

(a) Sequence alignment of a native *VvCeb1* sequence to codon-optimized *VvCeb1* (*VvCeb1_{opt}*) for ectopic overexpression in *Arabidopsis*. Green shading or dots in codon-optimized *VvCeb1* (lower line) indicate the modified or unmodified nucleotides relative native *VvCeb1*, respectively.

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Native VvCeb1  ATG GCA GCC TTT TCT CAG CAG TCT CAC CAC CT CAC CC CAC AAA AAT TTA AGG CTG GAC TCG ACC ATT GTG CCA ATG ATG TCT GCA GT 90
Translation +1 M A A F S Q Q S H H L H P H K N L R L D S T I V P S M S A V 90
Codon optimized VvCeb1
Translation +1 M A A F S Q Q S H H L H P H K N L R L D S T I V P S M S A V 90

Native VvCeb1  TTT GAT GAT GAG AAG AAA CC ACC ACC TC ATC TC TGT TTT TCT GAT GAT CCT GTG AAG AAG ATC AC CAT TGT TCA TC ATG GGG GCT 180
Translation +1 F D D E K K P T T S I S C F S D D P V K K I T H C S S M G A 180
Codon optimized VvCeb1
Translation +1 F D D E K K P T T S I S C F S D D P V K K I T H C S S M G A 180

Native VvCeb1  GAG CTT GGG GCT CCA GGG ATG GC AGG AAG AGA AAG AAG GCT GAT TTT GAG GAA GAG AGG GAC GTG GAG GAG AAG AAG GGT AAA GCT GAG 270
Translation +1 E L G A P G M A R K R K K A D F E E E R D V E E K K G K A E 270
Codon optimized VvCeb1
Translation +1 E L G A P G M A R K R K K A D F E E E R D V E E K K G K A E 270

Native VvCeb1  AAG AAG AGG AAG AAG AAA GTG GTG AAA GAG GTC CCA AGT GGG TTT GTT CAT GTG AGA GCA AGG AGG GGT GAA GCA ACA GAC AGT CAC GGT 360
Translation +1 K K R K K K V V K E V P S G F V H V R A R R G E A T D S H S 360
Codon optimized VvCeb1
Translation +1 K K R K K K V V K E V P S G F V H V R A R R G E A T D S H S 360

Native VvCeb1  CTT GCT GAA AGG GCA AGA GAG AAA ATC AGT GAA AGA ATG AA CTT TTG CAA TCA CTT GTT CCT GGT TGT GAC AA CT ATT GG AAG 450
Translation +1 L A E R A R R E K I S E R M K L L Q S L V P G C D K L I G K 450
Codon optimized VvCeb1
Translation +1 L A E R A R R E K I S E R M K L L Q S L V P G C D K L I G K 450

Native VvCeb1  ACT CT GT CT GAT GAG ATA ATC AAT TAT GTC AAG TC CT CAG AAT CAA GT GAG TTC CTT GTG GG AA CT TCT TCT ATA AG CCT 540
Translation +1 T L V L D E I I N Y V K S L Q N Q V E F L V G K L A S I S P 540
Codon optimized VvCeb1
Translation +1 T L V L D E I I N Y V K S L Q N Q V E F L V G K L A S I S P 540

Native VvCeb1  ATG CT ATT GGA CAT GAA GCA AAN L D S S T L Q S E N L C S F G P P L P S L L 630
Translation +1 M L I G H E A N L D S S T L Q S E N L C S F G P P L P S L L 630
Codon optimized VvCeb1
Translation +1 M L I G H E A N L D S S T L Q S E N L C S F G P P L P S L L 630

Native VvCeb1  GCA TGC AAC TCT ACC CAG CTT AAT TCT TAT GCA GAA ACA TCA CT ACT TCT TCA TTT TCA CT CAA CAG GAC CAC CT TCT AGT GTT GTT 720
Translation +1 A C N S T Q L N S Y A E T S L T S S F S L Q Q Q D H L S S V V 720
Codon optimized VvCeb1
Translation +1 A C N S T Q L N S Y A E T S L T S S F S L Q Q Q D H L S S V V 720

Native VvCeb1  TCT CAG AAT GAT GGG ATC ATC TT TGG GAC ATG GAT GAT CAA GAA CAG CT CTT CTT GAT CAG TAT GGT TTC AG AAG AGA TAC TCT TTT 810
Translation +1 S Q N D G I I L W D M D D Q E Q S L L D Q Y G F S N R Y S F 810
Codon optimized VvCeb1
Translation +1 S Q N D G I I L W D M D D Q E Q S L L D Q Y G F S N R Y S F 810

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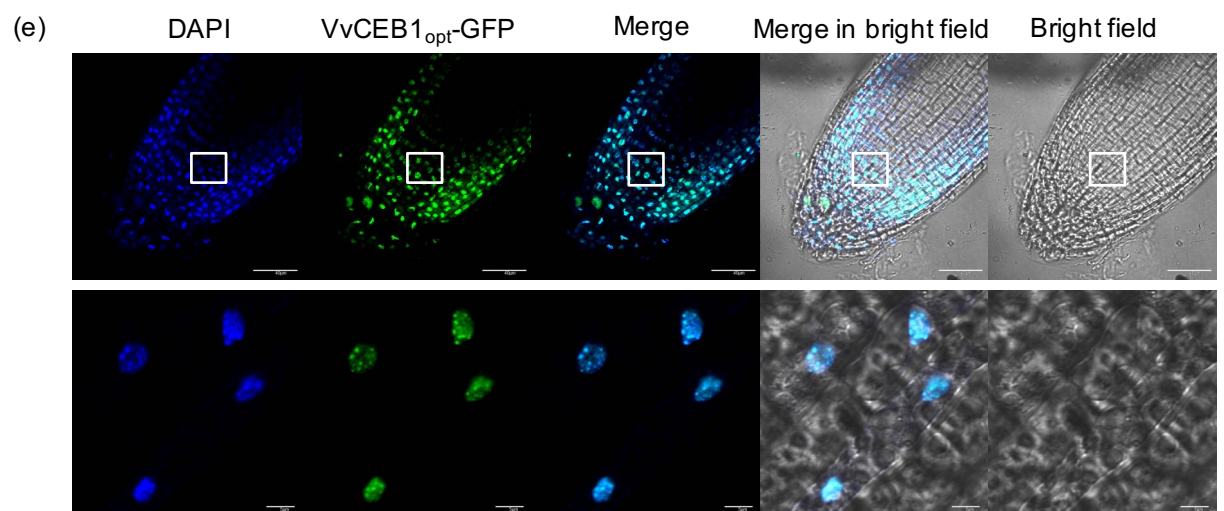
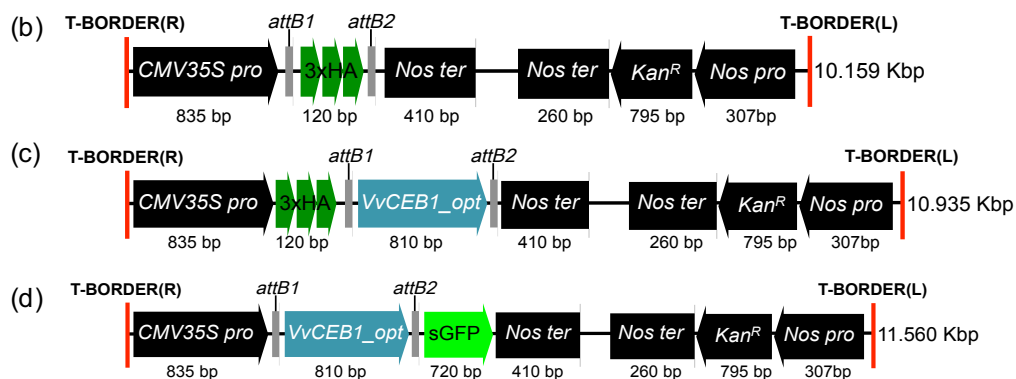


Figure S1. Subcellular localization of codon-optimized *VvCeb1*-*sGFP* fusion protein in *A. thaliana*. (a) Sequence alignment of a native *VvCeb1* sequence to codon-optimized *VvCeb1* (*VvCeb1_{opt}*) for ectopic overexpression in *Arabidopsis*. Green shading or dots in codon-optimized *VvCeb1* (lower line) indicate the modified or unmodified nucleotides relative native *VvCeb1*, respectively. Schematic representation of binary vector constructs used in this study. (b) The codon-optimized sequence of *VvCeb1* was synthesized and cloned into the ImpGWB415 vector containing the CaMV 35S promoter for transformation of *Arabidopsis*. (c) The synthesized 3xHA tag was cloned into the ImpGWB402 vector and

transformed into *Arabidopsis* for the 35S::3xHA empty-vector control. (d) The VvCeb1_{opt} fragment was cloned into the ImpGWB405 vector containing the CaMV 35S promoter and C-terminal synthetic green fluorescent protein (sGFP) to study subcellular localization. Kanamycin (Kan^R) was used as the selectable marker. T-border (R) and T-border (L) indicate T-DNA right border and T-DNA left borders, respectively. (e) Nuclear localization of the VvCEB1_{opt}-sGFP fusion protein. The 35S::VvCEB1_{opt}-sGFP construct was transformed into *Arabidopsis*. Seven-day-old seedlings (T₁) were used to analyze subcellular localization. Images in the lower panel correspond to magnification of the regions indicated by the white squares in the upper panel. Scale bars, 40 μm (top panels) and 5 μm (bottom panels).

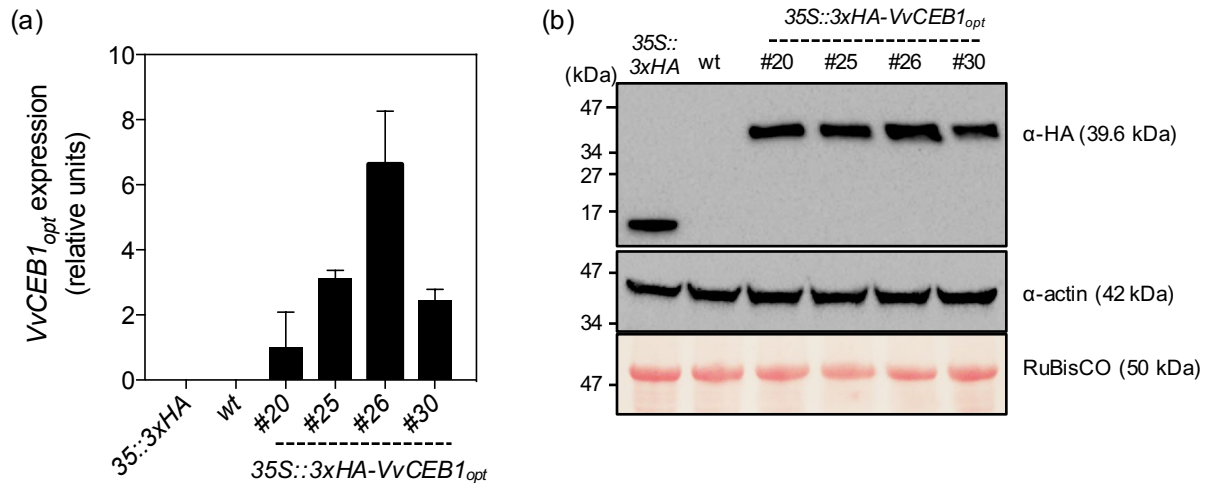


Figure S2. Characterization of the wild-type, 35S::3xHA empty-vector control line and *VvCEB1_{opt}*-overexpressing *Arabidopsis* lines. (a) Quantitative real-time PCR analysis of *VvCEB1_{opt}* transcript abundance in 35S::3xHA empty-vector control lines, wild-type *A. thaliana* ecotype Col-0, and four independent *VvCEB1_{opt}*-overexpressing lines (#20, #25, #26, and #30). Transcript levels of *VvCEB1_{opt}* in four different lines were quantified using *TIP41-like* (AT4G34270) expression as a normalization standard. Values represent means \pm s.d. of three biological replicates. (b) Immunoblot analysis with anti-HA antibody (clone 3F10, Roche Applied Science, Indianapolis, IN, USA) performed to measure protein abundance within the 3xHA-*VvCEB1_{opt}* transgenic lines. Immunodetection of actin and Ponceau S staining of RuBisCO were used as loading controls.

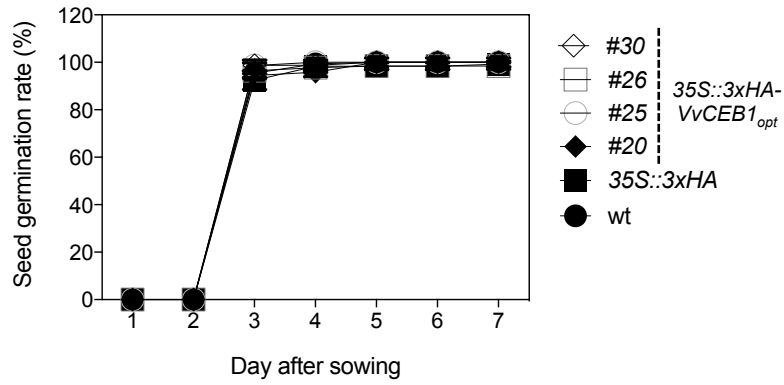


Figure S3. Seed germination rates of wild-type, 35S::3xHA empty vector control, and VvCEB1_{opt}-overexpressing *Arabidopsis* lines. Seeds were germinated and grown on MS agar medium for 7 days under a 16-h photoperiod. Seed germination rates of four independent VvCEB1_{opt}-overexpressing lines (#20, #25, #26, and #30), wild-type (wt) *A. thaliana* ecotype Col-0, and the 35S::3xHA empty-vector control line were scored for 7 days ($n = 3$).

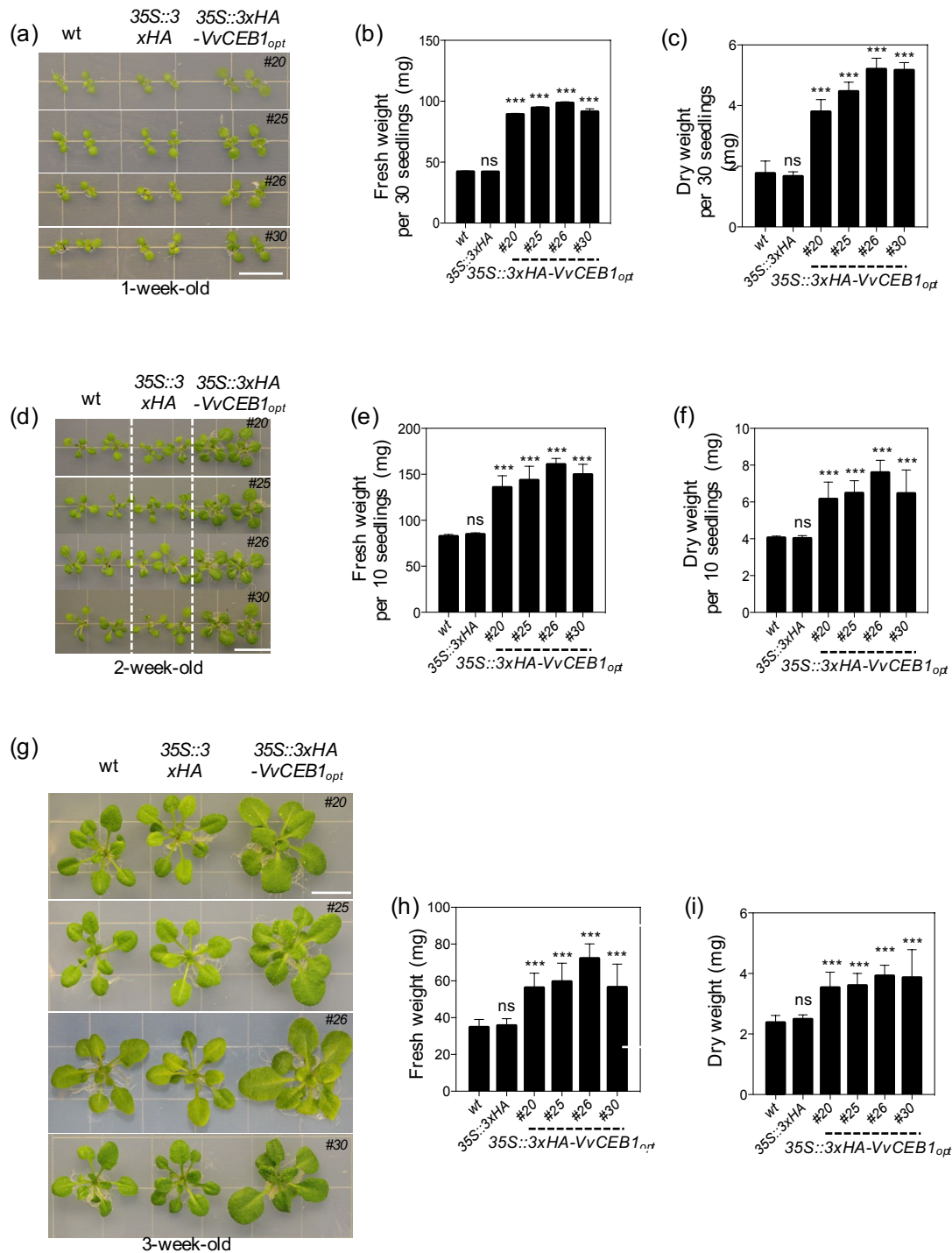


Figure S4. VvCEB1_{opt} overexpression in *Arabidopsis* increases shoot biomass. Seeds were germinated and grown on MS agar medium for 7 days for quantification of the seedling biomass. (a) Representative images of one-week-old seedlings of VvCEB1_{opt}⁻ overexpressing lines, and the Col-0 wild type, and the 35S::3xHA empty-vector control lines. Scale bar, 1 cm. (b) Quantification of fresh weight of 30 seedlings (*n* = 3 replicates). (c) Dry weight of 30 seedlings (*n* = 3 replicates). Seeds were germinated and grown on MS agar medium for 2

weeks for quantification of shoot biomass. (d) Representative images of two-week-old seedlings of four independent *VvCEB1_{opt}*-overexpressing lines, Col-0 wild type, and the *35S::3xHA* empty-vector control line. Scale bar, 1 cm. (e) Quantification of fresh weight of 10 seedlings ($n = 3$ replicates). (f) Dry weight of 10 seedlings ($n = 3$ replicates). Seeds were germinated and grown on MS agar medium for 3 weeks for quantification of plant biomass. (g) Representative images of three-week-old seedlings of four independent *VvCEB1_{opt}*-overexpressing lines, Col-0 wild type, and the *35S::3xHA* empty-vector control line. Scale bar, 1 cm. (h) Quantification of fresh weight per plant ($n = 10$). (i) Dry weight of three-week-old seedlings ($n = 10$). Values represent means \pm s.d., ns = non-significant, *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

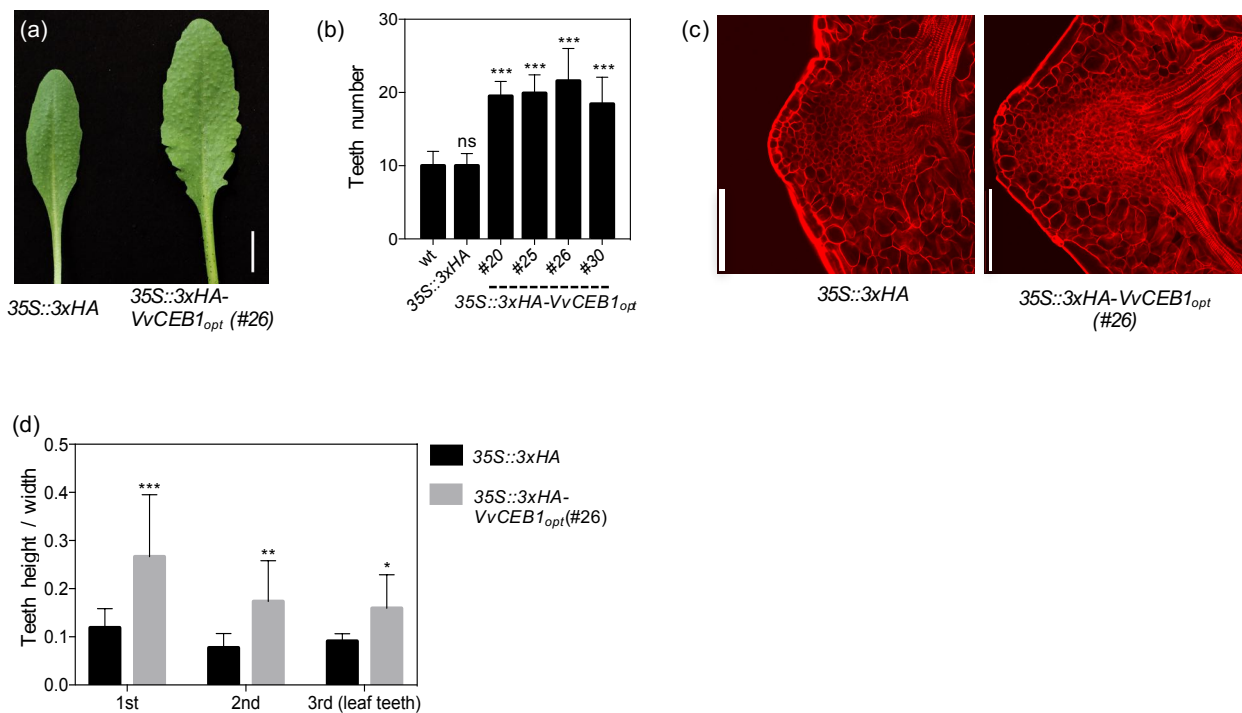


Figure S5. VvCEB1_{opt}-overexpressing *Arabidopsis* plants exhibit increased leaf teeth number and height along the leaf margin. Seeds of Col-0 wild-type (wt), the 35S::3xHA empty-vector control, and VvCEB1_{opt}-overexpressing lines (#20, #25, #26, and #30), were germinated and grown in soil mix for 4 weeks under a 12-h photoperiod. (a) Representative images of fifth leaves of VvCEB1_{opt}-overexpressing line and the 35S::3xHA empty-vector control line. Scale bar, 1 cm. (b) Quantification of teeth number per leaf ($n = 12$) (c) Confocal laser scanning images of 1st leaf teeth from petiole. Fifth leaves of four-week-old plants were stained with propidium iodide (PI). Scale bar, 100 μm. (d) Quantification of leaf teeth height/length ratio ($n = 14$). Values represents means \pm s.d., ns = non-significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

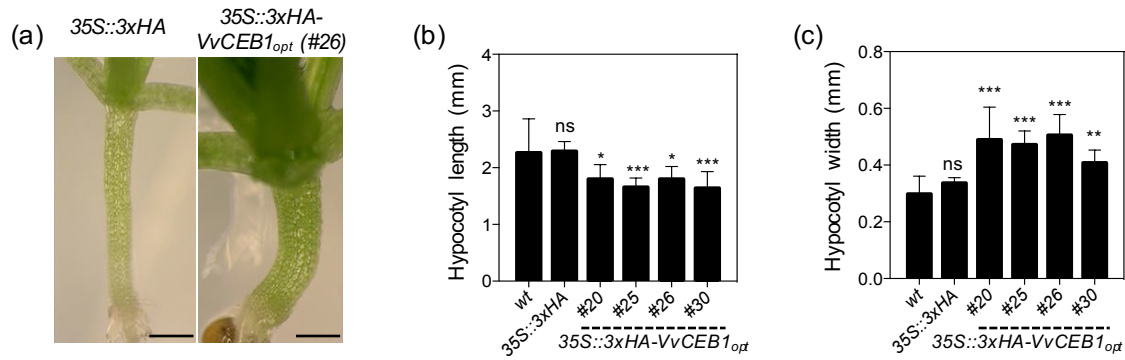


Figure S6. *VvCEB1_{opt}* overexpressing *Arabidopsis* plants exhibit hypocotyls with decreased length and increased width. Seeds of Col-0 wild-type, the 35S::3xHA empty-vector control line, and four *VvCEB1_{opt}*-overexpressing lines were germinated and grown vertically on half-strength Murashige and Skoog (MS) agar medium for 14 days under a 16-h photoperiod. (a) Images of hypocotyls from *VvCEB1_{opt}*-overexpressing line (#26) and the 35S::3xHA empty-vector control. Scale bar, 0.5 mm. (b) Comparison of hypocotyl length ($n = 10$). (c) Hypocotyl width ($n = 10$). Values represent means \pm s.d., ns = non-significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

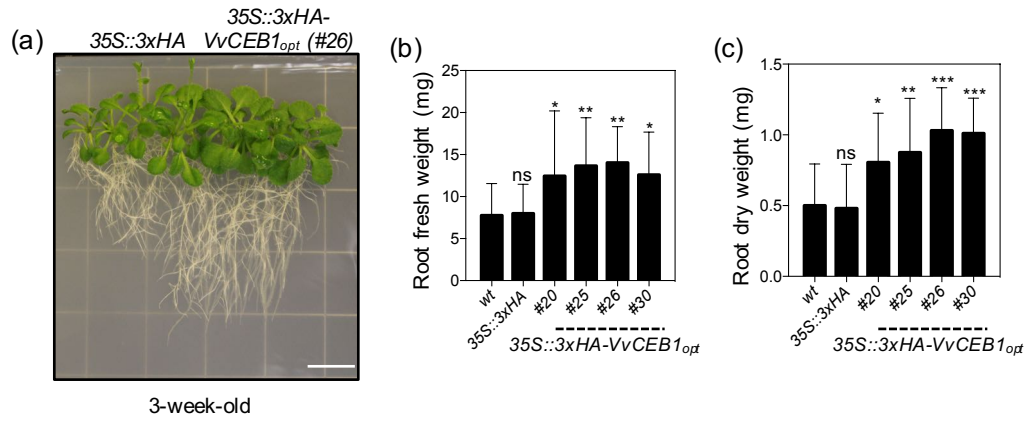


Figure S7. *VvCEB1_{opt}*-overexpressing *Arabidopsis* plants exhibit increased root biomass. Seedlings were grown vertically on half-strength MS medium for 3 weeks under a 16-h photoperiod (16 hours of light per day). (a) Representative images of root biomass of *35S::3xHA-VvCEB1_{opt}* line (#26), and the *35S::3xHA* empty-vector control line. Scale bar, 1 cm. (b) Quantification of root fresh weight of three-week-old seedlings ($n = 20$). (c) Root dry weight of three-week-old seedlings ($n = 20$). Values represent means \pm s.d., ns = non-significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

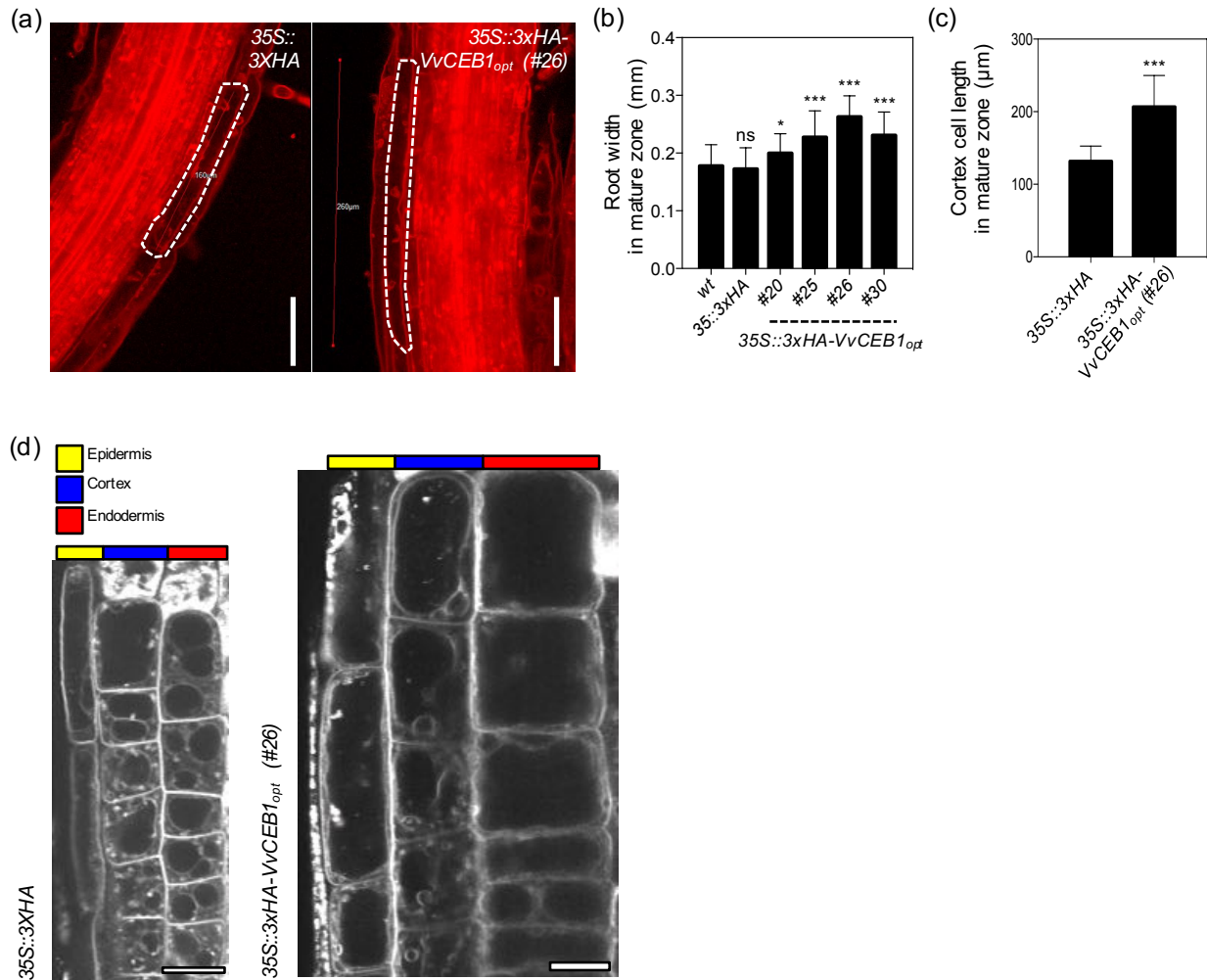


Figure S8. VvCEB1_{opt}-overexpression in *Arabidopsis* plants increases root cell size.

Seeds of Col-0 wild-type (wt), the 35S::3xHA empty-vector control line, and the four VvCEB1_{opt}-overexpressing lines, were germinated and grown vertically on half-strength MS agar medium for 14 days under a 16-h photoperiod (16 hours of light per day). (a) Confocal laser scanning images of root cells stained with FM4-64 (2 h) and PI in mature zone. White broken lines indicate the outlines of representative cortical cells. Scale bar, 60 μ m. (b) Quantification of primary root width in the maturation zone ($n = 30$). (c) Quantification of cortical root cell length in mature zone ($n = 60$). (d) Images of cells and vacuoles in basal meristem zone stained with FM4-64 (5 h) captured by confocal laser scanning microscopy. Yellow, blue, and red lines indicate the epidermis, cortex, and endodermis, respectively. Scale bar, 10 μ m. Values represent means \pm s.d., ns = non-significant, * $p < 0.05$, and *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test (b) and Student's t-test (c).

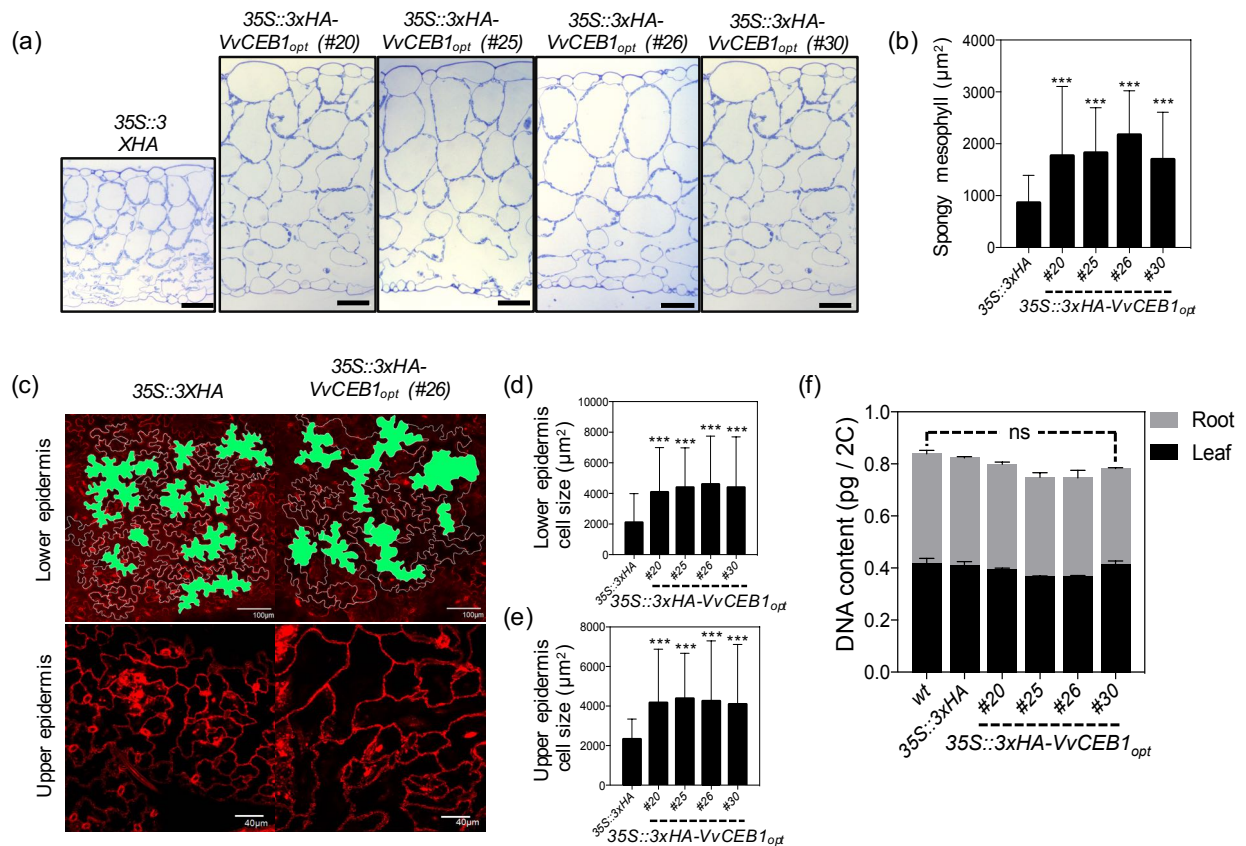


Figure S9. *VvCEB1_{opt}*-overexpressing *Arabidopsis* plants exhibit increased leaf thickness and cell size, but lack increased ploidy. Seeds of the *35S::3xHA* empty-vector control and the *VvCEB1_{opt}*-overexpressing lines (#20, #25, #26, and #30) were germinated and grown in soil mix for 4 weeks under a 12-h photoperiod. (a) Transverse sections of leaves from four *VvCEB1_{opt}*-overexpressing lines and the *35S::3xHA* empty-vector control line stained with toluidine blue O. Scale bar, 100 μm . (b) Quantification of the size of spongy mesophyll cells ($n = 350$). (c) Representative images of tangential leaf sections from *VvCEB1_{opt}*-overexpressing line (#26) and the *35S::3xHA* empty-vector control. Upper and lower epidermis cell layers of four-week-old plants were stained with propidium iodide (PI). Images were captured using laser scanning confocal microscopy. Green shading indicates representative areas of individual cells of the lower epidermis. Scale bars, 100 μm (upper panels) and 40 μm (lower panels). (d) Quantification of the size of cells in the lower epidermis ($n = 90$). (e) Quantification of the size of cells in the upper epidermis ($n = 50$). (f) Roots and leaves of three-week-old *VvCEB1_{opt}*-overexpressing lines, Col-0 wild-type, and the *35S::3xHA* empty-vector control line were separately sampled and DNA contents were analyzed by flow cytometry ($n = 3$ replicates). Values represents means \pm s.d., ns = non-significant, $**p < 0.01$, and $***p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

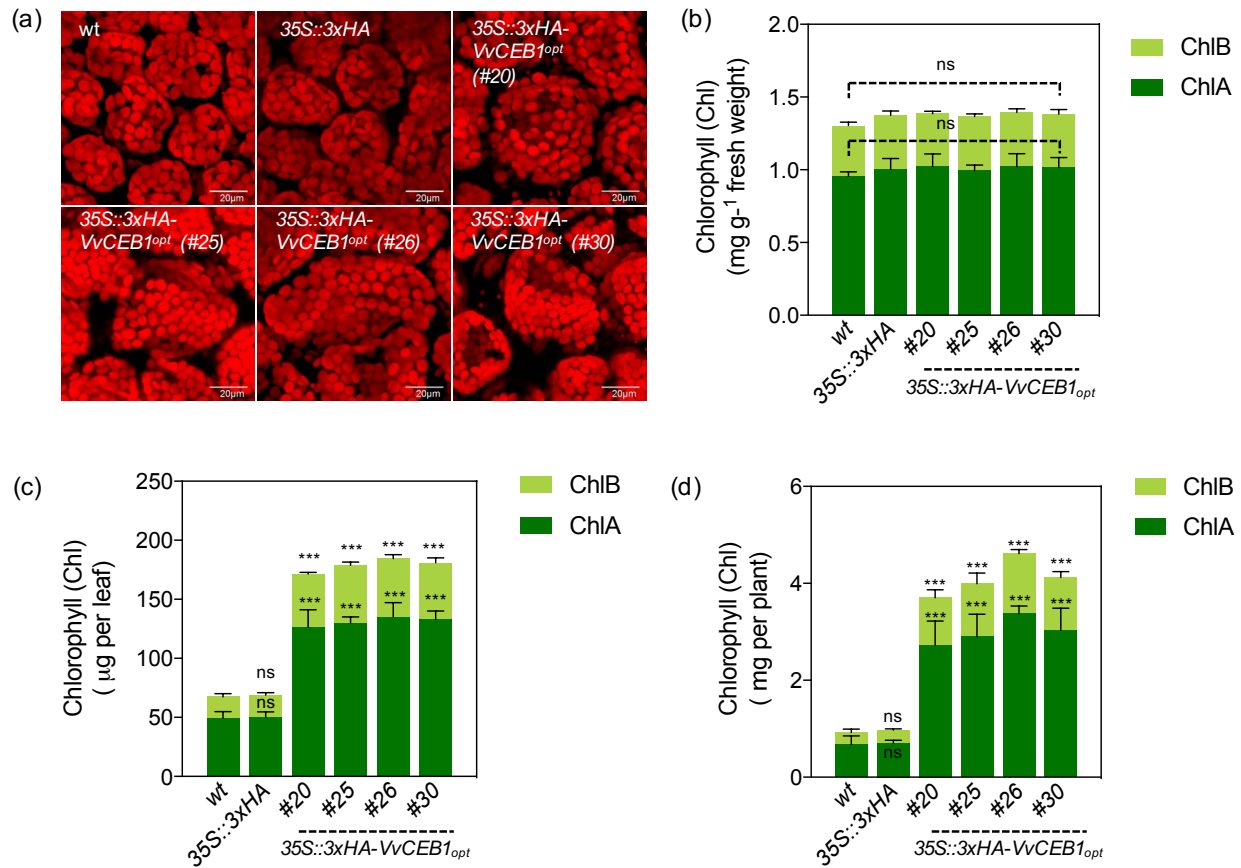


Figure S10. *VvCEB1*_{opt}-overexpressing *Arabidopsis* plants have increased chlorophyll contents on a per leaf or per plant basis. Seeds of wild-type *A. thaliana* ecotype Col-0, the 35S::3xHA empty-vector control line, and the four *VvCEB1*_{opt}-overexpressing lines were germinated and grown in soil mix for 4 weeks under a 12-h photoperiod (12 hours of light per day). (a) Confocal laser scanning images of palisade mesophyll cells and chloroplasts of *VvCEB1*_{opt}-overexpressing lines and control lines. Chlorophyll auto-fluorescence in the leaf palisade mesophyll cells is shown in red. The 3D-projection was generated by combining a Z-stack of images. Scale bar, 20 μm. Fifth fully expanded true leaves were sampled and measured for chlorophyll contents ($n = 4$ replicates). (b) Chlorophyll contents per gram fresh weight. (c) Chlorophyll contents per leaf. (d) Chlorophyll contents per plant. Values represent means \pm s.d., ns = non-significant, *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

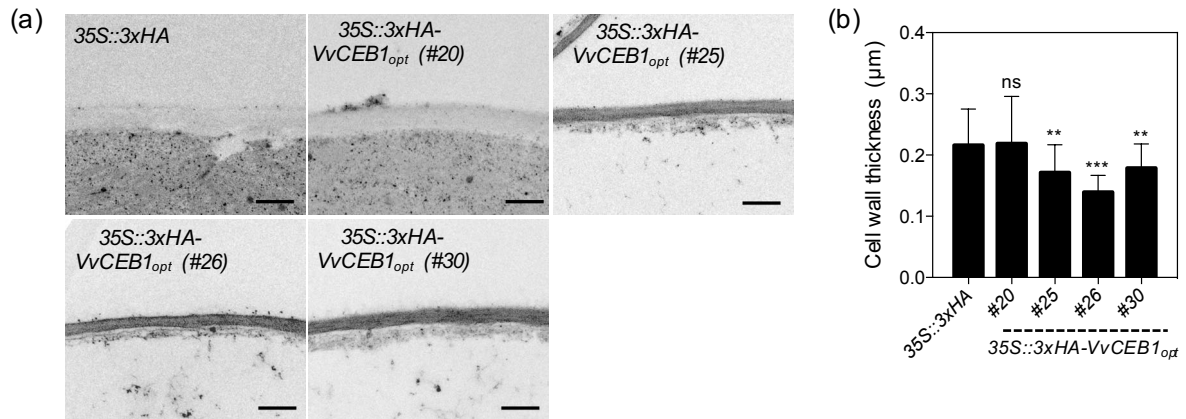


Figure S11. *VvCEB1_{opt}*-overexpressing *Arabidopsis* plants exhibit decreased cell wall thickness. (a) Representative cell wall images of the *35S::3xHA* empty-vector control line and the four *VvCEB1_{opt}*-overexpressing lines and the from four-week-old, soil mix-grown plants grown under a 12-h photoperiod. Images were captured by transmission electron microscopy. Scale bar, 0.5 μm . (b) Quantification of cell wall thickness ($n = 45$). Values represents means \pm s.d., ns = non-significant, ** $p < 0.01$, and *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

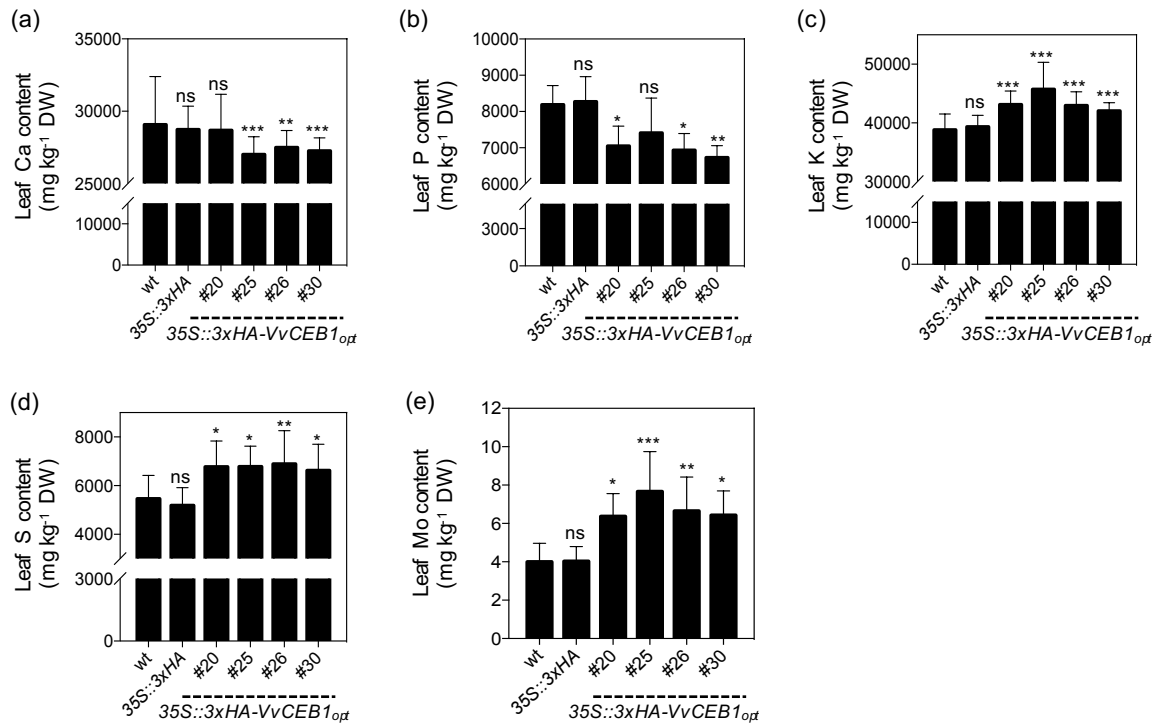


Figure S12. *VvCEB1*_{opt} overexpression alters the concentration of Ca, K, P, S, and Mo in *Arabidopsis* leaves. Determine the concentrations of inorganic ions in wild-type plants, 35S::3xHA empty-vector control line, and *VvCEB1*_{opt}-overexpressing lines. (a) Calcium, (b) Potassium, (c) Phosphorus, (d) Sulfur, and (e) Molybdenum content on a dry weight basis ($n = 6$). Values represent means \pm s.d., ns = non-significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, one-way ANOVA with Dunnett's multiple comparison test.

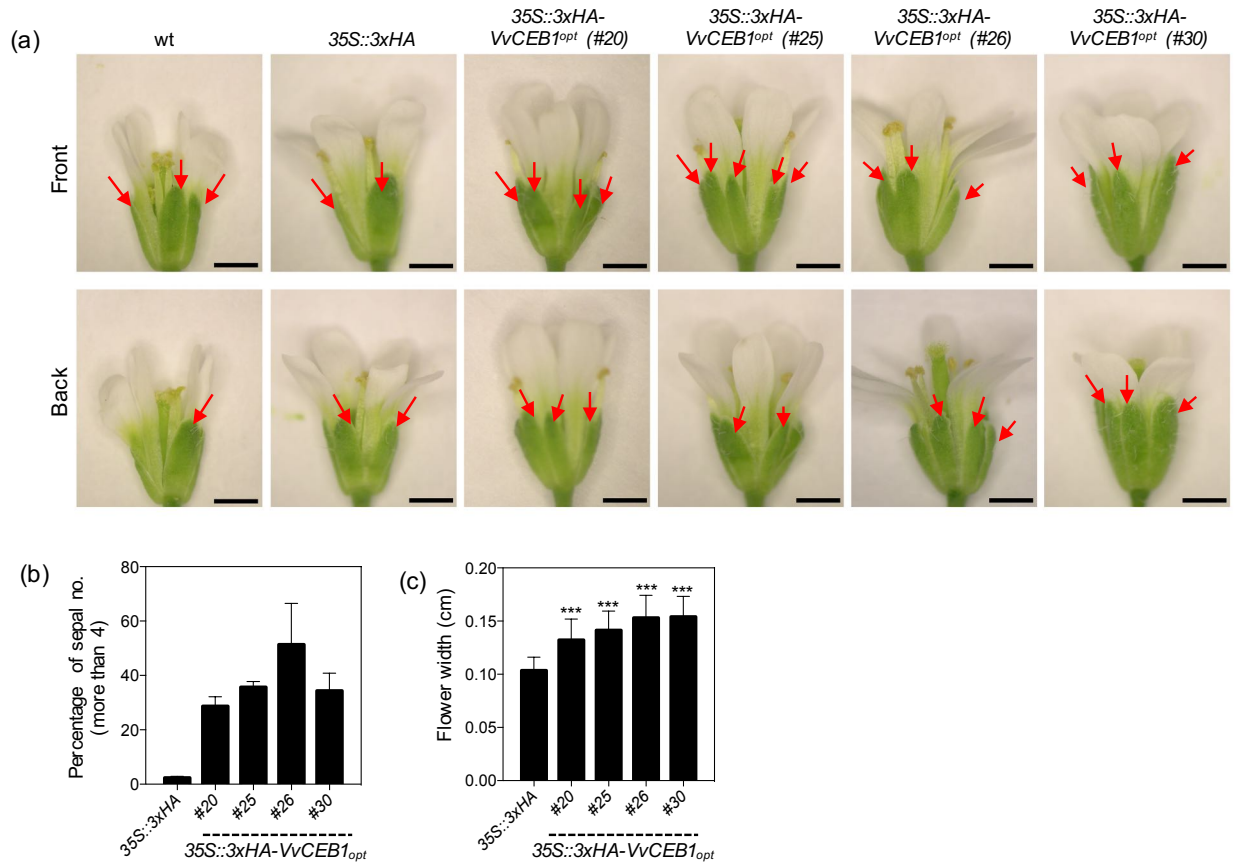


Figure S13. *VvCEB1_{opt}*-overexpressing *Arabidopsis* plants increase sepal number in flowers and flower width. (a) Representative flower images of Col-0 wild type (wt), the 35S::3xHA empty-vector control line, and the four *VvCEB1_{opt}*-overexpressing lines. Arrows indicate sepal number. Scale bar, 1 mm. (b) Quantification of percentage of flowers with sepal number greater than 4 ($n = 3$ replicates). (c) Quantification of flower width measured horizontally ($n = 63$). Values represent means \pm s.d., *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

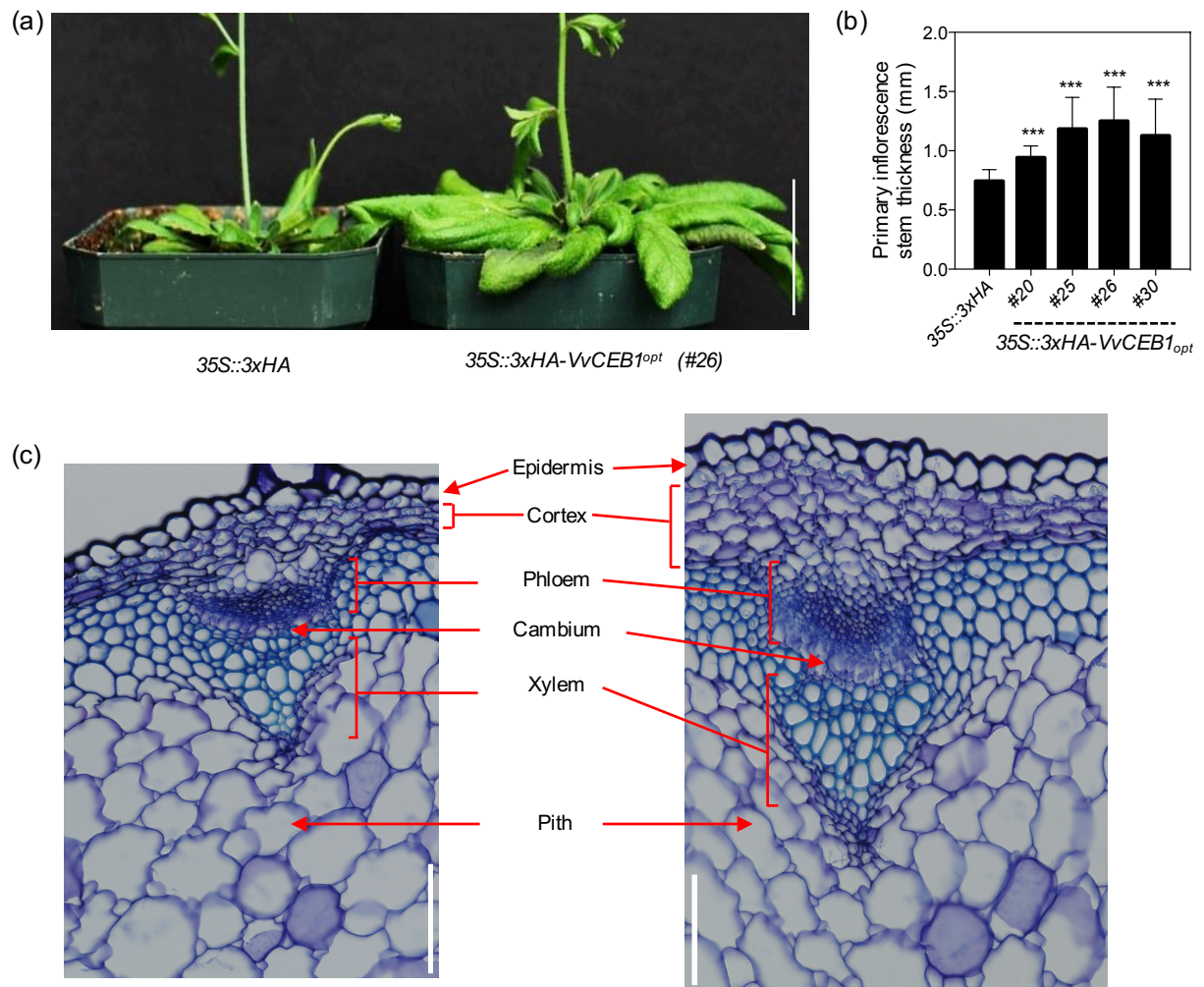


Figure S14. *VvCEB1*_{opt}-overexpressing *Arabidopsis* plants exhibit increased inflorescence stem thickness by increasing cell size. Seeds of 35S::3xHA empty-vector control line and the four *VvCEB1*_{opt}-overexpressing lines were germinated and grown in soil mix under a 12-h photoperiod (12 hours of light per day). (a) Representative images of the primary inflorescence stem of the 35S::3xHA empty-vector control line and the *VvCEB1*_{opt}-overexpressing line (#26) at 2 weeks after bolting. Scale bar, 5 cm. (b) Quantitation of the diameter of the primary inflorescence stem ($n = 30$). (c) Representative images of stem cross sections. Primary inflorescence stems were stained with toluidine blue O. Scale bar, 100 μ m. Values represent means \pm s.d., *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

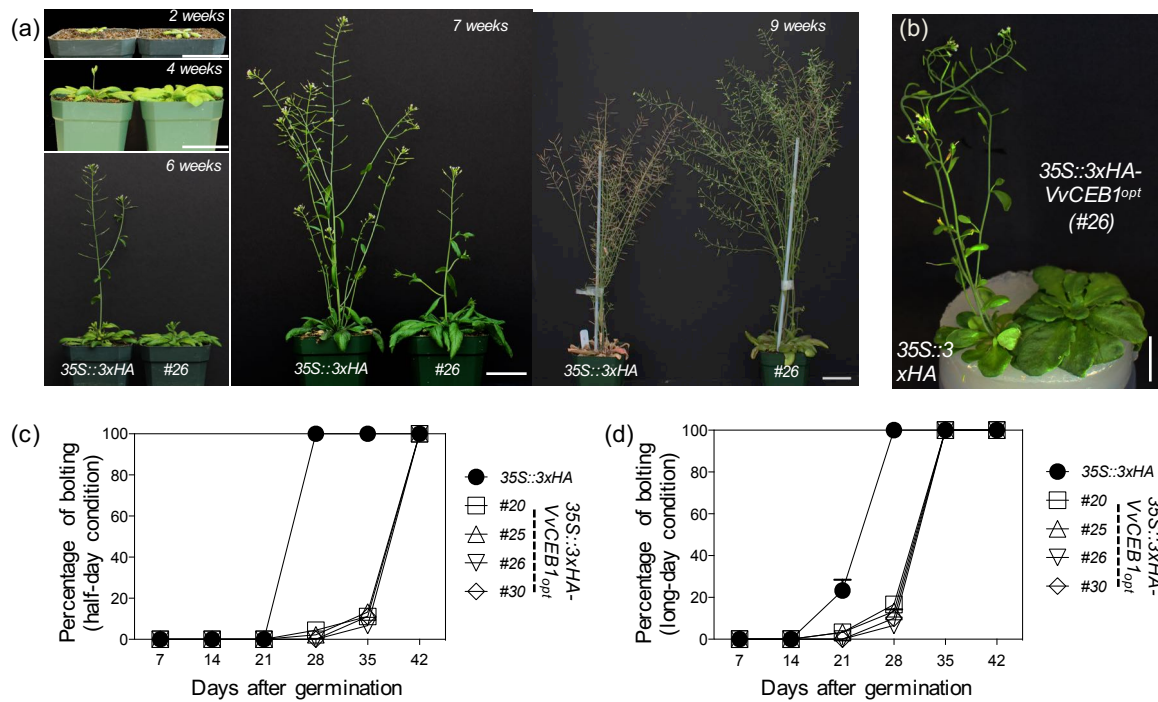


Figure S15. *VvCEB1_{opt}* overexpressing *Arabidopsis* plants exhibit delayed bolting under half-day and long-day conditions. (a) Representative images of delayed flowering and leaf senescence of the *35S::3xHA* empty-vector control line and the *VvCEB1_{opt}*-overexpressing line (#26) under half-day condition. Seeds of *35S::3xHA* (empty-vector control) line and the four independent *35S::3xHA-VvCEB1_{opt}* lines (#20, #25, #26, and #30) were germinated and grown in soil for nine weeks under a 12-h photoperiod. Scale bar, 5 cm. (b) Representative images of delayed flowering of the *35S::3xHA* empty-vector control line and the *VvCEB1_{opt}*-overexpressing line (#26) under long-day condition. Seeds of the *35S::3xHA* empty-vector control line and the four independent *VvCEB1_{opt}*-overexpressing lines were germinated and grown on MS agar medium under a 16-h photoperiod. Scale bar, 5 cm. Quantification of flowering time under (c) half-day and (d) long-day conditions ($n = 3$ replicates).

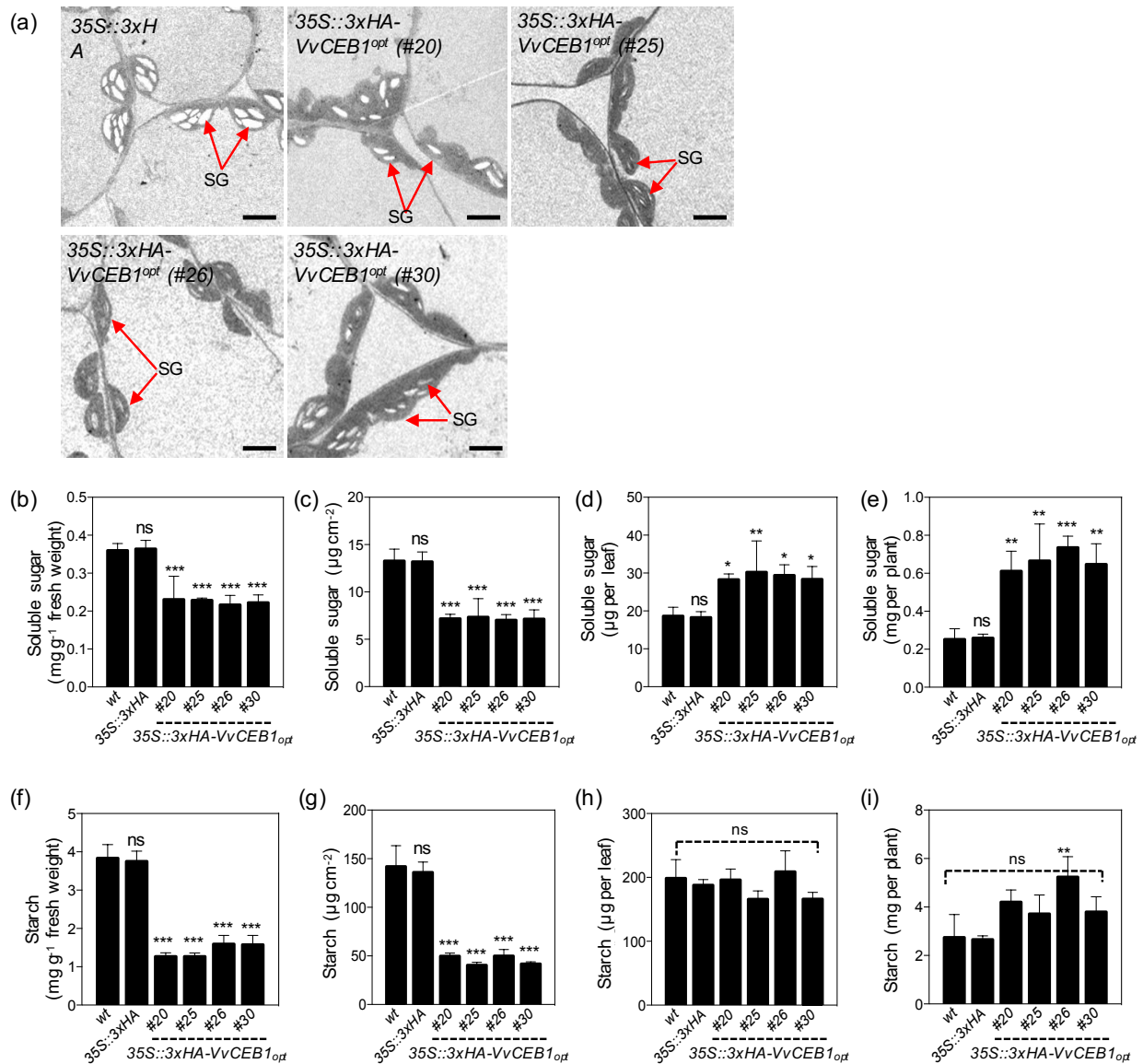


Figure S16. *VvCEB1*_{opt}-overexpressing *Arabidopsis* plants have increased soluble sugar and decreased starch contents. Seeds of wild-type (wt) *A. thaliana* ecotype Col-0, the 35S::3xHA empty-vector control line, and the four *VvCEB1*_{opt}-overexpressing lines were germinated and grown in soilless growth medium for 4 weeks under a 12-h photoperiod. Fifth fully expanded true leaves were sampled at noon for carbohydrate assays. (a) Representative images of chloroplasts in *VvCEB1*_{opt}-overexpressing lines, Col-0 wild type, and 35S::3xHA empty-vector control lines. Images were captured by transmission electron microscopy. Arrows indicate starch granules (SG). Scale bar, 5 μm. Soluble sugar contents in leaves ($n = 4$ replicates). (b) Soluble sugar contents per gram fresh weight. (c) Soluble sugar contents per leaf area (cm²). (d) Soluble sugar contents per leaf. (e) Soluble sugar contents per plant. Starch contents in leaves ($n = 4$ replicates). (f) Starch contents per gram fresh weight. (g) Starch contents per unit leaf area (cm²). (h) Starch contents per leaf. (i) Starch contents per plant.

Values represent means \pm s.d., ns = non-significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, One-way ANOVA with Dunnett's multiple comparison test.

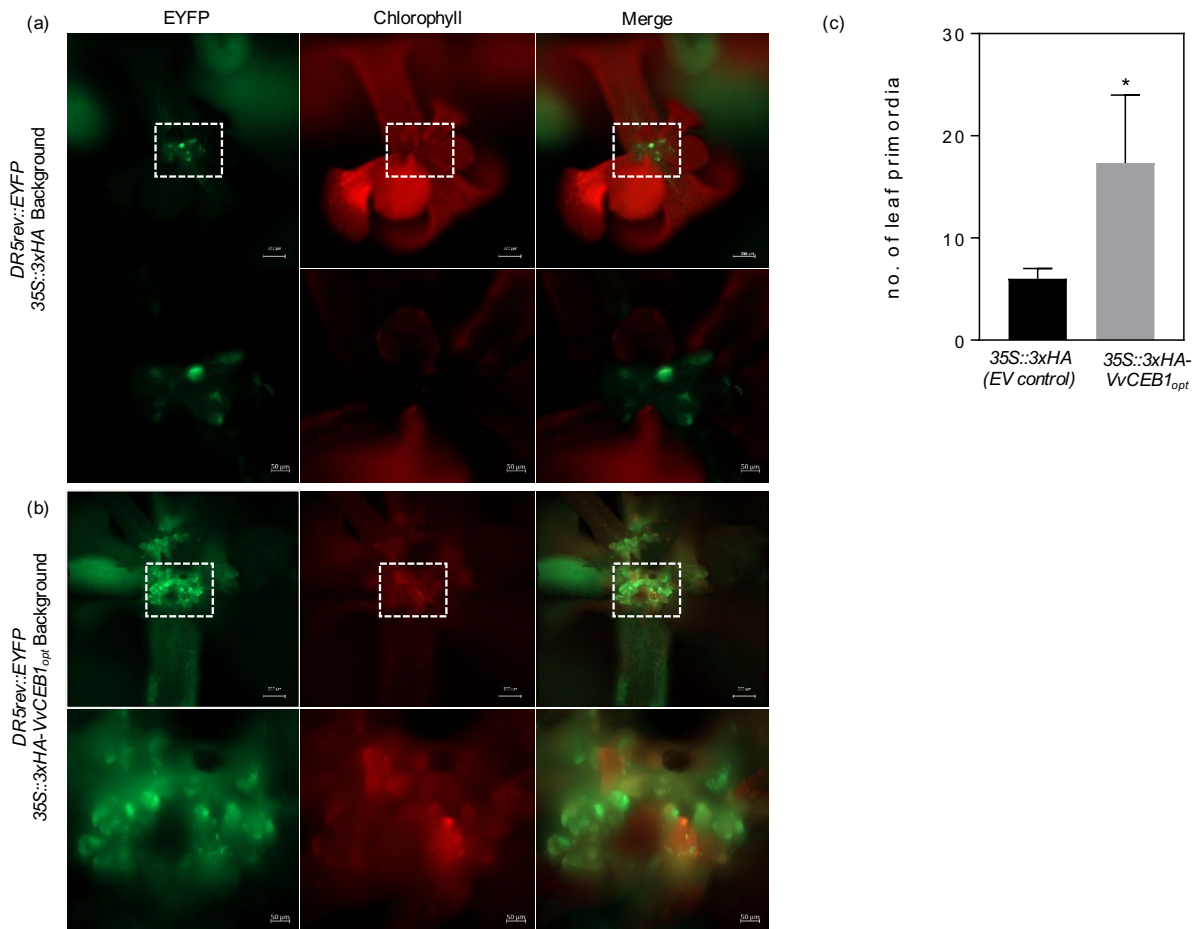


Figure S17. VvCEB1opt overexpression increases leaf primordia number in the shoot apical meristem (SAM) in Arabidopsis. (a), Expression of DR5rev::EYFP in the SAM region of empty vector (EV) control and (b) Ox-VvCEB1opt (#26) transgenic background lines (Size bars = 200 μ m or 50 μ m (insets)). (c) Quantification of leaf primordia number ($n = 3$). Values represents means \pm s.d. and * $p < 0.05$. Student's t-test.