# The role of Ime4 RNA methyltransferase in the muscle function of *D. melanogaster*

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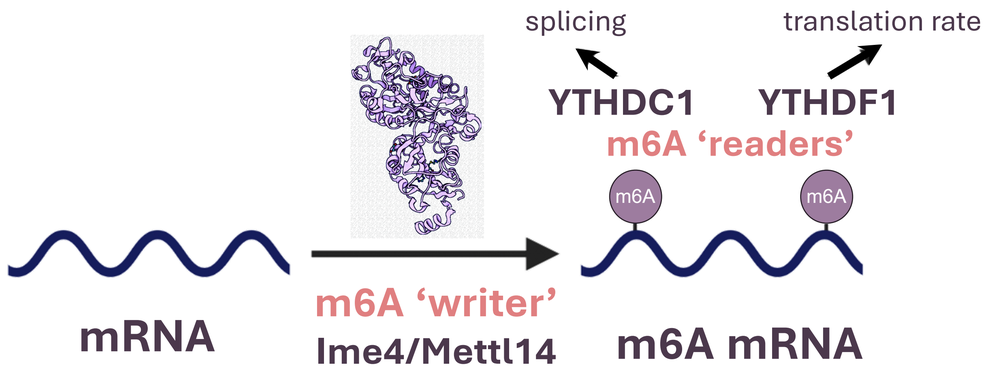
## Introduction

- Correct **mRNA regulation** crucial for **proper muscle function and development**
- **mRNA splicing** is a key step as production of different **protein isoforms** influences muscle protein properties
- **mRNA methylation** implicated in regulating **splicing** and **translation rate**[1]
- **Ime4 RNA methyltransferase** crucial in this process – knock-down and rescue experiments used to study its function
- m6A nuclear reader protein **YTHDC1**[2] and cytoplasmic reader protein **YTHDF1**[3] act downstream of Ime4 – knock-out flies expected to exhibit similar phenotypes




## Ime4 expression in muscles or neurons partially rescues mutant phenotype

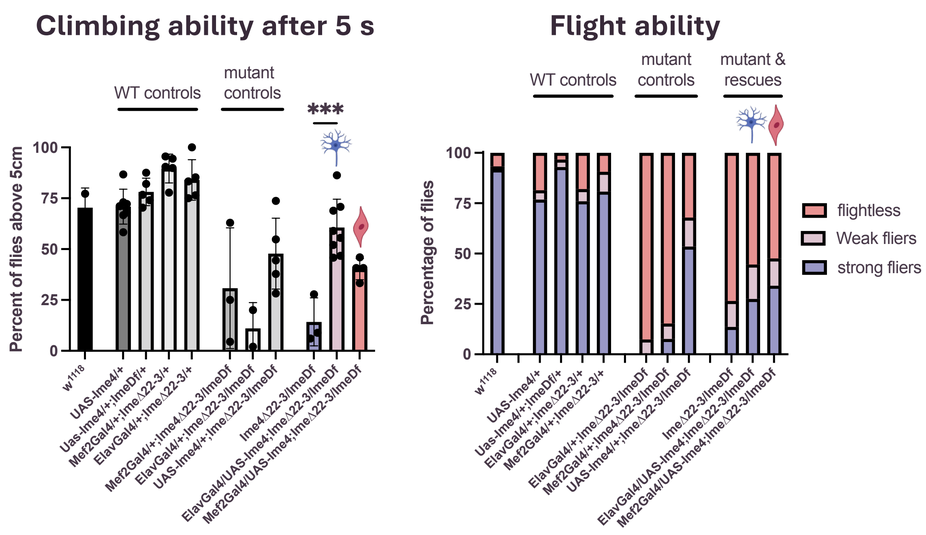



**Figure 1** Flies developed at 27°C tested for flight ability. Phenotype rescue evident in neuronal Ime4 expression.




**Figure 2** Flies developed at 27°C tested for climbing ability. No significant phenotype rescue observed.

## Ime4 regulates the splicing pattern and the expression level of flight muscle proteins

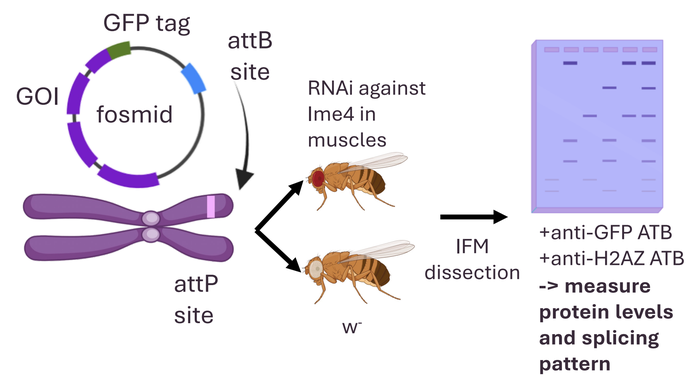



**Figure 3** Genes of interest fused with GFP tag at the most C-terminal exon. Fosmids inserted into the chromosome of wild type and RNAi line against Ime4 in muscles. Indirect flight muscles (IFMs) were dissected and run on a Western blot. Anti-GFP antibody was used to visualise the amount of protein together with its splicing pattern. Anti-H2AZ antibody was used to verify the equal sample loading for each lane.

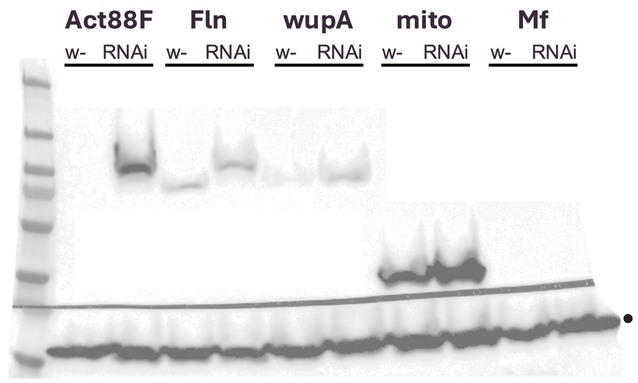



**Figure 4** Western blot of flight muscle proteins – Actin (Act88F), Flightin (Fln), Troponin I (wupA), mito (mitochondrial tag), and Myofilin (Mf). Wild type and RNAi line present for all proteins. Change in protein content evident in Fln, wupA, and mito. There is an apparent change in splicing pattern for Act88F and Fln. Dot marks anti-H2AZ antibody control.

## Cytoplasmic and nuclear m6A reader proteins exhibit varied phenotype

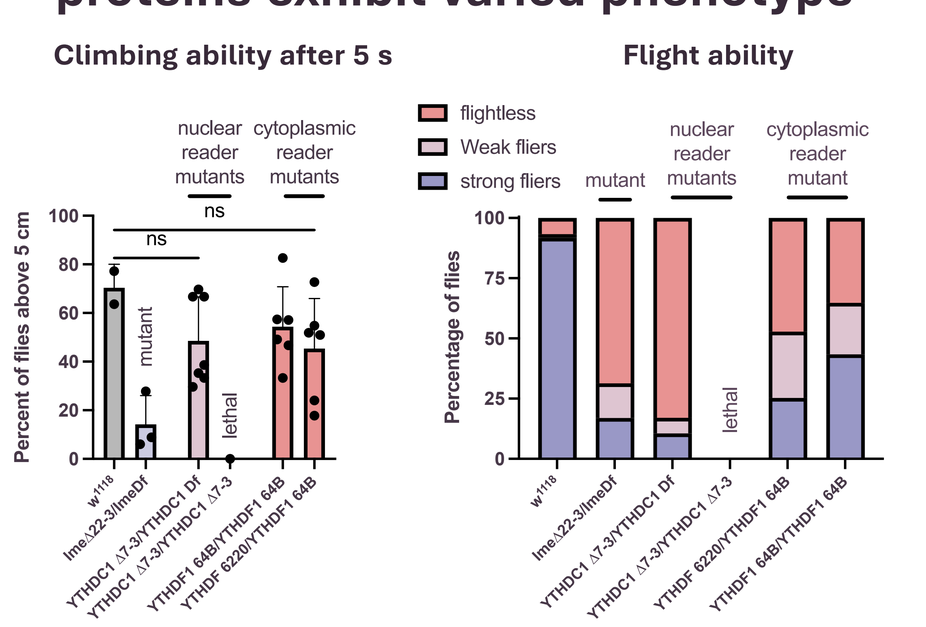



**Figure 5** Flies developed at 27°C tested for flight ability. m6A reader protein mutation seems to affect climbing ability only mildly compared to the wild type.




**Figure 6** Flies developed at 27°C tested for climbing ability. Both reader protein mutants exhibit strong phenotype similar to the mutant line. Nuclear reader mutants exhibit stronger phenotype than cytoplasmic readers.

## Cytoplasmic and nuclear m6A reader proteins modulate splicing and expression level of flight muscle proteins

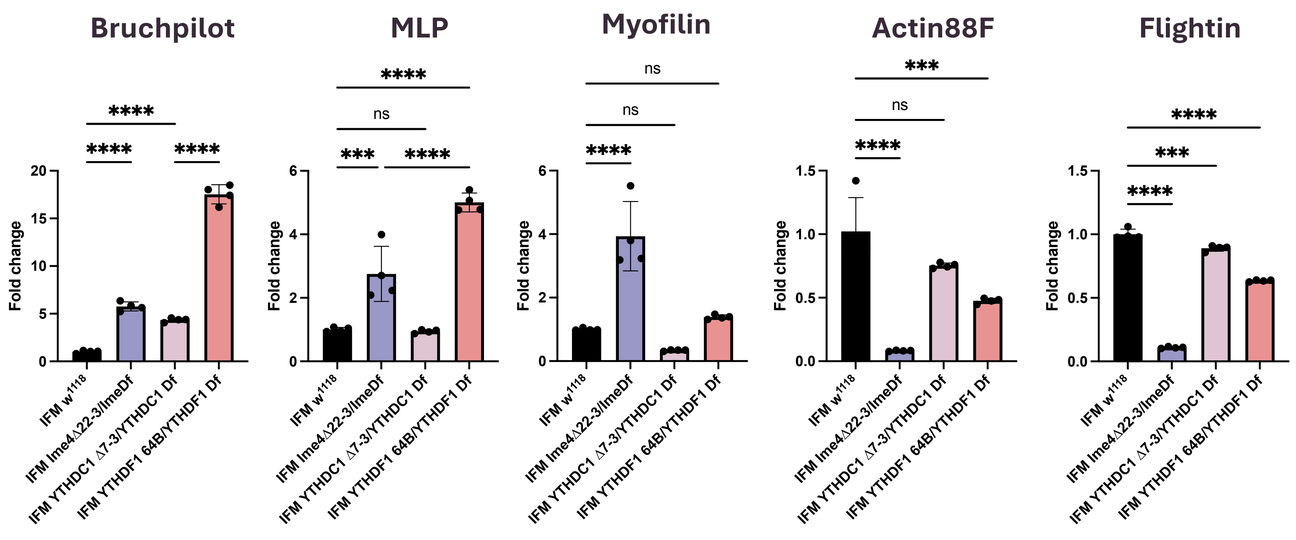



**Figure 7** qPCR of IFM mRNA coding for muscle proteins. mRNA levels measured in wild type, Ime4 mutant, nuclear m6A reader protein mutant, and cytoplasmic m6A reader protein mutant (lanes from left to right). Increased expression evident in Bruchpilot, MLP, and Myofilin, while Actin88F and Flightin are decreased.

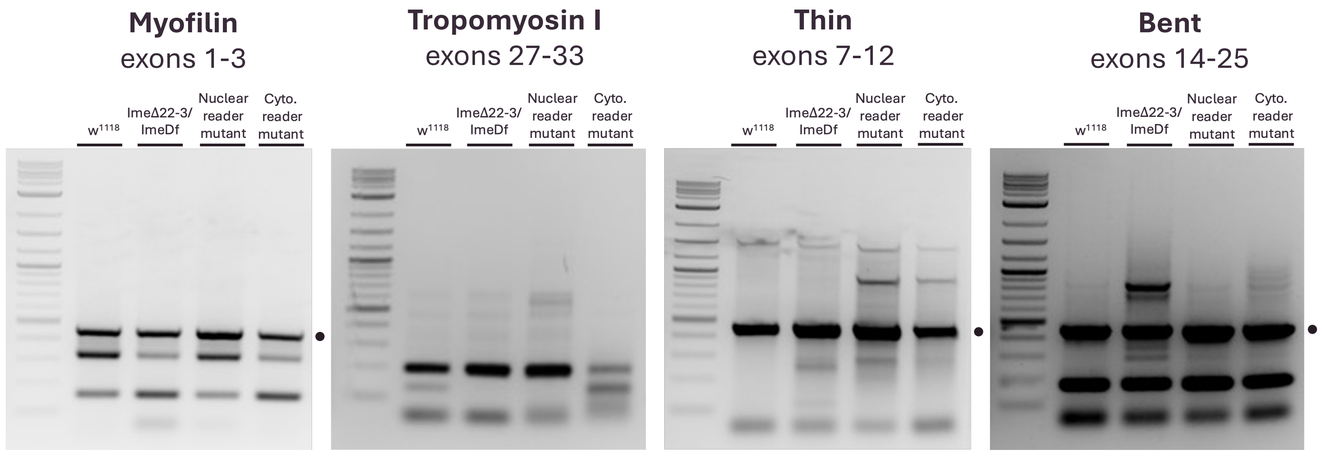



**Figure 8** RT-PCR of mRNA coding for muscle proteins extracted from IFMs. mRNA collected from wild type, Ime4 mutant, nuclear m6A reader protein mutant, and cytoplasmic m6A reader protein mutant (lanes from left to right). In all of the proteins change in splicing pattern is evident. Dot marks RP49 acting as an internal control.

## Conclusion

- Expression of Ime4 in neurons of Ime4 mutants partially rescues impaired climbing ability
- Ime4 regulates the splicing pattern and expression levels of flight muscle proteins
- Cytoplasmic and nuclear m6A reader protein mutants exhibit similar deficiencies as Ime4 mutants, but there is differential requirement for reader proteins in flight
- m6A reader proteins modulate splicing and expression levels of flight muscle proteins

**References**:
[1] Wang Y, Zhao JC. Update: Mechanisms Underlying N6-Methyladenosine Modification of Eukaryotic mRNA. Trends Genet. 2016 Dec;32(12):763–73.
[2] Xiao W, Adhikari S, Dahal U, Chen YS, Hao YJ, Sun BF, Sun HY, Li A, Ping XL, Lai WY, et al. Nuclear m(6)A reader YTHDC1 regulates mRNA splicing. Mol Cell 2016; 61:507; PMID:26876937; https://doi. org/10.1016/j.molcel.2016.01.012
[3] Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, et al. 5′ UTR m(6)A Promotes Cap-Independent Translation. Cell. 2015 Nov 5;163(4):999–1010.

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