Phosphate, a key plant nutrient, is perceived through inositol polyphosphates (InsPs) by SPX domain-containing proteins. SPX1 an inhibit the PHR2 transcription factor to maintain Pi homeostasis. How SPX1 recognizes an InsP molecule and represses transcription activation by PHR2 remains unclear. Here we show that, upon binding InsP\textsubscript{6}, SPX1 can disrupt PHR2 dimers and form a 1:1 SPX1-PHR2 complex. The complex structure reveals that SPX1 helix \(\alpha_1\) can impose a steric hindrance when interacting with the PHR2 dimer. By stabilizing helix \(\alpha_1\), InsP\textsubscript{6} allosterically decouples the PHR2 dimer and stabilizes the SPX1-PHR2 interaction. In doing so, InsP\textsubscript{6} further allows SPX1 to engage with the PHR2 MYB domain and sterically block its interaction with DNA. Taken together, our results suggest that, upon sensing the surrogate signals of phosphate, SPX1 inhibits PHR2 via a dual mechanism that attenuates dimerization and DNA binding activities of PHR2.
Phosphorus is a fundamental element of all living organisms and represents a key building block of many cellular molecules, such as the genetic material DNA and the energy carrier ATP. Therefore, all cells need to maintain a sufficient concentration of phosphate (Pi) in their cytoplasm. For plants, the availability of inorganic Pi in soil is poor due to its low mobility. To cope with the fluctuating levels of Pi, plants have evolved sophisticated strategies to modulate Pi uptake and remodeling. The Pi sensing and regulatory mechanisms that control Pi acquisition and homeostasis are crucial for plant developmental and reproductive processes. Understanding these mechanisms can provide insight into plant responses to growing environmental stressors.

For plants, Pi is a critical nutrient for growth and development. The availability of Pi in the soil can affect plant physiology and morphology. To cope with fluctuating Pi levels, plants have evolved sophisticated strategies to regulate Pi uptake and homeostasis. Understanding these mechanisms can provide insight into plant responses to environmental stressors and can inform sustainable agricultural practices.

The Pi-sensing transduction pathway in Arabidopsis involves the Pi-responsive transcription factors, PHR1 and PHR2. These transcription factors bind to a Pi-responsive cis-regulatory element in the promoter regions of Pi starvation-induced (PSI) genes. By activating the expression of PSI genes, PHR1 and PHR2 enhance Pi uptake under Pi-deficient conditions. To avoid Pi toxicity caused by excessive Pi accumulation in plants, SPX proteins, on the other hand, bind to PHR proteins and inactivate PHR-induced transcription under Pi-replete conditions.

Recent structural analysis of the SPX-domain, in conjunction with in vivo studies, has provided compelling evidence for the unique dual mechanism by which SPX1 mediates Pi sensing and signaling. SPX1 (SPX11–259) interacts with full length and a C-terminally truncated fragment of PHR2 (PHR230–426), which has been previously reported to interact with full length and a C-terminally truncated fragment of SPX1 (SPX11–259) (Supplementary Fig. 1a). To verify whether InsP molecules mediate SPX1-PHR2 complex formation, we used the SPX-domain in complex with InsP6, a commercially available substitute of InsP6, and representative of the cellular Pi status.

The SPX1-PHR2 complex contains two copies of the InsP6-bound PHR2 complex, with a molecular weight of about 50.1 kDa. Using ITC assay, we further analyzed the effect of InsP6 on the binding affinity of PHR2. Individual InsP6 or SPX1 did not affect the ability of PHR2 to bind DNA. However, when both InsP6 and SPX1 were mixed with PHR2, the DNA binding activity of the transcription factor was completely abolished (Fig. 1e). In line with the documented importance of PHR2 dimerization, we noticed that PHR2 monomeric mutant exhibited lower DNA binding and transcriptional activity compared to wild type in ITC and dual-LUC transient transcriptional activity assays. In contrast to the wild type PHR2, the transcriptional activity of the PHR2 monomeric mutant could be inhibited by SPX1 under Pi starvation conditions in accordance with its constitutive interaction with SPX1. Based on these results and our aforementioned analysis shown in Fig. 1a, we conclude that PHR2 monomerization is a critical step in PHR1- and InsP6-induced inactivation of the transcription factor.

**Overall structure of SPX1-PHR2.** The SPX1-PHR2 complex formation mediated by InsP6 initiates downstream transcriptional inhibition of PSI genes. To understand how SPX1 responds to InsP6 binding, we determined the InsP6-SPX1-PHR2 complex structure at 2.6 Å resolution using single-wavelength anomalous diffraction. The model was refined to a final Rwork and Rfree value of 22.6 and 27.4%, respectively (Supplementary Table 1). There are two amino acids located in the outlier region of Ramachandran plot probably due to poor accuracy associated with the electron density in the local region. The final model of the InsP6-SPX1-PHR2 complex contains two copies of the InsP6-SPX1-
A highly crystallizable T4 lysozyme (T4L) was fused to the C-terminus of SPX1 to facilitate crystallization and improve the quality of crystals. The two T4 lysozyme molecules in the asymmetric unit adopt different orientations with respect to SPX1 due to crystal packing (Supplementary Fig. 1c).

The SPX domain of SPX1 consists of two long core helices $\alpha_3$ and $\alpha_4$, and two shorter C-terminal helices, $\alpha_5$ and $\alpha_6$. Two N-terminal helices $\alpha_1$ and $\alpha_2$ form a helical hairpin. The two SPX1 molecules in the asymmetric unit, which share high structural homology with documented SPX domain structures, can be superimposed with each other with a Ca RMSD of 1.36 Å (Supplementary Fig. 1d, e)\(^1\). The PHR2 MYB domain contains three consecutive $\alpha$ helices, which give rise to the conserved fold of the MYB proteins. The MYB domains of rice PHR2 and Arabidopsis PHR1 can be superimposed with a RMSD of 1.40 Å (Supplementary Fig. 1f)\(^2\). The PHR2 CC motif long helix closely aligns with the CC motif of Arabidopsis PHR1 with a Ca RMSD of 0.89 Å (Supplementary Fig. 1g)\(^3\). Interestingly, both the MYB domain and CC motif of PHR2 make direct contact with SPX1. They approach SPX1 from two different directions and sandwich SPX1 in between with extensive protein–protein interfaces (Fig. 2a).
**SPX1-PHR2 CC motif interface.** SPX1 interacts with the PHR2 CC motif via an extensive and elongated interface. Specifically, the PHR2 CC motif long helix interacts with three helices of SPX1 (α4, α5, and α6) through several ionic interactions and hydrogen bonds (Fig. 2b). SPX1 E135 and R161 form salt-bridges with PHR2 R356 and E363 respectively. In addition, SPX1 E135, R161, and Y180 interact with PHR2 H349, E363, E353, and R356 through hydrogen bonds.

To verify the structural observations, we individually mutated residues in the SPX1-PHR2 CC motif interface into alanine. The R161A mutation in SPX1 and the PHR2 mutants, H349A and R356A, all compromised SPX1-PHR2 stable complex formation, whereas other SPX1 mutants, E135A and Y180A, and PHR2 mutants, E353A and E363A, failed to alter SPX1-PHR2 binding (Fig. 2c and Supplementary Fig. 3b, d). Moreover, hydrophobic residues, such as V125 and V132 in SPX1 and L352, L342 of PHR2 also contribute to the interface formation (Fig. 2b). Substitution of V125 and V132 in SPX1 and L342, L352 in PHR2 with alanine abolished SPX1-PHR2 interaction (Supplementary Fig. 3a, b). The L360 of PHR2 looks like participate the hydrophobic patch formation, however, PHR2 single mutation L360A retained the ability to bind SPX1. Collectively, SPX1-PHR2 CC interface, which is largely stabilized by hydrogen bonds, salt-bridges and hydrophobic interactions, is critical for complex formation.

The crystal structure of the Arabidopsis PHR1 CC dimer has recently been determined. Surprisingly, superposition analysis indicates that the PHR CC dimer interface is fully compatible with SPX1 binding (Fig. 2d), suggesting that the SPX1 binding via the SPX1-PHR2 CC interface itself does not interfere with PHR2 dimerization. In order to disrupt the PHR2 dimer, InsP6 must act through a mechanism outside the SPX1-PHR2 CC interface.

**InsP₆-binding site.** Our structure reveals three InsP₆ molecules in the asymmetric unit (Supplementary Fig. 4a). One InsP₆ molecule participates in crystal-packing interactions, while the other two are each captured by an SPX1 molecule via a highly basic surface groove formed between helices α1, α2, and α4 (Figs. 2a, 3a and Supplementary Fig. 4c). Importantly, this binding mode of these two InsP₆ molecules to SPX1 is very similar to the reported InsP₆-SPX domain structures.
Mutations of InsP₆-binding sites affect SPX1-PHR2 complex formation and Pi signaling. a A close-up view of the SPX1-InsP₆ interactions. InsP₆ was represented as a stick model with a stimulated annealing omit map and contoured at 1.1σ. Residues of SPX1 interacting with InsP₆ were shown as sticks and labeled. The yellow dashed lines represent hydrogen bonds involved in the interaction of SPX1 and InsP₆. b Binding affinities of InsP₆ with SPX1-259 (orange) and SPX1-259 Y25F/K29A/K151A (SPX1₃M, purple) were measured by ITC. NDB, no detectable binding. c Mutations of InsP₆-binding sites in SPX1-259 impaired the SPX1-259-PHR2 interaction. The assays were performed as described in (Fig. 1a). Experiments were independently repeated three times with similar results. Uncropped gel images are available as source data. d Growth phenotype of WT, spx1 spx2, and two kinds of transgenic plants were grown under Pi-sufficient condition (SPX1/YWT/spx1 spx2 and SPX1/Y25F/K29A/K151A/spx1 spx2). e Phenotype of phosphate excess in leaves of plants shown in (d). f Pi contents of plants shoots shown in (d). Error bars, mean ± s.d. The number of biologically independent samples of WT, spx1 spx2, SPX1/YWT/spx1 spx2 and SPX1₃M/spx1 spx2 is 4, 5, 3 and 4, respectively. Sets of data were analyzed by independent-samples T test (two-tailed), where *** represents a statistically significant difference at p < 0.001. g PSI genes (OsIPS1 and OsPT2) expression in the root of the plants shown in (d). Error bars, mean ± s.d. The bar graphs show the results from three biologically independent samples (n = 3). Sets of data were compared by paired-samples T-test (two-tailed), where *** represents a statistically significant difference at p < 0.001.
the PHR2 CC motif of our InsP₆-SPX1-PHR2 ternary complex structure onto the Arabidopsis PHR1 CC dimer structure. Remarkably, the resulting model readily revealed a severe collision between the α₁ helices of the two SPX1 molecules (Fig. 4b). This observation strongly suggests that InsP₆ stabilizes SPX1 α₁ helix and reinforces a steric hindrance to prevent the SPX1-bound PHR2 to maintain its dimeric state. To validate this idea, we tested the ability of the SPX1 mutant missing its N-terminal α₁ helix to disrupt the PHR2 dimer in the presence of InsP₆. Although this SPX1 mutant retains its InsP₆-binding activity as shown in Fig. 4a, it indeed failed to separate the PHR2 dimer (Fig. 4c and Supplementary Fig. 8). To visualize the effect of InsP₆ on the SPX1 α₁ helix, we obtained Small Angle X-ray Scattering (SAXS) data for SPX1 and InsP₆-SPX1. The comparison of scattering curves between SPX1 and InsP₆-bound SPX1 shows that InsP₆-bound state appears to be different. It seems that InsP₆ makes SPX1 pack tightly according to the smaller Guinier radius of gyration (Rg) and Dmax for SPX1 in presence of InsP₆ (Supplementary Fig. 9 and Supplementary Table 2). Further, we calculated the respective molecular bead model using SAXS profiles, and reconstructed the SPX1 model by SASREF. SPX1 and InsP₆-bound SPX1 show similar architecture except α₁ helix. In addition, the Ab initio molecular envelope shows a difference in the α₁ helix area (Supplementary Fig. 10). Further, the thermal shift assay showed that InsP₆ could increase the melting temperature (Tm) of SPX1 greatly but could not change the Tm of SPX1ΔN₁₇ (N-terminal α₁ deletion of SPX1ΔN₁₇) obviously (Supplementary Fig. 11a, c, d). The comparison of Circular Dichroism (CD) spectra of SPX1 and InsP₆-SPX1 indicated that InsP₆ could increase the helical content of SPX1 (Supplementary Fig. 11a, b). Therefore, InsP₆ could stabilize SPX1 α₁ helix. Notably, the corresponding helix α₁ truncation mutation in PHO1, an SPX1 domain-containing protein, exhibited a similar phenotype as SPX1 InsP₆-binding site triple mutation. Collectively, the location of the InsP₆-binding site distant from the SPX1-PHR2 CC interface, the involvement of the SPX1 α₁ helix in InsP₆ binding, and the steric hindrance imposed by SPX1 α₁ helix in the context of the PHR2 dimer, provide a structural mechanism by which InsP₆ allosterically uncouples the PHR2 dimer and promotes the formation of an SPX1-PHR2 1:1 heterodimeric complex.

**Interface between SPX1 and PHR2-MYB.** In addition to breaking the PHR2 dimer apart, our structure shows that SPX1 forms a second interface with PHR2 via its MYB domain. The helices α₁ and α₃ of the PHR2 MYB domain act as a clamp to hold the helix α₃ of SPX1 through a salt-bridge and side-chain hydrogen bond network, which is formed among E89, E92, K99, E100 of SPX1 and K297, H294, R248, E257, R302 of PHR2 (Fig. 5a). To verify the structure, we individually mutated residues in the SPX1-PHR2 MYB interface into alanine. SPX1 mutants E89A, K99A, E92A, and E100A impaired the interaction with PHR2 and PHR2 mutants K297A, H294A, R302A attenuated the binding with SPX1. Other mutants PHR2 R248 and E257 had little effect on the SPX1-PHR2 interaction. These results suggest that this second SPX1-PHR2 interface is also important for SPX1-PHR2 complex assembly (Fig. 5b and Supplementary Fig. 3c, d).

Remarking superposition analysis of our structure and the Arabidopsis PHR1 MYB-DNA complex structure reveals a severe steric hindrance for DNA binding by the SPX1-bound PHR2-MYB. In fact, the PHR2 MYB domain contacts with SPX1 through the same helix α₃ and Loop1 region, which is used by Arabidopsis PHR1 to dock onto DNA (Fig. 5c). This feature of the InsP₆-stabilized SPX1-PHR2 complex provides the structural basis explaining the previous observation that SPX1 competes with DNA for PHR2 MYB binding in vitro and ultimately inhibits the transcriptional activation of PHR2-induced PSI genes. The functional importance of this SPX1-PHR2 MYB interface is further underscored by the conservation of the majority of the interface residues in plants (Supplementary Fig. 6a, b). In fact, Arabidopsis PHR1 mutants K325A/R335A and K325A/H328A/R335A, which have residues at the predicted SPX1-binding interface (R335 and H328 are the PHR1 residues corresponding to rice PHR2 R356 and H349), showed a Pi excess phenotype.

**Discussion**

Our InsP₆-SPX1-PHR2 structure predicates the incompatibility of PHR2 homodimerization and SPX1 binding due to the collision of two SPX1 helices α₁ in superimposition analysis and mechanistically explains the ability of SPX1 in disrupting PHR2

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**Fig. 4 SPX1 helix α₁ interferes the PHR2 dimer.** a Binding affinity of InsP₆ with SPX1ΔN₁₇ (N-terminal helix α₁ deletion of SPX1ΔN₁₇) was measured by ITC. b PHR2 dimer model constructed by superimposing PHR2 CC in InsP₆-SPX1-PHR2 ternary complex structure with AtPHR1 CC dimer structure. The obvious clash occurred between the two helices α₁ was shown in the close-up view. Two helices α₁ of SPX1 were represented in orange and blue respectively. c SPX1ΔN₁₇ impaired the SPX1-PHR2 MYB-binding interaction. SPX1 helix α₁ is essential for PHR2 dimer dissociation. The assays were performed as described in (Fig.1a). Experiments were independently repeated three times with similar results. Uncropped gel images are available as source data.
dimer in the presence of InsP₆. Interestingly, the InsP₆ binding site is not situated at the SPX1-PHR2 interface. But the loss of InsP₆ binding ability clearly affects the stable interaction between SPX1 and PHR2. Based on these observations, we conclude that InsP₆ plays an important role in SPX1-mediated PHR2 dimer dissociation. A similar effect on PHR2 attenuation was observed in SPX1 helix α₁, whose deletion did not affect InsP₆ binding greatly, but prevented the dimer separation despite of helix α₁ absence on the SPX1-PHR2 interaction interface. However, neither InsP₆ nor SPX1 itself could abolish the PHR2 dimer (Fig. 1a and Supplementary Fig. 12a, b). Since helix α₁ stabilizes SPX1-InsP₆ interaction, InsP₆ is expected to elicit the same effect on SPX1 helix α₁, which leads to the destabilization of the PHR2 dimer. We hypothesize that the InsP molecule is sensed by SPX1 and binds to SPX1 with the increase of InsP concentration. Each InsP molecule from two SPX1 molecules probably stabilizes SPX1 helix α₁ to disrupt PHR2 dimer. Concurrently with the dimer dissociation, PHR2 MYB domain is occupied by SPX1, and its DNA binding is blocked and hence loses the ability to promote expression of PSI genes (Fig. 6a).

Fig. 6 InsP₆-induced and SPX1-mediated PHR2 dimer dissociation and DNA binding inhibition. A proposed model for InsP molecule induced recognition and inhibition of PHR2 by SPX1. Under Pi-deficient condition, PHR2 existed as a dimer to bind to the P1BS, and activated the transcription of PSI genes. Under Pi-sufficient condition, InsP molecule could be recognized by SPX1 and stabilized the helix α₁ of SPX1 with InsP molecule concentration increasing. Then, PHR2 dimer was disrupted, allowing SPX1 interacted with MYB and CC domains of PHR2 simultaneously. Thus, the transcriptional activation of PSI genes was repressed. The red octagon in the model represented InsP molecule.
CL2 loop of PYL1 and weakens the dimer interface. However, our InsP₅–SPX1–PHR2 ternary complex structure and assays indicate that InsP₅ may attenuate PHR2 dimer by stabilizing SPX1 helix α₁, which is different from the conformational changes induced by ABA. By deciphering the InsP sensing and signaling mechanism of SPX1, our findings shed light on a key process of plant adaptations to variations in nutrient availability and pave the road for engineering phosphate efficiency in crops.

**Methods**

**Expression and purification.** The truncations and mutants of SPX1 or PHR2 were cloned into the modified pET21a vector (Novagen) providing a tobacco etch virus (TEV) cleavable N-terminal 10×His-MsyB tag. Sequences for primers are listed in Supplementary Table 3. The proteins were overexpressed in E. coli BL21 (DE3). Cells were grown to OD₆₀₀nm of 0.6 at 37 °C, then reduced to 16 °C and etched virus (TEV) cleavable N-terminal 10×His-MsyB tag. Sequences for primers are listed in Supplementary Table 3. The proteins were collected at beamlines BL17U1 and BL19U1 at the Shanghai Synchrotron Radiation Facility. InsP₆-mediated transformation. Sequences for primers are listed in Supplementary Table 3.

**Crystallization, data collection, and structure determination.** The crystals of PHR2 (residues 230-380) and SPX1 (residues 1-198) complex showed obvious amorphous, and the T₄ lysozyme was fused to SPX1 for improving the crystallization quality. The complex crystals were grown at 4 °C by the hanging-drop vapor diffusion method, using 1.5 μL protein complex sample mixed with an equal volume of reservoir solution (0.1 mM sodium phosphate dibasic/citric acid pH 4.2, 20 mM NaCl, 0.1% w/v PEG3350, 20% glycerol). These procedures improved the resolution of the crystal to 2.6 Å. The target proteins were further purified by size exclusion chromatography (SEC, Superdex 26/60 GL, GE Healthcare) with the buffer A containing 20 mM Tris/HCl pH 8.0, 200 mM NaCl, 1 mM TCEP to the same buffer containing 1 M NaCl through size exclusion chromatography (SEC, Superdex 200 Increase 10/300 GL, GE Healthcare) with the buffer B (0.979 Å). All data sets were indexed, integrated, and merged, and rebuilding statistics are summarized in Table 3.

**Plant materials and growth conditions.** The spx1 spx2 mutant was kindly offered from Dr. Chuanzao Mao (Zhejiang University, China). Hydroponic experiments were conducted using rice culture nutrient solution containing 500 μM KH₂PO₄. The nutrient solution was adjusted to pH 5.5 by KOH and replaced every 3 days. Experiments were carried out in a greenhouse with 16 h day (30 °C)/8 h night (22 °C) photoperiod and photon density 200 μmol·m⁻²·s⁻¹.

**Generation of transgenic plants.** The coding sequence of OsSPX1 was amplified and subcloned into pmD19-T vector. Mutations of InsP binding sites (Y25F/K29A/K151A) in OsSPX1 were generated by PCR. The OsSPX1 and mutated OsSPX1 sequences were cloned into the pCAMBIA2301 vector. The 35S promoters were replaced by the OsSPX1 native promoter. These two constructs were confirmed by sequencing and transformed into spx1 spx2 mutant through Agrobacterium-mediated transformation. Sequences for primers are listed in Supplementary Table 3.

**Quantification of Pi content.** The Pi content of 3-week-old seedlings was measured using the phosphomolybdate colorimetric assay. The shoots were collected after fresh weight measurement and ground into fine powder in liquid nitrogen. Inorganic phosphate (Pi) was extracted in 1% acetic acid by repeated freezing and thawing twice. The supernatant was collected and mixed with ammonium molybdate and ascorbic acid, in which Pi concentration was measured by colorimetric assay at OD = 820 nm.

**Real-time quantitative PCR.** Total RNA was extracted from root of 3-week-old rice plants using the RNeasy Plant Kit (Qiagen). 1 μg total RNA was used for cDNA synthesis with PrimeScript RT reagent Kit (TaKaRa). Real-time qPCR was performed using a QuantStudio SYBR Green PCR Kit (Qiagen) on CFX96 real-time PCR detection system (Bio-Rad). Relative transcripts level was calculated by 2⁻ΔΔCt method according to the Cq values. Rice OsACP2 was used as the internal reference. Sequences for primers are listed in Supplementary Table 3.

**Dual-LUC transient transcriptional activation assay.** Protoplast isolation and transactivation assays are based on a previous report. Hydroponic culture system was used for phr1phl1 mutant plants growth as described. The phr1phl1 mutant plants were grown in ½ Hoagland nutrient solution for 4 weeks and transferred to Pi-depleted condition for 24 h. About four-week-old plants were used for protoplast isolation. After transferring the plasmids, the protoplasts from spx1 spx2 mutant plants was incubated under Pi-replete condition for 12 h. The harvested protoplasts were quantified with luciferase assay kit (Promega, USA), and LUC luminescence was measured with a plate reader (Perkin Elmer, USA). β-galactosidase (GUS) reporter plasmid was used as internal control to normalize transfection efficiency in protoplast assays. Sequences for primers are listed in Supplementary Table 3.

**Crosslinking experiment.** Purified OsPHR230–426 protein was concentrated to 4 mg ml⁻¹ in 25 mM HEPES, pH 7.0, 150 mM NaCl. About 2 mg of crosslinker DSS (diuccimimidyl suberate) was dissolved in 108 μl DMSO to a final concentration of 50 μM. 2 μl crosslinker DSS was added into 18 μl protein sample at increasing concentration (0.02, 0.04, 0.06, 0.08, and 0.10 mM) according to the manufacturer’s protocol. The reaction was carried out for 30 min at room temperature and stopped by the addition of 50 mM Tris-HCl buffer, pH 8.0. After incubation for the mixture for 15 min at room temperature, the oligomeric state of samples was analyzed by SDS-PAGE electrophoresis.
Thermal shift assay. Thermal shift assays were conducted with 15 μM of SPX11–1 and SPX12–1 bound to 25 nM InsP6 in 20 mM HEPES/NaOH pH 7.5, 200 mM NaCl, and 5 mM EDTA in a SYPRO Orange dye (S6550, Thermo Fisher Scientific). The concentration of InsP6 ranges from 0 to 250 μM. Protein samples were heated with a 0.5 °C per 10 s increasing gradient from 10 to 95 °C, the melt curve and melt temperature were recorded by the CFX Connect Real-Time PCR detection system (Bio-Rad).

Circular dichroism measurements. The CD spectra of the protein samples were recorded by a Chirascan+® CD Spectrometer at the room temperature. The protein samples were concentrated to 25 μM in 20 mM HEPES/NaOH pH 7.5, 300 mM NaCl in the absence or presence of 1 mM InsP6.

SAXS data collection and analysis. The small angle X-ray scattering data were collected at the BL19U2 beamline at National Facility for Protein Science Shanghai (NCPSS) and Shanghai Synchrotron Radiation Facility (SSRF), 60 μL of SPX11–1 and SPX12–1 bound to 1.8 mg/mL InsP6 in 1 mM MgCl2 were loaded in a quartz capillary. 2D scattering images were converted to 1D SAXS curves by BioXTAS RAW. The matching buffer scattering was subtracted from the sample scattering by PRIMUS. Pair distribution functions of the particles P(r) and the maximum sizes Dmax were calculated by the program GNOM. Low-resolution shapes were determined from solution scattering data using DAMMIF, from the ATAS suite of programs. Twenty independent calculations were performed by DAMMIF programs for each data set, using default parameters and no symmetry constraints. Then twenty independent reconstructions were then averaged and filtered to a final consensus model using the DAMAVER suite. Rigid body modeling was performed using the program SASREF. We used SASREF to find relative positions of the helix α1 and the remaining part of SPX1 by inputting both models separately.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Structural coordinates and structural factors have been deposited in the Protein Data Bank under accession number 7H4O. Source data are provided with this paper.

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Author contributions
Experimental design: J.Z., Q.H. and W.X.; different constructs for protein purification: J.Z., Q.H.; manuscript writing: J.Z., Q.H., F.M., M.L., and W.X. with help from all of the co-authors.

Competing interests
The authors declare no competing interests.
