

SUPPLEMENTAL MATERIAL

LYN- and AIRE-mediated tolerance checkpoint defects synergize to trigger

organ-specific autoimmunity

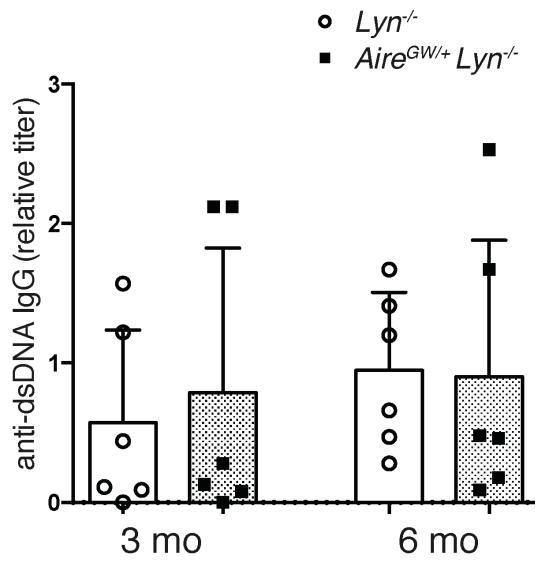
Irina Proekt,¹ Corey N. Miller,² Marion Jeanne,³ Kayla J. Fasano,² James J. Moon,⁴ Clifford A. Lowell,⁵ Douglas B. Gould,³ Mark S. Anderson,^{2*} Anthony L. DeFranco^{1*}

¹Department of Microbiology and Immunology, ²Diabetes Center, ³Departments of Ophthalmology and Anatomy, Institute for Human Genetics, UCSF, San Francisco, California, USA. ⁴Center for Immunology and Inflammatory Diseases and Division of Pulmonary and Critical Care Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA. ⁵Department of Laboratory Medicine, UCSF, San Francisco, California, USA.

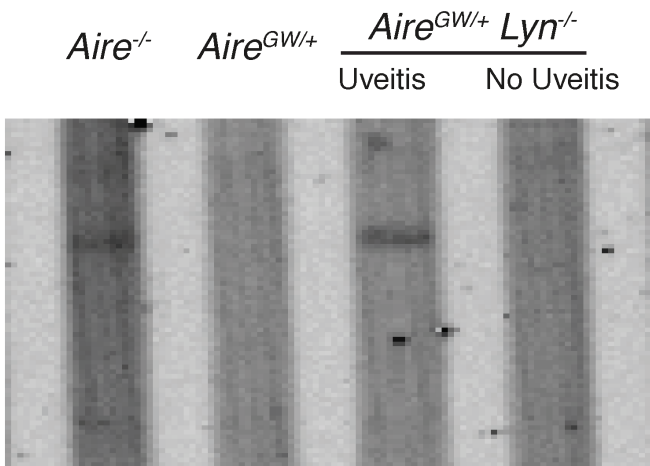
*Corresponding Authors:

Mark S. Anderson
UCSF Diabetes Center
513 Parnassus Ave
San Francisco, CA 94143
(415) 502-8052
manderson@diabetes.ucsf.edu

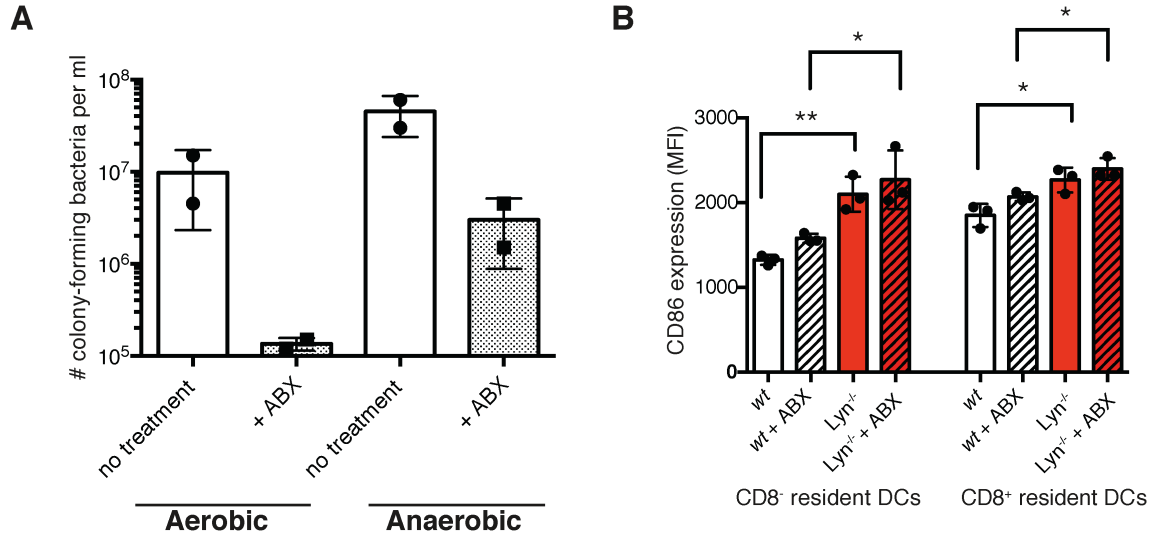
Anthony L. DeFranco
Dept. of Microbiology and Immunology
513 Parnassus Ave
San Francisco, CA 94143
(415) 476-5488
Anthony.DeFranco@ucsf.edu



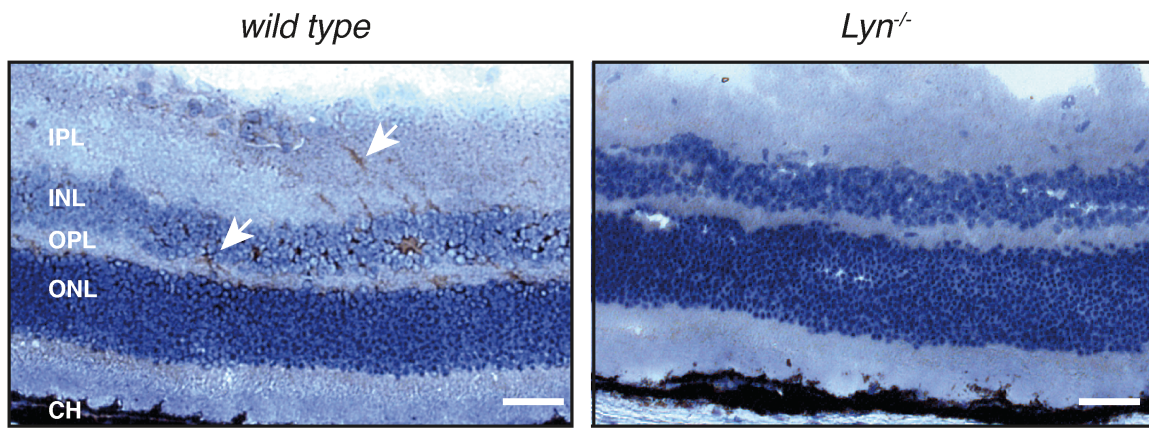
Supplemental Figure 1. Titers of anti-dsDNA antibodies in *Lyn*^{-/-} and *Aire*^{GW/+} *Lyn*^{-/-} mice. Levels of serum anti-dsDNA antibodies were measured in a cohort of mice at 3 and 6 months of age. Titers were normalized to pooled sera from 8 month old *MRL/lpr* mice. Each data point represents an individual mouse and bars show mean \pm SD. Data are representative of two independent experiments with 6 mice per group.



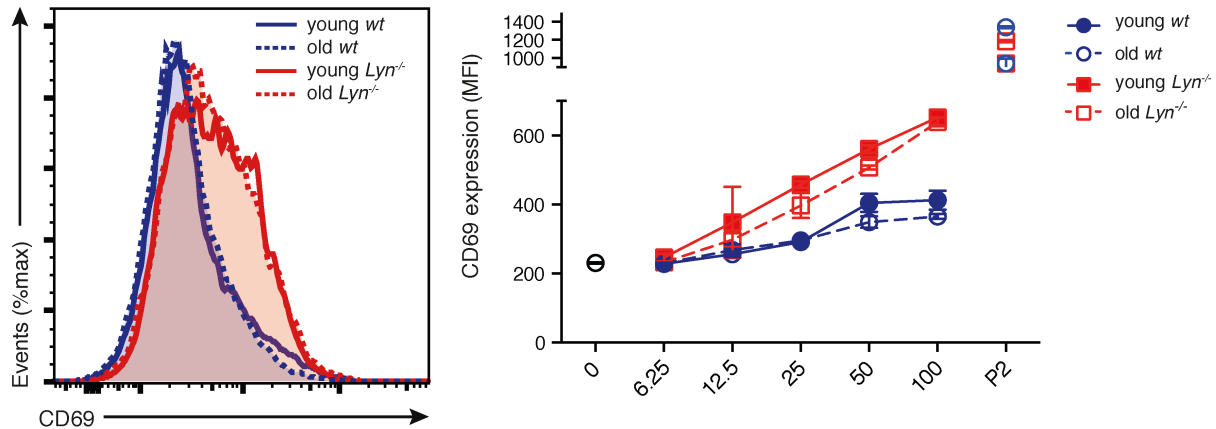
Supplemental Figure 2. Sera from double mutant mice predominantly recognize a single eye antigen, IRBP. Whole mouse eye extracts were resolved by SDS-PAGE, transferred to filters and immunoblotted from sera collected from 3-8 month-old mice of indicated genotypes. Presence of uveitis was confirmed by histology. At least 5 mice for each genotype were analyzed and representative reactivities are shown.



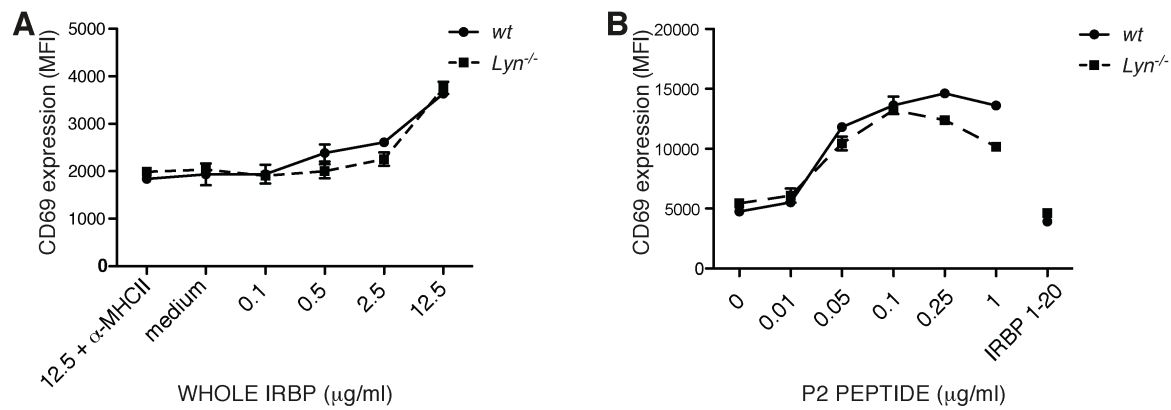
Supplemental Figure 3. Depletion of commensal microbes does not reduce DC activation in *Lyn*^{-/-} mice. *wt* and *Lyn*^{-/-} mice were treated with a broad spectrum antibiotic cocktail (ABX) from birth until the endpoint at 12 weeks of age. Two separate cohorts of 3-5 animals per group were analyzed. **(A)** Representative colony-forming unit counts of serial dilutions of fecal contents were performed under aerobic and anaerobic conditions. **(B)** CD86 expression on cervical LN DCs from antibiotic-treated mice and controls. A representative experiment is shown.



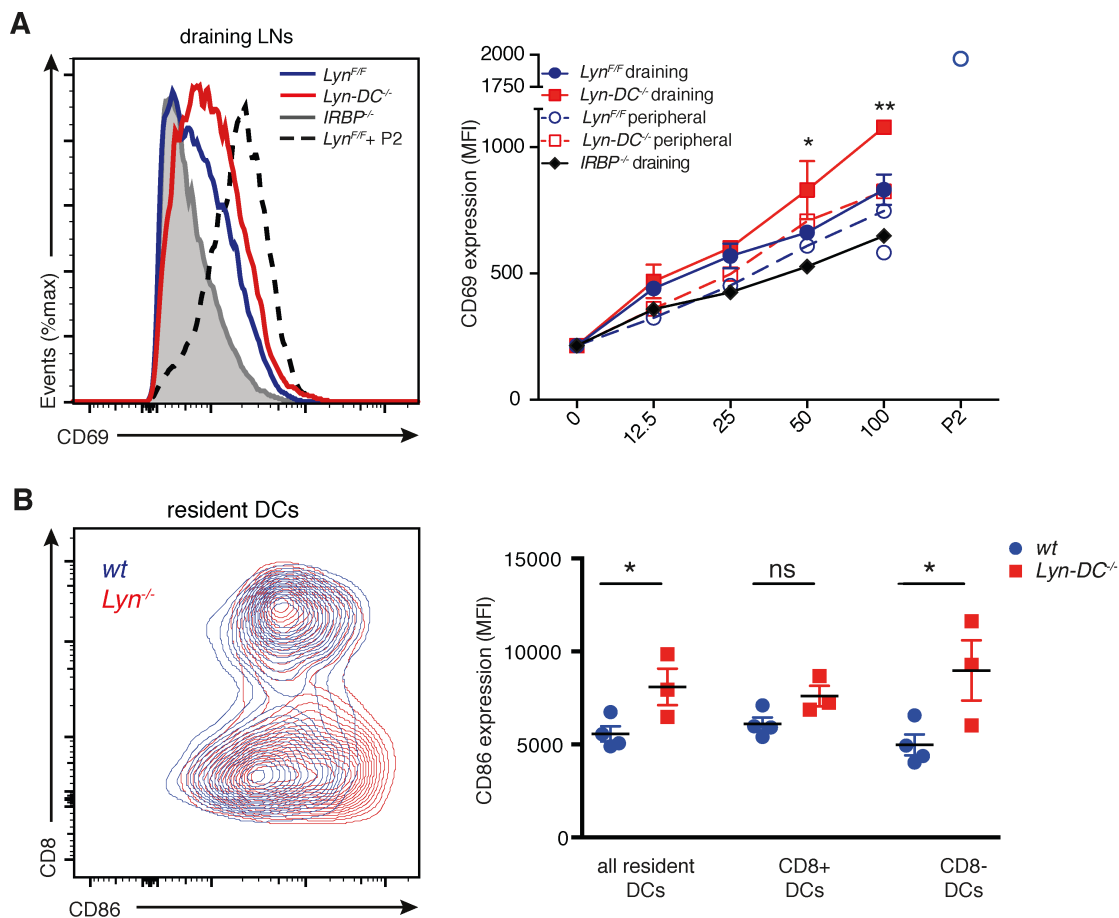
Supplemental Figure 4. Lyn expression in the retina. Immunohistochemistry staining for Lyn (brown) and hematoxylin (blue) of frozen eye sections from wild type and Lyn-deficient mice. Lyn-expressing cells possessed a ramified morphology (arrows) and were predominantly localized to the inner plexiform layer (IPL), inner nuclear layer (INL) and outer plexiform layer (OPL). A representative image is shown, n=3 mice per group. Scale bar = 50 μ M. CH=choroid.



Supplemental Figure 5. Stimulation of IRBP-specific hybridomas by *Lyn*^{-/-} DCs from the eye-draining lymph nodes can be blocked by addition of anti-MHCII antibody and is independent of mouse age. 50,000 P2-specific T-cell hybridoma cells were co-cultured with DCs from draining LNs without addition of exogenous peptide, except where indicated. Hybridoma stimulation was measured by upregulation of CD69. Data is representative of two independent experiments. (Left) Flow cytometry plot showing representative CD69 levels on hybridoma cells co-cultured with 100,000 DCs from draining LNs of mice of indicated genotypes. (Right) CD69 induction on hybridoma cells co-cultured with varying numbers of draining lymph node DCs from either 2 month old (solid symbols) or 6 month old (open symbols) *wt* and *Lyn*^{-/-} mice. As a positive control, hybridomas were co-cultured with 12,500 *wt* DCs with addition of 0.1 ug/ml P2 peptide. ***P* < 0.01, ****P* < 0.001, 1-way ANOVA.



Supplemental Figure 6. Similar abilities of wt and *Lyn*^{-/-} DCs to present exogenous IRBP to the P2-specific T-cell hybridoma. 50,000 P2-specific hybridoma cells were co-cultured with either wild type or *Lyn*^{-/-} DCs. Shown are CD69 levels on hybridoma cells after overnight incubation with DCs. **(A)** 30,000 splenic DCs from wild type or *Lyn*^{-/-} mice were cultured for 24 hours in the presence of whole mouse IRBP at the indicated concentrations, followed by addition of hybridoma cells and subsequent overnight incubation. **(B)** 100,000 DCs pooled from spleen and cervical LNs of wild type or *Lyn*^{-/-} mice were cultured with hybridoma cells in the presence of indicated concentrations of P2 peptide or IRBP 1-20 peptide as a negative control. Data are representative of two independent experiments.



Supplemental Figure 7. DCs from *Lyn-DC*^{-/-} mice present more endogenous IRBP and are more activated. (A) 50,000 P2-specific T-cell hybridoma cells were co-cultured overnight with DCs from either eye-draining cervical LNs or non-draining pooled axillary and inguinal LNs. Cells were incubated overnight without addition of exogenous peptide, except where indicated. Hybridoma stimulation was measured by upregulation of CD69. Left panel: Flow cytometry plot showing representative CD69 levels on hybridoma cells co-cultured with 100,000 DCs from draining LNs of mice of indicated genotypes. Dashed histogram corresponds to added 0.1 ug/ml P2 peptide. Right panel: Quantification of CD69 expression (MFI) on hybridoma cells co-cultured with varying numbers of DCs from either eye-draining (solid symbols) or peripheral (open symbols) LNs of *Lyn*^{F/F}, *Lyn-DC*^{-/-} or IRBP^{-/-} mice in the absence of exogenous peptide. As a positive control, hybridomas were co-cultured with 12,500 *Lyn*^{F/F} DCs with addition of 0.1 ug/ml P2 peptide. As a negative control, anti-MHCII blocking antibody was added to hybridomas co-cultured with 200,000 wt DCs (open circle). Data are representative of two independent experiments. **P* < 0.05, ***P* < 0.01, 1-way ANOVA. (B) Left: Representative flow cytometry plots showing CD86 and CD8 expression by resident (CD11c⁺ MHCII^{int}) cervical LN DCs from *Lyn*^{F/F} and *Lyn-DC*^{-/-} mice. Right: Quantification of CD86 expression (MFI) of indicated DC populations from *Lyn*^{F/F} and *Lyn-DC*^{-/-} mice. Each circle or square represents an individual mouse and the horizontal lines show mean ± SD. Data are representative of two independent experiments. **P* < 0.05, unpaired 2-tailed student's T test.