Published in final edited form as:

Cell Signal. 2011 May ; 23(5): 785–793. doi:10.1016/j.cellsig.2011.01.001.

Alteration of Golgi Structure in Senescent Cells and its Regulation by a G protein γ subunit

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Abstract

Cellular senescence is a process wherein proliferating cells undergo permanent cell cycle arrest while remaining viable. Senescence results in enhanced secretion of proteins that promote cancer and inflammation. We report here that the structure of the Golgi complex which regulates secretion is altered in senescent cells. In cells where senescence is achieved by replicative exhaustion or in cells wherein senescence has been induced with BrdU treatment dependent stress, the Golgi complex is dispersed. The expression of a G protein γ subunit, $\gamma 11$, capable of translocation from the plasma membrane to the Golgi complex on receptor activation increases with senescence. Knockdown of $\gamma 11$ or overexpression of a dominant negative $\gamma 3$ subunit inhibits Golgi dispersal induced by senescence. Overall these results suggest that in cellular senescence an upregulated G protein gamma subunit mediates alterations in the structure of the Golgi.

1. Introduction

Replicatively senescent cells or cells that have undergone genotoxic stress exhibit increased secretion of a number of factors including cytokines, growth factors, metalloproteinases and extracellular matrix proteins [1]. The enhanced secretion of these factors is known to induce inflammation and has been demonstrated to facilitate epithelial mesenchymal transition which promotes tumorigenesis [1]. Since cellular secretion is mediated by the Golgi complex, we examined the status of the Golgi in senescent cells resulting from stress or replicative exhaustion. Based on previous reports, 5-bromo 2-deoxyuridine (BrdU) exposure to cells was used as a stress induced model for senescence [2-4] which mimics the properties of replicative senescence. It has been shown that BrdU treatment induces cellular senescence likely by inducing the DNA–damage response [5]. DNA damage has been shown to trigger senescence [6]. It has been shown that it induces senescence in stem cells and inhibits proliferation of cancer cells [15, 16]. We also confirmed a previous finding that a heterotrimeric G protein subunit, $\gamma 11$ (GNG11) is upregulated in senescent cells [7]. The $\gamma 11$ subunit is capable of translocation from the plasma membrane to the Golgi on receptor activation as a $\beta\gamma$ complex [8, 9] and regulates the structure of the Golgi [10]. We therefore

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examined the possibility that the G protein $\gamma 11$ subunit plays a role in the regulation of Golgi structure in senescence.

2. Materials and Methods

2.1. Constructs, cell lines and chemicals

The tagged and untagged G protein constructs, various Golgi markers and PH-mCh used in this study have been previously described [9-12]. Mammalian expression vector containing cDNA encoding $\gamma 11$ shRNA and control scrambled shRNAs were from the TRC library of Broad Institute (Sigma) and CFP-tubulin from E. Bertrand (CNRS, Montpellier, France). HeLa cell line was from ATCC; WI-38 and IMR90 cell lines were from NIA Aging Cell Repository at Coriell Institute for Medical Research (Camden, NJ). Antibodies to Golgi network marker, TGN46 were obtained from Sigma; antibodies to *cis* Golgi marker, GM130 were from A. Lindstedt (Carnegie Mellon University, Pittsburgh, PA) and were used at a dilution of 1:100. TRITC – conjugated goat anti – rabbit secondary antibody was from Sigma and was used at a 1:1000 dilution. 5-bromo deoxyuridine was procured from Sigma and was dissolved in DMSO to prepare a 200 μ M solution. The solution was prepared just before use.

2.2. Cell culture, transfections and lentiviral transduction

HeLa cells were cultured in DMEM (Cellgro, Manassas, VA) containing 10% dialyzed FBS (Atlanta Biologicals), while WI38 and IMR90 cells were grown in MEM containing 10% non-dialyzed FBS, at 37°C, 5% CO₂. Cells were transfected with Lipofectamine 2000 (Invitrogen) as per the manufacturer's protocol. To obtain stable knock down HeLa cell lines, lentiviral particles containing specific shRNAs were used as per the protocol provided by Sigma. The cells were transduced in a 96-well plate and after 48 hours, 2 μ g/ml of puromycin was added to screen for cells expressing shRNAs. The cells were cultured for several generations and the reduction in the expression of $\gamma 11$ was monitored to evaluate the stability of knock down cell line. For all experiments, the cell line was evaluated for knock down before use by real time PCR.

2.3. Quantitative real time–PCR

Total cellular RNA was isolated from various cells lines using the RNeasy Plus Mini Kit (QIAGEN). Reverse transcription of RNA was performed using Themascript RT-PCR system (Invitrogen, Carlsbad, CA) as per manufacturer's instructions and as previously described [10]. Quantitative real time PCR was performed using SYBR Green PCR master mix (Applied Biosystems) in 20 μ l reaction volume as per manufacturer's instructions. Melting curve analyses were performed on all reactions to check for specificity of the amplicons. Expression levels of β -actin were used to normalize the data. The following primer pairs were used for quantitative RT-PCR analysis.

Fibronectin - 5'GGTGGCTGTCAGTCAAAGC3' and 5'CGCATTGCCTAGGTAGGTC3'
p21 - 5'GGAGCAGGCTGAAGGGTC3' and 5'CCGGCGTTTGGAGTGGTAG3'
 $\gamma 10$ - 5'TGCCTTCAAGCACAAAGTGA3' and 5'TATAGGACCAGGCCACAGGA3'
 $\gamma 11$ - 5'GTGCCCTTACATCGAAGAT3' and 5'CACTTGTTGTCTCTGCAACTTCA3'
 β -actin - 5'CCAACCGCGAGAAGATGAC3' and 5'CAGAGGCGTACAGGGATAGC3'

2.5. IL-8 secretion

HeLa cells were seeded in 6 – well plates and were grown overnight. Next day the cells were treated with 200 μ M BrdU and after 24 hours the growth media was replaced with serum free media (DMEM-F12 1:1 mix with Peprotech serum free mix 1) containing 200 μ M BrdU for another 48 hours. The media and cells were collected and IL-8 levels were

estimated in the media using an ELISA kit (R&D systems). The cells were lysed with M-Per protein extraction reagent (Pierce) and total protein was estimated using BCA protein estimation kit (Pierce). The IL-8 levels were then calculated per μg total protein.

2.6. Immunofluorescence microscopy

Immunofluorescence microscopy was performed as described previously [10]. Briefly, cells grown on cover slips were subjected to BrdU treatment and fixed with 4% freshly prepared paraformaldehyde for 10 min, followed by permeabilization with 0.5% Triton X-100 for 10 min. The permeabilized cells were blocked with 2% BSA in PBS for 1 hr followed by treatment with primary antibodies at recommended dilution for 2 hrs followed by incubation with goat anti-rabbit-TRITC conjugate Ab (Sigma, MO; 1:1000) for 1 hr. The coverslips were washed and mounted on a slide with antifade solution (Calbiochem, CA) and imaged using Nikon TE2000 widefield fluorescence microscope using a 60 $\times$ Plan-Apochromat objective (1.4 NA) as described previously [10].

2.7. Live cell imaging

Live cell imaging was performed essentially as described [8, 9, 11, 13]. An inverted wide field epifluorescence microscope with 1.4 NA 60 $\times$ Plan Apochromat objective (Nikon TE2000) was used. The microscope was equipped with Exfo X-Cite light source containing a 103W Hg-Arc lamp for illumination, Sutter lambda 10-2 optical filter wheel controller for wavelength selection (Sutter Instrument Company), specific wavelength filters (Chroma), Orca-ER CCD camera (Hamamatsu Corp.) for image acquisition, 8 – channel automated fluid delivery system (Automate Scientific) and Metamorph 6.3.7 software (Molecular Devices) for controlling all the devices. Imaging was performed at room temperature at variable exposure times with variable binning. Filters used to acquire specific fluorescence were as follows: for CFP, 436/10 excitation and D465/30 emission; YFP, D492/18 excitation and D535/30 emission; DsRed, D580/20 excitation and D630/60 emission. Endomembrane regions for intensity measurements were selected using MetaMorph. The images were enhanced for brightness and/or contrast uniformly post acquisition either using Metamorph, ImageJ or Microsoft Word image editing tool.

3. Results

3.1. BrdU (5 - bromo deoxyuridine) induces cellular senescence

We used BrdU treated HeLa cells as a rapidly assayable model for cellular senescence. It has been observed that BrdU induces cellular senescence by activating the DNA damage response [5, 14]. Consistent with previous reports [2, 4, 5, 14, 15, 17] we found that BrdU induces cellular senescence. HeLa cells treated with 200 μM BrdU for 24-48hrs showed several characteristics similar to replicatively senescent IMR90 cells. BrdU treated cells slowed down in growth, changed shape (Fig 1A and Fig 1B) and showed enhanced transcription of an inhibitor of cyclin dependent kinase 4, p21 (Fig. 1C) [18]. BrdU treated HeLa cells and replicatively senescent IMR90 and WI38 cells also expressed higher levels of fibronectin [19] and a G protein $\gamma 11$ subunit type (Fig. 1C and 1D) consistent with previous reports [7]. In our experiments, G protein $\gamma 10$ subunit served as an internal control that did not show any change in expression when exposed to BrdU (Fig. 1C and 1D). To further confirm that BrdU treatment mimics replicative senescence, we monitored the secreted levels of the pro-inflammatory cytokine IL-8 which has been shown to be an integral part of the senescence associated secretory phenotype [1, 20]. As expected we observed increased secretion of IL-8 levels from BrdU treated cells (Fig. 1E). These observations together confirmed that BrdU treatment mimics cellular senescence induced by replicative exhaustion.

3.2. The G protein $\beta\gamma$ complex translocates from the plasma membrane to the Golgi complex on receptor activation

Recent results show that the $\gamma 11$ subunit type is capable of translocating from the plasma membrane to the Golgi complex on receptor activation [8, 9], regulating the structure of the Golgi [10]. Here, we examined the potential role of the G protein $\gamma 11$ subunit in regulating the structure of the Golgi in senescent cells since its transcript levels are upregulated in these cells (Fig. 1C and 1D). First, we examined whether the upregulation of the $\gamma 11$ transcript was reflected in its translocation properties in senescent cells. The $\beta 1$ subunit tagged with a fluorescent protein (FP) was cotransfected with the M3 muscarinic receptor and αq into HeLa cells. We utilized this strategy based on our previous observations wherein we have detected co-translocation of tagged $\beta 1$ in cells which endogenously contain a translocation proficient γ subunit [10]. Receptor mediated translocation was examined before and after BrdU treatment. In untreated cells, agonist stimulation (100 μ M carbachol) did not evoke significantly detectable translocation of the YFP- $\beta 1$ subunit to the inside of the cell (Fig. 2A upper panel). In contrast, when cells were treated with BrdU, significant YFP- $\beta 1$ translocation to the intracellular region was detected (Fig. 2B). The translocation was reversible and rapid (Fig. 2C) as reported previously [8-10]. To confirm that the observed effect was due to activation of the M3 muscarinic receptor, we also monitored the activation of PLC β by monitoring the generation of IP3 inside the same cell using the PH-mCh sensor as previously [11] (Fig 2A lower panels and 2D).

3.3. The Golgi complex is dispersed in senescent cells

To observe the status of the Golgi complex in HeLa cells, a marker for the Golgi region, Galactosyl transferase tagged with red fluorescent protein, DsRed (GalT-DsRed) [21] was transiently expressed in the cells. Senescence was induced post transfection by treating the cells with BrdU. While untreated cells contained compact *trans* Golgi, BrdU treated senescent cells contained dispersed Golgi (Fig.3A). Similarly, when replicatively senescent IMR-90 and WI-38 cells were examined by immunofluorescence microscopy using antibodies specific to the endogenous *trans* Golgi network marker, TGN46 [21], early PDL cells contained predominantly compact TGN and late PDL cells contained TGN that was dispersed (Fig.3B). The cells were scored for compact versus dispersed Golgi. For both IMR-90 and WI-38 cells, ~70% of cells exhibited compact Golgi in the pre-senescent (early PDL) stage and the profile was reversed in senescent cells (late PDL) where more than 70% of cells had dispersed Golgi (Fig. 3C). These results suggested that (i) the Golgi structure is altered in senescent cells and (ii) the changes in the Golgi structure alternations were not peculiar to BrdU treatment but were common to cellular senescence.

3.4. The *cis* and medial Golgi are also altered in senescent cells

We examined whether the alterations in the Golgi structure were restricted to the *trans* Golgi and *trans* Golgi network. A specific antibody for GM130 which localizes to the *cis* Golgi [22] was used to examine the *cis* Golgi structure in control and BrdU treated HeLa cells as well as early and late PDL IMR-90 and WI-38 cells. Similarly, the structure of the medial Golgi was examined by expressing CFP-mannosidase II which is known to be localized specifically to this region of the Golgi complex [23]. The results showed that both the *cis* and medial Golgi were dispersed in senescent cells (Fig. 4).

3.5. Golgi alterations in senescent cells are not due to microtubule disassembly

Microtubule disassembly is known to disrupt the structure of the Golgi [24]. We examined whether the senescence associated changes are due to microtubule disassembly during senescence. Tubulin tagged with CFP was coexpressed with GalT-DsRed and their distribution was examined in HeLa cells before and after treatment with BrdU. Cells treated

with BrdU showed GalT-DsRed scattered over the cells suggesting disruption of the *trans* Golgi. But the microtubule distribution in these cells was unaltered and similar to the control untreated cells (Fig. 5A and 5B, right panels). When HeLa cells were treated with nocodazole, an agent known to lead to the depolymerization of microtubules [24, 25], the microtubule polymers were no longer detectable (Fig. 5C). In these cells, GalT-DsRed was distributed in dispersed structures indicating that the *trans* Golgi was broken down as expected from previous reports [24]. The pattern of *trans* Golgi distribution was distinctly different in nocodazole treated cells compared to BrdU treated cells (left panels of Fig. 5B & 5C) suggesting that the Golgi dispersal which occurs during senescence induction is distinct from the Golgi fragmentation due to microtubule depolymerization. Overall, these results suggested that the structural changes observed in the Golgi in senescent cells are not likely to be a result of microtubule depolymerization.

3.6. Knockdown of $\gamma 11$ inhibits senescence associated Golgi dispersal

Previous results have shown that receptor activated translocation of the G protein $\gamma 11$ subunit from the plasma membrane to the Golgi complex mediates the fragmentation of the Golgi complex [10]. As mentioned in section 3.2, we also observed upregulation of the $\gamma 11$ subunit in senescent cells as visualized by $\beta 1$ -cotranslocation (Fig. 2B). To examine further whether the translocation of $\beta 1$ in response to M3 activation in BrdU treated cells was due to co-translocation with endogenous $\gamma 11$, we knocked down the $\gamma 11$ subunit expression in HeLa cells using a $\gamma 11$ specific shRNA. Introduction of $\gamma 11$ shRNA into HeLa cells reduced the transcript specific to $\gamma 11$ by about ~70% (Fig. 6A). Cells expressing shRNA specific to $\gamma 11$ were then treated with BrdU and the translocation of $\beta 1$ examined by activation of the M3 receptors as described in section 3.2. Significant $\beta 1$ translocation was not detected in these cells (Fig. 6B, upper panels) in comparison to cells expressing a shRNA with a scrambled sequence (not shown). The presence of receptors in the cells was confirmed by detecting PH-mCh translocation as above (Fig. 6B, lower panel). This result suggests that endogenous $\gamma 11$ is a major mediator of the receptor dependent translocation of the $\beta 1$ subunit in senescent cells. This result also provides support for the $\gamma 11$ knockdown effect reducing $\gamma 11$ protein levels.

To evaluate if the changes in the Golgi structure observed here are mediated through the $\gamma 11$ subunit, we knocked down the G protein $\gamma 11$ subunit stably using a specific shRNA targeted against it [10]. HeLa cells were stably transduced with lentiviruses expressing $\gamma 11$ specific shRNA which were previously confirmed to be capable of significantly reducing $\gamma 11$ subunit expression [10]. Quantitative real time PCR analysis was used to determine the expression level of $\gamma 11$ in the cells expressing the shRNA. $\gamma 11$ mRNA expression was reduced by ~70-80% compared to cells in which a control scrambled shRNA was expressed (Fig. 6A). When these cells were exposed to BrdU to induce senescence and the Golgi complex examined as above, dispersal of the *trans* Golgi was significantly inhibited compared to control cells (Fig 6C).

To examine whether the effect of the $\gamma 11$ shRNA was specific, we introduced the mouse $\gamma 11$ cDNA into the knockdown cells tagged with YFP. Since there was a mismatch in the shRNA targeting the human $\gamma 11$ and mouse $\gamma 11$, the $\gamma 11$ shRNA had no effect on the mouse $\gamma 11$. When the Golgi structure in the cells expressing YFP was examined they were similar to wild type cells (Fig. 6D). These results showed that the effect of the $\gamma 11$ shRNA was specifically due to the depletion of the G protein $\gamma 11$ subunit and not due to non-specific effects and the Golgi structure is indeed regulated by the presence of the G protein $\gamma 11$ subunit in these cells.

3.7. Translocation of the G protein $\beta\gamma$ complex is likely required to mediate the Golgi structural changes associated with senescence

Receptor stimulated translocation of the G protein $\beta\gamma$ complex from the plasma membrane to the Golgi complex has been shown to lead to structural changes in the Golgi complex [10]. We examined if a mechanism similar to that seen here was at the basis of the dispersal of Golgi elements in cellular senescence. We have previously reported that activation of the muscarinic receptor leads to the translocation of six members of the G protein γ subunit family while translocation of the remaining six members was not detected under the conditions used [9]. We have also shown that one of these γ subunits, $\gamma 3$ whose translocation could not be detected, is dominant negative and reduces the translocation of an endogenous γ subunit so that it is not detectable on receptor activation and inhibits the breakdown of the Golgi complex mediated by the translocation [10]. Using a similar strategy, we expressed YFP- $\gamma 3$ in HeLa cells and induced senescence with BrdU. The structure of the Golgi complex in these cells in comparison to control cells in which YFP alone was expressed was then observed using TGN46 as a marker for the *trans* Golgi network (Fig. 7). A significant decrease in the number of cells containing dispersed Golgi was noted in cells expressing the dominant negative $\gamma 3$ subunit. These observations suggest that the translocation ability of the $\gamma 11$ subunit is a likely requirement for Golgi dispersal during senescence.

4. Discussion

The structure of the Golgi complex in senescent cells has not been examined before to our knowledge. Results here show that the Golgi complex is compact in non-senescent or pre-senescent cells but is dispersed in senescent cells. The consistently altered appearance of the Golgi in both stress induced senescent cells and replicatively senescent cells suggests that the Golgi structure could serve as a useful marker for cellular senescence. Senescent cells show significantly increased secretion of cytokines such as IL-6, IL-8; growth factors such as VEGF and extracellular matrix proteins such as fibronectin [1, 19, 27]. One potential reason for the alteration in the structure of the Golgi complex could be that it is a result of the increased secretion of various proteins which traffic from the Golgi to the plasma membrane.

It has been shown that the G protein $\gamma 11$ subunit is upregulated in stress induced and replicatively senescent cells [7] and we have confirmed this finding here. We show here that the upregulated G protein $\gamma 11$ subunit in senescent cells mediates the translocation of the $\beta\gamma 11$ complex from the plasma membrane to the Golgi in response to receptor activation. The translocation is mediated by the upregulated $\gamma 11$ subunit because in the presence of the $\gamma 11$ shRNA, translocation is not seen. Recent evidence shows that receptor mediated translocation of the $\beta\gamma$ complex breaks down the Golgi complex [10]. The changes seen in the structure of the Golgi complex in senescent cells are similarly mediated by the $\beta\gamma 11$ complex because the $\gamma 11$ shRNA inhibits the dispersal of *trans* Golgi and *trans* Golgi network. Furthermore, the ability of a known γ subunit whose translocation could not be detected, $\gamma 3$, to inhibit senescence associated Golgi structure alterations suggests strongly that it is mediated by the translocation of the $\beta\gamma$ complex from the plasma membrane to the Golgi.

The results here suggest that G protein $\gamma 11$ upregulation plays a role in senescence and are consistent with an earlier report that overexpression of $\gamma 11$ shortens the number of PDLs required to reach replicative senescence [7]. Cellular senescence is thought to be cancer protective since cell division is arrested. Consistent with such a role $\gamma 11$ transcription levels have been reported to be reduced in medullary thyroid carcinoma [29] and splenic marginal zone lymphoma [30] indicating that its downregulation is accompanied by cell proliferation.

It is expected that secretory changes in a cell will be primarily reflected at the level of the *trans* Golgi network and *trans* Golgi regions. However, the structurally altered *cis* and medial Golgi seen here in senescent cells may be because of the increased secretion of a large number of proteins from senescent cells [1]. There is previous evidence that the entire Golgi can be broken down when regulators of secretory vesicle formation such as the translocating $\beta\gamma$ complexes and PKD are overactive [10, 26]. An alternative possibility is that the Golgi structure changes seen in senescent cells are due to the cell division arrest which is a consequence of senescence induction. It is known that during cell division and specifically mitosis, the Golgi complex breaks down completely and is then reassembled in daughter cells [31]. It is possible that during senescence the initial changes in the Golgi that occur in mitosis are observed but the process does not continue further than the dispersal of the Golgi elements.

Is it possible that Golgi structural changes accompany senescence but do not directly play a role in regulating senescence? Previous results show that overexpression of the G protein $\gamma 11$ protein induces senescent growth arrest at an earlier PDL compared to control mortal cells [7]. This result suggests that the translocation of the $\gamma 11$ subunit from the plasma membrane to the Golgi likely plays a direct role in regulating senescence. The results here also suggest that a G protein coupled receptor (GPCR) regulates senescence through the translocation of the $\beta\gamma 11$ complex. GPCRs are the single most important target for therapeutic drugs because they are accessible to extracellular ligands and modulate an enormous variety of physiological processes. The results here suggest that it may be possible to target a specific GPCR to regulate senescence.

Acknowledgments

This work was supported by NIH grants (GM69027 and GM080558) to N.G. and an AHA postdoctoral fellowship (D.K.S.).

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Fig. 1. Characterization of BrdU stress induced cellular senescence in immortal HeLa cells and replicative senescence in mortal WI38 cells

HeLa cells were exposed to 200 μ M BrdU to induce cellular senescence. Non senescent PDL17 and close to senescence PDL46 WI38 cells were used. **A. Inhibition of cell proliferation on treatment with BrdU.** Equal number of cells (0.2 million/well) were seeded in triplicate in six well plates out of which three wells were exposed to 200 μ M of BrdU for 72 hours. Cell numbers were counted and ratio of the number of cells at 72 hours to the initial number at seeding was calculated. This ratio for BrdU treated cells is distinctly different from untreated cells ($p < 0.01$) ($n = 3$). **B. Change in morphology of cells exposed to BrdU.** DIC images of HeLa cells before and after exposure to BrdU are shown. Cells were imaged in glass bottom dishes using Leica DMI6000 in DIC mode using 1.4 N.A. 60 $\times$ plan apochromat objective. **C. Changes in gene expression in BrdU treated senescent cells.** Quantitative real time PCR was performed for measuring mRNA levels for p21, fibronectin, G protein $\gamma 10$ and $\gamma 11$ subunits as described in Materials and Methods. Cells were treated with 200 μ M BrdU for 24 hours and 48 hours. Levels of RNAs were normalized using β -actin as an internal control, and relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method. Error bars represent SEM ($n = 3$). **D. Changes in gene expression in replicatively senescent cells.** Early PDL and late PDL WI38 cells were used for expression analysis as above. **E. Induction of IL-8 secretion from senescent cells.** IL-8 was measured as described in Materials and Methods. Error bars represent SEM ($n = 3$).

Fig. 2. Cotranslocation of YFP-β1 in HeLa cells

HeLa cells expressing M3, α_q , YFP-β1 and PH-mCh were used. The cells were treated with 200 μ M BrdU for 48 hours. The cells were mounted on a 25 μ l volume perfusion chamber (RC30, Warner Instruments) and imaged in a continuous flow of HBSS at a rate of 0.5ml/ml. The cells were stimulated with M3 receptor agonist, 100 μ M carbachol followed by antagonist, 100 μ M atropine. The images show distribution of YFP-β1 and PH-mCh before and after agonist treatment in the same cell. Representative images are shown ($n > 4$). **A. YFP-β1 translocation is not detectable in basal state HeLa cells.** Images from basal HeLa cells (not exposed to BrdU) depicting no translocation of YFP-β1 on receptor activation (upper panel). Lower panel shows translocation of PH-mCh in the same cells to confirm the presence of the receptor. **B. YFP-β1 translocation is detectable in BrdU treated HeLa cells.** Images were taken at 5 sec interval. Representative images of cell before and after

agonist treatment. The images shown were acquired at 10 sec (basal) and at 2'20 sec (agonist treated). The intracellular region used for intensity measurements is shown (white circle). **C. Plot showing translocation of YFP- β 1 to the endomembranes.** The intensity measurement from the endomembranes is plotted as a function of time (sec). Arrows indicate the point at which agonist and antagonist were added. **D. Plot showing translocation of PH-mCh to the endomembranes** (as reported previously) [11]. The intensity measurement from the endomembranes (white circle) is plotted as a function of time (sec). Arrows indicate the point at which agonist and antagonist were added. The translocation of PH domain confirmed that the introduced receptor is present in the cells and is active.

Fig. 3. Dispersal of *trans* Golgi and *trans* Golgi network (TGN) in senescent cells

A. Dispersal of Golgi in stress induced senescence in HeLa cells. For *trans* Golgi labeling, HeLa cells were transfected with GalT- DsRed using lipofectamine 2000 (Invitrogen, Carlsbad, CA). The localization of GalT in live cells was monitored by epifluorescence microscopy. For *trans* Golgi network labeling, HeLa cells were grown on coverslips, treated for 24 hrs with BrdU to induce senescence, fixed and stained using the anti-TGN46 antibody (Sigma, St.Louis, MO). Top panels, basal cells, bottom panel, senescent cells ($n>3$). **B. Replicative senescence in IMR90 and WI-38 cells.** Early (16-17 PDL) and late PDL (46 PDL) IMR90 and WI38 cells were fixed and stained for TGN46 distribution. Images were acquired using widefield microscopy. Top panels, early PDL cells, and bottom panels, late PDL cells. **C.** Bar graph depicting the number of cells with compact or dispersed TGN in late and early PDL IMR90 and WI38 cells. Average plot of two experiments. The percentage variability across the experiments was $<10\%$.

Fig. 4. Redistribution of *cis* and medial Golgi in senescent cells

A. Control and BrdU treated senescent HeLa cells. B. Early and late PDLs of WI38 and IMR90 cells. In A & B *cis* Golgi structure was observed using anti-GM130 antibodies. Representative cells with compact or dispersed *cis* Golgi (n=2). **C. Redistribution of α mannosidase, medial Golgi marker, in senescent HeLa cells.** HeLa cells were transfected with CFP - α mannosidase. The distribution of α mannosidase was monitored by fluorescence microscopy (n=2).

Fig. 5. Tubulin independent redistribution of trans Golgi in stress induced senescent HeLa cells
 HeLa cells transfected with GalT-DsRed and CFP-tubulin were treated with 200 μ M BrdU to induce senescence. The cells were then visualized for GalT and tubulin distribution using epifluorescence microscopy. **A.** Basal HeLa cells. **B.** BrdU treated senescent HeLa cells. **C.** Nocodazole treated HeLa cells, which leads to both depolymerization of microtubules as well as Golgi redistribution (note that tubulin distribution is distinctly absent in the nuclear region due to depolymerization) (n=2).

C

Fig. 6. Effect of G protein $\gamma 11$ subunit knockdown on Golgi structure in HeLa cells
A. Specificity of $\gamma 11$ -shRNA on expression of G protein $\gamma 10$ and $\gamma 11$ subunits in HeLa cells. HeLa cells stably transfected with $\gamma 11$ -shRNA and control scrambled shRNAs were used. RNA was isolated from stable cell lines and mRNA expression of the $\gamma 10$ and $\gamma 11$ subunits was analysed by real time PCR. The expression levels were normalized to β -actin expression levels (n=3). **B. YFP- $\beta 1$ translocation is not detectable in HeLa cells expressing $\gamma 11$ -shRNA.** HeLa cells expressing M3, αq , YFP- $\beta 1$, PH-mCh and $\gamma 11$ -shRNA were treated with 200 μ M BrdU for 24 hours. Representative images of cells at basal level and after agonist treatment are shown. Upper panel shows images of YFP- $\beta 1$ indicating absence of translocation on receptor activation. Lower panel shows translocation of PH-

mCh in the same cells to confirm the presence of receptor in the same cell. **C. Knock down of $\gamma 11$ in HeLa cells reduces senescence induced Golgi dispersal** (n=4). Bar graphs from representative experiments depict percentage of cells with compact or dispersed *trans* Golgi detected by TGN46 staining. Stable knock down of the $\gamma 11$ subunit was generated using lentiviral transduction containing specific shRNA particles (Sigma) and Golgi structure was analysed using TGN46 staining as mentioned above. A scrambled shRNA was used as a control. Representative images of cells expressing control shRNA and $\gamma 11$ -shRNA are shown. An averaged plot of all the experiments is shown. Golgi structure in wild type BrdU treated cells was significantly different from basal cells and similarly BrdU treated $\gamma 11$ -shRNA were significantly different from control-shRNA expressing cells (t test) ($p < 0.01$). Representative images of BrdU treated cells expressing various shRNAs immunostained with TGN46 antibodies are shown. **D. Effect of introducing the mouse YFP- $\gamma 11$ subunit into $\gamma 11$ knock down HeLa cells.** Mouse YFP- $\gamma 11$ was introduced transiently into cells expressing $\gamma 11$ shRNA or scrambled shRNA. Only cells transfected with YFP were counted for compact or dispersed Golgi (n=2). Plots are mean of two experiments. Percentage variability across experiments was $< 30\%$. Representative images of basal and BrdU treated cells expressing $\gamma 11$ -shRNA and mouse $\gamma 11$ -cDNA are shown.

Fig. 7. Inhibition of Golgi dispersal in BrdU treated senescent HeLa cells by the $\gamma 3$ subunit
Trans Golgi dispersal induced by incubation of HeLa cells with 200 μ M BrdU for 48 hours is inhibited in HeLa cells transiently transfected with G protein $\gamma 3$ subunit. The Golgi structure was observed by immunofluorescence using an antibody specific to the *trans* Golgi network, TGN46. Bar diagram representing the percentage of cells ($\pm$ SEM) that contained either compact or dispersed Golgi. Total cells analyzed range from 100-250 (n=3). Representative images of cells expressing YFP- $\gamma 3$ are shown. Upper panel, control untreated cells and lower panel, BrdU treated cells.