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Fig. 1. Isolation and molecular characterization of *early bud-break 1 dominant (ebb1D)* poplar mutant. **(A)** Precocious bud-break of the *ebb1D* in the field during the start of the second growing season. Mutant plants showed advanced bud-break compared to neighboring transgenic and WT-717 (wild-type) trees. Arrows point to two *ebb1D* ramets that show accelerated bud-break compared to majority of neighboring other activation tagging events and WT- 717 plants. Precocious bud-break in *ebb1D* mutant (left) compared to WT-717 (right) plants **(B)** after growth chamber photoperiodic induction of dormancy followed by 11 weeks of chilling, **(C)** and average number of days to bud-break(see Materials and methods for detailed description of inductive treatments). Bars show one standard error over means of at least 10 ramets per genotype. Significance of differences tested by Student t-test (***) - $P < 0.001$. **(D)** Genome position of activation tag insertion in *ebb1D*, Enh – Enhancer derived from the CaMV 35S promoter. **(E)** Expression of AP2/ERF tagged gene in WT-717 and *ebb1D* mutant plants. **(F)** Unrooted neighbor-joining tree of proteins from *Arabidopsis*, poplar, rice and maize that belong to the same AP2/ERF gene subfamily. Numbers in the branch nodes indicate % bootstrap support of 1,000 iterations. Poptr= *Populus trichocarpa*; FZP = FRIZZLE PANICLE (rice); BD1= BRANCHED SILKLESS 1 (maize).

Overexpression of EBB1 accelerate bud break

Knockdown of EBB1 delays bud break

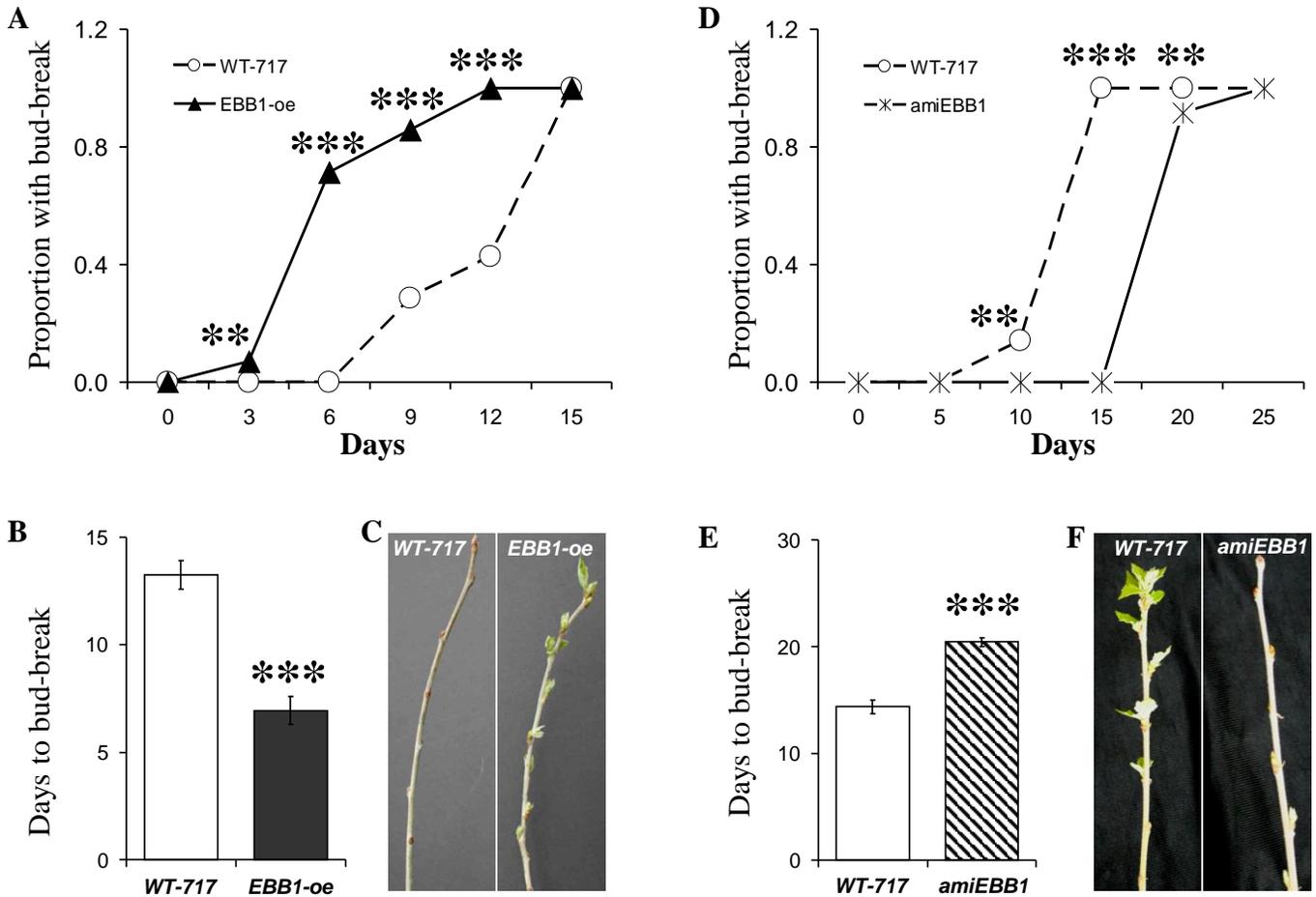


Fig. 2. Transgenic modification of EBB1. **(A)** to **(C)** overexpression of EBB1 from 35S promoter (denoted *EBB1-oe*), **(D)** to **(F)** gene knockdown via artificial micro-RNA (denoted *amiEBB1*). **(A)** and **(D)** Dynamics of bud-break in *EBB1-oe* (A) *amiEBB1* (B) and wild type controls - WT-717. **(B)** and **(D)** Average number of days to bud-break in *EBB1-oe* (B), *amiEBB1* (D) compared to WT-717. **(C)** and **(F)** Bud-break in a typical *EBB1-oe* (C) and *amiEBB1* (F) control plants after 1 week (C) or 2 weeks (F) following a chilling treatment (see Materials and Methods). Typical WT-717 (left in C and D), *EBB1-oe* (right, C) *amiEBB1* (right, F) plants. Bars in **(B)** and **(D)** show one standard error over genotypes' means (n= 10-15 in (A) and (B), 7-12 in (D) and (E)). Significance of differences tested by Fisher's Exact Test in (A) and (B) or Student t-test in (B) and (E), (** P<0.01, *** P<0.001).

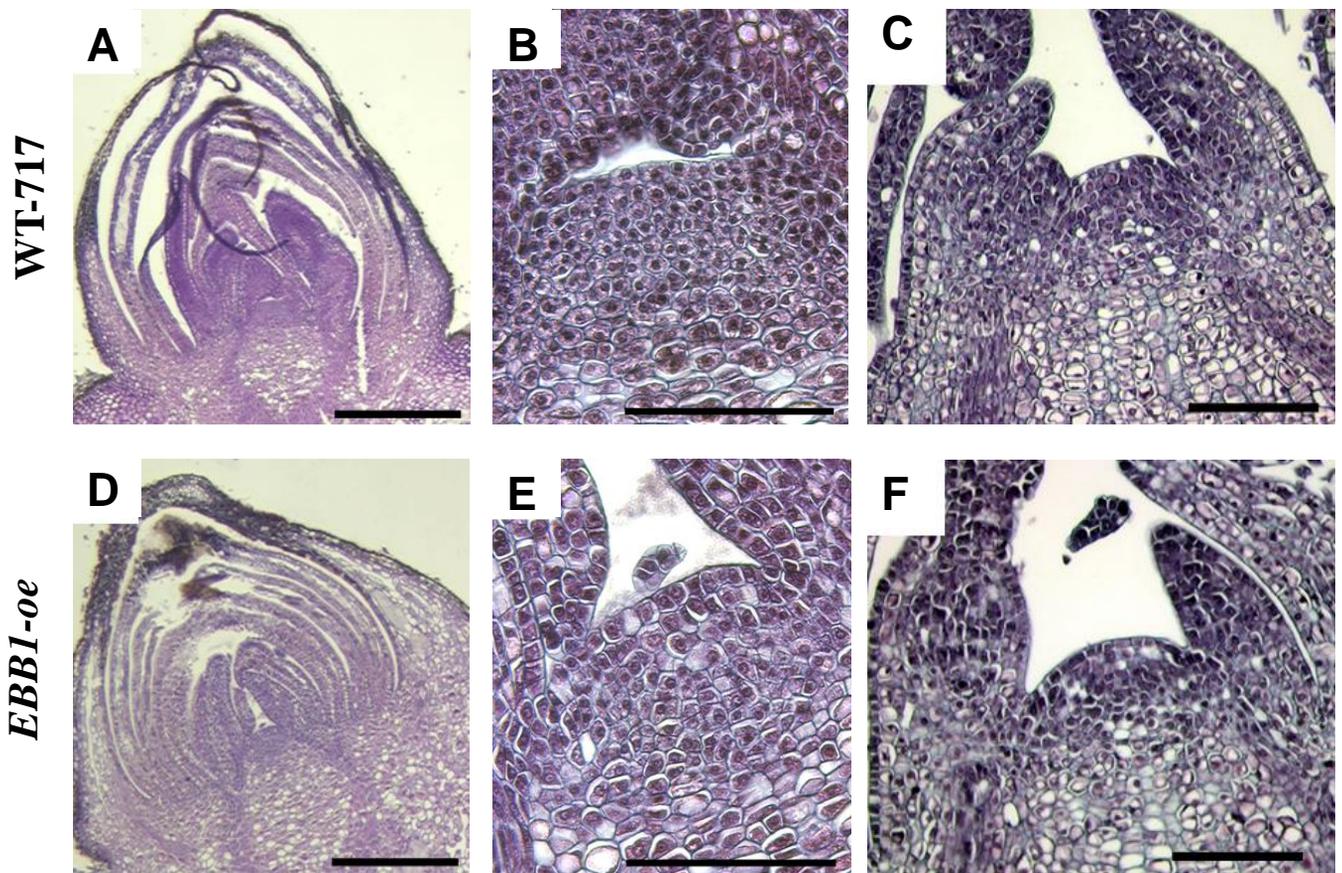


Fig. 3. Bud and apex morphology of *EBB1-oe* transgenics. Dormant bud (A, B, D, E) and actively growing vegetative SAM (C, F) in WT-717 (A, B, C) and transgenic *EBB1-oe* (D, E, F) plants. Note the difference in scales' shape in transgenic line, which form more open area around meristem. In wild-type buds, meristem is more compactly surrounded by buds scales. (B) and (E) represent close-up magnification of the same sections shown on (A) and (D). Scale bars = 500 μ m (A and D) and 100 μ m (B, C, E and F).

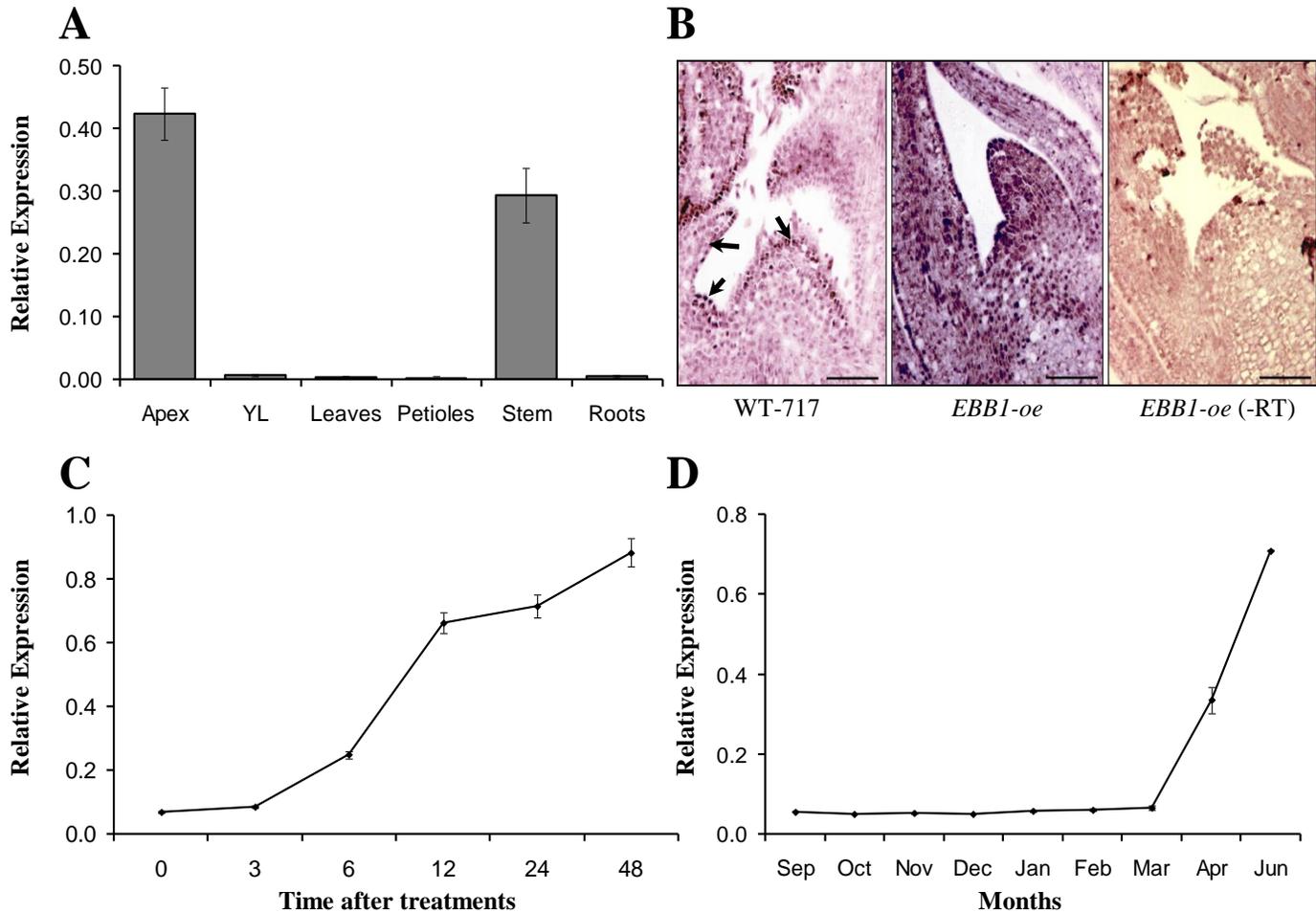


Fig. 4. *EBB1* expression and localization. **(A)** *EBB1* expression in various organs. Tissues were collected from WT-717 plants at the same time of the day and correspond to as follows: 1 cm roots tips (Roots); 2-3mm apical shoot including meristem and subtending leaf primordia (Apex); unexpanded young LPI 1-2 leaves (YL); fully-expanded LPI 5-10 leaves (Leaves); petioles of fully-expanded leaves (Petioles); whole stem collected from LPI5-10 (Stem). **(B)** *In situ* RT-PCR localization of *EBB1* transcript in actively growing apices of WT-717 (left), *EBB1-oe* plants (middle). Negative -RT control was performed on *EBB1-oe* apices (right). Arrows indicate the localization of the *EBB1* transcript in the L1/L2 layers of the meristem and leaf primordia. Scale bars = 50 μ m **(C)** *EBB1* is induced by a combination of cytokinin and auxin treatment (see Materials and Methods for more details). **(D)** Expression of *EBB1* in vegetative buds of wild aspen (*Populus tremuloides*) trees. Relative expression for all experiments was normalized for loading differences using ubiquitin gene (UBI) as previously described. Bars and data points show means \pm one standard error of at least three independent biological replicates for all experiments except for **(D)** where two individual trees were used as biological replications..

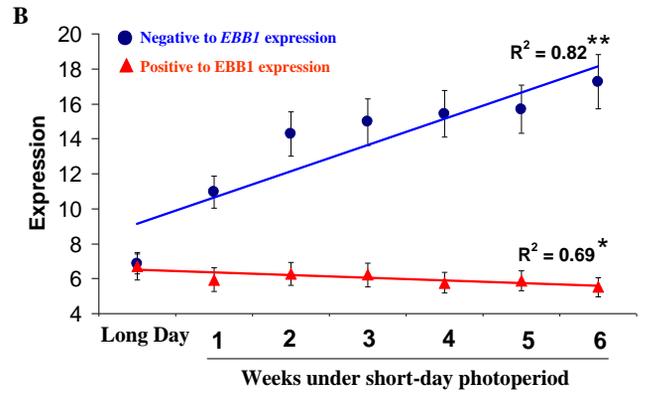
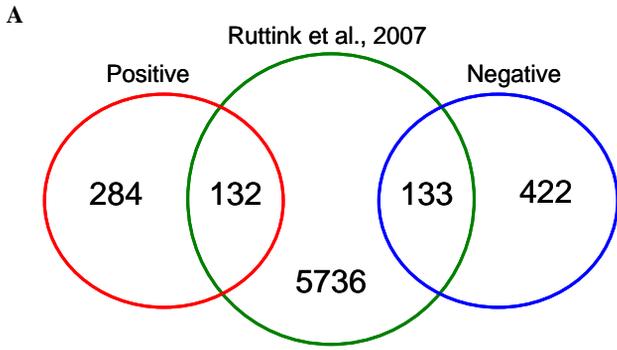


Fig. 5. Dormancy induction and *EBB1-oe* share common and opposing regulons. **(A)** Venn diagram of common gene set between differentially expressed genes in *EBB1* transgenic apices and genes that are differentially expressed in apices of the same genotype during SD-induced dormancy (Ruttink et al. 2007). **(B)** Trends in the expression of the common gene set during the 5 weeks of the dormancy induction period. Data points and error bars represent the mean and stand error over the averaged expression of all upregulated and downregulated genes. R^2 represents coefficient of determination for goodness of fit for the calculated linear trendline, linear regression significance are denoted as *- $p < 0.05$, and ** - $p < 0.01$.

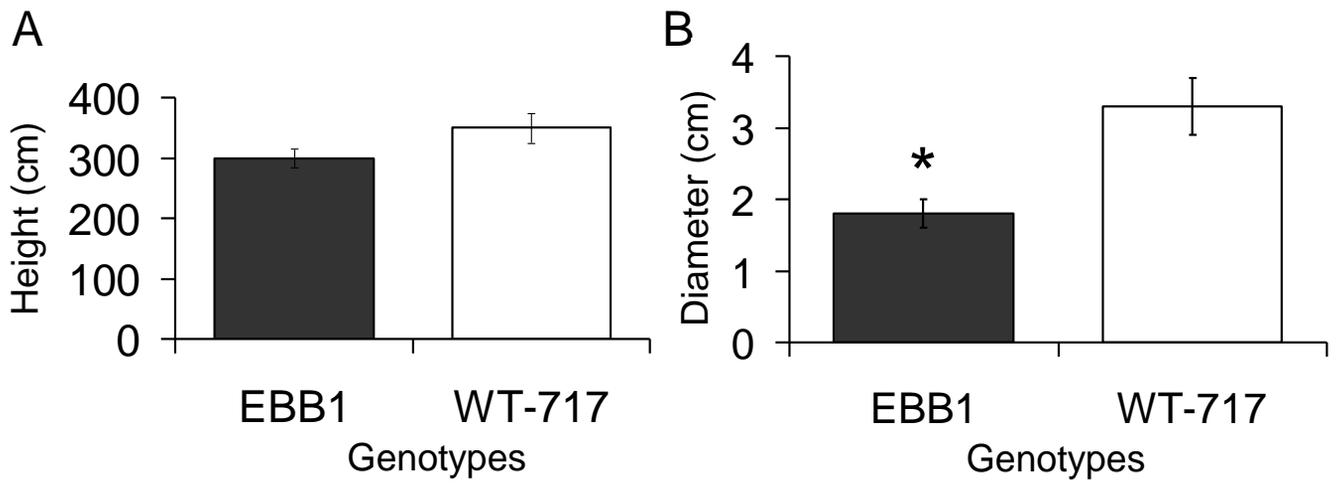


Figure S1. *EBB1* growth and morphology in the field. Height (**A**) and diameter (**B**) of 3-year-old field-grown *EBB1* and WT plants. Error bars represent one standard error over ramet values. * - indicates $P < 0.05$ as determined by Student's t-test. Adaxial (**C**) and abaxial (**D**) sides of leaves from field-grown *EBB1* (left) and WT (right) plants. **E**. Whole-plant view of *EBB1* trees in the field (in front of light colored cloth screen).

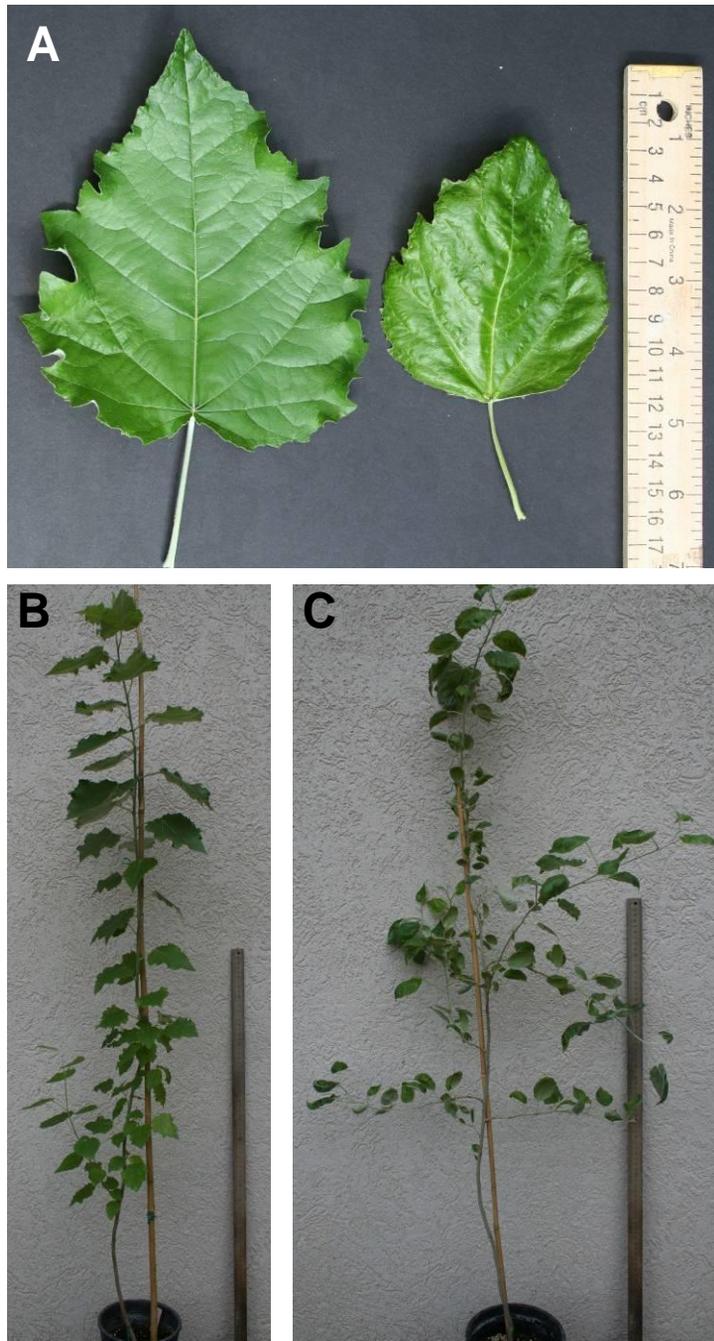


Figure S2. Overexpression of *EBB1* causes a range of phenotypic variation. **A.** Leaf sizes and form observed in *EBB1*-oe transgenic plants. Branching and size of WT (**B**) and *EBB1*-oe transgenic event (**C**) grown in a greenhouse for 4 months.

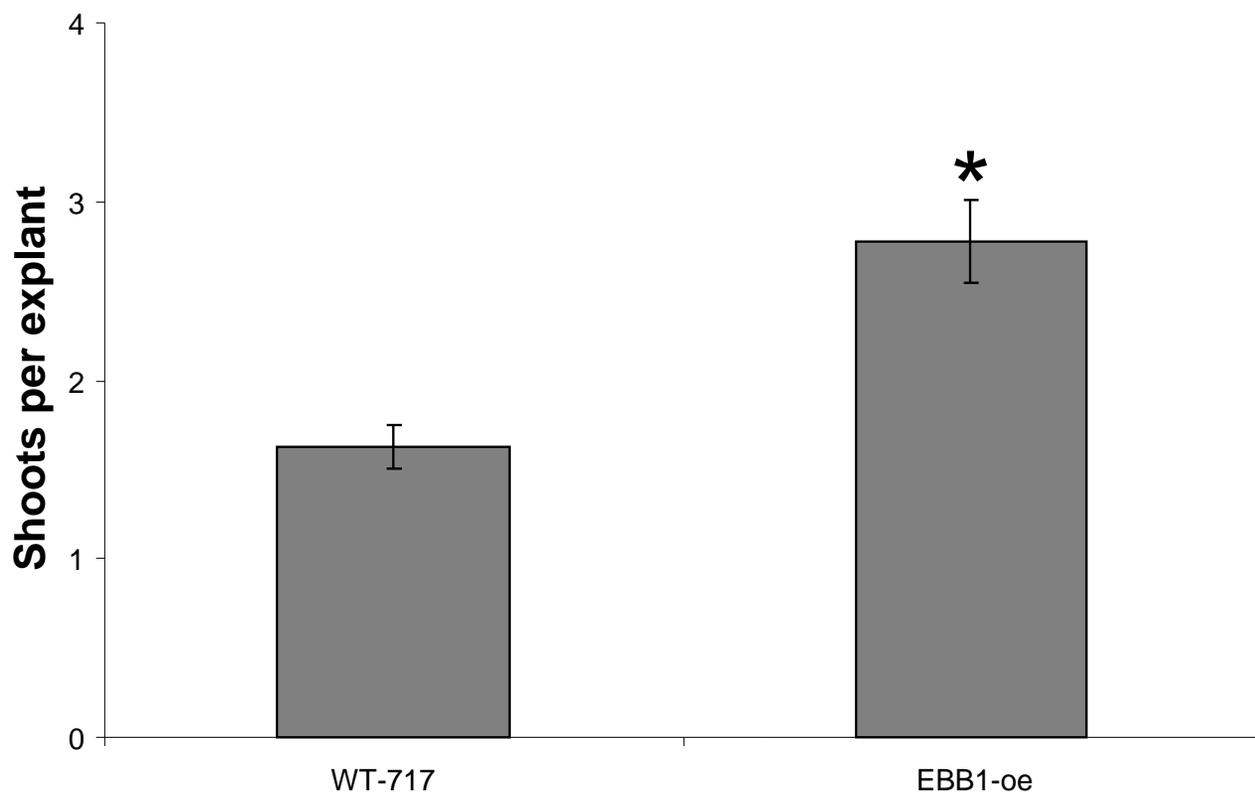


Figure S3. Shoot regeneration from leaf segments. Leaf segments from WT-717 and *EBB1-oe* events (+3) were cultivated on callus induction media (CIM) media (Han, K. H. et al. 2000) for three weeks, transferred on shoot induction media (SIM) media for four weeks, and regenerated shoots were recorded. Bars represent mean and standard errors from five biological replicates with 20 explants each. (*) denote t-test difference, $p < 0.05$

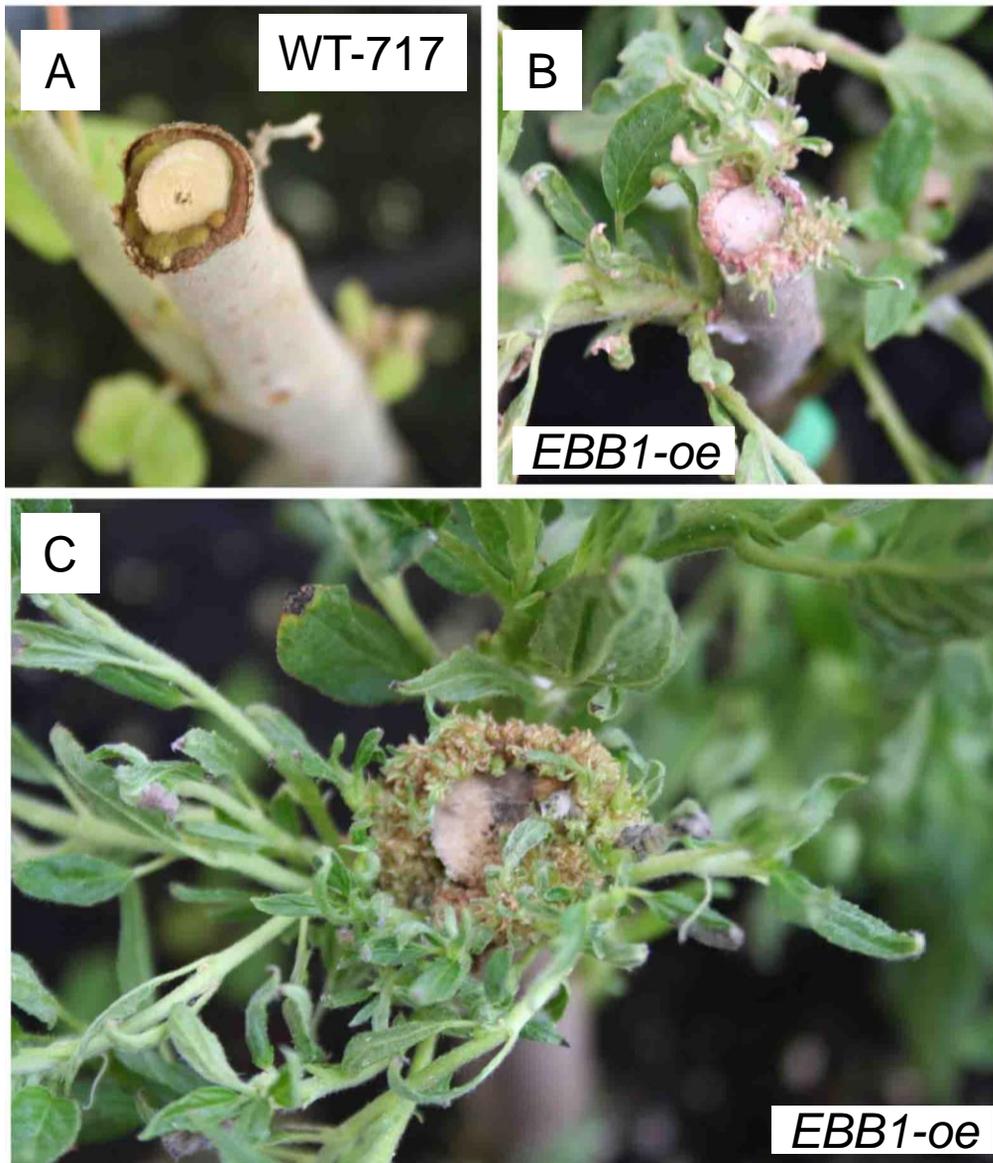


Figure S4. Spontaneous shoot regeneration from cambium-derived callus in *EBB1-oe* plants. **(A)** WT-717 plants. **(B)** and **(C)** *EBB1-oe* plants. Stems were cut approximately a foot from the soil and photos taken three weeks after cutting. Similar responses were seen in approximately half of the *EBB1-oe* events.

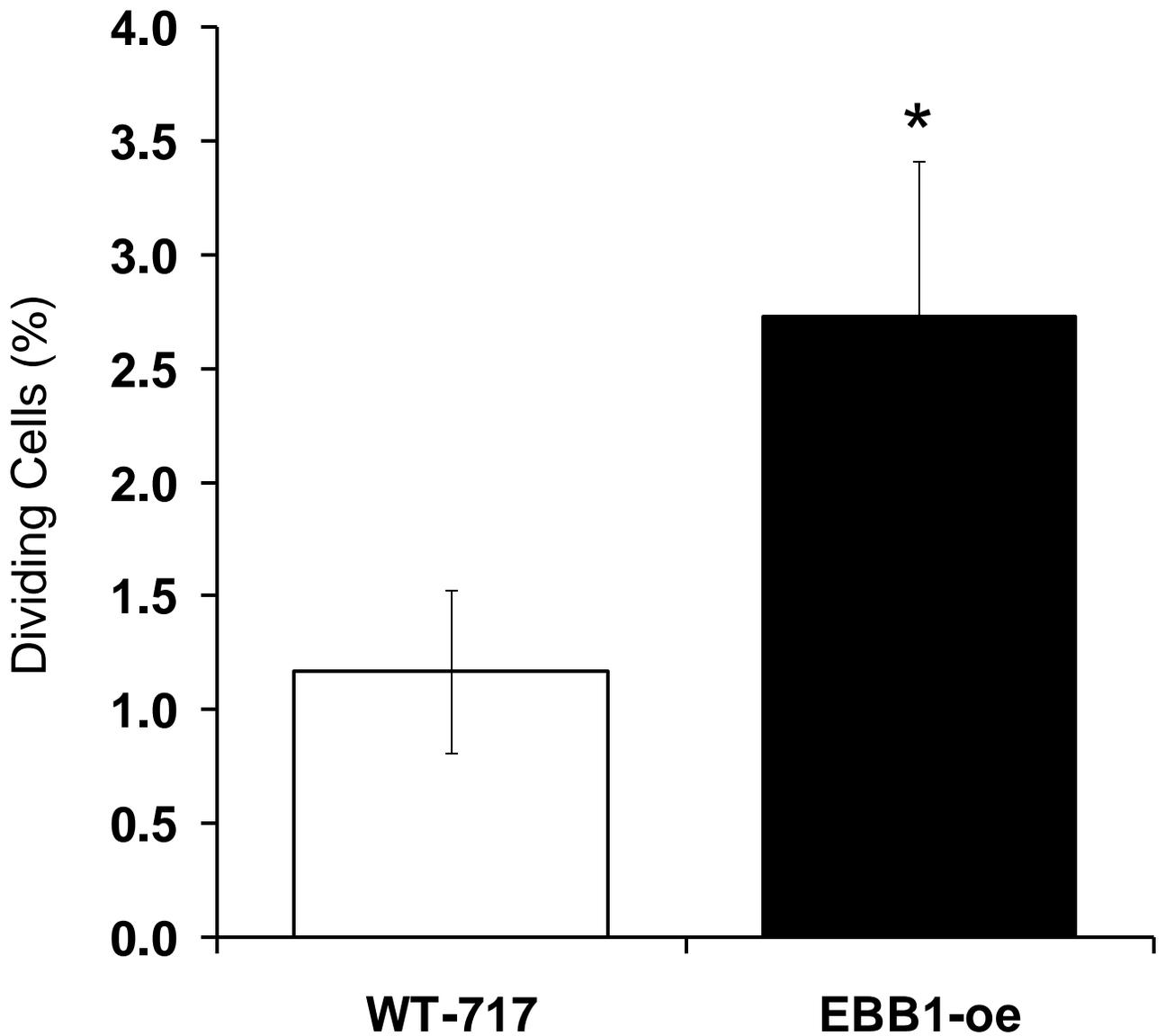


Figure S5. Increased cell division rate in the apex of *EBB-oe* transgenics. Cells in meta-, ana- and telophase were counted as dividing. The graph presents mean and standard errors of 10-12 acid-carmine-stained apices and approximately 2000 cells. (*) denote t-test difference, $p < 0.05$

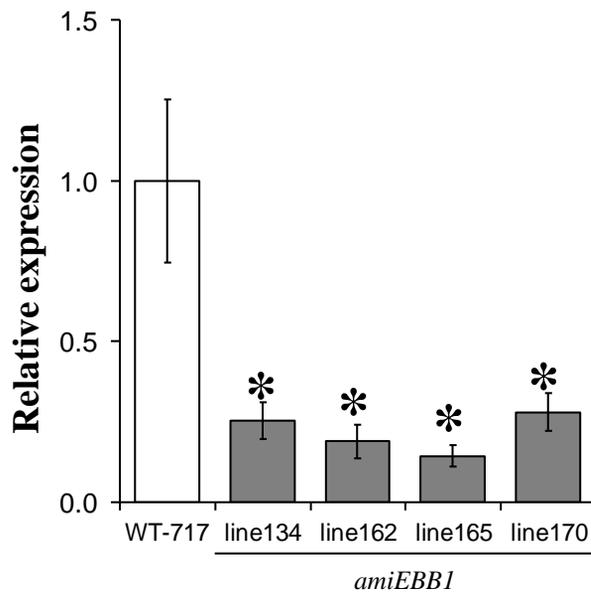


Figure S6. Suppression of EBB1 in 4 independent amiEBB1 lines. Bars show one standard error over genotypes' means (n= 3). Significance of differences tested by Student t-test (* P<0.05).

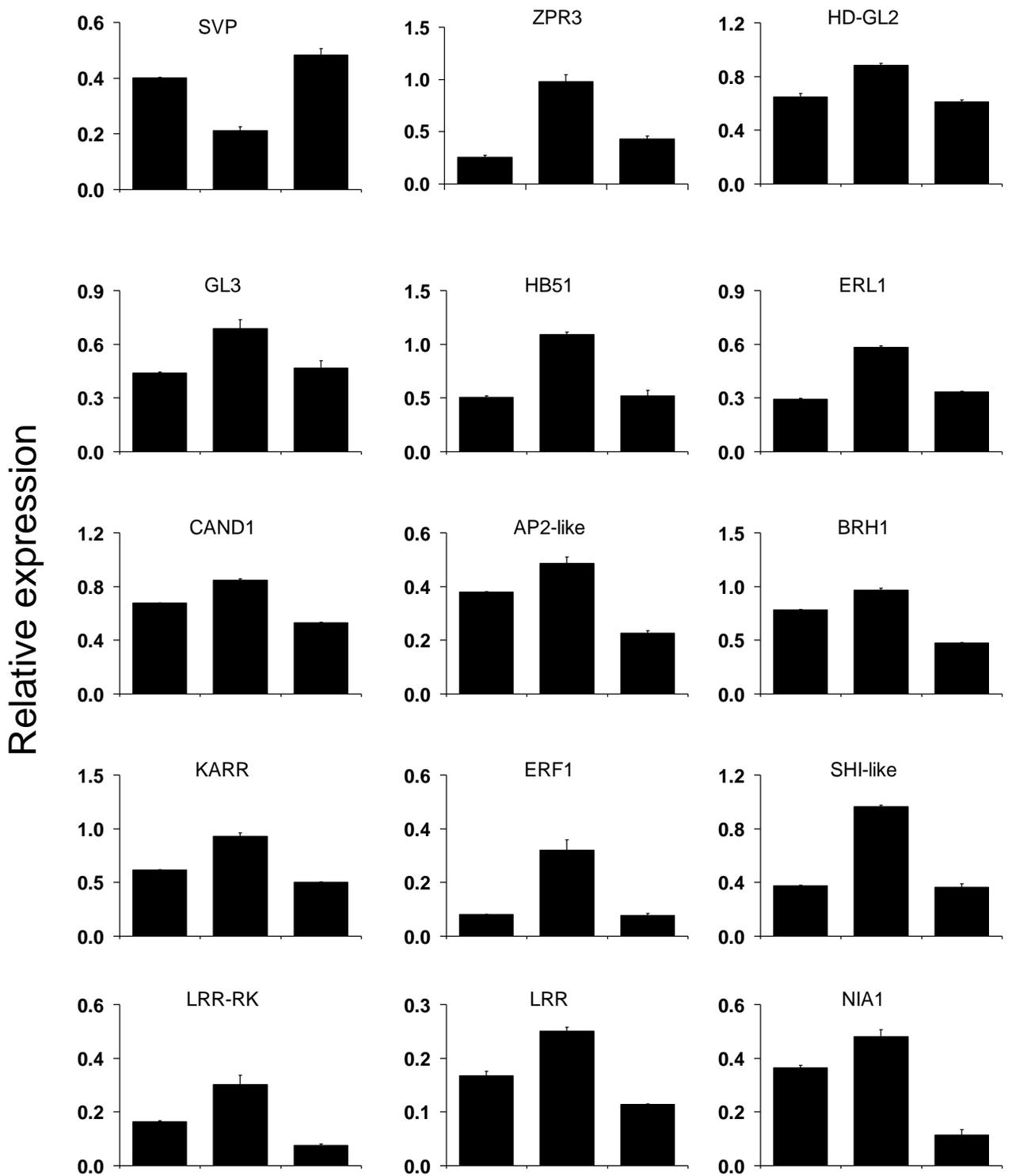


Figure S7. Validation of microarray results. Bars represent mean and standard errors over three independent biological replications. Abbreviations used in the figure correspond to the names and gene models as specified in Table S2. All expression estimates were normalized using ubiquitin gene expression as described above.