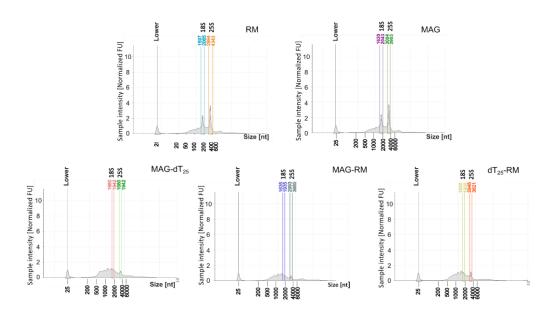
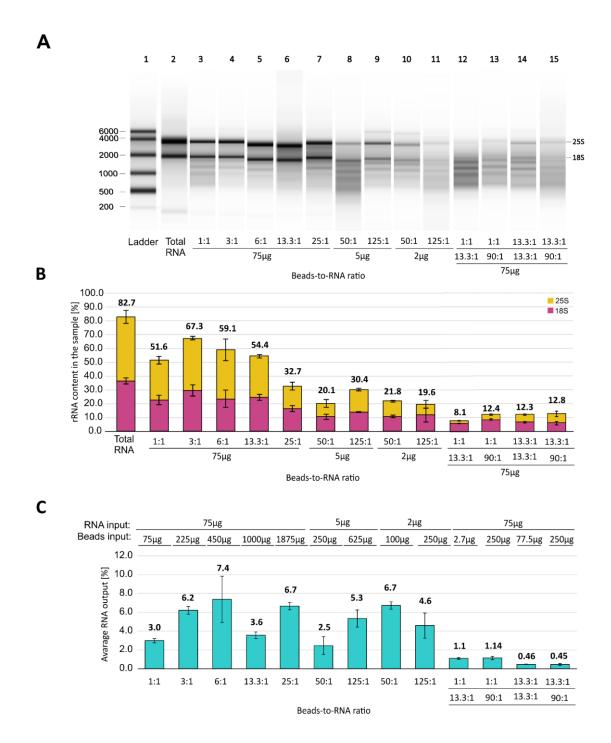
## **SUPPLEMENTARY FIGURES**

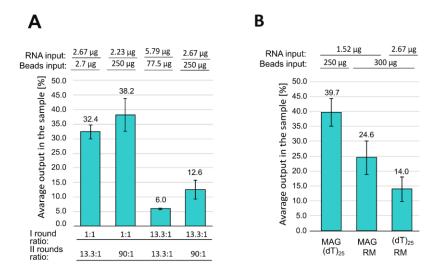
A comparative analysis of mRNA enrichment strategies and guidance for improving their efficiency.



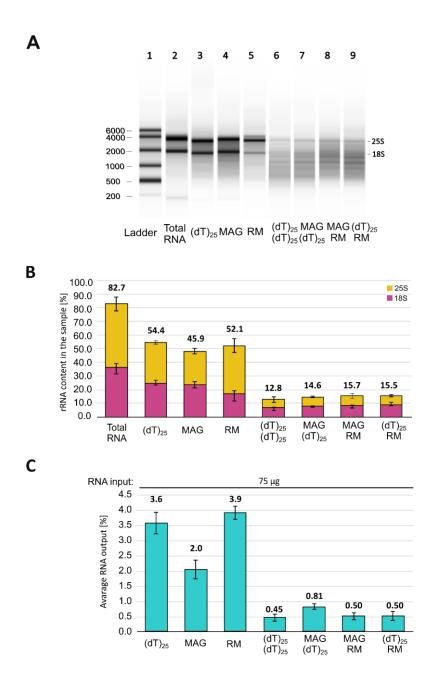
**Supplementary Fig. 1.** The electropherograms are generated by the TapeStation platform. The graphs represent samples after a single round of mRNA enrichment (RiboMinus and MAG kits) and after two rounds of mRNA enrichment in different combinations (MAG kit -  $(dT)_{25}$  beads, MAG kit - RiboMinus kit,  $(dT)_{25}$  beads – RiboMinus kit).



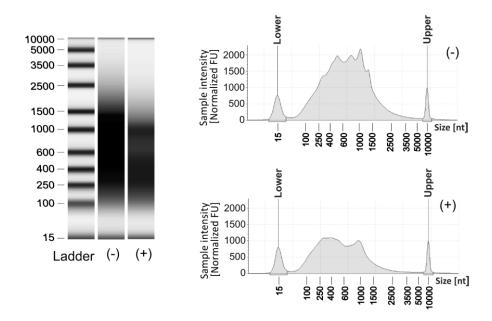
**Supplementary Fig. 2.** Results of a one- and two-round mRNA enrichment using different (dT)<sub>25</sub> beads-to-RNA ratios. **(A)** The electrophoretic results from the TapeStation platform. Lanes represent (1) RNA ladder; (2) total RNA input; (3-11) RNA samples after one round of mRNA enrichment; (12–15) RNA samples after two rounds of mRNA enrichment. Applied beads-to-RNA ratios are provided below the lanes. **(B)** Bar plot summary of rRNA content in the samples. **(C)** Bar plot summary of the average RNA output after mRNA enrichment. The RNA input for the first round was shown above the bars.



**Supplementary Fig. 3.** The bar plot summary of the average relative RNA output after the second round of mRNA enrichment **(A)** using (dT)<sub>25</sub> beads in different beads-to-RNA ratios and **(B)** using different method combinations. The RNA and bead inputs are shown above the corresponding bars.



**Supplementary Fig. 4.** Results of the one- and two-round mRNA enrichment with different method combinations. **(A)** The electrophoretic results from the TapeStation platform. Lanes represent (1) RNA ladder; (2) total RNA input; (3–5) RNA samples after one round of purification; (6–9) RNA samples after two rounds of purification. **(B)** Bar plot summary of rRNA content in the final samples. **(C)** Bar plot summary of the final RNA output.



**Supplementary Fig. 5.** The electrophoretic result from the TapeStation platform for dsDNA samples obtained after the SHAPE-MaP procedure combined with two rounds of mRNA enrichment with (dT)<sub>25</sub> beads. RNA samples were treated with DMSO only (-) or 100 mM NAI (+).