Zebrafish models of BAG3 myofibrillar myopathy suggest a toxic gain of function leading to BAG3 insufficiency

Avnika A. Ruparelia¹, Viola Oorschot², Raquel Vaz¹, Georg Ramm²³, Robert J. Bryson-Richardson¹*

¹ School of Biological Sciences, Monash University, Melbourne, Victoria, 3800, Australia.
²Monash Micro Imaging, Monash University, Melbourne, Victoria, 3800, Australia.
³Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria, 3800, Australia.

*Corresponding author:
E-mail: robert.bryson-richardson@monash.edu
Telephone: (+61) 3 99024629
Fax: (+61) 3 99055613
Supplementary Figure S1 *bag3* expression is restricted to the muscle. Lateral, dorsal, and cross-section views of *bag3* expression at the 10-somite (a), 16-somite (b), 24-somite (c), and 28-somite (d) stage. At the 10-somite stage *bag3* is expressed in precursors of both slow and fast muscle cells. At the 16-, 24- and 28-somite stages *bag3* expression is seen throughout the skeletal muscle.
Supplementary Figure S2: *bag3* is expressed in the adult zebrafish heart. RT-PCR using cDNA obtained from adult hearts reveals presence of *bag3* transcript in the heart. Lane 1 and 2: *β-actin* and *bag3* in cardiac cDNA respectively; Lane 3 and 4: No template control with *β-actin* and *bag3* primers respectively.
Supplementary Figure S3: Increased temperatures exacerbate the BAG3\textsuperscript{P209L} aggregate phenotype.
(a) Incubation of 48-hpf embryos expressing BAG3\textsuperscript{wt}-eGFP at 37\textdegree C has no effect on its localization or aggregation. (b) 37\textdegree C incubation of 48-hpf BAG3\textsuperscript{P209L}-eGFP expressing embryos however, results in the formation of larger aggregates.
Supplementary Figure S4: BAG3\textsuperscript{P209L}-eGFP aggregates are not contained within autophagic vesicles.

With the exception of a few BAG3\textsuperscript{P209L}-eGFP aggregates (arrowheads) most aggregates are not contained within autophagic vesicles as demonstrated by lack of overlap between GFP and LysoTracker (red).
**Supplementary Figure S5:** *bag3* morpholino injected embryos display fiber dissolution, similar to that seen in MFM patients.

Muscle fibers of 48-hpf un-injected control embryos and intact fibers in the *bag3* splMO injected embryos have normal Myosin and Actinin striation patterns (a-b). However, in the *bag3* splMO injected embryos where the fiber has broken and Myosin striation is lost, Actinin striation is also lost (arrowhead). The myosepta, as seen by Dystrophin labeling (c-d), in the *bag3* splMO embryo is indistinguishable from that in the un-injected control embryo. Fast muscle fibers, identified by their orientation, absence of slow Myosin staining (F59), and presence of Myosin labeling (A4.1025), are also affected in 48-hpf *bag3* splMO injected embryos (e-f).
Supplementary Figure S6: bag3 morphants have decreased bag3 mRNA. RT-PCR revealed a dramatic reduction of bag3 transcript levels in the bag3 splMO embryos, indicative of nonsense mediated decay. Lane 1 and 3: β-actin in un-injected control and bag3 splMO embryos respectively; Lane 2 and 4: bag3 levels in un-injected control and bag3 splMO embryos respectively.
Supplementary Figure S7: Knockdown of bag3 using a translational blocking morpholino results in fiber failure.

(a) Injection of a translational blocking bag3 morpholino (bag3 ATGMO) results in fiber failure at the 26-somite stage as seen by Myosin antibody label. (b) Cascade blue and eGFP expression in embryos injected with RNA in which the translation initiation site of eGFP was replaced with the bag3 translational blocking morpholino target sequence (RNA^{MO Target-eGFP}) (c) Injection of bag3 ATGMO with RNA^{MO Target-eGFP} results in loss of eGFP expression.
Supplementary Figure S8: Loss of BAG3 does not result in protein aggregation.
(a) Incubation of 48-hpf FLNC-eGFP and bag3 splMO co-injected embryos at 37°C does not result in aggregate formation. (b) Knockdown of endogenous Bag3 using bag3 splMO in BAG3\textsuperscript{wt}-eGFP or BAG3\textsuperscript{P209L}-eGFP injected embryos has no effect on the percentage of aggregate containing cells. Error bars indicate standard error.
Supplementary Figure S9: BAG3\textsuperscript{wt} and BAG3\textsuperscript{P209L} are capable of rescuing the bag3 morphant myofiber disintegration phenotype at 37\textdegree C.

(a) Embryos injected with bag3 splMO and bag3 ATGMO and incubated at 37\textdegree C display severe myofiber disintegration phenotype. Fibers expressing BAG3\textsuperscript{wt}-eGFP (b) or BAG3\textsuperscript{P209L}-eGFP (c) on the double morpholino background are intact (arrowhead) despite incubation at 37\textdegree C. (d) In an affected somite of a double morpholino injected embryo 32\% of slow muscle fibers undergo myofibrillar disintegration. In an affected somite 100\% of BAG3\textsuperscript{wt}-eGFP or BAG3\textsuperscript{P209L}-eGFP expressing slow muscle fibers are intact.