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SUPPLEMENTARY INFORMATION

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Untethering the TIR1 auxin receptor from the SCF complex increases its stability and inhibits auxin response

Plasmid constructs and generation of transgenic line

The *pTIR1/AFB:GUS* and *pTIR1/AFB:TIR1/AFB-GUS* lines were described in the study[1]. The *pTIR1:tir1/afb1-GUS* constructs were made by introducing a 2-kb 5' upstream region of the *TIR1* gene with the *tir1/afb1* cDNA into *pMDC163* vector [2]. The constructs were transformed into the *tir1-1* mutant. Site-specific mutagenesis was performed using the QuikChange II XL mutagenesis kit (Agilent, cat # 200521) All primers can be found in Supplemental Table 1 online.

Generation of the mutant library and mutant screen

Full-length *TIR1* complementary DNA (cDNA) was mutagenized by error prone PCR with a mutation ratio of approximately two mutations per *TIR1* molecule. The PCR products were ligated into the *pGILDA* vector and transformed into *Escherichia coli* competent cells to generate a library of approximately 1.3 x 10^4 colonies. The library was transformed into the *pB42AD:IAA7* yeast (Saccharomyces cerevisiae) strain (EGY48), and yeast colonies were screened on synthetic dropout medium lacking uracil, His, Trp, and Leu, supplemented with 10 μM IAA. The number of total yeast colonies screened was about 2.5 x10^5. Yeast colonies that grew fastest were isolated and tested on 5-bromo-4-chloro-indolyl-b-D-galactopyranoside plates. *The pGILDA:tir1-Myc* plasmids from positive colonies were extracted and sequenced.

GUS staining and measurement of β-galactosidase activity

Seedlings were collected in GUS staining solution (100 mM Na_2PO_4 pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 1.0 mM $K_3Fe(CN)_6$ and 2 mM X-Gluc), vacuum-infiltrated for 20 min, and stained overnight at 37°C. The seedlings were cleared in 70%

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(v/v) ethanol and imaged with a Nikon SMZ1500 dissecting microscope. MUG assays were performed as described[3]. To measure β-galactosidase activity, total yeast protein extract was prepared by Y-PER Reagent (Thermo) and the yield was quantified by Bradford assay. The reaction was set up by incubation of 100μl protein extract, 200ul 4mg/ml ONPG with 700μl Z-buffer (16.1 g $Na_2HPO_4 \cdot 7H_2O$, 5.5g $NaH_2PO_4 \cdot H_2O$, 0.75g KCl, 0.246g $MgSO_4 \cdot 7H_2O$ in 1L water, pH 7.0) at 37°C. Reaction were stopped by adding 400μl of 1M Na_2CO_3 and OD_{420} was measured. Specific activity was calculated as

$$Activity = \frac{OD_{420} \times 1.4}{0.0045 \times [protein] \times extractvolume \times time}.$$

Root and hypocotyl growth assays

For root growth assays, 5-day old seedlings were transferred onto fresh ATS medium with different concentrations of 2,4-D. Root length was measured after 2 days growth. For hypocotyl elongation assay, 5-day old seedlings grown in short day condition were transferred onto fresh ATS medium with different concentrations of IAA for another 2 days growth. To measure the gravitropic response, 5-day old seedlings were rotated 90° clockwise and root tip bending was recorded by a Nikon SMZ1500 dissecting microscope every 2hr for 12hr. All measurements were performed using ImageJ software.

Phylogenetic analysis. Complementary DNA sequences for each gene were obtained from the Joint Genome Institute plant genome website (http://www.phytozome.org, accessed 14 Jan, 2014) except for those from *Cleome hassleriana* (GenBank Accessions AOUI01033804.1, AOUI01006529.1, and AOUI01008893.1). The alignment was generated using T-Coffee [4](Notredame et al 2000) followed by manual editing. The tree was inferred using MrBayes (v3.2.2 x64)[5] with the following parameters: partition =

by_codon, ratepr = variable, nst = 6, rates = invgamma, nruns = 4, nchains = 4, and ngen = 1000000. The consensus tree was visualized and exported using FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

1 Parry, G. *et al.* Complex regulation of the TIR1/AFB family of auxin receptors. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 22540-22545, doi:10.1073/pnas.0911967106 (2009).

2 Curtis, M. D. & Grossniklaus, U. A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant physiology* **133**, 462-469, doi:10.1104/pp.103.027979 (2003).

3 Ge, L. *et al.* Arabidopsis ROOT UVB SENSITIVE2/WEAK AUXIN RESPONSE1 is required for polar auxin transport. *The Plant cell* **22**, 1749-1761, doi:10.1105/tpc.110.074195 (2010).

4 Notredame, C., Higgins, D. G. & Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* **302**, 205-217, doi:10.1006/jmbi.2000.4042 (2000).

5 Ronquist, F. *et al.* MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* **61**, 539-542, doi:10.1093/sysbio/sys029 (2012).

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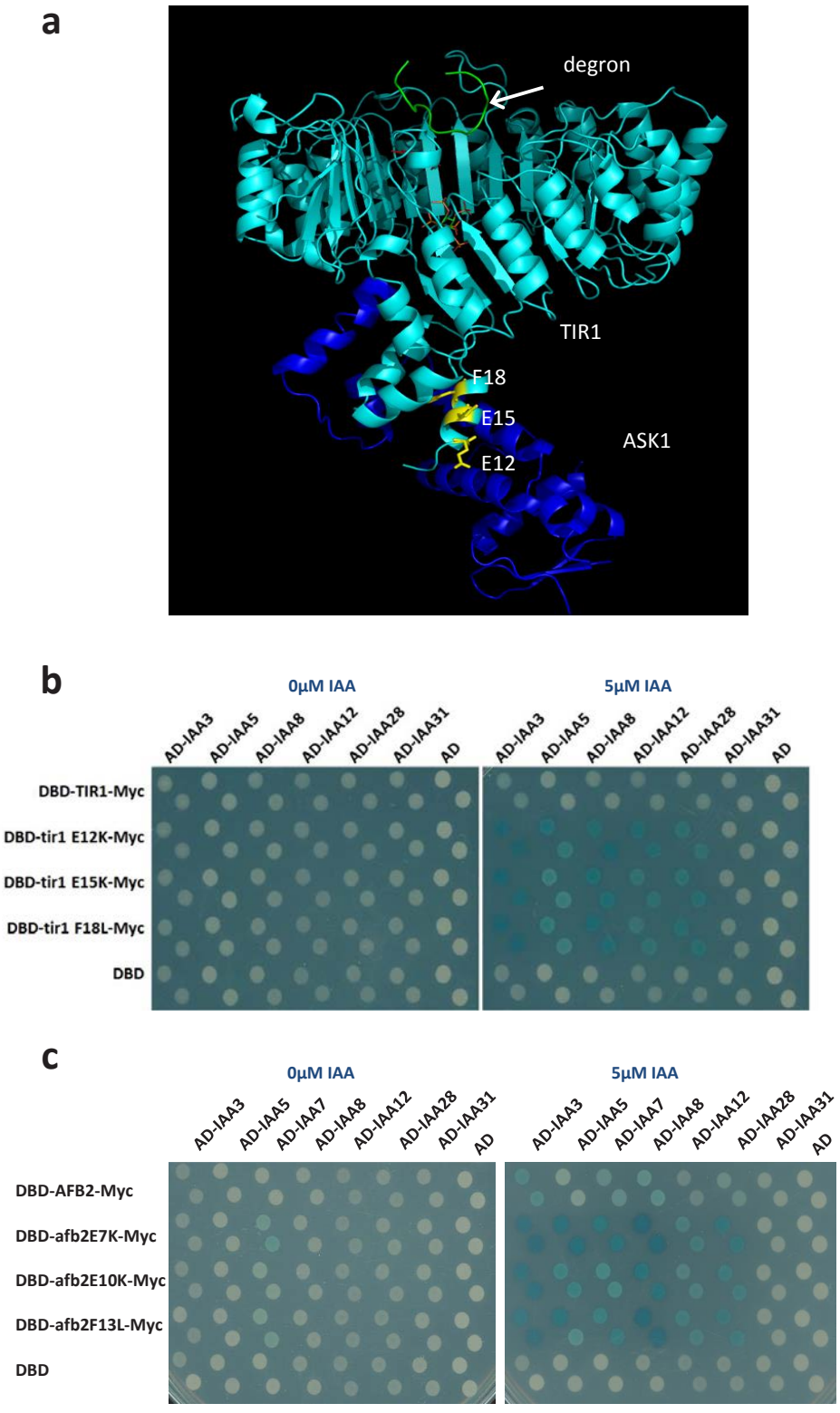
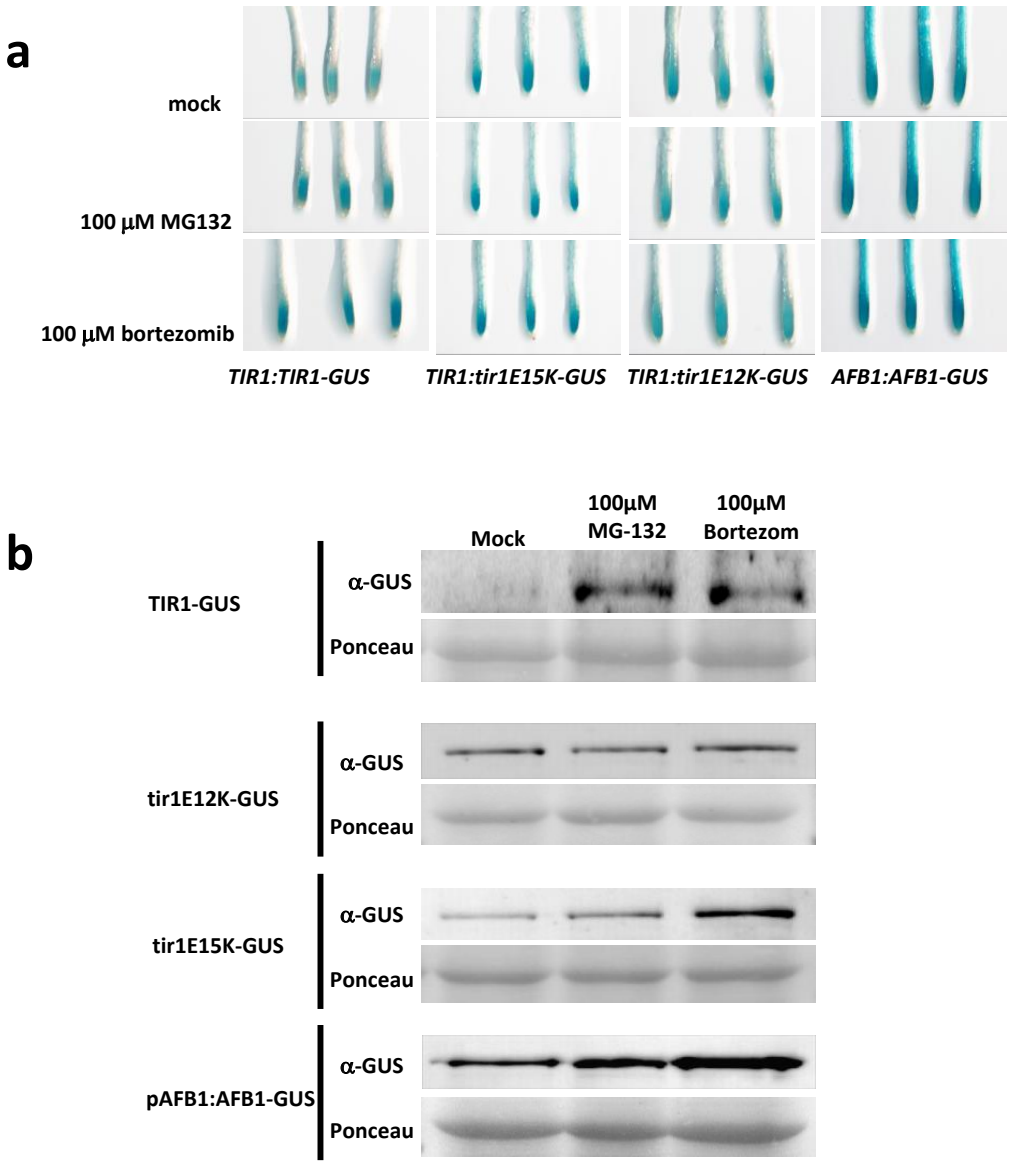


Supplemental Fig 1. Behavior of *tir1* and *afb2* mutants in yeast two-hybrid tests.
(a) Structure of TIR1-ASK1 showing position of mutated residues.
(**b**,**c**) Yeast-two-hybrid test of the interaction between wild type and mutant TIR1 and AFB2 and different Aux/IAA proteins.



Supplemental Fig 2. TIR1-GUS is degraded by the proteasome.

(a) Four-day-old seedlings were treated with proteasome inhibitor for 3 h and stained for GUS.
(b) Seven-day-old seedlings were treated as in (**a**) and analyzed by protein blot.

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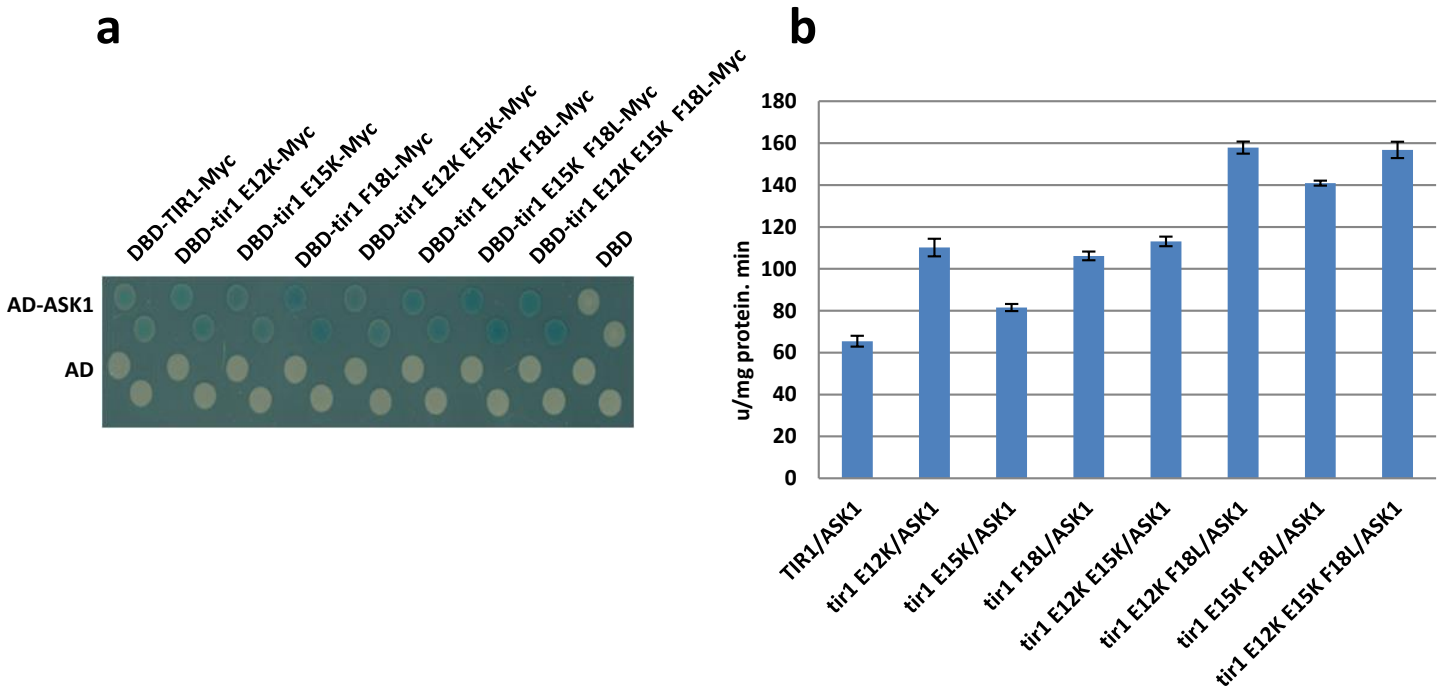
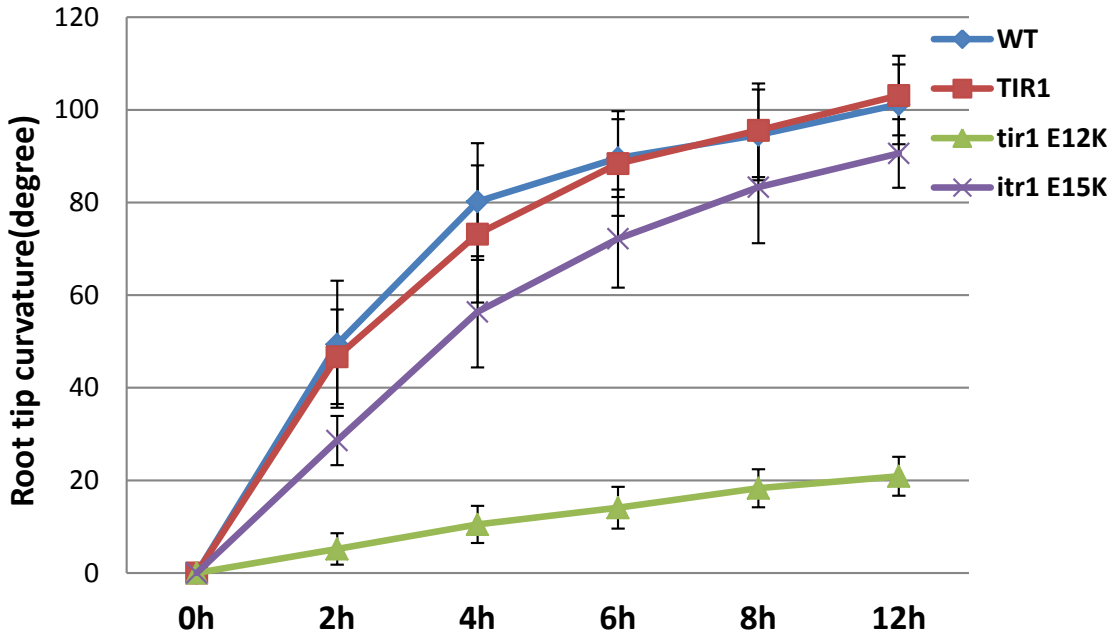


Supplemental Fig 3. Interaction of TIR1 with ASK1 in yeast
(**a**) Wild type and mutant TIR1 proteins interact with ASK1 in a Y2H assay.
(**b**) β-galactosidase activity in extracts from yeast strains in (**a**) Error bars represent SE.



Supplemental Fig 4. Gravitropic response of transgenic plants expressing mutant tir1 proteins. *Col-0* (WT), *TIR1:TIR1-GUS, TIR1:tir1E12K-GUS* , and *TIR1:tir1E15K-GUS*. **Error bars represent SE.**

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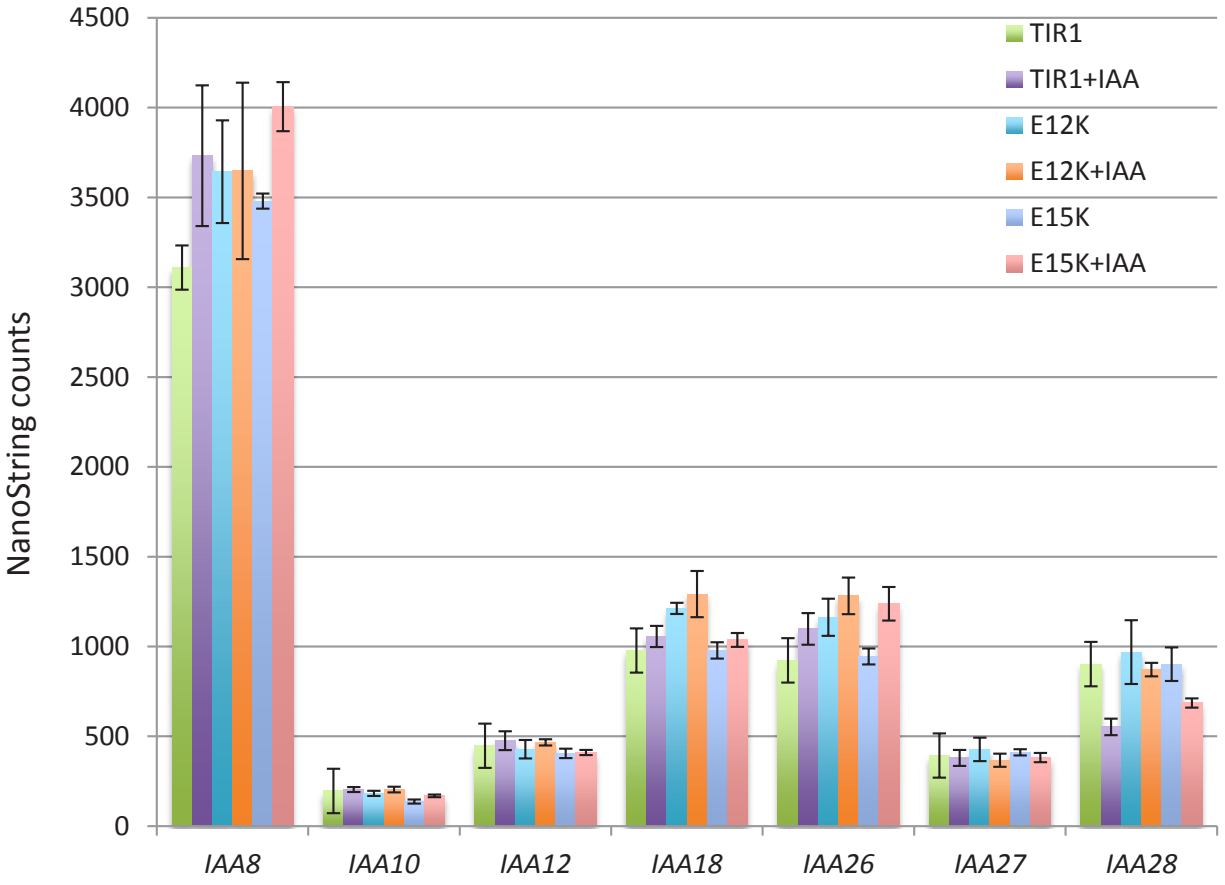
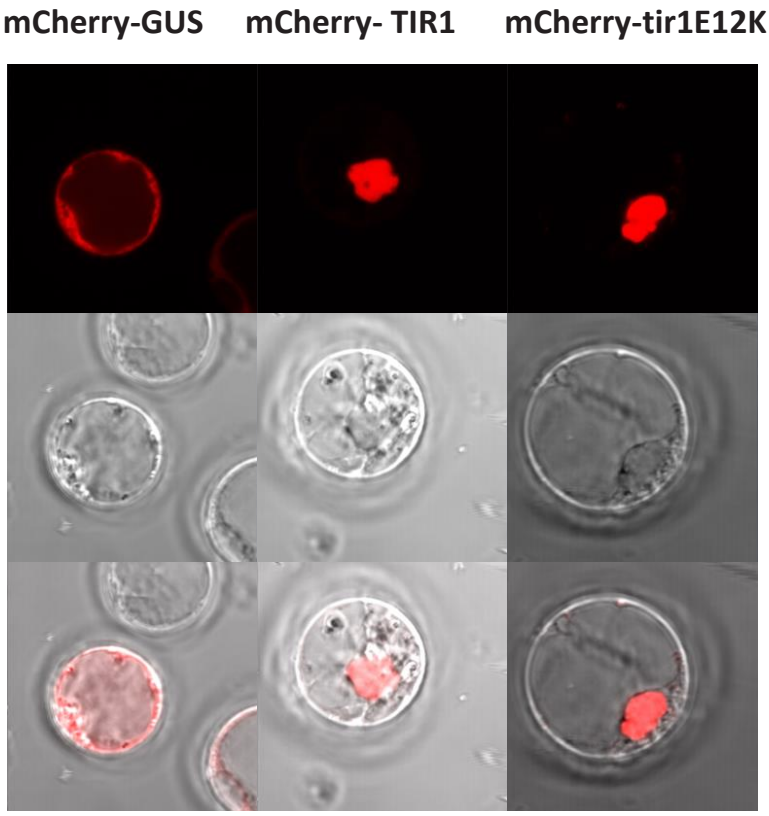


Supplemental Fig 5. Expression of selected Aux/IAA genes in response to auxin. Experiment was performed as described in Figure **5b**.



Supplemental Fig 6. The E12K mutation does not affect nuclear localization of TIR. Protoplasts were transiently transformed with mCherry-tagged TIR1 proteins or an mCherry-GUS control.

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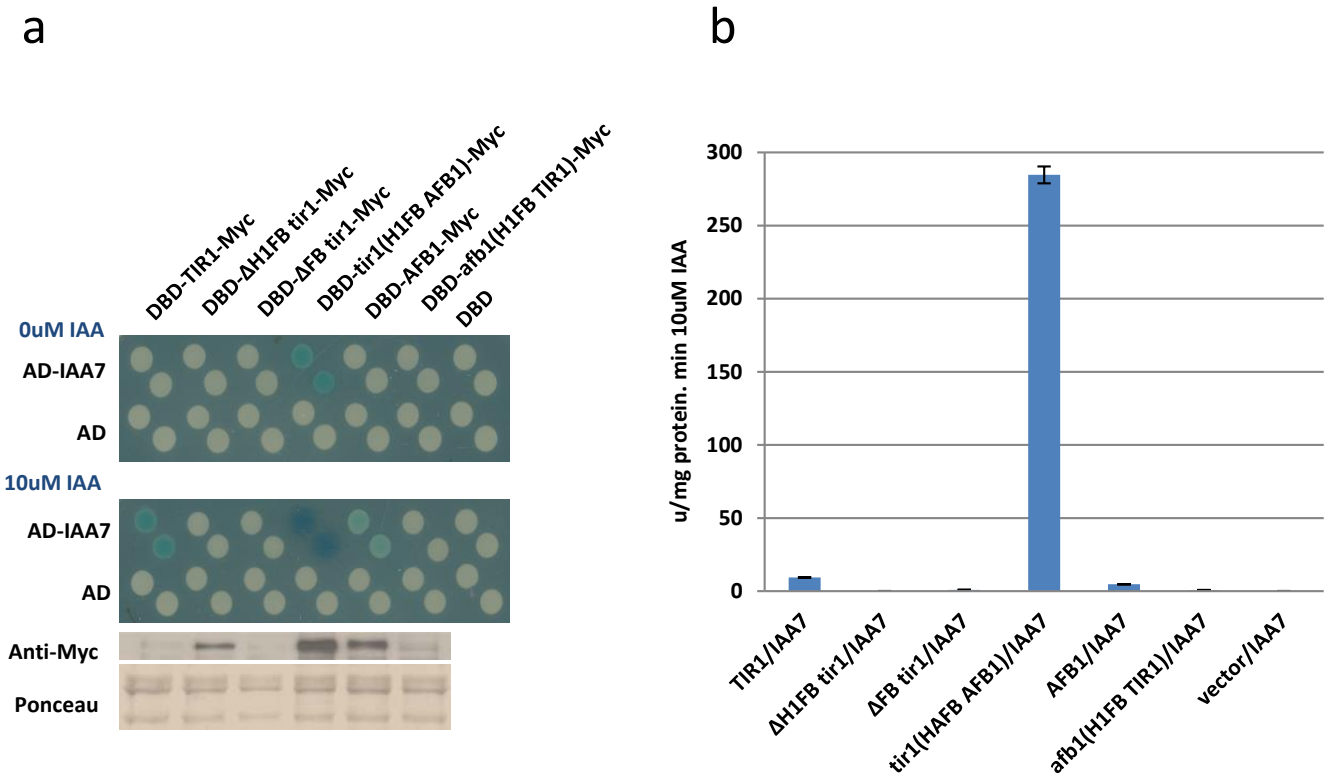
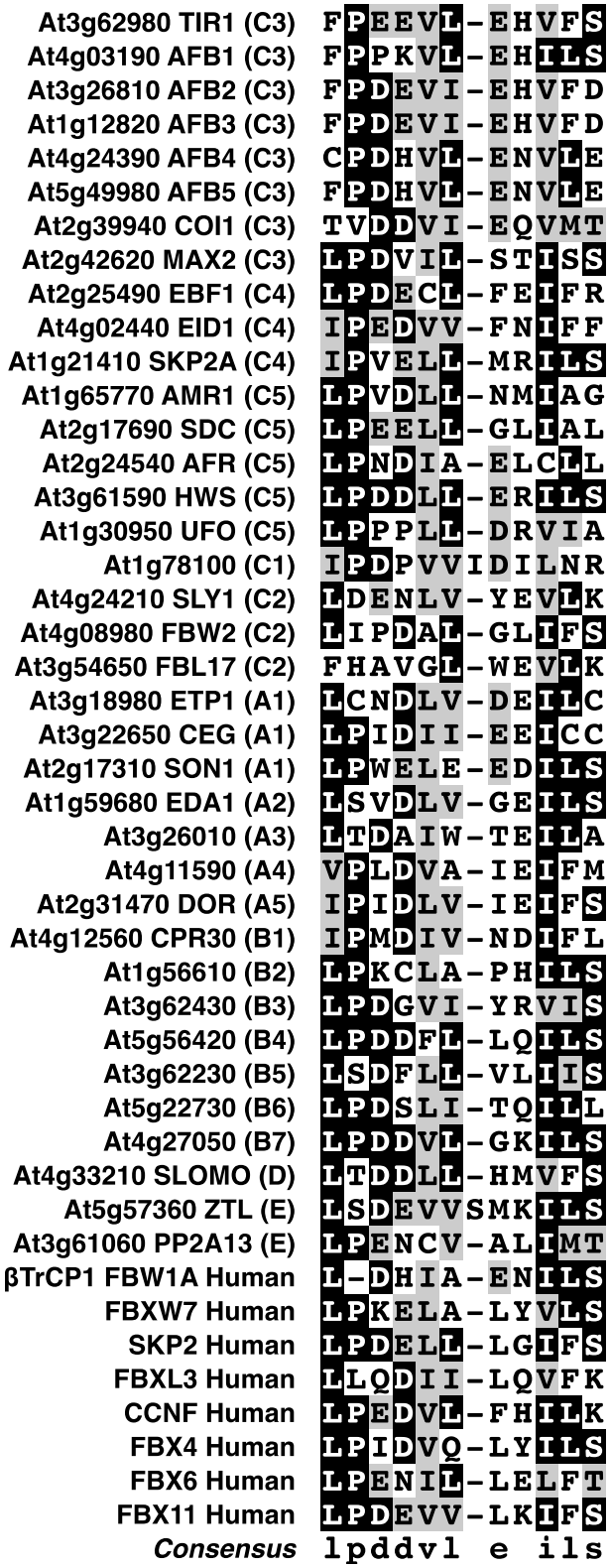


Supplemental Fig 7. Y2H analysis of the interaction between mutant tir1 and Aux/IAA proteins.
(**a**) Yeast-two-hybrid test of the interaction between IAA7 and mutant derivatives of TIR1.
(**b**) β-galactosidase activity of yeast strains in (**a**). Error bars are SE.



Supplemental Fig 8. Alignment of the H1 helix from selected Arabidopsis and human F-box proteins. Representatives of different Arabidopsis FBP subfamilies[3] were selected for comparison. The TIR1E12 position is conserved and typically occupied by E or D.

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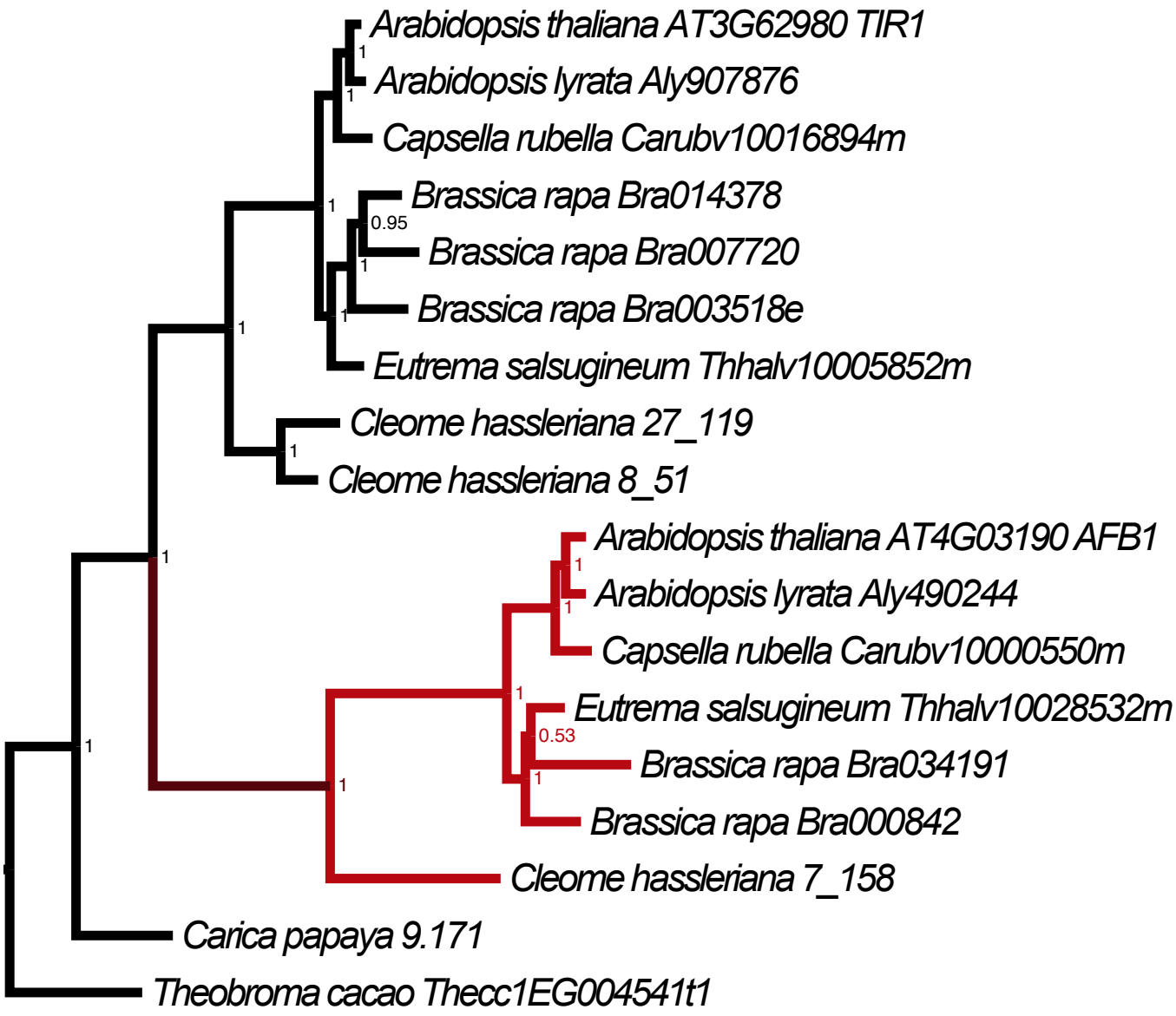

Supplemental Fig 9. Phylogeny of the TIR1 and AFB1 proteins. The E-to-K substitution is shared by AFB1 homologs from members of the Brassicaceae and Cleomaceae families. The Bayesian-inferred phylogenetic tree illustrates the relationships between the TIR1 and AFB1 homologs from members of the Brassicaceae and Cleomaceae families in relation to outgroup homologs from papaya and cacao tree. The clade sharing the E-to-K substitution is colored red.

K_5	IAA7	IAA28
TIR1	0.033	0.024
tir1E12K	0.003	0.002
tir1E15K	0.018	0.013

Supplemental Table 1. Degradation rates (k_5 value) for various IAA7 and IAA28 in the presence of wild-type and mutant TIR1 proteins. k_5 values were calculated as described in the Materials and Methods.

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Supplemental Table 2. Primers used in this study

Name	Sequence
tir1E12K F1	5'gccttgtcgtttccagaaaaggtactagagcatgtgtt 3 '
tir1E12K F2	5' cacatgctctagtacctttctggaaacgacaaggctatt 3 '
tir1E15KF1	5' cgtttccagaagaggtactaaagcatgtgttctcgtttat 3 '
tir1E15KF2	5' ctgaataaacgagaacacatgctttagtacctcttctggaa 3 '
tir1F18LF1	5' gaggtactagagcatgtgttatcgtttattcagctggata 3 '
tir1F18LF2	5' ccagctgaataaacgataacacatgctctagtacctcttct 3 '
tir1 E12K E15K F	5' cgtttccagaaaaggtactaaagcatgtgttctcgtttatt 3 '
tir1 E12K E15K R	5' gctgaataaacgagaacacatgctttagtacctttctggaaa 3 '
tir1 E12K F18L F	5' ggtactagagcatgtgttatcgtttattcagctggataa 3 '
tir1 E12K F18L R	5' ccagctgaataaacgataacacatgctctagtacctttt 3 '
tir1 E15KF18L F	5' gaggtactaaagcatgtgttatcgtttattcagctggata 3 '
tir1 E15K F18L R	5' ccagctgaataaacgataacacatgctttagtacctctt 3 '
tir1 E12KE15KF18L F	5' ggtactaaagcatgtgttatcgtttattcagctggataa 3 '
tir1 E12KE15KF18L R	5' ccagctgaataaacgataacacatgctttagtacctttt 3 '
ΔH1FB TIR1 F	5' caccatgtcgtttattcagctggataagga 3 '
H1(AFB1)-tir1 F	5' ggtgttggaacatatcctctcgtttattcagctggataaggata 3 '
H1(AFB1)-tir1 R	5' ccttatccagctgaataaacgagaggatatgttccaacacctt 3 '
H1(TIR1)-afb1 F	5' ggaagcgagtctttgtcgggaactgctacgccgtgagt 3 '
H1(TIR1)-afb1 R	5' cggcgtagcagttcccgacaaagactcgcttcctagtctt 3 '
afb2 E7K F	5' cccagataaagtaatagagcatgtattcgactttgtaa 3 '
afb2 E7K R	5' cgaatacatgctctattactttatctgggaaataattcat 3 '
afb2 E10K F	5' cccagatgaagtaataaagcatgtattcgactttgtaa 3 '
afb2 E10K R	5' aagtcgaatacatgctttattacttcatctgggaaat 3 '
afb2 F13L F	5' gtaatagagcatgtactcgactttgtaacatctcacaa 3 '
afb2 F13L R	5' gagatgttacaaagtcgagtacatgctctattacttcat 3 '
GUS Q-PCR F	5' acgttagccgggctgcactc 3 '
GUS Q-PCR R	5' tcggtttgcggtcgcgagtg 3 '
NmCherryF	5' agcaagactagtatggtgagcaagggcg 3'
NmCherryR	5' tctgagactagtcttgtacagctcgtccatgc 3'
CmCherryF	5' tgagcaccgcgggatggtgagcaagggcg 3'
CmCherryR	5' agatctccgcggttacttgtacagctcgtccatgc 3 '
tir1S438E F	5' aaggatctccgtcgcctcgaactatctggcctcttgacc 3 '
tir1S438E R	5' ggtcaagaggccagatagttcgaggcgacggagatcctt 3 '