A

mature form

1 2 3

human prepro-catZ

1, 2, 3 catZr-peptides

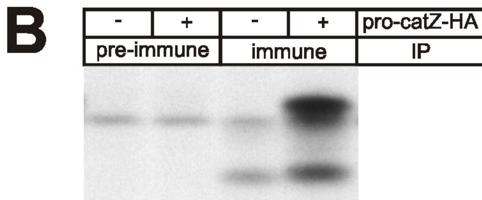
RT-PCR with degenerate primers

probe 1

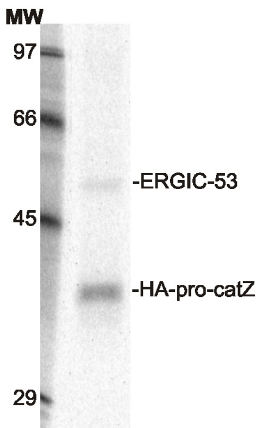
probe 2

screening of CHO cDNA library

chinese hamster prepro-catZ cDNA

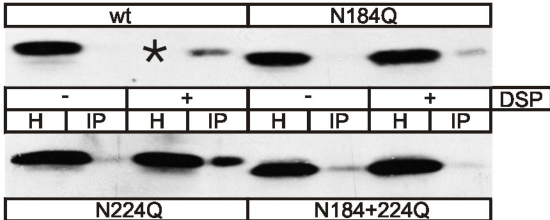


Appenzeller-Herzog et al. Figure S1



Appenzeller-Herzog et al. Figure S2

IP:αERGIC-53
WB:αHA



Appenzeller-Herzog et al. Figure S3

Supplementary figure legends

Fig S1: *Cloning of Chinese hamster prepro-catZ*

(A) Schematic representation of the cloning of Chinese hamster prepro-catZ. Two pairs of degenerate primers corresponding to the microsequenced catZr-peptides (Appenzeller *et al.*, 1999) were designed for subsequent RT-PCR on total RNA from GMAA cells. Two fragments of approximately 650 and 550 bp length were obtained and further used as probes to screen a CHO Lambda cDNA library. Sequence analysis of the isolated Lambda clones yielded a partial cDNA encoding Chinese hamster prepro-catZ (Fig. 1A). The sequence information has been deposited at NCBI (accession number: AJ303074).

(B) HA-tagged hamster pro-catZ was expressed in GM cells and, after metabolic labeling with ^{35}S-methionine for 3 h, isolated by immunoprecipitation with anti-HA (not shown) or a rabbit antiserum (immune) raised against two peptides derived from hamster pro-catZ (Fig. 1A). In non-transfected cells a band was detected that represents endogenous catZ after lysosomal maturation, i.e. after the proteolytic removal of its propeptide (Fig. 1A, see also Fig. 1C). Pro-catZ is hidden by a 40 kDa contaminating band that is also present in precipitates obtained with pre-immune serum.

Fig S2: *Pro-catZ binds to endogenous ERGIC-53 in HeLa cells.*

HA-pro-catZ-transfected HeLa cells were pulsed for 10 min with ^{35}S-methionine, treated with DSP, and subjected to sequential immunoprecipitation using anti-ERGIC-53 and anti-HA. Note that the successful crosslinking of transfected pro-catZ to endogenous ERGIC-53 excludes the possibility that the data in CHO cells arose from an artefact caused by tagged and overexpressed ERGIC-53.

Fig S3: *The distinct binding intensities of HA-pro-catZ glycosylation-site mutants to ERGIC-53 are not a result of steric hindrance by the HA-epitope.*

DSP-crosslinking and co-immunoprecipitation of GMAA-ERGIC-53 and alternatively tagged pro-catZ glycosylation-site mutants (see Materials and Methods) from transiently transfected CHO cells. Shown are pro-catZ bands visualized by anti-HA immunoblotting (WB) after crosslinking and anti-ERGIC-53 immunoprecipitation (IP). 1% of the total homogenate (H) was loaded as expression control. Note that DSP-dependent co-isolation is decreased for the N184Q mutant, but unaffected in the case of the N224Q mutant. The asterisk denotes sample loss.