Supplementary Video Legends

Supplementary Video 1. Brefeldin A (BFA) induces Golgi absorption in wild-type cells, but not in GBF1^{M832L} cells

The Golgi apparatus is shown before (0 min) and after BFA administration in wild-type (left) and GBF1^{M832L} cells (right). The image shows the *cis*-Golgi marker (Rb::GM130) in red, the *medial* Golgi marker (ManII::EGFP) in green, and the *trans*-Golgi marker (GalT::iRFP) in blue.

Scale bars: 10 μm

Supplementary Video 2. Dynamics of recycling endosomes (REs) are reduced by BFA administration in MT-disrupted GBF1^{M832L} cells

RE dynamics in MT-disrupted GBF1^{M832L} cells before (left) and after (right) BFA administration are shown. GalT::iRFP is shown in magenta, and the RE marker (NG::Rab11a) is shown in green. Each frame is a volume-rendered 3D image captured at 5-second intervals and captured using confocal live imaging microscopy (SCLIM).

Grid: 1.51 (left) or 1.57 (right) μm

Supplementary Video 3. Accumulation of vesicles on the *trans* side of Golgi stacks in BFA-treated GBF1^{M832L} cells

Scanning electron micrographs of serial sections with a 50-nm interval of a Golgi stack in a GBF1^{M832L} cell after 60 min of incubation with (right) or without (left) 10 μM BFA. Arrows indicate hemifused vesicles. Arrowheads indicate constricted connections between vesicles.

Scale bar: 500 nm

Supplementary Video 4. Cargo transport in BFA-treated GBF1^{M832L} cells

Time-lapse movies showing NG::GPI, NG::VSVG, and TNFα::NG transport initiated by the BME-RUSH system in untreated (left), Golgicide A (GCA)-treated (middle), and BFA-treated (right) cells. The cargoes are shown in green, and GalT::iRFP is shown in magenta.

Scale bar: 10 μm

Supplementary Video 5. Double cargo transport

Time-lapse movies showing the transport of NG::VSVG (green) and Sca::GPI (red), or NG::VSVG (green) and TNFα::Sca (red), initiated by the BME-RUSH system. GalT::iRFP is indicated in blue. Grid: 2.12 μm

Supplementary Video 6. Glycosylphosphatidylinositol-anchored protein (GPI-AP) transport in nocodazole- and BFA-treated GBF1^{M832L} cells

The time-lapse movies show NG::GPI transport initiated by the BME-RUSH system in untreated (left), BFA-treated (middle), and BFA-treated cells with washout (right). NG::GPI is shown in green, and GalT::iRFP is shown in magenta.
Scale bar: 10 μm

Supplementary Video 7. Cargo transport in BFA-treated GBF1^{M832L} cells

Time-lapse movies showing the recruitment of ARF1::NG or ARF3::NG (green) to the Golgi/RE unit and the efflux of Sca::GPI (red) from the Golgi/RE unit after BFA washout. BFA was added 5 min after BME administration, and washed 55 min later. GalT::iRFP is indicated in blue.
Grid: 2.12 μm

Supplementary Video 8. Sca::GPI transport into REs after BFA-washout in GBF1^{M832L} cells

The high-speed time-lapse movies show the RUSH cargo Sca::GPI (red) transported into the REs after BFA washout. GalT::iRFP is shown in magenta, and NG::Rab11a is shown in green. Numbers in the bottom-right corner represent the time after BFA washout. Each frame is a volume-rendered 3D image captured every 5 s and collected using SCLIM.
Scale bar: 1 μm

Supplementary Video 9. GPI-AP localization before and after BFA-washout in GBF1^{M832L} cells

Scanning electron micrographs of serial sections of a Golgi stack at 50-nm intervals in GBF1^{M832L} cells expressing APEX2::GPI before and after BFA washout. The frames are shown in Fig. 6a–h and Extended Data Fig. 4a,b.
Scale bar: 200 nm

Supplementary Video 10. AP1 and clathrin are diffused and recruited by BFA administration and washout in GBF1^{M832L} cells

Time-lapse videos showing NG::Clc (red), AP1M1::Sca (green), and GalT::iRFP (blue) before and after BFA treatment and BFA washout.

Grid: 2.11 μm

Supplementary Video 11. AP1 is recruited to the membrane with GPI-AP after BFA washout

Time-lapse movies show AP1M1::HT-SF650T (red, left), NG::GPI (green), GalT::iRFP (blue), and Sca::Rab11a (red, right) after BFA washout.

Grid: 2.11 μm

Supplementary Video 12. Cargo transport using RudLOV

Time-lapse movies showing GPI-AP or TNFα::HT-SF650T transport. Cargo transport was initiated with illumination at 445 nm for 5 min using RudLOV.

Grid: 2.11 μm

Supplementary Video 13. RE dynamics are reduced in MT-disrupted AP1G-DKO and AP1-QKO cells

Dynamics of REs in MT-disrupted wild-type, AP1G-DKO, and AP1-QKO cells. GalT::iRFP is shown in magenta, and NG::Rab11a is shown in green. Each frame is a volume-rendered 3D image captured at 5-second intervals and collected using SCLIM. The movie was run at eight frames per second.

Scale bar: 2 μm

Supplementary Video 14. GPI-AP transport in nocodazole-treated and untreated AP1G-DKO cells

The time-lapse movies show NG::GPI transport initiated by the BME-RUSH system in both nocodazole-treated and untreated wild-type, AP1G-DKO, and AP1-QKO cells. NG::GPI is shown in green, and GalT::iRFP is shown in magenta.

Scale bar: 10 μm

Supplementary Video 15. GPI-AP transport in nocodazole-treated and -untreated AP1G-DKO cells

The high-speed time-lapse movies show the RUSH cargo HT::GPI-JF549 (red) transported into REs after BFA washout. GalT::iRFP is shown in magenta, whereas NG::Rab11a is shown in green. Numbers in the bottom-right corner represent the time after BFA washout. Each frame is a volume-rendered 3D image captured every 5 s.

Grid: 2.17 μm

Supplementary Video 16. GPI-AP localization in AP1G-DKO and AP1-QKO cells

Scanning electron micrographs of serial sections of a Golgi stack at 50-nm intervals in AP1G-DKO and AP1-QKO cells expressing APEX2::GPI after BFA washout or without BFA treatment. The frames are shown in Fig. 8f–h and Extended Data Fig. 8c–g.

Scale bar: 200 nm