Supplementary Materials

Methodology:

**Antibodies:**

- APC-conjugated anti-CD3 (clone UCHT1), PE-conjugated anti-CD3 (clone HIT3a), PerCP/Cy5.5-conjugated anti-CD3 (clone UCHT1), FITC-conjugated anti-TCR Vδ2 (clone B6), PE-conjugated anti-TCR Vδ2 (clone B6), PerCP/Cy5.5-conjugated anti-TCR Vδ2 (clone B6), PE-conjugated anti-TCR Vδ (clone B1), FITC-conjugated anti-TCR Vδ (clone B1), APC-conjugated anti-CCR1 (clone 5F10B29), APC-conjugated anti-CCR2 (clone K036C2), PE-conjugated anti-CCR3 (clone 5E8), APC-conjugated anti-CCR4 (clone L291H4), APC-conjugated anti-CCR5 (clone J418F1), PE-conjugated anti-CCR6 (clone G034E3), APC-conjugated anti-CCR7 (clone G043H7), PE-conjugated anti-CCR8 (clone L263G8), APC-conjugated anti-CCR9 (clone L053E8), PE-conjugated anti-CCR10 (clone 6S88-5), APC-conjugated anti-CXCR1 (clone 8F1/CXCR1), PE-conjugated anti-CXCR2 (clone 5E8/CXCR2), APC-conjugated anti-CXCR3 (clone G025H7), PE-conjugated anti-CXCR4 (clone 12G5), PE-conjugated anti-CXCR5 (clone J252D4), PE-conjugated anti-CXCR6 (clone K041E5), PE-conjugated anti-CX3CR1 (clone 2A9-1), PerCP/Cy5.5-conjugated anti-IL-17A (clone B1168), APC-conjugated anti-TNF-α (clone Mab11), APC-conjugated anti-IFN-γ (clone 4S.B3), Brilliant Violet 421-conjugated anti-CD45 (clone HI30), Zombie UV™ Fixable Viability Kit, APC Mouse IgG1, κ Isotype Ctrl, PE Mouse IgG2a, κ Isotype Ctrl, PerCP/Cy5.5 Mouse IgG1, κ Isotype Ctrl, FITC Mouse IgG1, κ Isotype Ctrl, PE Mouse IgG1, κ Isotype Ctrl, APC Mouse IgG2a, κ Isotype Ctrl, PE Mouse IgG2b, κ Isotype Ctrl, PE Armenian Hamster IgG Isotype Ctrl, PERat IgG2b, κ Isotype Ctrl, Brilliant Violet 421 Mouse IgG1, κ Isotype Ctrl were obtained from Biolegend. PE Mouse Anti-p38 MAPK (pT180/pY182), Alexa Fluor® 647 Mouse anti-NF-κB p65 (pS529), PE Mouse anti-JNK.
Flow cytometric analysis

PBMCs were washed with PBS containing 1% bovine serum albumin (BSA) and were incubated in various combinations of monoclonal antibodies (mAbs) for 30 min at 4°C. Then, the cells were washed and suspended in PBS. The stained cells were immediately analyzed with a BD Accuri C6 flow cytometer (Becton Dickinson) or were fixed in 1% paraformaldehyde and analyzed within 24h. Data analysis was performed using FlowJo Software (Tree Star Inc.).

For the detection of intracellular IFN-γ, TNF-α and IL-17 in Vδ2 T cells, the cells were pretreated with PMA (0.5 mg/ml), Ion (1 mg/ml) and Golgiplug (1ug/ml) for 5 h. Then, the cells were washed twice with PBS containing 1% BSA and stained for surface molecules of CD3 and Vδ2. After washing twice with PBS containing 1% BSA, the cells were fixed and permeabilized with BD cytofix/cytoperm solution. The cells were then washed with permeabilization solution before staining with anti-human IFN-γ, TNF-α and IL-17 antibodies. The cells were incubated at room temperature (25°C) in the dark for 30 min, then washed twice with permeabilization solution before analysis using a BD Accuri C6 flow cytometer (Becton Dickinson). Data analysis was performed using FlowJo Software (Tree Star Inc.).

CFSE proliferation assay PBMCs were labeled with CFSE and used as the responder cells. The cells were cultured in RPMI-1640 medium containing 10% FBS and
200 IU/ml IL-2 in 24-well culture plates coated with 1 μg/ml anti-pan-TCR γδ mAb in the dark. After 5 days in culture, the cells were collected, washed twice with PBS containing 1% BSA, and stained with a primary antibody against Vδ2 for 1 h at 4°C. Then, the cells were stained with DyLight™ 649-conjugated goat anti-mouse IgG for 30 min at 4°C following a thorough washing. The cells were analyzed using a BD Accuri C6 flow cytometer (Becton Dickinson) with gating of the Vδ2-positive cells. Data analysis was performed using FlowJo Software (Tree Star Inc.).

**Apoptosis assay**

First, 1.0×10⁷ cells were washed twice with cold PBS containing 1% BSA, and then suspended with 100μl Annexin V Binding Buffer. Then, 5 μl of APC/Annexin V, 5 μl of 7-AAD Viability Staining Solution and 5 μl of FITC-conjugated anti-human TCR Vδ2 antibody were added. The cells were gently vortexed and incubated for 15 min at room temperature (25°C) in the dark. A total of 400 μl of Annexin V Binding Buffer was added to each tube. The stained cells were immediately analyzed with a BD Accuri C6 flow cytometer (Becton Dickinson).

**Immunohistochemistry assay**

To block endogenous peroxidase activity, the samples were treated with 1.5% H₂O₂ for 20 minutes. Then, the slides were washed with TBS 3 times for 3 minutes each. Non-specific binding sites were blocked with 10% diluted goat serum in TBS for 20 hours. The samples were incubated overnight at 4°C in the appropriate antibody dilution (1:5). The slides were washed 5 times in TBS and incubated in the anti-mouse IgG peroxidase antibody for 1 hour, then washed 5 times in TBS. Avidin-biotin-
peroxidase reagents were added and the slides were incubated in a 0.5 mg/mL HRP substrate solution (DAB+H2O2 prepared in distilled water), washed 5 times in TBS and counterstained for 1 minute with hematoxylin. The slides were dehydrated by washing the slides in an ethanol series for 1 minute each in 75%, 80% and, finally, 100% ethanol.

**Synovium Digestion and T cell isolation:**

Fresh synovium was cleaned of fat tissue, cut into small pieces and incubated in Hank's Balanced Salt Solution (HBSS) supplemented with 0.4U/ml Collagenase NB 4 standard grade (SERVA) for 2 h at 37 °C with gently shaking. The digested tissue was meshed and passed through cell strainers (BD). The cell suspensions were washed with HBSS, and mononuclear cells were isolated with Ficoll gradient.

Peripheral blood mononuclear cells were stained with anti-CD3 and anti-Vδ2 antibodies, and mononuclear cells from synovium were stained with viability dye (zombie), anti-CD45, anti-CD3 and anti-Vδ2 antibodies, then processed by flow cytometry.

Peripheral Vδ2 T cells were stained with anti-CD3 and anti-Vδ2 antibodies, and sorted with BD FACS Aria II cell sorter.
**Supplementary figures:**

Fig. S1. Decreased peripheral Vδ2 T cells in RA patients did not result from abnormal proliferation or apoptosis.

Fresh PBMC from RA, OA and HC were (A) stimulated with immobilized anti-pan-TCRγδ mAb for 5 days. Vδ2 T cells were labeled and proliferations were detected with CFSE dilution or (B) stained with an anti-Vδ2 mAb, 7-AAD and Annexin V to detect the rate of apoptosis. Data are representative of three independent experiments. Results are expressed as mean ± SEM. ns, no significance by one way ANOVA with Tukey-Kramer post hoc test.
Fig. S2. Vδ2 T cells of RA patients produced increasing amounts of IFN-γ and IL-17.

Flow cytometry analysis of the intracellular staining of (A) IFN-γ, (B) TNF-α and (C) IL-17 in Vδ2 T cells from PBMCs of RA, OA and HC were performed. Cytokines ELISA detection of the cultured supernatant of (D) total CD3⁺, CD3⁺ cells that were depleted of Vδ2⁺ (CD3⁺Vδ2⁻) and CD3⁺Vδ2⁺ cells from peripheral blood of RA, (E) CD3⁺ and CD3⁺Vδ2 cells sorted from RA synovial fluid. The right panels show bar graphs of the percentage of positively stained cells [A-C]. Results are expressed as mean ± SEM.
ND: Not Detectable * , P< 0.05; ** , P<0.01; ns, no significance by one way ANOVA with Tukey-Kramer post hoc test (A-D) and Student’s t-test (E).

Fig. S3. IL-6 upregulated the expressions of CCR5 and CXCR3 on Vδ2 T cells. Flow cytometry analysis of CCR5 and CXCR3 expression on HC Vδ2 T cells stimulated with (A)RA serum or RA serum in combination with neutralizing antibody of IL-6 for 3 days; or (B)stimulated with medium in the absence or presence of IL-6 after indicated days. Data were pooled from three independent experiments. Results are expressed as mean ± SEM. *, P< 0.05, **, P<0.01 by Student’s t-test (A) or two-way ANOVA (B).
Fig. S4. Peripheral Vδ2 T cells in RA patients treated with IL-6R antagonist.

Treatment-naïve RA patients (n=3) were treated with IL-6R antagonist in combination with methotrexate for 3 months. The percentage of (A) peripheral Vδ2 T cells and the expressions of (B) CCR5 and (C) CXCR3 on Vδ2 T cells were evaluated by flow cytometry before and after the treatment. The solid plots represent isotype control, and the open plots represent Vδ2 T cells staining. Results are expressed as mean ± SEM. ns, no significance by paired t-test.