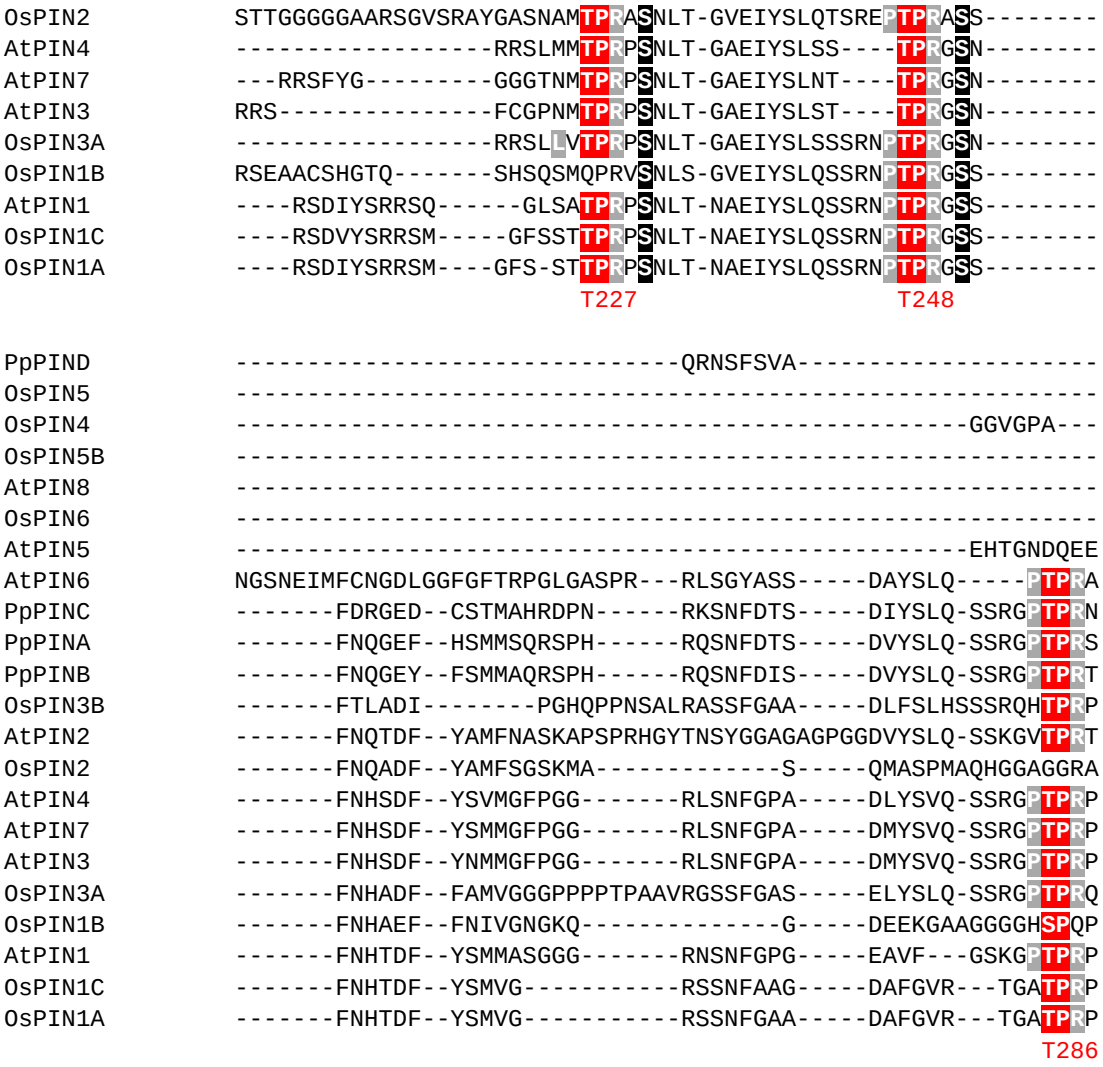
Supporting Information

Dory *et al*. – Co-evolving MAPK and PID Phosphosites Indicate an Ancient Environmental Control of PIN Auxin Transporters in Land Plants

```
PpPIND          MLTGGQFYDVLCAVVPLYVALFLGYGSLKWWGVVTPEQSAGISRFNALIAMPPLIFEIIA
OsPIN5          MITGSEVYQVVEAMAPLYTAAALGYGSVRWLKAFSNEQCAGINHFVALYAVPVLIFDMVS
OsPIN4          MIGWGDVYKVVGAMAPLYFALGLGYGSVRWWRFFTAEQCAAINTMVVYFSMPFFTFDFVV
OsPIN5B         MIGWGDVYKVVAAMAPLYFALGLGYGSVRWWRLFTADQCDAVNRLVACFAVPFFAFDFAA
AtPIN8          MINCGDVYKVIEAMVPLYVALILGYGSVKWWHIFTRDQCDAINRLVCYFTLPLFTIEFTA
OsPIN6          MIGWGDVYKVVAATVPLYFALFLGYGSVRWWRIFTREQCDAVNRLVAFFALPFFTFEFTL
AtPIN5          MISWLDIYHVVSATVPLYVSMTLGFLSARHLKLFSPEQCAGINKFVAKFSIPLLSFQIIS
AtPIN6          MITGNEFYTVMCAMAPLYFAMFVAYGSVKWCKIFTPAQCSGINRFVSVFAVPVLSFHFIS
PpPINC          MITGHDMYNVLSAMVPLYVAMMLAYASVKWWGILTPQQCDGINRFVSIFAVPLLSFQFVS
PpPINA          MINGHDIYNVLSAMVPLYVAMMLAYGSVKWWGILTPQQCGGINRFVSIFAVPLLSFQFIS
PpPINB          MINGHDIYNVLSAMVPLYVAMMLAYGSVKWWGILTPQQCGGINRFVSIFAVPLLSFQFIS
OsPIN3B         MISWHELYMVLSAVVPLYVAMMVAYGSVRWWGVLTPEQCSGINRFVAVIAVPLLSFHFIS
AtPIN2          MITGKDMYDVLAAMVPLYVAMILAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
OsPIN2          MITGRDIYDVLAAIVPLYVAMFLAYGSVRWWGIFTPDQCSGINRFVAVFAVPLLSFHFIS
AtPIN4          MITWHDLYTVLTAVVPLYVAMILAYGSVQWWKIFSPDQCSGINRFVAIFAVPLLSFHFIS
AtPIN7          MITWHDLYTVLTAVIPLYVAMILAYGSVRWWKIFSPDQCSGINRFVAIFAVPLLSFHFIS
AtPIN3          MISWHDLYTVLTAVIPLYVAMILAYGSVRWWKIFSPDQCSGINRFVAIFAVPLLSFHFIS
OsPIN3A         MISGHDFYTVMAAVVPLYVAMFLAYGSVRWWGIFTPDQCSGINRFVAIFAVPLLSFHFIS
OsPIN1B         MITVVDLYHVLTAVVPLYVAMTLAYASVRWWRIFSPDQCSGINRFVALFAVPLLSFHFIS
AtPIN1          MITAADFYHVMTAMVPLYVAMILAYGSVKWWKIFTPDQCSGINRFVALFAVPLLSFHFIA
OsPIN1C         MITGADFYHVMTAMVPLYVAMILAYGSVKWWRIFTPDQCSGINRFVALFAVPLLSFHFIS
OsPIN1A         MITAADFYHVMTAMVPLYVAMILAYGSVKWWRIFTPDQCSGINRFVALFAVPLLSFHFIS
                *:   :.* *: *  *** :  :.: * ..   .:  *. .:. :    ::* : : :
                                                 T35

PpPIND          FNNPYTMNNRLIAAYCLSNGIVLVGLCAWV-----WCT-------KCG----N--LD-WV
OsPIN5          TNNVYKMNGRLIAADTLQKAVLLLGLMAWAL----WERSRARGAGAKAKAAVSSPLQ-WV
OsPIN4          RTDPFAMNYRVIAADAVSKAIAIAAMAAWAR------T-------RCGCAAAKAGAQSWS
OsPIN5B         RIDPFALSYRVLAADALSKLAVALALAACAAAASTR-CCGSGGGKRGG----GGGFS-WC
AtPIN8          HVDPFNMNYRFIAADVLSKVIIVTVLALWAK----Y-S-------NKG----S--YC-WS
OsPIN6          HTDPFQVNYRAVAADVISKAVIVAVIGAWAR----F-M-------SKG----GCAVS-WS
AtPIN5          ENNPFKMSPKLILSDILQKFLVVVVLAMVLR----FWH-------PTGGRGGK--LG-WV
AtPIN6          QNNPYKMDTMFILADTLSKIFVFVLLSLWAV----F-F-------KAG----G--LD-WL
PpPINC          GNNPYEMNFRFIAADAVSKVFVLSCLGLWVR----F-S-------KRG----S--LE-WV
PpPINA          GNNPYAMNFKFIAADAVSKVLVLLCLGLWAR----Y-A-------KRG----S--LE-WM
PpPINB          GNNPYAMNFRFIAADAVSKVFVLLCLGLWAR----Y-S-------KRG----S--LE-WM
OsPIN3B         SSDPYAMNLRFVAADTLQKVLVLAALAAWSRFPARF-V-------PPA----WPPLD-CS
AtPIN2          SNDPYAMNYHFLAADSLQKVVILAALFLWQA----F-S-------RRG----S--LE-WM
OsPIN2          TNDPYSMNYRFLAADSLQKLVILAALAVWHNLLSRY-R-------RNGGAAAS--LD-WT
AtPIN4          TNDPYAMNFRFVAADTLQKIIMLVLLALWAN----L-T-------KNG----S--LE-WM
AtPIN7          SNNPYAMNLRFIAADTLQKLIMLTLLIIWAN----F-T-------RSG----S--LE-WS
AtPIN3          TNNPYAMNLRFIAADTLQKIIMLSLLVLWAN----F-T-------RSG----S--LE-WS
OsPIN3A         TNDPYAMNLRFLAADTLQKLLVLAGLAAWSR----LPS-------RTG----APRLD-WS
OsPIN1B         TNNPFAMNLRFLAADTLQKLIVLALLALWCR----L-S-------ARG----S--LD-WL
AtPIN1          ANNPYAMNLRFLAADSLQKVIVLSLLFLWCK----L-S-------RNG----S--LD-WT
OsPIN1C         TNNPYTMNLRFIAADTLQKLIVLALLTLWSH----L-S-------RRG----S--LE-WT
OsPIN1A         TNNPYTMNLRFIAADTLQKLMVLAMLTAWSH----L-S-------RRG----S--LE-WT
                  : : :.   : :  :.:      :                     .

PpPIND          ITLFQLSVMPNTIIVGIPVLSPLYS------VTESGIAAIFIGQVLWLFPTLFLYELKEV
OsPIN5          ITCFSVASLPNTIIMGVPLLNGMYGPV----SKDLMKQIVVMQFCIWYNVIIFLYEYMAA
```


OsPIN4 ITGFSLAALNNTLVVGVPLLDAMYGRW----AQDLVVQIAVVQSMVWFPLLLMAFELRKA
OsPIN5B ITGFSLATLNNTLVVGVPLLDAMYGKW----ARDLIVQISVVQTIVYFPLLLLAFEVRRA
AtPIN8 ITSFSLCTLTNSLVVGVPLAKAMYGQQ----AVDLVVQSSVFQAIVWLTLLLFVLEFRKA
OsPIN6 ITSFSLSTLTNSLVVGVPMARAMYGEW----AQQLVVQLSVFQAIVWLTLLLFVLEVRKA
AtPIN5 ITGLSISVLPNTLILGMPILSAIYGDE----AASILEQIVVLQSLIWYTILLFLFELNAA
AtPIN6 ITLFSIATLPNTLVMGIPLLQAMYGDY----TQTLMVQLVVLQCIIWYTLLLFLFELRAA
PpPINC ITLFMLTTIPNTLVIGTPLLAAMYGSK----PGQLTVQAVVLQCIIWYTLLLVMYEYRAA
PpPINA ITLFVLITIPNTLVMGTPLLAAMYGAG----PGDLTVQAVVLQCIIWYTLLLVMYEYRAA
PpPINB ITLFVLITIPNTLVMGTPLLAAMYGPG----PGDLTIQAVVLQCIIWYTLLLLMYEYRAA
OsPIN3B ITLFSVSTLPNTLVMGIPLLVSMYGPY----SGDLMVQIVVLQSIVWYTLLLFLFEFRAA
AtPIN2 ITLFSLSTLPNTLVMGIPLLRAMYGDF----SGNLMVQIVVLQSIIWYTLMLFLFEFRGA
OsPIN2 ITLFSLSTLPNTLVMGIPLLRAMYGDF----SGSLMVQIVVLQSVIWYTLMLFLFEYRGA
AtPIN4 ITIFSLSTLPNTLVMGIPLLIAMYGTY----AGSLMVQVVVLQCIIWYTLLLFLFEYRGA
AtPIN7 ITIFSLSTLPNTLVMGIPLLIAMYGEY----SGSLMVQIVVLQCIIWYTLLLFLFEYRGA
AtPIN3 ITIFSLSTLPNTLVMGIPLLIAMYGEY----SGSLMVQIVVLQCIIWYTLLLFLFEFRGA
OsPIN3A ITLFSLSTLPNTLVMGIPLLIAMYGPY----SGSLMVQIVVLQCIIWYTLMLFLFEFRAA
OsPIN1B ITLFSLSTLPNTLVMGIPLLKGMYAAAADVDSGSLMVQIVVLQCIIWYTLMLFLFEYRGA
AtPIN1 ITLFSLSTLPNTLVMGIPLLKGMYGNF----SGDLMVQIVVLQCIIWYTLMLFLFEYRGA
OsPIN1C ITLFSLSTLPNTLVMGIPLLKGMYGEF----SGSLMVQIVVLQCIIWYTLMLFMFEYRGA
OsPIN1A ITLFSLSTLPNTLVMGIPLLKGMYGEF----SGSLMVQIVVLQCIIWYTLMLFMFEYRGA
** : : : *::::* *: :*. .. :: :. * .
PpPIND RKM----------
OsPIN5 RRSASAPPPASSE--------GSAKISP------SSPVKAAAAAADT----------
OsPIN4 WVV----------
OsPIN5B TTAAAAPPPPPTG----------
AtPIN8 GF----------
OsPIN6 AIGMYVDGAEAAA----------
AtPIN5 RAL-----PSSGA----------SL----------
AtPIN6 RLLIRAEFPGQAAGSIAKIQVDDDVISL---DG-MDPLRTETETDVNGRIRLRIRRSVSS
PpPINC RILIMHRFPENAA-SIVSFKVESDVMSL---DG-PDPVLTEAEFRNDGKLHVRVRRSVSS
PpPINA KILIMQQFPENAA-SIVSFKVDSDVMSL---DG-REPVLTEAEIGDDGKLHVKVRRSVSS
PpPINB KILIMQQFPENAG-SIVSFKVDSDVMSL---DG-REPVLTEAEIGDDGKLHVKVRRSVSS
OsPIN3B RVLIAAQFPDTAA-SIAAVHVDPDVVSL---EGSQA--EAHAEVAPDGRLRMVVCRSSVS
AtPIN2 KLLISEQFPETAG-SITSFRVDSDVISL---NG-REPLQTDAEIGDDGKLHVVVRRSSAA
OsPIN2 KALISEQFPPDVGASIASFRVDSDVVSL---NG-REALQADAEVGRDGRVHVVIRRSASA
AtPIN4 KLLIMEQFPETGA-SIVSFKVESDVVSL---DG-HDFLETDAEIGNDGKLHVTVRKSNAS
AtPIN7 KILIMEQFPETGA-SIVSFKVESDVVSL---DG-HDFLETDAQIGDDGKLHVTVRKSNAS
AtPIN3 KMLIMEQFPETAA-SIVSFKVESDVVSL---DG-HDFLETDAEIGDDGKLHVTVRKSNAS
OsPIN3A RMLIADQFPDTAA-SIVSLHVDPDVVSL---EGGHA--ETEAEVAADGRLHVTVRRSSVS
OsPIN1B RLLVMEQFPDTAA-SIVSFRVDSDVVSLAGGGGGAAELQAEAEVGDDGRMRVTVRKSTSS
AtPIN1 KLLISEQFPDTAG-SIVSIHVDSDIMSL---DG-RQPLETEAEIKEDGKLHVTVRRSNAS
OsPIN1C RILITEQFPDTAG-AIASIVVDADVVSL---DGRRDMIETEAEVKEDGKIHVTVRRSNAS
OsPIN1A RMLITEQFPDTAA-NIASIVVDPDVVSL---DGRRDAIETETEVKEDGRIHVTVRRSNAS
PpPIND ----------GQPAVGSVA----------
OsPIN5 ----------NGNAVAADRPQEVAVNIEITEMAAS---TARDGV--------
OsPIN4 ----------GGG--------
OsPIN5B ----------
AtPIN8 ----------SSNNIS-DVQVDNI----------
OsPIN6 ----------
AtPIN5 ----------
AtPIN6 --------VPDSVM----SSSLCLTPRASNLS-NAEIFSVNTPNNRFFHGGGGSGTLQFY
PpPINC R--SQGVHSANHSI----PSSKALTPRASNLS-NAEIYSMNSSVNLTPRGSS--------
PpPINA R--SQGMHSAHHSM----PSSKALTPRPSNLT-GAEIYSMHSSVNLTPRDSS--------
PpPINB R--SQGMHSAHHSM----PSSKALTPRPSNLT-GAEIYSMHSSVNLTPRDSS--------
OsPIN3B ---RRSAAA----------AATPRASNLT-GVEIYSISSSRNATPRGST--------
AtPIN2 SSMISSFNKSHGGG----LNSSMITPRASNLT-GVEIYSVQSSREPTPRASS--------

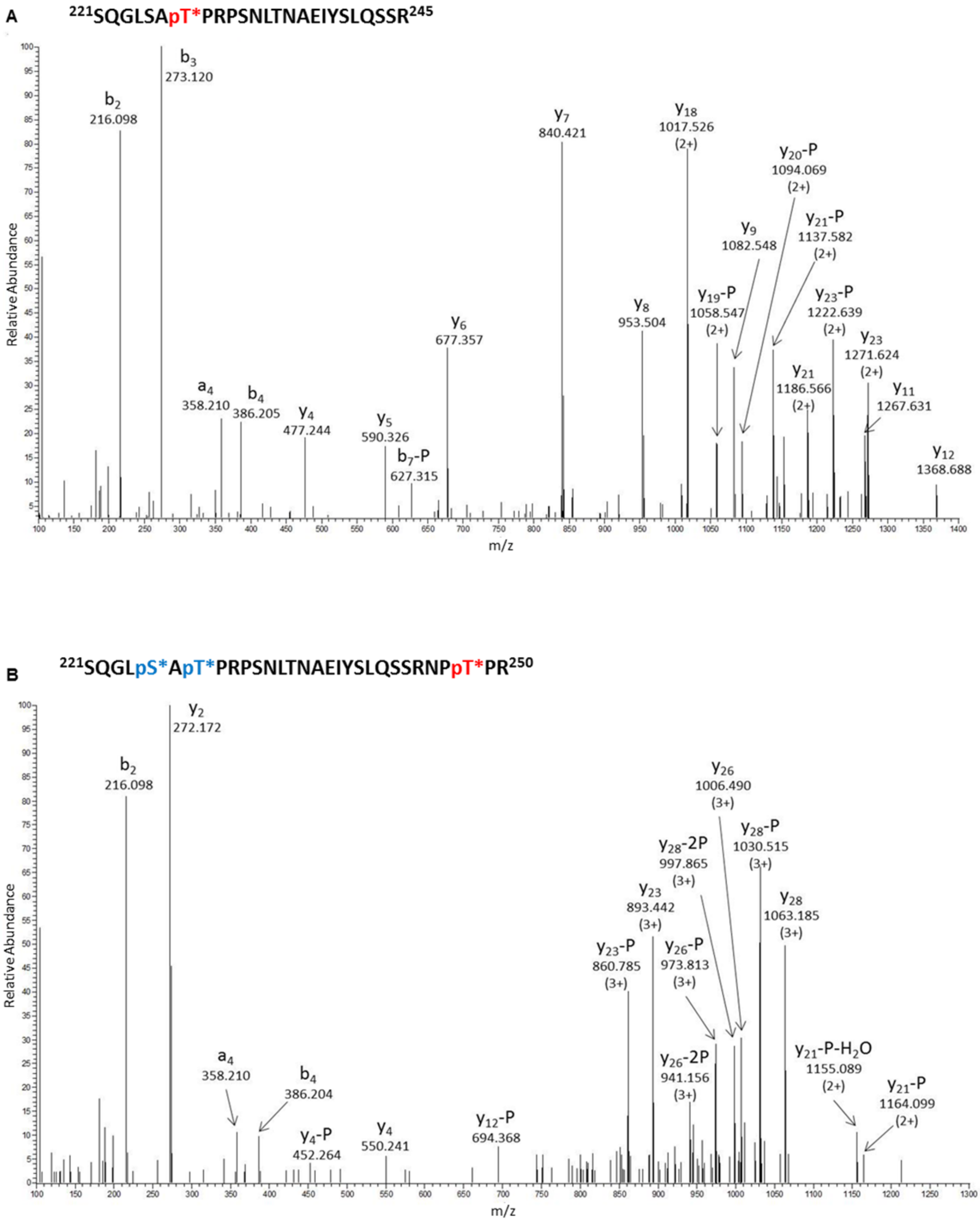

OsPIN2 STTGGGGGAARSGVSRAYGASNAMTPRASNLT-GVEIYSLQTSREPTPRASS--------
AtPIN4 ----------RRSLMMTPRPSNLT-GAEIYSLSS----TPRGSN--------
AtPIN7 ---RRSFYG---------GGGTNMTPRPSNLT-GAEIYSLNT----TPRGSN--------
AtPIN3 RRS----------FCGPNMTPRPSNLT-GAEIYSLST----TPRGSN--------
OsPIN3A ----------RRSLVTPRPSNLT-GAEIYSLSSSRNPTPRGSN--------
OsPIN1B RSEAACSHGTQ-------SHSQSMQPRVSNLS-GVEIYSLQSSRNPTPRGSS--------
AtPIN1 ----RSDIYSRRSQ------GLSATPRPSNLT-NAEIYSLQSSRNPTPRGSS--------
OsPIN1C ----RSDVYSRRSM-----GFSSTTPRPSNLT-NAEIYSLQSSRNPTPRGSS--------
OsPIN1A ----RSDIYSRRSM----GFS-STTPRPSNLT-NAEIYSLQSSRNPTPRGSS--------
T227
T248
PpPIND ----------QRNSFSVA----------
OsPIN5 ----------
OsPIN4 ----------GGVGPA---
OsPIN5B ----------
AtPIN8 ----------
OsPIN6 ----------
AtPIN5 ----------EHTGNDQEE
AtPIN6 NGSNEIMFCNGDLGGFGFTRPGLGASPR---RLSGYASS-----DAYSLQ-----PTPRA
PpPINC -------FDRGED--CSTMAHRDPN------RKSNFDTS-----DIYSLQ-SSRGPTPRN
PpPINA -------FNQGEF--HSMMSQRSPH------RQSNFDTS-----DVYSLQ-SSRGPTPRS
PpPINB -------FNQGEY--FSMMAQRSPH------RQSNFDIS-----DVYSLQ-SSRGPTPRT
OsPIN3B -------FTLADI--------PGHQPPNSALRASSFGAA-----DLFSLHSSSRQHTPRP
AtPIN2 -------FNQTDF--YAMFNASKAPSPRHGYTNSYGGAGAGPGGDVYSLQ-SSKGVTPRT
OsPIN2 -------FNQADF--YAMFSGSKMA------------S-----QMASPMAQHGGAGGRA
AtPIN4 -------FNHSDF--YSVMGFPGG-------RLSNFGPA-----DLYSVQ-SSRGPTPRP
AtPIN7 -------FNHSDF--YSMMGFPGG-------RLSNFGPA-----DMYSVQ-SSRGPTPRP
AtPIN3 -------FNHSDF--YNMMGFPGG-------RLSNFGPA-----DMYSVQ-SSRGPTPRP
OsPIN3A -------FNHADF--FAMVGGGPPPPTPAAVRGSSFGAS-----ELYSLQ-SSRGPTPRQ
OsPIN1B -------FNHAEF--FNIVGNGKQ--------------G-----DEEKGAAGGGGHSPQP
AtPIN1 -------FNHTDF--YSMMASGGG-------RNSNFGPG-----EAVF---GSKGPTPRP
OsPIN1C -------FNHTDF--YSMVG-----------RSSNFAAG-----DAFGVR---TGATPRP
OsPIN1A -------FNHTDF--YSMVG-----------RSSNFGAA-----DAFGVR---TGATPRP
T286
PpPIND ----------
OsPIN5 ----------
OsPIN4 ----------
OsPIN5B ----------
AtPIN8 ----------
OsPIN6 ----------
AtPIN5 ANIEDEP----------
AtPIN6 SNFNELD----------VNGNGTPVWMKSPAA----------GR--
PpPINC SNFNEENSKEVHNHRGALNVNIPRFAPPLYRNGSGGRLFMARSDLGGVGALSFEPAAH--
PpPINA SNFNEENSKDIHTHHRGLNMNSPRFAPPLYRNGMGARMFTPRPGLGGIGVPGTDCTGHGT
PpPINB SNFNEENSKDMHTHHRGLNLTSPRFVPPLYRNVAGGRMFMPRTGLGGLPVHGNDPTGHGS
OsPIN3B SSFDEHA----------AA--
AtPIN2 SNFDEEV----------MKTAKKAGRGGRSMSGEL----------
OsPIN2 QGLDEQV----------TNKFASGKAADPPS----------
AtPIN4 SNFEENN----------AVKYGFYNNTNSSVPAAGS----------
AtPIN7 SNFEESCAMA----------SSPRFGYYPGGAPGS----------
AtPIN3 SNFEENCAMA----------SSPRFGYYPGGGAGS----------
OsPIN3A SNFDEHSA----------
OsPIN1B ----------
AtPIN1 SNYEEDGGPA----------KPTAAGTAAGAGRFHYQSG----------
OsPIN1C SNYEEDAA----------APNKAGSKYGQ----------
OsPIN1A SNYEDDA----------SKPKYPLPASNAAPMAGH----------

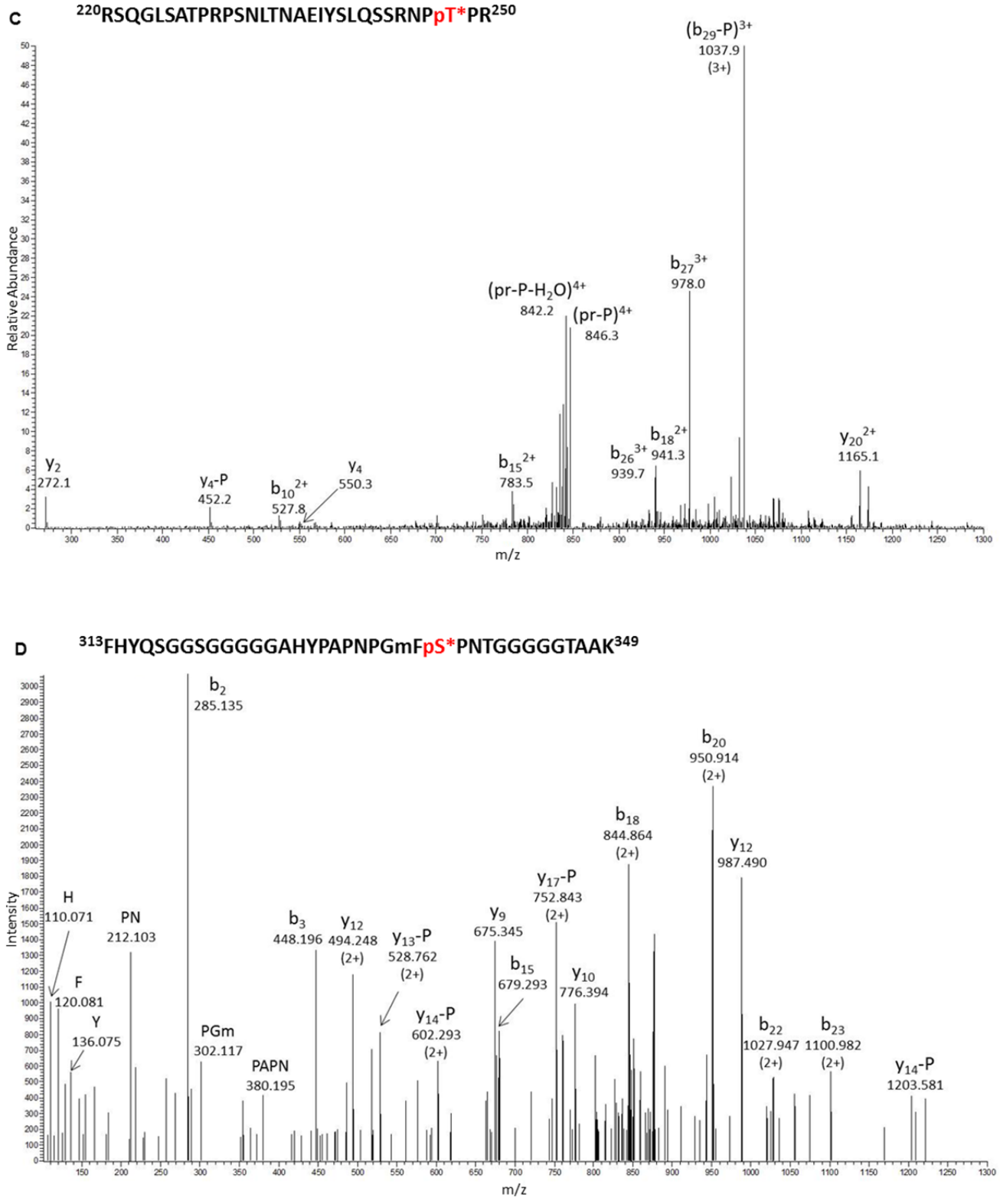
```
PpPIND          ------------------------------------------------------------
OsPIN5          ------------------------------------------------------------
OsPIN4          ------------------------------------------------------------
OsPIN5B         ------------------------------------------------------------
AtPIN8          ------------------------------------------------------------
OsPIN6          ------------------------------------------------------------
AtPIN5          ------------------------------------------------------------
AtPIN6          --------------IYRQSSPKM-------------------------------------
PpPINC          -------SMGPDGRTIYPGITVVTNSVAA---------VPASGVSTHIINPVFSPLVSQV
PpPINA          LSTLGAPGMGPDGRTIYPGSQTAINILTLGGAANVNATAPSTAVNTQIVNPVYSPQASQI
PpPINB          LSTLGTPGMGPDGRTIYPGSQTAISLVTPGGTGNIATPL-SSSLNTQIVNPVYSPRASQI
OsPIN3B         ---------------RARASATV-------------------------------------
AtPIN2          --------YNNNSVPSYPPPNPMFTGSTSGASGVKKKESGGGGSGGG-------------
OsPIN2          ----------------YPAPNPGMMPAPRKKELG--------------------------
AtPIN4          ----------------YPAPNPEFSTGTGVSTKPNKIPKENQQQLQEKD-----------
AtPIN7          ----------------YPAPNPEFSTGNKTGSKAPKENHHHVG-----------------
AtPIN3          ----------------YPAPNPEFSSTTTSTANKSVNKNPKDVNTNQQTTLPTGG-----
OsPIN3A         ----------------RPPKPPATTT-----------------------------------
OsPIN1B         ------------------------------------------------------------
AtPIN1          -------GSGGGGGAHYPAPNPGMFSPNTGGGGGTAAKGNAPVVGG--------------
OsPIN1C         ----------------YPAPNPAMAAPPKPKKAANG------------------------
OsPIN1A         ----------------YPAPNPAVSSAPKGAKKAA------------------------
                                      S337

PpPIND          ------------------------------------------------------NGESG
OsPIN5          ------------------------------------------------------SGETT
OsPIN4          ------------------------VMSSSSPP----------------------EKQSD
OsPIN5B         ------------------------------------------------------TDDDD
AtPIN8          ------------------------------------------------------NIESG
OsPIN6          ------------------------------------------------------AAGKD
AtPIN5          ------------------------------------------------------KEEED
AtPIN6          ------------------------MWESGQRHAAK----DINGSVPE--------KEISF
PpPINC          AKKVNDPRASIPKTDEEAKELHMFVSSANPTSVSE-GELHVFGGSDISINL----QQSVN
PpPINA          AKKVKDPKAS-PRADEDAKELHMFVWSANASPVSE-AGLHVFGGNDTSANL----QQRFD
PpPINB          AKKVKDTRTS-PKSDEDAKELHMFVWSANASPVSE-AGLHVFGGNDTSANL----HQSFD
OsPIN3B         ------------APTNDLKDTHMIEWSSGASAASEVTGLPVFRSGRETRRLVP--SDAPS
AtPIN2          -----------VGVGGQNKEMNMFVWSSSASPVSEANAKNAMTRGSS--------TDVST
OsPIN2          -----------GSNSNSNKELHMFVWSSSASPVSE---ANLRNAVNHAAST----DFASA
AtPIN4          -----------SKASHDAKELHMFVWSSSASPVSD-----VFGGGAGDNVATE--QSEQG
AtPIN7          -----------KSNSNDAKELHMFVWGSNGSPVSDRAGLQVDNGANEQVGK----SDQGG
AtPIN3          -----------KSNSHDAKELHMFVWSSNGSPVSDRAGLNVFGGAPDNDQGG---RSDQG
OsPIN3A         -----------GALNHDAKELHMFVWSSSASPVSEVSGLPVFSGGGGGG------ALDVG
OsPIN1B         -------------VVGKRKDLHMFVWSSSASPVSER------------------AAAAA
AtPIN1          -----------KRQDGNGRDLHMFVWSSSASPVSD-----VFGGGGGNHHADYSTATNDH
OsPIN1C         -----------QAKGEDGKDLHMFVWSSSASPVSD-----VFGNGAEY-------NDAAA
OsPIN1A         -----------TNGQAKGEDLHMFVWSSSASPVSD-----VFGGGAPDY------NDAAA
                                            S377

PpPIND          TRE------NGTEHGHE-------------------------------------------
OsPIN5          AAAKEVSSGEVAPVEEEEA-----------------------------------------
OsPIN4          VEMNGAVVAAPGGGGGVRL-----------------------------------------
OsPIN5B         VEDGAAAAATAAAA----------------------------------------------
AtPIN8          KRETVVV-----------------------------------------------------
OsPIN6          VEAAGAAAAAGTVVVAAAA-----------------------------------------
AtPIN5          EEEVAI------------------------------------------------------
AtPIN6          RDALKAAPQATAAGGGASM---------------E-EGAAGK-----------------
PpPINC          PKELHVHVHPQSEHHLPGAANHKTQ-----DEHARQ-GFSFGNRR----DLKVEDVDNNG
PpPINA          PKEVRMLVHPQLDRGLAAA-SPRTY-----DEYTRE-DFSFGNRN----DLKLEDLDKDG
PpPINB          PKEVRMLVHPQSDLRHPEA-NPRTY-----DNYAQE-DFSFGNRN----DLKLEDLDKDG
```

```
OsPIN3B         IASSRVIRPPPGATGGERA--------------ASF-NKAVGGQ---------DELAKLE
AtPIN2          DPKVSIPPHDNLATKAMQN--------------LIE-NMSPGRKG----HVEMDQDGNNG
OsPIN2          PPPAAVPVGGATPKGVSGSVTPAAK-----NGGGEL-EIEDG-------------LKSPA
AtPIN4          AKEIRMVVSDQPRKSNARG----GG-----DDIGGL-DSGEGER----------EIEKAT
AtPIN7          AKEIRMLISDHTQNGENKA--------------GPM-NGDYGG------EEESERVKEVP
AtPIN3          AKEIRMLVPDQSHNGETKAVAHPAS-----GDFGGEQQFSFAGK-----EEEAERPKDAE
OsPIN3A         AKEIHMVIPADLPQNNGSGKEHEEYGAVALGGGGGGENFSFGGGKTVDGAEAVDEEAALP
OsPIN1B         AGAVHVFGGGGADHGDAKG------------AQAYD-EYSFGNKN-----------EKDG
AtPIN1          QKDVKISVPQGNSNDNQYV--------------ERE-EFSFGNKDDDSKVLATD------
OsPIN1C         VKEVRMAVASPRKADGVER----------------D-DFSFGNRGVAERDAEAGDEKSVA
OsPIN1A         VKSPRKMDGAKDREDYVER----------------D-DFSFGNRG----VMDRDAEAGDE


PpPIND          ---------------------------------------MAPSQMNLKEMAIKVAKKMV
OsPIN5          ---------------------------------------SAPAPSMKHVIWMA-VKKLL
OsPIN4          ----------------------------------------PFWATARTV----GLKLA
OsPIN5B         ---------------------------------------RRSLWPLVRAV----WLKVA
AtPIN8          ---------GEKSFLE-------------------------------VMSLVWLKLA
OsPIN6          ---------G-------------------------------KPSLWALVKVV----AHKLA
AtPIN5          ---------------------------------------VRTRSVGTMKILLKAWRKLI
AtPIN6          ---------DTTPVAAIGKQE------------------MPSAIVMMRLILTVVGRKLS
PpPINC          SKLDKKF--RSILTAELAPKHPMDEG---KTS--------MPPSSVMIKLICVMTFRKLT
PpPINA          PRLDKF---GSTSTAELTPKLAEDEA---KKS--------MPPSAVMIKLIAVMTFRKLV
PpPINB          PRLDNKF--GSTSTAELTPKVPEDEA---KKS--------MPPSAVMIKLIAVMTFRKLV
OsPIN3B         AGAKTE---QQTTAVTTTTKGGGAAG---AER---ARGQQNAPAGVMLRLILTTVWRRLI
AtPIN2          ---------GKSPYMGKKGSDVEDGGPGPRKQQ-------MPPASVMTRLILIMVWRKLI
OsPIN2          AGLAAKFPVSGSPYVAPRKKGGGADV---PGL--AEAAHPMPPTSVMTRLILIMVWRKLI
AtPIN4          AGLNKM---GSNSTAELEAAGGDGGG---NNGTH------MPPTSVMTRLILIMVWRKLI
AtPIN7          NGLHKL---RCNSTAELNPKEAIETG---ETV----PVKHMPPASVMTRLILIMVWRKLI
AtPIN3          NGLNKL---APNSTAALQSKTGLGGAEASQRKN-------MPPASVMTRLILIMVWRKLI
OsPIN3A         DGLTKM---GSSSTAELHPKVVDVDGPNAGGGAAGAGQYQMPPASVMTRLILIMVWRKLI
OsPIN1B         PTLSKL---GSNSTAQLRPKDDGEGM---AAA--------MPPASVMTRLILIMVWRKLI
AtPIN1          ---------GGNNISNKTTQAKV-----------------MPPTSVMTRLILIMVWRKLI
OsPIN1C         AAVS-----GEHGKPGLTPAPTA-----------------MPPTSVMTRLILIMVWRKLI
OsPIN1A         KAAAAA---GADPSKAMAAPTA-----------------MPPTSVMTRLILIMVWRKLI
                                                                :      .:


PpPIND          QLPLTHATVMGIVYSLIAGRWGFDPLRILRNSLDIMGRITLGLTMYSIGLFMAGQKKLVA
OsPIN5          QIPNTYASFLGLIWSLIAFKCGFSMPKIVEDSLFTIRTTAVGLSMFSSGTFIARQSRFVP
OsPIN4          RNPNVYASVLGVVWACIAYRWHLSLPGIVTGSLQVMSRTGTGMSMFSMGLFMGQQERVIA
OsPIN5B         RNPNVYAGVLGVAWACVTNRWHVETPSIIEGSVLIMSKTGVGLSMFSMGLFMALQDKIIV
AtPIN8          TNPNCYSCILGIAWAFISNRWHLELPGILEGSILIMSKAGTGTAMFNMGIFMALQEKLIV
OsPIN6          RNPNTYASFVGITWACLANRLHIALPSAFEGSVLIMSKSGTGMAMFSMGLFMAQQEKIIA
AtPIN5          INPNTYATLIGIIWATLHFRLGWNLPEMIDKSIHLLSDGGLGMAMFSLGLFMASQSSIIA
AtPIN6          RNPNTYSSLLGLVWSLISFKWNIPMPNIVDFSIKIISDAGLGMAMFSLGLFMALQPKMIP
PpPINC          RNPNTYSSLLGVVWSLISFKCHLDMPLILYKSYHIISDAGIGMAMFSLGLFMGMGDRIIA
PpPINA          WNPNTYSSLLGVIWSLVANRWHLSMPLILYKSVHILSDAGLGMAMFSLGLFMGLGDRIIV
PpPINB          WNPNTYSSLLGVIWSLVANRWHFTMPLILYKSVHILSDAGLGMAMFSLGLFMGLGDRIVV
OsPIN3B         RNPNTYASLIGLTWSLIAFRFHITMPIIVAKSISILSDAGLGMAMFSLGLFMATQPKIIA
AtPIN2          RNPNTYSSLFGLAWSLVSFKWNIKMPTIMSGSISILSDAGLGMAMFSLGLFMALQPKIIA
OsPIN2          RNPNTYSSLIGLVWSLVSFRWNIQMPSIIKGSISILSDAGLGMAMFSLGLFMALQPKIIS
AtPIN4          RNPNTYSSLIGLIWALVAYRWHVAMPKILQQSISILSDAGLGMAMFSLGLFMALQPKIIA
AtPIN7          RNPNTYSSLIGLIWALVAFRWDVAMPKIIQQSISILSDAGLGMAMFSLGLFMALQPKLIA
AtPIN3          RNPNTYSSLIGLIWALVAFRWHVAMPKIIQQSISILSDAGLGMAMFSLGLFMALQPKLIA
OsPIN3A         RNPNTYSSLLGLAWSLVAFRWHVSMPAIVEKSISILSDAGLGMAMFSLGLFMALQPSIIA
OsPIN1B         RNPNTYSSLLGVIWSLVSYRWGIEMPAIIARSISILSDAGLGMAMFSLGLFMALQPRIIA
AtPIN1          RNPNSYSSLFGITWSLISFKWNIEMPALIAKSISILSDAGLGMAMFSLGLFMALNPRIIA
OsPIN1C         RNPNTYSSLIGLIWSLVCFRWNFEMPAIILKSISILSDAGLGMAMFSLGLFMALQPRIIA
OsPIN1A         RNPNTYSSLIGLIWSLVCFRWNFEMPAIVLKSISILSDAGLGMAMFSLGLFMALQPHIIA
```

```
                 *  :: ..*: :: :  .       .  *   :    * :*:. * *:.    .:

PpPIND           S-WWVAFYGAFCRFIVGPGTMGVASLLLGLRGDTLRFAFLQAALPQAVISFVFAKDYNLH
OsPIN5           CGYKIASFSMVIKFLIGPVVMLFASLVIGMHGTLLHIAVVQAALPLAVTSFVYAEEYKVH
OsPIN4           CGAGLTALGMALRFVAGPLATLVGAAALGLRGDVLHLAIIQAALPQSIASFVFAKEYGLH
OsPIN5B          CGAGLTVLGMALRFVAGPAATAVGAFALGLRGDLLRLAIIQAALPQSITTFVFAKEYGLH
AtPIN8           CGTSLTVMGMVLKFIAGPAAMAIGSIVLGLHGDVLRVAIIQAALPQSITSFIFAKEYGLH
OsPIN6           CGTSFAALGLVLKFALGPAAMAIGSIAVGLRGDVLRVAIIQAALPQSITSFIFAKEYGLH
AtPIN5           CGTKMAIITMLLKFVLGPALMIASAYCIRLKSTLFKVAILQAALPQGVVPFVFAKEYNLH
AtPIN6           CGAKKATMGMLIRFISGPLFMAGASLLVGLRGSRLHAAIVQAALPQGIVPFVFAREYNLH
PpPINC           CGTKHALFAMLLRFLVGPAVFAAASYLVGLRGVSLNVSTVQAALPQGIVPFVFAKEYNVH
PpPINA           CGTKMAVFGMALRFLAGPAVFAAASYLVGLRGVPLKVSIVQAALPQGIVPFVFAKEYGVH
PpPINB           CGRKMAIFGMSLRFLAGPAVFAAASYLVGLRGVPLKVSIVQAALPQGIVPFVFAKEYGVH
OsPIN3B          CGYSVAAASMGVRFFFGPAIMAAASAAVGIRGTLLRIAIVQAALPQGIVPFVFAKEYNLH
AtPIN2           CGKSVAGFAMAVRFLTGPAVIAATSIAIGIRGDLLHIAIVQAALPQGIVPFVFAKEYNVH
OsPIN2           CGKTVATFAMAVRFLTGPAVIAATSIAIGLRGVLLHVAIVQAALPQGIVPFVFAKEYNCH
AtPIN4           CGNSVATFAMAVRFITGPAIMAVAGIAIGLHGDLLRIAIVQAALPQGIVPFVFAKEYNVH
AtPIN7           CGNSTATFAMAVRFFTGPAVMAVAAMAIGLRGDLLRVAIVQAALPQGIVPFVFAKEYNVH
AtPIN3           CGNSVATFAMAVRFLTGPAVMAVAAIAIGLRGDLLRVAIVQAALPQGIVPFVFAKEYNVH
OsPIN3A          CGKSAAVVSMAVRFLAGPAVMAAASIAIGLRGTLLHVAIVQAALPQGIVPFVFAKEYNVH
OsPIN1B          CGNSLASYAMAVRFLVGPAVMAAASIAVGLRGVLLHIAIVQAALPQGIVPFVFAKEYNVH
AtPIN1           CGNRRAAFAAAMRFVVGPAVMLVASYAVGLRGVLLHVAIIQAALPQGIVPFVFAKEYNVH
OsPIN1C          CGNKVATFAMAVRFLTGPAVMAAASIAVGLRGTLLHVAIVQAALPQGIVPFVFAKEYSVH
OsPIN1A          CGNKVATYAMAVRFLAGPAVMAAASFAVGLRGTLLHVAIVQAALPQGIVPFVFAKEYSVH
                 .    :      .*  **      .  : :..  :. : :***** .: .*::* :*  *

PpPIND           TDVFTTAVSLQTIVFMPIVLVYYTLLEL--
OsPIN5           ADIMSTGVILGIFISLPVTIVYYILLGL--
OsPIN4           ADVLSTAVIFGTLISLPILIAYYAVLGFV-
OsPIN5B          AEILSTAVIFGTLASLPVLIVYYIVLGFIR
AtPIN8           ADVLSTAVIFGMLVSLPVLVAYYAALEFIH
OsPIN6           ADVLSTAVIFGMLVSLPLLVGFYIVLELIR
AtPIN5           PEIISTGVIFGMLIALPTTLAYYFLLDL--
AtPIN6           PDLLSTLVIFGMIVSLPVTILYYVLLGL--
PpPINC           PEILSTAVIFGMIVTLPTALLYYILLGL--
PpPINA           PEILSTAVIFGMLIALPITMVYYILLGL--
PpPINB           PEMLSTAVIFGMLIALPITMVYYILLGL--
OsPIN3B          ATILCTFVIFGMLIALPITLVYYIILGL--
AtPIN2           PDILSTAVIFGMLVALPVTVLYYVLLGL--
OsPIN2           PQILSTAVIFGMLIALPITILYYVLLGI--
AtPIN4           PTILSTGVIFGMLIALPITLVYYILLGL--
AtPIN7           PAILSTGVIFGMLIALPITLVYYILLGL--
AtPIN3           PAILSTGVIFGMLIALPITLVYYILLGL--
OsPIN3A          PAILSTAVIFGMLIALPITLLYYILLGL--
OsPIN1B          PNILSTAVIFGMLIALPITLVYYILLGL--
AtPIN1           PDILSTAVIFGMLIALPITLLYYILLGL--
OsPIN1C          PDILSTAVIFGMLIALPITLVYYILLGL--
OsPIN1A          PSILSTAVIFGMLIALPITLVYYILLGL--
                 . :: * * :  :  :*  : :*  * :
```

Supp. Fig. 1. Sequence alignment of Arabidopsis, rice and *Physcomitrella patens* members of the UNIPROT PIN auxin efflux protein family. Multiple sequence alignment was performed by the MUSCLE algorithm (v3.8). Potential MAPK phosphorylation sites (S/TP motifs) and their positions in PIN1 are indicated underneath with red font. MAPK and PID phosphorylation sites and preferred MAPK phosphorylation flanking residues are highlighted in red, black and grey backgrounds, respectively.

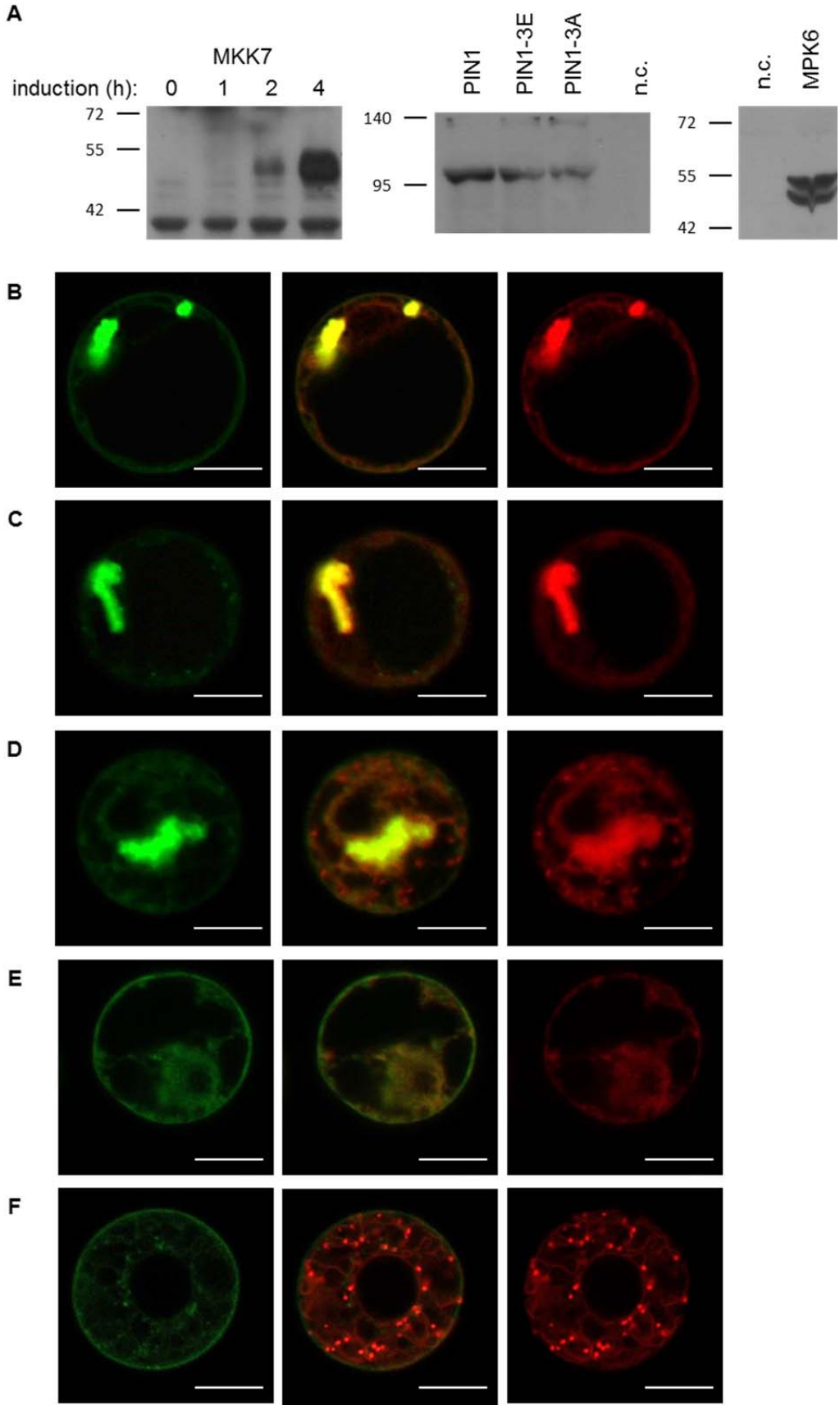

A
221SQGLSApT*PRPSNLTNAEIYSLQSSR245
b2 216.098
b3 273.120
y7 840.421
y18 1017.526 (2+)
y20-P 1094.069 (2+)
y9 1082.548
y21-P 1137.582 (2+)
y19-P 1058.547 (2+)
y8 953.504
y23-P 1222.639 (2+)
y6 677.357
y23 1271.624 (2+)
y21 1186.566 (2+)
y11 1267.631
a4 358.210
b4 386.205
y4 477.244
y5 590.326
b7-P 627.315
y12 1368.688
Relative Abundance
m/z
B
221SQGLpS*ApT*PRPSNLTNAEIYSLQSSRNPpT*PR250
y2 272.172
b2 216.098
y26 1006.490 (3+)
y28-P 1030.515 (3+)
y28-2P 997.865 (3+)
y23 893.442 (3+)
y28 1063.185 (3+)
y23-P 860.785 (3+)
y26-P 973.813 (3+)
y26-2P 941.156 (3+)
y21-P-H2O 1155.089 (2+)
y21-P 1164.099 (2+)
a4 358.210
b4 386.204
y4-P 452.264
y4 550.241
y12-P 694.368
Relative Abundance
m/z



Supp. Fig. 2. Spectra for representative identified phosphopeptides derived from PIN1 hydrophilic loop. HCD spectrum representing T286 phosphorylation is presented as Fig. 4C in the main text.

(A-D) Sequence of the precursor peptides are indicated above the spectra. Asterisk indicates phosphorylated Serine/Threonine. Peptide fragments are labelled according to the

nomenclature by Biemann (1990). -P stands for the 98-Da neutral loss of phosphoric acid characteristic to Ser/Thr phosphorylation. (A) HCD spectrum of m/z: 919.7788 (3+) representing SQGLSApTPRPSNLTNAEIYSLQSSR, [221-245] of PIN1. Site of phosphorylation is Thr-227 as proven by unmodified y_{18} and y_{19}-P fragment ions (m/z 1108.1 representing y_{19} was observed in the CID spectrum of the same precursor, data not shown). (B) HCD spectrum of m/z: 851.400 (4+) representing the doubly phosphorylated SQGLSATPRPSNLTNAEIYSLQSSRNPTPR, [221-250] of PIN1. One phosphorylation site is Thr-248 as proven by the y_4 fragment ion. The other site is either Ser-225 or Thr-227 proven by the (singly phosphorylated) y_{23} and the (doubly phosphorylated) y_{26} fragment ions (indicated by blue font). (C) CID spectrum of m/z: 870.4335 (4+) representing RSQGLSATPRPSNLTNAEIYSLQSSRNPpTPR, [220-250] of PIN1. Site of phosphorylation is Thr-248 as proven by y_4 and b_{27} fragment ions. (D) HCD spectrum of m/z: 876.1229 (4+) representing FHYQSGGSGGGGGAHYPAPNPGmFpSPNTGGGGGTAAK,
[313-349] of PIN1. m denotes oxidised Met. Site of phosphorylation is Ser-337 as proven by unmodified y_{12} and b_{23} fragment ions.


A
MKK7
induction (h): 0 1 2 4
72
55
42
PIN1
PIN1-3E
PIN1-3A
n.c.
140
95
n.c.
MPK6
72
55
42
B
C
D
E
F

Supp. Fig. 3. MAPK phosphorylation status at T227, T248 and T286 influences intracellular trafficking of PIN1. (A) Immunodetection of transfected proteins. PIN1:GFP MPK6:HA and myc:MKK7 were detected by commercial antibodies recognising the respective fusion tags. n.c. denotes non-transformed control. Molecular weight (kDa) markers are indicated. Protoplasts were transformed with all constructs simultaneously, rested overnight then treated with ß-estradiol for 4h if transfected with pER8:MKK7 prior to protein isolation or microscopy. (B-F) Co-localisation of PIN1 variants of differential phosphorylation status (green, left panels) with intracellular organelle markers (Nelson et al., 2007) (red, right panels) and merge images (middle panels). (B) PIN1 co-transformed with MPK6 and *pER8:MKK7*, induced with 1 µM β-estradiol for 4h with RFP:ER marker. (C) Phosphomimetic T227E, T248E, T286E (PIN1-3E) PIN1 mutant version with RFP:ER marker. (D) PIN1-3E with RFP:Golgi marker. (E) Non-phosphorylatable T227A, T248A, T286A (PIN1-3A) with RFP:ER marker. (F) PIN1-3A with RFP:Golgi marker. Scale bar: 10 µm.