Supplemental Materials

Molecular Biology of the Cell

Tang et al.

Mena-GRASP65 interaction couples actin polymerization to Golgi ribbon linking

Supplemental Materials

Online supplemental materials include 5 Supplementary Figures and 1 Supplementary Table. Figure S1 shows the biochemical properties of the cytosolic factors that enhance GRASP65 oligomerization. Figure S2 shows Biochemical enrichment of cytosolic proteins that enhance GRASP65 beads aggregation. Figure S3 shows that Mena depletion does not impact VSV-G transport. Figure S4 shows that actin filaments are required for Golgi ribbon linking but not for Golgi stacking. Figure S5 shows that depolymerization of F-actin inhibits Golgi reassembly after nocodazole washout. Table S1 shows the identification of Mena and actin as GRASP65-interacting proteins by mass spectrometry.

Figure S1

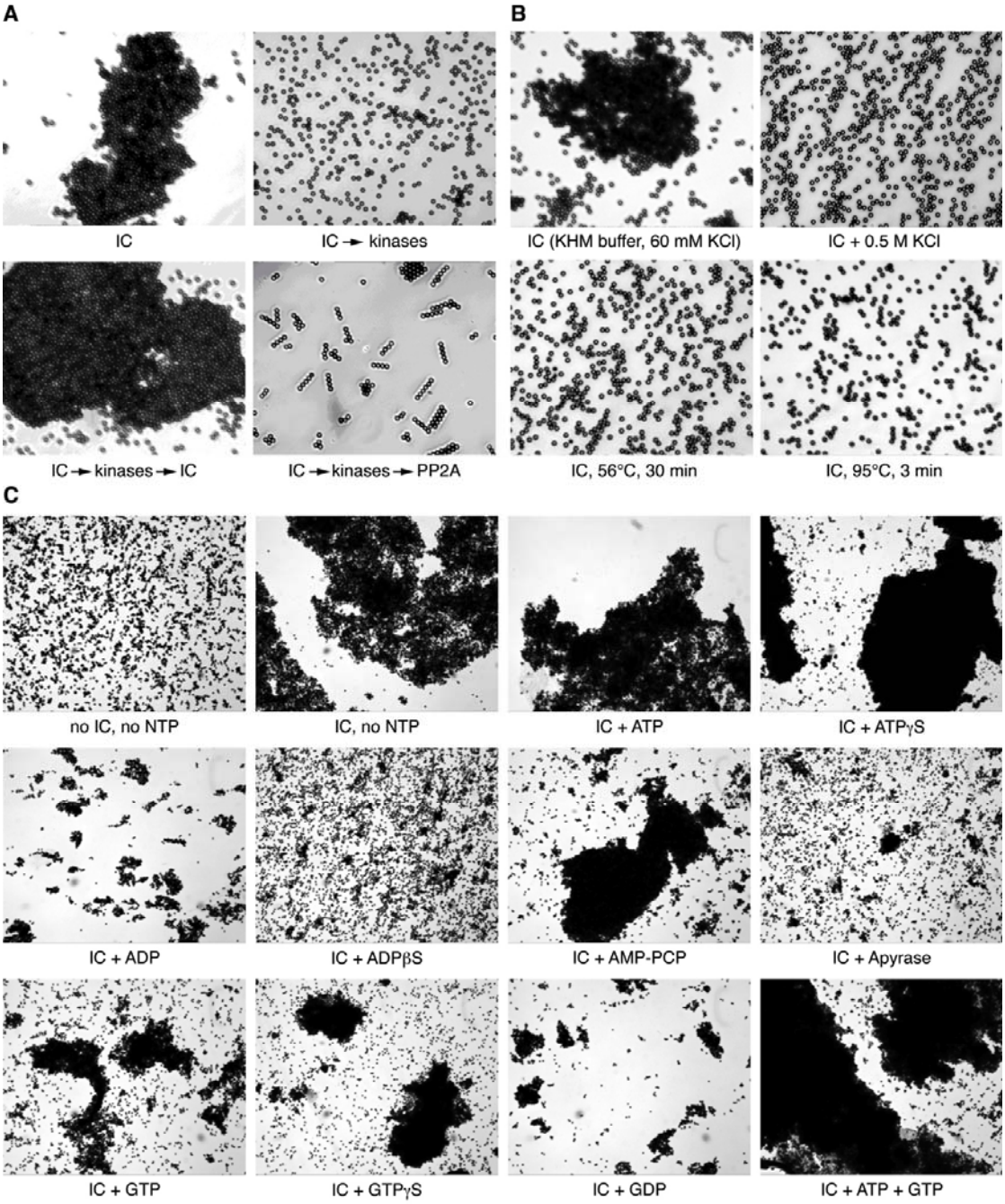
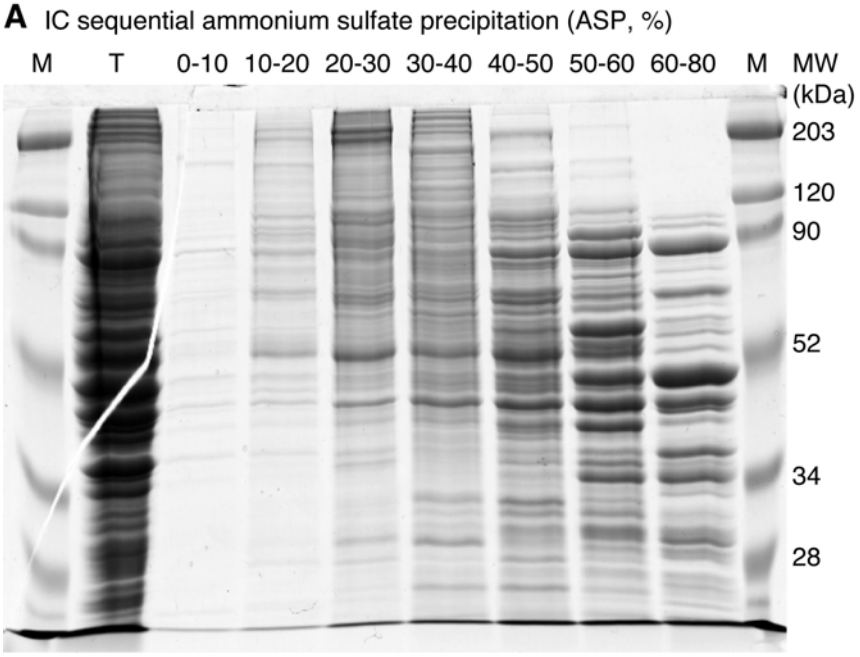


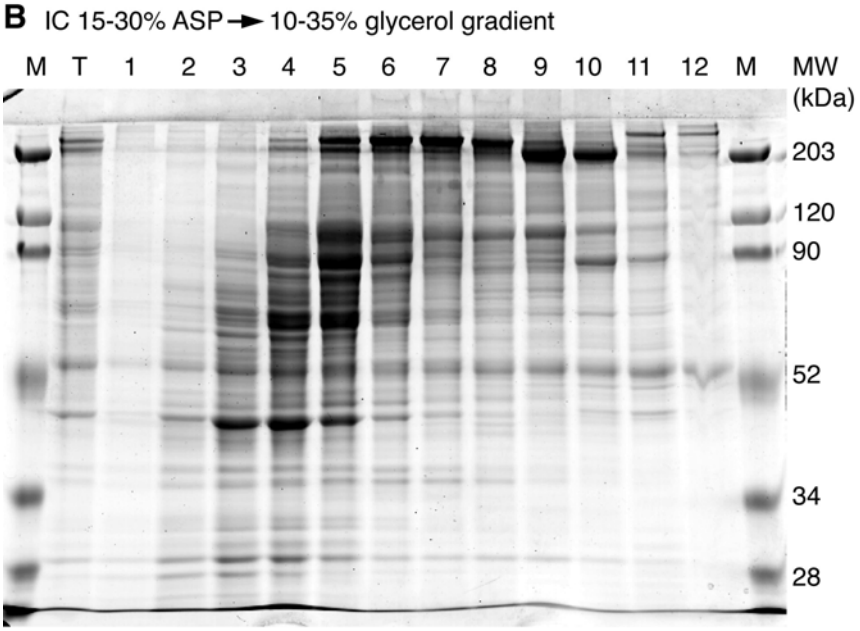
Figure S1. Biochemical properties of the cytosolic factors that enhance GRASP65 oligomerization.

(A) GRASP65 coupled beads incubated with interphase cytosol (IC), re-isolated and treated with mitotic kinases (IC→kinases), followed by incubation with either interphase cytosol (IC→kinases→IC) or PP2A (IC→kinases→PP2A). Shown are microscopy images of the beads. (B) GRASP65 coupled beads incubated with interphase cytosol that was pre-treated by adding 0.5 M NaCl or by heat (56ºC for 30 min or 95ºC for 3 min). Note both high salt and heat treatment abolished the activity of interphase cytosol. (C) GRASP65 coupled beads incubated under the indicated conditions, with or without interphase cytosol, plus ATP, GTP or their analogs (2 mM), or apyrase (0.02 U/μl) as indicated.

Figure S2



%ASP	Activity
Total	++++
0-10	+
10-20	+++
20-30	++++
30-40	++
40-50	+
50-60	+/-
60-80	-



Fraction	Activity
Total	+++
1	+
2	+
3	+/-
4	+/-
5	+/-
6	+++
7	++
8	++
9	++++
10	++
11	+
12	+

Figure S2. Enrichment of cytosolic proteins that enhance GRASP65 beads aggregation.
(A) Proteins from interphase cytosol were fractionated by sequential ammonium sulfate precipitation. Proteins that precipitated at indicated ammonium sulfate cut were dissolved and dialyzed into KHM buffer, analyzed by SDS-PAGE and Coomassie blue staining (left panel) and tested for activity in the GRASP65 bead aggregation assay (results summarized in the right panel). (B) Cytosolic protein that were precipitated by 15-30% ammonium sulfate cut were dissolved, dialyzed, and further separated by a 15-35% glycerol gradient. The gradient was fractionated from top to the bottom into 12 fractions. Proteins in each fraction were analyzed by SDS-PAGE PAGE and Coomassie blue staining (left panel) and tested for activity in the GRASP65 bead aggregation assay (right panel).

Figure S3

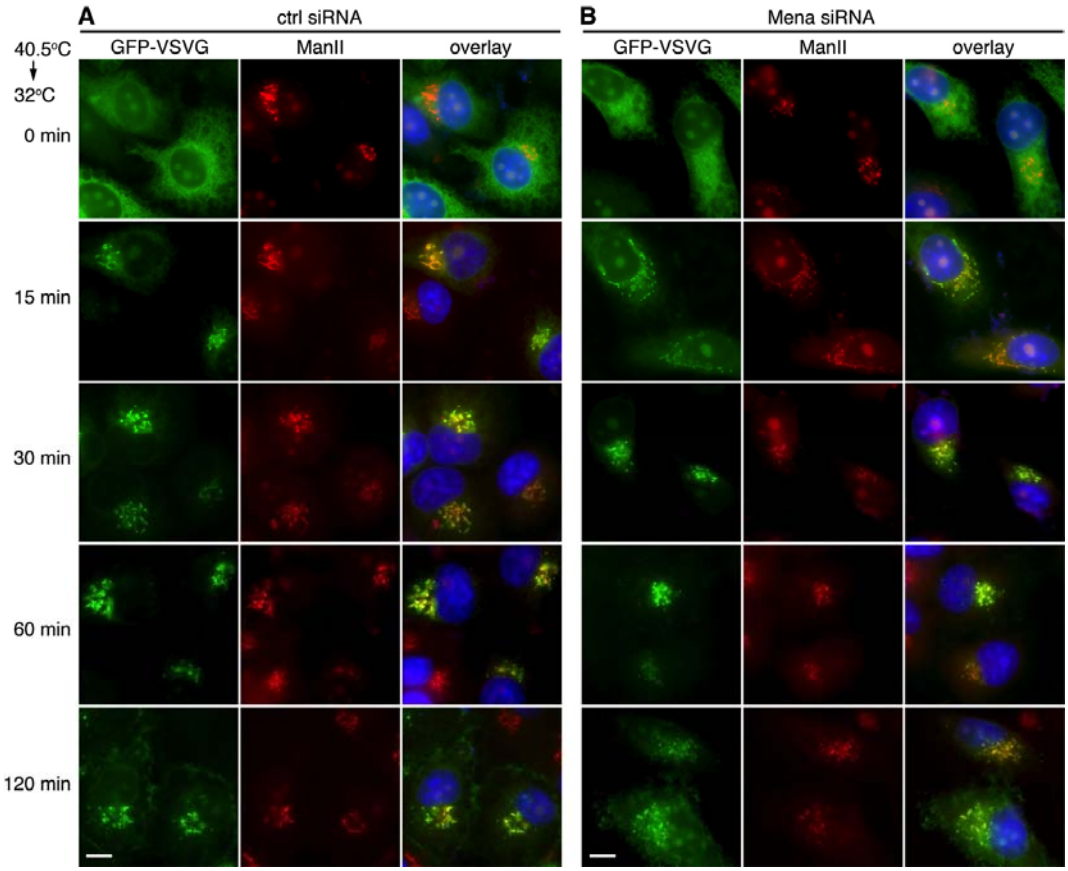


Figure S3. Mena depletion does not impact VSV-G transport.
(A, B) HeLa cells transfected with control or Mena siRNA were infected with VSV-G-ts045-GFP virus at 40.5ºC for 16 h to accumulate VSV-G in the ER. Cells were then moved to 32ºC for indicated times. Cells were fixed and stained for ManII. Bars, 10 µm.

Figure S4

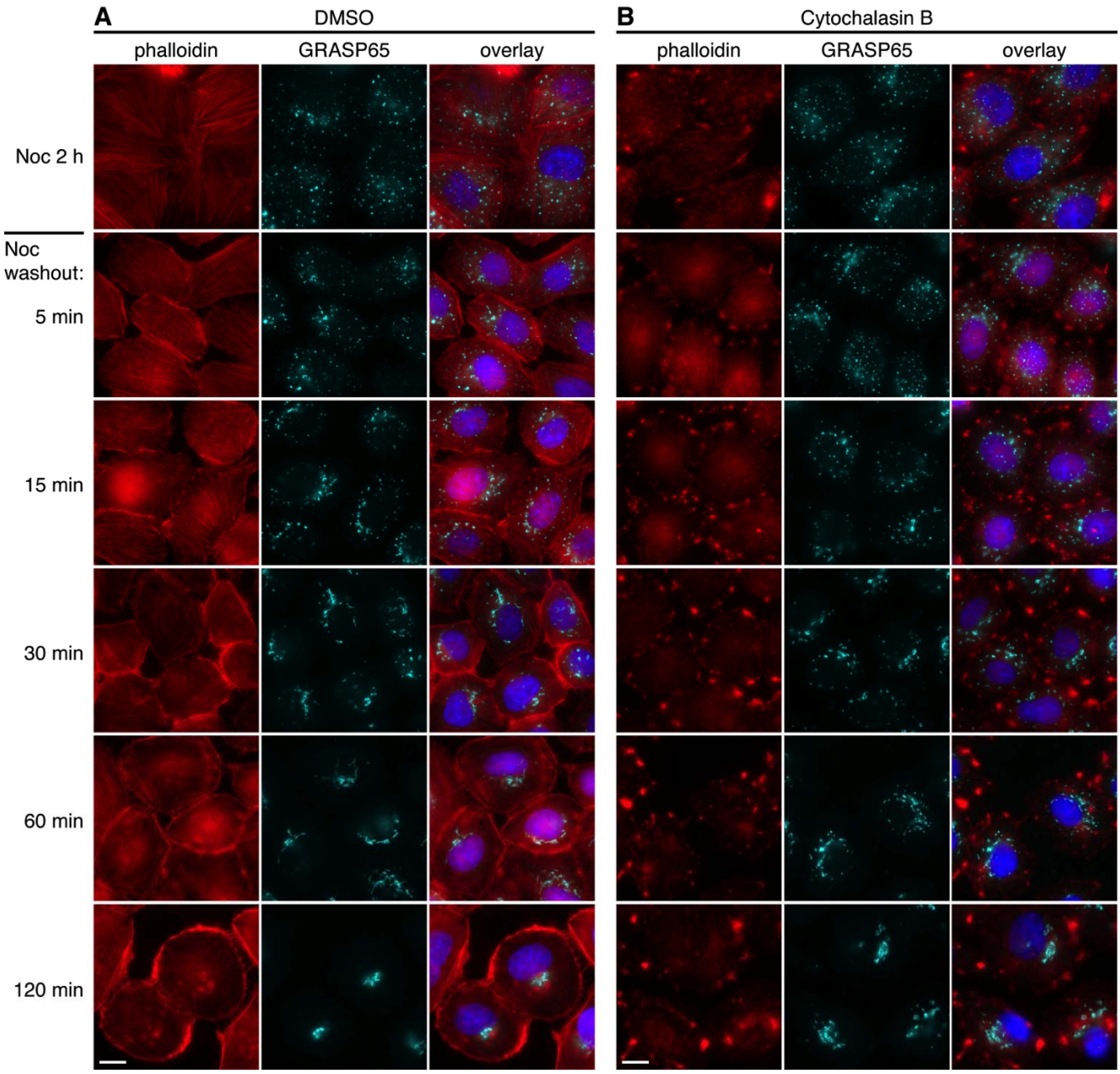


Figure S4. Depolymerization of F-actin inhibits Golgi reassembly after nocodazole washout. (A, B) HeLa cells were incubated with nocodazole for 2 h followed by the addition of DMSO or cytochalasin B for another 30 min. For nocodazole washout, cells were washed by PBS and kept in growth medium in the presence of DMSO or cytochalasin B but no nocodazole for the indicated times, fixed and stained by phalloidin and GRASP65 antibodies. Bars, 10 μm.

Figure S5

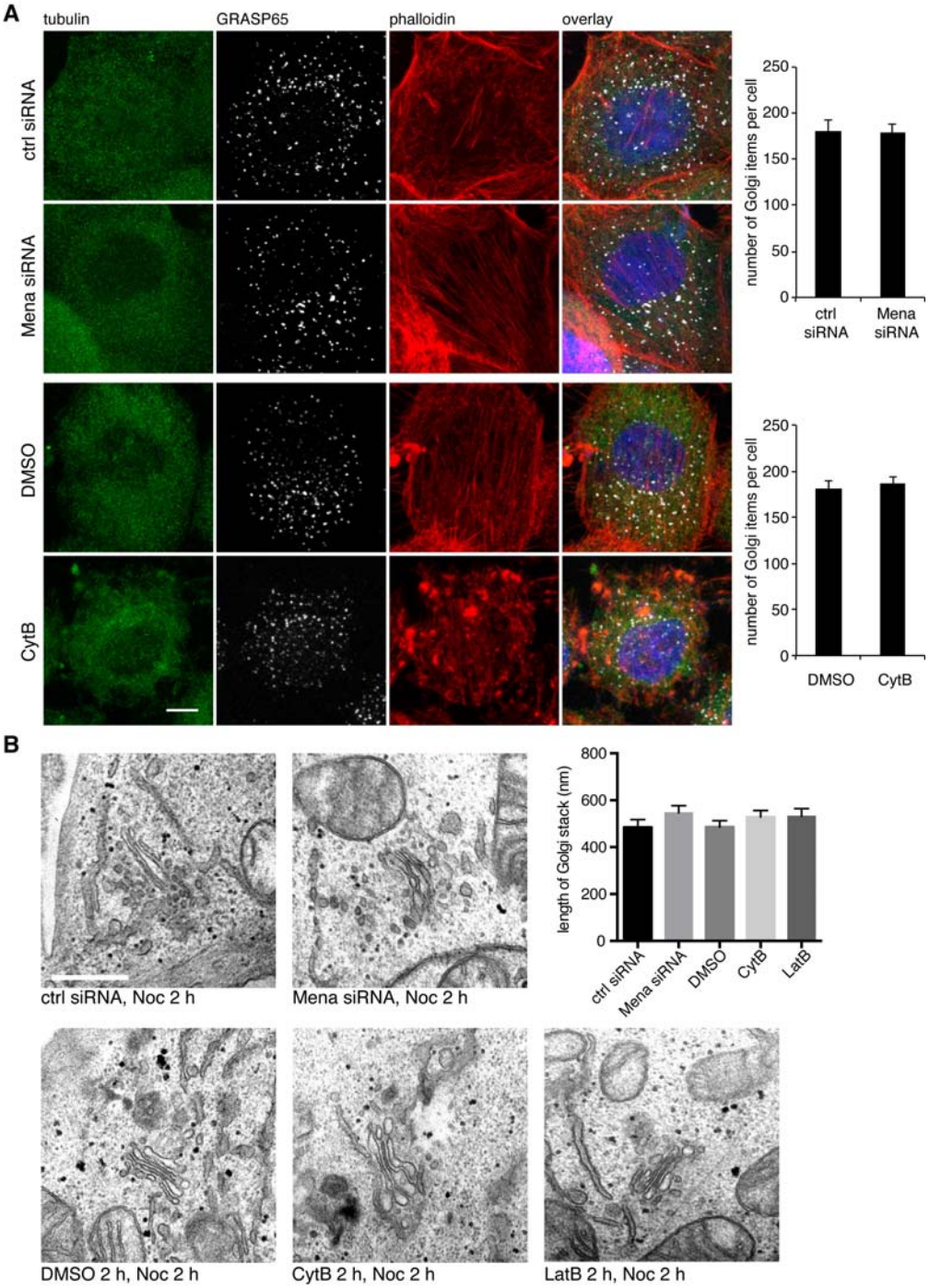


Figure S5. Actin filaments are required for Golgi ribbon linking but not stacking.

(A) Cells transfected with indicated siRNA followed with nocodazole treatment for 2 h, or treated with nocodazole together with DMSO or cytochalasin B for 2 h were fixed and stained. The average number of Golgi particles in the cells was quantified. Bar, 10 μm. (B) Cells with the indicated combined treatments as in (A) were imaged by EM and quantified. Note that the length of the Golgi stacks in cells had no significant difference between different treatments. Bar, 500 nm.

Table S1

Protein Name: Gene_Symbol=**ENAH** 89 kDa protein
Accession No.: IPI00010740
Protein Score: 79
Protein Score C. I. %:99.909

Calc. Mass	Obsrv. Mass	± da	Start Seq.	End Seq.	Sequence	Modification
929.4799	929.4857	0.0058	165	171	QLQEQQR	
989.4686	989.475	0.0064	273	279	EQLEWER	
1221.6011	1221.6102	0.0091	23	34	WVPAGGSTGFSR	
1329.6757	1329.6808	0.0051	205	214	YNQATQTFHQWR	
1579.7401	1579.7523	0.0122	70	81	VHIYHHTGNNTFR	
1595.7826	1595.788	0.0054	35	47	STPLSQPSANGVQTEGLDYDR	
2235.0525	2235.0679	0.0154	763	783	STPLSQPSANGVQTEGLDYDRLK	
2476.2314	2476.219	-0.0124	763	785	QNSQLPAQVQNGPSQEELEIQR	
2493.2329	2493.2329	0	142	163	QNSQLPAQVQNGPSQEELEIQR	

Protein Name: Gene_Symbol=PSPHL;**ACTG1** Actin, cytoplasmic 2
Accession No.: IPI00021440
Protein Score: 90
Protein Score C. I. %:99.994

Calc. Mass	Obsrv. Mass	± da	Start Seq.	End Seq.	Sequence	Modification
976.4482	976.4932	0.045	19	28	AGFAGDDAPR	
1132.527	1132.575	0.048	197	206	GYSFTTTAER	
1198.7054	1198.7531	0.0477	29	39	AVFPSIVGRPR	
1499.6761	1499.7498	0.0737	360	372	QEYDESGPSIVHR	Pyro-glu (N-term Q)[0]
1515.7491	1515.7872	0.0381	85	95	IWHHTFYNELR	
1516.7026	1516.774	0.0714	360	372	QEYDESGPSIVHR	
1954.0643	1954.1265	0.0622	96	113	VAPEEHPVLLTEAPLNPK	
2215.0698	2215.1379	0.0681	292	312	DLYANTVLSGGTTMYPGIADR	
2231.0649	2231.1433	0.0784	292	312	DLYANTVLSGGTTMYPGIADR	Oxidation (M)[14]
2730.4324	2730.377	-0.0554	336	359	KYSVWIGGSILASLSTFQQMWISK	
2746.4272	2746.375	-0.0522	336	359	KYSVWIGGSILASLSTFQQMWISK	Oxidation (M)[20]
3183.6143	3183.6658	0.0515	148	177	TTGIVMDSGDGVTHTVPIYEGYALPHAILR	
3199.6091	3199.6812	0.0721	148	177	TTGIVMDSGDGVTHTVPIYEGYALPHAILR	Oxidation (M)[6]

Protein Name: Gene_Symbol=**ACTB** Actin, cytoplasmic 2
Accession No.: IPI00848058
Protein Score: 87
Protein Score C. I. %:99.986

Calc. Mass	Obsrv. Mass	± da	Start Seq.	End Seq.	Sequence	Modification
976.4482	976.4932	0.045	48	57	AGFAGDDAPR	
1132.527	1132.575	0.048	226	235	GYSFTTTAER	
1198.7054	1198.7531	0.0477	58	68	AVFPSIVGRPR	
1499.6761	1499.7498	0.0737	389	401	QEYDESGPSIVHR	Pyro-glu (N-term Q)[0]
1515.7491	1515.7872	0.0381	114	124	IWHHTFYNELR	
1516.7026	1516.774	0.0714	389	401	QEYDESGPSIVHR	
1954.0643	1954.1265	0.0622	125	142	VAPEEHPVLLTEAPLNPK	
2215.0698	2215.1379	0.0681	321	341	DLYANTVLSGGTTMYPGIADR	
2231.0649	2231.1433	0.0784	321	341	DLYANTVLSGGTTMYPGIADR	Oxidation (M)[14]
2730.4324	2730.377	-0.0554	365	388	KYSVWIGGSILASLSTFQQMWISK	
2746.4272	2746.375	-0.0522	365	388	KYSVWIGGSILASLSTFQQMWISK	Oxidation (M)[20]
3183.6143	3183.6658	0.0515	177	206	TTGIVMDSGDGVTHTVPIYEGYALPHAILR	
3199.6091	3199.6812	0.0721	177	206	TTGIVMDSGDGVTHTVPIYEGYALPHAILR	Oxidation (M)[6]

Table S1. Identification of Mena and actin as GRASP65-interacting proteins by mass spectrometry.