

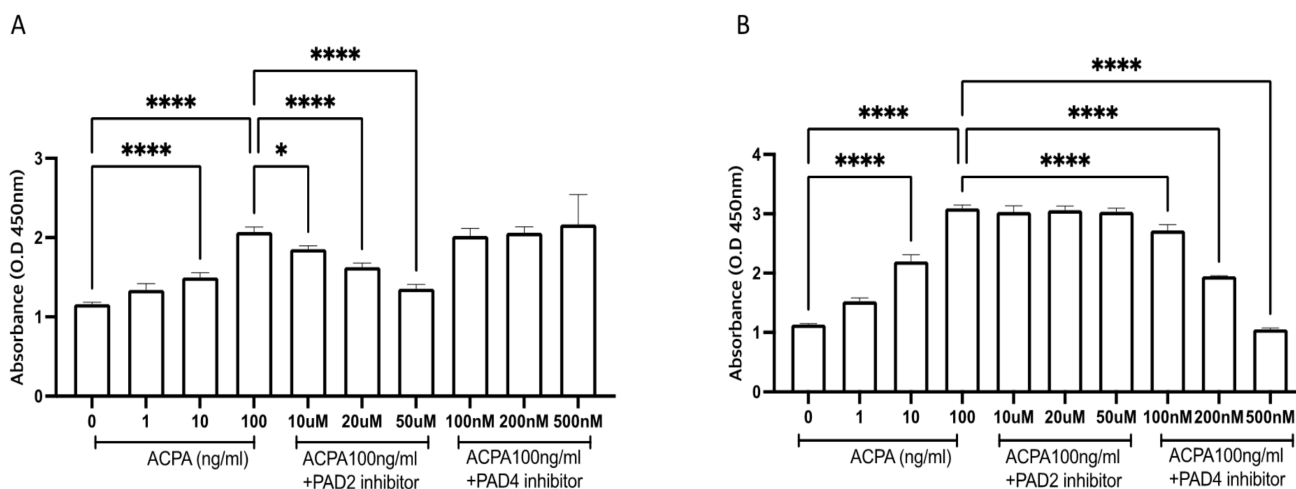
Supplementary Fig. 1. Effect of control monoclonal IgG1 Fc on RA-FLSs activation.

RA-FLSs were obtained from four patients with RA, and RA-FLSs (1×10^6 /mL) were cultured in a 24-well plate with 1, 10, and 100 ng/mL of control monoclonal IgG1 Fc protein.

A: The proportion of RANKL or TNF- α positive RA-FLSs was measured using flow cytometry.

B: ELISA was used to measure IL-6, IL-1 β , and IL-17 levels in culture media.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

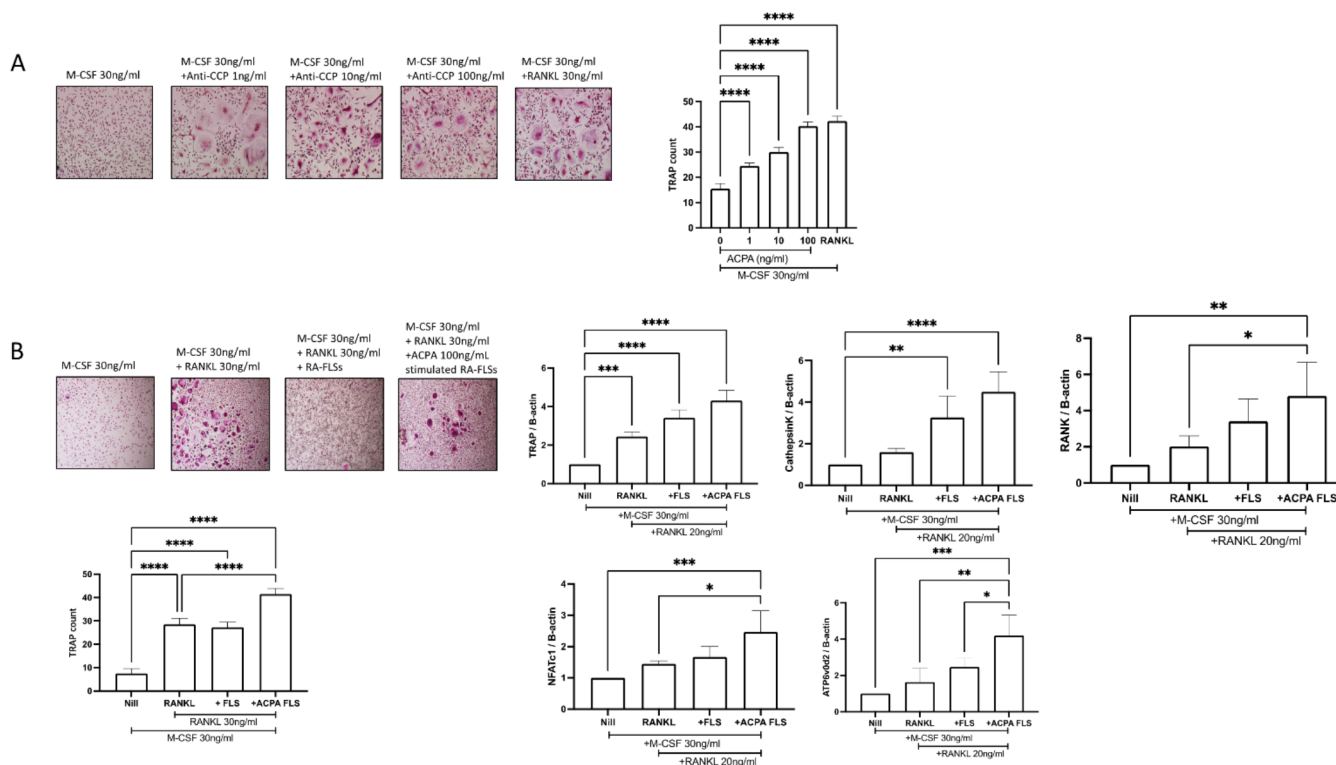


Supplementary Fig. 2. PAD activities on RA-FLSs.

RA-FLSs were obtained from four patients with RA, and RA-FLSs (1×10^6 /mL) were cultured in a 24-well plate with 1, 10, and 100 ng/mL of ACPA, 100 ng/mL ACPA + 10, 20, and 50 μ M PAD-2 inhibitor, and 100 ng/mL ACPA + 100, 200, and 500 nM PAD-4 inhibitor.

A: The PAD-2 activities, and **B:** PAD-4 activities were measured.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplementary Fig. 3. Effect of ACPA on osteoclast differentiation.

PBMCs were obtained from four patients with RA patients, and CD14+ monocytes were extracted. CD14+ monocytes (8×10^5 /well) were plated in a 24-well plate and stimulated with 100 ng/mL M-CSF for 72 h to induce osteoclast precursor.

A: To evaluate the direct effect of ACPA on osteoclastogenesis, 30 ng/mL M-CSF, 30 ng/mL M-CSF with 1, 10, and 100 ng/mL ACPA, and 30 ng/mL M-CSF + 30 ng/mL RANKL were added to osteoclast precursor.

B: A co-culture of ACPA-stimulated RA-FLSs with osteoclast precursor was used to evaluate the indirect effect of ACPA on osteoclastogenesis. Co-culture conditions were as follows; 1) 30 ng/mL M-CSF, 2) 30 ng/mL M-CSF + 30 ng/mL RANKL, 3) 30 ng/mL M-CSF + 30 ng/mL RANKL + unstimulated RA-FLSs (8×10^2 /well), and 4) 30 ng/mL M-CSF + 30 ng/mL RANKL + 100 ng/mL ACPA pre-stimulated RA-FLSs (8×10^2 /well). TRAP-positive multinucleated cells were counted after 10–14 days of culture. Gene expression levels were normalised to expression levels of β -actin.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.