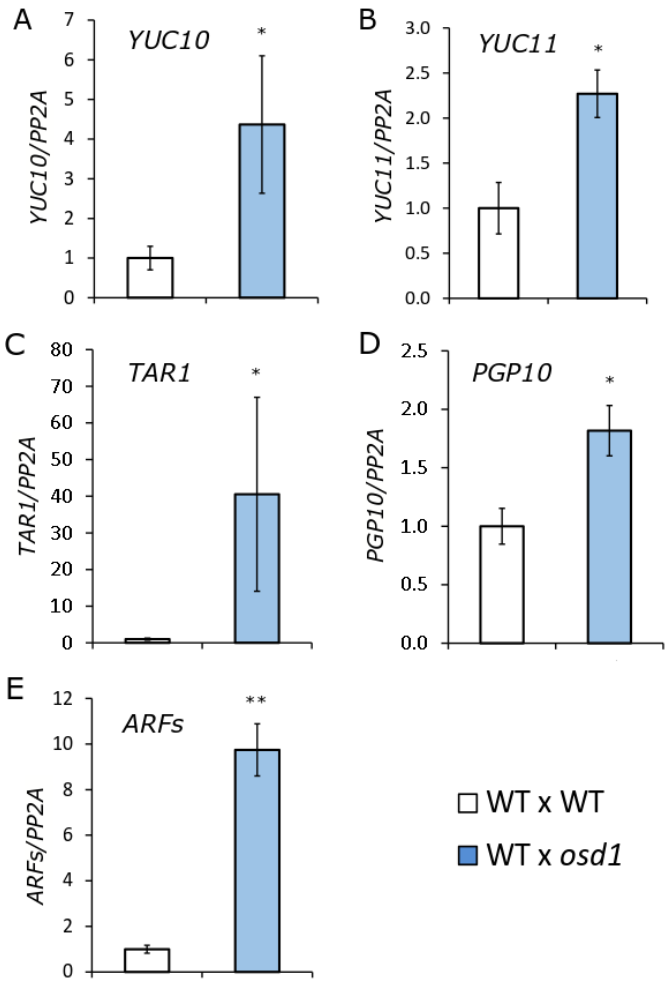
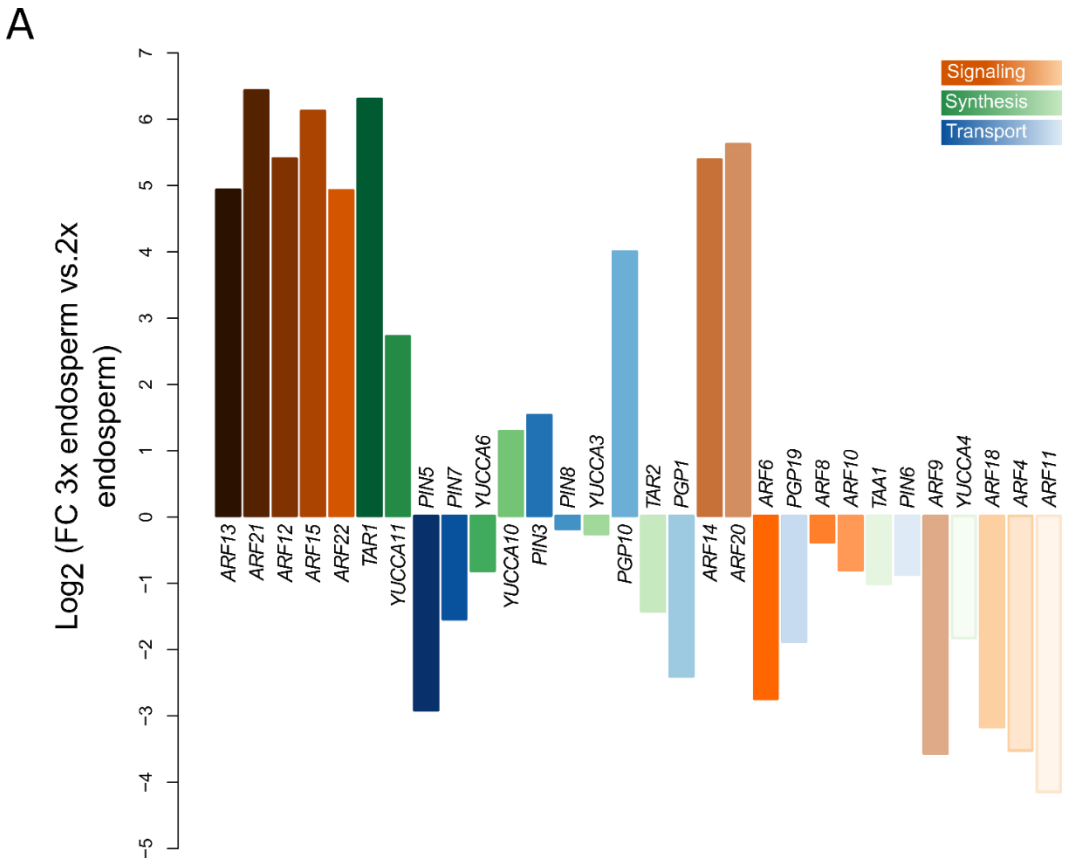
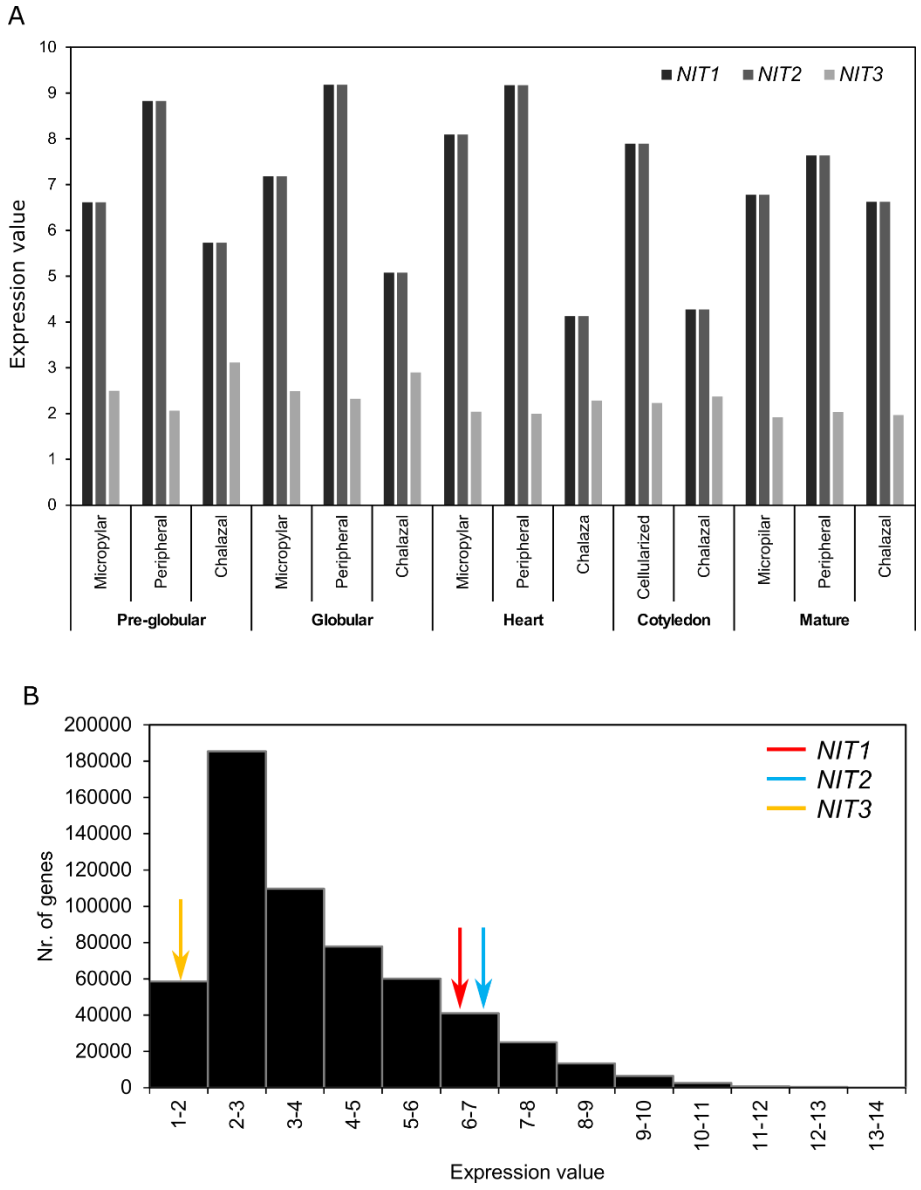


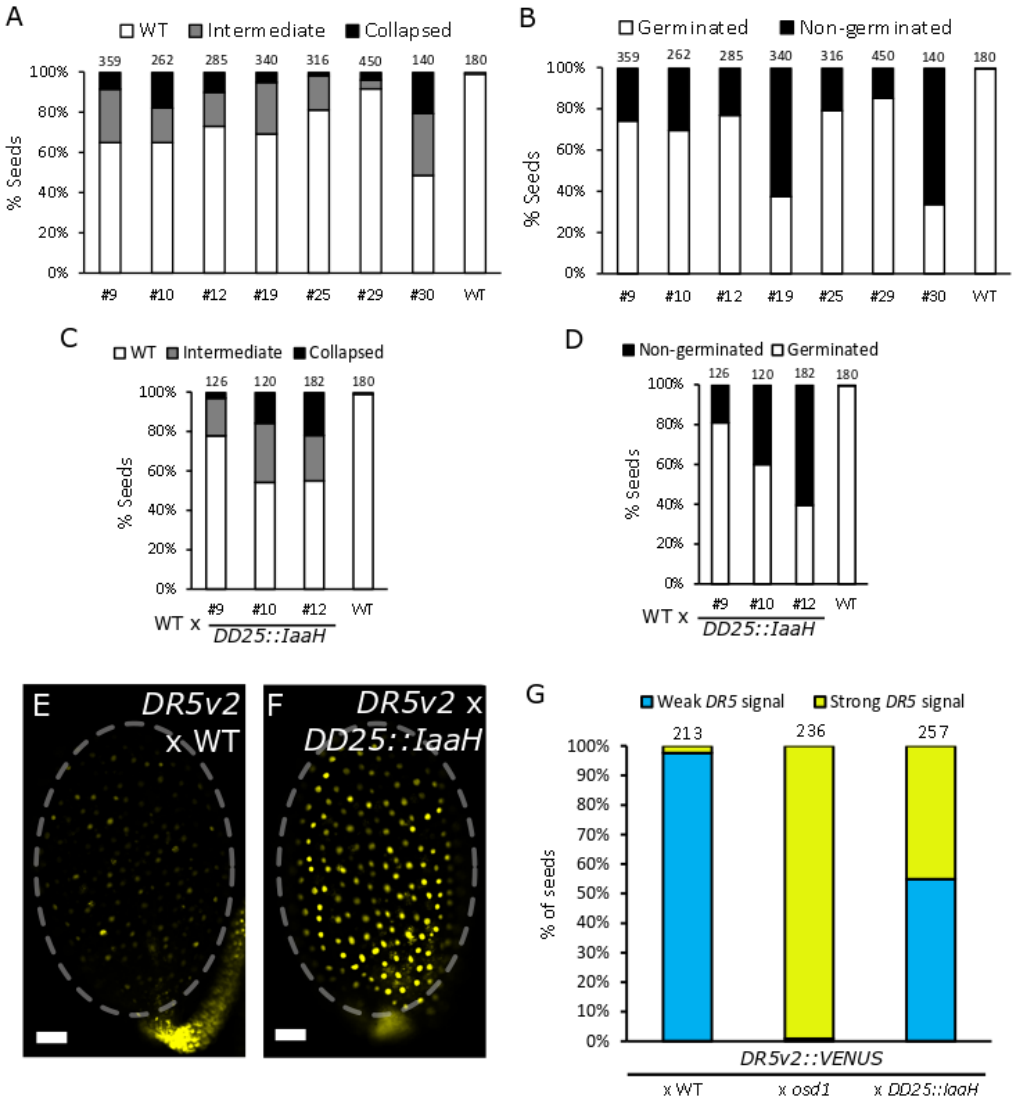
Supplemental Figure S1. Validation of auxin-related gene expression from Fig. 1A. Relative gene expression in 2x and 3x seeds 6 days after pollination (6 DAP), as determined by RT-qPCR for *YUC10* (A), *YUC11* (B), *TAR1* (C), *PGP10* (D) and *ARFs* (E). Each figure panel shows a representative biological replicate. *ARF12*, *13*, *14*, *15*, *21* and *22* were assayed together due to high sequence similarity. Three technical replicates were performed and error bars indicate standard deviation. Differences are significant for Student's T-test for $p<0.05$ (*) or $p<0.001$ (**).



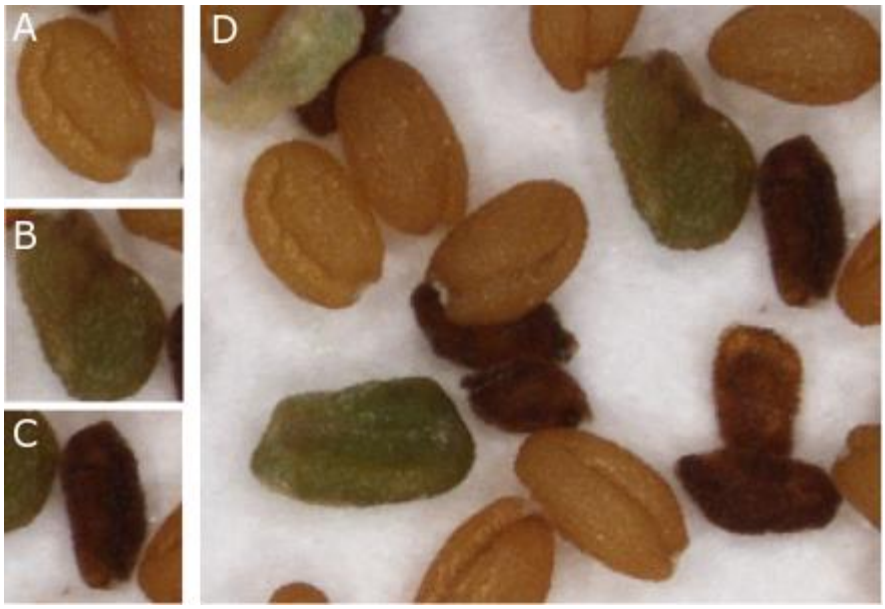
Supplemental Figure S2. Auxin-related genes are upregulated in the endosperm of 3x seeds. Log2-fold expression change between genes expressed in the endosperm of 3x and 2x seeds. Genes coding for auxin signaling, biosynthesis, and transport proteins are indicated by orange, green and blue colors, respectively.



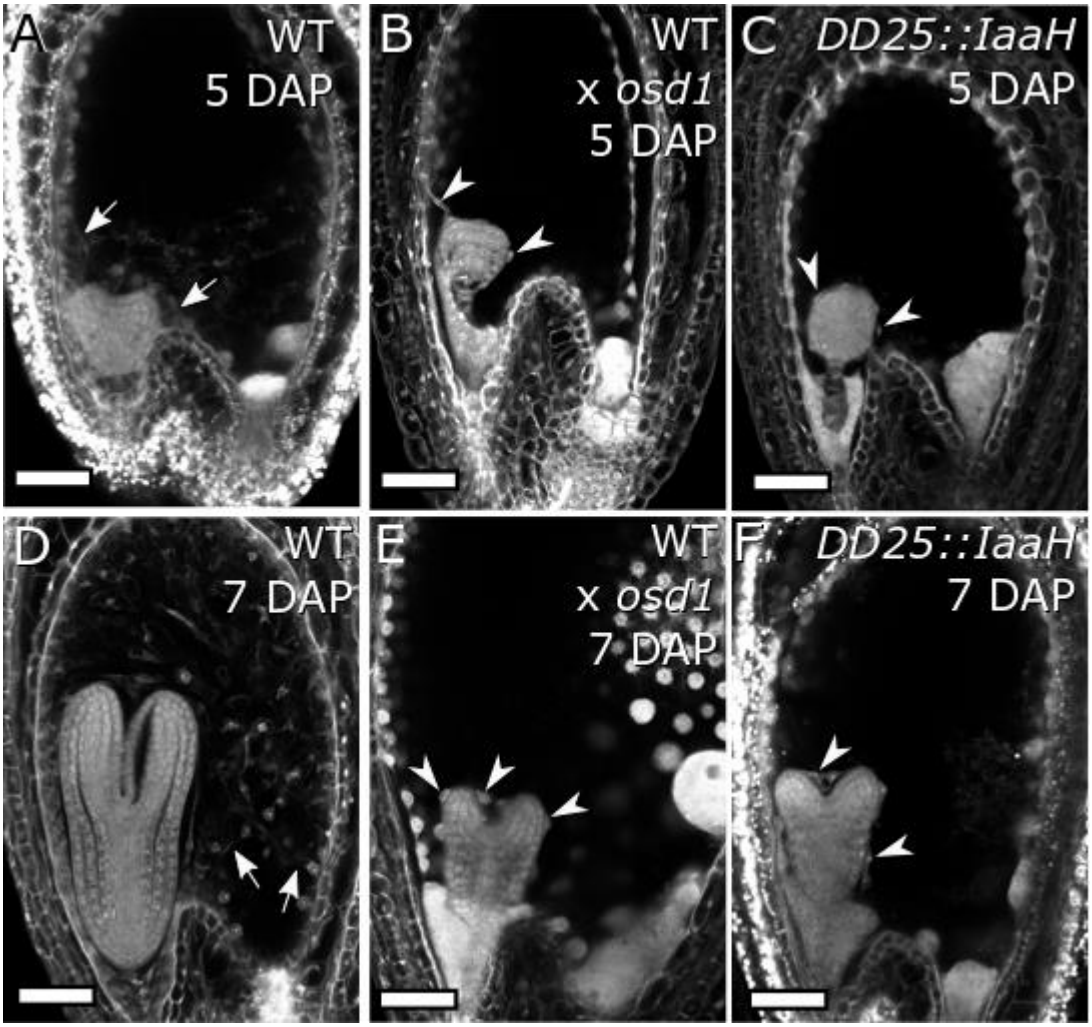
Supplemental Figure S3. Relative expression of NITRILASE (NIT) coding genes. (A) Expression of genes coding for NIT IAM-synthetizing enzymes in each endosperm domain along seed development. Gene expression values indicate normalized microarray signal intensity according to Belmonte et al. (2013). (B) Relative expression of *NIT1-3* genes, compared to the global distribution of gene expression in different subdomains and time-points of endosperm development, according to Belmonte et al. (2013). Arrows indicate the gene expression category for each *NIT* gene.



Supplemental Figure S4. Expression of *DD25::IaaH* induces seed abortion. (A) Quantification of seed phenotypes in independent lines expressing *DD25::IaaH*. The seeds were classified in three distinct classes, as shown in Supplemental Fig. S5. (B) Seed germination rates in lines expressing *DD25::IaaH*. (C, D) Same as for (A) and (B), but using *DD25::IaaH* as pollen donor crossed to WT. Numbers on top indicate number of seeds analyzed. (E-F) Activity of maternal *DR5v2* at 5 days after pollination with WT (E) or *DD25::IaaH* (F) pollen. Pictures show representative seeds of three independent siliques per cross. Scale bars indicate 50 µm. (G) Percentage of seeds showing increased *DR5v2::VENUS* expression after pollination with a WT, *osd1* or *DD25::IaaH*-expressing father. For *DD25::IaaH*, line #10 was used. The seeds were categorized as showing weak or strong *DR5* signals according to panels (E) and (F), respectively. WT, wild type.

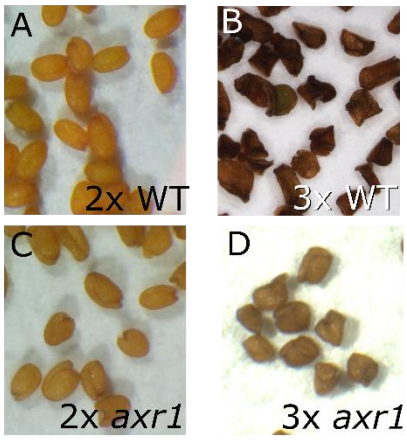


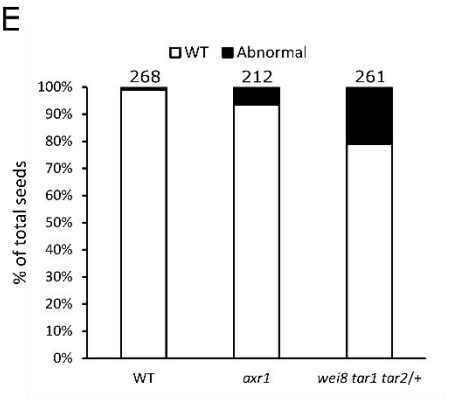
Supplemental Figure S5. Seed classification used for the phenotypic quantification of *DD25::IaaH* phenotypes in Fig. 4 and Supplemental Figs. S4 and S7. (A) WT-like seed, (B) Intermediate phenotype, misshapen seeds, (C) Fully collapsed and shriveled seed. (D) Overall view of the progeny of a *DD25::IaaH* transgenic line.

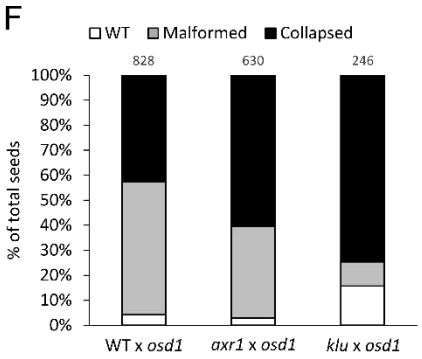


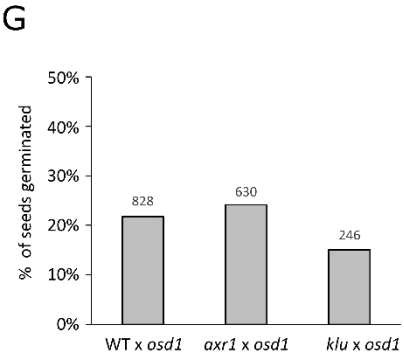
Supplemental Figure S6. Endosperm cellularization as determined by Feulgen staining. (A-C) Seeds at 5 days after pollination (DAP) of WT 2x (A), WT 3x (B) and *DD25::IaaH* 2x (C). (D-F) Same as for (A-C), but for 7 DAP seeds. Pictures show representative seeds of 10 independent siliques per cross. Arrows indicate cellularized endosperm and arrowheads indicate free endosperm nuclei surrounding the embryo. Scale bars indicate 50 µm. WT, wild type.

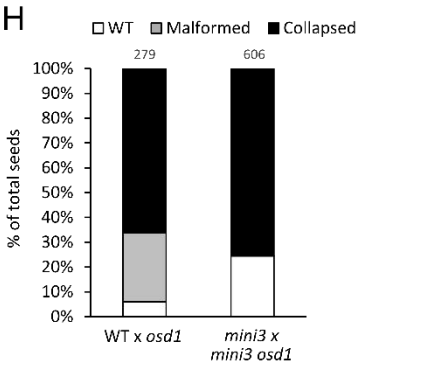
Supplemental Fig. S7. Expression map of commonly upregulated genes in 3x seeds and *DD25::IaaH* seeds. Gene expression was assessed in the endosperm and in the seed coat at different stages of seed development (containing pre-globular, globular and heart-stage embryos). Plotted are the genes belonging to GO-terms related with endosperm cellularisation (Table 2).

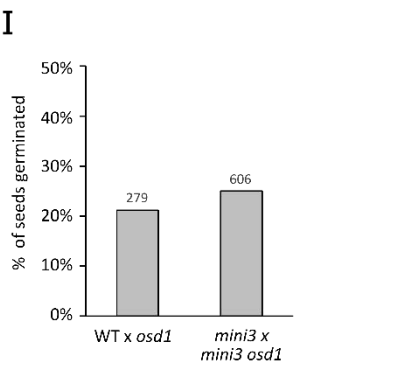

A
2x WT
B
3x WT
C
2x axr1
D
3x axr1

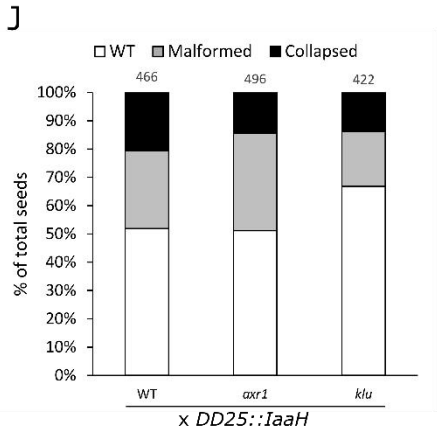

E
WT
Abnormal
268
212
261
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of total seeds
WT
axr1
wei8 tar1 tar2/+

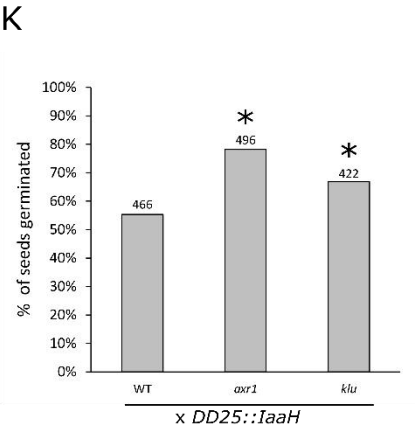

F
WT
Malformed
Collapsed
828
630
246
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of total seeds
WT x osd1
axr1 x osd1
klu x osd1


G
50%
40%
30%
20%
10%
0%
% of seeds germinated
828
630
246
WT x osd1
axr1 x osd1
klu x osd1

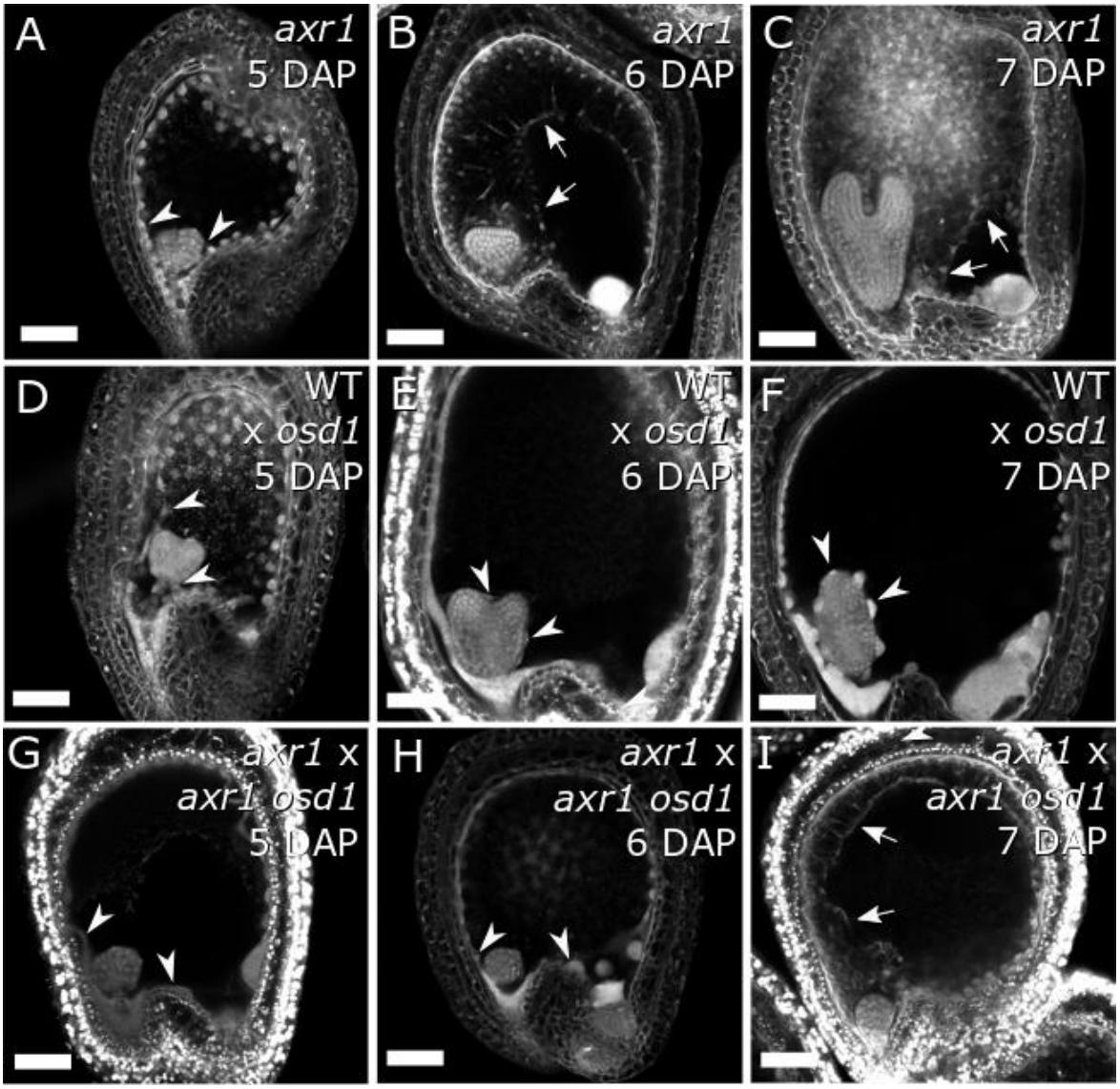

H
WT
Malformed
Collapsed
279
606
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of total seeds
WT x osd1
mini3 x
mini3 osd1


I
50%
40%
30%
20%
10%
0%
% of seeds germinated
279
606
WT x osd1
mini3 x
mini3 osd1

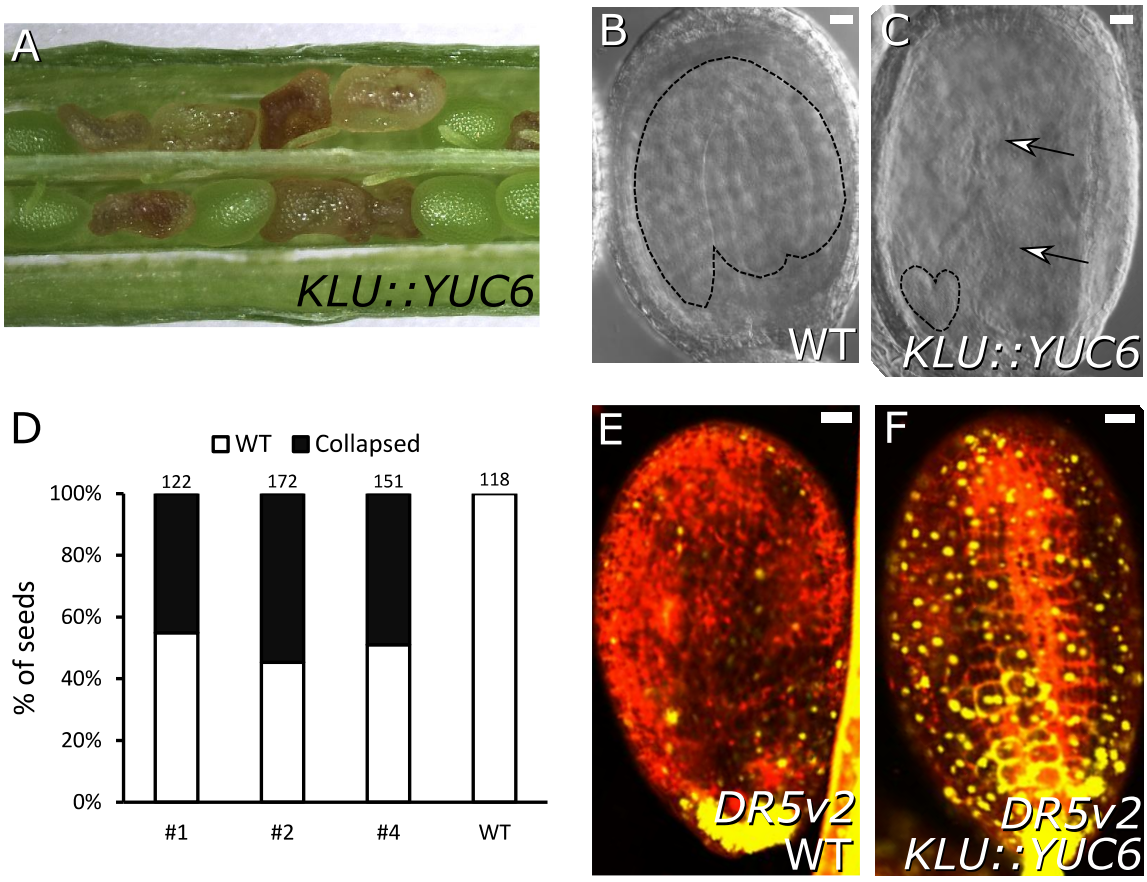

J
WT
Malformed
Collapsed
466
496
422
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of total seeds
WT
axr1
klu
x DD25::IaaH


K
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of seeds germinated
*
496
*
422
466
WT
axr1
klu
x DD25::IaaH

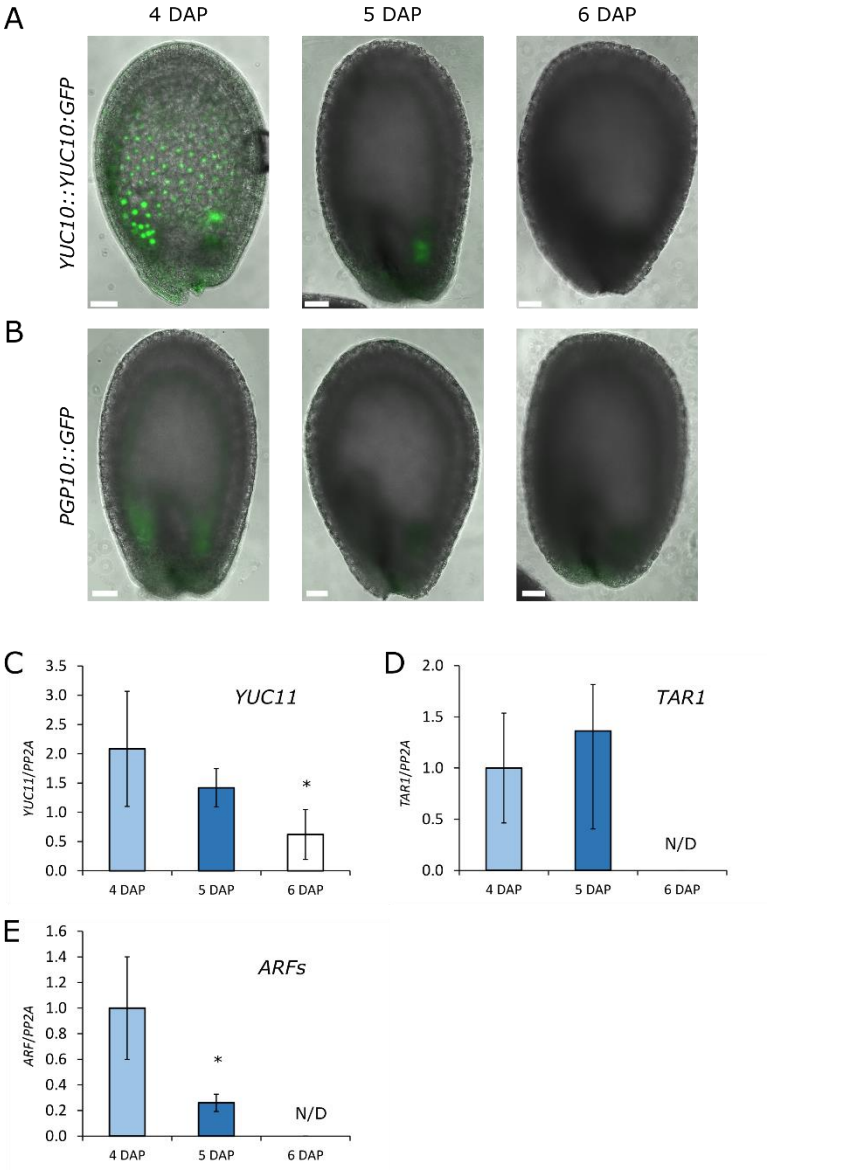
Supplemental Figure S8. Seed phenotypes of 2x and 3x seeds. Mature seeds of WT Col-0, 2x (A) and 3x (B), and *axr1* mutant, 2x (C) and 3x (D). (E) Quantification of seed malformation in 2x seeds of wild-type (WT), *axr1* and *wei8 tar1 tar2*/+ plants. (F-G) Phenotypic classification of 3x seeds resulting from crosses with WT, *axr1* and *klu* mutant mothers (F), and their corresponding germination rate (G). (H-I) Phenotypic classification of 3x seeds resulting from crosses with the *mini3* mutant (F), and their corresponding germination rate (I). (J-K) Phenotypic classification of 2x seeds resulting from crosses of WT, *axr1* and *klu* mutant mothers to a father expressing *DD25::IaaH* (H), and their corresponding germination rate (I). Differences between WT and mutant seed germination in (I) are significant for Chi-square test for $p<0.05$ (*). Numbers on top indicate number of seeds assayed.



Supplemental Figure S9. Loss of *AXR1* function restores endosperm cellularization in 3x seeds. (A-C) 2x *axr1* seeds at 5, 6 and 7 days after pollination (DAP). (D-F) Same as for (A-C), but for 3x WT seeds. (G-I) Same as (A-C), but for 3x *axr1* seeds. Pictures show representative seeds of 10 independent siliques per cross. Arrows indicate cellularized endosperm and arrowheads indicate free endosperm nuclei surrounding the embryo. Scale bars indicate 50 µm. WT, wild type.



Supplemental Figure S10. Over-production of auxin in the seed coat results in a triploid-like seed phenotype. (A) Representative photo of seeds aborting in a transgenic line expressing *KLU::YUC6*. (B-C) Seeds expressing *KLU::YUC6* show embryo retardation and the endosperm shows signs of continued proliferation (arrows). (D) Quantification of seed phenotypes in independent lines expressing *KLU::YUC6*. Numbers on top indicate number of seeds assayed. (E-F) The transgenic *KLU::YUC6* seeds show increased auxin activity, as measured by the *DR5v2* auxin sensor. Scale bars indicate 50 µm. WT, wild type.



Supplemental Figure S11. Downregulation of auxin-related genes coincides with the timing of endosperm cellularization. (A-B) Reporter activity for *YUC10::YUC10:GFP* (A) and *PGP10::GFP* (B) during seed development (4 to 6 days after pollination, DAP). Scale bars indicate 50 µm. (C-E) Relative gene expression in seeds at 4, 5 and 6 DAP, as determined by RT-qPCR for *YUC11* (C), *TAR1* (D), and *ARFs* (E). Each figure panel shows a representative biological replicate. *ARF12*, *13*, *14*, *15*, *21* and *22* were assayed together due to high sequence similarity. Three technical replicates were performed and error bars indicate standard deviation. Differences are significant for Student's T-test for $p<0.05$ (*). N/D, transcript not detected.

Supplemental Table S1 Significantly enriched biological processes for genes upregulated in 3x seeds (L*er* × *osd1* cross) but not in *DD25::IaaH* 2x seeds, at 6 days after pollination (Log2FC >1, p-value ≤ 0.05). GO-terms that are similar to those enriched for genes commonly upregulated in 3x seeds and *DD25::IaaH* seeds (Table 2) are marked in bold.

GO-term	p-value	Number of genes	Description
GO:0031640	**2.24E-07**	**25**	**killing of cells of other organism**
GO:0001906	**2.24E-07**	**25**	**cell killing**
GO:0050832	**1.00E-06**	**41**	**defense response to fungus**
GO:0080162	1.00E-02	2	intracellular auxin transport
GO:0042891	1.00E-02	2	antibiotic transport
GO:0055073	1.00E-02	2	cadmium ion homeostasis
GO:0000032	**1.00E-02**	**2**	**cell wall mannoprotein biosynthetic process**
GO:0009448	1.00E-02	3	gamma-aminobutyric acid metabolic process
GO:0048462	1.00E-02	3	carpel formation
GO:0051090	**1.00E-02**	**4**	**regulation of sequence-specific DNA binding transcription factor activity**
GO:0009404	1.00E-02	7	toxin metabolic process
GO:0010043	1.00E-02	8	response to zinc ion
GO:0098754	1.00E-02	8	detoxification
GO:0042631	1.00E-02	12	cellular response to water deprivation
GO:0009636	1.00E-02	10	response to toxic substance
GO:0005996	**1.00E-02**	**11**	**monosaccharide metabolic process**
GO:0010119	1.00E-02	13	regulation of stomatal movement
GO:0042545	**1.00E-02**	**13**	**cell wall modification**
GO:0006357	**1.00E-02**	**21**	**regulation of transcription from RNA polymerase II promoter**

Supplemental Table S2. Primer list

Name	Gene reference	Used for	Primer sequence (5'-3')*
Promoter *DD25*	AT3G04540	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAGCTCCTTCCTACGTTTTGTCACTA
			GGGGACCACTTTGTACAAGAAAGCTGGGTACTAGTAGACAACGAAGCAGCCATTAT
Promoter *KLU*	AT1G13710	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTGAGCTCCAGTGTGCAATTTTCGTTAGGA
			GGGGACCACTTTGTACAAGAAAGCTGGGTGCTAGCGGCAGGAGAGAGAGAGTGTTG
YUC6	AT5G25620	Cloning	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGAAGAGAGAGATGGAAGGTAAAC
			GGGGACCACTTTGTACAAGAAAGCTGGGTGATCATCAATTCCCACCACA
PP2A	AT1G69960	qPCR	TAACGTGGCCAAAATGATGC
			GTTCTCCACAACCGCTTGGT
PHE1	AT1G65330	qPCR	TCCAACACCGAAAACTCCAT
			CGCATGTGCGGTCATCC
YUC10	AT1G48910	qPCR	GCGAGATTCAGGTTATCAATGG GTAACCAGTAGCAAACACAATCG
YUC11	AT1G21430	qPCR	GTTTGCAGTGCCTCTCTGTG TGTGGCGTATTCGTCAAGGT
TAR1	AT1G23320	qPCR	GTTCCTTATTACTCCACATACGTG CATCAGGGTTGTTCGGTGAC
PGP10	AT1G10680	qPCR	ACAACAAGCATTGGATCGGC TTGATCGTCGATAGCCGGTG
ARF12,13, 14,15,21,22	AT1G34310 AT1G34170 AT1G35540 AT1G35520 AT1G34410 AT1G34390	qPCR	AAGTTCAAATGCAAGGTG TGCAGAATTCAGGCCATGG
AGL62	AT5G60440	qPCR	CCTCCTCACCAACACAACAA
			ACCTTTGAACCCCTCGAGTT
AGL36	AT5G26650	qPCR	GTGCTCTCATCTACAGTCCA
			CATCATCTTCTTGGTTCGGG
PEG2	AT1G49290	qPCR	GGAAGTGATAGAAGCGGTAGAG
			TAAACCTCGCACTCACAATCTC
PEG9	At5g15140	qPCR	ACAACAAGACGACGAATAATCTG
			GCATACCTTAGTAGCAAACCG
ADM	AT4G11940	qPCR	TTGAAAGAGTTTGCGGATGTG
			AGGACCAACATTATGGTCATACC

*primer adaptors are underlined