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## Supplementary information

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# TRAM promotes Toll-like-receptor-free myddosome signal transduction

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## **TRAM promotes Toll-like receptor-free myddosome signal transduction**

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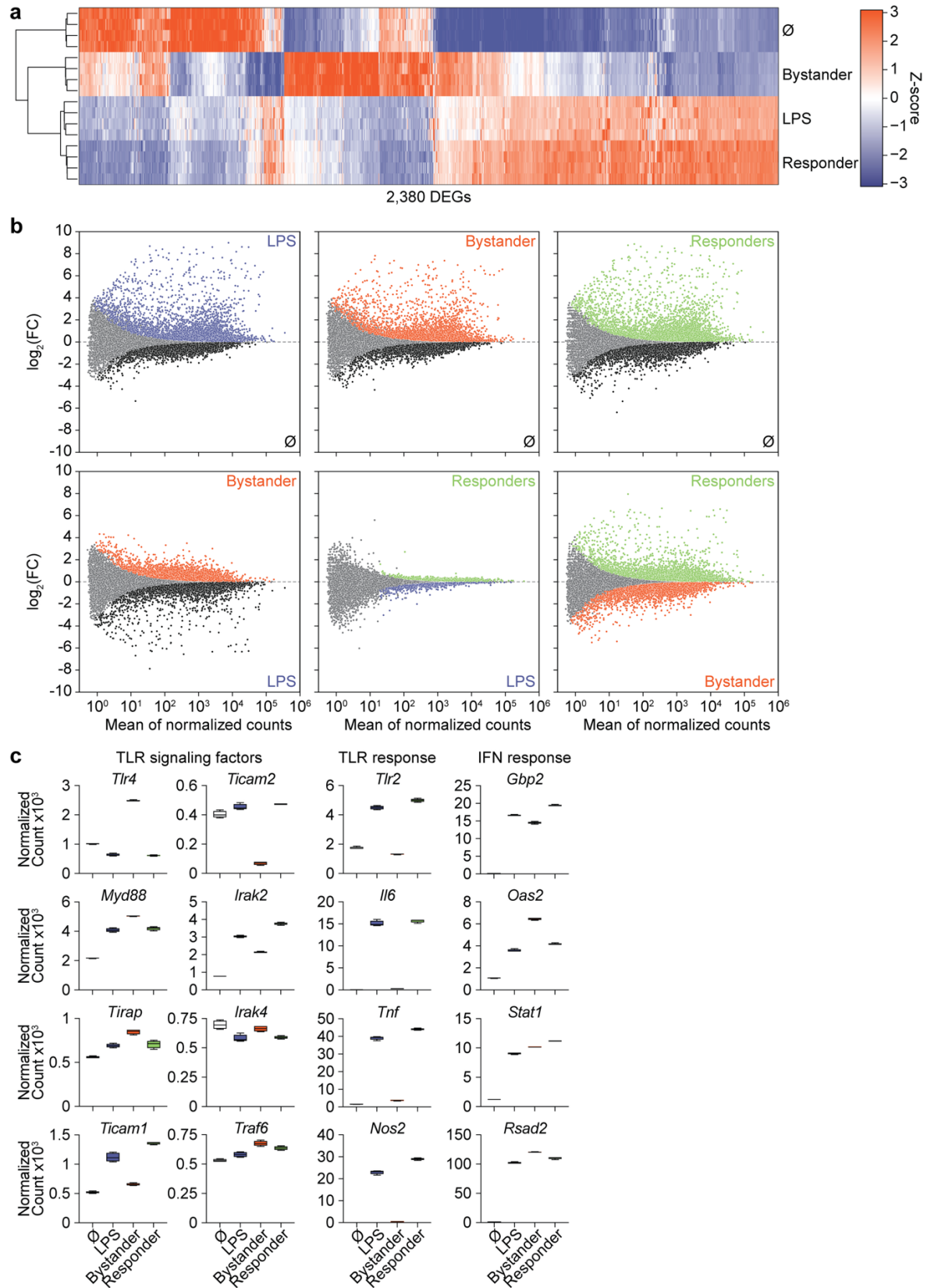
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## **CONTENTS**

Supplementary Figures 1-8

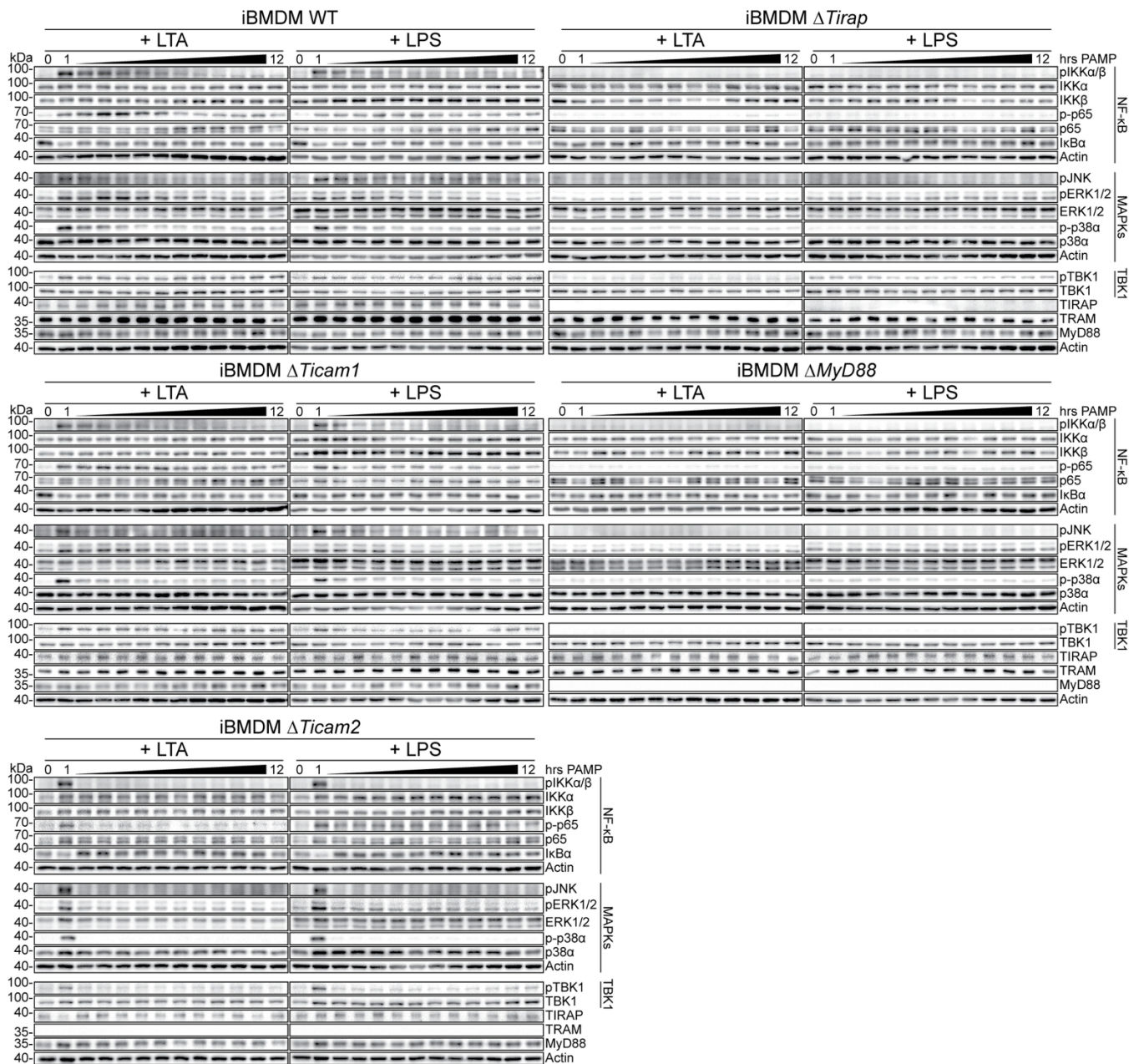
Supplementary Tables 1-7

# SUPPLEMENTARY FIGURES



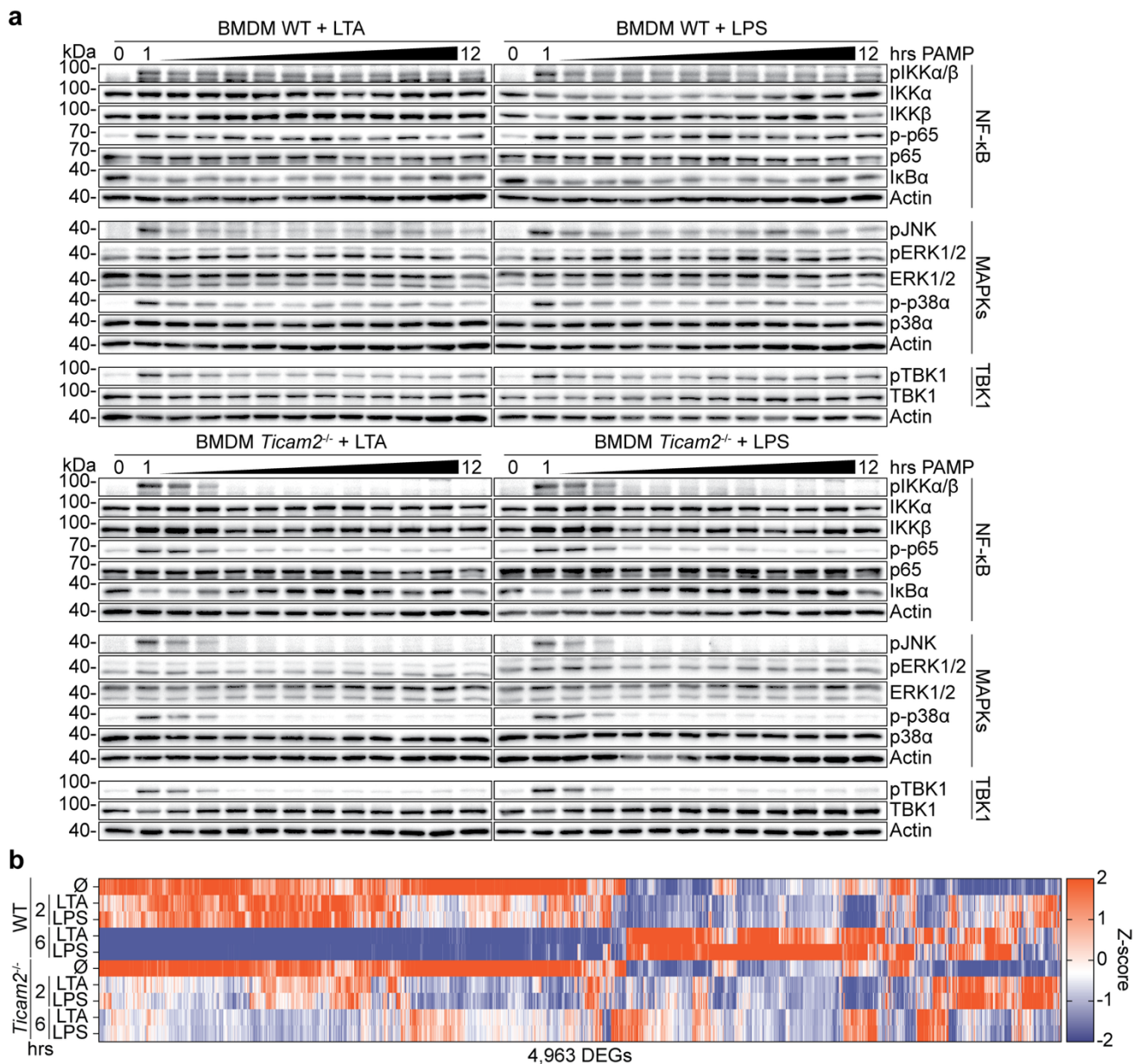
**Supplementary Fig. 1: RNA-Seq of LPS-stimulated iBMDM MyD88-AGF cells.**

**(a)** Heatmap and clustering analysis of differentially expressed genes (DEGs) from RNA-Seq of iBMDM MyD88-AGF cells treated with 10 ng/mL LPS for 6 hours and sorted for cytosolic myddosome (Responder) or absence of cytosolic myddosomes (Bystander). Untreated or LPS-treated but unsorted pools were used as controls and for comparison. **(b)** MA plot generated with DESeq2 showing  $\log_2$  fold change (FC) versus mean normalized gene expression. Each point represents a gene, with significantly differentially expressed genes for all individual comparisons  $P_{\text{adj}} < 0.05$  highlighted. **(c)** Normalized RNA-seq counts for selected TLR signaling factors, response genes, and interferon-stimulated genes in untreated cells ( $\emptyset$ ), bulk LPS-stimulated cells (LPS), bystander cells, and responder cells. Bystander cells displayed reduced *Ticam2* and increased *Tirap* expression, resulting in an elevated *Tirap*:*Ticam2* ratio. **Data information:** Heatmap in **(a)** shows per gene Z-score of all DEGs in this dataset (threshold  $P_{\text{adj}} < 0.01$  and  $|\text{LFC}| > 1$ ) from  $N = 4$  replicates per condition. Graphs in **(b)** show means of four replicates. Box plots in **(c)** depict the distribution of normalized gene expression values, with the center line indicating the median and the box representing the interquartile range.



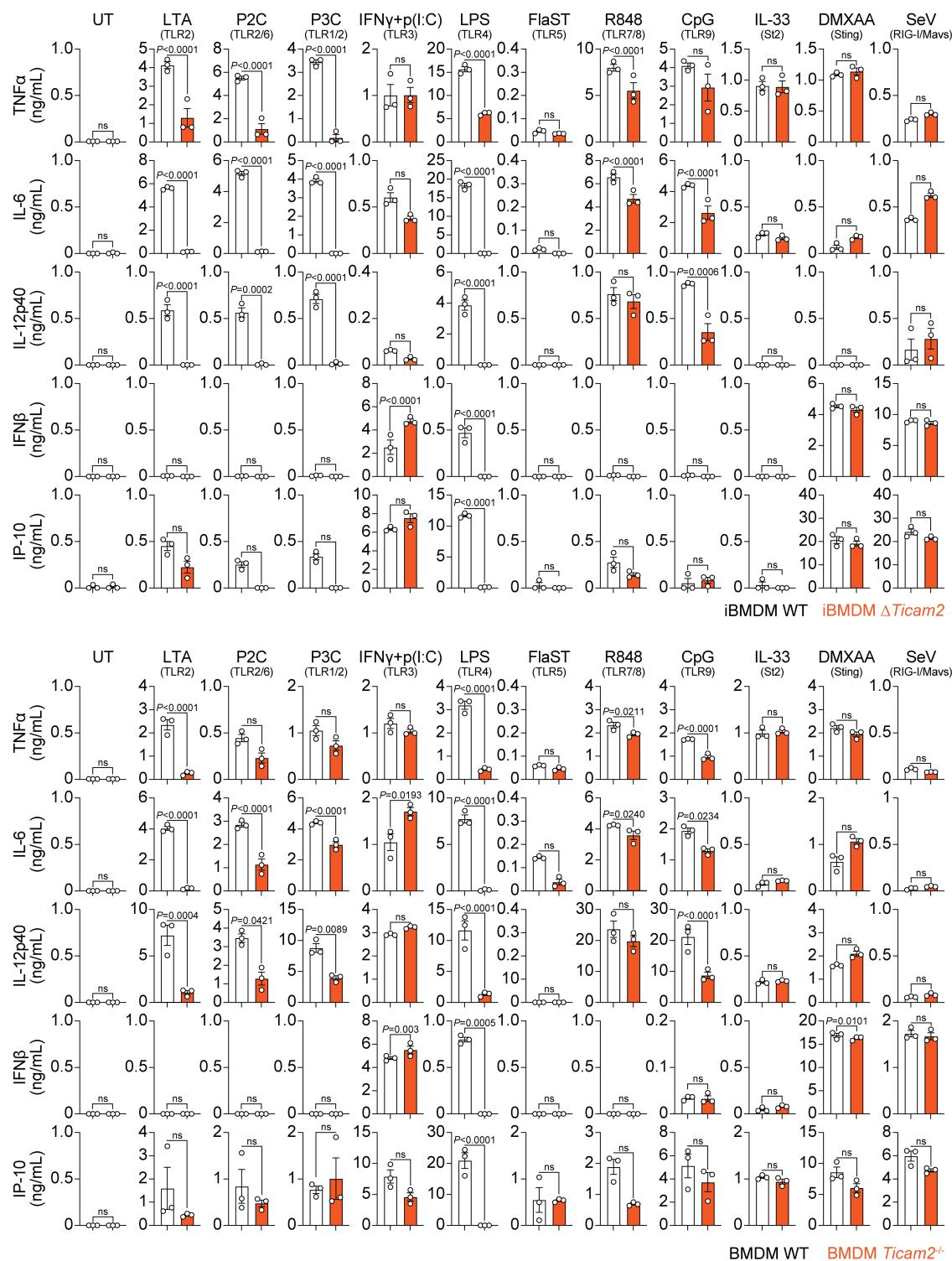
**Supplementary Fig. 2: Kinetic analysis of TLR-responsive signaling pathway activation.**

Immunoblots for phosphorylated and holo-enzymes in the NF- $\kappa$ B, MAPK and TBK1 signaling pathways in iBMDM WT,  $\Delta$ *MyD88*,  $\Delta$ *Tirap*,  $\Delta$ *Ticam1* or  $\Delta$ *Ticam2* cells stimulated with 100 ng/mL LTA or 10 ng/mL LPS for the indicated times. **Data information:** Images representative of n = 2 experiments.



**Supplementary Fig. 3: Kinetic analysis of TLR-responses in *Ticam2*<sup>-/-</sup> BMDMs.**

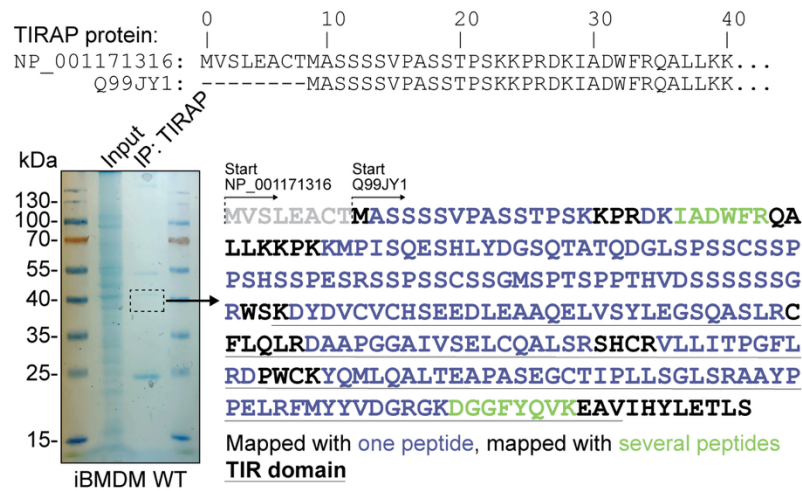
**(a)** Immunoblots for phosphorylated and holo-enzymes in the NF- $\kappa$ B, MAPK and TBK1 signaling pathways in BMDM WT or *Ticam2*<sup>-/-</sup> cells stimulated with 100 ng/mL LTA or 10 ng/mL LPS for the indicated times. **(b)** Heatmap and clustering analysis of differentially expressed genes (DEGs) from RNA-Seq of BMDM WT or *Ticam2*<sup>-/-</sup> cells treated with 10 ng/mL LPS or 100 ng/mL LTA for 2 or 6 hours. **Data information:** Images in **(a)** representative of N = 3 mice per genotype. Heatmap in **(b)** shows averaged per gene Z-score of all DEGs in this dataset (threshold  $P_{\text{adj}} < 0.05$  and  $|\text{LFC}| > 1$ ) from N = 3 mice per genotype.



**Supplementary Fig. 4: Cytokine responses to diverse innate immune stimuli in WT and TRAM-deficient macrophages.**

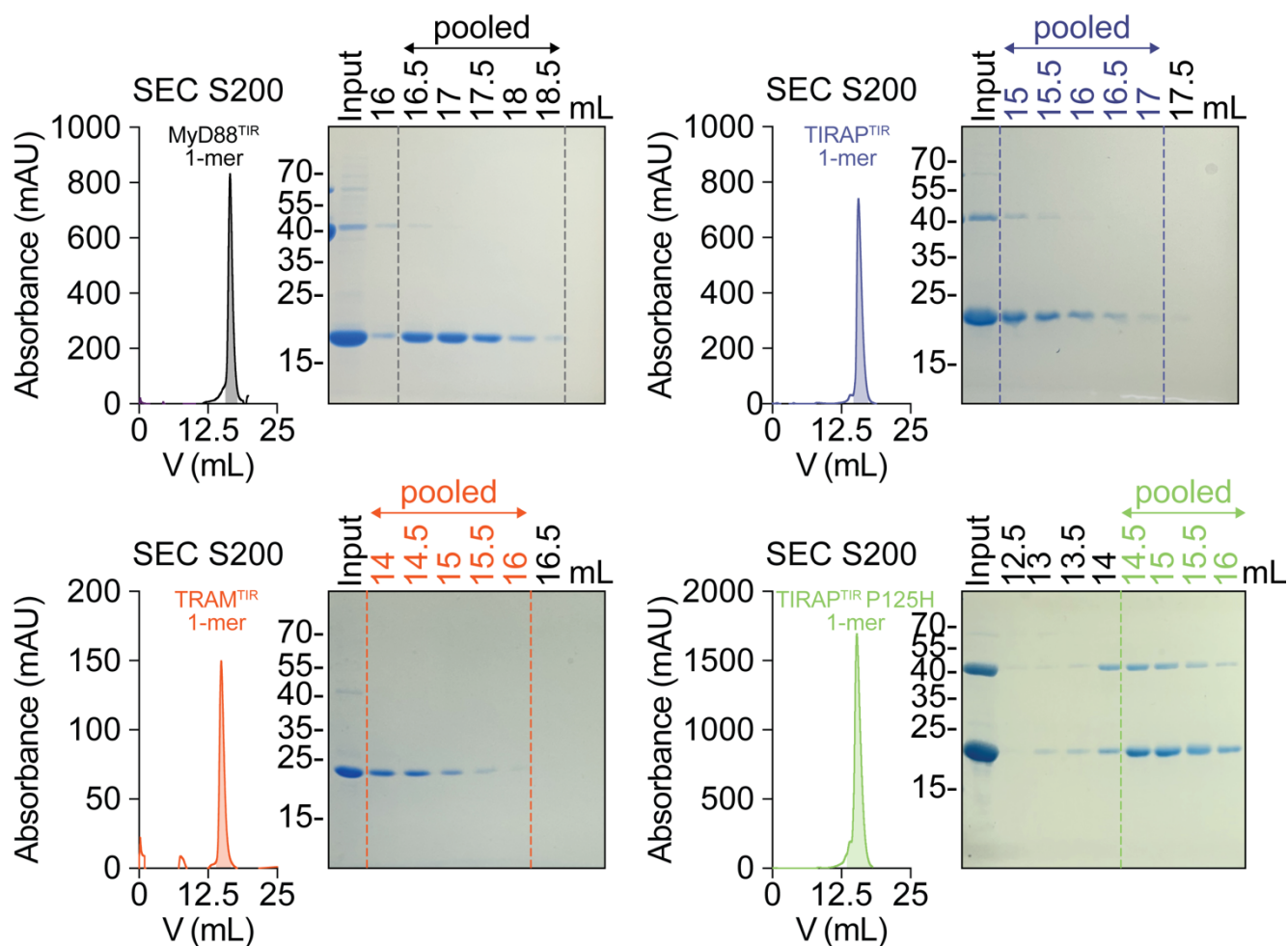
ELISA for TNFα, IL-6, IL-12p40, IFNβ and IP-10 secretion from primary BMDM WT or Ticam2<sup>-/-</sup> (Top) or immortalized iBMDM WT or  $\Delta$ Ticam2 (Bottom) cells treated with the indicated innate immune receptor agonists for 24 h. **Data information:** Graphs show mean  $\pm$  SEM from n = 3 experiments. P values for indicated comparisons from two-way ANOVA; ns: not significant.





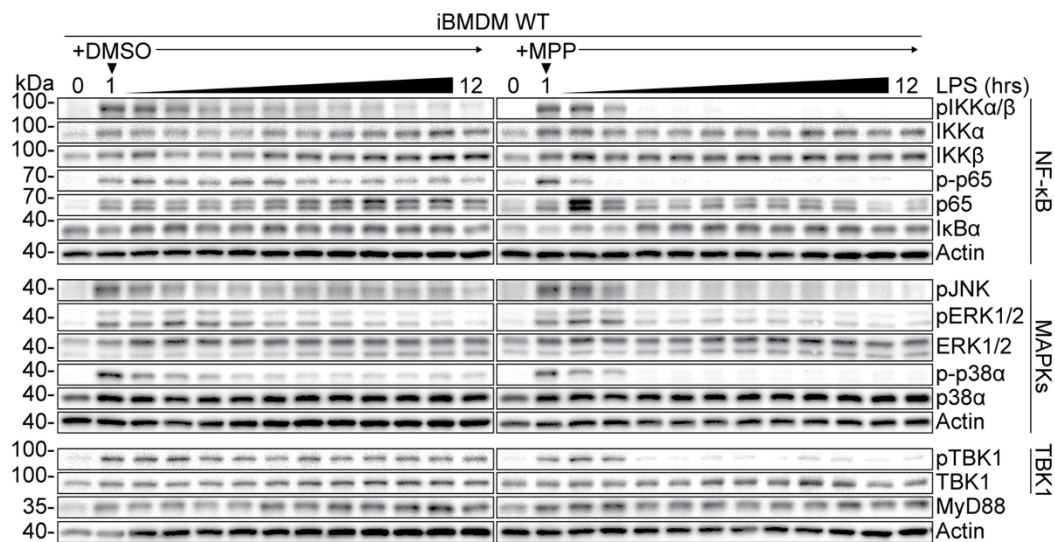
### Supplementary Fig. 6: Mapping murine TIRAP by mass spectrometry.

Alignment of TIRAP protein sequences from NCBI Protein (NP\_001171316) or Uniprot (Q99JY1) databases showing 8 additional amino acids at the N-terminus of murine TIRAP (**Top**). Image of Coomassie stained SDS-PAGE gel of TIRAP immunoprecipitation from iBMDM WT cells with excised TIRAP band indicated by the dashed box (**Bottom left**) and annotated TIRAP sequence with mass spectrometry peptide mapping showing no additional residues at the N-terminus (**Bottom right**). For mass spectrometry data see **Supplementary Data 4**.



**Supplementary Fig. 7: Production of recombinant MyD88, TIRAP and TRAM TIR-domains.**

Chromatograms from S200 size exclusion chromatography (SEC) and Coomassie stain of SDS-PAGE from corresponding fractions for the purification of MyD88 (**Top Left**), TIRAP (**Top Right**), TRAM (**Bottom Left**) and TIRAP<sup>P125H</sup> (**Bottom Right**) TIR domains following expression in *E. coli* (BL21). Fractions pooled as final product are indicated in the figure. **Data information:** Images and chromatograms representative of N > 4 purifications for each TIR domain.



**Supplementary Fig. 8: Analysis of MPP effects on TLR-responsive signaling pathways.**

Immunoblots for phosphorylated and holo-enzymes in the NF-κB, MAPK and TBK1 signaling pathways in iBMDM WT stimulated with 10 ng/mL LPS for the indicated times. 10 μM MPP was added 1 hour after LPS stimulation addition. **Data information:** Images representative of n = 2 experiments.

## SUPPLEMENTARY TABLES

**Supplementary Table 1: Cell lines, bacteria and virus strains.**

| Cell line                                                                                     | Source                    |
|-----------------------------------------------------------------------------------------------|---------------------------|
| HEK 293T                                                                                      | CRL-11268, ATCC           |
| iBMDM $\Delta$ MyD88                                                                          | Jonathan Kagan Laboratory |
| iBMDM $\Delta$ MyD88 +mScarlet3-p65                                                           | This study                |
| iBMDM $\Delta$ Tirap/Ticam2                                                                   | This study                |
| iBMDM $\Delta$ Tirap                                                                          | This study                |
| iBMDM $\Delta$ Tirap +mScarlet3-p65                                                           | This study                |
| iBMDM $\Delta$ Ticam2                                                                         | This study                |
| iBMDM $\Delta$ Ticam2 +mScarlet3-p65                                                          | This study                |
| iBMDM $\Delta$ Ticam1                                                                         | This study                |
| iBMDM $\Delta$ Ticam1 +mScarlet3-p65                                                          | This study                |
| iBMDM MyD88-AGF $\Delta$ Chuk                                                                 | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Ikbb                                                                 | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Ikkg                                                                 | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Irak1/Irak2                                                          | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Irak2                                                                | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Irak4                                                                | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Irf5                                                                 | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Mapk14                                                               | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Rela                                                                 | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Tbk1                                                                 | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF $\Delta$ Ticam1                                                               | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2                                                               | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2<br>+2PH-PLC $\delta$ -mScarlet3                               | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +EV                                                           | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +EV +PH-PLC $\delta$ -mScarlet3                               | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +Fyn-TRAM-HA                                                  | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +Fyn-TRAM-HA<br>+PH-PLC $\delta$ -mScarlet3                   | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +Lyn-TRAM-HA                                                  | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +Lyn-TRAM-HA<br>+PH-PLC $\delta$ -mScarlet3                   | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +TIRAP <sup>PBM</sup> -TRAM-HA                                | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +TIRAP <sup>PBM</sup> -TRAM-HA<br>+PH-PLC $\delta$ -mScarlet3 | This study                |
| iBMDM MyD88-AGF $\Delta$ Ticam2 +TRAM-HA WT                                                   | This study                |

|                                                                                             |                           |
|---------------------------------------------------------------------------------------------|---------------------------|
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA 3X                                          | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA 3X<br>+PH-PLC $\delta$ -mScarlet3           | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA C114H                                       | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA C114H<br>+PH-PLC $\delta$ -mScarlet3        | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA DE88/89AA                                   | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA DE88/89AA<br>+PH-PLC $\delta$ -mScarlet3    | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA E180A                                       | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA E180A<br>+PH-PLC $\delta$ -mScarlet3        | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA G2A                                         | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA G2A<br>+PH-PLC $\delta$ -mScarlet3          | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Ticam2</i> +TRAM-HA WT<br>+PH-PLC $\delta$ -mScarlet3           | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Tirap</i>                                                       | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Tirap/Ticam2</i> +EV                                            | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Tirap/Ticam2</i> +Fyn-TRAM-HA                                   | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Tirap/Ticam2</i> +Lyn-TRAM-HA                                   | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Tirap/Ticam2</i> +TIRAP <sup>PBM</sup> -TRAM-HA                 | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Tlr2</i>                                                        | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Tlr4</i>                                                        | This study                |
| iBMDM MyD88-AGF $\Delta$ <i>Traf6</i>                                                       | Jonathan Kagan Laboratory |
| iBMDM MyD88-AGF +dCas9                                                                      | This study                |
| iBMDM MyD88-AGF +dCas9 +EV                                                                  | This study                |
| iBMDM MyD88-AGF WT                                                                          | This study                |
| iBMDM MyD88-AGF +5xNFkB-RE-mScarlet3 +Cas9<br>+Julianna library = "Julianna cells"          | This study                |
| iBMDM MyD88-AGF +TIRAP-HA                                                                   | This study                |
| iBMDM MyD88-AGF +TIRAP <sup><math>\Delta</math>TIR</sup> -HA                                | This study                |
| iBMDM MyD88-AGF +TIRAP-HA +PH-PLC $\delta$ -mScarlet3                                       | This study                |
| iBMDM MyD88-AGF +TRIAP <sup><math>\Delta</math>TIR</sup> -HA<br>+PH-PLC $\delta$ -mScarlet3 | This study                |
| iBMDM MyD88-AGF +dCas9 +Tirap-sgRNA 1                                                       | This study                |
| iBMDM MyD88-AGF +dCas9 +Tirap-sgRNA 2                                                       | This study                |
| iBMDM MyD88-AGF +dCas9 +Tirap-sgRNA 3                                                       | This study                |
| iBMDM MyD88-AGF +dCas9 +Tirap-sgRNA 4                                                       | This study                |
| iBMDM MyD88-AGF +dCas9 +Tirap-sgRNA 5                                                       | This study                |
| iBMDM MyD88-AGF +dCas9 +Tirap-sgRNA 6                                                       | This study                |
| iBMDM MyD88-AGF +dCas9 +Tirap-sgRNA 1                                                       | This study                |

|                                                         |                                    |
|---------------------------------------------------------|------------------------------------|
| iBMDM MyD88-AGF +dCas9 +Tram-sgRNA 2                    | This study                         |
| iBMDM MyD88-AGF +dCas9 +Tram-sgRNA 3                    | This study                         |
| iBMDM MyD88-AGF +dCas9 +Tram-sgRNA 4                    | This study                         |
| iBMDM MyD88-AGF +dCas9 +Tram-sgRNA 5                    | This study                         |
| iBMDM MyD88-AGF +dCas9 +Tram-sgRNA 6                    | This study                         |
| iBMDM WT                                                | This study                         |
| iBMDM WT +mScarlet3-p65                                 | This study                         |
| <b>Bacteria strain</b>                                  | <b>Source</b>                      |
| <i>Lactococcus lactis</i> (NCTC 6681)                   | 19435, ATCC                        |
| <i>Staphylococcus aureus</i> (SA113)                    | 35556, ATCC                        |
| <i>Escherichia coli</i> K12 (MG1655)                    | Jonathan Kagan Laboratory          |
| <i>Salmonella enterica</i> serovar Typhimurium (SL1344) | Jonathan Kagan Laboratory          |
| <i>Listeria monocytogenes</i> (10403S)                  | Darren Higgins Laboratory          |
| <b>Virus strain</b>                                     | <b>Source</b>                      |
| Sendai virus, Cantell strain                            | 10100774, Charles River Laboratory |

**Supplementary Table 2: List of CRISPR guide crRNA sequences for CRISPR/Cas9 knockouts and CRISPR activation.**

| <b>Guide</b>              | <b>Sequence 5' → 3'</b> |
|---------------------------|-------------------------|
| <i>MyD88</i>              | TATATCCTCACGGTCTAACA    |
| <i>Ticam1</i>             | CACCACGGAGCGAAGGCCCT    |
| <i>Ticam2</i>             | AGTGGATCAGAGCCACCAAC    |
| <i>Tirap</i>              | TCTCCCCGTGACTGGTTC      |
| <i>Tlr2</i>               | TAAGAGATACTAACTTGGCC    |
| <i>Tlr4</i>               | TCTGACGAACCTAGTACATG    |
| <i>Tirap</i> activation 1 | AGCTGCCTGGTTACGACCCG    |
| <i>Tirap</i> activation 2 | GAATCTTGAGCGACGCGGAC    |
| <i>Tirap</i> activation 3 | GCTGGGAATCTTGAGCGACG    |
| <i>Tirap</i> activation 4 | ACCCGCCGGCCGGACCCTAG    |
| <i>Tirap</i> activation 5 | CGGACTGGCGAGCGCCCCCT    |
| <i>Tirap</i> activation 6 | GGACTGGCGAGCGCCCCCTA    |
| <i>Tram</i> activation 1  | AAACAAAGCAGAAATTTTCAG   |
| <i>Tram</i> activation 2  | CCGTGGGGGAACTGCCACCC    |
| <i>Tram</i> activation 3  | GGAGGAGGGGAAACGCCGTG    |
| <i>Tram</i> activation 4  | ACTGCCACCCAGGCAAATGC    |
| <i>Tram</i> activation 5  | AGCCCAGCCAGCATTTCCT     |
| <i>Tram</i> activation 6  | GAGGAGGGGAAACGCCGTGG    |

**Supplementary Table 3: Primers for genotyping and assessing CRISPR/Cas9 editing.**

| <b>Genotyping primer</b> | <b>Sequence 5' → 3'</b> |
|--------------------------|-------------------------|
| <i>Ticam1</i>            | CTACTCCTGCAGGTCTGTGC    |
|                          | GACACAGGTTCTCCTCAGCC    |
| <i>Ticam2</i>            | GACAGTGTGGATGCCGATCA    |
|                          | CGGCCTGATACCAAAGTCGT    |
| <i>Tirap</i>             | CAAGAGAGCTCAGGGGTGTG    |
|                          | GTGGCGAGGTAGGTGACATT    |
| <i>Tlr2</i>              | AAAGTCGATCCGCGACATCC    |
|                          | GTTTCTACTTTACCCAGCTCGC  |
| <i>Tlr4</i>              | TTTAGAGAATCTGGTGGCTGTGG |
|                          | TCAACCGATGGACGTGTAAACC  |

**Supplementary Table 4: List of antibodies for flow cytometry.**

| <b>primary Antibody</b>                 | <b>Isotype</b>        | <b>Source</b>     | <b>RRID</b> |
|-----------------------------------------|-----------------------|-------------------|-------------|
| PE anti-mouse CD284 (TLR4)              | rat IgG2a, $\kappa$   | 145404, Biolegend | AB_2561874  |
| PE anti-mouse CD282 (TLR2)              | rat IgG2a, $\kappa$   | 148604, Biolegend | AB_2564120  |
| PE anti-mouse CD317 (BST2)              | Rat IgG2b, $\kappa$   | 127009, Biolegend | AB_1953284  |
| PE Rat IgG2a, $\kappa$ ; isotype Ctrl   | rat IgG2a, $\kappa$   | 400508, Biolegend | AB_326530   |
| APC Rat IgG1, $\kappa$ ; Isotype Ctrl   | rat IgG1, $\kappa$    | 400412, Biolegend | AB_326518   |
| PE Rat IgG1, $\kappa$ ; Isotype Ctrl    | rat IgG1, $\kappa$    | 400408, Biolegend | AB_326514   |
| PE Mouse IgG2a, $\kappa$ ; isotype Ctrl | mouse IgG2a, $\kappa$ | 400212, Biolegend | AB_326460   |
| PE anti-mouse TNF- $\alpha$             | rat IgG1, $\kappa$    | 506306, Biolegend | AB_315427   |
| APC anti-mouse IL-6                     | rat IgG1, $\kappa$    | 504508, Biolegend | AB_10694868 |

**Supplementary Table 5: List of primary antibodies.**

*IB: immunoblot, IF: immunofluorescence, IP: immunoprecipitation. Ms: mouse, Gt: goat, Rb: rabbit.*

| primary Antibody                     | IB | IF | IP |    | Source               | RRID        |
|--------------------------------------|----|----|----|----|----------------------|-------------|
| $\beta$ -Actin                       | x  |    |    | Rt | 664802, Biolegend    | AB_2721349  |
| spCas9                               | x  |    |    | Rb | #65832, CST          | AB_2799695  |
| pERK1/2<br>(Thr202/Tyr204)           | x  |    |    | Rb | #4370, CST           | AB_2315112  |
| ERK1/2                               | x  |    |    | Rb | #9102, CST           | AB_330744   |
| Flag(M2)                             |    |    | x  | Ms | F3165, Sigma-Aldrich | AB_259529   |
| HA                                   | x  | x  | x  | Rb | #3724, CST           | AB_1549585  |
| pIKK $\alpha$ / $\beta$ (Ser176/180) | x  |    |    | Rb | #2697, CST           | AB_2079382  |
| IKK $\alpha$                         | x  |    |    | Rb | #2682, CST           | AB_331626   |
| IKK $\beta$                          | x  |    |    | Rb | #8943, CST           | AB_11024092 |
| pIKK $\epsilon$ (Ser172)             | x  |    |    | Rb | #8766, CST           | AB_2737061  |
| IKK $\epsilon$                       | x  |    |    | Rb | #3416, CST           | AB_1264180  |
| IL-1 $\beta$                         | x  |    |    | Rb | GTX130021, Genetex   | AB_2886157  |
| pJNK<br>(Thr183/Tyr185)              | x  |    |    | Rb | #9251, CST           | AB_331659   |
| IRAK2                                | x  |    |    | Rb | 3595, ProSci         | AB_735436   |
| IRAK4                                | x  |    |    | Ms | ab119942, abcam      | AB_10901245 |
| I $\kappa$ B $\alpha$                | x  |    |    | Rb | #9242, CST           | AB_331623   |
| Myc-Tag                              | x  |    |    | Rb | #2278, CST           | AB_490778   |
| MyD88 (mouse)                        | x  |    | x  | Gt | AF3109, R&D Systems  | AB_2146703  |
| p-p38 $\alpha$<br>(Thr180/Tyr182)    | x  |    |    | Rb | #4511, CST           | AB_2139682  |
| p38 $\alpha$                         | x  |    |    | Rb | #9212, CST           | AB_330713   |
| p62                                  | x  |    |    | Ms | ab56416, abcam       | AB_945626   |
| p-p65 (Ser536)                       | x  |    |    | Rb | #3033, CST           | AB_331284   |
| p65                                  | x  |    |    | Rb | #8242, CST           | AB_10859369 |
| pSTAT1 (Ser727)                      | x  |    |    | Rb | #9177, CST           | AB_2197983  |
| STAT1                                | x  |    |    | Rb | #9172, CST           | AB_2198300  |
| pTBK1 (Ser172)                       | x  |    |    | Rb | #5483, CST           | AB_10693472 |
| TBK1                                 | x  |    |    | Ms | #51872, CST          | AB_2799403  |
| TIRAP                                | x  |    | x  | Rb | ab17218, abcam       | AB_779101   |

|                     |   |  |  |    |                           |            |
|---------------------|---|--|--|----|---------------------------|------------|
| TLR4 (mouse)        | x |  |  | Rb | Jonathan Kagan laboratory | N/A        |
| TRAF6               | x |  |  | Ms | 654501, Biolegend         | AB_2561867 |
| TRAM                | x |  |  | Rt | MAB4348, R&D Systems      | AB_3658292 |
| TRAM (TIR-specific) | x |  |  | Rb | ab17221, abcam            | AB_443726  |
| Viperin             | x |  |  | Rb | #13996, CST               | AB_2734772 |

**Supplementary Table 6: List of secondary antibodies.**

*Ms: mouse, Gt: goat, Rb: rabbit, Dk: donkey*

| <b>secondary Antibody</b>                  | <b>Source</b>                       | <b>RRID</b> |
|--------------------------------------------|-------------------------------------|-------------|
| Gt anti-mouse IgG (H+L), HRP               | 115-035-003, Jackson ImmunoResearch | AB_10015289 |
| Gt anti-rabbit IgG (H+L), HRP              | 111-035-003, Jackson ImmunoResearch | AB_2313567  |
| Dk anti-goat IgG (H+L), HRP                | 705-035-003, Jackson ImmunoResearch | AB_2340390  |
| Dk anti-rat IgG (H+L), HRP                 | 712-035-150, Jackson ImmunoResearch | AB_2340638  |
| Dk anti-rabbit IgG (H+L), Alexa Fluor™ 488 | A21206, Invitrogen                  | AB_2535792  |
| Gt anti-rat IgG (H+L), Alexa Fluor™ 488    | A11006, Invitrogen                  | AB_141373   |
| Gt anti-rabbit IgG (H+L), Alexa Fluor™ 647 | A21245, Invitrogen                  | AB_2535813  |

**Supplementary Table 7: List of qPCR primer sequences.**

| qPCR primer            | Sequence 5' → 3'         | Transcript ID (& amplicon size) |
|------------------------|--------------------------|---------------------------------|
| <i>Cxcl10</i>          | CACGTGTTGAGATCATTGCCA    | NM_021274.2 (214 bp)            |
|                        | CCATCCACTGGGTAAAGGGG     |                                 |
| <i>Hprt1</i>           | CTGGTGAAAAGGACCTCTCGAAG  | NM_013556.2 (146 bp)            |
|                        | CCAGTTTCACTAATGACACAAACG |                                 |
| <i>Ifnb1</i>           | CAGCTCCAAGAAAGGACGAAC    | NM_010510.2 (138 bp)            |
|                        | GGCAGTGTAACCTCTTCTGCAT   |                                 |
| <i>Il12b</i>           | TGGTTTGCCATCGTTTTGCTG    | NM_001303244.1 (123 bp)         |
|                        | ACAGGTGAGGTTCACTGTTTCT   |                                 |
| <i>Il6</i>             | CTGCAAGAGACTTCCATCCAG    | NM_031168.2 (131 bp)            |
|                        | AGTGGTATAGACAGGTCTGTTGG  |                                 |
| <i>Il6</i> (pre-mRNA)  | TCTGCTCACTTGCCGGTTTT     | NC_000071.7 (162 bp)            |
|                        | AGTGGTATAGACAGGTCTGTTGG  |                                 |
| <i>MyD88</i>           | CTGCTACTGCCCCAACGATA     | NM_010851.3 (233 bp)            |
|                        | GGCTGAGTGCAAACCTTGGTC    |                                 |
| <i>Stat1</i>           | TCACAGTGGTTCGAGCTTCAG    | NM_001205313.1 (155 bp)         |
|                        | GCAAACGAGACATCATAGGCA    |                                 |
| <i>Ticam2</i>          | GACAGTGTGGATGCCGATCA     | NM_173394.3 (212 bp)            |
|                        | TCGTCCGGTGTCTCTTCTGC     |                                 |
| <i>Tirap</i>           | CAGGCAGGCTCTGTTGAAGA     | NM_001177845.1 (132 bp)         |
|                        | GAAGTGTGACTCGGGGGTG      |                                 |
| <i>Tirap</i> (TIR)     | CGCTGGAGCAAAGACTACGA     | NM_001177845.1 (166 bp)         |
|                        | CCTGGCATAGCTCCGAAACA     |                                 |
| <i>Tnfa</i>            | CCACGTCGTAGCAAACCAC      | NM_013693.3 (209 bp)            |
|                        | TAGCAAATCGGCTGACGGTG     |                                 |
| <i>Tnfa</i> (pre-mRNA) | AAGAGGGTGACACTGACTCAATC  | NC_000083.7 (238 bp)            |
|                        | TAGCAAATCGGCTGACGGTG     |                                 |