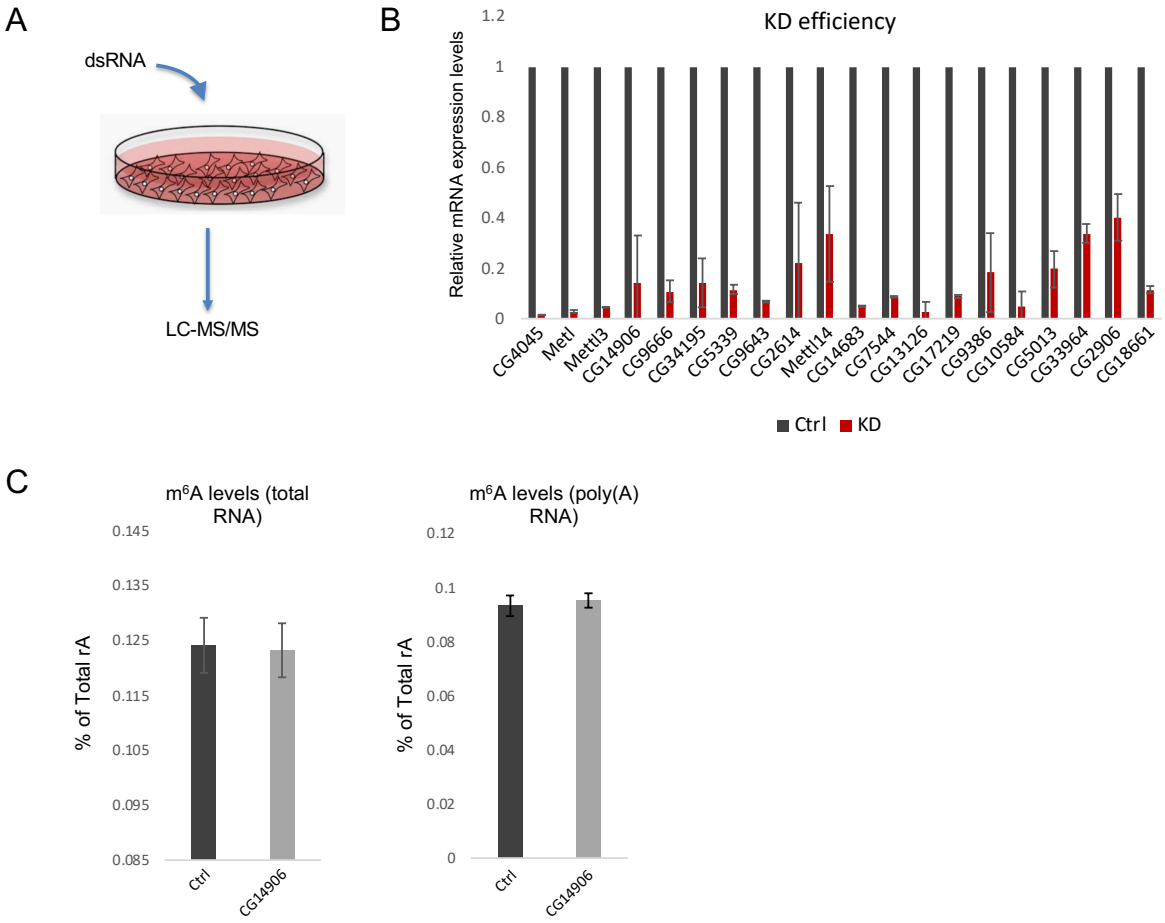
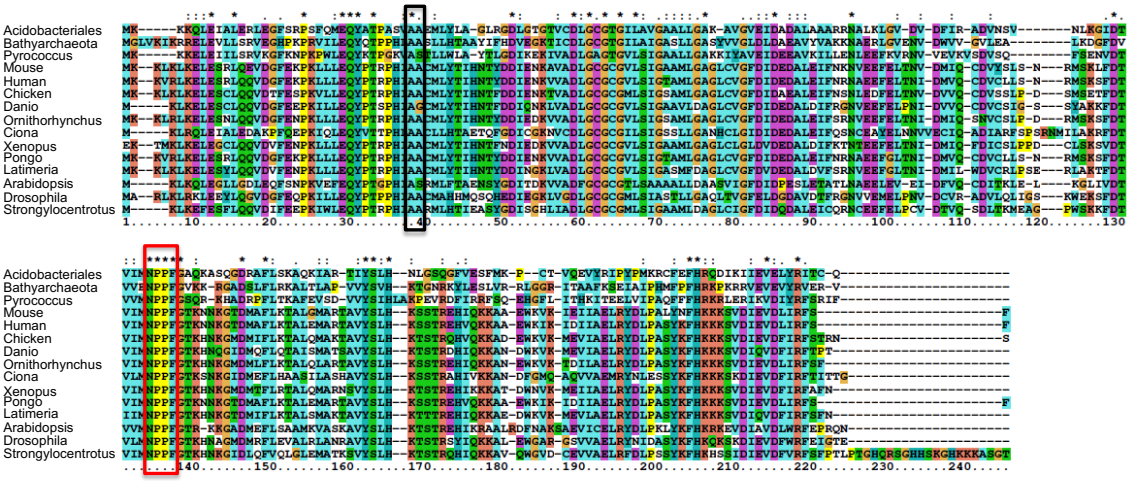
Table of Content



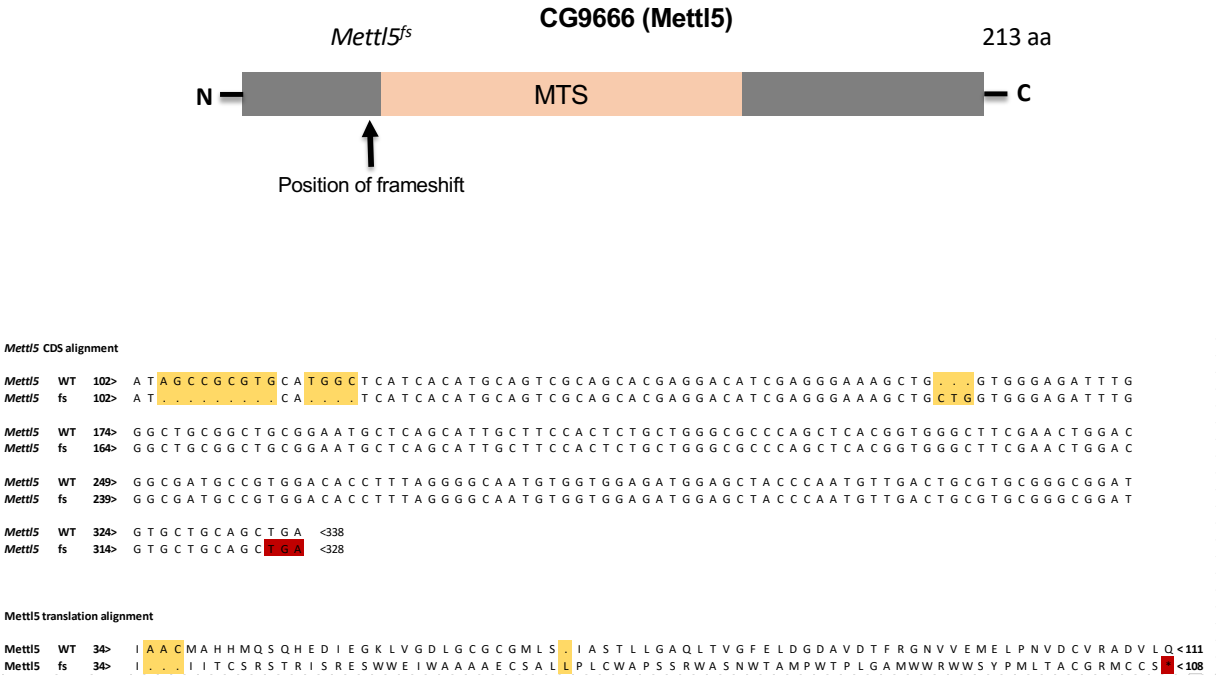
Appendix Figure S1: Design and validation of the candidate-based RNAi screen

(A) Experimental scheme of the RNAi screen performed in *Drosophila* S2R+ cells. After KD prolonged for 6 days, RNA was extracted and submitted to mass spectrometry analysis. (B) Efficiency of the knock down quantified by RT-qPCR. Bar chart represents the mean ± standard deviation of three technical measurements from three biological replicates. (C) LC-MS/MS measurements of m^6A levels in total RNA (left) or in poly(A)+ RNA (right) in S2R+ cells upon depletion of CG14906. Bar charts represent the mean± standard deviation of three technical measurements from three biological replicates.



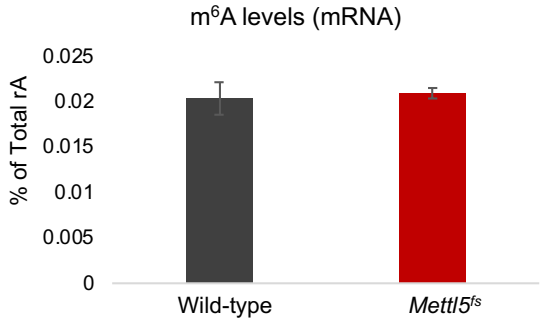
Appendix Figure S2: Phylogenetic analysis

Multiple sequence alignment of Mettl5 (UniProt: Q8MSW4_DROME) and representative orthologs from selected species (see Methods for details and sequence identifiers). Prokaryotic exemplary sequences from archaea (Pyrococcus, Bathyarchaeota) and bacteria (Acidobacteriales) are included as outliers. The red rectangle indicates the conserved NPPF motif and the black rectangle the position of the two amino acids deleted in the *Mettl5*$^{\Delta 2AA}$ allele.



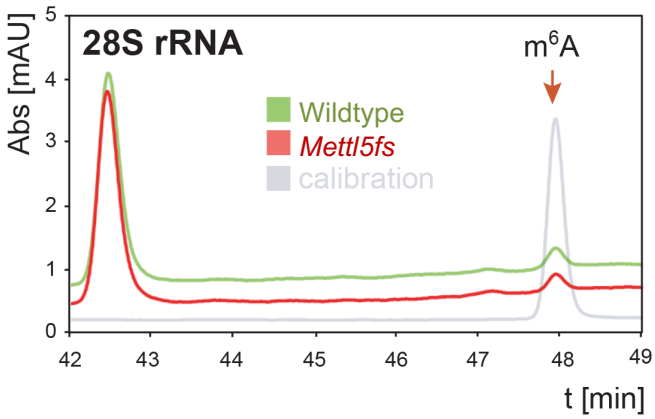
Appendix Figure S3: Description of the *Mettl5*^{fs} mutation

(Top) Representation of the *Mettl5*^{fs} mutant allele generated in this work, consisting of a frameshift mutation leading to a premature stop codon. (Bottom) Nucleotide and protein sequence alignments of WT *Mettl5* and *Metll5*^{fs}. Yellow indicates the changes in the mutant allele. Red indicates the premature stop codon.



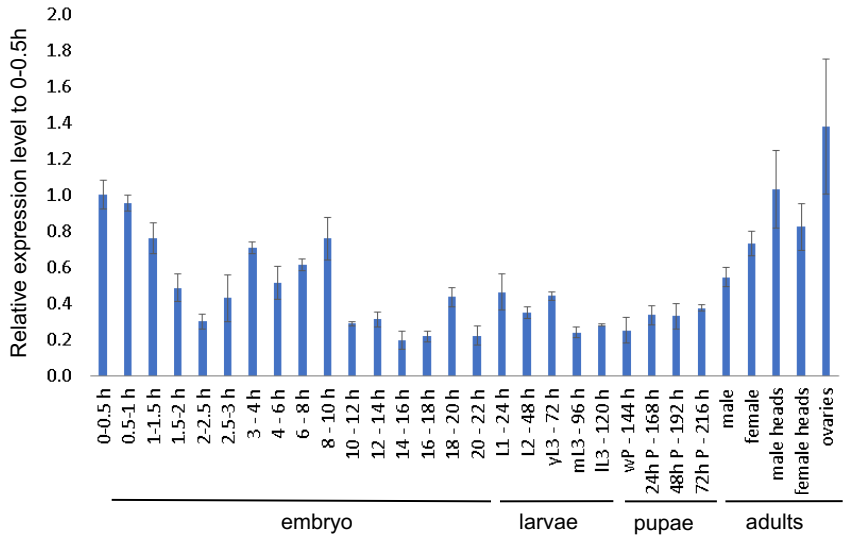
Appendix Figure S4: Methylation levels analysis

LC-MS/MS measurements of m^6A levels in mRNA of WT and *Mettl5*^{fs} mutant flies. Bars represent mean ± standard deviation of measurements of three technical measurements from three biological replicates.



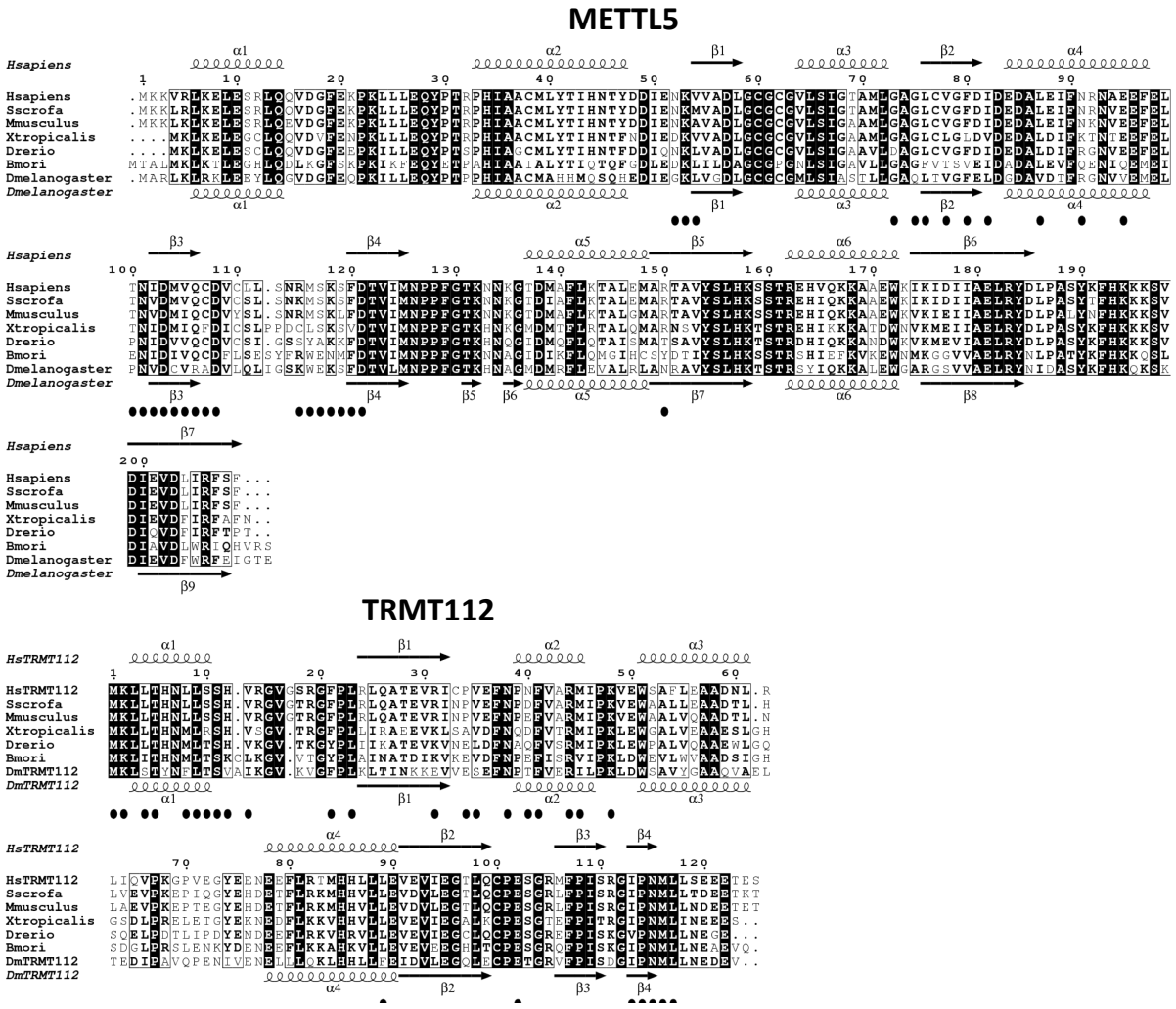
Appendix Figure S5: Analysis of m^{6}A levels on 28S rRNA by quantitative HPLC

Purified 28S rRNA analyzed for its m^{6}A content by quantitative HPLC. The 28S RNA was extracted from 60S subunits isolated on sucrose gradients. The calibration control is a commercial source of m^{6}A (in grey). m^{6}A elutes at 48 min.



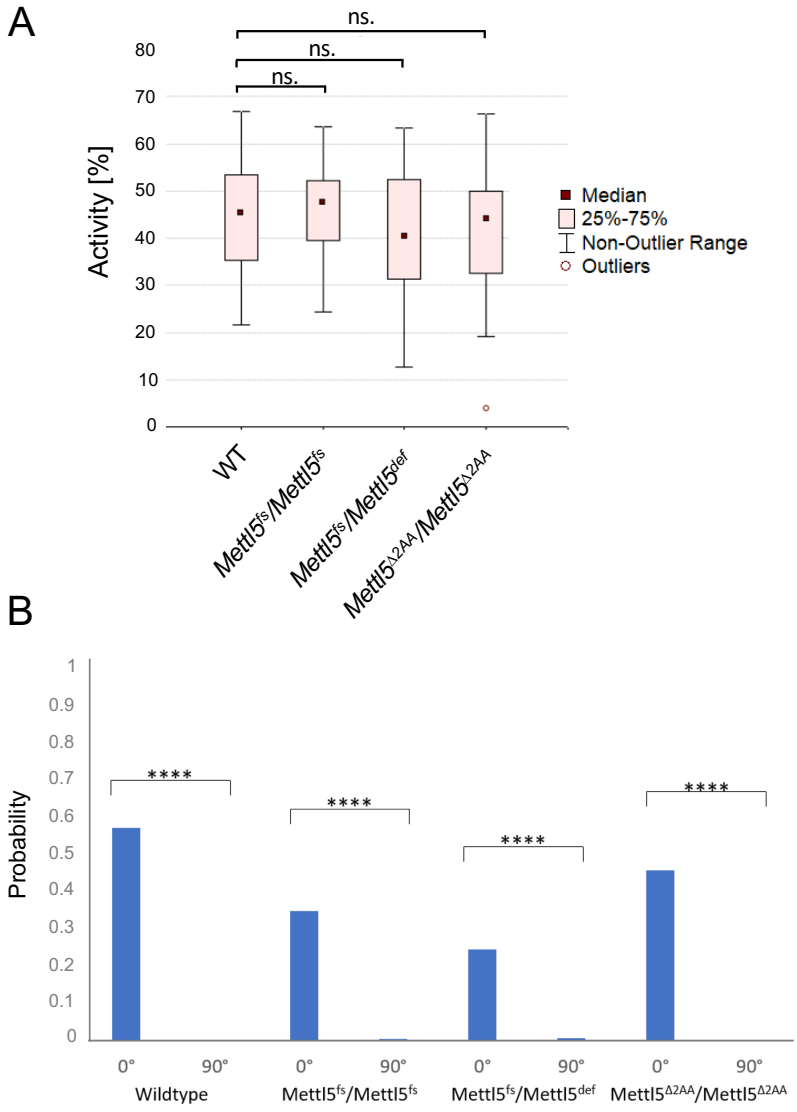
Appendix Figure S6: Expression of *Trmt112* transcript during development

Developmental expression of *Trmt112* transcript assayed by RT-qPCR analysis. The bar chart represents mean± standard deviation of three technical measurements.



Appendix Figure S7: Secondary structure element analysis of fly Mettl5 and Trmt112

Strictly conserved residues appear in white font on a black background. Partially conserved amino acids are indicated with a grey font. Secondary structure elements assigned on the basis of the *H. sapiens* crystal structure and our fly model (Fig. 4D) are indicated above and below the alignments, respectively. Black circles underneath the multiple sequence alignment indicate residues involved in complex formation. Display generated using the ESpript server (Robert & Gouet, 2014).



Appendix Figure S8: Statistical analysis of fly activity

(A) Fly activity evaluated with the help of the Buridan's paradigm. Shapiro-Wilk test was used to test for normal distribution in each group. Normally distributed groups were tested by t-test. Due to multiple comparison Bonferroni correction was applied. Number of flies tested: 30 per genotype. (n.s. = not significant). (B) Fixation index analyzed by Buridan's paradigm. 0° indicates area around black stripes, 90° indicates area 90° to the black stripes. Number of flies tested: 30 per genotype. ****P<0.0001 (t-test)