Supplementary information, Data S1 Methods

Protein expression and purification

The LRR domains of BRI1 (residues 24-784; BRI1LRR) and BAK1 (residues 1-220; BAK1LRR) from Arabidopsis with an engineered C-terminal 6xHis tag were generated by standard PCR-based cloning strategy and their identity were confirmed by sequencing. BRI1LRR (with a modified N-terminal Hemolin peptide) and BAK1LRR were expressed in high five cells at 22 ℃ using the vector pFastBacTM 1 (Invitrogen). One litre of cells (1.8×10^6 cells ml^{-1}) was infected with 20 ml of baculovirus and the media was harvested after 48 h of infection. The protein was purified using Ni-NTA (Novagen) and size-exclusion chromatography (Hiload 200, GE Healthcare) in buffer containing 10 mM citric acid pH 4.0, 100 mM NaCl. To crystallize the BRI1LRR-BAK1LRR-brassinolide (BL) complex, the purified BRI1LRR and BAK1LRR proteins together with BL were mixed and incubated on ice for 1 h. The mixture was concentrated and subjected to a gel filtration analysis (Hiload200; GE Healthcare) in buffer containing 10 mM citric acid pH 4.0, 100 mM NaCl. For crystallization, the purified complex was concentrated to about 20 mg ml^{-1}.

Crystallization, data collection, structure determination and refinement

Crystals of BRI1LRR-BAK1LRR-BL complex were generated by the hanging-drop vapor-diffusion method. The drops were set up with 1 μl protein plus 1 μl reservoir solution at 18 °C. Diffraction quality crystals of the BRI1LRR-BAK1LRR-BL complex were obtained at buffer containing 0.1 M Hepes pH 7.5, 2.0 M ammonium sulfate (the pH of the mixture was ~5.6). All the diffraction data sets were collected at the Shanghai Synchrotron Radiation Facility (SSRF) on beam line BL17U1 using a CCD detector. The data were processed using HKL2000 [1]. The crystal structure of BRI1LRR-BAK1LRR-BL complex structure was determined by molecular replacement with PHASER [2] using the structure of BRI1 LRR (PDB code: 3RGZ) as a search model. The model from MR was built with the program COOT [3] and

subsequently subjected to refinement by the program PHENIX [4]. At this stage, the electron density for BAK1LRR was sufficient for model building. The structure of the complex was finally refined to a resolution of 3.6 Å with R_{work}=22.4% and R_{free}=27.7%. All the structure figures were prepared using PYMOL [5]. The coordinates and diffraction data have been deposited into the Protein Data Bank with accession code 4M7E.

Gel filtration assay

The BRI1LRR and BAK1LRR proteins purified as described earlier were subjected to a gel filtration analysis (Hiload200; GE Healthcare) in the presence of BL (20 μM). BRI1LRR, BAK1LRR and BL were incubated on ice for 1 h before the gel filtration analysis. Buffer containing 10 mM Tris pH 8.0, 100 mM NaCl or 10 mM citric acid pH 4.0, 100 mM NaCl was used for Hiload200. Samples from relevant fractions were applied to SDS-PAGE and visualized by Coomassie blue staining.

References

1. Otwinowski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. *Methods in Enzymology* 1997; **276**:307-326.
2. Collaborative Computational Project, Number 4. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr* 1994; **D50**:760–763.
3. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* 2004; **60**:2126–2132.
4. Adams P, Grosse-Kunstleve R, Huang LW, *et al*. PHENIX: building new software for automated crystallographic structure determination. *Acta Crystallogr.* 2002; **D58**:1948–1954.
5. DeLano WL. PyMOL molecular viewer. 2002; (http://www.pymol.org/).