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

P. Alan[1*], K. Vandevoorde[1*], B. Joshi[1*], B. Cardoen[2*], G. Gao[1], Y. Mohammadzadeh[1], G. Hamarneh[2], I. R. Nabi[1];
1 University of British Columbia, VANCOUVER, BC,CANADA,
2 Simon Fraser University, Burnaby, BC, CANADA.
* equal contribution

Mitochondria are major sources of cytotoxic reactive oxygen species (ROS) that contribute to cancer progression. Mitophagy, the selective elimination of mitochondria by autophagy, monitors and maintains mitochondrial health and integrity, eliminating ROS-producing mitochondria. However, mechanisms underlying mitophagic control of mitochondrial homeostasis under basal conditions remain poorly understood. Gp78 E3 ubiquitin ligase is an endoplasmic reticulum membrane protein that induces mitochondrial fission and mitophagy of depolarized mitochondria. Here, we report that CRISPR/Cas9 knockout of Gp78 in HT-1080 fibrosarcoma cells increased mitochondrial volume and rendered cells resistant to carbonyl cyanide m-chlorophenyl hydrazone (CCCP)-induced mitophagy. These effects were phenocopied by knockdown of the essential autophagy protein ATG5 in wild-type HT-1080 cells. Use of the mito-Keima mitophagy probe confirmed that Gp78 promoted both basal and damage-induced mitophagy. Application of a spot detection algorithm (SPECHT) to GFP-mRFP tandem fluorescent-tagged LC3 (tfLC3)-positive autophagosomes reported elevated autophagosomal maturation in wild-type HT-1080 cells relative to Gp78 knockout cells, predominantly in proximity to mitochondria. Mitophagy inhibition by either Gp78 knockout or ATG5 knockdown reduced mitochondrial potential and increased mitochondrial ROS. Live cell analysis of tfLC3 in HT-1080 cells showed the preferential association of autophagosomes with mitochondria of reduced potential. Basal Gp78-dependent mitophagic flux is therefore selectively associated with reduced potential mitochondria promoting maintenance of a healthy mitochondrial population and limiting ROS production.

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Parsa Alan*[1], Kurt R. Vandevoorde[1*], Bharat Joshi[1*], Ben Cardoen[2*], Guang Gao[1], Yahya Mohammadzadeh[1], Ghassan Hamarneh[2], Ivan R. Nabi[1]

[1]Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

[2]School of Computing Science, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

*equal contribution

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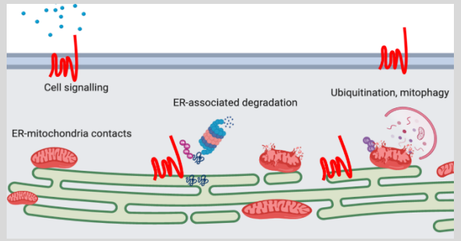
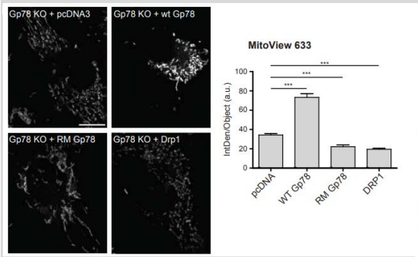
Background

Gp78 Functions

- Major E3 ubiquitin ligase in **ER**-**a**ssociated **d**egradation (ERAD)
- Regulates rough **m**itochondria-**ER** **c**onnect**s** (MERCs)
- Acts as a receptor and stimulates cell motility at the plasma membrane
- Regulates Parkin-independent **mitophagy**

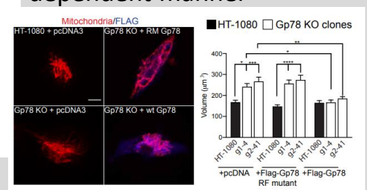


Mitochondrial fission fails to rescue mito potential

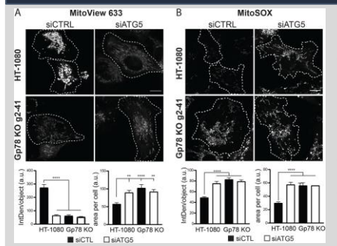


DRP1 does not restore potential

Gp78 rescues volume phenotype in a RING-domain dependent manner



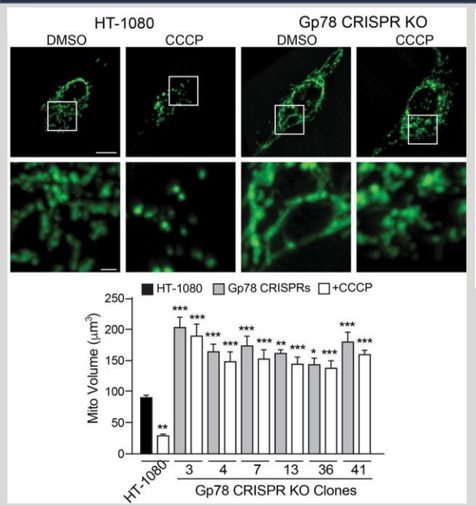
Gp78 basal mitophagy promotes mitochondrial health and limits ROS

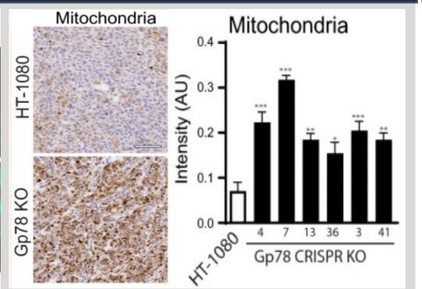


Upon loss of Gp78…

- Mitochondrial membrane potential-dependent dye MitoView 633 shows reduced signal
- Mito ROS dye shows increased signal
- Both phenocopied by loss of an essential autophagy gene (ATG5)

Gp78 knockout (KO) cells have increased mitochondrial abundance

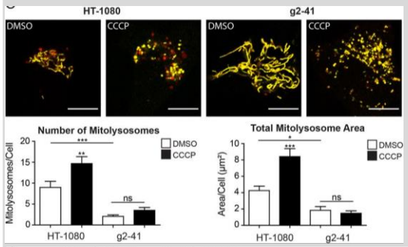




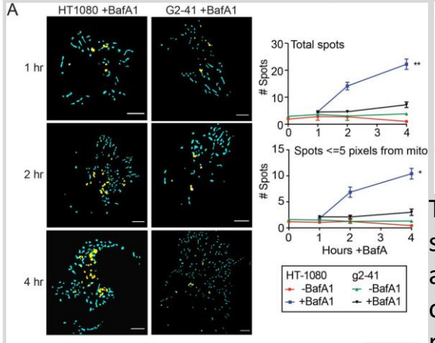
- Tumor sections from mice with xenograft treatment of Gp78 KO clones exhibit increased mitochondrial staining compared to wildtype HT-1080

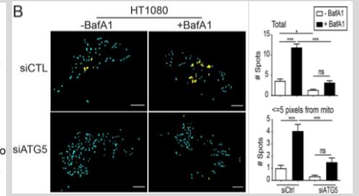
- Gp78 CRISPR/Cas9 clones exhibit increased mitochondrial volume and resistance to damage-induced mitophagy

Loss of Gp78 abrupts autophagic flux and basal mitophagy



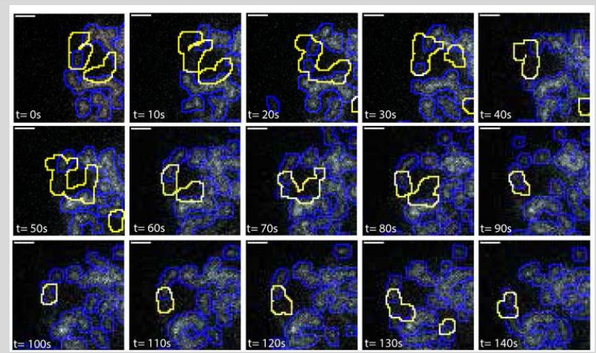
- Bi-flourescent mitophagy probe Mito-Keima shows defunct basal mitophagy and damage-induced mitophagy upon loss of Gp78

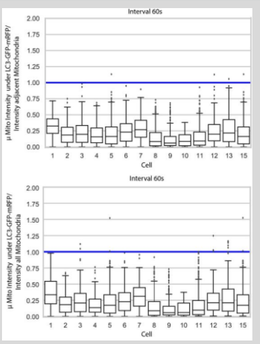




Time-course experiment showing Gp78-dependent autophagic and mitophagic flux of LC3 and LC3 association to mitochondria

Autophagosomes preferentially associate with low potential mitochondria





- Autophagosomes preferentially associate with regions of mitochondria that show reduced potential

Autophagosomes/**MitoView 633**

Future Directions

- Identify the mitochondrial substrates of Gp78
- Mito-Keima TG mouse to determine tissue-specific Gp78-dependent basal mitophagy

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Correspondence: Ivan R. Nabi
Email: irnabi@mail.ubc.ca