## METHODS

**Cell isolation/purification.** Cells were purified by positive selection using magnetic beads (Miltenyi Biotech) (typically >90% purity), or by sorting on a MoFlo cell sorter (DAKO) (typically >98% purity). $CD11c^+$ dendritic cells were purified from spleens by digestion with collagenase D (2 mg $ml^{-1}$, Roche) and DNase I (50 U $ml^{-1}$, Roche) for 30 min at 37 °C, and subsequent positive selection using anti-CD11c magnetic beads (Miltenyi Biotech). Dendritic cells used were typically 90–95% pure.

**Flow cytometry.** For extracellular flow cytometry, cells were re-suspended in 100 µl PBS plus 4% FCS, containing 5 µg $ml^{-1}$ Fc block (BD Pharmingen), and incubated with 1.5 µg $ml^{-1}$ antibody for 20 min on ice. Cells were analysed on a FACS Calibur or FACSort (BD Pharmingen). Data were analysed using Flow-Jo (Treestar).

For intracellular flow cytometry, extracellular antigens were stained as above, and cells fixed and permeabilized using fixation/permeabilization buffer (eBioscience) for 30 min at 4 °C. Cells were washed twice and stained for 30 min at 4 °C with antibodies diluted in permeabilization buffer (at 1.5 µg $ml^{-1}$). Cells were analysed as described above.

The following antibodies were used in experiments, and were purchased from BD Pharmingen or eBioscience unless otherwise stated: anti-CD4 (clone RM4-5), anti-CD8 (clone 53-6.7), anti-CD62L (clone MEL-14), anti-CD44 (clone IM7), anti-IL4 (clone 11B11), anti-IFN-γ (clone XMG1.2), anti-B220 (clone RA3-6B2), anti-CD86 (clone GL1) and anti-MHC II (clone NIMR, Southern Biotech).

**Primers used for RT–PCR and qRT–PCR.** To detect expression of mouse *Itgb8* RNA by RT–PCR, the following primers were used: forward, 5′-CATTCTTGATCGGGTTGCTT-3′; reverse, 5′-CAGGCTTTTCTCGTCGGTAG-3′. To detect mouse *Itgb8* RNA by qRT–PCR, the following primers were used: forward, 5′-CTGAAGAAATACCCCGTGGA-3′; reverse, 5′-AGACTGTATGCCTCCCCAT-3′.

**ELISA.** For detection of auto-antibodies (against double-stranded DNA and ribonuclear proteins), mouse serum was diluted and analysed using a REAADS ANA ELISA kit (Corgenix) according to the manufacturer's instructions.

Levels of IgG1, IgG2a, IgG2b, IgG3, IgA and IgM in mouse serum were analysed using a Clonotyping ELISA kit (Southern Biotech) according to the manufacturer's instructions. Levels of IgE in mouse serum were measured by sandwich ELISA, using anti-mouse IgE as a capture antibody (clone R35-72, BD Pharmigen, 2 µg $ml^{-1}$ coating concentration) and biotinylated anti-mouse IgE antibody (clone R35-118, BD Pharmigen, 1 µg $ml^{-1}$) as a detection antibody. Wells were incubated with streptavidin-HRP (BD Pharmingen, 1:1,000), and wells were subsequently developed using TMB solution (BD Pharmigen), and absorbance read at 450 nm.

For detection of IL-4 and IFN-γ by ELISA, all antibodies were purchased from eBioscience. Anti-IL-4 antibody (clone 11B11, 2 µg $ml^{-1}$) or anti-IFN-γ antibody (clone AN-18, 1 µg $ml^{-1}$) was coated in 96-well ELISA plates. Supernatant from activated splenocytes (see below) was diluted and added to wells. Cytokine was detected by incubating wells with biotinylated anti-IL-4 antibody (clone BVD6-24G2, 1 µg $ml^{-1}$) or biotinylated anti-IFN-γ antibody (clone R4-6A2, 1 µg $ml^{-1}$) followed by streptavidin-HRP (BD Pharmigen, 1:1,000 dilution). Wells were developed with TMB solution, and absorbance measured at 450 nm. Concentrations of immunoglobulin/cytokine were calculated from standard curves using purified cytokine or specific immunoglobulin isotypes.

**Regulatory T-cell induction assay.** $CD4^+GFP–Foxp3^-$ T cells from GFP–Foxp3 mice[25] were purified to greater than 99.8% purity on a MoFlo sorter. Splenic $CD11c^+$ dendritic cells from 2–4-month-old control or *(Vav1-cre)Itgb8$^{fl/fl}$* mice were purified as described above. A total of 2,500 dendritic cells were incubated with 50,000 $CD4^+GFP–Foxp3^-$ T cells in the presence of 1 µg $ml^{-1}$ anti-CD3 antibody (clone 2c11, BD Pharmigen) and either 40 µg $ml^{-1}$ mIgG (Sigma), 40 µg $ml^{-1}$ anti-TGF-β antibody (clone 1d11, hybridoma purchased from ATCC), or 2 ng $ml^{-1}$ active TGF-β (R&D Systems). After 72 h, cells were stained with CD4 antibody, and analysed by flow cytometry for $CD4^+GFP–Foxp3^+$ cells (dead cells, identified by positive staining for 7-amino-actinomycin D, were excluded from analysis).

**TGF-β activation assay.** To determine TGF-β activity by dendritic cells, purified dendritic cells were cultured for 72 h in 96-well plates. A total of 100 ng $ml^{-1}$ lipopolysaccharide was added for the last 48 h, and mink lung epithelial cells stably transfected with a plasmid containing firefly luciferase cDNA downstream of a TGF-β-sensitive portion of the plasminogen activator inhibitor 1 promoter[30] were added for the last 24 h. TGF-β activity was calculated as the difference in luciferase activity between untreated wells and wells treated with a blocking antibody to TGF-β (1D11).

**Colon lamina propria cell preparation.** Colonic lamina propria lymphocytes were isolated using a previously described method[29], with slight modification. Briefly, colons were extracted and placed on ice in CMF buffer (Hank's Balanced Salt Solution (HBSS) plus 2% FCS plus 15 mM HEPES, pH 7.4). The colon was washed, chopped into small (0.5 mm) pieces with a razor blade, and washed six more times with ice-cold CMF. Colon pieces were then incubated with CMF plus 5 mM EDTA for 4 × 15 min at 20 °C with gentle stirring (100 r.p.m.) to remove intraepithelial lymphocytes. Remaining colon pieces were washed with complete RPMI (RPMI plus penicillin and streptomycin plus 10% FCS plus 10 mM HEPES) to remove residual EDTA, then incubated for 3 × 1 h in complete RPMI plus 0.5 mg $ml^{-1}$ collagenase D (Roche). Supernatants containing lamina propria cells were collected and analysed by flow cytometry for CD4 and Foxp3. Live cells were gated using forward and side scatter.