



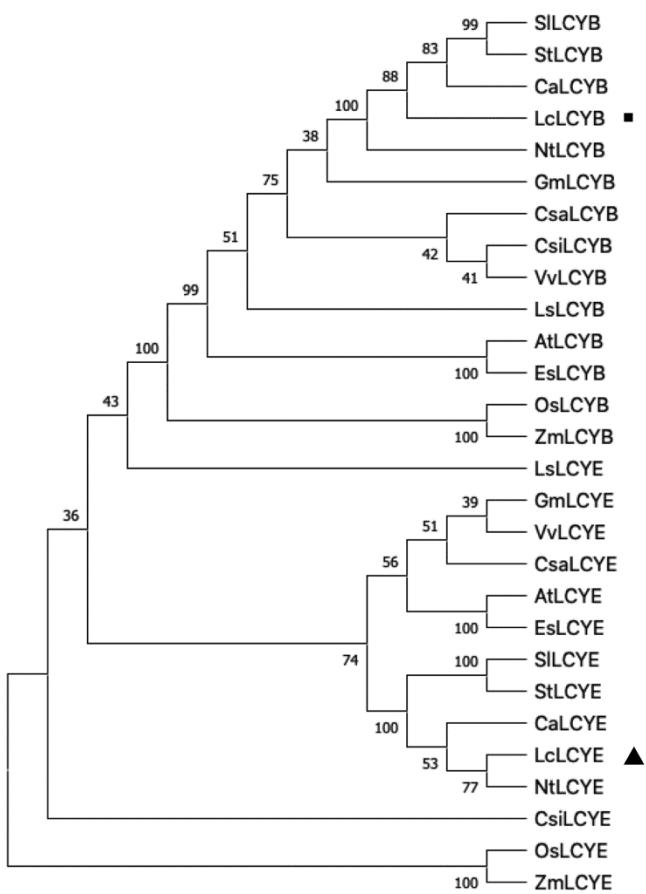
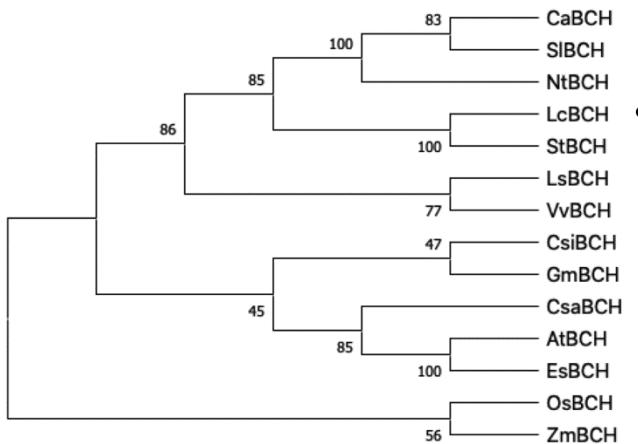
Supplementary Figure 1. Different ripening stages of wolfberry fruits. The genes were isolated from the stage (D), the red ripened fruits.

Supplementary Figure 2. Multiple sequence alignments of LcLCYB with the selected corresponding proteins. AtLCYB (*Arabidopsis thaliana*, NP_187634), CaLCYB (*Capsicum annuum*, PHT71462), CsaLCYB (*Cucumis sativus*, XP_004150761), CsiLCYB (*Citrus sinensis*, AAM21152), EsLCYB (*Eutrema salsugineum*, XP_006407592), GmLCYB (*Glycine max*, XP_003554132), LsLCYB (*Lactuca sativa*, XP_023755637), NtLCYB (*Nicotiana tabacum*, NP_001311716), OsLCYB (*Oryza sativa*, XP_015627234), SILCYB (*Solanum lycopersicum*, XP_010312096), StLCYB (*Solanum tuberosum*, XP_006351264), VvLCYB (*Vitis vinifera*, XP_002275769) and ZmLCYB (*Zea mays*, NP_001169155).

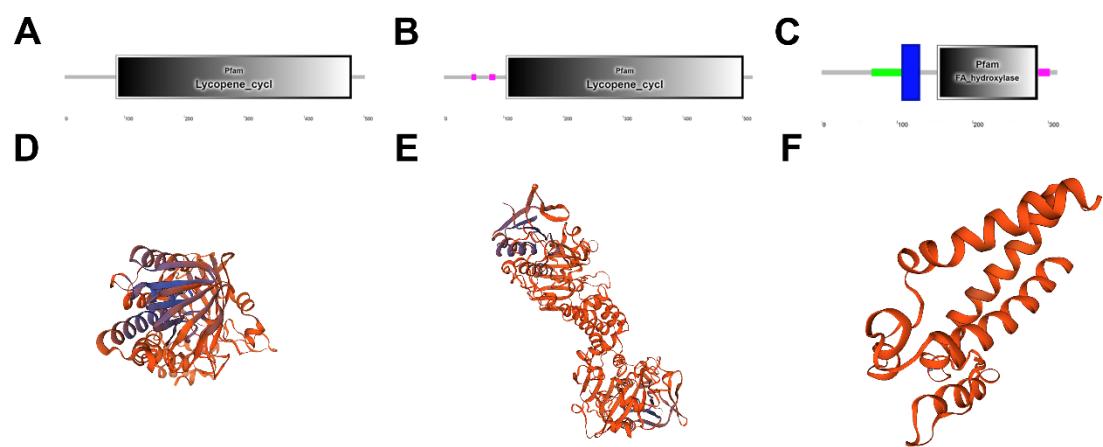
Supplementary Figure 3. Multiple sequence alignments of LcLCYE with the selected corresponding proteins. AtLCYE (*Arabidopsis thaliana*, NP_200513), CaLCYE (*Capsicum annuum*, PHT73568), CsaLCYE (*Cucumis sativus*, XP_004141172), CsiLCYE (*Citrus sinensis*, AAS48096), EsLCYE (*Eutrema salsugineum*, XP_006401252), GmLCYE (*Glycine max*, XP_003533775), LsLCYE (*Lactuca sativa*, XP_023756930), NtLCYE (*Nicotiana tabacum*, XP_016467034), OsLCYE (*Oryza sativa*, XP_015622198), SILCYE (*Solanum lycopersicum*, NP_001234337), StLCYE (*Solanum tuberosum*, XP_006353544), VvLCYE (*Vitis vinifera*, RVW43956) and ZmLCYE (*Zea mays*, AQK96213).

LcBCH		MAAGI S.G. I AIPRTFCLQPNFLWPKPA. STAPPVLFESPLTRNFGTILRSQEKP. . RLEKVCF	59
AtBCH		MAAGI S.T. STSFTSIVNRRSPFLPQKPTTTSVVPITPSNLGSILRCRRP. SFTVCF	60
CaBCH		MAAGLSA. STSFTSIVNRRSPFLPQKPTTTSVVPITPSNLGSILRCRRP. SFTVCF	60
CsaBCH		MAAGLSA. ATLPDKPHFLTSSSP. . KPDSPFLPPTVRNSHPQMKARRKT. LLTTCF	57
CsiBCH		MAAGLST. AVTEKFPLHRSFSTFSNSFLRHPKSVAFDPS. LRFKQY. . SVCF	50
EsBCH		MAAGLST. AVTEKFPLHRSFSTFSNSFLRHPKSVAFDPS. LRFKQY. . SVCF	50
GmBCH		MAASRI SF. TSSSQIYFRIGPFLCPNP. . VFPISSFSENRLGTLRSRKRP. . SFTVCF	54
LsBCH	NWSSSNLHHHFLLPPSNFTFSSRRPSI RLLPPNSLSSLTNPTNI TPQRTLCLHSSWSMAAAAAI AV. ASSSRSFRLRTMPFLGQKPT. . KSI PTTLPESMRI FHHTASPRTKVS. TFTVCF	58	
NtBCH		MAASRI SF. TSSSQIYFRIGPFLCPNP. . VFPISSFSENRLGTLRSRKRP. . SFTVCF	54
OsBCH		MAAVARLV. . AAR. . APLSPA. . AVAAAHRSPPALLR. . LAFAP	36
SIBCH		MAAAARI SA. SSTSRTFYFIRSPFLGPCKPT. STTSHSVSPISPFSLNLGPILRSRKRP. . SFTVCF	61
StBCH		MAAGI SA. SAGSRTI CLRNPFRGPKSA. STAPVVLFFSLTRNFGAI LLSRKRP. . RLAFCF	59
VvBCH		MAVEI SV. ATTCSR. . LGRNPLFLGKPT. SPFTPTSLIFI PSLRQENI FBCRKKT. . RLTVCF	56
ZmBCH		Consensus	0
LcBCH	VLAEDGK. . LDARKTEI ETI K. .	DEI EAEI NEEK SLAAR. . LAEKLAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFGAAVGMEWVA	164
AtBCH	VVEE. . RRKQSPPMDDNK.	PESTSSSEI. . LMTSR. . LKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVSVELMFCTALSFVAAGVMEWVA	151
CaBCH	VLBD. . DRKFTQPEAKT.	EDENMRI EEQ. . ISATR. . LAKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	160
CsiBCH	LVBG. . NQSSSGRVNDNEED.	EDENMRI EEQ. . ISATR. . VSERLAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	157
EsBCH	VVBE. . VVQKTTTID.	EDENMRI EEQ. . ISATR. . VSERLAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	161
GmBCH	VVBE. . DRQGSPPI DNDER.	PESTSSSTTAI DAEFIAILR. . LAKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	154
LmQD	PKQQTHNEI EFQ.	EQOPPPPPPPAQQLVSPK. . LAKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	162
VAGD	QKLETQI EEFQ.	EEI EMKRI EEQ. . ISATR. . LAKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	233
NtBCH	VLEDKEIINTLFDKLNAQLETGT.	EEI EMKRI EEQ. . ISATR. . LAKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	161
OsBCH	LPAR. . RLAVPLRVAVG.	EEPEEED. . IAR. . AVAERAQSE. . EDPEKHKI EEQ. . I LATR. . LAKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	133
SIBCH	VLIED. . EKLKPQFDDEA..	DQI VEI NEEK SLAACW. . LAKKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	165
StBCH	VLKNKEL. . NSTI QS ECEVI Q.	S EETVAS. . QI SAAR. . VAEKAKRKSERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEVPFS EMFTCTALSFVAAGVMEWVA	154
VvBCH	VVEE. . EKLSTEVENR.	MAKAPRSEERTTYLVAAVMSLIGITSNAVALAVYRISWQMEG. GEAVPVS EMFTCTALSFVAAGVMEWVA	67
ZmBCH		Consensus	k ser tyl aa n s g ts a v y rf wqmg g e m t f l s gaavgmewva
LcBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALSISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYFRVAAAQLHHSQDFGPVGLFLGPKEV	289
AtBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAICLISYOFFHKGLI PGLCFGAGLGI TMFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAQLHHTDKFKGPVGLFLGPKEV	276
CaBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	285
CsaBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	282
CsiBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	286
EsBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	279
GmBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	237
LsBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	358
NtBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	286
OsBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	258
SIBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	286
StBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	290
VvBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	TNAVPAIALISYOFFHKGLI PGLCFGAGLGI TVFGNAYMFVHDGLVHRRFPVGPVANPVYLRKAAAHSLSKEFNGPVGLFLGPKEV	279
ZmBCH	RVAHRALWIA. . SLWNHESHHHRPREGPEELNDVFAI	Consensus r wh al wh s l w n h e s h h g fe nd fai navpa ll g r p g	190
LcBCH	EVVCGLEELKEKVNRYVHKSKL. .		312
AtBCH	EVVCGLEELKEKJ SBRKLYNK. . GSSTS.		303
CaBCH	EVVCGLEELKEKJ SBRKLYNK. . TRIKOS.		308
CsaBCH	EVVCGLEELKEKJ SBRKLYNK. . KNTARKSI DGS.		310
CsiBCH	EVVCGLEELKEKJ SKRISNRVPK.		311
EsBCH	EVVCGLEELKEKJ SBRKLYKKSOGSSSS		309
GmBCH	EVVCGLEELKEKJ SBRKLYKKSOGSSSS		314
LeBCH	EVVCGLEELKEKJ KERIKMYNK. .		380
NtBCH	EVVCGLEELKEKJ KERIKMYNK. .		309
OsBCH	EVVCGLEELKEKJ KERIKMYNK. .		285
SIBCH	EVVCGLEELKEKJ KERIKMYNK. .		309
StBCH	EVVCGLEELKEKJ KERIKMYNK. .		314
VvBCH	EVVCGLEELKEKJ KERIKMYNK. .		303
ZmBCH	QKOFELDARA. .		200
Consensus	g		

Supplementary Figure 4. Multiple sequence alignments of LcBCH with the selected corresponding proteins. AtBCH (*Arabidopsis thaliana*, NP_200070), CaBCH (*Capsicum annuum*, KAF3668875), CsaBCH (*Cucumis sativus*, XP_004140758), CsiBCH (*Citrus sinensis*, NP_001275830), EsBCH (*Eutrema salsugineum*, XP_006413280), GmBCH (*Glycine max*, NP_001242409), LsBCH (*Lactuca sativa*, XP_023769464), NtBCH (*Nicotiana tabacum*, NP_001313021), OsBCH (*Oryza sativa*, XP_015628773), SIBCH (*Solanum lycopersicum*, NP_001234348), StBCH (*Solanum tuberosum*, ADF28628), VvBCH (*Vitis vinifera*, RVW41947) and ZmBCH (*Zea mays*, NP_001105865).

A**B**

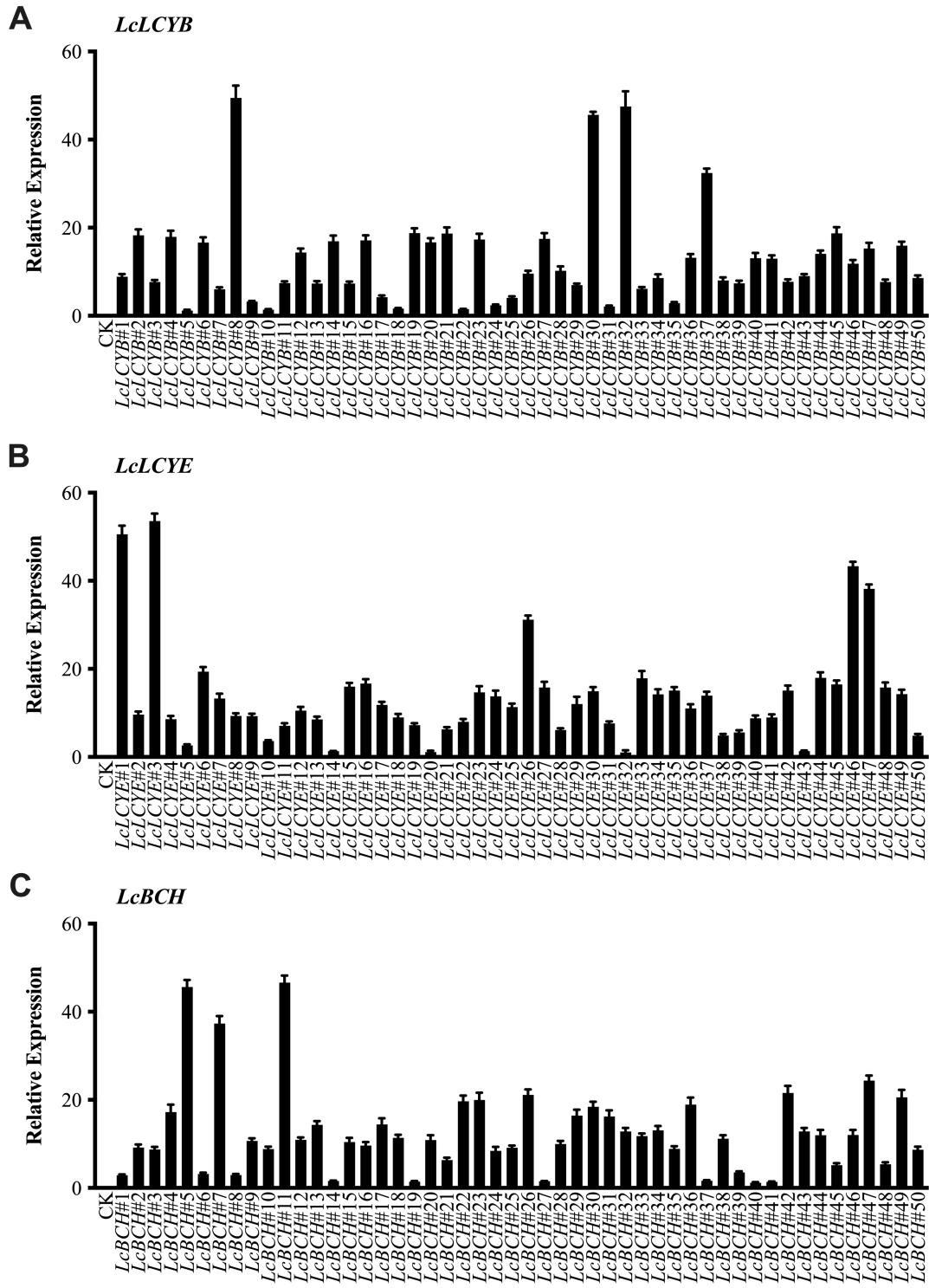
Supplementary Figure 5. Phylogenetic analysis of (A) LCYB and LCYE and (B) BCH were constructed using the Neighbor-Joining method with MEGA X software. The LcLCYB, LcLCYE, and LcBCH were indicated with ■, ▲ and ●, respectively.



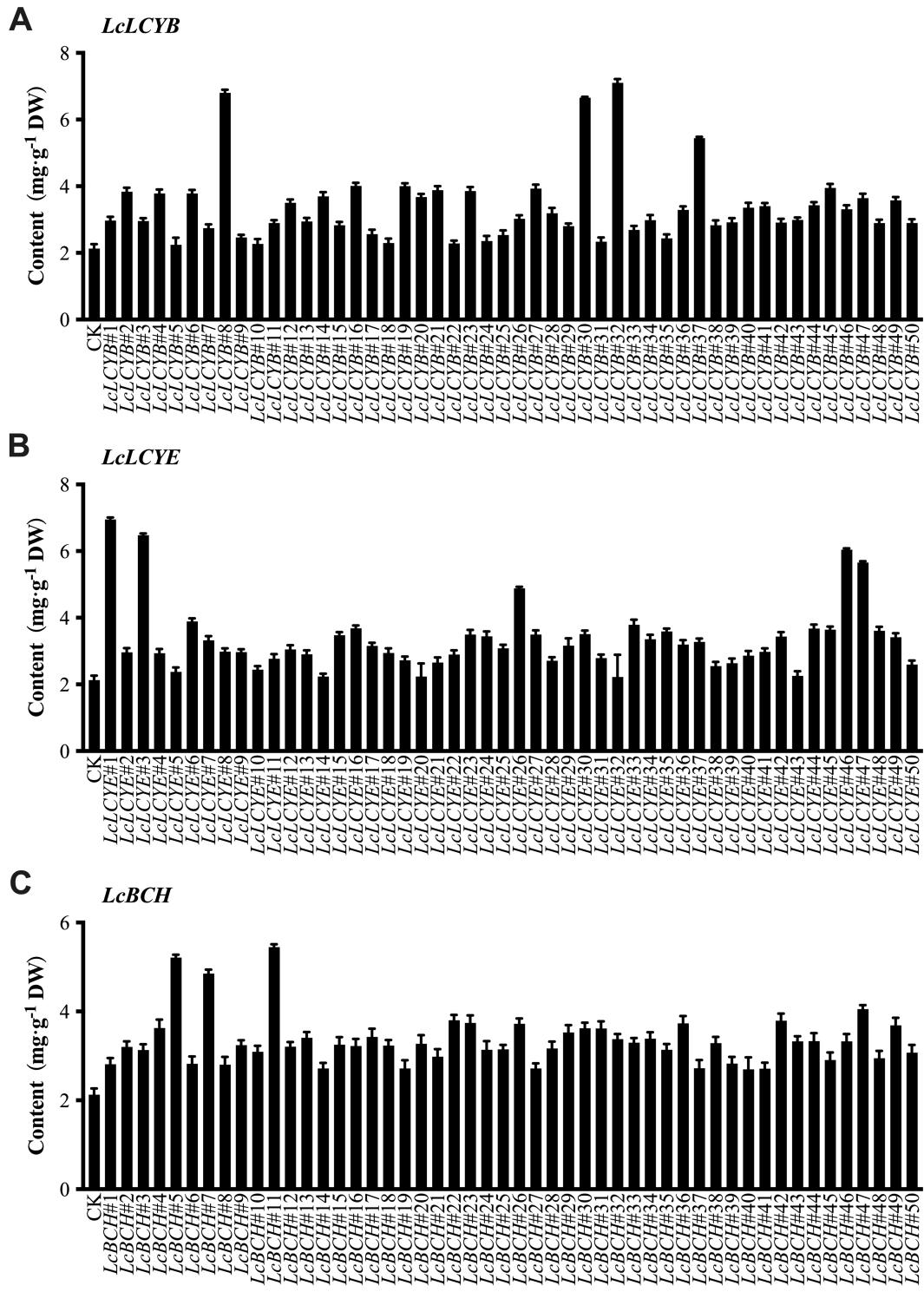
Supplementary Figure 6. Conserved domain analysis of **(A)** LcLCYB, **(B)** LcLCYE, and **(C)** LcBCH were accomplished with PFAM online tools (<http://pfam.xfam.org/>), and 3D structure prediction of **(D)** LcLCYB, **(E)** LcLCYE, and **(F)** LcBCH were applied with SWISS-MODEL Workspace (<https://swissmodel.expasy.org/>).



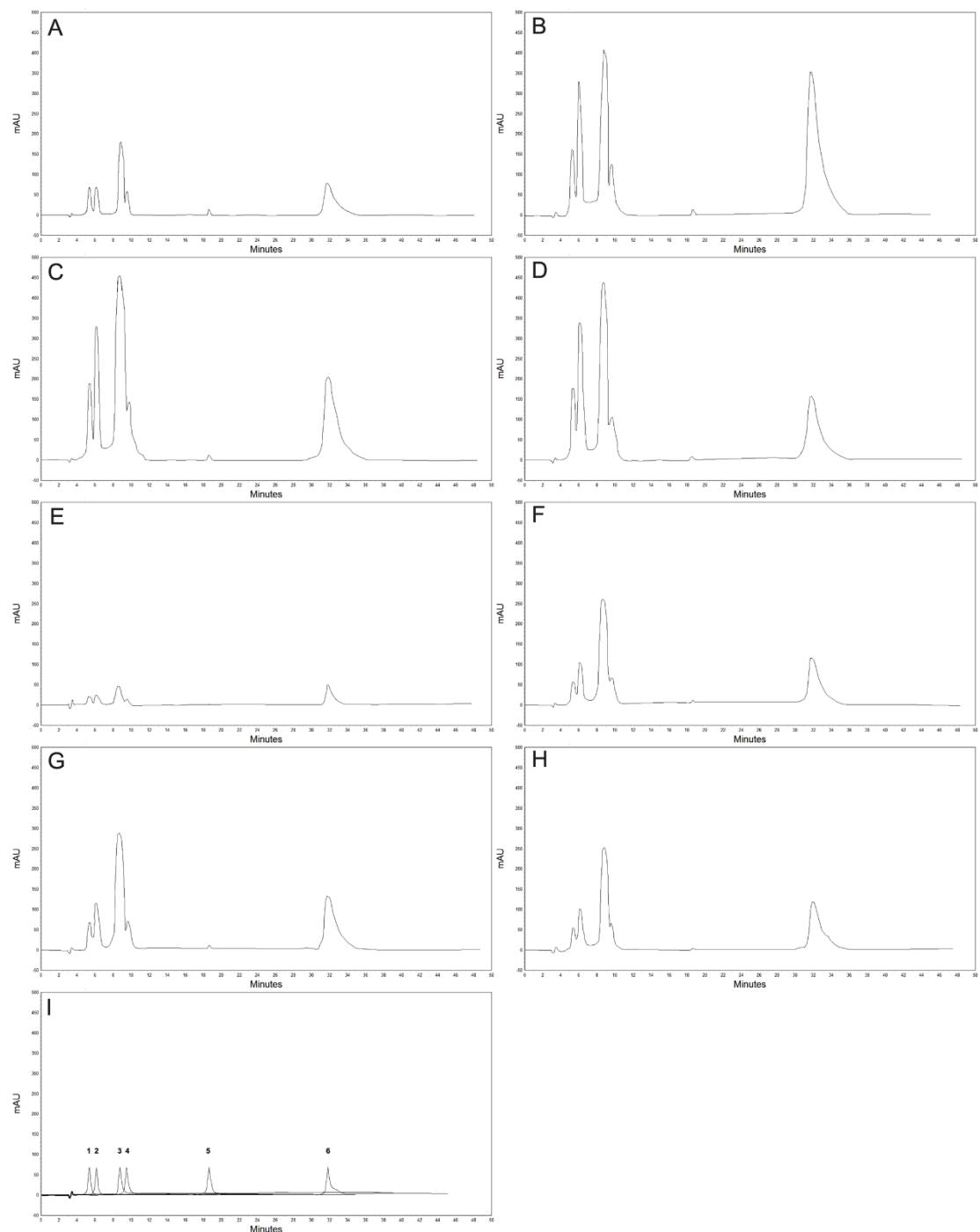
Supplementary Figure 7. The color complementation of *LcLCYB*, *LcLCYE*, and *LcBCH* in engineered *E.coli*. Recombined *E.coli* strains containing plasmid **(A)** pACCRT-*EBI* (*carring genes coding geranylgeranyl diphosphate synthase, 15-cis-phytoene synthase, and phytoene desaturase*), **(B)** pACCRT-*EBI* and pET-28a-*LcLCYB*, **(C)** pACCRT-*EBI* and pET-28a-*LcLCYE*, and **(D)** pACCRT-*EBI*, pET-28a-*LcLCYB* and pET-28a-*LcBCH* were displayed.



Supplementary Figure 8. Expression levels of **(A)** *LcLCYB*, **(B)** *LcLCYE*, and **(C)** *LcBCH* in transgenic tobacco were measured by Real-time PCR, and results were normalized to reference gene *NtActin*. Tobacco overexpression lines were named as *Gene#Number*. Data were obtained from three independent experiments and performed as means ± standard deviation.



Supplementary Figure 9. Total carotenoid contents in **(A)** *LcLCYB*, **(B)** *LcLCYE*, and **(C)** *LcBCH* in transgenic tobacco leaves. [Tobacco overexpression lines were named as Gene#Number](#). Data were obtained from three independent experiments and performed as means ± standard deviation.



Supplementary Figure 10. The chromatogram figures of HPLC analysis. The chromatogram figures of (A) control plants and tobacco over-expressing (B) *LcLCYB*, (C) *LcLCYE*, and (D) *LcBCH* growing under normal condition; (E) control plants and tobacco over-expressing (F) *LcLCYB*, (G) *LcLCYE*, and (H) *LcBCH* growing under salt stress. The chromatogram figures of standard samples were shown in (I), and peaks labeled with numbers were standard samples of (1) neoxanthin, (2) violaxanthin, (3) lutein, (4) zeaxanthin, (5) lycopene, and (6) β -carotene.

Supplementary Table 1. Physiochemical properties and predicted subcellular localizations

Gene	Accession No.	Length of ORF (bp)	Number of amino acids (aa)	Molecular weight (Da)	pI of protein	Predicted Subcellular location
<i>LcLCYB</i>	KJ624406	1272	423	47807.6	8.06	chloroplast
<i>LcLCYE</i>	KJ143993	1749	582	64949.0	6.50	chloroplast
<i>LcBCH</i>	KF430643	939	312	34850.3	8.47	chloroplast

Supplementary Table 2. Information of the protein for the sequence analysis

Protein	Species	Accession number
AtBCH	<i>Arabidopsis thaliana</i>	NP_200070
CaBCH	<i>Capsicum annuum</i>	KAF3668875
CsaBCH	<i>Cucumis sativus</i>	XP_004140758
CsiBCH	<i>Citrus sinensis</i>	NP_001275830
EsBCH	<i>Eutrema salsugineum</i>	XP_006413280
GmBCH	<i>Glycine max</i>	NP_001242409
LsBCH	<i>Lactuca sativa</i>	XP_023769464
NtBCH	<i>Nicotiana tabacum</i>	NP_001313021
OsBCH	<i>Oryza sativa</i>	XP_015628773
SIBCH	<i>Solanum lycopersicum</i>	NP_001234348
StBCH	<i>Solanum tuberosum</i>	ADF28628
VvBCH	<i>Vitis vinifera</i>	RVW41947
ZmBCH	<i>Zea mays</i>	NP_001105865
AtLCYB	<i>Arabidopsis thaliana</i>	NP_187634
CaLCYB	<i>Capsicum annuum</i>	PHT71462
CsaLCYB	<i>Cucumis sativus</i>	XP_004150761
CsiLCYB	<i>Citrus sinensis</i>	AAM21152
EsLCYB	<i>Eutrema salsugineum</i>	XP_006407592
GmLCYB	<i>Glycine max</i>	XP_003554132
LsLCYB	<i>Lactuca sativa</i>	XP_023755637
NtLCYB	<i>Nicotiana tabacum</i>	NP_001311716
OsLCYB	<i>Oryza sativa</i>	XP_015627234
SILCYB	<i>Solanum lycopersicum</i>	XP_010312096
StLCYB	<i>Solanum tuberosum</i>	XP_006351264
VvLCYB	<i>Vitis vinifera</i>	XP_002275769
ZmLCYB	<i>Zea mays</i>	NP_001169155
AtLCYE	<i>Arabidopsis thaliana</i>	NP_200513
CaLCYE	<i>Capsicum annuum</i>	PHT73568
CsaLCYE	<i>Cucumis sativus</i>	XP_004141172
CsiLCYE	<i>Citrus sinensis</i>	AAS48096
EsLCYE	<i>Eutrema salsugineum</i>	XP_006401252
GmLCYE	<i>Glycine max</i>	XP_003533775
LsLCYE	<i>Lactuca sativa</i>	XP_023756930
NtLCYE	<i>Nicotiana tabacum</i>	XP_016467034
OsLCYE	<i>Oryza sativa</i>	XP_015622198
SILCYE	<i>Solanum lycopersicum</i>	NP_001234337
StLCYE	<i>Solanum tuberosum</i>	XP_006353544
VvLCYE	<i>Vitis vinifera</i>	RVW43956
ZmLCYE	<i>Zea mays</i>	AQK96213

Supplementary Table 3. Primers used for vector construction in this study

Gene	Primer sequences for vector construction (5'-3')
<i>LcLCYB</i>	F: ATCGGGATCCATGGATACTTGTGAAAACCCCAAATA R: ATCGGTCGACTTATTCTGAATCCTGTAACAAATTG
<i>LcLCYE</i>	F: ATCGGGATCCATGGAATGTGTTGGAGTTCAAAATTG R: ATCGGTCGACTTAAATGTAAGATAAGTTCTTATC
<i>LcBCH</i>	F: ATCGGGATCCATGGCTGCCGGAATTTCAGGCATTG R: ATCGGTCGACTCATAATCCCTAGAAATTAACT

Supplementary Table 4. Information of carotenoids analyzed with HPLC

Peak Number	Chemical Name	CAS Number	Retention Time (second)
1	neoxanthin	14660-91-4	322.37
2	violaxanthin	126-29-4	369.71
3	lutein	127-40-2	525.26
4	zeaxanthin	144-68-3	570.35
5	lycopene	502-65-8	1118.2
6	β -carotene	7235-40-7	1909.4

Supplementary Table 5. Primers used for Real-time PCR in this study

Gene	Primer Sequence (5'-3')	Accession number
<i>NtActin</i>	F: CCTGAGGTCTTTCCAACCA R: GGATTCCGGCAGCTTCATT	XM_016658252.1
<i>NtIPI</i>	F: AAGAGCATTGAAGAGGACGAG R: AACAAATGACCAATAACTCCAGGC	NM_001326211.1
<i>NtGGPS</i>	F: CTGTCAATCGAGCCTAGATGC R: AAACCCGCTTGCCTTCG	NM_001325671.1
<i>NtPSY</i>	F: TGTGCTTGTTGTGGGTTG R: TTTGTCTCCGCCTTCA	NM_001325140.1
<i>NtPDS</i>	F: AGTCAGACTAAACTCACGAATAAA R: CTCCCCTAGCTTCTCAA	XM_016642616.1
<i>NtZDS</i>	F: GGGAGCTTGATTCCGATT R: CCCGCACCACTGGACTAA	XM_016658599.1
<i>NtCRTISO</i>	F: GCAGGACCAGATTCAGCG R: GATGGATAAAGGAGGTAGAGCC	NM_001325775.1
<i>NtLCYB</i>	F: AAGAGCATTGAAGAGGACGAG R: TGAGGGATGAACCAGACCAG	NM_001324787.1
<i>NtLCYE</i>	F: GCTCTTGCTGCCGAGTC R: GAATTGGATCGGCATCGT	NM_001325477.1
<i>NtBCH</i>	F: CTTGGCAAATGGAGGGTG R: AAGAAGAGCAATGGCTGGAA	NM_001326092.1
<i>NtVDE</i>	F: CTGAAAGAGTGCAGGTTAGAGC R: TGGAGACAGGCAACATTAGC	NM_001324805.1
<i>NtZEP</i>	F: CAACAGCAGAAACTGAGCCAT R: AACAAATGACCAATAACTCCAGGC	XM_016582459.1
<i>NtNSY</i>	F: AAGCCAAAGTAGGTGCCAA R: AACGGTAAGATGGAGAACATGGCT	XM_016625496.1
<i>NtAPX</i>	F: GGAAGAGTTGGAGTGGTGG R: ACAGCTGCCAATTCAAATCCT	NM_001324874.1
<i>NtCAT</i>	F: GTCTTGACAGGAAGGCGTGA R: AACGGAAGACAGAGTAGCAGC	NM_001325412
<i>NtP5CR</i>	F: AAGAGCGTGGCACAATCACT R: AGCTTTCTCGAGTTTCTTCTGC	XM_016610055.1
<i>NtPOD</i>	F: GGACAAATGGAGAGATTGTTCA R: TGCTGACTTGGCCTACCAAC	XM_016632447.1
<i>NtSOD</i>	F: TCCTCCCCAGGTTCGGAATA R: TCGCTACATTGGCAAGAG	XM_016631233.1
<i>LcLCYB</i>	F: ACATCCCTGTAGCTCGTCTGG R: ACCATGCCAGCAGTACCAACCA	KP262047.1
<i>LcLCYE</i>	F: AACGTTGGGCTCGTTGGGCCT R: CTCCCTCGTGCAGTAAATGGCGACT	KF768738.2

LcBCH

F: AGGCTGGCGGAAAAATTGGCGA
R: GCAGCGCCAACGAGAGAGCGA

KF430643.1
