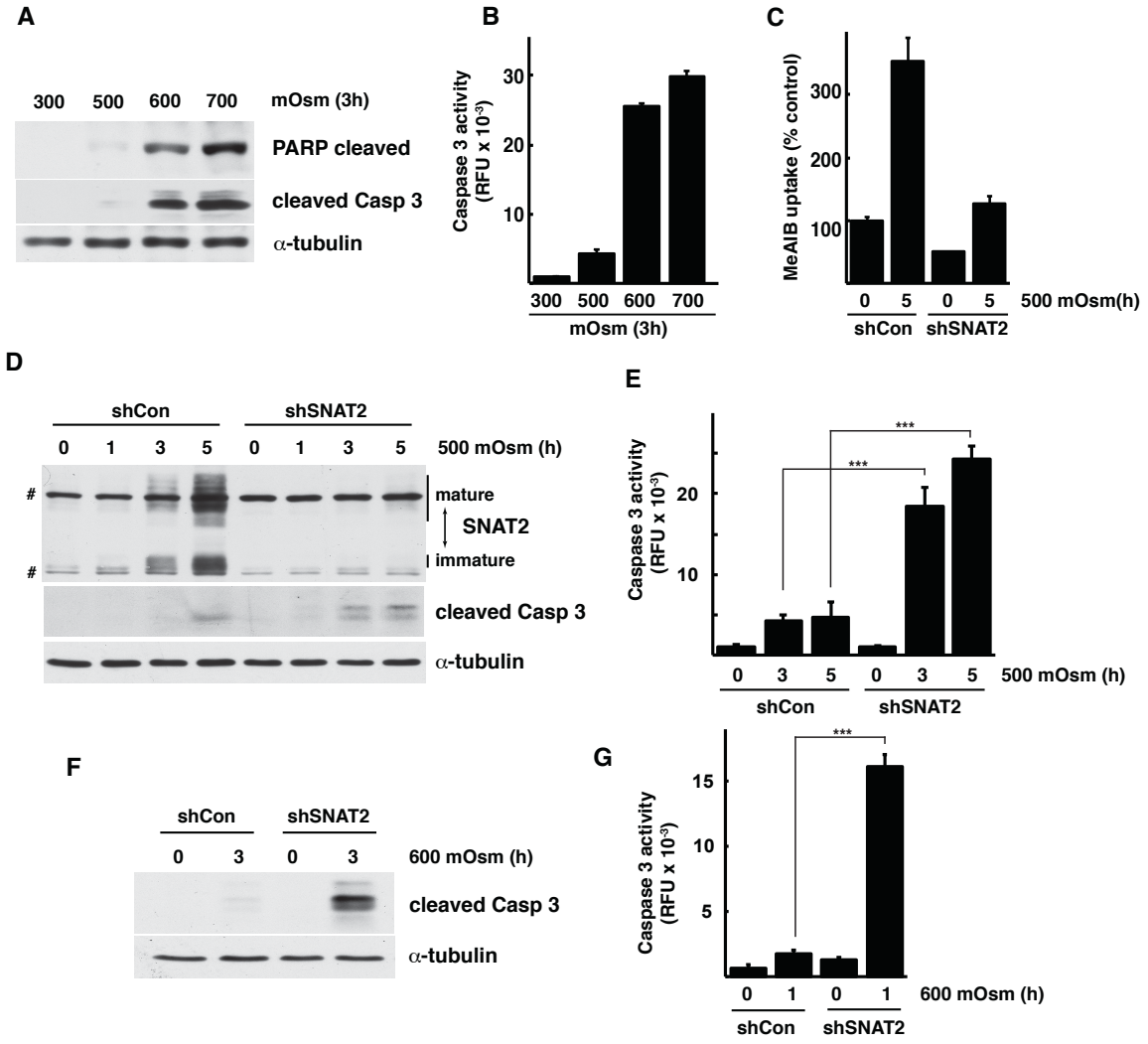


Fig. S1. Induction of SNAT2 inhibits apoptosis in corneal epithelial cells exposed to hyperosmotic stress. **Related to Figure 1.** A. Western blot analysis of extracts from cells treated with media of the indicated osmolarity. B. Caspase 3 activity in extracts of cells treated with the indicated media. C. SNAT2 activity, measured by ^{14}C-MeAIB uptake in cells expressing shRNA against SNAT2 exposed to hyperosmotic media. D. Western blot analysis of extracts from cells expressing shRNA against SNAT2 treated with hyperosmotic media (500 mOsm) for the indicated times. A nonspecific band on the immunoblot is indicated (#). E. Caspase 3 activity in extracts of cells expressing shRNA against SNAT2 treated with hyperosmotic media. F. Western blot analysis of extracts from cells expressing shRNA against SNAT2 treated with hyperosmotic media (600 mOsm) for 3h. G. Caspase 3 activity in extracts from cells expressing shRNA against SNAT2 treated with hyperosmotic media (600 mOsm) for the indicated times. Data in panels B,C E and G are represented as mean of 3 independent experiments ± SD.

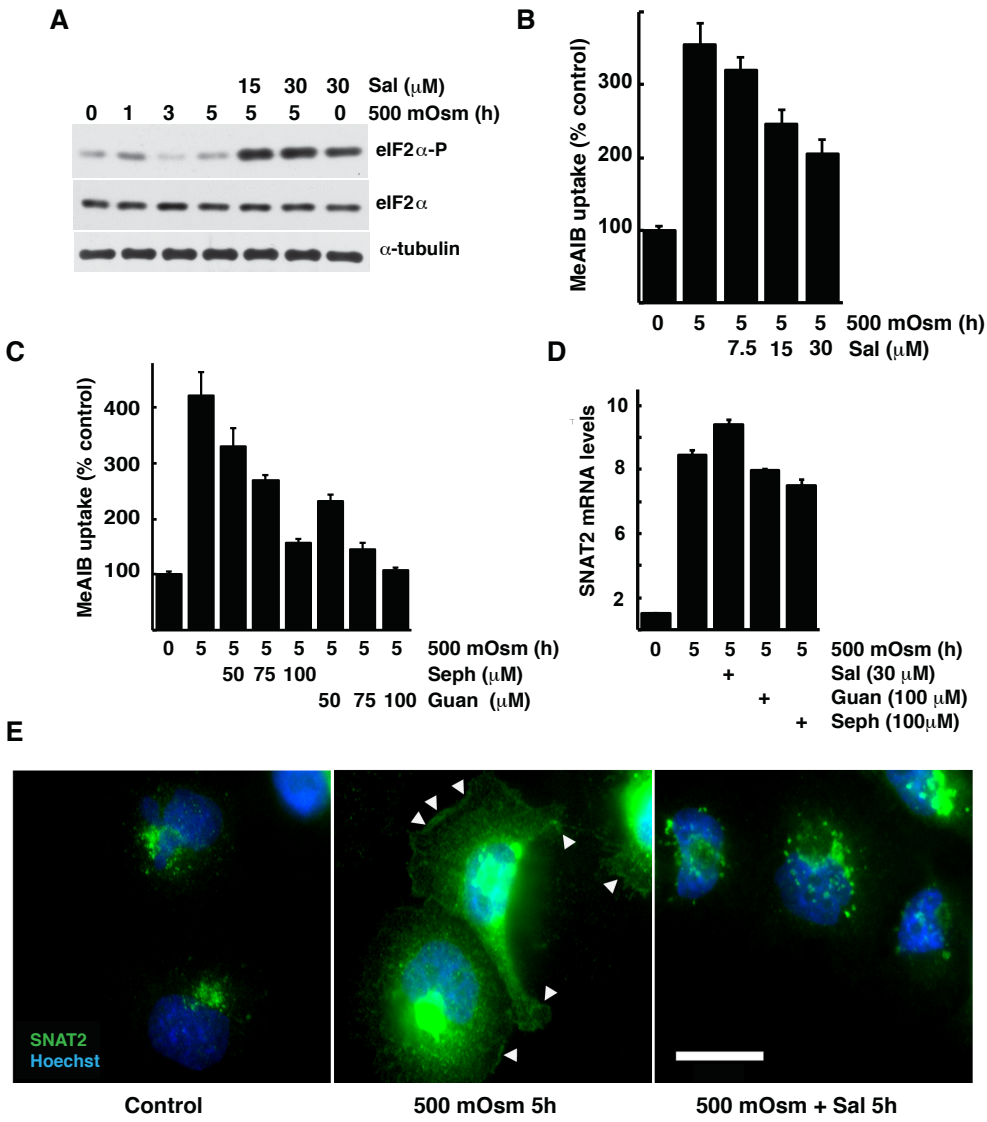


Fig. S2. Inhibition of GADD34/PP1 phosphatase activity during hyperosmotic stress in human corneal epithelial cells decreases SNAT2 activity not mRNA levels. Related to Figure 1. A. Western blot analysis of extracts from cells treated with 500 mOsm media in the presence of indicated concentration of Sal003. B. SNAT2 activity, measured by ^{14}C-MeAIB uptake in cells exposed to 500 mOsm media for 5h with the indicated concentrations of Sal003. C. SNAT2 activity, measured by ^{14}C-MeAIB uptake by cells treated with 500 mOsm media for 5h with the indicated concentrations of Guanabenz (Guan) or Sephin 1 (Seph). D. RT-qPCR analysis of SNAT2 mRNA levels from cells treated with 500 mOsm media for 5h in the presence of GADD34 inhibitors, Sal003 (30 μM), Guanabenz (Guan, 100 μM) or Sephin 1 (Seph, 100 μM). Values were normalized to GAPDH mRNA and plotted as a fold of induction over control. E. Fluorescence microscopy of cells treated with hyperosmotic media (500 mOsm) for 5h with or without Sal003 (Sal, 25 μM). Nuclei are stained with Hoechst. Cellular distribution of SNAT2 (green channel), also includes its PM localization, which is indicated by white arrowheads. Scale bar is 10 μm. Data in panels B-D are represented as mean of 3 independent experiments ± SD.

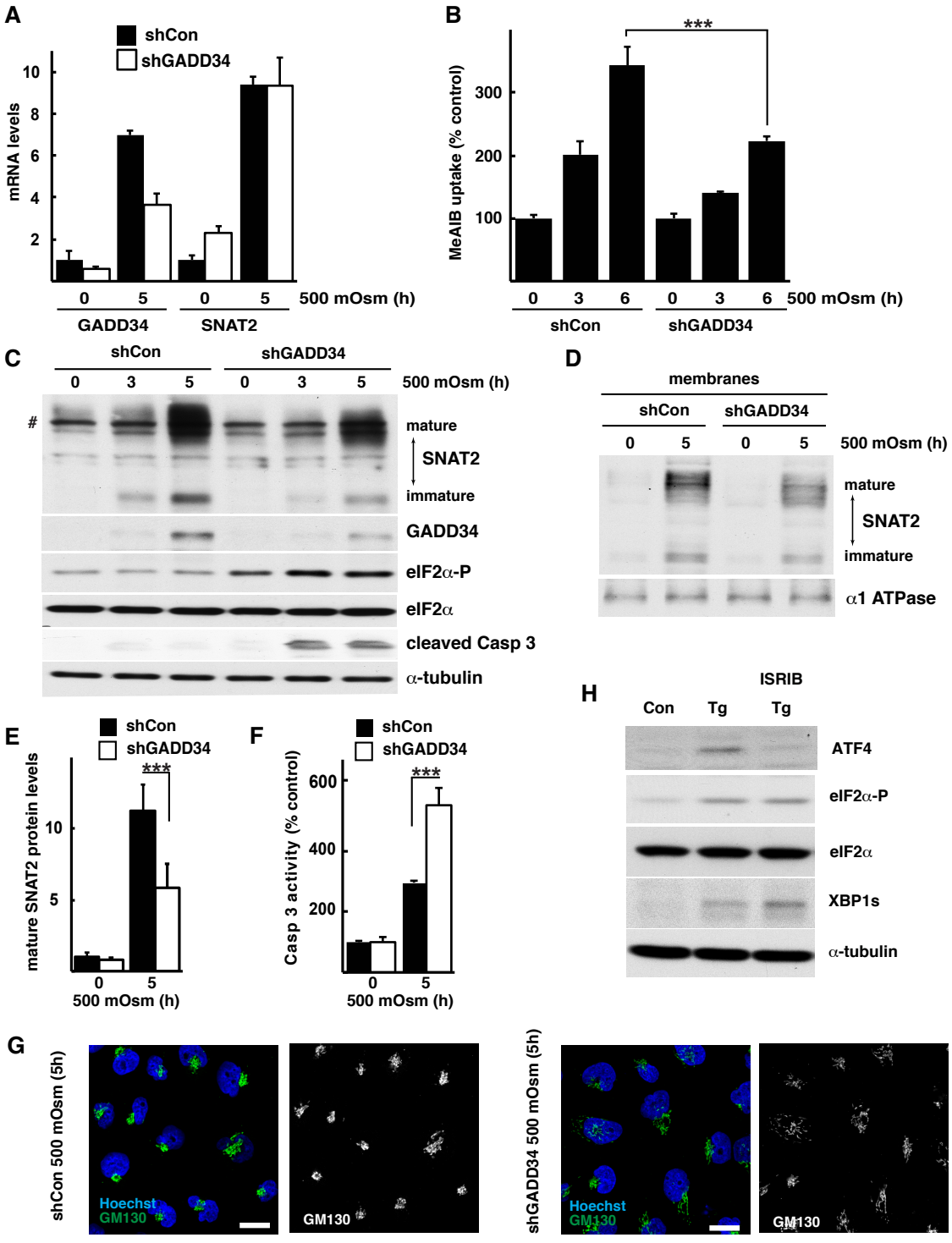


Fig. S3. Depletion of GADD34 in corneal epithelial cells decreases SNAT2 levels and inhibits adaptation to hyperosmotic conditions. Related to Figure 3. A. RT-qPCR analysis of SNAT2 and GADD34 mRNA levels in cells expressing shRNA against GADD34 treated with 500 mOsm media for 5h. Values were normalized to GAPDH mRNA and are plotted as a fold of induction over untreated cells expressing shCon. B. ^{14}C-MeAIB uptake in cells expressing shRNA against GADD34 exposed to 500 mOsm media. C. Western blot analysis of extracts from cells expressing shCon or shRNA against

GADD34 treated with 500 mOsm media. D. Western blot analysis of membrane fractions from cells expressing shRNA against GADD34 treated with 500 mOsm media. E. Quantification of mature SNAT2 from data in Panel D by densitometry. SNAT2 signal intensities were normalized to α-1 ATPase and represent a fold induction over untreated cells expressing shCon. Data are the mean of 3 independent experiments. Error bars represent SD. F. Caspase 3 activity in extracts from cells expressing shRNA against GADD34 treated with hyperosmotic media. Data are plotted as a mean of 3 independent determinations normalized to values of untreated cells. G. Immunofluorescent staining of Golgi marker GM130 (green) in cells expressing shRNA against GADD34 exposed to hyperosmotic (500 mOsm) media for 5h. Nuclei are stained with Hoechst. Images in the gray scale are also shown. Scale bar is 20 μm. H. Integrated stress response inhibitor (ISRIB) reverses eIF2α-P-dependent ATF4 expression during ER stress in corneal epithelial cells. Western blot analysis of extracts from cells treated with Thapsigargin (Tg, 100 nM) for 4h with or without ISRIB. ISRIB (25 nM) was added the last hour of Tg-treatment. Data in panels A,B,E and F are represented as mean of 3 independent experiments ± SD.

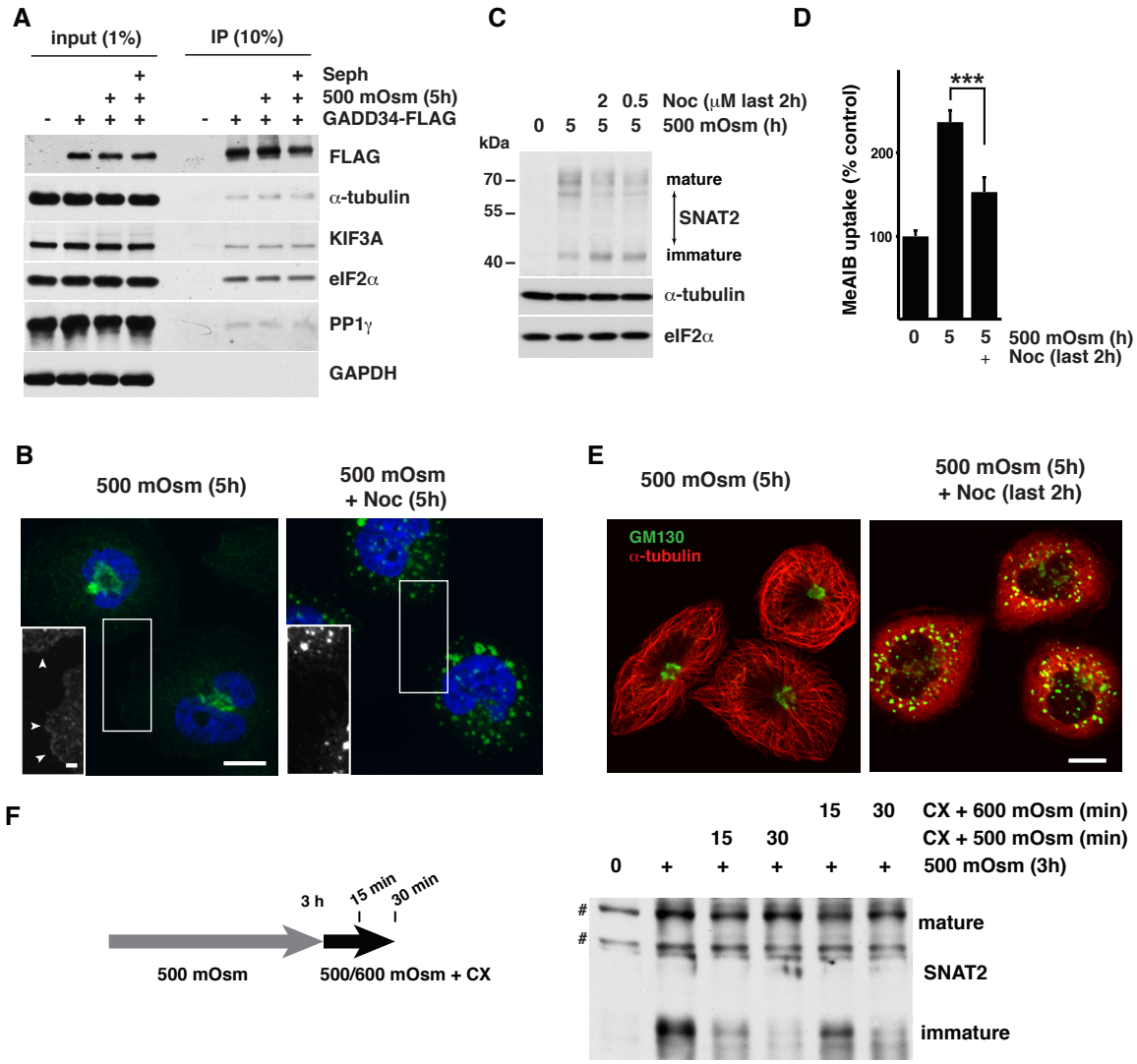


Fig. S4 Microtubule-dependent Golgi fragmentation prevents maturation and trafficking of SNAT2. Related to Figure 4. A. IP of Flag-tagged GADD34 from cells grown in control or 500 mOsm media with or without Sephin 1 (Seph, 50 μM). B. The sucellular distribution of SNAT2 in cells exposed to mild stress (500 mOsm) for 5h with or without nocodazole (Noc, 2 mM) was visualized using confocal fluorescence microscopy (green channel). Nuclei are stained with Hoechst. Boxed images show in grey-scale a magnification of the PM localization of SNAT2 (white arrows). Scale bar is 10 μm (main image) and 2 μm (insert). C. Immunodetection of SNAT2 protein in extracts from cells treated with stress (500 mOsm) and nocodazole present during the last 2h. eIF2α was used as loading control. Positions of protein size markers are indicated. D. SNAT2 activity, measured by ^{14}C-MeAIB uptake in cells exposed to hyperosmotic media with or without nocodazole (Noc, 2 μM) added for the last 2h of treatment. Data are represented as mean of 3 independent experiments ± SD. E. Subcellular distribution of GM130 (green channel) and α-tubulin (red channel) in cells exposed to 500 mOsm media with or without nocodazole (Noc, 2 μM) for last 2h of treatment. Nuclei are stained with Hoechst. Scale bar is 10 μ. F. Schematic of experimental design and western blot analysis of extracts from cells treated with 500 mOsm media for 3h, followed with the addition of cx for the indicated times. Artifact bands are indicated with #.

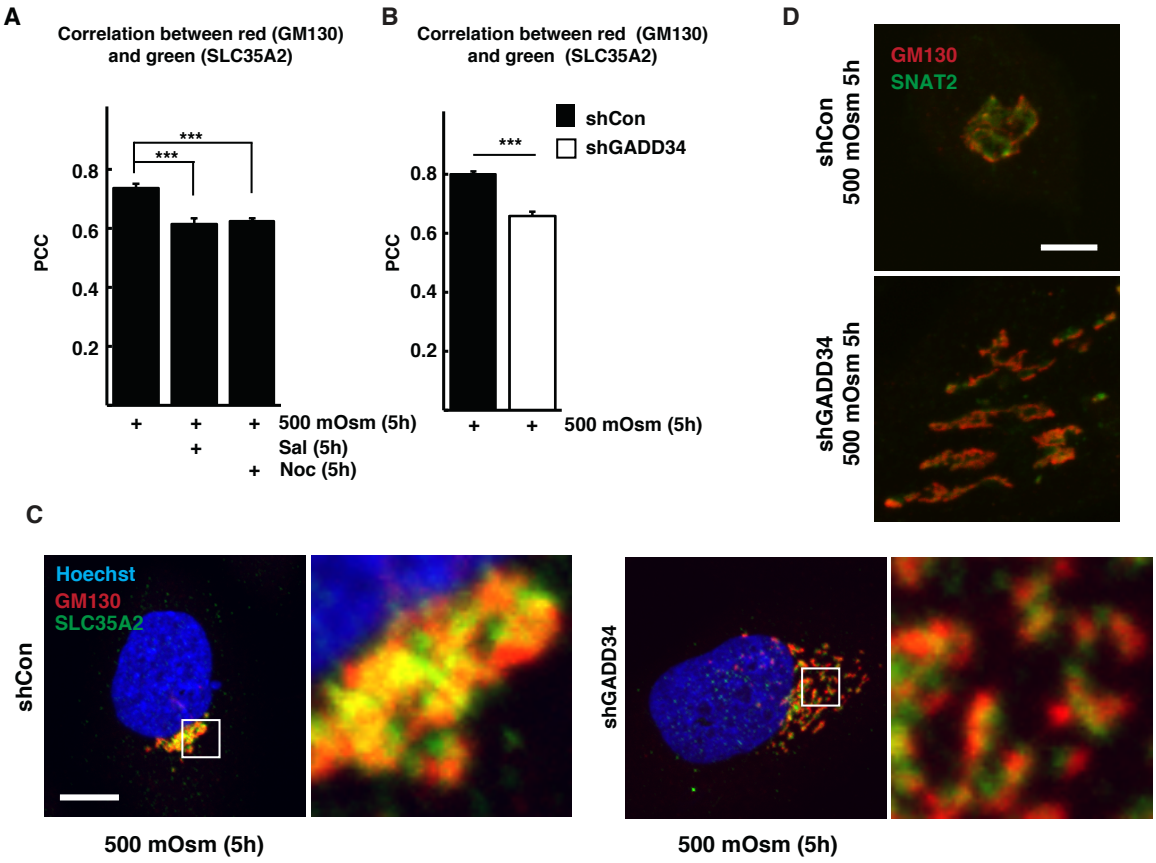


Fig. S5. Inactivation of GADD34 in corneal epithelial cells increases the separation of *cis*- and *trans*-Golgi during hyperosmotic conditions. Related to Figure 5. A. Quantification of the correlation between GM130 signal (red channel) and SLC35A2 (green channel) presented as Pearson's correlation coefficient (PCC). Cells were exposed to hyperosmotic stress (500 mOsm) for 5h in the presence or absence of Sal003 (Sal, 30 μM) or nocodazole (Noc, 500 μM). B. Quantification of the overlap between the GM130 signal (red channel) and SLC35A2 (green channel) presented as PCC. Cells expressing shRNA against GADD34 were treated with hyperosmotic stress (500 mOsm) for 5h. The signal overlap was measured in at least 7 consecutive confocal planes, 0.3 μM each (number of cells, n=9) Data are represented as mean of 3± SD. C. Immunofluorescent staining of *cis*- and *trans*-Golgi markers GM130 (red) and SLC35A2 (green) in cells expressing GADD34 shRNA and exposed to hyperosmotic stress (500 mOsm) for 5h. Nuclei are stained with Hoechst. The right panel shows magnification of boxed image areas. Scale bars are 20 μm. D. Subcellular distribution of the *cis*-Golgi marker GM130 (red) and of SNAT2 (green) in corneal epithelial cells expressing shCon or shGADD34 RNAs exposed to hyperosmotic stress (500 mOsm). Proteins were visualized by confocal fluorescence microscopy. Scale bar is 5 μm.

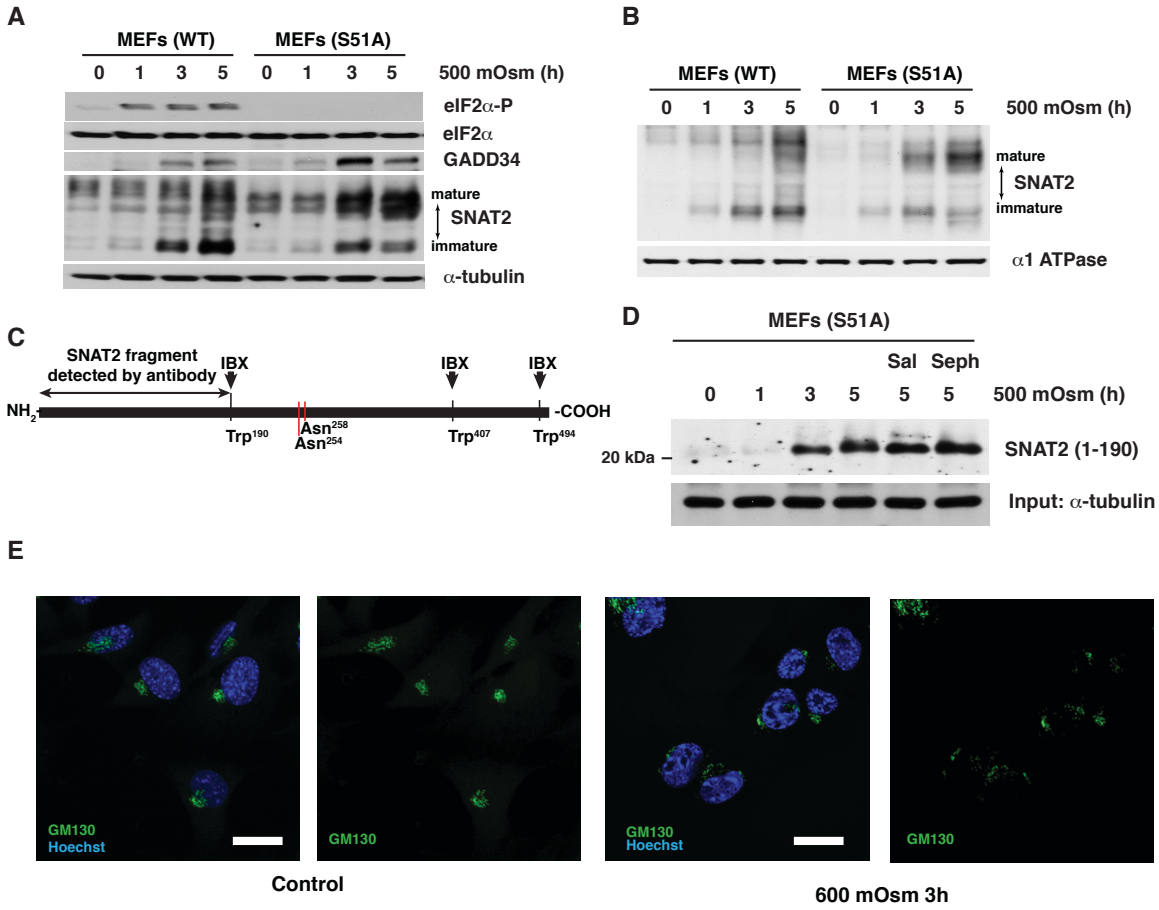
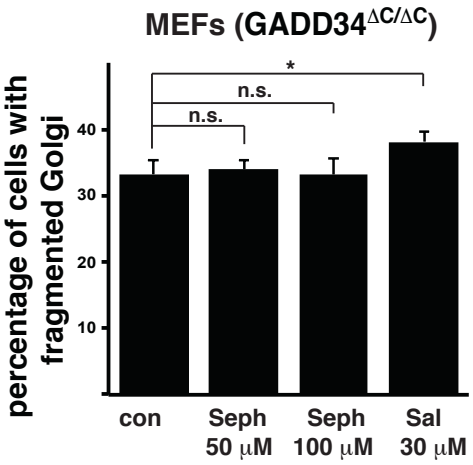


Fig. S6. Induction of SNAT2 and GADD34 during hyperosmotic stress in MEFs is independent of the eIF2α-P signaling. Related to Figure 6. A. Western blot analysis of cell extracts from WT and S51A MEFs treated with 500 mOsm media for the indicated times. B. Western blot analysis of membrane fractions from WT and eIF2α S51A MEFs treated with 500 mOsm media for the indicated times. C. Schematic representation of the positions of Trp and Asn residues in the SNAT2 protein (mouse). Arrows indicating the Trp residues represent the *o*-iodoxybenzoic acid (IBX) cleavage sites. Indicated Asn residues are known N-glycan modification sites. D. Inhibition of GADD34/PP1 in S51A MEFs, does not change stress-induced SNAT2 protein levels. Western blot analysis for SNAT2 of IBX-treated cell extracts from S51A MEFs treated with 500 mOsm media for 5 h with or without Sal003 (Sal, 30 μM) or Sephin 1 (Seph, 50 μM). The levels of α-tubulin were measured in non IBX-treated cell extracts. E. Immunofluorescent staining of GM130 in S51A MEFs incubated in control or hyperosmotic media, indicates stress-induced Golgi fragmentation.

A



B

40 min chase (% cells)

GPI-EGFP distribution	MEFs (WT)		MEFs (GADD34$^{\Delta C/\Delta C}$)	
	untreated	500 mOsm 3h	untreated	500 mOsm 3h
Golgi, PM	50	55	46	64
Golgi, vesicles, PM	42	36	46	27
PM	8	9	9	9

60 min chase (% cells)

GPI-EGFP distribution	MEFs (WT)		MEFs (GADD34$^{\Delta C/\Delta C}$)	
	untreated	500 mOsm 3h	untreated	500 mOsm 3h
Golgi, PM	3 +/- 5	3 +/- 5	3 +/- 6	10 +/- 10
Golgi, vesicles, PM	22 +/- 8	19 +/- 1	38 +/- 8	42 +/- 3
PM	75 +/- 7	78 +/- 5	58 +/- 10	49 +/- 10

Fig. S7. Quantification of GPI-EGFP distribution in WT and GADD34 $^{\Delta C/\Delta C}$ MEFs. Related to Figure 7. A. Effect of GADD34/PP1 inhibitors on Golgi integrity in GADD34$^{\Delta C/\Delta C}$ MEFs. Cells were treated with the indicated concentration of Sal003 (Sal) and Sephin 1 (Seph) for 2h. Data are represented as mean± SD. B. Cells were incubated in hyperosmotic media for 3h and GPI-EGFP was released from the ER by the addition of biotin. Specific subcellular distributions of GPI-EGFP were evaluated 40 or 60 min after biotin addition. Data after 60 min of biotin treatment are presented as a graph in Fig. 7B.

Supplemental Experimental Procedures

Western blotting. Cell lysates, whole membrane fractions and biotinylated surface proteins were prepared as described (Krokowski et al., 2015). Specific cleavage of proteins at tryptophan residues with *o*-iodosobenzoic acid in presence of *p*-cresol was performed according to published protocol (http://cshprotocols.cshlp.org/content/2007/3/pdb.prot4700.long). Protein concentrations were measured with the DC protein assay kit (Bio-Rad) with BSA as a standard and protein samples (20 μg protein) were resolved by SDS/PAGE and transferred to PVDF membranes. These were probed with primary antibody, followed by secondary immunoglobulin-HRP (Calbiochem) and detection with Western Lightning Plus (Perkin Elmer). Detection of proteins was performed using antibodies recognizing the following proteins: eIF2α (sc-13327), GORASP2 (sc-271840), PP1γ (sc-6108) and mouse GADD34 (sc-825) from Santa Cruz Biotechnology; human GADD34 (10449-1-AP) from Proteintech; GAPDH (ab-9485) from Abcam; cleaved caspase 3 (9661) from Cell Signaling; phospho-eIF2α (NB110-56949) from Novus Biologicals; α-tubulin (T9026) and anti-Flag M2 (F1804) from Sigma-Aldrich; SNAT2 (BMP081) from Medical and Biological Laboratories or (HPA035180) from Sigma-Aldrich; KIF3A (A302-708A) from Bethyl; Na^+,K^+-ATPase α-1 subunit (a6F) from the Developmental Studies Hybridoma Bank (deposited by D. M. Fambrough) and ubiquitin from Dako (Z0458). Densitometric quantification of proteins on Western blots was performed with ImageJ and data were normalized to the signal from a-tubulin or Na^+,K^+-ATPase α-1 subunit

Immunoprecipitation of Flag-tagged GADD34. Corneal epithelial cells were transfected with a plasmid expressing human GADD34 with N-terminal fusion of the Flag-tag sequence (pXJ40 GADD34, a gift from Shirish Shenolikar; Addgene #75478) using X-tremeGENE HP DNA Transfection Reagent (Roche) according to the manufacturer's instructions. After 24h cells were subjected to hyperosmotic treatment, then washed with warm PBS. Protein interactions were stabilized by treatment with 0.5% paraformaldehyde in PBS for 5 min at 37°C, and quenched by addition of TRIS to final concentration of 50 mM. Cells were collected and lysed in RIPA buffer. Cell extracts (800 μg) were incubated with 50 μL of anti-Flag M2 Affinity Gel (Sigma Aldrich) for 3h at 4ºC with constant rotation. Following washes (3X)

with TBS, the GADD34 protein was eluted with 150 ng/µl of 3xFlag peptide (Sigma Aldrich) in TBS for 30 min at room temperature. 10% of IP was analyzed by Western blotting after SDS/PAGE separation.

Caspase 3 activity. The activity of caspase 3 was determined by measuring the cleavage of the fluorescent substrate, Acetyl-Asp-Glu-Val-Asp–7-amino-4-trifluoromethylcoumarin (EMD Millipore). Cell lysates were prepared without protease inhibitors and assayed as described (Saikia et al., 2014).

mRNA analysis. Total RNA was isolated with Trizol according to the manufacturer's protocol (Invitrogen). cDNA libraries were synthesized with SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific). The mRNA abundance quantification was performed with the StepOnePlus Real-Time PCR System (Applied Biosystems) and One-Step RT-PCR Kit (Roche) as described (Krokowski et al., 2015). The following primers were used: human *GAPDH*, CCC ATC ACC ATC TTC CAG G (forward) and CAC AGT CTT CTG GGT GGC AG (reverse); human *GADD34*, CGA CTG CAA AGG CGG C (forward) and CAG GAA ATG GAC AGT GAC CTT CT (reverse); human *SNAT2*, AGT TGC CTT TGG TGA TCC AG (forward) and AAG GCC ACT GGT ATA TCC CAA (reverse); mouse *SNAT2*, TAA TCT GAG CAA TGC GAT TGT GG (forward) and AGA TGG ACG GAG TAT AGC GAA AA (reverse); mouse *GAPDH*, CGC CTG GAG AAA CCT GCC AAG TAT G (forward) and GGT GGA AGA ATG GGA GTT GCT GTT G (reverse).

Immunofluorescence staining. Cells were grown on cover slips coated with fibronectin, type I bovine collagen, and bovine serum albumin (corneal epithelial cells) or collagen (S51A MEFs). After incubation in experimental media, cells were fixed with 4% paraformaldehyde in PBS for 20 min at room temperature. Cells were permeabilized and all incubations were performed in 0.05% saponin-PBS. Non-specific staining was blocked by addition of 5 % goat or donkey sera (specific to the species of the secondary antibody) for 1h at room temperature. Primary antibodies (mouse anti-GM130 antibody, 1:200, Santa Cruz Biotechnology sc-55591; rabbit anti-GM130, 1:400, Abcam ab-52649; anti-SNAT2, 1:400, Medical and Biological Laboratories BMP081; anti-KDEL 1:100, Enzo, SPA-827; anti-TGN46 1:400, LifeSpan BioSciences, LS-C348748; anti-α-tubulin, 1:4000, Sigma-Aldrich, T9026; anti-SLC35A2, 1:300, Sigma-Aldrich, HPA036087) were added for 2h at room temperature. Cells were washed 3X with PBS, then

incubated with species-specific secondary antibodies (anti-mouse conjugated to Alexa Fluor 568; anti-rabbit conjugated to Alexa Fluor 488; anti-sheep conjugated to Alexa Fluor 647, from Molecular Probes) and washed 3X. For F-actin staining, Phalloidin Alexa Fluor 488 (Molecular Probes) was used during incubation with secondary antibodies, 1:500. Nuclei were stained using Hoechst (Sigma-Aldrich). Cells were mounted using Gelvatol mounting media (doi:10.1101/pdb.rec10252 Cold Spring Harb Protoc 2006). For Golgi fragmentation 100 cells stained with GM130 were inspected from at least 5 different images for each condition and plotted as mean from 3 independent determinations, with error bars representing SD.

Confocal Microscopy: Optical sections of 0.3 μm were acquired using either an Olympus IX81 Fluoview FV1000 with 60X, 1.4 NA planapochromat oil-immersion objective (Olympus America) controlled by FV10-ASW3.1 software, or UltraVIEW VoX spinning disk confocal system (PerkinElmer, Waltham, MA, USA) which is mounted on a Leica DMI6000B microscope (Leica Microsystems, Inc., Bannockburn, IL, USA) equipped with a HCX PL APO 63x/1.4 and 100x/1.4 oil immersion objectives controlled by Velocity software. Individual experiments were performed with identical laser output levels, exposure times, and scaling. Final images were produced using MetaMorph (Molecular Devices Corporation) and Adobe Photoshop (Adobe Systems Incorporated).

Quantification of protein co-localization: MetaMorph software was utilized to calculate co-localization of SNAT2 with *cis*-Golgi specific marker (GM130) in five consecutive 0.3 μm optical sections for each condition. To define individual red and green structures inside the cell, the program was trained to apply an inclusive threshold to what was considered a positive signal in each channel. Next, to quantify co-localization, a binary mask was generated for each color. Areas of overlap representing co-localization were recorded and displayed as percent area. The number of cells per field was determined manually. To establish the Pearson correlation coefficient (PCC), as a measurement of correlation between the intensities of red (Gm130) and green (SLC35A2) channels in each pixel, Image J software with Coloc2 plug-in was used (https://imagej.net/Coloc_2).

Determination of intra-Golgi localization of SNAT2: Linescan function of MetaMorph software was used to determine intra-Golgi localization of SNAT2 as described in (Dejgaard et al., 2007). Briefly, *cis*-Golgi

was labeled with GM130 (Alexa-568), *trans*-Golgi with TGN46 (Alexa-647), and SNAT2 with Alexa-488. Linescans were obtained manually from the Golgi regions in which *cis*- and *trans*- markers showed visible separation (see Fig. 6B and C). Linescans of 4 pixels wide were taken manually through the axis of separated *cis*- and *trans*-Golgi regions. Only linescans with separated *cis*- and *trans*-Golgi peaks were chosen for further analysis. Afterwards, the fluorescence profile along the linescan was visualized for all three channels and imported into Microsoft Excel program. 3-6 linescans were taken for each individual cell. Location of the maximum pixel value in blue channel (Alexa-647) was defined as 1 on a coordinate system representing the *cis*- to *trans*- axis of Golgi apparatus, whereas the maximum value of red channel was defined as 0. Location of the maximum pixel value of the peaks for green channel was plotted between 1 and 0 according to their relative location between maximum values of *cis*- and *trans*-Golgi markers.

GPI-EGFP expression, chase, fluorescence microscopy and image processing. RUSH plasmid expressing GPI-EGFP was a gracious gift from the Perez laboratory (Institut Curie, Paris, France) (Boncompain et al., 2012). Wild type and GADD34/PP1 activity deficient MEFs ($GADD34^{\Delta C/\Delta C}$) (Novoa et al., 2003) growing on glass coverslips were transfected with a plasmid expressing GPI-EGFP using FuGene HD transfection reagent (Promega) according to the manufacturer's protocol. Transfected cells were then incubated overnight at 37°C in the presence of avidin (1×10-7 M, Sigma-Aldrich) as a biotin scavenger to ensure efficient retention of GPI-EGFP in the ER. To create a mild osmotic stress, cells were pre-treated with 500 mOsm for 3h in complete culture medium. ER accumulated GPI-EGFP protein was then released from the ER by the addition of 40 μM biotin (Sigma-Aldrich). During the subsequent biotin chase period, cells were incubated at 37°C for 40 or 60 min in the presence of cycloheximide to prevent further protein synthesis. 4% paraformaldyhyde was used to fix the cells. Confocal image stacks were collected with a 63x/1.40 numerical aperture objective and a LSM880 Zeiss inverted microscope with confocal optics. Images were processed with Zen software (Zeiss). Confocal image stacks were taken at the same exposure time, and the image stacks were condensed into a single plane using a maximum intensity projection protocol (MIP). For the quantification of GPI-EGFP distribution, at least 10 cells were analyzed for each condition and experiment. Release of GPI-EGFP at 60 min was determined experimentally for three independent experiments and the results were averaged.

Supplemental References.

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