

SUPPLEMENTAL MATERIAL

Ohmichi et al., <http://www.jem.org/cgi/content/full/jem.20101786/DC1>

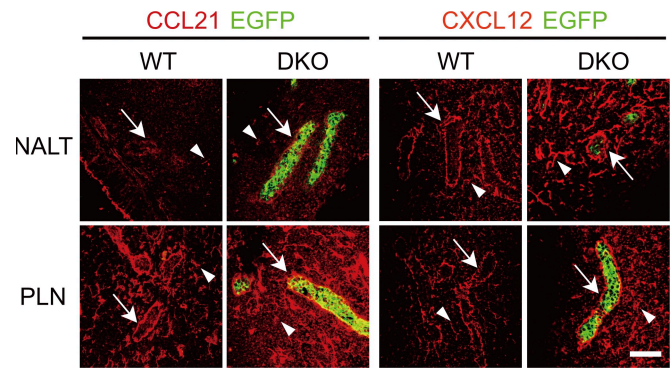


Figure S1. Expression of chemokines in NALT and PLNs in WT and DKO mice. Detection of CCL21 and CXCL12 in lymphoid organs. Frozen sections of NALT and PLNs were stained with polyclonal antibodies against CCL21 and CXCL12 (red). Green fluorescence is from the GlcNAc6ST-2-EGFP chimeric protein. Arrows indicate the expression of CCL21 or CXCL12 in HEVs. Arrowheads indicate CCL21 or CXCL12 in stromal cells. Bar, 50 μ m. Data are representative of three independent experiments.

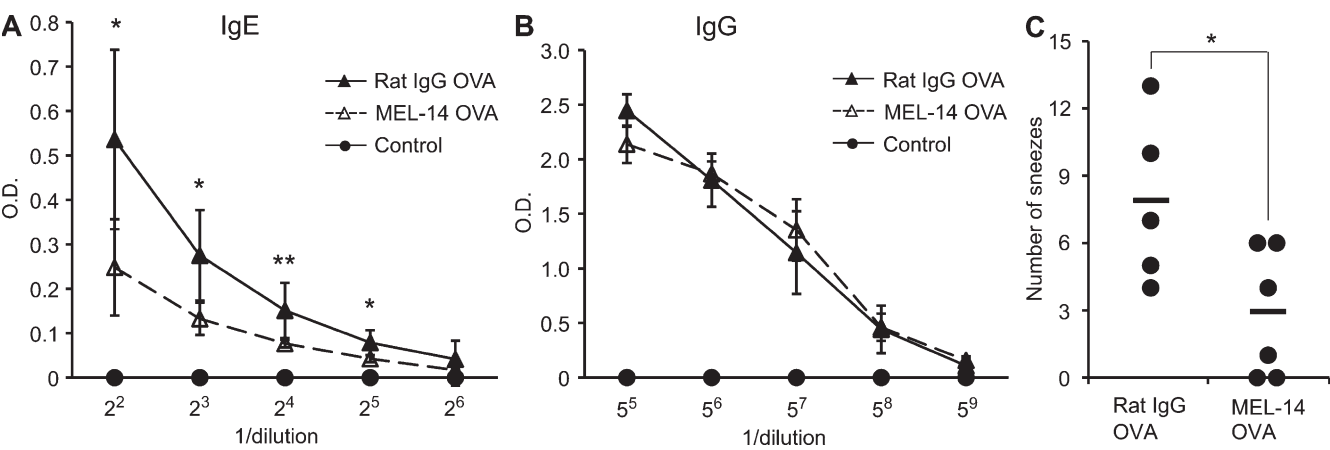


Figure S2. Anti-L-selectin mAb reduced OVA-specific IgE production and attenuated nasal symptoms. (A and B) WT mice injected intraperitoneally with 25 μ g/mouse of control rat IgG (Rat IgG OVA, closed triangles) or MEL-14 (MEL-14 OVA, open triangles) on days -1, 2, 6, 9, 13, 16, and 20 were immunized intranasally with OVA and CT on days 0, 7, and 14 (closed or open triangles) or left untreated (Control, closed circles). OVA-specific IgE (A) and IgG (B) were measured by ELISA on day 21 using 1-Step Ultra TMB-ELISA (Thermo Fisher Scientific) as a substrate. Error bars represent the SD ($n = 3-6$). *, $P < 0.05$; **, $P = 0.055$. (C) WT mice treated with rat IgG (Rat IgG OVA) or MEL-14 (MEL-14 OVA) and immunized with OVA and CT were intranasally challenged with OVA and CT on day 21. The number of sneezes was counted for 7 min. Each horizontal bar represents the mean of the values obtained from five or six animals. *, $P < 0.02$.

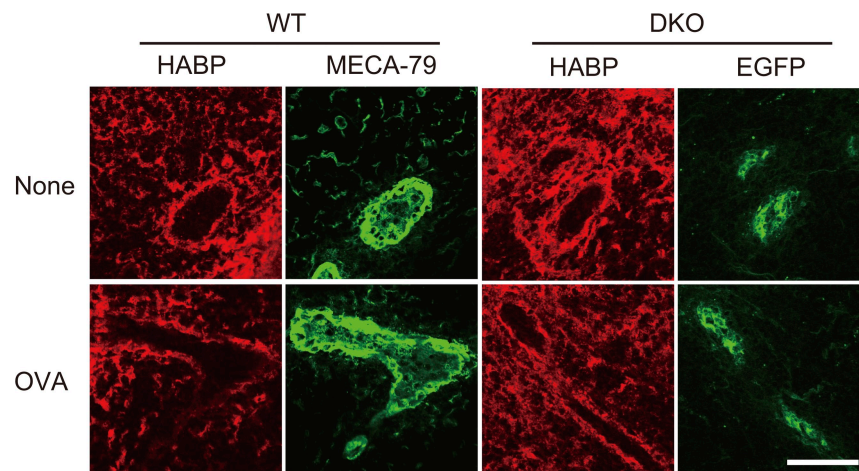


Figure S3. Expression of HA in NALT HEVs in WT and DKO mice. Frozen sections of NALT collected on day 21 from WT and DKO mice that had been intranasally immunized with OVA and CT on days 0, 7, and 14 (OVA) or left untreated (None) were stained with HABP and Alexa Fluor 594-conjugated streptavidin (red). The sections of WT mice were costained with DyLight 488-conjugated MECA-79 (green). Green fluorescence in the sections of DKO mice is from the GlcNAc6ST-2-EGFP chimeric protein that had been knocked-in to the GlcNAc6ST-2 locus. Bar, 50 μ m. Data are representative of two independent experiments.

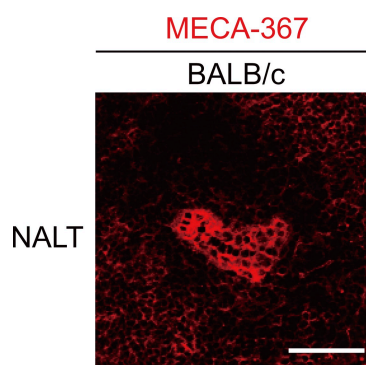


Figure S4. Expression of MECA-367 antigens in NALT HEVs in BALB/c mice. Frozen sections of NALT from BALB/c mice were stained with MECA-367 (red). Bar, 50 μ m. Data are representative of three independent experiments.

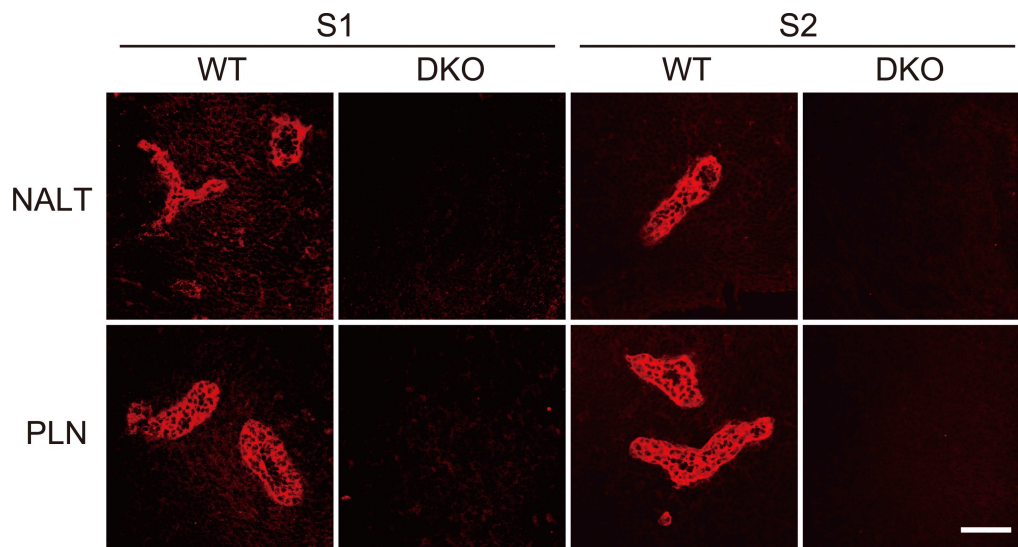


Figure S5. Expression of GlcNAc-6-*O*-sulfated glycans in NALT and PLN HEVs in WT and DKO mice. Frozen sections of NALT and PLNs from WT and DKO mice were incubated with 5 $\mu\text{g}/\text{ml}$ antisulfated glycan mAbs S1 and S2, which recognize GlcNAc-6-*O*-sulfated, α 2,3-sialylated *N*-acetylactosamine structures (Hirakawa et al., 2010), followed by 0.5 $\mu\text{g}/\text{ml}$ Alexa Fluor 594-conjugated streptavidin (red). Bar, 50 μm . Data are representative of two independent experiments.

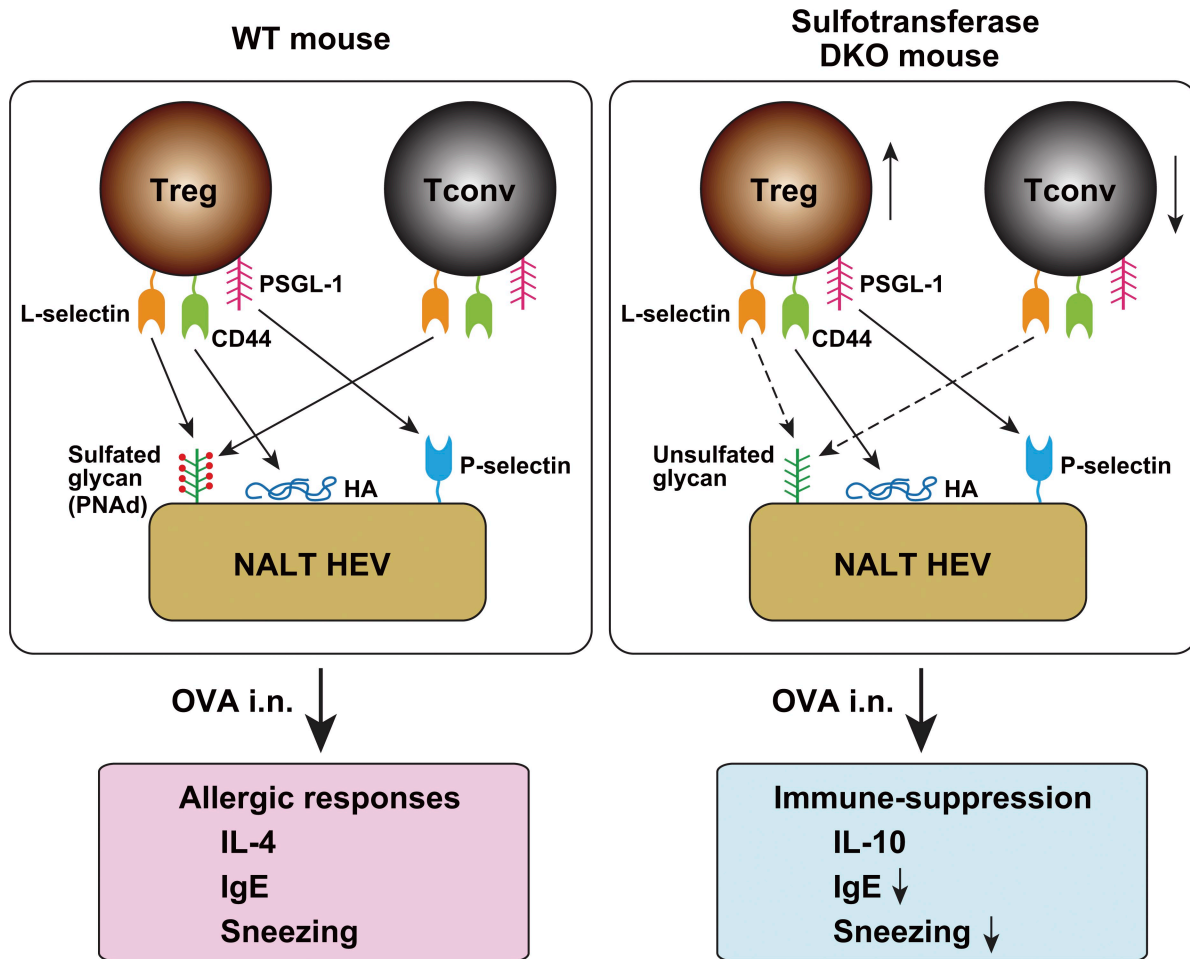


Figure S6. A proposed model for the molecular mechanisms of T_{reg} and T_{conv} cell homing to NALT and the reduction of allergic responses in DKO mice. In WT mice (left), homing of T_{reg} cells to NALT is mediated by L-selectin–PNAd, CD44–HA, and PSGL-1–P-selectin interactions, whereas that of T_{conv} cells is mainly mediated by an L-selectin–PNAd interaction (arrows). In DKO mice (right), GlcNAc-6-*O*-sulfation of PNAd (red symbols) is eliminated and the L-selectin–PNAd interaction is weakened (dashed arrows). As a result, T_{reg} cells are enriched in NALT in DKO mice, creating an immunosuppressive environment in NALT. After intranasal immunization (OVA i.n.), allergic responses, including IgE production and sneezing, are reduced in DKO mice, possibly through a shift in cell populations and the cytokine environment in NALT.

REFERENCE

Hirakawa, J., K. Tsuboi, K. Sato, M. Kobayashi, S. Watanabe, A. Takakura, Y. Imai, Y. Ito, M. Fukuda, and H. Kawashima. 2010. Novel anti-carbohydrate antibodies reveal the cooperative function of sulfated *N*- and *O*-glycans in lymphocyte homing. *J. Biol. Chem.* 285:40864–40878. doi:10.1074/jbc.M110.167296