



**Supplemental Figure. 1 | Mettl3 knockout versus wild-type direct RNA sequencing quality**

A) Mettl3 knockout target site located in exon 2. The image is generated using Synthego Inference of CRISPR edits. The inference was started from the 180 bp region where the sgRNA target site was located. The upper layer represents Sanger sequencing signal for Mettl3 knockout, and the bottom layer represents sequencing signal from wild-type. The PAM sequence is underlined with a red dash line, and the target site is underlined with a black line. B) The distribution of modification probabilities from two mESC WT replicates (green) and Mettl3 knockout (red). C) Modification ratios and locations of m6A sites with high probability ( $p > 0.85$ ) of being modified were plotted. The relative m6A positions within the transcript body were determined. The color scale indicates the number of sites. D) Comparison of motif sequence preference before (gray) and after (colored) selection of sites with a high-probability ratio.