

Supplementary Materials

Table S1. The primers used in this study

Primer	Sequence(5'-3')
C19S1	F: GATATCGAGCAGCATT <u>AG</u> TTCATTTGACGAATG
C19S2	R: CATTTCGTCAAATGACTAATGCTGCTCGATATC
C110S1	F: GTGAAGAGCGCATT <u>AG</u> TCTTGTGACAACCAAC
C110S2	R: GTTGGTTGTCACAAGACTAATGCGCTCTTCAC
C130S1	F:GTGTCAGACACTAGT <u>AG</u> CACATTCCTTCAG
C130S2	R:CTGAAGGGAATGTGCTACTAGTGTCTGACAC
mPex1	F: CTC <u>GATCC</u> CAACTCATGGTCGGATACTACAAG
mPex2	R: CAGTCTC <u>GAGT</u> TAGGACCTGCTGTTGACCTTGC

F: forward primer. R: reverse primer. The restrictive enzyme cut sites are indicated by the underlines. The mutated bases in the forward primers are marked in red.

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mHRP      -----QLTPTFYDNSCP NVSN
mPex      MTTSKLAVLTLFALLGSVSCQSGGYFYPTPQQPSPTSPSPSSPQLMVGYYKDKCAAYVD
                                         **  :*.:.*.  :

mHRP      IVRDTIVNELRSDPRIAASILRLHFHDCFVNGCDASILLD---NTTSFRTEKDAFGNANS
mPex      VEAIVKKHKVKTADAGMQAGLVRLLFHDCFVRGCDGSVLLDTFSNDSLTPKFGVFNFP
:   .   :   :*. : *.:** *****.***. *:***  * **: .** .. * *

mHRP      ARGFPVIDRMKAAVESACPRTVSCADLLTIAAQQS--VTLAGGPSWRVPLGRRDSLQAF
mPex      LRGFEVIDAAKAEIEAACPGTVSCADIVAFARDASYFLSGGGISFAMPAGRYDGNVSLA
*** **  *  :*:*** *****:>:::***:::  .  .** *: :* ** * .  ::

mHRP      DLANANLPAPFFTLPLQLKDSFRNVGLNRSSDLVALSGGHTFGKNQCRFIMDRLYNFSNTG
mPex      SETLPNLPSPTGFDQLVKVFADKGLD-AFDMITLSGAHSIGRSHCSSFTRDRLPSPNTT
.  :  .***:**  : ** . * : ** : *:::***.***:*.:.:*  :      ***

mHRP      LPDPTLNTTYLQTLRGLCPLNGNLSALVDFDLRTP TIFDNKYYVNLEEQKGLIQSDQELF
mPex      DIDP----AFAATLQASCASPNGTDNTVMQDFKTPDVLDNQYYKNVLAHKVLF TSDAALT
**      ::  **: . * .  . . *  *:** :***:** * : :* * : ** *

mHRP      SSPNATDTIPLVRSFANSTQTFFN-AFVEAMDRMGNITPLTGTQGQIRLNCRVNSNS
mPex      TNFTSNN---LVRAYADFPYLWQQKFAKAMVKMAGVEIKTAANGEIRKTCRKVNSRS
.  .: :  ***:*. :  ::: *.:** :*..:  *.:*:** .** **.*

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Fig. S1. Alignment of the deduced amino acid sequences of mature HRP (mHRP) with mPex. Identical amino acid residues are represented by asterisks. The alignment was performed using CLUSTALW (<https://www.genome.jp/tools-bin/clustalw>).

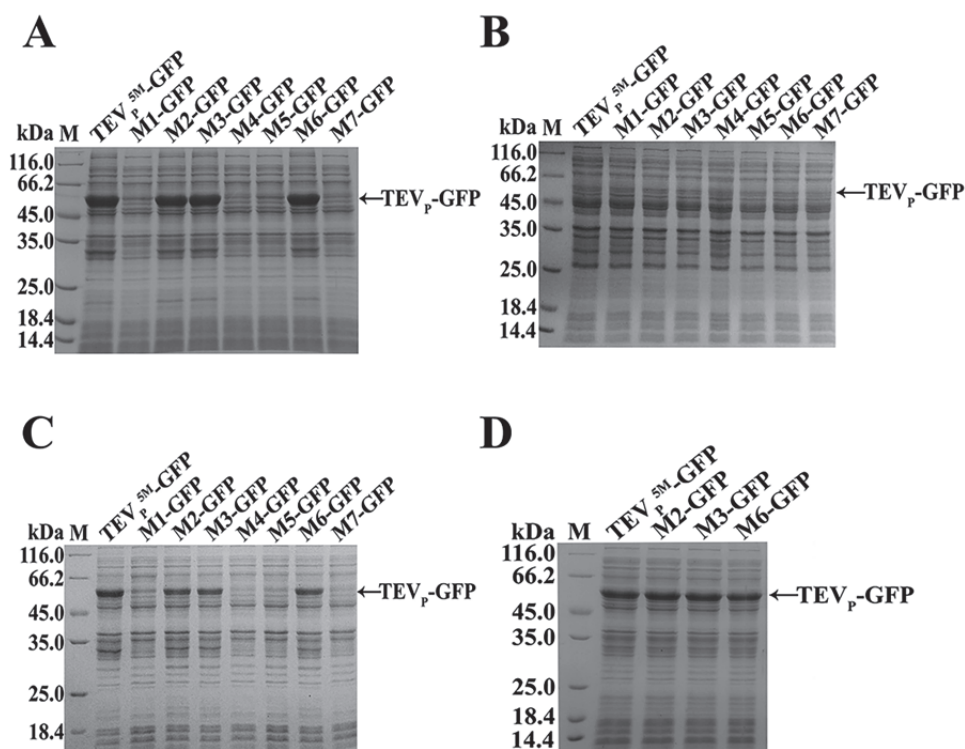


Figure S2 SDS-PAGE analysis of the C-terminally EmGFP tagged TEVp^{5M} and its variants. About 10 μ g soluble proteins expressed in BL21(DE3) (a), BL21(DE3)pLysS (b), Rosetta (DE3) (c) and Origami (DE3) (d) were separated by 12% SDS-PAGE.

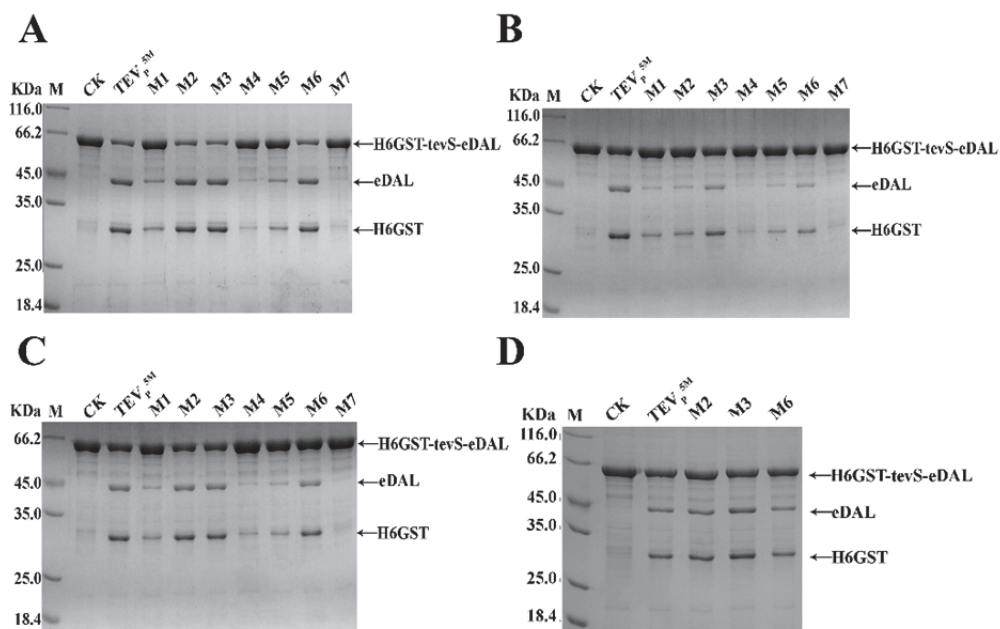


Figure S3 SDS-PAGE analysis of the purified fusion protein incubating with the TEVp construct in crude extract. CK: the fusion protein incubating with the soluble extract from the correspondent cells carrying the pET-28b or pET-22b plasmids. The fusion protein substrate and cleaved products were indicated by arrows.

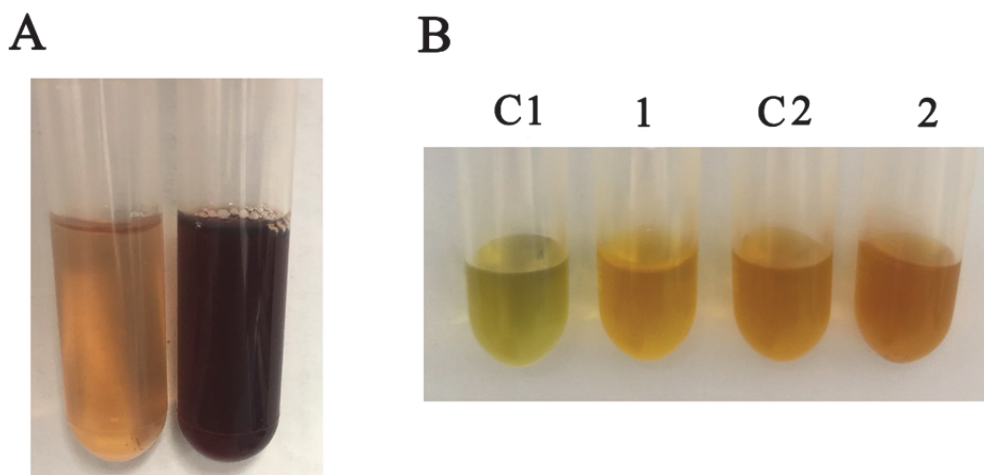


Figure S4 **The activity assay of the bEK and mPex.** A: the **refolded tag-free** bEK cleaved the fusion protein for the sDAL, rendering the released sDAL with the obviously increased activity for transformation of DL-DAP and coloration of pyruvate as the catalytic product with DNP. B: The activity assay of the **refolded tag-free** mPex. C1 and C2 represented the reaction mixture containing 10 mM or 30 mM H_2O_2 with addition of the heat-inactive mPerx (100 °C for 10 min). 1 and 3 represented the mixture with addition of the refolded mPex and OPA. **Activity assays** of the bEK and mPex were described in Material and Methods section.