SUPPLEMENTARY ONLINE MATERIALS

for

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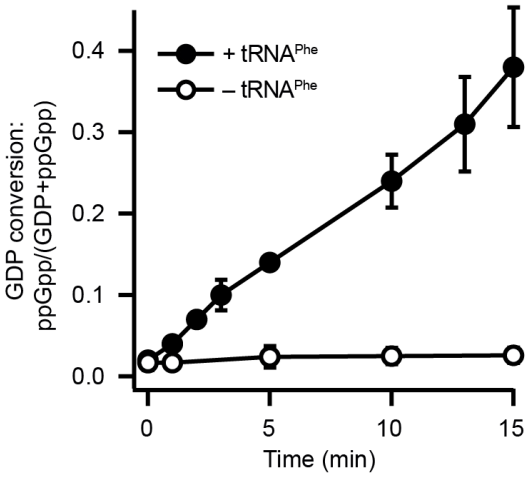

Figure S1 Activity of *E. coli* RelA to synthesize ppGpp. Cognate deacylated tRNA strongly activates ppGpp synthesis by RelA. Time courses of ^{3}H-ppGpp synthesis by RelA in the presence (filled circles) or absence (empty circles) of 1.5 µM deacylated $tRNA^{Phe}$. In both cases the reaction mixture contained 0.5 µM 70S programmed with 1 µM MF-mRNA and 1.5 µM $tRNA_f^{Met}$, 100 nM RelA, 100 µM ppGpp, 0.3 mM ^{3}H-GDP and 1 mM ATP. Results are shown as mean values of 3 replicates and error bars indicate standard error of the mean.

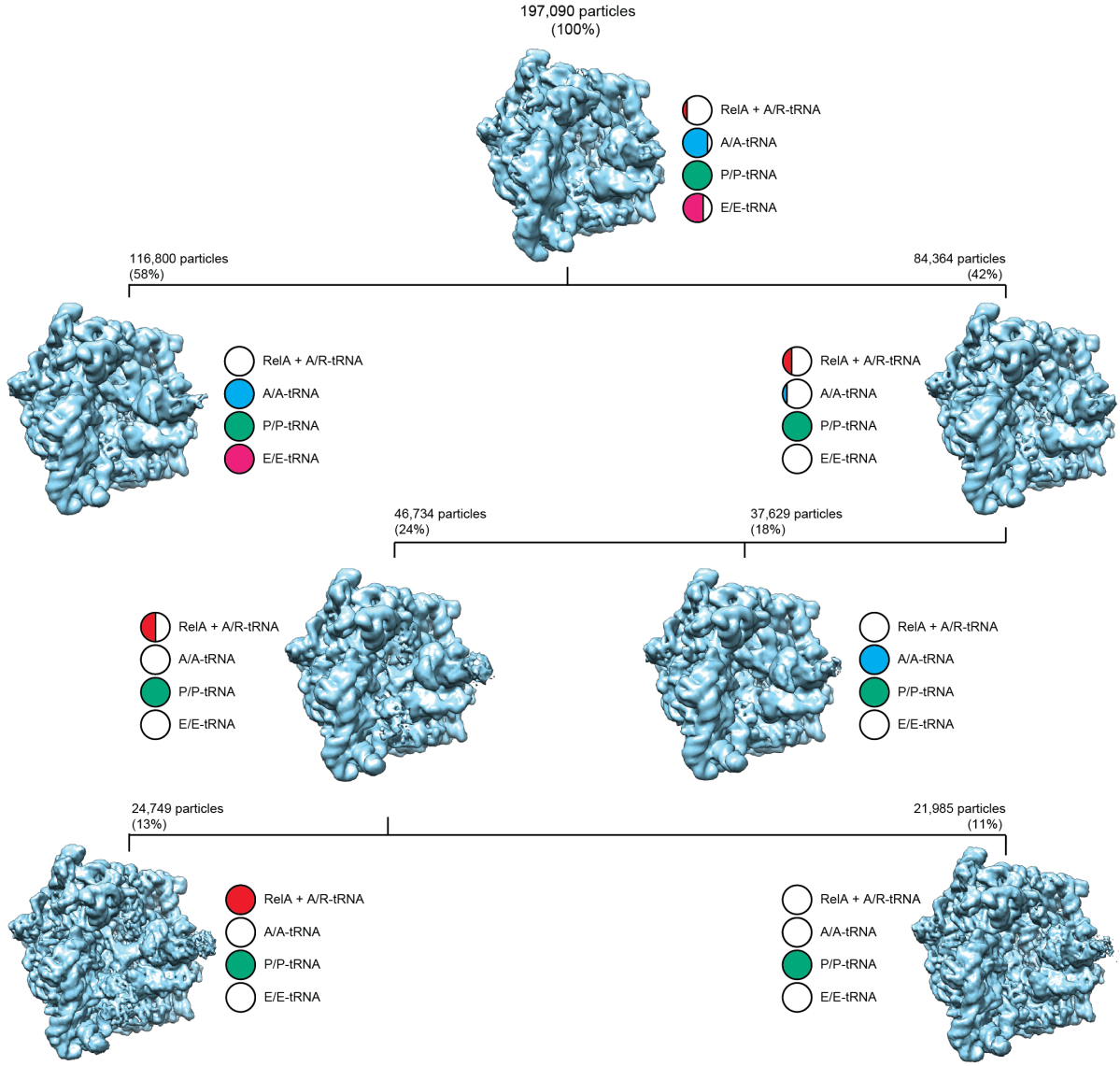

Figure S2: *In silico* sorting scheme of the RelA-SRC cryo-EM dataset. After removal of non-aligning and edge particles, sorting of the dataset yielded four homogenous sub-datasets. The first (58%; 116,800 particles) contained stoichiometric density for A-, P- and E-tRNAs, the second (18%; 37,629 particles) contained stoichiometric density for A- and P-tRNAs, the third (11%; 21,985 particles) contained stoichiometric density for P-tRNA and the fourth sub-dataset (13%; 24,749 particles) contained stoichiometric density for RelA, A/R-tRNA and P-tRNA (RelA-SRC).

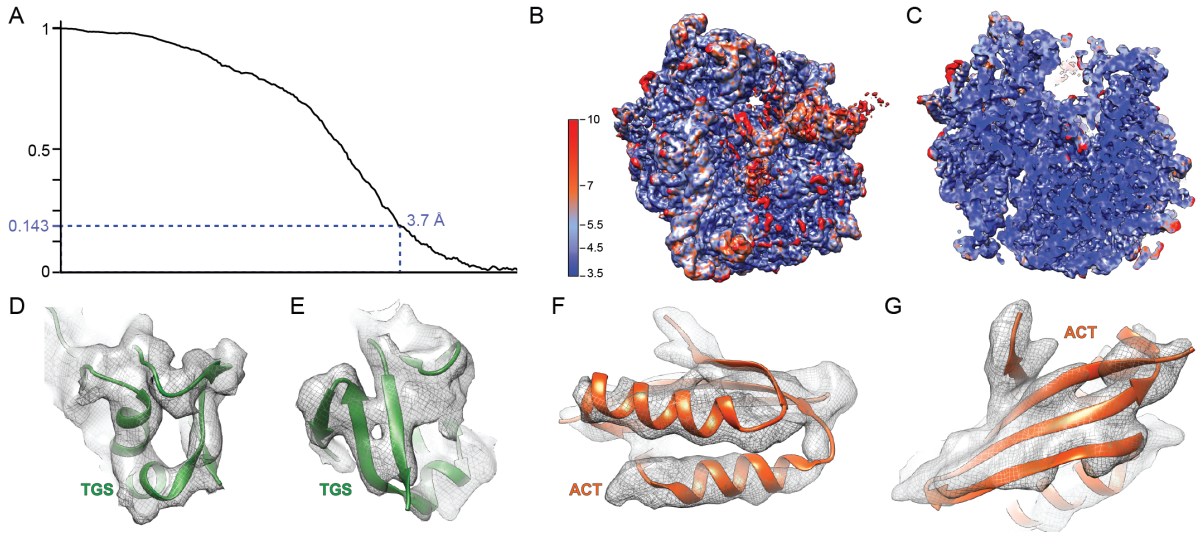

Figure S3: Resolution of the cryo-EM reconstruction of the RelA-SRC. (A) Fourier-shell correlation curve of the refined final map, indicating the average resolution of the RelA-SRC is 3.7 Å. (B,C) (B) Overview and (C) slice through of the RelA-SRC colored according to the local resolution as calculated using ResMap (1). (D,E) Different views showing the rigid body-fitted homology model of the TGS subdomain of RelA (green, PDB2EKI) into the cryo-EM density (grey mesh). (F,G) Different views showing the rigid body-fitted homology model of the ACT subdomain of RelA (orange, PDB2KO1) into the cryo-EM density (grey mesh).

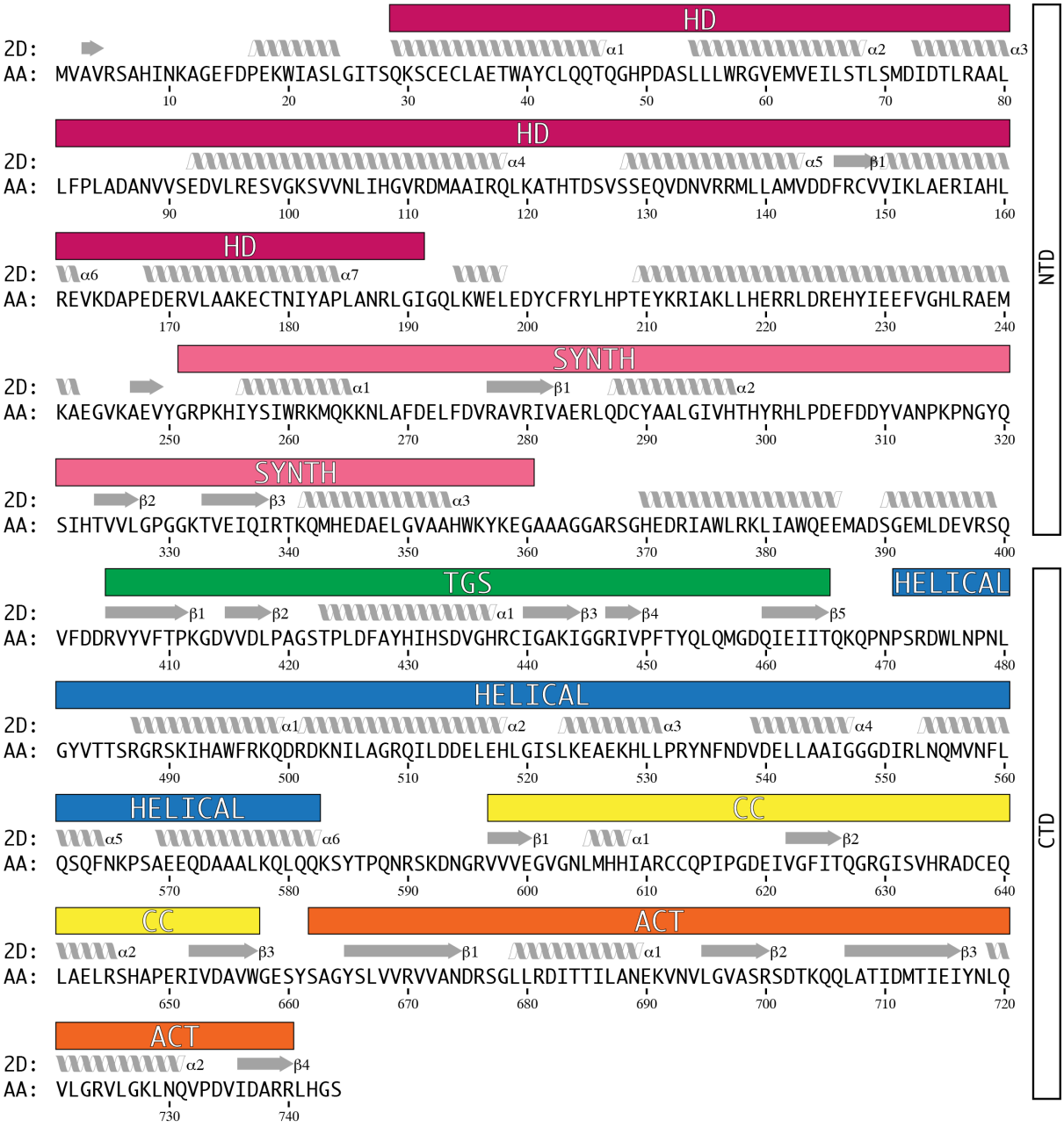

Figure S4: Secondary structure prediction for subdomains of *E. coli* RelA. The amino acid (AA) sequence of *E. coli* RelA with the HD (magenta), SYNTH (pink), TGS (green), HELICAL (blue), CC (gold) and ACT (orange) subdomains is shown together with secondary structure predictions for α-helices and β-strands (2D) based on PSIPRED (2). The secondary structures predictions for TGS and ACT domains were adjusted based on homology models generated from HHPred (3) and Modeller (4).

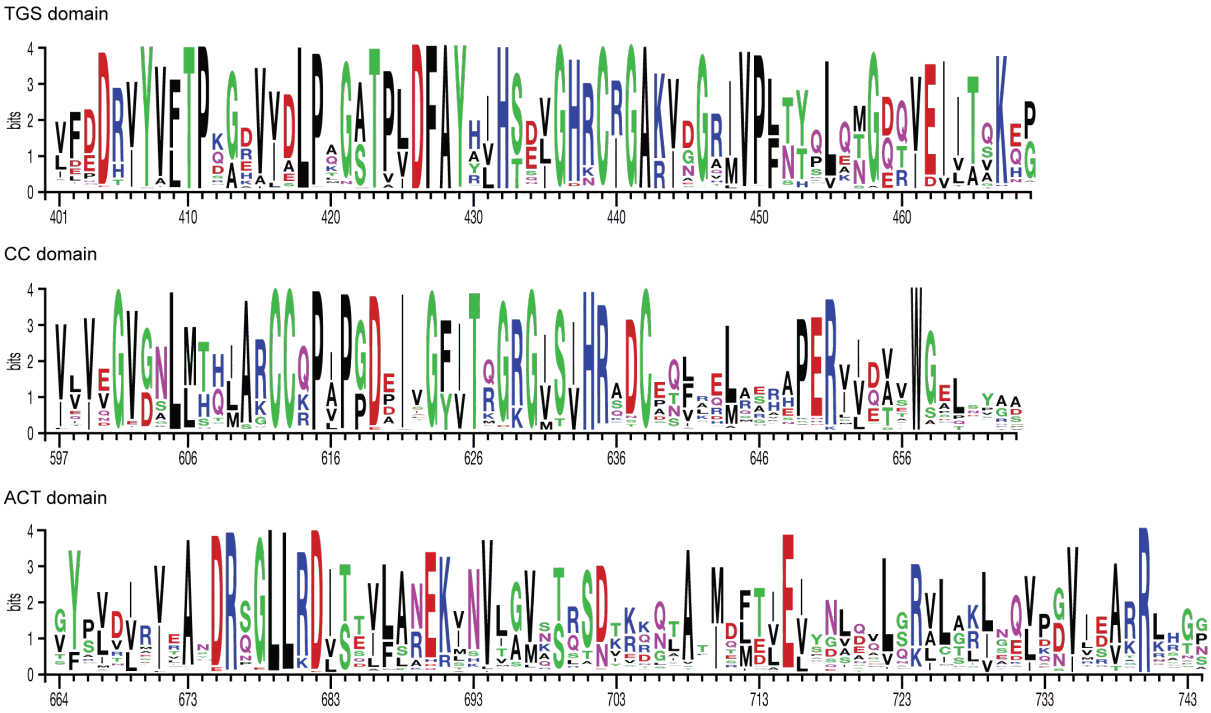

Figure S5: Weblogo plots showing conservation for TGS, CC and ACT subdomains of *E. coli* RelA. The amino acid number for *E. coli* RelA subdomains is shown on the x-axis and the figure was generated using the Weblogo server (5).

SUPPLEMENTARY REFERENCES

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4. Eswar, N., Eramian, D., Webb, B., Shen, M.Y. and Sali, A. (2008) Protein structure modeling with MODELLER. *Methods Mol Biol*, **426**, 145-159.
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