SUPPLEMENTAL FIGURES, MOVIES AND TABLE LEGENDS Figure S1

Figure S1

Alignment of the transmembrane and the cytoplasmic domains of calnexin and calmegin from different species, highlighting the conservation of the juxtamembranous cysteine and the transmembrane proline residues. The fully conserved amino acids are highlighted in blue. The palmitoylated cysteines are indicated by the green rectangle. The proline residue in the transmembrane region is indicated by the red rectangle.

Figure S2

A: HeLa cells were transfected for 72hrs with either the control siRNA or different DHHC siRNA. DHHC RNA levels were analyzed by quantitative RT-PCR. The figure shows that silencing was efficient for all DHHC enzymes. This is one representative experiment out of n>3.

B: HeLa cells were transfected with a shRNA targeting the DHHC6 non-coding region or a control shRNA for 6 days. During the last 2 days, the cells were recomplemented with either the empty vector or a plasmid expressing human DHHC6 cDNA. The cells were labeled with ^{3}H-palmitic acid for 2 h followed by immunoprecipitation with anti-calnexin. Immunoprecipitates were migrated on a SDS-PAGE and further analyzed by autoradiography.

C: HeLa cells were transfected for 72hrs with WT or mutant calnexin-HA and different DHHC siRNAs. Cells were incubated with ^{3}H-palmitic acid for 2hrs prior to immunoprecipitation using anti-HA antibodies. Immunoprecipitates were split into two, run on SDS-PAGE and analyzed either by autoradiography (^{3}H -palmitate) or Western blotting (anti-HA). Quantification is

shown in Fig. 2C. Error bars correspond to standard deviation (n=4)

D: HeLa cells were transfected 48hrs with DHHC5 or DHHC6 cDNAs. The cells were fixed with methanol and immunostained with either anti-flag or anti-myc antibodies. Bar: 10 µm. DHHC5 shows a typical Golgi staining, whereas DHHC6 shows a typical ER staining, including the nuclear membrane.

E: Hela cells were cotransfected 24hrs with WT or mutant calnexin-HA and with either DHHC6-myc or DHHC3myc- human cDNA. Cells were incubated with ^{3}H -palmitic acid for 2 h prior to immunoprecipitation using anti-HA antibodies. Immunoprecipitates were split into two, run on SDS- PAGE and analyzed either by autoradiography (^{3}H-palmitate) or Western blotting against HA and myc.

F: Autoradiograms from panel E were quantified using the Typhoon Imager (Image QuantTool, GE Healthcare). Errors correspond to standard deviations (n=4).

Figure S3

A: Calnexin and DHHC6 mRNA expression was measured by quantitative real time PCR in different human tissues. Both messengers are ubiquitously expressed (n=1).

B: HeLa cells were transfected 24hrs with or without myc-LRP6 or TEM-HA, and with human DHHC6 cDNA. Cells were incubated with ^{3}H-palmitic acid for 2hrs prior to immunoprecipitation using anti-transferrin receptor, anti-myc-LRP6, anti- CMG2 or anti-TEM8-HA antibodies. Immunoprecipitates were split into two, run on SDS PAGE and analyzed either by autoradiography (^{3}H-palmitate) or Western blotting (anti-tfR, anti-TEM8-HA, anti-CMG2 and anti-

MYC-LRP6).

Figure S4

A: HeLa cells were transfected with either the control siRNA or siRNA against DHHC6 for 72h. The cells were washed and fixed in methanol followed by immunostaining either with anti-calnexin, anti-TRAPα or anti-BiP antibody. Each image represents the summation of a z-stack. Bar: 10 μm

B: HeLa cells were transfected with either control shRNA or shRNA against DHHC6 for 8 days. On day 6, the cells were split and transfected either with an empty vector or human DHHC6 cDNA bearing a myc tag. The cells were washed, fixed and stained first with anti-calnexin, anti-nucleoporin and anti-DHHC6-myc. Bar: 10 μm

Figure S5

A: The footprint of control vs. DHHC6 RNAi treated cells was monitored using transferrin labeled cells, quantified in an automated manner, for 40 cells/ experiment in 3 independent experiments. The error bars represent the standard deviation.

B: HeLa cells were transfected with either the control siRNA or siRNA against TRAPα for 72h. The cells were washed and fixed in methanol followed by immunostaining with anti-calnexin antibody. The z-stacks were captured and each image represents the summation of the z-stack. Calnexin staining was insensitive to TRAPα silencing. Bar: 10 μm.

Figure S6

Total cell extracts for the experiment performed in Figures 5BC (**A**) and in 5D (**B**) were analyzed by SDS-PAGE and Western blotting against calnexin, TRAPα, Sec61α, L12, Sec61ß, DHHC6-myc and GAPDH, which was used as a loading control. Equal amounts of proteins (40µg) were loaded per lane.

C: HeLa cells were transfected with shRNA against DHHC6 for 8 days. On day6 the cells were retransfected either with an empty vector or a plasmid expressing human DHHC6 cDNA. The cells were lysed and immunoprecipitated with anti rabbit calnexin antibody followed by migration of the immunoprecipitates on the SDS-PAGE. The immunoprecipitates were analyzed by western blotting for calnexin and actin. Upon recomplementation with DHHC6, the interaction between calnexin and actin is restored.

Figure S7

A,B: Glycoprotein and the cytosolic protein fraction obtained from the glycoprotein analysis described in Figure 7A was migrated on SDS-PAGE and the gel was fixed and exposed to autoradiography. The radiolabeled products were visualized by using Typhoon Imager (Image QuantTool, GE Healthcare). Production of cytosolic proteins was unaffected by the silencing of DHHC6, DHHC3, TRAPα or calnexin.

C: HeLa cells were transfected with either the control siRNA or the siRNA against DHHC6, TRAPα and the shRNA against calnexin. These cells were cotransfected with a plasmid expressing bovine preprolactin (PPL) and in case of calnexin shRNA, the PPL transfection was done at 96h. The cell medium was replaced with fresh medium to analyze the protein secreted in 24h. The volume of medium loaded on the gels was normalized to the amount

of protein extracted from the cells. Western blot was performed and the membrane was blotted with anti Flag antibody to reveal the PPL expression in the medium. The blots were quantified using Image J software and the error bars represent the standard deviation (n=3).

D: HeLa cells were transfected with either the control shRNA or the shRNA against calnexin. At 96h post transfection, cells were transfected with the pSEAP2 vector and either empty vector or the vector expressing WT or mutant calnexins. At 144h post initial transfection, cell lysates were analyzed by western blotting against calnexin, HA tagged WT or mutants and GAPDH as a loading control.

E: Autoradiograms from Figure 7D (bottom panel) were quantified using Typhoon Imager (Image QuantTool, GE Healthcare). Errors correspond to standard deviations (n=3). Synthesis of PrP was unaffected by the silencing of DHHC6, TRAPα or calnexin.

Movies S1

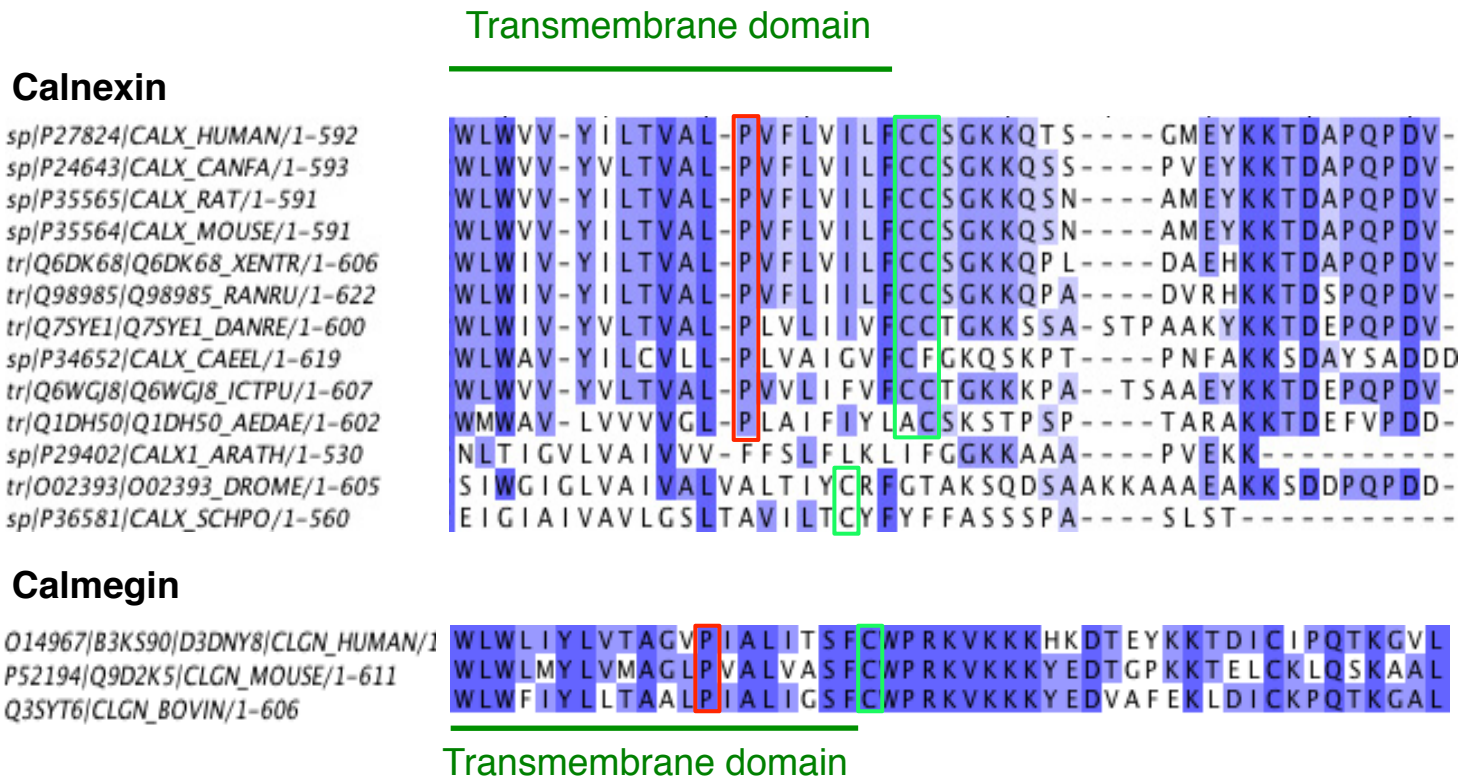
A: HeLa cells were transfected with control siRNA for 72h followed by methanol fixation and immunostaining with anti-calnexin antibody. The movie illustrates that at some point through the stacks, the nuclear membrane staining becomes strikingly apparent, showing rings around the nucleus.

B: HeLa cells were transfected with siRNA against DHHC6 followed by methanol fixation and immunostaining with anti-calnexin antibody. For both the movies, Z-stacks were generated for a lawn of cells using confocal microscopy and the movie was generated from the stacks using Image J software. The movie A illustrates that at some point through the stacks, the

nuclear membrane staining becomes strikingly apparent in control cells, showing rings around the nucleus. These rings are not observed in movie B.

Legend of Table S1

The table summarizes the siRNA and shRNA target sequences and the q-RT-PCR oligos used for each of the DHHC genes.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sp|P36581|CALX_SCHPO/1-560
Calmegin
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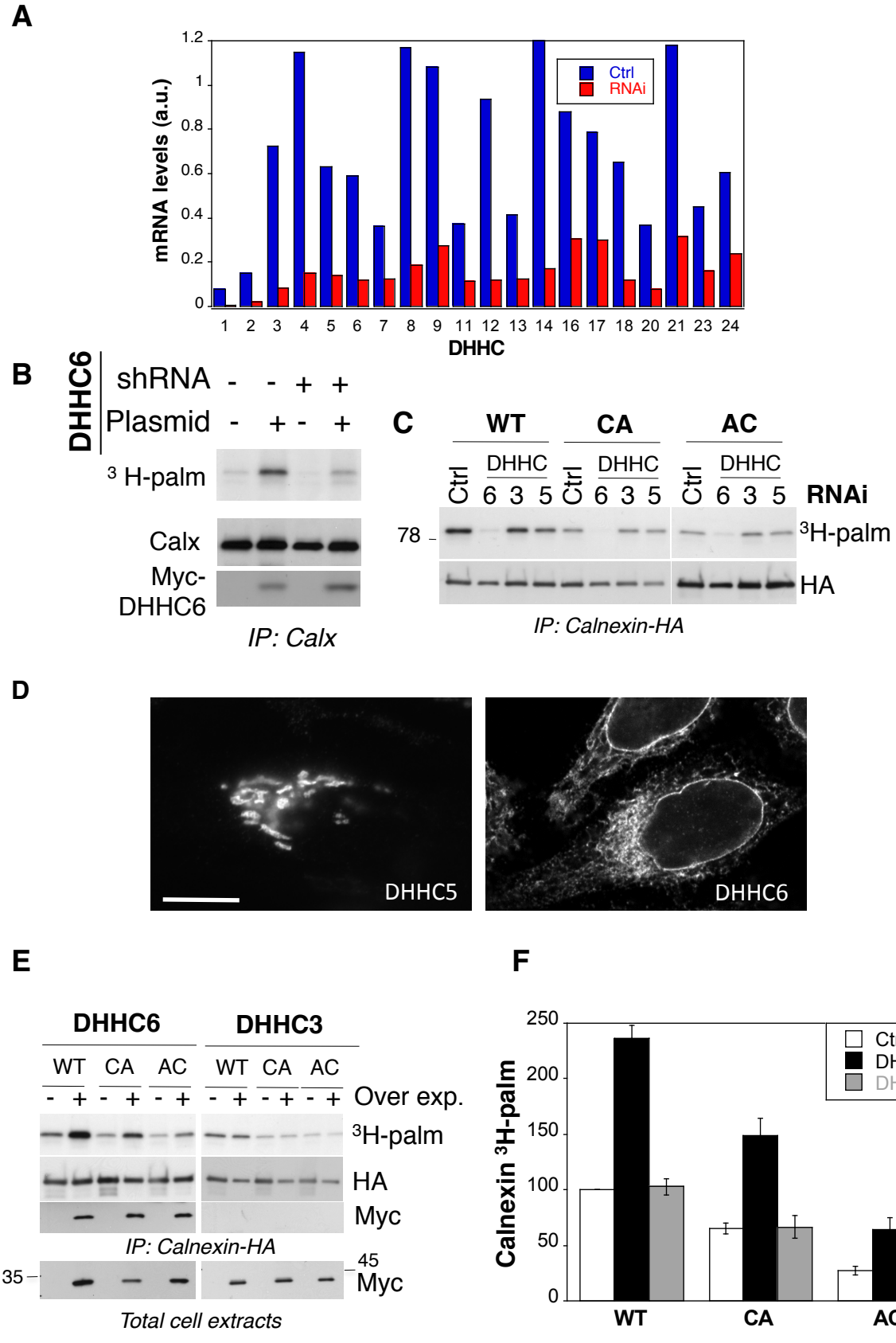
Transmembrane domain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DHHC
B
DHHC6
shRNA - - + +
Plasmid - + - +
3 H-palm
Calx
Myc-DHHC6
IP: Calx
C
WT
CA
AC
Ctrl
DHHC
6 3 5
RNAi
78
3H-palm
HA
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D
DHHC5
DHHC6
E
DHHC6
DHHC3
WT CA AC WT CA AC
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Over exp.
3H-palm
HA
Myc
IP: Calnexin-HA
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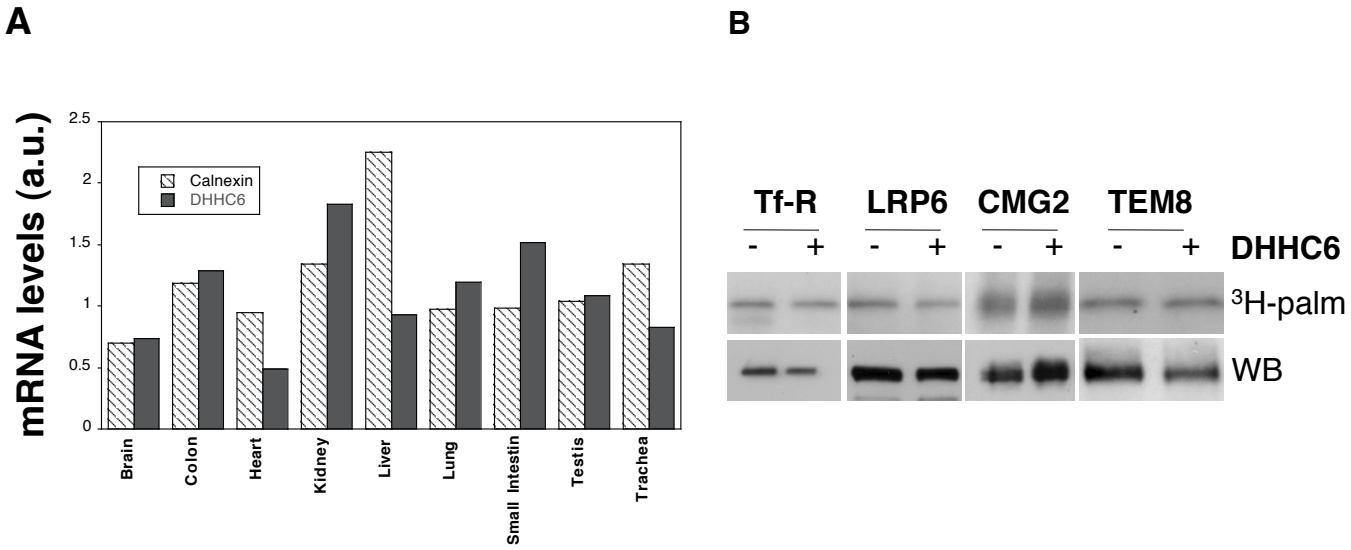
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Liver
Lung
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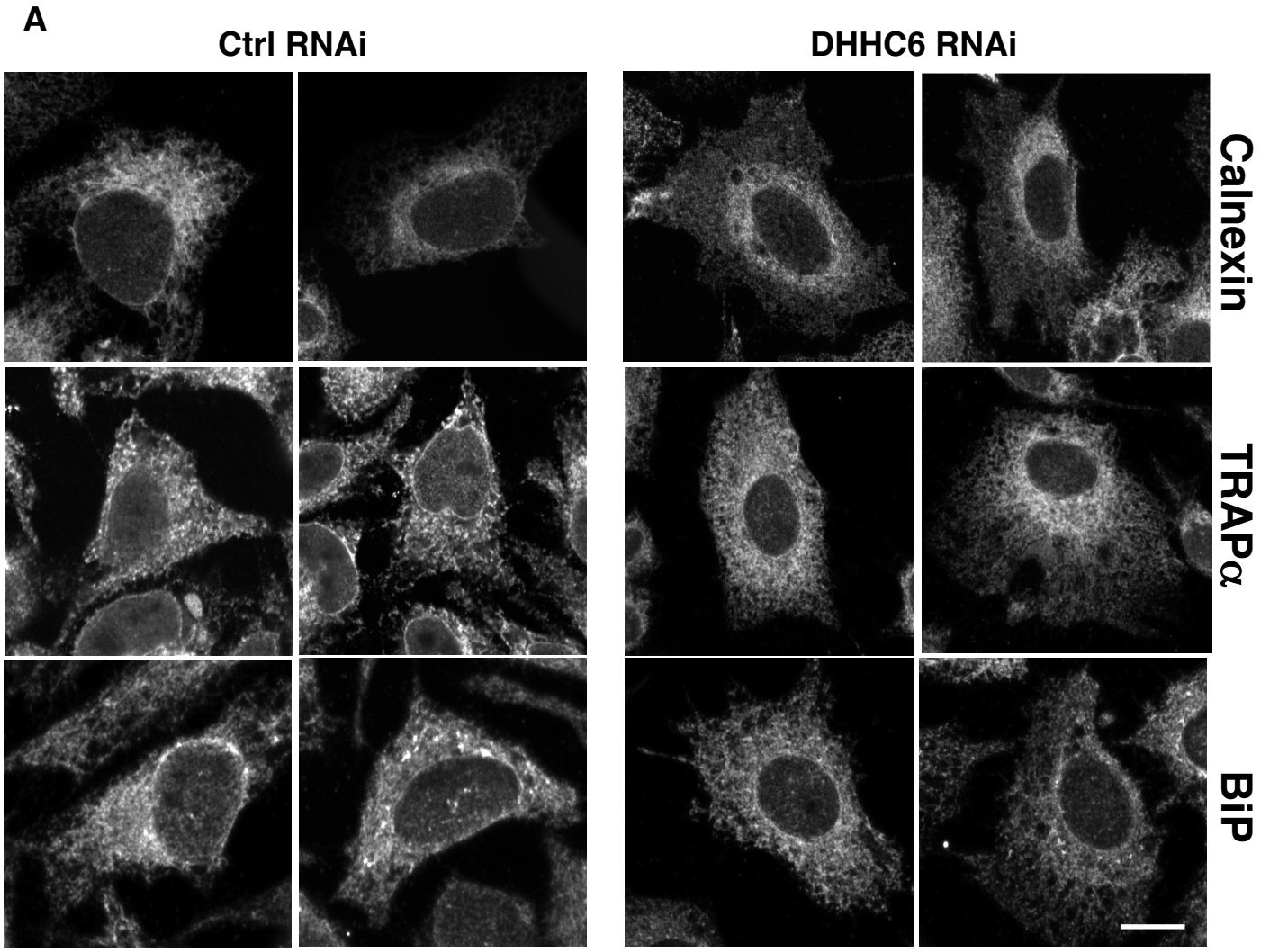
WB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TRAPα
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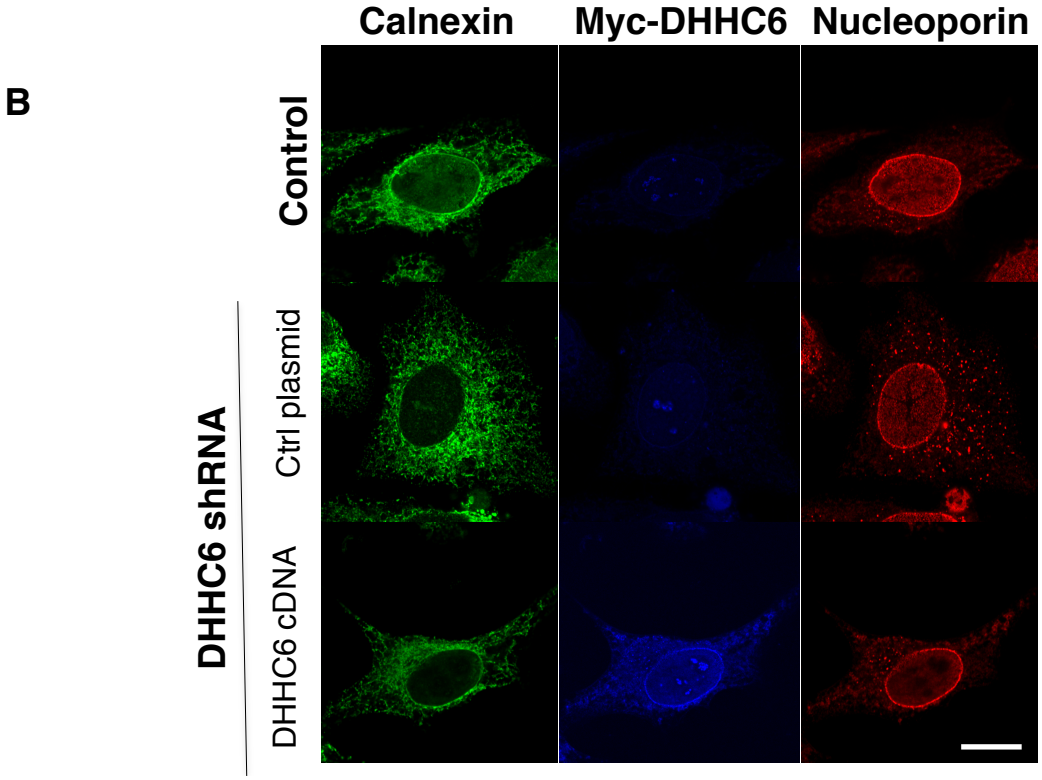
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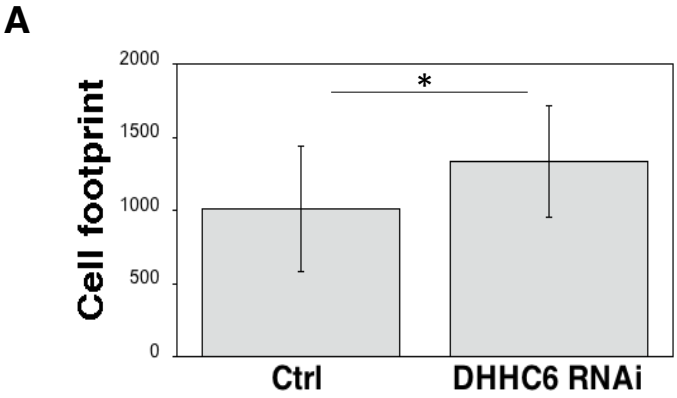
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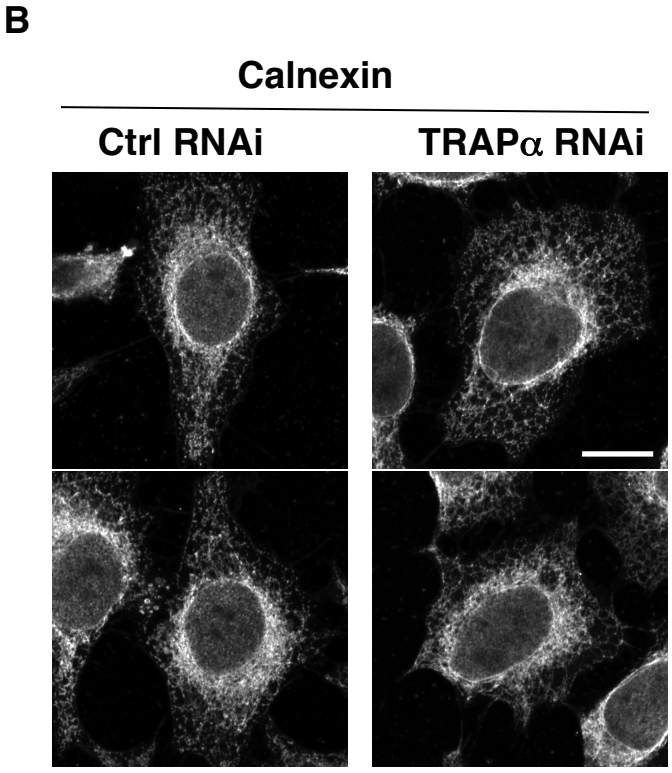
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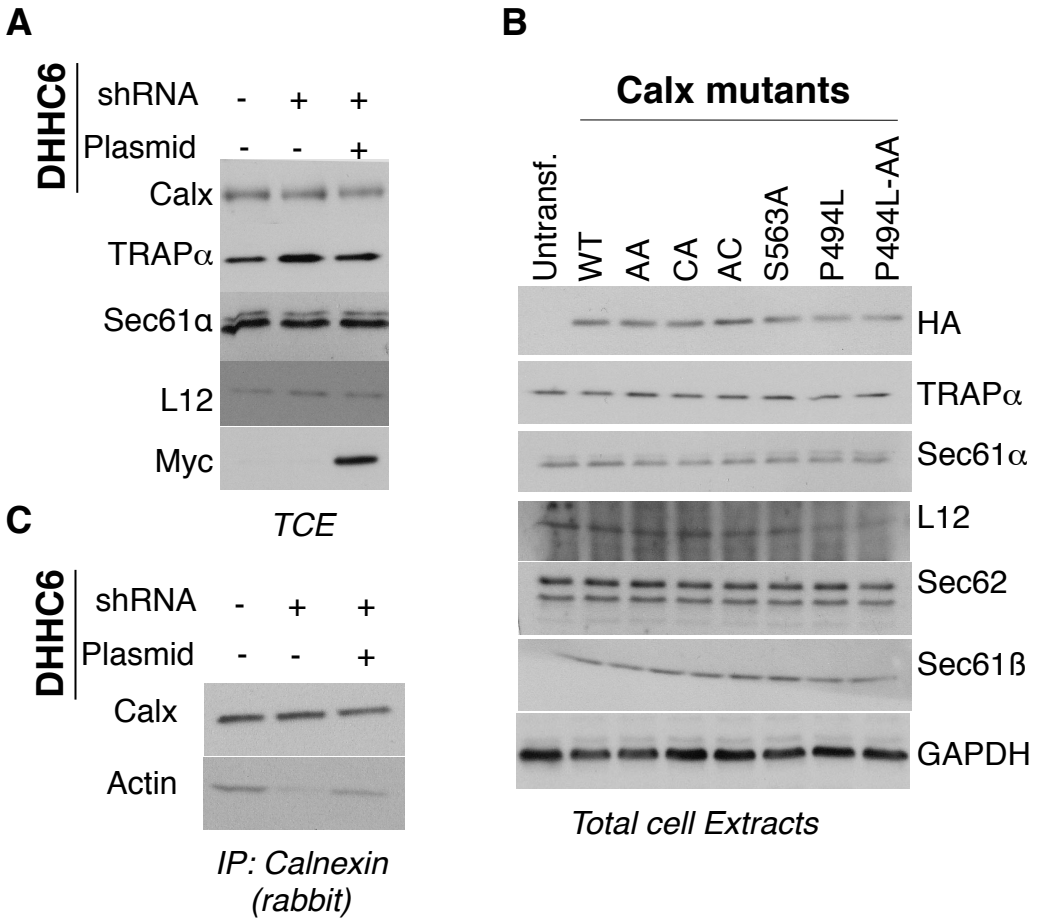
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Plasmid - - +
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Myc
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CA
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P494L
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HA
TRAPα
Sec61α
L12
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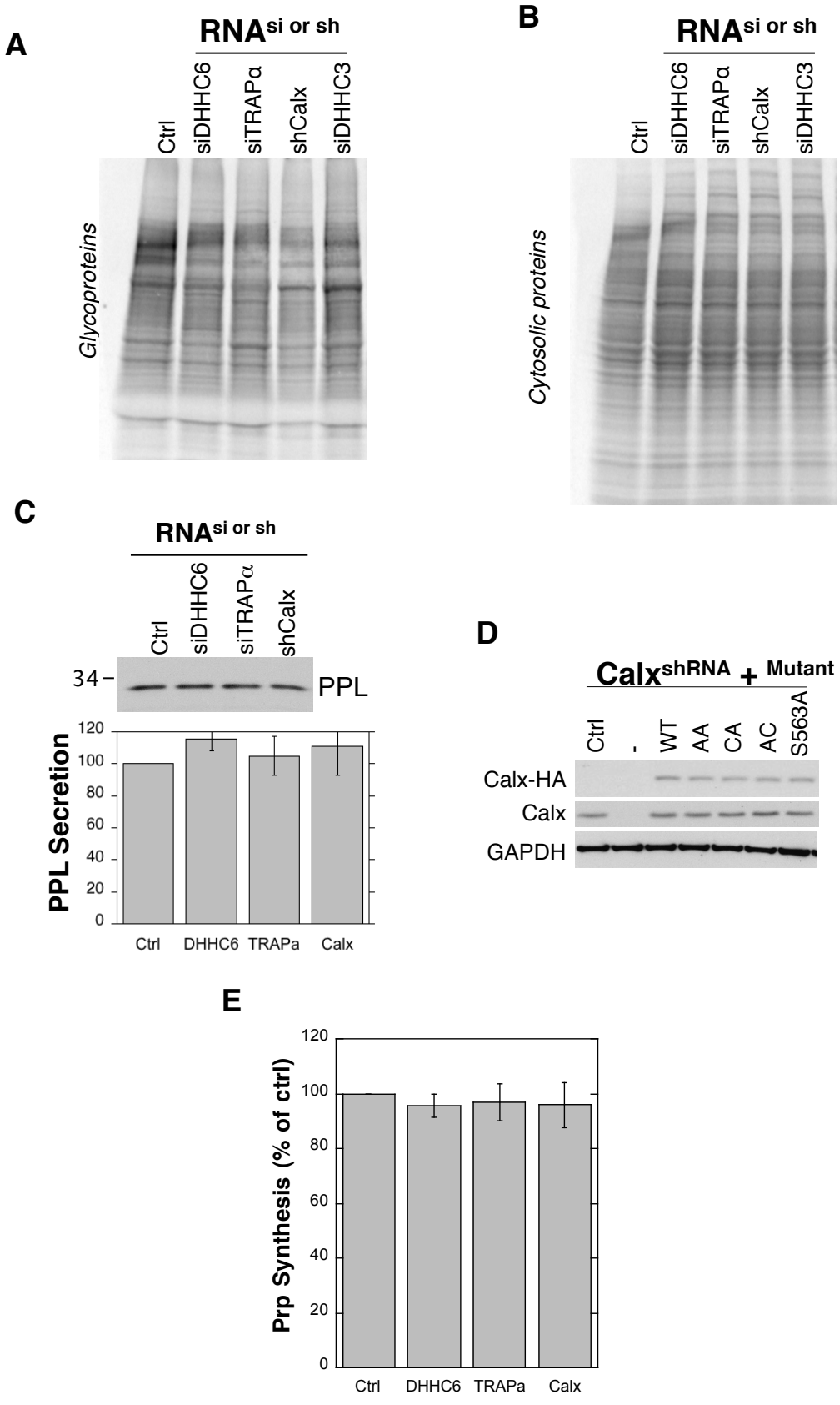
Calx
Actin
IP: Calnexin
(rabbit)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siDHHC3
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shCalx
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PPL
PPL Secretion
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100
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60
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Ctrl
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TRAPa
Calx
D
Calxshrna + Mutant
Ctrl
-
WT
AA
CA
AC
S563A
Calx-HA
Calx
GAPDH
E
Prp Synthesis (% of ctrl)
120
100
80
60
40
20
0
Ctrl
DHHC6
TRAPa
Calx

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DHHC2	TAGCTACTGCTAGAAGTCTTA	CTC AGT CTT GGA CGG AGA GCA G GTA TGA GCA ATC CTG CAT TAA CCA TGG
DHHC3	TCCGTTCTCATGAATGTTTAA	CTG CAG CAT CAA GCC CGA CC CGG TGC ATT CGG AAG ATG GAC C
DHHC4	ATGGATTGCTTCATTACCTTT	CCA GCT CGA TCC AAG CAC TGC CTG CAT CGG GGC CTG GAA CA
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ZDHHC6	GAGGTTTACGATACTGGTTAT	GCA CCA CTG GGT TGT ATC CAT GC GGG TGG AAC ACA GTG AAG ATC GAC
ZDHHC7	CCCGTGGTTACTATGAATGTA	CCT GTG CCT TGA GGG TCT TCT G GAA GCC CAC ATG GGA GCG G
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ZDHHC12	CAGATACTGCCTGGTGCTGCA	CTC ATG GAC CCT GGC TAC GTG CAG CCA TGG TTC CTC CAG CC
ZDHHC13	CAGCATAGTAGCCTTTCTATA	GCC TGC AGA AGC AGA GCA AGC CAG TGT GGC TGC TTT GGC TTG G
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Gene	shRNA Target sequence 5'-3'	Q-RT-PCR-oligos
CALNEXIN	GAGCTTGATCTGTGATTTC	CCG GAA GCC CGA GGA TTG GG GCC CCT GCT AAG ATT CCA GAT GAA G
ZDHHC6	CCTAGTGCCATGATTTAAA	GCA CCA CTG GGT TGT ATC CAT GC GGG TGG AAC ACA GTG AAG ATC GAC