

SUPPLEMENTARY DATA

CARMA1 is Necessary for Optimal T Cell Responses in a Murine Model of Allergic Asthma^{1, 2, 3}

Ravisankar A. Ramadas,^{*†} Marly I. Roche,^{*†} James J. Moon,^{*†} Thomas Ludwig,[‡] Ramnik J. Xavier^{§¶||} and Benjamin D. Medoff^{*†4}

*Pulmonary and Critical Care Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, 02114 USA

†Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, 02129 USA

‡Institute for Cancer Genetics, Columbia University, New York, NY 10032

§ Center for Computational and Integrative Biology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, 02114 USA

¶Gastrointestinal Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, 02114 USA

||The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, 02142 USA

Running title: CARMA1 in memory T cells.

⁴Address correspondences to:
Benjamin D. Medoff MD
Pulmonary and Critical Care Unit
Massachusetts General Hospital
55 Fruit St
Bulfinch 148
Boston, MA 02114
Email: bmedoff@partners.org
Tel: 617-643-8692
Fax: 617-726-5651

SUPPLEMENTARY FIGURE 1. *Deletion of CARMA1 after T cell activation using tamoxifen-induced Cre recombinase activity reduces T cell reactivation in vitro.* A) CD4⁺ T cells isolated from mice were cultured with OVA₃₂₃₋₃₃₉ pulsed APCs, treated with 4-hydroxytamoxifen and restimulated with anti-CD3 and anti-CD28 coated beads. B) Transcript expression of CARMA1 in CD4⁺ T cells three days following activation and after 5 days of culture with tamoxifen or vehicle, as analyzed by real-time quantitative PCR. RNA was isolated from pooled cells from 2 mice per genotype at the indicated time points. Expression was normalized to baseline CARMA1 expression in cells following activation with OVA loaded irradiated splenocytes for 3 days. Baseline expression did not differ between the two genotypes. C) Representative histograms demonstrating CD69 expression on CD4⁺ T cells isolated from wild-type OT-II and Rosa26^{CreERT2/+}/CARMA1^{F/F} OT-II mice following activation with OVA, tamoxifen or vehicle treatment for 5 days, and with or without 24 h restimulation with anti-CD3 and anti-CD28. Plots are representative of 2 experiments done with pooled cells from 2 mice per genotype.

SUPPLEMENTARY FIGURE 2. *In vivo OX40^{+Cre} activity is induced in CD4⁺ T cells with OVA immunization and challenge.* A) OX40^{+Cre}mT/mG mice were either sensitized or sensitized and challenged with OVA as depicted. Lung and BAL tissues were collected. B) Expression of OX40-Cre driven GFP in CD4⁺ T cells from the lungs of mice following OVA immunization. C) Expression of OX40-Cre driven GFP in CD4⁺ T cells from the lungs of mice following OVA immunization and challenge. D) Expression of OX40-Cre

driven GFP CD4⁺ T cells recovered from the BAL of mice following OVA immunization and challenge.

SUPPLEMENTARY FIGURE 3. *Deletion of CARMA1 by Ox40-Cre does not impair the development of memory T cells in the thoracic lymph nodes.* Presented data are representative flow cytometry plots that indicate the percentage of CD4⁺/tetramer⁺/CD8⁻/CD11c⁻/CD19⁻ cells following bead selection for tetramer-bound lymphocytes from the TLN of mice. TLNs within each genotype were pooled for each experiment. Depicted plot is representative of 1 such experiment. The experiment was repeated twice with similar results.