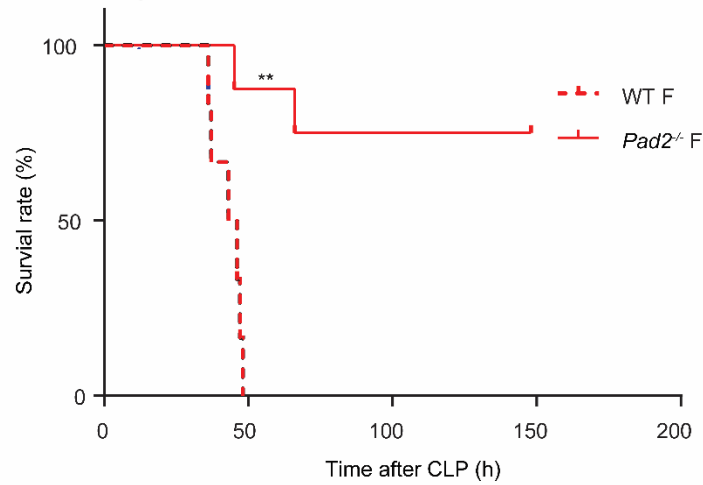


# Supplemental data

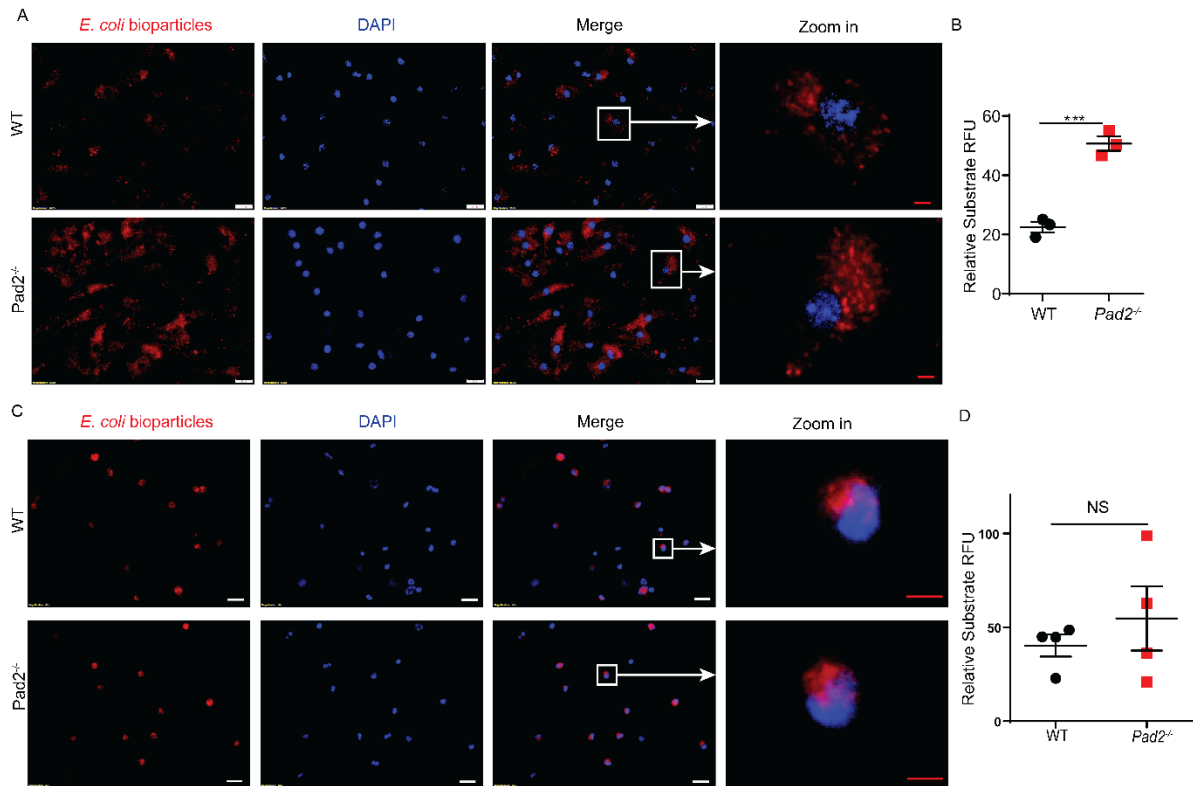
## Peptidylarginine Deiminase 2: Mechanistic insights into its role in sepsis

Supplemental figure 1



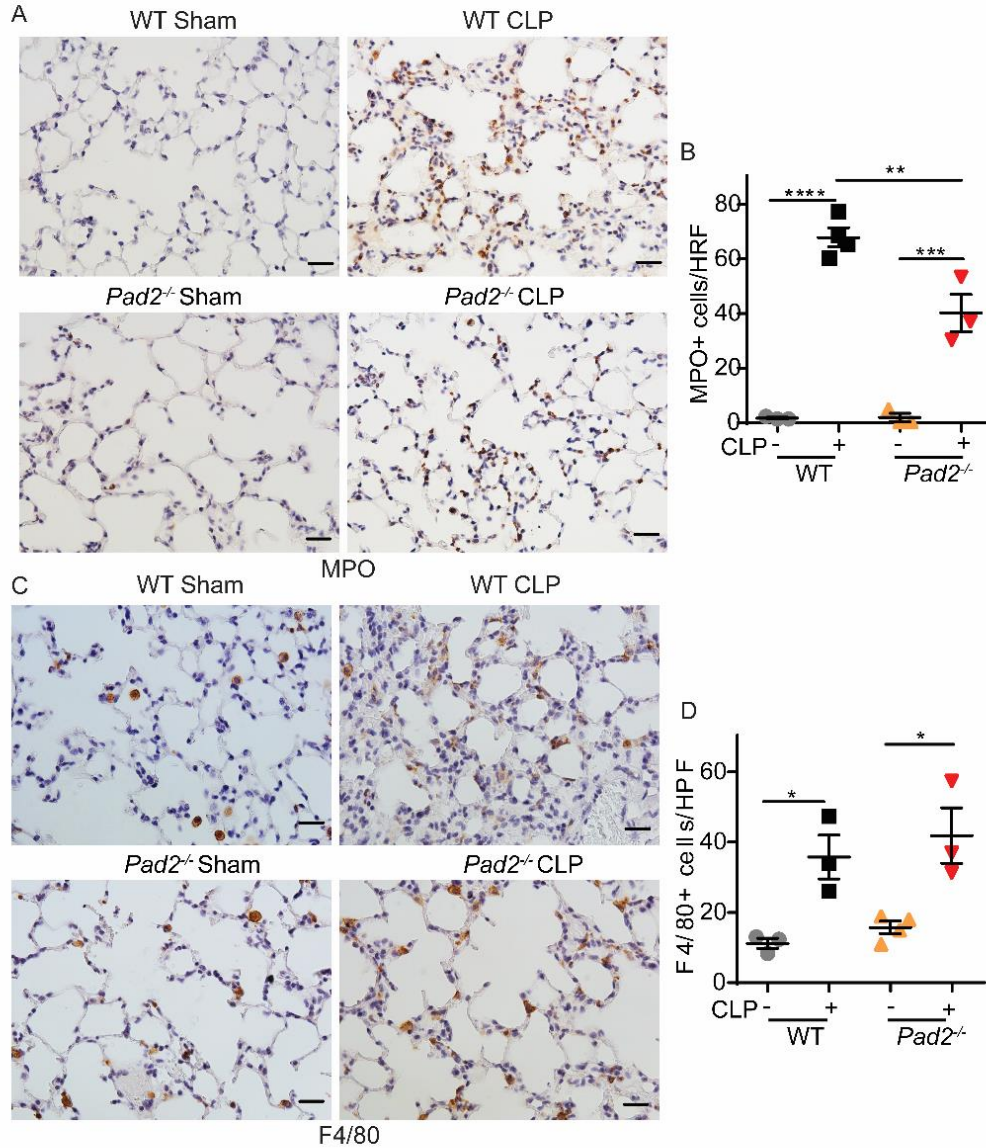
**Supplemental figure 1: Absence of the *Pad2* gene improves survival in a female mouse model of sepsis.** Kaplan-Meier survival curves of WT and *Pad2*<sup>-/-</sup> female mice subjected to **cecal-ligation and puncture (CLP)** (n=6 for female WT group and n=8 for female *Pad2*<sup>-/-</sup> group). Values are expressed as survival percentage. \*\*p<0.01 by log-rank test; NS: not significant; F: female.

Supplemental figure 2



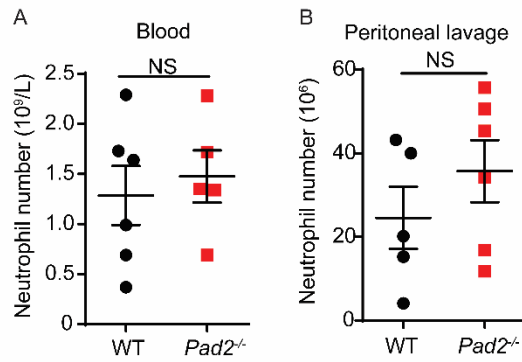
**Supplemental figure 2: Absence of the *Pad2* gene enhances phagocytosis in bone marrow derived macrophages (BMDMs), not neutrophils.** (A) Representative images show the engulfed *E. coli* bioparticles in BMDMs and (B) the fluorescence signal detected by a plate reader. (C) Representative images show the engulfed *E. coli* bioparticles in neutrophils and (D) the fluorescence signal detected by a plate reader. Values are relative fluorescence units (RFU), calculated from 3-4 wells per group. The results are representative of three independent experiments. Graphs are expressed as mean  $\pm$  SEM. Data with two groups were analyzed using an unpaired Student's *t* test. NS: not significant. White scale bar: 20  $\mu$ m, red scale bar: 5  $\mu$ m.

### Supplemental figure 3



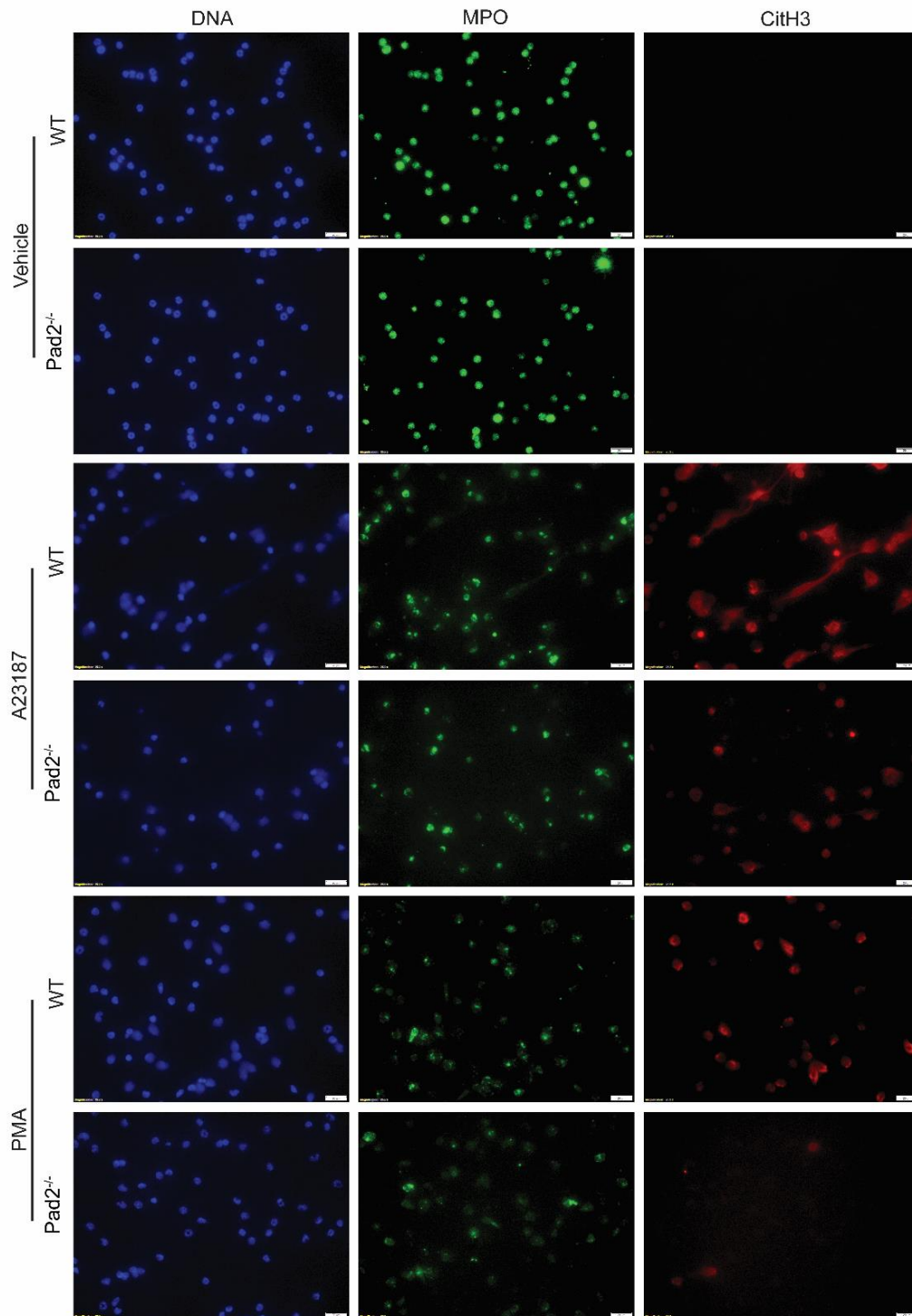
**Supplemental figure 3: Absence of the *Pad2* gene ameliorates CLP-induced neutrophil infiltration in lung tissues.** WT and *Pad2*<sup>-/-</sup> mice were subjected to CLP. (A) Immunohistochemical staining and (B) quantification of myeloperoxidase (MPO), a biomarker of neutrophil, in lung tissues. (C) Immunohistochemical staining and (D) quantification of F4/80, a biomarker of macrophage, in lung tissues. Graphs are expressed as mean ± SEM (n = 3-4/group). Data were analyzed using two-way ANOVA. \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001. Scale bar: 20 μm.

### Supplemental figure 4



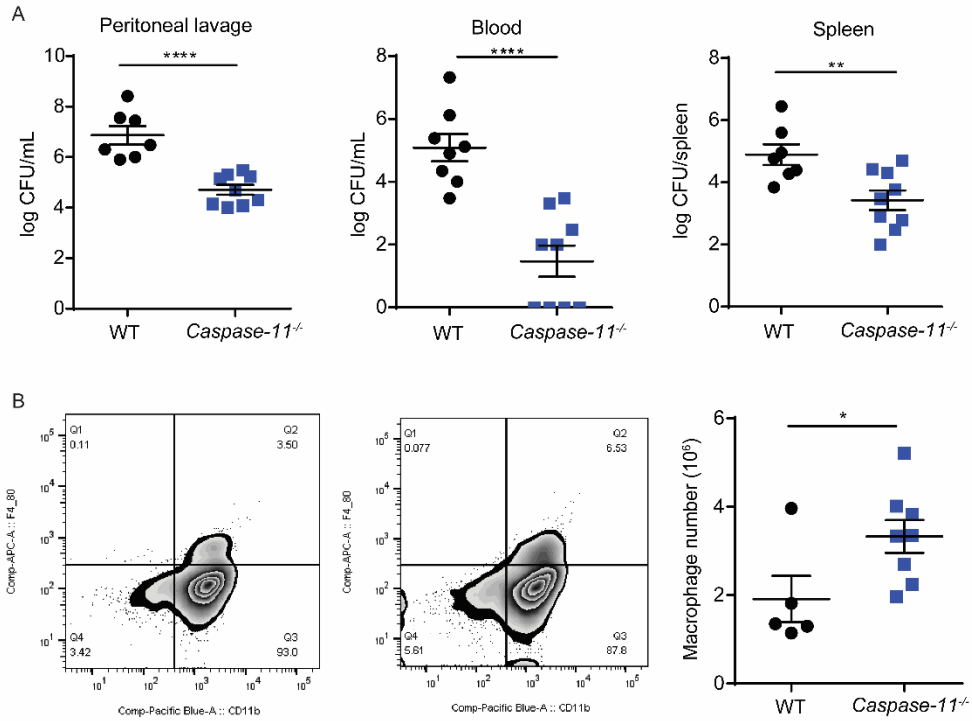
**Supplemental figure 4: Absence of the *Pad2* gene does not affect neutrophil number in a cecal-ligation and puncture (CLP) murine model of sepsis.** WT and *Pad2*<sup>-/-</sup> mice were subjected to CLP. Neutrophil numbers in (A) blood and (B) peritoneal cavity were measured 24 h after CLP. Graphs are expressed as mean ± SEM (n=5-6/group). Data were analyzed using an unpaired Student's *t* test. NS: not significant.

Supplemental figure 5



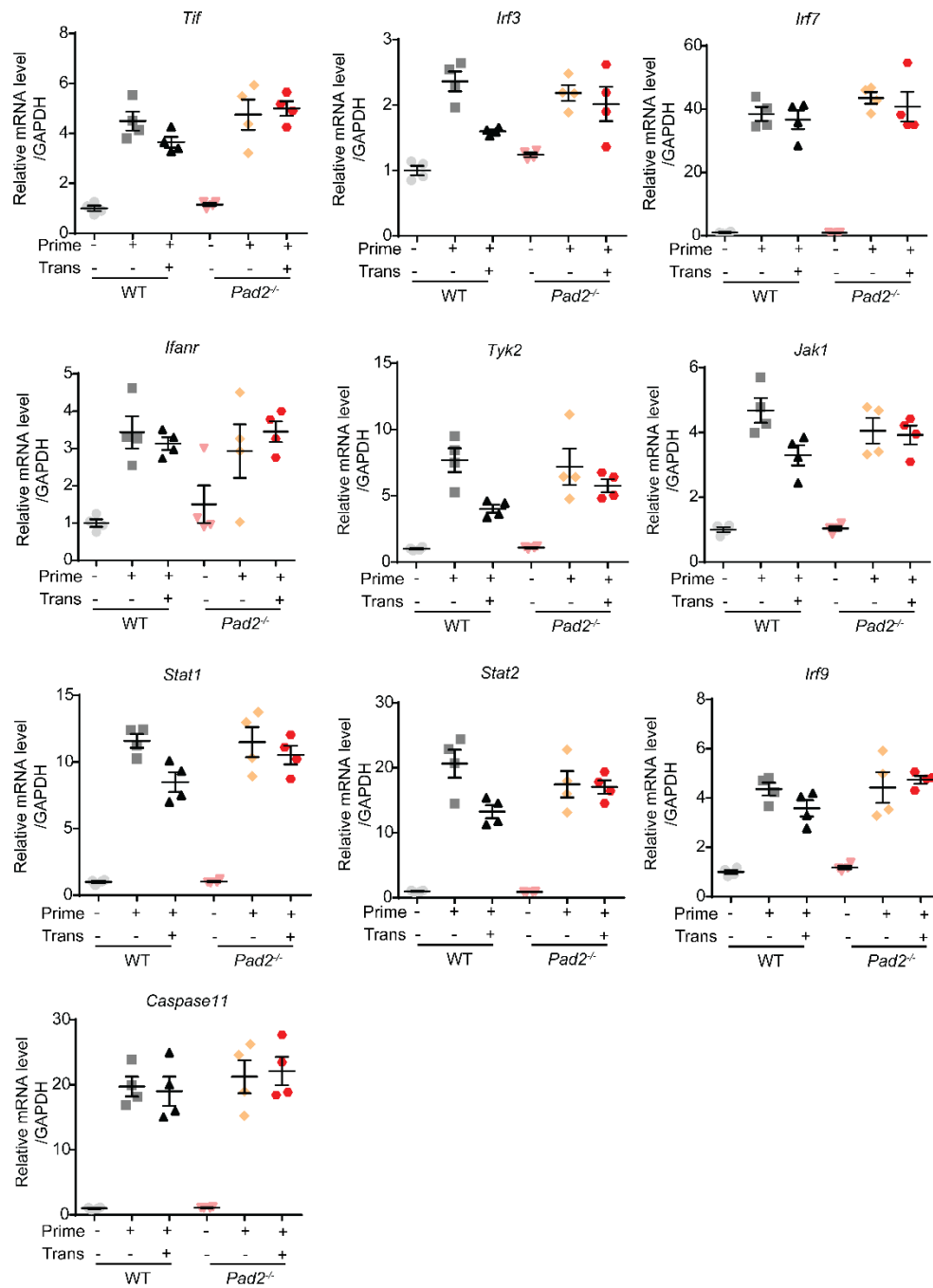
Supplemental figure 5: Single color staining images of NETs showed in Figure 5D. Scare bar: 20  $\mu$ m.

Supplemental figure 6



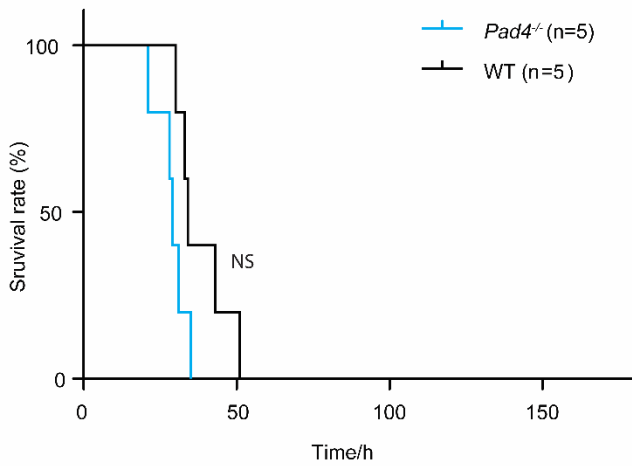
**Supplemental figure 6. Absence of the *Caspase-11* gene decreases bacterial loads and increases macrophage number in mice following cecal-ligation and puncture (CLP).** WT and *Caspase-11*<sup>-/-</sup> mice were subjected to CLP. **(A)** Bacterial loads in peritoneal lavage, blood and spleen 24 h after CLP (n=7-9/group). **(B)** Macrophage number in peritoneal cavity after 24 h (n=5-8/group). Graphs are expressed as mean ± SEM. Data were analyzed using Unpaired *t* test **(A, B)**. \*p<0.05, \*\*p<0.01, \*\*\*\*p<0.0001.

Supplemental figure 7



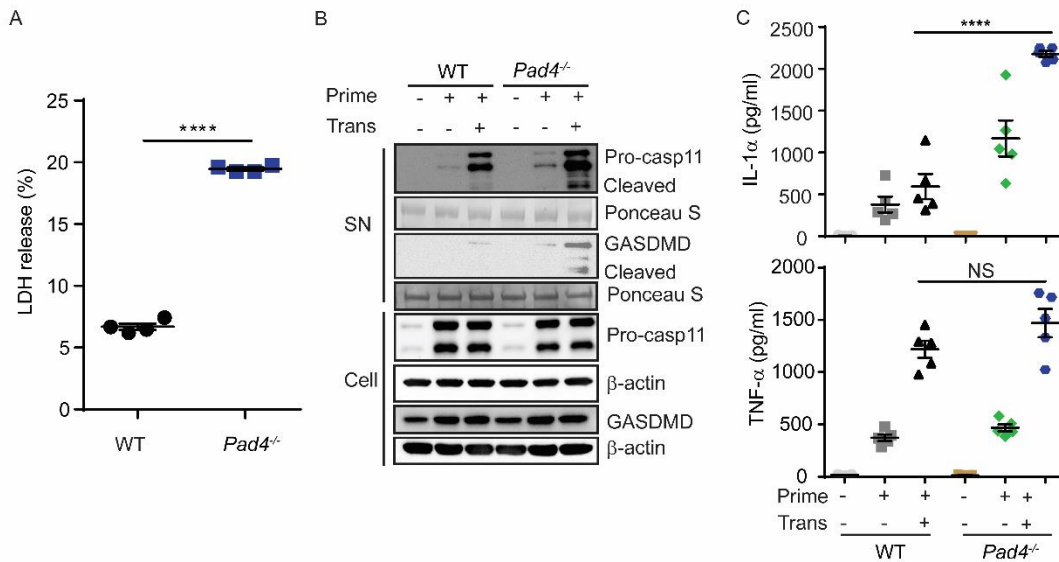
**Supplemental figure 7. Absence of the *Pad2* gene does not affect gene expression in non-canonical pyroptosis pathway.** Bone marrow derived macrophages from WT and *Pad2*<sup>-/-</sup> mice were treated with or without non-canonical pyroptosis stimulation. Gene expression were detected in cell lysate. Results are representative of three independent experiments. Graphs are expressed as mean  $\pm$  SEM (n=4/group). Data were analyzed using two-way ANOVA with Bonferroni's multiple comparisons test.

Supplemental figure 8



**Supplemental figure 8. Absence of the *Pad4* gene does not improve survival in mice following cecal-ligation and puncture (CLP).** Kaplan-Meier survival curve of WT and  $Pad4^{-/-}$  CLP mice. Values are expressed as survival percentage (n=5/group).  $p > 0.05$  by log-rank test. NS: not significant.

Supplemental figure 9



**Supplemental figure 9. Absence of the *Pad4* gene enhances Caspase-11-dependent non-canonical pyroptosis in bone marrow-derived macrophages (BMDMs).** (A) LDH release in supernatant of BMDMs after non-canonical pyroptosis treatment (n=4/group). (B) Representative Western blot images show Caspase-11 and GASDMD activation in supernatant and cell lysate of BMDMs with or without non-canonical pyroptosis treatment. Concentrations of (C) IL-1 $\alpha$  and TNF- $\alpha$  in the supernatant of BMDMs with or without non-canonical pyroptosis treatment (n=5/group). Results are representative of at least three independent experiments (A-C). Graphs are expressed as mean  $\pm$  SEM. Data with two groups were analyzed using Unpaired *t* test (A), and three or more groups were analyzed by two-way ANOVA analysis with Bonferroni's multiple comparisons test (C). \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . SN, supernatant.



**Supplemental table 1:** Primer information for qPCR

Primer name	Sequence
<i>Trif</i> -F	CGATCAAGACGGCCATGAGTC
<i>Trif</i> -R	CTCGTCGGTGTCATCTTCTGC
<i>Irf3</i> -F	GAGAGCCGAACGAGGTTCCAG
<i>Irf3</i> -R	CTCCAGGTTGACACGTCCG
<i>Irf7</i> -F	GCGTACCCTGGAAGCATTTTC
<i>Irf7</i> -R	GCACAGCGGAAGTTGGTCT
<i>Ifnar</i> -F	GACAACTACACCCTAAAGTGGAG
<i>Ifnar</i> -R	GCTCTGACACGAAACTGTGTTTT
<i>Jak1</i> -F	AGTGCAGTATCTCTCCTCTCTG
<i>Jak1</i> -R	GATTCGGTTCGGAGCGTACC
<i>Tyk2</i> -F	AGCTTCAAGAACTGCATCCCT
<i>Tyk2</i> -R	CACACAGGTATGCGCTCTGAG
<i>Irf9</i> -F	CACCATGCAAGCGAAGTATCA
<i>Irf9</i> -R	CGCCATTGGTCCTCCCATT
<i>Stat1</i> -F	TCACAGTGGTTCGAGCTTCAG
<i>Stat1</i> -R	CGAGACATCATAGGCAGCGTG
<i>Stat2</i> -F	GCATAACTTGCGAAAATTCAGCC
<i>Stat2</i> -R	GGATCTCACCAAGAACTCAATGT
<i>pro-Caspase-1</i> -F	TGGCAGGAATTCTGGAGCTT
<i>pro-Caspase-1</i> -R	CTTGAGGGTCCCAGTCAGTC
<i>pro-Caspase-11</i> -F	AGCGTTGGGTTTTTGTAGATGC
<i>pro-Caspase-11</i> -R	CCTTGTGAACTCTTCAGGGGA