



## Supplementary Information for

Patronus is the elusive plant securin, preventing chromosome separation by antagonizing separase

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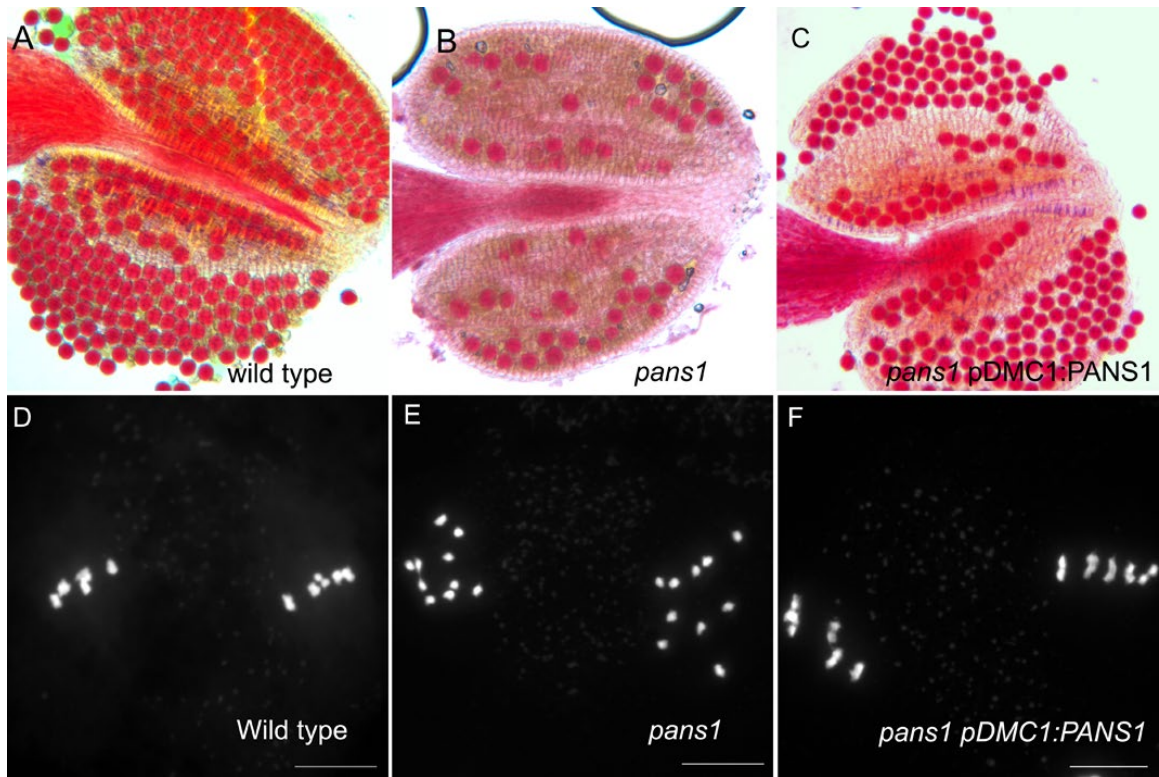
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### **This PDF file includes:**

Figs. S1 to S6  
Tables S1 to S5

### **Other supplementary materials for this manuscript include the following:**

Movies S1 to S3



**Figure S1. *pDMC1::PANS1* complements the *pans1* mutant**

(A-C) Alexander staining of anthers, with viable pollen appearing in red. In contrast to the wild type (A), a large proportion of pollen grains were dead in *pans1* anthers (B). *pans1* plants transformed by *pDMC1::PANS1* (C) have restored pollen viability (n=2/4). (D-F) Chromosome spreads at metaphase II. In the wild type (D), the two metaphase plates contain five pairs of chromatids. In *pans1* (E), the two metaphase plates contain ten free chromatids. In *pans1* transformed by *pDMC1::PANS1* (F), chromatid cohesion is restored (n=2/4).



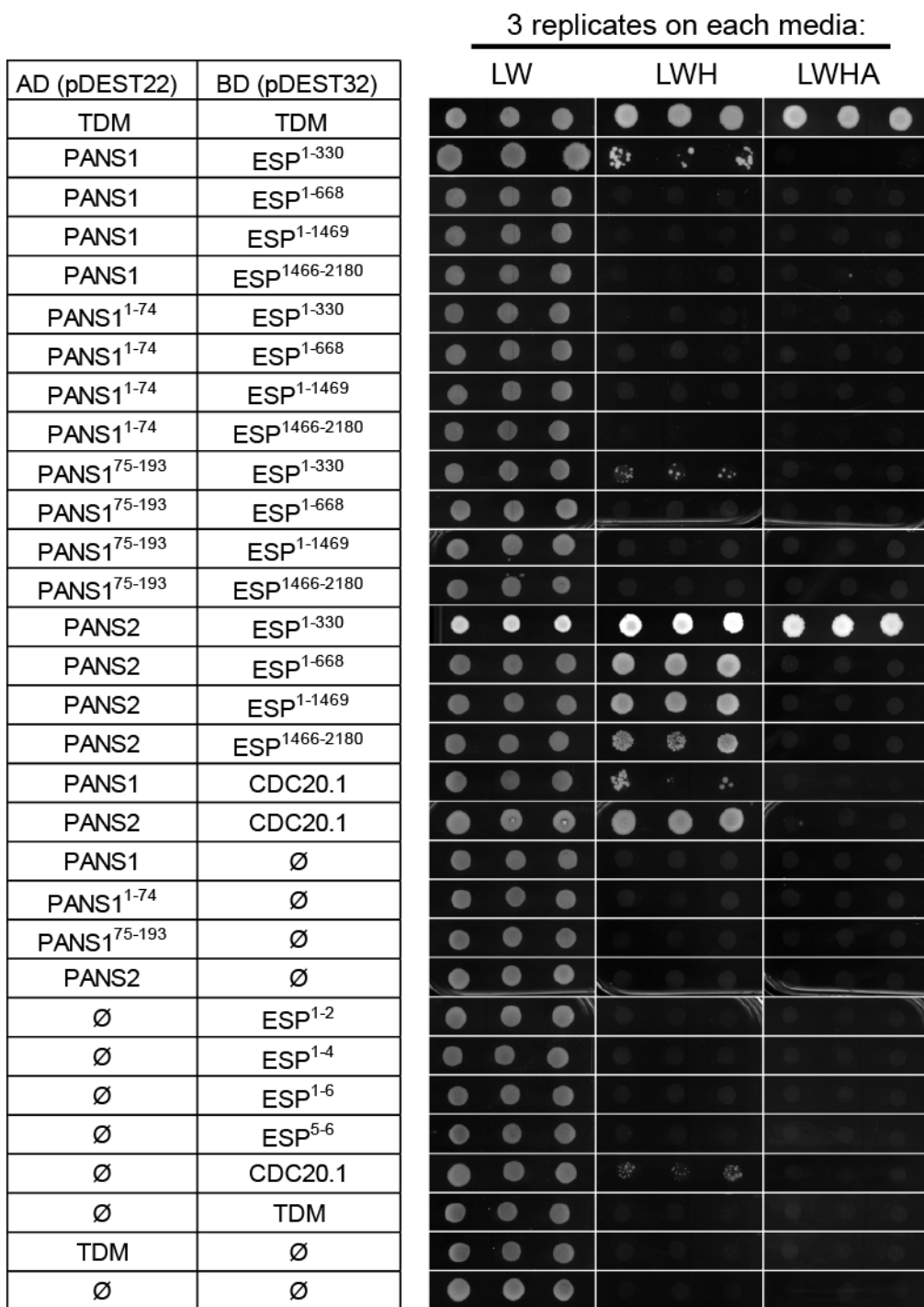
**FIGURE S2. *pDMC1::PANS1ΔD* have variable growth defects**

*pDMC1::PANS1ΔD* was transformed in wild type. These seven primary transformants were grown in parallel for five weeks, and show a range of growth defects.



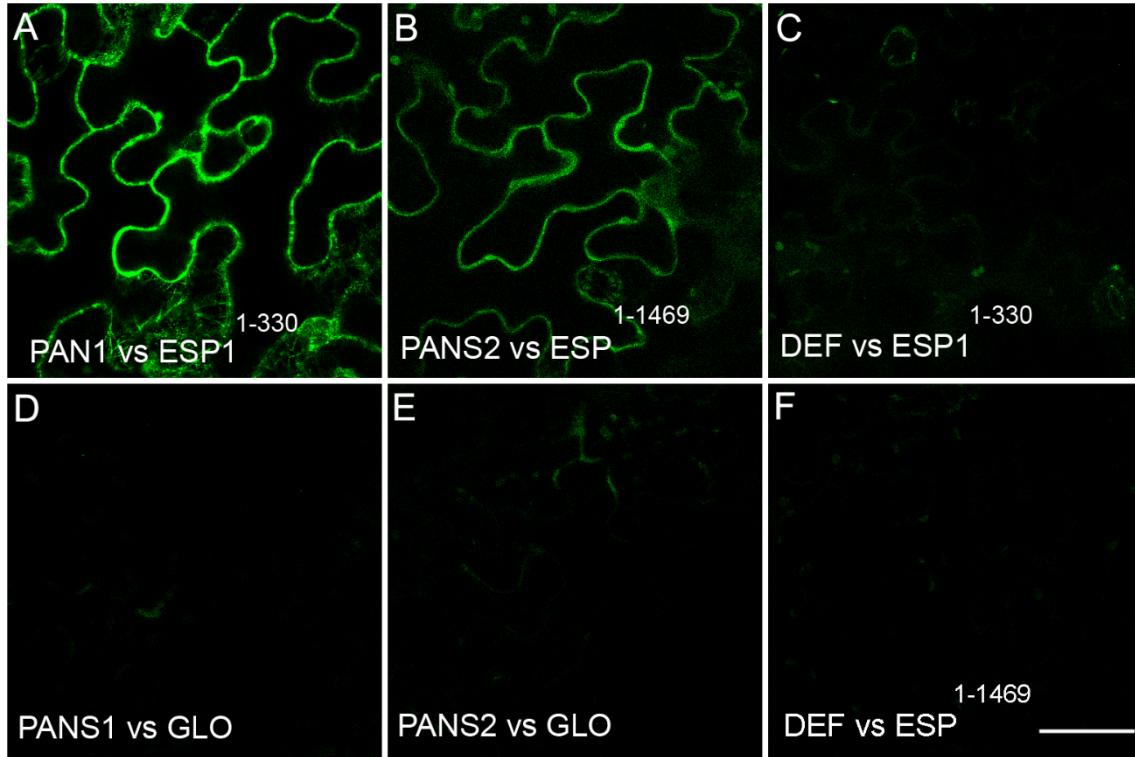






**Figure S4. Yeast two-hybrid assays**

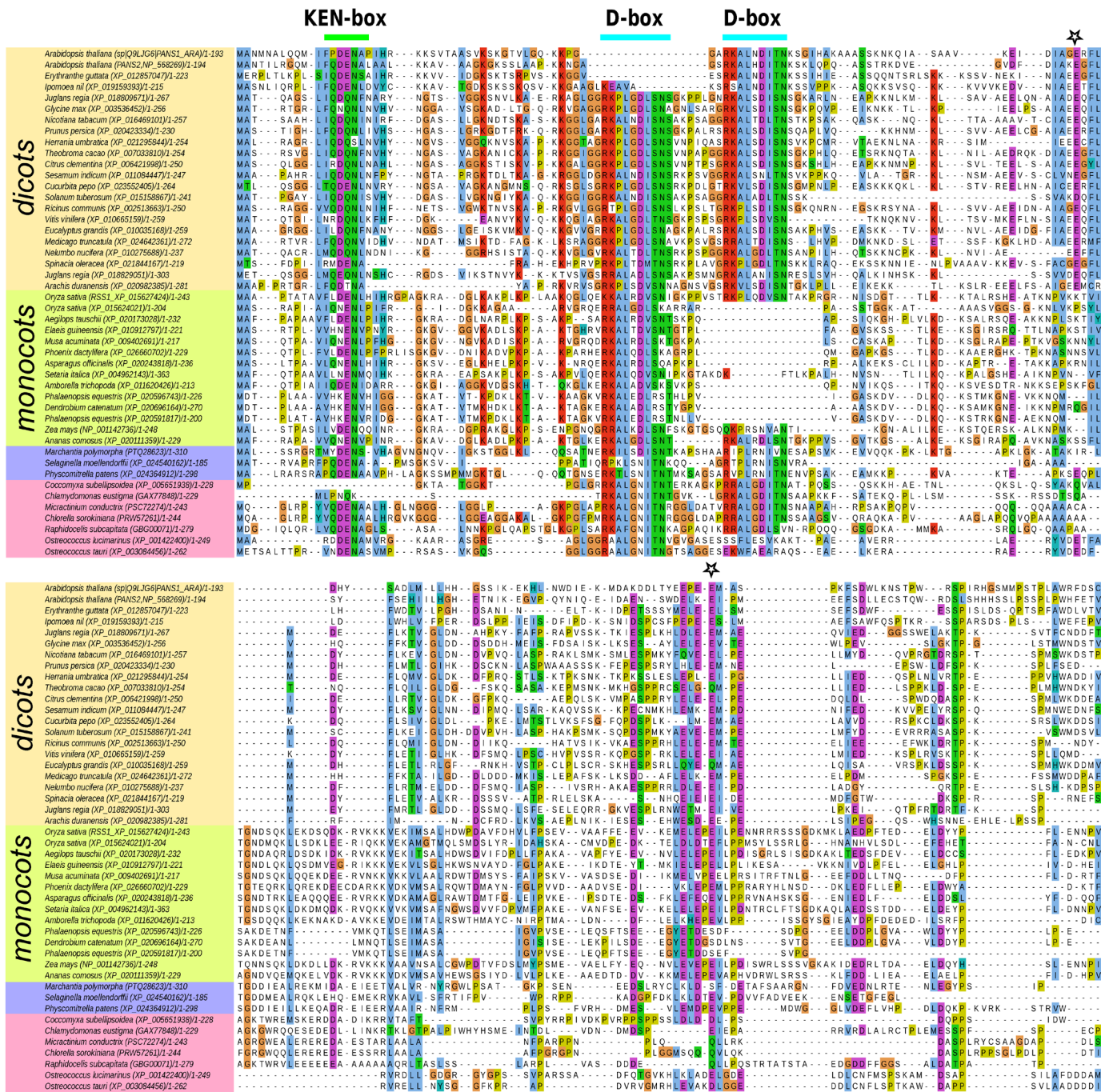
Proteins of interest were fused with the GAL4 activating domain (AD) or the GAL4 binding domain (BD). TDM self-interaction was used as positive controls (Cifuentes et al., 2016). Protein interactions were assayed by growing diploid cells on SD-LW (control), SD-LWH (weak interaction) and SD-LWHA (strong interaction).



**FIGURE S5. Bimolecular fluorescence complementation (BiFC)**

Protein interactions were tested using BiFC assays in leaf epidermal cells of *Nicotiana benthamiana* plants. (A) Expression of pBIF2-PANS1 and pBIP3-ESP1<sup>1-330</sup> results in a nuclear YFP signal. (B) Expression of pBIF2-PANS2 and pBIP3-ESP1<sup>1-1469</sup> results in a nuclear YFP signal. The four corresponding negative controls (C) pBIF2-DEF/pBIP3-ESP1<sup>1-330</sup>, (D) pBIF2-PANS1/pBIP3-GLO, (E) pBIF2-PANS2/pBIP3-GLO and (F) pBIF2-DEF/pBIP3-ESP1<sup>1-1469</sup> do not generate YFP signals. GLOBOSA (GLO) and DEFICIENS (DEF) are two interacting components of the *Anthirrium majus* MADS box transcription factor. Scale bar=50  $\mu$ m.





**FIGURE S6. Multiple sequence alignment of PANS1 homologs in plants**  
 Multiple sequence alignment of the homologs of AtPANS1 in plants built from the full-length sequences retrieved after the PSI-BLAST procedure described in Methods, realigned using MAFFT algorithm and represented using Jalview. Headers are coloured with respect to the clade to which the species belong (i.e. with dicots, monocots, mosses or green algae in yellow, green purple and pink, respectively). For the sake of concision, the columns of the alignment containing gaps in AtPANS1 and OsRSS1 sequences were trimmed.

Name of original mutant	Number of candidate mutations in the line	Genomic mutation in <i>ESP</i> (TAIR10)	Amino acid change in <i>ESP</i>
<i>spans25</i>	206	Chr4: 12040670 C>T	S606N
<i>spans41</i>	201	Chr4:12034602 G>A	P1946L
<i>spansD22</i>	62	Chr4:12034107 C>T	A2047T
<i>spansC4</i>	389	Chr4:12033780 G>A	P2156S

**Table S1. ESP mutations identified in this study**

genotype of the progeny		Expected %	Expected % if gametophyte lethal	observed % (n) in the progeny of:		
<i>PANS1</i>	<i>PANS2</i>			<i>pans1 +/- pans2 +/-</i>	<i>pans1 +/- pans2 +/- esp-P2156S -/-</i>	<i>pans1 +/- pans2 +/- esp-S606N -/-</i>
+/-	+/-	25	22	21 (36)	23 (39)	26 (41)
+/-	+/+	12.5	22	23 (38)	24 (41)	20 (32)
+/+	+/-	12.5	22	25 (41)	19 (32)	18 (29)
+/+	-/-	6.25	11	15 (25)	10 (17)	13 (21)
-/-	+/+	6.25	11	9 (15)	13 (22)	8 (13)
+/+	+/+	6.25	11	7 (12)	12 (21)	13 (21)
-/-	+/-	12.5	0	0	0	0
+/-	-/-	12.5	0	0	0	0
-/-	-/-	6.25	0	0	0	0

**Table S2. *esp* mutations do not restore the gametophytic lethality of *pans1 pans2***

Among the selfing progeny of a *pans1 +/- pans2 +/-* plants, independent segregation of these unlinked loci predicts 6.25% of *pans1 -/- pans2 -/-* plants, 12.5% of both *pans1 +/- pans2 -/-* and 12.5% of *pans1 -/- pans2 +/-*. However, these genotypes were absent in the observed progeny, as previously described (Cromer et al, 2013), suggesting that the *pans1 pans2* gametophytes are not viable. Introducing the *esp-P2156S* or the *esp-S606N* mutation in the parental plant did not modify this segregation, the three genotypes being still absent. This shows that the *esp-P2156S* or the *esp-S606N* mutation is not able to restore the viability of neither male nor female *pans1 pans2* gametophytes.

Panicum hallii var. hallii	(PUZ76441.1)	Triticum urartu	(EMS53671.1)
Panicum hallii	(XP_025791674.1)	Amborella trichopoda	(XP_011620426.1)
Panicum miliaceum	(RLN07740.1)	Brachypodium distachyon	(KQK12869.2)
Panicum miliaceum	(RLM78791.1)	Hordeum vulgare subsp. vulgare	(BAK00762.1)
Oryza sativa Japonica Group	(XP_015627424.1)	Aegilops tauschii subsp. tauschii	(XP_020182268.1)
Oryza brachyantha	(XP_006647477.1)	Oryza sativa Japonica Group	(BAD82353.1)
Dichantherium oligosanthes	(OEL17437.1)	Sorghum bicolor	(XP_021302849.1)
Dichantherium oligosanthes	(OEL20438.1)	Zostera marina	(KMZ64751.1)
Oryza brachyantha	(XP_015689052.1)	Dendrobium catenatum	(XP_020696164.1)
Zea mays	(NP_001132520.1)	Musa acuminata subsp. malaccensis	(XP_009379917.1)
Panicum miliaceum	(RLM99188.1)	Physcomitrella patens	(XP_024364912.1)
Zea mays	(ACG45876.1)	Ostreococcus tauri	(XP_003084456.2)
Sorghum bicolor	(XP_002454142.1)	Phalaenopsis equestris	(XP_020591817.1)
Oryza sativa Indica Group	(EAY86615.1)	Dendrobium catenatum	(PKU68131.1)
Phoenix dactylifera	(XP_008791773.1)	Pomacea canaliculata	(XP_025093964.1)
Asparagus officinalis	(XP_020261507.1)	Musa acuminata subsp. malaccensis	(XP_018675059.1)
Asparagus officinalis	(XP_020243818.1)	Phalaenopsis equestris	(XP_020596743.1)
Setaria italica	(XP_004953025.1)	Oryza sativa Indica Group	(EAY76834.1)
Phoenix dactylifera	(XP_026656827.1)	Musa acuminata subsp. malaccensis	(XP_018675061.1)
Elaeis guineensis	(XP_010912113.1)	Crassostrea virginica	(XP_022340323.1)
Zea mays	(NP_001142736.1)	Zostera marina	(KMZ59965.1)
Panicum miliaceum	(RLN29265.1)	Physcomitrella patens	(PNR27411.1)
Zea mays	(AQK85829.1)	Apostasia shenzhenica	(PKA64779.1)
Elaeis guineensis	(XP_010912797.1)	Marchantia polymorpha	(PTQ28623.1)
Brachypodium distachyon	(XP_003575314.1)	Micractinium conductrix	(PSC72275.1)
Elaeis guineensis	(XP_019703807.1)	Oryza brachyantha	(XP_015690666.1)
Musa acuminata subsp. malaccensis	(XP_009402692.1)	Micractinium conductrix	(PSC72274.1)
Zea mays	(PWZ27380.1)	Raphidocelis subcapitata	(GBG00071.1)
Hordeum vulgare subsp. vulgare	(BAK07257.1)	Chlorella sorokiniana	(PRW57261.1)
Panicum hallii var. hallii	(PUZ66070.1)	Chlamydomonas eustigma	(GAX77848.1)
Setaria italica	(XP_004962143.1)	Marchantia polymorpha subsp. ruderalis	(OAE27187.1)
Ostreococcus lucimarinus CCE9901	(XP_001422400.1)	Picea sitchensis	(ABK26784.1)
Triticum turgidum subsp. durum	(AIP89948.1)	Coccomyxa subellipsoidea C-169	(XP_005651938.1)
Panicum hallii	(XP_025808982.1)	Elaeis guineensis	(XP_010930199.1)
Musa acuminata subsp. malaccensis	(XP_009402691.1)	Elaeis guineensis	(XP_010930200.1)
Zea mays	(XP_008647649.1)	Acropora digitifera	(XP_015753966.1)
Triticum urartu	(EMS47367.1)	Ostreococcus tauri	(OUS44868.1)
Aegilops tauschii subsp. tauschii	(XP_020173028.1)	Orbicella faveolata	(XP_020600834.1)
Zea mays	(NP_001158919.1)	Crassostrea gigas	(EKC30626.1)
Zea mays	(AQK85830.1)	Asparagus officinalis	(ONK57046.1)
Apostasia shenzhenica	(PKA64761.1)	Zea mays	(ACG33508.1)
Elaeis guineensis	(XP_019702503.1)	Crassostrea gigas	(XP_011441394.1)
Phoenix dactylifera	(XP_026660708.1)	Pocillopora damicornis	(RMX43805.1)
Musa acuminata subsp. malaccensis	(XP_009404273.1)	Selaginella moellendorffii	(XP_024519951.1)
Musa acuminata subsp. malaccensis	(XP_009404272.1)	Selaginella moellendorffii	(XP_024540162.1)
Zea mays	(PWZ17396.1)	Zea mays	(ACG37554.1)
Asparagus officinalis	(ONK79989.1)	Stylophora pistillata	(XP_022807112.1)
Amborella trichopoda	(ERM98247.1)	Orbicella faveolata	(XP_020600847.1)
Oryza sativa Japonica Group	(XP_015624021.1)	Lottia gigantea	(XP_009062812.1)
Phoenix dactylifera	(XP_026660705.1)	Apostasia shenzhenica	(PKA58368.1)
Ananas comosus	(XP_020111360.1)	Saccoglossus kowalevskii	(XP_002742390.1)
Phoenix dactylifera	(XP_026660702.1)	Pyrus x bretschneideri	(XP_009363238.1)
Ananas comosus	(OAY72878.1)	Lingula anatina	(XP_013412898.1)
Asparagus officinalis	(ONK72469.1)	Biomphalaria glabrata	(XP_013082550.1)
Ananas comosus	(XP_020111359.1)	Ostreococcus tauri	(OUS44952.1)
Mizuhopecten yessoensis	(XP_021355525.1)	Exaiptasia pallida	(XP_020908213.1)
Zostera marina	(KMZ64759.1)	Biomphalaria glabrata	(XP_013082549.1)
Acropora digitifera	(XP_015760873.1)	Priapulius caudatus	(XP_014665187.1)

**Table S3.** Homologues detected by PSI-BLAST with the green alga *Ostreococcus lucimarinus* CCE9901 (XP\_001422400.1) as the input sequence. The hits have been ordered by increasing e-values ranging from  $4e^{-57}$  to  $1e^{-05}$  after 10 iterations.

Absolute read counts. Read counts normalized by Med. Format: average value.

	AT5G12360 <i>PANS2</i>	AT3G14190 <i>PANS1</i>	ratio <i>PANS1/PANS2</i>
Seedling meristem	126	2097	16.6
Meristem at 11 days after germination	177	1988	11.2
Inflorescence meristem at 15 days after germination	173	1811	10.5
Seeds 1	369	1648	4.5
Inflorescence meristem at 14 days after germination	147	1538	10.5
Inflorescence meristem at 16 days after germination	131	1519	11.6
Flower 19+	110	1517	13.8
Carpels of the young flower	127	1515	11.9
Meristem at 10 days after germination	200	1440	7.2
Meristem at 12 days after germination	136	1314	9.7
Flower 9-11	102	1310	12.8
Ovules from 6th and 7th flowers	136	1287	9.5
SAM at 8 days after germination	132	1272	9.6
SAM at 9 days after germination	130	1266	9.7
Young seeds 5	119	1266	10.6
Flower 15-18	102	1235	12.1
Inflorescence meristem at 13 days after germination	135	1226	9.1
SAM at 7 days after germination	414	1200	2.9
Leaf blade of the young leaf	137	1148	8.4
Petiole of the young leaf	58	1144	19.7
Axis of the inflorescence	81	1056	13.0
Young seeds 4	111	908	8.2
Carpels of the mature flower (before pollination)	95	899	9.5
Young seeds 3	139	897	6.5
Flower 12-14	91	879	9.7
Young seeds 2	106	872	8.2
Seeds 3	289	864	3.0
Petiole, intermediate 1	80	856	10.7
Seeds 5	319	769	2.4
Carpel of 6th and 7th flowers	66	696	10.5
Young seeds 1	98	693	7.1
Flower 6-8	83	617	7.4
Silique 2	172	568	3.3
Silique 4	157	563	3.6
Flower 2	51	562	11.0
Pedicele	76	525	6.9
Flower 4	66	500	7.6
Flower 1	70	496	7.1
Flower 5	72	492	6.8
Seeds 7	150	488	3.3
Anthers of the young flower	93	457	4.9
Leaf blade, intermediate 1	68	432	6.4
Flower 3	60	378	6.3
Silique 6	118	348	2.9
Root without apex	90	343	3.8
Root apex	102	337	3.3
Germinating seeds 3 (third day after soaking)	78	332	4.3
Silique 8	68	289	4.3
Seedling root	84	251	3.0
Sepals of the young flower	63	248	3.9
Seedling cotyledons	71	242	3.4
Internode	56	237	4.2
Pod of the silique 1	71	232	3.3
Pod of the silique 3	96	226	2.4
Petals of the mature flower	32	194	6.1
Pod of the silique 5	79	168	2.1

Petiole, intermediate 2	55	158	2.9
Dry seeds	118	147	1.2
Leaf vein, intermediate 2	53	143	2.7
Pod of the silique 7	56	129	2.3
Germinating seeds 2 (second day after soaking)	57	122	2.1
Seedling hypocotyl	66	118	1.8
Stamen filaments of the mature flower	26	116	4.5
Leaf blade, intermediate 2	52	113	2.2
Anthers of the mature flower (before opening).	75	90	1.2
Petiole of the mature leaf	36	85	2.4
Opened anthers	20	79	4.0
Stigmatic tissue	57	72	1.3
Vein of the mature leaf	40	71	1.8
Seeds of first yellowing silique.	79	66	0.8
Sepals of the mature flower	21	65	3.1
Germinating seeds 1 (first day after soaking)	124	59	0.5
Whole mature leaf	26	55	2.1
Senescent silique 2	64	53	0.8
Vein of the senescent leaf	30	33	1.1
Leaf blade of the mature leaf	28	29	1.0
Petiole of the senescent leaf	10	28	2.8
Senescent internode.	30	27	0.9
Pod of the senescent silique 1.	13	16	1.2
<b>Total</b>	<b>7898</b>	<b>49529</b>	<b>6.3</b>

**Table S4. Expression levels of PANS1 and PANS2.**

Absolute read counts. Read counts normalized by Med. Format: average value.

Data from Klepikova et al, BMC genomics 2015. Retrieved from

<http://travadb.org/>



**Movie S1. Time course of male meiosis I in wild type.**

Anthers at meiotic stages were observed in plant expressing the TUBULIN BETA CHAIN 4 fused with the RED FLUORESCENT PROTEIN (RFP:TUB4) and the cohesin REC8 fused with the GREEN FLUORESCENT protein (REC8:GFP). Scale bar= 10  $\mu$ M.

**Movie S2 and S3. Time course of male meiosis in *pDMC1::PANS1 $\Delta$ D*.**

Anthers at meiotic stages were observed in plants expressing RFP:TUB4, REC8:GFP and *pDMC1::PANS1 $\Delta$ D*. Scale bar= 10  $\mu$ M.