
Supplementary information

A viral ADP-ribosyltransferase attaches RNA chains to host proteins

In the format provided by the
authors and unedited

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SUPPLEMENTARY INFORMATION

A viral ADP-ribosyltransferase attaches RNA chains to host proteins

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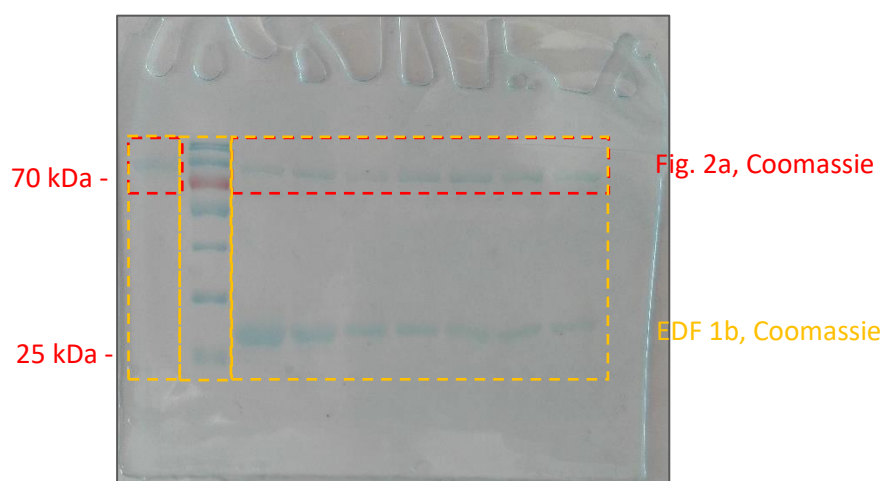
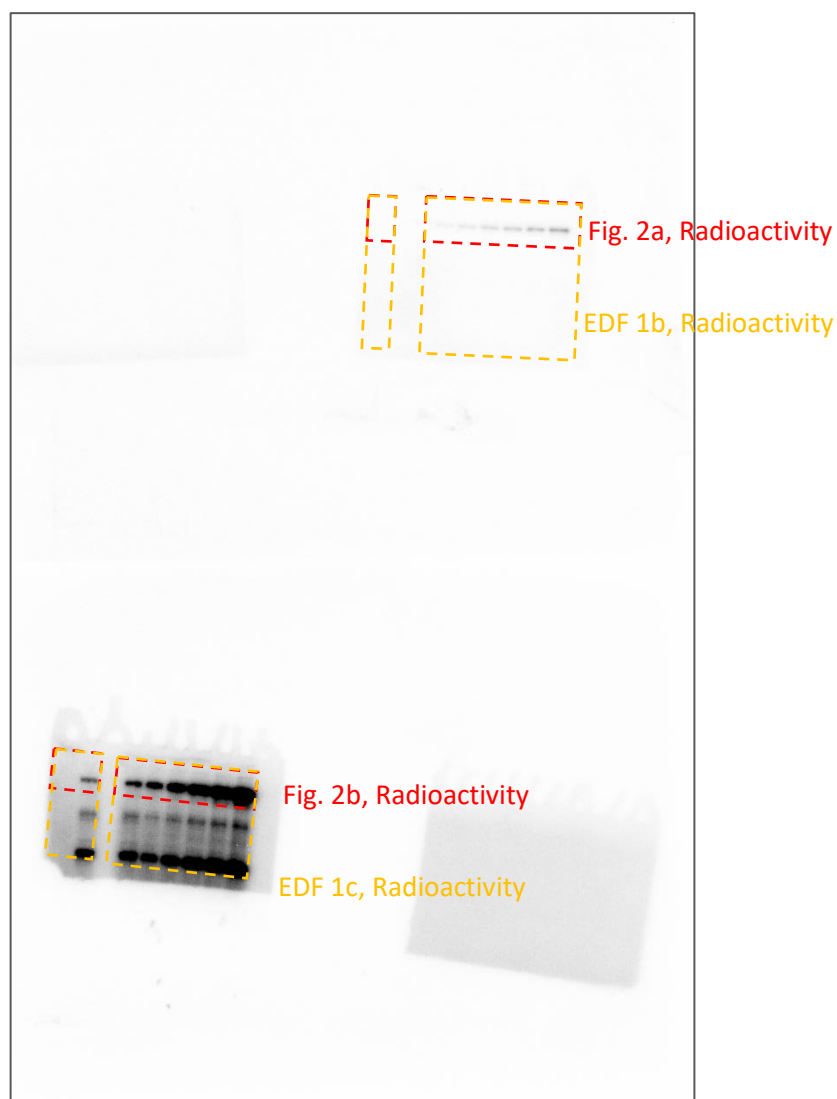
+ These authors contributed equally to this work.

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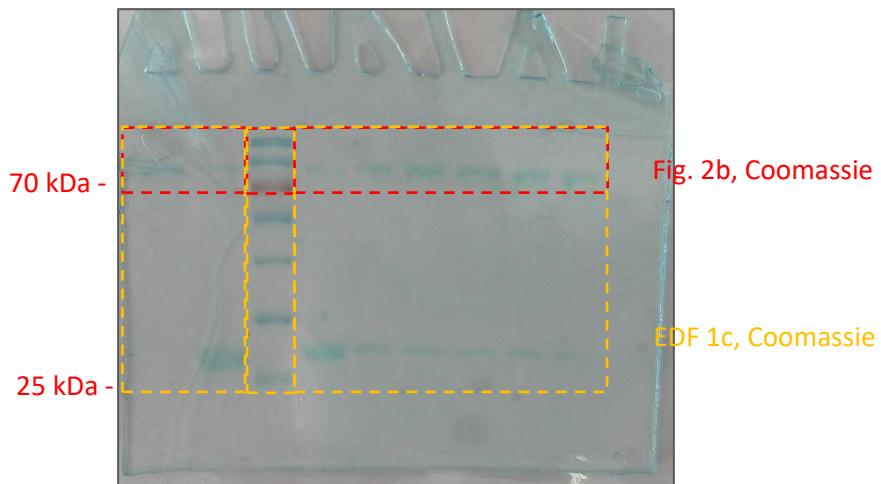
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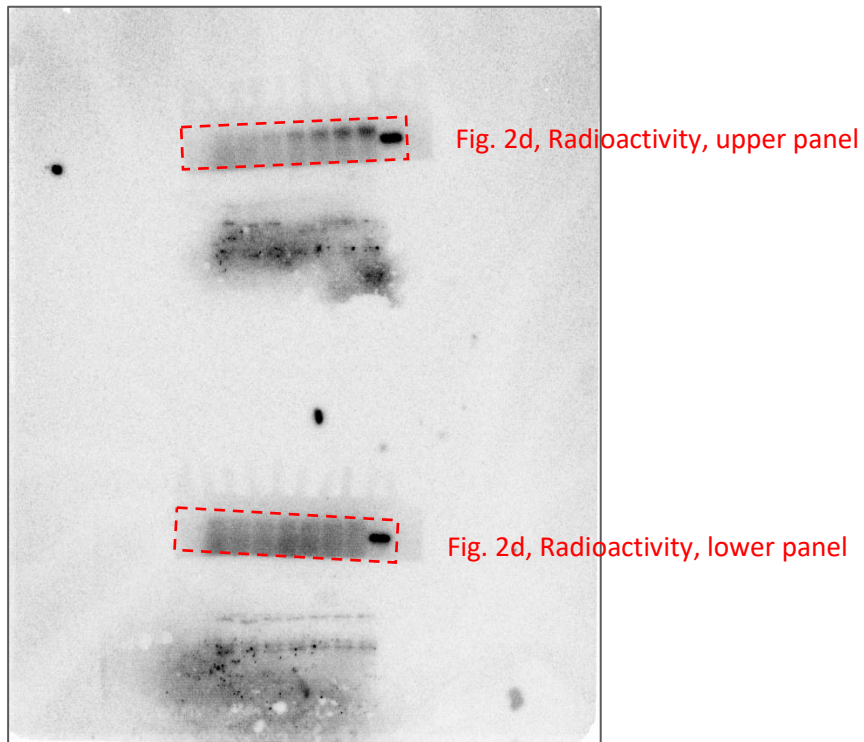
Supplementary Figure 1: Raw gel and blot images used for figures in this study.

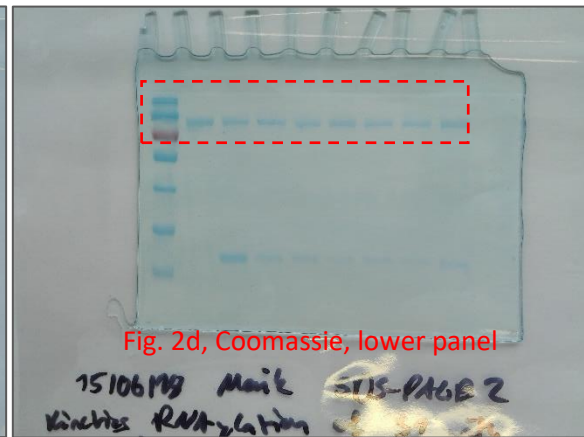
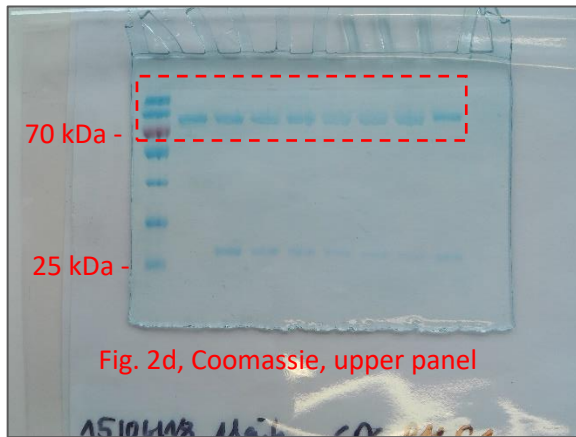


Used in Fig. 2a,b (red) and Extended Data Fig. 1b,c (yellow)

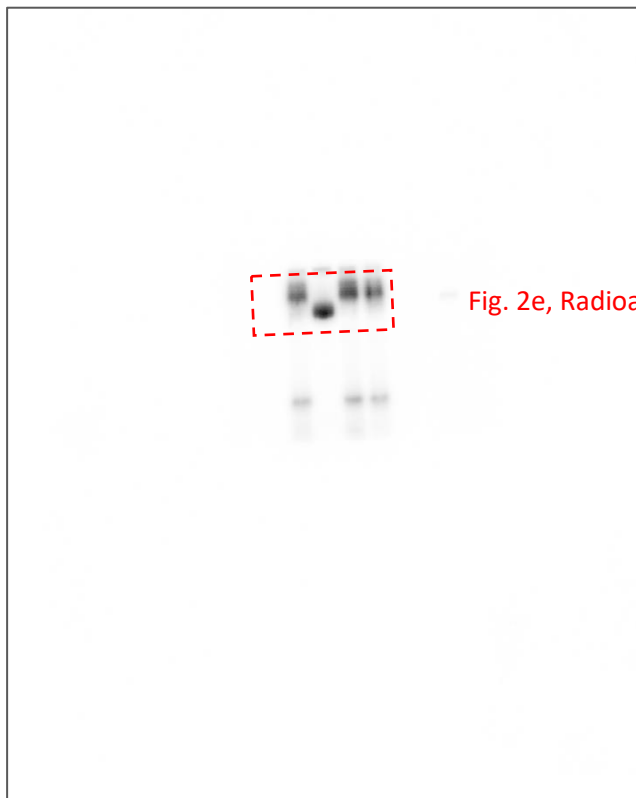


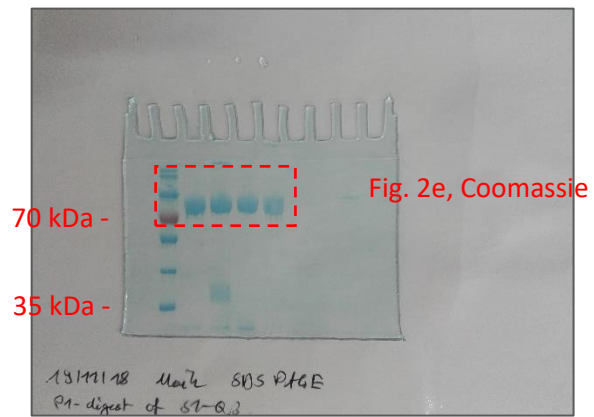
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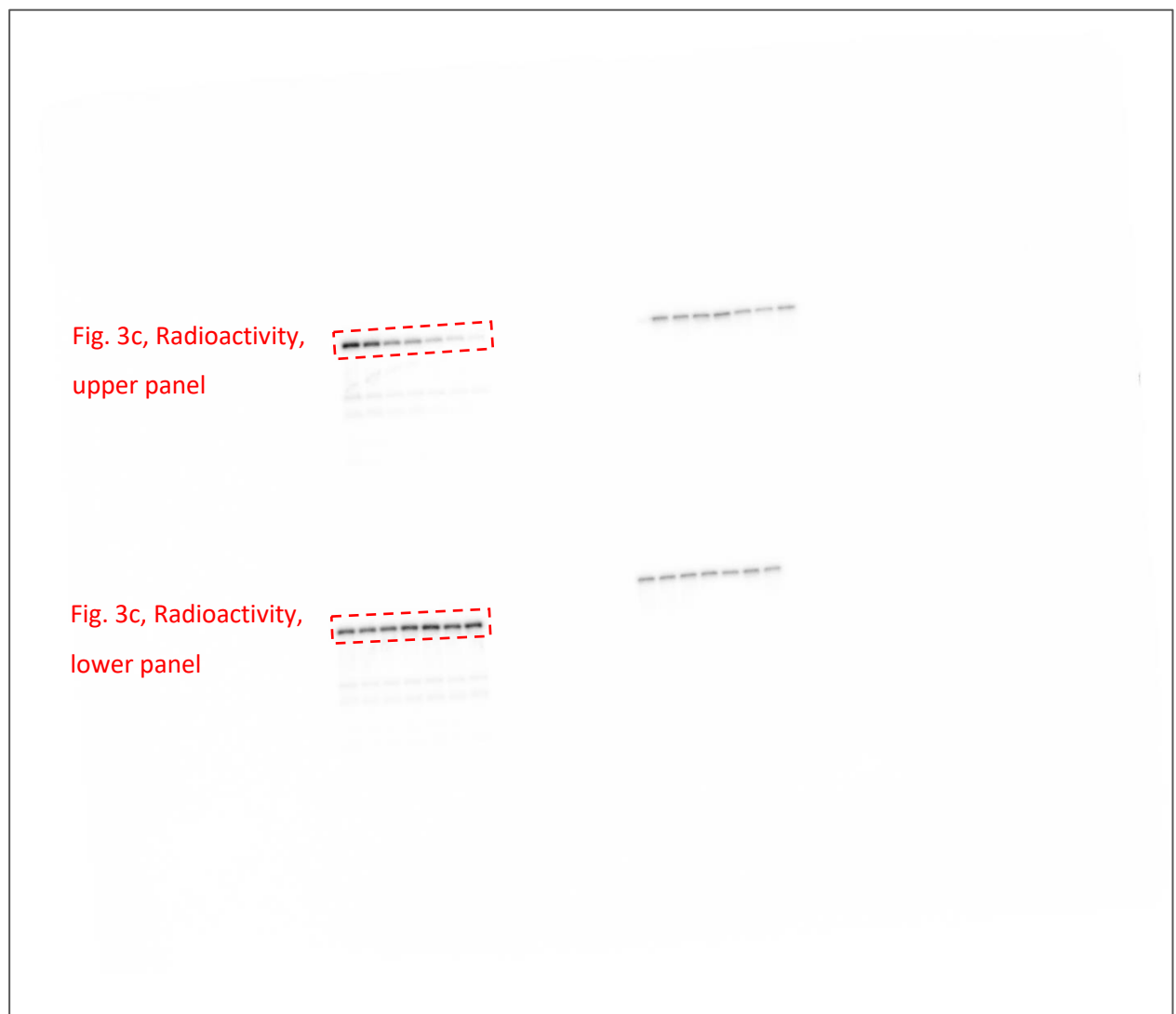


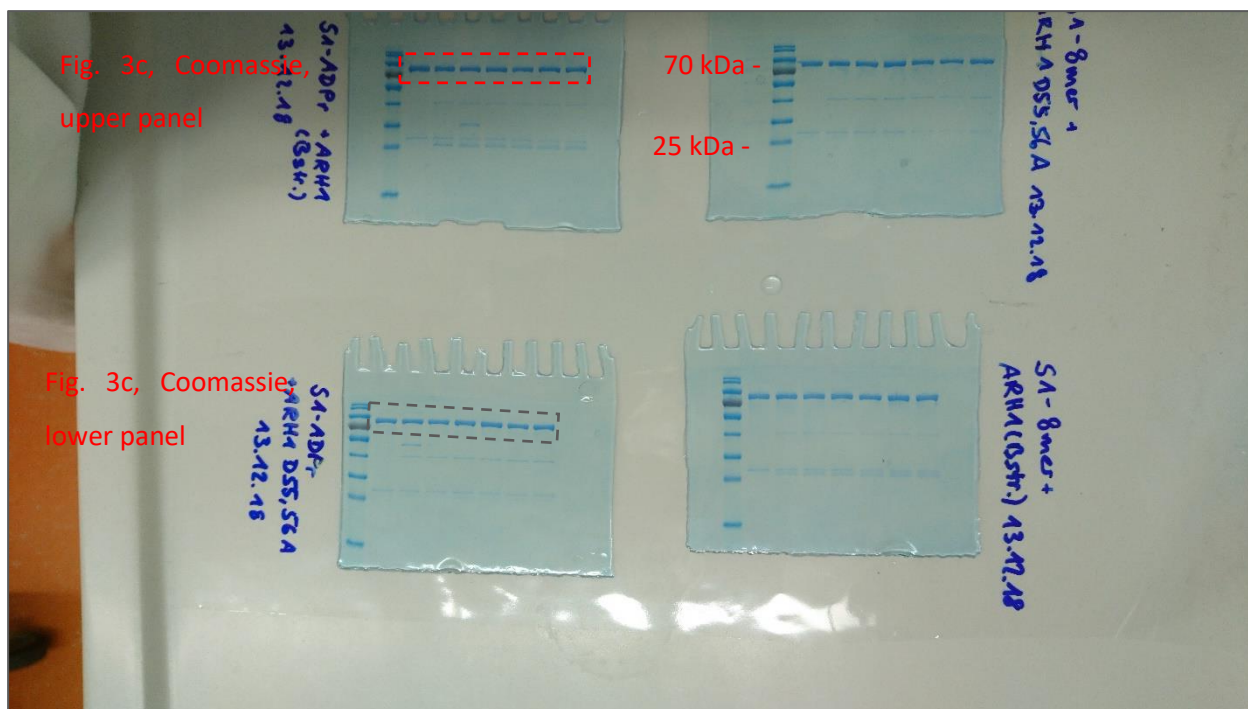
Used in Fig. 2d





Used in Fig. 2e, right panel





Used in Fig. 3c

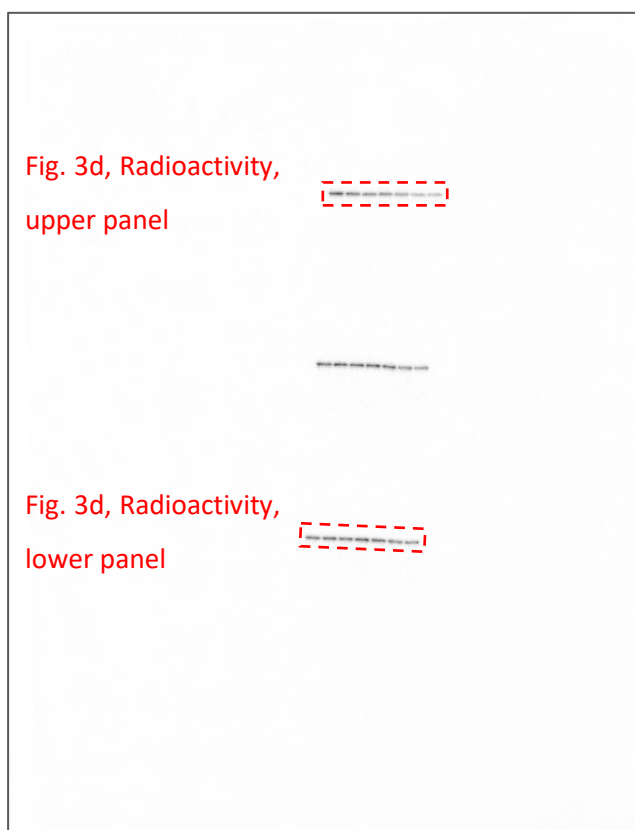


Fig. 3d, Coomassie,
upper panel

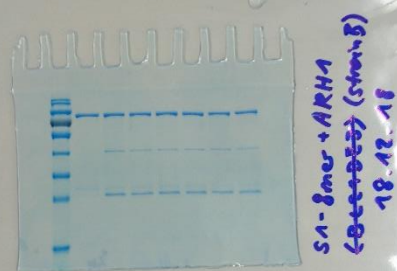
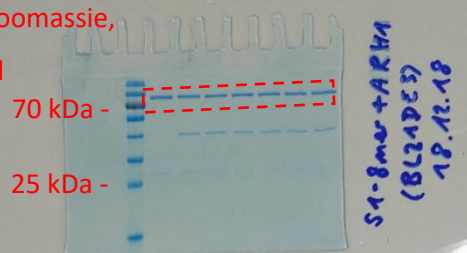
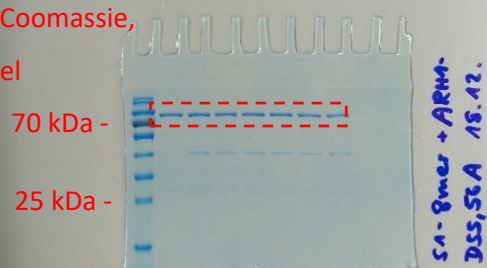
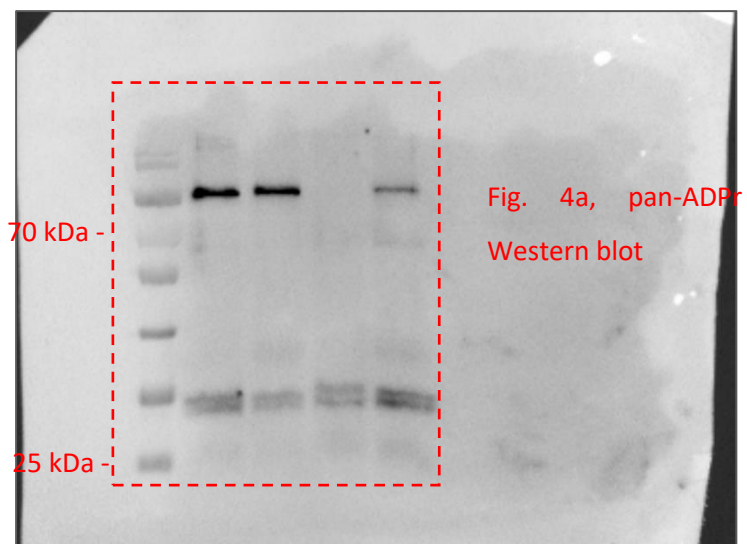


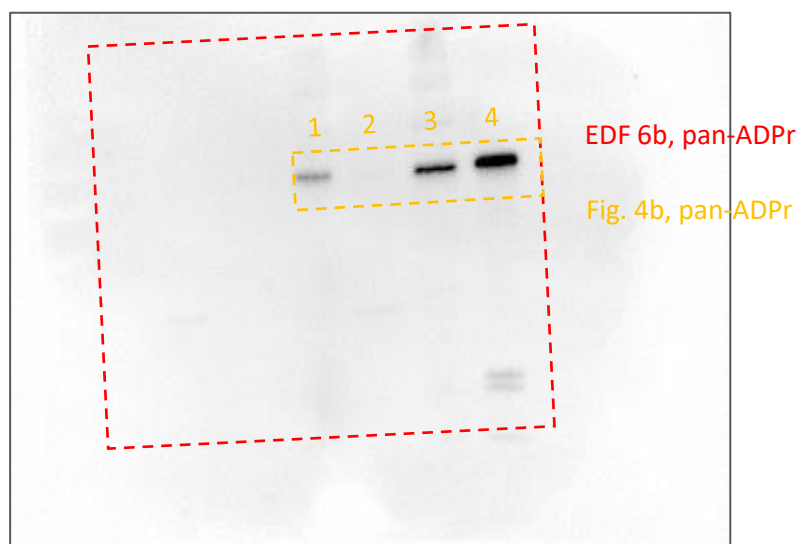
Fig. 3d, Coomassie,
lower panel



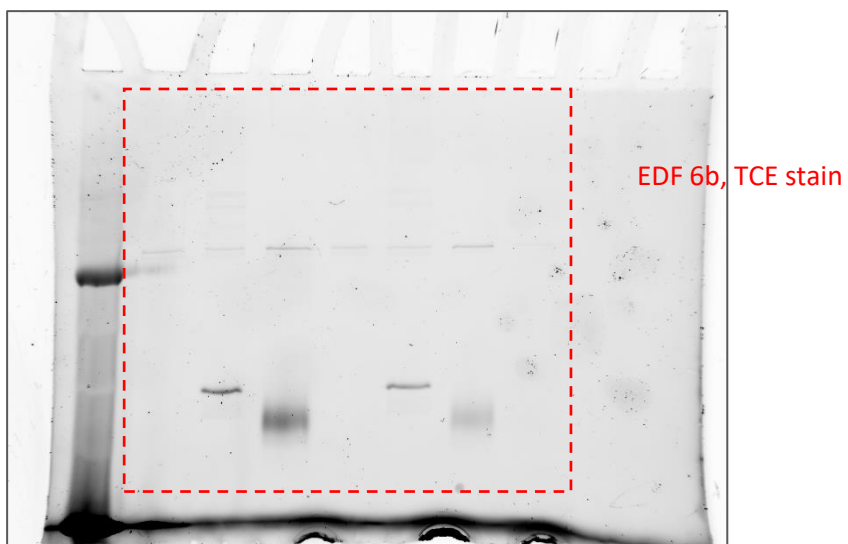
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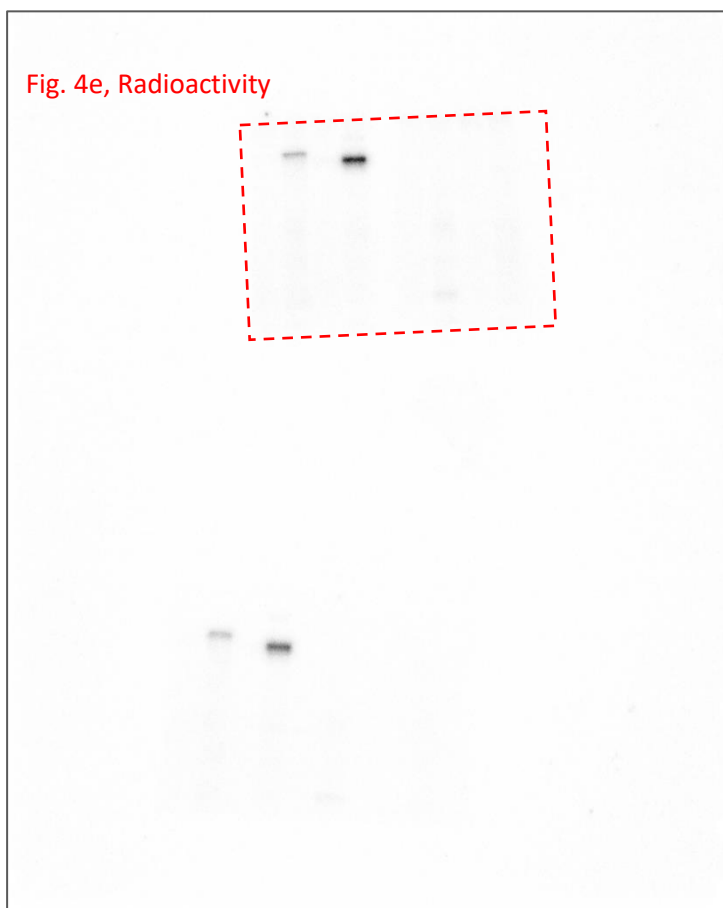
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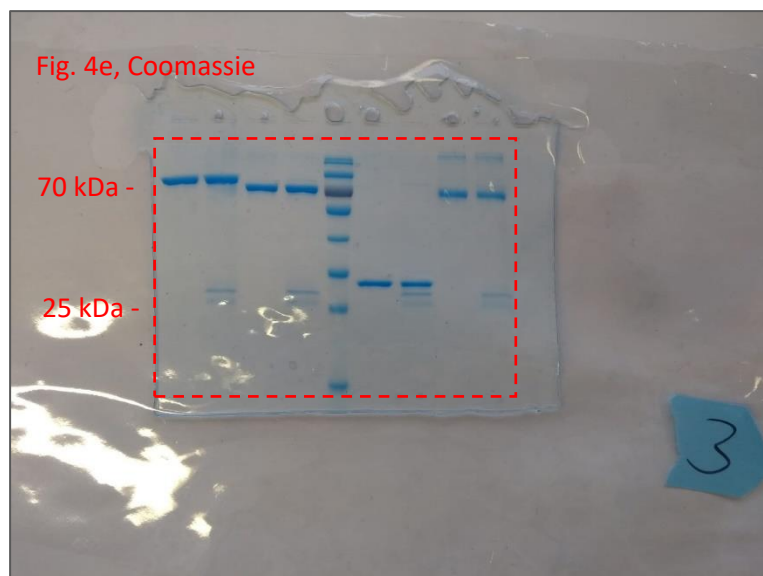
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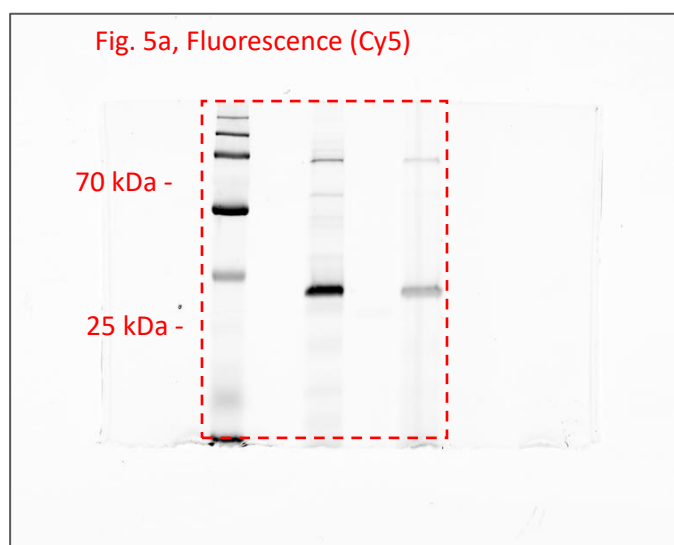
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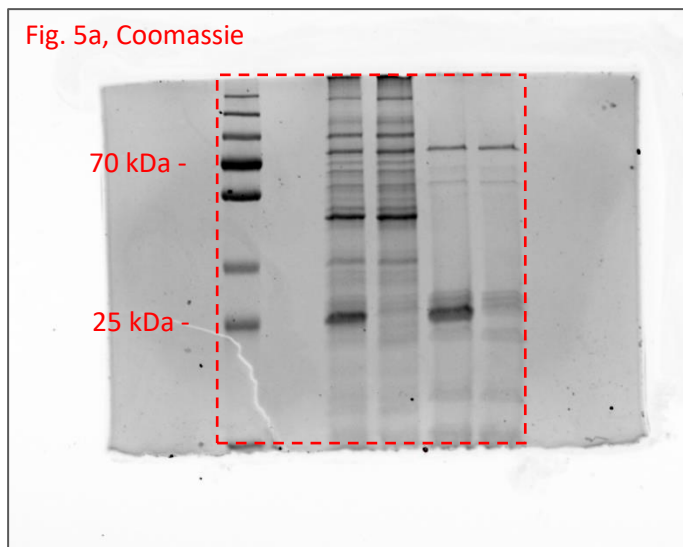


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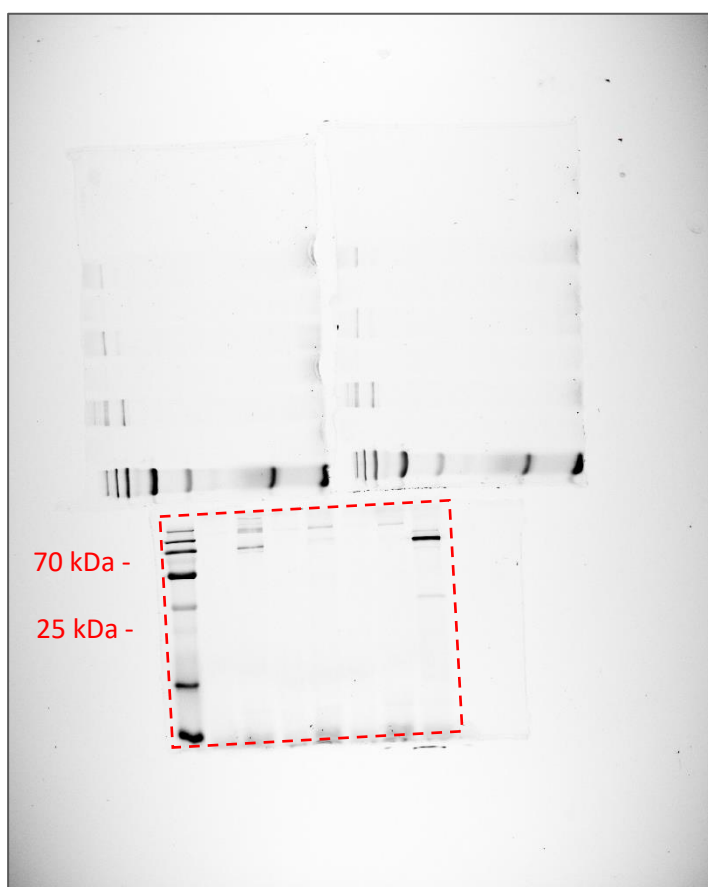


Used in Fig. 5a

Fig. 5a, Coomassie

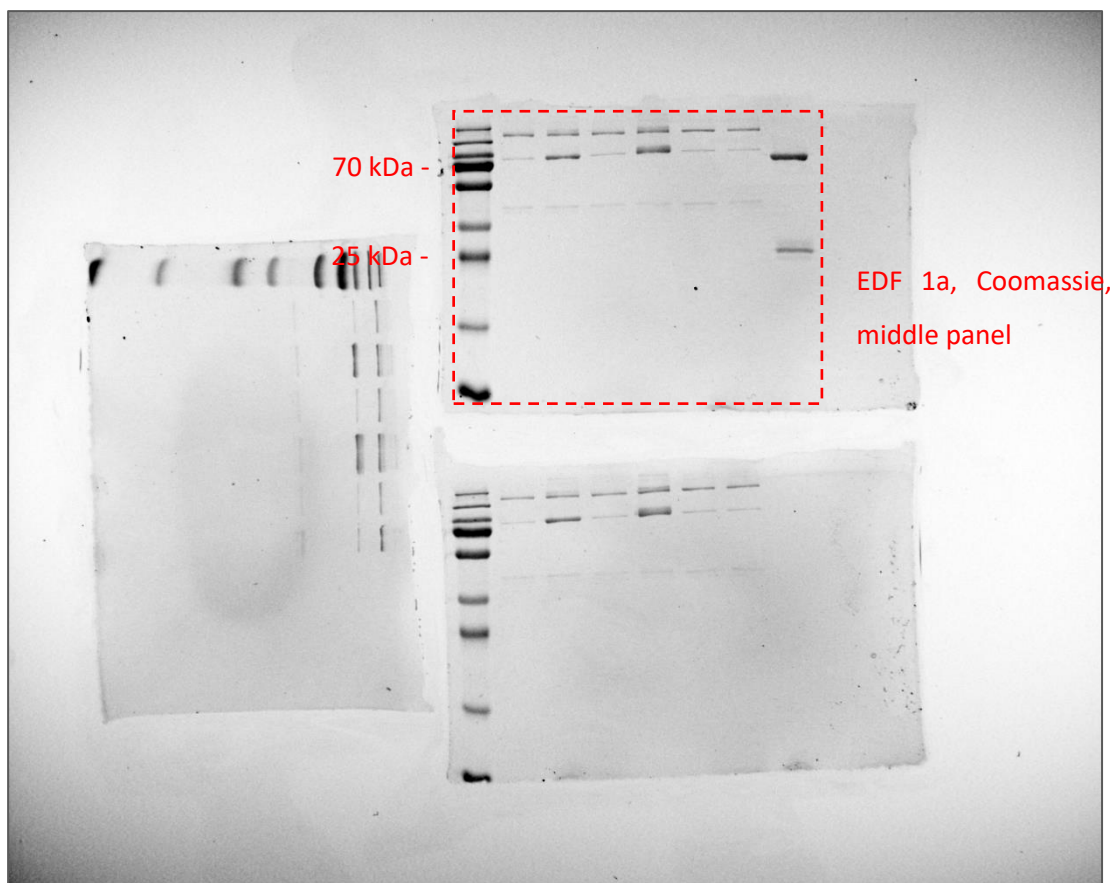


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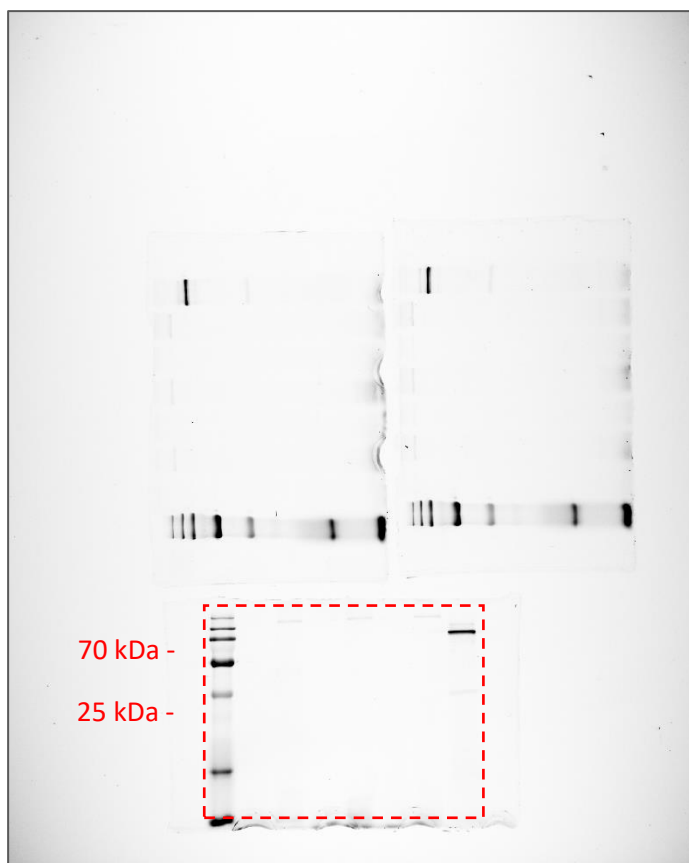


EDF 1a, Fluorescence (Cy5),
middle panel

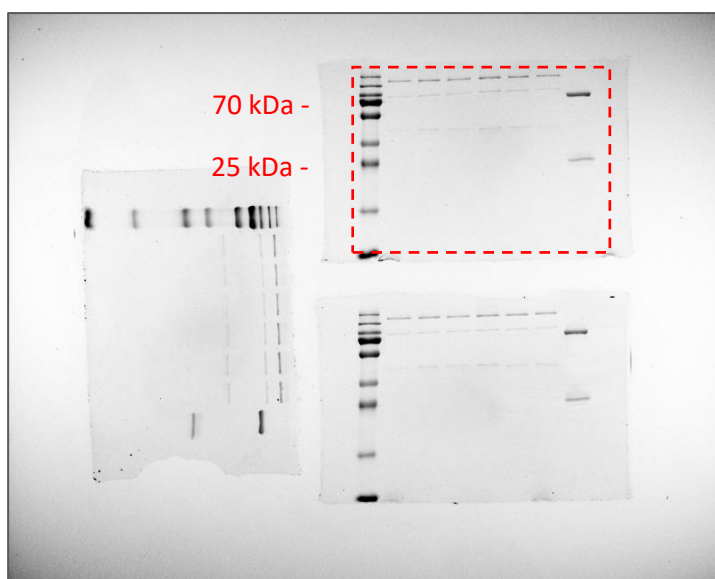
Used in Extended Data Fig. 1a, middle panel



Used in Extended Data Fig. 1a, middle panel

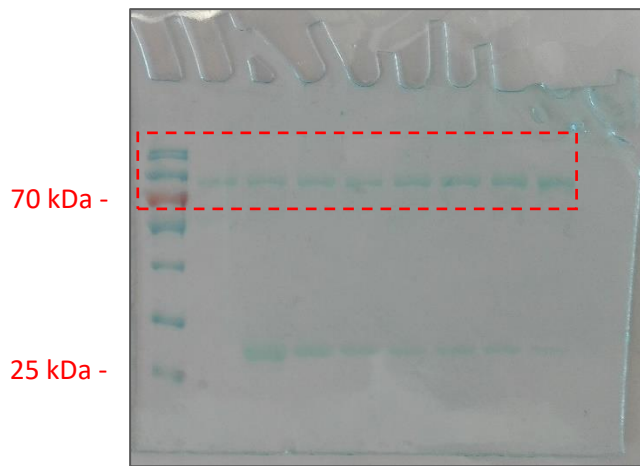


EDF 1a, Fluorescence (Cy5),
lower panel

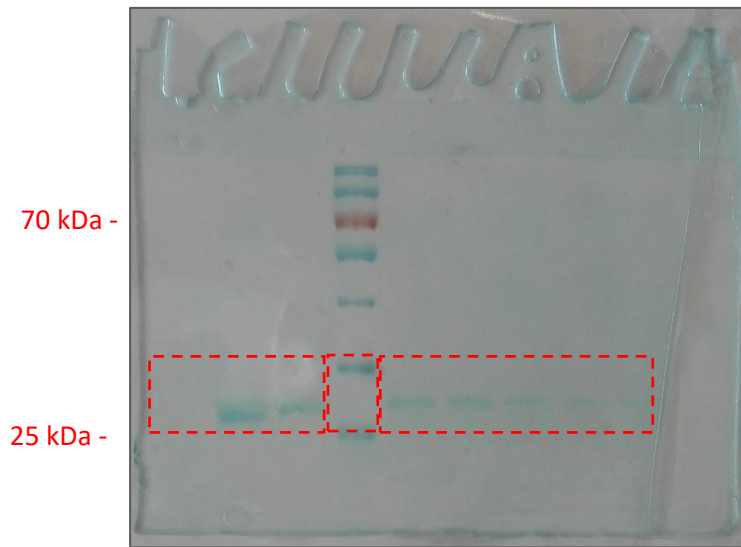


EDF 1a, Coomassie,
lower panel

Used in Extended Data Fig. 1a, lower panel

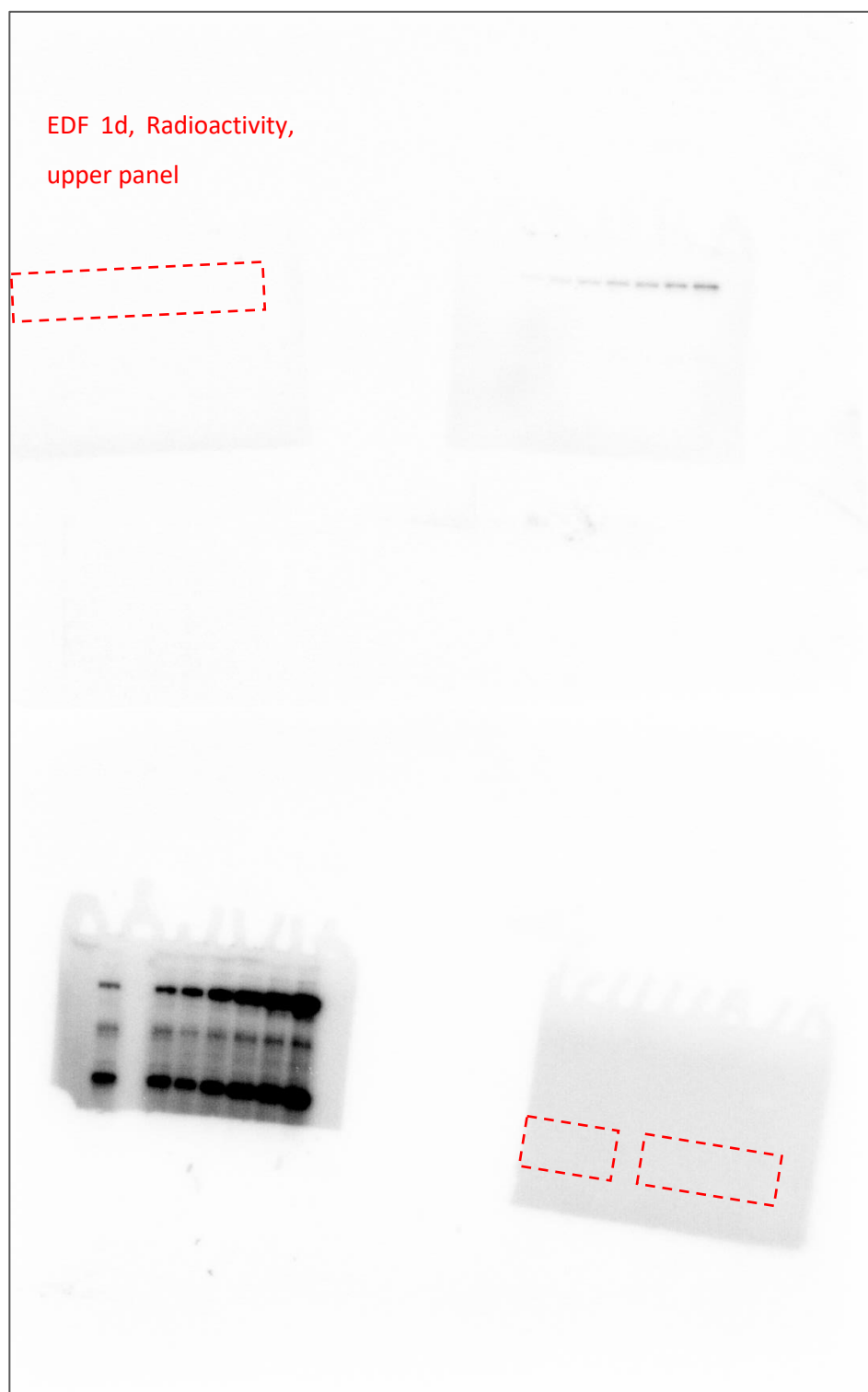


EDF 1d, Coomassie,
upper panel

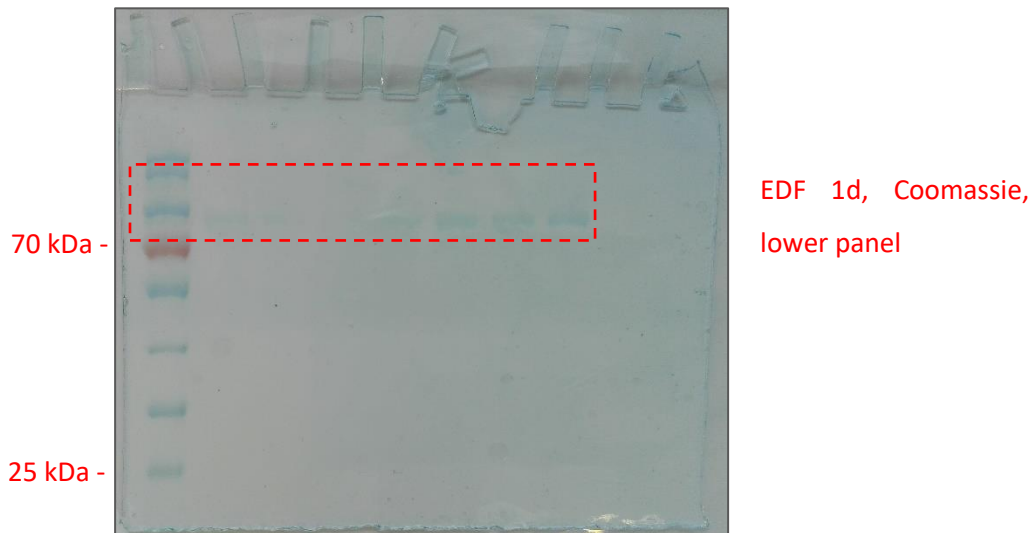
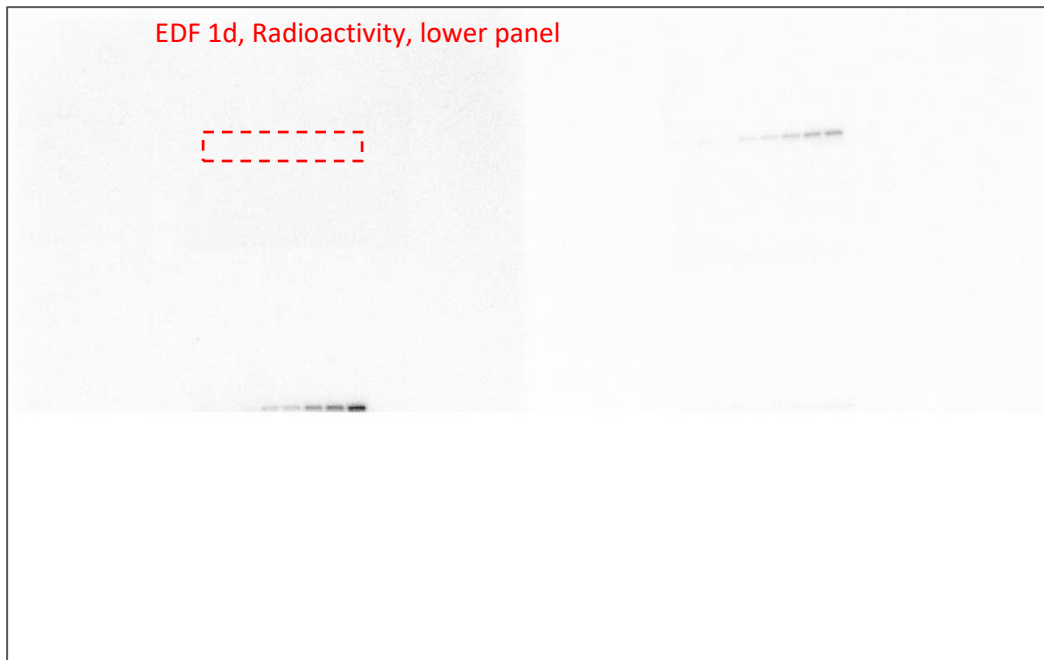


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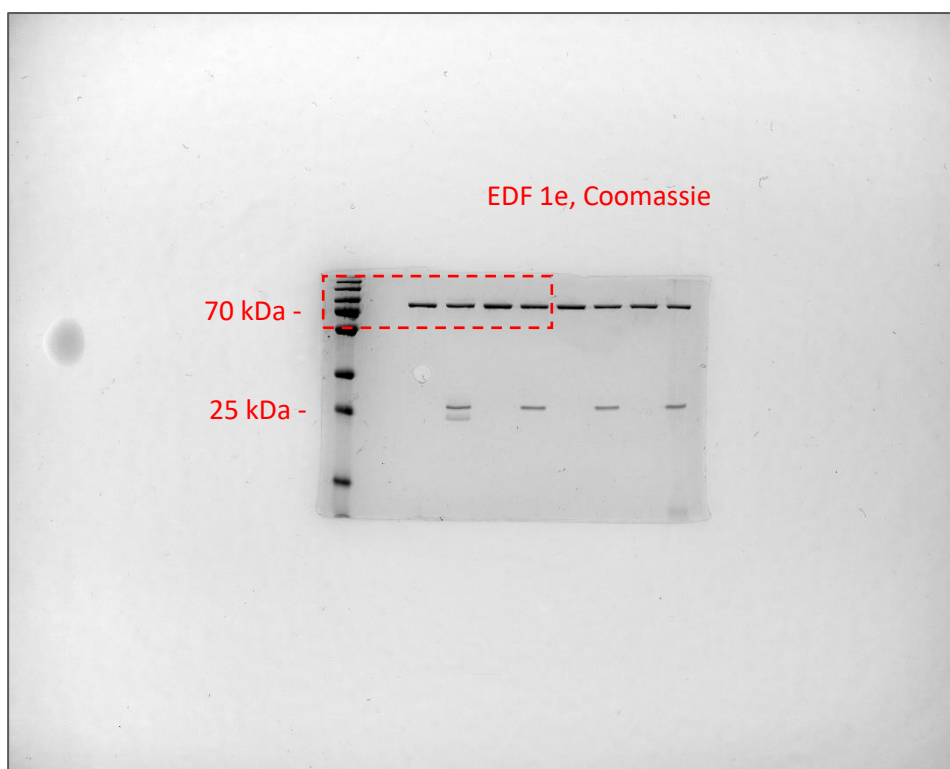
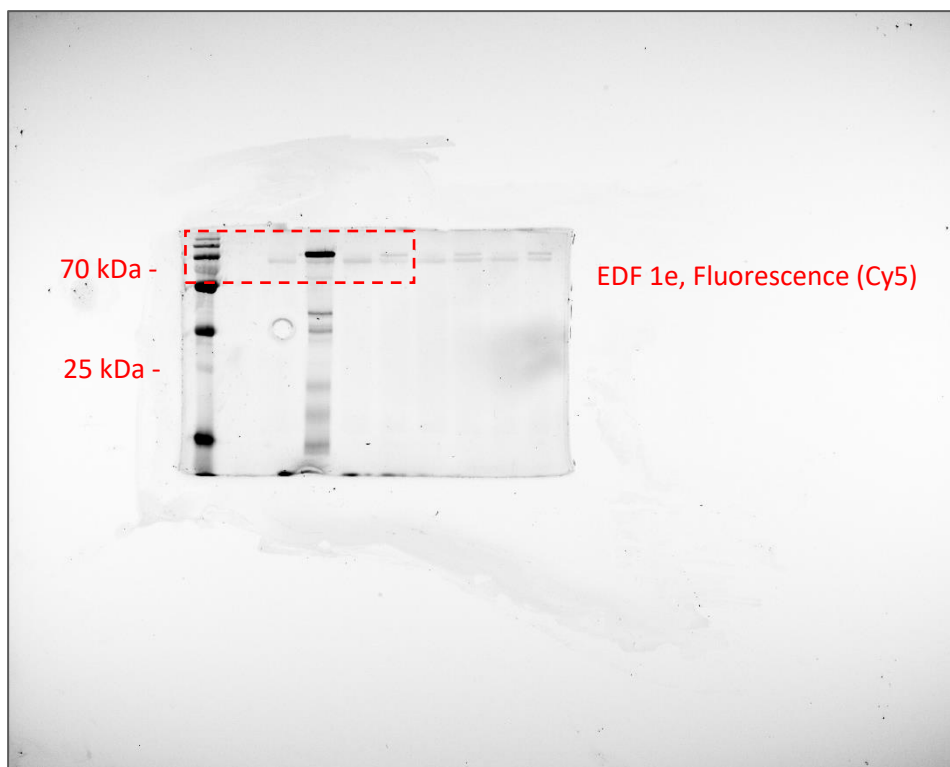
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Used in Extended Data Fig. 1d, upper and middle panel

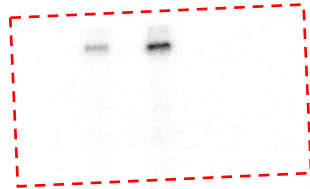


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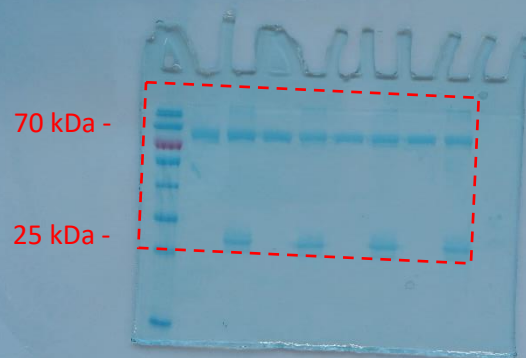


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EDF 2a, Radioactivity



EDF 2a, Coomassie

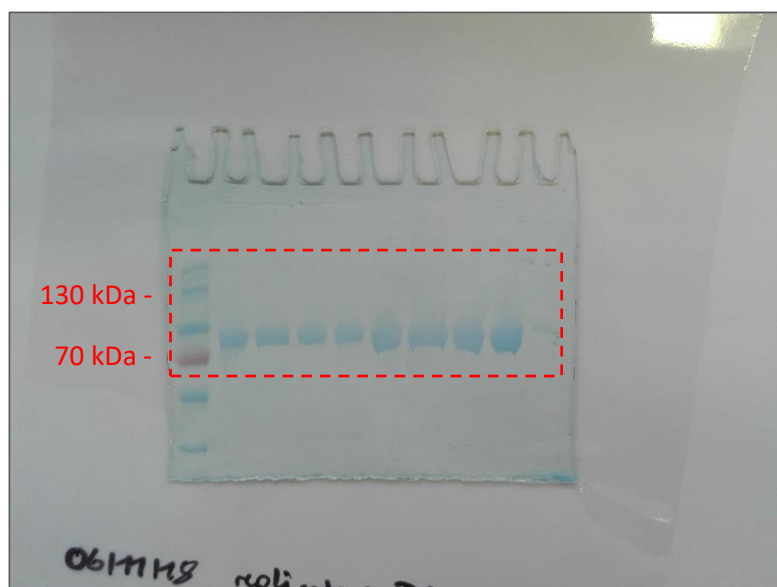


OSMMS with SDS-PAGE
Inhibition of R/R

Used in Extended Data Fig. 2a

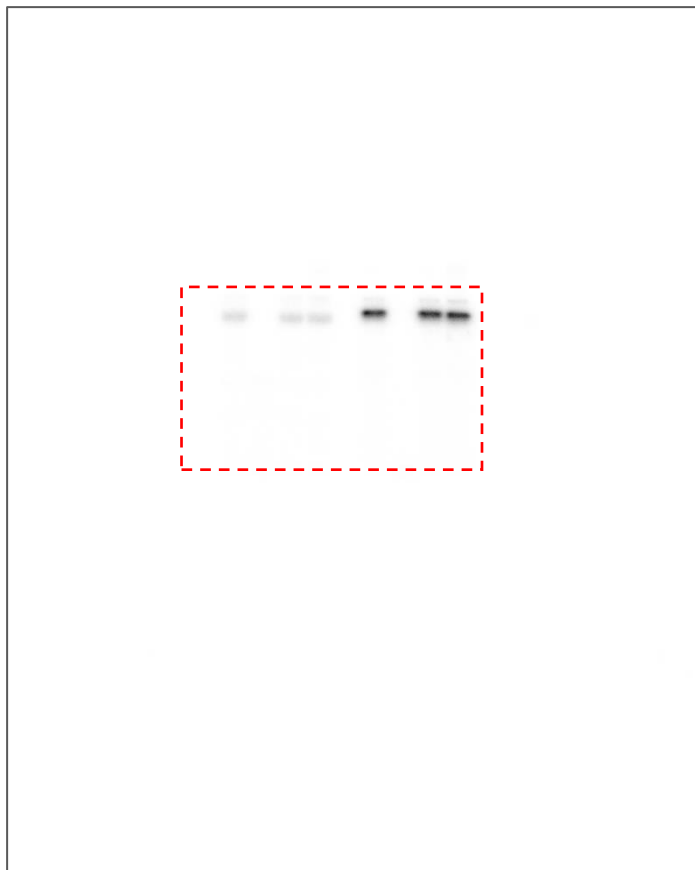


EDF 2b, Radioactivity

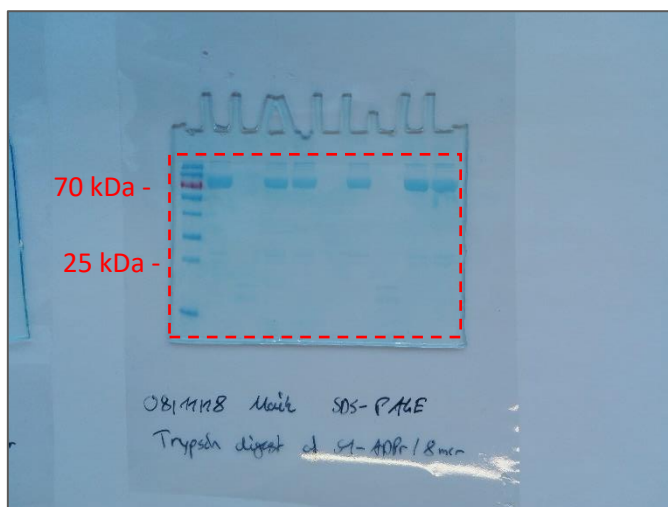


EDF 2b, Coomassie

Used in Extended Data Fig. 2b

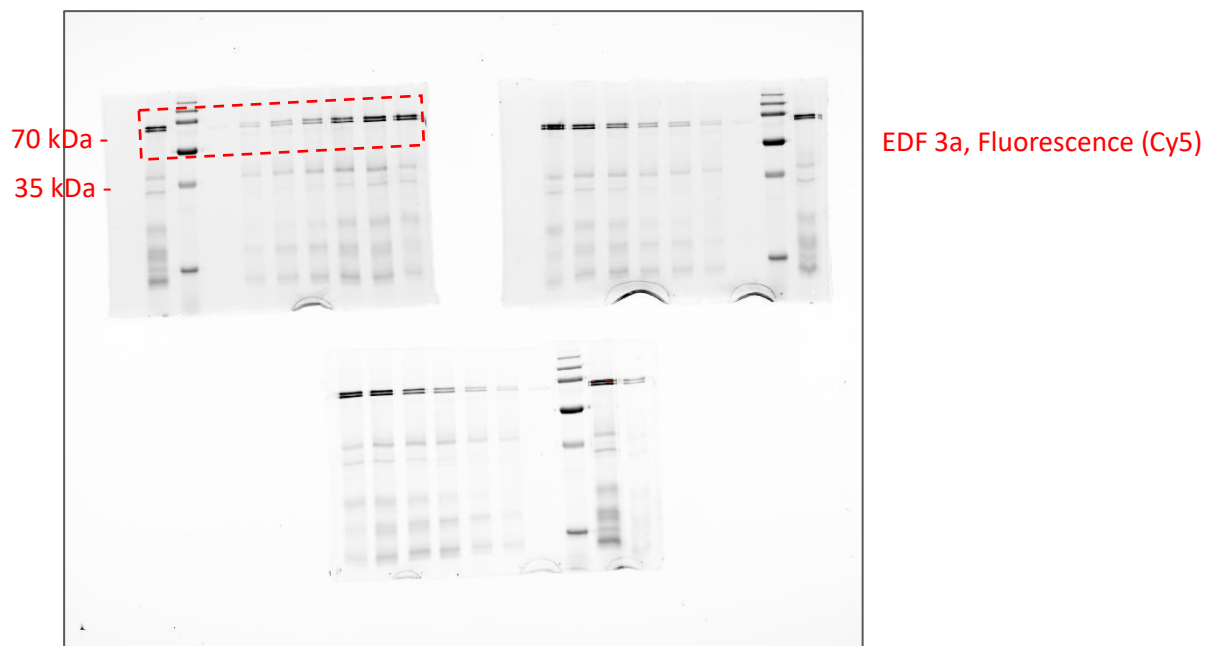


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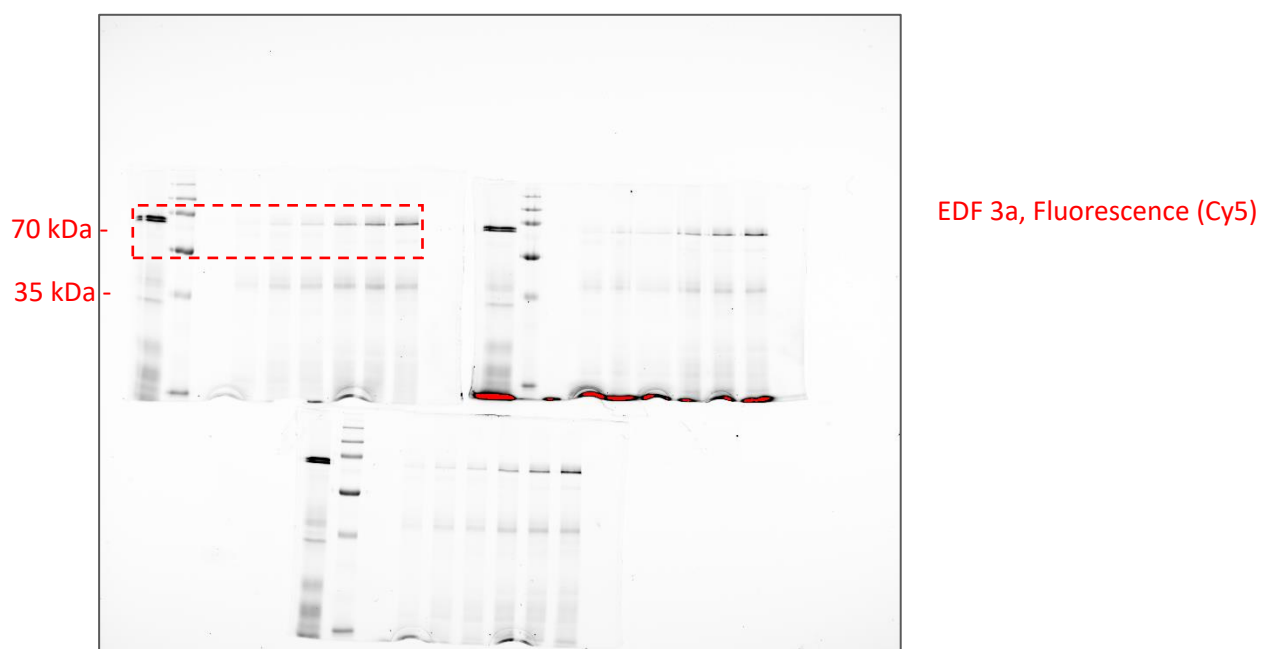


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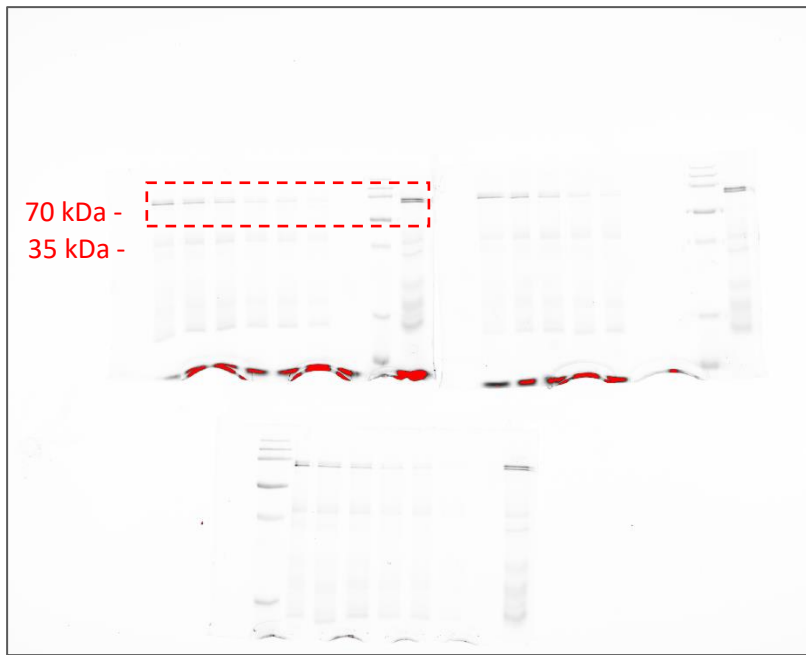
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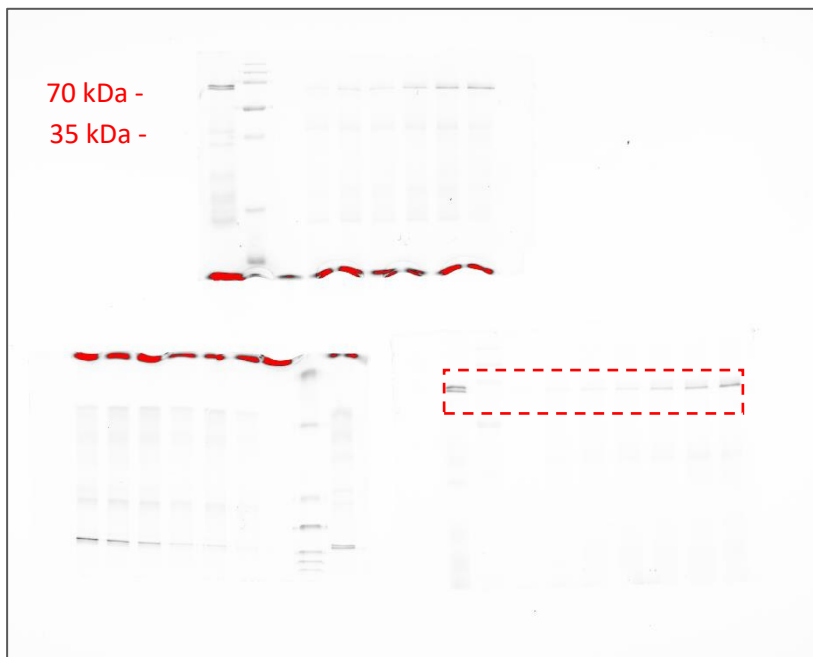
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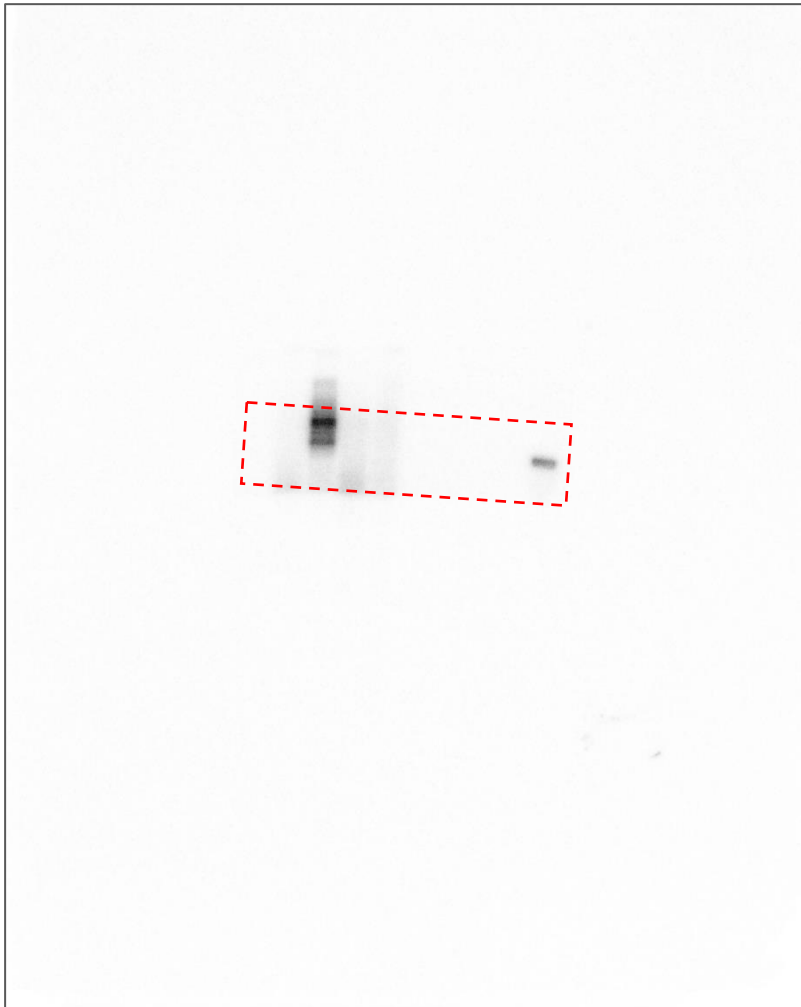
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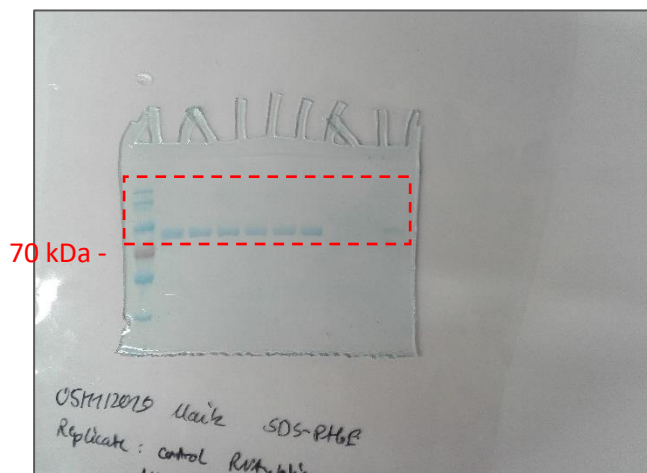
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Used in Extended Data Fig. 3a, lower right panel

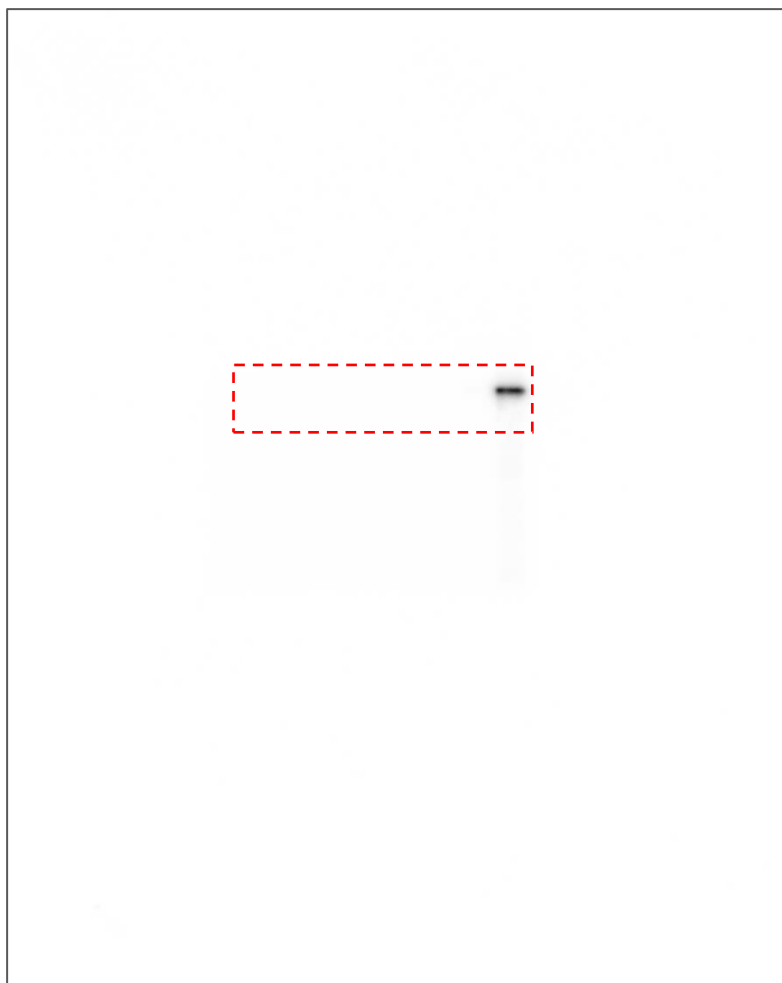


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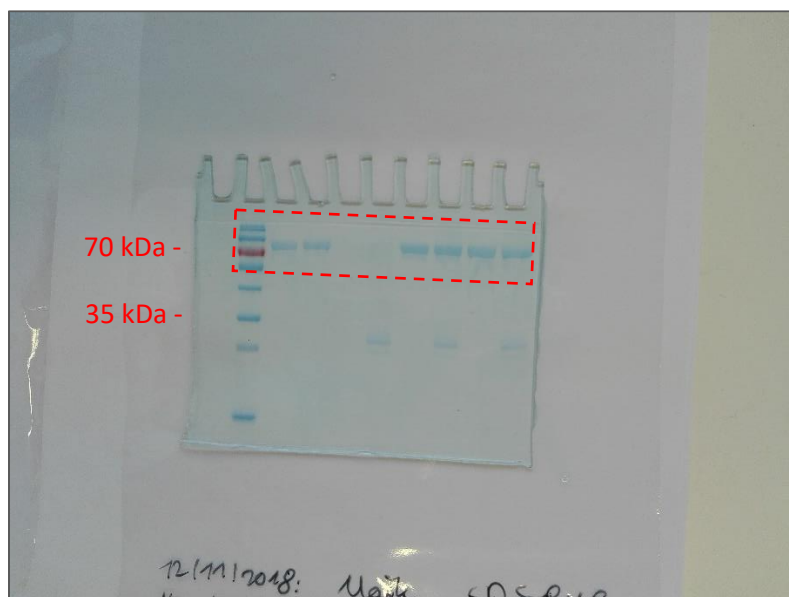


EDF 3b, Coomassie

Used in Extended Data Fig. 3b

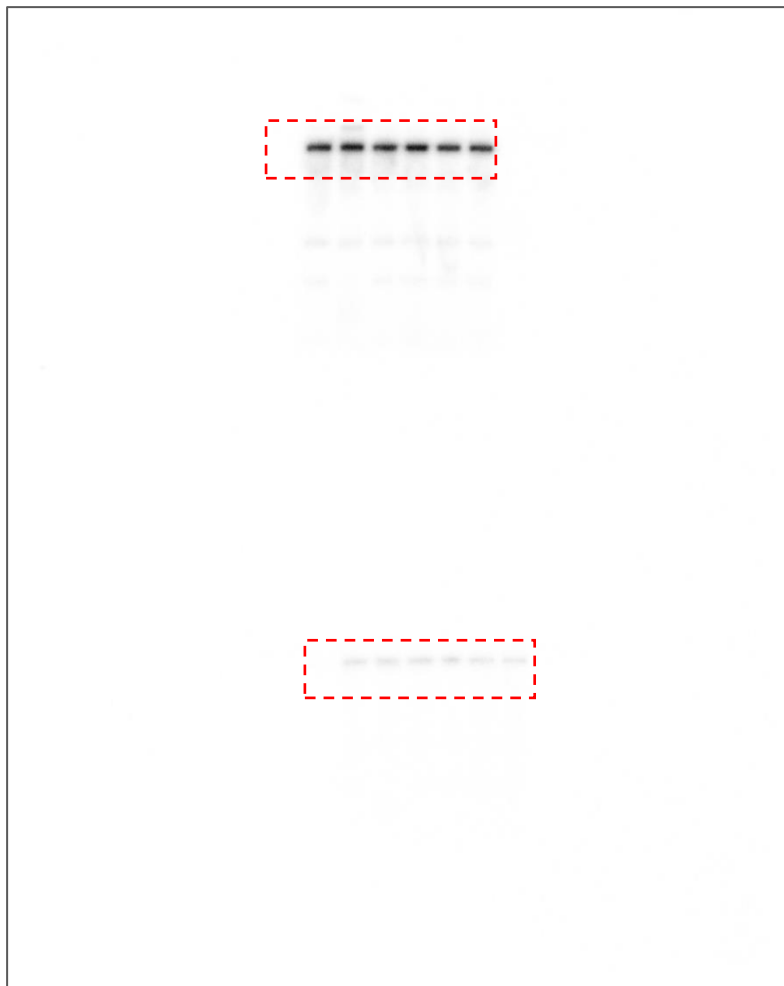


EDF 3c, Radioactivity



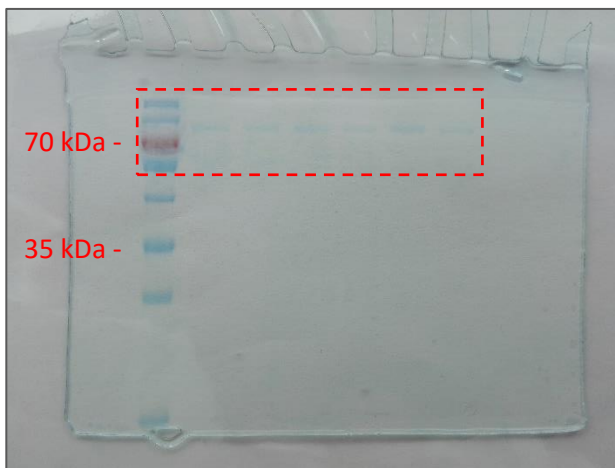
EDF 3c, Coomassie

Used in Extended Data Fig. 3c



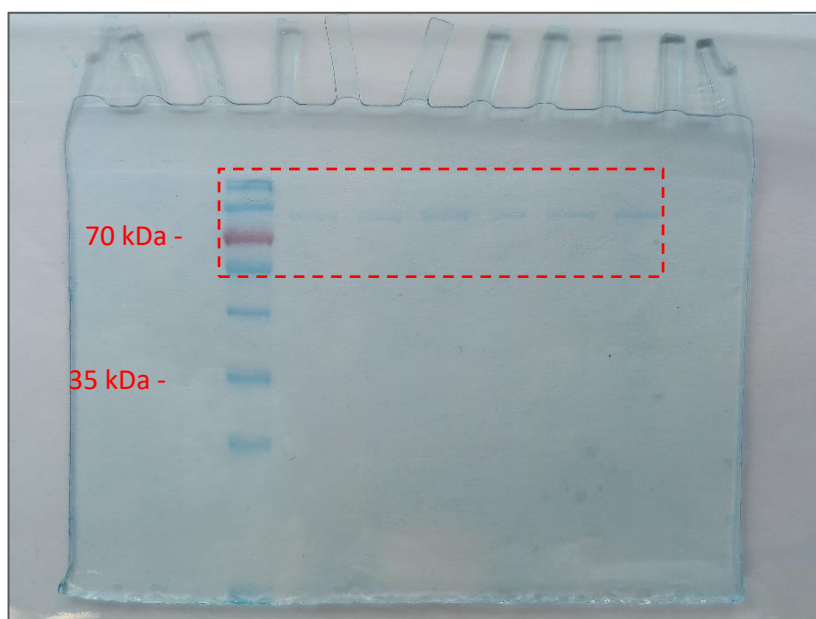
EDF 4a, Radioactivity,
ADP-ribosylated rS1

EDF 4a, Radioactivity,
RNAylated rS1



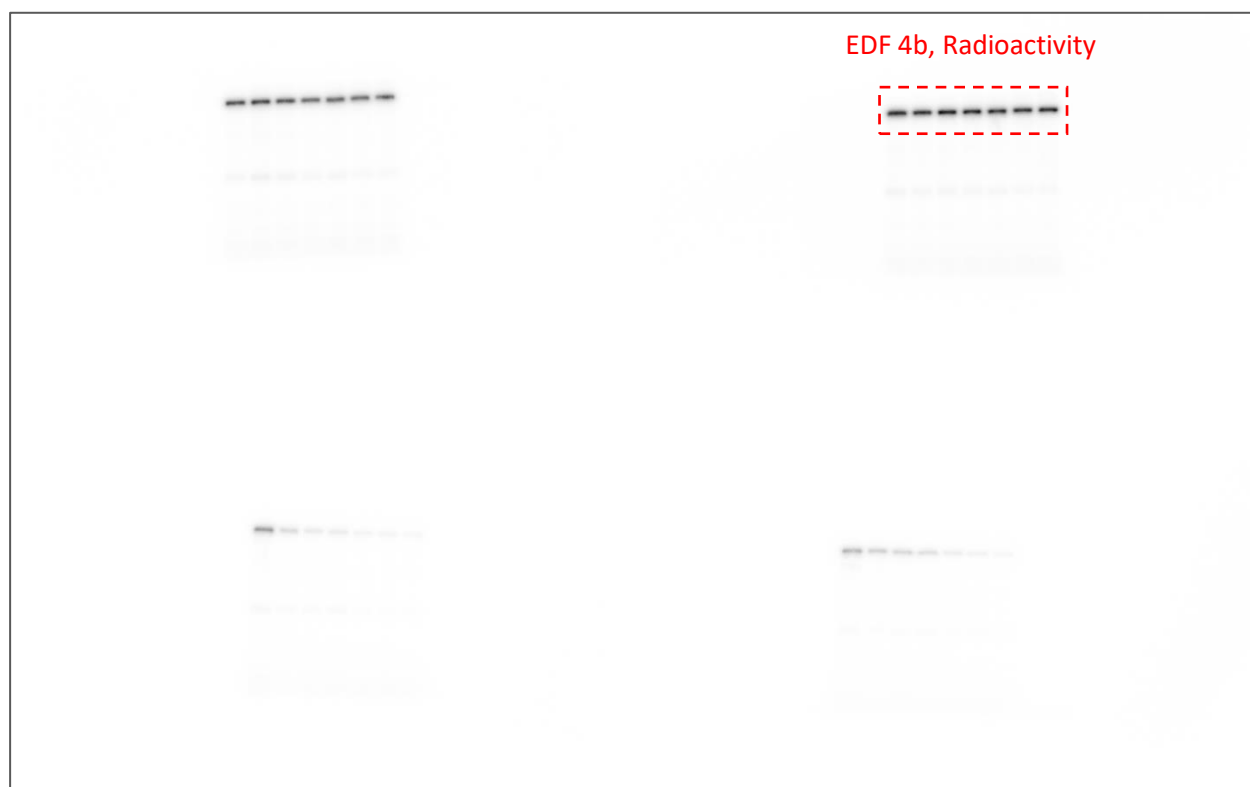
EDF 4a, Coomassie,
ADP-ribosylated rS1

Used in Extended Data Fig. 4a

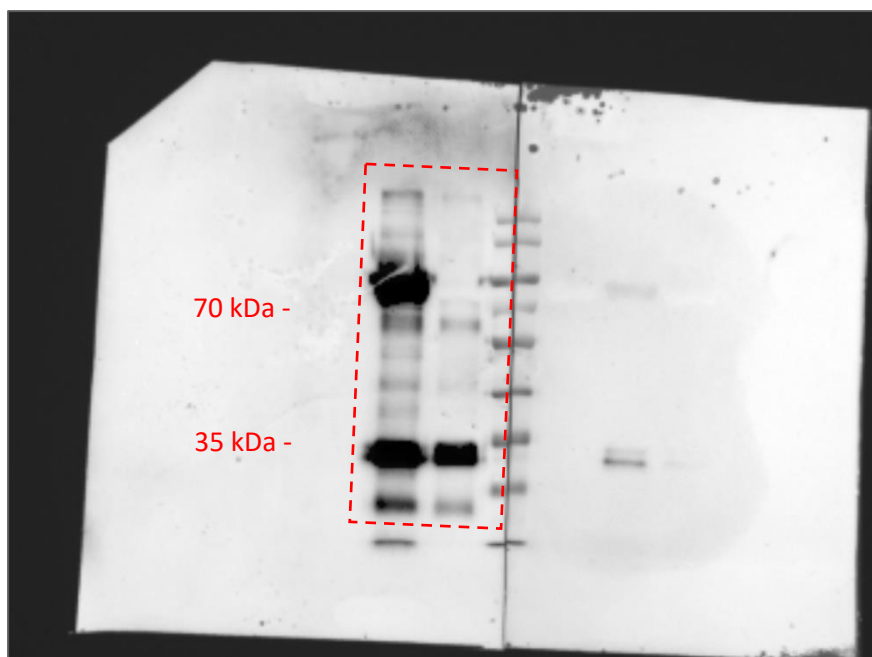


EDF 4a, Coomassie,
RNAylated rS1

Used in Extended Data Fig. 4a

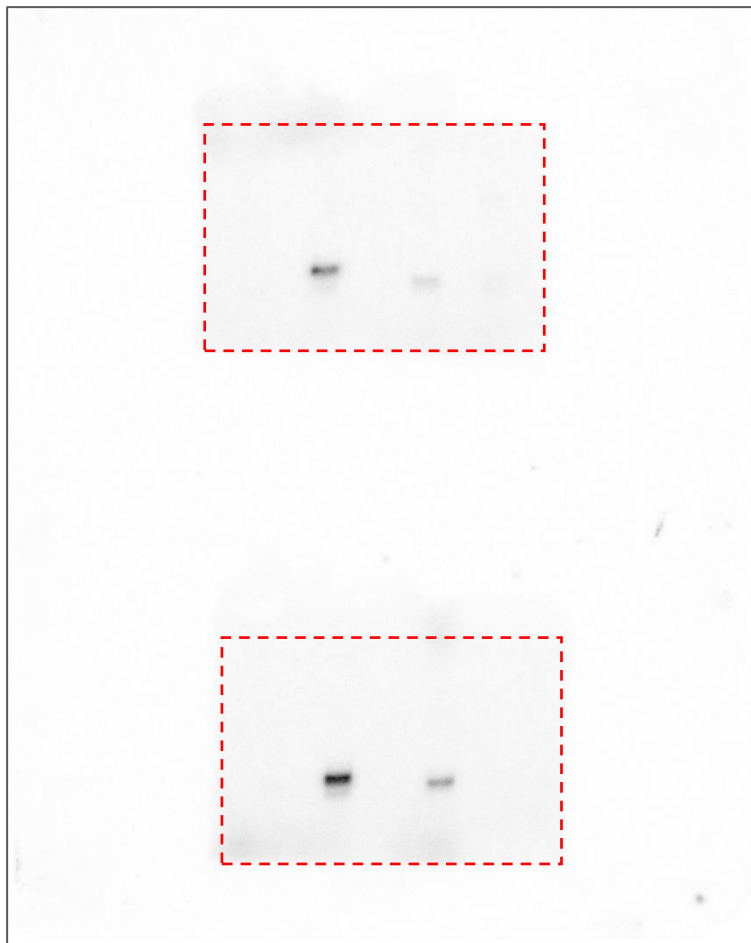


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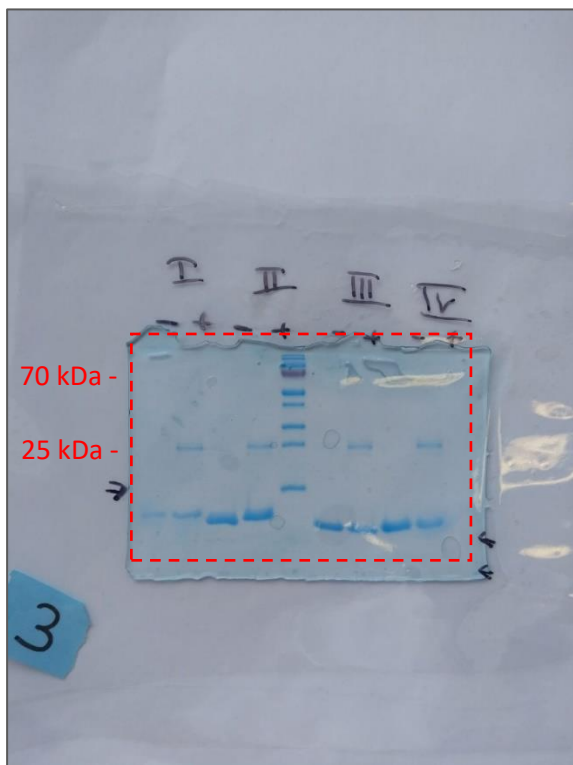
EDF 6a, pan-ADPr merge
with Colorimetric scan

Used in Extended Data Fig. 6a



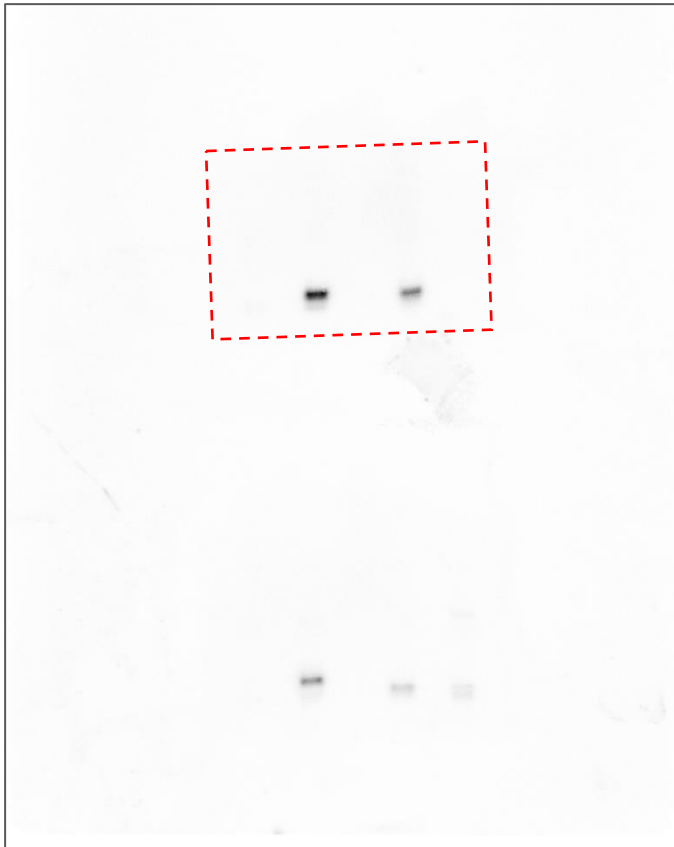
EDF 7b, Radioactivity,
upper panel

EDF 7b, Radioactivity,
lower panel

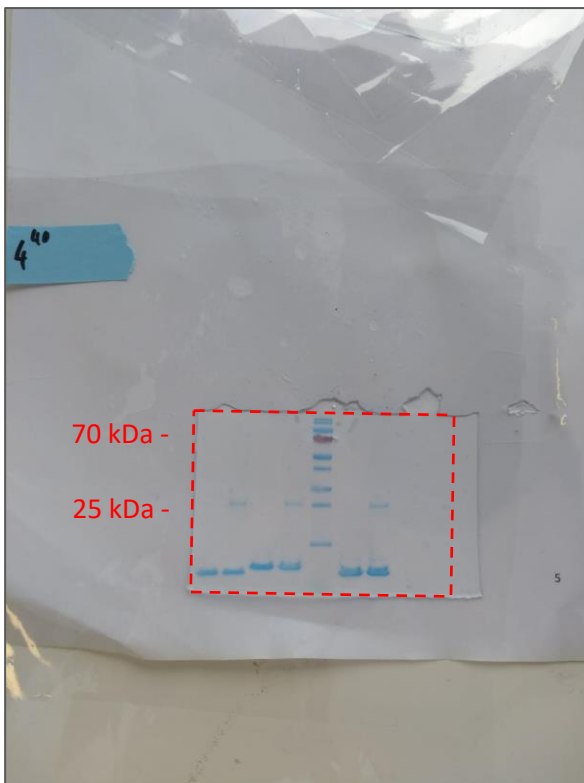


EDF 7b, Coomassie,
upper panel

Used in Extended Data Fig. 7b



EDF 7b, Radioactivity,
lower panel

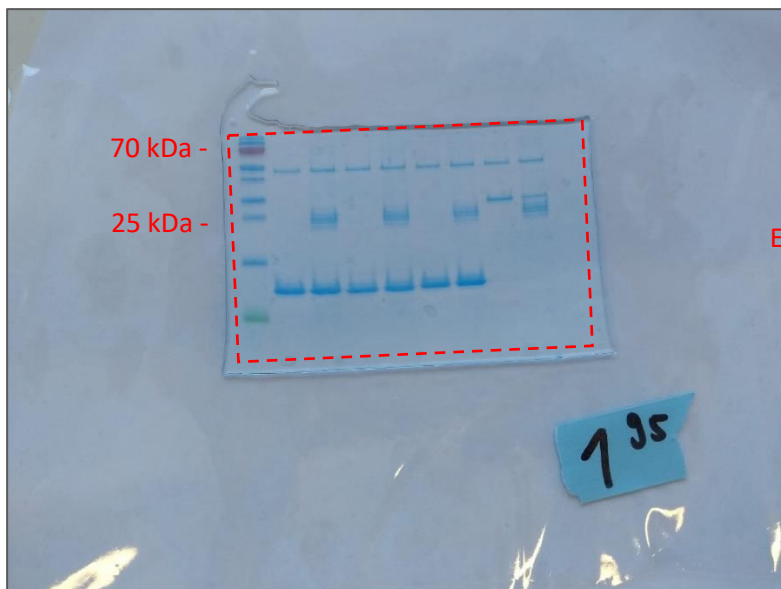


EDF 7b, Coomassie,
lower panel

Used in Extended Data Fig. 7b

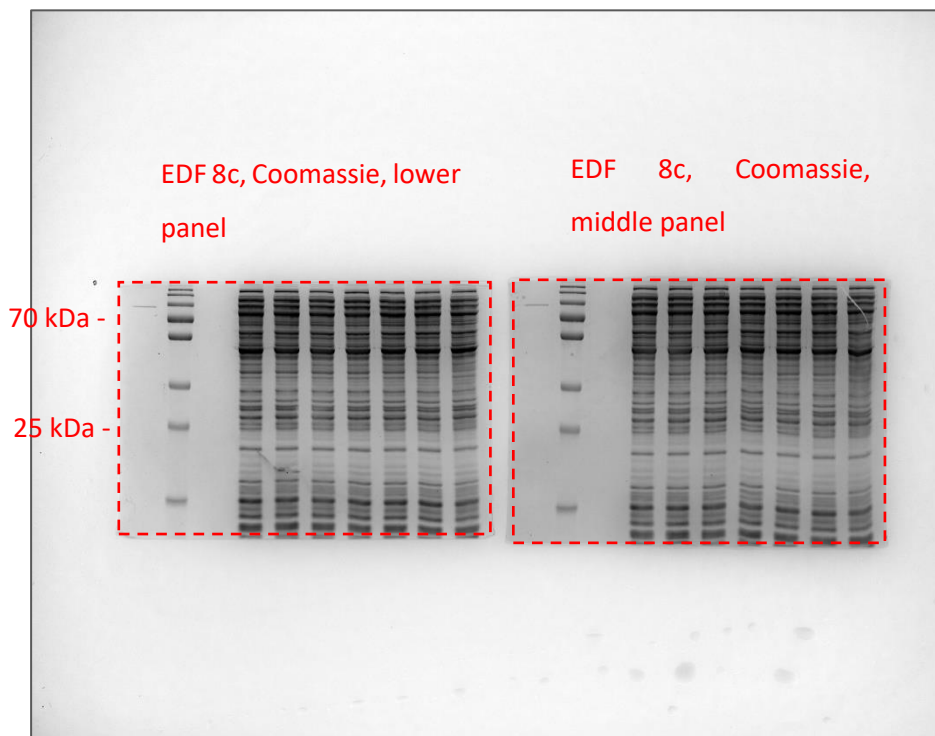
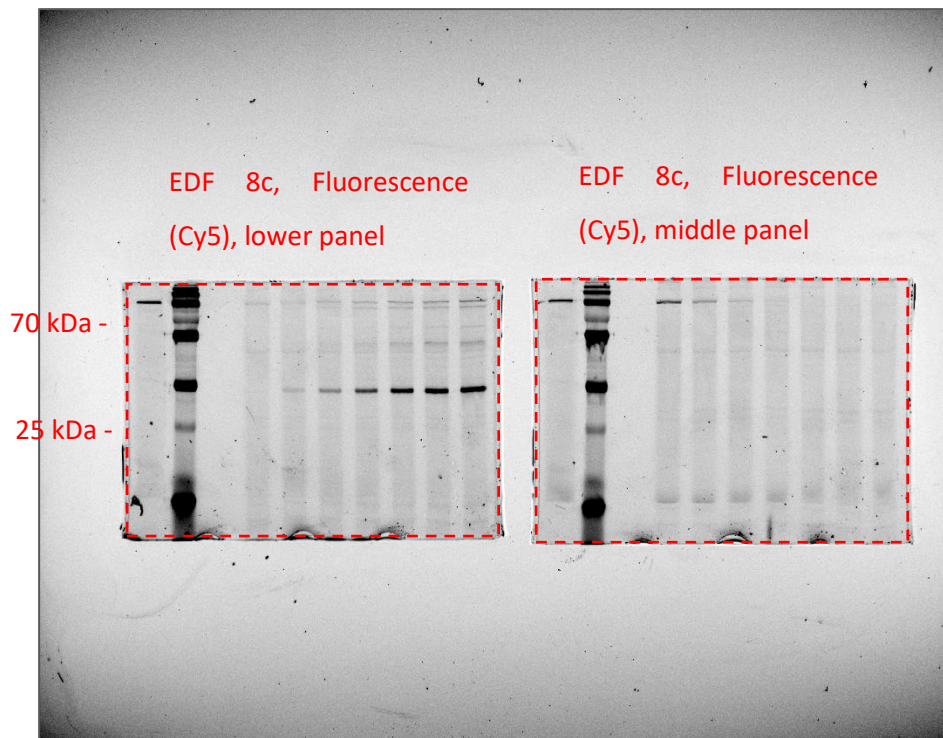


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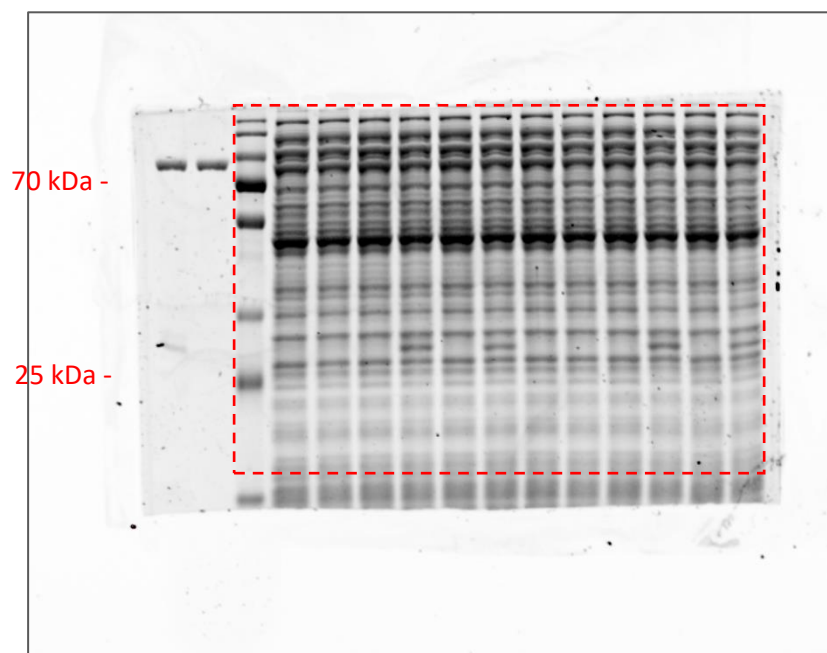
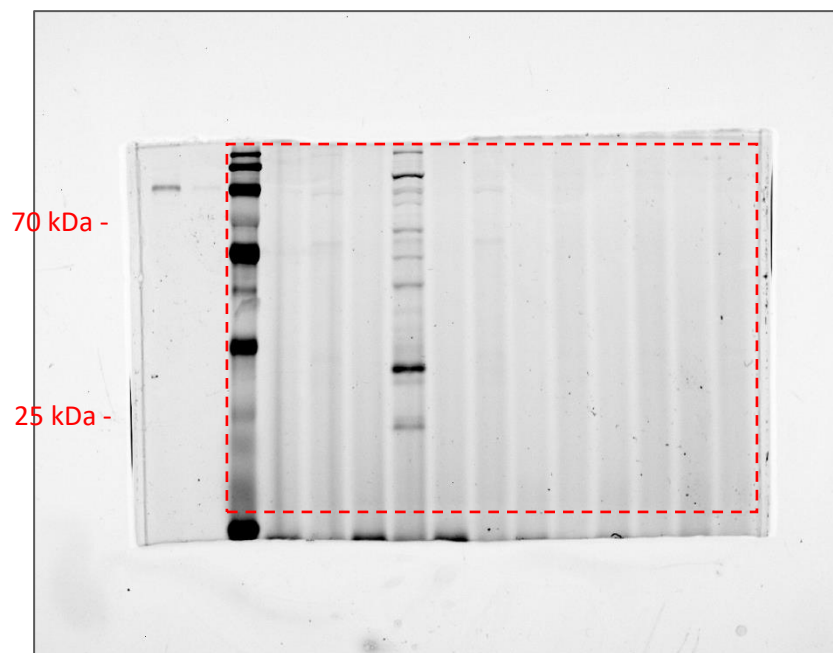


EDF 8a, Coomassie

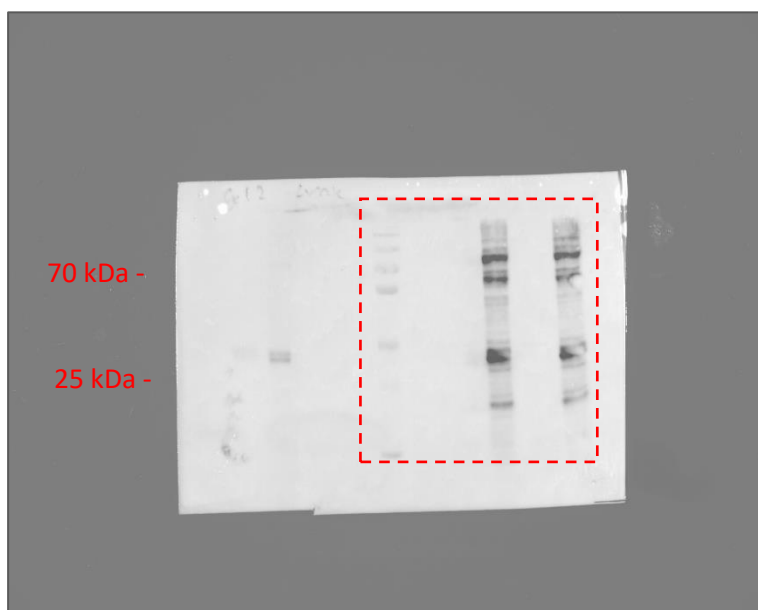
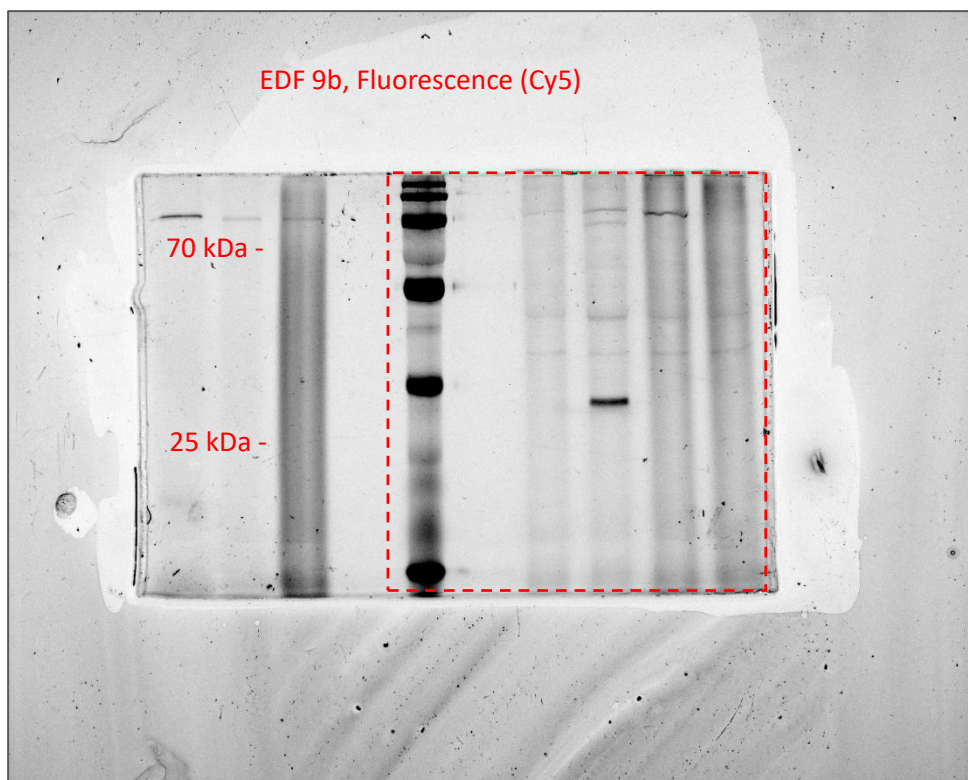
Used in Extended Data Fig. 8a



Used in Extended Data Fig. 8c

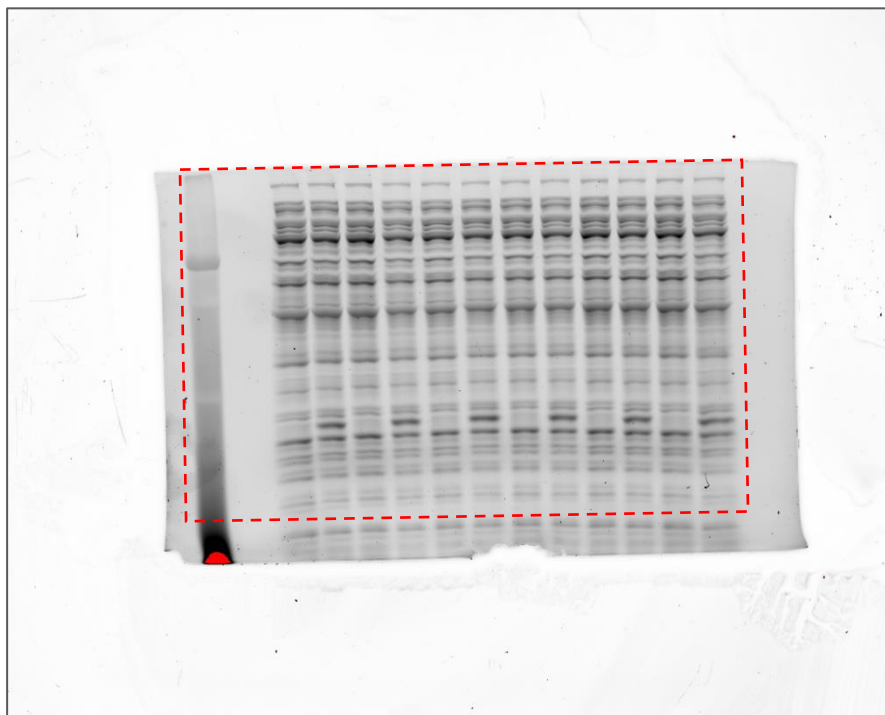
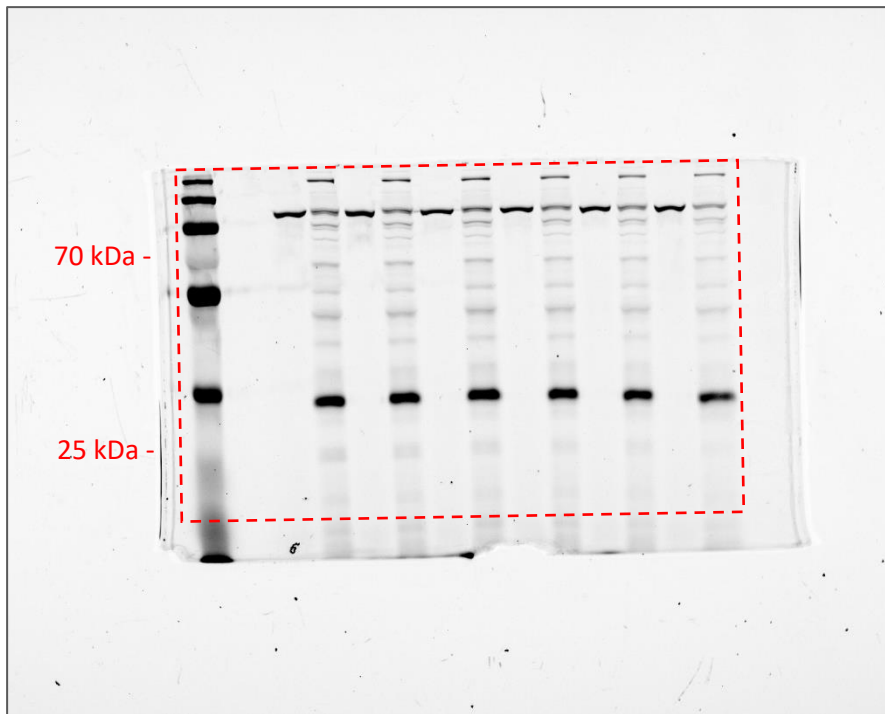


Used in Extended Data Fig. 9a

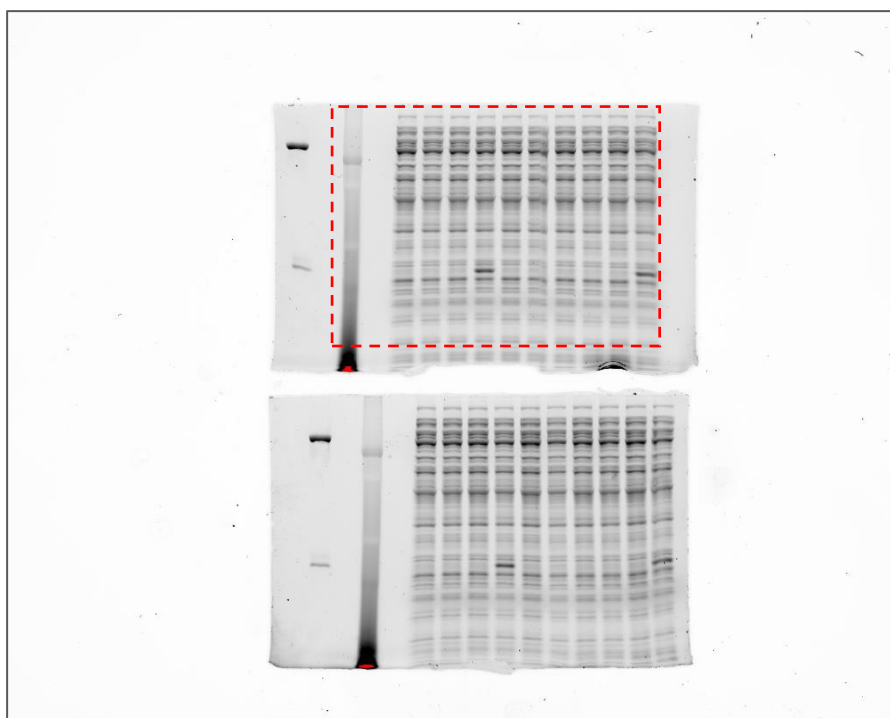


EDF 9b, pan-ADPr Western blot
merged with Colorimetric scan

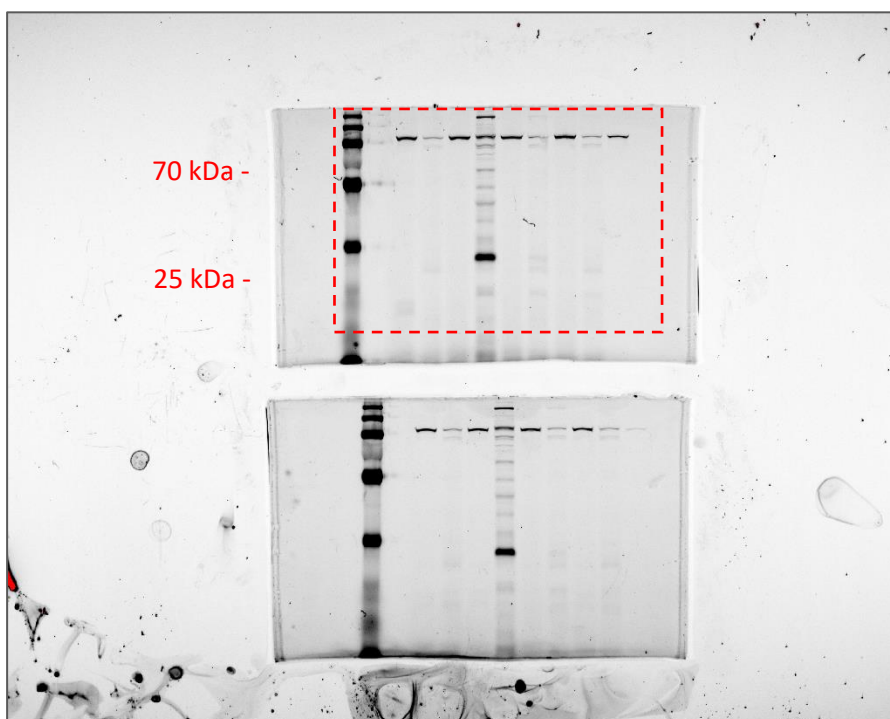
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Used in Extended Data Fig. 9c

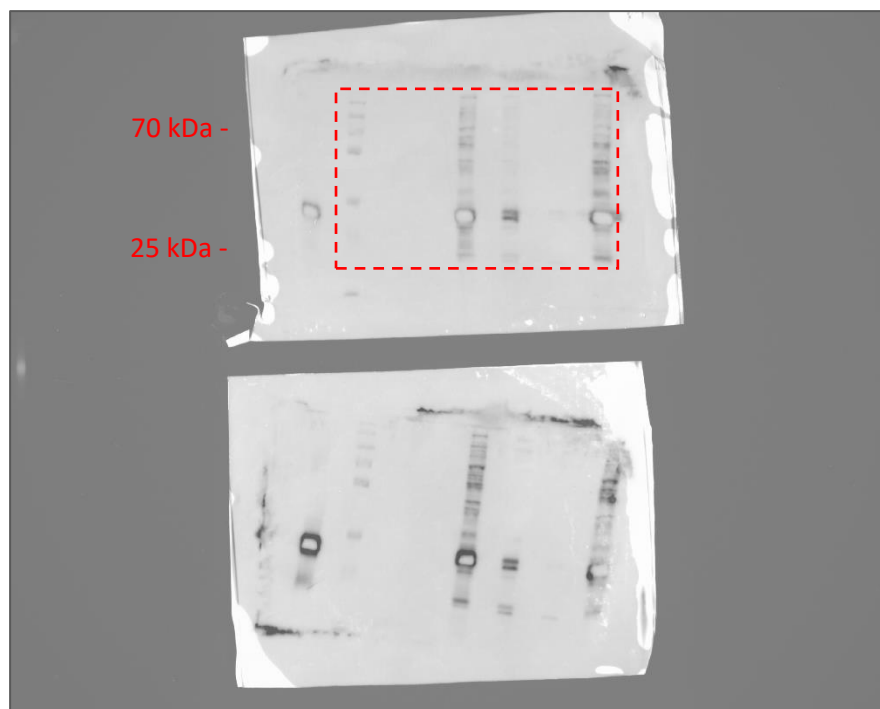


EDF 9d, TCE stain



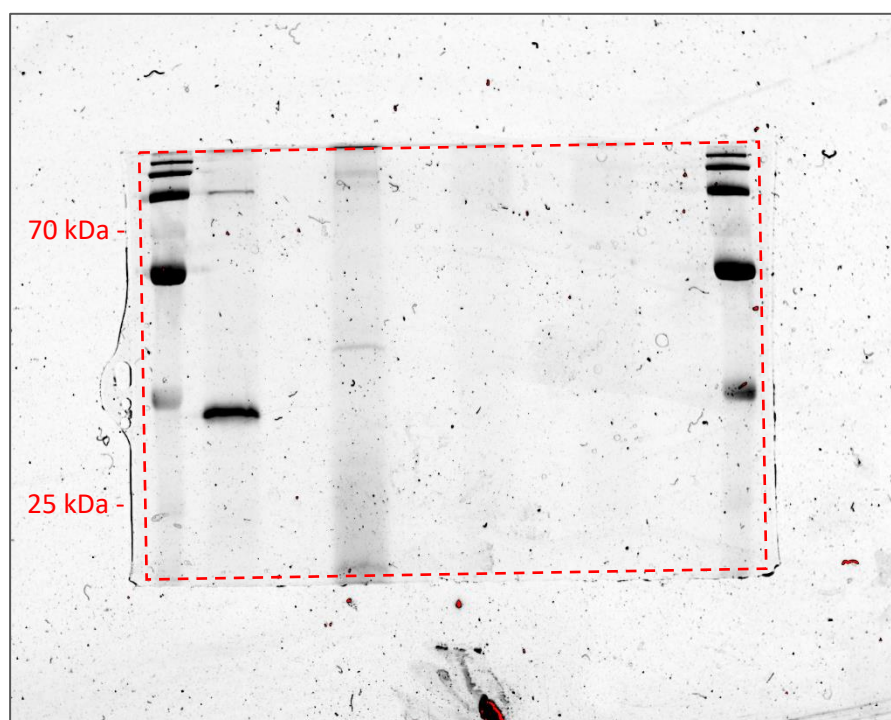
EDF 9d, Fluorescence (Cy5)

Used in Extended Data Fig. 9d



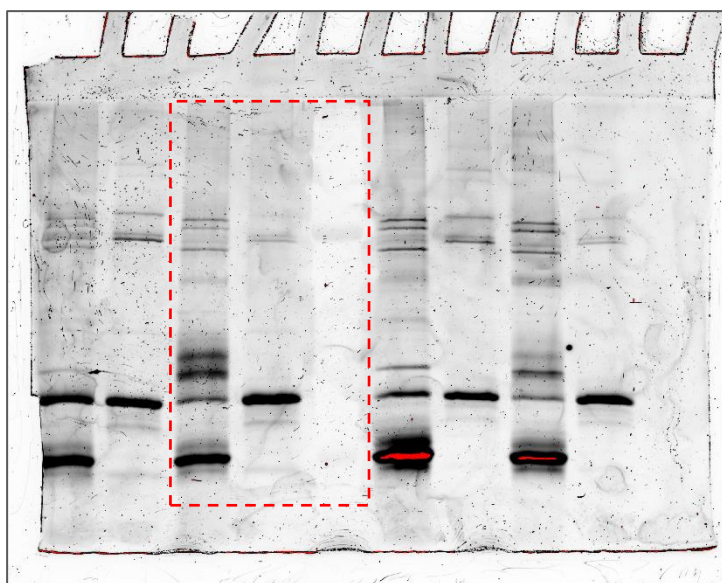
EDF 9d, pan-ADPr Western blot
merged with Colorimetric scan

Used in Extended Data Fig. 9d

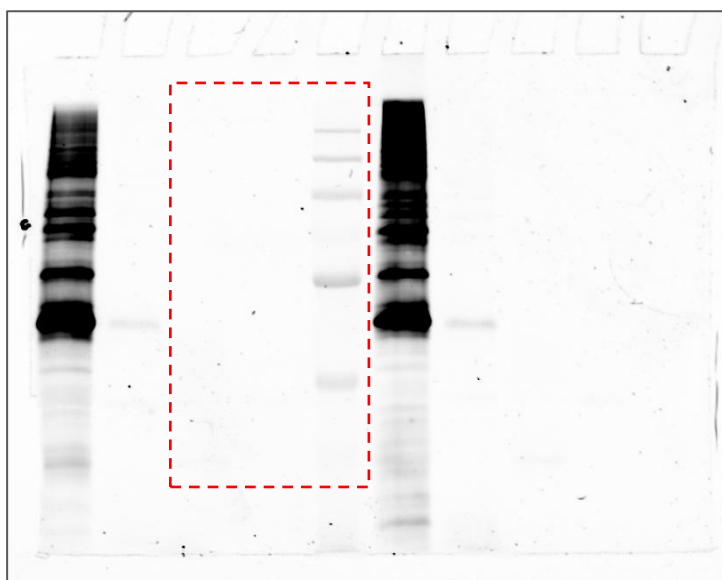


EDF 10a, Fluorescence (Cy5)

Used in Extended Data Fig.10a

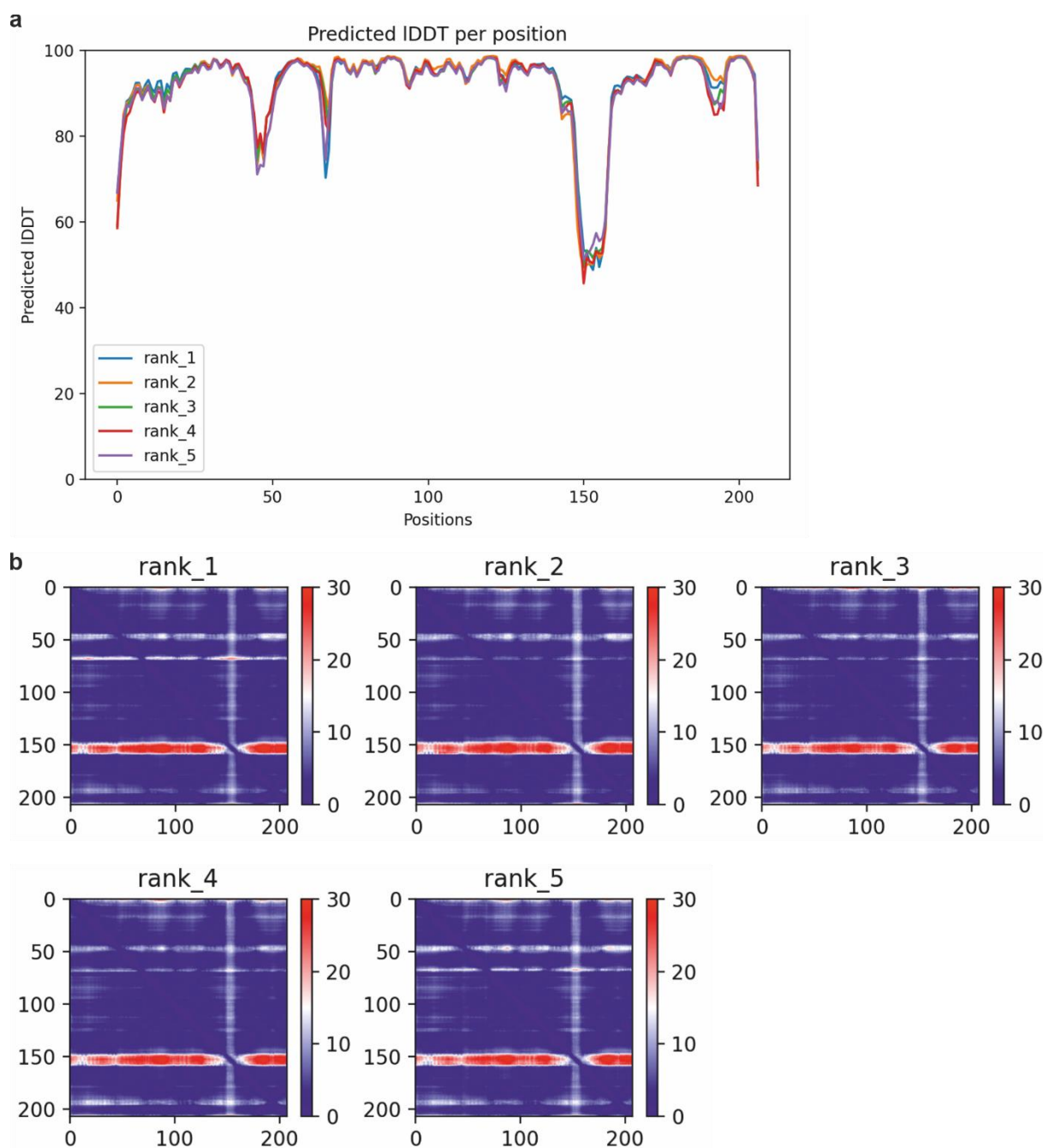


EDF 10c, Flamingo stain

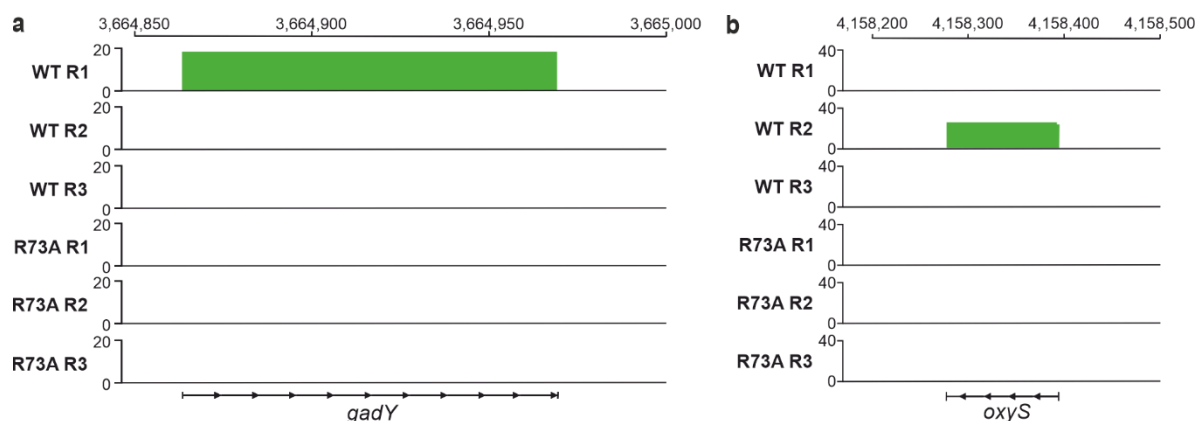


EDF 10c, Coomassie

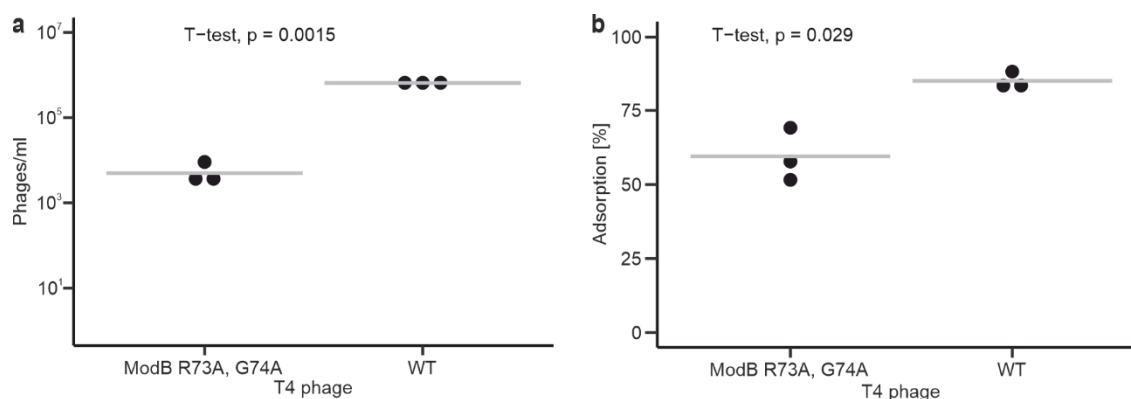
Used in Extended Data Fig. 10c



Supplementary Figure 2: Confidence metrics of the AlphaFold prediction model of ModB structure shown in Extended Data Fig. 1f. Plots of predicted local-distance difference test (pLDDT) (a) and predicted aligned error (PAE) (b) are shown as AlphaFold prediction metrics⁶³. Confidence metrics for the model used here are represented by “rank_1”.



Supplementary Figure 3: IGV coverage plots for RNAylomeSeq data. Read coverage on identified RNAylated RNAs as analysed in IGV is exemplarily shown for *gadY* (a) and *oxyS* (b) depicting reads in T4 WT samples (green) vs. T4 ModB R73A, G74A samples (red) for each replicate. RNAylomeSeq merely identifies 5'-termini of mRNAs or, if 200nt or smaller, entire sRNA sequences. This is due to the application of single-end Illumina-Seq which automatically only captures the 5'-end of the respective read/transcript.



Supplementary Figure 4: Statistical tests for phenotype of T4 phage ModB R73A, G74A mutant shown in Fig. 5 c-e. Dotplots are shown for burst size 140 min post-infection (a) and phage adsorption to the host cell 8 min post-infection (b). Grey bars represent mean, black dots individual data points of n=3 biologically independent replicates each. Two-sided t-tests found both differences in burst size (140 min post infection; t-test, two-sided, p-value = 0.0015 at $p_{\text{signif.}} < 0.05$) and phage adsorption between T4 phage WT and T4 phage R73A, G74A (8 min post infection; t-test, two-sided, p-value = 0.029 at $p_{\text{signif.}} < 0.05$) to be statistically significant on a significance level of 0.05. T4 phage ModB R73A, G74A produces less progeny and adsorbs less efficiently to the host cell.

Supplementary Table 1: MaxQuant Output for LC-MS/MS analysis of endogenously His-tagged rS1 from T4 phage-infected *E. coli* B strain. Endogenously His-tagged rS1 was isolated from T4 phage-infected (inf) and -uninfected *E. coli* B strain and subjected to LC-MS/MS analysis in biological triplicates (n=3). Intensities from MaxQuant are only shown for rS1 (1A; modificationSpecificPeptides). ADP-ribosylation is detected only for a small subset of rS1 peptides from T4 phage-infected samples whilst absent in uninfected samples. Predominantly, R139/R142 and R485/R487 were identified as ADP-ribosylation sites in all three replicates. ADP-ribosylated peptides are listed by rS1 domain and with the respective arginine residues in 1B; ADPr peptides. Modifications occur at R485/R487 (domain 6) and R139/R142 (domain 2) in all three replicates. Comparing intensities of ADP-ribosylated and unmodified peptides (1C; ADPr vs. unmodified peptides) shows ratios varying from 1.4 % to 6.6 %. Based on this data, one may speculate that R139/R142 and R485/R487 might be major ADP-ribosylation sites on rS1 *in vivo*. MaxQuant parameters for the presented data are presented in 1D; Parameters MaxQuant.

Supplementary Table 2: MaxQuant Output for LC-MS/MS analysis of His-tagged rS1-WT, -R139A and -R139K mutants from T4 phage-infected *E. coli*. MaxQuant Output filtered for rS1 protein and sorted according to ADP-ribosylation (ADP-ribosylwoDP) is presented (2A; modificationSpecificPeptides). T4 phage-infected samples (T4 phage) and -uninfected control (LB control) per rS1 version (WT, R139A or R139K mutant) are presented. A total of three biological replicates (n=3) were analysed per rS1 version. Peptides which were found ADP-ribosylated are listed and are assigned to their respective location (rS1 domain) and the modified arginines in the rS1 protein (2B; ADPr peptides). Intensities of ADP-ribosylated peptides are compared to their unmodified counterpart each by dividing the respective intensities (2C; ADPr vs. unmodified peptides). The peptide AFLPGSLVDVR(K/A)PVRDTLHLEGK is found ADP-ribosylated for rS1 WT, rS1 R139A and R139K only in T4 phage-infected samples across all three replicates. It becomes obvious that for the WT peptide high intensities of the ADP-ribosylated peptide relative to the unmodified peptide are detected across all three T4 phage-infected replicates. For the mutant peptides R139A and R139K, these intensities are at least 3-fold lower. Based on this finding, one may speculate that R139 mutation might reduce ADP-ribosylation of the AFLPGSLVDVRPVRDTLHLEGK peptide in rS1. MaxQuant parameters for the presented data are presented in 2D; Parameters MaxQuant.

Supplementary Table 3: *In vitro* ADP-ribosylation and RNylation sites in rS1 protein as identified by LC-MS/MS analysis. Peptide spectrum match (PSM) information for ADP-ribosylated and/or RNylated rS1 peptides are given in summarised form (pivot) and as complete output from OpenMS tool RNPxl (PSMs). Results were filtered for 1 % FDR on PSM level and q-values (scores) are given. Spectrum IDs, precursor m/z values, charge states, best localisation of modification within the peptide sequence, localisation score and mass errors (in ppm) are provided in "PSMs" sheet.

Supplementary Table 4: Genes identified to contribute to the RNAlome by RNAlomeSeq. An excerpt from the counts table is presented. Hits are calculated based on the mean read counts for each gene among T4 WT and R73A, G74A (MUT) samples for each replicate individually. For a hit, the log2 Fold Change (LFC) between WT and MUT sample is to be greater than 1.5 and the log2 transformed mean expression greater than -0.5. Hits are indicated as "+" for individual replicates. Read distribution for hits is presented in column "IGV" and the existence of corresponding NAD-capped transcripts is indicated in column "NAD-capped RNA?". Raw read counts are shown in WT_R1 – MUT_R3. Some hits are present in all replicates, some in one or two replicates only. Importantly, for the majority of protein_coding and ncRNA genes, reads initiate with an adenosine or contain an adenosine no more than 2 nt away from the read start. tRNA and rRNA (which more likely represent the background) hits are more abundant in replicates 2 and 3. Especially in replicate 3, the fraction of RNAI reads varies comparing WT and MUT samples, which may explain this variation from the background.

Supplementary Table 5: MaxQuant Output for LC-MS/MS analysis of *E. coli* cell lysate with addition of ModB to various concentrations. Samples 1 and 3 represent lysates without ModB, whilst samples 2 and 4 contain 85

nM and 8.5 nM ModB, respectively. The data serve as raw data for values shown in Extended Data Table 3, where log2 transformed protein intensity values are shown for each sample.

Supplementary Table 6: *In vitro* ADP-ribosylation and RNAylation sites in rL2 protein as identified by LC-MS/MS analysis. Peptide spectrum match (PSM) information for ADP-ribosylated and/or RNAylated rL2 peptides are given in summarised form (pivot) and as complete output from OpenMS tool RNPxl (PSMs). Results were filtered for 1 % FDR on PSM level and q-values (scores) are given. Spectrum IDs, precursor m/z values, charge states, best localisation of modification within the peptide sequence, localisation score and mass errors (in ppm) are provided in "PSMs" sheet.

Supplementary Table 7: RNAs used in this study.

RNA	RNA sequence
8mer	ACAGUAUU
RNAI	ACAGUAUUUGGUAUCUGCGCUCUGCUGAAGCCAGUUACCUUCGGAAAAAGAG UUGGUAGCUCUUGAUCCGGCAAACAAACCACCGCUGGUAGCGGUGGUUUUU UUGUU
100nt-RNA (Qβ)	AUCUUGAUACUACCUUUAGUUCGUUUAAACACGUUCUUGAUAGUAUCUUUU UAUUAACCCAACGCGUAAAGCGUUGAAACUUUGGGUCAAUUUGAUGAUG
10mer-Cy5	ACAGUAUUUG
2nt-5'overhang- Cy5	ACAGACUUCGGUCU-Cy5
3'overhang-Cy5	AGACUUCGGUCUA-Cy5
5'P-blunt-Cy5	AGACUUCGGUCU-Cy5
linear-Cy5	AGACUUCGAC-Cy5
40mer-Cy5	ACAGUAUUUGGUAUCUGCGCUCUGCUGAAGCCAGUUACUU-Cy5

Supplementary Table 8: Genomic DNA sequence of ARTs, rS1 variants and ADP-ribose hydrolases.
Start codon in italic; thrombin cleavage site in bold; mutations in red and bold; restriction sites underlined.

Gene [5', 3' restriction site]	DNA sequence
Alt [<i>NcoI</i> , <i>XhoI</i>]	<u>CCATGGG</u>GAGAACTTATTACAGAATTATTTGACGAAGATACTACTCTTCCAATTACAACTTAT ATCCAAAGAAGAAAATACCGCAAATTTTTTCAGTTCATGTTGATGATGCAATTGAACAACCG GCTTTCGTTTATGTACCTATACATCTGGAGGTGATACTAATCGTGATTTAAAGATGGGCGATA AAATGATGCATATTGTTCTTTTACATTAAGTCTAAAGGTTCAATTGCTAAATTTAAAGGTCT TGGTCCAAGCCCAATTAATTATATCAATTCAGTTTTTACTGTTGCAATGCAAACAATGCGCCA GTATAAAATTGATGCCTGTATGCTCCGTATTCTTAAGTCTAAAAGTCTGGCCAAGCTCGACA AATTCAAGTTATTGCTGATAGACTTATCCGTAGTCGTTCAAGGTGGTAGATACGTCCTTCTTAA GGAAGTCTGGGATTACGATAAAAAGTATGCATATATTCTTATACATCGCAAAAATGTATCACT AGAAGACATTCCAGGAGTTCGGGAAATTAGTACCGAGCTCTTTACTAAAGTTGAATCGAAGG TCGGTGATGTTTATATCAATAAAGATACTGGGGCTCAAGTAATAAAAATGAGGCAATTGCA GCATCTATTGCGCAAGAAAATGATAAACGTTCTGACCAAGCTGTAATCGTTAAAGTTAAATTT TCCCGTAGAGCAATTGCGCAAAGTCAGTCATTGGAATCTTCTAGATTGAAACACCAATGTTT CAAAAATTTGAGGCTTCAGCGGCCGAATTAATAAACAGCGGACGCGCCTTTAATTTCTGAT TCTAATGAATTAACGGTAATTTCTACTTCAGGATTTGCACTAGAGAATGCTCTTAGCAGTGTT ACAGCTGGGATGGCATTACAGAGAAGCTTCTATAATTCCTGAAGATAAAGAATCCATTATTAAC GCAGAAATAAAAAATAAAGCTTTAGAAAGATTACGAAAAGAATCTATTACTTCAATAAAAAAC CTTAGAACTATTGCTTCTATCGTCGATGATACTTTAGAAAAATATAAGGGTGCTTGGTTTGA AAGAAATATTAACAAACATTCGCATTTAAACCAAGATGCTGCAAATGAGTTAGTACAAAATTC TTGGAATGCAATAAAAAACAAAGATTATTCGAAGAGAATTACGTGGATATGCTCTTACCGCTG GATGGTCATTACATCCTATAGTCGAAAATAAAGATTCATCTAAATACACACCAGCGCAAAAAC GCGGAATTCGTGAATACGTAGGTTCAAGATATGTAGACATAAATAATGCTCTTTTGGGATTAT ATAATCCAGATGAGCGTACAAGTATTTTGACAGCATCTGACATAGAAAAAGCTATTGATAATT TAGATTACGCCTTTAAAAATGGTGAACGATTACCAAAAGGTATTACTTTGTATCGTTACAAAC GAATGTTACCTTCAATATACGAAGCAATGGTAAAAAATCGAGTTTTTTATTTTAGAACTTTGT GTCAACATCATTATATCCAAATATTTTGGTACTTGGATGACTGATTCATCTATAGGTGTTTTA CCAGACGAAAAGCGTTTAAGCGTTTCTATTGATAAACTGATGAAGGACTTGTAATTTCTAGC GATAATTTAGTTGGAATTGGATGGGTTATTACTGGGGCTGATAAGGTCAATGTTGTTTTACCC GGTGAAGTTTAGCGCCTTCAAATGAAATGGAAGTCATTTTGCCACGTGGATTAATGGTCAA AGTTAATAAAATAACCGATGCATCTTACAATGATGGAACAGTTAAACTAACAACAAGCTTAT TCAAGCTGAAGTTATGACCACAGAAGAAGTACCGAATCGGTAATCTATGACGGAGACCATT TAATGGAACTGGTGAATTGGTTACAATGACAGGTGATATAGAAGATAGAGTTGACTTTGCA TCATTTGTTTCATCAAATGTTAAACAGAAAGTAGAATCATCTCTTGAATATTGCGTCTTGCA TAGATATTGCAACATGCCTTACAAGTTCTGTTCAAGGACTGGTGCCGCGCGGCAGCCTCGAG
ModA [<i>NcoI</i> , <i>XhoI</i>]	<u>CCATGGG</u>GAAAAATACTCAGTAATGCAACTAAAAGATTTTAAATAAAATCAATGGATGCATCG GTGCGTGCTTCTATTCGTGAAGAATTACTTTCTGAAGGGTTTAATTTATCTGAAATTGAACTTT TAATTCATTGTATTACTAATAAACAGATGACCATTCTTGGTTAAATGAAATAATCAAATCTCG TTTGGTTCCAAACGATAAACCTCTTTGGAGAGGTGTTCCAGCTGAGACTAAACAAGTATTA TCAAGGAATTGATATTATTACATTTGATAAAGTCGTATCAGCTTCATATGATAAAAAATATAGC

	TCTACATTTTGCTTCTGGTTAGAGTATAACACACAAGTTATTTTTGAATTCAAAGCTCCTATG GTATTCAATTTCCAGGAGTATGCTATAAAAGCTCTACGCTGTAAAGAATACAATCCAACTTT AAGTTTCCGGATAGTCATCGTTATCGTAATATGGAATTAGTTTCAGATGAACAAGAAGTAATG ATACCAGCTGGAAGTGTATTTAGAATTGCAGATAGATATGAGTATAAAAAAGTGTTCAACATA CACTATCTATACTCTTGATTTTGAAGGATTTAATCTACTGGTGCCGCGCGGCAGCCTCGAG
ModB [NcoI, XhoI]	<u>CCATGGGA</u> ATTATTAATCTTGCAGATGTTGAACAGTTATCTATAAAAGCTGAAAGCGTTGATT TTCAATATGATATGTATAAAAAGGTCTGTGAAAAATTTACTGACTTTGAGCAGTCTGTTCTTTG GCAATGTATGGAAGCCAAAAAGAATGAAGCTCTTCATAAGCATTAAATGAAATCATTAAAA AGCATTAACTAAATCGCCTTATCAATTATATCGTGGTATATCAAATCGACAAAAGAAGTCA TTAAAGATTTACAAGTTGGAGAAGTGTTCACGAACAGGGTAGATTCAATTTACTACTAGTT TGCATACAGCGTGTCTTTTTCTTATGCTGAATATTTCACTGAAACAATACTTCGTTTAAAAAC TGATAAAGCTTTTAATTATTCTGACCATATCAGCGATATTATACTTTCTTCTCCTAATACTGAGT TTAAGTACACGTATGAAGATACTGATGGATTAGATTCAGAGCGTACTGATAACTTAATGATG ATTGTGCGTGAACAAGAATGGATGATTCCAATTGGAAAGTATAAAATAACTTCTATTTCAAAA GAAAAATTACACGATTCATTTGGAACATTTAAAGTTTATGATATTGAGGTAGTTGAACTGGTG CCGCGCGGCAGCCTCGAG
ModB R73A, G74A [NcoI/XhoI]	<u>CCATGGGA</u> ATTATTAATCTTGCAGATGTTGAACAGTTATCTATAAAAGCTGAAAGCGTTGATT TTCAATATGATATGTATAAAAAGGTCTGTGAAAAATTTACTGACTTTGAGCAGTCTGTTCTTTG GCAATGTATGGAAGCCAAAAAGAATGAAGCTCTTCATAAGCATTAAATGAAATCATTAAAA AGCATTAACTAAATCGCCTTATCAATTATAT CGCGCA ATATCAAATCGACAAAAGAAGTCA TTAAAGATTTACAAGTTGGAGAAGTGTTCACGAACAGGGTAGATTCAATTTACTACTAGTT TGCATACAGCGTGTCTTTTTCTTATGCTGAATATTTCACTGAAACAATACTTCGTTTAAAAAC TGATAAAGCTTTTAATTATTCTGACCATATCAGCGATATTATACTTTCTTCTCCTAATACTGAGT TTAAGTACACGTATGAAGATACTGATGGATTAGATTCAGAGCGTACTGATAACTTAATGATG ATTGTGCGTGAACAAGAATGGATGATTCCAATTGGAAAGTATAAAATAACTTCTATTTCAAAA GAAAAATTACACGATTCATTTGGAACATTTAAAGTTTATGATATTGAGGTAGTTGAACTGGTG CCGCGCGGCAGCCTCGAG CACCACCACCACCACCCTGA
pET28-rS1 [NcoI, XhoI]	<u>CCATGGGA</u> ACTGAATCTTTGCGGCATGCTCAACTCTTTGAAGAGTCCTTAAAGAAATCGAA ACCCGCCCCGGTTCTATCGTTCTGTGGCGTTGTTGTTGCTATCGACAAAGACGTAGTACTGGTT GACGCTGGTCTGAAATCTGAGTCCGCCATCCCGGCTGAGCAGTTCAAAAACGCCAGGGCGA GCTGGAAATCCAGGTAGGTGACGAAGTTGACGTTGCTCTGGACGCAGTAGAAGACGGCTTC GGTGAAACTCTGCTGTCCCGTGAGAAAGCTAAACGTCACGAAGCCTGGATCACGCTGGAAAA AGCTTACGAAGATGCTGAAACTGTTACCGGTGTTATCAACGGCAAAGTTAAGGGCGGCTTCA CTGTTGAGCTGAACGGTATTCTGTGCGTTCCTGCCAGGTTCTCTGGTAGACGTTCTGCCGGTGC GTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAATTTAAAGTAATCAAGCTGGATCAGAAG CGCAACAACGTTGTTGTTTCTCGTCTGTGCCGTTATCGAATCCGAAAACAGCGCAGAGCGCGA TCAGCTGCTGGAAAACCTGCAGGAAGGCATGGAAGTTAAAGGTATCGTTAAGAACCTCACTG ACTACGGTGCATTCGTTGATCTGGGCGGCGTTGACGGCCTGCTGCACATCACTGACATGGCC TGGAACGCGTTAAGCATCCGAGCGAAATCGTCAACGTGGGCGACGAAATCACTGTTAAAGT GCTGAAGTTCGACCGCAACGTACCCGTGTATCCCTGGGCCTGAAACAGCTGGGCGAAGATC CGTGGGTAGCTATCGCTAAACGTTATCCGGAAGGTACCAAAGTACTGGTTCGCGTGACCAAC CTGACCGACTACGGCTGCTTCGTTGAAATCGAAGAAGGCGTTGAAGGCCTGGTACACGTTTC CGAAATGGACTGGACCAACAAAACATCCACCCGTCCAAAGTTGTTAAGCTTGCGCATGTAG TGGAAGTTATGGTCTGGATATCGACGAAGAACGTCGTCGTATCTCCCTGGGTCTGAAACAG

	TGCAAAGCTAACCCGTGGCAGCAGTTCGCGGAAACCCACAACAAGGGCGACCGTGTTGAAG GTAATCAAGTCTATCACTGACTTCGGTATCTTCATCGGCTTGGACGGCGGCATCGACGGCC TGGTTCACCTGTCTGACATCTCCTGGAACGTTGCAGGCGAAGAAGCAGTTCGTGAATACAAA AAAGGCGACGAAATCGCTGCAGTTGTTCTGCAGGTTGACGCAGAACGTGAACGTATCTCCCT GGGCGTTAAACAGCTCGCAGAAGATCCGTTCAACAACCTGGGTTGCTCTGAACAAGAAAGGC GCTATCGTAACCGGTAAAGTAACTGCAGTTGACGCTAAAGGCGCAACCGTAGAACTGGCTGA CGGCGTTGAAGGTTACCTGCGTGCTTCTGAAGCATCCCGTGACCGCGTTGAAGACGCTACCC TGGTCTGAGCGTTGGCGACGAAGTTGAAGCTAAATTCACCGGCGTTGATCGTAAAAACCGC GCAATCAGCCTGTCTGTTCTGCGGAAAGACGAAGCTGACGAGAAAGATGCAATCGCAACTGT TAACAAACAGGAAGATGCAAACCTCTCCAACAACGCAATGGCTGAAGCTTCAAAGCAGCTA AAGGCGAGCTGGTGCCGCGCGGAGCCTCGAG
pET28-rS1 R139A [NcoI, XhoI]	<u>CCATGGGA</u>ACTGAATCTTTGCGGCATGCTCAACTCTTTGAAGAGTCCTTAAAAGAAATCGAA ACCCGCCCCGGGTTCTATCGTTCGTGGCGTTGTTGTTGCTATCGACAAAGACGTAGTACTGGTT GACGCTGGTCTGAAATCTGAGTCCGCCATCCCGGCTGAGCAGTTCAAAAACGCCAGGGCGA GCTGGAAATCCAGGTAGGTGACGAAGTTGACGTTGCTCTGGACGCAGTAGAAGACGGCTTC GGTGAAACTCTGCTGTCCCGTGAGAAAGCTAAACGTCACGAAGCCTGGATCACGCTGGAAAA AGCTTACGAAGATGCTGAAACTGTTACCGGTGTTATCAACGGCAAAGTTAAGGGCGGCTTCA CTGTTGAGCTGAACGGTATTCTGTGCGTTCCTGCCAGGTTCTCTGGTAGACGTTGGCCCGGTGC GTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAATTTAAAGTAATCAAGCTGGATCAGAAG CGCAACAACGTTGTTGTTTCTCGTCGTGCCGTTATCGAATCCGAAAACAGCGCAGAGCGCGA TCAGCTGCTGGAACCTGCAGGAAGGCATGGAAGTTAAAGGTATCGTTAAGAACCTCACTG ACTACGGTGCAATTCGTTGATCTGGGCGGCGTTGACGGCCTGCTGCACATCACTGACATGGCC TGGAACGCGTTAAGCATCCGAGCGAAATCGTCAACGTGGGCGACGAAATCACTGTTAAAGT GCTGAAGTTGACCGCGAACGTACCCGTGTATCCCTGGGCCTGAAACAGCTGGGCGAAGATC CGTGGGTAGCTATCGCTAAACGTTATCCGGAAGGTACCAAACCTGACTGGTCGCGTGACCAAC CTGACCGACTACGGCTGCTTCGTTGAAATCGAAGAAGGCGTTGAAGGCCTGGTACACGTTTC CGAAATGGACTGGACCAACAAAAACATCCACCCGTCCAAAGTTGTTAACGTTGGCGATGTAG TGGAAGTTATGGTTCTGGATATCGACGAAGAACGTGTCGTATCTCCCTGGGTCTGAAACAG TGCAAAGCTAACCCGTGGCAGCAGTTCGCGGAAACCCACAACAAGGGCGACCGTGTTGAAG GTAATCAAGTCTATCACTGACTTCGGTATCTTCATCGGCTTGGACGGCGGCATCGACGGCC TGGTTCACCTGTCTGACATCTCCTGGAACGTTGCAGGCGAAGAAGCAGTTCGTGAATACAAA AAAGGCGACGAAATCGCTGCAGTTGTTCTGCAGGTTGACGCAGAACGTGAACGTATCTCCCT GGGCGTTAAACAGCTCGCAGAAGATCCGTTCAACAACCTGGGTTGCTCTGAACAAGAAAGGC GCTATCGTAACCGGTAAAGTAACTGCAGTTGACGCTAAAGGCGCAACCGTAGAACTGGCTGA CGGCGTTGAAGGTTACCTGCGTGCTTCTGAAGCATCCCGTGACCGCGTTGAAGACGCTACCC TGGTCTGAGCGTTGGCGACGAAGTTGAAGCTAAATTCACCGGCGTTGATCGTAAAAACCGC GCAATCAGCCTGTCTGTTCTGCGGAAAGACGAAGCTGACGAGAAAGATGCAATCGCAACTGT TAACAAACAGGAAGATGCAAACCTCTCCAACAACGCAATGGCTGAAGCTTCAAAGCAGCTA AAGGCGAGCTGGTGCCGCGCGGAGCCTCGAG
pET28-rS1 R139K [NcoI, XhoI]	<u>CCATGGGA</u>ACTGAATCTTTGCGGCATGCTCAACTCTTTGAAGAGTCCTTAAAAGAAATCGAA ACCCGCCCCGGGTTCTATCGTTCGTGGCGTTGTTGTTGCTATCGACAAAGACGTAGTACTGGTT GACGCTGGTCTGAAATCTGAGTCCGCCATCCCGGCTGAGCAGTTCAAAAACGCCAGGGCGA GCTGGAAATCCAGGTAGGTGACGAAGTTGACGTTGCTCTGGACGCAGTAGAAGACGGCTTC GGTGAAACTCTGCTGTCCCGTGAGAAAGCTAAACGTCACGAAGCCTGGATCACGCTGGAAAA

	AGCTTACGAAGATGCTGAAACTGTTACCGGTGTTATCAACGGCAAAGTTAAGGGCGGCTTCA CTGTTGAGCTGAACGGTATTCTGTCGTTCTGCCAGGTTCTCTGGTAGACGTTAAACCGGTG CGTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAATTTAAAGTAATCAAGCTGGATCAGAA GCGCAACAACGTTGTTGTTTCTCGTCGTGCCGTTATCGAATCCGAAAACAGCGCAGAGCGCG ATCAGCTGCTGGAACCTGCAGGAAGGCATGGAAGTTAAAGGTATCGTTAAGAACCTCACT GACTACGGTGCATTGTTGATCTGGGCGGCGTTGACGGCCTGCTGCACATCACTGACATGGC CTGGAAACGCGTTAAGCATCCGAGCGAAATCGTCAACGTGGGCGACGAAATCACTGTTAAA GTGCTGAAGTTGACCGCGAACGTACCCGTGTATCCCTGGGCTGAAACAGCTGGGCGAAG ATCCGTGGGTAGCTATCGCTAAACGTTATCCGGAAGGTACCAAATGACTGGTCGCGTGACC AACCTGACCGACTACGGCTGCTTCGTTGAAATCGAAGAAGGCGTTGAAGGCCTGGTACACGT TTCCGAAATGGACTGGACCAACAAAAACATCCACCCGTCCAAAGTTGTTAACGTTGGCGATG TAGTGGAAGTTATGGTTCTGGATATCGACGAAGAAGTCGTCGTATCTCCCTGGGTCTGAAA CAGTGCAAAGCTAACCCGTGGCAGCAGTTCGCGGAAACCCACAACAAGGGCGACCGTGTTG AAGGTAAATCAAGTCTATCACTGACTTCGGTATCTTCATCGGCTTGGACGGCGGCATCGAC GGCCTGGTTCACCTGTCTGACATCTCTGGAACGTTGCAGGCGAAGAAGCAGTTCGTGAATA CAAAAAAGGCGACGAAATCGCTGCAGTTGTTCTGCAGGTTGACGCAGAACGTGAACGTATCT CCCTGGGCGTTAAACAGCTCGCAGAAGATCCGTTCAACAATGGGTTGCTCTGAACAAGAAA GGCGCTATCGTAACCGGTAAAGTAACTGCAGTTGACGCTAAAGGCGCAACCGTAGAACTGG CTGACGGCGTTGAAGGTTACCTGCGTGCTTCTGAAGCATCCCGTGACCGCGTTGAAGACGCT ACCCTGGTCTGAGCGTTGGCGACGAAGTTGAAGCTAAATTCACCGCGTTGATCGTAAAAA CCGCGCAATCAGCCTGTCTGTTCTGTGCGAAAGACGAAGCTGACGAGAAAGATGCAATCGCA ACTGTTAACAACAGGAAGATGCAAATCTCCAACAACGCAATGGCTGAAGCTTCAAAGC AGCTAAAGGCGAGCTGGTGCCGCGCGGCAGCCTCGAG
pTAC-rS1 [XhoI, SphI]	ATGAAGCTTCCTCGAGAGACTGAATCTTTTGCTCAACTCTTTGAAGAGTCCTTAAAAGAAATCGAAACC CGCCCGGGTCTATCGTTCTGGCGTTGTTGTTGCTATCGACAAAAGACGTAGTACTGGTTGACGCTGGT CTGAAATCTGAGTCCGCCATCCCGGCTGAGCAGTTCAAAAACGCCAGGGCGAGCTGGAATCCAGGT AGGTGACGAAGTTGACGTTGCTCTGGACGCAGTAGAAGACGGCTTCGGTGAAACTCTGCTGTCCCGTG AGAAAGCTAAACGTCACGAAGCCTGGATCAGCTGGA AAAAGCTTACGAAGATGCTGAAACTGTTACC GGTGTATCAACGGCAAAGTTAAGGGCGGCTTCACTGTTGAGCTGAACGGTATTCGTGCGTTCTCGCC AGGTTCTCTGGTAGACGTTCTGTCGGTGCGTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAATTTA AAGTAATCAAGCTGGATCAGAAGCGCAACAACGTTGTTGTTTCTCGTCGTGCCGTTATCGAATCCGAAA ACAGCGCAGAGCGGATCAGCTGCTGGAACCTGCAGGAAGGCATGGAAGTTAAAGGTATCGTTAA GAACCTCACTGACTACGGTGCATTGTTGATCTGGGCGGCGTTGACGGCCTGCTGCACATCACTGACAT GGCCTGGAACGCGTTAAGCATCCGAGCGAAATCGTCAACGTGGGCGACGAAATCACTGTTAAAGTGC TGAAGTTCGACCGCGAACGTACCCGTGTATCCCTGGGCTGAAACAGCTGGGCGAAGATCCGTGGGTA GCTATCGCTAAACGTTATCCGGAAGGTACCAAATGACTGGTCGCGTGACCAACCTGACCGACTACGG CTGCTTCGTTGAAATCGAAGAAGGCGTTGAAGGCCTGGTACACGTTTCCGAAATGGACTGGACCAACA AAAACATCCACCCGTCCAAAGTTGTTAACGTTGGCGATGTAGTGGAAGTTATGGTTCTGGATATCGACG AAGAACGTCGTCGTATCTCCCTGGGTCTGAAACAGTGCAAAGCTAACCCGTGGCAGCAGTTCGCGGAA ACCCACAACAAGGGCGACCGTGTGAAGGTAAAATCAAGTCTATCACTGACTTCGGTATCTTCATCGGC TTGGACGGCGGCATCGACGGCCTGGTTCACTGTCTGACATCTCCTGGAACGTTGCAGGCGAAGAAGC AGTTCGTGAATACAAAAAGGCGACGAAATCGCTGCAGTTGTTCTGCAGGTTGACGCAGAACGTGAAC GTATCTCCCTGGGCGTTAAACAGCTCGCAGAAGATCCGTTCAACAATGGGTTGCTCTGAACAAGAAA GGCGCTATCGTAACCGGTAAAGTAACTGCAGTTGACGCTAAAGGCGCAACCGTAGAACTGGCTGACG GCGTTGAAGGTTACCTGCGTGCTTCTGAAGCATCCCGTGACCGCGTTGAAGACGCTACCTGGTCTGA GCGTTGGCGACGAAGTTGAAGCTAAATTCACCGGCGTTGATCGTAAAAACCGCGCAATCAGCCTGTCT

	GTTCTGCGAAAGACGAAGCTGACGAGAAAGATGCAATCGCAACTGTAAACAAACAGGAAGATGCAA ACTTCTCCAACACGCAATGGCTGAAGCTTTCAAAGCAGCTAAAGGCGAGTGCATGCACGTAGAG
S1 D1 [NcoI, XhoI]	<u>CCATGG</u> AGTCCTTAAAGAAATCGAAACCCGCCGGTTCTATCGTTCGTGGCGTTGTTGTTG CTATCGACAAAGACGTAGTACTGGTTGACGCTGGTCTGAAATCTGAGTCCGCCATCCCGGCT GAGCAGTTCAAAAACGCCAGGGCGAGCTGGAAATCCAGGTAGGTGACGAAGTTGACGTTG CTCTGGACGCAGTAGAAGACGGCTTCGGTGAAACTCTGCTGTCCCGTGAGAAAGCTAAACGT CACGAAGCC CTGGTGCCGCGCGGCAGCCTCGAG
S1 D2 [NcoI, XhoI]	<u>CCATGG</u> CCTGGATCACGCTGGAAAAAGCTTACGAAGATGCTGAAACTGTTACCGGTGTTATC AACGGCAAAGTTAAGGGCGGCTTCACTGTTGAGCTGAACGGTATTCGTGCGTTCCTGCCAGG TTCTCTGGTAGACGTTTCGTCCGGTGCGTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAAT TTAAAGTAATCAAGCTGGATCAGAAGCGCAACAACGTTGTTGTTTCTCGTCGTGCCGTTATCG AATCCGAAAACAGCGCAGAG CTGGTGCCGCGCGGCAGCCTCGAG
S1 D2 R139A [NcoI, XhoI]	<u>CCATGG</u> CCTGGATCACGCTGGAAAAAGCTTACGAAGATGCTGAAACTGTTACCGGTGTTATC AACGGCAAAGTTAAGGGCGGCTTCACTGTTGAGCTGAACGGTATTCGTGCGTTCCTGCCAGG TTCTCTGGTAGACGTT GGC CCGGTGCGTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAAT TTAAAGTAATCAAGCTGGATCAGAAGCGCAACAACGTTGTTGTTTCTCGTCGTGCCGTTATCG AATCCGAAAACAGCGCAGAG CTGGTGCCGCGCGGCAGCCTCGAG
S1 D2 R139K [NcoI, XhoI]	<u>CCATGG</u> CCTGGATCACGCTGGAAAAAGCTTACGAAGATGCTGAAACTGTTACCGGTGTTATC AACGGCAAAGTTAAGGGCGGCTTCACTGTTGAGCTGAACGGTATTCGTGCGTTCCTGCCAGG TTCTCTGGTAGACGTT AAA CCGGTGCGTGACACTCTGCACCTGGAAGGCAAAGAGCTTGAAT TTAAAGTAATCAAGCTGGATCAGAAGCGCAACAACGTTGTTGTTTCTCGTCGTGCCGTTATCG AATCCGAAAACAGCGCAGAG CTGGTGCCGCGCGGCAGCCTCGAG
S1 D3 [NcoI, XhoI]	<u>CCATGG</u> CCCGCGATCAGCTGCTGGAAAACCTGCAGGAAGGCATGGAAGTTAAAGGTATCGT TAAGAACCTCACTGACTACGGTGCAATTCGTTGATCTGGGCGGCGTTGACGGCCTGCTGCACA TCACTGACATGGCCTGGAAACGCGTTAAGCATCCGAGCGAAATCGTCAACGTGGGCGACGA AATCACTGTTAAAGTGCTGAAGTTTCGACCGCGAACGTACCCGTGTATCCCTGGGCCTGAAAC AGCTGGGCGAAGATCCG CTGGTGCCGCGCGGCAGCCTCGAG
S1 D4 [NcoI, XhoI]	<u>CCATGG</u> CCTGGGTAGCTATCGCTAAACGTTATCCGGAAGGTACCAAACCTGACTGGTCGCGTG ACCAACCTGACCGACTACGGCTGCTTCGTTGAAATCGAAGAAGGCGTTGAAGGCCTGGTACA CGTTTCCGAAATGGACTGGACCAACAAAAACATCCACCCGTCAAAGTTGTTAACGTTGGCG ATGTAGTGGAAGTTATGGTTCTGGATATCGACGAAGAACGTCGTCTATCCCTGGGTCTG AAACAGTGCAAAGCTAACCCG CTGGTGCCGCGCGGCAGCCTCGAG
S1 D5 [NcoI, XhoI]	<u>CCATGG</u> CCTGGCAGCAGTTCGCGGAAACCCACAACAAGGGCGACCGTGTGAAGGTAAAT CAAGTCTATCACTGACTTCGGTATCTTCATCGGCTTGACGGCGGCATCGACGGCCTGGTTCA CTGTCTGACATCTCCTGGAACGTTGCAGGCGAAGAAGCAGTTCGTGAATACAAAAAGGCG ACGAAATCGCTGCAGTTGTTCTGCAGGTTGACGCAGAACGTGAACGTATCTCCCTGGGCGTT AAACAGCTCGCAGAAGATCCG CTGGTGCCGCGCGGCAGCCTCGAG
S1 D6 [NcoI, XhoI]	<u>CCATGG</u> CCTCAACAACCTGGGTTGCTCTGAACAAGAAAGGCGCTATCGTAACCGGTAAAGTA ACTGCAGTTGACGCTAAAGGCGCAACCGTAGAACTGGCTGACGGCGTTGAAGGTTACCTGC GTGCTTCTGAAGCATCCCGTGACCGCGTTGAAGACGCTACCCTGGTTCTGAGCGTTGGCGAC

	GAAGTTGAAGCTAAATTCACCGGCGTTGATCGTAAAAACCGCGCAATCAGCCTGTCTGTTCCG TGCGAAAGACGAAGCTGACGAGAACT CTGGTGCCGCGCGGCAGCCTCGAG
S1 domain of PNPase [NcoI, XhoI]	<u>CCATGGC</u> CAGAAATCGAAGTGGGCGCGTCTACACTGGTAAAGTGACCCGTATCGTTGACTTT GGCGCATTTGTTGCCATCGGCGGCGGTAAAGAAGGTCTGGTCCACATCTCTCAAATCGCTGA CAAACGCGTTGAGAAAGTGACCGATTACCTGCAGATGGGTGAGGAAGTACCGGTGAAAGTT CTGGAAGTTGATCGCCAGGGCCGTATCCGTCTGAGCATTAAAGAAGCGACTGAGCAGTCTCA ACCTGCTGCA CTGGTGCCGCGCGGCAGCCTCGAG
pET28 ARH1 [NcoI, XhoI]	<u>CCATGG</u> AAAAATACGTCGCCGCGATGGTTTTGTCAGCTGCTGGCGATGCTTTGGGATATTAT AATGGAAAGTGGGAATTTCTTCAGGACGGGGAGAAAATTCATCGTCAACTGGCTCAATTAGG GGGGCTGGATGCTCTGGACGTTGGCCGTTGGCGTGTGTCTGATGATACTGTCATGCACTTGG CAACAGCCGAGGCTTTGGTCGAGGCCGAAAGGCTCCAAACTGACTCAGCTTTATTATTG TTAGCCAAGCACTATCAGGATTGCATGGAAGATATGGACGGTCGCGCACCCGGGGGTGCGT CTGTACACAACGCGATGCAGCTTAAACCTGGGAAACCGAATGGCTGGCGTATCCCATTAAAC TCGCATGAAGGAGGGTGTGGCGCGGCGATGCGCGCGATGTGTATCGGTTTGCGTTTTCCGC ATCACTCTCAATTAGACACACTGATCCAAGTATCGATCGAGTCAGGACGTATGACCCATCATC ACCCGACAGGGTACCTTGCGCACTTGCGTCCGCCTTATTCACGGCCTATGCGGTAAATAGCC GCCCTCCATTGCAGTGGGGTAAGGGACTTATGGAGCTTTTGCCAGAGGCTAAAAAATACATT GTCCAATCCGGGTACTTTGTGGAAGAAAATTTACAGCATTGGTCTTATTTCAAACGAAGTGG GAAAACTATCTTAAACTGCGTGGAATCTTGACGGCGAGAGTGCTCCAACATTCCCTGAATCT TTTGCGGTAAAGAGCGCGACCAGTTCTACACTTCGTTGTCATATAGTGGCTGGGGCGGTTT ATCTGGGCATGATGCCCCATGATCGCGTATGACGCGGTGCTGGCGGCGGGAGACTCCTGG AAAGAGCTTGCGCACCGCGCCTTCTTTCACGGAGGTGACTCGGATTCGACCGCAGCCATTGC TGGATGTTGGTGGGGCGTCATGTACGGATTTAAGGGCGTCAGCCCCAGCAACTACGAAAAA TTAGAGTATCGCAATCGCCTTGAGGAAACAGCTCGCGCACTTTACTCGCTGGGTAGTAAAGA AGACACTGTTATCTCGCTG CTGGTGCCGCGCGGCAGCCTCGAG
pET ARH1 D55A, D56A [NcoI, XhoI]	<u>CCATGG</u> AAAAATACGTCGCCGCGATGGTTTTGTCAGCTGCTGGCGATGCTTTGGGATATTAT AATGGAAAGTGGGAATTTCTTCAGGACGGGGAGAAAATTCATCGTCAACTGGCTCAATTAGG GGGGCTGGATGCTCTGGACGTTGGCCGTTGGCGTGTGTCT GCGGCG ACTGTCATGCACTTGG CAACAGCCGAGGCTTTGGTCGAGGCCGAAAGGCTCCAAACTGACTCAGCTTTATTATTG TTAGCCAAGCACTATCAGGATTGCATGGAAGATATGGACGGTCGCGCACCCGGGGGTGCGT CTGTACACAACGCGATGCAGCTTAAACCTGGGAAACCGAATGGCTGGCGTATCCCATTAAAC TCGCATGAAGGAGGGTGTGGCGCGGCGATGCGCGCGATGTGTATCGGTTTGCGTTTTCCGC ATCACTCTCAATTAGACACACTGATCCAAGTATCGATCGAGTCAGGACGTATGACCCATCATC ACCCGACAGGGTACCTTGCGCACTTGCGTCCGCCTTATTCACGGCCTATGCGGTAAATAGCC GCCCTCCATTGCAGTGGGGTAAGGGACTTATGGAGCTTTTGCCAGAGGCTAAAAAATACATT GTCCAATCCGGGTACTTTGTGGAAGAAAATTTACAGCATTGGTCTTATTTCAAACGAAGTGG GAAAACTATCTTAAACTGCGTGGAATCTTGACGGCGAGAGTGCTCCAACATTCCCTGAATCT TTTGCGGTAAAGAGCGCGACCAGTTCTACACTTCGTTGTCATATAGTGGCTGGGGCGGTTT ATCTGGGCATGATGCCCCATGATCGCGTATGACGCGGTGCTGGCGGCGGGAGACTCCTGG AAAGAGCTTGCGCACCGCGCCTTCTTTCACGGAGGTGACTCGGATTCGACCGCAGCCATTGC TGGATGTTGGTGGGGCGTCATGTACGGATTTAAGGGCGTCAGCCCCAGCAACTACGAAAAA TTAGAGTATCGCAATCGCCTTGAGGAAACAGCTCGCGCACTTTACTCGCTGGGTAGTAAAGA AGACACTGTTATCTCGCTG CTGGTGCCGCGCGGCAGCCTCGAG

pET rL2 [NcoI, XhoI]	CCATGGGCGCAGTTGTTAAATGTAAACCGACATCTCCGGGTCGTCGCCACGTAGTTAAAGTG GTTAACCCCTGAGCTGCACAAGGGCAAACCTTTTGCTCCGTTGCTGGAAAAAACAGCAAATC CGGTGGTCGTAACAACAATGGCCGTATCACCCTCGTCATATCGGTGGTGGCCACAAGCAGG CTTACCGTATTGTTGACTTCAAACGCAACAAAGACGGTATCCCGGCAGTTGTTGAACGTCTTG AGTACGATCCGAACCGTTCCGCGAACATCGCGCTGGTTCTGTACAAAGACGGTGAACGCCGT TACATCCTGGCCCCTAAAGGCCTGAAAGCTGGCGACCAGATTCAGTCTGGCGTTGATGCTGC AATCAAACCAGGTAACACCCTGCCGATGCGCAACATCCCGTTGGTTCTACTGTTCATAACGT AGAAATGAAACCAGGTAAAGGCGGTCAGCTGGCACGTTCCGCTGGTACTTACGTTCAGATCG TTGCTCGTGATGGTGCTTATGTCACCCTGCGTCTGCGTTCTGGTGAAATGCGTAAAGTAGAAG CAGACTGCCGTGCAACTCTGGGCGAAGTTGGCAATGCTGAGCATATGCTGCGCGTTCTGGGT AAAGCAGGTGCTGCACGCTGGCGTGGTGTTCTGTCGACCGTTTCGCGGTACCGCGATGAACCC GGTAGACCACCCACATGGTGGTGGTGAAGGTCGTAACCTTTGGTAAGCACCCGGTAACCTCGT GGGGCGTTCAGACCAAAGGTAAGAAGACCCGCAGCAACAAGCGTACTGATAAATTCATCGT ACGTCGCCGTAGCAAACTCGAG
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Supplementary Table 9: Primers used in this study. Corresponding restriction site in bold, underlined; mutation in bold and red

Primer	Sequence (5' to 3')
Fwd Q β T7	TAATACGACTCACTATTATCTTGATACTACCTTTAG
Rev Q β	CATGATCAAATTGACCCAAAGTTTCAACGCTTTACGCG
Fwd RNAI T7	TAATACGACTCACTATAACAGTATTTGGTATC
Rev RNAI	ACAAAAAACCACCGCTACCAGCGGTGGTTTGTTC
Fwd Alt NcoI	ATCGAC <u>CCATGG</u> GAGAACTTATTACAGAATTATTTGACG
Rev Alt XhoI	ATTCGA <u>CTCGAG</u> GCTGCCGCGCGGCACCAGTCCTTGAACGAACTTGTAAAGCATG
Fwd ModA NcoI	ATCGAC <u>CCATGG</u> GAAAAATACTCAGTAATGCAACTAAAAG
Rev ModA XhoI	ATCGTA <u>CTCGAG</u> GCTGCCGCGCGGCACCAGTAGATTAAATCCTTCAAAATCAAG
Fwd ModB NcoI	ATCGAC <u>CCATGG</u> GAAATTATTAATCTTGCAGATGTTG
Rev ModB XhoI	ACTTAG <u>CTCGAG</u> GCTGCCGCGCGGCACCAGTTCAACTACCTCAATATCATAAAC
Fwd rS1 NcoI	ATCGAC <u>CCATGG</u> GAACTGAATCTTTGCTCAACTCTTTGAAGAGTCC
Rev rS1 XhoI	ATTCGA <u>CTCGAG</u> GCTGCCGCGCGGCACCAGCTCGCCTTTAGCTGCTTTG
Fwd rS1-pTAC XhoI	ATGAAGCTTC <u>CTCGAG</u> AGACTGAATCTTTGCTCAACTCTTTGAAGAGTCC
Rev rS1-pTAC SphI	CTCTACGT <u>GCATGC</u> ACTCGCCTTTAGCTGCTTTGAAAGCTTCAGCC
Fwd NcoI rS1 D1	ATCGAC <u>CCATGG</u> AGTCCTTAAAAGAAATCGAAACCCGCCGGG
Rev XhoI rS1 D1	TGGTG <u>CTCGAG</u> GCTGCCGCGCGGCACCAGGGCTTCGTGACGTTTAGCTTTCTCACGGG
Fwd NcoI rS1 D2	ATCGAC <u>CCATGG</u> CCTGGATCACGCTGGAAAAAGCTTACGAAGATGCTGAAAC
Rev XhoI rS1 D2	GGTG <u>CTCGAG</u> GCTGCCGCGCGGCACCAGCTCTGCGCTGTTTTCGGATTCGATAACGGCAC
Fwd NcoI rS1 D3	ATCGAC <u>CCATGG</u> CCCGCGATCAGCTGCTGGAAACCTGCAGGAAGG
Rev XhoI rS1 D3	TGGTG <u>CTCGAG</u> GCTGCCGCGCGGCACCAGCGGATCTTCGCCAGCTGTTTCAGGCCAGG
Fwd NcoI rS1 D4	ATCGAC <u>CCATGG</u> CCTGGGTAGCTATCGCTAAACGTTATCCGGAAGG

Rev XhoI rS1 D4	TGGTG CTCGAG GCTGCCGCGCGGCACCAGCGGGTTAGCTTTGCACTGTTTCAG ACCCAGGGAG
Fwd NcoI rS1 D5	ATCGAC CCATGG CCTGGCAGCAGTTCGCGGAAACCCACAACAAGGGCGACCG TGTTG
Rev XhoI S1 D5	TGGTG CTCGAG GCTGCCGCGCGGCACCAGCGGATCTTCTGCGAGCTGTTTAAC GCCCAGGGAGATACG
Fwd NcoI rS1 D6	ATCGAC CCATGG CCTTCAACAACCTGGGTTGCTCTGAACAAGAAAGGCGCTATC G
Rev XhoI rS1 D6	TGGTG CTCGAG GCTGCCGCGCGGCACCAGTTTCTCGTCAGCTTCGTCTTTCGCA CGAACAGACAGG
Fwd NcoI PNPase rS1 binding	ATCGAC CCATGG CAGAAATCGAAGTGGGCCGCGTCTACACTGGTAAAGTGACC CG
Rev XhoI PNPase rS1 binding	TGGTG CTCGAG GCTGCCGCGCGGCACCAGTGCAGCAGGTTGAGACTGCTCAG TCGCTTC
Fwd ARH1 NcoI	TGCAG CCATGG AAAAATACGTCGCCGCGATG
Rev ARH1 XhoI	GTGGTG CTCGAG GCTGCCGCGCGGCACCAG
Fwd rS1 R139A	CTGGTAGACGTT CCCC GGTGCGTGACACTC
Fwd rS1 R139K	CTGGTAGACGTT AAAC CGGTGCGTGACACTC
Rev rS1 R139	AGAACCTGGCAGGAACGCACGAATACCG
Fwd ARH1 D55,56A	GGCCGTTGGCGTGTGTCT GCGGCG ACTGTCATGCACTTGGC
Rev ARH1 D55,56A	AACGTCCAGAGCATCCAGCCCCCTAA
Fwd ModB R73A	CCTTATCAATTATAT GCG GGTATATCAAATCG
Rev ModB R73A	CGATTTAGTTAAATGCTTTTTAATGATTTC
Fwd ModB G74A	GACAAAAGAACTCATTAAAGATTTC
Rev ModB G74A	GATTTTGATAT TGCCGC ATATAATTGATAAGGCG
Fwd ModB DS_SPCas	AATTATATCGGTTTTAGAGCTATGCTGTTTTGAATGGTCC
Rev ModB DS_SPCas	GATAAGGCGAGCTAGCACTGTACCTAGGACTGAGC
Fwd ModB amplification T4 genome	CCAAGAATGGTCATCTGGTTTATTAG

Rev ModB amplification T4 genome	CCGCCTGGGCTCCCTGG
Fwd sequencing ModB T4 genome	CAGTTATCTATAAAAGCTGAAAG
Rev sequencing ModB T4 genome	CTTTCCAATTGGAATCATCCATTC
rpsA homologous downstream fwd	TTCTCTGACTCTTCGGGATTTTATTC
rpsA homologous downstream rev	AGGCAAATTAAGCGGCTGCTG
Terminator region fwd	TTCTCTGACTCTTCGGGATTTTATTC
Terminator region rev	AGGACGAAACCTGCAATCTGTC
FRT pKD4 fwd	TCGGAATAAAAATCCCGAAGAGTCAGAGAAGTCCATATGAATATCCTCCTTAG TTC
FRT pKD4 rev	GTTTACTTGACAGATTGCAGGTTTCGTCCTGTGTAGGCTGGAGCTGCTTC
5_70 left rev rpsA	AGGACGAAACCTGCAATCTGTC
5_fwd_rS1 amplification	GGCGTTGATCGTAAAAACCGC
Fwd NcoI rL2	<u>CCATGG</u> GCGCAGTTGTAAATGTAAACCG
Rev XhoI rL2	<u>CTCGAG</u> TTTGCTACGGCGACGTACGATG
adenylated RNA-3'- adapter	/5rApp/CNNNNNNAGATCGGAAGAGCACACGTCTG/3SpC3/
RT primer	CAGACGTGTGCTCTTCCGAT
cDNA anchor fwd	ACACGACGCTCTTCCGATCTGGG
cDNA anchor rev	/5Phos/CAGATCGGAAGAGCGTCGTGTCCC/3SpC3/
qPCR acpP fwd	CGTGGTAAGACCTGCCGG
qPCR acpP rev	CTCAACGGTGTCAAGAGAATCCAAAAC

qPCR gadY fwd	GAGCACAAAGTTTCCCGTGC
qPCR gadY rev	AAACCCGGCATAGGGGACC
qPCR mcaS fwd	AAAATAGAGTCTGTGACATCCGC
qPCR mcaS rev	CACCGGCGCAGAGGAGAC
qPCR oxyS fwd	AAAAGCGGATCCTGGAGATCC
qPCR oxyS rev	GAAACGGAGCGGCACCTC
qPCR rnaC fwd	CGTTGCGGCAACCTTGTC
qPCR rnaC rev	AAAAATATTGAGTAGCGTCAACTAC

Supplementary Table 10: Strains and plasmids used in this study.

Name	Description	Reference or resource
<i>E. coli</i> strain B	<i>E. coli</i> strain applied for bacteriophage T4 infection	DMSZ, <i>Escherichia coli</i> (Migula 1895) Castellani and Chalmers 1919 (DSM 613, ATCC 11303)
<i>E. coli</i> strain B pTAC rS1	<i>E. coli</i> strain B expressing His-tagged rS1 under the control of <i>E. coli</i> RNA polymerase promoter	This study
<i>E. coli</i> BL21 (DE3) pET16 RNase E (1-529)	<i>E. coli</i> strain expressing His-tagged catalytic domain of RNase E (1-529)	Plasmid was a kind gift from Prof. Dr. Ben Luisi ⁶⁷
<i>E. coli</i> BL21 (DE3) pET 28 NudC V157A, E174A, E177A, E178A	<i>E. coli</i> strain expressing His-tagged inactive Mutant of NudC	⁶⁸
<i>E. coli</i> BL21 (DE3) pET 28 rS1	<i>E. coli</i> strain expressing His-tagged rS1	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 R139K	<i>E. coli</i> strain expressing His-tagged rS1 R139K variant	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 R139A	<i>E. coli</i> strain expressing His-tagged rS1 R139A variant	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 D1	<i>E. coli</i> strain expressing His-tagged rS1 D1	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 D2	<i>E. coli</i> strain expressing His-tagged rS1 D2	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 D2 R139K	<i>E. coli</i> strain expressing His-tagged rS1 D2 R139K	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 D2 R139A	<i>E. coli</i> strain expressing His-tagged rS1 D2 R139A	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 D3	<i>E. coli</i> strain expressing His-tagged rS1 D3	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 D4	<i>E. coli</i> strain expressing His-tagged rS1 D4	This study
<i>E. coli</i> BL21 (DE3) pET 28 rS1 D5	<i>E. coli</i> strain expressing His-tagged rS1 D5	This study

<i>E. coli</i> BL21 (DE3) pET 28 rS1 D6	<i>E. coli</i> strain expressing His-tagged rS1 D6	This study
<i>E. coli</i> BL21 (DE3) pET 28 Alt	<i>E. coli</i> strain expressing His-tagged Alt	This study
<i>E. coli</i> BL21 (DE3) pET 28 ModA	<i>E. coli</i> strain expressing His-tagged ModA	This study
<i>E. coli</i> BL21 (DE3) pET 28 ModB	<i>E. coli</i> strain expressing His-tagged ModB	This study
<i>E. coli</i> BL21 (DE3) pET 28 ModB R73A, G74A	<i>E. coli</i> strain expressing His-tagged ModB with point mutations R73A and G74A	This study
<i>E. coli</i> BL21 (DE3) pET 28 NudC	<i>E. coli</i> strain expressing His-tagged NudC	⁶⁸
<i>E. coli</i> BL21 (DE3) pET 28 PNPase S1 domain	<i>E. coli</i> strain expressing His-tagged PNPase S1 domain	This study
<i>E. coli</i> BL21 (DE3) pET 28 ARH1	<i>E. coli</i> strain expressing His-tagged ARH1	This study
<i>E. coli</i> BL21 (DE3) pET 28 ARH1 D55A, D56A	<i>E. coli</i> strain expressing His-tagged ARH1 D55A, D56A	This study
<i>E. coli</i> DHα DS_SPCas_ModB	<i>E. coli</i> strain expressing CRISPR-Cas9 system for cleavage of <i>modB</i>	This study
<i>E. coli</i> DHα DS_SPCas_ModB pET28 ModB R73A, G74A	<i>E. coli</i> strain for editing of <i>modB</i> within T4 phage genome	This study
<i>E. coli</i> BL21 (DE3) pET28 rL2	<i>E. coli</i> strain expressing His-tagged rL2	This study
<i>E. coli</i> B strain with endogenously His-tagged rS1	<i>E. coli</i> strain with endogenous expression of rS1 with a His-tag fusion at the C-terminus	This study
T4 WT	Wild-type bacteriophage T4	Escherichia phage T4, DSM 4505, DSMZ, Braunschweig, Germany)
T4 ModB R73A, G74A	T4 phage mutant carrying inactive ModB version ModB R73A, G74A	This study

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- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
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Data collection	Manufacturer's Software for: MiniSeq (Illumina), ChemiDoc (Bio-Rad), Typhoon scanner (GE Healthcare), Orbitrap Exploris 480 Mass Spectrometer (Thermo Fisher Scientific), Ultimate 3000 nanoLC-system (Thermo Fisher Scientific), Dionex Ultimate 3000 RSLCnano system (Thermo Fisher Scientific), Tecan plate reader (Spark)
Data analysis	OpenMS pipeline RNPxl and OpenMS TOPPASViewer (OpenMS Version NuXL-2022-04-27), Skyline (v 21.2.0.369), MaxQuant (v 1.6.17.0 and v 2.0.3.0), Scaffold 5.2.2 for LC-MS/MS data analysis bcl2fastq (v 2.20.0, Illumina), FastQC (v 0.11.9), cutadapt (v 1.18), hisat2 (v 2.2.1), samtools (v1.7), featureCounts (v 2.0.1 from Subread package), R (v 4.1.2), Integrative Genomics Viewer (IGV, v 2.4.9) for RNAseq data analysis; the custom R Script is available (https://doi.org/10.5281/zenodo.7977386) AlphaFold2 (v 1.3.0 via ColabFold: https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb) ImageLab (v 6.1) and ImageQuant (v 5.2) for gel analysis and signal quantification Microsoft Excel 2016 (v 16.0.5395.1000) for basic calculations and normalisations including in-gel digest spectrum count data from Scaffold Origin Pro (v 2020b) and R (v 4.1.2 and 4.2.2) for plotting data and statistical tests

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The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. NGS data is accessible via GEO record GSE214431. LC-MS/MS raw data for measurements of rS1 ADP-ribosylation in vivo, in-gel digest and estimation of ModB abundance have been deposited in PRIDE under the accession PXD041714. LC-MS/MS raw data for measurements of in vitro ADP-ribosylated and RNAylated rS1 and rL2 have been deposited in PRIDE under the accession PXD038910. Reference genomes for E. coli (U00096.3, <https://www.ncbi.nlm.nih.gov/nucleotide/U00096.3>) and T4 phage (NC_000866.4, https://www.ncbi.nlm.nih.gov/nucleotide/NC_000866.4) were retrieved from NCBI. Protein structures (2MFI, 2MFL, 2KHI, 5XQ5, 2KHJ, 7K00, 6H4N) were downloaded from PDB using the indicated accession code (<https://www.rcsb.org/>). E. coli K12 pan proteome (UP000000625) and selected protein sequences were retrieved from Uniprot (<https://www.uniprot.org/>). Supplementary information is available including raw gel and blot images. Source data are provided with this paper.

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Sample size	No sample size calculation was performed. Assays were conducted in at least technical duplicates for in vitro prepared RNAs or biological triplicates (for quantification of modification) or duplicates (for qualitative assessment of effects). Sample size was determined based on similar studies in the field: Sharma S. et al. (2022) Xrn1 is a deNADding enzyme modulating mitochondrial NAD-capped RNA. Nat Commun.
Data exclusions	No data were excluded from analysis.
Replication	All statistical data shown in this manuscript is derived from at least three biologically independent replicates. Biochemical analyses shown were replicated at least twice (for qualitative effect) or three times (for quantitative effects). Mass spectrometry and RNA-Seq experiments were conducted in at least biological duplicates except for the assessment of ModB abundance and determination of RNAylation sites. All attempts of technical or biological replication were successful.
Randomization	No randomisation was necessary. Samples for biochemistry and mass spectrometry were measured sequentially, RNA-Seq was performed with all replicates at once. Images were acquired with state-of-the-art devices in an automated fashion. Humans, animals or individuals of different genders were no subjects of this study.
Blinding	No blinding was applied in this study. Blinding could not be performed, as samples were analysed or compared in a pairwise fashion or in groups using suitable controls for each experiment. In all assays of this study, the treatment cannot be disguised from the scientist.

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☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

1. Anti-pan-ADP-ribose binding reagent (Merck, MABE1016)
2. Goat-anti-rabbit HRP-conjugated secondary antibody (Advansta, 541088)

Validation

- please see manufactures specifications:
1. https://www.merckmillipore.com/DE/de/product/Anti-pan-ADP-ribose-binding-reagent,MM_NF-MABE1016
 2. <https://products.advansta.com/protein-HRP-conjugates/>

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Escherichia coli (DSM 613, ATCC 11303), Bacteriophage T4 (DSM 4505)

Wild animals

Does not apply

Reporting on sex

Does not apply

Field-collected samples

Does not apply

Ethics oversight

Does not apply

Note that full information on the approval of the study protocol must also be provided in the manuscript.