Supporting Information (SI)

Supplementary Figure Legends

Fig. S1. Signaling from osteoclast precursor cells is not impaired in GIT1 KO mice

WT BM cells were differentiated into OC with MCSF (20 ng/ml) and RANKL (50 ng/ml). Cell lysates were harvested at indicated time points and protein expression probed using GIT1 antibody. Actin serves as a loading control. (B) GIT1 WT and KO pre OC (day 5) were starved for 3-4 hrs and stimulated with RANKL (100ng/ml) for the indicated time points. Lysates were blotted for phosphorylated forms of JNK ERK1/2, p38, AKT and IkBα. Total JNK, ERK2, p38, AKT and actin served as loading controls.

Fig. S2. GIT1 functions downstream of RANK signaling in RAW 264.7 cells

RAW 264.7 cells were treated with RANKL (30 ng/ml) for 4 days to induce OC formation. (A) Cells were harvested at indicated times and GIT1 expression probed. Src expression was used as a positive control. Actin serves as loading control. Cells were starved for 6 hrs, stimulated with RANKL (100 ng/ml) for the indicated time points. (B) GIT1 phosphorylation (97kd band, arrow) was probed using 4G10 antibody. Blot was reprobed for GIT1 expression. (C) Immunoprecipitation [IP] was done using 4G10 phosphotyrosine specific antibody and immunoblotted [IB] for GIT1 expression. (D) Cells were pretreated with Src inhibitor, PP2 (10 μM) for 1 hr, IP with 4G10 antibody and IB for GIT1 expression.

Fig. S3. Src, GIT1, PLCγ2 localization in GIT1 KO osteoclast

GIT1 KO BM cells were differentiated into OC by treatment with MCSF (20 ng/ml) and RANKL (50 ng/ml) for 7 days, fixed and stained for (A) Src, (B) GIT1, (C) PLCγ2 antibodies. (D) GIT1 KO OC were serum starved for 3-4 hrs and stimulated with RANKL (100ng/ml) for 5 mins, fixed and stained for phosphorylated form of PLCγ2 using phospho- PLCγ2 (Y759) antibody. Rhodamine-phalloidin was used to visualize actin rings. (Scale bar: 15 μm)

Fig. S4. Src kinase activity is unaltered in GIT1 KO osteoclast

GIT1 WT and KO cells (day 7) were starved for 5 hrs and stimulated with RANKL (100 ng/ml) at indicated time points. Phosphorylated Src was identified by its phosphorylation at Y416 [p-Src (Y416)]. Total Src served as a loading control.

Fig. S5. Osteoblast activity is normal in GIT1 KO mice

(A) GIT1 WT and KO BM were cultured with 10 mM β-lycerophosphate and 50 μg/ml ascorbic acid to induce OB formation. Cell lysates were harvested at indicated time points and protein expression was probed using GIT1 antibody. Brain and VSMC lysate serves as positive controls. Actin served as a loading control. (B) WT and KO BM cells were differentiated into OB and stained for (B) ALP on day 14 and (D) alizarin-red on Day 21. Quantitative analysis of (C) number of ALP and (E) alizarin-red positive colonies expressed as percentage relative to control. (F) Images of fluorescent calcein labelled images of GIT1 WT and KO, Scale bar: 20 μm (G-I) Measurements of fluorescent calcein labeling of newly formed bone. (G) MS is the mineralized surface calculated as the percentage of calcein labeled trabecular surface (H) MAR is calculated as the average distance between two calcein labelled lines divided by the time interval between two calcein injections. (I) BFR is the total bone formation rate calculated as the product of MAR and MS. Values are expressed as mean ± SEM. (n=3). All data analysis was done by two-tailed unpaired student's t-test. A $p<0.05$ was considered statistically significant.

Fig. S6 GIT2 expression is unaltered in GIT1 KO osteoclast

BM cells from GIT1 WT and KO were differentiated into OC with MCSF (20 ng/ml) and RANKL (50 ng/ml). Cell lysates were harvested on day 7 and protein expression probed using GIT2 antibody. Actin served as a loading control.

Supplementary Table legend

Supplementary Table 1. Bone Morphometric data on the femur bones of GIT1 WT and KO mice

Bone morphometric analysis on the femur bones of 10-12 week old sex matched GIT1 WT and KO mice. Parameters include percentage (%) of bone volume (BV) to total volume (TV) [BV/TV %], trabecular number per mm [Tb.N/mm], trabecular thickness in mm [Tb.thickness (mm)], trabecular space in mm [Tb. space (mm)] and trabecular connectivity density per mm^3 [Connectivity density ($1/mm^3$)]. Values are expressed as mean ± SEM. (WT n=9, KO n=8). All data analysis was done by two-tailed unpaired student's t-test. A $p<0.05$ was considered to be statistically significant.