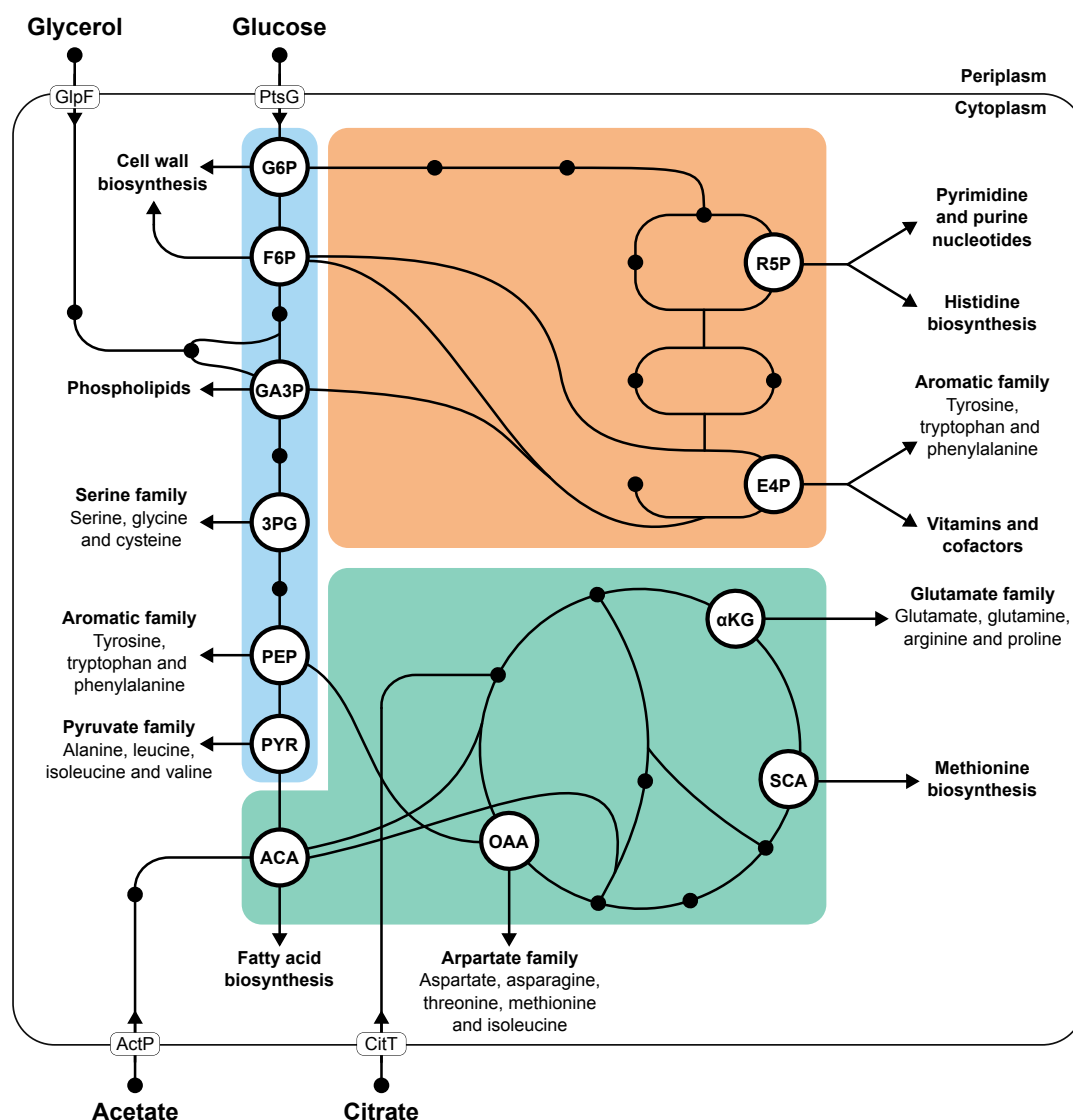


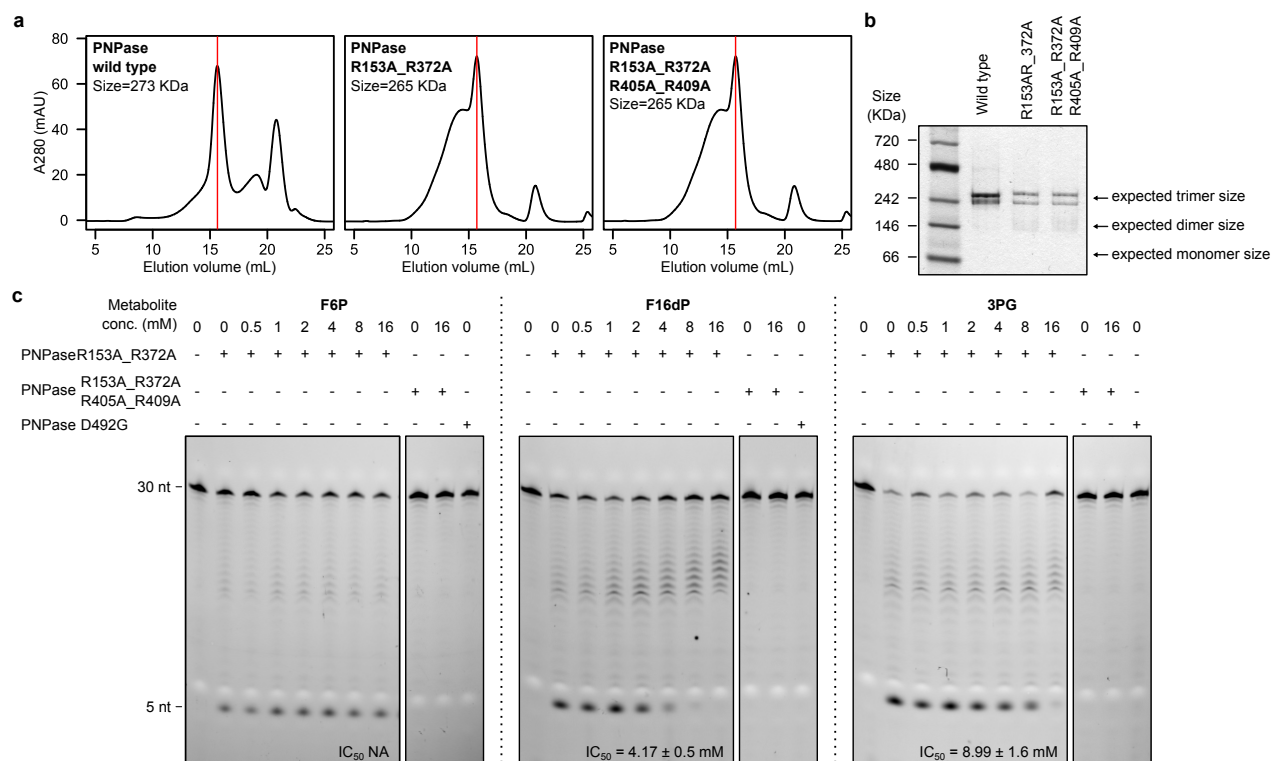
Supplementary Information

A glycolytic intermediate gatekeeps global mRNA decay in *Escherichia coli*

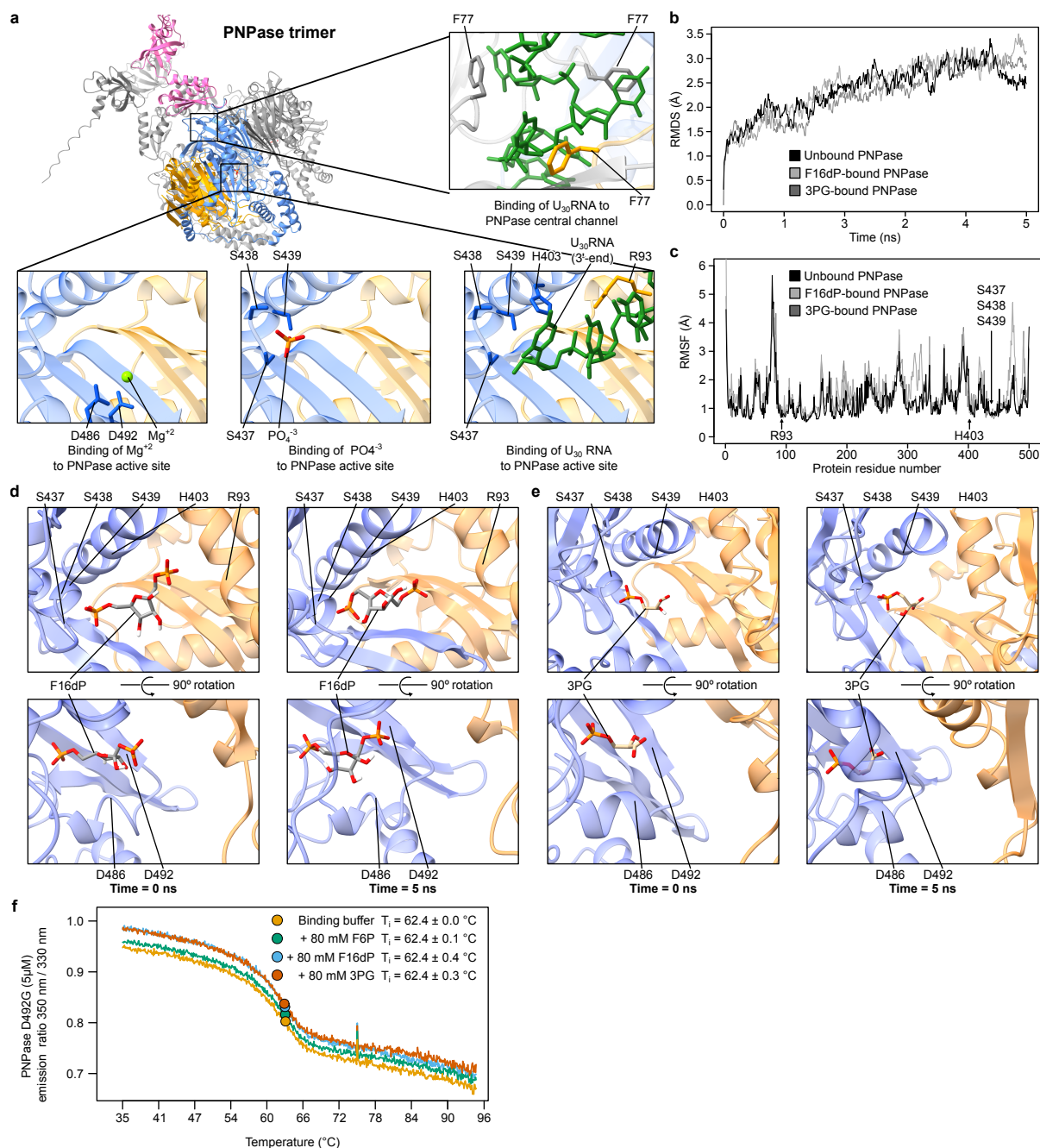
Ander Hernández Plágaro¹, Oleg N Murashko^{1,7}, Chen-Hsin Yu¹, Kun-Hai Yeh¹, Pankaj Leonard Tirkey¹, Mikel Irastortza-Olaziregi^{2,8}, Vladimir R Kaberdin^{2,3,4}, Yelina Ortiz Pérez⁵, Félix Javier Sangari^{5,6}, and Sue Lin-Chao^{1*}



Supplementary Fig. 1: Schematic overview of central carbon metabolism, entry point of different carbon sources and the diversion of precursor metabolites to biosynthetic pathways, adapted from the Ecocyc database ²⁵. Blue, orange and green indicate reactions within glycolysis, the pentose phosphate pathway and the tricarboxylic acid cycle, respectively. Specific periplasmic transporters for glycerol, glucose, acetate or citrate are indicated. Large circles represent the 12 precursor metabolites and small circles represent other metabolites. Abbreviations are as follows: glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), glyceraldehyde 3-phosphate (GA3P), 3-phosphoglycerate (3PG), phosphoenolpyruvate (PEP), pyruvate (PYR), acetyl-CoA (ACA), oxaloacetate (OAA), alfa-ketoglutarate (α KG), succinyl-CoA (SCA), erythrose 4-phosphate (E4P), ribose 5-phosphate (R5P).

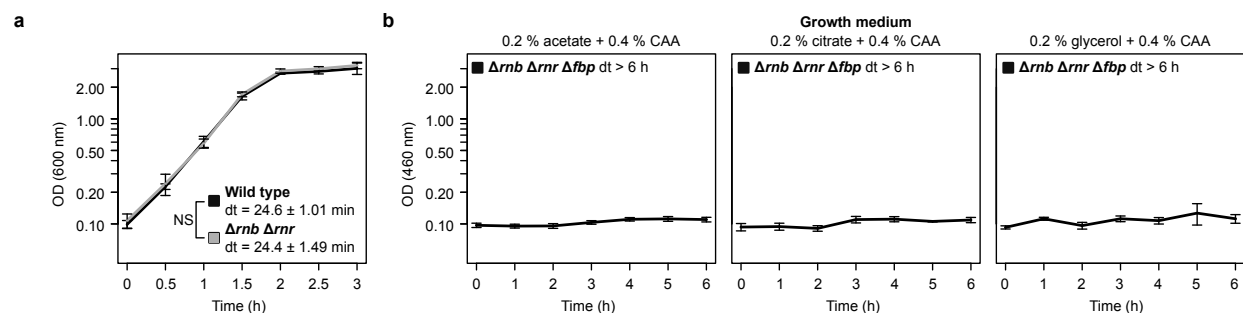


Supplementary Fig. 2: Characterization of PNPase variants by gel filtration, native gel electrophoresis and determination of their enzymatic activity in the presence of glycolytic metabolites. **a**, Size exclusion chromatography of PNPase variants in a Superose 6 column. Red lines indicate each fraction corresponding to the PNPase trimer that was used for further experiments. **b**, Native polyacrylamide gel of the indicated PNPase variants. Since the PNPase samples originated from single size-exclusion chromatography fractions, the two bands migrating far above the expected dimer size likely correspond to different trimeric conformations. **c**, Denaturing polyacrylamide gels from end-point ribonuclease assays showing the degradation of a 5'-FAM-U₃₀ single-stranded RNA substrate by the PNPase R153A_R372A double mutant, PNPase R153A_R372A_R405A_R409A quadruple mutant and PNPase D492G null mutant in the presence of various glycolytic metabolites. Results are representative of three independent replicates. All values are shown as the mean $\pm$ SD. Source data are provided as a Source Data file.

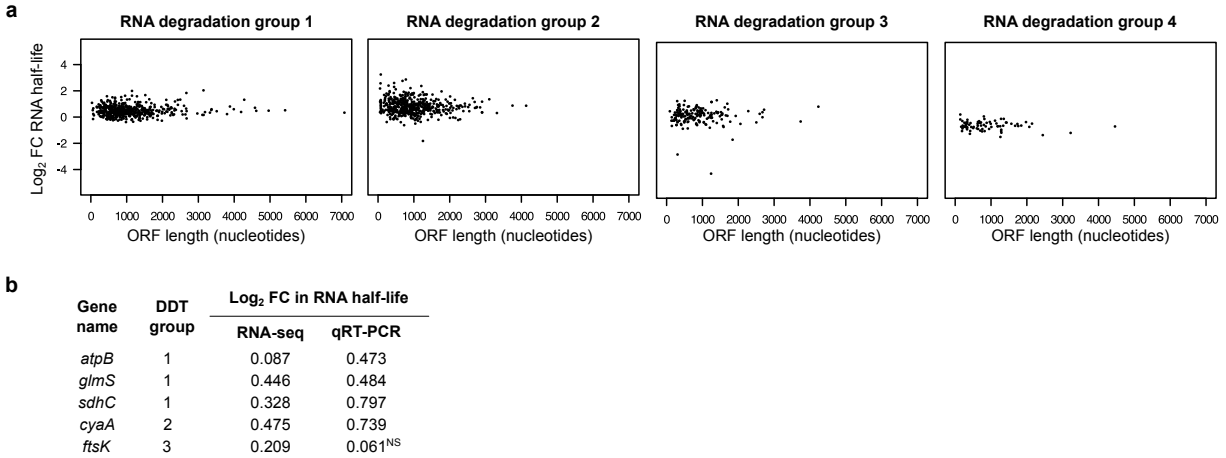


Supplementary Fig. 3: Docking in AlphaFold 3, molecular dynamics simulation of the 3D models with GROMACS and nanoDSF. **a**, AlphaFold 3 model for trimeric PNPase, one monomer is colored to highlight the different domains: the PH1 domain is orange, the PH2 domain is blue, and the KH and S1 domains are pink. Magnified views show docking for U_{30} RNA (in green) in the central channel, and dockings for magnesium, phosphate or U_{30} RNA in the active site, respectively. Residues predicted to be involved in binding are indicated. **b**, RMSD plots of

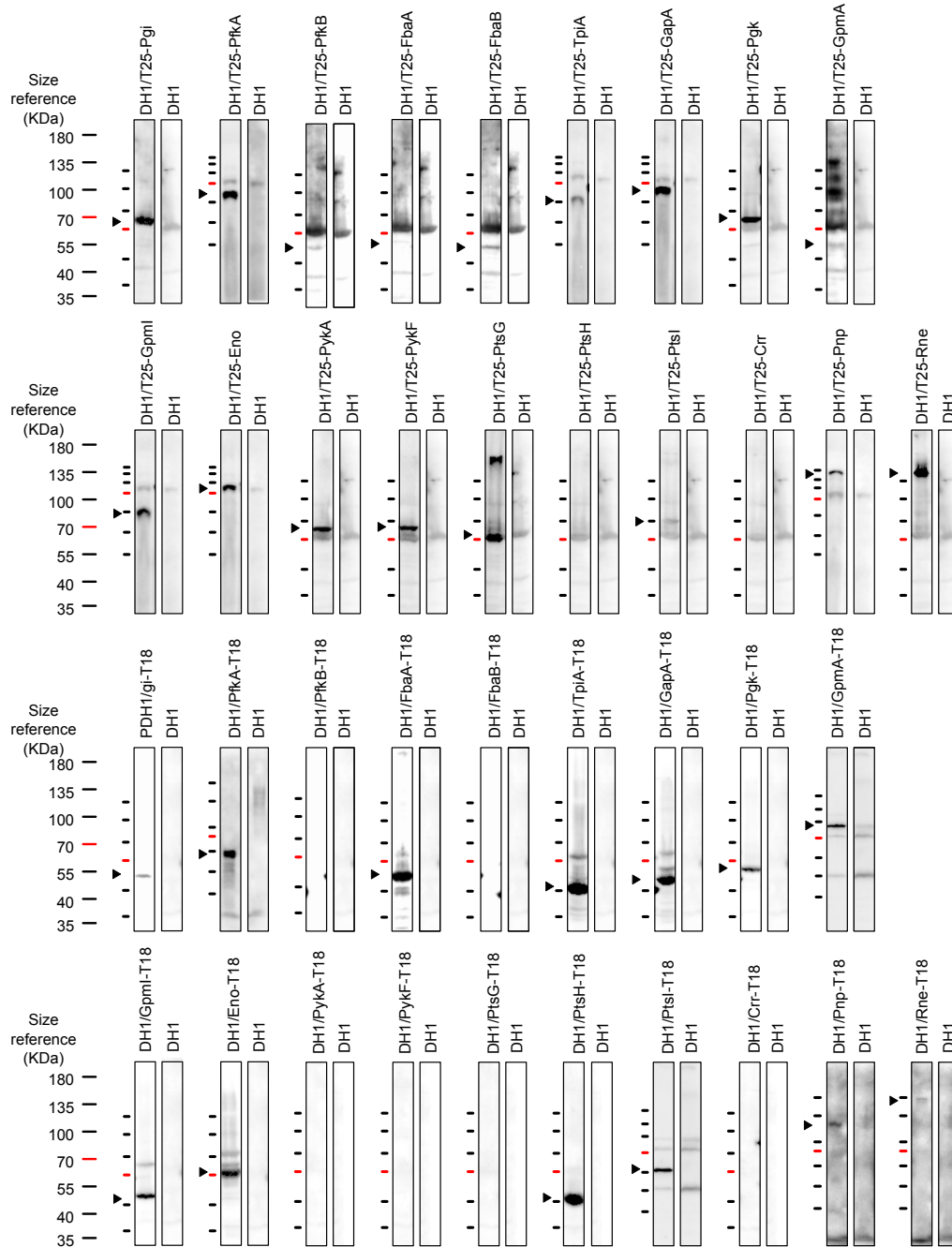
unbound, F16dP-bound and 3PG-bound PNPase during molecular dynamics simulation. **c**, RMSF plot of residues 1-500 of unbound, F16dP-bound and 3PG-bound PNPase. Residues predicted to be involved in binding F16dP or 3PG are indicated by arrows. **d**, Structure of F16dP bound to PNPase active site at the beginning (time = 0 ns) and the end (time = 5 ns) of molecular dynamics simulation, and 90° longitudinal rotation view. **e**, Structure of 3PG bound to PNPase active site at the beginning (time = 0 ns) and the end (time = 5 ns) of molecular dynamics simulation, and 90° longitudinal rotation view. Solvent particles have been omitted for visualization of GROMACS simulations. **f**, NanoDSF profiles showing thermal stability of PNPase D492G incubated with different glycolytic metabolites. NanoDSF results are representative of three independent replicates and values are shown as the mean $\pm$ SD. Source data are provided as a Source Data file.



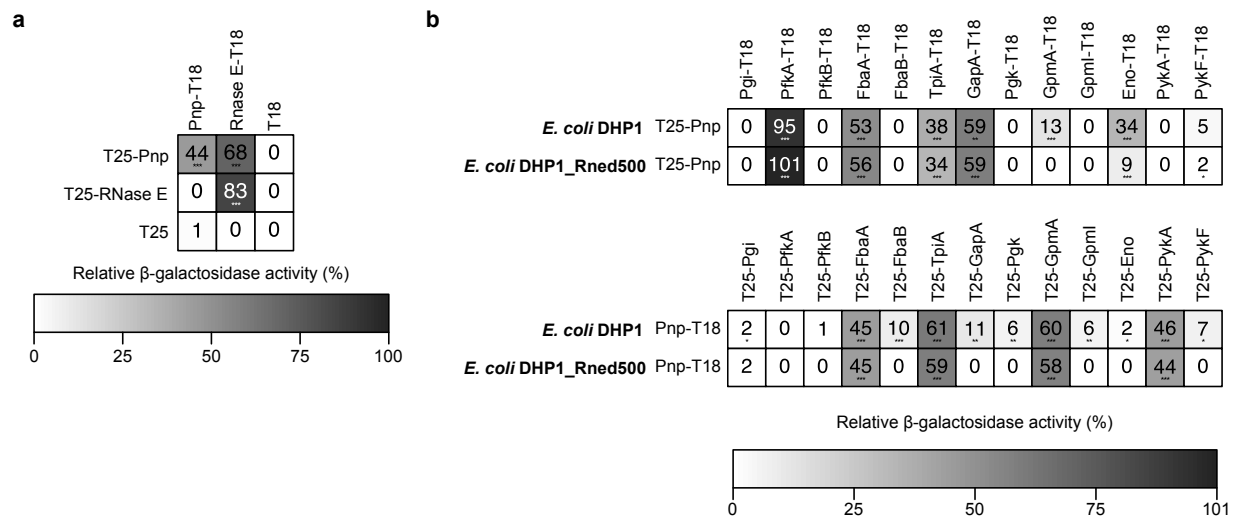
Supplementary Fig. 4. Growth analysis of PNP-essential, PNP-essential *fbp*-deletion and isogenic wild type strains. **a**, Growth curves of PNP-essential and isogenic wild type strains in LB and the corresponding doubling times. **b**, Growth curves of PNP-essential *fbp*-deletion strain on alternative carbon sources. The differences in mean values, as determined by a two-sided, unpaired, parametric t-test, are indicated (NS = not significant). Results are representative of three independent replicates. All values are shown as the mean $\pm$ SD. Source data are provided as a Source Data file.



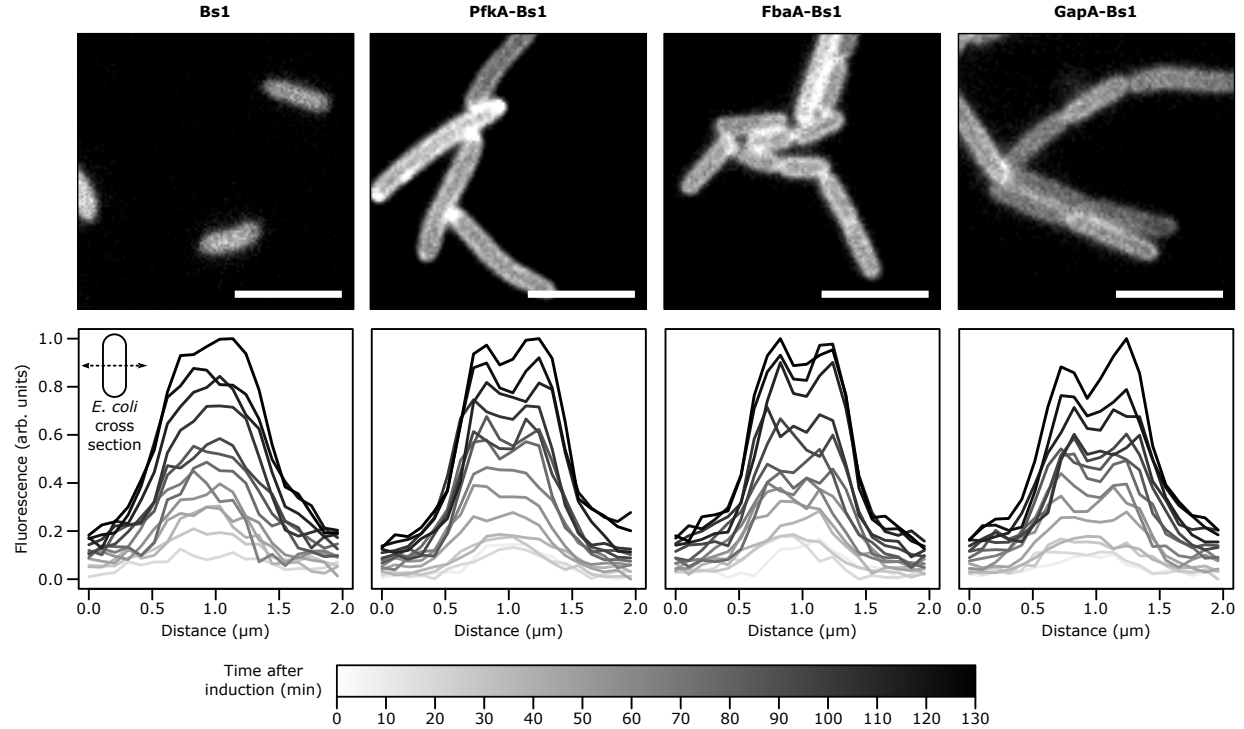
Supplementary Fig. 5: Fold-change in RNA half-life **a**, Scatterplot of log₂ fold-change in median mRNA half-life against length of the corresponding ORF in each of the four RNA degradation groups. **b**, Log₂ fold-change in RNA half-life determined by RNA-seq and qRT-PCR for genes selected based on their different RNA degradation group and the detection in complex with PNPase D492G. Represented data was obtained from four independent experiments for panel **a** and three independent experiments for qRT-PCR in panel **b**. NS indicates non-significant fold-change (p-value > 0.05) as determined by a one-sided, unpaired, parametric t-test. Source data are provided as a Source Data file.



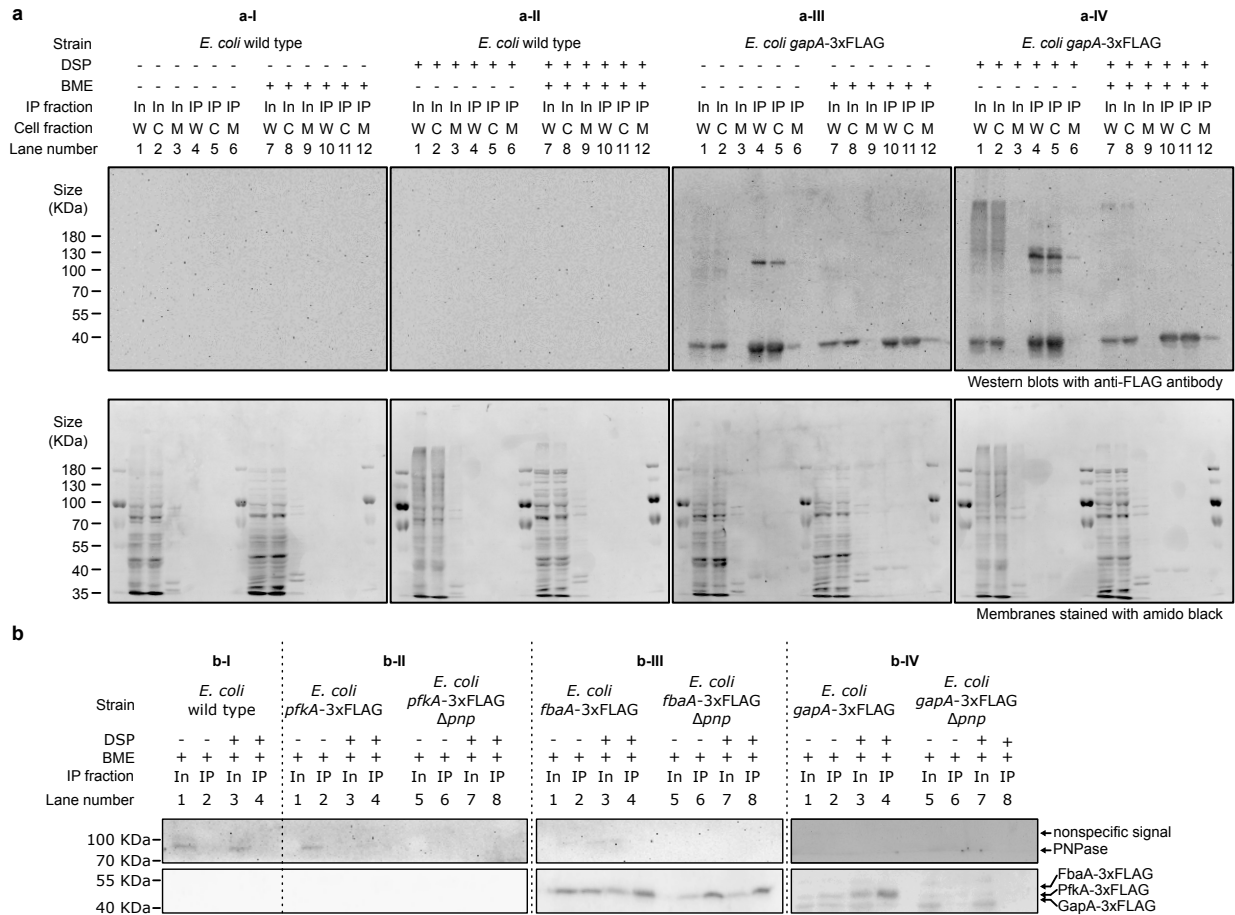
Supplementary Fig. 6: Western blot detection of T25- and T18-derived fusion proteins in *E. coli* DHP1 transformed with the indicated pT25 or pT18 plasmids. Anti-T25 and anti-T18 antibodies were used to probe for the respective fusion proteins. The expression levels were compared to the untransformed *E. coli* DHP1 wild type strain. These results allowed us to exclude interactions in the BACTH assay pertaining to proteins for which no specific signal (indicated by black triangles) was detected.



Supplementary Fig. 7: Determination of additional protein-protein interactions by BACTH assays. **a**, Interaction between PNPase and RNase E. **b**, Interactions between PNPase and glycolytic enzymes in *E. coli* DHP1 or *E. coli* DHP1_Rned500, in which the region encoding the C-terminal domain of RNase E was deleted. β -galactosidase activity was determined relative to the positive control. Statistical significance of the interaction compared to the negative control, as determined by a two-sided, unpaired, parametric t-test, is indicated by asterisks (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). All values presented are the mean of four independent replicates. Source data are provided as a Source Data file.



Supplementary Fig. 8: Live imaging of the distribution of fluorescently-labelled glycolytic enzymes in *E. coli*. Images show the distribution of fluorescently tagged enzymes PfkA-L-Bs1, FbaA-L-Bs1, and GapA-L-Bs1 130 min after induction. Imaging of Bs1 serves as a negative control for fluorescence. The lower panel shows fluorescence intensity profiles across a 2 µm perpendicular section of a representative cell for each construct, illustrating the similar spatial localization of glycolytic proteins, with lower fluorescence intensity towards the middle of the cell in comparison to Bs1. Scale bars represent 5 µm. Images are representative of three independent replicates and plots are representative of three randomly selected cells in each replicate. Source data are provided as a Source Data file.



Supplementary Fig. 9: Western blot detection of proteins after *in vivo* crosslinking and FLAG-immunoprecipitation. **a**, Detection of GapA-3xFLAG in crosslinked complexes by western blotting with anti-FLAG antibodies. Different cellular fractions (W: whole-cell lysate, C: cytoplasm, M: membrane) before (In: input) and after immunoprecipitation (IP) were analyzed. Where indicated, proteins were crosslinked *in vivo* with DSP and crosslinking was reversed by treatment with BME. **b**, Western blots of affinity purified membrane fractions of *E. coli* cells with anti-FLAG and anti-PNPase antibodies. Where indicated, proteins were crosslinked *in vivo* with DSP, crosslinking was reversed by BME before electrophoresis. Samples were analyzed before (In) and after immunoprecipitation (IP). Results are representative of three independent experiments. *E. coli* wild type strains correspond to the negative control for the anti-FLAG antibody. *E. coli* Δpnp strains correspond to the negative control for the anti-PNPase antibody.

