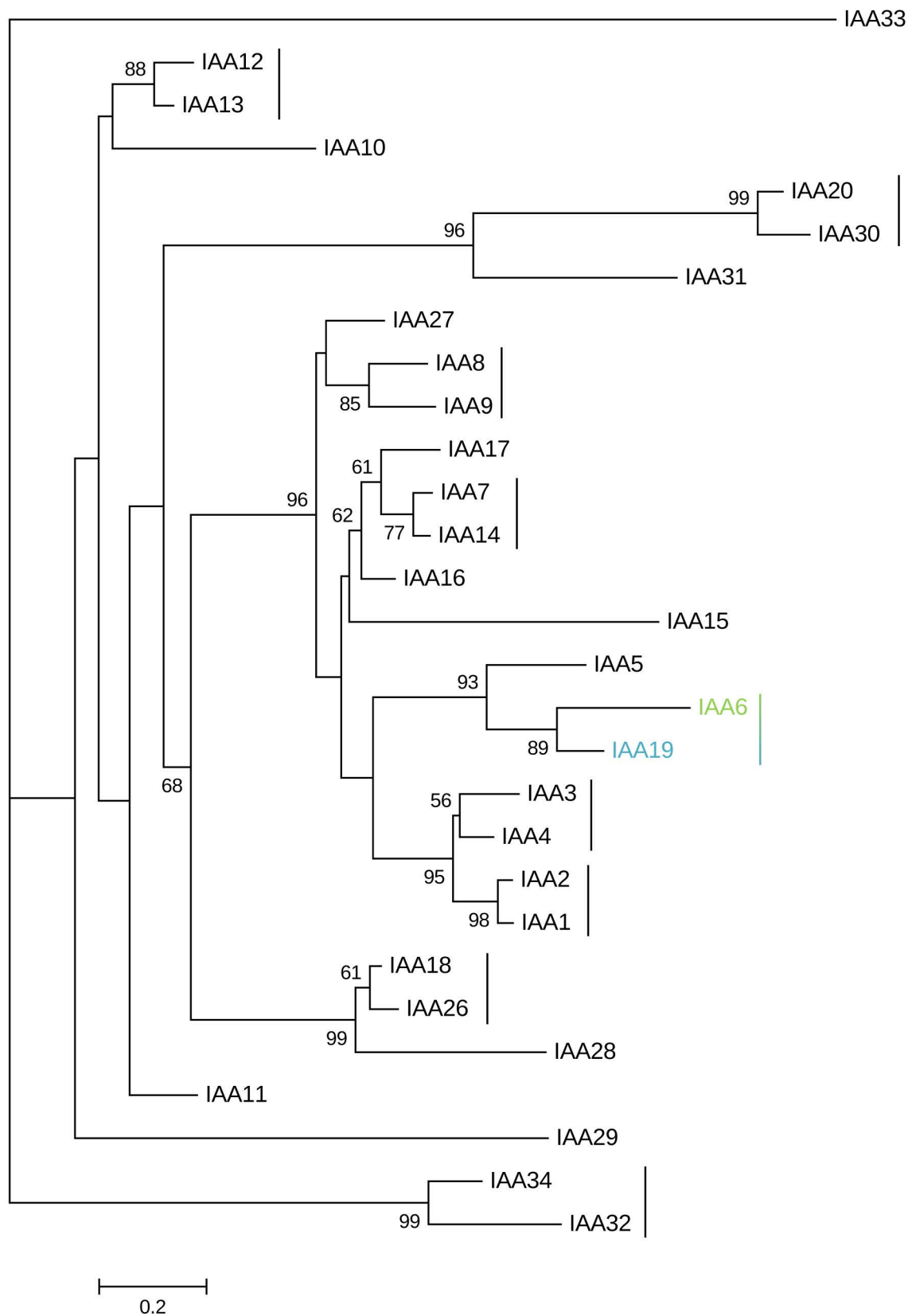
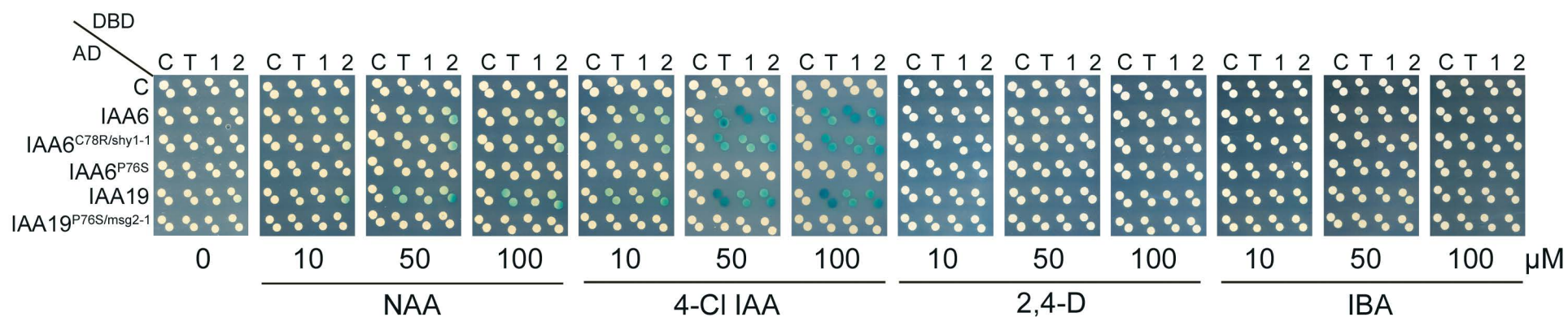


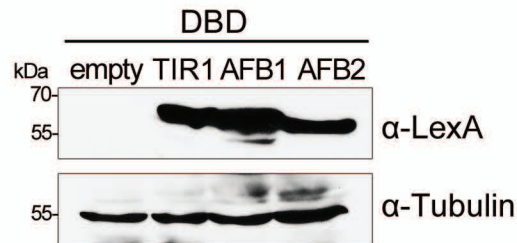


Supplementary Figure 1. Phylogeny of *A. thaliana* AUX/IAA proteins. The unrooted phylogenetic tree was created with the full-length protein sequences of 29 AUX/IAA proteins based on maximum likelihood. Vertical bars correspond to AUX/IAA sister pairs (ohnologs). IAA6 (green) and IAA19 (blue) ohnologs are highlighted. Bootstrap values greater than 50% are shown at the corresponding node. Scale bar 0.2 denotes 0.2 amino acid substitution per site.

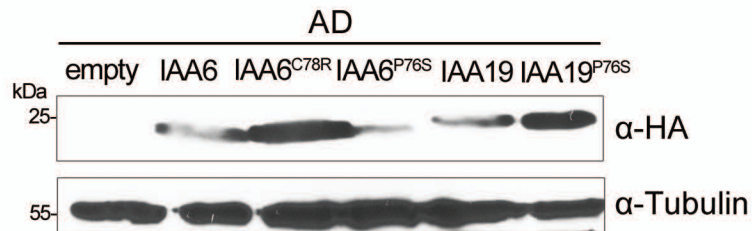


Supplementary Figure 3. LexA yeast two-hybrid interaction experiments of empty control (C), TIR1(T), AFB1 (1), or AFB2 (2) against IAA6, IAA6^{C78R/shy1-1}, IAA6^{P76S}, IAA19 and IAA19^{P76S/msg2-1} on 10, 50 or 100 μ M 1-naphthaleneacetic acid (NAA), 4-chloroindole-3-acetic acid (4-Cl IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), or indole-3-butyric acid (IBA). β -galactosidase reporter expression indicates auxin-induced TIR1/AFB1/AFB2-AUX/IAA interactions.

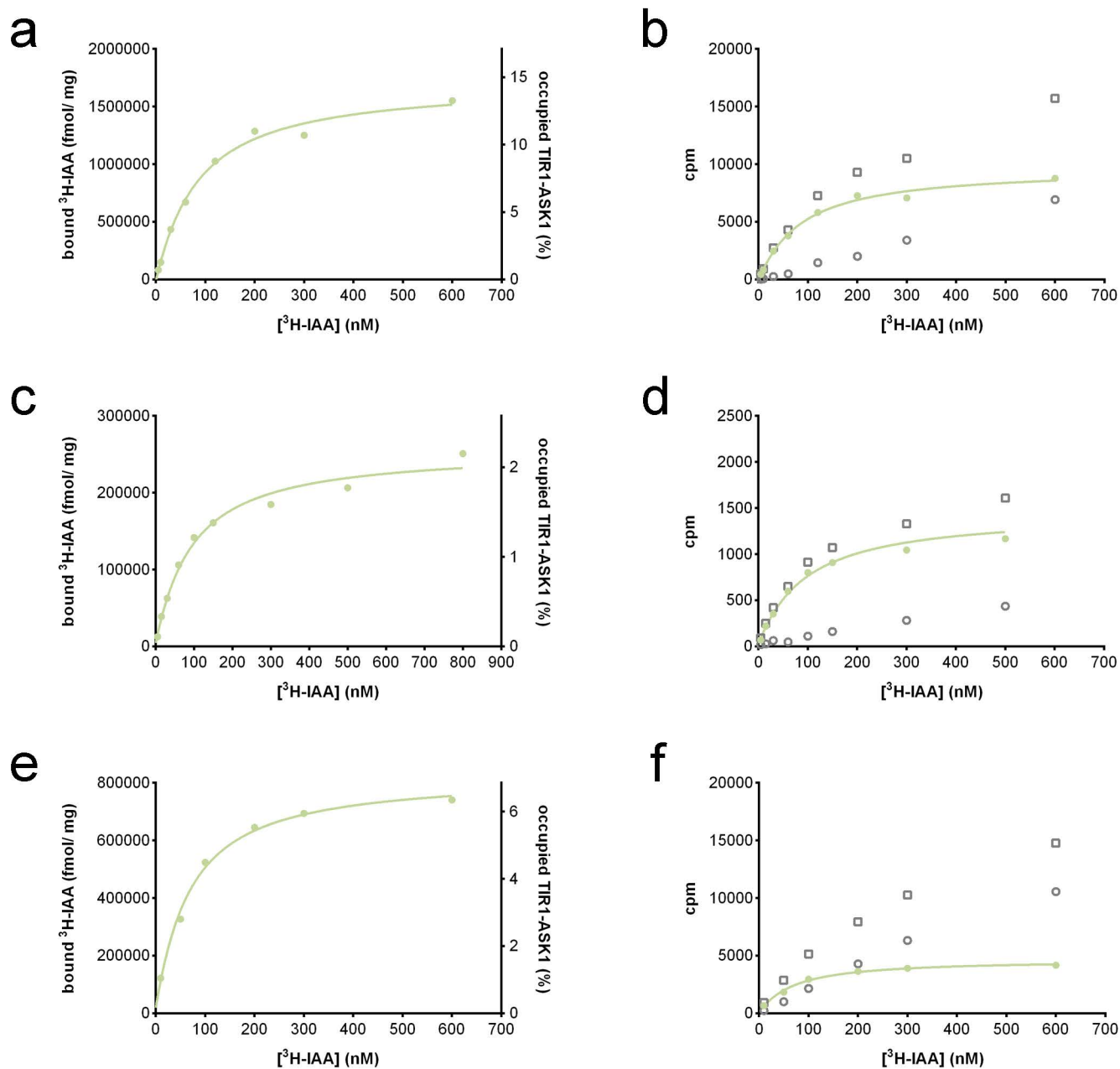
a



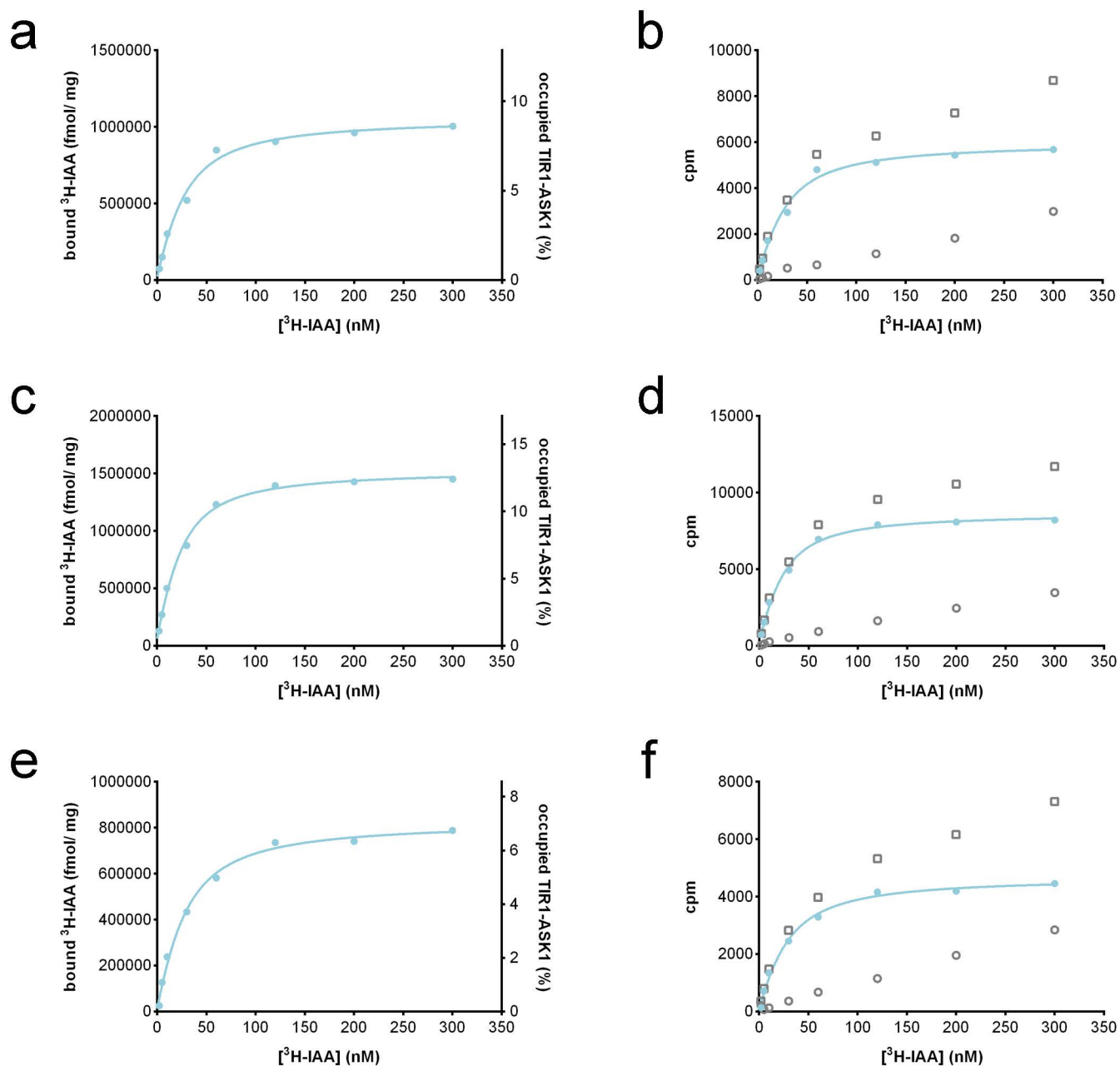
b



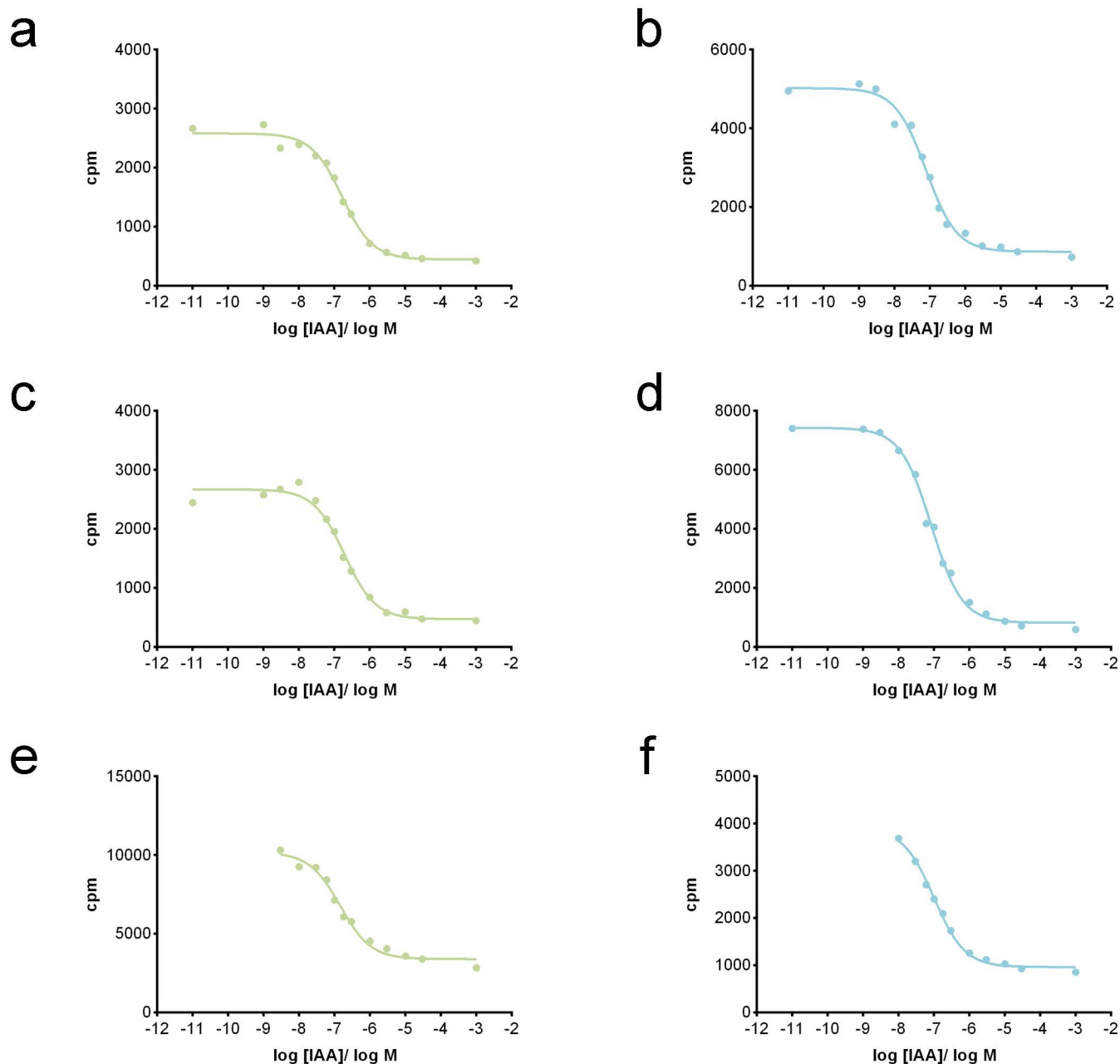
Supplementary Figure 4. (a) Immunoblots of LexA tagged -TIR1, -AFB1, -AFB2; and (b) HA tagged -IAA6, -IAA6^{C78R/shy1-1}, -IAA6^{P76S}, -IAA19, and -IAA19^{P76S/msg2-1} proteins. TIR1, AFB1, AFB2, as well as AUX/IAA fusion proteins screened for auxin dependent interactions in yeast diploids are well expressed, and repetitive experiments ($n > 4$) consistently showed that interactions (see Fig. 1c and Supplementary Fig. 3) are unlikely to be dependent upon fusion protein expression levels in yeast. Total proteins were extracted from yeast diploids grown on Gal/Raff -Ura-His-Trp and detected using either anti-HA (F-7) (Santa Cruz, SC7392) or anti-LexA (Abcam, AB14553) antibodies. Yeast α -Tubulin was detected with anti-Tubulin antibody (Abcam, AB6160) and used as loading control.



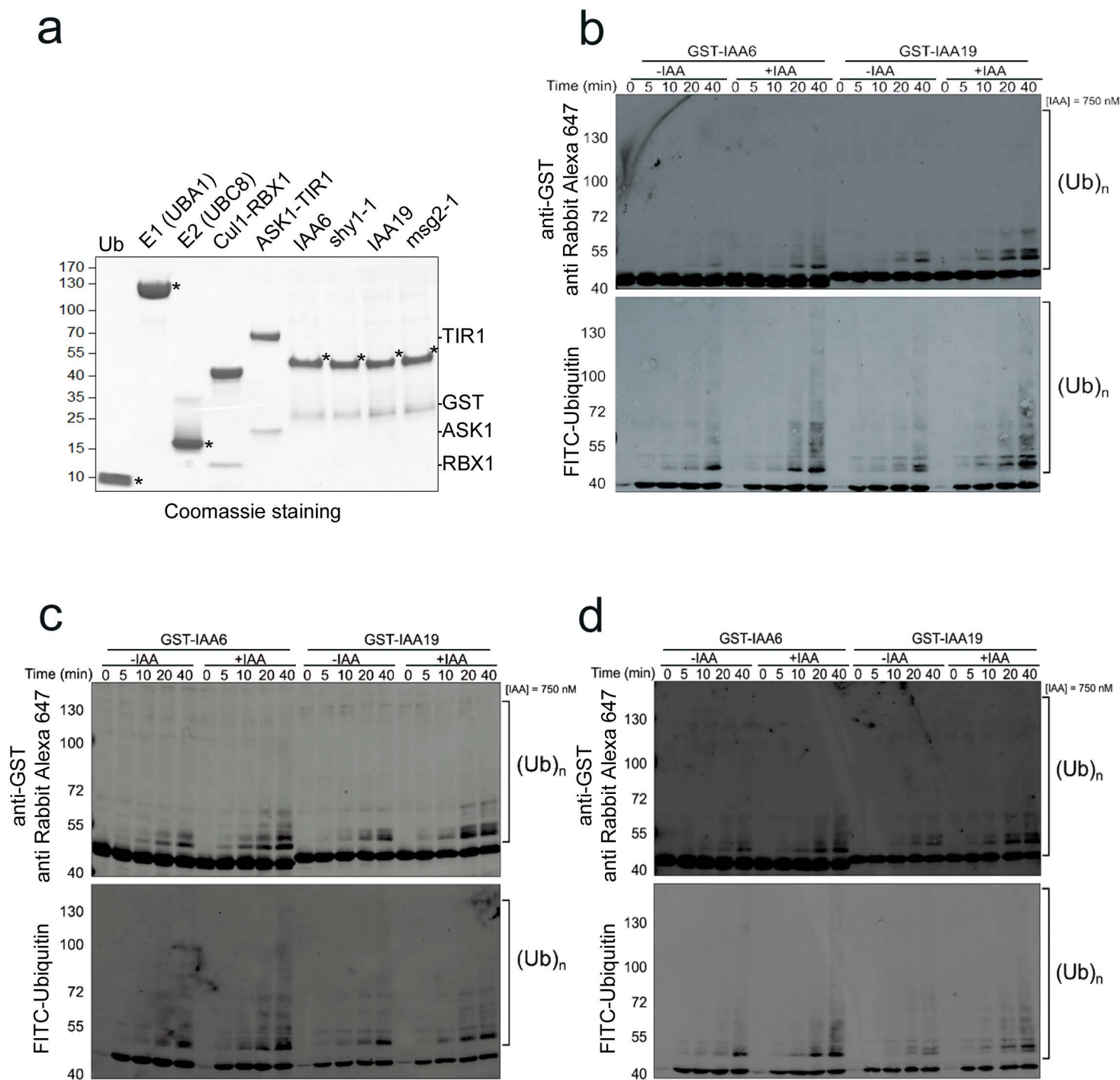
Supplementary Figure 5. Individual saturation binding experiments for the TIR1-IAA6 auxin co-receptor system. (a, c, e) Specific binding of IAA to TIR1-ASK1, expressed as either femtomol IAA bound per one milligram TIR1-ASK1 (left Y-axis) or fraction of TIR1 stably binding IAA (right Y-axis), was calculated assuming a 60% efficiency of the liquid scintillation counter and a molecular weight of 85.0 kDa for TIR1-ASK1. (b, d, f) Total, non-specific and specific binding (empty squares, empty circles and dots) shown as mean raw cpm data from two technical replicates. Specific binding was fitted to the Morrison model (a-f). Obtained K_d values are (74.0 ± 19.2) nM (a, b), (83.7 ± 18.3) nM (c, d) and (58.2 ± 13.6) nM (e, f).



Supplementary Figure 6. Individual saturation binding experiments for the TIR1-IAA19 auxin co-receptor system. (**a, c, e**) Specific binding of IAA to TIR1-ASK1, expressed as either femtomol IAA bound per one milligram TIR1-ASK1 (left Y-axis), or fraction of TIR1 stably binding IAA (right Y-axis), was calculated assuming a 60% efficiency of the liquid scintillation counter and a molecular weight of 85.0 kDa for TIR1-ASK1. (**b, d, f**) Total, non-specific and specific binding (empty squares, empty circles and dots, respectively) shown as mean raw cpm data from two technical replicates. Specific binding was fitted to the Morrison model (**a-f**). Obtained K_d values are (16.4 ± 4.4) nM (**a, b**), (12.8 ± 3.0) nM (**c, d**) and (17.6 ± 2.9) nM (**e, f**).

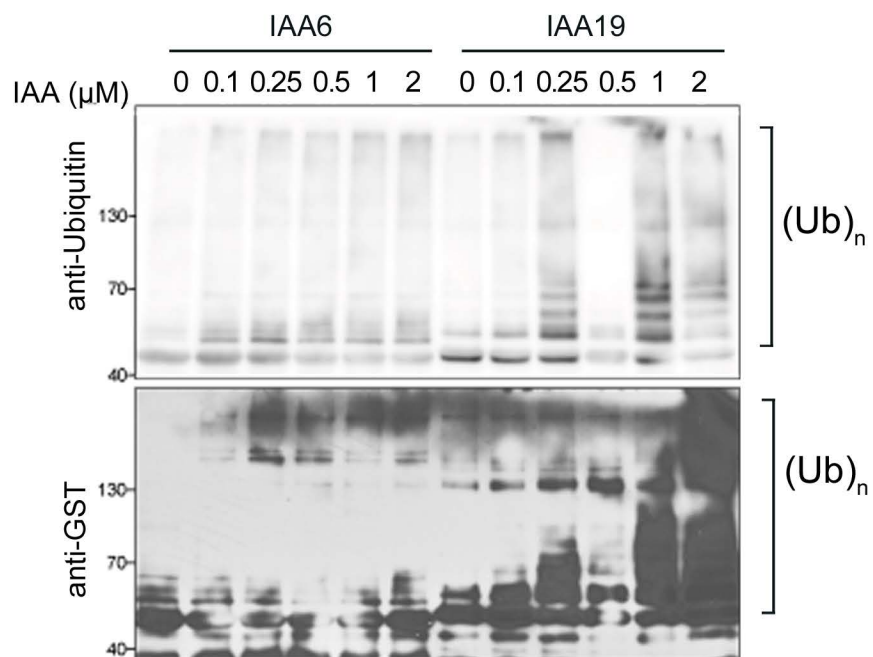


Supplementary Figure 7. Individual homologous competition binding experiments for TIR1-IAA6 and TIR1-IAA19 auxin co-receptors. Non-radioactive IAA was titrated to pre-formed TIR1-IAA6 (**a, c, e**) or TIR1-IAA19 (**b, d, f**) co-receptors bound to radioactive IAA. Resulting data are depicted as mean raw cpm data from two technical replicates were fitted to the built-in one site - $\log/C_{50}$ equation (GraphPad, Prism). K_i values were calculated according to the Cheng-Prusoff equation. K_i values for TIR1-IAA6 (**a,c,e**), and TIR1-IAA19 (**b,d,f**) are: 98.0 nM (**a**), 114.5 nM (**c**), 85.4 nM (**e**); and 29.7 nM (**b**), 32.3 nM (**d**), 38.5 nM (**f**).

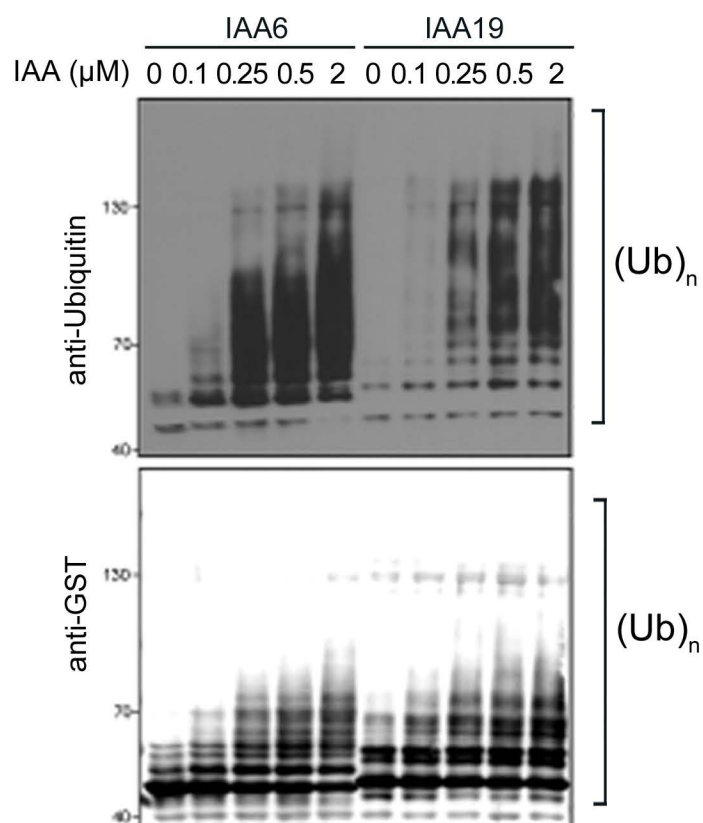


Supplementary Figure 8. (a) Coomassie staining of an SDS-gradient 4-12% gel depicting quality of proteins (~5 μ g) incorporated in IVU reactions. (b-d) Replicate blots from 3 independent IVUs used for quantification in Figure 2c. Fluorescein-labeled ubiquitin signals of individual lanes were quantified using ImageQuant TL software. Shown are immunoblots for either direct ubiquitin detection (bottom) or AUX/IAA detection using α -GST and α -rabbit Alexa Fluor Plus 647 antibodies (see **Methods** for additional information). (d) Immunoblots correspond to Figure 2c in gray scale. (e and f) continued in next page.

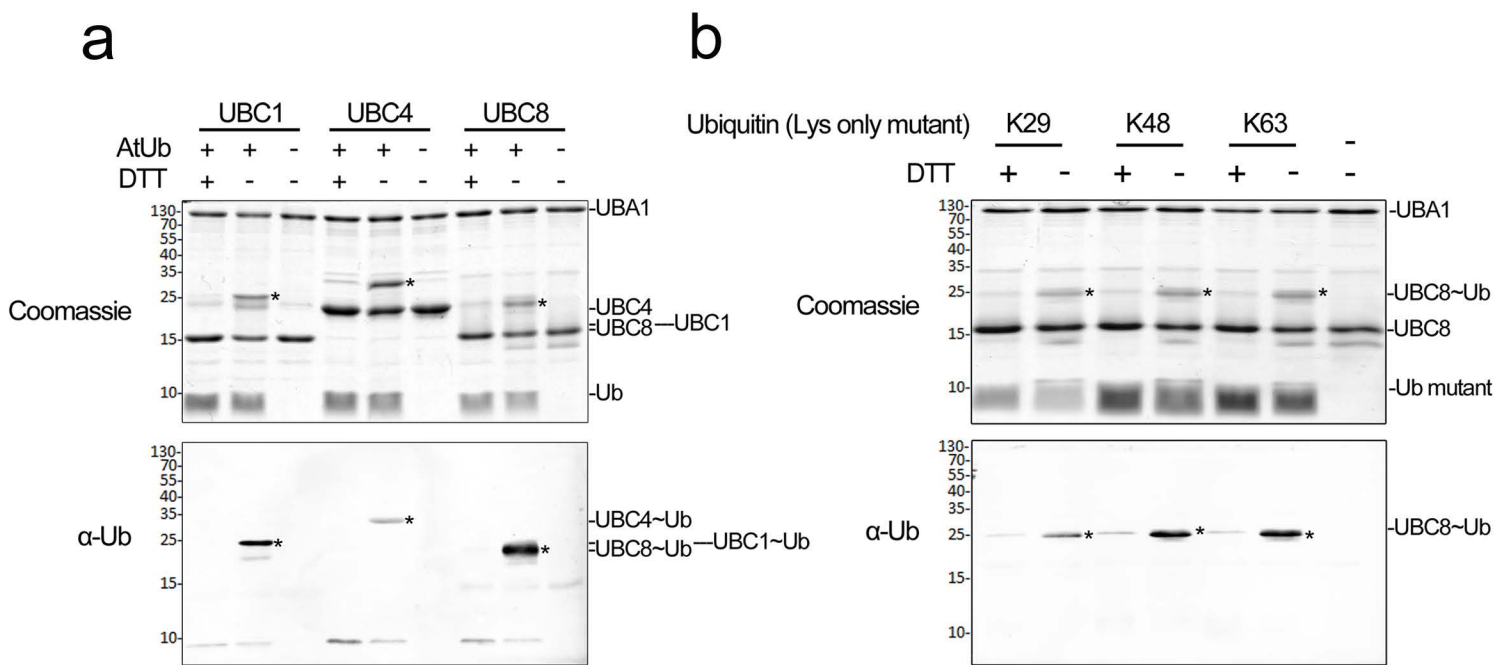
e



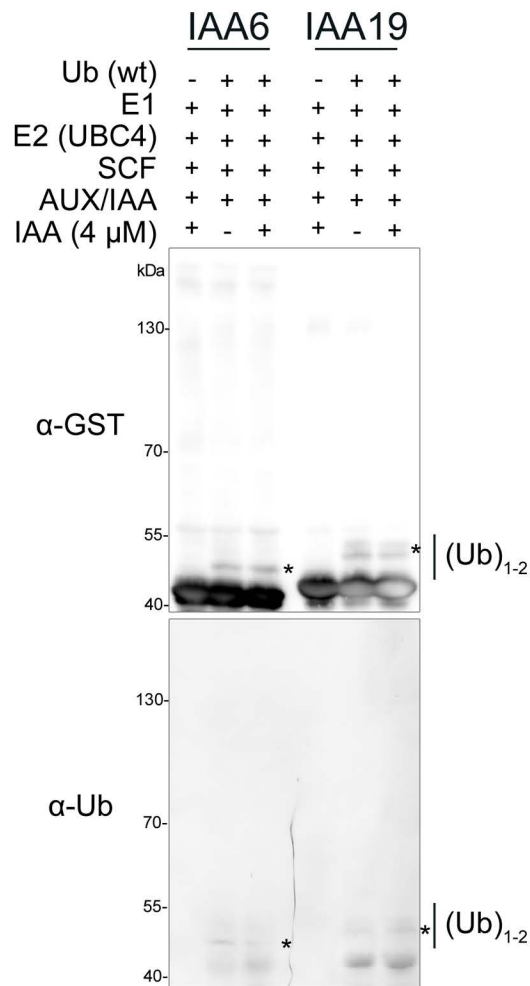
f



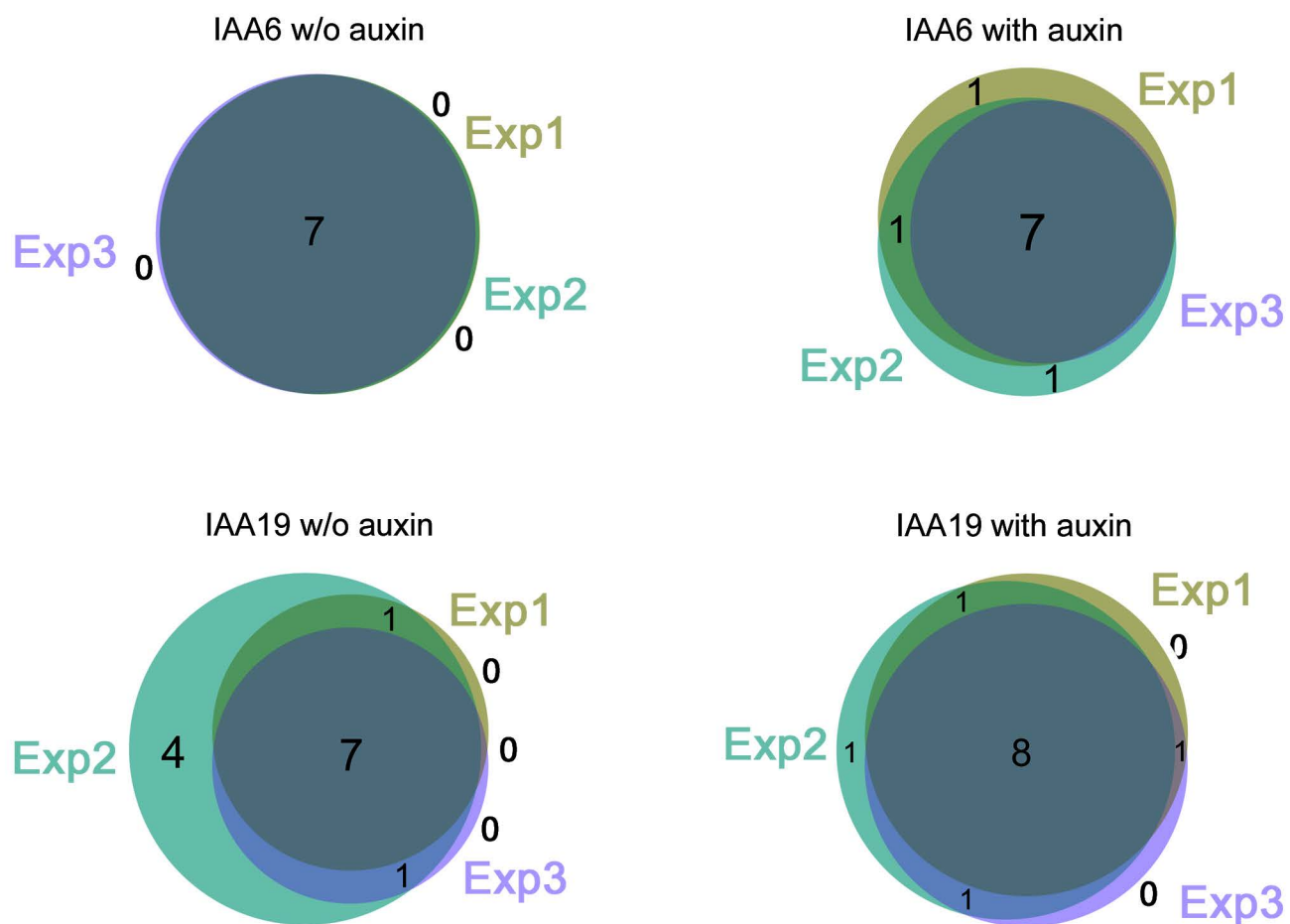
Supplementary Figure 8 continued. (e and f) Replicates showing auxin induced ubiquitylation of GST-IAA6 and GST-IAA19 (see Figure 2d). AUX/IAA ubiquitylated species were detected using α -Ub (P4D1) antibody and α -GST antibody.



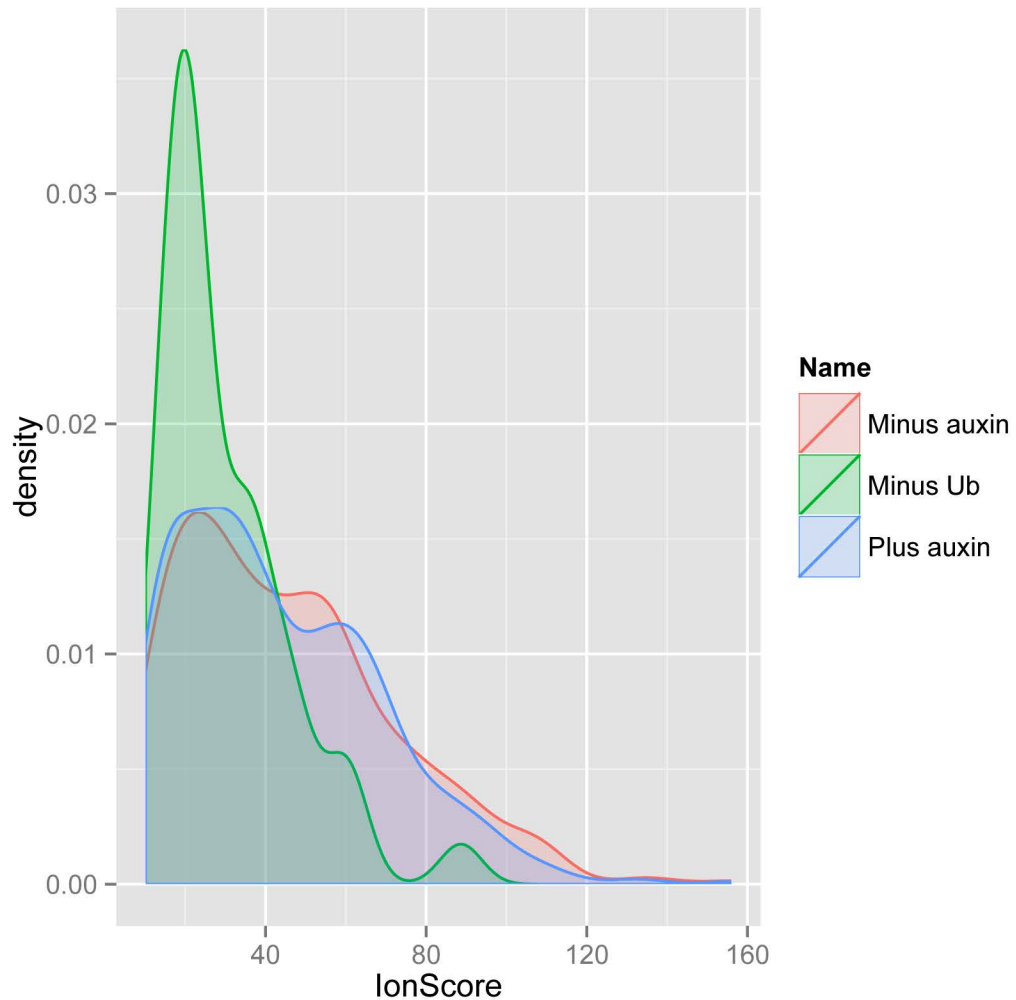
Supplementary Figure 9. ATP-dependent ubiquitin charging assays of Arabidopsis E2-ubiquitin conjugating enzymes used in this study. **(a)** UBC1, UBC4, and UBC8 charging assays show recombinantly expressed AtE2s are functional and can be Ub-charged *in vitro*. Asterisks indicate Ub-charged E2s (UBCx~Ub). **(b)** UBC8 can form a thioester bond with *Hs*Ubiquitin mutants containing only one lysine (Lys) residue available, namely K29, K48 or K63, while other Lys have been mutated to Arg. E2 (UBC8)-charging reactions were performed as described in the **Methods** section.



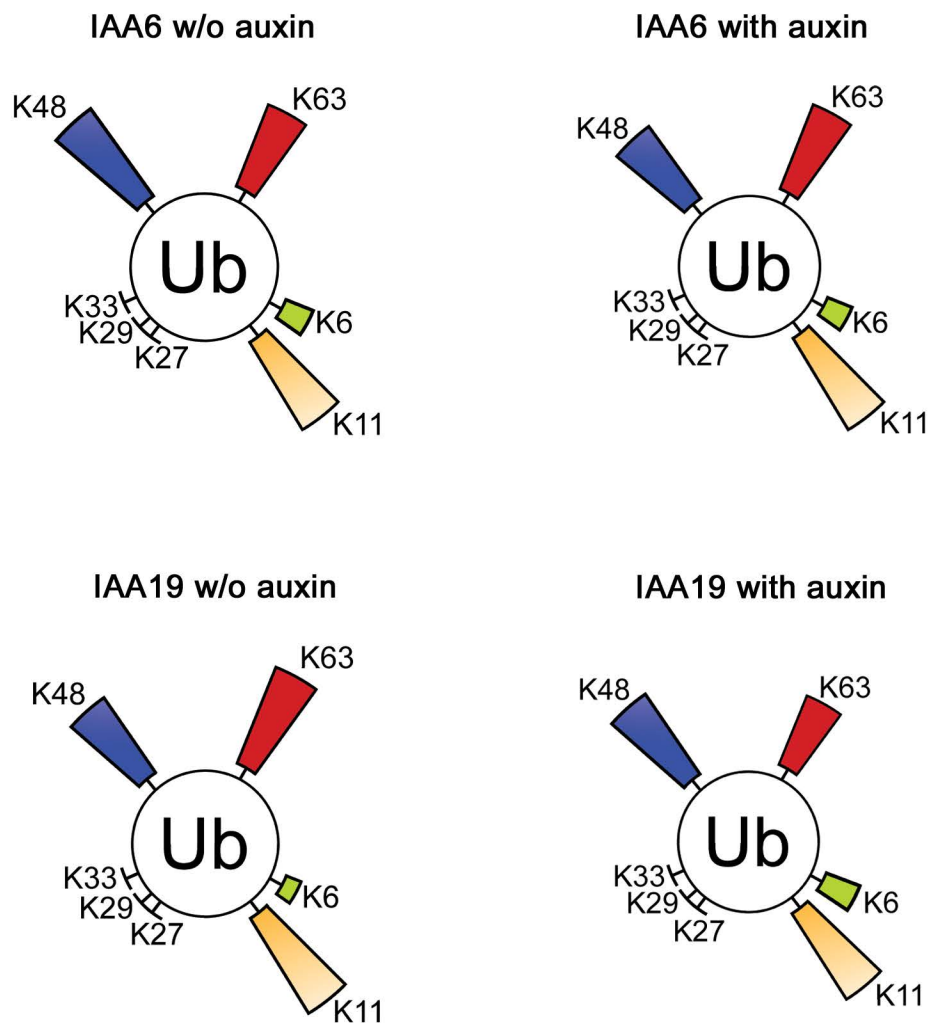
Supplementary Figure 10. UBC4 Ub-conjugating enzyme (E2) does not mediate poly-Ub-conjugation of IAA6 or IAA19 *in vitro*. As a result of 30 min IVU assays for IAA6 and IAA19, only low molecular ubiquitin conjugates, possibly monoubiquitin or diubiquitin (Ub_{1-2}), have been consistently detected with anti-GST and anti-Ub antibodies (*).



Supplementary Figure 11. Venn diagrams indicating the reproducibility in the identification of IAA6 and IAA19 ubiquitylated peptides upon three independent biological IVU reactions (Exp 1-3) with or without auxin followed by LC-MS analyses.

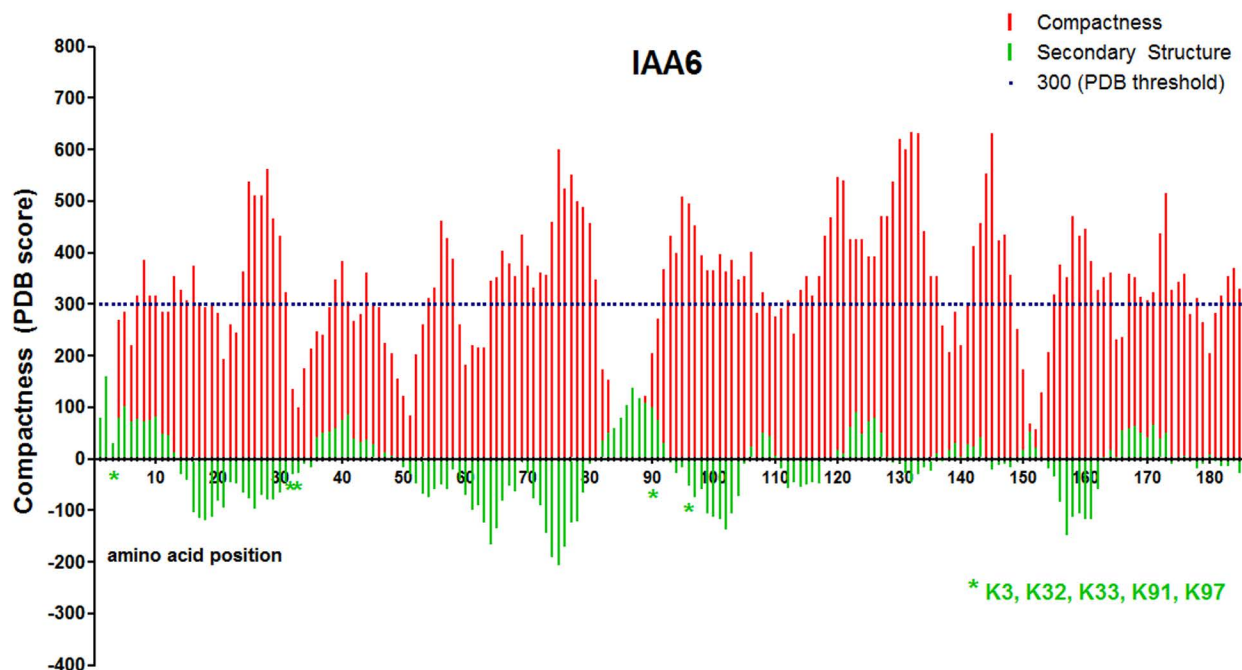


Supplementary Figure 12. Calculated false discovery rate (FDR) for IAA6 or IAA19 ubiquitylated peptides identified via LC-MS. Kernel density estimation of the PSMs (see **Supplementary Tables 1-2**) with a GG- modification identified in IVUs lacking Ub (Minus Ub), or containing Ub (supplemented with AUX/IAA or not, Plus Auxin, Minus Auxin respectively). FDR was calculated as described in the Methods section. An ion score of below 20 corresponded to an FDR threshold of 0.05 ($q < 0.05$) for IVUs containing Ub and supplemented with auxin. The same ion score corresponded to an FDR threshold of 0.07 for IVUs containing Ub and not supplemented with auxin.

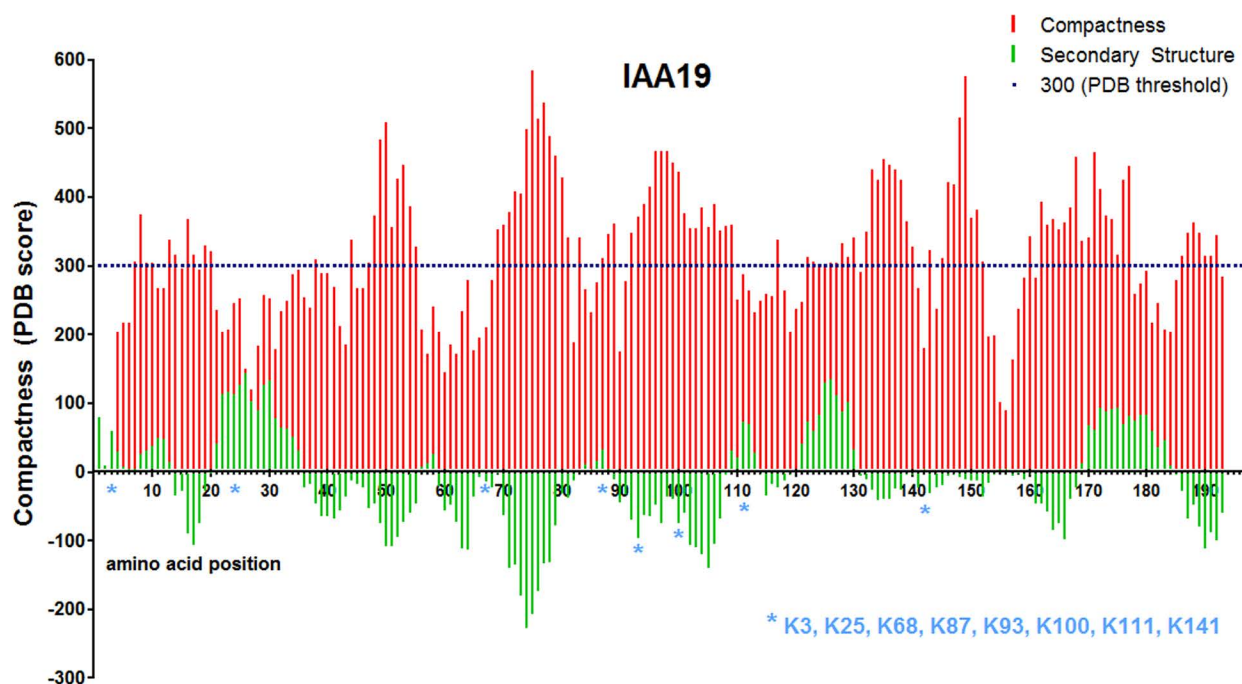


Supplementary Figure 13. Distribution of identified ubiquitin linkage types. *In vitro* ubiquitylation reactions for IAA6 (top) and IAA19 (bottom) were analyzed via LC-MS, and ubiquitin peptides corresponding to different ubiquitin linkage types were identified. A comparison of the number of peptide spectrum matches (PSMs) (see **Supplementary Tables 1-2** and PRIDE repository via ProteomeXchange with identifier PXD004027) for each of the ubiquitylated lysine residues in ubiquitin was used as a semi-quantitative index for determining the abundance of each linkage type in the reactions without or with auxin (4 μ M IAA). Depicted values for a specific linkage type were calculated as the percentage of the total number of PSMs corresponding to diGly-/LRGG-modified ubiquitin peptides. All peptides assigned to contain the Ub remnant (diGly or LRGG) on non-canonical residues were excluded. No K27, K29 nor K33 linkages were detected in these reactions using UBC8 as E2-ubiquitin conjugating enzyme.

a



b



Supplementary Figure 14. (a-b) Meta structure analyses for Arabidopsis IAA6 (a) and IAA19 (b), exhibiting features of intrinsically disordered proteins (IDPs) with regions of lower compactness. The threshold level in compactness is 300 (DisProt data base), while sequence regions that are significantly lower are flexible and open, amino acid segments above threshold can be considered classically folded. In secondary structure positive values denote alpha-helical regions, negative values beta sheets. Asterisks depict ubiquitylated sites identified via MS upon IVU reactions (for reference see Supplementary Tables 1-2).

c

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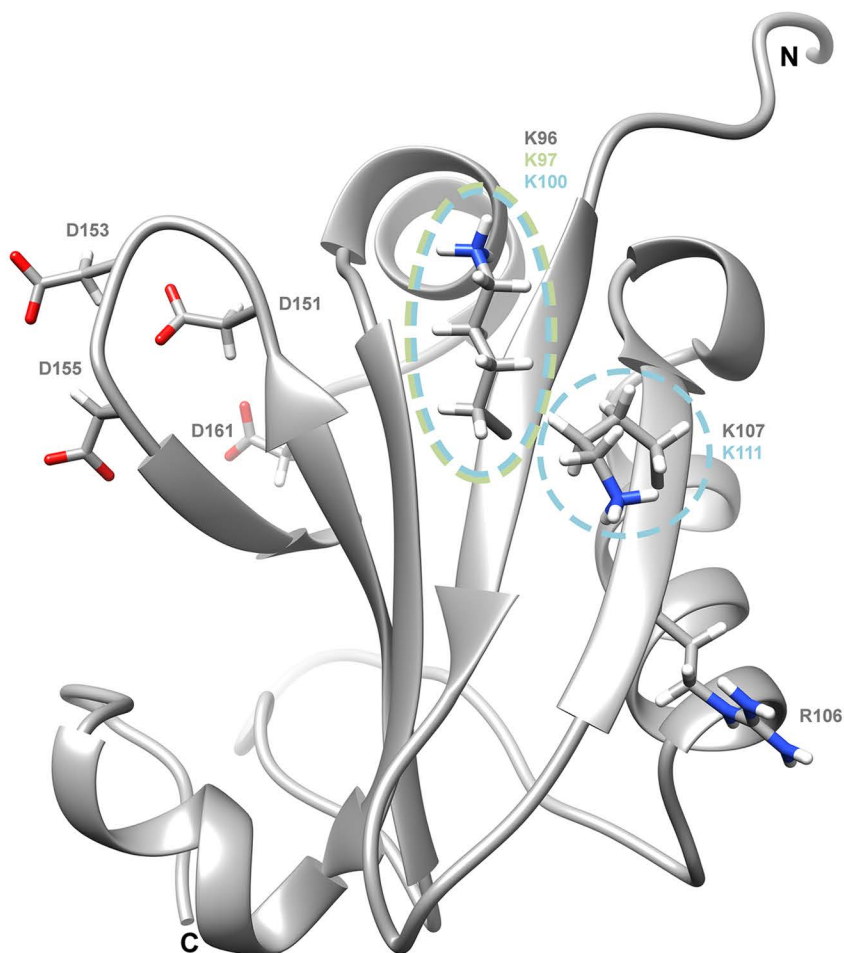
      3           32-33
AtIAA6 |MAKEGLALEITELRLGLPGDNYSEIS--VCGSSKKK---KRVLSDM-MTSSALDT-ENENSVVSSVED--
AtIAA19|MEKEGLGLEITELRLGLPGRDVAE-----KMMK---KRAFTENMTSSGSNSDQCESGVVSSGGDAE
PsIAA4 |ME-----FKATELRLGLPGITEEEKKIIHGSSVVKNNKRQLPQTSEESVSISKVTNDEHIVESS----
      3           25

      91 97 basic patch
AtIAA6 |--ESLPVVKSQAVGWPPVCSYRRKKNNEEAS---KAIGYVKV/SMDGVPIYRKIDLGSSNSYINLVTVLEN
AtIAA19|KVNDSPAAKSQVVGWPPVCSYRRKNSCKEASTTKVGLGYK/VMDGVPIYRKIDLGSSQGYDDLAFALDK
PsIAA4 |--SAAPPAAKAKIVGWPPIRSIR-KNSLHEAD---VGGIFV/VMDGAPYIRKIDLRVYGGYSELLKALET
      68      87 93 100 111

      acidic patch
AtIAA6 |LFGCLGIG-VA-KEG-KKCEYIIYYDDDDMLVGDVPWQMFKESCKRLRIVKRSDATGFGQLQQ----D
AtIAA19|LFGFRGIG-VALKDG-DNCEYVTIYYDDDDMLAGDVPWGMFLESCKRLRIMKRSDATGFGQLQPRGVDE
PsIAA4 |MFK-LTIGEYSEREYKGYKSEYAPTYYDDDDMLVGDVPWDMFVTSCKRLRIMKGTEAKGLGCGV----
      141

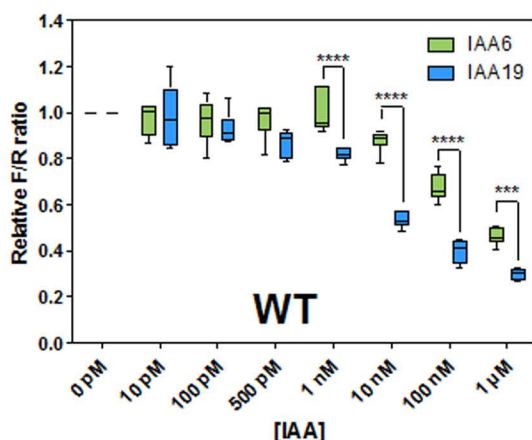
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d

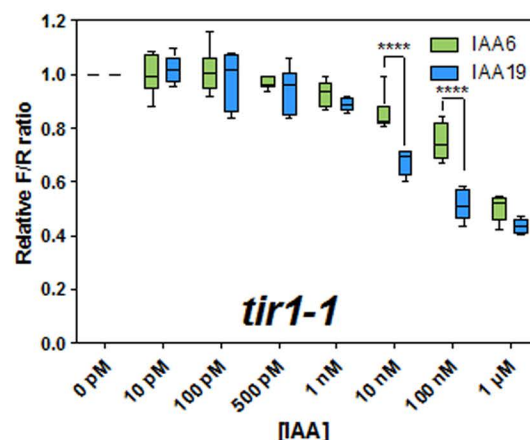


Supplementary Figure 14 continued. (c-d) Interface residues crucial for AUX/IAA and ARF homo- and hetero-dimerization are targeted for ubiquitylation *in vitro*. (c) MAFFT alignment of *A. thaliana* AtIAA6, AtIAA19 and *Pisum sativum* PsIAA4. IAA6 and IAA19 residues found to be ubiquitylated by UBC8 *in vitro* are highlighted in green and blue, respectively. K97 of IAA6, and K100 as well as K111 of IAA19 are part of the basic patch in the AUX/IAA PB1 domain (Dinesh et al. PNAS 2015). (d) Structural representation of the acidic (D151, D153, D155, D161) and basic (K96, R106, K107) patches in the PB1 domain of PsIAA4, PDB: 2M1M). Ubiquitylation of K97 of IAA6 (green ellipse) and K100 and K111 of IAA19 (blue ellipses) could interfere with proper dimerization of IAA6 and IAA19 with other AUX/IAAs and/or ARFs.

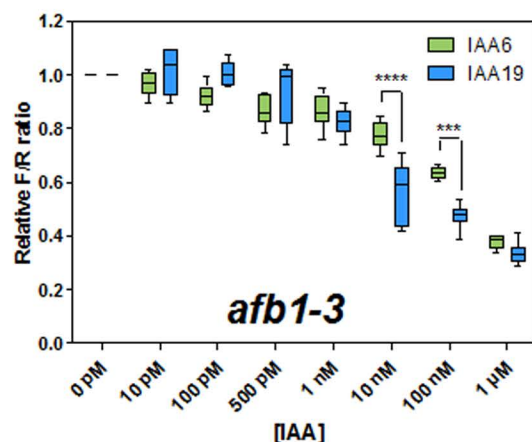
a



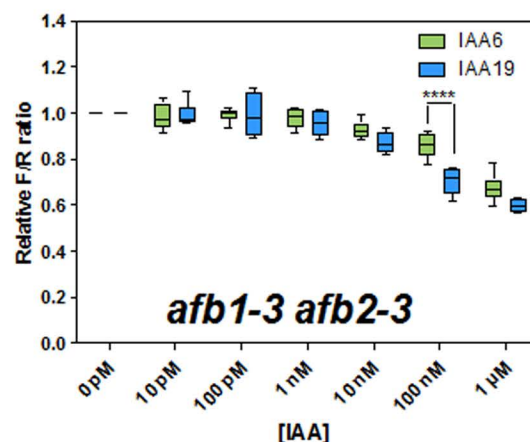
b



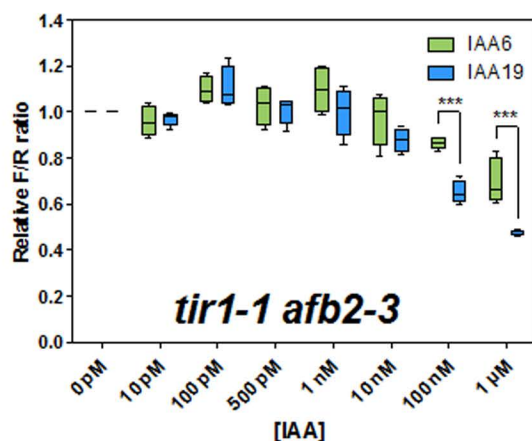
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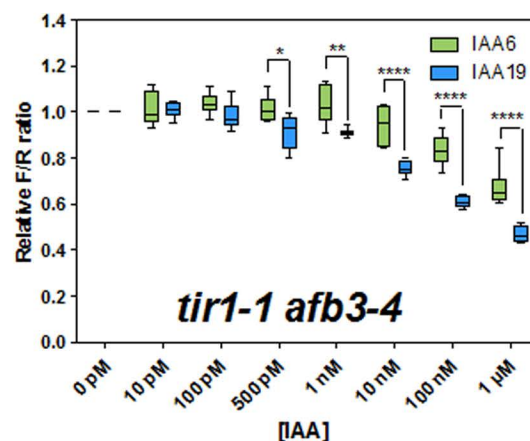
d



e

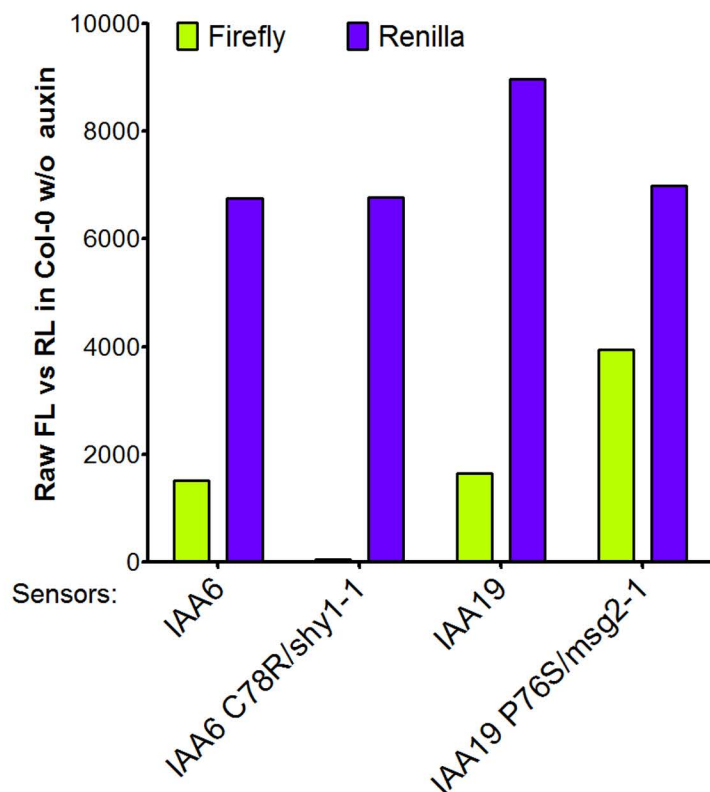


f

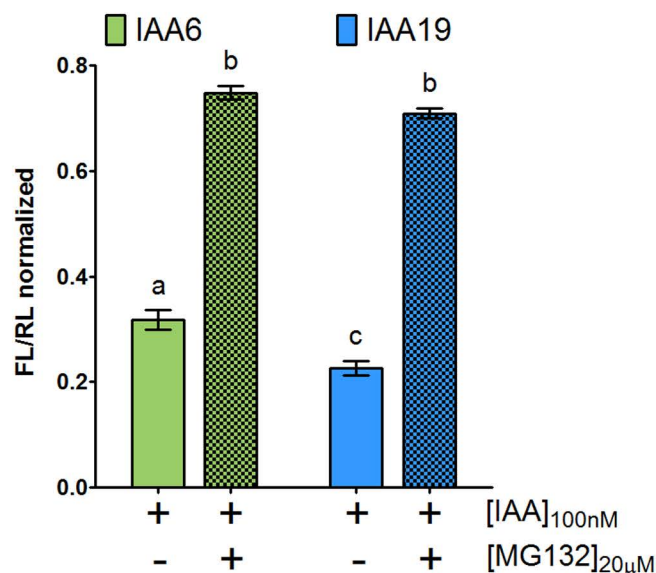


Supplementary Figure 15. Quantitative characterization of IAA6 and IAA19 sensor variants in different genetic backgrounds of *Arabidopsis* leaf protoplasts, namely from *Col-0* (WT) (a), as well as *tir1-1* (b), *afb1-3* (c), *afb1-3 afb2-3* (d), *tir1-1 afb2-3* (e), and *tir1-1 afb3-4* (f) mutant plants. Decrease in F/R ratios is a measure of sensor degradation after 30 min incubation with various IAA concentrations. Results are means $\pm$ s.e.m. of biological replicates ($n=6$). Statistical significances were calculated via two-way ANOVA with $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***), and $P<0.0001$ (****).

a



b



Supplementary Figure 16. (a) Raw values of Firefly luciferase (FL) -fused IAA6, IAA6^{C78R/shy1-1}, IAA19, IAA19^{P76S/msg2-1} sensors and Renilla luciferase (RL) fluorescence of the normalization element in *Col-0* protoplasts without exogenous IAA treatment. While IAA6, IAA19, and IAA19^{P76S/msg2-1} sensors are similarly expressed, IAA6^{C78R/shy1-1} sensor is highly unstable and not suitable for ratiometric analyses. (b) Ratiometric analysis of IAA6 and IAA19 sensors in protoplasts of *Col-0* (WT) plants are degraded in response to 100 nM IAA and stabilized upon combined treatment of IAA and 20 μ M proteasome inhibitor MG132. Error bars, s.e.m. Different letters denote statistically significant differences as assessed by two-way ANOVA with $P < 0.001$, $P > 0.05$ (n.s.).

Supplementary Table 1. IAA6 ubiquitylated peptides identified in LC-MS analyses

IAA6 w/o Auxin		Mascot			MaxQuant			MH+ [Da]	Charge	m/z [Da]	RT	Exp
Sequence	# PSMs	Modifications	IonScore	Exp Value	Modifications	Score	PEP*					
AGFMAKEGLALEITELR	6	K6(di-GI)	78	3.14019E-07	K6(di-GI)	101	1.53E-16	1963.0252	3	655.0132	67.64	1
AIGYVKVSMGVPYMR	1	K6(di-GI)	58	3.29577E-05	K6(di-GI)	69	3.50E-07	1899.9508	3	633.9885	57.37	1
KAGFMAKEGLALEITELR	2	K7(di-GI)	26	0.044766853	K7(di-GI)	43	7.78E-05	2091.1251	4	523.5367	57.96	1
LGLPGDNYSEISVCGSSKK	4	C14(Carbamidomethyl); K19(di-GI)	77	3.82786E-07	C14(Carbamidomethyl); K18(di-GI)	71	2.88E-24	2125.0210	2	1063.0142	47.49	1
LGLPGDNYSEISVCGSSKKK	6	C14(Carbamidomethyl); K20(di-GI)	37	0.003597134	C14(Carbamidomethyl); K18(di-GI)	83	5.54E-27	2253.1139	3	751.7130	41.33	1
NNEEASKAIGYVK	3	K7(di-GI)	92	1.31207E-08	K7(di-GI)	102	4.29E-14	1536.7666	2	768.8856	30.79	1
AGFMAKEGLALEITELR	4	K6(di-GI)	78	2.99886E-07	K6(di-GI)	108	2.79E-18	1963.0272	3	655.0139	67.44	2
AIGYVKVSMGVPYMR	1	K6(di-GI)	46	0.00055457	K6(di-GI)	50	0.00012455	1899.9364	3	633.9836	57.71	2
KAGFMAKEGLALEITELR	1	K7(di-GI)	14	0.856952141	n.d.	n.d.	n.d.	2091.1228	4	523.5362	58.53	2
LGLPGDNYSEISVCGSSKK	4	C14(Carbamidomethyl); K19(di-GI)	105	6.20807E-10	C14(Carbamidomethyl); K18(di-GI)	104	5.47E-37	2125.0191	2	1063.0132	48.02	2
LGLPGDNYSEISVCGSSKK	2	C14(Carbamidomethyl); K18(LRGG)	19	0.279226459	n.d.	n.d.	n.d.	2394.2069	4	599.3072	43.57	2
LGLPGDNYSEISVCGSSKKK	3	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	21	0.155223177	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	43	7.86E-17	2367.1559	4	592.5444	41.21	2
LGLPGDNYSEISVCGSSKKK	6	C14(Carbamidomethyl); K18(di-GI)	48	0.000305462	C14(Carbamidomethyl); K18(di-GI)	83	7.86E-17	2253.1149	3	751.7099	41.27	2
NNEEASKAIGYVK	3	K7(di-GI)	92	1.30004E-08	K7(di-GI)	101	2.05E-13	1536.7624	2	768.8840	31.13	2
AGFMAKEGLALEITELR	4	K6(di-GI)	72	1.34573E-06	K6(di-GI)	63	5.08E-07	1963.0292	3	655.0146	68.24	3
AIGYVKVSMGVPYMR	1	K6(di-GI)	29	0.02582002	K6(di-GI)	69	3.92E-07	1899.9406	3	633.9850	58.05	3
KAGFMAKEGLALEITELR	2	K7(di-GI)	25	0.069655685	K7(di-GI)	n.d.	n.d.	2091.1278	4	523.5374	58.55	3
LGLPGDNYSEISVCGSSKK	4	C14(Carbamidomethyl); K19(di-GI)	83	9.54897E-08	C14(Carbamidomethyl); K18(di-GI)	92	1.94E-16	2125.0210	3	709.0118	48.10	3
LGLPGDNYSEISVCGSSKK	2	C14(Carbamidomethyl); K18(LRGG)	38	0.003012705	C14(Carbamidomethyl); K18(LRGG)	62	1.94E-16	2394.2083	3	798.7410	43.84	3
LGLPGDNYSEISVCGSSKKK	6	C14(Carbamidomethyl); K18(di-GI)	45	0.000642623	C14(Carbamidomethyl); K18(di-GI)	65	5.26E-20	2253.1203	3	751.7128	41.66	3
LGLPGDNYSEISVCGSSKKK	2	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	16	0.519943997	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	42	5.26E-20	2367.1513	4	592.5433	41.57	3
NNEEASKAIGYVK	2	K7(di-GI)	92	1.23867E-08	K7(di-GI)	122	9.80E-13	1536.7674	2	768.8873	31.29	3

IAA6 with 4 µM IAA		Mascot			MaxQuant			MH+ [Da]	Charge	m/z [Da]	RT	Exp
Sequence	# PSMs	Modifications	IonScore	Exp Value	Modifications	Score	PEP*					
AGFMAKEGLALEITELR	7	K6(di-GI)	83	9.95306E-08	K6(di-GI)	106	2.77E-16	1963.0272	3	655.0139	71.26	1
AIGYVKVSMGVPYMR	1	K6(di-GI)	62	1.2559E-05	K6(di-GI)	77	5.87E-07	1899.9461	3	633.9869	57.51	1
KAGFMAKEGLALEITELR	2	K7(di-GI)	30	0.019676895	n.d.	n.d.	n.d.	2091.1239	4	523.5364	57.94	1
KIDLGSNSYINLVTLENLFGLGIGVAK	1	K1(di-GI); C23(Carbamidomethyl)	37	0.004374784	n.d.	n.d.	n.d.	3308.7418	3	1103.5854	120.04	1
KNNEEASKAIGYVK	2	K8(di-GI)	37	0.003908018	K8(di-GI)	70	6.75E-06	1664.8572	3	555.6239	24.18	1
LGLPGDNYSEISVCGSSKK	6	C14(Carbamidomethyl); K19(di-GI)	74	7.85157E-07	C14(Carbamidomethyl); K18(di-GI)	84	9.73E-23	2125.0242	2	1063.0157	47.74	1
LGLPGDNYSEISVCGSSKKK	3	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	24	0.088299159	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	60	3.87E-25	2367.1574	4	592.5448	41.06	1
LGLPGDNYSEISVCGSSKKK	3	C14(Carbamidomethyl); K18(di-GI)	38	0.003364779	C14(Carbamidomethyl); K18(di-GI)	119	3.87E-25	2253.1144	4	564.0341	41.18	1
NNEEASKAIGYVK	4	K7(di-GI)	81	1.62913E-07	K7(di-GI)	93	1.15E-08	1536.7672	2	768.8866	30.86	1
AGFMAKEGLALEITELR	5	K6(di-GI)	90	1.90527E-08	K6(di-GI)	103	3.30E-45	1963.0240	2	982.0156	67.84	2
AIGYVKVSMGVPYMR	1	K6(di-GI)	45	0.000617955	K6(di-GI)	80	3.15E-08	1899.9382	3	633.9843	58.23	2
KAGFMAKEGLALEITELR	4	K7(di-GI)	85	6.0528E-08	K7(di-GI)	84	7.35E-09	2091.1244	4	523.5366	58.60	2
KCEYIIYEDKDR	1	K1(di-GI); C2(Carbamidomethyl)	20	0.185761867	K1(di-GI); C2(Carbamidomethyl)	47	7.72E-12	1858.8969	3	620.3038	38.53	2
LGLPGDNYSEISVCGSSKK	8	C14(Carbamidomethyl); K19(di-GI)	85	6.71362E-08	C14(Carbamidomethyl); K19(di-GI)	57	2.06E-10	2125.0218	2	1063.0145	48.14	2
LGLPGDNYSEISVCGSSKKK	11	C14(Carbamidomethyl); K19(di-GI)	39	0.002517425	C14(Carbamidomethyl); K19(di-GI)	95	6.45E-14	2253.1163	3	751.7095	41.23	2
LGLPGDNYSEISVCGSSKKK	3	C14(Carbamidomethyl); K18(LRGG)	22	0.13211635	C14(Carbamidomethyl); K18(LRGG); K19(di-GI)	63	6.45E-14	2522.3004	5	505.2659	38.40	2
LGLPGDNYSEISVCGSSKKK	9	C14(Carbamidomethyl); K18(di-GI); K20(di-GI)	43	0.000924606	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	95	6.45E-14	2367.1516	3	789.7233	41.15	2
NNEEASKAIGYVK	3	K7(di-GI)	92	1.36131E-08	K7(di-GI)	141	5.06E-16	1536.7632	2	768.8849	31.41	2
AGFMAKEGLALEITELR	6	K6(di-GI)	84	8.78935E-08	K6(di-GI)	84	1.36E-11	1963.0259	3	655.0135	69.77	3
KAGFMAKEGLALEITELR	4	K7(di-GI)	72	1.27338E-06	K7(di-GI)	81	1.30E-09	2091.1288	3	697.7151	58.42	3
KNNEEASKAIGYVK	2	K8(di-GI)	30	0.02055685	n.d.	n.d.	n.d.	1664.8606	3	555.6251	24.30	3
LGLPGDNYSEISVCGSSKK	6	C14(Carbamidomethyl); K18(di-GI)	83	1.01148E-07	C14(Carbamidomethyl); K18(di-GI)	88	2.89E-13	2125.0206	3	709.0117	47.75	3
LGLPGDNYSEISVCGSSKK	2	C14(Carbamidomethyl); K18(LRGG)	42	0.00130004	C14(Carbamidomethyl); K18(LRGG)	34	2.89E-13	2394.2085	3	798.7410	43.56	3
LGLPGDNYSEISVCGSSKKK	6	C14(Carbamidomethyl); K18(di-GI)	42	0.001180203	C14(Carbamidomethyl); K18(di-GI)	63	7.78E-10	2253.1177	3	751.7108	41.33	3
LGLPGDNYSEISVCGSSKKK	3	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	21	0.170591178	C14(Carbamidomethyl); K18(di-GI); K19(di-GI)	63	7.78E-10	2367.1601	4	592.5455	41.13	3
LGLPGDNYSEISVCGSSKKK	3	C14(Carbamidomethyl); K18(LRGG)	21	0.146877939	C14(Carbamidomethyl); K18(LRGG)	17	7.78E-10	2522.3024	5	505.2663	37.97	3
NNEEASKAIGYVK	1	K7(di-GI)	72	1.29705E-06	K7(di-GI)	142	3.71E-37	1536.7665	3	512.9270	31.29	3
NNEEASKAIGYVK	1	K7(LRGG)	16	0.509279938	K7(LRGG)	58	3.71E-37	1805.9509	4	452.2432	29.27	3

*PEP: Posterior error probability
n.d. : not detected

Supplementary Table 2. IAA19 ubiquitylated peptides identified in LC-MS analyses

IAA19 w/o Auxin

Sequence	# PSMs	Mascot			MaxQuant			MH+ [Da]	Charge	m/z [Da]	RT	Exp
		Modifications	IonScore	Exp Value	Modifications	Score	PEP					
AGFMEKEGLGLEITELR	1	M4(Oxidation); K6(di-GI)	43	0.001016147	M4(Oxidation); K6(di-GI)	56	1.40E-24	2023.0063	3	675.0070	59.26	1
AGFMEKEGLGLEITELR	1	K6(di-GI)	53	9.93E-05	K6(di-GI)	69	1.40E-24	2007.0161	3	669.6769	64.69	1
EASTTKVGLGYVK	1	K6(LRGG)	29	0.023656831	K6(LRGG)	20	0.00095458	1735.9674	4	434.7473	29.65	1
KAGFMEKEGLGLEITELR	2	K7(di-GI)	51	0.000167478	n.d.	n.d.	n.d.	2135.1066	3	712.3737	55.77	1
KMDLGSSQGYDDLAFAFDK	1	K1(LRGG)	66	5.31E-06	K1(LRGG)	81	1.75E-246	2457.2028	4	615.0562	57.59	1
KMDLGSSQGYDDLAFAFDK	2	K1(di-GI); M2(Oxidation)	74	8.09E-07	K1(di-GI); M2(Oxidation)	67	1.75E-246	2204.0151	3	735.3432	58.72	1
KMDLGSSQGYDDLAFAFDK	4	K1(di-GI)	156	5.12E-15	K1(di-GI)	190	1.75E-246	2188.0196	2	1094.5134	63.66	1
LGLPGRDVAEKMMK	1	K11(di-GI)	16	0.558414348	K11(di-GI)	50	2.86E-06	1658.8687	3	553.6278	43.92	1
VGLGYVKVSMGVPYLR	1	K7(di-GI)	64	8.63E-06	K7(di-GI)	128	3.75E-31	1967.0354	3	656.3500	65.19	1
VNDSPAAKSQVVGWPPVCSYR	2	K8(di-GI); C18(Carbamidomethyl)	60	1.87E-05	K8(di-GI); C18(Carbamidomethyl)	75	1.64E-13	2431.1748	3	811.0631	51.52	1
AGFMEKEGLGLEITELR	1	M4(Oxidation); K6(di-GI)	33	0.009659543	M4(Oxidation); K6(di-GI)	29	9.57E-80	2023.0147	3	675.0098	59.56	2
AGFMEKEGLGLEITELR	2	K6(di-GI)	53	9.79E-05	K6(di-GI)	62	9.57E-80	2007.0192	3	669.6779	65.13	2
DVAEKMMK	4	K5(di-GI); M7(Oxidation)	16	0.497687311	K5(di-GI); M7(Oxidation)	79	6.03E-07	1081.5008	2	541.2540	16.86	2
DVAEKMMK	2	K5(di-GI); K8(di-GI)	20	0.190966227	K5(di-GI); K8(di-GI)	52	0.00067398	1307.6402	2	654.3237	20.18	2
EASTTKVGLGYVK	2	K6(LRGG)	29	0.02666592	K6(LRGG)	40	4.98E-06	1735.9709	4	434.7476	29.79	2
EASTTKVGLGYVK	1	K6(di-GI)	14	0.751547732	K6(di-GI)	32	4.98E-06	1466.7848	3	489.5998	33.28	2
GIGVALKDGDNCEYVTIYEDK	1	K7(di-GI); C12(Carbamidomethyl)	85	6.68E-08	K7(di-GI); C12(Carbamidomethyl)	54	4.69E-40	2473.1561	3	825.0569	58.68	2
KAGFMEKEGLGLEITELR	2	K1(di-GI); K7(di-GI)	59	2.58E-05	K1(di-GI); K7(di-GI)	68	3.73E-44	2249.1555	3	750.3900	55.76	2
KMDLGSSQGYDDLAFAFDK	1	K1(LRGG)	64	8.41E-06	K1(LRGG)	87	7.38E-113	2457.2072	4	615.0573	58.01	2
KMDLGSSQGYDDLAFAFDK	3	K1(di-GI); M2(Oxidation)	94	7.89E-09	K1(di-GI); M2(Oxidation)	95	7.38E-113	2204.0151	3	735.3432	59.09	2
KMDLGSSQGYDDLAFAFDK	4	K1(di-GI)	134	8.43E-13	K1(di-GI)	148	7.38E-113	2188.0218	2	1094.5145	64.18	2
LGLPGRDVAEKMMK	3	K11(di-GI)	19	0.250009533	K11(di-GI)	82	1.44E-10	1658.8703	4	415.4730	44.53	2
NSCKEASTTKVGLGYVK	1	C3(Carbamidomethyl); K4(di-GI); K10(di-GI)	72	1.16E-06	n.d.	n.d.	n.d.	2070.0320	3	690.6822	27.69	2
NSCKEASTTKVGLGYVK	2	C3(Carbamidomethyl); K10(di-GI)	24	0.082216043	n.d.	n.d.	n.d.	1955.9884	4	489.7526	26.8	2
VGLGYVKVSMGVPYLR	1	K7(LRGG)	16	0.463400575	n.d.	n.d.	n.d.	2236.2211	4	559.8107	59.82	2
VGLGYVKVSMGVPYLR	1	K7(di-GI)	57	3.97E-05	K7(di-GI)	97	1.26E-18	1967.0398	3	656.3514	65.51	2
VNDSPAAKSQVVGWPPVCSYR	2	K8(LRGG); C18(Carbamidomethyl)	32	0.013550539	n.d.	n.d.	n.d.	2700.3656	4	675.8469	46.98	2
VNDSPAAKSQVVGWPPVCSYR	3	K8(di-GI); C18(Carbamidomethyl)	60	1.95E-05	K8(di-GI); C18(Carbamidomethyl)	82	1.65E-17	2431.1762	3	811.0636	51.56	2
AGFMEKEGLGLEITELR	2	K6(di-GI)	102	1.15E-09	K6(di-GI)	139	4.29E-175	2007.0162	2	1004.0117	65.28	3
EASTTKVGLGYVK	1	K6(di-GI)	34	0.007942488	K6(di-GI)	27	1.35E-09	1466.7843	3	489.5996	32.31	3
EASTTKVGLGYVK	1	K6(LRGG)	15	0.587430604	n.d.	n.d.	n.d.	1735.9710	4	434.7482	29.98	3
KMDLGSSQGYDDLAFAFDK	2	K1(di-GI)	136	4.99E-13	K1(di-GI)	165	8.41E-97	2188.0228	2	1094.5150	64.22	3
KMDLGSSQGYDDLAFAFDK	1	K1(LRGG)	47	0.000386328	K1(LRGG)	116	8.41E-97	2457.2079	4	615.0574	58.04	3
LGLPGRDVAEKMMK	1	K11(di-GI)	16	0.534510903	K11(di-GI)	80	3.17E-06	1658.8698	3	553.6281	44.54	3
NSCKEASTTKVGLGYVK	2	C3(Carbamidomethyl); K10(di-GI)	29	0.022592098	n.d.	n.d.	n.d.	1955.9857	4	489.7519	26.69	3
VGLGYVKVSMGVPYLR	1	K7(di-GI)	45	0.000612289	K7(di-GI)	74	6.70E-09	1967.0350	3	656.3499	65.59	3
VNDSPAAKSQVVGWPPVCSYR	2	K8(di-GI); C18(Carbamidomethyl)	58	3.20E-05	K8(di-GI); C18(Carbamidomethyl)	110	1.82E-22	2431.1788	3	811.0645	52.07	3

IAA19 with 4 µM IAA

Sequence	# PSMs	Mascot			MaxQuant			MH+ [Da]	Charge	m/z [Da]	RT	Exp
		Modifications	IonScore	Exp Value	Modifications	Score	PEP					
AGFMEKEGLGLEITELR	1	M4(Oxidation); K6(di-GI)	73	1.10E-06	M4(Oxidation); K6(di-GI)	58	1.13E-50	2023.0136	3	675.0094	58.99	1
AGFMEKEGLGLEITELR	2	K6(LRGG)	23	0.111932594	K6(LRGG)	36	1.13E-50	2276.2140	4	569.8090	58.88	1
AGFMEKEGLGLEITELR	3	K6(di-GI)	84	7.55E-08	K6(di-GI)	92	1.13E-50	2007.0154	2	1004.0114	64.48	1
DVAEKMMK	8	M6(Oxidation); K8(di-GI)	22	0.115068531	n.d.	n.d.	n.d.	1081.4987	2	541.2537	17.58	1
EASTTKVGLGYVK	2	K6(LRGG)	26	0.053574308	K6(LRGG)	27	8.78E-07	1735.9676	4	434.7473	29.53	1
GIGVALKDGDNCEYVTIYEDK	3	K7(di-GI); C12(Carbamidomethyl)	104	7.87E-10	K7(di-GI); C12(Carbamidomethyl)	38	2.63E-41	2473.1471	3	825.0539	57.72	1
KAGFMEKEGLGLEITELR	4	K7(di-GI)	65	6.44E-06	K7(di-GI)	113	2.26E-40	2135.1125	4	534.5336	55.69	1
KAGFMEKEGLGLEITELR	2	K1(di-GI); K7(di-GI)	100	1.81E-09	K1(di-GI); K7(di-GI)	142	2.26E-40	2249.1522	3	750.3889	55.6	1
KMDLGSSQGYDDLAFAFDK	1	K1(LRGG)	59	2.71E-05	K1(LRGG)	100	2.01E-194	2457.2045	4	615.0566	57.49	1
KMDLGSSQGYDDLAFAFDK	6	K1(di-GI); M2(Oxidation)	94	7.13E-09	K1(di-GI); M2(Oxidation)	79	2.01E-194	2204.0136	3	735.3427	58.56	1
KMDLGSSQGYDDLAFAFDK	5	K1(di-GI)	156	5.03E-15	K1(di-GI)	204	2.01E-194	2188.0203	2	1094.5138	63.62	1
NSCKEASTTKVGLGYVK	2	C3(Carbamidomethyl); K4(di-GI); K10(di-GI)	46	0.000493124	C3(Carbamidomethyl); K4(di-GI); K10(di-GI)	57	1.03E-06	2070.0229	3	690.6791	27.3	1
VGLGYVKVSMGVPYLR	1	K7(di-GI); M10(Oxidation)	26	0.053945667	K7(di-GI); M10(Oxidation)	24	1.34E-39	1983.0351	3	661.6832	58.18	1

Supplementary Table 2 continued. IAA19 ubiquitylated peptides identified in LC-MS analyses

IAA19 with 4 µM IAA

Sequence	# PSMs	Mascot			MaxQuant			MH+ [Da]	Charge	m/z [Da]	RT	Exp
		Modifications	IonScore	Exp Value	Modifications	Score	PEP					
VGLGYVKVSMGVPYLR	2	K7(di-GI)	88	3.03E-08	K7(di-GI)	142	1.34E-39	1967.0392	2	984.0233	64.91	1
VNDSPAAKSQVVGWPPVCSYR	5	K8(di-GI); C18(Carbamidomethyl)	66	4.61E-06	K8(di-GI); C18(Carbamidomethyl)	89	1.64E-17	2431.1762	3	811.0636	51.35	1
AGFMEKEGLGLEITELR	1	K6(di-GI)	62	1.39E-05	K6(di-GI)	114	2.15E-113	2007.0187	3	669.6777	65.17	2
EASTTKVGLGYVK	2	K6(di-GI)	45	0.0058608	K6(di-GI)	39	4.04E-07	1466.7840	3	489.5995	32.11	2
EASTTKVGLGYVK	2	K6(LRGG)	26	0.051045395	K6(LRGG)	26	4.04E-07	1735.9694	4	434.7478	29.75	2
GIGVALKDGDNCEYVTIYEDK	1	K7(di-GI); C12(Carbamidomethyl)	62	1.27E-05	K7(di-GI); C12(Carbamidomethyl)	37	3.63E-104	2473.1556	3	825.0567	58.26	2
KMDLGSSQGYDDLAFAFDK	2	K1(di-GI)	117	3.89E-11	K1(di-GI)	194	2.38E-114	2188.0218	2	1094.5145	64.21	2
KMDLGSSQGYDDLAFAFDK	2	K1(di-GI); M2(Oxidation)	93	8.95E-09	K1(di-GI); M2(Oxidation)	101	2.38E-114	2204.0167	3	735.3438	59.03	2
KMDLGSSQGYDDLAFAFDK	2	K1(LRGG)	84	8.15E-08	K1(LRGG)	122	2.38E-114	2457.2045	3	819.7397	58.12	2
LGLPGRDVAEKMMK	2	K11(di-GI)	13	0.988454239	K11(di-GI)	52	0.00017596	1658.8719	2	829.9396	44.6	2
NSCKEASTTKVGLGYVK	5	C3(Carbamidomethyl); K10(di-GI)	55	6.81E-05	C3(Carbamidomethyl); K10(di-GI)	80	8.73E-10	1955.9851	4	489.7523	26.77	2
NSCKEASTTKVGLGYVK	1	C3(Carbamidomethyl); K4(di-GI); K10(di-GI)	14	0.79059956	C3(Carbamidomethyl); K4(di-GI); K10(di-GI)	26	8.73E-10	2070.0285	4	518.2626	27.89	2
VGLGYVKVSMGVPYLR	1	K7(di-GI)	60	2.00E-05	K7(di-GI)	105	5.87E-17	1967.0425	3	656.3524	65.5	2
VNDSPAAKSQVVGWPPVCSYR	2	K8(di-GI); C18(Carbamidomethyl)	60	1.91E-05	K8(di-GI); C18(Carbamidomethyl)	120	8.62E-28	2431.1806	3	811.0651	51.98	2
VNDSPAAKSQVVGWPPVCSYR	1	K8(LRGG); C18(Carbamidomethyl)	15	0.608074188	K8(LRGG); C18(Carbamidomethyl)	18	8.62E-28	2700.3666	4	675.8471	46.77	2
AGFMEKEGLGLEITELR	1	K6(di-GI)	60	1.79E-05	K6(di-GI)	89	1.19E-96	2007.0157	3	669.6768	65.49	3
EASTTKVGLGYVK	1	K6(di-GI)	58	3.31E-05	K6(di-GI)	39	1.12E-05	1466.7834	3	489.5993	32.81	3
EASTTKVGLGYVK	2	K6(LRGG)	24	0.074637411	K6(LRGG)	28	3.94E-28	1735.9707	4	434.7481	30.39	3
GIGVALKDGDNCEYVTIYEDK	1	K7(di-GI); C12(Carbamidomethyl)	65	7.08E-06	K7(di-GI); C12(Carbamidomethyl)	48	1.48E-63	2473.1545	3	825.0563	58.65	3
KMDLGSSQGYDDLAFAFDK	2	K1(di-GI)	130	1.88E-12	K1(di-GI)	191	5.11E-115	2188.0247	2	1094.5160	64.52	3
KMDLGSSQGYDDLAFAFDK	1	K1(di-GI); M2(Oxidation)	66	4.93E-06	K1(di-GI); M2(Oxidation)	71	5.11E-115	2204.0083	3	735.3409	59.74	3
NSCKEASTTKVGLGYVK	4	C3(Carbamidomethyl); K10(di-GI)	37	0.003980674	C3(Carbamidomethyl); K10(di-GI)	45	0.00046473	1955.9811	3	652.6652	27.23	3
NSCKEASTTKVGLGYVK	1	C3(Carbamidomethyl); K4(di-GI); K10(di-GI)	36	0.004644688	C3(Carbamidomethyl); K4(di-GI); K10(di-GI)	31	0.00046473	2070.0324	3	690.6823	28.22	3
VGLGYVKVSMGVPYLR	1	K7(di-GI)	63	1.01E-05	K7(di-GI)	93	1.22E-11	1967.0387	3	656.3511	65.95	3
VNDSPAAKSQVVGWPPVCSYR	3	K8(di-GI); C18(Carbamidomethyl)	60	2.04E-05	K8(di-GI); C18(Carbamidomethyl)	91	8.32E-15	2431.1812	3	811.0653	52.53	3

Supplementary Note 1

Population genetic and gene expression analyses

AtGenExpress¹ (<http://jsp.weigelworld.org/AtGenExpress/resources/>), and *Arabidopsis* eFP² (<http://www.bar.utoronto.ca/>) browsers were used to retrieve and compare *A. thaliana* expression profiles for *IAA6* and *IAA19* in different natural accessions³, and developmental stages, as well as different tissues including: root cells types^{4, 5}, microgametogenesis⁶, embryo development⁷, flowers³, xylem & cork⁸, guard & mesophyll cells⁹, stem epidermis¹⁰, stigma & ovaries¹¹, pollen germination¹², shoot apical¹³, trichomes^{14, 15}.

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