

Figure S1. Autophagy induction by caloric restriction mimetics in epithelial U2OS cells.

Human osteosarcoma U2OS cells stably expressing GFP-LC3 were treated with the indicated agents at the indicated concentrations for 6 hours. After fixation and staining with Hoechst 33342, images were acquired and the formation of GFP-LC3 puncta as well as the cytoplasmic translocation of nuclear GFP-LC3 were assessed by confocal microscopy. Representative images are depicted in (A). Size bar equals 10 μ m. Numerical values were statistically evaluated, normalized to control and are shown as bar-charts in (B,C) using a pseudo-logarithmic scale. For the assessment of autophagy, the average number of GFP-LC3 positive dots per cell was quantified and is expressed as fractional increase of untreated control, while cytoplasmic translocation of GFP-LC3 was calculated as loss of nuclear fluorescent signal. Data are represented as median $\pm$ MAD and significance were tested by means of a Mann–Whitney U-test. P-values are indicated. Rapamycin (Rapa), torin-1 (Tor1), bafilomycin A1 (BafA1), serum deprivation/starvation (Starv), triethylenetetramine (TETA), spermidine (Sper), 3,4-dimethoxychalcone (3,4-DC), 4,4-dimethoxychalcone (4,4-DC).

Figure S2. Nuclear cytoplasmic GFP-LC3 translocation induced by caloric restriction mimetics in HUVEC cells and RAW macrophages.

Human vascular endothelial HUVEC cells and murine RAW 264.7 macrophages stably expressing GFP-LC3 were treated with the indicated agents at the indicated concentrations for 6 hours. After fixation and staining with Hoechst 33342, images were acquired, the cytoplasmic translocation of nuclear GFP-LC3 was assessed by confocal microscopy and calculated as loss of nuclear fluorescent signal. Both were normalized to control and are shown as bar charts using a pseudo-logarithmic scale (A,B). Data are represented as median $\pm$ MAD and significance were tested by means of a Mann–Whitney U-test. P-values are indicated. Rapamycin (Rapa), torin-1 (Tor1), bafilomycin A1 (BafA1),

serum deprivation/starvation (Starv), triethylenetetramine (TETA), spermidine (Sper), 3,4-dimethoxychalcone (3,4-DC), 4,4-dimethoxychalcone (4,4-DC).

Figure S3. Mass spectrometric assessment of triglycerides in plasma and liver from 3,4-DC treated mice. Mice were treated with 230 mg/kg 3,4-dimethoxychalcone (3,4-DC) intraperitoneally, samples were prepared at the indicated timepoints and then subjected to mass spectrometric analysis. Data are depicted as heatmaps and resemble an excerpt of the larger metabolomic assessment depicted in Figure 4. Fatty acids annotated with ** have been controlled with available standards.

Figure S4. Statistical assessment of triglycerides in plasma and liver from 3,4-DC treated mice. Heatmaps show plasma (A) and liver (B) metabolites, as fold change of the mean. Test of significance was conducted with a Kruskal Wallis and Dunn's post-hoc test with Benjamini-Hochberg adjustment, and results are depicted as asterisks (* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.005$).

Figure S5. LC3 puncta formation in neointimal lesions of vein grafts. (A) Vein segments were grafted into the carotid artery and locally treated with vehicle (Ctrl) or 3,4-dimethoxychalcone (3,4-DC) and harvested 4 weeks after grafting. The sections were subjected to immunofluorescence staining with anti-LC3 antibody (red) and DAPI (blue). Size bars equal 10 μm . Positive cells in the neointimal lesions were enumerated and data is summarized as bar chart in (B). Data are represented as mean $\pm$ SEM of the indicated number of repeats ($n=6$). Data were statistically evaluated by means of a Mann–Whitney U-test. P-value is indicated.

Figure S6. Representation of plasmatic parameters determination in *Apoe*^{-/-} mice treated with vehicle (n = 15) or with 3,4-DC (n = 15) at day 15 and 30 after treatment and HFD.

Twelve-week-old *Apoe*^{-/-} mice were feed with HFD for 4 weeks in the presence of 3,4-dimethoxychalcone (3,4-DC) or vehicle (Ctrl). (A) Free cholesterol (mg/dL), (B) total cholesterol (mg/dL), (C) pancreatic Lipase (U/L), (D) High-density lipoprotein (HDL) (mg/L), (E) low-density lipoprotein (LDL) (mg/dL), (F) triglycerides (mg/dL) and (G) non-esterified fatty acids (NEFA) (mmol/L) were determined. Data are represented as mean $\pm$ SEM of the indicated number (n) of repeats. Data were statistically evaluated by Student's t test.

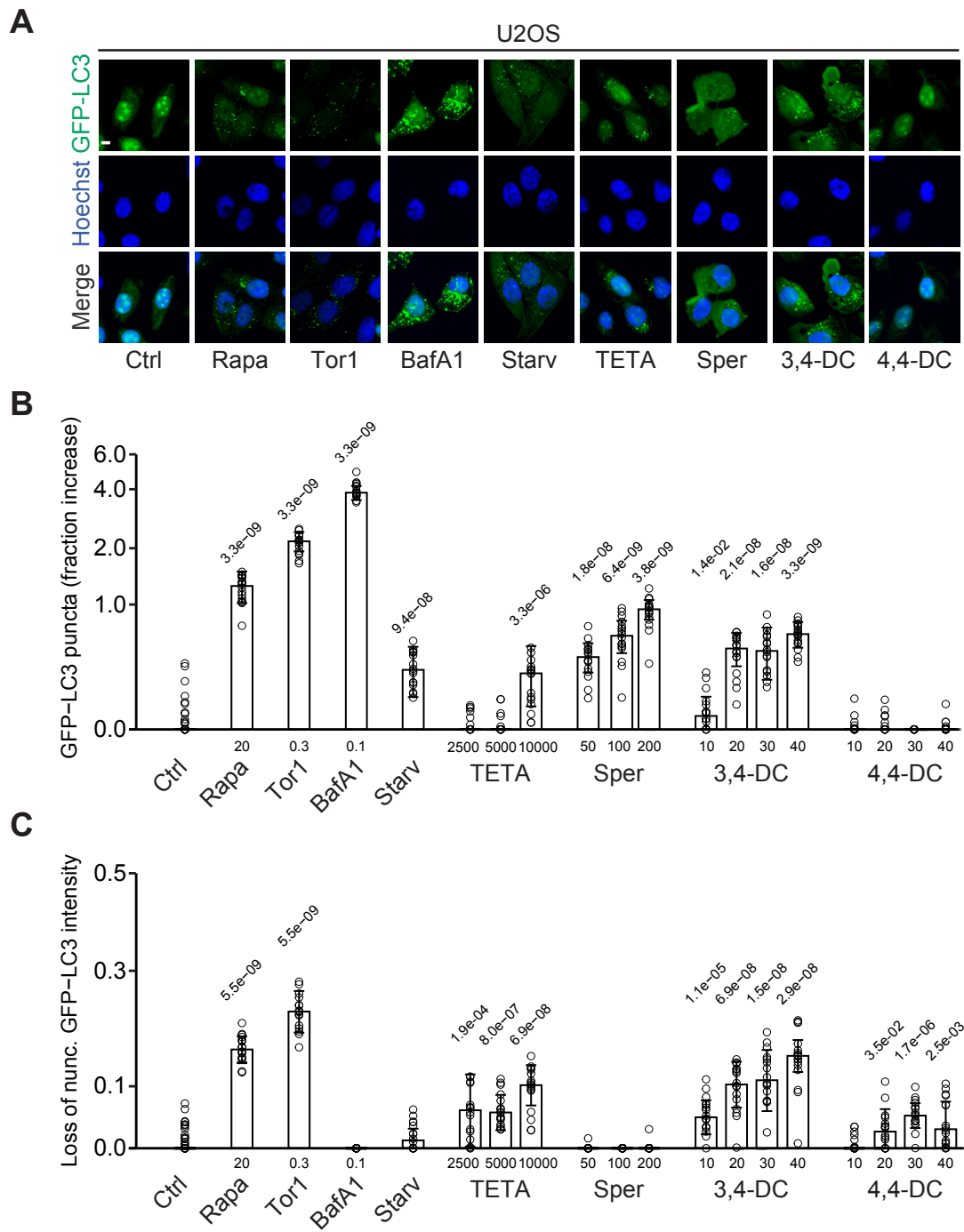


Figure S1

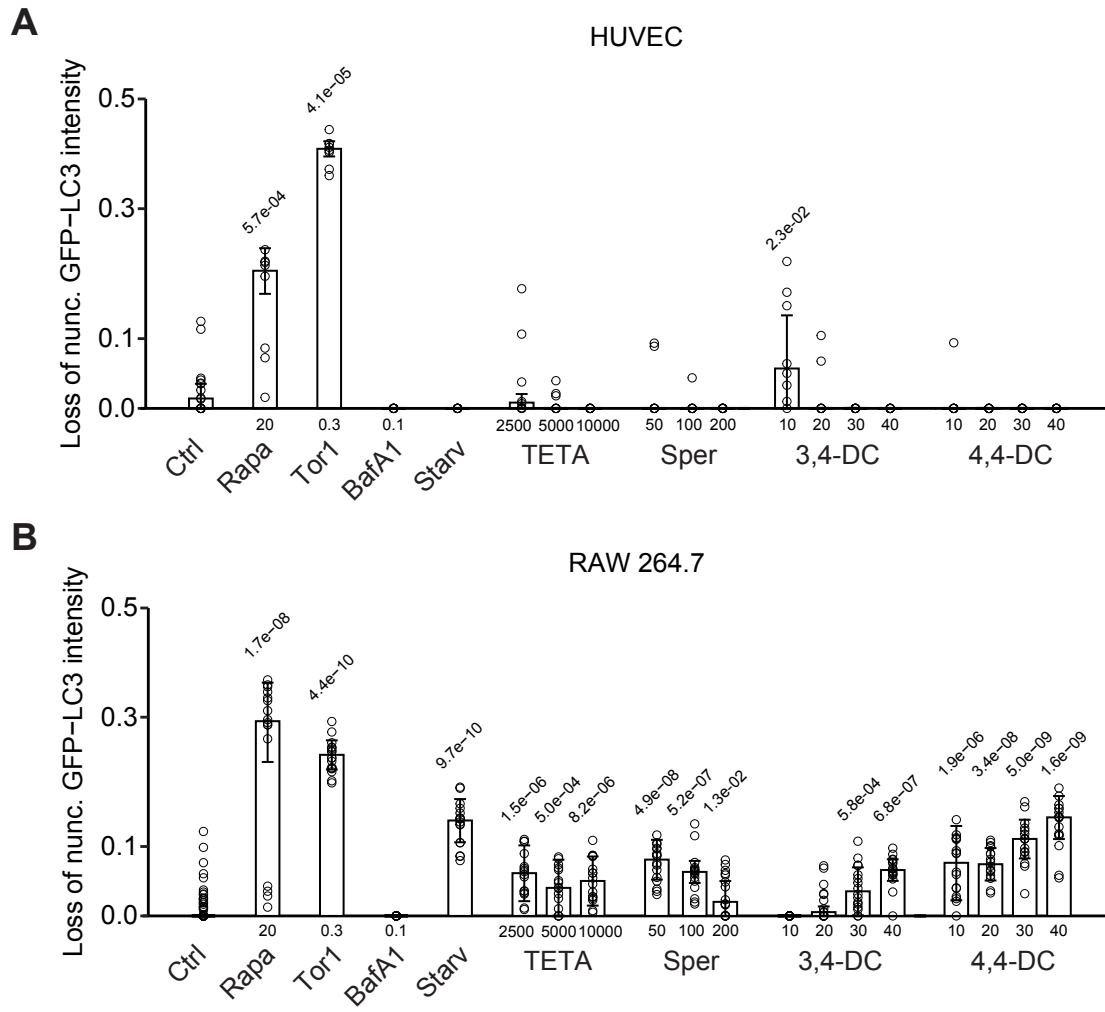


Figure S2

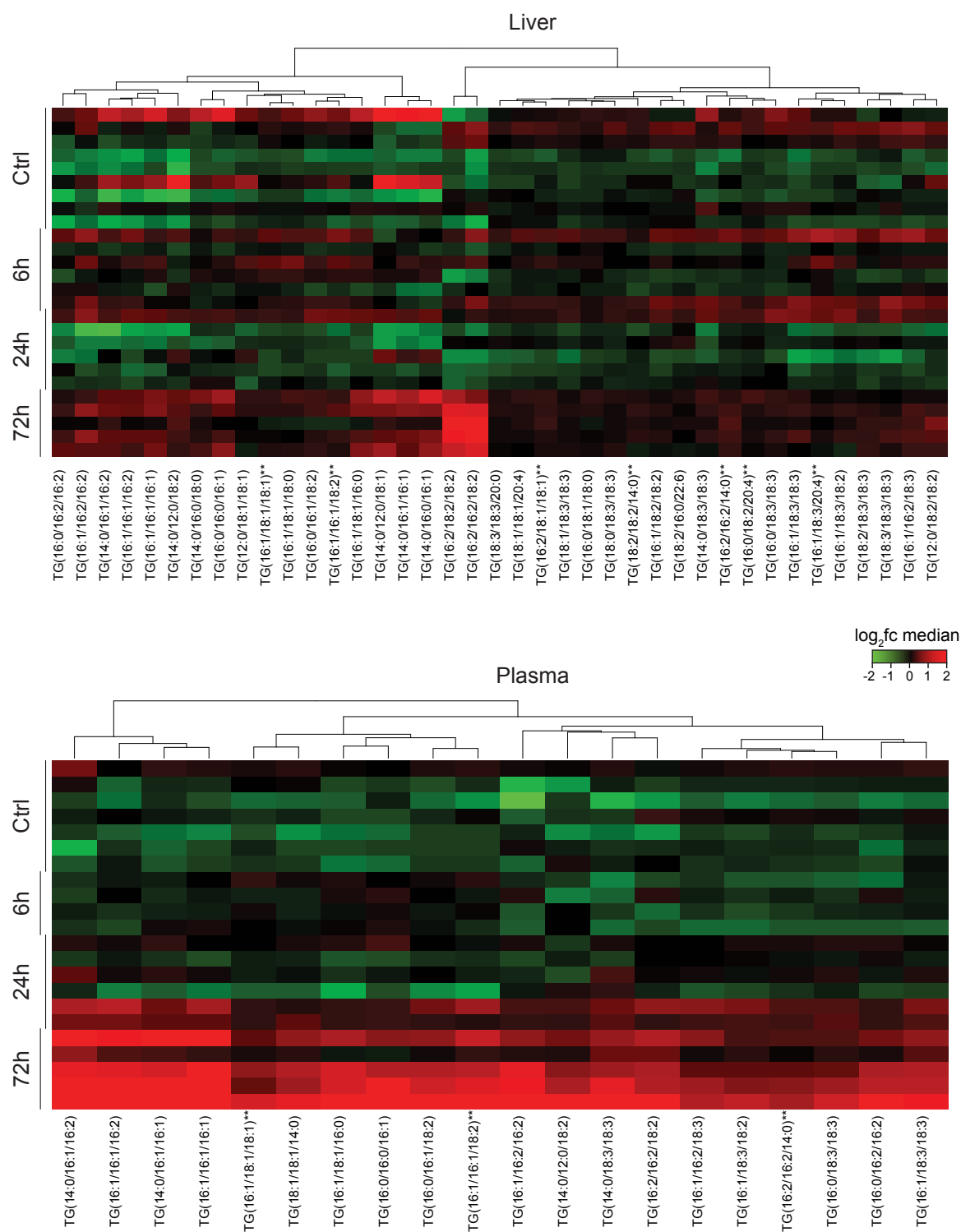


Figure S3

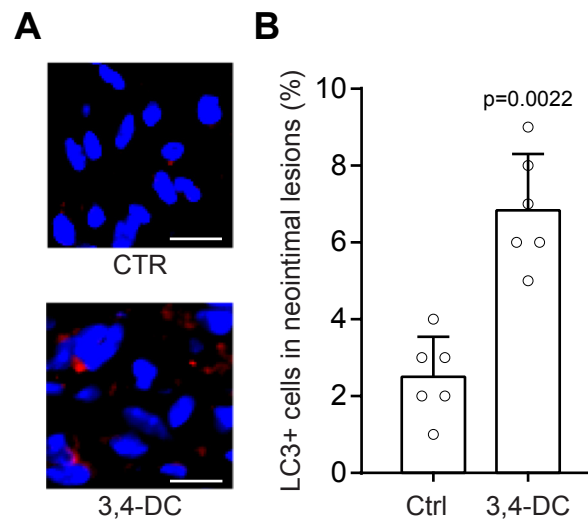


Figure S5

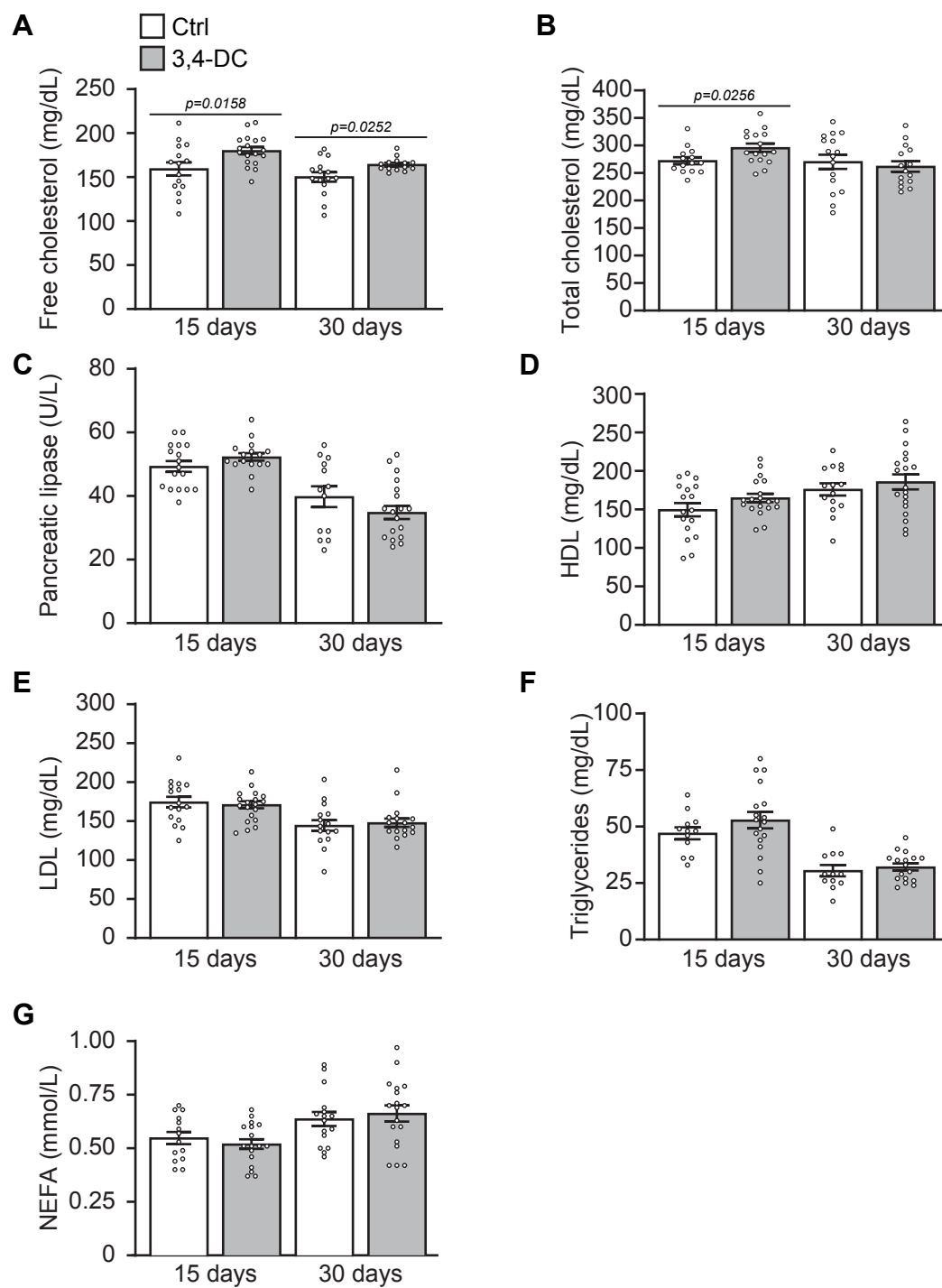


Figure S6