T cell mediated humoral immune responses to type 3 capsular polysaccharide of *Streptococcus pneumoniae*

Supplemental Figures



Supplementary Figure 1. Purification of Glycoconjugates (**A**) Superdex 200 (OVA) and (**B**)Sephacryl 300 (KLH) elution profiles of glycoconjugates. Fractions containing the conjugates were collected, desalted, and lyophilized for long-term storage.



Supplementary Figure 2. Carrier specific IgG in conjugate immunizations. KLH IgG titers in BALB/c mice primed (day 0) and boosted (day 14) with different antigen combinations, as measured by ELISA in serum obtained on day 14 (pre-boost) and 21. n=4/group. Serum titers are reported as the reciprocal dilution that results in an OD of 0.5 at 405nm. ND=Not Detected. Statistical significance was determined with the two-tailed student's t-test.



Supplementary Figure 3. Pn3P specificity in heterologous carrier immunizations. (A) Whole fixed Pn3 cell ELISA detecting Pn3P IgG titers in BALB/c mice primed (day 0) and boosted (day 14) with different antigen combinations, as measured by ELISA in serum obtained on day 21. n=4/group. Serum titers are reported as the reciprocal dilution that results in an OD of 0.5 at 405nm. (B) Competition experiment using increasing doses of soluble Pn3P to compete for Pn3P specific IgGs in BALB/c mice primed (day 0) and boosted (day 14) with different antigen combinations, as measured by ELISA in serum obtained on day 21. Statistical significance was determined with the two-tailed student's t-test. P<0.001. NS=not significant



Supplemental Figure 4. Presence of CD4+ T cells that recognize Pn3P epitopes after Pn3P-OVA immunization. CD4+ T cells were isolated from lymph nodes of Pn3P-OVA immunized mice and stimulated *in vitro* in the presence of irradiated APCs pulsed with Pn3P, homologous (Pn3P-OVA) or heterologous (Pn3P-TT) conjugates and carrier proteins. IL-2 production was detected by ELISPOT assay. Representative results are shown from one of three independent experiments performed. Data were shown as mean+SD. *P<0.05, **P<0.01,