# **Supplementary Material**

# Coiled-coil irregularities of the M1 protein structure promote M1-fibrinogen interaction and influence group A *Streptococcus* host cell interactions and virulence

Satoshi Uchiyama, Federica Andreoni, Claudia Zürcher, Katrin Schilcher, Miriam Ender, Jerzy Madon, Ulrich Mattı, Partho Ghosh, Victor Nizet, Reto A. Schuepbach, Annelies S. Zinkernagel

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### **Materials and methods Supplement**

#### **Bacterial growth curves**

Overnight cultures were diluted to  $OD_{600}$  0.15 in THB, grown at 37°C in a static incubator to mid-log phase and diluted again to an  $OD_{600}$  of 0.05 in THB, at which point growth assessment was started. Cell growth was measured by assessment of the  $OD_{600}$  every 30 minutes.

# **D**Nase activity measurement

The DNase activity was tested as previously described [1].

#### SLO activity measurement

The SLO activity of mid-logarithmic phase supernatants was measured using a haemolysis assay as described [2]. Haemolytic activity was quantified as follows: 100% haemolysis was reached by incubating red blood cells in deionised water. The reciprocal of the highest dilution of the supernatants that resulted in 50% or more haemolysis was used to quantify SLO activity.

#### **Supplemental Figures**

S 1

#### A Scheme of M\* protein



Fig S1. Scheme of the M1\* protein domains with position of the mutations introduced in the B domain and schematic drawing for precise in-frame allelic replacement of the *emm1* gene by *emm1*<sup>\*</sup> in M1 GAS



Fig S2. Characterization of the GAS M1\* strain. (A) Bacterial growth curves were performed over 4 hours for GAS M1,  $\Delta$ M1 and M1<sup>\*</sup> strains. (B) GAS DNase activity was assessed by agarose gel electrophoresis. One representative experiment out of 3 independent experiments is shown. (C) SLO activity was quantified by measuring human red blood cell hemolysis, data were pooled (means ± SD) from three independent experiments each done in duplicate.

# **References**

1. Buchanan JT, Simpson AJ, Aziz RK, Liu GY, Kristian SA, Kotb M, Feramisco J, Nizet V (2006) DNase expression allows the pathogen group A Streptococcus to escape killing in neutrophil extracellular traps. Curr Biol 16: 396-400. DOI S0960-9822(06)01021-9 [pii] 10.1016/j.cub.2005.12.039

2. Ruiz N, Wang B, Pentland A, Caparon M (1998) Streptolysin O and adherence synergistically modulate proinflammatory responses of keratinocytes to group A streptococci. Mol Microbiol 27: 337-346