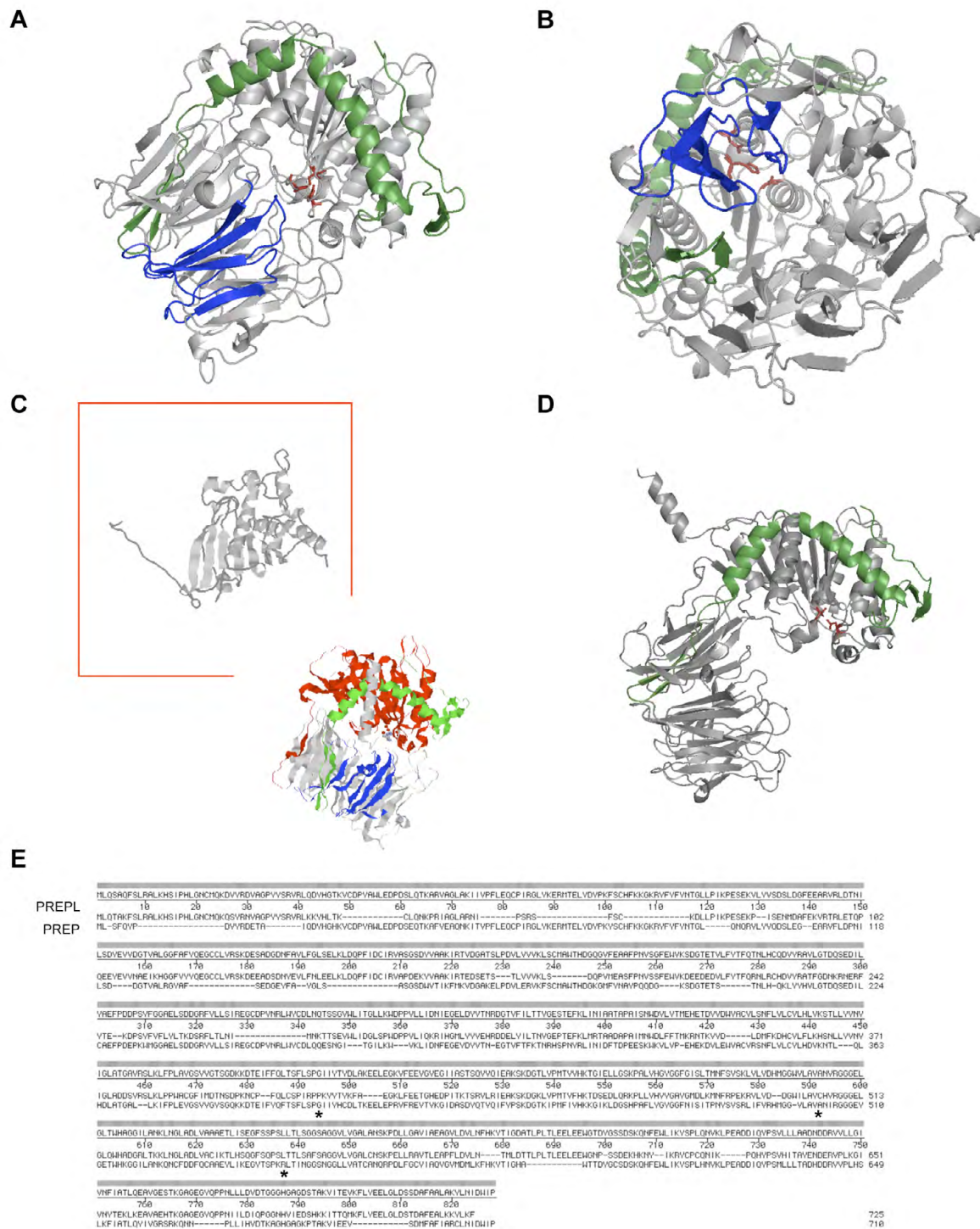
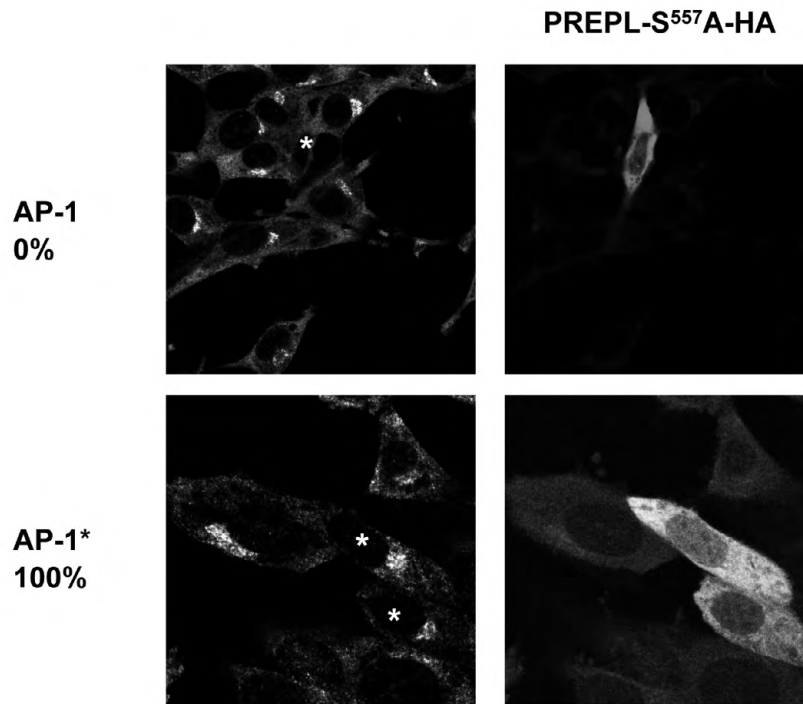


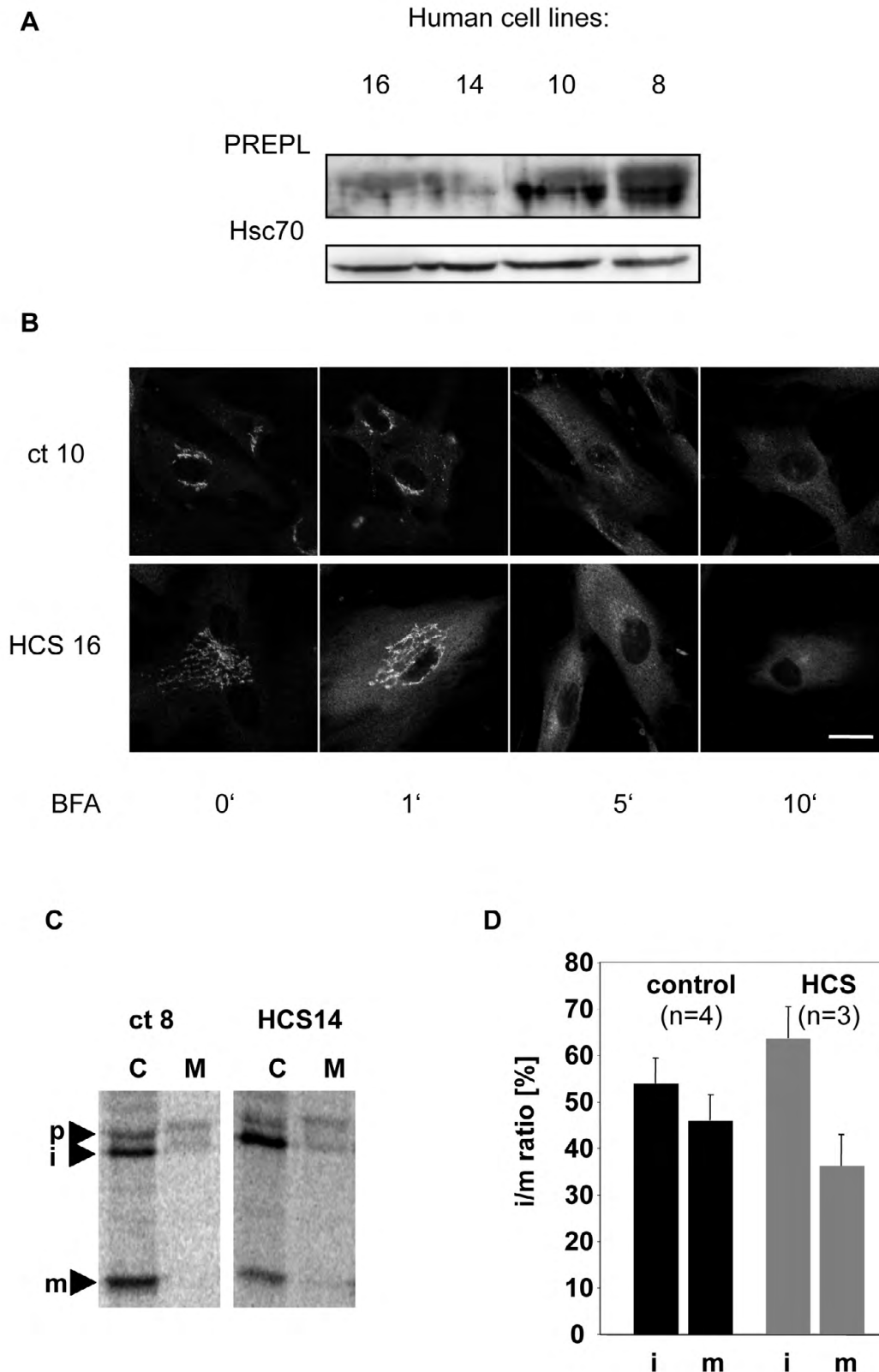
**Fig. S1.** (A) Crosslinking of AP-1 and AP-1\* MEF cell lines and PREPL with DSP (extracts of two independent experiments are shown). Western-blots were developed after non-reducing (left panel) and reducing SDS-PAGE (right panel) with anti-γ1 (AP-1) and anti-PREPL antisera. These experiments demonstrated efficient AP-1 as well as PREPL crosslinking to proteins. (B) Proteins crosslinked to PREPL. Crosslinking of AP-1 and AP-1\* MEF cell lines after <sup>35</sup>S-metabolic labelling for 16 h and anti-PREPL immunoprecipitation show several crosslinked proteins (7.5% SDS-PAGE) and did not reveal a different protein pattern.



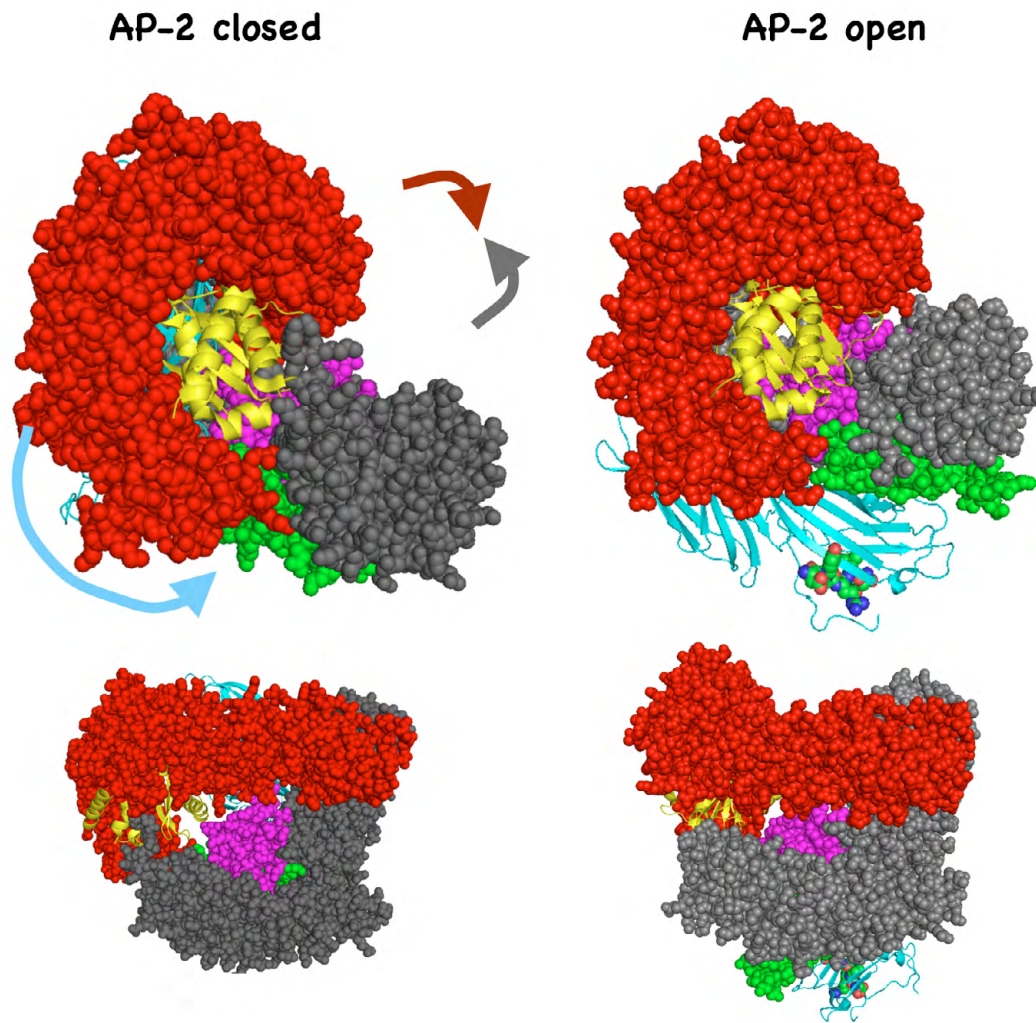
**Fig. S2.** The PREPL homologous porcine prolyl-oligopeptidase PREP structure, with coloured subdomains as in Fig. 5. **(A)** Side view with the C-terminal peptidase S9 family  $\alpha/\beta$ -fold domain at the top and the N-terminal  $\beta$ -propeller domain below. Sequence corresponding to the PREPL  $\mu$ 1A-adaptin binding domain is shown in blue. The residues Ser-Asp-His of the catalytic center are shown in as red sticks. N-terminal helix domain (aa 1-88) is coloured green. **(B)** View through the center of the  $\beta$ -propeller domain towards the catalytic center. **(C)** Modelling of PREPL structure on porcine PREP yielded only a fragment of the C-terminal domain (red domain in porcine PREP). Catalytic triad is not part of the modelled structure (software: SWISS-Modeller, Basel, CH). **(D)** Prolyl-oligopeptidase family member, which crystallised in the open conformation, with C-terminal helix sticking out and an exposed catalytic center (residues in red) and N-terminus (green) (Shan, L. et al. Proc.Natl.Acad.Sci.USA v102 pp.3599, 2005); (cartoons by software: Pymol). **(E)** Sequence alignment of PREPL and PREP with catalytic triad marked by asterisks.



**Fig. S3.** AP-1 and AP-1\* expressing MEF cells with transient expression of the PREPL-HA mutant with the Ser to Ala exchange in the putative catalytic triad. AP-1 can completely removed from membranes, with 0% AP-1 signal intensity (see also Fig. 6), whereas AP-1\* membrane binding is 100% and thus not effected.



**Fig. S4.** (A) Confirmation of the PREPL-deficient human cell lines using our rabbit polyclonal antisera. (B) Comparison of brefeldin A induced redistribution of AP-1 in control (ct 10) and PREPL-deficient (HCS 16) human cell lines. The experiment with the HCS14 and the second control cell line as well as the quantification of AP-1 membrane binding are shown in Fig. 7. (C,D) Cathepsin sorting in human PREPL-deficient cell lines. (C) Cathepsin D sorting by pulse-chase experiment (1h pulse, 6h chase). Shown are data from the human ct 8 control and HCS14 PREPL-deficient cell line. No increase in the amount of secreted Golgi-precursor form (p) in the medium of HCS14. Small amounts of the early endosome intermediate precursor form (i) in the medium of both cell lines demonstrates some cell lysis. (D) Processing of cathepsin D from intermediate to mature would indicate delayed TGN to endosome and endosome to lysosome transport. This has been demonstrated to be affected by either delayed endosome-maturation or altered endosome positioning. A slowed down pro-cathepsin D transport is indicated, but the pro-forms are not secreted.



**Fig. S5.** Conformational changes between the cargo-free -closed- and the cargo-loaded -open- AP complexes according to Jackson et al., 2010. The two large adaptins (red & grey) of AP-1 (see Fig. 1) and of AP-2 (shown here) form a cleft on the cytoplasmic face.  $\mu$ -adaptin N-termini are located at the bottom of this cleft. A dramatic reorientation of  $\mu$ 1A and  $\mu$ 2 C-terminal domains is required to open up the cargo binding domain (top cartoons). The transition to the open conformation is stabilised by  $\mu$ -adaptin phosphorylation. We have previously shown that the membrane bound pool of AP-1\* is in the closed conformation, indicating that it is not bound to the membrane by cargo proteins (Medigeschi et al. Traffic 2008). Recently the structure of an open, cargo-loaded AP-2 complex was solved (Jackson et al. Cell 2010) (shown on the right). The side view (top) and cytoplasmic face (bottom) of the cargo-loaded, open conformation of AP-2 show also a conformational change in both large adaptins. This alteration leads to the closure of the cleft formed by the two, preventing interactions with the  $\mu$ -adaptin N-terminus. The conformation of the  $\mu$ -adaptin N-terminal domain is not altered by these transitions. Given the high structural conservation between AP-1 and AP-2, the large adaptins of AP-1 are expected to undergo comparable reorientations. These data support our model of PREPL-induced redistribution of cargo-free AP-1 into the cytoplasm.