

Description of Additional Supplementary Files

Supplementary Movie 1 and 2: *Mettl14* deletion leads to slower growth cone dynamics.

Outgrowing growth cones of WT (Supplementary Movie 1) and *MI4* cKO (Supplementary Movie 2) mouse primary neuron were monitored over 3 hours at DIV6 (scale bar: 20 μ m). Related to **Fig. 1i-k**.

Supplementary Movie 3 and 4: *Mettl14* deletion reduces anterograde RNA dynamics. MS2

system visualize BFP-Actb-3'UTR-MS2x24 reporter on WT (Supplementary Movie 3) and *MI4* cKO mouse primary neuron (Supplementary Movie 4), acquired for 5minutes with 500ms intervals (scale bar: 10 μ m). Related to **Fig. 3i-k**.

Supplementary Movie 5 and 6: m⁶A point mutation in the reporter construct reduces

anterograde RNA dynamics. MS2 system visualize BFP-Actb-3'UTR-MS2x24 reporter (Supplementary Movie 5) and BFP-Actb-3'UTR Δ m⁶A-MS2x24 reporter (Supplementary Movie 6) mouse primary neuron, acquired for 5minutes with 500ms intervals (scale bar: 10 μ m). Related to **Fig. 3l-n**.

Supplementary Movie 7 and 8: *Mettl14* depletion does not affect to lysosome transport.

Lysotracker live imaging of WT (Supplementary Movie 7) and *Mettl14* cKO (Supplementary Movie 8) were observed for 30 minutes with 10 seconds intervals at DIV6 (scale bar: 10 μ m). Related to **Supplementary Fig 5.e-h**

Supplementary Movie 9 and 10: *Ythdf2* deletion leads to slower growth cone dynamics.

Outgrowing growth cones of *DF2 Het* control (Supplementary Movie 9) and *DF2* cKO (Supplementary Movie 10) mouse primary neuron were monitored over 3 hours at DIV6 (scale bar: 20 μ m). Related to **Fig. 4d-f**

Supplementary Movie 11 and 12: *Ythdf2* deletion reduces anterograde RNA dynamics. MS2 system visualize BFP-Actb-3'UTR-MS2x24 reporter on WT (Supplementary Movie 11) and *DF2* cKO mouse primary neuron (Supplementary Movie 12) acquired for 5minutes with 500ms intervals (scale bar: 10 μ m). Related to **Fig. 4I-n**

Supplementary Data 1. The list of *Mettl14*-dependent m⁶A peaks identified by m⁶A-SAC-seq. The table includes the chromosome, chromosomal position, original genome sequence, mutated sequence, mutation ratio among transcripts, gene symbol, and the genomic location of m⁶A peaks identified using m⁶A-SAC-seq by comparing P0 WT and *M14* cKO mouse forebrain.

Supplementary Data 2. The results of the RNA stability assay in *Mettl14* and *Ythdf2* cKO primary cortical neurons. The table includes the gene symbol, the log2 fold-change ratio in the 0-hour sample, the log2 fold-change ratio in the 5-hour sample, the difference between the 0-hour and 5-hour samples, and the presence of m⁶A from the m⁶A-SAC-seq results. The first sheet shows the stability assay results for the *M14* cKO condition compared to the control. The second sheet shows the stability assay results for the *DF2* cKO condition compared to the control. The third sheet compares the *M14* cKO and *DF2* cKO stability assay results. Results were filtered according to the standard described in the Methods section (provided as a separate Excel spreadsheet).

Supplementary Data 3. The list of mRNAs with significantly altered abundances between the corpus callosum of WT and *M14* cKO mice. The table includes the Ensembl transcript ID, gene symbol, baseMean count, log2 fold-change ratio, and adjusted p-value. Results were filtered according to the standard described in the Methods section (provided as a separate Excel spreadsheet).

Supplementary Data 4. The list of protein interactors of YTHDF2 identified by Co-IP LC-MS/MS experiments. The table includes the gene symbol, the normalized PSM values for WT and *M14* cKO

with IgG pull-down samples, and the log2 fold-change of PSM values in WT compared to *M14* cKO (provided as a separate Excel spreadsheet).

Supplementary Data 5. Summary of statistical analyses for all experiments. The table includes p-values, sample sizes, statistical tests used, and additional parameters (provided as a separate Excel spreadsheet).