SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure 1

Characterization of TMX/TMX4 antibodies used in this study. Left: Top and bottom: Immunofluorescence quality control of the rabbit anti-TMX antibody (Sigma) and the mouse anti-TMX4 antibody (middle). Right: Western blot quality controls of the rabbit anti-TMX antibody (Sigma), showing depletion with siRNA and a large section of a lysate gel.

Supplemental Figure 2

Quality control of the Optiprep protocol with HeLa cell homogenates. Cell homogenates were fractionated on a discontinuous 10-30% Optiprep gradient. ERp57 and Ribophorin-2 indicate the position of the rER (fractions 3-5), the peaks of the ACAT1 and calnexin signals indicates the MAM (fraction 6) that overlaps with mitochondrial complex 2.

Supplemental Figure 3

Analysis of the ER domain distribution of TMX family proteins in CaCo2 cells. Cell homogenates were fractionated on a discontinuous 10-30% Optiprep gradient and probed for the four TMX proteins.

Supplemental Figure 4

Percoll gradient quality control and electron microscopy of Percoll gradient fractions of MAM, mitochondria and microsomes. HeLa homogenates were fractionated into cytosol (Cyt.), microsomes (Micro), crude mitochondria (MC), purified mitochondria (MP) and MAM according to Materials and Methods. Marker proteins indicate mitochondria (complex 2), MAM (Ero1α) and rER membranes (ribophorin-2, eIF2α). Equal cell equivalents have been loaded. The bottom electron micrographs show the isolated fractions. Microsomes (left) contain vesicles and ribosomes (present as clusters). Mitochondria (center) and MAM (right) are also shown.

Supplemental Figure 5

Additional immunofluorescence microscopy. A. HeLa cells were processed and imaged as in Figure 2 using a rabbit TMX and a mouse calnexin antibody. A zoomed area is boxed. Extensive overlap is seen. B. A375P cells were processed and imaged as in Figure 2 using a rabbit TMX and a mouse TMX4 antibody.

Supplemental Figure 6

A375P cells were treated for TMX and TMX4 immunogold labeling as described in Materials & Methods, a representative image is shown. 10nm anti-mouse immunogold particles (anti-TMX4) are highlighted with black arrowheads; 15nm anti-rabbit immunogold particles (anti-TMX) are highlighted with white arrowheads. Scale bar = 200 nm.

Supplemental Figure 7

Expression level analysis of TMX/TMX4 chimera.

Supplemental Figure 8

Optiprep gradient fractionation of CD25/Tac-TMX and CD25/Tac-calnexin chimera. HeLa cells transfected with chimera of luminal CD25/Tac fused to the transmembrane and cytosolic domain of TMX, TMX CCAA, TMX4, calnexin and calnexin CCAA were fractionated on a discontinuous 10-30% Optiprep gradient. Detection by Western blot with a rabbit anti-CD25/Tac antiserum (n=3).

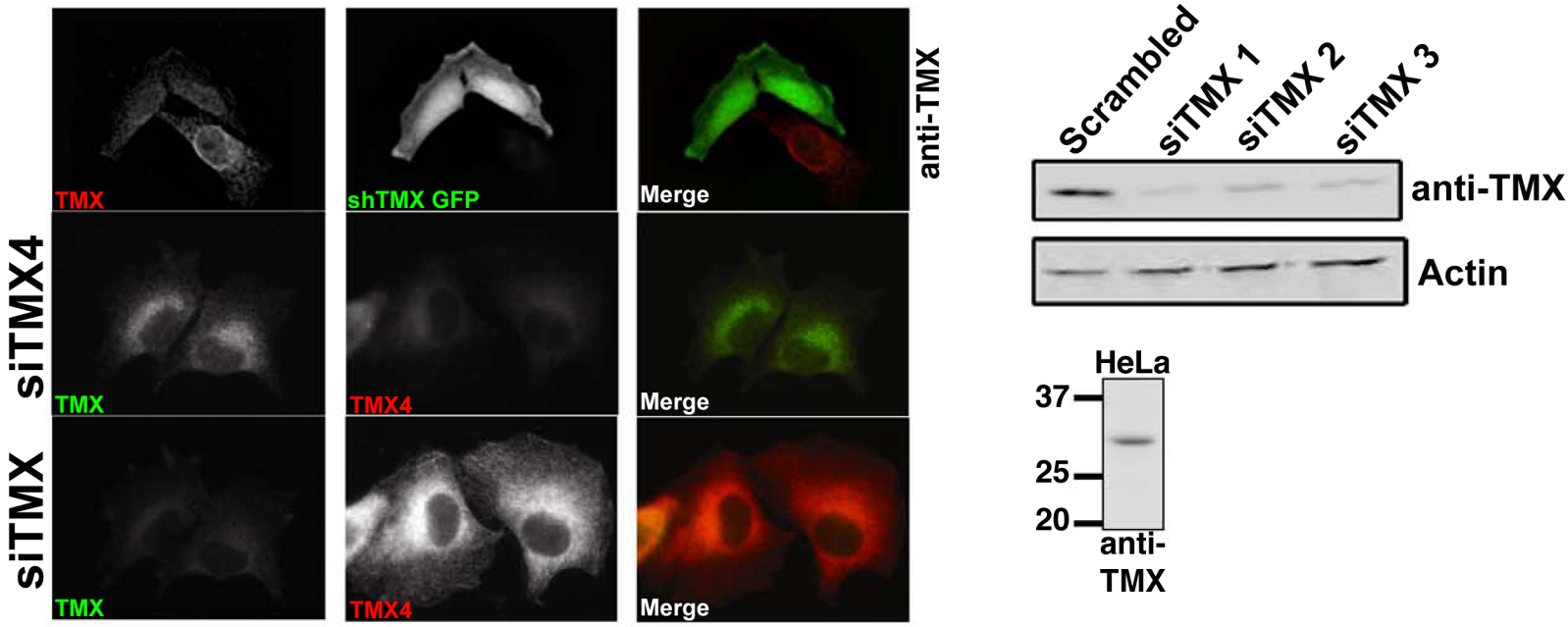
Supplemental Figure 9

Detection of palmitoylation on TMX sorting signal mutants (labeled as in Figure 4E). Top gel shows the alkynyl-palmitate detection with HRP-conjugated neutravidin. Bottom gel shows the input, as evidenced by the anti-FLAG Western blot signal.

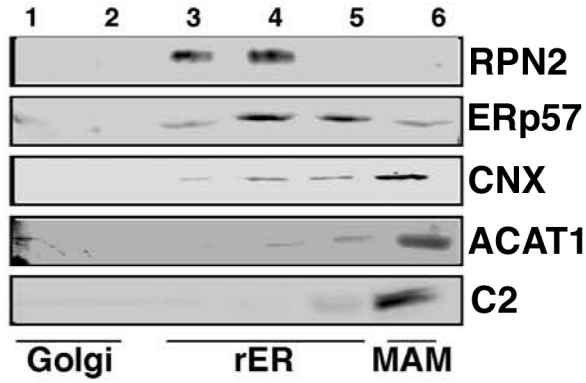
Supplemental Figure 10

β-methyl-cyclodextrin does not influence the apposition of the TMX and calnexin signals with mitochondria. HeLa cells depleted of cholesterol with β-methyl-cyclodextrin

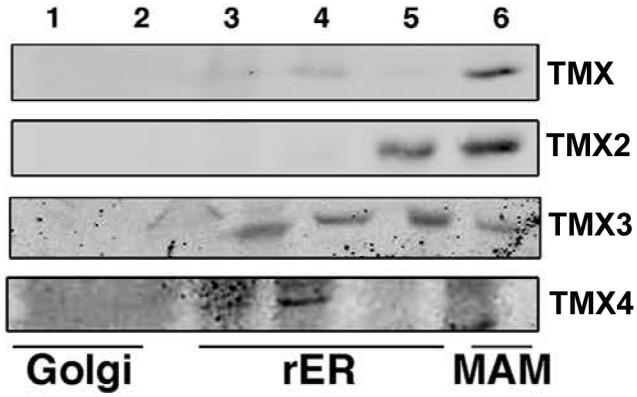
were fractionated on a discontinuous 10-30% Optiprep gradient, and enrichment of TMX on the MAM was determined by Western blot. Mitochondrial complex 2 serves as control for MAM integrity.



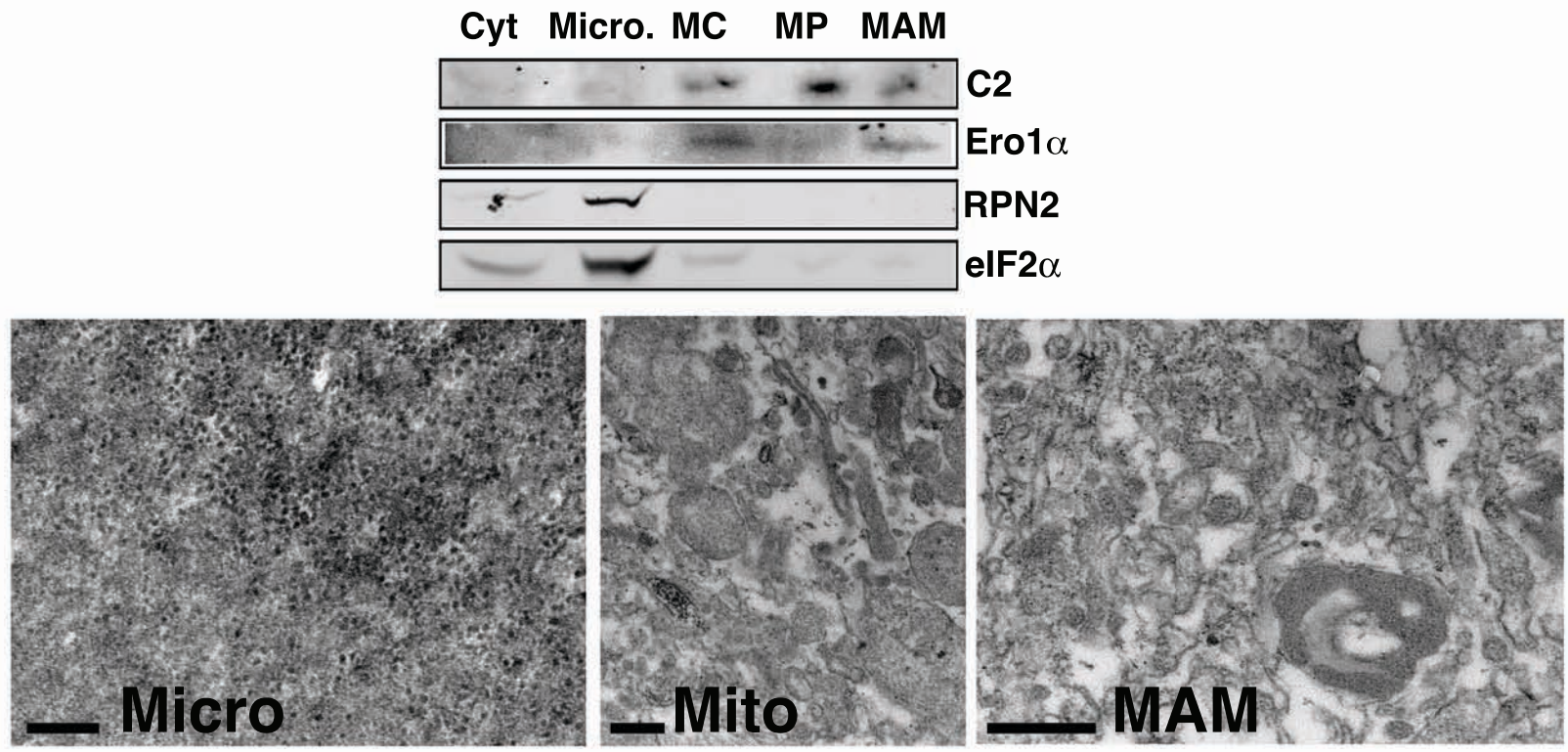
Lynes et al., Supplemental Figure 1



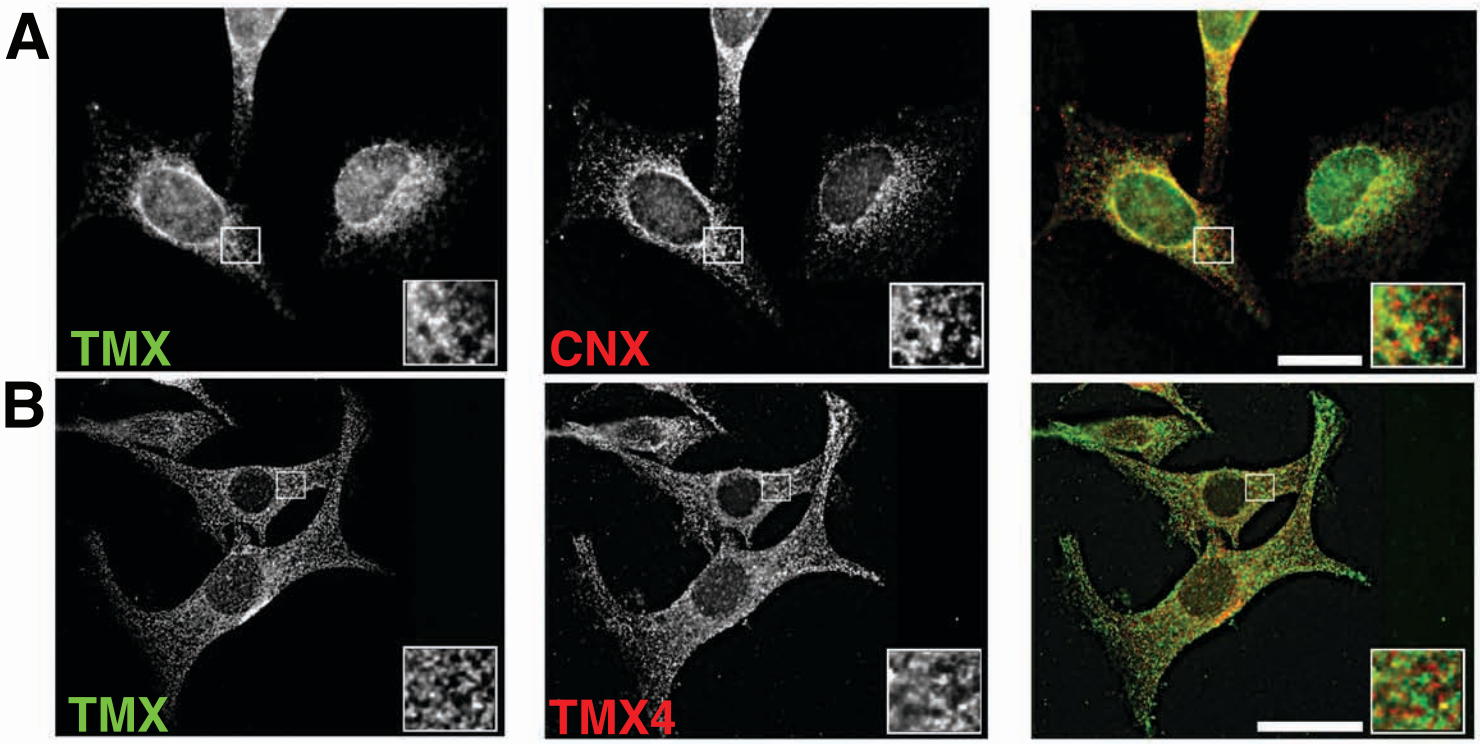
Lynes et al., Supplemental Figure 2



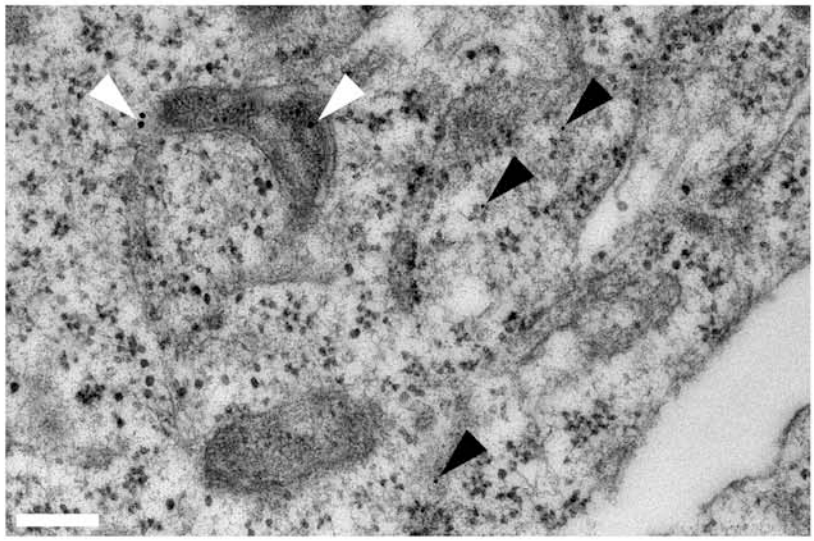
Lynes et al., Supplemental Figure 3



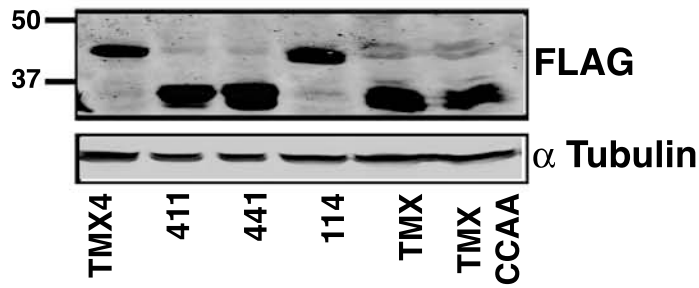
Lynes et al., Supplemental Figure 4



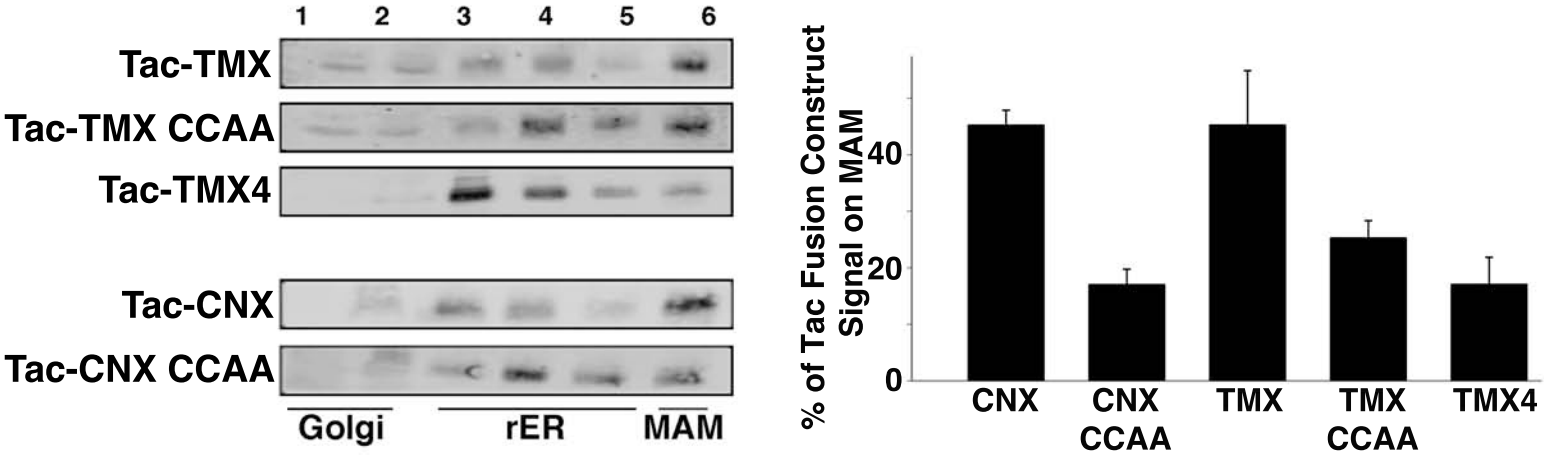
Lynes et al., Supplemental Figure 5



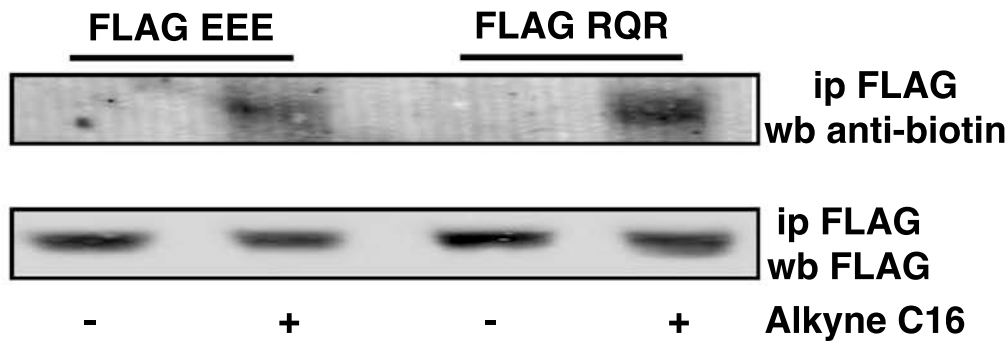
Lynes et al., Supplemental Figure 6



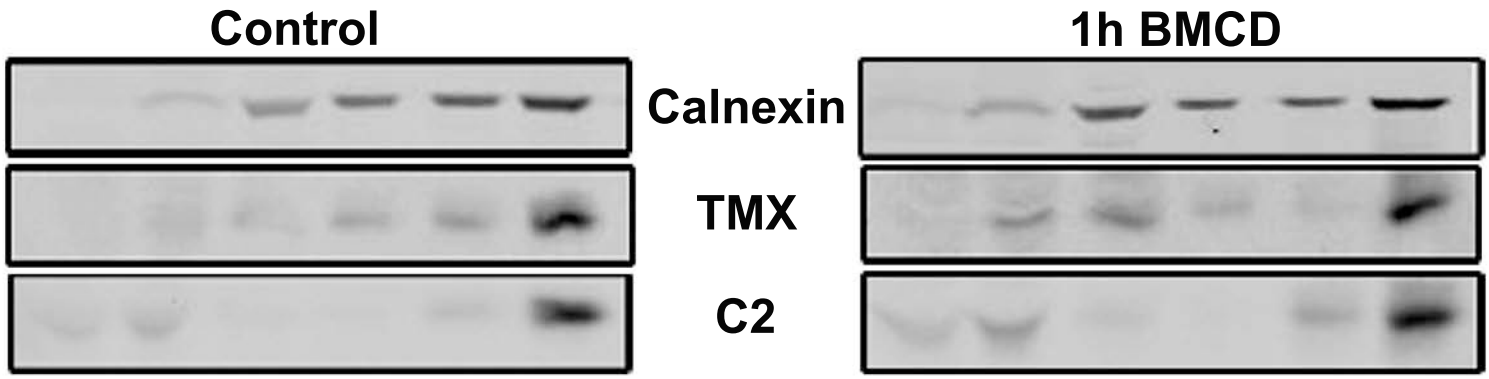
Lynes et al., Supplemental Figure 7



Lynes et al., Supplemental Figure 8



Lynes et al., Supplemental Figure 9



Lynes et al., Supplemental Figure 10