Supplemental Figures and Legends

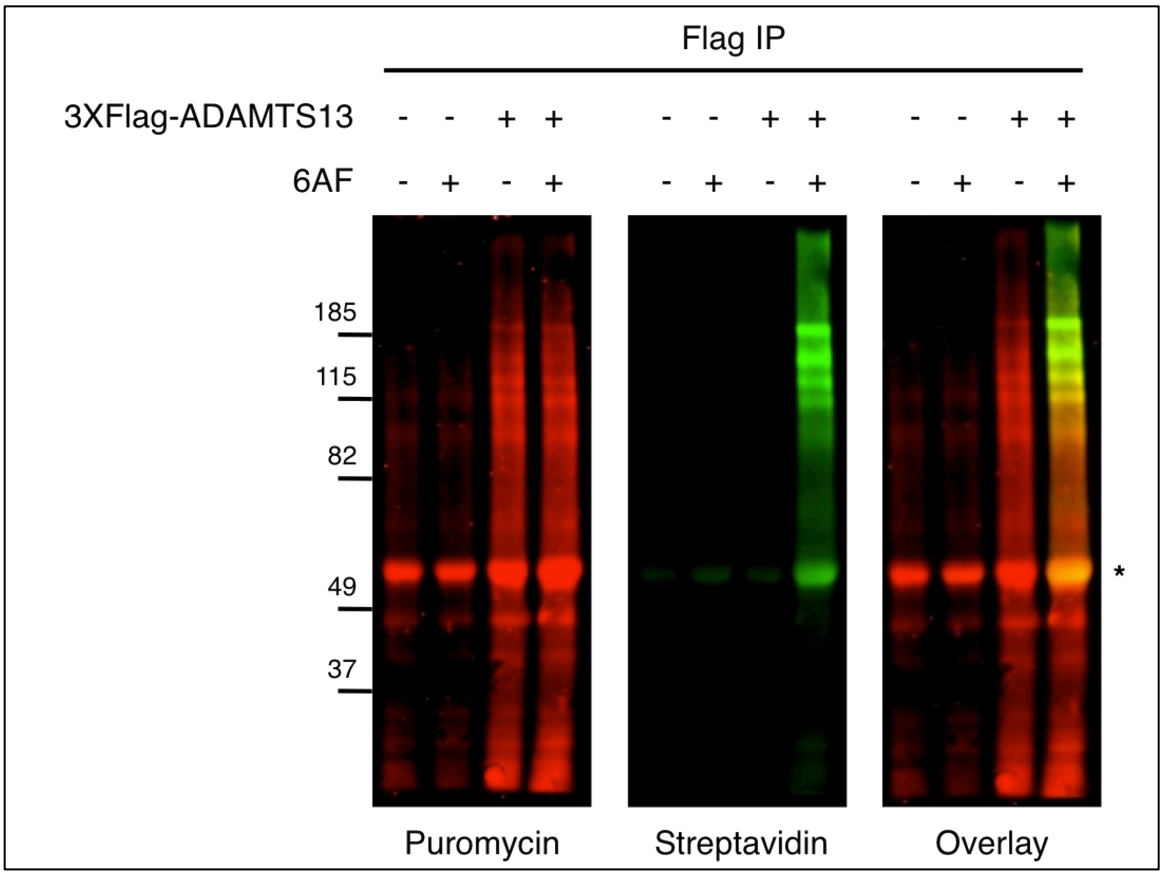

Figure S1 (related to Figure 2)

Puromycin-labeled ADAMTS13 nascent polypeptides are fucosylated

HEK293T cells were transfected with or without plasmid encoding 3X-FLAG-ADAMTS13 and metabolically labeled with or without 6AF. Cells were treated with 3 μM puromycin for 20 min before washing and lysis. ADAMTS13 was immunoprecipitated with anti-FLAG antibody and analyzed by Western blot with anti-puromycin (red, to detect nacent polypeptide chains) and Streptavidin (green, to detect 6AF). EV=empty vector control. * indicates band also appears in EV control.

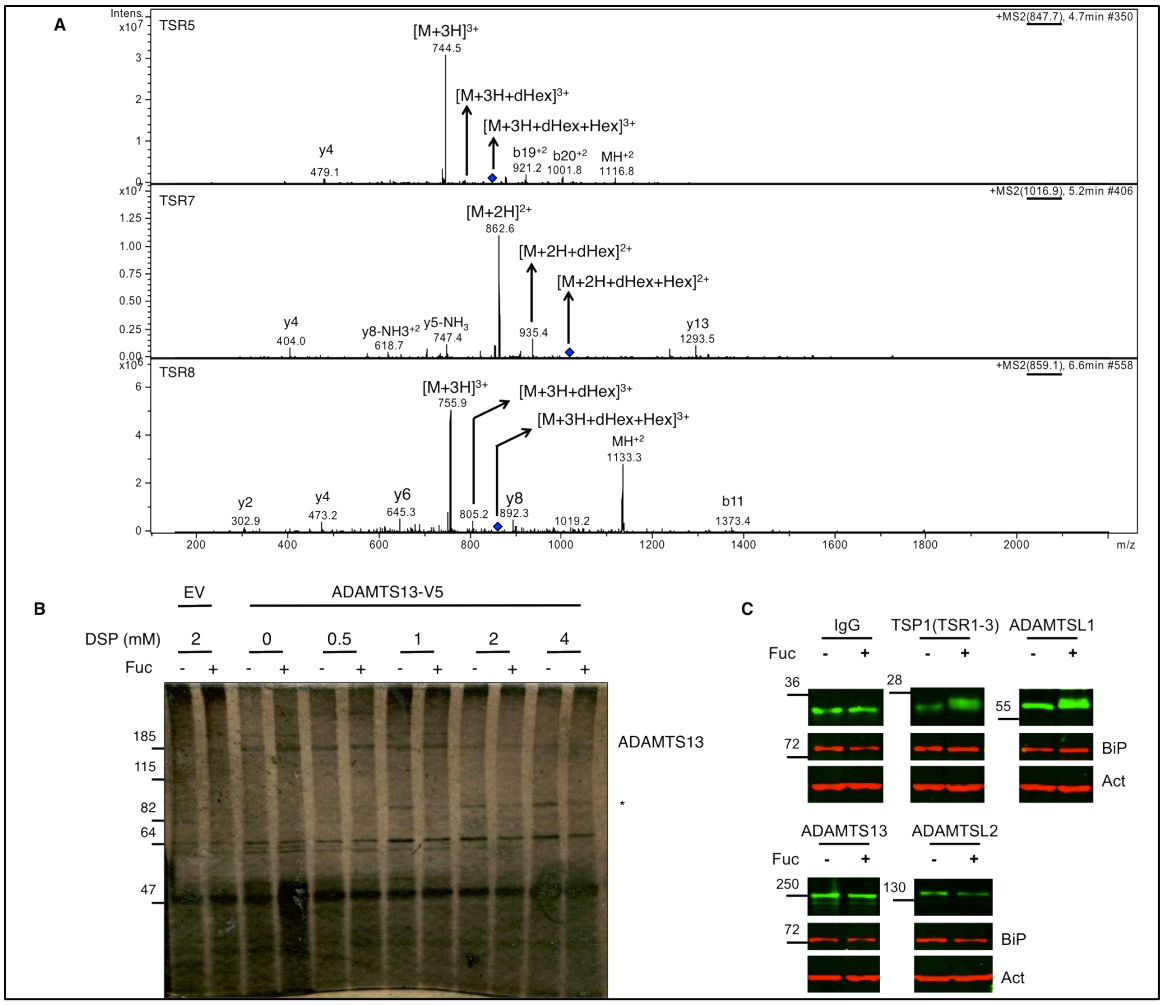

Figure S2 (related to Figure 3)

A) MS2 spectra for peptides described in Table S1 showing constant neutral loss of glucose and fucose from ADAMTS13 media samples

Top panel shows collision-induced dissociation fragmentation pattern of the ion corresponding the tryptic peptide from TSR5 of ADAMTS13. The parent ion selected for fragmentation is labeled as $[M+3H+dHex+Hex]^{3+}$ with a blue diamond. The ion, m/z 744.5 ($[M+3H]^{3+}$), matches the predicted mass for the triply charged, unglycosylated peptide. The difference between the mass of the parent ion (m/z 847.7) and the product (m/z 744.5) matches the losses of a hexose (Hex, presumably a glucose, 162 Da) and a

deoxyhexose (dHex, presumably a fucose, 146 Da) during the fragmentation of a triply charged peptide (103.2 Da x 3 = 309.6 Da). Confirmation of the peptide as ^{899}TGAQAAHVWTPAAGSCSVSCGR^{910} comes from detection of a number of *b* and *y* ions. Similarly, the middle and bottom panel show fragmentation of tryptic peptides from TSR7 (^{1018}VMSLGPCSASCGLGTAR^{1034}) and TSR8 (^{1076}WHVGTWMECSVSCGDGIQR^{1094}). Table S1 lists the calculated and observed masses of all ions.

B) Identification of chaperones involved in ER-retention of unfolded TSRs or lectins involved in ER-exit of folded TSRs

Lec13 CHO cells were grown in the presence or absence of 1 mM fucose and transfected with plasmid encoding ADAMTS13-V5-His6. The cells were treated with DSP prior to lysis. ADAMTS13 was immunoprecipitated from cell lysates and analyzed by SDS-PAGE followed by silver staining under reducing conditions. The band that was specifically enriched (marked by *) was excised and identified as BiP using mass spectrometry.

C) Expression of TSR containing proteins does not lead to increase in BiP levels.

Western blot showing input fraction of samples from Figure 3D. Actin (Act) was used as a loading control.

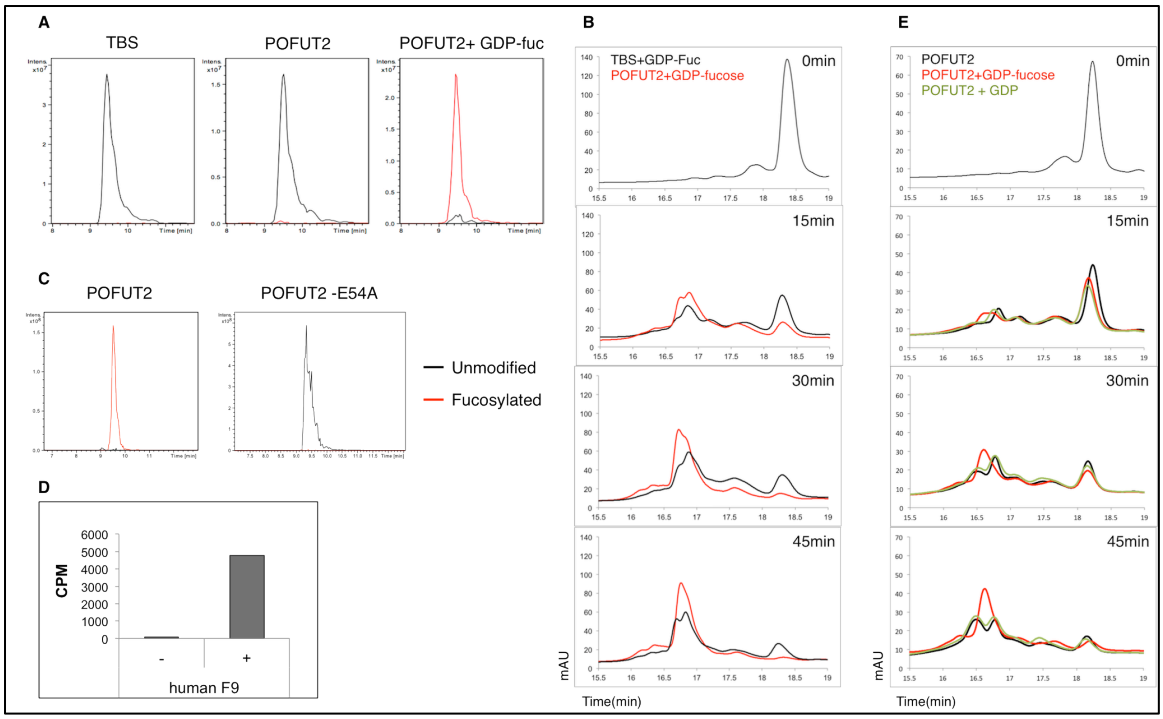

Figure S3 (related to Figure 5)

A) Majority of TSP1-TSR3 is *O*-fucosylated in the folding reaction

Aliquots from folding reactions (90 minute time point, Figure 5A) were spin filtered and analyzed by mass spectrometry. EICs of unmodified and *O*-fucosylated TSP1-TSR3 were prepared using masses listed in Table S2.

B) GDP-fucose alone is not sufficient to accelerate TSR folding

HPLC traces of refolding assay with TSR3-TSP1 in the presence of redox agents, GDP fucose and either TBS or POFUT2.

C) Pofut2-E54A mutant does not have fucosyltransferase activity

EICs from MS analysis of TSP1-TSR3 subjected to overnight fucosyltransferase assays using *wt* and the E54A mutant of Pofut2.

D) POFUT1 modifies EGF-like repeat from human factor 9 (hF9)

Affinity purified POFUT1 used in Figure 5B was tested for fucosyltransferase activity in an *in-vitro* metabolic labeling assay using hF9 as a acceptor substrate and GDP-[H^3]fucose as the donor substrate.

E) GDP-fucose is necessary for Pofut2 to accelerate TSR folding

HPLC traces of *in vitro* folding assays with redox agents and *wt* Pofut2 supplemented with either GDP or GDP-fucose. Performed as described in Figure 1.

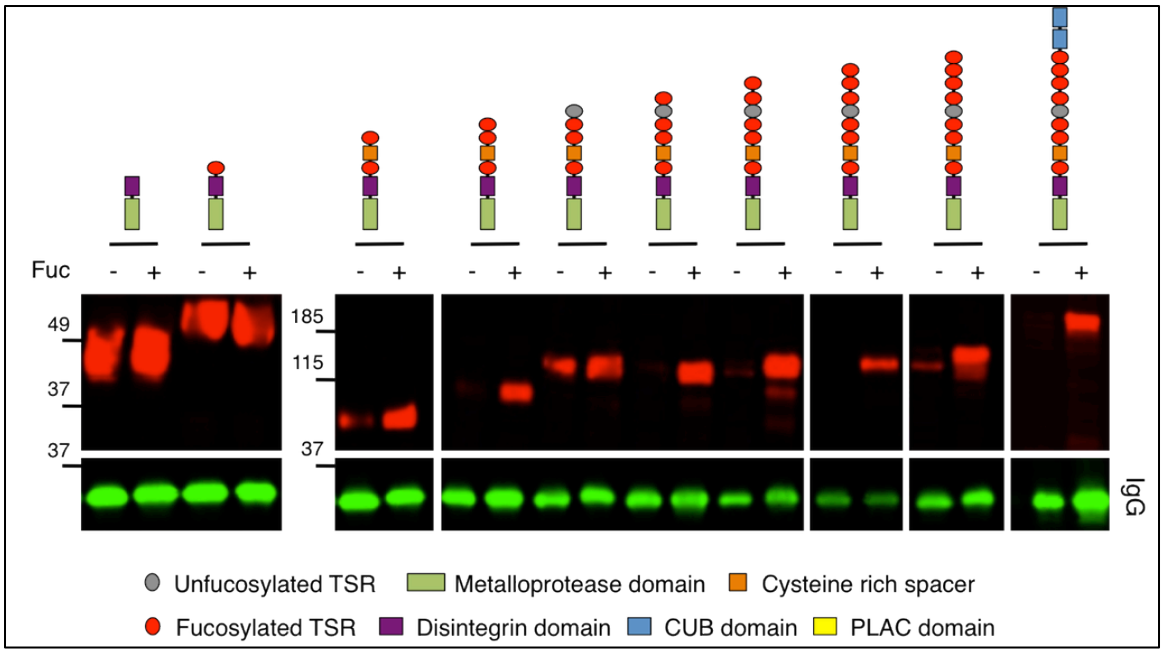

Figure S4 (Discussion).

Fucose-dependent secretion defect increases with number of tandem TSRs

Lec13 CHO cells were grown in the presence or absence of fucose and transfected with plasmids encoding truncated forms of ADAMTS13 varying in the number of TSRs or human IgG heavy chain as control. Media fractions were collected and analyzed by Western blot (red= anti-V5; green= anti-IgG).

Supplemental Tables

TSR #	Sequence	Charge (n)	$[M+nH]^{n+}$ (calculated)	$[M+nH]^{n+}$ (observed)	$[M+nH+Hex]^{n+}$ (observed)	$[M+3H+dHex+Hex]^{3+}$ (observed)
5	^{899}TGAQAAHVWTPAAGSCSVSCGR^{910}	3	744.8	744.9	793.5	847.5
7	^{1018}VMSLGPCSASCGLGTAR^{1034}	2	862.4	862.8	935.9	1017.1
8	^{1076}WHVGTWMECSVSCGDGIQR^{1094}	3	756.1	756.1	805.1	859.1

Table S1 (related to Figures 3 and S2)

Peptides identified in the MS analysis of ADAMTS13.

Calculated and observed masses of the ions derived from the (glyco)peptides from ADAMTS13 shown in Figures 3A and 3C. Three different glycoforms (unmodified, modified with Fuc monosaccharide or Glc-Fuc disaccharide) were detected.

Glycoform	$[M+7H]^{7+}$ (calculated)	$[M+7H]^{7+}$ (observed)
Unmodified	1173.9	1173.8
Fucosylated	1194.8	1194.7

Table S2 (Related to Figures 5 and S3)

Masses of unmodified and fucosylated TSP1-TSR3.

Calculated and observed masses of the +7 charge state of TSP1-TSR3 seen in Figures S3A and B. The average mass of unmodified TSP1-TSR3 is 8210.6 mass units.

Supplemental Experimental Procedures

Secretion assays in Lec13 CHO cells: The Lec13 CHO cells were cultured in α-MEM (GIBCO) containing bovine calf serum and penicillin/streptomycin (Life Technologies) either in the presence or absence of 1mM L-fucose (Spectrum). Post-transfection with plasmids encoding human IgG heavy chain or truncation mutants of ADAMTS13, the cells were incubated in Opti-MEM (Life Technologies) with or without 1 mM L-fucose for 24 h. Media fractions were collected and analyzed by western blotting with anti-V5 (Life Technologies) and anti-mouse Alexafluor680 (Life Technologies), or anti-IgG IRDye 800 (Rockland Immunochemicals).

Puromycin experiments: HEK293T cells were transiently transfected with plasmid encoding 3X-Flag-ADAMTS13 and incubated with 200 μM 6AF overnight. The cells were then treated with 3 μM puromycin for 20 min at 37°C. Following lysis, the samples were clicked, immunoprecipitated and analyzed by western blotting (Streptavidin, green; anti-puromycin, red).

DSP crosslinking and mass spectrometry: Lec13 CHO cells were transfected with ADAMTS13-V5-His_6 plasmid in the presence or absence of 1mM fucose. 24 h after transfection, the cells were treated with indicated concentrations of DSP (Pierce) for 2 h at 4°C according to the manufacturer's protocol. Cells were washed with TBS and lysed. Post lysis, ADAMTS13 was immunoprecipitated and eluates were analyzed on a reducing gel. Bands were visualized and prepared for mass spectrometric analysis using a silver stain kit (Life Technologies). Bands that changed in the presence or absence of fucose were excised and subjected to in gel digestion with trypsin as described, and proteins were identified by nano-LC-MS/MS analysis as described [S1].

Fucosyltranferase assay: POFUT1 activity was assayed as described before [S2]. Briefly, affinity purified POFUT1 was incubated with or without bacterially expressed EGF-like repeat from human F9 (10 nM), 10 μ M GDP-fucose containing 1 μ Ci $[H]^3$-GDP-fucose for 1 h at 37°C in buffer containing 50 mM HEPES pH 6.8, 10 mM $MnCl_2$. The reaction was stopped with 500 mM EDTA and unreacted GDP-fucose was removed using a C18-column. The human F9 was eluted with 80% methanol and counter on a Beckman scintillation counter.

Supplemental References

S1. Ricketts, L.M., Dlugosz, M., Luther, K.B., Haltiwanger, R.S., and Majerus, E.M. (2007). O-fucosylation is required for ADAMTS13 secretion. The Journal of biological chemistry 282, 17014-17023.

S2. Luo, Y., and Haltiwanger, R.S. (2005). O-fucosylation of notch occurs in the endoplasmic reticulum. The Journal of biological chemistry 280, 11289-11294.