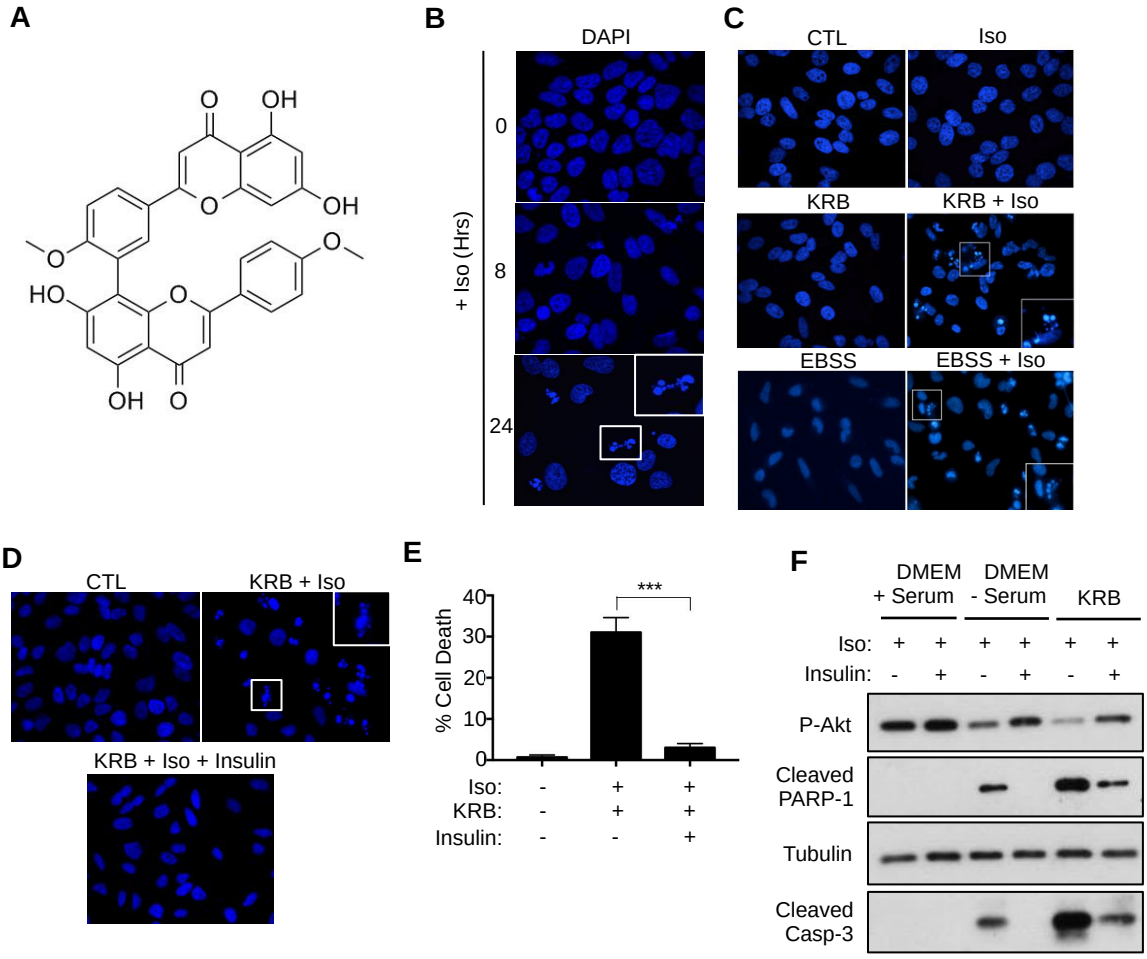

Figure S1: Growth factors and amino acid withdrawal sensitize cells to apoptosis via isoginkgetin. (A) Chemical structure of isoginkgetin. (B) DAPI stain of HeLa cells treated with 10 μM isoginkgetin for 0, 8 or 24 h. (C) DAPI stain of HeLa cells treated with 10 μM isoginkgetin either alone or in combination with KRB or EBSS for 6 h. (D-E) DAPI stain (D) and quantification (E) of HeLa cells treated with KRB and isoginkgetin, with or without insulin (5 ug/mL) (n=3, approximately 200 cells per condition, ***p<0.001). (F) Western blot analysis of tubulin, cleaved PARP-1, phospho-AKT and caspase-3 in cells treated with isoginkgetin in either complete DMEM, serum-free DMEM or KRB.

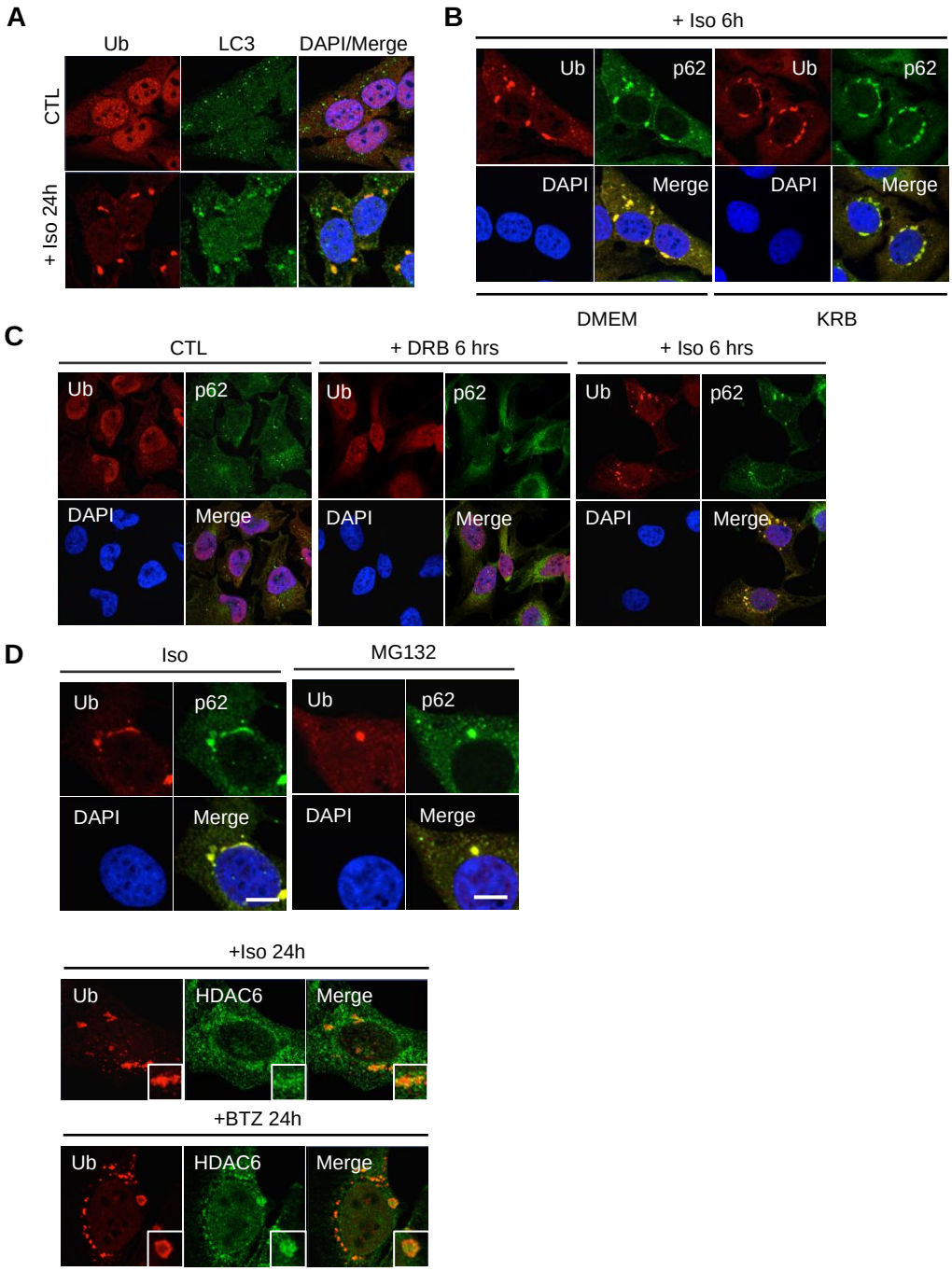

Figure S2: Isoginkgetin induces formation of perinuclear aggregates of ubiquitinated protein cargo that are distinct from previously reported aggresome. (A) Confocal immunofluorescence microscopy analysis of HeLa cells treated with 10 μM isoginkgetin for 24 h, stained with LC3, ubiquitin (Ub), and DAPI nuclear stain. (B) Confocal immunofluorescence microscopy analysis of HeLa cells treated with 10 μM isoginkgetin for 6 h in either nutrient-rich DMEM or KRB, stained with p62, Ub or nuclear stain DAPI. (C) Confocal immunofluorescence microscopy analysis of HeLa cells treated with 10 μM isoginkgetin or 10 μM 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside (DRB) for 6 h and stained with Ub, p62 antibodies, and DAPI nuclear stain. (D) Confocal immunofluorescence analysis of HeLa cells stained for p62, ubiquitin, HDAC6 and DAPI upon 24-h treatment with 10 μM isoginkgetin or 10 μM MG132 (scale bar = 10 μM).

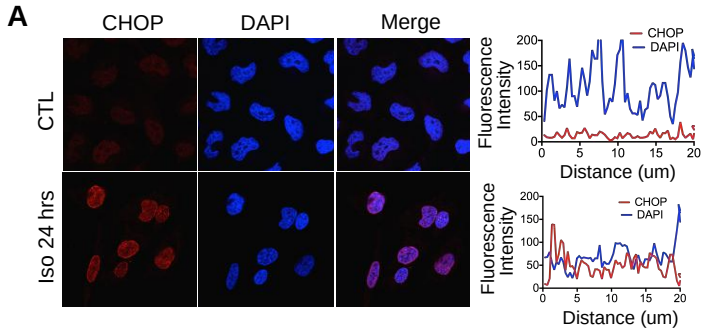

Figure S3: Isoginkgetin induces ER stress in HeLa cells. (A) Confocal microscopy analysis of HeLa cells stimulated with 10 μM isoginkgetin for 24 h, stained with CHOP and DAPI nuclear stain.

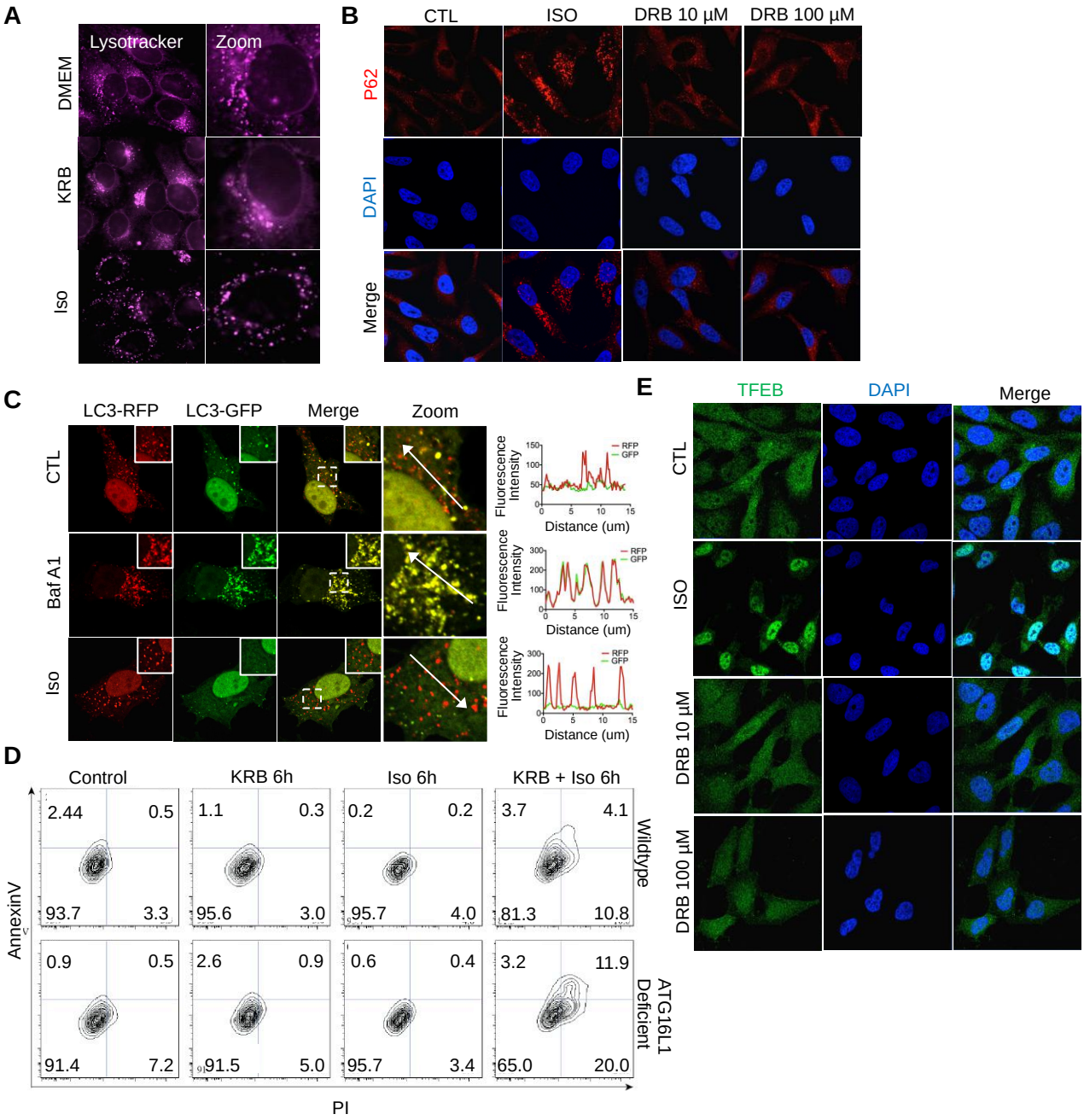

Fig S4. Autophagy activation and lysosomal repositioning occur in response to treatment with isoginkgetin. (A) HeLa cells stained with Lysotracker-DND 99 for 1 h following treatment with isoginkgetin or KRB for 6 h, visualized by immunofluorescence. (B) HeLa cells treated with isoginkgetin (10 µM) or DRB (10 µM or 100 µM) for 6h stained with a p62 antibody and DAPI nuclear stain. (C) Confocal immunofluorescence analysis of HeLa cells stimulated with either 1 µM bafilomycin A1 or 10 µM isoginkgetin for 6 h, followed by staining with LAMP2 and LC3 antibodies and DAPI nuclear stain. Co-localization plots show the fluorescence intensity over the distance indicated by the white arrow. Scale bar= 5 µm. (D) Wildtype and CRISPR-generated ATG16L1 knockout HCT 116 cells lines were stimulated with KRB or 10 µM isoginkgetin for 6 h, followed by staining with AnnexinV-FITC and propidium iodide and analyzed via flow cytometry. (E) HeLa cells treated with isoginkgetin (10 µM) or DRB (10 µM or 100 µM) for 6h stained with a TFEB antibody and DAPI nuclear stain.

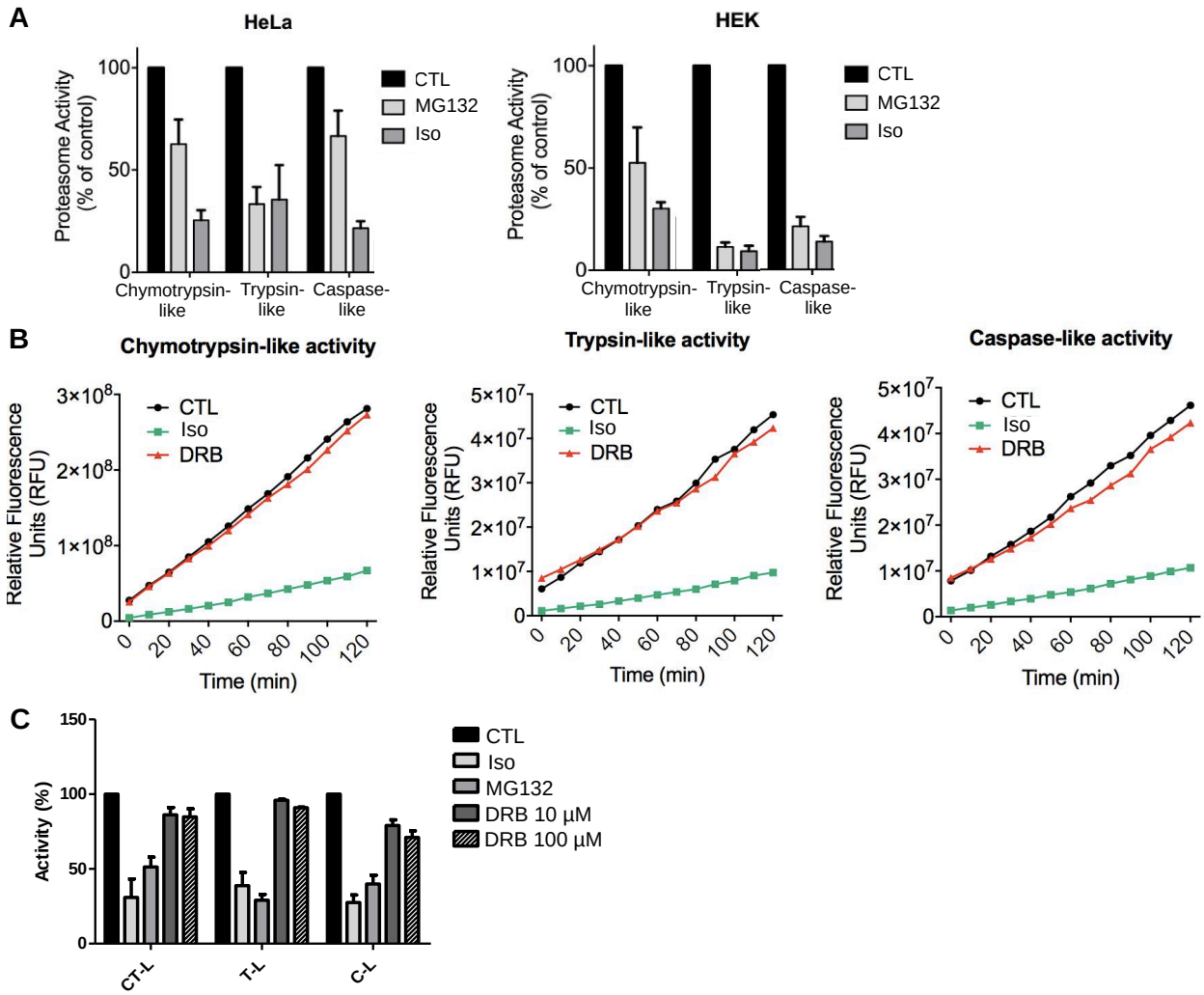

Figure S5: Isoginkgetin inhibits the proteolytic activities of the 20S proteasome and cancer cell lysates. (A) Analysis of the proteasome activity in HEK or HeLa cell lysates, measured by cleavage of fluorogenic substrates of the chymotrypsin-, trypsin- and caspase-like enzymatic activity over a 2-h period upon treatment with 10 µM isoginkgetin or 10 µM MG132. Representative data of three independent experiments. (B) Activity of purified 20S proteasome measured by cleavage of fluorogenic substrates of the chymotrypsin-, trypsin- and caspase-like enzymatic activity over a 2-h period upon treatment with 10 µM isoginkgetin or 10 µM DRB (representative data from three independent experiments). (C) Proteasome activity measured by cleavage of fluorogenic substrates of the chymotrypsin- (CT-L), trypsin- (T-L), and caspase-like (C-L) enzymatic activity after 2 h in HeLa cells treated with 10 µM isoginkgetin, 10 µM MG132, 10 µM DRB, or 100 µM DRB (representative data from three independent experiments).

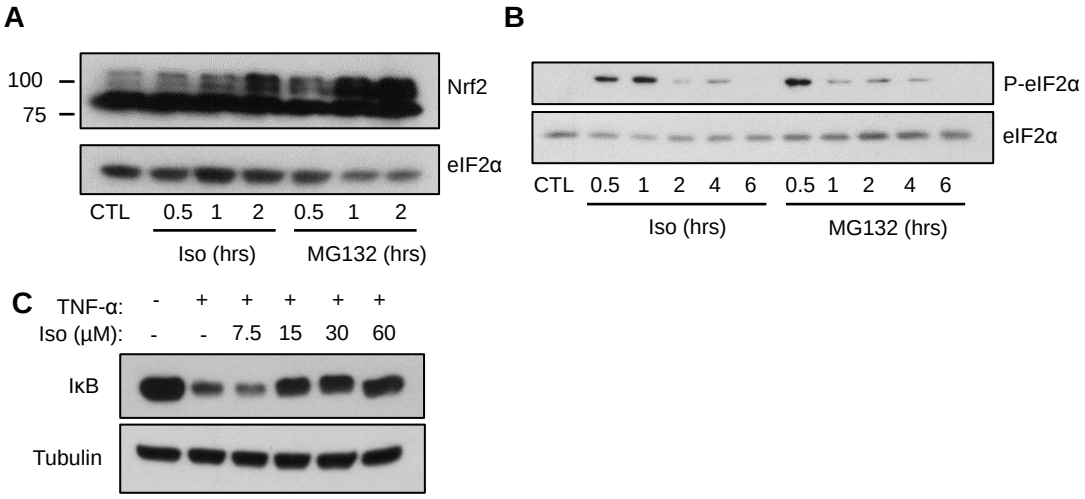

Figure S6. Isoginkgetin treatment leads to an accumulation of proteasomal targets. (A-B) Western blot analysis of the expression levels of Nrf2, eIF2α, and phopho-eIF2α in HeLa cells treated with isoginkgetin or MG132 for the indicated time periods. (C) Western blot analysis of IκB protein levels in HeLa cells pre-treated with the indicated doses of isoginkgetin for 6 h followed by a 30 min TNFα stimulation, compared to tubulin loading control.