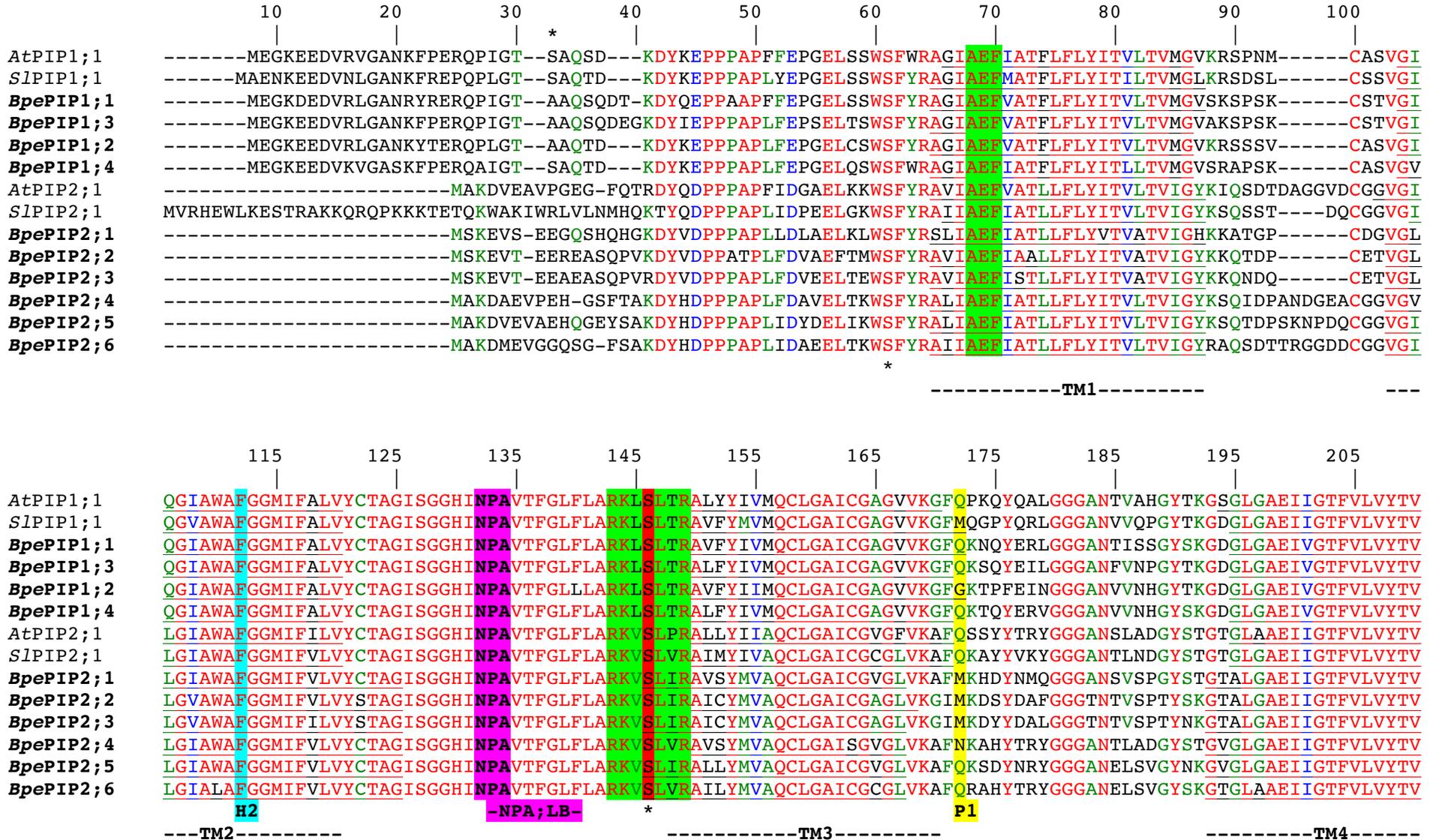
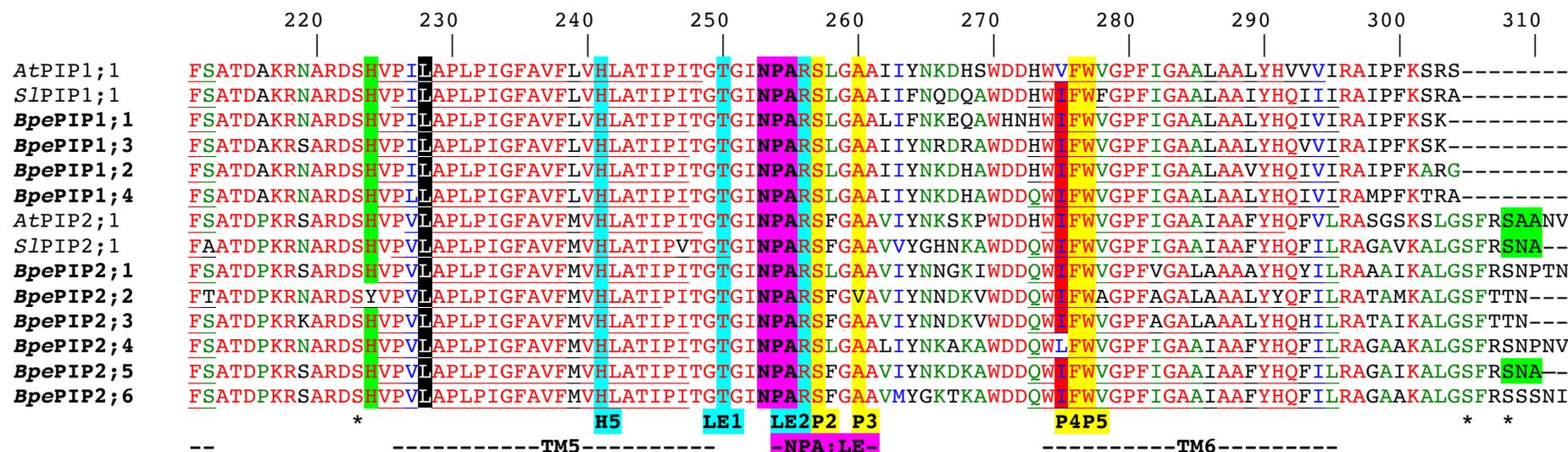


**Figure S6. Amino acid sequence alignments per subfamily of the *Bpe*AQP members from *Betula pendula*. S6A, PIP; S6B, TIP; S6C, XIP; S6D, NIP; S6E, SIP. Legends are detailed at the end of each subfamily alignment.**

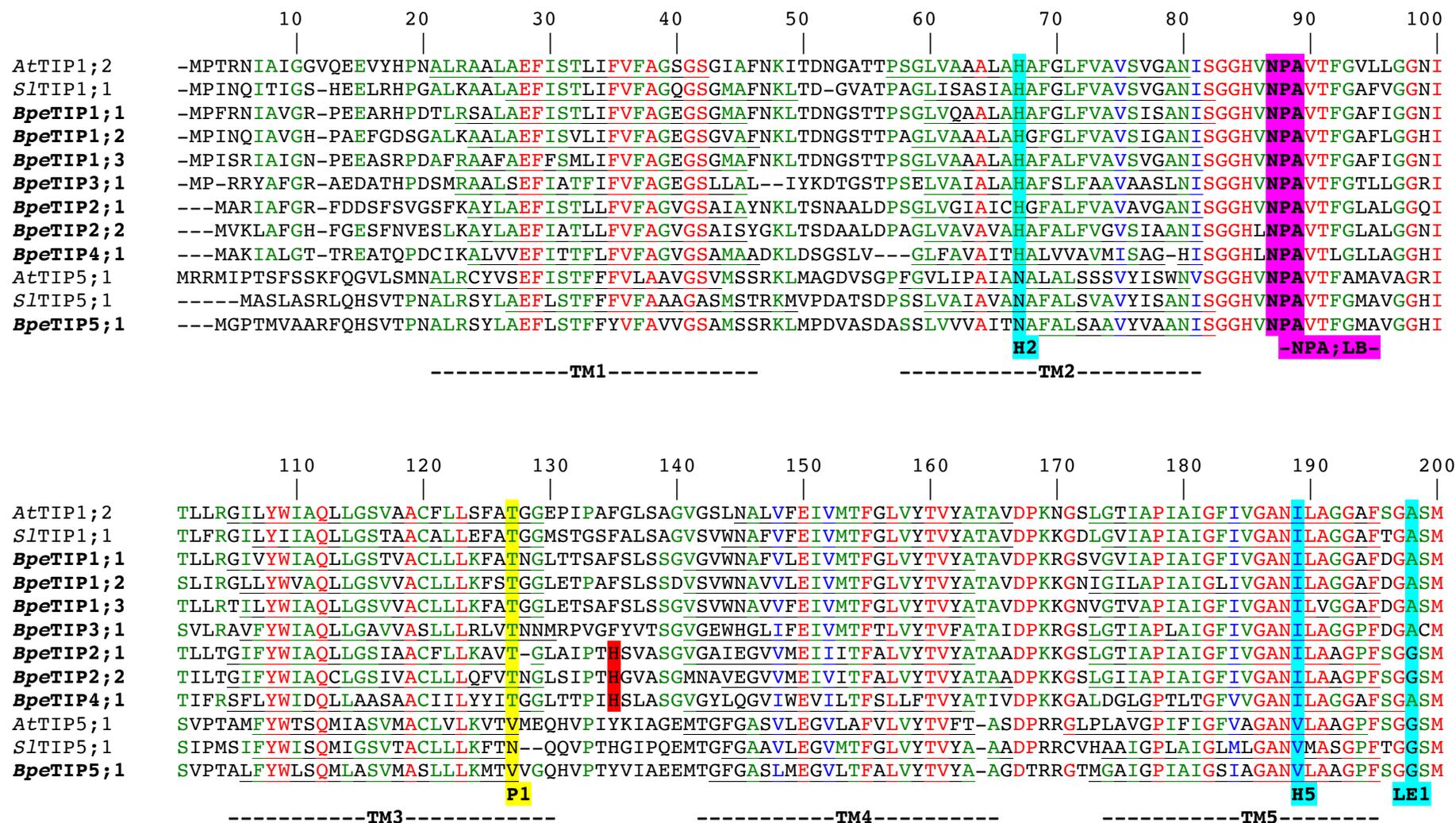
# Plasma membrane intrinsic proteins (PIPs)

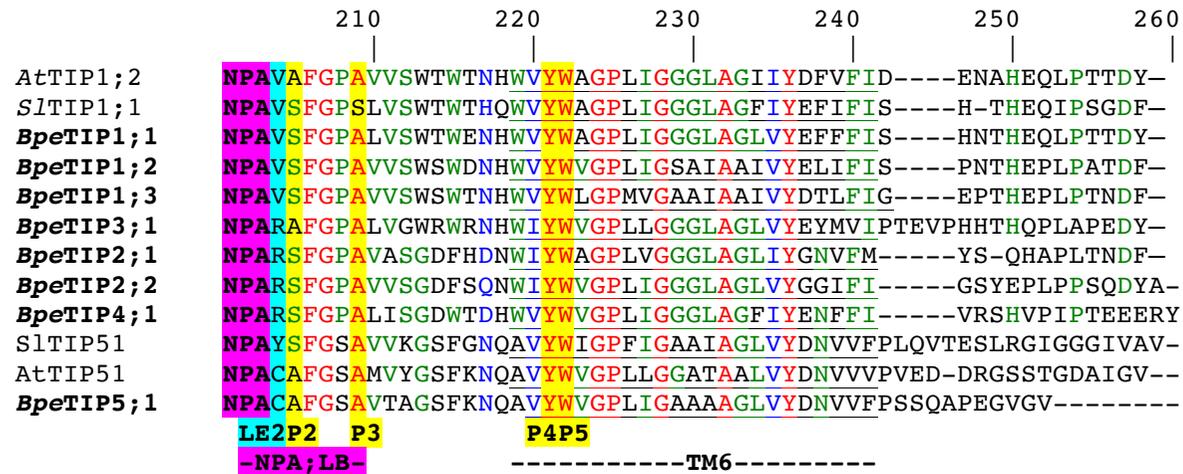




**Figure S6A. Amino acid sequence alignment of the *BpePIP* subfamily members from *Betula pendula*.** *S/PIP1;1* and *S/PIP2;1* (Solyc10g054840.1.1; Reuscher et al., 2013) from Tomato, and *AtPIP1;1* (At3g61430) and *AtPIP2;1* (At3g53420) (Muries et al., 2011) from *Arabidopsis thaliana* were used as references. The highest residue similarities between MIP members are shown in red (100%), and green (>80%). Residues with similar physicochemical behaviors are shown in blue. The six transmembrane regions (TM1-TM6) and the two hemi-helicoidal regions that include the «NPA» motives «NPA1» (in Loop B) and «NPA2» (in Loop E) (framed in purple) are underlined. The amino acids residues constituting the ar/R constriction region are shown with blue highlights and labeled H2, H5, LE1 and LE2. Residues constituting the Froger's positions are shown with yellow highlights and labeled P1 to P5. In addition, AEF motif, the His residue involved in gating, the RKXSXXR motif, the SxA motif for putative phosphorylation by PKC are marked in green, the KdvE motif for putative conserved methylation sites are marked in red, and the putative conserved blocking residue (L) is marked in black. Lastly, the Cysteines (C) involved in the tetramerization of PIPs are mentioned in purple. Columns or regions with conserved putative phosphorylation sites are marked with an asterisk. Isoleucine (I) at the position 275 on the alignment and surlined in red and placed in loop E would be key for the transport of CO<sub>2</sub>. Serine (S) at the position 146 on the alignment and surlined in red would be key for the transport of H<sub>2</sub>O<sub>2</sub>.

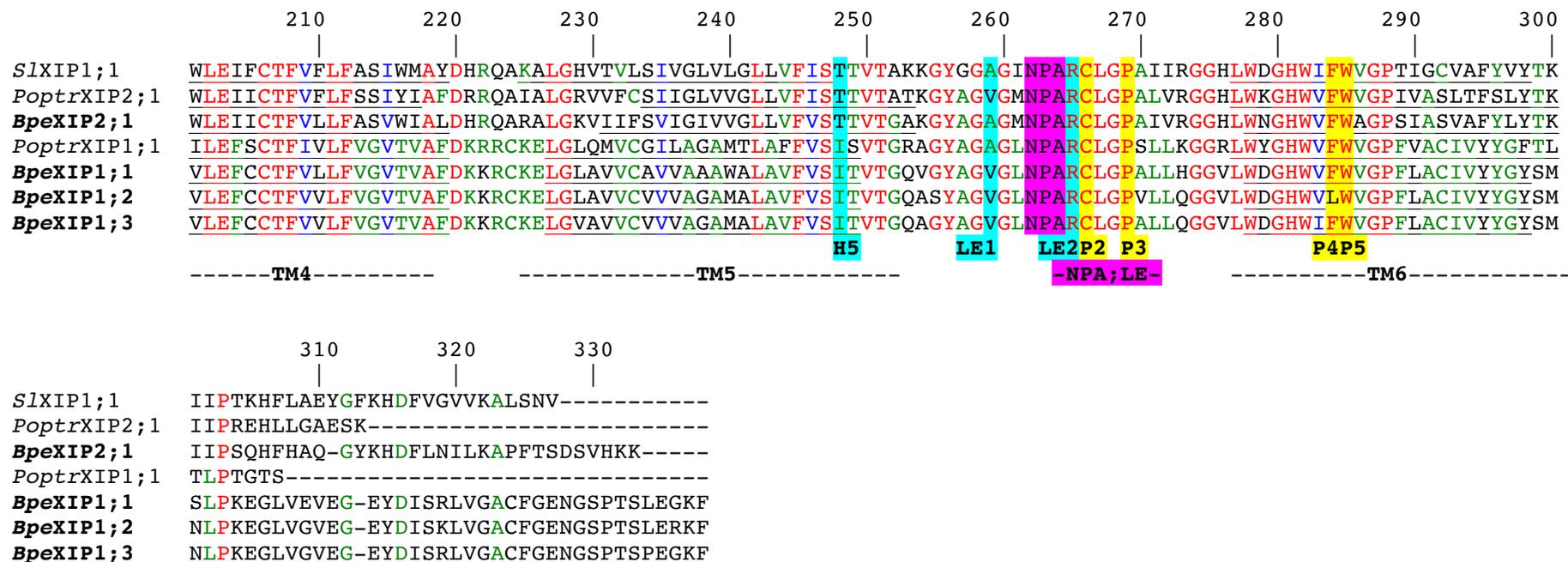
## Tonoplast intrinsic proteins (TIPs)





**Figure S6B. Amino acid sequence alignment of the *BpeTIP* subfamily members from *Betula pendula*.** *SlTIP1;1* (Solyc06g074820.1) and *SlTIP5;1* (Solyc03g093230.1) (Reuscher et al., 2013) from Tomato and *AtTIP1;1* (At2g36830) and *AtTIP5;1* (At3g47440) from *Arabidopsis thaliana* were used as references. The highest residue similarities between MIP members are shown in red (100%), and green (>80%). Residues with similar physicochemical behaviors are shown in blue. The six transmembrane domains (TMH1-6) and the two hemi-helicoidal regions that include the «NPA» motives «NPA1» (in Loop B) and «NPA2» (in Loop E) (framed in purple) are underlined. The amino acids residues constituting the ar/R constriction region are shown with blue highlights and labeled H2, H5, LE1 and LE2, and residues constituting the Froger's positions are shown with yellow highlights and labeled P1 to P5. Histidine (H) surligned in red and placed in loop C would be key for the de-protonation of  $\text{NH}_4^+$ , which would allow the transport of  $\text{NH}_3$  independent of pH.



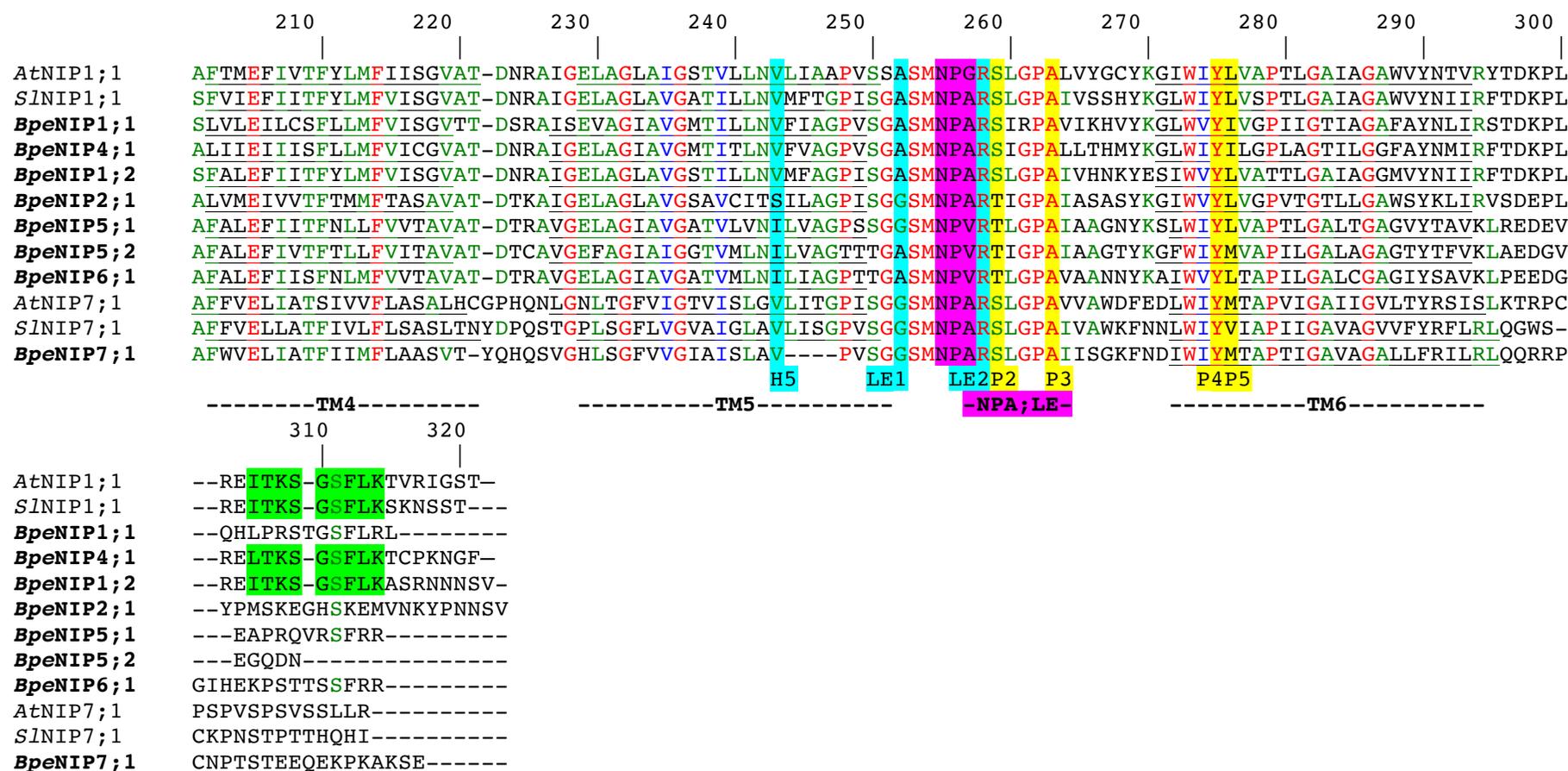


**Figure S6C. Amino acid sequence alignment of the *BpeXIP* subfamily members from *Betula pendula*.** *SlxIP1;1* (Solyc10g054840.1; Reuscher et al., 2013) from Tomato and *PoptrXIP2;1* and *PoptrXIP1;1* (Potri.009G128500.1, Potri.009G128100, resp.; Lopez et al, 2012) from Poplar were used as references. The highest residue similarities between MIP members are shown in red (100%), and green (>80%). Residues with similar physicochemical behaviors are shown in blue. The six transmembrane domains (TMH1-6) and the two hemi-helical regions that include the «NPA» motives «NPA1» (in Loop B) and «NPA2» (in Loop E) (framed in purple) are underlined. The amino acids residues constituting the ar/R constriction region are shown with blue highlights and labeled H2, H5, LE1 and LE2, and residues constituting the Froger's positions are shown with yellow highlights and labeled P1 to P5.

# Nodulin-26 like intrinsic proteins (NIPs)

	10	20	30	40	50	60	70	80	90	100						
<i>AtNIP1</i> ;1	-----	-----	MADISGNGYGNAREE	VVMVNLKDEVEHQ	EMEDIHNPRLKQD	SLLSVSVFPFLQKLI	AEFLGTYFLV	FTGCASVVVM	-----	-----						
<i>SlNIP1</i> ;1	-----	-----	MADHQINVNGNINHG	VSL-NIKEDHDLN	NHKESST----SS	-----	LTVPFIQKVI	AEEMIGTYFLI	FAGCGSVVN	-----						
<i>BpeNIP1</i> ;1	-----	-----	MGIEELEISKVEEG	VGTSAAATKTNSD	TVTAFCSASVVT	ITQKLI	AEVIGTYFVI	FAGCGAVAVN	-----	-----						
<i>BpeNIP4</i> ;1	-----	-----	MSSQDITKVEEGN	SSSSNNNGNSG	V----CNSSEI	VQLVQKVI	AEVIGTYFLI	FAGCGSVVN	-----	-----						
<i>BpeNIP1</i> ;2	-----	-----	MAENSGSNGSQS	ITL-NVKECEAN	HNQASSASK---	TNHGGGFCV	SVPFMQKLV	AEVLGTYFLI	FAGCGAVVNL	-----						
<i>BpeNIP2</i> ;1	-----	-----	MATDPKPKNLAE	ANEFVLENPISR	KPKP-GLLRAL	LEEHYP	PGFLSKV	VAETIATYLLV	FVTCGSAALSA	-----						
<i>BpeNIP5</i> ;1	-----	-----	-----	-----	-----	MYYFFFGGKSL	GFIIYLF--LS	---AFYLGAE	FVGTFFILIF	FMATAGPIVNQ						
<i>BpeNIP5</i> ;2	-----	-----	-----	-----	-----	MILGLNIFAS	MDQCLLIEI	IKVLKFI	FILNDMH---	NSTLGAEFVGT	FIMVFSAAA	APIVNE				
<i>BpeNIP6</i> ;1	MENEEVPSAP	STPATPGTPGAP	LFGGFKAERSG	NGRRSLLKNC	SKCFSDQEWAL	EETLPLKLS	CSLPPPI	IPLAKKVG	AEFIGTLLIF	FAGTATAMVNQ	-----	-----				
<i>AtNIP7</i> ;1	-----	-----	-----	-----	-----	MNGEARSRV	VDQEAGSTP	TLRDEDH	PSRQRLF	GCLPYDIDL	NPLRIV	MAELVGT	FILMF	FSVCGVIS	STQ	
<i>SlNIP7</i> ;1	-----	-----	-----	-----	-----	MKLPSYENGL	SVEFQVDAS	ASEQSTYD	QETTSNVEM	LERRNVCNS	ILGIDP	IFLRMV	LAEALGT	FLLMFC	ICGMMASME	
<i>BpeNIP7</i> ;1	-----	-----	-----	-----	-----	MKGQPHHLD	HITDHAPT	SGQSKDDQ	EMGVNAM	SKSDVLKNS	PFSCFP	QGM	DLNLGRV	VLAELVGT	FILMF	CVCGIIACTQ
																-----TM1-----

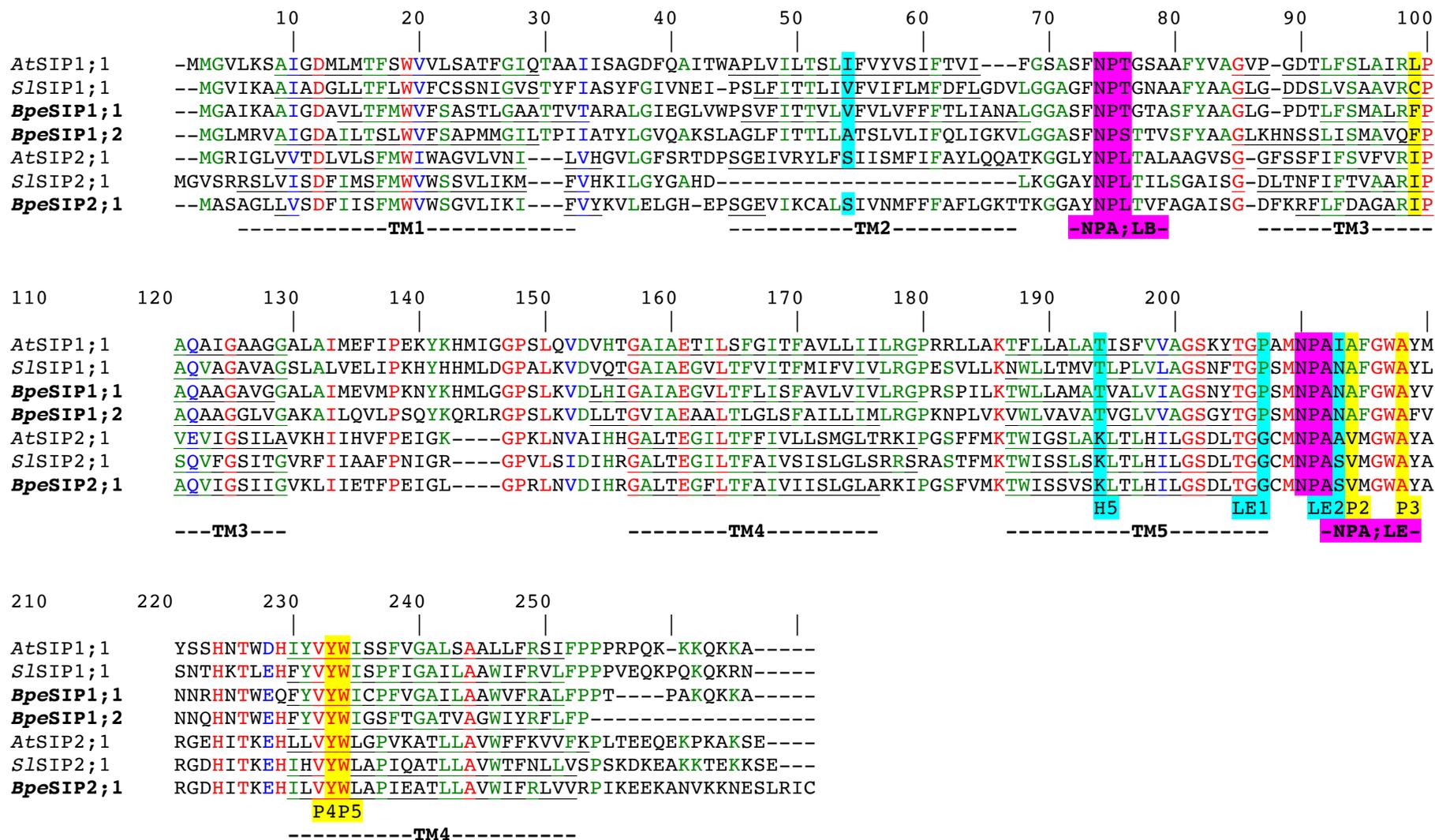
	110	120	130	140	150	160	170	180	190	200							
<i>AtNIP1</i> ;1	QNDNVV	TLPGIAIV	WGLTIM	VLIYSLGHIS	GAHINPA	VTIAFAS	CGRFPLKQ	VPAYVISQ	VIGSTL	AAATLRL	LFGLDHDV	CSGKHD	VFIGSS	PVGS	DLQ		
<i>SlNIP1</i> ;1	ADKGMIT	FPGVAIT	WGLVVM	VMVYSVGHIS	GAHFNPS	VTIAFAS	VKRFPWKQ	VPAYVA	AAQVLG	ATLASG	TLRLIF	-----	NGKHDH	FAGTLP	SGTDFQ		
<i>BpeNIP1</i> ;1	KIYGSV	TFPGICV	TWGLIV	TVMIYTVGHIS	GAHFNPA	VTIITSA	IFRRFPYKE	VPLYIA	AQLMGS	ILASG	TLALVF	-----	DVTPDAY	FGTVP	TGSNSQ		
<i>BpeNIP4</i> ;1	KIYGSV	SFPGICV	VWGLIV	VMVIYSVGHIS	GAHFNPA	VTIITFA	IFRRFPLKQ	VPLYIVA	QMIGST	LASATL	CALF	-----	PVDKKS	FFGTAP	VGSNFQ		
<i>BpeNIP1</i> ;2	GNDKVV	TAPGVS	IVWGLAV	MVLVYSVGHIS	GAHFNPA	VTIAFAT	CRRFPWKQ	VPAYVSA	QVLGSTM	ASGTL	RLIF	-----	NGHENR	FVGTIP	PAGSNLQ		
<i>BpeNIP2</i> ;1	ADENKV	SKLAAS	VAGGLIV	TVMIYAVGHIS	GAHMNPA	VTLFAA	AVRHFPWKQ	VPLYAA	AQLAGAI	SAF	TLRVIL	-----	NPIKEL	GTTSPT	GTETIE		
<i>BpeNIP5</i> ;1	KYNGAES	LIGNAAC	AGLAVM	VVILSTGHIS	GAHLNPS	LTIAFAT	LRHFPWLQ	VPAYIA	AQVSGS	ICASF	ALKGVF	-----	HPYMSG	GVTV	PSVTEGQ		
<i>BpeNIP5</i> ;2	KYQGAET	LIGSAAC	AGLAVM	MVLSLSTGHIS	GAHFNPS	VTIAFAL	RHFPWVQ	VPYLAQ	VSA	ICAF	TLKGVF	-----	HPFMSG	GVTV	PAVGIGQ		
<i>BpeNIP6</i> ;1	KTHGSET	LLGCAAS	AGLAVM	VVILSTGHIS	GAHLNPA	LTIAFAL	KHFPWKH	VPMYI	GAQVVA	SFCA	FALKGIF	-----	HPIMGG	GVTV	PSGSYGO		
<i>AtNIP7</i> ;1	LSGGHV	GLLEAV	TAGLSV	VVYVYSIGHIS	GAHLNPS	ITIAFA	VFGGFPWS	QVPLYI	TAQTLG	ATA	TLVGVSVY	-----	GVNADI	MATK	PALSCVS		
<i>SlNIP7</i> ;1	IMGVQV	GLMEYAT	TAALTV	VVVVFSIG	PISGAHIN	PAVTLFA	AAVGHFPWS	KVPLYI	VVAQV	GGSIL	ATYTGKLVY	-----	GLKAEF	VTK	PLHSCTS		
<i>BpeNIP7</i> ;1	LSKGEV	GLLEAA	TAGLTV	VVVFISIGHIS	CAHVNPA	VTLFAA	TFGHFPWS	KVPLYI	LAQTLG	SVL	ATCIGQSVY	-----	DVKSEF	MTR	PLRGCSS		
			H2			NPA; LB				P1						-----TM2-----	-----TM3-----



**Figure S6D. Amino acid sequence alignment of the *BpeNIP* subfamily members from *Betula pendula*.** *SlnIP1;1* (Solyc03g0059.2) and *SlnIP7;1* (Solyc03g0059.1) (Reuscher et al., 2013) from Tomato and *AtNIP1;1* (AT4G19030) and *AtNIP7;1* (AT3G06100) from *Arabidopsis thaliana* were used as references. The highest residue similarities between MIP members are shown in red (100%), and green (>80%). Residues with similar physicochemical behaviors are shown in blue. The six transmembrane domains (TMH1-6) and the two hemi-helical regions that include the «NPA» motives «NPA1» (in Loop B) and «NPA2» (in Loop E) (framed in purple) are underlined. The amino acids residues constituting the ar/R constriction region are shown with blue highlights and labeled H2, H5, LE1 and LE2, and residues constituting the Froger's positions are shown with yellow highlights and labeled P1 to P5. The putative conserved Calcium-dependent Protein Kinase recognition sites in the C-terminus are highlighted in green. Tyrosine (Y) surlined in red and localized in the TM2 would allow the transport of Urea and Glycine.



## Small basic intrinsic proteins (SIPs)



**Figure S6E. Amino acid sequence alignment of the *BpeNIP* subfamily members from *Betula pendula*.** *S/SIP1;1* (Solyc12g0196.1), *S/SIP1;2* (Solyc10g0784.1) and *S/SIP2;1* (Solyc01g0567.1) (Reuscher et al., 2013) from Tomato, and *AtSIP1;1* (AT3G04090), *AtSIP1;2* (AT5G18290) and *AtSIP2;1* (AT3G56950) from *Arabidopsis thaliana* were used as references. The highest residue similarities between MIP members are shown in red (100%), and green (>80%). Residues with similar physicochemical behaviors are shown in blue. The six transmembrane domains (TMH1-6) and the two hemi-helical regions that include the «NPA» motives «NPA1» (in Loop B) and «NPA2» (in Loop E) (framed in purple) are underlined. The amino acids residues constituting the ar/R constriction region are shown with blue highlights and labeled H2, H5, LE1 and LE2, and residues constituting the Froger's positions are shown with yellow highlights and labeled P1 to P5.