Il pesce zebrafish: utile modello per testare possibili composti atti a migliorare la malattia di Pompe

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CONVEGNO A.I.G. - 2019

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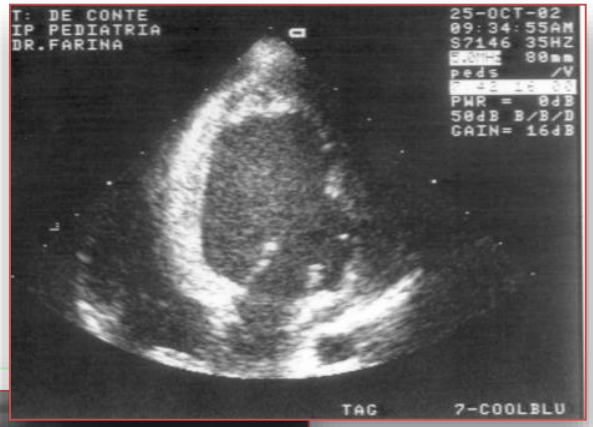
AIG:
ASSOCIAZIONE ITALIANA
GLICOGENOSI ONLUS

Malattia di Pompe

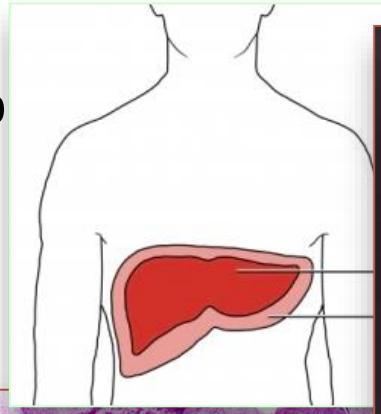
Glycogen storage type II

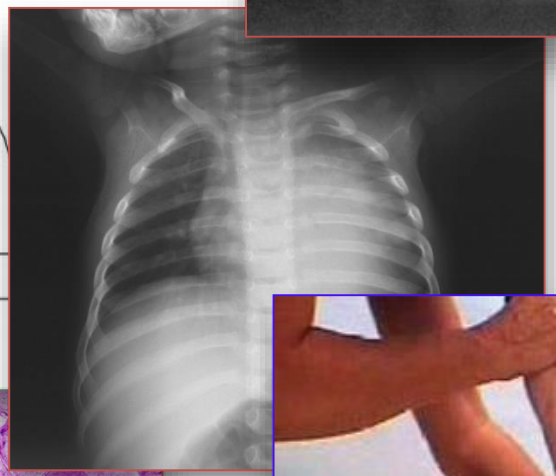
Incidenza: 1: 40.000
Deficit enzimatico: α-glucosidasi acida (GAA)
Difetto genetico: Mutazioni nel gene *GAA*
Età di esordio: Infantile, giovanile, adulta.

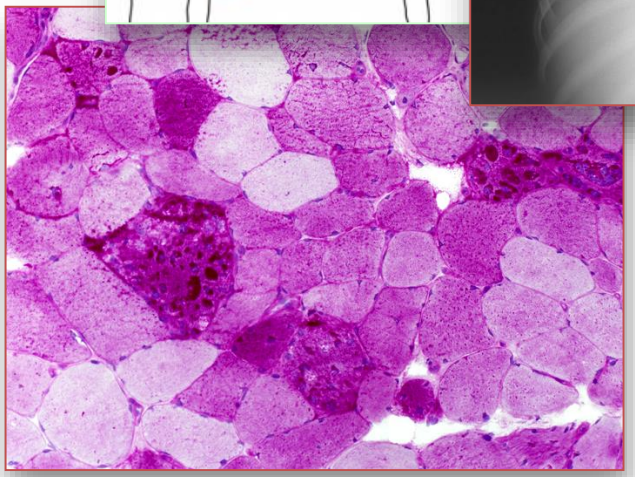


Accumulo generalizzato di glicogeno, prevalentemente in:

- **cuore**
- **muscolo scheletrico**
- **fegato**
- **vasi sanguigni**
- **cervello**



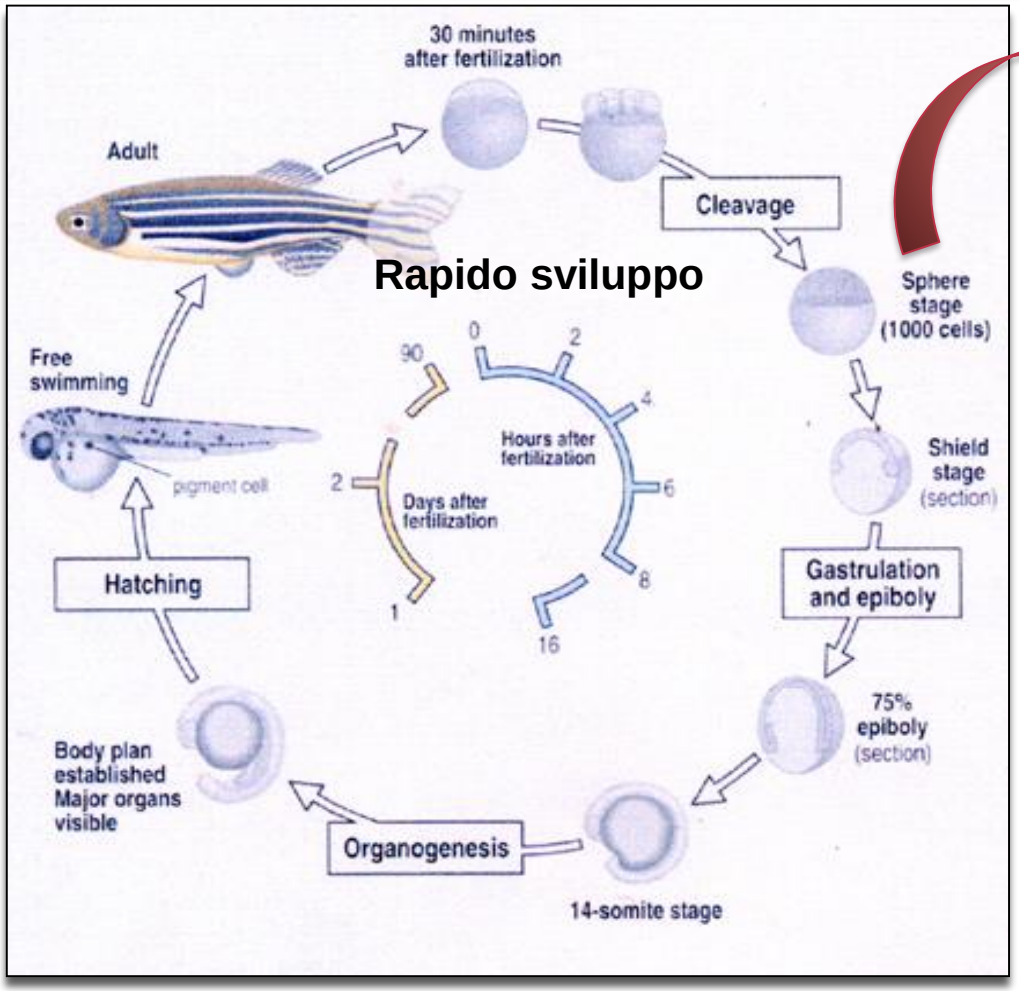


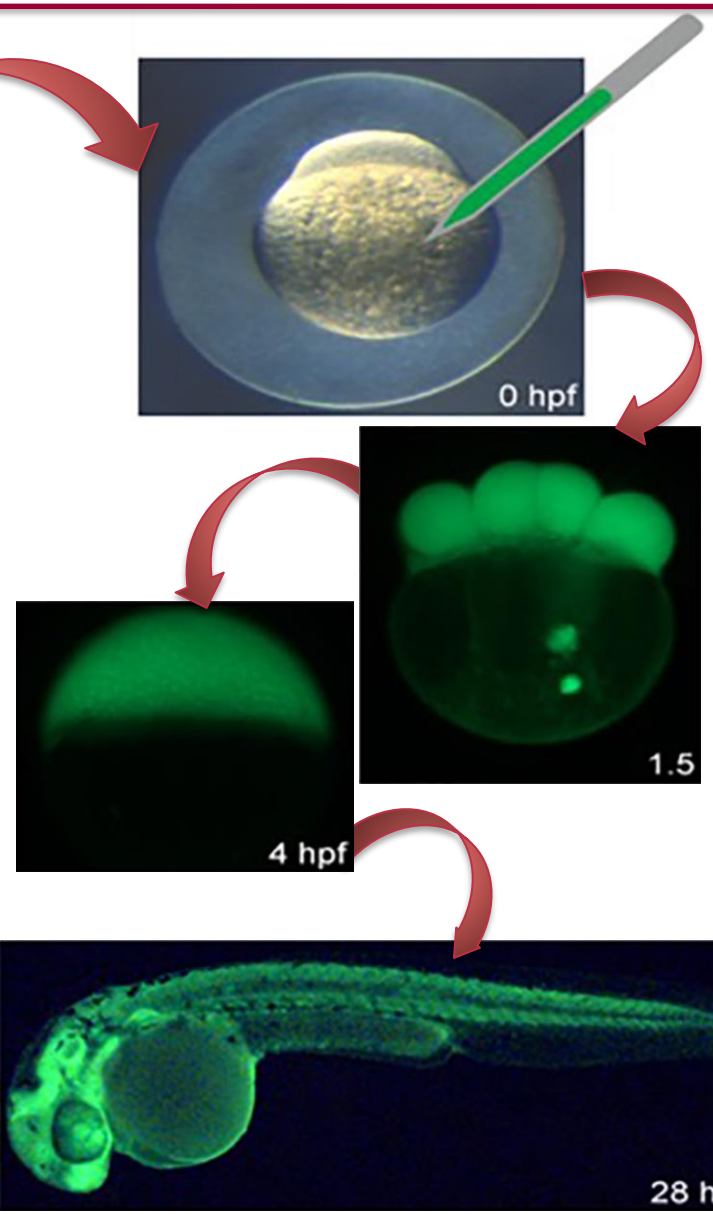


Zebrafish (*Danio rerio*)

- Vertebrato
- Rapido sviluppo
- Prolifico
- Fecondazione esterna
- Sempre disponibile durante l'anno
- Trasparente
- Embriologia ben conosciuta
- Facile da iniettare
- Facile da manipolare geneticamente

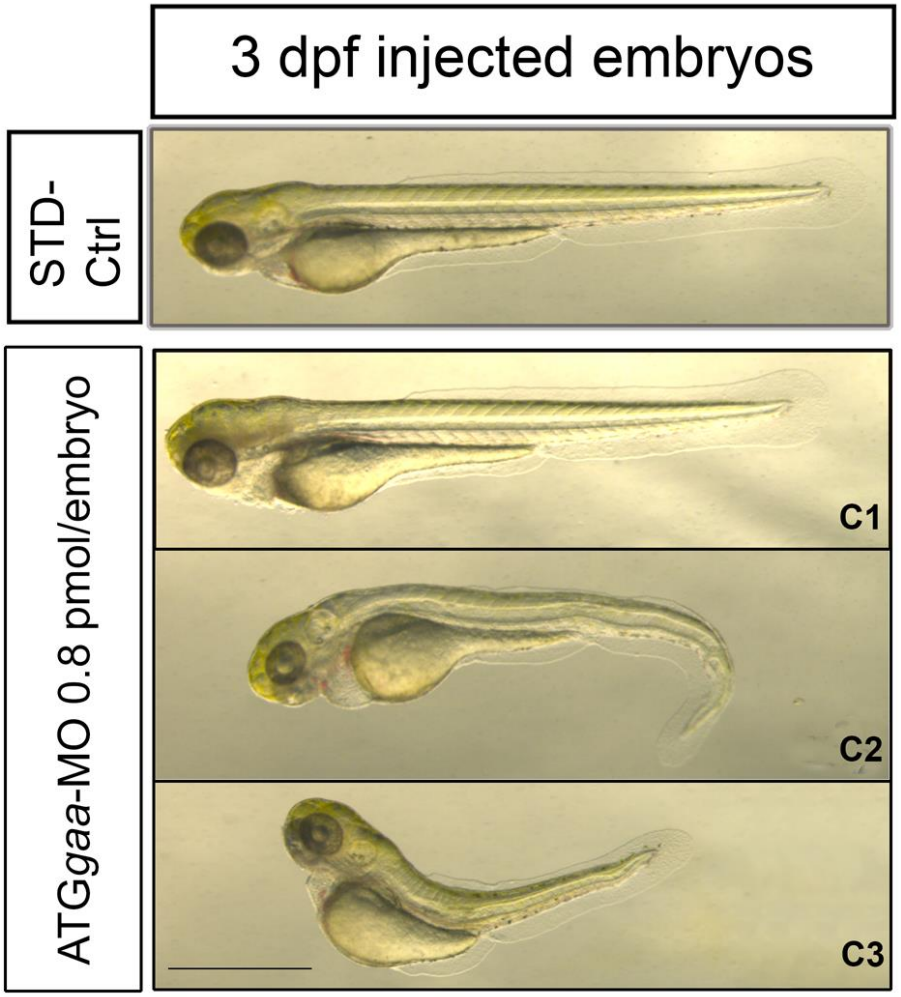
Ciclo vitale dello Zebrafish

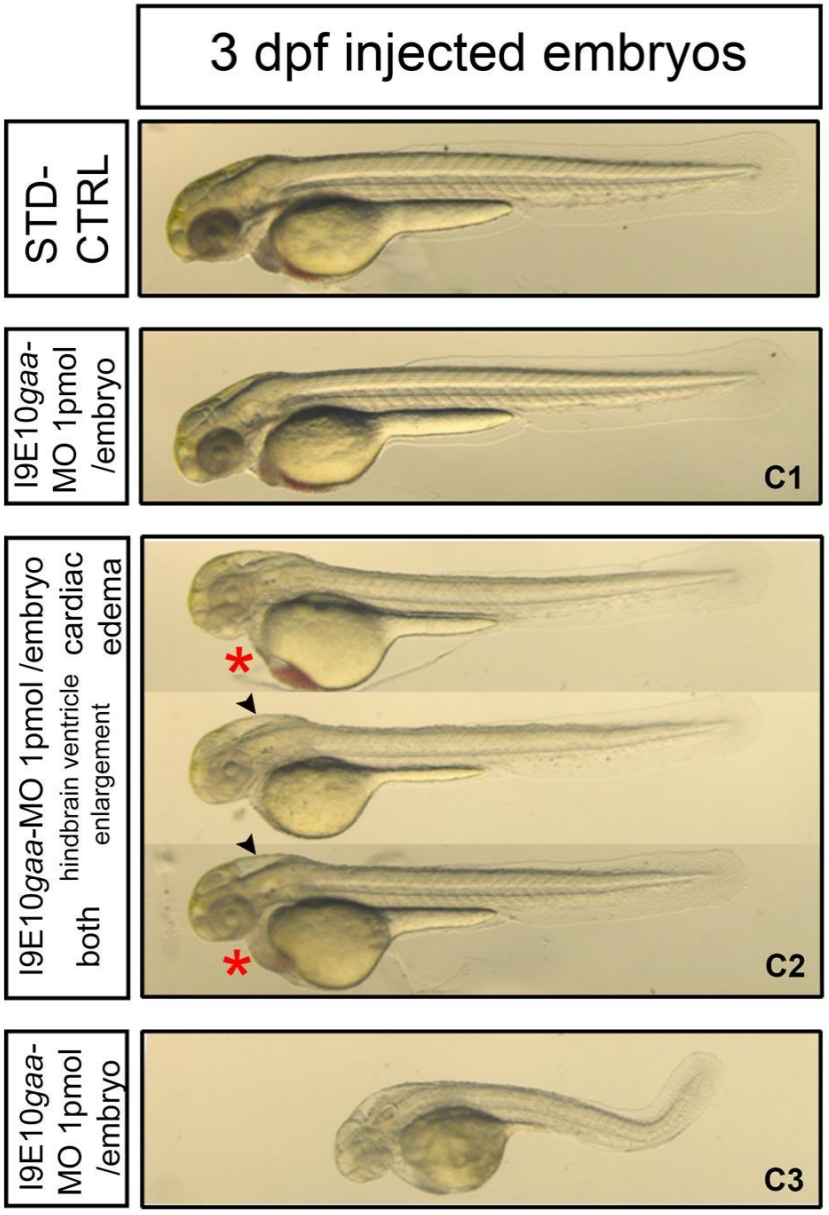




- Morpholino iniettato nel tuorlo allo stadio di una cellula;
- Co-iniezione di un tracciante per identificare gli embrioni correttamente iniettati;
- Come controllo negativo si usa un morpholino senza target.

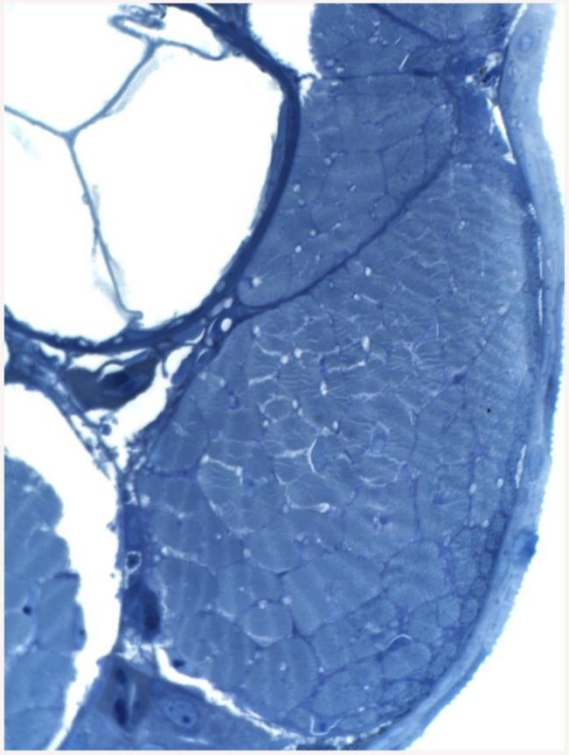
Fenotipi nello zebrafish Pompe



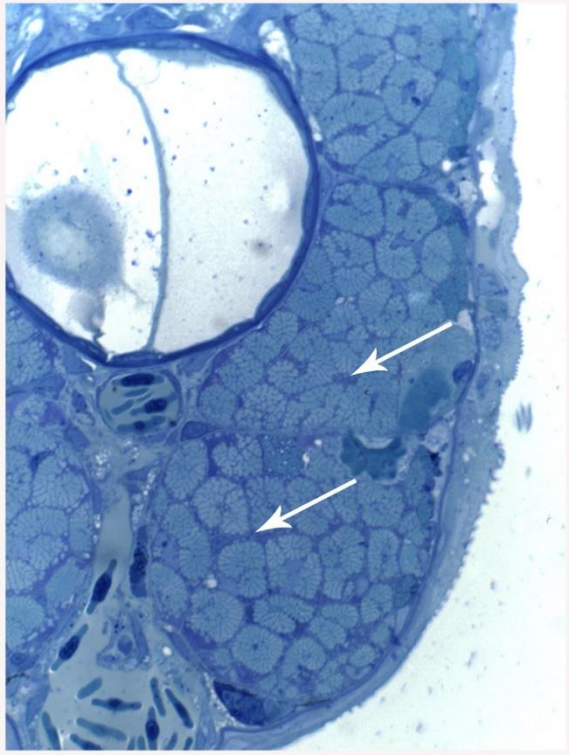


Sezioni trasverse semifini

Sezioni trasverse colorate con blu di toluidina, a 4 dpf



STD-MO



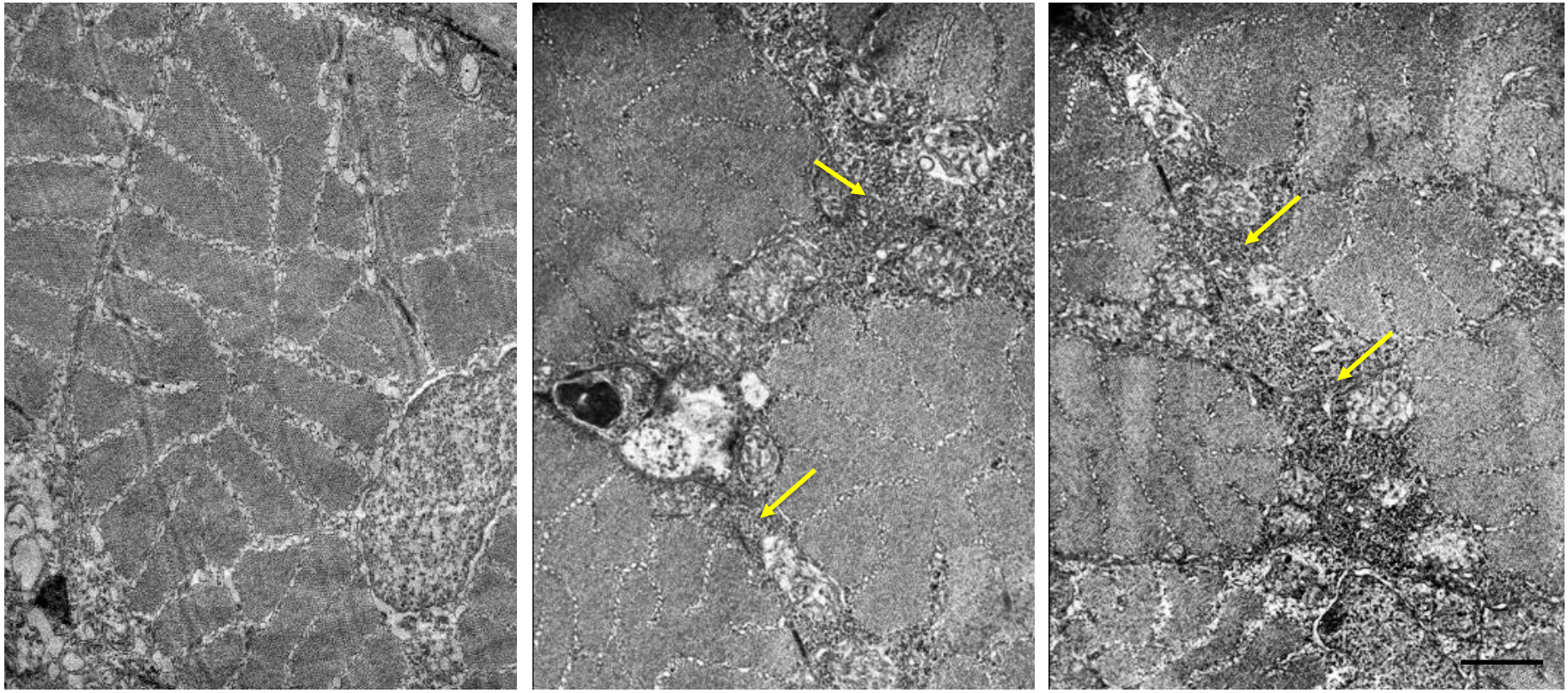
ATG*gaa*-MO

I9E10*gaa*-MO

Scale bar = 40 um

Microscopia elettronica

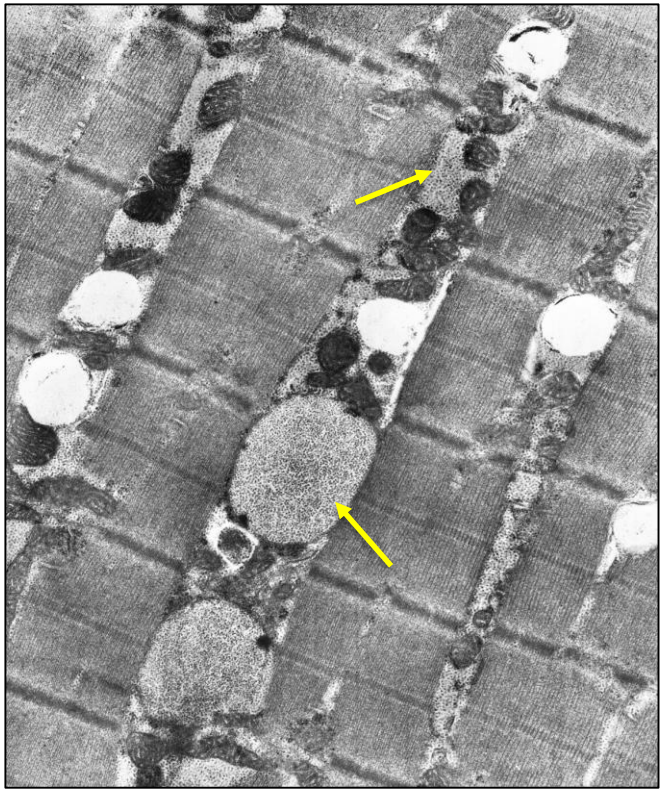
STD-MO	ATG*gaa*-MO	I9E10*gaa*-MO

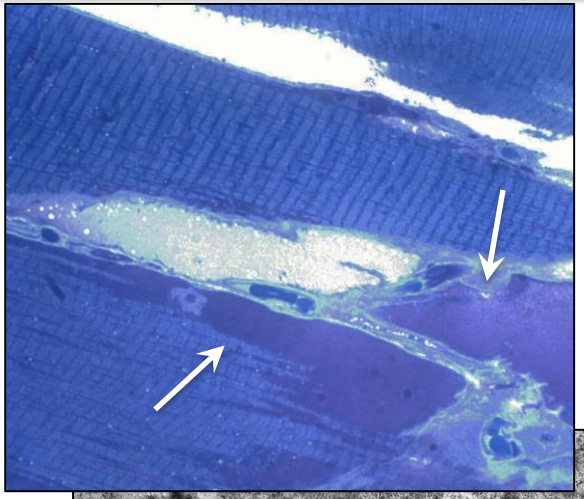


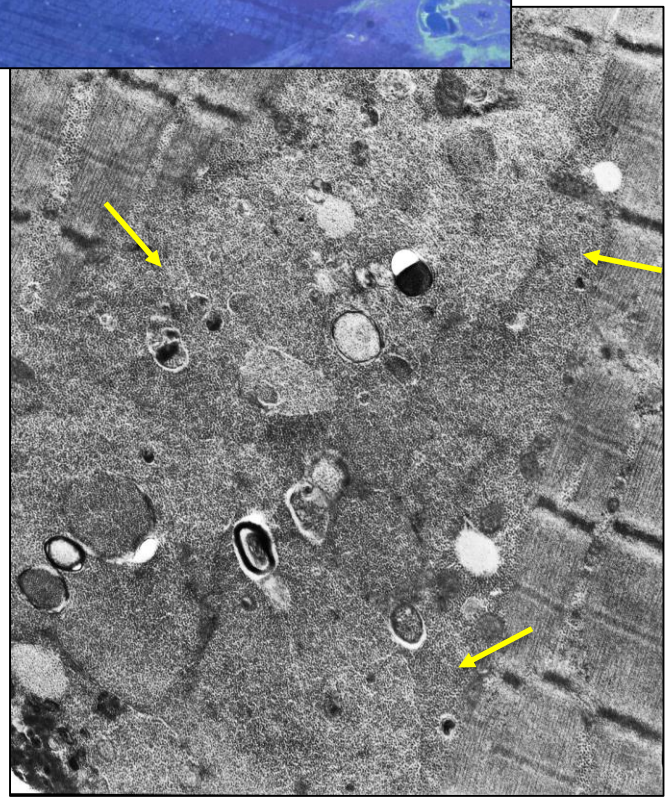
Scale bar = 2 um

Microscopia elettronica

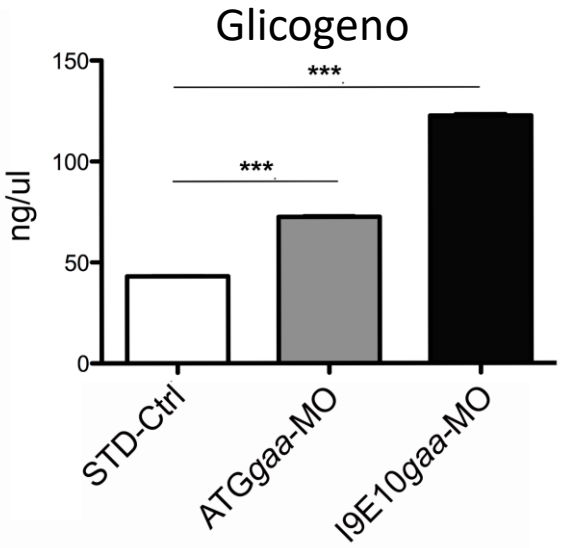
Muscolo di pazienti con malattia di Pompe

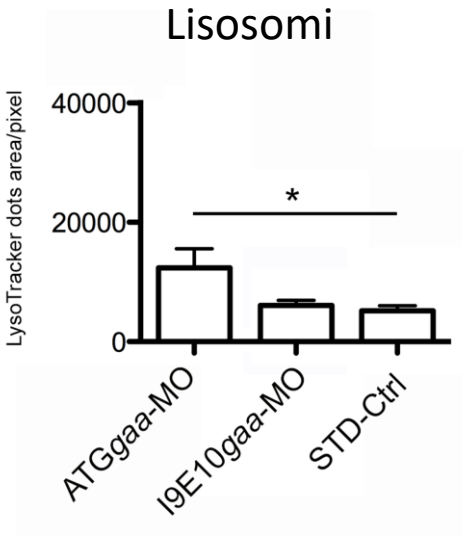


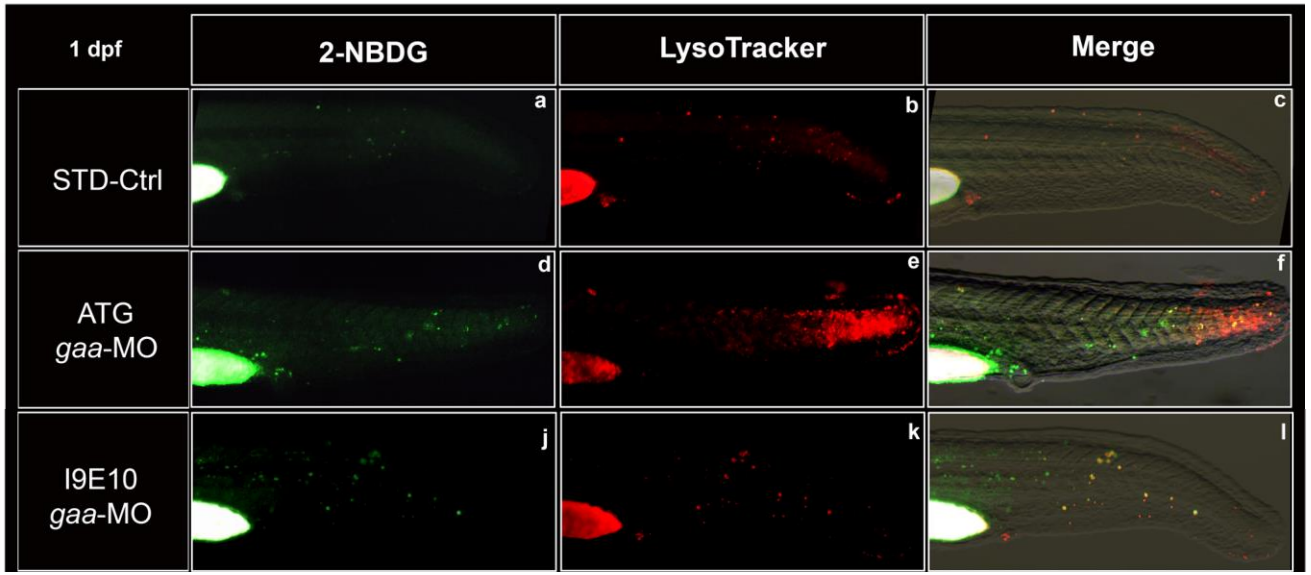




Glicogeno e lisosomi



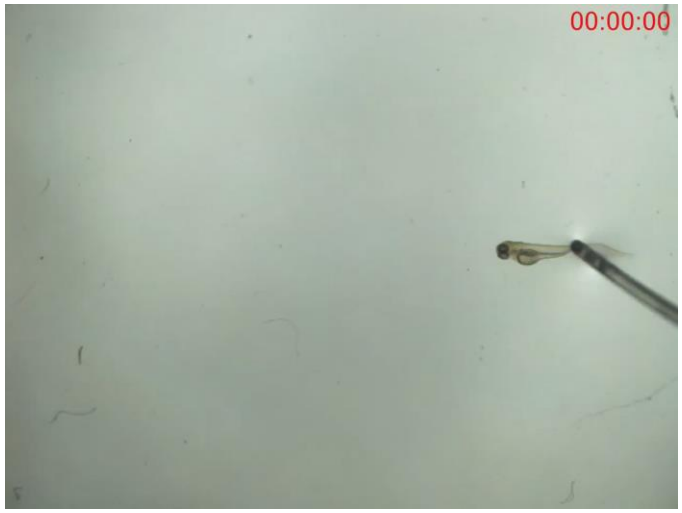




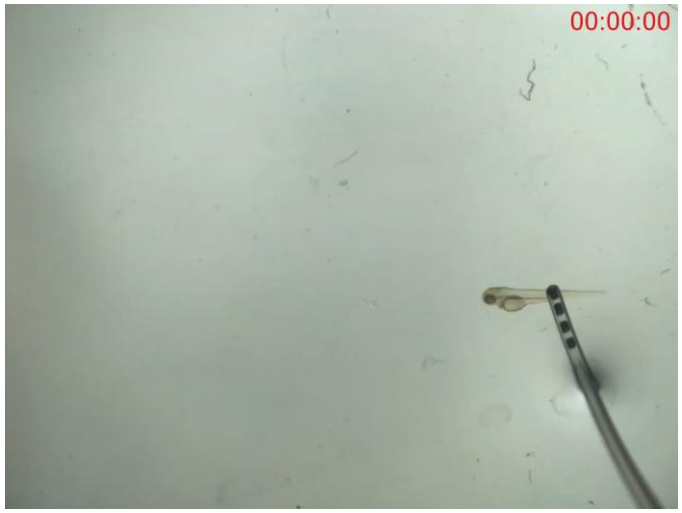
Touch-evoked response test

Embrioni a 3 dpf

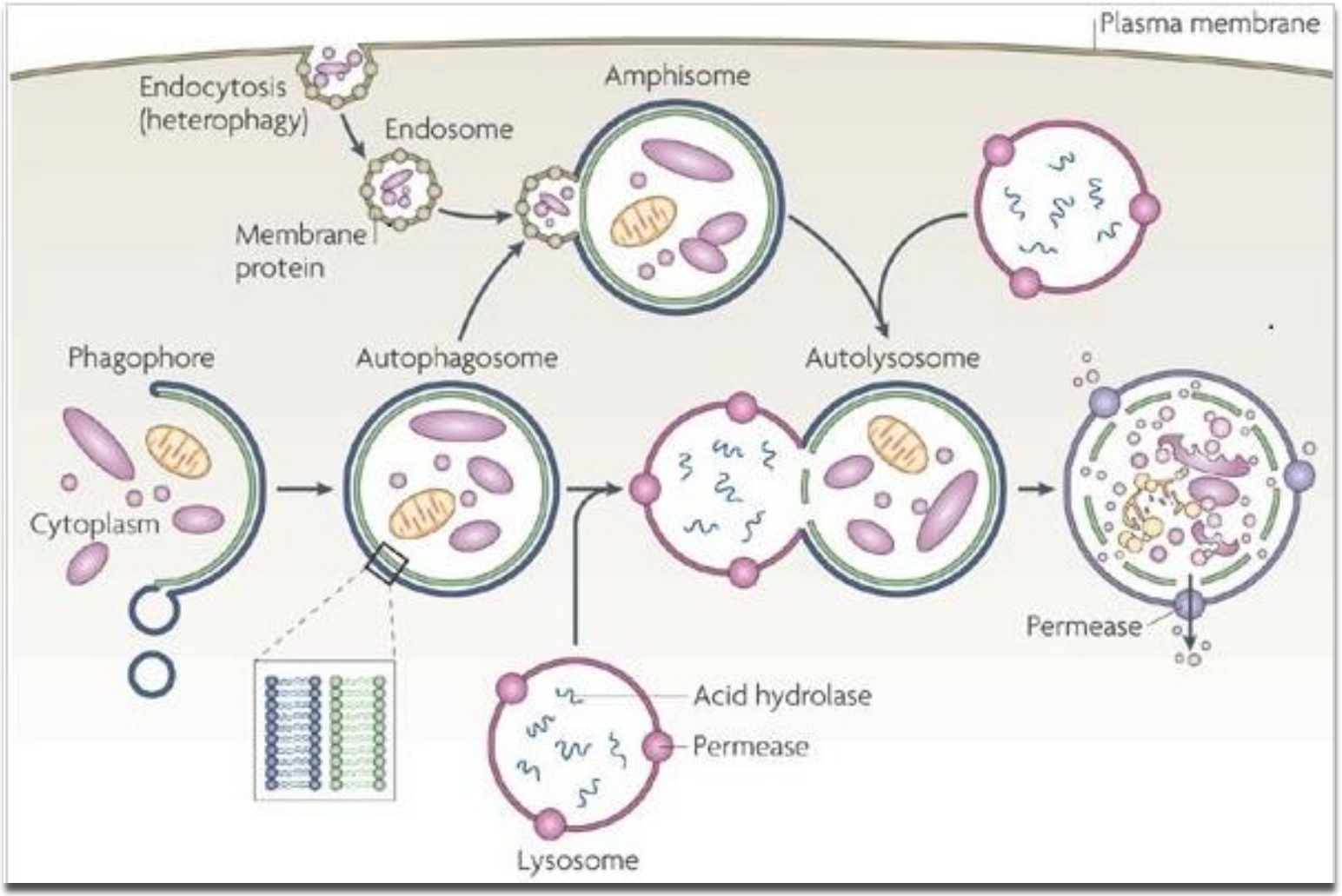
Standard Control



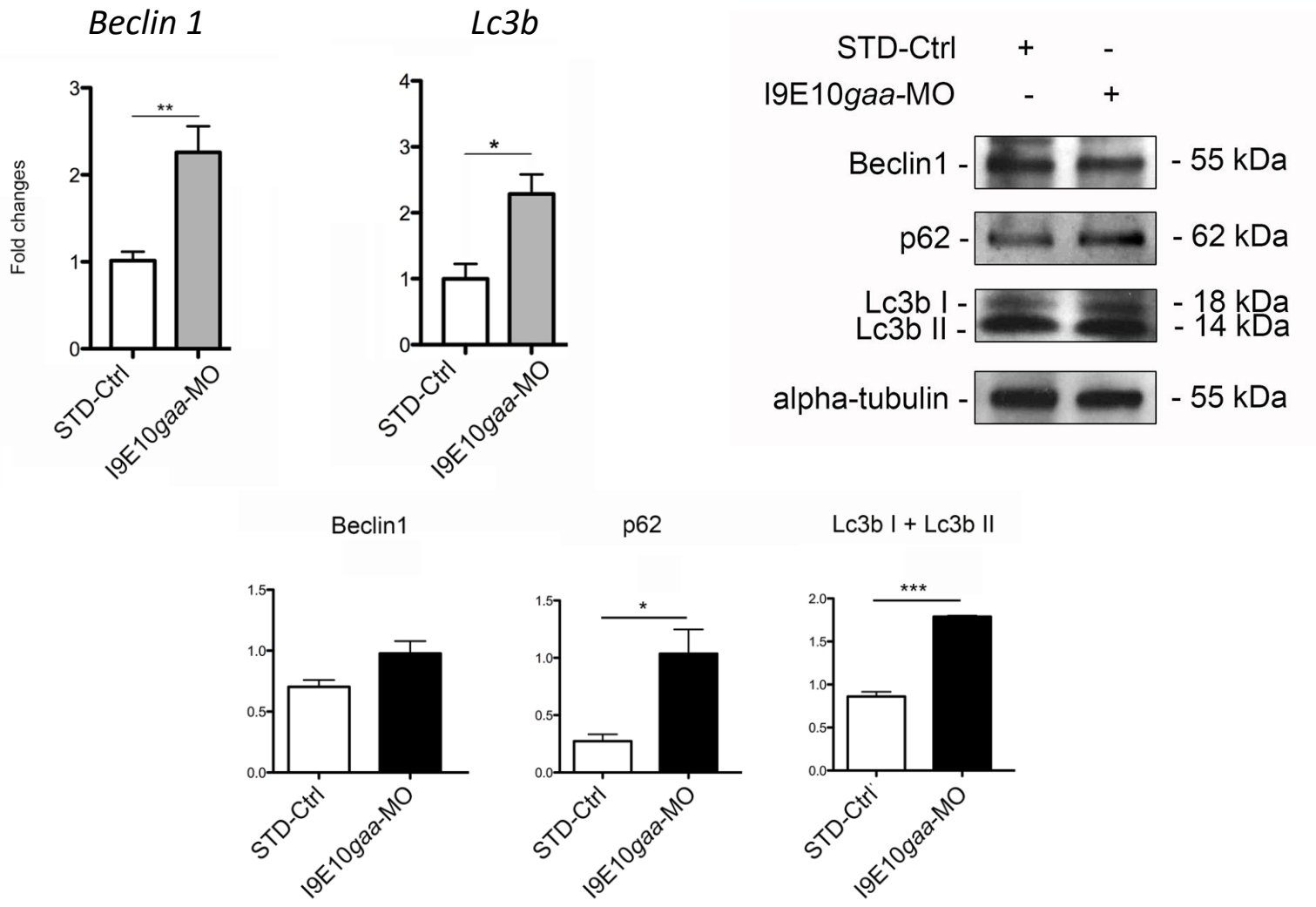
Morfante



Autofagia


Plasma membrane
Endocytosis
(heterophagy)
Endosome
Amphisome
Membrane
protein
Phagophore
Autophagosome
Autolysosome
Cytoplasm
Permease
Acid hydrolase
Permease
Lysosome

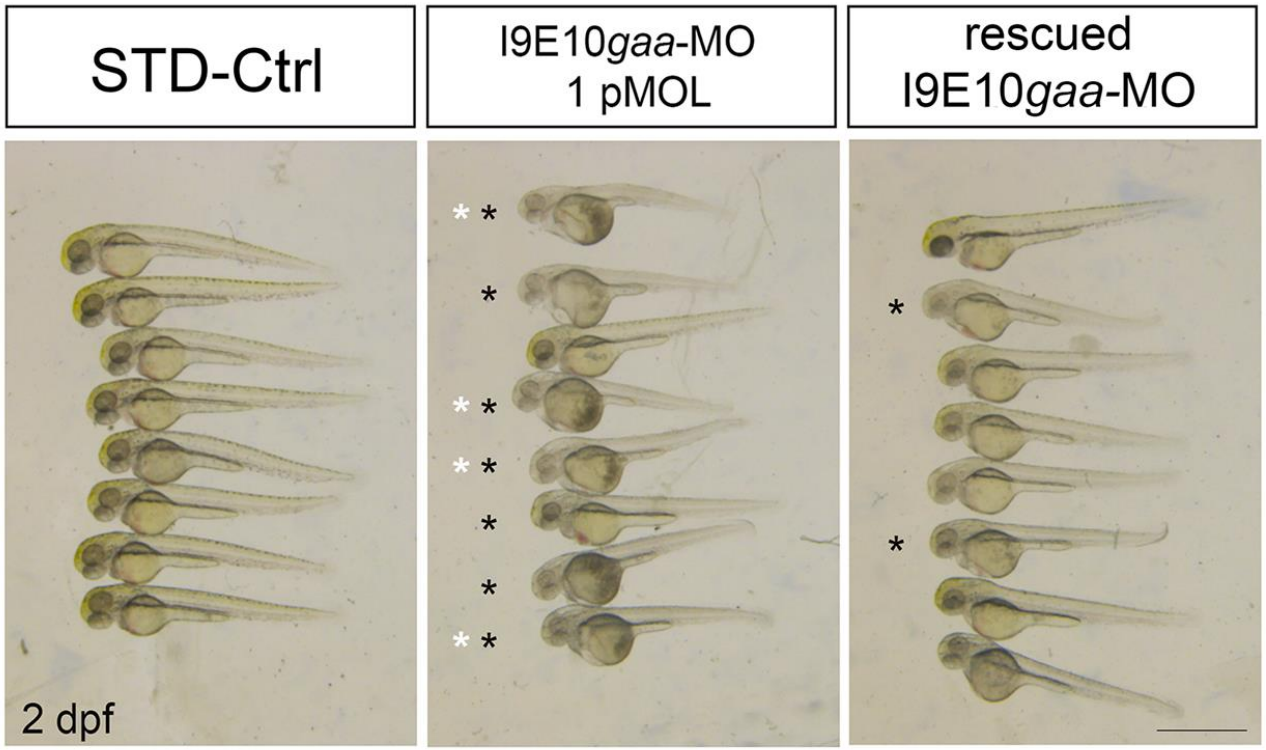
Autofagia



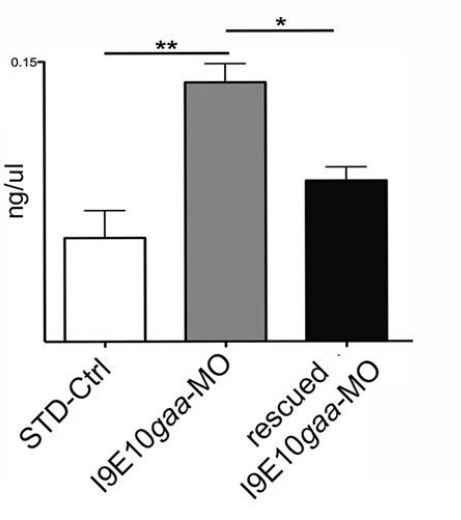
Nei morfanti trascritti e proteine dell'autofagia sono alterati

Rescue

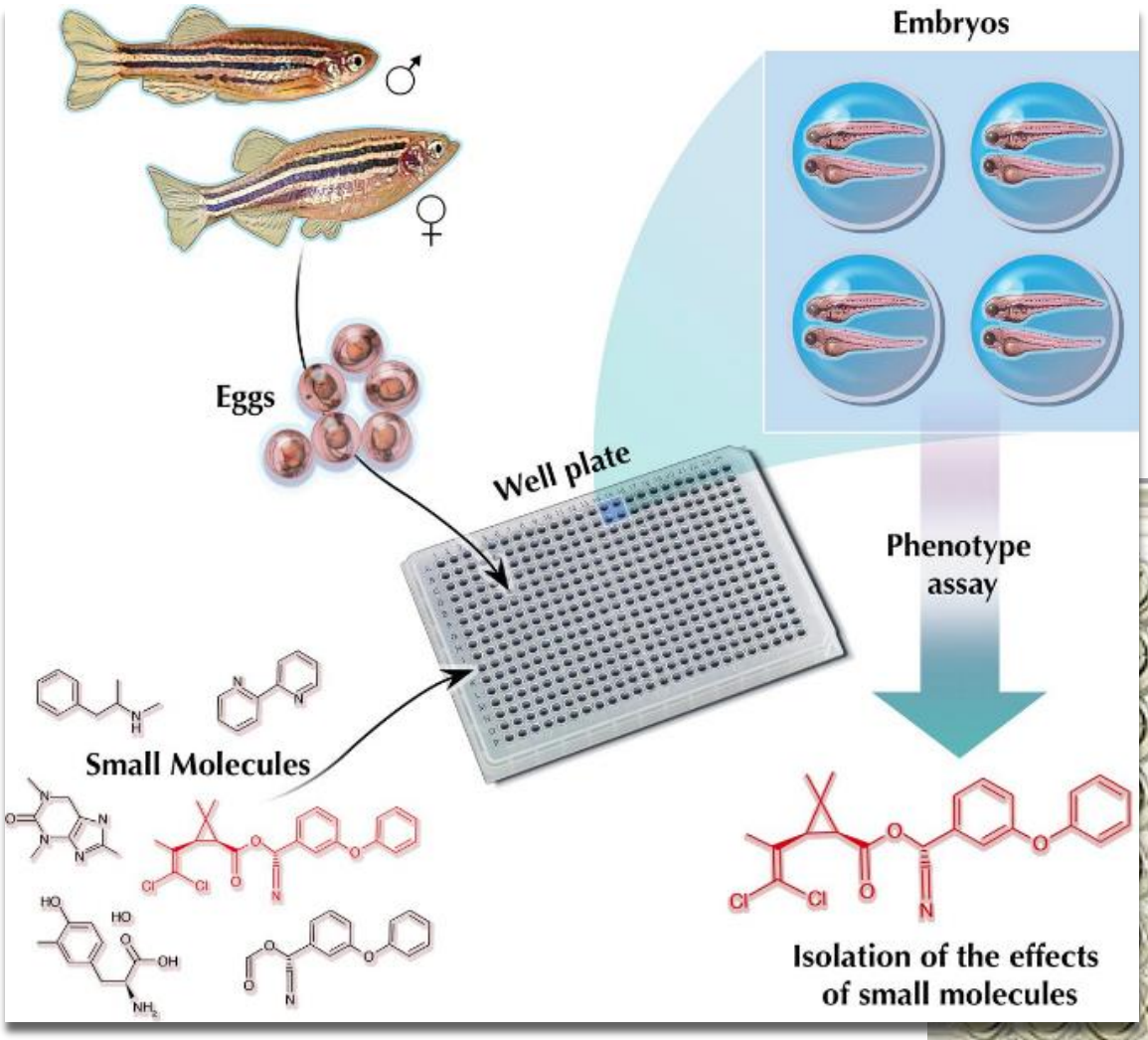
L'iniezione di *gaa m*RNA wild type ripristina il fenotipo sano

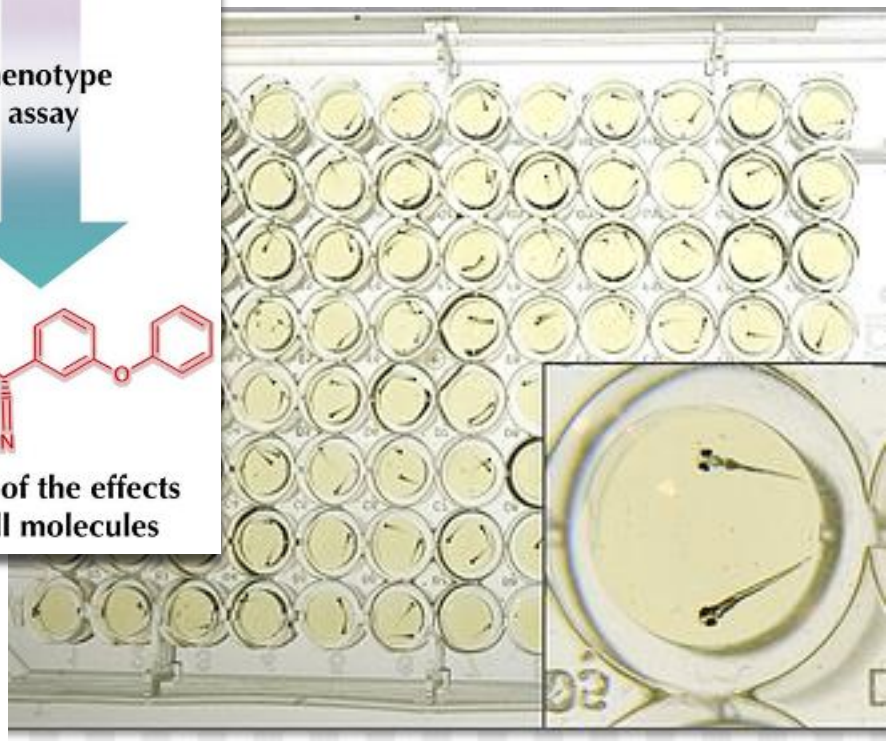


Glicogeno



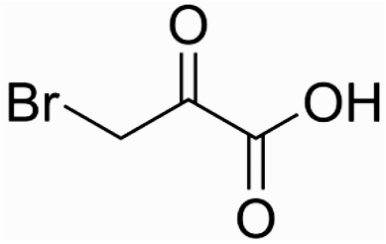
Test di composti chimici





Trattamento

Acido 3-Bromo piruvico

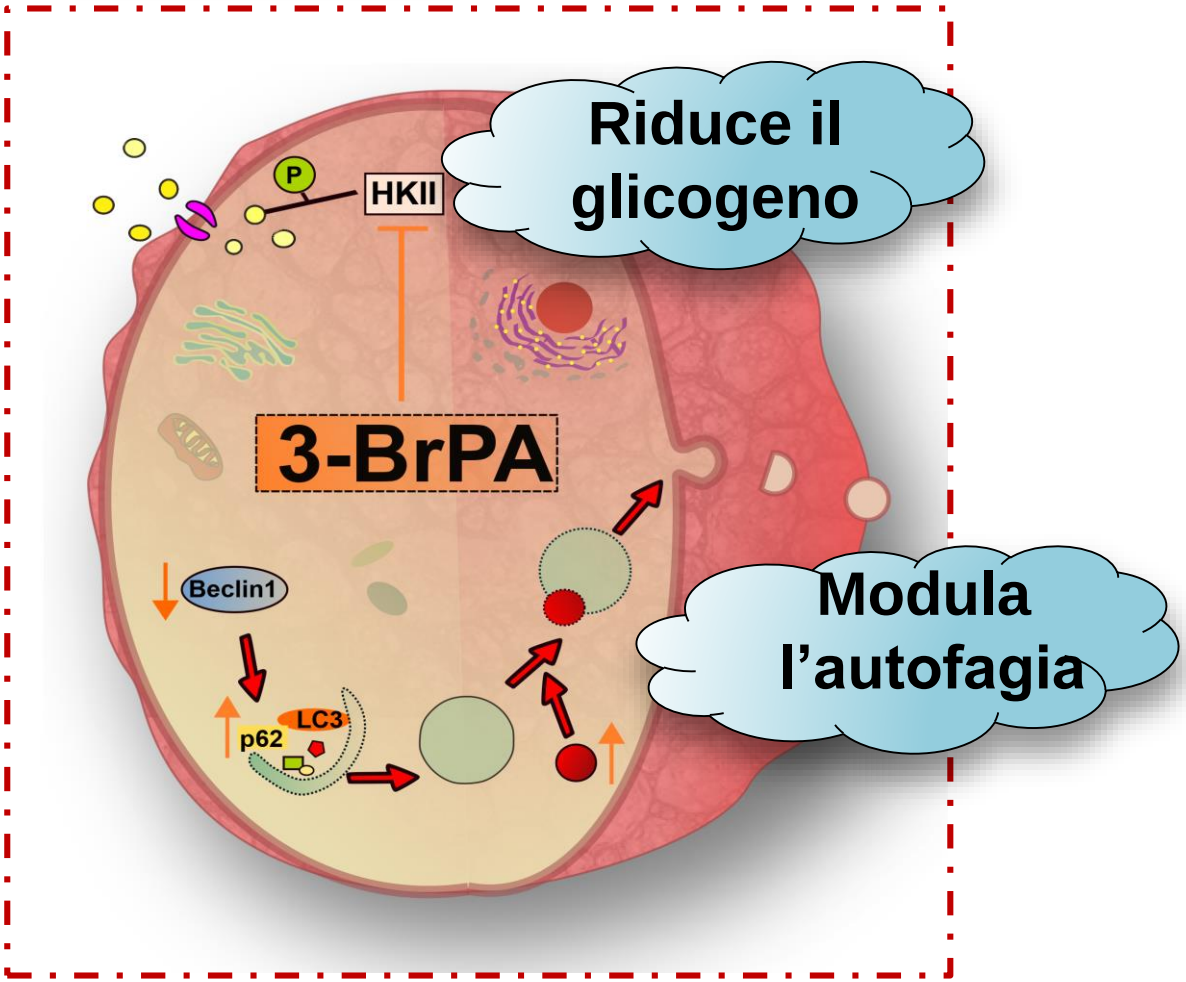


3-BrPA

3-BrPA è efficace come antitumorale nei tumori che dipendono dalla glicolisi per produrre ATP.

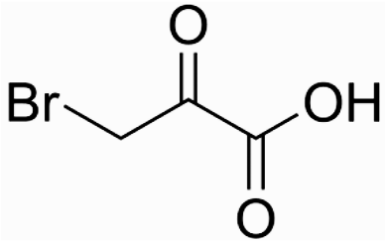
Esochinasi II è uno dei maggiori target di **3-BrPA**

3-BrPA ha una struttura simile al lattato ed è verosimilmente trasportato all'interno delle cellule attraverso un trasportatore monocarbossilato



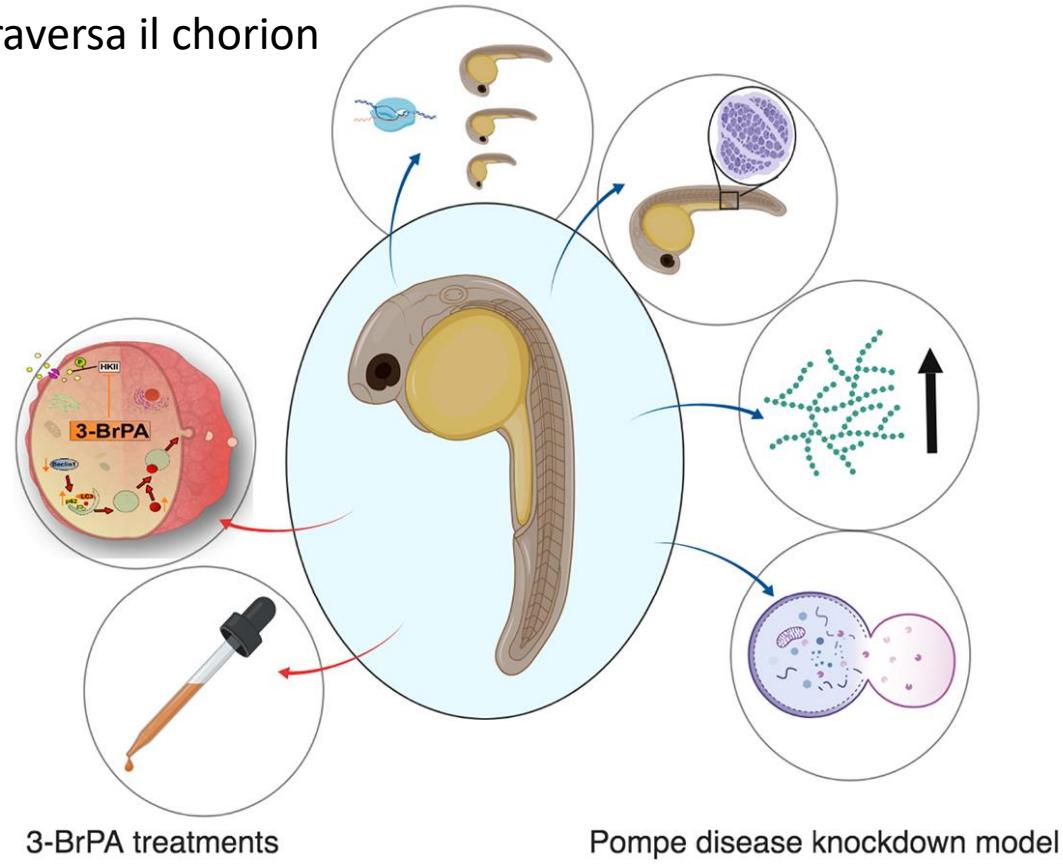
Trattamento

Acido 3-Bromo piruvico



3-BrPA

3-BrPA passa attraversa il chorion



Trattamento

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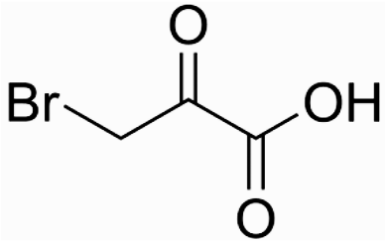
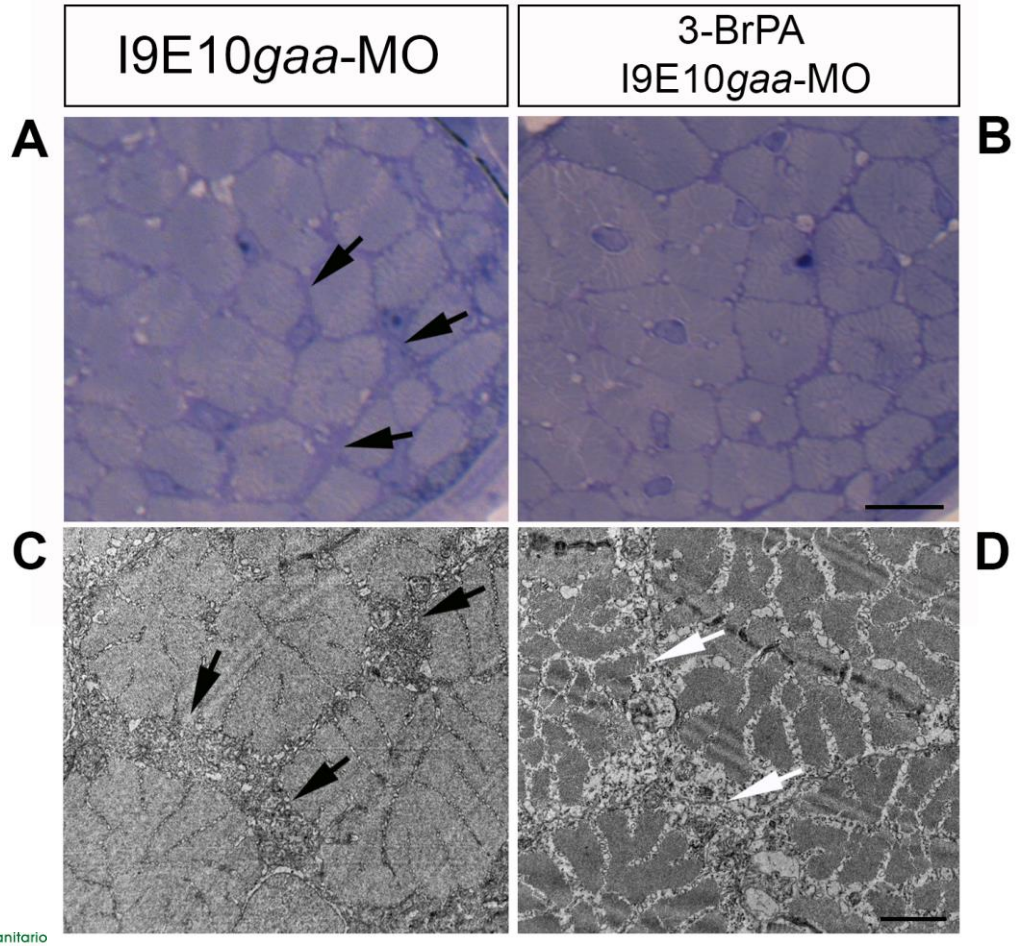
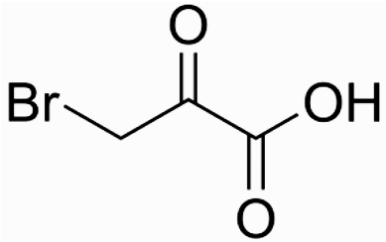


3-BrPA



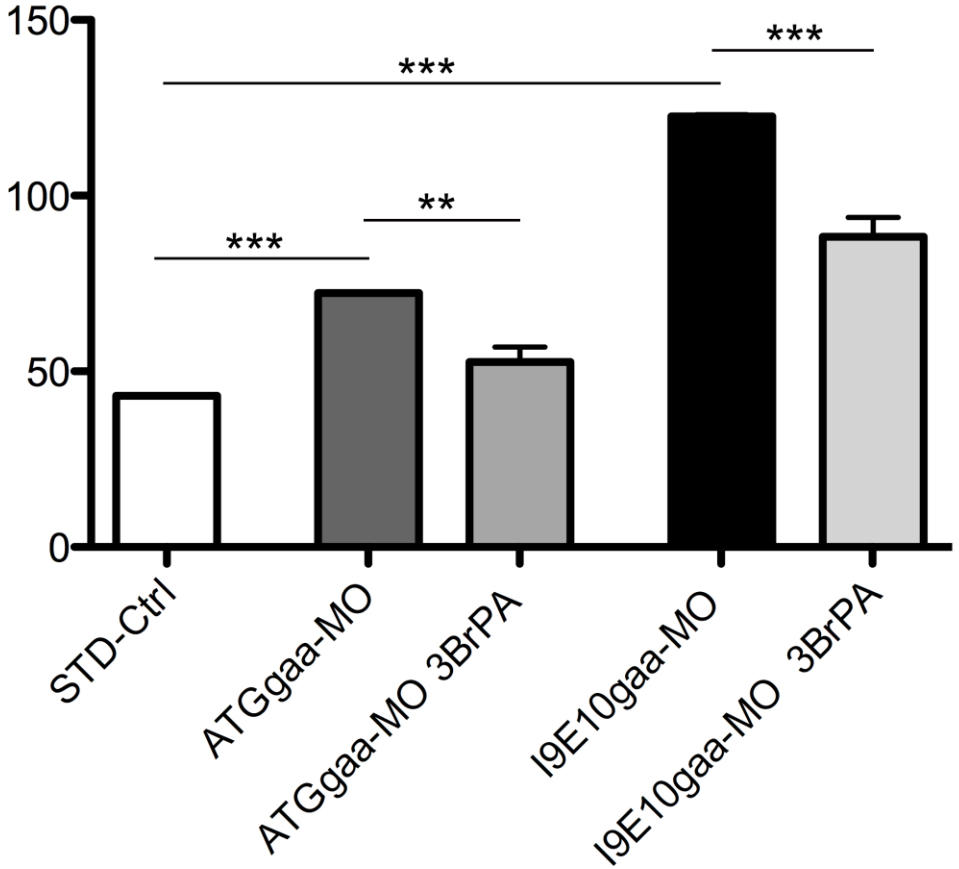
Trattamento

Acido 3-Bromo piruvico



3-BrPA

Glicogeno



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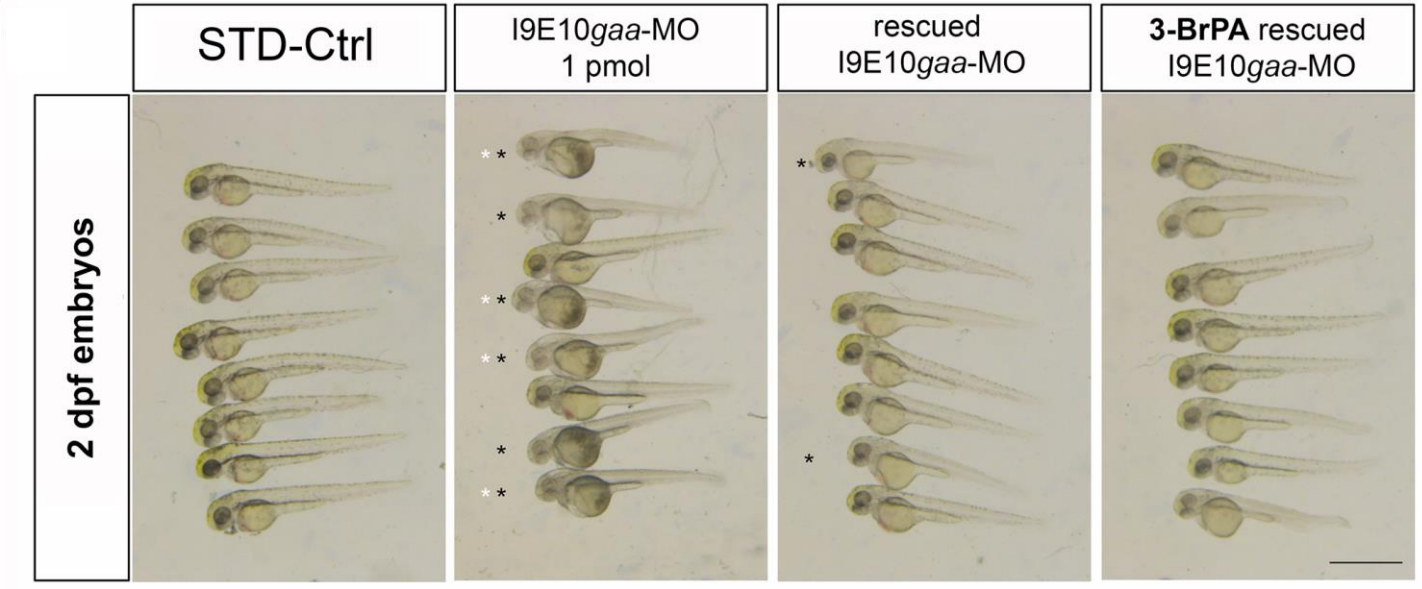
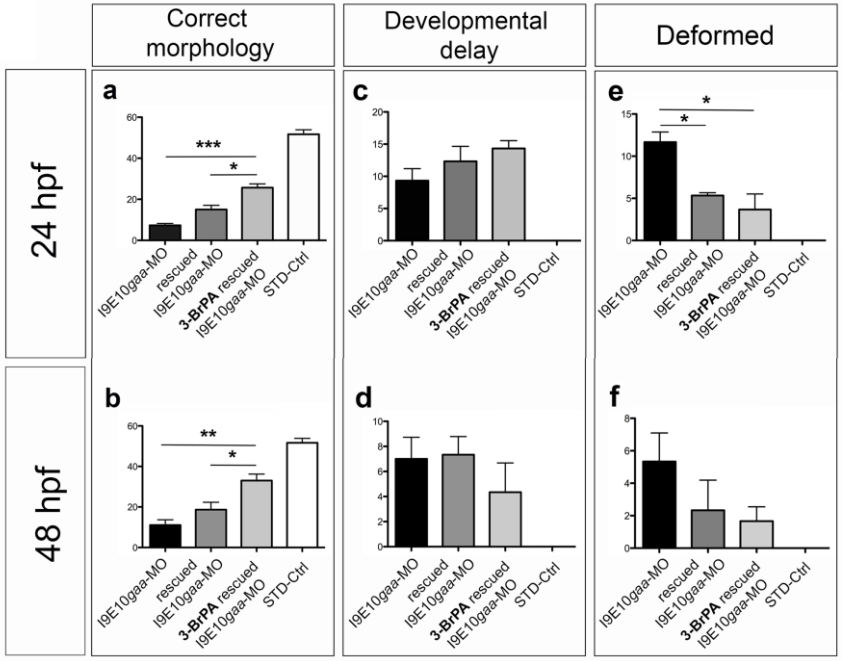


3-BrPA migliora
l'effetto rescue



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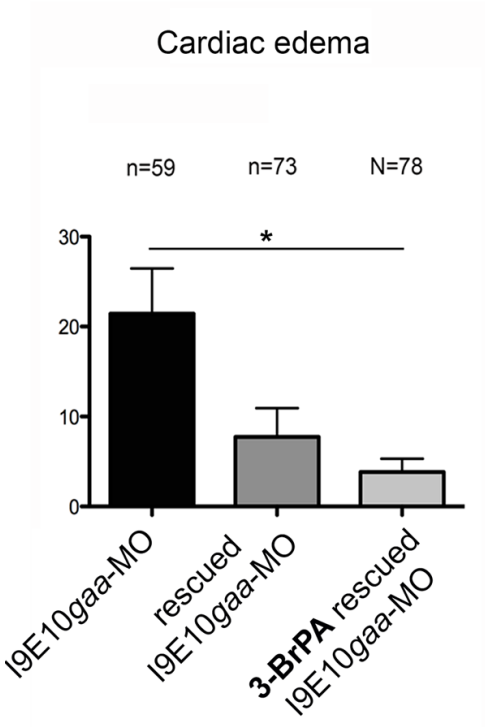
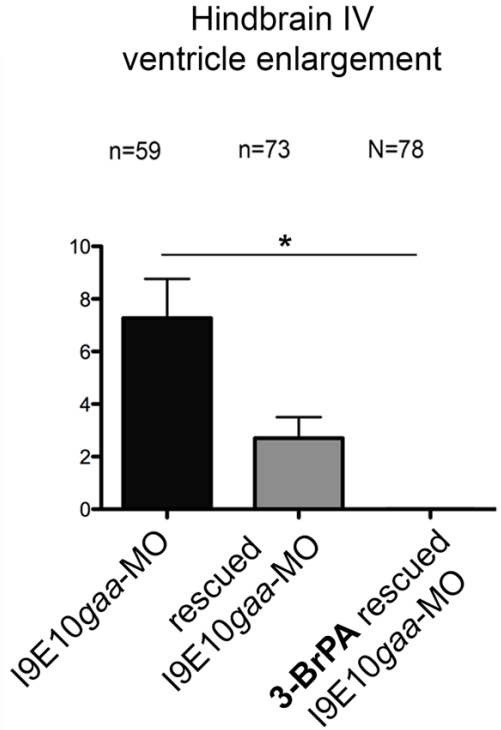
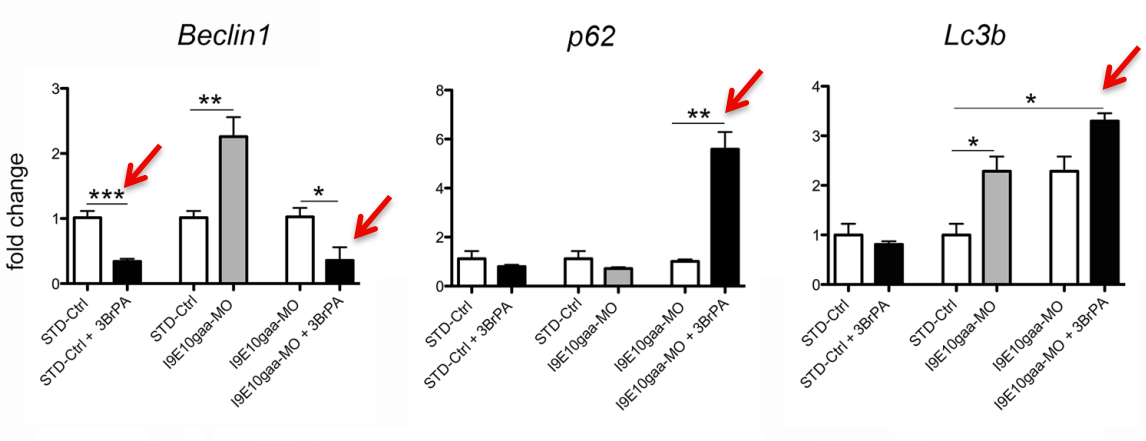


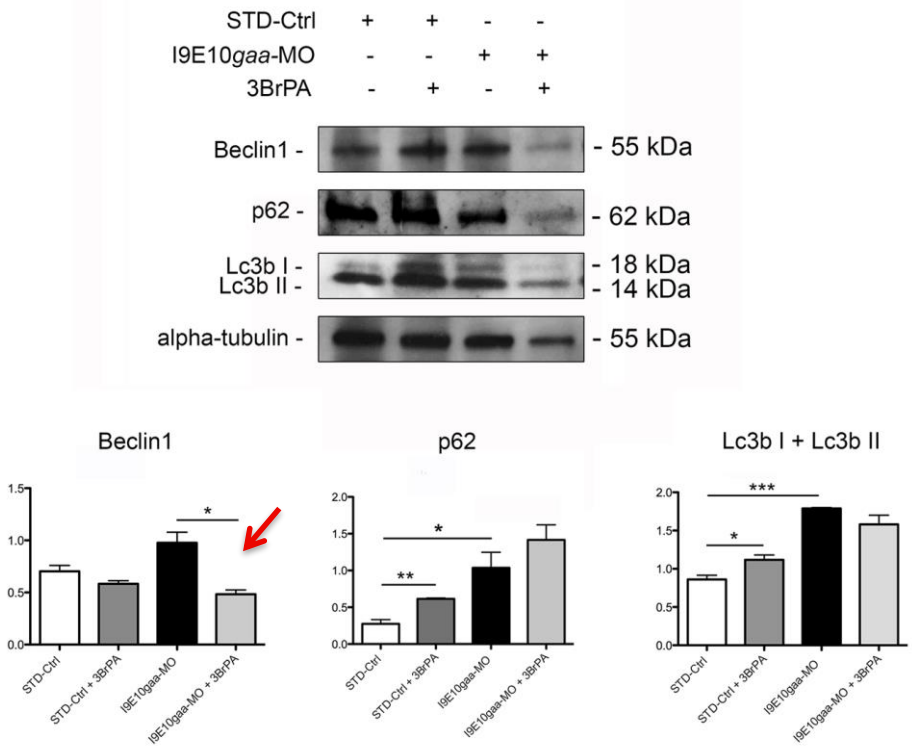


Trattamento

TRANSCRIPT LEVELS



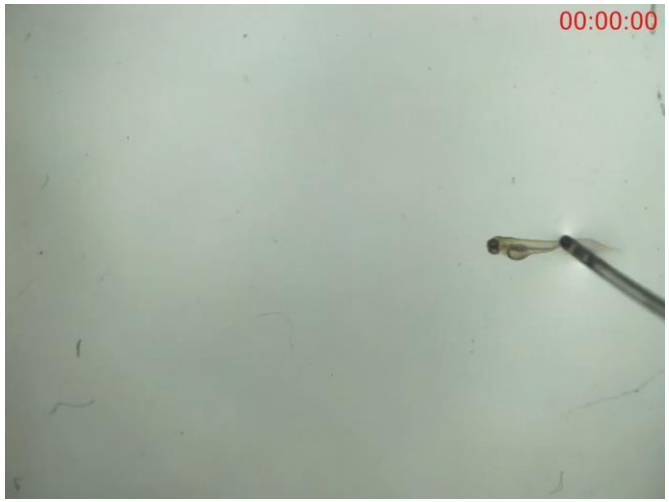
PROTEIN LEVELS



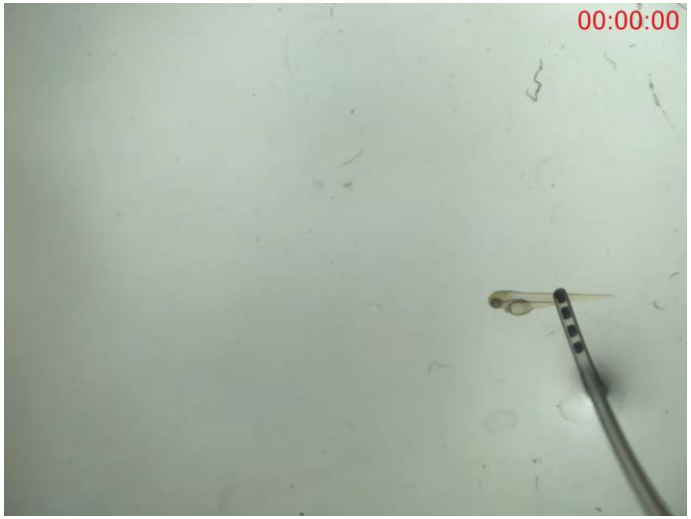
Touch-evoked response test

Embrioni a 3 dpf

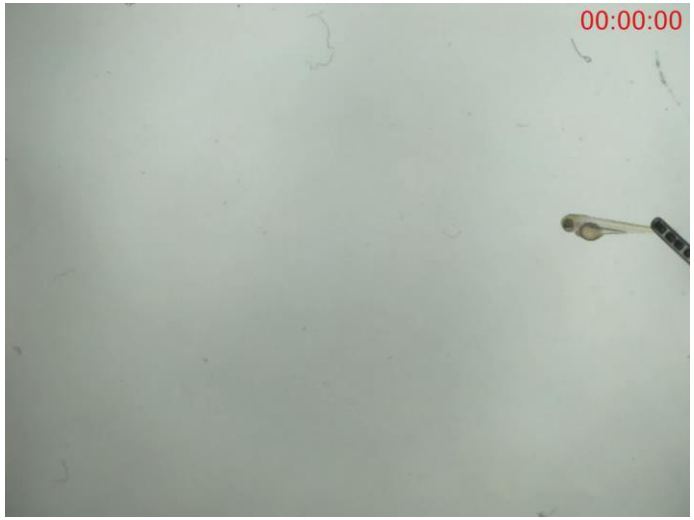
Standard



Morfante



Morfante trattato con 3-BrPA

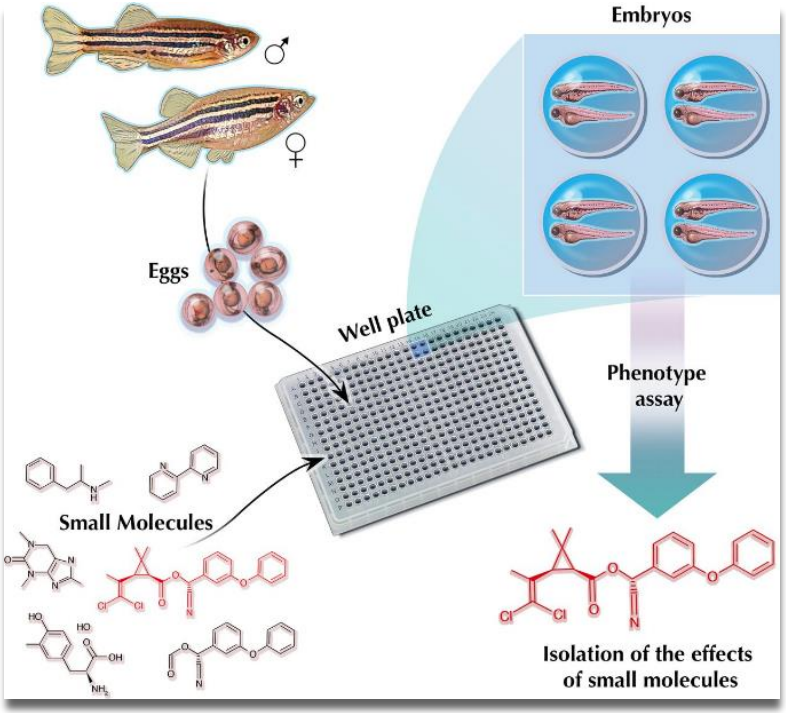


Conclusioni

- Il modello zebrafish ricapitola le caratteristiche patologiche della malattia di Pompe
- Lo zebrafish rappresenta un tool efficace e poco costoso per testare in modo veloce composti con potenziale terapeutico
- 3-BrPA potrebbe essere un adiuvante della terapia enzimatica

Prospettive future

- Validare i nostri risultati nel modello stabile fino all'adulto
- Verificare l'effetto del trattamento a lungo termine
- Utilizzare il modello transiente per selezionare altri composti



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GRAZIE!

Alessia Brix

AIG: Associazione Italiana Glicogenosi Onlus