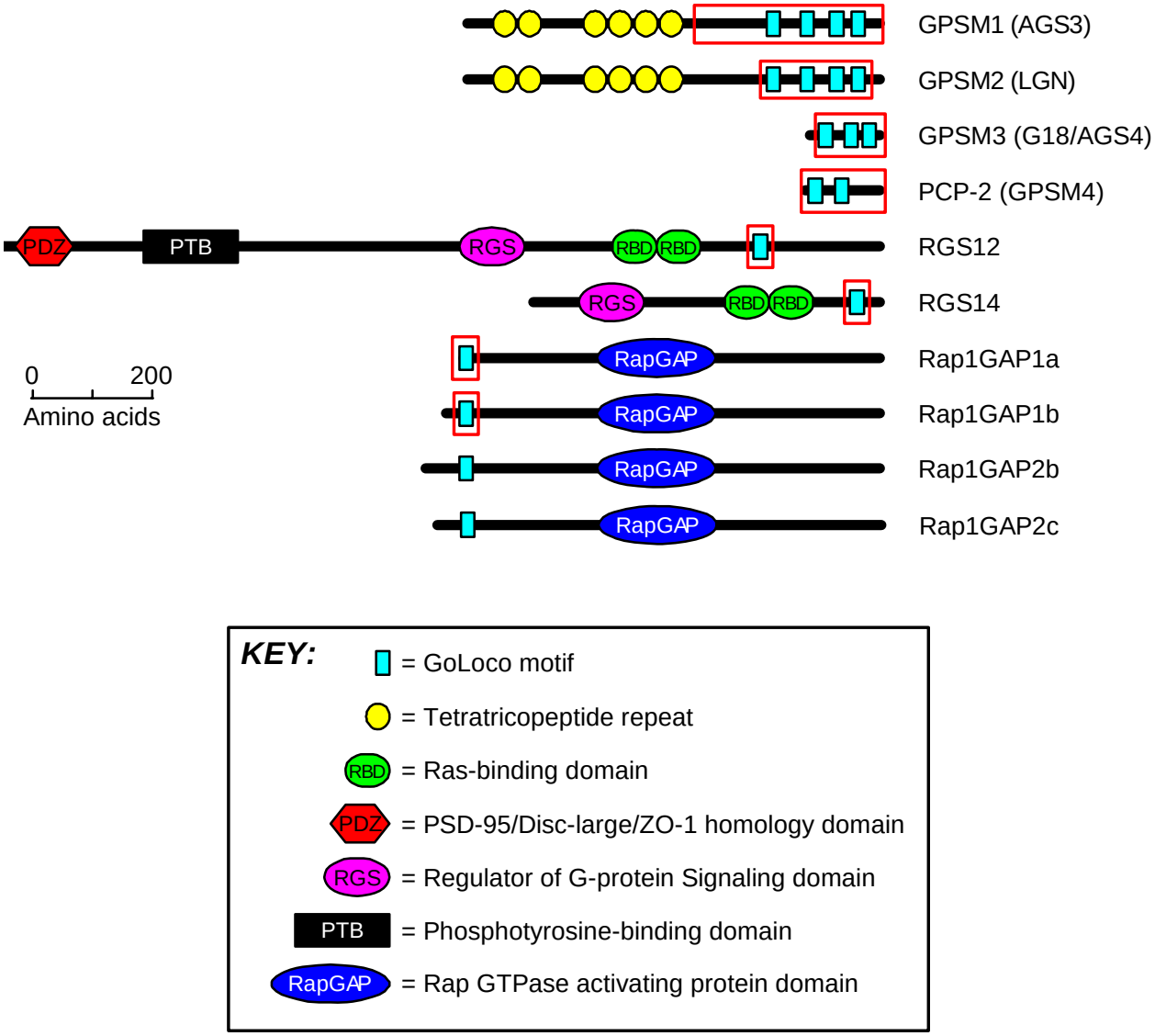
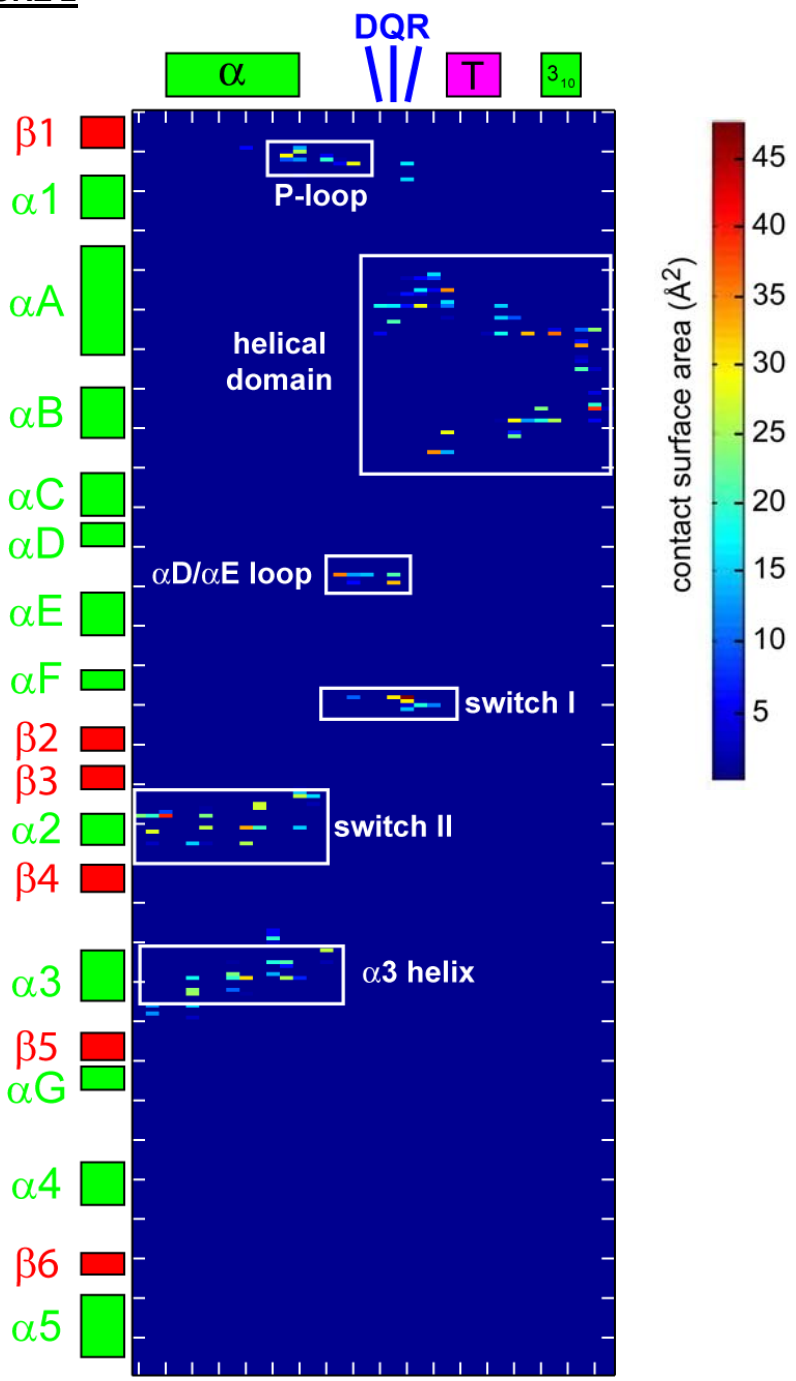
SUPPLEMENTAL FIGURE 1



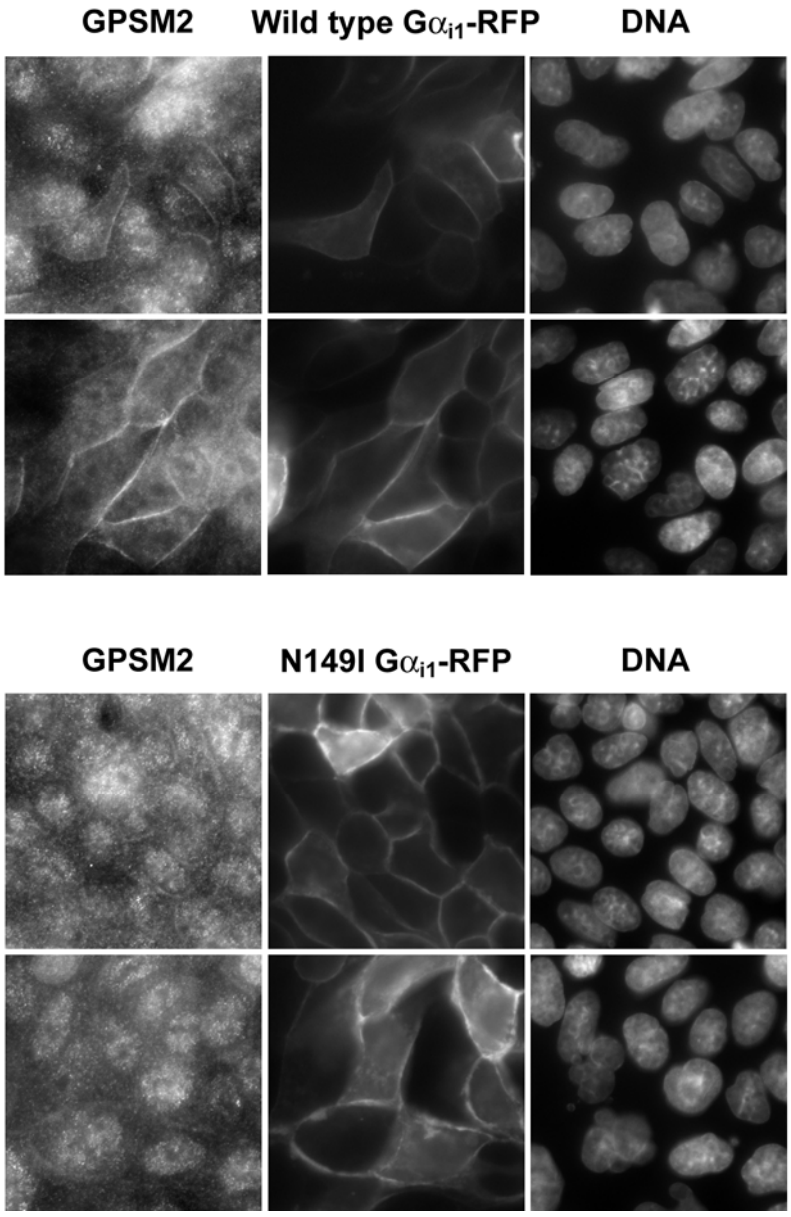
The mammalian complement of GoLoco motif-containing proteins: the GoLoco'ome. Known protein domains were determined using BLAST sequence similarity searches (1), the SMART database of protein domains (2) and published literature (3-6). Protein nomenclature is presented consistent with the HUGO human gene nomenclature committee (7). Protein domain annotations are described in the 'KEY'. Glutathione S-transferase fusion proteins derived from the GoLoco'ome are denoted by red boxes to indicate the borders of the respective proteins. Specific amino acid boundaries of individual proteins are presented in the 'Experimental Procedures' section. *Note 1*: Rap1GAP1a and Rap1GAP1b are generated by alternative splicing of a single gene (8). Similarly, Rap1GAP2b and Rap1GAP2c are generated by alternative splicing of a single gene (6). *Note 2*: Rap1GAP2b and Rap1GAP2c proteins were not used in experiments for this manuscript. We have previously demonstrated that the Rap1GAP2b/c GoLoco motif is non-functional (5). *Note 3*: It has been claimed that the protein WAVE1 contains a GoLoco motif (9); however, it has been subsequently demonstrated that WAVE1 merely contains a region of fortuitous amino acid similarity with GoLoco motifs (10).

SUPPLEMENTAL FIGURE 2



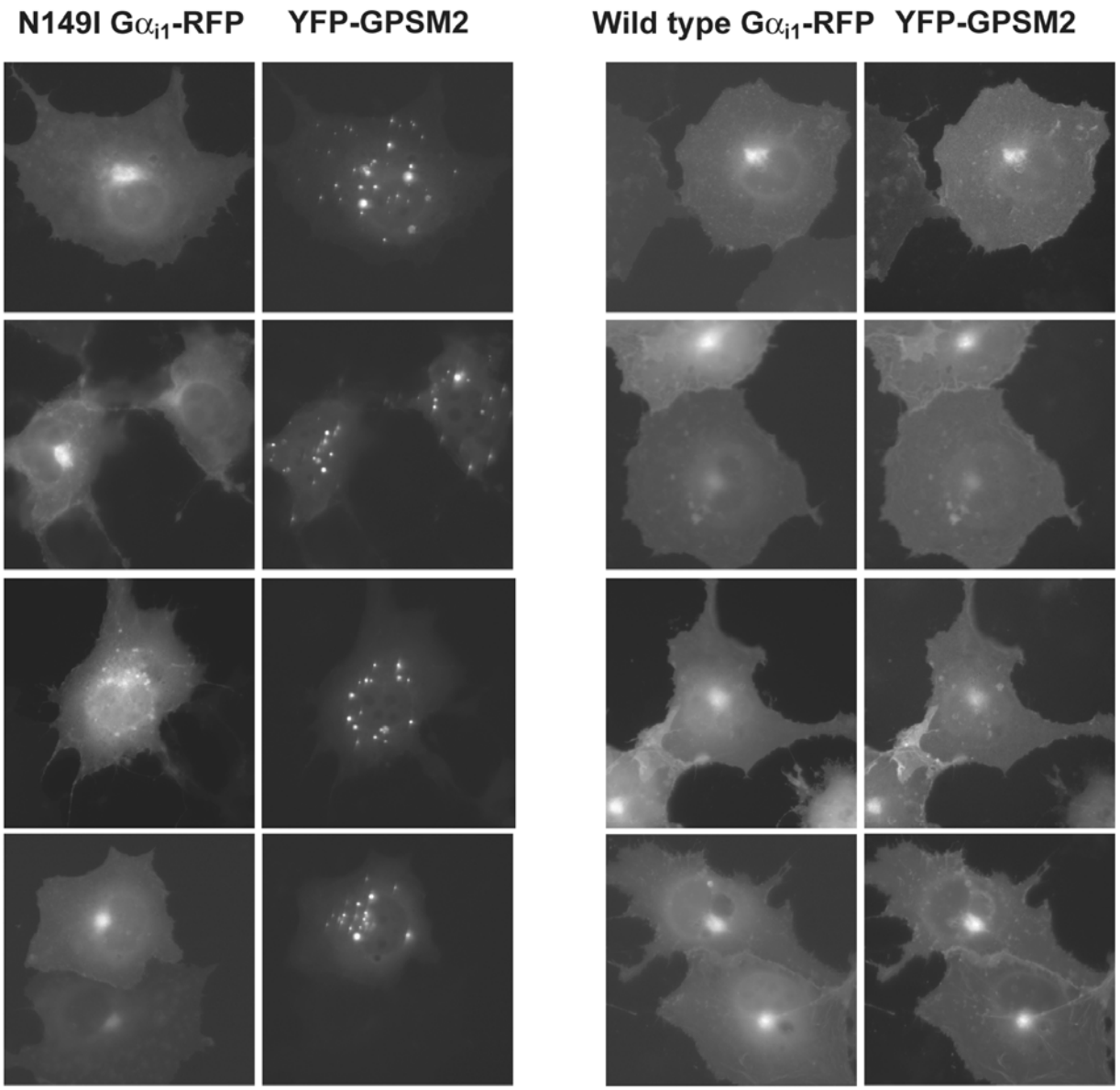
Identification of contact residues between $G\alpha_{i1}$·GDP and the GoLoco motif of RGS14. Contact distances between amino acid residues of RGS14 (abscissa) and $G\alpha_{i1}$ (ordinate) were determined using the program CMA (11). Data were plotted as a heat map using MATLAB with a threshold cutoff of 4 $Å^2$ inter-residue surface area. The observed secondary structures of $G\alpha_{i1}$ and RGS14 GoLoco motif are denoted on the ordinate and abscissa, respectively. Nomenclature: α (alpha helix; green), β (beta strand; red), DQR (conserved Asp-Glu-Arg triad; blue), T (turn; magenta), 3_{10} (3_{10} helix). The main contact areas between $G\alpha_{i1}$ and the RGS14 GoLoco motif are boxed in white.

SUPPLEMENTAL FIGURE 3



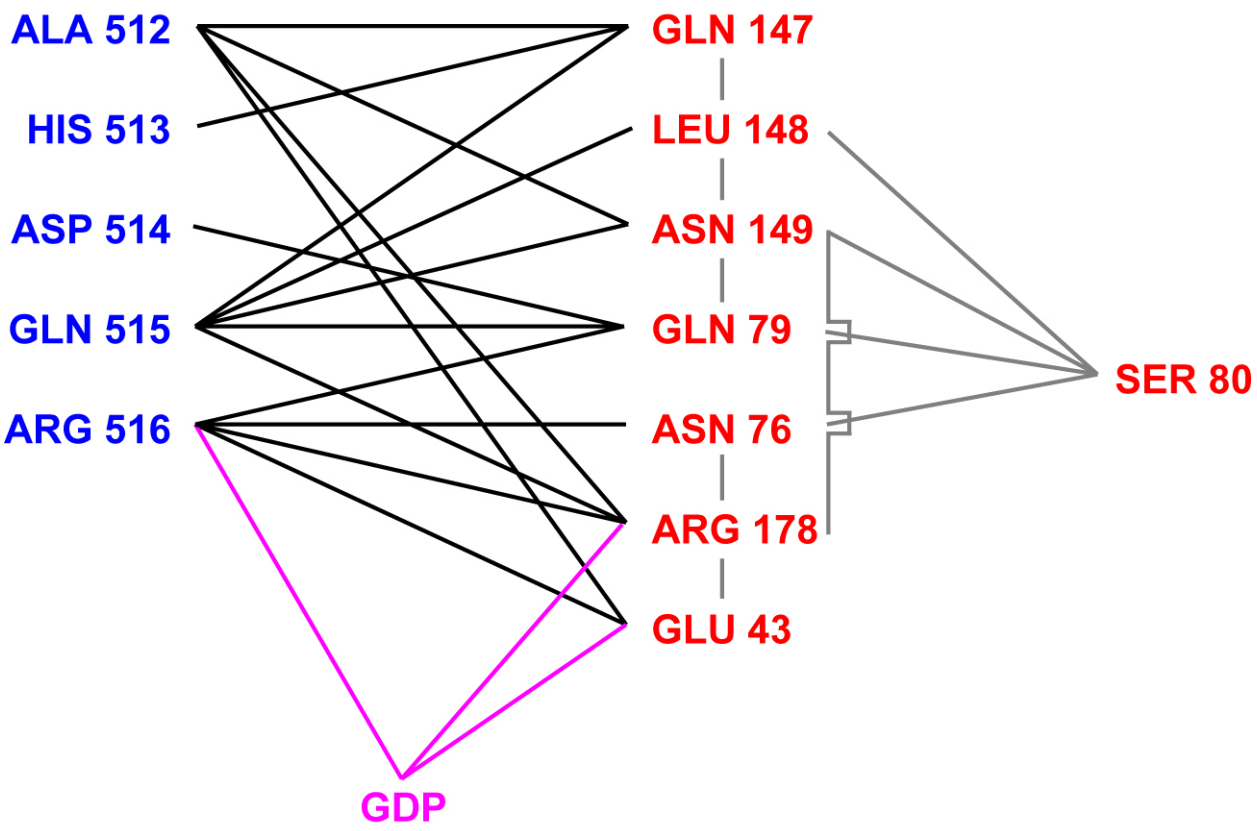
The plasma membrane localization of endogenous GPSM2 (LGN) is regulated by $G\alpha_i$ subunit interaction with GoLoco motifs. MDCK cells were transfected with either wild type (top panels) or N149I mutant (lower panels) $G\alpha_{i1}$ subunits tagged with red fluorescent protein (RFP). Cells were fixed after twenty four hours and DNA was stained using DAPI and endogenous GPSM2 was stained using indirect immunofluorescence, as described in (12). Images were obtained using confocal microscopy.

SUPPLEMENTARY FIGURE 4



The accumulation of exogenously-expressed GPSM2 (LGN) in vesicle-like intracellular puncta is disrupted by $G\alpha_i$ subunit interaction with GoLoco motifs. COS-7 cells were transfected with yellow fluorescent protein (YFP) tagged GPSM2 and either wild type (right panels) or N149I (left panels) red fluorescent protein (RFP) tagged $G\alpha_{i1}$ subunits. Cells were fixed after twenty four hours and images were obtained using confocal microscopy.

SUPPLEMENTARY FIGURE 5



Identification of amino acid residues in $G\alpha_{i1}$ that functionally interact with asparagine-149 of $G\alpha_{i1}$ to facilitate GoLoco motif-mediated GDP dissociation inhibitor (GDI) activity. Interactions between amino acid residues within $G\alpha_{i1}$, the RGS14 GoLoco motif, and GDP were calculated as described in Supplemental Figure S2 (11). A threshold cutoff distance of 4 Å was used. RGS14 GoLoco motif residues are denoted in blue, $G\alpha_{i1}$ amino acid residues are denoted in red, and GDP is denoted in purple. Interacting moieties are annotated by lines. *Note:* In addition to directly contacting Ala_{512} and Gln_{515} of the GoLoco motif, Asn_{149} of $G\alpha_{i1}$ is an important node among amino acid residues that (a) contact the GoLoco motif and (b) contact GDP.

References for Supplemental Materials

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