

Supplemental Figure 1

Sce MSITNGTSRSVSAMGHPAVERYTPGHIVCVGTHKVEVVNYLAEGGFAQIYVVKFLEYLNEFDNTASV-PLKIGDVAACLKRVLVQDENGLNEMRNEVEVMKKLKGAPNIVQYFD
Spa MSITNGTSRSVSAMGHPAVERYTPGHIVCVGTHKVEVVNYLAEGGFAQIYVVKFLEYLNEFDNTASV-PLKIGDVAACLKRVLVQDENGLNEMRNEVEVMKKLKGAPNIVQYFD
Sku MSITNGTSRSVSAMGHPAVERYTPGDIVCVGTHKVEIVNYLAEGGFAQIYVVKFLEYLNEFDNAASV-PLKIGDLACLKRVLVHIDENGLNEMRNEVEVMKKLKGAPNIVQYFD
Smi MSITNSTSRVSAMGHPAVERYTPGHIVCVGTHKVEIVNYLAEGGFAQIYVVKFLEYLNEFDNAASV-PLKIGDVAACLKRVLVQDENGLNEMRNEVEVMKKLKGAPNIVQYFD
Sba MSITNGTSRSVSAMGHPAVERYTPGKIVCVGTHKVEIVNYLAEGGFAQIYAVVKFLEYLNEFDNTASL-PLKIGDVAACLKRVLVHIDENGLNEMRNEVEVMKKLKGAPNIVQYFD
Sar MSITNGTSRSVSAMGHPAVERYTPGHIVCVGAHKVEVVNYLAEGGFAQIYVVKFLEYLNEFDNTASV-PLKIGDVAACLKRVLVHIDENGLNEMRNEVEVMKKLKGAPNIVQYFD
Sca MSTTSGPVSNNNTTVKPNNERYPGTQVAVGAHKVEIIKYIAEGGFAQIYAVKFIIEFLNEFENNRMKPKLQMGDVAACLKRVLVQDENGLNEMRNEVEVMKQLQGAPNIVQYFD
Cgl MERPN--SRSMTSLNSPNEEKYPNGOMISVGAHRVEIVSYLAEGGFAQIYVVKFVEYLNEFESLGSKSAITVGDIAACLKRVIVNDEMGLNEMRNEVEVMKKLKSSPNIVQYFD
Ago MSKRH (11) PGTGALTGPLEMLQAGSTVLVGVHQUEVIEYLAEGGFAHIYKVSFVGYTNELD-RQDR-ILQPGDTVCLKRVRVSDENGLNELRNEVEVMKKLRNCSNIVQYFD
Sk1 MANS (14) SASSASPTLEKLPGLIIVGSHKVEIVKYLAEGGFAHIYVVKFVEFSNELE-TPSS-SLKEGDLACLKRVLVTDENGLNELRNEVEVMKQLKNSDNIVQYFD
Kwa M-ITG (54) SSRSGTLPVLEQLKTGSQVIVGNHRVEIVKYLAEGGFAHIYVVRFIEYANELEQVPTI-KLEVGDIAACLKRVLVTDENGLNEMRNEVSMKQLSGCPNIVQYFD
Kla MS (12) HTAATSAM-IPNSHLLSPNTQVVVGTHRCEILEHLAEGGFANIYKVKFLELTNEMDAGIDSKLLKAGDIAACLKRVIVPDEENGLNELRNEVEVMKQLRGSPNIVQYFD

Sce SNASRRRDGVQGFVLLLMELCPNKSLLDYMNQRLSTKLTEAEIVKIMYDVALSISQMHYLPVSLIHRDIKIENVLVDAKNNFKLADFGSTSTCFPIVTTTHQDIALLTQNIYV
Spa SNASRRRDGVQGFVLLLMELCPNKSLLDYMNQRLSTKLTESEIVKIMYDVALSISQMHYLPVPLIHRDIKIENVLVDAKNNFKLADFGSTSTCFPIVTTTHQDIALLTQNIYV
Sku SNASRRRDGVQGFVLLLMELCPNKSLLDYMNQRLSTKLTESEIVKIMYDVVLSISQMHYLPVPLIHRDIKIENVLVDAKNNFKLADFGSTSTCFPIVTTTHQDIALLTQNIYV
Smi SNASRRRDGVQGFVLLLMELCPNKSLLDYMNQRLSTKLTESEIVKIMYDVVLSISQMHYLPVPLIHRDIKIENVLVDAKNNFKLADFGSTSTCFPIVTTTHQDIALLTQNIYV
Sba SNASRRRDGVQGFVLLLMELCPNKSLLDYMNQRLSTKLSESEIVKIMYDVALSISEMHYLPVPLIHRDIKIENVLVDAENNFKLADFGSTSTCFPIVTTTHQDIALLTQNIYV
Sar SNASRRRDGVQGFVLLLMELCPNKSLLDYMNQRLSTKLTESEIVKIMYDVALSISEMHYLPVPLIHRDIKIENVLVDGENNFKLADFGSTSTCFPIVTTTHQDIALLTQNIYV
Sca SNASRRHNGFPGFVLLLMELCPNKSLLDYMNQRLATKLTEKEILKIMYDVYAVSOMHYLPTPLIHRDIKIENVLVDAQNNFKLADFGSTSTCFPIVTTTHQDIAVLTQNIYV
Cgl SNASRRIDGKPGGFVLLLMELCPNKSLLDYMNQRLKTKLSESEILKIMYDVSIKSNMHYLDQPLIHRDIKIENVLVDAKNNFKLADFGSTSTCFPIVTTTHQDIAVLTQNIYV
Ago SNASRLGDGKPGGFVLLLMELCPNKSLLDYMNQRLATKLTSEAEVLKIMYDITVGLSHMHYQRTPLIHRDIKIENVLVDAKNNFKLADFGSTSTCFPIVTTTHQDIAVLTQNIYV
Sk1 SNASRRRDGSPGYEVLLLMELCPNKSLLDYMNQRLATKLTSEKEVLKIMYDVTKAVAQMHFLPTPLIHRDIKIENVLVDSENNFKLADFGSTSTCFPIVTTTHQDIALLTQNIYV
Kwa SHASRRDGSSEGFVLLLMELCPNKSLLDYMNQRLATKLTSEQEILKIMYDVTTRALAQMHYLPVPLIHRDVKIENVLVDAKNNFKLADFGSTSTCFPIVTTTHQDIAVLTQNIYV
Kla SNASRHPDGSSEGFVLLLMELCPNKSLLDYMNQRLATKLTTEAEILKIMYDVSNATIAQMHYLPVPLIHRDIKIENVLVDKDDNFKLADFGSTSTCFPIVTTTHQDIAVLTQNIYV

Sce HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYAEGAYNF
Spa HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYTEGAYNF
Sku HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYAEGAYNF
Smi HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYTEGAYNF
Sba HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYSEGAYDF
Sar HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYVEGAYNF
Sca HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYAEGPYDF
Cgl HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYGLGYPNF
Ago HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYGLGYPNF
Sk1 HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYGLGYPNF
Kwa HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYGLGYPNF
Kla HTTPQYRSPEMIDLRYRCLPINEKSDI WALGIFLYKLLFFTTTPFEMTGQFMAILHSHKYEFVVKYSSKLINLI IIMLAENPNLRPNYQVLYHLC EILNVEVP IEDKYGLGYPNF


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Sce  NDSPAPNSHHSYRVSPHASTAITENKRHSTGHELSTRSNKGKETHRTGSKORHDLERYRHSKDKDSNSSITISTSTPSEMRKSFARARQSLDDLERVREAMAS--SASSSGGS
Spa  NDSSAPOPPHHSYRVPPHASTAISENKRHSTGHELSTRSSKGKETHRTGSKORHDLERYRQSKDKDSNSSITISTSTPSEMRKSFARARQSLDDLERVREAMAS--NGSNSGGS
Sku  NDSSAPOSHHSYRVPPHTSTAISENKRHSTGHDSSTRLSAKHEAHRTSSKPRHDLERYRQSKDRDSNSSITISTSTPSEMRKSFARARQSLDDLERVRETMAS--NGTSSSGGS
Smi  NESLAPOSHHSYRAPSHASTAISENKRHSTGHELSTRSTGKQDTHRTSSKORHDLERYRHSKDKDSNSSITISTSNSSEMRKSFARARQSLDDLERVREAMAS--NGSSSGGS
Sba  TGSPAPOSHHSYRPPHSTTISENKRHSTGHE-STRSSKGKEMHRTNSKPRHDLERYRQSKDRDSNSSITISTSTPSEMRKSFARARQSLDDLERIRREATTANNGS----GS
Sar  NDSQASQSHHSYRGPPHSTTIMTENKRHSTGHE-STRSSKGKETHRTNSKPRHDLERYRQSKDRDSNSSITISTSTPSEMRKSFARARQSLDDLERVREAMASSG--SGSGSS
Sca  STSVHHISSIRE ( 19 )OROQPRHHQHTFDSSSNLNSKGANRSSSRGNIRGKODLESYKHSTKNNSSSIPISTTNTNEMKKSFAKARQSLDDLERVREALL-----NSD
Cgl  NTHRPGIKGMS ( 20 )QSSKVISOSTNARISLDRQRORHIEPRDSKSRSRS-HGLEEY-TSSNG-SNSSINISTSNKFEMKRSFAKARQSLDDLERARRDAM----SRSNSGHE
Ago  -----PVSASKTSSKAHLQPNRSGTANCGTSN-----SSSVVSGVRKSFHRGRKSVDLDVSKE-----SKEPTNSG--
Skl  -----PKESSQRHQHKESSORHQHKKI-----PSS-SEMRKSLSRARKSLDLEGVKRE-----SAGGSD-T
Kwa  -----PDIKLQTKTKVSHQOR-----KSNVAAEPPKSFSRARKSLDLERTKRD-----TPSNGENT
Kla  -----KTSF-----SRTSVRRSVEMERMKHDSNSTSNSNARDETK

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Sce  NGKRRSFFSVFRSEK.
Spa  NGKRRSFFSVFRSEK.
Sku  SGKRRSFFSVFRSEK.
Smi  NGKRRSFFSVFRSEK.
Sba  SGKRRSIFSVFRSEK.
Sar  G GKRRSIFSVFRSEK.
Sca  NGKRRSIFSMFRGDKK.
Cgl  TGKRKSLFSMFK.
Ago  SGKRRSIFGVFKS.
Skl  TSKRKSFFGVFKS.
Kwa  SSKRKSFFGGFKG.
Kla  ETKRRSFFGVFK.

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Figure S1. Comparison of Alk1 orthologs from twelve yeast species. The amino acid sequence of *Saccharomyces cerevisiae* Alk1 (top line) was aligned with the primary structures of the Alk1 orthologs from eleven other yeast species, including the *sensu stricto* group, *Saccharomyces paradoxus* (Spa), *Saccharomyces kudriavzevii* (Sku), *Saccharomyces mikatae* (Smi), *Saccharomyces bayanus* (Sba), and *Saccharomyces arboricola* (Sar), as well as more divergent species, *Saccharomyces castellii* (Sca), *Candida glabrata* (Cgl), *Ashbya gossypii* (Ago), *Saccharomyces kluyveri* (Sk1), *Kluveromyces waltii* (Kwa), and *Kluveromyces lactis* (Kla). As a means to emphasize their degree of relatedness to *S. cerevisiae* Alk1, only identities between the indicated ortholog and *S. cerevisiae* Alk1 are indicated (white letters on black boxes). Gaps (hyphens), as well as the positions of insertions of the indicated length (in parentheses), introduced to maximize the alignment in certain regions are also indicated. Period (.) indicates the end of the open-reading-frame. Matches to the consensus Fpk1 phospho-acceptor site motif (-R-x-S-L/V/I-D/E-) (yellow boxes with phosphorylation site in bold red); additional site in, respectively, Sca and Clg (yellow underline). The sources of the sequences shown were: Sce, strain S288C from the *Saccharomyces* Genome Database (<http://www.yeastgenome.org/locus/S000000263/protein>); Spa, strain CBS432 derived from the data of Liti *et al.* (2009) and Bergström *et al.* (2014); Sku, Smi, Sba, Sca and Sk1 as reported by Cliften *et al.* (2003) and Kellis *et al.* (2003); Sar from GenBank entry EJS44833.1; and, Cgl, Ago, Kwa and Kla from the Fungal Orthogroups database at the Broad Institute (https://portals.broadinstitute.org/cgi-bin/regev/orthogroups/show_orthogroup.cgi?orf=YBR059C).

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