

## Supplementary Information

### Discovery of fungal surface NADases predominantly present in pathogenic species

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**Supplementary table 1. Data Collection, Phasing, and Refinement Statistics**

Structure	AfNADase:apo	AfNADase:ADPR-Nam	AfNADase:BAD
PDB accession code	6YGE	6YGF	6YGG
<b>Data collection</b>			
Synchrotron/beamline	PETRAIII, EMBL/P13	PETRAIII, DESY/P11	DLS/I04-1
Space group	P 32 2 1	P 32 2 1	P 21 21 21
Cell dimensions a, b, c (Å)	63.79, 63.79, 257.99	63.82, 63.82, 257.72	51.05, 64.32, 161.01
Angles $\alpha$ , $\beta$ , $\gamma$ (°)	90, 90, 120	90, 90, 120	90, 90, 90
Resolution (Å) <sup>a</sup>	54.0-1.6 (1.657-1.6)	42.9-1.7 (1.76-1.7)	48.6-1.85 (1.916-1.85)
	(1.657-1.6)	(1.76-1.7)	(1.916-1.85)
Total reflections	672818 (50757)	136212 (12968)	303366 (30838)
Unique reflections	75947 (7267)	68236 (6579)	45536 (4447)
Multiplicity	8.9 (7.0)	2.0 (2.0)	6.7 (6.9)
Completeness (%)	92.8 (90.3)	99.7 (97.8)	98.5 (97.7)
I/ $\sigma$ (I)	16.5 (0.7)	15.2 (1.0)	9.3 (0.95)
Wilson B-factor	29.5	27.5	25.7
R-merge	5.9 (168.8)	2.5 (64.4)	14.3 (165.8)
R-pim	1.9 (61.0)	2.5 (64.4)	5.9 (67.5)
CC 1/2	0.99 (0.39)	0.99 (0.48)	0.998 (0.51)
<b>Refinement</b>			
R <sub>work</sub>	17.8 (33.3)	16.9 (34.3)	18.4 (34.0)
R <sub>free</sub>	21.1 (35.4)	20.1 (36.1)	22.5 (35.70)
Number of non-hydrogen atoms	3970	4173	3998
Macromolecules	3322	3346	3302
Ligands	171	263	237
Solvent	477	564	459
R.m.s.d (bonds/angles)	0.007/0.88	0.007/0.86	0.008/0.96
Ramachandran favored (%)	98.30	98.79	98.55
Ramachandran allowed (%)	1.70	1.21	1.21
Ramachandran outliers (%)	0	0	0.24
Clashscore	2.95	3.29	3.68
Average B-factor	39.8	35.2	32.7
Macromolecules	37.4	31.2	30.0
Ligands (including carbohydrates and substrates)	59.7	69.4	54.2
		<i>ADPR only: 73.1</i> <i>Nam only: 33.1</i>	<i>BAD only: 36.8</i>
Solvent	49.1	43.3	41.0

<sup>a</sup> Data for the highest resolution shell are given in parentheses

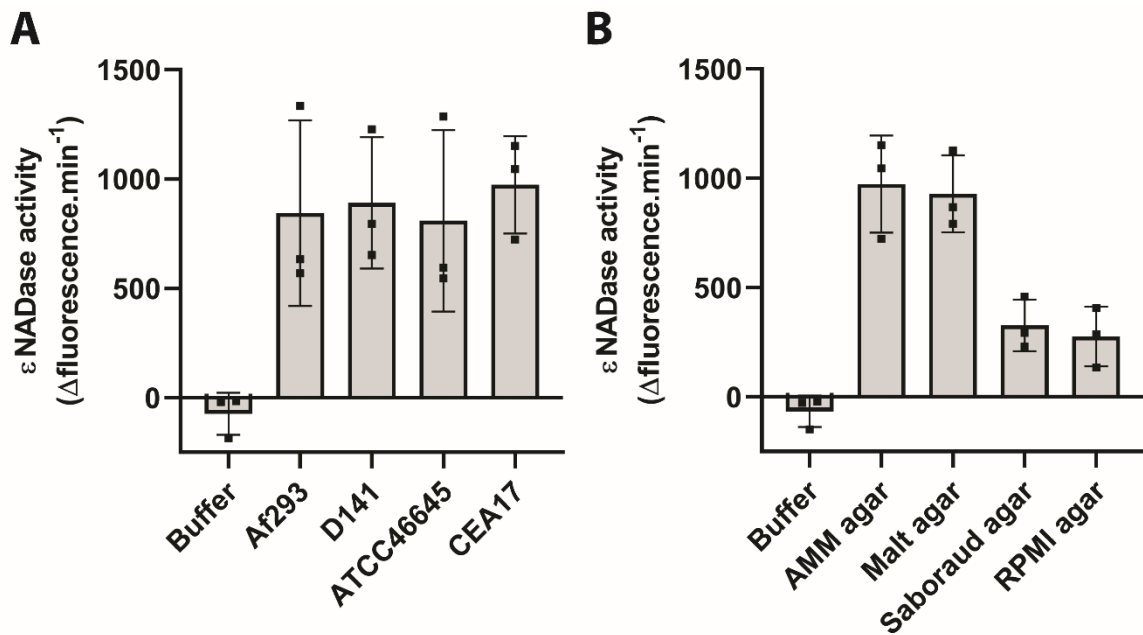
**Supplementary Table 2. Structural alignment of *Af*NADase to various NADases and ADP-ribose transferase using PDBeFOLD.**

<b>Protein</b>	<b>PDB</b>	<b>Z score</b>	<b>RMSD (Å)</b>	<b>Nalgn</b>	<b>Seq. ID (%)</b>
Mtb CpnT TNT	4QLP	10.7	1.93	134	27
Tse6	4ZV0	8.5	2.13	90	21
Diphtheria toxin	1TOX	5	2.71	71	11
Cholera toxin	1XTC	4.3	3.26	57	9
SPN	3PNT	3.6	3.61	73	5
PARP1	4DQY	2.9	3.48	75	9

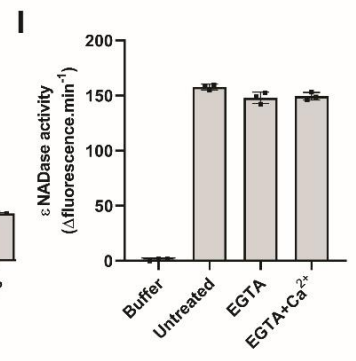
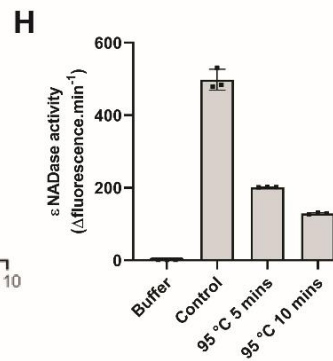
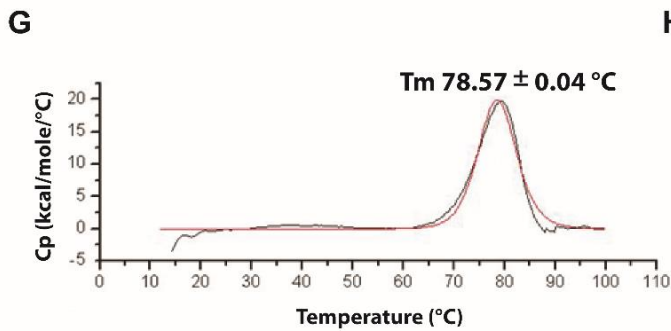
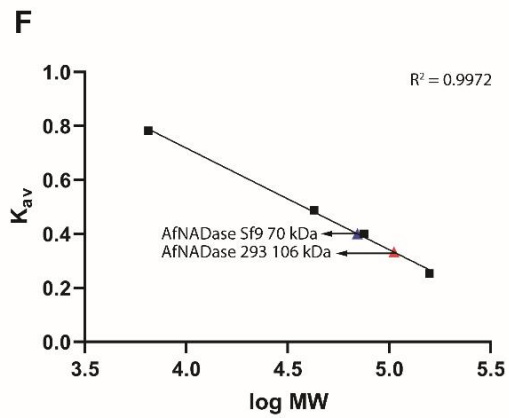
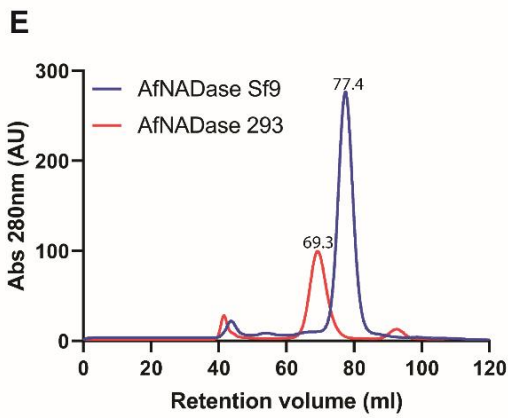
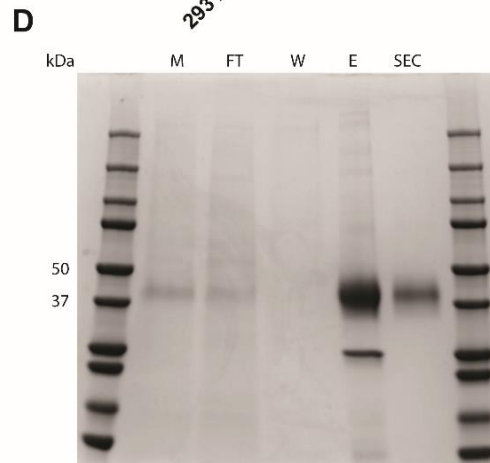
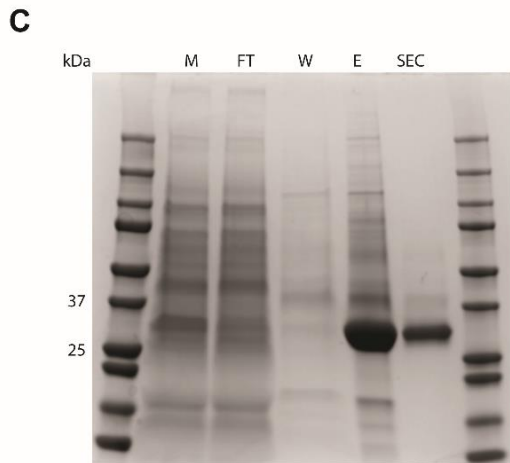
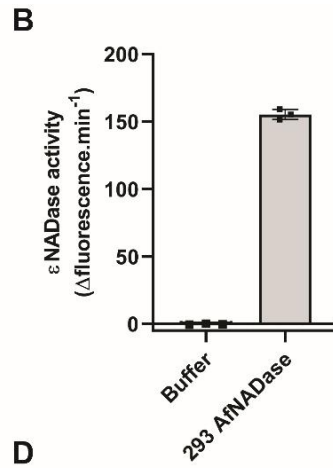
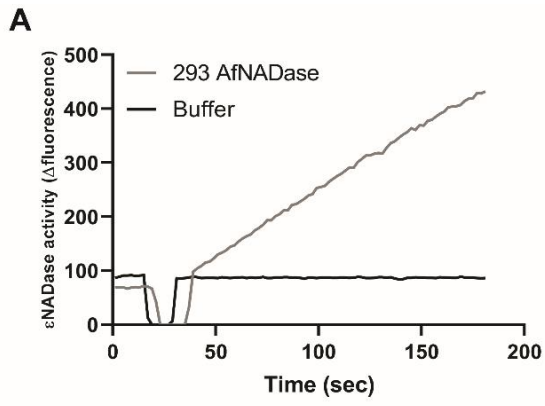
The table shows the summary of PDBeFOLD results of pairwise structural comparison of *Af*NADase and selected NADases and ADPR transferases. The Z-score measures the significance of a match in terms of Gaussian statistics. The root-means-square deviation in angstrom (RMSD), the length of the aligned segment (Nalgn) and the fraction of identical residues in the aligned segment (Seq. ID (%)) are shown.

**Supplementary Table 3. Primers used in the study.**

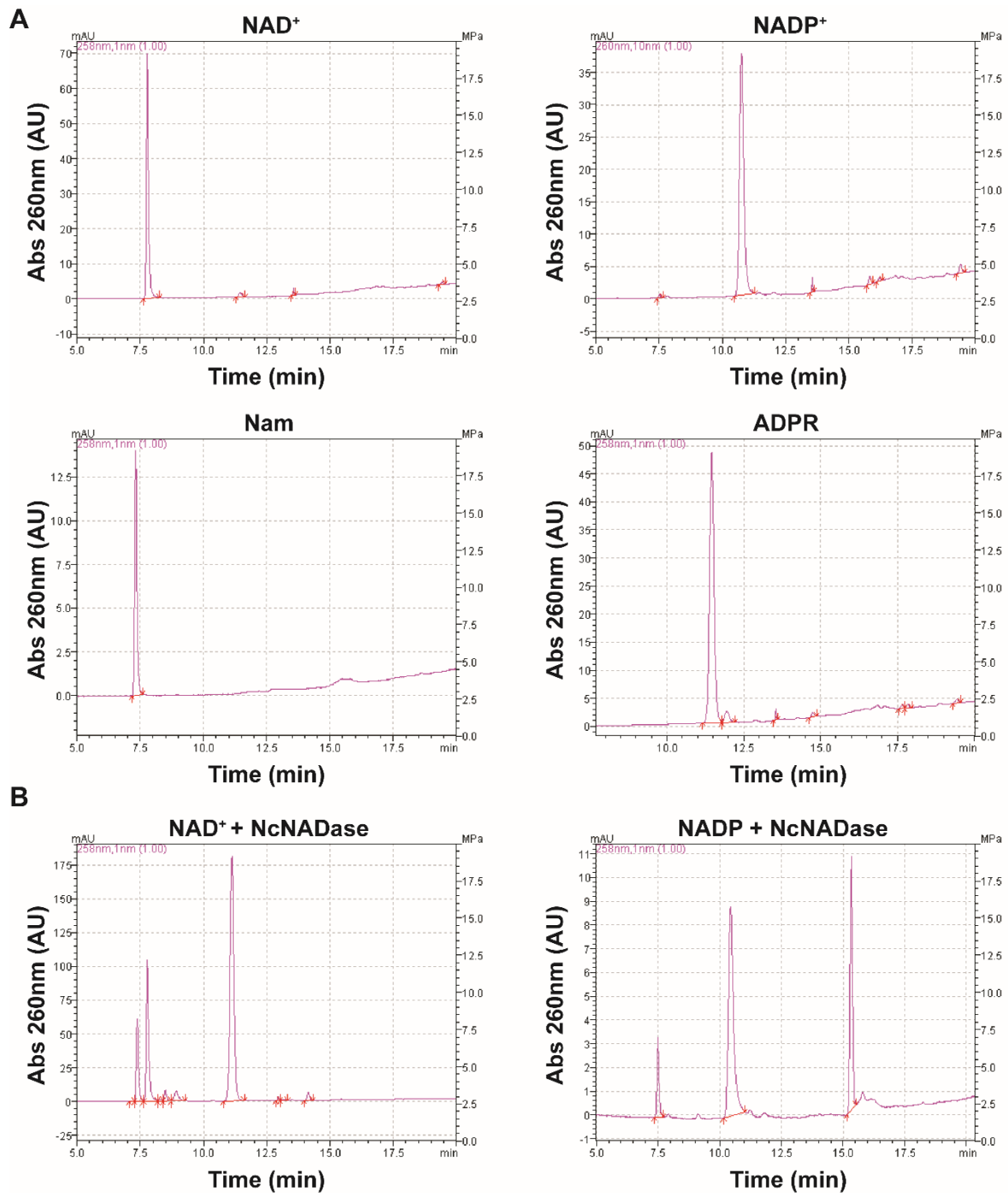
<b>Primer</b>	<b>Sequence (5'-3')</b>
<b>Generation of <i>A. fumigatus</i> strains</b>	
6G14470_5for	GGTCATTGTAAATATCTGGG
6G14470_ptrArev	GGCCTGAGTGGCCATCGAATTCCGCCGTGTAATACTGA GAAG
6G14470_ptrAfor	GAGGCCATCTAGGCCATCAAGCCTTATGGGAAGTGGAT CTTG
6G14470_3rev	GTAGTGGATAACGAAGATTCCG
ptrA-for	GAATTCGATGGCCACTCAGGCC
ptrA-rev	GCTTGATGGCCTAGATGGCCTC
14470_fwd	AGGCGTATCACGAGGCCCTTTCGTGCGTCATTGTAAAT ATCTGGG
14470_rev	CAATAGTGCCACGCTATTGGGATCACTGGC
hph_fwd	TGATCCCAATAGCGTGGCACTATTGATCATCC
hph_rev	GGCCATCGAATTCGCCAGTGTGCTGGAATTC
compl_ptrA_fwd	CAGCACACTGGCGAATTCGATGGCCACTCAG
compl_ptrA_rev	TCACCGTCATCACCGAAACGCGCGAGCTTGATGGCCTA GATGG
<b>Generation of AfNADase insect expression plasmid</b>	
AfNADaseFw_1	CAGGGACCCGGTATGATCTTCACCAACGCCATTCTGGT GATTAGCGCC
AfNADaseRv_233	GAAACAGCACTGCCTGATTCCGCCCCCGGAGTATACGGA TTTCG
<b>Generation of AfNADase 293 expression plasmid</b>	
AfNADase293Fw	GTTGGATCCCCACCATGATCTTCACCAAC
AfNADase293Rv	GCATAGAATTCCTAGTGATGGTGATGGTGATGCTGATT CGGCCCCGGAGTATAC
<b>Generation of AfNADase mutants</b>	
F137AFw	GTATGGCACCGCTCTGGCGCCGC
F137ARv	TCCGATCCGAAACGGTCAAG
Q194AFw	GATGGGGACGGCTTTCGTGACATATACCAATG
Q194ARv	CCTGGCTGCTCAAACCAA
Q194KFw	GATGGGGACGAAGTTCGTGACAT
Q194KRv	CCTGGCTGCTCAAACCAAG
R129AFw	GAAGCTTGACGCGTTCCGGATCGGAGTATGG
R129ARv	ATGCCAACCGGTAAGGTC
F130AFw	GCTTGACCGTGCGGGATCGGAGTATG
F130ARv	TTCATGCCAACCGGTAAG
D219A/E220AFw	GAGCGAGTATGCTGCCAAGGTGGAATACTC
D219A/E220ARv	TCATCCAACCGTCGCAAG



**Supplementary Figure 1. NADase activity of conidia from clinical *A. fumigatus* strains and conidia grown on different media.** (A) NADase activity of *A. fumigatus* conidia from the clinical isolates Af293, D141, ATCC46645 and the laboratory strain CEA17 $\Delta$ *akuB* demonstrated by a fluorometric assay using  $\epsilon$ NAD, n=3. Data are presented as mean values  $\pm$  SEM. (B) NADase activity of *A. fumigatus* conidia from the strain CEA17 $\Delta$ *akuB* cultivated on different media demonstrated by a fluorometric assay using  $\epsilon$ NAD, n=3. Data are presented as mean values  $\pm$  SEM. Experiments in A and B were performed independently three times with similar results. Source data are provided as a Source Data file.

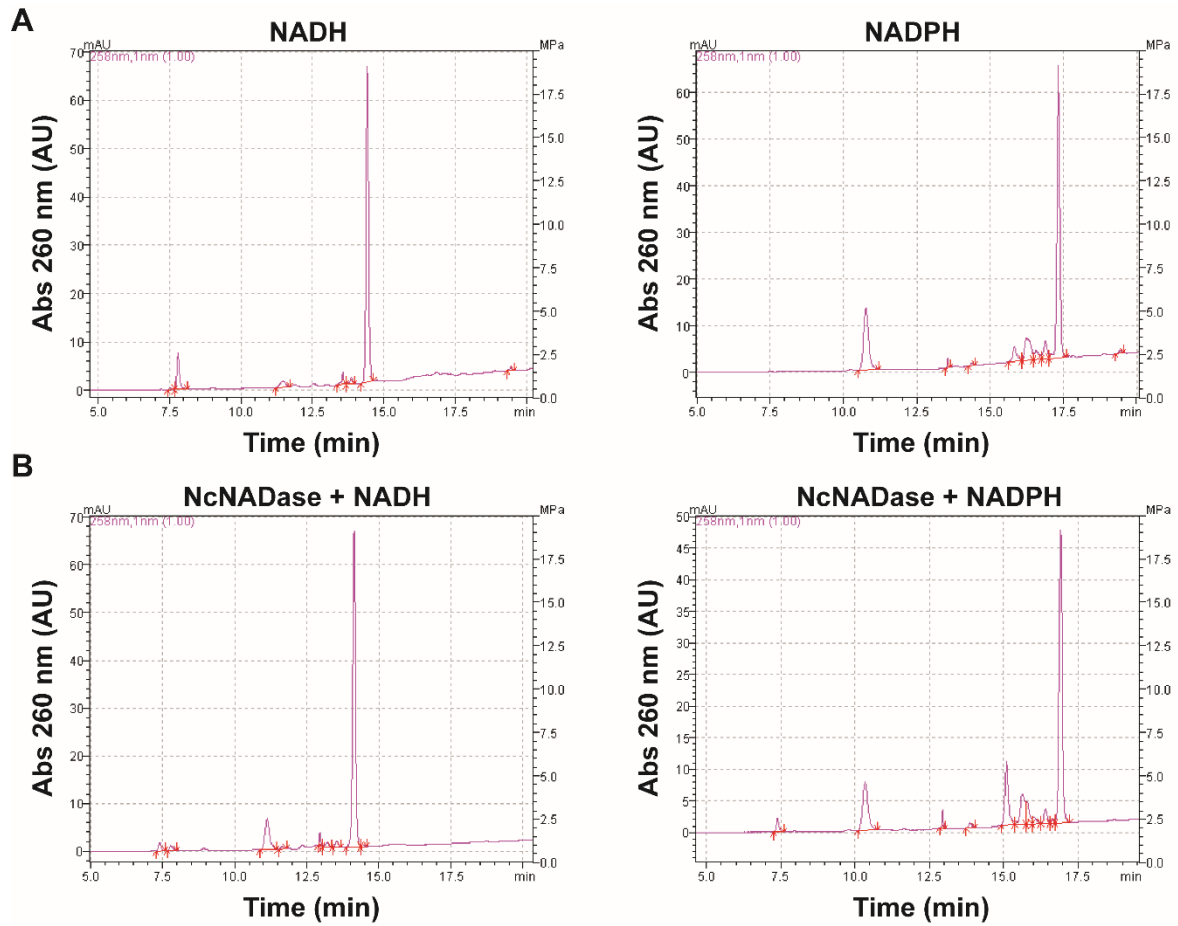


**Supplementary Figure 2. Purification and characterization of *Af*NADase expressed in 293 and Sf9 insect cells.** (A and B). NADase activity of *Af*NADase purified from 293 cells measured using  $\epsilon$ NAD, n=3. Data are presented as mean values +/- SEM. (C) Coomassie blue-stained SDS-PAGE of *Af*NADase expressed in Sf9 insect cells purified by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). M: medium, FT: flow through, W: wash, E: Elution from IMAC column, SEC: pooled fraction from the SEC column n=3. (D) Coomassie blue-stained SDS-PAGE showing *Af*NADase expressed in 293 cells purified by IMAC and size exclusion chromatography. M: medium, FT: flow through, W: wash, E: Elution from IMAC column, SEC: pooled fraction from the SEC column n=3. (E) Size exclusion chromatography elution profiles of *Af*NADase purified from 293 and Sf9 cells. (F) The size of *Af*NADase purified from 293 and Sf9 cells was determined by comparing their partition coefficient ( $K_{av}$ ) to a standard of proteins with known sizes (E and F). NADase activity of *Af*NADase purified from 293 cells measured using  $\epsilon$ NAD, n=3. Data are presented as mean values +/- SEM. (G) Differential scanning calorimetry of *Af*NADase purified from Sf9 cells, black: experimental data, red: fitted model. Experiments in A-G were performed independently three times with similar results. (H) NADase activity of *Af*NADase following incubation at 95 °C for 5 and 10 minutes, n=3. Data are presented as mean values +/- SEM. (I) NADase activity of recombinant *Nc*NADase from S293 cells treated with EGTA or EGTA and calcium chloride, n=3. Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

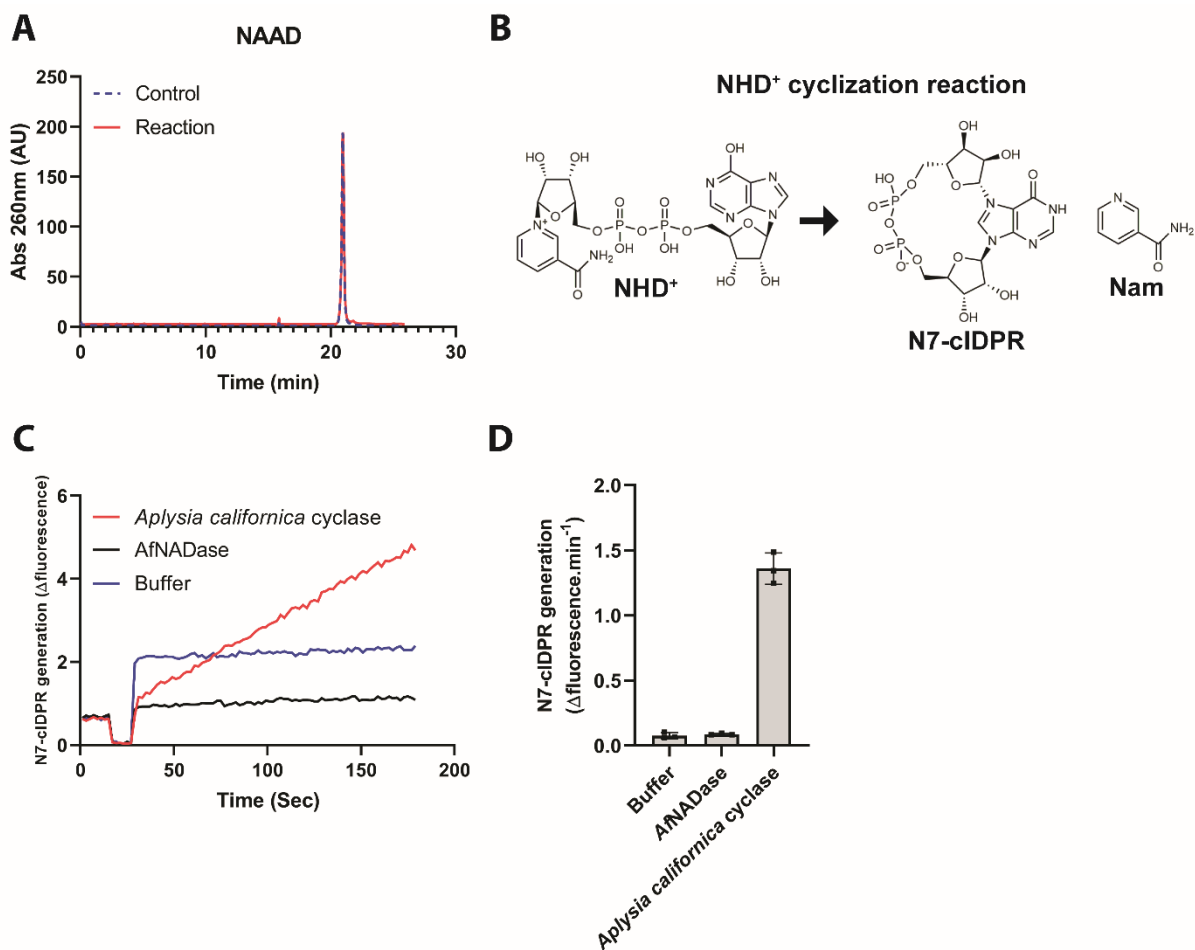


**Supplementary Figure 3. HPLC elution profile of NAD<sup>+</sup>, NADP<sup>+</sup> and NcNADase hydrolysis products.** (A) NAD<sup>+</sup>, NADP<sup>+</sup>, Nam and ADPR standards (B) NAD<sup>+</sup> and NADP<sup>+</sup> hydrolysis mediated by recombinant NcNADase expressed and purified from 293 cells. The experiment was repeated independently three times with similar results.

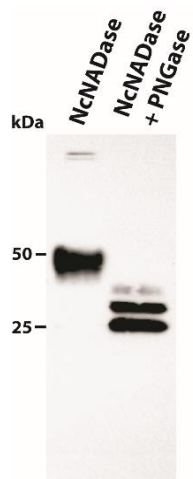




**Supplementary Figure 4. HPLC elution profiles of NADH, NADPH and *NcNADase* hydrolysis products.** (A) NADH and NADPH standards (B) NADH and NADPH incubated with *NcNADase* expressed and purified from 293 cells. The experiment was repeated independently three times with similar results.



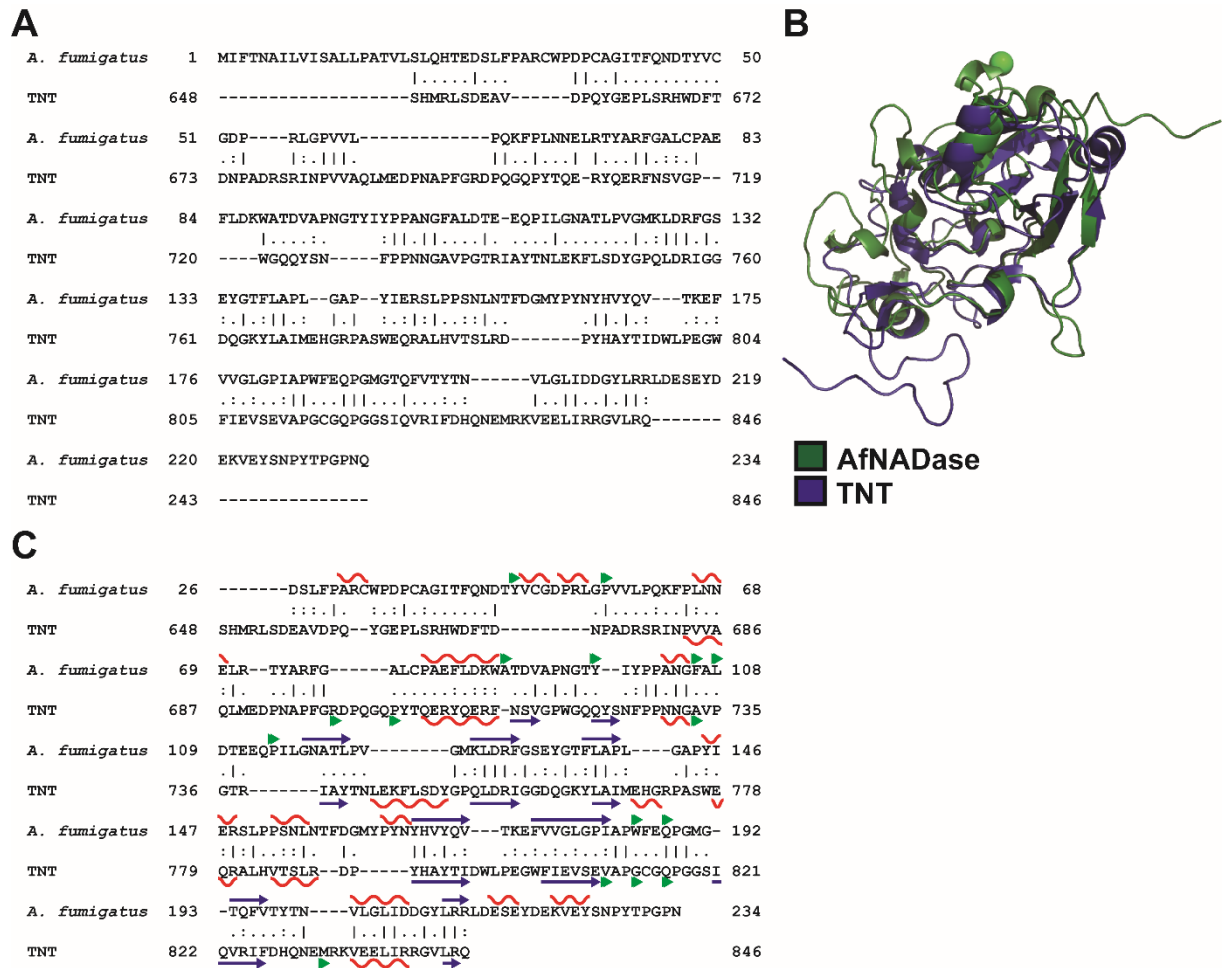
**Supplementary Figure 5. *Af*NADase does not cleave NAAD or catalyse ADPR cyclization.** (A) No *Af*NADase mediated reaction is observed with NAAD as shown by HPLC analysis. (B) Cyclization of NHD<sup>+</sup> leads to the formation of Nam and the fluorescent molecule N7-cIDPR. (C) N7-cIDPR is produced by *Aplysia californica* cyclase but not by *Af*NADase, as evidenced by fluorescence measurements, n=3. (D) Rate of N7-cIDPR formation by *Af*NADase and *A. californica* cyclase. Data are presented as mean values +/- SEM. The *A. californica* cyclase was used as a positive control. Experiments in A, C and D were performed independently three times with similar results. Source data are provided as a Source Data file.



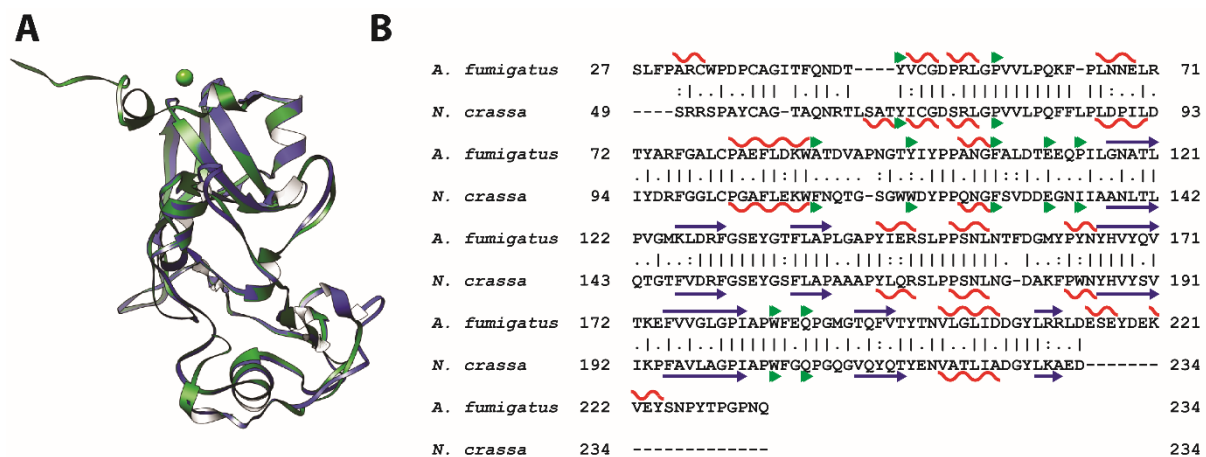
**Supplementary Figure 6. Recombinant *N. crassa* NADase is N-glycosylated.** Deglycosylation of purified FLAG-tagged NcNADase expressed in 293 cells with Peptide-N-Glycosidase F (PNGase) reduces the molecular mass of the protein, shown by western blotting using Anti-FLAG M2 antibody. The experiment was repeated independently three times with similar results. Source data are provided as a Source Data file.

<i>Aspergillus fumigatus</i> /1-234	1	-----MIFTNAILVISALLPATVLSL-----	21
<i>Aspergillus ruber</i> /1-212	1	-----MF-----	2
<i>Neurospora crassa</i> /1-243	1	-----MKFT-LLSTAVALLTSTAVALPTSSSSA-----G-----SLLNER	34
<i>Fusarium sp_AF-&amp;/1-225</i>	1	-----MRISFFLSSL--LWLSTASALPTS-----LDE-	25
<i>Streptomyces glauciniger</i> /1-2	1	--MKRRQRIS--LSTLPALLVLLVGTLLGTVTSAHAEDWGGP-----AQLAAP	44
<i>Mycobacterium tuberculosis</i> /1	1	MRFRRRGTIPAAATLGFTILLTGTAQAAQSSGAQP-----GGP-----CTAPLAES	46
<i>Bacillus cereus</i> /1-218	1	-----MPGSMKNV-----GEKIKNIEIPNVFPEP	24
<i>Lentzea guizhouensis</i> /1-212	1	-MILRKHVLSLLVLVSTMLGLAAAPVAHATPSAP-----	33
<i>Aspergillus fumigatus</i> /1-234	22	-----QHTEDSLFP-----ARCWPDPCCAGITFQ	44
<i>Aspergillus ruber</i> /1-212	3	-----ARMQQPTY-----DRCKDNPCAGIPSQ	25
<i>Neurospora crassa</i> /1-243	35	-----SYVNASSTATTCP-----YSRRSPAYCAG-TAQ	61
<i>Fusarium sp_AF-&amp;/1-225</i>	26	-----RKAVGCDCTG-TRN	38
<i>Streptomyces glauciniger</i> /1-2	45	PGRPAAHGPGPGAAGHGPGREADTPGRVP-----GGPENPRICIGATFT	89
<i>Mycobacterium tuberculosis</i> /1	47	TAKIIKQAG-----DCSAQAHRPQAVPATAHAAAAAKDALHARPHACG-----	90
<i>Bacillus cereus</i> /1-218	25	SAADVGNVG-----ERKTLGELFSVV-----KSETKDTGNVKGAS	60
<i>Lentzea guizhouensis</i> /1-212	34	TAEVV-----CE-----	40
<i>Aspergillus fumigatus</i> /1-234	45	N----DTYVCGDPRLGPVVLPPQKF-PLNNELRTYARFG-ALCPAEFLDKWATDVA-P	94
<i>Aspergillus ruber</i> /1-212	26	N----ASYVCGDSRLGPGVGLPSKF-PLSTETSTYARFG-NLCPKEWLDKWTS----	72
<i>Neurospora crassa</i> /1-243	62	NRTLSATYICGDSRLGPGVVLPPQFFLPDLDIYDRFG-GLCPGAFLEKWFNQTG--	115
<i>Fusarium sp_AF-&amp;/1-225</i>	39	GGPSAKEFICRDSRLGPKVLPKKL-PLDNLVENYDRFG-GLTPGQFLDKWTD-----	89
<i>Streptomyces glauciniger</i> /1-2	90	D----PKFFCGDPRLGPRLPQAKG-LLGAMLTDYRRLD-GQNANGFLRTWDFDA----	137
<i>Mycobacterium tuberculosis</i> /1	91	----PPYINGDPRLGPVNLPPQNGYF-GYLLRQYKRYG-GLTPSTFLYQYWDEAKTP	140
<i>Bacillus cereus</i> /1-218	61	E----AGKHVDDVKI-PENIKKWNYPPEEL--YKKYE-DVYKNP---KYDQE---	103
<i>Lentzea guizhouensis</i> /1-212	41	----KPFHLGDERFGKDLPPSPSHVAGKLLIGYKRFGEKASPQAFVTEYWKGK---	89
		Y100 R129 F130 L138 R148	
<i>Aspergillus fumigatus</i> /1-234	95	NGTYIYPPANGFA---LDTEEQPLIGNATLPVGMKLDLRFGEYGFLLAPLGAPYIER	148
<i>Aspergillus ruber</i> /1-212	73	DGNLRYPPQDGF---LDNDKNIWGNYTLTAGSKVDRFGSEYKYLTTLGAPYIER	126
<i>Neurospora crassa</i> /1-243	116	SGWWDYPPQNGF---VDDEGNIIAANLTQTGTFVDRFGSEYGSFLAPAAAPYLQR	169
<i>Fusarium sp_AF-&amp;/1-225</i>	90	KGNFIYPPQNGFQ---LDKNGNAINGTMELOKQALVDRFGSEYGSFISAAAAPYSQR	143
<i>Streptomyces glauciniger</i> /1-2	138	ANSYKFPDDGF---PGGPVAVI---NLAVGQRIDRFGGEGGRFLAPAGSPYAQR	187
<i>Mycobacterium tuberculosis</i> /1	141	TPDWRYPDDGFVHQLKIDNSRPARYKVTLRVGGQVDRFGAEAGRFIAPGGASFGSR	197
<i>Bacillus cereus</i> /1-218	104	TGEIHWPPNDGFV-----SGTKQVETLHPGMKLDRYGNPTGSFLAPESDSFPSR	152
<i>Lentzea guizhouensis</i> /1-212	90	--GWKYPENDGFI-----GRPT--TEVLAPGKLLDRFYGGSGRFLSPVGTPTFAQR	135
		F158	
<i>Aspergillus fumigatus</i> /1-234	149	SLPPSNLNT--FDGMY-----PYNHYVYQVTKEFVVGGLPIAPWFEQPGMGT	193
<i>Aspergillus ruber</i> /1-212	127	ALPPGNLDT--YDGKY-----PYNHYVYEVLKDQVNVVGPVAAWFEQPGMGT	171
<i>Neurospora crassa</i> /1-243	170	SLPPSNLNG--DAKF-----PWNYHVSIVIKPFAVLAGPIAPWFGQPGQV	213
<i>Fusarium sp_AF-&amp;/1-225</i>	144	ALPPSNLATNPPSPNF-----PYNHYVYRVLKALPVVGGPIAPWFGQPGGLGA	190
<i>Streptomyces glauciniger</i> /1-2	188	SIAPSNLNT--FDPGY-----PFNYHLYKVVRSFAVSAAGPVAPFYGQPGNGL	232
<i>Mycobacterium tuberculosis</i> /1	198	ALPPNLLNTRSDDP SHLCN-----YHLYRVSKRFSVDGGPTTEPAFQPGPGRGL	244
<i>Bacillus cereus</i> /1-218	153	ALAPHSEQA-----P--YVYEV IADFEVTSK IAPWFDQPGGGT	190
<i>Lentzea guizhouensis</i> /1-212	136	SLPPQSLNT-----CEYEQGVMPYGYRYKVEKQFEVLGQPAAKWFGQTGGGR	184
		Q194	
<i>Aspergillus fumigatus</i> /1-234	194	GFVITY-----NVLGLIDDGYLR-----RLDESEYDEKVEYSNPYTPGPNQ-	234
<i>Aspergillus ruber</i> /1-212	172	OIYTSK-----AVNELLEGGFLR-----RLSEDEYDERNEYAEPAPRGQDA-	212
<i>Neurospora crassa</i> /1-243	214	OYQTYE-----NVATLIADGYLKAEDPQRLVPRNY-----	243
<i>Fusarium sp_AF-&amp;/1-225</i>	191	QFFTGEV-----GNIMALIEKGYLESVDPSVLI-----FRGKGCA	225
<i>Streptomyces glauciniger</i> /1-2	233	OYVLP LG-----DSVANHITNGDLI-----RLN-----	255
<i>Mycobacterium tuberculosis</i> /1	245	OYVLSAYVPGAPHLTVKWLADKGYLQ-----RVY-----	275
<i>Bacillus cereus</i> /1-218	191	OIKYK---PNG-RTYSIEELEIELEIK-----QIKP-----	218
<i>Lentzea guizhouensis</i> /1-212	185	OYKVTS---PNPPERRNVAVLVENGYLS-----VVR-----	212

**Supplementary Figure 7. Multiple sequence alignment of fungal and bacterial TNT domain-containing proteins.** Multiple sequence alignment of selected TNT-containing proteins using MAFFT. Residues involved in BAD and/or product interaction in AfNADase are highlighted.



**Supplementary Figure 8. Pairwise and structural alignment of AfNADase and TNT.** (A) Global pairwise alignment of AfNADase and the TNT domain of *Mtb* CpnT using EMBOSS Needle. (B) Structural alignment of AfNADase and the TNT domain of *Mtb* CpnT. (C) Structure-based sequence alignment of AfNADase and the TNT domain of *Mtb* CpnT. Secondary structure elements were assigned using the DSSP algorithm, the red waves indicate helical secondary structure while the blue arrows and green arrowheads indicate beta strands and beta bridges, respectively.



**Supplementary Figure 9. Structural alignment of AfNADase and NcNADase** (A) Structural alignment of AfNADase (green) and the model of NcNADase (blue). (B) Structure-based sequence alignment of AfNADase and the model of NcNADase. Secondary structure elements were assigned using the DSSP algorithm; the red waves indicate helical secondary structure while the blue arrows and green arrowheads indicate beta strands and beta bridges, respectively.