

List of Supplemental material

Supplementary Figures

Supplemental Figure S1. METTL5 catalyses m⁶A formation. Related to Figure 1d.

Supplemental Figure S2. METTL5 methylates 18S rRNA in mESCs. Related to Figure 2.

Supplemental Figure S3. *Mettl5* KO affects pluripotency of mESCs. Related to Figures 3.

Supplemental Figure S4. Analysis of apoptotic markers and embryonic body formation in *Mettl5* KO mESCs. Related to Figures 3.

Supplemental Figure S5. Levels of ribosome components are largely unaltered in *Mettl5* KO mESCs. Related to Figure 4.

Supplemental Figure S6. *Mettl5* KO mice show multiple phenotypes. Related to Figure 5.

Supplementary Tables

Table S1. METTL5 interactome data. Related to Figure 1e.

Table S2a. LC-MS/MS parameters for Methyl-NAIL-MS. Related to Figure 2a,b.

Table S2b. LC-MS/MS parameters for quantification of modified nucleosides. Related to Figure 2c and Supplemental Figure S2b.

Table S3. List of genes, differentially expressed genes in *Mettl5* KO mESC. Related to Figure 3c and Supplemental Figure S3a.

Table S4. Transcripts table with Log₂ Fold Change values (*Mettl5* KO / WT) for RNA and RBF. Includes separate lists of 4 groups of transcripts used for generating the heatmap (Figure 4c) with RBF changes for each group displayed as violin plots. Related to Figure 4c,d.

Table S5. Results of body weight measurements of wt and *Mettl5* KO mice. Related to Figure 5b and Figure S6b.

Table S6. Results of the open field test in wt and *Mettl5* KO mice. Related to Figure 5f.

Table S7. Oligonucleotides and plasmids used in the study.