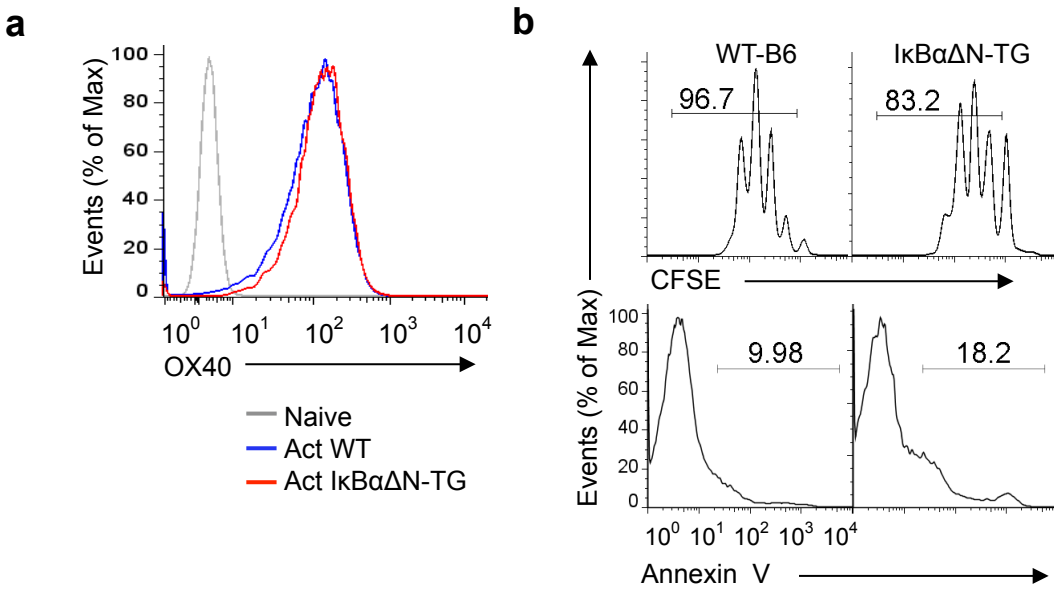
Supplementary Information

OX40 signaling favors the induction of T_H9 cells and airway inflammation

Xiang Xiao[1], Savithri Balasubramanian[2], Wentao Liu[1], Xiufeng Chu[1], Haibin Wang[3], Elizabeth J. Taparowsky[4], Yang-Xin Fu[5], Yongwon Choi[6], Matthew C. Walsh[6], and Xian Chang Li[1]

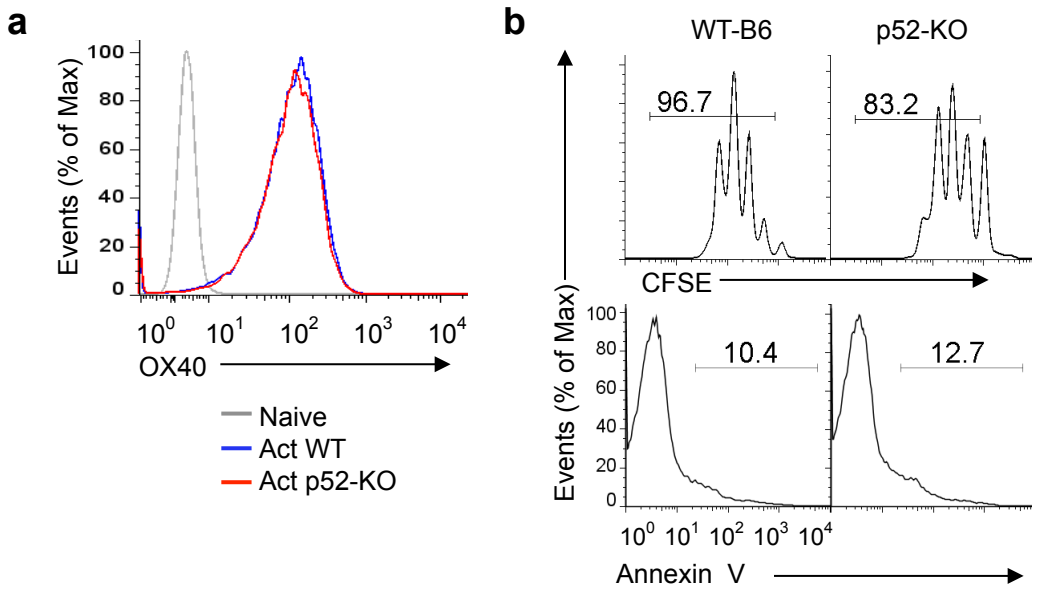
1. Transplant Research Center, Brigham and Women's hospital and Children's Hospital Boston, Harvard Medical School, Boston, MA 02115
2. The Transplant Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215
3. Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215
4. Department of Biological Sciences, Purdue University, Lafayette, IN 47907
5. Department of Pathology, University of Chicago, Chicago, IL 60637
6. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

Correspondence should be addressed to XCL (xli28@partners.org).



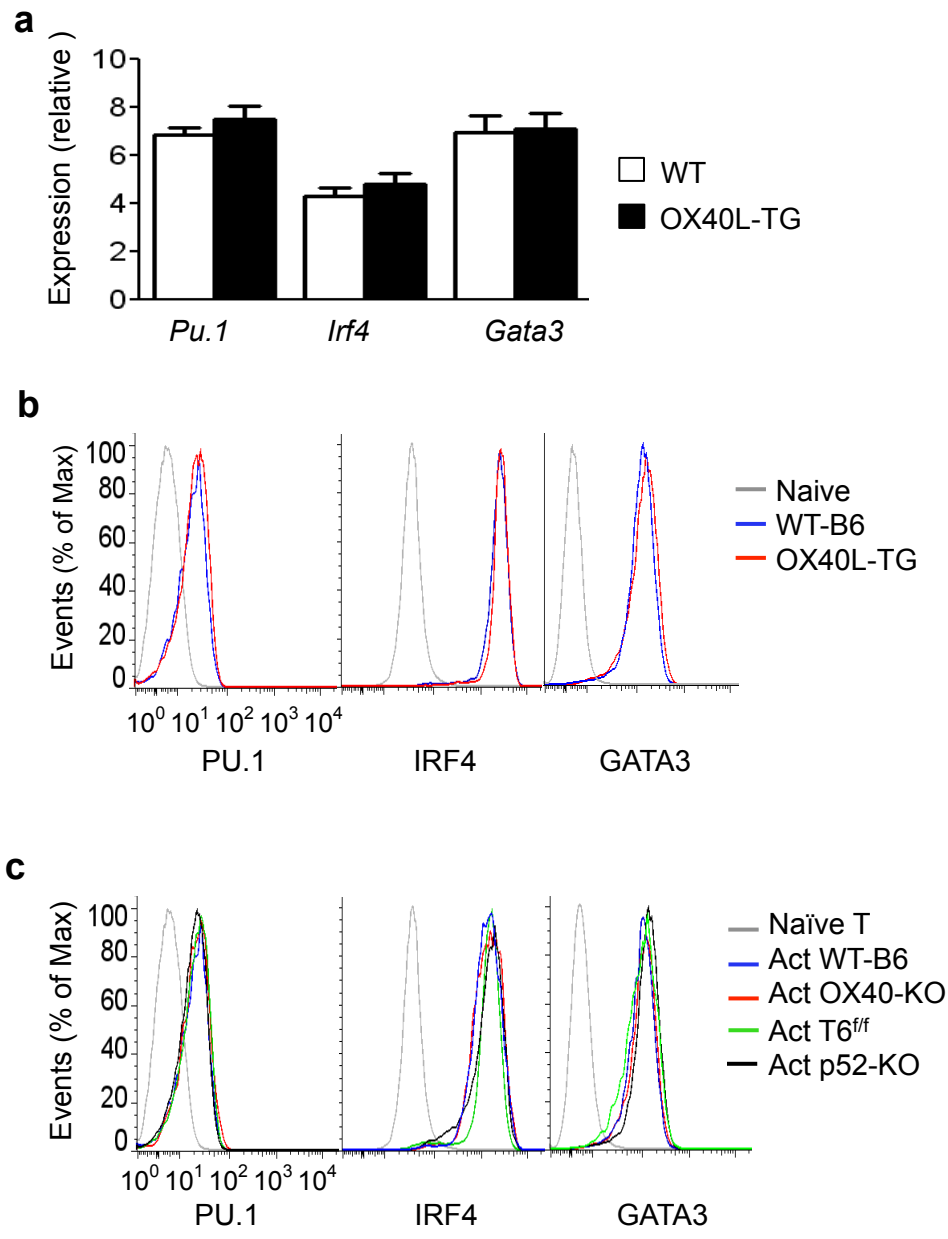
Supplemental Figure 1. Comparison of wt B6 and IκBαΔN-TG CD4⁺ T cells in OX40 expression, proliferation, and survival following activation.

(a). Naive $CD4^+$ T cells were activated with anti-CD3 and APCs for 3 days and expression of OX40 on the cell surface was examined by flow cytometry. Naive un-stimulated cells were used as controls. The plot shown is 1 of 3 independent experiments. (b). Naïve $CD4^+Foxp3^-$ T cells were flow sorted from WT B6 and IκBαΔN-TG mice, labeled with CFSE, and stimulated with anti-CD3 plus APCs for 3 days; cell proliferation and survival were determined by dilutions of the CFSE dye and Annexin V staining, respectively. One of 3 experiments is shown.



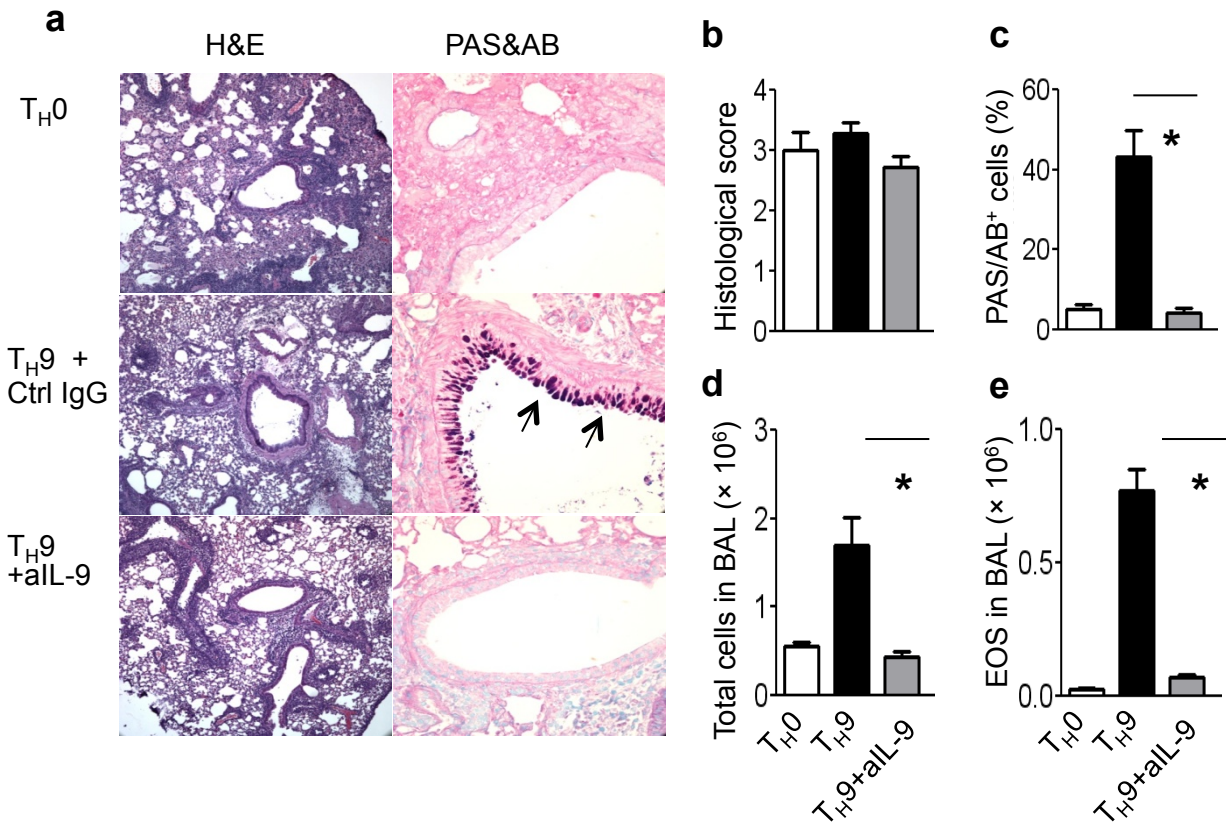
Supplemental Figure 2. OX40 expression, cell proliferation and survival of wt B6 and p52-KO CD4⁺ T cells after activation.

(a). Flow sorted naïve CD4⁺Foxp3⁻ T cells were activated with anti-CD3 plus APCs for 3 days and expression of OX40 on the cell surface was examined by flow cytometry. Naive un-stimulated cells were used as controls. The plot shown is 1 of 3 independent experiments. (b). Naïve CD4⁺Foxp3⁻ T cells were flow sorted from WT B6 and p52-KO mice, labeled with CFSE, and then stimulated with anti-CD3 plus APCs for 3 days. Cell proliferation and survival were determined by CFSE dilutions and Annexin V staining. One of 3 experiments is shown.



Supplemental Figure 3. Analysis of PU.1, IRF4, and Gata3 expression in activated CD4⁺ T cells.

(**a**). Naïve CD4⁺Foxp3⁻ T cells were activated with anti-CD3 plus either wt APCs (WT) or OX40Ltg APCs (OX40L-TG) for 48 hrs, and expression of *Pu.1*, *Irf4*, and *Gata3* was quantified with Real-Time PCR and shown. Data shown are mean ± SEM of 3 experiments. (**b**). Naïve CD4⁺Foxp3⁻ T cells were activated as described above, and expression of PU.1, IRF4, and Gata3 was assessed by intracellular staining with specific Abs and analyzed by flow cytometry. Naive un-stimulated CD4⁺ T cells were included as controls. (**c**). Simultaneous assessment of PU.1, IRF4, and Gata3 expression in activated CD4⁺Foxp3⁻ T cells from WT-B6, OX40-KO, p52-KO, and *Traf6*$^{f/f}$ CD4-Cre (T6$^{f/f}$) mice. The FACS plot shown denotes 48 hr stimulation with anti-CD3 plus APCs. One of 3 experiments is shown.



Supplemental Figure 4. Airway inflammation induced by passively transferred OVA specific Th9 cells.

(a). OVA specific OT-II T cells were polarized to Th9 cells in vitro under conditions of OX40 stimulation, and then adoptively transferred into syngeneic Rag-$1^{-/-}$ mice (10^6/mouse). The host mice were challenged with OVA via the airway for 2 days, and the lung inflammation is shown. Activated OT-II cells without Th9 polarizing cytokines were transferred as controls(T_H0). The arrows show mucin-producing cells. (**b**). Pathology scores of the lung parenchyma. (**c**). Mucin-producing cells among bronchial epithelial cells. (**d**). The absolute cell number in the bronchial alveolar lavage (BAL). (**e**). The absolute number of eosinophils (EOS) in the BAL. Each group included 5 animals. * $p<0.05$