Análisis de la actividad mitocondrial y su uso en el desarrollo de compuestos bioactivos (Plataforma MTox)

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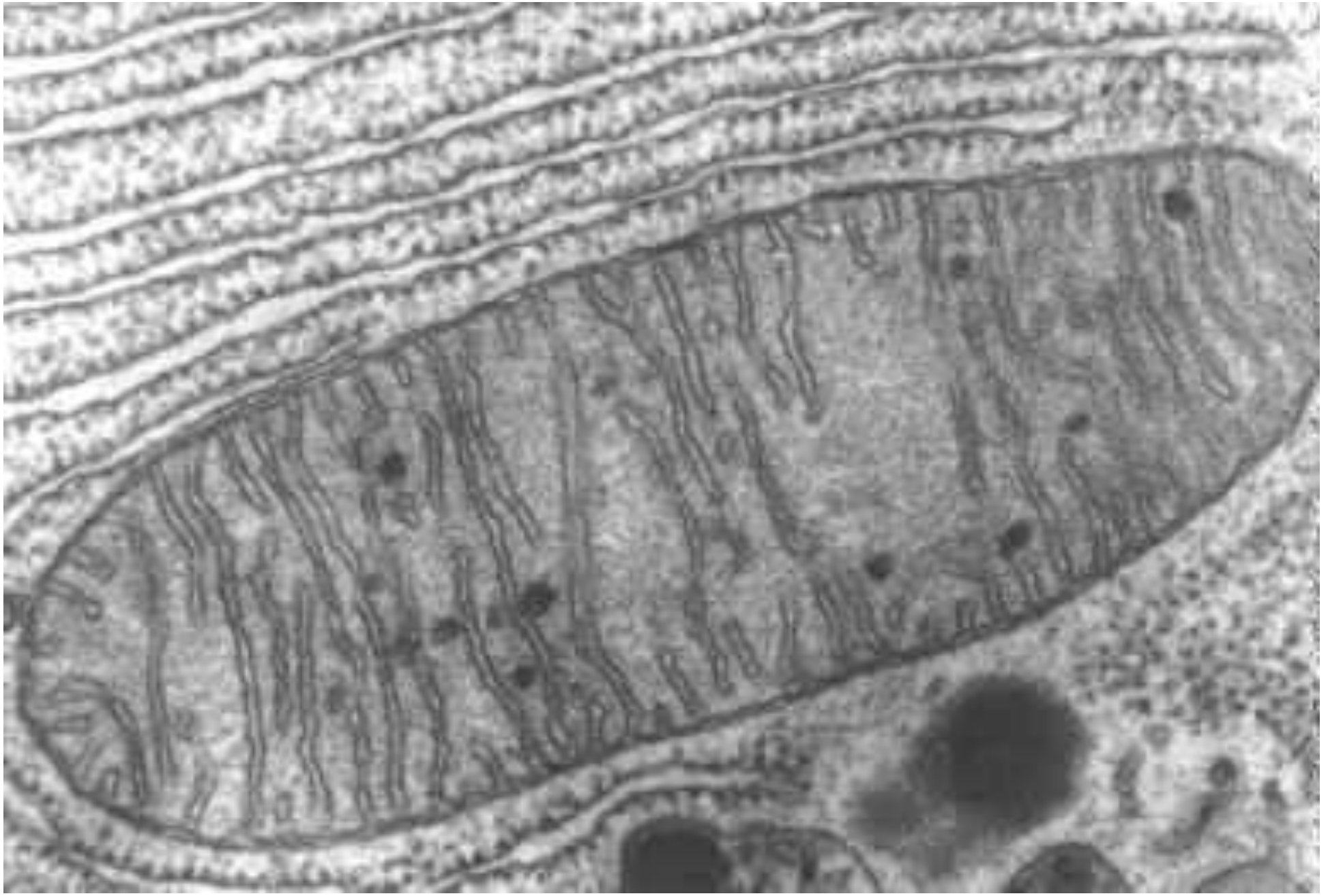
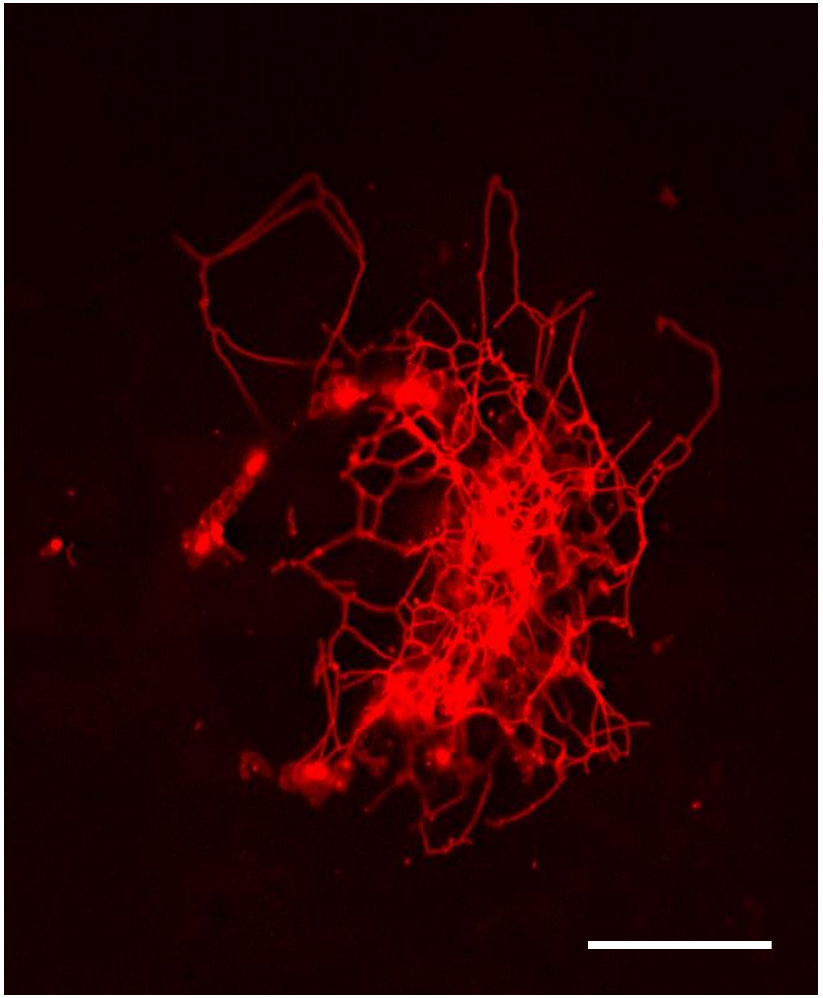
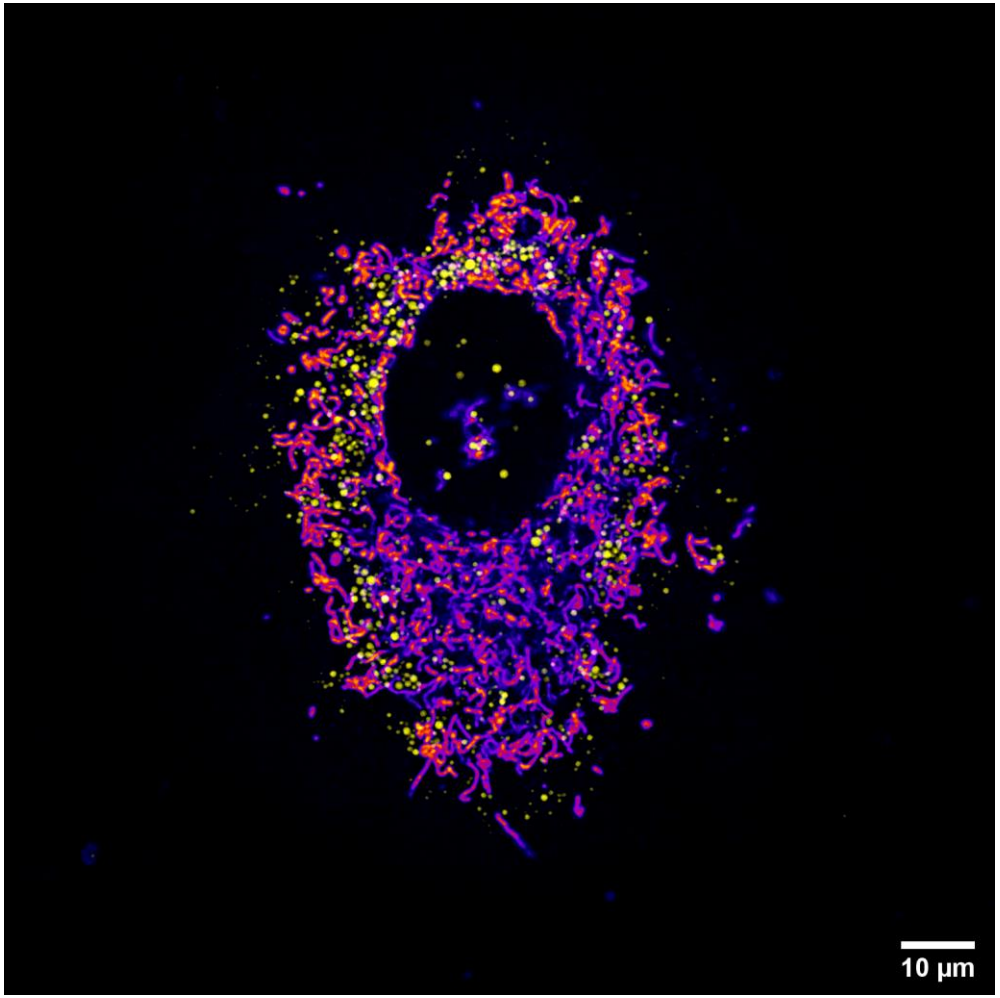
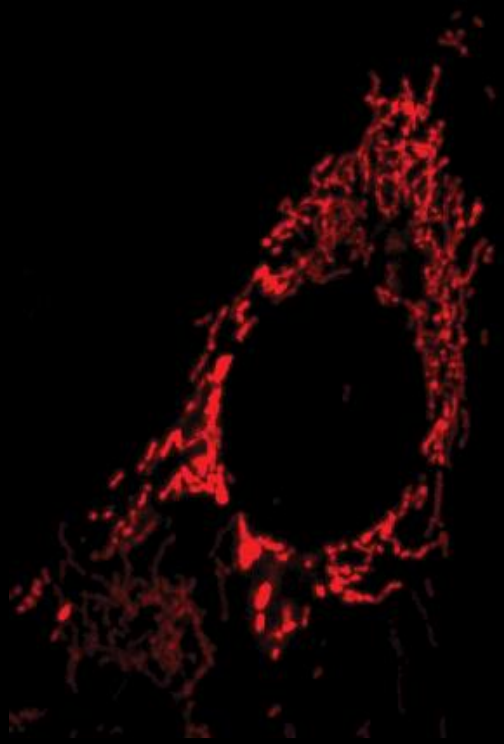
Cosmetorium, 23-24 Octubre, 2019



Mitochondria



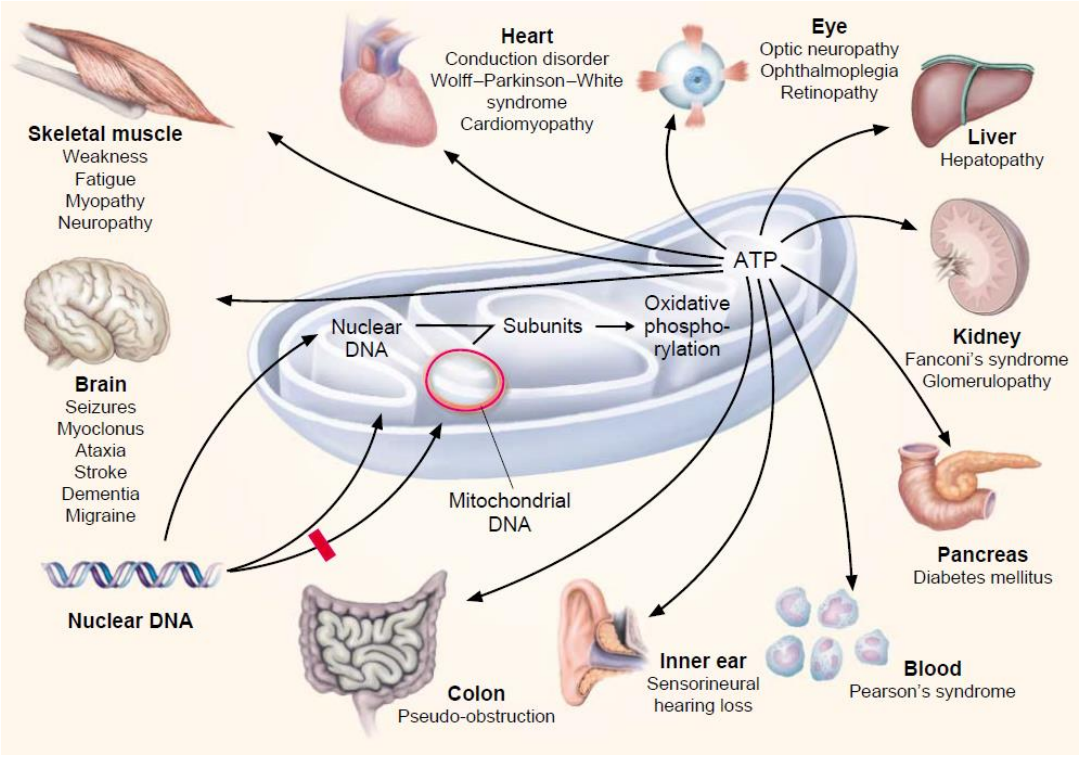


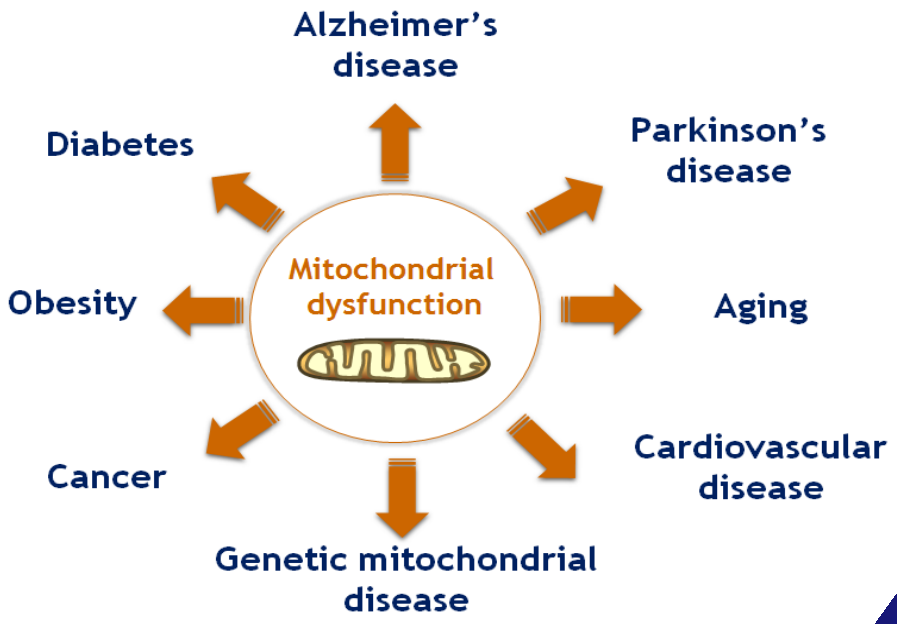


Mitochondria: essential organelles in human health and disease

- major energy generator
- cell signalling
- interorganellar communication
- apoptosis
- interconversion of carbohydrates, lipids and amino acids
- haem biosynthesis
- iron-sulphur cluster biogenesis
- innate immunity

Alterations in mitochondrial proteins have an impact in critical functions in cells and tissues leading to disease





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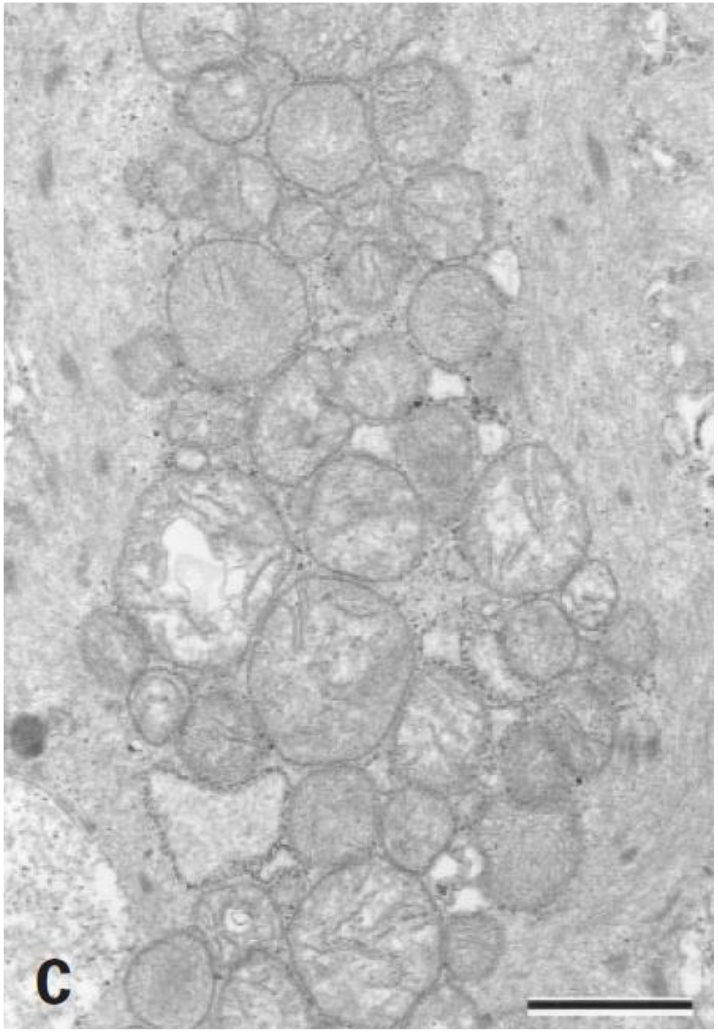
Mitochondrial diseases show skin involvement

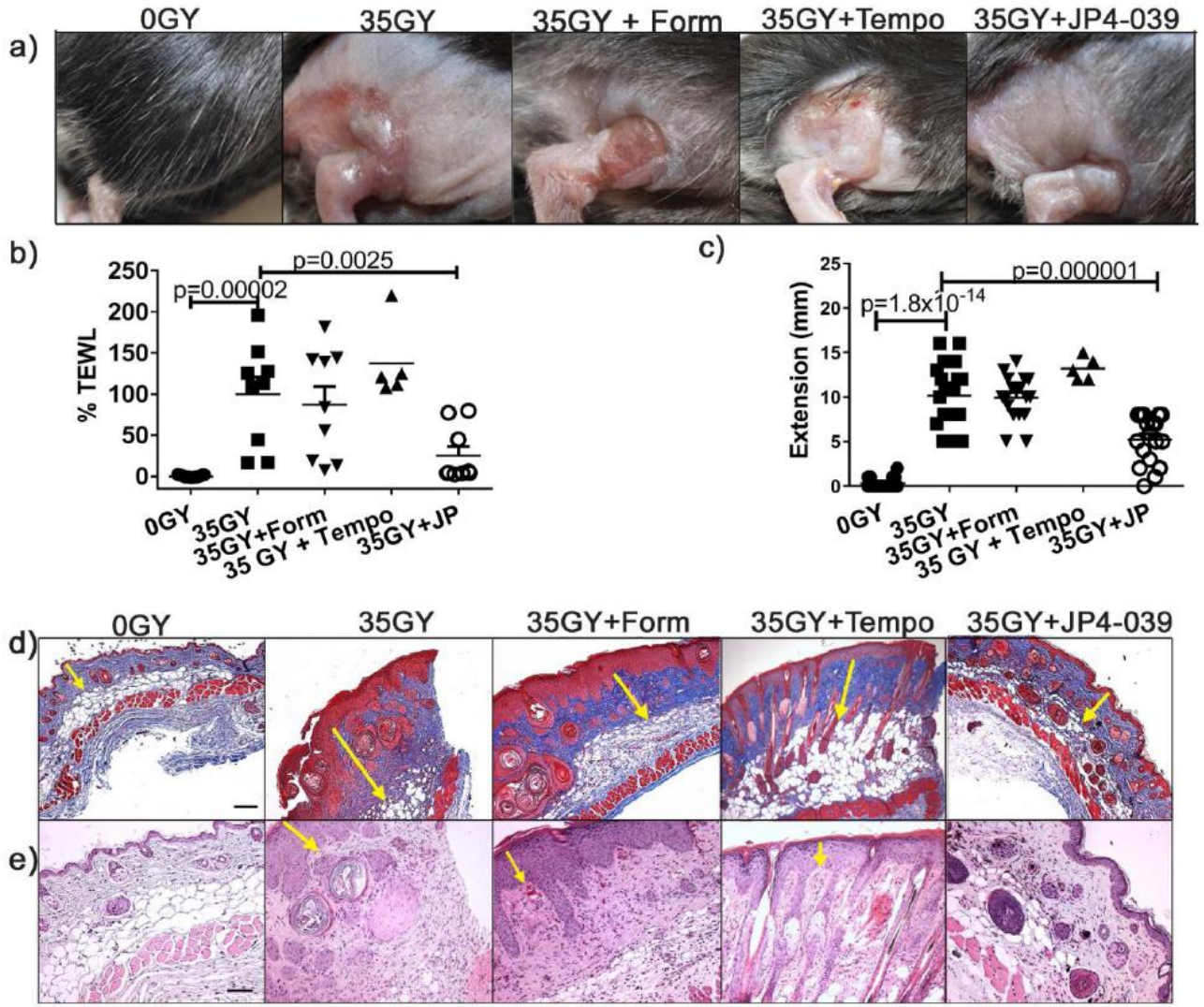


10% of patients with primary mitochondrial disorders present skin manifestations (hair abnormalities, rashes, pigmentation abnormalities, and acrocianosis).

Skin alterations have been reported in patients with Leigh síndrome, Pearson síndrome, or MELAS síndrome.

(Kubota et al., J. Am. Acad. Dermatol. 1999)

Mitochondria-targeted antioxidant prevents skin damage induced by oxidative stress



Topical JP4-039 mitigates skin damage from radiation induced oxidative stress

(Brand et al., J. Invest. Dermatol. 2017)

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MTox: Platform of Mitochondrial Pharmacology and Toxicology

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In vitro efficacy and early identification of mitochondrial toxicity to improve your Drug or Compound Discovery process

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Key Facts about mitochondrial toxicity

- ✓ Many drugs have received black box warnings due to mitochondrial impairment.
- ✓ Over 80% of the drugs pulled from the market or given black box warnings were flagged due to hepatotoxicity or cardiotoxicity complications resulting from mitochondrial toxicity.
- ✓ A retrospective analysis of more than 500 pharmaceutically relevant molecules indicates that about 35% directly and acutely impair mitochondrial function.

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Examples of Drugs that have been withdrawn or early replaced since 2000 due to effects related with mitochondrial toxicity

Cerivastatin (Bayer) - Cholesterol
market withdraw 4 years after launch due to rhabdomyolysis

Troglitazone (Pfizer) - Antidiabetic
market withdraw 3 years after launch due to hepatoxicity

Rofecoxib (Merk) - NSAID
market withdraw 5 years after launch due to cardiovascular risk

Sibutramine (Abbott) - Anorexigen
Market withdraw in several countries in 2010 due to cardiovascular risk

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Mitochondria and drug discovery

- ✓ Precise analysis of mitochondrial function facilitates the identification of the MoA of compounds designed for Mitochondrial Medicine Programs.
- ✓ The first opportunity to prevent mitochondrial toxicity arises in the early stages of drug development.
- ✓ Development of specific drugs (antivirals or antibacterial compounds) requires the monitoring of mitochondrial function.
- ✓ Monitoring of compounds for mitochondrial toxicity allows appropriate selection in drug screening programs.

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(Dykens J.A. et al., Expert Rev Mol Diagn. 2007)
(FDA Guidance for Industry 2004 and 2006)

We can help you to select compounds of interest

- ✓ Early evaluation of the toxicity or activity of your compounds at the mitochondrial level
- ✓ Identification/characterization of MoA of new compounds
- ✓ We can advise and help the client on experimental design
- ✓ Top level/internationally recognized expertise in mitochondria function/dysfunction

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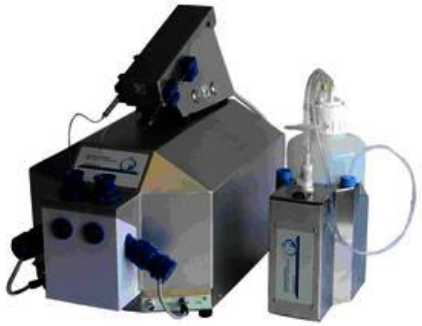
In vitro/in vivo analysis of mitochondrial function/dysfunction



- ✓ **Analysis of mitochondrial energy metabolism**
  - Mitochondrial respiration
  - Mitochondrial membrane potential
  - ATP production
- ✓ **Radical oxygen species (ROS) production**
  - ROS production
  - Antioxidant defense
- ✓ **Mitochondrial homeostasis and cell death**
  - Mitochondrial morphology
  - Mitochondrial biogenesis
  - Mitophagy
  - Analysis of apoptotic cell death

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A glance on techniques and assays available at MTox Platform

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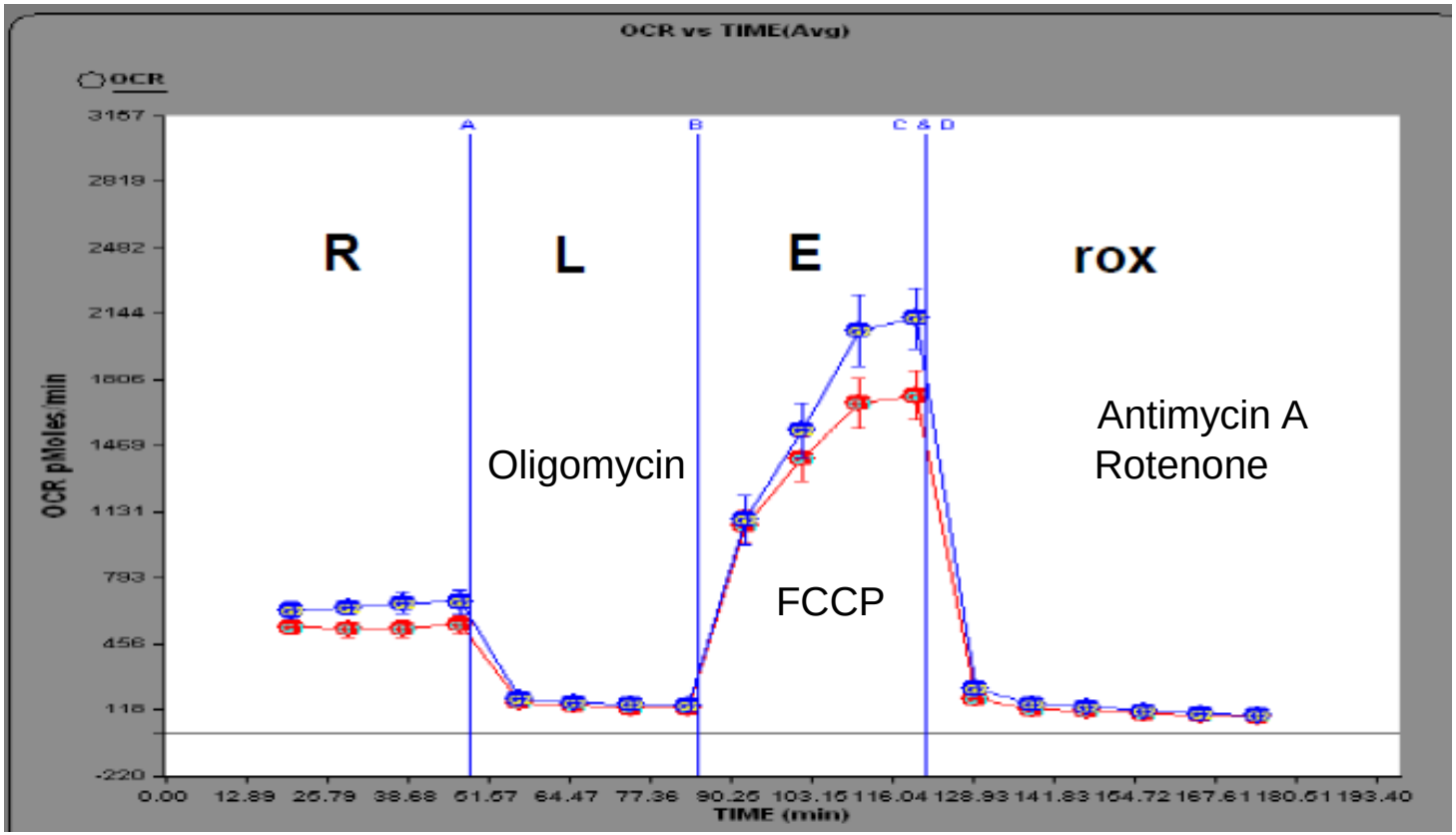
Mitochondrial Respiration

Analysis of mitochondrial respiration in living culture cells allows to identify conditions/compounds that cause mitochondrial dysfunction



Analysis of major mitochondrial respiratory parameters (basal and maximal respiration, ATP-coupled respiration, proton leak) by using validated inhibitors

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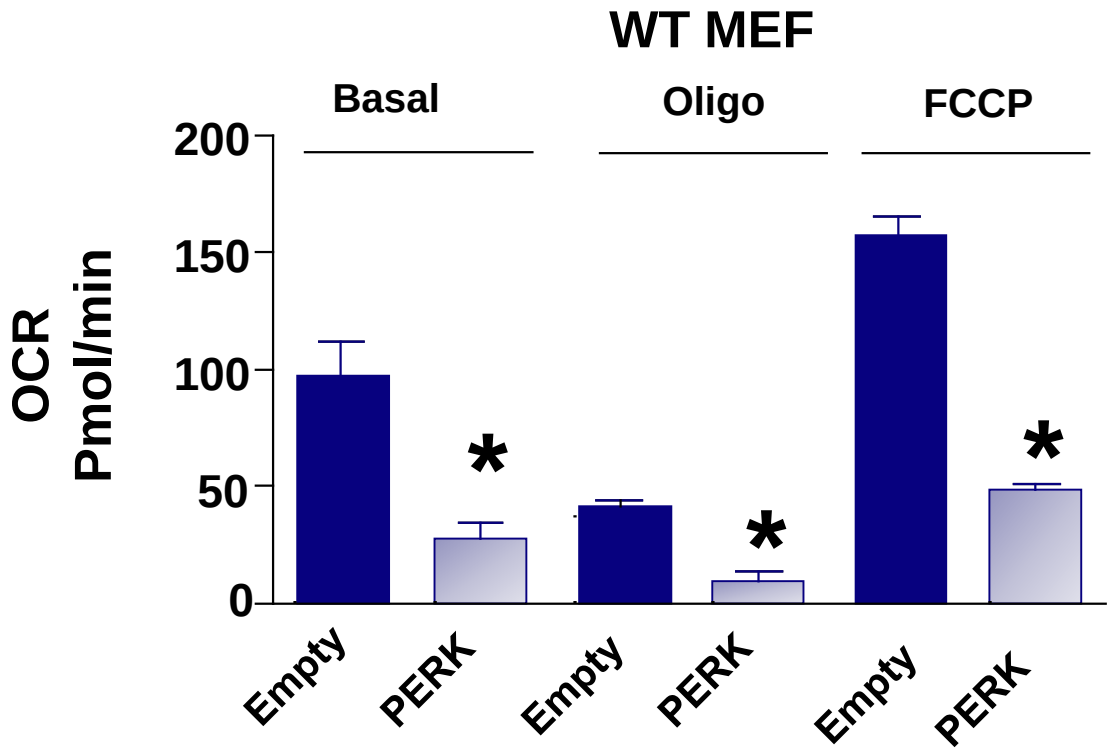
Mitochondrial Respiration

Analysis of mitochondrial respiration in cultured cells allows to identify compounds or proteins that upregulate mitochondrial function or that induce mitochondrial dysfunction



The protein kinase PERK reduces basal and maximal mitochondrial respiration as well as proton leak in MEF cells

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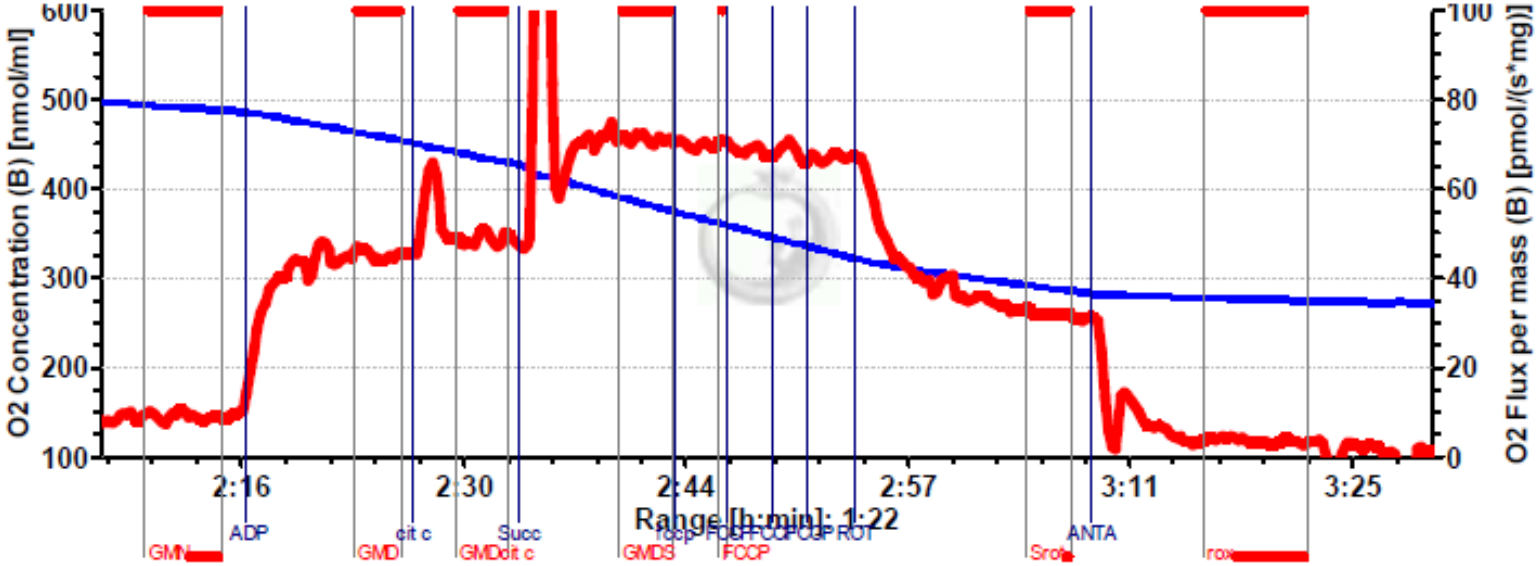
Mitochondrial Respiration

Analysis of mitochondrial respiration in tissue extracts or in permeabilized tissues allows to identify conditions/compounds that cause mitochondrial dysfunction

Respiration in permeabilized skeletal muscle



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Mitochondrial respiration is optimally measured in permeabilized skeletal muscles by using the Oroboros platform.

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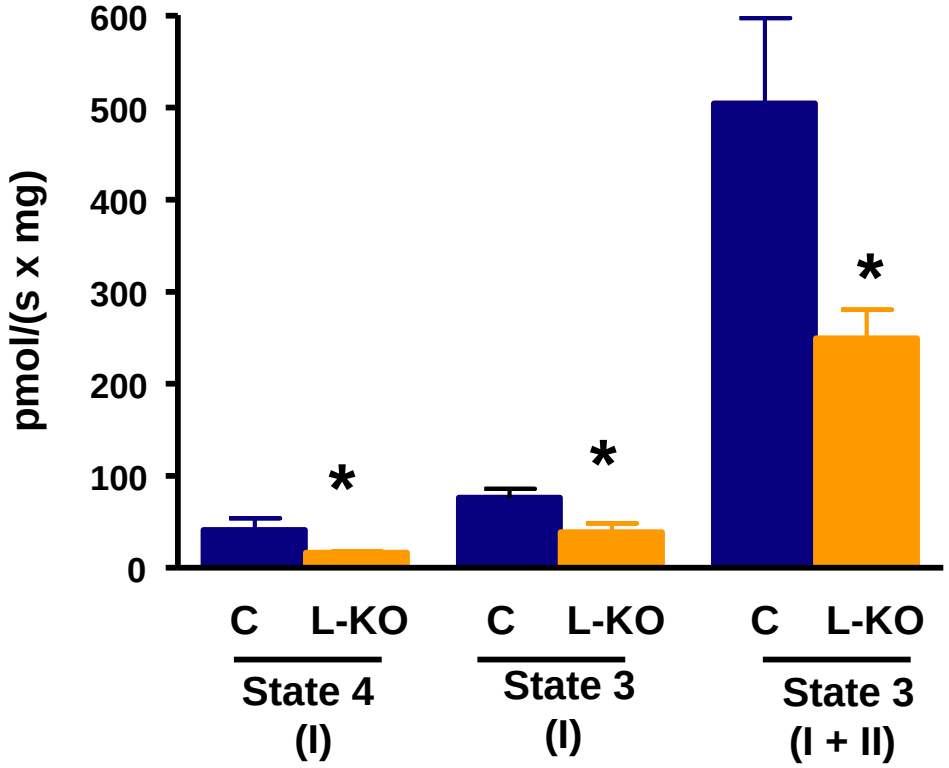
Mitochondrial Respiration

Deficiency of certain mitochondrial proteins (such as Mitofusin-2) causes reduced hepatic mitochondrial respiration



Mitofusin 2 is a major regulator of mitochondrial respiration in mouse liver. This is an excellent model to search for compounds that rescue a healthy mitochondrial metabolism.

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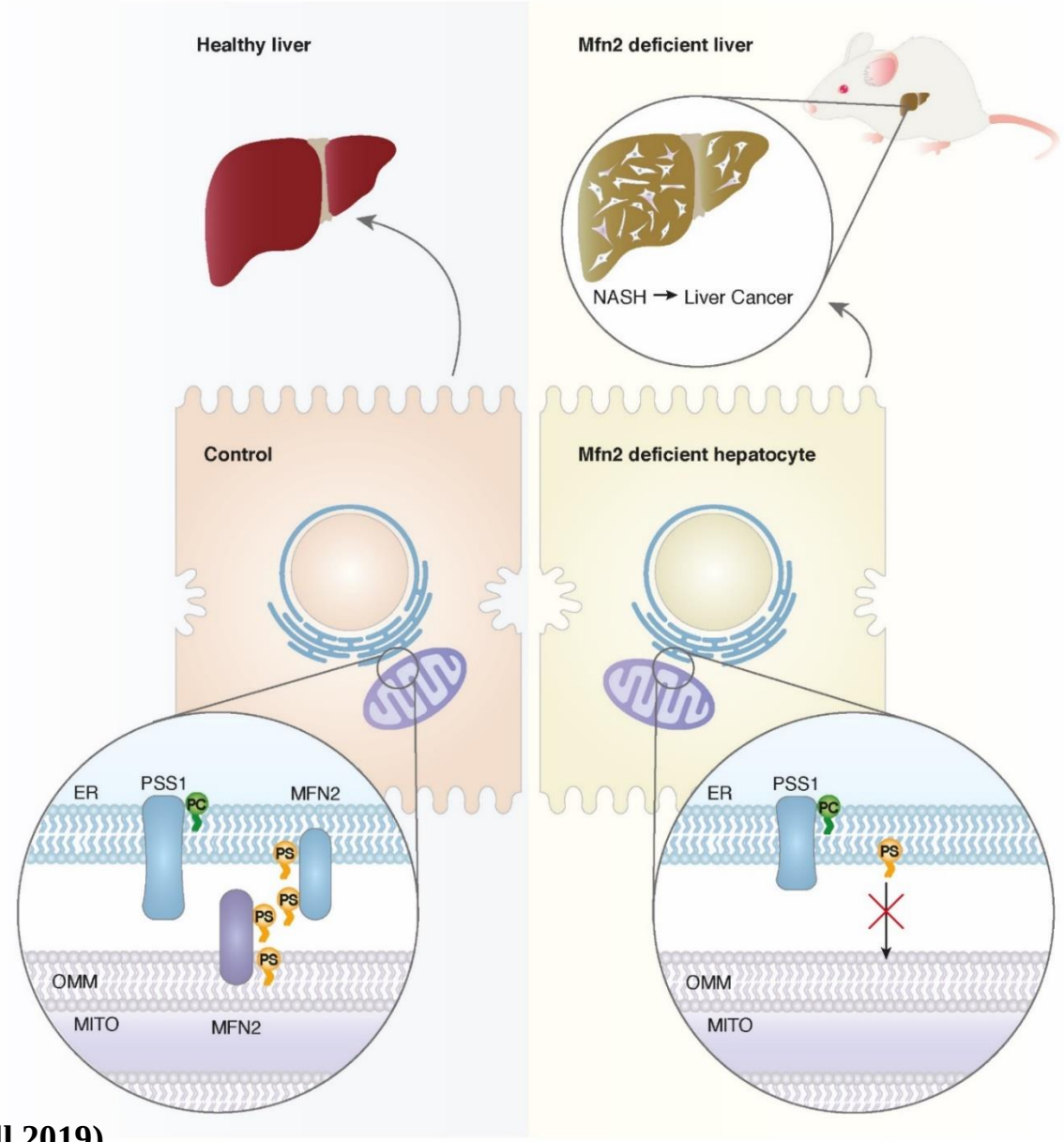
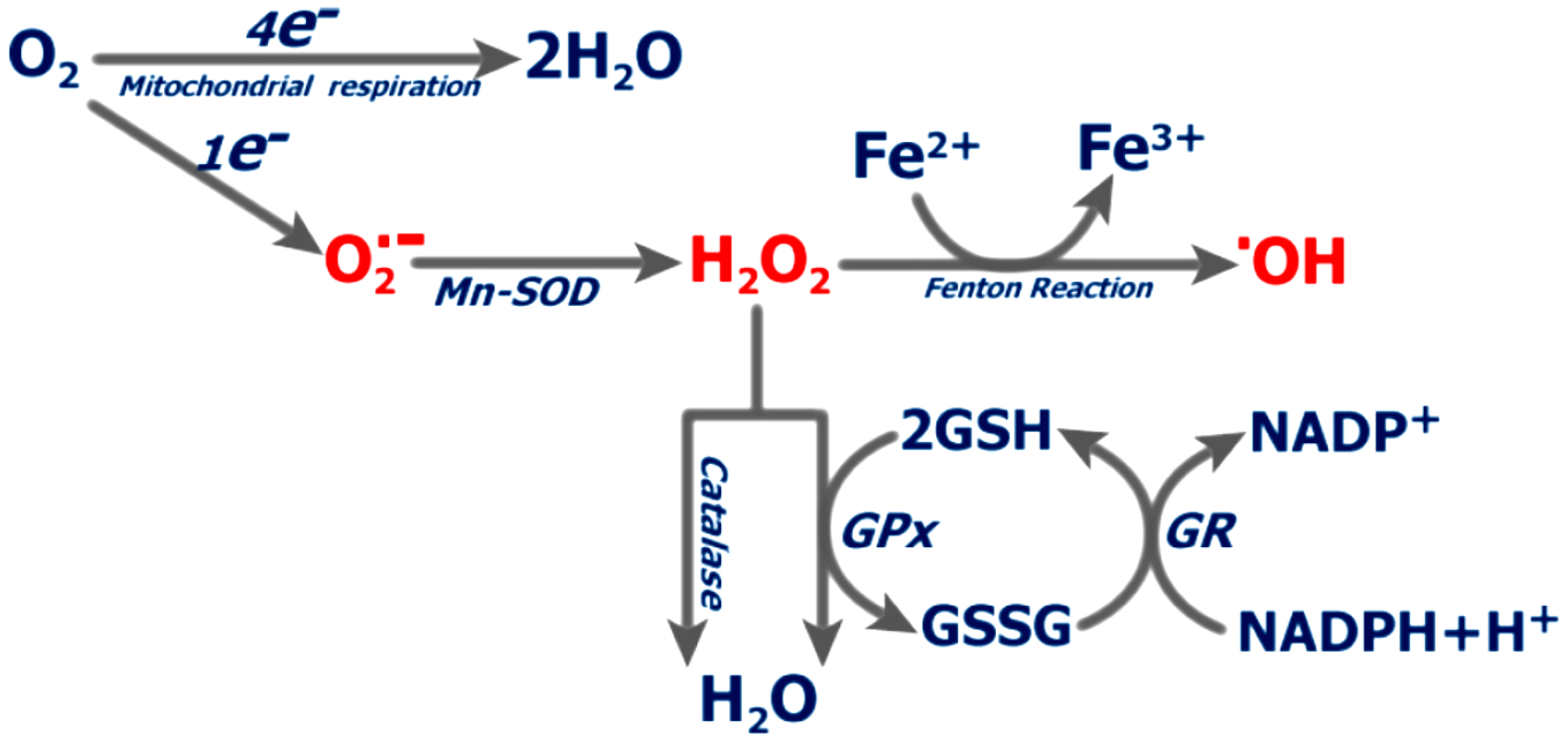
Mfn2 binds and transfers phosphatidylserine across mitochondria-ER contacts, and perturbation of this process leads to aberrant lipid metabolism and liver diseases



(Hernandez-Alvarez et al., Cell 2019)

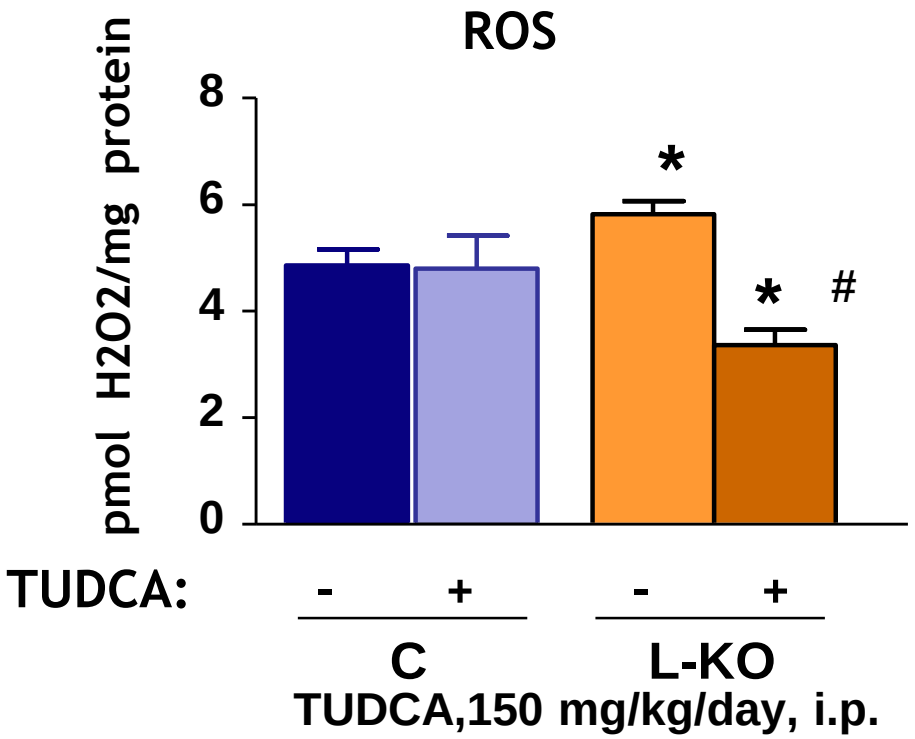
Reactive oxygen species (ROS) production



(Valle et al., Cancers 2010)

Reactive oxygen species (ROS) production

Chronic treatment with the compound TUDCA (tauroursodeoxycholic acid) reduces ROS production in Mfn2-deficient livers



TUDCA rescues a normal ROS production in Mfn2-deficient mouse livers.

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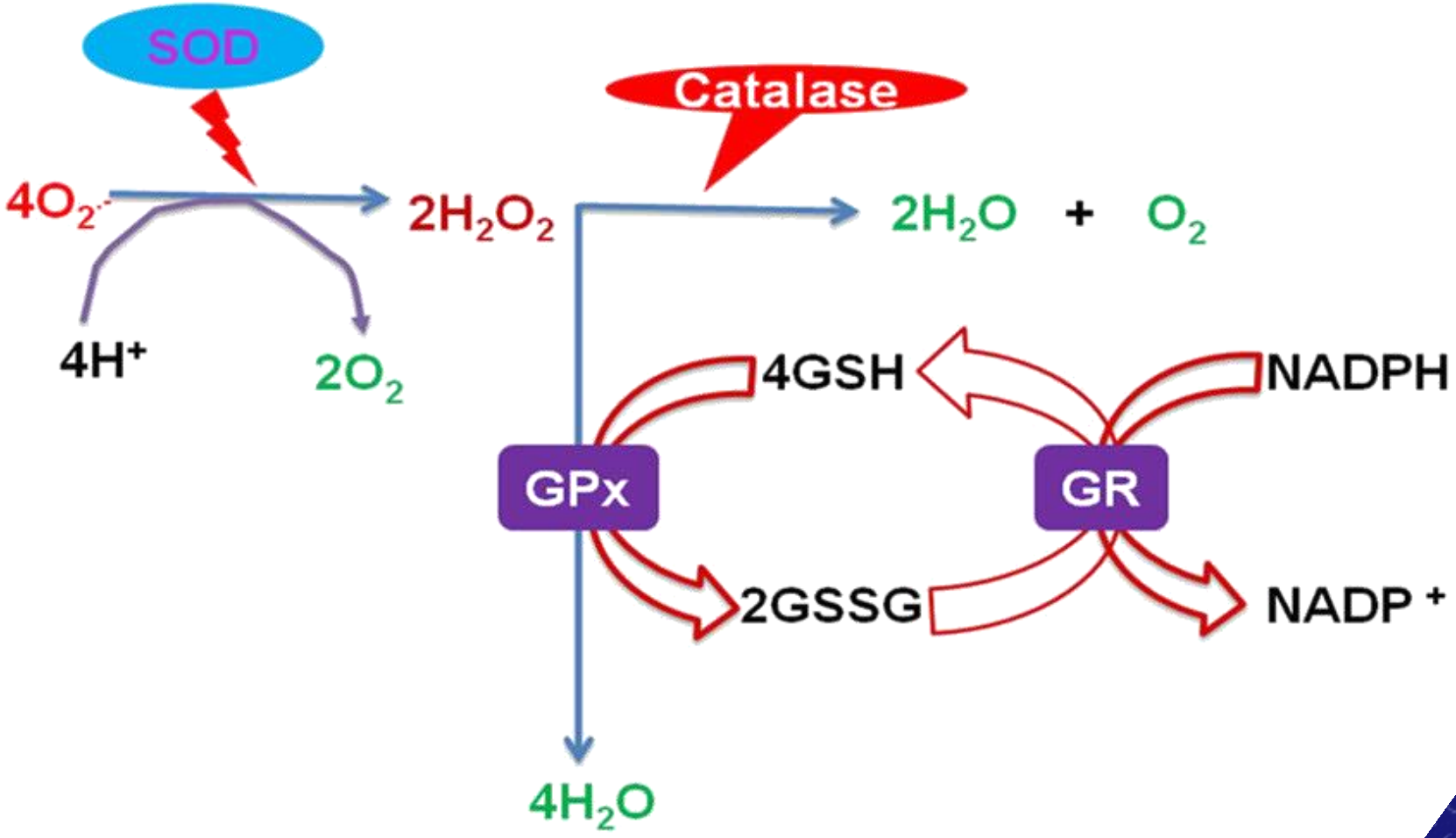
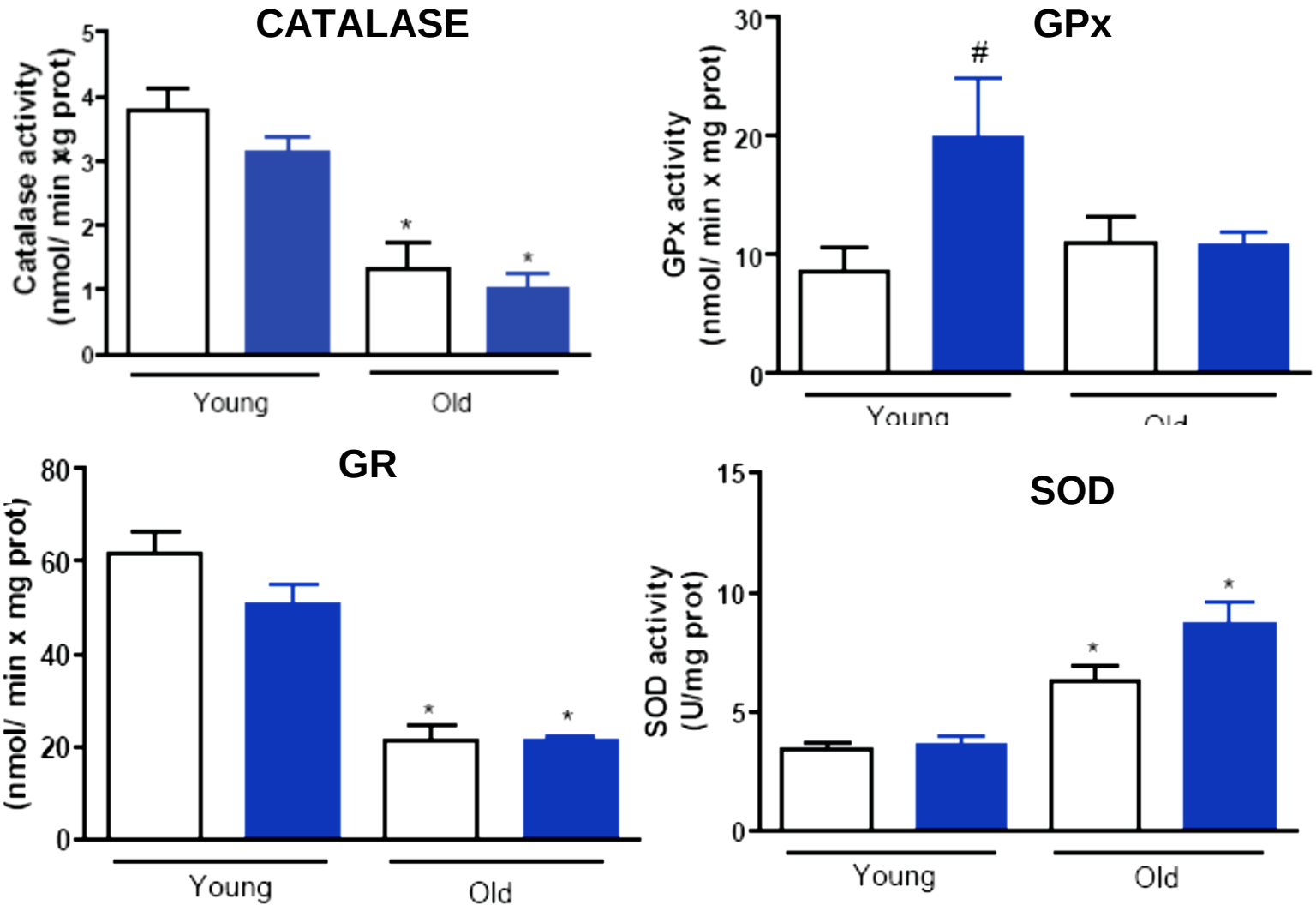
Cellular antioxidant defense



Cellular antioxidant defense



Enzymes that participate in the antioxidant defense are reduced in muscle during aging.

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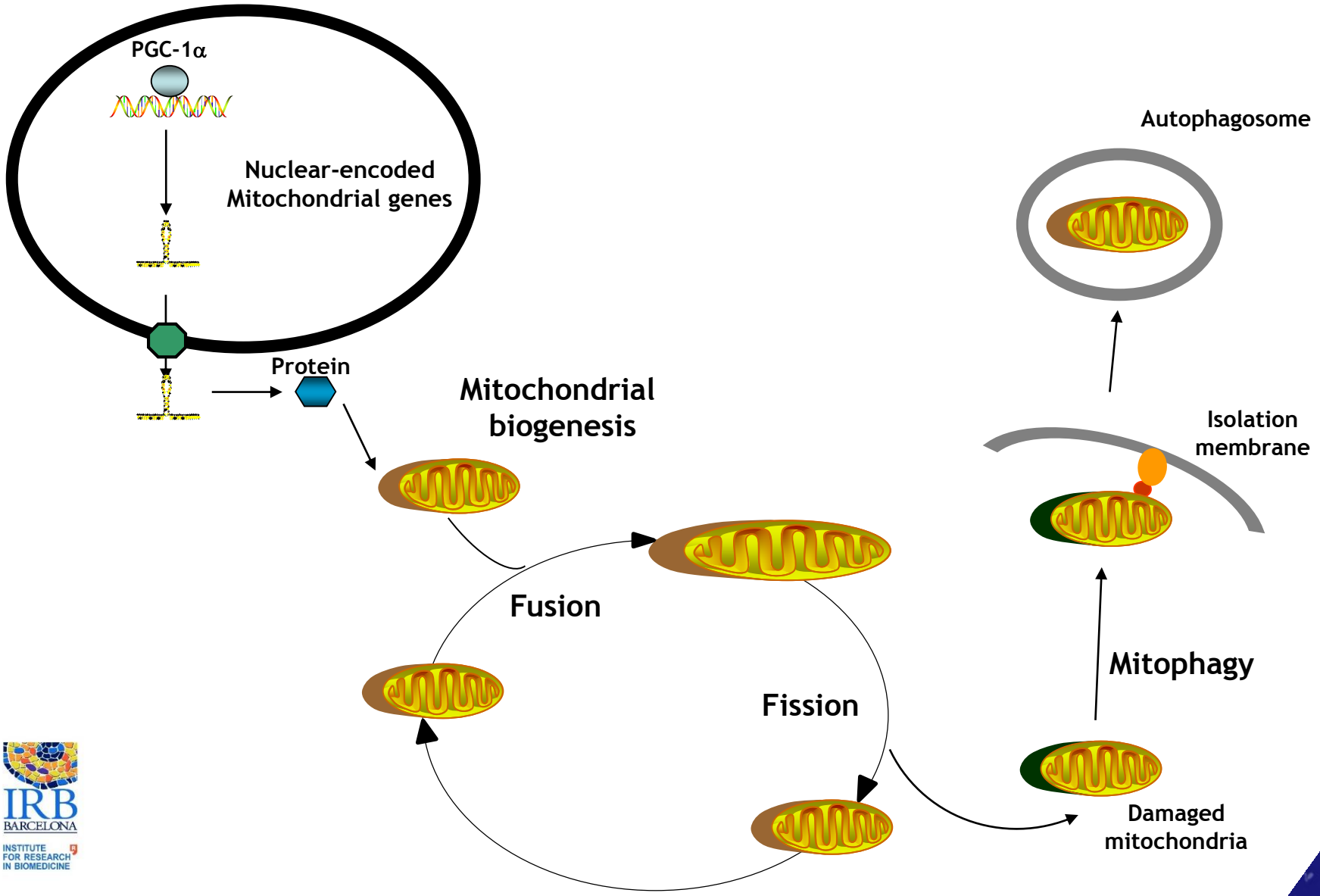
Mitochondrial homeostasis: mitochondrial biogenesis, dynamics and autophagy



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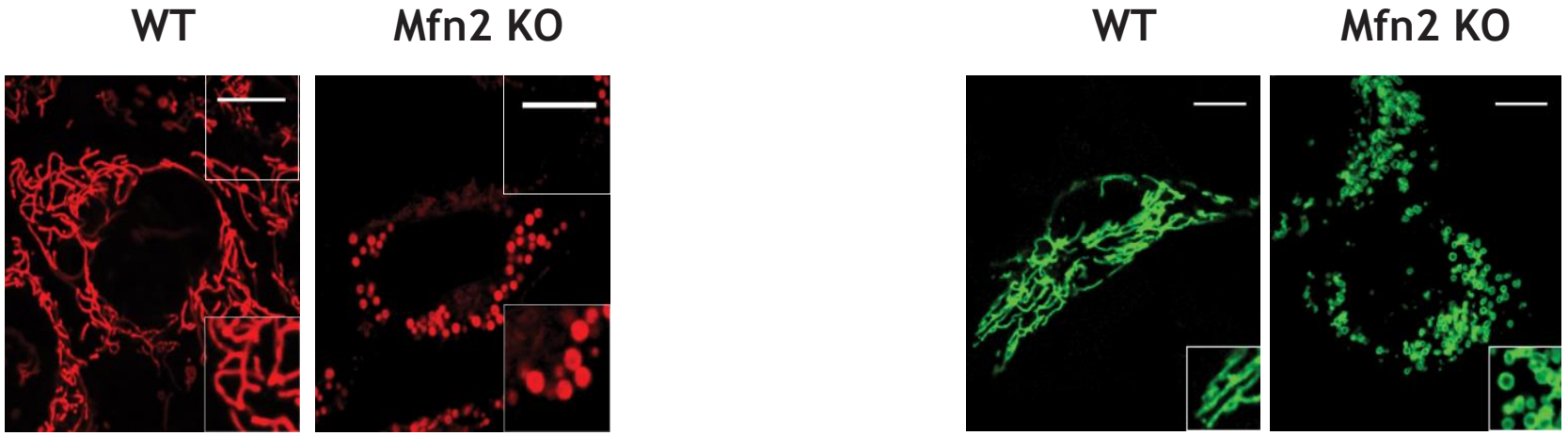
Mitochondrial morphology

Different compounds or proteins disrupt mitochondrial morphology in cultured cells



Different cell models are available to ameliorate altered mitochondrial morphology.

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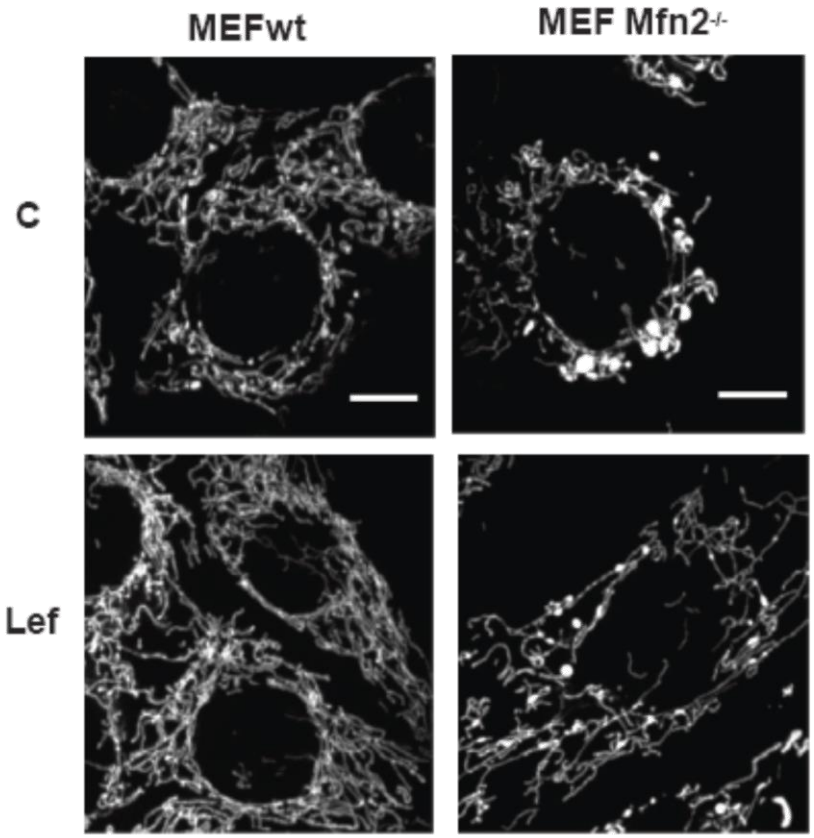
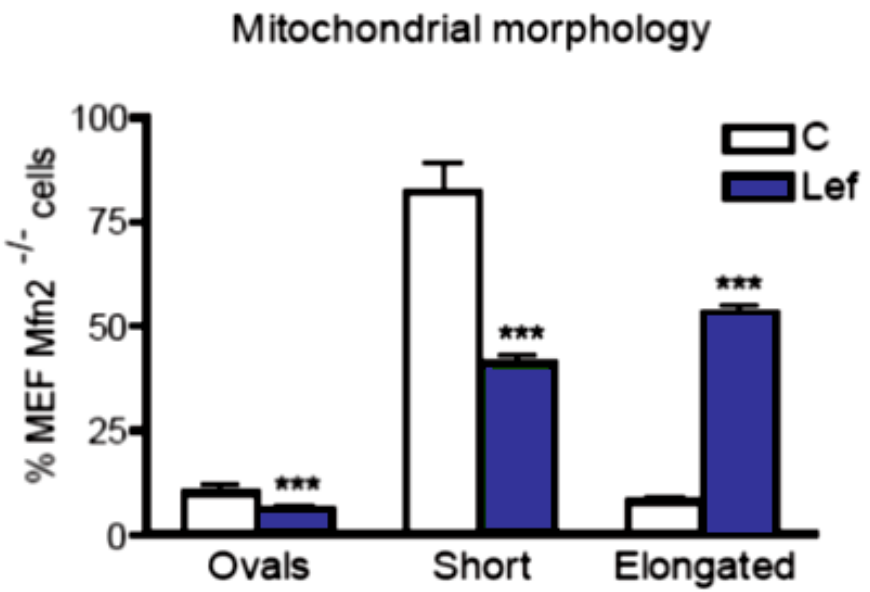
Mitochondrial morphology

Leflunomide induces mitochondrial elongation in MEF cells





Leflunomide, an inhibitor of dihydroorotate dehydrogenase, ameliorates mitochondrial morphology under conditions of lack of Mitofusin 2

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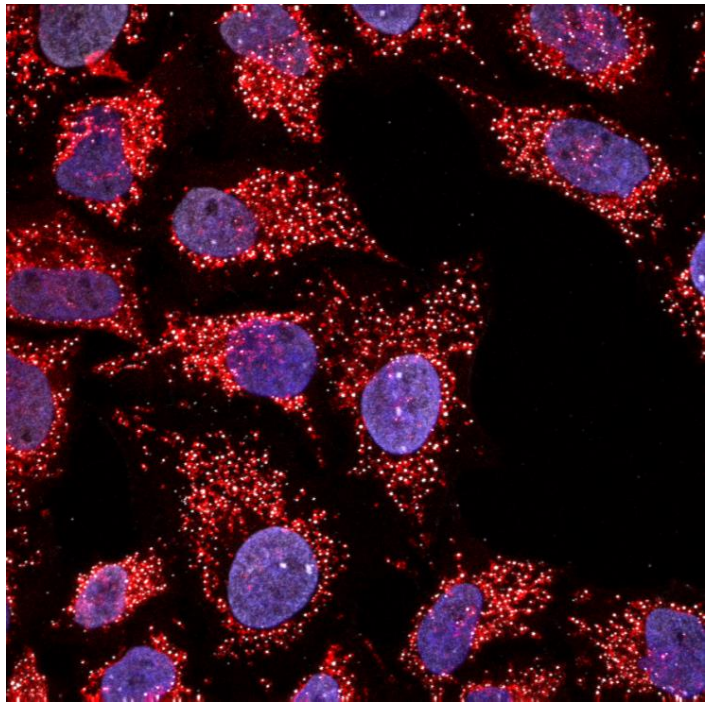
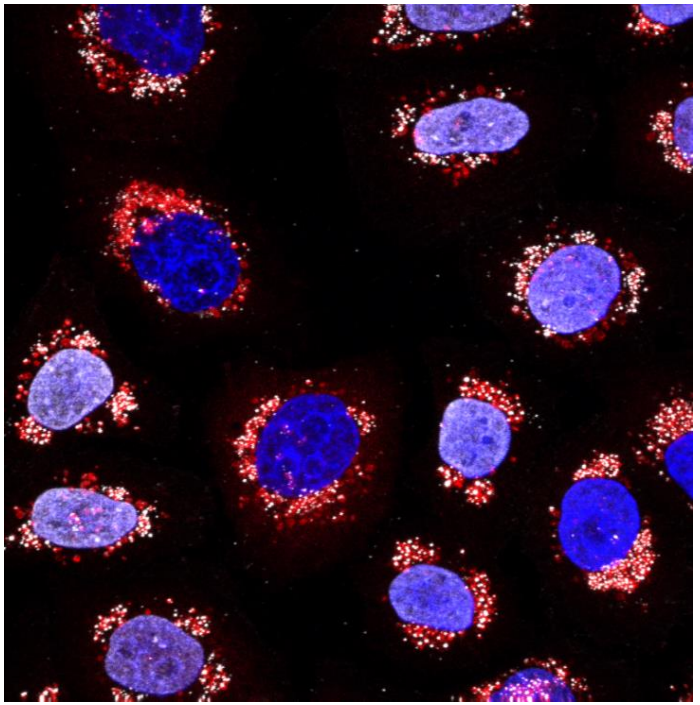
Mitophagy

The uncoupler compound CCCP triggers mitophagy in HeLa cells

Control



CCCP treatment



Mitophagy can be monitored by following the clustering and further disappearance of mitochondrial nucleoids.

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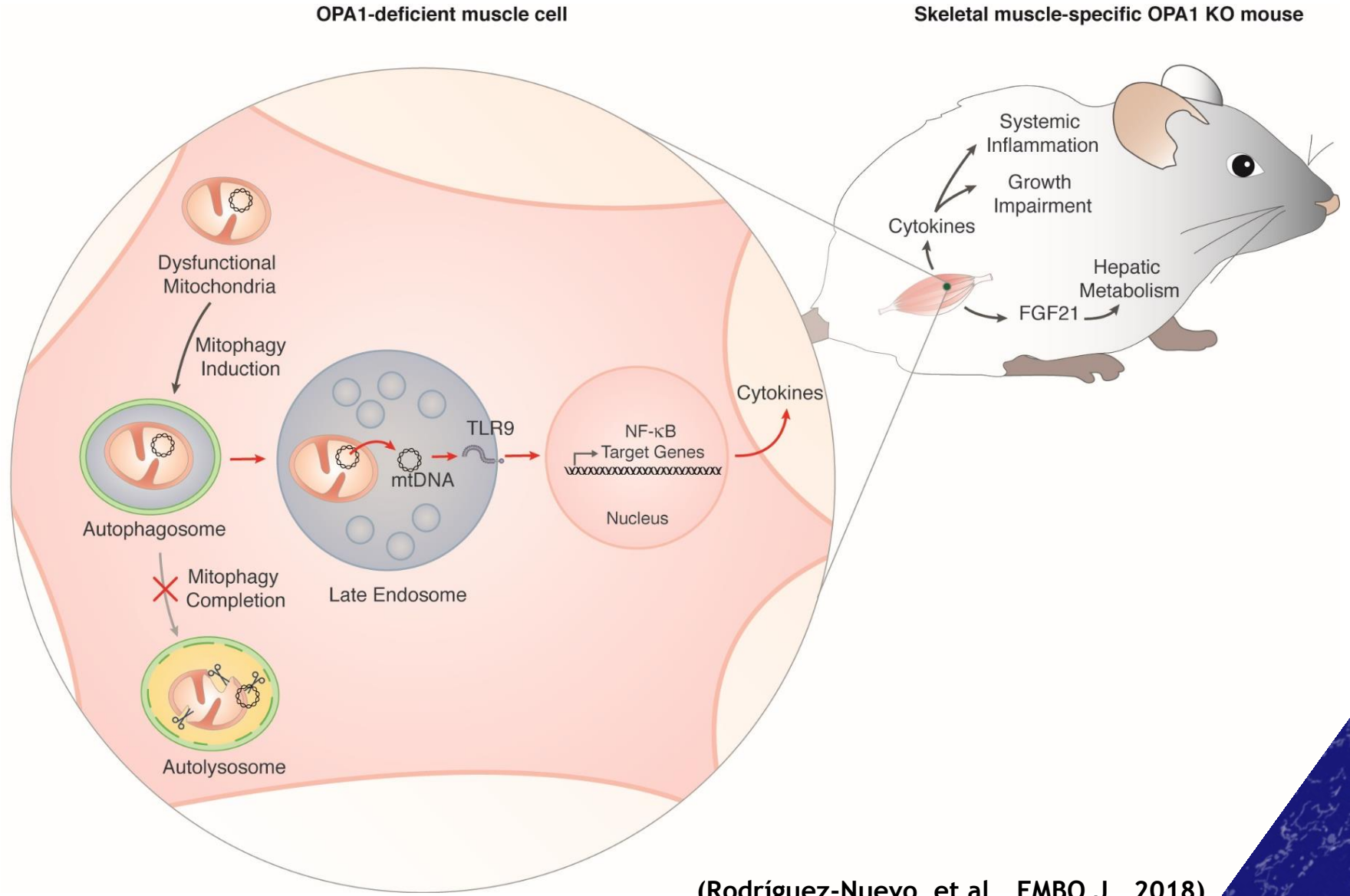
Mitochondrial DNA and TLR9 dive muscle inflammation upon Opa1 deficiency



(Rodríguez-Nuevo et al., EMBO J. 2018)

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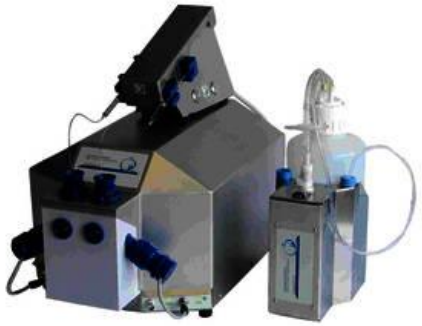
In vitro/in vivo analysis of mitochondrial function/dysfunction



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  - Mitophagy
  - Analysis of apoptotic cell death

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Gracias!

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