

Supplemental Data for

**“Expansion of protein farnesyltransferase specificity using “tunable” active site interactions:  
Development of bioengineered prenylation pathways”**

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**Table S1.** List of vectors used in fluorescent protein localization studies.

**Figure S1.** pCAF plasmid map

**Figure S2.** Polyacrylamide gel expression analysis of WT and variant FTases.

**Figure S3.** Representative secondary screening reactions of 102/106 library variants with dns-GCVLS

**Figure S4.** Representative secondary screening reactions of 102/106 library variants with dns-GCVDS

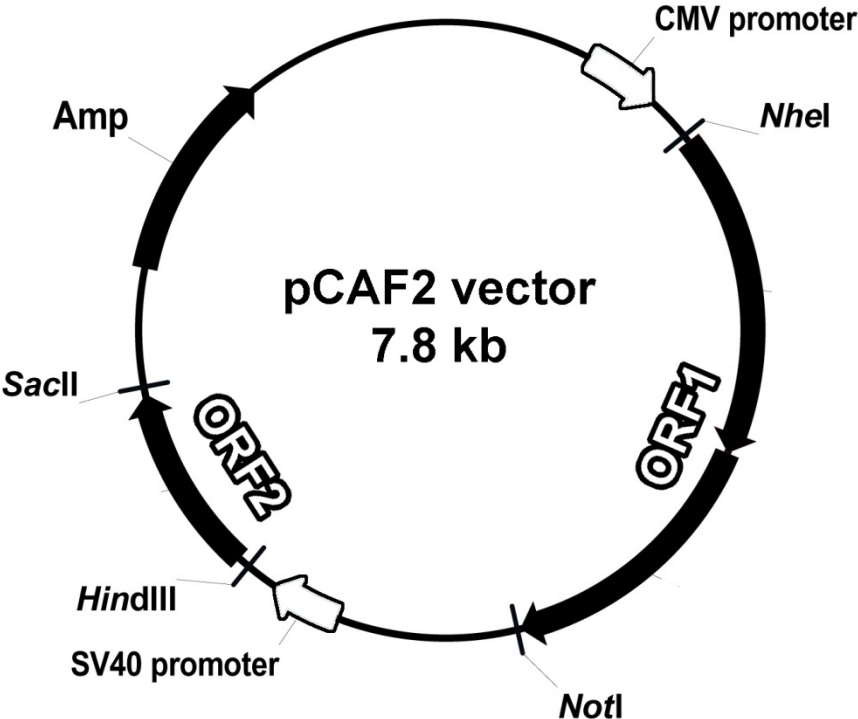
**Figure S5.** Representative secondary screening reactions of 102/106 library variants with dns-GCVKS

**Figure S6.** Alignment of protein farnesyltransferase beta subunits

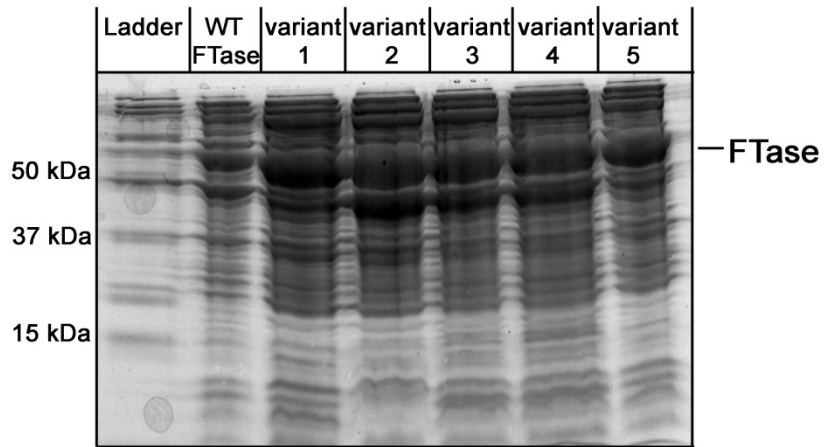
**Table S1.** List of vectors used in fluorescent protein localization studies

Vector	Open reading frame 1	Open reading frame 2
pPA-TagRFP-N	TagRFP	None
pCAF2 WW CVLS	WT FTase	TagRFP-CVLS
pCAF2 WW SVLS	WT FTase	TagRFP-SVLS
pCAF2 WW CVDS	WT FTase	TagRFP-CVDS
pCAF2 WW CVKS	WT FTase	TagRFP-CVKS
pCAF2 RL CVDS	W102R / W106L FTase	TagRFP-CVDS
pCAF2 FE CVKS	W102F / W106E FTase	TagRFP-CVKS

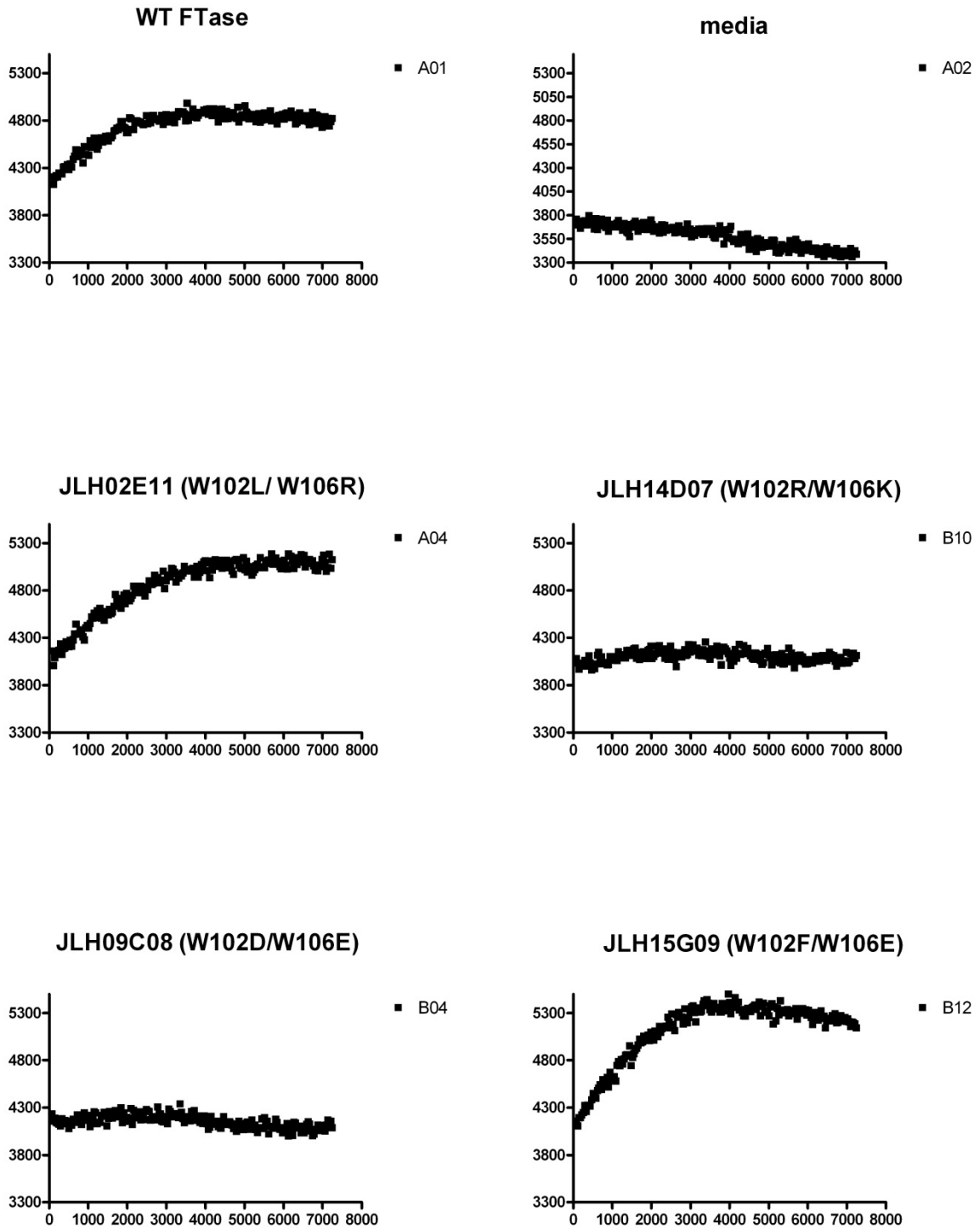
Figure S1. pCAF plasmid map



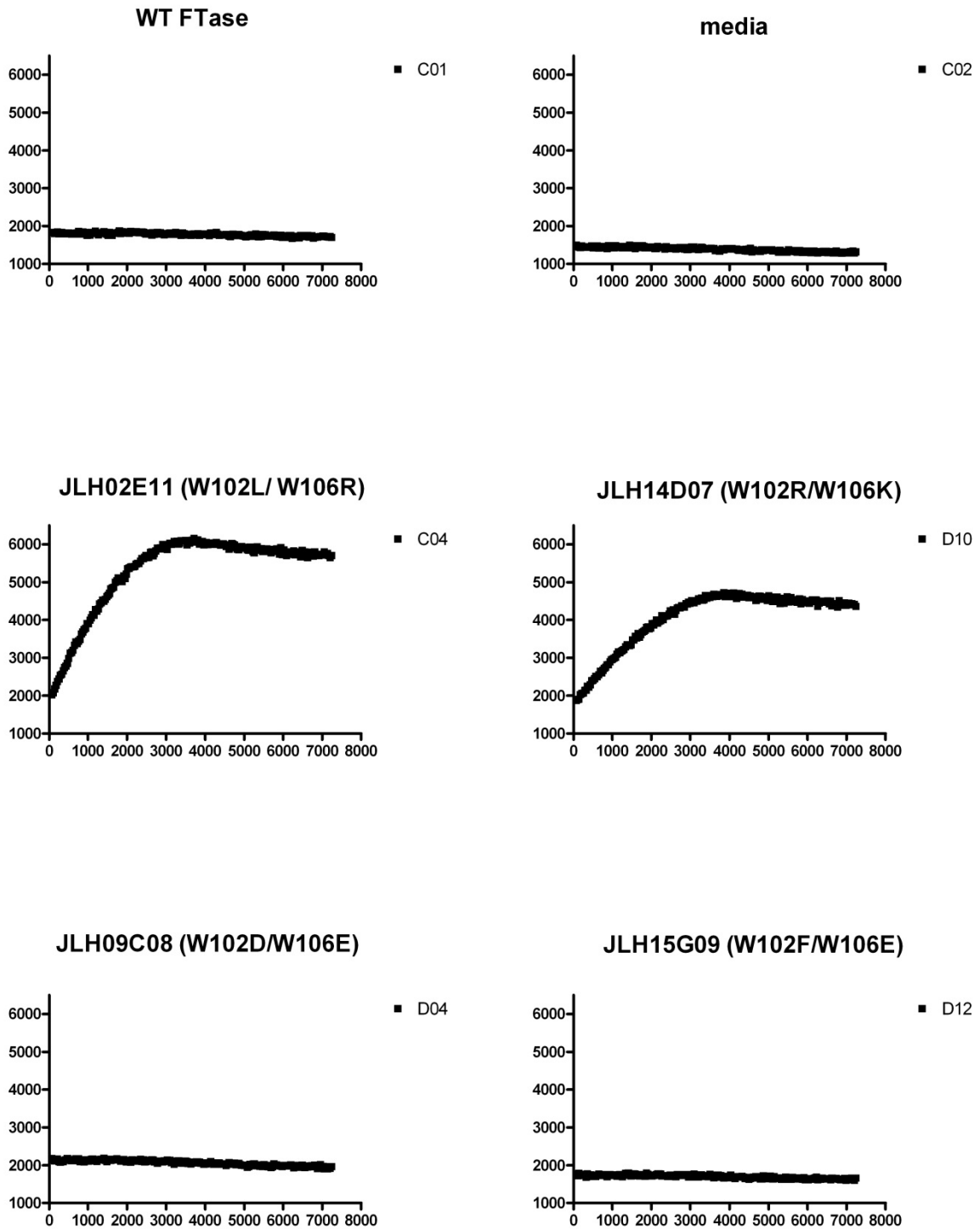
**Figure S2.** Polyacrylamide gel expression analysis of WT and variant FTases; variant FTase plasmids were chosen at random to check FTase expression compared to WT FTase.



**Figure S3.** Representative secondary screening reactions of 102/106 library variants with dns-GCVLS



**Figure S4.** Representative secondary screening reactions of 102/106 library variants with dns-GCVDS



**Figure S5.** Representative secondary screening reactions of 102/106 library variants with dns-GCVKS

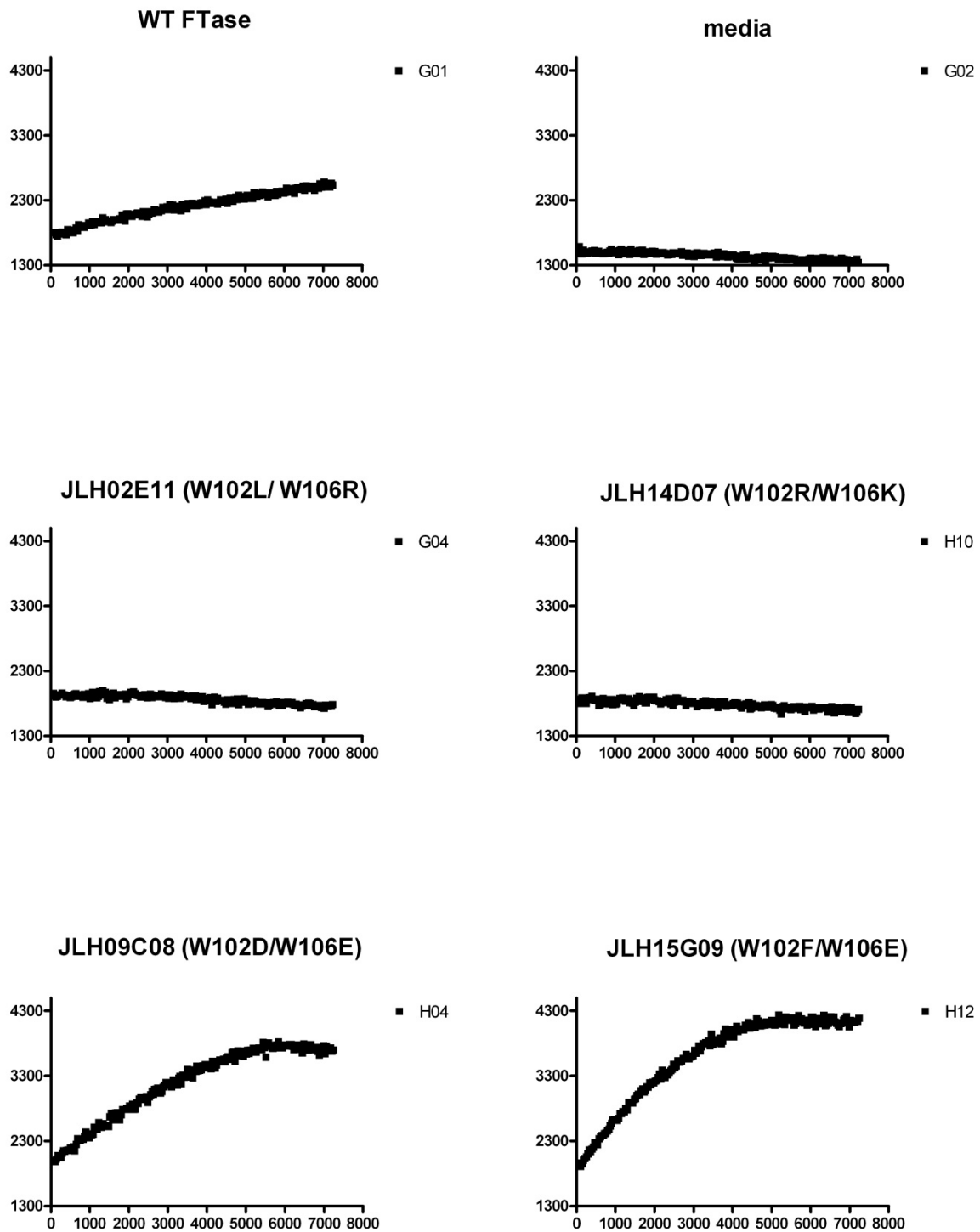


Figure S6. Alignment of protein farnesyltransferase beta subunits.

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Clustal-W alignment of protein farnesyltransferase beta subunits
59 total sequences

W102: 96% conservation
W106: 100% conservation

Homo_sapiens          -----RLVLQREKHFHYLKRGLRQ-LTDAYECLDASRPWLCYWILHSLEL 113
Pan_troglodytes      -----RLVLQREKHFHYLKRGLRQ-LTDAYECLDASRPWLCYWILHSLEL 67
Macaca_mulatta       -----RLILQREKHFHYLKRGLRQ-LTDAYECLDASRPWLCYWILHSLEL 113
Bos_taurus           -----RLVLQREKHFHYLKRGLRQ-LTDAYECLDASRPWLCYWILHSLEL 113
Rattus_norvegicus    -----RLVLQREKHFHYLKRGLRQ-LTDAYECLDASRPWLCYWILHSLEL 113
Mus_musculus         -----RLILQREKHFHYLKRGLRQ-LTDAYECLDASRPWLCYWILHSLEL 113
Canis_lupus_familiaris -----RLILQREKHFHYLKRGLRQ-LTDAYECLDASRPWLCYWILHSLEL 113
Xenopus_laevis       -----QLVLERESHAYLRKGLRY-LSDSYECLDSSRPWICYWIVHSMAL 98
Xenopus_tropicalis   -----QLVLEREPHTHYLRKGLRY-LSDSYECLDSSRPWICYWIVHSMGL 98
Danio_rerio          -----QPALLREQHYHYLKKGLRH-LSDAYECLDASRPWLCYWILHSLEL 98
Esox_lucius          -----QPTLLRDQHYQYLKGLRH-LSDSYECLDASRPWLCYWILHSLEL 98
Drosophila_melanogaster -----LTQIFRLEHQYYLDAMLR-LPSNYECLDSSRAWCVYWILQAAQL 100
Anopheles_gambiae    -----LPKLLRGDHARYLQLSLER-LSTAYESLDSSRPWMVYWILNAASI 100
Aedes_aegypti        -----LPKLLRTEHARYLETSLER-LSCGYECLDSSRPWLVYWIMNAASV 97
Arabidopsis_thaliana -----DANRPWLCYWILHSIAL 43
Oryza_sativa         -----MLELWREQHVEYLTRGLKH-LGPSFHVLDANRPWLCYWIHALAL 136
Pisum_sativum        -----I-----RPWLCYWIHSIAL 58
Ricinus_communis     -----MLELQRDKHMEYLTRGLKQ-LSSSFVLDANRPWLCYWILHSIAL 87
Nicotiana_glutinosa  -----HLETSTEKHFHYLKRGLRQ-LGPSFVLDANRPWLCYWILHSIAL 76
Catharanthus_roseus -----YLELQRDNHIEYLTNGLRK-LGPSFVLDANRPWLCYWILHPIAV 83
Triticum_aestivum    -----MLELWRDQHVKYLTGLRH-LAPSFHVLDANRPWLCYWMVHGLAL 105
Caenorhabditis_elegans -----QKHASYLLRYLKN-CPSSYATLDASRSWMCYWGVMALKI 101
Naegleria_gruberi    -----LVLFIEEHTKFAKGLRT-LPSYFDSLDASRPWFCFWCCNALSM 93
Plasmodium_falciparum -----LQLEKQLHFKFCFLDIFFL-KNMKLI-SLEASKPWIFWCIHSIHI 463
Leishmania_major     ---YTEPRLYRAAHVHFLMENLSV-APQGFSSLYPSRPWIVYWALQAAV 132
Leishmania_infantum ---YTEPRFYRAAHVHFLMENLSV-TPQGFSSLYPSRPWIVYWALQAAV 132
Leishmania_brasiliensis -----CEWPRFHRAAHVHFLMENLNA-APQGMGLYPSRPWIVYWALQAAV 135
Trypanosoma_brucei_brucei -----GIVHSLNRESHEKYLKSRLVK-LPEYARQLYNAQFWMVYWTLQAAEM 109
Trypanosoma_cruzi    ---DHLRLHRELHDSYVQGRFLF-LGESTQGLYSSQPWLAFWALQAAV 111
Entamoeba_histolytica -----NPEIHIKWLTLSIH--KP-LPSGFMSLDSSTPWILYWTLNPLRL 84
Brugia_malayi        -----YENVTTEYLR--MSG-----IYWCLQAMDI 63
Encephalitozoon_cuniculi -----FLYLLTEPFR--LNT-----IYWSVNALSM 83
Toxoplasma_gondii    -----AKLRTSAHIAFAQRYLEKPFNGMMELDASRCWLWVYWMVHALDL 211
Saccharomyces_cerevisiae -----FHKMYLDVAFEISLPPQMTALDASQPWMLYWIANSKLV 119
Schizosaccharomyces_pombe -----QKHLKYLTKMLDP-LPSPTVLDASRAWMVYWELSSLA 69
Schizosaccharomyces_japonicus -----DAHIFLESSLKP-FPAPYTVLDASKTWIYIYEWLVSLAL 67
Candida_albicans     -----DAHLKYILSSLIDPMPSGYQVLDVNHNSWMIYWLLNSYYL 205
Candida_dubliniensis -----DAHLKYILSSLTPMSSGYQVLDVNHNSWMIYWLLNSYYL 214
Pichia_stipitidis    -----LHLAYVRKSLQSQLPHYNSLDANHPWMMYWLANPQSL 125
Pichia_pastoris      -----QHRSFVKYFLETNLPAGFIALDASHWMIYFVLVNSFLL 124
Kluyveromyces_lactis -----SHKMFLEYWLNPLPSGFKSLDASQPWLLYWIGNAFKT 118
Dictyostelium_discoideum -----NIIKKKILNPLMNGIEK-IPMSHQGLDSSKVVWISFWILNGMDM 96
Eremothecium_gossypii -----AHQKLVEWPLRSMPAQPTTLDAAQPWVLYWTANALT 119
Aspergillus_fischerianus -----KDHIEYLYDSLED-YPASFVALDASRPWVYWALAGLCL 164
Aspergillus_fumigatus -----KDHLELYDSLED-YPASFVALDASRPWVYWALAGLCL 164
Aspergillus_clavatus -----DDHVAYLFDSDLED-YPASFVAMDASRPWVYWALAGLSL 164
Aspergillus_oryzae   -----DDHIAYLYDSLED-YPGSFVALDASRPWVYWALAGLAL 102
Aspergillus_flavus   -----DDHIAYLYDSLED-YPGSFVALDASRPWVYWALAGLAL 102
Magnaporthe_grisea   -----RKKHVRFLRNMLRQ-LPAPPIAADASRPWFLYWSLNAAMI 110
Neurospora_crassa    -----REKHIKFLKQSLGP-LPGRFVAVDASRPWLYWCLSGLTM 145
Blastomyces_dermatitidis -----DEHVSFLYDSLES-YPERFVGLDSSRPWVYWALAGLHF 227
Histoplasma_capsulatum -----DAHISFLYDSLES-YPERFVGLDSSRPWVYWALTGLYL 241
Paracoccidioides_brasiliensis -----DAHISFLYDSLES-YPDRFVGLDSSRPWVYWALAGLHM 198
Coccidioides_posadasii -----DVHVAYLYDALEE-YPGKFVGLDASRPWVYWALTGLYL 104
Penicillium_marneffeii -----DQHIEYLYDSLED-YPEGFVTMDSSRPWMSYWALAGLTL 99
Penicillium_stipitatum -----DEHIEYLYDSLEL-YPAGEFVAMDSSRPWMSYWALAGLTL 154
Microsporium_canis   -----QLHVDYLLDALGQ-YPASFVGLDASRPWVYWALAGLAL 143
Verticillium_albo-atrum -----SKNHTVSHKQLGK--LPAPYLIADASRPWFLEWSLNGLAL 103
Pyrenophora_tritici-repentis -----QRHEAMLKKILGD-YPSGAAMDAARPWLWVYWALQSMTA 142

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