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Supplementary Information for

Drosophila model of myosin myopathy rescued by overexpression of a TRIM-protein family member

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1. Supplementary Methods

Rapid iterative negative geotaxis (RING) assay

Four-day-old, 2-week-old and 5-week-old flies were evaluated for negative geotaxis, an innate escape response driven by mechanical stimulation of the flies. The RING assay was performed as previously described by Gargano, et al. (1). Briefly, the flies' positions in the tubes were captured in digital images taken 3s after initiating the behaviour/tapping. The RING was assessed in a total of 5 consecutive trials separated by 1 min of rest for each genotype and age group at room temperature in a transparent vial (9 cm high, internal diameter 2.6 cm). After completed trials, captured images were used to score the height climbed for each fly. Climbing ability was calculated as the average of five sequential trials. Twenty flies per genotype were assessed in 5 replicates.

Construction of UAS-Mhc

The *pUASTaAttB* vector, FlyBase ID: FBmc0003002, was used as the construct backbone. Available *AttB* sites flanking the *UAS* promoter and cDNA fragment allow site-directed insertion into the *Drosophila* genome upon injection into a specific strain that carries corresponding *AttP*-sites at a given genomic location. Two plasmids covering

the embryonic *Mhc* cDNA, a 5' part (covering exons 1-12) and a 3' part (covering exons 12-19) both carried in the *BlueScript-KS* vector, was kindly provided by S.I. Bernstein (San Diego State University). The two *Mhc* cDNA fragments were combined by excision of the 5' part with *XbaI* and *ApaI,* followed by sub-cloning into the *BlueScript-KS* vector containing the 3' fragment cut with the same restriction enzymes. The resulting full length embryonic *Mhc* cDNA was excised using *NotI* and *KpnI* restriction enzymes and ligated into the *pUASTaAttB* vector which was digested with the same enzymes. The *K1728del* mutation was introduced by QuikChange II (Agilent Technologies) into the full-length wild type embryonic *Mhc* cDNA fragment (GenScript, Piscataway, NJ, USA). The wild-type and mutated *Mhc* constructs were validated by sequencing of the entire cDNAs. Transgenic lines carrying *UAS-Mhc or UAS-Mhc*^{K1728del} on the second chromosome were generated in a *w*^{1118} background (w^{1118-}*;UAS-Mhc* and *w*$^{-}$*;UAS-Mhc*^{K1728del}) (BestGene). The muscle-specific *Mef2-Gal4* driver was used to induce *UAS-Mhc* or *UAS-Mhc*^{K1728del} overexpression.

2. Supplementary Figures

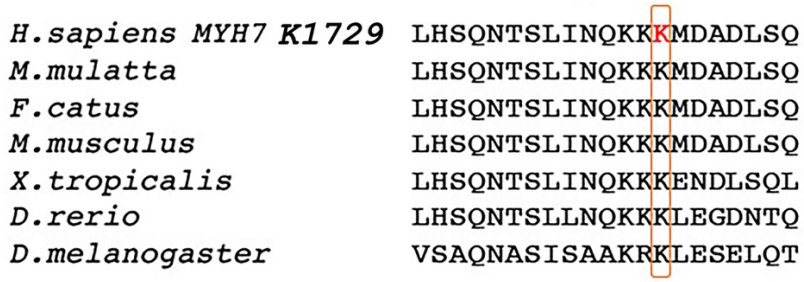

Fig. S1. Alignment of myosin heavy chain from human (*MYH7*) and several species including *Drosophila* (*Mhc*)

Multiple sequence alignment showing evolutionary conservation in the region surrounding residue K1729 (highlighted in red) of human *MYH7* that is associated with Laing distal myopathy.

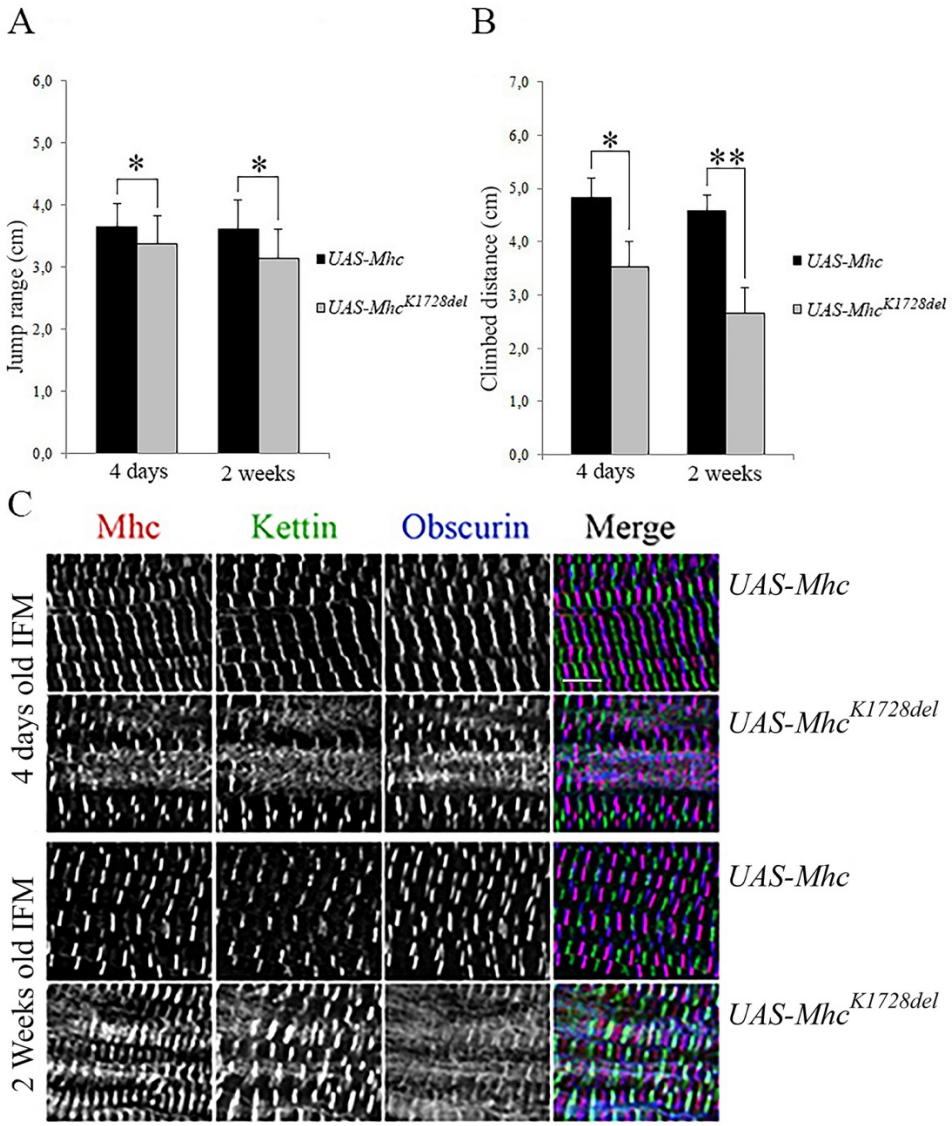

Fig. S2. Impaired muscle function and structure on expression of *UAS-Mhc^{K1728del}* in muscle

(A) Adult jump ability at 4 days and 2 weeks of age: *UAS-Mhc^{K1728del}* overexpressing flies showed reduced jump ability, compared to controls overexpressing wild type Mhc (*UAS-Mhc*) ($p<0.037$ at 4 days and $p<0.003$ at 2 weeks of age). No significant difference in jump ability was observed in either 4-day- or 2-week-old flies of a given genotype. (**B**) Climbing ability at 4 days and 2 weeks of age: climbing ability of *UAS-Mhc^{K1728del}* overexpressing flies was severely impaired compared to *UAS-Mhc*

expressing flies at both time-points measured ($p<0.003$ at 4-day-old and $p<0.0001$ at 2-week-old flies). There is no significant difference in climbing ability between 4-day- and 2-week-old flies, as observed with jump ability. (**C**) IFMs were labelled for Mhc, Kettin/Titin and Obscurin in 4-day-old, and 2-week-old adult flies with overexpression of wildtype Mhc or $Mhc^{K1728del}$. Flies overexpressing wildtype Mhc (*UAS-Mhc*) show parallel periodic striations across the IFMs at both time-points investigated. $Mhc^{K1728del}$ expressing (*UAS-*$Mhc^{K1728del}$) flies show progressive disruptions in sarcomeric structure. The 4-day-old adult myofibrils of *UAS-*$Mhc^{K1728del}$ flies show sarcomere fragmentation and unstructured Z-disks, myosin-containing A-bands and M-band. Severe undefined structure of Z-disks and M-bands were observed in IFMs of 2-week-old adult *UAS-*$Mhc^{K1728del}$ flies. A-bands appeared less separated. Scale bar 5 μm.

3. Supplementary Tables

Table S1. Table listing the genotypes used in the current study

The table shows the abbreviated genotypes used in text and figures (left column) together with the corresponding full genotype (right column).

Table S1

Abbreviated genotype	Full genotype
Control	w^{1118};*Sco*/*CyO, Dfd>YFP*
***Mhc*^{K1728del}/+**	w^{1118};*Mhc*^{K1728del}/*CyO, Dfd>YFP*
***Mhc*^{K1728del}/*Mhc*^{K1728del}**	w^{1118};*Mhc*^{K1728del}/Crispr-*Mhc*^{K1728del}
***Mhc*^{K1728del}/*Mhc*^{10}**	w^{1118};*Mhc*^{K1728del}/*Mhc*^{10}
Mef2>Abba	w^{1118};*UAS-Abba*/*Mef2-Gal4*
Mhc*^{K1728del}/+;*Mef2>Abba	w^{1118};*Mhc*^{K1728del}/+;*UAS-Abba*/*Mef2-Gal4*
Mhc*^{K1728del}/*Mhc*^{10};*Mef2>Abba	w^{1118};*Mhc*^{K1728del}/*Mhc*^{10};*UAS-Abba*/*Mef2-Gal4*
***abba*^{MJO0348}/+**	*Abba*^{MJO0348}/*CyO, Twi>GFP*
***Mhc*^{K1728del}/*abba*^{MJO0348}**	w^{1118}/+;*Mhc*^{K1728del}/*abba*^{MJO0348}
Mef2>Mhc	w^{1118};*UAS-Mhc, Mhc*^{1}/+;*Mef2-Gal4*/+
***Mef2>Mhc*^{K1728del}**	w^{1118};*UAS-Mhc*^{K1728del}*, Mhc*^{11}/+;*Mef2-Gal4*/+
Mhc*^{K1728del}/*Mhc*^{1}; *Mef2>Mhc	w^{1118};*Mhc*^{K1728del}/*UAS-Mhc, Mhc*^{1};*Mef2-Gal4*/+

4. Supplementary References

1. Gargano JW, Martin I, Bhandari P, & Grotewiel MS (2005) Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in Drosophila. *Experimental gerontology* 40(5):386-395.