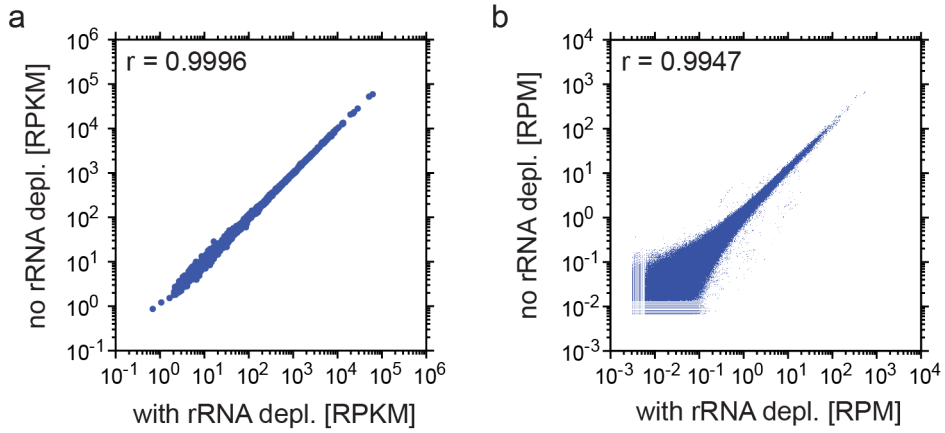

Comparison of translatome data including and excluding an rRNA depletion step during the library preparation.

E. coli MC4100 Δ*tig::Kan* + pTrc-tig-TEV-Avi cells were grown in LB medium, harvested according to the rapid protocol (step **1**, option C), and *ex vivo* crosslinked with EDC (step **7**, option B). After polysome digest ribosomes were isolated in sucrose gradient ultracentrifugation (step **18**, option B). Isolated footprint fragments were used to prepare a sequencing library including ('with rRNA depl.') or excluding ('no rRNA depl.') an rRNA depletion step. Footprint fragments were sequenced (step **96** of the Supplementary Methods) and data were analyzed in the basic and specific analysis (steps **35**–**59**). (a) Gene expression analysis and (b) read densities along protein coding regions were determined as described in the legend to Fig. 2a and c, respectively.