0

medium



CCL20

CXCL12

Supplemental Figure 1

Development of CCR6-deficient mice. (A) Targeting strategy. Schematic representation of the *Ccr6* gene corresponding to positions 73,2125-73,8664 in GenBank record NT_039638 (and shown with numbering 1-6539 for this BamHI fragment, top panel), the pPNT-Ccr6 targeting vector (middle panel), and the disrupted/deleted Ccr6 gene (bottom panel). Hatched regions on the diagrams indicate the predicted ORF for Ccr6. Positions 3094-3611 in the ORF were deleted in targeted allele and a BamHI site was introduced at the 3' end of the 5' Ccr6 fragment. The location of the 5' probe used for analyzing DNA for the presence of the disrupted/deleted Ccr6 allele is indicated ("probe"). (B) A representative Southern blot from either a WT (+/+) or heterozygous (+/-) colony of ES cells. DNAs from the ES cells were digested with BamHI, and probed with the 5'-radiolabeled probe indicated in (A). BamHI digestion of the WT and successfully targeted alleles yielded fragments of approximately 6 kb and 3 kb, respectively. Data are representative of 220 individual ES cell lines analyzed after transfection with pPNT-Ccr6. (C) CCR6 expression analyzed by flow cytometry on B cells from WT or CCR6KO mice. B cells were either used immediately after isolation from spleens ("Fresh") or after stimulating with $10 \,\mu g/ml$ goat anti-mouse IgM F(ab')₂ for 48 hours ("Anti-IgM"). Data are from one of two experiments. GFP was detectable in the anti-IgM-activated B cells, but not in other cells such as T cells or dendritic cells (data not shown), possibly due to low levels of IRES-initiated translation. As a result, GFP expression was not routinely analyzed in the experiments that follow. (D) Mouse B cells treated as in (C) were used in Transwell chemotaxis assays with either medium

alone or medium containing 1 μ g/ml of either CXCL12 or CCL20 in the lower wells. Bars show means from duplicate wells from one of two experiments



Β.



Supplemental Figure 2

Intracorneal neutrophilic microabscesses in WT and RAG-1KO mice injected with

IL-23. H&E-stained sections of IL-23-injected ears from WT (A) and RAG-1KO (B)

mice at Day 5. (k), intracorneal neutrophilic pustules with hyper-parakeratosis, (a),

acanthosis, (d), dermal mononuclear cell infiltrate, and (v), telangiectasia of dermal blood

vessels. Original magnification, 400x. Sections are representative of two experiments.