FIG. S1. 2D SDS-PAGE of cytoplasmic proteins from *S. pneumoniae* Rx1 (strain Sp1). Cytoplasmic proteins were separated using IPG strips pH 3-5.6 in the first dimension (1D) and 12,5% acrylamide gels in the second dimension (2D). Gels were stained with colloidal Coomassie blue G-250. Rectangle borders the part of the gel containing phosphoproteins PP1, PP2 and PP3 (Fig. 3A).

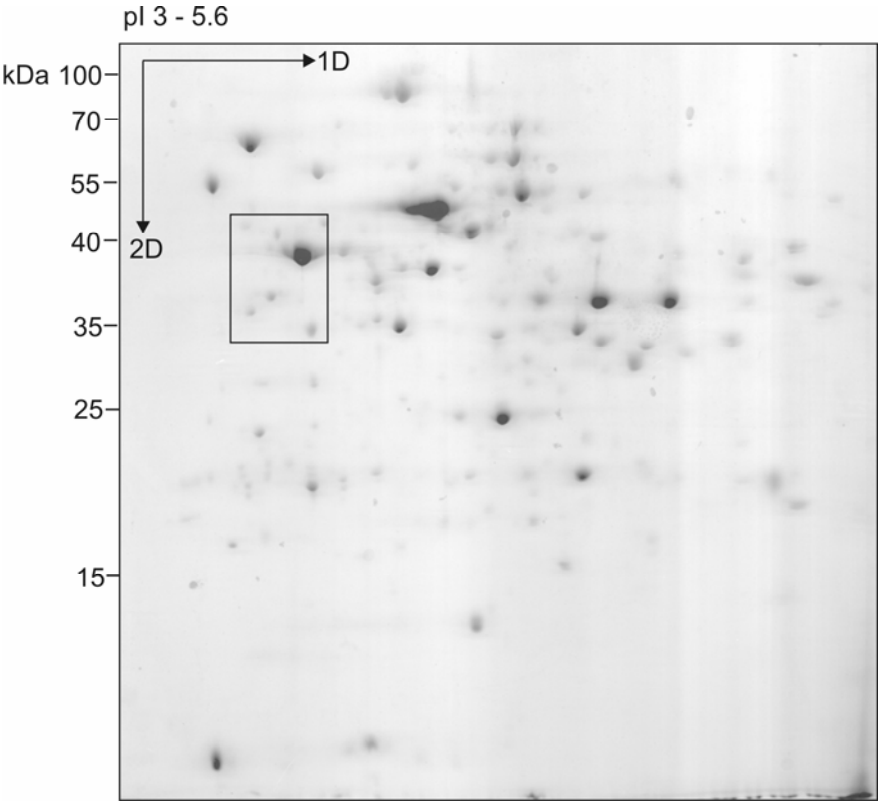

FIG. S2. 2D SDS-PAGE of membrane proteins from *S. pneumoniae* Rx1 (strain Sp1). Membrane proteins were solubilized in lysis buffer containing 4% CHAPS and separated using IPG strips pH 3-5.6 in the first dimension (1D) and 12,5% acrylamide gels in the second dimension (2D). Gels were stained with colloidal Coomassie blue G-250. Rectangle borders the part of the gel containing phosphoproteins PP1, PP2 and PP3 (Fig. 3B).

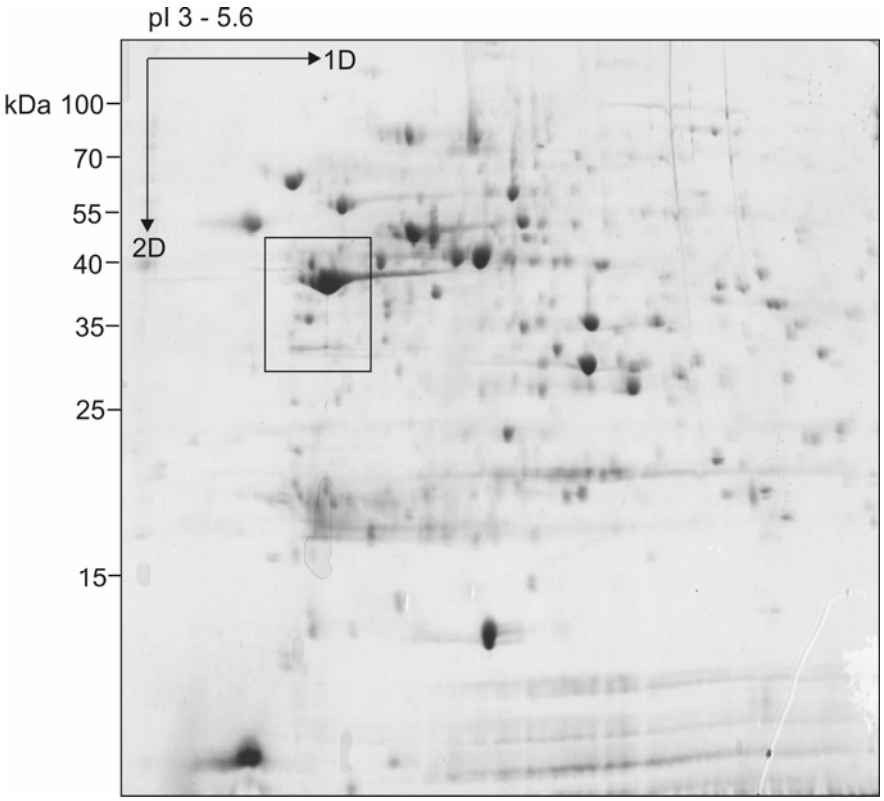

FIG. S3. 2D SDS-PAGE of membrane proteins from *S. pneumoniae* Rx1 (strain Sp1). Membrane proteins were solubilized in lysis buffer containing 4% CHAPS, 1% ASB14, 1% Triton X-100 and separated using IPG strips pH 3-10 in the first dimension (1D) and 12,5% acrylamide gels in the second dimension (2D). Gels were stained with colloidal Coomassie blue G-250. Rectangle borders the part of the gel containing phosphoproteins PP1, PP2 and PP4 (Fig. 3C).

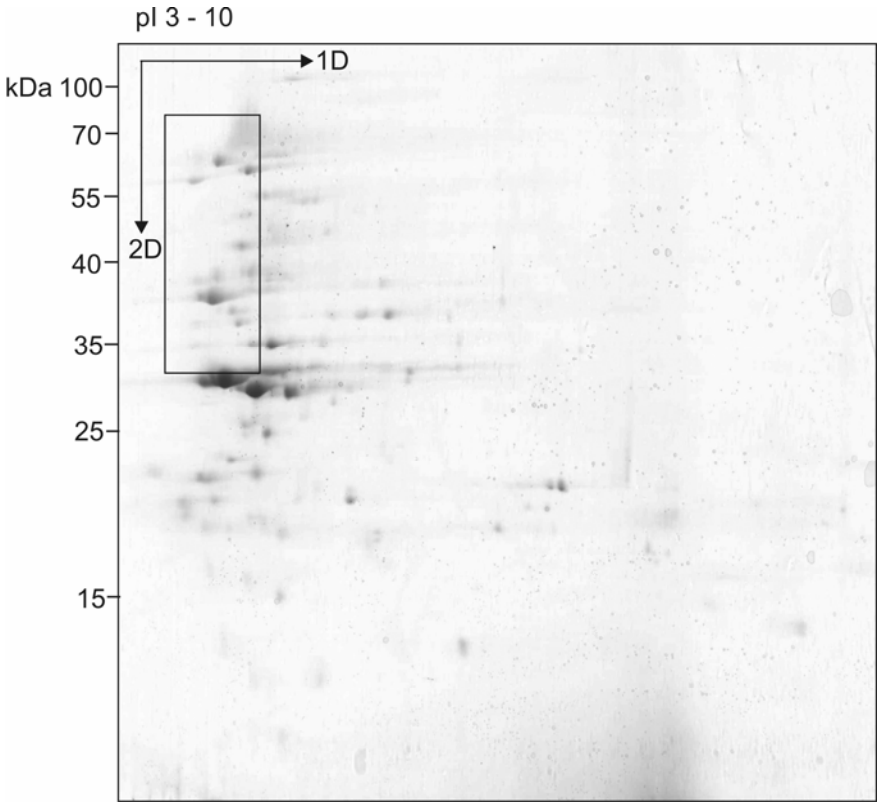

FIG. S4. Immunodetection of DivIVA and RpoA. Cytoplasmic proteins from *S. pneumoniae* Rx1 were separated on 2D SDS-PAGE using 13 cm IPG strips pH 4-7 in the first dimension and 12% acrylamide gels in the second dimension. Gels were immunoblotted and probed with antibody against phospho-threonine (anti-pThr), DivIVA (anti-DivIVA) or RpoA (anti-RpoA). Black arrows indicate proteins corresponding to phosphoproteins PP1 and PP2; white arrow indicates protein spot corresponding to enolase.

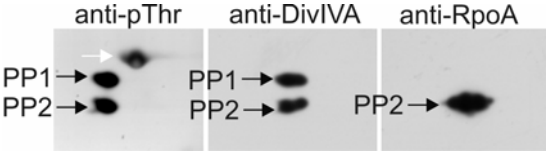

TABLE S1: Descriptive statistical analysis of the cell sizes (μm)

Strain	**minimum**	**1. quantile**	**median**	**average**	**3. quantile**	**maximum**
Sp1 (WT Rx1)	0.920	1.590	1.945	2.047	2.397	4.290
Sp10 (Δ*stkP*)	0.920	1.820	2.200	2.360	2.667	8.280