Supporting information

Mimicking a solvent interface at the substrate access channel of nylonase accelerates nylon degradation

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Contents

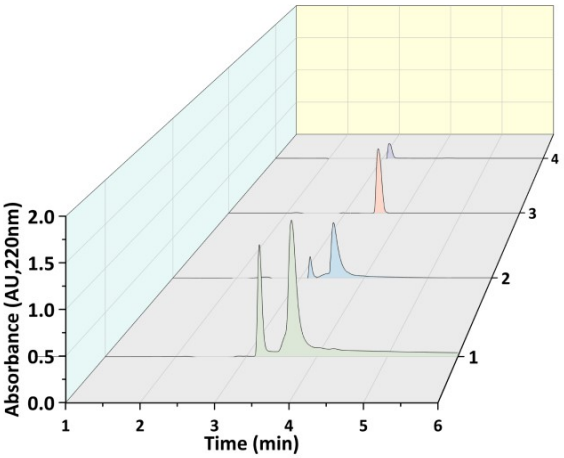

Figure S1. Detection of PA6 film hydrolyzed treated with NylC-V3 and WT by HPLC. The experimental conditions were as follows: purified NylC-V3 and WT (each at 0.1 μM) were incubated with PA6 film (90 mg/mL in PBS buffer) at 60 ℃ for 24 h to facilitate degradation. The standard products, 6-AHA monomer and dimer, were both prepared at a concentration of 1 mM in PBS buffer.

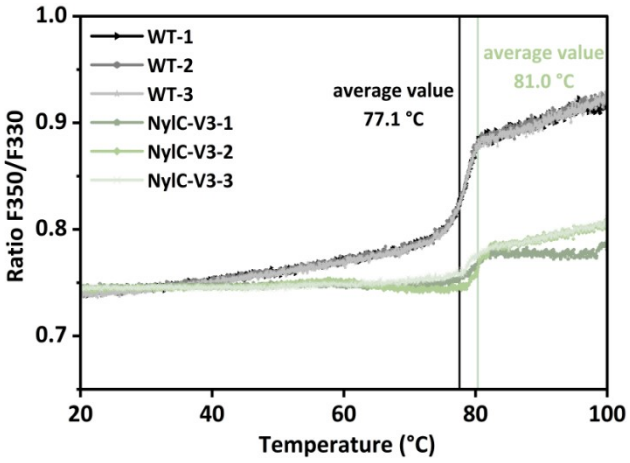

Figure S2. The T_m value of NylC-V3 and WT. The temperature ranged from 20 °C to 100 °C in increments of 1 °C min^{-1} by DSF. Numbers 1, 2 and 3 represented three parallel experiments.

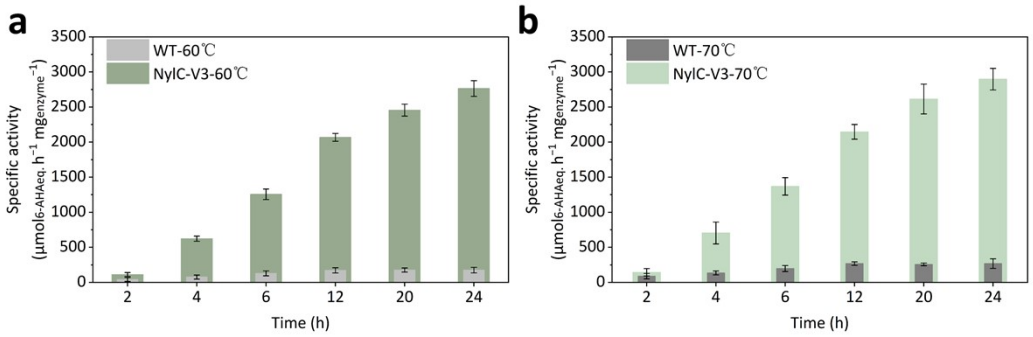

Figure S3. The time scale of PA6 degradation by NylC-V3 at different temperature. a). The specific activity of NylC-V3 and WT was analyzed in different time ranges at 60 °C; **b).** the specific activity of NylC-V3 and WT was analyzed in different time ranges at 70 °C. All the experiments were performed in triplicates.

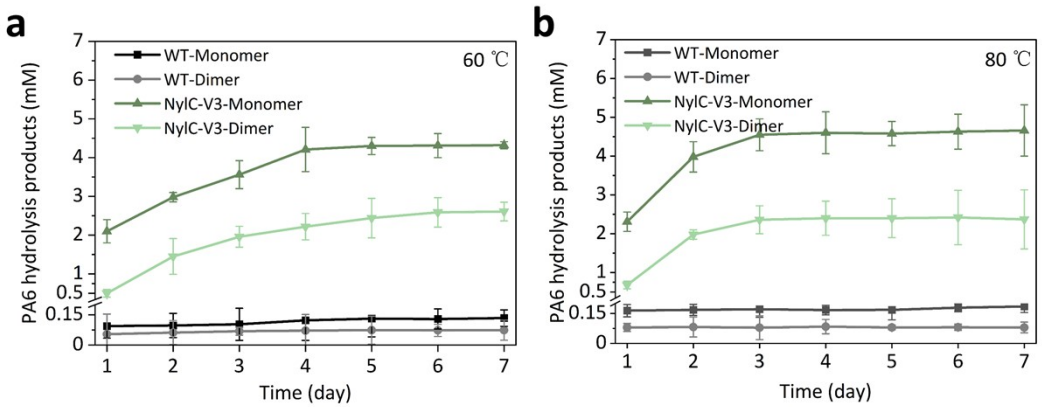

Figure S4. The PA6 hydrolysis products (6-AHA monomer and dimer) treated with NylC-V3 and WT for 7 days at 60 ℃ **and 80** ℃**.** All the experiments were performed in triplicates.

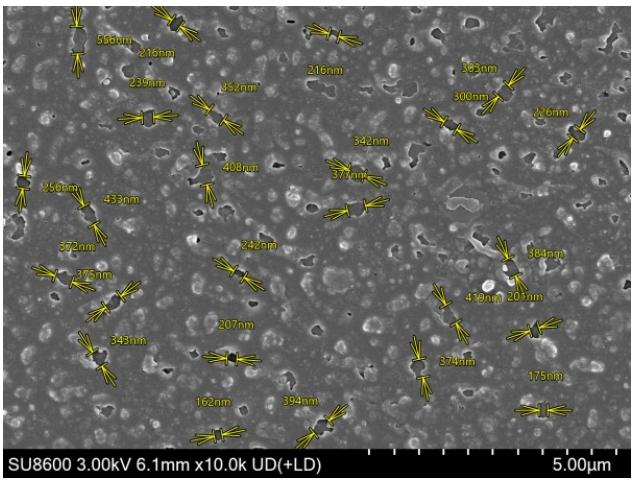

Figure S5. FESEM image of PA6 film treated with NylC-V3. The PA6 film treated with NylC-V3 at 60 °C for 7 days. The yellow arrowss indicated the size of the hole.

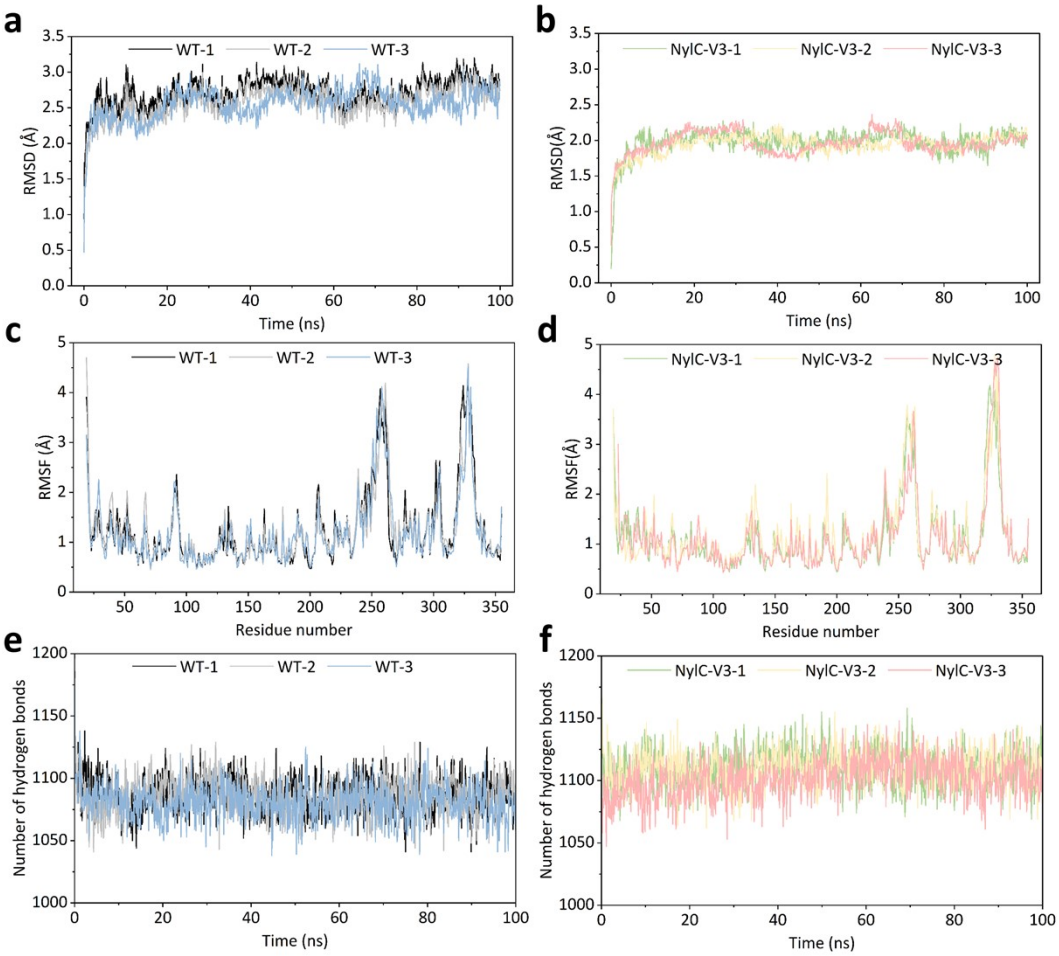

Figure S6. Parallel parameters of molecular dynamics simulation of WT and NylC-V3. a & b). Parallel datas of RMSD of WT and NylC-V3; **c & d).** parallel datas of RMSF of WT and NylC-V3; **e & f).** parallel datas of number of hydrogen bonds of WT and NylC-V3. 1, 2, and 3 respectively represent three parallel experiments.

Figure S7. Solvent-accessible surface area of WT and NylC-V3. 1, 2, and 3 respectively represent three parallel experiments.

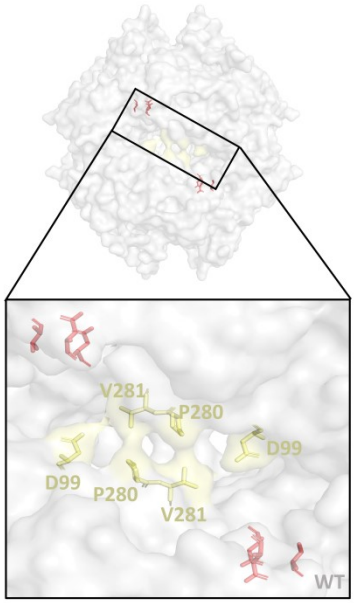

Figure S8. The tetramer conformation of WT. The red sticks represent catalytic triad. The yellow cartoon structures represent the key amino acid in the tetramer center of WT that block the substrate from entering the catalytic center.

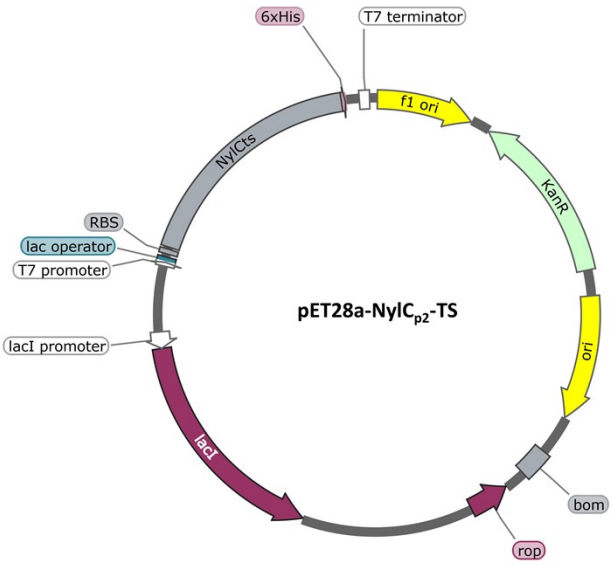

Figure S9. Gene map of pET28a-NylC$_{p2}$-TS.

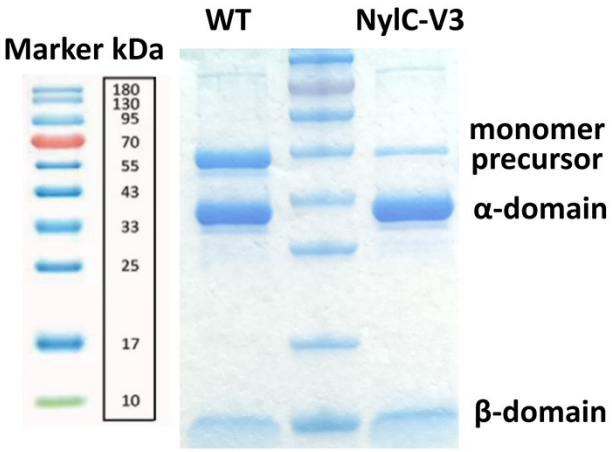

Figure S10. SDS-PAGE diagram of purified WT and NylC-V3. The molecular weight of nylonase monomer is 36 kDa, which is divided into α-domain (27 kDa) and β-domain (9 kDa) after auto cleavage.[1]

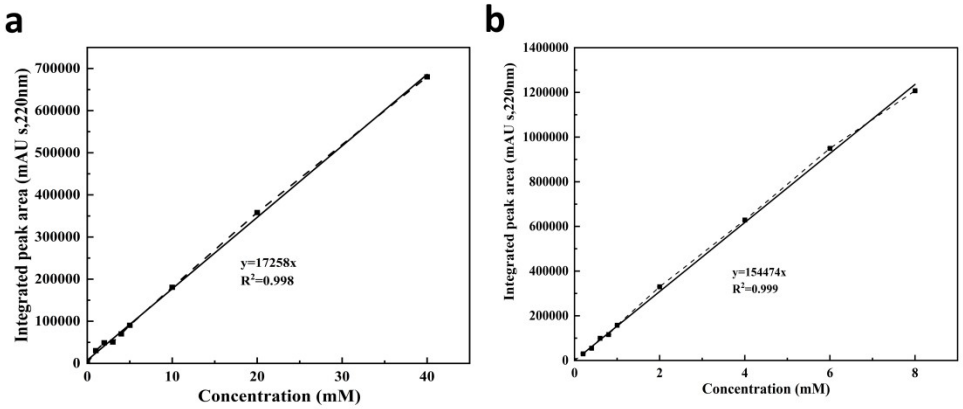

Figure S11. The curves of monomer and dimer of standard 6-AHA. a). The curve of monomer of 6-AHA; **b).** the curve of dimer of 6-AHA. The standard products were analyzed by HPLC (Waters, USA) equipped with an AlphaHybird C18 column (150 Å, 5 µm). The mobile phase was 70% v v^{-1} deionized water and 30% v v^{-1} acetonitrile. The flow was isocratic and fixed at 0.5 mL min^{-1}. The detection wavelength was 220 nm. All the experiments were performed in triplicates.

Table S1. Sequences result of 60 randomly picked clones from the smart library

	Variant position
Clone 1	F134W/V258H
Clone 2	V258F
Clone 3	G89Y/G93F/H302Y
Clone 4	G89Y/G93Y/H302Y
Clone 5	G89stop
Clone 6	G89Y/G93W/H302Y
Clone 7	A91W/P220F/D304Y
Clone 8	P220F
Clone 9	G93C
Clone 10	A91W/P220Y/D304Y
Clone 11	A91W/P220C
Clone 12	A91W/P220W/D304Y
Clone 13	V92F/G222F/T303F
Clone 14	D304F
Clone 15	V92F/D304Y
Clone 16	A91W/G22F/T303stop
Clone 17	T303C
Clone 18	V92F/G222W/T303F
Clone 19	R88K/F134W/V190F
Clone 20	D304W
Clone 21	P220C
Clone 22	D304F
Clone 23	V92F/G222F/T303W
Clone 24	A91W/D304F/M305F
Clone 25	G89W/G93W/G222L/M305Y
Clone 26	A91W/D304W/M305W
Clone 27	A91W/D304F/M305Y
Clone 28	P220F/P300F/M305W
Clone 29	G90W
Clone 30	M305stop
Clone 31	A91W/D304F/M305W

Clone 32	G89W/G93W/G222F/M305Y
Clone 33	N219C
Clone 34	N219W
Clone 35	P220W/G222W
Clone 36	A91F/V92C/D304W
Clone 37	G89stop/A91W
Clone 38	G89W/G93W/G222Y/M305Y
Clone 39	/
Clone 40	G90F
Clone 41	/
Clone 42	M305F
Clone 43	V221F/P300F/D304W
Clone 44	G89W/G93W/G222W/M305Y
Clone 45	/
Clone 46	M305L
Clone 47	P220F/D304W
Clone 48	G222F/T303F
Clone 49	G222F
Clone 50	M305stop
Clone 51	G89W/V92F/P220W/P300F/M305F
Clone 52	G222F/T303W
Clone 53	P220W/P300Y
Clone 54	G89Y/T303W
Clone 55	M305F
Clone 56	A91F/D304Y
Clone 57	G89Y/N219F/G222F
Clone 58	G222Y/M305W
Clone 59	G93W
Clone 60	G222F/P300Y/D304F

Note：The “/” indicates no amino acid change.

Table S2. The kinetic parameters of NylC-V3 and WT

Enzyme	K_m (g L^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (L g^{-1} s^{-1})
WT	5.6±0.6	4.9±0.8	0.8750±0.6362
NylC-V3	3.0±0.2	9.3±1.1	3.1000±0.7421

Table S3. GPC and DSC analysis of different treatment methods of PA6 film

	Mw (g mol^{-1})	Melting peak area (mJ mg^{-1})
Control	109210±23.5	65.9±1.4
WT	104851±56.1	65.6±2.0
NylC-V3	79568±39.8	58.7±1.3

Table S4. Average binding energy in 100ns of NylC-V3 and WT

	Average binding energy(kcal mol^{-1})
WT	-37.8±1.30
NylC-V3	-43.5±1.43

Table S5. The nucleotide and amino acid sequences of WT and NylC-V3

enzyme	nucleotide sequence	amino acid sequence
WT	ATGCACCACCACCACCACCACGGCGCAGGTGCTAACACTACTCCA	MHHHHHHGAGANTTPVH
	GTACACGCTCTGACCGATATCGATGGTGGCATCGCGGTAGATCCA	ALTDIDGGIAVDPAPRLAGP
	GCACCACGTCTGGCAGGTCCACCGGTGTTTGGTGGTCCGGGTAAC	PVFGGPGNAAFDLAPVRST
	GCGGCATTCGACCTGGCTCCGGTTCGTTCCACTGGCCGTGAAATG	GREMLRFDFPGVSIGAAHY
	CTGCGTTTCGACTTTCCAGGTGTTTCTATCGGTGCAGCGCACTACG	EEGPTGATVIHIPAGARTAV
	AAGAAGGTCCGACTGGTGCTACTGTTATCCACATTCCGGCTGGTG	DARGGAVGLSGGYDFNHAI
	CTCGTACCGCTGTAGATGCACGTGGTGGTGCTGTAGGTCTGTCCG	CLAGGAGYGLEAGAGVSG
	GTGGTTACGACTTCAACCACGCTATCTGCCTGGCTGGCGGTGCTG	ALLERLEYRTGFAELQLVSSA
	GTTACGGTCTGGAAGCTGGTGCTGGTGTTAGCGGTGCACTGCTGG	VIYDFSARSTAVYPDKALGR
	AACGTCTGGAATACCGTACCGGCTTTGCTGAACTGCAGCTGGTAT	AALEFAVPGEFPQGRAGAG
	CTTCTGCTGTGATCTACGACTTCTCTGCTCGTAGCACCGCGGTTTAT	MSASAGKVDWDRTEITGQ
	CCGGACAAAGCGCTGGGTCGTGCTGCACTGGAATTCGCGGTACCG	GAAFRRLGDVRILAVVVPN
	GGTGAATTTCCACAGGGTCGTGCTGGCGCTGGTATGTCCGCTAGC	PVGVIVDRAGTVVRGNYDA
	GCAGGTAAAGTTGACTGGGACCGTACCGAAATCACCGGTCAGGG	QTGVRRHPVFDYQEAFAE
	TGCTGCTTTCCGTCGTCTGGGCGATGTGCGTATCTTGGCAGTAGTT	QVPPVTQAGNTTISAIVTN
	GTTCCAAATCCGGTTGGTGTTATCGTAGATCGTGCTGGTACCGTTG	VRMSPVELNQFAKQVHSS
	TTCGTGGTAACTACGATGCACAGACTGGTGTTCGTCGTCATCCGGT	MHRGIQPFHTDMDGDTLF
	GTTCGATTACCAAGAAGCATTCGCTGAACAGGTTCCGCCGGTTACT	AVTTDEIDLPTTPGSSRGRL
	CAGGCTGGTAACACCACCATCTCTGCAATCGTAACCAACGTTCGTA	SVNATALGAIASEVMWDA
	TGAGTCCGGTTGAACTGAACCAGTTCGCGAAACAGGTTCACAGCT	VLEAGK
	CTATGCACCGTGGTATCCAGCCGTTCCACACTGACATGGATGGCG	
	ACACTCTGTTCGCGGTAACTACCGACGAAATCGACCTGCCAACCAC	
	TCCGGGTAGCTCTCGTGGTCGTCTGAGCGTGAACGCAACCGCTCT	
	GGGTGCAATCGCGTCTGAAGTTATGTGGGATGCGGTTCTGGAAGC	
	CGGTAAGTAACTCGAG	
NylC-V3	ATGCACCACCACCACCACCACGGCGCAGGTGCTAACACTACTCCA	MHHHHHHGAGANTTPVH
	GTACACGCTCTGACCGATATCGATGGTGGCATCGCGGTAGATCCA	ALTDIDGGIAVDPAPRLAGP
	GCACCACGTCTGGCAGGTCCACCGGTGTTTGGTGGTCCGGGTAAC	PVFGGPGNAAFDLAPVRST
	GCGGCATTCGACCTGGCTCCGGTTCGTTCCACTGGCCGTGAAATG	GREMLRFDFPGVSIGAAHY
	CTGCGTTTCGACTTTCCAGGTGTTTCTATCGGTGCAGCGCACTACG	EEGPTGATVIHIPAGARTAV
	AAGAAGGTCCGACTGGTGCTACTGTTATCCACATTCCGGCTGGTG	DARGG**W**VGLSGGYDFNH
	CTCGTACCGCTGTAGATGCACGTGGTGGT**TGG**GTAGGTCTGTCCG	AICLAGGAGYGLEAGAGVS
	GTGGTTACGACTTCAACCACGCTATCTGCCTGGCTGGCGGTGCTG	GALLERLEYRTGFAELQLVS
	GTTACGGTCTGGAAGCTGGTGCTGGTGTTAGCGGTGCACTGCTGG	SAVIYDFSARSTAVYPDKAL
	AACGTCTGGAATACCGTACCGGCTTTGCTGAACTGCAGCTGGTAT	GRAALEFAVPGEFPQGRAG
	CTTCTGCTGTGATCTACGACTTCTCTGCTCGTAGCACCGCGGTTTAT	AGMSASAGKVDWDRTEIT
	CCGGACAAAGCGCTGGGTCGTGCTGCACTGGAATTCGCGGTACCG	GQGAAFRRLGDVRILAVVV
	GGTGAATTTCCACAGGGTCGTGCTGGCGCTGGTATGTCCGCTAGC	PN**W**VGVIVDRAGTVVRGN
	GCAGGTAAAGTTGACTGGGACCGTACCGAAATCACCGGTCAGGG	YDAQTGVRRHPVFDYQEAF
	TGCTGCTTTCCGTCGTCTGGGCGATGTGCGTATCTTGGCAGTAGTT	AEQVPPVTQAGNTTISAIVT
	GTTCCAAAT**TGG**GTTGGTGTTATCGTAGATCGTGCTGGTACCGTTG	NVRMSPVELNQFAKQVHS

	TTCGTGGTAACTACGATGCACAGACTGGTGTTCGTCGTCATCCGGT	SMHRGIQPFHT**Y**MDGDTL
	GTTCGATTACCAAGAAGCATTCGCTGAACAGGTTCCGCCGGTTACT	FAVTTDEIDLPTTPGSSRGR
	CAGGCTGGTAACACCACCATCTCTGCAATCGTAACCAACGTTCGTA	LSVNATALGAIASEVMWD
	TGAGTCCGGTTGAACTGAACCAGTTCGCGAAACAGGTTCACAGCT	AVLEAGK
	CTATGCACCGTGGTATCCAGCCGTTCCACACT**TAT**ATGGATGGCGA	
	CACTCTGTTCGCGGTAACTACCGACGAAATCGACCTGCCAACCACT	
	CCGGGTAGCTCTCGTGGTCGTCTGAGCGTGAACGCAACCGCTCTG	
	GGTGCAATCGCGTCTGAAGTTATGTGGGATGCGGTTCTGGAAGCC	
	GGTAAGTAACTCGAG	

Note：Bold fonts were mutation sites.

Table S6. Primer names and primer sequences

primer	primer sequences (5'-3')
F-R88FYW	TGTAGATGCATDBGGTGGTGCTGTAGGTCTG
R-R88FYW	CCVHATGCATCTACAGCGGTACGAGCACCAG
F-G89FYW	GATGCACGTTDBGGTGCTGTAGGTCTGTCCGG
R-G89FYW	CACCVHACGTGCATCTACAGCGGTACGAGCAC
F-G90FYW	CAGCVHAACCACGTGCATCTACAGCGGTACGAG
F-G90FYW	GCACGTGGTTDBGCTGTAGGTCTGTCCGGTG
F-A91FYW	CACGTGGTGGTTDBGTAGGTCTGTCCGGTGG
R-A91FYW	CVHAACCACCACGTGCATCTACAGCGGTACG
F-V92FYW	GGTGGTGCTTDBGGTCTGTCCGGTGGTTACG
R-V92FYW	GACCVHAAGCACCACCACGTGCATCTACAGC
F-G93FYW	GGTGCTGTATDBCTGTCCGGTGGTTACGAC
R-G93FYW	CAGVHATACAGCACCACCAGTGATCTAC
F-N219FYW	GTAGTTGTTCCATDBCCGGTTGGTGTTATCG
R-N219FYW	VHATGGAACACACTACTGCCAAGATACGCAC
F-P220FYW	TAGTTGTTCCAAATTDBGTTGGTGTTATCGTAG
R-P220FYW	AATTTGGAACAACTACTGCCAAGATACGCAC
F-V221FYW	GTTCCAAATCCGTDBGGTGTTATCGTAGATCG
R-V221FYW	VHACGGATTTGGAACAACTACTGCCAAGATAC
F-G222FYW	CAAATCCGGTTTDBGTTATCGTAGATCGTGCTG
R-G222FYW	CVHAACCGGATTTGGAACACTACTGCCAAG
F-P300FYW	GGTATCCAGTDBTTCCACACTGACATGGATG
R-P300FYW	GGAAVHACTGGATACCACGGTGCATAGAGCTG
F-F301FYW	GGTATCCAGCCGTDBCACACTGACATGGATGG
R-F301FYW	GVHACGGCTGGATACCACGGTGCATAGAGCTGTG
F-H302FYW	CCAGCCGTTCTDBACTGACATGGATGGCGAC
R-H302FYW	GTVHAGAACGGCTGGATACCACGGTGCATAG
F-T303FYW	CAGCCGTTCCACTDBGACATGGATGGCGACAC
R-T303FYW	CVHAGTGGAACGGCTGGATACCACGGTGCATAG
F-D304FYW	GCCGTTCCACACTTDBATGGATGGCGACACTC
R-D304FYW	VHAAGTGTCGAACGGCTGGATACCACGGTGC
F-M305FYW	GTTCCACACTGACTDBGATGGCGACACTCTGTTC
R-M305FYW	VHAGTCAGTGTGGAACGGCTGGATACCACGGTGC

References:

1 S. Negoro, N. Shibata, D. Kato, Y. Tanaka, K. Yasuhira, K. Nagai, S. Oshima, Y. Furuno, R. Yokoyama, K. Miyazaki, M. Takeo, K. Hengphasatporn, Y. Shigeta, Y. Lee and Y. Higuchi, *The FEBS Journal*, 2023, **290**, 3400–3421.