**Figure supplements**

**Figure1-figure supplement 1** GPP130 displays rim-localization in a majority of native Golgi stacks by EM. (A) Transmitted light image of NRK cells expressing GPP130-APEX2-GFP after APEX2-catalyzed labeling reaction and plastic embedding. (B) Representative overview EM image of a cell expressing GPP130-APEX2-GFP. Scale bar, 2 µm. (C) Categorization of GPP130-APEX2-GFP distribution patterns. The experiment is the same as in Figure 1G. 57 GPP130-APEX2 positive Golgi stacks from 25 cells were imaged. Side and en face views of Golgi stacks are further categorized as rim and non-rim-localization according to their APEX2 staining patterns. Scale bar, 200 nm.

**Figure 1-figure supplement 2** The N and C-terminus of Giantin, GCC185 and GM130 coincide on the Golgi mini-stack. (A) The ratio of the ring diameter of Giantin N (N-to-N) and C-antibody (C-to-N) to that of N-antibody. In N-to-N, cells were stained by Giantin N-terminus antibody with both Alexa Fluor 488 and 647. In C-to-N, cells were stained by Giantin N and C-terminus antibody conjugated with Alexa Fluor 647 and 488, respectively. Two diameters were measured from Alexa Fluor 488 and 647 channels by line intensity profiles for each Golgi mini-stack and ratios were calculated and plotted. Green line represents the mean. *P*-value is from *t*-test. (B) The N and C-terminus of GCC185 are indicated by GFP and mCherry tag, respectively. (C) The N and C-terminus of GM130 are labeled by corresponding antibodies. The intensity profile is acquired as in Figure 1I. Scale bar, 500 nm.

**Figure 2-figure supplement 1** Typical en face and side view images of Golgi transport machinery components. (A-E) ERES, ERGIC and *cis*-Golgi proteins (LQ < 0). (F) GRASP55-GFP, a *cis*-Golgi protein (0 ≤ LQ < 0.25). (G) En face averaged image of GRASP55-GFP and the corresponding radial mean intensity profile. n, the number of averaged Golgi mini-stack images. Scale bar, 500 nm.

**Figure 3-figure supplement 1** En face and side view images of the medial and *trans*-Golgi SNAREs, including GS15 and GS28, showed their rim localization (A and C). (B and D) Corresponding en face averaged images and radial mean intensity profiles. n, the number of averaged mini-stack images. Scale bar, 500 nm.

**Figure 3-figure supplement 2** The lateral localization of components of the *trans*-Golgi and TGN transport machinery in the Golgi mini-stack. (A, C and E-M) En face and side view images of *trans*-Golgi and TGN transport machinery components (LQ ≥ 1.0). (B and D) En face averaged images and radial mean intensity profiles corresponding to (A) and (C). n, the number of averaged mini-stack images. Arrows in (H) indicate colocalization between CLCB and Furin-GFP. Scale bar, 500 nm.

**Figure 4-figure supplement 1** Golgi enzymes that primarily display central disk localization at the interior of medial and *trans*-Golgi cisternae. (A, C, E, G, I, K, M, O, Q and S) En face view images of Golgi enzymes and SLC35C1. Side view images are also shown in (A). Dotted arrows and boxes and line intensity profiles are used or acquired as in Figure 1K. (B, D, F, H, J, L, N, P, R and T) Corresponding en face averaged images and radial mean intensity profiles. n, the number of averaged mini-stack images. (U) MGAT2-APEX2 biotinylated proteins mainly localize to the cisternal interior. Cells expressing MGAT2-APEX2-GFP were subjected to APEX2-catalyzed reaction to biotinylate its neighboring proteins. The biotinylated proteins were detected by Alexa Fluor 594 conjugated streptavidin (streptavidin-594). The line intensity profile is generated as in Figure 1K. Scale bar, 500 nm.

**Figure 4-figure supplement 2** Golgi enzymes, Man1B1, ManII and TPST2, display ring-pattern localization. (A, C and E) En face view images. (B, D and F) Corresponding en face averaged images and radial mean intensity profiles. n, the number of averaged mini-stack images. The line intensity profile is generated as in Figure 1K. Scale bar, 500 nm.

**Figure 4-figure supplement 3** MGAT2 mainly localizes to the cisternal interior in native Golgi stacks by EM. (A) Transmitted light image of NRK cells expressing MGAT2-APEX2-GFP after APEX2-catalyzed labeling reaction and plastic embedding. (B) Representative overview EM image of a cell expressing MGAT2-APEX2-GFP. Scale bar, 2 µm. (C) Categorization of MGAT2-APEX2-GFP distribution patterns. The experiment is the same as in Figure 4I. 58 MGAT2-APEX2 positive Golgi stacks from 14 cells were imaged. Side and en face views of Golgi stacks are further categorized as interior and rim-localization according to their APEX2 staining patterns. Scale bar, 200 nm.

**Figure 5-video supplement 1** Live-cell imaging showing the interior localization of mCherry-GPI during its transition through the Golgi mini-stack. This movie corresponds to the boxed region of Figure 5C. The time lapse was acquired every 3 min after the addition of biotin. mCherry-GPI and GFP-Golgin84 are shown in red and green respectively.

**Figure 5-figure supplement 1** Conventional or small size secretory cargos can localize to the cisternal interior during their intra-Golgi transport. (A-D) En face view images of E-cadherin, VSVG, secretory GFP and CD8a-Furin during the chase after ER synchronization. Experiments were similar to Figure 5A. Similar to Figure 5B, the right of each panel displays corresponding LQ vs time plot measured from the same experiment. Arrows indicate budding membrane carriers. Scale bar, 500 nm. Error bar, mean ± SEM. n, the number of Golgi mini-stacks used for the calculation.

**Figure 5-figure supplement 2** ST6Gal1 mainly localizes to the cisternal interior under VSVG traffic wave. HeLa cells co-expressing VSVG-SBP-Flag (a RUSH reporter), ST6Gal1-moxGFP and GalT-mCherry were subjected to biotin to chase VSVG along the secretory pathway. At various chase time, cells were fixed, immuno-stained and imaged. (A) En face averaged images of ST6Gal1 and endogenous Giantin during the chase. The dotted red circle indicates the interior region of interest (ROI), which has the radius of ST6Gal1 at steady-state transport (Figure 4J). (B) The plot of VSVG’s LQ vs chase time. VSVG-SBP-Flag and endogenous GM130 were immuno-stained and, in combination with GalT-mCherry, LQs of VSVG were calculated by GLIM to demonstrate the intra-Golgi trafficking status of VSVG. (C) The percentage of interior ST6Gal1 within the interior ROI during the chase of VSVG. Error bar, mean ± SEM. n, the number of Golgi mini-stacks used for the calculation.

**Figure 5-figure supplement 3** Bulky secretory cargos are restricted to the cisternal rim during their intra-Golgi transport. (A) The image of GFP-Nup133-mut-substituted nuclear pores and the histogram of the total intensity of a nuclear pore. Cells were depleted of endogenous Nup133 by shRNA and allowed to simultaneously express shRNA-resistant GFP-Nup133-mut. A typical image was shown above and the total intensity of a nuclear pore was plotted in the histogram below. (B-D) Estimating copy numbers of GFP-collagenX, GFP-FM4-CD8a and FM4-moxGFP in a Golgi punctum. Left, the image of typical Golgi puncta; right, the histogram of the copy number in a Golgi punctum. (E) FM4-moxGFP partitions to the cisternal-rim upon aggregation. The organization of these panels is similar to Figure 5G. Scale bars represent 500 nm unless specified otherwise.

**Supplementary files**

**Supplementary file 1**

Mouse Genome Informatics (MGI) and Human Genome Organization Gene Nomenclature Committee (HGNC) official full names of glycosylation enzymes used in this study. The official full name of ManII is from MGI while the rest are from HGNC. Except GalT and ManII, all names are official symbols.

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| --- | --- | --- |
| name | GenBank Accession No.: | official full name |
| β3GalT6 | BC082998 | beta-1,3-galactosyltransferase 6 |
| β4GalT3 | BC009985.2 | beta-1,4-galactosyltransferase 3 |
| β4GalT7 | NM\_007255 | beta-1,4-galactosyltransferase 7 |
| GALNT1 |  | polypeptide N-acetylgalactosaminyltransferase 1 |
| GALNT2 |  | polypeptide N-acetylgalactosaminyltransferase 2 |
| GalT | HG765101 | beta-1,4-galactosyltransferase 1 |
| MGAT1 | M61829 | mannosyl(alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase |
| MGAT2 | BC006390 | mannosyl(alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase |
| MGAT4B | AB000624 | alpha-1,3-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase B |
| Man1B1 | BC006079.1 | mannosidase alpha class 1B member 1 |
| ManII | BC138372 | mannosidase2, alpha 1 |
| POMGNT1 | NM\_017739 | protein O-linked mannose N-acetylglucosaminyltransferase 1 (beta 1,2-) |
| ST6Gal1 | BC040009 | ST6 beta-galactoside alpha-2,6-sialyltransferase 1 |
| SLC35C1 | NM\_018389 | solute carrier family 35 member C1 |
| TPST1 | NM\_003596.3 | tyrosylprotein sulfotransferase 1 |
| TPST2 | NM\_001008566.1 | tyrosylprotein sulfotransferase 2 |

**Supplementary file 2**

Review of previous EM literature that directly addressed lateral localizations of Golgi residents and secretory cargos in comparison with this study.

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| Golgi proteins | | this study | literature | | |
| localization | localization | results or data | reference |
| machinery components | COPI (β-COP) | rim and surrounding | rim and surrounding | interior (15), coated buds (116) and vesicles (89) (labeling density, gold particles/µm2) | Table 2 (Orci et al., 1997) |
| Giantin | rim | interior | interior (58 %), rim + coated buds + vesicles (42 %) (percentage of gold particles) | Table II (Martinez-Menarguez et al., 2001) |
| rim | interior (< 5%), rim + coated buds + vesicles (45 %) (percentage of gold particles) | Fig. 1A,B (Koreishi et al., 2013) |
| KDEL receptor | rim and surrounding | rim and surrounding | interior (36 %), rim + coated buds + vesicles (64 %) (percentage of gold particles) | Table II (Martinez-Menarguez et al., 2001) |
| rim | rim to interior ratio = 2.83 (number of gold particles) | Table III (Cosson et al., 2002) |
| rim and surrounding | interior (81), coated buds (160) and vesicles (103) (labeling density, gold particles/µm2) | Table 2 (Orci et al., 1997) |
| GS27 (membrin) | rim and surrounding | rim | rim to interior ratio = 4.6 (number of gold particles) | Table I (Cosson et al., 2005) |
| GS15 | rim | rim | rim to interior ratio = 2.1 (number of gold particles) | Table I (Cosson et al., 2005) |
| Golgi enzymes | Man1B1 | rim | rim | rim to interior ratio > 1.5 (linear density of gold particles) | Fig. 2b,c,e (Rizzo et al., 2013) |
| ManII | interior | interior | interior (91), coated buds (17) and vesicles (7) (labeling density, gold particles/µm2) | Table I (Orci et al., 2000) |
| interior | interior (69 %), rim + coated buds and vesicles (31 %) (percentage of gold particle) | Table II (Martinez-Menarguez et al., 2001) |
| interior | rim to interior ratio = 0.79 (number of gold particles) | Table III (Cosson et al., 2002) |
| rim | localization to cisternal perforated zone | Fig. 4 (Kweon et al., 2004) |
| interior | rim to interior ratio = 0.79 (number of gold particles) | Table I (Cosson et al., 2005) |
| MGAT1 (NAGTI) | interior | interior | interior (214), coated buds (24) or vesicles (17) (in label density, gold particles/µm2) | Table I (Orci et al., 2000) |
| ST6Gal1 | interior | rim | cisternal perforated zone | Fig. 4 (Kweon et al., 2004) |
| GalT | interior | rim | The ratio of the cisternal perforated zone to interior is 2.16:1 (linear density of gold particles). | Table 2 and Fig. 4 (Kweon et al., 2004) |
| interior | rim to interior ratio = 0.55 (number of gold particles) | Table I (Cosson et al., 2005) |
| Secretory cargo | VSVG | interior | interior | interior (1.34), coated buds (0.2) and vesicles (0.16) (linear density of gold particles) | Table I(Mironov et al., 2001) |
| interior | interior (94 %), rim + coated buds + vesicles (6 %) (percentage of gold particle total labeling) | Table II (Martinez-Menarguez et al., 2001) |
| FM4 soluble aggregate | rim | rim | rim connecting magavesicles | Fig.4 and 5 (Volchuk et al., 2000) |

**Supplementary file 3**

The source and cloning method of DNA plasmids used in this study.

**Supplementary file 4**

Fiji macro “Gyradius and intensity normalization.ijm”.

**Supplementary file 5**

Fiji macro “Golgi mini-stack alignment.ijm”.

**Supplementary file 6**

Fiji macro “Radial mean intensity profile.ijm”.

**Supplementary file 7**

Protocol for en face averaging and radial mean intensity profile

I install the following three macros in Fiji (Plugins->Macros->Install).

1. Macro “Gyradius and intensity normalization.ijm”
2. Macro “Golgi mini-stack alignment.ijm”
3. Macro “Radial mean intensity profile.ijm”

II Steps to average en face view images:

1. Acquire 2D multi-color images of Golgi mini-stacks. Giantin must be co-stained.
2. Crop an en face view of a Golgi mini-stack in a square in Fiji.
3. Subtract the background so that all background pixel values are 0.
4. Save it into a working folder.
5. Select Giantin channel image.
6. Launch the macro “gyradius and intensity normalization.ijm” in Fiji.
7. The macro prompts you to choose the directory to export processed images; once it is selected, the macro runs. A Log window appears to display processing information. The multi-channel image is split into individual channel images and their sizes and intensities are normalized. These processed images are saved in the directory specified by the macro.
8. The procedure can be repeated to process more images of Golgi mini-stacks.
9. Open all images of a specific Golgi marker that are processed by the macro “gyradius and intensity normalization.ijm”.
10. Convert them to an image stack in Fiji.
11. Launch the macro “Golgi mini-stack alignment.ijm” and Golgi mini-stacks are aligned to the center of the canvas.
12. The en face averaged Golgi mini-stack image is acquired by using z-projection in Fiji (Image->Stacks->Z projection).

III Steps to generate radial mean intensity profile:

1. Open the en face averaged image of a Golgi marker.
2. Launch the macro “Radial mean intensity profile.ijm”.
3. In the Results window, copy the first (distance from the center; in pixel) and fourth (mean intensity) column to a spreadsheet processing software, such as Excel and Origin.
4. Determine the radius of the half maximum of the outer slope.
5. The normalized radius is calculated as the radius of a Golgi marker divided by that of corresponding Giantin.