Supplemental Data

Deficiency of PspA or PspC reduces the severity of pneumococcal infection. An infection study was performed to confirm that the Psp knockout strains used in this study have limited virulence in vivo. Mice were infected intra-nasally with 2.5x106 cfu of the parent D39 strain and its PspA-, PspC-, and PspA-/C- derivatives. 90% of the mice infected with D39 succumbed to the infection. Mortality was reduced to 30% with the PspA- strain and 50% with the PspC- strain, while the PspA-/C- knockout strain proved to be avirulent (fig. S1).

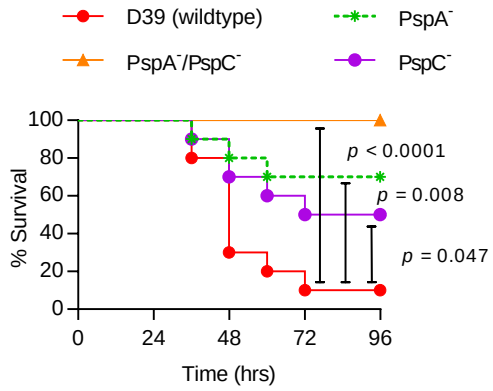

Fig S1: Deficiency of PspA or PspC reduces the severity of pneumococcal infection. C57BL/6 mice were intra-nasally infected with 2.5 x 10^6 CFU of either *S. pneumoniae* D39 wildtype, *pspA*, *pspC* or *pspA/C* mutant strains. Mice were monitored for the development of the signs of diseases and euthanized once they reached the lethargic stage. Deficiency of either PspA or PspC significantly increases the survival of mice (Mantel-cox test; n=10/group).

Supplemental Methods

Ethics Statement

Animal experiments performed in this study were authorized by the UK Home Office (Animals Scientific Procedures Act 1986; Home Office project licence 60/4327) and

approved by the animal welfare committee of the University of Leicester. Every effort was made to minimise suffering of mice. During infection, mice were humanely culled when they displayed signs of lethargy.

***In vivo* Infection**

Ten to twelve week old female wildtype C57BL/6 mice were purchased from Charles River, UK. Groups of ten mice were infected with *S. pneumoniae*: D39 (wildtype), PspA$^-$, PspC$^-$ or PspA$^-$/C$^-$. 50µl of PBS containing 2.5 x 10^6 CFU of bacteria was intranasally administered, under a light anesthesia with 2.5% (v/v) fluothane (AstraZeneca), as described previously (8). The administered dose of each bacterial strain was subsequently confirmed by colony counting on blood agar plates overnight at 37^{o}C. After infection, mice were observed for 96 hours by monitoring their clinical signs. If mice became lethargic, they were euthanized by cervical dislocation under full anesthesia. This time was recorded as the survival time of each mouse. Mice alive at 96 hours were deemed to have survived the infection.