The Zinc Finger Transcription Factor *SlZFP2* Negatively Regulates Abscisic Acid Biosynthesis and Fruit Ripening in Tomato

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SUPPLEMENTAL MATERIALS

Fig. S1-S11

Table S1-S5

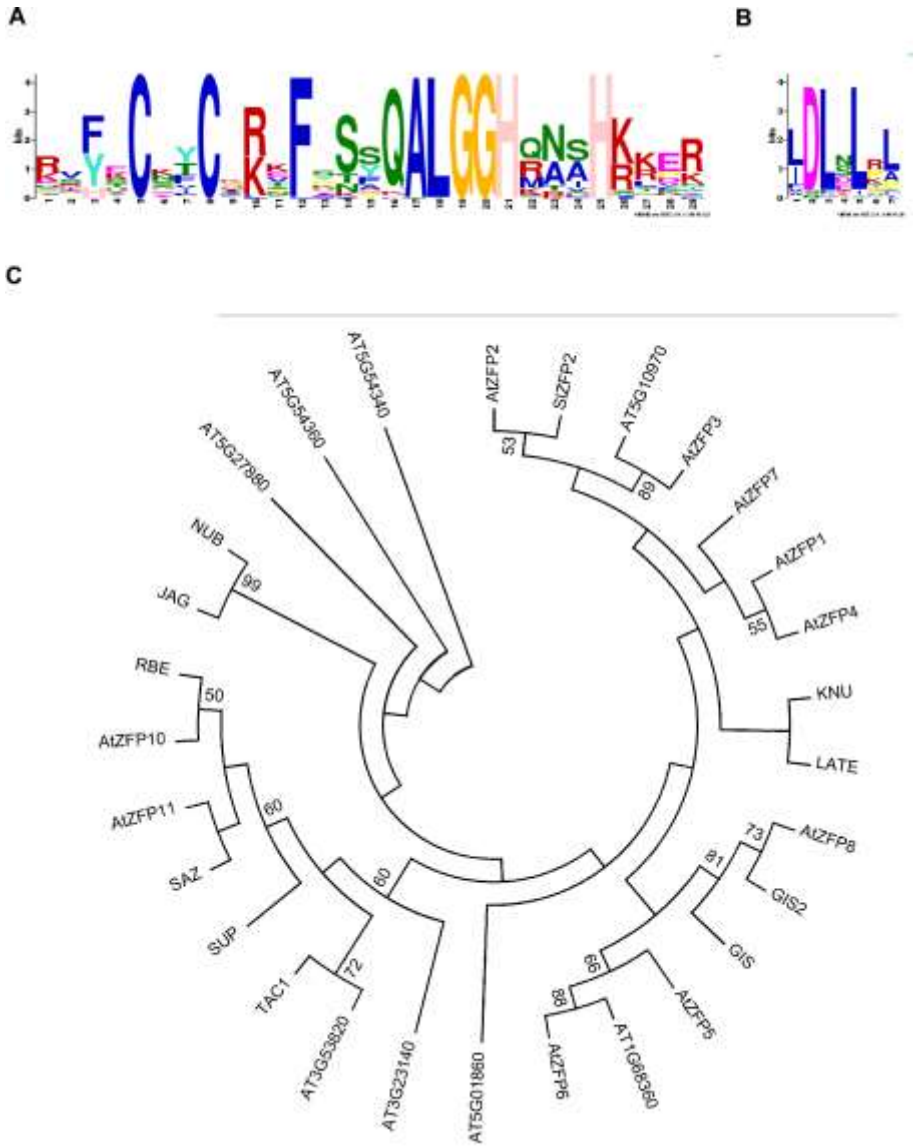

Fig. S1 ***SlZFP2*** **encodes a C_2H_2-type zinc finger protein mainly expressed in fruits**

SlZFP2 contains two conserved motifs -- C_2H_2 zinc finger and EAR-like -- predicted by MEME program (Bailey et al., 2006, Nucl. Acids Res. **34:** W369-W373). (**A**) Weblog of C_2H_2 zinc finger motif. (**B**) Weblog of EAR like motif 2. (**C**) Maximum parsimony tree of SlZFP2 and its homologs from Arabidopsis by MEGA5 (Tamura et al., 2011, Mol Biol Evol **28:** 2731-2739). The bootstrap consensus tree was inferred from 1000 replicates using MEGA5. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed.

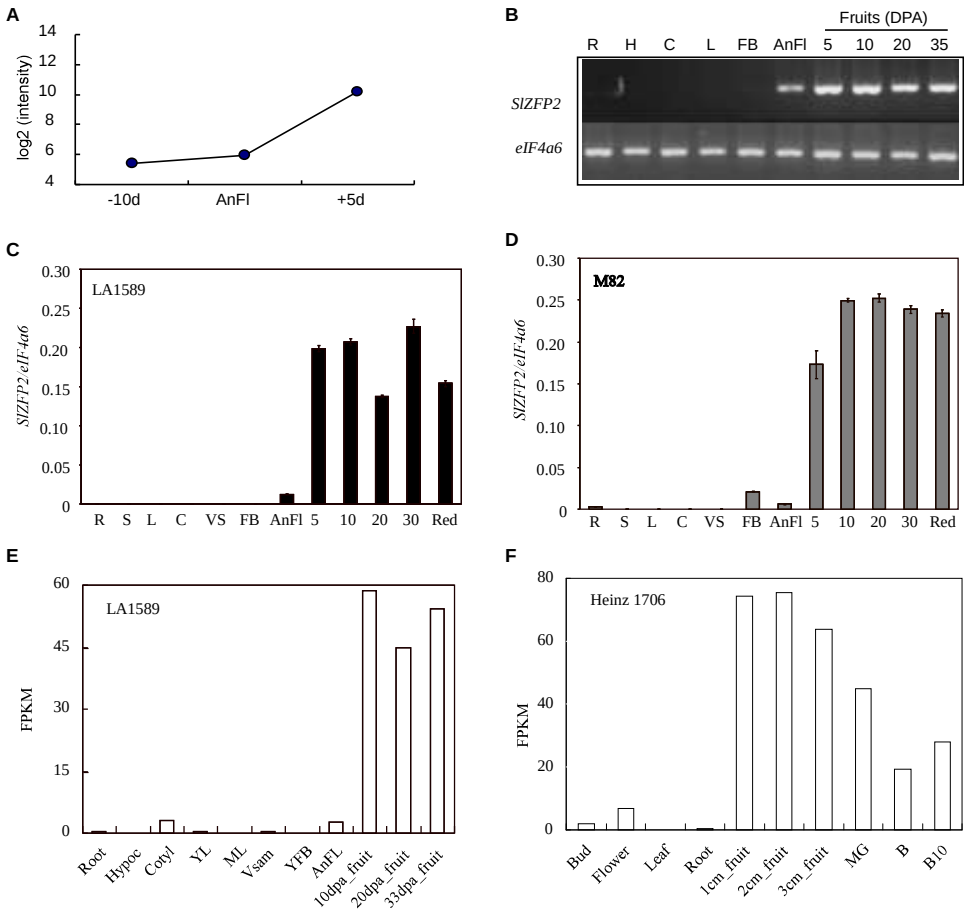

Fig. S2 *SlZFP2* expression in various tissues revealed by RT-PCR, qRT-PCR, microarray and RNA-seq

(**A**) Expression of *SlZFP2* in flowers and fruits of LA1589. The expression values (log2) were from dataset previously described (Xiao et al 2009, BMC Plant Biol. 9,49). (**B**) RT-PCR analysis of *SlZFP2* expression in different tissues of LA1589. (**C**) qRT-PCR analysis of *SlZFP2* expression in different tissues of LA1589. (**D**) qRT-PCR analysis of *SlZFP2* in various tissues of M82. (**E**) *SlZFP2* expression in different tissues of LA1589 determined by RNA-seq. (**F**) *SlZFP2* expression in different tissues of Heinz1706 determined by RNA-seq. Expression value in (**E**) and (**F**) were from public database Tomato Functional Genomics Database (http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/ home.cgi. D006 and D004, respectively). The vegetative tissues, and flowers and fruits (in **C** and **D**) were collected from 7-10 days-old seedlings of two months-old plants, respectively. -10d, flower bud at 10 days preanthesis; AnFl, anthesis flower; +5d, 5 DPA fruit. C/Cotyl, cotyledon; FB, unopened flower; H/Hypoct, hypocotol; L, leaf; YL, young leaf; ML, mature leaf; R, Root; S, stem; VS/Vsam, vegetative shoot apex, YFB, young flower buds. FPKM, number of fragments per kilobase of transcript sequence per millions base pairs sequenced.

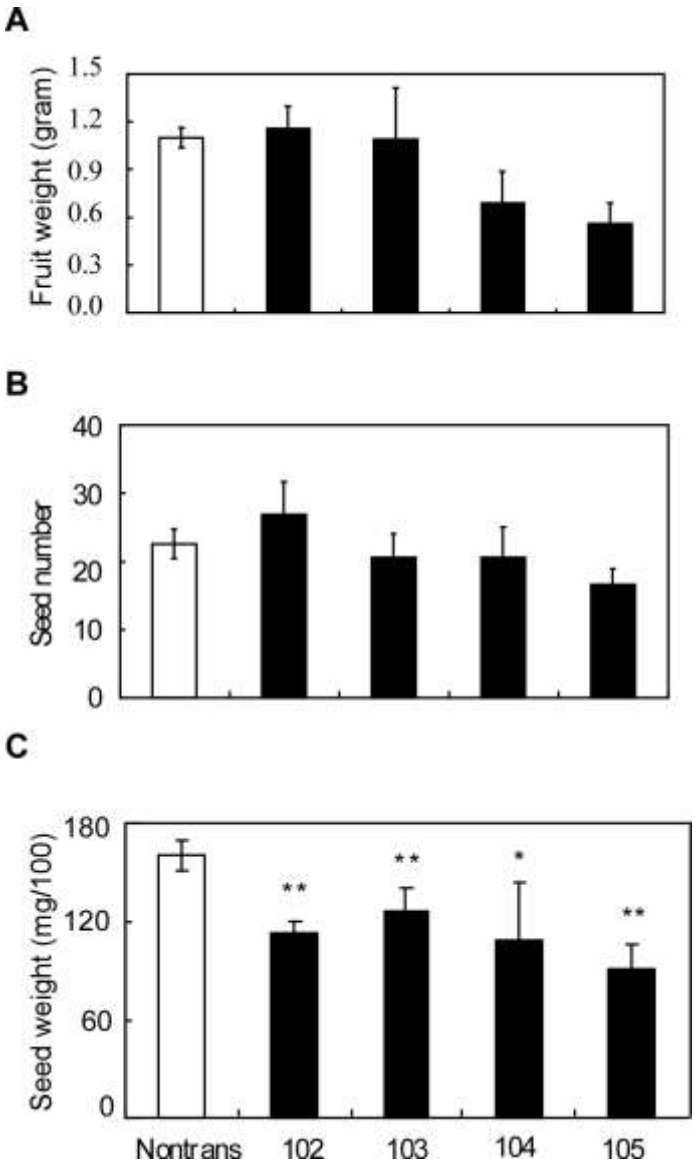

Fig. S3 Fruit and seed morphology of *HA-SlZFP2* overexpression lines in LA1589 background

(**A**) Fruit weight of *HA-SlZFP2* overexpression lines and their nontransgenic siblings. (**B**) Seed number of *HA-SlZFP2* overexpression lines and their nontransgenic siblings. (**C**) Seed weight of *HA-SlZFP2* overexpression lines and their nontransgenic siblings. 15-20 fruits per plant from total of five T2 plants for each line and nontransgenic sibling were used to measure fruit size, seed number and seed weight (100-seed). Statistical significance of *p*-values is based on Student's t-test. *, $p<0.05$; **, $p<0.01$. Data are means ± SD.

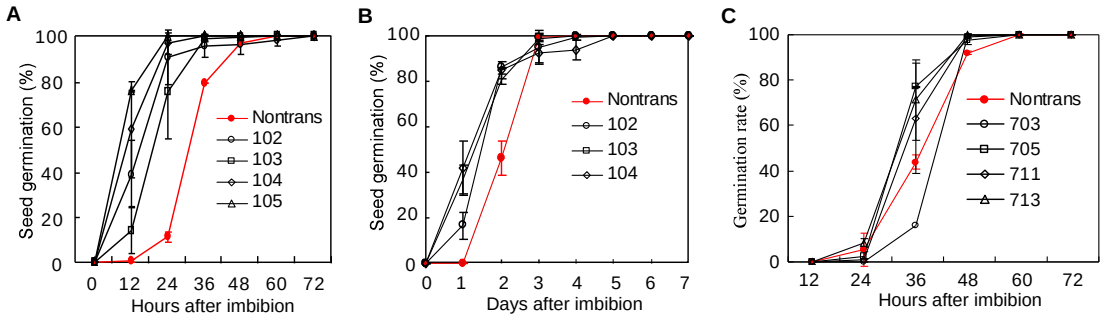

Fig. S4 Seed germination of *SlZFP2* overexpression lines

(**A**) Germination of fleshly harvested seeds of four *HA-SlZFP2* overexpression lines from LA1589and their nontransgenic siblings. (**B**) Germination of stored dry seeds of three *HA-SlZFP2* overexpression lines from LA1589 and their non-transgenic siblings. (**C**) Germination of stored dry seeds of *SlZFP2* overexpression lines and their non-transgenic siblings from LA1589. For each line, more than 150 seeds per line from total of three plants were tested and germination was based on radical emergence. Seeds (in **B** and **C**) have been stored for one month under at room temperature.

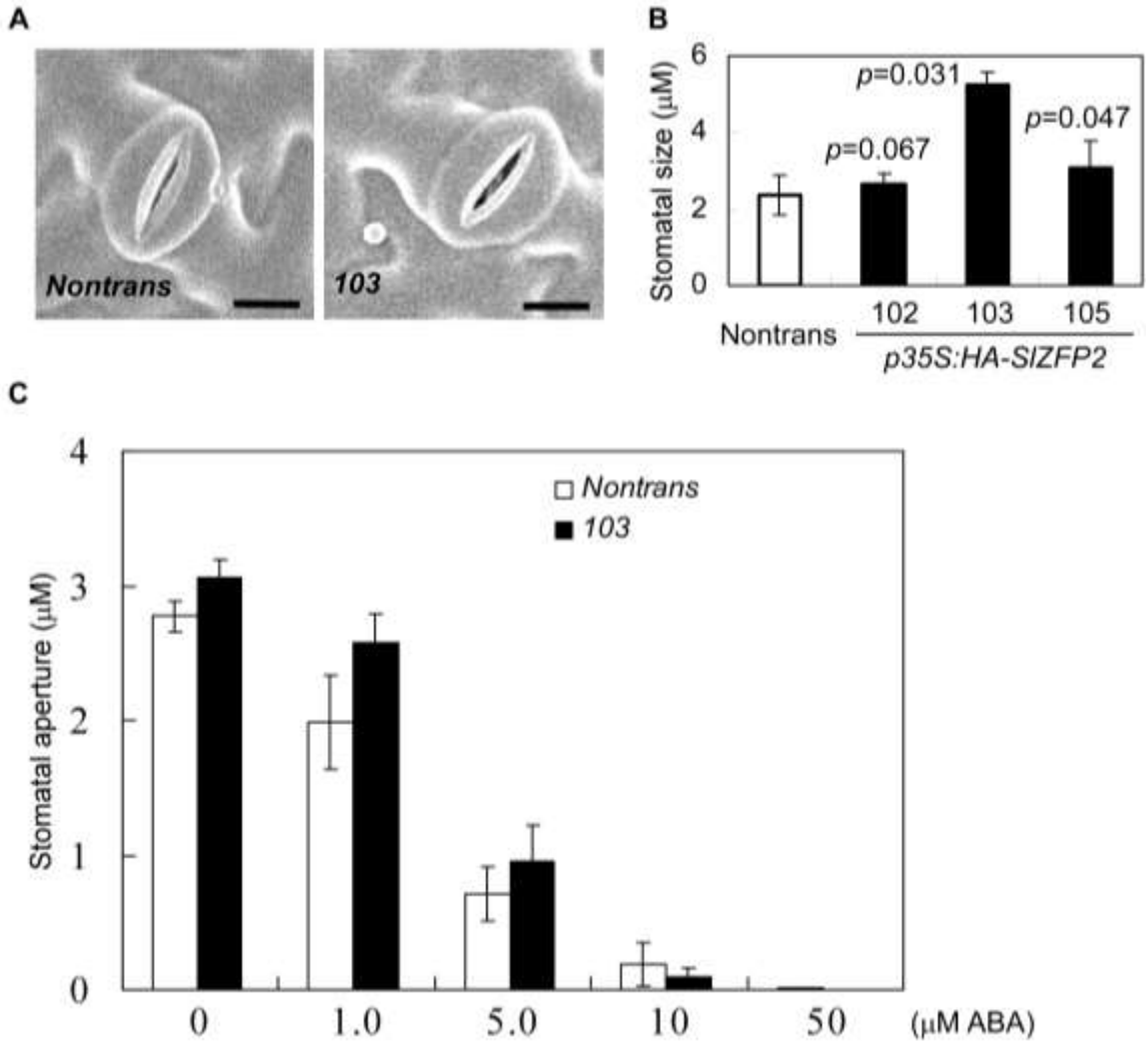

Fig. S5 Overexpression of *HA-SlZFP2* affects stomata aperture

(**A**) Representative stomata of a *HA-SlZFP2* overexpression line (103) from LA1589 and its nontransgenic sibling. The images were taken from leaves of plants grown in greenhouse under normal condition. (**B**) Quantification of stomata sizes for three *HA-SlZFP2* overexpression lines from and their nontransgenic siblings. At least 30 stomata per leaf were measured, and five leaves were taken from three plants per line. (**C**) Sensitivity of stomatal aperture to ABA. Statistical significance of *p*-values is based on Student's *t*-test.

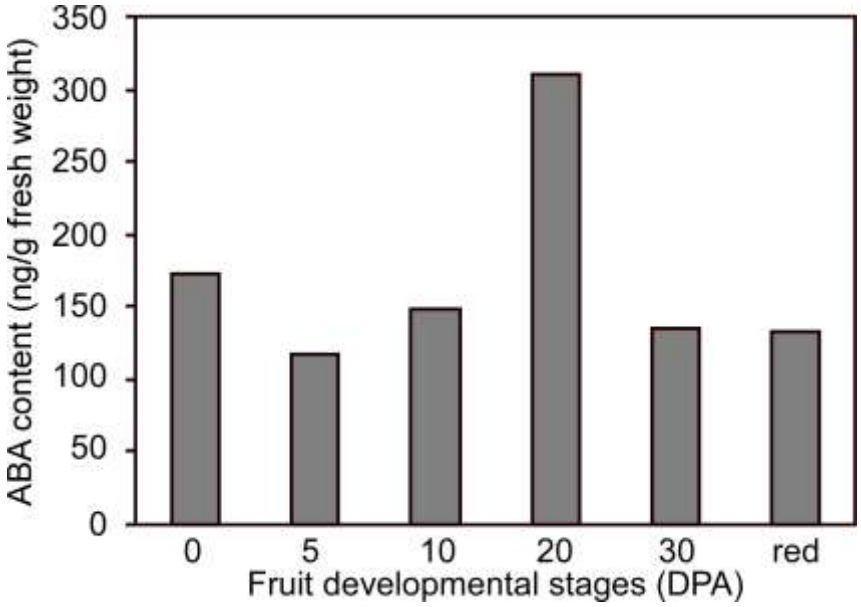

Fig. S6 Changes of ABA levels in M82 fruits after pollination
ABA was assayed on pooled 5-10 fruits per time point from 3-5 plants.

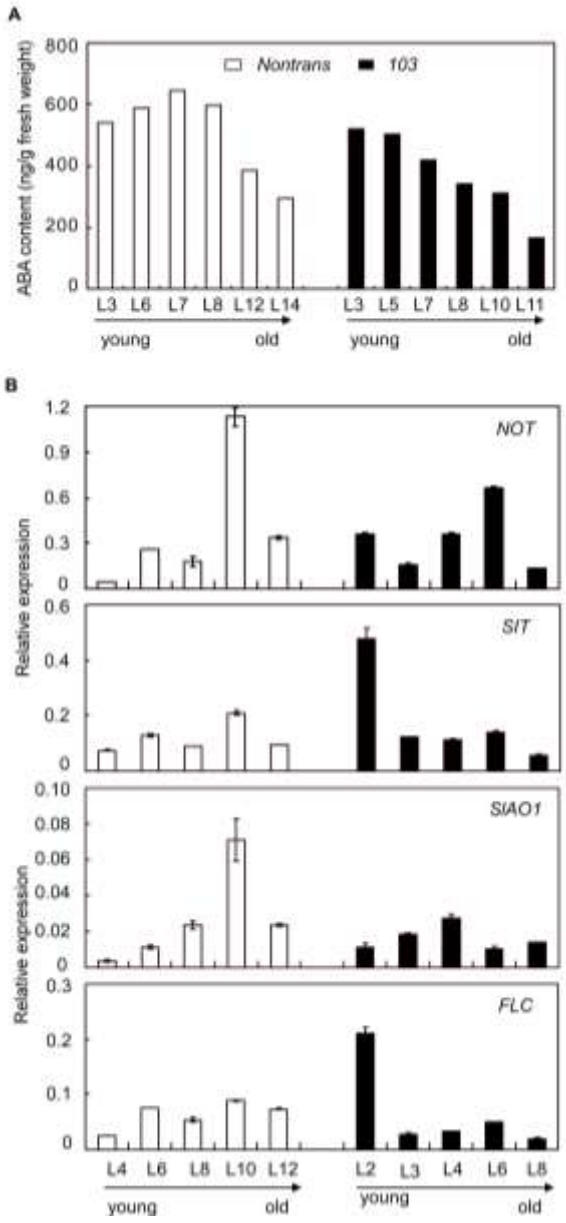

Fig. S7 Repressed ABA biosynthesis in leaf by overexpression of HA-SlZFP2

(**A**) ABA levels in the consecutive leaves of a representative transgenic line103 overexpressing *HA-SlZFP2*. (**B**) Transcript levels of ABA biosynthetic genes in the consecutive leaves of the *HA-SlZFP2* overexpression line 103. ABA and total RNA was extracted from pooled five leaves of five plants at the same developmental stages (leaves at the same nodes). The plants were 45 days old with 12 and 16 leaves for the transgenic line and its nontransgenic sibling, respectively. Expression levels relative to *SleIF4a6* were determined by qRT-PCR. Data in Fig. S7B are means ± SD.

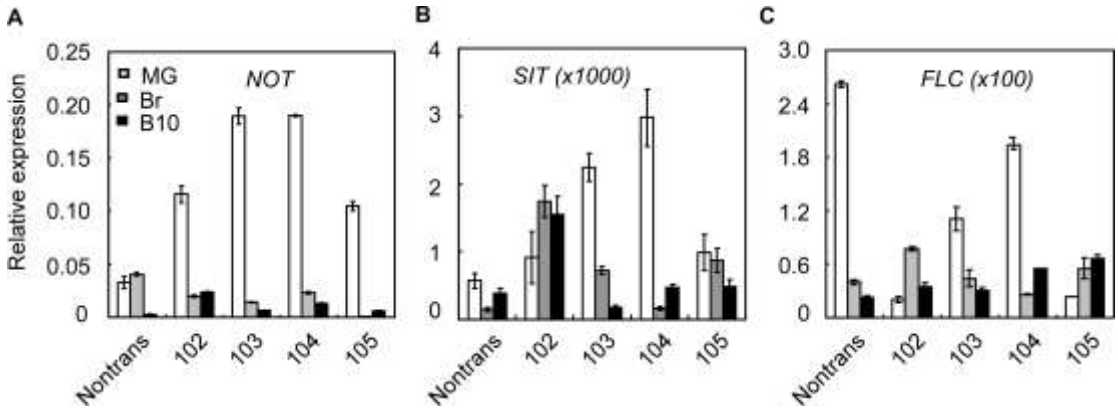

Fig. S8 Transcript levels of ABA biosynthetic genes in maturing seeds overexpressing *HA-SlZFP2*

(**A**) *NOT* expression in seeds extracted from fruits at stages of mature green (MG), breaker (Br) and red ripen (B10, breaker plus 10 days). (**B**) *SIT* expression in seeds extracted from fruits at MG, Br and B10 stages. (**C**) *FLC* expression in seeds extracted from fruits at MG, Br and B10 stages. Expression relative to *SleIF4a6* was determined by qRT-PCR in three technical replicates. Data are means ± SD, N=3.

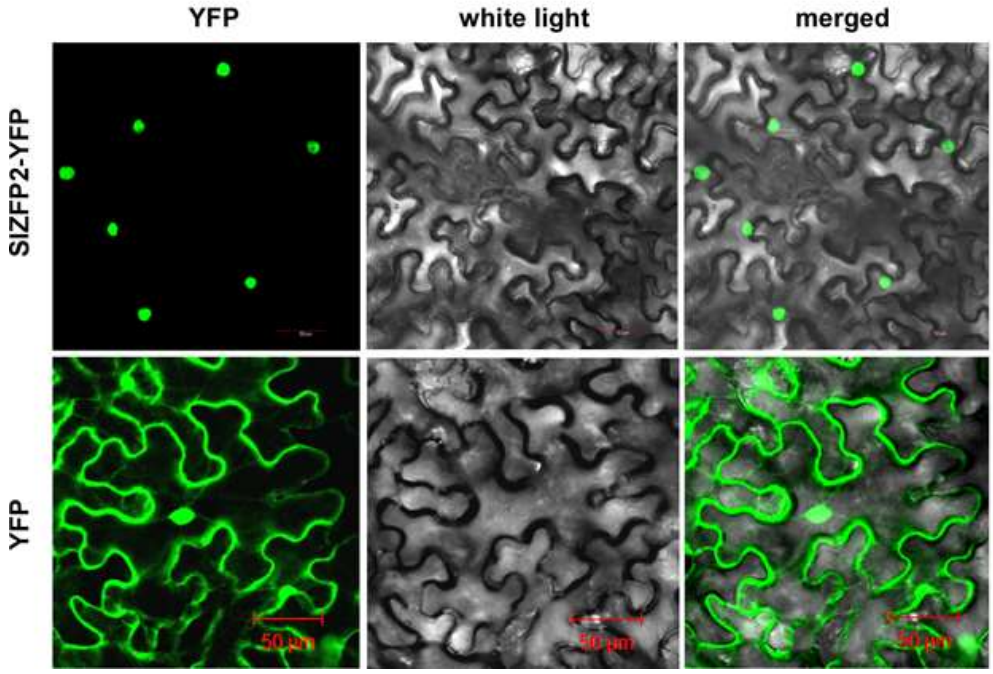

Fig. S9 Subcellular localization of SlZFP2 protein.
After infiltration with *A. tumefaciens* GV3101 containing the constructs, transient expression of SlZFP2-YFP fusion protein and YFP alone under 35S promoter in *N. benthamiana* leaves was monitored after three days post infiltration under confocal laser scanning microscope.

A. *NOT* promoter (355bp)

CAAAAAATGTAGGGACACCCACCCCTTCTCCCCACACCCACATGGGTCAT
TTGTTGTATTAGTAGTAATATATAGTAGTAGTAAAGAGTTTAATCCTCCAA
ATATGCATTTTTCACATATCCAACACGTGGTCTTCTCTTACATGTTAGAAG
TGGACCATAATTCCACACTCTCCCCAACTCTATATATACAAATCCTCCCCT
TCTCTCTTCTATAAACTCAACTCAAAAGGAAACTAAACATAAACTCTCCT
CATTTCCCACCTCTTTTCTCTCTACAAAAAAAAAAATCCCCTTTCCACAAC
ACCAAATTGTACTATTAACATCCAAAAAAAAGAAAAAGAAATAGGTAG
CT**ATG**

B. *SIT* promoter (472bp)

TACAAATGGAGGAAACACTGAAAAATGAAAAATCTTGAATACCCAATTG
AAGTCAAGACTGGTCAGTGTATGCTTTTTCAGCCTAAAGAACACATTTTTG
CCTTGTTTGAAGTTTATTGCTTGACCCCAGAATCTTGTTTGGAGTGTCTAA
ATGGAGAAGGCACTGAAAAATGAAAAATCTTGAATAACCCAATTGAAGT
CAAGATTGGTTAGTGTATATTTTTCAGCCAAAAAACACAAAAAGATAAGT
TTTTTTGTCTTATTTGAAGTTTATTGCTTGACCCCAGAATCTTGTTTGGAGT
GTATAGATGGAGAAGGCACTGAAAAAATGAAATCTTTTGAATACTCAATT
GAAGTCAAGATTGGTATGTGTGCATTTTTCAGCCAAAAGAACAGAAAAAG
ACTAAGGTTTTTGGAGTACATTTAATTGAAGTTTGTTGATTGAGAACAGA
GTGTTGTTTGGAGATTACAA**ATG**

C. *Solyc11g071620/SlAO1* promoter (838bp)

AAAGTTAACTGTTTCAGCTAGCAATGAACTTAAACTATTCAAGATTCTTGC
TTTTTTCTTTCATTTCTGTTATTTTTCAGCTCAGTAGGTTGAAAAAAAGTTG
AAAAAGATGAGATTTTTGATAAAGGGTGTGTGTCAAAGTTAACTTCTTCA
GTGTTTTCAGCTAGCCAAGAATCATGATTGAAAAAGCTTTTAGACATCCAA
TTCAATGATTCTTTTTTTTTCAGTTCTGGTAATATTTCAGCTCAGTATATAGA
AAAATTTTGAAAAAGATTGAGATTTTTGATAAAGGGTATGTGTCAAAGTC
AACTGCTGTCAGTGTTATAGCTAGATAAGAATTTTGATTCAAGAAGCTTTT
GTGTTAAAAAGTCAAGTTTTTGGATACCAAATTTGTATAATTCAAGATTCT
AGTTTTTTTTTCTAATTCTGGTAATTGTTCAGCTCAGTAAGAGAAAAAAG
TTGAAAAAATGTGTCATTGCTTCAGCTAGCCAAGAATCTTGATCTAAGAA
GCTTTTGTGTAAAAAGTCAAGCTTTGGACACCCAATTCAAGATTCTTGTTT
TTTTTTTCCAGTTCTGGTAATTTTTCAGCTCTGTAAGTTGAAAAAAGTTGA
AAAAGACTAGATTTTTGGTGAAAGGTATGTGTCAAAGTTAACTGCTTCAG
TATTACAGCTAGCCAAGAATTTTTTATTCAACAATCTTGTGTTAAAAAGTC
AAGTTTTTGGACTCCTAATTCAATTCAAGATTCAGTTTCTTCTTTCACTTCT
GATATTTTAAAAGACAATATTTTTTTGTGAAGGAGCTAACCATAGAATTTT
GATTTTAAGGAGGCTATTGTAGTA**ATG**

D. *Solyc01g088170/SlAO2* (490bp)

TGTGTATTTGGACACCCAATTCAATTCAAAGATTCTTGCTTTTTTTCTTTCAG
TTCTGATATTTTTTAGCTGAGGAAGTATGAAAAACTATTTTGATAAAGGAT

TTGTGTCTATTTAATTGCTTCACAGTTTCAGCTAGCAAAGAATCTTGATCT
CAGAAGTTCTTTGTGCTAAAAAGTAAGTATTTGGACACCCAATTCAAGTT
CAAGATTCTTGATTTTTCTGTCAGTTCTGTTATTTTCCACCTGAGGAAGTA
GAAAAGAAGTTGAAAAAGATTAAGTTTTTGAGACAGGGTTTGACTAAAGT
TAAGTGTTTCAGCTAACCAAGAATCTTGATTTAAGAAGCTTTTTGTGTTGA
ACAGTAAATATTTGGAGACCAAAATCAATTCAAGGTTCATGCTTTTTCTTT
CAGTTGGGATATTGTTCAGCTCAGGAAGTAGAAAAAGATAAGATTTTGA
TAAAGGGACTATGTGAAAGGGGCTATTATATTA**ATG**

E. *FLC* promoter (282bp)

GCGCAATATTAAACAATTTGTAAATAGAGGTGGCAACGATTGCCATTTCC
ACGTGAAAAATGTCTTCAAATCTGACGACGTGCATCTCGCCGGTGTCATA
TTATAATGAATTCTTTTTGCCACCATAATATATCAACTAATTTAGTTTCTA
AGAATATTCTAGTAGTAGTATAATATTTTTAATCGGCTAAGTAAGAGGAC
ATAAAAAAGTGTGTTTTCTTCTTCATTCATTTGAGTTTGTTCCGGGCTTGA
GTAAGAAATTCCAATCGCAACCGCTGCAAA**ATG**

F. *CNR* promoter (1475bp)

AATGAGGAATATACAAATAAGGTCATTTTGGGGAACCATTAAGCTATAAA
ACAATAATACACACTTATGAATTACCGATATATAATTTAATTTGGAATTTC
ATTCATATGGTTAATAGCAACAGAGTTGTCTTTGTATTAGTGCACTATCAA
TTTAATACCTAGCTGTGACACTAAAAAGCTAGGTGCCCACAATTATTAAA
ACAAAAGTGTATCCACCTCAAGAAGAAGAAAAAAGGCAAATATGATATA
AAACCATTTAACAAAGTCCATATCACAAAAATTAGACGGCAAAATCATAC
ACGACTAATTTATAGATTCACTGAACCATGCAATTCTATACCGTTCACTTC
CAATAAATAAAACATAATACACTATGTTTAGAGTACAAGACTCTCCTTGC
TTGAAAAGGACTACCAAGTAGGGGTTACTGCAGTGACTACCAAGTAGGGG
TTACTGCAGTCATTTGTTAATTCATTTTGAGTAATGTAACTGTGCAAGATA
ATGATGTTTTTTTTCCTTTTTGGTTAACTAAGATAATGATGGATTTAGGTA
GATGAATTAGACATCTAGTGATAATGGAGAGCCAGTGCAACAATTGAGCA
CTCTACTGGACCGACATGGACAACTGAGAGACCAACTTGCAGTATTATAA
TAGTGCAAATTATAGTTTAGTCGACTCCCTTCGGAATCTACTACATAAAGA
ACTACCATAAACTATGTTAGATGGCTATTACGGAGTTTAAATTAAACTCG
AAAATATCAGAAAAAGAAGTAACTTCAACCAATTACAATGCATACCCTTA
TCACAAGTGAAAAAGAGTAAACGTGCCAAACTCTTTTGATCCCTCCAAAG
CTAGAGGAAAAGAGTGAGCAATTCACTACAAACCACTGGCTTTGGTCTAT
GGTTGACACAACTCCTCGGCTAATTGGTCAAAATATCTTGTGACCACCAA
CCAGCAAGCACTAAATTGGATGTTCTATCAGCTTCTTTACATCATAAAACA
GTGAATACTGAACGCTGAGAGGCTAACTGACTGCCCAAAAAAACCTTGAC
AAAAAGTTAGTGGAGTAACTACCTAGGAGTAAATTCAATAGTAGACCTTG
AAAAGAACTTTAGCAAAGTCATCATAAATGCTCTTCACGTCTCATGTACT
ATGTTAAGGAATGGTCACATTTCTCTCTGCATTAAAGCTAGTTCATGTTAA
AAGTTGAGGCCGGTAGTAGTTTCAACTTTCAATTTAATTCCACCTTTCCTG
GCCCACTTCTGTACGGAACACCAATCAGAATCTTTAGTTCATCTTAACACC

AAAGCATCTCCACTTAGACACTTACTAGACTTCACATAGGAGGAAAAATA
TGGAACTGGTGGTCCTCACAC**GT**ACTTACCTTTCTTTTTTTACCTTTGTTCAAGTTTCATACTCTT
TTATCTGGCTTCCTCACTCTATTTTGGCCCAAT**AG**GTTCTCCTCACAGGG**ATG**GAAACTA

Fig. S10 SlZFP2 binding sites found in the promoters of *NOT*, *SIT*, *SlAO1*, *SlAO2*, *FLC* and *CNR*

Promoter sequences of the ABA biosynthetic genes *NOT*, *SIT*, *SlAO1*, *SlAO2* and *FLC*, and the ripening regulator *CNR* were manually checked to identify SlZFP2 binding motifs. SlZFP2 binding motifs are highlighted in shade and the putative binding sites are boxed. Translation start codon ATG for each gene is boxed and bolded. Sequences in red are of the first *CNR* exon.

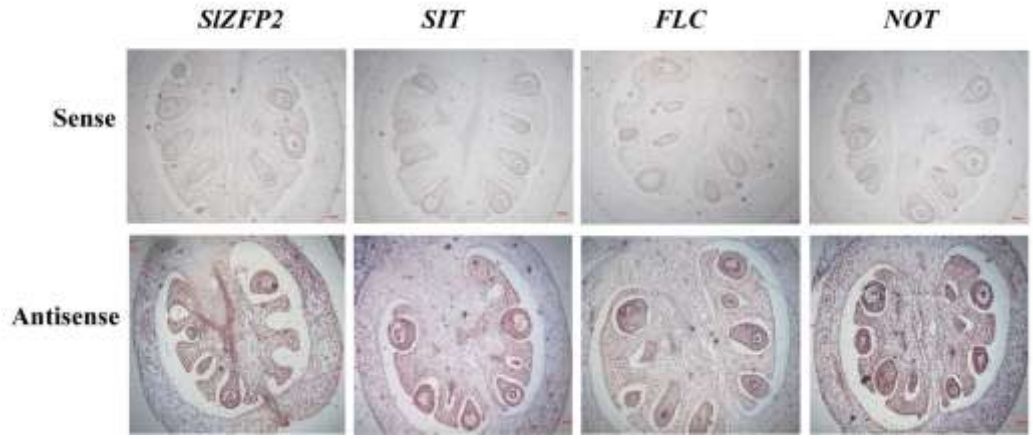

Fig. S11 Expression of *SlZFP2* and the ABA biosynthetic genes revealed by *in situ* hybridization.

In situ hybridization was performed on 5 dpa fruits of LA1589 with sense and antisense probes made from cDNA fragments of *SlZFP2* and the ABA biosynthetic genes *NOT*, *SIT* and *FLC*.