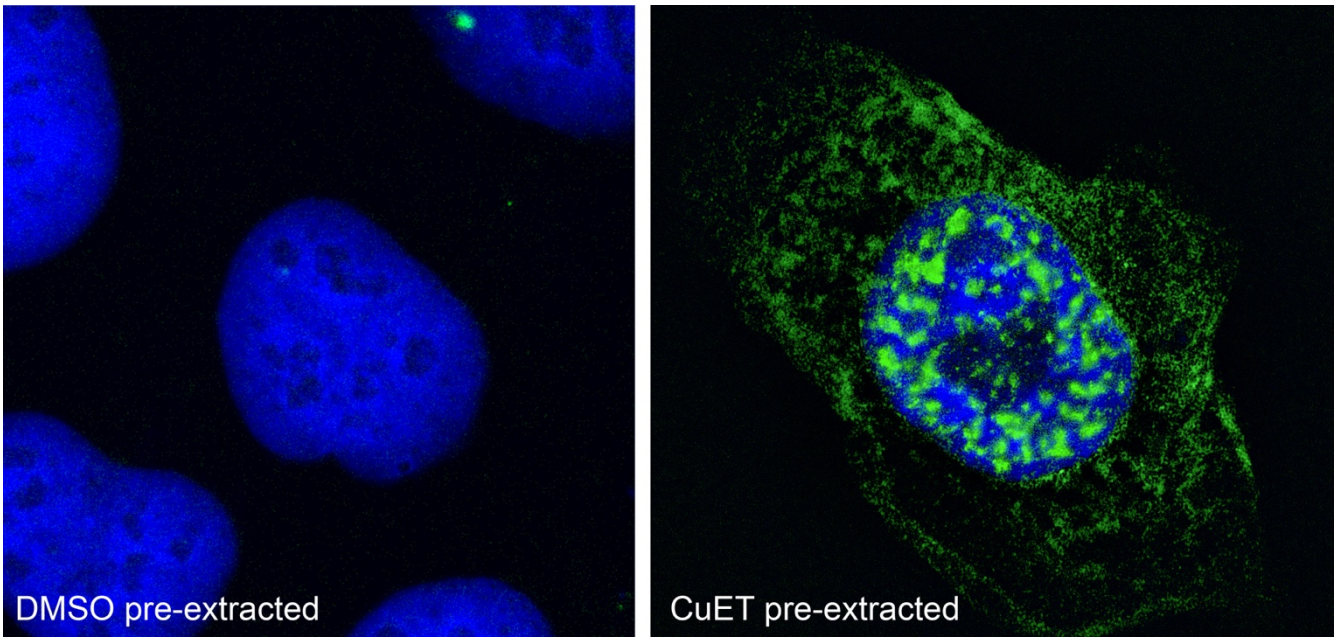
Annual highlight 1

The alcohol abuse drug disulfiram targets cancer via the key component of the cellular recycling machinery: the p97/NPL4 segregase

We have identified the NPL4 protein, a cofactor of the p97VCP segregase as an unexpected target for treatment of a wide spectrum of human tumors using the strategy of drug repurposing. This complex is a central element upstream of the proteasome in the cellular protein recycling machinery, and it plays roles in multiple cellular pathways including autophagy. It recognizes ubiquitylated cellular proteins in various organelles and structures, extracts these proteins and delivers the cargo to proteasome for degradation and recycling. Our results showed that the FDA-approved old and safe drug disulfiram (known as Antabuse and used to treat alcoholism) is rapidly metabolized to a complex of ditiocarb with copper, and this is the active anti-cancer 'compound' that preferentially accumulates in tumors *in vivo* and binds to the NPL4 protein's putative zinc-finger domain thereby triggering aggregation and immobilization of the otherwise extremely dynamic and mobile p97-NPL4 complex. This leads to severe endogenous stress and cell death due to inability to recycle damaged or misfolded proteins. Our experimental data were further strengthened by epidemiological evidence from the Danish nation-wide cancer and drug registries, methods to detect the active metabolite in blood and tissues, and identification of candidate biomarkers to help guide upcoming clinical trials with combination of the disulfiram with a copper supplement to enhance the formation of the active anti-cancer metabolite. Several such clinical trials are under preparation in Denmark and other countries. This major study provides a significant step towards achieving one of the major goals of the CARD project, to identify and exploit innovative strategies to treat cancer by targeting vulnerabilities in the autophagy/recycling machinery.

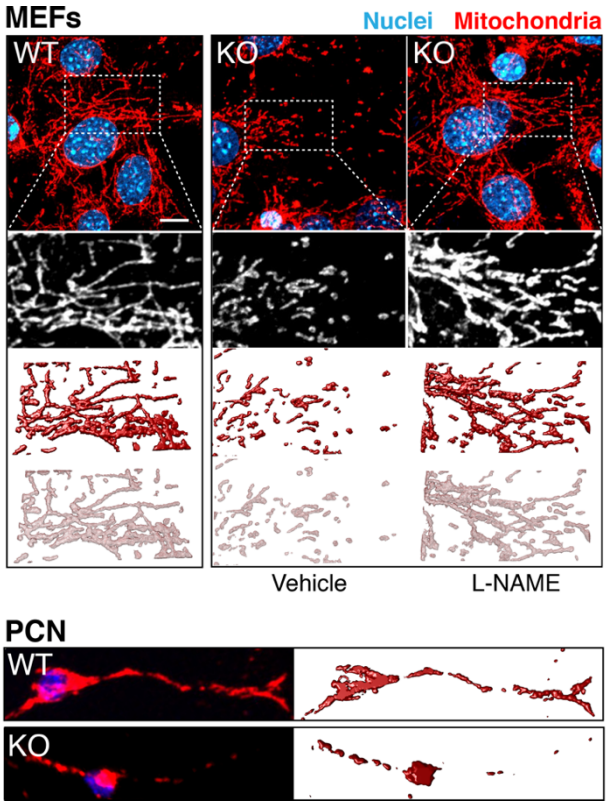


Aggregation of NPL4 complexes in cells treated with CuET, the active metabolite of disulfiram. In human cancer cells that have been pre-extracted to remove free cellular proteins, and their DNA (nuclei) stained in blue, the fluorescently labeled NPL4 protein (green) is removed/lost from cells that are untreated and grow under control conditions (left, marked DMSO), in sharp contrast to the same cells that have been treated with the active anti-cancer metabolite of disulfiram/Antabuse (named CuET, right) which results in insoluble aggregates of NPL4 (green) that block the cellular protein recycling machinery and trigger cancer cell death.

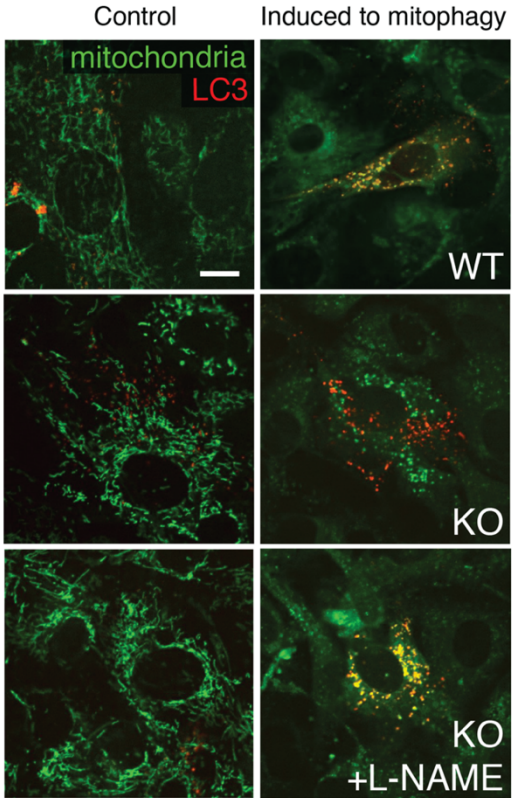
Annual highlight 2

S-nitrosylation regulates mitochondrial dynamics and mitophagy, and impacts on aging

S-nitrosylation is a prototypic redox-based posttranslational modification, which is induced by nitric oxide and frequently dysregulated in disease. *S*-nitrosoglutathione reductase (GSNOR) regulates protein *S*-nitrosylation by functioning as a protein denitrosylase. Deficiency of GSNOR results in tumorigenesis and neuromuscular disorders. Using spinning disk technology coupled with 3-D reconstruction, we observed that GSNOR-deficient cells have fragmented mitochondria that are not properly recognized for removal due to the selective *S*-nitrosylation of proteins regulating mitochondrial dynamics (Drp1) and mitophagy (Parkin). Accumulation of mitochondrial damage is commonly accepted as an age-related phenomenon associated with the inescapable side effects of oxidative metabolism. Indeed, *Gsnor*-KO mice show phenotypes that typify aging. We also observed that GSNOR expression decreases during cell senescence and ageing, but not change in exceptionally long-lived individuals. Our findings implicate GSNOR in mammalian longevity and suggest a molecular link between protein *S*-nitrosylation and mitochondria quality control in aging with direct therapeutic implications, especially in cancer, where aging is a major risk factor for **cancer** development.



***S*-nitrosylation enhances mitochondrial fragmentation.** Confocal microscope analyses of *wild-type* (WT) and *Gsnor*-KO mouse embryonic fibroblasts (MEFs, *Top*) or primary cortical neurons (PCN, *Bottom*) stained for nuclei (blue) and mitochondria (Red). 3D rendering of acquired z-stacks highlights differences in mitochondrial network, which are highly fragmented in conditions of *Gsnor*-KO (excessive *S*-nitrosylation). Incubation with L-NAME – a chemical compound used to counteract *S*-nitrosylation – restores a correct (elongated) mitochondrial morphology.



***S*-nitrosylation impairs mitophagy.** Still images of *wild-type* (WT) and *Gsnor*-KO cells performed with a Spinning Disk confocal microscope. Cells were transfected with LC3-cherry (red) to visualize autophagosomes and, contextually, stained with Mitotracker Green, then treated with an uncoupling molecule to induce mitophagy. *Gsnor*-KO cells are incapable of engulfing damaged mitochondria in the autophagosomes (yellow fluorescence). Incubation with L-NAME restores a correct mitophagy.