Gene targeting
A targeting vector pACN-TV was used that included a *loxP*-flanked ACN cassette containing both the *neo*^{r} gene and the *Cre*-recombinase gene under the control of the sperm-specific ACE promoter.[1] The ACN-cassette was deleted in chimeras during spermatogenesis. A 1.2-kb ScaI/SacI fragment containing exons 26 and 27 and a 7.4-kb SacI/EcoRI fragment containing exon 28 and UTR were subcloned into the upstream regions and downstream of the *lox*P-flanked ACN cassette, respectively. An engineered XbaI site was introduced downstream of exon 27 such that a targeted allele from WT was identified by Southern blot analysis. The exonic sequences in the targeting vector were confirmed by DNA sequencing.

The targeting vector was transfected into ES cells of C57BL/6J origin (Bruce-4). G418-resistant ES cell clones were screened for homologous recombination by Southern blot analysis. Homologous integrant ES cell cones were injected into Balb/c blastocytes and the resulting chimeric males were bred to C57BL/6 females for germline transmission. Offspring were screened for heterozygosity by PCR to confirm a germline transmission and an *in vivo* deletion of the ACN cassette. After intercrossing heterozygotes with each other, the resulting F2 mice were screened to select the homozygote. F5 to F10 generations were used in all experiments.

Mice
C57BL/6J (Charles River Laboratories), Balb/c (Taconic), and C57BL/6J-CD45.1 (The Jackson laboratory) were obtained for this study. All mice were fed a PicoLab Diet 20 (PMI nutrition) and maintained in a specific pathogen-free animal facility in the Warren Alpert Building at Harvard Medical School. All animal experiments were approved by the Institutional Review Board of the Immune Disease Institute.

Antibodies and Reagents
The following mAbs were used in this study: CD3e (145-2C11), CD3 (17A2), CD28 (37.51), B220 (RA3-6B2), α4 (9C10), αL (2D7), β2 (C71/16), and β7 (M293) (BD Biosciences). CBL 1333 mAb (β1) was obtained from Chemicon. DATK32 (α4β7) and PS/2 (α4) mAbs were purified from hybridomas (ATCC). Murine MAdCAM-1-, VCAM-1and ICAM-1-Fc fusion proteins, fibronectin, TNF-α, CXCL12, and CCL21 were acquired from R&D Systems. Calcein-AM, CFSE (Carboxyfluorescein diacetate, succinimidyl ester) and CMTMR (5-(and-6)-(((4-chloromethyl) benzoyl) amino) tetramethylrhodamine) were obtained from Molecular Probes. Dextran sodium sulphate (DSS) was from MP Biomedicals.

Cell isolation and flow cytometry
Mononuclear cells were isolated from the spleen (SP), peripheral blood (PB), peripheral lymph node (PLN), mesenteric lymph node (MLN), Peyer's patch (PP), intraepithelial lymphocyte (IEL), lamina propria lymphocyte (LPL), and bone marrow (BM) as previously described.[2] Multicolor immunofluorescent cytometry was performed as previously described [3] using a FACSCalibur flow cytometer (BD Biosciences). For intracellular staining of integrins, cells were fixed and permeabilized using Fix & Perm (Caltag) prior to staining with PE-conjugated anti-α4 antibody (9C10). Data were analyzed using CellQuest software (BD Bioscience).

Real-time quantitative RT-PCR (qRT-PCR)

Total RNA from splenocytes was isolated with RNeasy total RNA isolation kit (Qiagen). A SYBR GreenER Two-Step qRT-PCR Kit was used. qRT-PCR was performed with iCycler (Biorad) using the following primer pairs: α4, 5′CCCCTCAACACGAACAGATAGG-3′ (forward) and 5′GCCTTGTCCTTAGCAACACTGC-3′ (reverse); X-box binding protein-1 (XBP-1), 5′ACACGCTTGGGAATGGACAC-3′ (forward) and 5′-CCATGGGAAGATGTTCTGGG-3′ (reverse);[4] glucose-regulated protein 78 (GRP78), 5′-TGACTGCTGAGCGACTGGTC-3′ (forward) and 5′-GCTGCTGTAGGCTCATTGATG-3′ (reverse);[5] GAPDH, 5′CCAGGTTGTCTCCTGCGACTT-3′ (forward) and 5′-CCTGTTGCTGTAGCCGTATTCA3′ (reverse). Standard curves were generated and a threshold was set in the linear part of the amplification curve. Melting curve analysis and agarose gel electrophoresis were performed to assess the purity of the amplified bands.

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