

Supplementary Materials for

Cytotoxicity of Oleandrin Is Mediated by Calcium Influx and by Increased Manganese Uptake in *Saccharomyces cerevisiae* Cells

Table S1. Effect of oleandrin on the growth of various single-gene knockout mutants of *Saccharomyces cerevisiae*. Strains were inoculated (initial cell density 5×10^5 cells/mL) in SD media containing 100 ng/mL oleandrin (final concentration). Growth was assessed spectrophotometrically (OD₆₆₀) after 16 hours of incubation with shaking (200 rpm) at 30 °C and expressed (%) as the ration between cell density in the presence of oleandrin and cell density in the absence of oleandrin.

Strain	Gene Deleted	Gene Function	Growth in the Presence of Oleandrin (%)
Wild type ¹	None	Control strain	49.6 ± 2.8
<i>Genes involved in monovalent cation transport</i>			
<i>ena1Δ</i>	<i>ENA1</i>	P-type ATPase sodium pump; involved in Na ⁺ and Li ⁺ efflux.	51.2 ± 1.8
<i>nha1Δ</i>	<i>NHA1</i>	Na ⁺ /H ⁺ antiporter; involved in sodium and potassium efflux through the plasma membrane	54.6 ± 2.5
<i>tok1Δ</i>	<i>TOK1</i>	Outward-rectifier potassium channel of the plasma membrane	47.2 ± 3.4
<i>trk1Δ</i>	<i>TRK1</i>	High affinity K ⁺ transporter	53.1 ± 2.4
<i>trk2Δ</i>	<i>TRK2</i>	Trk1 paralog; K ⁺ transporter	51.6 ± 1.9
<i>Genes involved in calcium homeostasis</i>			
<i>cch1Δ</i>	<i>CCH1</i>	Voltage-gated high-affinity Ca ²⁺ channel involved in calcium influx in response to some stresses	78.8 ± 6.9 *
<i>mid1Δ</i>	<i>MID1</i>	Stretch-activated Ca ²⁺ -permeable cation channel; interacts with Cch1	89.6 ± 7.9 **
<i>pmc1Δ</i>	<i>PMC1</i>	Vacuolar Ca ²⁺ -ATPase involved in depleting cytosol of Ca ²⁺ ions.	21.6 ± 1.7 **
<i>vcx1Δ</i>	<i>VCX1</i>	Vacuolar membrane antiporter with Ca ²⁺ /H ⁺ and K ⁺ /H ⁺ exchange activity	43.6 ± 2.9
<i>yvc1Δ</i>	<i>YVC1</i>	Vacuolar cation channel; mediates release of Ca ²⁺ from the vacuole	57.6 ± 4.9
<i>pmr1Δ</i>	<i>PMR1</i>	High affinity Ca ²⁺ /Mn ²⁺ P-type ATPase; required for Ca ²⁺ and Mn ²⁺ transport into Golgi	16.8 ± 2.1
<i>Genes Involved in Heavy Metal Transport</i>			

<i>ctr1Δ</i>	<i>CTR1</i>	High-affinity copper transporter of plasma membrane	48.6 ± 3.4
<i>ftr1Δ</i>	<i>FTR1</i>	High-affinity iron transporter of the plasma membrane	49.2 ± 4.3
<i>fet4Δ</i>	<i>FET41</i>	Low-affinity iron transporter of the plasma membrane	54.6 ± 4.5
<i>smf1Δ</i>	<i>SMF1</i>	Divalent metal ion transporter	91.6 ± 4.9 ***
<i>pho84Δ</i>	<i>PHO84</i>	High-affinity inorganic phosphate (Pi) transporter and low-affinity divalent metal ion transporter	54.3 ± 3.7
<i>ztr1Δ</i>	<i>ZTR1</i>	High-affinity Zn ²⁺ transporter of the plasma membrane	53.6 ± 3.9
<i>ztr2Δ</i>	<i>ZTR2</i>	Low-affinity Zn ²⁺ transporter of the plasma membrane	54.5 ± 4.2
<i>Genes involved response to oxidative stress</i>			
<i>yap1Δ</i>	<i>YAP1</i>	Transcription factor; required for oxidative stress tolerance	41.6 ± 5.2
<i>skn7Δ</i>	<i>SKN7</i>	Transcription factor required for optimal induction of heat-shock genes in response to oxidative stress	43.8 ± 6.1
<i>msn2Δ</i>	<i>MSN2</i>	Stress-responsive transcriptional activator	50.6 ± 6.8
<i>sod1Δ</i>	<i>SOD1</i>	Cu,Zn-Superoxide dismutase	48.6 ± 4.2
<i>sod2Δ</i>	<i>SOD2</i>	Mn-Superoxide dismutase	47.3 ± 4.6
<i>ahp1Δ</i>	<i>AHP1</i>	Thioredoxin peroxidase with alkyl hydroperoxide reductase activity	28.3 ± 3.2 **
<i>ccp1Δ</i>	<i>CCP1</i>	Citochrome c peroxidase	48.6 ± 5.1
<i>cta1Δ</i>	<i>CTA1</i>	Catalase A	47.3 ± 4.8
<i>ctt1Δ</i>	<i>CTT1</i>	Calalase T	48.6 ± 5.2
<i>gpx1Δ</i>	<i>GPX1</i>	Phospholipid hydroperoxide glutathione peroxidase	513 ± 4.9
<i>gpx2Δ</i>	<i>GPX2</i>	Phospholipid hydroperoxide glutathione peroxidase	43.4 ± 4.1
<i>hyr1Δ</i>	<i>HYR1</i>	Thiol peroxidase that functions as a hydroperoxide receptor to sense intracellular hydroperoxide levels and transduce a redox signal to the Yap1p transcription factor	49.3 ± 5.7

<i>prx1Δ</i>	<i>PRX1</i>	Mitochondrial peroxiredoxin with thioredoxin peroxidase activity	44.6 ± 6.3
<i>trr1Δ</i>	<i>TRR1</i>	Cytoplasmic thioredoxin reductase	54.6 ± 7.5
<i>tsa1Δ</i>	<i>TSA1</i>	Thioredoxin peroxidase; acts as both ribosome-associated and free cytoplasmic antioxidant	48.3 ± 4.9
<i>tsa2Δ</i>	<i>TSA2</i>	Stress inducible cytoplasmic thioredoxin peroxidase	41.6 ± 6.2

¹ Wild type is strain BY4741 (*MATa*; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*). The isogenic knock-out mutants had the phenotype BY4741 *yfg*: *kanMX4* except for a null mutation of one single gene deletion. All strains were purchased from EUROSCARF (www.euroscarf.de).