This section contains a list of the supplementary materials included on the attached CD-ROM.

FOLDERS

Database All samples were searched against the *S. pneumoniae* D39 database from UniProt (1,918 proteins, 2017) included in this folder. For TPP based spectral library construction the FASTA-header were shortened and replaced by the corresponding NCBI identifier.

MaxQuant For classical database search, spectra were searched separately for each strain with MaxQuant (1.6.1.0, Max Planck Institute of Biochemistry, (Cox and Mann, 2008)) and its implemented search engine Andromeda (Cox et al., 2011) against the *S. pneumoniae* D39 database from UniProt (2017).

The specific search parameters can be found in the mqpar files. The search output including the proteinGroups.txt file is attached as well.

- MaxQuant_search parameters
- MaxQuant_output

Perseus For shot gun proteomic data analyses the software Perseus 1.6.1.1 (Tyanova et al., 2016) was used.

The corresponding .sps files including the unique peptide identifications and the MaxQuant LFQ intensities are presented in this folder. Data were normalized over the median in Microsoft Excel and re-imported in Perseus.

- D39_RPMI_Unique peptides_and_LFQ
- D39_THY_Unique peptides_and_LFQ

SAM Statistical evaluation of quantified proteins was performed using RStudio (version 3.5.0) and the SAM (Significance analysis of microarrays (Tusher et al., 2001)) script by Michael Seo (https://github.com/MikeJSeo/SAM) with the implemented two-class unpaired test, which is analogous to a t-test between subjects. The input data tables and the original output tables (.xlsx files) can be found in the sub folders. Differentially expressed proteins with a minimum fold change of two and a q-value ≤ 0.01 were considered as significantly regulated.

- Input
- Original_output

Spectral library *S. pneumoniae* D39

The validated spectral library *S. pneumoniae* D39 was constructed using the Trans Proteomic Pipeline (TPP, 5.1.0-rc1 Sysygy, 2017) and can be applied for phosphorylation-centered searches.

- Final_Sp_D39_combined_splib_SILAC_fwd_decoy_CHi

DATA SHEETS

Annotation table - *Streptococcus pneumoniae* D39

Data Sheet 1 - Overview of data sets and raw files

Data Sheet 2 - LFQ results

Data Sheet 3 - ON_OFF_protein_identifications

Data Sheet 4 - STY phospho identifications_RPMI_exp

Data Sheet 5 - Summary LFQ and phosphoproteome results_RPMI_exp

Voronoi treemap collection