## Supplementary Information

A sex-inducing pheromone triggers cell cycle arrest and mate attraction in the diatom Seminavis robusta

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Supplementary Figure S1. The cytostatic pheromone has no effect on cells above the SST. Means $\pm$ s.e. ( $\mathrm{n}=3$ ) of proportions of mitotically dividing $\mathrm{MT}^{+}$or $\mathrm{MT}^{-}$cells above the $\mathrm{SST}, 9 \mathrm{~h}$ after illumination, depending on the administration of fresh medium (blank), medium from a mating culture or medium from the opposite mating partner (above or below the SST).


Supplementary Figure S2. Cytostatic effect of MT medium. Light microscopy images of $\mathrm{MT}^{+}$(85B) cells treated for 8 h with control medium (i-ii), or with filtered medium of $\mathrm{MT}^{-}$( 85 A ) cells below the SST (iii-iv). Cells are shown in the girdle (i, iii) or valve (ii, iv) view. Scale bar $=10 \mu \mathrm{~m}$.


Supplementary Figure S3. Mass spectral analysis of SIP ${ }^{+}$. The $\mathrm{MS}^{2}$ spectrum of $[\mathrm{M}-\mathrm{H}]^{-}=842$ acquired by LC-ESI-Orbitrap mass spectrometry indicated a sulphated molecule.


Supplementary Figure S4. Differential expression of meiosis-related genes after treatment with SIP ${ }^{+}$. Mean $\pm$s.e. ( $\mathrm{n}=3$ ) counts per million (cpm) for MRE11, MCM8, RAD50 and MSH4 in untreated (blue) and treated (red) cultures.
a
BT_AC1_C1 291 RIFHKIYIQRHDNVSILFADIVGFTGLASQCT. . . AQELVKLLNELFGKFDELATE. . .NHCRRIKILGDCYYCVSGLT BT_AC1_C2 857 PRNMDLYYQSYSQVGVMFASIPNFNDFYIELDGNNMGVECLRLLNEIIADFDELMDKDFYKDLEKIKTIGSTYMAAVGLA BT_AC2_C2 872 LKNEELYHQSYDCVCVMFASIPDFKEFYTESDVNKEGLECLRLLNEIIADFDDLLSKPKFSGVEKIKTIGSTYMAATGLS DD ACG 382 ENGEDVVAERSNNACVFFLDIAGFTRFSSIHS....PEQVIQVLIKIFNSMDLLCAK...HGIEKIKTIGDAYMATCGIF BT_sGCa 468 WQGHAVQAKRFGNVTMLFSDIVGFTAICSQCS....PLQVITMLNALYTRFDRQCGE...LDVYKVETIGDAYCVAGGLH BT_sGCb 407 RHKRPVPAKRYDNVTILFSGIVGFNAFCSKHASGEGAMKIVNLLNDLYTRFDTLTDSRKNPFVYKVETVGDKYMTVSGLP HS rGC1 866 KTGTPVEPEYFEQVTLYFSDIVGFTTISAMSE....PIEVVDLLNDLYTLFDAIIGS... HDVYKVETIGDAYMVASGLP
$\frac{\text { SR NC/PDE } 582 \text { NDSMKPIADLFPNTTVMFADISGFIAWSSTRD. ...PSQVFMLLQRIYGEFDEVARRRN...VFKVETIGDCYVACAGLP }}{* *}$

| BT_AC1_C1 |  |
| :---: | :---: |
| BT_AC1_C2 | .PTAGTRAKKSISSHLSTLADFAIDMFDVLDEINYQSY. .NDFVLRVGINVGPVVAGVI . . . . . . GARRPQYDIWGNTVNVASRMD |
| BT_AC2_C2 | AIPSQEHAQEPERQYMHIGTMVEFAYALVGKLDAINKHSF. .NDFKLRVGINHGPVIAGVI. . . . . GAQKPQYDIWGNTVNVASRMD |
| DD ACG | P........KCDDIRHNTYKMLGFAMDVLEFIPKEMSFHL . . GLQVRVGIHCGPVISGVI. . . . . SGYAKPHFDVWGDTVNVASRME |
| BT_sGCa | KESDTHAVQIALMALKMMELSHEVVSPHG. . EPIKMRIGLHSGSVFAGVV. . . . . . GVKMPRYCLFGNNVTLANKFE |
| BT_sGCb | EPCIHHARSICHLALDMMEIAGQVQVD.G..ESVQITIGIHTGEVVTGVI.......GQRMPRYCLFGNTVNLTSRTE |
| HS rGC1 | .QRNGQRHAAEIANMSLDILSAVGTFRMRHMPEVPVRIRIGLHSGPCVAGVV. . . . . .GLTMPRYCLFGDTVNTASRME |
| SR NC/PDE |  |
| b | *** |
| HS_PDE4A | LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYH |
| HS_PDE7B | LSKVGMWDFDIFLFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVQEDYHSQNPYHNAVHAADVTQAMHCYLKEPK |
| HS_PDE8B1 | LDNEESWDFNIFELEA ITHKRPLVYLGLKVFSRFGVCEFLNCSETTLRAWFQVIEANYHSSNAYHNSTHAADVLHATAFFLGKER |
| BT_PDE5 | TLKITDFSFSDFELS----DLETALCTIRMFTDLNLVQNFQMKHEVLCKwILSVKKNYRKNVAYHNWRHAFNTAQCMFAALKAGK |
| HS_PDE6 | SAELYEFRFSDFPLT----EHGLIKCGIRLFFEINVVEKFKVPVEVLTRWMYTVRKGYR-AVTYHNWRHGFNVGQTMFTLLMTGR |
| HS_PDE9 | IEALRKPTFDVWLWE----PNEMLSCLEHMYHDLGLVRDFSINPVTLRRWLFCVHDNYR-NNPFHNFRHCFCVAQMMYSMVWLCS--------LQ |
| SR_NC/PDE | VEAIKLPDFDDNAVT--------------EEDLSFVHIPEVVVDQLRAHVTAIAESYQ-DNPFHNFEHACHVTMSTTKFLGRIASMEIDLPTLQGKDARAAVAS |
|  | : . . : . : * : ** * . : |
| HS_PDE4A | AIHDVDHPGVSNQFLINTNSELALMYNDESVLENHHLAVGFKLL-QEDNCDI---FQNLS--------AVFTDLEILAALFAAKRQRQSLRKMVIDMVLATDMSK |
| HS_PDE7B | AAHDVDHPGVNQPELIKTNHHLANLYQNMSVLENHHWRSTIGML-RESR--L---LAHLP--------SFLTPLDIMLGLLAAKEMTQDIEQQLGSLILATDINR |
| HS_PDE8B1 | TVHDVDHPGRTNSFLCNAGSELAVLYNDTAVLESHHTALAFQLTVKDTKCNI---FKNID--------GSLDQLDEVAALIAARNHYRTLRQAIIDMVLATEMTK |
| BT-PDE5 | LSHDLDHRGVNNSYIQRSEHPLAQLYC-HSIMEHHHFDQCLMIL-NSPGNQI---LSGLS--------KRLTDLEILALLIAAIEEYKTTLKIIKQAILATDLAL |
| HS_PDE6 | FCHDIDHRGTNNLYQMKSTSPLARLHG-SSILERHHLEYSKTLL-QDESLNI---FQNLN--------KYYTDLEAFAMLAAAKRQFETVIHLFEVAIIATDLAL |
| HS_PDE9 | ICHDLDHPGYNNTYQINARTELAVRYNDISPLENHHCAVAFQIL-AEPECNI---FSNIP--------EKFSQTDILILMTAAPDGFKQIRQGMI TLILATDMAR |
| SR_NC/PDE | LIHDCDHAGVGNAQTIKENPKMGEKYQGKSVAEQNSLDIAWNLLMSERFAELRALLFANDHIHDYTHGILSDPLTLFGIVYSSKAEMARFRQVVVNIVLATDIFD |
|  | ** ** : . : : : * : . : |
| HS_PDE4A | HMTLLADLKTMVETKKVTSS-------GVLLLDNYSDRIQVLRNMVHCADLSNPTKPLELYRQWTDRIMAEFFQQGDRERERGME-ISPMCDKHT-AS |
| HS_PDE7B | QNEFLTRLKAHLHNK-------------DLRLEDAQDRHFMLQIALKCADICNPCRIWEMSKQWSERVCEEFYRQGELEQKFELE- ISPLCNQQK-DS |
| HS_PDE8B | HFEHVNKFVNS INKPMAAEIEGSDCECNPAGKNFPENQILIKRMMIKCADVANPCRPLDLCIEWAGRISEEYFAQTDEEKRQGLPVVMPVFDRNT-CS |
| BT_PDE5 | YIKRRGEFFELIMK------------NQFNLEDPHQKELFLAMLMTACDLSAITKPWPIQQRIAELVATEFFDQGDRERKELNIEPADLMNREKKNK |
| HS_PDE6 | YFKKRTMFQKIVDACE---QMQTEEEAIKYVTVDPTKKEIIMAMMMTACDLSAITKPWEVQSQVALMVANEFWEQGDLERTVLQQQP |
| HS_PDE9 | HAEIMDSFKEKMENF--------------DYSNEEHMTLLKMILIKCCDISNEVRPMEVAEPWVDCLLEEYFMQSDREKSEGLP-VAPFMDRDK-VT |
| SR_NC/PDE | KE--LNGLRKGRWNKA--------FEESAEGSNSHLRATIVLEHIIQASDVSHTMQHWHIYRKWNRRLFLEMYSAYKQGRMGAD--PS--------SF |

Supplementary Figure S5. Conservation of the cyclase and phosphodiestase domains of GC/PDE. (A) Sequence alignment of the catalytic core of the following adenylyl/guanylyl cyclases: BT_ACI_C1 (NP_776654.1), BT_ACI_C2 (NP_776654.1), BT_ACII_C2 (NP_112269.1), DD_ACG (Q03101.2), BT_sGC $\alpha$ (NP_786972.1), BT_sGC $\beta$ ( $\overline{\mathrm{N}}$ _ $7 \overline{7} 7066.1$ ), ${ }^{-}$HS_rGC1 (AAI48422.1) and SR_NC/PDE ( $\mathrm{BT}=$ Bos Taurus, $\mathrm{DD}=$ Dictyostelium discoideum, $\mathrm{HS}=$ Homo sapiens, $\mathrm{SR}=$ Seminavis robusta).The first four proteins are adenylyl cyclases, the next three are guanylyl cyclases. Adenine/guanine binding residues are indicated in yellow in SR_NC/PDE and catalytic sites are indicated in red, conversed residues are indicated with * (adapted from Liu et al. (1997)). (B) Sequence alignment of part of the following PDE proteins: three cAMP-specific PDEs (PDE4, PDE7 and PDE8), three cGMP-specific PDEs (PDE5, PDE6 and PDE9) and the bifunctional $S$. robusta GC/PDE. The eight amino acids conserved only in cAMP-hydrolyzing PDEs are indicated in red.

Table S1: General statistics on the S. robusta transcriptome

| total number of transcripts | 50231 |
| :--- | :--- |
| total transcriptome length | 71.10 Mbp |
| minimal transcript length | 501 bp |
| maximal transcript length | 17339 bp |
| average transcript length | 1416 bp |
| N50 | 1730 bp |

Table S2: See file: TableS2.xlsx
Table S3: Primers used in the RT-qPCR on CYCA/B1, CYCB1, P5CS and GC/PDE.

| gene | forward primer | reverse primer |
| :--- | :--- | :--- |
| $\mathbf{V 1}^{1}$ | CAAGGCAAGAAGGATGGCAAG | GCGACAAGCAAATTAGCAAACC |
| $\mathbf{V 4}^{1}$ | AGGCTACCGTGGGACTTGG | GATCTGGACTGCGCTGGTTC |
| $\mathbf{C Y C A / B 1}$ | GCTTGGGTCGATGCAATACT | ATCCGAGAGGGTCAAGAGGT |
| $\mathbf{C Y C B 1}$ | GGGATGTAACAAACGCCAAT | CCTCTCATCCATGACGGACT |
| $\mathbf{P 5 C S}$ | AATCGAAAACGGGGTAGCTT | CCCTCGACAATCTCAACCAT |
| GC/PDE | TCCTTCCCAAGTTTTCATGC | CATGGTTTGGTTGCTTGTTG |
|  |  |  |
| ${ }^{1}$ Normalization genes. |  |  |

