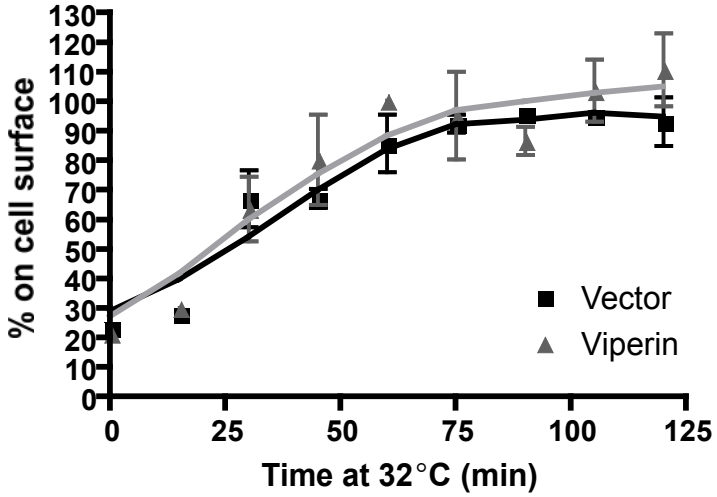
SUPPLEMENTAL FIGURE 1. Viperin does not alter the transport of membrane bound proteins.
(A) 293T cells transiently expressing ts045 VSV-G GFP and either viperin or the vector control were incubated at 39°C overnight to misfold VSV-G and retain the glycoprotein in the ER. Cells were moved to the permissive temperature (32°C) and harvested at the noted times, fixed and stained for surface VSVG. The level of cell surface VSV-G was normalized to the total amount of VSV-G GFP. The graph represents an average of three independent experiments.
(B) 293T cells transiently expressing PLAP together with vector control, viperin, or Sar1 dominant negative (dn), were treated with phospholipase C to remove cell surface PLAP. Cells were washed and incubated for the noted times. The surface arrival kinetics of newly synthesized PLAP was determined by harvesting cells, retreating with phospholipase C to remove PLAP, and then quantitating the released PLAP using the SeAP assay.
(C) 293T cells expressing the noted constructs and PLAP were analyzed as in Figure 2B for PLAP trafficking by measuring the acquisition of Endo H resistance.

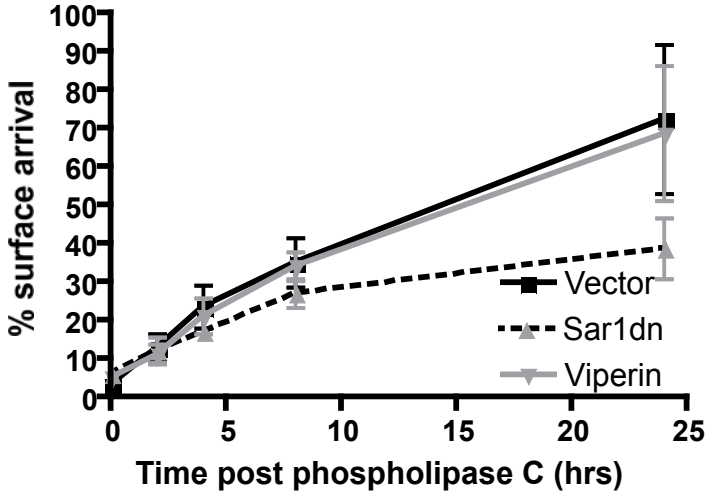
SUPPLEMENTAL FIGURE 2. Point mutants in the N-terminal amphipathic 〈-helix do not alter ER localization. 293T cells over-expressing the noted viperin helical wheel point mutants were fixed, permeabilized with Triton X-100, and then stained with MaP.VIP and anti-calnexin to examine ER localization.

SUPPLEMENTAL FIGURE 3. Progressively deleting helical turns in the N-terminal amphipathic 〈-helix reduces viperin ER association. (A) 293T cells over-expressing the vector control (Cnt), wildtype (WT) viperin, or viperin mutants with one (T1, residues 4-8 deleted), two (T2, residues 4-13 deleted), three (T3, residues 4-18 deleted), *etc.,* sequential helical turns deleted were analyzed by immunofluoresence for localization with the ER resident protein calnexin. (B) 293T cells over-expressing the noted helical turn mutants in (A) plus GFP as a transfection control and cytosolic marker were separated into membrane and cytosol fractions by freeze-thaw and ultracentrifugation. The membrane pellet was washed and solubilized in 1% Triton X-100. Equal amounts of proteins were subjected to SDS-PAGE and then blotted for viperin, GM130 as a membrane marker, and GFP as a cytosolic marker.

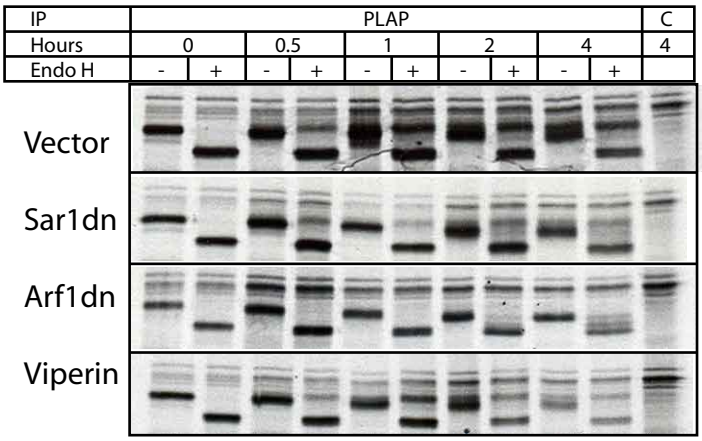
A

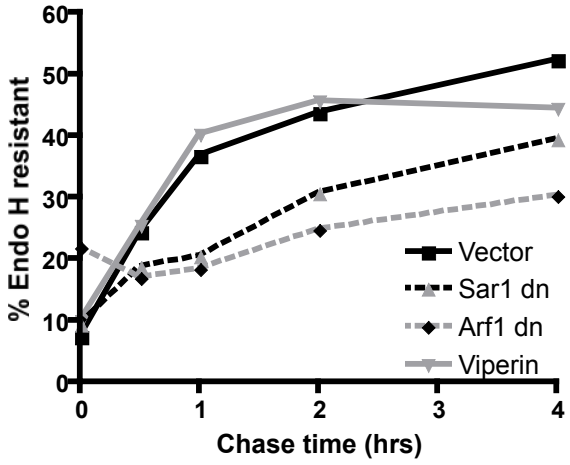

% on cell surface
Time at 32°C (min)
Vector
Viperin

B

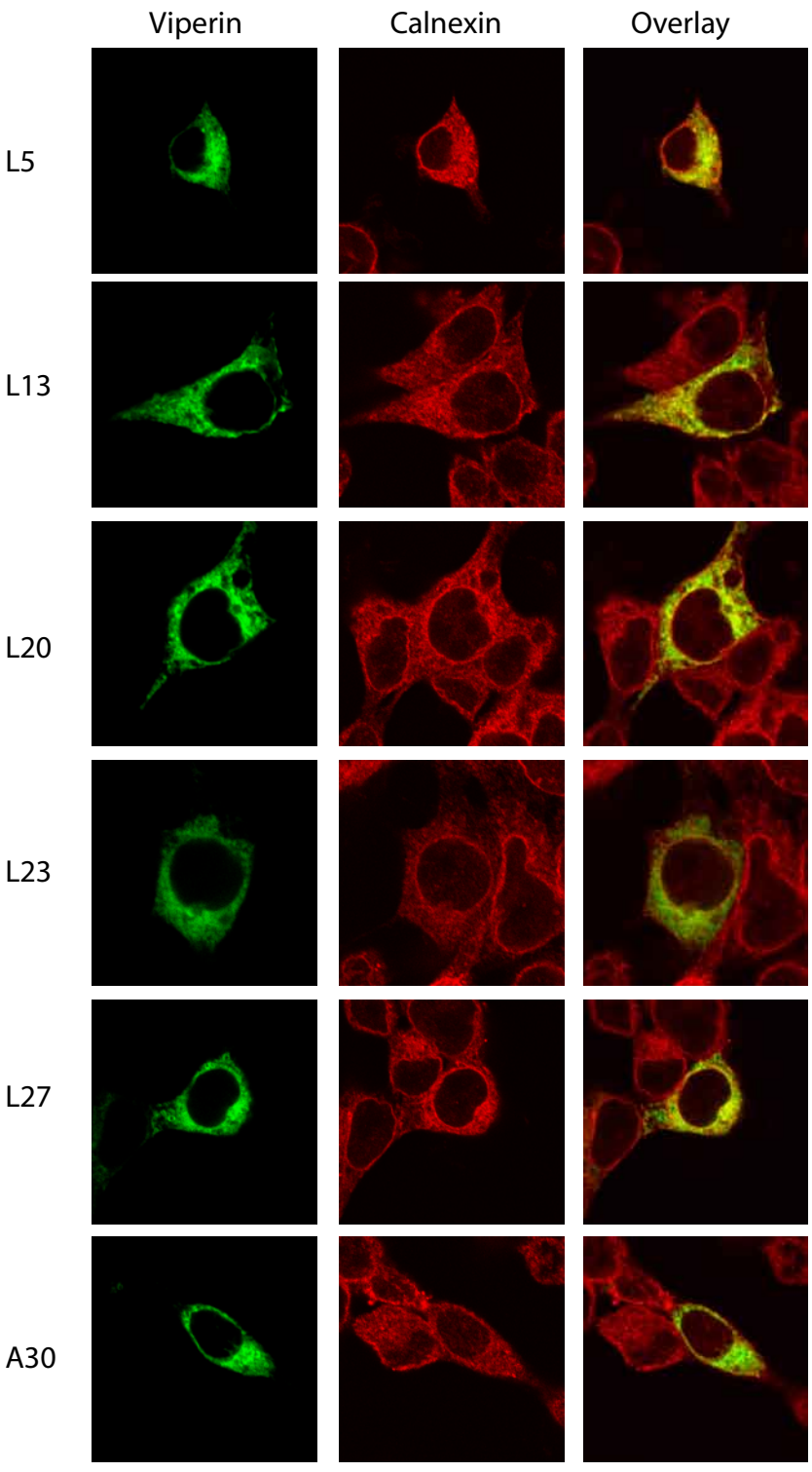

% surface arrival
Time post phospholipase C (hrs)
Vector
Sar1dn
Viperin

C


IP
PLAP
C
Hours
0
0.5
1
2
4
4
Endo H
Vector
Sar1dn
Arf1dn
Viperin

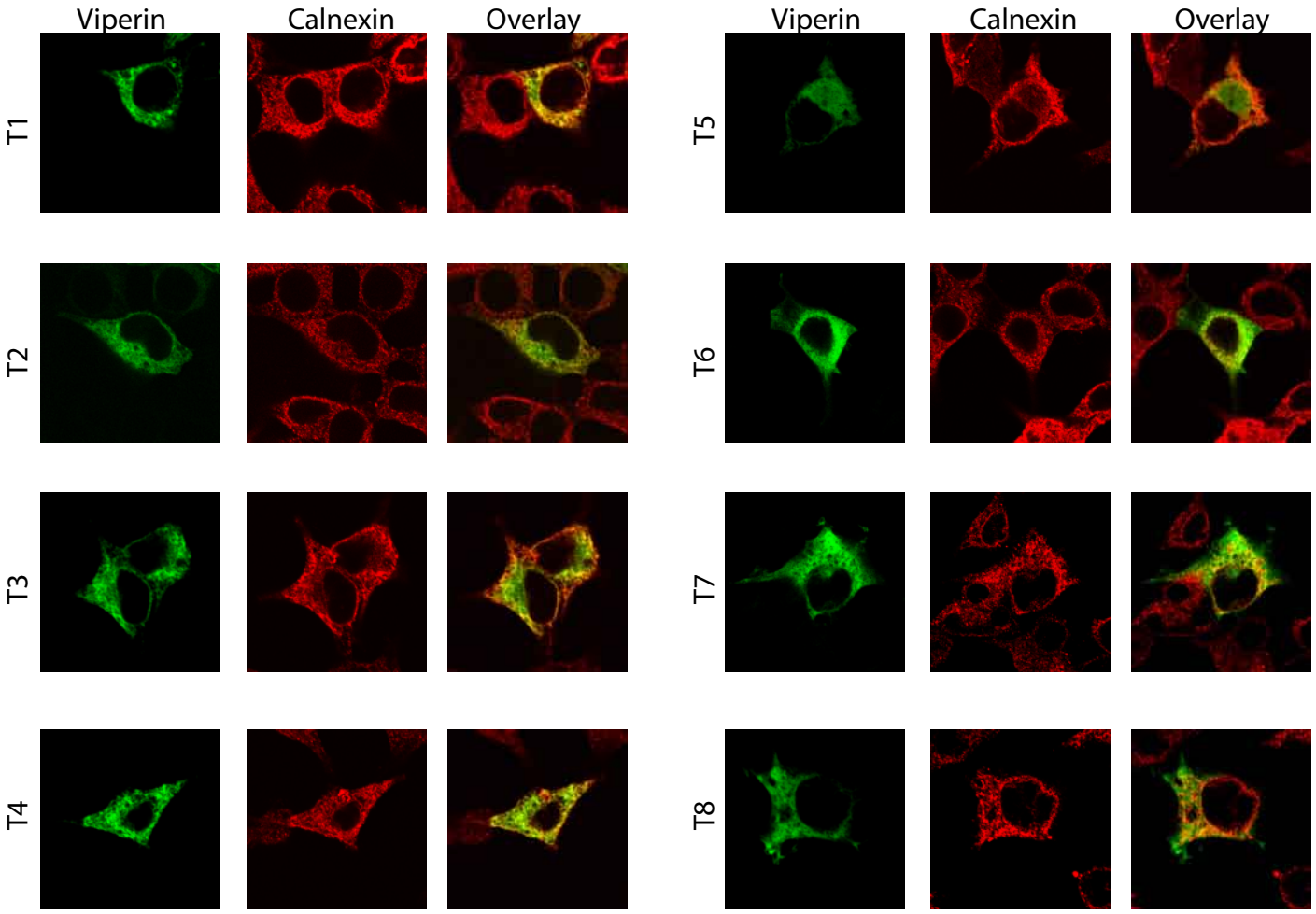

% Endo H resistant
Chase time (hrs)
Vector
Sar1 dn
Arf1 dn
Viperin

Supplemental Figure 2



Supplemental Figure 3

A



B