

1 **Supplemental Material and Methods**

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3 *Analysis of total protein and IL-10 in Bronchoalveolar lavage fluid (BALF)*

4 Mice were sacrificed before or at different time points after the challenge through
5 injection of a lethal dose of urethane (15 mg per 10 g of body weight) and BALF
6 samples were collected as described before. Cell free fluid samples were analyzed
7 through sandwich ELISA for the presence of IL-10 (PeproTech Inc., Rocky Hill, NJ,
8 USA) according to the instructions of the manufacturers. Total protein was quantified
9 by using the Bradford Bio-Rad Protein Assay kit (Bio-Rad laboratories, Hercules, CA,
10 USA).

11 *Analysis of lymphocyte infiltration and antibodies in the respiratory tract of immunized* 12 *mice, 10 days after the challenge*

13 BALF samples were collected as described before from mice that survived the challenge
14 (10 days after the challenge, 3 mice per group). Cells were collected by centrifugation at
15 100 g for 10 min and suspensions of 10^6 cells in 100 μ L of PBS were incubated for 30
16 min on ice with either one of the following antibodies: APC conjugated anti- mouse
17 CD4 (RM4-5), PerCP conjugated anti-mouse CD8 (53-67) or PE conjugated anti-B220
18 (clone RA3-6B2) (BD Biosciences, Franklin Lakes, NJ, USA). Samples were washed
19 twice with PBS, suspended in 200 μ L of cytofix (BD Biosciences) and stored at 4°C
20 until analysis. Flow cytometry was performed using FACS Canto II (BD Biosciences),
21 with 10,000 gated lymphocytes. Samples were analyzed using the Flow Jo 7.6.1
22 software (Tree Star, Ashland, OR, USA). Cell free fluids were analyzed for antibody
23 levels by ELISA in plates coated with PspA5. The assay was performed using goat anti-
24 mouse IgG or IgA and rabbit anti-goat conjugated with HRP (Southern Biotech,

25 Birmingham, AL, USA). Standard curves were generated using mouse IgG or IgA
26 (Southern Biotech).