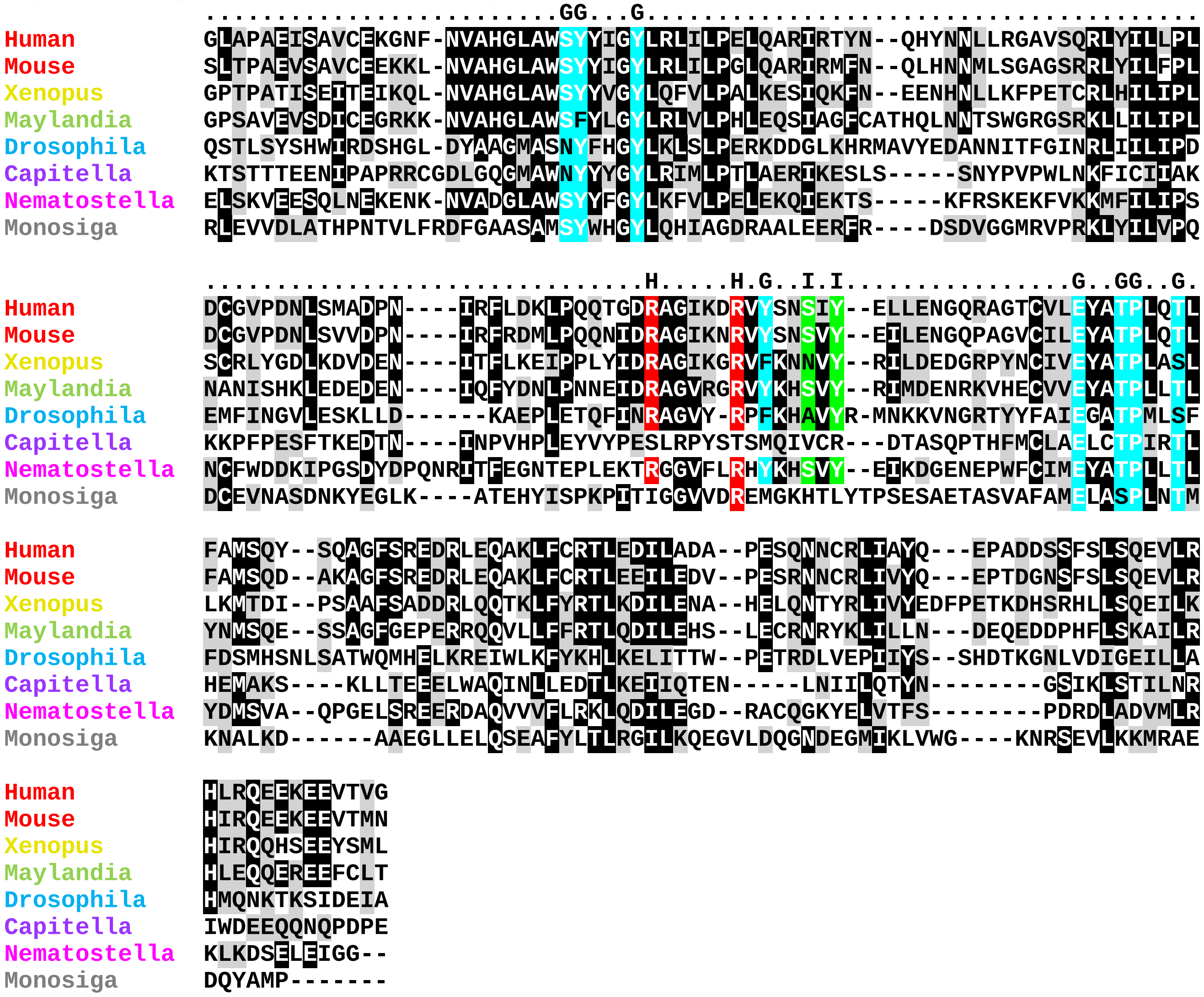
SUPPLEMENTAL FIGURES

Figure S1. Alignment of STING homologs



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                 .........................GG...G.......................................
Human            GLAPAEISAVCEKGNF-NVAHGLAWSYYIGYLRLILPELQARIRTYN--QHYNNLLRGAVSQRLYILLPL
Mouse            SLTPAEVSAVCEEKKL-NVAHGLAWSYYIGYLRLILPGLQARIRMFN--QLHNNMLSGAGSRRLYILFPL
Xenopus          GPTPATISEITEIKQL-NVAHGLAWSYYVGYLQFVLPALKESIQKFN--EENHNLLKFPETCRLHILIPL
Maylandia        GPSAVEVSDICEGRKK-NVAHGLAWSFYLGYLRLVLPHLEQSIAGFCATHQLNNTSWGRGSRKLLILIPL
Drosophila       QSTLSYSHWIRDSHGL-DYAAGMASNYFHGYLKLSLPERKDDGLKHRMAVYEDANNITFGINRLIILIPD
Capitella        KTSTTTEENIPAPRRCGDLGQGMAWNYYYGYLRIMLPTLAERIKESLS-----SNYPVPWLNKFICIIAK
Nematostella     ELSKVEESQLNEKENK-NVADGLAWSYYFGYLKFVLPELEKQIEKTS-----KFRSKEKFVKKMFILIPS
Monosiga         RLEVVDLATHPNTVLFRDFGAASAMSYWHGYLQHIAGDRAALEERFR----DSDVGGMRVPRKLYILVPQ

                 ...............................H.....H.G..I.I................G..GG..G.
Human            DCGVPDNLSMADPN----IRFLDKLPQQTGDRAGIKDRVYSNSIY--ELLENGQRAGTCVLEYATPLQTL
Mouse            DCGVPDNLSVVDPN----IRFRDMLPQQNIDRAGIKNRVYSNSVY--EILENGQPAGVCILEYATPLQTL
Xenopus          SCRLYGDLKDVDEN----ITFLKEIPPLYIDRAGIKGRVFKNNVY--RILDEDGRPYNCIVEYATPLASL
Maylandia        NANISHKLEDEDEN----IQFYDNLPNNEIDRAGVRGRVYKHSVY--RIMDENRKVHECVVEYATPLLTL
Drosophila       EMFINGVLESKLLD------KAEPLETQFINRAGVY-RPFKHAVYR-MNKKVNGRTYYFAIEGATPMLSF
Capitella        KKPFPESFTKEDTN----INPVHPLEYVYPESLRPYSTSMQIVCR---DTASQPTHFMCLAELCTPIRTL
Nematostella     NCFWDDKIPGSDYDPQNRITFEGNTEPLEKTRGGVFLRHYKHSVY--EIKDGENEPWFCIMEYATPLLTL
Monosiga         DCEVNASDNKYEGLK----ATEHYISPKPITIGGVVDREMGKHTLYTPSESAETASVAFAMELASPLNTM

Human            FAMSQY--SQAGFSREDRLEQAKLFCRTLEDILADA--PESQNNCRLIAYQ---EPADDSSFSLSQEVLR
Mouse            FAMSQD--AKAGFSREDRLEQAKLFCRTLEEILEDV--PESRNNCRLIVYQ---EPTDGNSFSLSQEVLR
Xenopus          LKMTDI--PSAAFSADDRLQQTKLFYRTLKDILENA--HELQNTYRLIVYEDFPETKDHSRHLLSQEILK
Maylandia        YNMSQE--SSAGFGEPERRQQVLLFFRTLQDILEHS--LECRNRYKLILLN---DEQEDDPHFLSKAILR
Drosophila       FDSMHSNLSATWQMHELKREIWLKFYKHLKELITTW--PETRDLVEPIIYS--SHDTKGNLVDIGEILLA
Capitella        HEMAKS----KLLTEEELWAQINLLEDTLKEIIQTEN-----LNIILQTYN--------GSIKLSTILNR
Nematostella     YDMSVA--QPGELSREERDAQVVVFLRKLQDILEGD--RACQGKYELVTFS--------PDRDLADVMLR
Monosiga         KNALKD------AAEGLLELQSEAFYLTLRGILKQEGVLDQGNDEGMIKLVWG----KNRSEVLKKMRAE

Human            HLRQEEKEEVTVG
Mouse            HIRQEEKEEVTMN
Xenopus          HIRQQHSEEYSML
Maylandia        HLEQQEREEFCLT
Drosophila       HMQNKTKSIDEIA
Capitella        IWDEEQQNQPDPE
Nematostella     KLKDSELEIGG--
Monosiga         DQYAMP-------
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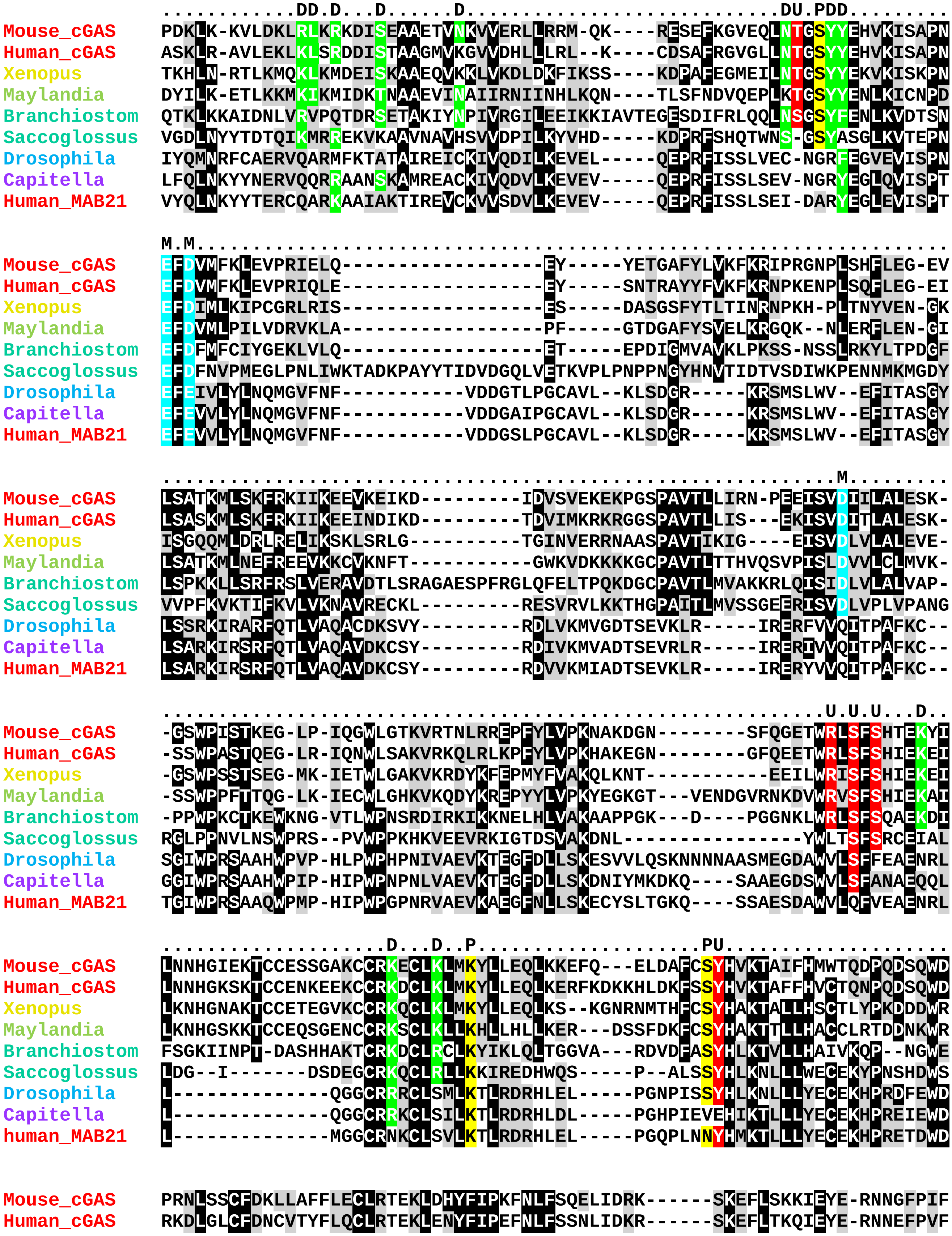
Homologs of human STING were retrieved by BlastP search of genomes from vertebrate and invertebrate phyla as indicated in the legend to figure 1. Sequences were aligned using M-coffee and the segment corresponding to the structurally analyzed STING-C domain (1-5) (6) is shown above, with the name of the species from which the sequence is derived color-coded to represent phylum as in figure 1A.

Residues that are identical or similar in at least half of the aligned proteins are highlighted in black and grey, respectively. Amino acid residues that interact with both c-di-GMP and 2’3’cGAMP are highlighted in blue and marked above the sequence with “G”. Residues that specificially interact with 2’3’cGAMP are highlighted in red and marked with “H”, and residues that mediate interdomain interactions induced by 2’3’cGAMPS are highlighted in green and marked with “I”.

Genbank ids. *Homo sapiens*: NP_938023; *Mus musculus*: NP_082537; *Xenopus tropicalus*: NP_001106445; *Maylandia zebra*: XP_004563256; *Drosophila erecta*: XP_001969143; *Capitella teleta*: ELT98160; *Nematostella vectensis*: XP_001627385; *Monosiga brevicollis*: EDQ90889. Additional sequences used in phylogeny (Fig. 1A): *Gallus gallus*: XP_001232171; *Anolis carolinensis*: XP_003220124; *Pimephales promelas*: CCI55628; *Danaus plexippus*: EHJ74437; *Camponotus floridanus*: EFN63910; *Daphnia pulex*: EFX66211; *Hydra magnipapillata*: XP_004207409.

Figure S2. Alignment of cGAS homologs



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              ............DD.D...D......D............................DU.PDD.........
Mouse_cGAS    PDKLK-KVLDKLRLKRKDISEAAETVNKVVERLLRRM-QK----RESEFKGVEQLNTGSYYEHVKISAPN
Human_cGAS    ASKLR-AVLEKLKLSRDDISTAAGMVKGVVDHLLLRL--K----CDSAFRGVGLLNTGSYYEHVKISAPN
Xenopus       TKHLN-RTLKMQKLKMDEISKAAEQVKKLVKDLDKFIKSS----KDPAFEGMEILNTGSYYEKVKISKPN
Maylandia     DYILK-ETLKKMKIKMIDKTNAAEVINAIIRNIINHLKQN----TLSFNDVQEPLKTGSYYENLKICNPD
Branchiostom  QTKLKKAIDNLVRVPQTDRSETAKIYNPIVRGILEEIKKIAVTEGESDIFRLQQLNSGSYFENLKVDTSN
Saccoglossus  VGDLNYYTDTQIKMRREKVKAAVNAVHSVVDPILKYVHD-----KDPRFSHQTWNS-GSYASGLKVTEPN
Drosophila    IYQMNRFCAERVQARMFKTATAIREICKIVQDILKEVEL-----QEPRFISSLVEC-NGRFEGVEVISPN
Capitella     LFQLNKYYNERVQQRRAANSKAMREACKIVQDVLKEVEV-----QEPRFISSLSEV-NGRYEGLQVISPT
Human_MAB21   VYQLNKYYTERCQARKAAIAKTIREVCKVVSDVLKEVEV-----QEPRFISSLSEI-DARYEGLEVISPT

              M.M...................................................................
Mouse_cGAS    EFDVMFKLEVPRIELQ------------------EY-----YETGAFYLVKFKRIPRGNPLSHFLEG-EV
Human_cGAS    EFDVMFKLEVPRIQLE------------------EY-----SNTRAYYFVKFKRNPKENPLSQFLEG-EI
Xenopus       EFDIMLKIPCGRLRIS------------------ES-----DASGSFYTLTINRNPKH-PLTNYVEN-GK
Maylandia     EFDVMLPILVDRVKLA------------------PF-----GTDGAFYSVELKRGQK--NLERFLEN-GI
Branchiostom  EFDFMFCIYGEKLVLQ------------------ET-----EPDIGMVAVKLPKSS-NSSLRKYLTPDGF
Saccoglossus  EFDFNVPMEGLPNLIWKTADKPAYYTIDVDGQLVETKVPLPNPPNGYHNVTIDTVSDIWKPENNMKMGDY
Drosophila    EFEIVLYLNQMGVFNF-----------VDDGTLPGCAVL--KLSDGR-----KRSMSLWV--EFITASGY
Capitella     EFEVVLYLNQMGVFNF-----------VDDGAIPGCAVL--KLSDGR-----KRSMSLWV--EFITASGY
Human_MAB21   EFEVVLYLNQMGVFNF-----------VDDGSLPGCAVL--KLSDGR-----KRSMSLWV--EFITASGY

              ............................................................M.........
Mouse_cGAS    LSATKMLSKFRKIIKEEVKEIKD---------IDVSVEKEKPGSPAVTLLIRN-PEEISVDIILALESK-
Human_cGAS    LSASKMLSKFRKIIKEEINDIKD---------TDVIMKRKRGGSPAVTLLIS---EKISVDITLALESK-
Xenopus       ISGQQMLDRLRELIKSKLSRLG----------TGINVERRNAASPAVTIKIG----EISVDLVLALEVE-
Maylandia     LSATKMLNEFREEVKKCVKNFT-----------GWKVDKKKKGCPAVTLTTHVQSVPISLDVVLCLMVK-
Branchiostom  LSPKKLLSRFRSLVERAVDTLSRAGAESPFRGLQFELTPQKDGCPAVTLMVAKKRLQISIDLVLALVAP-
Saccoglossus  VVPFKVKTIFKVLVKNAVRECKL---------RESVRVLKKTHGPAITLMVSSGEERISVDLVPLVPANG
Drosophila    LSSRKIRARFQTLVAQACDKSVY---------RDLVKMVGDTSEVKLR-----IRERFVVQITPAFKC--
Capitella     LSARKIRSRFQTLVAQAVDKCSY---------RDIVKMVADTSEVRLR-----IRERIVVQITPAFKC--
Human_MAB21   LSARKIRSRFQTLVAQAVDKCSY---------RDVVKMIADTSEVKLR-----IRERYVVQITPAFKC--

              ...........................................................U.U.U...D..
Mouse_cGAS    -GSWPISTKEG-LP-IQGWLGTKVRTNLRREPFYLVPKNAKDGN--------SFQGETWRLSFSHTEKYI
Human_cGAS    -SSWPASTQEG-LR-IQNWLSAKVRKQLRLKPFYLVPKHAKEGN--------GFQEETWRLSFSHIEKEI
Xenopus       -GSWPSSTSEG-MK-IETWLGAKVKRDYKFEPMYFVAKQLKNT-----------EEILWRISFSHIEKEI
Maylandia     -SSWPPFTTQG-LK-IECWLGHKVKQDYKREPYYLVPKYEGKGT---VENDGVRNKDVWRVSFSHIEKAI
Branchiostom  -PPWPKCTKEWKNG-VTLWPNSRDIRKIKKNELHLVAKAAPPGK---D----PGGNKLWRLSFSQAEKDI
Saccoglossus  RGLPPNVLNSWPRS--PVWPPKHKVEEVRKIGTDSVAKDNL----------------YWLTSFSRCEIAL
Drosophila    SGIWPRSAAHWPVP-HLPWPHPNIVAEVKTEGFDLLSKESVVLQSKNNNNAASMEGDAWVLSFFEAENRL
Capitella     GGIWPRSAAHWPIP-HIPWPNPNLVAEVKTEGFDLLSKDNIYMKDKQ----SAAEGDSWVLSFANAEQQL
Human_MAB21   TGIWPRSAAQWPMP-HIPWPGPNRVAEVKAEGFNLLSKECYSLTGKQ----SSAESDAWVLQFVEAENRL

              ....................D...D..P....................PU....................
Mouse_cGAS    LNNHGIEKTCCESSGAKCCRKECLKLMKYLLEQLKKEFQ---ELDAFCSYHVKTAIFHMWTQDPQDSQWD
Human_cGAS    LNNHGKSKTCCENKEEKCCRKDCLKLMKYLLEQLKERFKDKKHLDKFSSYHVKTAFFHVCTQNPQDSQWD
Xenopus       LKNHGNAKTCCETEGVKCCRKQCLKLMKYLLEQLKS--KGNRNMTHFCSYHAKTALLHSCTLYPKDDDWR
Maylandia     LKNHGSKKTCCEQSGENCCRKSCLKLLKHLLHLLKER---DSSFDKFCSYHAKTTLLHACCLRTDDNKWR
Branchiostom  FSGKIINPT-DASHHAKTCRKDCLRCLKYIKLQLTGGVA---RDVDFASYHLKTVLLHAIVKQP--NGWE
Saccoglossus  LDG--I-------DSDEGCRKQCLRLLKKIREDHWQS-----P--ALSSYHLKNLLLWECEKYPNSHDWS
Drosophila    L--------------QGGCRRRCLSMLKTLRDRHLEL-----PGNPISSYHLKNLLLYECEKHPRDFEWD
Capitella     L--------------QGGCRRKCLSILKTLRDRHLDL-----PGHPIEVEHIKTLLLYECEKHPREIEWD
human_MAB21   L--------------MGGCRNKCLSVLKTLRDRHLEL-----PGQPLNNYHMKTLLLYECEKHPRETDWD

Mouse_cGAS    PRNLSSCFDKLLAFFLECLRTEKLDHYFIPKFNLFSQELIDRK------SKEFLSKKIEYE-RNNGFPIF
Human_cGAS    RKDLGLCFDNCVTYFLQCLRTEKLENYFIPEFNLFSSNLIDKR------SKEFLTKQIEYE-RNNEFPVF
```

```
Xenopus        PKDLAACFDRYIEDFMKCLKDARLPNFFIPSLNLFSEELIPKK------CLDYLLRKLDKQ-KKLQYTLF
Maylandia      VSDLSDCFQVLLQDFEGYLENGQLCNFFIPSQNLFSGIRVCKG---------LSDAIREE-RRKGFPIF
Branchiostom   NDNLVTCIVYVIDELVQCIIKRHLPHFFIPGYNLFDPAVFRGLRHLDAVKNHFLRLNCVLQKYKRRSSLL
Saccoglossus   RDMLGQRFVGLVGSLLKCLRGQNCPHYFIPSINLFRNKENSKLKE---PENLLLIADKVQEFYENPCGFI
Drosophila     ESCIADRINGIFLQLISCLQYRRCPHYFLPGLDMFKGKSPSALEN---AAKQVWRLTREMLTNANAFEKL
Capitella      ETCLGDRINGILLQMISCLQNRRCPYYFLPNMDLFRGKSASSMDA---AAKQAWRILRELLTNSRSLEKL
Human_MAB21    ESCLGDRLNGILLQLISCLQCRRCPHYFLPNLDLFQGKPHSALES---AAKQTWRLAREILTNPKSLDKL
```

Sequences of homologs of mouse cGAS were retrieved, aligned and presented with color-coded species names as described above. The segment corresponding to the crystallized mouse cGAS domain is shown (7).

Residues that are identical or similar in at least half of the aligned proteins are highlighted in black and grey, respectively. Amino acid residues that interact with DNA are highlighted in green and marked with “D” above the sequence. Residues that interact with the purine moiety of the substrates GTP and/or ATP are highlighted in red and marked with “U”. Residues that interact the γ-phosphate of ATP or GTP are highlighted in yellow and marked “P”, while residues that interact via a Me^{2+} ion with the α- or β- phosphate are highlighted in blue and marked “M”.

Genbank ids: *Mus musculus* cGAS: NP_775562; *Homo sapiens* cGAS: NP_612450; *Xenopus tropicalis*: XP_002937200; *Maylandia zebra*: XP_004553495; *Branchiostoma floridae*: XP_002596738; *Saccoglossus kowalevski*: XP_002740936; *Drosophila ananassae*: XP_001966298; *Capitella teleta*: ELT95908; *Homo sapiens* MAB21L2: AAG09800. Additional sequences used in phylogeny (Fig. 1B): *Gallus gallus*: XP_419881; *Anolis carolinensis*: XP_003226105; *Metaseiulus occidentalis*: XP_003738072; *Crassostrea gigas*: EKC35425; *Nematostella vectensis*: XP_001634789; *Mus musculus* MAB21L2: AAD40478.

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