

Supplementary Material

Group B streptococcal hemolytic pigment impairs platelet function in a two-step process

Kristin Jahn ¹, Patience Shumba ¹, Phoenicia Quach ², Mathias Müsken ³, Jan Wesche ⁴, Andreas Greinacher ⁴, Lakshmi Rajagopal ^{2,5,6}, Sven Hammerschmidt ^{1*}, and Nikolai Siemens ^{1*}

- 1 Department of Molecular Genetics and Infection Biology, Interfaculty Institute for Genetics and Functional Genomics, Center for Functional Genomics of Microbes, University of Greifswald, Germany; kristin.jahn@uni-greifswald.de (K.J.); patience.shumba@uni-greifswald.de (P.S.); sven.hammerschmidt@uni-greifswald.de (S.H.); nikolai.siemens@uni-greifswald.de (N.S.)
 - 2 Department of Global Health, University of Washington, Seattle, Washington, USA; pjfquach@gmail.com (P.Q.); lakshmi.rajagopal@seattlechildrens.org (L.R.)
 - 3 Central Facility for Microscopy, Helmholtz Centre for Infection Research, Braunschweig, Germany; mathias.muesken@helmholtz-hzi.de (M.M.)
 - 4 Department of Transfusion Medicine, Institute of Immunology and Transfusion Medicine, University Medicine Greifswald, Germany; wesche@uni-greifswald.de (J.W.); andreas.greinacher@med.uni-greifswald.de (A.G.)
 - 5 Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, Washington, USA
 - 6 Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington, USA
- * Correspondence: nikolai.siemens@uni-greifswald.de; Tel.: +4938344205711 (N.S.); sven.hammerschmidt@uni-greifswald.de; Tel.: +4938344205701 (S.H.)

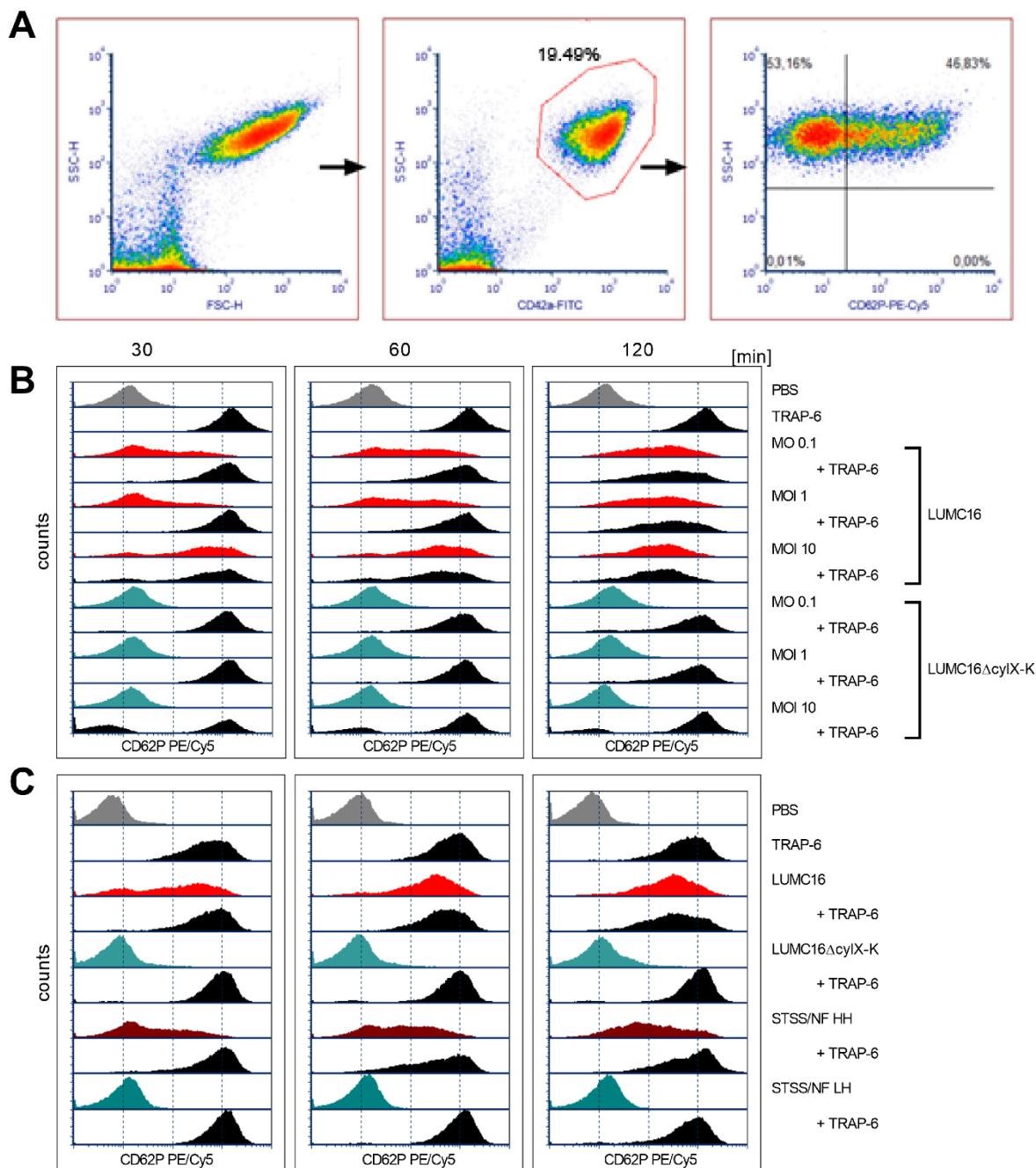


Figure S1. Washed human platelets were infected with the pigmented LUMC16 and the non-pigmented LUMC16 Δ cylX-K GBS strains at MOI 0.1, MOI 1.0, and MOI 10 or with the pigmented STSS/NF HH and the non-pigmented STSS/NF HH at MOI 0.1. (A) Gating strategy of infected platelets. To exclude overlapping of bacteria and platelets in SSC/FSC scatter plot, platelets were labelled with CD42a. Activation of CD42a-positive cells was analyzed via CD62P PE/Cy5 staining. (B and C) Representative histograms for each platelet treatment/infection condition.

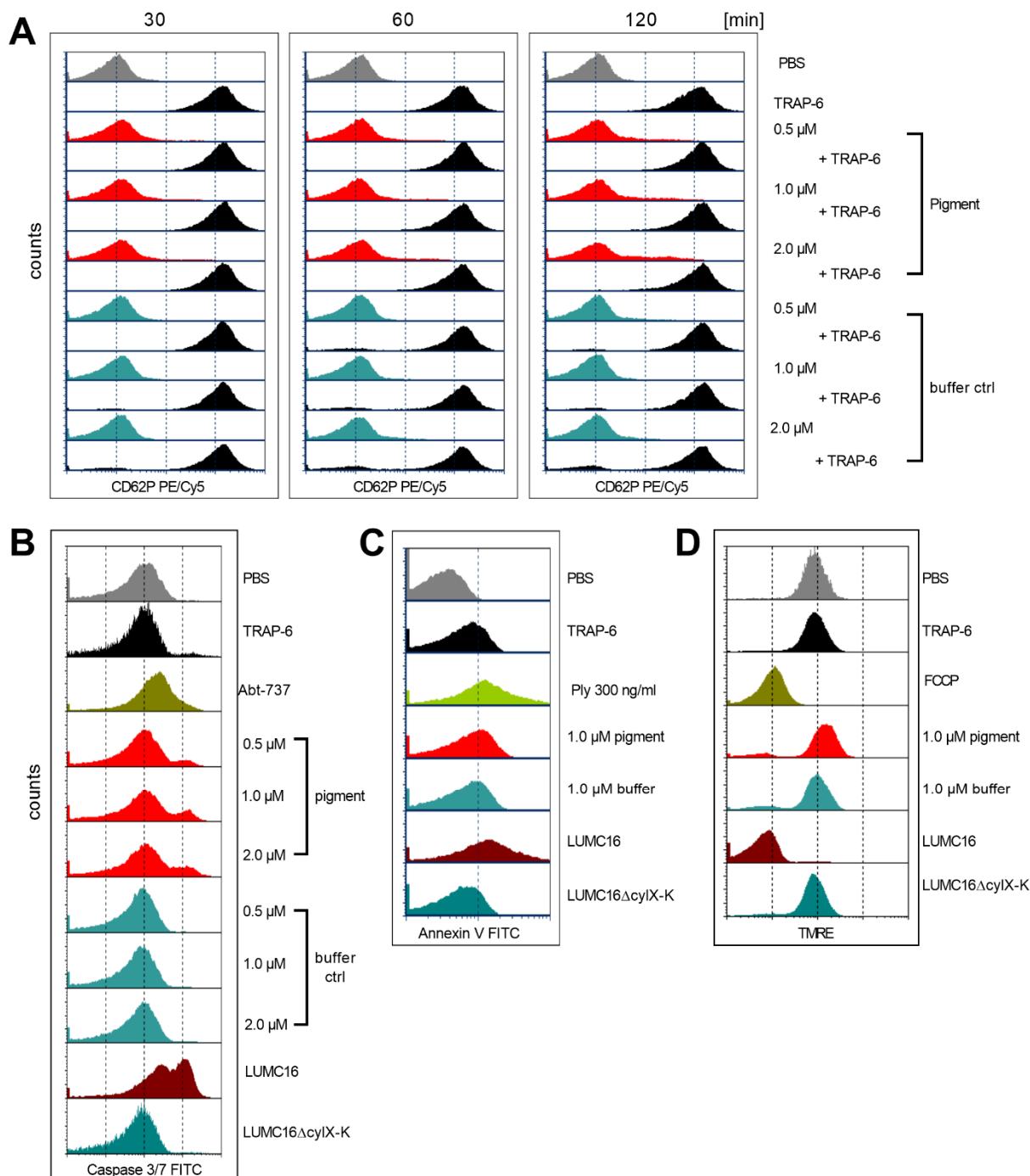


Figure S2. Washed human platelets were infected with the pigmented LUMC16 and the non-pigmented LUMC16 Δ cylIX-K GBS strains at MOI 0.1 or treated with increasing concentration of pigment and/or the respective buffer control. Shown are representative histograms for (A) CD62P dependent platelet activation, (B) caspase 3/7 activity, (C) phosphatidylserine positivity, and (D) changes of the inner mitochondrial membrane potential.