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SUPPLEMENTARY INFORMATION

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Maternal auxin supply contributes to early embryo patterning in *Arabidopsis*

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Supplementary Material

Materials

Plant material

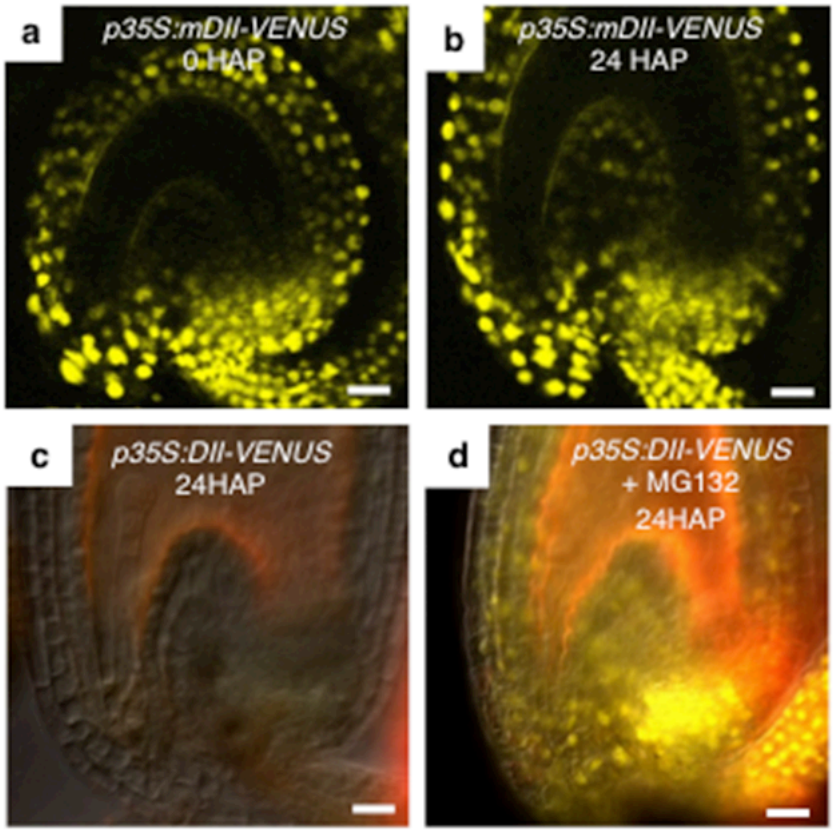
pTAA1:GFP-TAA1, *wei8-1*, *wei8-3, wei8-11, pDR5:GFP wei8-1*, *wei8-1 tar1-1*, *pDR5:nls-3xGFP wei8-1 tar1-1, pDR5rev:GFP, pYUC1:nls-3xGFP, pYUC4:nls-3xGFP, pYUC8:eGFP-GUS, pYUC9:eGFP-GUS, pPIN3:PIN3:GFP, pAUX1:AUX1-YFP, pLAX1:LAX1-YFP, pABCB1:ABCB1-GFP, pABCB19:ABCB19-GFP, p35S:DII-VENUS, p35S:mDII-VENUS* and *R2D2* (*pAtRPS5:DII/mDII*) were previously described[1-9].

Figure supplements

Figure 1 has six figure supplements (Supplementary Fig. 1. Expression controls for *p35S:DII-VENUS*; Supplementary Fig. 2. Representative pictures for fluorescent signal quantification in Fig. 1; Supplementary Fig. 3. Auxin response in the tip region of the inner integuments and micropylar nucellus cells of maize; Supplementary Fig. 4. Expression dynamics of *YUC* gene; Supplementary Table 1. Auxin and metabolites quantification in ovules; Supplementary Table 2. Fluorescent signal intensities in the embryo attachment region)

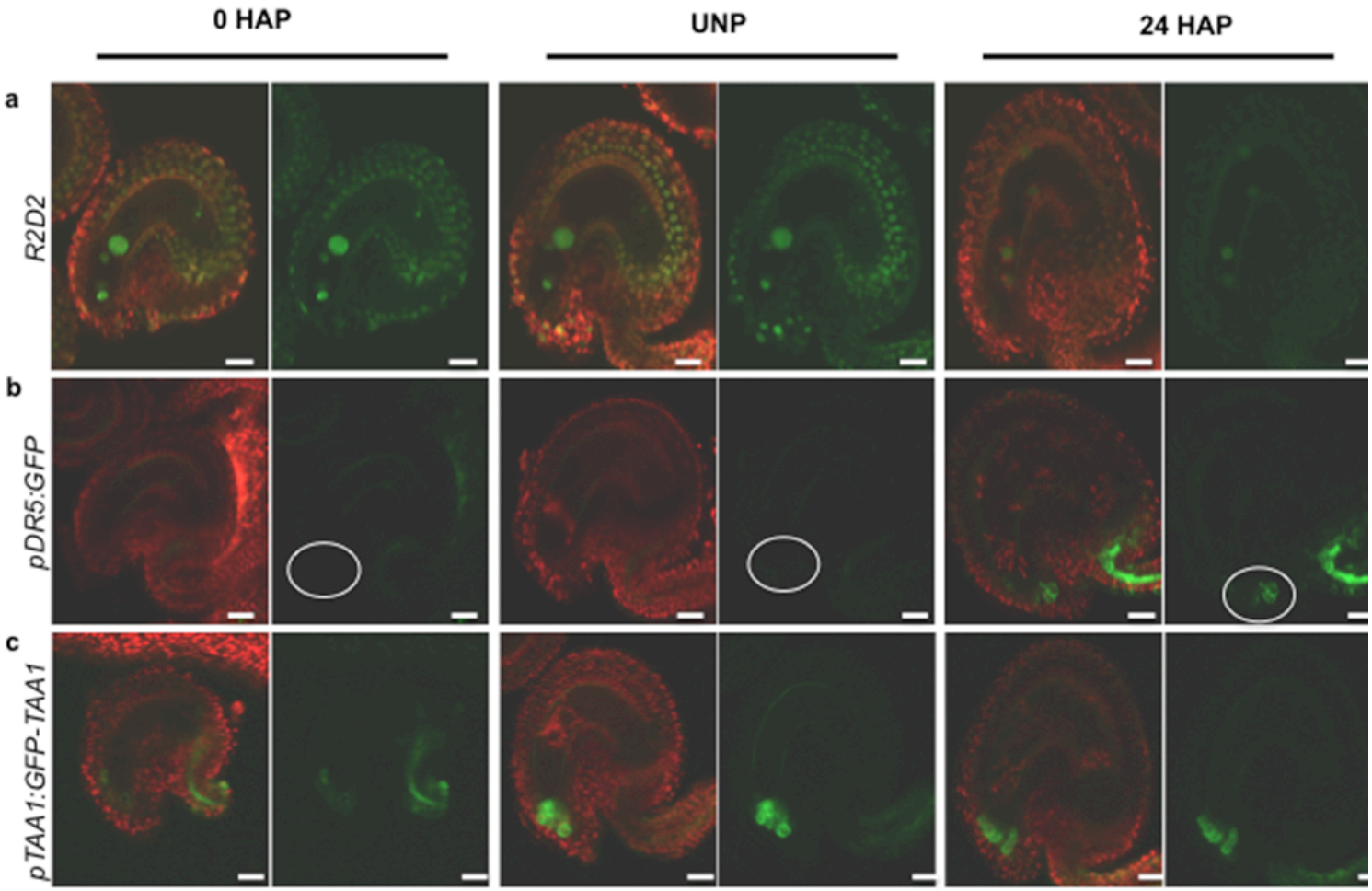
Figure 2 has one figure supplement (Supplementary Fig. 5. Phenotypes of the *wei8* mutant)

Figure 3 has one figure supplement (Supplementary Fig. 6. Localization of auxin transport proteins)



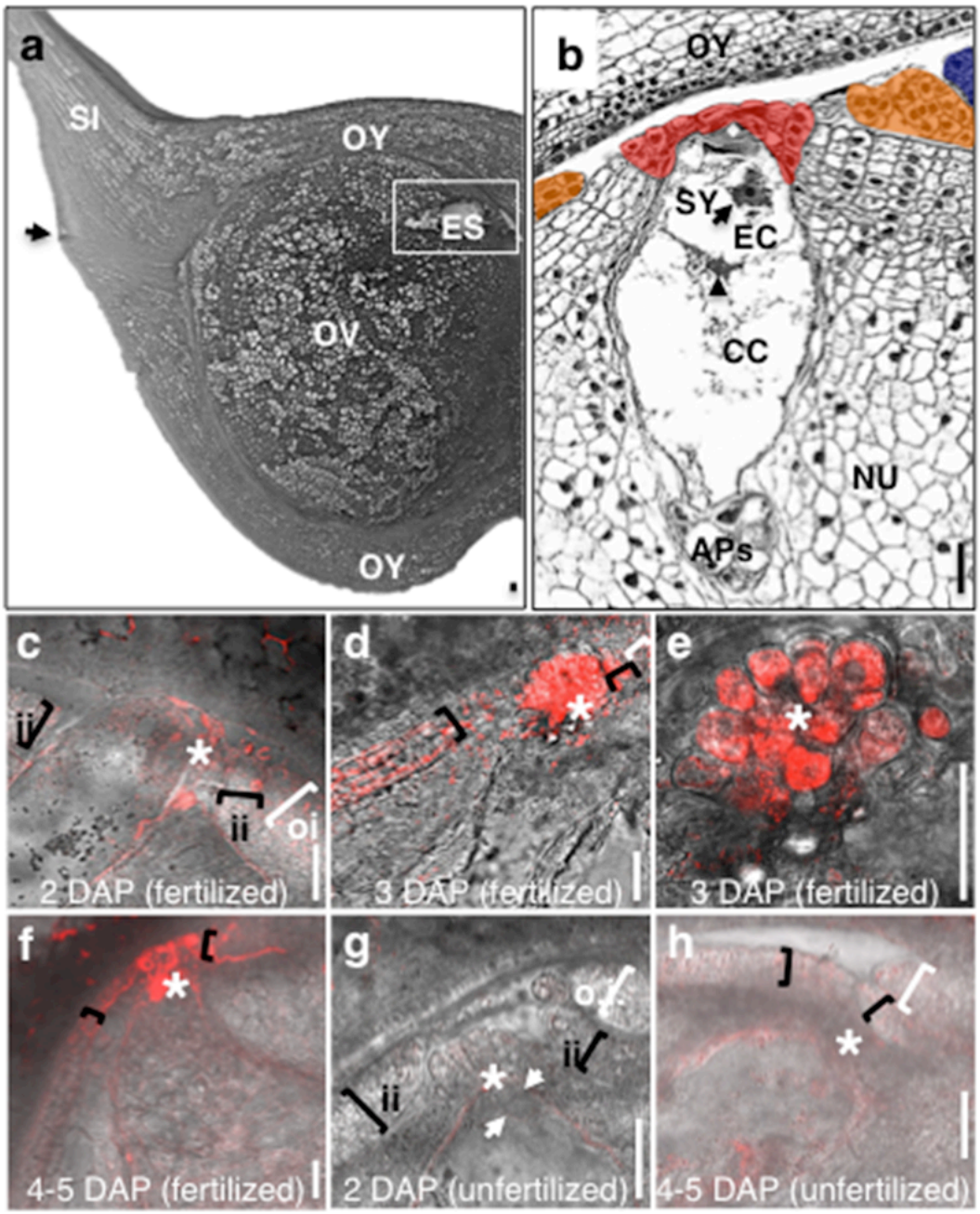
Supplementary Fig. 1: Expression controls for *p35S:DII-VENUS*.

Expression of *p35S:mDII-VENUS* (**a, b**) and *p35S: DII-VENUS* (**c, d**) (yellow signal) as indicated. Autofluorescence of the embryo sac appears as a red signal (**c**, **d**). Bright field images are merged (**c**, **d**). Scale bars represent 20 µm. These observations were repeated independently twice with similar results.



Supplementary Fig. 2: Representative pictures for fluorescent signal quantification in Figs 1e and 1f.

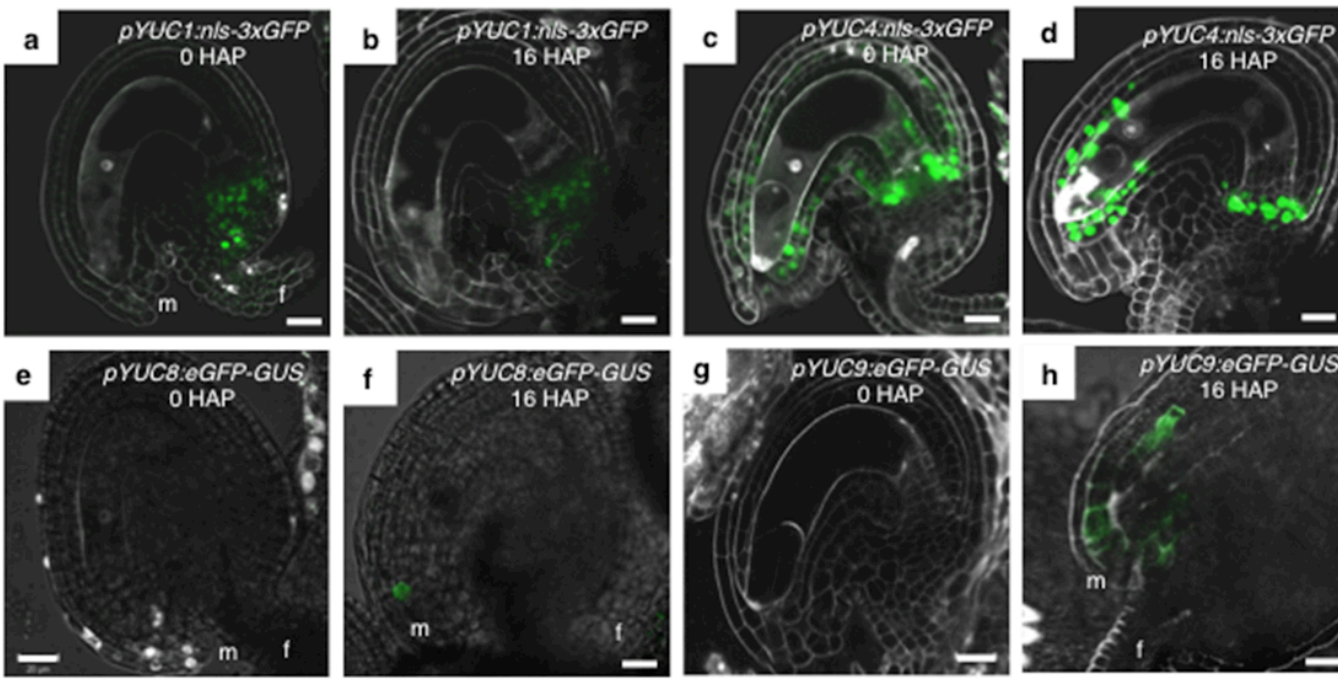
Expression pattern of *R2D2* (**a**), *pDR5:GFP* (**b**) and *pTAA1::GFP-TAA1* (**c**) in ovules at 0 HAP, UNP and 24 HAP as indicated. The region of embryo attachment is indicated by a white oval for illustration. Fluorescent signal (GFP, DII) is green; autofluorescence and mDII signal appears red; and merged DII/mDII signal is yellow. Merged fluorescence and autofluorescence images are shown in the left panels for each time point, and the separate fluorescence signal of the corresponding reporters in the right panels. HAP, hours after pollination; UNP, unpollinated ovules at same time corresponding to 24 HAP of pollinated ovules Scale bars represent 20 μm.



Supplementary Fig. 3: Auxin response in the tip region of the inner integuments and micropylar nucellus cells of maize.

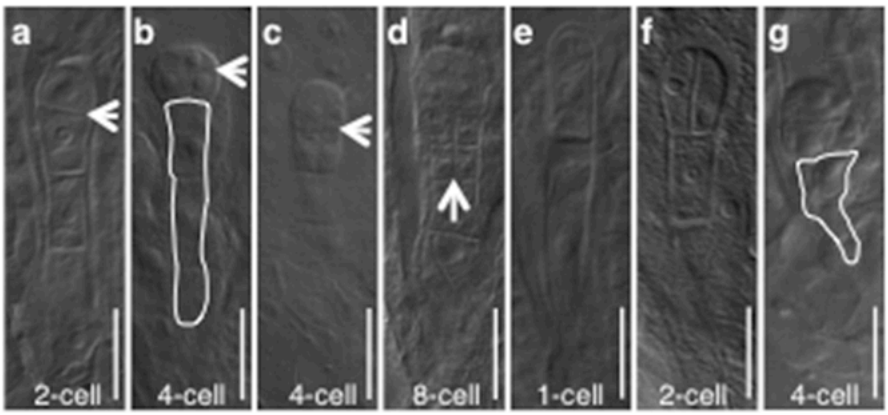
a, Longitudinal section through the ovary (OY) and embryo sac (ES) of maize. An ovary connected to the silk (SI) contains a single ovule (OV). The stylar channel is indicated by an arrow. **b**, Enlargement of the embryo sac region shown by a frame in (**a**). The image is turned 90º anti-clockwise. The arrow points towards the sectioned egg cell (EC) nucleus and the arrowhead towards the polar nuclei of the central cell (CC). Two synergids (SY) form the

filiform apparatus (diamond) and antipodal cells (APs) are located at the opposite pole. Inner integuments are indicated in orange, outer integument in blue and the region of embryo attachment consisting of micropylar nucellus (NU) cells in red. **c-h**, *pDR5:mRFP.er* ovaries were sectioned longitudinally along the embryo sac axis. Stages as indicated. Inner (ii) and outer integuments (oi) are indicated by black and white brackets, respectively. Maternal micropylar nucellus cells are located around the asterisks. The egg cell (in **g**) is located between arrows. Fertilized ovule 2 days after pollination (DAP), n = 8/8 biologically independent samples (**c**); Fertilized ovule 3 DAP (**d**, enlargement of micropylar nucellus cells in **e**), n = 7/9 biologically independent samples (**d, e**); Fertilized ovule 4-5 DAP, n = 7/8 biologically independent samples (**f**); Unfertilized ovule 2 DAP (the egg cell is located between arrows), n = 7/10 biologically independent samples (**g**); Unfertilized ovule 4-5 DAP, n = 6/10 biologically independent samples (**h**). Scale bars 50 μm. The similar results were independently observed on 10 independent biological samples.



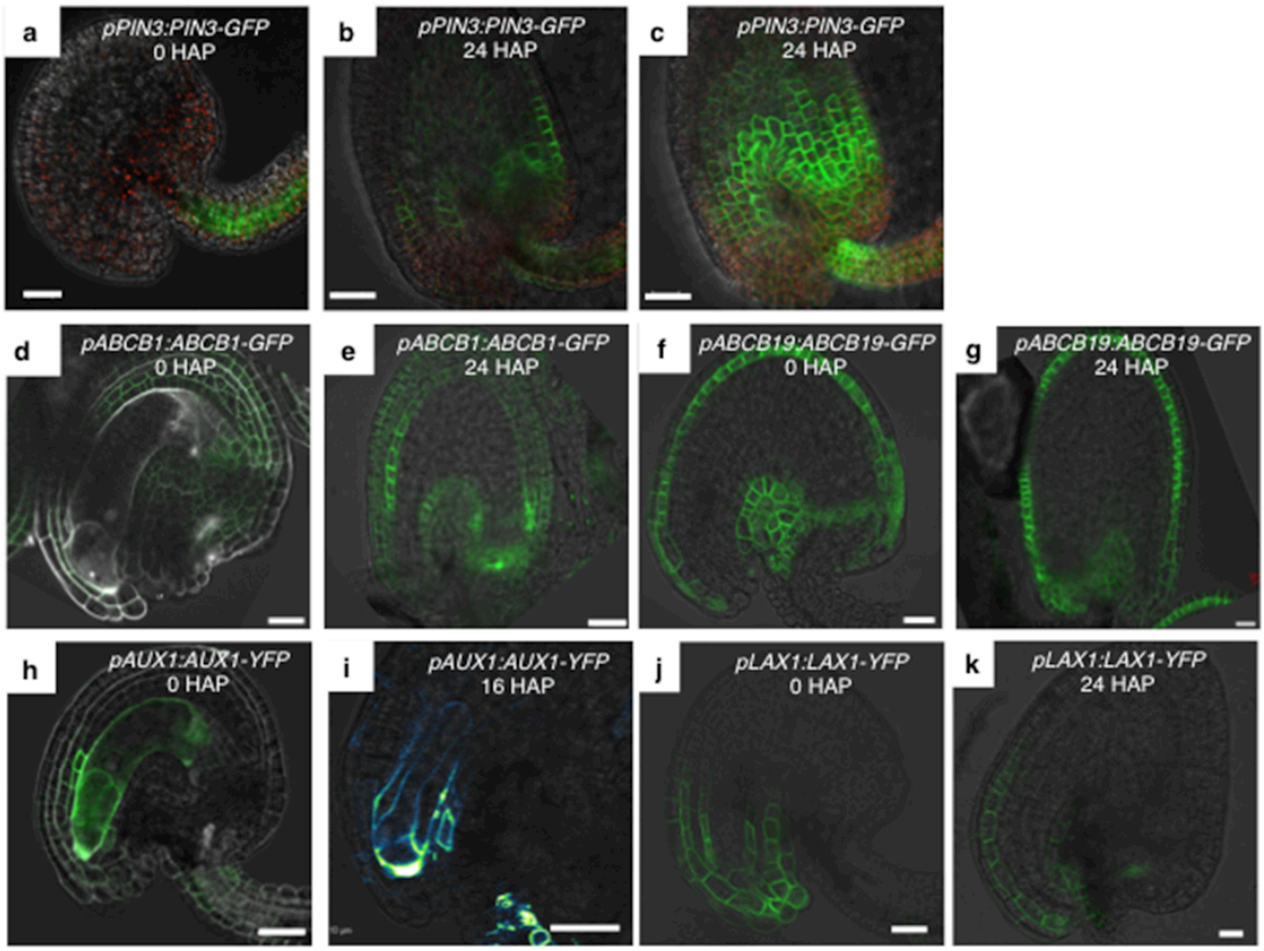
Supplementary Fig. 4: Expression pattern of *YUC* genes after pollination.

Expression patterns of *YUC* gene reporters (green signal) in ovules at 0 HAP and 16 HAP as indicated. Propidium iodide is used as counter-stain and gives a white signal. Merged fluorescent and bright field images are shown. Micropyle (m) and funiculus (f) are indicated in (**f**). HAP, hours after pollination. Scale bars represent 20 μm. These observations were repeated independently at least three times with similar results.



Supplementary Fig. 5: Representative pictures for the embryonic phenotypes of the *wei8* mutant.

a-d, Defects in cell division pattern both in the apical and basal cell lineage. **e**, Symmetric cell division of the zygote. **f**, Long apical cell. **g**, Short suspensor (compare to b). Arrows indicate abnormal cell divisions. The suspensors are outlined in (**b, g**). Scale bars represent 20μm. These observations were repeated independently at least three times with similar results.



Supplementary Fig. 6: Localization of auxin transport proteins.

Expression patterns of reporter genes for auxin transport proteins as indicated. Fluorescence signal is green (**a-h, j-k**), or color-coded blue-to-white to highlight graded signal (**i**). Autofluorescence is visible in red. Propidium iodide is used as counter stained in white (**d, h)**. HAP, hours after pollination. Scale bars represent 20µm. These observations were repeated independently three times with similar results.

Supplementary Table 1: Auxin and metabolites quantification in ovules, presented in Fig. 1b.

				Levels in pmol / 1000 ovules					
samples				IAA		oxIAA		IAAsp	
#	# sil.	# ov.	stage genot.	level	mean ± s.d.	level	mean ± s.d.	level	mean ± s.d.
1	19	766	0 HAP Col	0,127	0.109 ± 0.026	0,274	0.231 ± 0.153	0,021	0.011 ± 0.007
2	18	779		0,094		0,166		0,008	
3	16	784		0,082		0,113		0,003	
4	16	757		0,091		0,091		n.d.	
5	15	748		0,152		0,509		0,011	
6	13	754	24 HAP Col	1,040	0.852 ± 0.192	6,886	4.690 ± 2.109	0,160	0.119 ± 0.062
7	13	776		0,913		4,893		0,169	
8	14	747		0,924		5,732		0,132	
9	14	749		n.d.		n.d.		n.d.	
10	16	779		0,531		1,248		0,014	
11	17	771	0 HAP *wei8 tar1*	0,068	0.102 ± 0.022	0,097	0.130 ± 0.022	0,001	0.004 ± 0.003
12	16	749		0,086		0,135		0,004	
13	15	749		0,124		0,144		0,008	
14	17	786		0,123		0,113		0,004	
15	16	780		0,108		0,159		0,006	
16	15	769	24HAP *wei8 tar1*	0,166	0.155 ± 0.022	0,295	0.489 ± 0.216	0.011	0.009 ± 0.002
17	16	747		0,167		0,428		n.d.	
18	16	769		0,117		0,401		n.d.	
19	15	767		n.d.		0,411		n.d.	
20	16	793		0,171		0,912		0.007	

Significance of differences between fertilized Col ovules (24 HAP) and 0 HAP Col, 0 HAP *wei8 tar1*, 24 HAP *wei8 tar1* ovules is $p<0.0001$ for oxIAA measurements by Two-way ANOVA followed by Tukey's multiple comparison test. n.d.: not detected.

This experiment was repeated independently twice with similar results.

Supplementary Table 2: Fluorescent signal intensities in the embryo attachment region

Genotype	stage	n	Percentage of ovules with different signal strengths		
			Strong	Weak	Undetectable
p35S:DII-Venus	24 HAP	157	0.00	0.00	100.00
p35S:DII-Venus + MG132	24 HAP	76	30.26	69.74	0.00
p35S:mDII-Venus	0 HAP	78	100.00	0.00	0.00
p35S:mDII-Venus	24 HAP	83	100.00	0.00	0.00
pYUC1:nls-3xGFP	16 HAP	32	37.50	40.63	21.87
pYUC4:nls-3xGFP	16 HAP	51	80.39	13.73	5.88
pYUC8:eGFP-GUS	16 HAP	100	68.00	20.00	12.00
pYUC9:eGFP-GUS	16 HAP	38	0.00	73.68	26.32
pDR5:GFP	0 HAP	157	0.00	100.00	0.00
pDR5:GFP	24 HAP	199	100.00	0.00	0.00
pDR5:GFP wei8	24 HAP	219	0.00	14.61	85.39
pDR5:nls-3xGFP	24 HAP	149	100.00	0.00	0.00
pDR5:nls-3xGFP wei8 tar1	24 HAP	198	0.00	0.00	100.00
pDR5:GFP pBAN:iaaL #7	24 HAP	168	26.19	39.29	34.52
pDR5:GFP pBAN:bdl #2	24 HAP	156	19.87	28.21	51.92
pDR5:GFP pBAN:bdl #3	24 HAP	172	20.35	22.67	56.98

HAP, hours after pollination; UNP, unpollinated ovules at same time corresponding to 24 HAP of pollinated ovules. n, number of ovules analyzed.

Supplementary Table 3. Cloning primer sequences used in this study.

Primer	Sequence
iaaL F	ATGACTGCCTACGATATG
iaaL R	TCAGTTTCGGCGGTCGAT
iaaM F	ATGTCAGCTTCACCTCTCCTT
iaaM R	CTAATTTCTAGTGCGGTAGTT
bdl F	CCCGGGATGCGTGGTGTGTCAGAATTG
bdl R	CCCGGGCTAAACAGGGTTGTTTCTTTG

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