Supplementary Information
Competence-induced protein Ccs4 facilitates pneumococcal invasion into brain tissue and virulence in meningitis
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Figure S1. Deduced transmembrane topology of Ccs4. Transmembrane regions of Ccs4 were predicted using the SOSUI algorithm. Pink-shaded area indicates extracellular region with an arginine-rich region (-RFRRSARSRRS-).
Figure S2. The percentage of Δccs4 containing Ccs4-expressing vector in blood and brain homogenates. In the intravenous infection model, we counted CFU in blood and brain homogenates which isolated from complement strain-infected mice on THY agar with or without erythromycin (n = 18). The percentage of erythromycin-resistant Δccs4 [pCcs4] = (CFU counts on THY agar with erythromycin / CFU counts on THY agar without erythromycin) ×100.
Figure S3. Effects of heparin pretreatment on association with and invasion into hBMECs by *S. pneumoniae*. Association rates were calculated by dividing the CFU value obtained at 1 hour after infection by the value for the original inoculum. Invasion rates were calculated by dividing the CFU value obtained at 1 hour after antibiotic addition by the value for the original inoculum. Values are presented as the mean of 6 wells from one of 3 independent experiments. Vertical lines represent the mean +S.E. Statistical differences between groups were analyzed using a Kruskal-Wallis test with Dunn's post hoc test. *p < 0.05 and **p < 0.01.
Materials and Methods

Similarity search

We searched for proteins similar to Ccs4 of *S. pneumoniae* TIGR4 using the BLASTP program (NCBI BLAST). Proteins with an E-value <2E-100, coverage of the query >99%, and identity >40% are listed. Evaluation criteria for capsule +/- were used as presented in literature or based on the existence of *cpsA–cpsD* encoding a putative polysaccharide capsule in *S. pneumoniae* TIGR4.
| E-value | Coverage | Identity | Product | Protein ID |
|---------|----------|----------|---------|------------|
| 1.00E-132 | 49%     |          | DORA_23_24 S. parasanguis | CP019562.1 |
| 1.00E-108 | 4.00E-124 |         | Competence-induced protein Ccs4 CBZ01433.1 | CP012646.1 |
| 1.00E-108 | 99%     |          | Damage-inducible protein CinA AMH89424.1 | CP014326.1 |
| 1.00E-108 | 91%     |          | Competence-induced protein Ccs4 CBZ01433.1 | CP000919.1 |
| 1.00E-108 | 99%     |          | Competence-induced protein Ccs4 AMH89424.1 | CP002176.1 |
| 1.00E-108 | 99%     |          | Putative membrane protein CP000936.1 | CP000936.1 |
| 99%     |          |          | Damage-inducible protein CinA AMH89424.1 | CP002176.1 |
| 99%     |          |          | Putative membrane protein CP000936.1 | CP002176.1 |
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| 99%     |          |          | Damage-inducible protein CinA AMH89424.1 | CP002176.1 |
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