Supplementary materials

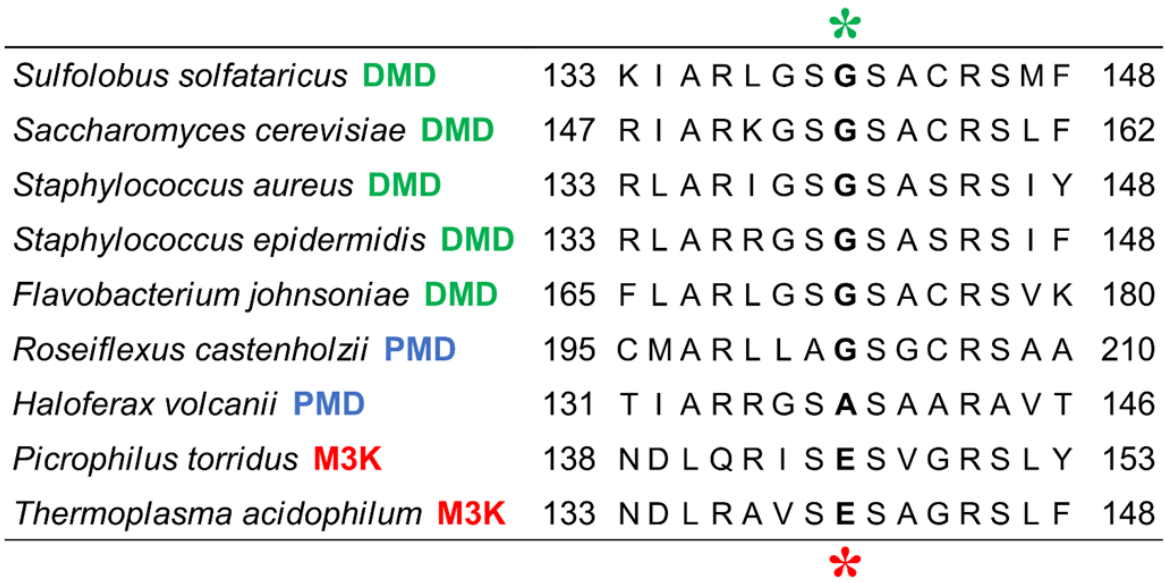

Fig. S1. A part of multiple alignment of amino acid sequences of the DMD homologues distributing in the MVA pathways.

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

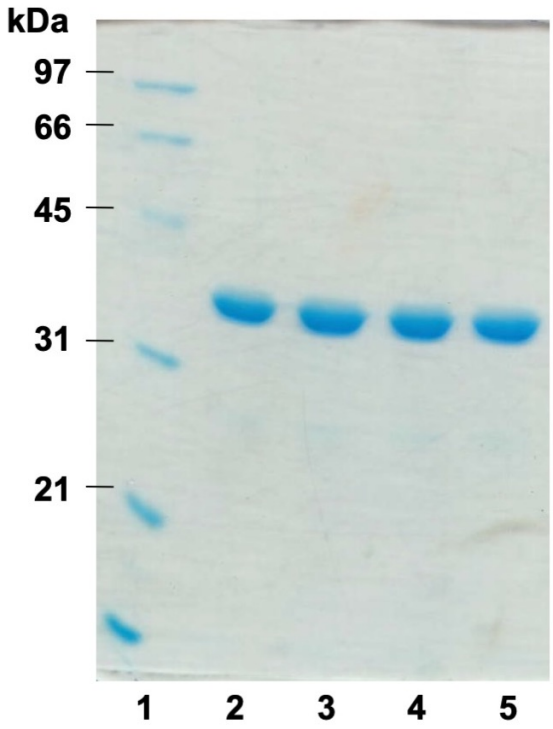

Fig. S2. SDS-PAGE analysis of the purified wild type TacM3K and E140 mutants.

Lane 1, molecular marker; lane 2, wild type (35.2 kDa); lane 3, E140A; lane 4, E140S; lane 5, E140G. The proteins were separated on 12.5% SDS-PAGE.

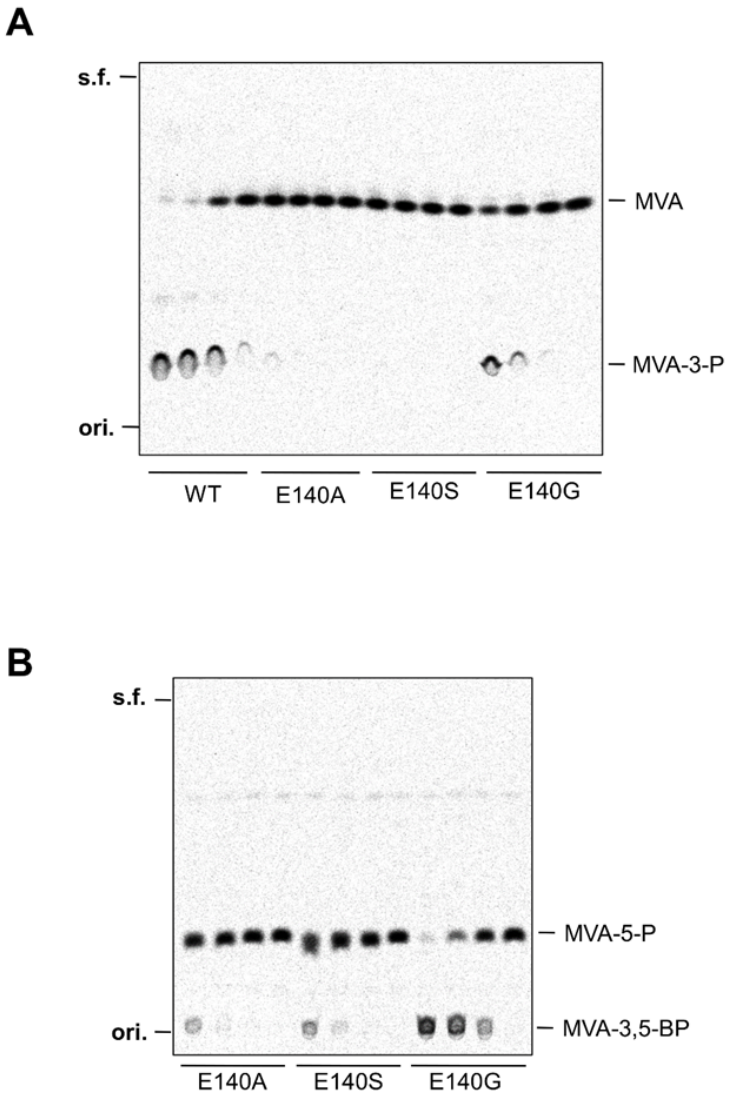

Fig. S3. Semi-quantitative assay of the E140 mutants.

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

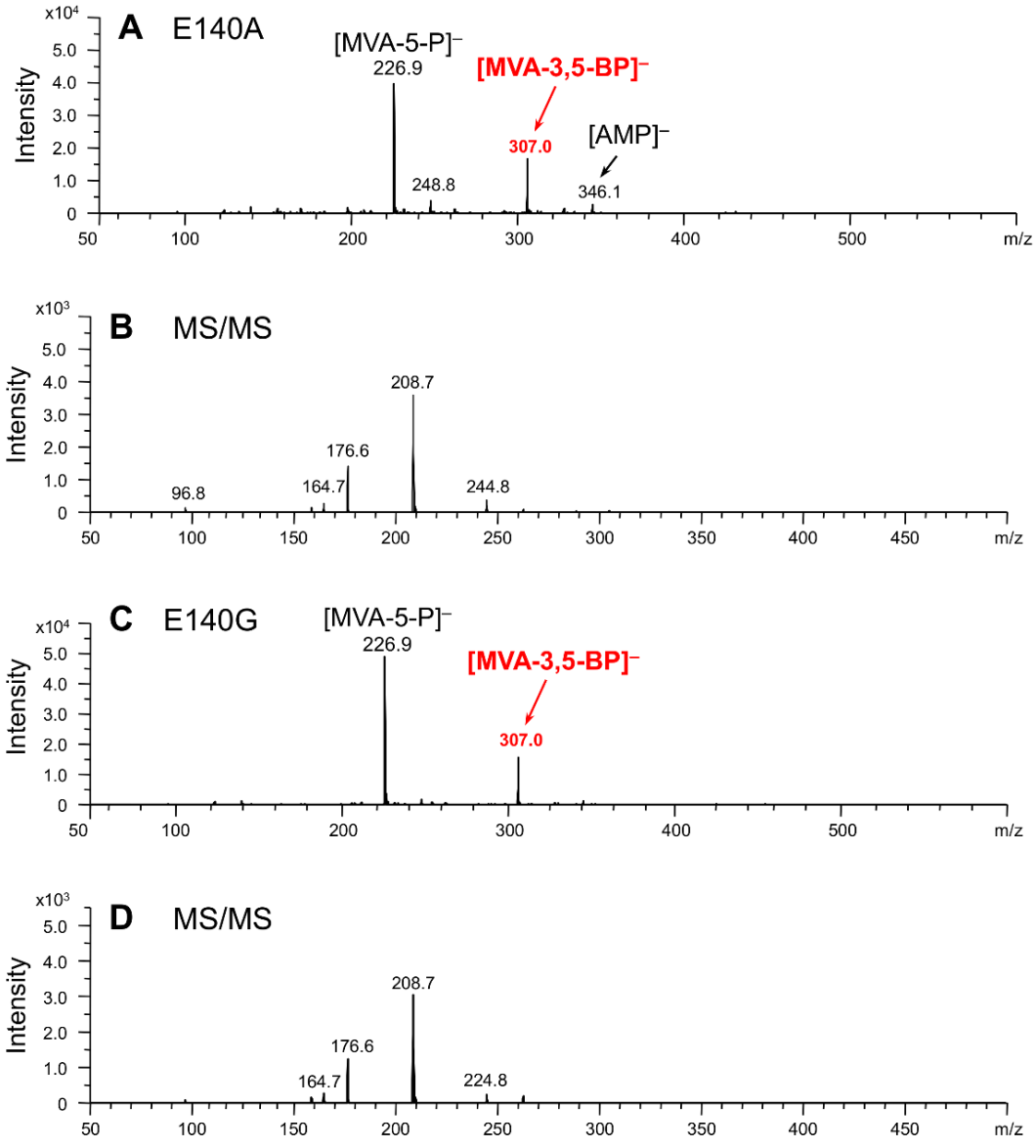

Fig. S4. Negative ESI-MS analysis of the E140A or E140G reaction product from (*R*,*S*)-MVA-5-P. (A and C) Mass spectrum of the sample from the reaction of the E140A (A) or E140G (C) mutant of TacM3K with (*R*,*S*)-MVA-5-P. (B and D) MS/MS analysis of the ion of m/z 307.0 from (A and C, respectively).