***Figure S1.* Involvement of the Golgi apparatus in oleate-induced autophagy.**

**(A)** *Colocalization of autophagic markers with the Golgi apparatus in cells exposed to unsaturated fatty acids.* GFP-GALT/RFP-LC3-expressing U2OS cells were maintained in control conditions (Co) or exposed to saturated (black) or unsaturated (blue) fatty acids (final concentration = 500 µM). After the indicated time, the colocalization between RFP-LC3+ dots and GFP-GALT+ structures was assessed by automated fluorescence microscopy. Data are normalized means±SEM of at least 3 independent experiments (\**p*<0.05, \*\**p*<0.01 *vs.* untreated cells).

**(B-E)** *Impact of oleate on the integrity and functionality of the Golgi apparatus.* Wild-type U2OS (**B,C**) or wild-type HeLa cells (**D**) were cultured in control conditions or treated with 500 µM palmitate (PA), 500 µM oleate (OL), 10 µM golgicide A (GCA) or 10 µg mL-1 brefeldin A (BFA) for 6 h. Thereafter, cells were processed for the quantification of GBF1, GOLGA2 and LMAN1 levels by immunoblotting (**B**), the assessment of protein glycosylation by means of a colorimetric reaction involving the oxidation of glycol moieties (**C**), or endocytosis, based on a pH-sensitive variant of dextran that becomes fluorescent within endosomes (**D**). Alternatively, HeLa cells transiently expressing EGFP-VSVG were cultured overnight at 41°C, maintained in control conditions or exposed to 500 µM PA or 500 µM OL for 6 h and then moved to 32 °C (T0). Thirty min later (T1), fluorescence microscopy was employed to quantify the transport of EGFP-VSVG to the Golgi apparatus (**E**). Scale bar = 10 μm. In **B**, β actin levels were monitored to ensure equal loading of lanes. In **C**, lane loading was monitored by Comassie blue (CB) staining. In **C-E**, data are means±SEM or normalized means±SEM of 2-3 independent experiments (\**p*<0.05, \*\*\**p*<0.001 *vs.* untreated cells).

***Figure S2.* Induction of non-canonical autophagy by oleate.**

**(A)** *Implication of AMPK, PKR, JNK1 and CPT1A in palmitate-induced, but not oleate-induced, autophagy.* GFP-LC3-expressing U2OS cells were transfected with a control siRNA (siUNR) or with siRNAs targeting AMPK (siAMPK), PKR (siPKR), JNK1 (siJNK1) or CPT1A (siCPT1A) for 48 h, followed by the immunoblotting-assisted quantification of residual AMPK, PKR, JNK1 or CPT1A levels. The abundance of β actin was monitored to ensure equal loading of lanes.

(**B,C**) *Upstream regulators of autophagy induced by saturated fatty acids.* GFP-LC3-expressing (**B**) or RFP-FYVE-expressing (**C**) U2OS cells were transfected with siUNR or with siRNAs specific for BECN1 (siBECN1) or PIK3C3 (siPIK3C3) for 48 h, and either kept in control conditions (Co) or treated with saturated (black) or unsaturated (blue) fatty acids (final concentration = 500 µM). Six h later, the number of GFP-LC3+ (**B**) or RFP-FYVE+ (**C**) dots was quantified by automated fluorescence microscopy. Data are normalized means±SEM of at least 3 independent experiments (#*p*<0.05 *vs.* siUNR-transfected cells treated with the same fatty acid). Scale bar = 10 μm.

***Figure S3.* Mechanistic characterization of oleate-induced autophagy.**

**(A)** *Impact of proteins involved in non-canonical autophagy on the autophagic response to oleate.* GFP-LC3-expressing U2OS cells were transfected with a control siRNA (siUNR) or with siRNAs specific for AMBRA1, ATG14, HMGB1, KIAA0226 (RUBICON), PINK1, SH3GLB1 (BIF1), UVRAG and VMP1 for 48 h, and either kept in control conditions (Co) or treated with 500 µM oleate (OL), alone or in combination with 10 g/mL E-64d and 10 g/mL pepstatin (Pep). Six h later, the number of GFP-LC3+ dots was quantified by automated fluorescence microscopy. Data are normalized means±SEM of at least 2 independent experiments.

**(B,C)** *Sensitivity of oleate-induced autophagy to MTORC1 signaling.* Wild-type U2OS cells were transfected with siUNR or with siRNAs specific for TSC2 (siTSC2) or PTEN (siPTEN) for 48 h, and either maintained in control conditions or treated with 500 µM palmitate (PA) or 500 µM OL. Six h later, LC3 lipidation was assessed by immunoblotting. TSC2 and PTEN expression levels were assessed to as indicators of siRNA-mediated target depletion. β actin levels were monitored to ensure equal loading of lanes.

***Figure S4.* Implication of PLD in oleate-induced autophagy.**

**(A,B)** *Impact of PLD inhibition on the autophagic response to oleate.* Wild-type U2OS cells were maintained in control conditions (Co) or treated with 500 µM oleate (OL) or 500 µM palmitate (PA), alone or in the presence of 10 µM VU0155069, 10 µM BML-279 or 10 µM BML-280, for 6 hrs (**A**). Alternatively, WT U2OS cells were transfected with a control siRNA (siUNR) or with a validated siRNA specific for PLD1 (siPLD1) for 48 hrs, then maintained in control conditions or treated with 500 µM OL or 500 µM palmitate PA for additional 6 hrs. In both settings, the lipidation of LC3 was assessed by immunoblotting, and β actin levels were monitored to ensure equal loading of lanes. Irr, irrelevant sample.

***Figure S5.* BECN1-independent autophagic responses to oleate in vivo.**

*Becn1-independent induction of autophagy by oleate in kidney.* Wild-type (WT) or *Becn1+/-* mice were injected i.p. with vehicle, 100 mg/kg palmitate (PA) or 100 mg/kg oleate (OL). Six h later, animals were euthanatized, followed by the immunoblotting-assisted assessment of LC3 lipidation and p62 degradation in kidneys. β actin levels were monitored to ensure equal loading of lanes, and densitometry was employed to quantify the abundance of lipidated LC3 (LC3-II) and p62, both normalized to β actin levels. Results are means±SD of 3 mice (n.s., non-significant, \**p*<0.05, \*\**p*<0.01 *vs.* untreated mice of the same genotype).