

## Supplemental Material

### Applied Microbiology and Biotechnology

#### New biomarkers underlying acetic acid tolerance in the probiotic yeast *Saccharomyces cerevisiae* var. *boulardii*

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**Table S1** Primers for RT-qPCR analysis used in this study

<b>Purpose</b>	<b>Gene</b>	<b>Primer sequence(s) (5' to 3')</b>
Endocytosis	<i>PAN1</i>	AGCAAGCACAGTCCTTGC and CACCGGAAACAGTATTCGAACC
	<i>SLA1</i>	ACCAAGCTCCAGATGAGGA and CCTCGATTTTCGGTAACGTTCC
	<i>END3</i>	GCAGACTTGCCTCCAAAGAA and CTCCGCCAGAGAATTTGGT
Signaling/MAPK	<i>TOR1</i>	GCAGCCTCATCTGGTTACG and GAGACATGCCCTGCATGAG
	<i>SMI1</i>	ATCACTCACGCAGAGGAAGA and GTGCATATAACCGTTGCACT
Fatty acid biosynthesis	<i>OLE1</i>	CATCTCCGAACAACCATGGA and ATCTCAATGGCCAGTGAGC
Glycerol biosynthesis	<i>GPD1</i>	GTGGGTGTTCGAAGAAGAG and ACAGGAGATAGCTCTGACG
	<i>GPP1</i>	GAAGTTCCAGGTGCTGTC and CGTTGTATTCACCGACTC
Transporter activity	<i>VBA5</i>	GGCTCGTTACAGGCTACAG and TCACCAATGGACGTGACC
	<i>FRE7</i>	GTCTGTTCGTGCAGGCATAA and
	<i>YPT53</i>	GTCTTCTAGCAATAGGCAGCG
	<i>OPT2</i>	AACCTACTATTGGTGCCGC and AGTCTTAGCGCTTGCTTCG
	<i>ADE17</i>	CGGAAGACGTTGCGTATATCC and CGGAATGTCTCCACTGGAAT GTGTGACTATTCCAGAAGCCG and TGTGGATTGGCACCATAACG

**Table S2** Mapped data stats of *S. boulardii*

<b>Strain</b>	<b>Ref. length</b>	<b>Mapped sites (<math>\geq 1x</math>)</b>	<b>Total reads</b>	<b>Mapped reads</b>	<b>Mapped bases</b>	<b>Mean depth</b>
<b>Enterol (ancestral)</b>	12,001,065	11,966,515 (99.71 %)	6,326,040	5,383,980 (85.11%)	751,552,975	62.62
<b>Enterol ev16</b>	12,001,065	11,966,699 (99.71 %)	6,691,164	6,223,777 (93.01%)	877,736,951	73.14
<b>Enterol ev17</b>	12,001,065	11,968,794 (99.73 %)	6,927,114	5,913,452 (85.37%)	831,147,204	69.26

**Table S3** Raw data stats of *S. boulardii* bases sequenced

<b>Strain</b>	<b>Total read base (bp)</b>	<b>Total reads</b>	<b>GC (%)</b>	<b>Q20 (%)</b>	<b>Q30 (%)</b>
<b>Enterol (ancestral)</b>	1,206,462,216	7,989,816	37.93	96.63	92.70
<b>Enterol ev16</b>	1,097,931,570	7,271,070	36.65	96.75	92.82
<b>Enterol ev17</b>	1,168,264,350	7,736,850	37.63	96.65	92.57

**Table S4** Filtered data stats of *S. boulardii* bases sequenced

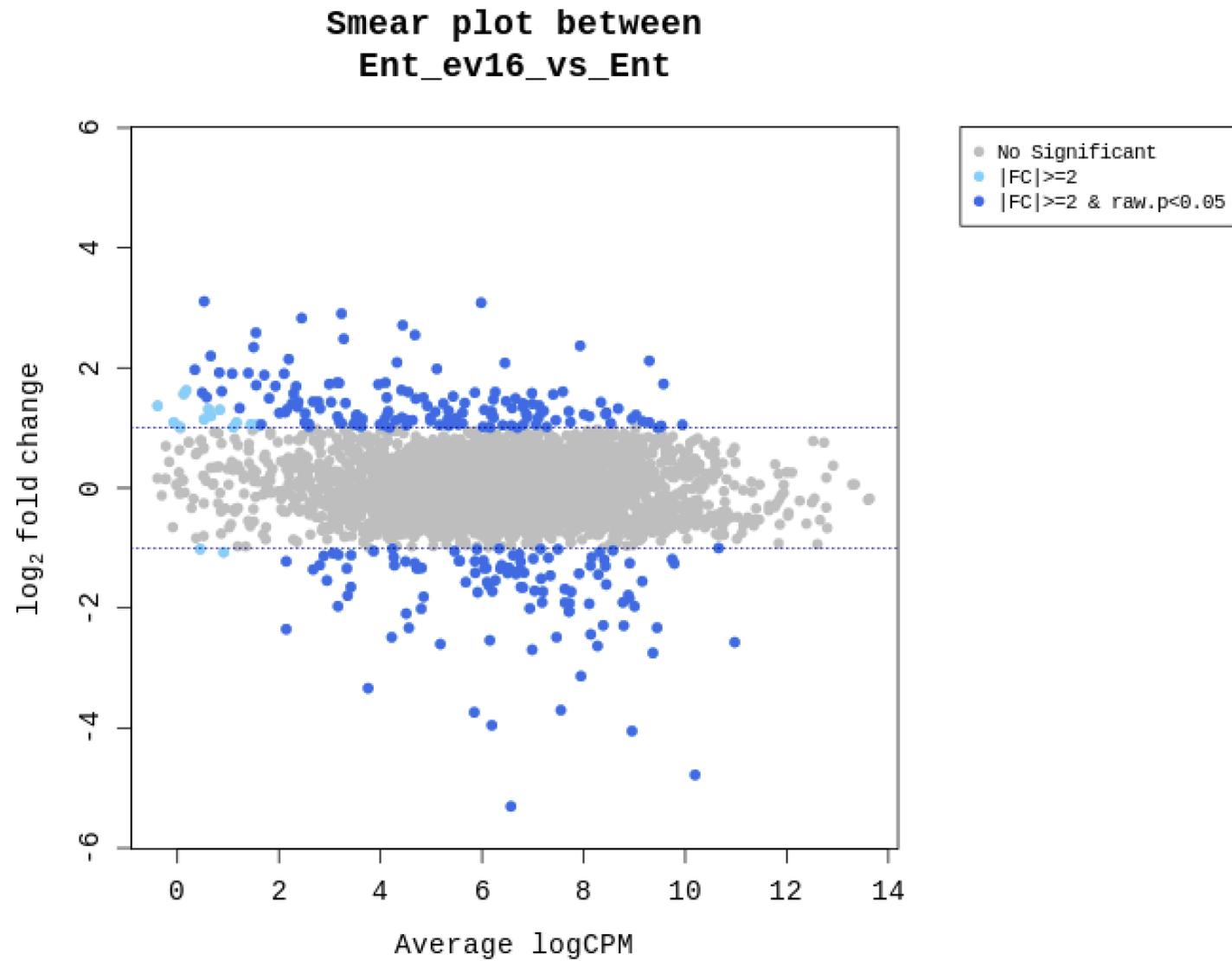
<b>Strain</b>	<b>Total read base (bp)</b>	<b>Total reads</b>	<b>GC (%)</b>	<b>Q20 (%)</b>	<b>Q30 (%)</b>
<b>Enterol (ancestral)</b>	1,007,457,971	6,927,114	37.55	99.11	97.07
<b>Enterol ev16</b>	918,284,036	6,326,040	36.27	99.09	97.00
<b>Enterol ev17</b>	970,684,577	6,691,164	37.34	99.07	96.95

**Table S5** Summary of variant count of *S. boulardii*

<b>Strain</b>	<b>Number of SNPs</b>	<b>Number of insertions</b>	<b>Number of deletions</b>
<b>Enterol (ancestral)</b>	1,942	565	122
<b>Enterol ev16</b>	1,898	573	130
<b>Enterol ev17</b>	1,965	576	125



**Fig. S1** The nucleotide and amino acid sequences of the *PAN1* gene in the yeast *S. boulardii* ATCC MYA-796. The ranges of sequence starting from nucleotide position 1,393 (amino acid at 465) were used to analyze the polymorphism in various evolved Enterol strains (black box) via polymerase chain reaction (PCR) amplification technique with specific oligonucleotides, which cover the mutation site.



**Fig. S2** The transcripts of DEGs in Enterol ev16 versus ancestral Enterol according to the overall average expression level (Smear); a smear plot is drawn. (X-axis: Average logCPM, Y-axis: log<sub>2</sub>-fold change)