Supplementary data:

Detailed experimental methods

Cell handling and transport protocols

HeLa cells (European Collection of Cell Cultures) were grown in minimum essential medium (MEM, invitrogen) supplemented with 10% FCS, L-glutamine, 1% non-essential amino acids and HeLa-myc cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) supplemented with 10% FCS, L-glutamine and 180 µg/ml geneticin (Invitrogen). HeLa cells were transfected using the JetPEI reagent (Polyplus transfection, New York, USA) according to the manufacturer instructions. VSV infection: cells were infected with VSV as described previously (Mironov et al, 2001). Transport-pulse protocols: PC-IV and VSVG transport pulses were performed in DMEM containing 1% calf serum and 20 mM HEPES, as described previously (Bonazzi et al, 2005; Mironov et al, 2001). Cycloheximide (100 µg/ml) was added at the temperature shift or 30 min before fixing the cells, as appropriate. Intoxication with pertussis toxin: HeLa cells were plated at a concentration suitable to have 70%-90% confluence. After 6 h, the cells were treated with 400 ng/ml pertussis toxin (Calbiochem, San Diego, CA) for 16 h in the presence of complete medium and then were subjected to a traffic pulse. The steady state transport of the endocytosis defective LDL receptor (LDLR-Y18A) was used as previously described (Cancino et al, 2007). Briefly the HeLa cells have been transfected with LDLR-Y18A-GFP; 24 h later the cells have been treated for 2 hours with cycloheximide (100 µg/ml), fixed and the intracellular distribution of LDLR analyzed by immunofluorescence microscopy. The steady state transport of albumin-GFP was performed as for LDL receptor. The GFP-albumin construct was prepared by PCR amplification and sequential cloning of the pre-pro-signal region of albumin, the GFP cDNA from the pEGFP-N2 vector (Clontech), and the albumin without the signal region, into the pcDNA-3B vector. Specifically, the construct was prepared by subcloning the HindIII-BamHI-digested, PCR-amplified (forward primer, CCCAAGCTTATGAAGTGGGTAACCTTTATTTCCC; reverse primer CGCGGATCCTCGACGAAACACCCCTGG) pre-pro-albumin region into the pcDNA-3B vector, followed by the sub-cloning of the BamHI-EcoRI-digested, PCR-amplified (forward primer, CGCGGATCCGAGCAAGGGCGAGGAGC; reverse primer, CCGGAATTCCTTATACAGCTCGTCCATGCCGAG) GFP cDNA into the pre-pro-albumin-containing construct, and in turn the sub-cloning of the EcoRI-XhoI-digested, PCR-amplified albumin (forward primer, CCGGAATTCCTTATACAGCTCGTCCATGCCGAG) GFP cDNA into the pre-pro-albumin-containing construct, and in turn the sub-cloning of the EcoRI-XhoI-digested, PCR-amplified albumin (forward primer, CCGGAATTCCTTATACAGCTCGTCCATGCCGAG).
CCGCTCGAGTTATAAGCCTAAGGCAGCTTGAC) into the previous construct. The final construct was verified by direct sequencing. RNA interference:

commercially available siRNAs directed towards the Gαs (duplex6: GCAAGUGGAUCCAGUCUUU; duplex7: GCAUGCAACCUCGUAGUAUU; duplex8: AUGAGGAUCCUGCAUGUAUU; duplex9: CAACCAAAGUGCAGGACAUUU) and siCONTROL non-targeting siRNA no. 2 (duplex1: AUGAACGUGAAUUGCUCAA; duplex2: UAAAGCUAUGAAGCUAC; duplex3: AUGUAUGGCUGUAAUAG; duplex4: UGGUUAUGUGUCUCUA) were purchased from Dharmacon (CO, USA); siRNA directed towards the Gαq/11 (GAUGUUCGUGGACCUGAAC corresponding to positions 932 to 950 relative to the start codon of human Gα1 and Gα11) (Krumins & Gilman, 2006) was purchased from Sigma-Aldrich. Briefly, HeLa cells were transfected with 50 nM of Gαq/11 or Gαs siRNAs using the Hyperfect (Qiagen) reagent according to the manufacturer instructions (see details below).

Preparation and uptake of membrane-permeant peptides

The labelling of CFFKDEL and CFFKDEA peptides (Gen Script, USA) was performed as previously described (Pap et al, 2001). Briefly, an iodacetamide derivative of C2-BODIPY581/591 fluorophore (Molecular Probes, Eugene, OR, USA) was coupled to iodoacetamide by adding 50 μl 200 mM iodoacetamide in bicine buffer (50 mM, pH 8.5) to 50 μl of a 15 mM C2-BODIPY581/591 SE solution in tetrahydrofuran. The reaction was carried out under subdued light for 1 h. After the reaction, the excess iodoacetamide was removed by acidic extraction. For this purpose 200 μl chloroform, 100 μl water and 5 μl acetic acid were added, the mixture was vortexed and allowed to partition into the organic and aqueous layers; the water phase was removed and discarded. The extraction was repeated four times by addition of a synthetic upper phase. The product in the chloroform phase was dried under a stream of nitrogen. Next, a 5-fold molar excess of BODIPY-iodacetamide in tetrahydrofuran was added to 5 μg peptide dissolved in 100 μl 0.1 M Tris (pH 7.4). The reducing agent Tris-(2-carboxyethyl) phosphine (0.1 mM) was added to prevent the formation of disulfide bonds. The mixture was stirred for 24 h at RT in the dark. Subsequently the reaction mixture was extracted as describe above. With this extraction the unreacted dye partitioned into the chloroform phase and the labelled peptide into the water phase. The extraction was repeated 3 times, as before. The purity of the fluorescent products was about 95%, as verified by thin layer chromatography on silica 60 plates using as eluent: chloroform: methanol: acetic acid: water (25 : 15 : 3 : 2 v/v). Finally the peptides were further purifies by Pierce C-18 Spin Columns according to the manufacturer instructions and used for cell treatment. Briefly, the cells were incubated with 1% FCS medium for 1 h, rinsed with enriched phosphate-buffered saline (PBS+: 137 mM NaCl, 2.7
mM KCl, 0.5 mM MgCl₂, 0.9 mM CaCl₂, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, supplemented with 5 mM glucose, pH 7.4) and then incubated in PBS++ containing the fluorescent peptides (5 μg/ml) for up to 35 min. After uptake of the peptides, the cells were washed with fresh PBS++, fixed and processed for immunofluorescence.

Approaches to impair Gaₐ₉/₁₁ signalling
Three independent approaches have been used to inhibit Gaₐ₉/₁₁ signalling in order to evaluate Golgi-SFKs activation and VSVG transport. Active SFKs (p-SFKs) was detected using an antibody that selectively recognizes pTyr at position 419 (Invitrogen); VSVG on the plasma membrane was selectively stained using an anti-VSVG luminal antibody in non-permeabilized cells (see microscopy). As first approach, HeLa cells were transfected with siRNAs directed towards the Gaₐ or Gaₐ₉/₁₁ (see above). Briefly, 50 nM of siRNA and 6 µl of the Hyperfect (Qiagen) were incubated in 100 µl of OptiMEM (Invitrogen) for 10 min, in order to facilitate complex formation. The siRNA transfection mix was added to the HeLa cells cultured in complete medium. Controls consisted of non-targeting siRNA no. 2. Two days later, the cells were trypsinized, counted and plated on glass coverslips. The following day, the cells were subjected to VSVG transport pulses, fixed and processed for immunofluorescence to evaluate Golgi-SFKs activation and VSVG arrival to the plasma membrane or harvested for immunoblot analyses. For rescue experiments, cells were transfected with siRNA directed towards the Gaₐ₉/₁₁ as described above, and after 24 h trypsinised, counted, and plated on glass coverslips. The day after, cells were ritransfected with mouse Ga₁₁ (Origene Technologies) and GFP for 24 h, then exposed to PC-IV traffic pulses, fixed and processed for immunofluorescence to evaluate Golgi-SFKs activation. For VSVG transport rescue experiments cells were processed as described above and ritransfected with mouse Ga₁₁ (Origene Technologies) and VSVG-GFP (kindly provided by J. Lippincott-Schwartz, NIH, Bethesda, USA), incubated at 40°C for 16 h in complete culture medium, then shifted to 32°C for 100 min in DMEM containing 1% calf serum, 20 mM HEPES and 100 µg/mL cycloheximide. After fixation, cells were processed for immunofluorescence to evaluate VSVG arrival to the plasma membrane. The expression of mouse Ga₁₁ was evaluated by immunoblot analyses using an antibody against Gaₐ₉/₁₁ (Santa Cruz Biotechnology).

As second approach, the Gaₐ₉/₁₁ subunit C-terminal peptide was used (Gaₐ₉/₁₁ CT). HeLa cells were co-transfected with this construct and the GFP vector (as a marker of transfected cells) for 24 h, then exposed to PC-IV transport synchronization protocols (see Cell handling and transport synchronization protocols), fixed and processed for immunofluorescence to monitor Golgi-SFKs activation. To evaluate the effect of Gaₐ₉/₁₁ CT on VSVG transport, cells were co-transfected with
VSVG-GFP and Gaq/11 CT and exposed to transport synchronization protocols as described above, fixed and stained for external VSVG.

The third approach was based on the use of the GTP-bound Gaq/11 signalling inhibitor GRK2-RGS. HeLa cells were transfected with GRK-2-RGS-GFP construct for 24 h, subjected to PC IV transport synchronization protocol, fixed and stained for p-SFKs to evaluate SFKs activation. Alternatively, cells transfected with GRK-2-RGS-GFP or with pcDNA3.1 as control for 24 h were incubated with 1% FCS medium for 1 h, rinsed with PBS++ supplemented with 5 mM glucose, and then incubated in PBS containing the membrane permeant KDEL-R agonist Bodipy-KDEL (3 µM) or the control peptide Bodipy-KDEA (3 µM) at 37°C for 15 min. The cells were washed with fresh PBS++, fixed and processed for immunofluorescence to monitor SFKs.

**Protein analysis and immunoprecipitation**

The cell lysate were prepared as previously described (Pulvirenti et al, 2008). Briefly, following siRNA treatment and/or the transport protocol, as described above, the cells were washed three times with ice cold PBS and collected immediately at 4 °C in lysis buffer (1% Triton X-100, 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM Na3VO4, 1 mM PMSF, 30 mM β-glycerophosphate, 10 mM NaF) and complete protease inhibitors (5×; Roche). Cell lysates were centrifuged at 18,000g for 5 min at 4 °C to eliminate nuclei. The postnuclear supernatant was immediately processed for SDS–PAGE and western blotting. Gaq/11, SFKs (Santa Cruz Biotechnology), Ga₅ (Millipore), Ga₃/0 (Upstate), p-SFKs (Invitrogen) and actin (Cell signalling) proteins, were detected using an enhanced chemiluminescence substrate mixture (ECL Plus, Amersham). Antibodies were used in combination with a secondary horseradish peroxidase-conjugate (Calbiochem). For immunoprecipitation, total cell lysates were prepared in lysis buffer containing 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 2 mM MgCl₂, 1 mM EDTA, 1 mM β-mercaptoethanol, 1% Triton or 15 mM CHAPS (Sigma-Aldrich) and a cocktail of protease inhibitors. Subsequently the total lysates were passed through a syringe and incubated with rotation for 1.5 h at 4 °C. The lysates were cleared by centrifugation for 15 min at 20,000 xg. Supernatants (4-6 mg) were incubated with an agarose-coupled rabbit polyclonal c-myc antibody (50 µl/mg) (Santa Cruz Inc.) or an agarose-coupled goat polyclonal GFP antibody (50 µl/mg) (Vector Laboratories, Inc, CA) overnight. Settled beads were extensively washed the lysis buffer, and the bound proteins were eluted with 0.1 M ammonium hydroxide pH 11 or directly in 2X SDS-sample buffer (resin: buffer 1:1 v/v). The eluted samples were concentrated by lyophilization and resuspended in 100 µl 2X SDS-sample buffer. The eluted samples were separated by SDS-PAGE. The amount of KDEL-R-myc was detected with an anti-myc antibody. To reveal co-immunoprecipitation between RGS-GFP and Gaq/11, HeLa cells
were transfected with RGS-GFP by using a Digital Bio MicroPorator system, according to the manufacturer instructions, and incubated at 37 °C in a humidified CO₂ incubator for 48 h. Subsequently, the cells were treated with a 30 mM NaF and 50 µM AlCl₃ mix in 1% FCS culture medium for 30 min, and harvested for immunoprecipitation (see above) and immunoblot analyses. The amount of RGS-GFP was detected with an anti-GFP antibody (Abcam, Cambridge, UK). In this case, Rabbit IgG TrueBlot (eBiosciences) was used as secondary antibody to detect target protein, without hindrance by interfering immunoprecipitating immunoglobulin heavy and light chains. To reveal possible changes in the co-immunoprecipitation between KDEL-R and Gα₁₁ upon KDEL-R activation, HeLa-myc cells were transfected with Gα₁₁ and incubated at 37°C in a humidified CO₂ incubator for 48 h. Subsequently, the cells were treated at 37°C with 3 µM Bodipy-KDEL or Bodipy-KDEA as control in DMEM containing 20 mM HEPES on a shaker incubator, and harvested for immunoprecipitation (see above) and immunoblot analyses. The amount of KDEL-R-myc and co-immunoprecipitated Gα₁₁ was detected with an anti-myc antibody and an anti-Gα₁₁ antibody respectively.

**Biotinylation assay for surface VSVG detection**

HeLa cells treated for 48 h with siRNAs directed towards the Gα₁₁ were transfected with VSVG-GFP plasmid, incubated for 16 h at 40°C in complete culture medium (to accumulate VSVG in the endoplasmic reticulum, ER), then shifted to 32°C in DMEM containing 1% calf serum, 20 mM HEPES and 100 μg/mL cycloheximide (to initiate transport from the ER towards the plasma membrane, PM). To detect surface VSVG, 100 min after the release of the temperature block cells were washed three times with ice-cold PBS++ pH 8, agitated gently at 4°C for 30 minutes in PBS++ containing 0.55 mg/mL sulfo-NHS-biotin, and then washed 10 times in PBS++ 100 mM glycine. The cells were lysed with 1% Triton X-100 in TBS-150 (50 mM Tris, 150 mM NaCl, pH 7.5) containing Complete Protease Inhibitor Cocktail (Roche). The lysates were cleared by centrifugation for 15 min at 20,000xg. Supernatants (1 mg) were incubated with and an agarose-coupled goat polyclonal GFP antibody (50 μl/mg) (Vector Laboratories, Inc, CA) overnight. Settled beads were extensively washed with the lysis buffer and the bound proteins were eluted in 2X SDS-sample buffer (resin: buffer 1:1 v/v). The eluted samples were separated by SDS-PAGE. The amount of VSVG-GFP on the cell surface was detected by peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, INC.), the total VSVG-GFP was detected with an anti-GFP antibody (Abcam, Cambridge, UK).
**Microscopy**

Immunofluorescence microscopy (LSM 510, LSM710 (Zeiss) and SP5 (Leica) laser scanning confocal microscopes) were as described previously (Pulvirenti et al, 2008). The Golgi G-proteins immunofluorescence staining in Supplementary Figure S1 was obtained from streptolysin-O-permeabilized cells, as described previously (Mironov et al, 1997). Quantification of immunofluorescence in the Golgi area was defined by using Golgi-marker images (with GM130, BDbiosciences or giantin, Abcam). Images were acquired with pinhole size and amplification gain optimized for intensity values between 1 and 254 (linear range). The total immunofluorescence within each area of interest was acquired, and the immunofluorescence intensity was calculated by integration of the immunofluorescence signal within the region of interest divided by the area. VSVG on the plasma membrane was selectively stained using an anti-VSVG luminal domain antibody in non-permeabilized cells. Total VSVG was stained using the P5D4 anti-VSVG antibody after the cells had been permeabilized (Polishchuk et al, 2004). All experiments were carried out three times, and immunofluorescence was quantified in at least 40 cells (from two wells) per point per experiment using the LSM510-3.2 software (Zeiss) or MetaMorph software (Molecular Devices) as previously described (Cancino et al, 2007). Sampling of cells was performed randomly. All samples were processed equally and evaluated in a blind fashion. The results are shown graphically on an arbitrary scale (AU).

**Isolation of Golgi-enriched membranes from rat liver and from HeLa cells**

The isolation of Golgi membranes was performed as previously described (Taylor et al, 1997). Briefly, six male Sprague-Dawley rats (180-200 g) were starved for 20 h and then killed by decapitation. Livers were finely minced and resuspended at 10 ml/ 6 g 0.5 M phosphate-buffered sucrose containing 100 mM KH2PO4/ K2HPO4, pH 6.8, 5 mM MgCl2, and 4 µg of a mixture of complete protease inhibitors (Roche). All sucrose solutions contained the same buffer and proteolytic inhibitors. Homogenization was carried out by Ultraturrax (T25, Janke and Kunkell). The homogenate was centrifuged at low speed (1500 xg for 10 min) to pellet unbroken cells, cell debris and nuclei. The resulting postnuclear supernatant (PNS, 12 ml) was loaded in the middle of a sucrose step gradient in an SW28 tube. The gradient was centrifuged at 100,000 xg in a swing-out rotor for 1 h with the brake off (Beckman Instruments, Palo Alto, CA). The following fractions were collected from the top of the gradient: SI, the 0.25- 0.5 M interface; SII, the 0.5- 0.86 M interface; SIII, the 0.86- 1.3 M interface. Small aliquots of SII fraction were frozen in liquid nitrogen and stored at −80 °C. Golgi fractions from HeLa cells were prepared following a modification of the method established by Balch et al. (Balch et al., 1984). Forty plates (15-cm
diameter) of HeLa cells were transfected with the empty vector (12 µg) or KDEL-RD193N-Myc constructs (12 µg) by microporation. The cells were cultured for 48 h. Subsequently, the cells were trypsinized and centrifuged 10 min at 500 xg, washed twice with DPBS (10 min at 500 xg), twice with homogenization buffer (250 mM sucrose in 10 mM Tris-HCl, pH 7.4; 10 min at 1,500 xg), and resuspended in four volumes of homogenization buffer. The cells were finally homogenised using the Balch ball-bearing device. The homogenate was brought to a sucrose concentration of 37% (wt/v) by the addition of 62% sucrose in 10 mM Tris-HCl, pH 7.4 (wt/v), and EDTA (1 mM, final concentration). Twelve milliliters of this solution was placed at the bottom of an SW28 tube and carefully overlaid with 15 ml 35% sucrose (wt/v) and 9 ml 29% sucrose (wt/v) in 10 mM Tris-HCl (pH 7.4). The gradients were centrifuged at 25,000 rpm for 2.5 h in the SW28 rotor. The Golgi-enriched membrane fraction was recovered at the 35%–29% sucrose interphase and the enrichment in Golgi proteins was evaluated comparing this fraction with total lysate by immunoblotting for TGN46, Gαq/11, and KDEL-R (Stressgene). The Golgi-enriched membrane fraction were frozen in aliquots in liquid nitrogen and stored at −80 °C. Protein concentrations were determined using the Bradford assay (Biorad).

**GTPγS binding assay**

The [35S]GTPγS binding assay follows the protocol previously described by Akam et al. (Akam et al, 2001). To detect [35S]GTPγS binding to Gαq/11, the membrane aliquots of Golgi enriched-membranes from rat liver or HeLa cells (see above) were diluted 1:4 in assay buffer (100 mM NaCl, 10 mM MgCl2, and 10 mM Hepes, pH 7.4) with complete protease inhibitors, and centrifuged at 18,000 xg for 10 min to remove excess sucrose. Subsequently, the membranes were resuspended in assay buffer to give a final protein concentration of 1 µg/µl, using 100 µg and 30 µg of Golgi enriched-membranes from rat liver and from HeLa cells, respectively. These membranes were added to 50 µl assay buffer containing 2 nM [35S]GTPγS (1,000 Ci/mmol) (PerkinElmer, USA), 2 µM GDP, without or with Bodipy-KDEL/KDEA (1-3 µM), and/or non-related IgGs (6 µg) or anti-KDEL-R Ab (2-6 µg) and then incubated at 30 °C for 5 min. Non-related IgGs and anti-KDEL-R Ab were preincubated for two hours at 4°C. The reactions were terminated by the addition of ice-cold assay buffer. The samples were then centrifuged at 20,000 xg for 10 min, and the pellets were solubilized by the addition of 100 µl ice-cold solubilization buffer (100 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, and 1.25% Igepal CA 630, pH 7.4 containing 0.2% SDS). Once the pellets were completely solubilized, an equal volume of solubilization buffer without SDS was added to each tube. The solubilized pellets were precleared with 1 µg/ml rabbit IgG and 30 µl of protein A-Sepharose suspension (Sigma-Aldrich) (30% w/v in TE buffer [10 mM Tris-HCl, 10 mM EDTA,
pH 8.0]) for 30 min at 4 °C. After centrifugation at 20,000 xg for 5 min, 100 µl of the supernatant was transferred to a fresh tube containing 4 µl anti-Gα_{11}. The samples were allowed to stand for 60 min at 4 °C, and then added to 140 µl protein A-Sepharose suspension and rotated for 60 min at 4 °C. The protein A-Sepharose beads were pelleted at 20,000 xg and washed three times with 500 µl solubilization buffer (without SDS). The tubes containing the washed beads were put into vials, mixed with scintillant (Ultima Gold, Packard Bioscience), and the radioactivities determined via scintillation counter.To detect [{^35}S] GTPγS incorporated into the immunoprecipitated KDEL-R complex, HeLa cells stably expressing KDEL-R-myc were transfected with Gα_{11} or pCDNA3.1 as control. After 24 h, the protein extracts were prepared and incubated with an agarose-coupled rabbit polyclonal c-myc antibody (50 µl/mg) (Santa Cruz, Inc.) as described in protein analysis and immunoprecipitation. Agarose beads were extensively washed with the lysis buffer and then subjected to a modified [{^35}S] GTPγS binding assay. Briefly, the beads were resuspended in assay buffer containing 5 nM [{^35}S] GTPγS (2,500 Ci/mmol) (Perkin Elmer, USA), 2 µM GDP, 3 µM Bodipy-KDEL or Bodipy-KDEA and incubated at 30°C for 10 min. The reactions were terminated by the addition of ice-cold assay buffer. The samples were then washed five times with 1 mL ice-cold assay buffer to remove the unincorporated [{^35}S] GTPγS. The tubes containing the washed beads were put into vials, mixed with scintillant (Ultima Gold, Packard Bioscience), and the radioactivity determined via scintillation counter.

**Computational modelling of the KDEL-R**

The structural model of the KDEL-R was built by means of the comparative modelling software MODELLER (Sali & Blundell, 1993), by using the crystal structure of sensory rhodopsin II (i.e. PDB code: 1gue) as a template for the seven-helix bundle. Template selection privileged degree of completeness and quality of the structural model among the ones available in the PDB. Five of the six loops were modelled following an *ab initio* approach implemented in the same program. To allow for *ab initio* loop modelling, the following portions characterized by lack of sequence similarity between template and target were eliminated from the template: 27-34 (i.e., first intracellular loop), 57-70 (first extracellular loop), 92-121 (second extracellular loop), 181-195 (third extracellular loop 3 and first six amino acids of helix 7) and 213-219 (C-terminal of helix 7). During comparative modelling, α-helical restraints were assigned to the KDEL-R sequences 20-25, 58-69, 115-122, 166-173 and 177-189 (i.e., C-term of H1, N-term of H3, N-term of H5, C-term of H6 and N-term of H7, respectively). Moreover, β-strand restraints were assigned to the 206-209 amino acid stretch in the C-tail. Eleven different alignments and 9 different distance restraints were probed, leading to the building of 2000 structural
models. The alignment that led to the finally selected model is shown in Supplementary Figure S2A. Such model was finally subjected to refinement of the six loops (i.e. the 26-32, 52-57, 86-93, 141-145 and 173-177 amino acid segments), by means of the LOOP routine within the MODELLER software (Fiser et al, 2000), leading to other 100 models. Amongst the first 15 models holding the lowest violation of stereochemical restraints, the one was selected that was characterized by an optimal combination of goodness of main chain torsion angles, consistency with secondary structure predictions, and high 3D Profile score. In this model, the highest incongruence with secondary structure predictions concerns the second intracellular loop and helix 4 (Supplementary Figure S3A).
Supplementary Figure legends

Figure S1. Endogenous Gαq/11 and Gαs localise to the Golgi complex in HeLa cells. The cells were grown under standard conditions, permeabilised with streptolysin O, and stained with antibodies against the different Gα classes (left panels, red) and against giantin (marker for Golgi area definition; middle panels, green). Merged images of red and green signals are shown in the right panels (overlay). Scale bars, 10 μm.

Figure S2. Sequence and structural comparison between the sensory rhodopsin and the KDEL-R. (A) The sequence alignment between sensory rhodopsin (1GUE) and the KDEL-R (KDELR) used for comparative modelling is shown. Star, colon, and point indicate, respectively, identical, highly similar, and similar amino acids. (B) The Cα-atom superimposition between the KDEL-R model (violet) and the modified template (green) is shown; the helix-bundle is seen in a direction parallel to the membrane surface, with the intracellular side at the top.

Figure S3. Prediction of the KDEL-R secondary structure. (A) Secondary structure predictions have been performed by PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/), JPred (http://www.compbio.dundee.ac.uk/www-jpred/index.html) and PHD (http://www.predictprotein.org/) servers. The numbers give the reliability scores of each of the three predictions. The coloured and shadowed sequences indicate the regions in the α-helical conformation as computed for the predicted model. (B, C) Structural comparison between the KDEL-R model and bovine rhodopsin. The Cα-atom superimposition between the seven helices of the KDEL-R model (violet) and the transmembrane portions of the corresponding helices of bovine rhodopsin (green) is shown. In the panel B, the helix-bundles are seen in a direction parallel to the membrane surface, the intracellular side being on top. In the panel C, the helix bundles are seen, from the intracellular side, in a direction perpendicular to the membrane surface. The Cα-Root Mean Square Deviation relative to 147 superimposed pairs is 3.89 Å.

Figure S4. Transfection of exogenous Gα11 rescues SFKs activation and VSVG transport to the plasma membrane in Gαq/11 interfered cells. (A) HeLa cells were treated with non-targeting siRNAs (Ctrl) and siRNAs against Gαq/11 (Gαq/11siRNA) for 48 h, then transfected
with mouse Gα11 (Rescue) or with pCDNA3.1 as control (Ctrl and Gαq11SiRNA) for 24 h. The cells lysates were analysed by immunoblotting for Gαq, with actin as the loading control. (B) HeLa cells treated as in A, were co-transfected 48 h later with GFP and pCDNA3.1 (Ctrl and Gαq11siRNA), or with GFP and mouse Gα11 (Rescue) for 24 h, then exposed to traffic pulse as described in figure 3. The GFP vector was included to detect the transfected cells. After fixation, cells were stained for active SFKs (p-SFKs, red) and GM130 (marker for Golgi area definition, blue). Merged images are shown (pSFKs/GM130/GFP). Scale bars, 10 µm. (C) Quantification of data illustrated in B. The p-SFKs immunofluorescence intensities at the Golgi complex are expressed as arbitrary units (AU). Data are means (± SE) from three independent experiments. ***p<0.001, 32°C Ctrl compared to 40°C Ctrl; ***p<0.001, 32°C Gαq11siRNA compared to 32°C Ctrl; ***p<0.001, 32°C Rescue compared to 40°C Rescue and 32°C Gαq11siRNA (ANOVA analysis). (D) HeLa cells treated as in A were co-transfected 48 h later with VSVG-GFP and pCDNA3.1 (Ctrl and Gαq11siRNA) or with VSVG-GFP and mouse Gα11 (Rescue), then incubated at 40°C for 16 h (temperature block), and shifted to 32°C for 100 min (temperature-block release). Panels 30 min: control cells (Ctrl) and siRNAs-treated cells (Gαq11siRNA and Rescue, respectively) were fixed and stained for GM130 (marker for Golgi area definition, red). Merged images of green and red signals are shown (Total VSVG/GM130). Panels 100 min: total VSVG-GFP (green); immunostaining of external VSVG (red) and GM130 (blue). Merged images of green, red and blue signals are shown (VSVG/GM130). Scale bars, 10 µm. (E) Quantification of data illustrated in D. VSVG immunofluorescence intensities at the plasma membrane (PM) were calculated as the ratio of VSVG on the PM to the VSVG on the Golgi, and expressed as arbitrary units (AU). Data are means (± SE) from three independent experiments. ***p<0.001, Gαq11siRNA compared with Ctrl; ***p<0.001, Rescue compared with Gαq11siRNA (ANOVA analysis).

**Figure S5. Expression of Gαq11 minigene prevents traffic-pulse-dependent Golgi-SFKs activation and VSVG transport to the plasma membrane.** (A) HeLa cells were co-transfected with pcDNA3.1 and GFP as control (pcDNA3.1/GFP), or with the Gαq11 minigene and GFP (Gαq11CT/GFP) for 24 h. The cells were then exposed to traffic pulse as described in figure 3, then fixed and stained for active SFKs (p-SFKs, red) and GM130 (marker for Golgi area definition, blue). Merged images are shown (p-SFKs/GM130/GFP). Scale bars, 10 µm. (B) Quantification of data illustrated in A. The p-SFKs immunofluorescence intensities at the Golgi complex are expressed as arbitrary units (AU). Data are means (± SE) from a single representative experiment, which was performed four times. ***p<0.001, 32°C pcDNA3.1
compared with 40°C pcDNA3.1; ***p<0.001, Gaq11CT compared with 32°C pcDNA3.1 (ANOVA analysis). (C) HeLa cells were co-transfected with VSVG-GFP and pcDNA3.1 vector as control (VSVG-GFP/pcDNA3.1) or with VSVG-GFP and Gaq11CT subunit C-terminal peptide (VSVG-GFP/Gaq11CT) and incubated at 40°C for 16 h (temperature block), then shifted to 32°C for 100 min (temperature-block release). Panels 30 min: cells were fixed and stained for GM130 (marker for Golgi area definition, red). Merged images of green and red signals are shown (Total VSVG/GM130). Panels 100 min: total VSVG-GFP (green); staining of external VSVG (red) and GM130 (blue). Merged images of green, red and blue signals are shown (VSVG/GM130). Scale bars, 10 µm. (D) Quantification of data illustrated in C. VSVG immunofluorescence intensities at the plasma membrane (PM) were calculated as the ratio of VSVG on the PM to the VSVG on the Golgi, and expressed as arbitrary units (AU). Data are means (± SE) from three independent experiments. ***p<0.001, Gaq11CT versus control (Student’s t-test).

Figure S6. Cell-permeable KDEL ligands mimic the effects of a traffic pulse. (A), Bodipy-KDEL redistributes the KDEL receptor into the ER. HeLa cells were treated with the membrane permeant KDEL-R agonist Bodipy-KDEL (3 µM) or Bodipy-KDEA (3 µM; control peptide that cannot bind the KDEL-R) at 37 °C, for the indicated times. The cells were then fixed and stained for KDEL-R (upper panels, green) and giantin (marker for Golgi area definition, red). Merged images of green and red signals are shown (bottom panels). Scale bars, 10 µm. (B) Quantification of KDEL-R in the Golgi area (Golgi IF), as the ratio of Golgi to total cellular immunofluorescence, in cells treated with Bodipy-KDEA (white bars) and Bodipy-KDEL (black bars). Data are means (±SE) from three independent experiments. *** p<0.001 compared to Bodipy-KDEA 15 min (ANOVA analysis). (C) Bodipy-KDEL triggers Golgi-SFKs activation. HeLa cells were treated as in a, for the indicated times Then the cells were fixed and stained for active SFKs (p-SFKs, red, upper panels) and GM130 (green). Merged images of red and green signals are shown (bottom panels). Scale bars, 10 µm. (D) Quantification of the effects of Bodipy-KDEA (white bars) and Bodipy-KDEL (black bars) treatments on p-SFKs in the Golgi area. The p-SFKs immunofluorescence intensities at the Golgi complex are expressed as arbitrary units (AU). Data are means (±SE) from four independent experiments. *** p<0.01 compared to Bodipy-KDEA 10 min (ANOVA analysis).
Figure S7. KDEL-R stimulation activates Golgi-SFKs via Ga_q/11. (A) HeLa cells were transfected with pcDNA3.1 (Ctrl) or GRK2-RGS-GFP. After 24 h, the cells were treated with the membrane permeant KDEL-R agonist Bodipy-KDEL (3 µM) or the control peptide Bodipy-KDEA (3 µM) at 37°C for 15 min. The cells were then fixed and stained for active SFKs (p-SFKs, red) and GM130 (marker for Golgi area definition, blue). Merged images of red and blue signals or red, green and blue signals are shown for control (Ctrl) and GRK2-RGS-GFP respectively. Scale bars, 10 µm. (B) Quantification of data illustrated in A. The p-SFKs immunofluorescence intensities at the Golgi complex are expressed as arbitrary units (AU). Data are means (± SE) from a single representative experiment, which was performed two times. 100 cells were quantified. ***p<0.001, Ctrl Bodipy-KDEL compared with Ctrl Bodipy-KDEA; ***p<0.001 GRK2-RGS-GFP Bodipy-KDEL compared to Ctrl Bodipy-KDEL (ANOVA analysis).

Figure S8. Ga_q/11 is activated at the Golgi complex by traffic pulse KDEL-R stimulation. (A) HeLa cells were transfected with GRK2-RGS-GFP and after 24 h the cells were infected with VSV for 1h and incubated at 40°C for 3 h to accumulate VSVG on ER, then shifted to 32 °C for 10 min (temperature-block release) and fixed. The cells were stained for GM130 (marker for Golgi area definition, red) and DAPI (marker for nuclear area definition, blue). Merged images of green (GFP) and blue signals or green (GFP) red and blue signals are shown in the upper and lower panels respectively. Insets show the DAPI staining (blue) and the distribution of VSVG labelled in parallel samples (red). Scale bars, 10 µm. (B) Quantification of recruitment of GRK2-RGS-GFP to the Golgi complex, as the ratio of its Golgi to cytosol immunofluorescence. Data are means (±SE) from three independent experiments. *** p<0.001 compared with time 0 (Student’s t-test).
References


Supplementary Figure 1
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Supplementary Figure 4
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Supplementary Figure 8
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