## **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. (n = 3) of proportions of mitotically dividing MT<sup>+</sup> or MT<sup>-</sup> cells above the SST, 9 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<sup>-</sup> medium. Light microscopy images of MT<sup>+</sup> (85B) cells treated for 8 h with control medium (i-ii), or with filtered medium of MT<sup>-</sup> (85A) cells below the SST (iii-iv). Cells are shown in the girdle (i, iii) or valve (ii, iv) view. Scale bar = 10  $\mu$ m.



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



Supplementary Figure S4. Differential expression of meiosis-related genes after treatment with SIP<sup>+</sup>. Mean  $\pm$  s.e. (n=3) counts per million (cpm) for *MRE11*, *MCM8*, *RAD50* and *MSH4* in untreated (blue) and treated (red) cultures.

a	
BT_AC1_C1	291 RIFHKIYIQRHDNVSILFADIVGFTGLASQCTAQELVKLLNELFGKFDELATENHCRRIKILGDCYYCVSGLT
BT_AC1_C2	857 PRNMDLYYQSYSQVGVMFASIPNFNDFYIELDGNNMGVECLRLLNEIIADFDELMDKDFYKDLEKIKTIGSTYMAAVGLA
BT_AC2_C2	872 LKNEELYHQSYDCVCVMFASIPDFKEFYTESDVNKEGLECLRLLNEIIADFDDLLSKPKFSGVEKIKTIGSTYMAATGLS
DD ACG	382 ENGEDVVAERSNNACVFFLDIAGFTRFSSIHSPEQVIQVLIKIFNSMDLLCAKHGIEKIKTIGDAYMATCGIF
BT_sGCa	468 WQGHAVQAKRFGNVTMLFSDIVGFTAICSQCSPLQVITMLNALYTRFDRQCGELDVYKVETIGDAYCVAGGLH
BT_sGCb	407 RHKRPVPAKRYDNVTILFSGIVGFNAFCSKHASGEGAMKIVNLLNDLYTRFDTLTDSRKNPFVYKVETVGDKYMTVSGLP
HS rGC1	866 KTGTPVEPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYTLFDAIIGSHDVYKVETIGDAYMVASGLP
SR NC/PDE	582 NDSMKPIADLFPNTTVMFADISGFDAWSSTRDPSQVFMLLQRIYGEFDEVARRRNVFKVETIGDCYVACAGLP
	* * * * * * *
BT_AC1_C1	QPKTDHAHCCVEMGLDMIDTITSVAEATEVDLNMRVGLHTGRVLCGVLGLRKWQYDVWSNDVTLANVME
BT_AC1_C2	PTAGTRAKKSISSHLSTLADFAIDMFDVLDEINYQSY NDFVLRVGINVGPVVAGVIGARRPQYDIWGNTVNVASRMD
BT_AC2_C2	AIPSQEHAQEPERQYMHIGTMVEFAYALVGKLDAINKHSFNDFKLRVGINHGPVIAGVIGAQKPQYDIWGNTVNVASRMD
DD ACG	PKCDDIRHNTYKMLGFAMDVLEFIPKEMSFHLGLQVRVGIHCGPVISGVISGYAKPHFDVWGDTVNVASRME
BT_sGCa	KESDTHAVQIALMALKMMELSHEVVSPHGEPIKMRIGLHSGSVFAGVVGVKMPRYCLFGNNVTLANKFE
BT_sGCb	EPCIHHARSICHLALDMMEIAGQVQVD.GESVQITIGIHTGEVVTGVIGQRMPRYCLFGNTVNLTSRTE
HS_rGC1	QRNGQRHAAEIANMSLDILSAVGTFRMRHMPEVPVRIRIGLHSGPCVAGVVGLTMPRYCLFGDTVNTASRME
SR_NC/PDE	NKQPNHAAIMSRFAWECKSKLAVIL.KHLEVSLGPD <mark>I</mark> GDLSMRFGLHSGAVTAGVLRGDKA <mark>R</mark> FQLFGDTVNTAA <mark>R</mark> ME
	the shadow of th
h	*****
b	* *** ** *
b hs_pde4a hs_pde7b	* *** ** * LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIPVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD LSKVGMWDFDIFLFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVORDYHSONPYHNAVHAADVTOAMHCYLKEPKLA
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B1	* *** ** * LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD LSKVGMWDFDIFLFDRLTMGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVQEDYHSQNFYHNAVHAADVTQAMHCYLKEPKLA LDNEESWDFNIFELEAITHKRPLVYLGLKVFSRFGVCEFLNCSETTLRAWFQVIEANYHSSNAYHNSTHAADVLHATAFFLGKERVK
b HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6	* *** ** * LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD LSKVGMWDFDIFLFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVQEDYHSQNFYHNAVHAADVTQAMHCYLKEPKLA LDNEESWDFNIFELEAITHKRPLVYLGLKVFSRFGVCEFLNCSETTLRAWFQVIEANYHSSNAYHNSTHAADVLHATAFFLGKERVK
b HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9	* *** ** * LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDLDLSKVGMWDFDIFLFDRLTMGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVWVQEDYHSQNFYHNAVHAADVTQAMHCYLKEPKLA
b HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE	* *** ** * * LENLNKWGLNI FCVSDYAGGRSLTCIMYMI FQERDLLKKFRI PVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD
b HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE	* *** ** ** LENLNKWGLNI FCVSDYAGGRSLTCIMYMI FQERDLLKKFRI PVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDLDLSKVGMWDFDI FLFDRLTMGNSLVTLLCHLEMTHGL HHFKLDMVTLHRFLVMVQEDYHSQNFYHNAVHADVTQAMHCYLKSPKLA
b HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE4A HS_PDE4A	<pre>k **** ** ** LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD</pre>
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE4A HS_PDE4A HS_PDE7B HS_PDE8B1	LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTWVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B1 HS_PDE6 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 UC_PDE5	<pre>k **** ** ** LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTWVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDLSKVGMNDFDIFLFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVQEDYHNAVHAADVTQAMHCYLKEFKLA</pre>
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B1 HS_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9	<pre>k **** ** ** LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTWVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALDLSKVGMNDFDIFLFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVWVQEDYHSQNPYHNAVHAADVTQAMHCYLKEFKLA</pre>
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B1 HS_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE	LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTWVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE4A HS_PDE9 HS_PDE4A HS_PDE9 HS_PDE8B1 HS_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE	<pre>* *** ** ** ** ** ** ** ** ** ** ** **</pre>
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B11 BT_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE4A HS_PDE9B1 HS_PDE9B1 BT_PDE5 HS_PDE9B1 HS_PDE9 SR_NC/PDE HS_PDE9 SR_NC/PDE HS_PDE9 SR_NC/PDE	<pre>k **** ** ** LENLNKWGLNIFCVSDYAGGRSLTCIMYMIFQERDLLKKFRIFVDTWVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD</pre>
<b>b</b> HS_PDE4A HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE6 HS_PDE9 SR_NC/PDE HS_PDE7B HS_PDE8B1 BT_PDE5 HS_PDE8B1 HS_PDE9 SR_NC/PDE HS_PDE9 HS_PDE8 HS_PDE9 HS_PDE8 HS_PDE8B1	LENLNKWGLNI FCVSDYAGGRSLTCIMYMI FQERDLLKKFR I PVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD
b           HS_PDE4A           HS_PDE7B           HS_PDE8B1           ET_PDE5           HS_PDE6           HS_PDE9           SR_NC/PDE           HS_PDE8B1           BT_PDE5           HS_PDE7B           HS_PDE8B1           BT_PDE5           HS_PDE6           HS_PDE7B           HS_PDE8           RS_PDE4A           HS_PDE8           HS_PDE7B           HS_PDE7B           HS_PDE7B           HS_PDE7B           HS_PDE7	LENLNKWGLNI FCVSDYAGGRSLTCIMYMI FQERDLLKKFR I PVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD
b           HS_PDE4A           HS_PDE7B           HS_PDE8B1           BT_PDE5           HS_PDE6           HS_PDE6           HS_PDE6           HS_PDE6           HS_PDE6           HS_PDE8           HS_PDE4A           HS_PDE8B1           BT_PDE5           HS_PDE6           HS_PDE6           HS_PDE7B           HS_PDE6           HS_PDE8           HS_PDE5           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8	LENLNKWGLNI FCVSDYAGGRSLTCIMYMI FQERDLLKKFR I PVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD LSKVGMMDFDI FLFDRLTNGNSLVTLLCHLFNTHGLIHHFKLDMVTLHRFLVMVQEDYHSQNPYHNAVHAADVTQAMHCYLKEFKLA
b           HS_PDE4A           HS_PDE7B           HS_PDE8B1           BT_PDE5           HS_PDE6           HS_PDE6           HS_PDE6           HS_PDE6           HS_PDE8B1           BT_PDE8           HS_PDE8B1           BT_PDE8B1           BT_PDE6           HS_PDE6           HS_PDE6           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8           HS_PDE8B1           HS_PDE8B1           HS_PDE8B1           HS_PDE8B1           HS_PDE8           HS_PDE8           HS_PDE9           SR_NC/PDE	LENLNKWGLNI FCVSDYAGGRSLTCIMYMI FQERDLIKKFRI FVDTMVTYMLTLEDHYHADVAYHNSLHAADVLQSTHVLLATPALD

**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\_ACI\_C2 (NP\_112269.1), DD\_ACG (Q03101.2), BT\_sGCa (NP\_786972.1), BT\_sGC\beta (NP\_777066.1), HS\_rGC1 (AAI48422.1) and SR\_NC/PDE (BT = *Bos Taurus*, DD = *Dictyostelium discoideum*, HS = *Homo sapiens*, 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 PDE5 (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
V1 <sup>1</sup>	CAAGGCAAGAAGGATGGCAAG	GCGACAAGCAAATTAGCAAACC
V4 <sup>1</sup>	AGGCTACCGTGGGACTTGG	GATCTGGACTGCGCTGGTTC
CYCA/B1	GCTTGGGTCGATGCAATACT	ATCCGAGAGGGGTCAAGAGGT
CYCB1	GGGATGTAACAAACGCCAAT	CCTCTCATCCATGACGGACT
P5CS	AATCGAAAACGGGGTAGCTT	CCCTCGACAATCTCAACCAT
GC/PDE	TCCTTCCCAAGTTTTCATGC	CATGGTTTGGTTGCTTGTTG

<sup>1</sup>Normalization genes.