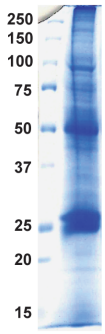
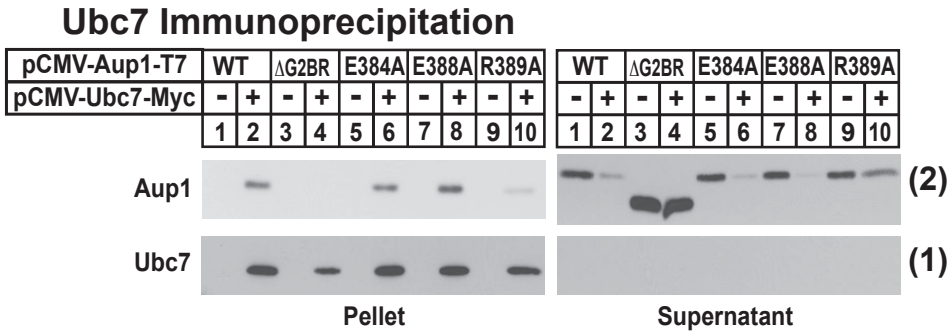
A



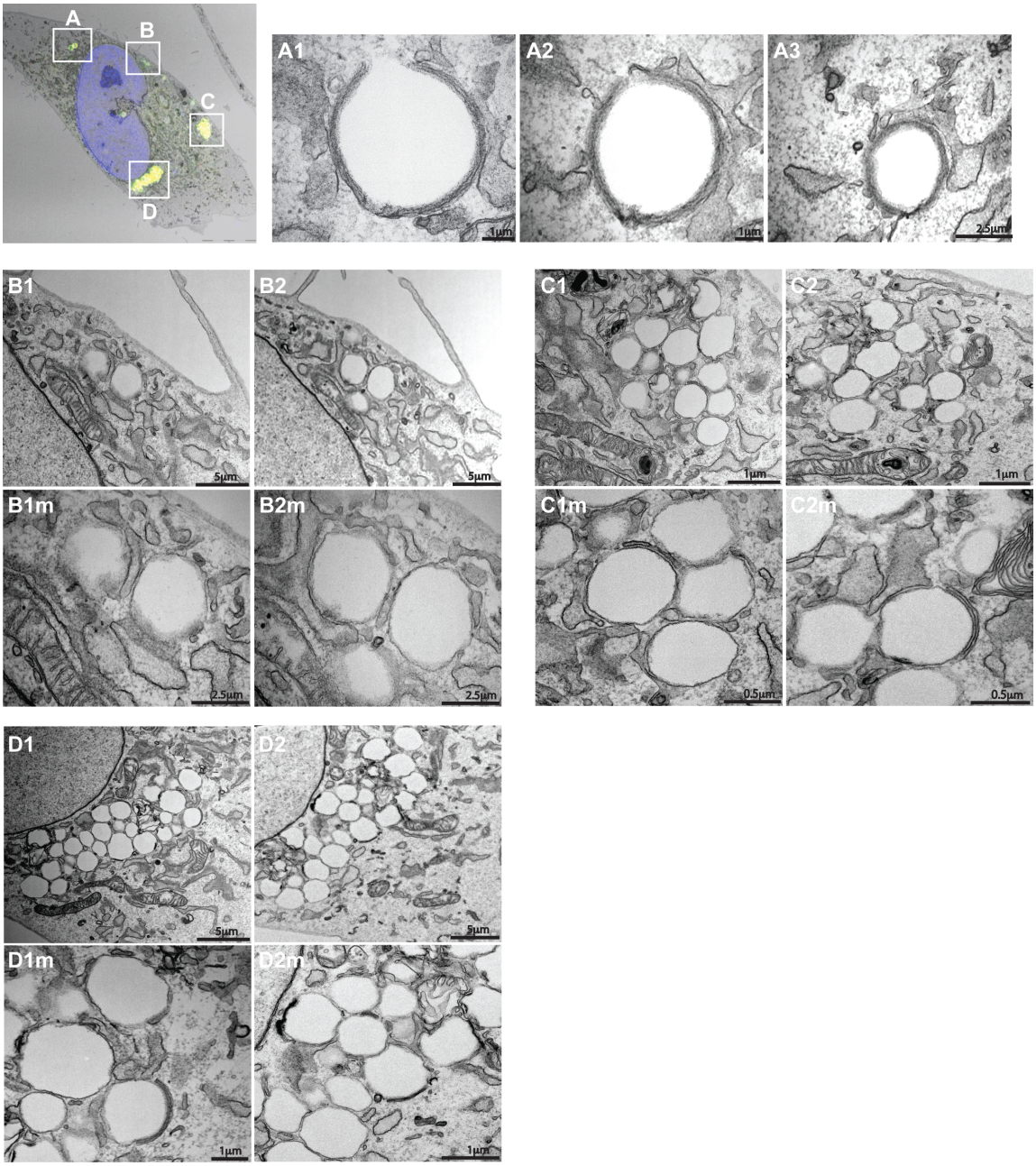
B

Partial list of gp78-associated proteins	Sequence coverage (%)
gp78	23.8
Aup1	4.4
Bat3	35.3
Bip	7.5
Cue-domain containing protein 1-like	4.0
p97/VCP	7.3
HMG CoA reductase	9.8
Protein disulfide isomerase	3.1
Proteasome (S4) regulatory subunit	3.6
Proteasome subunit p42	3.9
Proteasome subunit alpha type-2	6.0
Proteasome subunit beta type-1	7.5
proteasome subunit beta type -3	6.8
Rab1b	8.0
Rab5b	5.6
Rab5c	6.9
Rab7	6.3
Rab14	14.0
Rab-33b	14.0
Rab39	3.0
Rab6 interacting protein	1.2
Sec11-like 1	4.2
Sec22-like 1	6.5
SEL1L	5.0
SPFH2	9.7
Ufd2 (Ube4A)	1.3
Ubiquitin	17.1
Ubiquilin 4	2.7
Ubxd8	4.0
Ubxd2	3.1
USP19	11.5

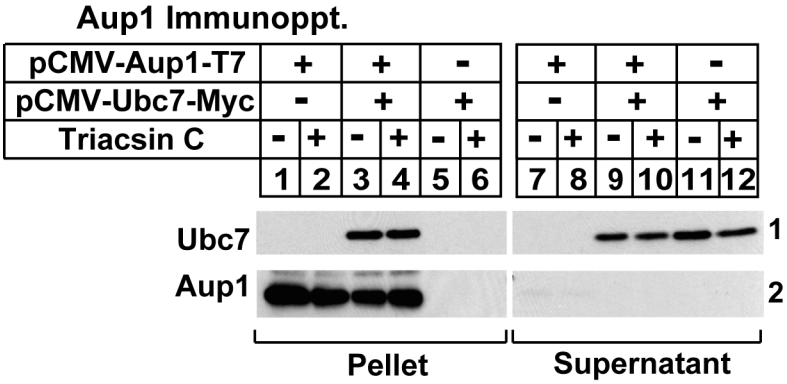
Supplementary Figure 1. Identification of gp78 - associated proteins through tandem affinity purification. (A) CHO/gp78-TAP cells were set up on day 0 at 7 X 10^5 cells/100-mm dish in medium A containing 5% LPDS. On day 2, the cells were depleted of sterols through incubation in medium A supplemented with 5% LPDS, 10 μM compactin and 50 μM mevalonate. After 16 h at 37 °C, the cells were pretreated for 2 h with 10 μM MG-132, after which they received 1 μg/ml of 25-hydroxycholesterol, 10 μg/ml cholesterol and 10 mM mevalonate and were incubated an additional 2 h at 37 °C. The cells were subsequently harvested, lysed, and subjected to affinity chromatography on human IgG-conjugated beads. Co-precipitated proteins were released from the beads by incubation with the TEV protease and the eluted material was precipitated with acetone, and resuspended proteins were fractionated by 4-20% gradient SDS-PAGE, followed by staining with colloidal blue. Visible bands were excised from the gel, digested with trypsin, and protein identities were determined by tandem mass spectrometry. Sequence coverage for identified proteins is summarized in the table (B).



Supplementary Figure 2. Association of Ubc7 with various mutants of Aup1. CHO-7 cells were set up for experiments on day 0, transfected on day 1 with 1.0 μg/dish pCMV-Ubc7-Myc together with 0.1 μg/dish wild type or mutant pCMV-Aup1-T7, and depleted of sterols as described in the legend to Figure 1C. After 16 h at 37 °C, cells were refed medium A containing 5% LPDS, 10 μM compactin, and 10 μM MG-132. After 2 h at 37 °C, the cells were subsequently harvested, lysed, and immunoprecipitated with anti-Myc. Resulting pellet and supernatant fractions were subjected to immunoblot analysis with anti-T7 IgG (against Aup1) and IgG-9E10 (against Ubc7).



Supplementary Figure 3. Close association of ER membrane and lipid droplets as revealed by transmission electron microscopy (TEM). Magnification of various regions (denoted A-D) of the TEM image shown in Figure 5B. Serial sections are denoted as A1-A3, B1, B2, C1, C2, D1, and D2. Magnification of these images are labeled as "m."



Supplementary Figure 4. The fatty acyl-CoA synthetase inhibitor Triacsin C does not inhibit Aup1-Ubc7 co-immunoprecipitation in transfected CHO cells. CHO-7 cells were set up for experiments on day 0, transfected on day 1 with 0.1 μg/dish of pCMV-Aup1-T7 and 1 μg/dish pCMV-Ubc7-Myc, and depleted of sterols as described in the legend to Figure 1. The cells were then refed medium A containing 5% LPDS and 10 μM compactin in the absence or presence of 10 μM Triacsin C. Following incubation for 4 hr at 37 °C, cells received 10 μM MG-132 and incubated for an additional 2 hr, after which they were harvested for anti-T7 immunoprecipitation. Pellet and supernatant fractions were subjected to immunoblot analysis with anti-T7 (against Aup1) and IgG-9E10 (against Ubc7).