Supplemental information

*Klebsiella pneumoniae* hijacks the Toll-IL-1R protein SARM1 in a type I IFN-dependent manner to antagonize host immunity

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Figure S1 related to Figure 1. SARM1 negatively regulates inflammation induced by carbapenem *K. pneumoniae* strains.

ELISA of TNFα, IL1β, CXCL10 secreted by wild-type (WT) and *sarm1<sup>−/−</sup>* macrophages following infection with KP35 and NJST258<sub>2</sub> for 16 h. Type I IFN levels determined in the supernatants of macrophages 16 h post infection.

In all infections, after 1 h contact, the medium was replaced with medium containing gentamicin (100 µg/ml) to kill extracellular bacteria.

In all panels, values are presented as the mean ± SD of three independent experiments measured in duplicate. ****P ≤ 0.0001; **P ≤ 0.01; for the indicated comparisons using unpaired t test.
Figure S2 related to Figure 1. SARM1 negatively regulates *K. pneumoniae*-induced inflammation.

A. ELISA of TNFα, IL1β, CXCL10 secreted by wild-type (WT) and *sarm1*<sup>−/−</sup> BMDMs non-infected (ni) or infected with Kp52145 for 6 and 16 h.

B. Efficiency of transfection of SARM siRNA (siSARM) in wild-type iBMDMs. mRNA levels were assessed 16 h post transfection as fold change against control non-silencing agents AllStars (siAS).

C. ELISA of IL1β, TNFα, and CXCL10 secreted by wild-type (WT) macrophages transfected with All Stars siRNA control (siAS), or SARM1 siRNA (siSARM) non-infected (ni) or infected with Kp52145 for 16 h.
D. ELISA of IL1β, TNFα, and CXCL10 secreted by wild-type (WT) and Sarm1em1.ITip macrophages non-infected (ni) or infected with Kp52145 for 6 and 16 h.

In all infections, after 1 h contact, the medium was replaced with medium containing gentamicin (100 µg/ml) to kill extracellular bacteria.

In panels A, C, and D, values are presented as the mean ± SD of three independent experiments measured in duplicate. ****P ≤ 0.0001; **P ≤ 0.01; *P ≤ 0.05; for the indicated comparisons using one way-ANOVA with Bonferroni contrast for multiple comparisons test. In panel B, **P ≤ 0.01 using unpaired t test.
Figure S3 related to Figure 1. Densitometry analysis.

Quantification of the relative intensity of phosphorylated bands of MAPKs ERK (A), JNK (B), and p38 (C) normalized to tubulin following infection of wild-type macrophages with Kp52145 for the indicated time points. The value of non-infected cells was set to 1.

Values are presented as the mean ± SD of three independent blots. ****P ≤ 0.0001; ***P ≤ 0.001; ** P ≤ 0.01; *, P ≤ 0.05; ns, P > 0.05 for the indicated comparisons using one way-ANOVA with Bonferroni contrast for multiple comparisons test.
Figure S4 related to Figure 2. *K. pneumoniae* induction of IL10 is controlled by p38 and it is negatively regulated by type I IFN.

A. ELISA of IL10 secreted by wild-type macrophages non-infected (ni) or infected with Kp52145 (Kp) 16 h. Cells were treated with the p38 inhibitor SB202190 or DMSO vehicle control.

B. Efficiency of transfection of SARM1 siRNA (siSARM) in *il10*–/– macrophages. mRNA levels were assessed 16 h post transfection as fold change against control non-silencing agents AllStars (siAS).

C. *ifit1* mRNA levels were assessed by qPCR, in wild-type (WT), and *ifnar1*–/– macrophages non-infected (ni) or infected with Kp52145 for the indicated time points.
D. Immunoblot analysis of phosphorylated p38 (P-p38), and tubulin levels in lysates of wild-type (WT) and ifnar1−/− macrophages non-infected (NI) or infected with Kp52145 for the indicated time points.

E. Immunoblot analysis of phosphorylated p38 (P-p38), and tubulin levels in lysates of wild-type (WT) and tlr4−/− macrophages non-infected (NI) or infected with Kp52145 for the indicated time points.

F. Immunoblot analysis of phosphorylated p38 (P-p38), and tubulin levels in lysates of wild-type (WT) and tram−/−trif−/− macrophages non-infected (NI) or infected with Kp52145 for the indicated time points.

G. il10 mRNA levels were assessed by qPCR, in wild-type (WT), tlr4−/−, tram−/−trif−/−, and ifnar1−/− macrophages non-infected (ni) or infected with Kp52145 for 6 and 16 h. In all infections, after 1 h contact, the medium was replaced with medium containing gentamicin (100 µg/ml) to kill extracellular bacteria.

In panels A, B and G, values are presented as the mean ± SD of three independent experiments measured in duplicate. In panel C, values are presented as the mean ± SD of two independent experiments measured in duplicate. In panels D, E and F the images are representative of three independent experiments. In panel A and C, ****P ≤ 0.0001; * P ≤ 0.05 for the indicated comparisons using one way-ANOVA with Bonferroni contrast for multiple comparisons test. In panel B, **P ≤ 0.01 using unpaired t test. In panel G, ****P ≤ 0.0001 for the comparison between infected knock-out and wild-type cells for 6 h; # P ≤ 0.0001 for the comparison between infected knock-out and wild-type cells for 16 h using one way-ANOVA with Bonferroni contrast for multiple comparisons test.
Figure S5 related to Figure 3. *K. pneumoniae* does not activate NLRP3 inflammasome.

A. ELISA of IL1β secreted by wild-type (WT), asc<sup>−/−</sup>, and gsdmd<sup>−/−</sup> macrophages non-infected (ni) or infected with Kp52145 (Kp) for 16 h.

B. Immunoblot analysis of processed pro-IL1β, and β-actin levels in lysates of wild-type macrophages (WT) and asc<sup>−/−</sup> and gsdmd<sup>−/−</sup> macrophages non-infected or infected with Kp52145 for 16h.
C. ELISA of IL1β secreted by wild-type (WT) macrophages non-infected (ni) or infected with Kp52145 (Kp) for 6 and 16 h. Cells were treated with the NLRP3 inhibitor MC950 or DMSO vehicle control.

D. ELISA of IL1β secreted by wild-type (WT) and nlrp3\(^{-/-}\) macrophages non-infected (ni) or infected with Kp52145 for 6 and 16 h.

E. Immunoblot analysis of processed pro-IL1β, and β-actin levels in lysates of wild-type macrophages (WT) and nlrp3\(^{-/-}\) macrophages non-infected or infected with Kp52145 for 16h.

F. Immunoblot analysis of NLRP3 and tubulin levels in lysates of wild-type macrophages (WT) and nlrp3\(^{-/-}\) macrophages non-infected (NI) or infected with Kp52145 for the indicated time points.

G. Efficiency of transfection of AIM2 siRNA (siAIM2) in sarm1\(^{-/-}\) macrophages. mRNA levels were assessed 16 h post transfection as fold change against control non-silencing agents AllStars (siAS).

In all infections, after 1 h contact, the medium was replaced with medium containing gentamicin (100 \(\mu\)g/ml) to kill extracellular bacteria.

In panels A, C and D values are presented as the mean ± SD of three independent experiments measured in duplicate. \(*\*\*P \leq 0.0001; \text{ns, } P > 0.05\) for the indicated comparisons using one way-ANOVA with Bonferroni contrast for multiple comparisons test. In panel G, values are presented as the mean ± SD of three independent experiments measured in duplicate. \(\**P \leq 0.01\) using unpaired t test. In panels B, E and F, images are representative of three independent experiments.
Cell viability was assessed by quantifying the binding of the supravital dye neutral red to the lysosomes.

A. Percentage of cell viability following infection with Kp52145 for 23 of wild-type (WT), caspase-1<sup>-/-</sup>, gsdmd<sup>-/-</sup>, nlrp3<sup>-/-</sup> and aim2<sup>-/-</sup> macrophages. The value of non-infected wild-type cells was set to 100%.

B. Percentage of cell viability following infection with Kp52145 for 23 of wild-type (WT), and sarm1<sup>-/-</sup> macrophages. The value of non-infected wild-type cells was set to 100%.

In all infections, after 1 h contact, the medium was replaced with medium containing gentamicin (100 µg/ml) to kill extracellular bacteria.

Values are presented as the mean ± SD of three independent experiments measured in triplicate.

****P ≤ 0.0001; ns, P > 0.05 for the indicated comparisons using unpaired t test.
Figure S7 related to Figure 6. Adhesion and phagocytosis of *K. pneumoniae* by *sarm1*<sup>−/−</sup> macrophages.

A. Adhesion in wild-type (WT) and *sarm1*<sup>−/−</sup> macrophages. Cells were infected with Kp52145 for 30 min, wells were washed and bacteria were quantified by lysis, serial dilution and viable counting on LB agar plates.

B. Phagocytosis of Kp52145 by wild-type (WT) and *sarm1*<sup>−/−</sup> macrophages. Cells were infected for 30 min, wells were washed, and it was added medium containing gentamicin (100 µg/ml) to kill extracellular bacteria. After 30 min, cells were washed and bacteria were quantified by lysis, serial dilution and viable counting on LB agar plates.

In panels A and B, values are presented as the mean ± SD of three independent experiments measured in triplicate. * P ≤ 0.05; ns, P > 0.05  for the indicated comparisons using unpaired t test.
A

B

cluster_1
cluster_7
cluster_30
cluster_10
cluster_24
cluster_3
cluster_21
cluster_23
cluster_27
cluster_25
cluster_22
cluster_18
cluster_20
cluster_8
cluster_31
cluster_28
cluster_4
cluster_14
cluster_11
cluster_12
cluster_13
cluster_15

2e+014
4e+014
6e+014
8e+014

Sarm1
Figure S8 related to Figure 7. Description of mouse immune populations following *K. pneumoniae* infection.

A. PhenoGraph cluster analysis of immune populations in the lungs wild-type (WT), and *sarm1*^-/-^ mice non-infected (ni) or infected intranasally with Kp52145 for 24. Graphs shows the combine results of all groups.

B. Heat map showing relative signal intensities of the indicated markers on the clusters identified in panel A. The heat map is coloured based on signal intensity of the indicated markers. Results are based on data from three mice per group.

C. PhenoGraph cluster analysis of immune populations in the lungs wild-type (WT), and *sarm1*^-/-^ mice non-infected (ni) or infected intranasally with Kp52145 for 24. Each graph represents an individual mouse.
**Figure S9.** *K. pneumoniae* exploits the immunomodulatory properties of SARM1 to antagonize cell intrinsic immunity.

Kp52145 activates the signalling pathway TLR4-TRAM-TRIF-IRF3 to induce the production of type I IFN, which signals through the IFNAR1 receptor (Ivin et al., 2017). Type I IFN stimulates the transcription of SARM1, and AIM2 via IRF3. SARM1 negatively regulates MyD88 and TRIF-governed inflammatory responses, the activation of the MAP kinases ERK and JNK, and the AIM2 inflammasome. In contrast, SARM1 is required for the activation of the MAP kinase p38, which controls the production of IL10. Kp52145 exploits IL10 to control inflammation. Absence of SARM1 impairs the intracellular survival of Kp52145, and *sarm1<sup>−/−</sup>* mice do control Kp52145 infection. Collectively, our findings illustrate the crucial role of SARM1 in *K. pneumoniae* immune evasion strategies.
Table S1. Antibodies used to characterize immune populations by mass cytometry.

| Marker  | Metal isotope | Clone | Reference                  |
|---------|---------------|-------|----------------------------|
| Ly6G    | 141Pr         | 1A8   | Fluidigm 3141008B          |
| SIRPa   | 143Nd         | P84   | BD 552371*                 |
| B220    | 144Nd         | RA3-6B2 | Fluidigm 3144011B       |
| CD4     | 145Nd         | RM4-5 | Fluidigm 3145002B          |
| F4/80   | 146Nd         | BM8   | Fluidigm 3146008B          |
| CD45    | 147Sm         | 30-F11 | Fluidigm 3147003C         |
| CD11b   | 148Nd         | M1/70 | Fluidigm 3148003C          |
| CD19    | 149Sm         | 6D5   | Fluidigm 3149002B          |
| CD24    | 150Nd         | M1/69 | Fluidigm 3150009B          |
| CD25    | 151Eu         | 3C7   | Fluidigm 3151007B          |
| CD3c    | 152Sm         | 145-2C11 | Fluidigm 3152004B     |
| PD-L1   | 153Eu         | 10F.9G2 | Fluidigm 3153016B        |
| CD103   | 155Gd         | 2E7   | BioLegend 121402*         |
| CD90.2  | 156Gd         | 30-H12 | Fluidigm 3156006B         |
| IL-10   | 158Gd         | JES5-16E3 | Fluidigm 3158002C   |
| TCRgd   | 159Tb         | GL3   | Fluidigm 3159012B          |
| CD62L   | 160Gd         | MEL-14 | Fluidigm 3160008C         |
| iNOS    | 161Dy         | CXNFT | Fluidigm 3161011B          |
| Ly6C    | 162Dy         | HK1.4 | Fluidigm 3162014B          |
| SiglecH | 163Dy         | 551   | BioLegend 129602*         |
| LAP/TGFb| 164Dy         | TW7-16B4 | Fluidigm 3164014B     |
| FOXP3   | 165Ho         | FJK-16S | Fluidigm 3165024A     |
| CCR2    | 166Er         | 475301R | R&D MAB55381R*      |
| CD335/NKp46 | 167Er | 29A1.4 | Fluidigm 3167008B |
| CD8a    | 168Er         | 53-6.7 | Fluidigm 3168003B         |
| MerTK   | 169Tm         | Polyclonal | R&D AF591*        |
| CD161/NK1.1 | 170Er | PK136 | Fluidigm 3170002C      |
| CD44    | 171Yb         | IM7   | Fluidigm 3171003C         |
| CD86    | 172Yb         | GL-1  | Fluidigm 3172016B         |
| CD117/c-kit | 173Yb | 2B8   | Fluidigm 3173004B         |
| MHC     | 174Yb         | M5/114.15.2 | Fluidigm 3174003B   |
| FcER1   | 176Yb         | MAR-1 | Fluidigm 3176006B         |
| CD11c   | 209Bi         | N418  | Fluidigm 3209005B         |

*Antibodies conjugated to the indicated metal isotype using Maxpar X8 Antibody Labelling Kit.
| Population                  | Subpopulation                        | Cluster | Markers                                      |
|-----------------------------|--------------------------------------|---------|----------------------------------------------|
| B cells                     | Naive B cells                        | 7       | B220⁺CD19⁻MHC-II⁻CD62L<sub>high</sub>        |
|                             | Mature B cells                       | 30      | B220⁺CD19⁻MHC-II⁻CD62L<sub>low</sub>        |
|                             | CD11c<sup>+</sup> B cells            | 10      | B220⁺CD19⁻MHC-II⁻CD62L<sub>low</sub>CD11c<sup>+</sup> |
|                             | Plasma cells                         | 2       | B220⁻CD19⁻MHC-II⁻                      |
| CD4 T cells                 | Naive CD4 T cells                    | 3       | CD90.2⁺CD3⁻CD4⁻CD62L<sub>high</sub>        |
|                             | Mature CD4 T cells                   | 21      | CD90.2⁺CD3⁻CD4⁻CD62L<sub>low</sub>        |
|                             | Ly6C<sup>+</sup> mature CD4 T cells  | 23      | CD90.2⁺CD3⁻CD4⁻CD62L<sub>low</sub>Ly6C<sup>high</sup> |
| CD8 T cells                 | Naive CD8 T cells                    | 27      | CD90.2⁺CD3⁻CD8⁻CD62L<sub>high</sub>        |
|                             | Mature CD8 T cells                   | 25      | CD90.2⁺CD3⁻CD8⁻CD62L<sub>low</sub>        |
| Double negative T cells     |                                      | 22      | CD90.2⁺CD3⁻CD4⁻/CD8⁻CD62L<sub>low</sub>   |
| Gamma delta T cells         |                                      | 18      | CD90.2⁺CD3⁻TCR<sup>gd</sup><sup>+</sup>   |
| Innate lymphoid cells       |                                      | 20      | CD90.2⁺CD3⁻CD62L<sub>low</sub>             |
| NK cells                    | CD90.2⁺ NK cells                     | 8       | CD90.2⁻/NK1.1⁻NKp46<sup>+</sup>            |
|                             | CD90.2⁺CD62L<sub>+</sub> NK cells   | 31      | CD90.2⁺NK1.1⁻NKp46<sup>⁻</sup>CD62L<sub>low</sub> |
|                             | CD90.2⁺CD62L<sub>+</sub> NK cells   | 28      | CD90.2⁺NK1.1⁻NKp46<sup>⁻</sup>CD62L<sub>low</sub> |
| Plasmacytoid dendritic cells| CD103+ dendritic cells               | 26      | MHC-II⁻CD11c<sup>⁺</sup>B220⁻SiglecH<sup>⁺</sup>F4/80<sub>low</sub> |
| Myeloid dendritic cells     | CD11b<sup>+</sup> dendritic cells    | 24      | MHC-II⁻CD11c<sup>⁻</sup>CD11b<sup>⁻</sup>CD103<sup>⁺</sup>F4/80<sup>⁺</sup> |
| Inflammatory monocytes      |                                      | 29      | MHC-II⁺Ly6G⁺Ly6C<sup>⁻</sup>CD11b⁺CD11c<sup>⁺</sup>CCR2<sup>high</sup> |
| Resident monocytes          |                                      | 9       | MHC-II⁻Ly6G⁺Ly6C⁺CD11b⁻CD11c<sup>⁺</sup>CCR2<sup>⁺</sup> |
| Interstitial macrophages    | CD11c<sup>⁺</sup> interstitial macrophages | 16    | MHC-II⁻Ly6G⁺Ly6C<sup>⁺</sup>CD11b⁻CD11c<sup>⁻</sup>CCR2<sup>⁺</sup> |
|                             | CD11c<sup>⁻</sup> interstitial macrophages | 17   | MHC-II⁻Ly6G⁺Ly6C<sup>⁻</sup>CD11b⁻CD11c<sup>⁻</sup> |
| Alveolar macrophages        | CCR2<sup>⁺</sup> alveolar macrophages | 5       | MHC-II⁻Ly6G⁺Ly6C⁺CD11b<sup>⁻</sup>CD11c<sup>⁺</sup>CCR2<sup>⁺</sup> |
|                             | CCR2<sup>⁻</sup> alveolar macrophages | 6       | MHC-II⁻Ly6G⁺Ly6C⁺CD11b<sup>⁻</sup>CD11c<sup>⁻</sup>CCR2<sup>⁻</sup> |
| Mast cells/basophils        |                                      | 19      | MHC-II⁻Ly6G⁺Ly6C<sup>⁺</sup>F4/80<sub>low</sub>CD90.2⁺CD11b⁺CD11c<sup>⁺</sup> |
| Eosinophils                 |                                      | 14      | MHC-II⁻Ly6G⁺Ly6C<sup>⁺</sup>F4/80<sub>⁺</sub> |
| Neutrophils                 | SiglecH<sup>⁺</sup>PD-L1<sup>⁺</sup> neutrophils | 11    | MHC-II⁻Ly6G⁺Ly6C<sup>⁺</sup>F4/80<sub>low</sub>SiglecH<sup>⁺</sup>PD-L1<sup>⁺</sup> |
|                             | SiglecH<sup>⁺</sup>PD-L1<sup>⁺</sup> neutrophils | 12    | MHC-II⁻Ly6G⁺Ly6C<sup>⁺</sup>F4/80<sub>low</sub>SiglecH<sup>⁺</sup>PD-L1<sup>⁻</sup> |
|                             | SiglecH<sup>⁻</sup>PD-L1<sup>⁺</sup> neutrophils | 13    | MHC-II⁻Ly6G⁺Ly6C<sup>⁺</sup>F4/80<sub>low</sub>SiglecH<sup>⁻</sup>PD-L1<sup>⁻</sup> |
|                             | SiglecH<sup>⁻</sup>PD-L1<sup>⁺</sup> neutrophils | 15    | MHC-II⁻Ly6G⁺Ly6C<sup>⁺</sup>F4/80<sub>low</sub>SiglecH<sup>⁻</sup>PD-L1<sup>⁻</sup> |
Table S3. Primers used in this study.

| Name       | Sequence (5’-3’)                                                                 |
|------------|----------------------------------------------------------------------------------|
| mSARM Forward | GGT GCA CAA GGA GAT TGT GAC                                                      |
| mSARM Reverse | CAT GGG ACC ATT TGA TGC CGT T                                                      |
| mIL1B-F1    | AGA TGA AGG GCT GCT TCC AAA                                                     |
| mIL1B-R1    | AAT GGG AAC GTC ACA GAC CA                                                      |
| mTNFα-F1    | TTC TGT CTA CTG AAC TCC GGG GTG ATC GGT CC                                      |
| mTNFα-R1    | GTA TGA GAT AGC AAA TCG GCT GAC GGT GTG GG                                      |
| mIL10-F1    | GGA CTT TAA GGG TTA CTT GGG TTG CC                                              |
| mIL10-R1    | CAT GTA TGC TTC TAT GCA GTG GAT GA                                              |
| mISG15-F    | GGG GCC ACA GCA ACA TCT AT                                                      |
| mISG15-R    | CGC TGG GAC ACC TTC TTC TT                                                      |
| m.Mx1_F1    | GAC TAC CAC TGA GAT GAC CCA GC                                                  |
| m.Mx1_R1    | ATT TCC TCC CCA AAT GTT TTC A                                                   |
| mIFIT1-F    | CAG GTT TCT GAG GAG TTC TG                                                     |
| mIFIT1-R    | TGA AGC AGA TTC TCC ATG AC                                                     |
| mIL12_p40_F1 | GGA AGC AGG GCA GCA GAA TA                                                   |
| mIL12_p40_R1 | AAC TTT AGG GAG AAG TAG GAA TGG                                               |
| AIM2_Fwd    | GTT GAA TCT AAC CAC GAA GTC C                                                   |
| AIM2_Rvr    | CTA CAA GGT CCA GAT TTC AAC TG                                                   |