

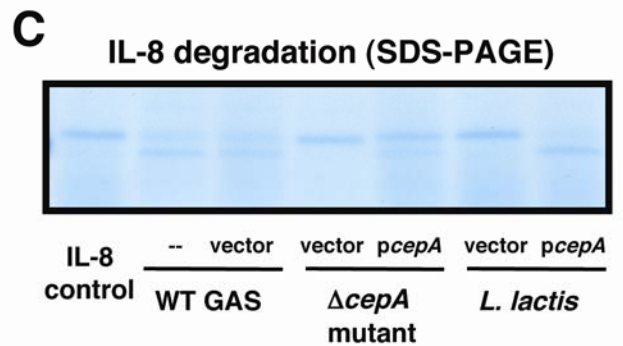
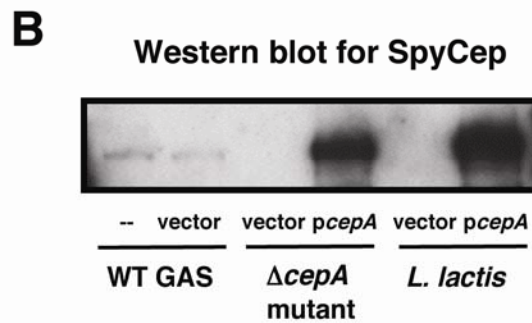
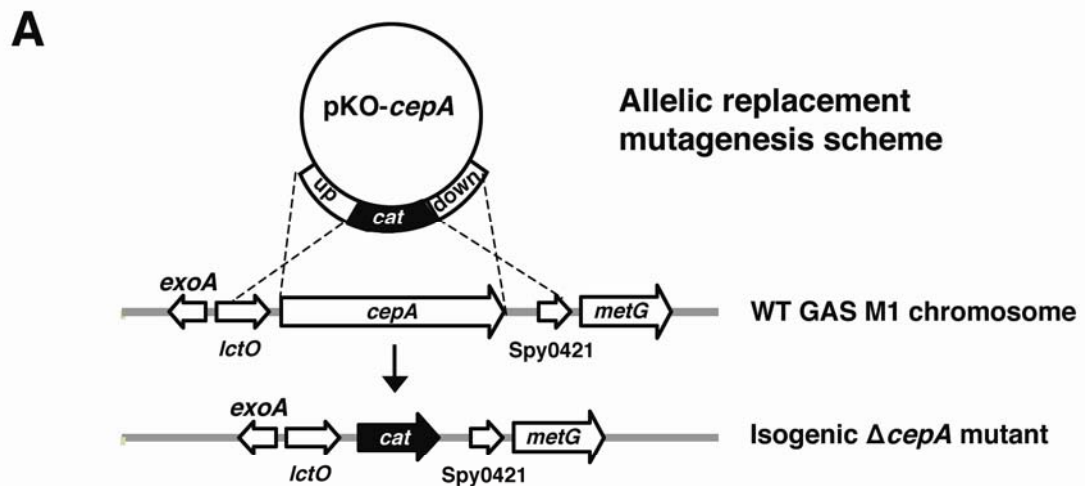
Supplemental Data

Cell Host & Microbe, Volume 4

### IL-8 Protease SpyCEP/ScpC Promotes Group A Streptococcal Resistance to Neutrophil Killing

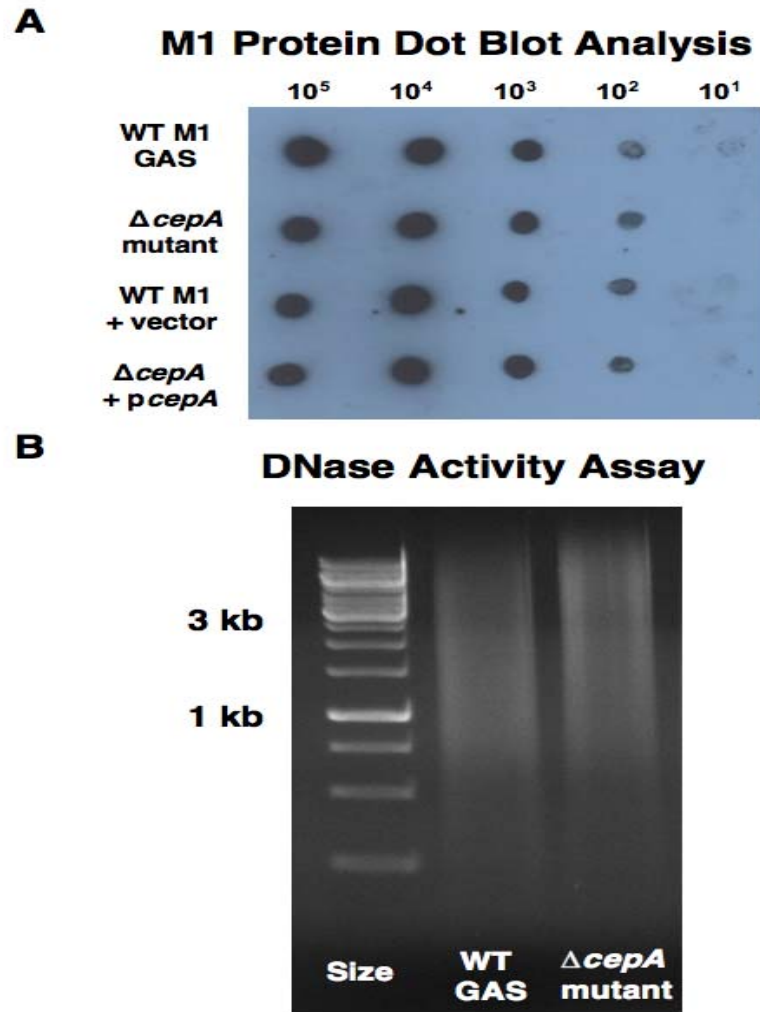
Annelies S. Zinkernagel, Anjuli M. Timmer, Morgan A. Pence, Jeffrey B. Locke, John T. Buchanan, Claire E. Turner, Inbal Mishalian, Shiranee Sriskandan, Emanuel Hanski, and Victor Nizet

Supplemental Figures



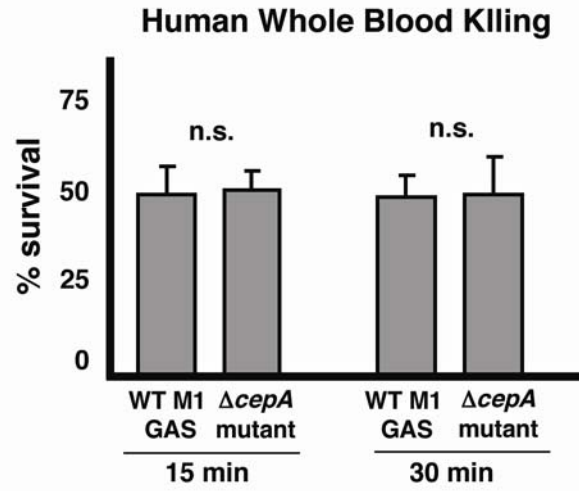
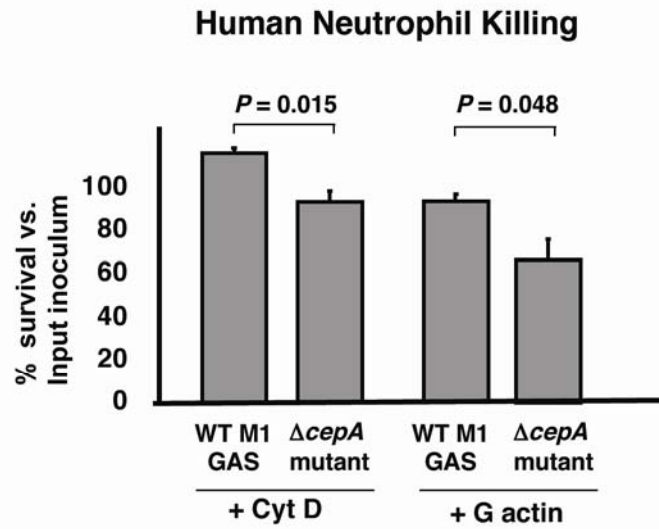
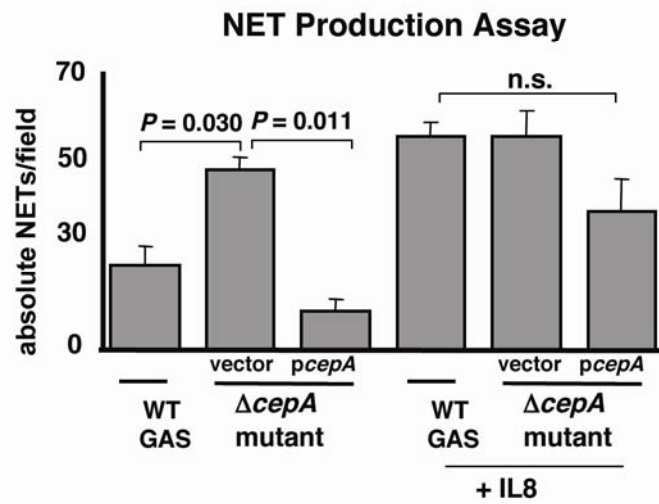
**Figure S1:** Mutagenesis and heterologous expression of IL-8 protease SpyCEP. (A) Scheme for precise, in-frame allelic replacement of the *cepA* gene in M1 GAS; (B) Western blot confirms loss of SpyCEP expression upon targeted mutagenesis in M1 GAS, complementation of the mutant *in trans*, and heterologous expression of SpyCEP in *Lactococcus lactis*; (C) Presence of SpyCEP correlates with IL-8 protease activity by SDS-PAGE .

## Supplementary Figure S2



**Figure S2:** Elimination of SpyCEP does not affect M1 protein or DNase expression. (A) M1 protein expression is quantified by dot blot for the WT M1 GAS parent strain (with and without the vector pDCerm), isogenic  $\Delta cepA$  mutant and the complemented  $\Delta cepA$  mutant. (B) DNase activity

assessed by degradation of calf thymus DNA by supernatants of WT M1 GAS parent vs. isogenic  $\Delta cepA$  mutant as compared by agarose gel electrophoresis.

**A****B****C**

**Figure S3:** Additional studies on the role of SpyCEP in GAS phagocyte resistance. (A) No difference in human whole blood survival observed between the WT M1 GAS strain and the isogenic  $\Delta cepA$  mutant. Experiments performed in triplicate and repeated three times with similar results; representative experiment, mean  $\pm$  SEM. (B) WT GAS parent strain shows enhanced neutrophil survival compared to the isogenic  $\Delta cepA$  mutant when phagocytosis is inhibited by cytochalasin D (Cyt D) or NET degradation is inhibited by G-actin. Experiments performed in triplicate and repeated three times with similar results; representative experiment shown, mean  $\pm$  SEM. (C) In the presence of excess exogenous IL-8 differences in induction of neutrophil extracellular traps (NETs) in response to bacterial exposure is abolished; quantitative enumeration of NETs, experiments performed in triplicate and repeated two to three times with similar results; representative experiment  $\pm$  SEM, two-sided T test.

## Amino acid alignment of Group A Streptococcal SpyCEP and the homologous *Streptococcus iniae* protease, Ceph

*S. iniae* Ceph MEKKEERFSLRKYKSGIVSVLIGTVFAGASQVSAADQLITVSL-----IAEETPSLTKVEVDGQTEENSNDKLTM-----SADQLTAEDLKELEESI  
 GAS SpyCEP MGKRRQFSLRKYKSGIVSVLIGSVLVMITTVAADELSTMSSEPTITNHAQQQAQHLTNTELSSAESKSDTTSQITPKTNREKEQSQDLVSEPTTTEADT  
 1.....100

*S. iniae* Ceph L S A D ----- S S A E K E E S V P L L D A P I N K D T H D W T Q V S G A W E N G Y K G Q G K V I A I I D T G I D V N H Q A M R I S D I S O A K F K T A E D M D Q Q K A K A K I N Y G K W I N Q K V  
 GAS SpyCEP D A A S M A N T G P D A T Q K S A S L P --- P V N T D I H D W V K T K G A W D N G Y K G Q G K V A V I D T G I D P A H Q S M R I S D V S T A K V K S K E D M L A R Q K A A G I N Y G S W I N D K V  
 101.....200

*S. iniae* Ceph I F A H N Y V E N N D K V K E V K E F D F D F D I E D D S I L D S I E S T L V Q S V D K K R Y R V P K S N S D K P K E T V I Q I N P D F S H I I D W P S H D D E S Q H E S H G M H V T G I A V G N  
 GAS SpyCEP V F A H N Y V E N S D N I K E N Q E F D F D E D W E N F E F D A E A E P K A I --- K K H K I Y R P Q S T Q A P K E T V I K T E E T D G S H D I D W T Q T D D D T K Y E S H G M H V T G I A V G N  
 201.....300

*S. iniae* Ceph P L E A S P I G E R F L G A P E A Q V I F M R V F A N D F M G T G E A L V I K A I E D A V A L G A D A I N L S L G G P N G S F L G G N A S L M A A I E K A K K A G V S V I V A A G N E R L F G S D H A  
 GAS SpyCEP S K E A A A T G E R F L G A P E A Q V N F M R V F A N D V M G S A E S L F I K A I E D A V A L G A D V I N L S L G T A N G A Q L S G S K P L M E A I E K A K K A G V S V I V A A G N E R M Y G S D H  
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 GAS SpyCEP D P L A T N P D Y G I V G S P S T G R T P T S V A A I N S K W V I Q R L M T V E E L E K R A D I N H G K A I V S E S W D F K N I K D S L G Y D K S H Q F A V V K S E T D A G Y N A Q D V K G K I A L I E  
 401.....500

*S. iniae* Ceph R H L D H P Y V E L I A N A K K H E V A G I L I F N H I P G Q S N R K M R L T S E G Q V I P S A F I S H E F G K A M S Q L N G N G T G R I R F E S K L S K A S N O R S Q Q M N H F S S W G L T S D G Y L  
 GAS SpyCEP R D P N K T Y D E M I A L A K K H G A L G V L I F N N K P G Q S N R S M R L T A N G M G I P S A F I S H E F G K A M S Q L N G N G T S L E F D S V V S K A P S Q R G N E M N H F S N W G L T S D G Y L  
 501.....600

*S. iniae* Ceph K P D I T A P G G D I Y S T Y N D N H Y G S Q T G T S M A S P Y I A G A S L L K Q O Y I E A Q H P D V K T E E M S D I V K Y L L M S N A S I H K D P K T Q L T T S P R R Q G A G L L N V Q A A V T S G L  
 GAS SpyCEP K P D I T A P G G D I Y S T Y N D N H Y G S Q T G T S M A S P Q I A G A S L L V K O Y L E K T Q P L P K E I A D I V K N L L M S N A Q I H V N P E T K T T T S P R Q Q A G L L N I D G A V T S G L  
 601.....700

*S. iniae* Ceph Y L T G S D N Y G S I S L G N L G E K I S F D V T V H N L S N H A K K L R Y V T D L M T D K V - - E D G R F T L S S V A L K S Y Q G H L V D V P A K G Q T I I R V S M D V S E F T K I L T K Q M P N G Y  
 GAS SpyCEP Y V T G K D N Y G S I S L G N I T D T M T F D V T V H N L S N K D K T L R Y D T E L L T D H V D P Q R G R F T L T S R S L K T Y Q G G E V T V P A N G K V T V R V T M D V S Q F T K E L T K Q M P N G Y  
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*S. iniae* Ceph Y L E G F V R F E N A T S T K D R N I P F V G F K G E F Q N L A V V E S I Y N L A A K K E K G F Y F E S K T P E I Y V G K H Y T G L V I T G A D A N V S T R I T S D N G I H T L G T Y R N K D  
 GAS SpyCEP Y L E G F V R F R D S Q D Q L N R V N I P F V G F K G Q F E N L A V A E E S I Y R L K S Q G K T G F Y F D E S G P R D D I Y V G K H F T G L V I L G S A T N V S T K T I S D N G L H T L G T F R N A D  
 801.....900

*S. iniae* Ceph G K F I L E K D Q S G N V L A I S P N G D K N Q D F V A F K G V F L R K Y K G L K A S Y R A D D H K R Q Q L L W T S - Q A H N G E K N V H S D I R F P Q S T I L L S I E F S G R S L S G E D L P D G  
 GAS SpyCEP G K F I L E K N A Q G N P Y L A I S P N G D N N Q D F A F K G V F L R K Y Q G L K A S Y Y H A S D K E H K N P L W V S P E S F K G D K N E S D I R F A K S T I L L G T A E S G K S L F G A E L P D G  
 901.....1000

*S. iniae* Ceph K Y Q Y V V S Y P D V I G A K S Q E M V F D V I V D R E K P L L T S A S F N P E T R E F K A L D V H D R G Q S G L L R D S V F Y L E K D G K P Y T I S I N Q G F K Y V S V A D N K V F V G K S K D G  
 GAS SpyCEP Y Y H Y V V S Y P D V V G A K R Q E M T F D M I I D R O K P V L S Q A F E D P E T N R E K P E L R D R G L A G V R K D S V F Y L E R K D N K P Y T V T I N D S Y K Y V S V E D N K T F E V E R Q A D G  
 1001.....1100

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 GAS SpyCEP S F I L P L D K A K L G D F Y Y M V E D F A G N V A I A K L G D H L P Q T L G K T P I K L K L T D G N Y Q T K E T L R D N L E M T Q S D T G L V T N Q A Q L A V V H R N Q P S Q L T K M N Q - D F F I  
 1101.....1200

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 GAS SpyCEP S P N E D G N R D F V A F R G L E N N V Y N D L I V N V V A K D D H Q K Q T P I W S S Q A G A G A S A E S T A W Y G I T A R G S K V M P G D Y Q V V T Y R D E H G K E H Q K Y T I S V N D K K P M  
 1201.....1300

*S. iniae* Ceph I T K A S F Q K D G D K E F F K P G K V L D L N Q V G I A R E E V F Y L E K E G R K Y D I A T V D D L V T I S D R R V L I P R N A D G S Y T I P K V E G V T P A D F F Y L V E D M A G N I V Y S S L L  
 GAS SpyCEP I T Q G R F D T I N G V D H F T P D K T K A L G S S G I V R E E V F Y L A K K N G R K E D V T E G K D G I T W S D N R V Y I P K N P D G S Y T I S K R D G V T L S D Y Y Y L V E D R A G N M S E A T L R  
 1301.....1400

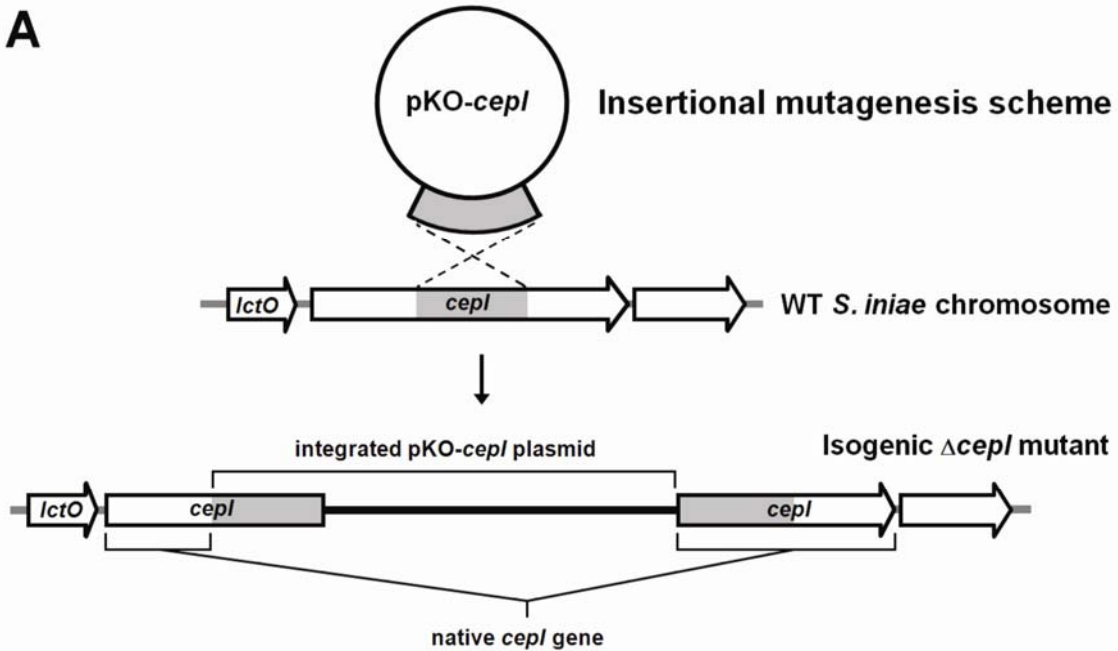
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 1401.....1500

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 GAS SpyCEP L A T S Q I T A H F D H L L P E G S R V S L K T A Q G Q L I P L E Q S L Y V P K A Y G K T V Q E G T E V V V S L P R G Y R I E G N T K V N T L P N E V H E L S E R L V K V G D A S D S T G D H K V M S  
 1501.....1600

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 GAS SpyCEP K N N S Q A L T A S A T P F K T T T P A T A K A L P S T G E K M G L K L R I V G L V L L G L T C V - E S R K R S T K - D  
 1601.....1650

**Figure S4.** Homologue of GAS SpyCEP identified in *Streptococcus iniae*. Amino acid alignment of CepA (M14 GAS strain JS95) and CepI (*S. iniae* strain 9117) with high level of similarity; identical amino acids shaded in black and similar amino acids shaded in gray. Alignment generated with ClustalW and shaded using BOXSHADE.



**A****B**

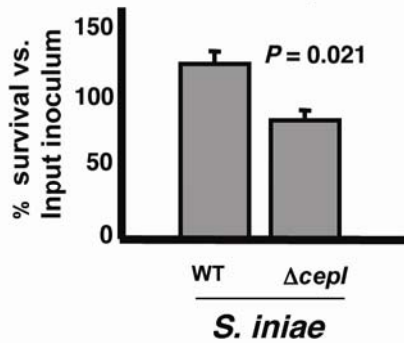
IL-8 Western Blot



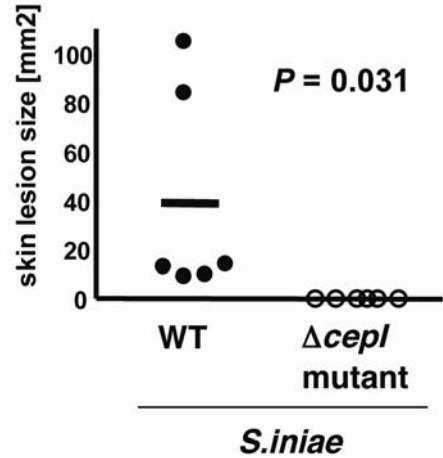
WT  $\Delta$ *cepl*  
mutant  
*S. iniae*

**C**

Neutrophil killing assay

**D**

Subcutaneous Infection



**Figure S5.** *Streptococcus iniae* CepI contributes to IL-8 degradation and neutrophil resistance. (A) Insertional mutagenesis scheme for the *S. iniae cepA* homologue, *cepI*. (B) Anti-IL-8 Western blot confirms cleavage of IL-8 by *S. iniae* reduced in  $\Delta cepI$  mutant. (C) SpyCEP contributes to *S. iniae* survival upon co-incubation with human neutrophils, experiments performed in triplicate and repeated twice with similar results; representative experiment  $\pm$  SEM. (D) C57Bl6 mice injected subcutaneously with equivalent inocula of WT *S. iniae* (left flank) or the the isogenic  $\Delta cepI$  mutant (right flank); skin lesion progression measured for 2 days.