Fig. S1

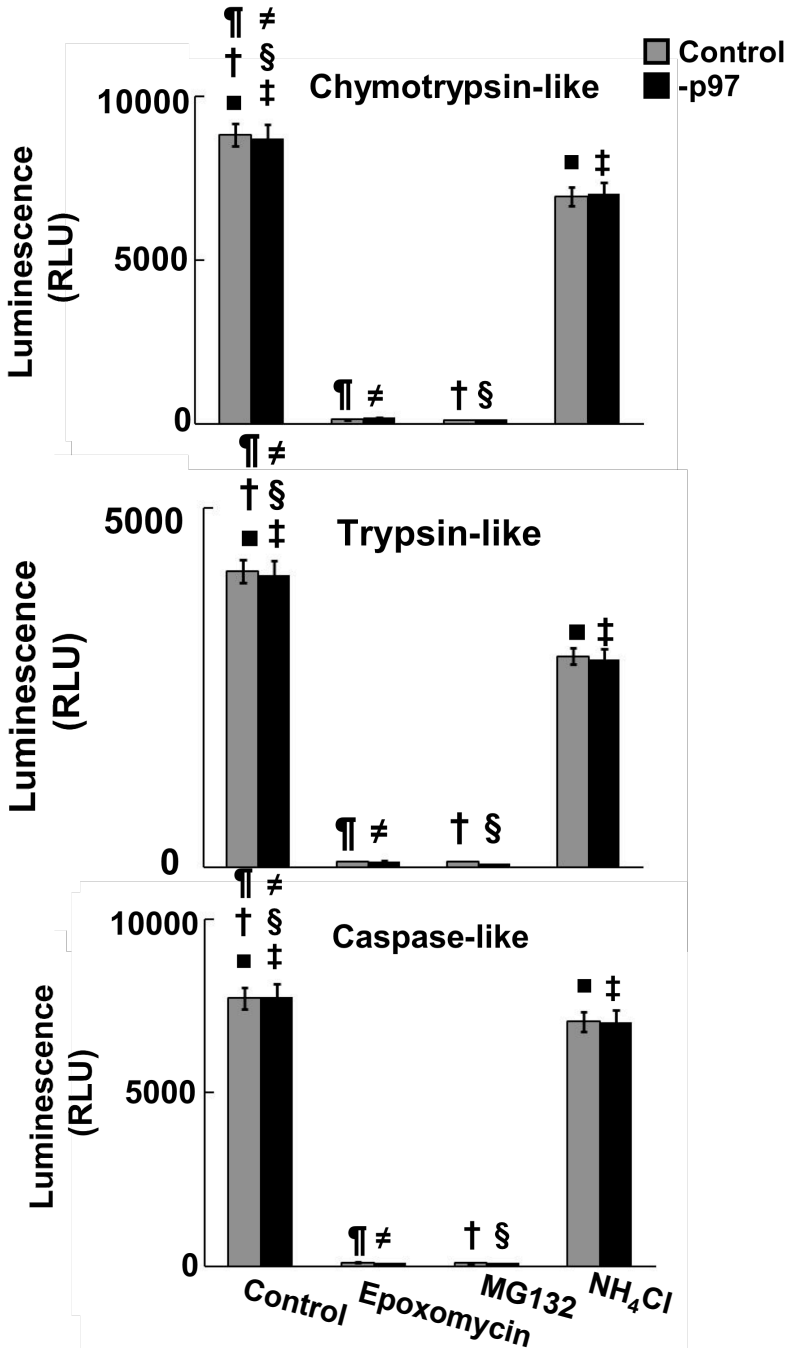
Control
-p97
Chymotrypsin-like
Trypsin-like
Caspase-like
Luminescence (RLU)
10000
5000
0
¶ ≠
† §
■ ‡
Control
Epoxomycin
MG132
NH4Cl

Fig. S2

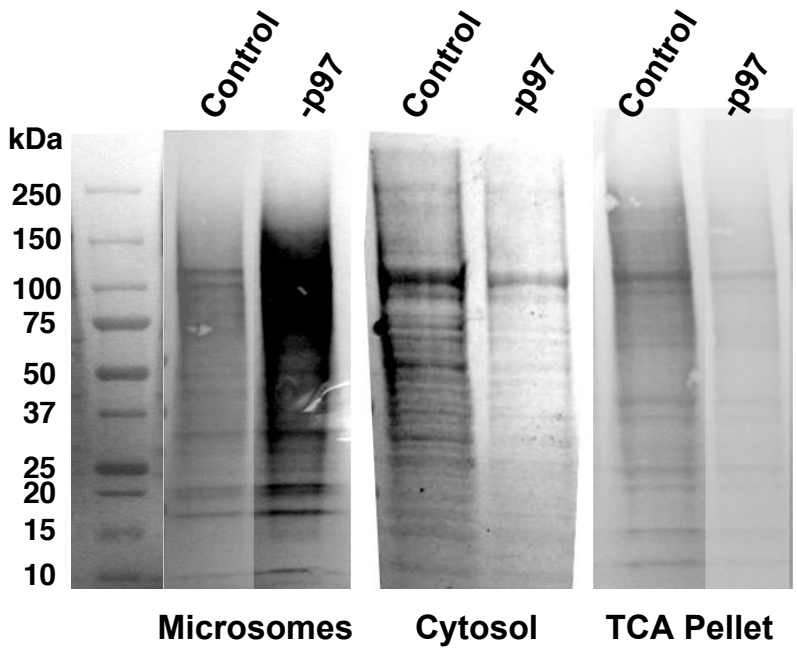
Control
-p97
Control
-p97
Control
-p97
kDa
250
150
100
75
50
37
25
20
15
10
Microsomes
Cytosol
TCA Pellet

Fig. S3

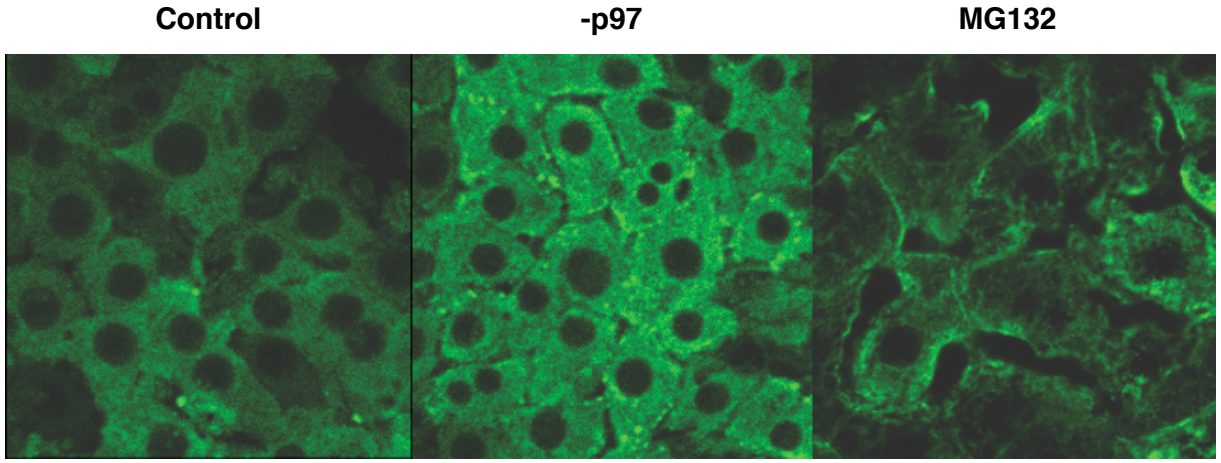

***Fig. S1. Relative contributions of hepatic proteasome and lysosomes to the chymotrypsin-like, trypsin-like and caspase-like activities of cultured rat hepatocytes as probed with specific inhibitors.*** Cells were infected with control shRNA or p97 shRNA 1+2 (-p97) for 7 days, and on the 8$^{th}$ day the effects of p97 knockdown on hepatic proteasomal function were assessed with the specific probes for the proteasomal chymotrypsin-like, trypsin-like and caspase-like activities in a cell-based bioluminescent assay in the presence of a proteasomal inhibitor (MG132 or epoxomycin) or a lysosomal inhibitor ($NH_4Cl$) as detailed (Experimental Procedures). Values are mean ± SD relative luminescence units (RLU) derived from three separate –p97 cultures relative to the values of corresponding activities in control shRNA treated cells. No statistically significant differences were observed between activities from control and -p97 cells. However, statistically significant differences were observed between the two mean ± SD values of activities each marked with the same symbol as follows: Chymotrypsin-like (≠), (¶), (†), and (§) at $p<0.0001$; (‡) and (■) at $p<0.001$. Trypsin-like (≠), (¶), (†), and (§) at $p<0.0005$; (‡) and (■) at $p<0.001$. Caspase-like (≠), (¶), (†), and (§) at $p<0.0001$; (‡) and (■) at $p<0.005$.

***Fig. S2. Relative levels of ubiquitinated proteins after p97 knockdown in cultured hepatocytes*** Relative total hepatic protein ubiquitination analyses of microsomes (20 μg protein), cytosol (50 μg protein) and $Na_2CO_3$-solubilized material (50 μg protein) obtained from the cells described in *Fig. 6A*. A representative immunoblot developed with alkaline-phosphatase-conjugated secondary antibody is shown.

***Fig. S3. Confocal immunofluorescence microscopy of control, -p97 or MG132-treated rat hepatocytes: Comparative analyses.*** Cells were infected with control shRNA or p97 shRNA 1+2 (-p97) for 7 days, and on the 8$^{th}$ day the effects of p97 knockdown were assessed as detailed (Experimental Procedures). Cells were also treated with the proteasomal inhibitor MG132 (300 μM) for 6 h as described (66). This treatment is known to trigger hepatic ER-stress, with consequent induction of PERK and GCN2 eIF2α kinases, and consequent translational shut-off of hepatic proteins. Thus even though proteasomal inhibition is expected to stabilize hepatic CYPs 3A, the CYP3A content of MG132-treated cells is reduced due to the concomitant ER-stress induced translational shut down. This is in clear contrast to the CYP3A stabilization observed in hepatocytes after -p97 knockdown.