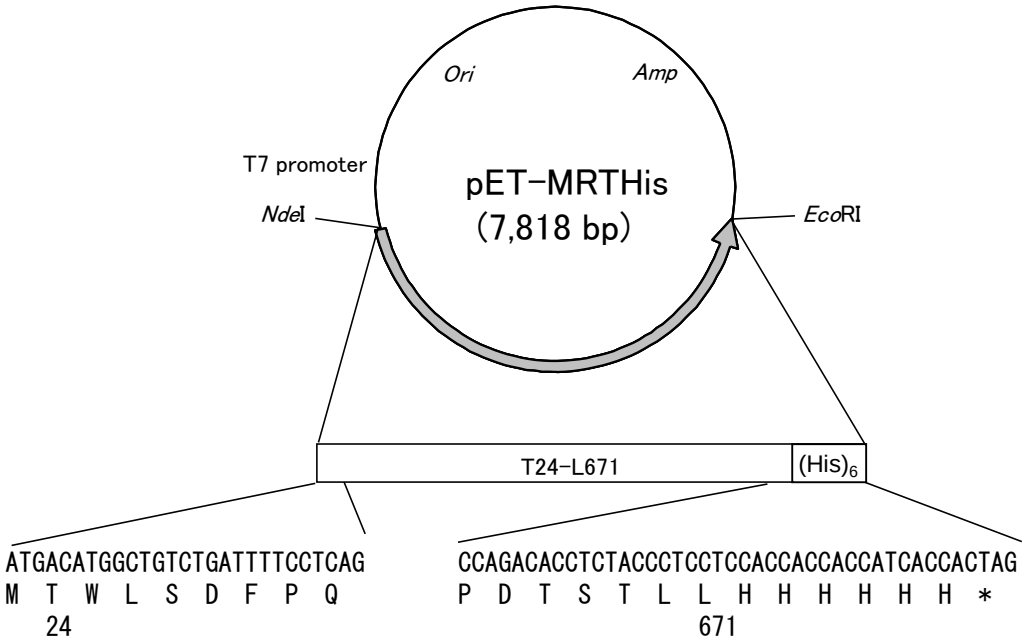

Fig. S1. Plasmid for the expression of MMLV RT in the cell-free system. The asterisk indicates the termination codon.

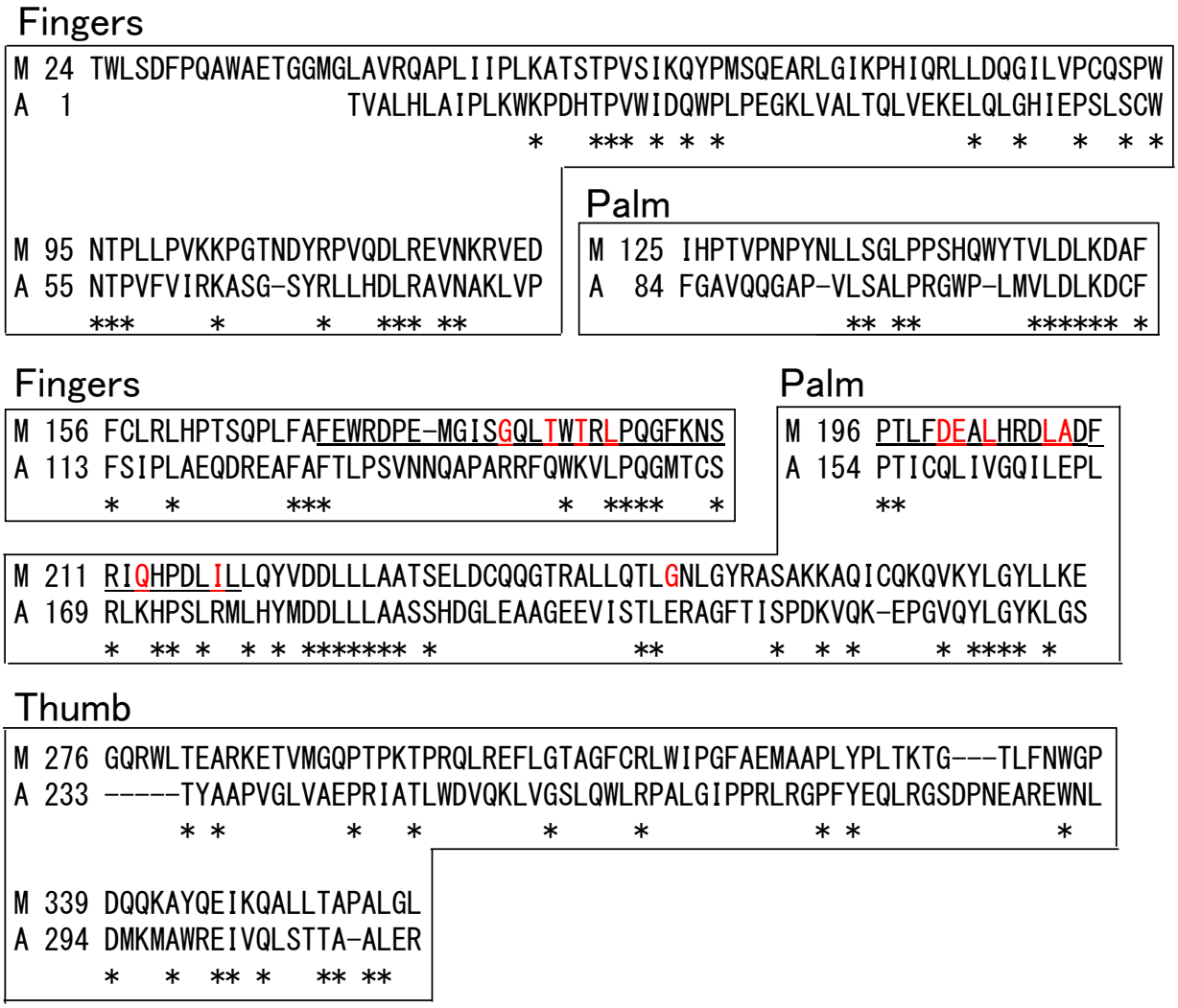

Fig. S2. Sequence alignment of MMLV RT and Aivian myeloblastosis virus (AMV) RT. Homology search was performed using the search program DDBJ CLUSTALW and revised based on the data of X-ray crystallographic analysis of MMLV RT (Das and Georgiadis., 2004). M and A indicate MMLV RT and AMV RT, respectively. Amino acid numberings of MMLV RT and AMV RT are according to GenBank accession codes J02255 and FJ041197, respectively. Asterisks show homologous amino acid residues. The polypeptide region corresponding to Region 3 (Phe170-Leu219) of MMLV RT is underlined. The amino acid residues identified as the mutational site for thermostabilization are marked in red.

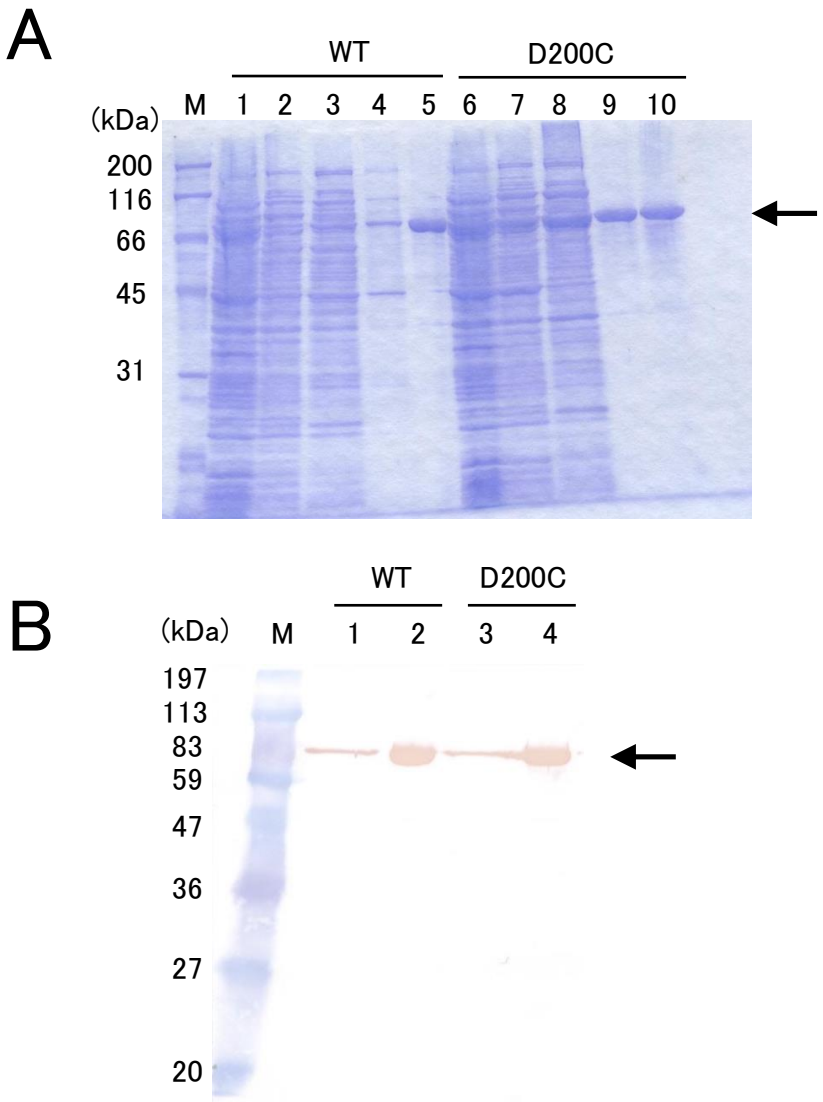

Fig. S3. Expression in *E. coli* and purification of MMLV RT. (A) 10% SDS-PAGE under reducing conditions. Active fractions of each purification stage for WT (lanes 1—5) and the thermostable MMLV RT variant D200C (lanes 6—10) were applied. Lanes: marker proteins (lane M), soluble fractions of the total extracts (lanes 1, 6), active fractions of ion-exchange chromatography (lanes 2, 7), the centrifuged pellets after fractionation by ammonium sulfate at 40% saturation (lanes 3, 8), active fractions of Ni^{2+} affinity chromatography (lanes 4, 9), and active fractions of gel filtration columns, which are the purified enzyme preparations (lanes 5, 10). (B) Western blot. Cell-free expression products (lanes 1, 3) and the purified enzymes (lanes 2, 4) of WT (lanes 1, 2) and D200C (lanes 3, 4) were applied to 10% w/v SDS-polyacryl amide gel followed by the Western blot with Anti-His-tag mAb-HRP DirectT. (A, B) The arrow indicates the band corresponding to MMLV RT. Pre-stained Protein Markers Broad Range (Nacalai Tesque) was used as a marker.