

# Short transmembrane domains with high-volume exoplasmic halves determine retention of Type II membrane proteins in the Golgi complex

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## Summary

It is still unclear why some proteins that travel along the secretory pathway are retained in the Golgi complex whereas others make their way to the plasma membrane. Recent bioinformatic analyses on a large number of single-spanning membrane proteins support the hypothesis that specific features of the transmembrane domain (TMD) are relevant to the sorting of these proteins to particular organelles. Here we experimentally test this hypothesis for Golgi and plasma membrane proteins. Using the Golgi SNARE protein Sft1 and the plasma membrane SNARE protein Sso1 from *Saccharomyces cerevisiae* as model proteins, we modified the length of their TMDs and the volume of their exoplasmic hemi-TMD, and determined their subcellular localization both in yeast and mammalian cells. We found that short TMDs with high-volume exoplasmic hemi-TMDs confer Golgi membrane residence, whereas TMDs with low-volume exoplasmic hemi-TMDs, either short or long, confer plasma membrane residence to these proteins. Results indicate that the shape of the exoplasmic hemi-TMD, in addition to the length of the entire TMD, determine retention in the Golgi or exit to the plasma membrane of Type II membrane proteins.

**Key words:** Transmembrane domain, Golgi, Traffic, Yeast, SNAREs

## Introduction

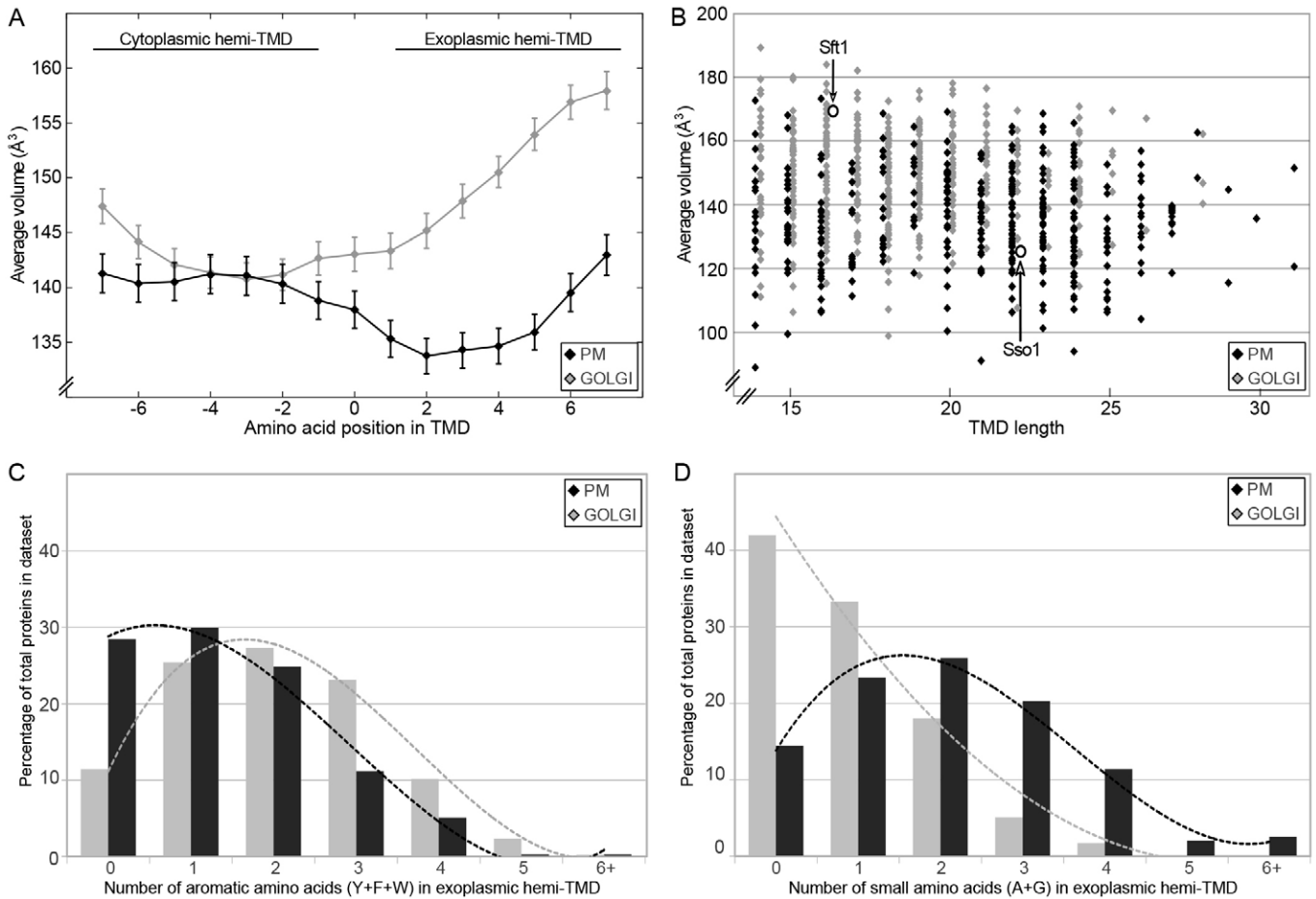
Proteins of the secretory pathway are transported by tubulo-vesicular carriers from their site of synthesis in the endoplasmic reticulum (ER) to the plasma membrane and the cell exterior. It is still an open question why Golgi-resident proteins concentrate in this organelle whereas other proteins do not, following the route towards the plasma membrane instead. For 16 yeast glycosyltransferases an FLxK amino acid motif in the cytoplasmic tail determines Golgi retention through interaction with the COPI-interacting protein Vps74 (Tu et al., 2008). Other reports assign importance to the TMD for localization of glycosyltransferases (GTs) in the Golgi (Opat et al., 2001; Tang et al., 1992; Teasdale et al., 1992). This capacity was attributed to particular amino acid residues in the TMD (Aoki et al., 1992; Sousa et al., 2003) or the length of the TMD (Bretscher and Munro, 1993; Rayner and Pelham, 1997; Ronchi et al., 2008). Recent analyses of a large protein dataset showed that TMDs from single-spanning membrane proteins of the Golgi and plasma membrane have distinctive geometric features: Golgi protein TMDs are on average four amino acid residues shorter than those of plasma membrane proteins in vertebrates, and seven residues shorter in fungi. Also, they have a higher concentration of amino acid residues with high-volume side chains in the exoplasmic hemi-TMD (Sharpe et al., 2010). Previous experimental evidence of these TMD features as determinants

of organelle residence only addressed the effect of TMD length (Munro, 1995; Ronchi et al., 2008). Here we tested whether both length and volume of TMDs are bona fide determinants for localization of Type II membrane proteins in the Golgi complex or the plasma membrane. The *S. cerevisiae* SNARE (soluble NSF attachment protein receptor) proteins Sft1 and Sso1, whose TMDs display representative geometric features of Golgi and plasma membrane proteins, respectively, were selected as model proteins. We show that modifying the length of the TMDs of these proteins or the volume of their exoplasmic hemi-TMDs, dramatically affects their subcellular localization, demonstrating that both features are major determinants for retention in the Golgi or exit to the plasma membrane.

## Results and Discussion

### Bioinformatic analyses reveals differences in length and shape of TMDs between Golgi and plasma membrane type II membrane proteins

The results of the analysis of our dataset (supplementary material Table S1), which consists of 472 Golgi and 395 pm type II metazoan membrane proteins, are shown in Fig. 1A. A plot of mean hydrophobicity for each TMD position of proteins in the dataset showed similar average hydrophobicity values for the hydrophobic core of plasma membrane and Golgi proteins. However, the length of the core was longer for plasma membrane



**Fig. 1. Geometric features of TMDs in metazoan type II Golgi and plasma membrane proteins.** (A) Average volume of amino acids of Type II Golgi and plasma membrane protein TMDs. Values are mean  $\pm$  2 s.e.m. calculated in a window of five amino acids at each position in the TMD for all proteins in the dataset. Because the volumes of the one to two flanking cytoplasmic and exoplasmic amino acids influence calculation of the volumes of the first two amino acids at these borders, their values were not included in the plot. Amino acid position is numbered from the centre of the TMD (0). Residues towards the cytoplasmic border are in negative numbers and those towards the exoplasmic border in positive numbers. Average volume of residues is plotted only for the central TMD core of 15 amino acids. (B) Plot of TMD length versus average volume of the exoplasmic hemi-TMDs. Volume was calculated as the average for the seven amino acids of the exoplasmic hemi-TMD closest to the centre of the TMD. Although not included in the dataset, values corresponding to the length and volume of the yeast model proteins Sso1 and Sft1 are indicated. (C) Percentage of Golgi and plasma membrane proteins present in the dataset bearing the indicated number of aromatic amino acids (F+Y+W). (D) Percentage of proteins bearing the indicated number of small amino acids (A+G).

proteins (supplementary material Fig. S1). TMD lengths of Golgi proteins (of which 79% are putative glycosyltransferases) had a median value of 18 aa ( $18.2 \pm 2.9$ ; mean  $\pm$  s.d.) and residues of the exoplasmic hemi-TMD had an average volume of  $150.9 \text{\AA}^3$ . TMD lengths of plasma membrane proteins had a median of 22 aa ( $21.1 \pm 3.8$ ; mean  $\pm$  s.d.) and residues of the exoplasmic hemi-TMD had an average volume of  $139.6 \text{\AA}^3$ . These results agree with a previous study (Sharpe et al., 2010). In Fig. 1B, the volume of the first seven amino acids of the exoplasmic hemi-TMD is plotted against TMD length for each protein in the dataset. Most Golgi proteins (74%) in the dataset were included in a region delimited by maximum TMD length of 21 amino acids and minimum average exoplasmic hemi-TMD volume of  $134 \text{\AA}^3$ , which excludes most (75%) plasma membrane proteins.

Analysis of the amino acid composition of Golgi and plasma membrane TMDs revealed similarities in their cytoplasmic hemi-TMDs but significant differences in their exoplasmic hemi-TMDs: Golgi proteins displayed increased numbers of aromatic

amino acids (particularly tyrosine, Y), whereas plasma membrane proteins display increased amounts of the small amino acids Ala and Gly (supplementary material Table S2). About 65% of Golgi protein exoplasmic hemi-TMDs had two or more aromatic, high-volume amino acids (Tyr, Phe, Trp), whereas 60% of plasma membrane protein exoplasmic hemi-TMDs had none or one (Fig. 1C). Additionally, 75% of Golgi proteins contained none or one small amino acid (Ala or Gly), whereas 65% of plasma membrane proteins had two or more (Fig. 1D). Both Golgi and plasma membrane proteins had diminished amounts of Leu and Ile in their exoplasmic hemi-TMDs.

#### TMD features determine Golgi or plasma membrane localization

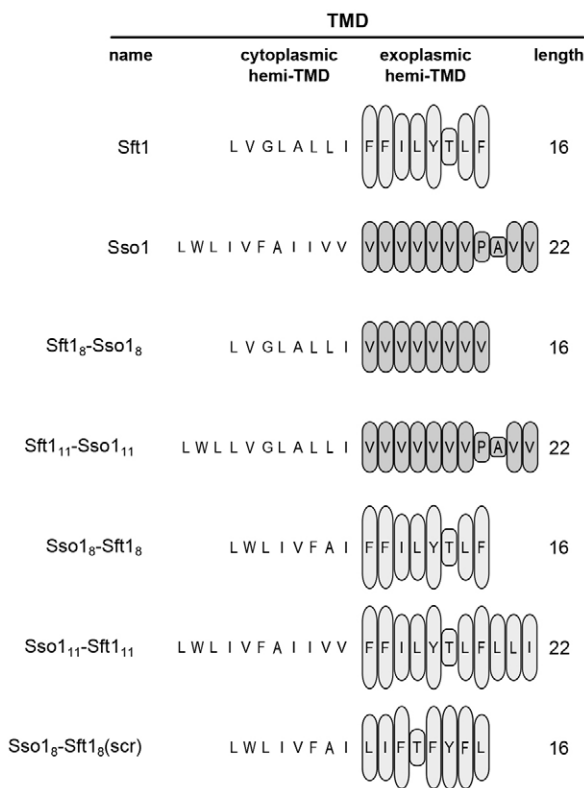
We experimentally tested whether TMD features determine Golgi or plasma membrane localization using wild-type or specific chimeric yeast SNARE proteins that differ only in a few residues of their TMDs and display robust localization

phenotypes. Sft1 is a Golgi protein with a short TMD (16 aa) and high-volume residues in its exoplasmic hemi-TMD. Sso1 is a plasma membrane protein with a longer TMD (22 aa) and with less-voluminous residues at its exoplasmic hemi-TMD (Fig. 1B and Fig. 2). The Sso1 TMD is sufficient for plasma membrane localization (Rayner and Pelham, 1997) and its cytoplasmic domain lacks endocytic signals (Valdez-Taubas and Pelham, 2003). Sft1 and Sso1 show a well-defined subcellular distribution in yeast even when overexpressed (Aalto et al., 1993; Banfield et al., 1995). Myc-tagged versions of the wild-type SNARE proteins or their chimeric variants (Fig. 2) were transformed into yeast and their subcellular localization was established by immunofluorescence (Fig. 3). Substituting the whole TMD of Sft1 for that of Sso1 [Sft1(Sso1)], resulted in a clear change of localization of Sft1 from the Golgi to the plasma membrane (Fig. 3A,B) and vice versa: substituting the Sso1 TMD for that of Sft1 [Sso1(Sft1)] led to Golgi localization of Sso1 (Fig. 3C,D). Colocalization experiments with the late-Golgi marker Chc1 confirmed the Golgi localization of the observed intracellular punctate structures (supplementary material Fig. S2). Results

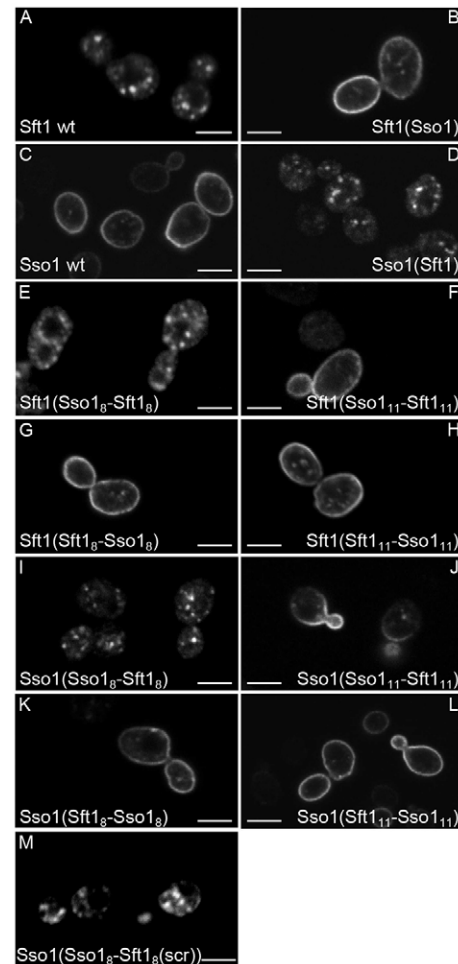
indicate that the TMDs of Sft1 and Sso1 act as determinants for Golgi and plasma membrane localization, respectively.

### Exoplasmic hemi-TMD volume is a subcellular localization determinant

We next examined whether determinants of subcellular localization were present in the exoplasmic or the cytoplasmic hemi-TMDs. Sft1 with the cytoplasmic hemi-TMD of Sso1 [Sft1(Sso1<sub>8</sub>-Sft1<sub>8</sub>)], remained in the Golgi complex (Fig. 3E), but localized at the plasma membrane when its exoplasmic hemi-TMD was substituted by the less voluminous, albeit shortened, version of the exoplasmic half of Sso1 [Sft1(Sft1<sub>8</sub>-Sso1<sub>8</sub>)] (Fig. 3G). Sft1 with the whole Sso1 exoplasmic hemi-TMD and an extended cytoplasmic Sft1 hemi-TMD [Sft1(Sft1<sub>11</sub>-Sso1<sub>11</sub>)] also localized to the plasma membrane (Fig. 3H). Noticeably, a long TMD with the cytoplasmic half of Sso1 and an extended exoplasmic half of Sft1 [Sft1(Sso1<sub>11</sub>-Sft1<sub>11</sub>)] left the Golgi and localized at the plasma membrane (Fig. 3F). Constructs bearing the cytoplasmic domain of Sso1, gave essentially the same results: a short TMD with a high-volume



**Fig. 2. Schematic representation of wild-type and chimeric TMDs.** The amino acid sequence of TMDs is indicated. The volume of each amino acid in the exoplasmic hemi-TMD is represented by the height of the boxes. Chimeric TMDs are named according to the origin of each hemi-TMD, with sub-indexes representing the number of amino acids. The last amino acid in Sft1<sub>8</sub>-Sso1<sub>8</sub> was retained as a Val residue to avoid ending in Pro. Sft1(scr) is the Sft1 exoplasmic hemi-TMD whose sequence has been scrambled. Each of these chimeric TMDs was cloned into vectors expressing the cytoplasmic domains of Sft1 or Sso1, N-terminally tagged with the Myc epitope or fluorescent protein. All constructs have a K at the cytoplasmic border of the TMD, and the Sso1 sequence KTR at the luminal border.



**Fig. 3. Geometric features of the TMD are determinants of Golgi or plasma membrane residence in yeast.** (A–M) Myc-tagged protein products (indicated within parenthesis and schematized in Fig. 2), were expressed in yeast, immunostained with anti-Myc and observed under the confocal microscope. Scale bars: 5 μm.

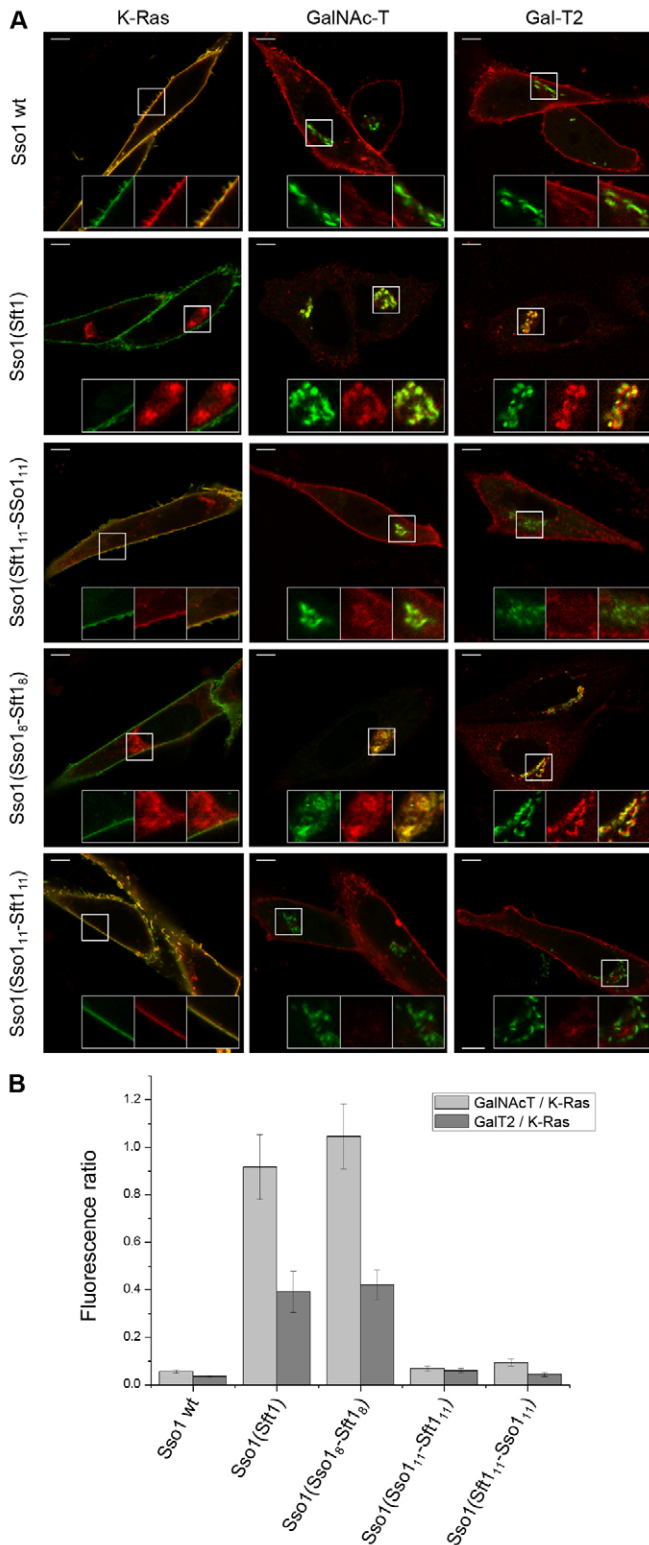
exoplasmic hemi-TMD caused Golgi retention of Sso1 [Sso1(Sso1<sub>8</sub>-Sft1<sub>8</sub>)] (Fig. 3I); and either a short TMD with less-voluminous exoplasmic hemi-TMD [Sso1(Sft1<sub>8</sub>-Sso1<sub>8</sub>)] (Fig. 3K) or longer TMDs of any volume, Sso1(Sso1<sub>11</sub>-Sft1<sub>11</sub>) and Sso1(Sft1<sub>11</sub>-Sso1<sub>11</sub>), resulted in plasma membrane

localization of the chimeras (Fig. 3H,J). The idea that the presence of an amino acid motif in the exoplasmic hemi-TMD of Sft1 acts as a specific Golgi-retention signal was discarded because a construct with scrambled residues in this region [Sso1(Sso1<sub>8</sub>-Sft1<sub>8</sub>scr)] (Fig. 2) still localized to the Golgi (Fig. 3M).

These experiments indicate that both short length and a high-volume exoplasmic hemi-TMD are crucial parameters to confer Golgi localization to the chimeras because replacement of Sft1 exoplasmic hemi-TMD with less-voluminous amino acid residues or extension of the exoplasmic hemi-TMD with residues of any volume results in localization at the plasma membrane.

#### Features of the exoplasmic hemi-TMDs define Golgi or plasma membrane localization in mammalian cells

To examine whether the geometric features of TMDs also act as determinants of localization in mammalian cells, CHO-K1 cells were transfected with mCherry-tagged Sso1 or fusions of the Sso1 cytoplasmic domain to the TMDs described in Fig. 2 and their subcellular localization was established by colocalization with the plasma membrane marker K-Ras (Gomez and Daniotti, 2005), the Golgi complex marker GalT2 or the trans-Golgi network marker GalNAcT (Giraud et al., 2001), all of which were tagged with YFP. Wild-type Sso1 colocalized with K-Ras, but when its TMD was replaced with that of Sft1, it colocalized partially with GalT2 and to a greater extent with GalNAcT (Fig. 4A). Longer TMDs, Sso1(Sso1<sub>11</sub>-Sft1<sub>11</sub>) and Sso1(Sft1<sub>11</sub>-Sso1<sub>11</sub>), resulted in plasma membrane localization of Sso1 whereas a short TMD with a high-volume exoplasmic hemi-TMD, Sso1(Sso1<sub>8</sub>-Sft1<sub>8</sub>), resulted in Golgi localization. Unexpectedly, the chimera with short and less-voluminous exoplasmic hemi-TMD [Sso1(Sft1<sub>8</sub>-Sso1<sub>8</sub>)] localized to mitochondria, where it colocalized with the Mitotracker marker (supplementary material Fig. S3A). The ratio of fluorescence of Sso1 in the Golgi or trans-Golgi network to that in the plasma membrane increased five- to ten-fold, respectively, when its TMD was either substituted for the whole Sft1 TMD [Sso1(Sft1)], or when just the exoplasmic hemi-TMD was substituted for that of Sft1 [Sso1(Sso1<sub>8</sub>-Sft1<sub>8</sub>)] (Fig. 4B). Chimeras bearing long TMDs, Sso1(Sso1<sub>11</sub>-Sft1<sub>11</sub>) and Sso1(Sft1<sub>11</sub>-Sso1<sub>11</sub>), displayed values comparable to that of wild-type Sso1 (Fig. 4B). It was verified that wild-type Sso1 traffics through the Golgi because it localized to the Golgi when exit was blocked at 20°C (supplementary material Fig. S3B). Therefore, results in *S. cerevisiae* are essentially reproduced in mammalian cells. Similar trends were found in the *Arabidopsis* membrane proteome (Nikolovski et al., 2012), suggesting that



**Fig. 4. Geometric features of the TMD are determinants of Golgi residence in mammalian cells.** (A) mCherry-Sso1 cytoplasmic domain fused to TMDs displayed in Fig. 2 (red) were co-expressed in CHO-K1 cells with the plasma membrane marker K-Ras, the Golgi complex marker GalT2 or the trans-Golgi network marker GalNAcT, fused to YFP (green). Live cells were observed under the confocal microscope. Insets show the red and green channels separately and the resulting merge. (B) Quantification of the subcellular localization shown in A. Values are means  $\pm$  s.e. of the ratio of the integrated fluorescence intensity in the Golgi (GalT2, dark grey) or trans-Golgi network (GalNAcT, light grey) to that in the plasma membrane (K-Ras) for each of the indicated chimeras. Approximately 50 cells were quantified for each condition. Scale bars: 5  $\mu$ m.

these TMDs features are indeed widespread determinants of Golgi or plasma membrane residence.

The results presented here highlight the relevance of the volume of the exoplasmic hemi-TMD, which might add to the geometric mismatch imposed by the length in determining Golgi or plasma membrane residence. An appealingly simple hypothesis is that the more voluminous exoplasmic hemi-TMDs of Golgi proteins negatively affect their fit into the highly curved membrane domains from which Golgi transport carriers enriched in cholesterol and sphingolipids originate (Duran et al., 2012; Klemm et al., 2009; Polishchuk et al., 2003). By contrast, TMDs from plasma membrane proteins could conform a complex, thermodynamically favourable association with these order-inducing lipids and exit the Golgi.

Large exoplasmic amino acid residues could also discourage Golgi proteins from loading into the curved membranes of COPI vesicles. However, these membranes are poor in order-inducing lipids (Klemm et al., 2009), and might accommodate voluminous exoplasmic hemi-TMDs, allowing Golgi proteins to be loaded into COPI vesicles. Interactions of cytoplasmic tail amino acid motifs such as FLxK in yeast with the COPI-coat-interacting VPS74 protein (Tu et al., 2008), might also promote loading into these vesicles. However, this motif was infrequent in Golgi proteins of our dataset (Maccioni et al., 2011).

Finally, interactions with unidentified cargo receptors that could recognize TMDs by their length and exoplasmic volume, similar to what has been described for the ER cargo receptor Erv14 (Herzig et al., 2012), could also lead to loading of Golgi proteins into COPI vesicles.

## Materials and Methods

### Plasmids and strains

Yeast (SEY6210) expression experiments were performed using pRS316-based plasmids containing the TP11 promoter, the Myc tag, wild-type or chimeric SNARE proteins and the PEP12 terminator. A plasmid bearing a fusion of Sft1t to the Sso1 TMD (pJV481) was used in *in vivo* gap repair experiments to generate all chimeric TMD constructs. DNA fragments encoding synthetic transmembrane domains and regions of homology to pJV481 were ordered from Genscript. A *HindIII* site in SSO1 was removed and this fragment was used to replace the Sft1 cytoplasmic domain in the plasmids described above. Golgi colocalization experiments in yeast were performed on the LCY1389 strain (kindly provided by Elizabeth Conibear), in which genomically encoded Chc1 (clathrin heavy-chain) was C-terminally tagged with RFP. Mammalian expression vectors were generated by introducing wild-type and chimeric SNARE proteins in pmCherry-C1 (Clontech). All plasmids were verified by DNA sequencing. Western blot analyses for both yeast and mammalian cells indicated that all chimeras displayed the expected electrophoretic mobility and expression levels comparable to those of the wild-type proteins (supplementary material Fig. S4).

### Protein dataset

193 human proteins with annotated subcellular location and Type II transmembrane topology were gathered from the SwissProt database. 2796 metazoan orthologs were gathered from the ORTHOMCL database; redundancy was reduced using CD-HIT to cluster sequences with more than 70% sequence identity; 842 Golgi proteins and 439 plasma membrane proteins were subjected to confirmation of Type II topology, carried out by TMD prediction using HMMTOP with constrained predictions (N-terminus cytoplasmic, C-terminus extracytoplasmic). A 12-amino-acid sliding window analysis, using the GES scale (Engelman et al., 1986), was used to identify the hydrophobic core of the TMDs. Positions of the edges of the hydrophobic core were used as starting points for an analysis using a hydrophobicity window of five amino acids with the GES scale, and a cut-off of  $-0.4$  to scan for TMD edges (PERL scripts written in our laboratory). When the sliding window average detected a TMD edge with a charged amino acid, the edge was moved one residue towards the core. Proteins for which a TMD edge could not be effectively determined were removed from the dataset. Amino acid volume analysis was performed with the volume scale TSAJ990102 (Tsai et al., 1999) from AA index, using PERL scripts written in the laboratory on the final dataset, which consists of 472 Golgi proteins and 397 plasma membrane type II membrane proteins.

### Immunofluorescence and microscopy

Exponentially growing yeast cells were fixed, spheroplasted, permeabilized and treated with anti Myc monoclonal antibody (Sigma). Mammalian and yeast cells were observed in an Olympus FluoView FV1000 confocal microscope.

### Fluorescence intensity quantification in CHO-K1 cells

Image processing was performed using ImageJ software (NIH, Bethesda, MD; <http://rsb.info.nih.gov/ij/>). Using the YFP channel, a threshold was applied to differentiate pixels corresponding to the particular organelle, and a binary mask was created. Fluorescence images of chimeric proteins were obtained by multiplication of this binary mask with the background-subtracted image of the mCherry channel. For each organelle marker, the ratio of integrated fluorescence intensity in this region relative to the integrated fluorescence intensity in the whole cell was calculated.

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### Author contributions

H.J.M. and J.V.T. designed and supervised the entire project and wrote the manuscript. R.Q. conducted the bioinformatics analyses, performed experiments, and wrote the manuscript. A.T. performed experiments in yeast and mammalian cells. A.G.M. assisted with bioinformatics and performed image quantification.

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Supplementary material available online at

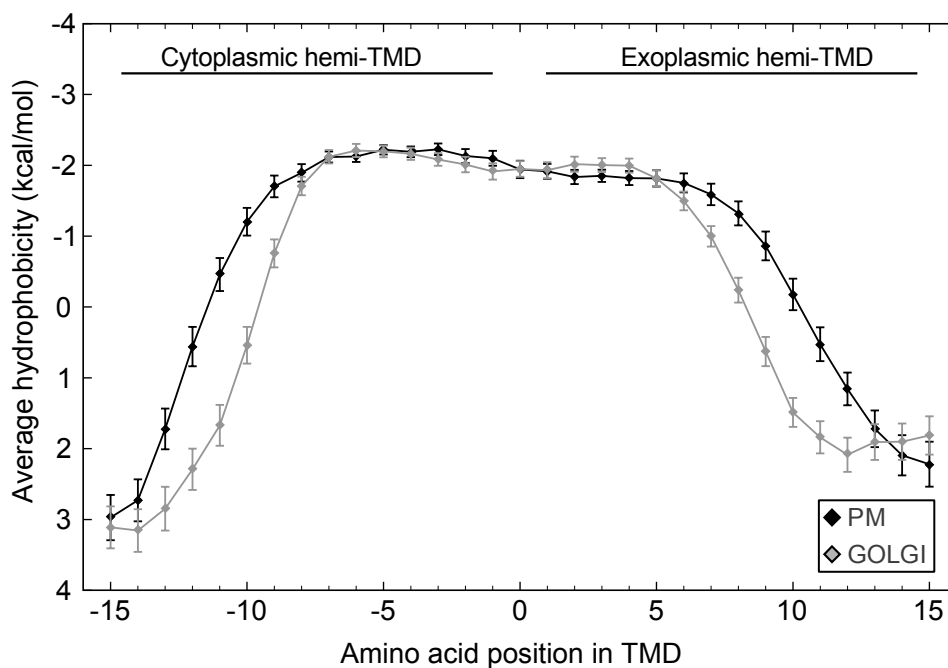
<http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.130658/-DC1>

### References

- Aalto, M. K., Ronne, H. and Keränen, S. (1993). Yeast syntaxins Sso1p and Sso2p belong to a family of related membrane proteins that function in vesicular transport. *EMBO J.* **12**, 4095-4104.
- Aoki, D., Lee, N., Yamaguchi, N., Dubois, C. and Fukuda, M. N. (1992). Golgi retention of a trans-Golgi membrane protein, galactosyltransferase, requires cysteine and histidine residues within the membrane-anchoring domain. *Proc. Natl. Acad. Sci. USA* **89**, 4319-4323.
- Banfield, D. K., Lewis, M. J. and Pelham, H. R. (1995). A SNARE-like protein required for traffic through the Golgi complex. *Nature* **375**, 806-809.
- Bretscher, M. S. and Munro, S. (1993). Cholesterol and the Golgi apparatus. *Science* **261**, 1280-1281.
- Duran, J. M., Campelo, F., van Galen, J., Sachsenheimer, T., Sot, J., Egorov, M. V., Rentero, C., Enrich, C., Polishchuk, R. S., Goñi, F. M. et al. (2012). Sphingomyelin organization is required for vesicle biogenesis at the Golgi complex. *EMBO J.* **31**, 4535-4546.
- Engelman, D. M., Steitz, T. A. and Goldman, A. (1986). Identifying nonpolar transbilayer helices in amino acid sequences of membrane proteins. *Annu. Rev. Biophys. Biophys. Chem.* **15**, 321-353.
- Giraud, C. G., Daniotti, J. L. and Maccioni, H. J. F. (2001). Physical and functional association of glycolipid N-acetyl-galactosaminyl and galactosyl transferases in the Golgi apparatus. *Proc. Natl. Acad. Sci. USA* **98**, 1625-1630.
- Gomez, G. A. and Daniotti, J. L. (2005). H-Ras dynamically interacts with recycling endosomes in CHO-K1 cells: involvement of Rab5 and Rab11 in the trafficking of H-Ras to this pericentriolar endocytic compartment. *J. Biol. Chem.* **280**, 34997-35010.
- Herzig, Y., Sharpe, H. J., Elbaz, Y., Munro, S. and Schuldiner, M. (2012). A systematic approach to pair secretory cargo receptors with their cargo suggests a mechanism for cargo selection by Erv14. *PLoS Biol.* **10**, e1001329.
- Klemm, R. W., Ejsing, C. S., Surma, M. A., Kaiser, H.-J., Gerl, M. J., Sampaio, J. L., de Robillard, Q., Ferguson, C., Proszynski, T. J., Shevchenko, A. et al. (2009). Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. *J. Cell Biol.* **185**, 601-612.
- Maccioni, H. J. F., Quiroga, R. and Spessott, W. (2011). Organization of the synthesis of glycolipid oligosaccharides in the Golgi complex. *FEBS Lett.* **585**, 1691-1698.
- Munro, S. (1995). An investigation of the role of transmembrane domains in Golgi protein retention. *EMBO J.* **14**, 4695-4704.
- Nikolovski, N., Rubtsov, D., Segura, M. P., Miles, G. P., Stevens, T. J., Dunkley, T. P. J., Munro, S., Lilley, K. S. and Dupree, P. (2012). Putative glycosyltransferases and other plant Golgi apparatus proteins are revealed by LOPIT proteomics. *Plant Physiol.* **160**, 1037-1051.

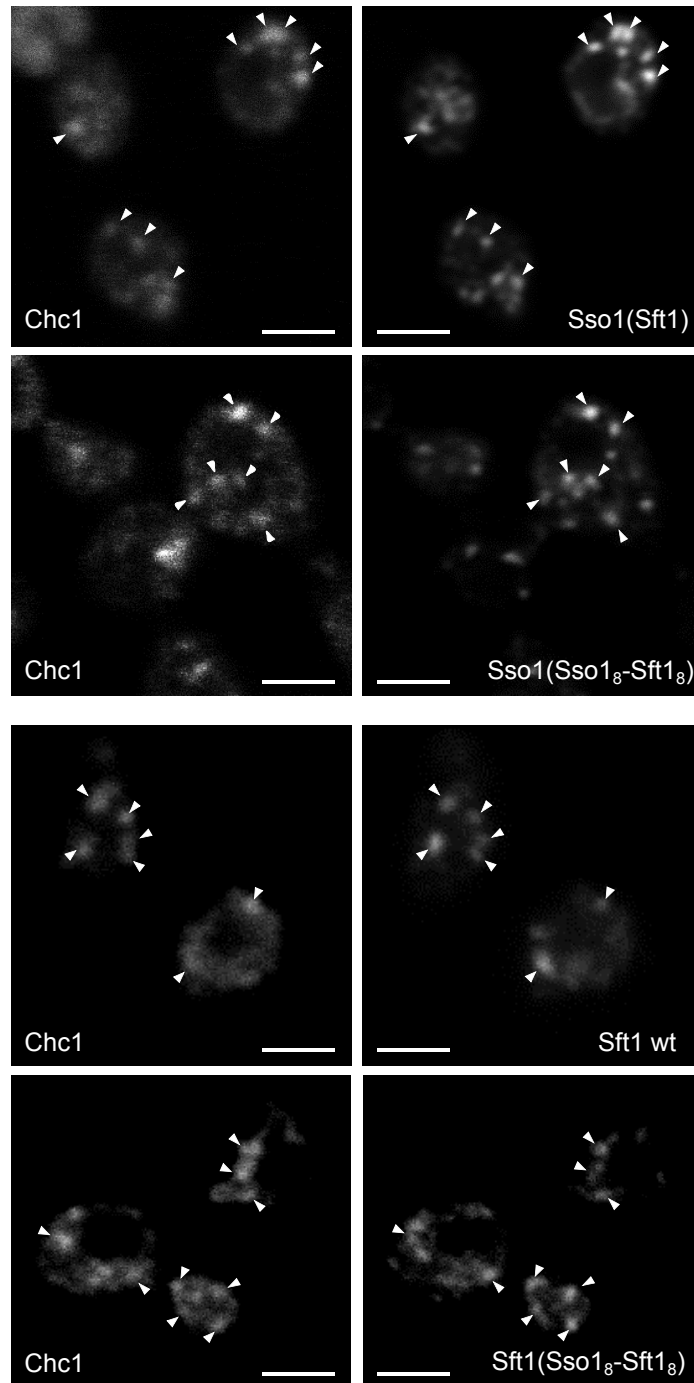
- Opat, A. S., van Vliet, C. and Gleeson, P. A.** (2001). Trafficking and localisation of resident Golgi glycosylation enzymes. *Biochimie* **83**, 763-773.
- Polishchuk, E. V., Di Pentima, A., Luini, A. and Polishchuk, R. S.** (2003). Mechanism of constitutive export from the golgi: bulk flow via the formation, protrusion, and en bloc cleavage of large trans-golgi network tubular domains. *Mol. Biol. Cell* **14**, 4470-4485.
- Rayner, J. C. and Pelham, H. R.** (1997). Transmembrane domain-dependent sorting of proteins to the ER and plasma membrane in yeast. *EMBO J.* **16**, 1832-1841.
- Ronchi, P., Colombo, S., Francolini, M. and Borgese, N.** (2008). Transmembrane domain-dependent partitioning of membrane proteins within the endoplasmic reticulum. *J. Cell Biol.* **181**, 105-118.
- Sharpe, H. J., Stevens, T. J. and Munro, S.** (2010). A comprehensive comparison of transmembrane domains reveals organelle-specific properties. *Cell* **142**, 158-169.
- Sousa, V. L., Brito, C., Costa, T., Lanoix, J., Nilsson, T. and Costa, J.** (2003). Importance of Cys, Gln, and Tyr from the transmembrane domain of human alpha 3/4 fucosyltransferase III for its localization and sorting in the Golgi of baby hamster kidney cells. *J. Biol. Chem.* **278**, 7624-7629.
- Tang, B. L., Wong, S. H., Low, S. H. and Hong, W.** (1992). The transmembrane domain of N-glucosaminyltransferase I contains a Golgi retention signal. *J. Biol. Chem.* **267**, 10122-10126.
- Teasdale, R. D., D'Agostaro, G. and Gleeson, P. A.** (1992). The signal for Golgi retention of bovine beta 1,4-galactosyltransferase is in the transmembrane domain. *J. Biol. Chem.* **267**, 13113.
- Tsai, J., Taylor, R., Chothia, C. and Gerstein, M.** (1999). The packing density in proteins: standard radii and volumes. *J. Mol. Biol.* **290**, 253-266.
- Tu, L., Tai, W. C. S., Chen, L. and Banfield, D. K.** (2008). Signal-mediated dynamic retention of glycosyltransferases in the Golgi. *Science* **321**, 404-407.
- Valdez-Taubas, J. and Pelham, H. R.** (2003). Slow diffusion of proteins in the yeast plasma membrane allows polarity to be maintained by endocytic cycling. *Curr. Biol.* **13**, 1636-1640.

## Supplementary Figure 1



**Figure S1.** Average hydrophobicity (GES scale) at each position in the aligned TMDs of Type II Golgi and PM proteins and their surrounding cytoplasmic and exoplasmic residues, relative to the centre position of the TMD. Hydrophobicity values in the GES scale correspond to the free energy for partitioning from water into a hydrophobic environment, and therefore positive values indicate preference for hydrophilic environments. Values represent the mean + 2 SEM calculated in a window of three amino acids at each position in the TMD and flanking residues for all proteins in the dataset. Amino acid position is numbered from the centre of the TMD (0). Residues towards the N-terminus (cytoplasmic) are in negative numbers and those towards the C-terminus (exoplasmic) in positive numbers.

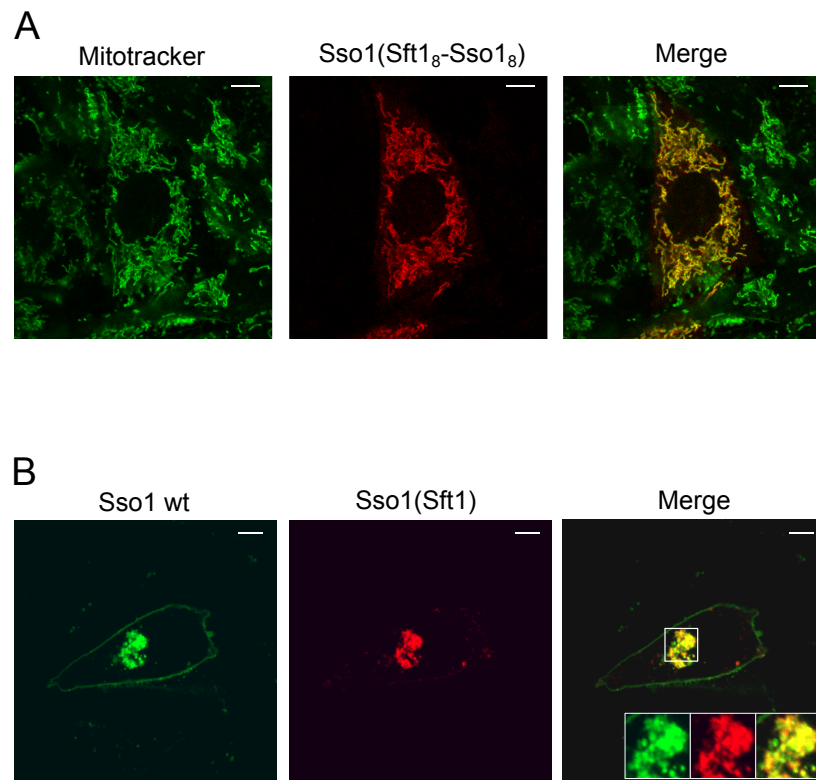
## Supplementary Figure 2



**Figure S2.** Co-localization of wt Sft1 and the indicated chimeric proteins with the late Golgi marker Chc1. The LCY1389 strain which expresses genomically encoded Clathrin heavy chain (Chc1) tagged with RFP, was transformed with plasmids expressing myc-tagged wt Sft1 and indicated chimeras. Cells were fixed, immunostained for c-myc and RFP, and observed under the confocal microscope. Arrowheads indicate identical positions for each pair of images. In similar experiments, partial co-localization with the early Golgi markers Cop1 and Sed5 was also observed (not shown). Scale bars correspond to 5 $\mu$ m.

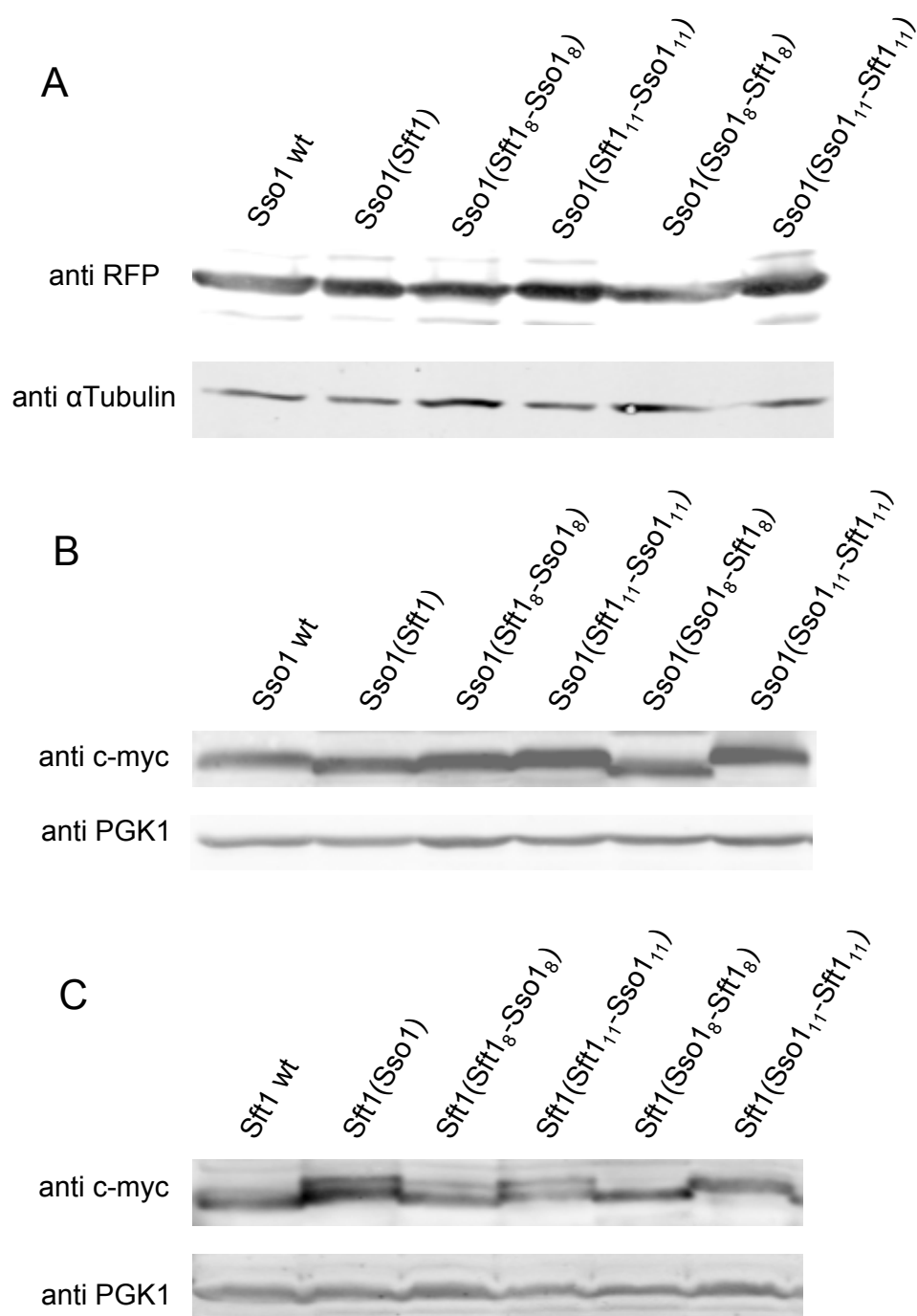


### Supplementary Figure 3



**Figure S3.** (A) Sso1(Sft18-Sso18) chimera localizes to mitochondria in mammalian cells. Sso1(Sft18-Sso18) with a mCherry tag was transfected into CHO-K1 cells. For mitochondrial staining, cells were incubated in DMEM without FBS supplemented with 200 nM MitoTracker Green FM (Molecular Probes) for 15 min at 37°C and observed in vivo under the confocal microscope. Note extensive co-localization of the chimera with Mitotracker stained mitochondria. (B) Chimeras that localize at the PM traffic through the Golgi complex. CHO-K1 cells expressing mCherry-Sso1-wt and GFP-Sso1(Sft1) were incubated for 2 hours post-transfection at 20 °C, to block protein exit from the Golgi. Note accumulation of wt Sso1 in the Golgi complex at this restrictive temperature. Scale bars correspond to 5µm.

## Supplementary Figure 4



**Figure S4.** Western Blot analyses of wt and chimeric SNAREs in mammalian and yeast cells. (A) CHO-K1 cells were transfected with plasmids encoding mCherry- tagged wt Sso1 and the indicated chimeric proteins. Loading was controlled using anti- $\alpha$ Tubulin. (B, C) Yeast cells were transformed with plasmids encoding c-myc- tagged wt Sso1 or Sft1 and the indicated chimeric proteins. Loading was controlled using anti-Pgk1.

**Table S1.** List of proteins included in dataset.

[Download Table S1](#)

**Table S2.** Amino acid composition (% of total amino acids) of cytoplasmic and exoplasmic hemi-TMDs for Golgi and PM proteins of the dataset

AA	Golgi			PM		
	Cytoplasmic (A)	Exoplasmic (B)	Ratio (B/A)	Cytoplasmic (A)	Exoplasmic (B)	Ratio (B/A)
<b>A</b>	8.5%	<b>5.4%</b>	<b>0.64</b>	10.0%	<b>11.0%</b>	<b>1.10</b>
C	4.5%	3.2%	0.71	4.1%	2.4%	0.59
<b>F</b>	9.8%	<b>10.9%</b>	<b>1.11</b>	7.7%	<b>7.4%</b>	<b>0.96</b>
<b>G</b>	5.4%	<b>4.4%</b>	<b>0.81</b>	5.6%	<b>8.2%</b>	<b>1.46</b>
H	0.5%	1.1%	2.29	0.4%	0.7%	1.74
M	2.2%	3.5%	1.62	2.8%	2.0%	0.72
N	0.6%	1.1%	1.79	0.5%	1.3%	2.64
P	1.3%	1.2%	0.94	1.6%	1.4%	0.86
Q	0.9%	0.8%	0.88	0.8%	1.0%	1.19
S	5.0%	6.5%	1.30	4.3%	5.0%	1.18
T	4.4%	5.0%	1.15	4.9%	6.0%	1.23
V	13.6%	11.8%	0.87	14.5%	13.2%	0.92
<b>W</b>	1.8%	<b>3.2%</b>	<b>1.84</b>	2.0%	<b>1.7%</b>	<b>0.85</b>
<b>Y</b>	1.3%	<b>7.6%</b>	<b>6.03</b>	1.6%	<b>3.6%</b>	<b>2.20</b>
<b>I+L</b>	39.8%	33.6%	0.84	38.7%	34.4%	0.89
<b>TOTAL *</b>	99.3%	99.2%		99.4%	99.3%	

\*Amino acids which represent less than 0.5% of total amino acids (D,E, R and K) are not included.

**Table S2.** Amino acid composition (% of total amino acids) of cytoplasmic and exoplasmic hemi-TMDs for Golgi and PM proteins of the dataset.