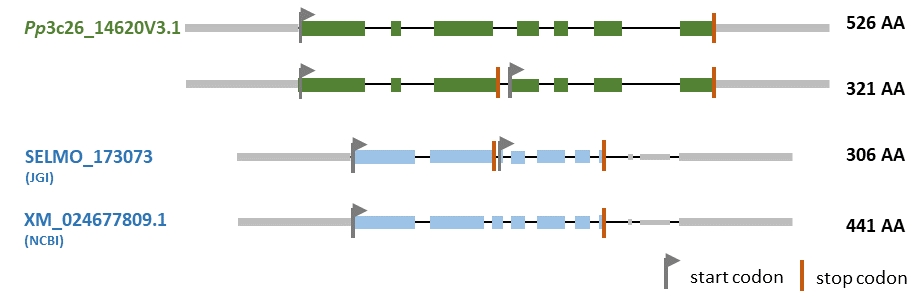
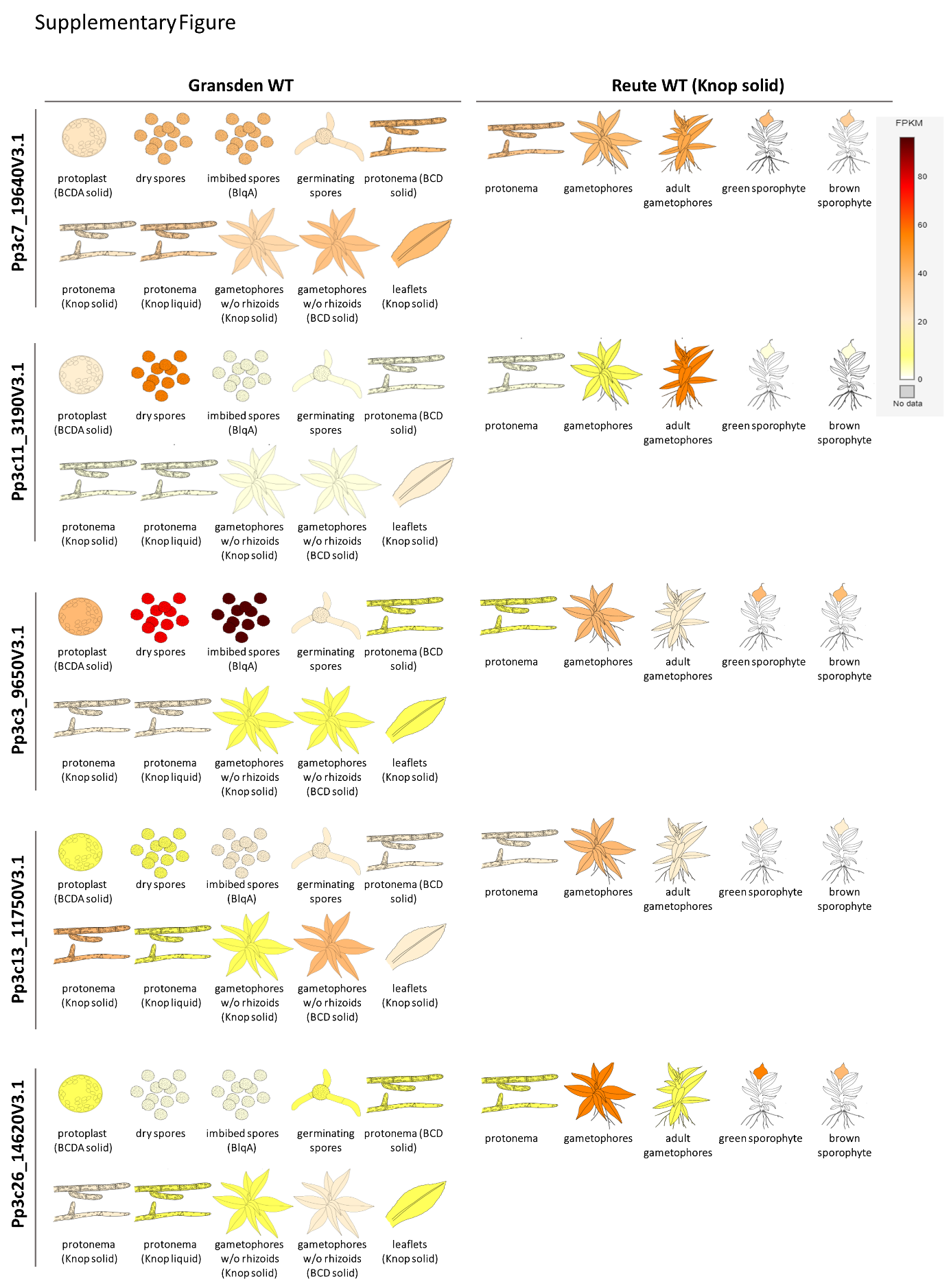
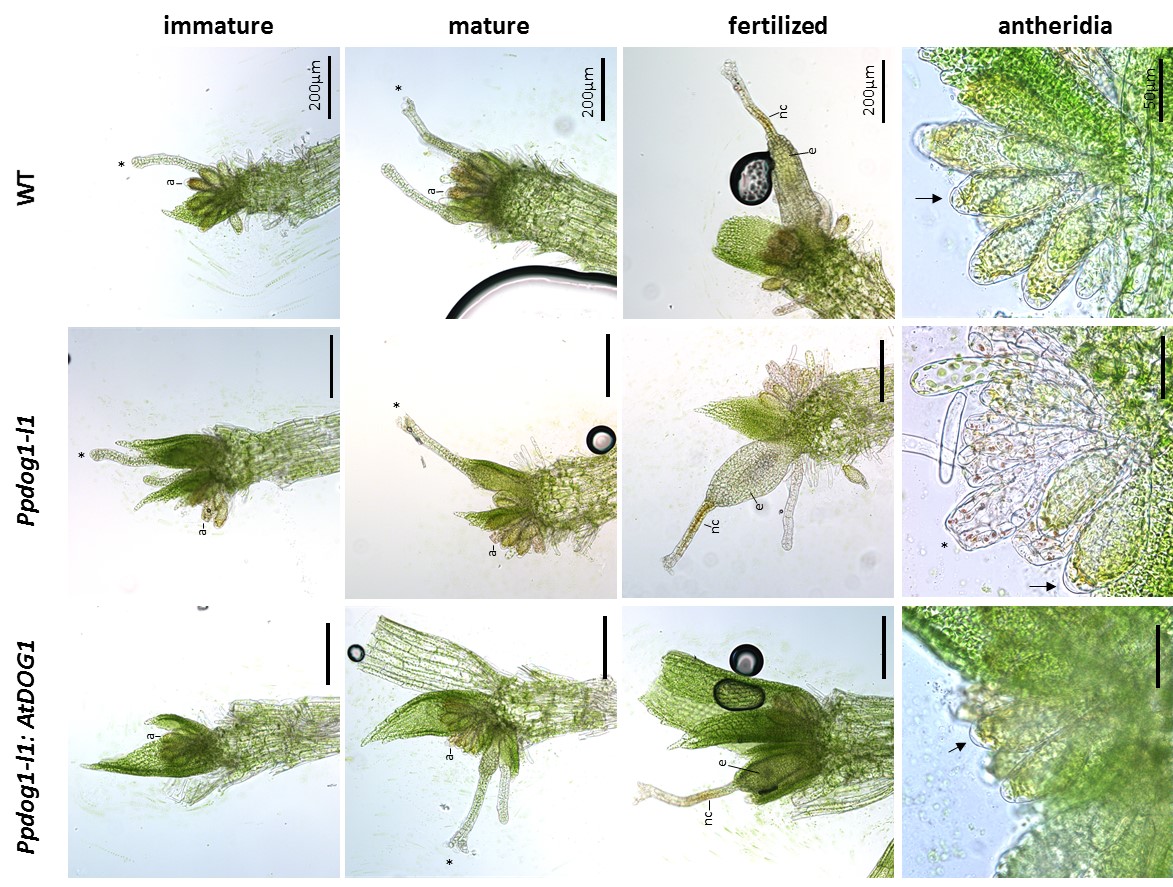
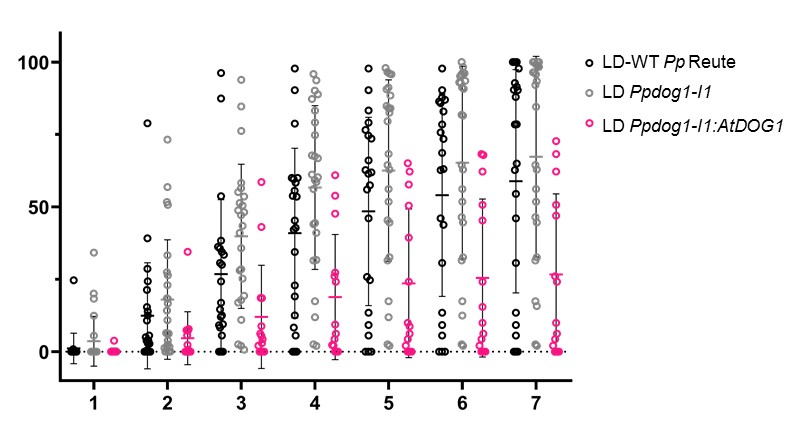
**Supporting information**

**Figure S1 Comparison of gene model/splice variants of *P. patens* and *S. moellendorffii* *DOG1* genes.** Variants w/ and w/o ANK domain maybe derived from intron retention. Schematic representation of *Pp*3c26\_14620V3.1 coding sequences compared to *Selaginella moellendorfii* (SELMO) gene 173073 (JGI, Joint Genome Institute) as well as its NCBI entry XM \_024677809.1. Green and blue bars represent coding sequences. AA, amino acid. Both variants of the *P. patens* transcript can be experimentally detected.

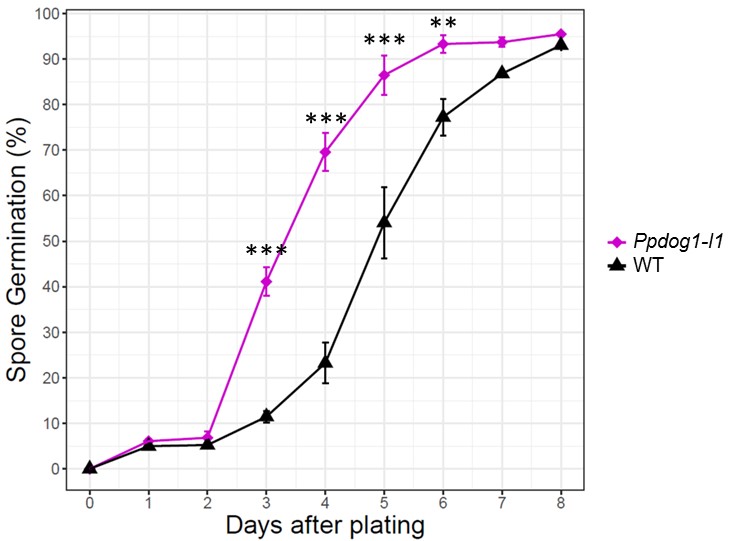
 **Figure S2** ***In silico* expression analyses of *P. patens* *DOG1* homologous genes.** PEATmoss (https://peatmoss.plantcode.cup.uni-freiburg.de/expression\_viewer/input; ([Fernandez-Pozo *et al.* 2020](#_ENREF_15)) expression images depict FPKM values of all five DOG1 homologous genes using RNAseq data of various developmental stages of *P. patens* ecotypes Gransden and Reute. WT = wild type.

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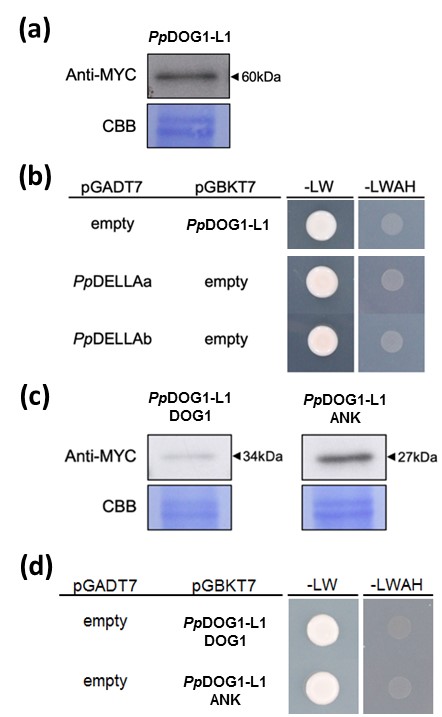
**Figure S3 Gametangia development of *Ppdog1-l1*and *Ppdog1-l1*:*AtDOG1*compared to wild type Reute (WT) 21 days after induction.** Immature: Apices with immature archegonia (female, tip cell closed, \*) and mature antheridia (male, yellow to brownish colour, swollen and/or burst tip cells, a). Mature: Apices possess at least one mature archegonium (tip cell open, \*) and mature antheridia (a). Fertilized: The archegonial neck canal (nc) cells are brownish, the venter is swollen and an embryo develops (e). Antheridia: Mature antheridia with swollen tip cell are visible for all strains (arrow). *Ppdog1-l1* antheridia are mainly empty and degrade after spermatozoid release (\*).



**Figure S4 Germination rate determination under long-day conditions for *Ppdog1-l1* and *Ppdog1-l1*:*AtDOG1* compared to wild type.** Diagram depicting germination rates of WT (black circles), knockout (grey circles), and replacement (pink circles) strains of *Pp*DOG1-L1 over seven days. For germination of spores, long-day conditions were tested using ≥3 repetitions and different light sources (LED and fluorescent bulbs for white light). For each independent repetition of the experiment, three to five replicates were tested. Statistical significance was calculated using CountSpores R package. Lines depict the estimated means +/- SEM; \*/§ p<0.05; \*\*/## p<0.01; ### p<0.001. X-axis: days; Y-axis: % germination.



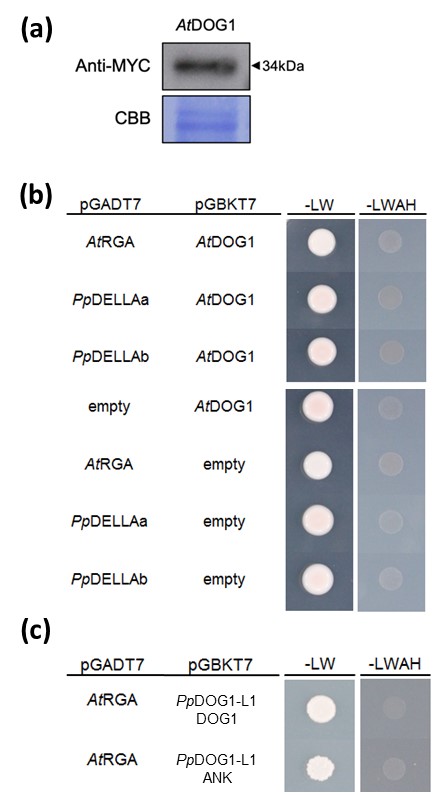
**Figure S5 *Ppdog1-l1* mutant spores germinate faster than wild type in a second lab environment.** Spores of the knockout strain *Ppdog1-l1* germinate significantly faster than wild type (WT, Reute ecotype) spores. Error bars, ± SEM. Significant differences between knockout strain KO\_*Pp*3c3\_9650 and WT were tested using CountSpores R package. Representative of 3 biological repeats; means are the average of 3 technical replicates. \*\* p<0.01, \*\*\* p<0.001.



**Figure S6 Immunoblot and yeast two hybrid approaches.**

Controls linked to Figure 5. **(a)** MYC-tagged *Pp*DOG1-L1 (60kDa) was expressed in yeast cells (anti-MYC western blot). CBB, Coomassie brilliant blue staining. **(b)** No autoactivation in the yeast system is seen with *Pp*DELLAs or *Pp*DOG1-L1 coexpressed with empty vector controls. **(c)** MYC-tagged truncations of the *Pp*DOG1-like protein *Pp*DOG1-L1 harbouring either the DOG1 or ANK domain were expressed in yeast as detected by an anti-MYC western blot. CBB, Coomassie brilliant blue staining.

(d) No autoactivation is seen in the yeast system with truncated *Pp*DOG1-L1 coexpressed with empty pGADT7 vector.

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**Figure S7 *At*DOG1 cannot interact with DELLA proteins and *At*RGA cannot interact with truncated DOG1 or ANK domains of *PpDOG1-L1*. (a)** Anti-MYC western blot showing that MYC-tagged *At*DOG1 (34kDa) is expressed in yeast cells. Myc-tagged *Pp*DELLAa and *Pp*DELLAb as well as *At*RGA expression was shown elsewhere ([Phokas *et al.* 2023](#_ENREF_60)). CBB, Coomassie brilliant blue staining. **(b)** Yeast two-hybrid assay between *At*RGA or *Pp*DELLAs in pGADT7 and *At*DOG1 in pGBKT7 plus empty vector controls. *At*RGA and *Pp*DELLAs do not interact with *At*DOG1. Representative results of three biological replicates are shown. **(c)** Yeast two-hybrid assay between *At*RGA in pGADT7 and truncated *Pp*DOG1-L1 in pGBKT7. Representative results of three biological replicates are shown. DOG1, DOG1 domain; ANK, ankyrin repeats domain.

**Table S1: Primers used**

|  |  |  |
| --- | --- | --- |
| Primer Name | Primer Sequence 5`-> 3` | Usage description |
| 9650\_HindIII\_LB\_FWD | AAGCTTAGCACAAGTGGGTATGGACCAG | Left border, knock out |
| 9650\_LB\_XhoI\_REV | CTCGAGTTACCCTGAATACCTTCCCTTCC | Left border, knock out |
| 9650\_SpeI\_RB\_FWD | ACTAGTACTGTACGGGTTAGGATGAGTAC | Right border, knock out |
| 9650\_RB\_AscI\_REV | GGCGCGCCTGTCTGGAGCAAGAGTAGTCACC | Right border, knock out |
| AT5G45830\_AscI FWD | GGCGCGCCATGGGATCTTCATCAAAGAACATCGAACAAGCTCAA | Complementation *AtDOG1* in *Pp* |
| AT5G45830\_AscI REV | GGCGCGCCTCAATTTCTCTCATTATTTGTCGTCTCCGTGGGGCG | Complementation *AtDOG1* in *Pp* |
| 9650\_ApaI\_LB FWD | GGGCCCAGCACAAGTGGGTATGGACCAG | Left border complementation in *Pp* |
| 9650\_LB\_AscI REV | CTCGAGGGCGCGCCTTACCCTGAATACCTTCCCTTCC | Left border complementation in *Pp* |
| 9650\_NdeI\_RB | CATATGACTGTACGGGTTAGGATGAGTAC | Right border complementation in *Pp* |
| 9650\_RB\_NotI REV | GCGGCCGCTGTCTGGAGCAAGAGTAGTCACC | Right border complementation in *Pp* |
| 9650\_outLB FWD | AGCTTCAGAATCCGACGTG | Genotyping |
| p35ss\_out REV | TCTGGTCTTCTGAGACTGTATC | Genotyping |
| CamvTer FWD | agggtttcgctcatgtgttgag | Genotyping |
| 9650\_outRB\_REV | TGTCTGGAGCAAGAGTAGTC | Genotyping |
| 9650\_midLB\_FWD | TGATCTGCAGTATCTGACAACG | Genotyping |
| 35s REV | ACCACTGTCGGCAGAGGCATC | Genotyping |
| *Pp*3c10\_17080V3.1 *ACTIN5* FWD | TCTGGCGCCTATCTCTCTCC | Transcript validation |
| *Pp*3c10\_17080V3.1 *ACTIN5*\_REV | GCATCATCTCCCGCGAAACC | Transcript validation |
| *Pp*3c3\_9650V3.1 9650\_FWD | TCGTAACAATGGAGCGAAACC | Transcript validation |
| *Pp*3c3\_9650V3.1 9650\_REV | CCTTCTGTCAACTCCATGTCC | Transcript validation |
| *M13\_F* | GTAAAACGACGGCCAG | Sequencing |
| *M13\_R* | CAGGAAACAGCTATGAC | Sequencing |
| *T7\_F* | TAATACGACTCACTATAGGG | Sequencing |
| *NdeI-Pp3c3\_9650\_F* | AAACATATGCGAGGGCCGGTGGTTCTGGATACGC | Transcripts, Y2H |
| *SalI-Pp3c3\_9650\_R* | AAAGTCGACTCAGCACATGGACCATTGATCACC | Transcripts, Y2H |
| *SalI-Pp3c3\_9650\_DOG\_R* | AAAGTCGACAGAAGACCGTGCATTCGCGTCTCC | Transcripts, Y2H |
| *NdeI-Pp3c3\_9650\_ANK\_F* | AAACATATGGGACATCCTGGAGTGAATATTTGG | Transcripts, Y2H |
| *NdeI-AtDOG1\_F* | AAACATATGGGATCTTCATCAAAGAACATCG | Transcripts, Y2H |
| *EcoRI-AtDOG1\_R* | ΑAAGAATTCTCAATTTCTCTCATTATTTGTCGTC | Transcripts, Y2H |
| *thioredoxin\_F* | TGCCCTCTTTTCAATTCCAC | qRT-PCR |
| *thioredoxin\_R* | ACAAAGTGCCGGTTTACGTC | qRT-PCR |
| *Pp3c3\_9650\_F* | CGAAGCAGGGATGATGTGGA | qRT-PCR |
| *Pp3c3\_9650\_R* | GAAGAAGTGCGTTGCTGCAT | qRT-PCR |
| *AtDOG1\_F* | GTGTGACTCAGTTTCTCCGC | qRT-PCR |
| *AtDOG1\_R* | CACAGTGGCGATGGGAATAT | qRT-PCR |