Online resource legends

Online Resource 1 Neither AXL1, nor AXR1, can catalyze UBQ-ECR1 thioester formation. Shown are various in vitro thioester assays with 6HIS-HA-UBQ, or 6HIS-3HA-RUB1, and recombinant UBQ E1, 6HIS-AXR1, 6HIS-AXL1, and GST-ECR1. Anti-HA immunoblot analysis was used to detect 6HIS-3HA-RUB1-GST-ECR1 (lanes 1 and 2) and 6HIS-HA-UBQ-UBQ E1 (lane 7) thioester formation, under non-reducing (-DTT, upper panel) conditions. 6HIS-3HA-RUB1 and 6HIS-HA-UBQ reactions were performed in parallel with 6HIS-AXR1 (lanes 1 and 5), or 6HIS-AXL1 (lanes 2 and 6). As negative controls, both 6HIS-AXR1 and 6HIS-AXL1 were omitted in 6HIS-3HA-RUB1 (lane 3) or 6HIS-HA-UBQ (lane 4) thioester reactions. Without prior stripping, blots were re-probed with anti-GST to indicate position of unmodified GST-ECR1 (indicated by asterisks), running at ~72 kDa. White spaces represent positions where unnecessary lanes were removed, or where lanes were moved for alignment purposes. Size markers are in kDa.

Online Resource 2 Spectra for single peptide-based identifications from LC-MS/MS analysis of 3HA-RUB1 co-immunoprecipitations.
Peptides identified are as listed for b-ion series. Identified amino acids are in boldface type. Masses for identified b- and y- ions are labeled on the respective peaks. For clarity, some masses are listed in line with the peak, above or below the b- or y- series, respectively. Index number corresponds to Table 2. The full ion fragmentation list is included in Online Resource 3. (a-c) peptides identified from CUL1, (d) peptide identified from CUL3a, (e) peptide identified from CUL4, (f-g) peptides identified from RCE2.

Online Resource 3 Recovered fragment ions from MS/MS on single peptide-based protein identification.
Full ion fragmentation list for spectra from Online Resource 2. Index number corresponds to Table 2.

Online Resource 4 Characterization of *axr1-30* null allele.
(a) Representation of *AXR1* transcribed region, with non-coding regions as lines (UTRs are grey, introns are black) and coding regions as grey boxes. For *axr1-30* (SAIL_904_E06), two proximate T-DNA inserts were identified and are represented by white boxes (not to scale). P1 (5'- TTGAAGGTTCTATACCAGATATGACC) and P2 (5'-CAATAACTGAGACTTGTGATCAATGC) indicate positions of PCR primers used to detect *AXR1* transcripts (not to scale). (b) *axr1-30* T-DNA insertion mutants do not produce wild-type *AXR1* mRNA. PCRs for *AXR1* (30 cycles) and *UBQ10* (25 and 35 cycles) transcripts are shown. RT-PCR was done with cDNA from *axr1-30* (lanes 1 and 2) and Columbia (lanes 3 and 4) with (lanes 1 and 3 for *AXR1*, all lanes for *UBQ10*), or without (lanes 2 and 4 for *AXR1*) reverse transcriptase (RT). Size markers indicate 1000 bp. (c) *axr1-30* T-DNA insertion mutants produce normal levels of *AXL1* mRNA . PCRs for *AXL1* (40 cycles; forward primer 5'-AACACACCGCTATTGAGACAAAG, reverse primer 5'- CAAAGACTGAGACTTGTGATCAATGC) and *UBQ10* (30 cycles) transcripts are shown. RT-PCR was done with cDNA from *axr1-30* (lanes 1 and 2) and Columbia (lanes 3 and 4), with (lanes 1 and 3) or without (lanes 2 and 4) RT. Size markers indicate 1000 bp.

Online Resource 5 Confirmation of 10MYC-AXR1 and 10MYC-AXL1 function.
Four- to six-week-old tobacco (*Nicotiana benthamiana*) leaves were infiltrated in various combinations with *35S:6HIS-3HA-RUB1*, *AXR1p:10MYC-AXL1*, and *AXR1p:10MYC-AXR1* by *Agrobacterium*-mediated transformation. Three days after infiltration, equal amounts of infiltrated tissue were collected from all samples. Soluble protein was then extracted, subjected to anti-MYC immunoprecipitation, and visualized with anti-MYC and anti-HA immunoblot analysis (αMYC IP, αMYC and αHA). Immunoprecipitated 10MYC-AXR1 and 10MYC-AXL1 interacted with similar amounts of 6HIS-3HA-RUB1, suggesting that both epitope-tagged proteins are functional (αMYC IP, αMYC and αHA). Expression of 6HIS-3HA-RUB1 was confirmed by anti-HA immunoblot analysis on a fraction of the total soluble protein (Input, αHA). 6HIS-3HA-RUB1

monomer is indicated by asterisk; slow-migrating forms represent 6HIS-3HA-RUB1-modified proteins (Input, αHA). Ponceau S staining is used to gauge protein extraction efficiency (Input, ponceau). White spaces represent positions where unnecessary lanes were removed. All experiments were repeated.

Online Resource 6 Test statistics for root length comparisons of each AXR1 and AXL line to controls.

Online Resource 7 Neither *AXR1*, nor *AXL1*, transgene corrects the auxin-resistant defect characteristic *of axr1-30* in nine-day-old seedling roots.
AXR1 and AXL lines were plated on GM agar, supplemented with 0, 0.025 or 0.05 μM 2,4-D and grown for nine days with germination marked on day two. On day nine, seedlings were removed from plates, photographed, and root length was measured. Root length was log-transformed to correct for heterogeneity of variance and plotted against auxin dose for each line. Linear contrasts were then performed to compare auxin response (i.e. slope) of each transgenic line to *axr1-30* and Columbia. Student's t-tests with Bonferonni adjustment (α = 0.00385) were performed on combined data from 3 replicates. Error bars represent SE with minimally n = 41 measurements per line per dose. Whereas auxin responses for Columbia and *axr1-30* are significantly different ($p = 3.27 \times 10^{-52}$), all AXR1 and AXL lines are distinguishable from Columbia and indistinguishable from *axr1-30*, regarding response to auxin. Test statistics presented in Online Resource 8.

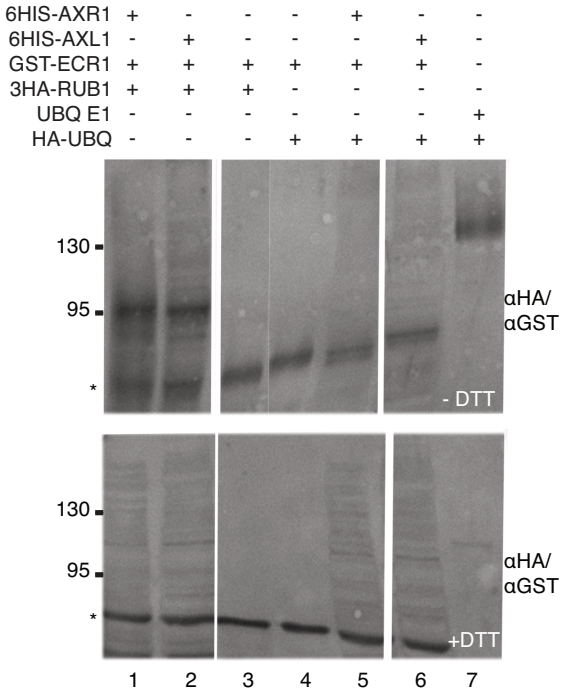
Online Resource 8 Linear contrasts to compare auxin response (i.e. slope) of each AXR1 and AXL line to controls.
Test statistics for data presented in Online Resource 7.

Online Resource 9 At day 28 post-plating, the *AXR1* transgene corrects the *axr1-30* rosette diameter defect more than *AXL1*.
AXR1 and AXL lines were grown for 28 days, then each was photographed and rosette diameter was measured. Representative pictures of AXL lines (a-d), AXR1 lines (e-f), *axr1-30* (g), and Columbia (h) are shown. Scale bar represents 1 cm.

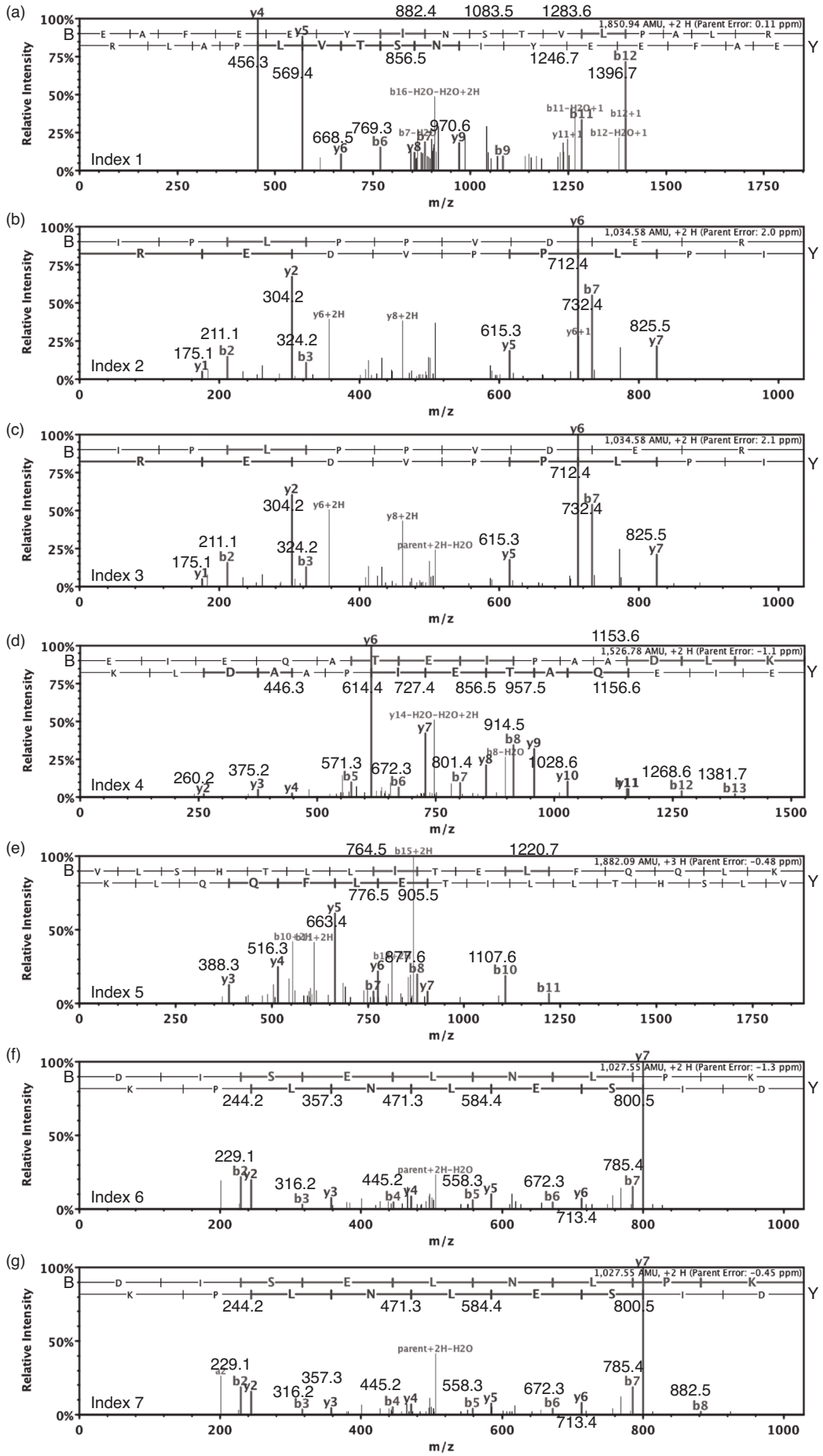
Online Resource 10 Test statistics for rosette diameter comparisons of each AXR1 and AXL line to controls.

Online Resource 11 Test statistics for inflorescence height comparisons of each AXR1 and AXL line to controls.

Online Resource 1



Online Resource 2



Online Resource 3 Recovered fragment ions from MS/MS on single peptide-based protein identification

Index1

B	B Ions	B +2H	B-NH3	B-H2O	AA	Y Ions	Y +2H	Y-NH3	Y-H2O	Y
1					E					16
2					A		862.0			15
3					F					14
4					E					13
5					E					12
6	769.3				Y	1246.7			1228.7	11
7	882.4			864.4	I					10
8					N	970.6				9
9	1083.5			1065.5	S	856.5				8
10				1166.5	T					7
11	1283.6			1265.6	V	668.5				6
12	1396.7			1378.7	L	569.4				5
13					P	456.3				4
14					A					3
15					L					2
16					R					1

Index2

B	B Ions	B +2H	B-NH3	B-H2O	AA	Y Ions	Y +2H	Y-NH3	Y-H2O	Y
1					I					9
2	211.1				P		461.8			8
3	324.2				L	825.5	413.2			7
4					P	712.4	356.7			6
5					P	615.3			597.3	5
6					V			501.2		4
7	732.4				D				401.2	3
8					E	304.2			286.2	2
9					R	175.1				1

Index3

B	B Ions	B +2H	B-NH3	B-H2O	AA	Y Ions	Y +2H	Y-NH3	Y-H2O	Y
1					I					9
2	211.1				P		461.8			8
3	324.2				L	825.5	413.2			7
4					P	712.4	356.7			6
5					P	615.3				5
6					V				500.3	4
7	732.4				D					3
8					E	304.2		287.1	286.2	2
9					R	175.1				1

Index4

B	B Ions	B +2H	B-NH3	B-H2O	AA	Y Ions	Y +2H	Y-NH3	Y-H2O	Y
1					E					14
2					I					13
3				354.2	E		643.3			12
4				482.2	Q	1156.6				11
5	571.3		554.3	553.3	A	1028.6			1010.6	10
6	672.3			654.3	T	957.5				9
7	801.4			783.4	E	856.5			838.5	8
8	914.5			896.4	I	727.4		710.4		7
9					P	614.4				6
10					A					5
11	1153.6				A	446.3				4
12	1268.6				D	375.2				3
13	1381.7				L	260.2				2
14					K					1

Index5

B	B Ions	B +2H	B-NH3	B-H2O	AA	Y Ions	Y +2H	Y-NH3	Y-H2O	Y
1					V					16
2					L					15
3					S		836.0			14
4					H					13
5					T					12
6					L					11
7	764.5				L		616.9			10
8	877.6	439.3		859.5	I		560.3			9
9		489.8			T		503.8			8
10	1107.6	554.3		1089.6	E	905.5				7
11	1220.7	610.9			L	776.5				6
12		684.4			F	663.4		646.4		5
13		748.4			Q	516.3				4
14		812.5			Q	388.3		371.2		3
15		869.0			L					2
16					K					1

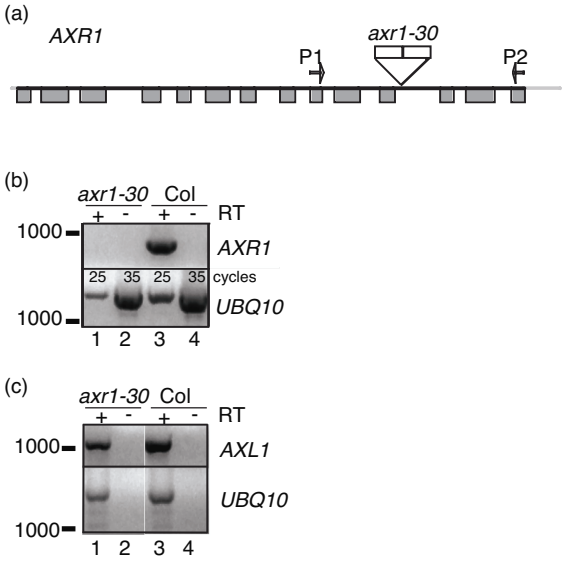
Index6

B	B Ions	B +2H	B-NH3	B-H2O	AA	Y Ions	Y +2H	Y-NH3	Y-H2O	Y
1					D					9
2	229.1				I					8
3	316.2				S	800.5	400.7	783.4		7
4	445.2			427.2	E	713.4				6
5	558.3				L	584.4				5
6	672.3				N	471.3				4
7	785.4			767.4	L	357.3				3
8					P	244.2		227.1		2
9					K					1

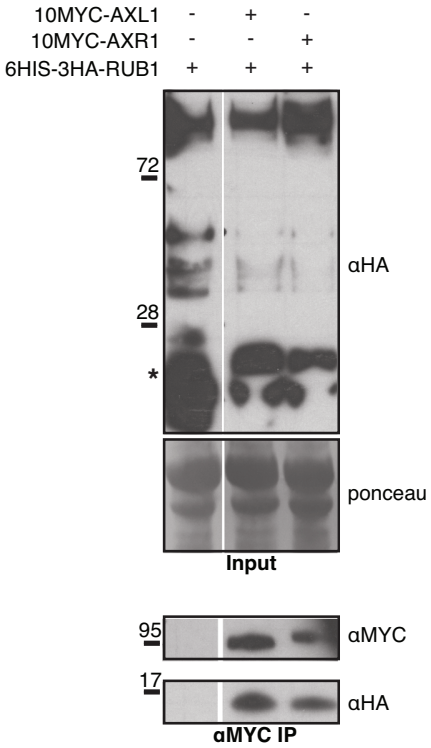
Index7

B	B Ions	B +2H	B-NH3	B-H2O	AA	Y Ions	Y +2H	Y-NH3	Y-H2O	Y
1					D					9
2	229.1				I		457.3			8
3	316.2				S	800.5	400.7		782.4	7
4	445.2			427.2	E	713.4				6
5	558.3				L	584.4				5
6	672.3			654.3	N	471.3				4
7	785.4			767.4	L	357.3				3
8	882.5				P	244.2				2
9					K					1

Online Resource 4



Online Resource 5



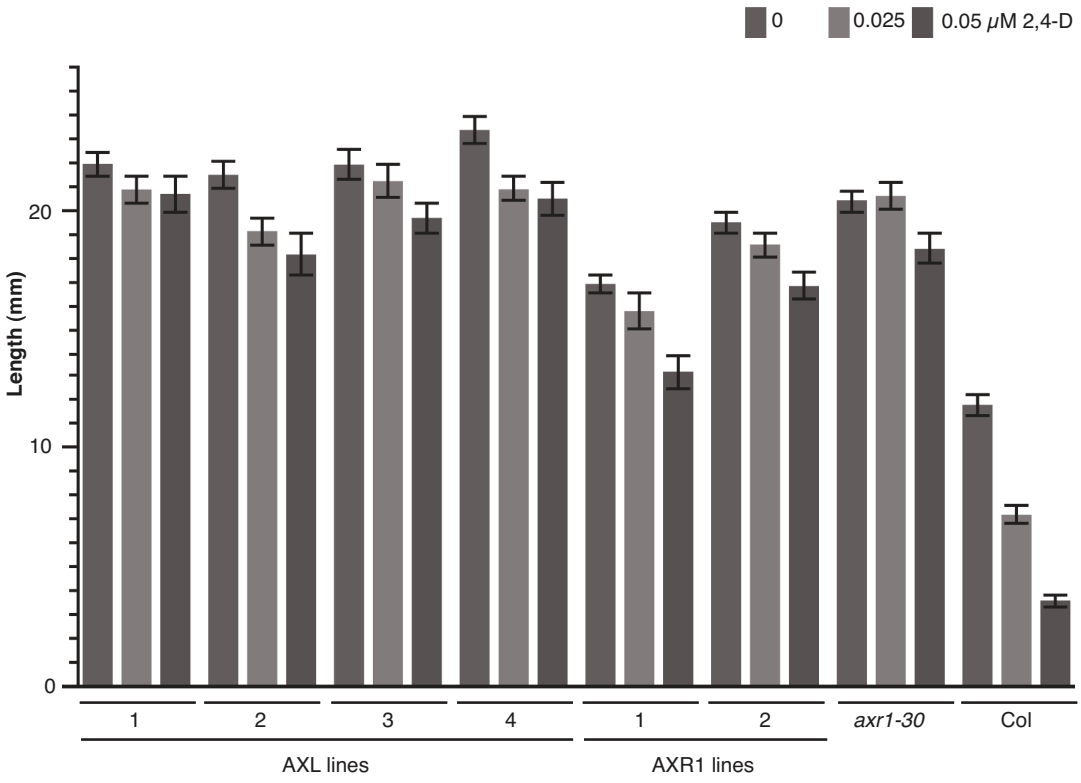
Online Resource 6 Test statistics for root length comparisons of each AXR1 and AXL line to controls

Plant Line	N	Mean Root Length (mm)	To Columbia[a]	To *axr1-30*[a]
AXL line 1	54	21.9	**<0.0001**	0.025
AXL line 2	54	21.4	**<0.0001**	0.1177
AXL line 3	54	21.8	**<0.0001**	0.0287
AXL line 4	54	23.3	**<0.0001**	**<0.0001**[b]
AXR1 line 1	54	16.8	**<0.0001**	**<0.0001**
AXR1 line 2	41	19.4	**<0.0001**	0.2101
axr1-30	60	20.3	**<0.0001**	n/a
Columbia	60	11.8	n/a	**<0.0001**

[a]p-value for Student's t-test with Bonferroni adjustment, α = 0.00385. Significance is indicated by boldface type.

[b]p-value for this line is significantly different from *axr1-30*, suggesting a potential ectopic phenotype.

Online Resource 7

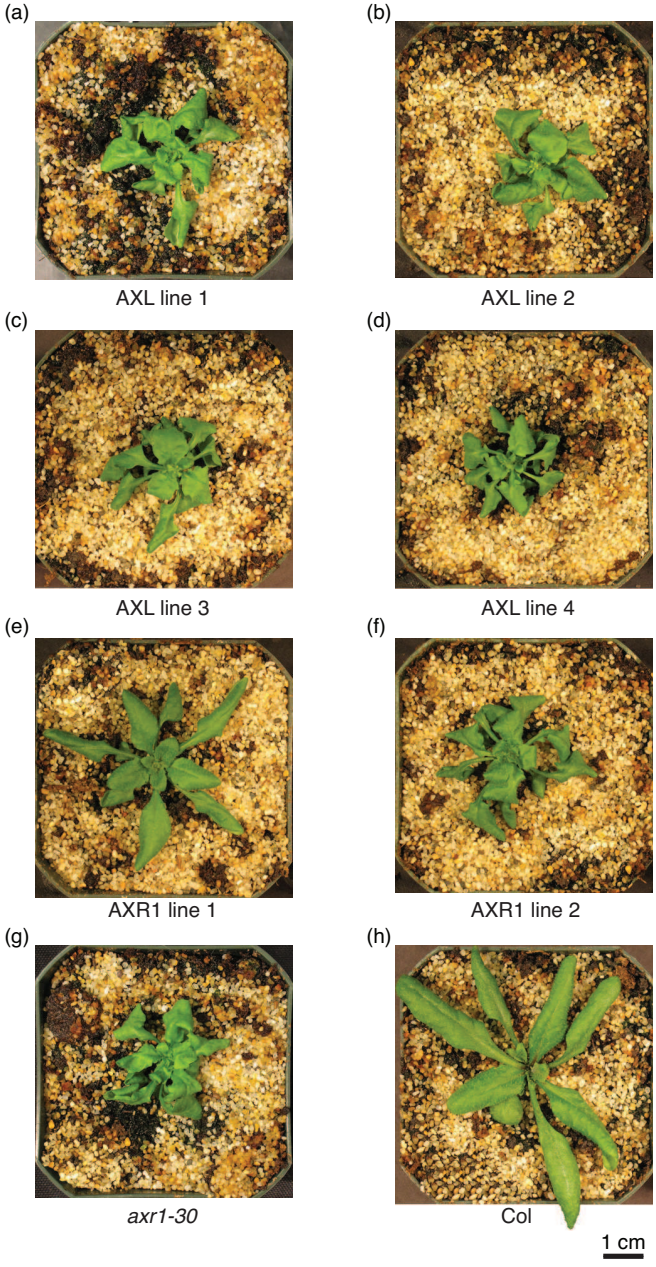


Online Resource 8 Linear contrasts to compare auxin response (i.e. slope) of each AXR1 and AXL line to controls

Plant Line	**To Columbia**[a]	**To *axr1-30***[a]
AXL line 1	**9.967×10^{-54}**	0.4987
AXL line 2	**2.0087×10^{-43}**	0.2639
AXL line 3	**7.9065×10^{-51}**	0.8600
AXL line 4	**1.2041×10^{-48}**	0.8642
AXR1 line 1	**4.6632×10^{-37}**	0.0239
AXR1 line 2	**4.6706×10^{-42}**	0.6702
axr1-30	**3.2727×10^{-52}**	n/a
Columbia	n/a	**3.2727×10^{-52}**

[a]p-value for Student's t-test with Bonferroni adjustment, $\alpha = 0.00385$. Significance is indicated by boldface type.

Online Resource 9



Online Resource 10 Test statistics for rosette diameter comparisons of each AXR1 and AXL line to controls

Plant Line	N	Mean Diameter (cm)	To Columbia[a]	To *axr1-30*[a]
AXL line 1	37	4.0	**<0.0001**	**<0.0001**
AXL line 2	35	3.6	**<0.0001**	0.2374
AXL line 3	39	3.6	**<0.0001**	0.1114
AXL line 4	29	2.9	**<0.0001**	**0.0023**[b]
AXR1 line 1	39	5.9	**<0.0001**	**<0.0001**
AXR1 line 2	36	4.0	**<0.0001**	**0.0002**
axr1-30	75	3.4	**<0.0001**	n/a
Columbia	77	7.5	n/a	**<0.0001**

[a]p-value for Student's t-test with Bonferroni adjustment, $\alpha = 0.00313$. Significance is indicated by boldface type.

[b]p-value for this line is significantly different from *axr1-30*, suggesting a potential ectopic phenotype.

Online Resource 11 Test statistics for inflorescence height comparisons of each AXR1 and AXL line to controls

Plant Line	N	Mean Height (cm)	To Columbia[a]	To *axr1-30*[a]
AXL line 1	34	29.3	**<0.0001**	**<0.0001**
AXL line 2	27	24.9	**<0.0001**	0.9374
AXL line 3	33	28.3	**<0.0001**	0.0035[b]
AXL line 4	24	24.7	**<0.0001**	0.5566
AXR1 line 1	39	45.8	**0.0014**	**<0.0001**
AXR1 line 2	35	37.3	**<0.0001**	**<0.0001**
axr1-30	72	25.2	**<0.0001**	n/a
Columbia	77	52.1	n/a	**<0.0001**

[a]p-value for Student's t-test with Bonferroni adjustment, α = 0.00313. Significance is indicated by boldface type.

[b]p-value for this line is not significantly different from *axr1-30*, but it is marginal.