SUPPLEMENTAL MATERIALS AND METHODS

Isolation of *ZmPIN1c* gene

The isolation of *ZmPIN1c* partial genomic sequence was carried out by means of PCR amplifications using degenerated primers (Fw, 5'-ATGATYACSGGSACGGACTTCTA-3'; Rev, 5'-TCCASACGAACATGTGKAGGTCCT-3'), designed on the *ZmPIN1a* (DQ836239) and *ZmPIN1b* (DQ836240) sequences (Carraro, et al., 2006). This approach leads to the identification of an amplicon of 1,055 bp, which was sequenced resulting a new putative member of the *ZmPIN1* family. On this sequence a specific forward primer (5'-GTCGTCTACTCGCGCCGCTCCG -3') was designed and used in combination with an oligo(dT)12–18 anchored primer to perform RT-PCR amplifications, which permitted identification of the corresponding partial cDNA. Sequencing of both the genomic DNA and cDNA partial clones allowed us to design new primers for the amplification of the *ZmPIN1c* full-length sequences (Accession n°. EU570251). The primer combination used for the amplifications of both the genomic and cDNA full-length *ZmPIN1c* sequences was: Fw, 5'-CGGAGCGTGAGATCGATCGAGCTTC-3' and Rev, 5'-GTCGTAGCAGCTCTATCTCCCGTCCT-3'.

All the PCR amplified products were cloned using the pCRII-TOPO TA-cloning kit (Invitrogen). Sequences were edited and aligned using EditSeq and Megalign (Lasergene DNASTAR) software. GeneSeqer (http://www.plantgdb.org/cgi-bin/GeneSeqer/PlantGDBgs.cgi) software was used to determine the exon/intron structure of the gene.

ZmPIN1 phylogenetic analysis

Neighbor-joining phylogenetic trees showing the predicted relationship between maize, Arabidopsis, rice, and wheat PIN proteins were prepared in according to Paponov et al., (2005). PIN sequences from *Arabidopsis thaliana*, *Triticum aestivum* and *Oryza sativa* were identified through a Blast search of the TAIR (http://www.arabidopsis.org) and TIGR (http://www.tigr.org) databases using AtPIN genes as query: AtPIN1, At1g73590; AtPIN2, At5g57090; AtPIN3, At1g70940; AtPIN4, At2g01420; AtPIN5, At5g16530; AtPIN6, At1g77110; AtPIN7, At1g23080; and AtPIN8, At5g15100. Data obtained were predicted amino acid sequences based on EST clones. The full cDNA sequences for wheat was unknown, therefore the EST at the 5'-end was used. The gene nomenclature corresponds to the following TIGR gene index: OsPIN1a, NP895789; OsPIN1b, TC250501; OsPIN2, Np897806; OsPIN4, TC259719; OsPIN5a, TC255589; OsPIN5b, TC272668; OsPIN9, TC256882; OsPIN10a, Tc260564; OsPIN10b, NP1102328; TaPIN1a, TC224207; TaPIN1b, Ck208849; TaPIN1c, Tc224208; TaPIN2, TC200857; TaPIN5a, BQ484087; TaPIN5b, CA722918; TaPIN8, CB307721; and TaPIN9, Cd895017. SEPIN (gi:57636762) from *Staphylococcus epidermidis* was selected as the outgroup.

The tree is based on an alignment prepared using ClustalX 1.81 [Blosum Weight Matrix; Gap Opening Penalty: 5; Gap Extension Penalty: 0.20; (Thompson, et al., 2002)], followed by analysis with Phylip

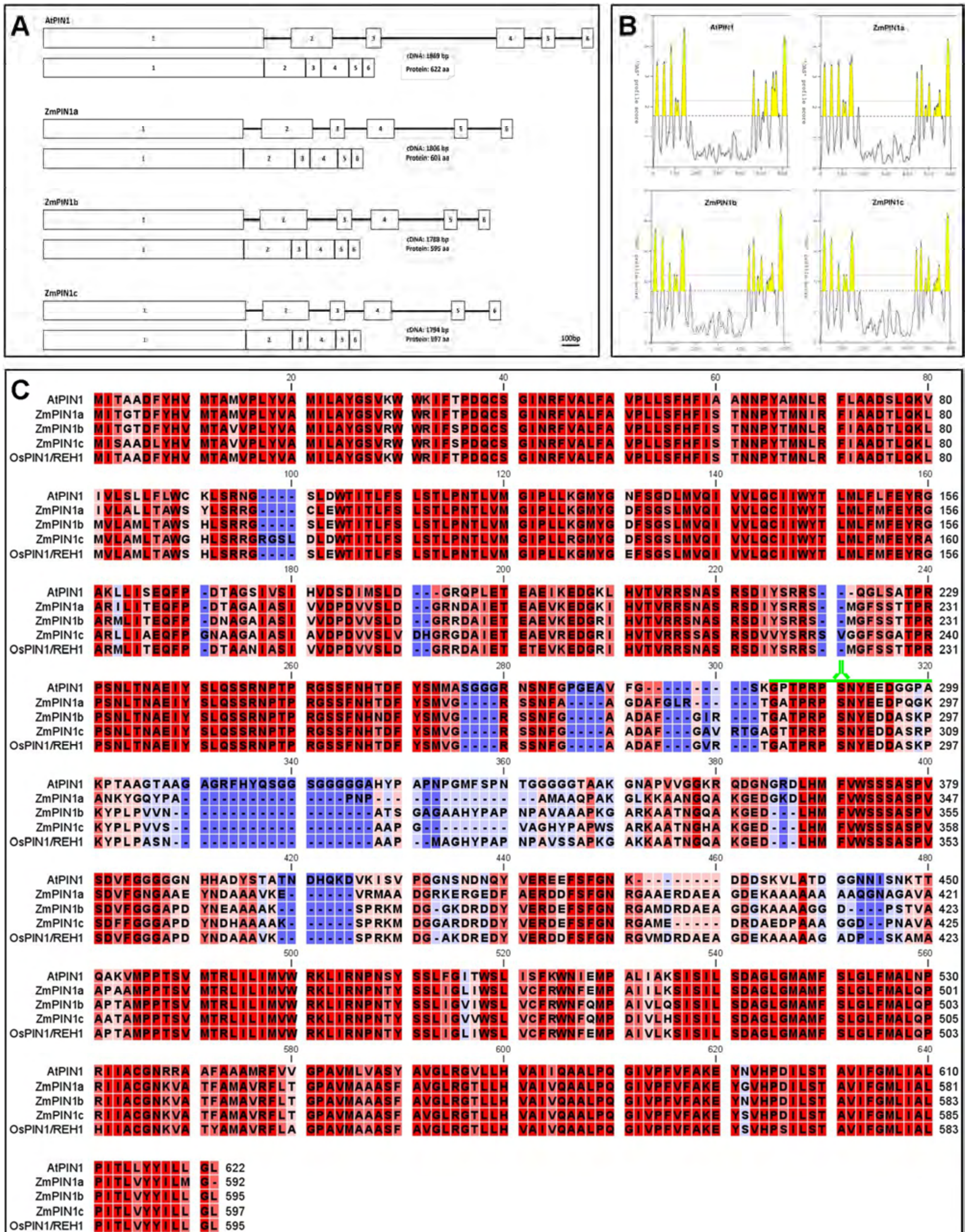
package (Felsenstein, 1989) using 100 bootstrap. The phylograms were drawn using Tree-View 1.6.6 (http://darwin.zoology.gla.ac.uk/wrpage/treeviewx).

LITERATURE CITED IN SUPPLEMENTAL MATERIALS & METHODS

Carraro N, Forestan C, Canova S, Traas J, Varotto S (2006) ZmPIN1a and ZmPIN1b encode two novel putative candidates for polar auxin transport and plant architecture determination of maize. Plant Physiol **142:** 254-264

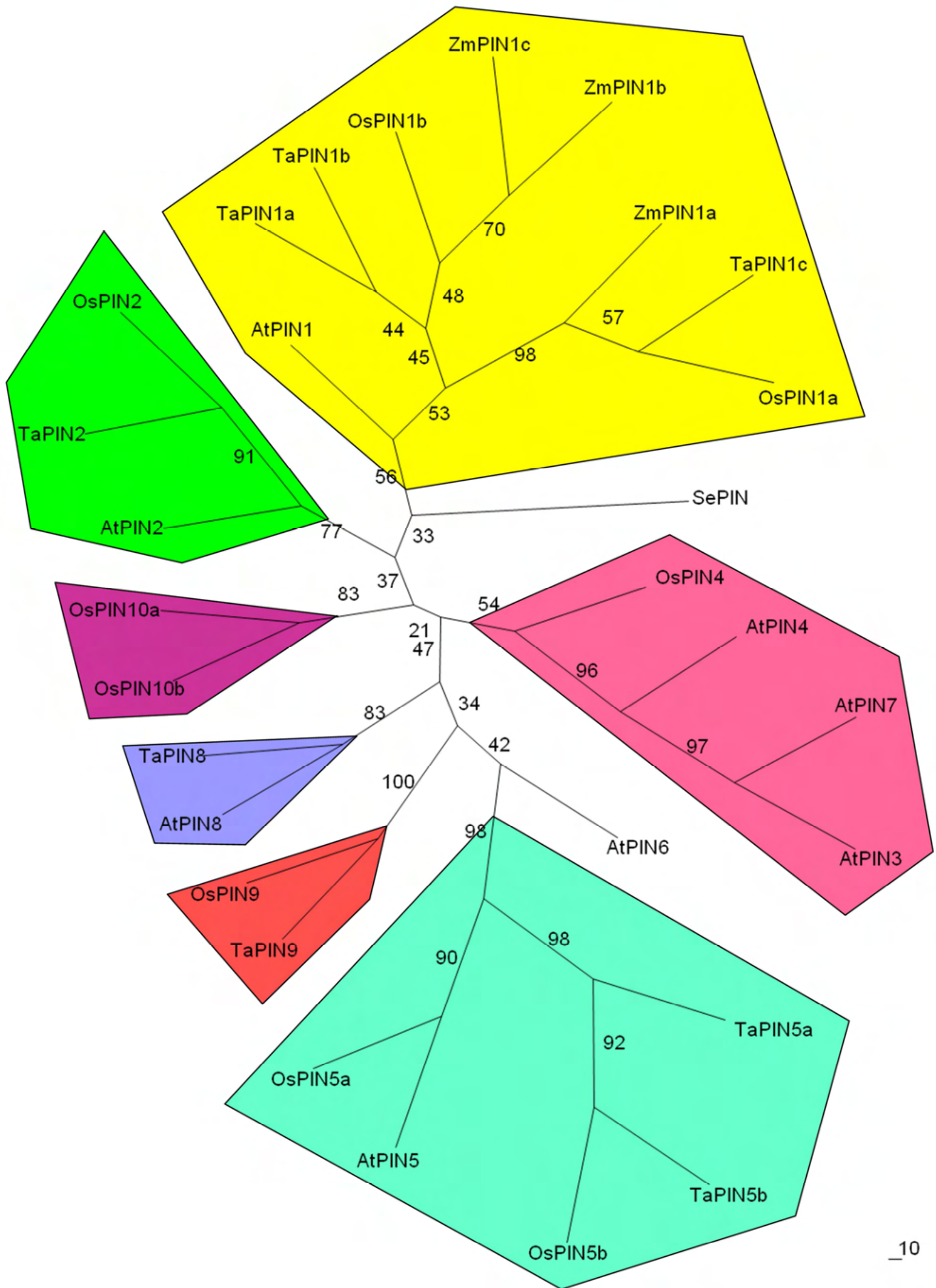
Felsenstein J (1989) PHYLIP - Phylogeny Inference Package. Cladistics **5:** 164-166

Thompson JD, Gibson TJ, Higgins DG (2002) Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinformatics **Chapter 2:** Unit 2.3



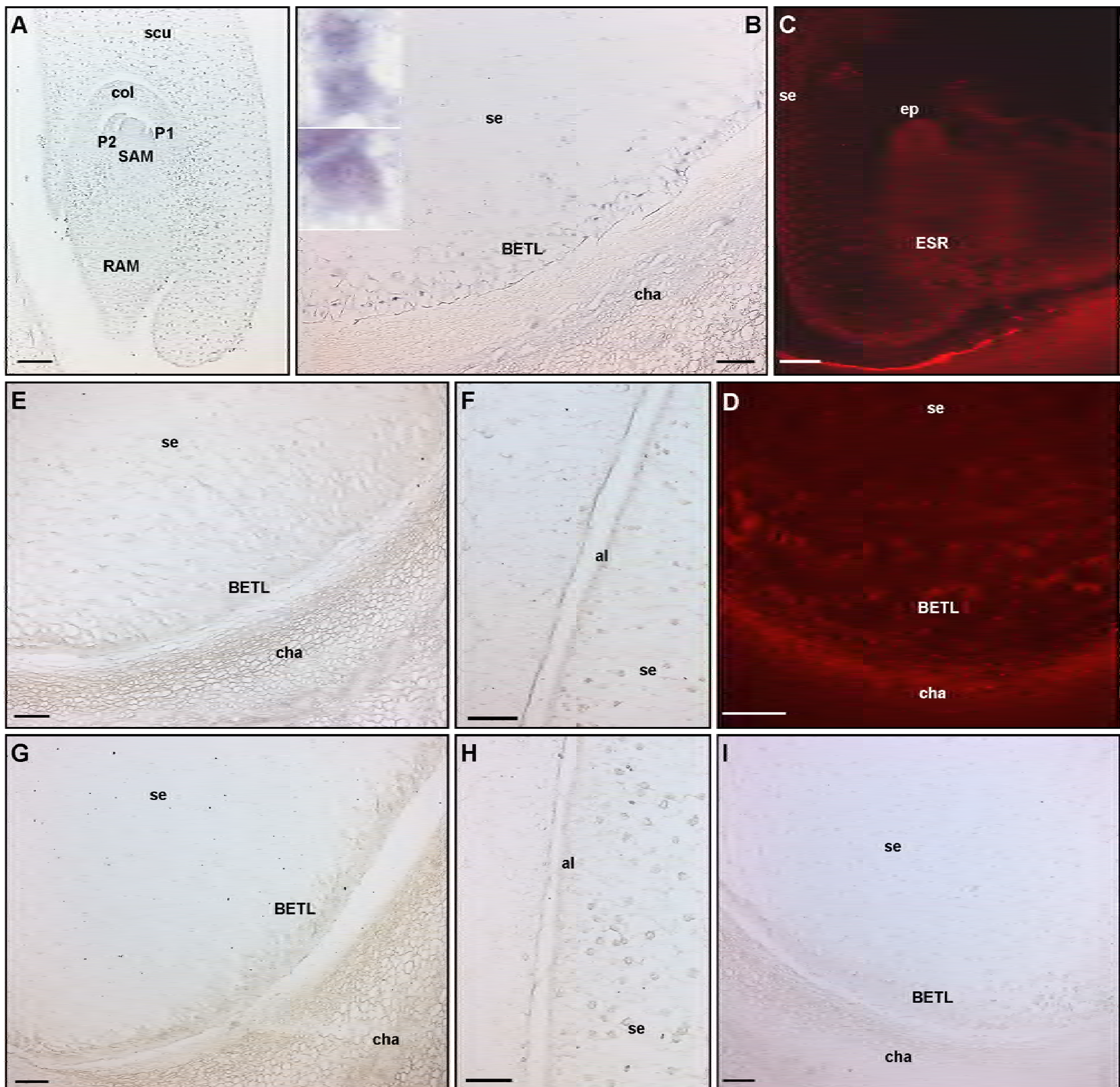
Supplemental Figure S1: Structural comparison between *AtPIN1* and *ZmPIN1* genes and proteins.
The new isolated *PIN1* gene was named *ZmPIN1c* (Accession n° EU570251). The full-length genomic sequence of *ZmPINc* is 2,756 bp long, while the corresponding open reading frame (ORF) is 1,794 bp long and encodes a putative protein of 597 amino acids (64.65 kDa).

(A) The *ZmPIN1c* gene shows an exon-intron structure comparable to that of *AtPIN1*, *ZmPIN1a* and *ZmPIN1b*. White boxed domains indicate translated exon sequence while introns are depicted as black lines. ORFs are also depicted, while the length of ORFs and encoded proteins are reported. Scale bar = 100 bp.
(B) ZmPIN1c protein shares a similar hydropathy profile with ZmPIN1a, ZmPIN1b and AtPIN1, with two putative lipophilic domains at the N and C ends and one central hydrophilic loop Transmembrane domains in PIN1 proteins were predicted using DAS algorithm [(Cserzo, et al., 1997) - http://www.sbc.su.se/~miklos/DAS/]. Yellow areas represent putative transmembrane domains.
(C) The ZmPIN1c protein shows an amino acid sequence identity of 83.8% and 91.9% with respect to ZmPIN1a and ZmPIN1b polypeptides, respectively. The amino acid identity between ZmPIN1c and AtPIN1 proteins is 72.9%, whereas the identity with other Arabidopsis PIN proteins is lower, spanning from 65.5% with AtPIN3 to 43.3% with AtPIN5. Amino acid sequence alignment of Arabidopsis, rice and maize putative PIN1 proteins. AtPIN1 (accession
n°: At1g73590), OsPIN1/REH1 (AF056027) and putative ZmPIN1a (DQ836239), ZmPIN1b (DQ836240) and ZmPIN1c (EU570251) were aligned using ClustalX 1.81: Blosum Weight Matrix; Gap Opening Penalty: 10; Gap Extension Penalty: 0.20, (Thompson, et al., 2002) and then edited with CLC Sequence Viewer 5. The number of amino acids is indicated on the right. Above the multiple sequence alignment a green bar indicated the oligopeptide GPTPRPSNYEEDGGPA targeted by an anti-AtPIN1 antibody monoclonal antibody that was demonstrated to recognize a maize protein with a similar molecular weight of AtPIN1 (Boutte, et al., 2006; de Reuille, et al., 2006).



Supplemental Figure S2: PIN1s phylogenetic analysis

Phylogenetic analysis showed that ZmPIN1c protein belongs in a cluster containing ZmPIN1a, ZmPIN1b, AtPIN1 and the putative PIN1 proteins of rice and wheat.
The phylogenetic analysis was performed using the PIN amino acid sequences from Arabidopsis (*Arabidopsis thaliana*), wheat (*Triticum aestivum*), and rice (*Oryza sativa*) and maize (*Zea mays*), as reported in (Paponov, et al., 2005). The tree is based on an alignment prepared using ClustalX 1.81: Blosum Weight Matrix; Gap Opening Penalty: 5; Gap Extension Penalty: 0.20, (Thompson, et al., 2002), followed by analysis with Phylip package (Felsenstein, 1989) using 100 bootstrap. The phylogram was drawn using Tree-View 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). For details see "Supplemental Materials and Methods''.

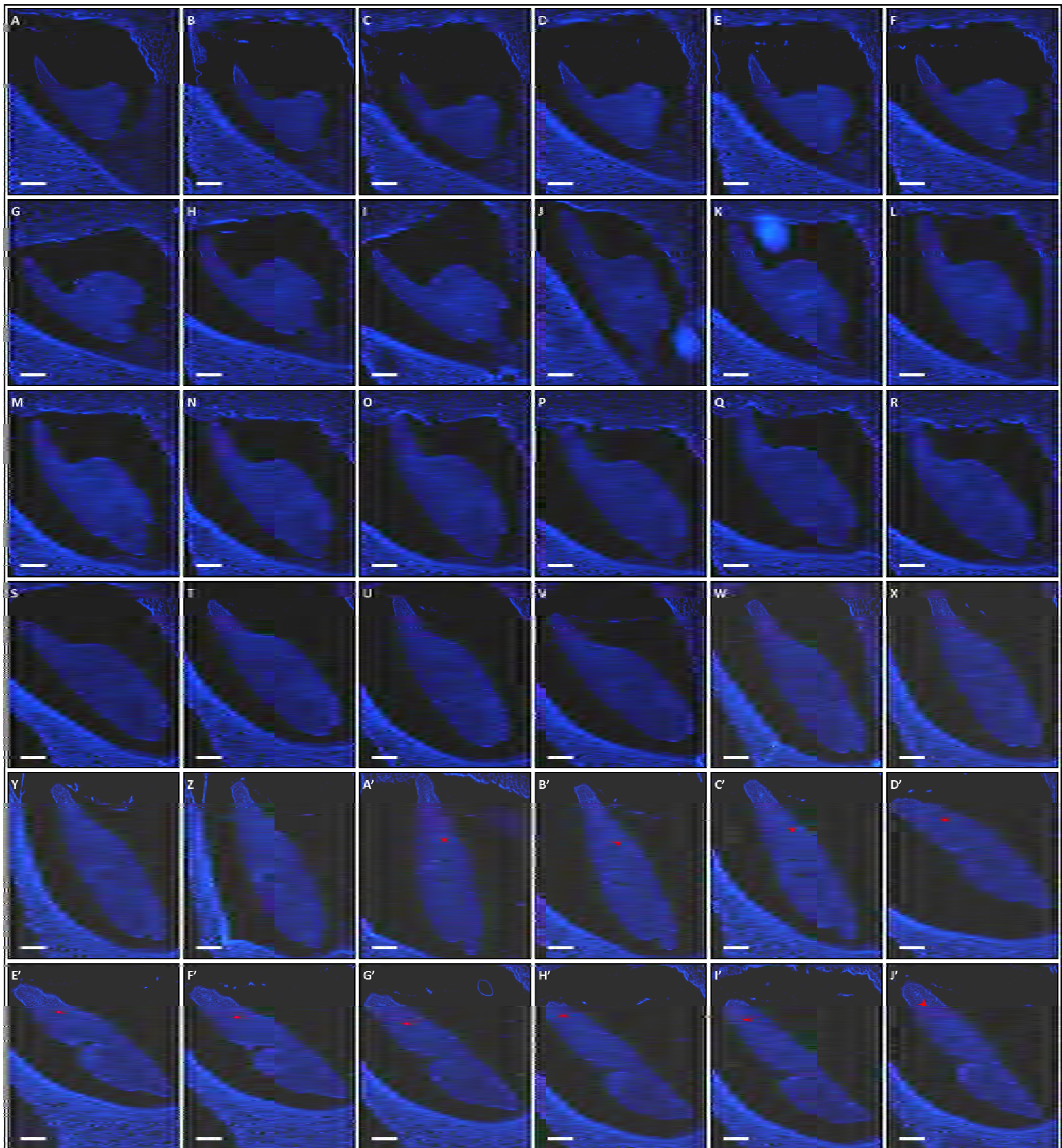


Supplemental Figure S3: *In situ* hybridization and immunolocalization negative controls

A sense RNA probe common to the three *ZmPIN1* genes was used as negative controls for *in situ* hybridization experiments; the longitudinal sections of embryo (**A**) and endosperm (**B**) hybridized with this probe are shown. Insets in (**B**) represent high magnifications of embryo sections labelled with *ZmPIN1* antisense probe: the differential staining of cytoplasm, which is confined around the nucleus, and the faint staining of the nucleus itself is visible.

Hybridization with secondary antibody only was used for negative control in anti-PIN1 immunolocalization assays; section of embryo (**C**) and BETL cells (**D**) labelled with the Alexa568 secondary antibody are shown. Three different negative controls were produced for anti-IAA immunolocalization experiments. For each series of slides derived from one kernel, two slides were hybridized with the primary (**E**) and (**F**) or secondary (**G**) and (**H**) antibody only. This control series show no staining in BETL (**E**) and (**G**) and aleurone cells (**F**) and (**H**), nor in the maternal chalazal region (**E**) and (**G**). In addition, immunolocalizations were also performed on kernels not prefixed with EDAC to test the specificity of antibody reaction (**I**). In samples not prefixed with EDAC the immune signal was not detectable and sections were as stained as the negative controls.

ESR: Embryo Surrounding Region; BETL: Basal Endosperm Transfer Layer; se: starchy endosperm; al: aleurone; ep: embryo proper; SAM: Shoot Apical Meristem; RAM: Root Apical Meristem; scu: scutellum; col: coleoptile; P1-2: leaf primordia. Scale Bars = 100 μm.



Supplemental Figure S4: Effects of NPA treatments on scutellum development and symmetry
NPA treatments, inhibiting polar auxin transport, cause aberrant scutellum development. Serial sections through a 15DAP embryo collected from a plant watered for two weeks with 120µM N-1-naphthylphthalamic acid (NPA) stained with 0.01% Calcofluor. All the panels present epifluorescent images acquired with a Leica DC300F camera.
The serial sections show the very irregular and asymmetric scutellum morphology and several vasculature defects in the scutellum vascular bundles. Red arrowheads indicate the abnormal, enlarged and irregular vascular tissues.
Scale Bars = 200 µm.