

Appendix for:

Aurora A Exacerbates Antibiotic-Resistant *Staphylococcus aureus* Infection through
ASC Phosphorylation

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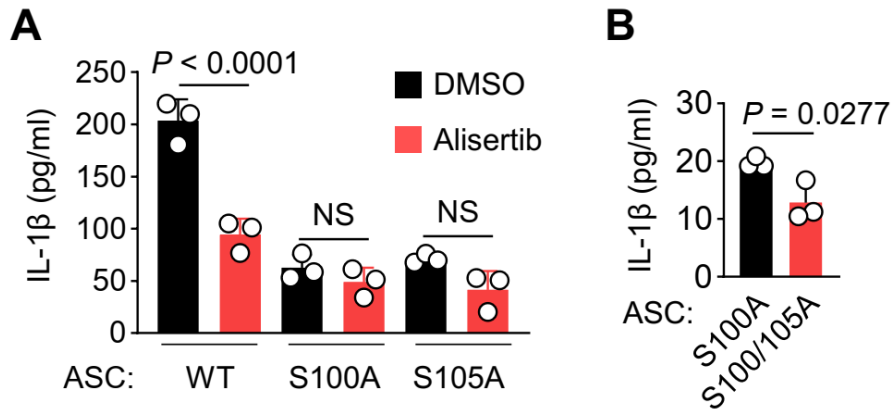
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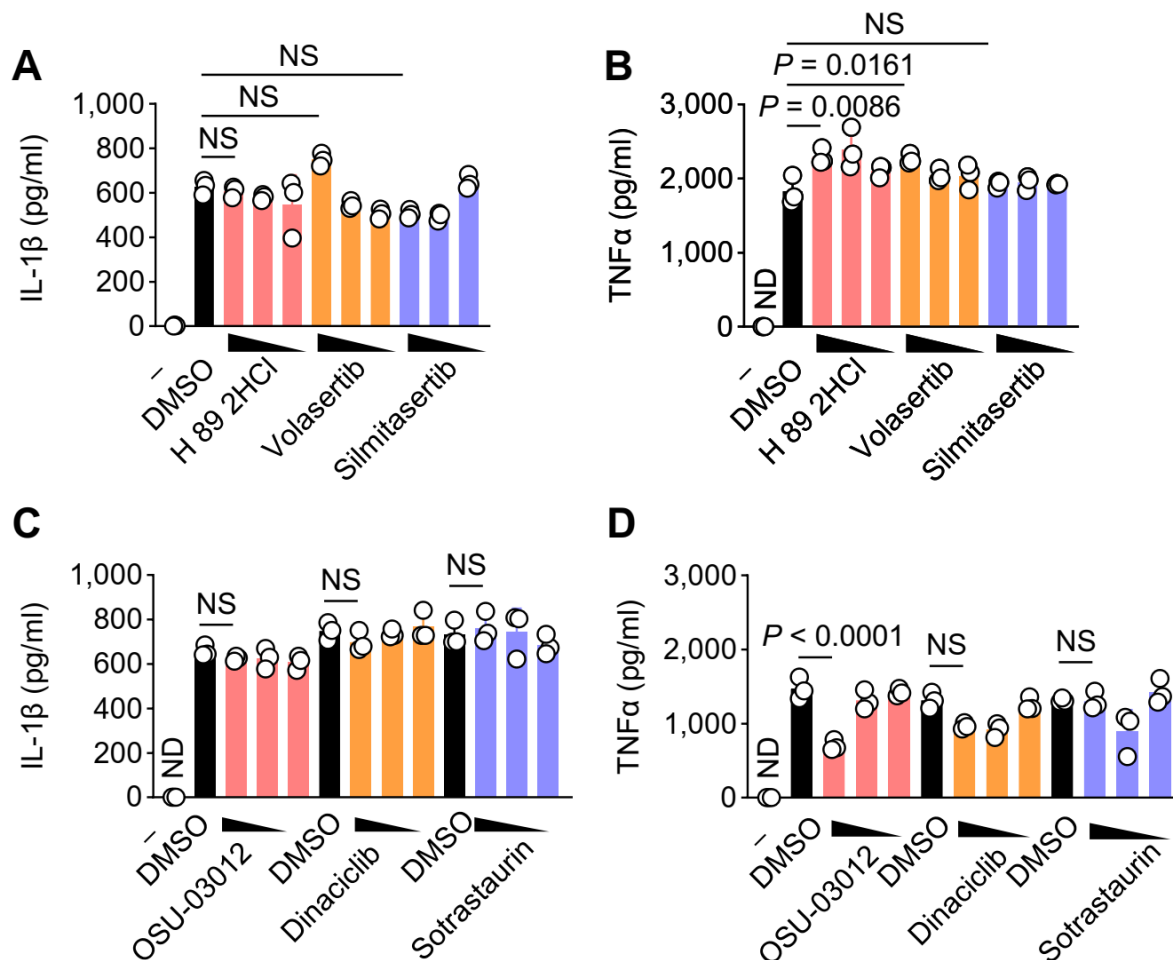
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Appendix Fig. S1. ASC S100 and S105 are involved in the IL-1 β production induced by *S. aureus* infection.

(A, B) Cytokine levels in cell supernatants of *Pycard*^{-/-} immortalized macrophages reconstituted with ASC wild-type, indicated mutants after infection with *S. aureus* NUMR101 at MOI = 10 for 12 h. (A) Cells were treated with 10 μ M Alisertib 30 min before infection. Bars represent the mean $\pm$ SD of three biological replicates. Statistical analysis was performed using (A) one-way ANOVA followed by Tukey's multiple comparison test or (B) two-tailed t-test. (A) $P < 0.0001$, (B) $P = 0.0277$. NS, no significant difference.



Appendix Fig. S2. Screening of kinases that affect IL-1 β production in response to *S. aureus* infection.

Cytokine levels in cell supernatants of peritoneal macrophages infected with *S. aureus* NUMR101 at MOI = 25 for 18 h. Cells were treated with H 89 2HCl (PKA inhibitor; 500, 50, and 5 nM), Volasertib (PLK inhibitor; 500, 50, and 5 nM), Silmitasertib (CK2 inhibitor; 500, 50, and 5 nM), OSU-03012 (PDK1 inhibitor; 20, 2, 0.2 μ M), Dinacilicb (CDK inhibitor;

35 500, 50, and 5 nM), and Sotrastaurin (PKC inhibitor; 500, 50, and 5 nM) 30 min before
36 infection. Results are representative of two biological replicates, and error bars denote SD.
37 Bars represent the mean $\pm$ SD of three biological replicates. Statistical analysis was
38 performed using one-way ANOVA followed by Tukey's multiple comparison test. **(B)** $P =$
39 0.0086, $P = 0.0161$, **(D)** $P < 0.0001$. NS, no significant difference. ND, not detected.

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Aurka gene sequence of Cas9 iBMDMs

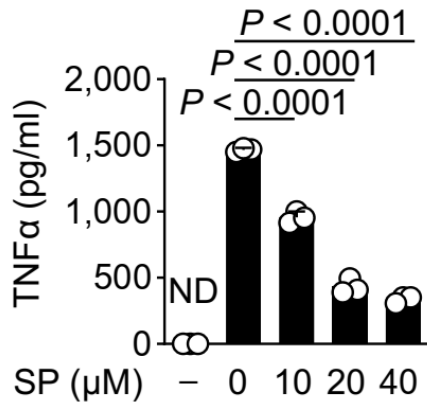
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61 ggtgacatggacagatgtaaagaaaactgtgtctccaggcctgttaagaccactgttccc 120
  G D M D R C K E N C V S R P V K T T V P
121 ttcggtccgaaacgcgtcttgggtgactgagcagattccgtctcagaacctaggatctgct 180
  F G P K R V L V T E Q I P S Q N L G S A
```

Aurka gene sequence of *Aurka*^{-/-} iBMDMs

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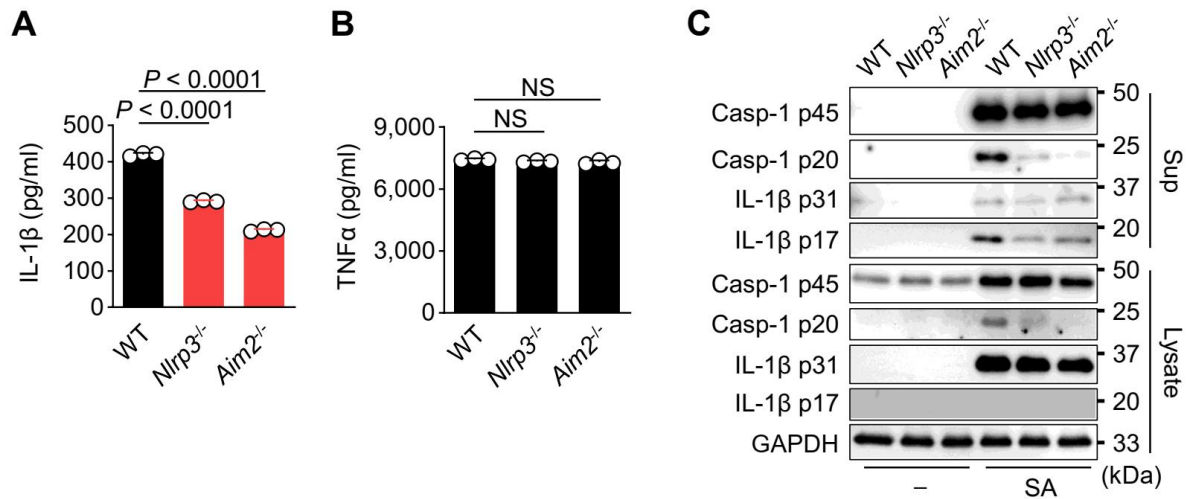
Appendix Fig. S3. Genome sequence of *Aurka*^{-/-} immortalized BMDMs.

Mutations in *Aurka* were determined by subcloning and DNA sequencing. * indicates stop codon.



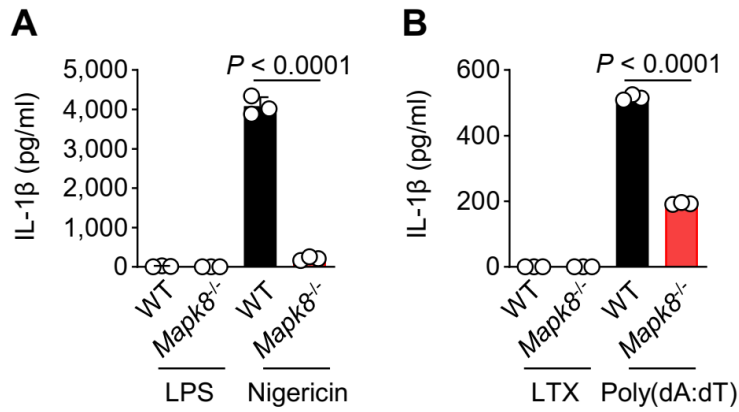
Appendix Fig. S4. Treatment with an JNK kinase inhibitor reduces TNFα production.

Cytokine levels in cell supernatants of peritoneal macrophages infected with *S. aureus* NUMR101 at MOI = 25 for 18 h. Cells were treated with SP600125 (SP, JNK inhibitor, 10, 20, and 40 μM) 30 min before infection. Bars represent the mean ± SD of three biological replicates. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. $P < 0.0001$, $P < 0.0001$, $P < 0.0001$. ND, not detected.



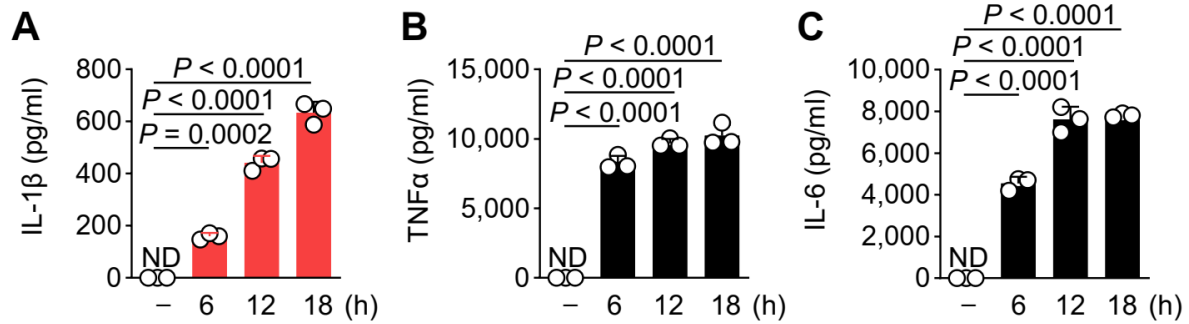
Appendix Fig. S5. *S. aureus* activates both NLRP3 and AIM2 inflammasomes.

(A, B) Cytokine levels in cell supernatants or (C) immunoblotting of wild-type, $Nlrp3^{-/-}$ and $Aim2^{-/-}$ primary BMDMs infected with *S. aureus* NUMR101 at MOI = 25 for 24 h. Bars represent the mean $\pm$ SD of three biological replicates. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. (A) $P < 0.0001$, $P < 0.0001$. NS, no significant difference.



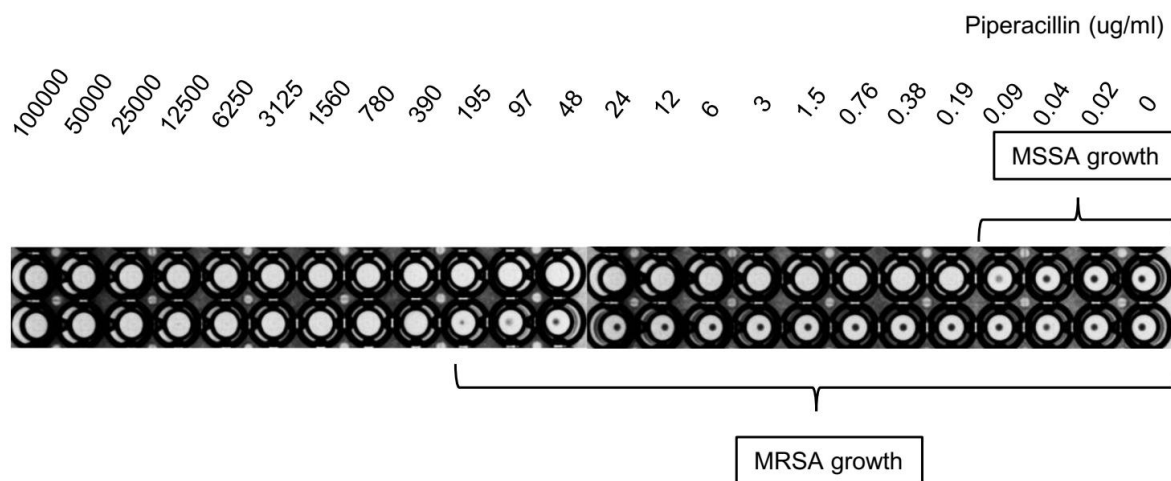
Appendix Fig. S6. JNK1 is required for both NLRP3 and AIM2 inflammasome activation.

Cytokine levels in cell supernatants of LPS-primed immortalized WT or *Mapk8*^{-/-} BMDMs stimulated with nigericin for 60 min or poly(dA:dT) plus Lipofectamine LTX for 120 min. Bars represent the mean $\pm$ SD of three biological replicates. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. (A) $P < 0.0001$, (B) $P < 0.0001$.



Appendix Fig. S7. Time-course analysis of cytokine production induced by *S. aureus* infection.

Cytokine levels in cell supernatants of immortalized wild-type BMDMs infected with *S. aureus* NUMR101 at MOI = 50 for indicated times. Bars represent the mean $\pm$ SD of three biological replicates. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. (A) $P = 0.0002$, $P < 0.0001$, $P < 0.0001$, (B) $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, (C) $P < 0.0001$, $P < 0.0001$, $P < 0.0001$. ND, not detected.



Appendix Fig. S8. Piperacillin sensitivity of MSSA and MRSA.

Piperacillin sensitivity of MSSA and MRSA (NUMR101) was determined by microdilution method. Bacterial precipitations were observed as dark spots at the center of the wells.

