Table S4 LC-MS/MS settings for analysis of samples of *in vitro* cultivated *S. pneumoniae* D39 wild-type and *S.p.* D39Δ*comDE* and *S.p.* D39Δ*aliB* mutants.

a. reversed phase liquid chromatography (RPLC)

instrument	Ultimate 3000 RSLC (Dionex, Idstein, Germany)
trap column	75 µm inner diameter, packed with 3 µm C18 particles (Acclaim PepMap100, Thermo Scientific)
analytical column	Accucore 150-C18, (Thermo Fisher Scientific), 25 cm x 75 µm, 2.6 µm C18 particles, 150 Å pore size
buffer system	binary buffer system consisting of 0.1% acetic acid (buffer A) and 0.1% acetic acid in 100% ACN in (buffer B)
flow rate	300 nl/min
gradient	linear gradient of buffer B from 5% up to 25% 1 – 2 min: increase from 2% to 5% 2 – 10 min: 5% 10 – 130 min: increase from 5% to 25% 130 – 135 min: increase from 25% to 40% 135 – 137 min: increase from 40% to 90% 137 – 142 min: 90% 142 – 145 min: decrease from 90% to 2% 145 – 160 min: 2%
gradient duration	120 min (in total 160 min)
column oven temperature	40°C

b. mass spectrometry (MS)

instrument	Q Exactive mass spectrometer (Thermo Scientific)
electrospray	via TriVersa NanoMate (Advion Biosciences, Norwich, UK)
operation mode	data-dependent
full MS resolution	70,000
full MS AGC target	3e6
full MS maximum ion injection time	120 ms
full MS scan range	300 to 1,650 m/z
dd-MS2resolution	17,500
dd-MS2AGC target	2e5
dd-MS2 selection for MS/MS	10 most abundant isotope patterns with charge ≥ 2 from the survey scan
dd-MS2 maximum ion injection time	120 ms
isolation window	3 *m/z*
dissociation mode	higher energy collisional dissociation (HCD)
normalized collision energy	27.5%
dynamic exclusion	30 s
MS/MS operation mode	centroid mode, peptide match: preferred
charge exclusion	1, > 6