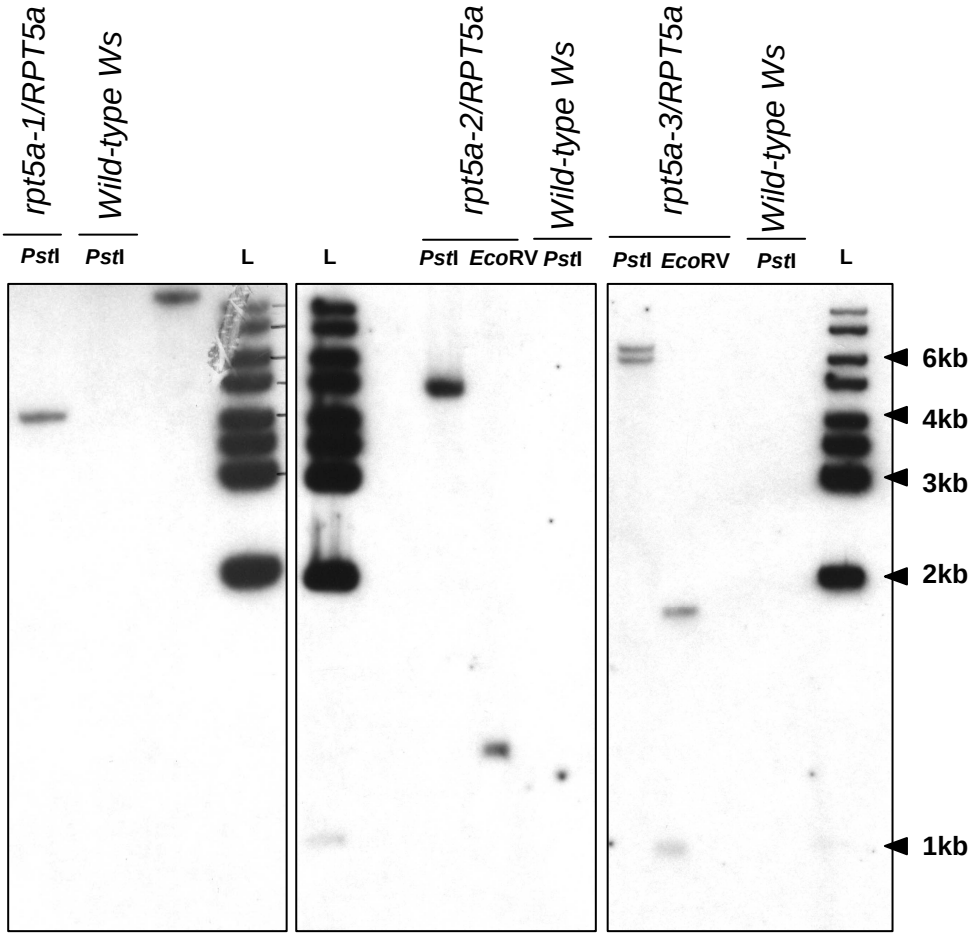
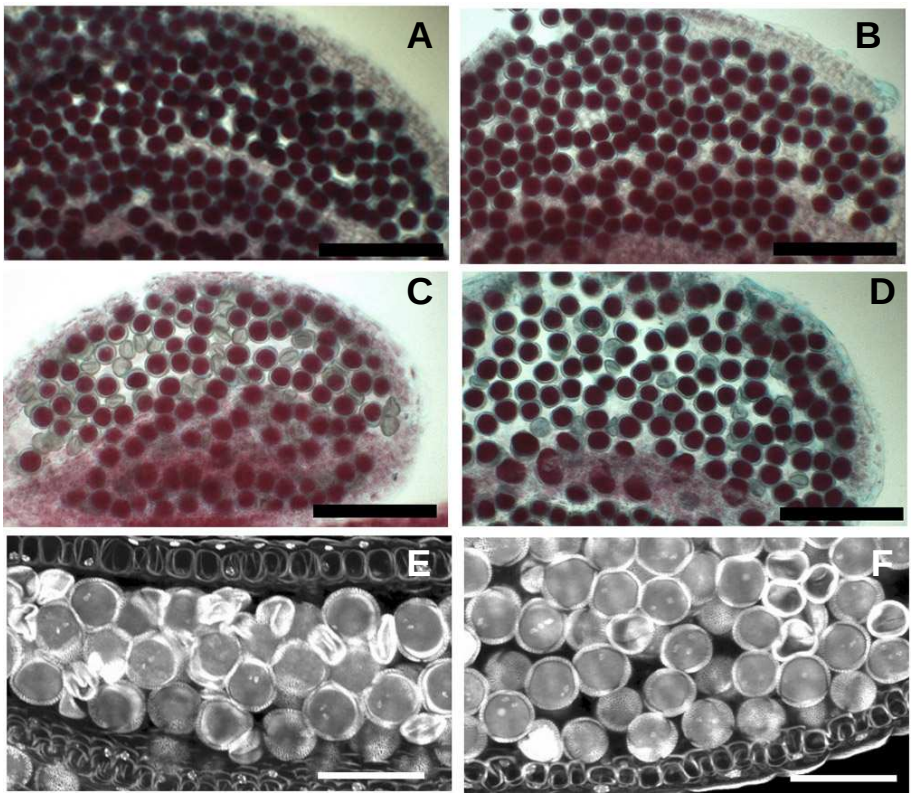
Supplemental data. Gallois et al. (2009). The *Arabidopsis* proteasome RPT5 subunits are essential for gametophyte development and show accession-dependent redundancy

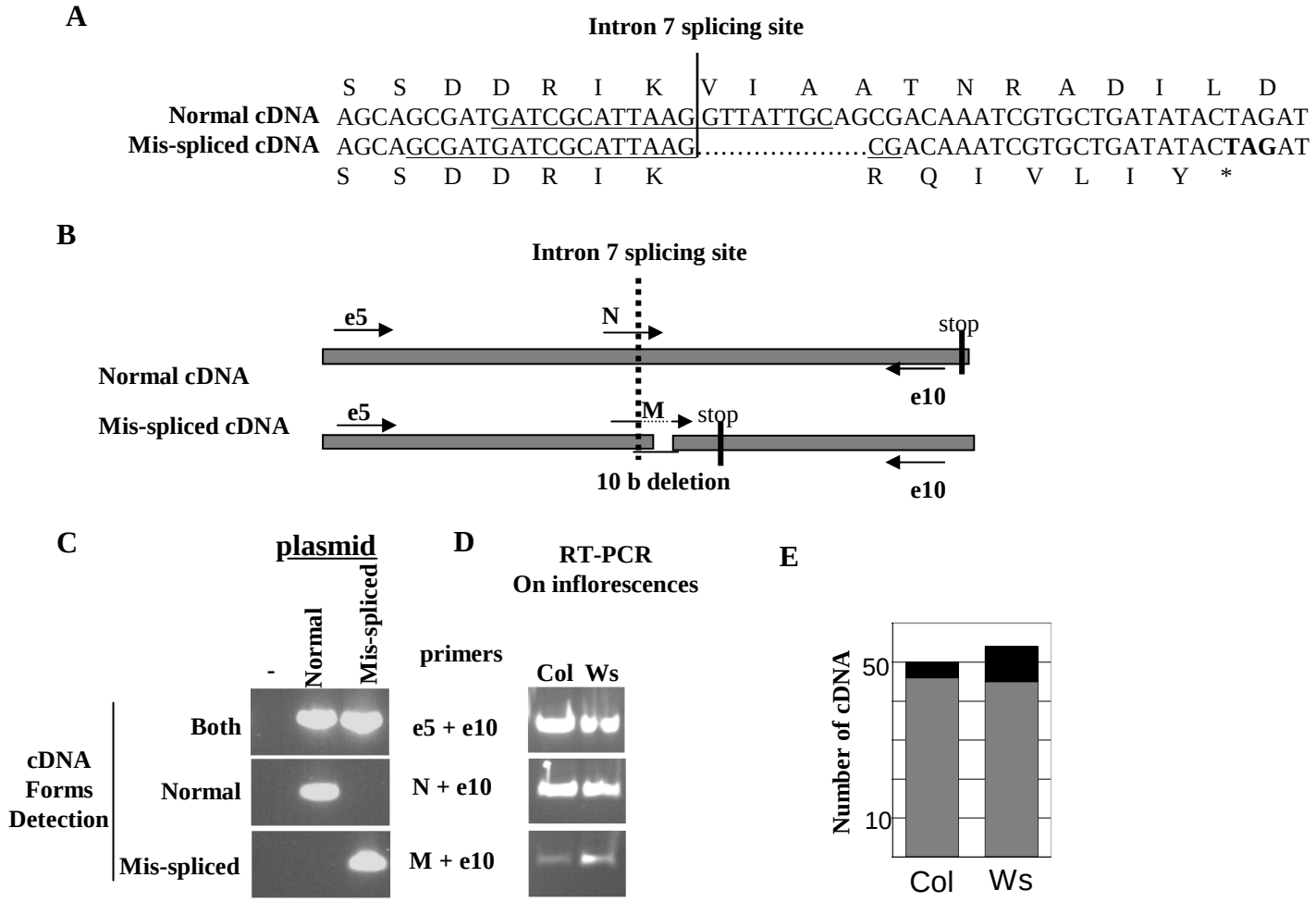


Supplemental Figure 1: single-locus T-DNA insertions in *rpt5a-1*, *rpt5a-2* and *rpt5a-3* mutants.
Southern blot hybridizations using a T-DNA Right Border probe on genomic DNA show that only one T-DNA copy is inserted in both *rpt5a-1* and *rpt5a-2* mutants. Southern blot and further Flanking Sequence Tag sequencing reveal a T-DNA triplex arrangement in *rpt5a-3* (in a LB-RB/LB-RB/RB-LB arrangement).

Additional Methods:
2,5µg of genomic DNA were digested with restriction enzymes as indicated, separated in a 0,8% agarose electrophoresis gel and transferred onto a Genescreen+ membrane (NEN Life Science Product). A 1,1kb T-DNA Right Border fragment (*BamH*I-*EcoR*I) from the pGKB5 binary vector (http://www-ijpb.versailles.inra.fr/en/sgap/equipes/cyto/ressources/pGKB5.html) was ^{32}P labelled using the Prime-a-Gene Labelling System (Promega) and used to probe the membranes in phosphate buffer. Time exposure was 2 days.
L is for ladder (Generuler 1kb DNA ladder-Fermentas).



Supplemental Figure 2: Male gametophyte development in *rpt5a-2*, *rpt5a-3* and *rpt5a-4* mutants.
(A-D) Mature pollen stained with Alexander's stain in anther locules from Columbia wild-type (A) and *rpt5a-4/RPT5a* (B) plants or from WS *rpt5a-2/RPT5a* (C) and *rpt5a-3/RPT5a* (D) plants.
(E, F) Optical section through a *rpt5a-2/RPT5a* (E) and a *rpt5a-3/RPT5a* (F) mature anther stained with propidium iodide. Both sections are showing a mix of mature and collapsed pollen grains.
Scale bars represent 100 μm (A-D) or 40 μm (E, F)



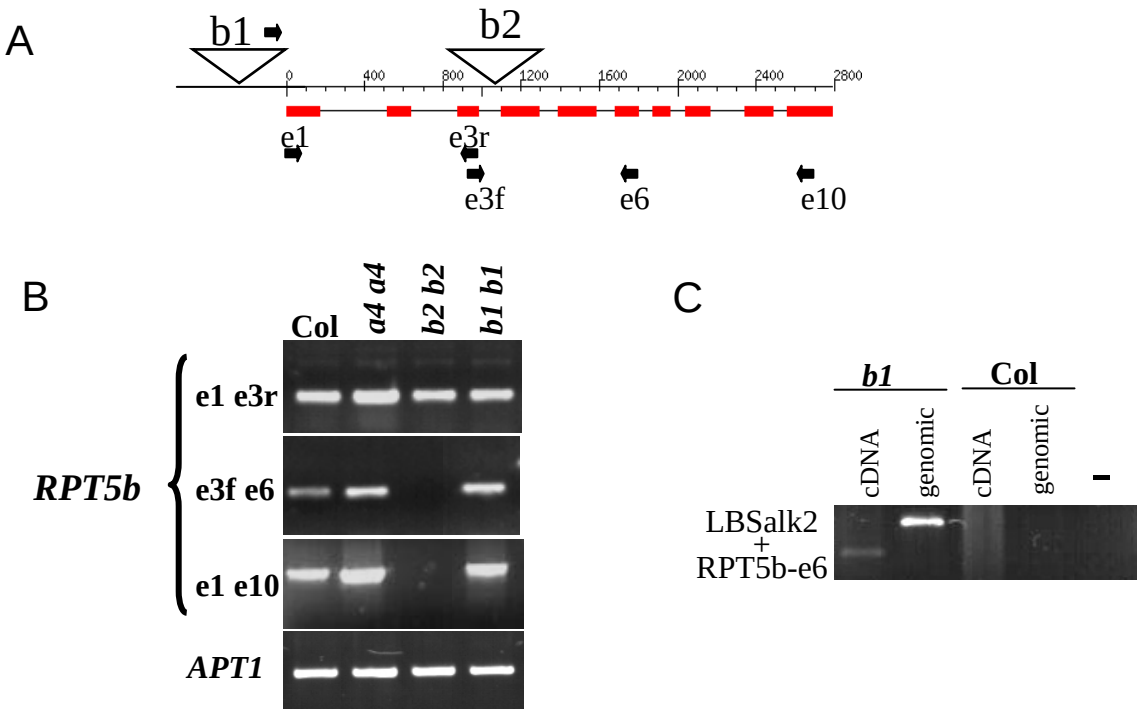
Supplemental Figure 3: Splicing at *RPT5b* intron 7 is not significantly affected between Col and Ws accession. Because we found a SNP between *RPT5b*^{Col} and *RPT5b*^{Ws} in intron 7, we investigated the intron 7 splicing and found that the *RPT5b* gave rise to an additional mispliced mRNA form with a 10 bp deletion (mispliced in **A**). The predicted sequence for the RPT5b protein is truncated due to premature stop codon (A, in bold character).

(A) Partial *RPT5b* cDNA sequences at intron 7 splicing site. Both normal and mis-spliced forms are represented with both predicted polypeptides. The mis-spliced cDNA deletion is represented by the dotted line.

(B, C) To assess the amount of both normal and mispliced form, we developed a PCR-based screen with N primer being specific for the normal form whereas M is specific for the mis-spliced form (N and M primers as underlined in (A)). Correct detection was checked on both forms subcloned into plasmids

(D) The mispliced form is present among both Col and Ws flower bud mRNA.

(E) To confirm the RT-PCR results, the flower bud *RPT5b* cDNAs were eventually subcloned into pGEMTeasy and the amount of normal (in grey) and mispliced form (in dark) form was checked. No significant difference could be found between correct vs mispliced form in both accessions

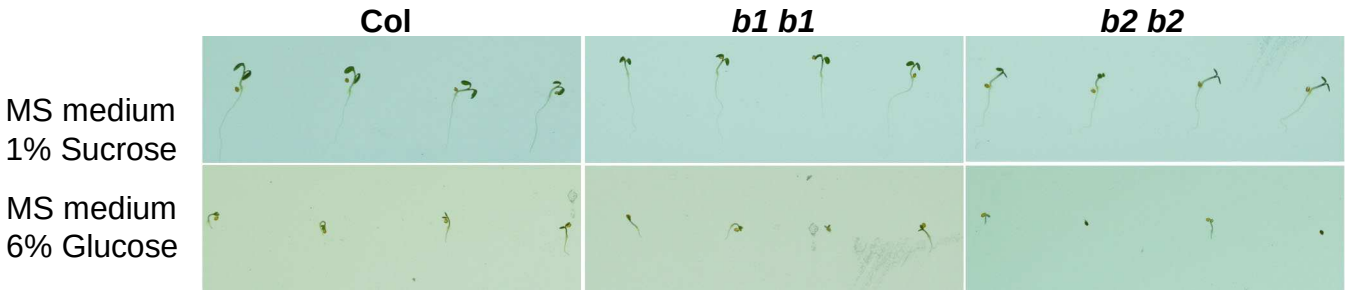


D

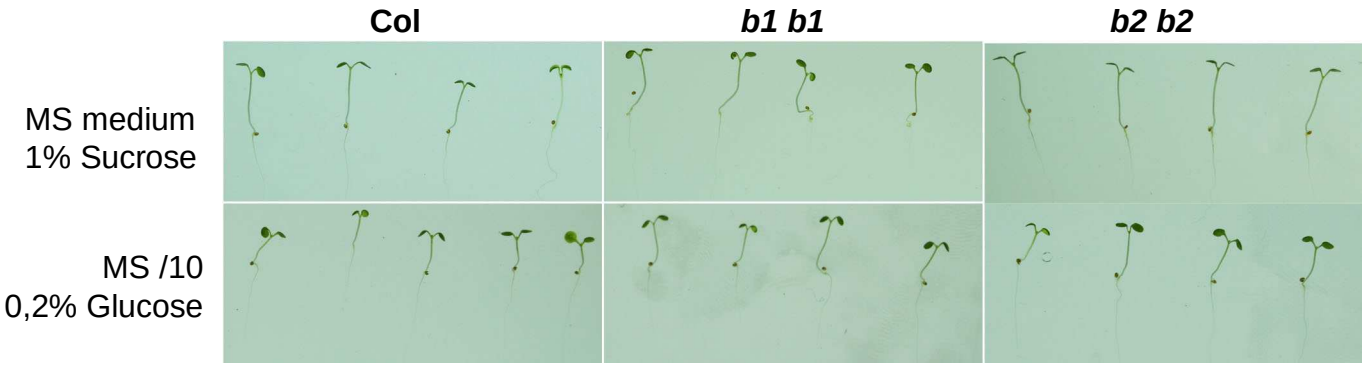
genotype	TE_M	TE_F
rpt5b-1/RPT5b	108 % (n=94)	88 % (n=92)
rpt5b-2/RPT5b	96 % (n=200)	113 % (n=182)

Supplemental Figure 4: *rpt5b* mutants characterization.
(A)T-DNA insertion location in the *RPT5b* gene for *rpt5b-1* (*b1*) and *rpt5b-2* (*b2*) mutant alleles. Primers used in (B) are depicted as well as the LB Salk2 primer, used in (C), which is located on the *b1* T-DNA insertion.
(B) RT-PCR on RNA isolated from wild-type and homozygous mutant plants inflorescence. Primers used are depicted in (A). A full-length *RPT5b* mRNA is still present in *b1 b1* plants (i.e. *b1* is not a nul allele) whereas only the first 3 exons of *RPT5b* are expressed in *b2 b2* plants.
(C) RT-PCR amplification on *b1 b1* plants using a LB-based primer shows that *RPT5b* transcription initiation is initiated within the T-DNA. Amplified cDNAs in *b1 b1* were cloned and sequenced, checking that they do contain a LB-*RPT5b* fusion transcript. PCR amplifications on genomic DNA are shown as controls.
(D) Neither male nor female transmission are affected for *rpt5b-1* and *rpt5b-2* mutant alleles.TE_M and TE_F are respectively male and female Transmission Efficiencies as in Table 1

A High Glucose conditions



B Low light Low Nutrient conditions



Supplemental Figure 5: *rpt5b* mutants display wild-type phenotype under high sugar and low nutrient conditions

A- Wild-type Col plants, *rpt5b-1/rpt5b-1* and *rpt5b-2/rpt5b-2* were grown *for* 4 days at 23℃ under constant light (75 µmol/m^2/s) on normal MS medium or in high glucose conditions (6% Glucose MS). Similar results were obtained on 5% Glucose MS.

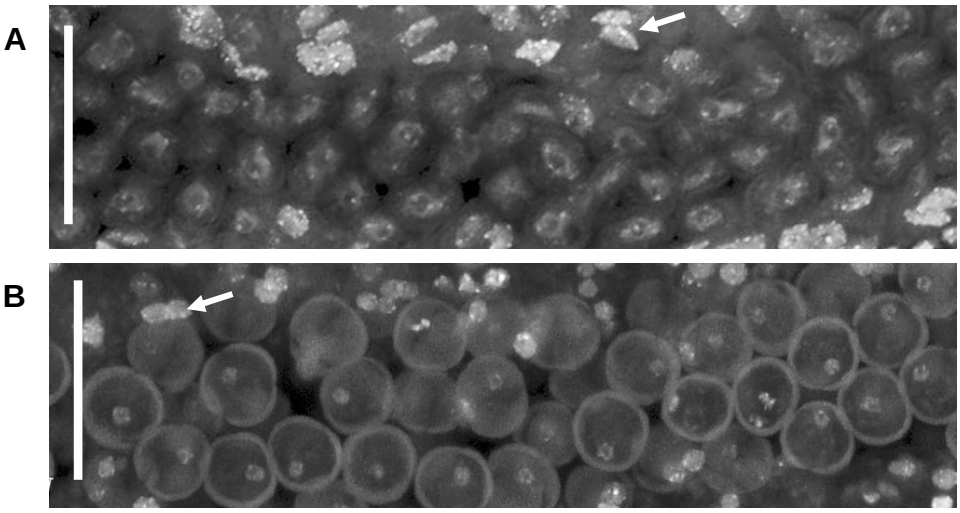
B- Wild-type Col plants, *rpt5b-1/rpt5b-1* and *rpt5b-2/rpt5b-2* were grown for 8 days at 23℃ under constant dim light (15 µmol/m^2/s) on normal MS medium or in low nutrient condition (MS/10 0,2% Glucose)

In our conditions, we were not able to see either insensitivity to glucose mediated arrest (A) or seedling growth retardation under low-light low-nutrient conditions (B) for both *rpt5b* homozygous mutants as compared with wild-type Columbia.

Conditions as reproduced from Cho et al. (2006) and Moore et al. (2003)

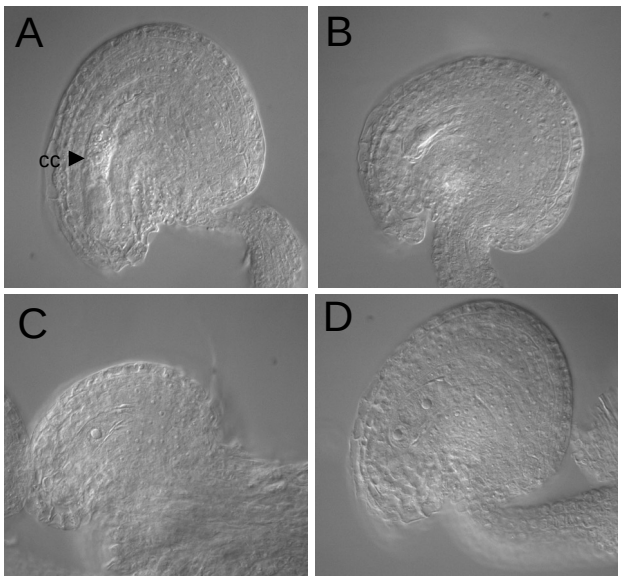
Additional reference:

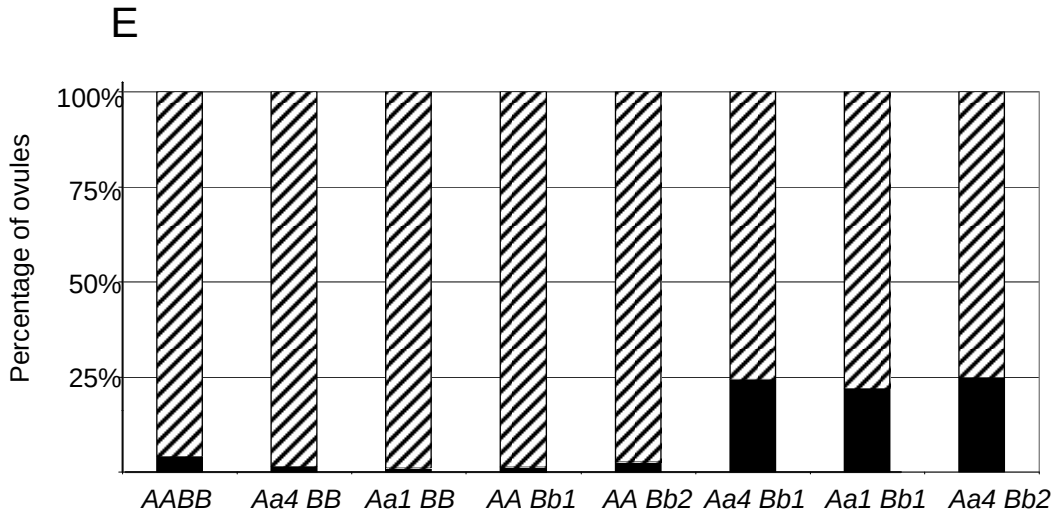
Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.H., Liu, Y.X., Hwang, I., Jones, T., and Sheen, J. (2003). Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science **300,** 332-336.



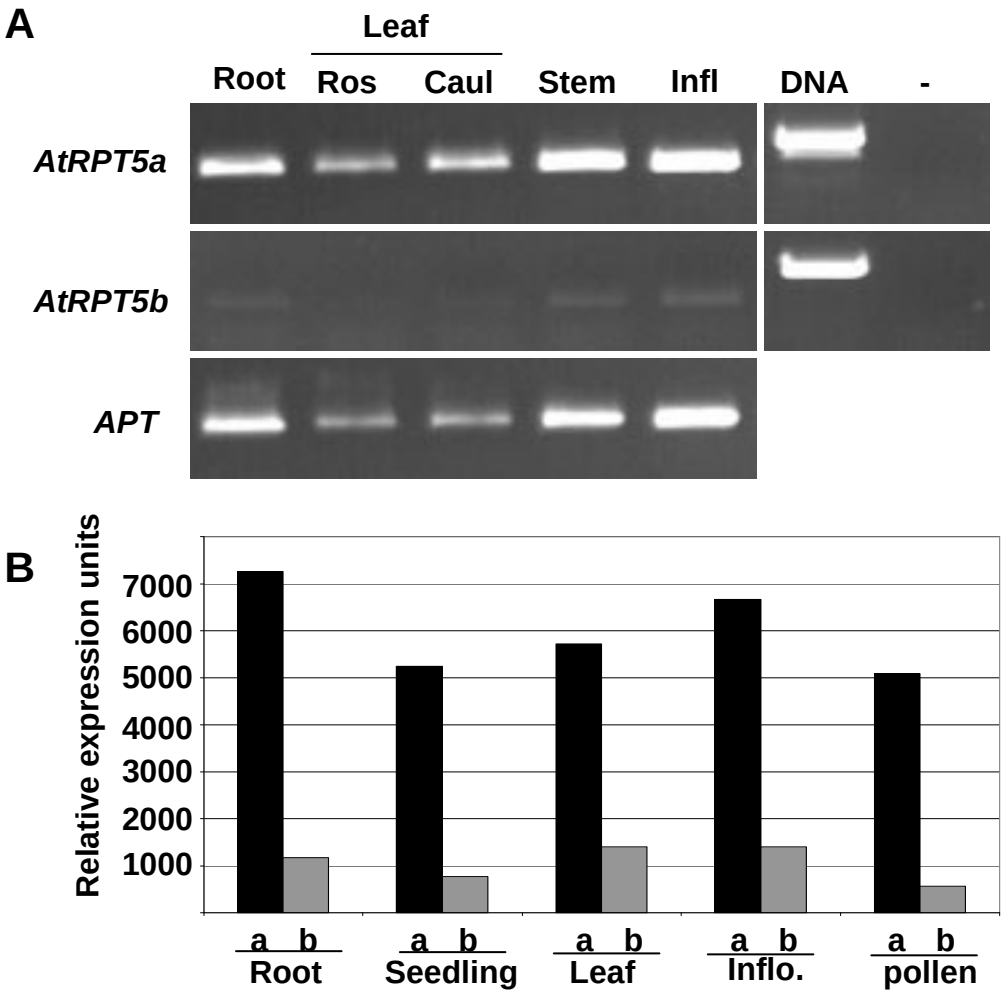
Supplemental Figure 6: Pollen Mitosis I occurs normally in *rpt5a*/*RPT5a* ; *rpt5b*/*RPT5b* plants.

Optical section through *rpt5a-4*/*RPT5a* ; *rpt5b-1*/*RPT5b* anthers stained with propidium iodide. All gametophytes develop as microspores (A) and then following PMI as Bi-Cellular Pollen grains (B). Bright nuclei appearing outside grains (arrows) are from sporophytic cells from the surrounding tissus. Scale bars represent 40 μm



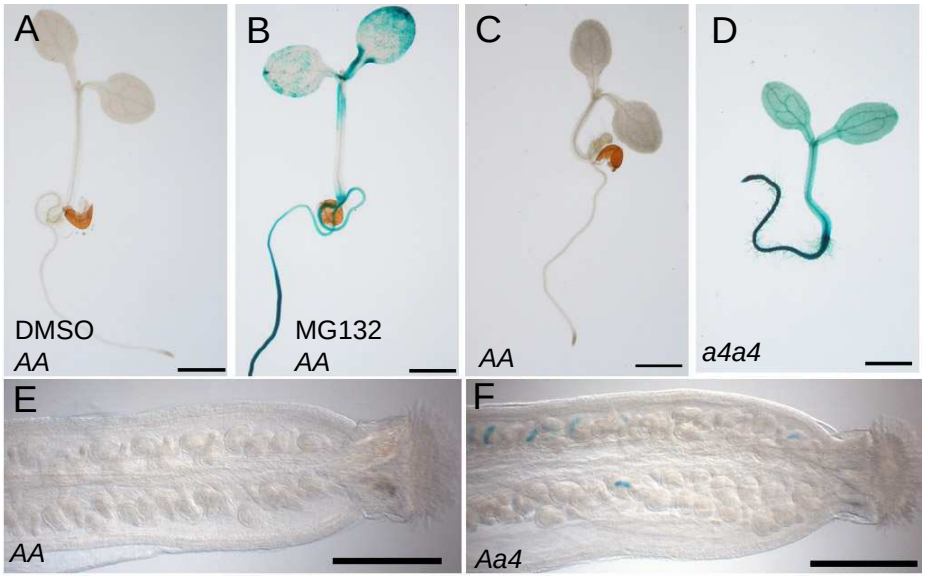


Supplemental Figure 7: ovule development in *rpt5a*/*RPT5a* ; *rpt5b*/*RPT5b* plants
DIC observation of mature ovules in *Aa1Bb1* plants. At the time of fertilization, siliques present 75% of ovules that are well-developed with an enlarged central cell nucleus (cc, arrow) (A). Other ovules display an altered structure and are blocked at one- (C) or two-nuclei stages (D). In a few cases, the whole embryo sac has degenerated (B).
(E) Counting of ovule development stages in 2 mm-long pistils using DIC in genotypes as indicated below the bars. All lines are in Col background. Fully developed ovules are represented as dashed bars whereas ovules arrested at the two-nuclei stage or earlier are in black bars. Results are shown in percentages. Between 500 and 600 ovules were counted per genotype.

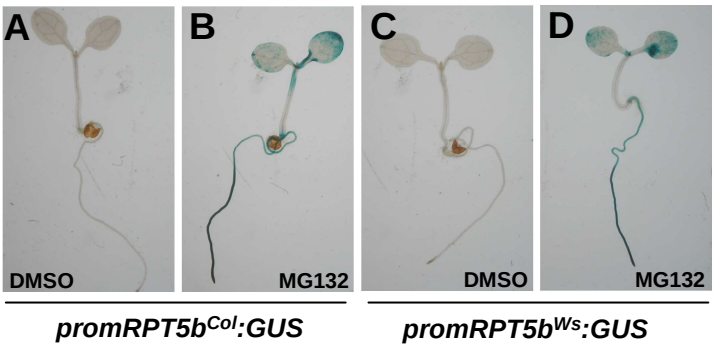


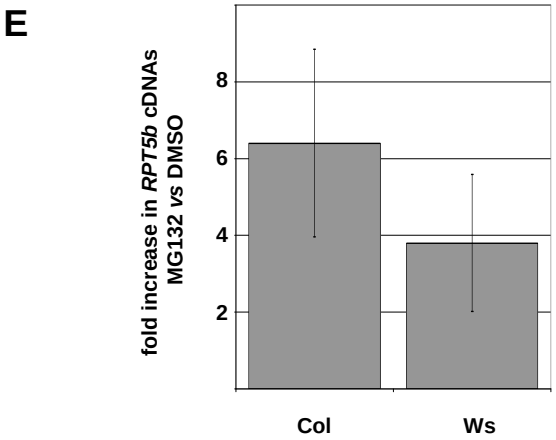
Supplemental Figure 8: *RPT5a* and *RPT5b* are expressed ubiquitously, with *RPT5a* being more strongly expressed.

(A) RT-PCR on Columbia tissues using primers specific for *RPT5a*, *RPT5b* and *APT* genes. All PCRs were carried out with 25 cycles of amplification. RNA extracted from Root, Rosette leaf (Ros), Cauline leaf (Caul), Stems and Inflorescences (Infl). Control amplifications were carried out on genomic DNA (DNA) and without template (-).
(B) Microarray expression data of *RPT5a* (a, black bars) and *RPT5b* (b, grey bars) as reported by genevestigator (*www.genevestigator.ethz.ch*)

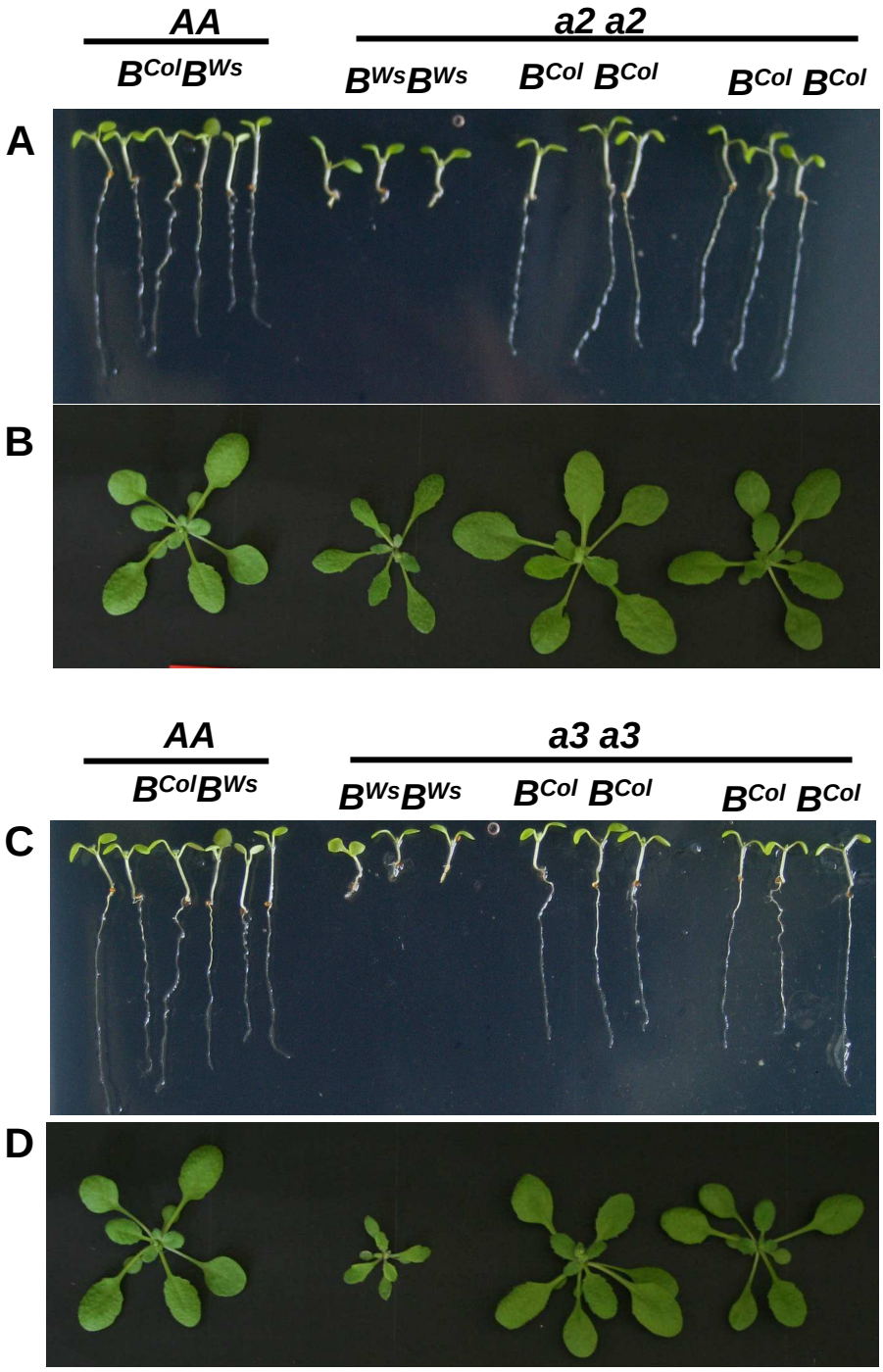


Supplemental Figure 9: The *RPT5b^{Ws}* promoter is up-regulated through the proteasome feedback loop in sporophyte and gametophytes.
GUS staining of Col plantlets that are transgenic for GUS constructs driven by the *RPT5b^{Ws}* promoter.
(A, B) For the proteasome inhibitor experiment, 4-day old plantlets have been submitted to a 36-hour treatment without MG132 (A: DMSO) or with MG132 100µM (B).
(C, D) 6-day old transgenics in wild-type (C) or *a4a4* (D) backgrounds, respectively.
(E-F) GUS staining on 2mm-pistils from an heterozygous *promRPT5b^{Ws}:GUS* transgene in a wild-type background (E) or in a *Aa4* background (F).
Scale bar represent 1 mm (A-D) and 100 µm (E, F).





Supplemental Figure 10: *RPT5b*^{Col} and *RPT5b*^{Ws} promoters react similarly to the proteasome feedback signal in both Col and Ws sporophyte.
(A-D) GUS staining of wild-type Ws plantlets that are transgenic for GUS constructs driven by the *RPT5b*^{Col} (A, B) or *RPT5b*^{Ws} (C,D) promoters. Four-day old plantlets have been submitted to a 36-hour treatment without (A, C: DMSO) or with the proteasome inhibitor MG132 100µM (B, D). Both Col and Ws *RPT5b* promoters respond to the proteasome feedback loop, in a similar way in Ws as they do in Col (as shown in Figure 8 and Supplemental Figure 4).
(E) Quantitative RT-PCR of *RPT5b* cDNA compared with *APT* cDNA accumulation from wild-type Col and Ws plants. Four-day old plantlets have been submitted to a 36-hour treatment without (DMSO) or with the proteasome inhibitor MG132 100µM prior RNA extraction. *RPT5b* cDNAs expression has been normalized to *APT* cDNAs expression. The data presents the relative increase of the *RPT5b* cDNAs upon MG132 treatment (MG132/DMSO). Endogenous *RPT5b* are thus subjected to the proteasome feedback loop in both Col and Ws accessions.



Supplemental Figure 11: *rpt5a-2/rpt5a-2* and *rpt5a-3/rpt5a-3* sporophytic phenotypes are rescued in a *RPT5b^{Col} / RPT5b^{Col}* background.
Wild-type F1 resulting from a Col X Ws backcross and *rpt5a-2/rpt5a-2* and *rpt5a-3/rpt5a-3* plants (pannels A and B, respectively) in *RPT5b^{Ws}/RPT5b^{Ws}* or *RPT5b^{Col}/RPT5b^{Col}* background. Two lines resulting from two independent F2 are shown for the latter genotype.
(A, C) 6-day old seedlings
(B, D) 20-day old rosettes from plants grown on soil.

Supplemental Table 1 :Primers used in this study:

(introduced restriction enzyme site are underlined)

Target sequence	primer name	sequence (5'->3')
APT	APT 5'	TCCCAGAATCGCTAAGATTGCCTCTT
APT	APT 3'	CGCAAGCACATTCAACAATCTTCACT
T-DNA	TAG6	CACTCAGTCTTTCATCTACGGC
T-DNA	LBSALK2	GCTTTCTTCCCTTCCTTTCTC
T-DNA	LB3Sail	TAGCATCTGAATTTCATAACCAATCTCGATACAC
RPT5a	EAT-U	TGGAAGAAATCTTTCTGTTGCAG
RPT5a	EAT-L	ATGTTGCCAACCAAGTAAGGC
RPT5a	EAT-A	GCGTGGTACCCATCCTTGTTC
RPT5a	EAT-D	TGAGTCGACAACTTAAAAAGA
RPT5a	DYI-U	TTGCAGCTACTAACCGTGCAG
RPT5a	DYI-L	GCTTGAAAGGATTAGTGCAACG
RPT5a	S46321-U	GTTGAAAACGTCTACACGGCA
RPT5a	S46321-L	CCTGTCGAAACGCTTTGTACC
RPT5a	RPT5a-5'	TCCCTAACTCTTTGCTCTTCT
RPT5a	RPT5-e4	CAAGTCACCAGGTTTCAAACT
RPT5a	EAT3194	CAAAGGGAGTGCTCTTGTATG
RPT5a	JLV100	GTAATACGACTCACTATATCAGGCGTAGTAGTTCAAG

Supplemental Table 1 :Primers used in this study (end).

Target sequence	primer name	sequence (5'->3')
RPT5b	RPT5b-e1	TTAACTCAGGCTGCTAAGGGA
RPT5b	RPT5b-e3r	AACTTCACCTGACGAGTTGATG
RPT5b	RPT5b-e3f	ATATCGATCTGGACTCTCAGA
RPT5b	RPT5b-e6	TTTGTCCCTATTGCGTCAATC
RPT5b	RPT5b-e10	ACCACACTTTGAAGGGGTTCT
RPT5b	RPT5b-3369	GCGTTTTGATAGGTACTCTAT
RPT5b	RPT5b-Hind3	GCACGATTTGTCGCTGCAATAACCTAAGCT
RPT5b	RPT5b-5'	TTTCTTACTTGTTTCCATTTG
RPT5b	RPT5b-e2	TATTTTCTCCTTCACTGATTC
RPT5b	RPT5b-e5	TCTTGTATGGTCCTCCTGGAA
RPT5b	JLV077	GTTGCCATGGCCCTTAGCAGCCTGAGTT
RPT5b	JLV083	AGACTGCAG GTCTAGTCTTAAGTTTTCTT
RPT5b	JLV085	TTCGTCGACCTCCATTGCTT
RPT5b	JLV086	AAGCAATGGAGGTCGACGAA
RPT5b	JLV087	AGAGGATCCGCACCACGCCAAGAGCGGCATTG
RPT5b	JLV094	AGAAAGCTTGTCTAGTCTTAAGTTTTCTT
RPT5b	JLV096	AGAACGCACGCTTGTATTAA
RPT5b	JLV097	TATTCAAATCGGAAGGGTGAA
RPT5b	JLV111	TGTAATACGACTCACTATAGGGCTTAGGCGTAG TAATTCAAG
RPT5b	N	GATCGCATTAAGGTTATTGC
RPT5b	M	GCGATGATCGCATTAAGCG

Supplemental Table 2. *RPT5b*^{Col} and *RPT5b*^{Ws} promoters both react similarly to the proteasome feedback signal in female gametophytes

Transgene	Line	Numbers of GUS-stained ovules	Total numbers of ovules	% GUS-stained ovules
promRPT5b^{Col} *:GUS*	# 7	43	176	24.5%
promRPT5b^{Col} *:GUS*	# 9	48	179	26.8%
promRPT5b^{Ws} *:GUS*	# 7	51	181	28.2%
promRPT5b^{Ws} *:GUS*	# 10	46	179	25.6%

Counting of GUS-stained ovules in pistils from F1 plants harbouring a *rpt5a4/RPT5a* ; *promRPT5b :GUS*/- genotype. If all ovules respond to the proteasome feedback regulation, 25% of the F1 ovules are expected to be GUS-stained as all transgenes segregated as a single locus. Ovules were counted in three independent pistils.