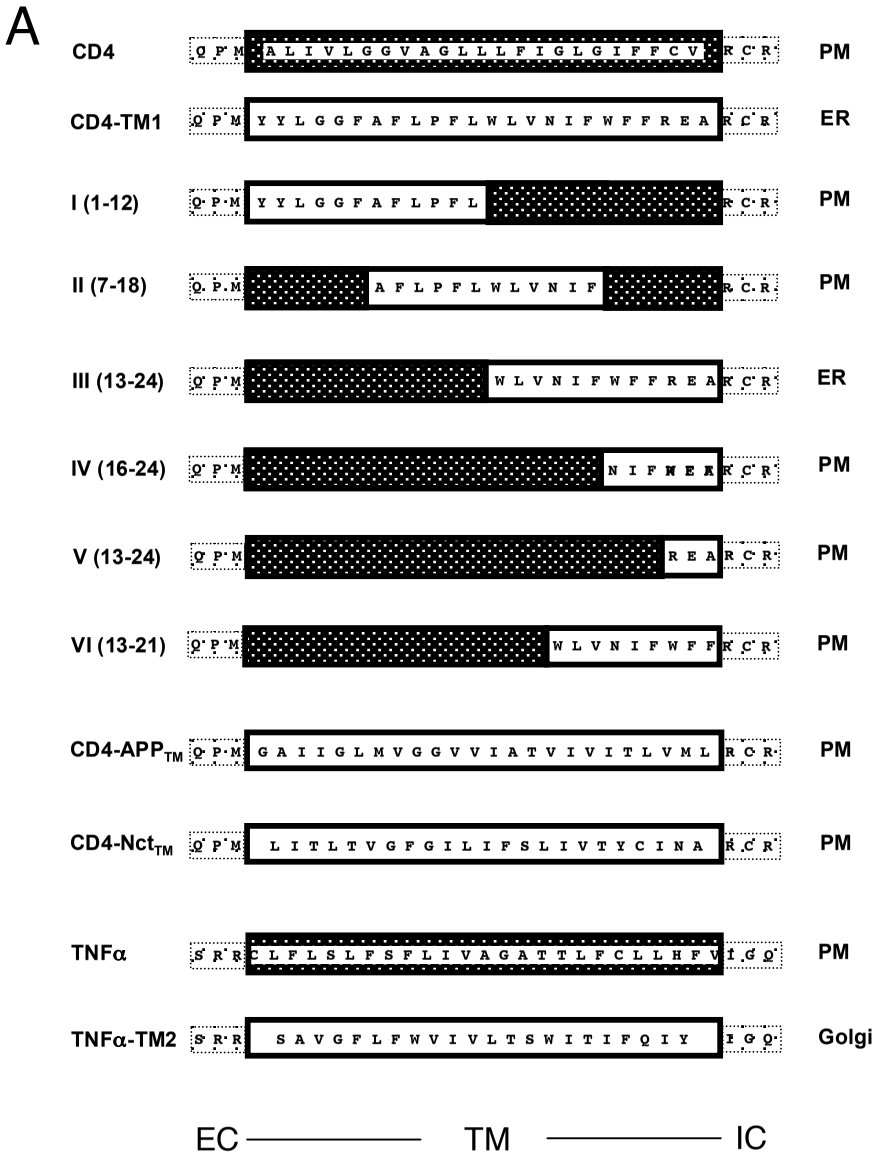
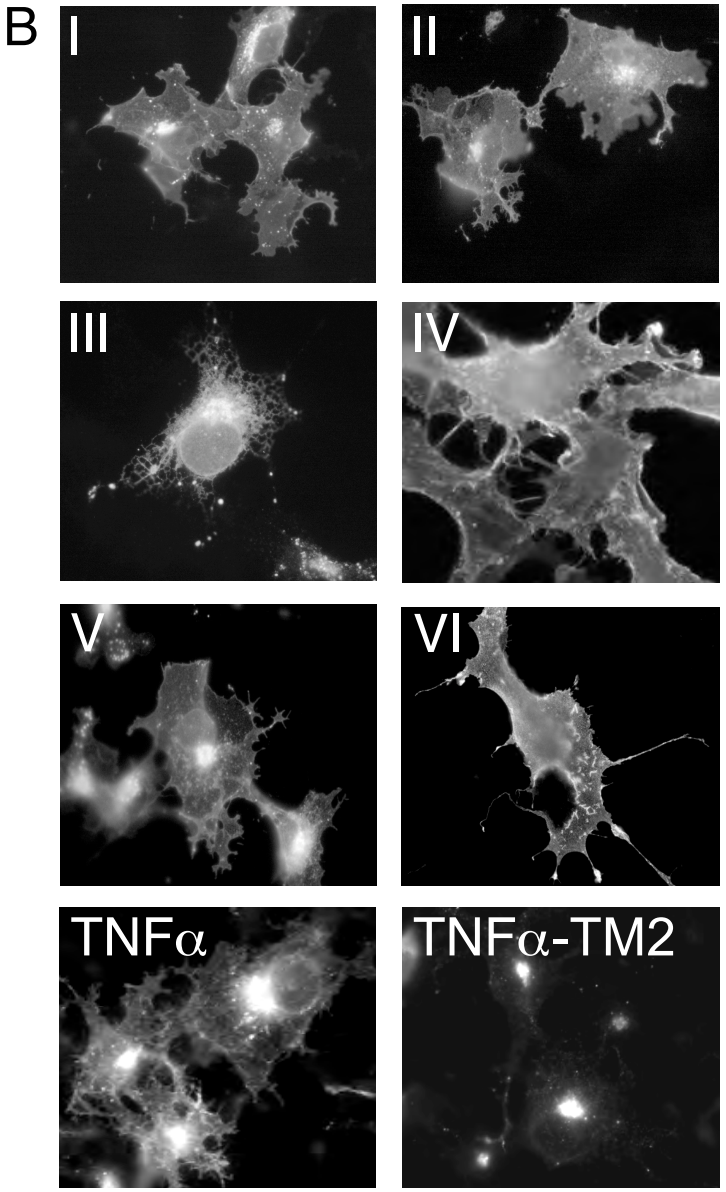
Supplemental data

Supplemental Fig. 1: ER-retention of Pen2 was mediated by the distal part of TM1. A) Scheme of the constructs used for determination of sorting signals in Pen2. Relevant amino acids are depicted. The dark box corresponds to CD4 or TNFα TMDs as indicated, the white blocks labels introduced amino acids derived from Pen2 or APP or Nct, as indicated. Dotted boxes refer to the flanking regions of the TMD at the N- and C-terminus, respectively. EC, extracellular domain, IC, intracellular domain. B) COS cells transiently transfected with the various constructs were processed for immunofluorescence using anti-CD4 antibodies or HA antibodies for TNFα-constructs (TNFα is HA-tagged at the C-term.). Only CD4-TM1 and construct III were retained/retrieved in the ER. TNFα-TM2 was clearly exported out of the ER and localized to the Golgi.

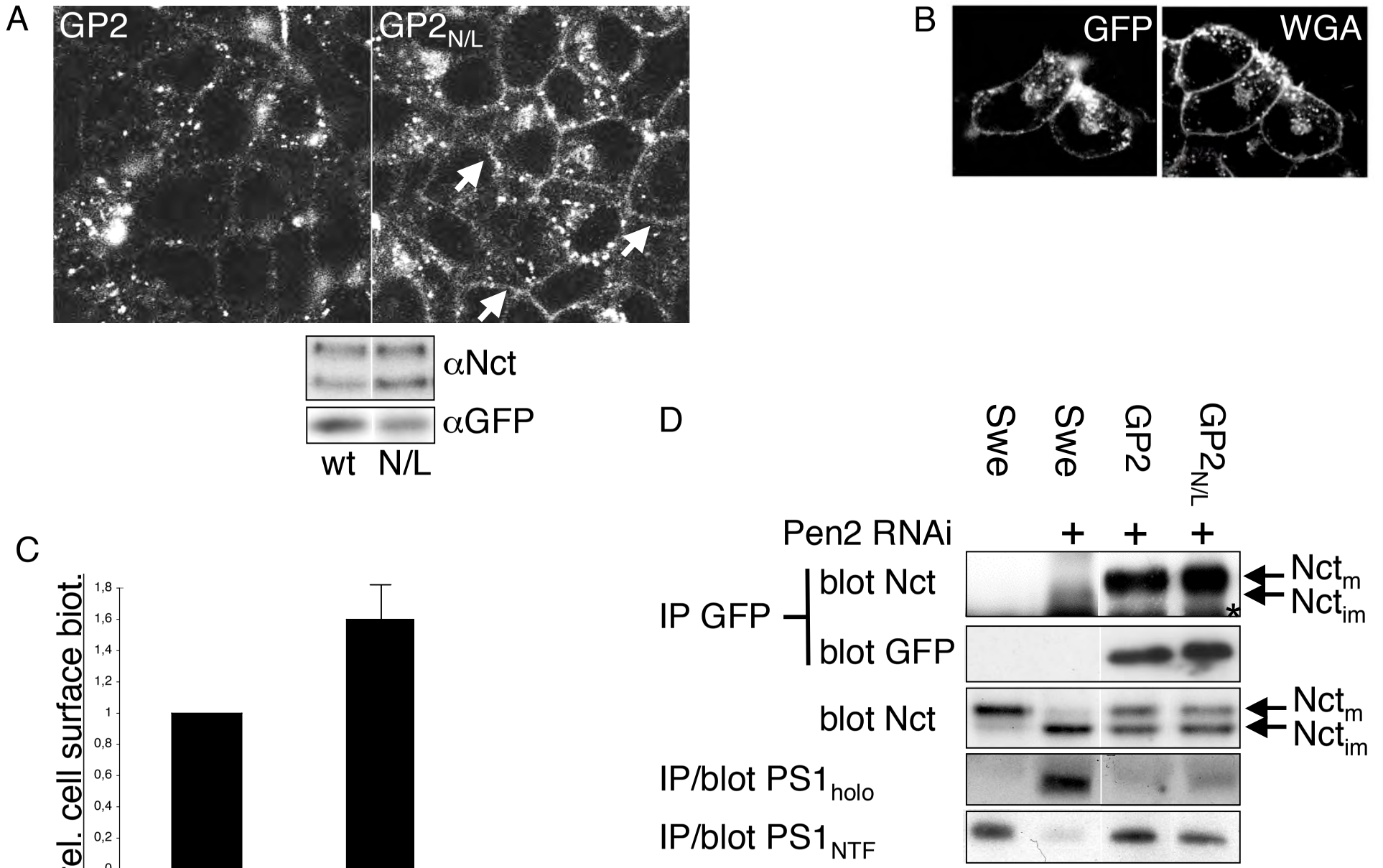
Supplemental Figure 2: Unassembled Pen2, which is deficient in Rer1 binding, escaped the retention machinery and was exported out of the ER. A) Living cells stably expressing GFP-Pen2 (GP2) or $GP2_{N/L}$ were imaged by confocal microscopy. Note the increased surface staining (arrows) in GFP-$Pen2_{N/L}$ expressing cells. When stably expressed, complex-associated GFP-Pen2 accumulates in endosomes/lysosomes and at the PM, similar to PS1-GFP (Kaether et al., 2002) and Nct-GFP (C.K., unpublished). The prominent ER staining seen in PS1-GFP expressing cells (Kaether et al., 2004; Kaether et al., 2002) is not observed in GFP-Pen2 stably expressing cells, presumably because most of the unassembled Pen2 is rapidly degraded (Bergman et al., 2004; Crystal et al., 2004). Both GFP-Pen2 and GFP-$Pen2_{N/L}$ were expressed at similar levels as shown in the western blots below the images. Nct served as loading control. B) GFP-$Pen2_{N/L}$ was at the plasma membrane as shown by the costaining of living cells with rhodamine-labeled wheat germ agglutinin (WGA). C) Cell surface biotinylation showed accumulation of GFP-$Pen2_{N/L}$ on the PM. The amount of GFP-Pen2 on the PM was set to 1, and the amount of GFP-$Pen2_{N/L}$ at the PM related to that (see

methods). Error bar indicate standard deviation, n=6 independent experiments. D) GFP-Pen2 and GFP-Pen2$_{N/L}$ assembled in a γ-secretase complex and were fully functional. Swe cells, Swe cells stably expressing a Pen2 RNAi and Swe cells stably expressing Pen2 RNAi and GFP-Pen2 or GFP-Pen2$_{N/L}$ were immunoprecipitated and blotted as indicated. Both Pen2 variants coprecipitated mature Nct and rescued Nct maturation and PS1 endoproteolysis, indicating full functionality. White lines indicate assembly of blot from different areas. The asterisk denotes an unspecific background band.


A
CD4
PM
CD4-TM1
ER
I (1-12)
PM
II (7-18)
PM
III (13-24)
ER
IV (16-24)
PM
V (13-24)
PM
VI (13-21)
PM
CD4-APP$_{TM}$
PM
CD4-Nct$_{TM}$
PM
TNFα
PM
TNFα-TM2
Golgi
EC
TM
IC


B
I
II
III
IV
V
VI
TNFα
TNFα-TM2

suppl. Fig. 1


A
GP2
GP2N/L
αNct
αGFP
wt
N/L
B
GFP
WGA
C
rel. cell surface biot.
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
wt
N/L
D
Swe
Swe
GP2
GP2N/L
Pen2 RNAi
+
+
+
IP GFP
blot Nct
blot GFP
blot Nct
IP/blot PS1holo
IP/blot PS1NTF
Nctm
Nctim
Nctm
Nctim
*

Suppl. Fig. 2