

APK1A: PIX15; AT1G07570  
 APK1B: PIX16; AT2G28930  
 APK2B: PIX14; AT2G02800  
 PIX17; AT5G01020  
 RIPK: PIX8; AT2G05940  
 PIX1; AT1G61590  
 PIX7; AT5G15080  
 PIX13; AT2G17220

```

MCIQLS---AQVKAFSSG-----ASTKYDAKDIG-SLGSKA-SS
MCIQLS---AQIKAVSPGKP-----GASPKYMSSEANDSLGSKS-SS
MCNQLD---SSAKVDSSSHSPHANSASLSSRVSSKTSRSTVPSSLSINSYSS
MCNGLT---RDEAVF---TQAQAQQ-LQKHS-----RVSVDLSLSD
MAYKKKVS-----WRSLIWCLGD---PETLMAS---SKPKRKNVDVIKQSS---FQRLSILDMSN
MRDSS TASTKSSP-----LWKPFASNCSS---VDDQTVFGLNLSRCRPSRSEFSPKHLGLPLSFRRLLSFADLSR
MGLDAVKAKGNWKSKEKPEENKNNHKKKNGDDNKS RNEEEEEGEASG CWVKFRFMEGICIPSKS DLLDASSSIYGSNCVTVTMESKSANRKNQDPVQGVSTTTTSSN
MGLCMGSSPSD---SPPTTTPSSSTGNISSVGTFKSSNNTTTGTSTRGSLSSNSGFS
  
```

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 PIX7; AT5G15080  
 PIX13; AT2G17220

```

I II III IV
VSVRPSRPRTEGDIAS-PNKKQSSFAELSSAPRNRPRDSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
VSI RTNP RRTGEGEISS-PNKKQSSFAELSSAPRNRPRDSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
VESLPTPRTEGDIAS-PNKKQSSFAELSSAPRNRPRDSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
PSTPRFRDDSRTPISY-AQVIPLFLHPLHVIKSFPRDYSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
PSSNTLSEDLSSISAG-SDTHVFLAELHVIKSFPRDYSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
SSSARINEDLAQTG--ADTHVFLAELHVIKSFPRDYSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
AESSSSTPVISSEHNISSHLRRLHPLHVIKSFPRDYSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
VASEGDAYPDGQIPI-PNTRISLAPURASAPRNRPRDSVIGEGGCGVFKQWIDKSLTASRPGTGLVFAVKLNQDQWQHCHQEPHAPVNYLGFPSHHLVKLTGY
  
```

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 PIX7; AT5G15080  
 PIX13; AT2G17220

```

V VI VII VIII
CLDDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
CLDDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
CLDDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
CCEDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
CCEDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
CCEDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
CCEDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
CCEDEHRLIVYFEMPRGSIENHFRKGLYFQPLSKWRLKVALGCAAKGTAFLHNAISST-VTYRDFKSNILLDSYNAKLSDFGLAKDEBEGDISHVSTRVMGTWGY
  
```

Minimal cDNA overlap in Y2H experiment

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 PIX1; AT1G61590  
 PIX7; AT5G15080  
 PIX13; AT2G17220

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VII
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
AAPPYVARGHLLKSDVYVGVVHPLTSCNRADKNEPSCERNLWENAKPYVYKRRKIFRVLNRRQDQYSMEHACKVATLSTRCTTPEIKRBNQNEVSSHHTL
  
```

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 PIX1; AT1G61590  
 PIX7; AT5G15080  
 PIX13; AT2G17220

```

QSLNAAIGGNMDKTDRLRR-----MRRRSDSVVS-KKVNAGFAHQTAVGS TVVAYPRPSA--SELYV*
QTLNAGGRNIDMVGRR-----MRRRSDSVAINQKPNAGFAHQTAVGV IATAYPRPSD--SELFV*
ESTKPGTGVGNRQAIID-----SPRGSNGSIVQKSPRRYSYDRPLLHITPGASPLPTHNHSRVR*
QCTGDALIPCATTTAG-----AAFAMGVV-PDYRMHRRFANVVGPGAICRSPNPNYSFGGAAACRVR*
KDYNDIIPMGTTPTYPNTPDNKEDDGRVGNK-PRKSSHHHHHQQQSNHPRS--SPSTTKSPSPAKSPRNSTENHRRTRNRGWNPSLRSEAGGERY*
IHYKDMAVSSGHWPLS-----PKSQGGKVSQVVRGDRSRGKKSAPGSLRS*
PHLKDMASSSYFQTMQAEARLKNKSGRSQGFGRNGCQPVFRTLSSPHGSSPYRHQIPSPKQKGAAT*
BAANNEKP-----LERRTTRASPISIRQQQGHYRPPQLSSFRPQNVNSRAH*
  
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### Supporting Figure S4. Protein alignment of the eight full-length PIX proteins belonging to the RLCK family identified by yeast two-hybrid assay as putative interactors with XopAC-H469A.

Alignment was performed using Geneious alignment default parameters. Black, grey and light grey highlighting of amino acids indicates, 100%, 80-100% and 60-80% of similarity, respectively. The two red asterisks indicate the Serine-Threonine residues of RIPK/PIX8 uridylylated by XopAC (Feng *et al.*, 2012). Amino acids corresponding to the borders of the cDNA clones identified by yeast two-hybrid assay are highlighted in red. A red frame represents the minimal overlap between these 11 different cDNA clones. The 8 conserved kinase domains are indicated with roman numerals.