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## Proteomic characterization of host-pathogen interactions using human cell lines infected with *Staphylococcus aureus* HG001 as a model

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### CONTENT OF SUPPLEMENTARY MATERIAL

1. Supplementary\_Material\_01\_Details of LC-MS-analysis.docx  
*Information on parameters for LC-MS/MS analysis for the NP and the cell line comparison project.*
2. Supplementary\_Material\_02\_Genedata\_Refiner\_Workflow\_identification\_NP.pdf
3. Supplementary\_Material\_03\_Genedata\_Refiner\_Workflow\_quantification\_NP.pdf
4. Supplementary\_Material\_04\_Voronoi-like\_treemaps.pdf  
*Voronoi-like treemaps of Figure 37, 52, 53, and 64 are provided as pdf-file to allow zooming into pathways.*
5. Supplementary\_Material\_Table\_01\_MRM\_transitions\_S\_aureus\_HG001.xlsx  
*MRM transitions for targeted S. aureus HG001 proteins. The finally acquired list is presented. Precursor and product m/z data as well as optimized collision energies are given together with start and end retention times as the instrument was run in scheduled mode.*
6. Supplementary\_Material\_Table\_02\_MRM\_transitions\_B\_pertussis\_Tohama\_I.xlsx  
*MRM transitions acquired to identify and relatively quantify proteins of B. pertussis Tohama I. A list of proteins, sequences, corresponding precursor-product pairs and collision energies is provided. All transitions were acquired to allow accurate identification. Only peptides detectable in all replicates were used for relative comparison in different samples and are highlighted in gray. For peptides written in bold letters additional MS/MS spectra are available for validation of peaks.*
7. Supplementary\_Material\_Table\_03\_S\_aureus\_DB\_NCTC8325\_BLAST\_HGW.xlsx  
*The database of S. aureus HG001 was updated by strain comparisons in order to find functions of proteins for similar sequences. Additionally the source of the new functional annotation is provided. This database was used as the new in-house database for studies on S. aureus HG001.*
8. Supplementary\_Material\_Table\_04\_S\_aureus\_proteins\_quantified\_from\_infected\_celllines.xlsx  
*All staphylococcal proteins which could be quantified with unique peptides after internalization by A549, S9 or HEK 293 cells are listed. Mean values of median-normalized protein intensities from three BR of internalized samples (2.5 h and 6.5 h after infection) were related on the non-adherent (non-ad) control. Ratio values to the control <0.5 are highlighted in blue, ratio values to the control >2 are highlighted in red. Data that were not available are labeled "na" and highlighted in gray.*

9. Supplementary\_Material\_Table\_05\_TEM\_slides\_A549\_S\_aureus\_cell\_counting.xlsx  
*A549 cells infected with S. aureus HG001 were prepared for transmission electron microscopy (TEM). At three sampling points (2.5 h, 4.5 h, and 6.5 h p.i.) each two slices were analyzed by TEM and infected and non-infected A549 cells as well as bacteria were counted. Average values, standard deviation and coefficient of variance (CV) are given. Values that were not available are labeled “na”.*
10. Supplementary\_Material\_Table\_06\_extracellular\_metabolome\_host.xlsx  
*The extracellular metabolome was measured by <sup>1</sup>H-NMR in the supernatant of A549 (four BR), S9 (three BR), and HEK 293 (four BR) cells one hour after exposure to the cells. Average, standard deviation and coefficient of variation (CV) are given for each metabolite.*
11. Supplementary\_Material\_Table\_07\_intracellular\_metabolome\_host.xlsx  
*The intracellular metabolome of A549, S9, and HEK 293 cells was analyzed by GC-MS and LC-MS one hour after treatment with sterile infection mix. Average values, standard deviations and coefficient of variation (CV) from each four BR were calculated.*
12. Supplementary\_Material\_Table\_08\_S\_aureus\_internalized\_by\_A549.xlsx  
*Mean values of median-normalized protein intensities from three BR of S. aureus HG001 pMV158GFP internalized by A549 cells are displayed. Data were acquired hourly between 1.5 h and 6.5 h after infection and are normalized on the 1.5 h time-point. Ratio values to the 1.5 h point in time <0.5 are highlighted in blue, ratio values to the 1.5 h point in time >2 are highlighted in red.*
13. Supplementary\_Material\_Table\_09\_the\_SEED\_functional\_categories\_S\_aureus\_HG001.xlsx  
*List of S. aureus HG001 proteins assigned to functional categories in three levels according to the SEED (Overbeek et al., 2005) are displayed. This list was used for physiological interpretation of S. aureus proteome response to internalization by human cell lines.*
14. Supplementary\_Material\_Table\_10\_S\_aureus\_HG001\_proteins\_in\_isolated\_phagosomes\_shotgun.xlsx  
*Five BR of proteome analysis of S. aureus HG001 pMV158GFP detected inside infected phagosomes from S9 cells are displayed. Shotgun results were complement with average values, standard deviation, coefficient of variation (CV), ratio data, fold changes, and p-values together with false discovery rate (FDR) determination. When no spectral counts were detected, “0” was replaced by “0.1”. P-values and FDR <0.05 are highlighted in yellow. Regulated proteins are labeled “yes” and highlighted in green. Fold change values 6.5 h/2.5 h <-1.5 are highlighted in blue, those >1.5 in red.*
15. Supplementary\_Material\_Table\_11\_PSORT\_NCTC\_8325\_protein\_localization.xlsx  
*Localization of S. aureus proteins detected inside isolated phagosomes was performed using the PSORTb algorithm (Nakai and Horton, 1999).*

16. Supplementary\_Material\_Table\_12\_PSORT\_S\_aureus\_isolated\_from\_phagosomes\_or\_sorted\_from\_S9\_cell\_debris.xlsx  
*Proteins of S. aureus HG001 pMV158GFP which were internalized by S9 cells were analyzed after isolation of phagosomes or sorting of bacteria from host cell debris. Information are provided if proteins were quantified in one of the categories or not (na). Further the localization prediction according to the PSORTb algorithm (Nakai and Horton, 1999) are given in order to compare differences in percentage of protein classes depending on the localization.*
17. Supplementary\_Material\_Table\_13\_B\_pertussis\_iron\_limitation\_spectral\_counting.xlsx  
*Spectral counts of B. pertussis Tohama I proteins measured during iron-supply and iron-limitation are displayed. Mean values and coefficient of variation (CV) of three BR are displayed as well as ratio data, p-values from student's t-test and q-values from multiple testing correction according to Benjamini-Hochberg are provided. P-values and q-values <0.05 are highlighted in yellow. Ratios between the conditions <0.66667 are highlighted in blue, ratios >1.5 are highlighted in red. Ratios that were not available were labeled "na" and highlighted in gray.*
18. Supplementary\_Material\_Table\_14\_B\_pertussis\_internalized\_spectral\_counting.xlsx  
*Spectral counts of B. pertussis Tohama I proteins measured from bacteria grown in DMEM and internalized bacteria 2 h and 48 h after infection are displayed. Mean values and coefficient of variation (CV) of three BR are displayed as well as ratio data, p-values from student's t-test and q-values from multiple testing correction according to Benjamini-Hochberg are provided. P-values and q-values <0.05 are highlighted in yellow. Ratios between the conditions <0.66667 are highlighted in blue, ratios >1.5 are highlighted in red.*
19. Supplementary\_Material\_Table\_15\_normalized\_SILAC\_ratios\_of\_A549\_proteins\_infected\_with\_S\_aureus.xlsx  
*Mean values of median-normalized protein intensities from each three BR of three different treatment groups of A549 cells (non-infected control, GFP-positive A549 cells that were infected with S. aureus HG001 pMV158GFP, A549 cells non-carrying bacteria after contact) are displayed. Mean ratio data from three BR of normalized intensities of the sample related to the heavy labeled standard A549 cells are listed. Ratios between internalized samples and the heavy labeled control <0.83333 are highlighted in blue, ratios >1.2 are highlighted in red.*
20. Supplementary\_Material\_Table\_16\_human\_proteins\_from\_isolated\_phagosomes\_of\_S\_aureus\_HG001-infected\_S9\_cells\_shotgun.xlsx  
*Five BR of proteome analysis of phagosomes isolated from human S9 cells infected with S. aureus HG001 pMV158GFP are displayed. Shotgun results were complemented with average values, standard deviation, coefficient of variation (CV), ratio data, fold changes, and p-values together with false discovery rate (FDR) determination. P-values and FDR <0.05 are highlighted in yellow. Regulated proteins are labeled "yes" and highlighted in green. Fold change values 6.5 h/2.5 h <-1.2 are highlighted in blue, those >1.2 in red.*