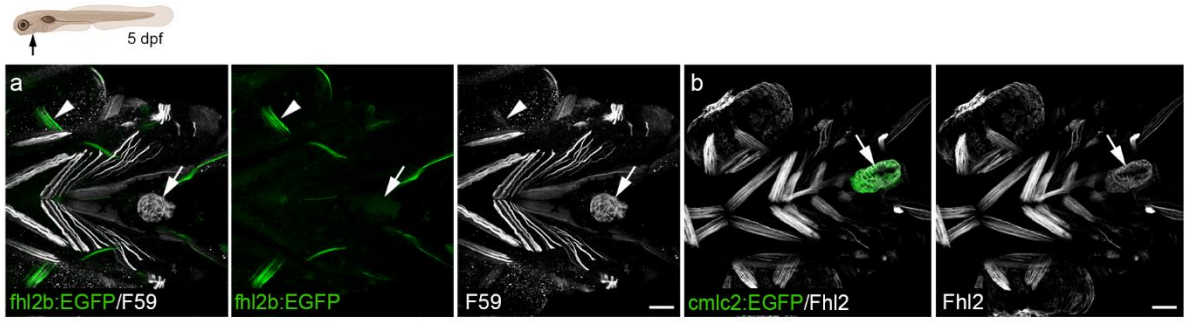
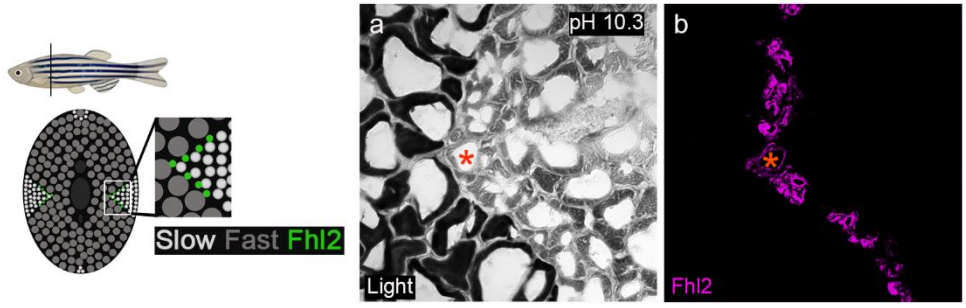
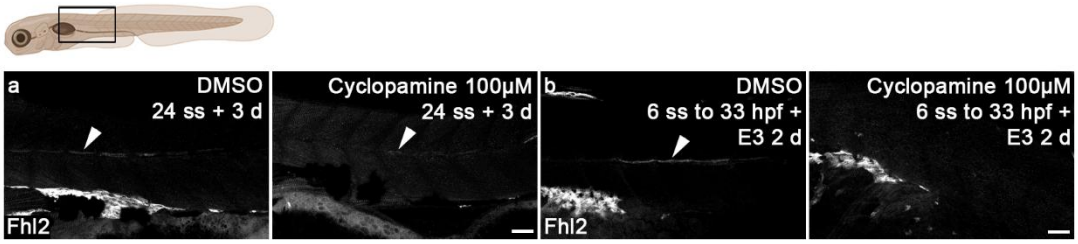
Supplementary figures and figure legends



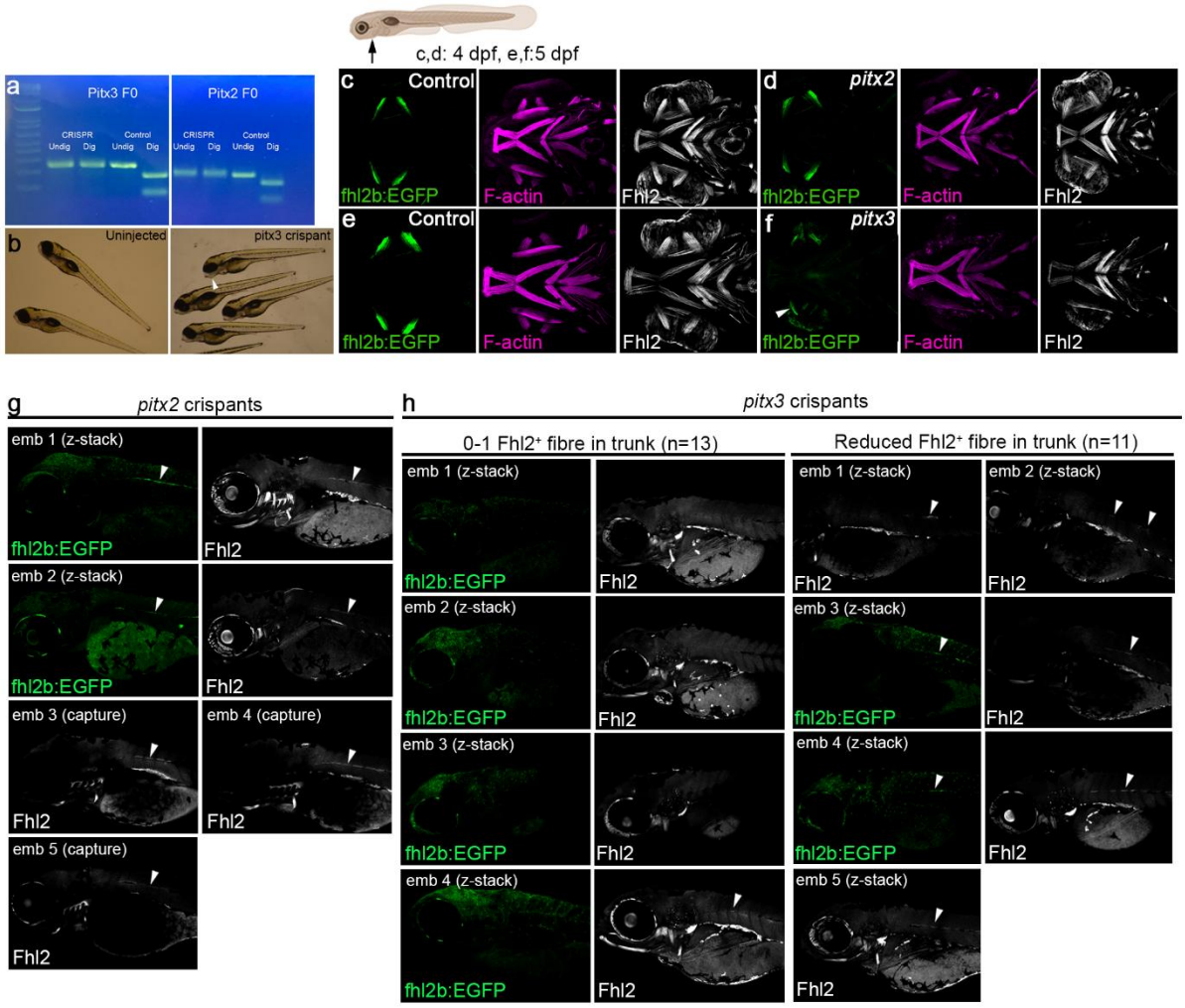
Supplementary figure 1. a) Ventral view of head of 5 dpf *Tg(fhl2b:EGFP)* embryo immunohistochemically stained with F59. b) Ventral view of head of 5 dpf *Tg(cmcl2:EGFP)* immunohistochemically stained with Fhl2. arrowhead indicate extraocular muscles and arrow indicated heart. Scale bar: 25 µm.



Supplementary figure 2. a) ATPase assay on transversal section of adult zebrafish performed at pH 10.3. b) Consecutive transversal section immunostained with Fhl2. Asterisk indicates the same fiber.



Supplementary figure 3. Fhl2 immunolabeling of embryos treated with DMSO or 100µM cyclopamine from a) 24 ss plus 3 days (n= 5 and 5 respectively) and b) 6 ss to 33 hpf plus 2 days in embryo medium (E3) (n=5 and 6 respectively), White arrowhead indicates $Fhl2^{+}$ fibres. Scale bar: 50 µm.



Supplementary figure 4. a) Undigested (undig) and digested (dig) PCR products of amplified target regions in *pitx2* (n=20, HindIII) and *pitx3* (n=20, PfoI) crispant embryos in comparison

to uninjected siblings (n=20). b) Uninjected siblings and *pitx3 crispant* embryos at 5 dpf. Arrowhead indicate malformed jaw. c) Lateral view of 4 dpf control *Tg(fhl2b:GFP)* and d) *Tg(fhl2b:GFP) pitx2* crispant embryo, F-actin is labelled to visualise cranial muscles. e) Lateral view of 5 dpf control *Tg(fhl2b:GFP)* and f) *Tg(fhl2b:GFP) pitx3* crispant embryo, F-actin is labelled to visualise cranial muscles. g) Examples of 5 dpf *pitx2* crispant embryos in *Tg(fhl2b:GFP)* and wt background immunolabeled with Fhl2. Arrowheads indicate $Fhl2^{+}$ fibres. h) Examples of 5 dpf *pitx3* crispant embryos in *Tg(fhl2b:GFP)* and wt background immunolabeled with Fhl2. Embryos are divided into two groups with very few (0-1) fibres (n=13) and reduced number (n=11) of fibres in trunk. Arrowheads indicate $Fhl2^{+}$ fibres.

Supplementary tables

Supplementary table 1. Fiber type proportions at different stages.

Fiber identity	**4 dpf** **Mean±SD**	**5 dpf** **Mean±SD**	**Adult** **Mean±SD**
Fhl2/fhl2b/myh7	16.829± 16.504	68.102± 13.171	63.197± 16.242
Fhl2/fhl2b	65.237± 19.501	27.269± 16.169	2.287± 4.484
Fhl2/myh7	4.079± 6.626	2.778± 6.804	18.092± 8.647
Fhl2	13.855± 11.186	1.852± 4.536	12.111± 11.894

Supplementary table 2. Fiber type proportions after dnPKA treatment.

Fiber identity	**Control** **Mean±SD**	**DnPKA** **Mean±SD**	**p-value**
Fhl2/myh7/fhl2b	0.56±0.40	0.78±1.12	0.2563
Fhl2/myh7	0.17±0.28	0.93±0.91	0.1221
Fhl2/fhl2b	2.44±0.83	0±0	0.0004
Fhl2	0.55±0.50	0.07±0.22	0.0409
myh7	0±0	6.52±1.95	<0.0001

Supplementary Table 3. Antibodies used.

Primary antibody	**Host**	**Dilution**	**Source**	**Productnr/clone**
F310	Mouse	1;10	DSHB	AB_531863
S58	Mouse	1;10	DSHB	AB_528377
MF20	Mouse	1;10	DSHB	AB_2147781
A4.1025	Mouse	1;10	DSHB	AB_528356
4D9 (engrailed)	Mouse	1;10	DSHB	AB_528224
Myomesin	Mouse	1;10	DSHB	AB_760349
F59	Mouse	1;10	DSHB	AB_528373
Syncoilin	Rabbit	1;100	Proteintech	25151-1-AP
Prox1	Rabbit	1;100	GeneTex	GTX128354
Desmin	Rabbit	1;100	Abcam	ab15200
Acetylated Tubulin	Rabbit	1;100	Sigma-Aldrich	T7451
Fhl2	Rabbit	1;100	Sigma-Aldrich	HPA005922
Fhl1	Rabbit	1;100	Proteintech	10991-1-AP
Fhl5	Rabbit	1;100	Invitrogen	PA5-21814
Secondary antibodies	**Against**	**Dilution**	**Source**	**Productnr/clone**
Alexa Fluor 633	Goat anti-mouse IgG	1;500	Invitrogen, Molecular probes	A21050
Alexa Fluor 633	Goat anti-rabbit IgG	1;500	Invitrogen, Molecular probes	A21070
Alexa Fluor 594	Goat anti-mouse IgG	1;500	Invitrogen, Molecular probes	A11005
Phalloidin rhodamine	Directly conjugated	1;500	Invitrogen, Molecular probes	R415
α-bungarotoxin Alexa Fluor 555	Directly conjugated	1;300	Invitrogen, Molecular probes	B35450
α-bungarotoxin Alexa Fluor 647	Directly conjugated	1;500	Invitrogen, Molecular probes	B35451
Phalloidin Alexa Fluor 647	Directly conjugated	1;100	Invitrogen, Molecular probes	A22287

Supplementary table 4. Oligonucleotide primers used for *in situ* probe synthesis.

myh7_F	TGAAGAGTCGCAAGCCGAGT
myh7_R	TCCTCGTGCTCCAGTGATGC
T7+myh7_R	CAGTGAATTGTAATACGACTCACTATAGGGAGATCCTCGTGCTCCAGTGATGC
myh7l_F	CAGGCTTGTGAAGGGCAAGC
myh7l_R	TCCAGTTGGCCCAGAAGACC
T7+myh7l_R	CAGTGAATTGTAATACGACTCACTATAGGGAGATCCAGTTGGCCCAGAAGACC
myh7ba_F	GAACCCGCCAAAGTTTGACC
myh7ba_R	TCAATGTCAGCAGAGGCCAG
T7+myh7ba_R	CAGTGAATTGTAATACGACTCACTATAGGGAGATCAATGTCAGCAGAGGCCAG
myh7bb_F	TGAGGGAGTTTGGAGAGGCG
myh7bb_R	AGTTTTGCCAGCACCGGATTC
T7+myh7bb_R	CAGTGAATTGTAATACGACTCACTATAGGGAGAAGTTTTGCCAGCACCGGATC
myhb_F	AGGGCAACTCAAGGATGCTC
myhb_R	TCAGCACCACGTCTCTGTTC
T7+ myhb_R	CAGTGAATTGTAATACGACTCACTATAGGGAGATCAGCACCACGTCTCTGTTC
fhl2b_F	GCAAAAAGCCCATTGGCTGC
fhl2b_R	CAGGTCTCATGCCAGCTGTTC
T7+ fhl2b_R	CAGTGAATTGTAATACGACTCACTATAGGGAGACAGGTCTCATGCCAGCTGTTC

Supplementary table 5. List of Oligonucleotide Primers used for RT-qPCR.

fhl2a_F	CTCTTCACACGACCAGCAG
fhl2a_R	CCAGTGGTGATGGGCTTCTT
fhl2b_F	AAGGGGAACAGCTGGCATGA
fhl2b_R	GTCGTGATAGGTCACGCCCC
myh7_F	GACAAGGCAATCATGGGGGA
myh7_R	GAGGGTTCTGGGGGTGAATG
β-actin_F	GCCTTCCTTCCTGGGTATGG
β-actin_R	CCAAGATGGAGCCACCGAT