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Supplemental Information

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Supplemental Figures and Legends

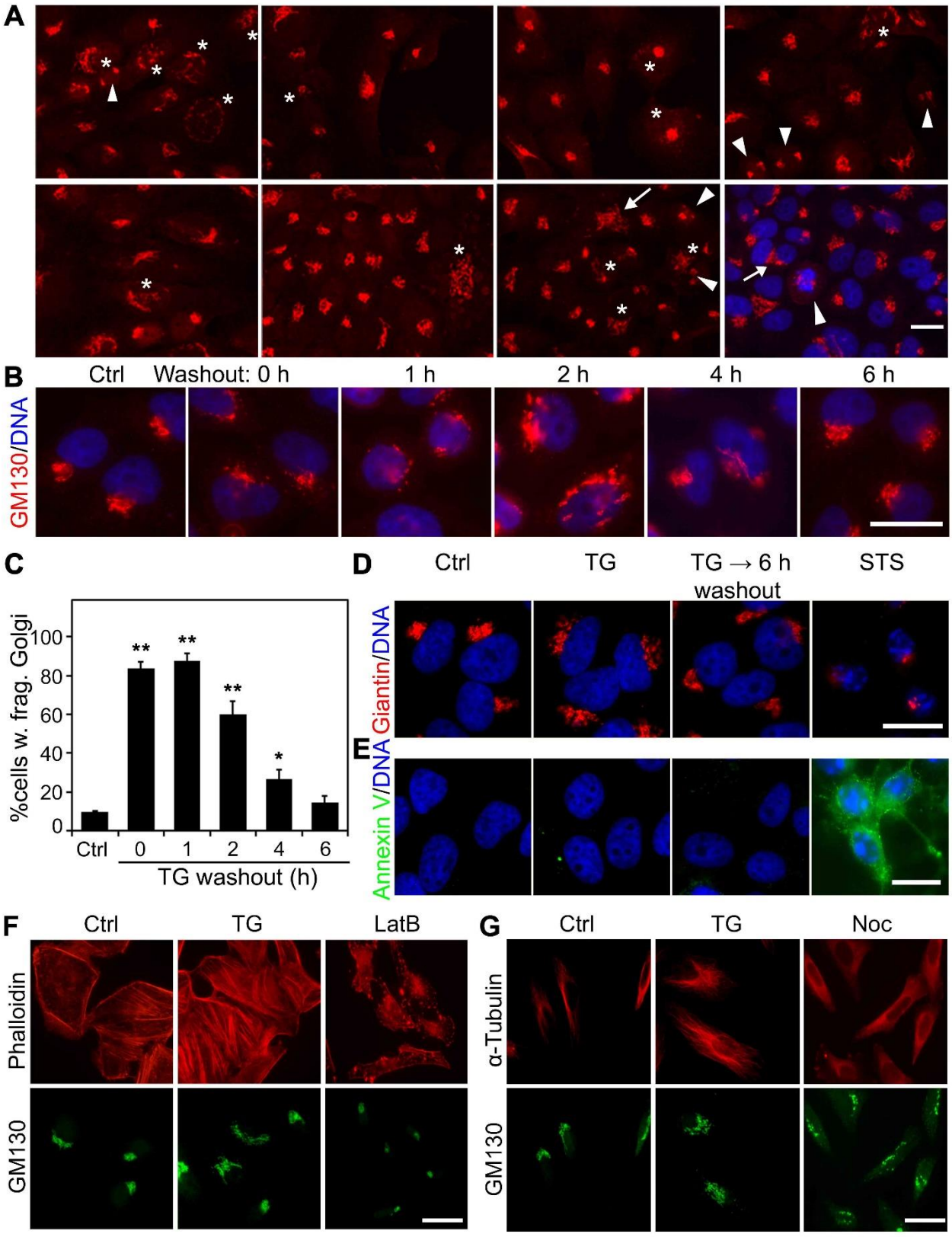

Figure S1. Related to Figure 1. TG-induced Golgi fragmentation is reversible

(A) A gallery of cells with intact or fragmented Golgi. Random images of HeLa cells treated with or without TG were labeled with a GM130 antibody. Asterisks (*) indicate fragmented Golgi, arrowheads

(►) are mitotic Golgi, arrows (→**)** are overlapping Golgi from two or more distinct cells. The last frame includes the DNA channel in blue to show a mitotic cell. **(B)** TG-induced Golgi fragmentation is reversible. Cells were treated with either DMSO or 100 nM TG for 1 h. After washing out TG, TG-treated cells were incubated in fresh growth medium for the indicated times and stained for GM130 and DNA. **(C)** Quantitation of (B) for cells with fragmented Golgi. For statistical analyses, treated cells were compared to the DMSO control (Ctrl). *, $p \leq 0.05$; **, $p \leq 0.01$. **(D)** Acute TG treatment does not cause apoptosis. Cells were treated with either DMSO or 100 nM TG for 20 min without or with 6 h washout, or with 2 μM staurosporine (STS) for 4 h, and stained for GM130 and DNA. **(E)** Cells in (D) were surface stained with Annexin V-EGFP. Scale bars in all fluorescent images, 20 μm. **(F)** Cells were treated with 0.5 μM latrunculin B for 2 h and 250 nM TG was added for the last 20 min. Cells were stained for F-actin with phalloidin (red) and GM130 (green). Scale bar, 20 μm. **(G)** Cells were treated with 1 μM Noc for 2 h and 250 nM TG was added for the last 20 min. Cells were stained for α-tubulin (red) and GM130 (green). Scale bar, 20 μm.

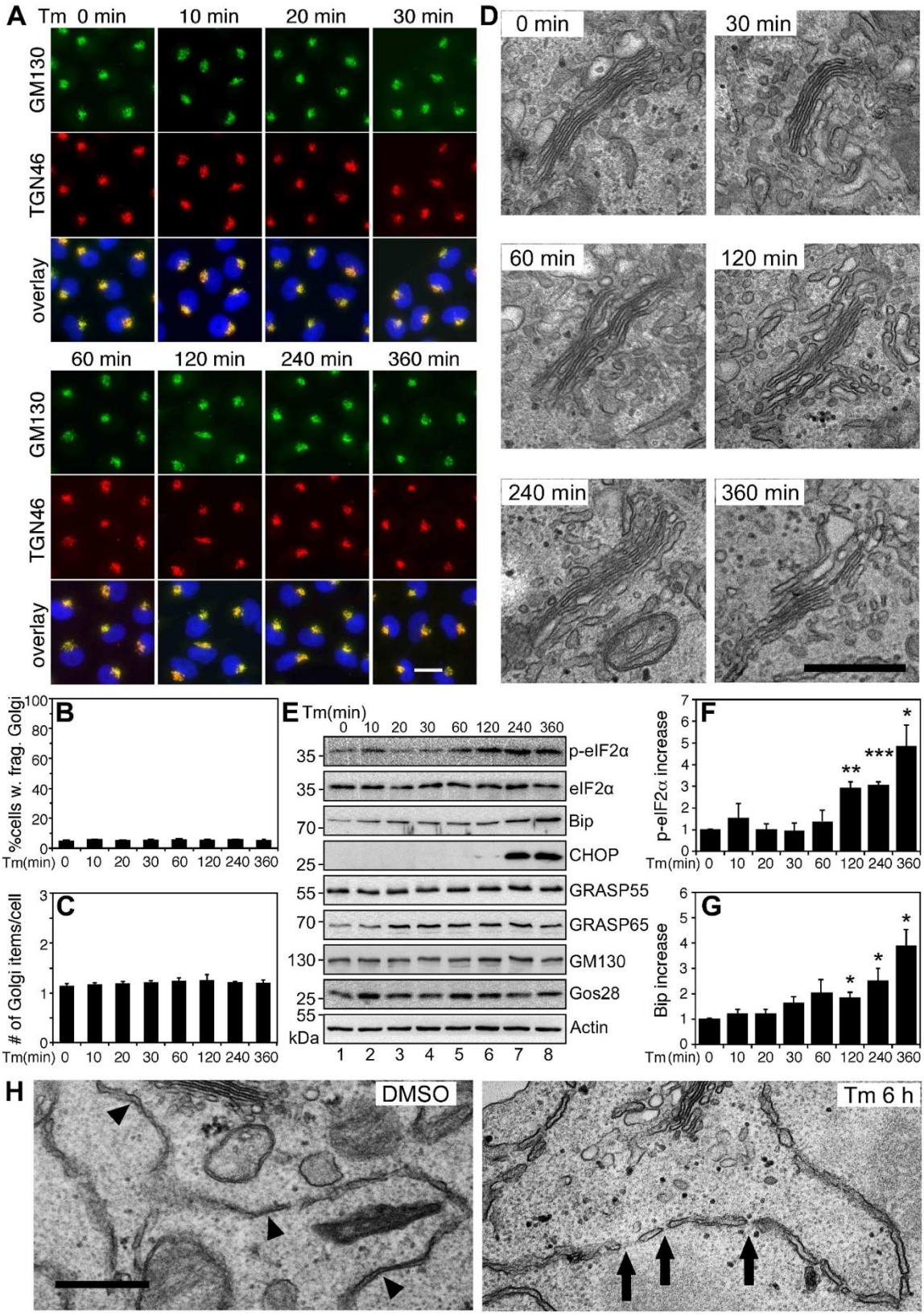

Figure S2. Related to Figure 1. Tm treatment induces UPR but not Golgi fragmentation

(A) Tm treatment has no effect on the Golgi morphology. HeLa cells were treated with 5 μg/ml Tm for indicated times and stained for GM130 and TGN46. Scale bar, 20 μm. **(B-C)** Quantitation of Golgi

fragmentation in Tm-treated cells in (A). **(D)** EM micrographs of the Golgi region in Tm-treated cells. Scale bar, 0.5 µm. **(E)** Tm-treated cells as in (A) were analyzed by Western blots. Note the increased levels of p-eIF2α, Bip and CHOP over time. **(F-G)** Quantitation of p-eIF2α/eIF2α and Bip in (E). Results are shown as Mean ± SEM from at least 3 independent experiments; statistical analyses were performed using two-tailed Student's *t*-tests (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$). **(H)** Representative EM images of ER cisternae in cells treated with DMSO (Ctrl) or tunicamycin (Tm, 5 µg/ml) for 6 h. ER cisternae in Ctrl cells, indicated by arrowheads (►), appear to have a narrow, intact structure; where in Tm treated cells, the ER cisternae appear to be swollen and fragmented, as indicated by arrows (→). Scale bar, 0.5 µm.

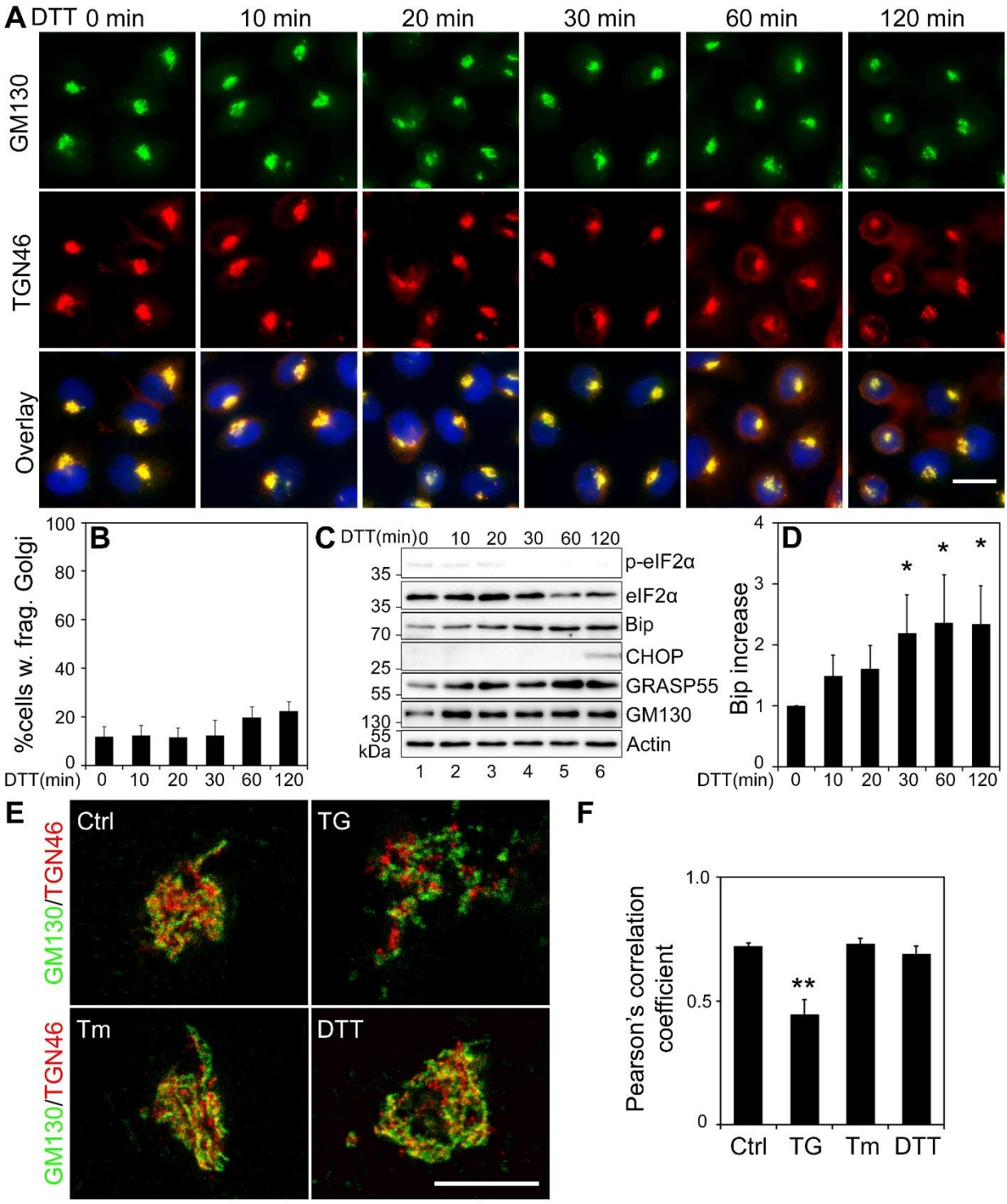

Figure S3. Related to Figure 1. DTT-treatment induces UPR but not Golgi fragmentation

(A) HeLa cells were treated with DMSO or 10 mM DTT for indicated times and stained for GM130 (green), and TGN46 (red). Scale bar, 20 µm. **(B)** Quantitation of (A) for cells with fragmented Golgi. **(C)** Western blots of ER stress and Golgi proteins showing UPR induction upon DTT treatment. **(D)** Quantitation of Bip levels from four independent experiments. Two-tailed Student's *t*-tests were used

to calculate statistical significance (*, $p \leq 0.05$). **(E)** HeLa cells were treated with DMSO, 250 nM TG for 20 min, 5 µM Tm for 6 h, or 10 mM DTT for 2 h. Cells were stained for GM130 (green) and TGN46 (red) and analyzed by super-resolution microscopy. **(F)** Pearson's correlation coefficients of GM130 and TGN46 signals in (E). Two-tailed Student's *t*-tests were used to calculate statistical significance (**, $p \leq 0.01$).

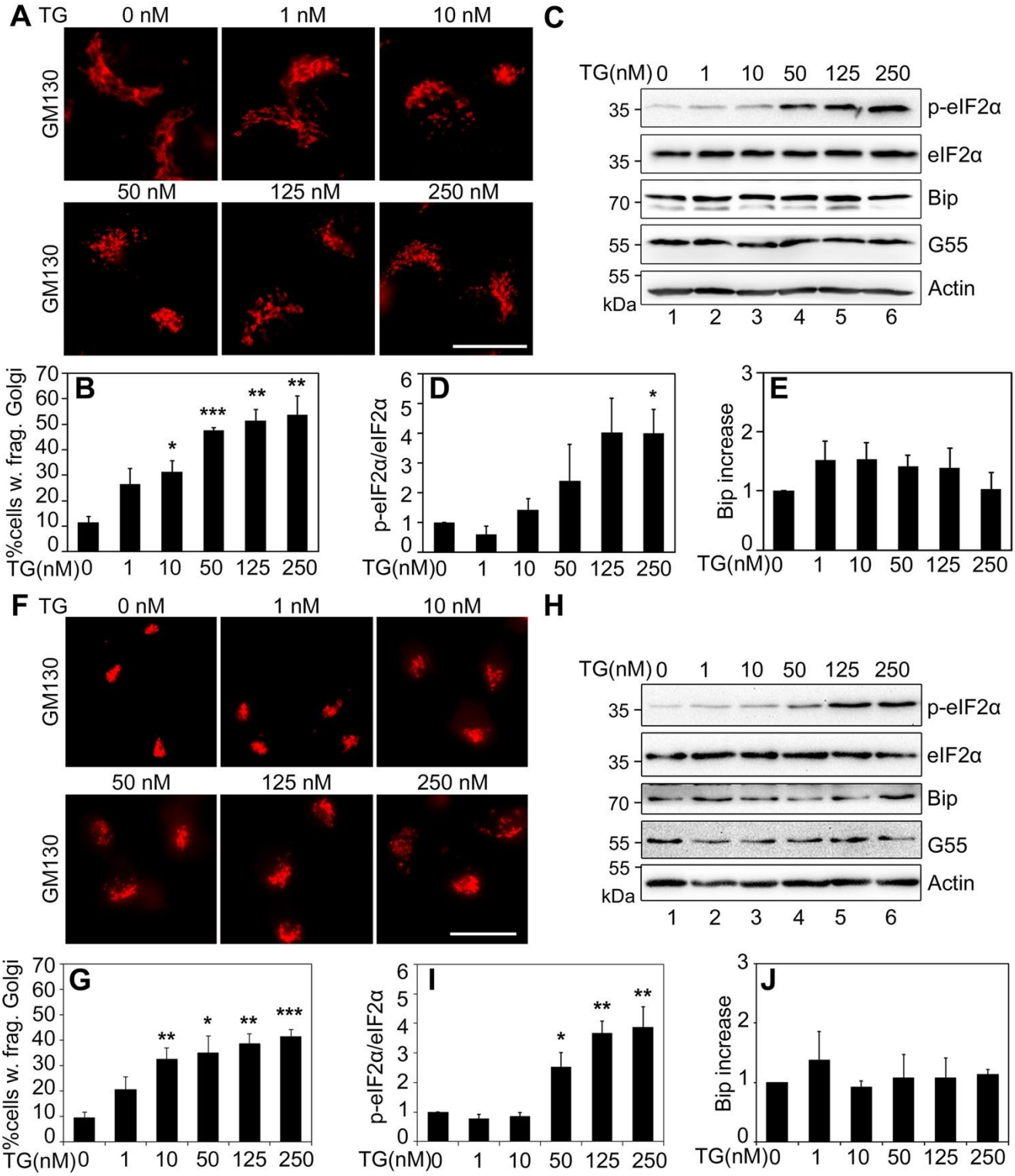

Figure S4. Related to Figure 2. Low concentration of TG induces Golgi fragmentation in NRK cells and RAW 264.7 macrophages

(A) NRK cells were treated with the indicated concentrations of TG for 20 min and stained for GM130. Scale bar, 20 μm. **(B)** Quantitation of cells with fragmented Golgi in (A). **(C)** Cells treated

with TG as in (A) were analyzed by Western blots. **(D-E)** Quantitation of p-eIF2α/eIF2α and Bip in (C) from 3 independent experiments. **(F)** RAW 264.7 murine macrophage cells were treated with the indicated concentrations of TG for 20 min and stained for GM130. Scale bar, 10 μm. **(G)** Quantitation of cells with fragmented Golgi in (F). **(H)** Cells treated with TG as in (F) were analyzed by Western blots. **(I-J)** Quantitation of p-eIF2α/eIF2α and Bip in (H) from 3 independent experiments. Two-tailed Student's *t*-tests were used to calculate statistical significance (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$).

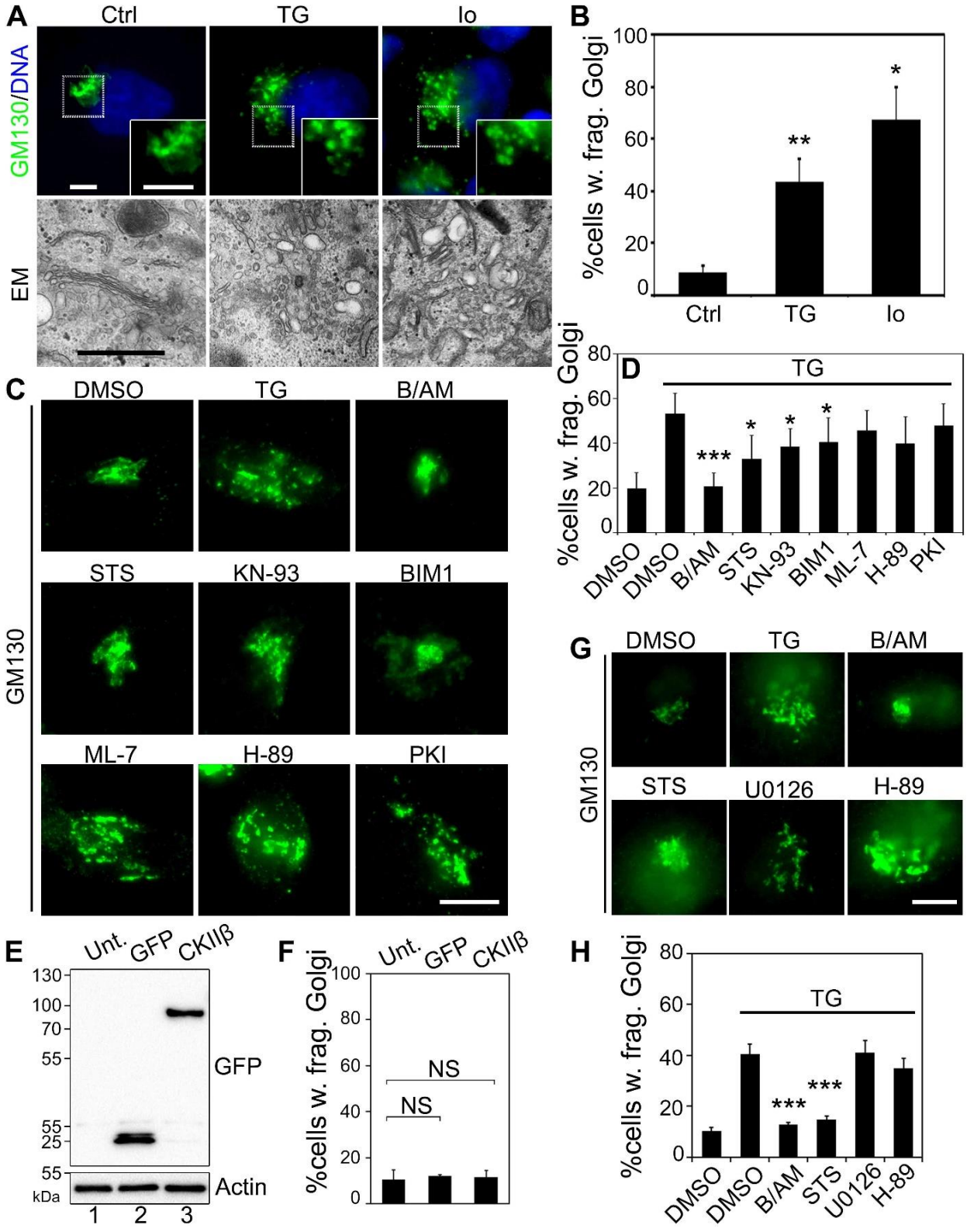

Figure S5. Related to Figure 2-3. Identification of PKC as the kinase for TG-induced Golgi fragmentation

(A) HeLa cells were treated with DMSO (Ctrl), 100 nM TG, or 1 μM ionomycin (Io) for 1 h, and analyzed by fluorescence microscopy (top panels) and EM (lower panels). Boxed regions are enlarged

in the insets. Scale bars on the fluorescence images, 5 µm; scale bar on EM micrographs, 0.5 µm. **(B)** Quantitation of cells with fragmented Golgi in (A) based on the GM130 pattern. **(C)** PKC inhibition reduces TG-induced Golgi fragmentation. Cells were pre-treated with 60 µM BAPTA-AM (B/AM), 2 µM staurosporine (STS), 5 µM KN-93, 2 µM BIM1, 5 µM ML-7, 30 µM H-89, or 10 µM PKI for 10 min, followed by the treatment with either DMSO or 250 nM TG for 20 min, and stained for GM130. Scale bar, 10 µm. **(D)** Quantitation of (C) for Golgi fragmentation. Experiments were quantified in a double-blinded fashion and results from three independent experiments were used to calculate means ± SEM. **(E)** Western blot of cells transfected with GFP or GFP-CAMKIIβ using a GFP antibody. **(F)** Quantitation of cells with fragmented Golgi in cells transfected with GFP or GFP-CAMKIIβ as in (E). For statistics, transfected cells were compared to control untransfected (Unt.) cells. **(G)** HeLa cells were treated with MAPK/ERK or PKA/PKD inhibitors, U0126 and H-89, respectively, followed by 100 nM TG treatment for 20 min. **(H)** Quantitation of Golgi fragmentation in (G). Two-tailed Student's *t*-tests were used to calculate statistical significance (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$).

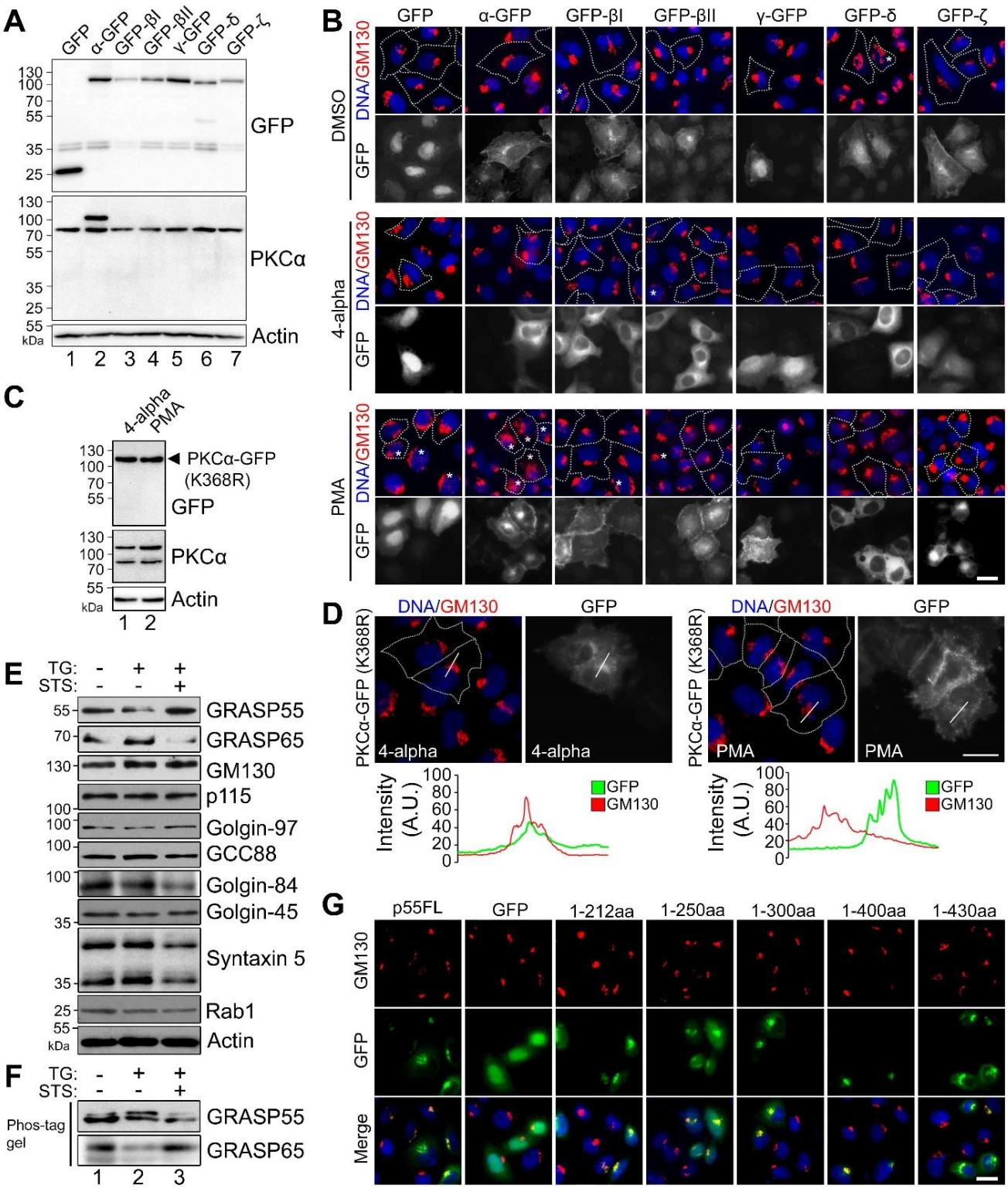

Figure S6. Related to Figure 4-5. PKCα localizes to the Golgi upon activation

(A) Expression of PKC isoforms. HeLa cells were transfected with indicated PKC isoforms and analyzed by Western blot for GFP or PKCα. **(B)** Fluorescent images showing the localization of expressed PKC isoforms after treatment with DMSO, 4-alpha, or PMA. PMA induces PKCα (α-GFP)

localization to the Golgi area, a similar but less dramatic effect was observed for PKCβII (GFP-βII). Scale bar, 20 μm. **(C)** PKCα-GFP (K368R) expressed in HeLa cells and treated with either 4-alpha or PMA and visualized by Western blotting. **(D)** Cells in (C) were probed for GM130 (red) and DNA (blue). To quantify the localization of PKC relative to the Golgi in these images, relative fluorescence intensity was plotted along a random line through the Golgi region. Note the agreement in peaks in control (left side) and relative disagreement in peaks in the PMA stimulated cell (right side). Scale bar, 20 μm. **(E)** TG treatment results in the phosphorylation of GRASP55 but not other Golgi proteins. HeLa cells were treated with 250 nM TG for 1 h, with or without 2 μM staurosporine (STS) pre-treatment for 10 min, and analyzed by Western blot. **(F)** Cell lysates in (E) were analyzed by phos-tag gels and Western blot. **(G)** Immunofluorescence images of cells expressing indicated GRASP55 truncation mutants.

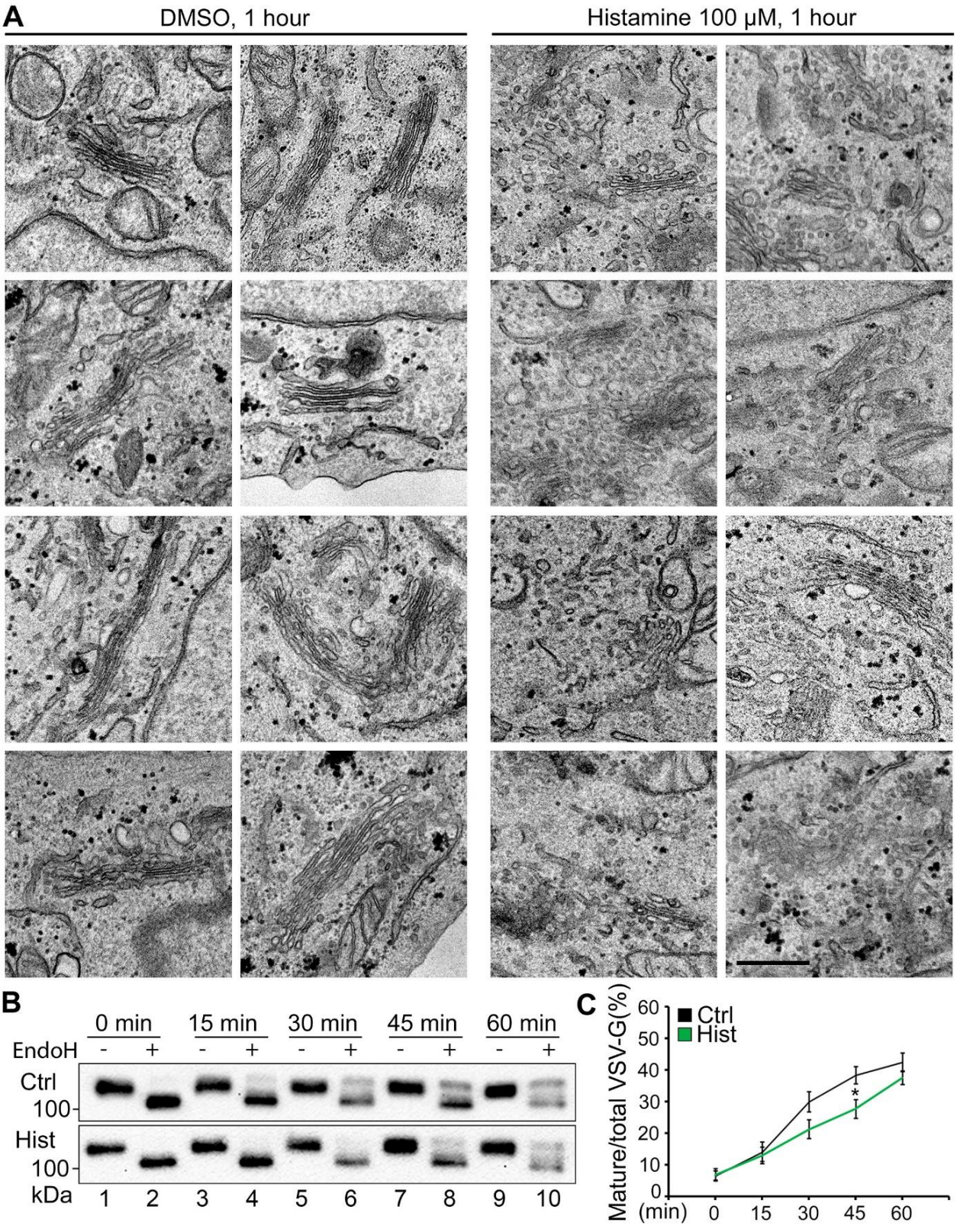

Figure S7. Related to Figure 6. Histamine treatment alters the Golgi structure

(A) HeLa cells treated with either DMSO (control, 1 h) or histamine (100 µM, 1 h) were analyzed by EM. Shown are collections of electron micrographs representing the two treatments. Consistent

aberrations in Golgi shape were frequently seen in histamine-treated cells, including reduced cisternae number and stack length, and increased number of vesicles. Scale bar, 0.5 µm. **(B)** Cells were transfected with the Str-li_VSVG wt-SBP-EGFP plasmid for 16 h and treated without (Ctrl) or with 100 µM histamine (Hist) at 37°C for 1 h. Cells were then incubated with complete medium containing 40 µM biotin and 100 µM of Hist, lysed at the indicated time points, and treated without (-) or with (+) EndoH, and analyzed by Western blot. **(C)** Quantification of (B) for the percentage of EndoH resistant VSV-G from 3 independent experiments. All quantitation results are shown as Mean ± SEM. Statistical analyses were performed using two-tailed Student's *t*-tests (*, $p \leq 0.05$).

TRANSPARENT METHODS

Reagents, Plasmids and siRNA

All reagents used were from Sigma-Aldrich (St. Louis, MO), Roche (Basel, Switzerland) or Calbiochem (EMD Millipore, Burlington, MA), unless otherwise stated. The Annexin V apoptosis detection kit was from BioVision Inc. (San Francisco, CA). PKCα-GFP and GFP-PKCβII plasmids were provided by Dr. Yusuf Hannun (Stony Brook Cancer Center). GFP-PKCβI, GFP-PKCδ, and GFP-PKCζ plasmids were provided by Dr. Hesham El-Shewy (Medical University of South Carolina). The CAMKIIβ plasmid was provided by Dr. Mohammed Akaaboune (University of Michigan). The Str-li_VSVGwt-SBP-EGFP plasmid was provided by Dr. Franck Perez (Institut Curie). PKCγ-GFP cDNA construct was purchased from Addgene (Cambridge, MA). The ManII-GFP HeLa cell line was made in house by transfecting HeLa cells with α-mannosidase II covalently linked to GFP. Control siRNA (Silencer Select Negative Control #1 siRNA) was purchased from Applied Biosystems (ThermoFisher). PKC-specific custom siRNA targeting to endogenous human PKCα (5'-CAGAAGAACTGTATGCAAT-3') was purchased from Ambien (ThermoFisher). To perform knockdowns, 200 nM of each oligo was used to transfect cells for 48 hours.

Antibodies

The following antibodies were used: monoclonal antibodies against β-actin and GFP (Sigma-Aldrich), Gos28 and GM130 (BD Biosciences, Franklin Lanes, NJ), PKCα (Santa Cruz Biotechnology, Dallas, TX), and α-tubulin (Developmental Studies Hybridoma Bank, University of Iowa); polyclonal antibodies against CHOP, p-eIF2α, eIF2α and p115 (Cell Signaling, Danvers, MA), Giantin, GRASP55 and GRASP65 (Proteintech), Bip (Santa Cruz), GM130 ("N73" from Dr. J. Seemann), and TGN46 (Bio-Rad). Secondary antibodies were purchased from Jackson Laboratory (Bar Harbor, ME). Secondary antibodies used for fluorescence microscopy include fluorescence-labelled goat anti-mouse, goat anti-rabbit and goat anti-sheep (for TGN46) antibodies, all used in 1:200 dilution. Secondary antibodies used for Western blot include HRP-conjugated goat anti-mouse and goat anti-rabbit antibodies, all used in 1:5000 dilution.

Molecular Cloning

Constructs for GRASP55 truncation mutants, aa1-212, aa1-250, aa1-300, aa1-400, aa1-430 were constructed in pEGFP-N1 vector using BamHI and HindIII sites (Zhang et al., 2018). Catalytically

inactive PKCα, PKCα-GFP (K368R), was made in-house using site-directed mutagenesis. The genetically encoded calcium indicator GRASP55-GCaMP7 was made in-house by inserting the GCaMP7a gene (kind gift from Dr. Haoxing Xu) into a pmCherry-N1-GRASP55 WT vector (Zhang et al., 2018) using BamH1 and NotI restriction sites. All cDNAs generated in this study were confirmed by DNA sequencing.

Cell Culture and Drug Treatments

For all experiments, mycoplasma-free HeLa were obtained from ATCC (Manassas, VA) and passaged ≤20 times prior to use in experiments. NRK cells were a gift from Dr. Peter Arvan (University of Michigan). HeLa and NRK cells were cultured in Dulbecco's modified Eagle's medium (DMEM; ThermoFisher, Waltham, MA) supplemented with 10% fetal bovine serum (FBS; Gemini Bio-Products, Sacramento, CA) and 100 units/ml penicillin-streptomycin at 37°C with 5% CO_2. RAW 264.7 murine macrophages were kindly provided by Dr. Kezhong Zhang (Wayne State University) and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 100 units/ml penicillin-streptomycin at 37°C with 5% CO_2. Cells were grown on glass coverslips according to standard tissue culture methods (Tang et al., 2012). Coverslips were pre-coated with poly-lysine (Gibco) to aid in cell attachment. For mitotic synchronization, cells were treated with 100 ng/ml nocodazole for 18 h and "shake-off" cells (Xiang et al., 2007) were collected and lysed for Western blot analysis. All drugs, except cAMP-dependent protein kinase inhibitor (PKI) that is a peptide and dissolved in water, were made in DMSO, aliquoted, and stored at -20ºC. Stocks were diluted into working solutions of DMEM at the time of the experiment as described in the text or figure legend. Upon the addition of the drug, cells were incubated at 5% CO_2 and 37°C for the indicated times. Cells were washed 3 times with ice cold phosphate buffered saline (PBS) and collected with a cell scraper.

Immunofluorescence Microscopy

For fluorescence microscopy, cells were rinsed 3 times in ice cold PBS, fixed with 4% (w/v) paraformaldehyde, quenched with 50 mM NH_4Cl, permeabilized in 0.2% v/v Triton X-100 in PBS, and blocked for 1 h with PBS with 1% w/v bovine serum albumin (BSA) Fraction V (ThermoFisher, Waltham, MA) (Tang et al., 2016). Cells were incubated with a primary antibody diluted in 1% BSA in PBS (PBSB) at room temperature for 1.5 h, washed with PBS, and incubated with an FITC- or TRITC-labeled secondary antibody (1:200 dilution) in PBSB for 45 min at room temperature. Cells were washed 3 times with PBS and stained with 1:5,000 Hoechst dye for 5 min, mounted on glass slides with Moviol, and images were captured with a Zeiss (Oberkochen, Germany) Observer

fluorescent microscope with a 63x oil objective lens with a numerical aperture of 1.4 and an Axiocam Mrm camera. TIF files were exported with AxioVision software (Zeiss).

For super-resolution microscopy, Alexa Fluor 647, and Alexa Fluor 488-labeled secondary antibodies (ThermoFisher) were used. After washing, coverslips were mounted to slides using ProLong Diamond antifade super-resolution imaging mountant (ThermoFisher). Super-resolution images were imaged using Leica (Wetzlar, Germany) TCS SP8 STED super-resolution microscope. Images were quantified using the NIH ImageJ software and assembled into figures with Photoshop Elements (Adobe, San Jose, CA). To clearly show the Golgi structure, brightness or contrast was adjusted linearly across all samples within each experiment.

For calcium imaging, GRASP55-GCaMP7 transfected cells were plated onto glass bottomed dishes and imaged by a Nikon C2-plus Laser Scanning Confocal Microscope System configured with a Ti2-E inverted microscope. Images were captured at 488 nm and 561 nm in sequential scanning mode. Z-stacks of 5 slices at 1 μm interval were acquired every 30 seconds for a total period of 10 min. The NIS-Elements C software was used for acquisition, analysis and visualization. The "+Histamine" symbol in Movie S1 was added in Adobe Premiere Pro 2020. For quantification, fluorescence intensity was measured every minute for 60 min. 20 cells were measured for each drug treatment.

To quantify Golgi fragmentation, cells were evaluated by eye under a microscope according to predefined fragmentation criteria, at least 300 cells were counted in each reaction. The following criteria are used to define whether a Golgi is intact or fragmented: 1) If the Golgi exists as a single piece of connected membrane, it is intact. 2) If a Golgi exhibits several items that are connected by visible membrane bridges, even though these bridges might be faint, the Golgi is considered intact. 3) If a Golgi exhibits ≥ 3 disconnected pieces (no visible bridges connecting them), then the Golgi is fragmented. 4) Mitotic cells, defined by the DNA pattern, and overlapping cells in which the Golgi pattern is difficult to define, are not counted. Hoechst was used to identify individual, mitotic and overlapping cells. In experiments where transfected proteins were employed, only transfected cells were counted, and 100 cells were counted per replicate. In experiments where an inhibitor screen was performed, an unbiased image thresholding method was used to extract fragmentation data from ≥40 cells per replicate.

Electron Microscopy (EM)

For EM, cells were fixed in pre-warmed serum-free DMEM, 20 mM Hepes, pH 7.4, 2% glutaraldehyde at room temperature for 30 min or 4°C overnight as previously described (Tang et al., 2010). Cells were washed 2 times with 0.1 M Sodium cacodylate (Electron Microscopy Sciences, Hatfield, PA), and post-fixed on ice in 1% v/v reduced Osmium tetroxide, 0.1 M Sodium cacodylate (w/v) and 1.5% cyanoferrate (w/v) in water. Cells were rinsed 3 times with 50 mM maleate buffer, pH 5.2, 3 times with water, scraped, and pelleted in microcentrifuge tubes for embedding. The EMBED 812 (EMD) protocol was used to embed cells and resin blocks were sectioned to 60 nm with a diamond knife and mounted on Formvar-coated copper grids. Samples were double contrasted with 2% uranyl acetate then with lead citrate and rinsed with copious amounts of water. Grids were imaged using a Philips (Amsterdam, Netherlands) transmission electron microscope. Golgi images were captured at 11,000x magnification. Golgi stacks were identified using morphological criteria and quantified using standard stereological techniques. A Golgi cisterna was identified as a perinuclear membrane within a Golgi stack ≥4 times longer than its width. Stack length was measured for the longest cisterna within a Golgi stack using the ruler tool in Photoshop Elements 13. For the number of cisternae per stack, the number of cisternae was counted. For the number of vesicles per stack, round objects no greater than 80 µm in diameter within 0.5 µm of a Golgi stack were counted. At least 20 cells were quantified in each experiment, and the EM results represent two independent experiments.

Protein Biochemistry

For immunoblotting, cells from a culture dish were pelleted and lysed with 30 µl lysis buffer (40 mM Hepes, pH 7.4, 200 mM KCl, 5 mM $MgCl_2$, 1% Triton X-100 (Bio-Rad, Hercules, CA), 50 mM beta-glycerol phosphate, protease inhibitor cocktail (Roche), and phosphatase inhibitors NaF and NaVan pH 8.0). Samples were mixed with 6X SDS-PAGE sample buffer (400 mM Tris-Cl pH 6.8, 15% SDS, 10 mM DTT, 50% glycerol, 0.05% bromophenol blue), denatured at 95°C for 4 min and then run on PAGE gels. For figures 5, subpanels A, C and D, 8% phos-tag gels were run at 4°C for 8 h. Protein was transferred to nitrocellulose membranes using semi-dry transfer (Bio-Rad, Hercules, CA) at constant 16 V. Membranes were blocked for 10 min with 3% milk in 0.2% Tween-20 in phosphate buffered saline (PBST) and immunoblotted. Western blots were captured with Enhanced Chemiluminescence (ECL) dye reagent (ThermoFisher), in a FluorChem M chemi-luminescent imager (ProteinSimple, San Jose, CA).

VSV-G Trafficking using RUSH system

VSV-G trafficking was performed as previously described (Li et al., 2019). Briefly, HeLa cells were transfected with the Str-li_VSVG wt-SBP-EGFP plasmid (Boncompain et al., 2012) and cultured at 37°C for 16 h. Cells were then incubated with 250 nM TG or 10 µM monensin in fresh medium for 0.5 h at 37°C before 40 µM D-biotin (VWR Life Science, Radnor, PA) was added. Cells were then lysed at indicated time points (chase), treated with or without EndoH, and analyzed by Western blotting for VSV-G-GFP using a GFP antibody. The percentage of EndoH resistant VSV-G was quantified using the ImageJ software.

In vitro Kinase Assay

Twenty µg/ml recombinant GRASP55 protein (Xiang and Wang, 2010) was incubated with 10 µg/ml recombinant PKCα (SignalChem, British Columbia, Canada) in the presence or absence of 2 mM ATP. Reactions were performed in kinase buffer (20 mM HEPES-NaOH, pH 7.4, 1 mM $CaCl_2$, 1 mM DTT, 10 mM $MgCl_2$, 200 µg/ml phosphatidylserine, 20 µg/ml diacylglycerol) at 30°C for 3 h. Reactions were terminated by adding SDS sample buffer and boiling. GRASP55 proteins were separated by Phos-tag SDS-PAGE and visualized by immunoblotting. In brief, 50 µM Phos-tag acrylamide and 100 µM $MnCl_2$ were included in the gel recipe according to the manufacturer's instructions. Phos-tag gels were washed three times in transfer buffer supplemented with 10 mM EDTA and twice in transfer buffer without EDTA before transferring to membranes. Proteins were visualized by Western blotting.

Quantitation and Statistics

All data represent the mean ± SEM (standard error of the mean) of at least three independent experiments unless noted. A statistical analysis was conducted with two-tailed Student's t-test in the Excel program (Microsoft, Redmond, WA). Differences in means were considered statistically significant at $p \leq 0.05$. Significance levels are: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Figures were assembled with Photoshop (Adobe, San Jose, CA). Pearson's colocalization coefficient values were computed using the "Coloc 2" function in ImageJ software.

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