

Data supplement #1

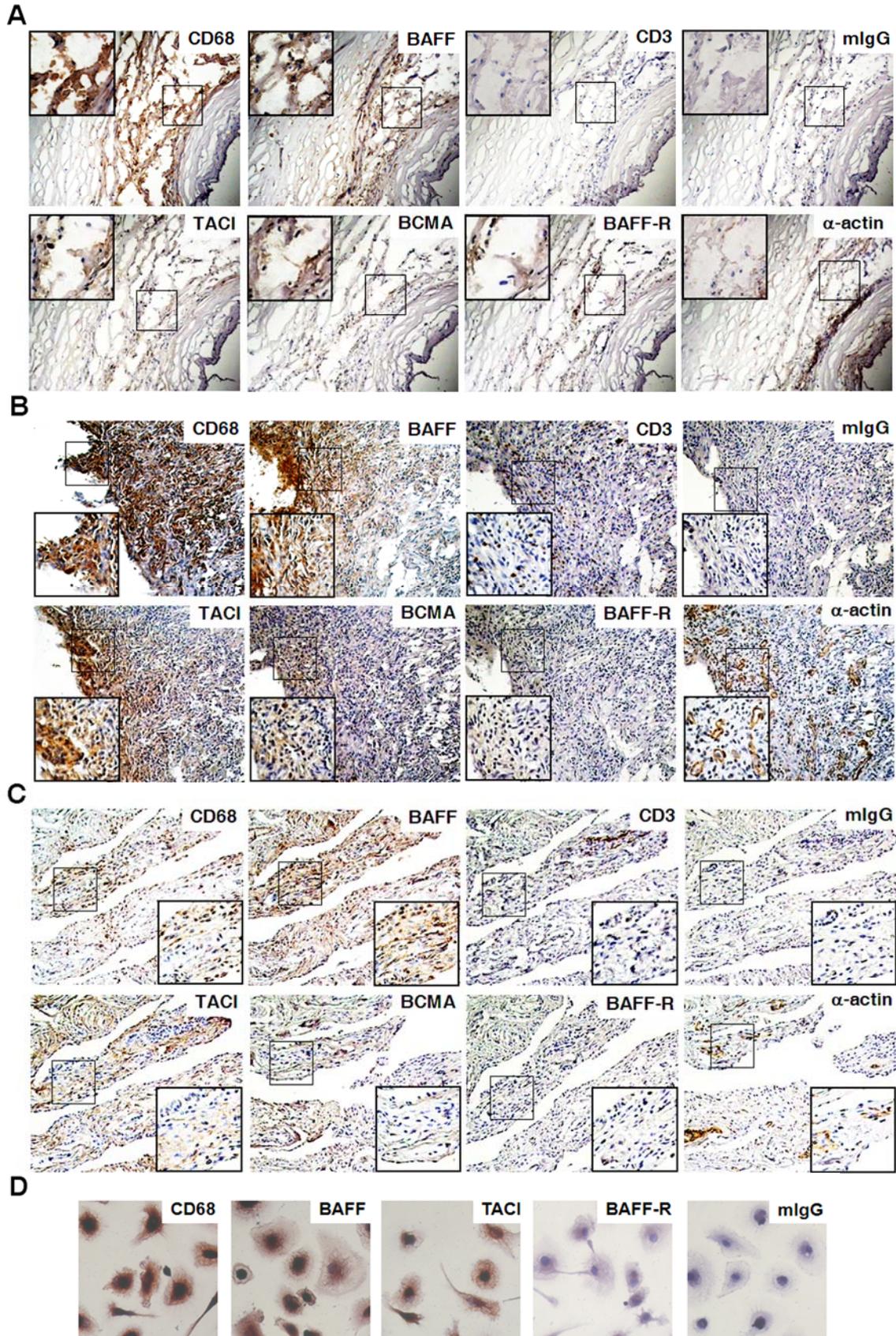
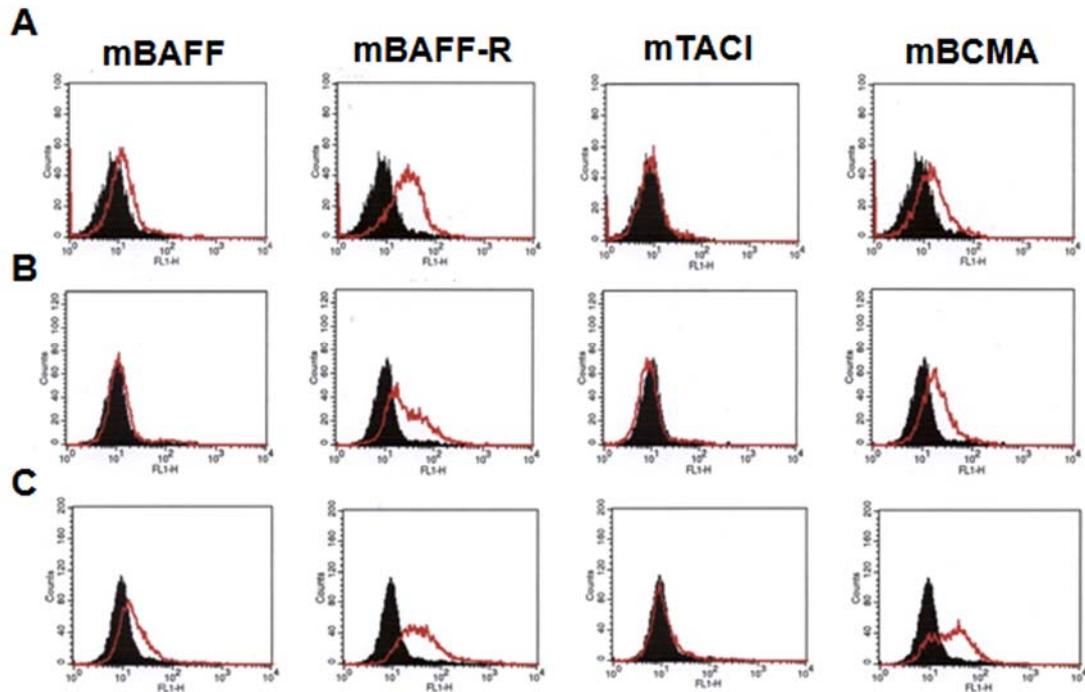


Figure legend. The expression of BAFF and its receptors in macrophages was detected at lesion areas of atherosclerosis and arthritis. **A.** Consecutive sections of an atherosclerotic plaque were stained with mAbs to CD68 (a marker for macrophages), BAFF, TACI, BCMA, BAFF-R, α -actin (a marker for smooth muscle cells), or CD3 (a marker for T-cells). Isotype-matching mouse immunoglobulins (mIgG) were used for staining as a negative control. Consecutive sections of RA (**B**) and OA (**C**) synovial tissue samples were stained as in **A**. Magnification: x40. Boxes indicate the region magnified in the inset (x400). The experiments were repeated for more than four tissue specimens in each case with essentially the same results. **D.** Primary macrophages derived from RA peripheral blood monocytes were stained with mAbs specific to CD68, BAFF, TACI, or BAFF-R for immunocytochemistry analysis. Mouse IgG (mIgG) was used to stain the section as a negative control.

Methods: *Primary culture of macrophages and histological analysis*

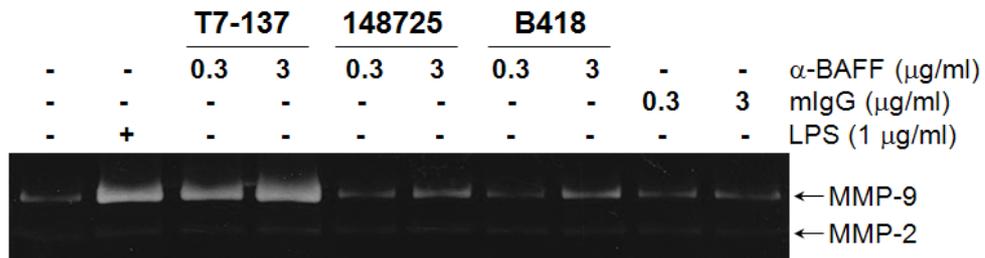
Carotid endarterectomy specimens, generously provided by Dr. Jeong-Euy Park, Sungkyunkwan University, School of Medicine, were obtained from patients, aged between 63 to 81, who had undergone surgery at the Samsung Seoul Hospital. RA and osteoarthritis (OA) synovial samples, generously provided by Dr. Eun-Mi Koh, Sungkyunkwan University School of Medicine, were obtained from RA patients during joint replacement therapy. Cases of RA/OA were diagnosed according to the criteria of the American College of Rheumatology. Mononuclear cells were isolated from peripheral blood by density gradient centrifugation, using a Histopaque (Sigma-Aldrich, St. Louis, Missouri). Adherent monocytes were isolated after one-hr incubation and the cells were incubated for one week to induce macrophage differentiation. The purity of the cells (>95% CD14 positive cells) was then confirmed using flow cytometry. These studies were approved by an institutional review committee and the subjects provided informed consent. Specimens were washed with saline and embedded in an optimal cutting temperature medium in order to make frozen sections. Standard 5- μ m sections were stained using a LSAB Kit (DAKO, Copenhagen, Denmark), according to instructions provided by the manufacturer.

Data supplement #2



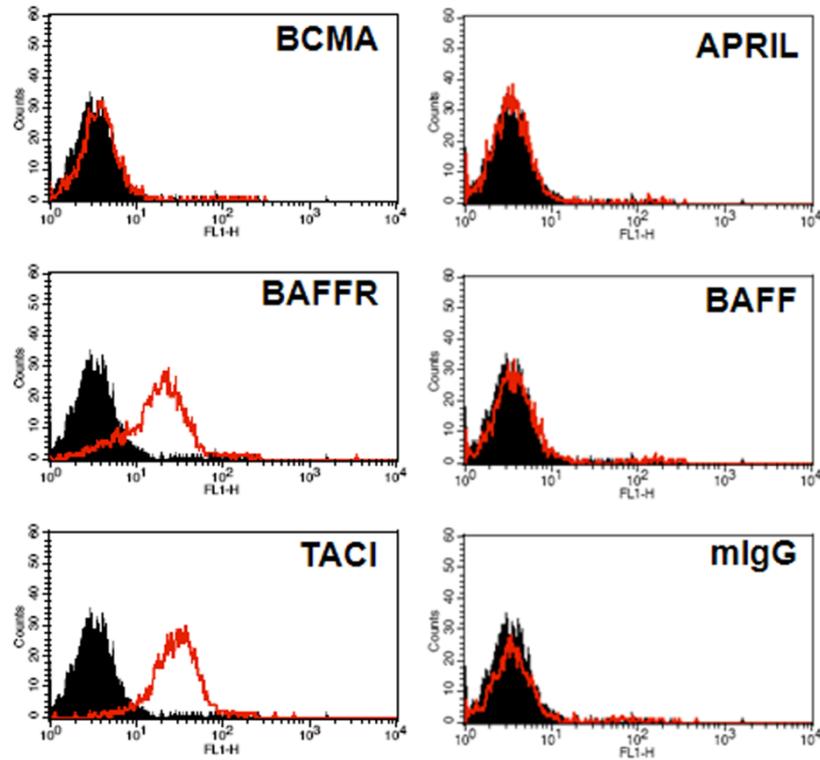
BAFF expression was detected in mouse primary macrophages and in the mouse macrophage cell line RAW264.7. Peritoneal macrophages (A), bone marrow-derived macrophages (B), and RAW264.7 cells (C) were stained for the expression of BAFF, BAFF-R, TACI, and BCMA. Histograms from specific staining (open area) and background staining (filled area, stained with rat IgG) are compared.

Data supplement #3



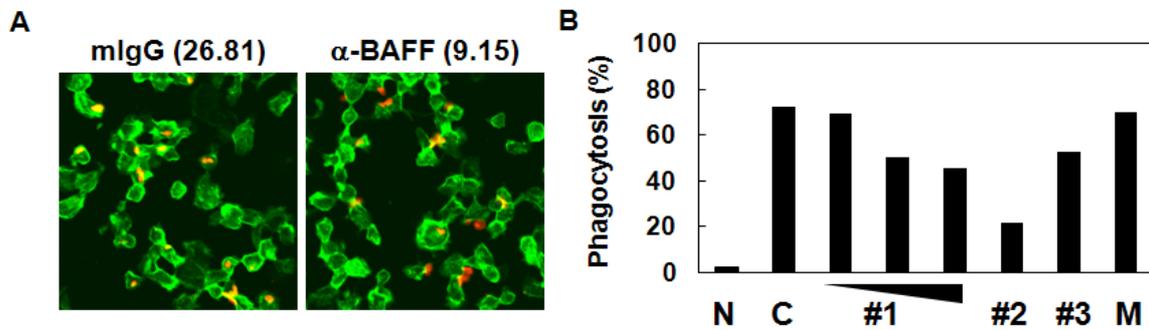
The treatment of THP-1 cells with three different anti-BAFF mAbs induces the expression of MMP-9 in THP-1 cells. THP-1 cells were treated with 0.3 or 3 $\mu\text{g/ml}$ of anti-BAFF mAbs (clone names: 148725, T7-137, and B418) or mouse IgG (mIgG). As a positive control, 1 $\mu\text{g/ml}$ of LPS was used. The experiment was repeated more than three times with essentially the same results.

Data supplement #4



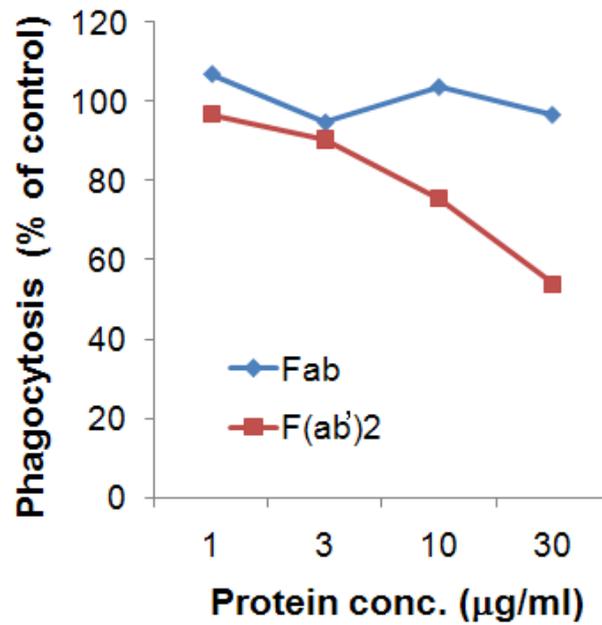
Ramos cells express TACI and BAFF-R. Ramos cells were stained for the expression of BAFF and its receptors. Histograms from specific staining (open area) and background staining (filled area, stained without primary antibody) are compared.

Data supplement #5



The stimulation of BAFF inhibited phagocytosis of opsonized zymosan in THP-1 cells. A. THP-1 cells were pre-treated for 30 min with 10 $\mu\text{g}/\text{ml}$ of mouse IgG (mIgG) or antibodies to BAFF. Cells were then incubated with 30 $\mu\text{g}/\text{ml}$ of opsonized zymosan-594 (red fluorescence). Three hours later, cells were fixed and F-actins were stained with Alexa Fluor-488 Phalloidin (green fluorescence). Confocal images of each sample are shown (upper panel). Note that internalized zymosan particles are yellow while those adhered to cell surface are red. More than 100 cells in five high power fields were analyzed for the fluorescence patterns and the numbers in the parenthesis represent the percentage of cells that internalized the zymosan particles. **B.** Phagocytosis assay was performed as in A with the addition of three different anti-BAFF mAbs (clone names were 148725, T7-137, and B418 for #1, 2 and 3 respectively) or mouse IgG (M). antibodies were added at 10 $\mu\text{g}/\text{m}$ concentrations except #1, which was added at 1, 3, and 10 $\mu\text{g}/\text{ml}$ concentration. N, no treatment control and C, treated with opsonized zymosan without any antibodies.

Data supplement #6



Stimulation of BAFF with F(ab')₂, but not with Fab, fragment of anti-BAFF mAb blocked phagocytosis in THP-1 cells. THP-1 cells were incubated with opsonized zymosan in the presence or absence of Fab or F(ab')₂ fragments of anti-BAFF mAb at indicated amounts. The percentage of cells internalized the zymosan particle was measured using flow cytometry.