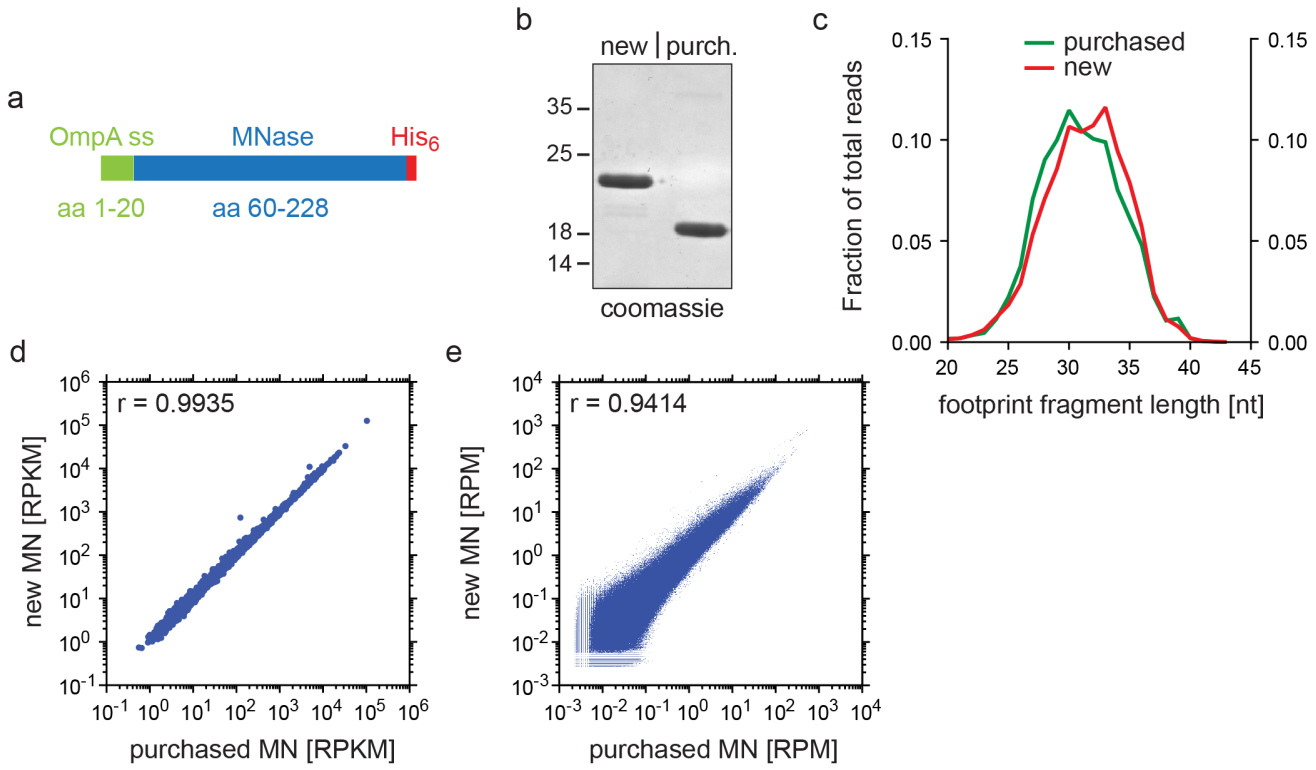

Purification and activity of recombinant MNase.

(a) Design of the MNase overexpression construct pET24a-ompA-nucB(MNase). Nt 178–684 (corresponding to amino acids (aa) 60–228) of the *nuc* gene from *Staphylococcus aureus* (687 nt) were codon-optimized for recombinant expression in *E. coli* and cloned into an expression vector to allow overexpression by T7-polymerase under IPTG-control. The protein was produced with the signal sequence of the precursor of outer membrane protein A (OmpA; aa 1–20) at its N-terminus to facilitate the export of the active, overproduced nuclease to the periplasm to avoid degradation of cytosolic nucleic acids. The signal sequence was cleaved off during translocation by endogenous signal peptidase. Furthermore, the protein was produced with a C-terminal His_6-tag for purification. (b) The new MNase has a slightly higher molecular weight compared to purchased MNase. 1.4 µg of each protein were loaded on 14% SDS-PAGE and the gel was stained with coomassie. While the new, mature, His_6-tagged protein has a molecular weight of 20.1 kDa (18.9 kDa without His_6-tag), the purchased protein has a slightly lower molecular weight and presumably consists of aa 80–228 from the *nuc* gene product[43]. (c–e) Comparison of translatomes derived from lysates treated with new or purchased MNase. *E. coli* MC4100 cells grown in LB medium were harvested according to step **1**, option C. The thawed lysate (according to step **7**, option A) was digested with 15 U/A_{260} of new or purchased MNase

and loaded onto sucrose gradients (step **18**, option B). Sequencing libraries (without rRNA depletion) were cloned (according to Supplementary Methods) and data were analyzed as described in steps **35**–**59**. (c) Read lengths of footprint fragments generated with different MNases according to analysis step **41**. (d,e) Gene expression levels (d) and read densities in protein coding regions (e) were calculated as described in the legend of Fig. 2a and c, respectively.