Article title: The R2R3 MYB transcription factor *PavMYB10.1* involves in anthocyanin biosynthesis and determines fruit skin colour in sweet cherry (*Prunus avium* L.)

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The following Supporting Information is available for this article:

**Figure S1.** Ripe fruit skin and flesh colour of ‘Big Dragon’, ‘Rainier’, and ‘Lapins’.
(a) Ripe fruit skin colour of ‘Big Dragon’. (b) Ripe fruit skin colour of ‘Rainier’. (c) Ripe fruit skin colour of ‘Lapins’. (d) Ripe fruit flesh colour of ‘Big Dragon’ (e) Ripe fruit flesh colour of ‘Rainier’. (f) Ripe fruit flesh colour of ‘Lapins’.

**Figure S2.** Southern blot of *PavMYB10.1* in three varieties ‘Big Dragon’, ‘Rainier’, and ‘Lapins’.

**Figure S3.** Genomic DNA sequence alignment of *PavMYB10.1a* and *PavMYB10.1b* gene between the dark red variety ‘Lapins’ and the blush ‘Rainier’. Introns shown in italics; black triangle indicates position of deletion, asterisk indicates stop codon.

**Figure S4.** Genomic DNA sequence alignment of *PavMYB10.2a* and *PavMYB10.2b* genes. Introns shown in italics and asterisk indicates stop codon.

**Figure S5.** Protein sequence alignment of PavMYB10.1, PavMYB10.2, and other genes from different species. Black arrows indicate Key amino acid residue difference. Double-sided arrow indicates R2 and R3 domains.
**Figure S6.** Alignment cDNA of *PavMYB10.1* transcript levels in sweet cherry ‘Lapins’ and ‘Rainier’. Coding sequence alignment of *PavMYB10.1a* and *PavMYB10.1b* between dark-red variety ‘Lapins’ and blush variety ‘Rainier’. Upwards arrow indicates position of deletion, asterisk marks stop codon.

**Figure S7.** Alignment cDNA of *PavMYB10.2a* and *PavMYB10.2b* gene. Coding sequence alignment of *PavMYB10.2a* and *PavMYB10.2b* between dark-red variety ‘Lapins’ and blush variety ‘Rainier’. asterisk marks stop codon.

**Figure S8.** Cellular localization of *PavMYB10.1* in onion epidermal cells. Onion bulb cells were bombarded with gold particles coated with plasmids. (a) The combination of cell morphology in dark field for green fluorescence and bright light for pEZS-NL-GFP. (b) Cell morphology in dark field for green fluorescence for pEZS-NL-GFP. (c) Cell morphology in bright light for pEZS-NL-GFP. (d) The combination of cell morphology in dark field for green fluorescence and bright light for pEZS-NL-PavMYB10.1-GFP. (e) Cell morphology in dark field for green fluorescence for pEZS-NL-PavMYB10.1-GFP. (f) Cell morphology in bright light for pEZS-NL-PavMYB10.1-GFP.

**Figure S9** Interaction of *PavMYB10.1* with PavbHLH and PavWD40. (a) Schematics of *PavMYB10.1a* and *PavMYB10.1b* constructs used in yeast two-hybrid assays. Solid circle marks stop codon. (b) Yeast two-hybrid assays of *PavMYB10.1a* and *PavMYB10.1b* with PavbHLH and PavWD40. Indicated combinations of bait (BD fusion) and prey (AD fusion) constructs were introduced into yeast reporter strain AH109. Transformants were streaked on selective medium (SD/-Leu-Trp) and then
the single clone on medium (SD/-Leu-Trp) were inoculated on selective medium (SD/-Ade-His-Leu-Trp). Empty vectors pGBK7 and pGADT7 were negative controls. Plates were photographed after incubation at 30°C for 7 days.

**Table S1** Correlations between anthocyanin content and relative expressions of *PavMYB10.1* and structural genes in ‘Big Dragon’, ‘Rainier’, and ‘Lapins’.

**Table S2** Correlations between relative expressions of *PavMYB10.1* and relative expressions of structural genes in ‘Big Dragon’, ‘Rainier’, and ‘Lapins’.

**Table S3** Sequences of oligonucleotide primers used in this work (F, forward; R, reverse).
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Intron shows in italics; asterisk indicates stop codon.
Figure S5 Protein sequence alignment of *PavMYB10.1*, *PavMYB10.2*, and other genes from different species. Black arrow indicates key amino acid residue difference. Double-sided arrow indicates R2 and R3 domains.
Figure S6 Alignment cDNA of *PavMYB10.1* in sweet cherry ‘Lapins’ and ‘Rainier’.

Coding sequence alignment of *PavMYB10.1a* and *PavMYB10.1b* between dark-red variety ‘Lapins’ and blush variety ‘Rainier’. Upwards arrow indicates position of deletion, asterisk marks stop codon.
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pEZS-NL-GFP. (b) Cell morphology in dark field for green fluorescence for 

pEZS-NL-GFP. (c) Cell morphology in bright light for pEZS-NL-GFP. (d) The combination of cell morphology in dark field for green fluorescence and bright light 

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### Table S1: Correlations between anthocyanin content and relative expressions of *PavMYB10.1* and structural genes in ‘Big Dragon’, ‘Rainier’, and ‘Lapins’.

| Gene name   | Big Dragon Correlation | p value | Rainier Correlation | p value | Lapins Correlation | p value |
|-------------|------------------------|---------|----------------------|---------|--------------------|---------|
| *PavMYB10.1* | –                      | –       | 0.913**              | 0.002   | 0.854*             | 0.014   |
| *PavPAL*    | –                      | –       | -0.416               | 0.306   | 0.288              | 0.488   |
| *PavC4H*    | –                      | –       | -0.401               | 0.325   | 0.272              | 0.515   |
| *Pav4CL*    | –                      | –       | -0.398               | 0.329   | 0.133              | 0.754   |
| *PavCHS*    | –                      | –       | -0.446               | 0.269   | 0.596              | 0.119   |
| *PavCHI*    | –                      | –       | -0.495               | 0.212   | 0.845**            | 0.008   |
| *PavF3H*    | –                      | –       | -0.46                | 0.252   | -0.057             | 0.894   |
| *PavF3’H*   | –                      | –       | -0.564               | 0.145   | 0.717*             | 0.045   |
| *PavANS*    | –                      | –       | -0.122               | 0.773   | 0.845**            | 0.008   |
| *PavUFGT*   | –                      | –       | 0.780*               | 0.022   | 0.857**            | 0.007   |
| *PavLAR*    | –                      | –       | -0.348               | 0.398   | -0.394             | 0.334   |
| *PavANR*    | –                      | –       | -0.447               | 0.267   | -0.332             | 0.422   |
| *PavFLS*    | –                      | –       | -0.34                | 0.41    | -0.13              | 0.758   |

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).
Table S2 Correlations between relative expression of PavMYB10.1 and that of structural genes in ‘Big Dragon’, ‘Rainier’, and ‘Lapins’.

| Genes | Big Dragon | Rainier | Lapins |
|-------|------------|---------|--------|
|       | Correlation | p value | Correlation | p value | Correlation | p value |
| PavPAL | – | – | -0.364 | 0.376 | 0.785* | 0.021 |
| PavC4H | – | – | -0.311 | 0.454 | 0.241 | 0.565 |
| Pav4CL | – | – | -0.298 | 0.473 | 0.023 | 0.957 |
| PavCHS | – | – | -0.35 | 0.396 | 0.271 | 0.517 |
| PavCHI | – | – | -0.413 | 0.31 | 0.458 | 0.254 |
| PavF3H | – | – | -0.366 | 0.373 | -0.185 | 0.661 |
| PavF3'H | – | – | -0.494 | 0.214 | 0.574 | 0.136 |
| PavDFR | – | – | -0.316 | 0.445 | 0.29 | 0.486 |
| PavANS | – | – | -0.087 | 0.838 | 0.902** | 0.002 |
| PavUFGT | – | – | 0.956** | 0 | 0.861** | 0.006 |
| PavLAR | – | – | -0.232 | 0.58 | -0.038 | 0.928 |
| PavANR | – | – | -0.35 | 0.395 | -0.547 | 0.161 |
| PavFLS | – | – | -0.226 | 0.591 | 0.446 | 0.269 |

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).
### Table S3

Sequences of oligonucleotide primers were used in this work (F, forward; R, reverse).

| Purpose       | Accession | Alias*       | Sequence (5→3)                                      |
|---------------|-----------|--------------|---------------------------------------------------|
| qRT-PCR       | JF748833  | *PavCHS*     | F: GCTCGTGTTCTTGGTGTGTCG<br>R: ACTGTCGGGAAAGTGTGTTTTG |
|               | JF740091  | *PavCHI*     | F: TCCACCGTACTAAACAC<br>R: CCTCAAATCACAGCCAAATC |
|               | JF740092  | *PavF3H*     | F: CATCGTCGACGAGCATTACAG<br>R: ATCAGCTGTCTGCTATCTCAG |
|               | KF974775  | *PavDFR*     | F: CATCCATGCAACACGGCTTCC<br>R: AAGTGAACGAACTGGCCCCC |
|               | KF974776  | *PavANS*     | F: GCCCTTTTTCGATCTTCCCAT<br>R: CTTCCTCCACCCCTTTC |
|               | KF974777  | *PavUFGT*    | F: ATGTGGGACCTCTTACCC<br>R: GGCAACCACTACCCATTT |
|               | GU990523  | *Pav4CL*     | F: CCAATGCAAGCTCATACC<br>R: GAGAAATGCAAGCAATTTCCTGG |
|               | GU990522  | *PavC4H*     | F: GAAGATCTGGCAGAAGGTC<br>R: GTCCCTCAGTATCCTCCC |
|               | GU938688  | *PavANR*     | F: GACCTGGTTGGCTGATTC<br>R: CGACACTGCAAGCTGGTAAT |
|               | GU938685  | *PavFLS*     | F: TTATACCCACCATGTCCC<br>R: ATGACCTAACGGTCTACC |
|               | GU938686  | *PavLAR*     | F: GGCGTACATCAGACAGGCT<br>R: ATCTCGGTCAATATTTG |
|               | AF036948  | *PavPAL*     | F: CATAGAGTTGGAGAC<br>R: CGAGGCTTCTGGCACCATCTG |
|               | JQ697494  | *F3'H*       | F: GTGGCTATTGTTGGAAATTG<br>R: GTGGCTATTGTTGGACATT |
|               | KP455680  | *PavMYB*     | F: GGTGGTCTGCTATTTT<br>R: GTGATGTTGCTGATGCTGTTG |
|               | FJ560908  | *PavACTIN*   | F: CCAGGGCTGTTTGCTTCTATT<br>R: ATGATCTGGCTGATCCTT |
| Cloning       | KP455680  | *PavMYB10.1* | F: ATGGAGGGCTATAACTTG<br>R: TTAGTCCCAGCTAATGGTACA |
|               | ABX71943  | *PavMYB10.2* | R: ATGGAGGGCTATAACTTG<br>F: CTATGGCTTCTCTGATATT |
| Yeast         | KP126521  | *PavHLH-EcoR1* | F: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC<br>R: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC |
| two-hybrid    | KP143539  | *PavWD40-NdeI* | F: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC<br>R: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC |
|               | KP455682  | *PavMYB10.1-FL-NdeI* | F: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC<br>R: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC |
|               | KP455683  | *PavMYB10.1-N-XhoI* | F: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC<br>R: GAAATTGATTTCTAGTGCTACGGGAAGCAGCAC |
| Subcellularly localized | KP455680 | PavMYB10.1-GFP-Xho I | F:GAAATTCATATGAAAAAGGTGAAAAGATAAACCACAGG |
|-------------------------|----------|----------------------|--------------------------------------------|
| Chromatin immunoprecipitation | KF974776 | Promoter of PavANS | F:TTGATAAAATTGAAAGAAACAT |
|                         |          | PavMYB10.1-GFP-Sac II | R:CAAGCATTGCTAATATTTTCAAA |
|                         | KF974777 | Promoter of PavUFGT | F:CGATATTGAGAGAGAAACAA |
|                         | AF036948 | Promoter of PavPAL | F:ATCCACAGATGATACCGTC |
| SNP marker              | Marker1637 | F:TCACACTGTAGATGACCTCATACTTTTGTAATTCAGTACTTG |
|                         | Marker3823 | F:GCTTGTGAAACTGTACTTTTGCTTGTGTTGTTGTAATGTTG |

SNP marker

Marker1637
F:TCACACTGTAGATGACCTCATACTTTTGTAATTCAGTACTTG
R:CTAATTTCCTAAACTGTTTTTGAATTCAATTGCAATG

Marker3823
F:GCTTGTGAAACTGTACTTTTGCTTGTGTTGTTGTAATGTTG
R:CTGCTGATATCTTTGATGCTAGTTTTCAATCATATTACCATTTT