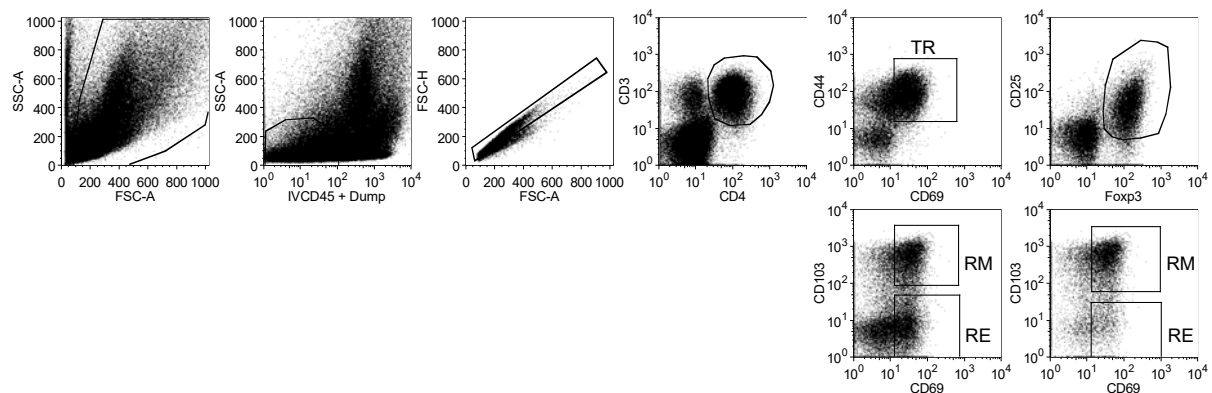
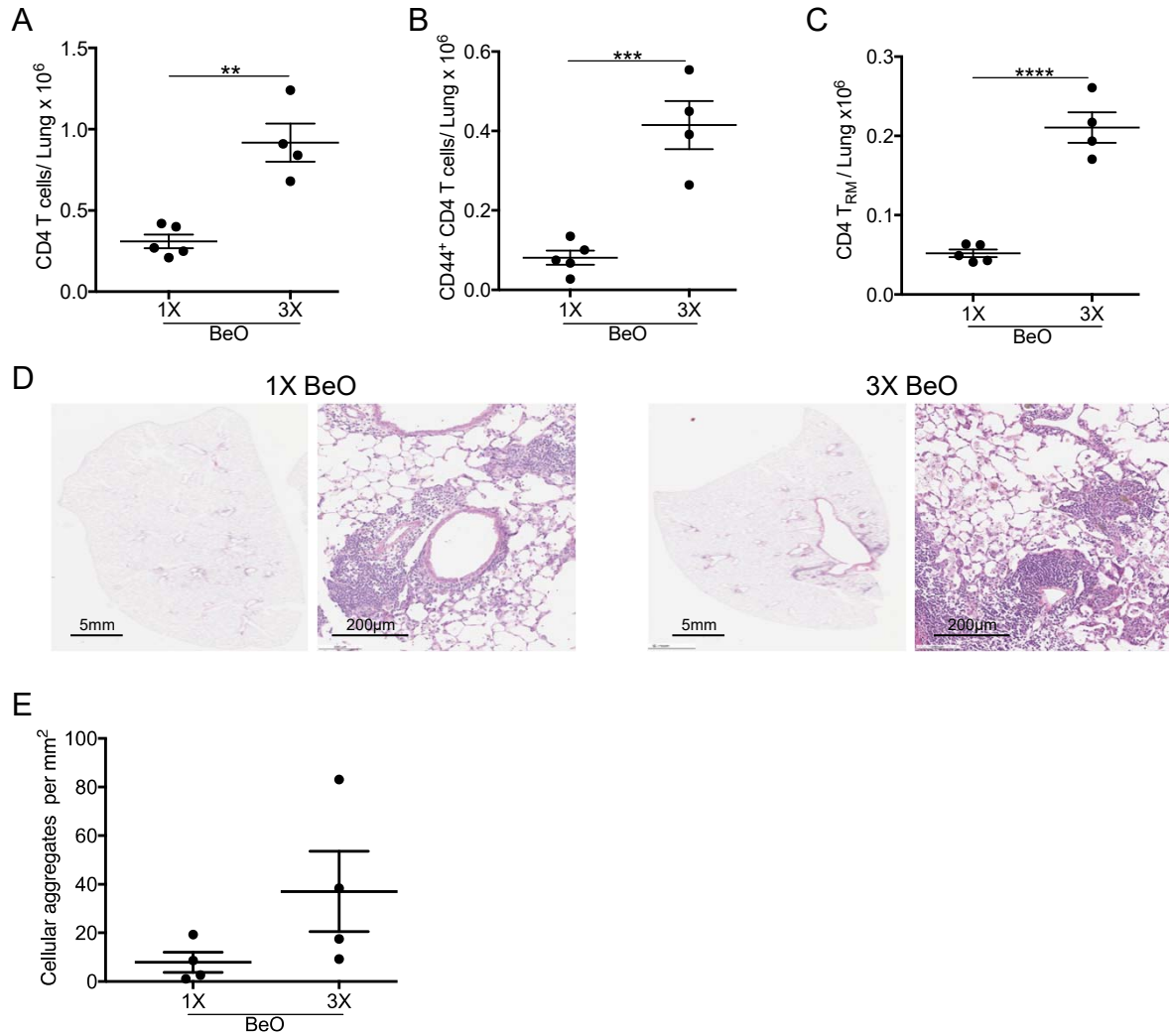


Supplementary Figure 1



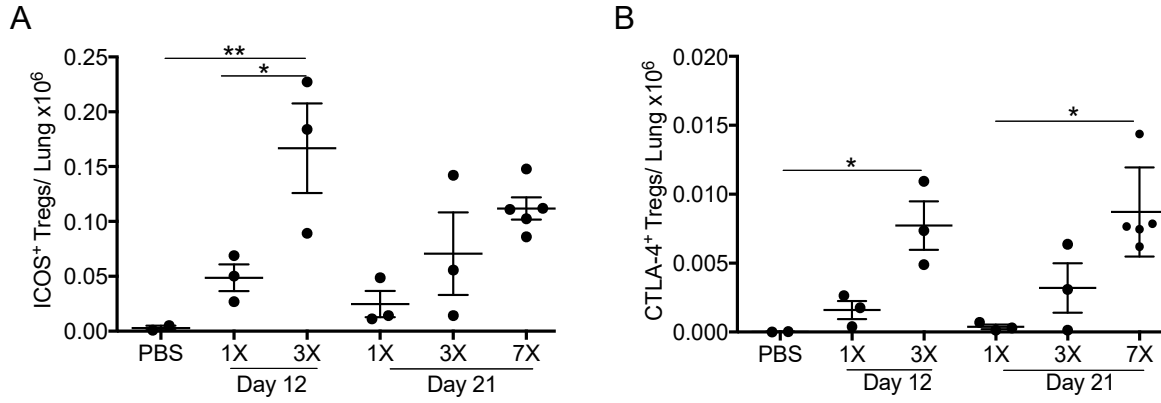
Supplemental Figure 1. Gating strategy for the identification of tissue-resident CD4⁺ T cells. Density plots show gating strategy for the identification of tissue-resident CD4⁺ T cells present in the CD45 negative fraction of the lungs of HLA-DP2 Tg mice sensitized and boosted with BeO (100 μ g) on days 0, 1, and 2, 14, 15, 18 and 19 and examined at day 21. TR denotes tissue-resident T cells while RM and RE denote resident memory and resident effector T cells, respectively.

Supplementary Figure 2



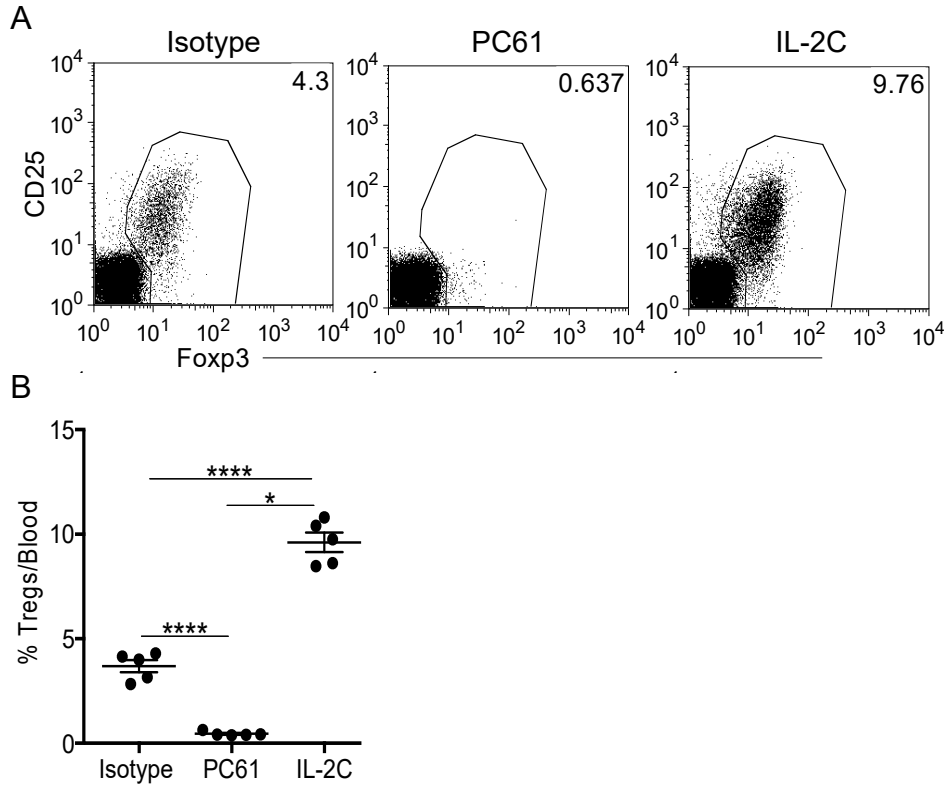
Supplemental Figure 2. BeO sensitization induces an increased accumulation of tissue-resident CD4⁺ T cells in the lungs of HLA-DP2 Tg mice on day 12. (A-C) The number of total CD4⁺ T cells (A), CD44⁺ effector T cells (B), and CD103⁺CD69⁺ resident memory CD4⁺ T cells (C) in the lungs at day 12. (D) Representative H&E staining of lung sections of HLA-DP2 Tg mice treated with 1 (1X, left) and 3 (3X, right) doses of BeO (100 µg) are shown at low and high magnification. A scale bar is shown in the lower left of each image. (E) Quantification of cellular aggregates in the lungs of HLA-DP2 Tg mice exposed to one (1X) or three (3X) doses of BeO. Data (mean ± SEM) are representative of three independent experiments (3-5 animals per group). Student's t test was used to test for differences. **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplementary Figure 3



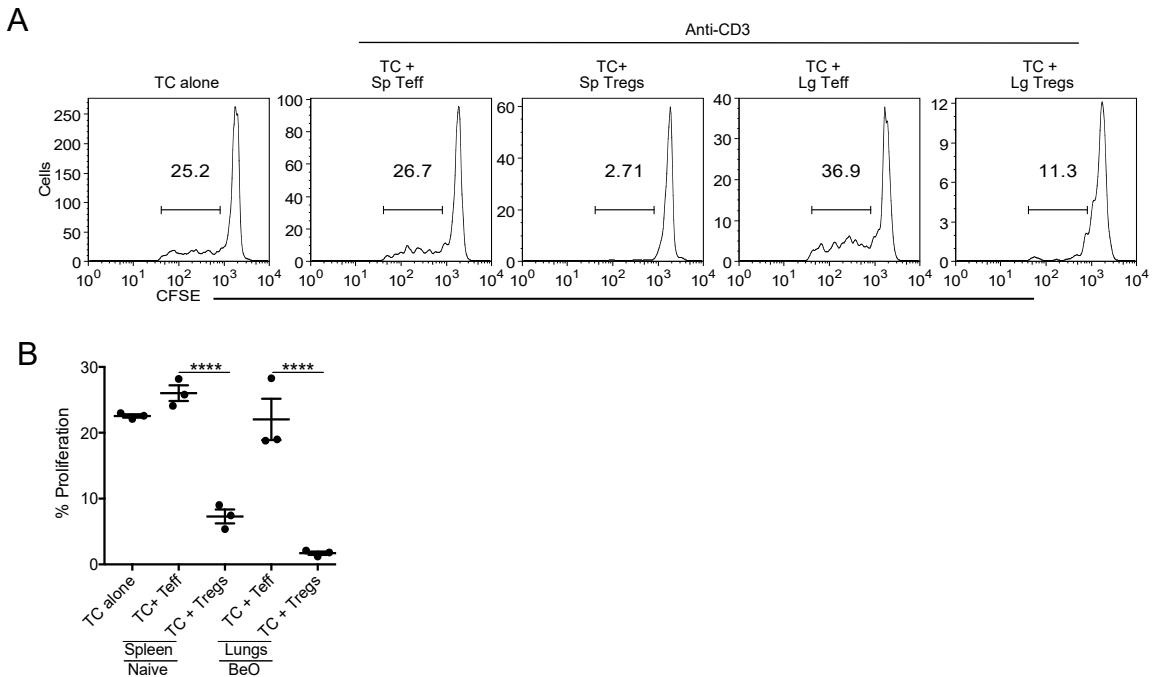
Supplemental Figure 3. Increased expression of ICOS and CTLA-4 on tissue-resident Tregs in the lungs of BeO-exposed HLA-DP2 Tg mice. (A-B) Number of ICOS (A) and CTLA-4 (B) expressing tissue-resident CD25⁺FoxP3⁺ regulatory CD4⁺ Tregs in the lungs of HLA-DP2 Tg mice exposed to one (1X), three (3X), and seven (7X) doses of BeO and analyzed at day 21. Data (mean ± SEM) are representative of three independent experiments. One-way ANOVA was used to test for differences. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplementary Figure 4



Supplemental Figure 4. Expression of CD4⁺ FoxP3⁺ T cells in the blood of BeO-exposed mice after antibody treatment. (A) Representative flow cytometric dot plots of CD4⁺ CD25⁺ FoxP3⁺ Tregs in blood on day 8 after HLA-DP2 Tg mice were sensitized to BeO on days 0, 1 and 2 and treated with either isotype control antibody, anti-CD25 mAb (PC61), or IL2/ α IL-2 complexes (IL-2C, IL-2/anti-IL2(JES6-1)). (B) Cumulative frequency of CD4⁺ Tregs in the blood at day 8. Data (mean \pm SEM) are representative of two independent experiments. One-way ANOVA was used to test for differences. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Figure 5



Supplemental Figure 5. Be-experienced Tregs suppress in vitro proliferation of anti-CD3 activated CD4⁺ T cells in a contact dependent fashion. (A) Representative histograms show proliferation of CFSE-labeled naive CD4⁺ T cells (TC) stimulated with anti-CD3 (1 μg/ml) and cultured in vitro in a 1:1 ratio with spleen (Sp) or lung (Lg)-resident effector (CD44⁺CD25⁻) or regulatory (CD25⁺FoxP3⁺) CD4⁺ T cells obtained on day 21 from BeO-sensitized/boosted HLA-DP2 Tg mice. Peripheral T effectors (CD44⁺CD25⁻) and T regs (CD25⁺FoxP3⁺) sorted from the spleen (Sp) of naive mice were used as control cells. **(B)** Cumulative frequency of proliferation of CFSE-labeled TC cells on day 5. Data (mean ± SEM) are representative of three independent experiments. One-way ANOVA was used to test for differences. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.