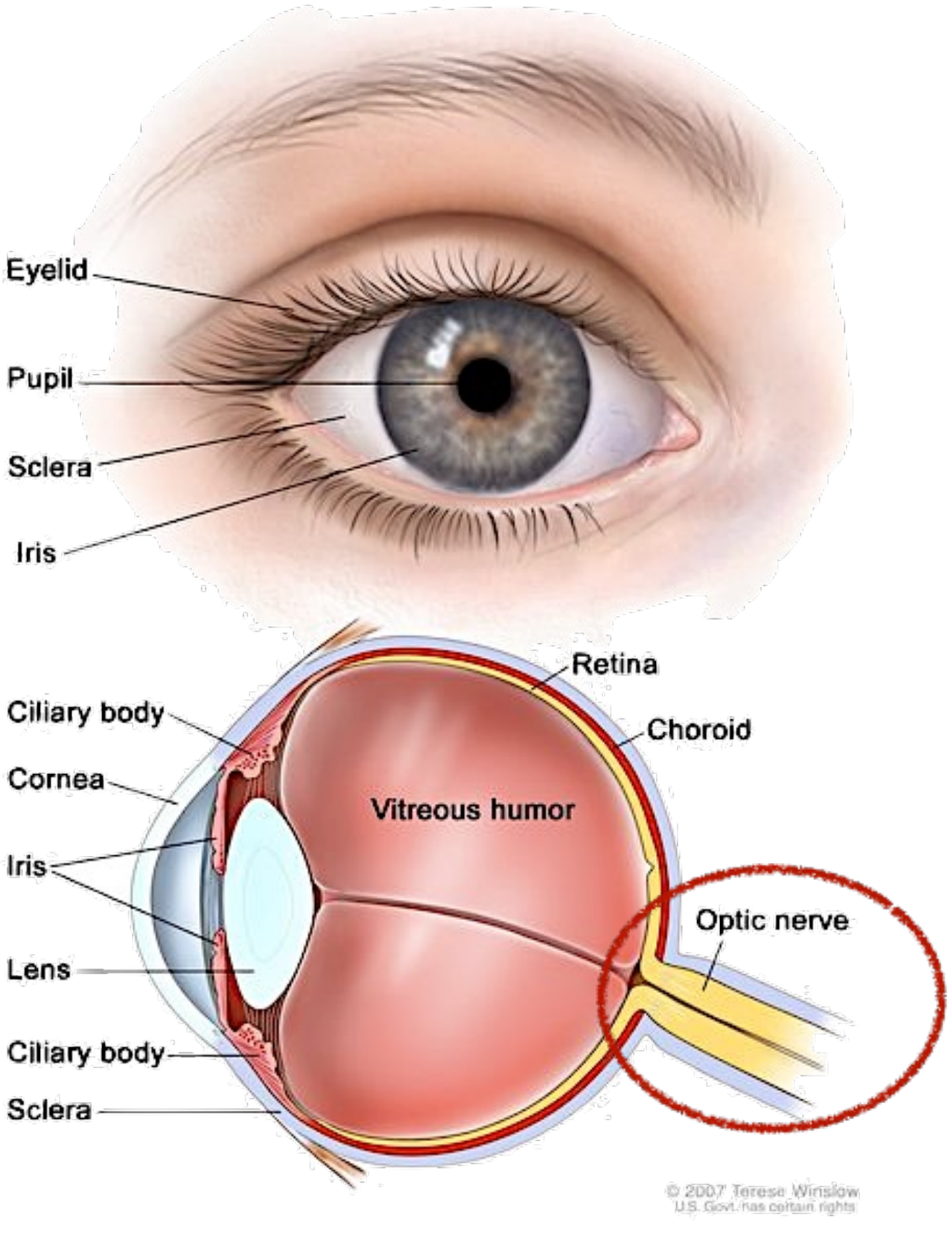
OPA 1
&
OPTIC ATROPHY TYPE 1

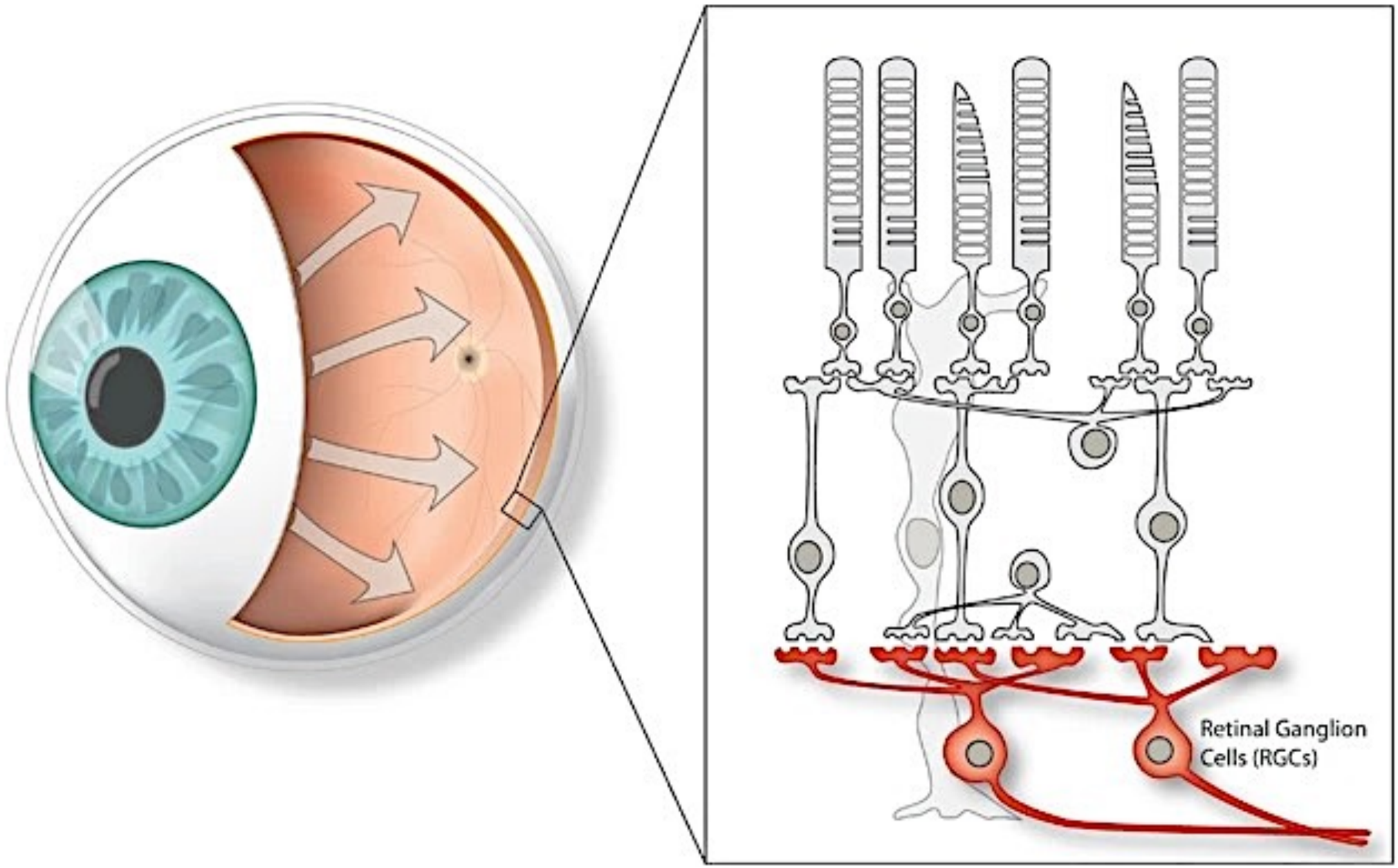
Dianna Xie

WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

What is optic atrophy type 1?



Defects in mitochondria lead to optic atrophy type 1



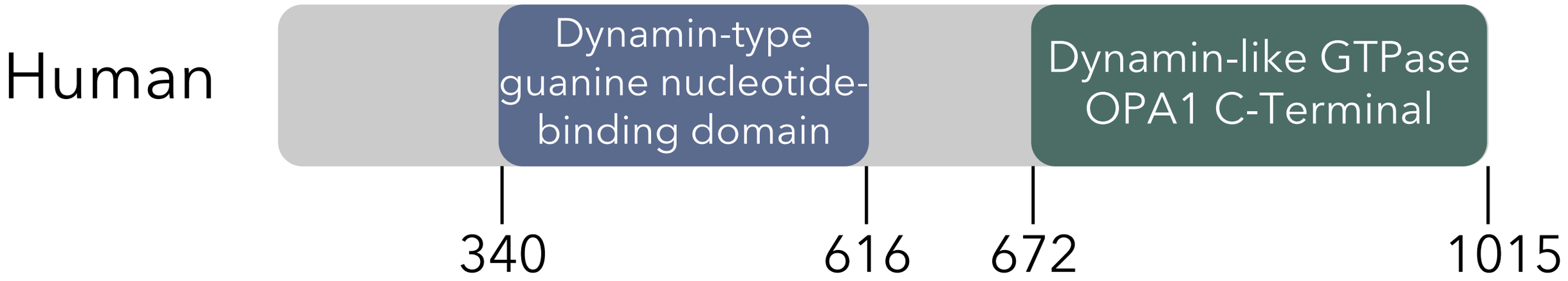
Optic atrophy type 1 causes impaired vision

Blurred

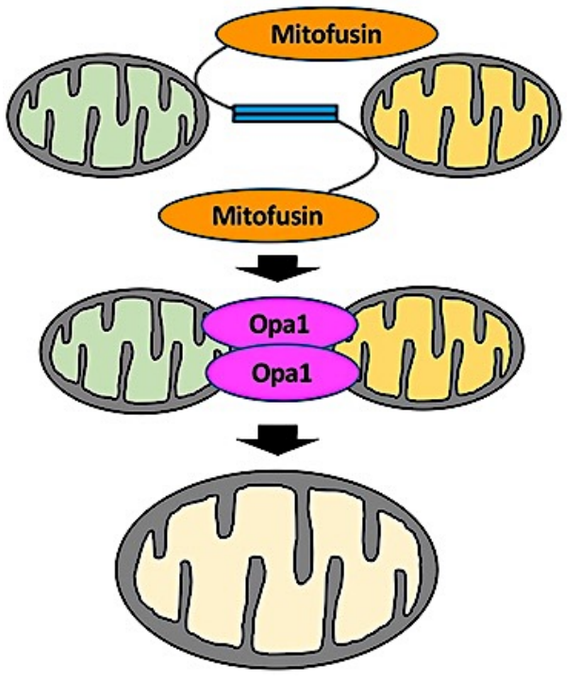
Blocked

Color vision deficiency

OPA1 is a dynamin-type protein found in mitochondria

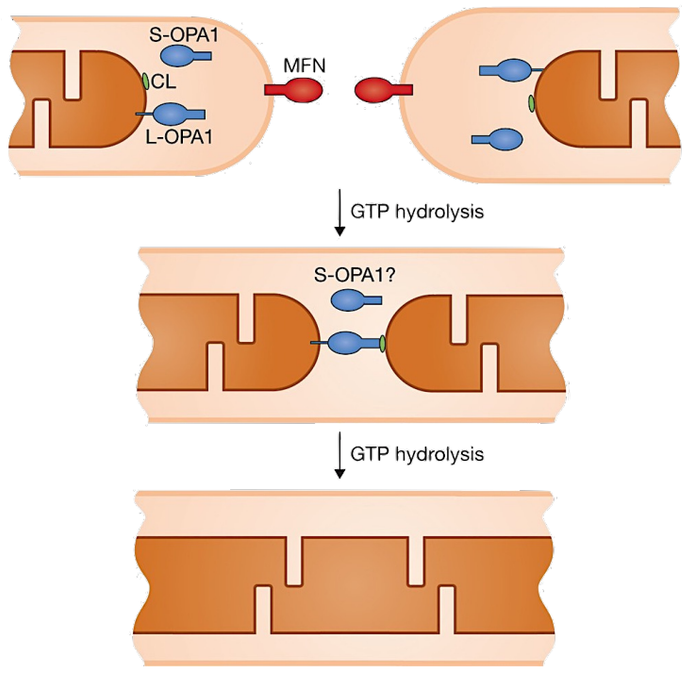


Biological process



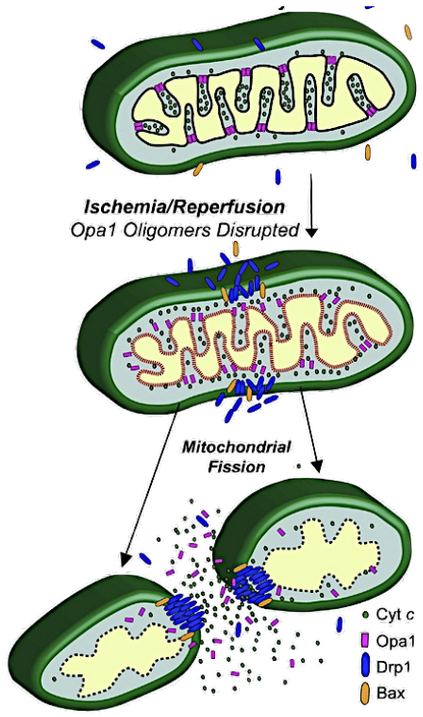
Mitochondria function

Molecular Function



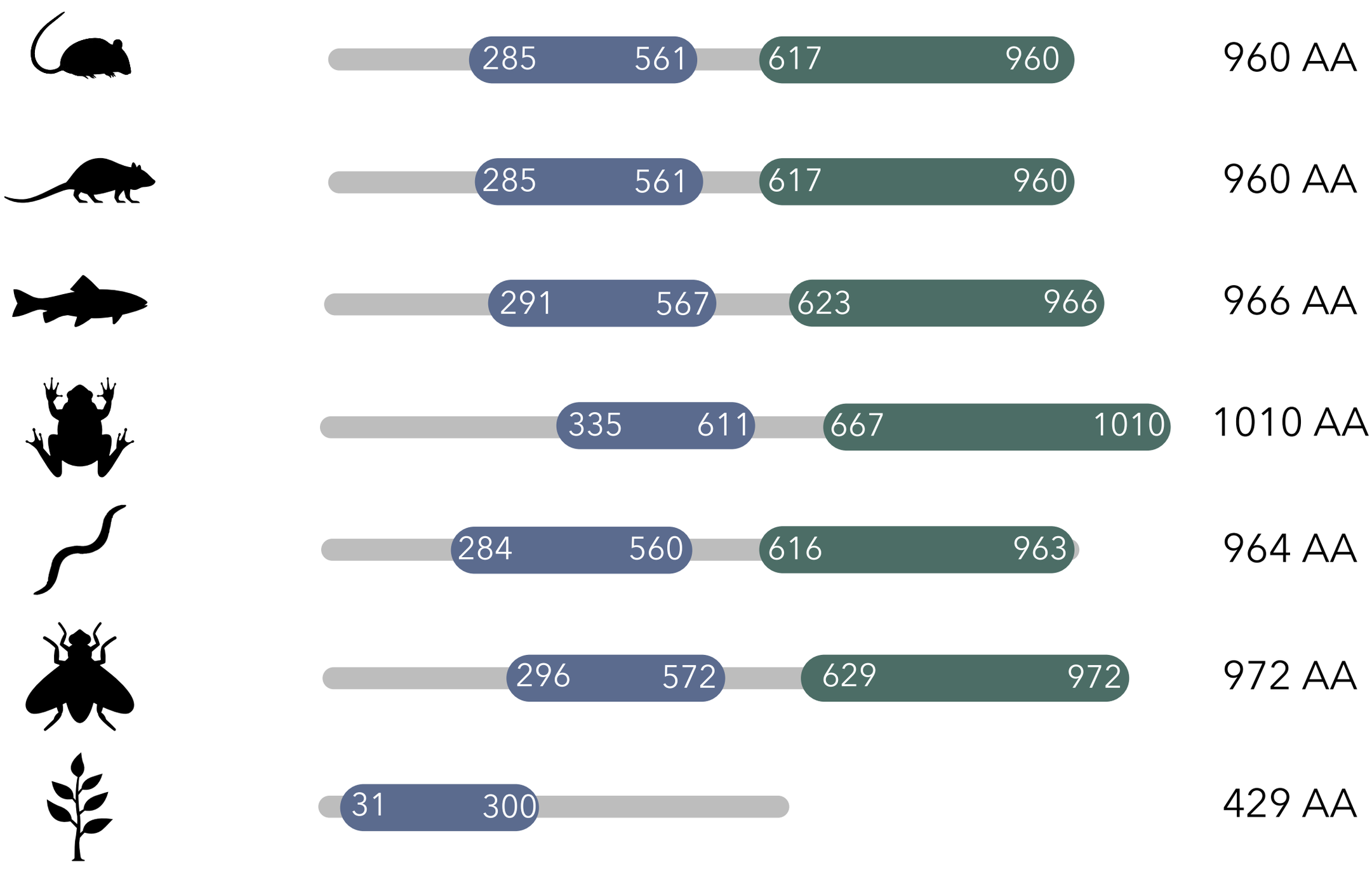
GTP binding

Cellular Component

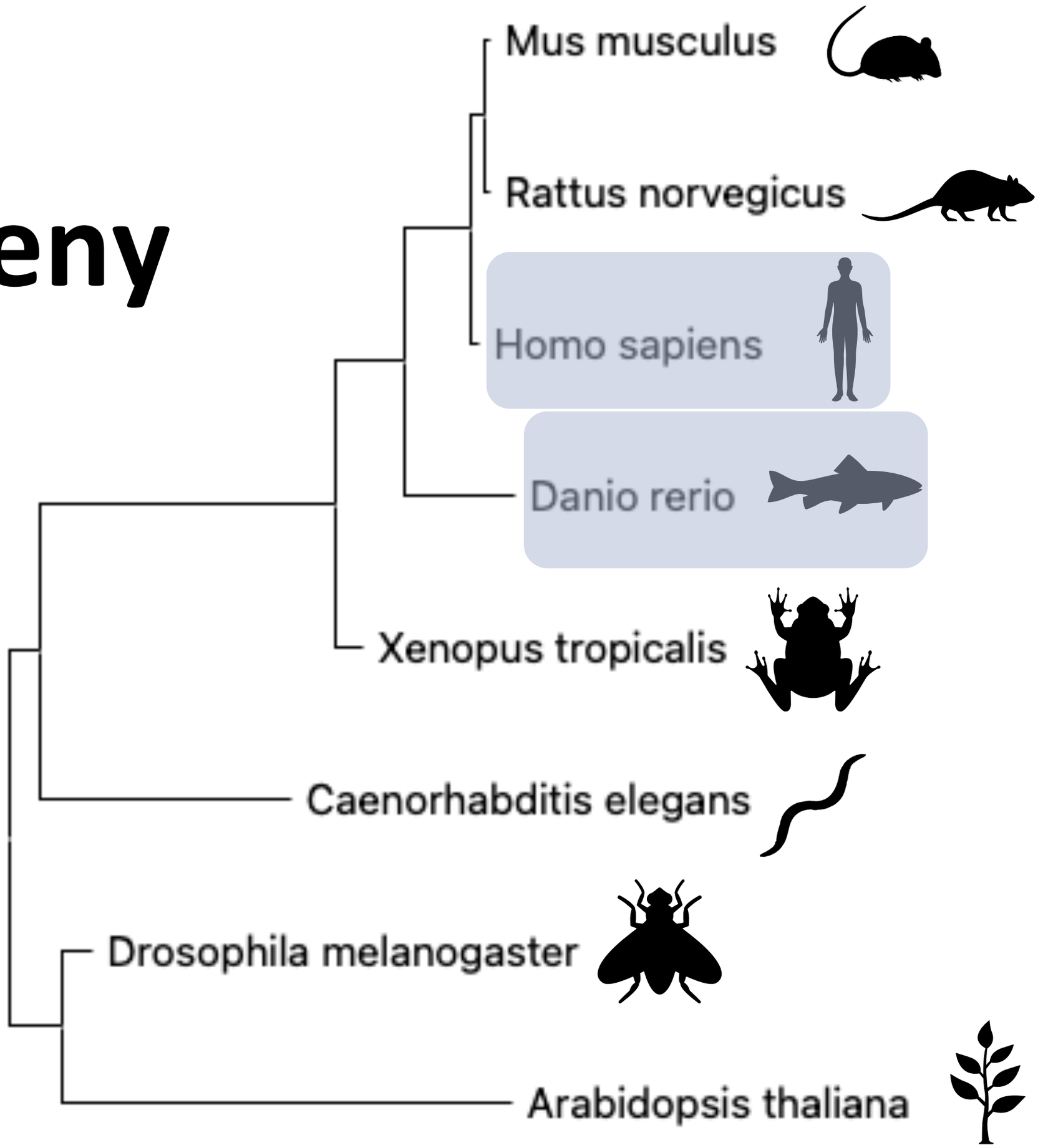


Mitochondrial membrane

OPA1 is well conserved across the animal and plant kingdoms

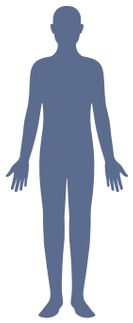


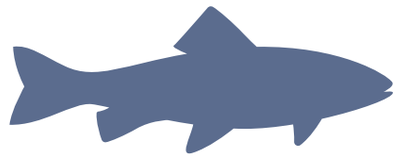
OPA1 Phylogeny

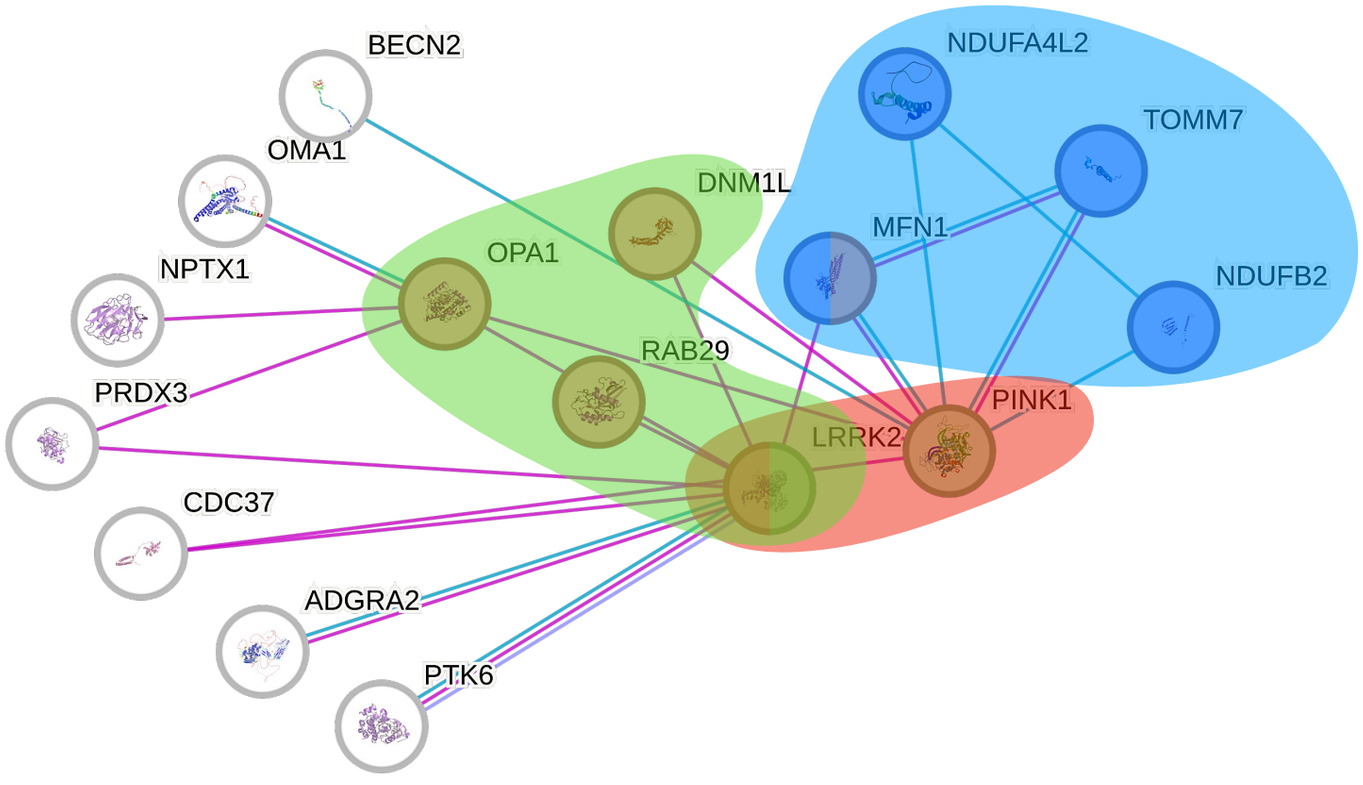


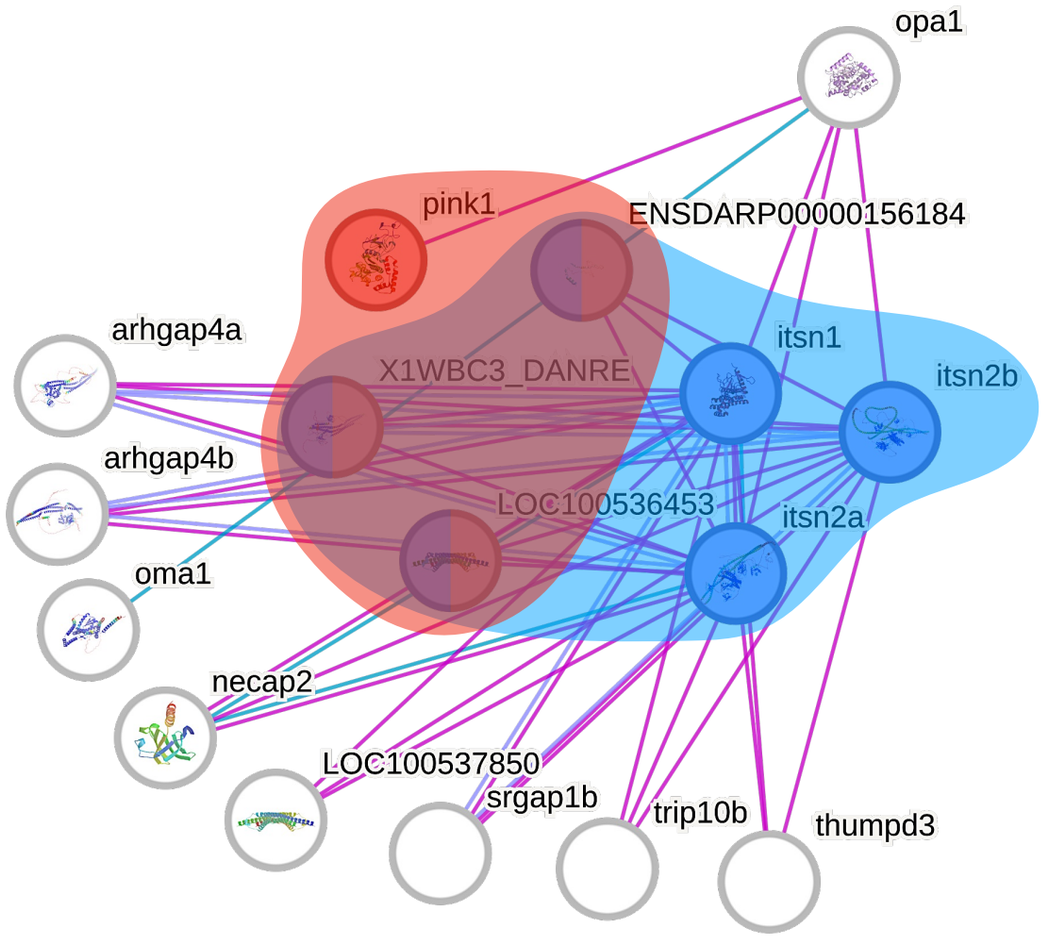
Muscle alignment:
maximum likelihood

OPA1 protein responsible for mitochondrial fusion and synapse transport









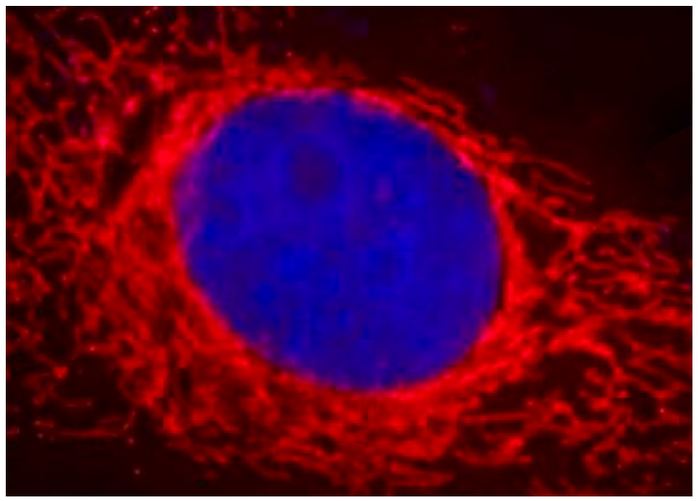
Mitochondria protein-containing complex

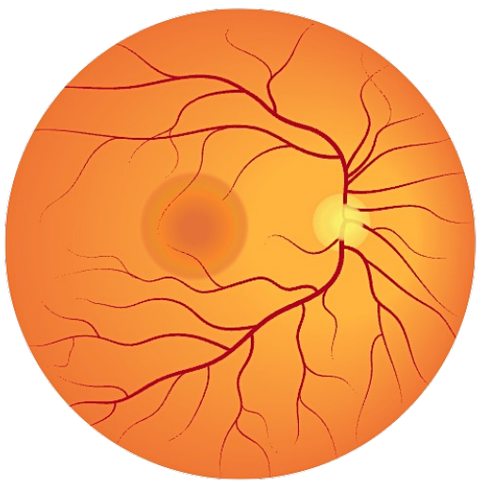
Synapse transport

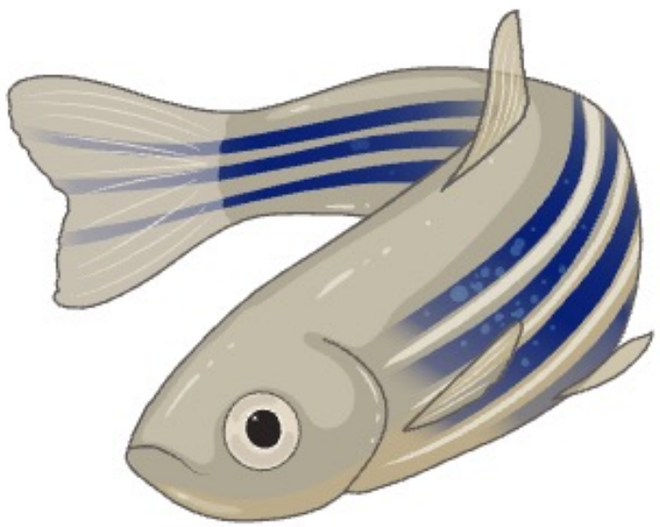
GTP Binding

Zebrafish as model organism for studying mitochondrial function

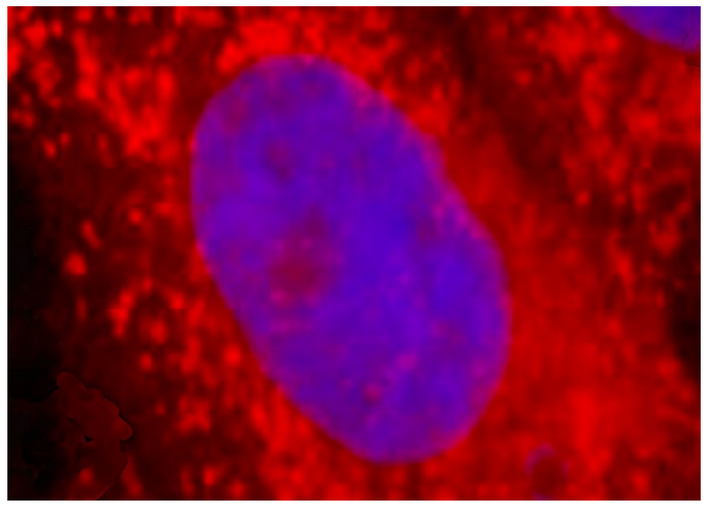
Normal

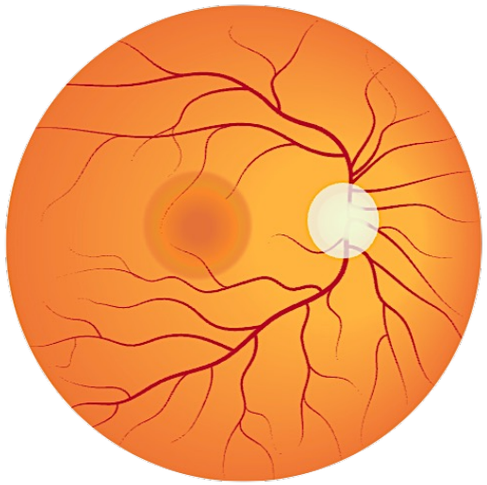


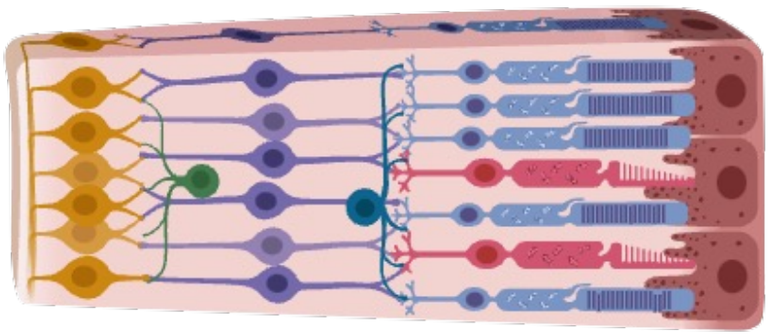




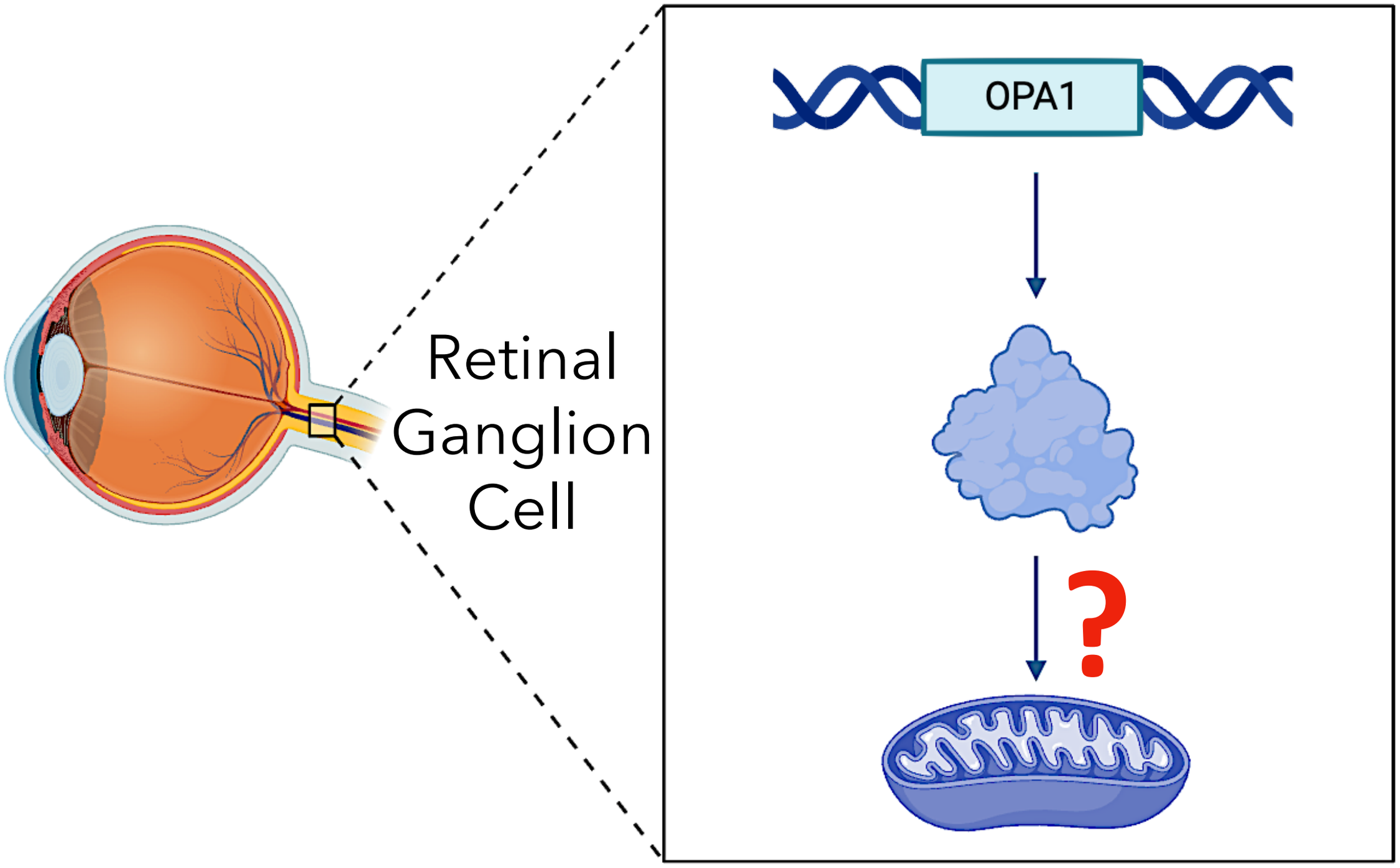
Atrophy







Gap: The role of OPA1 in the developmental stage of mitochondria is unknown

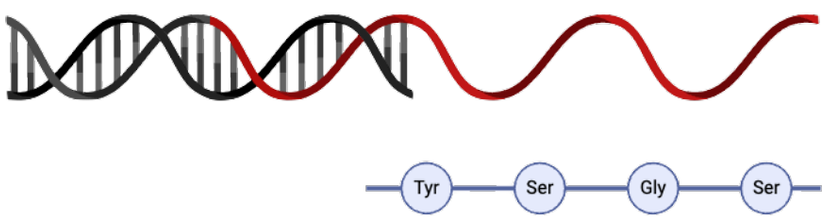


Primary Goal

Investigate when in the development of mitochondria is affected by the mutation of the OPA1 gene

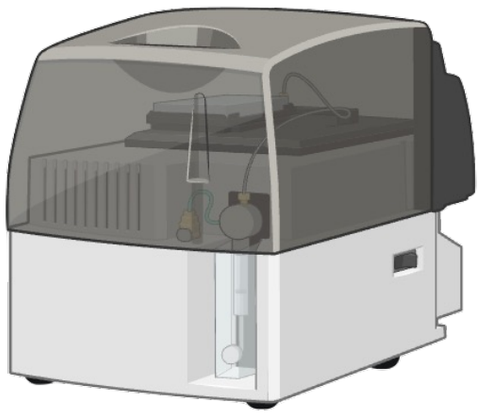
Aim 1

Identify OPA1 **gene domains** that are crucial for mitochondria development using **domain analysis**



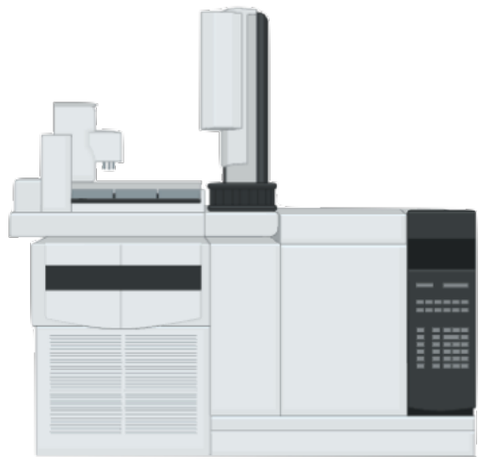
Aim 2

Identify **chemical compounds** that could rescue mitochondria fusion using **chemical screens**

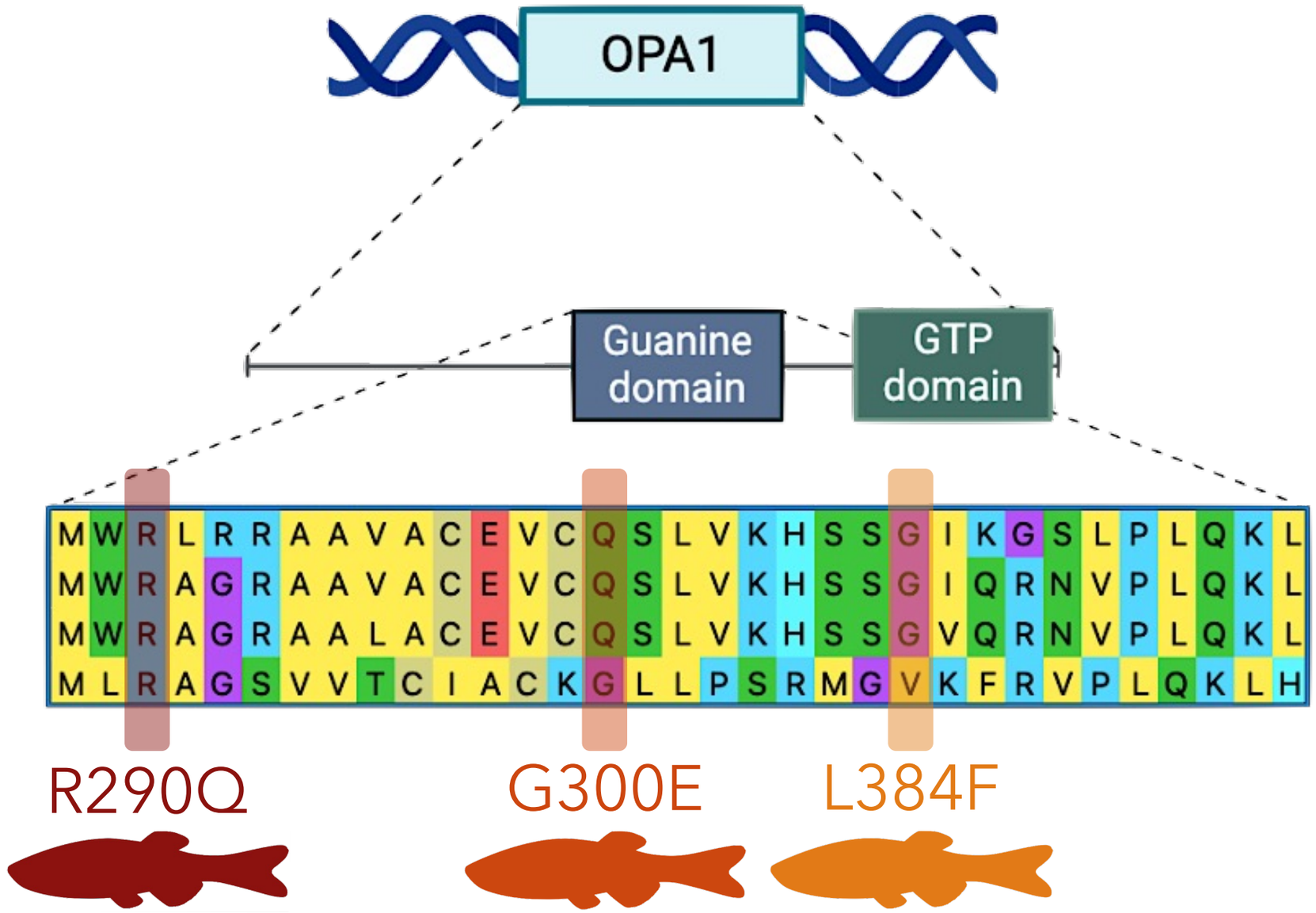


Aim 3

Identify **protein interactions** in wild type and mutant zebrafish using **iTRAQ**



Aim 1a Use domain analysis to identify OPA1 gene domains that are crucial for mitochondria development

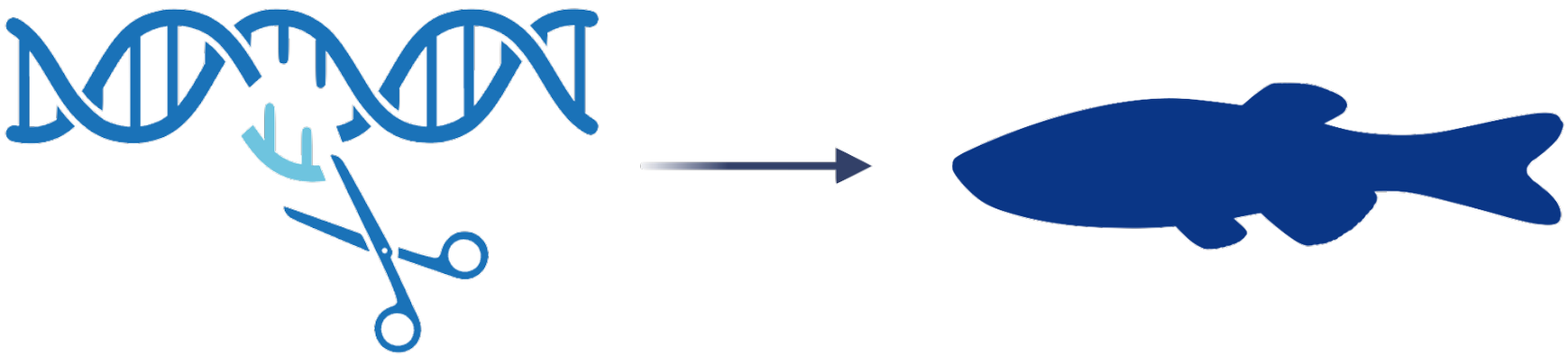


Rationale: Determine which gene domains affect protein function, then knock out using CRISPR and validate using chemical screens to see if phenotypes are rescued

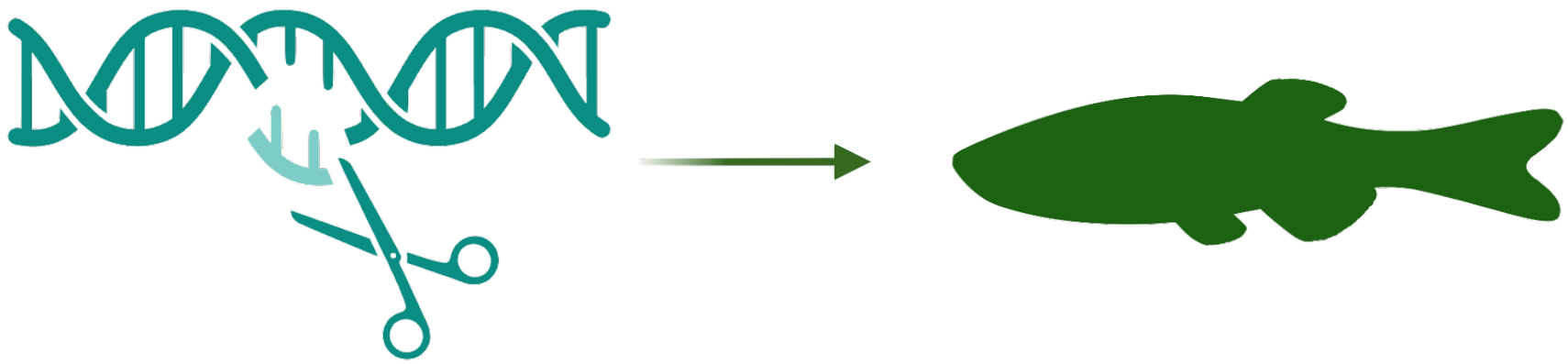
DOMAIN ANALYSIS → CRISPR → CHEMICAL SCREENS

Aim 1b Use CRISPR to knockout identified gene domains that potentially affect mitochondria development

Guanine nucleotide binding domain



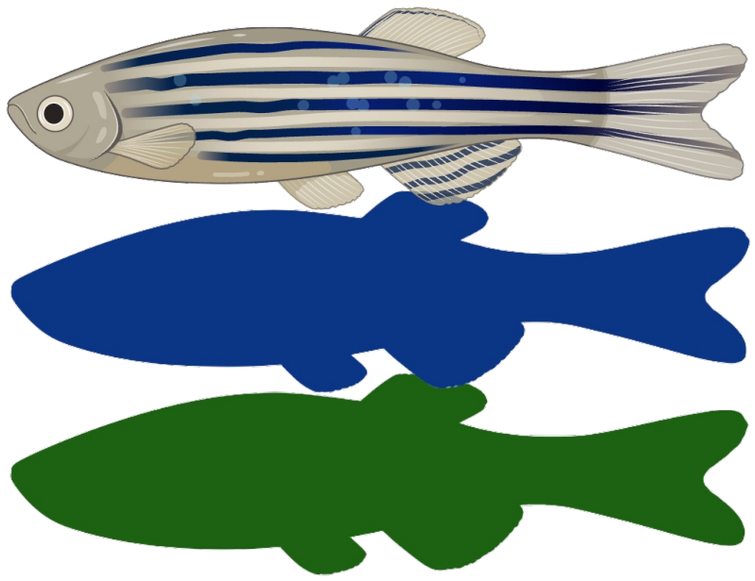
GTPase domain



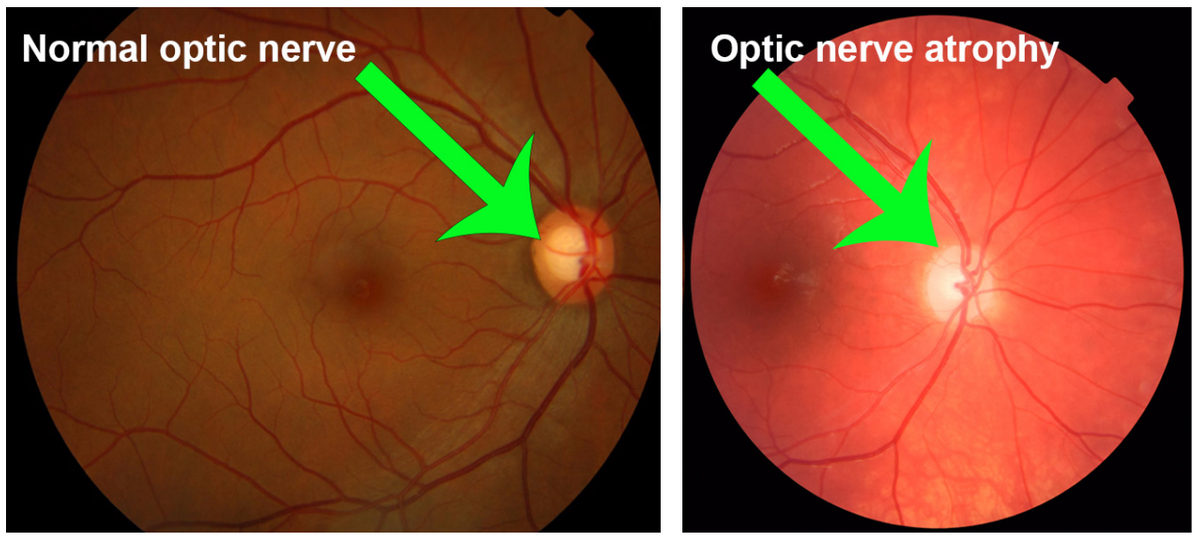
DOMAIN ANALYSIS | CRISPR | CHEMICAL SCREENS

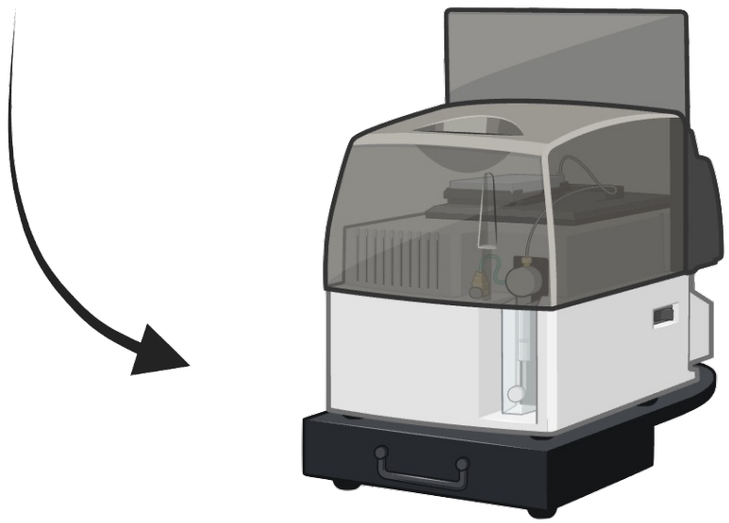
Aim 1c

Use chemical screens to validate optic atrophy type 1 phenotypes in wildtype and mutant zebrafish



EYE





Hypothesis: Deletion of guanine-binding domains will affect mitochondria functions

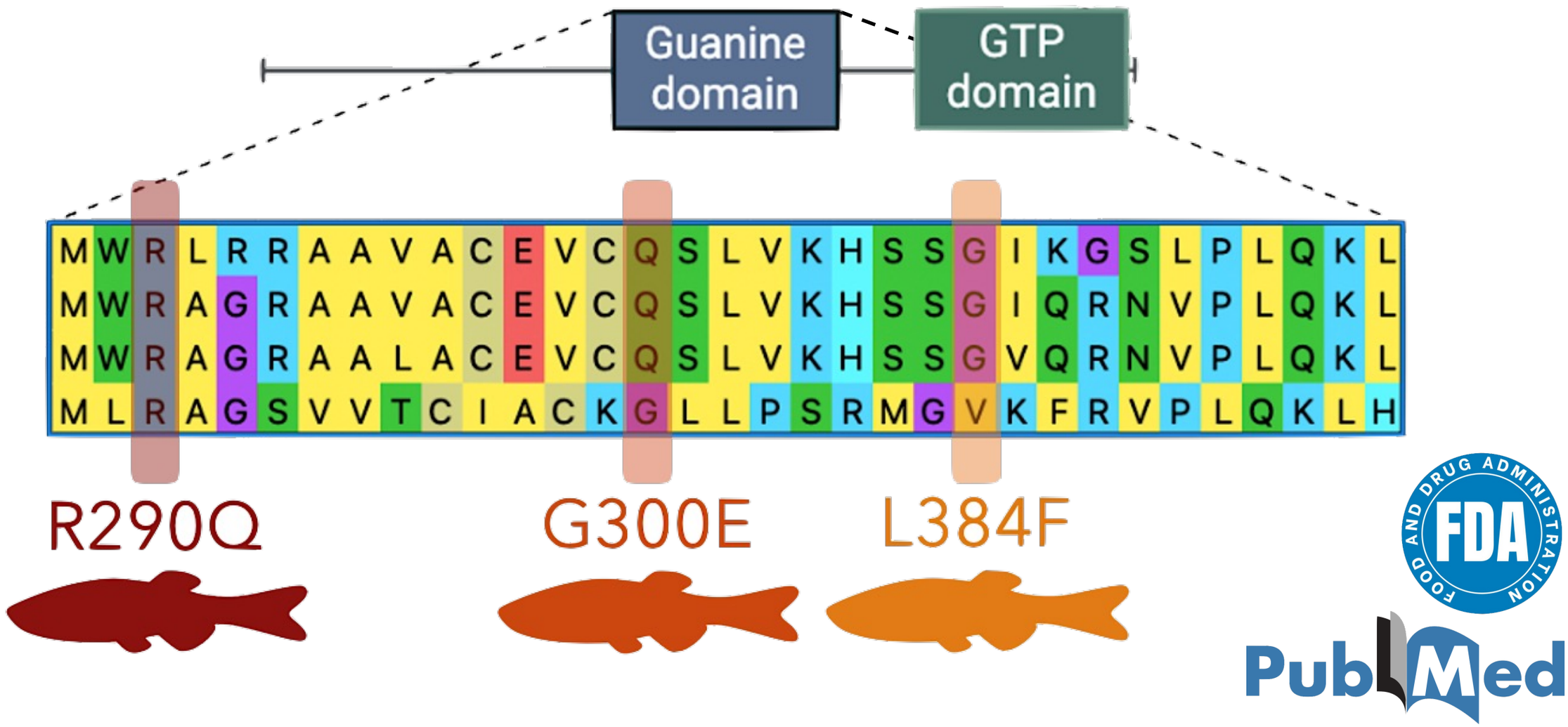
DOMAIN ANALYSIS

CRISPR

CHEMICAL SCREENS

Aim 2a

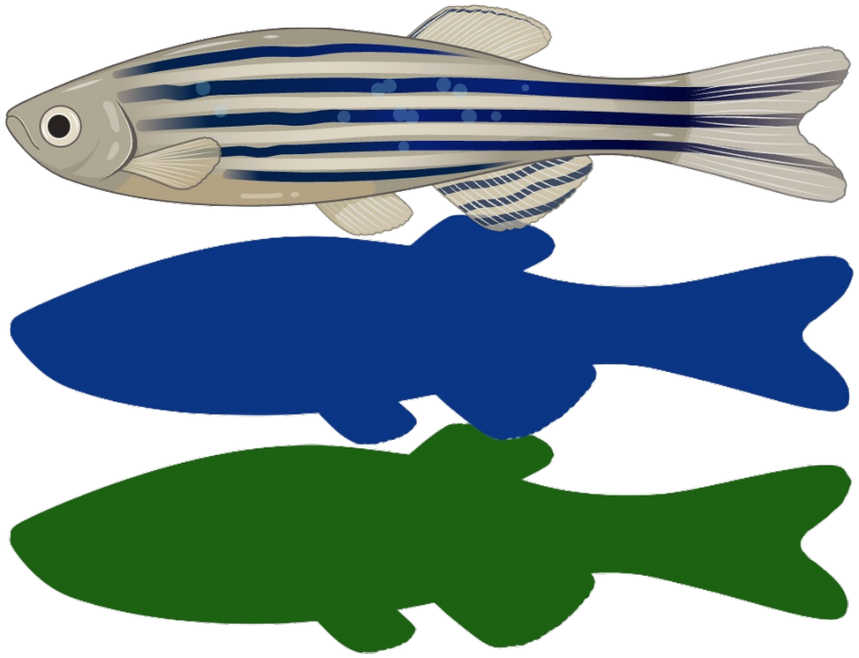
Identify chemical compounds that potentially rescue phenotypes using chemical libraries

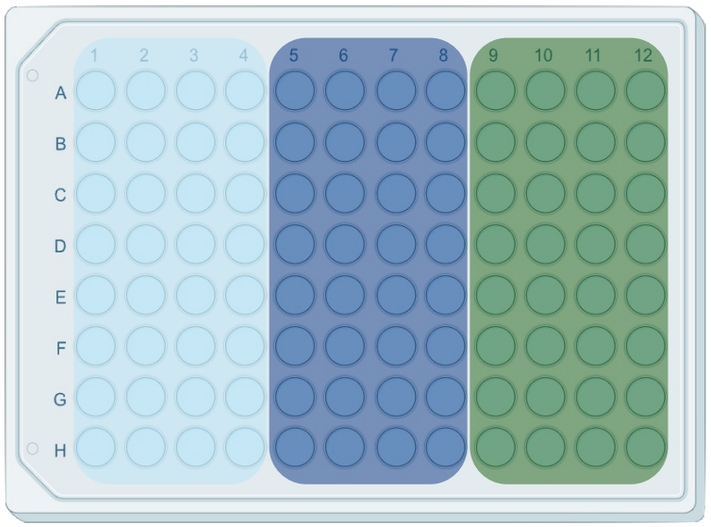


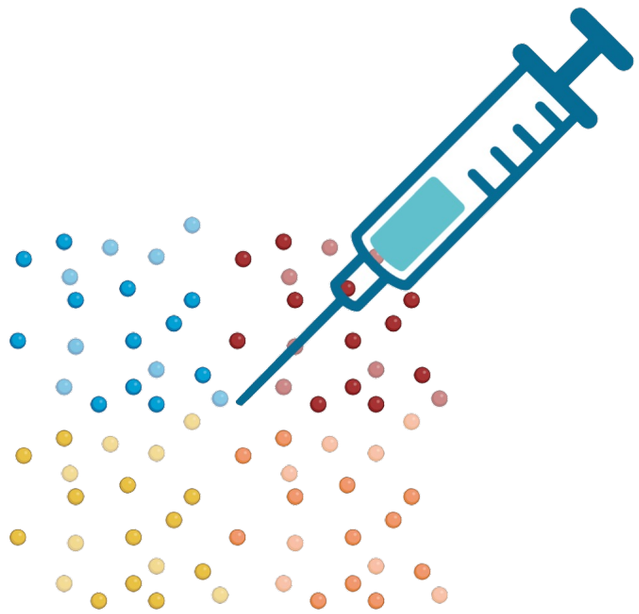
Rationale: Chemical screens visualize changes in the mitochondria and determine which chemical compounds is suitable for drug discovery

COMPOUND SELECTION	CELL STAIN & ASSAY	CHEMICAL SCREENS

Aim 2b Inject chemical compounds in both wildtype and mutant zebrafish





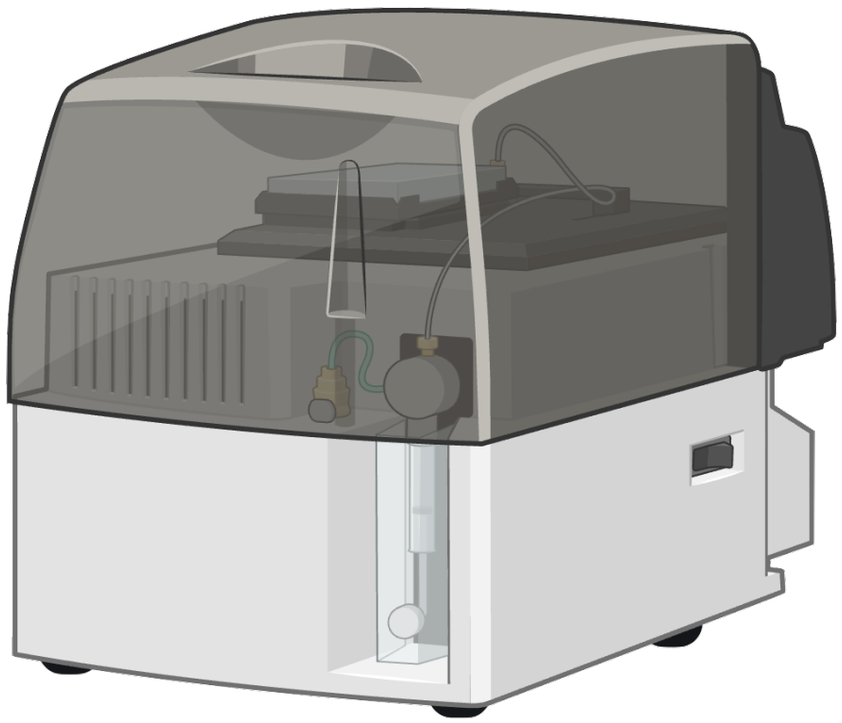


COMPOUND SELECTION

CELL STAIN & ASSAY

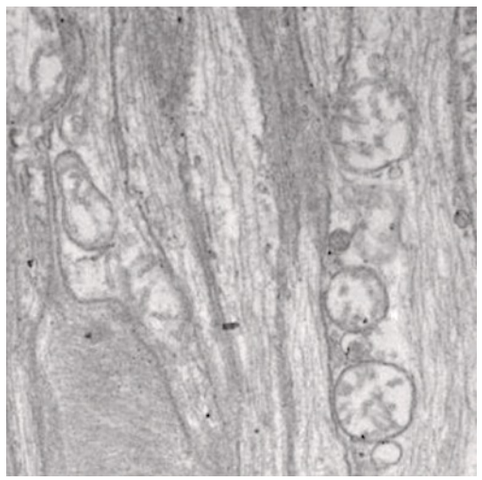
CHEMICAL SCREENS

Aim 2c Validate the phenotypes to see if those are rescued using chemical screens

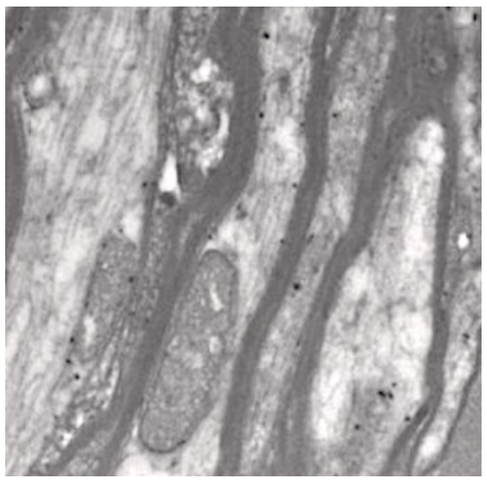


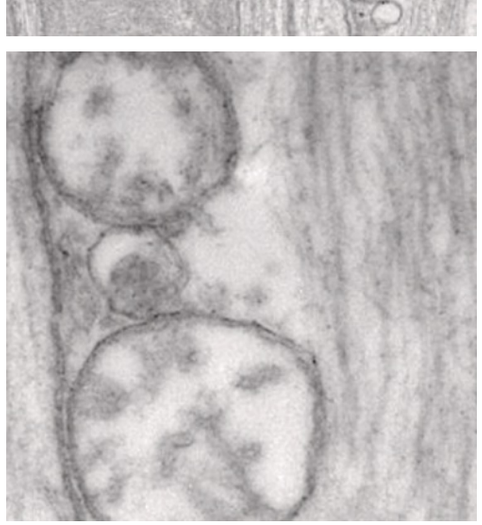
MITOCHONDRIA

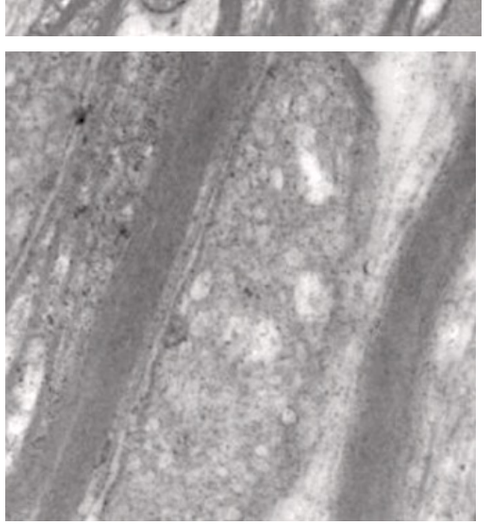
Before



After



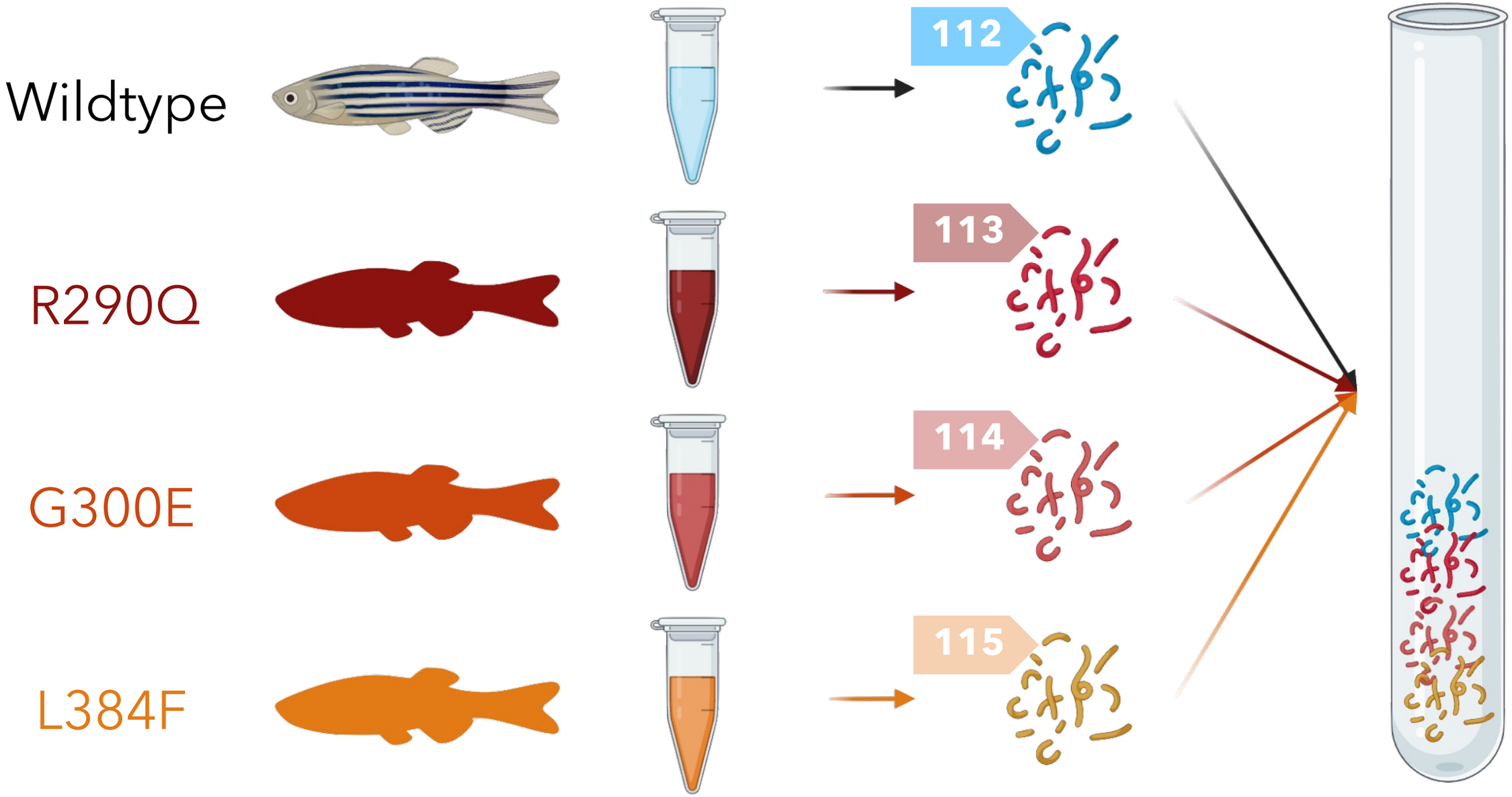




Hypothesis: Different compounds can rescue mitochondria fusion to different levels

COMPOUND SELECTION → CELL STAIN & ASSAY → CHEMICAL SCREENS

Aim 3a Quantify OPA1 binding proteins that is crucial for mitochondria function using iTRAQ

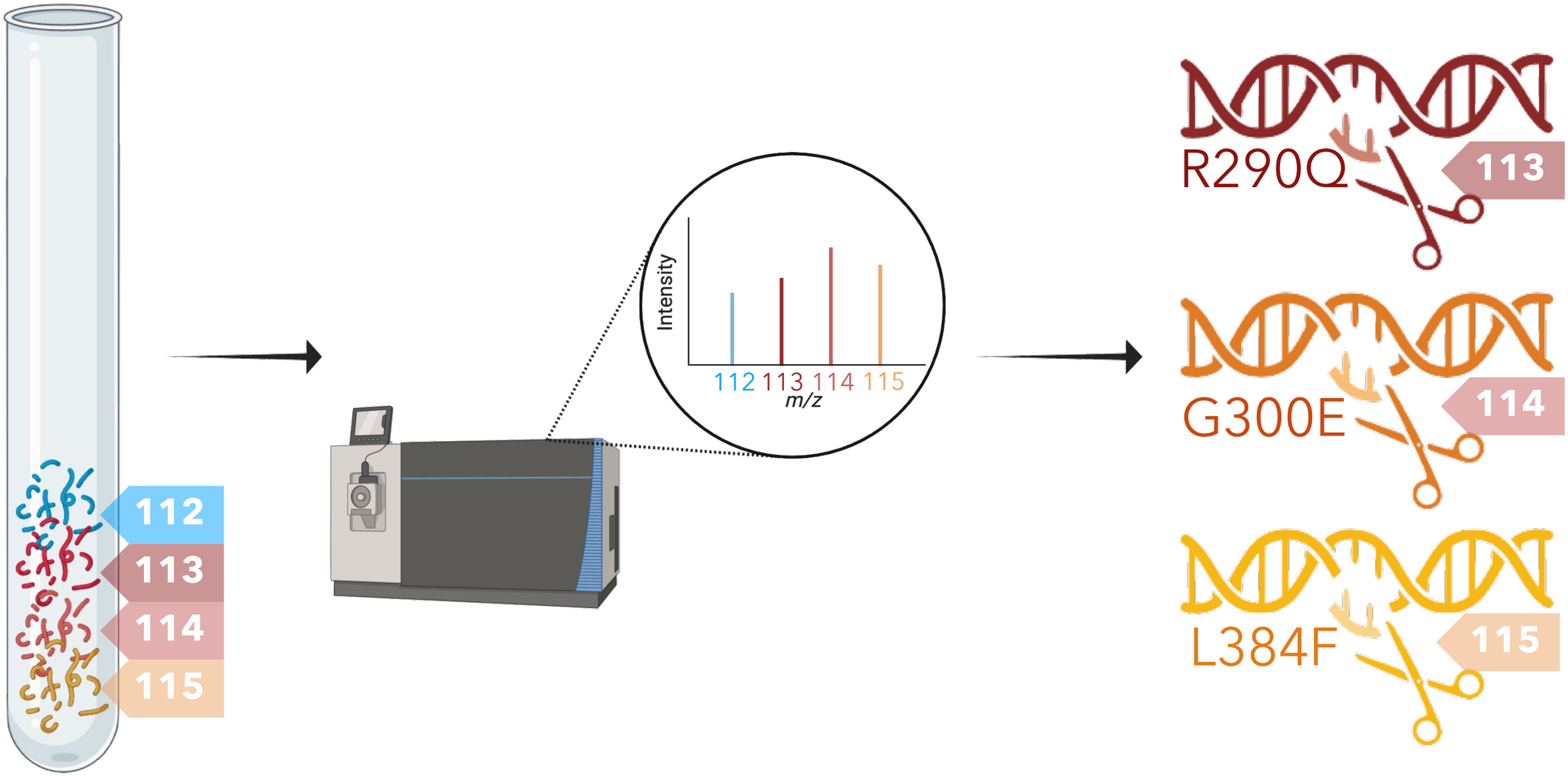


Rationale: Quantifying OPA1 protein could identify how protein interactions are affected by the mutation of OPA1 in mitochondria

iTRAQ	MASS SPEC + CRISPR	CHEMICAL SCREENS

Aim 3b

Obtain OPA1 protein level using mass spectrometry and knockout proteins with elevated abundance level using CRISPR

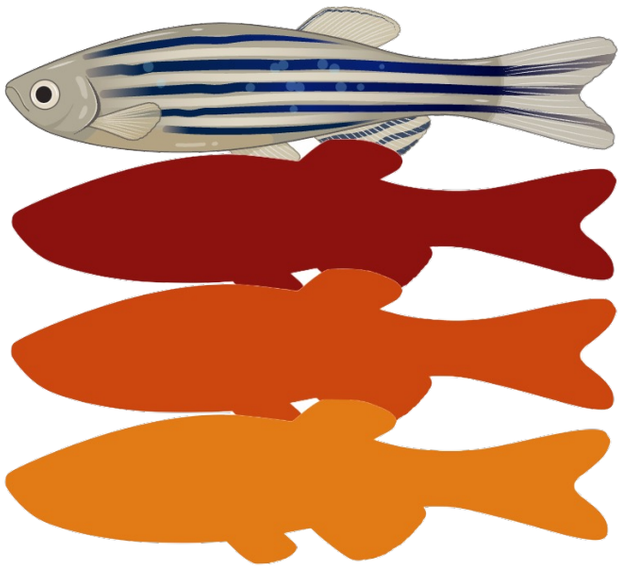


iTRAQ

MASS SPEC + CRISPR

CHEMICAL SCREENS

Aim 3c Validate OPA1 phenotypes after knocking out proteins using chemical screens



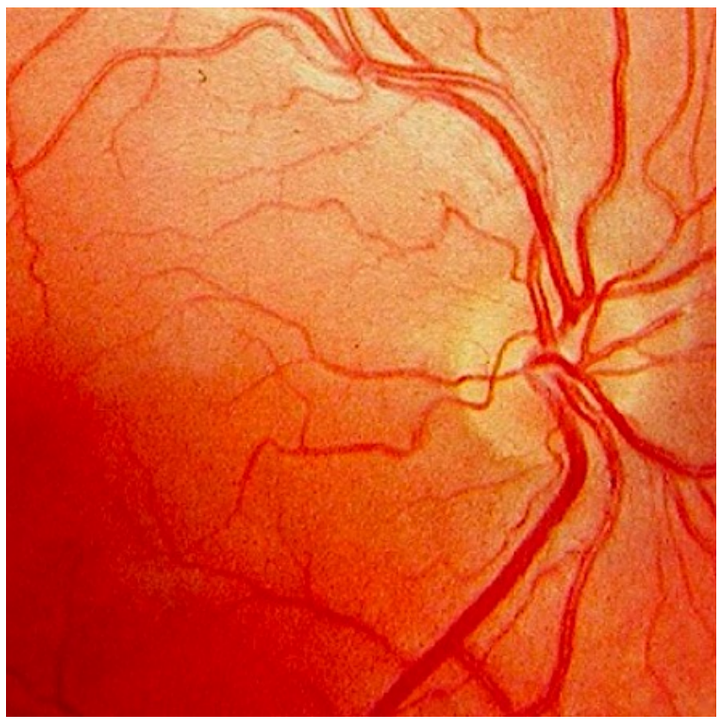
Wildtype

R290Q

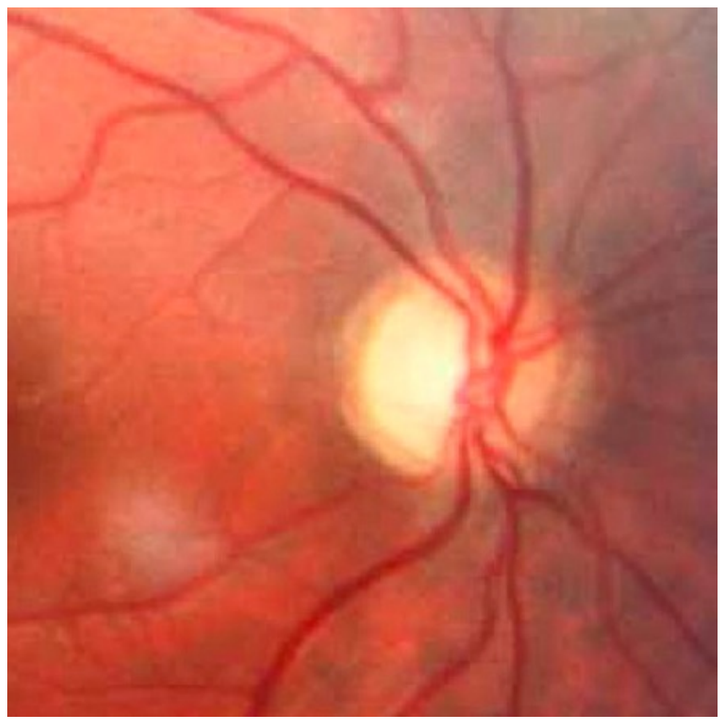
G300E

L384F

WT



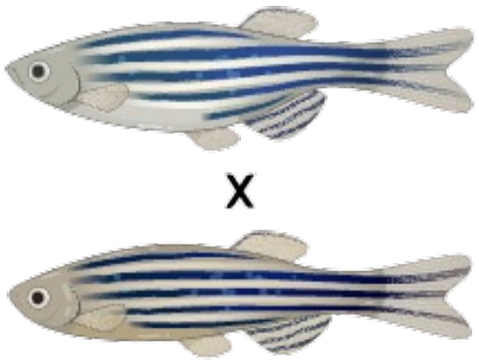
Mutants



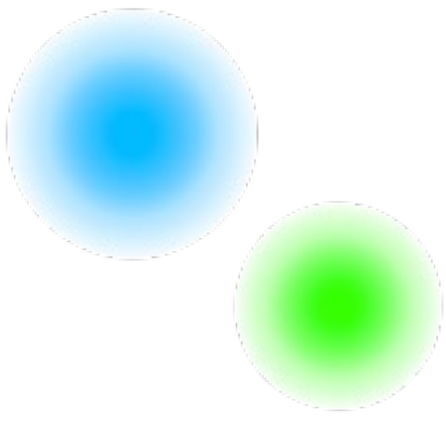
Hypothesis: Knockout of proteins with elevated protein level can rescue disease-like phenotypes

iTRAQ	MASS SPEC + CRISPR	CHEMICAL SCREENS

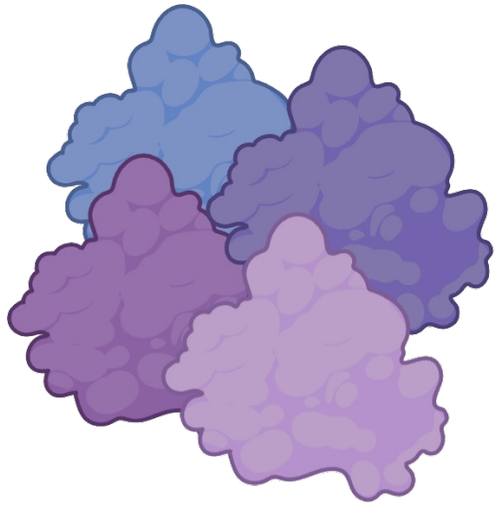
Conclusion



Mutations in OPA1 gene will lead to mitochondria dysfunction and cause optic atrophy type 1

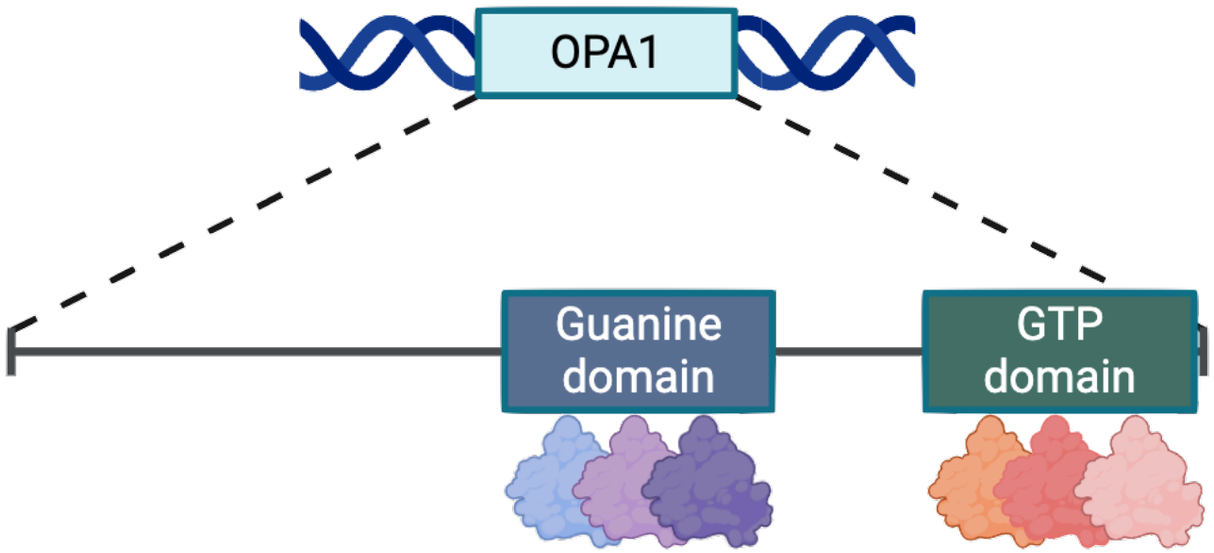


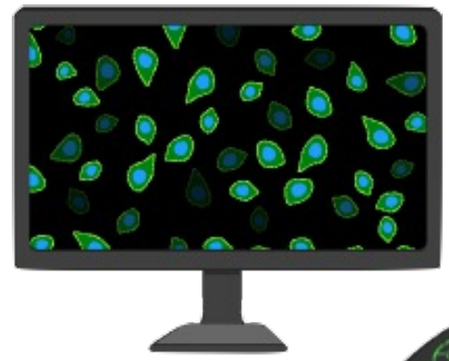
Chemical compounds could be identified for rescuing phenotypes of optic atrophy type 1

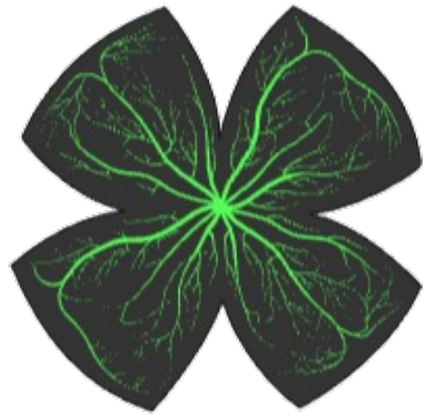


Analyzing OPA1 protein interactions may help identifying new therapeutical treatments

Future Direction







References

Article reference:

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Image reference:

[1] Biorender