A

MDCA (μM)	Radicle emergence (%)
0	99.65
50	99.32
75	98.96
100	99.39
125	97.03
150	98.02
175	97.78
200	98.10

B

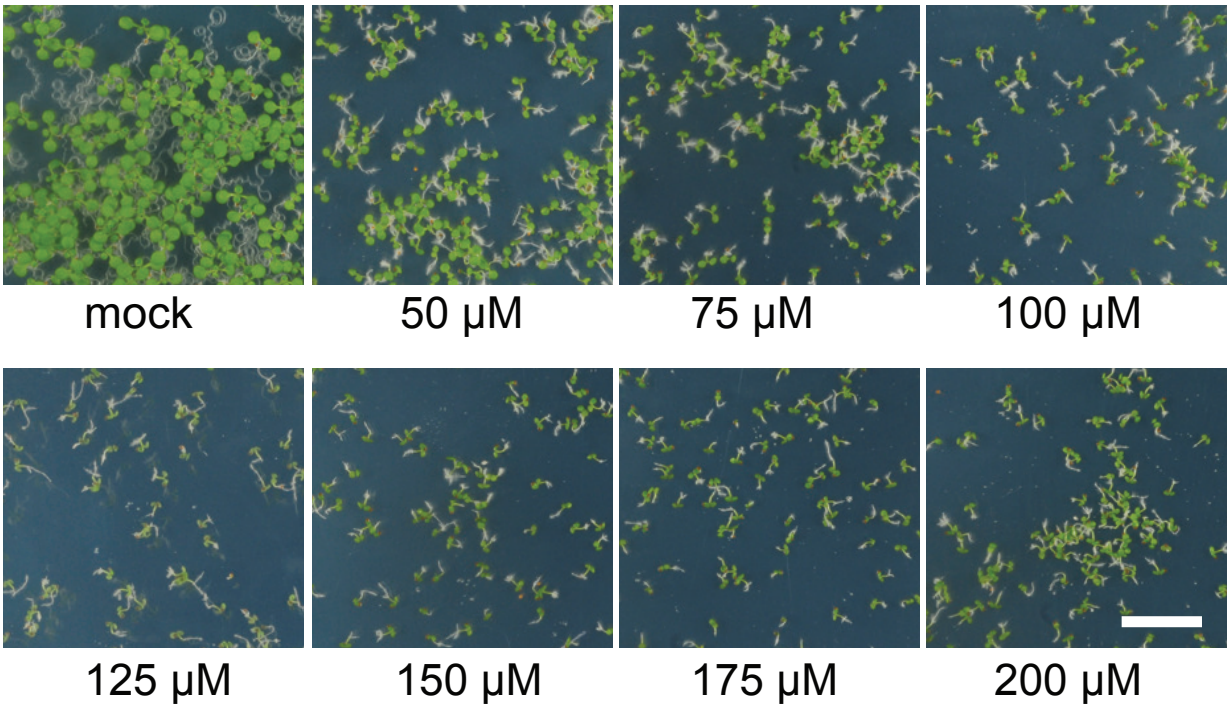


Figure S1. Effect of MDCA on germination of Arabidopsis.

(A) Radicle emergence (%) of seeds 2 DAG grown on 0.5xMS-medium supplemented with different concentrations of MDCA (n>250) (B) Phenotype of seedlings (12 DAG) grown on 0.5xMS-medium supplemented with different concentrations of MDCA (n>250) (scale bar: 0.5 cm).

Compound Top-10 UP	Retention time (min)	m/z	mock		10 µM MDCA		Fold change MDCA/mock
			Average	St. Dev.	Average	St. Dev.	
1. Cinnamoyl aspartate 1	10.21	262.07	0	0	168011	30020	UP
2. MDCA glutamate 1	8.93	320.07	0	0	147087	27639	UP
3. MDCA aspartate 1	8.30	306.06	82	49	130729	34993	1596.7
4. Cinnamoyl aspartate 2	7.49	262.07	1	2	76376	17493	99852.9
5. MDCA aspartate 2	10.09	306.06	1258	806	64036	18981	50.9
6. MDCA glutamate 2	10.76	320.07	0	0	49309	7942	UP
7. *Unknown*	7.17	319.09	0	0	28905	10706	UP
8. Cinnamoyl glutamate	10.96	276.08	0	0	27728	3120	UP
9. Cinnamoyl aspartate 1 (fragment)	10.22	218.08	0	0	27331	5474	UP
10. MDCA aspartate 2 (heterodimer)	8.32	900.23	0	0	23111	5081	UP
Compound Top-10 DOWN							
1. 4-methylthio-*N*-butyl-glucosinolate	3.43	420.04	298229	191900	24633	22996	0.0826
2. 1-methoxyindol-3-ylmethyl-glucosinolate	8.01	955.15	28706	17101	2325	3604	0.0810
3. G(8-*O*-4)ferulic acid + hexose + hexose	3.10	713.23	23577	4733	1323	331	0.0561
4. G(8-*O*-4)ferulic acid + hexose + hexose	3.22	713.23	21304	4272	1202	218	0.0564
5. 8-methylsulfinyloctyl-glucosinolate	12.92	492.10	20774	12097	845	1160	0.0407
6. Kaempferol + hexose + deoxyhexose	10.00	593.15	18628	3670	388	265	0.0208
7. 5-methylthio-*N*-pentyl-glucosinolate	5.44	434.06	17369	11795	1188	1413	0.0684
8. *Unknown*	2.17	338.08	12712	2986	575	259	0.0453
9. Quercetin + hexose + deoxyhexose	8.63	609.15	11396	2670	350	353	0.0307
10. 8-methylthio-*N*-octyl-glucosinolate (dimer)	14.10	953.24	10038	6169	184	375	0.0184

Figure S2. Metabolites with an altered abundance in 10 µM MDCA-treated Arabidopsis seedlings.

An overview of the top-10 significantly differential compounds with at least a 10-fold increase (UP) or decrease (DOWN) in 10 µM MDCA-treated seedlings as compared to mock-treated seedlings (n=6). The top-10 UP defined as those compounds with the highest normalized peak area in MDCA-treated seedlings, whereas the top-10 DOWN were defined as those compounds with the highest normalized peak area in mock-treated seedlings. The retention time is expressed in minutes. For each compound, normalized average peak areas (unitless) of mock- and 10 µM MDCA-treated seedlings are given. Peak areas are normalized relative to the dry weight of the pellet remaining after methanol extraction. The term UP implies that a peak could only be detected in 10 µM MDCA-treated seedlings and not in mock-treated seedlings.

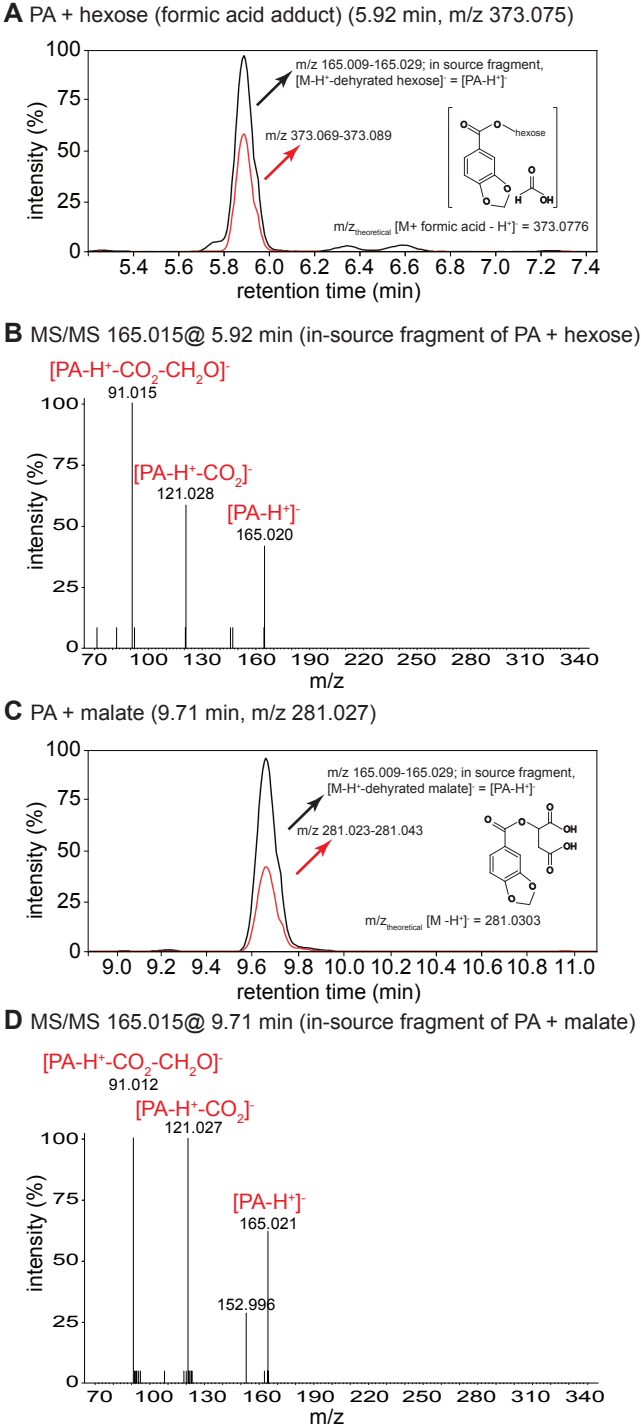


Figure S3. UHPLC-MS based detection of PA + hexose and PA + malate in MDCA treated Arabidopsis seedlings.

Structural identification of the compounds was done via accurate m/z match and MS/MS fragmentation spectra. (A) PA + hexose was detected at 5.92 min as formate adduct and as an in-source fragment. (B) The MS/MS of the in-source fragment of PA + hexose with m/z 165.015 eluting at 5.92 min showed characteristic fragmentation pattern of PA. (C) PA + malate was detected at 9.71 min as molecular ion and as an in-source fragment. (D) The MS/MS of the in-source fragment of PA + malate with m/z 165.015 eluting at 9.71 min showed characteristic fragmentation pattern of PA.

Compound	Retention time (min)	m/z	mock		10 µM MDCA		Fold change MDCA/mock
			Average	St. Dev.	Average	St. Dev.	
Cinnamic acid derivatives							
Cinnamoyl hexose (formic acid adduct)	9.83	355.10	0	0	10194	961	UP
Cinnamoyl malate 1	12.66	147.04	0	0	905	222	UP
Cinnamoyl malate 2	14.05	147.04	1	2	813	197	1018.7***
***p*-Coumaric acid derivatives**							
p-Coumaric acid 4-*O*-hexoside	3.02	325.09	138	63	204	91	1.4713
p-Coumaroyl hexose 1	4.67	325.09	5191	1536	2969	1543	0.5720*
p-Coumaroyl hexose 2	5.24	325.09	9094	1944	1379	177	0.1516***
Dihydro-*p*-coumaric acid + hexose	4.03	327.10	2437	617	1156	155	0.4744***
Caffeic acid derivatives							
Caffeic acid 1	4.90	179.03	0	0	0	0	0
Caffeic acid 2	5.80	179.03	0	0	0	0	0
Caffeic acid 3/4-*O*-hexoside 3	5.64	341.09	778	144	471	94	0.6058***
Caffeoyl hexose 1	3.45	341.09	895	680	1319	292	1.4732
Caffeoyl hexose 3/4-*O*-hexoside 1	3.12	503.14	115	69	519	124	4.5233***
Caffeoyl malate 1	5.65	295.05	0	0	0	0	0
Caffeoyl malate 2	5.91	295.05	0	0	0	0	0

Compound	Retention time (min)	m/z	mock		10 µM MDCA		Fold change MDCA/mock
			Average	St. Dev.	Average	St. Dev.	
Ferulic acid derivatives							
Ferulic acid	9.70	193.05	0	0	0	0	0
Feruloyl hexose 1	5.49	355.10	1887	284	950	271	0.5034***
Feruloyl hexose 2	6.00	355.10	161	68	10	19	0.0608***
Ferulic acid *O*-4-hexoside	3.85	355.10	1023	288	177	70	0.1733***
Feruloyl malate 1	9.06	309.06	122	126	26	41	0.2158
Feruloyl malate 2	8.50	309.06	0	0	0	0	0
Sinapic acid derivatives							
Sinapic acid	8.41	223.06	383	180	221	152	0.5772
Sinapoyl hexose 1	5.68	385.11	113076	28191	127022	27289	1.1233
Sinapoyl hexose 2	6.24	385.11	29797	7438	37333	8502	1.2529
Disinapoyl hexose 1	15.45	591.17	5457	1221	5627	1550	1.0311
Disinapoyl hexose 2	12.00	591.17	50334	10779	86872	20067	1.7259**
Sinapoyl malate 1	9.21	339.07	96825	11845	106291	20152	1.0978
Sinapoyl malate 2	9.50	339.07	48218	5230	47620	7285	0.9876
Varia							
Coniferin (formic acid adduct)	3.96	387.13	78987	15739	37617	4911	0.4762***
Scopolin (formic acid adduct)	4.62	399.09	30358	8264	7413	2109	0.2442***

Compound	Retention time (min)	m/z	mock		10 µM MDCA		Fold change MDCA/mock
			Average	St. Dev.	Average	St. Dev.	
Coniferyl alcohol - ferulic acid conjugates							
G(8-*O*-4) ferulic acid	8.96	389.12	2262	472	2060	569	0.9106
G(8-*O*-4) ferulic acid + hexose 1	6.09	551.18	1540	361	1345	265	0.8729
G(8-*O*-4) ferulic acid + hexose 2	6.21	551.18	1350	205	335	121	0.2479***
G(8-*O*-4) ferulic acid + hexose 3	6.59	551.18	13461	2904	23014	2409	1.7098***
G(8-*O*-4) ferulic acid + hexose 4	6.86	551.18	3802	965	3080	366	0.8101
G(8-*O*-4) ferulic acid + hexose 5	7.14	551.18	3313	670	3432	639	1.0359
G(8-*O*-4) ferulic acid + hexose 6	7.49	551.18	3421	602	760	201	0.2221***
G(8-*O*-4) ferulic acid + malate 1	9.58	505.14	2578	304	1593	429	0.6178**
G(8-*O*-4) ferulic acid + malate 2	9.83	505.13	1967	309	1328	433	0.6748*
G(8-*O*-4) ferulic acid + malate 3	10.43	505.13	1084	234	247	163	0.2275***
G(8-*O*-4) ferulic acid + malate 4	10.63	505.13	1497	209	575	187	0.3841***
G(8-*O*-4) ferulic acid + 224 Da 1	11.95	775.25	3434	581	154	88	0.0448***
G(8-*O*-4) ferulic acid + 224 Da 2	12.11	775.25	2661	519	144	92	0.0542***
G(8-5) feruloyl hexose 1	9.79	533.17	1229	302	100	96	0.0814***
G(8-5) feruloyl hexose 2	10.32	533.17	4024	731	986	255	0.2451***
G(8-5) feruloyl hexose 3	11.11	533.17	558	182	232	87	0.4154*
G(8-5) feruloyl malate	14.01	487.12	2865	640	1401	358	0.4891**
G(8-5) feruloyl malate *O*-4-hexoside 1	10.36	649.18	3118	310	995	341	0.3190***
G(8-5) feruloyl malate *O*-4-hexoside 2	9.99	649.17	119	46	65	61	0.5487

Compound	Retention time (min)	m/z	mock		10 µM MDCA		Fold change MDCA/mock
			Average	St. Dev.	Average	St. Dev.	
Hexosylated oligolignols							
G(8-5)G + hexose	9.36	519.19	2618	514	361	158	0.1380***
G 4-*O*-hexoside(8-5)G	10.94	519.19	2914	430	529	101	0.1814***
G(red8-5)G + hexose	8.41	521.20	4768	616	1015	227	0.2128***
G(red8-8)G 8/4-*O*-hexoside	9.26	521.20	1779	362	96	44	0.0542***
G(8-8)G hexoside 1	10.60	519.19	5654	886	2304	290	0.4075***
G(8-8)G hexoside 2	10.73	519.19	1620	326	3	7	0.0017***
G(8-8)G + hexose + hexose	7.26	681.24	2613	429	2640	487	1.0102
G(8-*O*-4)G(red8-5)G + hexose 1	10.20	717.28	2550	317	0	0	0.0000
G(8-*O*-4)G(red8-5)G + hexose 2	9.90	717.28	2916	263	225	109	0.0772***
Flavonol glycosides							
Isorhamnetin + hexose + deoxyhexose	7.11	623.16	1113	280	44	49	0.0394***
Isorhamnetin-3-*O*-rhamnoside-7-*O*-glucoside	8.68	623.16	38053	5356	16368	3157	0.4301***
Isorhamnetin + deoxyhexose + deoxyhexose	10.00	607.17	1546	309	1248	406	0.8070
Isorhamnetin + hexose + deoxyhexose	10.37	623.16	2872	792	11	26	0.0037***

Compound	Retention time (min)	m/z	mock		10 µM MDCA		Fold change MDCA/mock
			Average	St. Dev.	Average	St. Dev.	
Flavonol glycosides							
Kaempferol-3-*O*-rhamnosyl(1-->2)-glucoside -7-*O*-rhamnoside	6.40	739.21	87791	19898	90604	19866	1.0320
Kaempferol + deoxyhexose + deoxyhexose	6.72	593.15	11145	2022	9680	2755	0.8685
Kaempferol + hexose + hexose+ deoxyhexose	7.78	755.21	1447	313	2016	467	1.3932*
Kaempferol-3-*O*-rhamnoside-7-*O*-glucoside	8.35	593.15	92912	14139	65835	16130	0.7086**
Kaempferol + hexose + deoxyhexose + deoxyhexose	8.93	739.21	1038	130	389	323	0.3747**
Kaempferol-3-*O*-rhamnoside-7-*O*-rhamnoside	9.65	577.16	84357	10126	90086	20830	1.0679
Kaempferol + hexose + deoxyhexose	10.01	593.15	18628	3670	388	265	0.0208***
Kaempferol + hexose	10.41	447.09	9313	2054	360	699	0.0386***
Kaempferol + deoxyhexose	12.20	431.10	1848	351	62	83	0.0338***
Kaempferol + deoxyhexose	14.97	431.10	3917	721	1927	966	0.4919
Quercetin + hexose + deoxyhexose	5.65	609.15	4509	947	1805	940	0.4003**
Quercetin-3-*O*-rhamnosyl(1-->2)-glucoside -7-*O*-rhamnoside	7.72	755.21	23472	3643	14762	3284	0.6289**
Quercetin + hexose + hexose+ deoxyhexose	6.54	771.20	3301	602	226	142	0.0683***
Quercetin + hexose + deoxyhexose + deoxyhexose	7.17	755.21	9702	1770	4929	1274	0.5080**
Quercetin-3-*O*-rhamnoside-7-*O*-glucoside	7.23	609.15	64027	13075	43735	8181	0.6831*
Quercetin + hexose + deoxyhexose	8.63	609.15	11396	2670	350	353	0.0307***
Quercetin + deoxyhexose	12.72	447.09	2578	666	194	226	0.0754***

Figure S4. Metabolites with an altered abundance in 10 µM MDCA-treated Arabidopsis seedlings.

A targeted approach was used to investigate which of the identified compounds are altered in 10 µM MDCA-treated seedlings in comparison with mock-treated seedlings (n=6). For each compound, normalized average peak areas (unitless) of mock- and 10 µM MDCA-treated seedlings are given. Peak areas are normalized relative to the dry weight of the pellet remaining after methanol extraction. The retention time is expressed in minutes. Asterisks represent significant differences between 10 µM MDCA-treated and mock-treated plants as determined by Dunnett's test. Dunnett's test P-values: *$0.001 \leq P < 0.05$, **$0.0001 \leq P < 0.001$, *** $P < 0.0001$. The term UP implies that the compound could only be detected in 10 µM MDCA-treated seedlings and not in mock-treated seedlings.

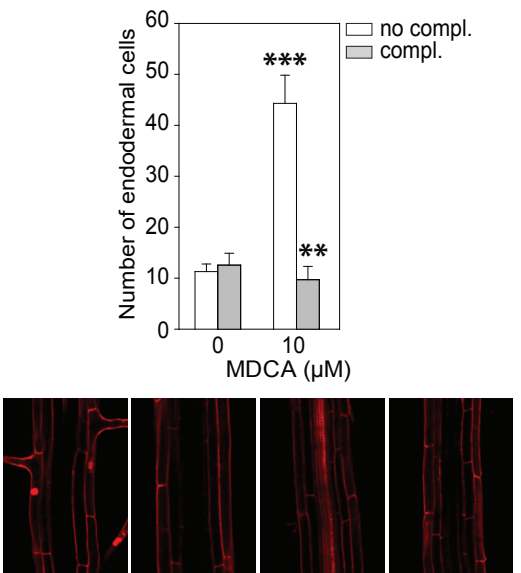


Figure S5. The impact of the MDCA-induced lignin reduction on the plant phenotype.

Visualization of the effect of MDCA treatment on the Casparian strip formation (white; no compl.) in Arabidopsis seedlings 5 DAG and complementation by exogenous application of two monolignols (grey; compl.): 50 µM of each coniferyl alcohol and sinapyl alcohol, which allows for the formation of a functional Casparian strip (n=10). See manuscript for additional explanation on this experiment. Error bars represent standard deviations and asterisks were used to indicate statistically significant differences compared to the corresponding mock-treated control sample as determined by Dunnett's test P-values: *P < 0.05, **P < 0.001, *** P <0.0001. (B)

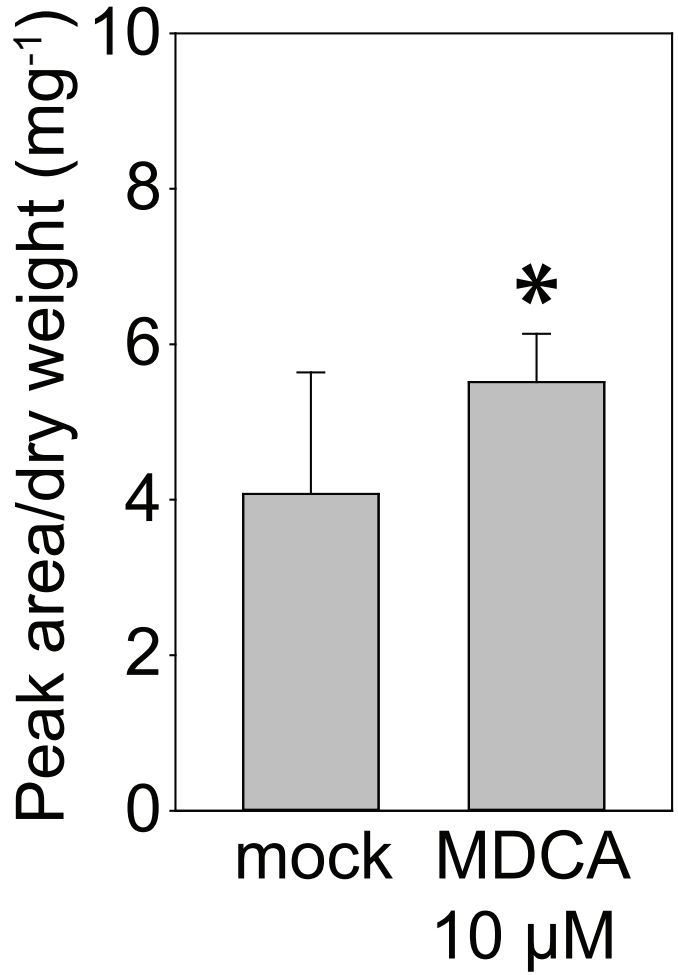
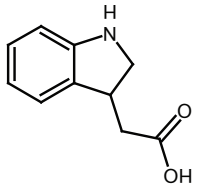


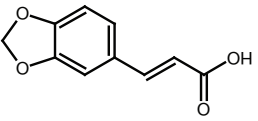
Figure S6. Detection of salicylic acid in 10 µM MDCA-treated Arabidopsis seedlings.

The average peak area of salicylic acid (SA) in mock- and 10 µM MDCA-treated seedlings 12 DAG (n=10). The unitless peak areas were normalized to the dry weight of the pellet remaining after methanol extraction (in mg). Error bars represent standard deviations. The asterisk represents significant difference in SA levels between 10 µM MDCA-treated and mock-treated plants ($0.01 < P\text{-value} < 0.05$) as determined by Dunnett's test.

A

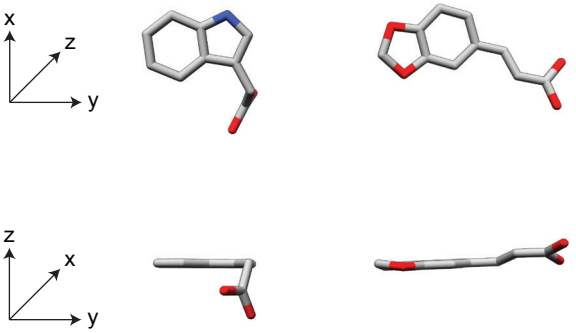


IAA



MDCA

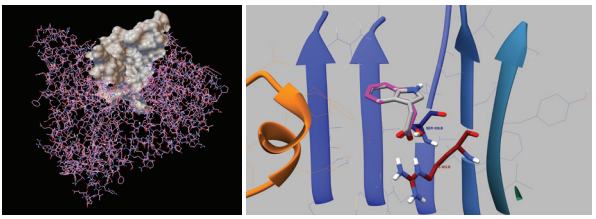
B



C

Physiochemical Properties	IAA	MDCA
MM	175.186	192.17
pKa	4.66	3.55
cLogP([octanol]/[water]) partition	1.1822	1.5853
#H-acceptors	3	4
#H-donors	2	1
Total surface area	166	168
Polar surface area	53.09	55.76
Rotable bonds	2	2
Aromatic rings	2	1

D



E

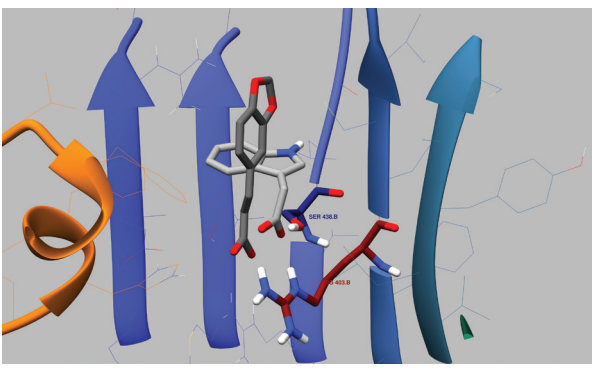


Figure S7. Chemical and physical characteristics and docking of MDCA for TIR1.

(A) The molecular structure of IAA and MDCA. (B) Top view of energy minimized structures represented as sticks with bicyclic ring structures planar. The IAA carboxylic acid group does not position in the plane of the aromatic ring, whilst for MDCA, the carboxylic acid is fixed planar to the ring due to the trans-alkene bond. (C) Physiochemical properties of IAA and MDCA. (D) The binding pocket of TIR1 shown as a surface representation in relation to the whole structure whereby the binding region is defined as an 18Å x 18Å x 18Å box. Docking of IAA from the crystal structure (purple) with that of the docked result (grey) showed almost identical and superimposable results. (E) The best possible pose for MDCA in the lower region of the TIR1 pocket.

	mock	MDCA 5 µM	MDCA 10 µM
Indole-3-actetic acid (IAA)	15.5 (2.50)	20.60 (2.30) *	27.80 (2.60) ***
Precursors			
Anthranilate	26.50 (6.4)	36.50 (7.80)	38.10 (9.30)
Tryptophan	2170.90 (508.90)	2202.90 (389.10)	3038.50 (648.90)
Tryptamine	0.18 (0.05)	0.28 (0.07) *	0.30 (0.08) *
Indole-3-acetamide	1.13 (0.26)	1.86 (0.37) **	2.71 (0.61) ***
Indole-3-acetonitrile	388.30 (79.50)	592.70 (126.70) *	1009.80 (189.10) ***
Indole-3-acetaldoxime	12.50 (2.40)	16.90 (3.00) *	20.20 (3.40) **
Indole-3-acetaldehyde	95.30 (24.60)	126.00 (20.80)	160.00 (43.10) *
Indole-3-pyruvic acid	129.50 (15.30)	121.50 (22.60)	149.00 (39.00)
Conjugates, catabolites			
2-oxindole-3-acetic acid	109.17 (26.10)	113.80 (20.22)	149.36 (26.82) *
IAA-glutamate	0.94 (0.16)	1.66 (0.36) **	2.24 (0.28) ***
IAA-aspartate	0.85 (0.20)	1.70 (0.37) **	2.66 (0.62) ***

Figure S8. Concentration of auxin and auxin metabolites after MDCA treatment of Arabidopsis seedlings.

The concentration of free IAA, IAA-precursors, IAA-amino acid conjugates and catabolites in seedlings (12 DAG) grown on 0.5xMS-medium supplemented with MDCA (5 µM and 10 µM). Each biological replicate represents ten seedlings that were pooled and analyzed (n=6). Standard deviations are mentioned inbetween brackets and asterisks represent statistically significant differences between MDCA-treated and mock-treated plants as determined by Dunnett's test. P-values: *P < 0.05, **P < 0.001, *** P <0.0001.

	WT	*ref3-2* mutant
Indole-3-actetic acid (IAA)	9.1 (0.7)	13.3 (1.9) ***
Precursors		
Anthranilate	26.8 (7.4)	15.6 (2.6) **
Tryptophan	5251.3 (1083.3)	4300.4 (1037.8)
Tryptamine	1.0 (0.3)	1.1 (0.2)
Indole-3-acetamide	1.2 (0.3)	2.2 (0.5) **
Indole-3-acetonitrile	333.1 (117.8)	578.2 (71.1) **
Indole-3-acetaldoxime	7.5 (1.4)	4.0 (0.9) ***
Indole-3-acetaldehyde	69.8 (10.7)	126.7 (42.7) *
Indole-3-pyruvic acid	218 (42)	344 (87) **
Conjugates, catabolites		
2-oxindole-3-acetic acid	65.2 (15.6)	93.4 (20.0) *
IAA-glutamate	2.5 (0.6)	9.5 (2.1) ***
IAA-aspartate	6.5 (1.8)	72.8 (13.9) ***

Figure S9. Concentration of auxin and auxin metabolites in the *c4h* mutant *ref3-2*.

The concentration of free IAA, IAA-precursors, IAA-amino acid conjugates and catabolites in the leaves of 2 months old *ref3-2* plants. Each biological replicate represents one plant (n=6). Standard deviations are mentioned inbetween brackets and asterisks represent statistically significant differences between wild type (WT) and *c4h* mutant *ref3-2* plants as determined by Dunnett's test. P-values: *P < 0.05, **P < 0.001, *** P <0.0001.

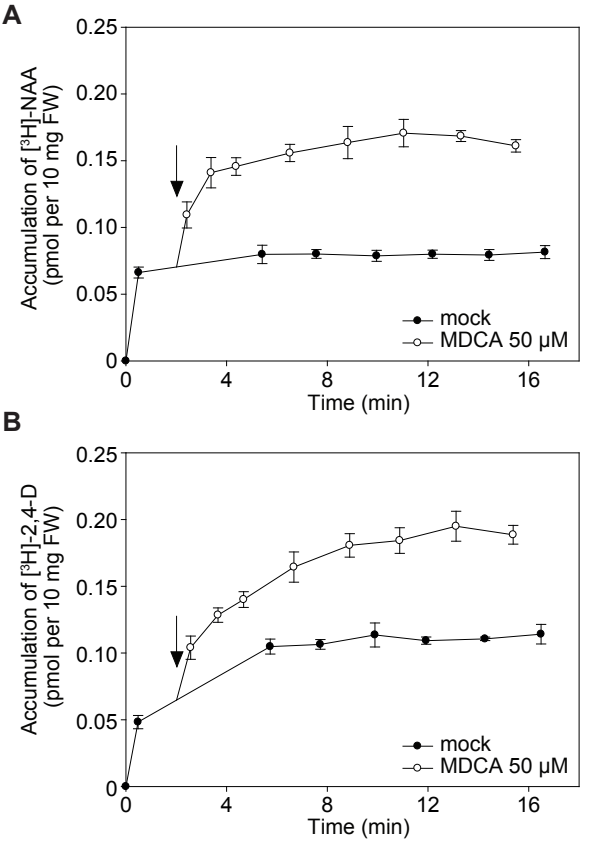


Figure S10. The impact of MDCA on auxin transport in suspension-grown Arabidopsis T87 cells.

(A-B) Effect of MDCA on the net accumulation of (A) [^{3}H]-NAA or (B) [^{3}H]-2,4-D in four-day old suspension-grown Arabidopsis cells (20 minute uptake period). Arrows point at time of application of MDCA. Error bars in (A-B) represent standard deviations (n=4).