Dynamic glycosylation governs the vertebrate COPII protein trafficking pathway

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Figure S1

Protein	Peptide sequence	Potentially modified residues on peptide	Number of O-GlcNAc moieties detected on peptide	Number of potentially modified residues on peptide	Odds of modification for each potentially modified residue
Sec23A	PQMPLIFLYVVDTCMEDEDLQALKE	T137	1	1	100%
Sec23A	VQVHELGCEGISK	S184	1	1	100%
Sec23A	PPSNRFLQPVQKIDMNLTDLLGELQR	S226, T241	2	2	100%
Sec23A	DAQTQIQNIAASFDQEAAAILMAR	T508, S516	2	2	100%
Sec23A	QPILYAYSFSGPPEPVLLDSSSILADR	S627, S629, S639, S640, S641	3	5	60%
Sec23A	PPSYAGISELNQPAELLPQFSSIEYVVLR	S97, S102, S115, S116	2	4	50%
Sec23A	TGLLEMKCCPNLTGGYMVMGDSFNTSLFK	T355, S367, S376, T379, S380	2	5	40%
Sec23A	PSQTHNNMYAWGQESGAPILTDDVSLQVFMDHLK	S725, T727, S738, T744, S748	1	5	20%
Sec23A	FSETFSLYPQFMFHLRRSSFLQVFNNSPDESSYYR	S571, T573, S575, S587, S588, S596, S600, S601	1	8	12.5%
Sec23B (assumed to be Sec23A)	IQPILYSYSFHGPPEPVLLDSSSILADR	S627, S629, S641, S642, S643	5	5	100%
Sec23B (assumed to be Sec23A)	QMSLSLLPPDALVGLITFGR	S154, S156, T168	2	3	T168 - 100%, S154, S156 - 50%
Sec23B (assumed to be Sec23A)	TQSLIMIQPILYSYSFHGPPEPVLLDSSSILADR	T615, S617, S627, S629, S641, S642, S643	3	7	42.3%
Sec23A (assumed to be Sec23A)	CANLTGGYMVMGDSFNTSLF	T369, S378, T381, S382	1	4	25%
Sec24C	FGAFYMSNTTDVELAGLDGDK	S773, T775, T776	3	3	100%

Figure S1 (continued)

Protein	Peptide sequence	Potentially modified residues on peptide	Number of O-GlcNAc moieties detected on peptide	Number of potentially modified residues on peptide	Odds of modification for each potentially modified residue
Sec24C	APPSSGAPPASTAQAPCGQAAYGQFGQGDVQNGPSS	S65, S66, S72, T73, S96, S97	5	6	83.33%
Sec24C	DTRETETVFVPVIQAGMEALK	T612, T615, T617	2	3	66.66%
Sec24C	GSYPQGQAPPLSQAQGHPGIQTPQR	S181, S191, T201	1	3	33.33%
Sec31A	QLQQAVQSQGFINYCQK	S451	1	1	100%
Sec31A	LTYAKPDEFSALCDLLGTR	T658, S666, T674	3	3	100%
Sec31A	LAIAWSMADPELLLSCGK	S269, S278	1	2	50%
Sec31A	DQVAQSDGEESPAAEEQLLGEHIK	S527, S532	1	2	50%
Sec31A	LTMHTHIVSTSNFSETSAFMPVLKV	T1187, T1190, S1194, T1195, S1196, S1199, T1201, S1202	3	8	37.5%
Sec31A	DVNIFQTNLVASGANESEIYIWDLNNFATPMTPGAK	T135, S140, S145, T157, T160	1	5	20%

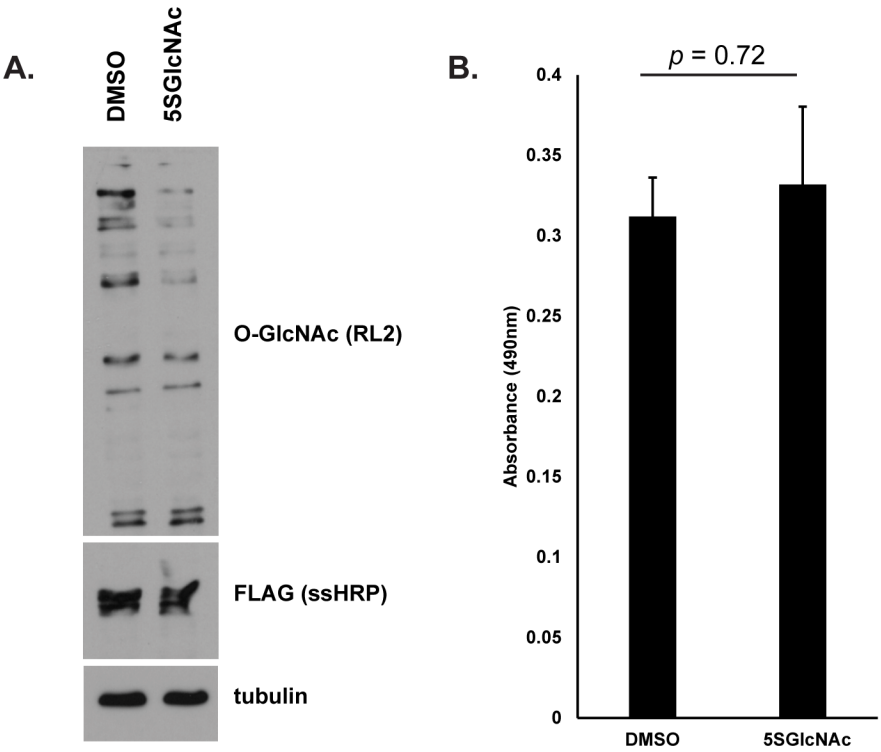
All O-GlcNAc-modified peptides and assigned or candidate O-GlcNAcylation sites are given. In most cases, the odds of modification were calculated by dividing the number of observed O-GlcNAc moieties by the number of modifiable residues on each peptide. In the case of SEC23A T168, O-GlcNAc was unambiguously assigned to the site upon manual inspection of MS/MS fragmentation data (see Figure 1). Note that peptides assigned by Mascot software to SEC23B were assumed to be the cognate SEC23A peptides, because SEC23A was expressed and purified to homogeneity (see Materials and Experimental Details).

Figure S1 (continued)

A scaffold file containing imported searched spectra from SEC23A, SEC24C, and SEC31A is also available for download. All searched spectra were imported into Scaffold (v4.7, Proteome Software) and scoring thresholds were set to achieve a peptide false discovery rate below 1% using the PeptideProphet algorithm.

In the SEC23A sample, peptides assigned by Mascot to SEC23B were assumed to be the cognate peptides in SEC23A, since the SEC23A was purified to homogeneity. Details of search parameters are available within the file. Scaffold viewer is available for free to download at http://www.proteomesoftware.com/products/free-viewer.

Figure S2



A. 5SGlcNAc treatment reduces global O-GlcNAc levels but does not reduce the intracellular expression of ssHRP. Cells were treated as in the secretion assay described in Figure 2B and whole-cell lysates were analyzed by WB.

B. 5SGlcNAc treatment does not affect cell viability. Cells were treated as in the secretion assay described in Figure 2B and were analyzed by MTS assay.

Figure S3

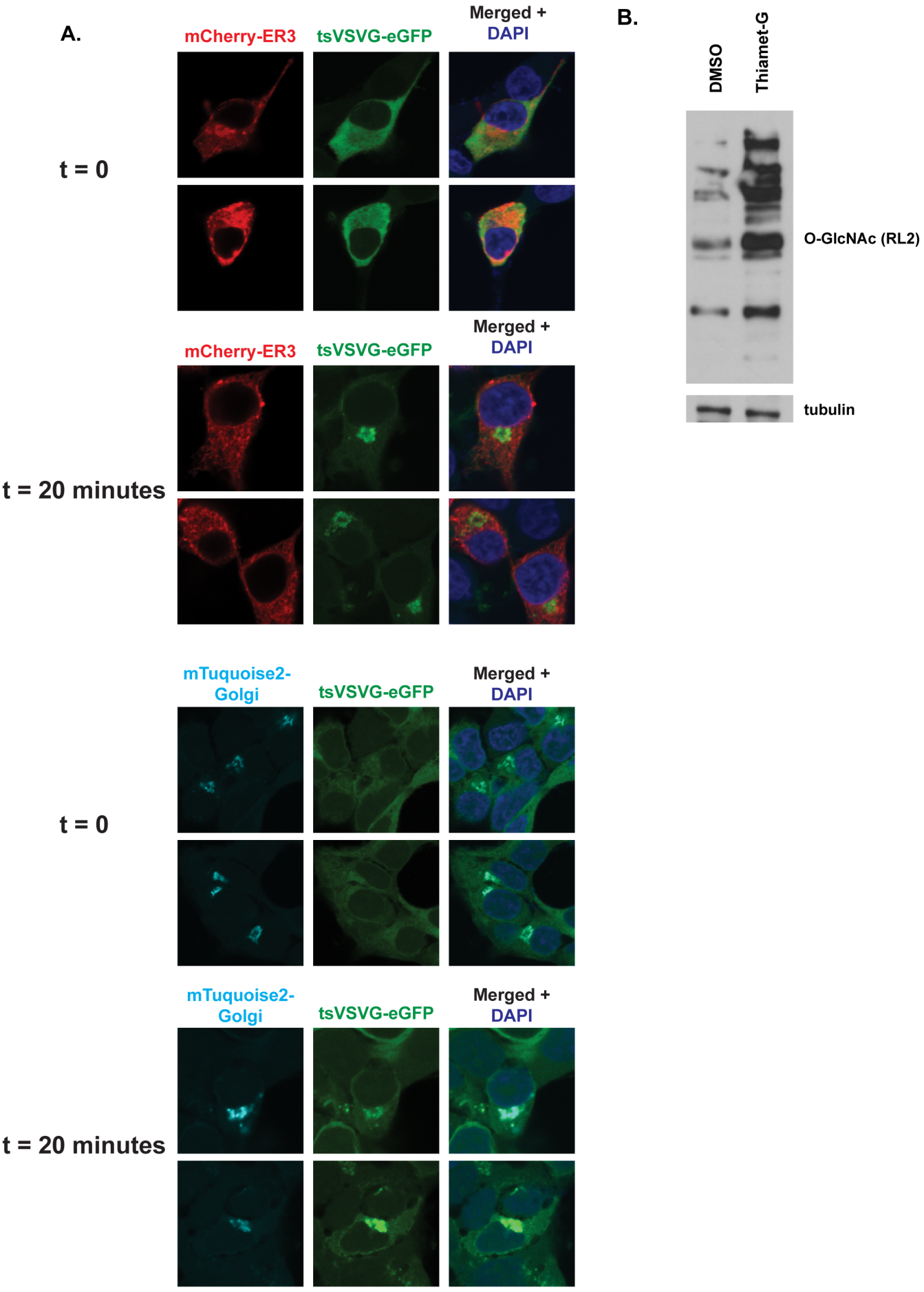
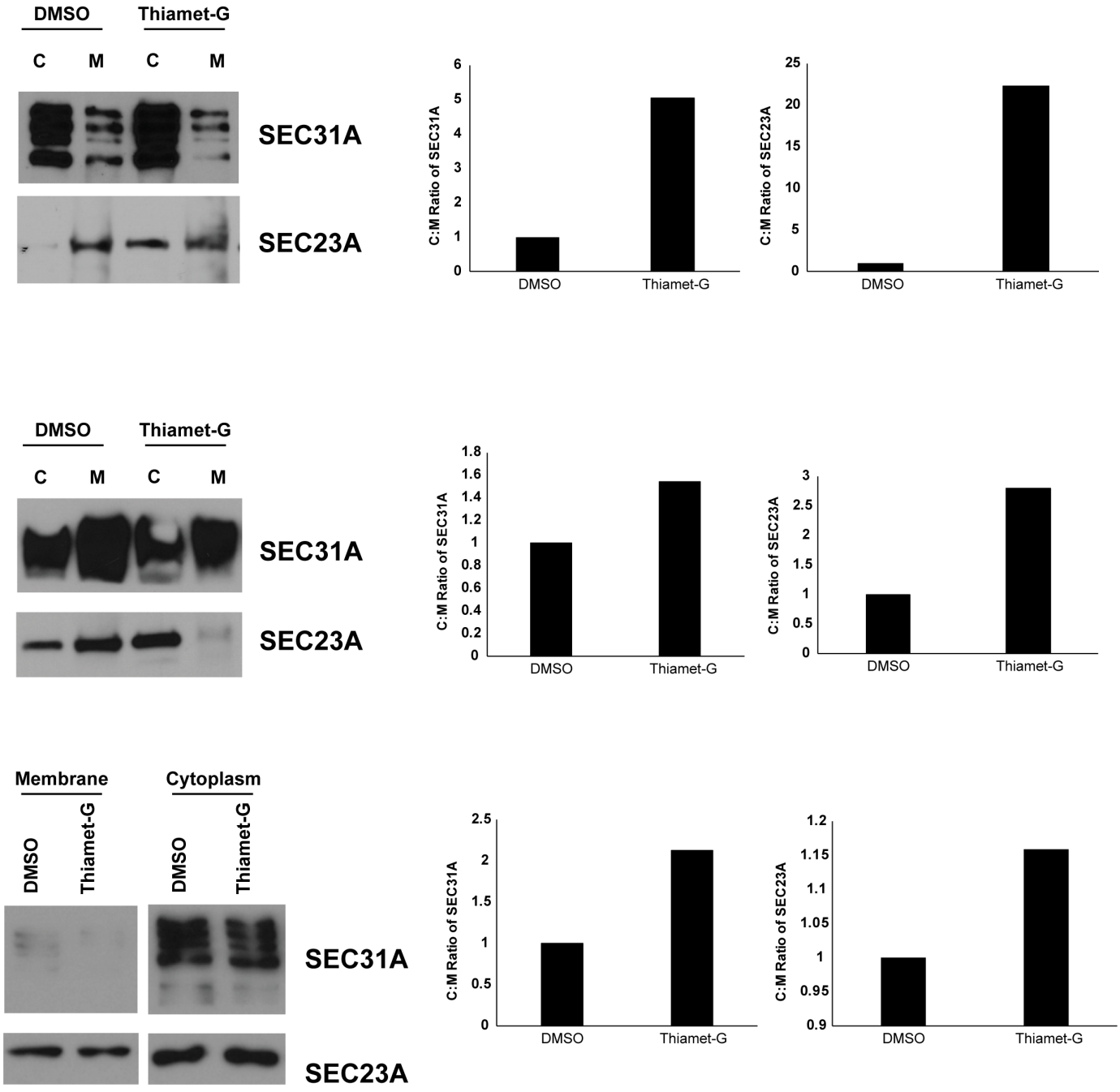

A.
mCherry-ER3
tsVSVG-eGFP
Merged + DAPI
t = 0
t = 20 minutes
mTuquoise2-Golgi
B.
DMSO
Thiamet-G
O-GlcNAc (RL2)
tubulin

Figure S3 (continued)

A. COS7 cells expressing tsVSVG-eGFP were transiently transfected with genetically encoded markers for the ER (mCherry-ER3, red) or Golgi (mTurquoise2-Golgi, cyan), subjected to the tsVSVG trafficking assay as described in Figure 2C, and then fixed and imaged at the indicated recovery times. As expected, tsVSVG-eGFP (green) initially colocalizes with the ER marker (t = 0) but traffics in a COPII-dependent manner to the Golgi (t = 20 minutes). Representative images from two biological replicates are shown.

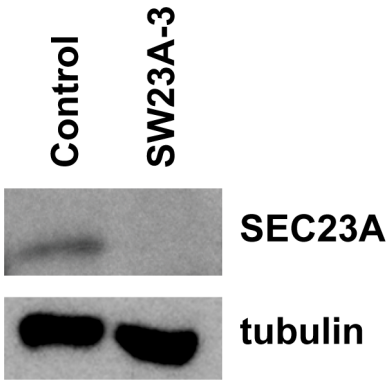
B. Thiamet-G treatment enhances global O-GlcNAc levels in COS7 tsVSVG-eGFP cells. Cells were treated as described in Figure 2C and whole cell lysates were analyzed by WB.

Figure S4



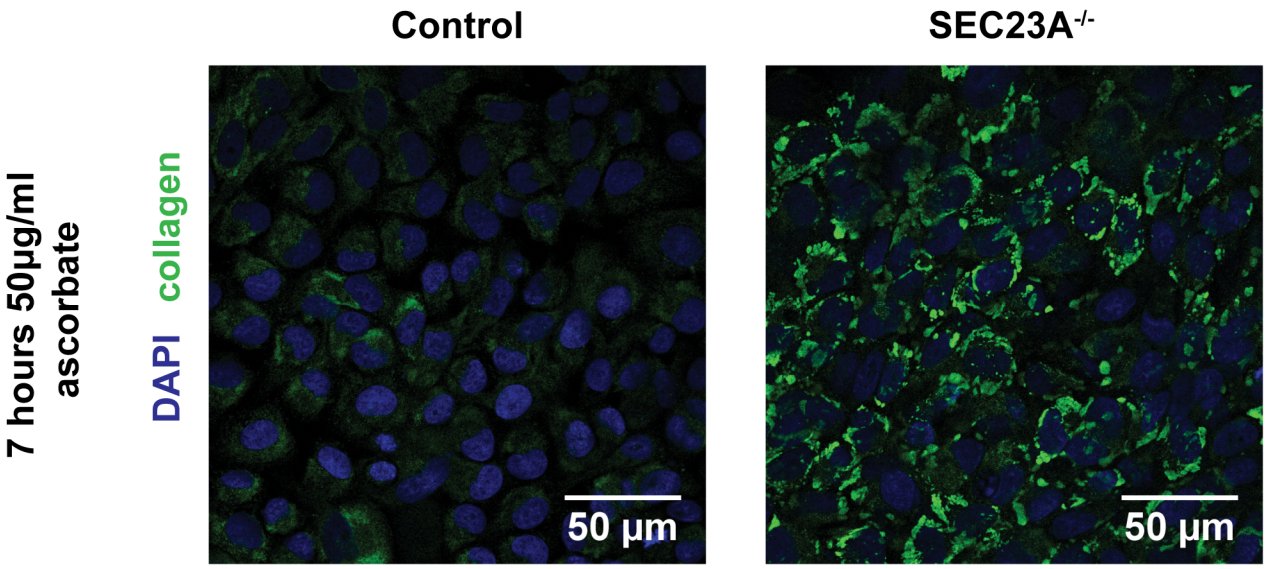
Three biological replicates of the digitonin fraction assay were performed as in Figure 2D and were analyzed by WB (left). The ratio of cytoplasmic (C) to membrane (M)-bound SEC23A or SEC31A (C:M) was calculated by densitometry for each independent experiment and was represented graphically (right). White rectangles indicate where irrelevant lanes have been cropped out of a single blot for one replicate (bottom panels).

Figure S5



Single-cell clones derived from SW1353 cells stably expressing Cas9 and an sgRNA targeting a “safe harbor” locus (Control) or the *SEC23A* locus (SW23A-3) were analyzed by WB.

Figure S6



SW1353 clones deleted for the "safe harbor" control locus (left, Control) or *SEC23A* (right, SEC23A$^{-/-}$) were treated with 50 µg/ml sodium ascorbate for 7 hours to stimulate collagen translation, as described[46, 47]. Then, cells were fixed and stained for endogenous collagen (LF68 anti-collagen, green) and DNA (DAPI, blue). Consistent with prior reports in other *SEC23A* loss-of-function systems[15, 21, 26], SEC23A$^{-/-}$ cells exhibit dramatic intracellular collagen accumulation and distended ER phenotype. See also Figure S7.

Figure S7

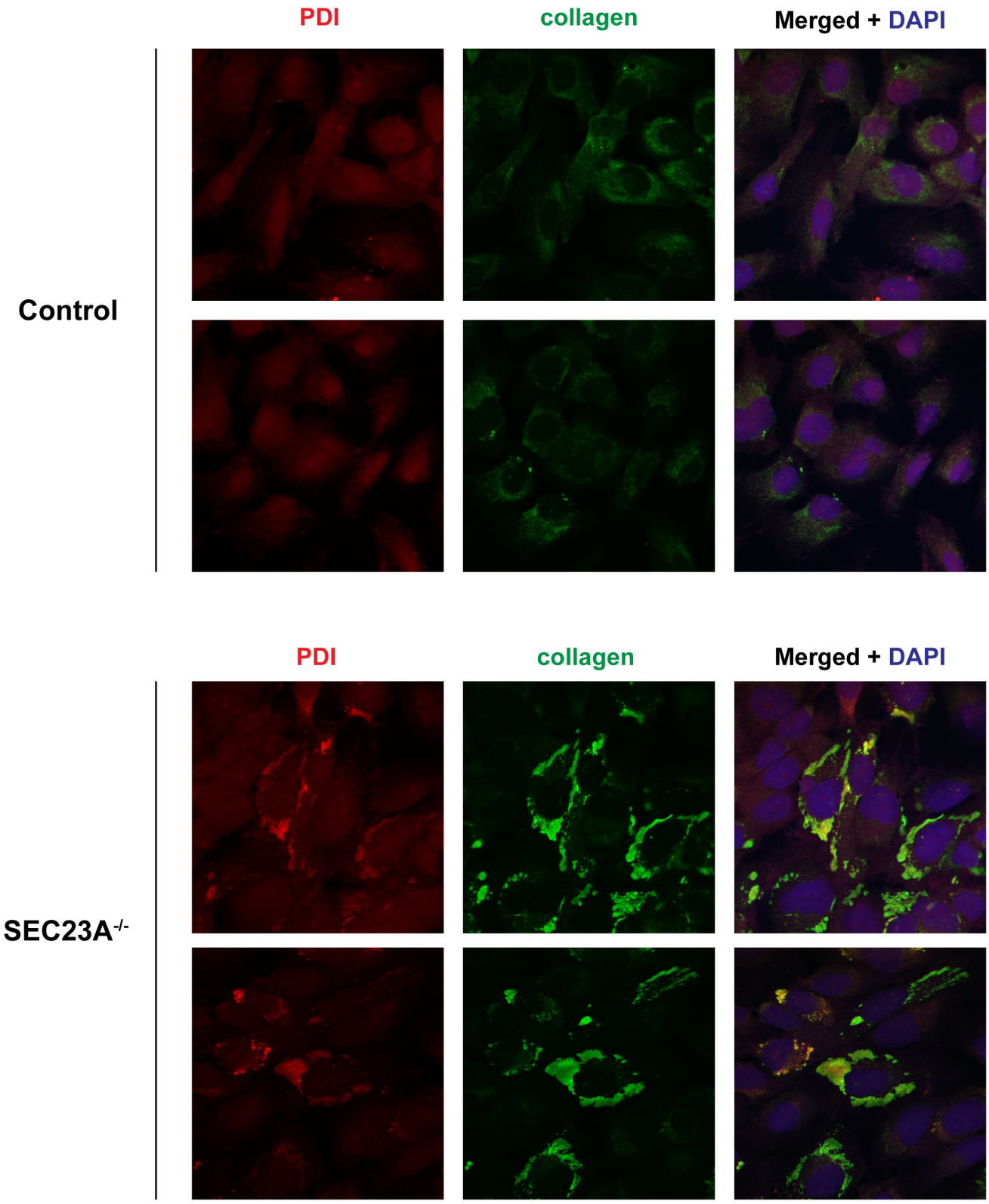
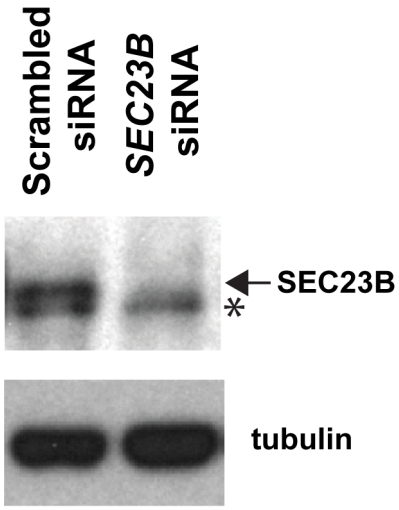

PDI
collagen
Merged + DAPI
Control
PDI
collagen
Merged + DAPI
SEC23A-/-

Figure S7 (continued)

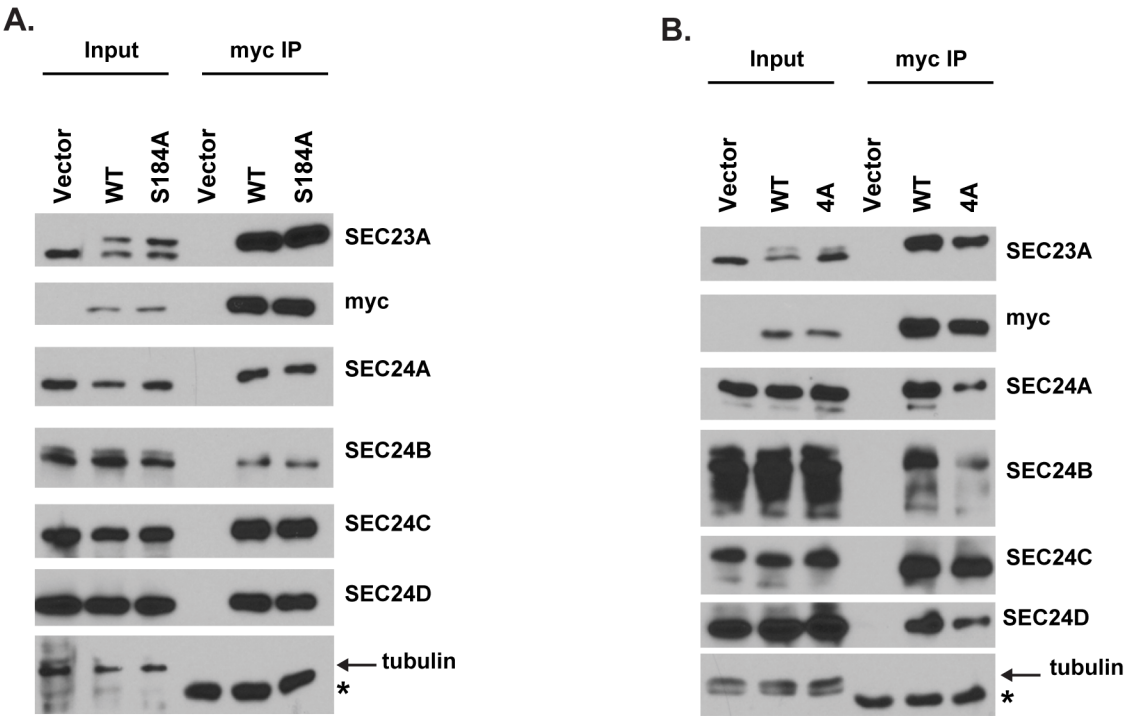
SW1353 clones deleted for the “safe harbor” control locus (top, Control) or *SEC23A* (bottom, SEC23A$^{-/-}$) were treated with 50 μg/ml sodium ascorbate for 7 hours to stimulate collagen translation, as described[46, 47]. Then, cells were fixed and stained for the endogenous ER marker proline disulfide isomerase (PDI, red), endogenous collagen (QED anti-collagen, green), and DNA (DAPI, blue). PDI and collagen colocalization confirms that the collagen signal in the SEC23A$^{-/-}$ cells represents retained protein in the ER, consistent with prior reports[15, 21, 26]. Representative images from two biological replicates are shown.

Figure S8



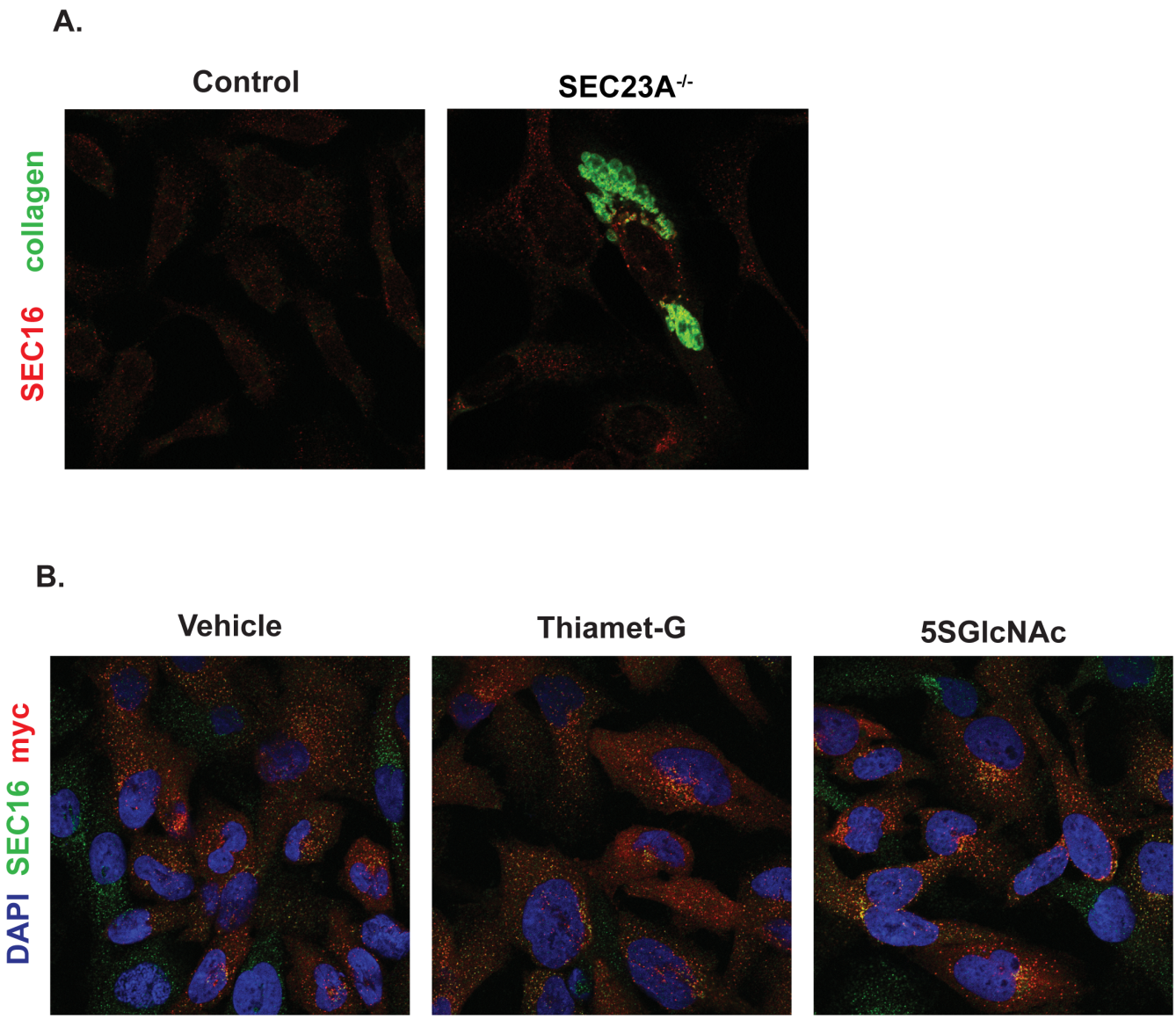
SEC23A$^{-/-}$ SW1353 cells stably reconstituted with wild type SEC23A-myc-6xHis were transfected with an siRNAs against *SEC23B* (right) or a scrambled control (left). After 72 hours, cell lysates were harvested and analyzed by WB. SEC23B is indicated by an arrow. Asterisk, nonspecific band. Tubulin is a loading control.

Figure S9



Epitope-tagged human S184A (**A**) or 4A (**B**) mutant SEC23A was stably expressed in SEC23A$^{-/-}$ SW1353 cells, IPed and analyzed by WB. Both mutants co-IP endogenous SEC24 paralogs with efficiency similar to that of wild type SEC23A. Tubulin (arrow) is a loading control. Asterisk, IgG heavy chain from IPing antibody.

Figure S10



A. SW1353 clones deleted for the "safe harbor" control locus (left, Control) or *SEC23A* (right, SEC23A$^{-/-}$) were stained for endogenous collagen (QED anti-collagen, green) and the ERES marker protein SEC16 (red). *SEC23A* deletion does not dramatically affect ERES number, morphology or distribution.

B. SEC23A$^{-/-}$ SW1353 cells stably reconstituted with wild type myc-6xHis-tagged SEC23A were treated with vehicle only, 50 μM Thiamet-G or 50 μM 5SGlcNAc for 8 hours as indicated and then stained for SEC23A (anti-myc, red) and endogenous SEC16 (green). Neither OGT inhibition nor OGA inhibition dramatically affects ERES number, morphology or distribution.