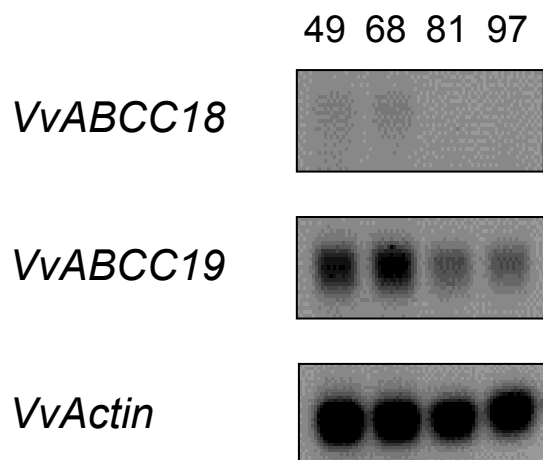


## ABCC1, an ATP-binding Cassette Protein from Grape Berry, Transports Anthocyanidin 3-O- Glucosides

The following material is available online:

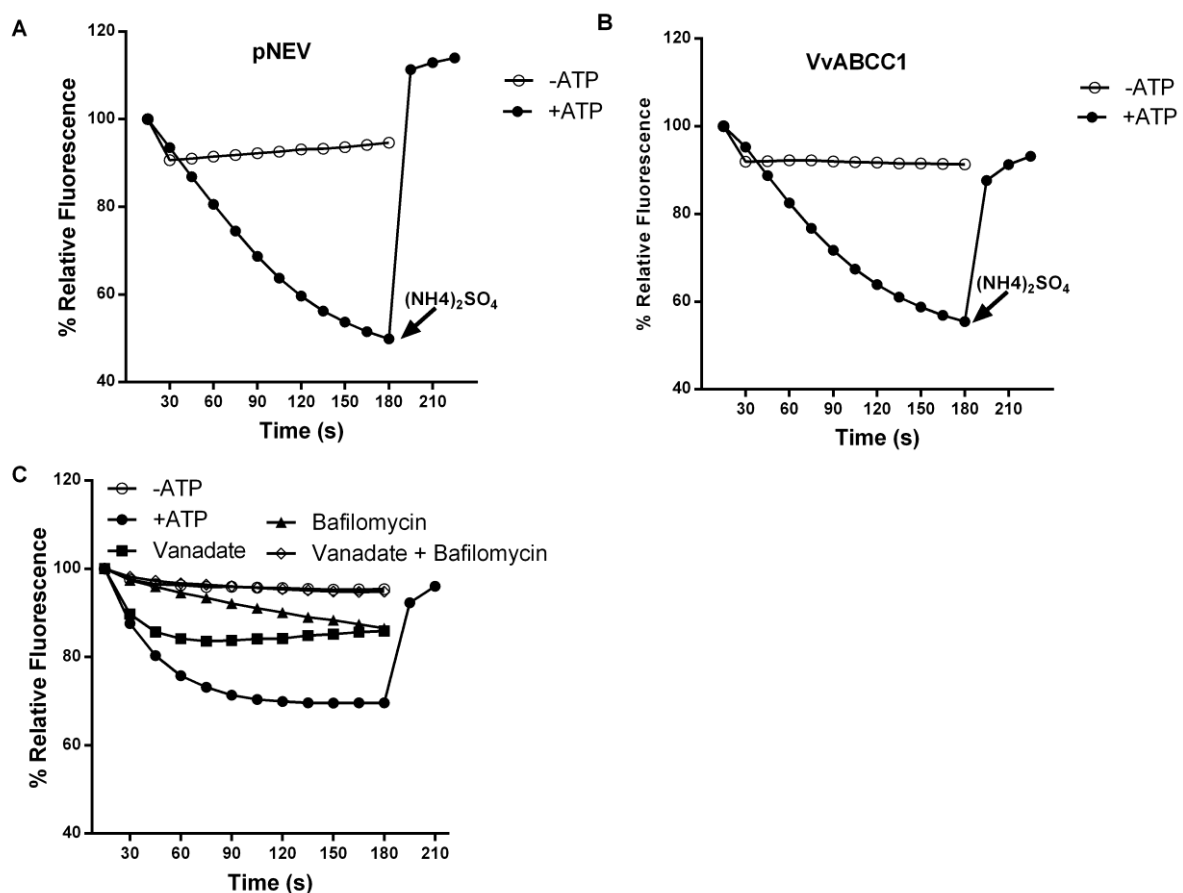
### Supplemental Figure 1. Expression analyses of the closest homologous of ABCC1/2

Standard RT-PCR was performed with 0.5 µg of total RNA isolated from berry exocarps during different fruit developmental/ripening stages (49, 68, 81 and 97 days after flowering). PCR analyses for analyzing the expression of *ABCC2*, *ABCC 14*, *ABCC16*, *ABCC18* and *ABCC19* were performed with gene-specific primers. PCR products with the actin primers were used as a loading control. PCR products were visualized on ethidium bromide-containing agarose gels. No amplification was observed for *ABC2*, *ABCC14* and *ABCC16*.



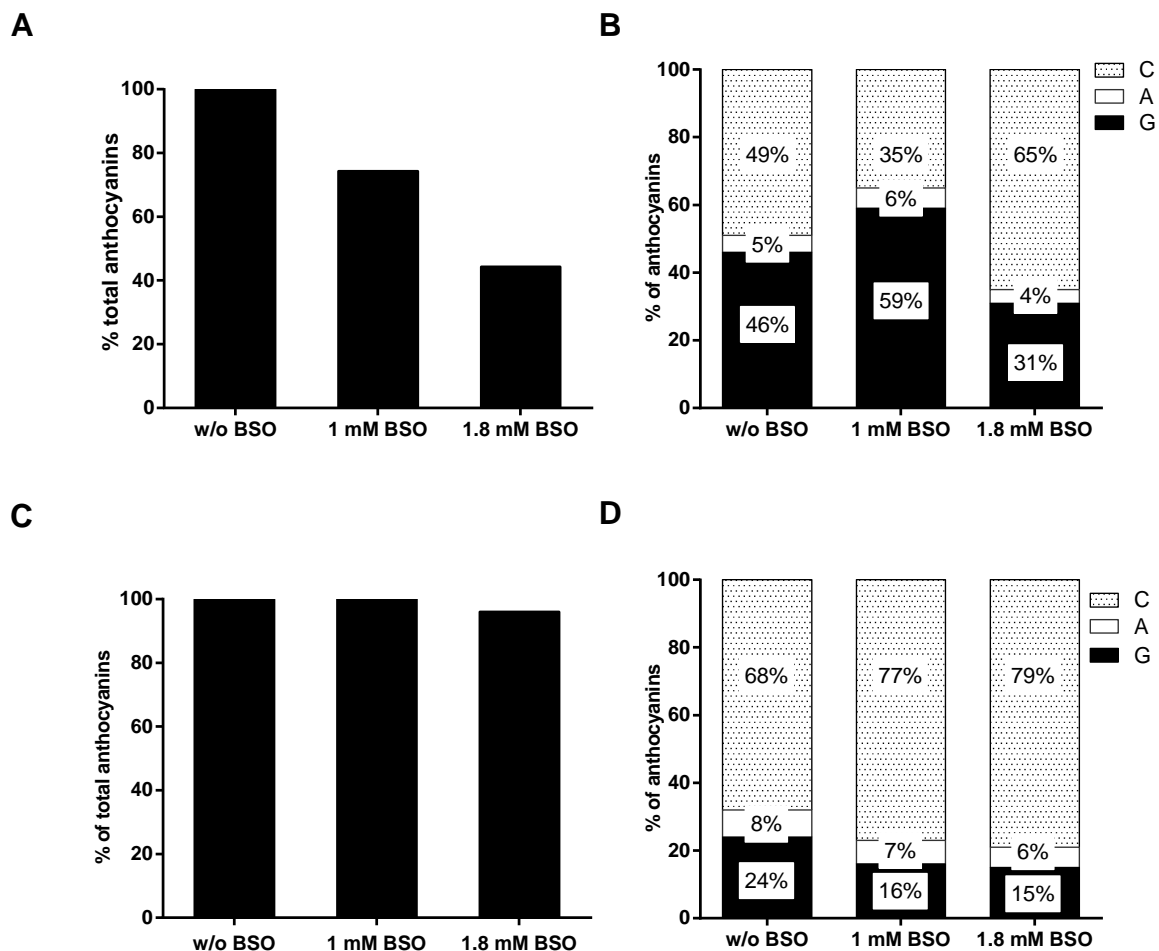
## Supplemental Figure 2. Assessment of physiological intactness of yeast microsomes

To assess the physiological intactness of the isolated vesicles, we chose the lumen acidification method. The acidification is the result of activity of various ATPases. The ATP-dependent quenching of the fluorescent dye 9-amino-6-chloro-2-methoxyacridin (ACMA) indicates that the vesicles are sealed and intact, and the pH gradient is generated. Addition of  $(\text{NH}_4)_2\text{SO}_4$ , restored the basic fluorescence, indicating that the proton gradient formed across the membranes collapsed, proof that the vesicles were intact. In **A**) results for the vector control-pNEV, in **B**) the results for pNEV-VvABCC1 are presented. **C**) To test whether both vacuolar and plasma membrane vesicles are present, and to what extent are they sealed, we performed the ACMA quenching experiment in the presence of vanadate (inhibitor of the plasma membrane ATPases), bafilomycin (inhibitor of vacuolar ATPases), and vanadate+bafilomycin. This is a representative figure; the test was performed for each of the used microsome preparation.



### Supplemental Figure 3. The effect of BSO on anthocyanin concentration and profile in hairy roots from Portan and Syrah cultivars,

Anthocyanin-producing Portan (A,B) and Syrah (C,D) hairy roots were grown on increasing concentrations of buthionine sulfoximine (BSO), a compound that inhibits glutamyl-cysteine synthetase, the enzyme responsible for the first step in glutathione synthesis. Root samples were harvested 14 days post BSO treatment. Anthocyanin content was determined using HPLC. A, C) Increasing BSO concentration led to a decrease in the total anthocyanin content. The anthocyanin content in roots grown on media without BSO was considered as 100%. B, D) Analysis of the anthocyanin profile in the root samples described in A, C) shows that BSO preferentially affects the proportions of the glycosylated fraction, and its effects are stronger in cultivars rich in glycosylated anthocyanins. Three independent experiments were performed. Here, the most representative data of three independent experiments are presented. G, glucosylated-, A, acylated-, and C, coumaroylated anthocyanins.



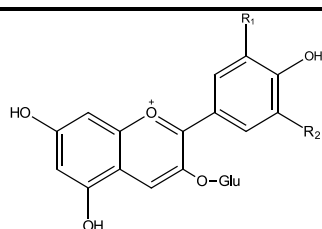
## Supplemental Table 1. *Vitis vinifera* ABCC protein inventory

Summary of the *Vitis vinifera* ABCC proteins with their NCBI RefSeq IDs, GENOSCOPE IDs and chromosomal locations. Remarks regarding gene models and annotations are detailed in the Methods section below. Chromosomal locations are given for the GENOSCOPE gene models. (\*) The protein and coding sequence of VvABCC1 were experimentally determined in this work (sequence submitted to GenBank under the accession number: JX245004) and deviated from sequences of corresponding gene models identified in the NCBI and GENOSCOPE databases (in brackets).

| Name      | NCBI ID           | GENOSCOPE ID                             | Location         | Start (bp) | End (bp) | Orient. | Length (aa) | Sequence based on |
|-----------|-------------------|--|------------------|------------|----------|---------|-------------|-------------------|
| VvABCC1 * | (XP_002267650.2)* | (GSVIVT01028722001)*                     | (GENOSCOPE_chr16 | 19447213   | 19454986 | +)*     | 1480        | This work         |
| VvABCC2   | XP_002266842.1    | GSVIVT01028744001                        | GENOSCOPE_chr16  | 19218947   | 19224345 | -       | 1525        | NCBI model        |
| VvABCC3   | XP_002266601.1    | GSVIVG01010326001                        | GENOSCOPE_chr1   | 19526696   | 19532390 | +       | 1462        | NCBI model        |
| VvABCC4   | XP_002265012.1    | GSVIVG01027990001                        | GENOSCOPE_chr7   | 3032722    | 3040092  | +       | 1509        | NCBI model        |
| VvABCC5   | XP_002269589.1    | GSVIVG01028440001                        | GENOSCOPE_chr7   | 7535201    | 7546064  | -       | 1540        | NCBI model        |
| VvABCC6   | XP_002280819.1    | GSVIVG01021589001                        | GENOSCOPE_chr10  | 7565358    | 7609701  | +       | 1623        | NCBI model        |
| VvABCC7   | XP_002281070.1    | GSVIVG01021595001                        | GENOSCOPE_chr10  | 7765400    | 7821944  | +       | 1624        | NCBI model        |
| VvABCC8   | XP_003632995.1    | GSVIVT01021593001 &<br>GSVIVT01021594001 | GENOSCOPE_chr10  | 7694908    | 7752675  | +       | 1550        | NCBI model        |
| VvABCC9   | XP_002265346.2    | GSVIVG01018403001                        | GENOSCOPE_chr15  | 9661841    | 9670181  | +       | 1528        | NCBI model        |
| VvABCC10  | XP_002264313.1    | GSVIVG01015960001                        | GENOSCOPE_chr9   | 16112767   | 16273585 | -       | 1305        | NCBI model        |
| VvABCC11  | XP_002276193.2    | GSVIVG01016879001-A                      | GENOSCOPE_chr9   | 2205746    | 2211753  | +       | 1502        | NCBI model        |
| VvABCC12  | XP_002276212.2    | GSVIVG01016879001-B                      | GENOSCOPE_chr9   | 2218514    | 2225094  | +       | 1509        | NCBI model        |
| VvABCC13  | XP_002276236.2    | GSVIVG01016880001                        | GENOSCOPE_chr9   | 2225152    | 2233977  | +       | 1505        | NCBI model        |
| VvABCC14  | XP_002271828.2    | GSVIVG01019467001                        | GENOSCOPE_chr2   | 870871     | 883981   | -       | 1530        | NCBI model        |
| VvABCC15  | XP_003631374.1    | GSVIVG01019469001                        | GENOSCOPE_chr2   | 886847     | 894005   | -       | 1524        | NCBI model        |
| VvABCC16  | XP_002271761.2    | GSVIVG01019471001                        | GENOSCOPE_chr2   | 899832     | 914539   | -       | 1537        | NCBI model        |
| VvABCC17  | XP_002271657.1    | GSVIVG01019476001                        | GENOSCOPE_chr2   | 952858     | 960480   | -       | 1391        | NCBI model        |
| VvABCC18  | XP_002271728.1    | GSVIVG01019473001                        | GENOSCOPE_chr2   | 916178     | 932015   | -       | 1490        | Manual annot.     |
| VvABCC19  | XP_002271694.2    | GSVIVG01019475001                        | GENOSCOPE_chr2   | 933100     | 947137   | -       | 1532        | NCBI model        |
| VvABCC20  | XP_002265605.2    | GSVIVG01037789001                        | GENOSCOPE_chr19  | 7910839    | 7917664  | -       | 1529        | NCBI model        |
| VvABCC21  | -                 | GSVIVG01014633001                        | GENOSCOPE_chr19  | 8023417    | 8033145  | -       | 1428        | GENOSCOPE model   |
| VvABCC22  | -                 | GSVIVT01014629001                        | GENOSCOPE_chr19  | 7936173    | 7941661  | -       | 1004        | Manual annot.     |
| VvABCC23  | XP_003634755.1    | GSVIVG01014632001                        | GENOSCOPE_chr19  | 7977107    | 7983555  | -       | 1532        | NCBI model        |
| VvABCC24  | XP_003634753.1    | GSVIVG01014628001                        | GENOSCOPE_chr19  | 7925931    | 7935919  | -       | 1530        | NCBI model        |
| VvABCC25  | -                 | GSVIVT01014631001                        | GENOSCOPE_chr19  | 7967803    | 7976826  | -       | 1314        | Manual annot.     |
| VvABCC26  | -                 | GSVIVG01028459001                        | GENOSCOPE_chr7   | 7912161    | 7916627  | +       | 287         | GENOSCOPE model   |

## Supplemental Table 2. Structure and distribution of the five major anthocyanins of grape berries

The table summarizes the general anthocyanin structure; R1 and R2 represent the substitution pattern specific to each anthocyanin. Cultivars are characterized by a distinct pattern of anthocyanins (represented as % of the total anthocyanin content).



|                                  | R1               | R2               | Tempranillo | Cabernet Sauvignon | Syrah |
|----------------------------------|------------------|------------------|-------------|--------------------|-------|
| <b>Delphinidin 3-O-glucoside</b> | OH               | OH               | 16.64       | 9.99               | 5.2   |
| <b>Cyanidin 3-O-glucoside</b>    | OH               | H                | 6.53        | 1.33               | 1     |
| <b>Petudinin 3-O-glucoside</b>   | OCH <sub>3</sub> | OH               | 12.33       | 6.07               | 6.1   |
| <b>Peonidin 3-O-glucoside</b>    | OCH <sub>3</sub> | H                | 12.53       | 5.27               | 8.3   |
| <b>Malvidin 3-O-glucoside</b>    | OCH <sub>3</sub> | OCH <sub>3</sub> | 36.68       | 42.57              | 36.4  |
| <b>Other forms</b>               | -                | -                | 15.29       | 34.77              | 43    |

Adapted from Mazza, 1995