Supplemental Materials

Molecular Biology of the Cell

Friedl *et al.*

Supplementary Figures

Supplementary Figure 1. Arg5,6 is internally processed by MPP *in vitro* and separate expression of Arg5 and Arg6 *in vivo* is non-toxic and can restore arginine prototrophy. (A) Radiolabeled Arg5,6 precursor was incubated with purified MPP for indicated times. EDTA was added as a control to inhibit the enzymatic activity of MPP. Reactions were analyzed by SDS-PAGE, Western blotting and autoradiography. **(B)** Radiolabeled Arg5,6 precursor was incubated with mitochondria isolated from wild type or *mas1*^{ts} cells. Mitochondria were incubated at 37°C for 20 min before the precursor was added to inactivate MPP. **(C-D)** The indicated Arg5,6 variants are expressed in yeast cells lacking *ARG5,6* and cells are streaked out on plates with minimal medium containing arginine. **(E)** Cells are grown in liquid minimal medium without arginine for 72 hours at 30°C. The optical density at 600 nm was measured every 10 min. **(F)** Radiolabeled Arg5$^{344\text{-}863}$ and Su9-Arg5$^{344\text{-}863}$ were incubated with purified MPP for 90 min. Its processing was analyzed by SDS-PAGE, Western blotting and autoradiography.

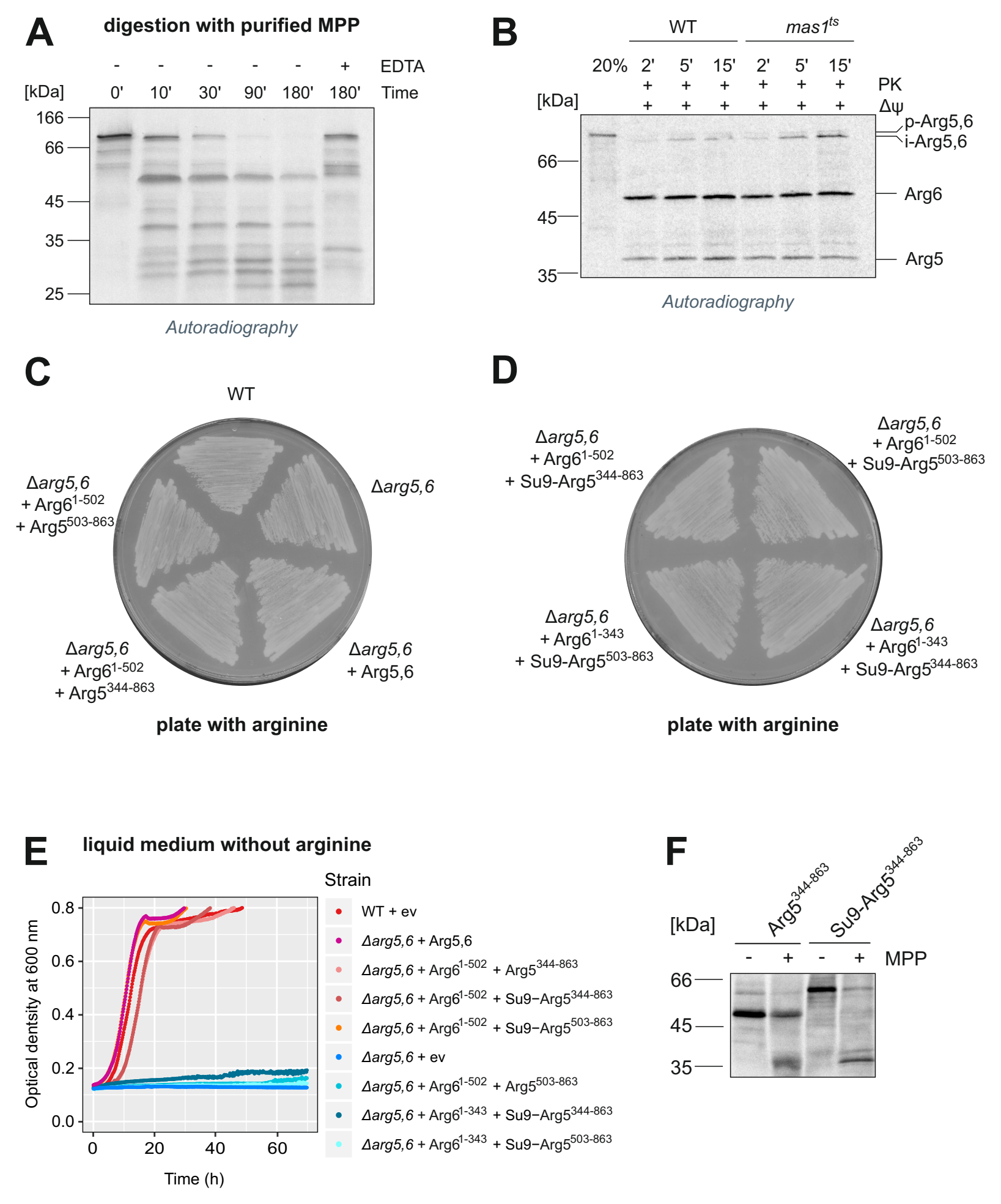
Supplementary Figure 2. Tandem organization of Arg5,6 is conserved in fungi, whereas algae synthesize two separate proteins. A database of 150 eukaryotes was searched for homologs of full length Arg5,6 and the separate Arg5 and Arg6 proteins of *S. cerevisiae*. Red, species in which the Arg5,6 homolog is encoded as fusion protein. Blue, Arg6 and Arg5 homologs are encoded as separate proteins. Black, no homolog of Arg5,6 found or unclear pattern.

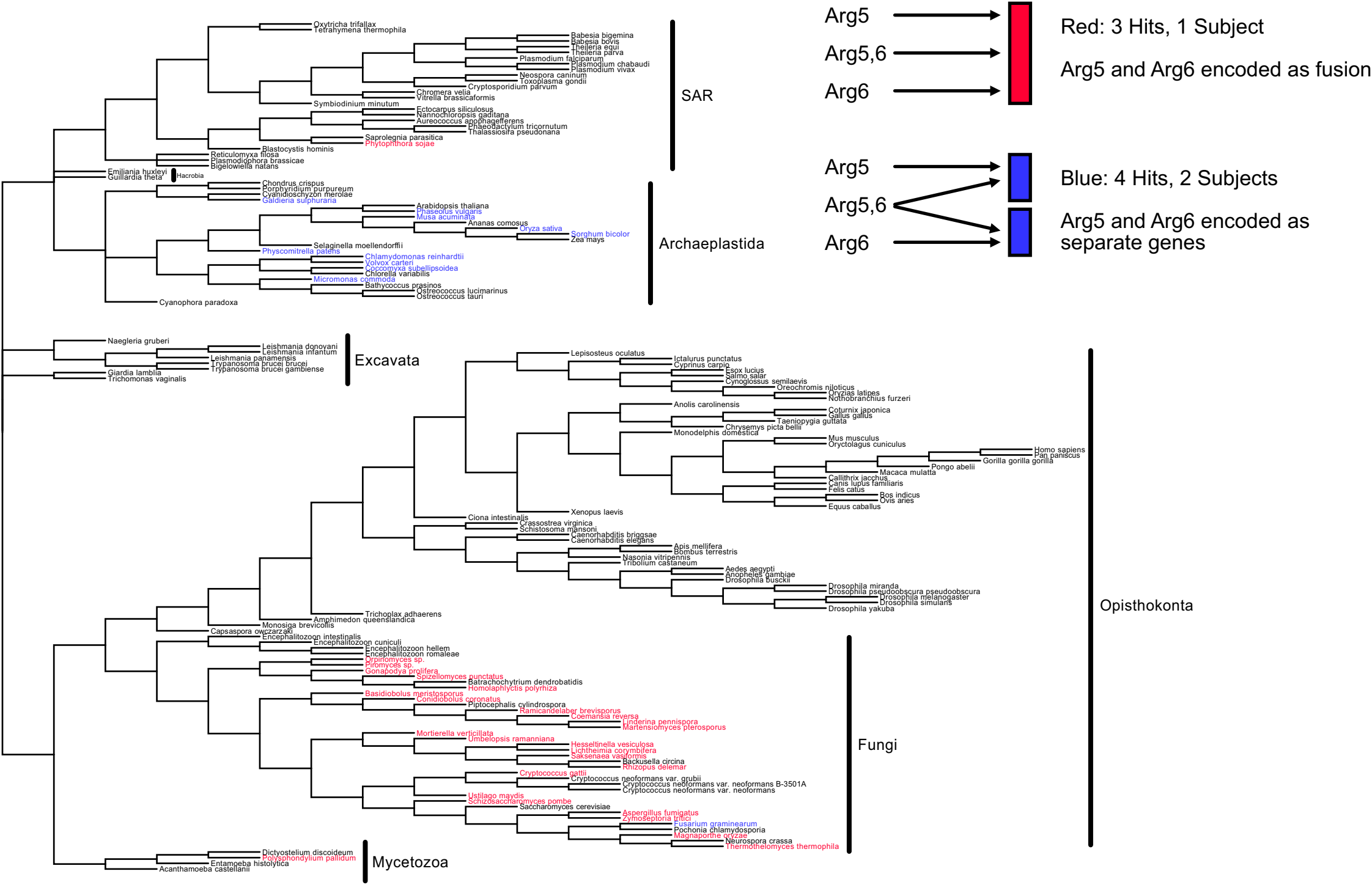
Supplementary Tables

Supplementary Table 1. Evolutionary conservation of the *ARG5,6* gene structure

Supplementary Table 2. Yeast strains and plasmids used in this study.

Supplementary Table 3. Numerical source data for Figures 2, 5 and 6.


A
digestion with purified MPP
- - - - - + EDTA
[kDa] 0' 10' 30' 90' 180' 180' Time
166
66
45
35
25
Autoradiography
B
WT
mas1ts
20% 2' 5' 15' 2' 5' 15'
+ + + + + + PK
+ + + + + + Δψ
[kDa]
66
45
35
p-Arg5,6
i-Arg5,6
Arg6
Arg5
Autoradiography
C
WT
Δarg5,6 + Arg6^1-502 + Arg5^503-863
Δarg5,6
Δarg5,6 + Arg6^1-502 + Arg5^344-863
Δarg5,6 + Arg5,6
plate with arginine
D
Δarg5,6 + Arg6^1-502 + Su9-Arg5^344-863
Δarg5,6 + Arg6^1-502 + Su9-Arg5^503-863
Δarg5,6 + Arg6^1-343 + Su9-Arg5^503-863
Δarg5,6 + Arg6^1-343 + Su9-Arg5^344-863
plate with arginine
E
liquid medium without arginine
Optical dentsity at 600 nm
0.0
0.2
0.4
0.6
0.8
0
20
40
60
Time (h)
Strain
WT + ev
Δarg5,6 + Arg5,6
Δarg5,6 + Arg6^1-502 + Arg5^344-863
Δarg5,6 + Arg6^1-502 + Su9−Arg5^344-863
Δarg5,6 + Arg6^1-502 + Su9−Arg5^503-863
Δarg5,6 + ev
Δarg5,6 + Arg6^1-502 + Arg5^503-863
Δarg5,6 + Arg6^1-343 + Su9−Arg5^344-863
Δarg5,6 + Arg6^1-343 + Su9−Arg5^503-863
F
[kDa]
Arg5^344-863
Su9-Arg5^344-863
- + - + MPP
66
45
35


Oxytricha trifallax
Tetrahymena thermophila
Babesia bigemina
Babesia bovis
Theileria equi
Theileria parva
Plasmodium falciparum
Plasmodium chabaudi
Plasmodium vivax
Neospora caninum
Toxoplasma gondii
Cryptosporidium parvum
Chromera velia
Vitrella brassicaformis
Symbiodinium minutum
Ectocarpus siliculosus
Nannochloropsis gaditana
Aureococcus anophagefferens
Phaeodactylum tricornutum
Thalassiosira pseudonana
Saprolegnia parasitica
Phytophthora sojae
Blastocystis hominis
Reticulomyxa filosa
Plasmodiophora brassicae
Bigelowiella natans
Emiliania huxleyi
Guillardia theta
Hacrobia
SAR
Chondrus crispus
Porphyridium purpureum
Cyanidioschyzon merolae
Galdieria sulphuraria
Arabidopsis thaliana
Phaseolus vulgaris
Musa acuminata
Ananas comosus
Oryza sativa
Sorghum bicolor
Zea mays
Selaginella moellendorffii
Physcomitrella patens
Chlamydomonas reinhardtii
Volvox carteri
Coccomyxa subellipsoidea
Chlorella variabilis
Micromonas commoda
Bathycoccus prasinos
Ostreococcus lucimarinus
Ostreococcus tauri
Archaeplastida
Cyanophora paradoxa
Arg5
Arg5,6
Arg6
Red: 3 Hits, 1 Subject
Arg5 and Arg6 encoded as fusion
Arg5
Arg5,6
Arg6
Blue: 4 Hits, 2 Subjects
Arg5 and Arg6 encoded as separate genes
Naegleria gruberi
Leishmania donovani
Leishmania infantum
Leishmania panamensis
Trypanosoma brucei brucei
Trypanosoma brucei gambiense
Giardia lamblia
Trichomonas vaginalis
Excavata
Lepisosteus oculatus
Ictalurus punctatus
Cyprinus carpio
Esox lucius
Salmo salar
Cynoglossus semilaevis
Oreochromis niloticus
Oryzias latipes
Nothobranchius furzeri
Anolis carolinensis
Coturnix japonica
Gallus gallus
Taeniopygia guttata
Chrysemys picta bellii
Monodelphis domestica
Mus musculus
Oryctolagus cuniculus
Homo sapiens
Pan paniscus
Gorilla gorilla gorilla
Pongo abelii
Macaca mulatta
Callithrix jacchus
Canis lupus familiaris
Felis catus
Bos indicus
Ovis aries
Equus caballus
Xenopus laevis
Ciona intestinalis
Crassostrea virginica
Schistosoma mansoni
Caenorhabditis briggsae
Caenorhabditis elegans
Apis mellifera
Bombus terrestris
Nasonia vitripennis
Tribolium castaneum
Aedes aegypti
Anopheles gambiae
Drosophila busckii
Drosophila miranda
Drosophila pseudoobscura pseudoobscura
Drosophila melanogaster
Drosophila simulans
Drosophila yakuba
Opisthokonta
Trichoplax adhaerens
Amphimedon queenslandica
Monosiga brevicollis
Capsaspora owczarzaki
Encephalitozoon intestinalis
Encephalitozoon cuniculi
Encephalitozoon hellem
Encephalitozoon romaleae
Orpinomyces sp.
Piromyces sp.
Gonapodya prolifera
Spizellomyces punctatus
Batrachochytrium dendrobatidis
Homolaphlyctis polyrhiza
Basidiobolus meristosporus
Conidiobolus coronatus
Piptocephalis cylindrospora
Ramicandelaber brevisporus
Coemansia reversa
Linderina pennispora
Martensiomyces pterosporus
Mortierella verticillata
Umbelopsis ramanniana
Hesseltinella vesiculosa
Lichtheimia corymbifera
Saksenaea vasiformis
Backusella circina
Rhizopus delemar
Cryptococcus gattii
Cryptococcus neoformans var. grubii
Cryptococcus neoformans var. neoformans B-3501A
Cryptococcus neoformans var. neoformans
Ustilago maydis
Schizosaccharomyces pombe
Saccharomyces cerevisiae
Aspergillus fumigatus
Zymoseptoria tritici
Fusarium graminearum
Pochonia chlamydosporia
Magnaporthe oryzae
Neurospora crassa
Thermothelomyces thermophila
Fungi
Dictyostelium discoideum
Polysphondylium pallidum
Entamoeba histolytica
Acanthamoeba castellanii
Mycetozoa