

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)

10 20 30 40 50 60 70 80 90 100

AtPIP1;1 -----MEGKEEDVRVGANKFPERQPIGT--SAQSD---KDYKEPPPAFFPELSSWSFWRAGIAETIATFLFLYITVLTVMGVKRSPNM-----CASVGI
SlPIP1;1 -----MAENKEEDVNLGANKFREPQPLGT--SAQTD---KDYKEPPPAFLYEPGELSSWSFYRAGIAETMATFLFLYITILTVMGLKRSDSL-----CSSVGI
BpePIP1;1 -----MEGKDEDVRLGANRYRERQPIGT--AAQSQDT-KDYQEPAAFFPELSSWSFYRAGIAETVATFLFLYITVLTVMGVSKSPSK-----CSTVGI
BpePIP1;3 -----MEGKEEDVRLGANKFPERQPIGT--AAQSQDEGKDYIEPPPAFLFEPSELTSWSFYRAGIAETVATFLFLYITVLTVMGVAKSPSK-----CSTVGI
BpePIP1;2 -----MEGKEEDVRLGANKYTERQPLGT--AAQTD---KDYKEPPPAFLFEPGELCSWSFYRAGIAETVATFLFLYITVLTVMGVKRSSSV-----CASVGI
BpePIP1;4 -----MEGKEEDVKVGASKFPERQAIGT--SAQTD---KDYKEPPPAFLFEPGELQSWSFWRAGIAETIATFLFLYITLLTVMGVSRAPSK-----CASVGV
AtPIP2;1 -----MAKDVEAVPGEF-FQTRDYQDPPPAFFIDGAELKKWSFYRAVIAETVATLLFLYITVLTVIGYKIQSDTDAGGVDCGGVGI
SlPIP2;1 MVRHEWLKESTRAKKQRPKKKTETQKWAKIWRLVNLNMHQKTYQDPPPAFLIDPEELGKWSFYRAVIAETIATLLFLYITVLTVIGYKQSST---DQCGVGI
BpePIP2;1 -----MSKEVS-EEGQSHQHKGKDYVDPPPAFLDLAELKLWSFYRSLIAETIATLLFLYITVATVIGHKKATGP-----CDGVGL
BpePIP2;2 -----MSKEVT-EEEREASQPVKDYVDPPPAFLFDVAEFTMWSFYRAVIAETIATLLFLYITVATVIGYKKQTDTP-----CETVGL
BpePIP2;3 -----MSKEVT-EEAEASQPVVDPPPAFLFDVEELTEWSFYRAVIAETIATLLFLYITVATVIGYKKQNDQ-----CETVGL
BpePIP2;4 -----MAKDAEVPPEH-GSFTAKDYHDDPPPAFLFDVAELTKWSFYRALIAETIATLLFLYITVLTVIGYKQIDPANDGEACGGVGV
BpePIP2;5 -----MAKDVEVAEHQGEYSAKDYHDDPPPAFLIDYDELTKWSFYRALIAETIATLLFLYITVLTVIGYKQTDPSKNPDQCGGVGI
BpePIP2;6 -----MAKDMEVGGQSG-FSAKDYHDDPPPAFLIDAEELTKWSFYRAVIAETIATLLFLYITVLTVIGYRAQSDTTRGGDDCGGVGI

-----TM1-----

115 125 135 145 155 165 175 185 195 205

AtPIP1;1 QGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTFALFYIVMQCLGAICGAGVVKGFQPKQYQALGGGANVVAHGYTKGSGLGAELIGTFVLVYTV
SlPIP1;1 QGVAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTFAVFYIVMQCLGAICGAGVVKGFQKQPYQRLGGGANVQPGYTKGDGLGAELIGTFVLVYTV
BpePIP1;1 QGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTFAVFYIVMQCLGAICGAGVVKGFQKNQYERLGGGANVSSGYSKGDGLGAELIGTFVLVYTV
BpePIP1;3 QGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTFALFYIVMQCLGAICGAGVVKGFQKQYERLGGGANVFNPGYTKGDGLGAELIGTFVLVYTV
BpePIP1;2 QGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLLLARKLSLTFAVFYIVMQCLGAICGAGVVKGFQKTPFEINGGGANVNVHGYTKGDGLGAELIGTFVLVYTV
BpePIP1;4 QGIAWAFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTFALFYIVMQCLGAICGAGVVKGFQKTQYERVGGGANVNVHGYTKGDGLGAELIGTFVLVYTV
AtPIP2;1 LGIAWAFGGMIFILVYCTAGISGGHINPAVTFGLFLARKVSLPRALLYIAQCLGAICGVGVKAFQSSYYTRYGGGANSLADGYSTGTGLAEELIGTFVLVYTV
SlPIP2;1 LGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKVSLVRAIMYIVAQCLGAICGCGLVKAFQKAYYVKYGGGANVTLNDGYSTGTGLAEELIGTFVLVYTV
BpePIP2;1 LGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKVSLIRAVSYMVAQCLGAICGVGLVKAFMKHDYNMQGGGANVSPGYSTGTALGAELIGTFVLVYTV
BpePIP2;2 LGVAWAFGGMIFVLVYSTAGISGGHINPAVTFGLFLARKVSLIRAIYMAQCLGAICGAGLVKGIMKDSYDAFGGGTNTVSPYTKGTALGAELIGTFVLVYTV
BpePIP2;3 LGVAWAFGGMIFILVYSTAGISGGHINPAVTFGLFLARKVSLIRAIYMAQCLGAICGAGLVKGIMKDYDALGGGTNTVSPYTKGTALGAELIGTFVLVYTV
BpePIP2;4 LGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKVSLVRAVSYMVAQCLGAISGVGLVKAFNKAHYTRYGGGANVTLADGYSTGVGLGAELIGTFVLVYTV
BpePIP2;5 LGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKVSLIRALLYMVAQCLGAICGVGLVKAFQKSDYNRYGGGANVSVGYNKGVGLGAELIGTFVLVYTV
BpePIP2;6 LGIALAFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKVSLVRAILYMAQCLGAICGCGLVKAFQRAHYTRYGGGANVSVGYNKGTGLAEELIGTFVLVYTV

H2 NPA;LB- P1

---TM2-----TM3-----TM4---

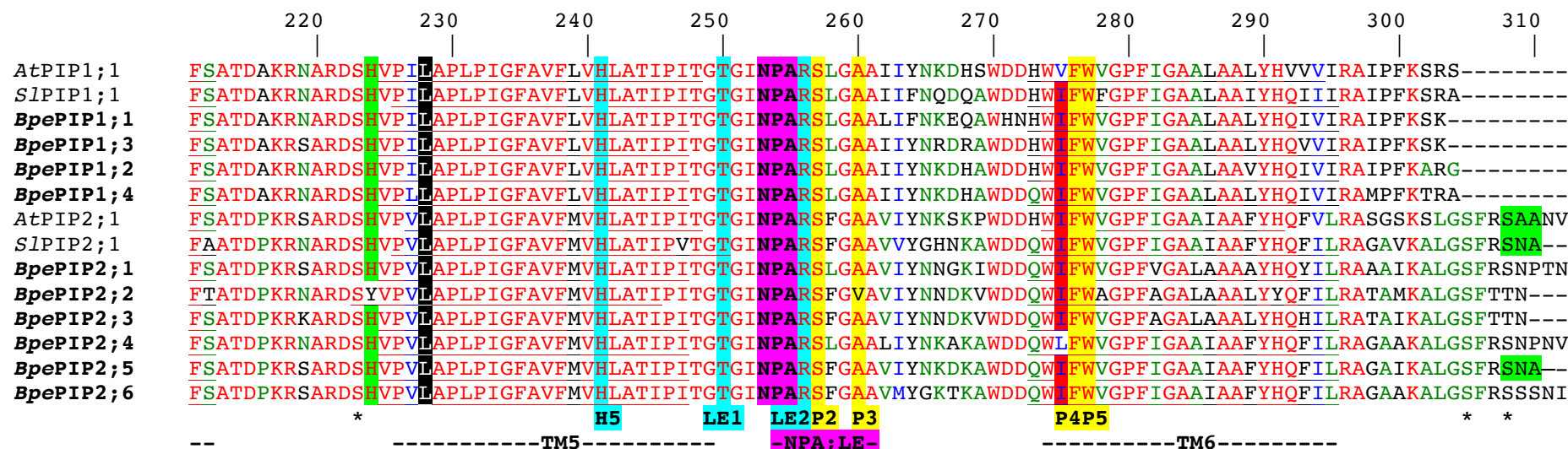
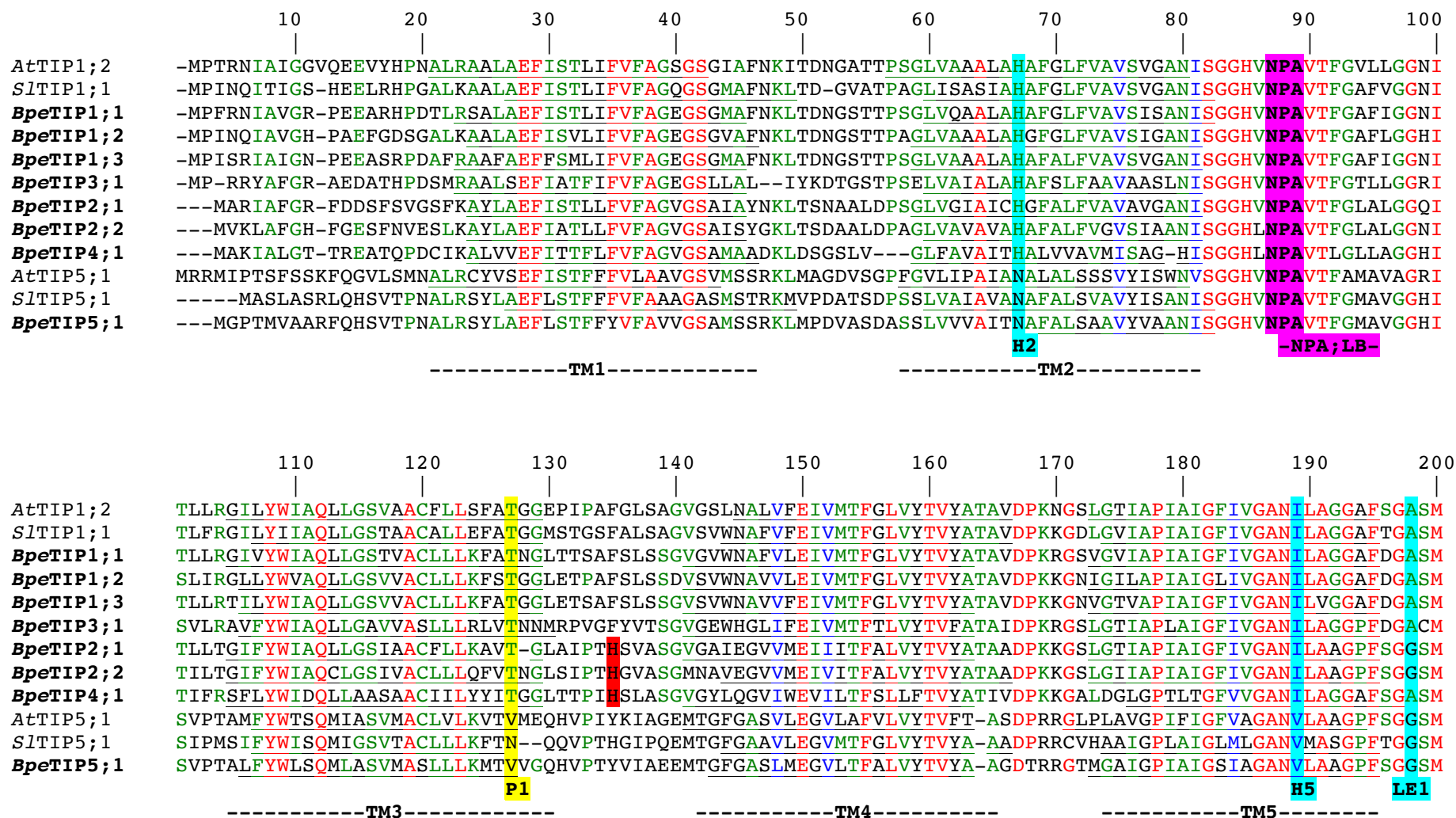


Figure S6A. Amino acid sequence alignment of the *BpePIP* subfamily members from *Betula pendula*. S/PIP1;1 and S/PIP2;1 (Solyc10g054840.1.1; Reuscher et al., 2013) from Tomato, and AtPIP1;1 (At3g61430) and AtPIP2;1 (At3g53420) (Muries et al., 2011) from *Arabidopsis thaliana* were used as references. 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 surligned in red and placed in loop E would be key for the transport of CO₂. Serine (S) at the position 146 on the alignment and surligned in red would be key for the transport of H₂O₂.

Tonoplast intrinsic proteins (TIPs)



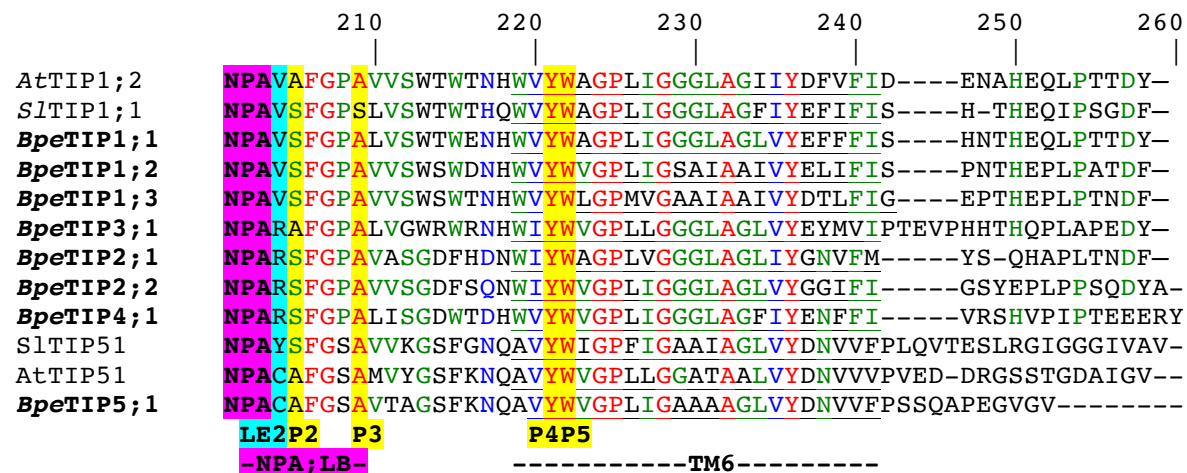
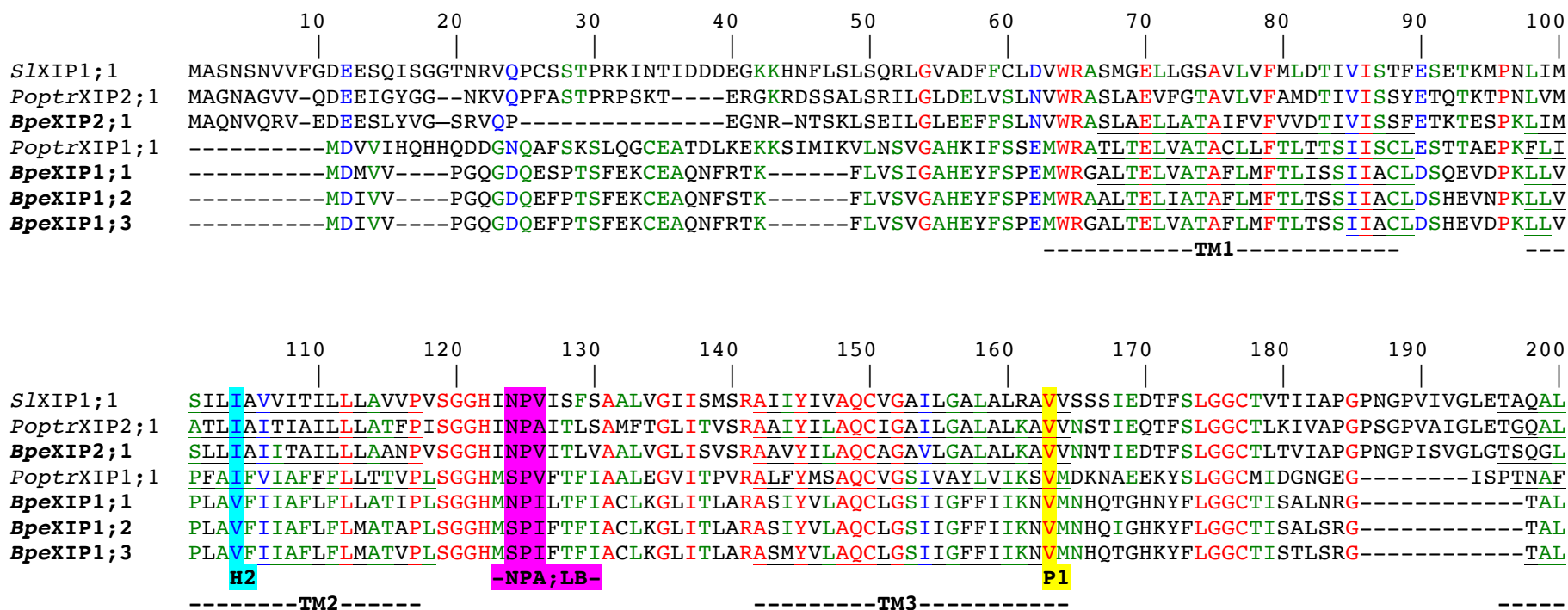


Figure S6B. Amino acid sequence alignment of the *BpeTIP* subfamily members from *Betula pendula*. *SlTIP1;1* (Solyc06g074820.1) and *SlTIP5;1* (Solyc03g093230.1) (Reuscher et al., 2013) from Tomato and *AtTIP1;1* (At2g36830) and *AtTIP5;1* (At3g47440) from *Arabidopsis thaliana* were used as references. 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 NH_4^+ , which would allow the transport of NH_3 independent of pH.

Uncharacterized *X* intrinsic proteins (XIPs)

The *BpeXIP2*;1 sequence used for the alignment was the redefined sequence (redefinition detailed in the Figure S6)



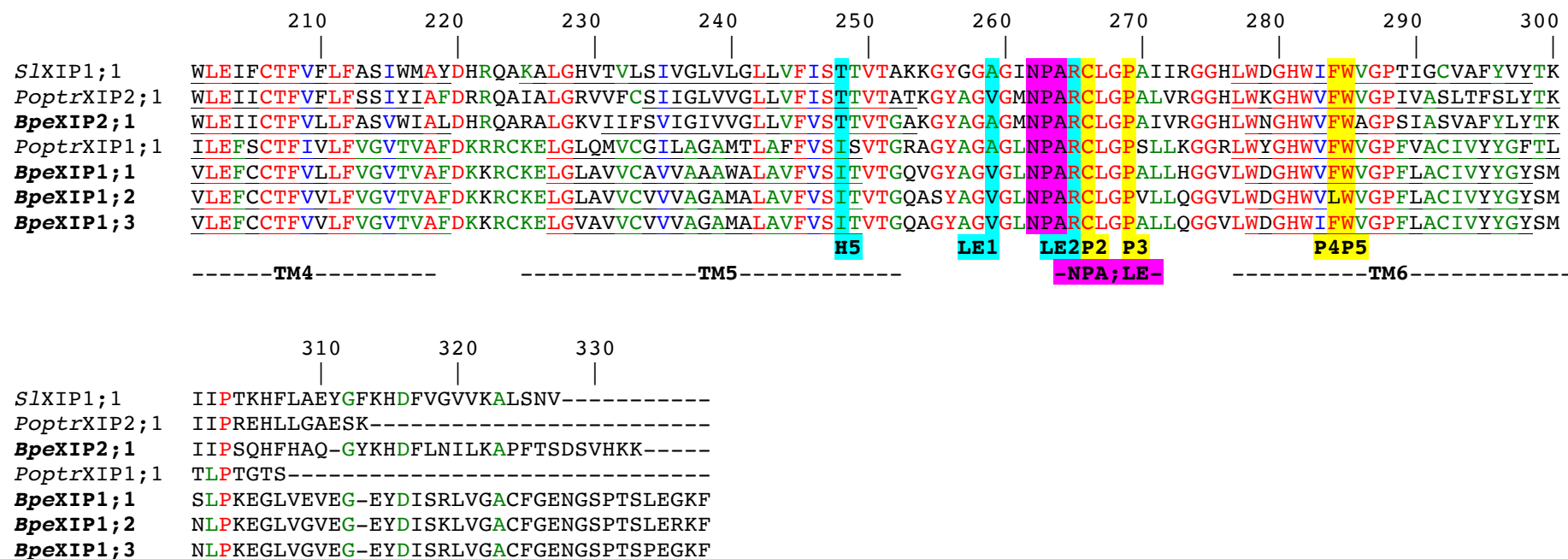


Figure S6C. Amino acid sequence alignment of the *BpeXIP* subfamily members from *Betula pendula*. *SlXIP1;1* (Solyc10g054840.1; Reuscher et al., 2013) from Tomato and *PoptrXIP2;1* and *PoptrXIP1;1* (Potri.009G128500.1, Potri.009G128100, resp.; Lopez et al, 2012) from Poplar were used as references. 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
 | | | | | | | | | |
 AtNIP1;1 -----MADISGNGYGNAREEVVMVNLKDEVEHQOEMEDIHNPRPLKKQDSLLSVSVPFLOKLI**AEFLGTYFLVFTGCASVVVNM**
 SlNIP1;1 -----MADHQINVNGNINHGVS-**NIKEDHDLNNHKESST**---SSF----LTPFPIQK**VI**AE**MI**GTY**FL**IFAGCGSVVVN****
BpeNIP1;1 -----MGIEELEISKVEEGVGTSAAKTNSDVTVAFCSASVVTITQKLI**AEVIGTYFVIFAGCGAVAVN**
BpeNIP4;1 -----MSSQDITKVEEGNSSSSNNNGNSGV---CNSSEIVQLVQK**VI**AE**VIGTYFL**IFAGCGSVVVN****
BpeNIP1;2 -----MAENSGSNGSQSITL-NVKECEANHNQASSASK---TNHGGGFCVSVPFMQKL**VAEVLGTYFLIFAGCGAVVVNL**
BpeNIP2;1 -----MATDPKPKNLAEANEFVSLNPISRKPKP-GLLRALLEEHPYPGFLSK**VVAETIATYLLVFVTCGSAALSA**
BpeNIP5;1 -----MYFFFFGGKSLGFIYLF--LS--AFYLG**AEFVGTFILIFMATAGPIVNQ**
BpeNIP5;2 -----MILGLNIFASMDQCLLIEIIKVLKFIFILNDMH--NSTLG**AEFVGTFIMVFSAAAAPIVNE**
BpeNIP6;1 MENEEVPSAPSTPATPGTPGAPLFGGFKAERSGNGRRSLLKNCSKCFSDQEWALEEGTLPKLSCSLPPPIPLAKK**VGAEFIGTLILIFAGTATAMVNQ**
 AtNIP7;1 -----MNGEARSRVVDQEAGSTPSTLRDEDHPSRQRLFGCLPYDIDLNPRI**VMAELVGTFILMFVSCGVISSTQ**
 SlNIP7;1 -----MKLPSYENGLSVEFQVDASASEQSTYDQETTSSNVEMLERNVNCNSILGIDPIFLRM**VLAELGTFLLMFCICGMMASME**
BpeNIP7;1 -----MKGQPHHLDDHTIDHAPTSQGSKDDQEMGVNAMS KSDVLKNSPFSCFPQGM DLNLGRV**VLAELVGTFILMF**CVCGGIIACTQ
 -----TM1

110 120 130 140 150 160 170 180 190 200

AtNIP1;1 QNDNVVTLPGIAIVWGLTIMVLIYSLGHISGAHINPAVNTIAFASCGRFPLKQVPAYVISQVIGSTLAAATLRLLFGLDHDVDCSGKHDVFIGSSPVGSDLQ

SlNIP1;1 ADKGMITFPGVAITWGLVVMVMVYSVGHISGAHFNPSVNTIAFASVKRFPPWKQVPAYVAAQVLGATLASGTLRLIF-----NGKHDHFAGTLPSTGDFQ

BpeNIP1;1 KIYGSVTFPGICVTWGLIVTVMIIYTVGHISGAHFNPAVNTITS AIFRRFPYKEVPLYIAAQLMGSI LASGTLALVF-----DVTTPDAYFGTVPTGNSNQ

BpeNIP4;1 KIYGSVSFPGICVVWGLIVMVMIIYSVGHISGAHFNPAVNTITFAIFRRFPLKQVPLYIVAQMIGSTLASATLCALF-----PVDKKSFFGTAPVGSNFQ

BpeNIP1;2 GNDKVVTAPGVSIVWGLAVMVLVYSVGHISGAHFNPAVNTIAFATCRRFPWKQVPAYVSAQVLGSTMASGTLRLIF-----NGHENRFVGTIPAGSNLQ

BpeNIP2;1 ADENKVSCLAASVAGGLIVTVMIIYAVGHISGAHFNPAVNTLAFAAVRHFPWKQVPLYAAAQLAGAISAFAATLRLVIL-----NPIKELGTTSPGTGTEIE

BpeNIP5;1 KYNGAESLIGNAACAGLAVMVVILSTGHISGAHLNPSLTIAFATLRFHPWLQVPAYIAAQVSGSICASFALKGVF-----HPYMSGGVTVPSVTEGQ

BpeNIP5;2 KYQGAETLIGSAACAGLAVMAMVLSTGHISGAHFNPSVNTIAFAALRHFPWVQVPGYLVAAQVSASICAFAATLKGVF-----HPFMSGGVTVPAVGIGQ

BpeNIP6;1 KTHGSETLLGCAASSGLAVMVVILSTGHISGAHLNPALENTIAFAALKHFPWKHVPMYIGAQVVASFCAAFALKGIF-----HPIMGGGVTVPSGSYGQ

AtNIP7;1 LSGGHVGLLEIAVTAAGLSVVVVYSIGHISGAHLNPSITIAFAVFGGFPWSQVPLYITAQTLGATAATLVGVSIV-----GVNADIMATKTPALSCVS

SlNIP7;1 IMGVQVGLMEYATTAALT VVVVVFSIGPISGAHINPAVNTLAFAAVGHFPWSKVPLYVVAQVGGSI LATYTGKLVY-----GLKAEFVTTKPLHSCTS

BpeNIP7;1 LSKGEVGLLEIAATAAGLT VVVVIFSI GHISCAHVNPAVNTIAFATFGHFPWSKVPLYILAQTLGSVLATCIGQSVY-----DVKSEFMTTRPLRGCSS

H2 H2 P1

-----TM2----- -NPA;LB- -----TM3-----

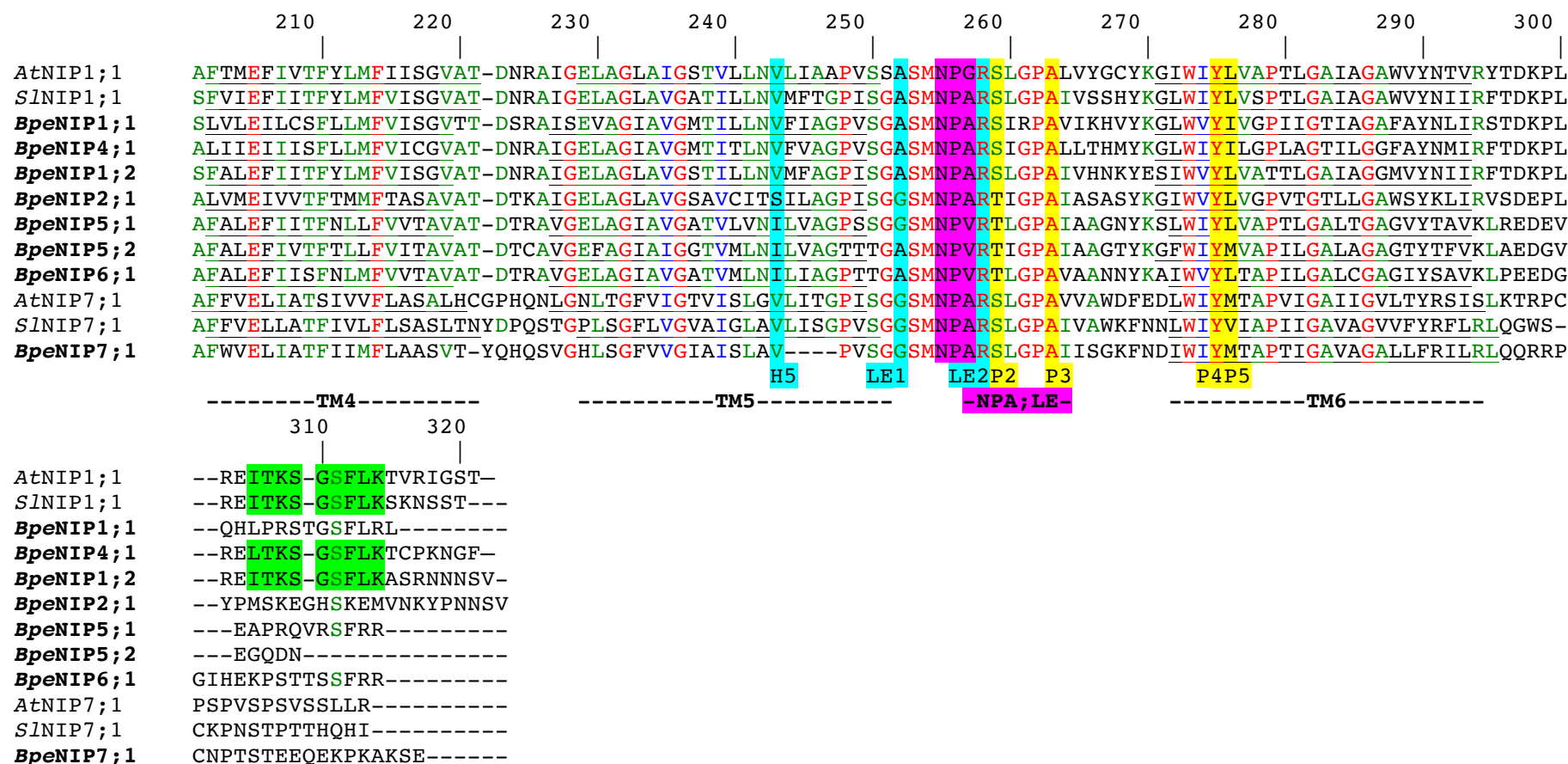


Figure S6D. Amino acid sequence alignment of the *BpeNIP* subfamily members from *Betula pendula*. SlnIP1;1 (Solyc03g0059.2) and SlnIP7;1 (Solyc03g0059.1) (Reuscher et al., 2013) from Tomato and AtNIP1;1 (AT4G19030) and AtNIP7;1 (AT3G06100) from *Arabidopsis thaliana* were used as references. The highest 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)

	10	20	30	40	50	60	70	80	90	100	
AtSIP1;1	-MMGVLSKSAIGDMLMTFSWVLSATFGIQTAALISAGDFQAITWAPLVILTSLIFVYVSIFTVI---FGSASFNPTGSAAFYVAGVPGDTLFSLAIRLP										
SlSIP1;1	--MGVIKAAIADGLLTFLWVFCSSNIGVSTYFIASYFGIVNEI-PSLFIITTLIVFVIFLMDFLGDVLGGAGFNPTGNAAFYAAAGLG-DDSLVSAAVRCP										
BpeSIP1;1	--MGAIKAAIGDAVLTFMWVFSASTLGAATTVTARALGIEGLVWPSVFIITTVLVFVLVFFFTLIANALGGASFNPTGTASFYAAAGLG-PDTLFSMALRFP										
BpeSIP1;2	--MGLMRVAIGDAILTSLWVFSAPMMGILTPIIATYLGVOAKSLAGLFITTLATSLVLIFQLIGKVLGGASFNPSTTVSFYAAAGLKHNSSLISMAVQFP										
AtSIP2;1	--MGRIGLVVTDLVLSFMWVWAGVLVNI---LVHGVLGFSRTDPSGEIVRYLFSIISMFIIFAYLQQATKGGLYNPLTALAAGVSG-GFSSFIISVFVRIP										
SlSIP2;1	MGVSRRSLVISDFIMSFMWVWSSVLIKM---FVHKILGYGAHD-----LKGAYNPLTILSGAISG-DLTNFIIFTVAARIP										
BpeSIP2;1	--MASAGLLVSDFIISFMWVWVSGVLIKI---FVYKVLLELGH-EPSGEVIKCALSIIVNMFFFAFLGKTTKGGAYNPLTVFAGAISG-DFKRFLFDAGARIP										
	-----TM1-----				-----TM2-----				-NPA;LB-		-----TM3-----
110	120	130	140	150	160	170	180	190	200		
AtSIP1;1	AQAI GAAGGALAIMEFIPEKYKHMIGGPSLQVDVHTGAIAETILSFGITFAVLLIILRGPRRLAKTFLALATISFVVAGSKYTGTPAMNPAIAFGWAYM										
SlSIP1;1	AQVAGAVAGSLALVELIPKHYHHMLDGPALKVDVQGTGAIAEGVLTFFVITFMIFVIVLRGPESVLLKNWLLTMVTLPLVLAGSNFTGTPSMNPANAFGWAYL										
BpeSIP1;1	AQAA GAVGGALAIMEVMPKNYKHMIGGPSLKVLDLHIGAIAGVLTFLISFAVLVIVLRGPRSPILKTWLLAMATVALVIAGSNYTGTPSMNPANAFGWAYV										
BpeSIP1;2	AQAA GGLVGAKAILQVLPSQYKQRLRGPSLKVLDLLTGVI EAALTLGLSFAILLIMLRGPKNPLVKVWLAVAVTVGLLVAGSGYTGTPSMNPANAFGWAFV										
AtSIP2;1	VEVIGSILAVKHIHVFPEIGK---GPKLNVAIHHGALTEGILTFFIVLLSMGLTRKIPGSFFMKTWIGSLAKLTLHLGSDLTGGCMNPAAVMGWAYA										
SlSIP2;1	SQVFGSITGVRFIIAAFPNIGR---GPVLSIDIHRGALTEGILTFAIVSISLGLSRRSRASTFMKTWISSLSKLTLLHLGSDLTGGCMNPASVMGWAYA										
BpeSIP2;1	AQVIGSIIGVKLI IETFPEIGL---GPRLNVDIHRGALTEGILTFAIVIISLGLARKIPGSFVMKTWISSVSKLTLLHLGSDLTGGCMNPASVMGWAYA										
	---TM3---		-----TM4-----				-----TM5-----				-NPA;LE-
210	220	230	240	250							
AtSIP1;1	YSSHNTWDHIYVYWISSFVGALS AALLFRSIFPPPRPQK-KKQKKA-----										
SlSIP1;1	SNTHKTLHFYVYWISPFIGAILAAWIFRVLFPFPVEQKPQKQKRN-----										
BpeSIP1;1	NNRHNTWEQFYVYWICPFVGAILAAWVFRALFPPT-----PAKQKKA-----										
BpeSIP1;2	NNQHNTWEHFYVYWIGSFTGATVAGWIYRFLFP-----										
AtSIP2;1	RGEHITKEHLLVYWLGPVKATLLAVWFFKVVFKPLTEEQEKPKAKSE----										
SlSIP2;1	RGDHIITKEHIHVYWLAPIQATLLAVWTFNLLVSPSKDKEAKKTEKKSE---										
BpeSIP2;1	RGDHIITKEHILVYWLAPIEATLLAVWIFRLVVRPIKEEKANVKKNESLRIC										
	P4P5		-----TM4-----								

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.