

Supporting Information for

High-resolution structure of RGS17 suggests a role for Ca^{2+} in promoting the GAP activity by RZ subfamily members

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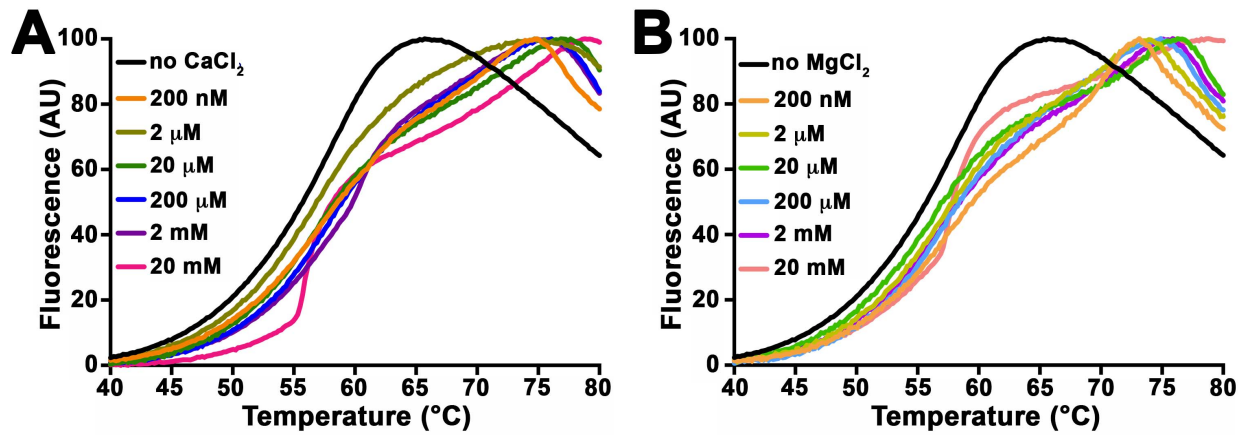
Running title: *Regulation of RGS17 by Ca^{2+}*

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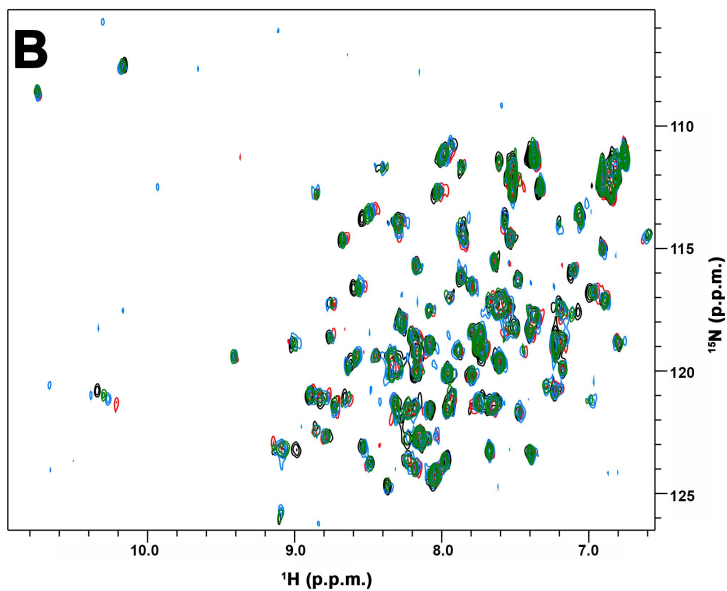
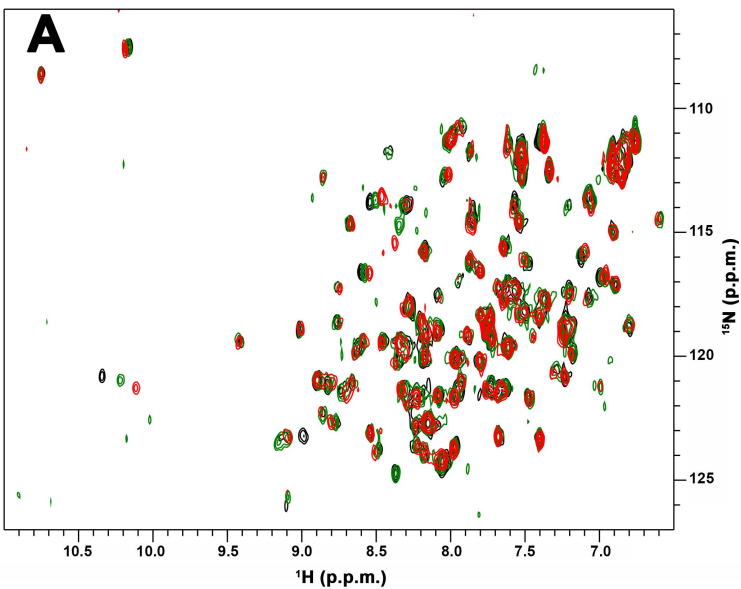
Supporting Table 1. Thermal Stability of RGS17

	RGS17 ($T_m \pm \text{SEM}$)	+ CaCl₂ ($T_m \pm \text{SEM}$)	+ MgCl₂ ($T_m \pm \text{SEM}$)
protein only	55.3 \pm 0.346		
+ 200 nM		56.1 \pm 0.63	58.3 \pm 0.870
+ 2 μ M		58.0 \pm 1.14	56.9 \pm 0.80
+ 20 μ M		56.3 \pm 1.07	56.1 \pm 1.05
+ 200 μ M		58.2 \pm 1.21	57.9 \pm 1.07
+ 2 mM		57.3 \pm 0.566	59.5 \pm 1.44
+ 20 mM		51.7 \pm 0.189	55.5 \pm 1.07

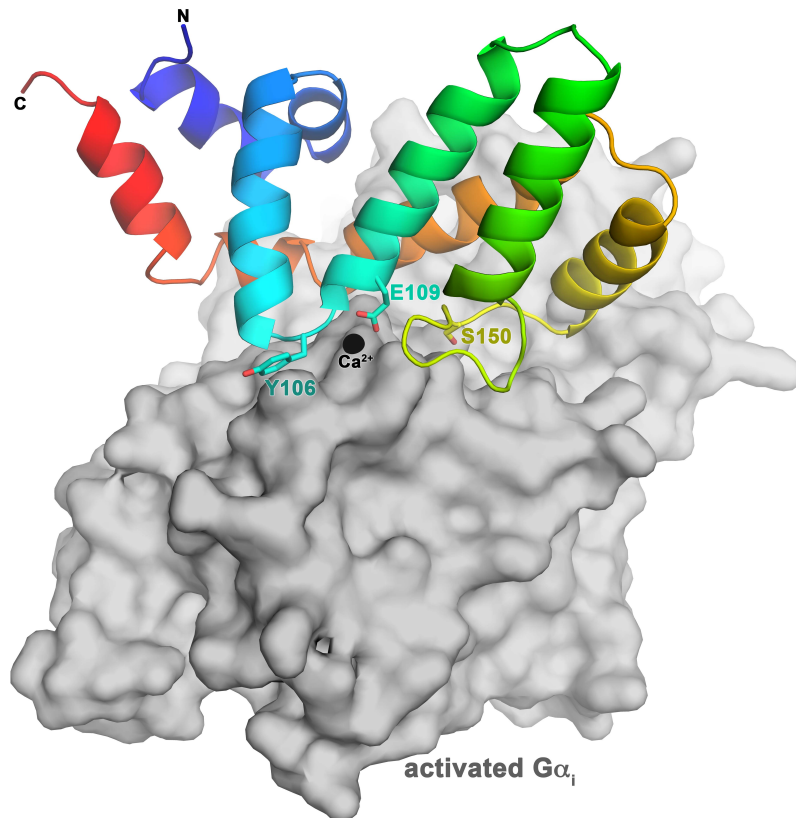
Data represents at least three independent experiments performed in triplicate.



Supporting Figure 1. RGS17 is not significantly thermally stabilized by the addition of CaCl₂ or MgCl₂. Differential scanning fluorimetry (DSF) was used to determine whether the binding of Ca²⁺ or Mg²⁺ perturbed the melting temperature (T_m) of RGS17. RGS17 was incubated with a fluorescent dye and increasing concentrations of CaCl₂ or MgCl₂, and the sample heated. As RGS17 denatures, the dye fluoresces, and the inflection point of the curve corresponds to the T_m (43).



Supporting Figure 2. ^1H - ^{15}N spectra of RGS2 in the presence of CaCl_2 or MgCl_2 . (A) ^1H - ^{15}N 2D HSQC spectra of RGS2 alone (black) and upon addition of 20 (green) or 250 molar excess of CaCl_2 (red). (B) ^1H - ^{15}N 2D HSQC spectra of RGS2 alone (black) and upon addition of 100 (green), 250 (blue), or 500 molar excess MgCl_2 (red).



Supporting Figure 3. Model of RGS17 bound to activated Gα_i. RGS17 is proposed to bind to the switch regions of Gα subunits to promote GTP hydrolysis, based on superposition of RGS17 with RGS4 in its complex with activated Gα_i (PDB ID 1AG4) (6). Ser150 is required for GAP activity, and is equivalent to Asn128 in RGS4 (9). Tyr106 and Glu109 coordinate a Ca²⁺ ion (shown as a black sphere) in the structure of RGS17, and are located on the predicted Gα_i binding surface. RGS17 is shown color-ramped from blue at the N-terminus to red at the C-terminus, and activated Gα_i is shown as a gray surface.