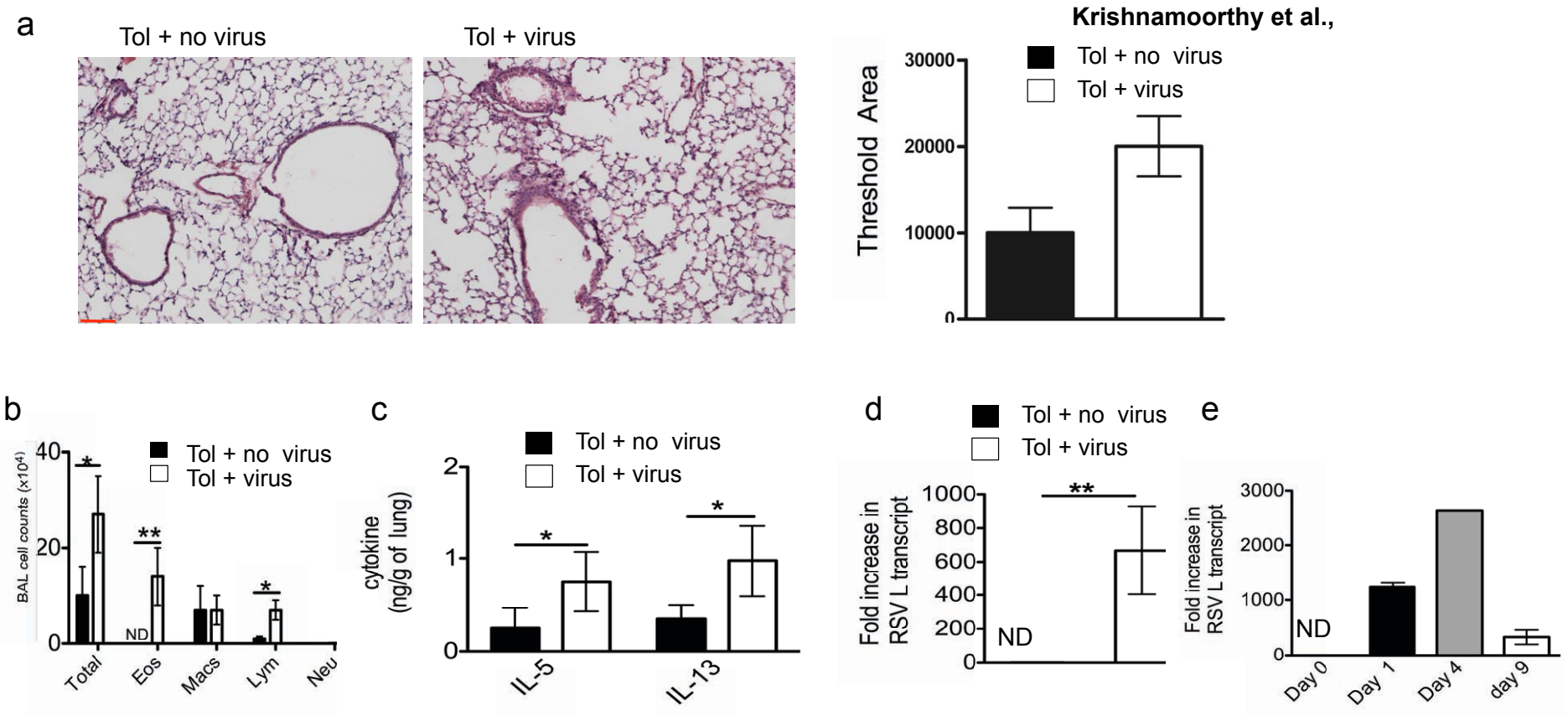
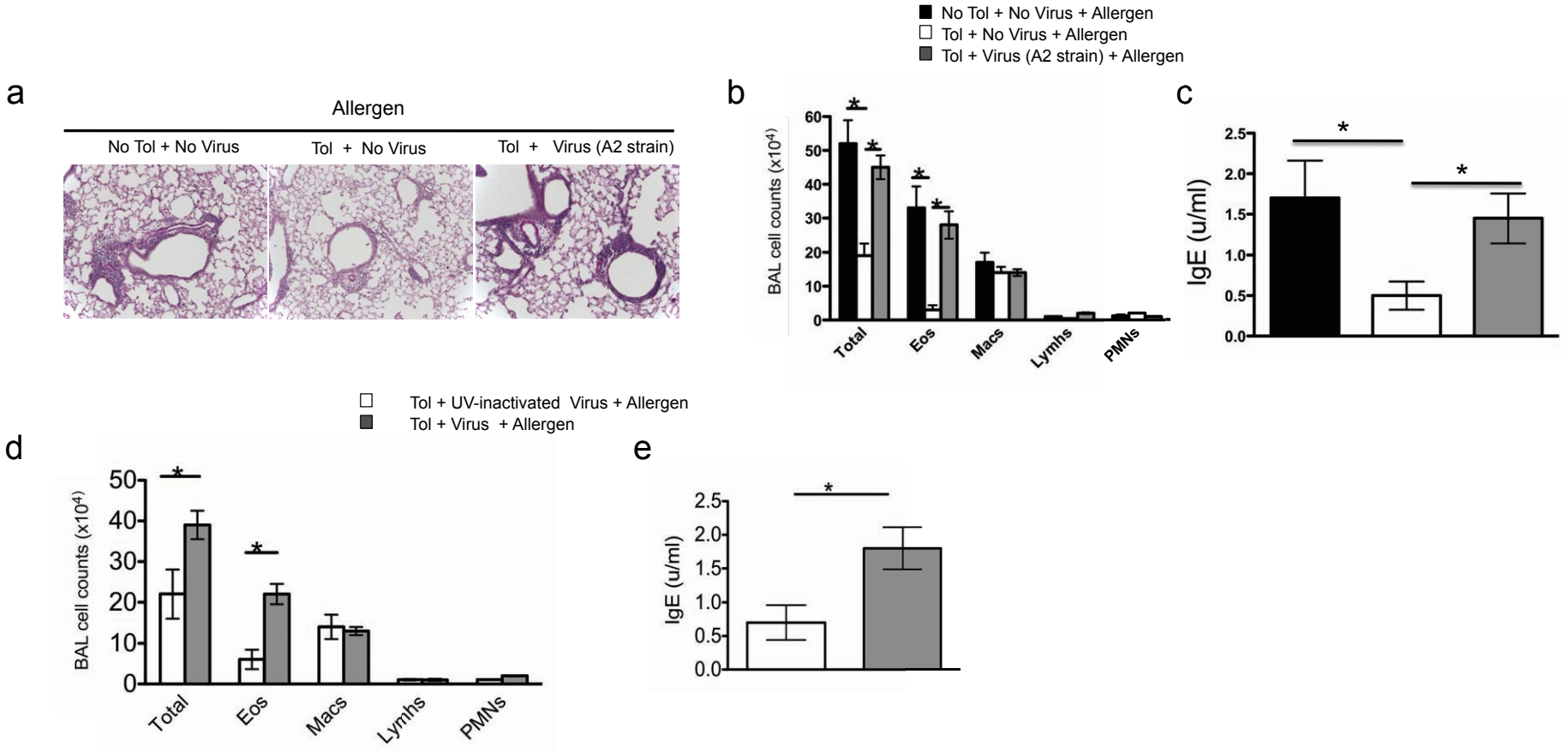
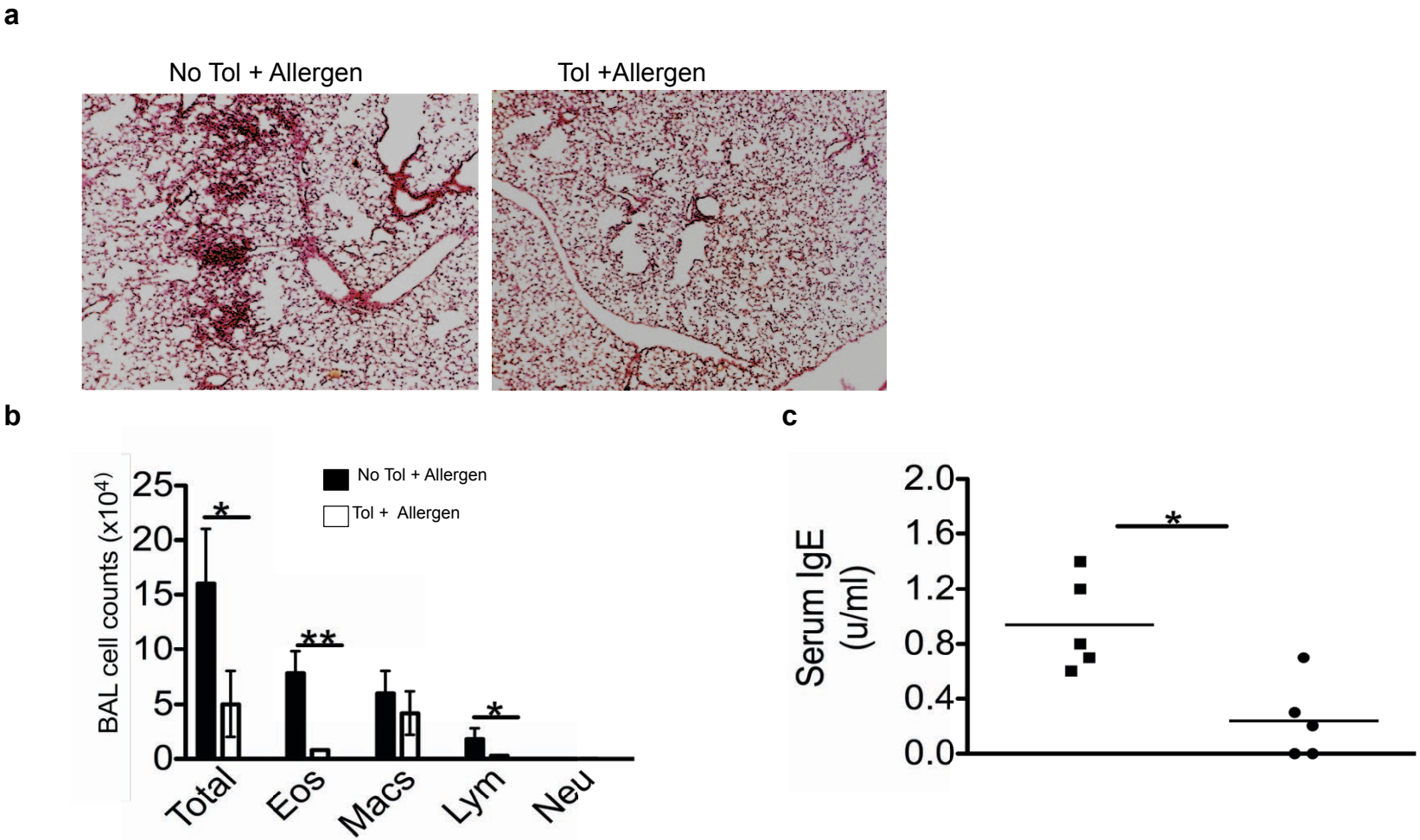


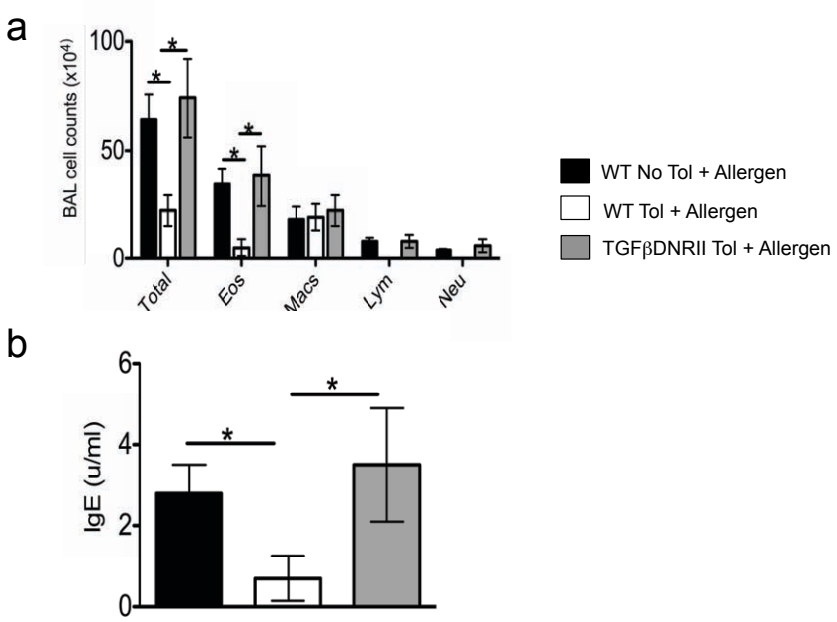
Supplementary Figure 1 RSV-induces inflammation even in the absence of allergen. Tolerized pups were either infected with RSV or not. The mice were sacrificed a week following the last infection and analyzed for different parametrs. (**a**) Histological examination of lung sections stained with H&E and the quantification based on threshold areas (Metamorph). The red scale bar is 100 μm. (**b**) Cell differentials in the BAL fluid. (**c**) Cytokines in lung homogenates assayed by ELISA. (**d**) Detection of RSV L transcript by qRT-PCR in tolerized mice after 3 successive infections. (**e**) Kinetics of expression of the RSV L transcript in infected mice. Data are represented relative to expression of the housekeeping gene GUS and calculated using the $2^{-\Delta\Delta Ct}$ method. Student's unpaired two-tailed t-test was used for analysis between the two groups. The error bars represent mean values ± SD, n=4-5 mice per group. * $p<0.05$, * *$p<0.005$. Data are representative of two independent experiments.



Supplementary Figure 2 RSV strain A2 also induces inflammation and breaches tolerance which requires live virus. Tolerized pups were either infected with RSV strain A2 or not and challenged with allergen. The mice were sacrificed and analyzed for different parameters. (**a**) Histological examination of lung sections stained with H&E. (**b**) Cell differentials in the BAL fluid. (**c**) Serum OVA-specific IgE levels assayed by ELISA. Two way ANOVA was used for data analysis in panels (**b**) and (**c**) to compare differences between groups. $p<0.01$. (**d**) BAL cell differentials obtained using live or UV-inactivated virus. (**e**) Serum OVA-specific IgE levels. Student's unpaired two-tailed t-test was used for analysis between the two groups. The error bars represent mean values ± SD, n=4-5 mice per group. * $p<0.05$. Data are representative of two independent experiments.



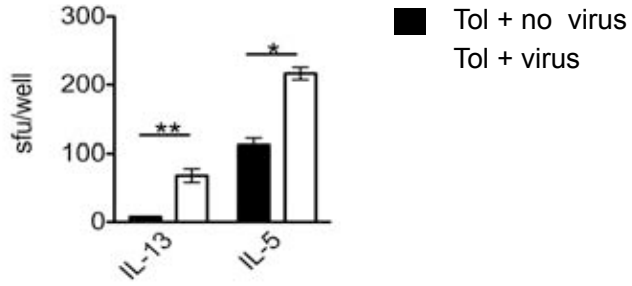
Supplementary Figure 3 Maternal transfer of tolerance is induced in the pups within the first 21 days. Pups were tolerized to OVA via breast-milk. Upon weaning, the mice were examined for tolerance by immunization with OVA/CT followed by OVA aerosol challenge. The mice were sacrificed a week following the last OVA aerosol and inflammation was analyzed. (**a**) Histological examination of lung sections stained with H&E. (**b**) OVA-specific IgE levels in serum. Student's unpaired two-tailed t-test was used for analysis between the two groups. The error bars represent mean values ± SD, n= 4-5 mice per group. This experiment was performed twice with similar results.



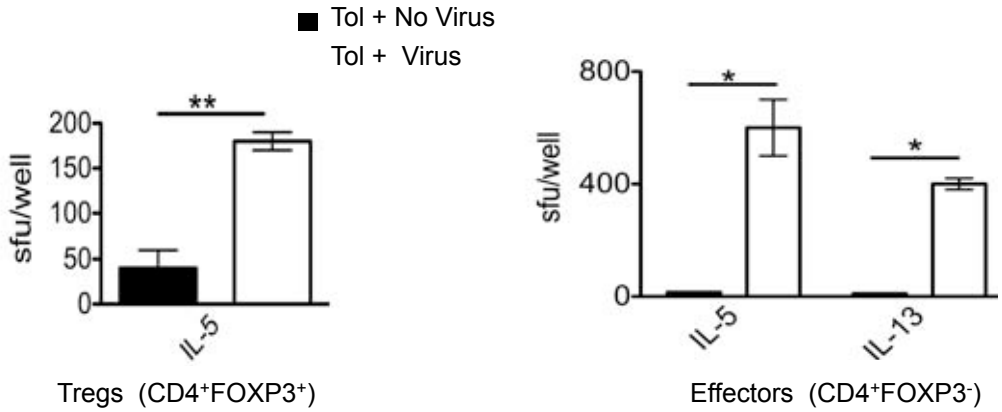
Supplementary Figure 4 TGF-β is required for maternal tolerance transfer. Newborn WT mice and those expressing TGF-βDNRII on CD4+ T cells were tolerized to OVA indirectly. Upon reaching 6 weeks, the mice were examined for tolerance by immunization with OVA/CT followed by OVA aerosol challenge. The mice were sacrificed a week following the last OVA aerosol and inflammation was analyzed. Pups nursed by non-tolerized mothers were used as positive controls. (**a**) Cell differentials in the BAL fluid. (**b**) OVA-specific IgE levels in the serum. The error bars represent mean values ± SD, n= 4-5 mice per group. * $p<0.05$, * *$p<0.005$. This experiment was performed twice with similar results.

a

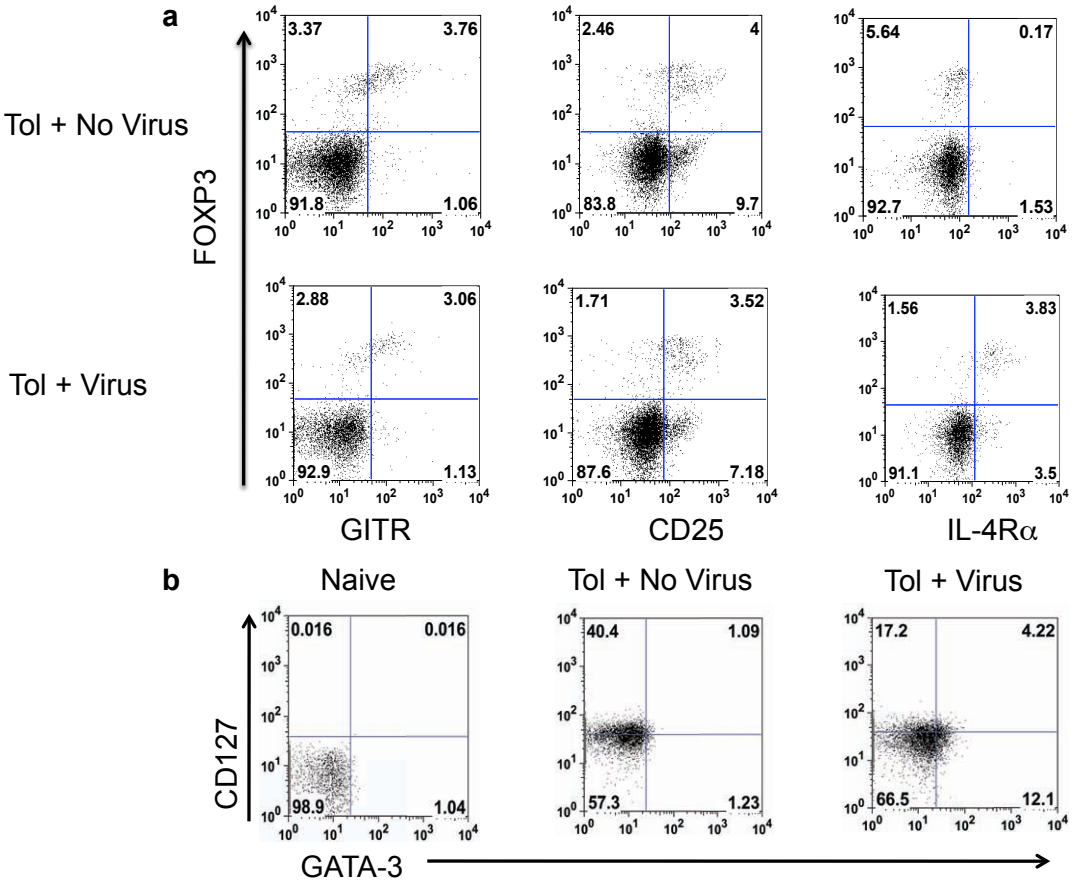
b



Supplementary Figure 5 Lung-draining lymph node cells from RSV-infected mice produce Th2 cytokines.
(**a**) The mediastinal LNs from the two groups were placed on a glass slide against a ruler with 1 mm spacing and photographed. (**b**) ELISPOT assay of LN cells stimulated with PMA/ionomycin for 24 h. The spot forming units (sfu)/well are shown. Data shown are mean values ± SD. Data shown are representative of two independent experiments, n=4-5 mice per group. Student's unpaired two-tailed t-test was used for analysis. * $p<0.05$ and ** $p<0.01$.

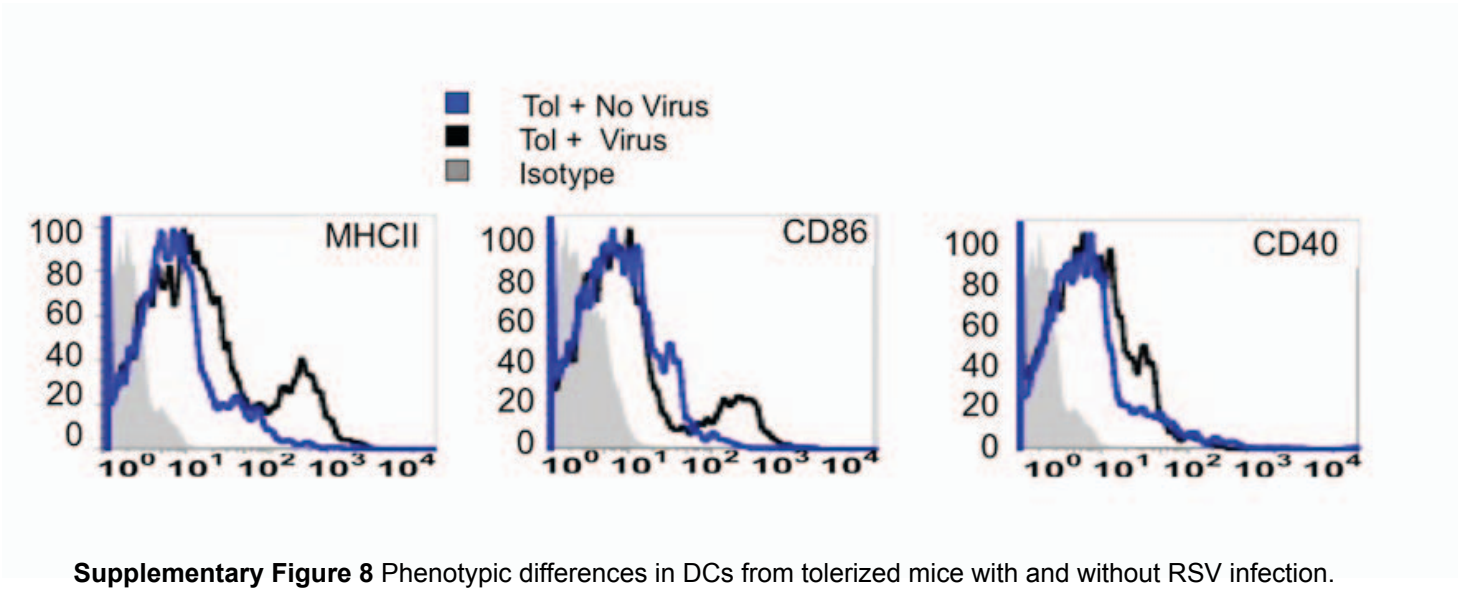


Supplementary Figure 6 Differential cytokine profile in Tregs and effector CD4+ T cells. Tregs ($CD4^+$ $FOXP3^+$) and the effector cells ($CD4^+$ $FOXP3^-$) were sorted from the lungs of virus infected and uninfected tolerized mice. The cells were plated for ELSIPOT assay with PMA/ionomycin stimulation for 24 h. The spot forming units (sfu)/well are shown. The error bars represent the mean values ± SD between wells. Student's unpaired two-tailed t-test was used for analysis ; * $p<0.05$. Similar results were obtained in two independent experiments.



Supplementary Figure 7 Expression of cell surface molecules on CD4$^+$ FOXP3$^+$ T cells in the lung.
(**a**) Lung cells from virus-infected or uninfected tolerized mice were analyzed for markers associated with Tregs and for IL-4Rα. The cells were gated on CD4 and the expression of FOXP3 in conjunction with other markers was analyzed. Data are representative of two independent experiments. (**b**) Expression of CD127 and GATA-3 was analyzed on lung cells gated on CD4$^+$ FOXP3$^+$ from virus-infected or uninfected tolerized mice.

Nature Medicine doi:10.1038/nm.2896



Supplementary Figure 8 Phenotypic differences in DCs from tolerized mice with and without RSV infection. WT pups were tolerized to OVA indirectly and upon weaning were either infected with RSV or not. A week after the last infection, the mice were sacrificed and dendritic cells in the lungs were analyzed for expression of MHC class II and co-stimulatory molecules. Similar results were obtained in two independent experiments.

Nature Medicine doi:10.1038/nm.2896