



**Figure S1.** Expression and localization of GFP-tagged native grapevine NIPs and their ar/R mutants in H2, H5, H2/H5 helices in the membrane of aqy-null *S. cerevisiae*. Left panel show images under phase contrast, whereas right panel pictures are taken under fluorescence microscopy at 100X magnification. Cytosolic accumulation of GFP was observed in yeast cells, transformed with empty plasmid pUG35.

**Table S1.** Primers used in the PCR for Site-directed Mutagenesis in ar/R selectivity filter of grapevine NIPs.

| Primer name      | Sequence   |
|------------------|--|
| NIP1;1 W86T Fwd  | GGAATATCCATAGTGACGGGACTGGTTGTGATG                |
| NIP1;1 W86T Rev  | CATCACAAACCAGTCCCGTCACTATGGATATTCC               |
| NIP1;1 V206I Fwd | GCTACTGTGTTACTTAATATCATGTTTGCAGGGCCA             |
| NIP1;1 V206I Rev | TGGCCCTGCAAACATGATATTAAGTAACACAGTAGC             |
| NIP2;1 G87A Fwd  | AAGCTTGGAGCATCGGTTGCAGCTGGACTGATAGTCACAGCTATG    |
| NIP2;1 G87A Rev  | CATAGCTGTGACTATCAGTCCAGCTGCAACCGATGCTCCAAGCTT    |
| NIP2;1 S206I Fwd | TCAGCAGTATGCATAACTATCATCTTGGCAGGACCAGTATCAGGT    |
| NIP2;1 S206I Rev | ACCTGATACTGGTCCTGCCAAGATGATAGTTATGCATACTGCTGA    |
| NIP5;1 A111G Fwd | ATAGGGAATGCGGCATGCGGCGGGCTTGCAGTATGATAGTGATT     |
| NIP5;1 A111G Rev | AATCACTATCATCACTGCAAGCCCGCCGCATGCCGCATTCCCTAT    |
| NIP5;1 I230S Fwd | ACTGTTATGCTGAACAGTCTTGTGGCAGGGCCGTCG             |
| NIP5;1 I230S Rev | CGACGGCCCTGCCACAAGACTGTTTCAGCATAACAGT            |
| NIP6;1 T118W Fwd | TCCTGGGGCTGGCAGCCTCCTGGGGGCTGGCGGTGATGGTCATAATAC |
| NIP6;1 T118W Rev | GTATTATGACCATCACCGCCAGCCCCAGGAGGCTGCCAGCCCCAGGA  |
| NIP6;1 I239V Fwd | ACTGTCATGCTCAATGTTCTCATCGCCGGGGAAACC             |
| NIP6;1 I239V Rev | GGTTTCCCCGGCGATGAGAACATTGAGCATGACAGT             |

\*Underlined base pairs are for single amino acid substitution.