Supplemental Notes

Methods

Phosphorylation of Pyk2, Syk and MAPK1/2 after BCR stimulation in +/+ and *ccr2*$^{-/-}$ mice.

B Lymphocytes were purified from spleens of +/+ and *ccr2*$^{-/-}$ mice using a negative selection column (R and D systems, Minneapolis, MN) and stimulated with F(ab')2 fragment of anti-IgM mAb and the phosphorylation of Pyk2, Syk, and MAPK1/2 was analyzed. Each of the tyrosine phosphorylation experiments were performed three times. Several time points starting from 1 minute to 10 minutes were analyzed.

Phosphorylation of PLCγ-1, ZAP-70, and MAPK1/2 after TCR stimulation in +/+ and *ccr2*$^{-/-}$ mice.

CD3+ T lymphocytes were purified from the spleen cells of wild-type and *ccr2*$^{-/-}$ mice using a negative selection column (R and D systems, Minneapolis, MN) and treated with anti-CD3 mAb. Cells were lysed and immunoprecipitated with antiphosphotyrosine mAb (4G10). The samples were fractionated by 10% SDS-PAGE and blotted on PVDF membrane. The filters were probed with 4G10, anti- PLCγ-1 mAb, anti-ZAP-70 Ab, and anti-MAPK1/2 Ab.