Specificity of RGS10A as a key component in the RANKL signaling mechanism for osteoclast differentiation

JCS008300 Supplementary Material

Files in this Data Supplement:

- **Supplemental Figure S1** -

  Fig. S1. RANKL- and M-CSF-induced NF-κB, Erk, and c-Fos signaling are unaltered in *RGS10A*-silenced cells. NF-κB activation in response to RANKL was assessed by western blot analysis of IκBα degradation (A) in BMMs infected with pLenti-R10a or pLenti-scrambled virus. β-actin levels were used as loading control (n=3). M-CSF signaling in BMMs infected with pLenti-R10a orpLenti-scrambled virus was determined by western blot analysis of the phosphorylated p42/p44 form of Erk at the indicated times (B). β-actin levels were used as loading control (n=3). Expression of c-Fos was determined by RT-PCR in day 2 cells stimulated with M-CSF in BMMs infected with pLenti-R10a or pLenti-scrambled virus for the indicated time (C). GAPDH levels were used as loading control (n=3).

- **Supplemental Figure S2** -

  Fig. S2. RGS10A has no interaction with the N-type calcium channel in RANKL-induced OCLs. Precipitation of RGS10A and RGS12 followed by western blotting with an antibody against the $α_{1B}$-subunit of the calcium channel. RGS12 directly interacts with the $α_{1B}$-subunit of the calcium channel; however, RGS10A has no interaction with the calcium channel in RANKL-induced OCLs.

- **Supplemental Figure S3** -

  Fig. S3. No interaction between RGS10A and calcium sensing receptor. Lane 1, positive controls. RANKL induced OCLs. Lane 2 and 3: RAW264.7 and *RGS10A*-silenced RAW264.7 cells were induced with RANKL for 96 hours. CaR is expressed in pre-osteoclasts and osteoclasts, and RGS12 binds with CaR (lane 2,3); however, RGS10A does not bind with CaR (lane 2,3), indicating RGS10A and RGS12 both play roles in regulating calcium oscillations, but they go through different pathways.

- **Supplemental Figure S4** -

  Fig. S4. Calmodulin binds with RGS10A but not with RGS12. RAW264.7 cells were induced with RANKL for 96 hours. Lane 1: RGS10 and RGS12 are both expressed in RANKL-induced OCLs. Lane 2: RGS10 interacts with CaM, but RGS12 does not bind with CaM.