

SUPPLEMENTAL MATERIAL

Table 1. Identification of Med8-associated Proteins by Tandem Mass Spectrometry

ORF	Peptides identified by mass spectrometry^a	~Calculated M_r (kDa)	Potential yeast homolog
TRAP230	KGTAETDQLAPIVPLNPGDLTFLGGEDGQK WAPEFMIDTLENPAAHTFTYTGLGK	250	Srb9 ^b
TRAP220	LSSSDSIGPDVTDILSDIAEEASK DNPAQDFSTLYGSSPLER GLSDALICTDDFIK HQVAYNTLIGSCVK	170	Med1 ^b
TRAP80	LSGPQAFDKNEINSIQSTEGLLER SAGSLFPHHGTFEVIK QAPDIGDLGTVNLFK FQPSLWPWDSVR LEAAQNVLLCK	73	Srb4 ^b
Cdk8	TSNPYHHDQLDR	53	Srb10 ^b
Cyclin C	SIDPVLMAPTCVFLASK	36	Srb11 ^b
TRAP37	AQPTTLVLPPQYVDDVISR TPLYSQLQAYK TLEAFHDTCRQ SFMTWLR	31	
Med8	TKPDPEVEEQEKQLTTDAAR QTFNPADTNALVA AVAFGK VPVFSHEVVPDHLR VLKHEKTPLFR	29	Med8 ^b
Med6	EAEPLPETVKSEEKESAK QRVDALLIDLR RKEEPSSIFQR	28	Med6 ^b
Med7	VIEMIQNCLASLPDDLPHSEAGMR LHPMQFDHKK	27	Med7 ^b
LCMR1	SLIEKPPILGGSFNPITGTMLSGFR STAGSGPFYLMR LHTGPLPEQCR	26	Rox3 ^c
p28b	GLCDNMEPETFLDHEMVFLK NCVDIATSENLTDFLMEMGFR NFAEQLKPLVHLEK YLGQPEMGDKNR GQQASPFVLR AGAPWHLR	24	Srb5 ^b

FLJ23445	LLIDGDGAGDDRRINLLVK WCNSGSQEEGYSQYQR MLSTLSQCEFSMGK IYKEIECSIAGAHEK TLLVYDMNLR	24	
TRFP	VGTVTMGPSAR SVQQTVELLTK QQQVPVAGIR YQYCDFLVK	23	Srb2 ^b
TRAP25	CNENCGGMDPIPVEQLIPYVDEDGSKNDDR NLIWDINAMLAMRN IGQETVQDIVYR TMEIFQLLR	20	Med11 ^b
AK007855	KPADMPQGSFLAFLEQASANIPAPLKQT LQLSVQKPDQVIKEDVSELR LRHWQQVLEDINVQHK FLDIAR	19	
Surf5	ATQGEQDNYEMHVR ETLLQSYNKR IEDETQVSR	16	Srb6 ^b
Soh1	LQQALAEQQQNTTAGK FIDEQQILHWQHYSR ELVNAQCAK	16	Soh1 ^b
Nut2	QQLHDITVPLEVFEYIDQGR QLGHIVSDFQPSSQAGLSQK LNFIVTGLQDIDKCR SIRGEDHPPS SLLIQELSK	16	Nut2 ^b
HSPC296	QAAAFTASVQHVEAELSAQIR YLTQVATGQPHEGSSYSSR LKLSDVAR	13	Med11 ^c

- a. The most highly enriched Med8-containing fractions from MonoQ chromatography (Brower, C.S., Sato, S., Tomomori-Sato, C., Kamura, T., Pause, A., Stearman, R., Klausner, R.D., Malik, S., Lane, W.S., Sorokina, I., Roeder, R.G., Conaway, J.W., Conaway, R.C. (2002) Proc. Natl. Acad. Sci. USA 99, 10353-10358) were fractionated by 1-dimensional SDS polyacrylamide gel electrophoresis in a 4 to 15% gradient gel. The gel was sectioned into uniformly sized slices. Proteins present in gel slices were subjected to in-gel reduction, *S*-carboxyamidomethylation, and tryptic digestion. Peptide sequences were determined by microcapillary reversed-phase HPLC coupled to the electrospray ionization source of a quadrupole ion trap mass spectrometer (Finnigan LCQ DECA XP, San Jose, CA). Identification of proteins present in the Med8-containing fractions was facilitated by the algorithm SEQUEST (Eng, J., McCormack, A.L., Yates, J.R., III (1994) J. Am. Soc. Mass Spectrom. 5, 976-989) and by programs developed in the Harvard Microchemistry and Proteomics Analysis Facility (Chittum, H.S., Lane, W.S., Carson, B.A., Roller, P.P., Lung, F.D., Lee, B.J., Hatfield, D.L. (1998) Biochemistry 37, 10866-10870).
- b. Boube, M., Joulia, L., Cribbs, D.L., Bourbon, H.-M. (2002) Cell 110, 143-151 and references therein.
- c. This study.

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Fig. 1. Multiple sequence alignments of LCMR1 and HSPC296 with yeast Rox3 and Med11.

A

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mouse (63) GPFYLMRELPGSTELTGSTNLIITHYNLEQAYNKFCG-----KKVKEKLSNFLPDLPGMIDLPGS-----HDNSSLRSLIEKPPI
human (80) GPFYLMRELPGSTELTGSTNLIITHYNLEQAYNKFCG-----KKVKEKLSNFLPDLPGMIDLPGS-----HDNSSLRSLIEKPPI
Dros (50) GPFYSMKEPPAKAELTGDKDLMTEYGLHHTLTKFKE-----KFKESLASFLQNIPIGINDLITHP-----VENSTLRSLIEKPPI
Cele (21) PFYTLKALLPPYSEIQGNHDLMSYELGPVEGGFSGS-----RRVKEKISSFLPHIIGEFHLDAT-----KEASSLRSLIEKPPI
Calbicans (1) YCFTPSIDIYQSPKPTPKDNLIKLYGLIPVTKSLARTNPDGSKGVKLRKSYKNHIQDLPGKHQISPAKP----IPPGLDPLIEQHPD
Spombe (7) YHYVGSVD-YQPTRPSAHQNLIELYGLTELAKVGRVDEFGNK-RKMRRSYKAYIQDLPGYNEILLRDN-----IKQWLTNPIREVPPI
Klactis (49) YYVDPVSLPVYEQQPFPVDDLIITTYGLEEVARQVARTNADGTVKAVKLRKSYKNQIQDLSGRFITIPSPRENG••NNPDMNQAKLVEGM
Scerev (14) YYYVDPETTYTYQQPNPLQDLISVYGLDDISRQVARTNLDGTVKAVKLRKSYKNQIQADLSGKFKSTIPPRENG••NPDMMIQPPQQGNM
Consensus (80) Y F ELY STEPTG NLITLYGLE V KKFART G K KKLKESYKNFIQDLPG DLI S DNSSLRSLIEKPPI

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B

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mouse (1) MATYSLANERLRALEDIEREIGAILQAGTAILELSKEKTNER---LLDRQAAAFVTSVQHVEAEIISAQ
human (1) MATYSLANERLRALEDIEREIGAILQAGTVILELSKEKTNER---LLDRQAAAFVTSVQHVEAEIISAQ
Stropicalis (1) MATYGMANERLRILEEIEREIAAILLNAGNVILELSKEKPNER---VLDKQATQFTASVQRVESEISGQ
Xlaevis (1) MATFGMANERLRALEEIEREIAAILLNAGNAILELSKEKPNER---MLDKQAAQFTASVQRVESEISGQ
Dros (1) ---MNPLDKIHALDEIEKEIILCMQSAQALQELGKEKSSQK---NAETQSQQFLKSLSSVESKISEQ
Cele (4) NPSDPVLTDRIQAIIVTTEKSIDEMMKCAREI IQDLGKEKQIGKN---KMEDNANNFKKLIITQVENEIISAQ
Calbicans (7) DKTENFIQERLDSLHEIDCKVVTLLDQFSSIFQSFYTK--SKE---DFSQQTSKIYSTLSKVAIDLIRKE
Sexigus (1) -MQPEYVKERLASLDEIDMKLCGMLQEASQVVFHAFSEVKSNGDAARPOFTKHVQGFYADLEIATVRLRNE
Kthermo (1) MPQPEFIQERLESLNAVNDQLLSTLHASQAVGTIEELKRGNEENMKSQFENHIRSFYGSLEEATVALRRE
Scerev (16) TMQPPYIQERLKS LNDIETQLCSMLQEASQVTFIFGELKRGNESVVKQFENHVKQFYERLDKSTTQLRKE
Consensus (16) M T LINERLRALEEIERE IAILQAGQVI ELSKEK R LDKQA QF ASL VESELSAQ

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Multiple sequence alignments were generated using the AlignX program of the Vector NTI Suite and edited manually to optimize the alignments. Panel A, LCMR1 and Rox3 alignments. LCMR1 accession numbers are AK010552, mouse; XP_058479, human; CG5546, *Drosophila melanogaster* (Dros); NP_497587, *Caenorhabditis elegans* (Cele). Rox 3 accession numbers are T40987, *Schizosaccharomyces pombe* (Spombe); AL426549, *Kluyveromyces lactis* (Klactis); S45409, *Saccharomyces cerevisiae* (Scerev). The sequence of *Candida albicans* (Calbicans) Rox3 is from the MEDB database and is available at <http://bio.lundberg.gu.se/medb/>. Panel B, HSPC296 and Med11 alignments. HSPC296 accession numbers are BAB25497, mouse; AAF28974, human; AL657682, *Silurana tropicalis* (Stropicalis); BJ03186, *Xenopus laevis* (Xlaevis); CG884, *Drosophila melanogaster*; NP_498066, *Caenorhabditis elegans*; AL407638, *Saccharomyces exiguus* (Sexigus); AL420292, *Kluyveromyces thermotolerans* (Kthermo); NP_013830, *Saccharomyces cerevisiae*. The sequence of *Candida albicans* Med11 is from the MEDB database.