Basal Gp78-dependent mitophagy promotes mitochondrial health and limits mitochondrial ROS

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Poster by Kurt Vandevoorde at UBC SBME, June 7th, 2022

Abstract

Gp78 is a major ER ubiquitin ligase in ER associated degradation, regulates rough ER-mitochondria contacts, and regulates Parkin independent mitophagy, the selective degradation of defective mitochondria. Understanding Gp78's role in mitophagy can elucidate how cells maintain mitochondrial health. Gp78 KO cells have increased mitochondrial volume and are resistant to damage (CCCP) induced mitophagy, suggesting defects in mitophagy, the removal of damaged mitochodnria. This is confirmed by Gp78 KO cells showing reduced autophagic flux, and Gp78 KO phenocopied by knockdown of essential autophagy protein ATG5. Gp78 mitophagy promotes mitochondrial health. We leverage SPECHT to robustly recover autophagosomes and autophagic flux. [1]

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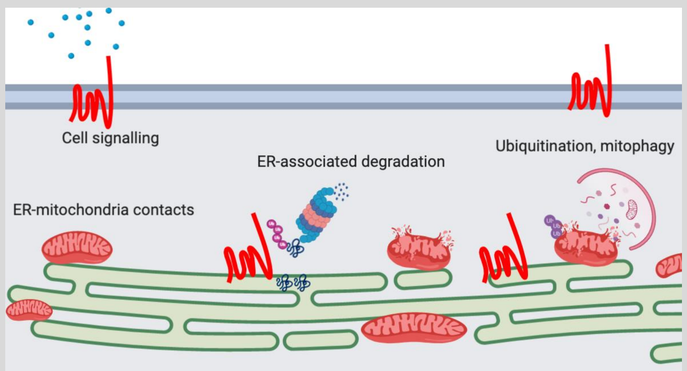
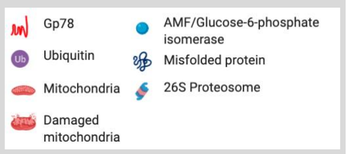
Background





Gp78 Functions:
- Major E3 ubiquitin ligase in ER-associated degradation
- Regulates rough ER-mitochondria contacts
- Acts as a receptor and stimulates cell motility at the plasma membrane
- Regulates Parkin-independent **mitophagy**

What is Mitophagy?
- The selective degradation of defective mitochondria
- Defects in mitophagy are associated with **cancer, neurodegenerative diseases, and metabolic disorders**
- Understanding Gp78's role in mitophagy can elucidate how cells maintain mitochondrial health

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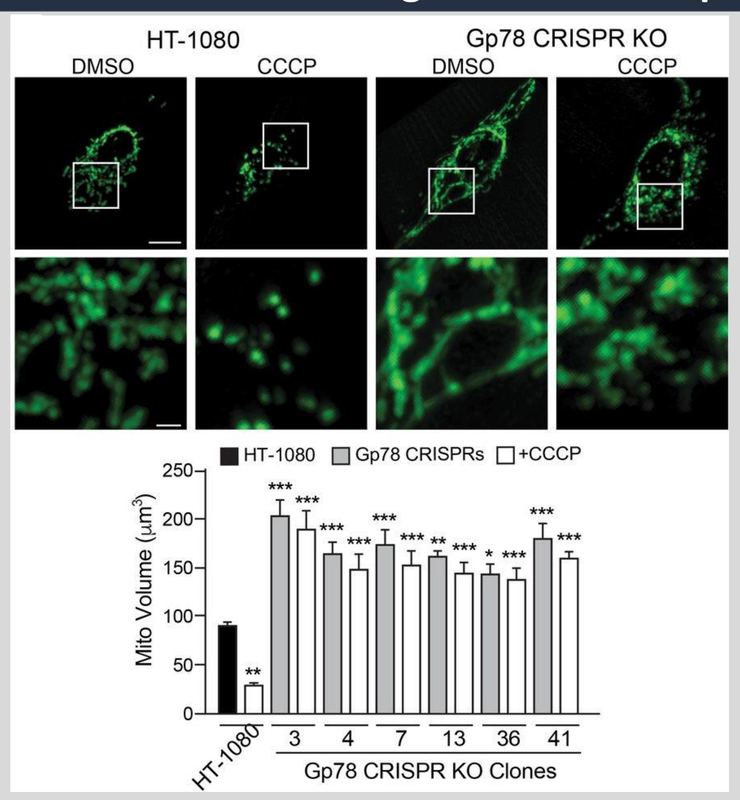
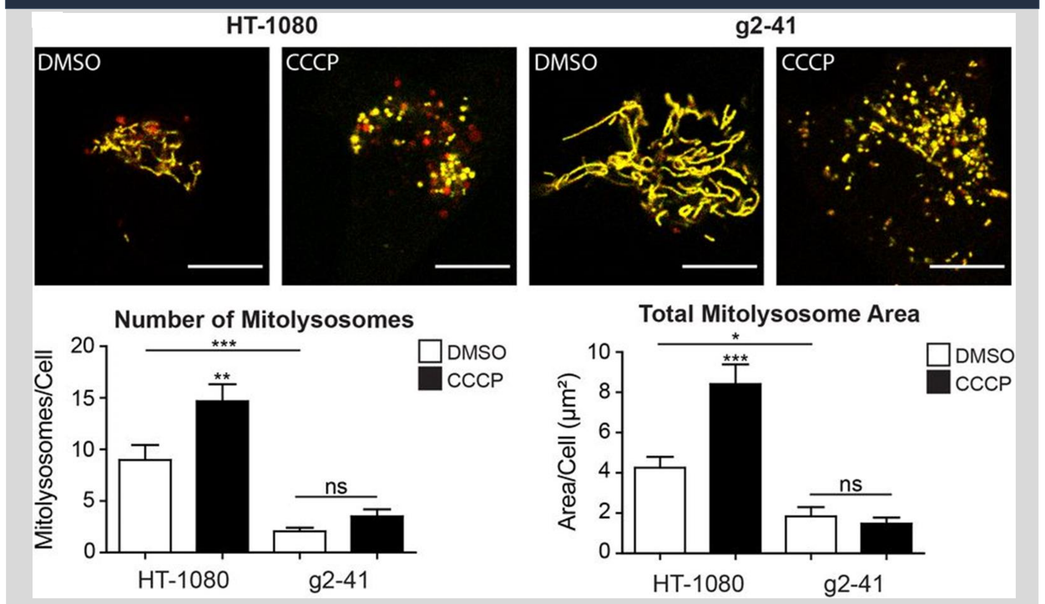
Gp78 KO cells have increased mitochondrial volume and are resistant to damage induced mitophagy



- Crispr/Cas9 KO of Gp78 in HT-1080 fibrosarcoma cells induces a mitochondrial phenotype characterized by a spread mitochondrial network and increase mitochondrial volume
- Treatment with the mitochondria-depolarizing drug Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) induces mitochondrial damage and mitophagy
- Gp78 KO cells retain their mitochondrial volume following depolarization suggesting defects in mitophagy

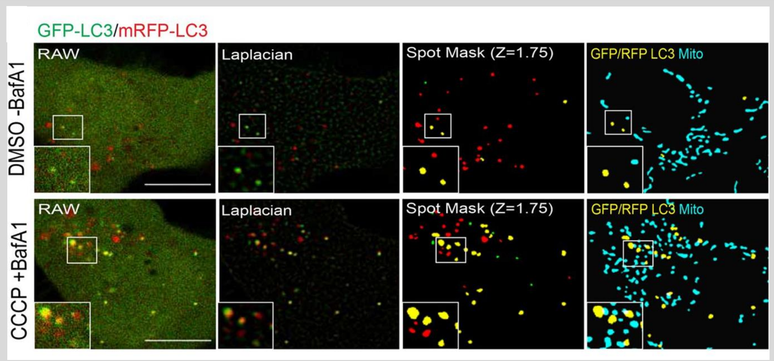
Mito-Keima confirms mitophagy defects in Gp78 KO cells

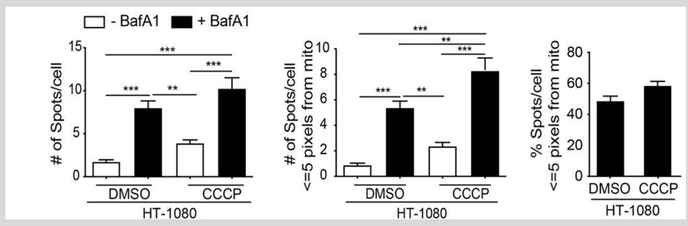


- Keima is a fluorescent protein which has different excitations based on pH
- Using Mito-Keima we confirmed that Gp78 KO cells show deficits in both basal and damage-induced mitophagy represented by the lack or red spots

All figures adapted from: Alan *et al.* 2021, bioRxiv

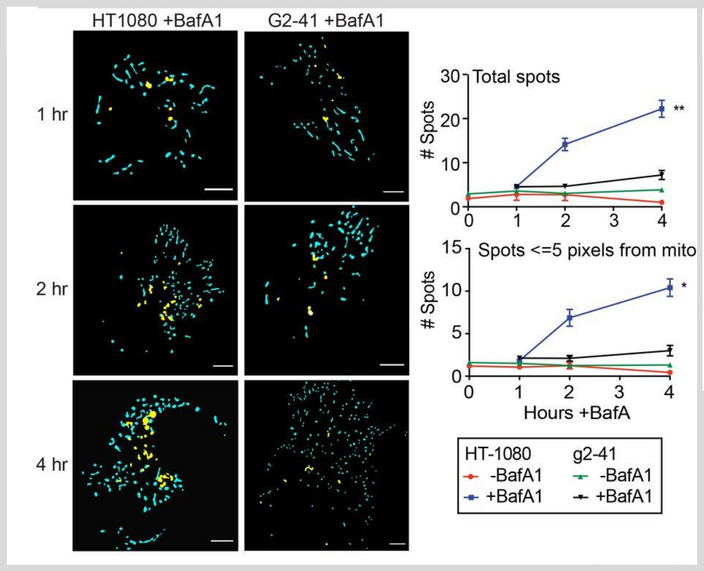
SPECHT algorithm detects autophagosomes and autophagic flux

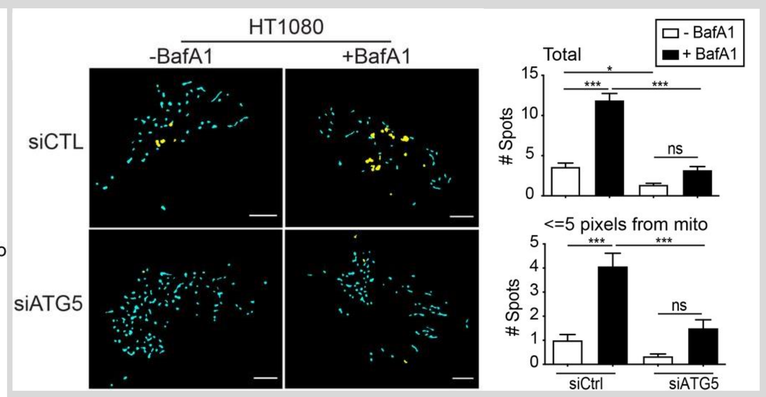




- LC3 B-II is a cytosolic protein which is incorporated into the phagophore during autophagy
- Because GFP is quenched in acidic environments and RFP is not, we can use tandem labelled LC3-GFP-RFP to detect early autophagosomes and autolysosomes
- The cytosolic signal of LC3-GFP-RFP makes segmentation of autophagosomes difficult so we use SPECHT to identify spots
- Treatment with BafA1 prevents lysosome binding and we see an accumulation of early autophagosomes in HT-1080 cells indicating high levels of autophagic flux
- In HT-1080 cells a high proportion of autophagosomes reside near mitochondria

Gp78 KO cells show reduced autophagic flux





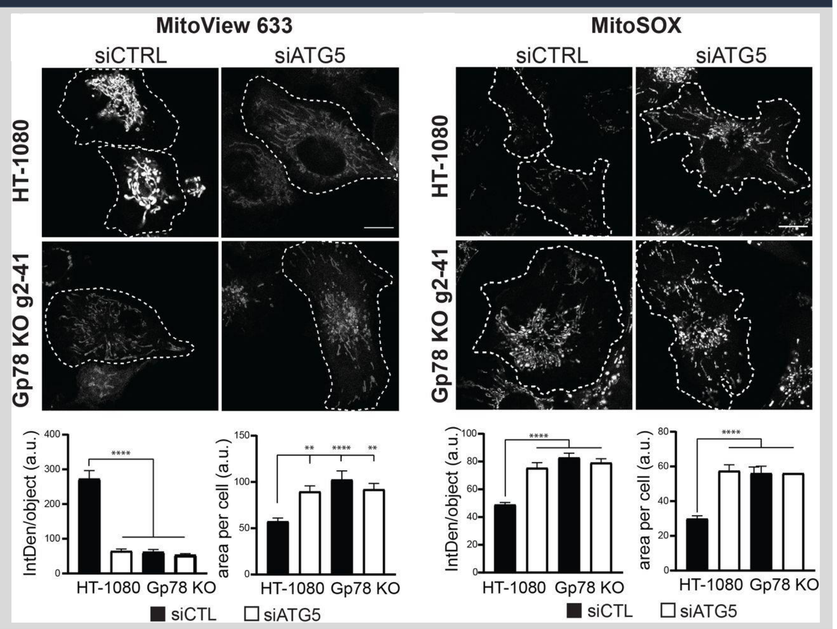
- Gp78 KO do not show an accumulation of early autophagosomes following BafA1 Treatment

- Gp78 KO is phenocopied by the knockdown of essential autophagy protein ATG5

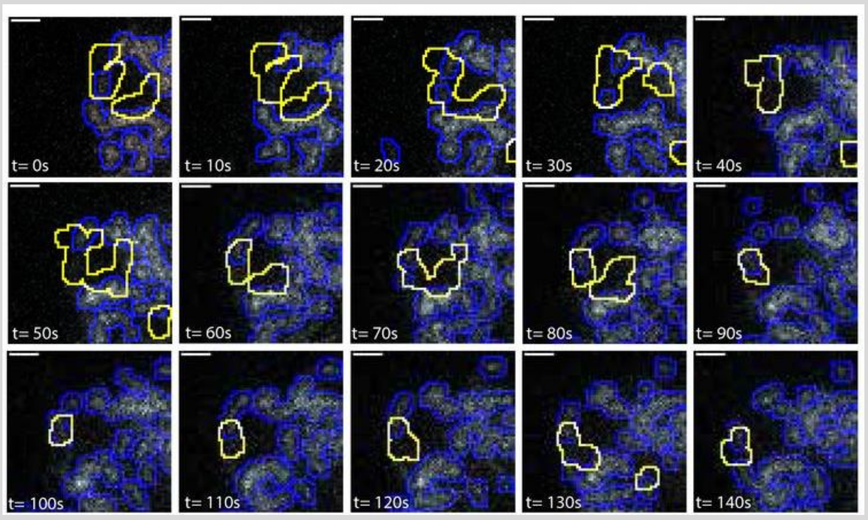
Gp78 mitophagy promotes mitochondrial health

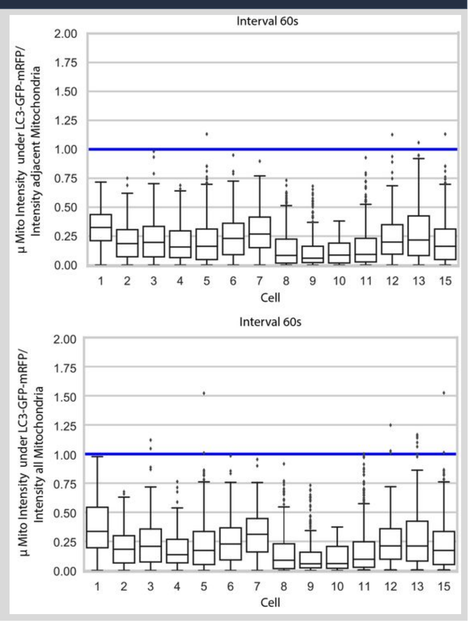
- MitoView633 labels mitochondria in a potential dependent manner
- More labelling indicates more robust proton gradient

- MitoSOX labels mitochondria derived superoxide (ROS)
- High levels of ROS can contribute to mitochondrial damage and ageing



Autophagosomes associate with low potential mitochondria





- Early autophagosomes preferentially associate with regions of mitochondria that show reduced potential

Future Directions

- Identify the mechanisms regulating Gp78 mitophagy
- Identify the mitochondrial substrates of Gp78
- Identify role of Gp78-dependent mitophagy in cancer progression

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