Modeling FSHD in zebrafish

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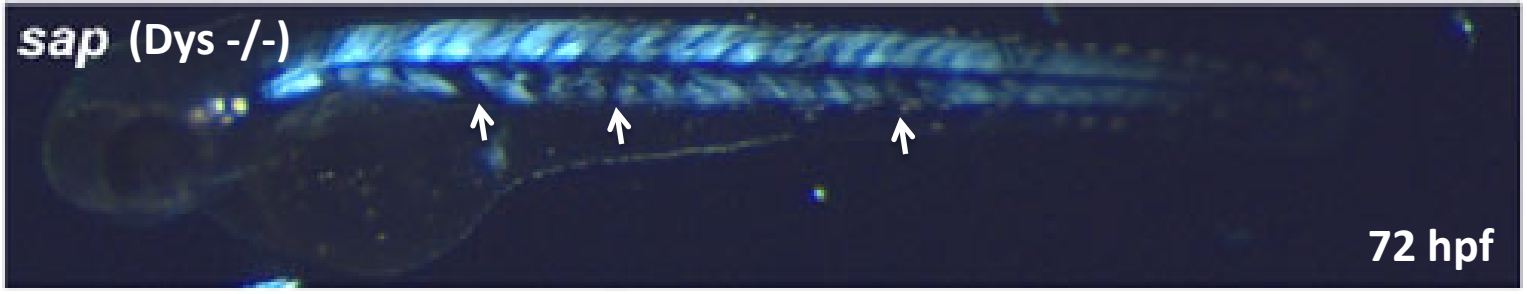
Advantage of zebrafish

- Many eggs : 100-300 eggs / pair
- Rapid development : Embryos hatch in 72 hours
- Muscular dystrophic phenotype is easily detected by birefringence
- Small compounds penetrate into zebrafish embryos



Jacoby et al., *Development* 2009

Final goal

Drug screening in zebrafish model of FSHD

Aim 1

How expression of DUX4 causes FSHD-like phenotype in zebrafish

Aim 2

To establish DUX4-transgenic fish

Chemical screens of Dystrophin Deficient Zebrafish for Functional Modifier

Chemical screening of small molecules using dystrophin null fish

Purpose:

Screening for effective chemicals to rescue the muscle phenotype

Methods:

1. Sapje and Sapje-like fish (heterozygous fish +/-)

(+/-) X (+/-)

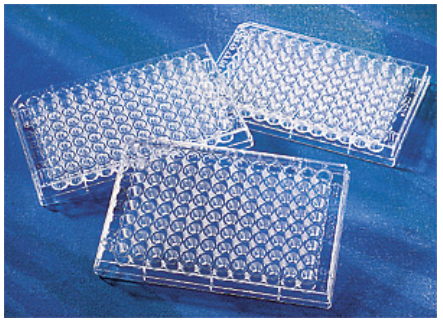
+/+ +/- -/-

25% 50% 25 %

2. Chemical treatment with 2.4 μg/ml from 1 to 4 dpf

Chemical library: Prestwick Collection 1120

- Chemicals are already approved by the FDA for treating disease
- The mechanism for drug action of the compounds is already known.



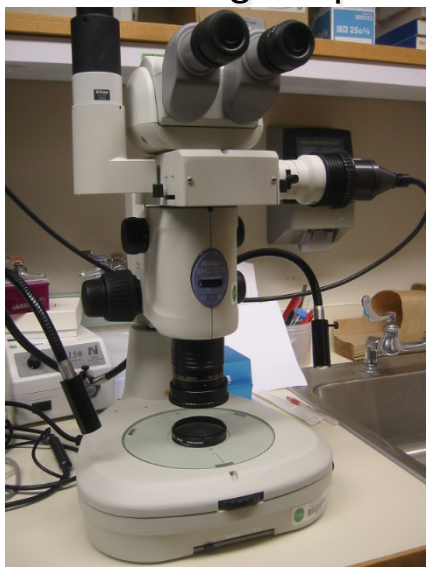
3. Birefringence assay

At 4 dpf, all fish were examined by birefringence and the number of affected fish were counted.

- 25% ➡ non effective
- less than 25% ➡ effective for decreasing affected fish

Observation of muscle by Birefringence

Dissecting scope

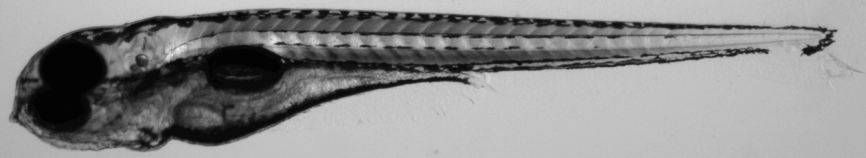


Polarizing filter

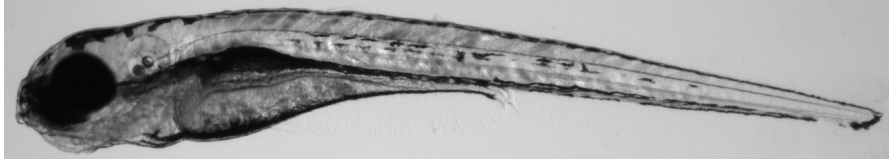
Anesthetized fish

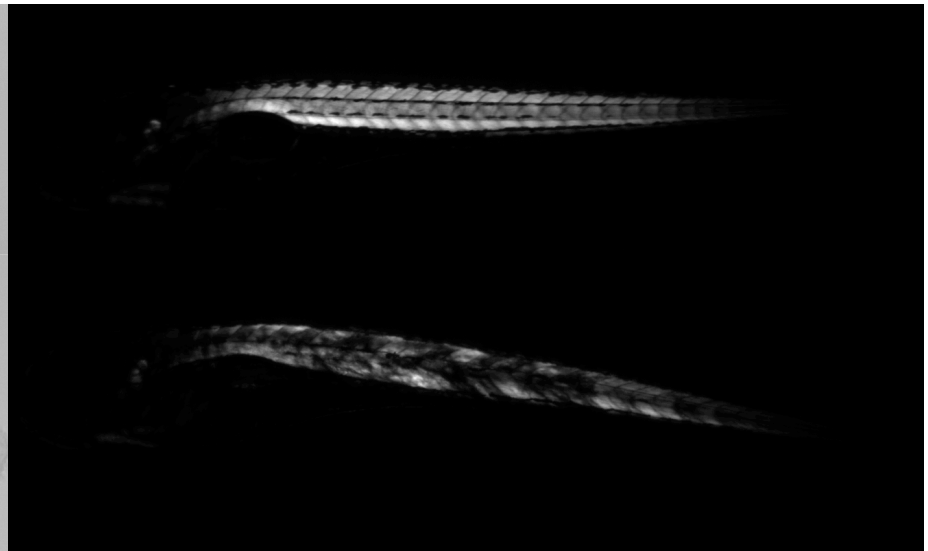
Polarizing filter

Wild type fish



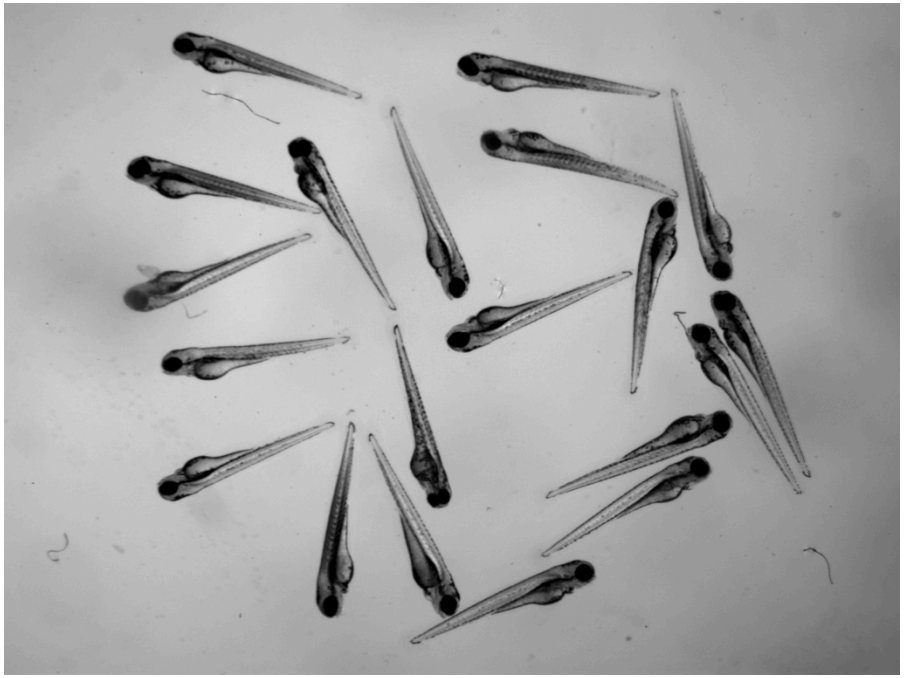
Sapje fish (DMD model fish)



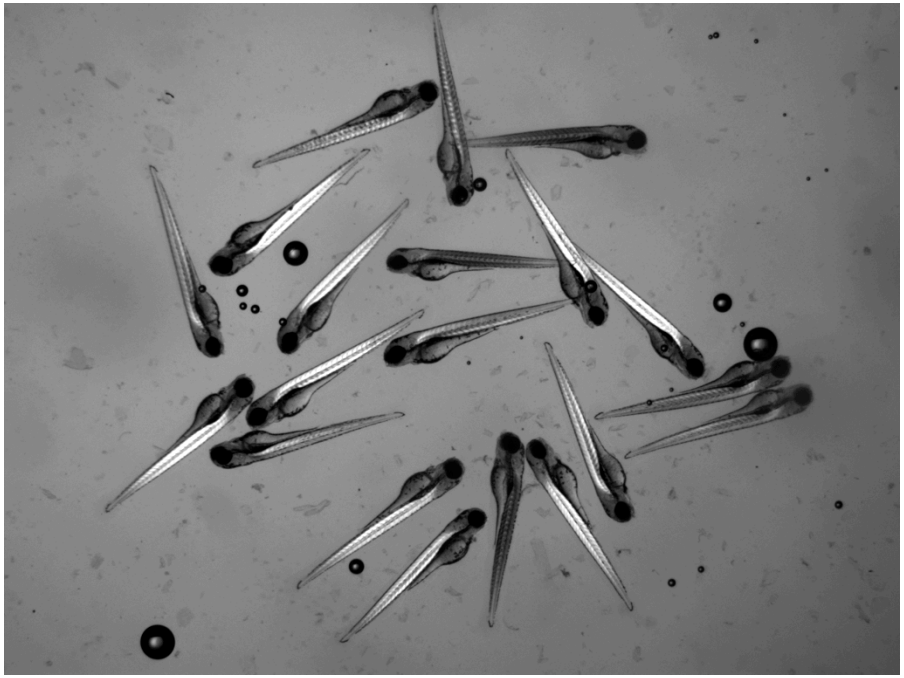


Examples of effective and ineffective chemicals

20 fish

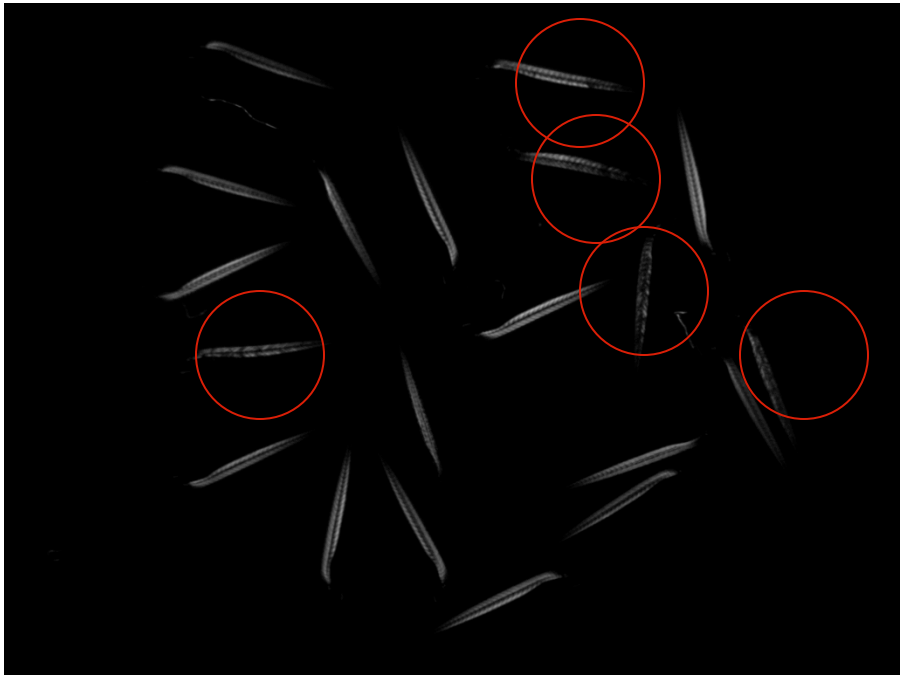


20 fish



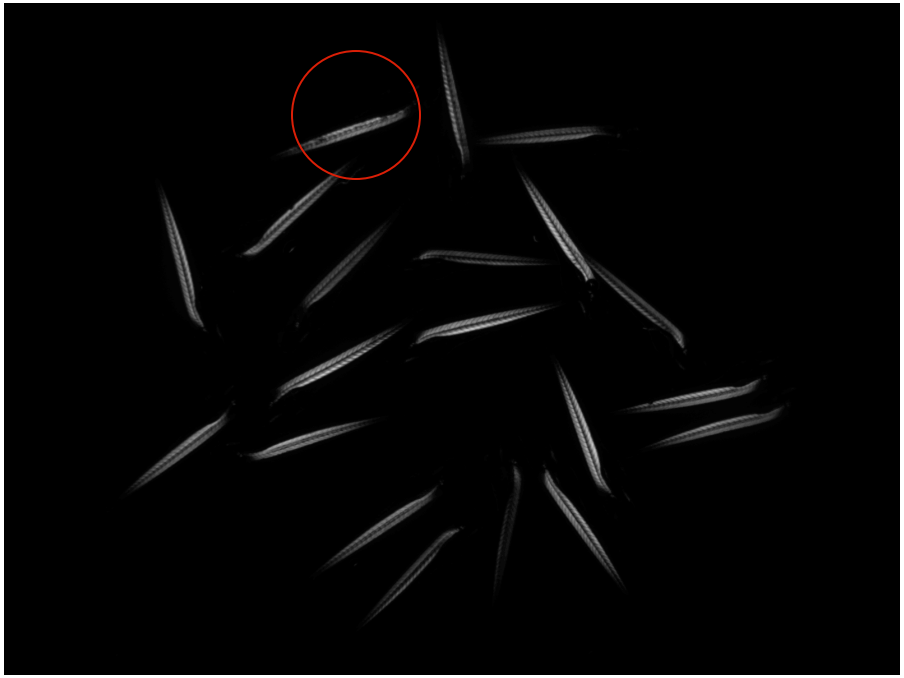
Examples of effective and ineffective chemicals

5 affected fish /20 fish (25%)



Non-effective

1 affected fish /20 fish (5%)



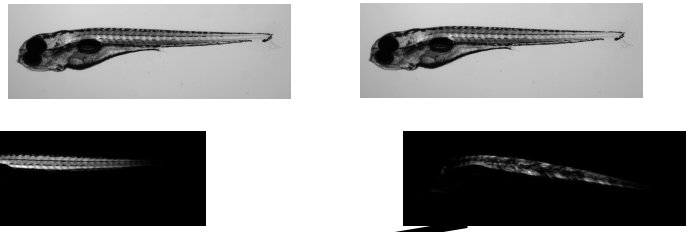
Effective

Candidate chemicals from our screens

No.	Chemicalname	Chemicallibrary
#1	Epirizole	Prestwickcollection1
#2	Homochlorcyclizine	Prestwickcollection1
#3	Conessine	Prestwickcollection1
#4	Aminophylline	Prestwickcollection1
#5	Equilin	Prestwickcollection1
#6	Penteticacid	Prestwickcollection1
#7	ProscillaridinA	Prestwickcollection1
#8	Nitromide	NINDS2Compoundlibrary
#9	Propanthelinebromide	NINDS2Compoundlibrary
#10	Androsteroneacetate	NINDS2Compoundlibrary
#11	Crassinacetate	NINDS2Compoundlibrary
#12	Pomiferin	NINDS2Compoundlibrary
#13	Cerulenin	ICCBKnownBioactivesLibrary
#14	9a,11b-ProstaglandinF2	ICCBKnownBioactivesLibrary

Affected Fish with Chemicals #1-7

Het (+/-) X Het (+/-)



75% 25%

Day 4 embryos -10 /cage

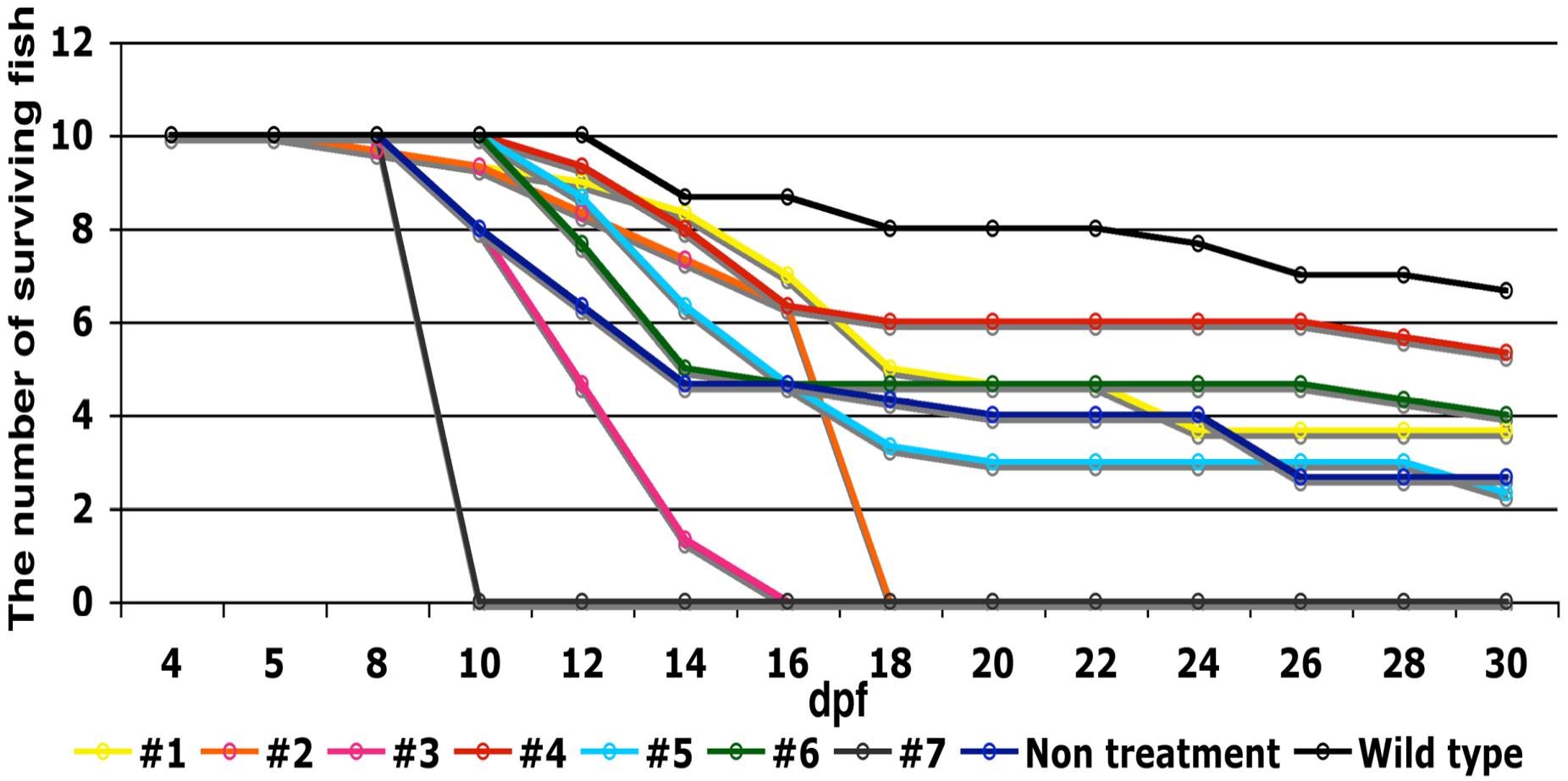
1. Treatment with individual chemicals (2.5 μg/ml)
2. Non treatment
3. Wild type

Culture fish from day 4 to day 30 in triplicate

Survival fish for 30 days

- Number of surviving fish
- Genotyping
- IHC

Some chemicals increase the life span of dystrophin null fish



Final goal

Drug screening in zebrafish model of FSHD

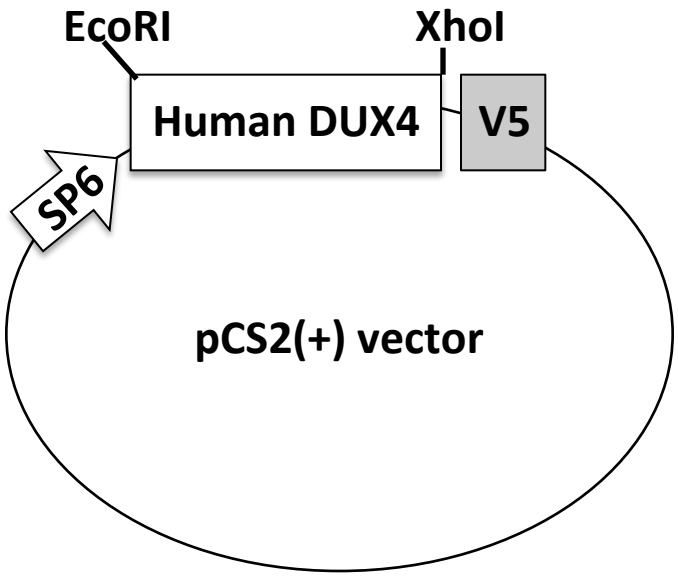
Aim 1

How expression of DUX4 causes FSHD-like phenotype in zebrafish

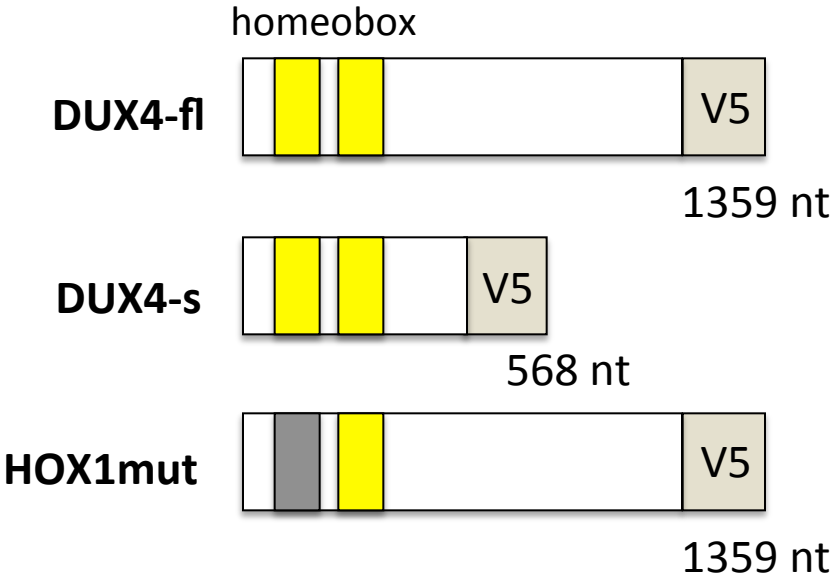
Aim 2

To establish DUX4-transgenic fish

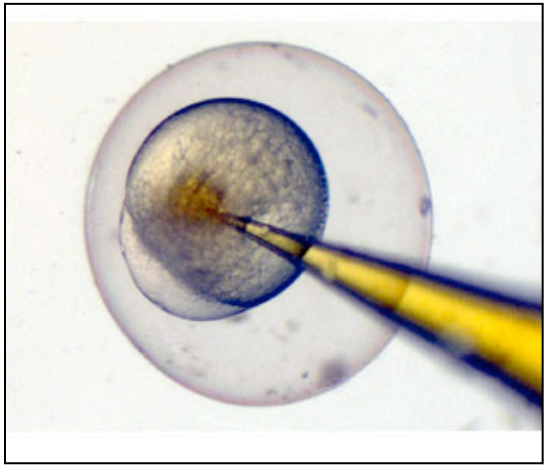
1. Cloning of human DUX4



2. Synthesize DUX4 mRNA *in vitro*



3. Injection into zebrafish embryos



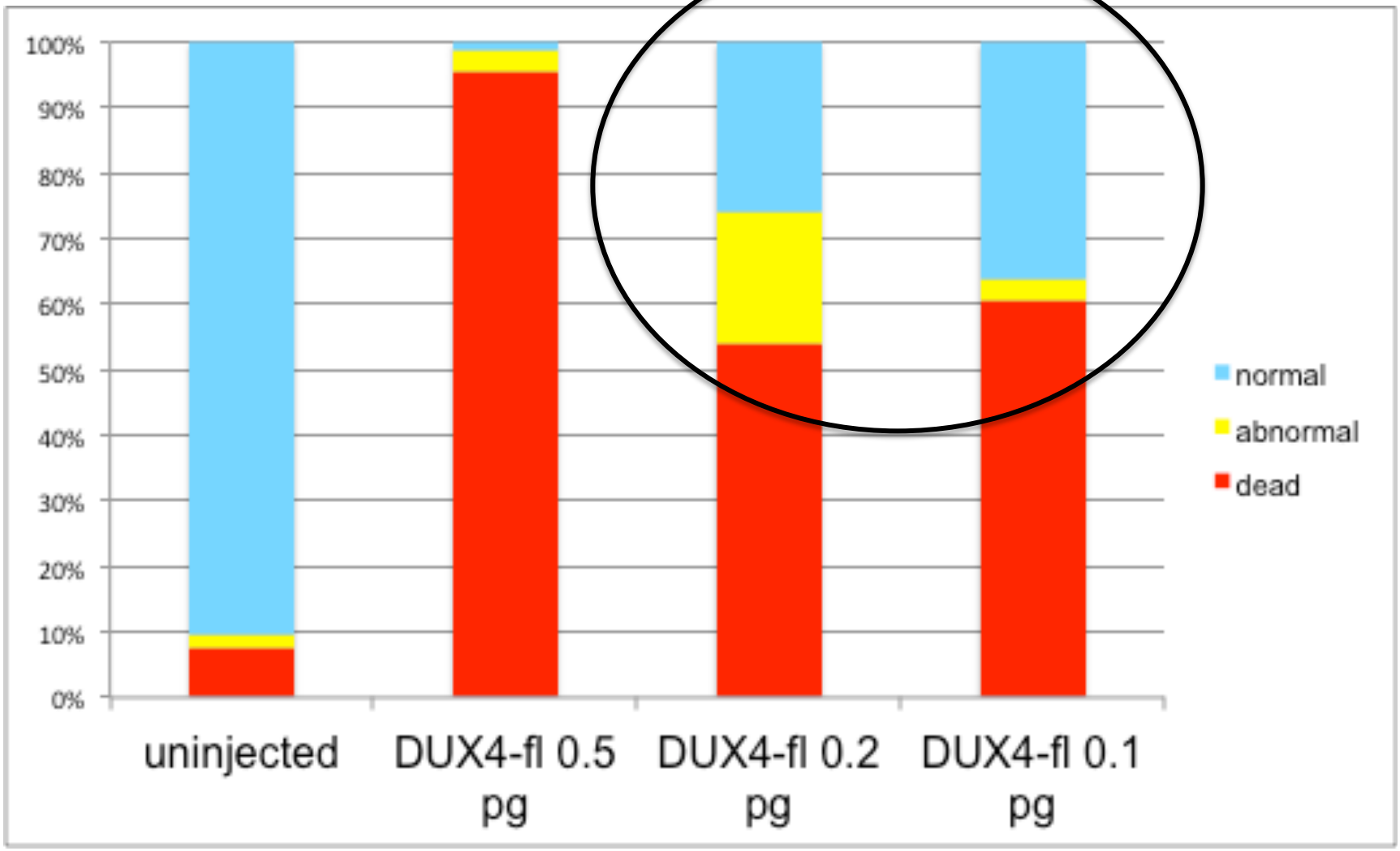
10, 0.5, 0.2, 0.1 pg mRNA per embryo

Only 1/1000 cells from FSHD patient express DUX4-fl

What will happen if we inject DUX4 less than 0.5 pg?

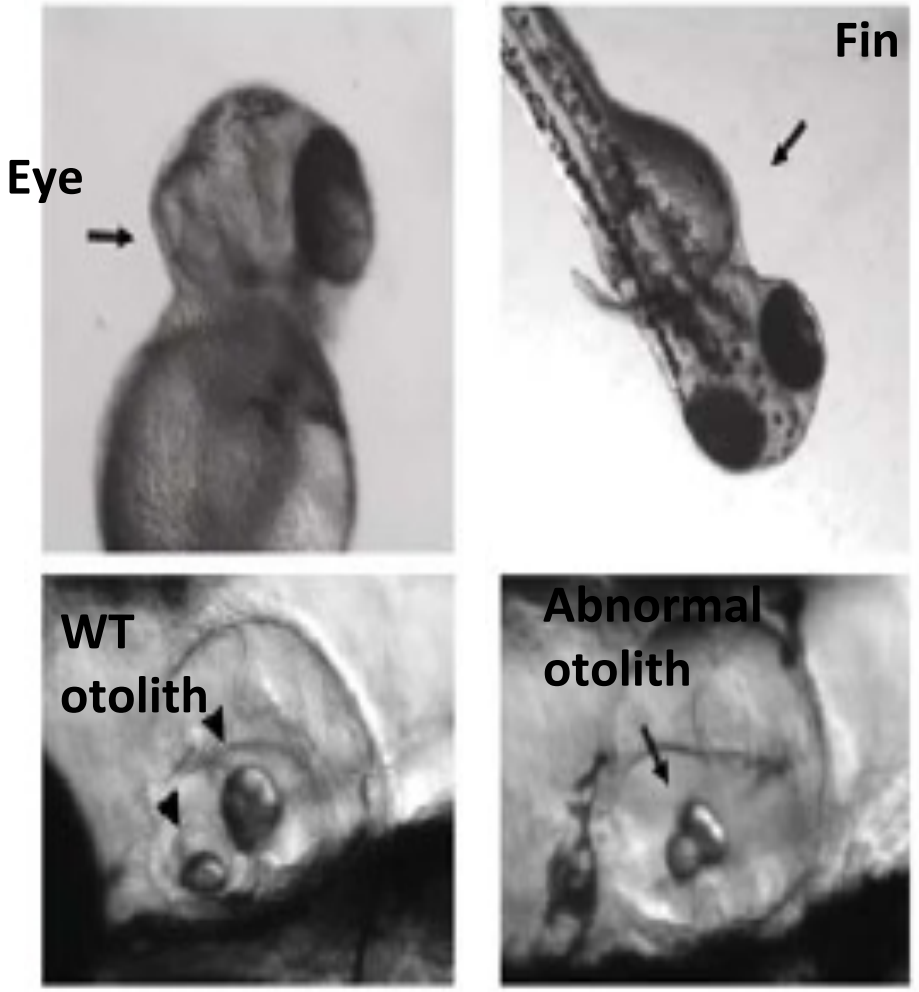
Day 4

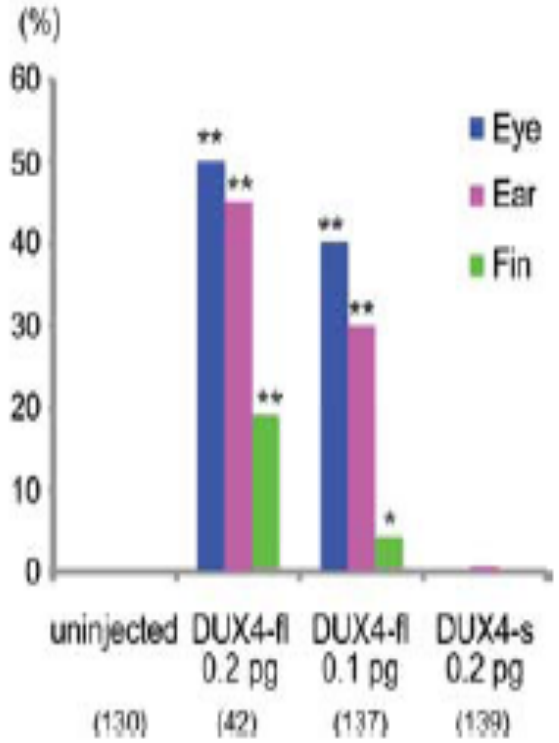
0.5, 0.2, 0.1 pg / embryo



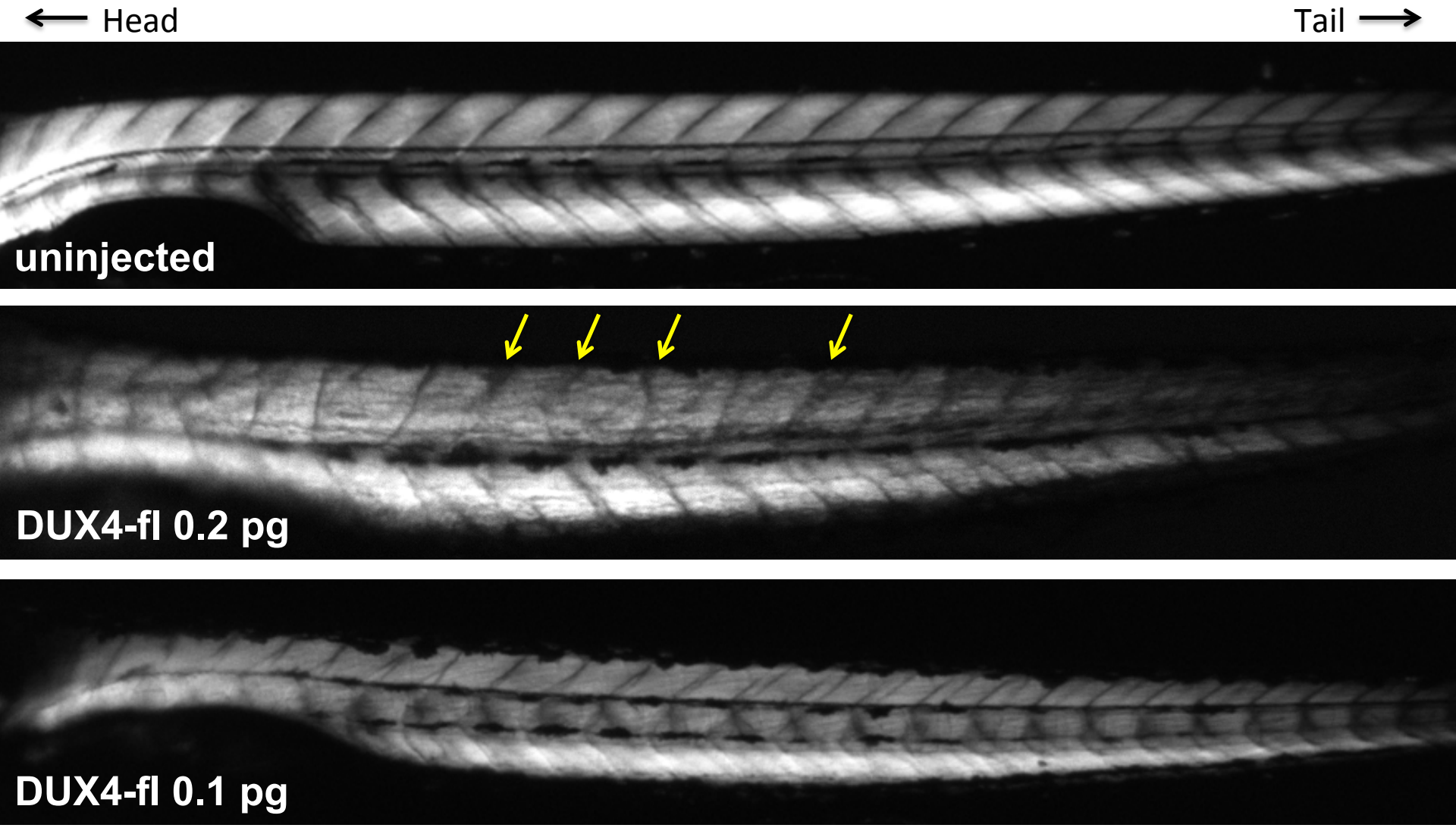
N = 150-200 embryos

- Low levels of DUX4-fl resulted in asymmetric abnormalities of the eyes, ears and fins in a dose-dependent manner
- Along with muscular dystrophy, FSHD patients experience hearing loss and retinal vasculopathy
- Is asymmetry caused by localization of DUX4-expressing cells to one side?





Birefringence was mildly affected in injected fish



Conclusions

- Very small amount of DUX4-fl (1 x 10^5 molecules) caused abnormal phenotypes on the eyes, face, and fin muscles in zebrafish
- DUX4-fl perturbed myogenesis of face and fin muscles in an asymmetrical manner.
- Zebrafish can start to model features of FSHD.

Final goal

Drug screening in zebrafish model of FSHD

Aim 1

How expression of DUX4 causes FSHD-like phenotype in zebrafish

Aim 2

To establish DUX4-transgenic fish

Aim 2: Generation of DUX4 Tg fish

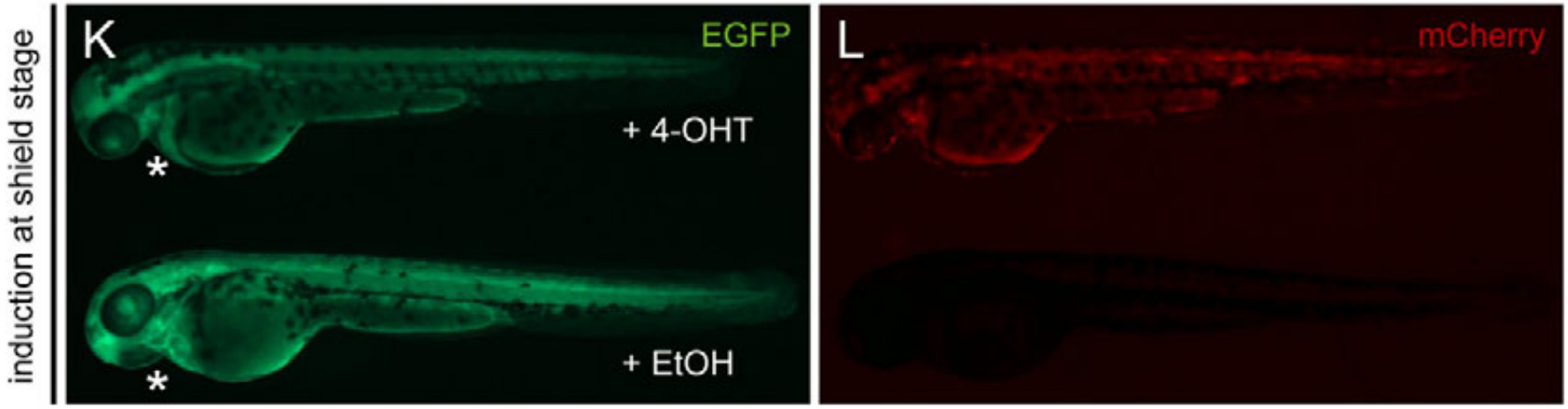
Only 1/1000 cells from FSHD patient express DUX4-fl

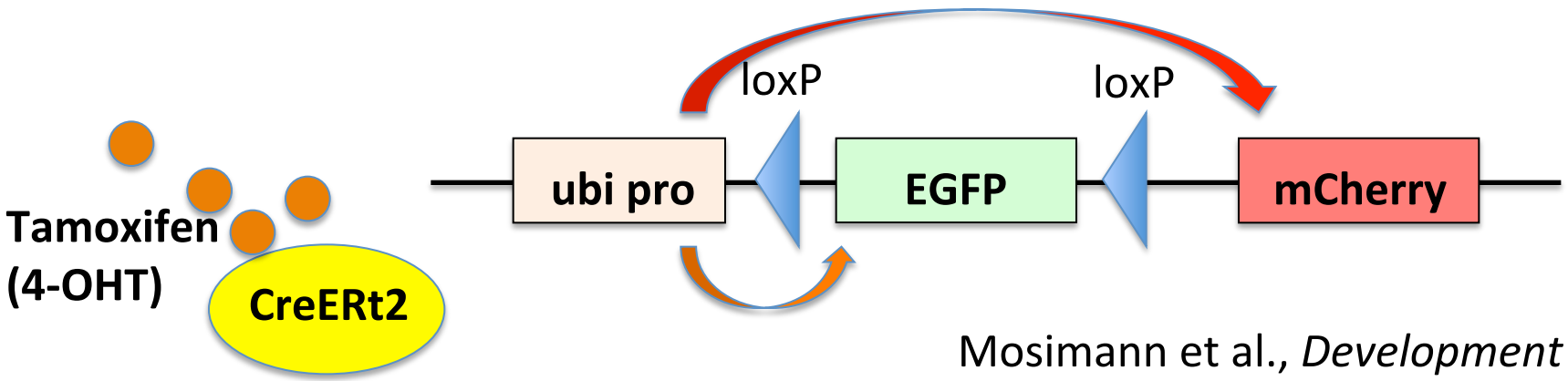
We need to express DUX4-fl in a small percent of cells.

Tamoxifen-inducible Cre-loxP system

Ubiquitous transgene expression and Cre-based recombination driven by the *ubiquitin* promoter in zebrafish

Christian Mosimann[1,2,3], Charles K. Kaufman[4], Pulin Li[1,2,3], Emily K. Pugach[1,2,3], Owen J. Tamplin[1,2,3] and Leonard I. Zon[1,2,3,4,*]

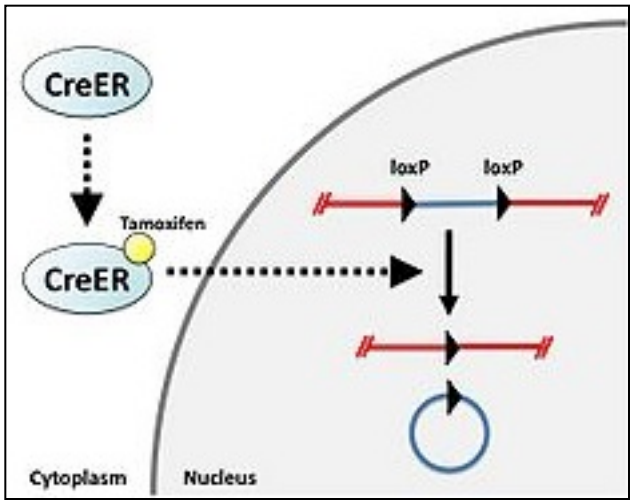


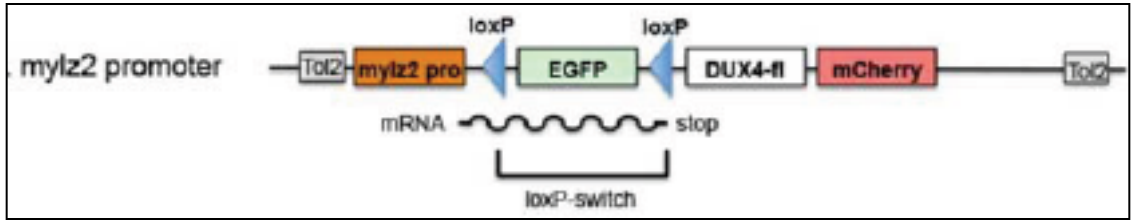


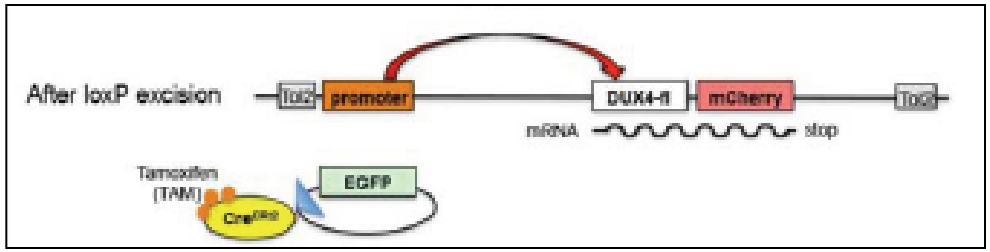
Mosimann et al., *Development* 2011

A transgenic zebrafish model of FSHD

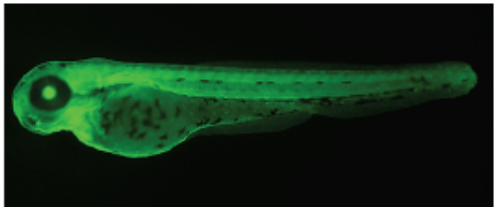
- Currently no widely accepted animal model of FSHD
- Regulating expression levels of DUX4 is key to creating a successful FSHD animal model
- We generated an inducible DUX4 transgenic zebrafish using a tamoxifen-controlled Cre^{ERt2}-loxP system
- Enables regulation of the dosage and timing of DUX4 expression







ubi:loxP-EGFP-loxP-DUX4-fl-mCherry (+/-) (F1)



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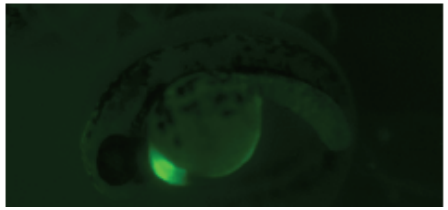
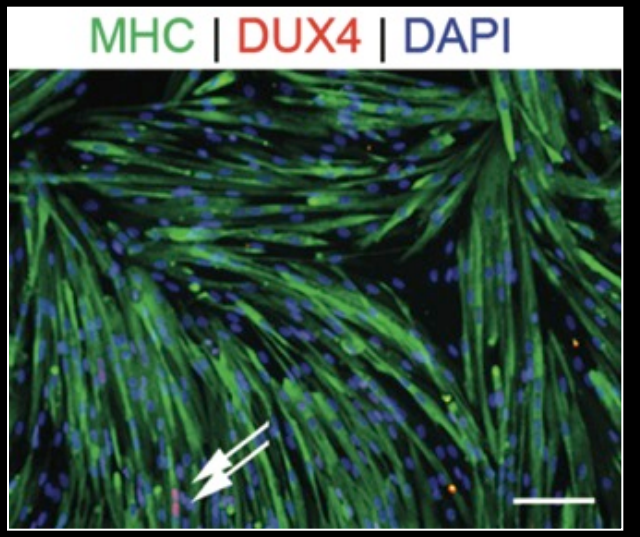
ubi: CreERt2 Tg (+/-)

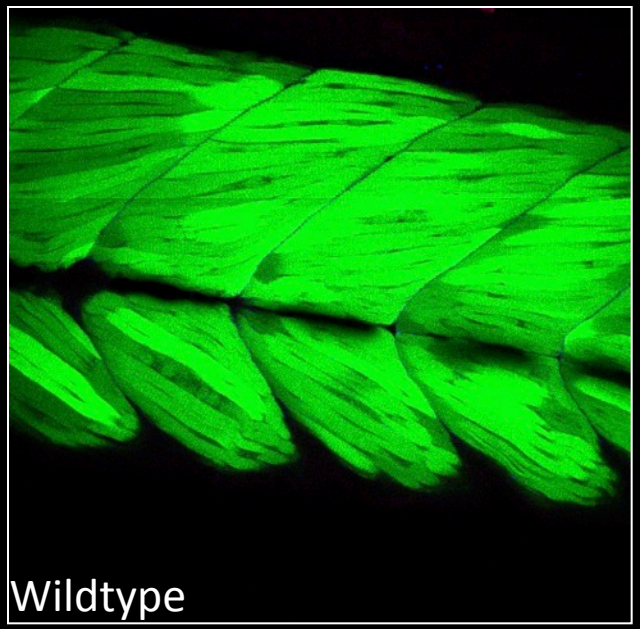


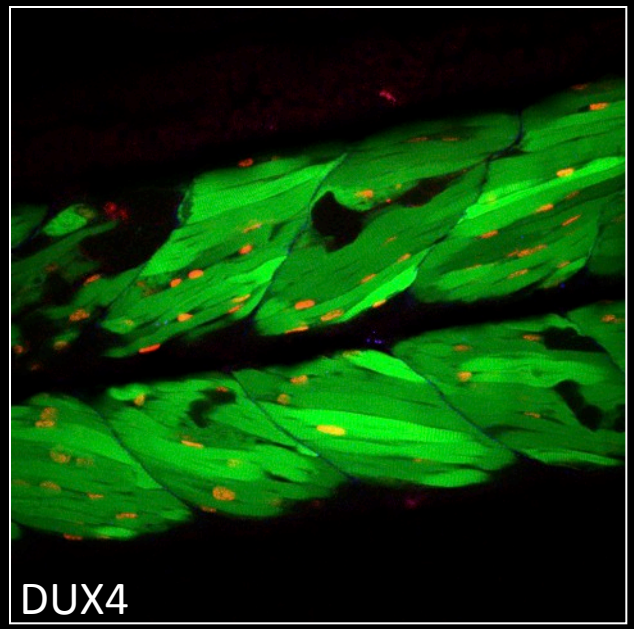
Mosaic expression of DUX4 in our transgenic model mirrors low DUX4 expression in human cells

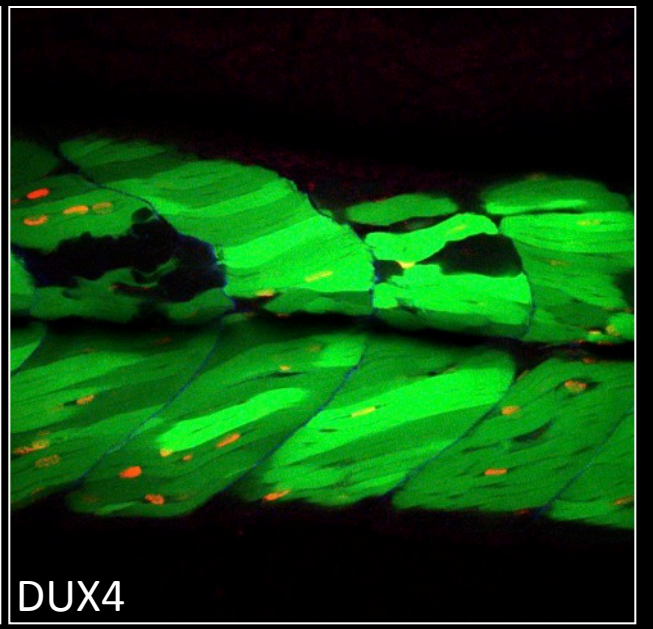
- Approximately 1 in 1000 myonuclei are DUX4 positive in primary human cultures
- Evidence of disorganized myofibers by day 3 of DUX4 induction in our transgenic model



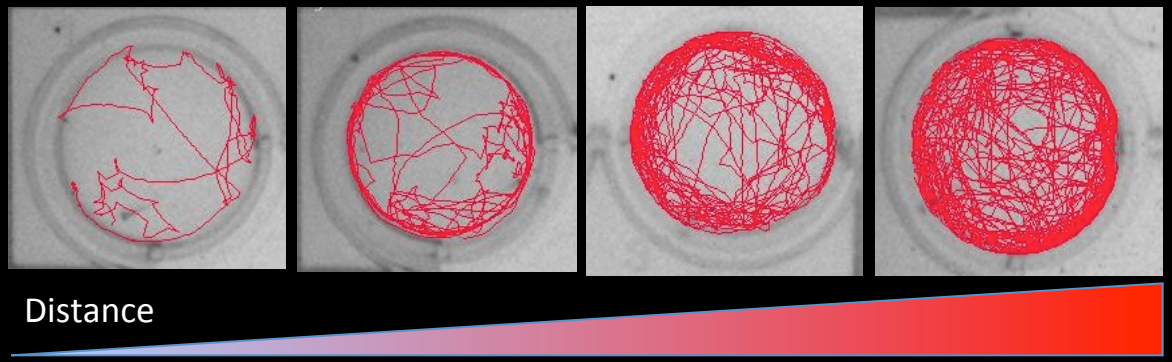
Block et al. 2013 Hum Mol Gen



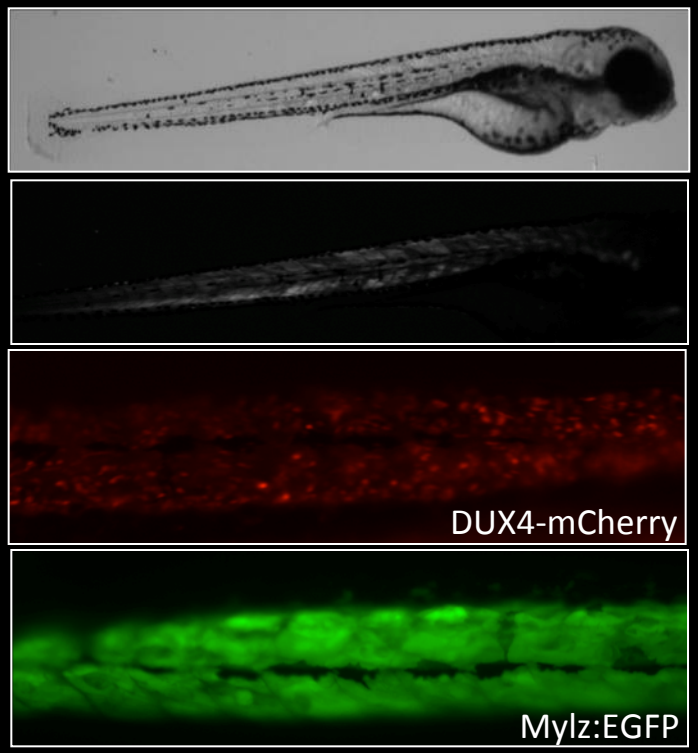




- Automated system to track movement of zebrafish larvae
- Enables high-throughput functional screening



Does altered muscle structure in DUX4 fish affect its function?



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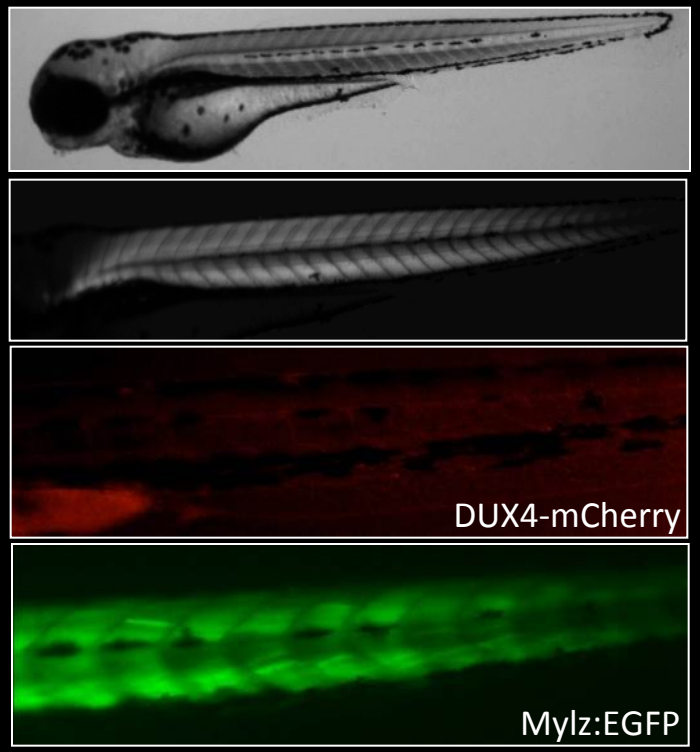
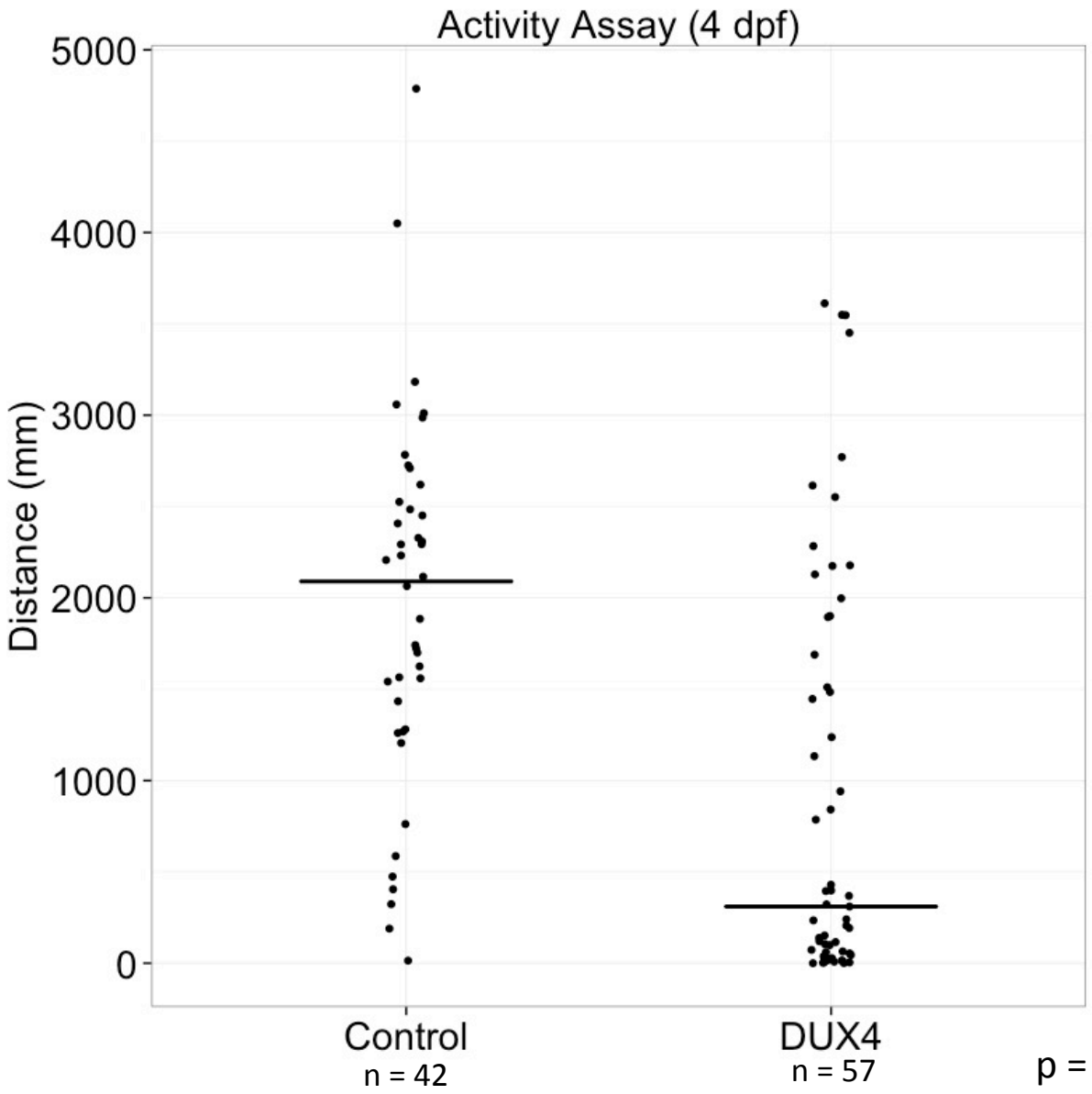


DUX4 transgenic fish significantly swim less than control in a 15 minute assay



p = 6.05358E-06

Conclusions

- Both our injection and transgenic model of DUX4 result in a muscular dystrophy phenotype
- Despite the primate-specific origins of DUX4, our zebrafish model is able to mimic FSHD patient phenotypes
- This suggests that misexpression of DUX4 results in a toxic disease pathway that is conserved across vertebrates
- We can use these fish to find important targets of DUX4
- Future work will be to use our transgenic model for drug screening

Acknowledgements

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Dr Hiroaki Mitsuhashi

Dr Fedik Rahimov

Members of the Kunkel Lab

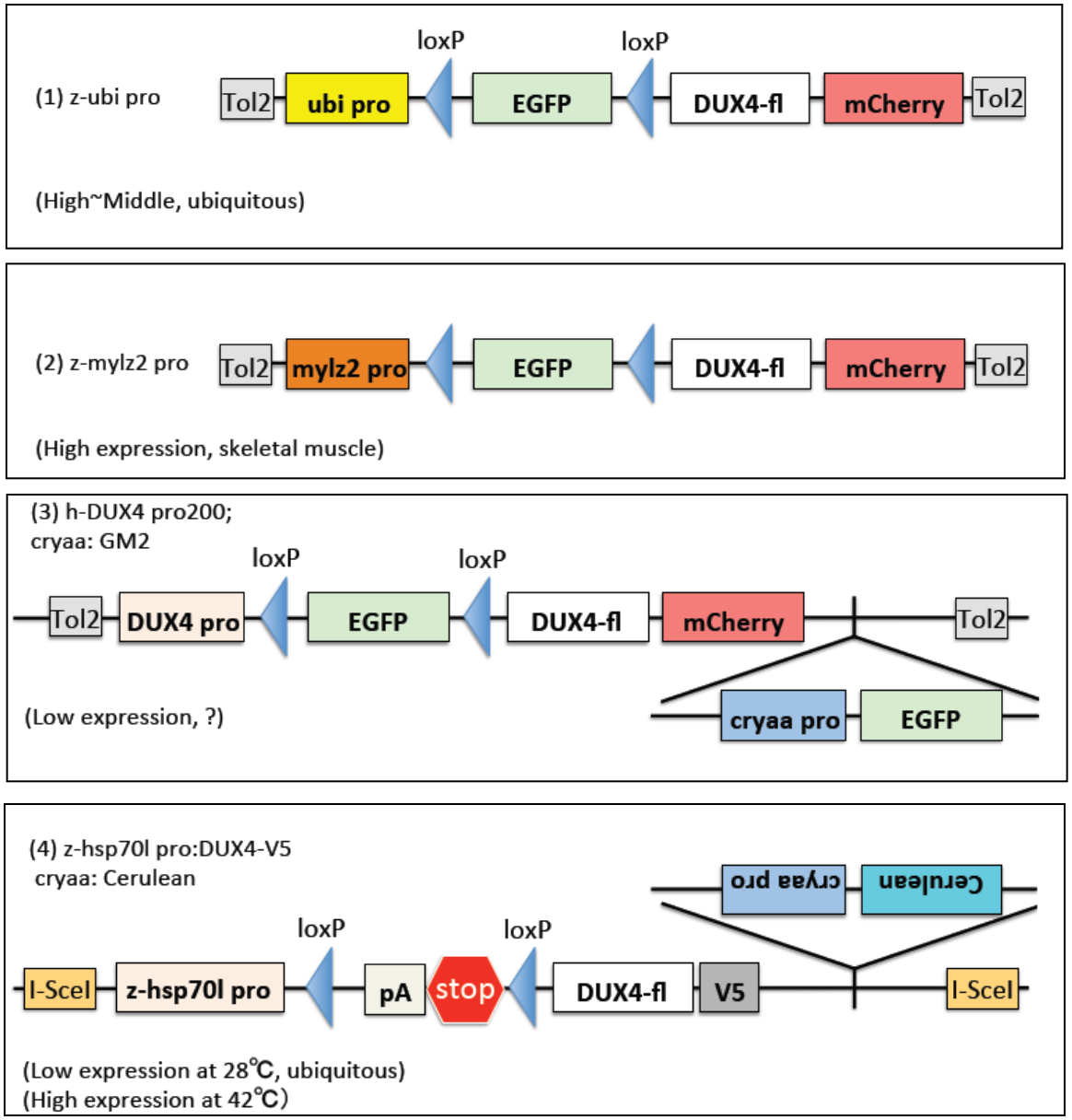
Jason Best and fish crew

Funding

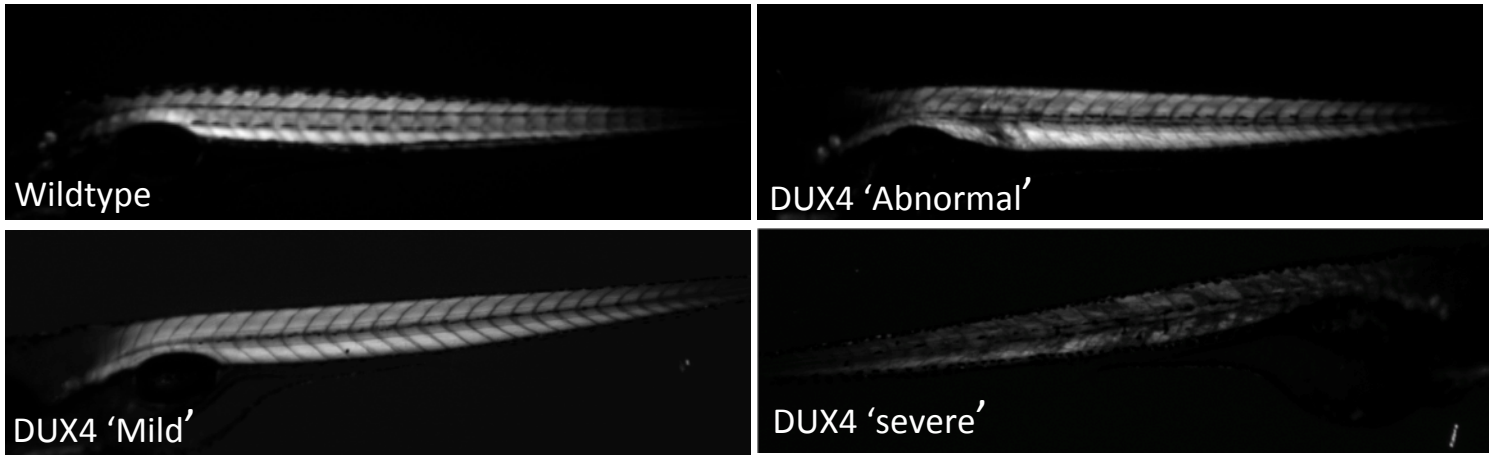
NICHD Wellstone Center for Muscular Dystrophy Research

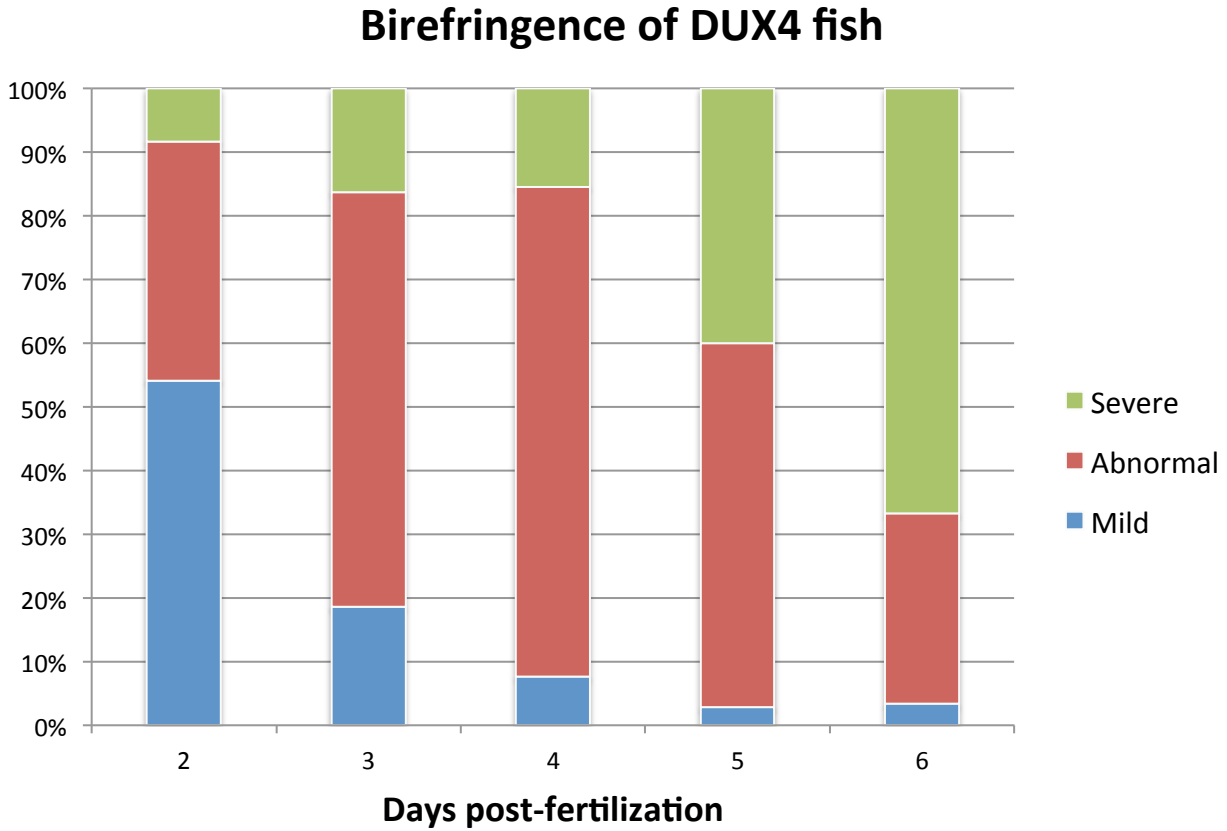
FSH Society

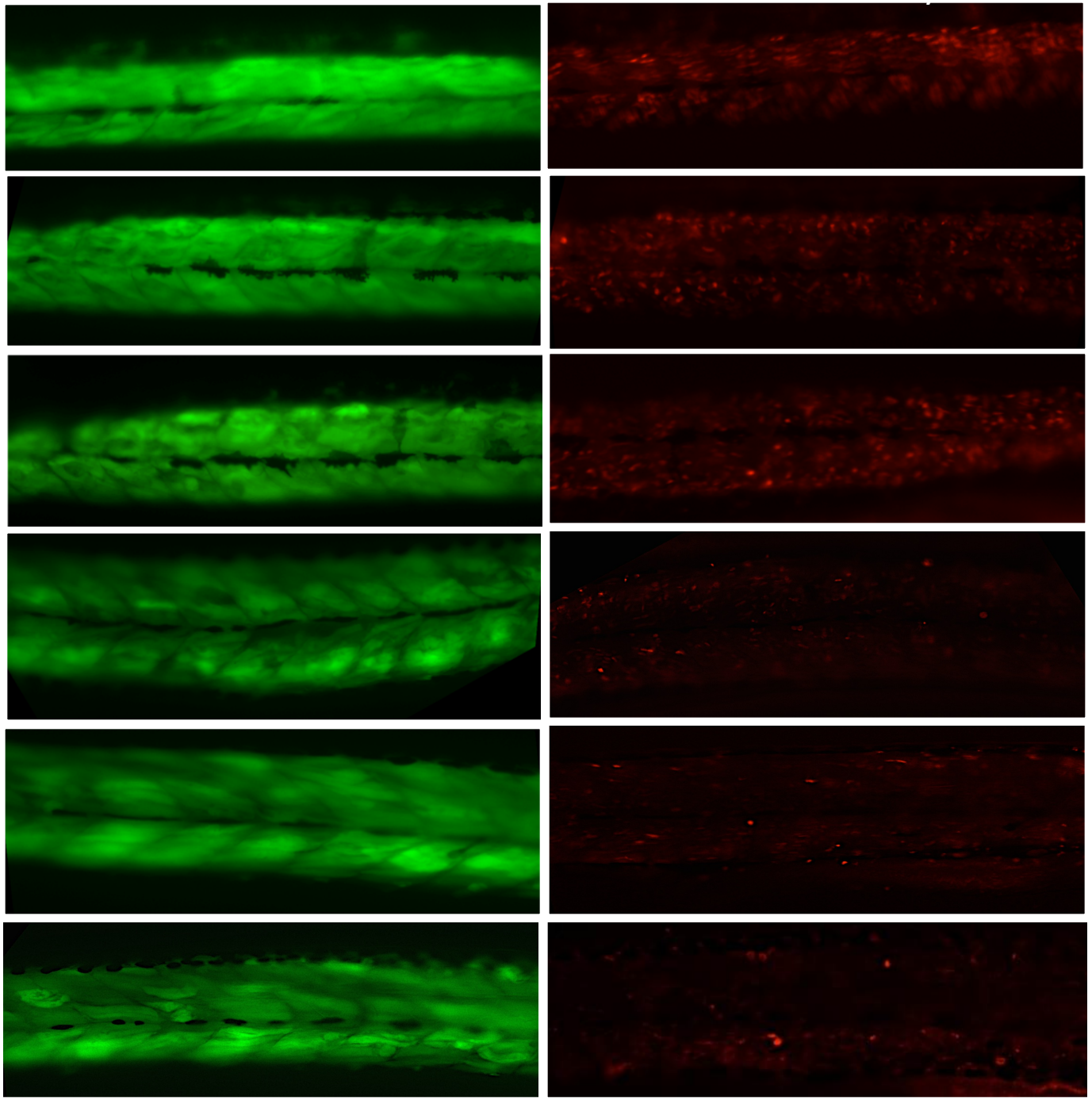
Several fish lines with different promoters to test varying levels and tissue specific expression of DUX4



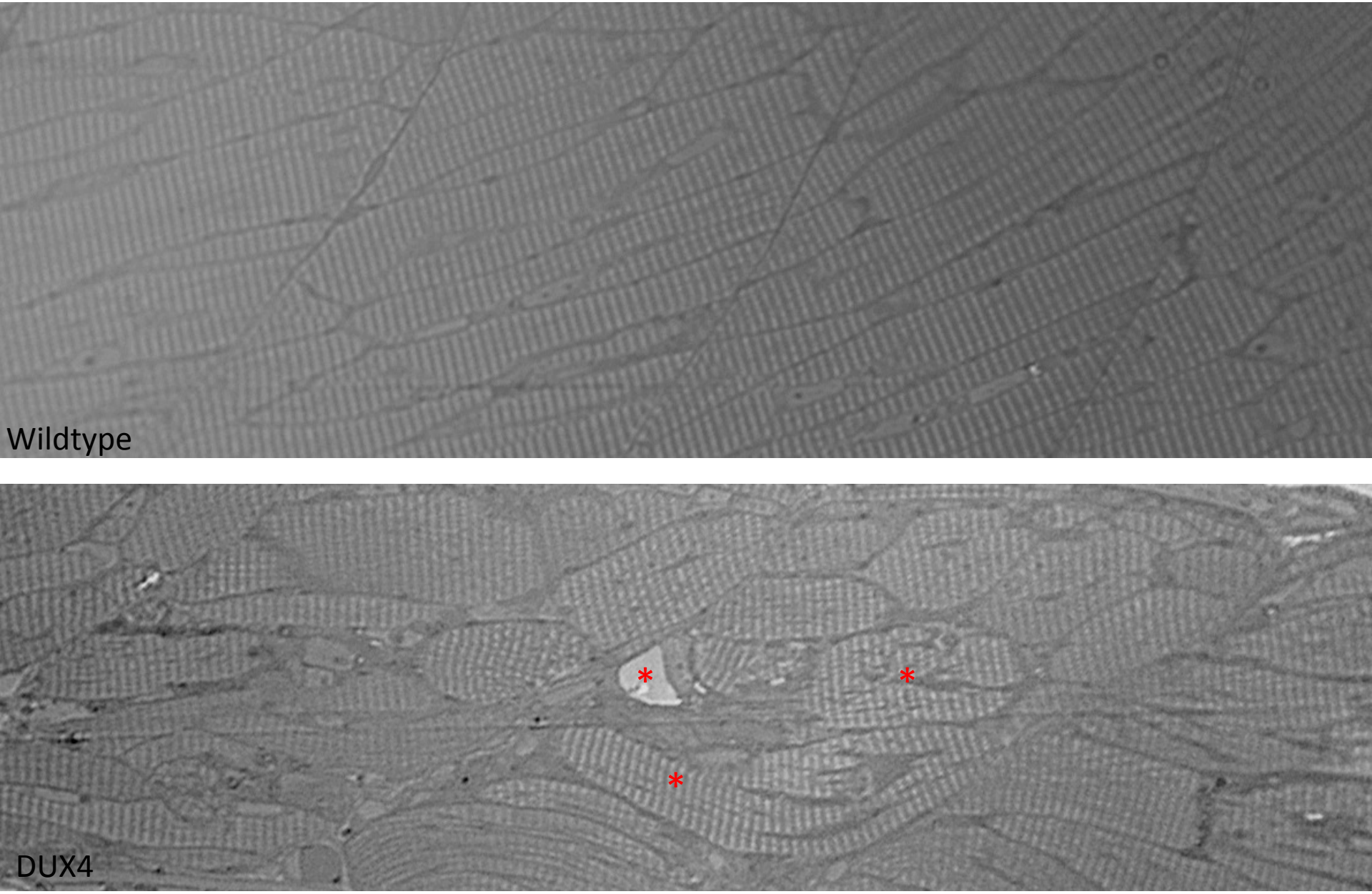
Transgenic induction of DUX4 is linked to a spectrum of abnormal birefringence phenotypes







Parasagittal section of DUX4 fish show areas of compromised muscle architecture



Nuclear spreading of DUX4