Supplementary Material

The long 5′UTR of *nrdAB* modulates transcription, mRNA stability, and virulence in *Pseudomonas aeruginosa*

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Table S1. Plasmids and strains.

Name	Reference name	Description	Source
Plasmids			
pJET1.2/blunt	pJET1.2	Blunt-end vector; Amp^R	Thermo Scientific
pETS130-GFP	pETS130	Broad host range, promoterless GFP; GmR	[1]
pETS134	pETS130-PA	pETS130 derivative carrying *nrdA* promoter; Gm^R	[2]
pETS257	pETS-PA- Δ5'UTR	pETS130 derivative carrying *nrdA* promoter without 5'UTR; Gm^R	This work
pETS258	pETS130-PA::*gfp*	pETS130 derivative carrying *nrdA* promoter translationally fused to *gfp*; Gm^R	This work
pETS259	pETS130-PA-Δ5'UTR::*gfp*	pETS130 derivative carrying *nrdA* promoter without Δ5'UTR translationally fused to *gfp*; Gm^R	This work
pJN105	pJN105	Expression vector with *araBAD* promoter. Gm^R	[3]
pJN106	pJN-5'UTRfor	pJN105 derivative carrying 5'UTR forward strand. Gm^R	This work
pJN107	pJN-5'UTRrev	pJN105 derivative carrying 5'UTR reverse strand. Gm^R	This work
pSH624-ssr	pSH624	Plasmid that expresses the gen coding for the Ssr recombinase from *Pseudomonas putida* DOT-T1E. Gm^R	Gift from Susanne Häußler (Addgene plasmid # 199241)[4]
pS148-CsR	pS148	Plasmid that expresses *Streptococcus pyogenes'* Cas9 and a tailored sgRNA for counterselection in gram-negative bacteria. Cb^R	Gift from Susanne Häußler (Addgene plasmid # 197858)[4]
pS149	pS148-Δ5'UTR	pS148-CsR derivative carrying sgRNA for 5'UTR mutant. Cb^R	This work
Strains			
E. coli			
DH5a	DH5a	*recA1 endA1 hsdR17 supE44 thi-1 relA1 Δ(lacZYA-argF) U169 deoR Φ80dlacZM15*	Laboratory
P. aeruginosa			
PAO1	PAO1 WT	Wild-type (ATCC 15692 / CECT 4122) - Spanish Type Culture Collection	Laboratory
PW9311	PAO1 Δ*rnr*	*P. aeruginosa* PAO1 *rnr::IS*phoA/hah; TcR	[5]
PW7510	PAO1 Δ*rhl*	*P. aeruginosa* PAO1 *rhl::IS*phoA/hah; TcR	[5]
PW5993	PAO1 Δ*rne*	*P. aeruginosa* PAO1 *rne*::*IS*phoA/hah; TcR	[5]
PAO1 Δ5'UTR	PAO1 Δ5'UTR	*P. aeruginosa* PAO1 5'UTR deletion mutant by CRISPR-cas9	This work

Table S2. Oligonucleotides.

Primer number	Name	Sequence (5 -> 3')	Application
1	PnrdA_GFP_up	AGGATCCGAATTCTTGCTCCACACAGCC TC	P*nrdA* promoter cloning/Sequencing
2	PnrdA_GFP_low	ACCCGGGTTCTCGCGTGTGGTGTCG	P*nrdA* promoter cloning/Sequencing
3	PnrdA_UTR_5'_low	TAGCGGGCATCAGAGTGGTCCGTGCG	P*nrdA*-Δ5'UTR promoter cloning/Sequencing
4	PnrdA_UTR 3'_up	ACTCTGATGCCCGCTAGCAAAACCATTA	P*nrdA*-Δ5'UTR promoter cloning/Sequencing and DNA absence test
5	PnrdA_SmaI_Rv	AAACCCGGGCTCGCGTGTGGTGTCGG	P*nrdA* promoter cloning/Sequencing
7	GFP_SmaI_Fw	AAACCCGGGAGTAAAGGAGAAGAACTT TTCACTG	GFP cloning
8	GFP_KpnI_Rv	AAGGTACCCCCCTCGAAGCTTGCAT	GFP cloning
9	pJN105_UTR_EcoRI_Fw	GAATTCCCCCATTAGCGCATTATCTTG	pJN105 cloning 5'UTR fw
10	pJN105_UTR_PstI_Rv	CTGCAGTGGGTATCTCCACGTTCTC	pJN105 cloning 5'UTR fw
11	pJN105_UTR_PstI_Fw	CTGCAGCCCCATTAGCGCATTATCTTG	pJN105 cloning 5'UTR Rv
12	pJN105_UTR_EcoRI_Rv	GAATTCGTGGGTATCTCCACGTTCTC	pJN105 cloning 5'UTR Rv
13	putORF-UTR up	TGACGTTGGAAAAGCGGTAC	pJN105 cloning check
14	putORF-UTR low	CGAATGCTGAGGGTGGGGAC	pJN105 cloning check
15	aaSSR-1F	TGAGCCAAGTAGCCAGGGTCG	Ssr amplification, curation plasmid test
16	aaSSR-1R	GATTTGTAGACGTTGGCAGCGCAG	Ssr amplification, curation plasmid test
17	aaCas9-1F	AAGCACAAGTGTCTGGACAAGG	Cas9 amplification, curation plasmid test
18	aaCas9-1R	TCACTCAAACCTCCACGTTCAGC	Cas9 amplification, curation plasmid test
19	SEVA-T0-F	GAACGCTCGGTTGCCGCC	Check guide cloning
20	5'UTR_PAO1(+)-Rec	GAGCGCACGAACGGCTACAACGGAACC AACCGCGCACGGACCACTCTGATGCCC GCTAGCAAAACCATTACGAGAACGTGG AGATACCCACCCATGCATA	Recombineering oligonucleotide CRISPR-cas9
21	sgRNA_UTR_Fw	gcgcgCCGTCGAACACAATACCTAG	Single guide cloning CRISPR-cas9
22	sgRNA_UTR_Rv	aaacCTAGGTATTGTGTTCGACGGc	Single guide cloning and check cloning CRISPR-cas9
23	PnrdA up	CTACGGACGCTTCCTAGG	UTR mutant PCR testing CRISPR-cas9
24	PA +1 low	GGTATGCATGGGTGGGTATC	UTR mutant PCR testing CRISPR-cas9 and SP2 in *nrdA* 5'RACE
25	PE1-nrdA	TCCTTGCGATGAGCGCCGTCG	Primer extension
26	PE2-nrdA	CAGCCGGCCTCGCGGCGGC	Primer extension
27	27f	AGAGTTTGATCMTGGCTCAG	Calibration curve 16S amplification
28	16sRNA 1306R	TTCACGCAGTCGAGTTGCA	Calibration curve 16S amplification
29	nrdATaqM2-low	TGTTCATGTCGTGGGTACG	Calibration curve *nrdA* amplification
30	qRTgreen_IVT_PAO-nrdA_fw	TCCCCTACACCGATGACAAG	Calibration curve *nrdA* amplification
31	qRTgreen_IVT_PAO-nrdjA_fw	CTCAAGAGCAAGGACGGTAC	Calibration curve *nrdJ* amplification
32	nrdjBamHIlow	GGATCCCGGCAGGCCGTTGATTTC	Calibration curve *nrdJ* amplification
33	T7 promoter	TAATACGACTCACTATAGGG	Calibration curve *cat* amplification
34	GFP mut3 Rv	GAATTGGGACAACTCCAGTG	Calibration curve *cat* amplification
35	greenQRT_PAO-nrdA_fw	ACCTGGAGAAACTGGGCAAG	qRT-PCR *nrdA*
36	greenQRT_PAO-nrdA_rv	TGTGGATGAAGTAGCGGTCG	qRT-PCR *nrdA*
37	greenQRT_PAO-nrdJA_fw	CGAATTCATCCGCGCCAAG	qRT-PCR *nrdJa*
38	greenQRT_PAO-nrdJA_rv	TCCACCGCCTGCATGAAC	qRT-PCR *nrdJa*
39	16S RNA qRTPCR fw	TGCCTGGTAGTGGGGGATAA	qRT-PCR 16S
40	16S RNA qRTPCR Rv	TCTGATAGCGTGAGGTCCGA	qRT-PCR 16S

41	qPCR_pETS130_cat_rv	AGCACAAGTTTTATCCGGCC	qRT-PCR *cat*
42	qPCR_pETS130_cat_FW	CATATCACCAGCTCACCGTC	qRT-PCR *cat*
43	IVT_PAO-nrdA_rv	ACCTGGTCCTGGATCTCTTC	DNA absence test
44	TSS_PAO_nrdA Rv	CTGGCCTGCGGGTTCTC	SP1 in *nrdA* 5'RACE
45	Oligo dT	GACCACGCGTATCGATGTCGACTTTTTTT TTTTTTTTT	PCR1 in 5'RACE
46	PCR Anchor Primer	GACCACGCGTATCGATGTCGAC	PCR2 in 5'RACE
47	TSS_PAO_UTRnrdA_rv	CGAATGCTGAGGGTGGGGAC	SP3 in *nrdA* 5'RACE
48	pJET 1.2 Fw	CGACTCACTATAGGGAGAGCGGC	Check cloning/Sequencing
49	pJET 1.2 Rv	AAGAACATCGATTTTCCATGGCAG	Check cloning/Sequencing
50	pUCP20T-up	CCTCTTCGCTATTACGCCAG	Check cloning/Sequencing

Figure S1. Riboswitch analysis. Results retrieved in 2021 by Riboswitch Scanner Software [6,7]

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Targeted riboswitch family: Cobalamin

MAIN STRAND (5'-3') :

No	Score	E-value	Region from	Region to
1	6.1	0.00058	249	378

COMPLEMENTARY STRAND (3'-5') :
No such riboswitch found

RIBOSWITCH SEQUENCES :
>seq:249-378
ATGACGTTGGAAAAGCGGTACGGGTGTTGCAACGGACGCTGTAAACAGACAGTCAACACC
ACTAGGTATTGTGTTCGACGGCAAAACGCCACGAACCCGCCAGGGCCAGGGAGGCACTCA
CAGTCCCCAC

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Figure S2. *nrdA* expression with and without the 5'UTR in PAO1 WT and RNA-processing mutants. (a) Transcriptional expression of *nrdA* in *P. aeruginosa* PAO1 WT pETS130-GFP derivative strains compared to chromosomal mutants in RNA-processing related genes. (**b)** Transcriptional expression of *nrdA* lacking the 5'UTR in PAO1 WT pETS130-GFP derivatives compared to RNA-processing related gene chromosomal mutants.

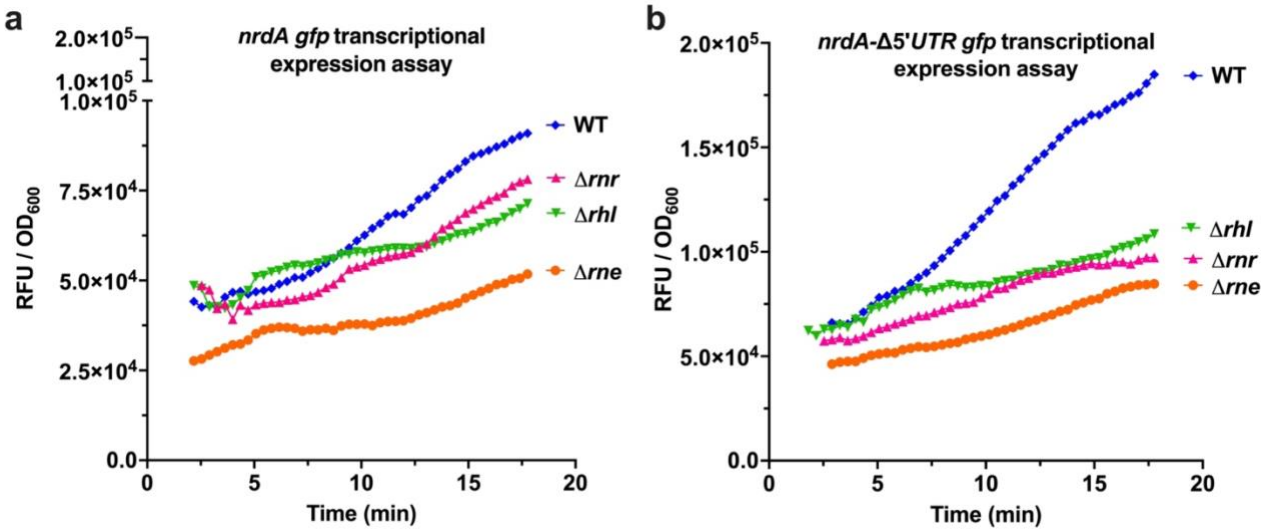


Figure S3. *nrdJ* expression under aerobic and anaerobic conditions. (a) Fold Change values of *nrdJ* expression measured by RT-qPCR in PAO1 Δ5'UTR (*nrdA*) (chromosomically deleted) compared to PAO1 WT, under both aerobic and anaerobic conditions during exponential and stationary growth phases. Significance: *p*-value <0.0001(****). **(b)** Fold Change values of *nrdJ* expression measured by RT-qPCR comparing PAO1 WT and PAO1 Δ5'UTR (*nrdA*) during *Galleria mellonella* infection **(c)** Western blot analysis of NrdJ protein levels in PAO1 WT and PAO1 Δ5'UTR (*nrdA*) during exponential and stationary growth under aerobic and anaerobic conditions. The cropped version is shown; the full-length and cropped blot is presented below in this Supplementary Figure S4 and is also available at https://doi.org/10.34810/data2361.

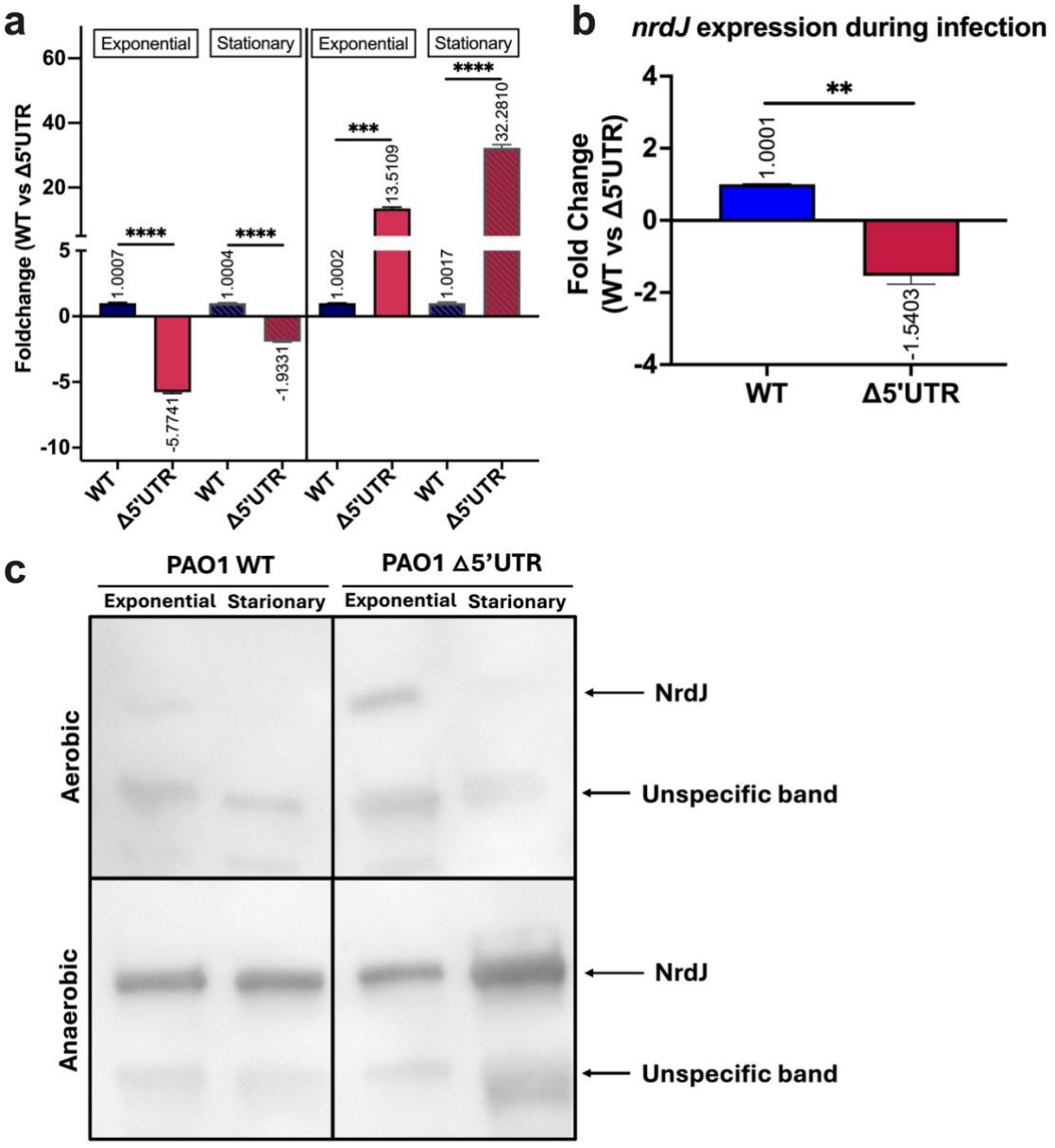
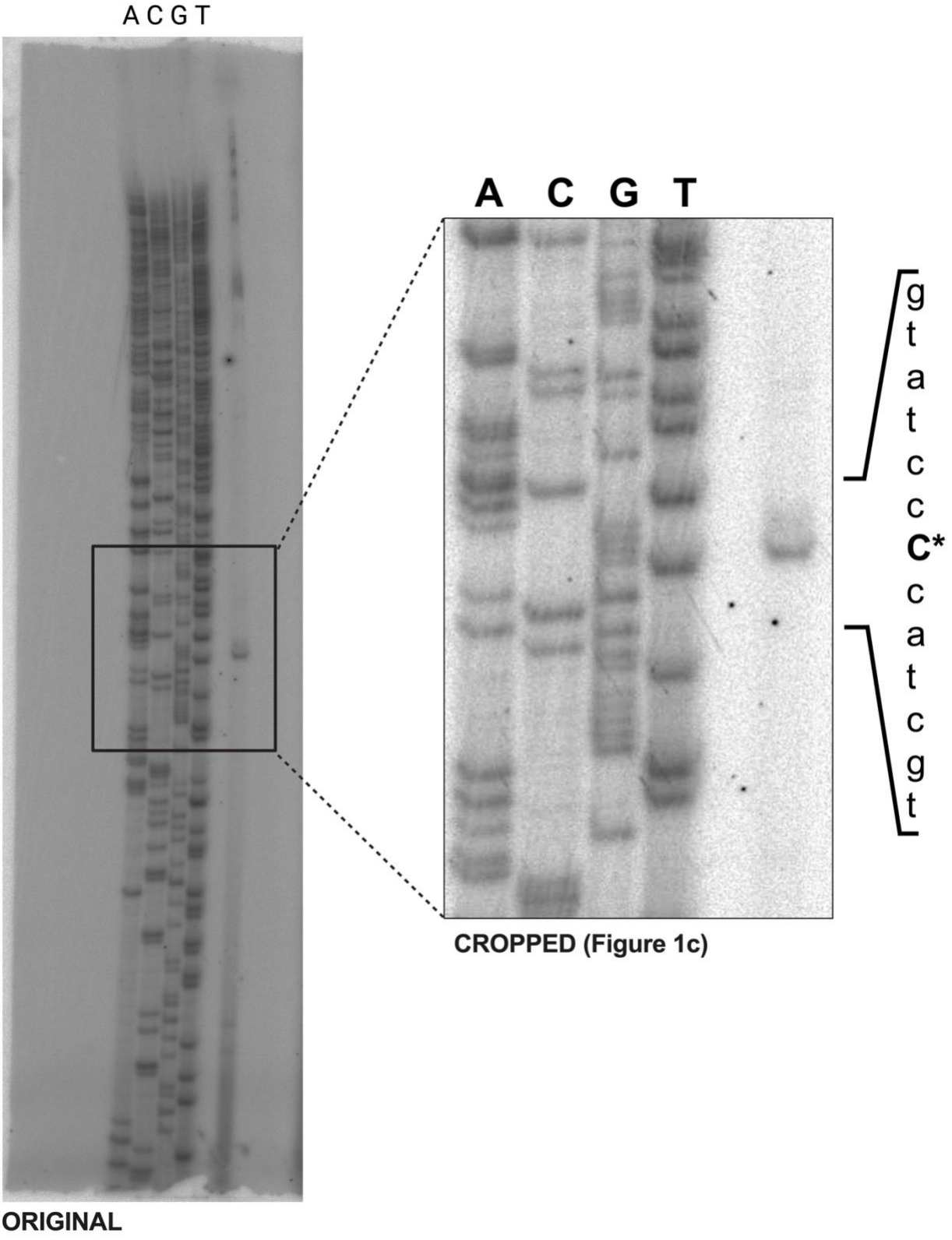
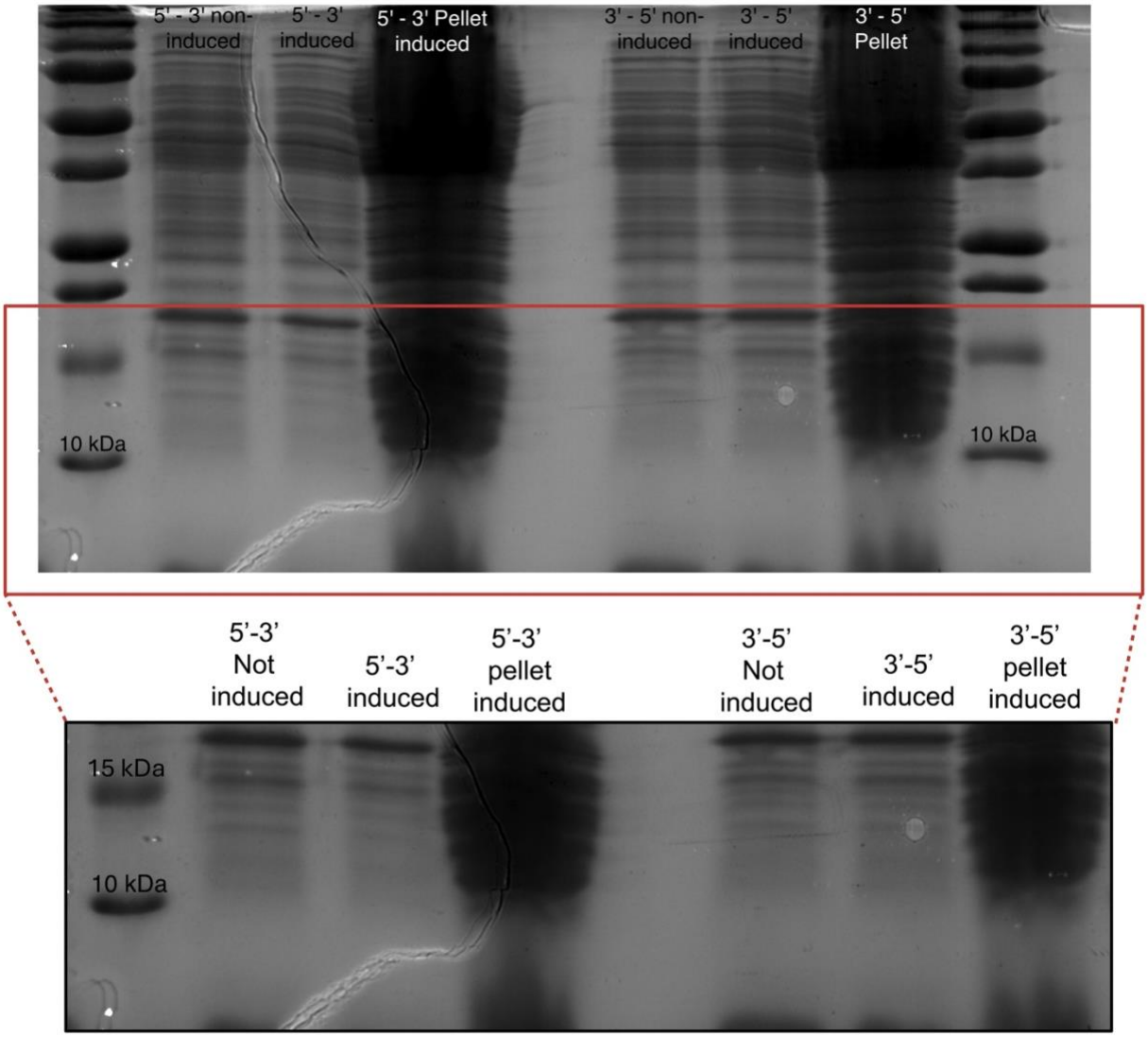


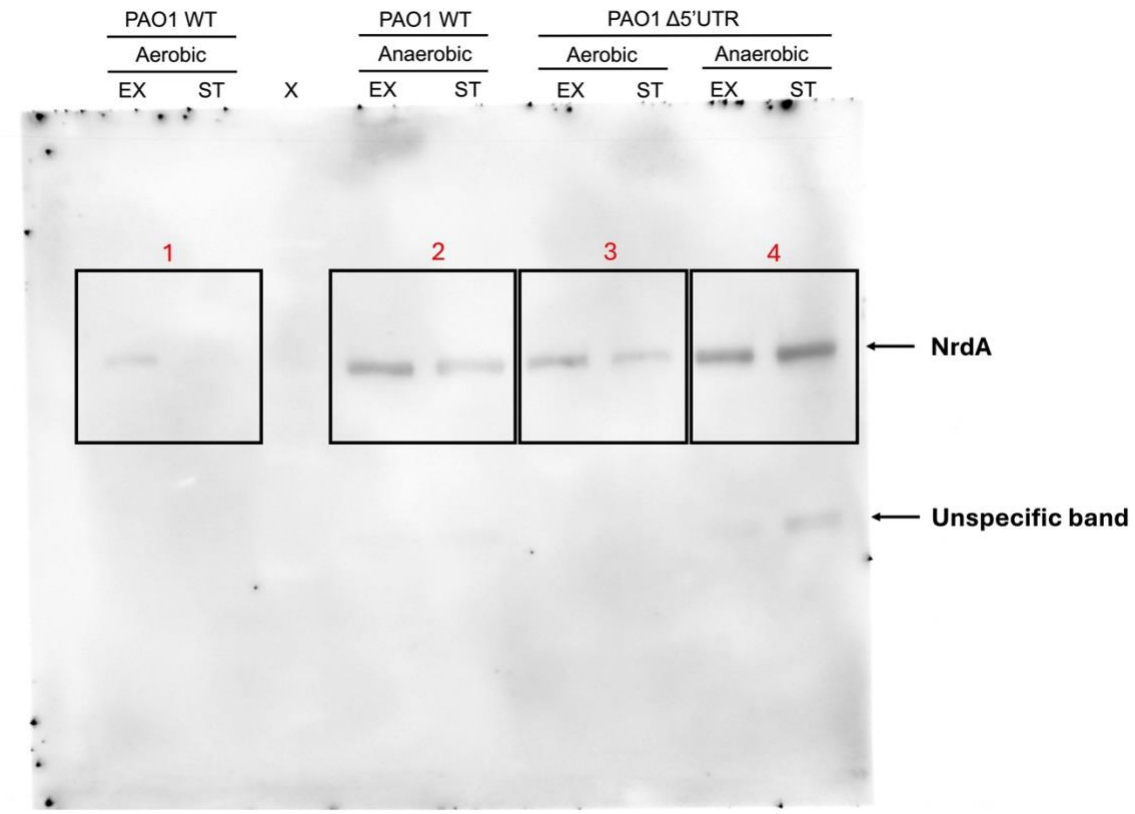
Figure S4. Original and cropped gels/blots from Figure 1c, Figure 3b, Figure 6c, Figure S3c



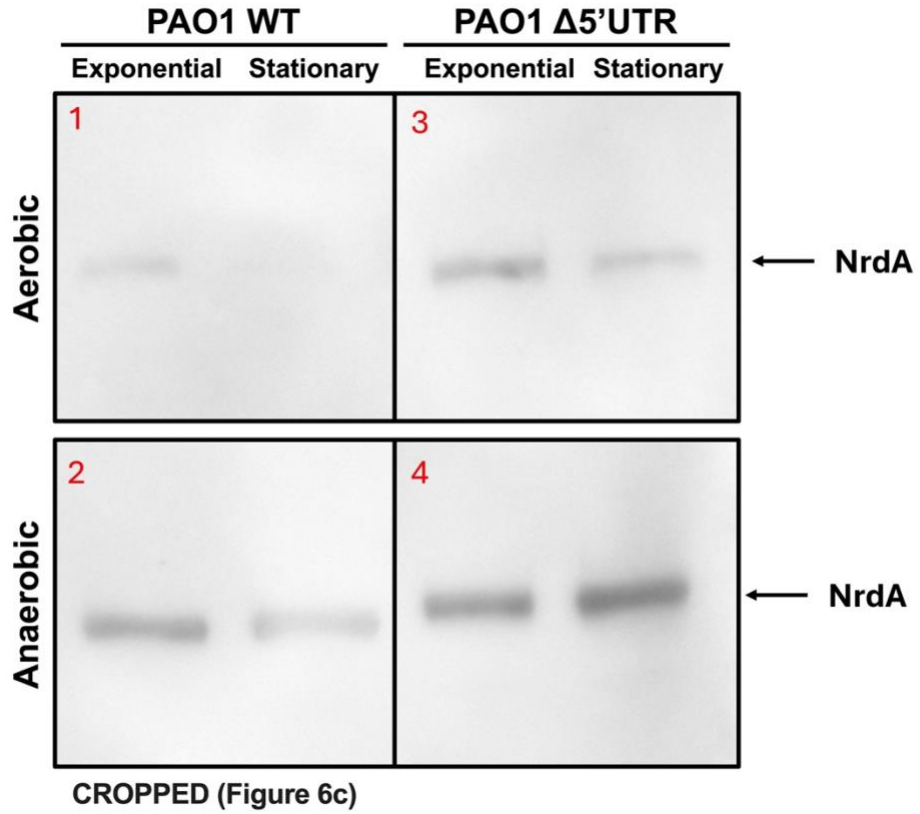
ORIGINAL



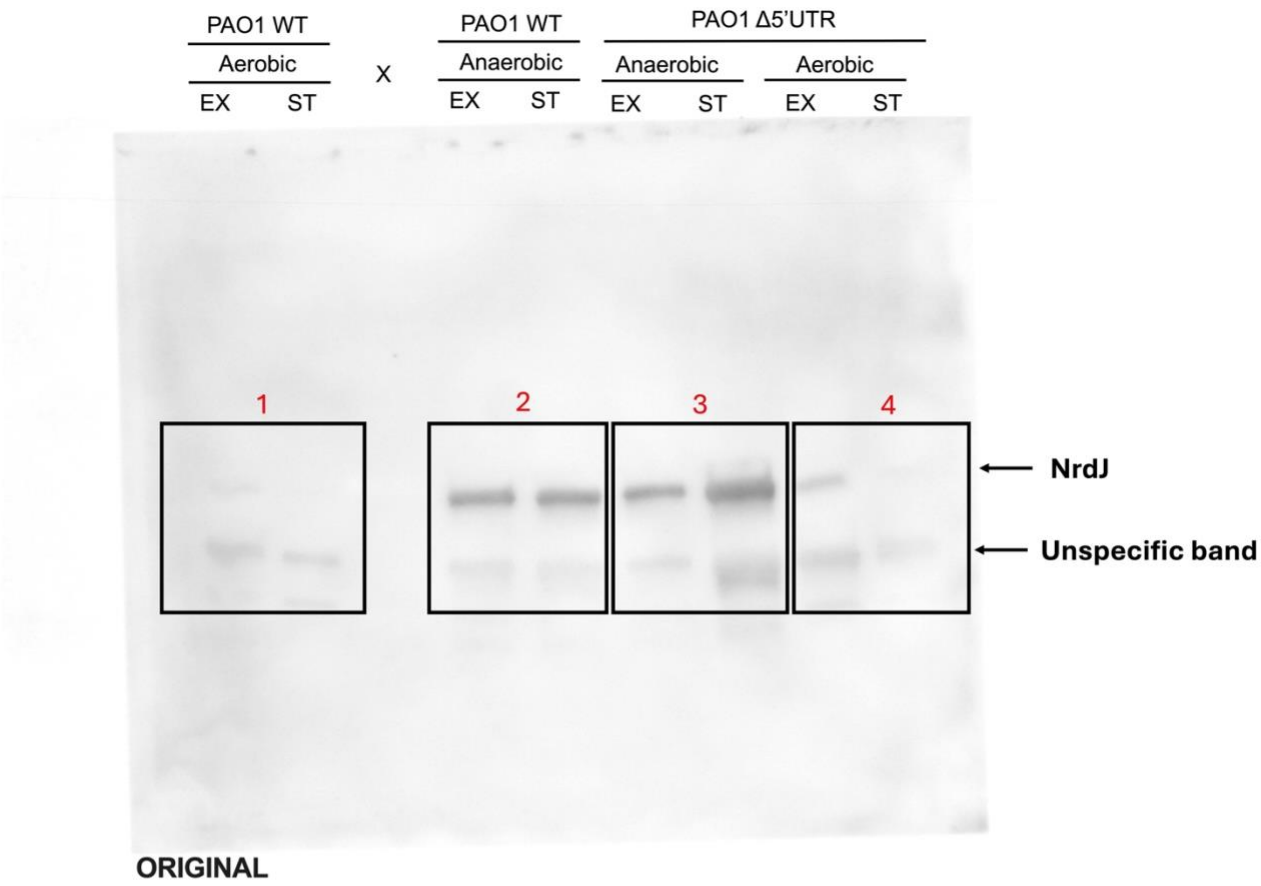
CROPPED (Figure 3b)



ORIGINAL



CROPPED (Figure 6c)



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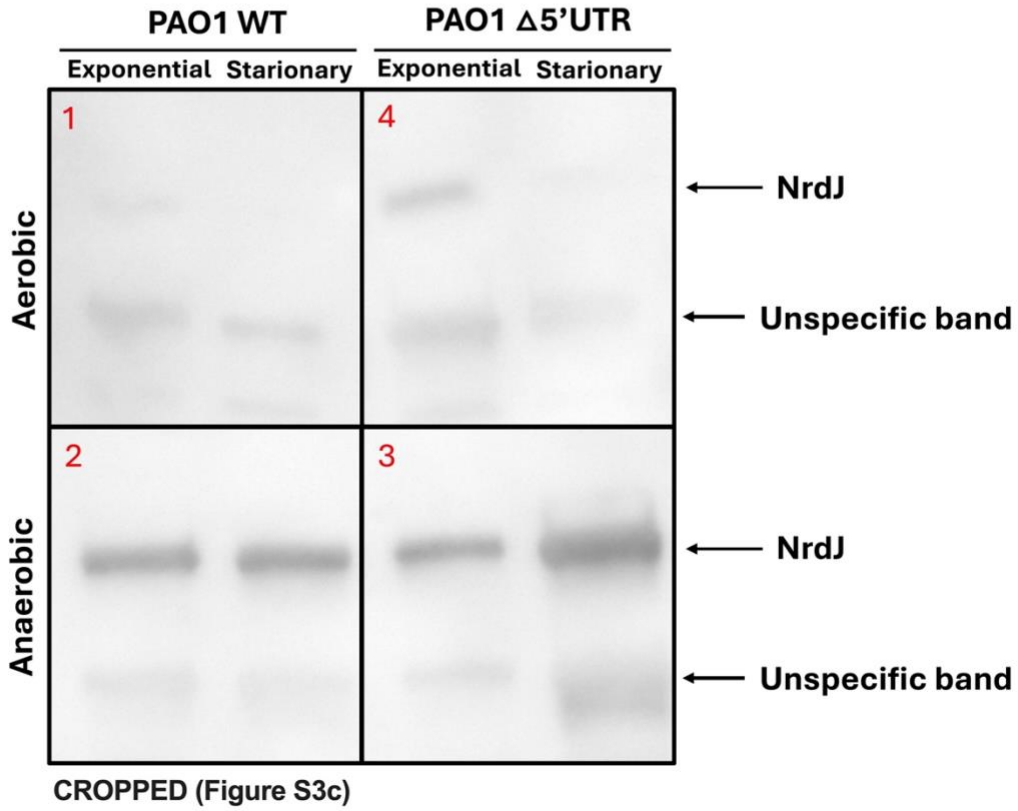

CROPPED (Figure S3c)

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