

Title: Resolvin D5 modulates Th17/Treg cell differentiation and suppresses osteoclastogenesis.

Hiroataka Yamada,¹ Jun Saegusa,^{1 2} Sho Sendo,¹ Yo Ueda,¹ Takaichi Okano,^{1 2} Masakazu Shinohara,^{3 4} Akio Morinobu¹

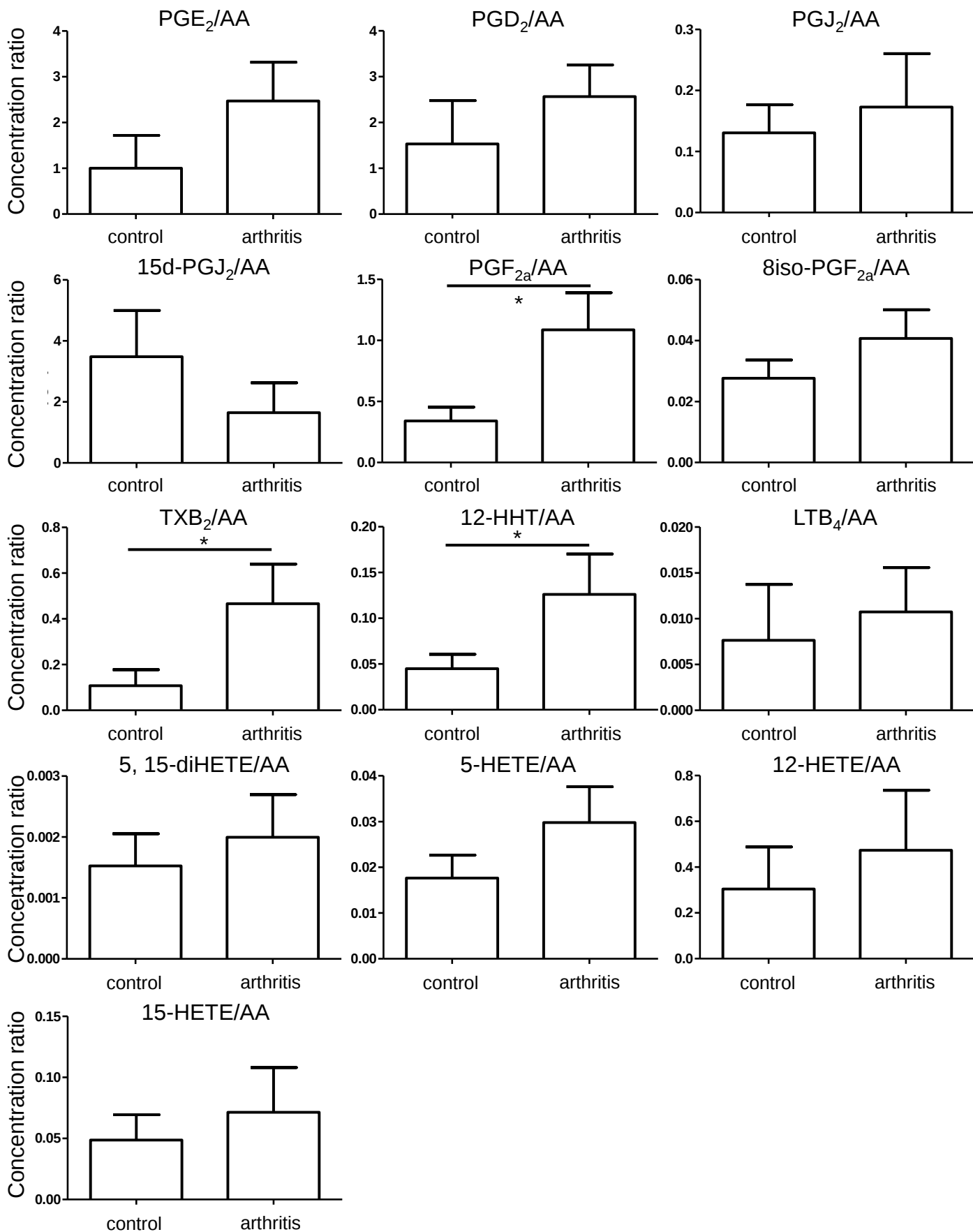
¹ Department of Rheumatology and Clinical Immunology, Kobe University Graduate School of Medicine, Kobe, Japan.

² Department of Clinical Laboratory, Kobe University Hospital, Kobe, Japan.

³ Division of Epidemiology, Kobe University Graduate School of Medicine, Kobe, Japan.

⁴ The Integrated Center for Mass Spectrometry, Kobe University Graduate School of Medicine, Kobe, Japan.

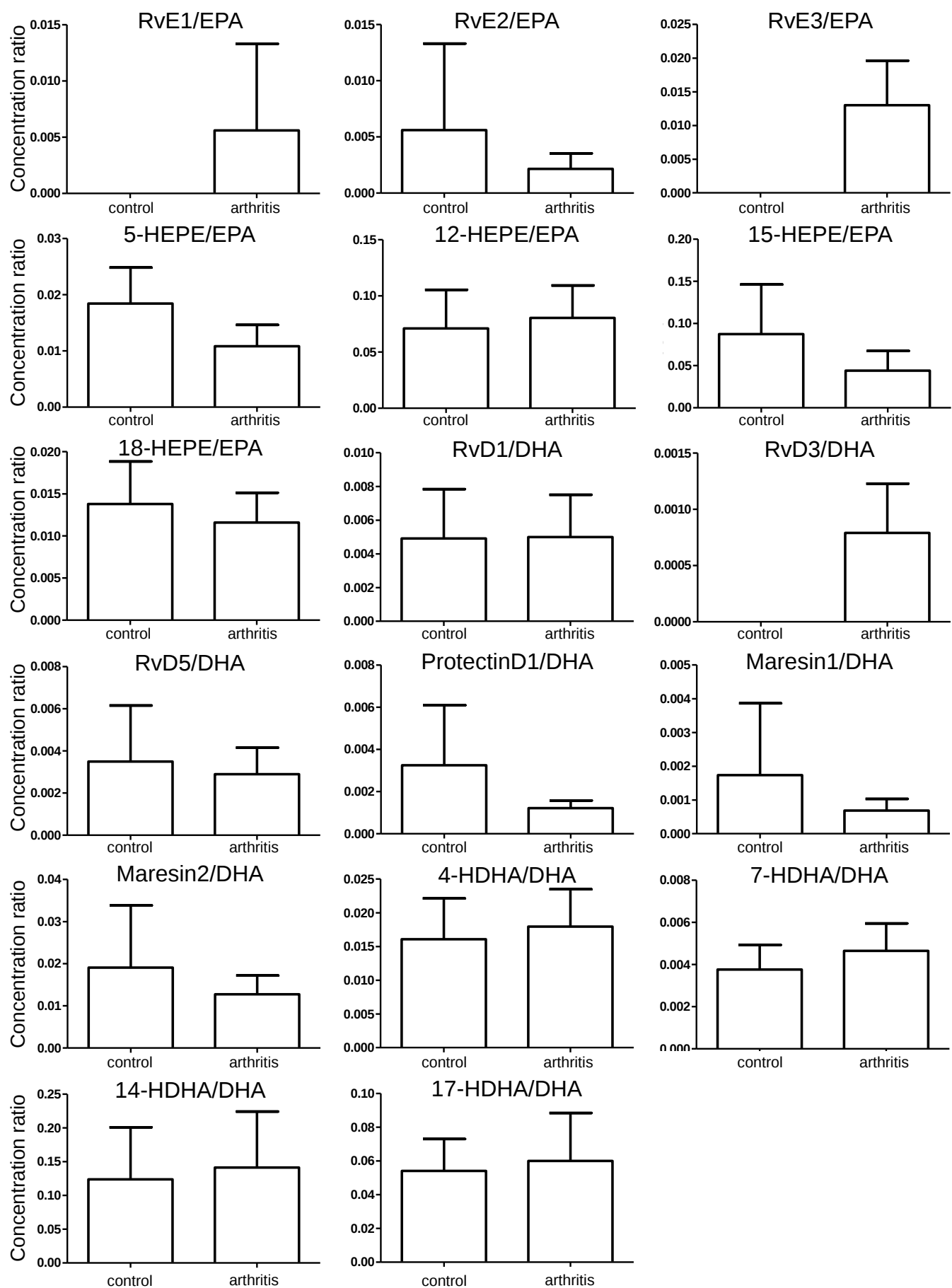
Supplemental figure 1



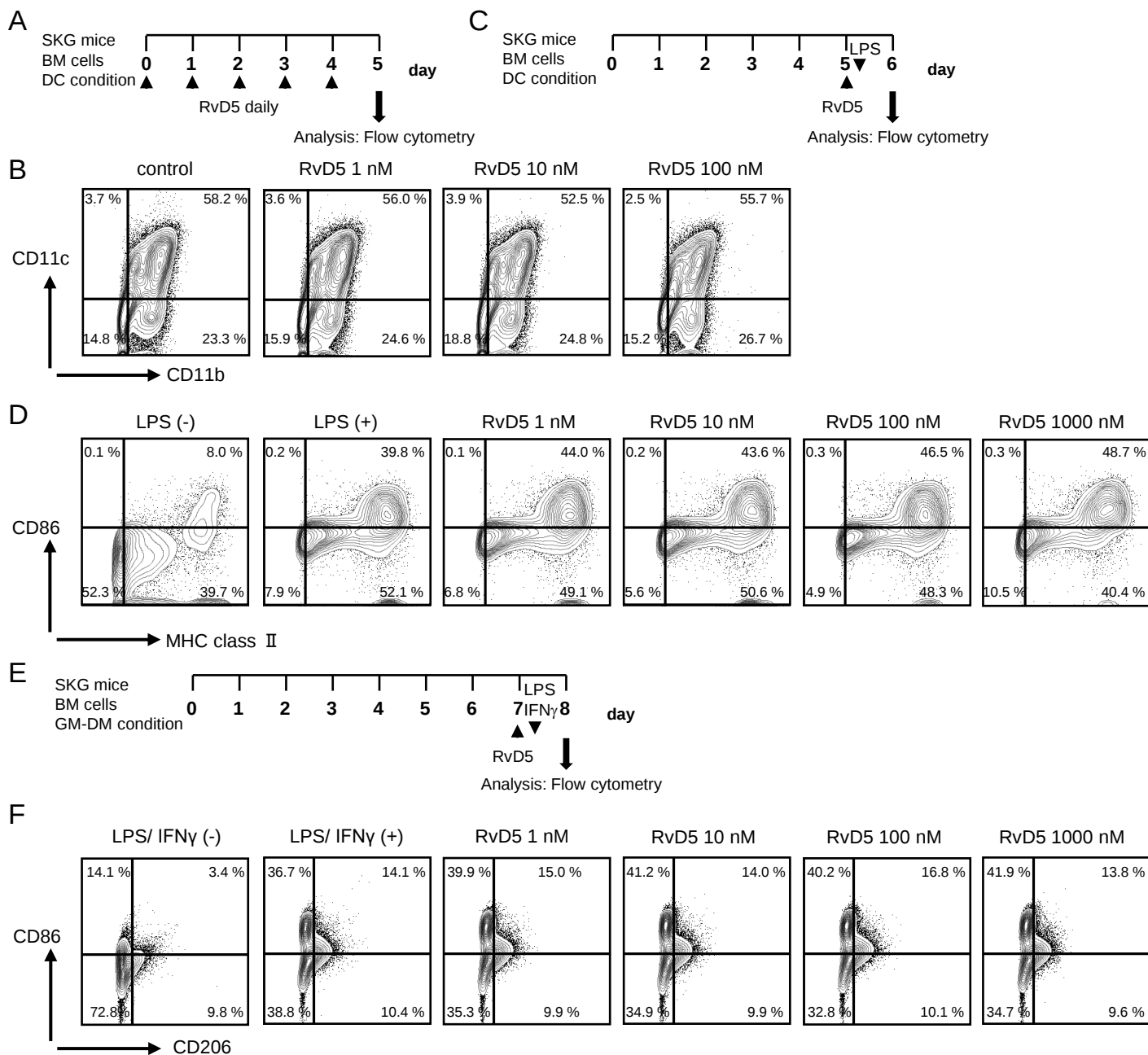
Supplemental figure 1. Biosynthesis of AA-derived pro-inflammatory mediators might be increased in the arthritic paws.

SKG mice were injected with ZyA (2 mg/body i.p.) on day 0. On day 112, paws were removed and LM quantified using LC/MS/MS-based LM profiling (control group, n = 5, arthritis group, n = 8). Concentrations ratios of LMs to their precursors. Bars represent mean $\pm$ SEM. **P* < 0.05, by Mann-Whitney U tests and FDR-BH correction.

Supplemental figure 1 (continue)

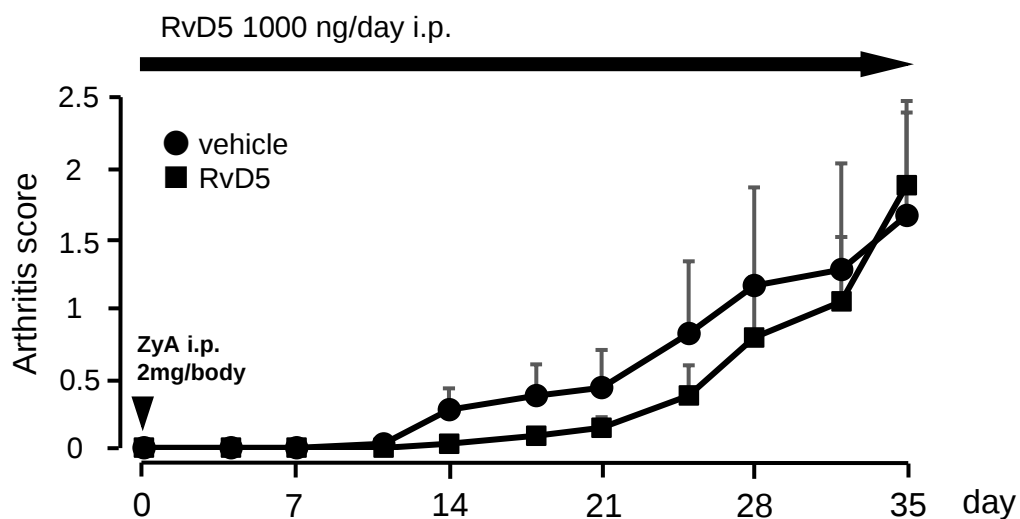


Supplemental figure 2



Supplemental figure 2. RvD5 doesn't suppress the differentiation and activation of DCs and doesn't polarize the M1 macrophages to M2 phenotype *in vitro*.

(A) Schematic representation of DC differentiation and experiment protocol. BM cells were isolated from 8-10-week-old SKG mice and culture for 5 days with GM-CSF and IL-4, with or without daily SPMs (RvD5: 1-100 nM). Cells were stained with anti-CD11b and anti-CD11c antibodies. **(B)** The frequency of CD11b + CD11c + cells were analyzed by flow cytometry. Data are representative of 5 independent experiments. **(C)** Schematic representation of DC activation and experiment protocol. On day5, cells were treated with or without RvD5 (1-1000 nM) and activated with or without LPS. One day after LPS activation, cells were stained with anti-CD11b, anti-CD11c, anti-CD86 and anti-MHC class II antibodies. **(D)** The frequency of CD86+MHC class II + cells among CD11b+CD11c+ cells were analyzed by flow cytometry. Data are representative of 4 independent experiments. **(E)** Schematic representation of M1 macrophages and experiment protocol. BM cells were isolated and culture for 7 days with GM-CSF. On day 7, cells were treated with or without RvD5 (1-1000 nM) and with or without LPS/ IFN γ to polarize to M1 phenotype. One day after LPS/ IFN γ polarization, cells were stained with anti-CD11b, anti-F4/80, anti-CD86 and anti-CD206 antibodies. **(F)** The frequency of M1 phenotype (CD86+) and M2 phenotype (CD206+) cells among CD11b+F4/80+ cells were analyzed by flow cytometry. Data are representative of 4 independent experiments.



Supplemental figure 3. RvD5 treatment tends to ameliorated ZyA-induced SKG arthritis. SKG mice were induced arthritis with ZyA (2 mg/body i.p.) on day 0 and were given daily RvD5 (1000ng) i.p. from day 0 (RvD5, n = 7). Vehicle group were treated with NS (vehicle, n = 7). We recorded clinical arthritis score up to day 35.