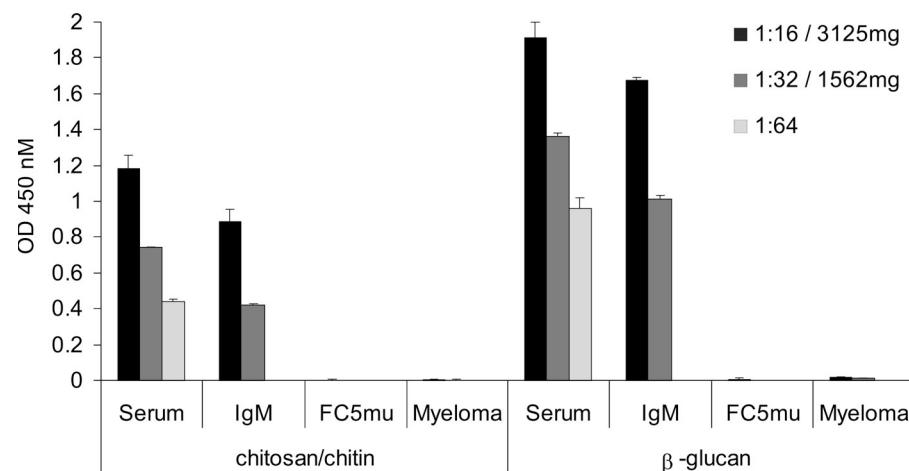
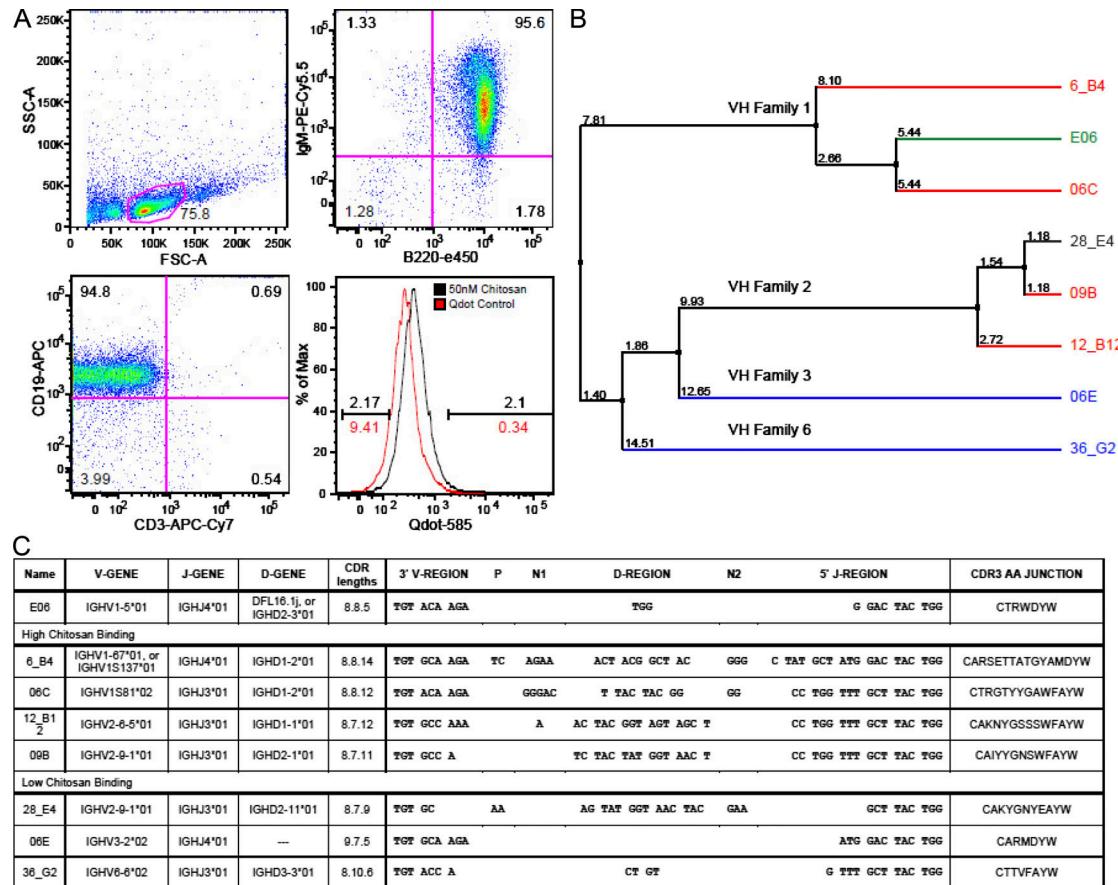


## SUPPLEMENTAL MATERIAL

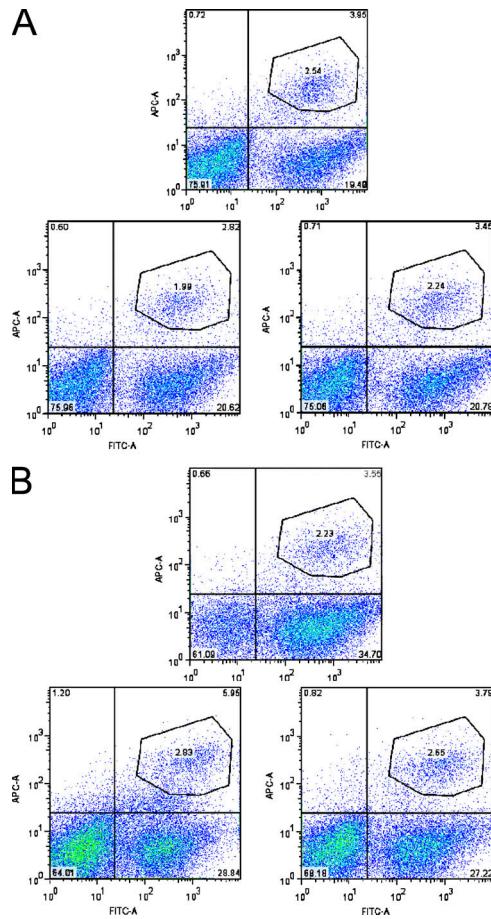
Rapaka et al., <http://www.jem.org/cgi/content/full/jem.20100034/DC1>



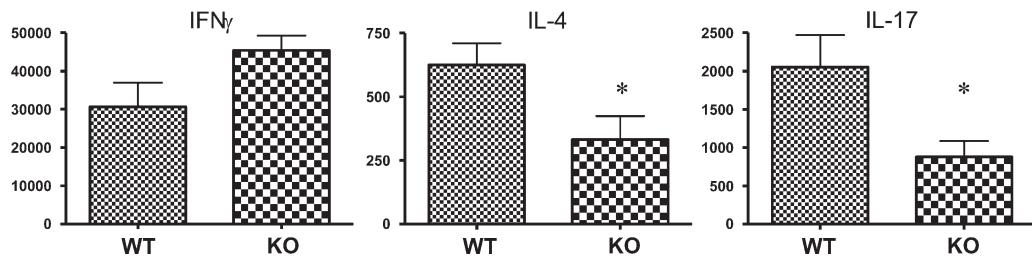
**Figure S1. Variable chain specificity of antipolysaccharide IgM.** Specificity of human antichitin/chitosan and antilaminarin IgM was determined by ELISA using dilutions of normal human serum (serum), purified IgM from normal (IgM) and a myeloma (myeloma) patient, and purified FC fragments from trypsin digests of human IgM (FC5mu). Error bars represent SEM.



**Figure S2.  $V_HDJ_H$  junctions of IgH genes expressed by high and low chitosan-binding B cells.** (A) Untouched B cells (>95% CD19, B220, IgM<sup>+</sup>) were isolated from spleen by negative selection, and single cells were sorted into a 96-well PCR plate based on binding to biotinylated chitosan. After staining with streptavidin-585 Qdots, the top and bottom 2% of fluorescence were used as cutoffs for sorting of high and low chitosan-binding cells. (B) A cladogram was generated by aligning amplified  $V_HDJ_H$  sequences using ClustalW2 software (European Bioinformatics Institute). Junctions were amplified by one-step RT-PCR followed by a second round of seminested PCR. Sequencing was performed using an M13 sequence incorporated into the nested reverse primer. Three to four representative rearrangements are shown for high (red) and low (blue) chitosan binding, as well as clone E06 (green), a nAb of the T15 idiotype described to bind oxidized phospholipids (Chou et al., 2009). (C) For each unique rearrangement, we report  $V_H$ , D, and  $J_H$  germline genes used, the amino acid (AA) length of the three complementary determining regions (CDR), and the nucleotide and amino acid sequences of CDR3. The nucleotide sequence of the junction includes the 3' V region starting with Cys = TGT (codon 104, ImMunoGeneTics information system numbering system), putative nucleotide (N) additions and palindromic (P) elements, the D segment sequence, and the 5' end of the J region ending with Trp = TGG (codon 118).



**Figure S3. Lung DC uptake of FITC-zymosan.** (A and B) C57BL/6J WT (A) or slgM KO (B) mice were intratracheally challenged with 500 µg FITC-zymosan delivered in 50 µl PBS. 18 h later, lungs were harvested and dissociated into single-cell suspensions. Cells were stained with CD11c-APC and assessed for FITC staining by flow cytometry. Representative analyses of individual lungs from three mice from WT (A) or slgM KO (B) are presented.



**Figure S4. Stimulated secreted IFN- $\gamma$ , IL-4, and IL-17 in mediastinal LNs 14 d after *Pneumocystis* infection.** C57BL/6J WT or slgM KO mice were intratracheally challenged with  $2 \times 10^5$  *P. murina* cysts and then sacrificed 14 d later. Single cells were obtained from mediastinal LNs and were cultured at 37°C in IMDM medium with 50 ng/ml PMA plus 750 ng/ml ionomycin for 5 h in the presence of BD Golgi plug (BD). Cells were then washed and stained with surface marker antibodies (eBioscience): CD4 e450, CD8 Alexa Fluor 700, GL3 PE-Cy5, DX-5 APC, and TCR-β APC-e780. After fixation and permeabilization with BD cytofix/cytoperm buffer, cells were stained with intracellular cytokine antibodies: IL-4 FITC, IL-17 PE, and IFN- $\gamma$  PE-Cy7. The y axis is total recovered IFN- $\gamma$ -, IL-17A-, or IL-4-positive CD4+ cells per lung ( $n = 5$  in each group; \* $P < 0.05$  by Student's *t* test). Error bars represent SEM.

**REFERENCE**

Chou, M.Y., L. Fogelstrand, K. Hartvigsen, L.F. Hansen, D. Woelkers, P.X. Shaw, J. Choi, T. Perkmann, F. Bäckhed, Y.I. Miller, et al. 2009. Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. *J. Clin. Invest.* 119:1335–1349. doi:10.1172/JCI36800