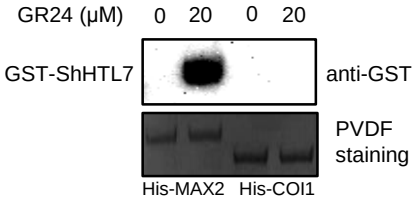
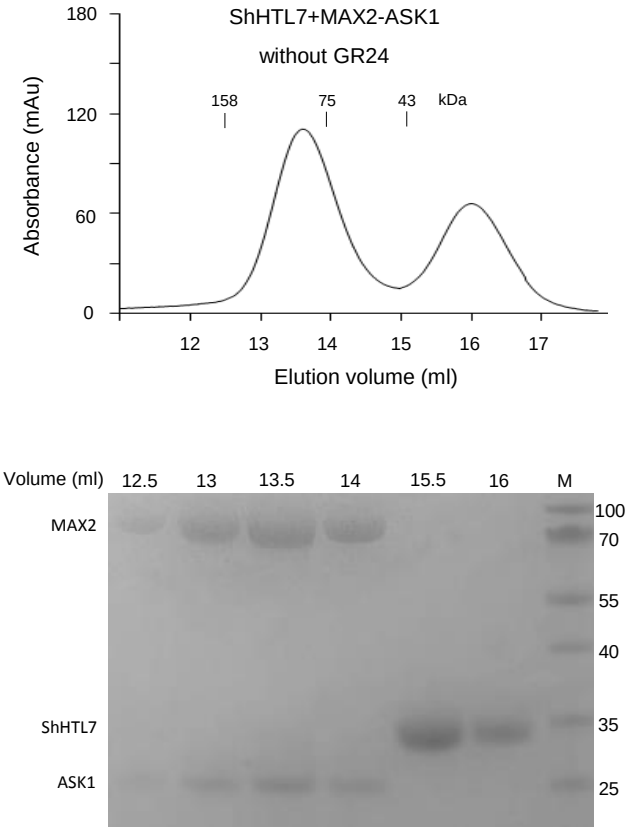


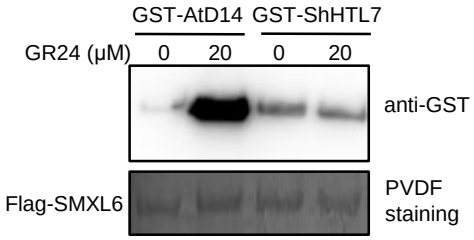
Supplementary information, Figure S1A ShHTL7 interacted with MAX2 but not another F-box protein COI1.

Pull-down assays using GST-ShHTL7 and His-MAX2-ASK1 or His-COI1-ASK1 (negative control) in the absence or presence of 20 μM GR24. GST-ShHTL7 was detected by anti-GST antibody and the PVDF membrane was stained with Memstain to show equal loading.



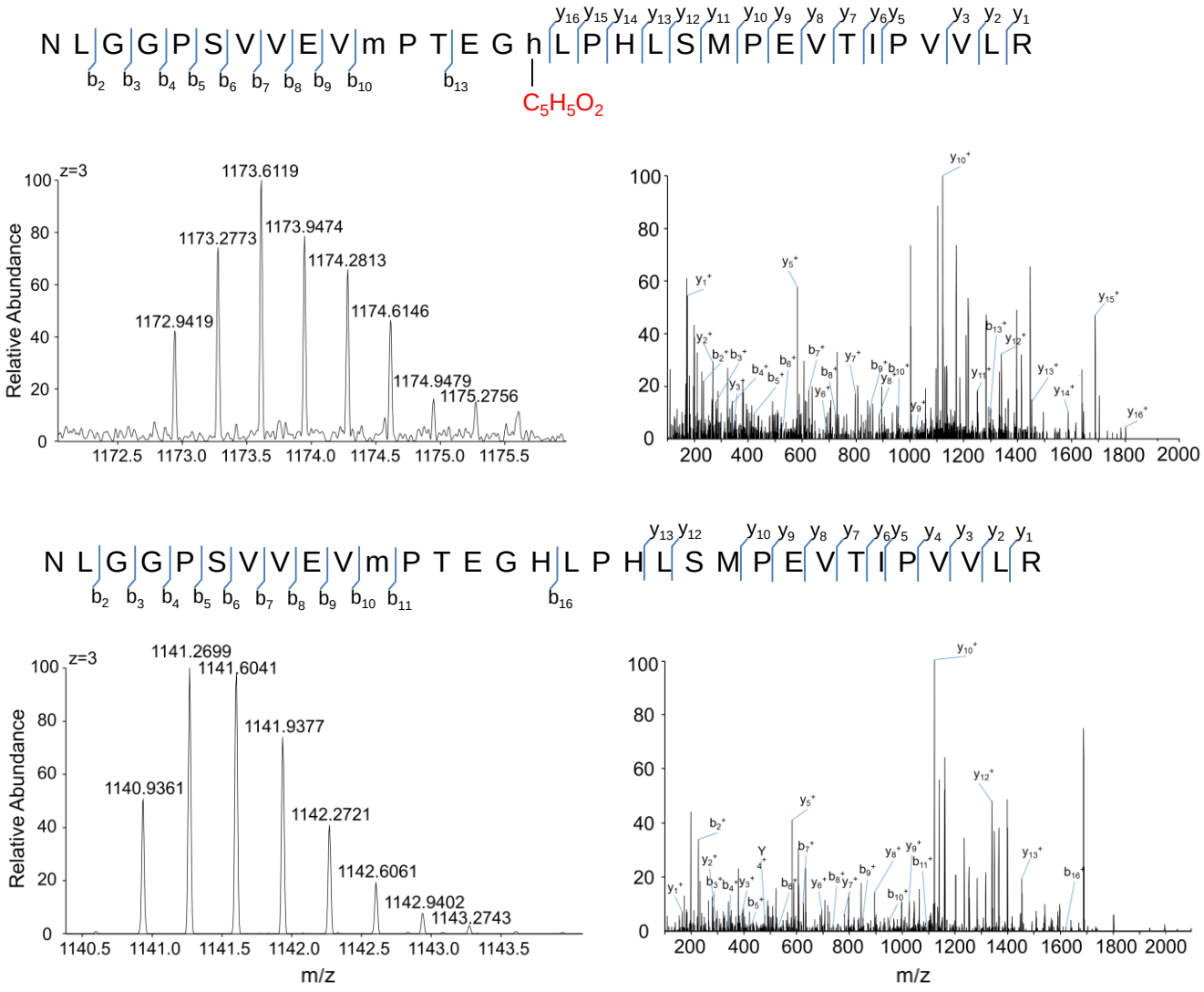
Supplementary information, Figure S1B ShHTL7 did not interact with MAX2 in the absence of GR24.

SEC analysis (upper panel) of the interaction between ShHTL7 and MAX2-ASK1 in the absence of GR24; the elution volumes of the molecular weight markers are indicated above the peaks. SDS-PAGE analysis (lower panel) of peak fractions from the upper panel; M, molecular weight ruler (kDa).



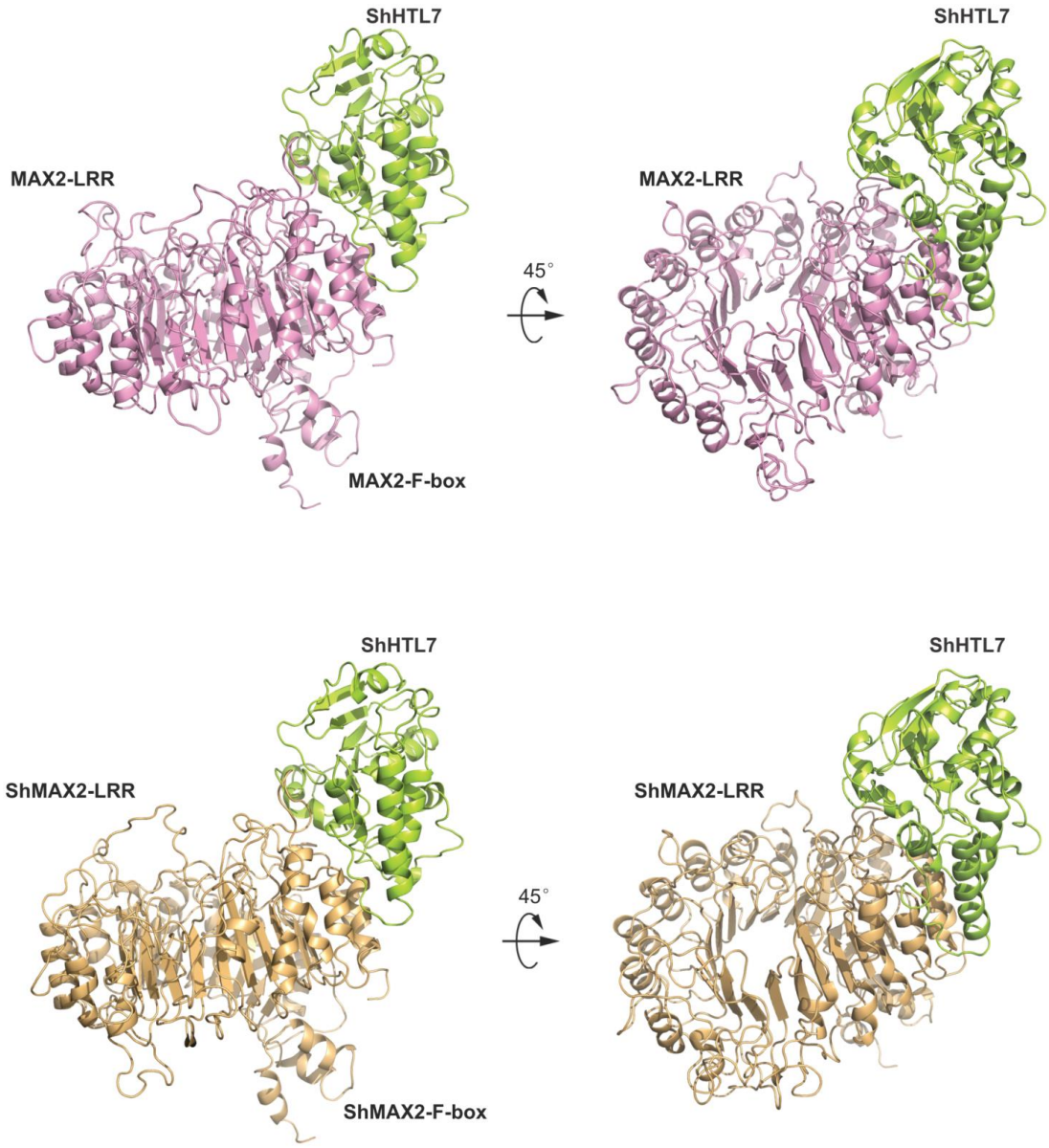
Supplementary information, Figure S1C GR24-enhanced interaction between ShHTL7 and SMXL6 was not detected in the pull-down assay.

Pull-down assays using GST-ShHTL7 and Flag-SMXL6 in the absence or presence of 20 μM GR24. GST-AtD14 protein was used as a positive control. GST-fused proteins were detected by anti-GST antibody and the PVDF membrane was stained with Memstain to show equal loading.



Supplementary information, Figure S1D ShHTL7 hydrolyzed 5DS and generated the $C_5H_5O_2$-modification on the catalytic residue H246.

A triply charged peptide (244-NLGGPSVVEVmPTEGHLPHLSMPEVTIPVVLR-262) of ShHTL7 with the 5DS-derived $C_5H_5O_2$-modification on H246 was identified by MS/MS (upper panel, m/z = 1172.9419; lowercase 'h' indicates the $C_5H_5O_2$-modified H246; lowercase 'm' indicates the oxidized M254); the modified peptide was isolated from the trypsin digestion products of ShHTL7 in the 5DS-induced ShHTL7-ShMAX2-ASK1 complex collected in SEC. As control, no $C_5H_5O_2$-modification on H246 of ShHTL7 was detected when ShHTL7 without pre-induction of GR24 was subjected to MS/MS analysis (lower panel, m/z = 1135.6088; lowercase 'm' indicates the oxidized M254). Labelled peaks in right column correspond to masses of y and b ions of the peptide displayed on the top, respectively.



Supplementary information, Figure S1E Overall structural models for the ShHTL7-MAX2 complex and the ShHTL7-ShMAX2 complex.

Structural models for the ShHTL7-MAX2 complex (upper panel) and the ShHTL7-ShMAX2 complex (lower panel), respectively. ShHTL7, MAX2 and ShMAX2 are shown as cartoon representation and coloured as limon, pink and light orange, respectively. The structure of ShHTL7 is a homology model based on AtD14 from the CLIM-AtD14-D3-ASK1 complex (PDB code: 5HZG; A chain); the structures of MAX2 and ShMAX2 are homology models based on D3 from the CLIM-AtD14-D3-ASK1 complex (PDB code: 5HZG; B chain). In each protein complex, the relevant homology models are structurally superimposed onto their corresponding templates in the CLIM-AtD14-D3-ASK1 complex.

Supplementary Information, Materials and Methods

Protein preparation

The full-length *Striga hermonthica* HTL7 (ShHTL7) was expressed in *E. coli* strain BL21 (DE3) as an N-terminal GST fusion protein. ShHTL7 was chosen from the ShHTL family due to its strongest response to GR24 and tested natural SLs [10]. After purified by Glutathione Sepharose 4B (GE Healthcare) affinity chromatography, GST-ShHTL7 was released by 10 mM GSH elution or on-column cleavage to remove GST tag, then further purified by Hitrap Q (GE Healthcare) followed by Superdex 200 10/300 (GE Healthcare) in a buffer containing 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM DTT. The preparation of GST-D14 is similar to that of GST-ShHTL7.

The full-length *Arabidopsis thaliana* MAX2 and ASK1 that stabilizes F-box protein [19] were co-expressed in sf9 insect cells as a His_6-Flag fusion protein and a Flag-tagged protein, respectively. After purified by Ni-NTA (Novagen) affinity chromatography, the His-MAX2-ASK1 complex was eluted and further purified by Hitrap Q (GE Healthcare) followed by Superdex 200 10/300 (GE Healthcare) in a buffer containing 20 mM MES, pH 6.5,150 mM NaCl and 5 mM DTT. The *Striga hermonthica* MAX2 (ShMAX2) was similarly co-expressed with ASK1.

The full-length *Arabidopsis thaliana* SMAX1 was expressed in sf9 cells with an N-terminal Flag tag and purified by anti-Flag beads (Sigma, A2220) according to the manual book. The *Arabidopsis thaliana* SMXL6 was prepared by using similar methods.

Pull-down assay

For the interaction between ShHTL7 and ShMAX2, ~20 μg His-ShMAX2-ASK1 was used as the bait and ~12 μg GST-ShHTL7 was used as the prey in the presence of 20 μM (+)-GR24 or its solvent DMSO as the control. The reaction mixtures were incubated with Ni-

NTA beads (Qiagen) at 4˚C for 1 h in the reaction buffer (50 mM Tris-HCl, pH 6.8, 100 mM NaCl, 25 mM imidazole, 10% (v/v) glycerol, 0.1% (v/v) Tween 20, 20 mM 2-mercaptoethanol). After extensive wash, the protein complexes on the beads were released and then subjected for western blot analysis. The pull-down assay of ShHTL7 with another F-box protein COI1 (the jasmonate receptor in *Arabidopsis thaliana*) was performed to serve as a negative control.

For the interaction between ShHTL7 and SMAX1, ~20 μg Flag-SMAX1 protein was used as the bait and ~12 μg GST-ShHTL7 was used as the prey in the presence of 20 μM (+)-GR24 or its solvent DMSO as the control. AtD14 was used as a control of ShHTL7. The reaction mixtures were incubated with anti-flag beads (Sigma) at 4˚C for 1 h in the reaction buffer (50 mM Tris-HCl, PH 7.0, 150 mM NaCl, 0.5% (v/v) Tween 20). After extensive wash, the protein complexes on the beads were released and then subjected for western blot analysis. The pull-down assay of ShHTL7 with SMXL6 was performed by using similar methods.

GST-fused proteins were detected by a monoclonal anti-GST antibody (Abmart). The PVDF membrane was stained with Memstain (Applygen) to show equal loading.

Size exclusion chromatography (SEC) assay

Purified ShHTL7 (roughly 10 μM) and MAX2/ShMAX2-ASK1 proteins (roughly 5 μM) were incubated with 200 μM GR24, or equal amount of DMSO as the solvent control, at 25˚C for one hour in the buffer containing 20 mM MES, pH 6.5, 150 mM NaCl, 5 mM DTT. The reaction mixture was then injected onto a Superdex 200 10/300 column for analysis at a flow rate of 0.3 ml/min. The fractions (0.5 ml/fraction) were analyzed by SDS-PAGE and visualized by coomassie brilliant blue staining.

Mass spectrometric analysis of covalent modification

The SEC-separated ShHTL7-MAX2/ShMAX2-ASK1 complex induced by GR24 or 5DS was analyzed by SDS-PAGE and visualized by coomassie brilliant blue staining. The gel bands of ShHTL7 were excised for mass spectrometric analysis as previously described [18].

MS/MS spectra from each LC-MS/MS run were searched against the ShHTL7 protein database using Proteome Discoverer (Version 1.4) searching algorithm. The search criteria were as follows: full tryptic specificity was required; two missed cleavages were allowed; carbamidomethylation was set as fixed modification; oxidation (on the residue of Met) were set as variable modifications; precursor ion mass tolerance was 10 ppm for all MS acquired in the Orbitrap mass analyzer; and fragment ion mass tolerance was 0.02 Da for all MS2 spectra acquired in the ion trap. High confidence score filter

(FDR < 1%) was used to select the “hit” peptides and their corresponding MS/MS spectra were manually inspected.

Homology modeling

Homology models for the ShHTL7-MAX2 complex and the ShHTL7-ShMAX2 complex were built on the SWISS-MODEL server (http://swissmodel.expasy.org/), using chain A (AtD14) and chain B (D3) of the CLIM-AtD14-D3-ASK1 complex structure (PDB code: 5HZG) as templates. Structural figures of the two protein complexes were generated in PyMOL [20]. Both structures of the two protein complexes were perfectly assembled with no obvious clashes observed in each interface. Models were refined in COOT [21] by satisfying stereochemical restraints of polypeptide chain configurations according to the Ramachandran plot. The quality of the obtained models was assessed by PROCHECK [22].

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