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SUPPLEMENTARY MATERIAL

corresponding to:

Overexpression of S-adenosylmethionine decarboxylase impacts polyamine homeostasis during development of *Dictyostelium discoideum*

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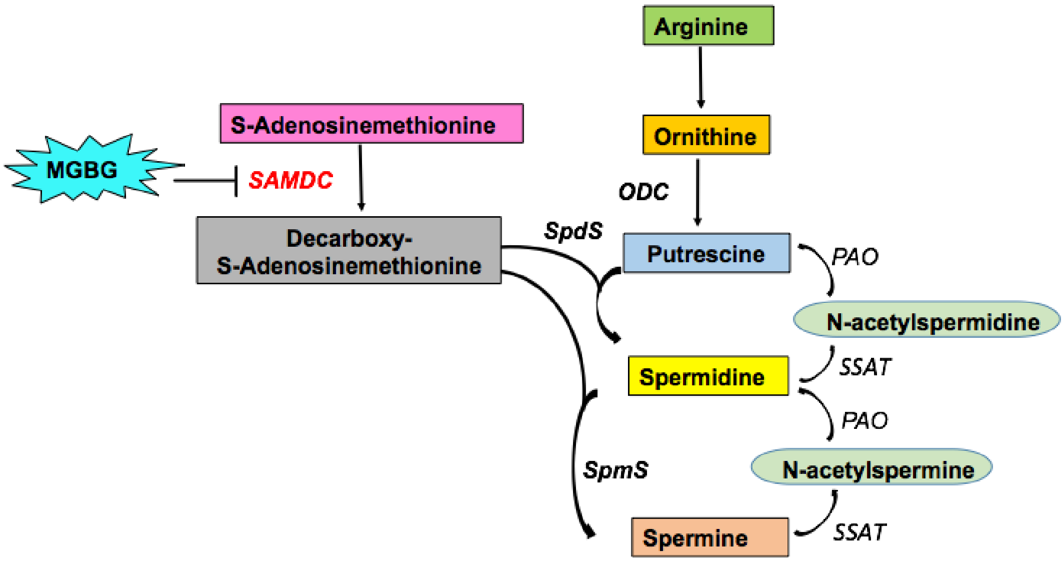
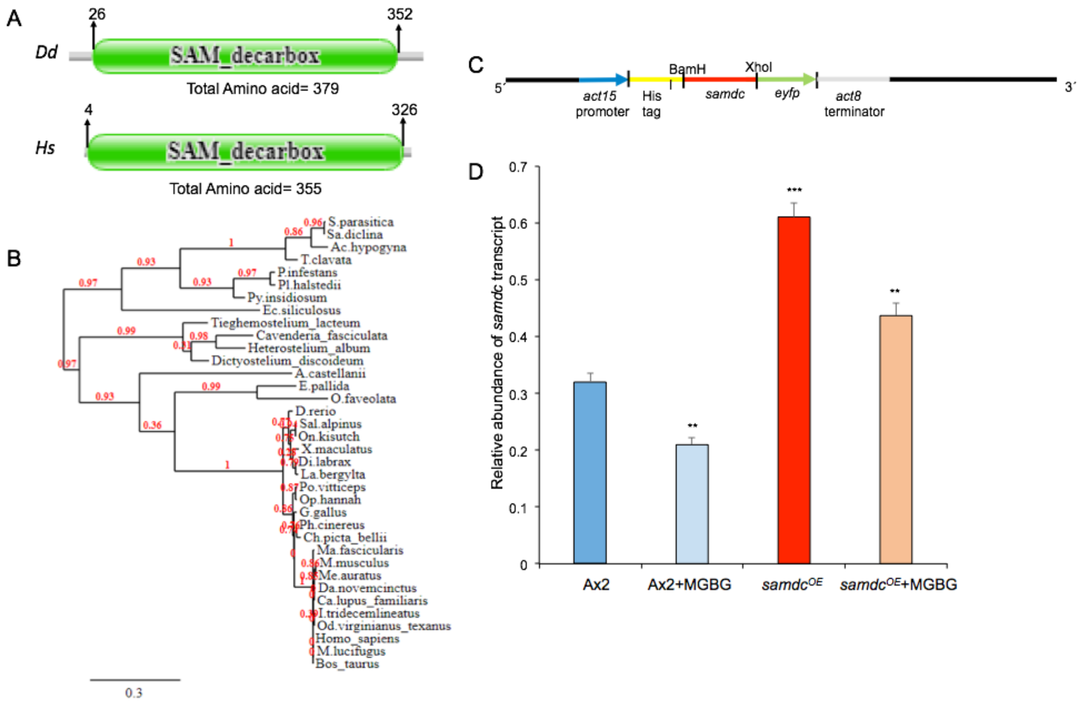
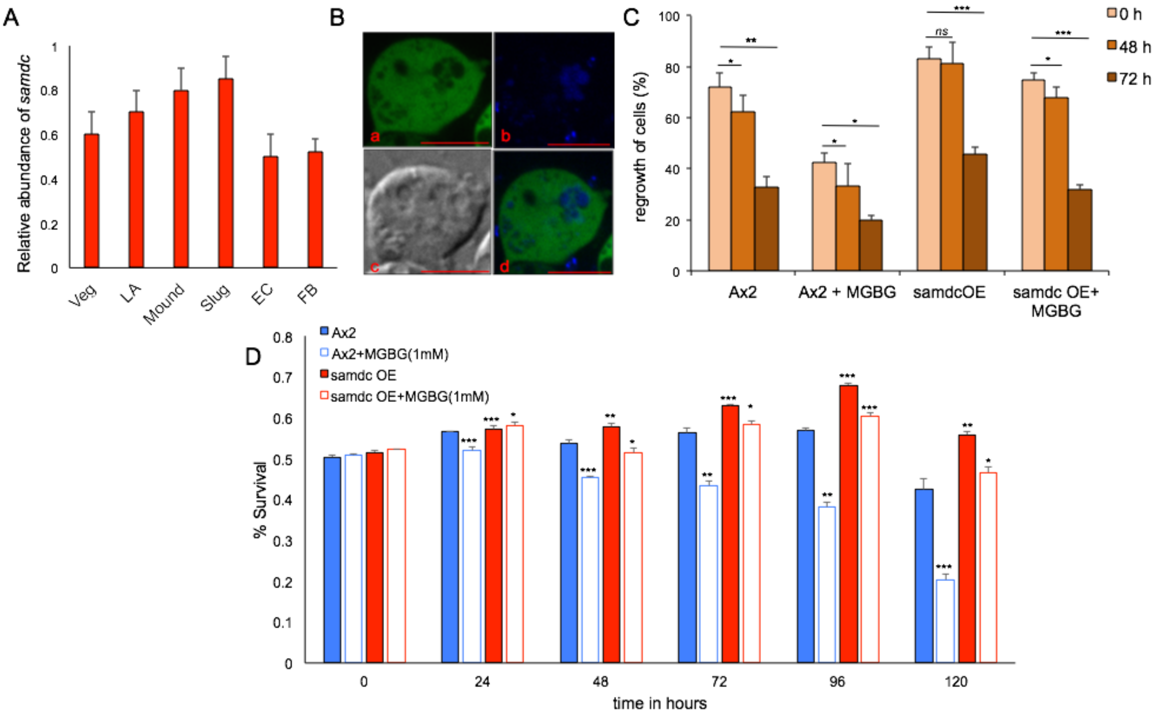


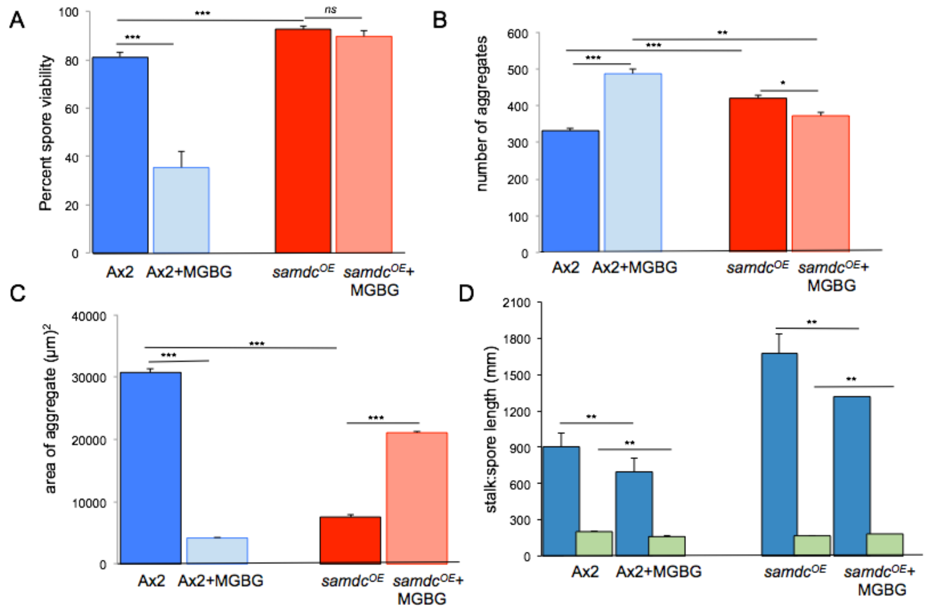
Supplementary Fig. S1. Pathways for polyamine metabolism and interconversions. *[ODC-Ornithine decarboxylase; SAMDC-S-adenosylmethionine decarboxylase, PAO polyamine oxidase, SSAT- spermidine/spermine N1-acetyltransferase]*.



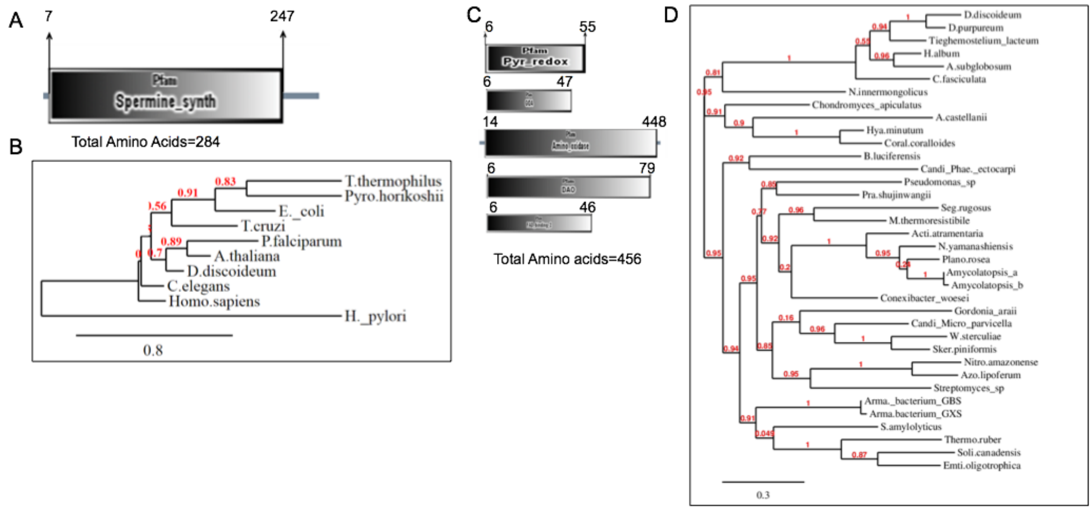
Supplementary Fig. S2. The domain, phylogenetic analysis and expression of SAMDC. (A) *The domain as predicted by the SMART programme.* **(B)** *Unrooted neighbor joining (NJ) tree of SAMDC from various organisms as listed and the candidate SAMDC of Dictyostelium discoideum, Heterostelium album, Tieghemostelium lacteum, Cavenderia fasciculate, Acytostelium subglobosum Exaiptasia pallida, Phytophthora infestans, Ectocarpus siliculosus, Pl. halstedii, Orbicella faveolata, Plasmopara halstedii, Saprolegnia parasitica, Pythium insidiosum, Saprolegnia diclina, Pogona vitticeps, Ophiophagus Hannah, hypogyna, Xiphophorus maculatus, Myotis lucifugus, Thraustotheca clavata, Danio rerio, Dasypus novemcinctus, Gallus gallus, Macaca fascicularis, Mus musculus,* *Salvelinus alpinus, Phascolarctos cinereus, Mesocricetus auratus, Dicentrarchus labrax, Labrus bergylta, Odocoileus virginianus texanus, Canis lupus familiaris, Homo sapiens, Bos Taurus, Oncorhynchus kisutch, Ictidomys tridecemlineatus,* **(C)** *Strategy for samdc overexpression in D. discoideum.* **(D)** *Transcript patterns of samdc in Ax2 and samdc*^{OE} *cells in presence and absence of MGBG (1mM). Expression pattern of samdc and rnlA (ig7) by RT-PCR using cDNA samples with or without treatment.*



Supplementary Fig. S3. Characterization of SAMDC. (A) *Relative samdc transcript during development. rnlA (ig7) was used as an internal control.* **(B)** *Subcellular localization of the recombinant fusion protein (SAMDC-eYFP) interspersed in the cytoplasm. a- green fluorescence due to the fusion protein, b-Nuclear stain with DAPI (blue), c-brightfield, d-merged image. [Scale bar, 10 µm].* **(C)** *Regrowth of cells monitored after 72h (stationary phase). Percent survival is measured in Ax2 and samdc*^{OE} *cells in presence and absence of MGBG.* **(D)** *Cell viability was measured by MTT assay. Percent survival is calculated in Ax2 and samdc*^{OE} *cells in presence and absence of MGBG. Values represent mean ± S.E.; n= 3; Student t-test, p-value ≤0.05, ≤0.01 and ≤0.001 has been represented as *, ** and ***, respectively].*

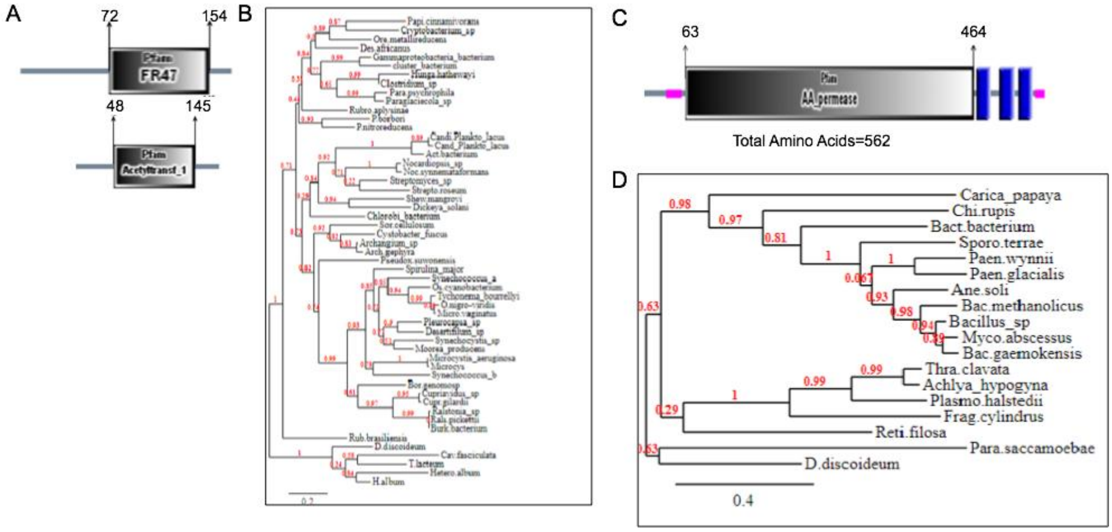


Supplementary Fig. S4. Morphological analyses of Ax2 and *samdc^{OE}* cells in the presence and absence of MGBG. (A) *Graph represents increased spore-viability upon overexpression of samdc.* **(B)** *The graph represents the number of aggregates formed by each strain with or without MGBG treatment.* **(C)** *The graph represents the size of aggregates (µm) formed by different cells as shown.* **(D)** *Graph represents the stalk: sorus ratio of fruiting bodies formed by each strain with or without MGBG treatment. samdc^{OE} formed stalky fruiting bodies. [The values representing mean ± S.E.; number of structures analyzed ~200; n = 3; Student t-test, p-value ≤0.05, ≤0.01 and ≤0.001 has been represented as *, ** and ***, respectively].*

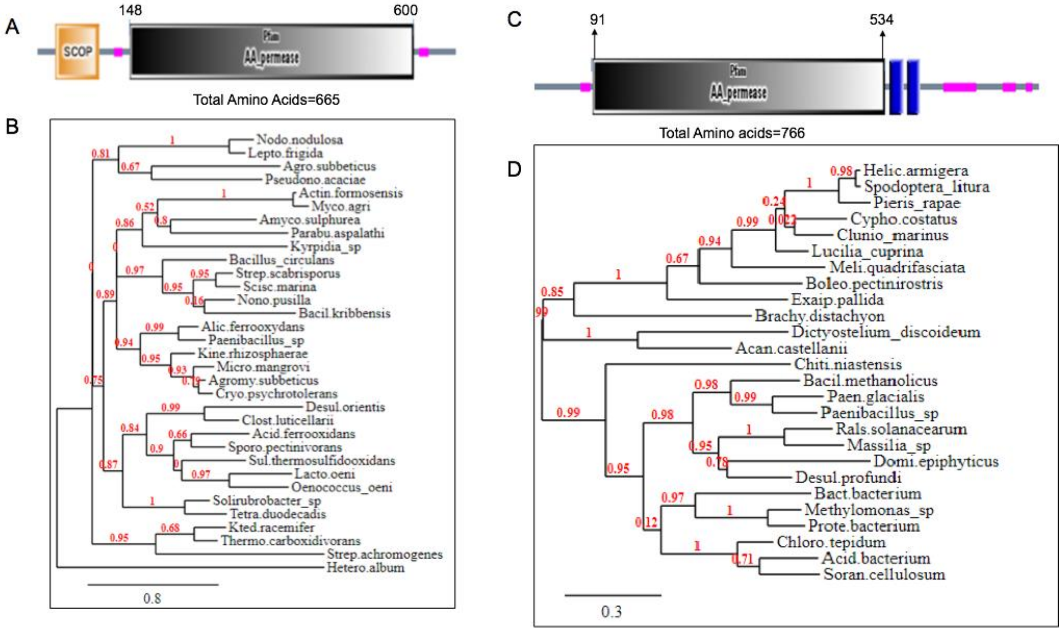


Supplementary Fig. S5. The domain and phylogenetic analyses of Spm/Spd synthase and Spd/Spm oxidase. (A) *The domain as predicted by the SMART programme for Spermidine synthase (DDB0191167).* **(B)** *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using PHYLIP. Dictyostelium discoideum, Homo sapiens, Caenorhabditis elegans, Arabidopsis thaliana, Plasmodium falciparum, Trypanosoma cruzi, E. coli, Thermus thermophilus, Pyrococcus horikoshii, Helicobacter pylori.* **(C)** *The domain as predicted by the SMART programme for Spermine oxidase (DDB0231707).* **(D)** *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using*

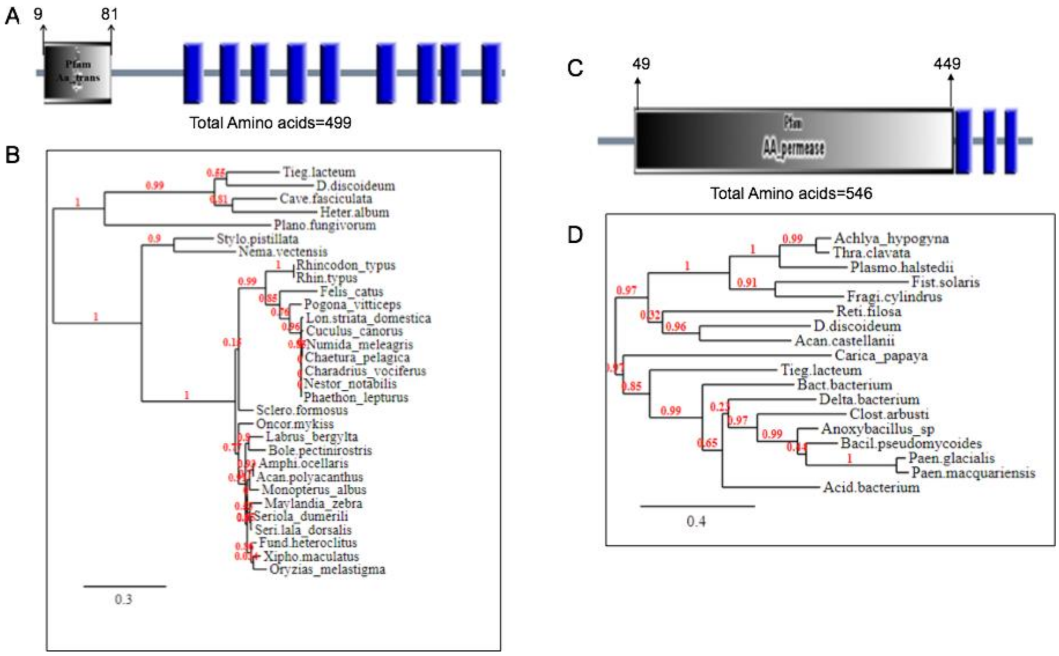
PHYLIP. Dictyostelium discoideum, Dictyostelium purpureum, Tieghemostelium lacteum, Heterostelium album, Cavenderia fasciculate, Bacillus luciferensis, Nocardia yamanashiensis, Chondromyces apiculatus, Acanthamoeba castellanii, Natronolimnobius innermongolicus, Sandaracinus amylolyticus, Hyalangium minutum, Pseudomonas sp, Thermoflexibacter ruber, Prauserella shujinwangii, Corallococcus coralloides, Amycolatopsis sp, Nitrospirillum amazonense, Mycobacterium thermoresistibile, Solitalea canadensis, Armatimonadetes bacterium, Candidatus Phaeomarinobacter ectocarpi, Conexibacter woesei, Segniliparus rugosus, Azospirillum lipoferum, Skermania piniformis, Emticicia oligotrophica, Planobispora rosea, Gordonia araii, Candidatus microthrix.



Supplementary Fig. S6. The domain and phylogenetic analyses of SSAT and DDB0185190. (A) *The domain as predicted by the SMART programme for SSAT domain1 (DDB0233628).* (**B)** *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using PHYLIP. Dictyostelium discoideum, Heterostelium album, Acytostelium subglobosum, Tieghemostelium lacteum, Cavenderia fasciculate, Rubinisphaera brasiliensis, Beta proteobacterium, Gammaproteobacteria bacterium, Gammaproteobacteria bacterium, Chlorobi bacterium, Microcystis aeruginosa, Tychonema bourrellyi, Bordetella genomosp, Sorangium cellulosum, Pseudomonas borbori, Oscillatoria nigro-viridis, Microcoleus vaginatus, Microcystis sp, Streptomyces sp, Paraglaciecola psychrophila, Burkholderiaceae bacterium, Pseudoxanthomonas suwonensis, Archangium sp, Cystobacter fuscus, Cupriavidus sp, Candidatus Planktophila lacus, Ralstonia sp, Betaproteobacteria bacterium, Pseudoxanthomonas, Streptosporangium roseum, Dickeya, Candidatus Planktophila lacus, Kamptonema, Nocardiopsis sp, Synechococcus sp, Moorea producens, Papillibacter cinnamivorans, Moorea bouillonii, Paraglaciecola sp, Shewanella mangrove, Hungatella hathewayi, Rubrobacter aplysinae, Desulfovibrio africanus, Nocardiopsis synnemataformans, Desertifilum sp, Pleurocapsa, Actinobacteria bacterium, Spirulina major, Orenia metallireducens.* **(C)** *The domain of Polyamine transporter DDB0185190 as predicted by the SMART programme.* (**D)** *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using PHYLIP. Dictyostelium discoideum , Carica papaya, Chitinophaga rupis, Bacteroidetes bacterium, Sporolactobacillus terrae, Paenibacillus wynnii, Paenibacillus glacialis, Aneurinibacillus soli, Bacillus methanolicus, Mycobacteroides abscessus, Bacillus gaemokensis, Thraustotheca clavata, Achlya hypogyna, Plasmopara halstedii, Fragilariopsis cylindrus, Reticulomyxa filose, Paramicrosporidium saccamoebae.*

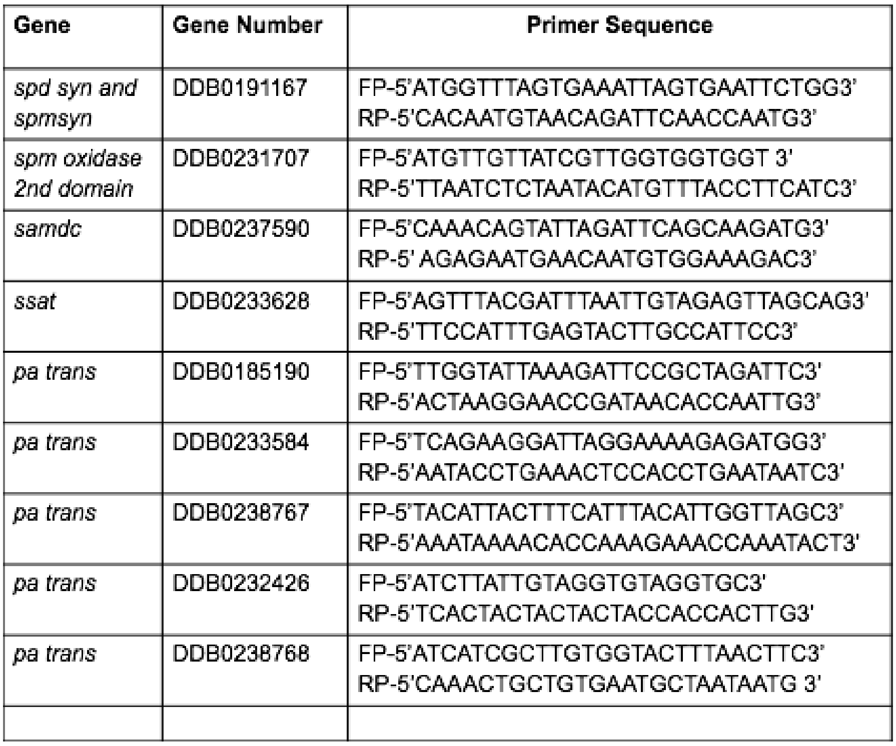


Supplementary Fig. S7. The domain and phylogenetic analyses of DDB0233584 and DDB0238767. (A) *The domain as predicted by the SMART programme for Polyamine transporter (DDB0233584).* (**B**) *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using PHYLIP. Heterostelium album, Paraburkholderia aspalathi, Sporolactobacillus pectinivorans, Agromyces subbeticus, Microbacterium mangrove, Alicyclobacillus ferrooxydans, Cryobacterium psychrotolerans, Ktedonobacter racemifer, Paenibacillus sp, Bacillus circulans, Alicyclobacillus ferrooxydans, Kineococcus rhizosphaerae, Solirubrobacter sp, Sulfobacillus thermosulfidooxidans, Tetrasphaera duodecadis, Thermogemmatispora carboxidivorans, Streptomyces scabrisporus, Tetrasphaera duodecadis, Nodosilinea nodulosa, Actinomadura formosensis, Agromyces subbeticus, Nonomuraea pusilla, Lobaria pulmonaria, Amycolatopsis sulphurea, Actinomadura oligospora, Microbacterium sp, Kyrpidia sp, Pseudonocardia acacia, Streptomyces achromogenes, Amycolatopsis taiwanensis, Leptolyngbya frigida, Sciscionella marina. The domain and phylogenetic analysis of DDB0238767.* **(C)** *The domain as predicted by the SMART programme for Polyamine transporter (DDB0238767).* (**D**) *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using PHYLIP. Dictyostelium discoideum, Acanthamoeba castellanii, Methylomonas, Desulfotomaculum profundi, Acidobacteria bacterium, Proteobacteria bacterium, Boleophthalmus pectinirostris, Paenibacillus glacialis, Domibacillus epiphytius, Melipona quadrifasciata, Lucilia cuprina, Exaiptasia pallida, Cyphomyrmex costatus, Clunio marinus, Helicoverpa armigera, Bacillus methanolicus, Ralstonia solanacearum, Chitinophaga niastensis, Spodoptera litura, Pieris rapae, Chlorobaculum tepidum, Bacteroidetes bacterium, Chlorobium limicola, Sorangium cellulosum, Massilia sp, Paenibacillus sp, Fictibacillus phosphorivorans, Brachypodium distachyon, Helicoverpa armigera.*



Supplementary Fig. S8. The domain and phylogenetic analysis of DDB0238768 and DDB0233426. (A) *The domain as predicted by the SMART programme for Polyamine transporter (DDB0238768).* (**B)** *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using PHYLIP. Dictyostelium discoideum, Acanthamoeba castellanii, Reticulomyxa filose, Achlya hypogyna, Thraustotheca clavata, Paenibacillus glacialis, Carica papaya, Clostridium arbusti, Acidobacteria bacterium, Fragilariopsis cylindrus, Tieghemostelium lacteum, Bacteroidetes bacterium, Deltaproteobacteria bacterium, Fistulifera solaris, Anoxybacillus, Paenibacillus macquariensis, Sciscionella marina, Nonomuraea pusilla, Lactobacillus oeni, Streptomyces indicus, Mycobacterium agri, Bacillus kribbensis, Oenococcus oeni, Acidihalobacter ferrooxidans.* **(C)** *The domain as predicted by the SMART programme for Polyamine transporter DDB0233426.* (**D)** *Neighbour joining (NJ) phylogenetic tree of the same showing branch length based on the alignment using CLUSTALW2 (bootstrap values out of 1000) was constructed using PHYLIP. Dictyostelium discoideum, Tieghemostelium lacteum, Cavenderia fasciculate, Heterostelium album, Stylophora pistillata, Seriola dumerili, Xiphophorus maculatus, Seriola lalandi dorsalis, Maylandia zebra, Planoprotostelium fungivorum, Monopterus albus, Oryzias melastigma, Scleropages formosus, Labrus bergylta, Fundulus heteroclitus, Nematostella vectensis, Amphiprion ocellaris, Oncorhynchus mykiss, Acanthochromis polyacanthus, Nestor notabilis, Charadrius vociferous, Cuculus canorus, Boleophthalmus pectinirostris, Pogona vitticeps, Chaetura pelagica, Rhincodon typus, Phaethon lepturus, Numida meleagris, Felis catus, Rhincodon typus, Lonchura striata domestica.*

Supplementary Table S1. *Oligos used in the present study.*



Gene	Gene Number	Primer Sequence
spd syn and spmsyn	DDB0191167	FP-5’ATGGTTTAGTGAAATTAGTGAATTCTGG3’ RP-5'CACAATGTAACAGATTCAACCAATG3’
spm oxidase 2nd domain	DDB0231707	FP-5’ATGTTGTTATCGTTGGTGGTGGT 3' RP-5'TTAATCTCTAATACATGTTTACCTTCATC3’
samdc	DDB0237590	FP-5’CAAACAGTATTAGATTCAGCAAGATG3' RP-5' AGAGAATGAACAATGTGGAAAGAC3’
ssat	DDB0233628	FP-5’AGTTTACGATTTAATTGTAGAGTTAGCAG3' RP-5'TTCCATTTGAGTACTTGCCATTCC3’
pa trans	DDB0185190	FP-5’TTGGTATTAAAGATTCCGCTAGATTC3' RP-5'ACTAAGGAACCGATAACACCAATTG3’
pa trans	DDB0233584	FP-5’TCAGAAGGATTAGGAAAAGAGATGG3’ RP-5'AATACCTGAAACTCCACCTGAATAATC3'
pa trans	DDB0238767	FP-5’TACATTACTTTCATTTACATTGGTTAGC3’ RP-5'AAATAAAACACCAAAGAAACCAAATACT3'
pa trans	DDB0232426	FP-5’ATCTTATTGTAGGTGTAGGTGC3' RP-5'TCACTACTACTACTACCACCACTTG3'
pa trans	DDB0238768	FP-5’ATCATCGCTTGTGGTACTTTAACTTC3’ RP-5'CAAACTGCTGTGAATGCTAATAATG 3'