Supporting Information

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SI Text

Materials. Plasmid DNAs for human galectins-3 and galectins-3C have been described (1). Recombinant cytokines were from Peprotech Inc. OVA323–339 peptide was from AnaSpec Inc. SEB was from Sigma, SEE was from Toxin Technologies. Unless otherwise specified, all antibodies against cell surface marker of mouse or human T cells and the cytokine ELISA sets were from eBioscience. Fluorescence-conjugated secondary antibodies and phalloidin were purchased from Jackson ImmunoResearch Laboratories. Anti-galectin-3 antibody has been published (2). Antibodies for signaling molecules were from Cell Signaling Technologies. A peptide, 21 residues in length, CPQQPSYPF-PQPPQQSYYPQQ, corresponding to a sequence in the C-terminal region of human Alix, was synthesized and conjugated to keyhole limpet hemocyanin via the cysteine. The conjugates were used to immunize a rabbit by standard procedures to obtain antiserum. The preimmune serum was obtained before immunization.

Gal3$^{-/-}$ mice were generated as described in ref. 3 and backcrossed to C57BL/6 for nine generations. Experiments with mice were approved by the Institutional Animal Care and Use Committee of the University of California-Davis (Sacramento, CA). OTII mice were purchased from Jackson Laboratory. Gal3$^{-/-}$ mice in C57BL/6 background were crossbred with OTII to obtain gal3$^{-/-}$ OTII mice. CD4$^+$ T cells were purified with anti-CD4-PE and EasySep PE Selection Kit from Stem Cell Technologies. Jurkat transfectants expressing wild-type galectin-3 and mutant galectin-3 without carbohydrate-binding activity were previously described in refs. 1 and 4. Activated T blast cells were prepared by stimulating purified CD4$^+$ T cells with anti-CD3/CD28-coated beads and subsequently cultured in medium containing IL-2 for 7–14 days before use. BMDC were generated as previously described in ref. 5.

Induction of the IS on Supported Lipid Bilayers. Preparation of supported lipid bilayers and induction of the IS has been described in detail elsewhere (6). Briefly, lipid bilayers were prepared in flow cells (Focht Chamber System 2, FCS2, Bioptechs Inc.) by using 0.01% Cap-biotin and 300 mol/μm^2 of mouse ICAM-1 with a glycophosphatidyl inositol anchor (ICAM-1-GPI) labeled with Cy5. After treatment with 5% casein, the flow cells were flushed with HBS/HSA (HEPES-buffered saline with 1% human serum albumin, 1 mM CaCl$_2$, and 2 mM MgCl$_2$) and coated with 8 μg/mL streptavidin for 20 min and then with 10 μg/mL biotin-anti-CD3-AF-568 for an-

other 20 min. The flow cells were prewarmed to 37 °C and injected with Jurkat cells. The cells were incubated for 15 min and then fixed with 2% paraformaldehyde, permeabilized, and stained with rabbit anti-galectin-3 (2.5 μg/mL) followed by (Fab)$_2$ goat anti-rabbit IgG-AF-488.

For mouse cells, gal3$^{-/-}$ and gal3$^{+/+}$ CD4$^+$ OTII T blast cells were mixed with AF-546-conjugated H57 Fab anti-TCRbeta and subsequently injected into prewarmed flow cells. The lipid bilayers in the flow cells were prepared with 17.5% DOGS-NTA and 300 mol/μm^2 cy5-labeled ICAM-1-GPI and subsequently coated with 100 nM NiSO$_4$ and then with 5 nM His-tagged I-Ab:OVA peptide complex. The cells were incubated for 30 min and then fixed, permeabilized, and stained with rabbit anti-galectin-3 (2.5 μg/mL), followed by (Fab)$_2$ goat anti-rabbit IgG-AF-488. The cells were also stained with biotinylated anti-phosphotyrosine followed by streptavidin-AF-488. The images were acquired with TIRFM and then processed and measured with Image J software (National Institutes of Health, Bethesda, MD). The integrated intensities of SMAC, cSMAC, and pSMAC were measured on immunofluorescence images of TCR or phosphotyrosine with region of interest plotted manually on TCR images.

Yeast Two-Hybrid Screening for Galectin-3-Binding Partners. All of the reagents for yeast two-hybrid screening, including yeast vectors, yeast strain EGY48, anti-HA, and anti-LexA antibodies, were generous gifts from Dr. Stephan Witte (La Jolla Institute for Allergy and Immunology). Construction of human galectin-3 bait plasmids for yeast two-hybrid screening: pGilda, a pEG202 derivative that contains an inducible GAL1 promoter, was used as an expression vector for constructing LexA-fused bait proteins (7). pGilda-Gal-3 expresses LexA fused to the full-length (1–250) coding sequence of human galectin-3.

Yeast two-hybrid screening was performed essentially according to (7). Briefly, *S. cerevisiae* EGY48 containing pJK103 and pGilda-Gal-3 was transformed with a Jurkat T lymphoma cDNA library constructed in the vector pJG4–5 (8). Approximately 6 $\times$ 10^7 transformants from 5 $\times$ 10^5 independent colonies were plated onto agar plates lacking uracil, histidine, tryptophan, and leucine after induction with galactose-containing medium. Colonies that appeared after 3 days were subjected to a filter assay for activation of the LacZ gene (β-galactosidase reporter gene). Library plasmids were rescued from those colonies that had both the leu2 and lacZ genes activated. Insert sizes were assessed by PCR, the PCR products were then used for direct sequencing to identify the inserts.

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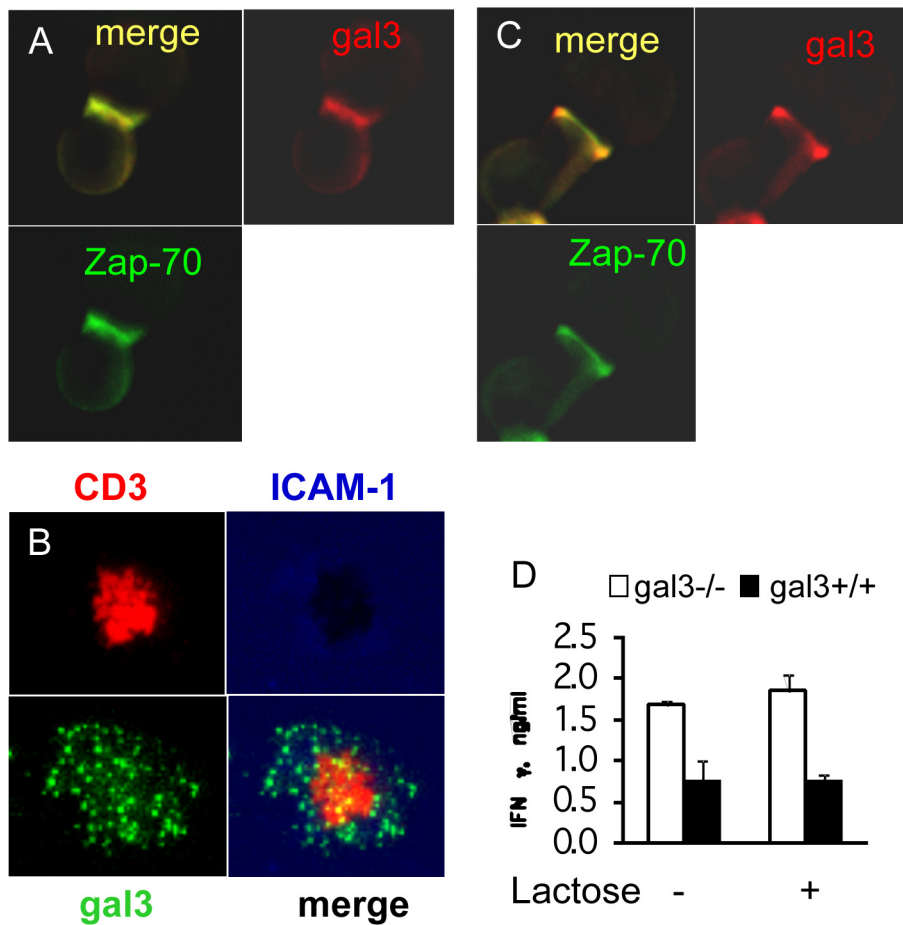
Fig. S1. (*A–C*) Galectin-3 is not dependent on its carbohydrate-binding activity to translocate to the IS. Jurkat cells transfected with galectin-3 mutant with disrupted carbohydrate-binding activity were (*A*) stimulated with SEE-pulsed RPMI 8866 B cells and analyzed by immunofluorescence staining, or (*B*) placed on supported lipid bilayers preloaded with fluorescence-labeled anti-CD3 and ICAM-1 to form the IS. (*C*) Gal3+ Jurkat cells were stimulated with SEE-pulsed B cells in the presence of lactose before staining. (*D*) Galectin-3's suppressive effect on T cells is not dependent on its binding to cell surface glycans. Gal3$^{-/-}$ or gal3$^{+/+}$ CD4$^+$ T cells were stimulated with anti-CD3/CD28 in the presence or absence of lactose. *P* < 0.01.