Supplemental Material

Applied Microbiology and Biotechnology

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

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| Purpose | Gene | Primer sequence(s) (5' to 3') |
|-------------------------|-------|---|
| Endoavtosis | DANI | |
| Endocytosis | PANI | CACCGGAAACAGTATTCGAACC |
| | SLA1 | ACCAAGCTCCAGATGAGGA and |
| | | CCTCGATTTCGGTAACGTTCC |
| | END3 | GCAGACTTGCCTCCAAAGAA and |
| | | CTCCGCCAGAGAATTTGGT |
| Signaling/MAPK | TOR1 | GCAGCCTCATCTGGTTACG and |
| | | GAGACATGCCCTGCATGAG |
| | SMI1 | ATCACTCACGCAGAGGAAGA and |
| | | GTGCATATACCGGTTGCACT |
| Fatty acid biosynthesis | OLE1 | CATCTCCGAACAACCATGGA and |
| | | ATCTCAATGGCCAGTGAGC |
| Glycerol biosynthesis | GPD1 | GTGGGTGTTCGAAGAAGAG and ACAGGAGATAGCTCTGACG |
| | GPP1 | GAAGTTCCAGGTGCTGTC and CGTTGTATTCACCGACTC |
| Transporter activity | VBA5 | GGCTCGTTACAGGCTACAG and TCACCAATGGACGTGACC |
| 1 5 | FRE7 | GTCTGTTCGTGCAGGCATAA and |
| | YPT53 | GTCTTCTAGCAATAGGCAGCG |
| | OPT2 | AACCTACTATTGGTGCCGC and AGTCTTAGCGCTTGCTTCG |
| | ADE17 | CGGAAGACGTTGCGTATATCC and |
| | | CGGAATGTCTCCACTGGAAT |
| | | GTGTGACTATTCCAGAAGCCG and |
| | | TGTGGATTGGCACCATAACG |

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

 Table S2 Mapped data stats of S. boulardii

| Strain | Ref. length | Mapped sites (≥ 1x) | Total reads | Mapped reads | Mapped bases | Mean depth |
|------------------------|----------------|-------------------------|----------------|-----------------------|-----------------|---------------|
| Enterol (ancestral) | 12,001,065 | 11,966,515 (99.71 %) | 6,326,040 | 5,383,980 (85.11%) | 751,552,975 | 62.62 |
| Enterol ev16 | 12,001,065 | 11,966,699 (99.71 %) | 6,691,164 | 6,223,777 (93.01%) | 877,736,951 | 73.14 |
| Enterol ev17 | 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

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

| Table S | 4 Filtered | l data | stats | of S. | boulardii | bases | sequenced |
|---------|------------|--------|-------|-------|-----------|-------|-----------|
|---------|------------|--------|-------|-------|-----------|-------|-----------|

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

| Strain | Number of SNPs | Number of insertions | Number of deletions |
|---------------------|-------------------|-------------------------|------------------------|
| Enterol (ancestral) | 1,942 | 565 | 122 |
| Enterol ev16 | 1,898 | 573 | 130 |
| Enterol ev17 | 1,965 | 576 | 125 |

| | 1,05 | 0 | 1,060 | 1,070 | | 1,080 | 1,090 | 1,1 | 00 | 1,110 | 1 | ,120 | 1,130 | | 1,140 |
|--|--|---|--|---|--|---|--|--|--|---|---|---|--|--|--|
| PAN1 Sb ATCC MYA | ATTGGATTCT | AAGACAAA | AAACGAA | GTTTĊAA | GTTTTAT | TGACGC | CATTAAT | TAAGCA | ΓΤGCAAA | CAĠGAT | TCTTCC | GCAAAC | GATGĊC | CCAAAA | ACTCCCT |
| Frame 1 | Leu Asp Ser | Lys Thr Lys | S Asn Glu | Val Ser S | Ser Phe Ile | e Asp Ala | lle Asn | Leu Ser II | e Ala Asn | Gln Asp | Ser Ser | Ala Asn | Asp Ala | Pro Lys | Thr Pro |
| | 350 | , , | 355 | | 36 | 0 | | 365 | | 370 | | 375 | | , | 380 |
| | 1,150 | 1,160 | 1 | 1,170 | 1,180 | 1, | 90 | 1,200 | 1,210 | | 1,220 | 1 | ,230 | 1,240 | |
| PAN1 Sb ATCC MYA | TTGATĠAATT | CATTACAG | CGGGCGT | ACAAAAT | ттбсаас | CTCAAC | AACAGG/ | ATATATG | Ι C T C A A A | CTAGTTT | TGGTAT | CCCATT | ACAGTC | ΤϹΑΑΑΤ | TACTGGA |
| Frame 1 | Phe Asp Glu Phe | e lle Thr A | Ala <mark>Gly V</mark> a | l Gln Asn | Leu Gln I | Pro Gln Pi | <mark>ro</mark> Thr Gly | Tyr Met | Pro Gln Th | hr Ser Ph | e Gly lle | Pro Lei | J Gln Sei | Gin lle | e Thr Gly |
| | 385 | 5 | 390 | D | | 395 | | 400 | | 405 | | 410 |) | | 415 |
| | 1,250 | 1,260 | 1,270 | 1,28 | 80 | 1,290 | 1,300 | | 1,310 | 1,320 | | 1,330 | 1 | ,340 | 1,350 |
| PAN1 Sb ATCC MYA | GGCGGTGTTG | CCTCGGCG | <u>TTGAATC</u> | CTCAATC | CACAGGA | TTTATG | CACCAA | CACTTT | AACATG | FCAAT GA | ATACCG | GAACTC | CCGGAT | TGAACC | CCCAAAT |
| Frame 1 | Gly Gly Val A | Ala Ser Ala | Leu Asn F | <mark>Pro Gln Se</mark> | r Thr Gly | Phe Met | Ala Pro T | hr Thr Phe | Asn Met | Ser Met / | Asn Thr C | Sly Thr F | <mark>ro Gly L</mark> | eu Asn I | Pro Gin lle |
| | 2 | 420 | | 425 | | 430 | | 435 | | 440 | | | 445 | | 450 |
| | 1,360 | 1,37 | 0 | 1,380 | 1,390 | | 1,400 | 1,410 | | 1,420 | 1,430 | | 1,440 | | 1,450 |
| PAN1 Sb ATCC MYA | | GCACCIGC | CICIAIG | | ACAIIAC | IGGCAA | IGCIIIGO | | | GGIAIG | AIGCCA | | ACIGGI The Chi | | |
| Frame | | Ald PIO Ald | a ser iviet | | ASIT ILE ITI | IT GIY ASI | Ald Leu | | | Giy iviet | Met Pro | | | wet wet | PIO GIII |
| | 4.450 | 400 | 4 400 | 400 | 4.400 | 405 | | 470 | 4 500 | 475 | 500 | 4 5 40 | 460 | 4.550 | 465 |
| | | | | тслотто | | 1,500 | | | | | | | | 1,550 | |
| Frame 1 | hr Ser Pho Glu | | | | | | GCAATC | CAGTATA | | | | Mot Pro | | | |
| FIGILIE I | The Sel Frie City | | eu diy Fit | 495 | | | | 505 | Thi diy d | 1 y 1yi G | y Jei va | | 515 | | 520 |
| | | 400 | | 455 | | 200 | | 505 | | 510 | 0 | | 515 | | 520 |
| | 1 57 | 0 | 1 5 0 0 | 1 500 | | 1 600 | 1.610 | 1 (| 20 | 1.620 | 1 | 640 | 1.650 | | 1.660 |
| | | | | | | 1,600 | | | | | | | | | |
| PAN1 Sb ATCC MYA | AGTATGCCCA Ser Met Pro | | 1,580 TTTAATC Phe Asn (| 1,590 AACAAGG | | 1,600 TCTCAG ⁻ Ser Gln | 1,610 TTAACCG Leu Thr G | 1,6 GTTGCAA | ACCCCAAC | ^{1,630} CCAACGG Pro Thr (| GTTTTC | ,640 TACCAC | 1,650 CATCTA | ACTTTA | 1,660 GTGCTAC Ser Ala Thr |
| PAN1 Sb ATCC MYA Frame 1 | 1,57 AGTATGCCCA Ser Met Pro A | 0 ATTTGTCC Asn Leu Ser 525 | ^{1,580} TTTAATC Phe Asn (| 1,590 AACAAGG GInGInGI 530 | ATTACAA y Leu Gln | 1,600 TCTCAG Ser GIN 535 | 1,610 FTAACCG Leu Thr G | 1,6 G T T G C A A ly Leu GIn 540 | ACCCCAAC Pro Gln | 1,630 CCAACGG Pro Thr (| 1 GTTTTC Gly Phe L 545 | ,640 TACCAC eu Pro F | 1,650 CATCTA ProSerA 550 | ACTTTA sn Phe S | 1,660 GTGCTAC Ser Ala Thr 555 |
| PAN1 Sb ATCC MYA Frame 1 | AGTATGCCCA Ser Met Pro A | ATTTGTCC ASTLEU Ser 525 | 1,580 TTTAATC Phe Asn (| 1,590 AACAAGG GIn GIn GI 530 | ATTACAA y Leu Gin | 1,600 TCTCAG Ser GIN 535 | 1,610 F T A A C C G (Leu Thr G | 1,6 G T T G C A A ly Leu Gln 540 1,720 | ACCCCAAC ProGIN | 1,630 CCAACGG Pro Thr (| 1 GTTTTC Gly Phe L 545 | ,640 TACCAC eu Pro F | 1,650 CATCTA Pro Ser A 550 | ACTTTA Asn Phe 1 | 1,660 GTGCTAC Ser Ala Thr 555 |
| PAN1 Sb ATCC MYA Frame 1 | AGTATGCCCA Ser Met Pro A | ATTTGTCC ATTTGTCC son Leu Ser 525 | 1,580 TTTAATC Phe Asn (A A A G A C A | 1,590 A A C A A G G Gin Gin Gin Gi 530 | ATTACAA y Leu Gin | 1,600 Ser Gin 535 1,7 C G A A A T | $\begin{array}{c} & 1,610 \\ & \Gamma T A A C C G G \\ & Leu Thr G \\ & 710 \\ & \Gamma T A T A C C G \\ \end{array}$ | 1,6 G T T G C A A ly Leu Gin 540 | Pro GIn 1,730 | 1,630 CCAACGG Pro Thr C | 1 GTTTTC Gly Phe L 545 1,740 TTAATT | ,640 TACCAC eu Pro F | 1,650 CATCTA Pro Ser A 550 7 CAAGT | ACTTTA ssn Phe 1 | GTGCTAC Ser Ala Thr 555 |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1,57 AGTATGCCCA Ser Met Pro A 1,670 CATGCCGTTG Met Pro Leu | ATTTGTCC AST Leu Ser 525 1,680 ACTGCCCA Thr Ala Gir | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr | 1,590 A A C A A G G Gin Gin Gi 530 G G A T T T G Gly Phe | ATTACAA y Leu Gln 1,700 GTAATAA Gly Asn As | 1,600 Ser Gin 535 1,7 CGAAA n Glu Ile | 1.610 TTAACCG Leu Thr G 710 TTATACC Tyr Thr | 1, GTTGCA y Leu Gļn 540 1,720 AATCCAA Lys Ser A | ACCCCAAC ProGIN ACTTTAA Sn Phe Asn | 1,630 CCAACGG ProThr CAATAAC Asn Asn | 1 GTTTTC Gly Phe L 545 1,740 TTAATT Leu Ile | ,640 TACCAC eu Pro F GATAAC Asp Asn | 1,650 CATCTA Pro Ser A 550 750 TCAAGT Ser Ser | ACTTTA sn Phe S 1,760 CAAGAC Gln Asp | GTGCTAC Ser Ala Thr 555 |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1.57 AGTATGCCCA Ser Met Pro A 1.670 CATGCCGTTG Met Pro Leu | ATTTGTCC AST Leu Ser 525 ACTGCCCA Thr Ala Gir 560 | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr | 1,590 A A C A A G G Gin Gin Gi 530 G G A T T T G Gly Phe 565 | ATTACAA y Leu Gln 1,700 GTAATAA Gly Asn As | 1,600 Ser Gin 535 C G A A A T n Glu Ile 570 | 1,610 TTAACCG Leu Thr G 710 TTATACC Tyr Thr | 1, G T T G C A A I,720 1,720 A A A T C C A A Lys Ser A 5' | ACCCCAAC Pro GIn ACTTTAA ACTTTAA SIN Phe Asn 75 | 1,630 C C A À C G G Pro Thr C F A A T A A C Asn Asn | 1 GTTTTC Gly Phe L 1,740 TTAATT Leu Ile 580 | GATAAC Asp Asn | 1,650 CATCTA ro Ser A 550 TCAAGT Ser Ser 585 | A C T T T A sn Phe 1 1,760 C A A G A C Gln Asp | AAAATTT Lys IIe |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1.57 AGTATGCCCA Ser Met Pro A 1.670 CATGCCGTTG Met Pro Leu 1.770 | ATTTGTCC AST Leu Ser 525 ACTGCCA Thr Ala Gir 560 1,780 | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 | 1,590 A A C A A G G Gin Gin Gi G G A T T T G Gly Phe 565 1,810 | ATTACAA y Leu Gln 1,700 GTAATAA Gly Asn As | 1,600 Ser Gin 535 C G A A A T n Glu Ile 570 1,810 | 1,610 TTAACCGC Leu Thr G 710 TTATACC Tyr Thr 1,820 | 1, G T T G C A A Iv Leu Gin 1,720 A A A T C C A A Lys Ser A 5 | 20 Pro GIn 1,730 A C T T T A A 5 1,830 | 1,630 CCAACGG Pro Thr C CAATAAC Asn Asn 1,840 | 1 GTTTTC Gly Phe L 545 1,740 TTAATT Leu IIe 580 | GATAAC ACAC eu Pro F GATAAC Asp Asn 1,850 | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 | A C T T T A sn Phe 1 1,760 C A A G A C Gin Asp 860 | 1,660 G T G C T A C Ser Ala Thr 555 A A A A A T T T Lys Ile 1,870 |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA | 1.57 AGTATGCCCA Ser Met Pro A 1.670 CATGCCGTTG Met Pro Leu 1.770 CCACGGAGGA | ATTTGTCC AST Leu Ser 525 ACTGCCCA Thr Ala Gir 560 1,780 AAAATCTT | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA | 1,590 A A C A A G G Gin Gin Gi G G A T T T G Gly Phe 565 1,89 T A A A A T T | ATTACAA y Leu Gln I,700 GTAATAA Gly Asn As | 1,600 Ser GIn 535 C G A A A T 1, C G A A A T 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, | 1,610 TTAACCG Leu Thr G TTATACC Tyr Thr 1,820 ATACTCA | 1,720 1, | ACCCCAAC ProGIN ACTTTAA ACTTTAA SIN Phe ASIN 75 1,830 GTTTGTT | 1,630 C C A A C G G Pro Thr C F A A T A A C Asn Asn 1,840 T A G A T T C | 1 GTTTTC Gly Phe L 545 1,740 TTAATT Leu IIe 580 | GATAAC ACTACAC eu Pro F GATAAC Asp Asn 1,850 TGCTGT | 1,650 CATCTA Yro Ser A 550 TCAAGT Ser Ser 585 GGAGAT | A C T T T A sn Phe 1 1,760 C A A G A C Gln Asp 1,760 C A A G A C Gln Asp 1,760 T T T T A G | 1,660 G T G C T A C Ser Ala Thr 555 A A A A T T T Lys Ile 1,870 A A A A T C T |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1.57 AGTATGCCCA Ser Met Pro A 1.670 CATGCCGTTG Met Pro Leu 1.770 CCACGGAGGA Ser Thr Glu Glu | ATTTGTCC AST Leu Ser 525 ACTGCCA Thr Ala Gir 560 1,780 AAAATCTT Lys Ser L | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA eu Phe Ty | 1,590 A A C A A G G Gin Gin Gi G G A T T T G G G A T T T G G G A T T T G 565 1,89 T A A A A T T r Lys IIe | ATTACAA y Leu Gln GTAATAA Gly Asn As 00 TTTGAAA Phe Glu | 1,600 Ser GIn 535 C G A A A T n Glu IIe 570 1,810 C T T T G / Thr Phe A | 1,610 TTAACCG Leu Thr G TTATACC Tyr Thr 1,820 ATACTCA sp Thr Gin | 1,720 A A A T C C A A Lys Ser A A A C A A A C A Sn Lys | ACCCCAAC ProGIN ACTTTAA ACTTTAA ACTTTAA A ACTTTAA A ACTTTAA A ACTTTAA A A A | 1,630 CCAACGG Pro Thr C CAATAAC Asn Asn 1,840 CAGATTC cu Asp Se | 1 GTTTTC Gly Phe L 545 1,740 TTAATT Leu IIe 580 CCCCAC r Pro Th | GATAAC ACTAAC GATAAC ASP ASN 1,850 TGCTGT r Ala Va | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 1 GGAGAT | A C T T T A sn Phe 1 1,760 C A A G A C GIn Asp 7 T T T T A G Phe Ar | 1,660 G T G C T A C Ser Ala Thr 555 A A A A A T T T Lys Ile 1,870 A A A A A T C T g Lys Ser |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1.57 AGTATGCCCA Ser Met Pro A 1.670 CATGCCGTTG Met Pro Leu 1.770 CCACGGAGGA Ser Thr Glu Glu | ATTTGTCC ATTGCCS Set ACTGCCCA Thr Ala Glr 560 1,780 AAAATCTT Lys Ser L 595 | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA eu Phe Ty | 1,590 A A C A A G G Gin Gin Gi G G A T T T G G G A T T T G G G A T T T G 565 1,80 T A A A A T T r Lys Ile 600 | ATTACAA y Leu Gln I,700 GTAATAA Gly Asn As 000 TTTGAAA Phe Glu | 1,600 Ser GIN 535 C G A A A T n Glu Ile 570 1,810 C T T T G A Thr Phe A 6 | 1,610 TTAACCG Leu Thr G TTATACC/ Tyr Thr 1,820 ATACTCA/ Sp Thr Gin 05 | 1,720 A A A T C C A A Lys Ser A A A C A A A C A A C A A A C Asn Lys | ACCCCAAC ProGIN 1,730 ACTTTAAT Sn Phe Asn 1,830 GGTTTGT Gly Leu Le 610 | 1,630 CCAACGG ProThrO CAATAAC AsnAsn 1,840 CAGATTC SuAspSe | 1 GTTTTC Gly Phe L 545 TTAATT Leu IIe 580 CCCCAC r Pro Thi 615 | GATAAC GATAAC Asp Asn 1,850 TGCTGT r Ala Va | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GGAGAT I GLU ILE 620 | A C T T T A I,760 C A A G A C GIN ASP I,760 C A A G A C I,760 C A A G A C I,770 C A A | 1,660 G T G C T A C Ser Ala Thr 555 A A A A A T T T Lys Ile 1,870 A A A A A T C T g Lys Ser |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1.57 AGTATGCCCA Ser Met Pro A 1.670 CATGCCGTTG Met Pro Leu 1.770 CCACGGAGGA Ser Thr Glu Glu 590 1.880 | 0 ATTTGTCC Asn Leu Ser 525 ACTGCCCA Thr Ala Gir 560 1,780 AAAATCTT Lys Ser L 595 1,89 | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA eu Phe Ty | 1,590 A A C A A G G Gin Gin Gi G G A T T T G G G A T T T G G G A T T T G 565 T A A A A A T T r Lys Ile 600 1,900 | A T T A C A A y Leu Gln i G T A A T A A Gly Asn As 00 T T T G A A A Phe Glu 1,910 | 1,600 Ser GIN 535 C G A A A T n Glu IIe 570 1,810 C T T T G A Thr Phe A 6 | 1,610 TTAACCG Leu Thr G 710 TTATACC Tyr Thr 1,820 1,920 | 1,720 AAATCCAA LysSerA AAACAAAC ASNLys 1,930 | ACCCCAAC ProGIN 1,730 ACTTTAAT Sn Phe Asn 1,830 GTTTGT GIV Leu Le 610 | 1,630 CCAACGG Pro Thr C CAATAAC Asn Asn 1,840 CAGATTC 20 Asp Se 1,940 | 1 GTTTTC Gly Phe L 545 1,740 TTAATT Leu IIe 580 CCCCAC r Pro Thi 615 1,950 | GATAAC GATAAC Asp Asn 1,850 TGCTGT r Ala Va | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GGAGAT GUU ILE 620 1,960 | A C T T T A I,760 C A A G A C GIN ASP C A A G A C GIN ASP C A A G A C C A A G A C C A A G A C GIN ASP C A A G A C C A A G A C GIN ASP C A A G A C C A A G A C GIN ASP C A A G A C C A A G A C GIN ASP C A A G A C C A A G A C | 1,660 G T G C T A C Ser Ala Thr 555 A A A A A T T T Lys Ile 1,870 A A A A A T C T g Lys Ser 1,970 |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA | 1.57 AGTATGCCCA Ser Met Pro A CATGCCGTTG Met Pro Leu 1.770 CCACGGAGGA Ser Thr Glu Glu 590 | ATTTGTCC ATTTGTCC ASN Leu Ser 525 ACTGCCCA Thr Ala Glr 560 1,780 AAAATCTT Lys Ser L 595 1,89 GTGCAGAT | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTA eu Phe Ty 0 TTGGAGC | 1,590 A A C A A G G GIn GIn GI 530 G G A T T T G G G A T T T G G G A T T T G 565 T A A A A T T r Lys IIe 600 1,900 A A A T T T G | A T T A C A A y Leu Gln G T A A T A A Gly Asn As T T T G A A A Phe Glu 1,910 G A A C C T T | 1,600 Ser GIN 535 CGAAAT 1,7 CGAAAT 1,810 CTTTTGA Thr Phe A 6 TGTGTGATA | 1,610 T A A C C G G Leu Thr G 710 T A T A C C / Tyr Thr 1,820 A T A A A C A / | 1,720 A A T C C A A Lys Ser A A A C A A A C A S C A C A A A C 1,930 A C A C C G G C | ACCCCAAC Pro GIn 1,730 ACTTTAAT ACTTTAAT ACTTTAAT I,830 GTTTGTT Gly Leu Le 610 CCAATTGA | 1,630 Pro Thr C FAATAAC Asn Asn 1,840 FAGATTC 20 Asp Se 1,940 AATAAAC | 1 GTTTTC Gly Phe L 545 1,740 TTAATT Leu IIE 580 CCCCAC r Pro Thi 615 1,950 AAGAAT | GAU TACCAC eu Pro F GATAAC Asp Asn 1,850 TGCTGT r Ala Va | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GU IIe 620 1,960 TAGGTA | A C T T T A I,760 C A A G A C GIN ASP C A A G A C GIN ASP C A C A C A T T T T A G Phe Ar T G C A C T | 1,660 GTGCTAC Ser Ala Thr 555 AAAATTT Lys Ile 1,870 AAAATCT g Lys Ser 1,970 TGGTTTA |
| PAN1 Sb ATCC MYA Frame 1 | 1.57 AGTATGCCCA Ser Met Pro A 1.670 CATGCCGTTG Met Pro Leu 1.770 CCACGGAGGA Ser Thr Glu Glu 590 GGCTTAAATC GJU Leu Asn A | A TTTGTCC A TTTGTCC A TTGCCA A CTGCCCA Thr Ala Gir 560 1,780 A A A A TCTT Lys Ser L 595 GTGCAGAT 1,89 GTGCAGAT | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTA eu Phe Ty 0 TTGGAGC Leu Glu (| 1,590 A A C A A G G G In GIn GI 530 G G A T T T G G G A T T T G 565 T A A A A A T T r Lys IIe 600 1,900 A A A T T T G GIn IIe Tr | A T T A C A A y Leu Gln 1,700 G T A A T A A Gly Asn As OU T T T G A A A Phe Glu 1,910 G A A C C T T p Asn Leu | 1,600 Ser GIN 535 CGAAAT 1,7 CGAAAT 1,810 CTTTTG Thr Phe A CTGTGAT Cys Asp | 1,610 TTAACCG Leu Thr G TTATACC/ Tyr Thr 1,820 ATACTCA/ sp Thr GIn 05 1,920 ATAAACA/ Ile Asn A | 1,720 A A A T C C A A Lys Ser A: A A C A A A C A A C A A A C A A C A A A C A Sn Lys 1,930 A C A C C G G C Sn Thr Gly | ACCCCAAC ProGIN ACTTTAA ACTTTAA ACTTTAA A ACTTTAA A ACTTTAA A A ACTTTAA A A A | 1,630 Pro Thr C FAATAAC Asn Asn 1,840 FAGATTC 20 Asp Se 1,940 ATAAAC Asn Lys C | 1 GTTTTC Sly Phe L 545 1,740 TTAATT Leu Ile 580 CCCCAC r Pro Thi 615 1,950 AAGAAT GIN GIU P | GAU TACCAC eu Pro F GATAAC Asp Asn 1,850 TGCTGT r Ala Va TTGCAC he Ala L | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GGAGAT GIU IIe 620 1,960 TAGGTA eu Gly M | A C T T T A I,760 C A A G A C GIN ASP C A A G A C T T T T A G Phe Ar T G C A C T Itet His I | 1,660 GTGCTAC Ser Ala Thr 555 AAAATTT Lys Ile 1,870 AAAATCT g Lys Ser 1,970 TGGTTTA eu Val Tyr |
| PAN1 Sb ATCC MYA Frame 1 | 1.57 AGTATGCCCA Ser Met Pro A CATGCCGTTG Met Pro Leu 1.770 CCACGGAGGA Ser Thr Glu Glu 590 A.880 GGCTTAAATC Gly Leu Asn A 625 | A T T T G T C C A T T T G T C C A T T G C C A A C T G C C C A Thr Ala Gir 560 1,780 A A A A T C T T Lys Ser L 595 G T G C A G A T Arg Ala Asp 630 | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA eu Phe Ty 0 TTGGAGC Leu Glu (| 1,590 A A C A A G G G In GIn GI 530 G G A T T T G G G A T T T G G G A T T T G 565 T A A A A T T r Lys II 600 A A A T T T G GIn II 61 G G I II 63 | ATTACAA y Leu Gln 1,700 GTAATAA Gly Asn As OO TTTGAAA Phe Glu 1 1,910 GAACCTT p Asn Leu 5 | 1,600 Ser Gin 535 CGAAAT n Giu Ile 570 1,810 CTTTTG Thr Phe A CTGTGAT Cys Asp | 1,610 ГТААСССС Leu Thr ГТАТАСС/ Туг Thr Туг Thr ХТАСТСА/ БР Thr БР Thr ГТААССС/ Туг Thr 1,820 ТАСТСА/ БР Thr БР Т АААСА/ ГЕ АSБ | 1,720 A A T C C A A Lys Ser A: A A C A A A C A A C A A A C A A C A A A C A Sn Lys 1,930 A C A C C G G C sn Thr Gly | ACCCCAAC Pro Gln 1,730 ACTTTAA ACTTTAA an Phe Asn 75 1,830 GITTGT Gly Leu Le 610 CCAATTGA 645 | 1,630 Pro Thr C FAATAAC Asn Asn 1,840 FAGATTC 20 Asp Se 1,940 ATAAAC Asn Lys C | 1 GTTTTC Sly Phe L 545 1,740 TTAATT Leu IIe 580 CCCCAC r Pro Thi 615 1,950 AAGAAT Sin Glu P 650 | GAU TACCAC eu Pro F GATAAC Asp Asn 1,850 TGCTGT r Ala Va TTGCAC he Ala L | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GGAGAT GU IIe 620 1,960 TAGGTA eu Gly N | A C T T T A I,760 C A A G A C GIN ASP R60 T T T T A G Phe Ar T G C A C T Act His I | 1,660 GTGCTAC Ser Ala Thr 555 AAAATTT Lys Ile 1,870 AAAATCT g Lys Ser 1,970 TGGTTTA _eu Val Tyr |
| PAN1 Sb ATCC MYA Frame 1 | 1.57 A G T A T G C C C A Ser Met Pro A (1,670 C A T G C C G T T G Met Pro Leu 1.770 C C A C G G A G G A Ser Thr Glu Glu 590 G G C T T A A A T C Gly Leu Asn A 625 | A TTTGTCC A TTTGTCC A TTGCCCA A CTGCCCA Thr Ala Gir 560 1,780 A A A A TCTT Lys Ser L 595 GTGCAGAT Arg Ala Asp 630 1,990 | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA eu Phe Ty 0 TTGGAGC Leu Glu (2,000 | 1,590 A A C A A G G G In GIn GI 530 G G A T T T G G G A T T T G G G A T T T G 565 T A A A A T T r Lys IIe 600 A A A T T T G GIn IIe Tr 63 | ATTACAA y Leu Gln 1,700 GTAATAA Gly Asn As OO TTTGAAA Phe Glu 1 1,910 GAACCTT p Asn Leu 5 2,010 | 1,600 Ser GIN 535 CGAAAT 1,7 CGAAAT 1,810 CTTTTGA Thr Phe A CTGTGAT Cys Asp 2,020 | 1,610 Leu Thr 710 T A A C C C T T A T A C C Tyr Thr 1,820 A T A C T C A A T A C T C A 1,920 A T A A A C A ILE ASN A A SN A | 1,4 G T T G C A A Iy Leu Gin 540 1,720 A A T C C A A Lys Ser A S A A C A A A C A Sn Lys 1,930 A C A C C G G C sn Thr Gly 2,030 | 20 Pro Gln 1,730 A C T T T A A T 5 1,830 G T T T G T T Gly Leu Le 610 C C A A T T G A Gln Leu 645 2,040 | 1,630 C C A A C G G Pro Thr C F A A T A A C Asn Asn 1,840 T A G A T T C 20 Asp Se 1,940 A T A A A C Asn Lys C | 1 GTTTTC Gly Phe L 545 1,740 TTAATT Leu Ile 580 CCCCAC r Pro Thi 615 1,950 AAGAAT Gln Glu P 650 | GAU TACCAC eu Pro F GATAAC Asp Asn 1,850 TGCTGT Ala Va TTGCAC he Ala L | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GGAGATA 620 1,960 TAGGTA eu Gly M | A C T T T A ssn Phe S 1,760 C A A G A C Gln Asp 860 T T T T A G Phe Ar T G C A C T Met His S 2,070 2,070 | 1,660 GTGCTAC Ser Ala Thr 555 AAAATTT Lys Ile 1,870 AAAATCT g Lys Ser 1,970 TGGTTTA eu Val Tyr |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1.57 A G T A T G C C C A Ser Met Pro A C A T G C C G T T G Met Pro Leu 1.770 C C A C G G A G G A Ser Thr Glu Glu 590 G G C T T A A A T C Gly Leu Asn A 625 1.980 C G G T A A A T T A | A T T T G T C C A T T T G T C C ASN Leu Ser 1,680 A C T G C C C A Thr Ala Gir 560 1,780 A A A A T C T T Lys Ser L 595 G T G C A G A T Arg Ala Asp 630 1,990 A A C G G G A A | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA eu Phe Ty 0 TTGGAGC Leu Glu (2,000 GCCAATC | 1,590 A A C A A G G G In GIn GI 530 G G A T T T G G G A T T T G G G A T T T G 565 T A A A A T T r Lys IIe 600 A A A T T T G GIN IIe Tr 63 C C C C A A T G | ATTACAA y Leu Gln 1,700 GTAATAA Gly Asn As 00 TTTGAAAA Phe Glu 1 1,910 GAACCTT p Asn Leu 5 2,010 TCCTACC | 1,600 Ser GIN 535 CGAAAT 1,7 CGAAAT 1,810 CTTTTGA Thr Phe A CTGTGAT Cys Asp 2,020 CTTCAAG | 1,610 TAACCG Leu Thr G 710 TATACC/ Tyr Thr 1,820 ATACTCA/ Sp Thr GIn 05 1,920 ATAAACA/ ILE ASN A 640 TTTAATTC | 1,720 A A T C C A A Lys Ser A: A A C A A A C A A C A A A C A A C C G G C A Sn Lys 1,930 A C A C C G G C Sn Thr Gly 2,030 C C C T C C A C | 20 A C C C C A A G Pro GIn 1,730 A C T T T A A 5 1,830 G T T T G T Gly Leu 610 C C A A T T G A Gin Leu 645 2,040 G C A A A A G C A A A A | 1,630 C C A A C G G Pro Thr C F A A T A A C Asn Asn 1,840 T A G A T T C 20 Asp Se 1,940 A T A A A C Asn Lys C | 1 GTTTTC GJy Phe L 545 TTAATT Leu Ile 580 CCCCAC r Pro Thi 615 1,950 AAGAAT GIN GIU P 650 COSO GACAAC | GAU TACCAC eu Pro F GATAAC Asp Asn 1,850 TGCTGT Ala Va TTGCAC he Ala L | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GGAGAT GGAGATA eu Gly N 60 AACCAA | A C T T T A sn Phe 1 1,760 C A A G A C Gln Asp 860 T T T T A G Phe Ar 7 T G C A C T Met His 1 555 2,070 T T A A A G | 1,660 GTGCTAC Ser Ala Thr 555 AAAATTT Lys Ile 1,870 AAAATCT g Lys Ser 1,970 TGGTTTA eu Val Tyr |
| PAN1 Sb ATCC MYA Frame 1 PAN1 Sb ATCC MYA Frame 1 | 1.57 A G T A T G C C C A Ser Met Pro A C A T G C C G T T G Met Pro Leu 1.770 C C A C G G A G G A Ser Thr Glu Glu 590 G G C T T A A A T C Gly Leu Asn A 625 1.980 C G G T A A A T T A Gly Lys Leu | A T T T G T C C A T T T G T C C ASN Leu Ser 1,680 A C T G C C C A Thr Ala Gir 560 1,780 A A A A T C T T Lys Ser L 595 G T G C A G A T Arg Ala Asp 630 1,990 A A C G G G A A Asn Gly Lys | 1,580 TTTAATC Phe Asn (AAAGACA Lys Thr 1,790 TGTTTTA eu Phe Ty 0 TTGGAGC Leu Glu (2,000 GCCAATC s Pro Ile | 1,590 A A C A A G G G In GIn GI 530 G G A T T T G G G A T T T G G G A T T T G 565 T A A A A T T r Lys II 600 A A A T T T G GIn II C C C A A T G Pro Asn C | ATTACAA y Leu Gln 1,700 GTAATAA Gly Asn As TTTGAAA Phe Glu 1,910 GAACCTT p Asn Leu 5 2,010 TCCTACC Val Leu Pr | 1,600 Ser GIN 535 CGAAAT 1,7 CGAAAT 1,810 CTTTTGA Thr Phe A CTGTGAT Cys Asp Cys Asp Cys Asp Cys Asp Cys Asp | 1,610 TTAACCG Leu Thr G TTATACC/ Tyr Thr 1,820 ATACTCA/ Sp Thr GIn 05 1,920 ATAAACA/ ILeu Asn A 640 TTTAATTC | 1,6 G T T G C A A ly Leu Gln 540 1,720 A A A T C C A A Lys Ser A A A C A A A C A Sn Lys 1,930 A C A C C G G C sn Thr Gly C C C T C C A C Pro Ser St | ACCCCAAC Pro Gln 1,730 ACTTTAA ACTTAA ACTTAA ACTTAA ACTTAA ACTTAA ACTTAA ACTTAA ACTTAA ACTTTAA ACTTTAA ACTTTAA ACTTTAA ACTTAA ACTTTAA ACTTAA | 1,630 C C A A C G G Pro Thr C F A A T A A C Asn Asn 1,840 T A G A T T C 20 Asp Se 1,940 A T A A A C Asn Lys C 20 C T T T T A Leu Leu | 1 GTTTTC Gly Phe L 545 TTAATT Leu Ile 580 CCCCAC r Pro Thi 615 1,950 AAGAAT GIN Glu P 650 GACAAC Asp Aşn | GAU TACCAC eu Pro F GATAAC Asp Asn 1,850 TGCTGT Ala Va TTGCAC he Ala L 2,060 TTAAAG Leu Lys | 1,650 CATCTA 'ro Ser A 550 TCAAGT Ser Ser 585 GGAGAT GGAGAT GGAGATA 620 1,960 TAGGTA eu Gly N 6 AACCAA Asn Gln | A C T T T A I,760 C A A G A C Gln Asp R60 T T T T A G Phe Ar T G C A C T Act His I S55 2,070 T T A A A G Leu Lys | 1,660 GTGCTAC Ser Ala Thr 555 AAAATTT Lys Ile 1,870 AAAATCT g Lys Ser 1,970 TGGTTTA eu Val Tyr 2,080 ACAGAGC Thr Glu |

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: log2-fold change)