

## Supporting Information

### Engineering the Enantioselectivity of Yeast Old Yellow Enzyme OYE2y in Asymmetric Reduction of (*E/Z*)-Citral to (*R*)-Citronellal

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Supplementary tables

**Table S1.** The primer information of site saturation mutation of P76 in OYE2y <sup>a</sup>

	Primer	Sequence
P76G	F	TACCTT <u>GG</u> CTCTCCACAATCTGGGGGTT
	R	TGGAG <u>AG</u> CAAAGGTACCCTCAGTGATAATC
P76A	F	TACCTT <u>G</u> CCTCTCCACAATCTGGGGGTT
	R	TGGAG <u>AGG</u> CAAAGGTACCCTCAGTGATAATC
P76V	F	TACCTT <u>G</u> TCTCTCCACAATCTGGGGGTT
	R	TGGAG <u>AGA</u> CAAAGGTACCCTCAGTGATAATC
P76L	F	TACCTT <u>CT</u> CTCCACAATCTGGGGGTT
	R	TGGAG <u>AGA</u> AAAGGTACCCTCAGTGATAATC
P76I	F	GTACCTT <u>AT</u> CTCCACAATCTGGGGGTT
	R	GTGGAG <u>AAA</u> AAAGGTACCCTCAGTGAT
P76F	F	TACCTT <u>TT</u> CTCCACAATCTGGGGGTT
	R	TGGAG <u>AGA</u> AAAAGGTACCCTCAGTGATAATC
P76Y	F	GTACCTT <u>TT</u> ATCTCCACAATCTGGGGGTT
	R	GTGGAG <u>A</u> AAAAGGTACCCTCAGTGATAATC
P76W	F	GTACCTT <u>TT</u> <u>GG</u> CTCCACAATCTGGGGGTT
	R	TGTGGAG <u>AC</u> AAAAGGTACCCTCAGTGAT
P76S	F	TACCTT <u>CC</u> CTCCACAATCTGGGGGTT
	R	GTGGAG <u>AGG</u> AAAAGGTACCCTCAGTGAT
P76T	F	GTACCTT <u>AC</u> CTCCACAATCTGGGGGTT
	R	GTGGAG <u>AGG</u> TAAGGTACCCTCAGTGATAATC
P76C	F	GTACCTT <u>TT</u> <u>G</u> CTCCACAATCTGGGGGTT
	R	GTGGAG <u>ACA</u> AAAGGTACCCTCAGTGATAATC

	F	GTACCTT <u>TAT</u> GTC <u>CTCCACA</u> AATCTGGGGGTT
P76M	R	GTGGAGAC <u>CATAAAGGTACCCTCAGTGATAATC</u>
	F	TACCTT <u>TA</u> ACTCTCCACAATCTGGGGGTT
P76N	R	GTGGAGAG <u>TTAAAGGTACCCTCAGTGATAATC</u>
	F	TACCTT <u>CAGT</u> CTCCACAATCTGGGGGTT
P76Q	R	GTGGAGAC <u>TGAAAGGTACCCTCAGTGATAATC</u>
	F	GTACCTT <u>GACT</u> CTCCACAATCTGGGGGTT
P76D	R	GTGGAGAG <u>CTCAAAGGTACCCTCAGTGATAATC</u>
	F	TACCTT <u>GAGT</u> CTCCACAATCTGGGGGTT
P76E	R	GTGGAGAC <u>CTCAAAGGTACCCTCAGTGATAATC</u>
	F	GTACCTT <u>AA</u> GTCTCCACAATCTGGGGGTT
P76K	R	GTGGAGAC <u>TTAAAGGTACCCTCAGTGATAATC</u>
	F	TACCTT <u>CGAT</u> CTCCACAATCTGGGGGTT
P76R	R	TGTGGAGAC <u>CGAAAGGTACCCTCAGTGAT</u>
	F	TACCTT <u>CA</u> CTCTCCACAATCTGGGGGTT
P76H	R	GTGGAGAG <u>TGAAAGGTACCCTCAGTGATAATC</u>

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<sup>a</sup> The code to introduce the substitution was underlined.

**Table S2.** The primer information of site saturation mutation of R330 in Oye2y <sup>a</sup>

	Primer	Sequence
R330F	F	GCTCTG <u>TTCCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>GAAC</u> CAGAGCAAAGTTACCAGCT
R330W	F	GCTCTG <u>TGCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>CCAC</u> AGAGCAAAGTTACCAGCT
R330P	F	GCTCTG <u>CCCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGGGG <u>GCAG</u> AGCAAAGTTACCAGCT
R330Y	F	GCTCTG <u>TACCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>TACAG</u> AGCAAAGTTACCAGCT
R330M	F	GCTCTG <u>ATGCC</u> CAGAAGTTGTCAGAGAAGAGGT
	R	TTCTGG <u>CATC</u> AGAGCAAAGTTACCAGCTC
R330G	F	GCTCTG <u>GGCCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>GCCCA</u> GAGCAAAGTTACCAGCT
R330A	F	GCTCTG <u>GGCCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>GGCCAG</u> AGCAAAGTTACCAGCT
R330V	F	GCTCTG <u>GGTCCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>GGACCAG</u> AGCAAAGTTACCAGCT
R330L	F	GCTCTG <u>CCTCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>GAGCAG</u> AGCAAAGTTACCAGCT
R330I	F	GCTCTG <u>ATCCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>GATCAG</u> AGCAAAGTTACCAGCT
R330S	F	GCTCTG <u>GAGCC</u> CAGAAGTTGTCAGAGAAG
	R	TTCTGG <u>GCTCAG</u> AGCAAAGTTACCAGCT

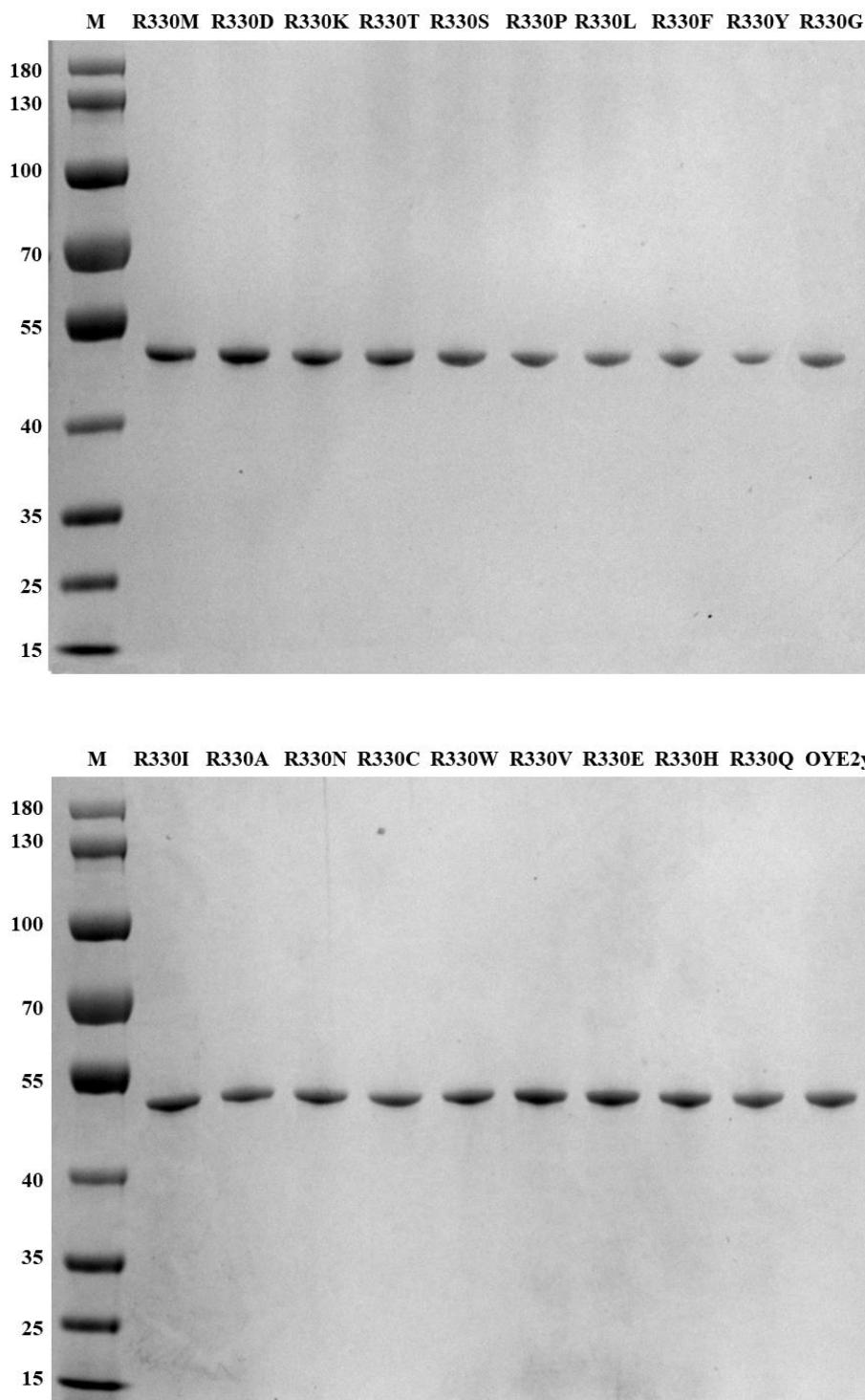
	F	GCTCTG <u>ACCCC</u> CAGAAC <u>TTGTC</u> CAGAGAAG
R330T	R	TTCTGG <u>GGT</u> CAGAGCAA <u>AGTTACCAG</u> CT
	F	GCTCTG <u>AACCC</u> CAGAAC <u>TTGTC</u> CAGAGAAG
R330N	R	TTCTGG <u>GGT</u> CAGAGCAA <u>AGTTACCAG</u> CT
	F	GCTCTG <u>GCAGC</u> CAGAAC <u>TTGTC</u> CAGAGAAG
R330Q	R	TTCTGG <u>CTGC</u> CAGAGCAA <u>AGTTACCAG</u> CT
	F	GCTCTG <u>GGACCC</u> CAGAAC <u>TTGTC</u> CAGAGAAG
R330D	R	TTCTGG <u>GGTCC</u> CAGAGCAA <u>AGTTACCAG</u> CT
	F	GCTCTG <u>GAACC</u> CAGAAC <u>TTGTC</u> CAGAGAAG
R330E	R	TTCTGG <u>GTCC</u> CAGAGCAA <u>AGTTACCAG</u> CT
	F	GCTCTG <u>GAAGCC</u> CAGAAC <u>TTGTC</u> CAGAGAAG
R330K	R	TTCTGG <u>CTTC</u> CAGAGCAA <u>AGTTACCAG</u> CT
	F	GCTCTG <u>TGCC</u> CAGAAC <u>TTGTC</u> CAGAGAAG
R330C	R	CTTCTGG <u>GCAC</u> CAGAGCAA <u>AGTTACCAG</u> CT

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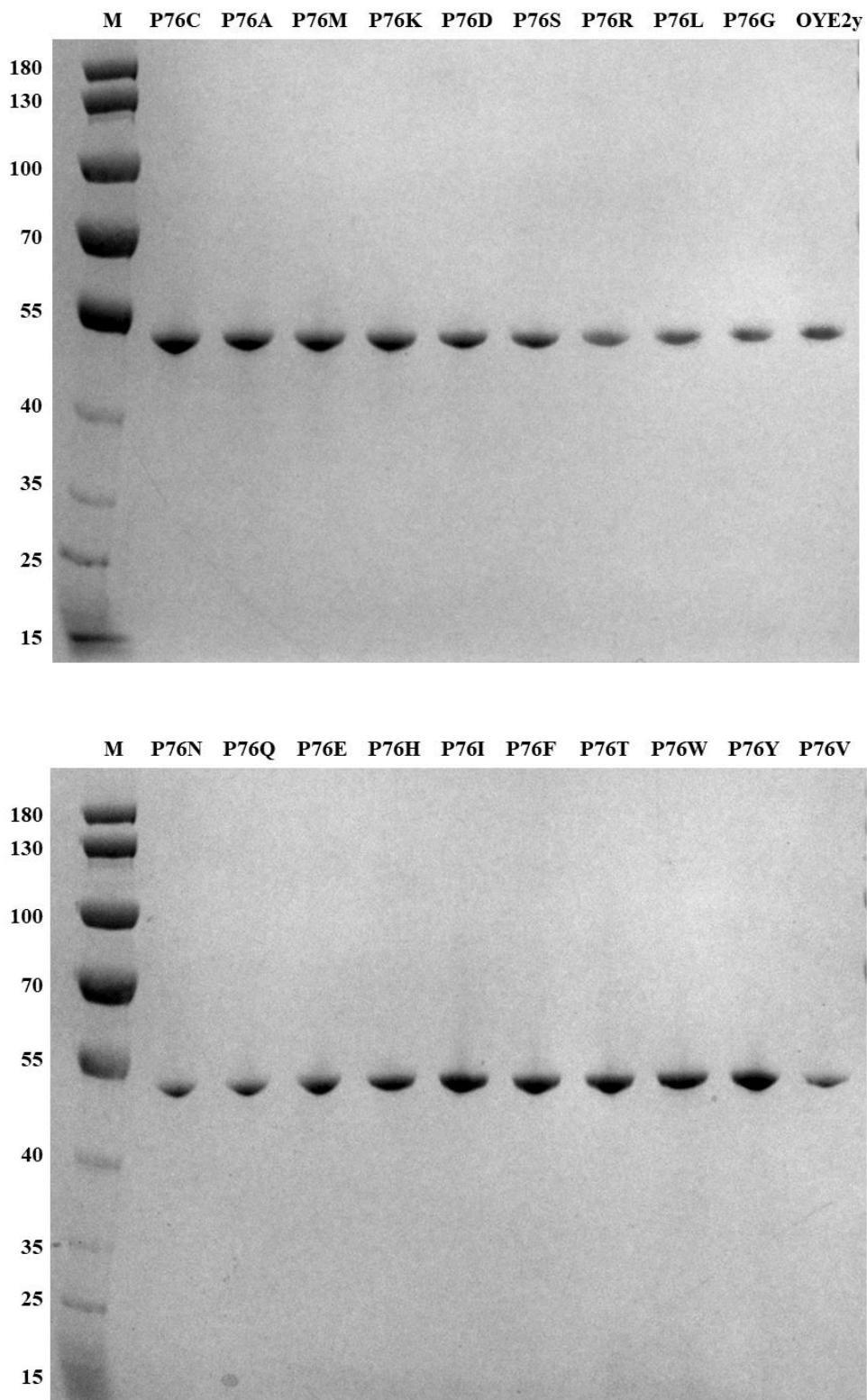
<sup>a</sup> The code to introduce the substitution was underlined.



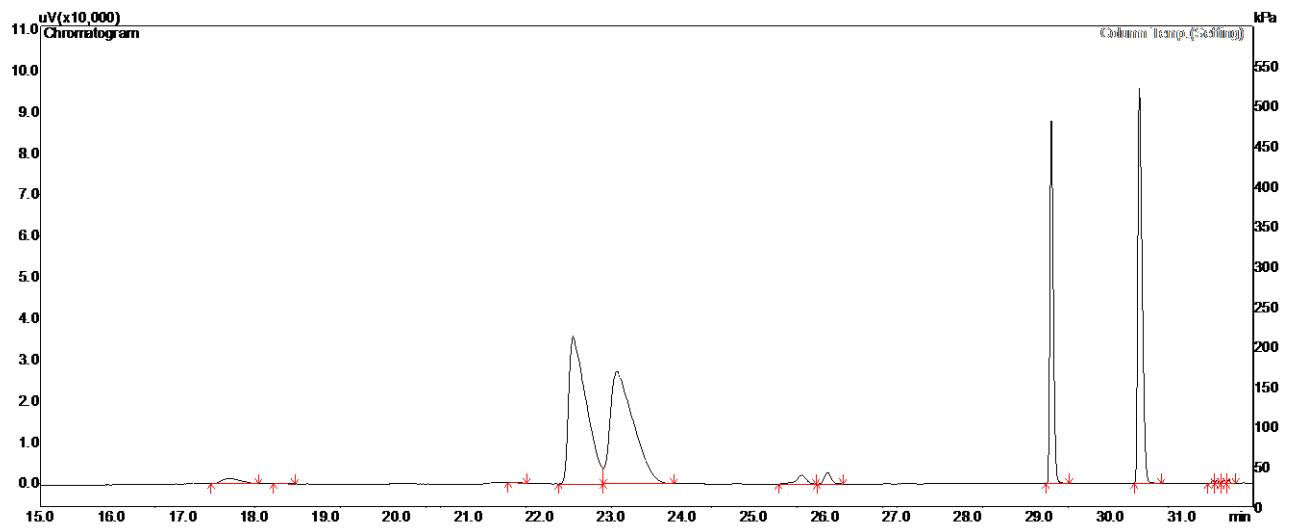
## Supplementary figures



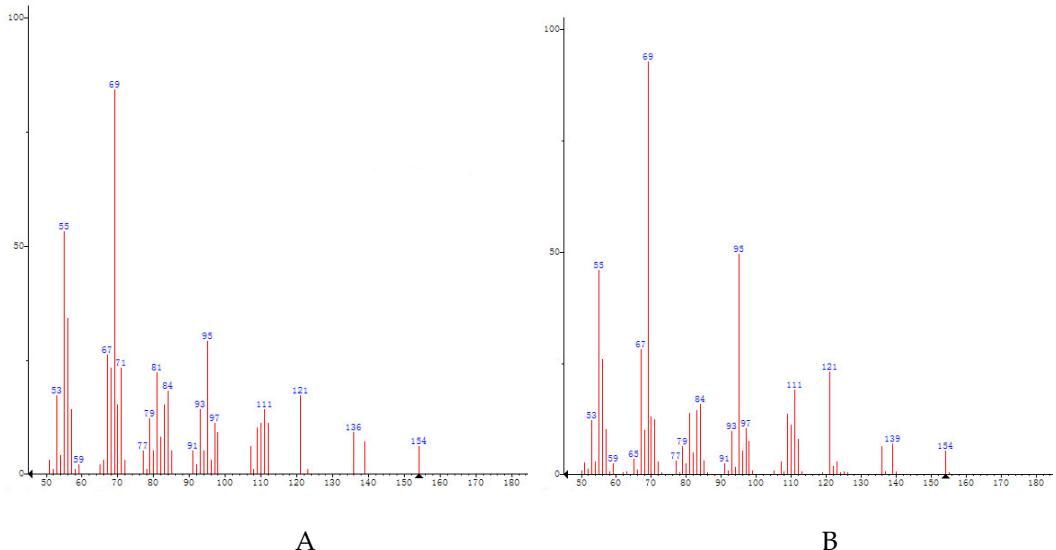
**Figure S1.** SDS-PAGE (12%) analysis of the purified OYE2y R330X variants. Lane M, standard molecular mass proteins. Other lanes are named according to the substitution for OYE2y. The proteins were visualized by staining with Coomassie brilliant blue R-250.



**Figure S2.** SDS-PAGE (12%) analysis of the purified OYE2y P76X variants. Lane M, standard molecular mass proteins. Other lanes are named according to the substitution for OYE2y. The proteins were visualized by staining with Coomassie brilliant blue R-250.

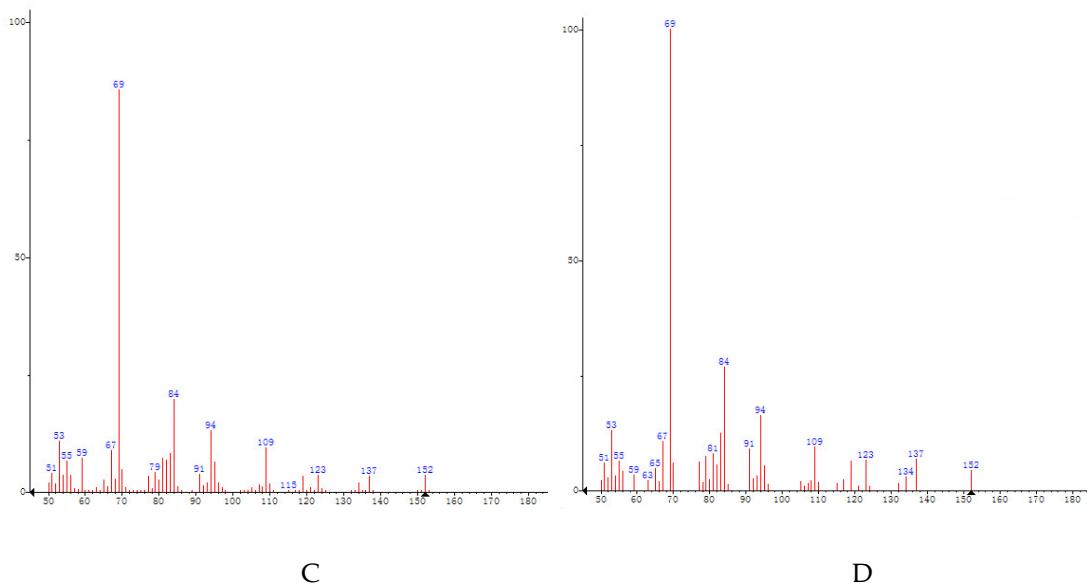


**Figure S3.** Gas chromatograph analysis for standards (*S*)-citronellal (22.459 min), (*R*)-citronellal (23.067 min), (*Z*)-citral (29.164 min) and (*E*)-citral (30.398 min).



A

B



C

D

**Figure S4.** Gas chromatograph-mass spectrometry analysis for (*S*)-citronellal (A), (*R*)-citronellal (B), (*Z*)-citral (C) and (*E*)-citral (D) in the asymmetric reduction of (*E/Z*)-citral.