

Supplementary Material:

List of supplementary Material:

Four supplemental figures, two supplemental tables

1. Figure S1. PIP strips. Associated with Figure 5. Shows that CCCP-1 is capable of binding to a number of different phospholipids with only weak binding specificity.
2. Figure S2. SEC of CCCP-1 fragments. Associated with Figure 6. Shows which domains of CCCP-1 are responsible for its large apparent molecular mass.
3. Figure S3. Alignment of CCCP-1. Shows that CC3 is the most conserved region of the protein.
4. Figure S4. Validation of CCCP1 antibody. Shows that the CCCP1 antibody recognizes CCCP1 in 832/13 cells.

Table S1. List of worm strains.

Table S2. List of plasmids.

Supplementary Figure Legends

FIGURE S1. **CCCP-1 binds to phosphatidylinositol lipids with a single phosphate group.** A, Equimolar amounts of GST-CCCP-1 or GST were incubated with membranes coated with different membrane phospholipids (PIP strips). Binding activity was detected using an antibody to the GST tag. PI(4,5)P2 Grip, a GST-tagged PLC- δ 1-PH domain protein, was used as a positive control. LPA: lysophosphatidic acid, LPC: lysophosphocholine, PI: phosphatidylinositol, PI(3)P: phosphatidylinositol (3) phosphate, PI(4)P: phosphatidylinositol (4) phosphate, PI(5)P: phosphatidylinositol (5) phosphate, PE: phosphatidylethanolamine, PC: phosphatidylcholine, S1P: sphingosine 1-phosphate, PI(3,4)P2: phosphatidylinositol (3,4) bisphosphate, PI(3,5)P2: phosphatidylinositol (3,5) bisphosphate, PI(4,5)P2: phosphatidylinositol (4,5) bisphosphate, PI(3,4,5)P3: phosphatidylinositol (3,4,5) trisphosphate, PA: phosphatidic acid, and PS: phosphatidylserine. B, CCCP-1 does not show obvious binding selectivity between phosphatidylinositol lipids. Equimolar amounts of GST-CCCP-1 or GST were incubated with membranes coated with different phosphatidylinositols of decreasing concentration (PIP arrays). Binding activity was detected using an antibody to the GST tag.

FIGURE S2. **The central CC2 domain is responsible for the large apparent molecular mass of CCCP-1.** Fractionation of CCCP-1 fragments by gel filtration on a Superose 6 column for large fragments (top) or Superdex 200 column for smaller fragments (bottom). 1 mL fractions were collected and analyzed by Western blot against the His₆ tag. IB: immunoblot.

FIGURE S3. **Alignment of the CCCP-1 protein.** Alignment of CCCP-1 proteins from *Capsaspora owczarzewski* (Capsaspora, CAOG_00459, accession # XP_004365330.2), *Monosiga brevicollis* (Choano, hypothetical protein, accession # XP_001745351.1), *Drosophila melanogaster* (Fly, golgin 104, accession # NP_648879.1), *Takifugu rubripes* (Fugu, Ccdc186, XP_011604585.1), *Homo sapiens* (Human, Ccdc186, accession # AAI03500.1), *Hydra vulgaris* (Hydra, Ccdc186-like, XP_012558241.1), *Rattus norvegicus* (Rat, Ccdc186, accession # KX954625), *Amphimedon queenslandica* (Sponge, Ccdc186-like, XP_011409991.1), *C. elegans* (Worm, CCCP-1b, accession # NP_499628.1). Identical residues are shaded in black and similar residues are shaded in grey. Alignment was made with T-Coffee¹, <http://www.ebi.ac.uk/Tools/msa/tcoffee/>, using default parameters and exhibited with BoxShade 3.21 (http://embnet.vital-it.ch/software/BOX_form.html). The coiled-coil domains of the worm protein (from SMART², <http://smart.embl-heidelberg.de>) are marked with blue bars. The CC3 domain of worm CCCP-1 is marked with a red bar. The presence of potential amphipathic helices in the CC3 domain was determined using the following settings in HeliQuest³ (<http://heliquet.ipmc.cnrs.fr>): Helix type: α , window size: 18 amino acids, Hydrophobic moment (μ H) peaks above 0.35. The predicted helices with a patch of five or more hydrophobic amino acids are numbered 1, 2 and 3. In the human ortholog, the region of helices #1 and #2 is still predicted to form amphipathic helices, but the region of helix #3 is not predicted to form an amphipathic helix.

FIGURE S4. **Validation of CCCP1 antibody.** Representative confocal image of 832/13 cells overexpressing CCCP1::GFP and costained with CCCP1 and GFP antibodies. The two signals are almost perfectly colocalized. Scale bar: 5 μ m

1. Notredame C, Higgins DG, Heringa J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol.* 2000;302(1):205-217.
2. Schultz J, Milpetz F, Bork P, Ponting CP. SMART, a simple modular architecture research tool: Identification of signaling domains. *Proc Natl Acad Sci.* 1998;95(11):5857-5864.
3. Gautier R, Douguet D, Antony B, Drin G. HELIQUEST: a web server to screen sequences with specific α -helical properties. *Bioinformatics.* 2008;24(18):2101-2102.

Table S1— Strain list

EG334 *cccp-1(ox334) III*
EG5627 *rab-2(nu415) I*
XZ1804 *yakEx101[Prab-3::cccp-1 cDNA::eGFP, Pmyo-3::mcherry]*
XZ1808 *yakEx99[Prab-3::CC2+3 cDNA::eGFP, Pmyo-3::mcherry]*
XZ1803 *yakEx100[Prab-3::CC3 cDNA::eGFP, Pmyo-3::mcherry]*
XZ1801 *yakEx98[Prab-3::CC1+2 cDNA::eGFP, Pmyo-3::mcherry]*
XZ1813 *rab-2(nu415) I; yakEx101[Prab-3::cccp-1 cDNA::eGFP, Pmyo-3::mcherry]*
XZ1812 *rab-2(nu415) I; yakEx99[Prab-3::CC2+3 cDNA::eGFP, Pmyo-3::mcherry]*
XZ1810 *rab-2(nu415) I; yakEx100[Prab-3::CC3 cDNA::eGFP, Pmyo-3::mcherry]*

XZ1809 *rab-2(nu415) I; yakEx98[Prab-3::CC1+2 cDNA::eGFP, Pmyo-3::mcherry]*
XZ1877 *oxls602[cb-unc-119(+), Pcccp-1::cccp-1 cDNA::eGFP] II ; cccp-1(ox334) III*
XZ1807 *yakSi21[Pcccp-1::CC1+2 cDNA::eGFP, cb-unc-119(+)] II ; cccp-1(ox334) III*
XZ1893 *yakSi23[Pcccp-1::CC2+3 cDNA::eGFP, cb-unc-119(+)] II ; cccp-1(ox334) III*
XZ1897 *yakSi25[Pcccp-1::CC3 cDNA::eGFP, cb-unc-119(+)] II ; cccp-1(ox334) III*
XZ2068 *yakEx132[Prab-3::cccp-1 cDNA::eGFP, Punc-129::tagRFP::rab-2, Pmyo-2::mcherry]*
XZ2070 *yakEx134[Prab-3::CC3 cDNA::eGFP, Punc-129::tagRFP::rab-2, Pmyo-2::mcherry]*

Table S2—Plasmid list

C. elegans vectors

pMA59 *Prab-3::cccp-1 cDNA::eGFP* in pCFJ150
pJC200 *Prab-3::CC2+3 cDNA::eGFP* in pCFJ150
pJC201 *Prab-3::CC1+2 cDNA::eGFP* in pCFJ150
pJC198 *Prab-3::CC3 cDNA::eGFP* in pCFJ150
pMA58 *Pcccp-1::cccp-1 cDNA::eGFP* in pCFJ150
pJC187 *Pcccp-1::CC2+3 cDNA::eGFP* in pCFJ150
pJC188 *Pcccp-1::CC1+2 cDNA::eGFP* in pCFJ150
pJC193 *Pcccp-1::CC3 cDNA::eGFP* in pCFJ150
pMA160 *Punc-129::tagRFP::rab-2* in pCFJ150

Bacterial expression vectors

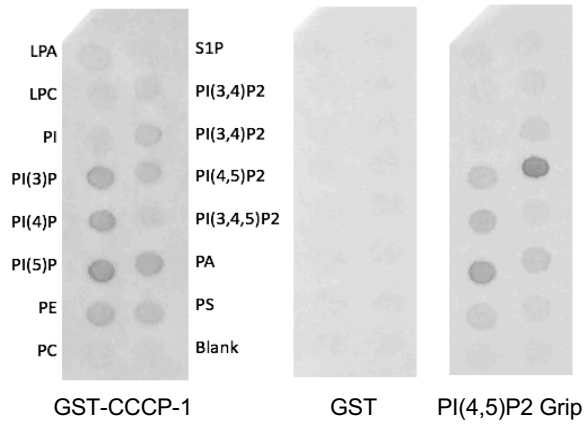
pGST parallel
pHIS parallel
pJC121 His₆-CCCP-1 in pHIS parallel
pJC158 His₆-CC1 in pHIS parallel
pJC159 His₆-CC1+2 in pHIS parallel
pJC160 His₆-CC3 in pHIS parallel
pJC161 His₆-CC2+3 in pHIS parallel
pJC190 His₆-CC2 in pHIS parallel
pJC164 GST-CCCP-1 in pGST parallel
pJC116 GST-RAB-2
pRK793 TEV protease S219V

Mammalian cell expression vectors

pET50 CCCP1::GFP (rat cDNA) in pEGFP-N1
pET159 CC1+2::GFP (rat cDNA) in pEGFP-N1
pJC218 CC3::GFP (rat cDNA) in pEGFP-N1

Figure S1

A



B

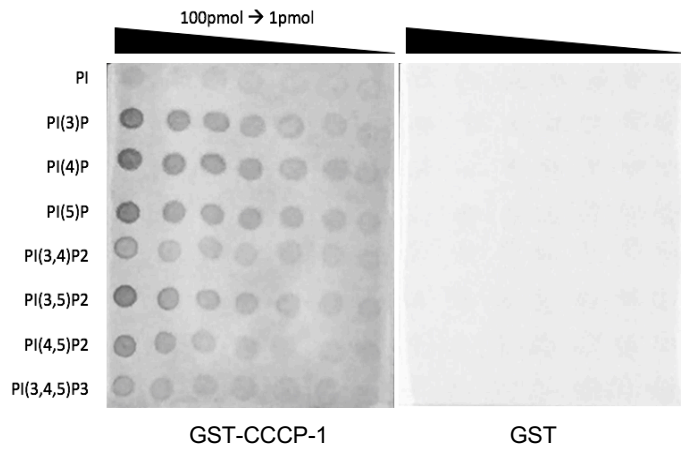


Figure S2

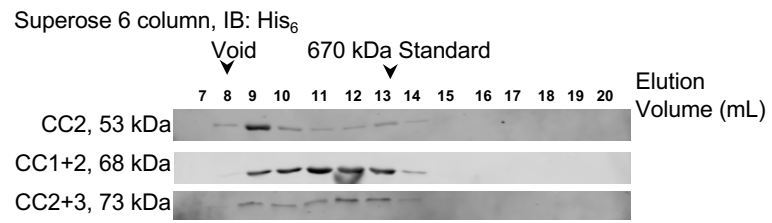


Figure S3

```

Capsaspora      1 MADLTI-----ASSAA-----
Choano          1 MDEDDD-----LLAELEA-----
Fly             1 MESAQATP-----PA-----
Fugu            1 MDPVSYSPTE--GSEGESLSKGSQDGRSANSPLNTEEEQQVCHNDQEEAEKCCQAHGTS
Human           1 MSETD-----
Hydra           1 MESFCSASDSE--LLEEYLK-----
Rat             1 MKIRSRFEEMQSELVAVS-----
Sponge          1 MMAEE-----EESIT-----
Worm            1 MEEDVV-----ESCEA-----

Capsaspora      12 -----SSDPAATSAATAL-TTDAAGAVAA-----
Choano          14 -----ELESAPDTGPDARA--DETSLATQAAMAA-----
Fly             11 -----EMENPASVEN-GDSG-RDSNHIEGKAIGD-----
Fugu            60 QALSEVMKTD2DIQTDTEVQTC-SQEQARDELHTDVISDEAFSTEGEKPSLSIQTDAA
Human           6 -----HIASTSSDKNVG-KTPELKEDSCNLFSGNESSKLENESKLLSLNTDKT
Hydra           19 -----KVQTELVVNDQNHIVTKKNLYEQGNINIQ-----
Rat             19 -----M-SE2EHIASISSDATTG-TTSELKDDSRISVSGDESSRLETGSELLSLNPDRV
Sponge          12 -----2GTNGVC-----L-KETGTGNGGSGNGL-----
Worm            12 -----2PTN-----STYG-TP-----

Capsaspora      39 -----PPSSSSS---SAA-----
Choano          41 -----IPQSGVSTEEFIRLQIQR-----QRA
Fly             38 -----VDMKADSIEQQLE-----
Fugu            119 SATPVDEV--VESSP-SVLEG-TLTFDDVKDSCAIHPSTDEHPFDLAADCTAAAPPHEDES
Human           53 LCQPNEHNNRIEAQENY----IPDHGGGEDSCAKTDIGS-----ENS
Hydra           49 -----
Rat             71 LCQTTEQCSQNEVQEDDVQEGRTPDCGSAEHSCAETDTCP-----EHS
Sponge          33 -----VNNGGTVNDPGVE-----
Worm            22 -----

Capsaspora      49 -----SSA-----SS2EP-----APPO2S2DASASA-SAATPAAPAARPNT---
Choano          62 EGTNNRSSR-----RS2MLGNMFR--GRSQ2NASVSE--SPAPSVKAVPPAE---
Fly             51 -----ESD-VK-----TE
Fugu            175 ASTVSPPSVQCGSEHSPTDSTT-TSGISNGLSTPSS2D-TVGSSPASSPQTSANTPVPLNS
Human           91 EQIANFPPSGNFAKHISKTN-2ET-EQKVTQILVELRSS-TFPE--SANEK-----TY
Hydra           49 -----TDFQVNG-----S-----
Rat             114 EQMDDCPGGNFAKPVSHTS-2EP-GHMVTQRLAEFKSS-APTE--AGDPK-----TT
Sponge          46 -----QEA-----DK2E-----G-----GTV-----
Worm            22 -----VRVASPLIHNEED-VIPT--TAVEN-----

Capsaspora      81 -----MTISIDEFAMMQHE2LVSLKTAK-YEF--AE-----
Choano          106 -----LEA--KD-----
Fly             58 SNGDQLTDQDEGKI--EQDLKAAVLEQVPI2EE2GLSLRF-----
Fugu            233 LSSPYDTCRKLMSQIQRSLSQESLLDELE2SELLACQLPAGVKSPTANGLAADQEGCVV
Human           137 SESPYDTCRKFISKIKSVSASEDLLEEIE2SELLSTEF AE-HRV--PNGMN-KGEHALV
Hydra           57 -----DSVSENLDIKKNI2EVN--LKILAEF-----
Rat             160 SASLYDTCRKLISKIKTVSASDLLGEIE2SELLSAELAEHQV--PNGVN-KGEHALA
Sponge          57 -----SY-----
Worm            44 -----S-----

Capsaspora      108 -----KEKR2VIG2OLAQ-----LEE2-QQIKDKKVKVAD2LA2EKTQ2KKR2DTLTD-----
Choano          111 -----KEIEK2LK2Q2RAQ-----QLEE-EVGLHKES-----KDQLLR-----
Fly             95 -----KDL2Q2AQEK-----VKE2Q2-QTPS-QPPQND2LSH-----
Fugu            293 VFER2CVQYN2VAQ2Q2EKA-----IQRI2LEENKRHQ2ELILG2TCSEK2DGMREE2LKKRTETE2KQH-
Human           193 LFER2CVQDK2YL2Q2Q2EHI-----IKK2L2IKENKKHQ2ELFVD2TCSEK2DN2IREE2LKKRTETE2KQH-
Hydra           80 ---KFLKEK2VS2OLENHVQLK2VQDFOLKTLEYE2TIQR2KTENIS2IVTQ2VNTEEFTE2KEST
Rat             217 LFER2CVHSR2YL2Q2Q2E2L2T-----IQ2Q2L2IKENKNHQ2ELILN2TCSEK2DN2IREE2LKKRTETE2KLH-
Sponge          59 -----EMYKTVLDERD2Q2LSL2LK2DIKQ2Q2DSF-----
Worm            45 LYS2KCN-----AV2Q2E2Q2E-----FER2ESQNAEYRE2KLLRTIRERDL-NEE2LK2KNV--Q2NQH-

```

Capsaspora 152 -----DFRNMDTELKKIRPESKANKTKID-ETLAAALTREQQAHTQAADKLSKLETHAAT
 Choano 140 -----QVKALQOLDLDDQRRVYKRSMAAINEKNSSLQAEIKR-----
 Fly 122 ---VHCLAQLE---EQRNRYEQQLEQIRTSNVQKDNMITL-----
 Fugu 348 ---MSSIKKLEGRVEELKELKESRDKLIQQDHAASALQM-----
 Human 248 ---MNTIKOLESRIEELNKEVKASRDKLIAQDVTAKNAVQQ-----
 Hydra 137 QILKKTIELEKELLQKCIKNDTIDKLSAHDAAKRAISI-----
 Rat 272 ---MNTIKOLESRIEELNKEVKASKDQIQAQDVTAKNAIQ-----
 Sponge 86 ---QAIARKLEVKCLSQQEEEMEELEERLQKE---KNTTTS-----
 Worm 93 ---KKELDAQVRRIRLELVQLKTTTDRGLAQEAHFNVTTKE-----

Capsaspora 206 AKQAATLQSALEQERLEKRALMAGSGTQATAALVDGAASSADAPSGDSTAAASAPSAAGN
 Choano 176 -----
 Fly 156 -----
 Fugu 386 -----
 Human 286 -----
 Hydra 178 -----
 Rat 310 -----
 Sponge 120 -----
 Worm 131 -----

Capsaspora 266 ADGSTASGGAVAELVLR--EMEQLRKSLEEARAAKDGLQADLDARQTEYAAAQALSKTSS
 Choano 176 -----LQONNVH--LEE---MAR-----
 Fly 156 -----IQRENAILGKEKQACRKEEMEMANKEKEATVIKFKAMKEKL-----
 Fugu 386 -----MQKEMTF--RLEQ--ANKKCEARQEKEAMVMKYVRGEKE-----
 Human 286 -----LHKEMAQ--RMEQ--ANKKCEEARQEKEAMVMKYVRGEKE-----
 Hydra 178 -----MQKENST--KIDQ--ITKMYDDCQKEKEDLNKISKVDE-----
 Rat 310 -----IHKEMAQ--RMDQ--ANKKCEARQEKEAMVMKYVRGEKE-----
 Sponge 120 -----LQEEELNK--KITE---LSQAIKNEETVMDRLSSLDQE-----
 Worm 131 -----MSQKFNL--ALQQL--ATKKAEQCDKEKNEAVVKYAMREGE-----

Capsaspora 323 QASETELA-----ALRATNEKQQKELAEIREAKESLETSEGELVAQTMRLSERV
 Choano 190 -----
 Fly 195 -----LI-----DAKKEKEAVEK---QIAEAKKEVKNVSTRFLA---VSEERK
 Fugu 422 -----AL-----DLRRDKEGLEK---RLREATKEVDRQALRGNQ---LAQDK
 Human 322 -----SL-----DLRKEKETLER---KLRDANKELEKNTNKIKQ---LSQEK
 Hydra 214 -----YIISKLESIIEDLQKCSVLQIK---KTAGOSQEVKLRGQLKS---KESDL
 Rat 346 -----AL-----DLRKEKETLER---KLRDASKELEKNTNKIKQ---LSQEK
 Sponge 152 -----RM-----KLLDEQVTLER---KTKELNRELEKAQAVVKN---QKVEL
 Worm 167 -----MM-----KLRDEISKKDS---NMKVIKEELEAAR-----K

Capsaspora 372 AGLDDKCATSVAECQTLTKTVADLTASLKQKTDEHEAATTOVKWMQTRLOSEVEAEDQVK
 Choano 190 -----EETSSDHMKPTQGGQTAS-----DSA
 Fly 231 SRMTY-----IIDEKCNEVRKYORECEKYKTEMGHLESKLYHINKLNIETEAKAVE
 Fugu 458 GRLLQQ-----LCDTKEAEVNRITREVEKMKEDTNSHLIKVQWAKLQKSEVDTHKTK
 Human 358 GRLLHQ-----LYETKEGETTRLIREIDKLKEDINSHVIKVKWAKLQKAEVDSHETK
 Hydra 261 TKLTT-----INENLENQVSKLNKKIDQKQEIDGYETKINWAKLQKTELEAKDSK
 Rat 382 GRLLQQ-----LYESKEGETTRLARETEKLKEEMNSHIKVKWAKLQKAEVDSHETK
 Sponge 188 TKLKT-----ANDKQETSLSSETTRELSKLEETSLSIKLQWAKLQKTELEGHDKTK
 Worm 194 AOSOE-----NLDDLEKTVONLKVETELKPKHERFDNFENRMKIAEKRVESLSSNLSK

Capsaspora 432 QNLAKSEKLAESKEETA AVRAT---YTSTLQ-SLQOTEKTKQOELEQRDREVSEHKAKL
 Choano 210 PGVANQPDSDATAQEEAEQRKGTVRECESTIK-QLKAT---ADQARTDLEVAQGDVAAL
 Fly 284 RKLEEEK-----NAPNKLEEK--ANEKLMFEANTI-----LLKHE-----
 Fugu 511 EKLRETTSKLTOAKEETEQIRKN---CQDMIR-TYQEEELKSNELDAKLRETKGELEKH
 Human 411 DKLKETTTLKTOAKEEADQIRKN---CQDMIK-TYQEEEEKSNELDAKLRVTKGELEKQ
 Hydra 314 KQISILTAKLQESKEEGSQIRQN---CQEMIE-RYQANEEIESVRLKIEAKEKDQRINEY
 Rat 435 DKLKETTTLKTOAKEEAEQIRSN---CQDMIK-TYQEEEEKSNELDAKLRVTKGELEKQ
 Sponge 241 SOLAATAKLLKOTKEESDQIRSD---LKKMIS-EYQEEEMRNSLQVKKLKKTEEBELRQQ
 Worm 247 QOGDMLRKOLIQAKDDKHILQ-----QYEVKLOTSTAELEERRLRESEHDVERL

Capsaspora 488 ---ERGQTESDLLWGRIKDATTLAETNAQQQFAQAQQTNTSMQQQIEQERETVTMLDNK
Choano 265 ---RQEIIVDLKLRADWATNNLEEARQ-----DAGSARDAMRKAQQEIKAEKEV
Fly 319 ---IT---SKTEALDKLTKE-----QOKLSAANKELQNOIQEITTEHNQIT---
Fugu 567 ---KQEQTDQLEVHRVKSKE-----LEDLKRYSYKESIDELRTTKLKCIEDER
Human 467 ---MOEKSDQLEMHHAKIKE-----LEDLKRFTFKEGMDERTRTKVKCKIEDER
Hydra 370 REIIESFNKKEEQYKAQIKI-----LE---RKSNDILEENLQYKDMVLKLNENN
Rat 491 ---MOEKSDQLEMHHAKIKE-----LEDLKRFTFKEGMDERTRTKTKCKIEDER
Sponge 297 ---EQEIAQQQQLLELTVKD-----LEINQASLEIANKEIQQLKNKVIHFENT-
Worm 295 ---RT-----SQLE-MAT---KFEASRENTDLLSKIDILQDQLSLEEDRR

Capsaspora 544 KLLSERVNV-----TLELVQATKFFELDSKLL---DANAKIQ
Choano 311 QRLRTAIDKEQKAM-----ADRLDTLTKAKSESQALASRLA---EIEQKHIE
Fly 359 ---EENRRLRELHNSVEGSYDELLNSAKLRGQLEELQLRTQNTINEEKLMDDQQRV
Fugu 613 PRWEDELISKYREIINRQKAE-I-----GRQEKVDEVTAEQHQQRDKQEVASIREEVE
Human 513 LRTEDELISKYKEIINRQKAE-I-----QNLLDKVKTADQLQEQQLRGKQEIENLKEEVE
Hydra 416 LSLENINNEKEKDCAEKCK-I-----DELNDKLEVLNNIKDNCNTRLEKELLDQLQDQ
Rat 537 LRTEDELISKYREIINRQKAE-I-----QNVWDKVKAAQQLQEQQLYSGKQEIENLKEEVE
Sponge 342 LEKQEEIDRMKVENAREKKE-Q-----QKLLNEKTAIQDMNKDIEKERLDYAAIKESHT
Worm 334 KLCSEQIDRLKGVESFVSS-S-----H-----RIEETEKERE

Capsaspora 576 GIEGTISTIEQNLTTANATIVALETDKQALETETVSTMTEEAATTAATLQEVGTGRDRLOA
Choano 354 SVVAEKNSIVOHNSQ-----LKTTLIEDMRLQL--DOTORESIEEQTKIQEQLAELEQ
Fly 415 KIEALVQDMETDLEQLKVKRQELLTINKEMSELIVQLONDI CLAKAKAQQGLDAE-NKLLK
Fugu 666 CLTNQMADYQHDVQGSREREAELLGFTTEKLSKNAQLOSESNAQALQDQLTSSFAELOA
Human 566 SINSLINDIQKDIEGSRKRESILLFTERTLSKNAQLOSESNSIQSQFDKVCSESQLOS
Hydra 469 INKKHINEENIEAFYKEKQOELMAYTQSLTENNVASKSVAELHAKQIEKE---DTLVK
Rat 590 SINSLINDIQKDIEGSRKRESILLFTTEKLSKNAQLOSESASSLOAVDSISCESQLOS
Sponge 395 HLQKTAQHIREVRSRRKKEAELLTFSDKMSSLNAELRTERDSLEQSVIQLE---NKLLK
Worm 366 TAEEDREQALEAAEYREQVEKMLKLTQELTERNMELQRKLDKEEGKNTSHNSTIEKLOV

Capsaspora 636 T-----LE-----ERDAKISDIEEQVSQLTATHAELOQAYATAVHDHETAITSNE
Choano 405 A-----LE-----DMTQAKAELALALEDERASATS EATYLT-----GQINEELO
Fly 474 Q-----EKL-----TYDTKYNQIEQQLSLEASEKNERLLIA-----KHISEKTK
Fugu 726 R-----LEGTT-----ELLDEKSRQLKQEEGIRROEVEGLQ-----EERTALQR
Human 626 Q-----CEQMK-----QTNINIESRLKKEEELRKEEVQTLQ-----AETACRQT
Hydra 526 Q-----SLLIS-----EKEAEIELLVNQRNEEL-HQVDEIK-----KTLIDQKNF
Rat 650 Q-----CEHMK-----QTNGDLESRLKKEEELRKEEVQSLQ-----AELSAVQT
Sponge 452 E-----VDNNE-----MIEGKLKEMADKQLQVERSCHKINSIM-----QALQDKSK
Worm 426 ELTTSLELCKSFEEENLKISEELENKTEMOKPVTLESLENFYR-----DKYDEASR

Capsaspora 682 QIQELTTKADGEVDKQILHRKMAAQKDFAKOLKIAQKRAADLDNP-----
Choano 444 QRQDMQQKTRDLEESIRLQKQKHAHTTRDMAKQLSQAQKKLASLSRQ-----
Fly 514 MYELTKQKLEEDVQGDFEATQKHAATVLRKELHRELNRYKRGITEPKTPI SYCSNCQQAING
Fugu 765 EVAQSNIRIEELKDELVTQRRKQANIKDLTKOLTQVRKRLEQVENC-----
Human 665 EVKALSTQVEELKDELVTQRRKHASSIKDLTKQLQQARRKLDQVESG-----
Hydra 564 SVIELSDLINQANDDLKIKKKNAAQLKDLQKHLVTTKIDKLESG-----
Rat 689 EARALSTQVEELKDELVTQRRKHASNVKDLKSKOLQQARRKLDQTEG-----
Sponge 494 AVEQLSVSIEEREQMVAIKRRKHLNNIKDMQRQIQLYSKRIEQLEAS-----
Worm 479 KLEQTEAKLAEKKNFSAFKKTSATLKEKSELKSGYRKNNGAGDSG-----

Capsaspora 729 -TAPHSPYSGSSLGRQS-----SFSSMSRTSEFTSSV PATPSH--HHPG
Choano 491 -PEED-AQSVASTGSMVDEPLHSPSNSSIHSR-DMNSMGGMGPTVSM LPATPEP-----
Fly 574 YPTENPQRS--HSRSSSHG-----SMHSGSRR-----ASES-----
Fugu 812 -GCDRDASSM--GSRSSSSGTTPGF-----GSLNARHGG--NGGVEE----
Human 712 -SYDKEVSSM--GSRSSSSG-----SLNARSS-----AED-----
Hydra 611 -STENLH--A--KSNISSNG-----SLEKLLSNPQ-----SSS----
Rat 736 -NYDKDVSSM--GSRSSSSG-----SLNARSS-----AED-----
Sponge 541 -TQSD-VATL--PVTSSGHM----TSGHMMR-SAHSHGSLDGSLNNIPLTNQTHST---
Worm 526 -AALGA-HVL--APPTSSDP-----SMSSRSRASSITSIDRV-----TS---

Capsaspora	770	LHDGYNGGSQRTSMLIEEDSVPPSP	SHNGR-P-----G---SMVIDDNTSE
Choano	542	-----LQPAPRTA-----	-----EVS-----
Fly	604	-----S-----	ESETV-A-S-----SATTVQQPPPO
Fugu	849	-----R-----	SPDGQMG-P-----SVVVVD-HF--
Human	739	-----R-----	SPENT-G-----SSVAVD-NF--
Hydra	640	-----D---PLMISDDHYISQPR	-----RG-----QNRSVG--
Rat	763	-----R-----	SPENT-S-----SSVAVD-NF--
Sponge	588	-----SLTPNRMSPDNFIPSP	QLOSSSPSHMHTVQGGGVSTGGG-VAAGIGL----
Worm	561	-----T-----	SREEEVS-S-----AAGEEAKRIEN----

Capsaspora	812	M-SVLEEDNKLFLQ-RVVDLQKRLDRRE	EKVSFLEDEAKALTDQVAQKTKILOHYFARED
Choano	553	--TAPSGLASLFGSRR	RAGEKQGPQORLOAKVDFLESEVNELTESIKNKNKLLQOQYFLREK
Fly	623	QDLQAVPSKRVLVE-RILRLQOQATARQ	TERIEFLENETAALVAEVQKSKVVOHYMLRDO
Fugu	866	----PEVDKSVLVD-RIVRLQKALARKQ	EKIEFMEDHIKOLVEEIRKTKIIQSYVLRRE
Human	754	----PQVDKAMLIE-RIVRLQKAHARKNE	KIEFMEDHIKOLVEEIRKTKIIQSYVLRRE
Hydra	663	----DIEIDKQVLIE-RICKLQRIHAKRNE	KIDFLNEHILHLTEDLQKKTRIIQFFLHKEE
Rat	778	----PEVDKAMLID-RIVRLQKAHARKNE	KIEFMEDHIKOLVEEIRKTKIIQSYVLRRE
Sponge	635	F-QTLDHEKTVLVD-KLCQMKRQLAKKE	EKIEFYEGEVQOLTEDIKSKSRLLIQHFIMREE
Worm	581	-EEQKLNMQIMID-KIVLQKRLARRTEK	CEFLIEEVRQCLEELQKTKIIQHFALREE

1 2

Capsaspora	870	-MGTLTPEKFDYDR-----	EORSKSK---GMMASMFGS-----KQDSTITLTL
Choano	611	-MCHLASPSTGASQ-----	POSSSAS---ARFKALLGA-----NLQOQ-HQAV
Fly	682	TAGALTTSRSDQNK-----	SELVYKG--NGIMAAIYGGGSSK--TGGENKAMSLEL
Fugu	921	-SGALSSEASDINK-----	VQLSRRG---GIMASLYTS-----HPADSGLTLDL
Human	809	-SGTSSSEASDFNK-----	VHLSRRG---GIMASLYTS-----HPADNGLTLEL
Hydra	719	-AGSLSPNLSDKIK-----	AKVSQEG---GVMASVYSA-----RPNDKAMTLEL
Rat	833	-SGTSSSEASDFNK-----	VHLSRRG---GIMASLYTS-----HPADSGLTLEL
Sponge	693	-AGALIPPEADANK-----	EQLSRRS---SSSIMGTVFKGGGVGGGASHKQAEMLEL
Worm	639	-ASLLMPSEGSLKLFANCEFVQVPIGR	KSAAYALMGAMFTS-----SGNEK-KQVOI

3

Capsaspora	910	CLEMNKRFOSLLEDMTLKNIRLQESMDT	MGAEVARIQGLLGVGDEPRTPDYATRFP
Choano	650	TQEAMAKLQEVLEDTMLQNI	ALKETIDALT-----
Fly	729	SLEINRKKLQAVLEDTLKNI	TLKENLDVGLQVDNITRKLK-----
Fugu	961	SLEINRKKLQAVLEDTLKNI	TLKENLQTLGAEIERLIKQO-----
Human	849	SLEINRKKLQAVLEDTLKNI	TLKENLQTLGTEIERLIKHO-----
Hydra	759	SLQINNKLQAVLEDTLKNI	MLKENLDTLGSEIDKLNEM-----
Rat	873	SLEINRKKLQAVLEDTLKNI	TLKENLQTLGTEIERLIKHO-----
Sponge	742	SLEINRKKLQAVLEDTLKNI	TLKESLNTLQGEVARLSAQIP-----
Worm	690	MTEVNSRLQAVLEDVIQKNILMRSSVD	TLSADNTRLSREN-----

3

Capsaspora	970	VLETASAPSTPARHPANEIPAQSLTDSAS	VLTVTDAARVPSSREHPHVDVSHPDSVEIVL
Choano	680	-----	-----
Fly	770	-----	-----
Fugu	1001	-----	-----
Human	890	-----	-----
Hydra	799	-----	-----
Rat	914	-----	-----
Sponge	783	-----	-----
Worm	731	-----	-----

Capsaspora	1030	TTATPLADAPVDLSLAAPAADDNASVP	VADLAPEQASVPVADLAPEQDSAQEPAAVEPAV
Choano	680	-----	-----
Fly	770	-----	-----
Fugu	1001	-----	-----
Human	890	-----	-----
Hydra	799	-----	-----
Rat	914	-----	-----
Sponge	783	-----	-----
Worm	731	-----	-----

Capsaspora	1090	AEQAPVEQAPVEPAAVEPAAVEPAAAAPEPAAAAEQPAPVEAPALESSPAEDQPIQQADA
Choanoflagellat	680	-----
Fly	770	-----
Fugu	1001	-----
Human	890	-----
Hydra	799	-----
Rat	914	-----
Sponge	783	-----
Worm	731	-----

Capsaspora	1150	PEIAPDSTIAAEPLPEQASDEAQPDPANPVTDAAAESAPLPDAVPAEETTPDVPSQDE---
Choanoflagellat	680	-----
Fly	770	-----SL---
Fugu	1001	-----
Human	890	-----EL---
Hydra	799	-----
Rat	914	-----EL---
Sponge	783	-----K---
Worm	731	-----LLSLS

Capsaspora	1207	-ASEDSL
Choanoflagellat	680	-----KA
Fly	772	---EGSCK
Fugu	1001	-----RS
Human	892	-EQRTKKT
Hydra	799	---RSSKK
Rat	916	-EQRTKKA
Sponge	784	-NTSTSNR
Worm	736	<u>QVRTTQDN</u>




-  CC3 fragment
-  Predicted Coiled-Coil domains
-  Predicted Amphipathic helix

Figure S4

