









Supplemental Figure S1 The intact phylogenetic tree of 156 putative bHLH proteins from *Acer palmatum* and eight reference bHLHs with known function of being involved in anthocyanin biosynthesis in other plants. The clade containing eight reference bHLHs and two putative targets was enclosed by the red dashed rectangle, which was presented as Figure 1 in the main text.

Supplemental Table S1 The name list of 156 putative bHLH proteins (longer than 60 amino acids) in *Acer palmatum* and 8 reference bHLHs

| | |
|-----------------------------|----------------------------|
| CL10105.Contig1_All.pro | CL3970.Contig1_All-rev.pro |
| CL1029.Contig27_All-rev.pro | CL3970.Contig2_All-rev.pro |
| CL1029.Contig29_All.pro | CL3970.Contig4_All.pro |
| CL10308.Contig2_All.pro | CL4150.Contig1_All.pro |
| CL10308.Contig3_All-rev.pro | CL4150.Contig2_All.pro |
| CL1035.Contig5_All.pro | CL4150.Contig3_All.pro |
| CL1035.Contig8_All.pro | CL4150.Contig4_All.pro |
| CL1292.Contig1_All-rev.pro | CL4150.Contig6_All.pro |
| CL1292.Contig2_All-rev.pro | CL418.Contig1_All.pro |
| CL1292.Contig3_All.pro | CL418.Contig2_All.pro |
| CL1616.Contig1_All.pro | CL4334.Contig1_All.pro |
| CL1616.Contig2_All.pro | CL4334.Contig2_All-rev.pro |
| CL1616.Contig3_All.pro | CL4514.Contig2_All.pro |
| CL1616.Contig4_All.pro | CL4738.Contig1_All-rev.pro |
| CL1616.Contig5_All.pro | CL4738.Contig2_All.pro |
| CL1833.Contig6_All-rev.pro | CL4849.Contig1_All-rev.pro |
| CL1833.Contig7_All-rev.pro | CL4849.Contig2_All-rev.pro |
| CL1848.Contig1_All.pro | CL4849.Contig4_All-rev.pro |
| CL1848.Contig3_All.pro | CL4849.Contig5_All-rev.pro |
| CL2907.Contig1_All-rev.pro | CL4857.Contig1_All.pro |
| CL2907.Contig2_All-rev.pro | CL4857.Contig2_All.pro |
| CL2929.Contig1_All.pro | CL5039.Contig2_All-rev.pro |
| CL2929.Contig2_All.pro | CL5039.Contig3_All.pro |
| CL3127.Contig1_All.pro | CL5039.Contig4_All-rev.pro |
| CL3127.Contig2_All.pro | CL5039.Contig5_All.pro |
| CL3151.Contig1_All.pro | CL5186.Contig2_All.pro |
| CL3151.Contig2_All.pro | CL5186.Contig3_All-rev.pro |
| CL3182.Contig1_All-rev.pro | CL5696.Contig5_All.pro |
| CL3182.Contig2_All.pro | CL572.Contig3_All.pro |
| CL3200.Contig1_All.pro | CL572.Contig35_All.pro |
| CL3200.Contig2_All.pro | CL572.Contig36_All.pro |
| CL3457.Contig1_All-rev.pro | CL572.Contig5_All.pro |
| CL3457.Contig3_All-rev.pro | CL572.Contig60_All.pro |
| CL3457.Contig5_All-rev.pro | CL572.Contig71_All.pro |
| CL3457.Contig6_All-rev.pro | CL6183.Contig1_All.pro |
| CL3641.Contig1_All-rev.pro | CL6183.Contig2_All.pro |
| CL3641.Contig2_All.pro | CL6183.Contig3_All.pro |
| CL3665.Contig3_All.pro | CL6463.Contig1_All.pro |
| | CL6463.Contig2_All.pro |
| | CL7127.Contig1_All-rev.pro |

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| CL7127.Contig2_All-rev.pro |
| CL7209.Contig1_All.pro |
| CL7209.Contig2_All.pro |
| CL7338.Contig4_All-rev.pro |
| CL7338.Contig5_All.pro |
| CL7338.Contig6_All-rev.pro |
| CL7338.Contig7_All.pro |
| CL7601.Contig1_All.pro |
| CL7601.Contig2_All.pro |
| CL7601.Contig3_All.pro |
| CL7601.Contig4_All-rev.pro |
| CL8408.Contig1_All.pro |
| CL8408.Contig2_All.pro |
| CL8881.Contig1_All.pro |
| CL8881.Contig2_All.pro |
| CL8881.Contig3_All.pro |
| CL9162.Contig1_All-rev.pro |
| CL9162.Contig2_All-rev.pro |
| CL9889.Contig2_All.pro |
| Unigene10591_All.pro |
| Unigene11120_All-rev.pro |
| Unigene11131_All.pro |
| Unigene1166_All.pro |
| Unigene11690_All.pro |
| Unigene11901_All.pro |
| Unigene1297_All-rev.pro |
| Unigene13498_All.pro |
| Unigene13561_All.pro |
| Unigene14361_All.pro |
| Unigene14501_All.pro |
| Unigene14587_All-rev.pro |
| Unigene14835_All.pro |
| Unigene16378_All.pro |
| Unigene17429_All-rev.pro |
| Unigene20219_All.pro |
| Unigene20480_All.pro |
| Unigene20542_All-rev.pro |
| Unigene20596_All-rev.pro |
| Unigene21161_All-rev.pro |
| Unigene21833_All.pro |
| Unigene23323_All-rev.pro |
| Unigene23324_All-rev.pro |
| Unigene23515_All-rev.pro |

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| Unigene23765_All-rev.pro |
| Unigene23773_All.pro |
| Unigene24001_All.pro |
| Unigene24403_All-rev.pro |
| Unigene24418_All.pro |
| Unigene24543_All-rev.pro |
| Unigene24875_All.pro |
| Unigene24922_All.pro |
| Unigene27722_All-rev.pro |
| Unigene29817_All-rev.pro |
| Unigene29818_All.pro |
| Unigene29948_All-rev.pro |
| Unigene30003_All-rev.pro |
| Unigene30513_All.pro |
| Unigene31035_All.pro |
| Unigene33000_All-rev.pro |
| Unigene33001_All-rev.pro |
| Unigene33002_All.pro |
| Unigene33003_All.pro |
| Unigene33203_All.pro |
| Unigene33566_All.pro |
| Unigene33670_All-rev.pro |
| Unigene34215_All-rev.pro |
| Unigene34283_All.pro |
| Unigene34478_All.pro |
| Unigene34840_All.pro |
| Unigene4107_All-rev.pro |
| Unigene47552_All.pro |
| Unigene527_All-rev.pro |
| Unigene7300_All-rev.pro |
| Unigene7426_All.pro |
| Unigene7478_All-rev.pro |
| Unigene7741_All.pro |
| Unigene842_All-rev.pro |
| Unigene9470_All.pro |
| At1g63650-EGL3.pro |
| At4g09820-TT8.pro |
| At5g41315-GL3.pro |
| MdbHLH3.pro |
| MdbHLH33.pro |
| MtTT8-KM892777.pro |
| PhAN1-AAG25928.pro |
| VvACC68685.pro |

Supplemental Table S2 Percentage of residue identity between two putative target bHLH proteins and five reference proteins

| | VvMYC1 | MdbHLH3 | MtTT8 | PhAN1 | AtTT8 |
|--------------------|--------|---------|-------|-------|-------|
| Unigene13498_All | 68.8 | 65.4 | 59.5 | 58.0 | 55.1 |
| CL8881.contig2_All | 54.4 | 52.8 | 47.9 | 47.2 | 51.1 |

The deduced protein sequences deduced from assembled transcripts of Unigene13498_All and CL8881.contig2_All were aligned with five reference bHLHs using ClustalW of MegAlign (DNASTAR, Madison, USA).

Supplemental Table S3 Open reading frame of *ApbHLH1* (2130 bp)

ATGGCTGCGCCGCCGAGTAGCCGGCTCAAAGTATGCTGCAGGAGGCGGT
 GCAATCAGTTCAGTGGACTTACAGTCTCTT
 TTGGCAAATCTGTCCACAACAAGGGATCCTAATATGGGGAGATGGGTATTAT
 AATGGAGCAATTAAAGACAAGGAAAACAG
 TGCAACCGATGGAGGTGAGTGCGGAGGAAGCGTCTCTGCAAAGAACCCA
 GCAGCTTAGAGAGCTTACGAGTCATTGTCG
 GCTGGAGAGACGAACCAGCCAGCAAGGAGGCCTGTGCTTCGTTGCGCC
 GGAGGACTTAACGGAGTCGGAGTGGTTCTA
 TTTGATGTGTGTCTTCTCTTCCCACCTGGCCTCGGGTTACCTGGAAAG
 GCATACGCAAGGCGGCAACATGTATGGC
 TTACAGGAGCGAACGAGGTTGACAGCAAAACCTCTAGAGCAATTCTT
 GCCAAGAGTGCTCGTATACTAGACTGTGTTA
 TGCATTCTCTTGACGGCGTGTGAATTGGGCTAACAGATAGGATAC
 AAGAGGACCTTGGATTGGTCCAACAAAT
 CAAGAGCTTCTCACAGACCAACACCACCCAAACCCCTAACACCACAAAC
 CAGCTCTCTCGAGCACTCCACCTCCAATC
 CCGCCACGTATCCGACCACCGCCCTCCACTCACCTCCAATTCCGGCCA
 TGTACGCCGCTGTTGACCCACCAGCCAAC
 CCCAACCAAGAACGAAAGACGACGAAGAAGAGGAGGAAGAGGAAGAA

GAAGAAGACGAAGAGGAGGAGCAAGAGTCTGA
CTCTGAAGCCGAAACGGGTGCGATCAGAACGCCAAGGC GCCGCTCAAAACC
CTCAATCCGTGGCTCCACCCATGGCTGAAC
CGAGTGAGCTCATGCAGCTGGAGATGTCTGAGGATATCCGAGTTGGATCCC
CCGACGATGCGTCGAATAATTGGACTCA
GATTTCATATGTTGCCGTGAGCCAAGCCGTGAACCCGGGTGATCACCAAG
CGGCGCTCTGACTCGTTCAGAGCTGAGTC
GAGTCGAAGGTGGCCGATGCTACAAGAACCAATTGACCGTAGTGGTCTTCC
ACCACCAACCTCCTTCAGCTTCAGGACAAC
TTGGAATGGAAGAATTGACACAAGAACACTCACTACTCCCCAAACCGTG
TCAACCATTATCCAAAAGCAGACAACACGG
TGGGCCGAATCATCGTCTACCGGCTACGTACGTACTCAACCCAGTCAGCG
TTTGCCAAGTGGACAAACCGGTCGGACCT
CCACCTCCAGATTCCCGTGGAAGGCACGTCTCAGTGGCTCCTCAAGTACAT
TTTGTTCAGCGTACCTTTCTACACAGCA
AATACCGTGATGAAAATCCCCAAGTCACGTGACGCCAGCACGCCGAC
GCGGCCTCCAAGTCCGTAAAGGGACCTCA
CAAGACGAGCTGAGTGCCAACCACGTGCTGGCGAGCGCCGCCGCG
AGAAGTTAACGAGAGGTTCATCATTAAAG
GTCATTAGTCCCCCTCGTCACTAAAATGGACAAGGCCTCCATATTGGGTGAC
ACTATCGAGTACGTCAAACAGTTGCGTA
AAAAGATTCAAGGATCTCGAGACACGTAACAAGCAGATGGAGACCGATCAA
CGGTCAAGATTGTCGGATTCTCCAAGGACT
AGTAGTGGTTGAAAGAGCAGAGAAGTGGGTTGACTGTTGGACCGGGC
ACGGGTGGGTCTGCACCCGGGTCTGATAA
GAGGAAGATGAGGATAGTGGAGACAAGTAGCGGCGTTGCAAAGCCTAAGA
CGGTGGAGTCACGCCACTGCCAACACCAT
TAACGACAACACCGACGGCGACAACAACGGCTAGTACGGTACAGGTGTCG
ATCATCGAGAGTGATGCGTTGGAGCTG
CAATGTGGGTATAGAGAAGGGTTGCTGATATCATGCTAATGCTAAGGG
AGCTTCTGATTGAAGTCACAGCAGTTCA
ATCTTCTCTAACTAATGGAGCTTGACTCAGCTAAGGGCAAAGGTGAA
AGATAATGTAAATGGAAAAAAAGCAAGCA
TTGTGGAAGTCAAGAGAGCAATACATCAACTGATAACCCAAACAATACTGA

Supplemental Table S4 Primers used in this study

| Name | Sequence (5' to 3') | Application |
|-------------|--|---|
| bH-f1 | CCACTGAAATTGGAAGTGAGA | Cloning <i>ApbHLH1</i> |
| bH-f2 | GAAGTGAGAATTCAATCCCAAGTTA | Cloning <i>ApbHLH1</i> |
| bH-f3 | TTCAATCCCAAGTTAGTGAAGAAG | Cloning <i>ApbHLH1</i> |
| bH-f4 | GTGAAGAAGATCTACCGTTGAATAC AG | Cloning <i>ApbHLH1</i> |
| bH-f5 | AGATCATTGCTAGGACTGCTTG | Cloning <i>ApbHLH1</i> |
| bH-f6 | TTTGTAAAGCACACATGGTGGAC | Cloning <i>ApbHLH1</i> |
| bH-f7 | AGGGTCAAACAGGGAGACAAC | Cloning <i>ApbHLH1</i> |
| bH-r1 | GGCGACTCGAATGATTAGAACAA | Cloning <i>ApbHLH1</i> |
| bH-r2 | TGCAAATTACCTGACACAGTTG | Cloning <i>ApbHLH1</i> |
| bH-r3 | ATTGGCATGCTTCTTGAGC | Cloning <i>ApbHLH1</i> |
| bH-r4 | CCTGATTACTCGTGAGTTGTGAAA | Cloning <i>ApbHLH1</i> |
| qAcUBQ-f | AGGCCAAAATTCAAGGACAAG | qPCR |
| qAcUBQ-r | CGAAGGACAAGATGCAGAGTAG | qPCR |
| qApbHLH1-f | CGTAACAAGCAGATGGAGACC | qPCR |
| qApbHLH1-r | CCAAAACAGTCAACCCACTTC | qPCR |
| PCCL-bHLH-f | ACGGGGGACGAGCT <u>CGGTACC</u> ATGGCTG CGCCGCCGAGT | Generating PCCL- <i>ApbHLH1</i> construct |
| PCCL-bHLH-r | CGCGTACGAGATCTGGTCGACGTATTGTT | Generating |

| | | |
|----------------|---|--|
| | GGGGTATCAGTTGATGTAT | PCCL-ApbHLH1 construct |
| PHNL-bHLH-F | ACGGGGGACGAGCTCGGTACCATGGCTG CGCCGCCGAGT | Generating PHNL-ApbHLH1 construct |
| PHNL-bHLH-R | AACATCGTATGGGTAGTCGACGTATTGTT GGGTATCAGTTGATGTAT | Generating PHNL-ApbHLH1 construct |
| PCCL-MYB-F | ACGGGGGACGAGCTCGGTACCATGACCA TGGTAGAGGGATTGTAG | Generating PCCL-ApMYB1 construct |
| PCCL-MYB-R | CGCGTACGAGATCTGGTCGACAATTGCAT ATTCTCTTCTGTGTTAAGA | Generating PCCL-ApMYB1 construct |
| PHNL-MYB-F | ACGGGGGACGAG <u>CTCGGTACCATGACCA</u> TGGTAGAGGGATTGTAG | Generating PHNL-ApMYB1 construct |
| PHNL-MYB-R | AACATCGTATGGGTAG <u>TCGACAATTGCAT</u> ATTCTCTTCTGTGTTAAGA | Generating PHNL-ApMYB1 construct |
| PCCL-bHLH1T-f | ACGGGGGACGAGCTCGGTACCATGGAG ATGGTATTATAATGGAG | Paired with PCCL-bHLH-r to generate PCCL-ApbHLH1T |
| PCCL-MYB1D-R1 | GTTCTCCAGGGACTTCATCTTCAGTGAA CTCTCCTCT | Paired with PCCL-MYB-f to get fragment before bHLH binding domain in <i>ApMYB1</i> |
| PCCL-MYB1D-2F1 | GATGAAGTCCCTGGAAGAACAGCAAATG ATGT | Paired with PCCL-MYB-r to get fragment after bHLH binding domain in <i>ApMYB1</i> |
| ApbHLH1-F | ttagat <u>ctGAATA</u> CAGTTCTAACAA <u>ATGGCT</u> G (underlined nucleotides as BglII site) | Generating pCAMIA1305-ApbH LH1 construct (<i>35S::ApbHLH1</i>) |
| ApbHLH1-R | tcgtt <u>aaac</u> TCAGTATTGTTGGGGTATCAGTT G (underlined nucleotides as PmeI site) | Generating pCAMIA1305-ApbH LH1 construct (<i>35S::ApbHLH1</i>) |