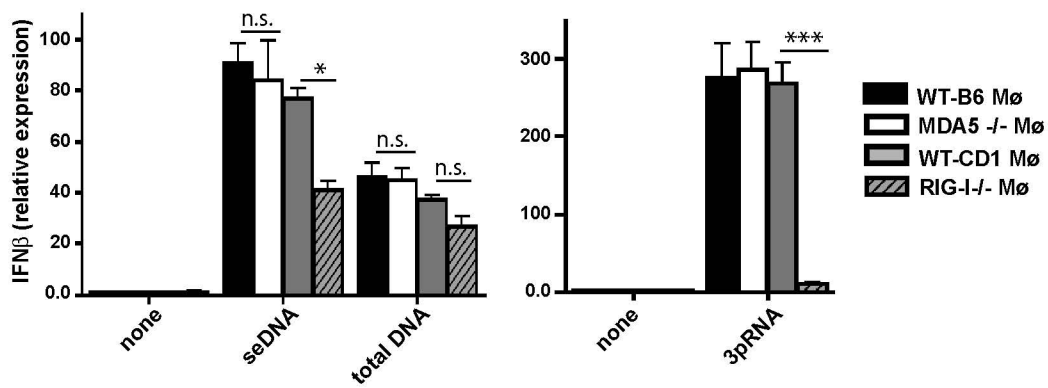
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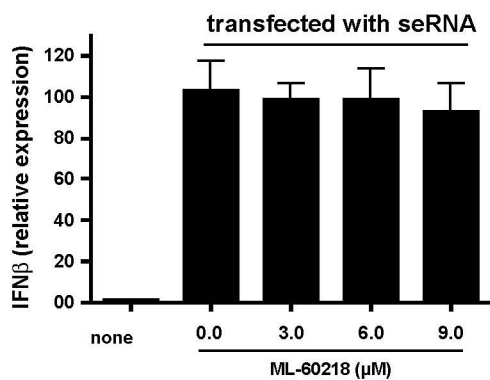
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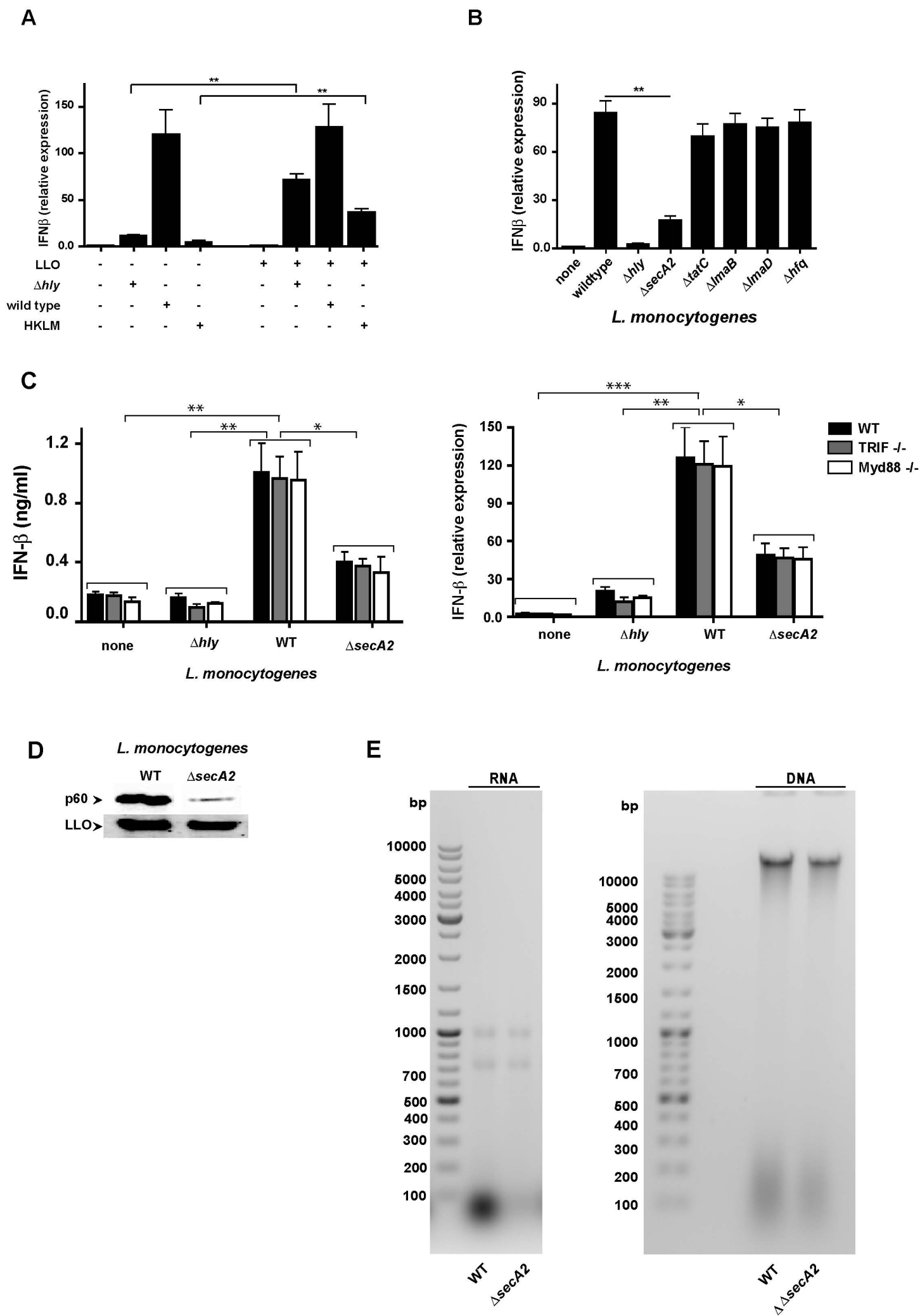


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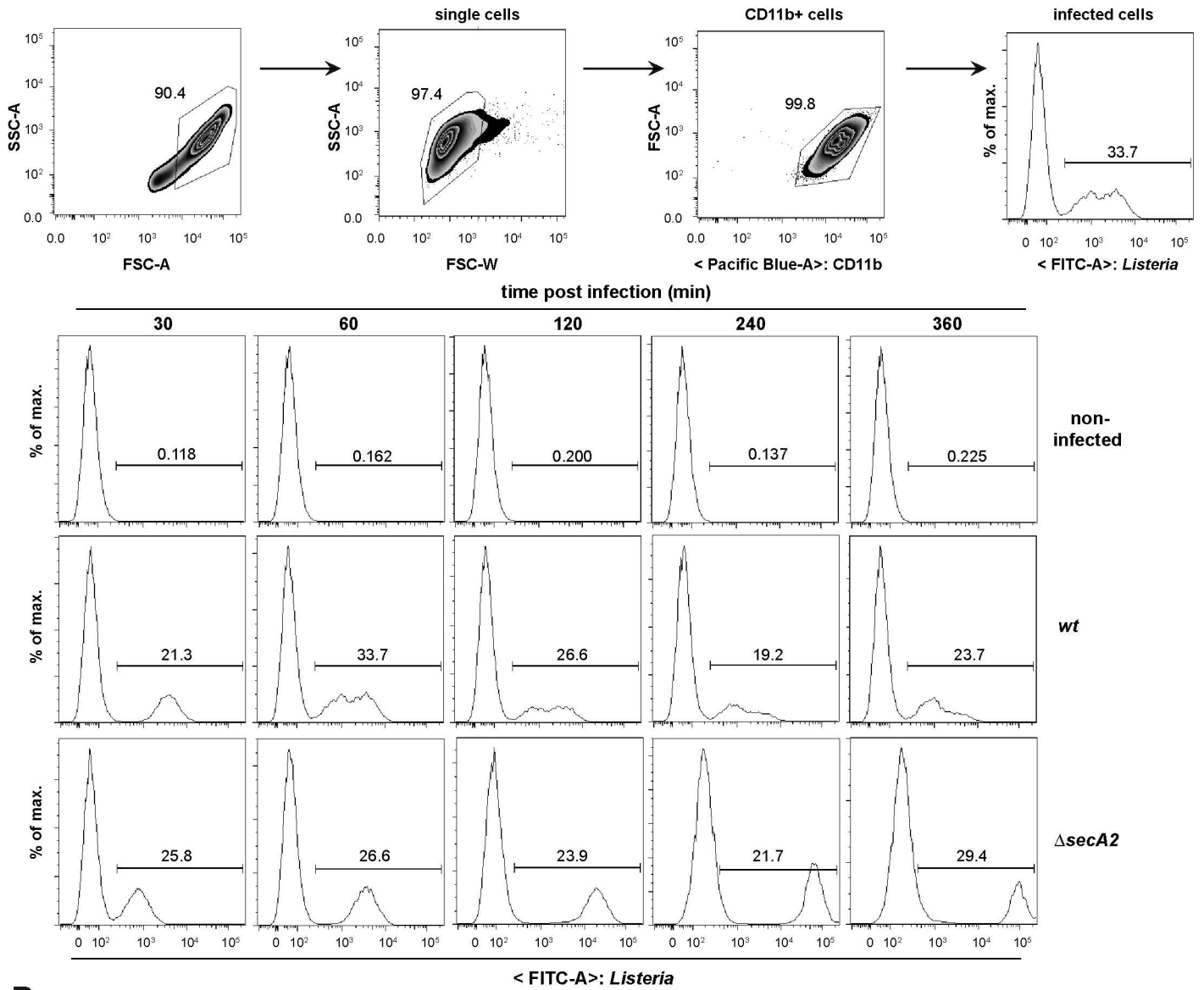


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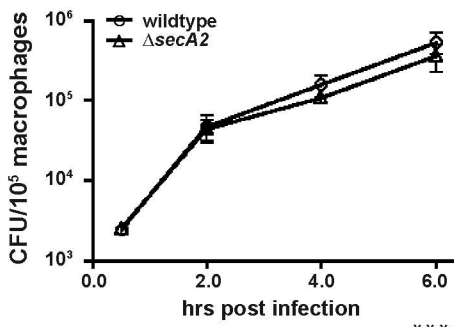




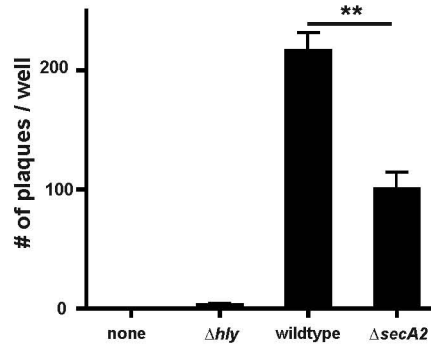
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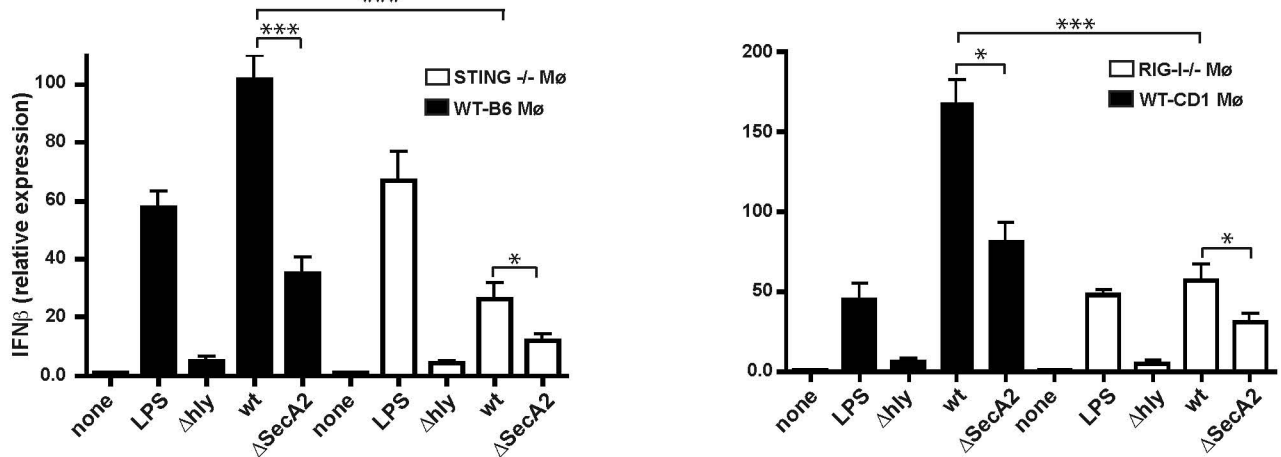
B



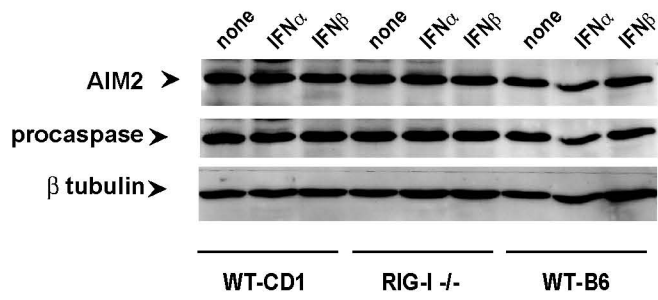
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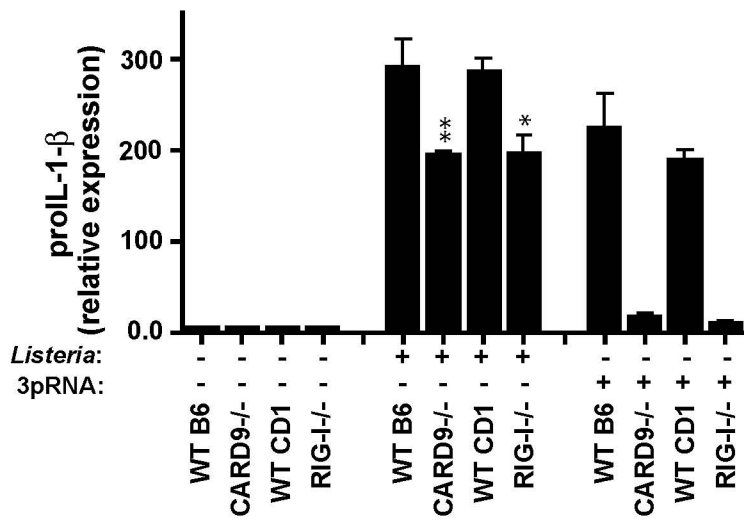
D



A



B



Legends to Supplementary Figure

Table S1: *Listeria* underwent exponential growth in BHI medium until they reached a concentration of 5.8×10^8 CFU/ml. Nucleic acids were extracted from cell free supernatant or from bacterial lysates and treated as described in methods. RNA extracts were treated with DNase and DNA extracts were treated with RNase. Photometric determination of RNA and DNA quantification was done with a Nanodrop instrument.

Figure S1: (A) IFN β mRNA assessed by qRT-PCR in wildtype, TRIF $^{-/-}$, MyD88 $^{-/-}$, TRIF / MyD88 $^{-/-}$ M ϕ infected for 6 hrs with wildtype *Listeria*, Δhly (MOI 10) or HKLM (MOI 100). (B) ELISA of IL-6 and TNF in cell culture supernatant of wildtype, RIG-I $^{-/-}$, STING $^{-/-}$, MDA5 $^{-/-}$ M ϕ 24 hrs after transfection with 3pRNA or stimulation with LPS (500 ng/ml). (C) IFN β mRNA in wildtype, STING $^{-/-}$ or RIG-I $^{-/-}$ M ϕ 6 hrs after infection with wildtype *Listeria*, $\Delta secA2$ or Δhly (MOI 10). LPS as positive control. Data are representative of at least three separate experiments (mean and s.d. of triplicates).

Figure S2: (D & E) ELISA of cell culture supernatant or IFN β mRNA in wildtype or MDA5 $^{-/-}$ M ϕ 18 hrs (D) or 6 hrs (E) after infection with wildtype *Listeria* or Δhly (MOI 10). LPS as positive control. ns, not significant. Data are representative of at least three separate experiments (mean and s.d. of triplicates).

Figure S3: (A-C) IFN β mRNA after transfection with seRNA (10ng/10 5 cells) or RNA from *Listeria* lysates (lysRNA) (1 μ g/10 5 cells) into (A) WT, TRIF $^{-/-}$ or MyD88 $^{-/-}$ M ϕ (B) WT (as co-transfection); (C) WT, STING $^{-/-}$, RIG-I $^{-/-}$ or MDA5 $^{-/-}$ M ϕ . (D) IFN β mRNA in WT, STING $^{-/-}$, RIG-I $^{-/-}$ or MDA5 $^{-/-}$ M ϕ after transfection with cyclic-di-GMP (200 ng/10 5 cells). (E) Fold increase of EC50 ATPase activity of seRNA or lysRNA compared to 3pRNA. Low EC50 represents high RIG-I ATPase activity. (F) IFN β mRNA after transfection of *Listeria* nucleic acids into WT M ϕ ; seRNA (10ng/10 5 cells), lysRNA (1 μ g/10 5 cells) or lysDNA (2 μ g/10 5 cells) treated with CIAP. Data are representative of at least three separate experiments.

Figure S4: (A) IFN β mRNA after transfection of *Listeria* nucleic acids into WT or RIG-I $^{-/-}$ M ϕ ; seDNA (20 ng/10 5 cells), lysDNA (2 μ g/10 5 cells), seRNA (10ng/10 5 cells) or lysRNA (1 μ g/10 5 cells) treated with CIAP. (B,C) IFN β mRNA after transfection of WT or RIG-I $^{-/-}$ M ϕ (A) or NOD1 $^{-/-}$ / NOD2 $^{-/-}$ M ϕ (B) with seRNA (10ng/10 5 cells) or seDNA (20 ng/10 5 cells) treated with

RNAse or DNase as indicated. ns, not significant. Data are representative of at least three separate experiments.

Figure S5: (A-C) IFN β mRNA in (A) WT, TRIF $^{-/-}$, MyD88 $^{-/-}$ M ϕ , (B) WT, STING $^{-/-}$, RIG-I $^{-/-}$, MDA5 $^{-/-}$ M ϕ or (C) WT, MDA5 $^{-/-}$ or RIG-I $^{-/-}$ M ϕ after transfection with seDNA (20 ng/10 5 cells) or lysDNA (2 μ g/10 5 cells) or 3pRNA as a control. (D) IFN β mRNA in WT M ϕ transfected with seRNA (10ng/10 5 cells) at increasing concentrations of the RNA polymerase III inhibitor (ML-60218). ns, not significant. Data are representative of at least three separate experiments.

Figure S6: (A-C) IFN β mRNA in (A) WT M ϕ after infection with wildtype *Listeria*, Δ hly (MOI 10) or heat-killed *Listeria monocytogenes* (HKLM, MOI 100) in the absence or presence of the pore-forming protein LLO, in (B) WT M ϕ after infection with wildtype or *Listeria* mutants (MOI=10) or (C) in TRIF $^{-/-}$, MyD88 $^{-/-}$ or WT M ϕ after infection with wildtype *Listeria*, Δ secA2 or Δ hly mutants (MOI 10). (D) Immunoblot of SecA2-dependent p60 and SecA2-independent listeriolysin (LLO) in protein extracts isolated from cell culture supernatant of wildtype *Listeria* or Δ secA2. (E) Visualization of secreted nucleic acids from wildtype *Listeria* or Δ secA2 isolated from the supernatant of the same number of bacteria.

Figure S7: (A) FACS analysis for WT M ϕ at indicated time points after infection with wildtype *Listeria* or Δ secA2 (upper panel shows the gating strategy) (B) Growth kinetic of wildtype *Listeria* or Δ secA2 mutant in infected macrophages, MOI=10. (C) Plaques formed in monolayers of L929 cells 72 hrs after infection with wildtype *Listeria* or Δ secA2. (D) IFN β mRNA in WT, STING $^{-/-}$, RIG-I $^{-/-}$ or MDA5 $^{-/-}$ M ϕ after infection with wildtype *Listeria*, Δ secA2 or Δ hly (MOI 10), LPS (500ng/ml) as positive control. *P=0.05, **P=0.01, ***P=0.001 (unpaired Student's t-test). Data are representative of at least three separate experiments (mean and s.d. of triplicates)

Figure S8: (A) Immunoblot of AIM2, procaspase 1 and β -tubulin as loading control in wildtype and RIG-I $^{-/-}$ macrophages 6hrs after treatment with 500 IU/ml of IFN α or IFN β . (B) Pro-IL1 β mRNA assessed by qRT-PCR in wildtype, CARD9 $^{-/-}$ and RIG-I $^{-/-}$ macrophages 6 hrs after infection with wildtype *Listeria* or 3pRNA.