

## Supplementary materials

**Table S1.** The list of single-point mutants and disulfide bond mutants.

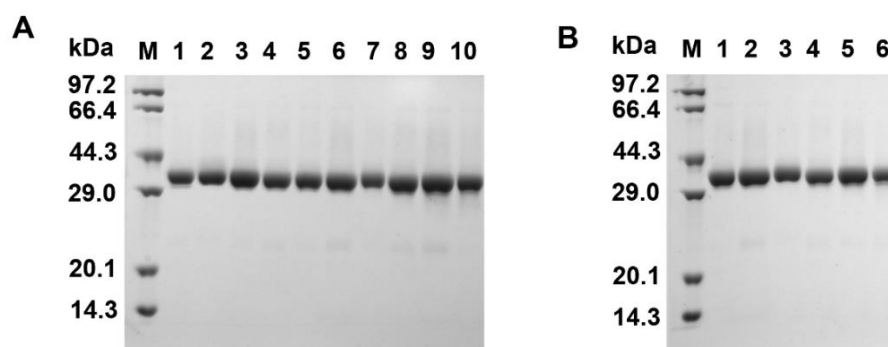
Single-point mutant			Disulfide bond mutant
G26D	W116K	Y193D	G28C-P206C
R27P	W116R	T195V	S31C-G262C
Q34P	W116Q	T195I	P35C-K258C
A37P	W116S	T195L	P35C-Y260C
N38V	Q117W <sup>a</sup>	T195F	N38C-A257C
N38L	N121D	G204V <sup>a</sup>	T42C-F286C
Y40W	I125R	G204F <sup>a</sup>	S46C-T288C
T42P	I125K	G204I	L47C-G67C
S46M	M135D	G204L	G50C-V301C
E61P	M135P <sup>a</sup>	S222Y <sup>a</sup>	M59C-T74C
N62P <sup>a</sup>	N143F	S222V	E61C-L64C
S69F	F150I	S222I	G67C-V301C
S69L <sup>a</sup>	Y156W	S222F	S69C-S89C
S69W	K160Y	S222L <sup>a</sup>	T74C-N85C
M75T	K160L	W229S	G76C-R81C
A80T	E162V	W229A	W116C-H136C
Q82P	M176V	R236P	Y127C-L186C
Q82L	M176I	D245K	Y128C-T132C
R83P	G177A	D245R <sup>a</sup>	K133C-N205C
H88E	C180V	A273P	A209C-Q241C
H88R	C180L	R274P	D213C-S244C
S91L	C180I	F278W	I249C-A257C
A95V	M182L	D279K	N253C-I284C <sup>a</sup>
H111E	D183F	Q289P <sup>a</sup>	Y260C-N265C
A113P	D183Y	M294R	N265-G268C <sup>a</sup>
W116E	R187F	A299P	H287C-H304C
W116D	D189P		

<sup>a</sup> the activity of the fermentation supernatant was too low, and thus the sample was not used for the following assay.

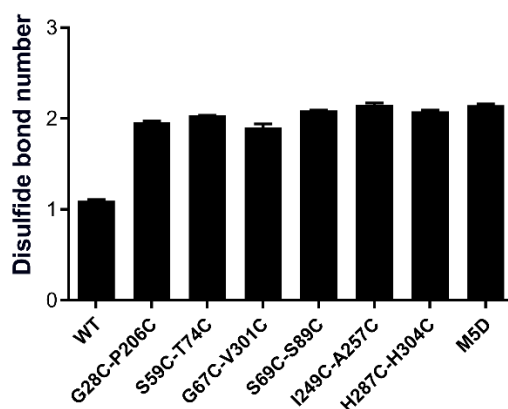
**Table S2.** Comparison of the DAG synthesis by using enzymes in this study and other reported enzymes via esterification.

enzyme	FA conversion (%)	MAG content (%)	1,3-DAG content (%)	1,2-DAG content (%)	Total DAG content (%)	TAG content (%)
Immobilized WT	71.40	31.17	26.57	13.66	40.23	\
Immobilized M5D	83.31	34.01	33.48	15.82	49.30	\
Novozyme 435 [38]	94.80	46.67	–	–	35.56	12.57
lipase G “Amano” 50 [39]	78.11	26.95	–	–	51.16	\
Immobilized PCL [8]	89.24	34.75	36.92	17.17	54.49	\

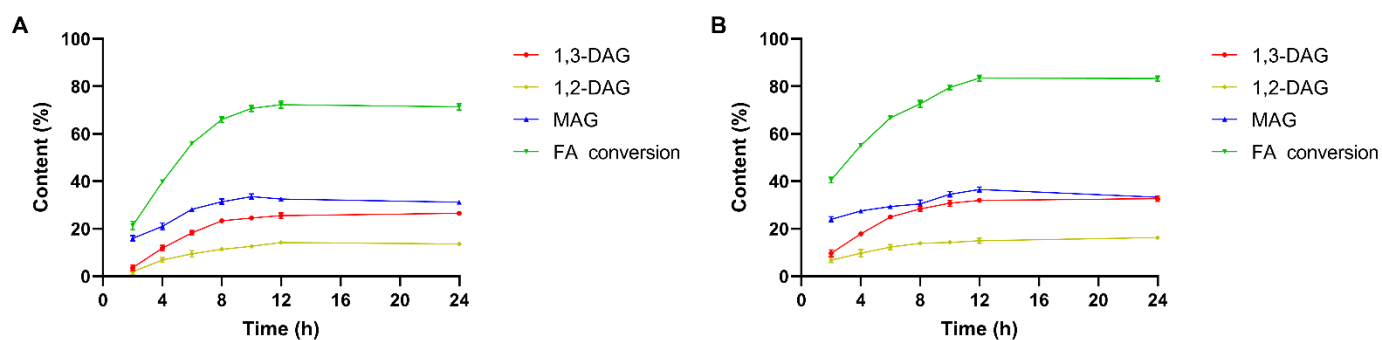
– represents that data are not reported. \ represents that TAGs cannot be detected.



**Figure S1.** SDS-PAGE analysis of purified beneficial mutants (A) Lane M is the molecular mass marker. Lane 1 and 12 are Q34P, A37P, Y40W, M176I, M176V, G177A, T195L, T195I, T195F, R274P, D279K and M294R, respectively. (B) Lane M is the molecular mass marker. Lane 1 and 6 are G28C-P206C, M59C-T74C, G67C-V301C, S69C-S89C, I249C-A257C and H287C-H304C, respectively.



**Figure S2.** Disulfide bond number of the WT and beneficial mutant enzymes. Error bars represent standard deviations between replicates.



**Figure S3.** Compositions of the DAG synthesis reaction catalyzed by immobilized (A) WT and (B) M5D mutant. Error bars represent standard deviations between replicates.