Supplementary Information for

T4 phage RNA is NAD-capped and alters the NAD-cap epitranscriptome of *Escherichia coli* during infection through a phage-encoded decapping enzyme

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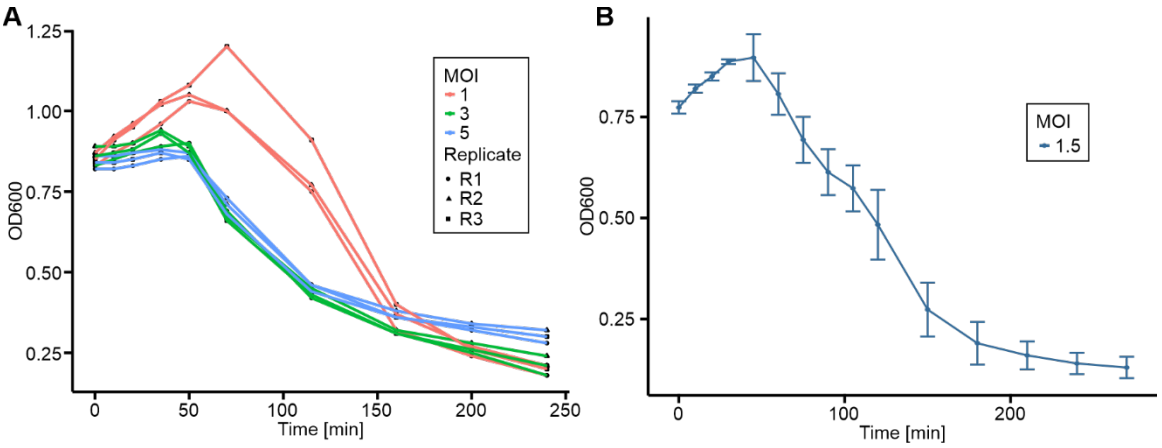
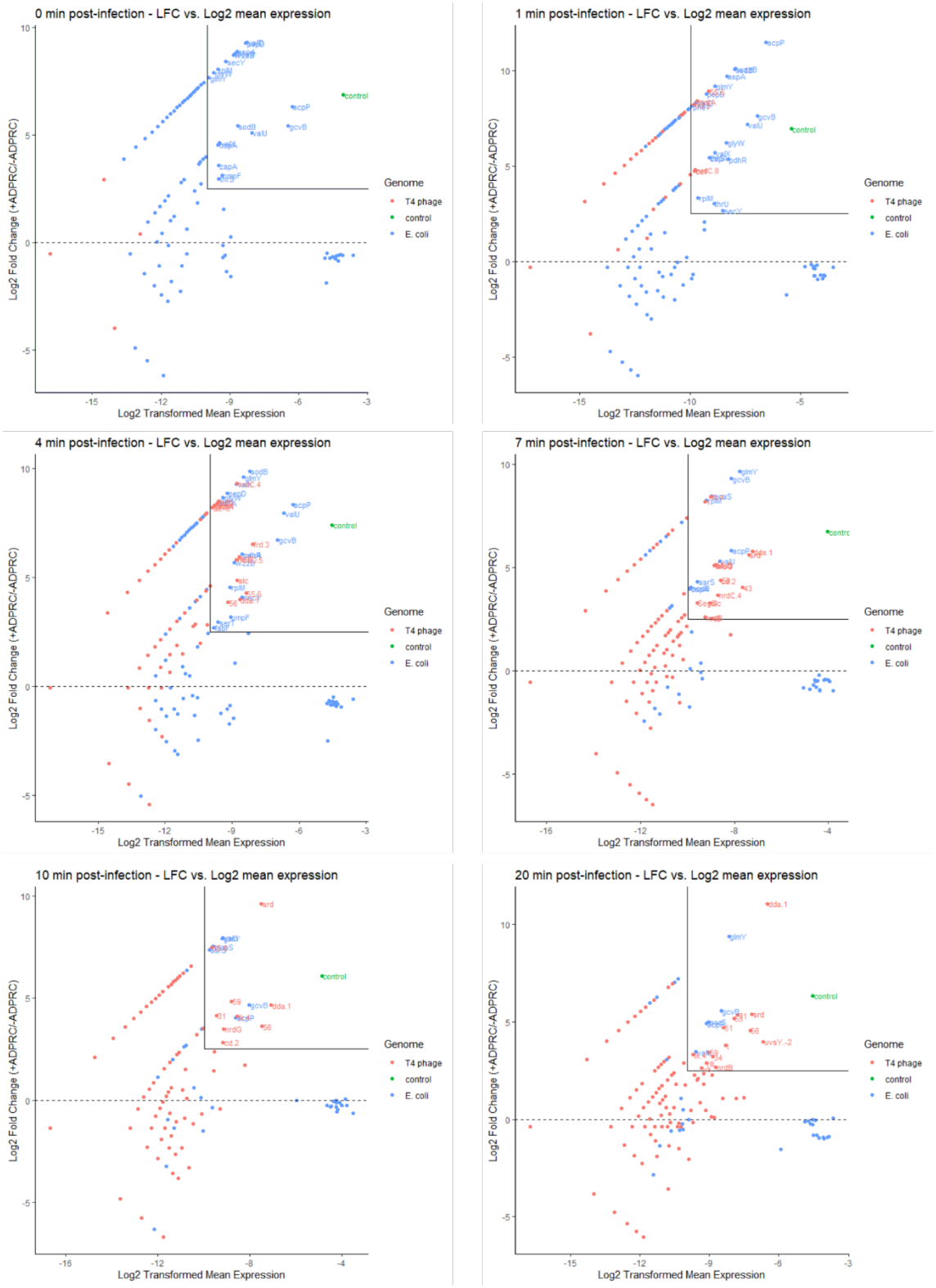
Content:



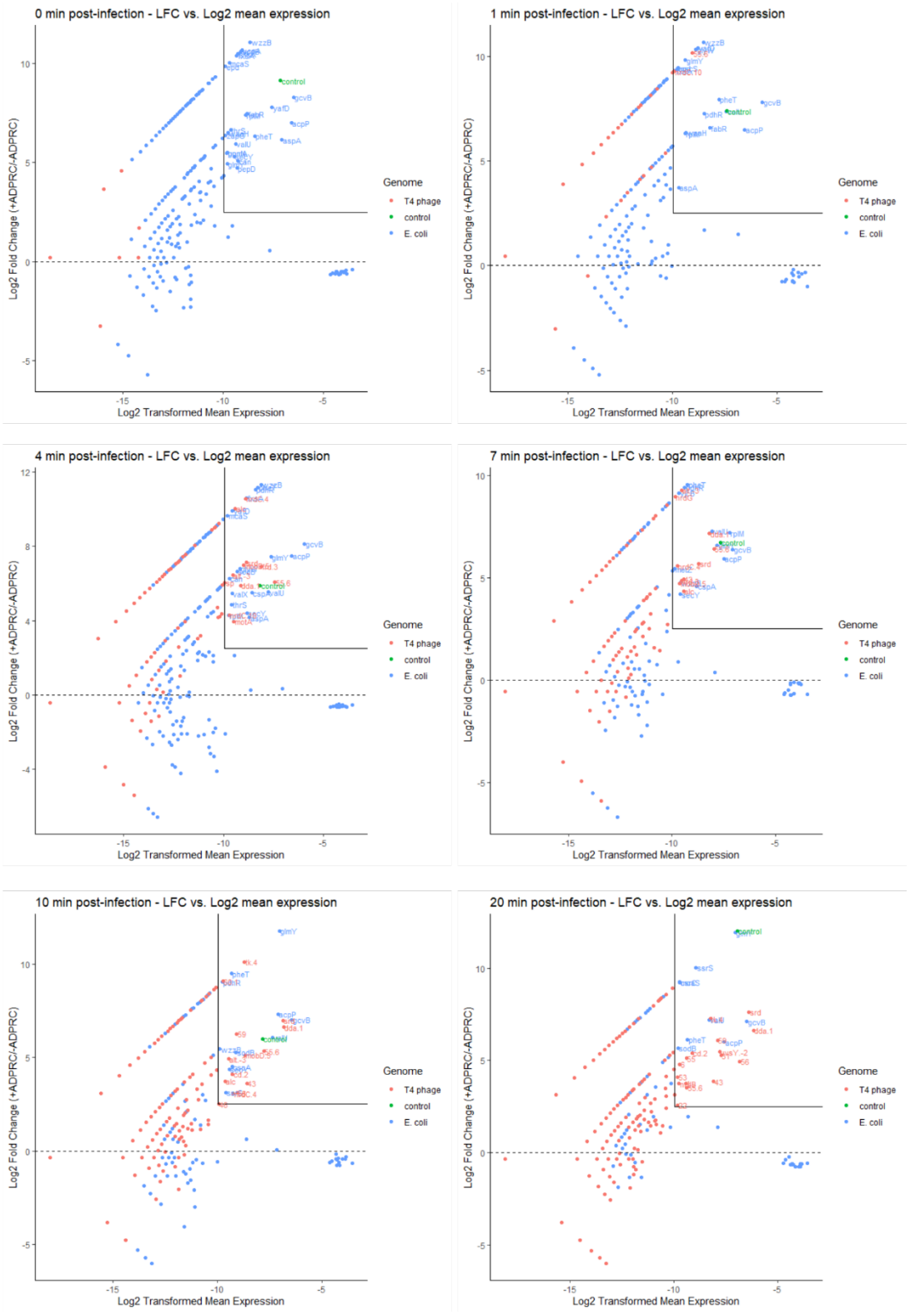
Supplementary Figure S1: Lysis curves of T4 phage infection depending on MOI.
(**A**) Lysis curves of *E. coli* upon T4 phage infection at multiplicity of infection (MOI) of 1, 3 and measured by optical density at 600 nm (OD_{600}) (n=3). (**B**) Lysis curve for *E. coli* infected by T4 phage at an MOI of 1.5, the condition used for NAD captureSeq experiments. Data points with error bars represent mean ± s.d. (n=3).

	Replicate 1	Replicate 2
Pass reads	220,028	499,383
Median read length [b]	324	318
Median PHRED score	10.239	10.796
Mean reads per barcode	14,777	36,255
Identified NAD-RNAs	116	110

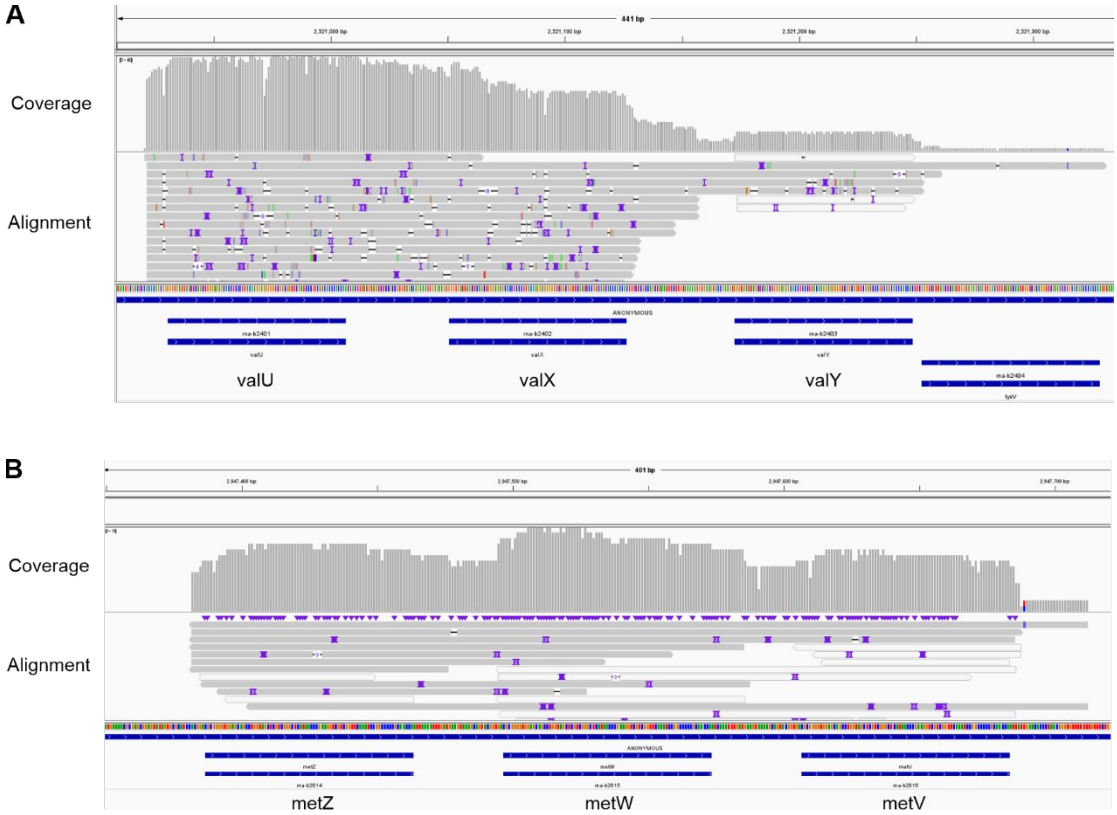
Supplementary Figure S2: Statistics of Nanopore sequencing runs for both biological replicates presented in this study.



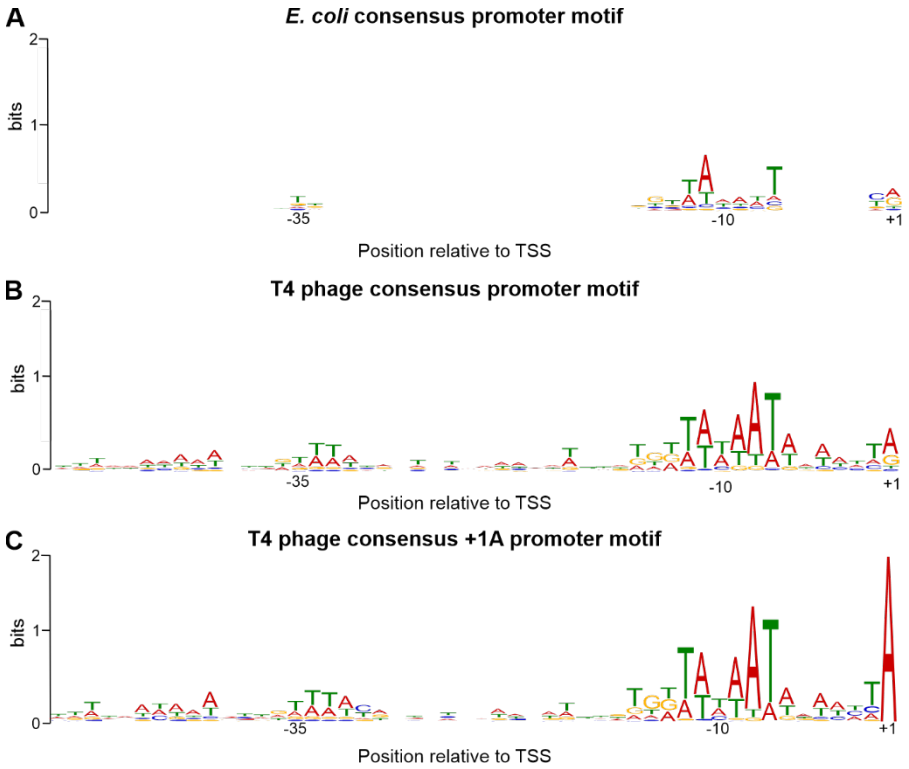
Supplementary Figure S3: NAD captureSeq analysis for all time points for replicate 1.
MA plots are presented for all 6 time points of infection (t0, t1, t4, t7, t10, t20) for replicate 1. y-axis represents the log2 fold change in normalized read counts comparing fully-treated sample (+ADPRC) and negative control (-ADPRC), x-axis shows log2 transformed mean normalized read counts for genes from + and -ADPRC samples. Enriched genes are labelled with their corresponding gene symbol and colored according to their genome (T4 phage, red; 100 nt control RNA, green; *E. coli*, blue).



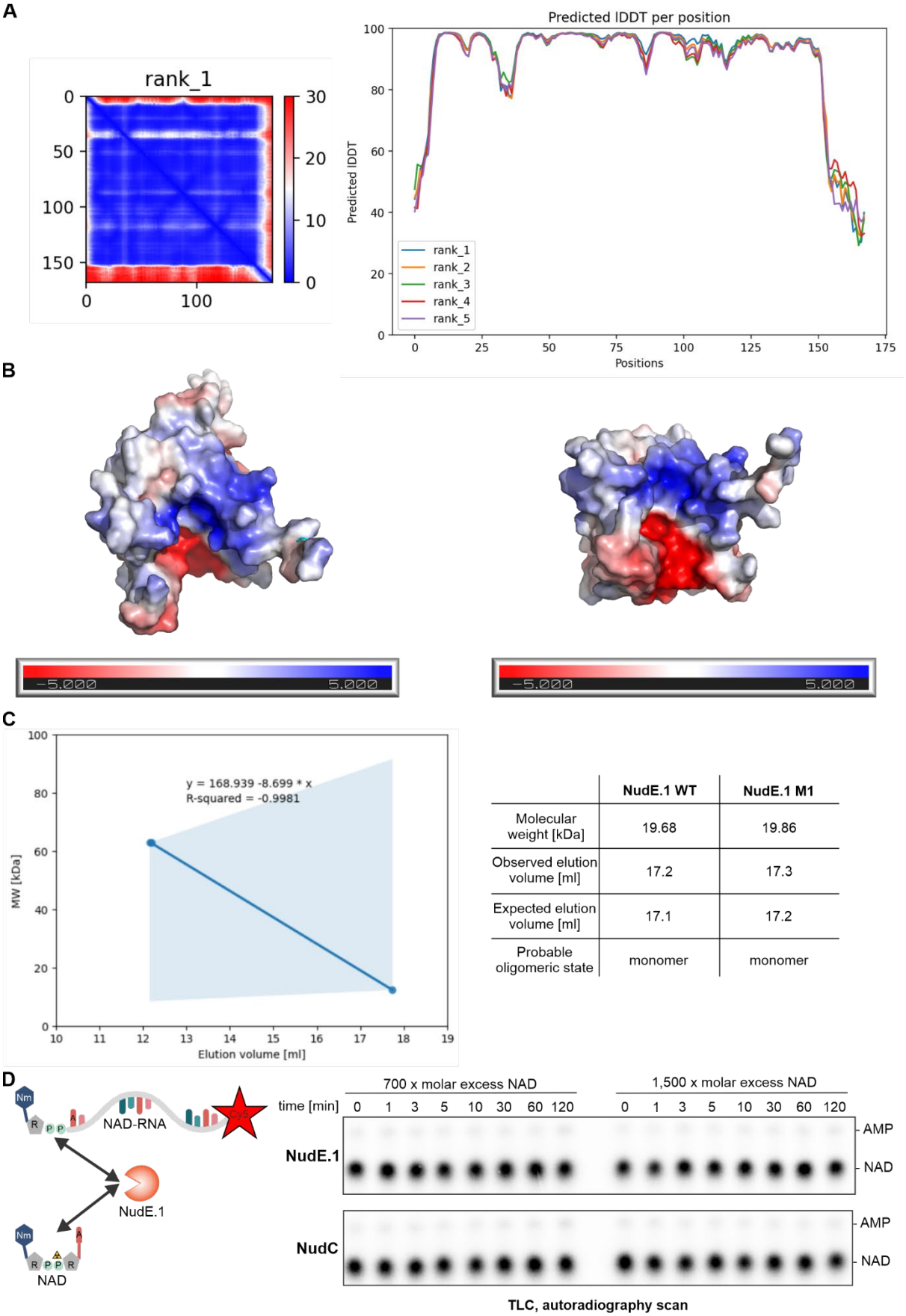
Supplementary Figure S4: NAD captureSeq analysis for all time points for replicate 2.
MA plots are presented for all six time points of infection (t0, t1, t4, t7, t10, t20) for replicate 2. y-axis represents the log2 fold change in normalized read counts comparing fully-treated sample (+ADPRC) and negative control (-ADPRC), x-axis shows log2 transformed mean normalized read counts for genes from + and -ADPRC samples. Enriched genes are labelled with their corresponding gene symbol and coloured according to their genome (T4 phage, red; 100 nt control RNA, green; *E. coli*, blue).



Supplementary Figure S5: tRNA coverage in NAD captureSeq data.
Coverage and alignment profiles for the valU/X/Y (**A**) and metZ/W/V (**B**) operons for the +ADPRC sample from time point t0, replicate 1 from the NAD captureSeq experiment. Reads clearly span across two or three tRNA genes in these operons indicating that the polycistronic tRNA precursors are NAD-capped in *E. coli*. The TSS is in good agreement with the TSS of primary transcripts derived from our dRNA-Seq data. .



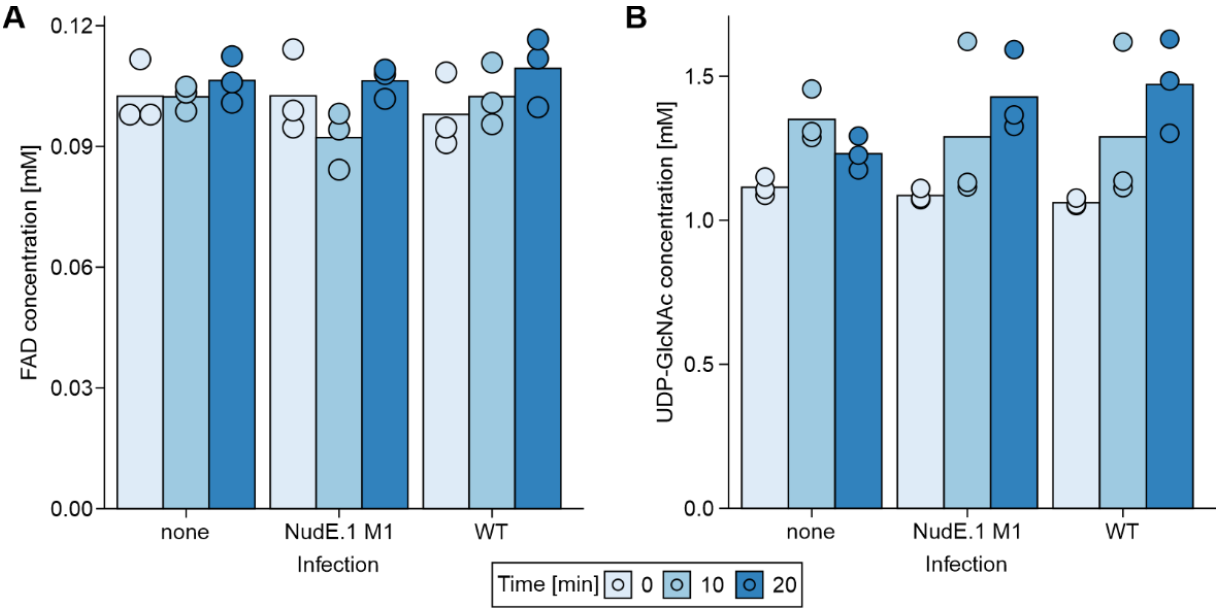
Supplementary Figure S6: Consensus promoter motifs of *E. coli* and T4 phage genes.
Consensus motifs of promoters for all *E. coli* genes (4380 promoters) (**A**), all T4 phage genes (150 promoters) (**B**) and +1A T4 phage promoters (90 promoters) (**C**) including the TSS (+1) and the -10 and -35 element as identified by dRNA-Seq. Motifs were created using Meme Suite (1).



	NudE.1 WT	NudE.1 M1
Molecular weight [kDa]	19.68	19.86
Observed elution volume [ml]	17.2	17.3
Expected elution volume [ml]	17.1	17.2
Probable oligomeric state	monomer	monomer

Supplementary Figure S7: Structure and oligomeric state of the Nudix hydrolase NudE.1.
(**A**) Alphafold prediction metrics for the Alphafold model of NudE.1 WT presented in Figure 6A. The model presented corresponds to rank 1. (**B**) Surface charge structural models of NudE.1 WT based on

the model presented in Figure 6A. Red color indicates negative charge, blue color represents positive charge. Left panel shows model in same orientation as in Figure 6A, right panel shows a tilted model enabling a view directly in the open cleft with the catalytic site (charged negatively, colored red). (**C**) Analytical size exclusion chromatography (SEC) to determine the oligomeric state of NudE.1. The SEC column was calibrated with monomeric protein standards of known molecular weight and a linear regression model was fit to calculate an expected elution volume for a given molecular weight. NudE.1 WT and E64,65Q migrate as apparent monomers during SEC. (**D**) NAD levels in the NAD vs. NAD-RNA competition assays as shown in Figure 6E. Autoradiography image of TLC analysis is shown. NAD levels are barely affected over the time course of infection in the presence of both NudE.1 WT and NudC WT independent of the fold molar excess of NAD over NAD-RNA.



Supplementary Figure S8: FAD and UDP-GlcNAc concentrations in during T4 phage infection. Cellular concentrations of FAD (**A**) and UDP-GlcNAc (**B**) in T4 WT, T4 NudE.1 E64,65Q infected and uninfected (none) *E. coli* over the time course of 20 minutes before infection (0 min) as well as 10 and 20 min post infection. Concentrations are derived from endometabolomics analysis of biological triplicates (n=3) by LC-MS.

Supplementary Table S1: List of bacteria and phages used in this study.

Strain	Purpose	Reference
E. coli BL21 DE3 NudE.1 WT	Overexpression of His-tagged NudE.1 WT via pET28-NudE.1 WT	This study
E. coli BL21 DE3 NudE.1 E64,65Q	Overexpression of His-tagged NudE.1 M1 via pET28-NudE.1 E64,65Q	This study
E. coli BL21 DE3 NudC WT	Overexpression of His-tagged NudC WT via pET28-NudC WT	(2)
E. coli B strain	Strain used for T4 phage infection	DSMZ
E. coli JM109 + pUC19	*E. coli* strain with NAD-capped RNAI	(3)
E. coli BL21 DE3 Cas13a_NudE.1	*E. coli* strain expressing Cas13a targeting NudE.1 WT RNA.	This study
E. coli BL21 DE3 pET28a_NgTET_NudE.1-M1 + pCpf1_NudE.1	*E. coli* expressing NgTET and Cas12 targeting NudE.1 WT, and providing NudE.1 M1 donor DNA	This study
Bacteriophage T4 WT	WT T4 phage	DSMZ
Bacteriophage T4 NudE.1 E64,65Q	T4 phage with NudE.1 E64,65Q (inactive)	This study

Supplementary Table S2: DNA sequences of probes, primers and splints.

Name	Sequence
Northern blot probes	
Northern probe RNAI	TGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGC AGAGCGCAGATACCAAATACTGT
Northern probe 5S rRNA	CCCCACACTACCATCGGCGCTACGGCGTTTCACTTCTGAGTTCGGCATGGGGTC AGGTGGGACCACCGCGCTACGGC
qPCR primers for NAD captureSeq data validation	
qPCR fwd primer 100 nt control RNA	ATACTACCTTTAGTTCGTTTAAACACG
qPCR rev primer 100 nt control RNA	CATGATCAAATTGACCCAAAGTTTC
qPCR fwd primer GcvB	GCCGGAACGAAAAGTTTTATCGG
qPCR rev primer GcvB	TGCTACCATGGTCTGAATCGC
qPCR fwd primer GlmY	GTGGCTCATTCACCGACTTATG
qPCR rev primer GlmY	CCCGATGGTTGATATAGCTACG
qPCR fwd primer ssrS	TCTCTGAGATGTTCGCAAGCG
qPCR rev primer ssrS	GTGTCGTCGCAGTTTTAAGGC
qPCR fwd primer acpP	TGAGCACTATCGAAGAACGCG
qPCR rev primer acpP	CTCAACGGTGTCAAGAGAATCC
qPCR fwd primer dda.1	GGTTTATGTATATGCGATAGTTTACCG

qPCR rev primer dda.1	TCTTTAAGAGTGGTAAATACTTTATCAGC
qPCR fwd primer motA	TGTCTAAAGTAACTTACATCATCAAAGC
qPCR rev primer motA	GCACCTCACGAACTTCTGC
qPCR fwd primer alc	TTTACAACTTATTACTACTGAAATGGTCG
qPCR rev primer alc	CTTAGCTAAATCTTTCTTAAGACCAGTG
qPCR fwd primer segG	GGTTTACATTTGAAGACCGTGTC
qPCR rev primer segG	CAGTATTCCTTTCCGGAAGAGTC
qPCR fwd primer nrdC.4	GAATATATCAAATCATTCAATAGCG
qPCR rev primer nrdC.4	CTTGCATCAACACTATTGAGCC
qPCR fwd primer 5S	TTGAAGAGTTTGATCATGGCTCAG
qPCR rev primer 5S	CAGTTTCCCAGACATTACTCACC
circNC-like protocol	
RT primer dda.1 circNC	CTTTTCAGTTTAAGTTTATCAATAAAAGAC
qPCR rev primer dda.1 circNC	CAAATTTTGATACCGCTCTACACC
qPCR fwd primer dda.1 circNC	GATATCCGCTAAATTGTTCAATTTTTAAAC
RT primer gcvB circNC	CAGAACACGCATTCCGATAAAAC
qPCR rev primer gcvB circNC	TTCGTTCCGGCTCAGGAAG
qPCR fwd primer gcvB circNC	TGAACTTTTGGCTTACGGTTGTG
RT primer RNAI circNC	CGAAGGTAACTGGCTTCAGC
qPCR rev primer RNAI circNC	AGAGCGCAGATACCAAATACTG
qPCR fwd primer RNAI circNC	AAAAAGAGTTGGTAGCTCTTGATC
RT primer 5S rRNA circNC	GGTCAGGTGGGACCACC
qPCR rev primer 5S rRNA circNC	CGCTACTGCCGCCAGG
qPCR fwd primer 5S rRNA circNC	TGCCGAACTCAGAAGTGAAACG
Cloning and mutagenesis of *nudE.1* gene	
NudE.1 fwd NcoI	ATCGACCCATGGGACAGGAAATTAAAATGAAAACATTATCAGC
NudE.1 rev XhoI	GTGCTCGAGGCCCTGAAAATAAAGATTCTCACCAAAGAGGTTGTTCATTATTCGGTAAAG
NudE.1 E64,65Q fwd	CGAAGAGAATGTTTACAACAGACTGGTTTTAGC
NudE.1 E64,65Q rev	TGCTGCATCTAATGCGCTTAAATC
NudE.1 screening fwd	CCAGTCACGACGTTGTAAAACGCATAATACCTCCTAAGTATTTATAGAAGG

NudE.1 screening rev	AGCGGATAACAATTTCACACAGGCTAGGGATATGGCGTATGTTCATTGAAATGCC
NAD captureSeq	
Adenylated RNA 3'-adapter	rAppCNNNNNNAGATCGGAAGAGCACACGTCTG-3SpC3
RT primer	CAGACGTGTGCTCTTCCGAT
cDNA anchor fwd	pCAGATCGGAAGAGCGTCGTGTCCC-SpC3
cDNA anchor rev	ACACGACGCTCTTCCGATCTGGG
BC1 fwd	CACAAAGACACCGACAACTTTCTTGGGACACGACGCTCTTCCGATCTG
BC1 rev	CACAAAGACACCGACAACTTTCTTCAGACGTGTGCTCTTCCGAT
BC2 fwd	ACAGACGACTACAAACGGAATCGAGGGACACGACGCTCTTCCGATCTG
BC2 rev	ACAGACGACTACAAACGGAATCGACAGACGTGTGCTCTTCCGAT
BC3 fwd	CCTGGTAACTGGGACACAAGACTCGGGACACGACGCTCTTCCGATCTG
BC3 rev	CCTGGTAACTGGGACACAAGACTCCAGACGTGTGCTCTTCCGAT
BC4 fwd	TAGGGAAACACGATAGAATCCGAAGGGACACGACGCTCTTCCGATCTG
BC4 rev	TAGGGAAACACGATAGAATCCGAACAGACGTGTGCTCTTCCGAT
BC5 fwd	AAGGTTACACAAACCCTGGACAAGGGGACACGACGCTCTTCCGATCTG
BC5 rev	AAGGTTACACAAACCCTGGACAAGCAGACGTGTGCTCTTCCGAT
BC6 fwd	GACTACTTTCTGCCTTTGCGAGAAGGGACACGACGCTCTTCCGATCTG
BC6 rev	GACTACTTTCTGCCTTTGCGAGAACAGACGTGTGCTCTTCCGAT
BC7 fwd	AAGGATTCATTCCCACGGTAACACGGGACACGACGCTCTTCCGATCTG
BC7 rev	AAGGATTCATTCCCACGGTAACACCAGACGTGTGCTCTTCCGAT
BC8 fwd	ACGTAACTTGGTTTGTTCCCTGAAGGGACACGACGCTCTTCCGATCTG
BC8 rev	ACGTAACTTGGTTTGTTCCCTGAACAGACGTGTGCTCTTCCGAT
BC9 fwd	AACCAAGACTCGCTGTGCCTAGTTGGGACACGACGCTCTTCCGATCTG
BC9 rev	AACCAAGACTCGCTGTGCCTAGTTCAGACGTGTGCTCTTCCGAT
BC10 fwd	GAGAGGACAAAGGTTTCAACGCTTGGGACACGACGCTCTTCCGATCTG
BC10 rev	GAGAGGACAAAGGTTTCAACGCTTCAGACGTGTGCTCTTCCGAT
BC11 fwd	TCCATTCCCTCCGATAGATGAAACGGGACACGACGCTCTTCCGATCTG
BC11 rev	TCCATTCCCTCCGATAGATGAAACCAGACGTGTGCTCTTCCGAT
BC12 fwd	TCCGATTCTGCTTCTTTCTACCTGGGGACACGACGCTCTTCCGATCTG
BC12 rev	TCCGATTCTGCTTCTTTCTACCTGCAGACGTGTGCTCTTCCGAT
***In vitro* transcription templates**	
Fwd ultramer IVT 100 nt control RNA	TAATACGACTCACTATTATCTTGATACTACCTTTAGTTCGTTTAAACACGTTCTTGATAGTATC*TTTTTATT*AACC
Rev ultramer IVT 100 nt control RNA	CATGATCAAATTGACCCAAAGTTTCAACGCTTTACGCGTTGGGTTAATAAAAAGATACTATCAAGAACGTG
Fwd primer IVT 100 nt control RNA	TAATACGACTCACTATTATCTTGATACTACCTTTAG
Rev primer IVT 100 nt control RNA	CATGATCAAATTGACCCAAAGTTTCAACGCTTTACGCG
Fwd ultramer IVT RNAI	TAATACGACTCACTATTACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTG
Rev ultramer IVT RNAI	AACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTC
Fwd primer IVT RNAI	TAATACGACTCACTATTACAG
Rev primer IVT RNAI	AACAAAAAAACCACCGCTACC

Supplementary Table S3: RNA sequences used in this study.

Name	Sequence
100 nt control RNA	AUCUUGAUACUACCUUUAGUUCGUUUAAACACGUUCUUGAUAGUAUCUUU UUAUUAACCCAACGCGUAAAGCGUUGAAACUUUGGGUCAAUUUGAUCAUG
RNAI	ACAGUAUUUGGUAUCUGCGCUCUGCUGAAGCCAGUUACCUUCGGAAAAAG AGUUGGUAGCUCUUGAUCCGGCAAACAAACCACCGCUGGUAGCGGUGGUU UUUUUGUU
10mer-Cy5	pACAGUAUUUG-Cy5
Linear-10mer-Cy5	pAGACUUCGAC-Cy5
2nt-5′-overhang-10mer-Cy5	pACAGACUUCGGUCU-Cy5
Blunt-end-10mer-Cy5	pAGACUUCGGUCU-Cy5
1nt-3′-overhang-10mer-Cy5	pAGACUUCGGUCUA-Cy5

Supplementary Table S4: DNA sequences of proteins as expressed from plasmids used in this study. Full plasmid maps are available at: https://github.com/MaikTungsten/PhageEpitranscriptomics.

Plasmid Name	Sequence	Reference
pET28-NudE.1 WT	ATGGGACAGGAAATTAAAATGAAAACATTATCAG CTGGTATTATCTTTATGACAGAAGATAAAGATTTA TTTATGGGTCGGGTTACTGGTTCTCGTAAGACTGG AATGATGGCACATCGTTGGGATATTCCAAAGGGC CGTGTAGAAAATTCTGATTTAAGCGCATTAGATG CAGCACGAAGAGAATGTTTAGAAGAGACTGGTTT TAGCAATTATAATCCAGACCTTCTAGAAGACCTA GGTGTATTTAAATATTCTAGTAATAAAGACCTACA GTTATTTTATTACACGATTCCAGTAGAGCATGAGA TGTTCAGAAATTGCCGTTGCGAGTCTTATTTTGAA AATAAAGATGGCGTTATGATTCCAGAGATGGACG CTTTTGCTCTTATTCCTCGTACTCAGTGGCAATATG TGATGGGTCCTTCACTTTACCGAATAATGAACAAC CTCTTTGGTGAGAATCTTTATTTTCAGGGCCTCGA GCACCACCACCACCACCACTGA	This study
pET28-NudE.1 E64,65Q	ATGGGACAGGAAATTAAAATGAAAACATTATCAG CTGGTATTATCTTTATGACAGAAGATAAAGATTTA TTTATGGGTCGGGTTACTGGTTCTCGTAAGACTGG AATGATGGCACATCGTTGGGATATTCCAAAGGGC CGTGTAGAAAATTCTGATTTAAGCGCATTAGATG CAGCACGAAGAGAATGTTTACAACAGACTGGTTT TAGCAATTATAATCCAGACCTTCTAGAAGACCTA GGTGTATTTAAATATTCTAGTAATAAAGACCTACA GTTATTTTATTACACGATTCCAGTAGAGCATGAGA TGTTCAGAAATTGCCGTTGCGAGTCTTATTTTGAA AATAAAGATGGCGTTATGATTCCAGAGATGGACG CTTTTGCTCTTATTCCTCGTACTCAGTGGCAATATG TGATGGGTCCTTCACTTTACCGAATAATGAACAAC CTCTTTGGTGAGAATCTTTATTTTCAGGGCCTCGA GCACCACCACCACCACCACTGA	This study

pET28-NudC WT	ATGGATCGTATAATTGAAAAATTAGATCACGGCT GGTGGGTCGTCAGCCATGAACAAAAATTATGGTT GCCGAAGGGAGAATTGCCATATGGCGAAGCGGC AAATTTCGATCTTGTGGGTCAGCGCGCACTACAA ATCGGCGAATGGCAGGGGGAACCTGTTTGGTTAG TACAACAGCAGCGGCGTCACGATATGGGGTCGG TACGTCAGGTCATTGATCTCGATGTTGGGCTGTTT CAACTGGCCGGACGAGGCGTACAACTGGCGGAG TTTTACCGATCGCATAAATACTGTGGTTACTGCGG GCATGAAATGTATCCGAGCAAAACCGAATGGGC GATGCTGTGCAGCCATTGCCGTGAGCGTTACTAC CCGCAAATCGCCCCCTGCATTATTGTTGCCATCCG TCGCGATGATTCGATCCTCCTCGCCCAGCATACC CGCCATCGTAACGGTGTCCATACAGTACTTGCCG GATTCGTCGAAGTGGGCGAAACCCTCGAGCAGG CAGTCGCGCGGGAAGTGATGGAAGAGAGCGGAA TTAAAGTTAAAAACTTGCGTTACGTGACTTCTCAG CCGTGGCCGTTTCCTCAGTCTTTAATGACCGCGTT TATGGCGGAATATGACAGCGGCGACATCGTGATC GACCCGAAAGAATTGCTCGAGGCGAACTGGTATC GCTATGACGATTTGCCGTTACTCCCGCCGCCCGG CACCGTAGCGCGCCGTCTGATAGAAGATACGGT GGCGATGTGTCGGGCAGAGTATGAGCTGGTGCC GCGCGGCAGCGCGGCCGCACTCGAGCACCACCA CCACCACCAC	(2)
pUC19	No insert.	(3)
pCpf NudE.1	Inserted guide RNA against NudE.1: GAAGAGACTGGTTTTAGCAA	This study
pBA560-Cas13a-NudE.1	Inserted guide RNA against NudE.1: GCTAAAACCAGTCTCTTCTAAACATTCTCTT	This study, (4)
pET28 NgTET_NudE.1 E64,65Q	NgTET insert: ATGGGAACGACATTTAAACAGCAGACGATTAAAG AAAAAGAGACAAAGCGTAAATACTGTATCAAAG GGACCACTGCGAATCTGACACAAACCCATCCCAA TGGGCCAGTGTGTGTTAACCGCGGGGAGGAAGT AGCAAATACGACTACTCTGTTGGACTCAGGGGGC GGGATTAACAAAAAATCGCTGTTGCAGAATCTGT TGTCCAAATGTAAAACTACATTTCAGCAGTCATTT ACAAACGCCAACATTACTTTAAAGGATGAAAAGT GGCTTAAAAACGTCCGTACTGCTTATTTTGTTTGC GATCATGACGGGAGCGTCGAACTTGCATACCTTC CTAACGTGCTTCCCAAAGAATTAGTCGAAGAATT CACCGAGAAATTTGAATCGATCCAGACCGGACGT AAGAAAGACACAGGTTACTCAGGTATTCTGGACA ACTCGATGCCGTTCAATTACGTCACTGCGGATTTA TCACAGGAGTTAGGACAGTATCTGTCTGAGATTG TGAATCCTCAGATCAACTATTACATCAGTAAATTG CTGACTTGCGTTAGTTCACGTACAATCAATTACCT GGTATCTTTGAATGACTCGTACTATGCCCTTAACA ACTGTTTGTATCCTTCAACCGCCTTCAACTCATTA AAGCCGTCCAACGACGGCCACCGCATCCGTAAA CCTCATAAGGACAATTTGGACATTACCCCGTCGA	This study, (5)

	GCCTTTTCTATTTTGGAAATTTTCAAAATACGGAA GGATATCTTGAGTTAACAGACAAGAATTGCAAAG TTTTCGTCCAACCGGGGGATGTATTATTTTTCAAA GGCAATGAATATAAACACGTCGTGGCCAATATCA CCTCGGGCTGGCGCATTGGATTGGTCTACTTCGC ACACAAGGGGAGTAAAACTAAACCGTATTATGAA GACACGCAGAAGAACTCCCTGAAAATTCATAAAG AAACGAAATAAC NudE.1 E64,65Q insert: GACAGGAAATTAAAATGAAAACATTATCAGCTGG TATTATCTTTATGACAGAAGATAAAGATTTATTTAT GGGTCGGGTTACTGGTTCTCGTAAGACTGGAATG ATGGCACATCGTTGGGATATTCCAAAGGGCCGTG TAGAAAATTCTGATTTAAGCGCATTAGATGCAGC ACGAAGAGAATGTTTACAACAGACTGGTTTTAGC AATTATAATCCAGACCTTCTAGAAGACCTAGGTG TATTTAAATATTCTAGTAATAAAGACCTACAGTTA TTTTATTACACGATTCCAGTAGAGCATGAGATGTT CAGAAATTGCCGTTGCGAGTCTTATTTTGAAAATA AAGATGGCGTTATGATTCCAGAGATGGACGCTTT TGCTCTTATTCCTCGTACTCAGTGGCAATATGTGA TGGGTCCTTCACTTTACCGAATAATGAACAACCTC TTT	

Supplementary Table S5: Mass transitions, collision energies, cell accelerator voltages and dwell times for LC-MS/MS analysis of compounds of interest. Parameters have been optimized using chemically pure standards.

Name	**Precurser Ion**	**Product Ion**	**Collision energy [V]**	**Fragmentor Voltage [V]**	**Cell Accelorator Voltage [V]**	**Dwell time [msec]**	**Polarity**
NAD	664.1	524 428	18 26	380	5	90	Positive
FAD	786.2	348.1 136	21 46	380	5	90	Positive
UDP-GlcNac	606.1	384.8 281.90	29 32	380	5	90	Negative

Captions for separate Supplementary Tables

Supplementary Table S6: NAD-RNAs identified by NAD captureSeq.
Data is presented for replicate 1 (**A**) and replicate 2 (**B**). For each time point, it is indicated, whether the transcript of the corresponding gene was found enriched (+) or not (-). Entity represent the species, which is either *E. coli* (U00096.3), T4 phage (NC_000866.4) or the 100 nt control RNA (spike-in).

Supplementary Table S7: qPCR to confirm enrichment of NAD-RNAs on cDNA level as reported by NAD captureSeq.
For each analyzed time point and target gene/cDNA the Ct-value of technical duplicates is presented. The Log2 Fold Change (LFC) is calculated as the difference of Ct-values for –ADPRC and +ADPRC sample. Negative LFCs are marked in red, ct (no template) indicates background signal. Both *E. coli* and T4 phage targets have been validated.

Supplementary Table S8: Statistics of Transcription Start Site Prediction for *E. coli* (A) and T4 phage (B).

Supplementary Table S9: Burst size of T4 phage WT and NudE.1 M1 mutant as a mean of technical triplicates.

Supplementary Table S10: Top 100 hits of protein blast search of NudE.1 protein sequence (Y06L).

References

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5. Pozhydaieva, N., Billau, F.A., Wolfram-Schauerte, M., Rojas, A.A.R., Paczia, N., Schindler, D. and Höfer, K. (2024) Temporal epigenome modulation enables efficient bacteriophage engineering and functional analysis of phage DNA modifications. *bioRxiv*, 2024.2001.2028.577628.