

SUPPLEMENTARY TABLES

Enzyme	K_m (μM)	k_{cat} (s^{-1})	K_{cat}/K_m ($\text{M}^{-1} \text{s}^{-1}$)	Reference
TNT	614 ± 43	51.7 ± 1.1	8.4×10^4	This work
SPN	188 ± 23	$8,390 \pm 360$	4.5×10^7	¹
Diphtheria Toxin	8.3 ± 2	8.3×10^{-4}	100	²
Diphtheria Toxin*	9	0.58	6.4×10^4	³
Diphtheria Toxin	85 ± 13	1.8×10^{-3}	21.6	⁴
Diphtheria Toxin*	6 ± 2	3.0 ± 0.25	5.0×10^5	⁴
Cholera Toxin	1.4×10^4	0.14	10	⁵
Cholix Toxin	67 ± 4	5.3×10^{-4}	7.9	⁶
Cholix Toxin*	45 ± 3	10 ± 3	2.3×10^5	⁶
Pertussis Toxin	15.3	1.8×10^{-2}	1161	⁷
CD38	18 ± 3	148 ± 8	8.2×10^6	¹

Supplementary Table 1. Enzymatic parameters of NAD⁺ glycohydrolases

* The enzymatic activities of ADP-ribosyltransferases was determined in the presence of the appropriate target proteins.

Sample	N	Live embryos	
		24 h.p.i.	48 h.p.i.
no injection	20	18 (90%)	17 (85%)
buffer	19	17 (89%)	15 (79%)
BSA	17	14 (82%)	12 (71%)
TNT	19	8 (42%)	4 (21%)

no injection	23	23 (100%)	22 (96%)
buffer	22	21 (95%)	21 (95%)
TNT _{H792N Q822K}	22	20 (91%)	20 (91%)
TNT-IFT complex	21	18 (86%)	18 (86%)

Supplementary Table 2. Quantification of zebrafish zygote survival

*Data were obtained from two separate experiments as indicated by the dotted line. h.p.i. hours post injection

Strain	Parent strain and relevant genotype	Source or reference
<i>E. coli</i> DH5α	<i>recA1; endA1; gyrA96; thi; relA1; hsdR17(rK-;mK+); supE44; φ80ΔlacZΔM15; ΔlacZYA-argF; UE169</i>	⁸
<i>E. coli</i> BL21(DE3)	F-; <i>ompT; hsdSB; (rB- mB-); gal; dcm lacYI; (DE3)</i>	⁹
<i>E. coli</i> DH5αZ1	DH5α derivative, <i>lacI</i> ^q , PN25-tetR, <i>Sp</i> ^R , <i>deoR</i> , <i>supE44</i>	¹⁰
<i>M. tuberculosis</i> H37Rv	Wild-type	ATCC 25618
<i>M. tuberculosis</i> mc ² 6230	H37Rv derivative; <i>ΔRD1 (Δesx-1)</i> and <i>ΔpanCD</i>	Dr. Jacobs
<i>M. tuberculosis</i> mc ² 6206	H37Rv derivative, <i>ΔleuCD</i> and <i>ΔpanCD</i>	¹¹
<i>M. tuberculosis</i> ML2003	mc ² 6206 derivative, <i>ΔcpnT::mycgfp2+</i> , <i>Hyg</i> ^R , marked <i>cpnT</i> deletion mutant	¹²
<i>M. tuberculosis</i> ML2004	ML2003 derivative, unmarked <i>cpnT</i> deletion mutant	¹²
<i>M. tuberculosis</i> ML2017	ML2004 derivative, L5::pML3009, <i>Kan</i> ^R	This work
<i>M. tuberculosis</i> ML2018	ML2004 derivative, L5::pML3167, <i>Kan</i> ^R	This work

Supplementary Table 3. Bacterial strains used in this work

ATCC: American Type Culture Collection

Oligonucleotide	Sequence (5' → 3')
CN605	TCAGTGGTGGTGGTGGTGGT
CN1394 [§]	<u>CGAAGCTT</u> CTACTGTCGCAACACCCCCGCGC
CN2811 ^{*‡}	TATGGCTCTAAAATACAGGTTCCCGAGGTTGTTATTGTTATTG TTGTTGTTGTT <u>CGAGCT</u>
CN2816 [†]	TTCCC <u>CTAGA</u> ACCAACAAGGACCATA <u>GATT</u> TGAAAAT
CN2939 [¶]	CC <u>AGAT</u> CTATGCGGTTATCGACGAAGCG
CN2940 [#]	CT <u>TTAATTAA</u> ACTGTCGCAACACCCCCGCGCC
CN2950 [☆]	GTA <u>AGGT</u> ACCATGGGGAGGCCACCATCACCAT
CN2951 [§]	AT <u>TAAGCTT</u> AATCACTGTCGCAACACCCCCGCGCC
CN2988 [*]	AT <u>CCGGTACCAT</u> GCGGTTATCCGACGAAGC
CN3075 [‡]	TTTC <u>CAGAGCC</u> CATATGACAATAGGTGTGGACCTGTCAA
CN3076 [§]	GGCC <u>CGCAAGCTT</u> AACCTTATAGTCCTCCAAAGGGT
CN3078 [‡]	CAG <u>AGCC</u> CATATGCGGTTATCCGACGAAGGGTTGAC
CN3129 [‡]	<u>GTCATATG</u> AAAATCCATCACCATCAC
CN3130	CTAGCGCTCTGAAAATACAGGTTTC
CN3146 [§]	CG <u>AAAGCTT</u> ATGGTGAGCAAGGGCGAG
CN3147 ^{**}	TC <u>GGTCGACCC</u> CTTGTACAGCTCGTCCAT
CN3185	CGCATTATCCGCATTAACGACGATGATGTTTCCGCCTGGCTCTCGC CAAAATCCATACCACCATCACCAT
CN3186	GGACCATAGATTATGAAAATAAAAACAGGTGCACGCATCCTCGCATTAT CCGCATTAACGAC
CN3187	<u>TGCATGCT</u> TCGAGTGC <u>GGCCGCAAGCTT</u>
CN3292	GC <u>GGATCC</u> CATCAA <u>AGTGC</u> GGATATT
CN3293	GAATATCCGC <u>ACTT</u> GATGGATCCGC
CN3357 ^{**}	GC <u>ATGTCGAC</u> CATGCGGTTATCCGACGAAGC
CN3358 ^{††}	CG <u>ATGAATT</u> CTCACTGTCGCAACACCCCCGCGCC

Supplementary Table 4. Oligonucleotides used in this work.

Restriction sites are underlined; *: ScaI, †: XbaI, ‡: NdeI, §: HindIII, ||: SphI, ¶: BglII, #: PstI, ☆: KpnI, **: SalI, ††: EcoRI

Plasmid	Parent vector, relevant genotype and properties	
pBAD/His-D-PATagRFP	pBAD/His-B derivative, pBR322 origin, <i>bla</i> , <i>araC</i> , p _{AraB} -His-RFP, 4701 bp	Dr. Verkhusha
pML3006	pBAD/His-B derivative, pBR322 origin, <i>bla</i> , <i>araC</i> , p _{AraB} -His-MCS, 4007 bp	This work
pML3007	pBAD/His-B derivative, pBR322 origin, <i>bla</i> , <i>araC</i> , p _{AraB} -His- <i>tnt</i> , 4601 bp	This work
pZE21MCS	coleE1 origin, <i>aph</i> , P _{LtetO-1} , MCS, 2254 bp	¹⁰
pML3030	pZE21MCS derivative, coleE1 origin, <i>aph</i> , P _{LtetO-1} -His- <i>tnt</i> , 2856 bp	This work
pML3031	pZE21MCS derivative, coleE1 origin, <i>aph</i> , P _{LtetO-1} - <i>tnt</i> , 2820 bp	This work
pML1993	pZE21MCS derivative, coleE1 origin, <i>aph</i> , P _{LtetO-1} -HIS- <i>malE</i> -POLYN-TEV- <i>tnt</i> , 4014 bp	This work
pET-21a(+)	pBR322 origin, <i>bla</i> , <i>lacI</i> ^q , p _{T7lac} , MCS, 5443 bp	Novagen
pML1947	pML1925, pMB1 origin, <i>rop</i> , <i>bla</i> , <i>lacI</i> ^q , p _{tac} -HIS- <i>malE</i> -POLYN-TEV-MCS, 5698 bp	¹²
pML1970	pET-21a(+), pBR322 origin, <i>bla</i> , <i>lacI</i> ^q , p _{T7lac} -HIS- <i>malE</i> -POLYN-TEV-MCS, 6618 bp	This work
pML1974	pML1970 derivative, pBR322 origin, <i>bla</i> , <i>lacI</i> ^q , p _{T7lac} -HIS- <i>malE</i> -POLYN-TEV- <i>rv3902c</i> , 7087 bp	This work
pML1995	pML1970 derivative, pBR322 origin, <i>bla</i> , <i>lacI</i> ^q , p _{T7lac} -HIS- <i>malE</i> -POLYN-TEV- <i>tnt rv3902c</i> , 7677 bp	This work
pML1997	pML1995 derivative, pBR322 origin, <i>bla</i> , <i>lacI</i> ^q , p _{T7lac} -SP _{<i>malE</i>} -HIS- <i>malE</i> -POLYN-TEV- <i>rv3902c</i> , 7752 bp	This work
pDP3615	pACYC184, p15A origin, <i>cam</i> , p _{tet} - <i>hly</i> , 5625 bp	¹³
pML1999	pDP3615, p15A origin, <i>cam</i> , p _{tet} - <i>rv3902c</i> , 4469 bp	This work
pML2123	ColE1 origin, hyg, oriM, psmyc-cpnTaa650-847 mutants (R757L, R757S, Q822K, G818V) translationally fused to HA-mycGfp2, 6861 bp	¹²
pML3331	pML1995 derivative, pBR322 origin, <i>bla</i> , <i>lacI</i> ^q , p _{T7lac} -HIS- <i>malE</i> -POLYN-TEV- <i>tnt_{H792N,Q822K} rv3902c</i> , 7677 bp	This work
pML3339	pML3331 derivative, pBR322 origin, <i>bla</i> , <i>lacI</i> ^q , p _{T7lac} -HIS- <i>malE</i> -POLYN-TEV- <i>tnt_{H792N,Q822K}</i> , 7152 bp	This work
pML3035	pML2093 ¹² derivative, CMV promoter- <i>egfp-tnt_{wt}</i> , SV40 origin, pBR322 origin, <i>bla</i> , 6050 bp	This work
pML3036	pML2138 ¹² derivative, CMV promoter- <i>egfp-tnt_{G818V}</i> , SV40 origin, pBR322 origin, <i>bla</i> , 6050 bp	This work
pML3059	pML3036 derivative, CMV promoter- <i>egfp-tnt_{mutants}</i> (H792N/Q822K, Q822A, R757L, Y765A, G818V), SV40 origin, pBR322 origin, <i>bla</i> , 5999 bp	This work
pMN437	ColE1 origin, hyg, oriM, Psmyc-mycGfp2+, 6236 bp	¹⁴
pML3009	PBR322 origin, <i>aph</i> , L5 attP, FRT-int-Pimyc-esxF-esxE-cpnT-rv3902c-FRT, 9790 bp	¹²
pML3167	PBR322 origin, <i>aph</i> , L5 attP, FRT-int-Pimyc-esxF-esxE-cpnT _{H792N/Q822K-} rv3902c-FRT, 9789 bp	This work

Supplementary Table 5. Plasmids used in this work.