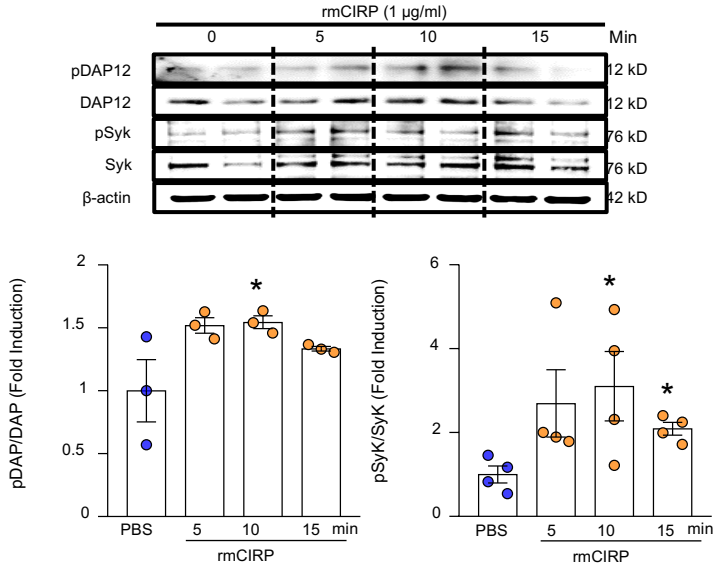
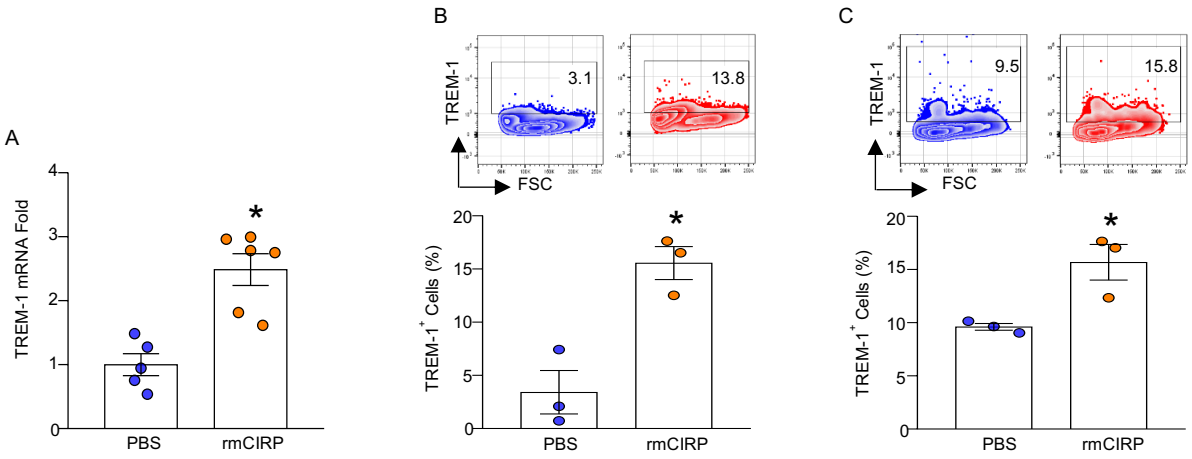
Supplemental Figure 1



Supplemental Figure 1: eCIRP activates TREM-1 downstream molecules DAP12 and Syk in macrophages

A total of 1 × 10^6/ml RAW264.7 cells were stimulated with rmCIRP (1 μg/ml) for various times. Proteins were immunoprecipitated by using anti-DAP12 Ab, followed by Western blotting using pTyr (4G10) and DAP12 Ab. Extracted proteins obtained from rmCIRP (1 μg/ml for various times) stimulated RAW264.7 cells (1 × 10^6/ml) were subjected to Western blotting using pSyk, Syk, and β–actin Abs. Representative Western blots for pTyr (4G10), DAP12, pSyk, Syk, and β–actin are shown. Each blot was quantified by densitometric analysis. Phosphotyrosine (pDAP12) and pSyk expression in each sample was normalized to DAP12 or Syk or β-actin expression and the mean values of PBS-treated groups were standardized as one for comparison. Data are expressed as means ± SE (n=5 samples/group). The groups were compared by one-way ANOVA and Tukey method (*$p<0.05$ vs. PBS). DAP12, DNAX activation protein of 12kDa; rmCIRP, recombinant mouse CIRP.

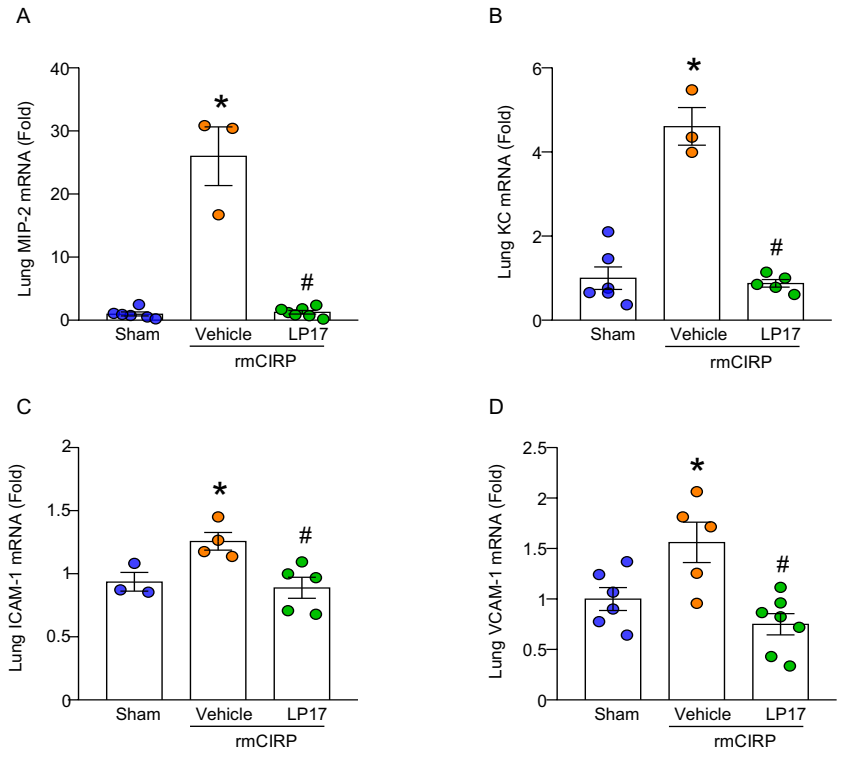
Supplemental Figure 2



Supplemental Figure 2: eCIRP induces TREM-1 expression in macrophages

(A) RAW264.7 cells were stimulated with PBS or rmCIRP (1 µg/ml). After 6 h, RT-PCR was done for TREM-1. Data are expressed as means ± SE, two independent experiment, n = 5/group, unpaired T-test, *p <0.05 vs PBS. **(B)** To detect TREM-1 expression on the surface of macrophages, a total of 1×10^6 RAW264.7 or **(C)** primary peritoneal macrophages were stained with APC anti-mouse TREM-1 Ab. Acquisition was performed on 10,000 events using a BD LSR Fortessa flow cytometer and data were analyzed with FlowJo software. Data are expressed as means ± SE, n = 3/group, unpaired T-test, *p <0.05 vs PBS. PBS, phosphate buffered saline; APC, allophycocyanin.

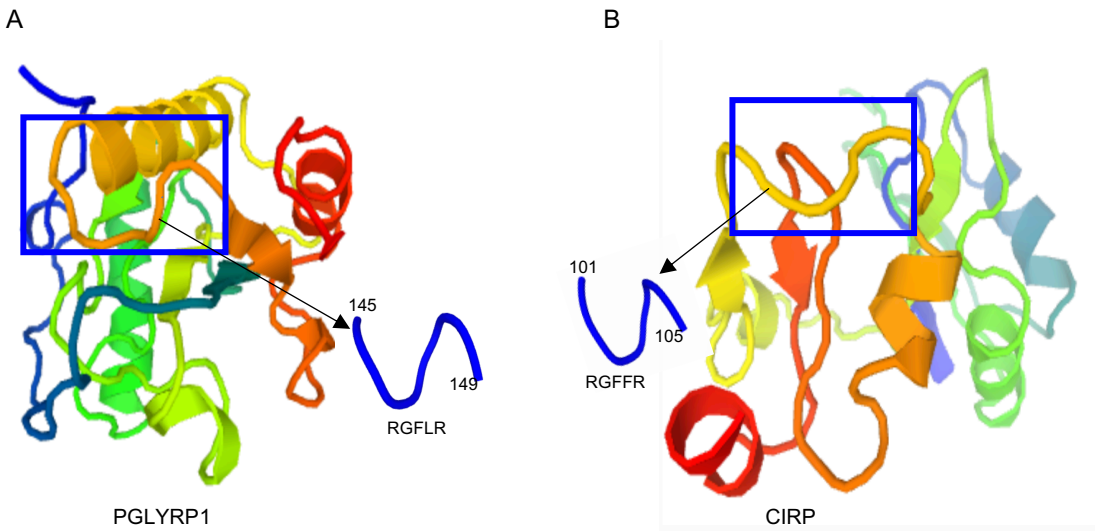
Supplemental Figure 3



Supplemental Figure 3: LP17 treatment inhibits lung chemokine and adhesion molecules following rmCIRP injection in mice

Adult C57BL/6 mice were randomly assigned to sham, vehicle, or treatment group. rmCIRP at a dose of 5 mg/kg BW or equivalent volume normal saline was administered *i.v.* via retro-orbital injection. LP17 at a dose of 5 mg/kg BW or vehicle (PBS) was given *i.p.* at the time of rmCIRP injection. At 5 h after rmCIRP injection, mice were euthanized, and tissue was collected for analysis. Lung mRNA levels of **(A)** MIP-2, **(B)** KC, **(C)** ICAM-1, and **(D)** VCAM-1 were measured by RT-PCR. Data are expressed as means ± SE. n = 5-8 mice/group. The groups were compared by one-way ANOVA and Tukey (*$p<0.05$ vs. sham and #$p<0.05$ vs. vehicle-treated mice). MIP-2, macrophage inflammatory protein-2; KC, keratinocyte chemoattractant; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion protein.

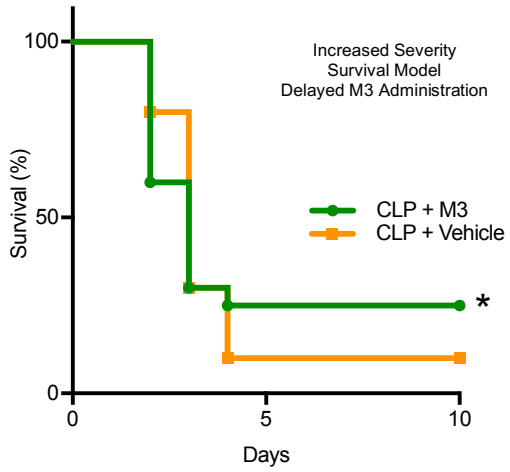
Supplemental Figure 4



Supplemental Figure 4: Development of the M3 peptide

Three-dimensional structures generated using the Protein Model Portal of **(A)** murine PGLYRP1 and **(B)** murine CIRP. Murine and human CIRP share 96% amino acid sequence homology. Boxed area highlights an area of structural similarity. PGLYRP1, peptidoglycan recognition protein 1.

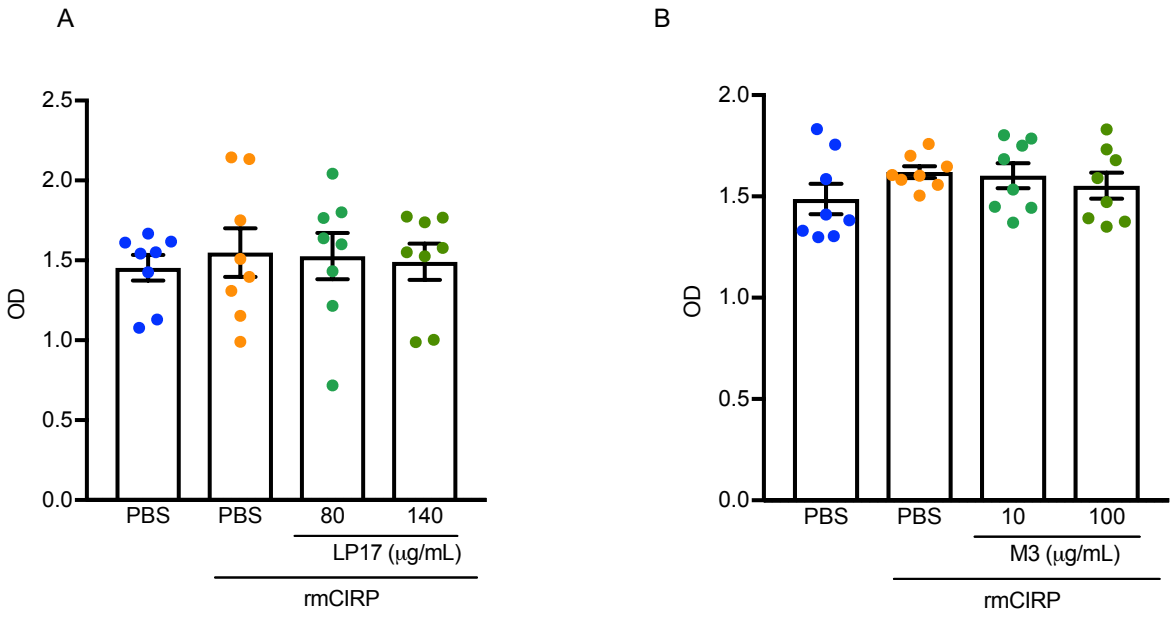
Supplemental Figure 5



Supplemental Figure 5: Delayed administration of M3 in sepsis mice improves survival

Kaplan-Meier survival curve generated from treatment (M3) and vehicle CLP mice during the 10-day monitoring period after increased severity CLP with delayed M3 treatment (90 minutes after sepsis induction) is shown. n=20 mice/treatment, n = 10 mice/vehicle, *$p<0.05$ vs. vehicle, determined by the log-rank test.

Supplemental Figure 6



Supplemental Figure 6: Treatment of macrophages with LP17 or M3 does not impact cell viability

RAW264.7 cells were treated with indicated doses of peptides LP17 and M3 for 30 min. Cells were then stimulated with rmCIRP (1 μg/ml). After 24 h, colorimetric determination of cell viability was determined according to manufacturer's instructions using the CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison WI). The quantity of formazan product, as measured by absorbance at 490 nm, is directly proportional to the number of living cells in culture. We found no significant changes in OD values with maximal doses of LP17 and M3 indicating no change in the number of living cells in culture. Data are expressed as means ± SE and analyzed using by one-way ANOVA and Tukey method.

Supplemental Table 1: Primer sequences for RTqPCR.

Gene	Ref Seq	Forward primer	Reverse primer
β-actin	NM_007393	CGTGAAAAGATGACCCAGATCA	TGGTACGACCAGAGGCATACAG
TREM-1	NM_021406	CTACAACCCGATCCCTACCC	AAACCAGGCTCTTGCTGAGA
IL-6	NM_031168	CCGGAGAGGAGACTTCACAG	CAGAATTGCCATTGCACAAC
IL-1β	NM_008361	CAGGATGAGGACATGAGCACC	CTCTGCAGACT-CAAACTCCAC
KC	NM_008176	GCTGGGATTCACCTCAAGAA	ACAGGTGCCATCAGAGCAGT
MIP-2	NM_009140	CCCTGGTTCAGAAAATCATCCA	GCTCCTC- CTTTCCAGGTCAGT
TNF-α	NM_012675	AGACCCTCACACTCAGATCATCTTC	TTG CTACGACGTGGGCTACA
ICAM-1	NM_010493	GGGCTGGCATTGTTCTCTAA	CTTCAGAGGCAGGAAACAGG
VCAM-1	NM_011693	GAACCCAAACAGAGGCAGAG	TGAGCAGCTCAGGTTCACAG