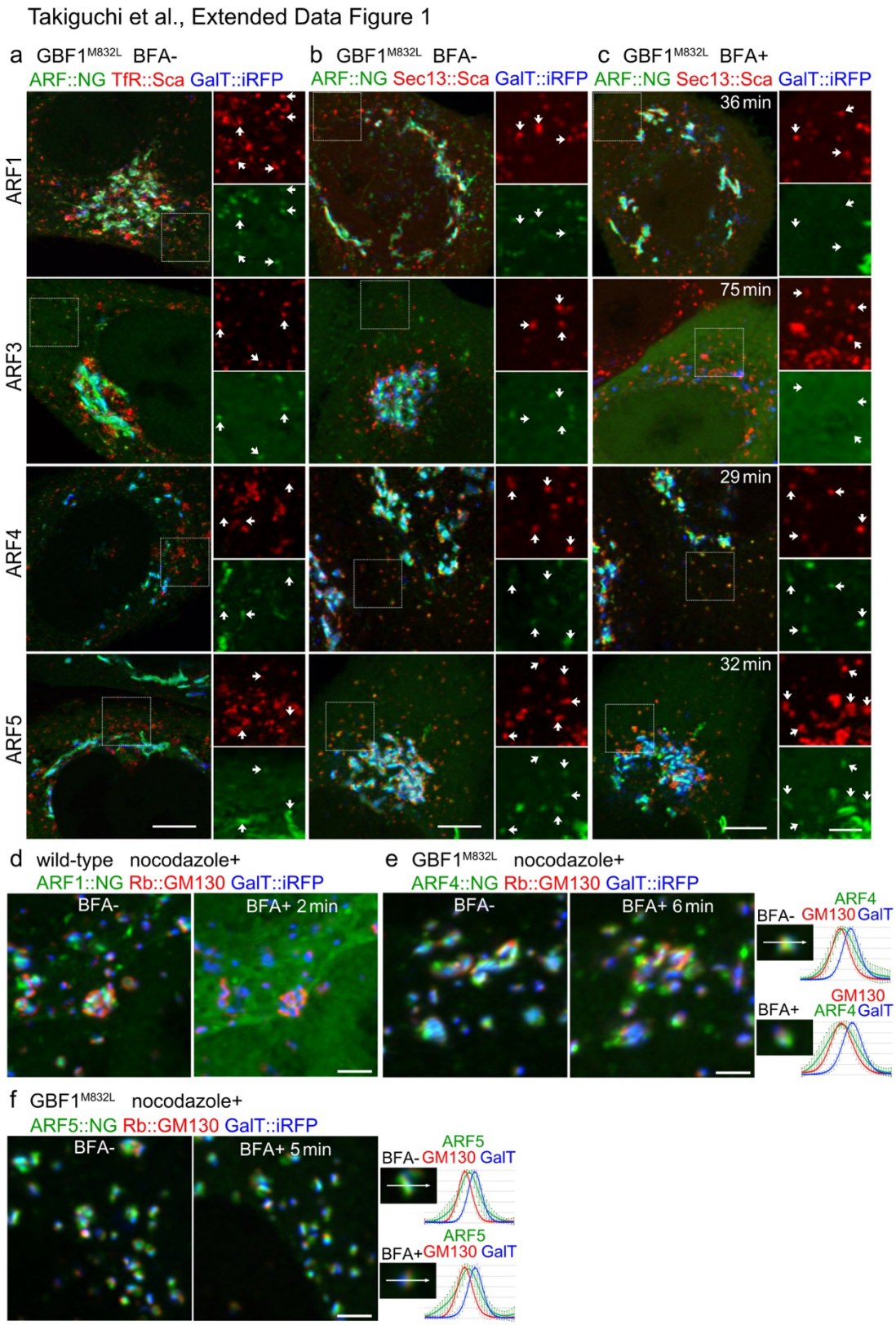
Inventory of Supporting Information



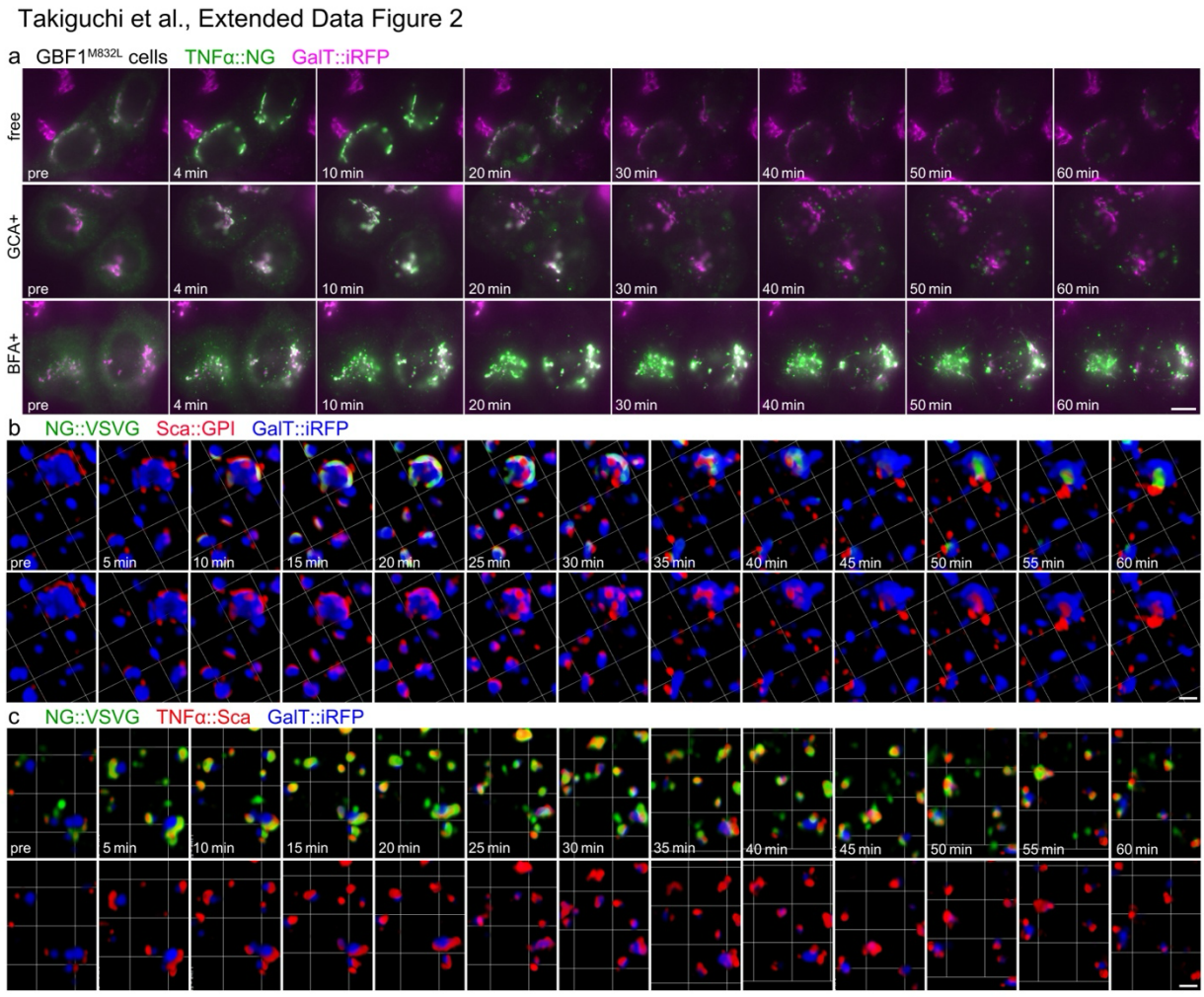
Extended Data Fig. 1. ARF1 and ARF3 are sensitive to brefeldin A (BFA) in GBF1^{M832L} cells

a–c Localization of ARF1::NG, ARF3::NG, ARF4::NG, and ARF5::NG (green) before (a, b) and after (c) BFA administration in GBF1^{M832L} cells. The recycling endosome (RE) marker (TfR::Sca) is shown in red in (a), and the endoplasmic reticulum (ER) exit site (ERES) marker (Sec13::Sca) is shown in red in (b, c). The *trans*-Golgi marker (GalT::iRFP) is indicated in blue. The time indicated in the upper-right corner represents the incubation time with BFA.

d ARF1 localization before (left) and 2 min after (right) BFA administration in nocodazole-treated wild-type cells. The *cis*-Golgi marker (Rb::GM130) is shown in red, GalT::iRFP is shown in blue, and ARF1::NG is shown in green.

e, f ARF::NG4 (e) and ARF5::NG (f) localization before (left) and 5 or 6 min after BFA administration (right) in nocodazole-treated GBF1^{M832L} cells (l, m). Rb::GM130 is shown in red and GalT::iRFP is shown in blue. ARF4::NG (e) and ARF5::NG (f) are shown in green. The plots show the normalized mean values for 15 Golgi markers and ARF line profiles across the Golgi stack. The image on the left shows a typical Golgi stack.

Scale bars: 5 μm (a–c), 2 μm (insets of a–c), and 2 μm (d–f)



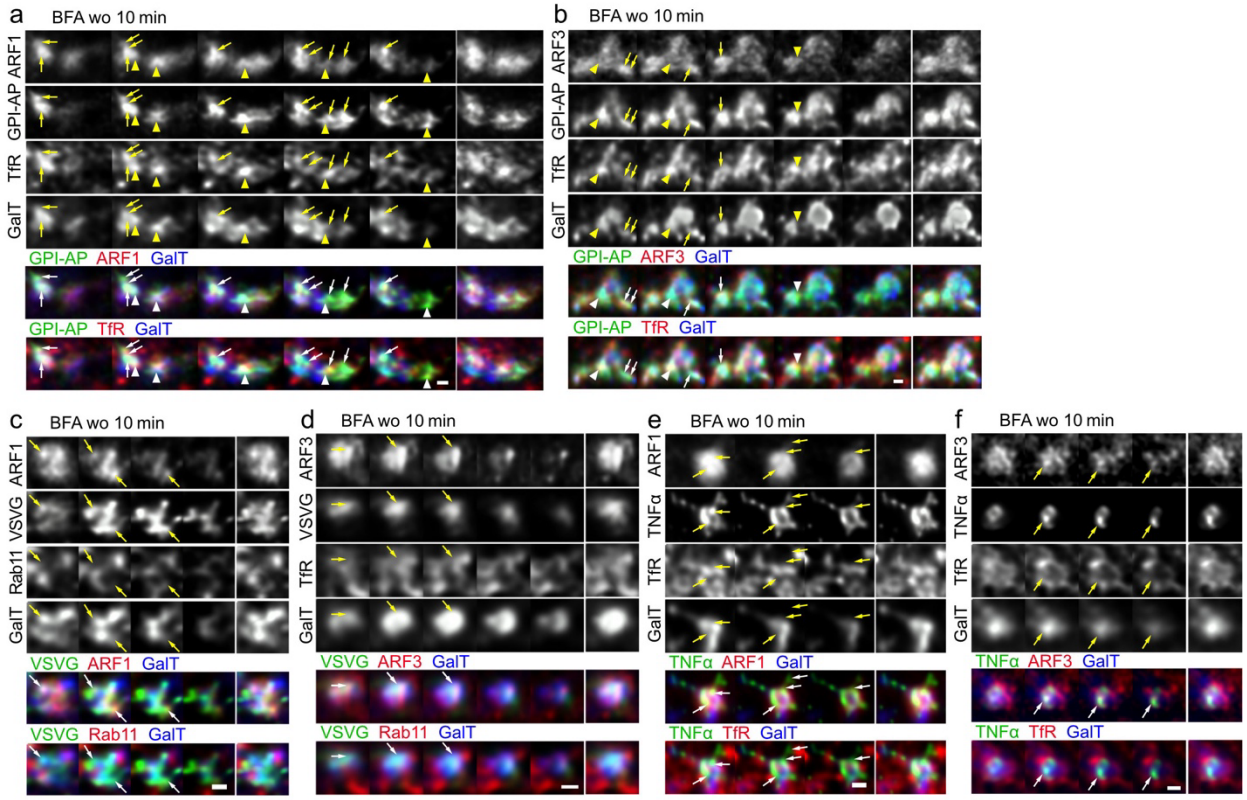
Extended Data Fig. 2. Vesicular stomatitis virus glycoprotein (VSVG) and tumor necrosis factor-alpha (TNFα) but not glycosylphosphatidylinositol-anchored protein (GPI-AP) strongly co-localize during transport

a Frames from a time-lapse movie showing TNFα::NG transport initiated by the BME-RUSH system in Golgicide A (GCA) (middle), BFA-treated (bottom), or untreated cells (top). TNFα::NG is shown in green, and GalT::iRFP is shown in magenta.

b, c Frames of the time-lapse movie showing NG::VSVG (green) and Sca::GPI (red) transport (b) or NG::VSVG (green) and TNFα::Sca (red) transport (c) initiated by the BME-RUSH system. GalT::iRFP is indicated in blue.

Scale bars: 10 μm (a) and 1 μm (b, c)

Takiguchi et al., Extended Data Figure 3



Extended Data Fig. 3. ARF1 and ARF3 co-localize with GPI-AP, VSVG, and TNFα at the *trans*-Golgi network (TGN)

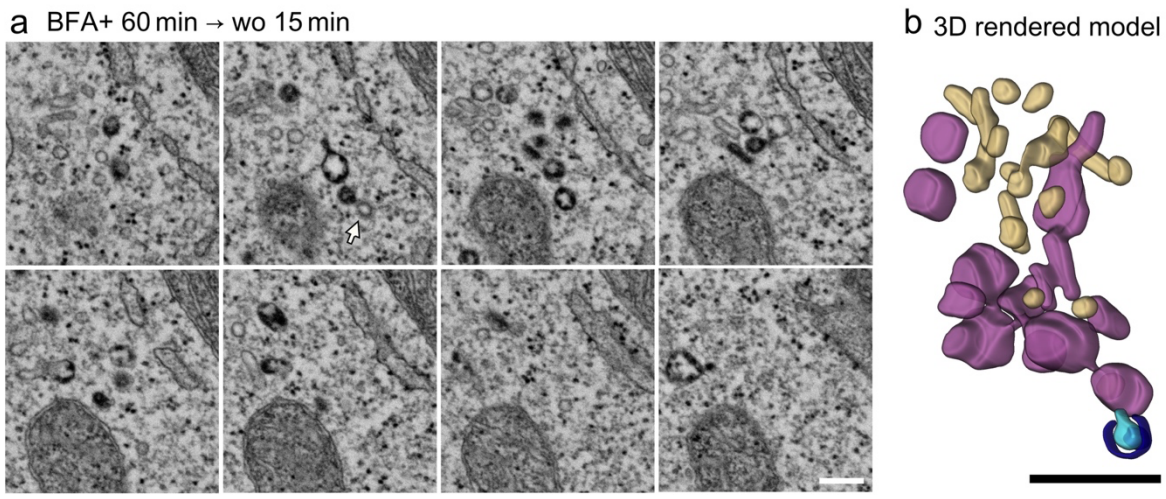
Localization of cargo and ARF1/3. Cargo transport from the ER was initiated using the BME-RUSH system. BFA was administered 5 min after the addition of BME, leading to the accumulation of NG::GPI around the TGN. BFA was washed out 60 min after BME treatment, and the cells were fixed for 10 min. Arrows indicate the co-localization of ARF1/3::tgB and cargo. Arrowheads indicate the localization of the cargo to the REs.

a, b AFR1::tgB (a) or ARF3::tgB (b) is shown in red. NG::GPI (green), GalT::iRFP (blue), and TfR, an RE marker, were detected using an anti-TfR antibody (Alexa Fluor 568) (red).

c, d AFR1::tgB (c) or ARF3::tgB (d) is shown in red. NG::VSVG is indicated in green, GalT::iRFP is shown in blue, and the RE marker Rb::Rab11a is presented in red (c). TfR was detected using an anti-TfR antibody (Alexa Fluor 568) (red).

e, f AFR1::tgB (e) or ARF3::tgB (f) is shown in red. TNFα::NG (green), GalT::iRFP (blue), and TfR were detected using an anti-TfR antibody (Alexa Fluor 568) (red).

Scale bars: 500 nm (a–f)



Extended Data Fig. 4. Clathrin bud without GPI-AP connected to GPI-AP-positive cisternae

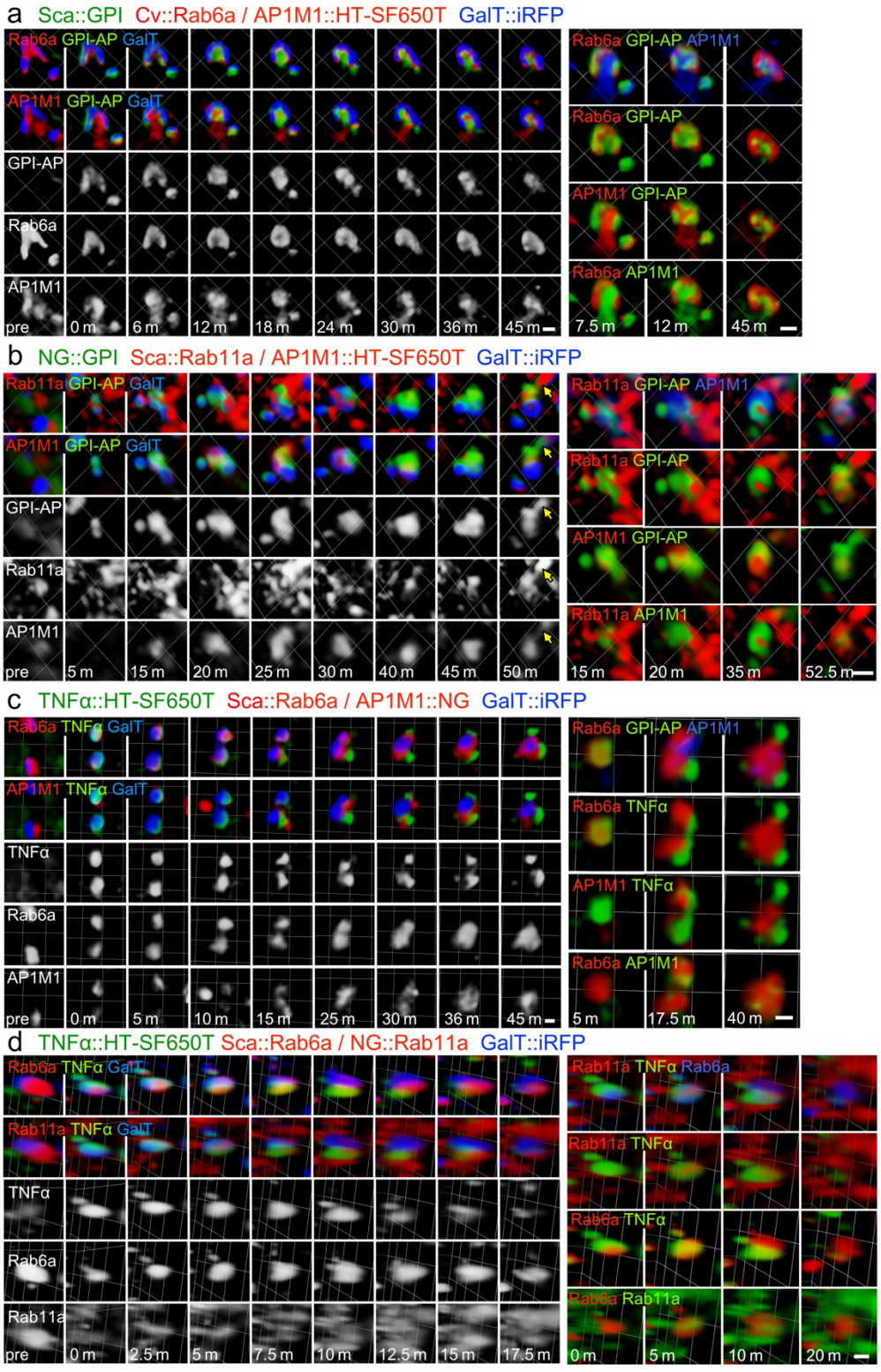
a Scanning electron micrograph of serial sections of APEX2::GPI-positive vesicle clusters at 50-nm intervals in a GBF1^{M832L} cell. BFA was added 5 min after BME administration, washed out after 60 min, and fixed after 15 min.

The white arrow indicates a clathrin bud without GPI-AP.

b 3D images constructed from serial sections. APEX2::GPI-positive vesicles and tubules are shown in purple; APEX2::GPI-negative vesicles are shown in yellow; and clathrin-coated buds without GPI-AP are shown in blue.

Scale bars: 500 nm (a, b)

Takiguchi et al., Extended Data Figure 5



Extended Data Fig. 5. GPI-AP and TNFα transport observed using RudLOV

Observation of cargo transport using RudLOV. Cargo transport was initiated via 5 min of illumination at 445 nm. Colors indicate the following four proteins:

a Sca::GPI, Cv::Rab6a, AP1M1::HT-SF650T, and GalT::iRFP.

b TNFα::HT-SF650T, AP1M1::NG, Sca::Rab6a, and GalT::iRFP.

c NG::GPI, AP1M1::HT-SF650T, Sca::Rab11a, and GalT::iRFP.

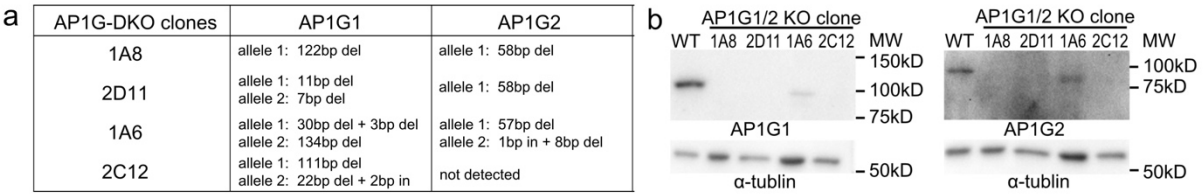
d TNFα::HT-SF650T, NG::Rab11a, Sca::Rab6a, and GalT::iRFP.

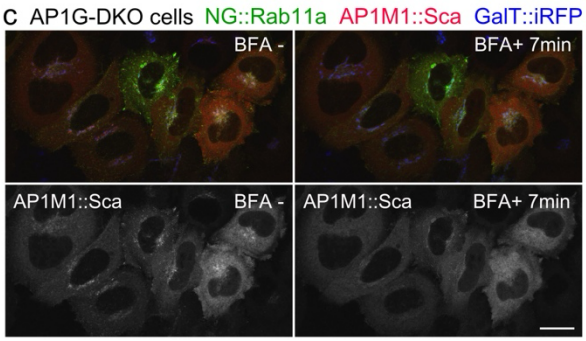
Scale bars: 1 μm (a–d)

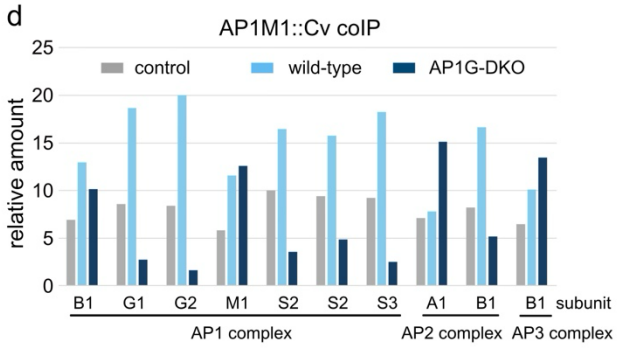
Takiguchi et al., Extended Data Figure 6

a

AP1G-DKO clones	AP1G1	AP1G2
1A8	allele 1: 122bp del	allele 1: 58bp del
2D11	allele 1: 11bp del allele 2: 7bp del	allele 1: 58bp del
1A6	allele 1: 30bp del + 3bp del allele 2: 134bp del	allele 1: 57bp del allele 2: 1bp in + 8bp del
2C12	allele 1: 111bp del allele 2: 22bp del + 2bp in	not detected

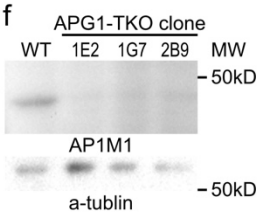






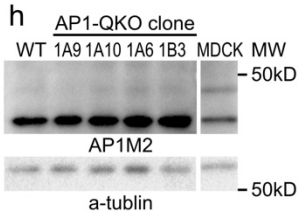
e

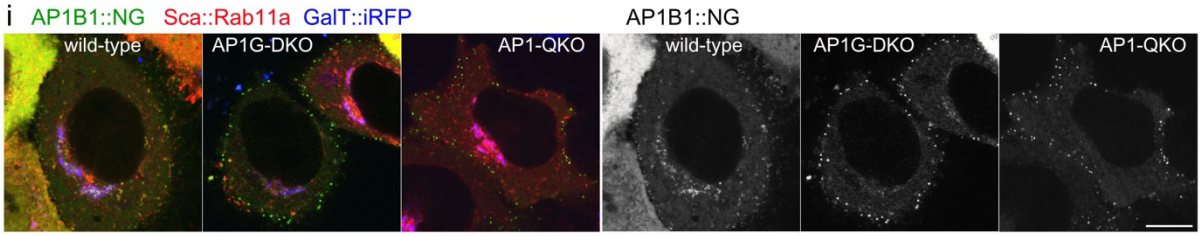
AP1G1/2M1-TKO clones	AP1M1
1E2	allele 1: 4bp del allele 2: 1bp in
1G7	allele 1: 20bp del allele 2: 1bp in
2B9	allele 1: 1bp del allele 2: 1bp in allele 3: 130bp in



g

AP1-QKO clones	AP1M2
1A9	allele 1: 11bp del
1A10	allele 1: 11bp del allele 2: 1bp in
1A6	allele 1: 1bp del allele 2: 1bp in
1B3	allele 1: 11bp del





Extended Data Fig. 6. Construction of AP1G-DKO cells and analysis of cargo transport

a List of AP1G-DKO candidate clones.

b Immunoblotting of extracts prepared from the wild-type and AP1G-DKO candidate clones using anti-AP1G1, anti-AP1G2, and anti-α-tubulin antibodies. 1A8 was selected as AP1G-DKO cells.

c AP1M1::Sca localization before and 7 min after BFA administration in AP1G-DKO cells. AP1M1::Sca is shown in red; GalT::iRFP is shown in blue; and NG::Rab11a is shown in green. Some AP1M1 was localized to the TGN in AP1G-DKO cells.

d Liquid chromatography-mass spectrometry analysis of the co-immunoprecipitated products of wild-type (light blue) and AP1G-DKO (dark blue) cells expressing AP1M1::Cv using an anti-GFP antibody. Wild-type cells not expressing AP1M1::Cv were used as controls (gray).

All AP1 subunits, as well as the B1 subunits of AP2 and AP3, co-immunoprecipitated in wild-type cells. However, only AP1B1, AP2A1, and AP3B1 co-immunoprecipitated in AP1G-DKO cells, whereas the other AP1s were detected at low levels only. The AP2A1 subunit was present in higher amounts in AP1G-DKO cells than in wild-type cells, suggesting that it formed an AP1-like complex instead of an AP1G1/2 complex. Thus, AP1G-DKO cells lack AP1G1/2 subunits, yet retain residual AP1 complex activity; however, this activity is likely limited.

e List of AP1G1/2M1-TKO candidate clones.

f Immunoblotting of extracts prepared from the wild-type and AP1G1/2M1-TKO candidate clones using anti-AP1M1 and anti-α-tubulin antibodies.

g List of AP1-QKO candidate clones.

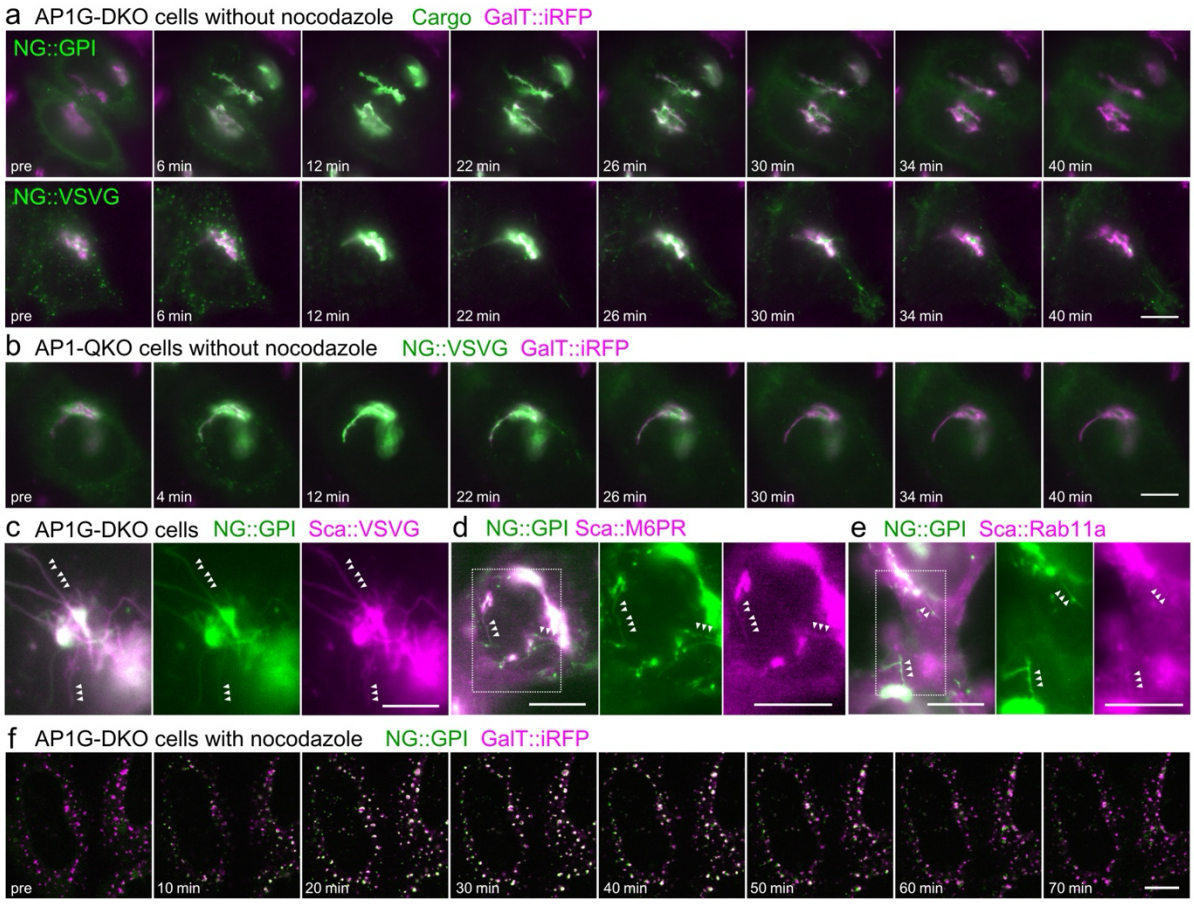
h Immunoblotting of extracts prepared from the wild-type and AP1-QKO candidate clones using anti-AP1M2 and anti-α-tubulin antibodies. 1A9 was selected as AP1-QKO cells.

i AP1B1::NG localization (green) in wild-type, AP1G-DKO, and AP1-QKO cells. Sca::Rab11a is shown in red; GalT::iRFP is shown in blue.

In wild-type cells, AP1B1 was localized on the *trans* side of the Golgi apparatus, presumably in the TGN. Notably, AP1B1 was also detected in the plasma membrane, suggesting that it was incorporated into the AP2 complex. AP1B1 was weakly detected in the TGN of AP1G-DKO cells but was absent in AP1-QKO cells. Localization to the plasma membrane was detected in both AP1G-DKO and AP1-QKO cells.

Scale bars: 20 μm (c) and 10 μm (i)

Takiguchi et al., Extended Data Figure 7



Extended Data Fig. 7. Cargo transport in AP1G-DKO and AP1-QKO cells

a Time-lapse images showing NG::GPI (top) and NG::VSVG (bottom) transport initiated by the BME-RUSH system in AP1G-DKO cells. The cargo is shown in green, and GalT::iRFP is shown in magenta.

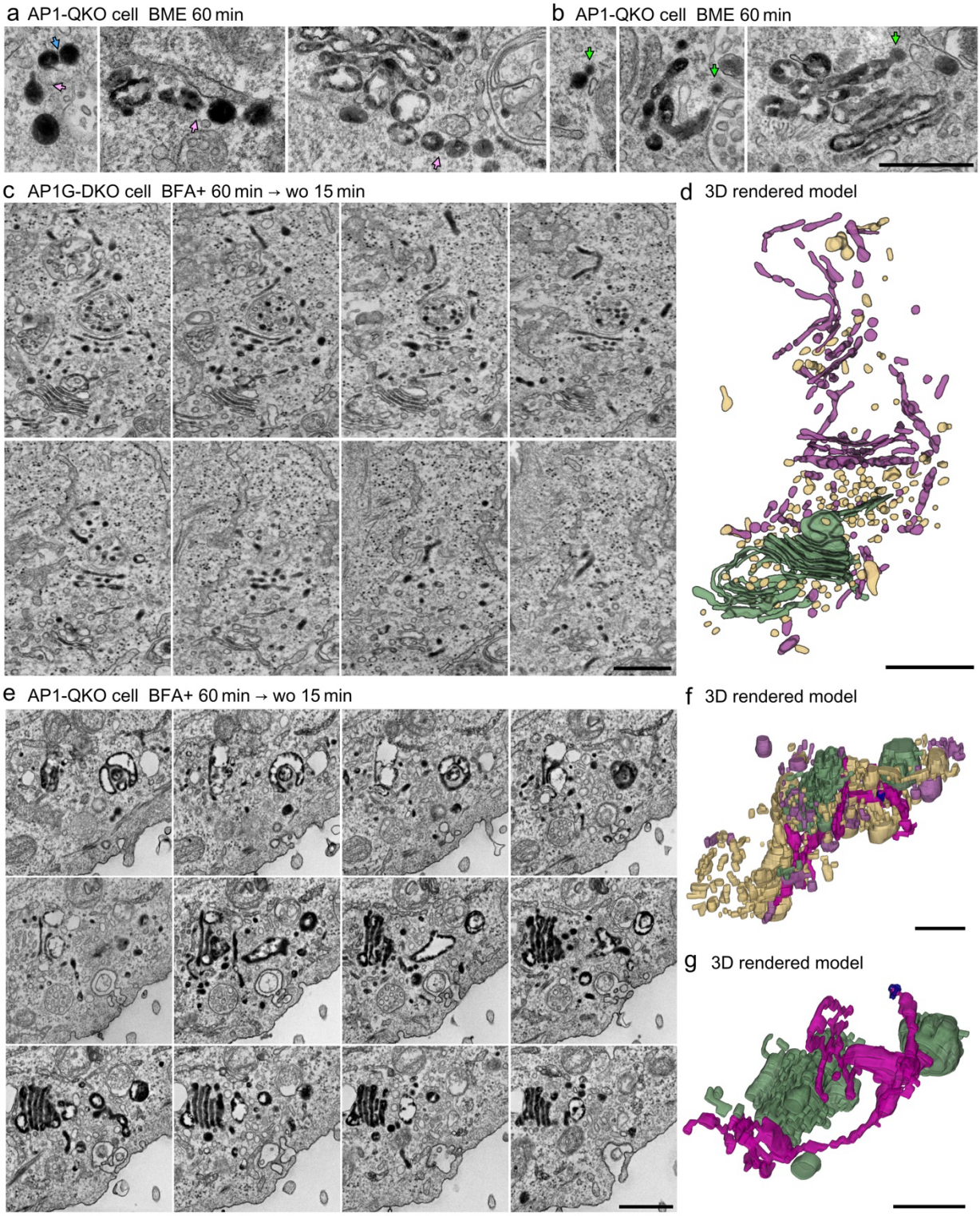
b Time-lapse images showing NG::VSVG (green) transport initiated by the BME-RUSH system in AP1-QKO cells. GalT::iRFP is shown in magenta.

c–e Co-localization studies in AP1G-DKO cells: NG::GPI (green) and Sca::VSVG (magenta) localization 36 min after BME administration (b), NG::GPI (green) and Sca::M6PR (magenta) localization 36 min after BME administration (c), NG::GPI (green) and Sca::Rab11a (magenta) localization 30 min after BME administration (d). The right panels in (c) and (d) show magnified single-color images of the insets of the left panels. Arrowheads indicate co-localization of cargo pairs (b, c) or cargo with Rab11a (d).

f Time-lapse images showing NG::GPI transport initiated by the BME-RUSH system in AP1G-DKO cells. BME treatment was initiated after 4 h of incubation with nocodazole. NG::GPI is shown in green, and GalT::iRFP is shown in magenta.

Scale bars: 10 μm (a, b), 5 μm (b–e), and 10 μm (f)

Takiguchi et al., Extended Data Figure 8



Extended Data Fig. 8. GPI-AP localization in AP1G-DKO and AP1-QKO cells

a, b Transmission electron micrographs of nocodazole-treated AP1-QKO cells treated with APEX2::GPI, in which transport was initiated using the BME-RUSH system and fixed 60 min after BME administration. Clathrin-coated buds containing APEX2::GPI (green arrows). Hemifused vesicles with and without APEX2::GPI are indicated by blue and green arrows, respectively. The pink arrows indicate candidate bead structures with APEX2::GPI.

c–g Scanning electron micrographs of serial sections at 50-nm intervals of Golgi stacks, tubules, and vesicles in nocodazole-treated AP1G-DKO (c) and AP1-QKO cells (e) 15 min after BFA washout, and their 3D images reconstructed from serial sections (d, f, g). APEX2::GPI transport was initiated using the BME-RUSH system, and BFA was added 5 min after BME administration. BFA was washed out after 55 min of incubation, and the cells were fixed 15 min after BFA washout. APEX2::GPI was visualized as electron-dense precipitates. In the 3D-rendered image, the Golgi stack is shown in green; APEX2::GPI-positive tubules connected to the Golgi stack are shown in purple; APEX2::GPI-positive tubules without connection to the Golgi stack are shown in pink; vesicles without GPI-AP are shown in light yellow; and the clathrin coat is shown in dark blue. Only the Golgi stack (green) and APEX2::GPI-positive tubules connecting the Golgi membrane (purple) are shown in (g), at different angles from those in (f).

Scale bars: 500 nm (a–g)