

Supplementary Information

Figure S1: Dock5 is essential for the formation of the sealing zone in osteoclasts.

(A) Western blot showing the expression of Dock5 and Gapdh in extracts from RAW264.7 cells expressing shRNAs against Luciferase (shLuc) or Dock5 (shDock5) at days 0, 3 and 5 of differentiation. (B) Osteoclasts differentiated from RAW264.7 cells expressing shLuc or shDock5 were fixed and stained to reveal the activity of Tartrate Resistant Acid Phosphatase (TRAP). Scale bars 200 μm (C) Osteoclasts differentiated on calcium-phosphate substrate were fixed and stained for actin to reveal the sealing zones (arrows). Scale bars 100 μm (D) Osteologic Biocoat matrices were stained with von Kossa to reveal resorbed areas (clear zones). Graph shows average and standard deviation (SD) resorbing activity per osteoclast obtained by measuring the resorbed area using ImageJ and dividing by the number of osteoclasts from two independent experiments performed in triplicates. (E) Osteoclasts differentiated from BMMs expressing shLuc or shDock5 on calcium-phosphate substrate were fixed and stained for actin to reveal the sealing zones (arrows). Scale bars 100 μm (F) Average and SD CTx concentration per osteoclast in one experiment performed in triplicates and representative of three experiments.