

Figure S1. IL-10 secretion in the respiratory tract of mice after the challenge with the ATCC6303 strain. BALF samples were collected from non immunized BALB/c mice (Non) or mice immunized with wP or PspA5-wP, at different time points after the intranasal challenge. Cells were removed by centrifugation and IL-10 was measured in the supernatants by sandwich ELISA. Results were represented as means of 4 animals per group, with the standard deviations. * represents significantly higher numbers of cells when compared with the same time point in non-immunized mice. Statistical analyses were performed by the Mann-Whiney U test. P \leq 0.05, for the comparisons marked.

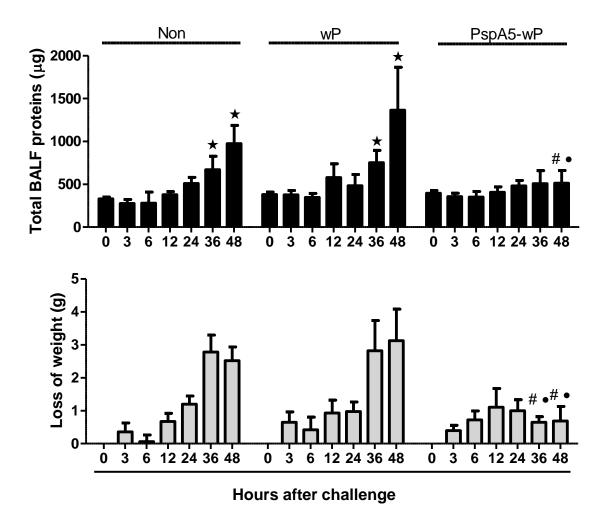


Figure S2. Total protein in the respiratory tract of mice and loss of body weight after the challenge with the ATCC6303 strain. BALF samples were collected from non-immunized BALB/c mice (Non) or mice immunized with wP or PspA5-wP, at different time points after intranasal challenge. Cells were removed by centrifugation and total protein was quantified by Bradford (A). Mice were weighted before and at different time points after the challenge, Differences in body weight were recorded (B). Results were represented as means of 4 animals per group, with the standard deviations. \star represents significantly higher levels of protein when compared to mice before the challenge (time point 0); # and • represents significant differences when compared with the same time point in non-immunized mice or mice immunized with wP, respectively. Statistical analyses were performed by the Mann-Whiney U test. P<0.05 for the comparisons marked.

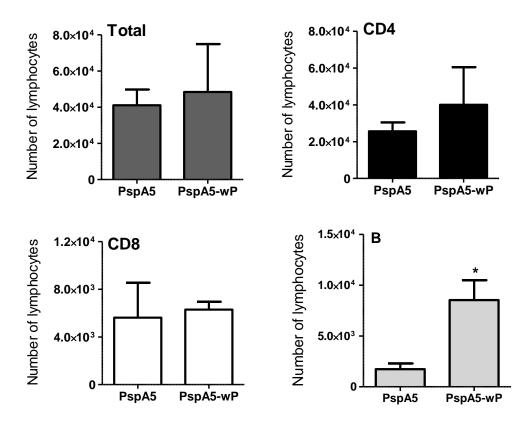


Figure S3. Characterization of the lymphocytes present in the respiratory tract of mice 10 days after the challenge with the ATCC6303 strain. Ten days after the challenge BALF samples were collected and $1X10^6$ cells were incubated with APC conjugated anti- mouse CD4 (upper right panel), PerCP conjugated anti-mouse CD8 (lower left panel) or PE conjugated anti-B220 (lower right panel). Samples were analyzed by Flow cytometry and results of 10.000 gated lymphocytes are represented as the means of total cells recovered (from groups of 3 mice) with the standard deviations. *Represents significantly different numbers of lymphocytes when compared with the group inoculated with PspA5. Statistical analyses were performed by Mann-Whitney *U* test, *P=0.05.

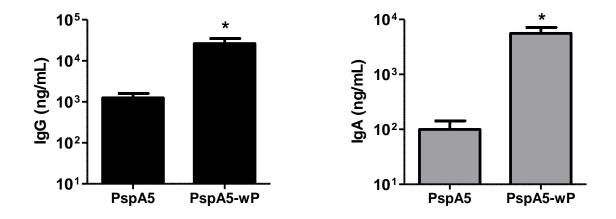


Figure S4. Evaluation of anti-PspA5 antibodies in the respiratory tract of mice 10 days after the challenge. BALF samples were collected 10 days after the challenge and anti-PspA5 IgG (right panel) or IgA (left panel) were detected through ELISA. Concentrations of antibodies (means of 3 to 6 animals) with the standard deviations are shown. *represents significant differences from the group immunized with PspA5. Statistical analyses were performed using the Mann-Whitney *U* test, *P=0.05.