## Supplemental Information for *Natural antibodies drive type 2 immunity in response to damage associated molecular patterns.*

Arlind B. Mara<sup>1\*</sup>, Kavita Rawat<sup>1\*</sup>, William T. King<sup>1</sup>, and Claudia V. Jakubzick<sup>1‡</sup>

<sup>1</sup>Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, NH, 03756, USA

\*Authors contributed equally to this work ‡Corresponding author

**Correspondence:** Claudia Jakubzick, Ph.D. Department of Microbiology and Immunology Geisel School of Medicine at Dartmouth College 626W Borwell One Medical Center Drive Lebanon, NH, 03756 claudia.jakubzick@dartmouth.edu Wild Type

IgHEL



Figure S1. Close-up micrographs of H&E lung sections taken from WT and IgHEL mice following induction of AAD using the Alum-OVA model. Note the lack of peribronchiolar and perivascular eosinophilic inflammation in IgHEL mice as compared to WT controls. Yellow arrows point to areas where eosinophils can be observed.



**Figure S2.** Raw flow cytometry data demonstrating the extent of adoptively transferred CFSE labeled OT-II cell proliferation in (A) WT and IgHEL, and (B), WT, CD19<sup>DTA</sup>, AID<sup>-/-</sup>, and B cell depleted WT (WT aCD20) mice stimulated with an injection of Alum-Ova.

В



Gated: CD4+CD8-Va2+ CFSE-labeled OT2 T cells





**Figure S3.** Adoptive transfer of WT B cells to IgHEL mice only marginally restores WT B cell niche and only partially rescues Alum-OVA AAD. (A) Representative flow cytometry plots and scatter plot graphs of eosinophils (defined as SSC<sup>hi</sup>,CD11b<sup>+</sup>,CD11c<sup>-</sup>,SiglecF<sup>+</sup>, Ly6G<sup>-</sup>) in BALF collected from WT, IgHEL, or IgHEL+WT B mice following induction of AAD with Alum + OVA. (B) Flow plots demonstrating B cell phenotype (WT: IgMa<sup>-</sup>, or HEL specific IgMa<sup>+</sup>) in WT, IgHEL, and IgHEL mice that received WT B cell transfer.



**Figure S4.** (A) Scatter plot displaying data demonstrating that the adoptive transfer of WT B cells in CD19<sup>DTA</sup> mice reconstitutes the B cell niche. (B) Flow dot plots demonstrating purity of negatively enriched B and CD4+ T cell fractions used to reconstitute mice.



**Figure S5.** Adoptive transfer of naïve WT serum and naïve Germ Free mouse serum rescues the development of Alum-OVA AAD in B cell deficient CD19<sup>DTA</sup> mice. Scatter plot representing the frequency of eosinophils in BALF of mice following induction of Alum-OVA AAD. Error bars indicate mean and standard error of the mean. Each point represents data from an individual animal, with data pooled from 2 independent experiments per graph. Statistical comparisons were performed in GraphPad Prism using a Kruskal Walis ANOVA on Ranks followed by a Dunn's *post hoc* test for multiple comparisons to control. Asterisks denote statistical significance with a p value of less than 0.5, ns indicates no statistical significance.