

Sengupta et al. <http://www.jcb.org/cgi/content/full/jcb.200902110/DC1>

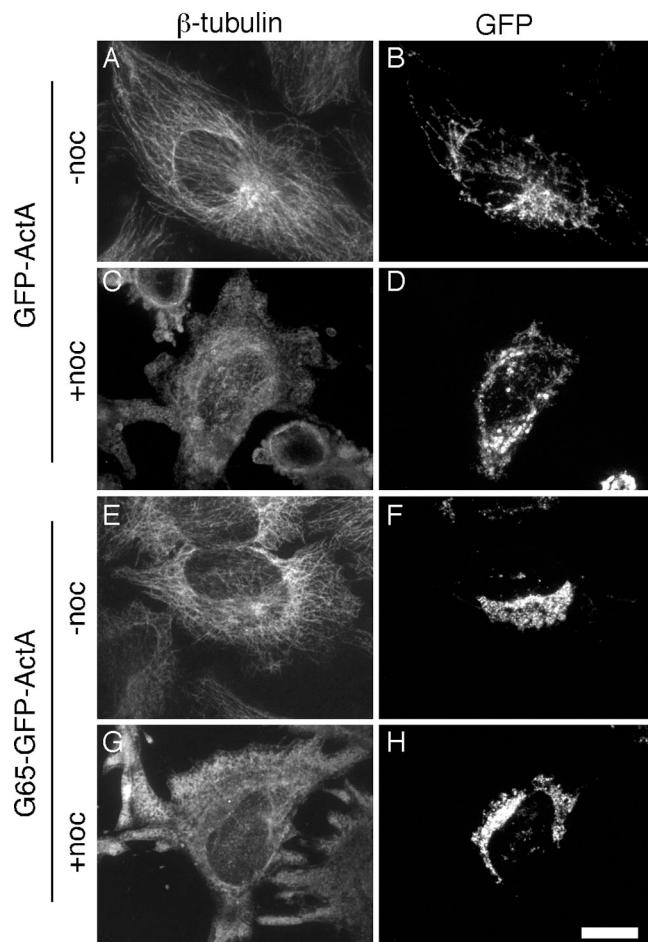


Figure S1. Clustering persists in the absence of microtubules. (A–H) HeLa cells (not BFA treated) expressing GFP-ActA (A–D) or G65-GFP-ActA (E–H) were either untreated (A, B, E, and F) or nocodazole treated to depolymerize microtubules (C, D, G, and H) and then stained and imaged to reveal β-tubulin and GFP patterns. Bar = 10 μm.

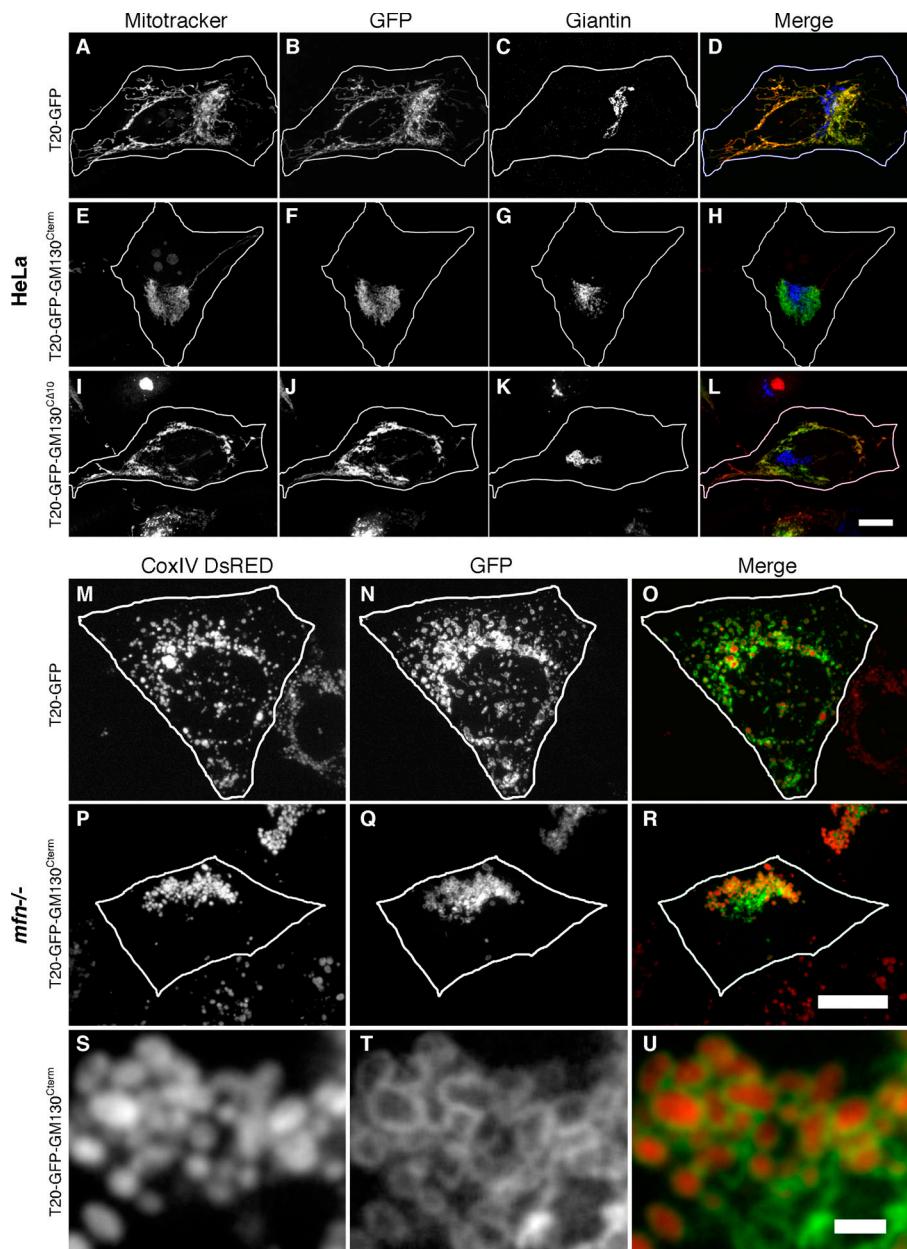


Figure S2. Clustering by the GRASP65-binding domain of GM130. (A–L) HeLa cells expressing T20-GFP (A–D), T20-GFP-GM130^{Cterm} (E–H), or T20-GFP-GM130^{CA10} (I–L) without BFA treatment were analyzed using Mitotracker to stain mitochondria, GFP fluorescence to localize the transfected proteins, and giantin staining to image the Golgi apparatus. A merged image is also shown (Mitotracker = red, GFP = green, giantin = blue). Bar = 10 μ m. (M–U) Mouse embryonic fibroblasts lacking the mitofusin-1 and mitofusin-2 genes and expressing the matrix marker COX-IV-DsRed were transfected with T20-GFP (M–O) or T20-GFP-GM130^{Cterm} (P–R), were BFA treated, and processed to reveal mitochondrial distribution (COX-IV-DsRed), the localization of the expressed proteins (GFP fluorescence), or a merged image (COX-IV-DsRed = red, GFP = green). Bar = 10 μ m. An enlarged view of a single optical section of the T20-GFP-GM130^{Cterm} expressing cells is also shown to illustrate the appearance of intact outer membranes surrounding intact matrix compartments (S–U). Bar = 1 μ m.

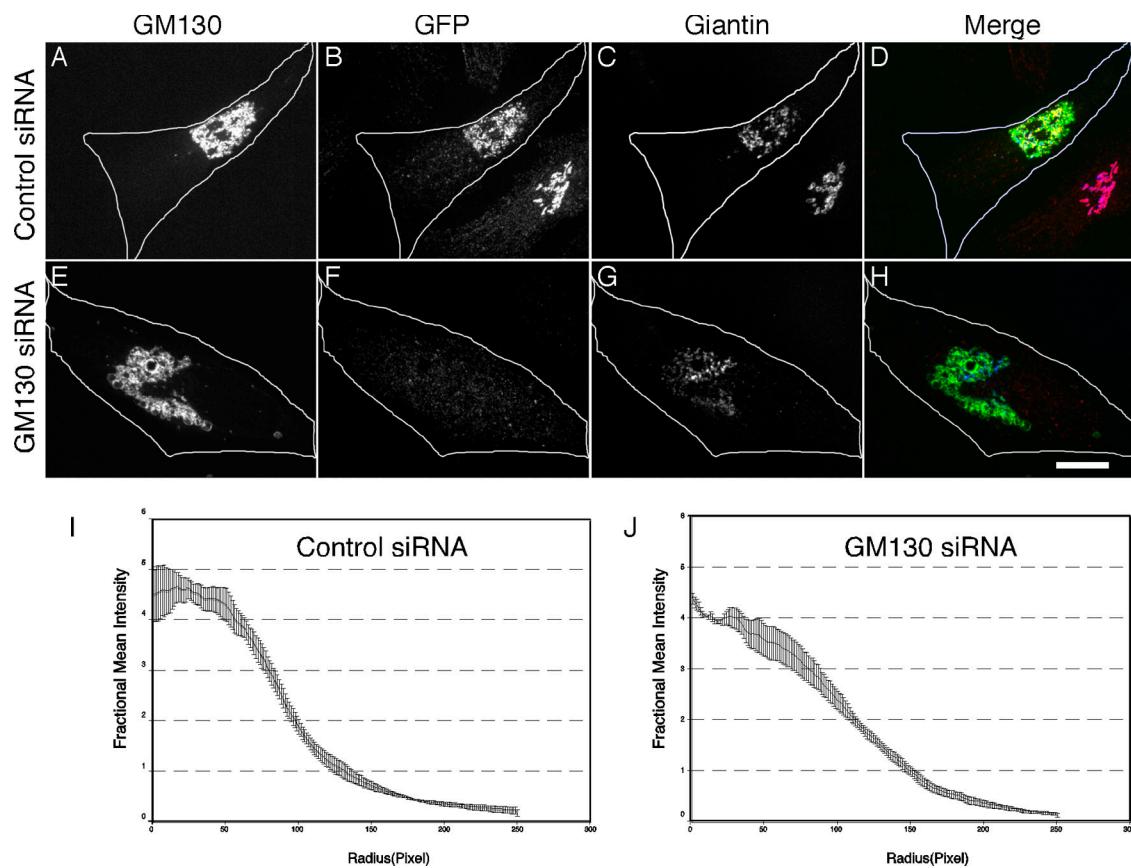


Figure S3. GRASP65 mediates clustering in cells lacking GM130. (A–H) HeLa cells were mock transfected (A–D) or transfected with siRNA targeting GM130 (E–H). After 48 h the cells were transfected with the G65-GFP-ActA construct and after a further 24 h the cells (not BFA treated) were processed to reveal GFP fluorescence, GM130 staining, giantin staining, and a merged image (GFP = green, GM130 = red, giantin = blue). Bar = 10 μ m. (I and J) The radial profile analysis is also shown ($n = 3$, \pm SEM, >15 cells/experiment).

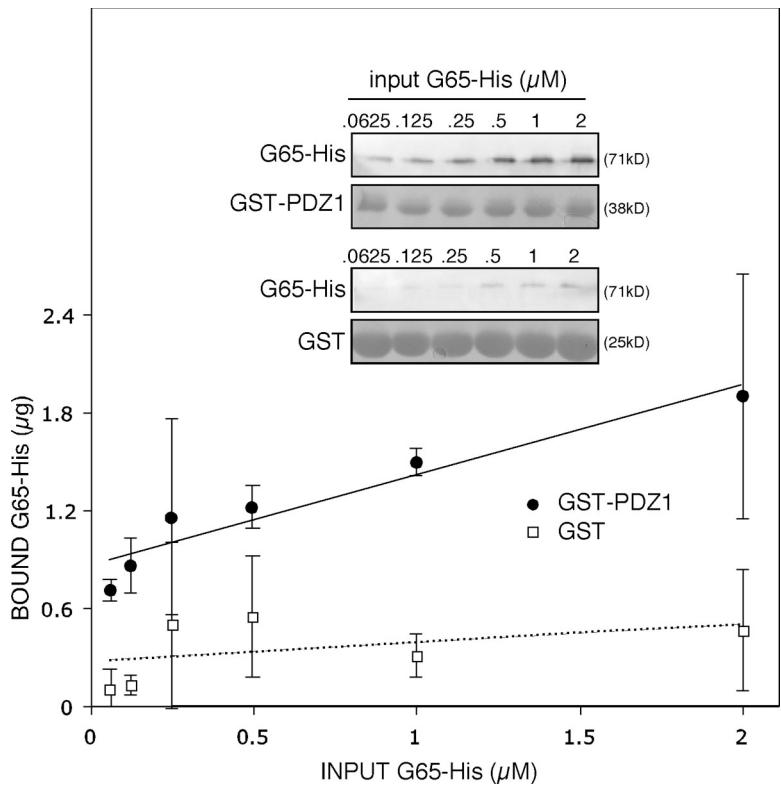


Figure S4. PDZ1 binds GRASP65 directly. Recovery of purified G65-His after incubation at various amounts with bead-attached GST (squares) and GST-PDZ1 (circles). The amount bound for each input amount is indicated ($n = 2, \pm SD$). Inset shows immunoblot assay detecting bound G65-His and corresponding Ponceau S staining of the GST and GST-PDZ1 present in each incubation.

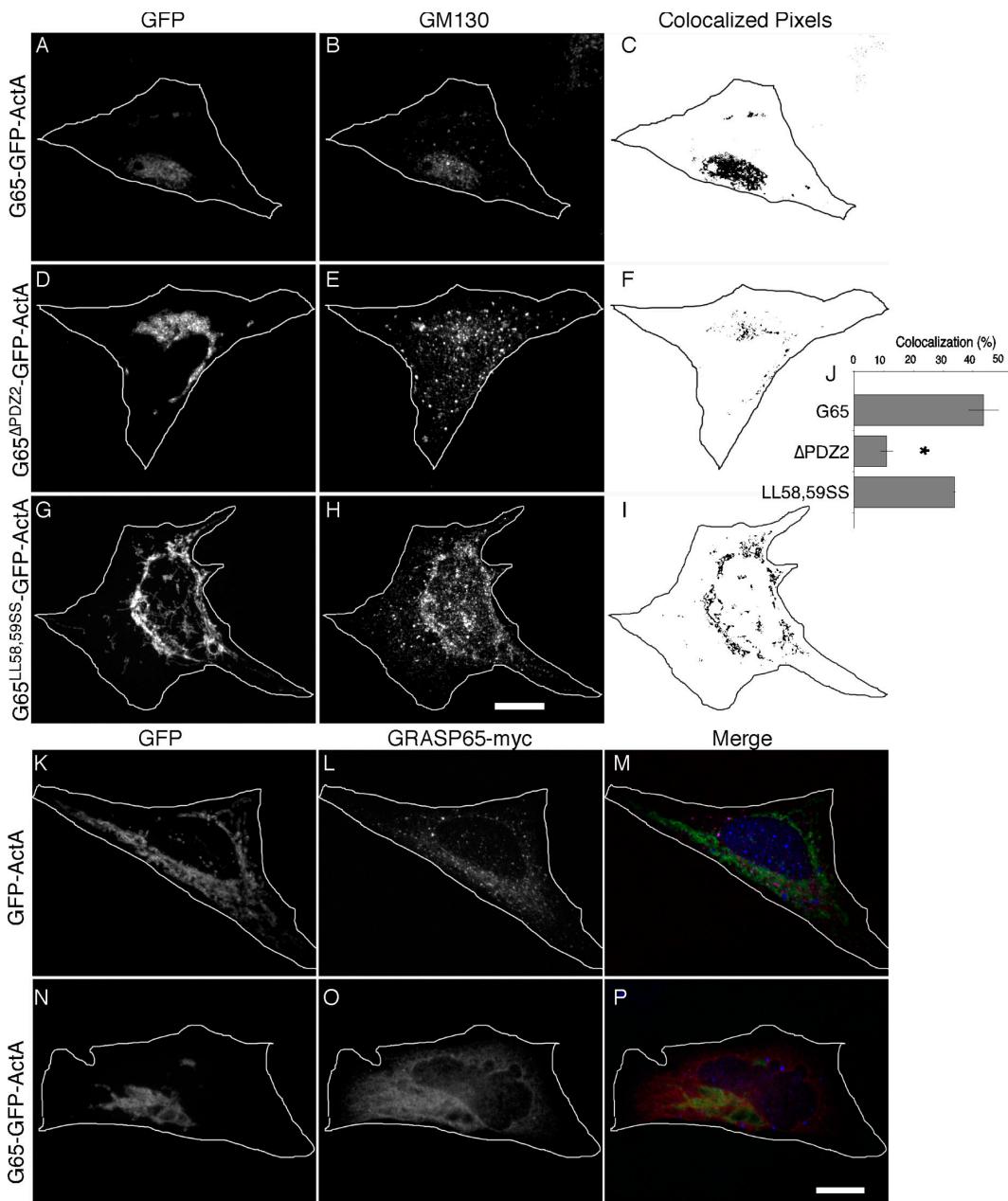
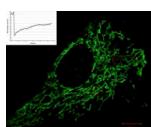
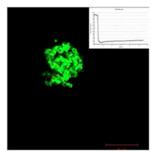


Figure S5. Recruitment of GM130 by G65-GFP-ActA depends on PDZ2, whereas soluble GRASP65-myc is not substantially recruited. (A–I) HeLa cells expressing G65-GFP-ActA (A–C), G65^{ΔPDZ2}-GFP-ActA (D–F), or G65^{LL58,59SS}-GFP-ActA (G–I) were BFA treated for 30 min to disassemble the Golgi apparatus and processed to reveal GFP fluorescence, GM130 staining, and representations of the colocalized pixels. Colocalized pixels shown are single optical sections. Bar = 10 μ m. (J) GM130 recruitment was assayed by determining the fraction of total GFP-positive pixels in single optical sections (chosen to maximize mitochondrial representation) that colocalized with GM130 staining ($n = 3$, \pm SEM, >15 cells/experiment; *, $P < 0.005$). (K–P) HeLa cells expressing GFP-ActA (K–M) or G65-GFP-ActA (N–P) were BFA treated for 30 min to disassemble the Golgi apparatus and processed to reveal GFP fluorescence, myc staining, and a merged image. Bar = 10 μ m.



Video 1. Fluorescence recovery of GFP-ActA after photobleaching. Time-lapse imaging of GFP-ActA-transfected HeLa cells upon photobleaching (fluorescence was bleached to 20%). Imaging was performed using a Meta/UV DuoScan Inverted Spectral Confocal Microscope system (LSM 510; Carl Zeiss, Inc.). The video shows 2-s frames starting immediately before bleaching and continuing after bleaching. Quantification is shown in the inset. Bar = 10 μ m.



Video 2. **Fluorescence recovery of G65-GFP-ActA after photobleaching.** Time-lapse imaging of G65-GFP-ActA-transfected HeLa cells upon photobleaching (fluorescence was bleached to 20%). Imaging was performed using a Meta/UV DuoScan Inverted Spectral Confocal Microscope system (LSM 510; Carl Zeiss, Inc.). The video shows 2-s frames starting immediately before bleaching and continuing after bleaching. Quantification is shown in the inset. Bar = 10 μ m.