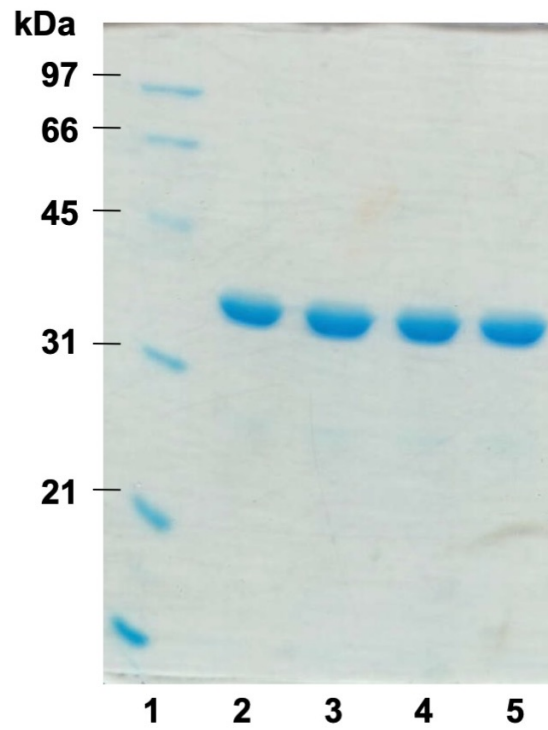


1 **Supplementary materials**

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<i>Sulfolobus solfataricus</i>	DMD	133	K I A R L G S	G S	A C R S M F	148	
<i>Saccharomyces cerevisiae</i>	DMD	147	R I A R K G S	G S	A C R S L F	162	
<i>Staphylococcus aureus</i>	DMD	133	R L A R I G S	G S	A S R S I Y	148	
<i>Staphylococcus epidermidis</i>	DMD	133	R L A R R G S	G S	A S R S I F	148	
<i>Flavobacterium johnsoniae</i>	DMD	165	F L A R L G S	G S	A C R S V K	180	
<i>Roseiflexus castenholzii</i>	PMD	195	C M A R L L A	G S	G C R S A A	210	
<i>Haloferax volcanii</i>	PMD	131	T I A R R G S	A S	A A R A V T	146	
<i>Picrophilus torridus</i>	M3K	138	N D L Q R I S	E S	V G R S L Y	153	
<i>Thermoplasma acidophilum</i>	M3K	133	N D L R A V S	E S	A G R S L F	148	
							*

2 **Fig. S1. A part of multiple alignment of amino acid sequences of the DMD homologues**
 3 **distributing in the MVA pathways.**

4 The accession numbers of DMD homologs from NCBI database; *S. solfataricus* DMD
 5 (AAK43094.1), *S. cerevisiae* DMD (NP_014441.1), *S. aureus* DMD (ACY10476.1), *S.*
 6 *epidermidis* DMD (NP_763917.1), *F. jhonsoniae* DMD (ABQ04421.1), *R. castenholzii*
 7 **PMD** (ABU57050.1), *H. volcanii* **PMD** (ADE02509.1), *P. torridus* **M3K** (AAT43063.1), *T.*
 8 *acidophilum* **M3K** (CAC12426.1). The colors of the names of the enzymes are in accord
 9 with those in Figure 1. The multiple alignment was performed using the Clustal W
 10 (Thompson JD, Higgins DG, Gibson TJ. 1994. Nucleic Acids Res 22:4673-4680. DOI:
 11 10.1007/978-1-4020-6754-9_3188). The asterisks indicate conserved glycine of DMDs
 12 (green) and 140th glutamate of TacM3K (red), which are the amino acid residues related to
 13 the recognition of substrates in active site.



14

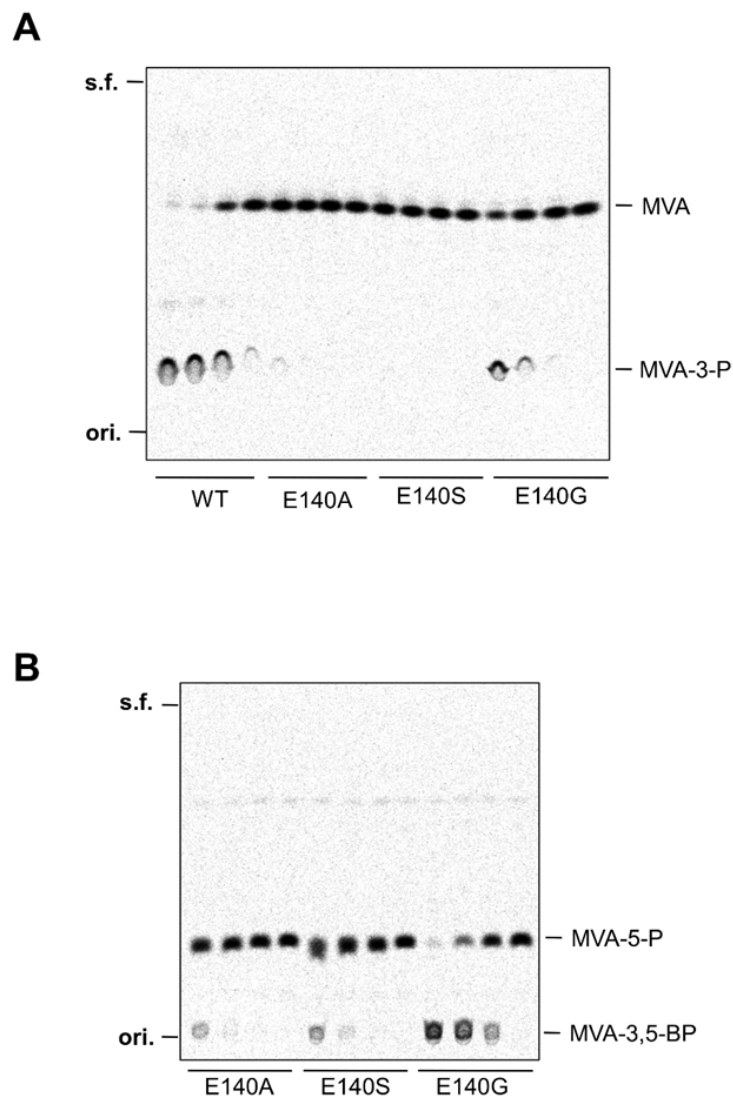
15 **Fig. S2. SDS-PAGE analysis of the purified wild type TacM3K and E140 mutants.**

16 Lane 1, molecular marker; lane 2, wild type (35.2 kDa); lane 3, E140A; lane 4, E140S; lane

17 5, E140G. The proteins were separated on 12.5% SDS-PAGE.

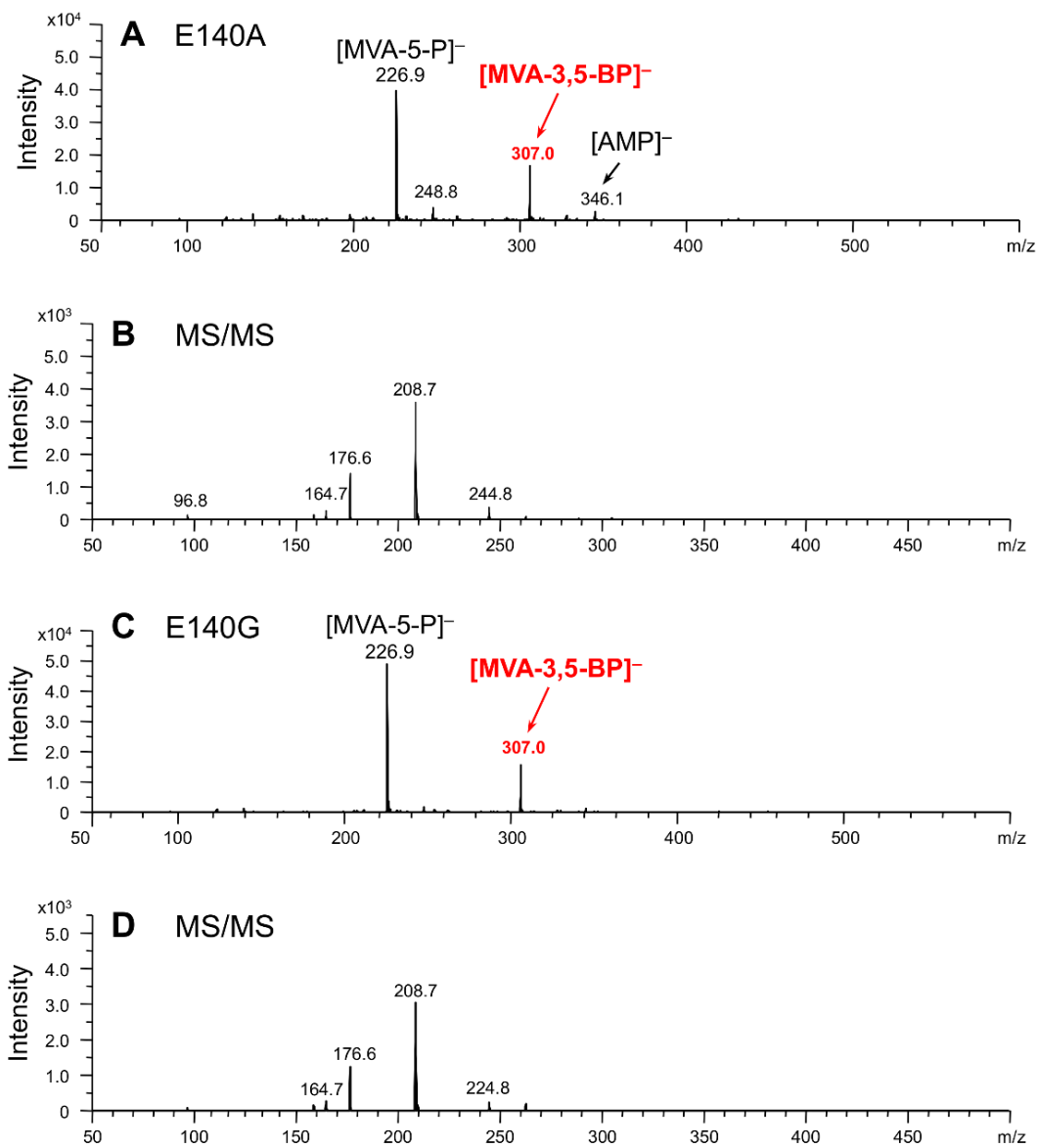
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20 **Fig. S3. Semi-quantitative assay of the E140 mutants.**

21 The mutated TacM3K, E140A, E140S, or E140G was reacted for 1 hour at 60 °C with 25
 22 pmol of [2-¹⁴C]MVA (A) or [2-¹⁴C]MVA-5-P (B), excepting the wild type in the presence of
 23 ATP and Mg²⁺. ori., origin; s.f., solvent front. 440, 220, 110 or 8.8 ng (12.5, 6.25, 3.125 and
 24 0.25 pmol, respectively) of each enzyme was used (from left to right lanes). An aliquot of
 25 reaction mixture was analyzed by normal-phase TLC.



26

27 **Fig. S4.** Negative ESI-MS analysis of the E140A or E140G reaction product from (*R,S*)-
 28 MVA-5-P. (A and C) Mass spectrum of the sample from the reaction of the E140A (A) or
 29 E140G (C) mutant of TacM3K with (*R,S*)-MVA-5-P. (B and D) MS/MS analysis of the ion
 30 of m/z 307.0 from (A and C, respectively).

31