



THE MANTON CENTER  
for Orphan Disease Research



# Drug discovery for *RYR-1* myopathies using zebrafish models

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# Boston Congenital Myopathy Study

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- Established 1992
- Identify and enroll patients and their families from around the world
- Includes >2700 individuals
  - almost 1000 with congenital myopathy
- Collect clinical records, DNA and muscle specimens
- Discover new myopathy genes, understand how mutations cause weakness, develop treatments

# Why use zebrafish as a disease model?

✓ Small vertebrate organism

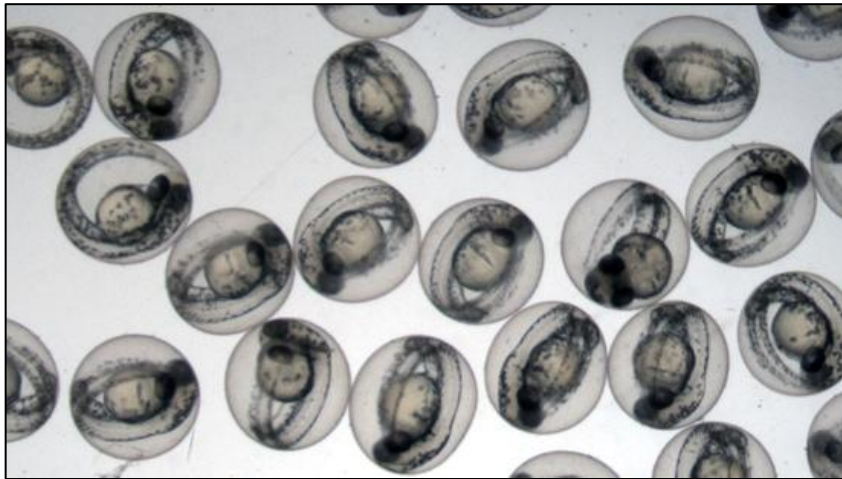


✓ Easy husbandry

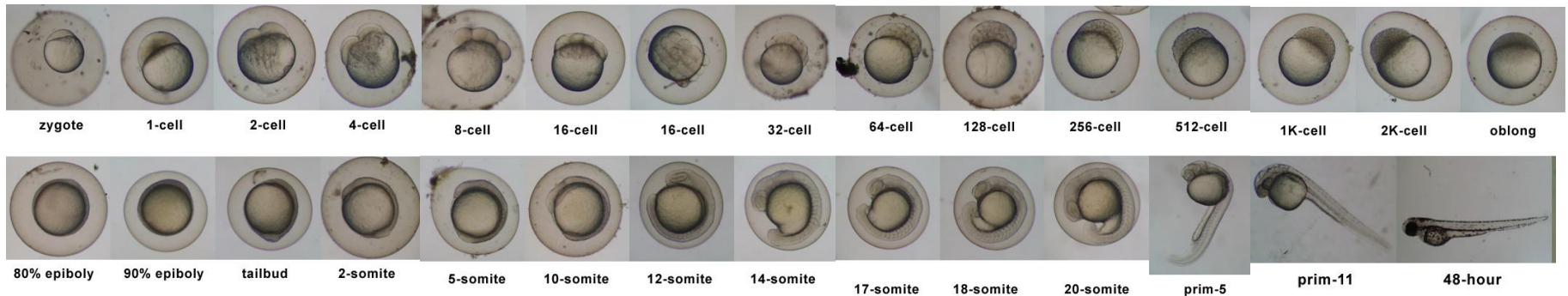
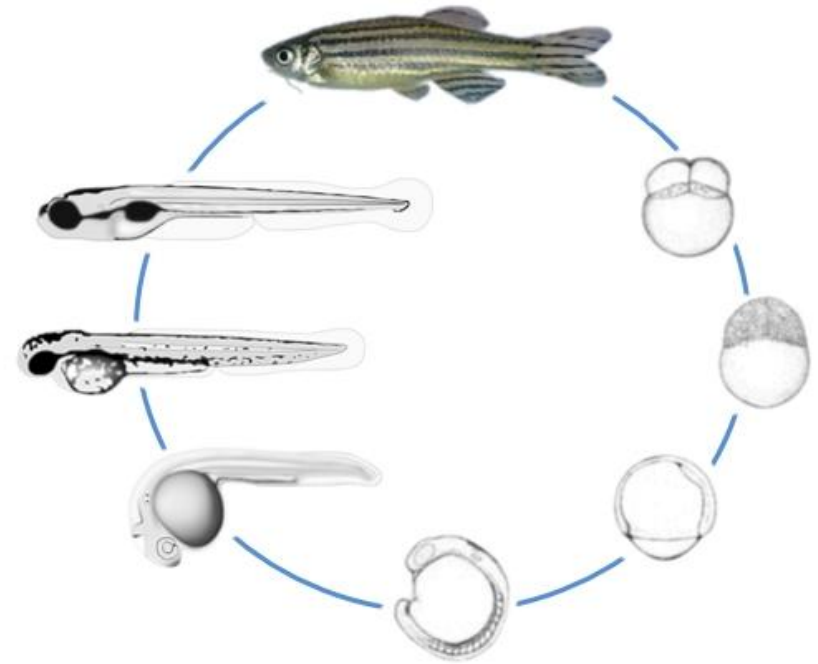


# Why use zebrafish as a disease model?

✓ High spawning productivity



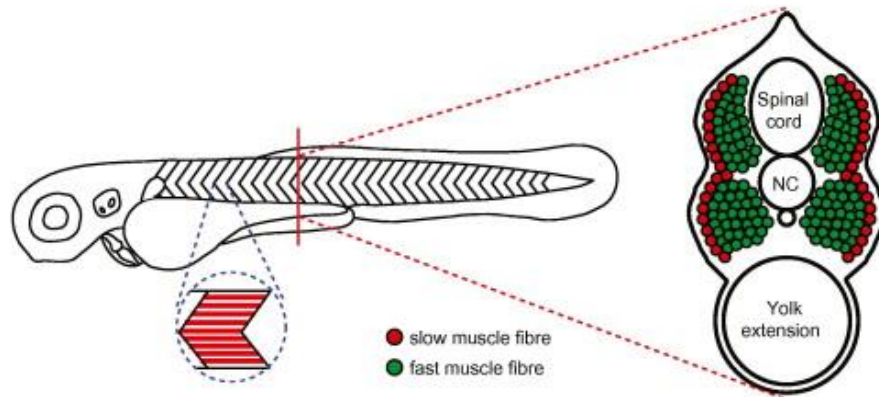
✓ Rapid *ex utero* development



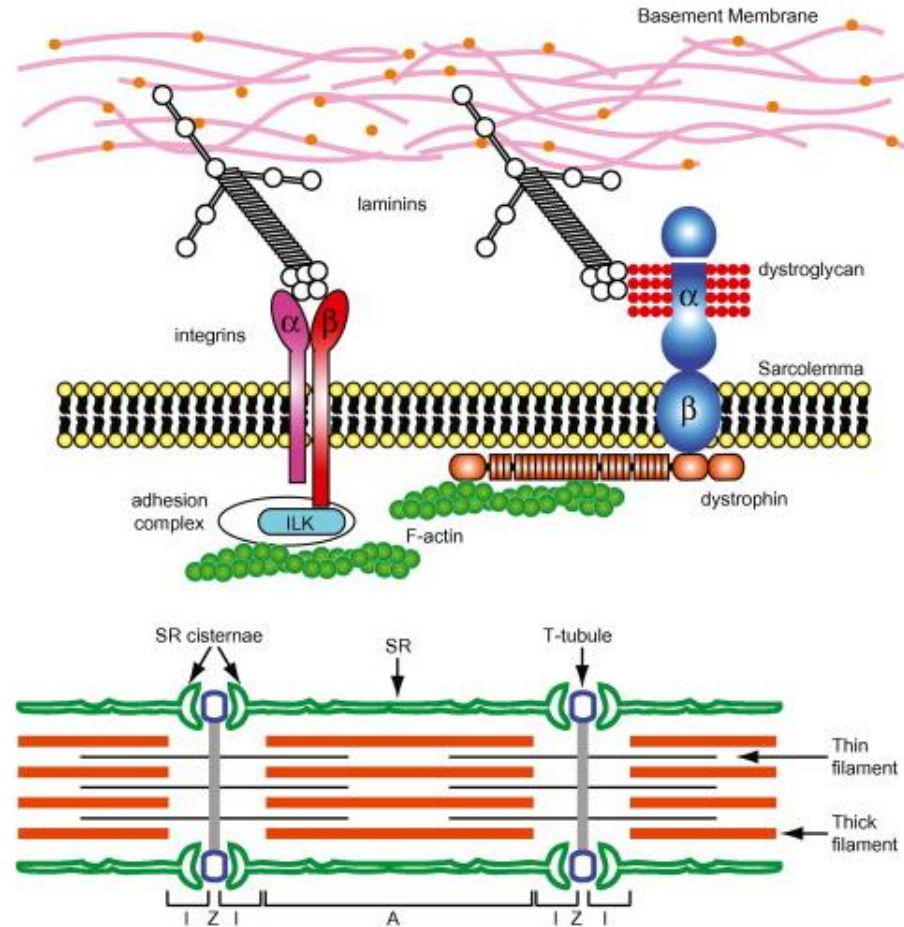
Watch them grow!



# Proteins and structures are highly conserved between human and zebrafish muscle



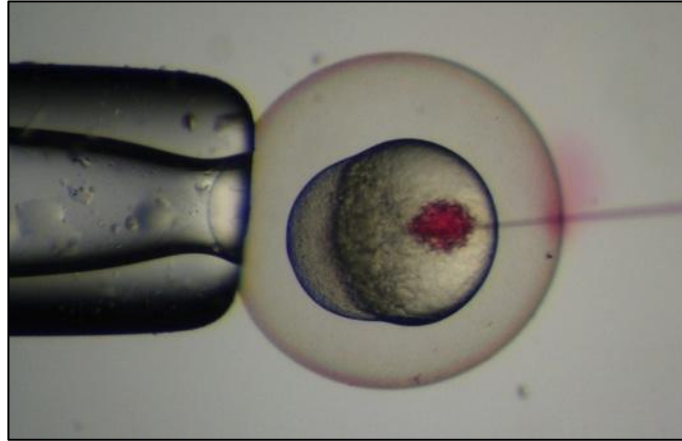
Fully differentiated  
slow & fast muscle fibers  
by 48 hours post fertilization



Similar dystrophin-glycoprotein complex  
& sarcomeric structure

# Disrupting gene expression in zebrafish embryos

Injection at the one-cell stage



Transiently reduce expression

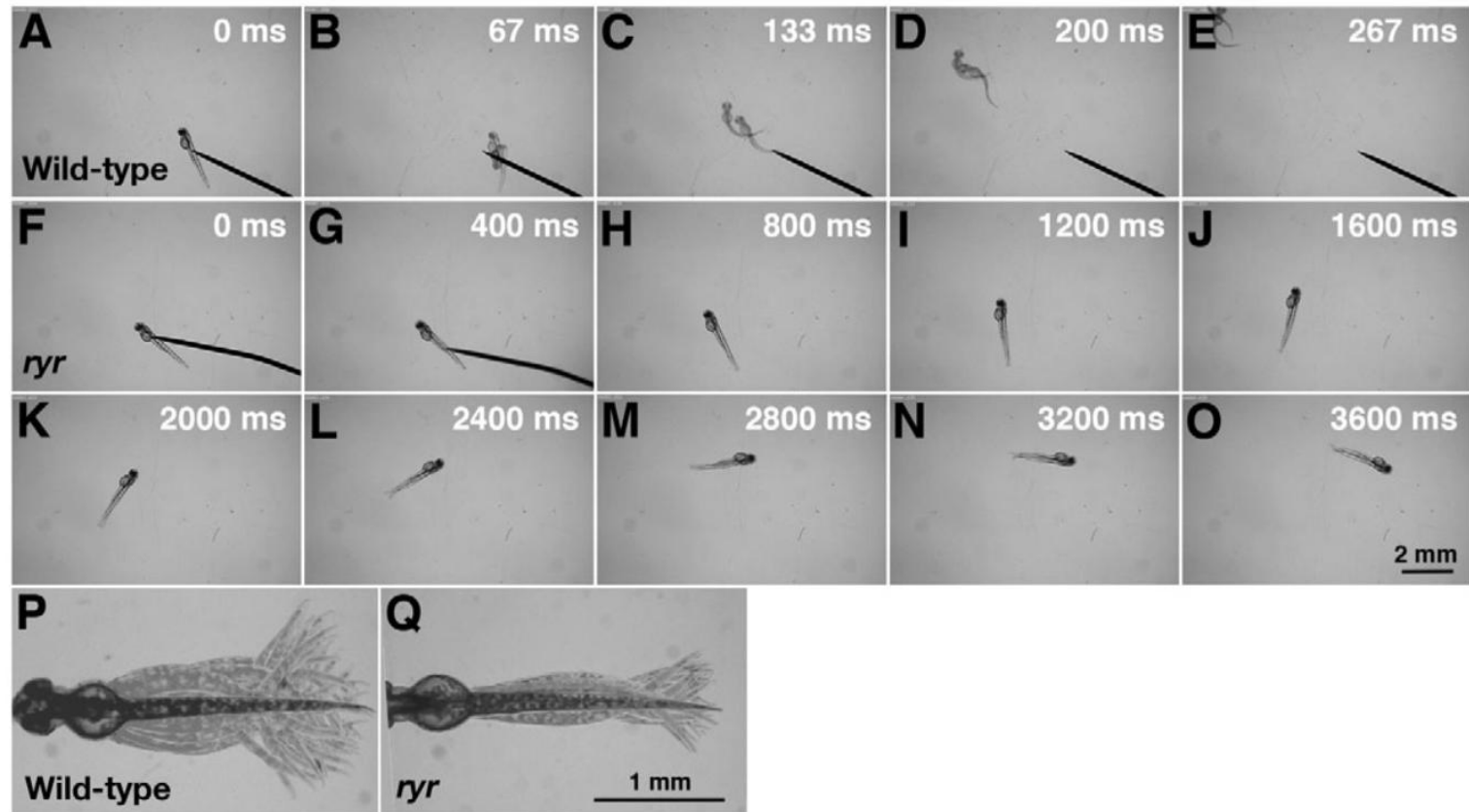


Create stable genetic knockouts

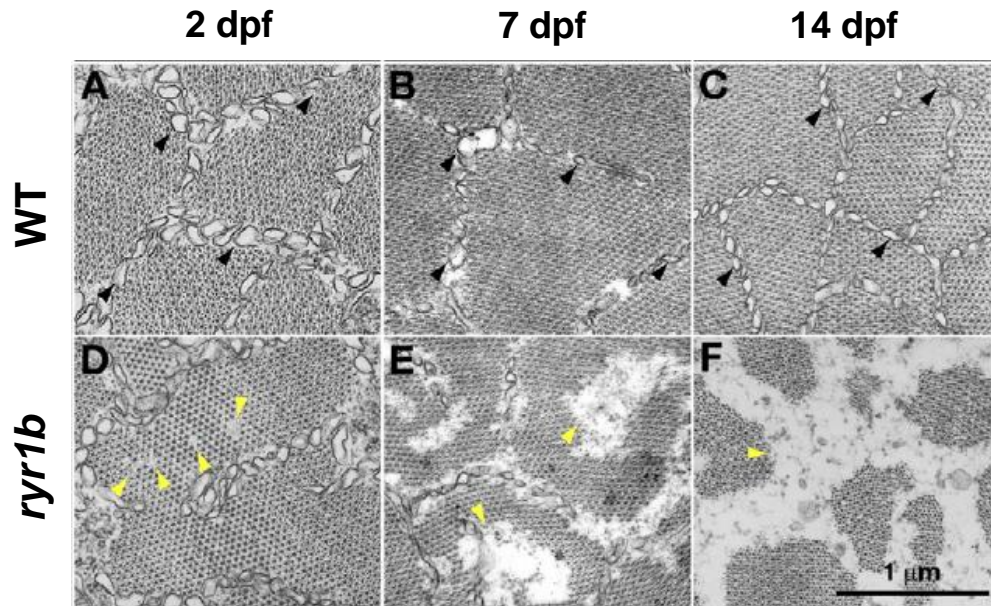
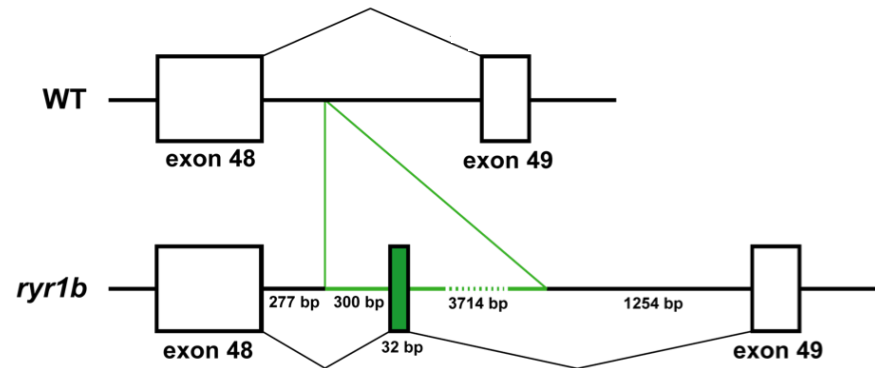


# Zebrafish *relatively relaxed* mutants have a ryanodine receptor defect, show slow swimming and provide a model of multi-minicore disease

Hiromi Hirata<sup>1,2,†</sup>, Takaki Watanabe<sup>1</sup>, Jun Hatakeyama<sup>3</sup>, Shawn M. Sprague<sup>2</sup>, Louis Saint-Amant<sup>2,\*</sup>, Ayako Nagashima<sup>2</sup>, Wilson W. Cui<sup>2</sup>, Weibin Zhou<sup>2</sup> and John Y. Kuwada<sup>2</sup>



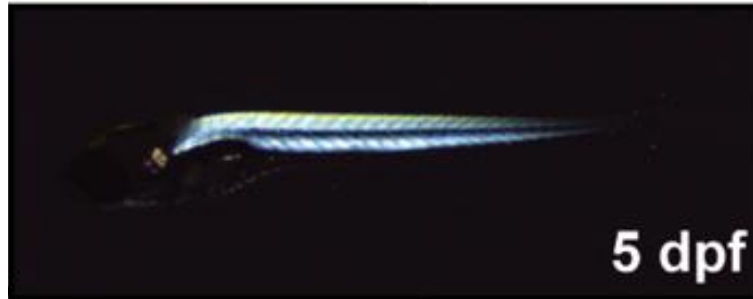
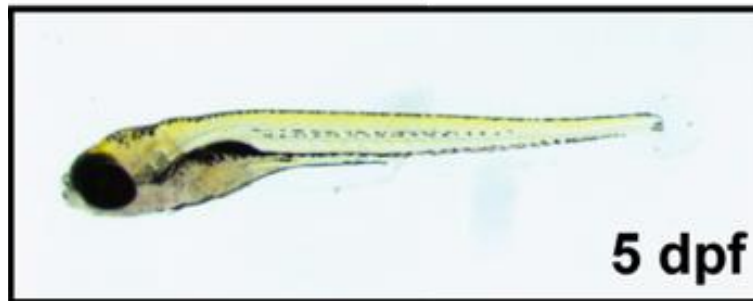
*Relatively relaxed* fish have a DNA insertion resulting in aberrant splicing of fast *ryr1b* mRNA and loss of protein



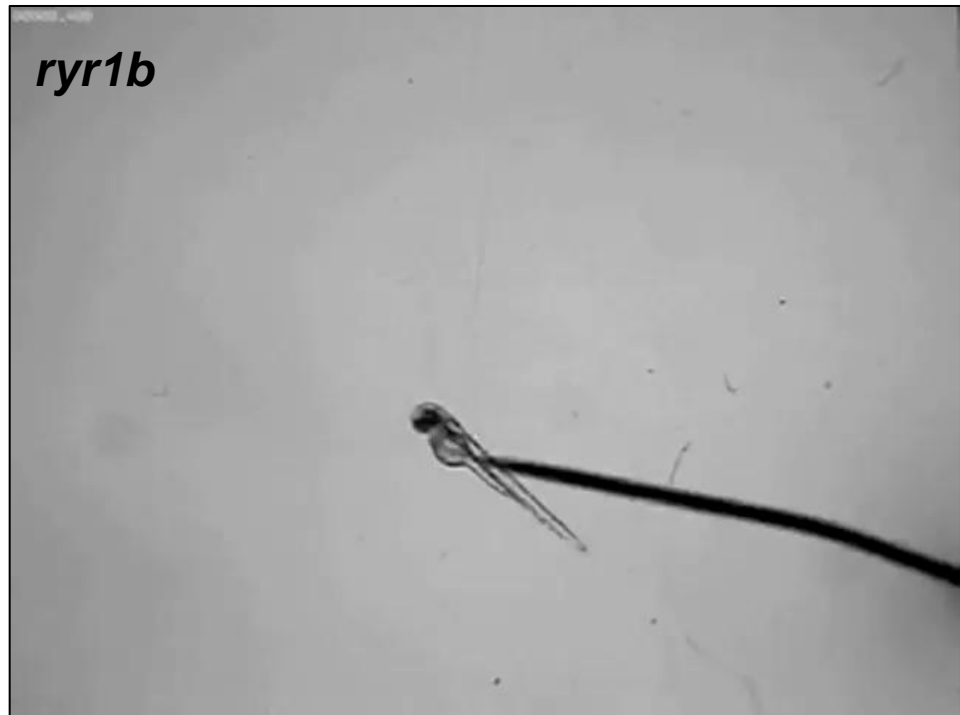
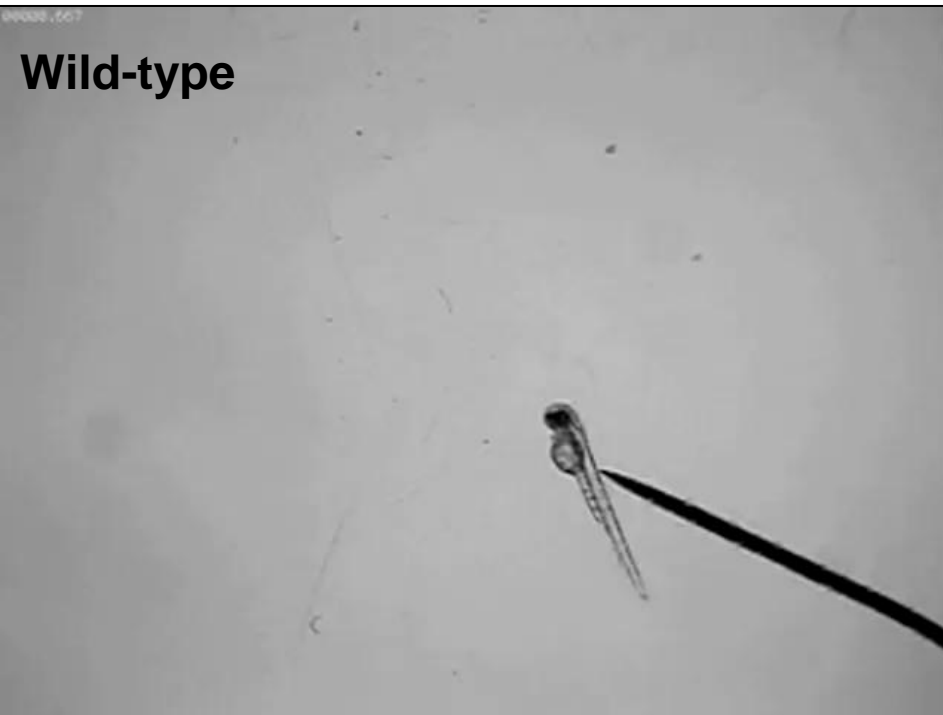


# Zebrafish *ryr1b* mutants

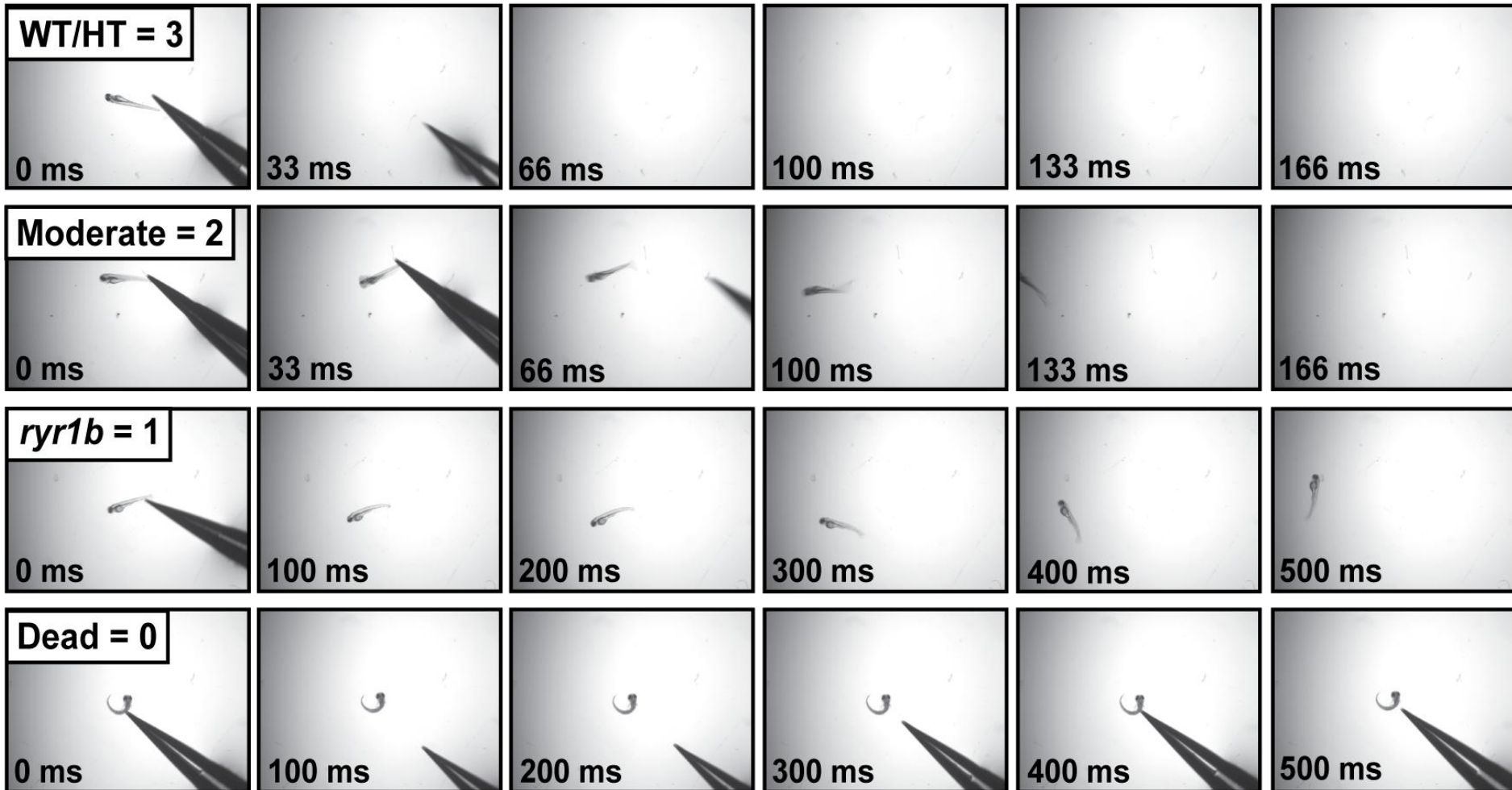
*ryr1b*<sup>-/-</sup>



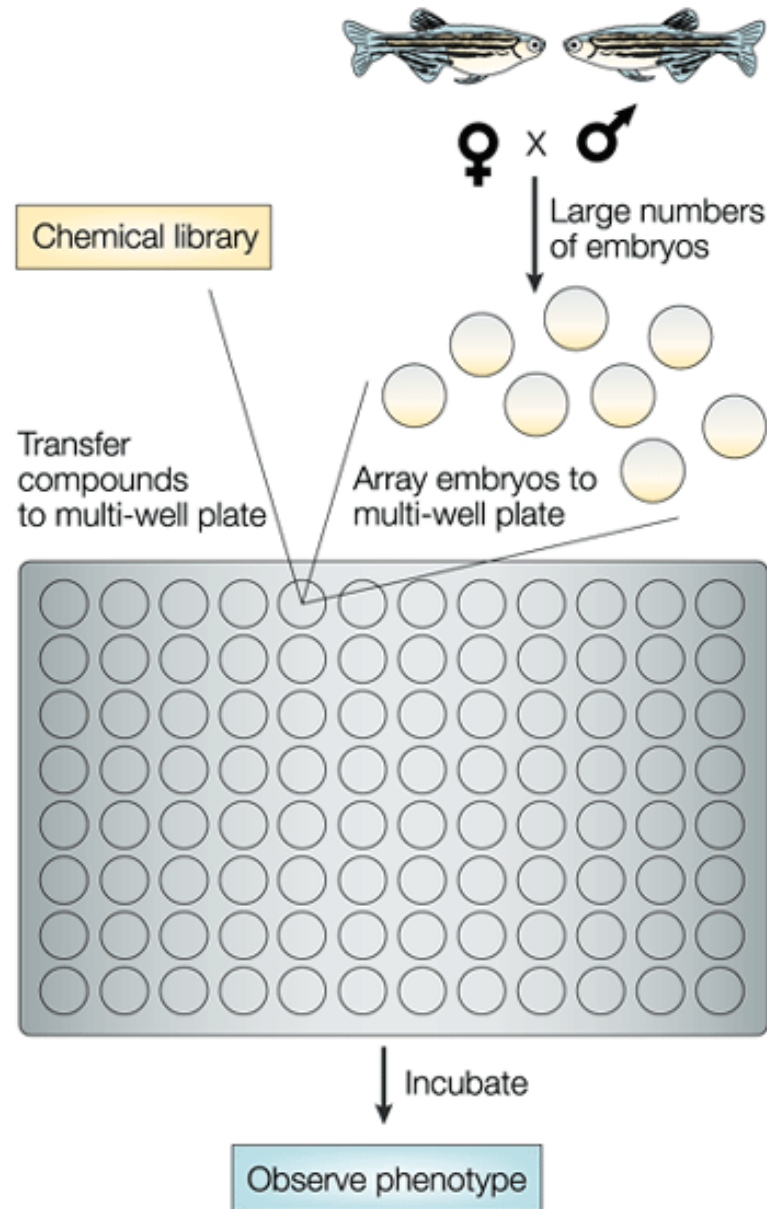
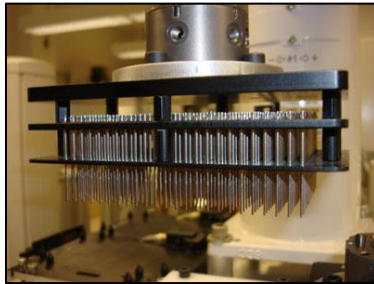
# Diminished touch-evoked swimming in *ryr1b* mutants



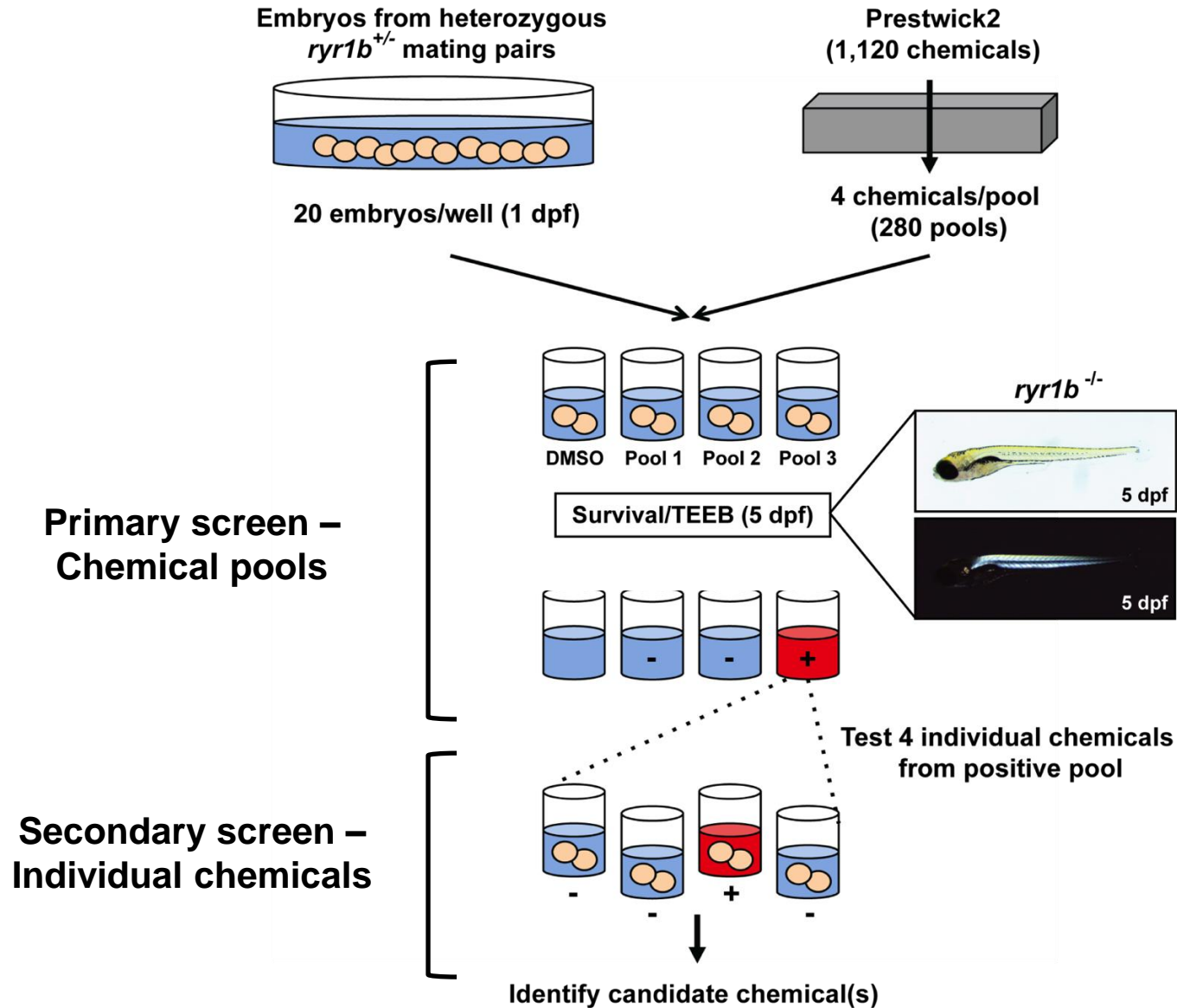
# Numerical rating system for zebrafish mobility



# Chemical screens in zebrafish



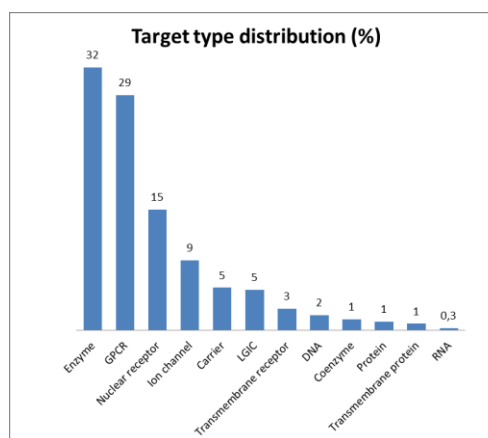
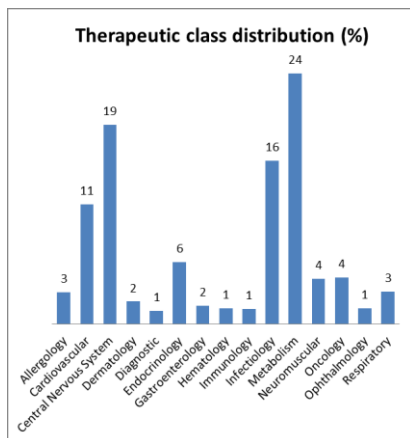
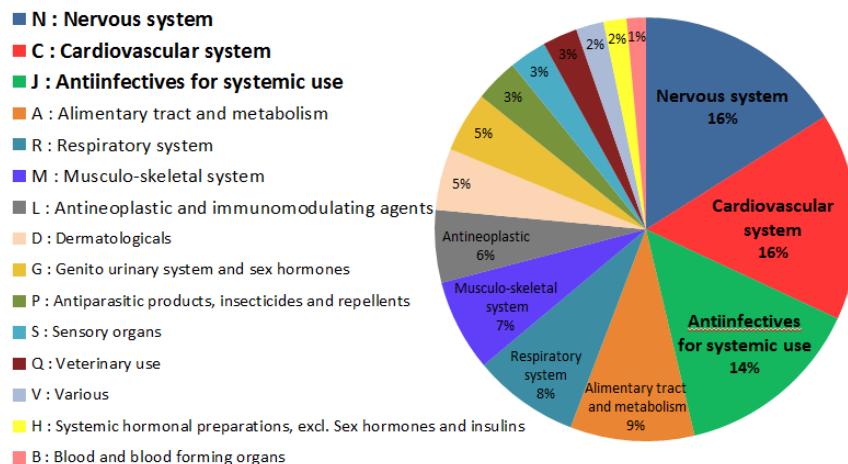
# Chemical screening strategy



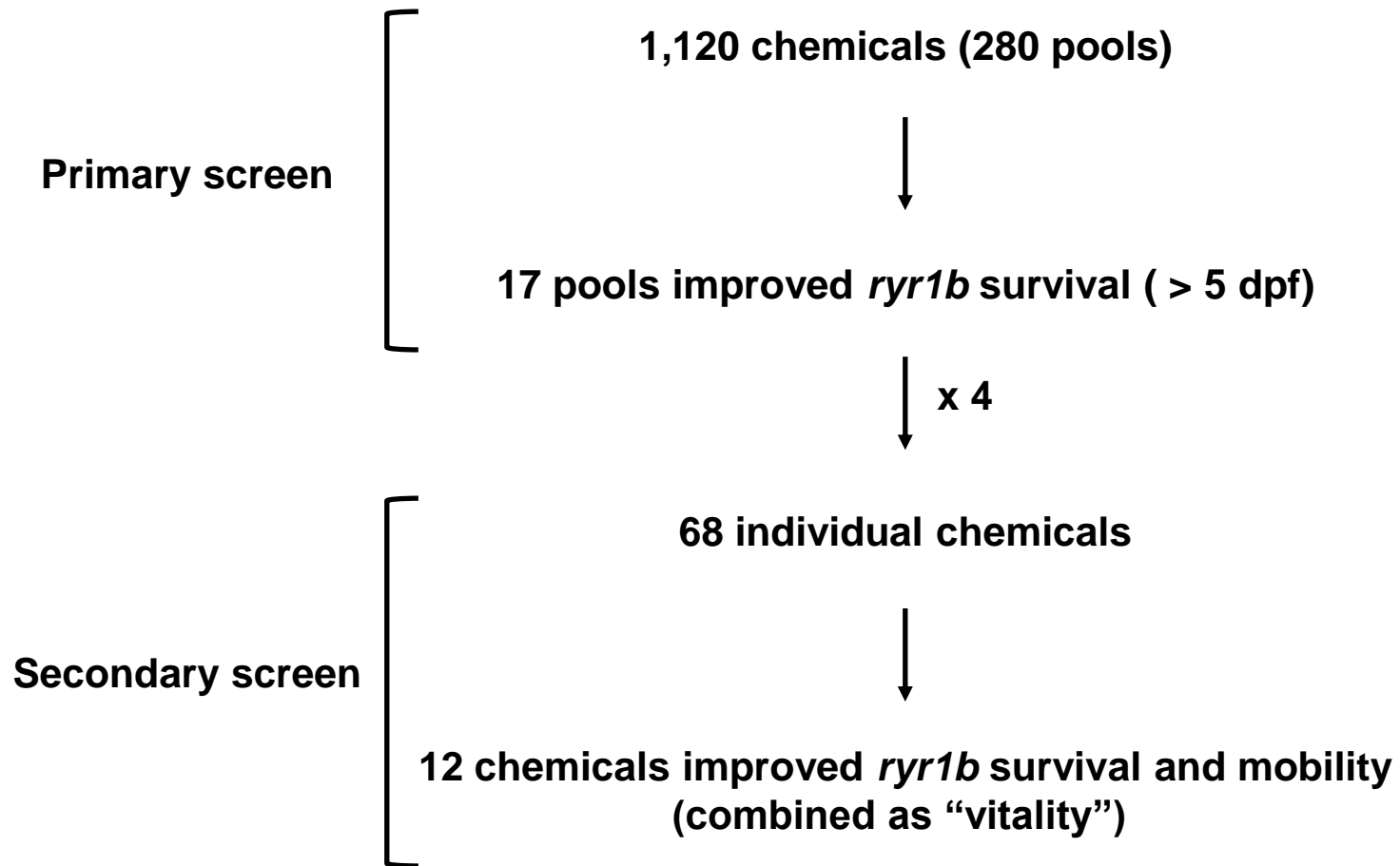


- 1120 off-patent compounds that have been selected for structural diversity, collective coverage of multiple therapeutic areas, and known safety and bioavailability in humans.
- 100% approved drugs. Over 85% of the Prestwick compounds are currently marketed.

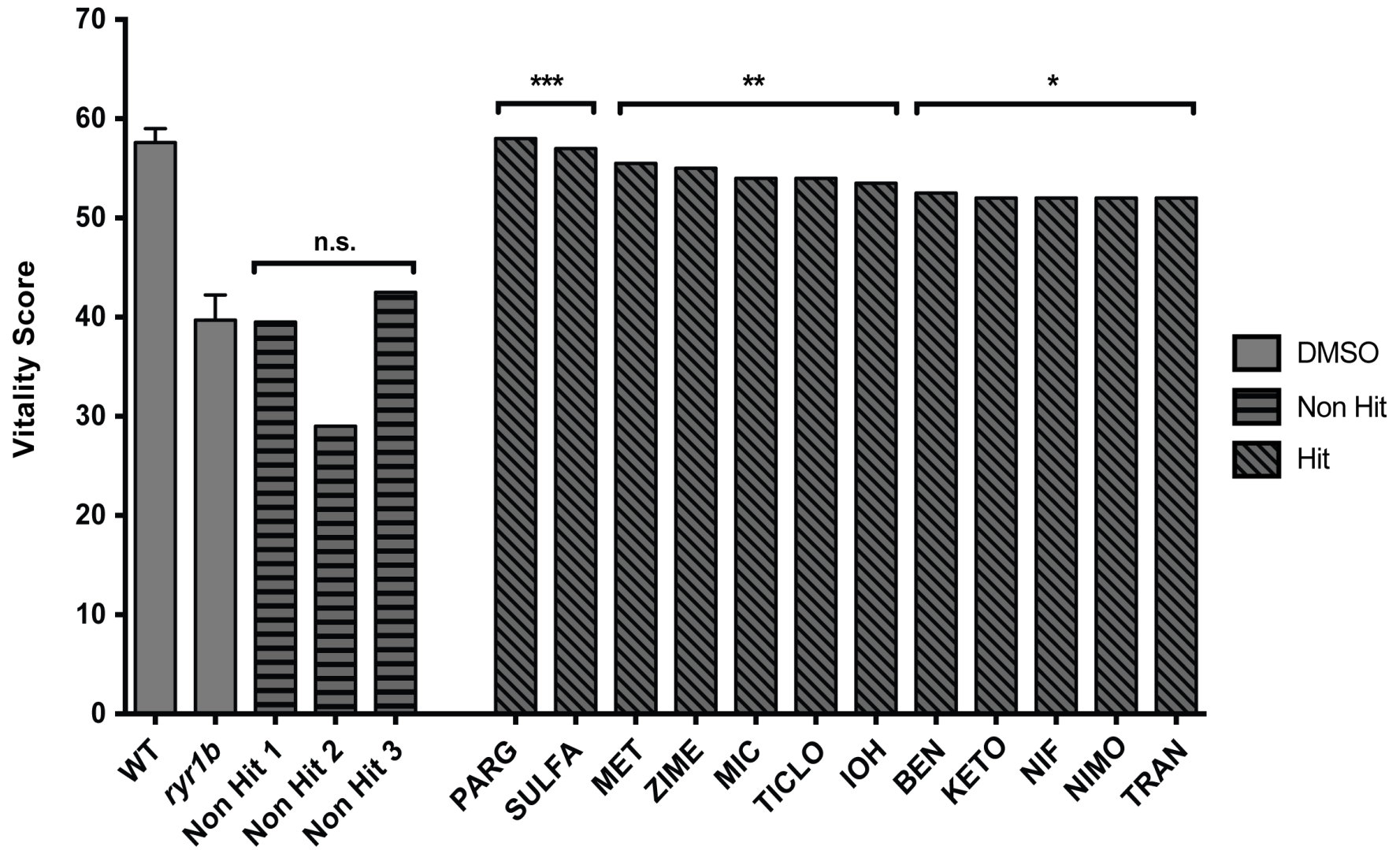
## ATC Classification



# Results by the numbers



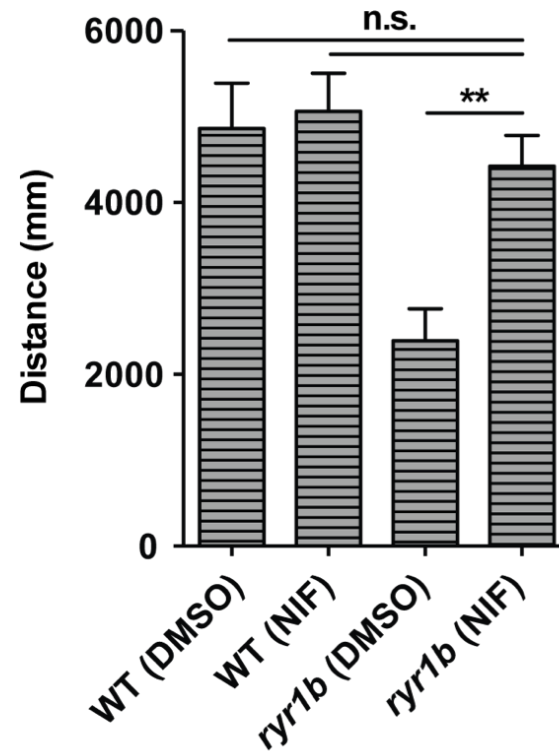
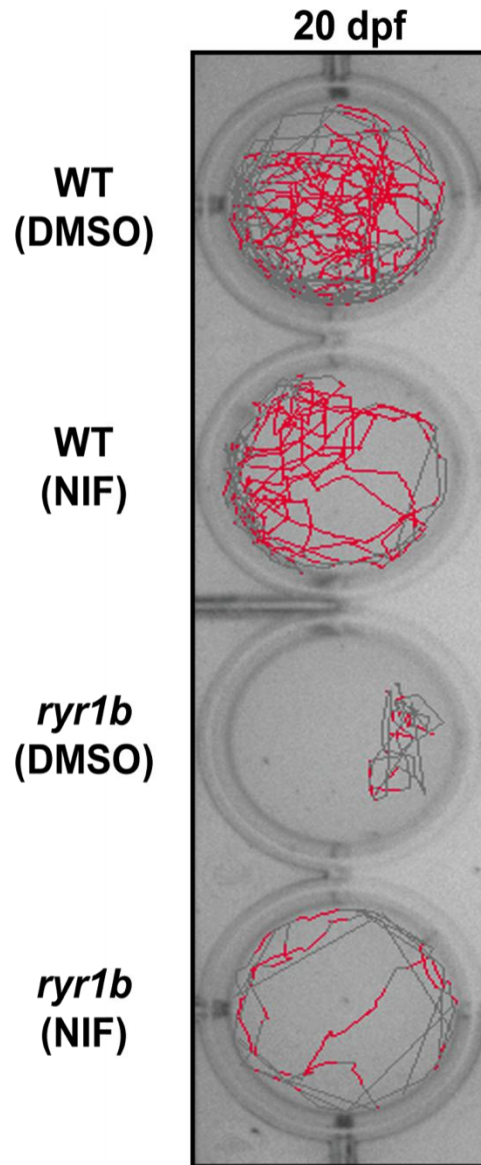
# Identifying candidates in the secondary screen



# Candidate compounds

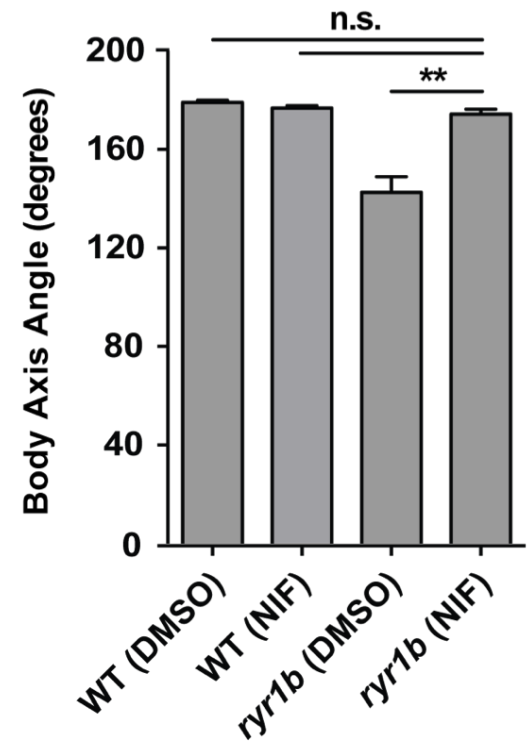
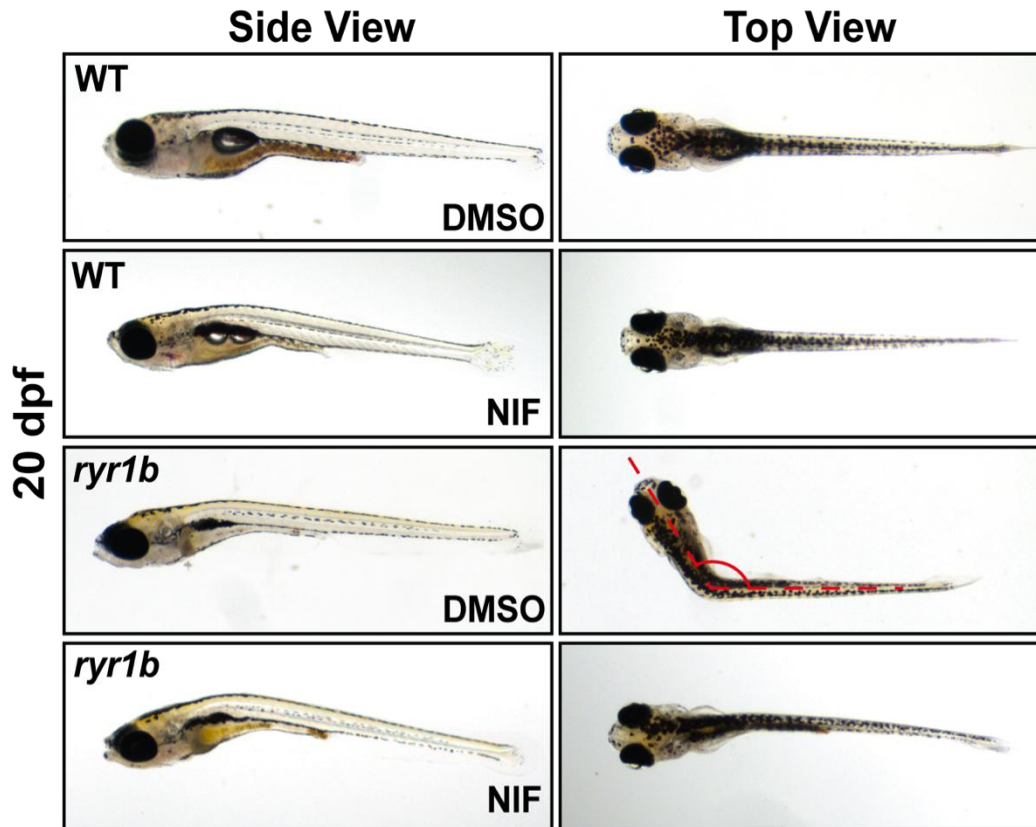
Vitality Score	Chemical Name	Formula	MW	Mechanism of Action
58.0	Pargyline hydrochloride *	C <sub>11</sub> H <sub>14</sub> ClN	195.69	Irreversible monoamine oxidase (MAO) inhibitor
57.0	Sulfasalazine	C <sub>18</sub> H <sub>14</sub> N <sub>4</sub> O <sub>5</sub> S	398.39	NF-KB inhibitor; anti-inflammatory
55.5	Metolazone **	C <sub>16</sub> H <sub>16</sub> ClN <sub>3</sub> O <sub>3</sub> S	365.83	Sodium-chloride channel inhibitor
55.0	Zimelidine dihydrochloride monohydrate **	C <sub>16</sub> H <sub>21</sub> BrCl <sub>2</sub> N <sub>2</sub> O	408.16	Selective serotonin reuptake inhibitor
54.0	Miconazole ***	C <sub>18</sub> H <sub>14</sub> Cl <sub>4</sub> N <sub>2</sub> O	416.13	Anti-fungal agent
54.0	Ticlopidine hydrochloride ***	C <sub>14</sub> H <sub>15</sub> Cl <sub>2</sub> NS	300.25	Inhibitor of platelet aggregation
53.5	Iohexol	C <sub>19</sub> H <sub>26</sub> I <sub>3</sub> N <sub>3</sub> O <sub>9</sub>	821.14	Low-osmolality contrast agent
52.5	Benoxinate hydrochloride ***	C <sub>17</sub> H <sub>29</sub> ClN <sub>2</sub> O <sub>3</sub>	344.88	Surface anaesthetic
52.0	Ketoprofen	C <sub>16</sub> H <sub>14</sub> O <sub>3</sub>	254.28	Cyclooxygenase inhibitor; anti-inflammatory
52.0	Nifuroxazide	C <sub>12</sub> H <sub>9</sub> N <sub>3</sub> O <sub>5</sub>	275.22	JAK/STAT signaling inhibitor
52.0	Nimodipine	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> O <sub>7</sub>	418.44	Dihydropyridine calcium channel blocker
52.0	Tranylcypromine hydrochloride *	C <sub>9</sub> H <sub>12</sub> ClN	169.65	Irreversible MAO inhibitor

# JAK-STAT inhibition improves swimming behaviors

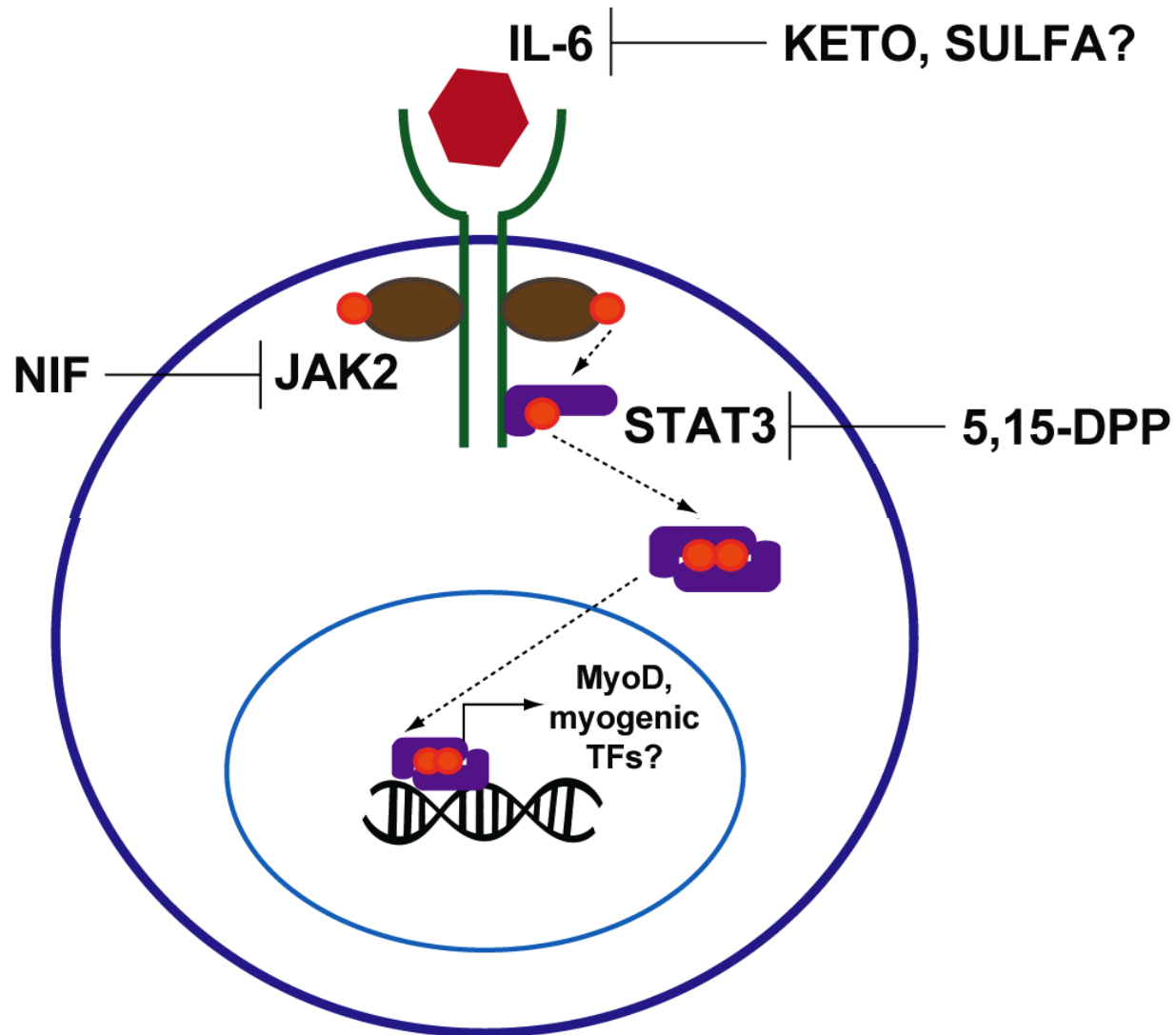




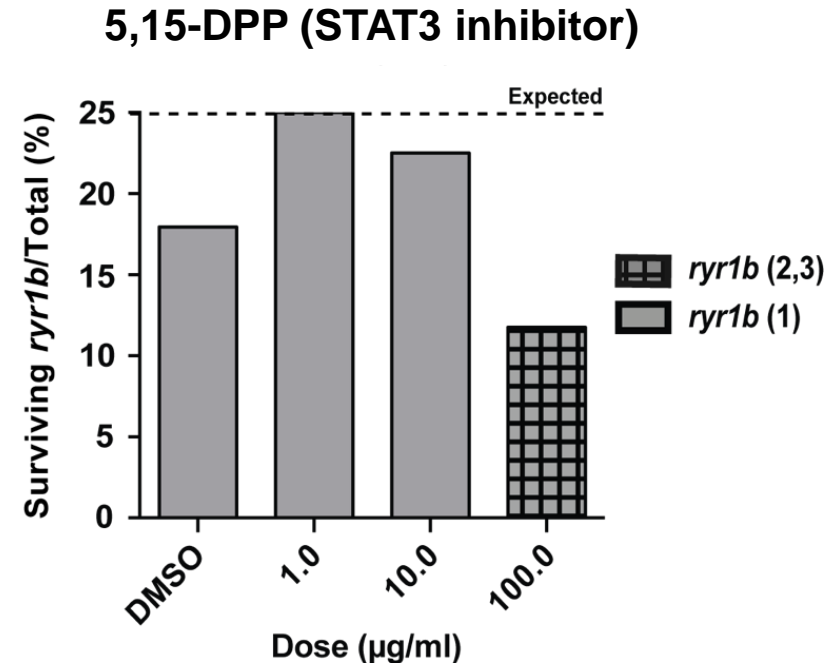
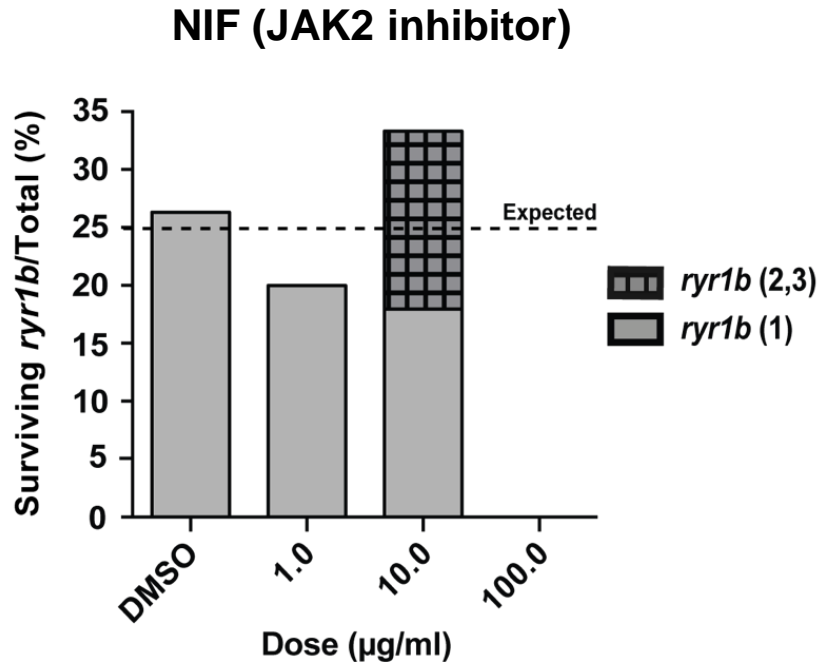
# JAK-STAT inhibition (10 ug/ml NIF) corrects mild morphological defects



# Inhibiting JAK-STAT as a therapeutic approach for *RYR1*-related congenital myopathy



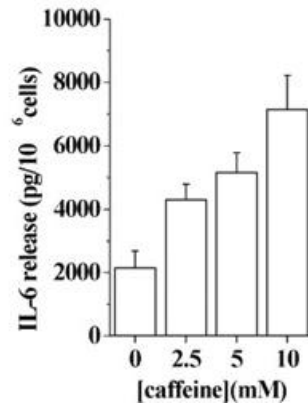
# JAK-STAT inhibitors show dose-response



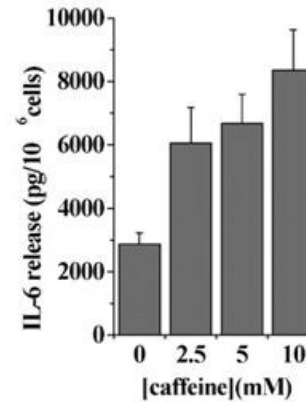
3 = wild-type swimming  
2 = moderate swimming  
1 = poor swimming  
at 5 dpf

# Increased caffeine-induced IL-6 release by myotubes with *RYR1* mutations

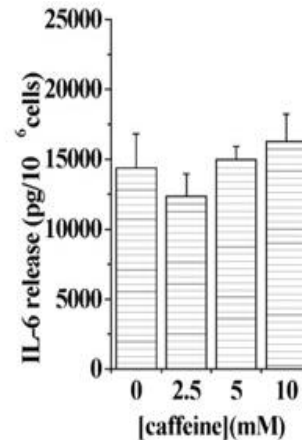
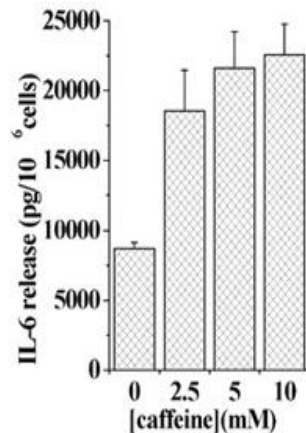
**Normal**



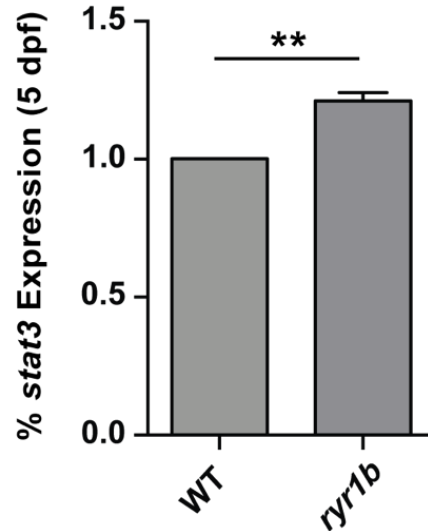
**MH *RYR1* mutant (non-CM)**



**CCD *RYR1* mutants (CM)**



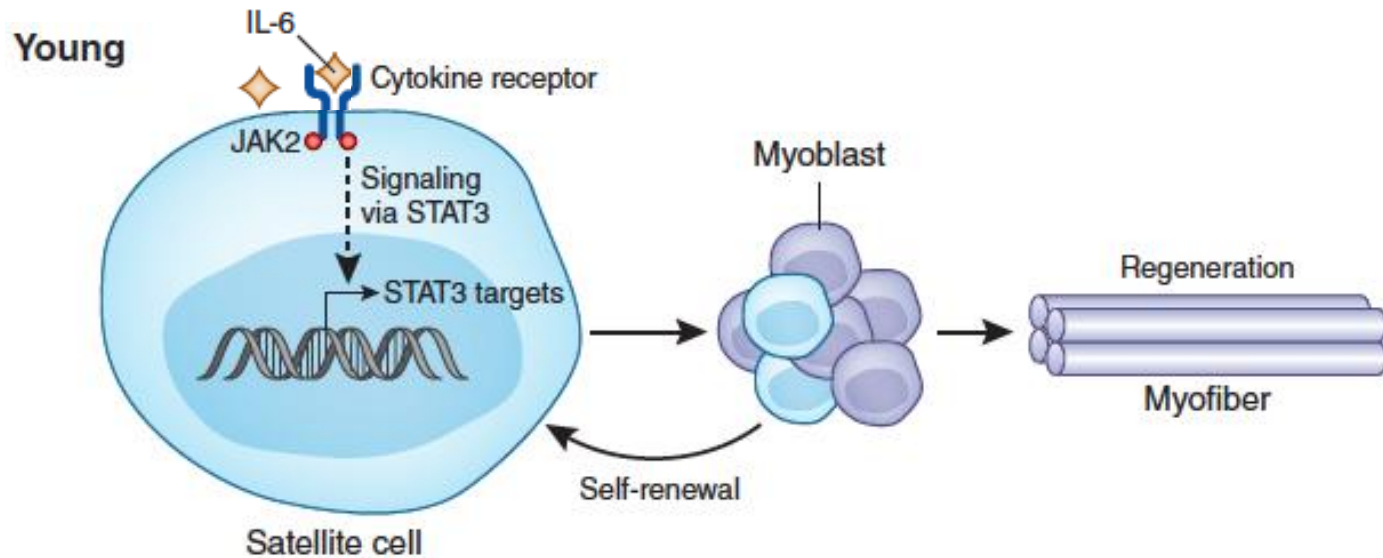
# Increased *stat3* expression and sensitivity to NIF treatment in 5dpf *ryr1b* mutants



qRT-PCR of *stat3* mRNA



# Potential mechanism of JAK-STAT in skeletal muscle



# Potential mechanism of JAK-STAT in skeletal muscle

