

**Supplementary Table S1:**

Summary of compound identification from blueberry tissues using LC-MS. RT: retention time; CAS: Chemical abstract service registry number; Equivalence: quantified in equivalence of the listed standard compound; ID confidence: Degree of confidence in accuracy identification.

Compound	Abbreviation	RT(min)	Formula	CAS	Exact Mass	Equivalence	ID confidence
(E)-caffeooyl 4-glucoside	E.Caf4glu	4.68	C15H18O9		342.0951	chlorogenic acid	2
4-coumaroylshikimic acid	X5.CouShA	0	C16H16O7	196496-50-1	320.0896	chlorogenic acid	3
5-caffeooylshikimic acid	X5.CafShA	7.37	C16H16O8	73263-62-4	336.0845	chlorogenic acid	2
caffeoic acid	CafA	5.42	C9H8O4	331-39-5	180.0423	chlorogenic acid	1
catechin	Cat	4.68	C15H14O6	154-24-4	290.079	catechin	1
chlorogenic acid	CGA	5.17	C16H18O9	327-97-9	354.0951	chlorogenic acid	1
cis-chlorogenic acid	cisCGA	6.21	C16H18O9	15016-60-1	354.0951	chlorogenic acid	2
epicatechin	epiCat	6.42	C15H14O6	490-49-0	290.079	epicatechin	1
ferulic acid	FA	8.85	C10H10O4	1135-24-6	194.0579	chlorogenic acid	1
gallic acid	GA	1.26	C7H6O5	149-91-7	170.0215	epicatechin	1
gallocatechin	GalCat	2.38	C15H14O7	970-73-0	306.0739	epicatechin	3
isorhamnetin 3-glucoside	Iso.glu	10.65	C22H22O12	5041-82-7	478.1111	quercetin 3-galactoside	2
isorhamnetin 3-glucuronide	Iso.gluc	10.86	C22H20O13	36687-76-0	492.0904	quercetin 3-galactoside	2
isorhamnetin 3-rhamnoside	Iso.rha	11.63	C22H22O11	67068-82-0	462.116	quercetin 3-galactoside	2
kaempferol 3-glucoside	K.glu	10.6	C21H20O11	480-10-4	448.1006	quercetin 3-galactoside	2
kaempferol 3-rutinoside	K.rut	10.3	C27H30O15	17650-84-9	594.1585	quercetin 3-galactoside	1
laricitrin 3-galactoside	Lar.gal	9.91	C22H22O13	93219-26-2	494.106	quercetin 3-galactoside	2
laricitrin 3-glucoside	Lar.glu	9.99	C22H22O13	39986-90-8	494.106	quercetin 3-galactoside	2
laricitrin 3-glucuronide	Lar.gluc	9.99	C22H20O14	1026666-46-5	508.0853	quercetin 3-galactoside	2
laricitrin 3-rhamnoside	Lar.rha	10.81	C22H22O12		478.1111	quercetin 3-galactoside	2
leucocyanidin	leucoCy	4.3	C15H14O7	480-17-1	306.074	epicatechin	1
leucodelphinidin	leucoDp	0	C15H14O8	491-52-1	322.0689	epicatechin	3
myricetin 3-arabinoside	My.ara	9.45	C20H18O12	26856-98-4	450.0798	quercetin 3-galactoside	2
myricetin 3-galactoside	My.gal	8.57	C21H20O13	15648-86-9	480.0904	quercetin 3-galactoside	2
myricetin 3-glucoside	My.glu	8.73	C21H20O13	19833-12-6	480.0904	quercetin 3-galactoside	2
myricetin 3-glucuronide	My.gluc	8.64	C21H18O14	77363-65-6	494.0697	quercetin 3-galactoside	2
myricetin 3-rhamnoside	My.rha	9.57	C21H20O12	17912-87-7	464.0955	quercetin 3-galactoside	2
naringenin	Nar	13.33	C15H12O5	480-41-1	272.0685	quercetin 3-galactoside	1
neochlorogenic acid	neoCGA	3.14	C16H18O9	202650-88-2	354.0951	chlorogenic acid	2
p-coumaric acid	pCouA	7.67	C9H8O3	7400-08-0	164.0473	quercetin 3-galactoside	1
procyanidin B1	PC.B1	4.28	C30H26O12	20315-25-7	578.1424	procyanidin B2	1
procyanidin B2	PC.B2	6.03	C30H26O12	29106-49-8	578.1424	procyanidin B2	1
procyanidin Cx	PC.Cx	5.37	C45H38O18	37064-30-5	866.2058	procyanidin B2	2
quercetin	Q	12.44	C15H10O7	117-39-5	302.0427	quercetin 3-galactoside	1
quercetin 3-acetyl-glucoside	Q.aceglu	10.28	C23H22O13	54542-51-7	506.106	quercetin 3-galactoside	2
quercetin 3-arabinopyranoside	Q.arapy	10.47	C20H18O11	22255-13-6	434.0849	quercetin 3-galactoside	2
quercetin 3-galactoside	Q.gal	9.71	C21H20O12	482-36-0	464.0955	quercetin 3-galactoside	1
quercetin 3-glucoside	Q.glu	9.86	C21H20O12	482-35-9	464.0955	quercetin 3-galactoside	2
quercetin 3-glucuronide	Q.gluc	9.79	C21H18O13	22688-79-5	478.0747	quercetin 3-galactoside	2
quercetin 3-rhamnoside	Q.rham	10.66	C21H20O11	522-12-3	448.1006	quercetin 3-galactoside	1
quercetin 3-rutinoside	Q.rut	9.63	C27H30O16	153-18-4	610.1534	quercetin 3-galactoside	1
quercetin 3-xyloside	Q.xyl	10.19	C20H18O11	549-32-6	434.0849	quercetin 3-galactoside	2
syringetin 3-galactoside	Sy.gal	10.77	C23H24O13	55025-56-4	508.1217	quercetin 3-galactoside	2
syringetin 3-glucoside	Sy.glu	10.82	C23H24O13	40039-49-4	508.1217	quercetin 3-galactoside	2
syringetin 3-glucuronide	Sy.glc	10.91	C23H22O14	1094607-09-6	522.101	quercetin 3-galactoside	2
syringetin 3-rhamnoside	Sy.rh	11.7	C23H24O12	93126-00-2	492.1268	quercetin 3-galactoside	2
trans-4-p-coumaroyl quinic acid	t4.CouQA	6.6	C16H18O8	1108200-72-1	338.1002	trans-4-p-coumaroyl quinic acid	1
cyanidin 3-(6-acetyl)glucoside	Cy.acglu	12.63	C23H23O12	784107-03-5	491.1184	cyanidin 3-glucoside	2
cyanidin 3-arabinoside	Cy.ara	7.96	C20H19O10	27214-72-8	419.0973	cyanidin 3-glucoside	2
cyanidin 3-galactoside	Cy.gal	6.62	C21H21O11	142506-26-1	449.1078	cyanidin 3-glucoside	1
cyanidin 3-glucoside	Cy.glu	7.31	C21H21O11	7084-24-4	449.1078	cyanidin 3-glucoside	1
delphinidin 3-(6-acetyl)glucoside	Dp.acglu	11.36	C23H23O13	753416-12-5	507.1133	cyanidin 3-glucoside	2
delphinidin 3-arabinoside	Dp.ara	6.8	C20H19O11	324533-67-7	435.0922	cyanidin 3-glucoside	2
delphinidin 3-galactoside	Dp.gal	5.5	C21H21O12	28500-00-7	465.1028	cyanidin 3-glucoside	2
delphinidin 3-glucoside	Dp.glu	6.08	C21H21O12	50986-17-9	465.1028	cyanidin 3-glucoside	2
malvidin 3-(6-acetyl)glucoside	Mv.acglu	14.37	C25H27O13	732279-31-1	535.1446	cyanidin 3-glucoside	2
malvidin 3-arabinoside	Mv.ara	11.24	C22H23O11	863107-21-5	463.1235	cyanidin 3-glucoside	2
malvidin 3-galactoside	Mv.gal	9.88	C23H25O12	104880-34-4	493.1341	cyanidin 3-glucoside	2
malvidin 3-glucoside	Mv.glu	10.49	C23H25O12	7228-78-6	493.1341	cyanidin 3-glucoside	1
peonidin 3-(6-acetyl)glucoside	Pn.acglu	14.05	C24H25O12	751447-22-0	505.1341	cyanidin 3-glucoside	2
peonidin 3-arabinoside	Pn.ara	10.3	C21H21O10	27214-74-0	433.1129	cyanidin 3-glucoside	2
peonidin 3-galactoside	Pn.gal	8.93	C22H23O11	260256-26-6	463.1235	cyanidin 3-glucoside	2
peonidin 3-glucoside	Pn.glu	9.65	C22H23O11	68795-37-9	463.1235	cyanidin 3-glucoside	2
petunidin 3-(6-acetyl)glucoside	Pt.acglu	13.91	C24H25O13	774148-75-3	521.129	cyanidin 3-glucoside	2
petunidin 3-arabinoside	Pt.ara	9.09	C21H20O11	749848-37-1	449.1078	cyanidin 3-glucoside	2
petunidin 3-galactoside	Pt.gal	7.78	C22H23O12	260256-23-3	479.119	cyanidin 3-glucoside	2
petunidin 3-glucoside	Pt.glu	8.37	C22H23O12	6988-81-4	479.119	cyanidin 3-glucoside	2

ID Confidence

1 High-tested against authentic standard

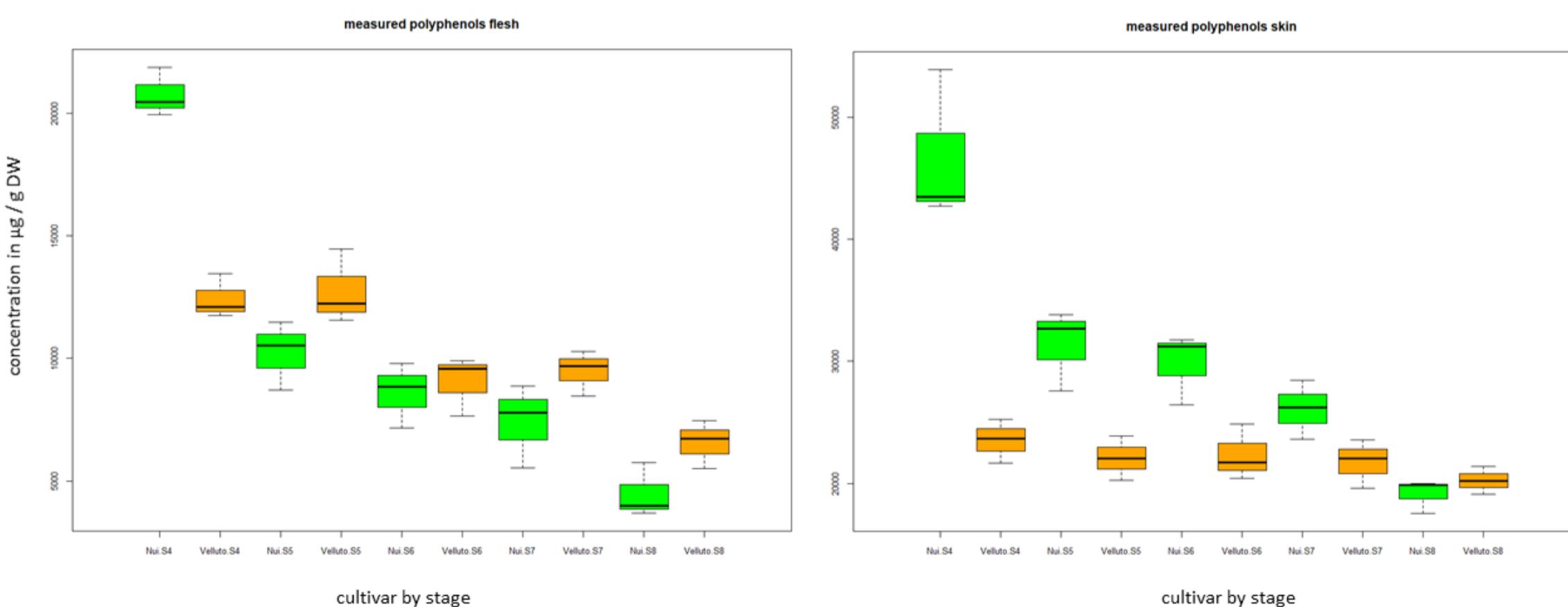
2 Good- exact mass

3 Reasonable- ambiguity possible

**Table S2:** Glycosylation patterns of anthocyanidins from blueberry (*Vaccinium*) skin.

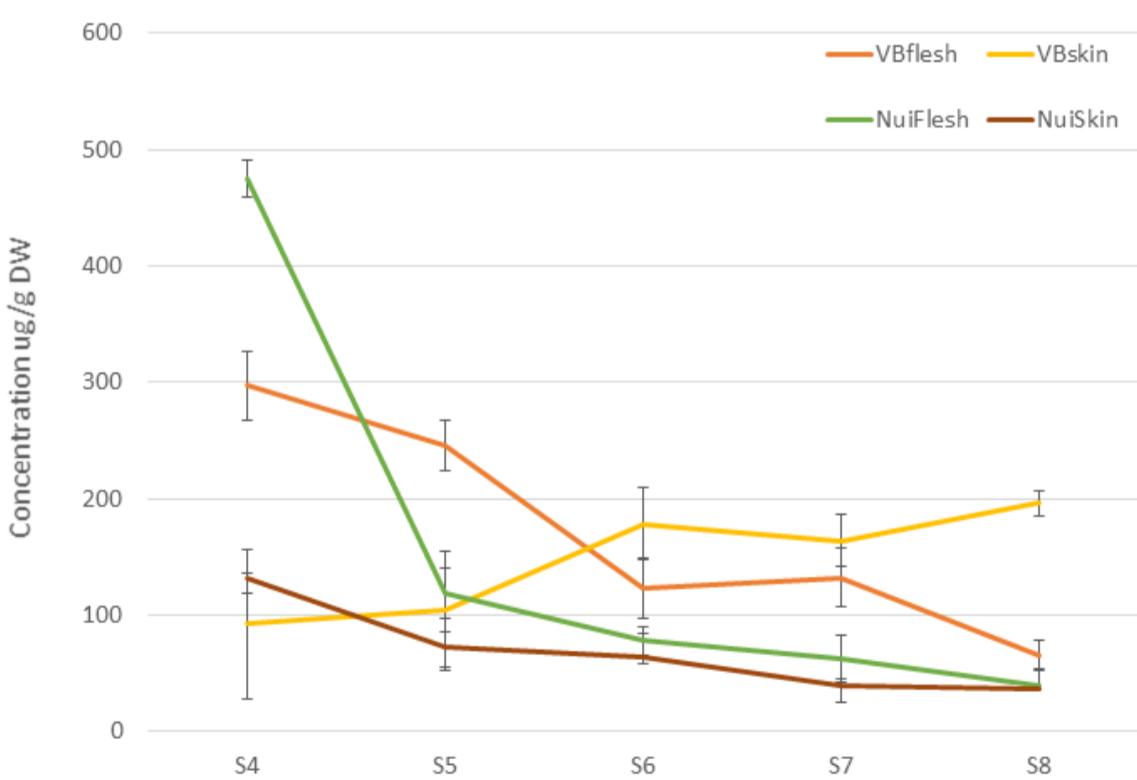
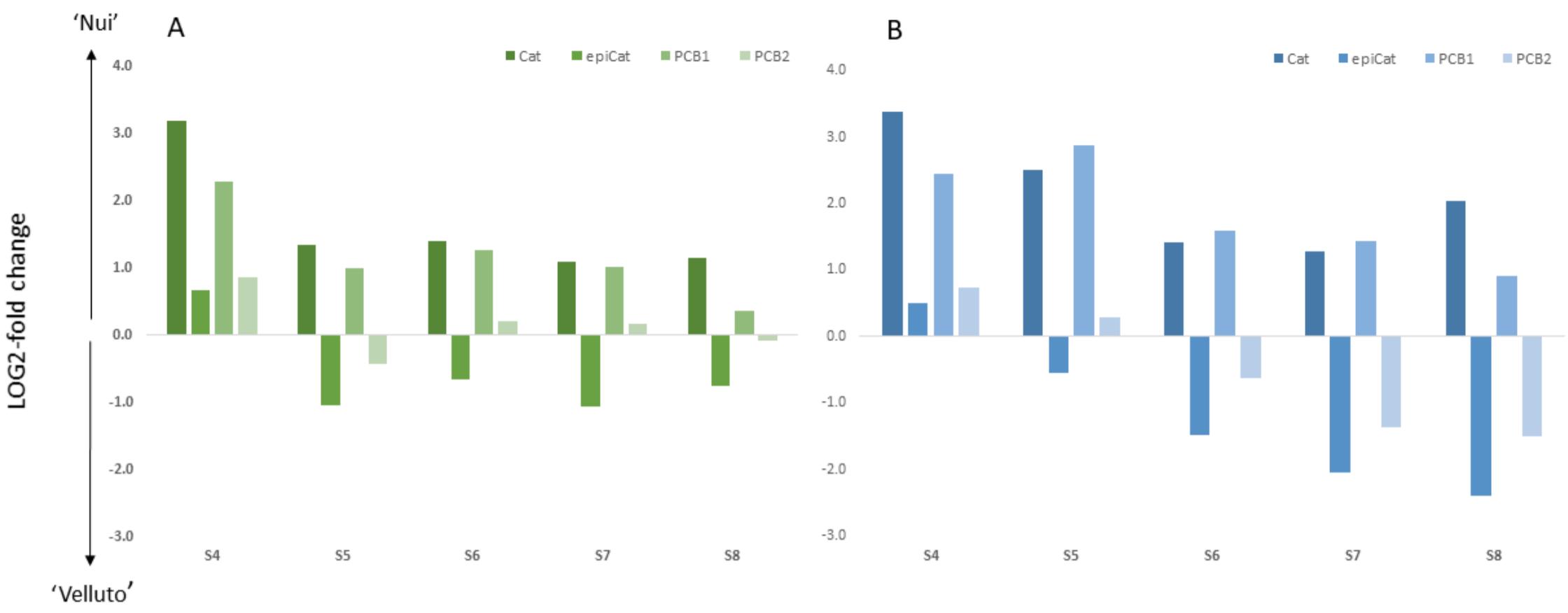
Proportions were averaged over development if time had no significant effect (Kruskal-Wallis test,  $\alpha=0.05$ ) on concentrations. If levels differed over development the stage producing maximum and minimum amounts is listed.

	<i>V. virgatum</i> 'Velluto Blue'			<i>V. corymbosum</i> 'Nui'			
	Average % $\pm$ Std			Average % $\pm$ Std (%)			
	<u>Arabinoside</u>	<u>Galactoside</u>	Glucoside	<u>AcetylGlucoside</u>	<u>Arabinoside</u>	<u>Galactoside</u>	Glucoside
<u>Cyanidin</u>	S4: $39.8 \pm 1.3$	S4: $60.2 \pm 1.3$	$6.0 \pm 1.7$	$28.9 \pm 2.9$	$22.8 \pm 5.3$	$19.4 \pm 3.3$	$29 \pm 1.9$
	S7: $18.6 \pm 1.9$	S7: $75.9 \pm 2.5$					
<u>Peonidin</u>	$20.9 \pm 0.1$	$63.4 \pm 0.5$	$15.7 \pm 0.8$	$31.2 \pm 1.6$	$12.7 \pm 2.5$	$15.4 \pm 0.4$	$40.8 \pm 3.7$
<u>Delphinidin</u>	S5: $41.2 \pm 9.3$	$66.2 \pm 5.7$	$4.2 \pm 0.8$	$11.8 \pm 1.4$	S6: $33.9 \pm 1.8$	S6: $21.2 \pm 0.9$	S6: $33.8 \pm 1.2$
	S8: $30.2 \pm 2.9$				S8: $24.4 \pm 2$	S8: $25.5 \pm 1$	S8: $39 \pm 1.7$
<u>Malvidin</u>	S6: $26.6 \pm 2.7$	$62.9 \pm 7.3$	$8.6 \pm 1.9$	S6: $11.3 \pm 0.2$	$16.7 \pm 1.6$	$20.4 \pm 1.7$	$39.4 \pm 3.65$
	S8: $38 \pm 2.3$			S7: $16.8 \pm 2.1$			
<u>Petunidin</u>	$29.5 \pm 4.8$	$65.6 \pm 2.5$	S6: $4.8 \pm 0.9$	$16.7 \pm 1.7$	$22.2 \pm 4.7$	$18 \pm 2.8$	$43.1 \pm 0.9$
			S8: $7.9 \pm 0.6$				



**Figure S1:**

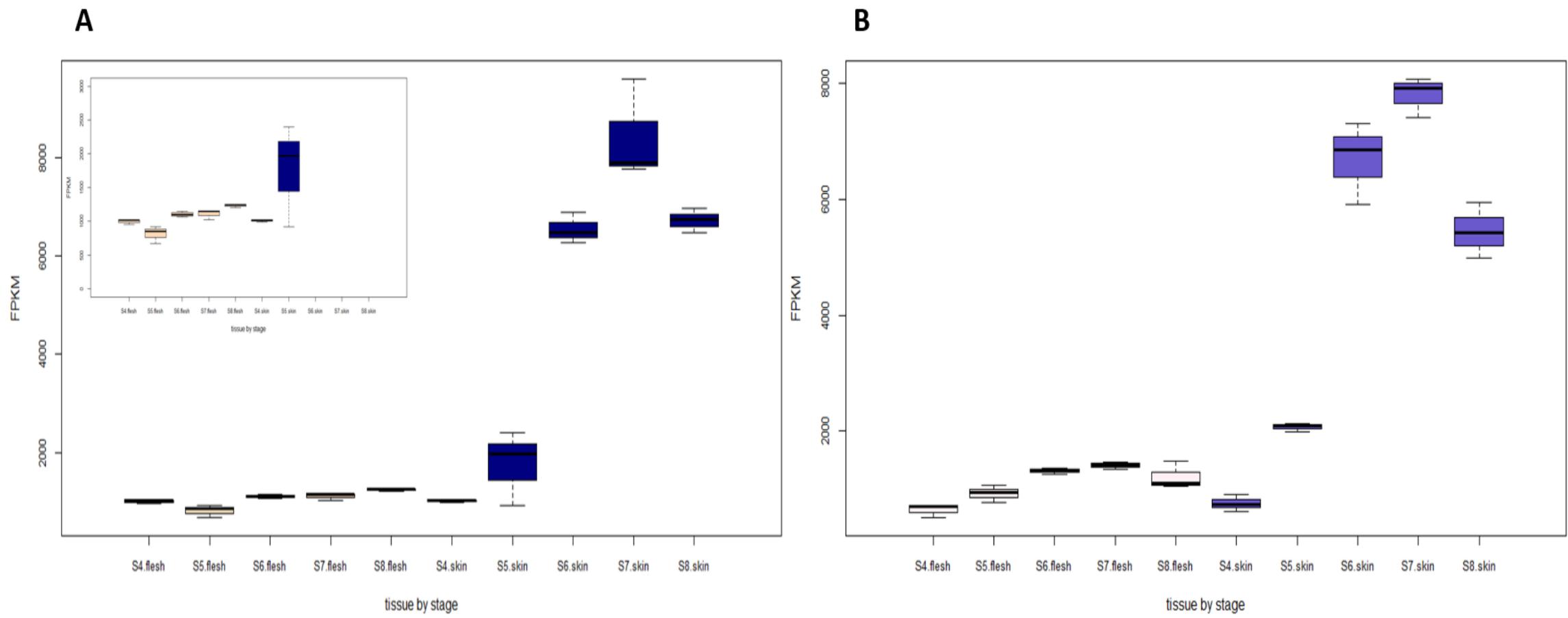
Concentrations of total measured polyphenols in blueberry (*Vaccinium virgatum* 'Velluto Blue' (orange); *V. corymbosum* 'Nui' (green)) fruit tissue types (left: flesh; right: skin) during maturation (S4-S8).



**Table S3:**

Overview of gene mapping results and functional annotation. Candidate genes were identified by mapping transcripts to *V. corymbosum* Reference Transcriptome (RefTrans V1) from the Genome Database For *Vaccinium* (GDV) and annotated using the KEGG/ KASS server. Genome location was then identified through reciprocal blast against the chromosome-scale genome assembly of tetraploid highbush blueberry from Michigan State University (MSU).

Name	Abbreviation	KEGG	reftrans ID	MSU ID
CL1	4CL	K01904	v.corymbosum_GDV_reftransV1_0009935	maker-VaccDscaff46-augustus-gene-86.25
CL2	4CL	K01904	v.corymbosum_GDV_reftransV1_0018642	maker-VaccDscaff47-augustus-gene-2.17
CL3	4CL	K01904	v.corymbosum_GDV_reftransV1_0023569	maker-VaccDscaff37-augustus-gene-303.53
CL4	4CL	K01904	v.corymbosum_GDV_reftransV1_0030312	augustus_masked-VaccDscaff50-processed-gene-21.6
CL5	4CL	K01904	v.corymbosum_GDV_reftransV1_0033876	maker-VaccDscaff47-augustus-gene-69.25
CL6	4CL	K01904	v.corymbosum_GDV_reftransV1_0020364	maker-VaccDscaff48-augustus-gene-108.40
A3GT1	UFGT	K12930	v.corymbosum_GDV_reftransV1_0000638	maker-VaccDscaff28-augustus-gene-290.34
A3GT2	UFGT	K12930	v.corymbosum_GDV_reftransV1_0001574	maker-VaccDscaff6-augustus-gene-420.36
A3GT3	UFGT	K12930	v.corymbosum_GDV_reftransV1_0012230	maker-VaccDscaff37-augustus-gene-300.29
A3GT4	UFGT	K12930	v.corymbosum_GDV_reftransV1_0018085	maker-VaccDscaff19-snap-gene-64.36
A3GT5	UFGT	K12930	v.corymbosum_GDV_reftransV1_0035590	maker-VaccDscaff34-augustus-gene-294.29
ANR	ANR	K08695	v.corymbosum_GDV_reftransV1_0028563	maker-VaccDscaff19-augustus-gene-256.19
ANS	ANS	K05277	v.corymbosum_GDV_reftransV1_0032663	maker-VaccDscaff43-augustus-gene-236.29
C3.H	C3'H	K09754	v.corymbosum_GDV_reftransV1_0034725	maker-VaccDscaff1-augustus-gene-373.37
C4.H1	C3'H	K00487	v.corymbosum_GDV_reftransV1_0032452	maker-VaccDscaff24-augustus-gene-58.38
C4.H2	C3'H	K00487	v.corymbosum_GDV_reftransV1_0005046	augustus_masked-VaccDscaff33-processed-gene-307.8
CAD1	CAD	K00083	v.corymbosum_GDV_reftransV1_0016328	maker-VaccDscaff3-augustus-gene-213.23
CAD2	CAD	K00083	v.corymbosum_GDV_reftransV1_0016712	maker-VaccDscaff22-augustus-gene-306.29
CAD3	CAD	K00083	v.corymbosum_GDV_reftransV1_0021895	maker-VaccDscaff49-snap-gene-1.40
CAD4	CAD	K00083	v.corymbosum_GDV_reftransV1_0022475	maker-VaccDscaff22-snap-gene-141.41
CAD5	CAD	K00083	v.corymbosum_GDV_reftransV1_0004320	maker-VaccDscaff149-snap-gene-2.68
cCoAOMT1	OMT	K00588	v.corymbosum_GDV_reftransV1_0036845	maker-VaccDscaff34-snap-gene-139.31
cCoAOMT2	OMT	K00588	v.corymbosum_GDV_reftransV1_0038613	augustus_masked-VaccDscaff42-processed-gene-262.6
CCR1	CCR	K09753	v.corymbosum_GDV_reftransV1_0024732	maker-VaccDscaff4-augustus-gene-398.17
CCR2	CCR	K09753	v.corymbosum_GDV_reftransV1_0024733	maker-VaccDscaff8-snap-gene-149.28
CCR3	CCR	K09753	v.corymbosum_GDV_reftransV1_0024734	augustus_masked-VaccDscaff36-processed-gene-33.3
CCR4	CCR	K09753	v.corymbosum_GDV_reftransV1_0007209	maker-VaccDscaff27-snap-gene-293.30"
CHI	CHI	K01859	v.corymbosum_GDV_reftransV1_0019487	maker-VaccDscaff26-snap-gene-196.29
CHS1	CHS	K00660	v.corymbosum_GDV_reftransV1_0007549	augustus_masked-VaccDscaff9-processed-gene-64.0
CHS2	CHS	K00660	v.corymbosum_GDV_reftransV1_0017397	maker-VaccDscaff13-augustus-gene-46.32
CHS3	CHS	K00660	v.corymbosum_GDV_reftransV1_0018037	maker-VaccDscaff2-augustus-gene-67.14
COMT1	OMT	K13066	v.corymbosum_GDV_reftransV1_0014435	maker-VaccDscaff20-augustus-gene-5.31
COMT2	OMT	K13066	v.corymbosum_GDV_reftransV1_0033992	maker-VaccDscaff20-snap-gene-377.34
DFR1	DFR	K13082	v.corymbosum_GDV_reftransV1_0001248	augustus_masked-VaccDscaff13-processed-gene-166.8
DFR2	DFR	K13082	v.corymbosum_GDV_reftransV1_0026455	maker-VaccDscaff12-snap-gene-66.28
F3.5.H1	F3'5'H	K13083	v.corymbosum_GDV_reftransV1_0036273	maker-VaccDscaff29-augustus-gene-305.28
F3.5.H2	F3'5'H	K13083	v.corymbosum_GDV_reftransV1_0036277	maker-VaccDscaff19-augustus-gene-162.21
F3.5.H3	F3'5'H	K13083	v.corymbosum_GDV_reftransV1_0001233	maker-VaccDscaff10-augustus-gene-348.25
F3.5.H4	F3'5'H	K13083	v.corymbosum_GDV_reftransV1_0007214	maker-VaccDscaff24-augustus-gene-336.30
F3.H	F3'H	K05280	v.corymbosum_GDV_reftransV1_0007478	maker-VaccDscaff32-augustus-gene-159.26
F3H1	F3H	K00475	v.corymbosum_GDV_reftransV1_0033001	maker-VaccDscaff32-augustus-gene-323.35
F3H2	F3H	K00475	v.corymbosum_GDV_reftransV1_0038468	snap_masked-VaccDscaff7-processed-gene-48.23
FLS1	FLS	K05278	v.corymbosum_GDV_reftransV1_0030204	maker-VaccDscaff6-augustus-gene-163.26
FLS2	FLS	K05278	v.corymbosum_GDV_reftransV1_0032793	maker-VaccDscaff25-augustus-gene-225.24
HCT1	HCT	K13065	v.corymbosum_GDV_reftransV1_0006514	augustus_masked-VaccDscaff38-processed-gene-5.8
HCT2	HCT	K13065	v.corymbosum_GDV_reftransV1_0006673	maker-VaccDscaff24-augustus-gene-299.21
HCT3	HCT	K13065	v.corymbosum_GDV_reftransV1_0035212	maker-VaccDscaff27-snap-gene-291.30
LAR1	LAR	K13081	v.corymbosum_GDV_reftransV1_0032700	maker-VaccDscaff25-augustus-gene-293.25
LAR2	LAR	K13081	v.corymbosum_GDV_reftransV1_0033478	maker-VaccDscaff28-augustus-gene-232.26
LAR3	LAR	K13081	v.corymbosum_GDV_reftransV1_0007959	maker-VaccDscaff9-augustus-gene-315.13
PAL1	PAL	K10775	v.corymbosum_GDV_reftransV1_0003392	maker-VaccDscaff19-augustus-gene-331.32
PAL2	PAL	K10775	v.corymbosum_GDV_reftransV1_0013816	maker-VaccDscaff10-augustus-gene-222.29
PAL3	PAL	K10775	v.corymbosum_GDV_reftransV1_0024098	maker-VaccDscaff29-augustus-gene-173.18
VcbHLH075	bHLH		v.corymbosum_GDV_reftransV1_0002395	maker-VaccDscaff17-augustus-gene-313.25
VcbHLH1	bHLH		v.corymbosum_GDV_reftransV1_0032614	maker-VaccDscaff22-snap-gene-21.29
VcbHLH2	bHLH		v.corymbosum_GDV_reftransV1_0008144	maker-VaccDscaff19-augustus-gene-381.30
VcMYB4	Repressor		v.corymbosum_GDV_reftransV1_0019415	maker-VaccDscaff37-augustus-gene-96.16
VcMYBA	Activator		v.corymbosum_GDV_reftransV1_0025517	maker-VaccDscaff1486-snap-gene-0.3
VcMYBC2	Repressor		v.corymbosum_GDV_reftransV1_0001734	maker-VaccDscaff28-augustus-gene-197.19
VcMYBPA1	Activator		v.corymbosum_GDV_reftransV1_0038996	maker-VaccDscaff39-snap-gene-168.25
VcMYBPA2a	Activator		v.corymbosum_GDV_reftransV1_0003384	maker-VaccDscaff32-augustus-gene-55.27
VcMYBPA2b	Activator		v.corymbosum_GDV_reftransV1_0015556	maker-VaccDscaff33-snap-gene-307.38
VcMYBR3	Repressor		unknown	maker-VaccDscaff4-snap-gene-174.23
VcWDR1	WDR		v.corymbosum_GDV_reftransV1_0019141	maker-VaccDscaff28-augustus-gene-346.33



**Figure S4:** Combined transcript counts (FPKM) of biosynthetic genes and transcription factors for *Vaccinium corymbosum* 'Nui' (A) and *V. virgatum* 'Velluto Blue' (B) fruit tissue (pink: flesh; purple: skin) during maturation development (S4 green/unripe- S8 purple/ ripe).

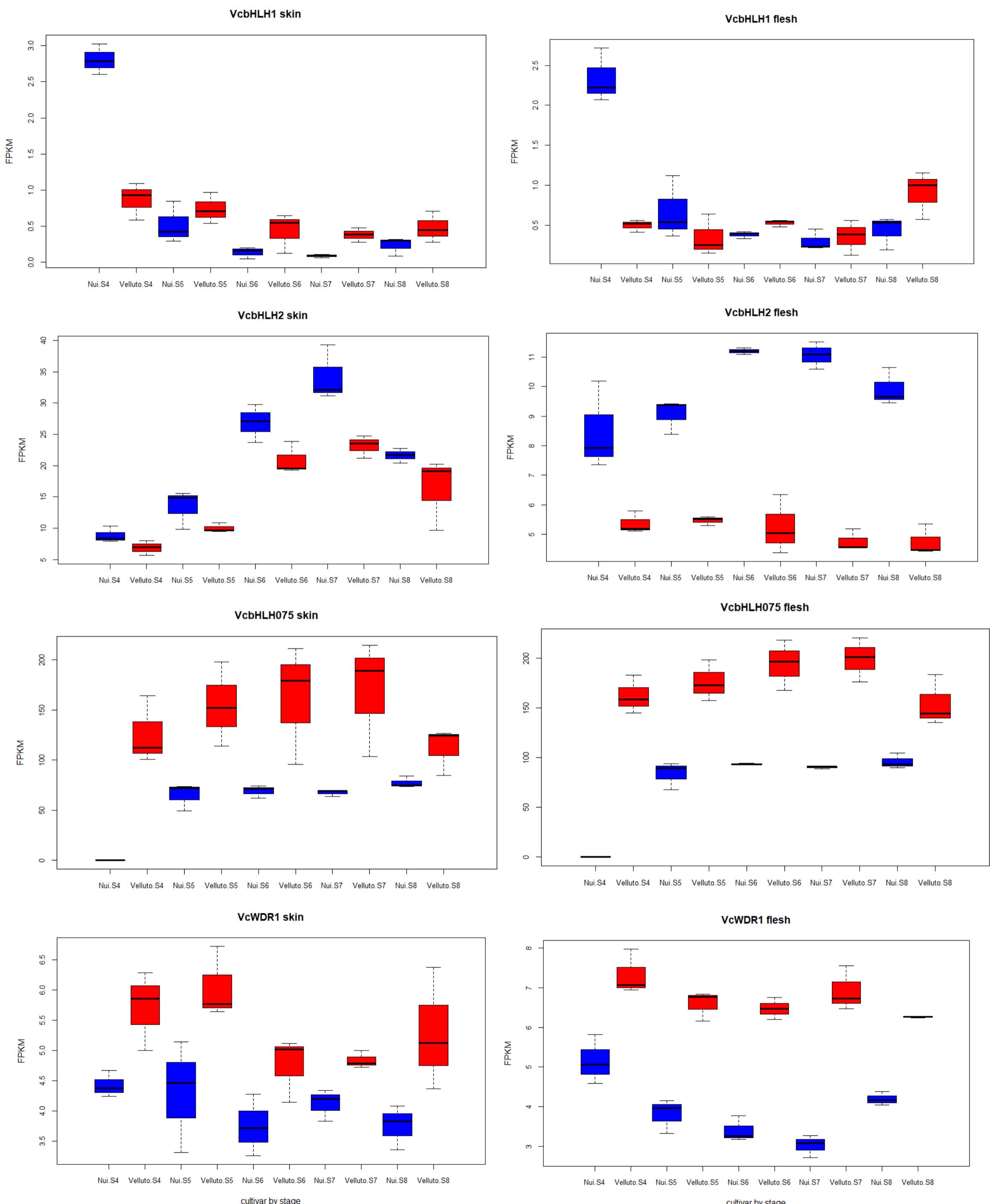
**Table S4:**

Summary of factorial Analysis of Variance testing the effect of blueberry cultivar (*Vaccinium virgatum* 'Velluto Blue' and *V. corymbosum* 'Nui'), tissue type (fruit skin, fruit flesh) and their interaction on gene expression of candidate transcription factors. Normalised (FPKM) values were Log10 –transformed for data normalisation and P-values adjusted (Padj) using Benjamini Hochberg method for False discovery rate correction. Significant ( $\alpha=0.05$ ) results are highlighted in red.

	Cultivar		Tissue		Interaction	
	F-value	P <sub>adj</sub> -value	F-value	P <sub>adj</sub> -value	F-value	P <sub>adj</sub> -value
bHLH075	16.10	<b>5.41 x 10<sup>-04</sup></b>	0.24	0.72	0.00	0.99
bHLH1	0.39	0.63	1.16	0.36	2.41	0.19
bHLH2	24.55	<b>3.3 x 10<sup>-05</sup></b>	71.32	<b>1.2 x 10<sup>-10</sup></b>	3.44	0.12
MYB4	7.45	<b>0.02</b>	22.28	<b>6.7x10<sup>-05</sup></b>	4.48	0.07
MYBA	5.07	0.06	20.66	<b>9.9 x10<sup>-05</sup></b>	0.13	0.8
MYBC2	5.23	0.05	47.50	<b>3.4 x10<sup>-08</sup></b>	1.45	0.33
MYBPA1	0.01	0.97	175.23	<b>2.3x 10<sup>-17</sup></b>	1.20	0.36
MYBPA2a	2.55	0.19	5.65	0.05	0.90	0.42
MYBPA2b	45.46	<b>5.1 x 10<sup>-08</sup></b>	106.35	<b>1.6x 10<sup>-13</sup></b>	1.35	0.35
MYBR3	5 x 10 <sup>-06</sup>	0.99	20.64	<b>9.9x 10<sup>-05</sup></b>	2.40	0.19
WDR1	120.10	<b>2.5 x 10<sup>-14</sup></b>	6.10	<b>0.04</b>	14.69	<b>8.9 x 10<sup>-04</sup></b>

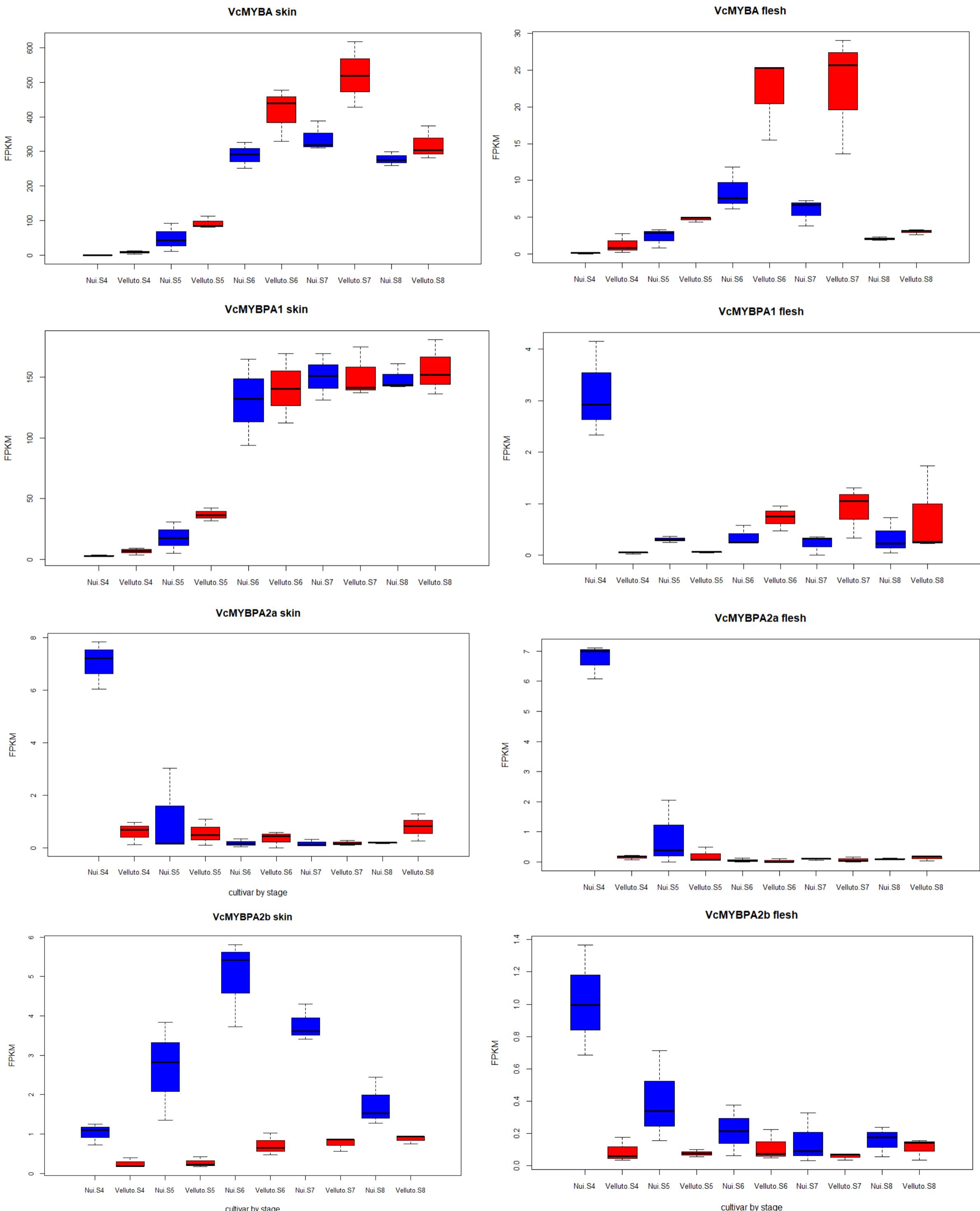
**Figure S5A:**

bHLH and WDR1 gene expression in *Vaccinium virgatum* 'Velluto Blue' (red) and *V. corymbosum* 'Nui' (blue) fruit tissue types over development (S4-S8)



**Figure S5B:**

MYB activator gene expression in *Vaccinium virgatum* 'Velluto Blue' (red) and *V. corymbosum* 'Nui' (blue) fruit tissue types over development (S4-S8)



**Figure S5C:**

MYB repressor gene expression in *Vaccinium virgatum* 'Velluto Blue' (red) and *V. corymbosum* 'Nui' (blue) fruit tissue types over development (S4-S8)

