Appendix

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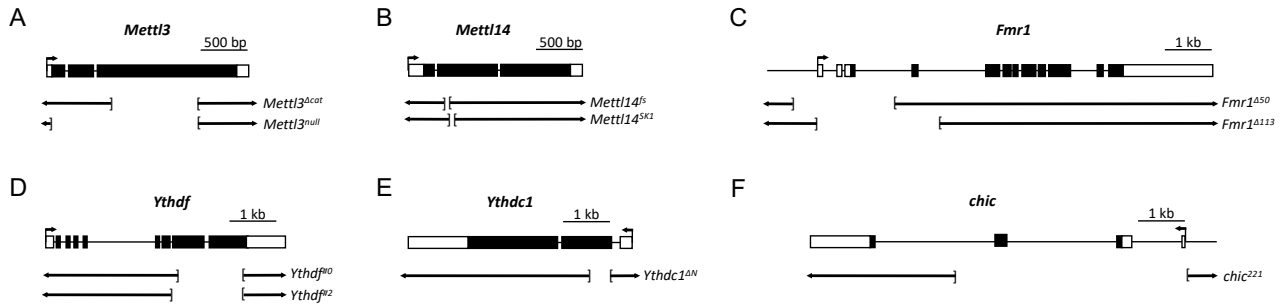

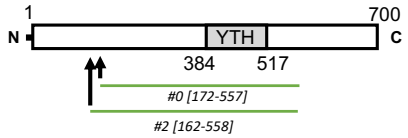

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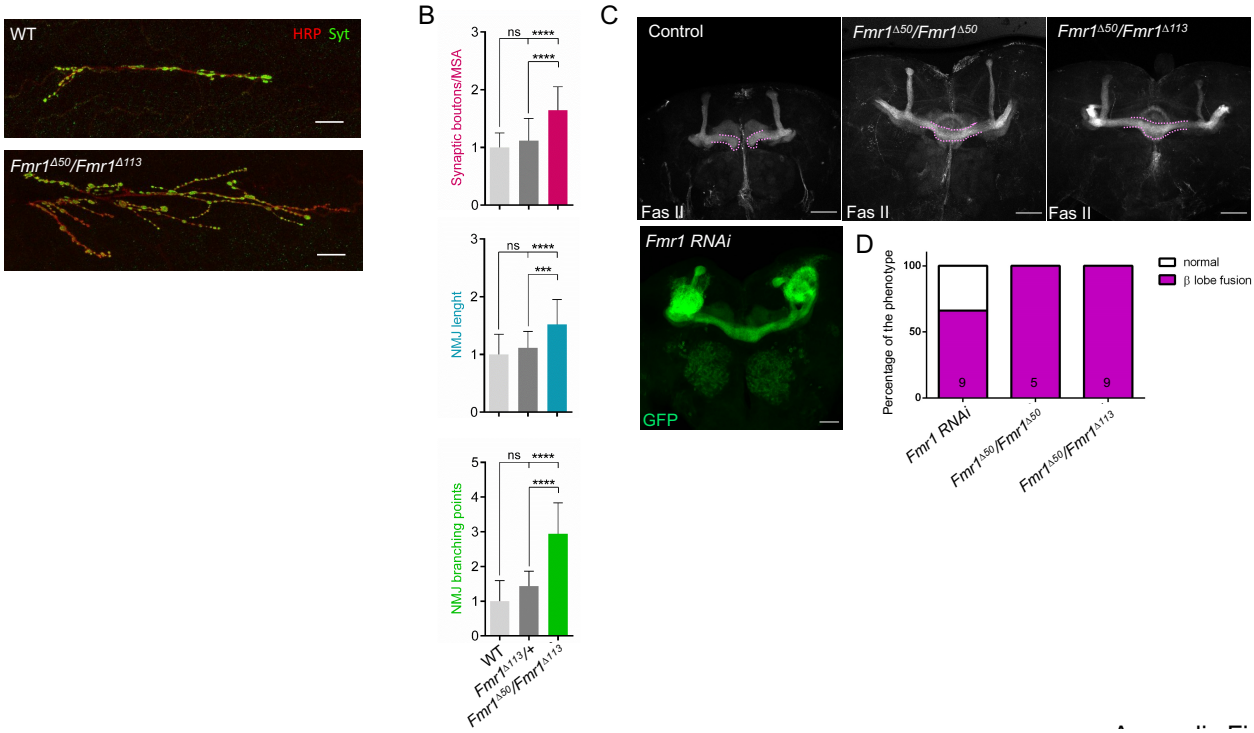
Mutant name	Original pubblication	Description
Mettl3$^{\Delta cat}$	Lence et al., 2016	Deletion 607–1,175 bp in chr3R:24032157..24034257
Mettl3^{null}	Lence et al., 2016	Deletion 1,876–3,166 bp in chr3R:24030157..24034257
Mettl14^{fs}	Lence et al., 2016	Frameshift (2 bp) after 227 aa leading to premature stop codon at aa position 89
Mettl14^{SK1}	Kan et al., 2017	Deletion (7 bp) leading to frameshift after 98 aa and premature stop codon after the addition of 10 novel aa
Fmr1$^{\Delta 50}$	Zhang et al., 2001	Deletion removing P{EP} and flanking DNA including the 5' UTR and the first coding exon
Fmr1$^{\Delta 113}$	Zhang et al., 2001	Deletion removing P{EP} and flanking DNA including the 5' UTR and the first two coding exons
Ythdf$^{\Delta 0}$	Soldano et al., 2020	Deletion of 1221 bp in the CDS, deleting aa 172 to 557
Ythdf$^{\Delta 2}$	Soldano et al., 2020	Deletion of 1319 bp in the CDS, deleting aa 162 to 558
Ythdc1$^{\Delta N}$	Lence et al., 2016	Deletion 133–734 bp in chr3L:3370451..3374170 (reverse complemented)
chic^{221}	Verheyen and Cooley, 1994	Deletion in chr2L:5,975,585..5,980,992
futsch^{N94}	Hummel et al., 2000	Hypomorphic allele

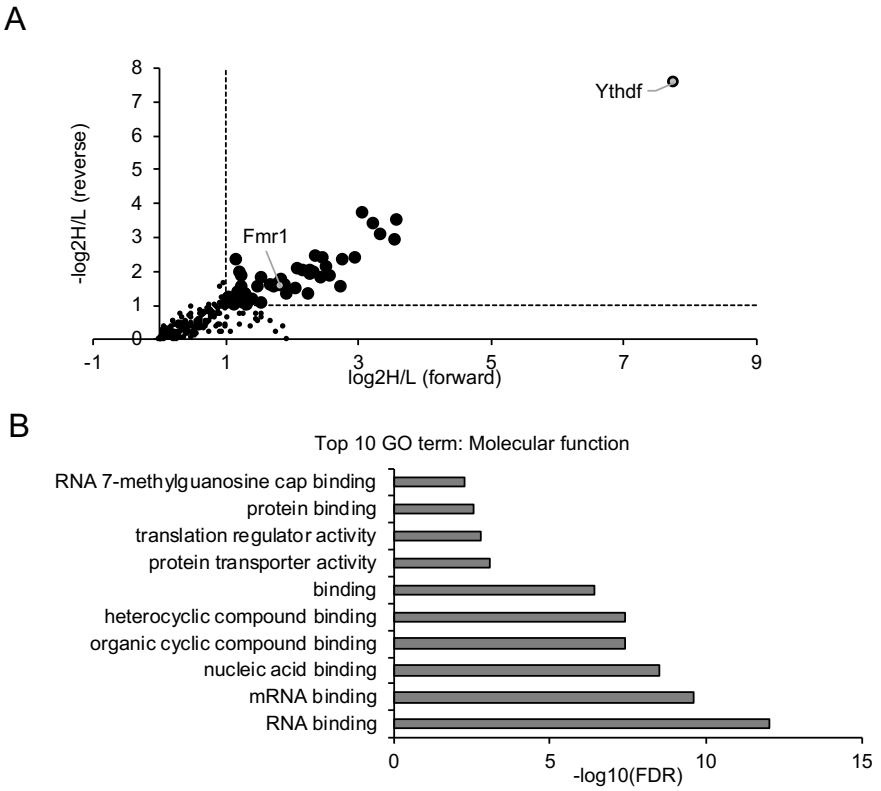
Appendix Figure S1: Molecular information of the alleles used in this study

(A-F) *Mettl3* (A), *Mettl14* (B), *Fmr1* (C), *Ythdf* (D), *Ythdc1* (E) and *chic* (F) loci with indicated deletions. (G) Schematic showing the deleted parts (in green) of the Ythdf protein (H) Table recapitulating the information about the origins and molecular deletions of the alleles used in this study.



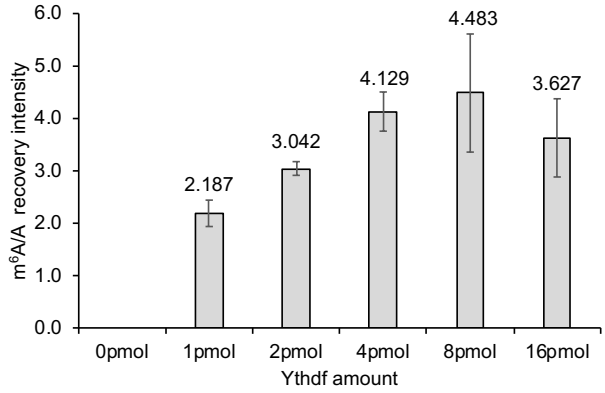
Appendix Figure S2: *Fmr1* loss of function is reminiscent to the m^{6}A loss

(A) Representative confocal images of muscle-6/7 NMJ synapses of abdominal hemisegments A2-A3 for the indicated genotypes labelled with anti-Synaptotagmin (green) and HRP (red) to reveal the synaptic vesicles and the neuronal membrane. Scale bar: 20 µm. (B) Quantification of normalized bouton number, normalized axon length and normalized branching of NMJ 6/7 in A2-A3 of the indicated genotypes. Error bars show mean ± s.e.m. Multiple comparisons were performed using one-way ANOVA with a post-hoc Sidak-Bonferroni correction. (n.s. = not significant; $p<0.001$= ***; $p<0.0001$=****). (C) Immunofluorescence analysis of adult control, *Fmr1*$^{\Delta 50}$*/Fmr1*$^{\Delta 50}$ and *Fmr1*$^{\Delta 50}$*/Fmr1*$^{\Delta 113}$ with anti FasII antibody. The *Fmr1*$^{\Delta 50}$*/Fmr1*$^{\Delta 50}$ brains were dissected few hours prior to eclosion since no homozygote viable adults were obtained. For *c772Gal:UAS-CD8,GFP:Fmr1 (8833) RNAi* brain, MBs are marked by membrane GFP (autofluorescence). Scale bar 50 µm. (D) Quantification of the penetrance of β-lobe fusion phenotype for the indicated genotypes.



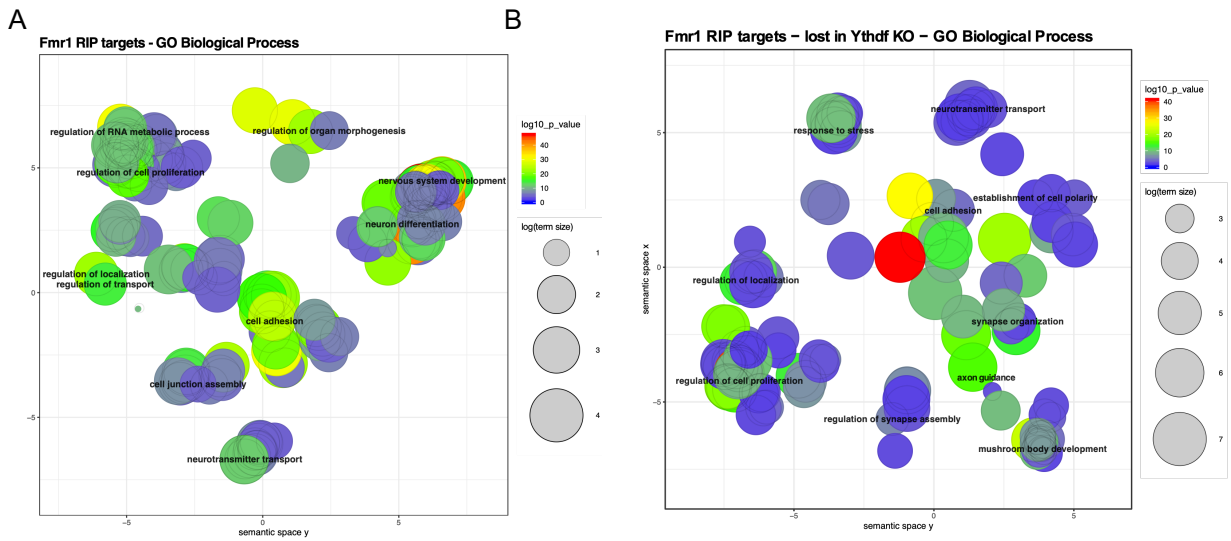
Appendix Figure S3: Ythdf interactome

(A) Quantitative proteomics upon pulldown of Flag-tagged Ythdf in S2R+ cells. Scatter plot of normalized forward versus inverted reverse experiments plotted on a log2 scale. The threshold was set to a 1-fold enrichment (dashed line). (B) Top 10 enriched Gene ontology (GO)-terms of Molecular function for Ythdf interactors.



Appendix Figure S4: Enrichment of Ythdf in m^{6}A probe pulldown fraction is not influenced by Fmr1

Quantification of the enrichment of GST-Ythdf upon pulldown of GGACU RNA probes incubated with 4 pmol of His-NT-Fmr1 and increasing amounts of GST-Ythdf, plotted as the median of the m6A/A signal intensity +/- SEM of all replicates.



Appendix Figure S5: Gene ontology analysis of FMR1-bound mRNAs

(A) Plot of significantly enriched Gene Ontology Biological Process terms in wild type Fmr1 RNA immunoprecipitation targets. Terms are plotted in semantic space, with functionally closer terms closer to one another in the plot. Representatives for each term group are indicated by their name. Adjusted p-values are indicated by the color scale, while term plot size is a function of the corresponding number of genes. (B) Plot of significantly enriched Gene Ontology Biological Process terms in genes lost in *Ythdf* KO samples. Terms are plotted in semantic space, with functionally closer terms closer to one another in the plot. Representatives for each term group are indicated by their name. Adjusted p-values are indicated by the color scale, while term plot size is a function of the corresponding number of genes.