

Table S1. Strains used in this study.

Yeast strain (AWRI number)	Selection ^a	Causative mutation ^b
AWRI796		
AWRI2936	PFP (spontaneous)	Tyr1p C108Y [†]
AWRI2940	PFP (spontaneous)	Tyr1p M197I
AWRI4124	PFP (mutagenized)	Tyr1p A200T
AWRI2965	OFP (spontaneous)	Aro4p Q166R
AWRI2969	OFP (mutagenized)	Tyr1p S398L

^aStrategy used to select strains from parent AWRI796. Untreated (spontaneous) or chemically mutagenized cells were selected in solid media containing either *p*-fluoro-DL-phenylalanine (PFP) or *o*-fluoro-DL-phenylalanine (OFP). ^bMutations in the amino acid sequence of proteins involved in aromatic amino acid metabolism ([†]mutation in heterozygosity).

Table S2. Higher alcohols and esters produced following alcoholic fermentation of Chardonnay using strains AWRI796 (parent) and five variants carrying mutations in Aro4p (AWRI2965) or Tyr1p (AWRI2936, 2940, 4124, and 2969). Concentrations of the different metabolites are shown in mg L⁻¹, unless otherwise indicated.

Metabolite	AWRI796	AWRI2936	AWRI2940	AWRI4124	AWRI2965	AWRI2969	P-value	F-value
Ethyl acetate	23.2 ± 1.5	25.0 ± 1.0	27.3 ± 0.88	27.6 ± 0.86	25.3 ± 1.0	24.2 ± 1.5	0.040	6.6
2-methylpropyl acetate (Val) (µg L ⁻¹)	89 ± 29 ^b	120 ± 34 ^{ab}	176 ± 3.8 ^a	112 ± 29 ^{ab}	85 ± 24 ^b	97 ± 17 ^{ab}	0.033	3.6
2-methylbutyl acetate (Ile) (µg L ⁻¹)	108 ± 5.2 ^d	160 ± 3.7 ^c	209 ± 10 ^a	195 ± 5.1 ^a	147 ± 3.6 ^c	178 ± 4.3 ^b	<0.001	118
3-methylbutyl acetate (Leu) (µg L ⁻¹)	2649 ± 102 ^c	2848 ± 87 ^c	4064 ± 213 ^a	3552 ± 70 ^b	2895 ± 37 ^c	3490 ± 110 ^b	<0.001	64
2-PEA (Phe)	0.28 ± 0.03 ^f	1.48 ± 0.05 ^e	9.88 ± 0.30 ^a	4.08 ± 0.07 ^c	3.55 ± 0.03 ^d	4.93 ± 0.02 ^b	<0.001	1973
2-methylpropanol (Val)	28.5 ± 5.0 ^{ab}	27.8 ± 0.6 ^{ab}	36.5 ± 3.0 ^a	32.8 ± 3.8 ^{ab}	22.3 ± 1.6 ^b	25.9 ± 2.1 ^{ab}	0.074	2.7
2-methylbutanol (Ile)	19.7 ± 1.5	23.3 ± 3.6	25.7 ± 2.0	25.3 ± 0.95	22.1 ± 4.9	22.8 ± 1.2	0.159	1.9
3-methylbutanol (Leu)	114.0 ± 9.1	110.7 ± 10.1	122.5 ± 7.4	121.2 ± 2.0	108.8 ± 20.1	119.0 ± 4.8	0.532	0.9
2-PE (Phe)	19.8 ± 2.9 ^e	53.9 ± 2.4 ^d	301.5 ± 12.7 ^a	138.9 ± 0.70 ^c	147.4 ± 2.3 ^c	164.8 ± 1.0 ^b	<0.001	966
TyrOH (Tyr)	13.6 ± 1.8 ^b	10.1 ± 0.1 ^c	7.1 ± 0.2 ^d	9.1 ± 0.2 ^{cd}	127 ± 1.6 ^a	7.5 ± 0.1 ^{cd}	<0.001	7205
TOL (Trp)	0.89 ± 0.02 ^e	2.66 ± 0.1 ^d	52.1 ± 0.5 ^a	6.3 ± 0.3 ^c	7.8 ± 0.4 ^b	5.7 ± 0.2 ^c	<0.001	11240
TOL-SO ₃ H	<LOQ	<LOQ	0.26 ± 0.13	<LOQ	<LOQ	<LOQ	-	-
Free SO ₂	<3	<3	<3	<3	<3	<3	-	-
Total SO ₂	20 ± 1 ^{ab}	22 ± 1 ^a	22 ± 1 ^{ab}	22 ± 1 ^a	23 ± 2 ^a	18 ± 2 ^b	0.009	5.2

Results are the average ± standard deviation of three independent replicates. In parenthesis, amino acid from which the corresponding metabolite is derived from in the Ehrlich pathway. Means with the same superscript letter are not significantly different from each other (Tukey's test, P < 0.05).

Table S3. Basic wine composition at bottling of the Chardonnay wines made using the yeast AWRI796 (parent), and five variants carrying mutations in Aro4p (AWRI2965) or Tyr1p (AWRI2936, 2940, 4124, and 2969).

strain	Ethanol (% v/v)	Sugars (g L ⁻¹)	pH	Titrateable acidity pH 8.2	Malic (g L ⁻¹)	Acetic acid (g L ⁻¹)	Glycerol (g L ⁻¹)	Free SO ₂ (mg L ⁻¹)	Total SO ₂ (mg L ⁻¹)	Molar ratio (Free SO ₂ / TOL)
AWRI796	13.3 ± 0.1	0.6 ± 0.3	3.42 ± 0.0	5.7 ± 0.2	2.1 ± 0.1	0.39 ± 0.02	5.9 ± 0.1	42 ± 0.6	109 ± 1.5	132
AWRI2936	13.5 ± 0.1	0.7 ± 0.1	3.42 ± 0.0	5.6 ± 0.1	2.1 ± 0.0	0.35 ± 0.01	5.7 ± 0.1	40 ± 1.2	112 ± 1.2	39
AWRI2940	13.4 ± 0.0	1.1 ± 0.1	3.40 ± 0.01	5.7 ± 0.0	2.2 ± 0.1	0.40 ± 0.02	6.1 ± 0.1	38 ± 0.6	109 ± 0.6	1.8
AWRI4124	13.4 ± 0.0	0.7 ± 0.1	3.43 ± 0.01	5.7 ± 0.0	2.0 ± 0.1	0.43 ± 0.01	6.4 ± 0.1	36 ± 0.6	109 ± 0.6	15
AWRI2965	13.4 ± 0.1	1.1 ± 0.1	3.43 ± 0.01	5.6 ± 0.0	2.0 ± 0.1	0.46 ± 0.01	5.9 ± 0.1	40 ± 1.0	113 ± 0.6	13
AWRI2969	13.4 ± 0.1	1.0 ± 0.2	3.43 ± 0.0	5.6 ± 0.0	2.1 ± 0.1	0.45 ± 0.01	5.9 ± 0.2	37 ± 0.6	105 ± 1.5	17

Results are the average ± standard deviation of three independent replicates.

Table S4. F-ratios, probability values[†], degrees of freedom (df) and mean square error (MSE) from the analysis of variance of the sensory attribute data after 3 months in bottle.

Attribute	Strain	FRep (Strain)	J*Strain	J*FRep (Strain)	PRep (Strain, FRep)	MSE
Yellow colour intensity	5.71***	1.75	1.54*	0.95	1.27	0.305
Tropical fruit A	1.42	0.99	0.72	0.81	0.75	1.190
Passionfruit A	0.10	1.69	1.43	0.72	1.33	2.061
Citrus A	0.74	0.66	1.26	0.88	1.06	1.338
Banana confection A	0.69	0.46	0.63	1.44*	1.30	1.954
Stone fruit A	0.57	0.99	0.77	0.95	0.59	1.908
Floral A	2.49*	1.91*	1.27	0.76	0.52	2.108
Grassy A	1.92†	1.47	1.04	1.16	0.72	1.126
Yeasty A	0.37	0.75	1.20	0.74	0.96	1.622
Flint A	1.08	1.94*	1.14	1.10	1.84*	1.855
Honey A	0.52	0.88	1.53*	1.01	0.71	1.699
Cooked veg/potato A	1.96†	0.54	1.49*	0.76	0.84	2.099
Pungent A	0.84	0.74	1.34	1.09	2.02*	0.573
Sweetness	2.34†	1.27	1.21	1.02	1.48	1.095
Sourness	0.48	0.77	1.25	1.01	0.80	0.517
Bitterness	1.74†	1.59	0.85	0.88	1.23	1.207
Astringency	0.67	1.02	0.68	1.36*	1.26	0.534
Viscosity	1.09	0.25	0.94	1.05	1.26	0.509
Hotness	1.24	0.43	0.71	0.73	0.99	1.175
Tropical fruit F	0.96	1.20	1.26	0.62	0.71	1.177
Citrus F	0.34	1.29	2.19*	0.47	1.14	1.043
Stone fruit F	2.24†	0.57	0.86	1.19	0.93	1.549
Banana confection F	0.41	0.75	0.92	0.87	0.84	1.780
Grassy F	2.63*	1.32	0.74	0.93	1.04	0.786
Flint F	1.98†	1.11	0.77	0.79	0.64	1.783
df	5	12	45	108	18	162

A: aroma, F: Flavour, AT: Aftertaste. [†]Statistical evidence levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; † $P \leq 0.15$. df = degrees of freedom. Evidence was found for Judge effects for all attributes ($P < 0.001$), Strain = Yeast strain treatment, FRep = Fermentation replicate, PRep = presentation replicate, J = Judge.

Table S5. Mean scores and Tukey's Honest Significant Difference (HSD) values for sensory attributes after 3 months in bottle.

Yeast Strain	AWRI796	AWRI2936	AWRI2940	AWRI2965	AWRI2969	AWRI4124	HSD
Yellow colour intensity	3.85	4.06	4.47	4.03	4.03	4.09	0.37
Tropical fruit A	5.36	5.13	5.41	5.32	5.40	5.14	ne
Passionfruit A	2.36	2.43	2.45	2.44	2.32	2.47	ne
Citrus A	4.16	3.83	4.10	4.16	4.01	3.91	ne
Banana confection A	3.33	3.44	3.37	3.05	3.37	3.44	ne
Stone fruit A	3.56	3.53	3.70	3.66	3.81	3.78	ne
Floral A	4.82	4.61	5.28	4.68	4.68	4.42	0.79
Grassy A	2.96	2.38	2.67	2.92	2.78	2.72	ne
Yeasty A	1.08	1.11	0.90	1.14	0.99	1.11	ne
Flint A	1.61	1.61	1.31	1.90	1.57	1.40	ne
Honey A	1.99	1.93	1.63	1.83	2.07	1.89	ne
Cooked veg/potato A	1.62	1.35	1.08	1.28	1.32	1.86	ne
Pungent A	4.87	4.99	5.05	5.02	4.78	4.85	ne
Sweetness	1.95	1.87	1.46	1.42	1.48	1.69	ne
Sourness	6.17	6.20	6.27	6.17	6.09	6.28	ne
Bitterness	4.90	4.92	5.19	5.26	5.21	5.21	ne
Astringency	3.43	3.59	3.57	3.61	3.59	3.64	ne
Viscosity	4.46	4.40	4.46	4.32	4.38	4.60	ne
Hotness	4.85	5.00	4.90	4.79	4.73	5.01	ne
Tropical fruit F	5.38	5.04	5.17	5.09	5.24	5.25	ne
Citrus F	5.12	5.08	5.29	5.21	5.20	5.11	ne
Stone fruit F	3.82	3.82	3.47	3.32	3.31	3.76	ne
Banana confection F	2.67	2.54	2.43	2.46	2.58	2.66	ne
Grassy F	3.31	3.12	3.53	3.33	3.54	3.36	0.41
Flint F	1.69	1.66	1.48	2.02	1.55	1.75	ne

HSD values calculated at 95% confidence level included for sensory attributes that showed statistical evidence ($P < 0.05$) for differing across strains, ne: no statistical evidence found.

Table S6. F-ratios, probability values[†], degrees of freedom (df) and mean square error (MSE) from the analysis of variance of the sensory attribute data after 15 months in bottle.

Attribute	Strain	FRep (Strain)	J*Strain	J*FRep (Strain)	PRep (Strain, FRep)	MSE
Yellow colour intensity	19.95***	3.5***	1.26	1.35*	2.12***	0.235
Pungent A	2.75*	1.01	1.46	0.68	0.75	0.592
Stone fruit A	1.81	1.89	0.62	1.49**	1.21	1.022
Rose A	2.68*	1.34	1.11	0.87	1.36	3.212
Citrus A	0.32	1.71	1.33	1.25	1.51*	0.407
Banana A	1.82	0.94	0.77	0.85	1.04	2.219
Grassy A	0.93	0.91	0.41	1.25	1.37	0.695
Flint A	0.92	1.50	1.85**	0.71	0.97	1.896
Cooked veg/potato A	12.75***	1.96*	1.18	1.01	0.95	2.383
Sourness	5.69***	0.73	0.85	0.86	0.83	0.651
Astringency	1.00	0.83	1.52	0.65	1.30	0.723
Bitterness	1.45	1.93	1.12	1.09	0.83	1.130
Viscosity	0.22	0.46	1.42	0.70	0.97	0.620
Hotness	0.68	0.47	1.54	0.82	1.44	1.141
Sweetness	7.41***	3.32***	1.08	0.96	1.10	1.255
Citrus F	1.41	1.55	1.48	0.63	1.51*	0.920
Rose F	2.49†	0.96	1.51	0.81	0.95	2.147
Stone fruit F	2.75*	0.88	1.19	1.06	0.79	1.627
df	4	10	36	90	30	270

A: aroma, F: Flavour, AT: Aftertaste. [†]Statistical evidence levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; † $P \leq 0.15$. df = degrees of freedom. Evidence was found for Judge effects for all attributes ($P < 0.001$), Strain = Yeast strain treatment, FRep = Fermentation replicate, PRep = presentation replicate, J = Judge.

Table S7. Mean scores and Tukey's Honest Significant Difference values for sensory attributes after 15 months in bottle.

Yeast Strain	AWRI796	AWRI2936	AWRI2940	AWRI2965	AWRI4124	HSD
Yellow colour intensity	4.05	4.13	4.79	4.19	4.40	0.27
Pungent A	4.41	4.56	4.37	4.20	4.47	0.33
Stone fruit A	3.24	3.45	3.28	3.51	3.19	ne
Rose A	3.72	3.94	4.55	4.03	4.06	0.75
Citrus A	2.68	2.62	2.63	2.56	2.57	ne
Banana A	2.40	2.54	2.55	2.72	2.27	ne
Grassy A	2.31	2.22	2.21	2.11	2.11	ne
Flint A	1.43	1.45	1.16	1.42	1.61	ne
Cooked veg/potato A	2.38	1.66	1.06	1.14	2.36	0.72
Sourness	5.04	5.09	5.46	5.10	5.29	0.29
Astringency	2.80	2.64	2.86	2.83	2.84	ne
Bitterness	3.44	3.42	3.78	3.47	3.47	ne
Viscosity	3.94	3.96	3.95	4.02	4.03	ne
Hotness	4.01	3.72	3.85	3.83	3.87	ne
Sweetness	1.44	1.64	0.78	1.46	1.37	0.49
Citrus F	4.32	4.18	4.46	4.19	4.34	ne
Rose F	2.07	2.19	2.77	2.24	2.31	ne
Stone fruit F	3.31	3.66	2.96	3.42	3.29	0.61

HSD values calculated at 95% confidence level included for sensory attributes that showed statistical evidence ($P < 0.05$) for differing across strains, ne: no statistical evidence found.

Table S8. Sensory attributes, definitions and composition of reference standards after 3 months in bottle.

Attribute	Definition/Synonyms	Standard
<i>Appearance</i>		
Yellow colour	The degree of yellow colour intensity in the sample	
<i>Aroma</i>		
Tropical fruit	Intensity of the aroma of tropical fruits; pineapple and melon	10 g cubes and juice of tinned pineapple (Golden Circle), 10 g cubes and juice of rockmelon fruit cup (SPC Ardmona)
Passionfruit	Intensity of the aroma of passionfruit, including sweaty odour	2 µL of 16.5 mL/L 3-mercaptohexyl acetate
Citrus	Intensity of the aroma of citrus fruits	5 g of fresh lemon, 5 g of fresh lime and 5g of fresh grapefruit including zest
Banana confection	Intensity of the aroma of banana lolly	5 g banana lolly (Black & Gold Brand) not in wine
Stone fruit	Intensity of the aroma of stone fruit including; apricot and peach	10 g apricots and 10 g peaches, in juice (Goulburn Valley)
Floral	Intensity of the aroma of orange blossom, rose and laundry powder	40 µL of 200 mg/L 2-phenylethanol
Grassy	Intensity of the aroma of freshly cut grass and hay	1g of fresh cut grass not in wine.
Yeasty	Intensity of the aroma of baker's yeast	1 g of baker's yeast and 1 g sugar in 30 mL 35 °C water
Flint	Intensity of the aroma of flint and struck match and mineral	20 µL of 1 mg/L benzyl mercaptan
Honey	Intensity of the aroma of honey	2.5 g honey (Beechworth's)
Cooked veg/potato	Intensity of the aroma of cooked veg water and boiled potato	20 µL of 3.3 mg/L methional
Pungent	Intensity of the aroma and effect of alcohol.	4 mL of 95% food grade ethanol (Tarac Technologies)
<i>Palate</i>		
Sweetness	Intensity of the perception of sweetness	6 g/L table sugar in water
Sourness	Intensity of acid taste in the mouth including aftertaste.	2 g/L L-(+)-tartaric acid (Chem-Supply) in water
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration.	15 mg/L quinine sulfate (Sigma Aldrich) in water

Attribute	Definition/Synonyms	Standard
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency.	0.43 g/L aluminium sulfate (Ajax fine Chem Supply Pty Ltd in water
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel.	1.5 g/L carboxymethylcellulose sodium salt (Sigma Aldrich) in water
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning.	8% food grade alcohol (Tarac Technologies) in water
Tropical fruit	Intensity of the flavour of tropical fruits; pineapple and melon	
Citrus	Intensity of the flavour of citrus fruits	
Stone fruit	Intensity of the flavour of stone fruit including apricot and peach	
Banana confection	Intensity of the flavour of banana lolly	
Grassy	Intensity of the flavour of freshly cut grass and hay	
Flint	Intensity of the flavour of flint, struck match and mineral	

All white wine standards were added to 30 mL of 2017 Yalumba premium selection bag-in-box unoaked Chardonnay unless otherwise noted.

Table S9. Sensory attributes, definitions and composition of reference standards after 15 months in bottle

Attribute	Definition/Synonyms	Standard
<i>Appearance</i>		
Yellow colour	The degree of yellow colour intensity in the sample	
<i>Aroma</i>		
Pungent	Intensity of the aroma and effect of alcohol.	4 mL of 95% food grade ethanol (Tarac Technologies)
Stone fruit	Intensity of the aroma of stone fruit including; apricot and peach	5 g apricots and 5 g peaches, in juice (Goulburn Valley)
Rose	Intensity of the aroma of floral roses	2 mL of rosewater (ANOUN Food Co.)
Citrus	Intensity of the aroma of citrus fruits	5 g of fresh lemon, 5 g of fresh lime and 5g of fresh grapefruit including zest
Banana	Intensity of the aroma of banana lolly	5 g banana lolly (Black & Gold Brand) not in wine
Grassy	Intensity of the aroma of freshly cut grass and hay	1g of fresh cut grass not in wine.
Flint	Intensity of the aroma of flint and struck match and mineral	20 µL of 1 mg/L benzyl mercaptan
Cooked veg/potato	Intensity of the aroma of cooked veg water and boiled potato	20 µL of 3.3 mg/L methional
<i>Palate</i>		
Sourness	Intensity of acid taste in the mouth including aftertaste.	2 g/L L-(+)-tartaric acid (Chem-Supply) in water
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency.	0.43 g/L aluminium sulfate (Ajax fine Chem Supply Pty Ltd in water
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration.	15 mg/L quinine sulfate (Sigma Aldrich) in water
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel.	1.5 g/L carboxymethylcellulose sodium salt (Sigma Aldrich) in water

Attribute	Definition/Synonyms	Standard
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning.	8% food grade alcohol (Tarac Technologies) in water
Sweetness	Intensity of the perception of sweetness	6 g/L table sugar in water
Citrus	Intensity of the flavour of citrus fruits	
Rose	Intensity of the flavour of floral roses and Turkish delight confection	
Stone fruit	Intensity of the flavour of stone fruit including apricot and peach	

All white wine standards were added to 30 mL of 2017 Yalumba premium selection bag-in-box unoaked Chardonnay unless otherwise noted.

Table S10. Composition of the wines after 3 months in bottle. Wines were produced using AWRI796 (parent strain) and five variants carrying mutations in Aro4p (AWRI2965) or Tyr1p (AWRI2936, 2940, 4124, and 2969). Concentrations of the different metabolites are shown in $\mu\text{g L}^{-1}$, unless otherwise indicated.

Metabolite	Label	AWRI796	AWRI2936	AWRI2940	AWRI4124	AWRI2965	AWRI2969	P value	F-value
Acetic acid (mg L^{-1})	AceAc	$226.8 \pm 20.7^{\text{ab}}$	$191.5 \pm 22.4^{\text{b}}$	$225.5 \pm 3.6^{\text{ab}}$	$237.6 \pm 24.5^{\text{ab}}$	$254.6 \pm 20.8^{\text{a}}$	$276.3 \pm 25.4^{\text{a}}$	0.006	5.7
Ethyl acetate (mg L^{-1})	EthAt	$23.2 \pm 0.26^{\text{b}}$	$25.0 \pm 0.44^{\text{b}}$	$27.3 \pm 0.78^{\text{a}}$	$27.6 \pm 1.5^{\text{a}}$	$25.3 \pm 1.7^{\text{b}}$	$24.2 \pm 1.1^{\text{b}}$	0.004	6.4
2-methylpropyl acetate	2MeProAt	$70 \pm 18^{\text{b}}$	$72 \pm 24^{\text{b}}$	$128 \pm 18^{\text{a}}$	$120 \pm 15^{\text{a}}$	$58 \pm 3.4^{\text{b}}$	$72 \pm 6.7^{\text{b}}$	0.001	10.2
2-methylbutyl acetate	2MeButAt	$108 \pm 5.2^{\text{d}}$	$160 \pm 3.7^{\text{c}}$	$209 \pm 10.2^{\text{a}}$	$195 \pm 5.1^{\text{a}}$	$147 \pm 3.6^{\text{c}}$	$178 \pm 4.3^{\text{b}}$	<0.001	118
3-methylbutyl acetate	3MeButAt	$1907 \pm 96^{\text{c}}$	$1918 \pm 47^{\text{c}}$	$2720 \pm 52^{\text{a}}$	$2415 \pm 59^{\text{b}}$	$1940 \pm 26^{\text{c}}$	$2341 \pm 14^{\text{b}}$	<0.001	111
2-PEA	2PheEthAt	$228 \pm 20^{\text{f}}$	$1096 \pm 26^{\text{e}}$	$7283 \pm 250^{\text{a}}$	$3047 \pm 25^{\text{c}}$	$2703 \pm 17^{\text{d}}$	$3709 \pm 37^{\text{b}}$	<0.001	1655
Ethyl 2-methyl propanoate	Et2MePro	29.6 ± 0.4	29.1 ± 1.0	31.9 ± 0.8	28.9 ± 1.0	31.4 ± 2.3	31.2 ± 0.8	0.030	3.7
Ethyl propanoate	EtPro	$25.1 \pm 0.4^{\text{ab}}$	$26.7 \pm 0.2^{\text{a}}$	$25.7 \pm 0.2^{\text{ab}}$	$24.5 \pm 0.7^{\text{b}}$	$25.8 \pm 0.8^{\text{ab}}$	$25.2 \pm 1.5^{\text{ab}}$	0.063	2.9
Ethyl 2-methylbutanoate	Et2MeBut	$10.8 \pm 0.6^{\text{b}}$	$11.3 \pm 0.1^{\text{ab}}$	$11.3 \pm 0.1^{\text{ab}}$	$11.0 \pm 0.6^{\text{b}}$	$11.4 \pm 0.1^{\text{ab}}$	$12.2 \pm 0.2^{\text{a}}$	0.010	5.1
Ethyl 3-methylbutanoate	Et3MeBut	$7.2 \pm 0.4^{\text{ab}}$	$7.2 \pm 0.2^{\text{ab}}$	$7.2 \pm 0.1^{\text{ab}}$	$7.0 \pm 0.3^{\text{b}}$	$7.1 \pm 0.7^{\text{ab}}$	$8.1 \pm 0.5^{\text{a}}$	0.045	3.2
Ethyl butanoate	EtBut	$206 \pm 7^{\text{bc}}$	$202 \pm 16^{\text{c}}$	$244 \pm 3^{\text{a}}$	$214 \pm 3^{\text{bc}}$	$208 \pm 10^{\text{bc}}$	$228 \pm 6^{\text{ab}}$	0.001	9.8
Ethyl hexanoate	EtHex	$315 \pm 14^{\text{d}}$	$361 \pm 3.4^{\text{a}}$	$360 \pm 9.9^{\text{a}}$	$327 \pm 3.7^{\text{cd}}$	$337 \pm 3.2^{\text{bc}}$	$356 \pm 3.9^{\text{ab}}$	<0.001	19.8
Ethyl octanoate	EtOct	$528 \pm 9.8^{\text{c}}$	$573 \pm 6.5^{\text{b}}$	$602 \pm 4.2^{\text{a}}$	$546 \pm 6.9^{\text{c}}$	$541 \pm 8.5^{\text{c}}$	$585 \pm 9.6^{\text{ab}}$	<0.001	40.5
Ethyl decanoate	EtDec	172 ± 13	175 ± 15	163 ± 11	167 ± 14	153 ± 16	154 ± 8	0.277	1.5
Hexyl acetate	HexAt	$215 \pm 11^{\text{d}}$	$218 \pm 6.7^{\text{cd}}$	$269 \pm 8.6^{\text{a}}$	$246 \pm 4.9^{\text{b}}$	$237 \pm 2.3^{\text{bc}}$	$249 \pm 4.5^{\text{b}}$	<0.001	25.9
butanol	BtOH	$446 \pm 87^{\text{b}}$	$644 \pm 60^{\text{ab}}$	$671 \pm 49^{\text{a}}$	$556 \pm 85^{\text{ab}}$	$610 \pm 20^{\text{ab}}$	$616 \pm 108^{\text{ab}}$	0.034	3.5
2-methylpropanol (mg L^{-1})	2MePrOH	$21.1 \pm 2.7^{\text{c}}$	$25.8 \pm 0.61^{\text{bc}}$	$32.1 \pm 0.67^{\text{a}}$	$27.1 \pm 3.6^{\text{ab}}$	$21.3 \pm 2.4^{\text{bc}}$	$24.1 \pm 0.61^{\text{bc}}$	<0.001	11.1
2-methylbutanol (mg L^{-1})	2MeBtOH	$29.4 \pm 0.74^{\text{ab}}$	$30.5 \pm 0.65^{\text{ab}}$	$32.9 \pm 3.5^{\text{a}}$	$35.2 \pm 3.3^{\text{a}}$	$26.5 \pm 0.75^{\text{b}}$	$30.1 \pm 1.7^{\text{ab}}$	0.006	5.9
3-methylbutanol (mg L^{-1})	3MeBtOH	$103.2 \pm 3.6^{\text{b}}$	$103.3 \pm 3.2^{\text{b}}$	$114.4 \pm 6.0^{\text{a}}$	$114.6 \pm 2.4^{\text{a}}$	$91.3 \pm 0.61^{\text{c}}$	$104.5 \pm 2.9^{\text{b}}$	<0.001	18.4
hexanol	HxOH	$1207 \pm 46^{\text{a}}$	$1105 \pm 41^{\text{ab}}$	$1029 \pm 40^{\text{b}}$	$1057 \pm 40^{\text{b}}$	$1121 \pm 31^{\text{ab}}$	$1098 \pm 26^{\text{b}}$	0.002	7.9
2-PE (mg L^{-1})	2PhenEtOH	$18.3 \pm 2.4^{\text{e}}$	$47.7 \pm 1.7^{\text{d}}$	$263.8 \pm 7.4^{\text{a}}$	$121.4 \pm 2.9^{\text{c}}$	$138.6 \pm 1.9^{\text{b}}$	$149.0 \pm 5.1^{\text{b}}$	<0.001	1320
TyrOH (mg L^{-1})	Tsol	$14.1 \pm 1.2^{\text{b}}$	$11.0 \pm 0.4^{\text{bc}}$	$6.6 \pm 0.1^{\text{c}}$	$8.7 \pm 0.1^{\text{c}}$	$122 \pm 4.2^{\text{a}}$	$8.9 \pm 1.4^{\text{c}}$	<0.001	1797
TOL (mg L^{-1})	Tphol	$0.08 \pm 0.1^{\text{b}}$	$0.28 \pm 0.1^{\text{b}}$	$39.2 \pm 1.3^{\text{a}}$	$1.01 \pm 0.2^{\text{b}}$	$0.98 \pm 0.2^{\text{b}}$	$1.02 \pm 0.3^{\text{b}}$	<0.001	2252
TOL-SO ₃ H (mg L^{-1})	Tsol_SO3	$1.25 \pm 0.1^{\text{e}}$	$3.6 \pm 0.1^{\text{d}}$	$19.9 \pm 1.1^{\text{a}}$	$7.6 \pm 0.5^{\text{c}}$	$9.6 \pm 0.2^{\text{b}}$	$7.2 \pm 0.5^{\text{c}}$	<0.001	298

Propanoic acid	ProAc	1077 ± 249	1748 ± 529	1729 ± 343	1349 ± 478	1768 ± 229	1782 ± 603	0.288	1.4
Butanoic acid	ButAc	1211 ± 82	1363 ± 110	1433 ± 131	1216 ± 78	1323 ± 85	1428 ± 166	0.114	2.3
3-methyl butanoic acid	3MeButAc	822 ± 34	834 ± 6.5	861 ± 60	859 ± 92	807 ± 14	846 ± 52	0.773	0.5
2-methyl butanoic acid	2MeButAc	402 ± 21	388 ± 4.9	397 ± 23	382 ± 6.6	394 ± 23	397 ± 20	0.785	0.5
Hexanoic acid	HexAc	5659 ± 283 ^c	6661 ± 191 ^{ab}	7026 ± 209 ^a	6071 ± 185 ^{bc}	6247 ± 21 ^{bc}	6743 ± 493 ^{ab}	0.001	10.3
Octanoic acid	OctAc	8024 ± 526 ^b	8692 ± 259 ^{ab}	8957 ± 365 ^{ab}	8106 ± 165 ^{ab}	8316 ± 381 ^{ab}	9000 ± 270 ^a	0.015	4.5
Decanoic acid	DecAc	1792 ± 92 ^a	1665 ± 123 ^{ab}	1601 ± 36 ^{ab}	1659 ± 112 ^{ab}	1460 ± 209 ^b	1.603 ± 27 ^{ab}	0.083	2.6
Free SO ₂ (mg L ⁻¹)	FSO ₂	23 ± 4.4	23 ± 3.6	23 ± 3.1	20 ± 1.0	21 ± 4.7	18 ± 1.0	0.355	1.2
Total SO ₂ (mg L ⁻¹)	TSO ₂	80 ± 7.6	86 ± 4.7	85 ± 6.1	83 ± 2.9	85 ± 8.3	76 ± 1.0	0.293	1.4
H ₂ S	H ₂ S	0.4 ± 0.10	0.4 ± 0.04	0.4 ± 0.05	0.4 ± 0.01	0.4 ± 0.0	0.4 ± 0.02	0.362	1.2
MeSH	MeSH	1.54 ± 0.19 ^a	1.31 ± 0.16 ^{ab}	1.19 ± 0.08 ^b	1.30 ± 0.05 ^{ab}	1.28 ± 0.11 ^{ab}	1.30 ± 0.12 ^{ab}	0.090	2.5
DMS	DMS	3.0 ± 0.1 ^b	1.8 ± 0.1 ^c	2.3 ± 0.3 ^c	2.0 ± 0.2 ^c	3.5 ± 0.2 ^{ab}	3.5 ± 0.1 ^a	<0.001	44.3
MeSAc	MeSAc	5.5 ± 0.4 ^a	4.2 ± 0.2 ^{bc}	4.8 ± 0.1 ^{ab}	4.0 ± 0.1 ^{cd}	3.5 ± 0.2 ^d	4.6 ± 0.5 ^{bc}	<0.001	19.1
Methionol	Metol	542 ± 75 ^d	671 ± 5 ^{cd}	2280 ± 198 ^a	1164 ± 39 ^b	537 ± 25 ^d	794 ± 49 ^c	0.000	163
Methional	Metal	1.71 ± 0.4 ^a	1.91 ± 0.2 ^a	0.84 ± 0.3 ^b	1.37 ± 0.4 ^{ab}	0.84 ± 0.2 ^b	1.25 ± 0.1 ^{ab}	0.001	8.5
2-methylpropanal	2MeProAl	5.4 ± 1.6 ^{ab}	6.2 ± 0.7 ^{ab}	7.3 ± 2.2 ^a	5.1 ± 1.3 ^{ab}	2.9 ± 0.3 ^b	5.5 ± 1.2 ^{ab}	0.042	3.3
3-methylbutanal	2MeButAl	4.4 ± 0.9 ^{ab}	5.8 ± 0.6 ^a	4.9 ± 1.3 ^{ab}	4.9 ± 0.5 ^{ab}	3.0 ± 0.1 ^b	4.7 ± 1.5 ^{ab}	0.066	2.8
furfural	Furfal	12.5 ± 3.6 ^b	19.6 ± 0.4 ^{ab}	38.5 ± 16 ^a	29.4 ± 7.1 ^{ab}	21.8 ± 0.7 ^{ab}	24.4 ± 5.4 ^{ab}	0.037	3.6
5-methylfurfural	MeFFAL	1.1 ± 0.4	1.5 ± 0.7	1.2 ± 0.6	1.1 ± 0.4	1.4 ± 0.6	1.2 ± 0.5	0.860	0.4
benzaldehyde	Benzal	61 ± 16	65 ± 4.0	57 ± 1.5	65 ± 8.1	55 ± 7.5	54 ± 6.4	0.448	1.0
2-phenylacetaldehyde	PAceAld	5.1 ± 1.0 ^c	6.7 ± 1.3 ^{bc}	10.9 ± 0.5 ^a	9.2 ± 1.2 ^{ab}	9.6 ± 1.0 ^{ab}	9.1 ± 0.8 ^{ab}	<0.001	11.5
linalool	linool	2.7 ± 0.4	3.7 ± 0.8	2.8 ± 0.1	3.9 ± 0.6	3.5 ± 0.1	3.1 ± 0.4	0.031	3.6
nerol	nerol	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.3	0.894	0.3
geraniol	gerol	3.4 ± 0.1 ^a	3.3 ± 0.1 ^a	2.1 ± 0.2 ^b	1.9 ± 0.1 ^{bc}	1.6 ± 0.3 ^c	2.0 ± 0.1 ^{bc}	<0.001	62
cis-rose oxide	RosOx	1.7 ± 0.1 ^a	1.6 ± 0.1 ^{ab}	1.5 ± 0.1 ^{ab}	1.5 ± 0.1 ^{ab}	1.4 ± 0.1 ^b	1.6 ± 0.1 ^a	0.006	5.7
α-terpineol	αTrpol	2.4 ± 0.1	2.4 ± 0.3	2.4 ± 0.2	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.2	0.924	0.3
β-damascenone	BDM	2.6 ± 0.4	2.8 ± 0.3	2.8 ± 0.2	3.3 ± 0.2	2.7 ± 0.5	3.1 ± 0.1	0.132	2.1

Results are the means ± SD of three independent fermentation replicates. Means with the same superscript letter are not significantly different from each other (Tukey's test, P = 0.05).

Table S11. Composition of the wines after 15 months in bottle. Wines were made using AWRI796 (parent strain) and five variants carrying mutations in Aro4p (AWRI2965) or Tyr1p (AWRI2936, 2940, 4124, and 2969). Concentrations of the different metabolites are shown in $\mu\text{g L}^{-1}$, unless otherwise indicated.

Metabolite)	Label	AWRI796	AWRI2936	AWRI2940	AWRI4124	AWRI2965	AWRI2969	P value	F-value
Acetic acid (mg L^{-1})	AceAc	434.3 ± 36.8^c	371.8 ± 7.7^d	408.1 ± 28.5^{cd}	441.9 ± 14.4^{bc}	505.7 ± 13.6^a	500.4 ± 17.3^{ab}	0.005	16.7
Ethyl acetate (mg L^{-1})	EthAt	38.1 ± 3.5^a	37.0 ± 0.79^{ab}	35.1 ± 2.8^{ab}	36.8 ± 0.80^{ab}	31.9 ± 1.2^b	30.7 ± 6.5^b	0.010	5.0
2-methylpropyl acetate	2MeProAt	28.9 ± 2.6^b	25.7 ± 6.7^b	52.4 ± 10.6^a	48.1 ± 6.4^a	28.6 ± 7.9^b	24.8 ± 2.5^b	0.001	9.8
2-methylbutyl acetate	2MeButAt	59 ± 0.1	52 ± 13	73 ± 15	77 ± 14	59 ± 12	58 ± 4.9	0.137	2.1
3-methylbutyl acetate	3MeButAt	890 ± 54	631 ± 196	$1,040 \pm 288$	996 ± 276	811 ± 231	725 ± 75	0.203	1.7
2-PEA	2PheEthAt	135 ± 12^d	497 ± 129^{cd}	4270 ± 794^a	1761 ± 394^b	1571 ± 381^{bc}	1836 ± 21^b	<0.001	40.1
Ethyl 2-methyl propanoate	Et2MePro	178 ± 7.8	209 ± 24	217 ± 26	192 ± 27	204 ± 29	192 ± 26	0.469	1.0
Ethyl propanoate	EtPro	89 ± 3.7	94 ± 3.3	93 ± 5.5	92 ± 5.0	89 ± 4.3	83 ± 9.7	0.249	1.5
Ethyl 2-methylbutanoate	Et2MeBut	14.4 ± 0.7	19.1 ± 3.4	16.5 ± 3.5	16.5 ± 4.0	16.5 ± 3.8	17.8 ± 3.0	0.626	0.7
Ethyl 3-methylbutanoate	Et3MeBut	27.3 ± 0.9	36.4 ± 7.9	33.8 ± 5.9	32.1 ± 6.4	30.1 ± 4.7	37.2 ± 4.3	0.283	1.4
Ethyl butanoate	EtBut	329 ± 16^b	331 ± 16^b	389 ± 3.1^a	328 ± 11^b	346 ± 10^{ab}	333 ± 38^b	0.014	4.6
Ethyl hexanoate	EtHex	646 ± 13^c	744 ± 12^{ab}	771 ± 10^a	682 ± 2.1^c	699 ± 10^{bc}	692 ± 50^{bc}	<0.001	12.1
Ethyl octanoate	EtOct	954 ± 20^b	1001 ± 40^{ab}	1030 ± 21^a	960 ± 18^{ab}	951 ± 22^b	982 ± 31^{ab}	0.020	4.2
Ethyl decanoate	EtDec	319 ± 17^{ab}	297 ± 8.2^{ab}	293 ± 11^{ab}	299 ± 24^{ab}	284 ± 37^b	351 ± 19^a	0.028	3.8
Hexyl acetate	HexAt	97 ± 7.0	66 ± 20	98 ± 23	96 ± 27	90 ± 26	76 ± 7.2	0.338	1.2
butanol	BtOH	733 ± 60^b	1036 ± 103^a	959 ± 111^a	865 ± 42^{ab}	949 ± 45^a	939 ± 6.0^a	0.004	6.4
2-methylpropanol (mg L^{-1})	2MePrOH	27.4 ± 0.56^d	30.4 ± 1.1^c	39.1 ± 0.84^a	35.6 ± 0.21^b	28.2 ± 0.60^d	29.1 ± 0.58^{cd}	<0.001	136
2-methylbutanol (mg L^{-1})	2MeBtOH	29.6 ± 1.8^b	35.3 ± 2.0^a	35.1 ± 1.4^a	37.0 ± 2.1^a	29.8 ± 0.91^b	33.4 ± 1.6^{ab}	0.001	9.9
3-methylbutanol (mg L^{-1})	3MeBtOH	144.0 ± 8.4^{ab}	144.2 ± 8.6^{ab}	157.4 ± 6.7^a	160.3 ± 8.0^a	131.1 ± 4.3^b	150.1 ± 4.3^{ab}	0.003	6.9
hexanol	HxOH	1982 ± 27^a	1773 ± 27^c	1753 ± 16^c	1800 ± 21^c	1902 ± 10^b	1802 ± 41^c	<0.001	35.0
2-PE (mg L^{-1})	2PhenEtOH	26.2 ± 3.2^f	66.6 ± 2.1^e	398.1 ± 7.5^a	177.7 ± 4.3^d	192.9 ± 4.6^c	211.6 ± 2.2^b	<0.001	2676
TyrOH (mg L^{-1})	Tsol	15.6 ± 0.7^b	12.0 ± 0.5^{bc}	6.8 ± 0.3^c	9.8 ± 0.5^{bc}	124 ± 5.1^a	9.5 ± 1.6^{bc}	<0.001	1312
TOL (mg L^{-1})	Tphol	0.05 ± 0.1^c	0.32 ± 0.1^c	39.7 ± 1.0^a	1.01 ± 0.3^{bc}	1.63 ± 0.3^b	0.58 ± 0.1^{bc}	<0.001	3814
TOL-SO ₃ H (mg L^{-1})	Tsol_SO3	1.29 ± 0.1^e	3.68 ± 0.1^d	22.6 ± 2.2^a	8.2 ± 0.7^c	9.5 ± 0.2^b	7.3 ± 0.3^c	<0.001	79

Propanoic acid	ProAc	1140 ± 102	1019 ± 252	1006 ± 249	925 ± 92	1066 ± 43	1080 ± 124	0.696	0.6
Butanoic acid	ButAc	846 ± 70 ^c	1100 ± 26 ^a	1085 ± 80 ^{ab}	901 ± 88 ^{bc}	1002 ± 75 ^{ab}	1135 ± 71 ^a	0.002	8.0
3-methyl butanoic acid	3MeButAc	645 ± 7 ^{ab}	638 ± 7 ^b	654 ± 13 ^{ab}	576 ± 9 ^c	637 ± 15 ^b	683 ± 30 ^a	<0.001	15.2
2-methyl butanoic acid	2MeButAc	371 ± 8	356 ± 30	352 ± 25	342 ± 15	378 ± 24	378 ± 35	0.397	1.1
Hexanoic acid	HexAc	7048 ± 397 ^c	8205 ± 316 ^{abc}	8636 ± 490 ^a	7408 ± 535 ^{abc}	7271 ± 354 ^{bc}	8421 ± 626 ^{ab}	0.005	6.2
Octanoic acid	OctAc	10423 ± 135 ^{bc}	11110 ± 302 ^a	11366 ± 229 ^a	10405 ± 44 ^{bc}	10261 ± 174 ^c	10942 ± 208 ^{ab}	<0.001	15.3
Decanoic acid	DecAc	2221 ± 80 ^a	1942 ± 134 ^{ab}	1934 ± 124 ^{ab}	1965 ± 134 ^{ab}	1858 ± 161 ^b	1857 ± 70 ^b	0.030	3.7
Free SO ₂ (mg L ⁻¹)	FSO ₂	15 ± 2.0	15 ± 4.9	17 ± 3.1	10 ± 0.6	14 ± 3.5	11 ± 0.6	0.128	2.2
Total SO ₂ (mg L ⁻¹)	TSO ₂	72 ± 4.9	80 ± 8.3	80 ± 7.4	69 ± 1.0	77 ± 6.7	68 ± 1.5	0.099	2.4
H ₂ S	H ₂ S	3.5 ± 0.9	3.6 ± 1.0	3.0 ± 1.1	4.0 ± 1.2	2.3 ± 0.9	1.8 ± 0.3	0.101	2.4
MeSH	MeSH	8.7 ± 0.8 ^a	7.6 ± 0.5 ^{ab}	7.4 ± 0.3 ^{ab}	7.8 ± 0.2 ^a	4.6 ± 0.5 ^c	6.3 ± 0.6 ^b	<0.001	22.1
DMS	DMS	15.9 ± 0.8 ^b	16.4 ± 1.9 ^b	16.5 ± 0.9 ^b	14.5 ± 0.5 ^b	26.9 ± 0.5 ^a	26.9 ± 0.4 ^a	<0.001	106
MeSAc	MeSAc	nd	nd	nd	nd	nd	nd		
Methionol	Metol	567 ± 57 ^c	679 ± 20 ^c	2102 ± 205 ^a	1104 ± 34 ^b	571 ± 12 ^c	807 ± 17 ^c	<0.001	133
Methional	Metal	26.1 ± 2.9 ^{ab}	28.0 ± 6.8 ^a	12.9 ± 3.1 ^c	29.4 ± 1.9 ^a	15.9 ± 3.7 ^c	19.9 ± 2.2 ^{bc}	0.001	9.8
2-methylpropanal	2MeProAl	128 ± 12	151 ± 31	153 ± 38	127 ± 2.7	128 ± 8.3	136 ± 7.1	0.477	1.0
3-methylbutanal	2MeButAl	26.3 ± 0.4 ^c	35.9 ± 3.3 ^b	43.4 ± 4.1 ^a	40.4 ± 1.3 ^{ab}	43.7 ± 1.6 ^a	40.5 ± 1.4 ^{ab}	<0.001	22.7
furfural	Furfal	257 ± 31	226 ± 14	222 ± 26	259 ± 4.5	236 ± 35	248 ± 24	0.361	1.2
5-methylfurfural	MeFFAL	7.7 ± 0.1 ^a	6.7 ± 0.7 ^{ab}	5.5 ± 0.4 ^b	6.7 ± 0.4 ^{ab}	6.7 ± 0.5 ^{ab}	6.0 ± 0.9 ^{ab}	0.021	4.3
benzaldehyde	Benzal	130 ± 18	152 ± 17	176 ± 39	142 ± 7.7	158 ± 16	158 ± 26	0.283	1.4
2-phenylacetaldehyde	PAceAld	25.8 ± 1.0 ^e	35.3 ± 1.6 ^d	107 ± 4.2 ^a	60.7 ± 1.7 ^c	83.9 ± 3.1 ^b	63.4 ± 3.2 ^c	<0.001	373

Results are the mean ± SD of three independent fermentation replicates (µg L⁻¹). Means with the same superscript letter are not significantly different from each other (Tukey's test, P = 0.05). n.d.: not detected

Figure S1

Relationship between 2-PE and 2-PEA production at the end of alcoholic fermentation in the Chardonnay wines. The wines were fermented by the parent AWRI796 (●) strain, and five variants carrying mutations in Aro4p (AWRI2965 (●) or Tyr1p (AWRI2936 (●), AWRI2940 (●), AWRI4124 (●) and AWRI2969 (●)). Results are the mean (in mg L⁻¹) of three independent fermentation replicates.

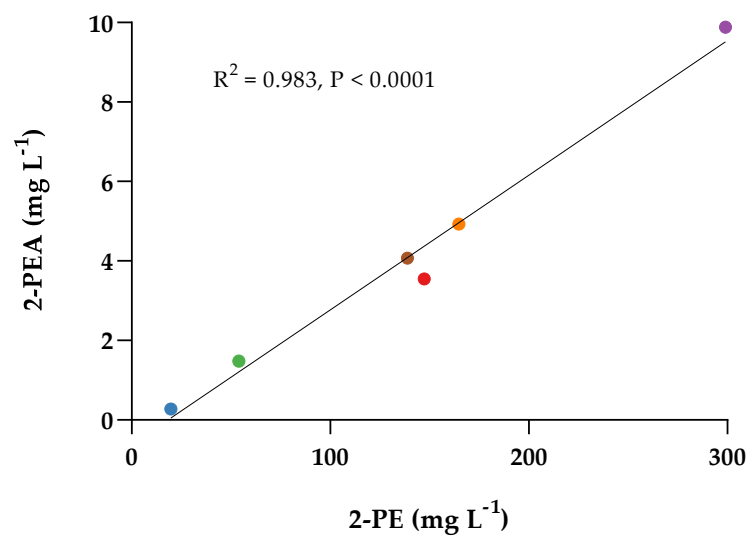


Figure S2

Relationship between concentrations of TOL (in mg L^{-1}) at the end of alcoholic fermentation and its maximum percentage of conversion into TOL- SO_3H in the Chardonnay wines during ageing. Wines were fermented by the parent AWRI796 (●) strain, or five variants carrying mutations in Aro4p (AWRI2965 (●) or Tyr1p (AWRI2936 (●), AWRI2940 (●), AWRI4124 (●) and AWRI2969 (●)). The graph is zoomed-in to highlight the relationship between these two variables at low to moderate concentrations of TOL ($< 10 \text{ mg L}^{-1}$). Results are the mean of three independent fermentation replicates.

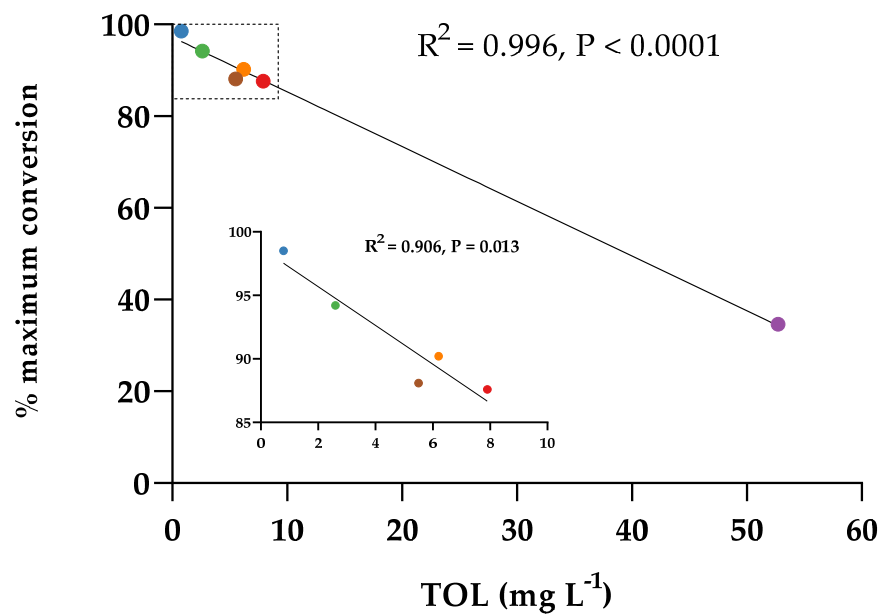


Figure S3

Relationship between the decrease in free (filled symbols) and total (empty symbols) SO₂ concentrations and TOL-SO₃H formation in the Chardonnay wines at different time points during ageing. Wines were fermented with either the parent AWRI796 (●) strain, and five variants carrying mutations in Aro4p (AWRI2965 (●) or Tyr1p (AWRI2936 (●), AWRI2940 (●), AWRI4124 (●) and AWRI2969 (●)). Results are expressed as the average of three independent triplicates. No correlation was observed between either free or total SO₂ and TOL-SO₃H levels in the wines at any given time point ($P < 0.05$).

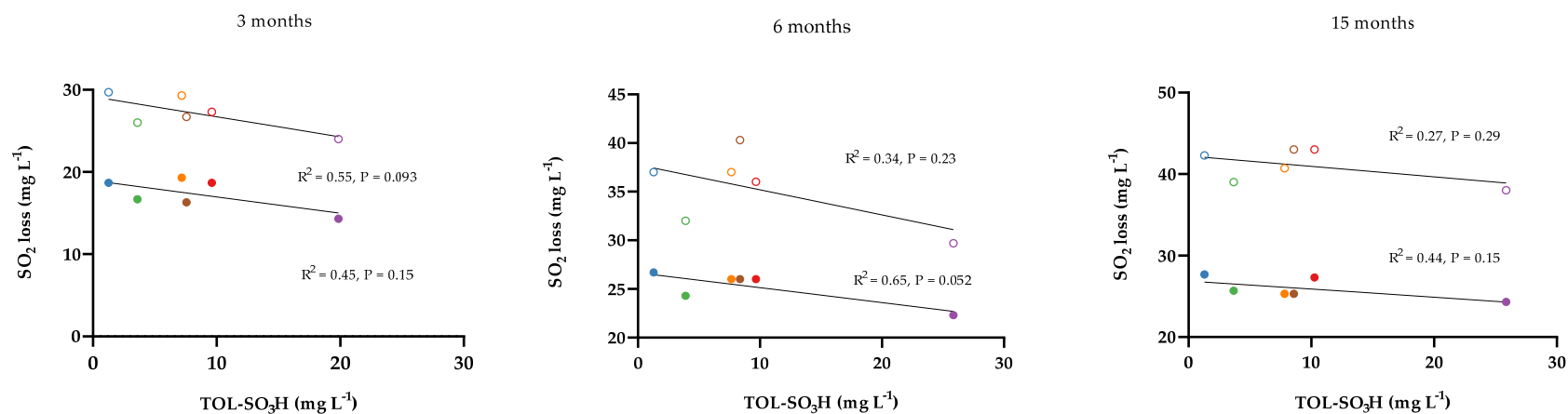


Figure S4

Mean blue fluorescence by flow cytometry for AWRI796 and two variants with an altered Ehrlich pathway (AWRI2940 and AWRI2965), with genomic integration of the blue fluorescence protein (BFP) under the control of the *S. cerevisiae* ARO9 promoter (Aro9pr). Results are the average of the mean blue fluorescence for three independent replicates after growth for 48 hours in liquid minimal media.

A DNA fragment of 3.4 kb was amplified by PCR from the pCV2-HO-Aro9pr-BFP plasmid, and approximately 200 µg of the purified DNA used to transform each of the different yeast strains. Transformants were selected in YPD-CloNAT plates, and genomic integration in the HO locus was confirmed by PCR. Mean blue fluorescence was acquired after growth in 96-well plates containing 600 µl of Yeast Nitrogen Base medium without amino acids and 2% glucose. Microplates were sealed with Breathe-Easy sealing membranes and incubated at 28°C for 48 hours. Cell cultures were then diluted in PBS buffer to ensure a concentration of about 5×10^5 cells/ml. Mean blue fluorescence was detected using a violet (405 nm solid state laser) excitation and a 448/50 nm detection filter by flow cytometry and using a Guava easyCyte 12HT instrument. The mean blue fluorescence of the transformed cells was subtracted from that obtained from untransformed cells. For all analysis, a minimum of 5000 events were acquired while cell throughput was kept under 500 cells/µL. Results were analysed with inCyte software version 3.2.

