Supplemental (SI) Appendix

SI Material and Methods

Molecular Cloning

Mammalian expression vectors. Human-Golgin97 E381Q/E386Q/E393Q, E558Q/E559Q/E565Q and E579Q/E585Q mutants were generated by site-directed mutagenesis reactions using the primers listed below:

- G97 E381Q/E386Q/E393Q for

5'_GCTGCCCAGCAAACTCAGATACAGCAGCTCGCTGCCGCCAACCAGCAGAGCAGCCA T-3

- G97 E381Q/E386Q/E393Q rev

5'_ATGGCTGCTCTGCTGGTTGGCGGCAGCGAGCTGCTGTATCTGAGTTTGCTGGGCAGC -3'

- G97 E558Q/E559Q/E565Q for

5'-CACCCTCAAGGCGCAGCAGGCTGCAGTGGTCGCGCAGCAGGAGGACCTG-3'

- G97 E558Q/E559Q/E565Q rev

5'-CAGGTCCTCCTGCTGCGCGACCACTGCAGCCTGCTGCGCCTTGAGGGTG-3'

- G97 E579Q/E585Q for

5'-CCATTGCAGGCCCAAGCACTCTCAGTCAATCAGTCGCACGTGACC-3'

- G97 E579Q/E585Q rev

5'-GGTCACGTGCGACTGATTGACTGAGAGTGCTTGGGCCTGCAATGG-3'

PCR reactions were carried out in a 50 µl final volume including the following components: 50 ng of DNA template, 200 µM of each dNTPs, 1x Pfu TurboCx Hotstart DNA Polymerase buffer (Agilent Technologies, #600414), 0.2 µM of each primer and 2.5 U of Pfu TurboCx Hotstart DNA Polymerase (Agilent Technologies, #600414). PCR conditions were as follows: a single denaturation step (95°C, 5 min) was followed by 20 cycles of denaturation (95°C, 1 min), annealing (60°C, 1 min) and elongation (68°C, 19 min). Then a final elongation step of 10 min at 72°C was carried out. The mutagenesis reactions products were treated with DpnI endonuclease to digest the parental DNA template and transformed into TOP-10 chemical competent cells (Invitrogen, C404006).

The constructs encoding the fusion proteins between hGolgin97 wild-type or its mutants and Tomato were generated amplifying the corresponding coding regions using the EcoRI hG97 for (5'-TAACCGGAATTCTATGTTTGCAAAACTGAAGAAGAAAATTGC-3') and hG97 XbaI rev (5'-ACTAGTCTAGACTAGGACCATGGTATCCGAGGGTTTG-3') primers and cloning the generated fragments into the ptdTomato-C1 vector (Addgene, 54653) previously digested with EcoRI and XbaI restriction enzymes. The sequences of all the constructs were checked by sequence analysis.

Bacterial expression vectors. The constructs encoding the His-tagged hGolgin97 wild-type or ADP-ribosylation defective mutant proteins were obtained digesting the corresponding constructs in ptdTomato-C1 vector and cloning them in pET-28b(+) vector (Novagen, 69865-3), downstream of the T7 promoter. Briefly, the hGolgin97 wild type and ADP-ribosylation-defective mutants coding regions were obtained by digestion with XbaI restriction enzyme, whose 3´recessed ends were filled with Klenow, followed by digestion with EcoRI restriction enzyme. Then, the generated fragments were cloned in pET-28b(+) vector, previously digested with XhoI restriction enzyme, whose 3´recessed ends were filled with Klenow, and then digested with EcoRI restriction enzyme. The sequences of all the constructs were verified by sequence analysis.

Cell Culture and treatments

HeLa cells (wild-type, ΔGolgin-97 or ΔGolgin-245 HeLa cells, kindly provided by Sean Munro, Shin et al., 2017) and MCF7 cells (from ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin at 37 °C and 5% CO2. All cell culture reagents were from Life Technologies. For treatment with PJ34 (50 μM), with IWR1 (25 μM) or with CRT0066101 (10 μM), cells were grown to 80% confluence and then treated with inhibitors for 2 h. Nocodazole treatment (33 μM) was performed for 3 h. All treatments were carried out in complete growth media.

Transient transfections

HeLa cells were transiently transfected with plasmids encoding FLAG-tagged or Tomato-tagged Golgin-97, mCherry-tagged Rab11, GFP-tagged E-cadherin, Myc-DDK-FIP1/RCP, thermosensitive mutant protein tsO45 YFP-VSVG or with RUSH-based constructs encoding E-cadherin and TNFα using TransIT-LT1 Reagent, according to the manufacturer's instructions.

siRNA-mediated knockdown

Commercially available siRNA oligos targeting hPARP12 (Dharmacon, L-013740-00-0005), hGolgin-97 (synthetized by Sigma-Aldrich; #1: AAGAUCACAGCCCUGGAACAA[dT]; #2: AAGUGCUUCUCCAGAAAGAGC[dT] as reported by Lock JG et al., Traffic, 2005), PKD1 (synthetized by Sigma-Aldrich; #1: CGGCAAAUGUAGUGUAUUAUU[T]; #2: GAACCAACUUGCACAGA GAUU[dT]; #3: GGUCUGAAUUACCAUAAGAUU[T]; #4: GGAGAUAGCCAUCCAGCA UUU[T]; PKD2 (synthetized by Sigma-Aldrich; #1: 1 UGAGACACCUUCACUUCAUU[T]; #2: CAAGAACAUUGUCCACUGUUU[T]; 3#: GGAAGAUGGGAGAGCGAUAUU[T] as reported by Capasso S et al., EMBO J, 2017) or non-targeting siRNAs (Dharmacon, D-001810-01-05) were transfected in HeLa and MCF7 cells at a final concentration of 100 or 50 nM using Lipofectamine RNAiMAX reagent (Invitrogen, 13778150) for 72 and 48 h, respectively, according to the manufacturer instructions. Where indicated, after 24 h, cells were transiently transfected with FLAG-tagged Golgin-97 or with constructs encoding E-cadherin and TNFα used for the RUSH system.

***In Vitro* ADP-ribosylation assay**

Purified GST-tagged PARP12 catalytic fragment (250 ng) was incubated with 1 μg purified recombinant wild-type Golgin-97 in ADP-ribosylation Buffer [50 mM Tris-HCl pH 7.4, 4 mM DTT, 500 μM MgCl2, 30 μM unlabeled NAD+ /4 μCi of 32P-NAD+] at 37 °C for 60 min. The incorporated [32P]-ADP-ribose was detected by autoradiography. Alternatively, purified GST-tagged PARP12 catalytic fragment (500 ng) was incubated with 3 μg purified recombinant Golgin-97 (WT and mutants) in ADP-ribosylation Buffer containing 100 μM unlabeled NAD+, at 37 °C for 60 min. At the end of the reaction, 1/10 of the reaction mixture was saved (input material); 10 μg of purified GST-tagged Af1521 macro domain were added to the remaining reaction mix and incubated at 4 °C. After 2 h, samples were recovered, glutathione Sepharose matrix added to the samples and further incubated at 4 °C. After 1 h incubation, samples were recovered and washed three times in reaction buffer. Samples were processed for SDS-PAGE and WB analysis. Signals were quantified using a Gel-Doc system (Bio-Rad). The percentage of MARylated Golgin-97 was calculated as ratio between Af1521-bound Golgin-97/total Golgin-97.

***In vitro* kinase assay**

The kinase reaction was performed as previously described (39). with some modifications. Briefly, 500 ng of GST-PKD1 or its kinase-dead mutant (GST-KD-PKD1) and purified GST-tagged PARP12 catalytic domain (600 ng) were incubated in 30 µl kinase buffer (50 mM Tris pH 7.4, 10 mM $MgCl_2$, 2 mM DTT, 100 µM ATP, in presence of phosphatase inhibitors), for 30 min at 37 °C. At the end of incubation, the reaction was stopped by adding 30 µl of 2x SDS-sample buffer. The samples were boiled at 95° C for 5 min and resolved by SDS-PAGE, blotted onto nitrocellulose and analyzed by western blotting using a phospho-Ser/Thr antibody (see Resource Table). Alternatively, the reaction was performed using immunoprecipitated full-length Myc-tagged PARP12. PARP12 was immunoprecipitated as previously described (21). Briefly, HeLa cells were transiently transfected with Myc-tagged PARP12. After 24 h, cells were lysate in complete RIPA buffer and 1 mg total lysates incubated ON with 5 µl of mouse anti-Myc antibody. The mixtures were then centrifuged (500 g, 5 min, 4 °C) and the beads were washed 3 times with RIPA buffer and further 5 times in the same buffer without detergents. The immunocomplexes were used in the kinase reaction as described above.

Where indicated, phosphorylated PARP12 was then used in *in vitro* MARylation assays in presence of His-tagged Golgin-97 (see below).

His-pull down assay

Histidine pull-down assays were carried out as previously described (46), with some modifications. Briefly, HeLa cells transfected with 5 µg PARP12 cDNA for 24 h were lysed in 700 µl lysis buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 5 mM MgCl2, with phosphatase/protease inhibitors) and incubated at 4 °C, for 30 min. Samples were clarified by centrifugation at 16,000× g for 10 min at 4 °C. Total lysates (700 µg) were incubated with 5 or 10 µg of purified recombinant His-Golgin-97 in His incubation buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 20 mM imidazole, 1% Triton X-100, protease inhibitors) at 4 °C. After an ON incubation, 30 µl Ni-NTA agarose beads were added, and samples incubated for 1 h at 4 °C. The beads were then washed three times with His wash buffer (50 mM Tris-HCl, pH 7.4, 300 mM NaCl, 20 mM imidazole, 1% Triton X-100, protease inhibitors) and twice with the same buffer without Triton X-100. Bound proteins were then eluted from the beads with His elution buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 300 mM imidazole, pH 7.4) and centrifuged at 700× g for 5 min at 4°C. The 5% and 30% of the input and eluted proteins, respectively, were processed for 7.5% SDS/PAGE and western blotting analysis.

***Macro* domain based pull-down assay**

The procedure to obtain the Af1521 *macro* domain resin was described previously (55). For pull-down assays, HeLa cells were washed three times in ice-cold PBS and solubilized in RIPA buffer (100 mM Tris HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, supplemented with protease inhibitors and 5 µM PJ34), under constant rotation for 30 min at 4 °C. The mixtures were clarified by centrifugation at 15,000 × *g* for 10 min at 4 °C, the supernatants were recovered and protein concentration evaluated using the BCA Protein Assay Kit. Total lysates (1 mg) were then incubated ON with 30 µl of a 10 µg/µl GST cross-linked wild-type *macro* domain resin at 4 °C or, for experiments performed with MCF7 cells, with 50 µg of free GST-*macro* domain. After ON incubation, glutathione Sepharose matrix (previously equilibrated in RIPA buffer) was added to lysates and further incubated at 4 °C for 1 h. Beads were centrifuged at 500× *g* for 5 min to recover

the proteins bound to the wild-type macro domain. Resins were then washed 3 times with RIPA buffer and another 2 times in the same buffer without detergents. At the end of the washing steps, the resins were resuspended in 100 µl SDS sample buffer, boiled, analyzed by 8% SDS/PAGE and transferred onto nitrocellulose for Western blotting. The membranes were blocked for 1 h at RT in Tris-Buffered Saline (TBS) with 0.05% Tween (TBS-T) containing 5% BSA. Primary antibodies were diluted (see Resource Table) in blocking solution and incubated with membranes ON at 4° C. After washing with TBS-T, the membranes were incubated with an appropriate HRP-conjugated secondary antibody diluted in 5% non-fat, dry milk in TBS-T for 45 min at RT. The membranes were washed extensively with TBS-T before chemiluminescent detection using the ECL western Blotting Detection Reagents and X-ray film or VersaDoc system.

Immunoprecipitation

HeLa cells in 10 cm plates were transiently transfected with 6 µg Myc-DDK-FIP1/RCP and 4 µg tomato-tagged WT-Golgin-97 or its mutants. After 24 h transfection, cells were washed with PBS and lysed with lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton-X100, 1 mM EDTA, protease and phosphatase inhibitors). Lysates were centrifuged (16,000 g, 20 min, 4 °C) and 1 mg of protein lysate incubated ON with 2 µg anti-FLAG antibody. The mixtures were centrifuged (700× g, 5 min, 4 °C) and the beads washed 3 times with lysis buffer and 3 times in the same buffer without detergents. Samples were processed for SDS-PAGE and Western blotting analysis.

Purification of His-tagged Golgin-97 from *Escherichia coli*

His-tagged Golgin-97 was purified as described below. BL21-DE3 cells transformed with the plasmid encoding the His-tagged Golgin-97 were grown in Luria broth (LB) containing 60 µg/ml kanamycin, ON at 37 °C under continuous shaking (200 rpm). The cultures were then diluted in 400 ml LB, and OD_{600} was monitored until 0.6. Bacteria were induced with 0.2 mM IPTG at 25 °C. After 4 h, the cultures were chilled on ice and centrifuged at 5,000× *g* for 10 min at 4 °C. The pellets were resuspended in 4 ml His-lysis buffer (50 mM Tris-HCl, pH 8, 1.5% Triton X-100, 300 mM NaCl, 10% glycerol, 5 mM imidazole, 10 mM 2-mercaptoethanol, protease inhibitor cocktail, 0.5 mg/ml lysozyme) and the lysates incubated at 4 °C for 30 min. Lysates were sonicated on ice three times for 30 sec and then centrifuged at 20,000× *g* for 20 min at 4 °C. The resulting supernatants were incubated with 0.5 ml Ni-NTA beads at 4 °C for 2 h, and then packed into a 10 ml chromatography column. The columns were washed 5 times with 10 ml His-wash buffer *plus* 20 mM imidazole, and the protein was eluted by adding aliquots of 0.5 ml His-elution buffer *plus* 300 mM imidazole. The elution step was repeated 5 times. The fractions were then dialyzed twice against 1,000 vol. of PBS *plus* 10% glycerol, flash frozen in liquid nitrogen, and stored in aliquots at -80 °C. The same procedure was used for the purification of wild-type Golgin-97 and Golgin-97 mutants.

Immunofluorescence and Confocal Microscopy

Cells were fixed in 4% paraformaldehyde for 10 min at RT, washed three times in PBS, and incubated for 30 min at RT in blocking solution (0.5% bovine serum albumin, 50 nM NH4Cl in PBS, pH 7.4, 0.1% saponin and 0.02% sodium azide). Cells were subsequently incubated with the indicated antibodies diluted in blocking solution (see Resource Table) for 2 h at RT or ON at 4 °C. After incubation with the primary antibody, cells were washed three times in PBS and incubated with a fluorescent-probe-conjugated secondary antibody, for 30 min at RT. Alexa Fluor 488-, 568- or 647-

conjugated anti-rabbit, anti-mouse or anti-goat donkey antibodies were used at a dilution of 1:400 in blocking solution. After immunostaining, cells were washed three times in PBS and twice in sterile water. The coverslips were then mounted on glass-microscope slides with Mowiol. Images were taken using a Zeiss-LSM 700 confocal microscope. Optical confocal sections were taken at 1 Air Unit.

Transport assays

All transport assays were performed in presence of cycloheximide (50 μg/ml). For RUSH-based transport, cycloheximide was added 15 min before the addition of biotin.

TGN-exit assay of VSVG

HeLa cells (wild-type and ΔGolgin-97) were transiently transfected with the thermosensitive mutant tsO45 YFP-VSVG or infected with vesicular stomatitis virus (VSV) and incubated overnight at 40 °C, following incubation at 20 °C for 90 min in presence of cycloheximide (50 μg/ml) to accumulate the G protein of VSV (VSVG) in the Golgi complex. To monitor VSVG exit from the TGN the temperature was shifted to 32 °C and the samples were fixed with 4% paraformaldehyde at different times (40 ° and 20 °C temperature blocks; 15, 30, 45 and 60 min of the 32 °C temperature release) and stained with the indicated antibodies, as reported. Quantification was performed by counting the number of cells showing VSVG at the Golgi complex.

RUSH system

HeLa cells were plated on coverslips in 24-well plates and transfected with the Retention Using a Selective Hook (RUSH)-based EGFP-tagged TNFα or E-cadherin constructs (Str-KDEL_SBP-EGFP-E-cadherin/TNFα). After 24 h incubation at 37 °C, biotin (40 μM) was added at different times (0, 30, 60, 120 min) to allow the cargo to undergo trafficking. Cells were then fixed and stained with the specific antibodies, as reported. For surface immuno-staining, cells were fixed with freshly-prepared 4% paraformaldehyde and incubated with blocking solution without saponin. After 30 min, non-permeabilized cells were incubated with an anti-GFP antibody for 1 h at room temperature, washed three times with PBS and then incubated for further 30 min with the Alexa-568 secondary antibody. Samples were then analyzed using a Zeiss-LSM 700 confocal microscope.

Live cell imaging using GFP-tagged E-cadherin

Wild-type or ΔGolgin-97 HeLa cells were grown on glass-bottomed 35-mm dishes and transfected with GFP-tagged E-cadherin alone or GFP-tagged E-cadherin/Tomato-tagged Golgin-97 (wild-type or Mut B). Three hours after transfection, the glass-bottomed dishes were mounted on a Zeiss LSM700 laser scanning microscope under controlled temperature and CO_2; cells showing GFP-E-cadherin at the Golgi complex were imaged for 2 h. Frames were taken every 3 min (488 for excitation; PMT: 510–550 nm; 512×512 pixels; frame average, 4).

Live cell imaging using SBP-EGFP- E-cadherin and quantifications

For live cell imaging, wild-type HeLa cells were grown on glass-bottomed 35-mm dishes and transfected with SBP-EGFP-E-cadherin alone or in combination with PARP12 siRNAs (25 nM) for 36 h. For experiments performed in ΔGolgin-97 cells, SBP-EGFP-E-cadherin was transfected alone or in combination with tomato-tagged Golgin-97 (WT, MutA, Mut B or MutC). After 24 h transfection, cells were incubated in complete media supplemented with 40 μM biotin. Time-lapse acquisitions were performed at 37 °C in a thermostat-controlled chamber using a Spinning Disk system (Nikon, Eclipse Ni-E). Images were acquired using a 63× objective; frames were taken every 1 min. Integrated intensity was measured at each time point in a region of interest (ROI) around the Golgi complex. The position of the Golgi complex was obtained using later time point and used for

the entire time-lapse quantification. The obtained values were then normalized to the maximum value and on the total fluorescence. A total number of 9 cells were quantified for each condition. Quantifications were made using the Fiji software; Prism 8 (GraphPad Software) was used for drawing the plots.

Quantitative fluorescence image analysis

Quantitative analysis was performed using the Image J software. In brief, to calculate the amount of cargo in the Golgi area, the integrated intensity fluorescence was measured for each cell area and for the Golgi area of that cell and the Golgi/Total ratio was calculated. For quantification of the GFP-surface staining (used for the RUSH system), the integrated intensity fluorescence was measured for each cell area and for the cell surface and the Surface/Total ratio was calculated. The co-localization analysis was performed using the JaCoP Plugin of the freeware ImageJ software according to the Pearson's Correlation Coefficient. The freeware ImageJ software was also used for the analysis of carrier size.

Sources of all antibodies and reagents used are listed in the Resource Table below

RESOURCES TABLE

Antibodies	Source	Identifier	Working dilution
PARP12	Abcam	Ab45873	IF 1:200
PARP12	Sigma Prestige Antibodies	HPA003584	WB 1:2000
Tankyrase-1/2 (H350)	Santa Cruz Biotechnology	sc-8337	WB 1:1000
Golgin-97	Sigma-Aldrich	HPA044329	WB 1:2000 IF 1:200
Golgin-97	ThermoFisher Scientific	A-21270	WB: 1:1000
p230/Golgin-245	BD Transduction Laboratories	611280	WB 1:1000 IF 1:100
TGN46	BioRad	AHP500GT	IF 1:200
GM130	BD Transduction Laboratories	160823	IF 1:100
Giantin	Enzo Life Sciences	ALX-804-600-C100	IF 1:1000
LAMP1	Abcam	Ab24170	IF 1:200
EEA1	BD Transduction Laboratories	610457	IF 1:100
Transferrin Receptor	ThermoFisher Scientific	13-6800	IF 1:200
E-cadherin	BD Transduction Laboratories	610182	WB 1:2000 IF 1:200
PKD1	Cell Signaling Technology	90039	WB 1:1000
PKD2	Santa Cruz Biotechnology	sc-100415	WB 1:2000
GFP	Abcam	Ab1218	IF 1:100
FLAG	Sigma-Aldrich	F1804	WB 1:5000 IF 1:500
Calnexin	Abcam	Ab2259	WB 1:2000
Phospho-(Ser/Thr)	Cell Signaling Technology	9631	WB 1:1000
VSVG (P5D4)	Sigma	SAB42000695	IF 1:400
VSVG	Bethyl	A190-231A	IF 1:400
Myc/c-Myc (E910)	Santa Cruz Biotechnology	sc-40	IP 2,5µg/500 µg total lysates
GST	Abcam	Ab111947	**WB 1:1000**
GFP	Abcam	Ab1218	**WB 1:1000**

Chemicals and recombinant proteins		
PJ34	Sigma-Aldrich	P4365
IWR-1	Sigma-Aldrich	I01061
CRT 0066101	TOCRIS	4975
Nocodazole	Sigma-Aldrich	M1404
Biotin	Sigma-Aldrich	B4501
[^{32}P]-β-NAD^{+}	PerkinElmer	BLU023X
β-NAD^{+}	Sigma-Aldrich	N0632
PARP12	Abcam	Ab193474
Golgin-97	See SI Methods	N/A
Af1521 *macro* domain	See SI Methods	N/A

ATP	Sigma-Aldrich	A-2383
Experimental Models: Cell lines		
HeLa wild-type	Shin et al., 2017	PMID: 29084197
HeLa ΔGolgin-97	Shin et al., 2017	PMID: 29084197
HeLa ΔGolgin-245	Shin et al., 2017	PMID: 29084197
MCF7	ATCC	HTB-22
Primers for molecular cloning		
EcoRI hG97 aa2 for	See SI Methods	N/A
hG97 aa660 SmaI rev	See SI Methods	N/A
EcoRI hG97 aa603 for	See SI Methods	N/A
EcoRI hG97 aa603 for	See SI Methods	N/A
G97 E381Q/E386Q/E393Q for	See SI Methods	N/A
G97 E381Q/E386Q/E393Q rev	See SI Methods	N/A
G97 E558Q/E559Q/E565Q for	See SI Methods	N/A
G97 E558Q/E559Q/E565Q rev	See SI Methods	N/A
G97 E558Q/E559Q/E565Q rev	See SI Methods	N/A
G97 E579Q/E585Q rev	See SI Methods	N/A
EcoRI hG97 for	See SI Methods	N/A
hG97 XbaI rev	See SI Methods	N/A
Recombinant DNA		
FLAG- Golgin-97 wild-type	De Matteis LAB	N/A
FLAG- Golgin-97 mutants	This study	N/A
Tomato- Golgin-97	This study	N/A
Myc-Golgin-245	Lock et al., 2005	N/A
GFP- E-cadherin	Lock and Stock, 2005	N/A
Str-KDEL_SBP-EGFP-E-cadherin	Boncompain et al., 2012	N/A
Str-KDEL_SBP-EGFP-TNFα	Boncompain et al., 2012	N/A
YFP-VSVG	Valente et. al, 2012	N/A
GST-PKD1	Hausser et al., 2005	N/A
Myc-DDK-Rab11FIP1	Origene	Rc209640
Myc-PARP12	Catara et al., 2017	N/A

Software

ADPredict	Lo Monte et al., 2018	https://www.adpredict.net/
Multicoil2	Trigg et al., 2011	http://cb.csail.mit.edu

SI Figures

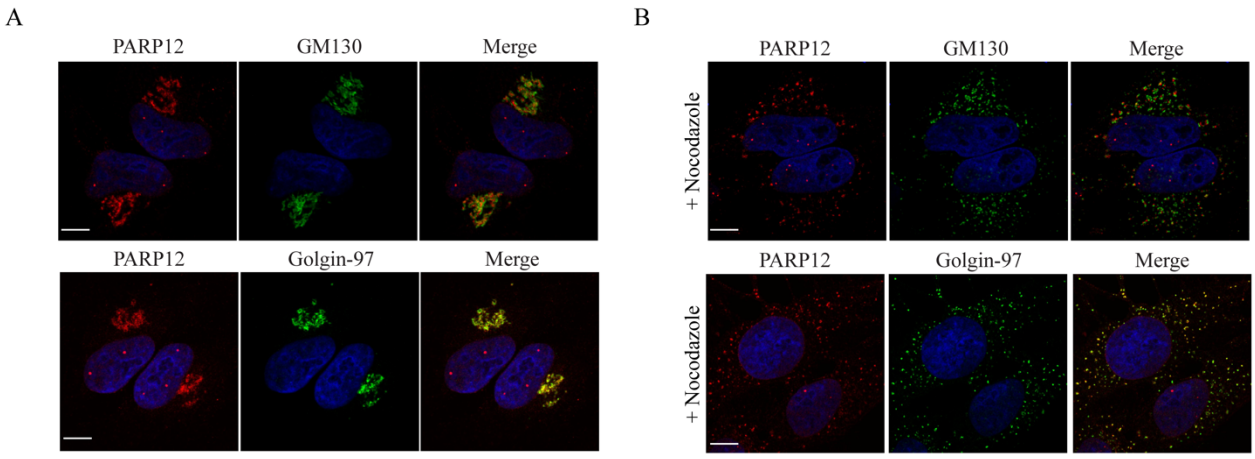


Figure S1. PARP12 co-localizes with Golgin-97 at the *trans*-Golgi network (TGN)

(A, B) Representative confocal microscopy images of HeLa cells showing PARP12 co-localization with Golgin-97. Hela cells were (A) untreated or (B) treated with nocodazole (33 μM, 3 h), fixed and labeled with anti-PARP12 (red), anti-GM130 (a cis-Golgi marker, green) and anti-Golgin-97 (a TGN-Golgi marker, green) antibodies. Scale bars, 10 μm.

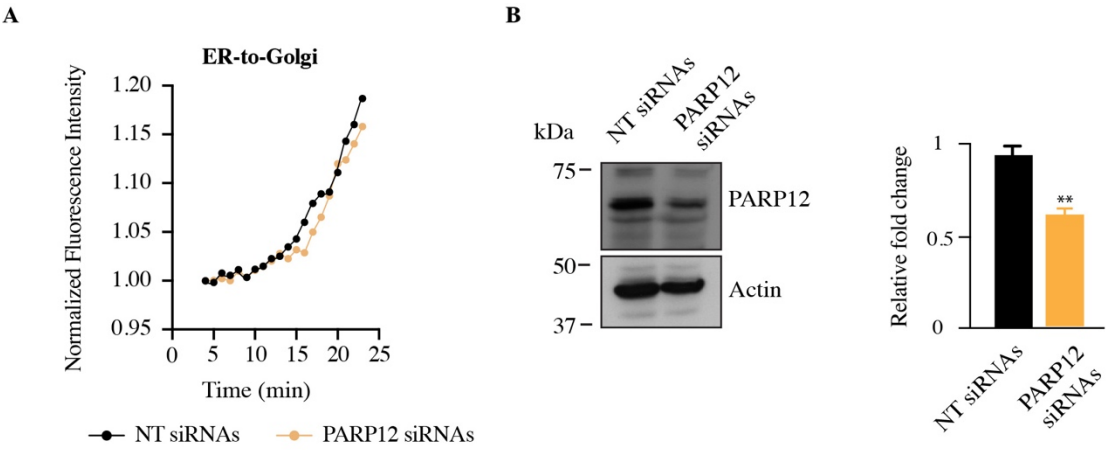


Figure S2. PARP12 depletion does not affect ER-to-Golgi transport of E-cadherin

(A) HeLa cells expressing SBP-EGFP-E-cadherin and depleted or not of PARP12 (NT siRNAs *vs* PARP12 siRNAs) were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals. Averaged (n=9 cells) and normalized time-based changes in fluorescence intensity of a ROI containing the Golgi complex are shown. **(B)** PARP12 depletion levels were monitored by Western blotting using a PARP12 antibody. Detection of actin levels was used as loading control. Blots are representative of three independent experiments. Statistical significance was calculated by unpaired Student's t-test; ***$p < 0.001$.

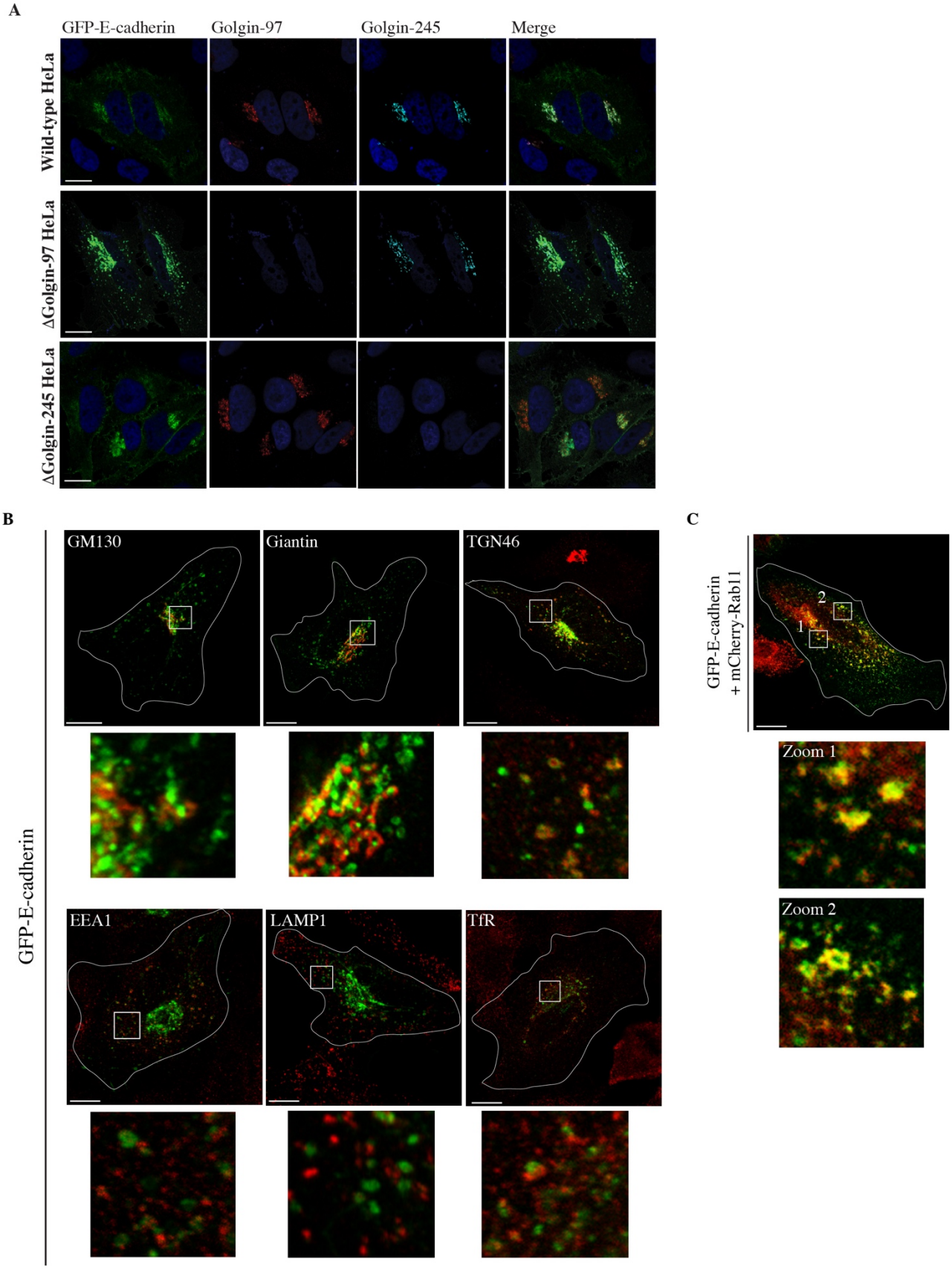

A
GFP-E-cadherin
Golgin-97
Golgin-245
Merge
Wild-type HeLa
ΔGolgin-97 HeLa
ΔGolgin-245 HeLa
B
GFP-E-cadherin
GM130
Giantin
TGN46
EEA1
LAMP1
TfR
C
GFP-E-cadherin
+ mCherry-Rab11
1
2
Zoom 1
Zoom 2

Figure S3. Absence of Golgin-97 causes E-cadherin-transport defect, showing carriers co-localizing with Rab11

(A) Representative confocal images of GFP-E-cadherin (green) localization in wild-type, ΔGolgin-97 and ΔGolgin-245 cells, showing E-cadherin at the PM in control and ΔGolgin-245, but not in ΔGolgin-97, cells. Golgin-97 is shown in red; Golgin-245 is in cyan. **(B)** ΔGolgin-97 HeLa cells were transiently transfected with GFP-tagged E-cadherin (green). After 8 h overexpression, cells were fixed and stained with markers of the Golgi apparatus (GM130, Giantin, TGN46), early endosomes (EEA1), lysosomes (LAMP1) and recycling endosomes (Transferrin receptor; TfR), as indicated. **(C)** To follow Rab11 localization, ΔGolgin-97 HeLa cells were co-transfected with mCherry-tagged Rab11 and GFP-tagged E-cadherin; after 8 h transfection cells were fixed and processed for immunofluorescence analysis. Insets: enlarged view of merged signals. Scale bars, 10 μm. Note that the pool of E-cadherin at the Golgi compartment did not co-localize with GM130 or Giantin, excluding the cargo from the cis/medial side of the Golgi complex (intra-Golgi defect); instead, it clearly co-localized with TGN46 (B). Of note, the E-cadherin-including carriers did not show any degree of colocalization with either EEA1 or LAMP1 (B), while they colocalized with Rab11 (C), defining a post-TGN, Rab11-positive compartment as the site of E-cadherin accumulation.

In addition, the E-cadherin-containing carriers did not co-localize with TfR (while they co-localized with Rab11). This suggests that these carriers define a post-TGN, Rab11-positive compartment.

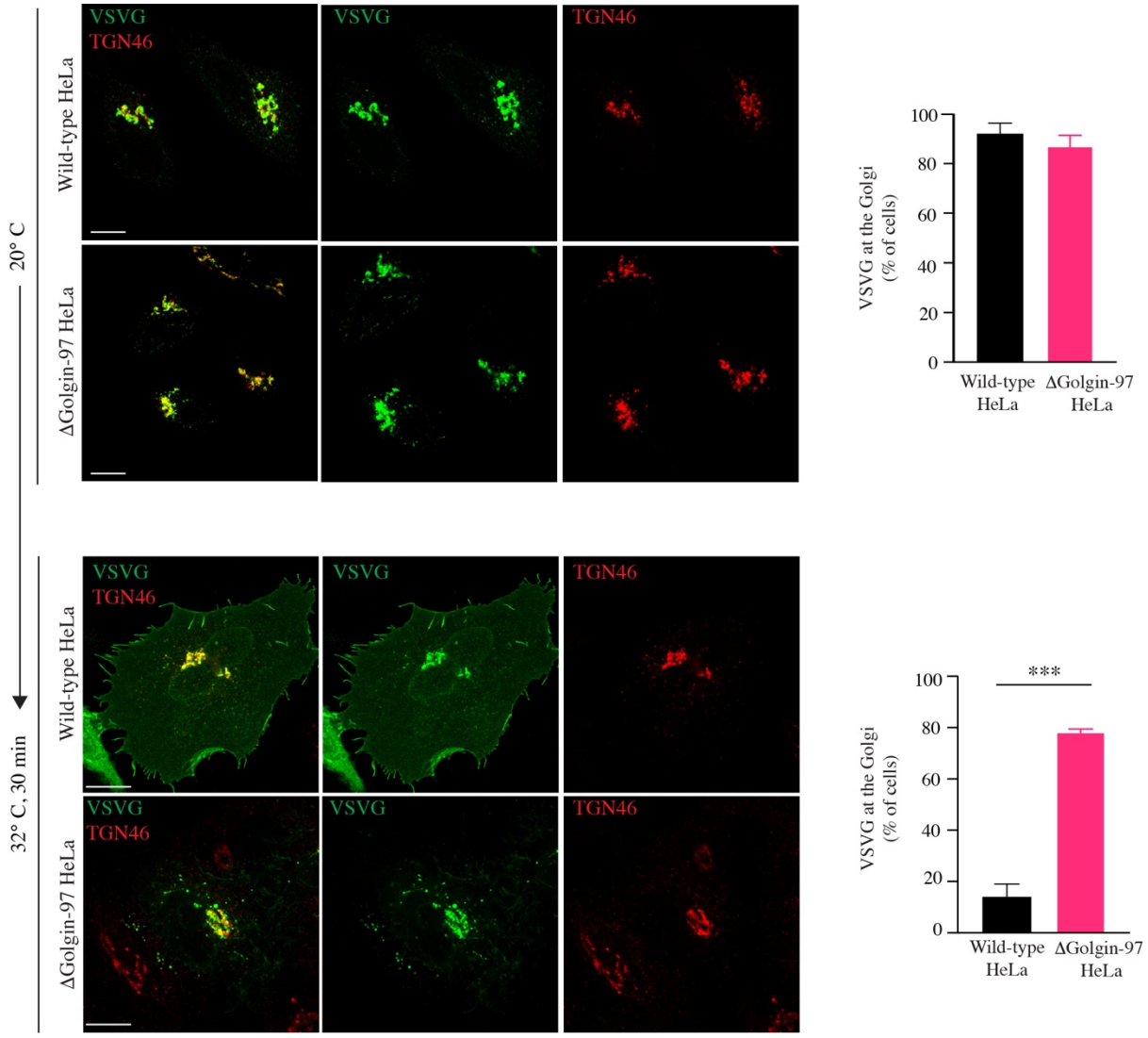


Figure S4. Golgin-97 depletion inhibits TGN-to-PM transport of VSVG cargo

Wild-type and ΔGolgin-97 HeLa cells were infected with VSV virus (see methods, TGN-exit assay of VSVG). After the 20 °C temperature-block, cells were incubated at 32 °C to allow the release of the cargo from the TGN, fixed and stained with VSVG (green) and TGN46 (red) antibodies. Representative confocal images of cells fixed at the end of the 20 °C temperature block and fixed at 30 min of the 32 °C temperature-block release are shown. Quantifications show the percentage of cells showing VSVG in the Golgi area at 20 °C or at 30 min of the 32 °C temperature-block release. Data are mean of n= 300 cells from three different experiments ± SD. Statistical significance was calculated by unpaired Student's t-test; ***$p < 0.001$. Scale bars, 10 μm.

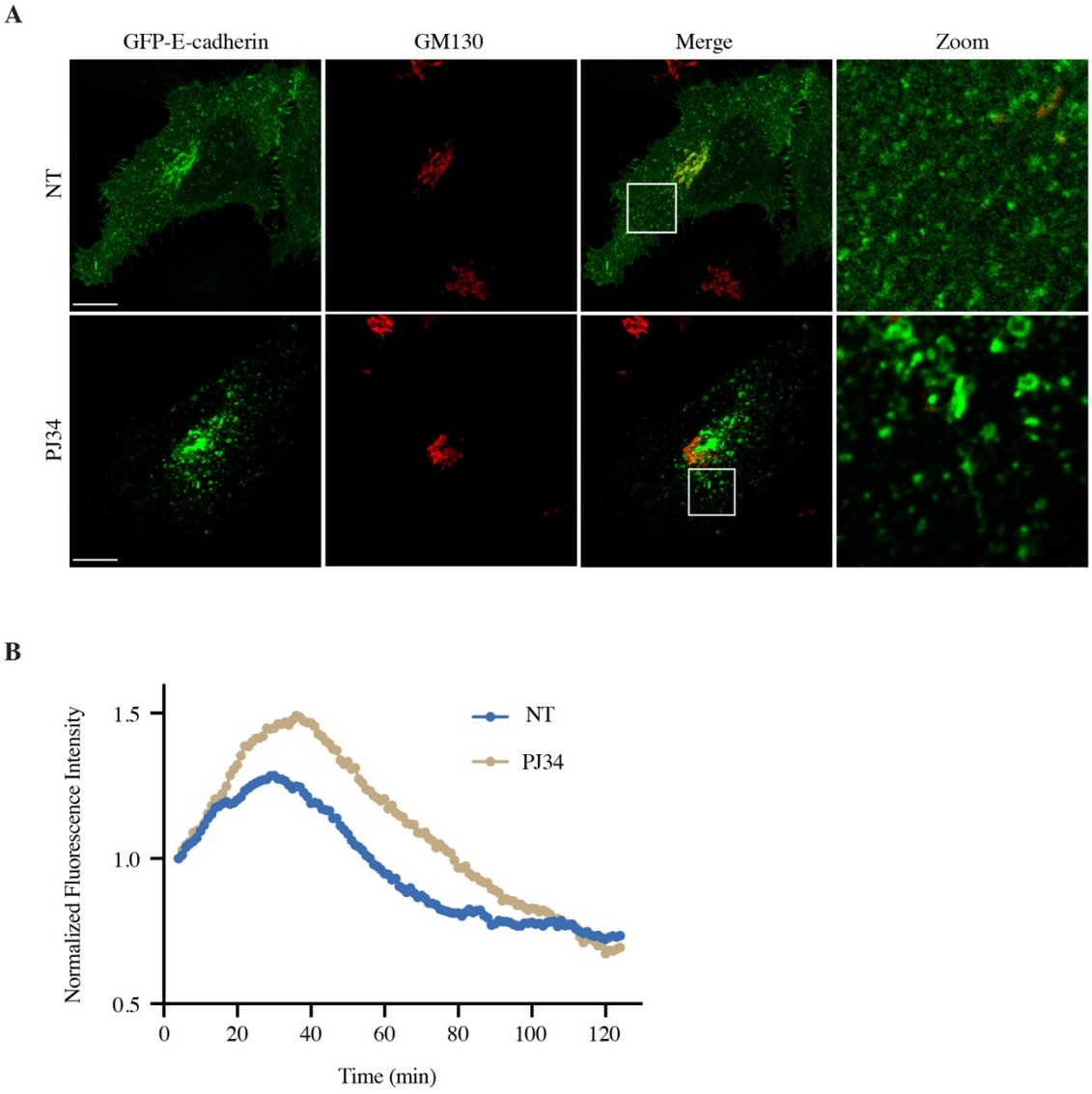


Figure S5. PJ34 treatment causes a mis-localization of E-cadherin and a delay of its exit from the TGN

(A) Wild-type HeLa cells, transiently transfected with GFP-E-cadherin, were treated or not (NT) with the general PARP inhibitor PJ34 (50 µM, 2 h), fixed and stained with GM130 antibody (used as Golgi marker). Representative confocal images showing E-cadherin accumulation in spots reminiscent of transport carriers upon PJ34 treatment, that do not co-localize with the Golgi marker. Scale bars, 10 µm. **(B)** Wild-type HeLa cells expressing SBP-EGFP-E-cadherin were treated or not (NT) with the general PARP inhibitor PJ34 (50 µM) and subjected to a biotin-based traffic pulse. PJ34 was added to the complete media 30 min before the addition of the biotin and left for all the duration of the experiment (2 h). Time-lapse confocal images were captured for 2 h at 1 min intervals. The plot shows averaged (n=5) integrated fluorescence intensity in the Golgi region at each time point, normalized to the first value and on the total fluorescence. See representative Movies 3 and 4.

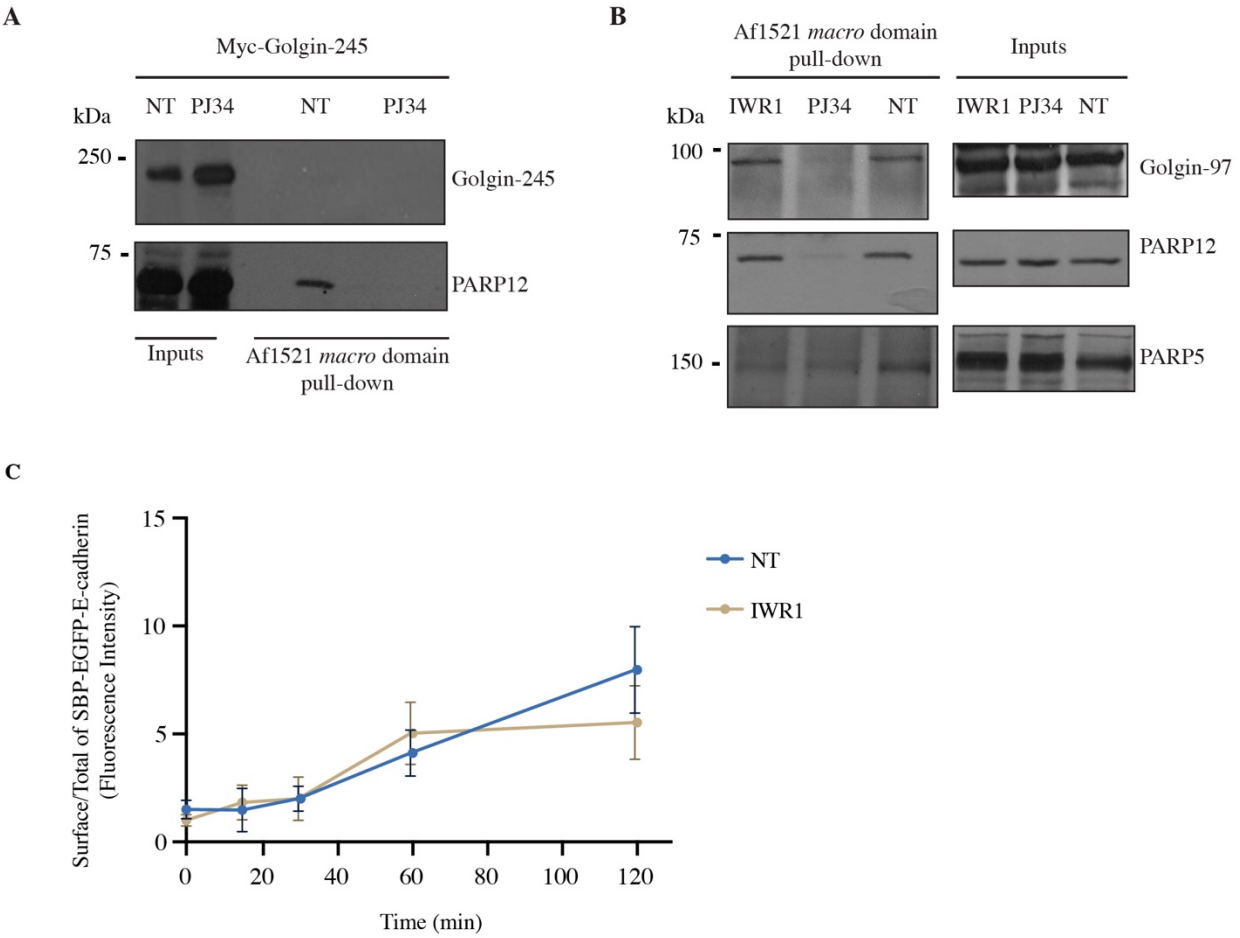
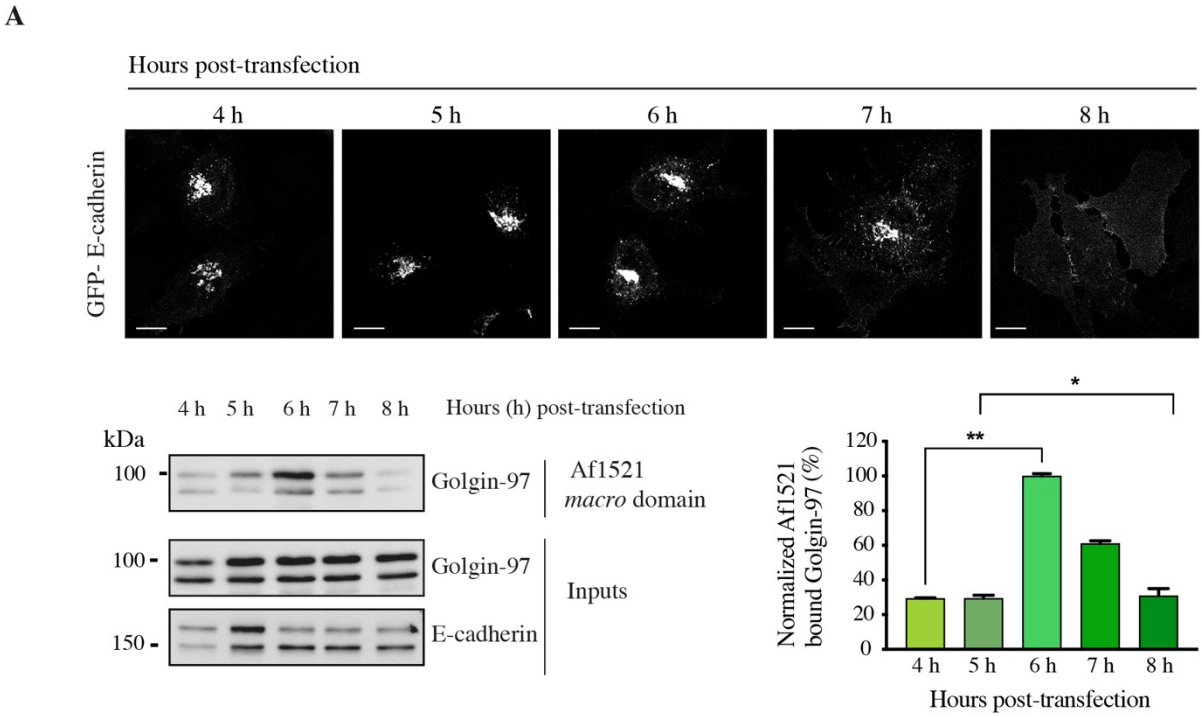
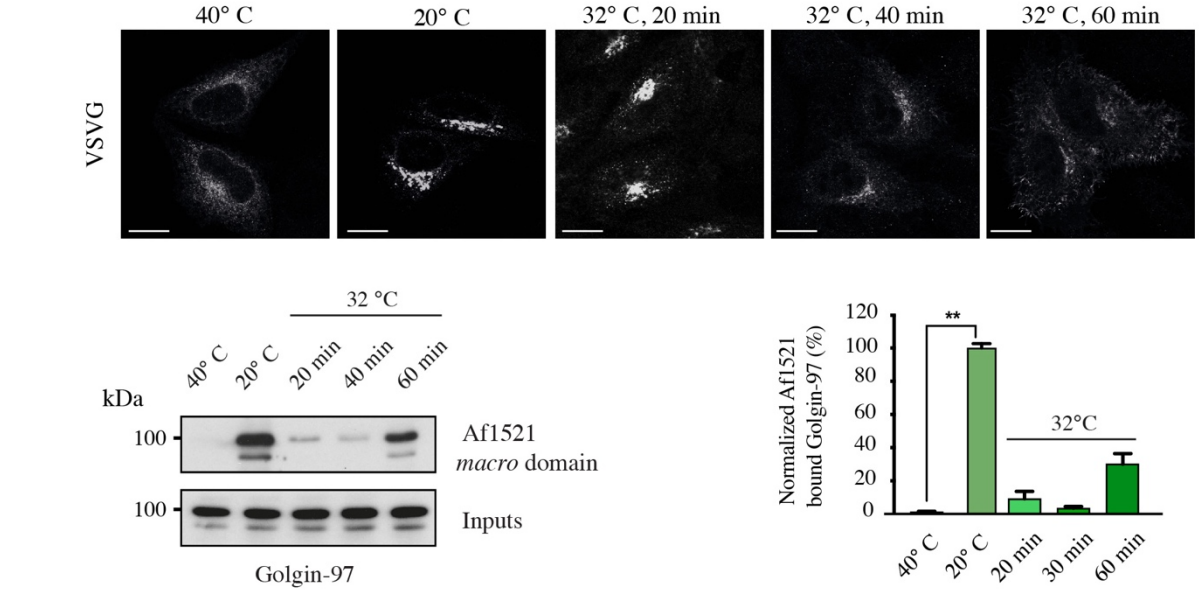


Figure S6. MARylation is specific for Golgin-97 and is independent of PARP5

(A) Af1521 *macro* domain pull-down assay of total cell lysates obtained from HeLa cells transiently transfected with Myc-tagged Golgin-245, untreated (NT) or treated with PJ34 (50 µM, 2 h) showing the absence of MARylation for Golgin-245. **(B)** Af1521 *macro* domain pull-down assay of total cell lysates obtained from HeLa cells untreated (NT) or treated with PARP5 inhibitor IWR1 (25 µM, 2 h) or with the general PARP inhibitor PJ34 (50 µM, 2 h) showing the absence of Golgin-97 MARylation upon PJ34 treatment, but not upon IWR1 treatment. Detection of PARP12 or PARP5 ADP-ribosylation was used as internal control. **(C)** Quantification of SBP-EGFP-E-cadherin surface staining in HeLa cells treated or not (NT) with the PARP5 inhibitor IWR1 (25 µM, 2 h) during biotin-based traffic pulse. Data are mean of n= 30 cells from three different experiments ± SD.


A
Hours post-transfection
4 h
5 h
6 h
7 h
8 h
GFP- E-cadherin
4 h 5 h 6 h 7 h 8 h Hours (h) post-transfection
kDa
100
Golgin-97
Af1521
macro domain
100
Golgin-97
Inputs
150
E-cadherin
*
**
Normalized Af1521 bound Golgin-97 (%)
120
100
80
60
40
20
0
4 h 5 h 6 h 7 h 8 h
Hours post-transfection


B
40° C
20° C
32° C, 20 min
32° C, 40 min
32° C, 60 min
VSVG
32 °C
40° C
20° C
20 min
40 min
60 min
kDa
100
Af1521
macro domain
100
Inputs
Golgin-97
**
32°C
Normalized Af1521 bound Golgin-97 (%)
120
100
80
60
40
20
0
40° C
20° C
20 min
30 min
60 min

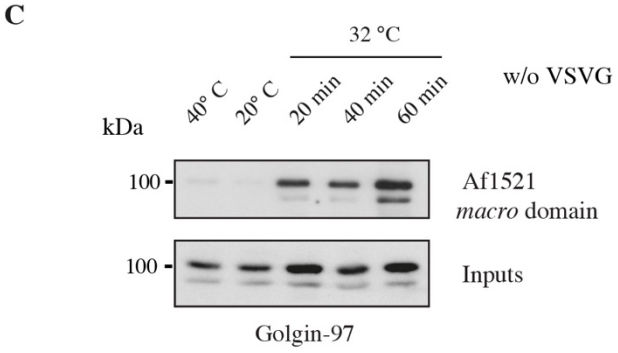

C
32 °C
40° C
20° C
20 min
40 min
60 min
w/o VSVG
kDa
100
Af1521
macro domain
100
Inputs
Golgin-97

Figure S7. Golgin-97 MARylation is specifically triggered during transport

(**A**) Representative confocal images of GFP-E-cadherin localization at different times post-transfection, as indicated. MARylation levels of Golgin-97 during transport were analyzed using Af1521 *macro* domain based pull-down assay of total cell lysates obtained from HeLa cells transfected with GFP-E-cadherin for different times. **(B)** Representative confocal images of VSVG localization during traffic pulse experiments at different times, as indicated. MARylation levels of Golgin-97 during transport were analyzed using Af1521 *macro* domain based pull-down assay of total lysates obtained from cells subjected to a VSVG traffic-pulse. Note that the increase in Golgin-97 MARylation observed after 60 min at 32 °C could be interpreted with the activation of endocytic processes that follows arrival to the PM of an export wave. The resulting endocytic wave could bring a load of PM proteins back into the endosomes down to the recycling endosomes, where these proteins could partially reactivate the MARylation reaction. (**C**) Af1521 *macro* domain based pull-down assay of total cell lysates obtained from HeLa cells incubated at 40 °C, at 20 °C (2 h) or at 32 °C (for the indicated times) in the absence of the cargo VSVG (w/o VSVG). Golgin-97 MARylation was not modulated in the absence of the VSVG synchronized transport. All assays were performed in the presence of cycloheximide 50 µg/mL. Blots are representative of three independent experiments. Quantifications from three different experiments ± SD are reported in the graphs. $^{**}p < 0.01$; calculated by Student's t test.

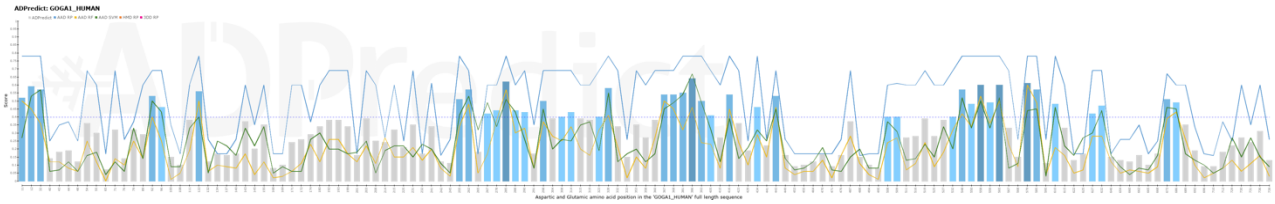


Figure S8. ADP-ribosylation site prediction for Golgin-97

Graphic visualization of predicted ADP-ribosylation sites for Golgin-97, as downloaded from ADPredict web application (www.adpredict.net). Histogram bars report the total score for each acidic residue, the higher the most prone to be modified.

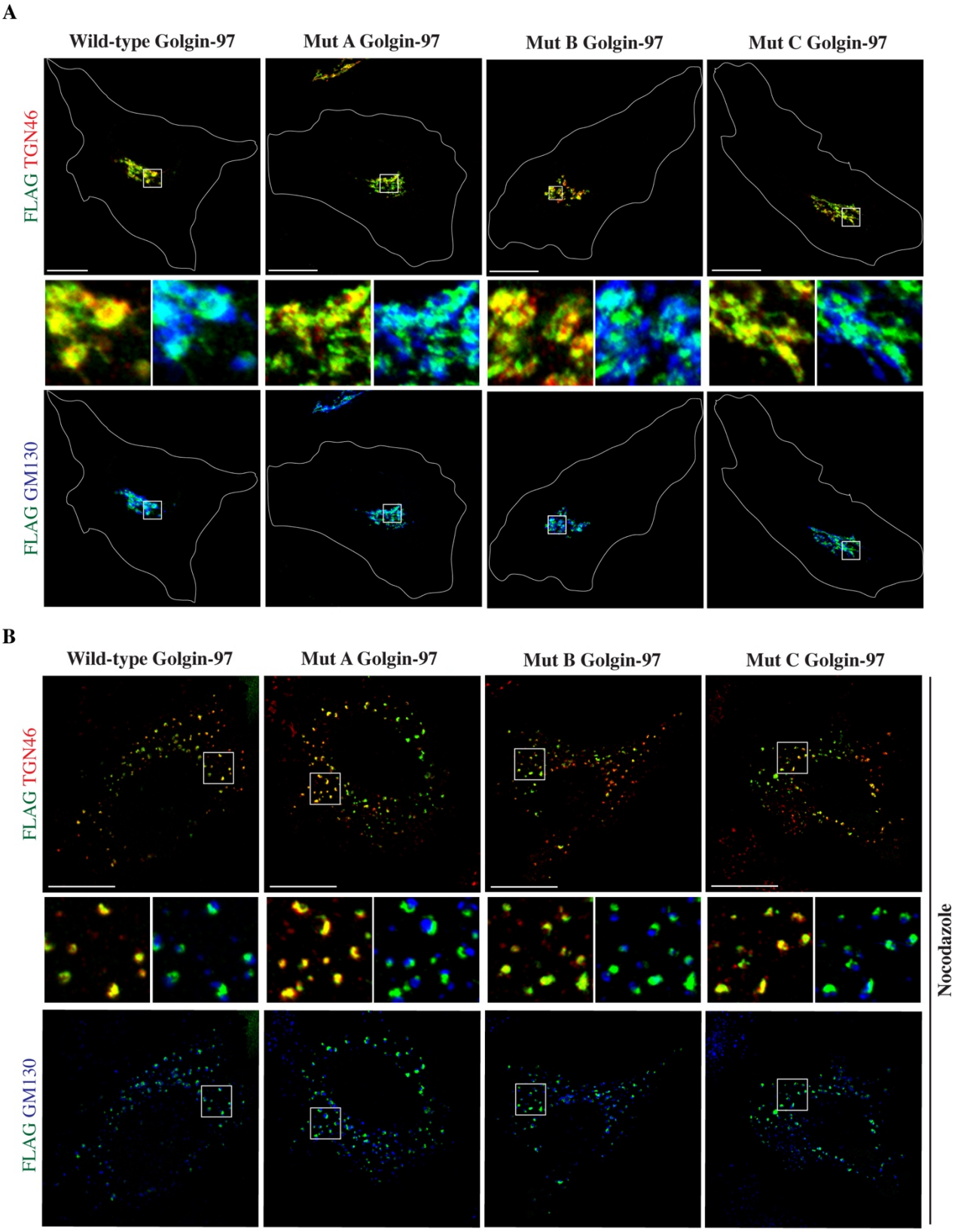

A
Wild-type Golgin-97
Mut A Golgin-97
Mut B Golgin-97
Mut C Golgin-97
FLAG TGN46
FLAG GM130
B
Wild-type Golgin-97
Mut A Golgin-97
Mut B Golgin-97
Mut C Golgin-97
FLAG TGN46
FLAG GM130
Nocodazole

Figure S9. Subcellular localization of Golgin-97 multiple point mutants

HeLa cells were transfected as indicated, and treated with (**A**) vehicle alone or (**B**) nocodazole (33 μM, 3 h); a correct TGN-localization of FLAG-tagged (green) Golgin-97 point mutants and of the wild-type Golgin-97 is shown as colocalization with the TGN marker TGN46, but not with the cis-Golgi marker GM130 (blue). Insets: enlarged view of merged signals at the Golgi membranes. Scale bars, 10 μm. The pictures are representative of three independent experiments.

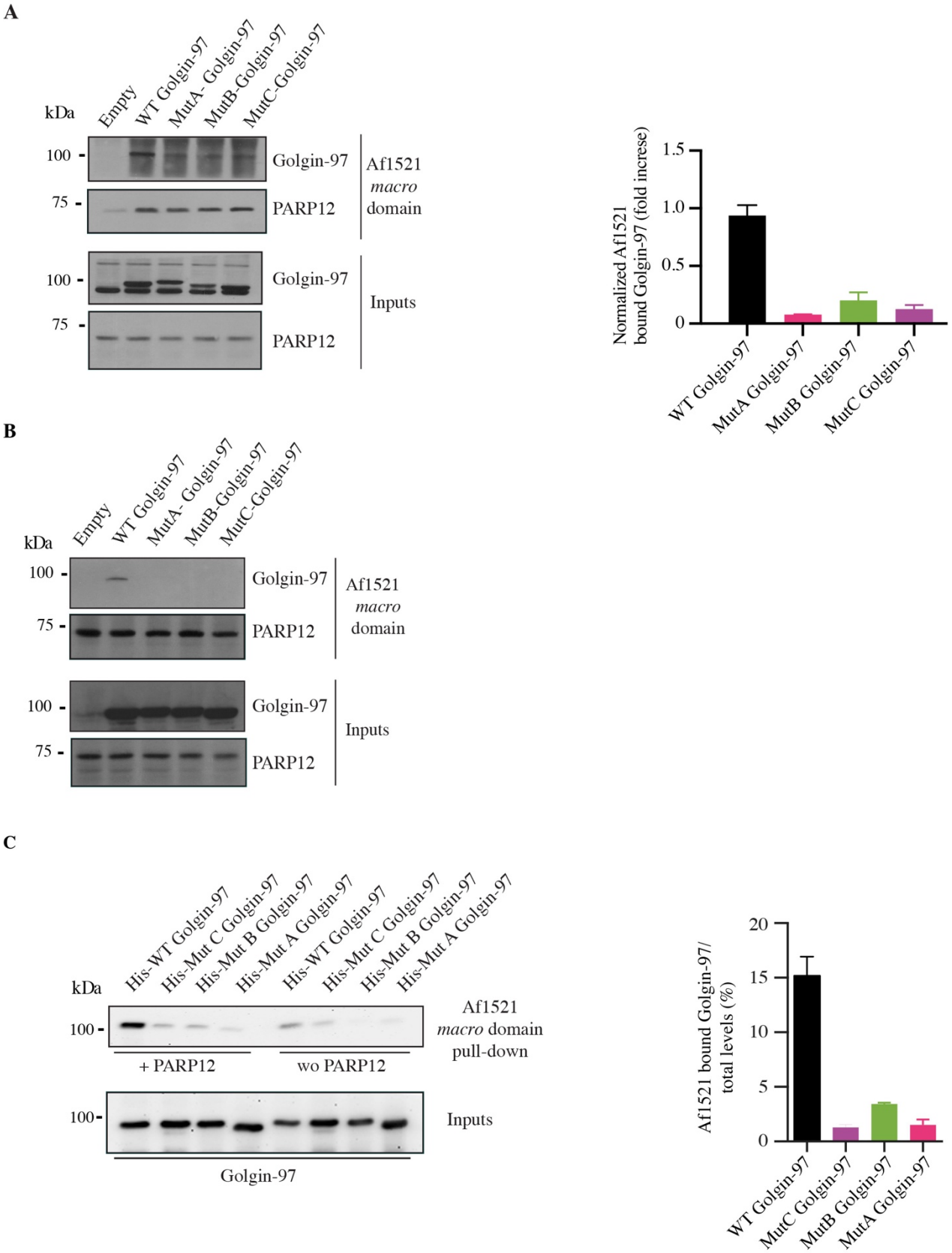
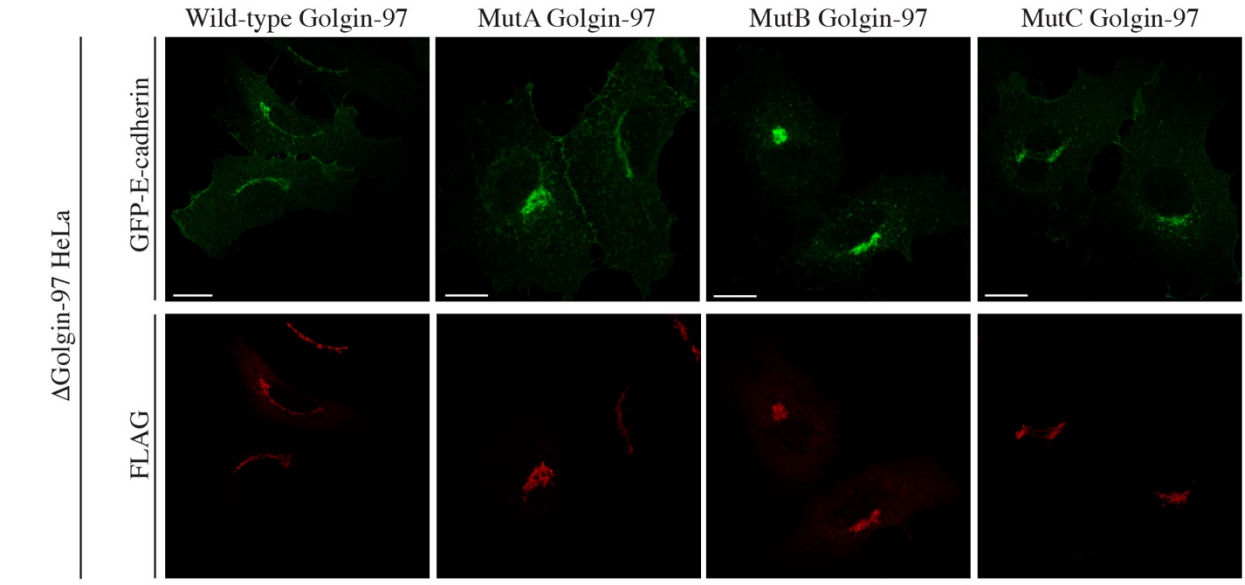

A
kDa
Empty
WT Golgin-97
MutA- Golgin-97
MutB-Golgin-97
MutC-Golgin-97
100
75
Golgin-97
PARP12
Af1521
macro
domain
Inputs
Normalized Af1521
bound Golgin-97 (fold increse)
1.5
1.0
0.5
0
WT Golgin-97
MutA Golgin-97
MutB Golgin-97
MutC Golgin-97
B
C
His-WT Golgin-97
His-Mut C Golgin-97
His-Mut B Golgin-97
His-Mut A Golgin-97
+ PARP12
wo PARP12
Af1521
macro domain
pull-down
Af1521 bound Golgin-97/
total levels (%)
20
15
10
5
0
MutA Golgin-97

Figure S10. Validation of Golgin-97 MARylation-defective mutants

(**A, B**) Af1521 *macro* domain pull-down assay of total cell lysates obtained from (A) wild-type and (B) ΔGolgin-97 HeLa cells transiently transfected with the full-length Golgin-97 or with its multiple point mutants. The bound proteins were eluted and detected by Western blotting (WB) with anti-Golgin-97 antibody. MARylation of PARP12 detected as internal control. (**C**) *In vitro* MARylation assay using GST-tagged purified PARP12 catalytic fragment and His-tagged purified Golgin-97 (wild-type and multiple point mutants) was followed by the Af1521 *macro* domain pull-down assay to recover the pool of MARylated Golgin-97. The bound proteins were eluted and detected by Western blotting with anti-Golgin-97 antibody. Signals deriving from samples incubated with the reaction buffer alone (wo PARP12) were considered as background signals. Lower panel shows input materials. Graphs show quantifications of MARylated Golgin-97.

A



Cell-cell contacts	48 % (SD ± 4,3)	38 % (SD ± 5,2)	6 % (SD ± 1,76)	40 % (SD ± 3)

B

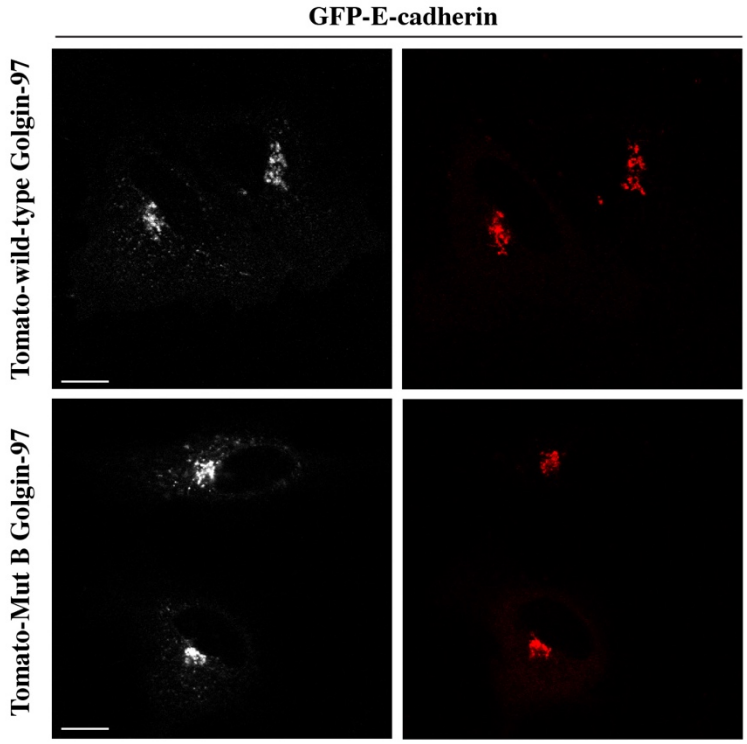


Figure S11. E-cadherin localization in ΔGolgin-97 cells following expression of wild-type Golgin-97 or its ADP-ribosylation defective mutants

(A) Representative confocal images of GFP-E-cadherin (green) localization in ΔGolgin-97 cells showing the rescue of E-cadherin localization and formation of adherens junctions with FLAG-tagged (red) wild-type Golgin-97 or MutA and MutC, but not with MutB. Pictures are representative of three independent experiments. The percentage (%) of cells showing cell-cell contacts in each condition is reported. **(B)** Representative confocal images of Movie 7 and Movie 8 for GFP-tagged E-cadherin (gray) transport (see methods, Live cell imaging section) in ΔGolgin-97 HeLa cells transiently transfected with the Tomato-tagged wild-type Golgin-97 or with its ADP-ribosylation defective mutant B (Mut B, red), as indicated. Scale bars, 10 μm.

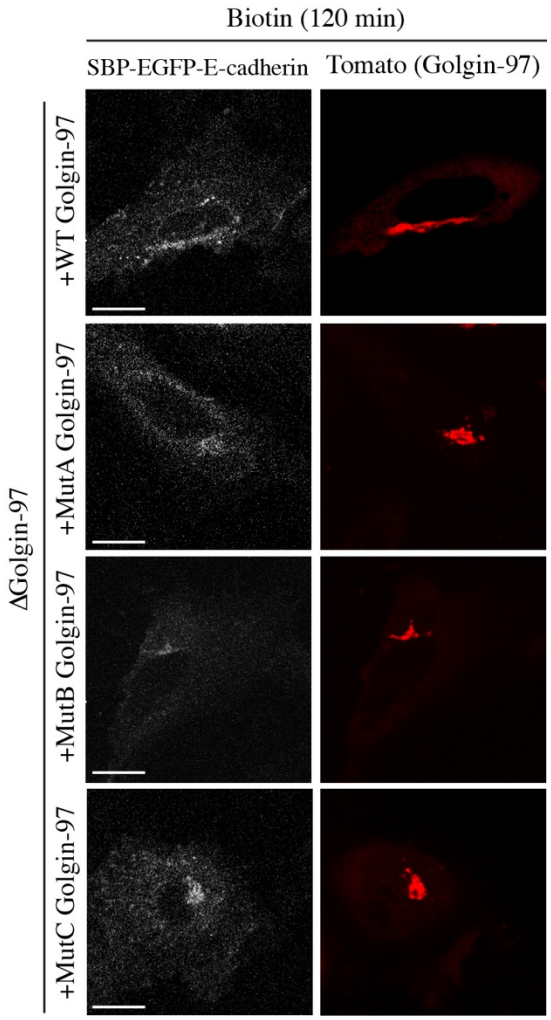


Figure S12. SBP-EGFP-E-cadherin transport in ΔGolgin-97 cells following expression of wild-type Golgin-97 or its ADP-ribosylation defective mutants

ΔGolgin-97 HeLa cells were transiently co-transfected with SBP-EGFP-E-cadherin and tomato tagged wild-type Golgin-97 or its ADP-ribosylation defective mutants. E-cadherin transport was imaged for 2 h upon biotin addition. Reported are frames at 120 min upon biotin addition related to Movies 10-13, showing SBP-EGFP-E-cadherin (gray) and Golgin-97 (red) localization. Scale bars, 10 μm.

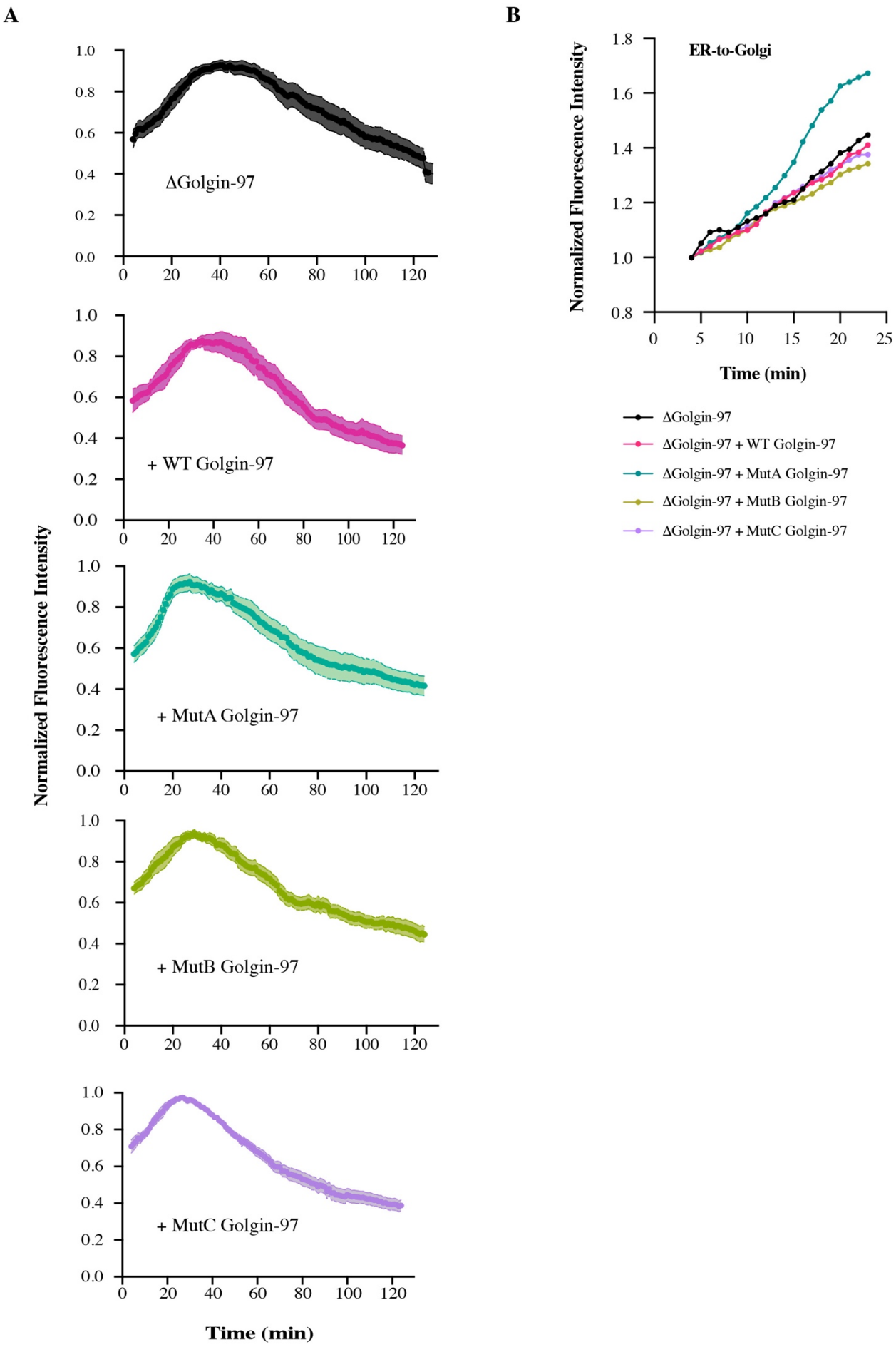

A
B
Normalized Fluorescence Intensity
ΔGolgin-97
+ WT Golgin-97
+ MutA Golgin-97
+ MutB Golgin-97
+ MutC Golgin-97
Time (min)
ER-to-Golgi
ΔGolgin-97
ΔGolgin-97 + WT Golgin-97
ΔGolgin-97 + MutA Golgin-97
ΔGolgin-97 + MutB Golgin-97
ΔGolgin-97 + MutC Golgin-97

Figure S13. Kinetics of SBP-EGFP-E-cadherin transport in ΔGolgin-97 cells following expression of wild-type Golgin-97 or its ADP-ribosylation defective mutants

(A, B) ΔGolgin-97 HeLa cells expressing SBP-EGFP-E-cadherin alone or in combination with tomato-tagged wild-type (WT) Golgin-97 or its ADP-ribosylation defective mutants (MutA, MutB and MutC) were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals, for all the indicated conditions. (A) The plots show averaged (n=9) integrated fluorescence intensity (with SEM) in the Golgi region at each time point, normalized to the maximum value and on the total fluorescence. (B) A detailed analysis of the ER-to-Golgi kinetics is reported.

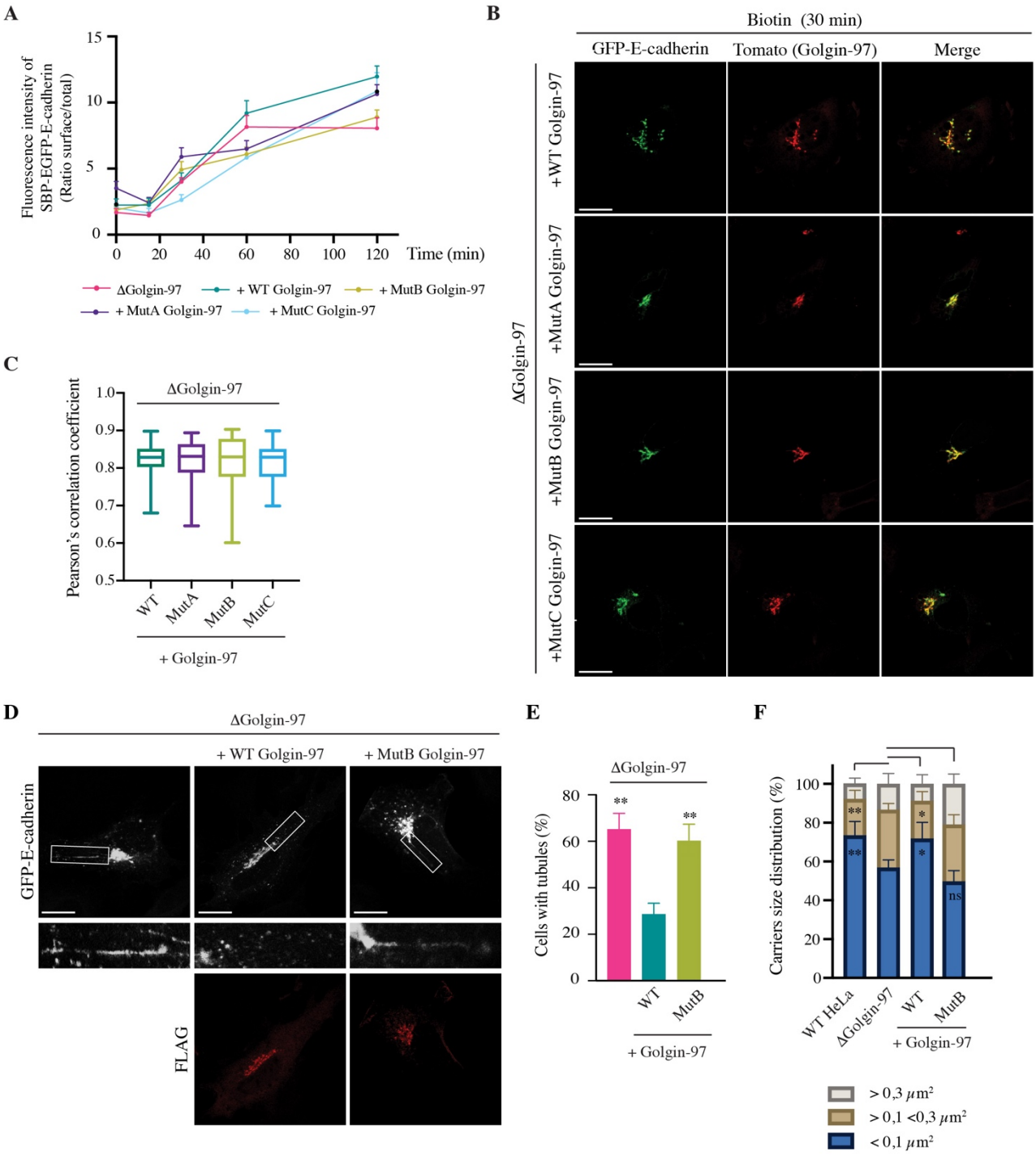


Figure S14. Analysis of Golgin-97 ADP-ribosylation mutants on E-cadherin transport

(A) Quantification of surface/total fluorescence intensities of SBP-EGFP-E-cadherin expressed in ΔGolgin-97 HeLa cells alone or in combination with tomato-tagged wild-type (WT) Golgin-97 or its ADP-ribosylation defective mutants (MutA, MutB and MutC). Upon biotin addition, cells were fixed at the indicated time points and E-cadherin pool at the PM detected by performing surface staining (see methods). Data are mean of n= 30 cells from three different experiments ± SD. **(B, C)** Co-localization analysis of Golgin-97 and E-cadherin in ΔGolgin-97 HeLa cells co-expressing SBP-

EGFP-E-cadherin and tomato-tagged wild-type (WT) Golgin-97 or its ADP-ribosylation defective mutants (MutA, MutB and MutC). Upon 30 min of biotin addition, cells were fixed and processed for immunofluorescence. (B) Representative confocal microscopy images of Golgin-97 (red) and the cargo E-cadherin (green) localization are shown. (C) Relative quantifications of Pearson's correlation coefficient of Golgin-97/SBP-EGFP-E-cadherin are reported in the graph. **(D)** Representative confocal microscopy images of tubulovesicular carriers containing GFP-E-cadherin in ΔGolgin-97 HeLa cells expressing FLAG-tagged (red) wild-type (WT) Golgin-97 or its ADP-ribosylation defective mutant (MutB). Note that longer tubules are formed in the absence of Golgin-97 or when its Mut B is over-expressed. **(E)** Quantification of ΔGolgin-97 cells showing tubules upon expression of WT or MutB Golgin-97. **(F)** Size analysis of transport carriers in wild-type HeLa cells expressing SBP-EGFP-E-cadherin or in ΔGolgin-97 HeLa cells expressing SBP-EGFP-E-cadherin alone or in combination with tomato-tagged wild-type (WT) Golgin-97 or its ADP-ribosylation defective mutant (MutB). Upon 60 min of biotin addition, cells were fixed and processed for immunofluorescence. A minimum of 100 carriers/cell were analyzed using Fiji Software. Data represent mean values from five different experiments ± SD. Statistical significance was calculated by unpaired Student's t-test; $^{**}p < 0.01$; $^{*}< 0.05$.

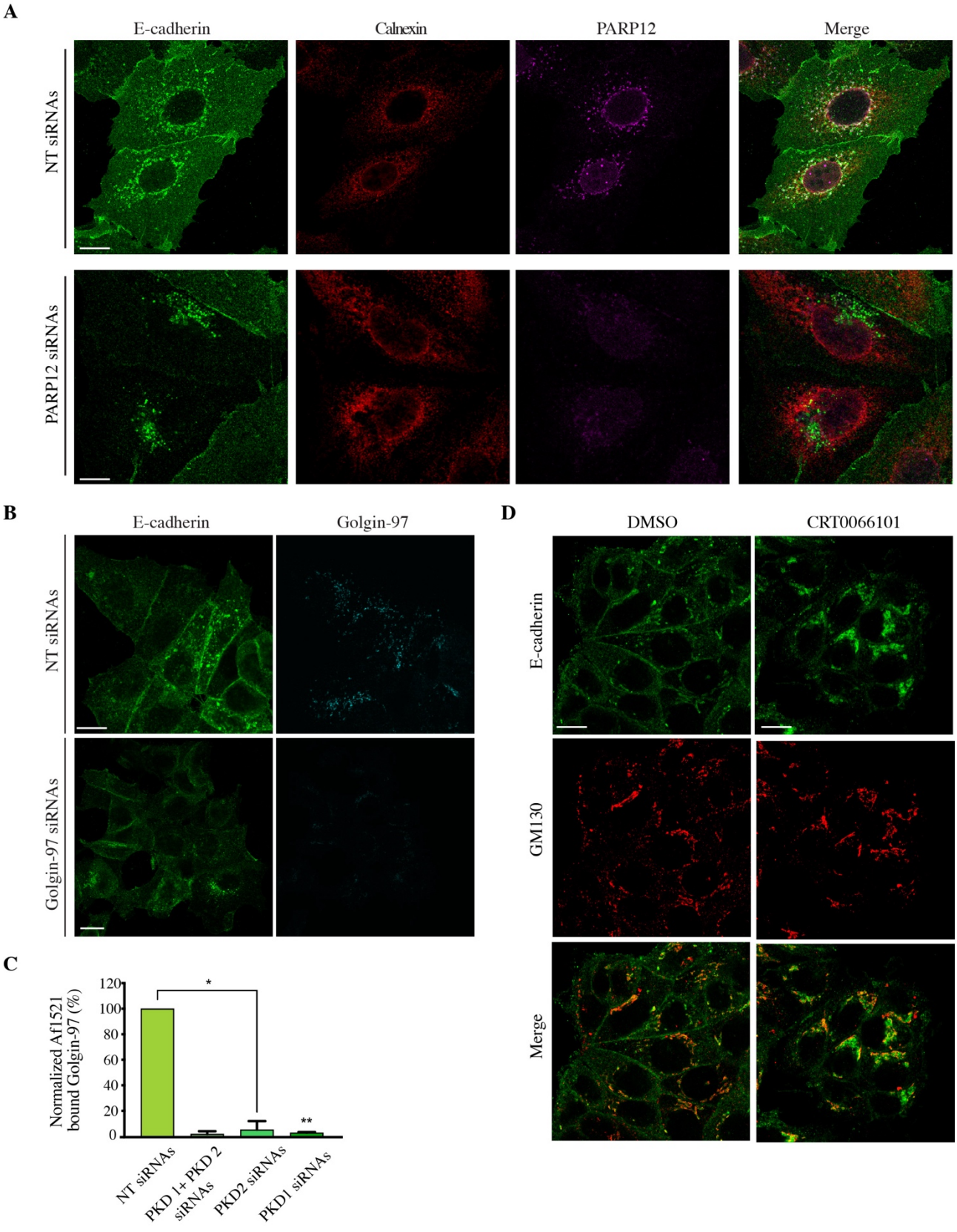

A
E-cadherin
Calnexin
PARP12
Merge
NT siRNAs
PARP12 siRNAs
B
E-cadherin
Golgin-97
NT siRNAs
Golgin-97 siRNAs
C
Normalized Af1521 bound Golgin-97 (%)
120
100
80
60
40
20
0
*
**
NT siRNAs
PKD 1+ PKD 2 siRNAs
PKD2 siRNAs
PKD1 siRNAs
D
DMSO
CRT0066101
E-cadherin
GM130
Merge

Figure S15. PARP12-driven Golgin-97 MARylation and PKD activity regulate the localization of endogenous E-cadherin in MCF7 cells

(A) Representative confocal images of MCF7 cells transiently depleted of PARP12 (PARP12 siRNAs) or not (NT siRNAs) and stained for endogenous E-cadherin (green), calnexin (red, used as ER marker) and PARP12 (magenta), showing absence of co-localization between E-cadherin and the ER compartment. Pictures are representative of three independent experiments. **(B)** Representative confocal images of the decreased endogenous E-cadherin (green) localization at PM in MCF7 cells depleted or not of Golgin-97 (NT siRNAs *vs* Golgin-97 siRNAs). **(C)** Quantification (relative to Figure 6D) of Golgin-97 MARylation in MCF7 cells depleted of PKD1 (PKD1 siRNAs), PKD2 (PKD1 siRNAs) or both (PKD1+PKD2 siRNAs) or not (NT siRNAs) showing the PKD dependence of PARP12-mediated MARylation of Golgin-97. (**D)** Representative confocal images of the decreased endogenous E-cadherin (green) localization at PM MCF7 cells treated with the PKD inhibitor (CRT0066101, 10 μM, 2 h) or vehicle alone (DMSO). Pictures are representative of three independent experiments. Scale bars, 10 μm.

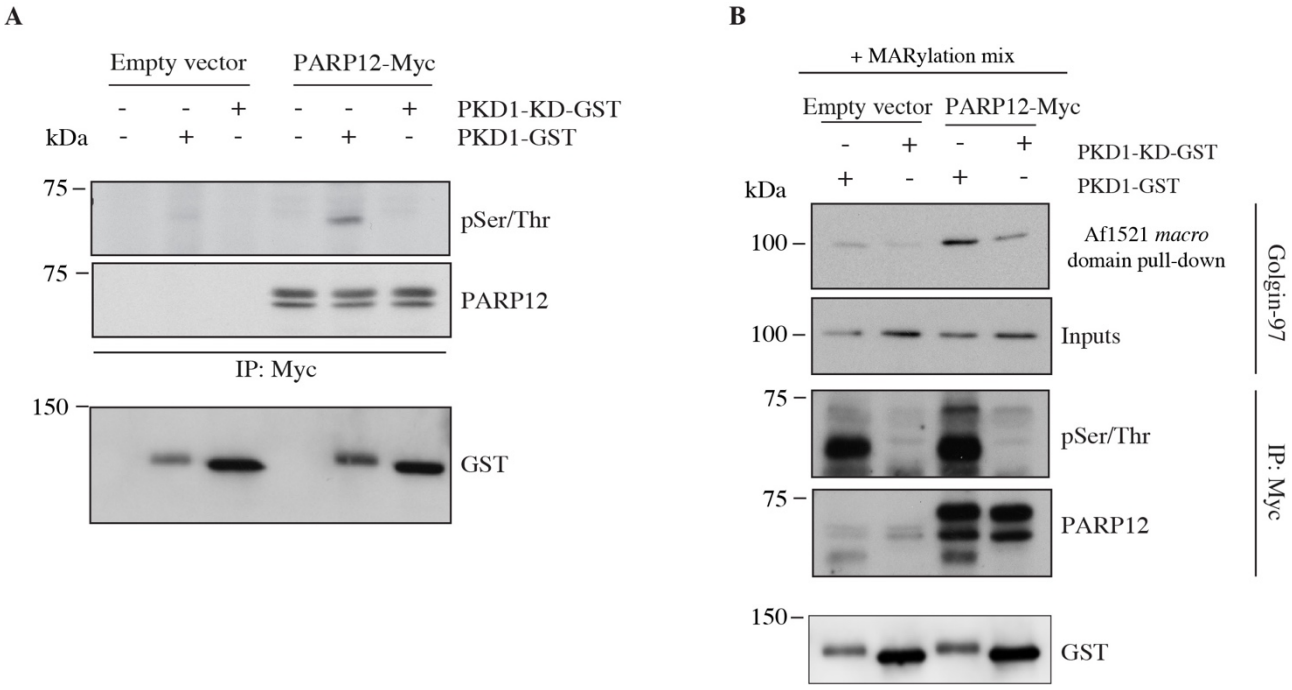


Figure S16. PARP12 catalytic activity is enhanced by PKD phosphorylation

(A) HeLa cells, transiently transfected with Myc-tagged PARP12 vector or its corresponding empty vector, were lysed and incubated with anti-Myc antibody to immunoprecipitate PARP12. Immunoprecipitated PARP12 was subjected to an *in vitro* kinase assay in presence or not of GST-PKD1 or its dead mutant (PKD1-KD-GST). Phosphorylated proteins were detected using an anti phospho-Ser/Thr antibody (see methods). **(B)** *In vitro* MARylation assay using His-tagged purified Golgin-97 and immunoprecipitated Myc-PARP12, previously phosphorylated by PKD1. The MARylation assay was followed by the Af1521 *macro* domain pull-down assay to recover the pool of MARylated Golgin-97. The bound proteins were eluted and detected by Western blotting with anti-Golgin-97 antibody. Phosphorylated immunoprecipitated PARP12 was detected using an anti phospho-Ser/Thr antibody. Input materials for Golgin-97 were also monitored. Total levels of immunoprecipitated PARP12 and PKD1 were monitored by Western Blotting using anti-PARP12 and anti-GST antibodies, respectively.

SI Movie Legends

Movie 1. Transport of SBP-EGFP- E-cadherin in wild-type HeLa cells

HeLa cells expressing SBP-EGFP-E-cadherin (NT siRNAs) were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals.

Movie 2. Transport of SBP-EGFP- E-cadherin in PARP12-depleted HeLa cells

HeLa cells expressing SBP-EGFP-E-cadherin and depleted of PARP12 (PARP12 siRNAs) were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals.

Movies 3-4. Transport of SBP-EGFP- E-cadherin in PJ34-treated HeLa cells

HeLa cells expressing SBP-EGFP-E-cadherin were subjected to a biotin-based traffic pulse, in the absence (Movie 3) or in the presence (Movie 4) of PJ34 (50 μM). Time-lapse confocal images were captured for 2 h at 1 min intervals.

Movie 5. Transport of GFP-E-cadherin in wild-type HeLa cells

Live cell imaging of GFP-E-cadherin transfected wild-type HeLa cells. Three hours after transfection, cells showing GFP-E-cadherin (gray) at the Golgi complex were imaged for the duration of the experiment (2 h). Frames were taken every 3 min.

Movie 6. Transport of GFP-E-cadherin in ΔGolgin-97 HeLa cells

Live cell imaging of GFP-E-cadherin transport in ΔGolgin-97 HeLa cells. Three hours after transfection, cells showing GFP-E-cadherin (gray) at the Golgi complex were imaged for the duration of the experiment (2 h). Frames were taken every 3 min.

Movie 7. Transport of GFP-E-cadherin in ΔGolgin-97 HeLa cells expressing WT Golgin-97

Transport of GFP-E-cadherin in ΔGolgin-97 HeLa cells co-transfected with tomato-WT Golgin-97. Three hours after transfection, cells showing GFP-E-cadherin at the Golgi complex (gray) were imaged for the duration of the experiment (2 h). Frames were taken every 3 min.

Movie 8. Transport of GFP-E-cadherin in ΔGolgin-97 HeLa cells expressing MutB Golgin-97

Transport of GFP-E-cadherin in ΔGolgin-97 HeLa cells co-transfected with tomato-Mut B Golgin-97. Three hours after transfection, cells showing GFP-E-cadherin at the Golgi complex (gray) were imaged for the duration of the experiment (2 h). Frames were taken every 3 min.

Movie 9. Transport of SBP-EGFP- E-cadherin in ΔGolgin-97 HeLa cells

ΔGolgin-97 HeLa cells expressing SBP-EGFP-E-cadherin were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals.

Movie 10. Transport of SBP-EGFP- E-cadherin in ΔGolgin-97 HeLa cells expressing WT Golgin-97

ΔGolgin-97 HeLa cells co-expressing SBP-EGFP-E-cadherin and WT Golgin-97 were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals.

Movie 11. Transport of SBP-EGFP- E-cadherin in ΔGolgin-97 HeLa cells expressing MutA Golgin-97

ΔGolgin-97 HeLa cells co-expressing SBP-EGFP-E-cadherin and MutA Golgin-97 were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals.

Movie 12. Transport of SBP-EGFP- E-cadherin in ΔGolgin-97 HeLa cells expressing MutB Golgin-97

ΔGolgin-97 HeLa cells co-expressing SBP-EGFP-E-cadherin and MutB Golgin-97 were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals.

Movie 13. Transport of SBP-EGFP- E-cadherin in ΔGolgin-97 HeLa cells expressing MutC Golgin-97

ΔGolgin-97 HeLa cells co-expressing SBP-EGFP-E-cadherin and MutC Golgin-97 were subjected to a biotin-based traffic pulse. Time-lapse confocal images were captured for 2 h at 1 min intervals.

Table S1: ADPredict output for human Golgin-97

Position	Sequence	Secondary Structure	PDB ID (CHAIN)	ADPredict	AAD RP	AAD RF	AAD SVM	HMD RP	3DD RP	EXP DATA
11	KKKIAEETAVA			0.52	0.78	0.50	0.27			
12	KKIAEETAVAQ			0.59	0.78	0.45	0.53			
32	RSVSKESVASM			0.57	0.78	0.36	0.57			
40	ASMGADSGDDF			0.14	0.25	0.12	0.06			
43	GADSGDDFASD			0.18	0.35	0.12	0.07			
44	ADSGDDFASDG			0.19	0.37	0.08	0.12			
48	DDFASDGSSSR			0.12	0.25	0.06	0.06			
54	GSSSREDLSSQ			0.36	0.69	0.25	0.16			
55	SSSREDLSSQL			0.30	0.60	0.12	0.18			
65	LLRRNEQIRKL			0.07	0.17	0.00	0.04			
71	QIRKLEARLSD			0.32	0.69	0.14	0.12			
76	EARLSDYAEQV			0.14	0.26	0.10	0.06			
79	LSDYAEQVRNL			0.32	0.37	0.25	0.33			
89	LQKIKEKLEIA			0.29	0.60	0.14	0.14			
92	IKEKLEIALEK			0.53	0.69	0.40	0.50			
96	LEIALEKHQDS			0.46	0.69	0.25	0.43			
100	LEKHQDSSMRK			0.15	0.35	0.01	0.09			
108	MRKFQEQNETF			0.10	0.17	0.05	0.09			
111	FQEQNETFQAN			0.38	0.60	0.19	0.33			
122	RAKMAEGLALA			0.56	0.78	0.50	0.40			
132	ALARKDQEWSE			0.12	0.26	0.06	0.05			
134	ARKDQEWSEKM			0.17	0.17	0.10	0.25			
137	DQEWSEKMDQL			0.16	0.17	0.09	0.22			
140	WSEKMDQLEKE			0.17	0.26	0.08	0.16			
143	KMDQLEKEKNI			0.37	0.60	0.17	0.33			
145	DQLEKEKNILT			0.20	0.35	0.04	0.22			
155	TAQLQEMKNQS			0.35	0.60	0.12	0.34			
168	LFQRRDEMDEL			0.08	0.17	0.02	0.05			
169	FQRRDEMDELE			0.10	0.17	0.03	0.09			
171	RRDEMDELEGF			0.24	0.60	0.06	0.06			
172	RDEMDELEGFQ			0.26	0.60	0.11	0.06			
174	EMDELEGFQQQ			0.29	0.37	0.23	0.26			
180	GFQQQELSKIK			0.34	0.60	0.12	0.30			
192	MLLKKEESLGK			0.38	0.69	0.26	0.20			
193	LLKKEESLGKM			0.38	0.69	0.26	0.20			
199	SLGKMEQELEA			0.34	0.69	0.17	0.17			

201	GKMEQELEART			0.16	0.17	0.12	0.18			
203	MEQELEARTRE			0.39	0.69	0.22	0.25			
208	EARTRELSRTQ			0.25	0.60	0.11	0.05			
214	LSRTQEELMNS			0.24	0.27	0.27	0.19			
215	SRTQEELMNSN			0.32	0.60	0.15	0.22			
225	NQMSSDLSQKL			0.21	0.25	0.15	0.22			
231	LSQKLEELQRH			0.35	0.69	0.21	0.15			
232	SQKLEELQRHY			0.17	0.17	0.13	0.23			
241	HYSTLEEQRDH			0.34	0.61	0.20	0.20			
242	YSTLEEQRDHV			0.12	0.16	0.08	0.11			
245	LEEQRDHVIAS			0.11	0.26	0.03	0.05			
255	SKTGAESKITA			0.51	0.78	0.34	0.41			
262	KITALEQKEQE			0.57	0.69	0.48	0.53			
265	ALEQKEQELQA			0.18	0.17	0.05	0.32			
267	EQKEQELQALI			0.42	0.60	0.17	0.49			
278	QQLSIDLQKVT			0.44	0.60	0.38	0.34			
285	QKVTAETQEKE			0.62	0.78	0.57	0.51			
288	TAETQEKEDVI			0.44	0.60	0.30	0.43			
290	ETQEKEDVITH			0.43	0.69	0.33	0.26			
291	TQEKEDVITHL			0.17	0.35	0.08	0.08			
298	ITHLQEKVASL			0.50	0.69	0.37	0.45			
304	KVASLEKRLEQ			0.39	0.69	0.28	0.20			
308	LEKRLEQNLSG			0.40	0.69	0.25	0.26			
314	QNLSGEEHLQE			0.43	0.69	0.34	0.25			
315	NLSGEEHLQEL			0.39	0.60	0.20	0.35			
319	EEHLQELLKEK			0.38	0.60	0.16	0.37			
323	QELLKEKTLAE			0.40	0.69	0.32	0.19			
328	EKTLAEQNLED			0.58	0.78	0.41	0.55			
332	AEQNLEDTRQQ			0.34	0.69	0.21	0.12			
333	EQNLEDTRQQL			0.15	0.26	0.02	0.17			
353	AINTLETRVRE			0.35	0.69	0.15	0.20			
358	ETRVRELEQTL			0.27	0.60	0.08	0.12			
360	RVRELEQTLQA			0.38	0.69	0.27	0.17			
367	TLQASEEQLQQ			0.54	0.69	0.50	0.45			
368	LQASEEQLQQS			0.54	0.69	0.45	0.49			
381	IVAAQETQIQE			0.55	0.78	0.32	0.54			
386	ETQIQELAAAN			0.64	0.78	0.46	0.67			

393	AAANQESSHVQ			0.50	0.78	0.24	0.49			
405	QALALEQQFLE			0.41	0.69	0.23	0.32			
410	EQQFLERTQAL			0.27	0.60	0.08	0.12			
416	RTQALEAQIVA			0.54	0.78	0.45	0.39			
423	QIVALERTRAA			0.36	0.69	0.24	0.14			
429	RTRAADQTTAE			0.19	0.25	0.10	0.21			
434	DQTTAEQGMRQ			0.46	0.78	0.29	0.32			
441	GMRQLEQENAA			0.22	0.26	0.15	0.25			
443	RQLEQENAALK			0.53	0.69	0.46	0.45			
449	NAALKECRNEY			0.16	0.26	0.08	0.14			
453	KECRNEYERSL			0.09	0.17	0.04	0.07			
455	CRNEYERSLQN			0.10	0.17	0.06	0.08			
464	QNHQFELKKLK			0.12	0.17	0.06	0.12			
470	LKKLKEEWSQR			0.17	0.17	0.13	0.21			
471	KKLKEEWSQRE			0.09	0.17	0.02	0.07			
476	EWSQREIVSVA			0.16	0.26	0.16	0.06			
487	MAQALEEVRKQ			0.37	0.69	0.28	0.15			
488	AQALEEVRKQR			0.15	0.16	0.11	0.20			
494	VRKQREEFQQQ			0.10	0.17	0.04	0.08			
495	RKQREEFQQQA			0.08	0.17	0.01	0.08			
508	LTAIIDEKEQN			0.40	0.60	0.24	0.37			
509	TAIIDEKEQNL			0.40	0.61	0.27	0.31			
511	IIDEKEQNLRE			0.27	0.60	0.07	0.13			
516	EQNLREKTEVL			0.28	0.60	0.11	0.14			
519	LREKTEVLLQK			0.39	0.69	0.24	0.23			
525	VLLQKEQEILQ			0.28	0.60	0.09	0.15			
527	LQKEQEILQLE			0.38	0.60	0.18	0.34			
532	EILQLERGHNS			0.40	0.69	0.32	0.20			
548	HQLQAELEALR			0.57	0.78	0.42	0.52			
550	LQAELEALRTL			0.48	0.78	0.34	0.33			
558	RTLKAEEAAVV			0.60	0.78	0.53	0.50			
559	TLKAEEAAVVA			0.49	0.78	0.36	0.33			
565	AAVVAEQEDLL			0.60	0.78	0.50	0.52			
567	VVAEQEDLLRL			0.33	0.69	0.23	0.08			
568	VAEQEDLLRLR			0.15	0.26	0.09	0.11			
579	GPLQAEALSVN			0.61	0.78	0.60	0.44			
585	ALSVNESHVTS			0.57	0.78	0.49	0.45			

595	SRAMQDPVFQL			0.11	0.26	0.04	0.03			
610	RTPNGEVGAMD			0.48	0.78	0.21	0.46			
615	EVGAMDLTQLQ			0.33	0.60	0.16	0.22			
622	TQLQKEKQDLE			0.13	0.17	0.05	0.16			
625	QKEKQDLEQQL			0.17	0.17	0.07	0.27			
627	EKQDLEQQLLE			0.42	0.69	0.28	0.30			
632	EQQLLEKNKTI			0.47	0.69	0.28	0.44			
646	QQRMLELRKTL			0.15	0.17	0.12	0.16			
654	KTLQKELKIRP			0.13	0.26	0.05	0.09			
660	LKIRPDNELFE			0.12	0.26	0.07	0.04			
662	IRPDNELFEVR			0.16	0.35	0.06	0.08			
665	DNELFEVREKP			0.10	0.17	0.05	0.07			
668	LFEVREKPGPE			0.17	0.25	0.09	0.16			
673	EKPGPEMANMA			0.51	0.67	0.39	0.46			
686	VTNNTDLTDAR			0.49	0.60	0.43	0.45			
689	NTDLTDAREIN			0.35	0.60	0.26	0.17			
692	LTDAREINFEY			0.19	0.34	0.10	0.13			
696	REINFEYLKHV			0.09	0.17	0.02	0.10			
710	FMSCRESEAFH			0.09	0.16	0.05	0.05			
712	SCRESEAFHLI			0.18	0.37	0.08	0.08			
729	LNFSQEEENML			0.22	0.25	0.13	0.27			
730	NFSQEEENMLK			0.27	0.60	0.06	0.15			
731	FSQEEENMLKE			0.24	0.35	0.11	0.27			
736	ENMLKETLEYK			0.31	0.60	0.16	0.15			
739	LKETLEYKMSW			0.13	0.26	0.03	0.09			

Table S1. ADP-ribosylation site prediction for Golgin-97

Predicted values of ADP-ribosylated acidic residues for Golgin-97, as downloaded from ADPredict web application (www.adpredict.net).

Position	Residue	Register (Dimer,Trimer)	Probability Dimer	Probability Trimer	Residue	Register (Dimer,Trimer)	Probability Dimer	Probability Trimer
381	E	e(e,e)	0,999	0,999	Q	e(e,e)	0,999	0,999
386	E	c(c,c)	0,999	0,999	Q	c(c,c)	0,999	0,999
393	E	c(c,c)	0,999	0,999	Q	c(c,c)	0,999	0,999
558	E	g(g,c)	0,977	0,977	Q	g(g,c)	0,975	0,975
559	E	a(a,d)	0,957	0,957	Q	a(a,d)	0,956	0,956
565	E	g(g,c)	0,918	0,918	Q	g(g,c)	0,93	0,93
579	E	g(g,g)	0,68	0,68	Q	g(g,g)	0,573	0,573
585	E	f(f,f)	0,648	0,648	Q	f(f,f)	0,529	0,529
	WT				Mutants			

Table S2. Table S2 resumes the predicted coiled-coil scoring for the indicated Golgin-97 residues, as computed by multicoil2. On the left half (WT), the predicted values of the native glutamic acids are reported in both the dimeric and trimeric possible state of multimerization; Golgin-97 is known to assemble in the dimeric form. On the right side (Mutants), the predicted values for the glutamine residues. Highlighted in red and green are the diminished or augmented values, respectively. As reported, even when the mutant affects negatively the predicted predisposition to coiled-coil fold, it barely lowers the values, suggesting a non-perturbing modification.