SUPPLEMENTARY METHODS

Flow cytometry and intracellular staining. We obtained red blood cell-free single cell suspensions from DLN and spleens, and subjected the lymphocytes (1 × 10^6 cells in 100 µl) to flow cytometric analysis at 4 °C after an initial blocking step with 1 µg unlabeled anti-FcγR Ab (BD PharMingen) with indicated fluorochrome-conjugated antibodies, followed by analysis by FACSCalibur (BD Biosciences). The following mAbs were purchased from BD PharMingen for flow cytometry analysis: FITC-, PE-, PerCP-, and biotin-anti-CD8 (53-6.7); PE-anti-CD4 (GK1.5); PE- and biotin-anti-CD11c (HL3); FITC- and biotin-anti-CD11b (M1/70); PE-anti-B220 (RA3-6B2); FITC-anti-IFN-γ (XMG1.2); FITC-anti-IL10 (JESS-16E3); PE-anti-IL12 (C15.6); biotin-anti-I-2K^d (KH14); biotin-anti-I-A^q (KH116); biotin-anti-CD80 (16-10A1); biotin-anti-CD86 (GL1); biotin-anti-CD40 (3/23); biotin-anti-CD45RA (14.8); purified anti-CD16/CD32 (2.4 G2); PE-, FITC-, and Cy-streptavidin; and a mouse Vβ TCR screening panel that contained FITC-conjugated mAb against Vβ2, 3, 4, 5.1/5.2, 6, 7, 8.1/8.2, 8.3, 9, 10b, 11, 12, 13, 14, and 17 TCRs. FITC-anti-DEX205 (NLDG-145) was purchased from Serotec. DX5 was purchased from eBioscience.

For intracellular cytokine staining, cells were stimulated with CII (50 µg/ml) or CII plus-PMA (50 ng/ml; Sigma) and ionomycin (500 ng/ml; Sigma) for 18 h. GolgiPlug (BD PharMingen) was added during the last 6 h. Cells were first stained for surface markers, fixed, permeabilized, and incubated with FITC-conjugated anti-IFN-γ, anti-TGF-β, anti-IL-4, anti-IL-10, or IL-12 mAb with the Cytofix/Cytoperm Kit (BD PharMingen), according to the manufacturer’s instructions.

The BrdU incorporation assay was performed according to the manufacturer’s instructions (BrdU Flow Kit, BD PharMingen). Briefly, DLN cells (2 × 10^5) were cultured in the presence of CII (50 µg/ml). The cells were stained with PE-conjugated anti-CD4, fixed, permeabilized, treated with DNase I, and further incubated with FITC-conjugated anti-BrdU (BD PharMingen). Samples were immediately analyzed by FACSCalibur (BD Biosciences).