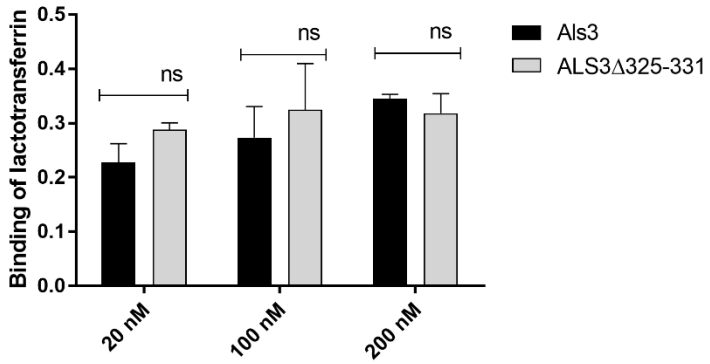
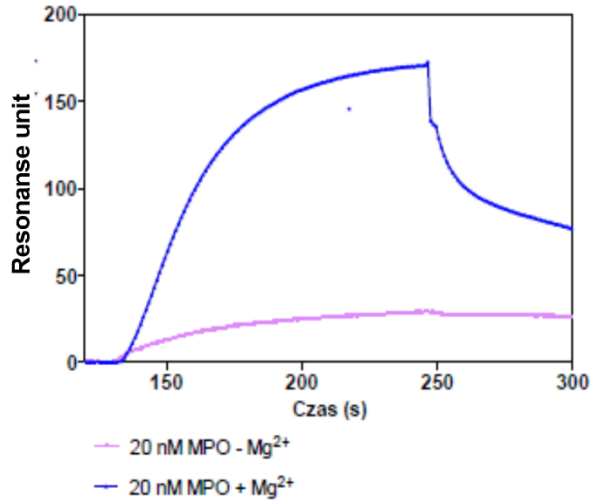
SUPPLEMENTARY MATERIALS

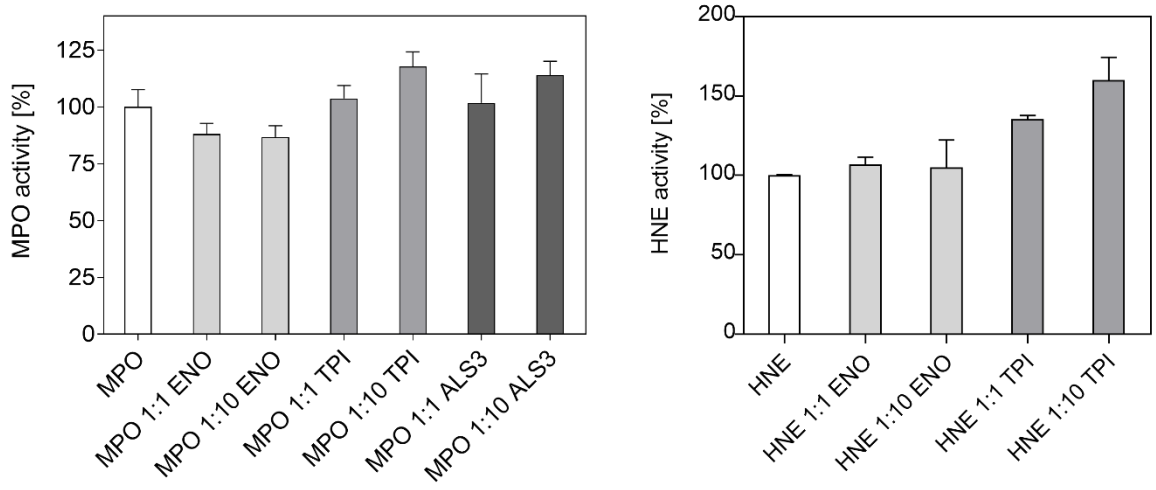
Figures:



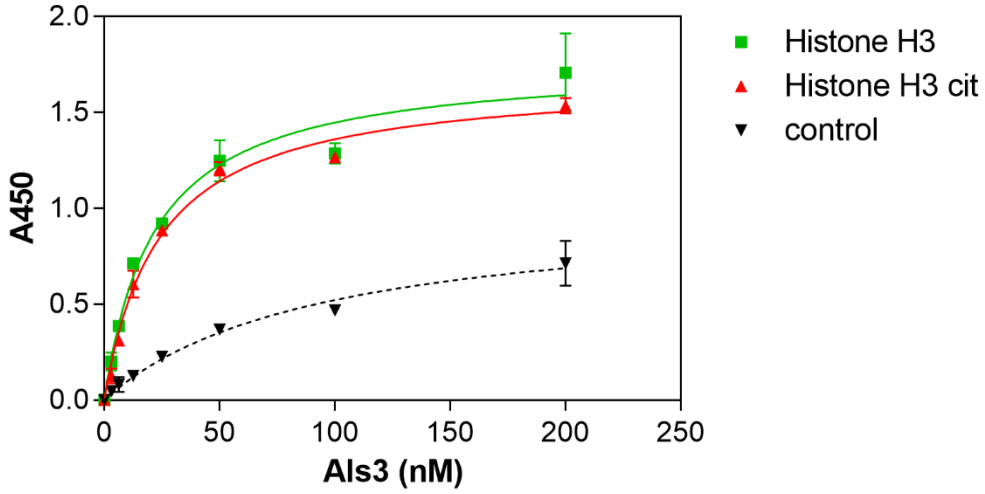
Supplementary Figure S1. Comparison of interaction properties between *S. cerevisiae* strains exposing Als3 and its mutated form Als3Δ325-331towards selected NET- composing protein – lactotransferrin.



Supplementary Figure S2. The influence of Mg++ on interaction properties of enolase and MPO – an example.



Supplementary Figure S3. The influence of formation of complex between selected fungal proteins and MPO or HNE on their enzymatic activities.



	Histone H3	Histone H3 cit	control
One site binding (hyperbola)			
Best-fit values			
Bmax	1.764	1.682	1.008
Kd	21.97	23.78	93.36
Std. Error			
Bmax	0.08213	0.05074	0.0955
Kd	3.323	2.282	18.76

Supplementary Figure S4. The comparable binding properties between Als3 and native or citrullinated histone H3.

Tables:

Supplementary Table S1. The list of the *Saccharomyces cerevisiae* strains used in this study.

Strain	Description	Reference
BY4742 UB2022	*MATα leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1*	(Brachmann et al., 1998)
UB2155	BY4742 with pBC542 (9.2 kb; ApR; pMB1 *ori*; *URA3*)	(Nobbs et al., 2010)
UB2157	BY4742 with pBC542-*ALS3lg*	(Nobbs et al., 2010)
UB2161	BY4742 with pBC542-*CWP1*	(Nobbs et al., 2010)
UB2316	BY4742 with pBC542-*ALS3Δ325-331*	(Bamford et al., 2015)

Brachmann CB, Davies A, Cost GJ, et al (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast Chichester Engl 14:115–132. https://doi.org/10.1002/(SICI)1097-0061(19980130).

Nobbs AH, Margaret Vickerman M, Jenkinson HF (2010) Heterologous expression of *Candida albicans* cell wall-associated adhesins in *Saccharomyces cerevisiae* reveals differential specificities in adherence and biofilm formation and in binding oral *Streptococcus gordonii*. Eukaryot Cell 9:1622–1634. https://doi.org/10.1128/EC.00103-10.Bamford CV, Nobbs AH, Barbour ME, et al (2015) Functional regions of *Candida albicans* hyphal cell wall protein Als3 that determine interaction with the oral bacterium *Streptococcus gordonii*. Microbiol Read Engl 161:18–29. https://doi.org/10.1099/mic.0.083378-0.

Supplementary Table S2. Mass spectrometric identification of human proteins associated to the fungal cell surface after interactions of *C. albicans* cells with isolated NETs.

To obtain the yeast-like form of *C. albicans*, cells were initially cultured at 30°C for 16 h in YPD medium, and to achieve filamentation $5x10^8$ cells were transferred to RPMI 1640 medium and further incubated at 37°C for 3 h. Then $5x10^8$ blastospores or cells in filamentous form wereincubatedat 37°Cfor 1 hinPBSbuffer with NETs isolated from $8x10^6$ neutrophils. After washing out of unbound proteins, cell surface shaving with trypsin was performed as described previously in Karkowska-Kuleta et al. 2019 andobtained peptideswere analyzed by LC-MS/MS with HCT Ultra ETD II mass spectrometer (Bruker, Bremen, Germany) or Q-Exactive mass spectrometer (Thermo Scientific, Waltham, MA) – the parameters obtained from the latter are in italics. The obtained lists of peaks were searched against the SwissProt protein database (SC – sequence coverage).

Accession Number	Protein Name	Score	Matches	SC [%]
Yeast-like Cells				
ACTB_HUMAN	Actin, cytoplasmic 1	252	15	37
		216	13	29
		430.50	*9*	*26.40*
ACTN1_HUMAN	Alpha-actinin-1	46	1	1
		55	1	1
		126.90	*4*	*4.82*
BPI_HUMAN	Bactericidal permeability-increasing protein	*199.66*	*2*	*4.31*
C5AR1_HUMAN	C5a anaphylatoxin chemotactic receptor 1	*35.40*	*1*	*2.86*
CAMP_HUMAN	Cathelicidin antimicrobial peptide	*90.85*	*3*	*13.53*

CATG_HUMAN	Cathepsin G	81	4	12
		98	4	12
		323.00	*5*	*19.61*
DEF1_HUMAN	Neutrophil defensin 1	*98.71*	*2*	*19.15*
ELNE_HUMAN	Neutrophil elastase	57	2	6
		96.48	*2*	*6.37*
GELS_HUMAN	Gelsolin	55	1	1
		67.67	*2*	*3.96*
H2AV_HUMAN	Histone H2A.V	89	3	20
		62	3	20
H2AZ_HUMAN	Histone H2A.Z	*70.87*	*2*	*12.50*
H2B1B_HUMAN	Histone H2B type 1-B	192	74	25
H2B1K_HUMAN	Histone H2B type 1-K	*472.69*	*5*	*40.48*
H2B1C_HUMAN	Histone H2B type 1-C/E/F/G/I	205	94	40
H31_HUMAN	Histone H3.1	*78.31*	*2*	*10.29*
H31T_HUMAN	Histone H3.1t	51	5	23
H33_HUMAN	Histone H3.3	41	6	24
H4_HUMAN	Histone H4	90	5	49
		103	4	29
		225.11	*4*	*40.78*
HBA_HUMAN	Hemoglobin subunit alpha	38	1	6
		38	1	6
		97.80	*1*	*10.56*
HBB_HUMAN	Hemoglobin subunit beta	68	3	19
		120	4	29
		231.34	*5*	*35.37*
KLH35_HUMAN	Kelch-like protein 35	35	2	1
LYSC_HUMAN	Lysozyme C	*74.36*	*1*	*8.11*
MNDA_HUMAN	Myeloid cell nuclear differentiation antigen	*105.30*	*2*	*5.90*
MRCKA_HUMAN	Serine/threonine-protein kinase MRCK alpha	44	1	0
MYH11_HUMAN	Myosin-11	48	2	0
MYH9_HUMAN	Myosin-9	47	4	3
		91.96	*3*	*2.24*
PERM_HUMAN	Myeloperoxidase	35	1	1
		361.88	*6*	*13.42*
PLSL_HUMAN	Plastin-2	*271.11*	*8*	*19.46*
PRTN3_HUMAN	Myeloblastin	96	4	17
		40	3	7
		214.97	*4*	*20.70*
RAP1A_HUMAN	Ras-related protein Rap-1A	79	3	12
RS27A_HUMAN	Ubiquitin-40S ribosomal protein S27a	41	1	10
		52	1	10

S10A6_HUMAN	Protein S100-A6	*34.86*	*1*	*8.89*
S10A8_HUMAN	Protein S100-A8	60	5	33
		64	4	26
		188.54	*4*	*32.26*
S10A9_HUMAN	Protein S100-A9	127	6	37
		113	6	50
		474.74	*6*	*56.14*
S10AC_HUMAN	Protein S100-A12	*95.71*	*3*	*38.04*
TRFL_HUMAN	Lactotransferrin	*68.13*	*2*	*2.68*
VIME_HUMAN	Vimentin	104	6	16
		152	6	12
		184.43	*5*	*12.23*
Hyphae				
ACTB_HUMAN	Actin, cytoplasmic 1	191	11	28
		312	13	29
		2927,79	*7*	*56.80*
ACTN1_HUMAN	Alpha-actinin-1	132	4	4
		72	2	3
		2057.64	*21*	*41.14*
BPI_HUMAN	Bactericidal permeability-increasing protein	*276.32*	*4*	*12.53*
C5AR1_HUMAN	C5a anaphylatoxin chemotactic receptor 1	49	1	2
CAMP_HUMAN	Cathelicidin antimicrobial peptide	38	2	10
		65.59	*2*	*10*
CATA_HUMAN	Catalase	*716.49*	*14*	*38.71*
CATG_HUMAN	Cathepsin G	122	5	12
		93	4	16
		96.12	*2*	*8.24*
DEF1_HUMAN	Neutrophil defensin 1	*119.15*	*3*	*52.13*
ELNE_HUMAN	Neutrophil elastase	75	2	6
		80	2	6
		84.05	*1*	*3.75*
GELS_HUMAN	Gelsolin	*1436.32*	*22*	*45.01*
H2A1A_HUMAN	Histone H2A type 1-A	56	3	17
H2AY_HUMAN	Core histone macro-H2A.1	*427.83*	*6*	*25.81*
H2AJ_HUMAN	Histone H2A,J	74	4	26
H2AZ_HUMAN	Histone H2A.Z	*258.28*	*2*	*31.25*
H2B1B_HUMAN	Histone H2B type 1-B	271	10	39
		332	8	33
H2B1J_HUMAN	Histone H2B type 1-J	*2578.11*	*7*	*51.59*
H33_HUMAN	Histone H3,3	47	5	19
		51	7	24
		121.08	*3*	*14.07*

H4_HUMAN	Histone H4	93	3	29
		88	3	29
		86.41	*3*	*31.07*
HBA_HUMAN	Hemoglobin subunit alpha	*386.01*	*7*	*66.90*
HBB_HUMAN	Hemoglobin subunit beta	113	3	21
		612.40	*8*	*60.54*
KLH35_HUMAN	Kelch-like protein 35	35	1	1
		35	1	1
LYSC_HUMAN	Lysozyme C	65	3	16
		65.66	*1*	*8.11*
MNDA_HUMAN	Myeloid cell nuclear differentiation antigen	*285.63*	*8*	*28.75*
MYH9_HUMAN	Myosin-9	41	1	0
		4415.28	*41*	*36.38*
PERM_HUMAN	Myeloperoxidase	*50.96*	*1*	*1.34*
PLSL_HUMAN	Plastin-2	*1947.91*	*29*	*58.69*
PROF1_HUMAN	Profilin-1	71	2	20
		874.17	*6*	*57.14*
PRTN3_HUMAN	Myeloblastin	71	1	9
		74	3	17
		213.36	*4*	*20.70*
RAP1B_HUMAN	Ras-related protein Rap-1b	150	4	20
		128	3	20
RL40_HUMAN	Ubiquitin-60S ribosomal protein L40	52	2	19
S10A4_HUMAN	Protein S100-A4	*129.15*	*2*	*18.81*
S10A6_HUMAN	Protein S100-A6	42	1	8
		86.13	*3*	*24.44*
S10A8_HUMAN	Protein S100-A8	42	5	33
		66	5	32
		2792.07	*15*	*97.85*
S10A9_HUMAN	Protein S100-A9	195	8	52
		197	9	67
		16150.64	*10*	*86.84*
S10AB_HUMAN	Protein S100-A11	*244.12*	*4*	*39.05*
S10AC_HUMAN	Protein S100-A12	*454.72*	*5*	*72.83*
STOM_HUMAN	Erythrocyte band 7 integral membrane protein	81	2	8
TRFL_HUMAN	Lactotransferrin	*57.28*	*1*	*1.41*
VIME_HUMAN	Vimentin	144	6	12
		206	9	16
		1322.36	*28*	*61.37*

Karkowska-Kuleta J, Satala D, Bochenska O, et al (2019) Moonlighting proteins are variably exposed at the cell surfaces of *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* under certain growth conditions. BMC Microbiol 19:149. https://doi.org/10.1186/s12866-019-1524-5.

Supplementary Table S3. Mass spectrometry identification of *C. albicans* cell wall-associated proteins binding particular NET proteins.

Chemical cross-linking was performed using trifunctional reagent sulfo-SBED (sulfo-N-hydroxysuccinimidyl-2-(6-[biotinamido]-2-(p-azido benzamido)-hexanoamido) ethyl-1,3'-dithioproprionate)(Thermo Fisher Scientific Inc., Woltham, MA, USA). Samples containing 50 µg of each NET-composing proteins in 20 mM HEPES buffer (pH 7.8) were treated with the 50-fold molar excess of cross-linker in the dark at 4 °C for 2 h. The samples were dialyzed against PBS at 4°C overnight in the dark to remove the unbound cross-linker. The particular labeled NET protein was incubated with a mixture of fungal CWP (300 µg in 300 µl PBS) at 37°C for 1 h in the dark. Then samples were placed on ice and exposed to UV radiation (365 nm) for 15 min. Covalently linked pairs of biotinylated proteins were adsorbed on MagnaBind Streptavidin Beads (Thermo Fisher Scientific Inc.). After 1 h of incubation with intense stirring, beads were washed five times with 1 ml of PBS to remove unbound proteins, followed by 1 h incubation with 40 µl of 50 mM DTT at 60°C. Samples were again washed 5 times with 1 ml of PBS. The fungal proteins were removed from the beads by boiling in 30 µl 2.5% β-mercaptoethanol and 2% SDS for 30 min. The released proteins were separated by SDS-PAGE, and protein bands were visualized by staining with Coomassie Brilliant Blue R-250 and analyzed by mass spectrometry as described in details in Seweryn et al. 2015.

NET Protein	*C. albicans* Protein	MW (kDa)	Score	Identified Peptides	SC (%)
MPO	Eft2	93.9	594	16	29
	Eno1	47.2	510	7	27
	Tpi1	26.9	386	8	56
LF	Eft2	93.9	302	10	17
	Eno1	47.2	237	5	15
	Gpm1	27.4	212	11	54
	Tpi1	26.9	113	4	23
H3.1	Eno1	47.2	444	9	24
	Eft2	93.9	385	10	13
	Gpm1	27.4	311	11	50
	Tpi1	26.9	112	4	17
H2A	Eno1	47.2	410	10	29
	Tpi1	26.9	196	8	38
	Gpm1	27.4	163	7	37
H4	Eno1	47.2	583	9	26
	Tpi1	26.9	271	11	56
	Gpm1	27.4	236	6	28
	Eft2	93.9	104	2	3
HNE	Eno1	47.2	544	10	29
	Tpi1	26.9	220	9	46

MW – molecular weight; SC – sequence coverage

Seweryn K, Karkowska-Kuleta J, Wolak N, et al (2015) Kinetic and thermodynamic characterization of the interactions between the components of human plasma kinin-forming system and isolated and purified cell wall proteins of *Candida albicans*. Acta Biochim Pol 62:825–35. https://doi.org/10.18388/abp.2015_1142.

Supplementary Table S4. Mass spectrometric identification of fungal cell wall-associated proteins present at the fungal cell surface during the interactions of *C. albicans* with neutrophils.

C. albicans cells ($4x10^9$), after washing with PBS were mixed with $4x10^7$ of human neutrophils suspended in 5 ml of fresh RPMI 1640 medium and co-incubated with gentle stirring for 4 h in a humidified 5% CO_2 atmosphere at 37˚C. Fungal cells incubated under the same conditions but without neutrophils served as a control. After incubation, human and fungal cells were collected by centrifugation at 2000 rpm for 3 min at 4˚C, re-suspended in 5 ml of PBS buffer with calcium and magnesium ions and the 750 µl of the solution of microccocal nuclease (MNase) (Roche, Basel, Switzerland) was added at concentration of 1U/ml for 15 min at 37°C.

After removing of the supernatant, 5 ml of PBS has been added to cells with 1 mM phenylmethanesulfonyl fluoride (PMSF) (Sigma). The human cells have been broken by drawing them up to 20 times in a syringe (21 gauge) and their remnants were removed from *C. albicans* cells suspension by extensive washing with 1 ml of distilled water containing 1 mM PMSF and centrifugation at 3 000 rpm for 5 min. Washed fungal cells were frozen at -80°C and after that transferred to the new vials with 0.5 ml of distilled water with 1 mM PMSF and disrupted with glass beads (diameter 0.5 mm) (Sigma) in Precellys Evolution homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) with 20 sec pulse, 6 300 rpm, repeated four times. The obtained cell wall fraction was harvested by centrifugation at 5 000 rpm for 3 minutes, washed two times with 0.5 ml of ice-cold distilled water with 1 mM PMSF and then four times with 0.5 ml of each of the following ice-cold solutions containing 1 mM PMSF: 5% NaCl, 3% NaCl, 1% NaCl and finally again with 0.5 ml of cold distilled water in order to remove cytoplasmic contaminants from the cell walls preparation. *C. albicans* cell wall proteins were extracted by boiling the cell walls in 150 µl of the solution of 2% SDS and 2% 2-mercaptoethanol in water for 0.5 h. Then, obtained protein sample was extensively dialyzed against water at 4˚C. To qualitative assessment of proteins present at the fungal cell surface the SDS-PAGE electrophoresis was performed followed by staining of the proteins with Coomassie Brilliant Blue R-250 and excision of all noticeable protein bands. The proteins were then in-gel digested with trypsin and identified with LC-MS/MS (Seweryn et al. 2015). The obtained lists of peaks were searched against the SwissProt protein database restricted for Fungi. Proteins detected in particular experimental conditions were indicated as "+".

Accession Number	Protein Name	*C. albicans* with Neutrophils	*C. albicans*
1433_CANAL	14-3-3 protein homolog Bmh1	+	+
ACEA_CANAX	Isocitrate lyase Icl1	+	+
ACON_CANAL	Aconitate hydratase, mitochondrial Aco1	+	+
ACS1_CANAX	Acetyl-coenzyme A synthetase 1 Acs1	+	+
ACS2_CANAL	Acetyl-coenzyme A synthetase 2 Acs2		+
ACT_CANAX	Actin Act1	+	+
ADH1_CANAX	Alcohol dehydrogenase 1 Adh1	+	+
ADH2_CANAL	Alcohol dehydrogenase 2 Adh2	+	+
ALF_CANAL	Fructose-bisphosphate aldolase Fba1	+	+
ALS1_CANAL	Agglutinin-like protein 1 Als1		+
ALS3_CANAL	Agglutinin-like protein 3 Als3	+	+
BGL2_CANAL	Glucan 1,3-beta-glucosidase Bgl2	+	+

CHI2_CANAL	Chitinase 2 Cht2	+	+
EF1A1_CANAL	Elongation factor 1-alpha 1 Tef1	+	+
EF2_CANAL	Elongation factor 2 Eft2	+	+
ENO1_CANAL	Enolase 1 Eno1	+	+
G3P_CANAW	Glyceraldehyde-3-phosphate dehydrogenase Tdh1	+	+
GBLP_CANAL	Guanine nucleotide-binding protein subunit beta-like protein Asc1	+	+
HSP60_CANAL	Heat shock protein 60, mitochondrial Hsp60	+	+
HSP71_CANAL	Heat shock protein Ssa1	+	+
HSP75_CANAW	Heat shock protein Ssb1	+	+
HSP90_CANAL	Heat shock protein 90 homolog Hsp90	+	+
IF4A_CANAL	ATP-dependent RNA helicase eIF4A Tif1		+
IPYR_CANAL	Inorganic pyrophosphatase Ipp1		+
KAD2_CANAL	Adenylate kinase Adk1	+	+
MCR1_CANAL	NADH-cytochrome b5 reductase 2 Mcr1	+	+
MDHC_CANAL	Malate dehydrogenase, cytoplasmic Mdh1	+	+
MIC60_CANAL	MICOS complex subunit MIC60 Mic60	+	
NCE2_CANAL	Non-classical export protein 102 Nce102		+
PCKA_CANAX	Phosphoenolpyruvate carboxykinase [ATP] Pck1	+	+
PDC1_CANAL	Pyruvate decarboxylase Pdc11	+	+
PGK_CANAL	Phosphoglycerate kinase Pgk1	+	+
PHR1_CANAL	pH-responsive protein 1 Phr1	+	
PMA1_CANAX	Plasma membrane ATPase 1 Pma1	+	+
PMGY_CANAL	Phosphoglycerate mutase Gpm1	+	+
PRA1_CANAL	pH-regulated antigen Pra1	+	
QCR2_CANAL	Cytochrome b-c1 complex subunit 2, mitochondrial Qcr2		+
RL10A_CANAL	60S ribosomal protein L10a Rpl10A		+
RS3A_CANAL	40S ribosomal protein S1 Rps1	+	+
RS4_CANAX	40S ribosomal protein S4 Rps4	+	+
SODM_CANAX	Superoxide dismutase [Mn], mitochondrial Sod2	+	+
SUR7_CANAL	Protein SUR7 Sur7	+	+
TPIS_CANAL	Triosephosphate isomerase Tpi1	+	+
TSA1_CANAL	Peroxiredoxin Tsa1	+	+
VDAC_CANAL	Mitochondrial outer membrane protein porin Por1	+	+
YWP1_CANAL	Yeast-form wall Protein 1 Ywp1	+	+

Seweryn K, Karkowska-Kuleta J, Wolak N, et al (2015) Kinetic and thermodynamic characterization of the interactions between the components of human plasma kinin-forming system and isolated and purified cell wall proteins of *Candida albicans*. Acta Biochim Pol 62:825–35. https://doi.org/10.18388/abp.2015_1142.