## **Supplemental Data**

SERUM CALCIUM-DECREASING FACTOR, CALDECRIN, INHIBITS OSTEOCLAST DIFFERENTIATION BY SUPPRESSION OF NFATc1 ACTIVITY Hiroya Hasegawa, Seisui Kido, Mineko Tomomura, Kengo Fujimoto, Michi Ohi, Masaru Kiyomura, Haruhide Kanegae, Akemi Inaba, Hiroshi Sakagami, and Akito Tomomura



Fig. S1. Caldecrin and various inhibitors inhibit RANKL-stimulated osteoclastogenesis. (A) BMCs were cultured for 3 days with M-CSF (10 ng/ml), and further cultured for 4 days with M-CSF alone or M-CSF plus various drugs, TPA (10 nM), RANKL (20 ng/ml), Wt caldecrin (5 µg/ml), FK568 (2 µM), CsA (1 µM), BAPTA-AM (20 µM), U73122 (10 µM), calphostin (100 nM), or ER-27319 (15 µM) as indicated. TRAP activities of culture media were measured (mean  $\pm$  S.D. of three experiments: \*\*P < 0.01 vs. M-CSF plus RANKL-treated group).



Fig. S2. Effects of caldecrin on osteoclastogenesis in the presence of osteoblastic cell line ST2. BMCs were cocultured for 7 days with ST2 cells in the presence of dexamethasone (10 nM) alone (D3(-)) or dexamethasone plus 1a,25-dihydroxy vitamin D<sub>3</sub> (10 nM) (positive control; PC) with or without FK506 (2  $\mu$  M), CsA (1  $\mu$  M), or Wt caldecrin (5  $\mu$  g/ml). At the end of culture, TRAP staining (left) and activity (right) were measured (mean  $\pm$  S.D. of three experiments: \*\**P* < 0.01, \**P* < 0.05 *vs.* positive control group). Scale bar shows 200 mm.