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Supplemental information

The *Polycomb* group protein MEDEA controls cell proliferation and embryonic patterning in *Arabidopsis*

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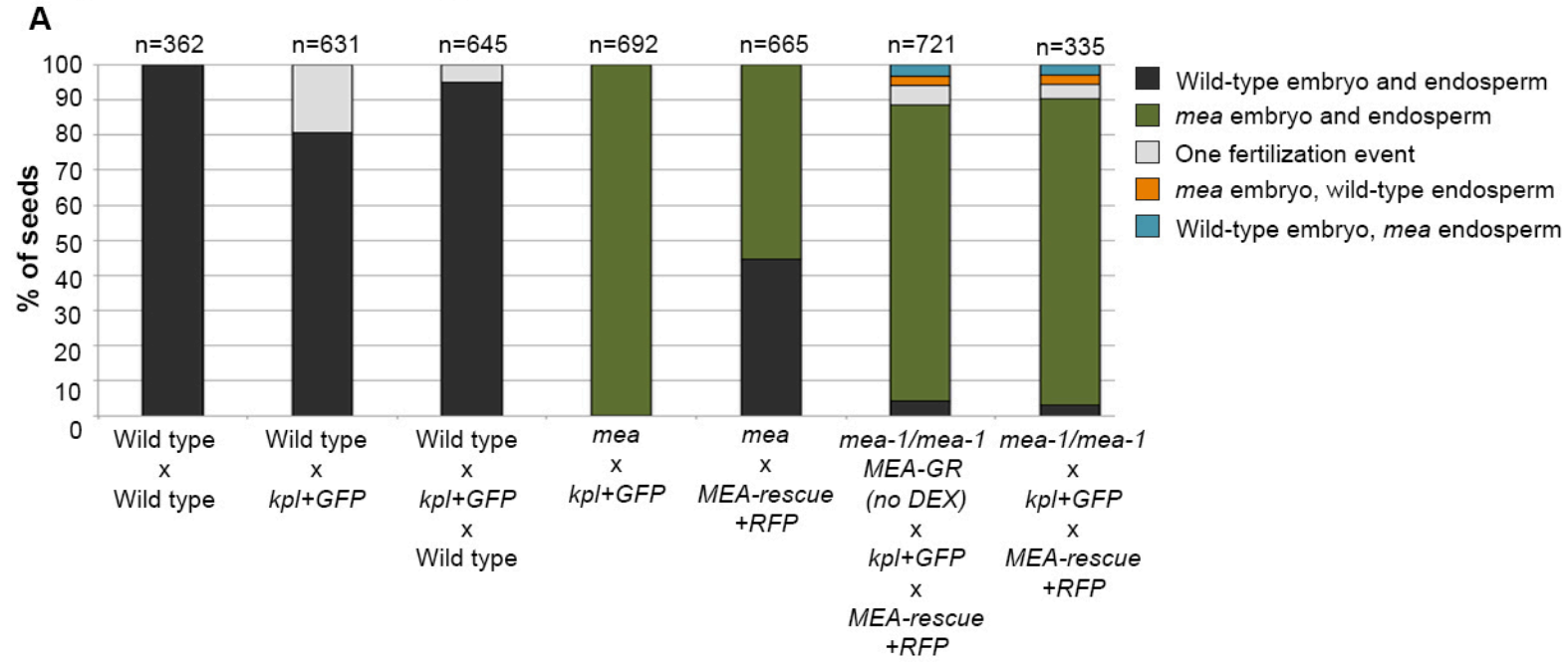
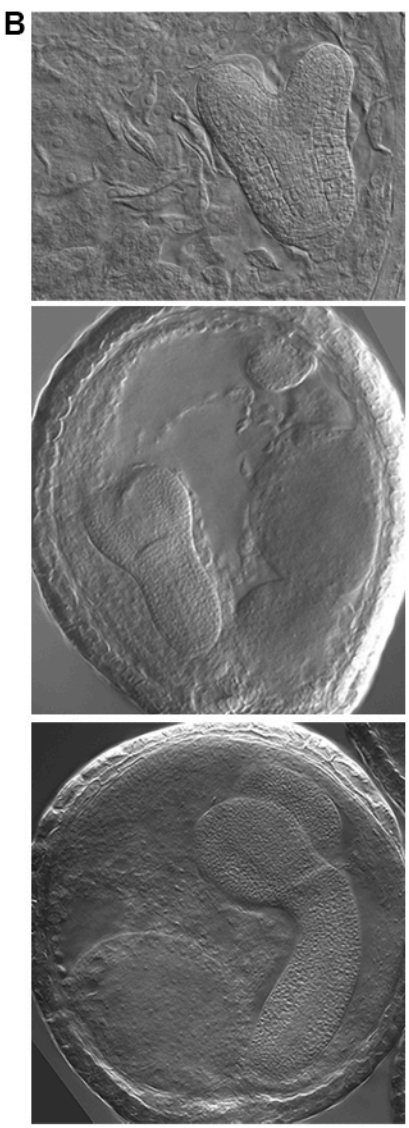
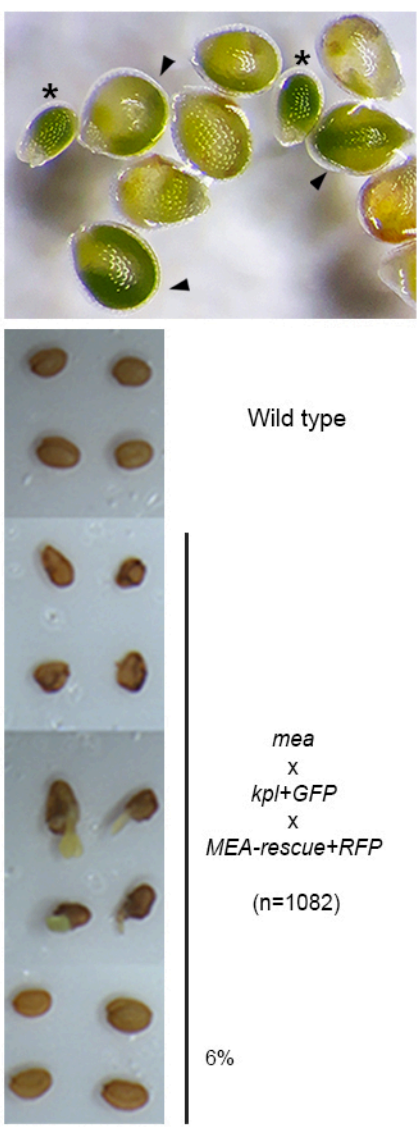
Figure S1. Related to Figure 1.







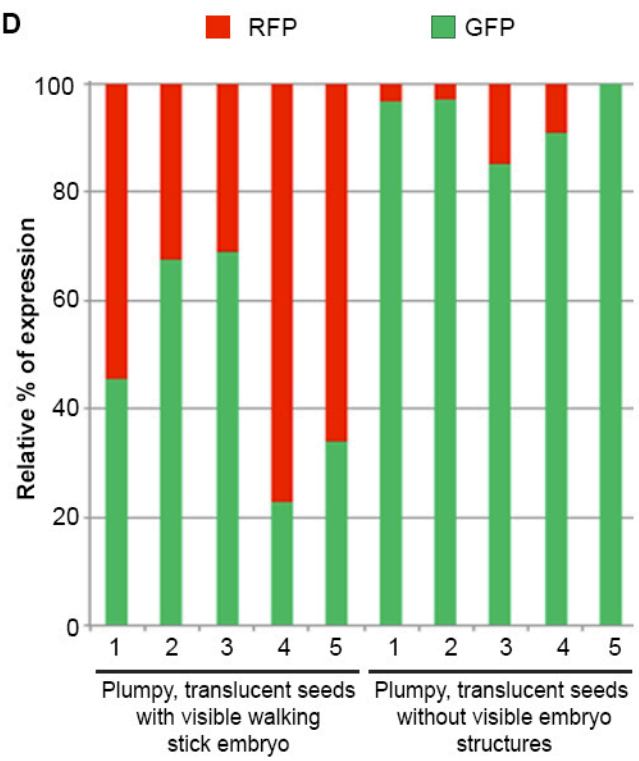


Figure S1: Rescue of mutant *mea* embryos and genotyping of rescued and non-rescued *mea* seeds, related to Figure 1.

A. Quantification of phenotypes observed in the single and double crosses.

B. Images of cleared seeds derived from the double pollination of *mea* pistils. Wild-type embryo is surrounded by uncellularized endosperm.

C. Appearance of developing seeds (top) and dried seeds (bottom) in double pollination of *mea* pistils. The developing embryo (arrow head) is visible in big and swollen seeds, a phenotype that is characteristic of *mea* seeds. Small seeds are the ones derived from full rescue by the *MEA-rescue+RFP* pollen (asterisk).

D. ddPCR analyses for presence of *GFP/RFP* transgenes in single seeds derived from the double pollination of *mea* pistils.

Figure S2. Related to Figure 2.

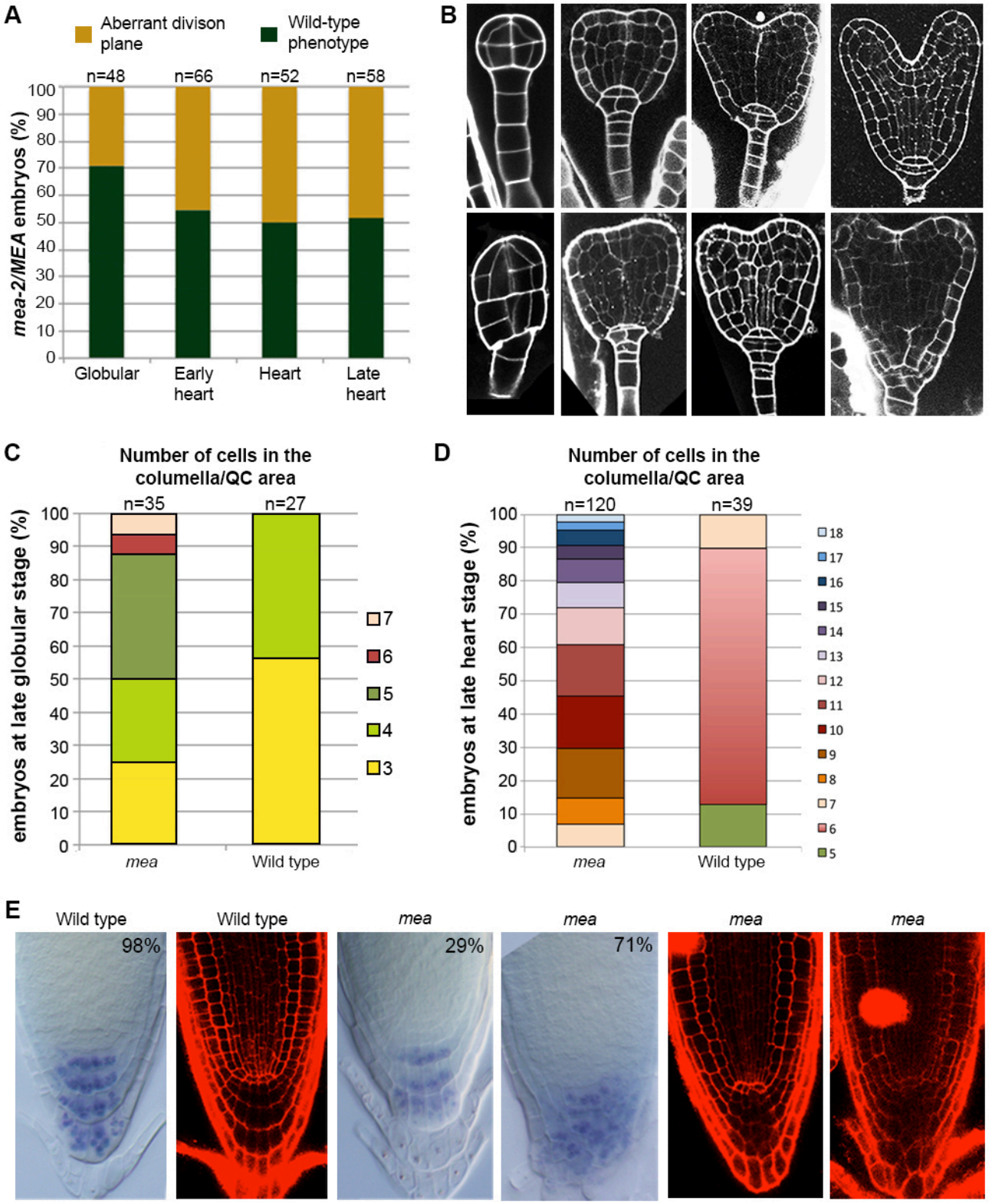


Figure S2: Morphological defects in *mea* embryos from early globular to late heart stages and in the QC-columella area of the primary root of *mea* seedlings, related to Figure 2.

A. Quantification of the number of embryos showing aberrant division planes in *mea/MEA* heterozygous offspring.
B. mPS-PI staining of *mea/MEA* seeds, showing examples picture of embryos from globular (left) to late heart (right) stages.
C. Quantification of the number of cells in the columella/QC area in embryos at late globular stage showing a wild-type phenotype or aberrant divisions.
D. Quantification of the number of cells in the columella/QC area in embryos at late heart stage showing a wild-type or a *mea* phenotype.
E. Lugol staining and Propidium Iodide staining of root tip of wild-type and *mea* homozygous seedlings. The percentage shows the frequency of the phenotype observed in the imaged seedlings (n=14).

Figure S3. Related to Figure 3

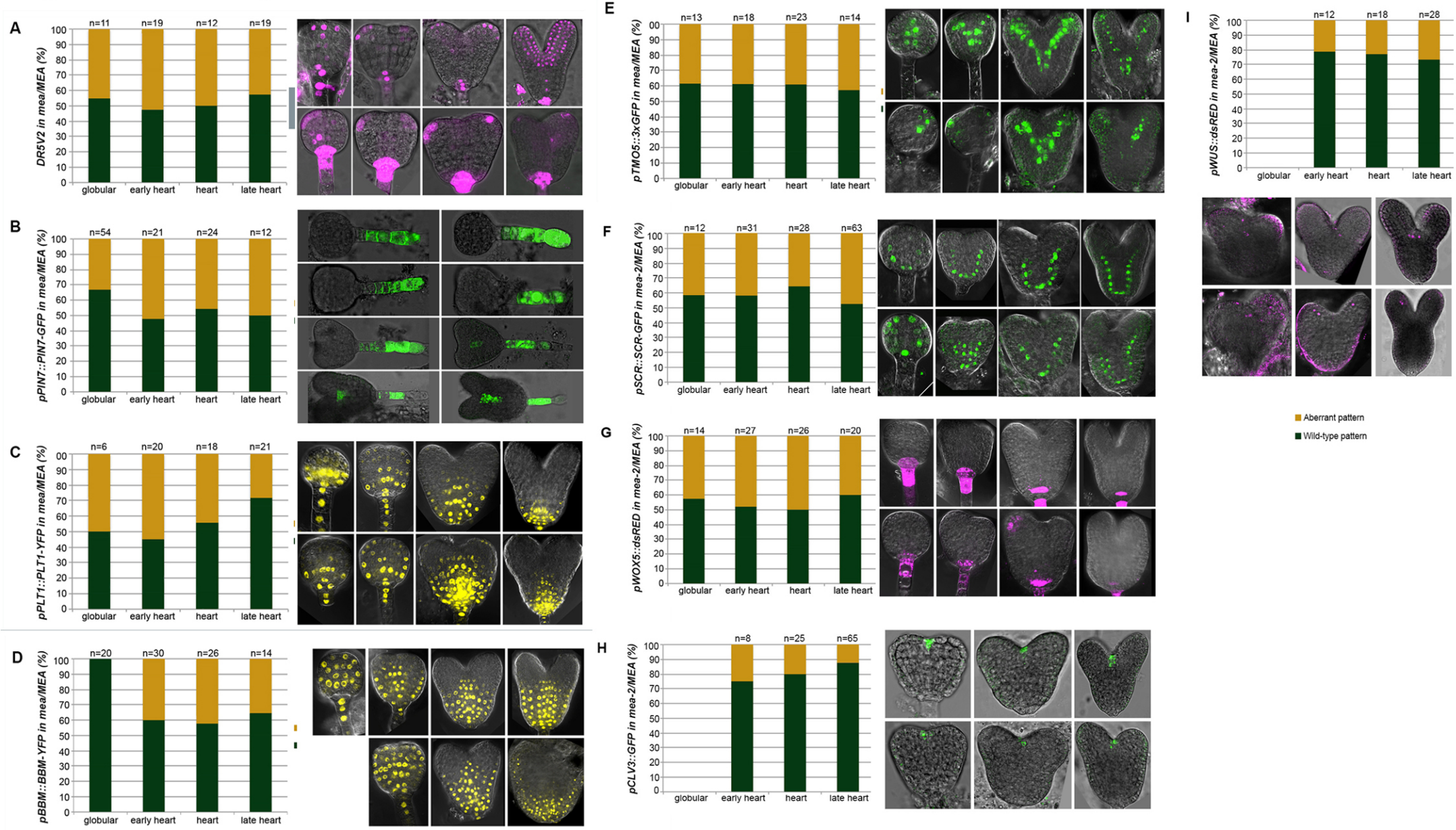


Figure S3. Expression patterns of cell fate markers and their quantification in seeds of *mea/MEA* plants, related to Figure 3.
For all markers, histograms of the quantification are shown on the left and pictures of representative embryos on the right; pictures show wild-type expression patterns in the top row and aberrant ones in the bottom row for all markers, except for (b) where wild-type and aberrant expression patters are shown in the left and right panels, respectively.

A. Quantification of aberrant versus wild-type expression patterns i*n mea/MEA DR5V2* seeds.
B. Quantification of aberrant versus wild-type expression patterns in *mea/MEA pPIN7::PIN7-GFP* seeds.
C. Quantification of aberrant versus wild-type expression patterns in *mea/MEA pPLT1::PLT1-YFP* seeds.
D. Quantification of aberrant versus wild-type expression patterns in *mea MEA pBBM::BBM-YFP* seeds.
E. Quantification of aberrant versus wild-type expression pattern in *mea/MEA pTMO5::3xGFP* seeds.
F. Quantification of aberrant versus wild-type expression patterns in *mea/MEA pSCR::SCR-GFP* seeds.
G. Quantification of aberrant versus wild-type expression patterns in *mea/MEA pWOX5::dsRED* seeds.
H. Quantification of aberrant versus wild-type expression patterns in *mea/MEA pCLV3::GFP* seeds.
I. Quantification of aberrant versus wild-type expression patterns in *mea/MEA pWUS::dsRED* seeds.

Figure S4. Related to Figure 5.

A

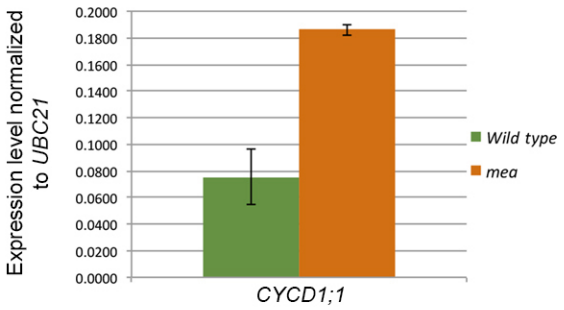


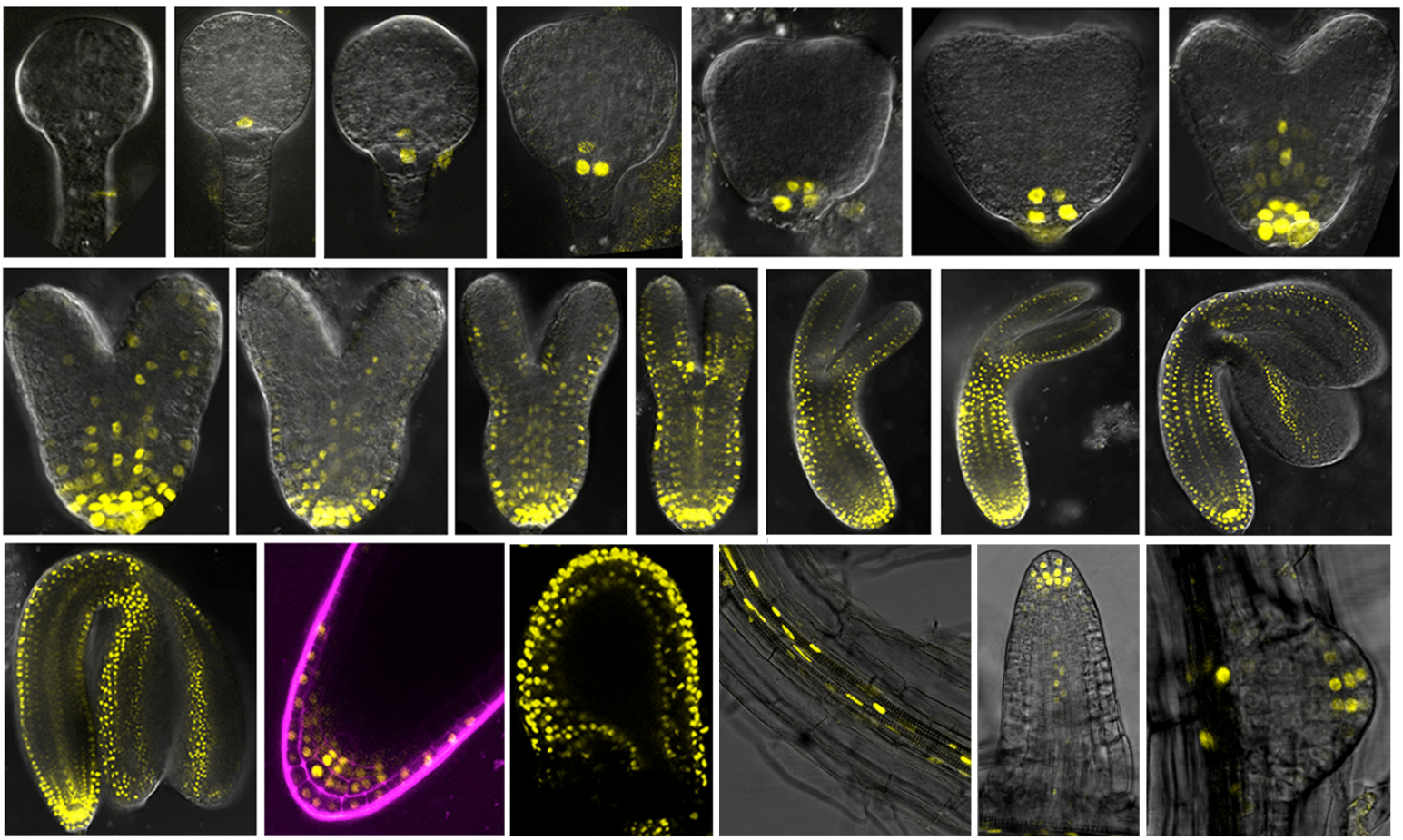
Figure S4: *CYCD1;1* expression in *mea* embryos and confocal analysis of *pCYCD1;1::NLS-3XVenus-3'UTR* in and *pCYCD1;1::CYCD1;1-GFP-3'UTR* marker lines in wild-type plants, related to Figure 5.

A. ddPCR analysis of *CYCD1;1* transcript level in wild-type versus *mea* embryos around early globular stage.

B. Confocal images of *pCYCD1;1::NLS-3xVenus-3'UTR* marker line showing its wild-type expression pattern in embryos from the globular to mature stages, a magnification of the root tip showing no expression in the QC, a seed showing expression only in the sporophytic tissues, the vasculature of the primary root, and two emerging lateral roots.

C. Confocal images of the *pCYCD1;1::CYCD1;1-GFP-3'UTR* reporter gene showing its wild-type expression pattern in embryos from the globular to heart stages, seed coat, vasculature of the primary root, and two emerging lateral roots.

B *pCYCD1;1::NLS-3xVenus-3'UTR (wild-type expression pattern)*



C *pCYCD1;1::CYCD1;1-GFP-3'UTR (wild-type expression pattern)*

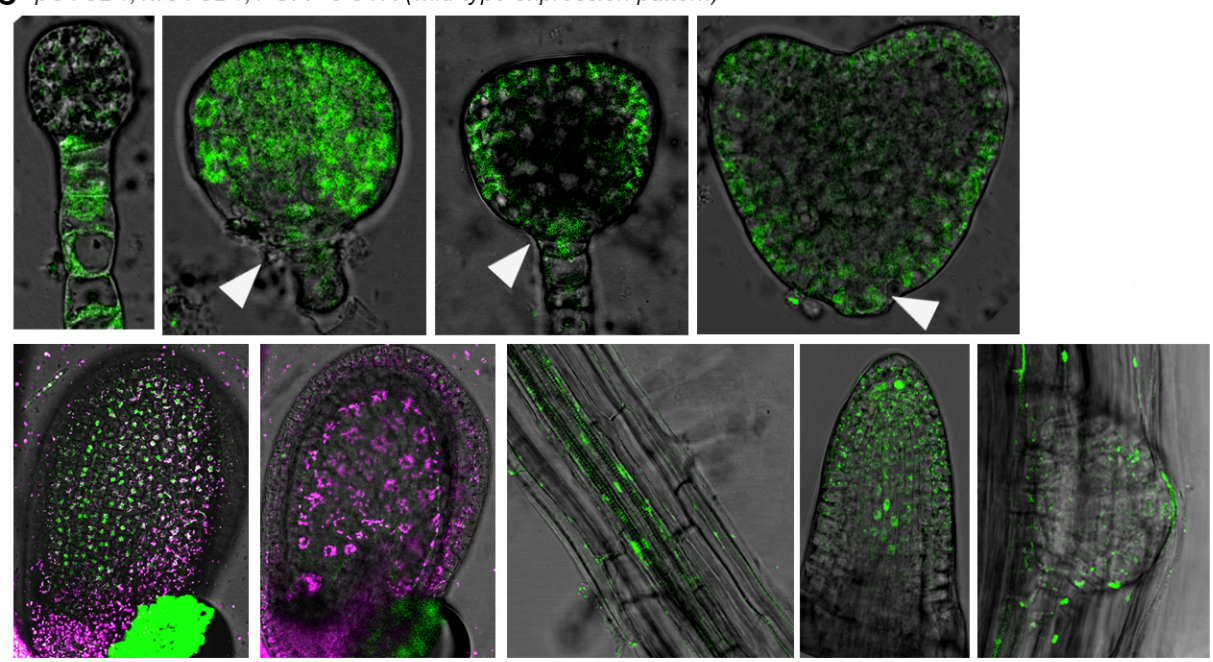
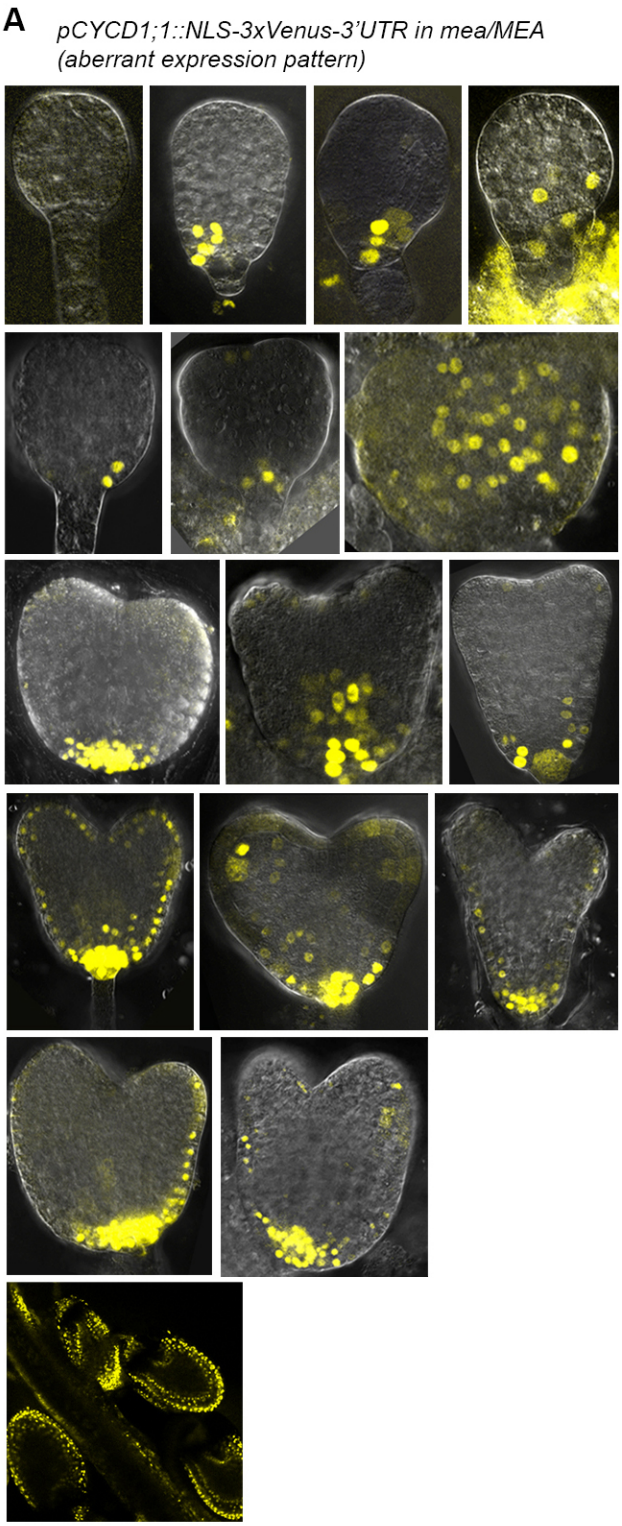
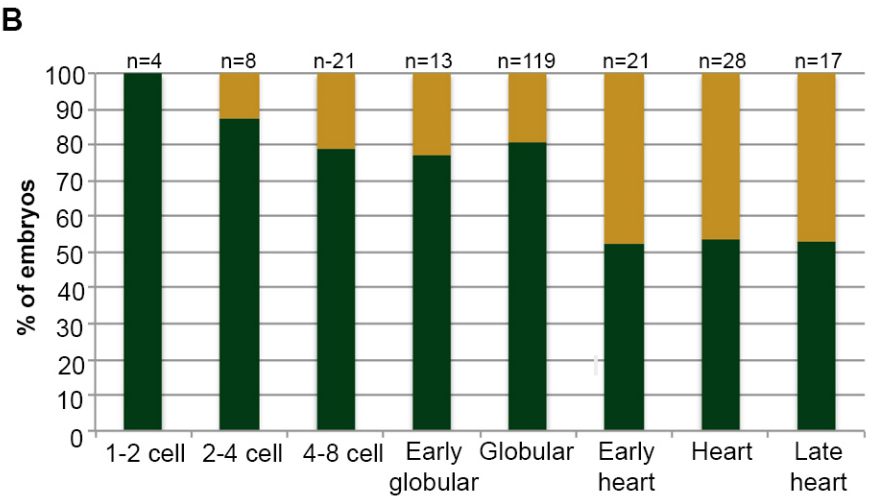


Figure S5. Related to Figure 5.





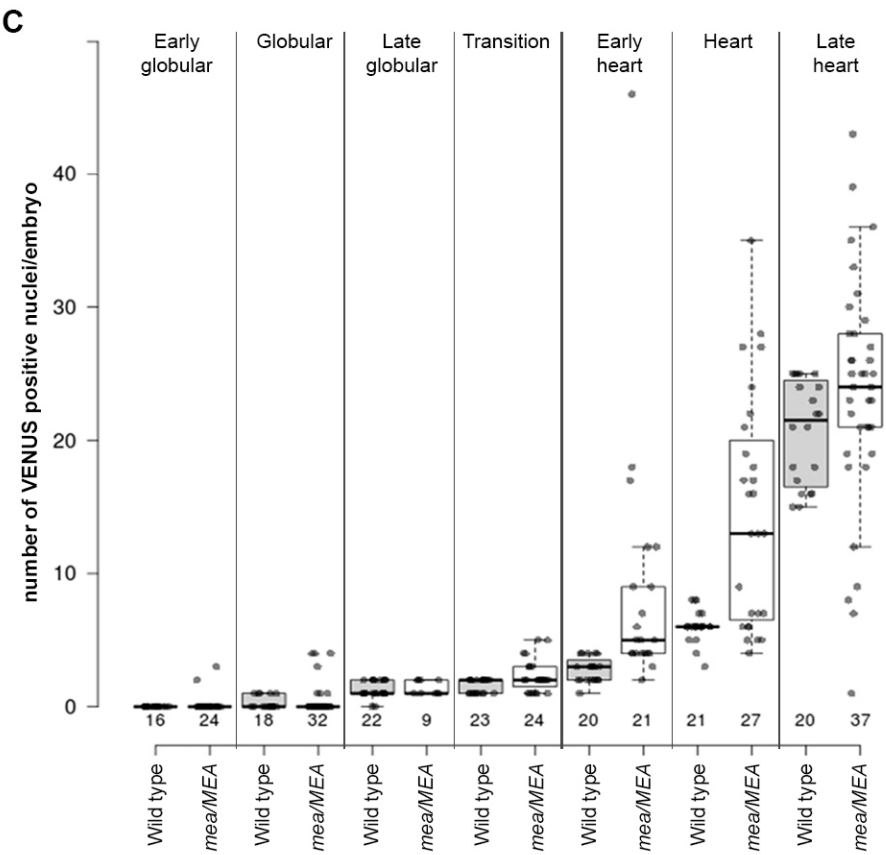


Figure S5: Expression pattern of *pCYCD1;1::NLS-3XVenus-3'UTR* and its quantification in embryos of *mea/MEA* plants at different developmental stages, related to Figure 5.

A. Confocal images of *pCYCD1;1::NLS-3xVenus-3'UTR* marker line showing aberrant expression in *mea/MEA* seeds, in embryos from the globular to late heart stages. In *mea/MEA* seeds, no expression of the marker is detected in the endosperm.

B. Quantification of the percentage of embryos showing an aberrant expression pattern of the *pCYCD1;1::NLS-3xVenus-3'UTR* reporter gene in the *mea/MEA* background.

C. Quantification of the number of nuclei expressing the *pCYCD1;1::NLS-3xVenus-3'UTR* reporter gene in embryos developing from wild-type and *mea/MEA* plants. Embryonic stages from the early globular to the late heart stage are shown.

Figure S6. Related to Figure 5.

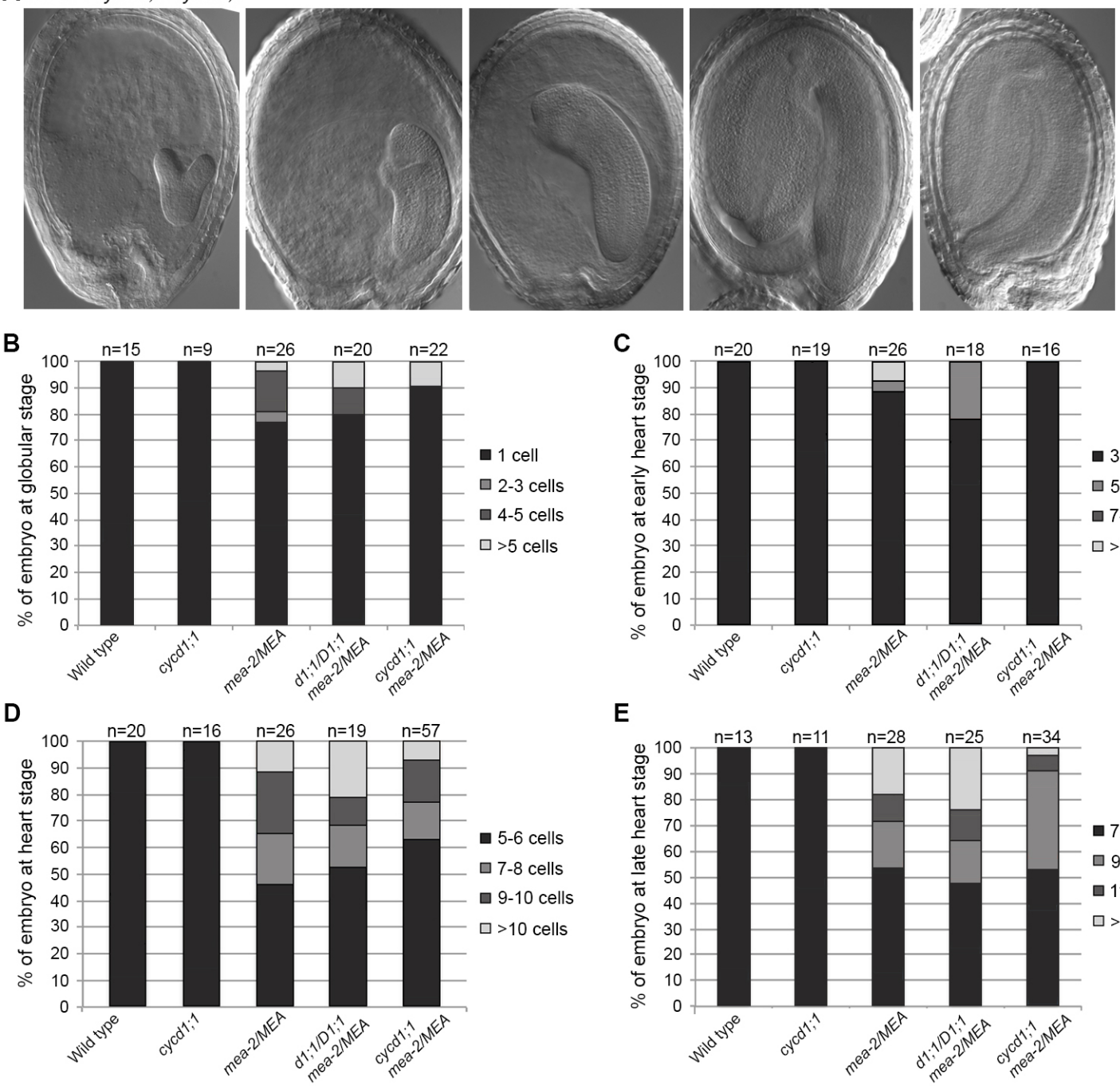
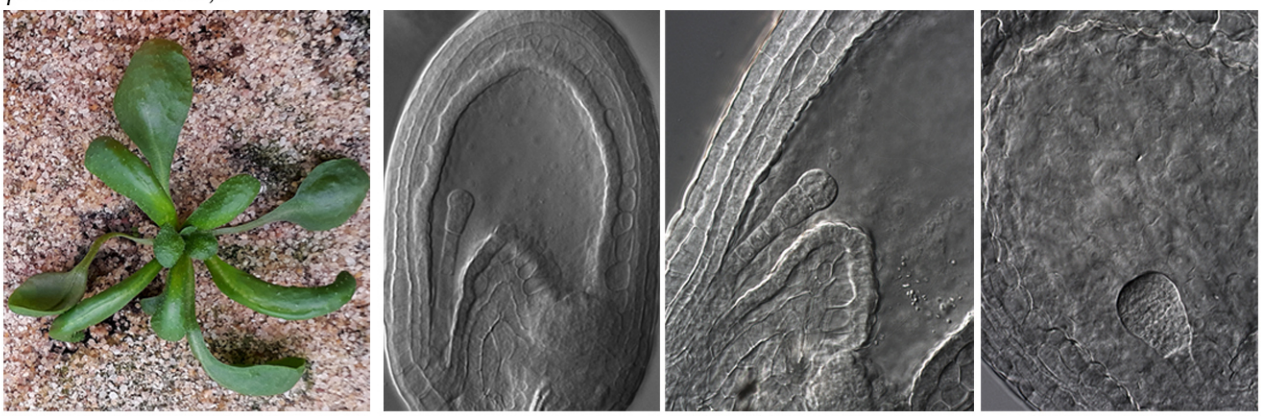


Figure S6. Characterization of *mea cycd1;1* seeds and embryos from globular to late heart stages, related to Figure 5.
A. DIC microscopy analysis of *mea cycd1;1* seeds, showing enlarged embryos surrounded by defective endosperm.
B-E Quantification of the number of cells in the columella/QC area in embryos at globular stage (b), early heart stage (c), heart stage (d), and late heart stage (e), of wild-type,*cycd1;1* mutant, *mea/MEA* mutant, *cycd1;1/CYCD1;1 mea/MEA* double heterozygous mutant, and *cycd1;1 mea/MEA* mutant.

Figure S7. Related to Figure 6.

A *pRPL18::NLS-3xVenus*

B *pRPL18::CYCD1;1*



C *pRPL18::NLS-3xVenus*

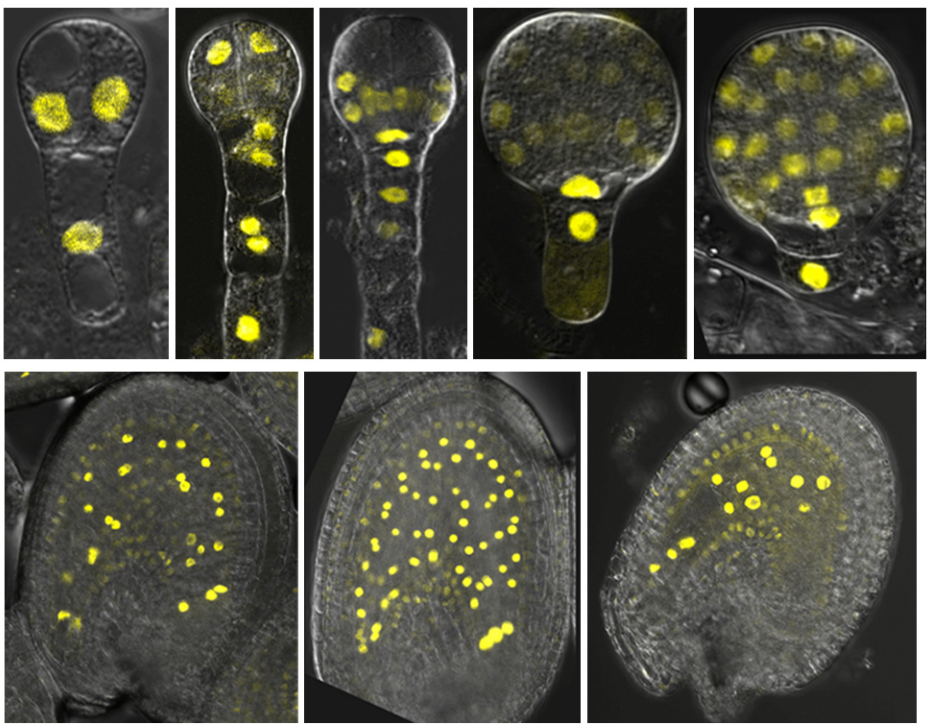


Figure S7. Phenotypes resulting from ectopic expression of *CYCD1;1* under control of the *pRPL18* promoter and reporter gene expression controlled by the latter in embryos and seeds, related to Figure 6.

A. Images of rosettes of *pRPL::NLS-3xVenus* (left) and embryo at globular stage by DIC analysis (right).

B. Images of rosettes of *pRPL18::CYCD1;1* (left), and by DIC microscopic analysis of seeds (right), showing embryos with abnormal shape surrounded by normal endosperm.

C. Confocal imaging of the *pRPL18::3xNLS-Venus* line showing the expression pattern conferred by the *pRPL18* promoter in the developing embryo and seed.

Supplementary Table S2. Primers used in this study related to STAR Methods

pCYCD1;1::3xNLS-Venus-3'UTR	
CYCD1;1 promoter	Fw gtggtctcaGGAGtagtcgcaacttgcaaac rev gtggtctcaCATttctctccccataaccggagatg
CYCD1;1 3'UTR	Fw gtggtctcaGCTTaatttggggagtgaaagtagag Rev gtggtctcaAGCGgatattgaaggagttgttcaatg
pCYCD1;1::CYCD1;1-GFP-3'UTR	
CYCD1;1 promoter	Fw gtggtctcaGGAGtagtcgcaacttgcaaac
	Rev gtggtctcaCATttctctccccataaccggagatg
CYCD1;1 gene	Fw gtggtctctaATGAGGAGTTACCGTTTTAGTG Rev gtggtctcTcaccATTAGAGGTAGATGTTTCATC
CYCD1;1 3'UTR	Fw gtggtctcaGCTTaatttggggagtgaaagtagag Rev gtggtctcaAGCGgatattgaaggagttgttcaatg
pMEA::MEA-GFP-3'UTR	
MEA fragment 1	Fw ggtctcaGGAGtcaggatatgtttaatataacc Rev ggtctcTCACAAAATCcAGACCCTCCA
MEA fragment 2	Fw ggtctcTTGTGAACTGGTGGATGTATTAC Rev ggtctcCAAACGAATGGGGAGGTTAAG
MEA fragment 3	Fw ggtctcCGTTTGGAGGGTCTgAATTTTG
	Rev ggtctcCCATtaaccactcgcctcttc
MEA fragment 4	Fw gtggtctctaATGGAGAAGgttagtttc Rev gtggtctcaaaccGGGCTCATACTTCTCATGCA
GFP fusion with MEA	Fw ggtctcaggttctatggtgagcaagggcgagga Rev ggtctcaggaactcttgtacagctcgtccatgc
MEA fragment 5	Fw gtggtctcattccGAGTCTAGATCCgtaagcatt Rev gtggtctcATGCTCACTGCATGGTTGTC
MEA fragment 6	Fw gtggtctcGAGCATTGTTACCTCAAAgtc Rev GTTTGGATCTGACACCACAA
MEA fragment 7	Fw TATCAAACAAGATTGTGGTGTC Rev gtggtctcTaagCTAACGAGCTGGACGGGCTTC
MEA fragment 8	Fw gtggtctcaGCTTtttgatctgaggagaagcagc Rev gtggtctctAGCGattccacaaatccaac
pRPL18::CYCD1;1	
RPL18 promoter	Fw ggtctcaGGAGattggtgatcaaagtcaatactc Rev ggtctcCCATttttcgtctggagagagac
CYCD1;1 CDS	Fw ggtctctaATGAGGAGTTACCGTTTTAGTG Rev g ggtctcTaagcTTAATTAGAGGTAGATGTTTC
CYCD1;1 GABI_214D10 genotyping	
Wild-type band	Fw ATTCATGGCCTGGTGATTCTATC Rev ttacaaagttttcaataaagccga
ddPCR	
GFP	Fw GCGGCACGACTTCTTCAAGAG Rev TCGATCCTGTTGACGAGGGT 5' FAM- ACGGGAACTACAAGACA –MGB 3' probe
RFP	Fw GACGGCTGCCTCATCTACAAC Rev CCCAGCCGAGTGTTTTCTTC FAM-TGAACTTCCCATCCAAC MGB 3' probe
Internal control (*At5g62150*)	Fw CAACGCTTCTAATTCGATTAGAGGT Rev GGATATGCGGGTTTCGCTC probe VIC ACCATCGGCGATAAA-MGB
CYCD1;1	Fw CGGGTACCTTTCTCGGGTTC Rev TCGCTACACAGAGAATCGCG Rev exonic GAAAGCTCGCTTCTTTTATGTTGG
UBC21	Fw ATGCTTGGAGTCCTGCTTGG Rev TGCCATTGAATTGAACCCTCTC

CUT&RUN and ChIP	
Region -2693	Fw TCAACCAAGACCAAAGTGGA Rev CAAGCACTTGCATGATGAGT
Region -2288	Fw AATGACTCGACCCACTTGTT Rev TAGCATAGTCAACTCGGACC
Region -2149	Fw TCAATAGGGTCCGAGTTGAC Rev TTCATGGGAGCTATTGCCTT
Region -1870 / Region A	Fw AGGTTGGGAGGTGCATATTT Rev TTATGGCTAGGTTGGGCTAC
Region -1226	Fw AAGGCACACGTGAAAGAAAG Rev CCTTTTCGTAGAACCGGAGA
Region -546	Fw CGACCAACAAAATGGTCCAA Rev ACGTTGCATGAGTGAGTACA
Region -336	Fw TCCTTGTTCCTAACCTACGC Rev CGGTTACCGTTTCCGTTATG
Region -75 / Region B	Fw CCTTCACTCTTCTCTCCACA Rev AACCGGAGCTAGATAAACCG
Region 197 / Region C	Fw TGATTTCTCGTCTTCCGAGG Rev ATAGGCTCGCCTTGAGAATC
Region 362	Fw ATTCTCAAGGCGAGCCTATT Rev GCCGTTAAAGGCTGAAAGTT
Region 1570	Fw AGAAGCGAGCTTTCTTGAGT Rev GGGGATTGACAACAGAGGAT
Region 1884	Fw AGCTTCGAGTGAGTGTAAGG Rev CACCTACCCATGAATAGCCA
Region 2057	Fw TGGGGAGTGAAAGTAGAGGA Rev TTCCCCTTGGTTGGAAAACT
Region 2521	Fw TAATGGATCGCAGCAGTTCA Rev GTCACGTGAAGTCACACACA
Region 2925	Fw TTTGATTTTGGTGTGGCCTG Rev TCCACAACACACATGTTCAC
Region 3345 / Region D	Fw CCACGAAACCATAACGTAGC Rev TGGCTAATGTTTGAGGGGTT
Region 3586	Fw CGACCAACAAAATGGTCCAA Rev ACGTTGCATGAGTGAGTACA
Region 4015	Fw ACAAAAGGTTGGCATGGTTT Rev AACTCCATCTGGTTCACGTT
Region 4309	Fw AATGACCAGCCCACACATTT Rev GCTTAGGTCCCTTTGTGTCC
Region 4514	Fw TACACGCGTTTGCCATTAGT Rev TTTGTCTCTACCCCATTCCCA
Mlu-like	Fw GATTTACAAGGAATCTGTTGGTGGT Rev CATAACATAGGTTTAGAGCATCTGC
MEA promoter	Fw ATCGCCCAAGCTTGTGCC Rev GGAGGTTAAGTCTATCCGCCGTAA
PHE1 promoter	Fw TTGTGGTCTTTAGAATCTGATGTTTAG Rev CAGTTGGATAGAAAGTAATGTTATGGC