Supplementary Information

Structural basis for ribosomal 16S rRNA cleavage by the cytotoxic domain of colicin E3

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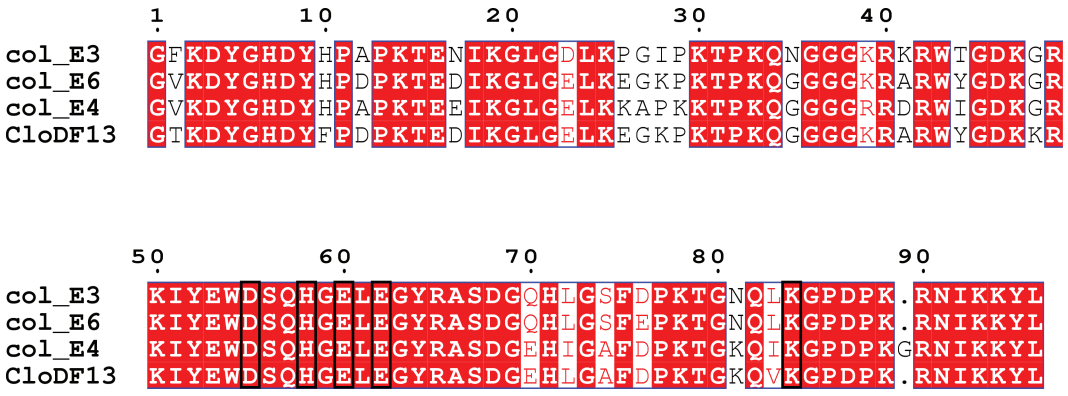
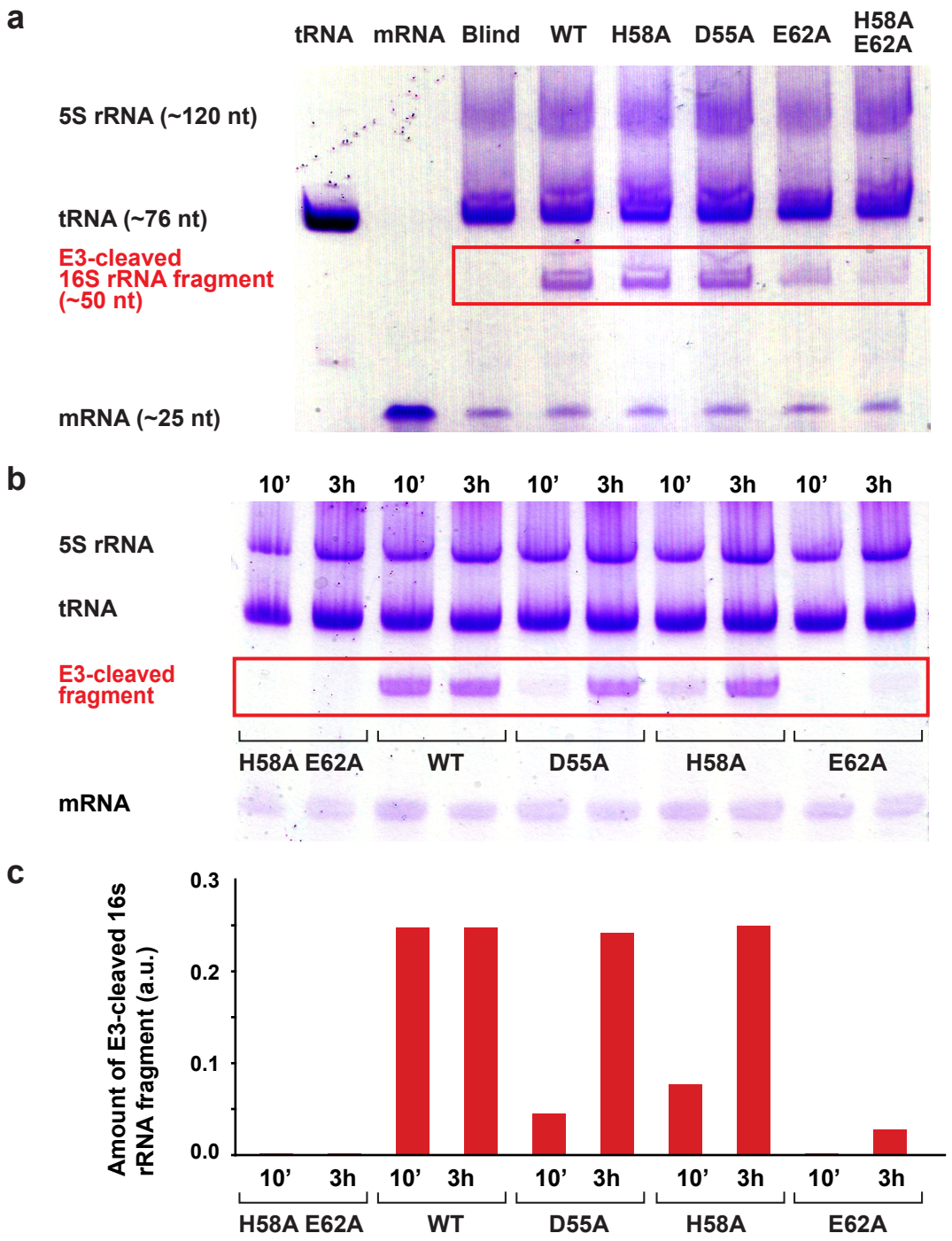
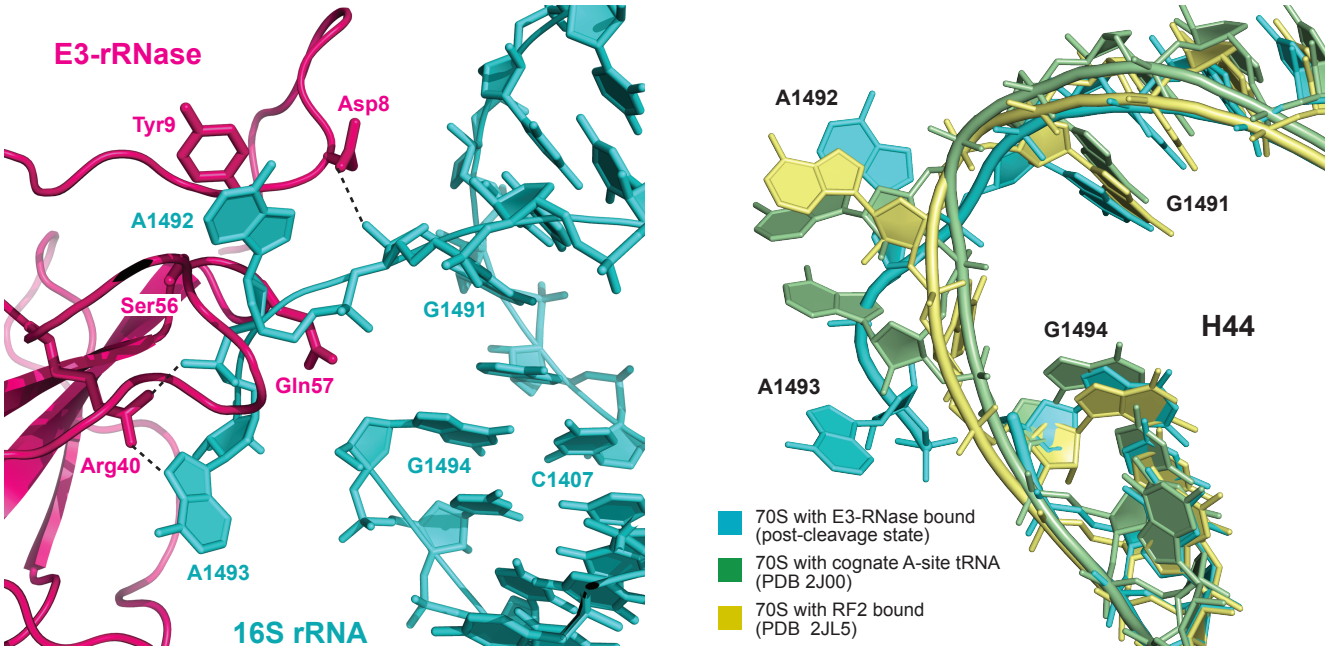


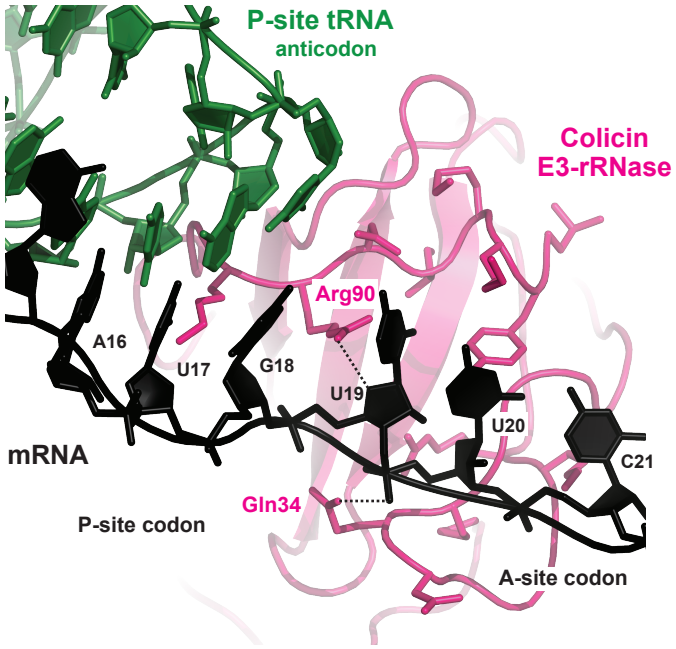
Supplementary Figure 1. Sequence alignment of E3-rRNase with rRNase domains of colicin E4, E6 and cloacin DF13. Fully conserved positions are boxed in red, putative catalytic amino acids Asp55, His58, Glu60, Glu62 and Lys84 are boxed in black. Sequences were obtained from the NCBI.



Supplementary Figure S2. *In vitro* cleavage assay. (**a**) 70S *Thermus thermophilus* ribosomes were incubated with $tRNA^{fMet}$, mRNA and a 5 fold excess of E3-rRNase mutants (wt, D55A, H58A, E62A, H58A E62A) in buffer G (5 mM HEPES pH 7.5, 50 mM KCl, 10 mM NH_4Cl, 10 mM magnesium acetate, 6 mM 2-mercaptoethanol) at room temperature for 48 hours. Complex formation was performed as for crystallization experiments and as described in the Methods and Materials section. At the indicated time point the reactions were stopped by addition of phenol/chloroform, concentrated by ethanol precipitation and separated by 16% urea denaturing PAGE; the bands were stained with toluidine. (**b**) 70S *Th. thermophilus* ribosomes were incubated with $tRNA^{fMet}$, mRNA and a 5 fold excess of E3-rRNase mutants in a more physiological buffer (polymix buffer: 5mM Mg Acetate, 0.5 mM $CaCl_2$, 5mM NH_4Cl, 95 mmM KCl, 8 mM putrescine pH 7.5, 1 mM spermidine, 5 mM potassium phospate pH 7.3, 1mM dithioerythriol) at 37°C and samples were taken at different time points. It can be clearly seen, that while the WT E3-rRNase shows full cleavage activity after 10’, the mutant where both catalytic residues His58 and Glu62 were mutated to alanine does not show any determinable cleavage activity also after 3 hours, although the E3-rRNase is present at a 5-fold excess. (**c**) The amounts of E3-cleaved 16S rRNA were determined by scanning the toluidine stained bands with a Typhoon imager and quantified with Image Quant TL (both GE Healthcare).



Supplementary Figure S3. Conformation of the decoding centre as a result of colicin E3-rRNase binding and cleavage. (**a**) Interactions of 16S rRNA residues in the decoding site with conserved residues of colicin E3-rRNase. Hydrogen bonds are depicted by dashed lines. (**b**) Conformation of the decoding centre adenosines A1492 and A1493 in a number of ribosome complexes including the 70S bound to colicin E3-rRNase (blue, this study), the 70S bound to A-site tRNA (PDB 2J00 (Selmer et al., 2006)), and the 70S bound to release factor 2 (PDB 2JL5 (Weixlbaumer et al., 2008)).



Supplementary Figure S4. Interactions of E3-rRNase with mRNA. P-site (nucleotides 16-18) and A-site (nucleotides 19-21) mRNA codons are shown. Hydrogen-bonds between universally conserved E3-rRNase residues Arg90 and Gln34 and A-site nucleotides U19 and U20 are depicted by dotted lines.