**Magyar et al. *Arabidopsis* E2FA stimulates proliferation and endocycle separately through RBR-bound and RBR-free complexes**

**Supplementary information**

**Supplementary materials and methods**

*Construction of the 35S::E2FA$^{\Delta RB}$/DPA and the 35S::E2FA/DPA$^{\Delta DB}$ lines*

The HA- tagged E2FA$^{\Delta RB}$ mutant (Magyar et al. 2000) was PCR amplified by using 5' and 3' end primers containing SalI or BamHI extension, respectively. The PCR product was digested by SalI and BamHI and cloned into the SalI and BamHI sites of pLBR19 plasmid, which contains the cauliflower mosaic virus (CaMV) 35S promoter with a double enhancer sequence (p70). The p70/HA-E2FA$^{\Delta RB}$/NOS terminator cassette was released by SacI and XbaI and cloned into the SacI and XbaI sites of pBINPLUS binary vector (van Engelen et al. 1995). To generate the mutant DPA (DPA$^{\Delta DB}$) 108 nucleotides was deleted (representing a 36 amino acid long region comprising the DNA binding domain from 100 to 135 amino acid position in the DPA protein) by using a chimeric primer (5'-ATCCTTTTGCAGGTAATCTCATTTTCATTCAAAGG-3') in a one tube PCR reaction according to the method as described (Pont-Kingdon 2003). The PCR product was cloned into the 2xMyc-epitope tagged pBluescript SK plasmid (Magyar et al. 2000) and the mutation was verified by sequencing of the cloned product. The Myc-tagged DPA$^{\Delta DB}$ was than released by HindIII and PstI and cloned into the HindIII and PstI sites of pLBR19 plasmid. Next the p70/Myc-DPA$^{\Delta DB}$ /NOS terminator was cloned into the SacI and XbaI sites of pBINPLUS binary vector.

*In vitro transcription-translation system, Electrophoretic Mobility Shift Assays (EMSA)*

The HA-E2FA$^{\Delta RB}$, Myc-DPA or Myc-DPA$^{\Delta DB}$ proteins were synthesized by using the TNT T7-coupled wheat germ extract kit (Promega) primed with appropriate plasmids. DNA probes were generated by annealing oligonucleotides spanning the regions of interest and by filling in the single-strand overhangs with α-$^{32}$P-dCTP using the Klenow fragment of DNA polymerase I. Complementary oligonucleotides were synthesized to generate a double-stranded DNA fragment containing the consensus E2F-binding site or its mutant version in 6 times repetition (wild type E2F binding site: top 5'-gTTTCCCGCCAAtg-3'; and bottom 5'-tcgaTTGGCGGGAAAtgca-3' or the mutated E2F-binding sequence 5'-gTTTCC*aa*CCAAtg-3'; bottom 5'-tcgaTTGG*tt*GGAAAtgca-3') where lower case letters represent overhangs or the mutated sites. The conditions for the DNA binding reaction and electrophoresis were as described (Kosugi and Ohashi 1997), except that E2F-probe was incubated with 1 µl of *in vitro* translation or co-translation products. The antibody supershift assay was carried out by using non-specific IgG or specific anti-HA or anti-Myc monoclonal antibodies as described before (Kosugi and Ohashi 1997).

**Supplementary References**


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# Magyar_Supplementary Table I.

| | | E2FA | | | RBR1 | |
|---|---|---|---|---|---|---|
| Gene ID | Correlation | Gene description | | Gene ID | Correlation | Gene Description |
| At2g36010 | 1.0000 | E2F transcription factor-A (E2FA) | 1 | At3g12280 | 1.0000 | retinoblastoma-related protein (RBR1) |
| At2g31270 | 0.8431 | CDT1a-cyclin-dependent protein kinase | 4 | At2g31270 | 0.7812 | CDT1a-cyclin-dependent protein kinase |
| At1g04730 | 0.8345 | AAA-type ATPase family protein-CTF18 | 12 | At1g04730 | 0.7502 | AAA-type ATPase family protein-CTF18 |
| At4g02060 | 0.8082 | prolifera protein (PRL) | 39 | At4g02060 | 0.7094 | prolifera protein (PRL) |
| At5g08020 | 0.8041 | replication protein, putative | 24 | At5g08020 | 0.7283 | replication protein, putative |
| At3g24495 | 0.7978 | DNA mismatch repair protein MSH6-2 (MSH7) | 22 | At3g24495 | 0.7291 | DNA mismatch repair protein MSH6-2 (MSH7) |
| At1g67630 | 0.7903 | DNA polymerase alpha subunit B family | 8 | At1g67630 | 0.7675 | DNA polymerase alpha subunit B family |
| At2g24490 | 0.7901 | replication protein, putative | 10 | At2g24490 | 0.7548 | replication protein, putative |
| At5g61000 | 0.7863 | replication protein, putative | | | | |
| At2g40550 | 0.7835 | ETG1; E2F target gene 1 | 13 | At2g40550 | 0.7495 | ETG1; E2F target gene 1 |
| At2g37570 | 0.7827 | SLT1; Sodium-lithium tolerant 1 | 86 | At2g37570 | 0.6824 | SLT1; sodium-lithium tolerant 1 |
| At4g28310 | 0.7747 | expressed protein | 23 | At4g28310 | 0.7290 | expressed protein |
| At5g63920 | 0.7699 | DNA topoisomerase III alpha, putative | 110 | At5g63920 | 0.6735 | DNA topoisomerase III alpha, putative |
| At5g41880 | 0.7695 | DNA primase small subunit family | 9 | At5g41880 | 0.7555 | DNA primase small subunit family |
| At3g18524 | 0.7694 | DNA mismatch repair protein MSH2 (MSH2) | 6 | At3g18524 | 0.7686 | DNA mismatch repair protein MSH2 (MSH2) |
| At5g10390 | 0.7647 | histone H3 | 128 | At5g10390 | 0.6682 | histone H3 |
| At3g27640 | 0.7590 | transducin family protein | | | | |
| At3g25100 | 0.7579 | cell division control protein-related | | | | |
| At3g59550 | 0.7576 | cohesion family protein SYN3 (SYN3) | 155 | At3g59550 | 0.6598 | cohesion family protein SYN3 (SYN3) |
| At5g43080 | 0.7569 | cyclin, putative | 64 | At5g43080 | 0.6909 | cyclin, putative |
| At1g78650 | 0.7564 | POLD; DNA directed DNA polymerase | 3 | At1g78650 | 0.7847 | POLD; DNA directed DNA polymerase |
| At1g02970 | 0.7561 | protein kinase, putative | | | | |
| At1g26330 | 0.7541 | DNA-binding protein | 103 | At1g26330 | 0.6765 | DNA-binding protein |
| At1g67320 | 0.7527 | DNA primase, large subunit family | 67 | At1g67320 | 0.6890 | DNA primase, large subunit family |
| At5g23420 | 0.7525 | high mobility group (HMG1/2) family protein | 38 | At5g23420 | 0.7120 | high mobility group (HMG1/2) family protein |
| At3g42660 | 0.7513 | WD-40 repeat family protein | 5 | At3g42660 | 0.7791 | WD-40 repeat family protein |
| At3g12170 | 0.7499 | DNAJ heat shock N-term domain-containing protein | 19 | At3g12170 | 0.7342 | DNAJ heat shock N-term domain-containing protein |
| At5g49010 | 0.7498 | DNA replication protein-related | | | | |
| At3g02820 | 0.7492 | zinc knuckle (CCHC-type) family protein | 47 | At3g02820 | 0.7014 | zinc knuckle (CCHC-type) family protein |
| At5g65360 | 0.7484 | histone H3 | | | | |
| At1g23790 | 0.7471 | expressed protein | | | | |
| At1g07370 | 0.7393 | proliferating cell nuclear antigen 1 (PCNA1) | 17 | At1g07370 | 0.7390 | proliferating cell nuclear antigen 1 (PCNA1) |
| At3g12280 | 0.6927 | retinoblastoma-related protein (RBR1) | 60 | At2g36010 | 0.6927 | E2F transcription factor-A (E2FA) |

# Magyar_Supplementary Table II.

## E2FA

| GO-ID | p-value | Description |
|---|---|---|
| 6996 | 2,68E-19 | organelle organization and biogenesis |
| 6260 | 7,01E-19 | DNA replication |
| 6259 | 1,16E-18 | DNA metabolic process |
| 6325 | 1,77E-17 | establishment and/or maintenance of chromatin architecture |
| 7001 | 4,74E-16 | chromosome organization and biogenesis |
| 6334 | 1,49E-15 | nucleosome assembly |
| 6323 | 3,81E-15 | DNA packaging |
| 7017 | 7,39E-15 | microtubule-based process |
| 7010 | 2,48E-14 | cytoskeleton organization and biogenesis |
| 6333 | 4,35E-14 | chromatin assembly or disassembly |
| 74 | 2,76E-13 | regulation of cell cycle |
| 7018 | 2,15E-12 | microtubule-based movement |
| 6263 | 2,16E-11 | DNA-dependent DNA replication |
| 7049 | 1,46E-10 | cell cycle |
| 6139 | 6,78E-09 | nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| 6270 | 2,34E-07 | DNA replication initiation |
| 8151 | 4,14E-07 | cellular process |
| 278 | 5,25E-06 | mitotic cell cycle |
| 6310 | 9,95E-06 | DNA recombination |

## RBR1

| GO-ID | p-value | Description |
|---|---|---|
| 6139 | 2,75E-25 | nucleobase, nucleoside, nucleotide and nucleic acid metabolic process |
| 6259 | 2,69E-23 | DNA metabolic process |
| 6260 | 1,40E-22 | DNA replication |
| 6263 | 1,47E-12 | DNA-dependent DNA replication |
| 6996 | 1,32E-11 | organelle organization and biogenesis |
| 6325 | 1,52E-10 | establishment and/or maintenance of chromatin architecture |
| 7001 | 1,42E-09 | chromosome organization and biogenesis |
| 6394 | 4,20E-08 | RNA processing |
| 7010 | 2,03E-07 | cytoskeleton organization and biogenesis |
| 7049 | 4,21E-07 | cell cycle |
| 74 | 4,80E-07 | regulation of cell cycle |
| 6323 | 1,30E-06 | DNA packaging |
| 8151 | 2,76E-06 | cellular process |
| 6333 | 4,41E-06 | chromatin assembly or disassembly |
| 7018 | 6,64E-06 | microtubule-based movement |
| 6397 | 8,73E-06 | mRNA processing |
| 6334 | 2,13E-05 | nucleosome assembly |
| 6310 | 1,32E-05 | DNA recombination |
| 6270 | 1,49E-05 | DNA replication initiation |

# Magyar_Supplementary Table III.

| Gene | forward primer | reverse primer |
|---|---|---|
| ***q-PCR*** | | |
| KRP3 (AT5G48820) | AAGACACAGCACAAGGGAGA | TCTTTGGTTGCTCTGCACAT |
| ORC2 (AT2G37560) | TCCCGAATCACAACAAACTC | CCACAATAATGGAGCGTTGA |
| MCM3 (AT5G46280) | TGGGCAGCACATGAGGAC | CACTTTGTTATCATCTTGCAGTTT |
| RNR2 (AT3G27060) | CGCTTGCGTCGAAGGTAT | GAGAATGTTAATCCAGGCATGAG |
| CYCB1;1 (AT4G37490) | TCAGCAATGGAAGCAACAAG | AGCAGATTCAGTTCCGGTCA |
| CYCA2;3 (AT1G15570) | TCTTGGGAGATCAGCTTCTACAGC | GGCATAGAGGCAGCACAGTAAAGG |
| CYCD3;1 (AT4G34160) | GCAGCATAAGTTCAAGTGTGTAGC | AAACCGTAAGAGGCAGCTCTGG |
| CDKB1;1 (AT3G54180) | TCTGTTGGTTGTATCTTTGCTGA | CATTGCTGCTCAGTTGGTGT |
| ACTIN (AT3G18780) | AAATCACAGCACTTGCACCA | TGAGGGAAGCAAGAATGGAA |
| PIP2 (AT2G16850) | GCTTCATTCCGCAGCAAC | TCCGATTACATCAAATTAAAACACA |
| E2FA (AT2G36010) | CAACCCAGAAACTGCTATTGTT | GTCCGACTCATCATTTTCAAC |
| E2FA3'(AT2G36010) | TGTATGAACCAATGGCAGGT | CTCGGTGCTCCTCTACAACA |
| E2FB (AT5G22220) | GGACCGAGCGACAACAAA | AGGTGATCTCGTAGCAGTGGA |
| E2FC (AT1G47870) | TGCCGTTATGACAGTTCTTTAGGG | AGTGTTCCATCCTCAGCTTCCT |
| DPA (AT5G02470) | CTCGACTTCAATAGCACACCTTT | TGAGCGGAGCTATGTTGAGA |
| DPB (AT5G03415) | TTTGATTTCAACAGCACTCCA | GCGTGAAATTGTGACAAAAC |
| DEL1 (AT3G48160) | TGCTGCCTCGAAATTAGGAGTGG | CCTTCAATGCTCCTGGAATTGCG |
| DEL2 (AT5G14960) | ACCATCTCCCAGACCTGACAAC | TCACAAAGTTCTGTGCAAGTAGCC |
| DEL3 (AT3G01330) | AAAGGCGGCGTATCTATGATGTG | TCCCTCTTCTTGCCACAATCCC |
| RBR1 (AT3G12280) | CGCTTCCATTTTGGTTTTGA | TGAACAACAGCAGCAGCAAC |
| EXPANSIN5 (At3g29030) | GTACTATTCCCGTTATGTATAGAAGG | TGACCAACACCAAGTTAAAGTATGAG |
| CER1 ( At1g02200) | AGGCGTTCAGGTCTCCACT | CCACCAGCCATACCTTGTTT |
| CCS52A1 (AT4G22910) | GACCAACTCAAGCTGGCTG | GGAACCAACATTCAACACAACCGG |
| CCS52A2 (AT4G11920) | CCTGATTTCGAGAATCATGTCAAGAC | CTGTGAACACGCCGCAGCAGTG |

| ***ChIP assay*** | ***E2F site*** | ***Length*** | forward primer | reverse primer |
|---|---|---|---|---|
| CCS52A1-pr1 | + | 135 bp | CCGCTTAACTTCACATGTCGTTTTCTG | CATCTCTTTGTCCTTTTTATTGTAGTTTGG |
| CCS52A1-pr3 | - | 182 bp | ACGCCTGCCATCTAAGATTC | GGTGAGGACCGTGAGGTTTA |
| CCS52A1-pr5 | - | 142 bp | CCACCTACACATCAACAAGCA | TGAAAGAAAAAGAAAAACAAAACC |
| CCS52A2-pr1 | + | 142 bp | TTTCGTTCCTCCAGATTTGA | TTCGCACCAATTCACTTGAC |
| CCS52A2-pr2 | + | 148 bp | CCAGATTTGAAGATTTGGGTTG | TGATCGCTCCCTTATTTTCG |
| CCs52A2-pr4 | - | 146 bp | TGCAAATGCAACTTTTAGAAAATA | TGCACTTTAAATGGTAAAAATCC |
| CCS52A2-pr5 | - | 100 bp | TTGTTATTAGAAATATCATTTTGGTC | TCGTTGACCGTTATTGTTCTATTT |
| PCNA1 | + | 162bp | AATGACCAAAAATATCCATCCAA | CGGCTATTTTGGAAAGTGGA |
| IR1(chr3) | - | | CATTGAACACCTATTGGTAGGAA | ACTGTTTTGGTCCAGATTTTCAG |

IR (intergenic region)

**Table SIII.** Primer sequences used for Q-RT-PCR and ChIP assay.

**Supplementary Figure legends**

**Supplementary Figure 1.** Root meristem sizes of pE2FA::gE2FA-GFP, pRBR1::gRBR1-GFP lines and wild type Columbia. **(A)** Confocal microscopy images of the root tip of *Arabidopsis* plants four days after germination (4dag) expressing either the translational GFP fusion of E2FA (pE2FA::gE2FA-GFP lines - left) or RBR1 (pRBR1::gRBR1-GFP - right) and the enlarged image of the boxed area next to their right side showing details of their localization around the transition from root meristem to elongation zone. **(B)** Confocal microscopy images after propidium iodide staining of the roots as indicated. Arrows indicate the first elongated cortex cell (transition zone), arrowheads mark the quiescent centre. **(C)** Root meristem size (expressed as the number of cortex cells marked in (*B*) by arrow and arrowhead) of wild type, the translational fusion of E2FA (pE2FA::gE2FA-GFP), and RBR1 (pRBR1::gRBR1-GFP) transgenic lines.

**Supplementary Figure 2**. Efficiency and specificity of the E2FA RNAi silencing. **(A)** Detection of HA-E2FA or HA-E2FB protein levels in protoplasts transiently expressing the 35S::HA-E2FA or 35S::HA-E2FB alone or in combination with either E2FA-RNAi or E2FB-RNAi constructs two days after transformation as indicated. Anti-HA antibody was used in Western blot, the same membrane was stained afterwards with Coomassie indicating protein loading. **(B)** Quantitative RT-PCR of *E2FA* transcript in the indicated E2FA RNAi (E2FAi) T2 homozygous lines.

**Supplementary Figure 3**. E2FAi have smaller cotyledons and root compared to WT. **(A)** Pictures of *in vitro* grown wild type and E2FAi line 2/1 seedlings were taken 4 days after germination. **(B)** Root length of wild type and E2FA-RNAi line 2/1 was determined three

days after germination. **(C)** Root meristems of wild type Columbia (right) and E2FA-RNAi line 2/1 (left) at 5 days post germination. Arrows represent epidermis meristem-elongation transition zone, and asterisks indicate the position of quiescent centre. **(D)** Comparison of RBR1, CDKB1;1 and CDKA;1 protein levels between wild type and E2FA-RNAi plants one week post germination. Molecular masses are indicated on the left. **(E)** Flow cytometry measurements of DNA content from cells in leaf pairs representing all the rosette leaves from the first (L1/2) to the sixth pair (L11/12) of control wild type and E2FAi line 2/1 plants grown on soil at 24 dag. **(F)** Flow cytometry measurements of DNA content from cells in cotyledons of two weeks old control wild type and E2FAi line 7/2 (on the left) and ten days old control wild type and E2FA knock out line (*e2fa-1*; on the right).

**Supplementary Figure 4.** Regulation of RBR1 phosphorylation during leaf development. **(A)** Immunoprecipitation (IP) with anti-phospho-Rb (P-RB/Ser807/811) specifically immunoprecipitates a protein detected with anti-RBR1 antibody on Western blot. P-RB form is most abundant at early stages, 9 dag, during leaf development. **(B)** RBR1 phosphorylation in CYCD3;1 and E2FA/DPA overexpression lines. Proteins were extracted from two weeks old seedlings of CyCD3;1 overexpressor-OE (Dewitte et al. 2003), E2FA/DPA-OE (De Veylder et al. 2002), and wild type, followed by IP with anti-phospho-Rb antibody and probed the IP for RBR1 and CDKA on Western blots. The inputs (1/10$^{th}$ of what was used for the IP from the extracts) and the P-RB IP are indicated. **(C)** RBR1 phosphorylation in the KRP2 overexpression line. Proteins were extracted from a week old seedlings of KRP2 overexpression line (De Veylder et al. 2001) or wild type control, immunoprecipitated with anti-phospho-Rb antibody and the IPs were probed with anti-RBR1 or anti-CDKA;1 antibodies on Western blot. **(D)** RBR1, but not the P-RB form interacts with E2FA-GFP. First leaf pair at eight days after germination of two E2FA-GFP lines (line 2/10 and line 81/11) were used to immunoprecipitate E2FA-GFP with anti-GFP antibody and test the presence of

phosphorylated RBR1 (P-RB) and RBR1 in complex with E2FA-GFP. **(E)** Reciprocal IP as to what was shown in D confirms that the P-RB form does not interact with E2FA. The line with the highest level of E2FA-GFP (81/11) was used to prepare extract from the first leaf pairs ten days after germination, IP was carried out with anti-P-RB antibody. We could detect RBR1 but not E2FA-GFP in the IP using anti-GFP and anti-RBR1 specific antibodies. **(F)** The P-RB antibody also specifically immunoprecipitates the RBR1-GFP fusion. Immunoprecipitation (IP) with anti-phospho-Rb (P-RB/Ser807/811) specific antibody from the first leaf pair of transgenic line expressing the translational GFP fusion of RBR1 and probed with anti-GFP antibody on Western blot at different time points during leaf development as indicated (dag). Arrow points to the GFP-tagged RBR1 protein. The molecular mass of RBR1-GFP protein is around 150 kD. 1/20$^{th}$ of the IP from the extract was loaded as input.

**Supplementary Figure 5**. Phosphorylation of RBR1 is regulated by CYCD3;1-KRP2 pathway in a sucrose dependent manner. **(A)** Six days old seedlings grown on plate in the presence of 1% sucrose of wild type *Arabidopsis* Columbia (Col-WT) and Landsberg (Ler-WT) and transgenic CYCD3;1 and KRP2 overexpressor lines (T0) were treated for six hours with liquid media containing no (0%) or 2% sucrose. Total proteins were extracted and blotted with anti-P-RB, anti-RBR1 and anti-CDKA;1 antibodies as indicated. **(B)** Flow cytometry measurements of DNA content from cells in the first leaf pair of wild type *Arabidopsis* Columbia seedlings grown on plate with 1% sucrose for six days after germination treated with liquid medium in the presence or in the absence of sucrose (2% or 0% respectively) for six, twelve and 24 hours (h) as indicated. **(C)** Expression analysis of CYCD3;1, CYCA2;3, CYCB1;1, and CDKB1;1 were carried out by Q-RT-PCR from six days old wild type *Arabidopsis* Columbia seedlings treated with medium supplemented with or without sucrose (2% and 0%, respectively) for the indicated time period.

**Supplementary Figure 6.** In the CycD3;1 overexpression line there is an increased amount of E2FA-RBR1 complex compared to WT, but there is no detectable E2FB-RBR1. **(A)** Interaction of E2FA with RBR1 in wild type *Arabidopsis* Landsberg (Ler) or CycD3;1 overexpression line (D3;1-OE). IP from protein extracts of one week old seedlings with anti-E2FA or anti-DPA antibodies as indicated. The co-IP of RBR1 was probed with anti-RBR1 antibody in Western blots. The intensity of the cross reacting bands were determined by using Image J software and indicated bellow the image **(B)** Cotyledons enter into endocycle early during development in WT, but this is inhibited by CYCD3;1 overexpression. The percentage of DNA ploidy levels were determined by flow cytometry in samples taken at four time points (3, 6, 8, 10 days after germination - dag) of cotyledon of wild type *Arabidopsis* ecotype Landsberg (Ler) and CycD3;1 overexpressor line (CYCD3;1-OE). **(C)** RBR1, E2FB, DPA and CDKB1;1 protein levels are elevated in the CYCD3;1 overexpression line, indicative of active cell proliferation. Proteins were extracted from the cotyledons of wild type (Ler) and the CycD3;1 overexpression line (CYCD3;1-OE) at three time points (days after germination - dag) and were probed by using specific antibodies against RBR1, E2FB, DPA, and CDKB1;1 in Western blot as indicated. Coomassie-stained proteins on the same membranes are shown as a loading control. **(D)** E2FA, but not E2FB interacts with RBR1 in the cotyledons of CYCD3;1 OE plants where cells abundantly proliferate. Co-immunoprecipitation assays of cell extract prepared from the cotyledons (cot) of wild type (Ler) and the CycD3;1 overexpressor line (CYCD3;1-OE) at the indicated time point (10 dag). Immunoprecipitations (IP) used anti-E2FA or anti-E2FB or anti-DPA antibodies as indicated. Co-IP of RBR1 was detected by anti-RBR1 antibody on Western blot. RBR1 protein is indicated by arrow. $1/25^{th}$ of the IP from the extract was loaded as input. **(E)** Flow cytometry measurement of the developing first leaf pair of wild type control and pRBR::gRBR1-GFP line 7 days after germination (DAG) when most leaf cells are dividing and at 16 DAG when endocycle is dominant over proliferation.

**Supplementary Figure 7**. E2FA can dimerise both with DPA and DPB proteins. Anti-DPA or anti-DPB antibodies were used for IP from leaf extract derived from E2FA-GFP line (line 5/1) and the Co-IP of E2FA-GFP was probed with anti-GFP antibody. The detected E2FA-GFP is marked by arrow on the right and molecular mass of the protein is indicated on the left.

**Supplementary Figure 8.** E2FA$^{\Delta RB}$ is a C-terminal deletion mutant that removes both the RB-binding and the overlapping transactivation domain and therefore cannot bind RBR1 and cannot transactivate genes, but still able to efficiently dimerise with DP and to bind to the E2F element on target promoters. **(A)** Graphical representation of domain organization of E2FA indicating the deletion of 65 amino acids from the C-terminal end which resulted in the E2FA$^{\Delta RB}$ mutant that lack the RB-binding domain, and has a shortened transactivation region. **(B)** Electrophoretic mobility shift assays (EMSA) show that the *in vitro* translated mutant HA-E2FA$^{\Delta RB}$ could bind to oligo DNA with E2F elements in the presence of Myc-DPA protein as indicated with arrow. **(C)** The binding of the E2FA$^{\Delta RB}$/DPA heterodimer to the E2F specific oligo DNA was further confirmed in the EMSA antibody suppershift assay where antibodies recognising the epitope tag (HA-and MYC) were added to cause supershifts as indicated by lines in bracket. **(D)** Co-immunoprecipitation with DPA- and DPB-specific antibodies from extracts of HA-E2FA$^{\Delta RB}$/DPA lines shows that the overexpressed HA-tagged E2FA$^{\Delta RB}$ protein can efficiently dimerise with the co-expressed DPA protein, but not with the endogenous DPB. As a control we have shown that endogenous E2FB can be equally well detected in complex with both DPA and DPB in the IPs. **(E)** We also tested whether the mutant E2FA could form complex with RBR1 protein. We immunoprecipitated E2FA$^{\Delta RB}$ protein through the N-terminal HA-epitope tag and as a positive control we have used HA-tagged E2FB protein. We could recover RBR1 protein with the HA-tagged E2FB but there

was no detectable amount of RBR1 with the E2FA$^{\Delta RB}$ protein. Asterisk indicates a non-specific cross-reacting protein with the antibody, while HA-tagged E2FB and E2FA$^{\Delta RB}$ proteins are marked with arrows. **(F)** To test whether the overexpression of mutant E2FA$^{\Delta RB}$ could block the interaction between RBR1 and the endogenous E2FA we have done an IP with anti-E2FA specific antibody from WT and HA-E2FA$^{\Delta RB}$/DPA lines and tested for the presence of RBR1 in the IP. The E2FA-RBR1 interaction was reduced in the HA-E2FA$^{\Delta RB}$/DPA line compared to WT. **(G)** The C-terminal part of E2FA contains both the RB binding and transactivation domains. To test the transactivation capacity of the HA-E2FA$^{\Delta RB}$ construct, either the full length E2FA and DPA or the mutant E2FA$^{\Delta RB}$ and DPA were co-transformed into protoplasts and measured their transactivation activities on the RNR2 promoter containing E2F sites and driving the GUS reporter construct (Horvath et al. 2006). There was an increase in the promoter activity of RNR2 in the presence of E2FA and DPA, while there was no increase in the GUS activity with the E2FA$^{\Delta RB}$ construct.

**Supplemetary Figure 9**. Co-expression of E2FA$^{\Delta RB}$ with DPA increases ploidy level through the activation of endocycle. **(A)** Proteins were extracted from seedlings of wild type control, DPA, HA-E2FA$^{\Delta RB}$, HA-E2FA$^{\Delta RB}$/DPA, and E2FA/DPA lines one week after germination, and the level of the HA-E2FA$^{\Delta RB}$, DPA, E2FB, RBR1, CDKB1;1, CDKA;1 were determined on Western blots by specific antibodies as indicated. **(B)** The HA-E2FA$^{\Delta RB}$/DPA seedlings are slightly smaller compared to wild type at five days after germination Bar= 2mm. **(C)** Root meristems of wild type Columbia (right) and HA-E2FA$^{\Delta RB}$/DPA (left) at 5 days post germination. In the lugol stained roots arrowheads indicate the position of quiescent centre, and straight line marks the size of the root meristem (RM). **(D)** Representative images from the adaxial epidermal cell layer of the first leaf pairs at two developmental time points (days after germination-dag) from wild type (WT), and the 35S::HA-E2FA$^{\Delta RB}$/DPA line as indicated. Bar=100 μM. **(E)** Representative images of the first leaf of wild type control

(Columbia) and the 35S::HA-E2FA$^{\Delta RB}$/DPA transgenic line shows significant size difference. Scale bar is 1 cm. **(F)** Trichomes are severely overbranched on the leaf surface of the HA-E2FA$^{\Delta RB}$/DPA plants. **(G)** Percentage of trichomes with number of branches from 3 to 10 in control wild type, HA-E2FA$^{\Delta RB}$, HA-E2FA$^{\Delta RB}$/DPA plants (n=150). **(H)** Flow cytometry measurements of DNA content from cells in the first leaf pair at 15 dag of control wild type, DPA, E2FA$^{\Delta RB}$, and E2FA$^{\Delta RB}$/DPA overexpression lines.

**Supplementary Figure 10**. Co-expression of E2FA or E2FA$^{\Delta RB}$ with DPA$^{\Delta DB}$ mutant missing the DNA-binding region do not lead to elevated ploidy level. **(A)** E2FA was detected by anti-E2FA antibody, Myc-DPA$^{\Delta DB}$ with anti-myc on Western blot, and the specific cross-reacting bands are labelled by arrows. Asterisks denote the non-specific crossreaction of antibody with proteins on the blot. **(B)** Flow cytometry measurements of DNA content of the first leaf pairs of DPA$^{\Delta DB}$, E2FA/DPA$^{\Delta DB}$, E2FA$^{\Delta RB}$/DPA, and wild type control. *(C)* Flow cytometry analysis of DNA content from cotyledon samples of transgenic seedlings including single and double overexpressor lines as indicated and the wild type control (WT-Col) eight days after germination.

**Supplementary Figure 11.** Expression of selected set of E2FA-regulated genes in the E2FA$^{\Delta RB}$/DPA line. **(A-C)** Quantitative RT-PCR analysis of the expression of *CER1, RNR2, MCM3* genes from the developing first leaf pair of E2FA$^{\Delta RB}$/DPA transgenic lines (7 and 8; A to C, respectively).

**Supplementary Figure 12.** Expression of all 6 E2Fs in the *e2fa-1* knock out mutant compared to wild type. Expression analysis of E2FA, E2FB, E2FC, DEL1, DEL2 and DEL3 genes by using Q-RT-PCR in leaf samples collected at the indicated time points days after germination (DAG) from wild type control and E2FA T-DNA insertion line (*e2fa-1*).

**Supplementary Figure 13.** Quantifications of the Co-IP experiments presented in Figure 3 and 4. The intensity of the specific protein bands were determined by Image J software.

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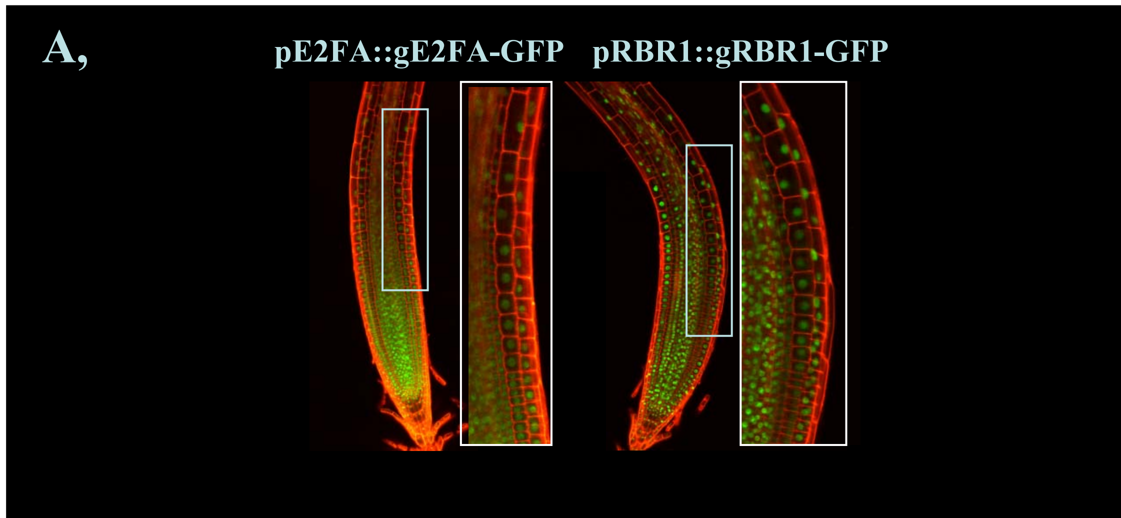


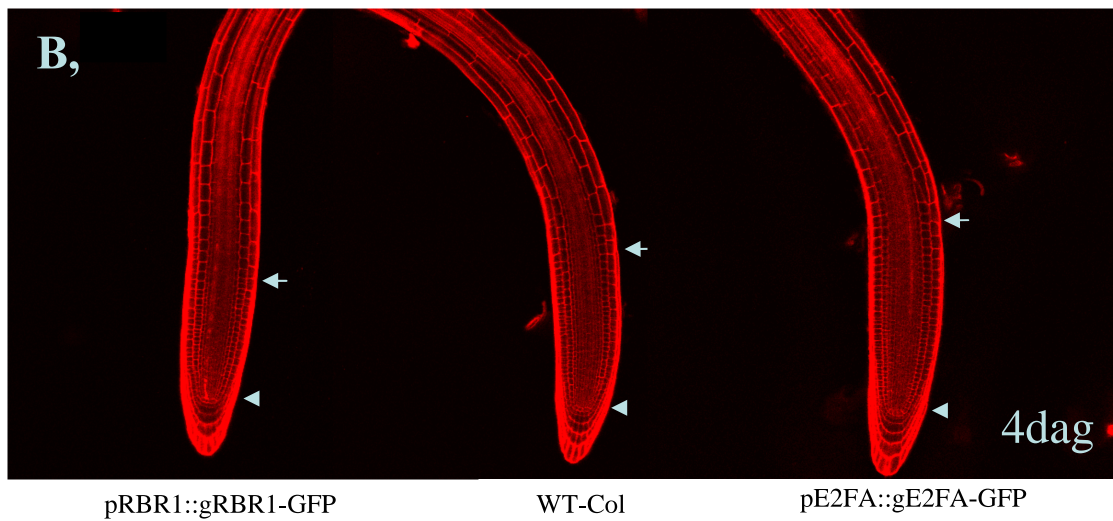


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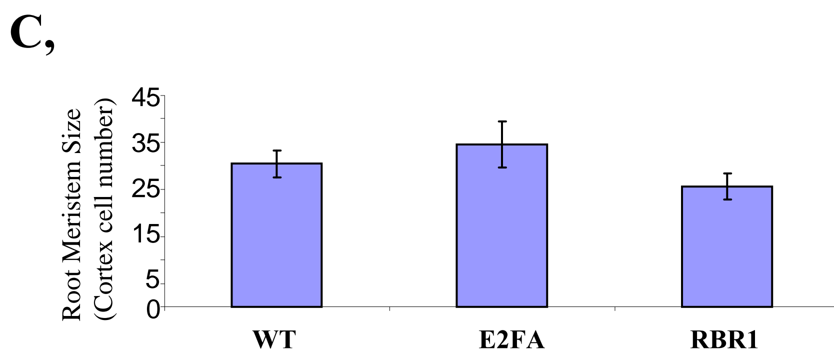

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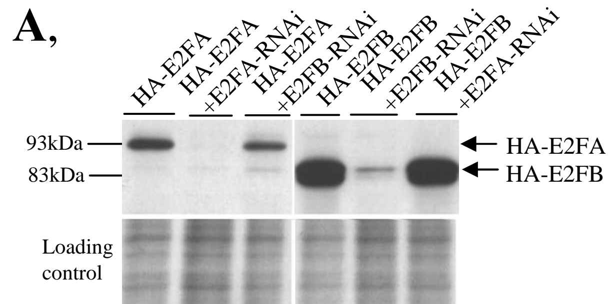


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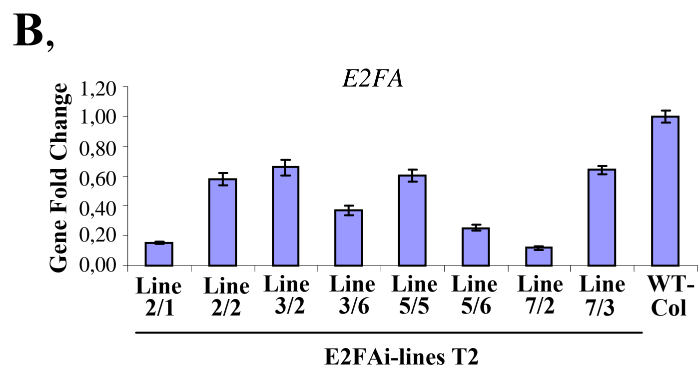

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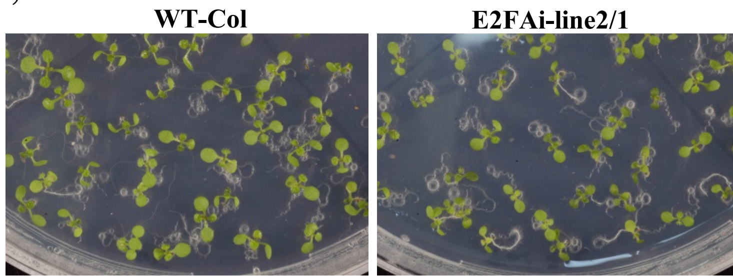


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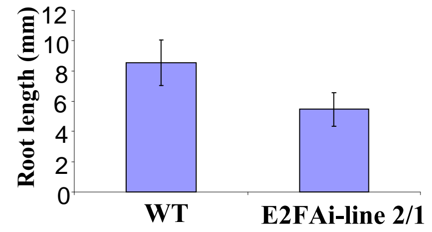


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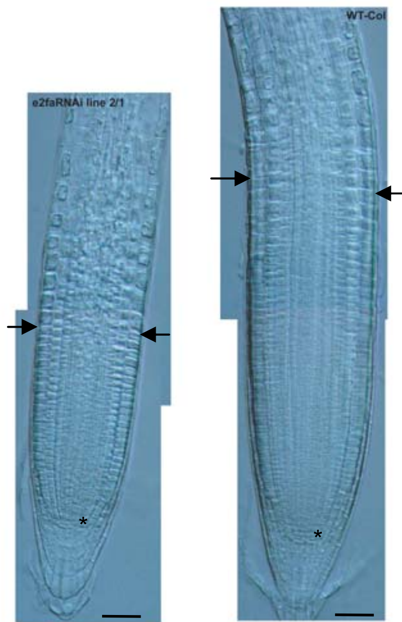


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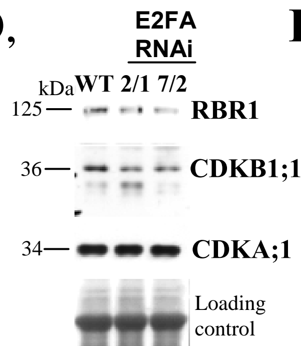


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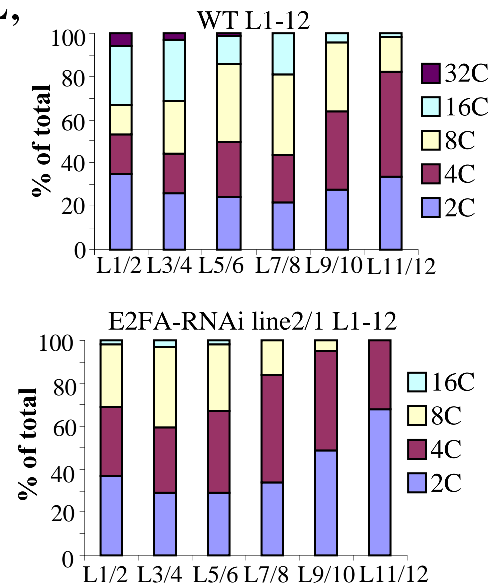


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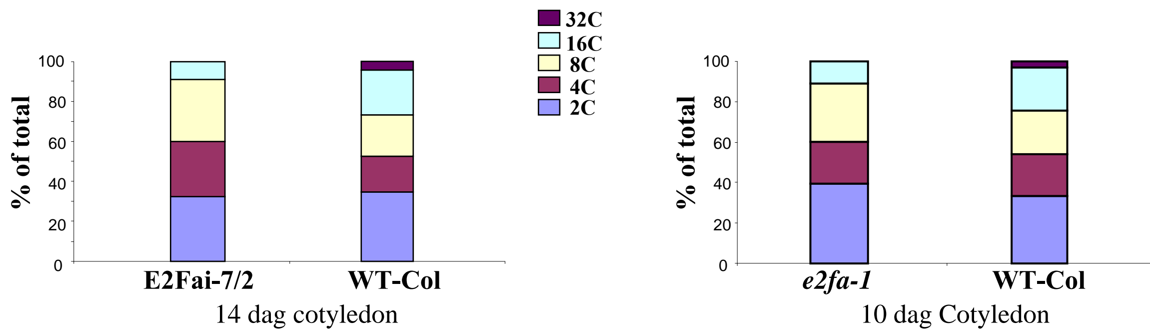

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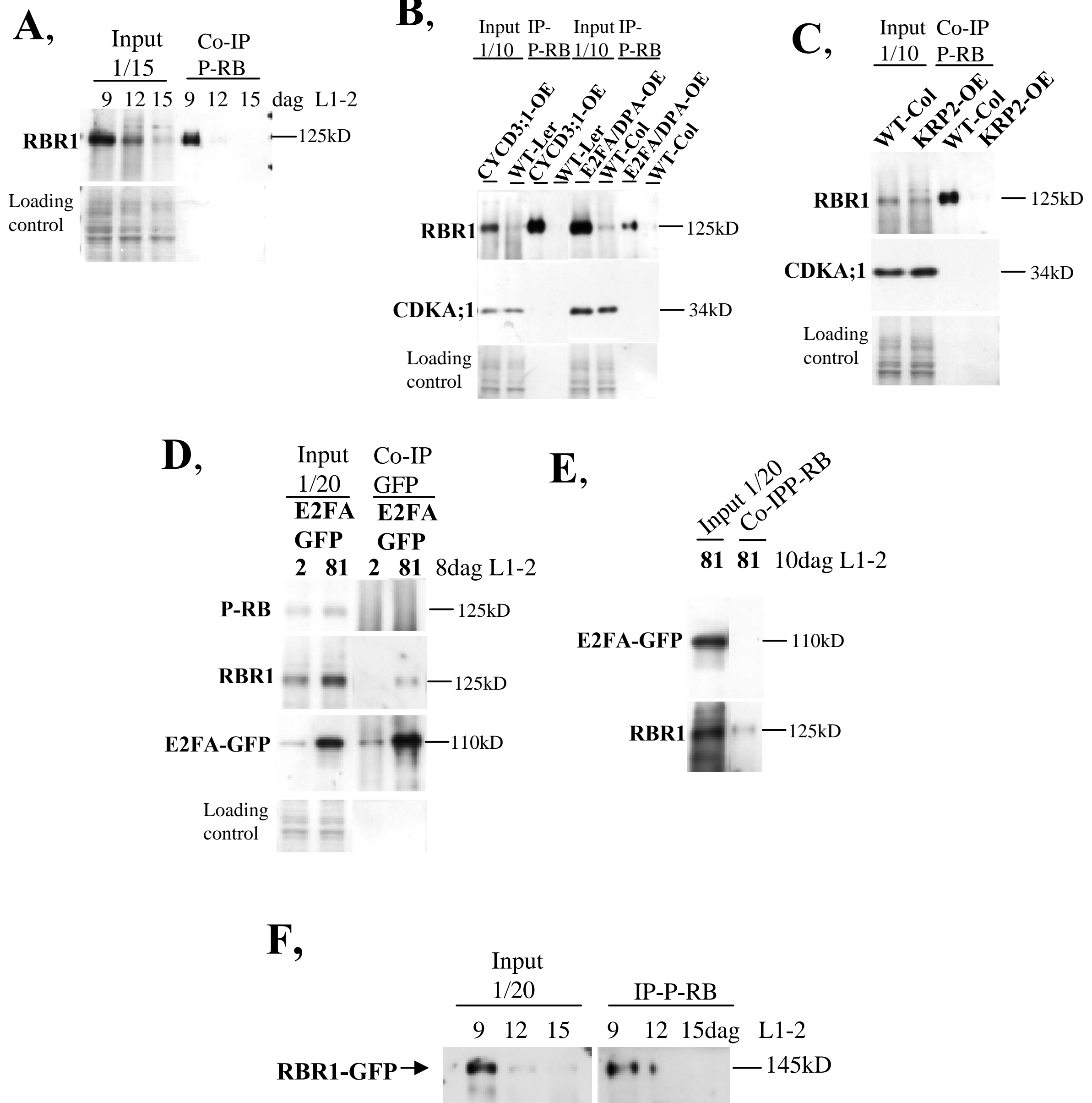

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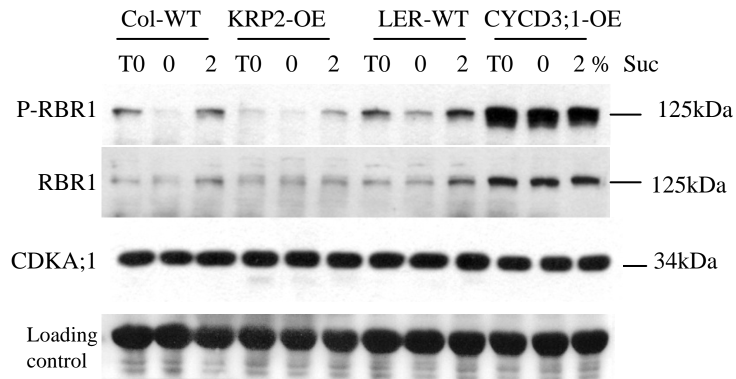


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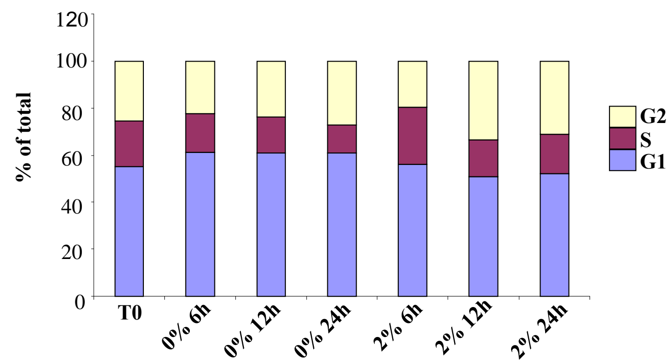


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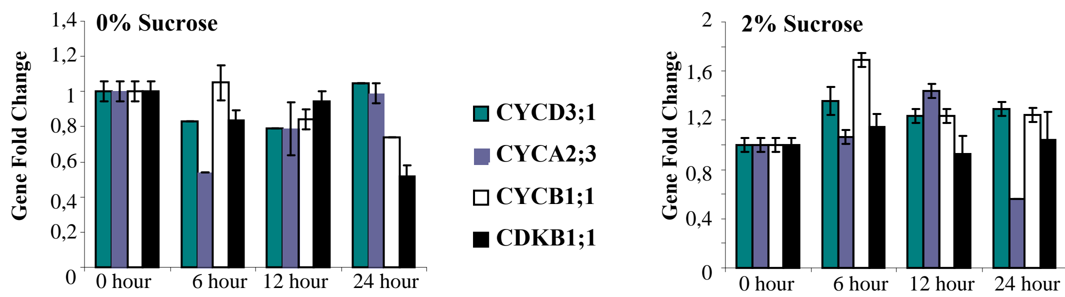

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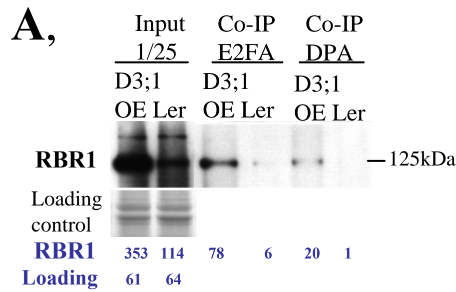


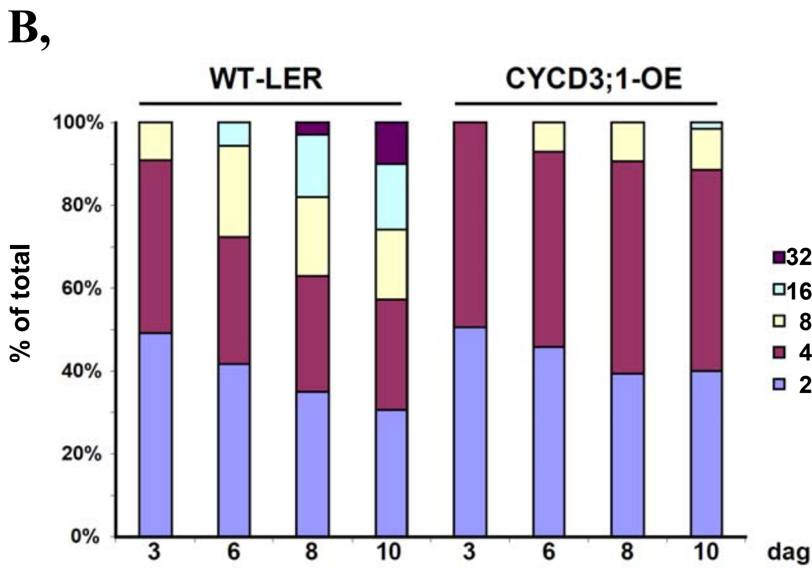


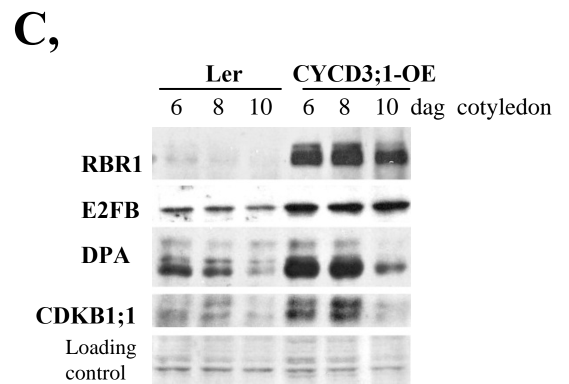


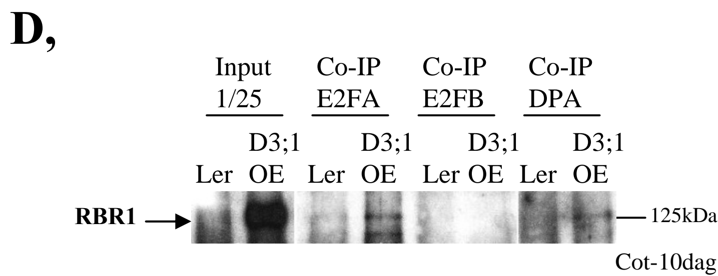


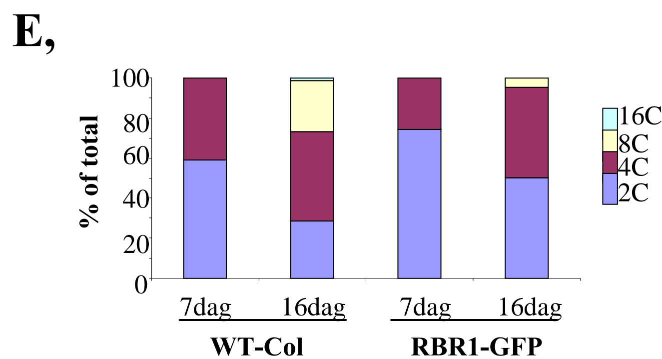

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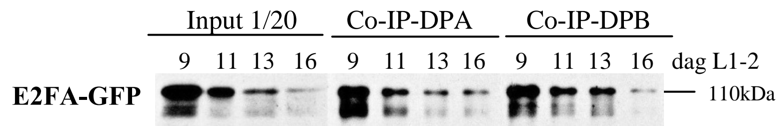

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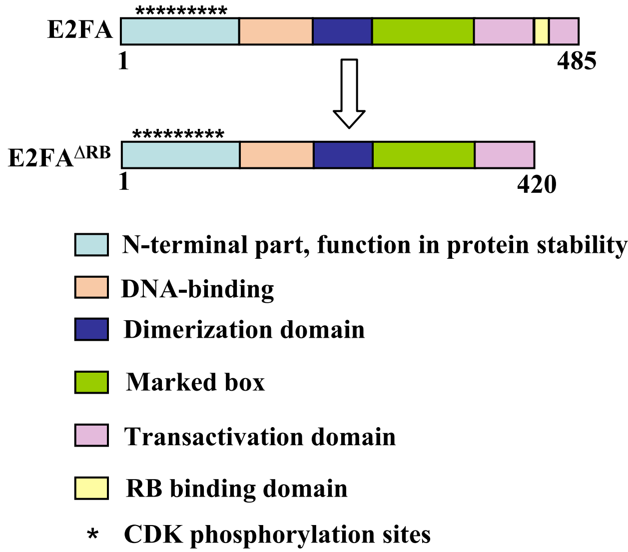


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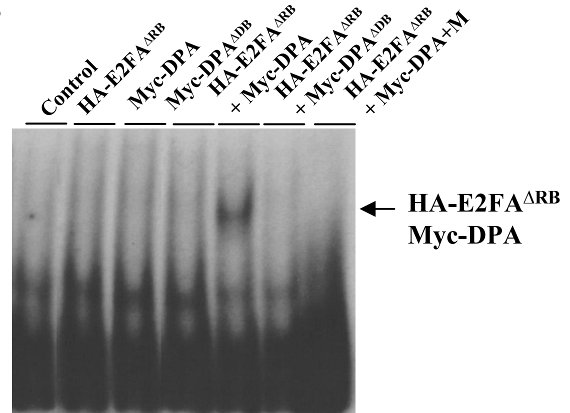


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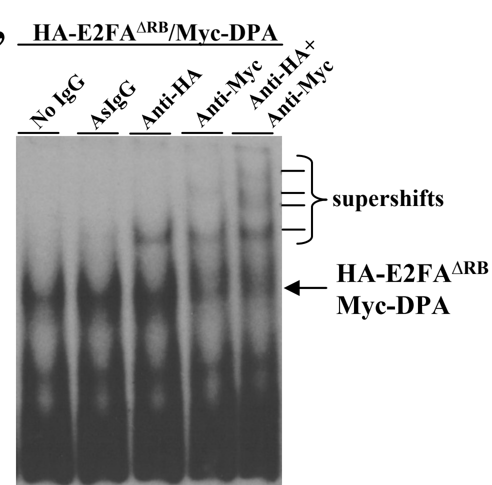


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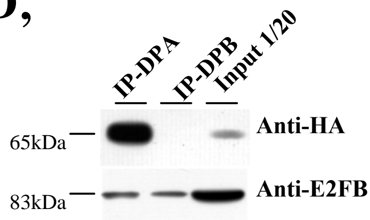


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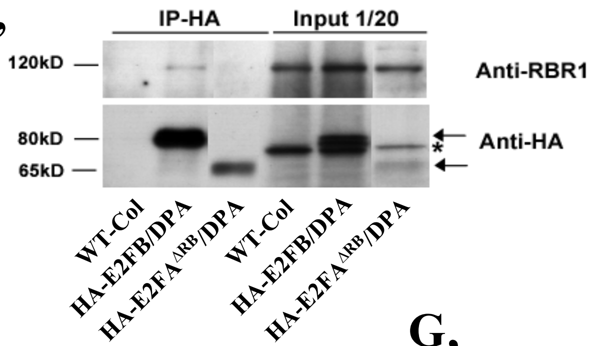


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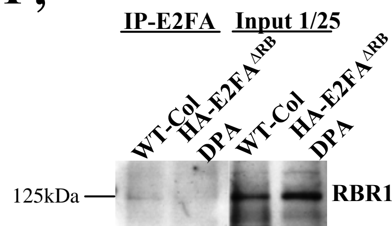


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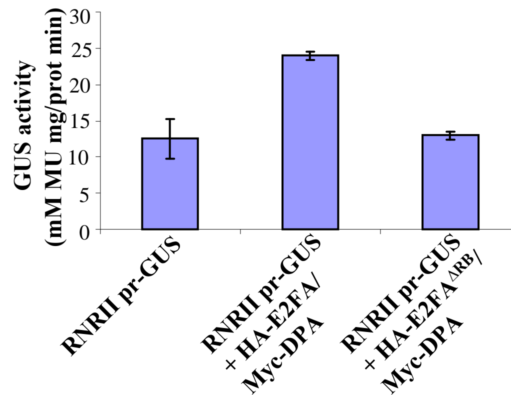

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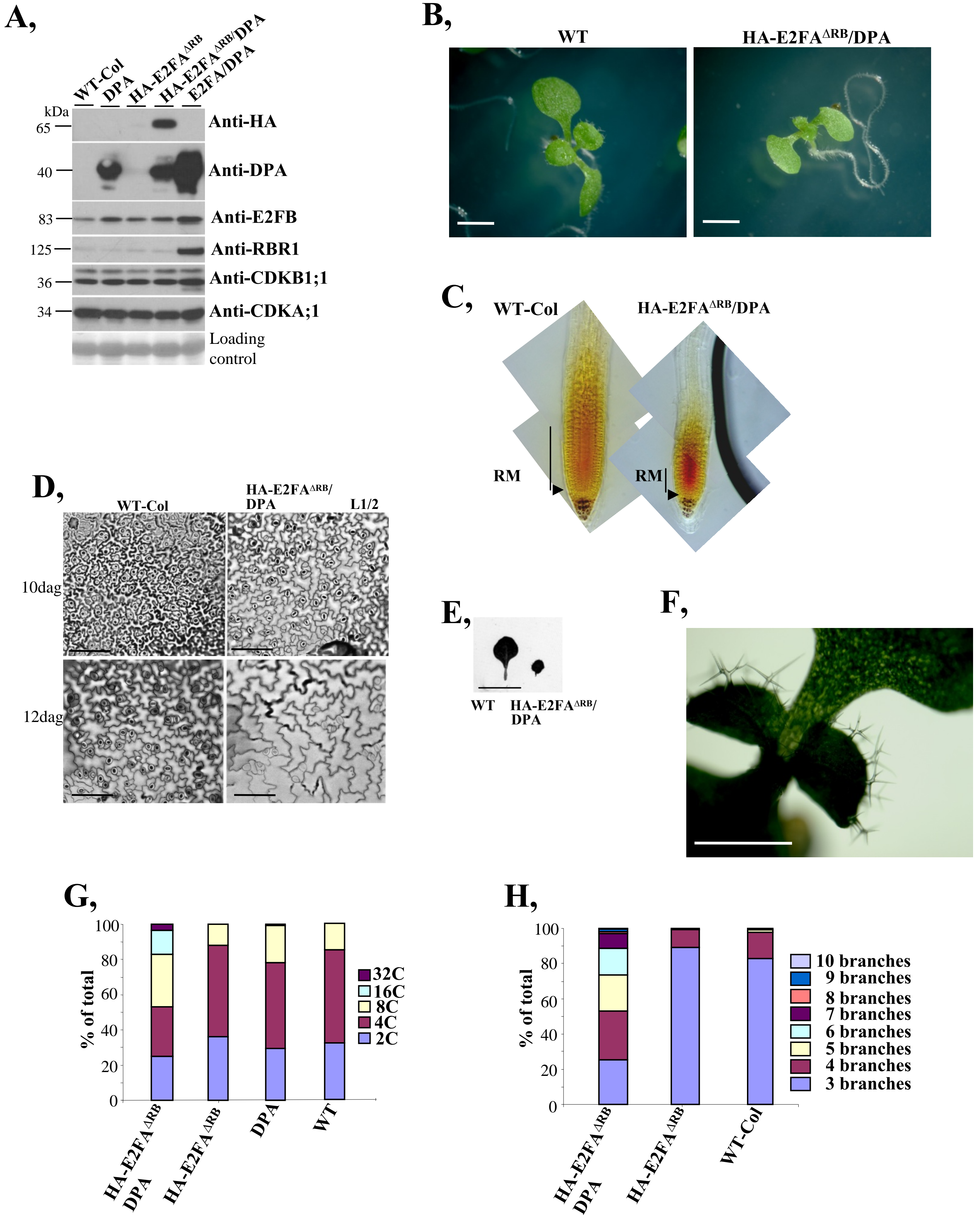

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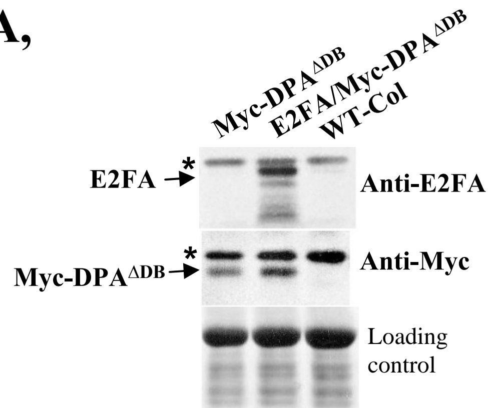


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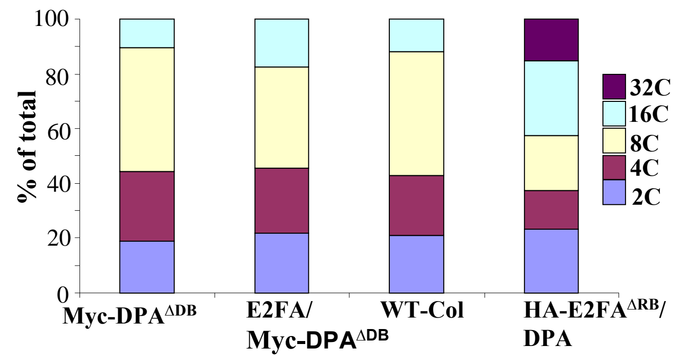


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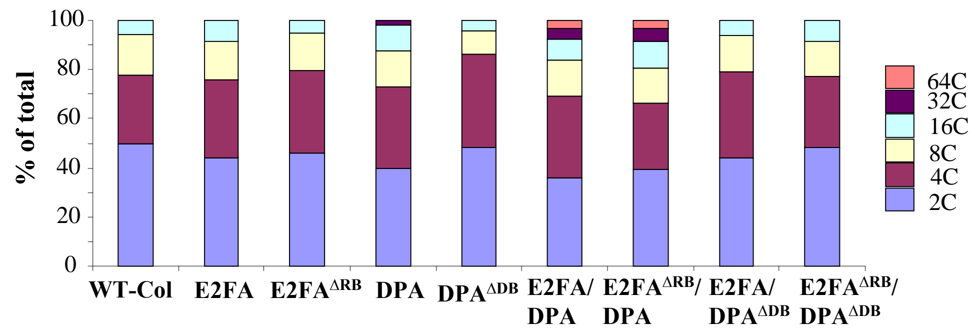

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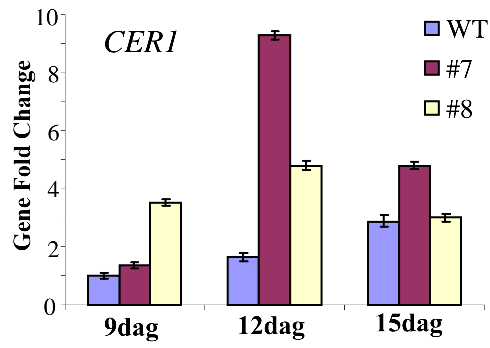


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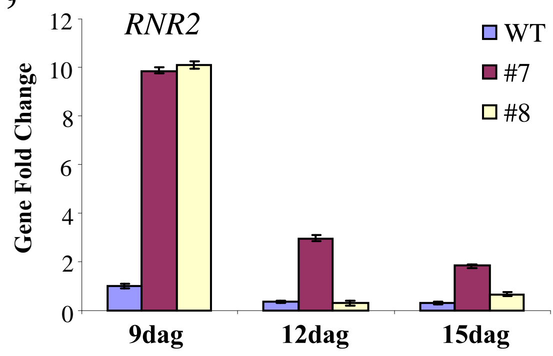


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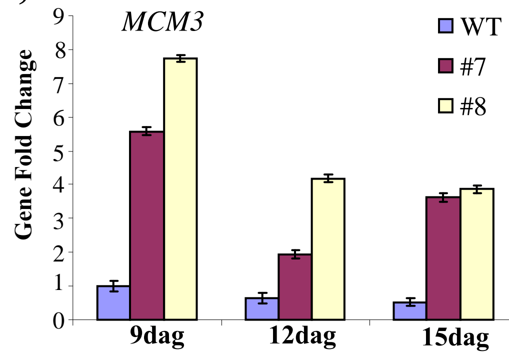

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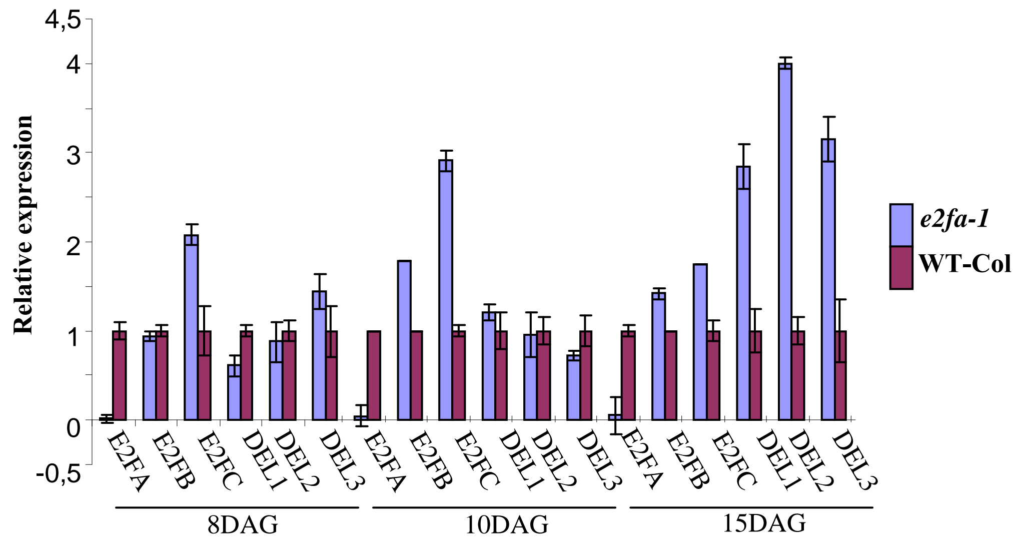

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