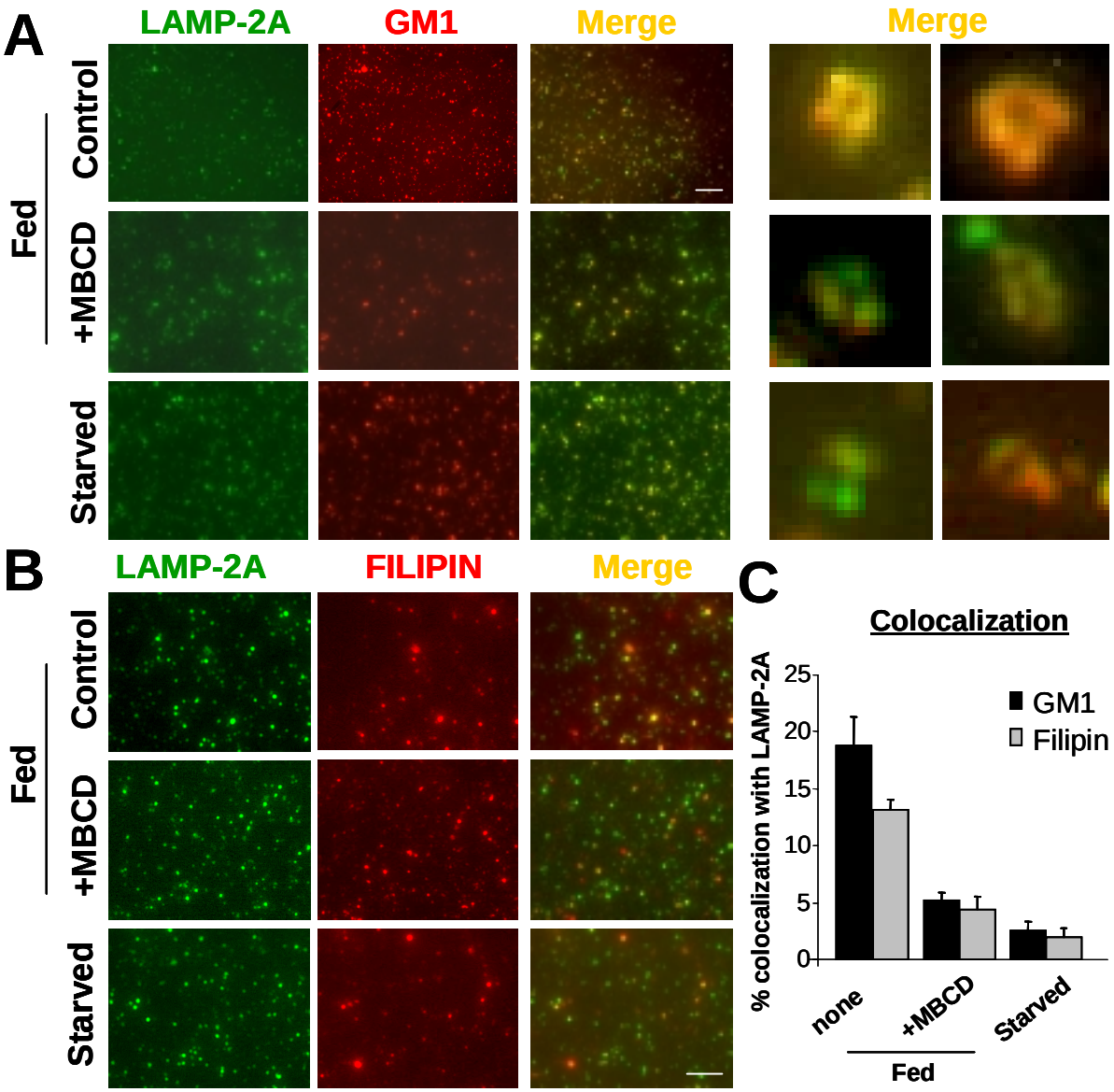
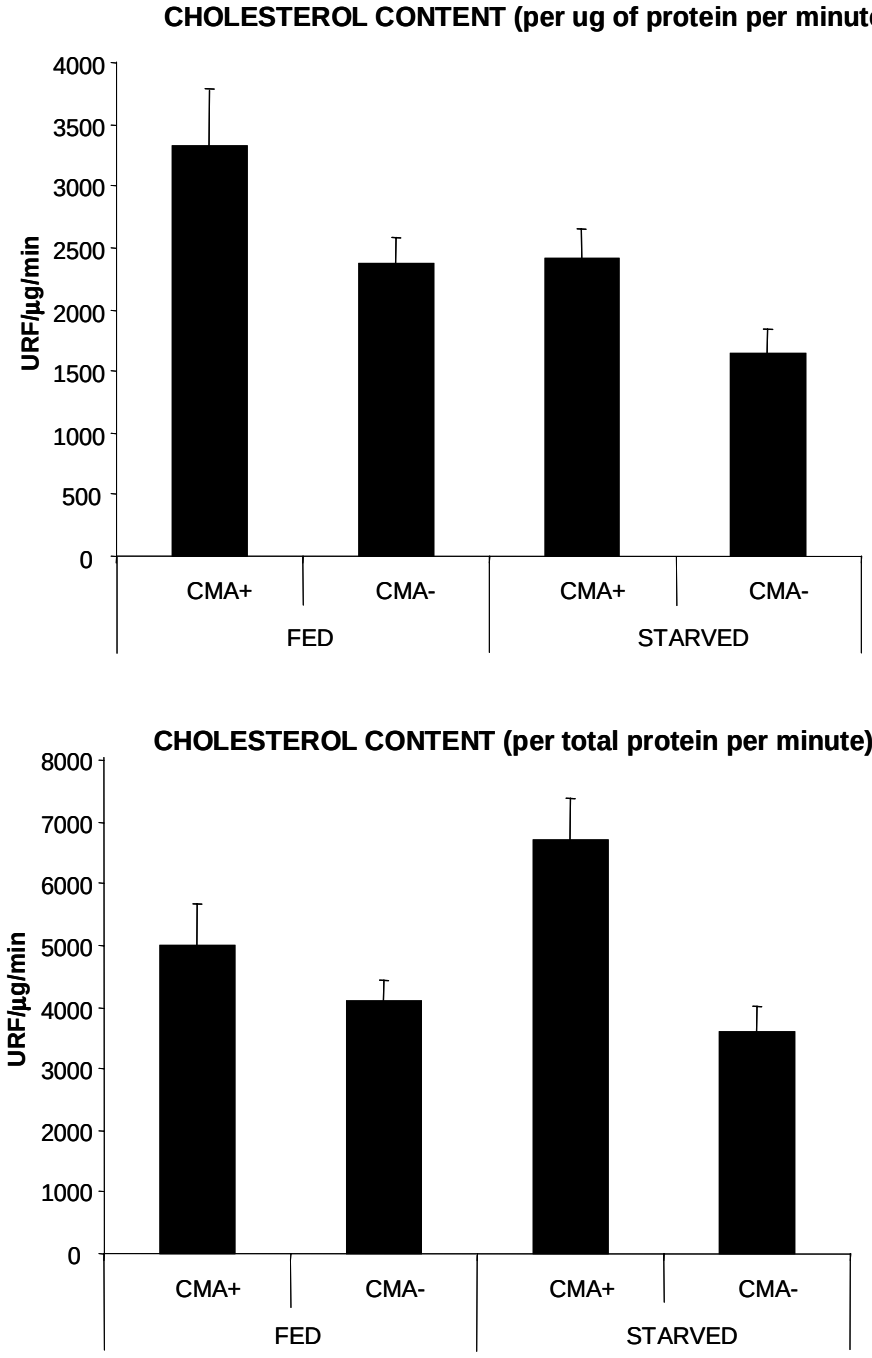


Suppl. Fig. 1. Distribution of lysosomal membrane proteins at the lysosomal membrane. Lysosomes isolated from livers of rats normally fed (Fed) or starved for 48h (Stv) were double labeled with Texas-Red-cholera toxin B subunit (**A**) or with filipin (**B**) and subjected to indirect immunofluorescence for LAMP-2A (red). Merged images are shown on the right. Where indicated lysosomes were treated with methylbetacyclodextrin (MBCD, 25mM) for 30 min before fixation Bar: 10 μm. Insets on the right show selected vesicles at higher magnification (100x + 1.6x Optivar). The quantification of colocalization of the two fluorophores is shown in **C**, and it is expressed as the mean + S.E. of percentage of colocalization in 5 different fields from 2 different lysosomal preparations.



Suppl. Fig. 2. Changes in lysosomal cholesterol content. Total cholesterol content was measured as indicated under Material and Methods in the membranes of lysosomes with high (CMA+) and low (CMA-) activity isolated from livers of normally fed (FED) or 24h starved (STARVED) rats. Values are mean + S.E. of three different experiments with triplicate samples and are expressed as cholesterol content per µg of lysosomal protein (**A**) or per total amount of lysosomal protein in each fraction (**B**).

IMMNOGOLD LABELING

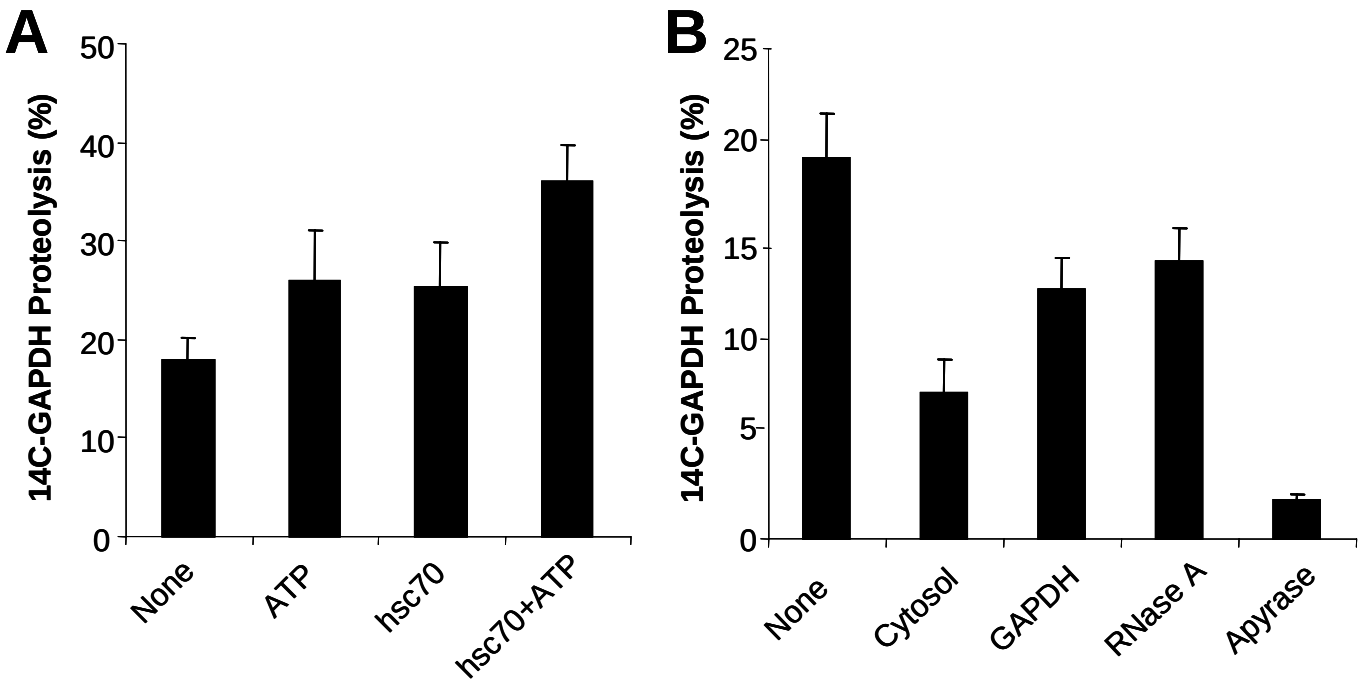
LYSOSOME LABELING		
	(Number)	(%)
Lysosomes counted	245	
Lysosomes with any labeling	202	82.4*
Lysosomes with both labels	142	70.3
COLOCALIZATION **		
LAMP-2A	(% of gold particles)	
Co-localized with Flot-1	45.5 ± 6.5	
Non co-localized with Flot-1	54.5 ± 3.2	
Flot-1		
Co-localized with LAMP-2A	35.4 ± 9.1	
Non co-localized with LAMP-2A	64.6 ± 9.2	
"CLUSTER" COMPOSITION ***		
Flot-1 (6nm) + LAMP-2A (15nm)	(Number gold particles)	
Flot-1 per cluster	6.7 ± 1.3	
LAMP-2A per cluster	2.3 ± 0.5	
Flot-1 per LAMP-2A	2.9 ± 0.6	
Flot-1 (15nm) + LAMP-2A (6nm)		
Flot-1 per cluster	1.7 ± 0.8	
LAMP-2A per cluster	6.0 ± 1.7	
LAMP-2A per Flot-1	3.5 ± 0.9	

Suppl. Fig. 3. Immunogold labeling of LAMP-2A and Flotillin-1 in lysosomes. Intact rat liver lysosomes were fixed and subjected to double immunogold labeling with specific antibodies against LAMP-2A and flotillin-1. Labeling for each protein and percentage of colocalization was calculated. Results are mean ± S.E. from 3 grids in 2 different experiments.

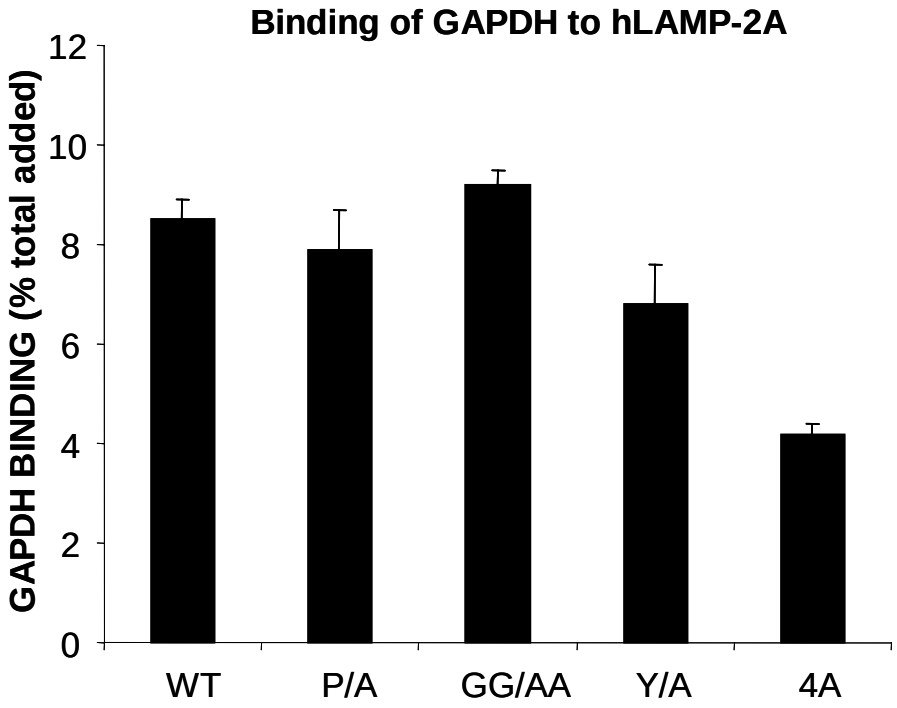
* 17% of lysosomes did not show any labeling probably as result of alterations of the antigen during the handling/processing of the samples.

** Colocalization refers to the presence of the label in close proximity (<2 diameters of distance) and not to presence of label in the same lysosome (lysosomes with both labels).

*** Calculations were done in parallel grids from the same sample labeled with both antibodies, but in which the size of the gold particle conjugated to the second antibody was switched, to eliminate artifacts resulting from steric hindrance.



Suppl. Fig. 4. Effect of different factors in the CMA ability of isolated lysosomes. Degradation of radiolabeled glyceraldehyde-3-phosphate dehydrogenase (^{14}C-GAPDH) by intact lysosomes isolated from fed rat livers (50 μg protein) incubated in an isotonic buffer without additions (none) or in the presence of ATP (5mM), GST-hsc70 (10 μg/ml), rat liver cytosol (25 μg), GAPDH (25 μg), ribonuclease A (RNase A) (25 μg), or apyrase (10 μg/ml). Proteolysis was calculated as the amount of acid precipitable radioactivity transformed in acid soluble at the end of the incubation. Agents with a stimulatory effect are shown on the top panel and agents with an inhibitory effect on the bottom.



Suppl. Fig. 5. Effect of changes in the amino acid sequence of the transmembrane and cytosolic tail of LAMP-2A on its ability to bind GAPDH. HA-tagged versions of the mutant forms of hLAMP-2A described in Fig. 8 were immobilized in Sulfolink containg anti-HA antibody and their ability to bind GAPDH present in a rat liver cytosol was analyzed. Briefly, cytosol was incubated in batch with the beads containing the immobilized protein, and the beads were collected by centrifugation, extensively washed and then subjected to SDS-PAGE and immunoblotted for GAPDH. Values are mean + S.E. of the densitometric quantification of three different experiments and are normalized to the amount of each HA-LAMP-2A immobilized in the beads.