

Figure S1. Neutrophils and recruited monocyte/macrophages are increased in the airways after repeated BeO exposures in HLA-DP2 Tg mice. FVB/N HLA-DP2 Tg mice were exposed to PBS or BeO as described in methods and BAL were harvested on day 21 and analyzed by flow cytometry. A. Gating strategy for analysis of myeloid populations is shown for representative PBS and BeO treated mice. Cells were gated to remove debris and doublets, gated for CD45<sup>+</sup> cells and separated for analysis based on CD64 expression. CD64 positive monocyte/macrophages were gated on alveolar macrophages (CD11c<sup>hi</sup>) and recruited macrophages (CD11b<sup>hi</sup>), and the scatter profile of these different macrophage population is shown in overlay. The CD64 negative cells were analyzed for the presence of neutrophils (Ly6G<sup>hi</sup> SigF<sup>lo</sup>) and eosinophils (Ly6G<sup>lo</sup> SigF<sup>hi</sup>). The remaining Ly6G/SigF negative cells were analyzed for MHCII and CD11c to gate on classical dendritic cells (cDCs). B. Total number of BAL cells from PBS or BeO treated HLA-DP2 Tg mice are shown. C and D. Percent (C) and total number (D) of eosinophils (Eos), neutrophils (NP), cells (DC) are shown for PBS- and BeO-exposed HLA-DP2 Tg mice. Dots on graphs indicate values for individual mice and lines on graphs indicate means. The data are combined from 2 separate experiments. Student's t test (2 tailed) (B) and 2-way ANOVA (C-D) were used to test for differences in each cell population between PBS and BeO-treated groups.



Figure S2. B cells derived from spleen, lung-draining lymph nodes (LDLNs) and bronchoalveolar lavage (BAL) co-express CD19 and B220. Representative density plots of B cells stained with mAbs directed against CD19 and B220 are shown. The numbers indicate the percentage of cells in either BAL, LDLNs or spleen that express CD19 and B220. Cells were obtained from BeO-exposed HLA-DP2 Tg mice at day 21.



**Figure S3. B cells in lung-draining lymph nodes (LDLNs) are not activated after BeO instillation.** Representative multicolor immunofluorescence images (200x) of LDLNs of mice instilled with PBS or BeO were taken with a Axioplan Zeiss microscope and recorded with a Hammatsu camera. **A** and **E**. Double immunofluorescent stain shows the presence of complex FDCM1/CD21-CD35<sup>+</sup> FDC networks in both BeO-exposed (**A**) and PBS-treated (**E**) mice. Fluorescent images show CXCL12<sup>+</sup>PNAd<sup>+</sup> high endothelial venules in BeO-exposed mice (**B**) and PBS-treated mice (**F**). In addition, slightly larger and more complex lymphatics in LDLNs of BeO-exposed mice is shown in **B**. Distinctive compartmentalization of CD3<sup>+</sup> T cells and B220<sup>+</sup> B cells, and spatial location of IgG<sup>+</sup> plasma cells (yellow arrows) in LDLNs of BeO-exposed (**C**) and PBS-treated (**G**) mice. Absence of proliferating B cell blasts (PCNA<sup>+</sup>PNA<sup>+</sup>B220<sup>Low</sup>) in PBS-treated (**D**) and BeO-exposed (**H**) mice is noted, suggesting a lack of B cell activation in the LDLNs after BeO instillation. Yellow scale bars represent 100 µM.







**Figure S5: Treatment with anti-CD20 mAb depletes B cells and modulates serum IgG levels.** HLA-DP2 Tg FVB/N mice were injected intraperitoneally with either anti-mouse CD20 mAb, 5D2, or a murine IgG2a isotype control antibody 2 days prior to BeO exposure, and then weekly for the duration of the experiment. (A) Total B cell numbers in BAL, lung, lung-draining lymph nodes and spleen of BeO-exposed HLA-DP2 Tg mice treated with either anti-CD20 mAb or the isotype control mAb are shown. Cumulative data from two independent experiments having five mice per group are shown. (B) Serum IgG concentration in unexposed mice as well as in anti-CD20 and isotype control mAb-treated HLA-DP2 Tg mice exposed to BeO are shown. (C) Serum concentrations of IgG isotypes (IgG1, IgG2a, IgG2b, and IgG3) in unexposed mice as well as in anti-CD20 and isotype control mAb-treated HLA-DP2 Tg mice exposed to BeO are shown. The data are representative of two separate experiments. Solid line and error bars depict the mean ± SEM. Student's t test (2 tailed) (A) and 2-way ANOVA (B and C) were used to test for statistical differences.