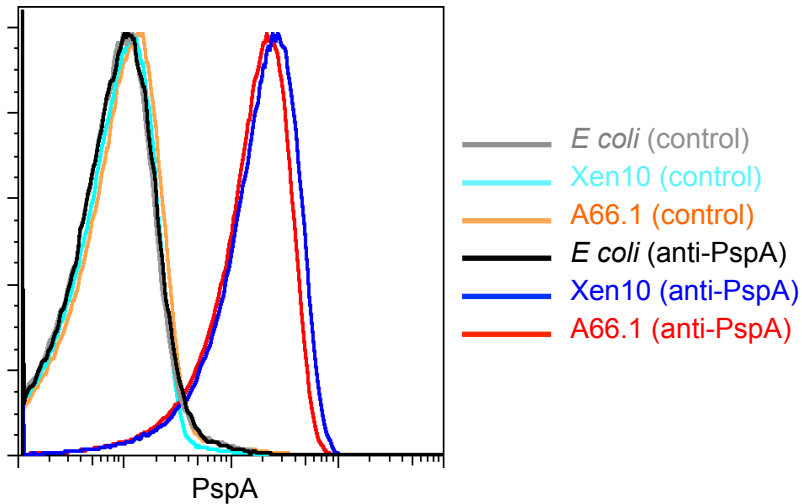
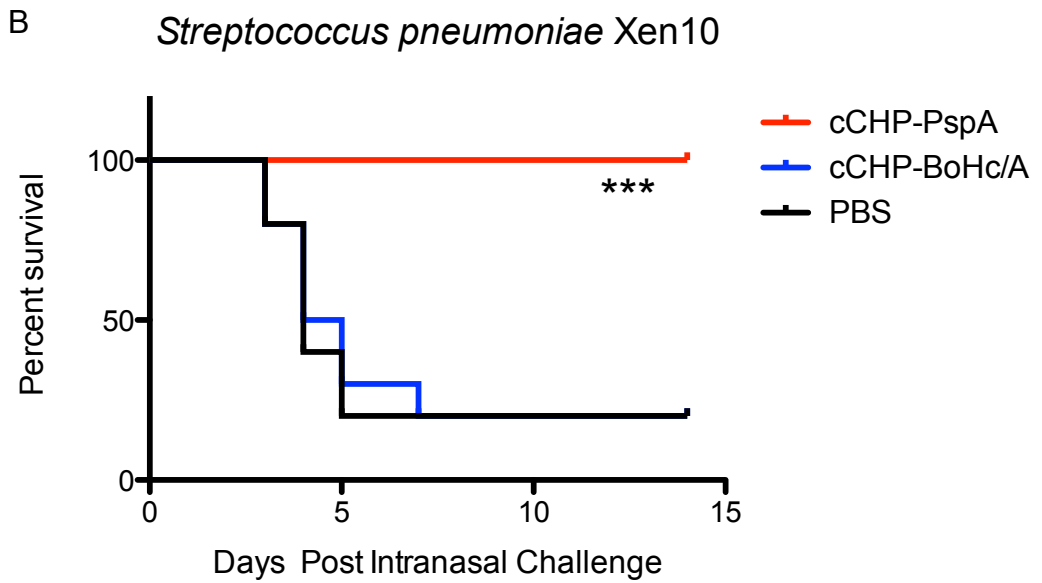
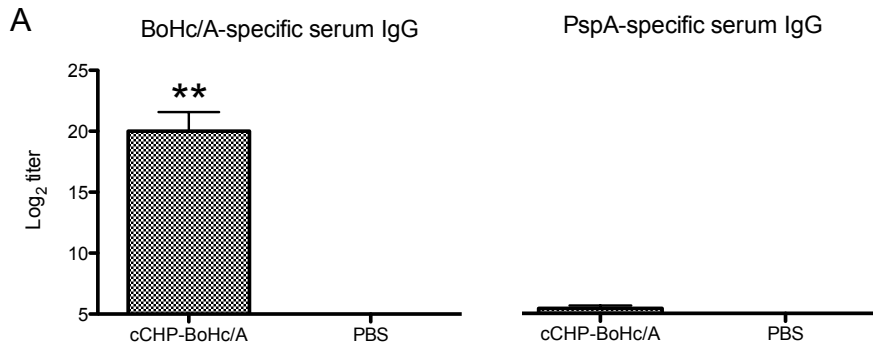


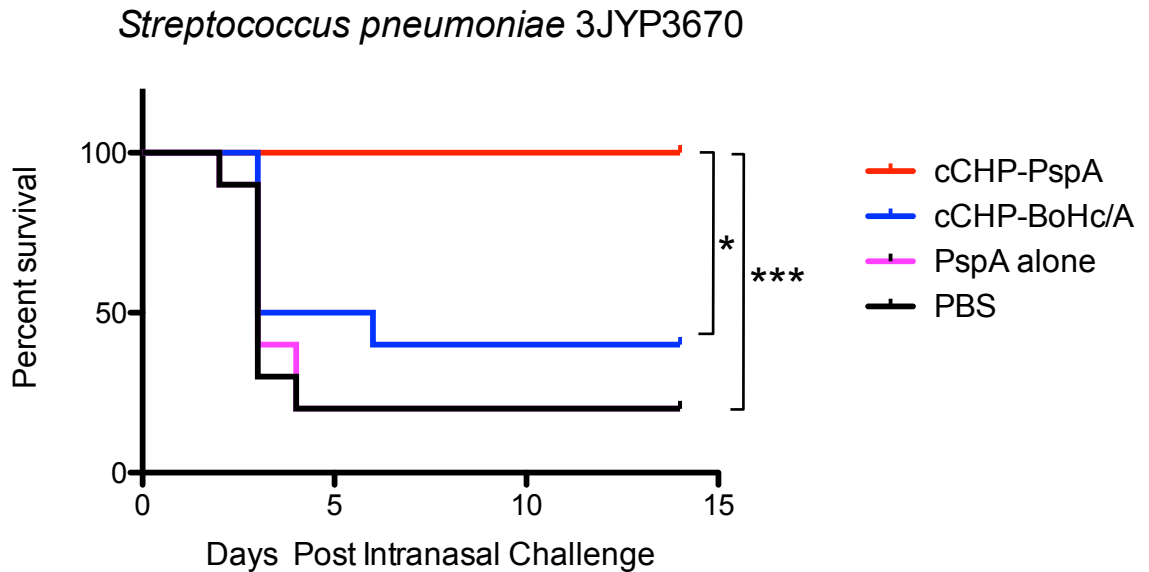
**FIG S1** Fluorescence resonance energy transfer analysis confirmed that TRITC-conjugated cCHP nanogel appropriately formed nanoparticles after the incorporation of FITC-conjugated PspA. Abbreviations: cCHP, cationic cholesteryl-group-bearing pullulan; PspA, pneumococcal surface protein A.



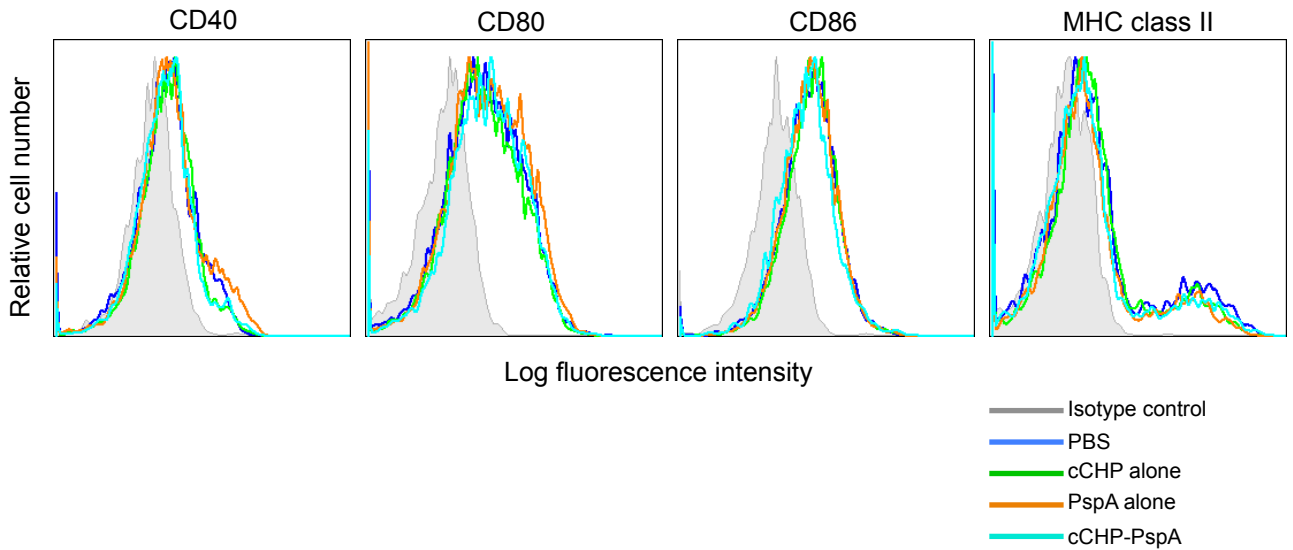
**FIG S2** PspA expression by *Streptococcus pneumoniae* Xen10 was comparable to that of its parental strain, *S. pneumoniae* A66.1. Each strain was cultured, stained with anti-PspA (or control) antibody, and treated with fluorescent anti-mouse IgG secondary antibody. Abbreviation: PspA, pneumococcal surface protein A.



**FIG S3** Induction of PspA-specific immunity was crucial to the protection of mice from lethal challenge with *Streptococcus pneumoniae* Xen10. Protective immunity against pneumococci was induced by intranasal vaccination with cCHP–PspA but not with cCHP-BoHc/A. (A) BoHc/A- and PspA-specific IgG levels in serum samples from the groups of mice immunized with cCHP-BoHc/A or PBS were analyzed 1 wk after final immunization. \*\*,  $P < 0.01$ . (B) Immunized mice were challenged with *S. pneumoniae* Xen10 ( $2 \times 10^5$  cfu/mouse), and survival was monitored. Data are representative of two independent experiments, and each group consisted of 10 mice.  $P$ -values were calculated by using Fisher’s exact test to compare the numbers of alive versus dead mice from each group. \*\*\*,  $P < 0.001$ , when compared with the groups immunized with cCHP-BoHc/A or PBS. Abbreviations: cCHP, cationic cholesteryl-group-bearing pullulan; PspA, pneumococcal surface protein A. BoHc/A, recombinant non-toxic receptor-binding fragment of *Clostridium botulinum* type-A neurotoxin subunit antigen Hc.



**FIG S4** Intranasal vaccination with cCHP-PspA induces cross-protective immunity against pneumococci. One week after final immunization, mice were challenged with *Streptococcus pneumoniae* 3JYP3670 expressing PspA of clade 4 ( $5 \times 10^4$  cfu/mouse), and survival was monitored. Data are representative of two independent experiments, and each group consisted of 10 mice. *P*-values were calculated by using Fisher exact test to compare the numbers of alive versus dead mice from each group. \*, *P* < 0.05, when compared with the group immunized with cCHP-BoHc/A. \*\*\*, *P* < 0.001, when compared with the groups immunized with PspA alone or PBS. Results after immunization with cCHP-BoHc/A, PspA-alone, or PBS were not significantly different (*P* > 0.05). Abbreviations: cCHP, cationic cholesteryl-group-bearing pullulan; PspA, pneumococcal surface protein A. BoHc/A, recombinant non-toxic receptor-binding fragment of *Clostridium botulinum* type-A neurotoxin subunit antigen Hc.



**FIG S5** Intranasal immunization with cCHP-PspA had no effect on the expressions of costimulatory molecules on dendritic cells. Nasal CD11c<sup>+</sup> cells spontaneously expressed the costimulatory molecules CD40, CD80, CD86, and MHC class II, and their expression levels remained unchanged 3 days after immunization. Abbreviations: cCHP, cationic cholesteryl-group-bearing pullulan; PspA, pneumococcal surface protein A.