Expanded View Figures

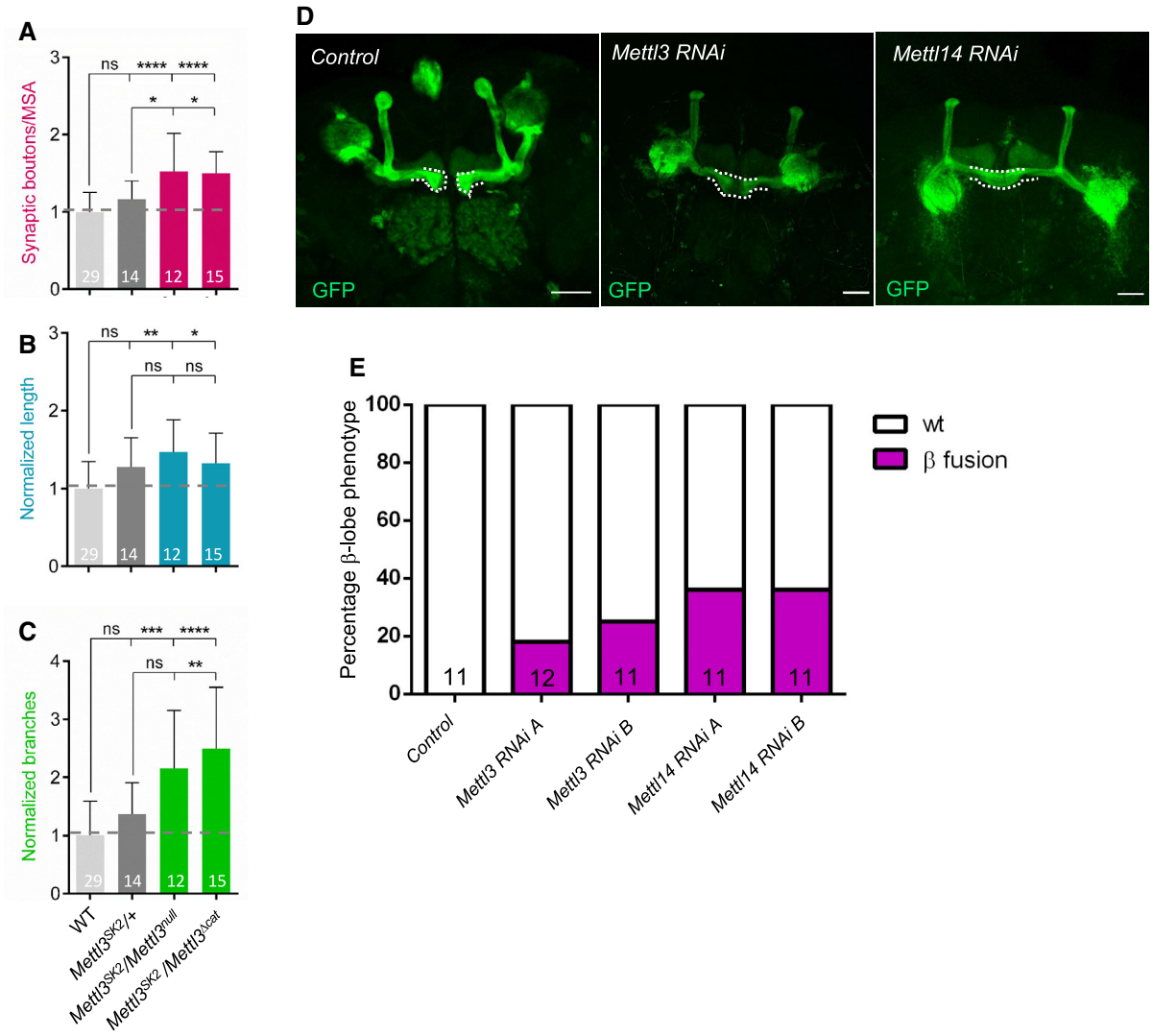

Figure EV1. m^{6}A writer components control axonal growth at NMJ and MB.

A–C Quantification of normalized bouton number (A, total number of boutons/muscle surface area (μm^2 × 1,000)), normalized axon length (B), and normalized branching (C) of muscle-6/7 NMJ in hemisegments A2-A3 of the indicated genotypes. 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 < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).

D Analysis of adult control *c772Gal:UAS-CD8,GFP, c772Gal:UAS-CD8,GFP:Mettl3 RNAi (υ20969)* and *c772Gal:UAS-CD8,GFP:Mettl14 RNAi (υ48560)* MB in the brains by visualization of the membrane GFP autofluorescence. White dashed lines highlight the normal and fused β-lobes. Scale bar 50 μm.

E Quantification of the penetrance of β-lobe fusion phenotype in the following genotypes: control (*c772Gal:UAS-CD8,GFP/+*), *c772Gal:UAS-CD8,GFP:Mettl3 RNAi (υ20968)* indicated as RNAi A, *c772Gal:UAS-CD8,GFP:Mettl3 RNAi (υ20969)* indicated as RNAi B, *c772Gal:UAS-CD8,GFP:Mettl14 RNAi (υ13542)* indicated as RNAi A and *c772Gal:UAS-CD8,GFP:Mettl14 RNAi (υ48560)* indicated as RNAi B.

Data information: In (A–C, E), bars are labeled with the number of replicates.

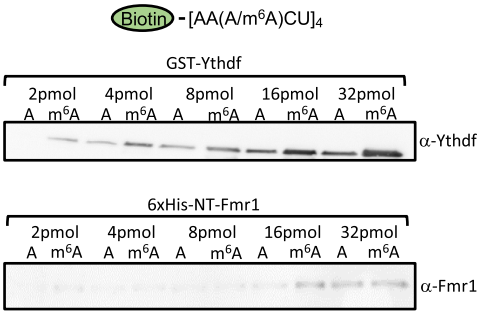

Figure EV2. Binding behavior of Ythdf and Fmr1 on RNA probe in the AAACU sequence context.
Results of m^{6}A RNA pulldown using RNA probes. Pulldown were performed using biotinylated probes containing four AAACU m^{6}A consensus sites, with or without the methylation and recombinant purified GST-Ythdf and His-NT-Fmr1 proteins. Ythdf binds more efficiently upon methylation, while Fmr1 does not show specificity for any of the probes.

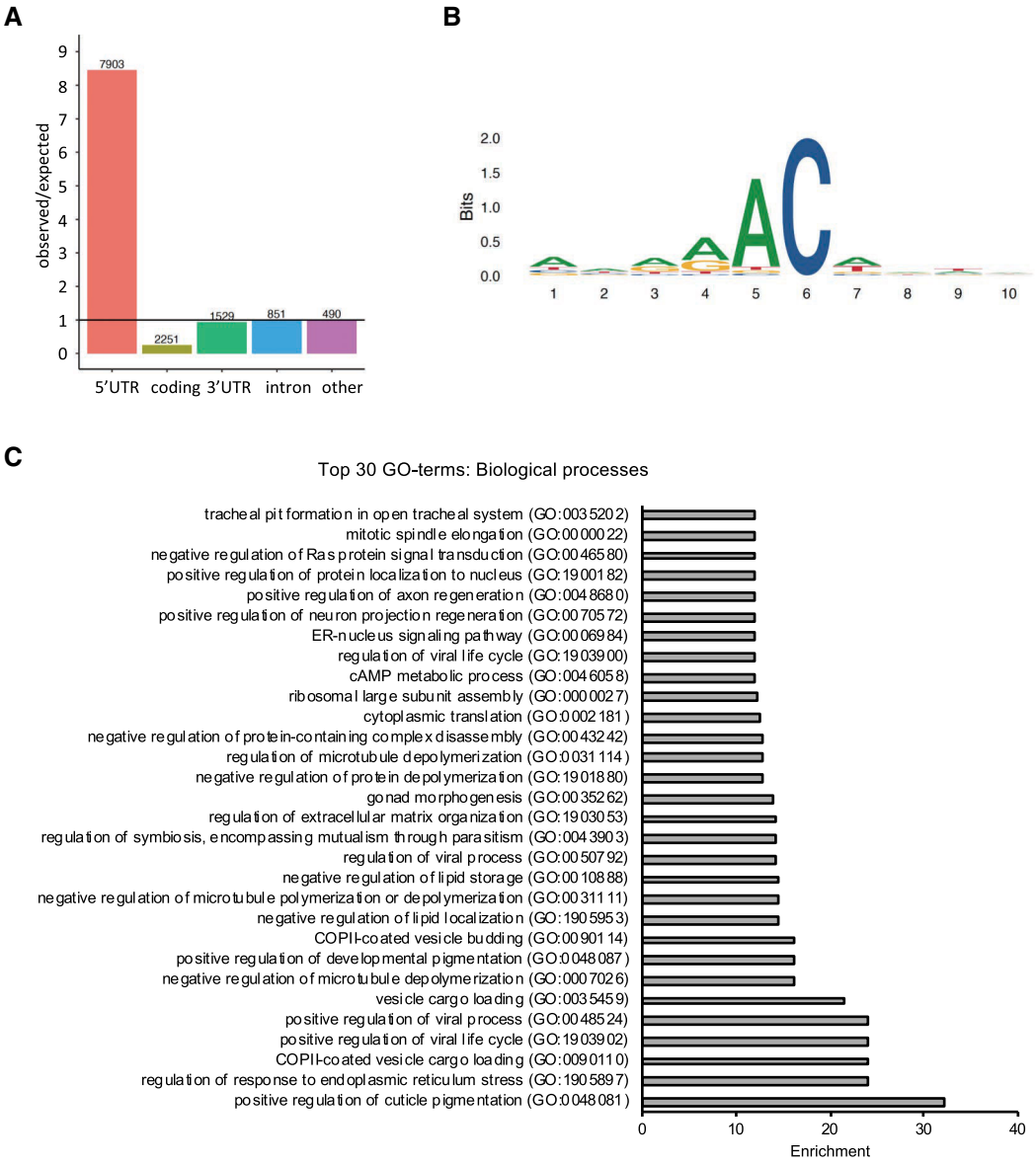

Figure EV3.

Figure EV3. miCLIP analysis from S2R$^+$ cells.

A Relative enrichment of m^{6}A peaks across transcript segments identified by miCLIP. The numbers above the bars correspond to the number of m^{6}A sites determined as described in the Materials and Methods section. In total, a set of 13024 C-to-T conversions CIMS and AC truncation CITS across 2464 genes was identified.

B Sequence logo of deduced consensus motif for m^{6}A peaks centered on the modified adenosine.

C Top 30 enriched Gene ontology (GO)-terms of biological processes for the gene set of m^{6}A modified and Fmr1- and Ythdf-bound transcripts.

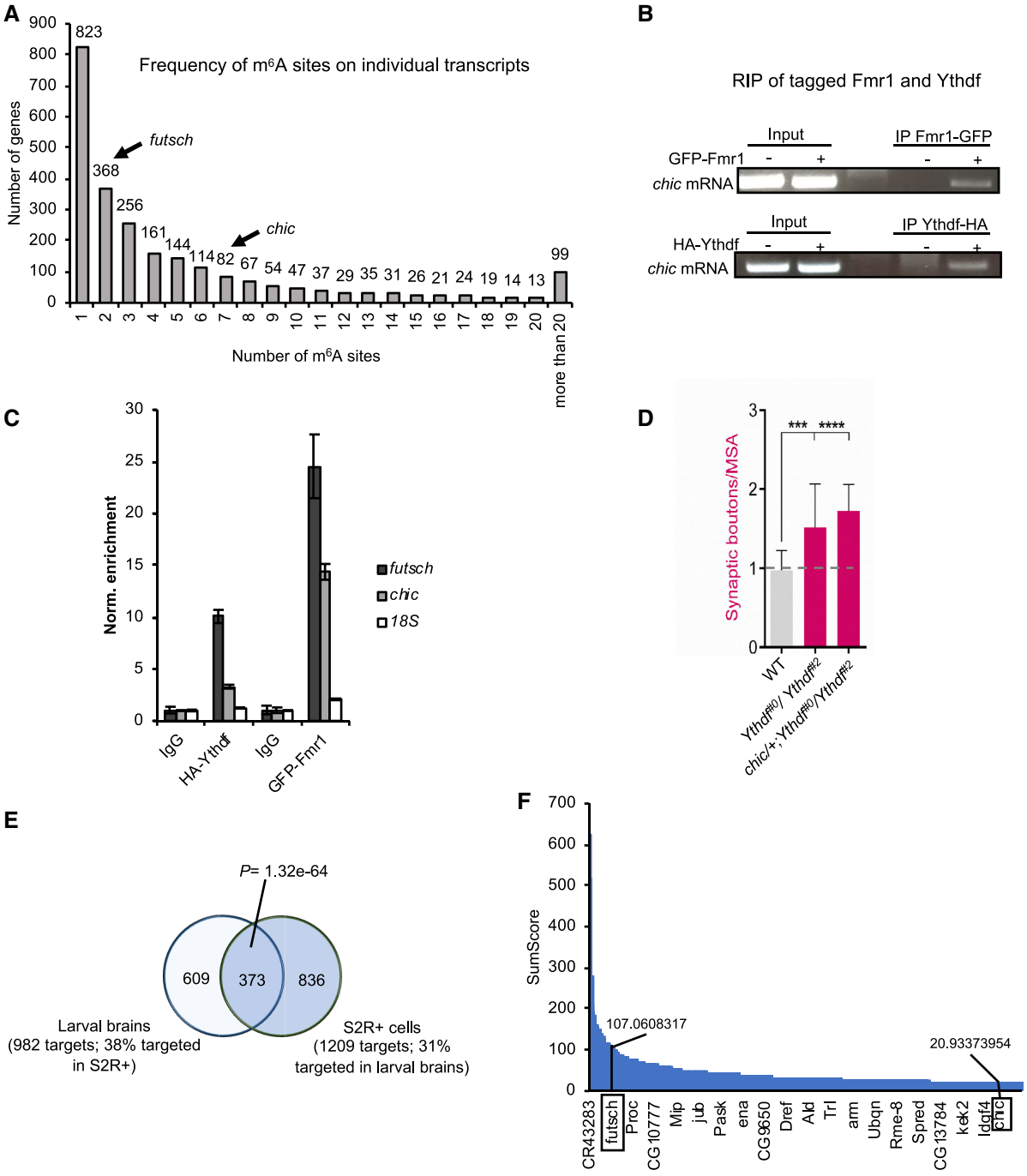

Figure EV4.

Figure EV4. Fmr1 and Ythdf bind *chic* and *futsch* transcripts.

A Graph showing the frequency of m^{6}A sites on individual transcripts identified by miCLIP-seq.

B RNA Immunoprecipitation of GFP-tagged Fmr1 (*Elav-Gal4: UAS-Fmr1*-GFP) or HA-tagged Ythdf (*Elav-Gal4:UAS-Ythdf-HA*) from 50 adult heads. The RNA was then reverse-transcribed and analyzed via PCR with primers designed on *chic* transcript.

C RNA immunoprecipitation assay. Quantification of *futsch* and *chic* RNA levels upon immunoprecipitation of pan-neuronally expressed GFP-tagged Fmr1 or HA-tagged Ythdf in 3rd instar larvae. Bars show average ± SD of technical triplicates. *18S* mRNA serves as a negative control for unspecific enrichment.

D Quantification of normalized bouton number (total number of boutons/muscle surface area (μm^2 × 1,000)) of muscle-6/7 NMJ in hemisegments A2–A3 of the indicated genotypes (n = 20). Bars show mean ± s.e.m. Multiple comparisons were performed using one-way ANOVA with a *post hoc* Sidak–Bonferroni correction (***P < 0.001; ****P < 0.0001).

E Vein diagram showing the overlap of Ythdf-TRIBE datasets produced in S2R+ cells and in larval brain. The P-value is calculated by hypergeometric test.

F Graph showing the distribution of SumScores of all identified Ythdf mRNA targets by TRIBE in the larval nervous system. The position and SumScores of *futsch* and *chic* are marked in the graph.

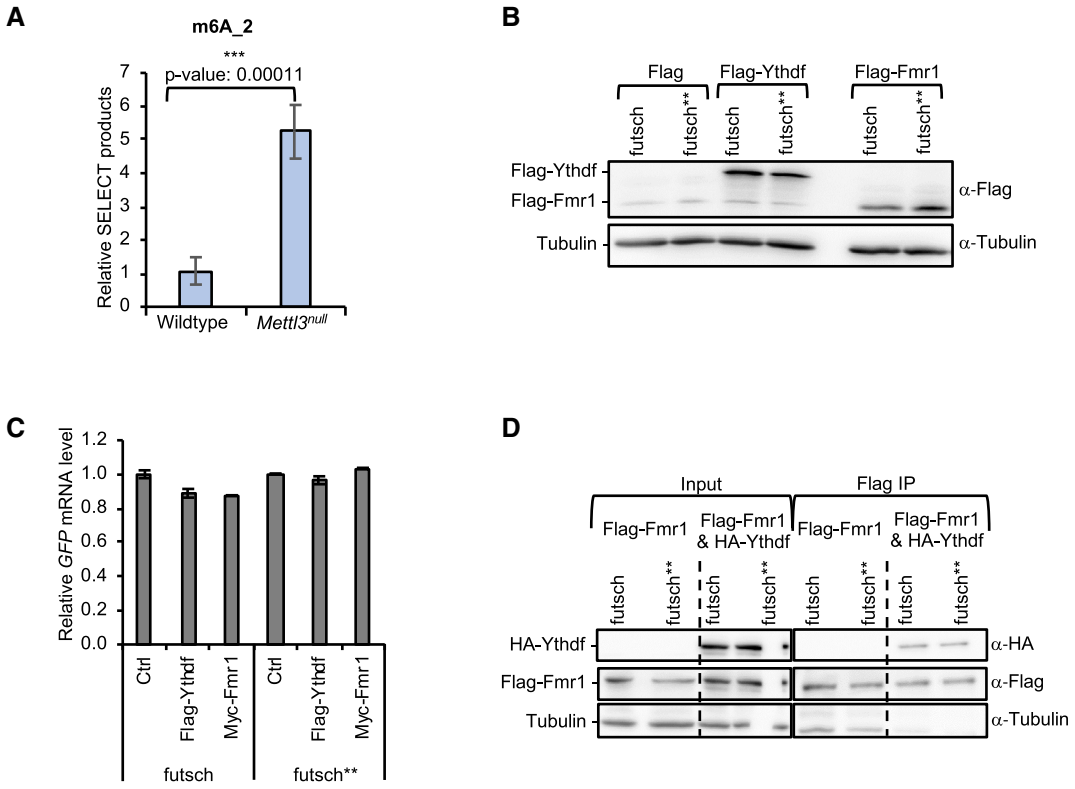

Figure EV5. Controls for assay with the *futsch* 5'UTR reporter.

A Relative amount of SELECT qPCR products targeting the m^{6}A_2 site on *futsch* using total RNA of Wild-type or *Mettl3^{null}* larval brains. Bars show average ± SD of biological triplicates. P values were determined with a Student's t-test. (***P < 0.001).

B Representative Western blot analysis showing the protein input level for RNA immunoprecipitation experiment of *futsch* 5'UTR GFP reporter.

C Relative *GFP* mRNA input level for RNA immunoprecipitation experiment of *futsch* 5'UTR GFP reporter. *Rpl15* served as a normalization control. Experiments were performed in triplicates. Bars show average ± SD.

D Representative Western blot analysis showing the protein input level and IP efficiencies for RNA immunoprecipitation experiment of *futsch* 5'UTR GFP reporter in the presence of HA-Ythdf and Flag-Fmr1.