

Table S1 Mutable sites predicted by HotSpot wizard for hydrolytic activity of DLFae4

Position	Residue	Mutable	In tunnel	In catalytic pocket	Codon	Encoded AAs
145	Thr	9	√	×	VDS	Asp:1 Glu:1 Gly:2 His:1 Ile:1 Lys:1 Leu:2 Met:1 Asn:1 Gln:1 Arg:3 Ser:1 Val:2
146	Pro	8	√	×	RNS	Ala:2 Asp:1 Glu:1 Gly:2 Ile:1 Lys:1 Met:1 Asn:1 Arg:1 Ser:1 Thr:2 Val:2
132	Trp	8	√	√	VNS	Ala:2 Asp:1 Glu:1 Gly:2 His:1 Ile:1 Lys:1 Leu:2 Met:1 Asn:1 Pro:2 Gln:1 Arg:3 Ser:1 Thr:2 Val:2
157	Arg	8	√	×	NHC	Ala:1 Asp:1 Phe:1 His:1 Ile:1 Leu:1 Asn:1 Pro:1 Ser:1 Thr:1 Val:1 Tyr:1

Figure Captions

Figure S1 Screening clones with enhanced activity. (a) Screening of random mutation clones using EFA as substrate. (b) Screening of site-directed saturation mutation clones using EFA as substrate

Figure S2 Comparison of relative enzyme activities between WT and mutants

Figure S3 Effects of pH and temperature on the activity of DLFae4 and mutants. (a) The effect of temperature on activity; (b) the effect of pH on activity; (c) the effect of temperature (40°C) on thermal stability; (d) the effect of pH on stability. The maximal activity was defined as 100% and the relative activity is shown as a percentage of maximal activity. Each point presented mean \pm standard deviation (n = 3)

Figure S4 Ramachandran plot of DLFae4(a) and mutants (b: DLFae4-m1; c: DLFae4-m2; d: DLFae4-m3; e: DLFae4-m4; f: DLFae4-m5). Note: The red area represented residues in the most favoured regions, the yellow area represented residues in additional regions, the light yellow area represented residues in generously allowed regions, and the white area represented residues in disallowed regions

Figure S5 Hydrophobic pocket graphics of DLFae4 (a) and mutants (b: DLFae4-m1; c: DLFae4-m2; d: DLFae4-m3; e: DLFae4-m4; f: DLFae4-m5). Note: The substrate MFA was shown in gray sticks (by element). The amino acids that differ between WT and mutants were shown by the abbreviation of amino acid and serial number. The hydrophobic amino acids that differ between WT and mutants marked with red dashed circles

Figure S6 DLFae4 catalyzed the formation of insoluble phenethyl acetate

Figure S7 GC-MS analysis of the structure of the product after the reaction of vinyl acetate and 2-phenylethanol

Figure S8 HPLC analysis of the product after the reaction of vinyl acetate and cyanidin-3-O-glucoside. Negative control: cyanidin-3-O-glucoside + enzyme, Blank control: cyanidin-3-O-glucoside + vinyl acetate + inactivating enzyme, Experimental group: cyanidin-3-O-glucoside+ vinyl acetate+ enzyme.

Figure S1

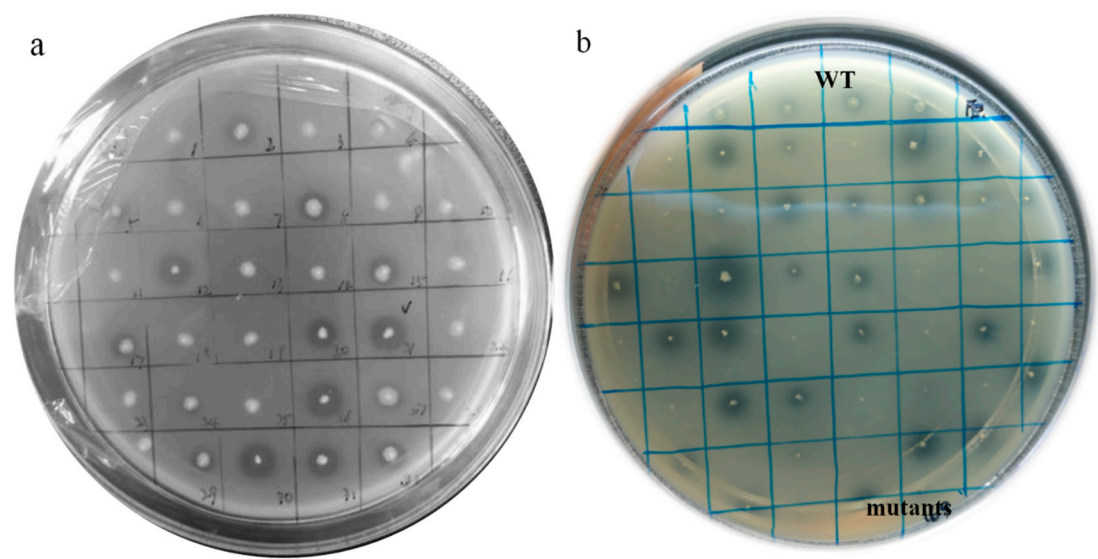


Figure S2

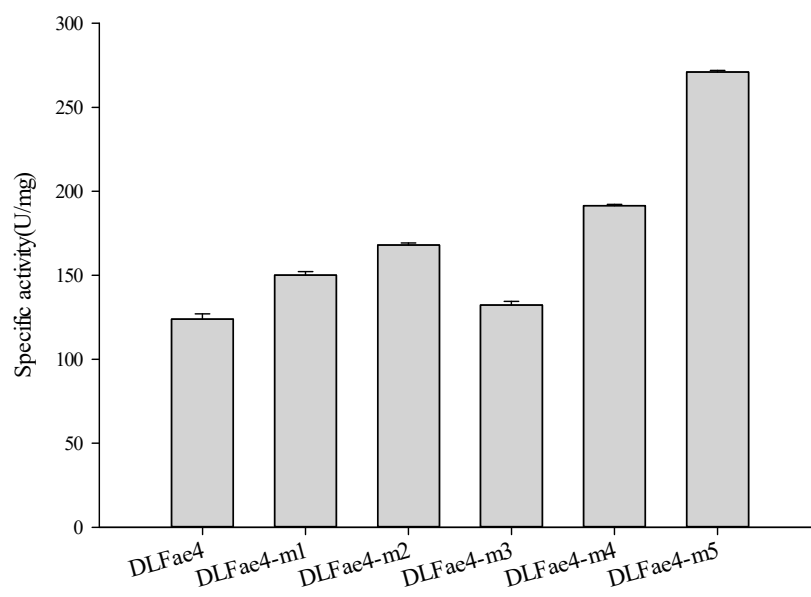


Figure S3

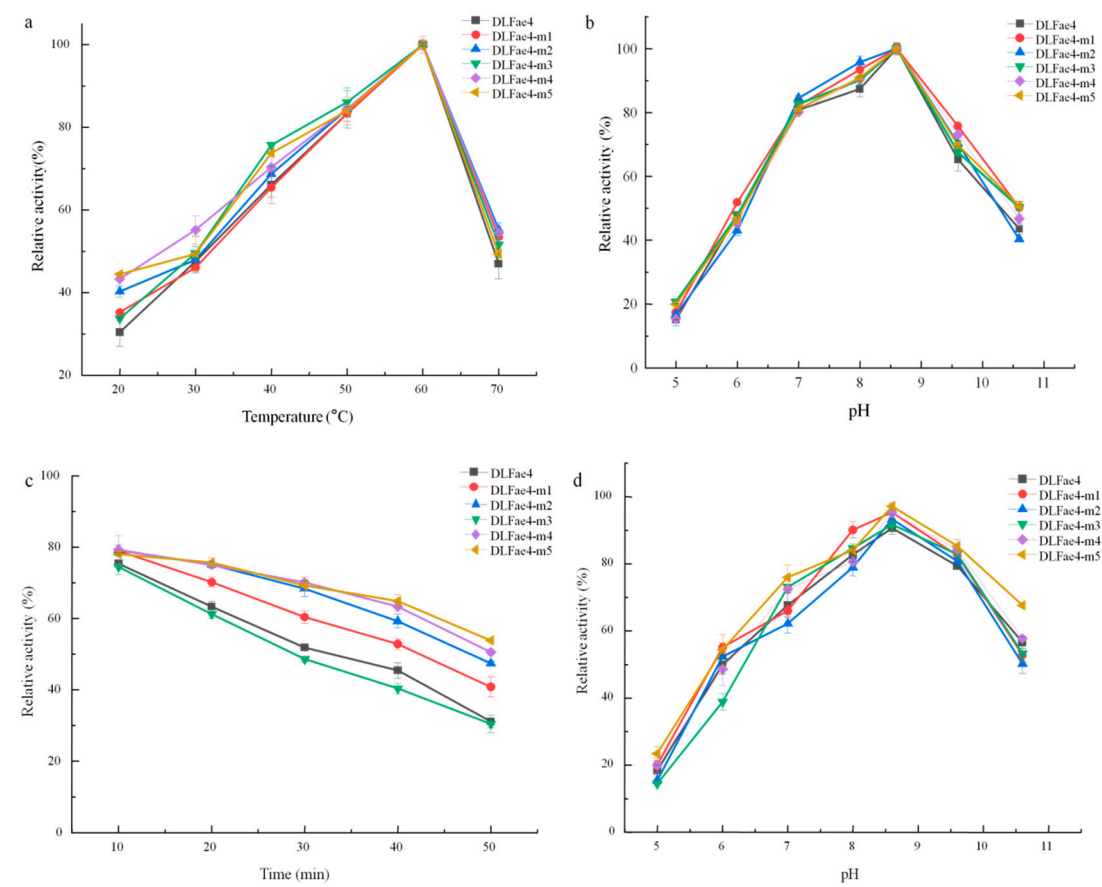


Figure S4

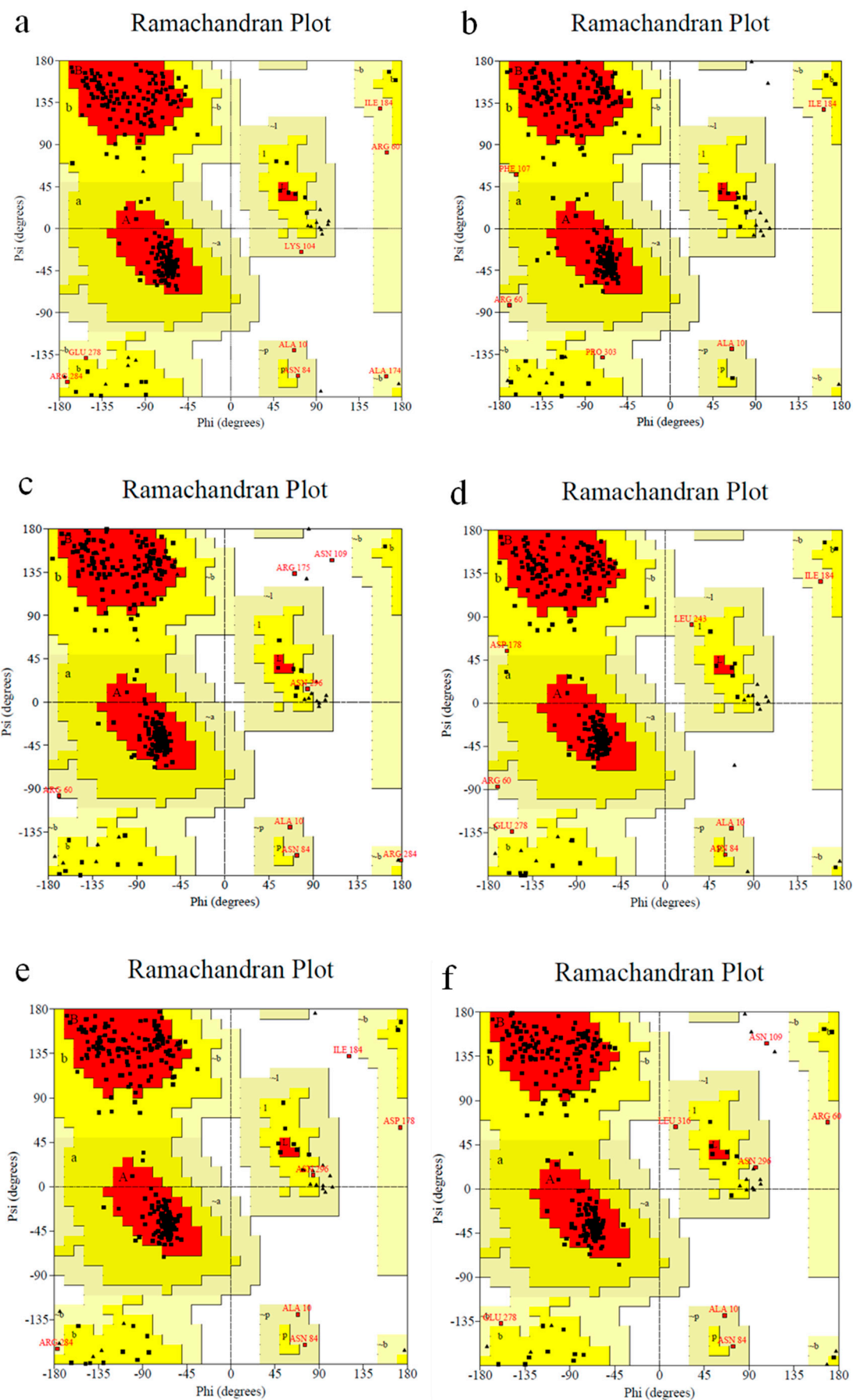


Figure S5

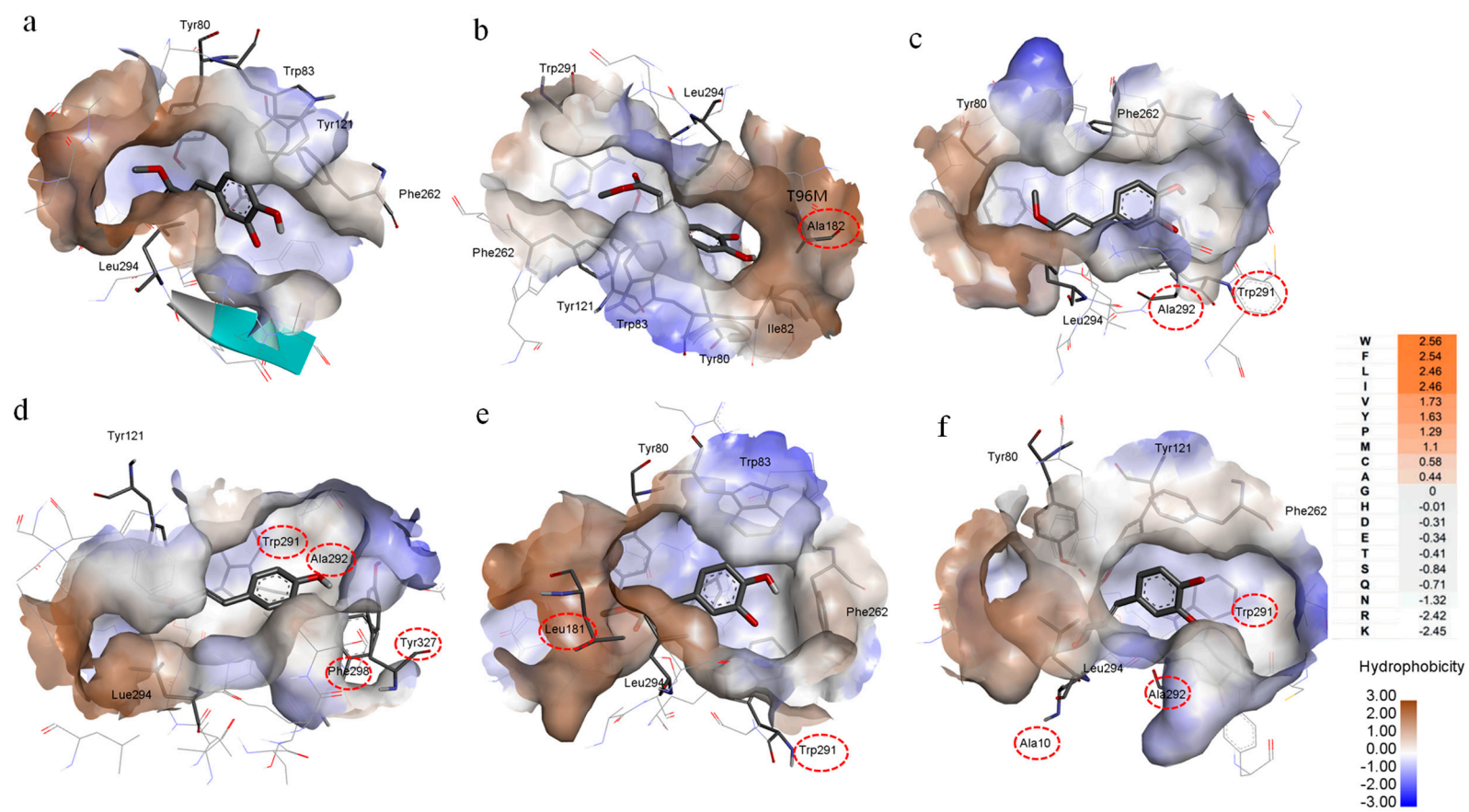


Figure S6

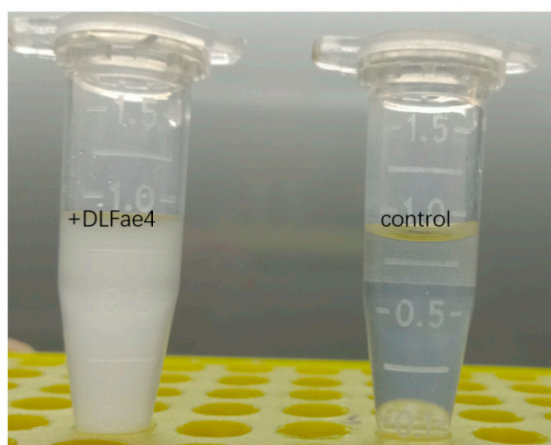


Figure S7

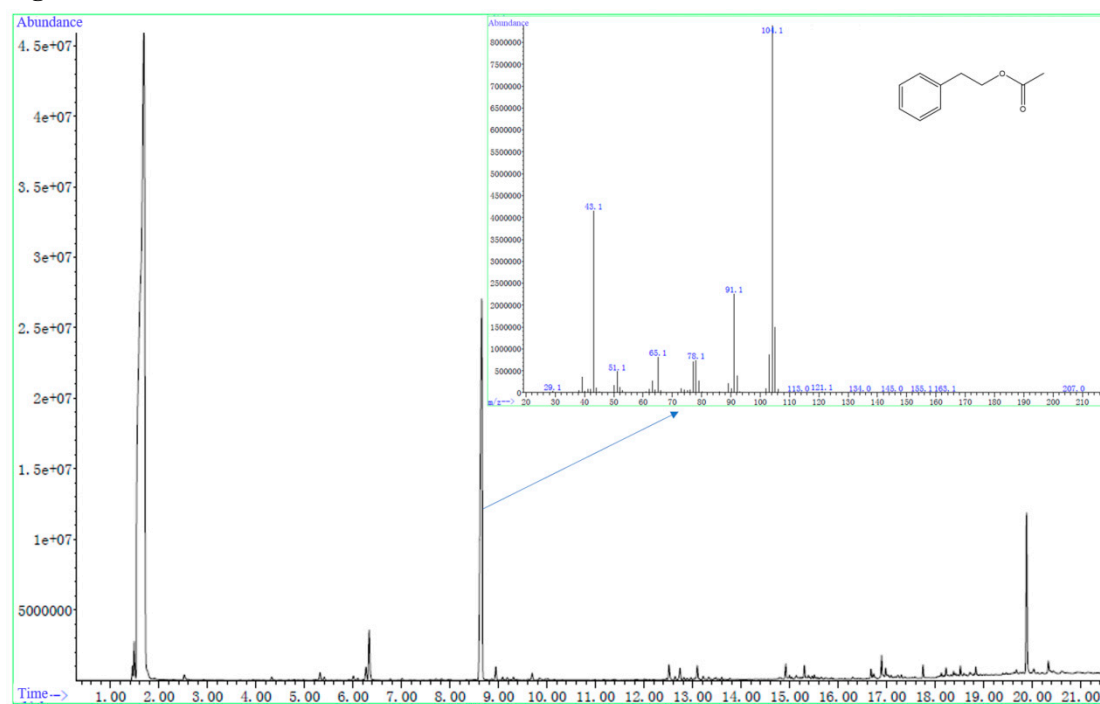


Figure S8

