The following Supplementary Material is available for this article:

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Tables

Table S1-1. List of qPCR primers used in this study.

Name	Sequence 5′-3′	Amplicon size, bp
CsEF1a FOR	ATGGGTAAGGAGAAGGTTCACATTAACATT	241
CsEF1a REV	CGAACTTCCACAAAGCAATATCAATT	
CsRALF34 FOR	CGTAGGGAAGGAGTGAAGAGGTGG	160
CsRALF34 REV	TGGATGAGGGAAGTGGTGGTGG	
CsGATA14 FOR	TTCCTTCAAGACCAAACCCCTGAT	280
CsGATA14 REV	CCTCCACCAGTTGTTCCTCCGAG	
CsGATA24 FOR	GAAGAAAATGGGAGGATCAGGAGG	164
CsGATA24 REV	ATAAAGCCATAAGAAGCAACGCTGC	
CsE2Fa FOR	AAGCCATCGGAGCCACTGC	186
CsE2Fa REV	GAGAGGTTTAACAACAATGGTATCAGG	
CsE2Fa-like FOR	GTCCATGCCGTTACGATAGTTCTCT	163
CsE2Fa-like REV	CCAATTCCCTCTAGGACATTTGTTATATC	
CsE2Fc FOR	GATCTCATCTTCCGCCACCG	141
CsE2Fc REV	CCGATGATACTGAAGACAACACTGAG	
CsE2Fe FOR	CCCTTGATGAAGCTGCTAAGCTCT	171
CsE2Fe REV	TCACTCCCAACCACCTAAACGC	
CsE2Fe-like FOR	GGACTCTTCTTCAAACGCTTACAGC	192
CsE2Fe-like REV	CTACACAGAATACCAACACTTTCTAAAACG	
CsDP FOR	GTTGCAGATGAACTTGTCGCAGA	177
CsDP REV	GCAGACCCTTCCATTGTATCTCCT	

Table S1-2. List of primers used for PCR in this study.

Name	Restriction enzyme	Sequence 5`-3`
CDS_CsRALF34_FOR	-	TTGAAAACCGACACTAAAAAACAAGAA
CDS_CsRALF34_REV	-	ATATAAAATAAGGAAATCCCCAAAACTACA
CDS_CsRALF34_FOR1	KpnI	AAAGGTACCATGGCTTCCAAATCCCTCCTCTT
CDS_CsRALF34_REV1	NotI	AAGCGGCCGCTCAGCGGCGGCAGCGA
gusA_FOR	KpnI	AAAGGTACCATGTTACGTCCTGTAGAAACCCCAAC
gusA_REV	NotI	AAGCGGCCGCTCATTGTTTGCCTCCCTGCTG
p35S_FOR	-	GCCGCCTAGAGCCAAGCTGA
TermAct_REV	-	CTCAAGCGAAATGGTGCGATCT

Restriction enzyme/att sites in adaptors are underlined and given in **bold** style.

Table S1-3. Combination of primers, used for different cloning steps.

Combination of primers	Application
CDS_CsRALF34_FOR/ CDS_CsRALF34_REV	PCR amplification of *CsRALF34* coding sequence using cucumber genomic DNA as a template for subsequent cloning to pJET1.2
CDS_CsRALF34_FOR1/ CDS_CsRALF34_REV1	PCR amplification of *CsRALF34* coding sequence for subsequent *Kpn*I-*Not*I cloning to pUC18-entry8
gusA_FOR/gusA_REV	PCR amplification of *gusA* coding sequence for subsequent *Kpn*I-*Not*I cloning to pUC18-entry8
p35S_FOR/ TermAct_REV	*p35S::CsRALF34-TermAct* and *p35S::gusA-TermAct* inserts verification by PCR in pKGW-RR-MGW vector

Table S1-4. Thermally stabile primary metabolites annotated by spectral similarity search and/or co-elution with authentic standards in *Cucumis sativus* root aqueous methanolic extracts from the roots with overexpression of *CsRALF34* or from roots of control group. Metabolite analysis relied on GC-EI-Q-MS after derivatization of the lyophilized extracts with methoxyamine hydrochloride (MOA) and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA).

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
1	Carbodiimide	2TMS	6.16	977.9	171
2	Ethoxyamine	2TMS	6.36	979.9	119
3	Boric acid	3TMS	6.5	988.8	221
4	Ethylene glycol	2TMS	6.63	997.2	191
5	Glyoxylic acid	2TMS	6.82	1007.1	117
6	1,3-Dimethoxybenzene	-	6.88	1009.5	140
7	4-Hydroxypyridine	1TMS	7.64	1043.4	152
8	RI_1057_Unknown	-	7.95	1057.6	234
9	Lactic acid	2TMS	8.12	1064.9	117
10	Glycolic acid_peak1	2TMS	8.42	1078.4	177
11	Glycolic acid_peak2	2TMS	8.52	1082.8	177
12	Glycine	-	8.68	1091.6	106
13	Valine_peak1	1TMS	8.85	1097.4	72
14	Glycine_peak1	3TMS	8.89	1099.6	116
15	1-Octanol	1TMS	9	1104.5	187
16	*L*-Alanine_peak1	3TMS	9.01	1105.1	116
17	Valine	1TMS	9.01	1111.81	72
18	*L*-Alanine_peak2	2TMS	9.09	1108.8	116
19	Hydroxylamine	3TMS	9.15	1111.9	249
20	Oxalic acid_peak1	2TMS	9.89	1148.5	133
21	Oxalic acid_peak2	2TMS	9.95	1151.4	220
22	β-Lactate	2TMS	10.06	1156.6	177
23	RI_1169_Unknown	1TMS	10.31	1168.9	89
24	*L*-Proline_peak1	TMS	10.78	1191.9	70
25	Methyl-phosphate	2TMS	10.82	1193.8	241
26	Isoleucine_peak1	1TMS	10.83	1194	86
27	Malonic acid_peak 1	2TMS	11.33	1218.5	130
28	Malonic acid_peak 2	2TMS	11.43	1223.5	174
29	*DL*-Glyceraldehyde	2TMS, MEOX	11.43	1224	133
30	Valine_peak2	1TMS	11.56	1230.3	144
31	*DL*-Norvaline	2TMS	11.64	1234.06	144
32	*DL*-Glyceraldehyde	2TMS, MEOX	11.68	1235.9	133
33	unknown_RI_1240	-	11.83	1243.49	156
34	Diethylene glycol	2TMS	11.84	1244.1	117
35	Urea	2TMS	12.37	1269.8	189
36	Benzoic acid	1TMS	12.42	1272.6	179

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
37	Serine	2TMS	12.6	1281.2	116
38	Ethanamine	3TMS	12.67	1284.5	174
39	Octanoic acid	2TMS	12.84	1292.1	201
40	Glycerol	3TMS	12.87	1294.2	205
41	*L*-Leucine	2TMS	12.87	1294.3	158
42	Phosphate	3TMS	12.88	1294.7	299
43	*L*-Isoleucine_peak2	2TMS	13.3	1311.2	158
44	*L*-Proline_peak2	2TMS	13.38	1313.7	142
45	*L*-Threonine_peak1	2TMS	13.43	1315.7	219
46	Nicotinic acid	TMS	13.55	1319.9	180
47	Glycine_peak2	2TMS	13.58	1320.8	248
48	Maleic acid	2TMS	13.68	1324.3	245
49	Succinic acid_peak 1	2TMS	13.7	1325	247
50	Butenedioic acid	-	13.82	1329.3	101
51	Succinic acid_peak 2	2TMS	13.91	1332.1	247
52	RI_1336_unknown	-	14.05	1337.1	57
53	*D*-Glyceric acid	3TMS	14.18	1341.6	189
54	Uracil	2TMS	14.41	1349.9	241
55	Citraconic acid	2TMS	14.66	1358.6	245
56	RI_1361_Unknown	-	14.73	1361	188
57	Fumaric acid	2TMS	14.75	1361.5	245
58	RI_1364_unknown	-	14.81	1363.4	188
59	*L*-Homoserine	2TMS	14.85	1365.1	146
60	α-methyl-serine_peak1	3TMS	14.85	1366.8	116
61	Nonanoic acid	1TMS	14.97	1369.2	215
62	3-Aminopropionitrile	2TMS	15	1361.3	245
63	Propanedioic acid	-	15.42	1385	131
64	1,1,1-Tris(hydroxymethyl)propane	3TMS	15.48	1386.1	191
65	*L*-Threonine_peak2	3TMS	15.48	1387.1	219
66	α-methyl-serine_peak2	3TMS	15.6	1391.8	116
67	Homocysteine	3TMS	15.88	1401.2	234
68	Glutaric acid (Pentanedioic acid)_1	2TMS	15.95	1403.9	261
69	3-Deoxytetronic acid	3TMS	16.14	1410.7	219
70	*L*-Methionine	TMS	16.2	1410.9	131
71	β-Alanine	3TMS	16.29	1416.1	248
72	*L*-Aspartic acid_peak 1	2TMS	16.32	1417.2	116
73	*L*-Homoserine	3TMS	16.75	1433.3	218
74	Erythrose_peak 1	3TMS, MEOX	16.99	1442.1	205
75	Decanoic acid	1TMS	17.09	1445.9	229
76	Erythrose_peak 2	3TMS, MEOX	17.17	1448.5	205
77	*D*(-)-Erythrulose_peak1	1MEOX_3TMS	17.21	1450.3	173
78	*D*(-)-Erythrulose_peak2	1MEOX_3TMS	17.39	1456.8	173
79	*L*-Glutamine	3TMS	17.4	1456.9	91
80	4-Ketoglucose	4TMS	17.4	1457	103

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
81	L-Hydroxyproline	2TMS	17.62	1465.2	158
82	Malic acid	3TMS	17.85	1472.9	233
83	meso-Erythritol	2TMS	17.9	1475.6	217
84	Salicylic acid	2TMS	18.02	1479.8	267
85	RI_1478_unknown	-	18.05	1480.9	58
86	Adipic acid	2TMS	18.06	1481.3	205
87	Hexadecanoic acid_peak2	-	18.13	1484	170
88	2-Phenylglycine	2TMS	18.24	1486	178
89	*L*-Aspartic acid_peak 2	3TMS	18.27	1490.2	232
90	*L*-Proline_peak3	2TMS	18.3	1490.3	156
91	*L*-Methionine	2TMS	18.32	1490.9	128
92	γ-Aminobutyric acid (=GABA)	3TMS	18.42	1495.7	174
93	Pyrogallol	3TMS	18.56	1499.6	239
94	*D*-Erythronic acid	4TMS	18.63	1504.9	292
95	Phenylalanine	-	18.86	1521.6	120
96	*L*-Cysteine	3TMS	18.96	1527.9	57
97	*L*-Threonic acid	4TMS	19.19	1544.8	292
98	Glutaric acid/Pentanedioic acid_2	2TMS	19.21	1546.2	221
99	Dodecanol	1TMS	19.29	1552.1	243
100	α-Hydroxyglutaric acid	3TMS	19.39	1559.4	247
101	Glutaric acid/Pentanedioic acid_3	2TMS	19.48	1565.7	198
102	RI_1571_Unknown (related to proline)	-	19.55	1571	142
103	RI_1588_Unknown	-	19.79	1588.8	239
104	Asparagine	2TMS	19.87	1593.2	159
105	RI_1598_unknown	-	19.94	1598.9	57
106	Cinnamic acid	1TMS	19.96	1600.5	131
107	Heptanedioate/Pimelic acid	2TMS	20.12	1608.6	75
108	Glutamic acid	3TMS	20.26	1615.7	246
109	L(-)-Phenylalanine	2TMS	20.33	1619.7	218
110	L-(+)-Tartaric acid (=L-Threaric acid)	4TMS	20.56	1631.7	292
111	D(+)- Xylose_peak1	4TMS, MEOX	20.57	1632.3	307
112	RI1640_Unknown	-	20.71	1639.8	229
113	Vanillin	1MEOX_1TMS	20.83	1645.9	223
114	Pyrophosphate	4TMS	20.98	1654.1	451
115	Dodecanoic acid	1TMS	21.16	1663.3	257
116	*D*(+)- Xylose_peak2	4TMS, MEOX	21.19	1665.2	307
117	d-Ribose	1TMS	21.21	1665.8	307
118	RI_1680_C5-sugar alcohol	-	21.49	1680.6	307
119	RI_1685_unknown	-	21.56	1684.4	221
120	RI_1688_hydroxyfatty acid	-	21.64	1688.6	97
121	l-(-)-Arabitol	5TMS	21.68	1690.7	307
122	RI_1693_C5-sugar alcohol	-	21.73	1693.2	217
123	Suberic acid	TMS	21.79	1696.6	303
124	*D*-glyceraldehyde-3-phosphate	1MEOX_3TMS	21.88	1701.3	328

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
	(gap)_peak1				
125	*L*-Rhamnose_peak1	nTMS	21.94	1704.6	160
126	*D*-glyceraldehyde-3-phosphate (gap)_peak2	1MEOX_3TMS	21.98	1706.7	328
127	Arabinose	1MEOX_4TMS	22.01	1708.7	307
128	*L*-Rhamnose_peak2	nTMS	22.01	1708.7	160
129	D-glyceraldehyde-3-phosphate (gap)_peak3	1MEOX_3TMS	22.19	1718.9	328
130	Putrescine (=1.4-butanediamine)	1MEOX_4TMS	22.28	1723.9	174
131	Ribitol	5TMS	22.29	1724	205
132	RI_1729_unknown	-	22.36	1728.2	57
133	Ribonic acid	3TMS	22.52	1736.7	333
134	Dihydroxyacetone phosphate dilithium salt_peak1	1MEOX_3TMS	22.59	1740.8	315
135	Ornithine hydrochloride anhydrous_peak1	3TMS	22.62	1742	174
136	cys-Aconitic acid	3TMS	22.72	1747.7	333
137	RI_1748_C5-sugar derived acid	-	22.73	1748.4	292
138	Dihydroxyacetone phosphate_peak2	1MEOX_3TMS	22.75	1749.5	315
139	Glycerol-alpha-phosphate	4TMS	22.82	1753.7	299
140	RI_1759_C5-sugar	-	22.92	1759.3	191
141	Vanillic acid	2TMS	22.96	1761.3	297
142	RI_1771_unknown_phosphate	-	23	1771.9	299
143	RI_1766_C5-sugar derived acid	-	23.05	1766.5	333
144	RI_1770_Amine	-	23.12	1770.3	174
145	RI_1784_unknown	-	23.35	1783.3	174
146	Terephthalic acid	2TMS	23.37	1784.1	295
147	1,4-Benzenedicarboxylic acid	2TMS	23.42	1786.9	333
148	RI_1788_C5-sugar derived acid	-	23.45	1788.4	333
149	*DL*-Isocitric acid	4TMS	23.59	1796.19	273
150	Shikimic acid	4TMS	23.6	1796.08	255
151	*D*(-)-Phospho-glyceric acid (gip)	4TMS	23.65	1799.4	299
152	Ornithine hydrochloride anhydrous_peak2	4TMS	23.74	1803.1	174
153	3,4-dihydroxybenzoic acid	nTMS	23.89	1807.7	370
154	RI_1823_Amino acid	-	24.25	1823.8	156
155	RI_1856_C5-6-sugar alcohol	-	24.29	1825.7	215
156	Quinic acid	5TMS	24.34	1827.4	345
157	Adenine	TMS	24.45	1831.5	165
158	Myristic acid	nTMS	24.47	1832.6	117
159	*D*-Fructose_peak1	1MEOX_5TMS	24.61	1838	307
160	RI_1841_C6-sugar acid	-	24.68	1841.2	275
161	*D*-Fructose_peak2	1MEOX_5TMS	24.78	1845	361
162	*D*-Mannose_peak1	1MEOX_5TMS	24.86	1848.3	319

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
163	*D*-(+)-Gluconic acid δ-lactone (=gluconolactone)	4TMS	24.93	1851.1	319
164	*D*-Mannose_peak2	1MEOX_5TMS	25.01	1857.7	319
165	*D*-(+)-Galactose	1MEOX_5TMS	25.02	1854.5	319
166	Erythrose-4-phosphate sodium_peak1	1MEOX_5TMS	25.29	1865.5	357
167	*L*-Histidine monohydrochloride monohydrate	3TMS	25.39	1869.3	154
168	Erythrose-4-phosphate sodium_peak2	1MEOX_5TMS	25.48	1873.1	357
169	*D*-Mannitol	6TMS	25.53	1874.8	319
170	Lysinee_peak1	3TMS	25.56	1876.1	174
171	*D*-Sorbitol(d-Glucitol)	6TMS	25.61	1878.1	319
172	Dulcitol (=Galactitol)	6TMS	25.73	1883	319
173	*D*-(+)-Galacturonic acid_peak1	1MEOX_5TMS	25.79	1885.4	333
174	Lysine_peak2	4TMS	25.79	1885.4	174
175	*D*-Glucuronic acid	1MEOX_4TMS	25.91	1890.2	333
176	*D*-Glucose_peak1	MEOX _5TMS	25.94	1891.5	205
177	*L*-Ascorbic acid_peak2	4TMS	26.04	1895.2	332
178	*D*-(-)-Isoascorbic acid	4TMS	26.13	1898.9	332
179	*D*-(+)-Galacturonic acid monohydrate_peak2	1MEOX_5TMS	26.13	1898.9	333
180	cys-Ferulic acid	2TMS	26.28	1914.8	338
181	*L*-Iditol	6TMS	26.29	1915.7	103
182	RI_1919_Phosphocarbohydrate	-	26.37	1919.5	299
183	5-Keto-D-Gluconic acid	nTMS	26.4	1927.9	149
184	*D*-Glucose_peak2	1MEOX_5TMS	26.43	1936.9	205
185	4-Coumaric acid	nTMS	26.57	1947.9	219
186	*D*-(+)-Galacturonic acid_peak3	1MEOX_5TMS	26.59	1949.4	333
187	RI_1951_C6_sugar	-	26.62	1951.9	217
188	Gulonic acid, γ-lactone	4TMS	26.66	1953	189
189	*D*-Glucaric acid	6TMS	26.83	1976.8	333
190	Pyridoxine	3TMS	26.91	1986.4	293
191	*L*-Ascorbic acid_peak2	4TMS	27.26	1949.1	332
192	Palmitoleic acid_1	nTMS	27.27	2015.6	129
193	Gallic acid	4TMS	27.29	1950.9	281
194	Mucic acid (=Galactaric acid)	6TMS	27.34	2020.2	333
195	RI_2034_Unknown	-	27.56	2034.4	203
196	Palmitic acid	TMS	27.71	2044.5	313
197	Myo-inositol	6TMS	28.08	2068.5	318
197	RI_2092_C6-sugar derived acid	-	28.45	2092.3	245
198	*D*-Ribose-5-phosphate	1MEOX_5TMS	28.47	2094	315
199	RI_2103_unknown	-	28.51	2096.6	174
200	RI_2108_Unknown	-	28.68	2107.8	299
201	*N*-Acetyl glucosamine_1	nTMS	28.71	2109.3	205
202	*N*-acetyl-d-hexosamine 1	nTMS	28.8	2114.81	319

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
203	RI_2122_unknown	-	28.89	2121.4	221
204	Hexanedioic acid	nTMS	28.95	2124.6	131
205	trans-Caffeic acid (=2-propenoic acid)	3TMS	29.07	2133.2	219
206	Heptadecanoic acid	nTMS	29.19	2141	204
207	Octadecanol	nTMS	29.39	2153.8	327
208	*α-D*-gluco-hexopyranose	nTMS	29.73	2175.7	204
209	Phytanic acid	nTMS	29.82	2181.7	120
210	RI_2185_C5-6-sugar-phosphate	-	29.88	2185.4	357
211	N-*α*-acetyl-*L*-Lysine	-	29.99	2193.6	174
212	RI_2194_inositol-phosphate	-	30	2194	299
213	*DL*-Tryptophan	2TMS	30.03	2195.3	202
214	Tryptamine_peak1	2TMS	30.06	2197.1	174
215	9,12-Octadecadienoic acid	TMS	30.21	2208	341
216	Tryptamine_peak2	3TMS	30.25	2210.3	174
217	Oleic acid		30.3	2214.2	341
218	Heptadecanoic acid	TMS	30.46	2225.3	204
219	RI_2242_unknown phosphate	-	30.69	2241.6	299
220	Stearic acid_peak1	TMS	30.69	2241.7	341
221	RI_2260_unknown	-	30.91	2257	290
222	Hexadecanoic acid butyl ester	TMS	30.93	2258.6	87
223	Flavone	TMS	30.94	2259.7	221
224	RI_2267_unknown	-	31.05	2267.4	165
225	RI_2274_unknown	-	31.14	2273.8	151
226	Glycerol-3-galactoside	TMS	31.22	2279.2	117
227	Fructose-6-phosphate	1MEOX_6TMS	31.33	2287.3	315
228	Mannose-1-phosphate_peak1	1MEOX_6TMS	31.5	2299.4	387
229	Mannose-1-phosphate_peak2	1MEOX_6TMS	31.75	2317	387
230	RI_2318_C5-6-sugar-phosphate	-	31.77	2318	299
231	RI_2323_unknown sugar	-	31.84	2322.9	319
232	*D*-Glucose 6-phosphate	6TMS	31.93	2329.4	204
233	Sinigrin	nTMS	32.08	2340.6	17
234	Lysine	nTMS	32.12	2342.7	204
235	Glucose-6-phosphate_peak1	1MEOX_6TMS	32.37	2360.6	387
236	*D*-Glucuronic acid	-	32.38	2360.8	103
237	Glucose-6-phosphate_peak2	1MEOX_6TMS	32.54	2372.6	387
238	RI_2377_Carbohydrate	-	32.6	2377.2	204
239	Myo-inositol	nTMS	32.72	2385.8	239
240	Hexanedioic acid	2TMS	32.74	2387.5	129
241	RI_2395_unknown	-	32.85	2394.9	221
242	Nonadecanoic acid	TMS	33.14	2416.1	130
243	RI_2438_Carbohydrate	-	33.43	2438.6	204
244	RI_2465_unknown	-	33.78	2465.4	87
245	Biotin	nTMS	33.95	2478.7	446
246	Monoferuloylglycerol	TMS	34.05	2486.4	249

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
247	RI_2493_Disacharide	-	34.14	2492.8	361
248	Ribulose-1.5-biphosphate_peak1	1MEOX_5TMS	34.2	2498.2	357
249	Ribulose-1.5-biphosphate_peak2	1MEOX_5TMS	34.57	2525.8	357
250	RI_2530_Disacharide	-	34.63	2530.2	361
251	Melibiose	8TMS	34.7	2535.8	149
252	RI_2545_unknown	-	34.82	2545.3	165
253	1-Docosanol	nTMS	34.84	2546.6	383
254	Salicyl alcohol-b-glucoside	nTMS	35.01	2559.6	361
255	Monopalmitoylglycerol	2TMS	35.29	2581.1	371
256	Sucrose	8TMS	35.62	2606.8	361
257	Lactose_peak1	nTMS	36.2	2654.4	361
258	RI_2662_Disacharide	-	36.29	2662.5	361
259	RI_2670_Disacharide	-	36.39	2670	361
260	Lactose_peak2	nTMS	36.42	2672.7	361
261	RI_2677_Disacharide	-	36.47	2676.6	361
262	Fructose-1.6-biphosphate_peak1	1MEOX_6TMS	36.61	2688.3	315
263	Fructose-1.6-biphosphate_peak2	7TMS	36.76	2700.8	315
264	*D*-(+)-Maltose monohydrate_peak1	1MEOX_8TMS	36.87	2709.2	361
265	*D*-(+)-Trehalose α,α' dihydrate	8TMS	36.91	2713.2	361
266	Turanose_peak1	8TMS	37.05	2724.2	361
267	Melibiose_peak1	8TMS	37.13	2730.6	361
268	*D*-(+)-Maltose monohydrate_peak2	1MEOX_8TMS	37.2	2736.6	361
269	Turanose_peak2	1MEOX_8TMS	37.4	2752.4	307
270	RI_2759_lysolipid derivate	-	37.47	2759	311
271	Stearic acid_peak2	2TMS	37.65	2773.8	399
272	RI_2786_Disaccharide	-	37.8	2785.9	361
273	RI_2793_unknown	-	37.87	2791.9	221
274	Squalene	nTMS	38.1	2811.5	69
275	RI_2813_Disacharide	-	38.13	2813.8	361
276	Turanose_peak3	7TMS	38.23	2822.7	361
277	RI_2835_Disacharide	-	38.37	2835	361
278	Melibiose_peak2	8TMS	38.37	2835.6	361
279	Isomaltose_peak1	1MEOX_8TMS	38.45	2842.8	361
280	RI_2849_unknown		38.52	2849	165
281	Isomaltose_peak2	1MEOX_8TMS	38.83	2876	361
282	Galactinol_peak1		39.34	2920.8	221
283	RI_2946_Disacharide	-	39.63	2945.9	361
284	Galactinol_peak2	9TMS	39.67	2950.1	204
285	RI_2975_Disaccharide	-	39.76	2975	204
286	Melibiose_peak2	8TMS	40.11	2985.1	361
287	RI_3005_Galactinol derivate	-	40.29	3005.2	204
288	RI_3037_Carbohydrate-phosphate	-	40.64	3037.4	299
289	RI_3064_unknown	-	40.92	3064.1	204
290	RI_3069_Unknown	-	40.98	3069.5	375

#	Name[a]	Derivative[b]	t_R exp[c]	RI[d]	*m/z* quant[e]
291	RI_3104_unknown	-	41.35	3104.8	482
292	RI_3147_unknown	-	41.8	3147.1	204
293	RI_3182_unknown	-	42.18	3182.4	204
294	Octacosanoic acid	nTMS	42.67	3230.8	117
295	RI_3249_unknown	-	42.86	3249.8	496
296	RI_3294_Trisacharide	-	43.3	3294	427
297	beta-Sitosterol	1TMS	43.62	3325.6	396
298	*α-D*-gluco-hexopyranose	8TMS	43.78	3341.7	361
299	RI_3378_Trisacharide	-	44.15	3378.6	204
300	Erlose	11TMS	44.16	3380.1	361
301	RI_3409_unknown	-	44.45	3409.3	204
302	RI_3451_Trisacharide	-	44.85	3451	204
303	Maltose_peak3	8TMS	45.31	3498.9	361
304	Turanose_peak4	7TMS	45.51	3519.8	361
305	RI_3533_unknown	-	45.65	3533.6	452
306	RI_3563_unknown	-	46	3570.2	204
307	RI_3570_Unknown	-	46	3570.1	647
308	Maltotriose	11TMS	46.09	3578.6	204
309	RI_3626_unknown	-	46.6	3626.7	204
310	RI_3644_Trisacharide	-	46.81	3644.2	204
311	RI_3679_Trisacharide	-	47.22	3679.8	204
312	RI_3721_unknown	-	47.71	3721.7	204
313	RI_3723_unknwon	-	47.73	3722.9	340
314	RI_3819_Trisacharide	-	48.93	3819.2	204
315	RI_3832_Trisacharide	-	49.14	3832.3	204
316	RI_3918_peak_340_unknown	-	50.5	3918.5	340
317	RI_4043_Trisacharide	-	52.48	4043.3	204
318	RI_4160_unknown	-	54.33	4160.1	340
319	RI_4168_unknown	-	54.33	4168.4	361
320	RI_4247_Trisacharide	-	55.54	4247.7	361
321	RI_4250_Trisacharide	-	55.56	4250.2	361

[a]All metabolites are arranged in order of increasing retention times (t_R). Metabolites having the same name and _1H, _1L, 2H, and 2L designations are interpreted as oxime isomers of sugar MeOx derivatives. In the abbreviations _1H, _1L, 2H, and _2L, the numbers denote the peak number of the metabolite and the letters denote the relative peak height L (low) or H (high) of the corresponding peak compared with the peak height of the second isomer of the metabolite.

Unidentified metabolites are labeled with the word *Unknown*, and their annotation contains the retention index (RI). The name of metabolites annotated to a certain chemical class (without exact annotated structure) also begins with RI, followed by the name of the corresponding chemical class. Annotation to specific chemical classes was confirmed by the presence of characteristic signals (*m/z* values) specific for the corresponding chemical class (for example, C5-6-sugar-phosphate - *m/z* 299, 315 and 387; disaccharide - *m/z* 361, 204 and 319; galactinol derivative - *m/z* 204, 361 and 433).

[b]The numbers and types of derivatization groups attached to the identified metabolites: TMS - trimethylsilyl group, MEOX - methyloxime group.

[c]Retention time of the metabolite,

[d]Retention index of the metabolite;

[e]The *m*/*z* value of the most characteristic ion in the EI spectrum (quantifier), from which the extracted ion chromatogram was reconstructed and the peak area integration was accomplished at the given t_R.

Table S1-5. Thermally labile primary metabolites annotated by co-elution with authentic standards and tandem mass spectrometric (MS/MS) information in *Cucumis sativus* root aqueous methanolic extracts from the roots with *CsRALF34* overexpression or from roots of control group. Metabolite analysis relied on the ion pair-reversed phase high-performance liquid chromatography coupled online to the triple quadrupole tandem mass spectrometer (RP-IP-HPLC-QqQ-MS/MS) without any derivatization.

#	Metabolite	t_R (min)[a]	Q1 (*m*/*z*)[б]	Q3 (*m*/*z*)[в]
1	histidine	0.79	154.1	93.0
2	proline	0.80	114.1	86.0
3	leucine + isoleucine	0.93	261.3	130.2
4	creatine	0.50	130.1	88.1
5	glutamine	0.79	145.1	108.9
6	ornithine	0.73	131.1	82.9
7	allantoin	0.82	157.0	97.2
8	γ-aminobutyric acid	0.70	102.1	84.0
9	alanine	0.80	88.0	41.9
10	citrulline	0.80	174.1	131.0
11	*L*-cysteine	1.11	120.0	79.8
12	lysine	0.80	145.1	99.0
13	arginine	0.77	173.1	131.0
14	serine	0.78	104.0	74.0
15	valine_1	0.80	233.3	116.0
16	valine_2	0.80	116.1	70.0
17	*L*-dicysteine	1.00	239.0	120.0
18	glyoxilic acid	0.89	73.0	45.0
19	methionine	0.89	148.0	47.0
20	uridine	0.88	243.1	109.9
21	glycine	0.79	74.0	74.0
22	threonine	0.79	118.1	73.9
23	*S*-adenosyl-*L*-homocysteine	0.84	383.1	133.9
24	asparagine	0.83	131.0	87.1
25	sucrose	0.85	341.1	89.0
26	dehydroascorbic acid	0.88	173.0	127.0
27	cytidine	0.87	242.1	108.9
28	*P*-choline	0.86	242.1	108.9
29	tyrosine	0.90	180.1	118.9
30	guanosine	1.32	282.1	149.9
31	2'-deoxyguanosine	1.44	266.1	150.0
32	phenylalanine	1.50	164.1	103.0
33	glucopyranonic acid/	2.40	193.0	113.0
34	galactopyranuronic acid	2.40	193.0	113.0
35	glutamic acid	2.44	146.0	102.0
36	adenosine	2.41	266.1	133.9
37	ribonic acid	2.57	165.0	75.0
38	*D*-galactonic acid/*D*-gluconic acid	2.47	195.1	129.0

#	Metabolite	t_R (min)[a]	Q1 (m/z)[б]	Q3 (m/z)[в]
39	glucosamine 6-phosphate	2.40	258.0	97.0
40	dihydroorotic acid	2.52	157.0	112.7
41	glucolate	2.56	75.0	47.0
42	shikimic acid	2.48	173.0	92.9
43	aspartic acid	2.45	132.0	88.0
44	quinic acid	2.50	191.1	85.0
45	uric acid	2.58	167.0	124.0
46	3-ureidopropionic acid/3-(carbamoylamino)propanoic acid	2.56	131.0	87.9
47	3-dehydroxyshikimic acid	2.59	171.0	127.0
48	ascorbic acid	2.50	175.0	115.0
49	glucosamine 1-phosphate	2.50	258.0	78.9
50	tryptophan	2.54	203.1	116.2
51	chloride	2.85	35.0	35.0
52	lactic acid	3.12	89.0	42.9
53	glutathione	3.80	306.1	143.0
54	phosphate	3.73	96.9	78.9
55	nicotinamide adenine dinucleotide	4.44	662.1	540.1
56	adenosine 2′,3'-cyclic mono-phosphate	6.79	328.0	134.0
57	orotic acid	4.11	155.0	110.7
58	fructose 6-phosphate/glucose 6-phosphate	5.26	259.0	96.9
59	2-keto-3-deoxy-6-phosphogluconate	5.28	257.0	97.0
60	cyclic guanosine monophosphate	5.60	344.0	150.0
61	2-deoxyribose 5-phosphate	6.45	229.0	96.8
62	glyceraldehyde 3-phosphate	5.77	169.0	97.0
63	dihydroxyacetone phosphate	7.60	169.0	97.0
64	sedoheptulose 7-phosphate/arginosuccinat	5.76	289.0	97.0
65	glycerophosphoric acid	5.83	171.0	78.8
66	glucose-1-phosphate	6.11	259.0	240.8
67	nicotinic acid	6.44	122.0	77.9
68	pantothenic acid	6.48	218.1	88.1
69	2-*C*-methylerythritol 4-phosphate	6.08	215.0	78.9
70	cytidine monophosphate	6.39	322.0	79.0
71	mevalonic acid lactone	6.85	147.1	59.1
72	erythrose 4-phosphate	5.25	199.0	96.8
73	ribulose-5-phosphate/xylulose-5-phosphate	6.45	229.0	96.8
74	uridine monophosphate	7.24	323.0	79.0
75	ribose-1-phosphate	7.25	229.0	211.0
76	2'-deoxyguanosine 5'-monophosphate	8.33	346.1	78.8
77	adenosine monophosphate	8.37	346.1	78.8
78	guanosine 5'-monophosphate	8.39	362.1	78.9
79	inosinic acid	8.40	347.0	134.8
80	1-deoxy-*D*-xylulose 5-phosphate	8.75	213.0	97.0

#	Metabolite	t_R (min)[a]	Q1 (m/z)[б]	Q3 (m/z)[в]
81	glutathione disulfide	8.61	611.1	306.1
82	2'-deoxyadenosine 5'-monophosphate	8.83	330.1	195.0
83	digalacturonic acid	8.77	369.1	175.0
84	thymidine-5'-phosphate	9.55	321.0	78.8
85	succinic acid	9.54	117.0	73.0
86	3-hydroxypyruvate	9.43	103.0	59.0
87	ureidosuccinic acid	9.53	175.0	131.8
88	fumaric acid	9.70	115.0	71.0
89	malate	9.73	133.0	115.0
90	uridine-5'-diphosphate-glucose	9.52	565.0	323.0
91	uridine-diphosphate-*N*-acetylglucosamine	9.60	606.1	384.8
92	sulfate	9.82	97.0	97.0
93	oxaloacetic acid-1	9.90	131.0	87.0
94	oxaloacetic acid-2	10.32	131.0	43.0
95	4-diphosphocytidyl-2-*C*-methyl-*D*-erythritol	5.57	520.1	78.9
96	adenosine diphosphoribose	10.26	558.1	346.0
97	adenosine diphosphate glucose	10.16	588.1	345.9
98	pentanoates	10.36	101.1	101.1
99	α-ketoglutaric acid	10.33	145.0	101.0
100	1,4-dihydronicotinamide adenine dinucleotide	10.80	664.1	78.9
101	(*R*)-5-phosphomevalonic acid	11.30	227.0	97.0
102	2*P*-glycolate	12.82	155.0	79.0
103	xanthosine-5'-phosphate	12.85	363.0	151.1
104	2-phosphoglyceric acid	12.83	185.0	79.0
105	3-phosphoglyceric acid	12.76	185.0	96.7
106	(2E)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate	12.91	261.0	79.0
107	uridine-5'-diphosphate	13.07	403.0	78.8
108	2'-deoxyadenosine-5'-diphosphate	13.20	410.0	78.9
109	isopentenyl pyrophosphate/	14.38	245.0	78.9
	delta3-isopentenyl pyrophosphate	14.38	245.0	78.9
110	/(dimethylallylpyrophosphat)	14.22	245.0	78.9
111	flavin adenine dinucleotide	12.83	784.1	79.0
112	aconitic acid	13.12	173.0	128.7
113	adenosine-5'-diphosphate	13.16	426.0	78.9
114	nicotinamide adenine dinucleotide phosphate	13.12	743.1	620.0
115	guanosine-5'-diphosphate	13.22	442.0	78.9
116	phosphoenolpyruvic acid	13.52	167.0	78.8
117	cytidine-5'-diphosphate	12.77	402.0	78.9
118	thymidine-5'-diphosphate	14.03	401.0	78.8
119	6-phosphogluconic acid	13.01	275.0	79.0
120	trigalacturonic acid	13.59	545.1	369.0
121	citric acid	14.05	191.0	87.0
122	isocitric acid	14.17	191.0	73.0

#	Metabolite	t_R (min)[a]	Q1 (m/z)[б]	Q3 (m/z)[в]
123	2-deoxyribose 5-phosphate	14.57	212.9	97.1
124	orotidine 5'-monophosphate	15.55	367.0	78.9
125	fructose-1,6-diphosphate	15.45	339.0	96.9
126	ribulose-1,5-bisphosphate	15.44	309.0	97.0
127	4-diphosphocytidyl-2-*C*-methyl-*D*-erythritol 2-phosphate	15.76	600.0	78.9
128	sedoheptulose-1,7-biphosphate	15.60	369.0	97.0
129	cytidine 5'-triphosphate	15.61	482.0	158.8
130	desoxyadenosintriphosphat	15.83	490.0	391.9
131	mevalonate-5-diphosphate	15.75	307.0	78.9
132	adenosine triphosphate	15.90	506.0	158.8
133	guanosine-5'-triphosphate	15.90	522.0	158.8
134	adenylosuccinic acid	16.00	462.1	133.9
135	uridine-5'-triphosphate	15.85	483.0	158.8
136	ADP-ribose-2`-phosphate	16.40	638.0	426.0
137	dihydronicotinamide adenine dinucleotide phosphate	16.44	744.1	79.0
138	coenzyme A	17.32	766.1	407.9
139	inositol triphosphate	17.30	419.0	320.8
140	deoxythymidine 5'-triphosphate	15.85	481.0	158.7
141	5-phosphoribosyl diphosphate	17.30	388.9	176.8
142	S-acetyl coenzyme A	17.57	808.1	407.9
143	geranyl diphosphate	18.33	313.1	78.9
144	methylmalonyl coenzyme A	18.35	866.1	408.0
145	isovaleryl coenzyme A	19.20	850.2	407.9
146	acetoacetyl coenzyme A	19.20	850.1	408.0
147	malonyl coenzyme A	19.20	852.1	408.0
148	β-hydroxy β-methylglutaryl-CoA	18.39	910.1	407.9
149	ent-copal-8-ol diphosphate	18.33	866.1	407.6
150	β-methylcrotonyl coenzyme A	19.04	848.1	407.8
151	inositol-1,3,4,5-tetraphosphate	18.80	498.3	400.7
152	1-diphosinositol pentakisphosphate	18.91	578.9	480.6
153	geranylgeranyl pyrophosphate	19.74	449.2	78.8
154	farnesyl diphosphate	19.70	381.1	78.9
155	phytic acid/inositol hexaphosphate	19.45	658.9	560.7
156	glucose	0.79	179.1	89.0
157	5-amino-4-imidazolecarboxamide ribotide	8.1	337.1	78.9

[a] The arrangement of the metabolites in the table corresponds to the order of increasing retention times (tR).

[b] m/z of quasi-molecular ions of standard substances

[c] m/z of fragment ion obtained by fragmentation of quasi-molecular ions of standard substances.

Table S1-6. Protein extraction yields, protein concentrations in the extracts and optical densities of individual SDS-PAGE lanes corresponding to individual samples of protein extracts isolated from the *Cucumis sativus* roots with overexpression of *CsRALF34* or from roots of control group.

Sample	Sample weight (g)	Protein concentration (mg/mL)	Protein recovery (mg/g fresh weight)	Optical densities [a]
Control (1)	0.250	1.53	4.28E-01	1595601
Control (2)	0.254	1.13	3.12E-01	1612235
Control (3)	0.255	1.25	3.43E-01	1525704
Control (4)	0.241	1.51	4.37E-01	1525891
Control (5)	0.236	1.35	4.00E-01	1572125
Control (6)	0.271	1.65	4.28E-01	1598280
Control (7)	0.233	1.38	4.13E-01	1625710
Control (8)	0.284	1.88	4.63E-01	1658598
RALF34+ (1)	0.253	1.47	4.07E-01	1727770
RALF34+ (2)	0.248	1.64	4.62E-01	1734730
RALF34+ (3)	0.273	1.50	3.85E-01	1823112
RALF34+ (4)	0.224	1.05	3.30E-01	1718919
RALF34+ (5)	0.284	0.95	2.34E-01	1736292
RALF34+ (6)	0.244	1.33	3.82E-01	1768621
RALF34+ (7)	0.260	1.51	4.05E-01	1768116
RALF34+ (8)	0.224	1.27	3.97E-01	1542560

[a] optical densities of individual SDS-PAGE lanes

Table S1-7. Eigenvalues (loadings) obtained in the principal component analysis, in particular PC1, of differentially expressed part of *Cucumis sativus* root proteome, associated with *CsRALF34* overexpression.

Protein name [a]	PC1[b]	Direction of alterations	log_2FC[c]	$p_{adjusted}$[d]
Thioredoxin	0.084	Up	-17.827	5.27E-35
CHP-rich zinc finger protein-like	0.081	Up	-17.201	6.81E-35
Glutathione peroxidase	0.081	Up	-17.163	4.37E-35
Ribosomal protein L19	0.081	Up	-17.026	5.64E-35
40S ribosomal protein S24	0.080	Up	-16.696	3.16E-34
Carnitine operon protein CaiE	0.079	Up	-16.568	5.64E-35
Wound/stress protein	0.078	Up	-16.523	4.37E-35
Porin/voltage-dependent anion-selective channel protein	0.078	Up	-16.462	5.64E-35
60S ribosomal protein L18a	0.077	Up	-16.422	6.81E-35
Putative villin 2 protein	0.075	Up	-16.340	8.87E-35
Eukaryotic translation initiation factor 3 subunit C	0.077	Up	-16.305	1.36E-32
3-oxoacyl-[acyl-carrier-protein] synthase 3	0.076	Up	-16.286	8.96E-35
Pleckstrin homology domain-containing family A member	0.077	Up	-16.272	5.00E-16
RNA-binding protein 8A	0.076	Up	-16.085	8.30E-35
Glycine cleavage system H protein 1	0.075	Up	-16.013	8.80E-30
Phosphofructokinase	0.077	Up	-15.994	8.70E-35
Acidic endochitinase	0.075	Up	-15.750	2.88E-35
Phosphomannomutase	0.074	Up	-15.747	9.47E-35
60S ribosomal protein L35	0.074	Up	-15.745	1.51E-29
TBCC domain-containing protein	0.071	Up	-15.704	9.47E-35
Tripeptidyl peptidase II	0.073	Up	-15.642	1.02E-34
40S ribosomal protein S29	0.075	Up	-15.619	9.47E-35
40S ribosomal protein S12	0.074	Up	-15.518	8.21E-35
THO complex subunit	0.073	Up	-15.495	3.51E-28
Ribosomal protein L15	0.074	Up	-15.455	1.01E-34
AP-1 complex subunit gamma-2	0.072	Up	-15.414	1.17E-34
Putative 3-dehydroquinate synthase	0.073	Up	-15.388	9.18E-35
37S ribosomal protein	0.073	Up	-15.386	8.92E-25
Lipid A export ATP-binding/permease protein MsbA	0.072	Up	-15.331	3.20E-19

Protein name [a]	PC1[b]	Direction of alterations	log_2FC[c]	$p_{adjusted}$[d]
Protein phosphatase 2c, putative	0.072	Up	-15.319	3.61E-34
Transmembrane 9 superfamily protein member	0.075	Up	-15.315	9.47E-35
Isocitrate dehydrogenase [NADP]	0.072	Up	-15.267	3.17E-23
Surface antigen (D15)	0.072	Up	-15.254	1.01E-34
Eukaryotic translation initiation factor 5A	0.072	Up	-15.246	3.68E-29
Transketolase	0.073	Up	-15.228	9.47E-35
Histone H2A	0.072	Up	-15.194	9.47E-35
Phosphoribosylformylglycinamidine cyclo-ligase	0.073	Up	-15.188	2.09E-34
5-methyltetrahydropteroyl triglutamate-homocysteine methyltransferase	0.072	Up	-15.187	2.53E-21
Beta-glucosidase	0.072	Up	-15.176	1.20E-34
Peroxidase	0.072	Up	-15.139	1.20E-34
Endo-1,3-1,4-beta-d-glucanase	0.071	Up	-15.124	9.47E-35
Calmodulin-binding transcription activator	0.071	Up	-15.101	6.45E-28
NADP-dependent D-sorbitol-6-phosphate dehydrogenase	0.071	Up	-15.099	6.12E-33
Nonsense-mediated mRNA decay NMD3 family protein	0.071	Up	-15.003	1.18E-34
UDP-glycosyltransferase 1	0.071	Up	-14.996	9.18E-35
Thioredoxin family protein	0.071	Up	-14.990	5.35E-33
Alpha-galactosidase 1	0.070	Up	-14.943	6.75E-34
Acyl-CoA-binding domain-containing protein	0.073	Up	-14.933	1.08E-33
Vacuolar-sorting receptor 7	0.070	Up	-14.912	1.20E-34
ATP-binding cassette	0.070	Up	-14.909	2.96E-19
Exocyst complex component	0.070	Up	-14.897	2.65E-23
Adenylosuccinate lyase	0.070	Up	-14.896	3.33E-28
Acylamino-acid-releasing enzyme	0.070	Up	-14.875	5.08E-30
Acetolactate synthase small subunit	0.070	Up	-14.851	1.29E-34
Ran GTPase activating protein 2	0.069	Up	-14.837	1.22E-34
Splicing factor 3B subunit	0.070	Up	-14.748	2.09E-22
60S ribosomal protein L14	0.069	Up	-14.672	1.18E-34
Pro-resilin	0.069	Up	-14.666	4.77E-29
Methyltransferase	0.069	Up	-14.651	5.89E-33
Serine/threonine protein kinase	0.068	Up	-14.599	2.14E-34
Peroxisomal membrane protein 11-1	0.068	Up	-14.586	2.43E-34
RuvB-like helicase	0.071	Up	-14.568	1.64E-34
Calcium dependent protein kinase 12	0.069	Up	-14.524	1.43E-34
Signal recognition particle 72 kDa protein	0.068	Up	-14.511	8.57E-33
Histidine decarboxylase	0.068	Up	-14.473	1.43E-34
Bifunctional protein folD	0.068	Up	-14.411	1.46E-34
Nuclear RNA binding protein	0.068	Up	-14.381	6.59E-21
Allene oxide cyclase 3	0.069	Up	-14.320	2.43E-34

Protein name [a]	PC1[b]	Direction of alterations	log_2FC[c]	$p_{adjusted}$[d]
Bifunctional protein folD	0.066	Up	-14.235	4.37E-35
Aladin	0.067	Up	-14.041	2.85E-34
40S ribosomal protein S25	0.066	Up	-13.980	1.47E-34
Serine/threonine protein phosphatase 2A, regulatory subunit	0.065	Up	-13.820	3.76E-21
Probable exocyst complex component 4	0.066	Up	-13.807	2.43E-34
SMP-30/Gluconolaconase/LRE-like region family protein	0.065	Up	-13.789	1.77E-34
Dihydroorotate dehydrogenase (quinone)	0.065	Up	-13.779	1.46E-25
Glutamine-fructose-6-phosphate aminotransferase [isomerizing] 2	0.065	Up	-13.730	1.72E-19
Phosphomevalonate kinase	0.064	Up	-13.618	4.98E-33
Protein transport protein SEC23	0.064	Up	-13.612	9.65E-34
Small nuclear ribonucleoprotein-associated protein	0.063	Up	-13.434	6.54E-27
Mannan synthase	0.063	Up	-13.399	4.94E-34
General vesicular transport factor p115	0.062	Up	-13.129	4.11E-34
Glucosidase II beta subunit	0.062	Up	-13.041	2.27E-19
ATP-dependent RNA helicase	0.061	Up	-12.961	1.05E-19
Early-responsive to dehydration	0.061	Up	-12.920	2.23E-16
LETM1 and EF-hand domain-containing protein 1	0.062	Up	-12.768	7.18E-35
RNA recognition motif-containing protein	0.017	Up	-4.239	4.50E-07
Brefeldin A-inhibited guanine nucleotide-exchange protein	0.009	Up	-2.074	2.03E-02
Chaperone protein htpG family protein	0.009	Up	-2.010	2.69E-02
Translational activator GCN1	0.008	Up	-1.933	2.69E-02
ADP-ribosylation factor family protein	0.009	Up	-1.874	5.45E-04
Ras protein Rab-2-B	0.008	Up	-1.872	1.23E-04
3.1.2 inositol monophosphatase	0.007	Up	-1.861	3.17E-04
UDP-glycosyltransferase 1	-0.008	Down	1.611	1.62E-03
Subtilisin-like serine protease	-0.008	Down	1.790	9.16E-04
2-oxoglutarate-dependent dioxygenase	-0.009	Down	1.956	4.49E-02
Transitional endoplasmic reticulum ATPase	-0.010	Down	2.072	2.96E-02
Myosin-6, (Protein of unknown function DUF827, plant)	-0.013	Down	3.669	5.22E-03
Phosphorylase	-0.057	Down	12.172	2.81E-24
Serine/threonine protein phosphatase 2A 57 kDa regulatory subunit B' beta	-0.058	Down	12.279	2.27E-25
UDP-glycosyltransferase 1	-0.059	Down	12.507	8.02E-34
Proteasome component ECM29	-0.061	Down	12.859	3.88E-34
Peroxidase	-0.062	Down	12.863	4.60E-34
Tetratricopeptide repeat protein	-0.062	Down	12.864	8.49E-34
ADP-ribosylation factor family protein	-0.061	Down	12.928	1.08E-32
Kinesin-4	-0.061	Down	13.012	8.22E-26

Protein name [a]	PC1[b]	Direction of alterations	log_2FC[c]	$p_{adjusted}$[d]
Hexokinase 1	-0.061	Down	13.057	1.16E-33
Armadillo/beta-catenin repeat family protein	-0.062	Down	13.133	1.63E-19
Oxidoreductase nad-binding rossmann fold protein	-0.064	Down	13.388	5.07E-34
Phosphatidylinositol 4-kinase	-0.064	Down	13.413	4.11E-34
AT5g06970/MOJ9_14	-0.064	Down	13.576	6.93E-35
Cytochrome P450	-0.064	Down	13.613	4.35E-29
Malic enzyme	-0.066	Down	13.615	1.82E-34
UDP-glycosyltransferase 74 F1	-0.064	Down	13.651	1.45E-34
Cystathionine gamma synthase	-0.065	Down	13.701	3.19E-34
AP-2 complex subunit beta-1	-0.065	Down	13.704	5.74E-34
Leukotriene A4 hydrolase/aminopeptidase	-0.067	Down	13.753	4.79E-35
Ubiquitin carboxyl-terminal hydrolase	-0.065	Down	13.793	4.11E-34
Inositol-tetrakisphosphate 1-kinase	-0.066	Down	13.991	4.60E-34
3,4-dihydroxy 2-butanone 4-phosphate synthase	-0.066	Down	13.993	3.03E-32
Thioredoxin domain-containing protein	-0.066	Down	14.081	2.85E-33
Beta-galactosidase	-0.068	Down	14.123	1.36E-34
Peroxidase (Haem peroxidase)	-0.068	Down	14.135	2.07E-34
Dynamin	-0.067	Down	14.232	4.37E-35
NADPH:quinone oxidoreductase	-0.069	Down	14.235	1.22E-34
Auxin F-box protein 5	-0.067	Down	14.261	1.89E-34
Lipoxygenase	-0.069	Down	14.299	1.77E-34
Phytochrome	-0.067	Down	14.312	1.16E-34
ATRAD3 (Putative S-adenosyl-L-methionine-dependent methyltransferase)	-0.066	Down	14.327	1.82E-34
Pentatricopeptide repeat-containing protein	-0.068	Down	14.342	2.10E-34
Signal recognition particle 19 kDa protein	-0.068	Down	14.389	3.64E-32
Argininosuccinate lyase	-0.068	Down	14.390	6.79E-28
AMP deaminase	-0.068	Down	14.398	4.84E-24
Acetyl-coenzyme A synthetase	-0.068	Down	14.410	2.35E-25
3,4-dihydroxy 2-butanone 4-phosphate synthase	-0.068	Down	14.478	4.09E-28
Sieve element occlusion protein 2	-0.070	Down	14.556	5.15E-34
Uridine kinase	-0.069	Down	14.690	4.37E-35
RNA-dependent RNA polymerase 1b	-0.069	Down	14.739	2.89E-31
Prolyl 4-hydroxylase alpha subunit	-0.070	Down	14.753	1.79E-25
50S ribosomal protein L1	-0.069	Down	14.753	1.36E-34
Lipoxygenase	-0.070	Down	14.756	5.15E-26
ATP phosphoribosyltransferase	-0.070	Down	14.780	1.42E-34
1-aminocyclopropane-1-carboxylate oxidase	-0.070	Down	14.782	8.73E-22
GDSL esterase/lipase	-0.070	Down	14.804	3.98E-34
5-oxoprolinase	-0.071	Down	14.809	1.22E-34
NADPH-cytochrome P450 reductase	-0.070	Down	14.837	6.56E-23

Protein name [a]	PC1[b]	Direction of alterations	log_2FC[c]	$p_{adjusted}$[d]
Beta-glucosidase	-0.070	Down	14.842	9.47E-35
Ankyrin repeat-containing protein	-0.070	Down	14.843	2.95E-25
Ubiquitin-protein ligase 4; contains IPR000569 (HECT), IPR016024 (Armadillo-type fold)	-0.068	Down	14.894	1.20E-34
Acyl-CoA dehydrogenase	-0.071	Down	14.894	1.20E-34
NADP-dependent D-sorbitol-6-phosphate dehydrogenase	-0.070	Down	14.933	2.06E-22
Sugar transporter	-0.071	Down	14.958	1.29E-34
Monoglyceride lipase	-0.070	Down	14.964	9.47E-35
Translin	-0.073	Down	14.993	2.25E-34
Malic enzyme	-0.071	Down	15.044	1.20E-34
Signal peptidase complex subunit 2	-0,072	Down	15.070	1.53E-34
Major facilitator superfamily domain-containing protein	-0.070	Down	15.083	1.23E-33
UDP-glycosyltransferase 1	-0.071	Down	15.095	8.51E-35
Deoxyuridine 5'-triphosphatenucleotido hydrolase	-0.073	Down	15.102	4.65E-34
Endo-1,4-beta-glucanase	-0.070	Down	15.134	8.48E-35
Cullin-1	-0.071	Down	15.155	9.47E-35
Cyclin dependent kinase A	-0.071	Down	15.157	4.06E-25
Putative vesicle-associated membrane protein family protein	-0.072	Down	15.172	1.20E-34
Ferredoxin	-0.072	Down	15.182	1.16E-34
Protein kinase	-0.071	Down	15.198	9.47E-35
1-pyrroline-5-carboxylate dehydrogenase	-0.072	Down	15.273	9.89E-35
Heat shock 70 kDa protein	-0.072	Down	15.274	9.47E-35
Catalase	-0.073	Down	15.317	1.26E-34
Coatomer subunit zeta-1	-0.074	Down	15.318	8.70E-35
Nitrogen regulatory protein P-II	-0.072	Down	15.337	7.83E-35
Pre-mRNA-processing factor-like protein	-0.073	Down	15.400	5.10E-27
Small nuclear ribonucleoprotein E	-0.073	Down	15.413	1.06E-34
Membrane protein insertase YidC	-0.072	Down	15.444	1.07E-34
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit	-0.073	Down	15.445	5.96E-30
Vacuolar protein sorting-associated	-0.073	Down	15.569	6.81E-35
Costars family protein ABRACL	-0.073	Down	15.585	3.90E-29
Putative 4-methyl-5(B-hydroxyethyl)-thiazol monophosphate biosynthesis enzyme	-0.073	Down	15.590	7.18E-35
Aquaporin	-0.074	Down	15.594	9.47E-35
60S ribosomal protein L38e	-0.075	Down	15.668	8.87E-35
Patatin-like protein	-0.074	Down	15.720	2.53E-34
Splicing factor U2AF subunit	-0.075	Down	15.745	9.18E-35

Protein name [a]	PC1[b]	Direction of alterations	log_2FC[c]	$p_{adjusted}$[d]
Eukaryotic translation initiation factor 3 subunit B	-0.074	Down	15.763	9.47E-35
Reticulon family protein	-0.074	Down	15.766	9.47E-35
Lysine/histidine transporter	-0.075	Down	15.767	9.18E-35
Mitochondrial uncoupling protein	-0.077	Down	15.850	8.87E-35
Vacuolar protein sorting-associated protein 27	-0.075	Down	15.927	9.47E-35
Glutathione S-transferase	-0.077	Down	15.949	9.12E-35
Glycylpeptide N-tetradecanoyltransferase	-0.075	Down	15.960	8.54E-29
Developmentally-regulated GTP-binding protein 2	-0.076	Down	16.013	3.89E-33
Histidinol-phosphate aminotransferase	-0.074	Down	16.017	9.47E-35
Clp protease ATP binding subunit	-0.076	Down	16.169	8.65E-35
Adenylate kinase	-0.076	Down	16.193	3.38E-30
Progesterone 5-beta-reductase	-0.076	Down	16.200	1.23E-34
Xylose isomerase	-0.077	Down	16.342	7.74E-35
Succinyl-CoA ligase [ADP-forming] subunit beta	-0.078	Down	16.504	7.18E-35
Splicing factor U2af 38 kDa subunit	-0.078	Down	16.556	1.56E-32
Proliferating cell nuclear antigen	-0.078	Down	16.569	1.90E-28
Translocon-associated protein	-0.078	Down	16.685	6.03E-35
NADH dehydrogenase 1 alpha subcomplex subunit 13	-0.079	Down	16.852	5.64E-35
Arginine/serine-rich splicing factor	-0.078	Down	16,986	4.37E-35
Protein MEMO1	-0.081	Down	17.238	6.81E-35
60S ribosomal protein L32	-0.082	Down	17.330	1.99E-30
Subtilisin-like serine protease	-0.081	Down	17.420	8.30E-35
Pentatricopeptide repeat-containing protein	-0.082	Down	17.517	1.70E-35
10 kDa chaperonin	-0.083	Down	17.580	4.79E-35
AP-4 complex accessory subunit tepsin	-0.083	Down	17.647	2.41E-34
Phloem lectin	-0.086	Down	18.212	4.79E-35
Ubiquitin-like domain-containing CTD phosphatase	-0.089	Down	18.696	4.49E-35
70 kDa heat shock protein	-0.100	Down	21.144	1.70E-35

[a]Identification of peptides and annotation of protein relied on a search against amino acid sequences of *Cucumis sativus* cv. Chinese Long v2 (obtained from the FTP-site of the Cucurbit Genomics Database v1) accomplished with SEQUEST algorithm, Mercator4 (v.2.0) was used for proteins annotation, subcellular localization of proteins was defined using WoLF PSORT. Data are available via ProteomeXchange with identifier PXD037725; [b]PC, ptincipal component; [c]FC, fold change; [d]$p_{adjusted}$, adjusted p value.

Table S1-8. Gas chromatographic (GC) separation conditions and electron ionization-quadrupole-mass spectrometry (EI-Q-MS) settings for analysis of primary thermally stabile metabolites from *Cucumis sativus* roots with GC-EI-Q-MS.

Parameters	**Settings**
	GC settings
Separation column	MEGA-5 MS capillary column (30 m × 0.32 mm ID, 0.25 μm film thickness, MEGA S.r.l., Legnano, Italy)
Carrier gas / carrier gas flow rate	Helium/1.5 mL/min
Injector operation mode	Splitless mode (90 s splitless time)
Injector temperature	280°C
Temperature program	1 min at 40°C ramp 15°C/min to 70°C 1 min at 70°C ramp 6°C/min to 320°C 12 min at 320°C
Parameters	**MS settings**
Ionization mode	Electron ionization (EI)
Electron energy	70 eV
Operation mode	Positive, scanning at 0.34 sec $scan^{-1}$
m/*z* range	50 - 700

The analysis relied on Shimadzu GCMS-QP2010 Ultra, equipped with an auto sampler AOC-5000 Plus (Shimadzu).

Table S1-9. The conditions of ion pair-reversed phase ultrahigh performance liquid chromatographic (IP-RP-UHPLC) separation and the settings for electrospray ionization-triple quadrupole-tandem mass spectrometry (ESI-QqQ-MS/MS) used for the analysis of anionic primary thermally labile metabolites from *Cucumis sativus* root with IP-RP-UHPLC- ESI-QqQ-MS/MS.

Chromatography	
ACQUITY Sample Manager (SM)	
Injection mode	PartialLoop
Injection volume	5 μL
Weak wash solvent	0.3 mmol/L aq. ammonium formate
Weak wash volume	800 μL
Strong wash solvent	Acetonitrile
Strong wash volume	400 μL
Target sample temperature	4.0 C
Needle overfill flush	Automatic

Column conditions	
Separation column	EC 150/2 NUCLEOSHELL RP 18 (150 x 2 mm, particle size 2.7 µm)
Target column temperature	40.0 C
ACQUITY Binary Solvent Manager (BSM)	
Eluent A	0.3 mmol/L aq. ammonium formate
Eluent B	Acetonitrile
Seal wash duration	5 min
Flow rate	0.4 mL/min
Elution program	2% eluent B isocratic - 2 min gradient to 36% eluent B – 16 min
Mass spectrometry	
General	
Mass analyzer type	triple quadrupole-linear ion trap (QqLIT, QTRAP, operated in QqQ mode)
Ion source	TurboIonSpray®
Experiment type	multiple reaction monitoring (MRM)
Operatinon mode	negative
Cycle time (s)	1.1
Pause between ranges (ms)	5.007
Settling time (s)	0
Duration	24 min
Ion source settings	
Nebulizer gas (psig)	60
Drying gas (psig)	70
Curtain gas (psig)	40
Ion spray voltage (kV)	-4.5
Ion source temperature (°C)	450
MS/MS settings	
Fragmentation mode	CAD
MS/MS experiment type	MRM
Collision gas	nitrogen
Collision gas pressure	3 psig (medium)

Entrance potential (V)	-10.0
Scheduled MRM	enabled
Scheduled MRM type	basic
MRM detection window (s)	500
Target scan time (s)	1
Dwell time	adjusted by scheduled MRM algorithm
Q1 resolution	unit
Q3 resolution	unit
Declustering potential (DP, V)	compound-specific (listed below)
Collision potential (CE, V)	compound-specific (listed below)
Exit potential (CXP, V)	compound-specific (listed below)

Analyte-specific settings

Analyte-specific combinations of Q1 and Q3 *m*/*z* ranges (transitions)

#	Analyte	t_R (min)	Q1 (*m*/*z*)	Q3 (*m*/*z*)	DP (V)	CE (V)	CXP (V)
1	2-deoxy-*D*-ribose 5-phosphate	N/A	212.9	97.1	-40	-20	-19
2	3-[(carboxylatovinyl)oxy]benzoate	N/A	207.1	179.0	-240	-38	-13
3	5-amino-4-imidazolecarboxamide ribotide	N/A	337.1	78.9	-85	-50	-5
4	5-formamido-1-(5-phospho-D-ribosyl)imidazole-4-carboxamide	N/A	365.0	78.9	-40	-35	-10
5	5-formyl-tetrahydrofolate	N/A	472.2	315.1	-40	-35	-10
6	5-methyl-tetrahydrofolate	N/A	458.2	329.1	-40	-35	-10
7	5′-phosphoribosyl-*N*-formylglycinamide	N/A	313.0	78.9	-40	-35	-10
8	5'-phosphoribosyl-5-aminoimidazole	N/A	294.0	78.9	-40	-43	-10
9	allantoic acid	N/A	175.0	132.0	-35	-32	-12
10	beta-nicotinamide mononucleotide	N/A	334.0	78.9	-25	-16	-13
11	carboxyaminoimidazole ribotide	N/A	338.0	78.9	-40	-35	-10
12	chorismate	N/A	225.0	179.0	-35	-25	-10
13	cytidine-5'-diphosphate choline	N/A	487.0	428.0	-10	-20	-23
14	glycineamide ribonucleotide	N/A	285.0	78.9	-40	-35	-10
15	nicotinamide	N/A	121.0	76.9	-40	-16	-9
16	nicotinamide mononucleotide	N/A	333.0	78.9	-50	-30	-13
17	nicotinamide riboside	N/A	253.1	121.0	-40	-35	-10
18	phosphoribosylamine	N/A	227.0	78.9	-40	-35	-10
19	riboflavin-5′-phosphate	N/A	455.1	97.0	-35	-25	-10
20	succinylaminoimidazole-carboxamide ribotide	N/A	453.1	78.9	-40	-35	-10
21	tetrahydrofolate	N/A	444.2	176.1	-40	-35	-10

22	histidine	0.5	154.1	93.0	-40	-24	-3
23	arginine	0.6	173.1	131.0	-50	-18	-7
24	glutamine	0.6	145.1	108.9	-30	-18	-5
25	ornithine	0.6	131.1	82.9	-60	-20	-5
26	proline	0.6	114.1	86.0	-55	-18	-3
27	4-aminobutanoic acid	0.7	102.1	84.0	-35	-14	-7
28	alanine	0.7	88.0	41.9	-20	-20	-13
29	allantoin	0.7	157.0	97.2	-60	-16	-1
30	asparagine	0.7	131.1	87.1	-75	-16	-11
31	citrulline	0.7	174.1	131.0	-35	-18	-7
32	creatine	0.7	130.1	88.1	-25	-14	-5
33	cysteine	0.7	120.0	79.8	-25	-32	-2
34	lysine	0.7	145.1	99.0	-65	-14	-5
35	cystine	0.8	239.3	120.0	-40	-32	-1
36	dehydroascorbic acid	0.8	173.0	127.0	-15	-18	-17
37	glycine	0.8	74.0	74.0	-36	-13	-3
38	methionine	0.8	148.0	47.0	-45	-24	-5
39	hexoses	0.8	179.1	89.0	-50	-12	-13
40	S-adenosyl-*L*-homocysteine	0.8	383.1	133.9	-80	-36	-7
41	serine	0.8	104.0	74.0	-20	-16	-3
42	sucrose	0.8	341.1	89.0	-240	-38	-13
43	threonine	0.8	118.1	73.9	-25	-18	-3
44	uridine	0.8	243.1	109.9	-65	-22	-5
45	valine	0.8	233.3	116.0	-25	-10	-5
46	valine	0.8	116.1	7.0	-25	-20	-5
47	cytidine	1.0	242.1	108.9	-70	-18	-5
48	leucine + isoleucine	1.0	261.3	130.2	-30	-10	-1
49	leucine + isoleucine	1.0	130.1	87.1	-25	-25	-10
50	tyrosine	1.0	180.1	118.9	-60	-24	-5
51	guanosine	1.4	282.1	149.9	-80	-26	-7
52	2'-deoxyguanosine	1.5	266.1	150.0	-115	-24	-3
53	adenosine	1.5	266.1	133.9	-70	-12	-1
54	phenylalanine	1.5	164.1	103.0	-55	-24	-5
55	aspartic acid	1.9	132.0	88.0	-40	-18	-13
56	glucopyranonic acid	1.9	193.0	113.0	-20	-16	-5
57	galactopyranuronic acid	2.0	193.0	113.0	-20	-16	-5
58	glyoxilic acid	2.0	73.0	45.0	-25	-25	-10
59	*D*-galactonic acid/ *D*-gluconic acid	2.1	195.1	129.0	-50	-18	-9
60	glucosamine 6-phosphate	2.1	258.0	97.0	-45	-24	-5
61	glutamic acid	2.1	146.0	102.0	-80	-18	-9
62	ribonic acid	2.3	165.0	75.0	-45	-20	-35
63	glucosamine 1-phosphate	2.4	258.0	78.9	-55	-42	-1
64	2'-deoxyadenosine	2.5	250.1	134.0	-115	-26	-9
65	glucolate	2.5	75.0	47.0	-30	-14	-13
66	shikimic acid	2.5	173.0	92.9	-15	-20	-5
67	3-dehydroxyshikimic acid	2.7	171.0	127.0	-25	-16	-15
68	quinic acid	2.7	191.1	85.0	-50	-28	-13
69	uric acid	2.7	167.0	124.0	-45	-20	-7
70	ascorbic acid	2.8	175.0	115.0	-25	-25	-5
71	carbamoyl-alanine	2.8	131.0	87.9	-10	-14	-13
72	chloride	2.8	35.0	35.0	-50	-10	-10
73	dihydroorotic acid	3.3	157.0	112.7	-40	-10	-5

74	tryptophan	3.4	203.1	116.2	-50	-22	-7
75	lactic acid	3.8	89.0	42.9	-15	-12	-5
76	glutathione	4.0	306.1	143.0	-5	-26	-7
77	phosphate	4.0	96.9	78.9	-40	-18	-15
78	cyclic guanosine monophosphate	4.5	344.0	150.0	-70	-34	-11
79	orotic acid	4.8	155.0	110.7	-25	-12	-5
80	pyruvic acid	4.9	87.0	43.0	-30	-12	-1
81	nicotinamide adenine dinucleotide	5.0	662.1	540.1	-45	-22	-15
82	glucose 6-phosphate	5.4	259.1	97.0	-65	-18	-13
83	glyceraldehyde 3-phosphate	5.4	169.0	97.0	-30	-12	-5
84	fructose 6-phosphate	5.5	259.0	96.9	-30	-20	-11
85	2-keto-3-deoxy-6-phosphogluconate	5.8	257.0	97.0	-30	-20	-9
86	erythrose 4-phosphate	5.9	199.0	96.8	-40	-12	-5
87	adenosine 2',3'-cyclic mono-phosphate	6.2	328.0	134.0	-125	-36	-5
88	ribulose-5-phosphate	6.3	229.0	96.8	-35	-20	-5
89	glucose-1-phosphate	6.4	259.0	240.8	-30	-16	-15
90	ribulose-5-phosphate/xylulose-5-phosphate	6.5	229.0	96.8	-45	-18	-15
91	mevalonic acid lactone	6.6	147.1	59.1	-45	-20	-7
92	sedoheptulose 7-phosphate	6.6	289.0	97.0	-50	-22	-5
93	2-C-methylerythritol 4-phosphate	6.8	215.0	78.9	-40	-56	-9
94	glycerophosphoric acid	6.8	171.0	78.8	-45	-24	-1
95	cytidine monophosphate	7.0	322.2	79.0	-65	-68	-5
96	nicotinic acid	7.0	122.0	77.9	-55	-16	-13
97	pantothenic acid	7.0	218.1	88.1	-55	-18	-5
98	adenosine 3',5'-cyclic mono-phosphate	7.2	328.0	134.0	-125	-36	-5
99	ribose-1-phosphate	7.7	229.0	211.0	-50	-14	-3
100	uridine monophosphate	7.8	323.0	79.0	-65	-68	-5
101	guanosine 5'-monophosphate	8.1	362.1	78.9	-65	-66	-5
102	inosinic acid	8.3	347.0	134.8	-70	-38	-7
103	2'-deoxyguanosine 5'-monophosphate	8.4	346.1	78.8	-80	-42	-3
104	dihydroxyacetone phosphate	8.5	169.1	97.0	-35	-14	-11
105	thymidine-5'-phosphate	8.7	321.0	78.8	-65	-58	-3
106	1-deoxy-D-xylulose 5-phosphate	8.8	213.0	97.0	-50	-18	-1
107	adenosine monophosphate	8.8	346.1	78.8	-70	-52	-3
108	glutathione disulfide	8.9	611.1	306.1	-35	-34	-7
109	2'-deoxyadenosine 5'-monophosphate	9.1	330.1	195.0	-85	-22	-17
110	digalacturonic acid	9.5	369.1	175.0	-75	-18	-17
111	phosphocreatine	9.6	210.0	78.9	-35	-22	-1
112	malate	9.8	133.0	115.0	-20	-16	-5
113	succinic acid	9.9	117.0	73.0	-25	-16	-7
114	3-hydroxypyruvate	10.0	103.0	59.0	-30	-22	-7
115	4-diphosphocytidyl-2-C-methyl-*D*-erythritol	10.0	520.1	78.9	-120	-108	-9
116	ureidosuccinic acid	10.1	175.1	131.8	-25	-16	-7
117	sulfate	10.2	97.0	97.0	-40	-18	-15
118	uridine-5'-diphosphate-glucose	10.2	565.0	323.0	-125	-36	-11
119	uridine-diphosphate-*N*-acetylglucosamine	10.2	606.1	384.8	-175	-36	-25
120	fumaric acid	10.4	115.0	71.0	-5	-12	-13
121	adenosine diphosphoribose	10.5	558.1	346.0	-170	-34	-19
122	oxaloacetic acid-1	10.5	131.0	87.0	-35	-10	-17

123	oxaloacetic acid-2	10.5	131.0	43.0	-35	-18	-11
124	adenosine diphosphate glucose	10.6	588.1	345.9	-140	-32	-19
125	α-ketoglutaric acid	10.7	145.0	101	-10	-12	-13
126	pentanoates	10.7	101.1	101.1	-50	-10	-5
127	2C-methyl-*D*-erythritol 2,4-cyclodiphosphate	10.9	277.0	79.0	-45	-64	-37
128	(R)-5-phosphomevalonic acid	11.2	227.0	97.0	-30	-35	-10
129	1,4-dihydronicotinamide adenine dinucleotide	11.4	664.1	78.9	-100	-124	-1
130	folate	11.5	440.1	311.1	-40	-35	-10
131	2-phosphoglyceric acid	12.7	185.0	79.0	-25	-20	-35
132	isopentenyl pyrophosphate	12.8	245.0	78.9	-15	-44	-37
133	2*P*-glycolate	12.9	155.0	79.0	-15	-36	-35
134	xanthosine-5'-phosphate	12.9	363.0	151.1	-60	-36	-5
135	guanosine-5'-diphosphate	13.0	442.0	78.9	-85	-70	-3
136	6-phosphogluconic acid	13.1	275.0	79.0	-60	-66	-5
137	flavin adenine dinucleotide	13.1	784.1	79.0	-60	-130	-1
138	uridine-5'-diphosphate	13.1	403.0	78.8	-75	-68	-3
139	cytidine-5'-diphosphate	13.1	402.0	78.9	-65	-70	-5
140	3-phosphoglyceric acid	13.2	185.0	96.7	-30	-22	-7
141	(2E)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate	13.3	261.0	79.0	-40	-52	-9
142	aconitic acid	13.3	173.0	128.7	-25	-10	-55
143	adenosine-5'-diphosphate	13.3	426.2	78.9	-75	-66	-3
144	nicotinamide adenine dinucleotide phosphate	13.4	743.1	620.0	-55	-22	-17
145	thymidine-5'-diphosphate	13.4	401.0	78.8	-70	-68	-3
146	2'-deoxyadenosine-5'-diphosphate	13.5	410.0	78.9	-60	-76	-3
147	trigalacturonic acid	13.8	545.1	369.0	-105	-24	-25
148	phosphoenolpyruvic acid	13.9	167.0	78.8	-20	-16	-9
149	isocitric acid	14.1	191.1	73.0	-45	-28	-31
150	citric acid	14.2	191.0	87.0	-35	-22	-15
151	dimethylallylpyrophosphat	14.2	245.0	78.9	-15	-44	-37
152	cytidine 5'-triphosphate	15.4	482.2	158.8	-85	-36	-9
153	4-diphosphocytidyl-2-C-methyl-*D*-erythritol 2-phosphate	15.5	600.0	78.9	-115	-126	-19
154	desoxyadenosintriphosphat	15.5	490.0	391.9	-90	-34	-25
155	sedoheptulose 1,7-bisphosphate	15.5	369.0	97.0	-35	-20	-27
156	adenosine triphosphate	15.6	506.2	158.8	-80	-38	-9
157	fructose-1,6-diphosphate	15.7	339.0	96.9	-35	-22	-11
158	ribulose-1,5-bisphosphate	15.7	309.0	97.0	-35	-20	-27
159	adenylosuccinic acid	15.8	462.3	133.9	-85	-62	-7
160	orotidine 5'-monophosphate	15.8	367.0	78.9	-50	-78	-1
161	mevalonate-5-diphosphate	15.9	307.0	78.9	-25	-35	-13
162	guanosine-5'-triphosphate	16.0	522.0	158.8	-90	-48	-9
163	deoxythymidine 5'-triphosphate	16.0	481.0	158.7	-80	-38	-9
164	dihydronicotinamide adenine dinucleotide phosphate	16.3	744.1	79.0	-40	-16	-9
165	ADP-ribose-2`-phosphate	16.5	638.0	426.0	-170	-34	-19
166	coenzyme A	17.0	766.1	407.9	-245	-50	-19
167	inositol triphosphate	17.4	419.0	320.8	-25	-28	-21
168	5-phosphoribosyl diphosphate	16.8	388.9	176.8	-55	-28	-9

169	S-acetyl coenzyme A	17.6	808.1	407.9	-220	-52	-27
170	methylmalonyl coenzyme A	17.8	866.1	408.0	-185	-58	-21
171	geranyl diphosphate	18.0	313.1	78.9	-65	-46	-1
172	β-hydroxy β-methylglutaryl-CoA	18.5	910.1	407.9	-220	-52	-27
173	malonyl coenzyme A	18.6	852.1	408.0	-185	-58	-21
174	ent-copal-8-ol diphosphate	19.0	467.2	78.8	-220	-52	-27
175	succinyl coenzyme A	19.0	866.1	407.6	-260	-56	-25
176	inositol-1,3,4,5-tetraphosphate	19.2	498.3	400.7	-100	-30	-27
177	β-methylcrotonyl coenzyme A	19.1	848.1	407.8	-185	-58	-21
178	1-diphosinositol pentakisphosphate	19.2	578.9	480.6	-25	-32	-31
179	geranylgeranyl pyrophosphate	19.2	449.2	78.8	-65	-68	-35
180	isovaleryl coenzyme A	19.2	850.2	407.9	-240	-58	-19
181	acetoacetyl coenzyme A	19.5	580.1	408.0	-220	-52	-27
182	Phytic acid	19.6	658.9	560.7	-145	-38	-31
183	farnesyl diphosphate	19.7	381.1	78.9	-50	-50	-5

The analysis relied Waters ACQUITY UPLC H-Class UPLC System (Waters GmbH, Eschborn, Germany) coupled online to a hybrid triple quadrupole-linear ion trap mass spectrometer (QqLIT) AB Sciex QTRAP 6500 (AB Sciex, Darmstadt, Germany).

Table S1-10. The conditions of ultrahigh performance liquid chromatographic (UHPLC) separation and the settings for electrospray ionization-quadrupole-time of flight mass spectrometry (ESI-QqTOF-MS) applied for the analysis of semi-polar secondary metabolites from *Cucumis sativus* root.

Chromatography	
SIL-30AC Autosampler	
Injection mode	Partial Loop
Injection volume	5 μL
Wash solvent	50% MeOH
Wash solvent volume	300 μL
Cooler temperature	4.0 C
Rinse type	Internal & external
Rinse mode	Before and after aspiration
Needle overfill flush	Rinse port + rinse pump
Rinse time	2 sec
Column conditions	
Separation column	ACQUITY UPLC BEH C18 Column (50 x 2.1 mm, particle size 1.7 μm)
Column oven temperature	40.0 C
LC separation parameters	
Eluent A	0.3 mmol/L aq. ammonium formate
Eluent B	acetonitrile
Flow rate	0.4 mL/min
Elution program	5% eluent B isocratic - 1 min gradient to 95% eluent B – 6 min 95% eluent B isocratic – 2.5 min gradient to 5% eluent B – 0.5 min 5% eluent B isocratic – 2.5 min (re-equilibration)
Mass spectrometry	

General	
Mass analyzer type	quadrupole-time of flight (QqTOF-MS)
Ionsource	ESI
Experiment type	Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra (DIA)
Operatinon mode	positive, negative
Cycle time (s)	1.108
Duration	6.5 min
Ion source settings	
Nebulizer gas (L/min)	3
Drying gas (L/min)	10
Ion spray voltage (kV)	4.0/-3.0 (positive/negative mode)
Ion source temperature (°C)	210
MS settings	
Experiment type	TOF-MS
m/*z* range	65 - 1250
Accumulation time (ms)	100
ID function	ON
MS/MS Settings	
Collision gas	Ar
MS/MS experiment type	DIA
SWATH window number	48
SWATH window width (*m*/*z*)	24.7
CE	10-80 (45±35V)
Accumulation time (ms)	21

ID function	OFF
Collision potential (V)	45/-45 (positive/negative mode)
Collision energy spread (V)	35 (positive/negative mode)

The analysis relied on the high-performance liquid chromatograph coupled on line to a QqTOF mass spectrometer Shimadzu LCMS-9030 System (Shimadzu, Kyoto, Japan).

Table S1-11. Parameters of the nanoHPLC separation method employed in the nanoLC-QqTOF-MS-based proteomics experiments.

Parameter	Settings	
Method parameters		
Injection volume	2 μL	
Injection mode	sample loading pressure 217.5 bar	
Column temperature	45^{0}C	
Eluents		
Solvent A	0.1% (v/v) aq. formic acid	
Solvent B	0.1% (v/v) formic acid in acetonitrile	
Elution regimen	Time (min)	% B
	0	2
	40	40
	40,5	85
	55,9	85
	57,9	2
	60	2
Trap Column	Thermo Trap Cartridge 5mm	
Volume	0.148 μL	
Equilibration pressure	217.5 bar	
Estimated equilibration time	0.65 min	
Equilibration volume (×10)	1.48 μL	
Separation Column	Bruker FORTY	
Volume	0.742 μL	
Equilibration pressure	600.0 bar	
Estimated equilibration time	7.91 min	
Equilibration volume (×4)	2.97 μL	

Table S1-12. Instrument settings applied for ESI-QqTOF-MS DDA experiments employed in the nanoLC-QqTOF-MS-based proteomics experiments.

Parameter	Settings
MS conditions	
Ionization mode	Positive
Mass to charge ratio (*m*/*z*) range	150 – 2200
Spectra rate	2 Hz
End plate offset	500 V
Capillary voltage	4500 V
Nebulizer	1.5 bar
Dry temperature	200°C
Dry gas	2.0 l/min
MS/MS conditions	
Scan mode	Auto MS/MS
Fragmentation type	Collision-induced dissociation
Isolation width	2 – 3
MS/MS spectra acquisition	8 – 32 Hz
Threshold (per 1000 sum.)	250 cts
Cycle time	3 sec
Collision energy	from 23 eV (m/z 300) to 65 eV (m/z 1300)
Scan mode	Auto MS/MS

Table S1-13. PEAKS Studio 10.6 parameters for database search settings.

Database search settings	
Analysis program	PEAKS Studio 10.6 build 20201221
Parent mass error tolerance:	10.0 ppm
Fragment mass error tolerance:	0.05 Da
Precursor mass search type:	Monoisotopic
Protease	Trypsin
Missed cleavage sites	2
FDR	2
Fixed modifications:	Carbamidomethylation: 57.02
Variable modifications	Oxidation (M): 15.99 Acetylation (Protein N-term): 42.01 Deamidation (NQ): 0.98
Max variable PTM per peptide	2
Filter charge	1 – 7

Figures

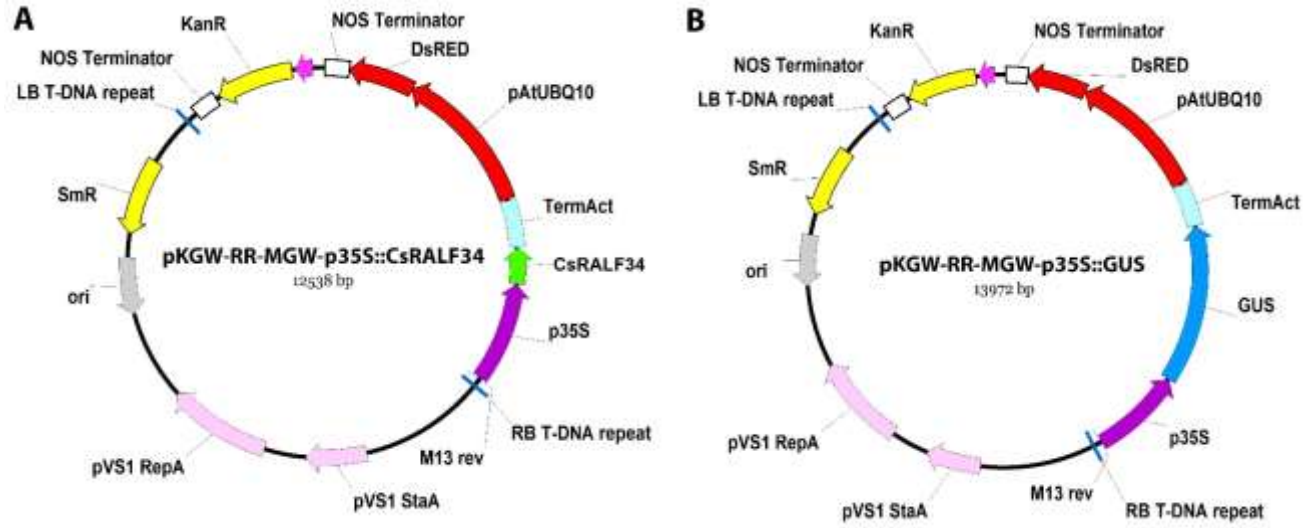


Figure S1-1. Map of vectors for *CsRALF34* overexpression.

Maps of binary vectors (A) pKGW-RR-MGW-*p35S::CsRALF34* and (B) pKGW-RR-MGW-*p35S::gusA* used for *R. rhizogenes*-mediated transformation of cucumber seedlings. Both vectors (A,B) contain DsRED1 under the control of the *AtUBQ10* promoter as a screenable marker within the T-DNA borders. (A) pKGW-RR-MGW-*p35S::CsRALF34* vector contains *p35S::CsRALF34* fusion for *CsRALF34* overexpression and (B) pKGW-RR-MGW-*p35S::gusA* contains *p35::gusA* fusion as a control against *CsRALF34* overexpression.

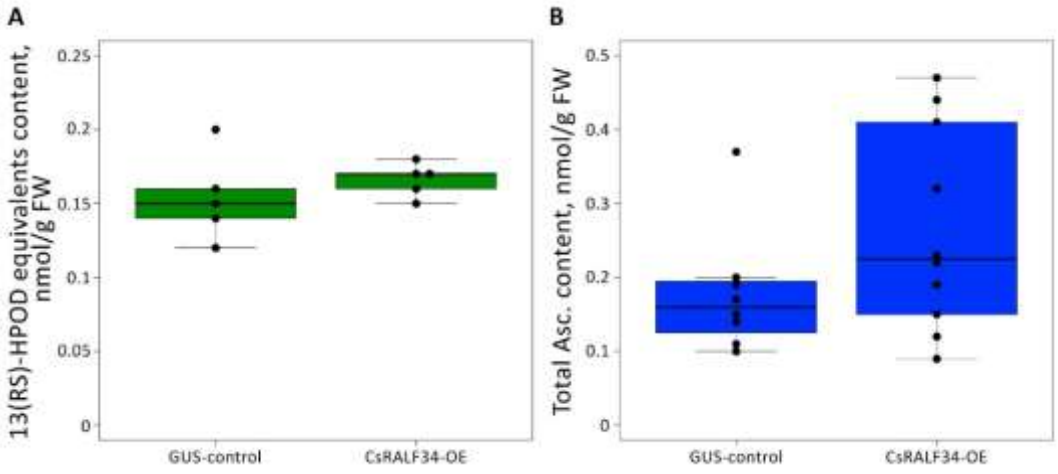


Figure S1-2. Biochemical characterization of *Cucumis sativus* transgenic roots overexpressing *CsRALF34*.

Graphs were drawn using R software default code for boxplot and stripchart functions. (**A**) The contents of hydroperoxides (expressed as 13S-hydroperoxy-9Z,11E-octadecanoic acid (13(RS)-HPOD) equivalents) in GUS-control roots (overexpression of β-glucuronidase gene) and with *CsRALF34* overexpression (RALF34-OE). (**B**) Total ascorbate (Asc) contents in GUS-control and in RALF34-OE. Statistical analysis using Student's t-test showed no significant differences ($p > 0.05$) in *CsRALF34* overexpression group compared to the GUS-control. The raw data are presented in Supplementary information 2.

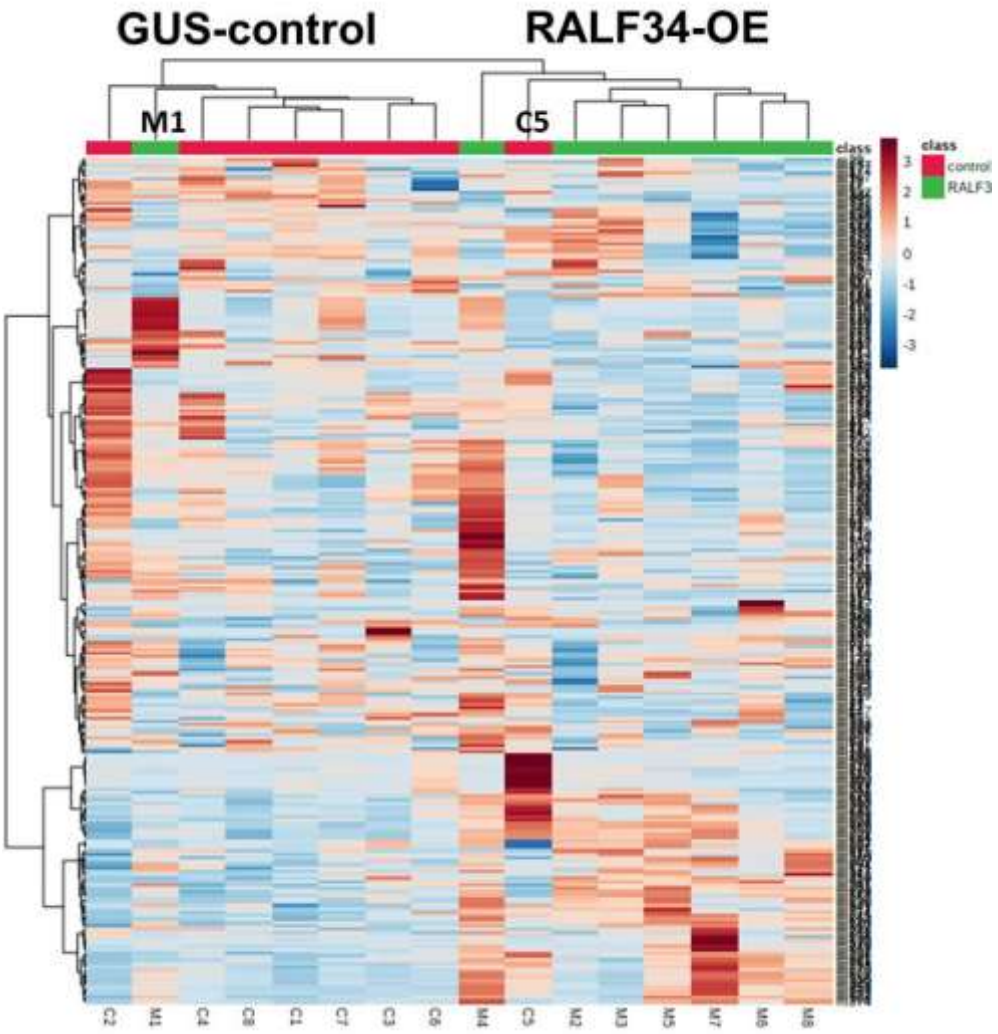
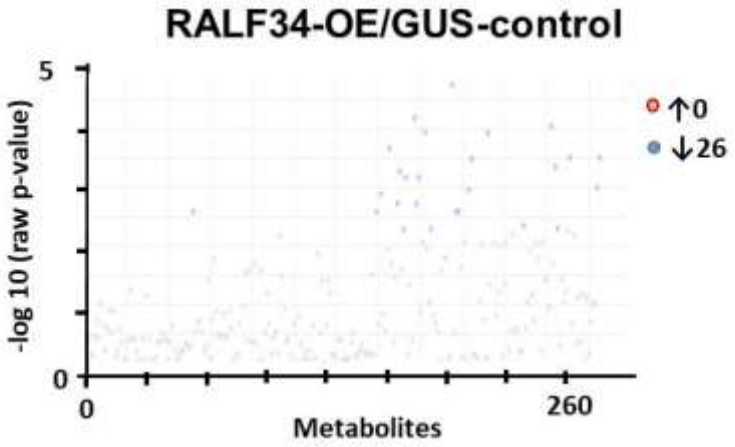


Figure S1-3. Results of hierarchical clustering with a heatmap representation of all primary metabolites.

Annotated with GC-EI-Q-MS and RP-IP-UHPLC-QqQ-MS/MS after merging together both result sets and processing the resulted data matrix by Metaboanalyst online tool. Based on the results of hierarchical clustering, the samples M1 and C5 can be treated as the outliers and were, therefore, excluded from the further analysis.

A

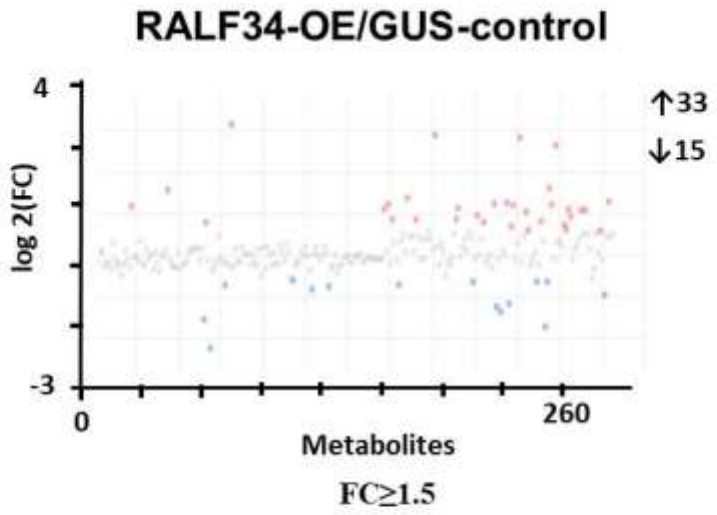


B

significan tdown metabolites	t.stat	p.value	-log10(p)	FDR
serine	-6.857	0.000	4.756	0.004
glycerophosphoric acid	-5.975	0.000	4.190	0.006
nicotinic acid	-5.766	0.000	4.049	0.006
isocitric acid	-5.612	0.000	3.943	0.006
citric acid	-5.225	0.000	3.671	0.009
sedoheptulose 7-phosphate	-4.999	0.000	3.509	0.009
arginosuccinat	-4.999	0.000	3.509	0.009
adenylosuccinic acid	-4.971	0.000	3.488	0.009
ornithine	-4.788	0.000	3.354	0.011
fructose 6-phosphate	-4.682	0.001	3.275	0.012
glycine	-4.545	0.001	3.173	0.013
glucose-6-phosphate	-4.539	0.001	3.168	0.013
glucose	-4.310	0.001	2.994	0.018
aconitic acid	-4.270	0.001	2.964	0.018
arginine	-4.173	0.001	2.888	0.020
erythrose 4-phosphate	-3.971	0.002	2.731	0.026
glutamine	-3.962	0.002	2.725	0.026
threonine	-3.801	0.003	2.597	0.029
sucrose	-3.787	0.003	2.587	0.029
6-phosphogluconic acid	-3.784	0.003	2.584	0.029
orotidine 5'-monophosphate	-3.430	0.005	2.302	0.049
malic acid	-3.418	0.005	2.293	0.049
glucose-1-phosphate	-3.408	0.005	2.285	0.049
gluconic/galacturonic acid	3.491	0.004	2.351	0.047
D-mannose	3.790	0.003	2.589	0.029
cGMP	5.595	0.000	3.932	0.006

Figure S1-4. Visualization of the results (A) and the corresponding statistical information (B) acquired in the t-test accomplished for the aqueous methanolic extracts obtained from the *Cucumis sativus* roots with *CsRALF34* overexpression or from the control group.

A



B

	Significant changed metabolites	Fold Change	log2(FC)
significant down	5-keto-*D*-gluconic acid	0.213	-2.231
	glutathione	0.306	-1.710
	D-mannose	0.343	-1.542
	cGMP	0.391	-1.356
	ATP	0.425	-1.235
	CTP	0.447	-1.161
	UTP	0.519	-0.947
	RI_2785_unknwon_disacharide	0.569	-0.814
	melibiose	0.598	-0.742
	1,2,3,4,6-*O*-glucopyranose, 5TMS	0.610	-0.714
	ureidosuccinic acid	0.614	-0.704
	ascorbic acid	0.643	-0.638
	glutathione disulfide	0.645	-0.633
	gluconic/galacturonic acid	0.648	-0.625
	sucrose	0.658	-0.603

	Significant changed metabolites	Fold Change	log2(FC)
significant up	UMP	1.507	0.592
	fructose-1,6-diphosphate	1.526	0.609
	NAD+	1.538	0.621
	dADP	1.620	0.696
	nicotinic acid	1.665	0.735
	decanedioic acid, 2TMS	1.736	0.796
	adenosine	1.745	0.803
	glycolate	1.766	0.821
	glucose-1-phosphate	1.813	0.858
	arginine	1.825	0.868
	ribose-1-phosphate	1.834	0.875
	orotidine 5'-monophosphate	1.916	0.938
	3-phosphoglyceric acid	1.941	0.957
	1-deoxy-*D*-xylulose 5-phosphate	2.069	1.049
	ribonic acid	2.128	1.089
	shikimic acid	2.130	1.091
	ornithine	2.151	1.105
	2-phosphoglyceric acid	2.157	1.109
	2-deoxyribose 5-phosphate	2.202	1.139
	serine, 2TMS	2.277	1.187
	dGMP	2.301	1.202
	inosinic acid	2.328	1.219
	6-phosphogluconic acid	2.346	1.230
	AMP	2.364	1.241
	CMP	2.401	1.264
	xanthosine-5'-phosphate	2.456	1.296
	2-deoxyribose 5-phosphate	2.596	1.376
	2-phenylglycine	2.974	1.573
	histidine	3.055	1.611
	delta3-isopentenyl pyrophosphate	6.249	2.644
	dimethylallylpyrophosphat	7.109	2.830
	isopentenyl pyrophosphate	7.439	2.895
	N-α-acetyl-*L*-lysine	8.881	3.151

Figure S1-5. Visualization of the results (A) and the corresponding statistical information (B) acquired in the fold-change (FC) analysis with the cut-off FC ≥ 1.5, accomplished for the aqueous methanolic extracts obtained from the *Cucumis sativus* roots with *CsRALF34* overexpression or from the control group.

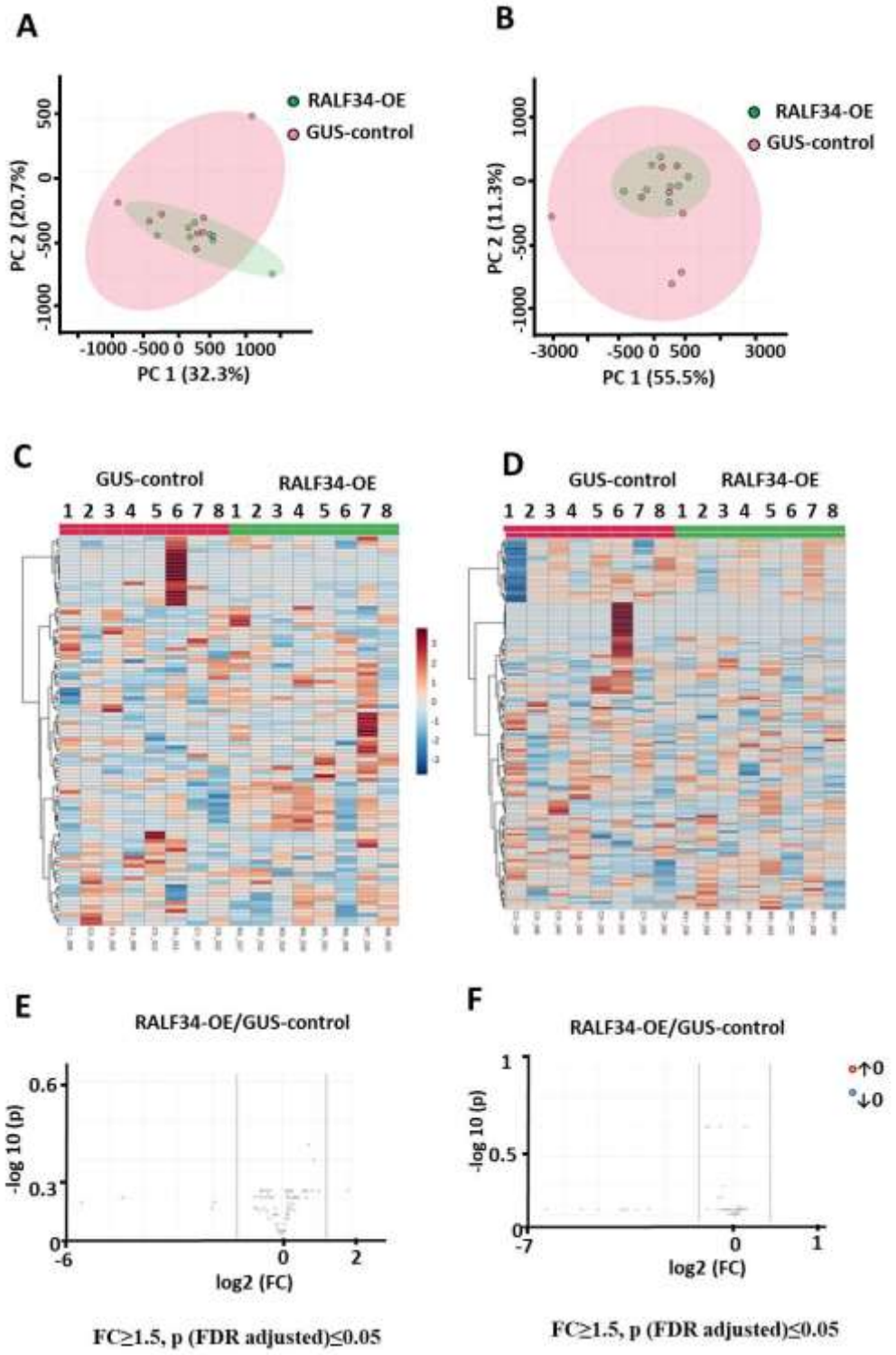


Figure S1-6. Analysis of the semi-polar secondary metabolites: post-processing of the RP-HPLC-QqTOF-MS data.

The negative (A, C and E) and positive (B, D and F) ion mode with principal component analysis, PCA (score plots, A and B), hierarchical clustering with the heatmap representation (C and D) and t-test visualization (Volcano plots, E and F).

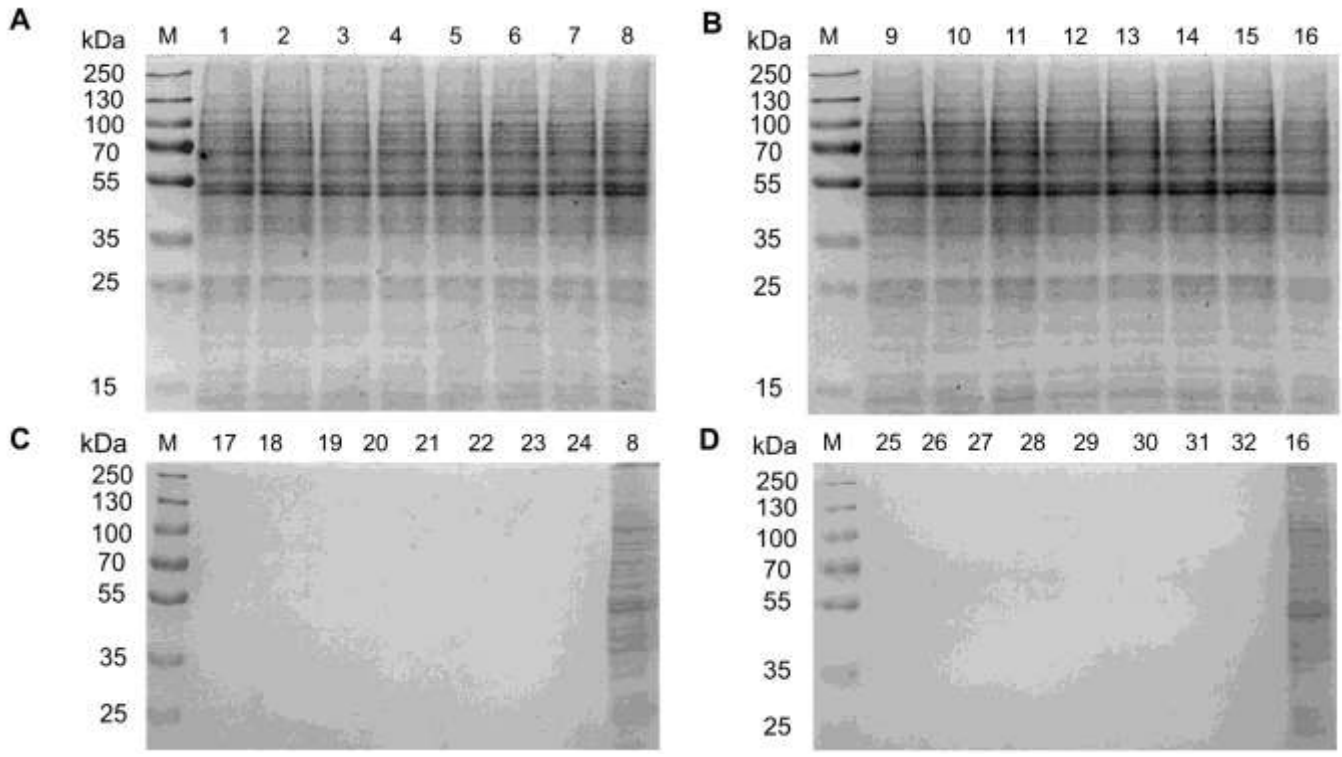


Figure S1-7. Electrophoregrams of total protein isolates and their tryptic hydrolysates from the *Cucumis sativus* roots from the control group (GUS-control) and with *CsRALF34* overexpression (CsRALF34-OE).

Isolates from 5 μg of total protein (panels A and B, lanes 1-16) and their tryptic hydrolysates (panels C and D, lanes 17-32) for control plants (A, C) and *CsRALF34* overexpression (B, D). M – marker of molecular weight (PageRuller Plus Prestained Protein Ladder, 10–250 kDa, Thermo Fisher Scientific). The gels were stained with 0.1% (w/v) Coomassie G-250 solution.

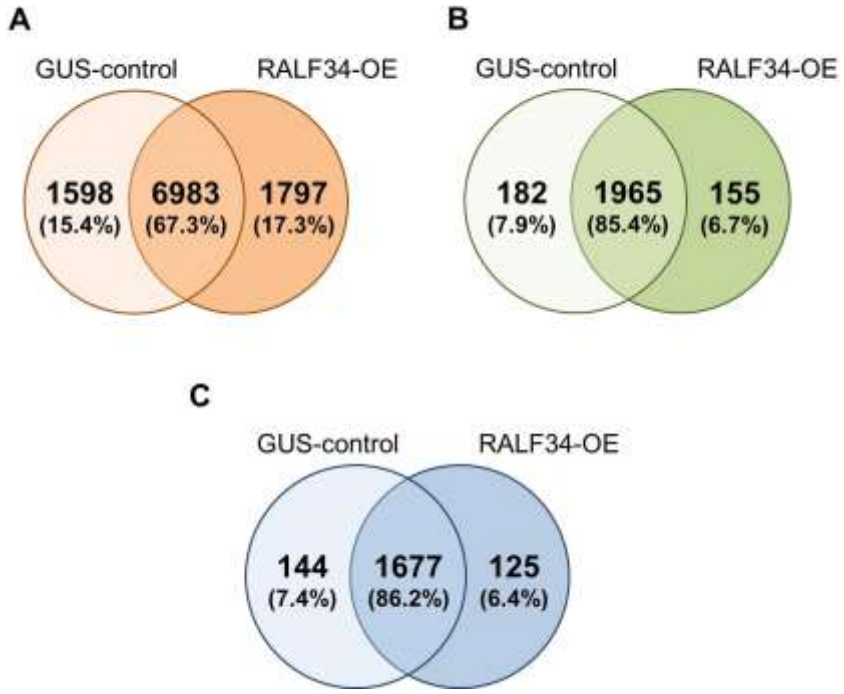


Figure S1-8. Number of peptides (A), proteins (B) and protein groups (C), identified in control (GUS-control) and CsRALF34-OE (overexpression of *CsRALF34*) *Cucumis sativus* roots.

Identification and annotation of peptides and proteins relied on *Cucumis sativus* sequence database in PEAKS Studio 10.6 software.

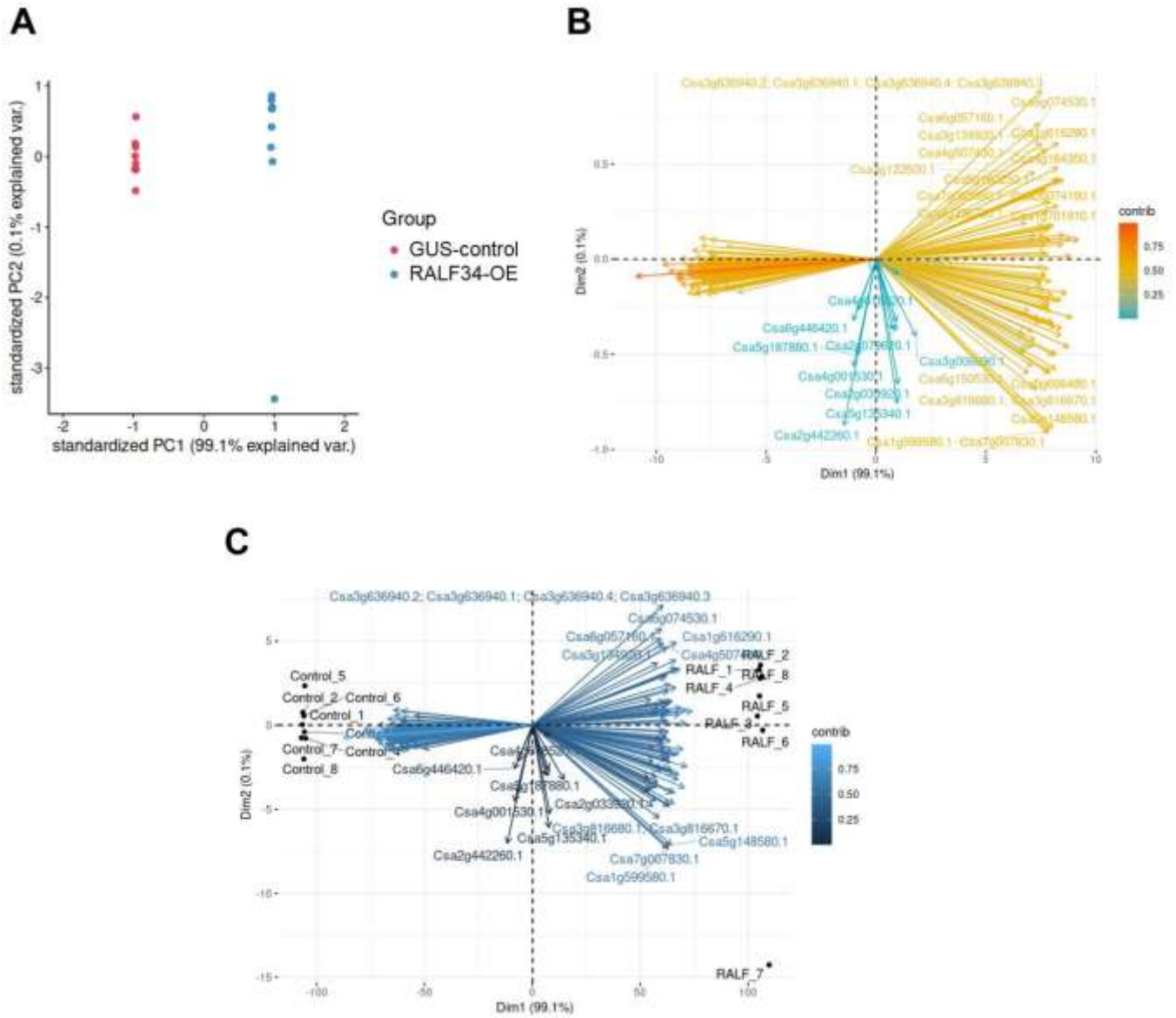


Figure S1-9. Principal component analysis (PCA) with a score plot (A), loadings plot (B) and a biplot illustrating correlation between principal components and source variables (C).

The complete list of the eigenvalues (loadings) for all differentially expressed proteins is provided in Table S1-4.

Protocols

Protocol S1-1. The full list of reagents.

Unless stated otherwise, materials were obtained from the following manufacturers. AMRESCO LLC (Cleveland, Ohio, USA): bis-acrylamide (ultra pure grade), tris-(2-carboxyethyl)-phosphine hydrochloride (ultra pure grade), 2-mercaptoethanol (biotechnology grade), phenylmethyl sulfonyl fluoride (high purity grade); Bioanalytical Technologies 3M Company (Saint Paul, Minnesota, USA): Empore™ solid phase octodecyl extraction discs; Calbiochem (Madison, Wisconsin, USA): glycine (molecular biology grade); Carl Roth Gmbh (Karlsruhe, Germany): glacial acetic acid (ROTIPURAN®, p.a. 100%); Dia-M (Moscow, Russia): phenol for molecular biology (99.5%); Ekos-1 (Moscow, Russia): acetone (extra pure); Helicon (Moscow, Russia): *N,N,N′,N′*-tetramethylethylenediamine (ultra pure), ammonium persulfate (ACS grade), sodium dodecyl sulfate (biotechnology grade), acrylamide 2K (standard grade, extra pure), Urea (USP grade), polyoxyethilene-20-sorbitan monolaurate (reagent grade), DL-dithiothreitol (biotechnology grade), potassium chloride (CP); LSU Research Institute (Saint Petersburg, Russia): chloroform; Merck KGaA (Darmstadt, Germany): acetonitrile (LC-MS grade), ethanol (LC grade), methanol (LC-MS grade); Reachem (Moscow, Russia): hydrochloric acid (GR for analysis), trichloroacetic acid, sodium carbonate (extra pure); SERVA Electrophoresis GmbH (Heidelberg, Germany): Coomassie Brilliant Blue G-250 (ultra pure), 2-mercaptoethanol (research grade), trypsin NB (sequencing grade, modified from porcine pancreas); Thermo Fisher Scientific (Waltham, Massachusetts, USA): PageRuller™ Plus Prestained Protein Ladder #26619 (10–250 kDa); Dichrom GmbH (Marl, Germany): Progenta™ adaptors for stage-tips; Sigma-Aldrich (Saint Louis, Missouri, USA): ammonium formate (for LC-MS, ≥ 99.0%), ascorbate oxidase from *Cucurbita sp.*, catalase from bovine liver, ethylenediaminetetraacetic acid, iodoacetamide (BioUltra), ammonium bicarbonate (≥ 99.0%), formic acid (≥ 98%) , xylenol orange tetrasodium salt (ACS grade), ammonium acetate (≥ 98%), sorbitol (GR for analysis), tributylamine (≥ 99%); Vekton (St. Petersburg, Russia): thiobarbituric acid (≥ 99%). Water was purified in-house (resistance 5–15 mΩ/cm) on water conditioning and purification systems Elix 3 UV (Millipore, Moscow, Russia) or Millipore Milli-Q Gradient A10 system (resistance 5–15 mΩ/cm, Merck Millipore, Darmstadt, Germany).

Protocol S1-2. Determination of hydrogen peroxide contents.

Analysis of H_2O_2 contents in cucumber roots relied on the method described by Chantseva, *et al.* [1] with modifications. In detail, 100 mg of frozen milled plant material were supplemented with 1 mL of ice-cold $HClO_4$ (0.4 mol/L), vortexed intensively (30 s) and centrifuged (13000x g, 10 min, 4°C). Then the supernatants were neutralized by 4 mol/L potassium hydroxide to achieve pH = 7 with sub-sequent centrifugation (13000x g, 10 min, 4°C). Next, 375 μL of sodium phosphate buffer (0.1 mol/L, pH 5.6), 1 UN of ascorbate oxidase (1.5 μL) and 125 μL of each sample extract were added to two UV grade cuvettes, and the mixtures were vortexed for 30 s. Afterwards, 1 μL of catalase solution (1 UN) was added to one of the cuvettes with further incubation (2 min, 25°C). Finally, 500 μL aliquots of FOX solution (0.2 mmol/L xylenol orange tetrasodium salt, 200 mmol/L sorbitol, 50 mmol/L sulphuric acid, 0.5 mmol/L ammonium ferrous sulphate hexahydrate) were added to both cuvettes (with and without supplemented catalase). The cuvettes were vortexed (30 s) and left in the dark for 30 min, after what the absorbance was measured at 575 nm (the length of the optical way – 1 cm) and calculations were accomplished as described previously [1].

Protocol S1-3. Determination of lipid peroxidation product contents.

Lipid peroxidation products were quantified as malondialdehyde equivalents according to the protocol described by Soboleva, *et al.* [2] with modifications. In detail, approximately 25 mg of frozen milled plant material were left on ice for 3 minutes, before addition of 300 μL 5% (w/v) trichloroacetic acid, the resulted suspensions were vortexed for 30 s and centrifuged (10000 x g, 20 min, 4°C). Afterwards, 1 mL of thiobarbituric acid (TBA) reagent (0.5% w/v TBA in 20% trichloroacetic acid) were supplemented to 250 μL of supernatant. The mixtures were incubated (30 min, 95°C). Afterwards, the mixtures were cooled on ice to stop the reaction, centrifuged (1900 x g, 10 min, 4°C) and 1 mL of each coloured supernatant was used to measure the absorbance at 532 nm against the proper blank (250 μL 5% w/v trichloroacetic acid and 750 μL TBA reagent). The non-specific absorbance at 600 nm was

subtracted from the absorbance acquired at 532 nm. The contents of malondialdehyde were calculated with ε = 155 $mM^{-1}cm^{-1}$. The length of optical way – 1 cm.

Protocol S1-4. Determination of lipid hydroperoxide contents.

Analysis of lipid hydroperoxide contents relied on the method of Frolov, *et al.* [3] with modifications. In detail, 10 mg of frozen milled plant material were transferred on ice and 3 min later supplemented with 750 μL of ice-cold chloroform-methanol mixture (ratio 1:2, v/v) and 150 μL of aquatiq acetic acid (0.15 mol/L), then vortexed for 30 s. The next step was addition of 225 μL of chloroform with 0.01% (w/v) butylated hydroxytoluene (BHT) and the same volume of distillated water, vortexing (30 s) and centrifugation (3000x g, 5 min, 4°C). Afterwards, the lower phase was collected, transferred to black polypropylene tubes and dried under reduced pressure (CentriVap Vacuum Concentrator, Labconco, Kansas City, Missouri, USA) for 120 min. After this, 100 μL of 0.01% (w/v) BHT in methanol was added and tubes were left on ice for 30 min. Further addition of 900 μL of ferrous ion oxidation xylenol orange (1.0 mmol/L xylenol orange and 2.5 mmol/L ammonium ferrous sulphate in 250 mmol/L H_2SO_4 – 0.01% (w/v) BHT in methanol, 1:9, v/v) was also accompanied by 30 min incubation on ice and, afterwards, the absorption at 560 nm (the length of the optical way – 1 cm) against pure FOX reagent was assessed. The hydroperoxide contents were calculated as 13S-hydroperoxy-9Z, 11E-octadecanoic acid equivalents, $\varepsilon = 6.0 \times 10^4\ M^{-1}cm^{-1}$.

Protocol S1-5. Determination of ascorbic acid contents.

Determination of the ascorbate contents relied on the method described by Shumilina, *et al.* [4] with modifications. In detail, 50 mg of frozen milled plant material were supplemented with 500 μL of ice-cold $HClO_4$ (2.5 mol/L), were vortexed intensively for 30 s and centrifuged (13000 x g, 10 min, 4°C). Afterwards, the supernatants were neutralized by saturated solution of Na_2CO_3 to achieve pH 7.0 with further centrifugation (13000x g, 10 min, 4°C). To assess the contents of reduced ascorbate (ascorbic acid), 1.8 μL of sodium phosphate buffer (0.1 mol/L, pH 5.6) and 200 μl of each supernatant were supplemented to a UV grade cuvette. The suspensions were mixed by the vortex for 30 s, and the optical density (A1) of the samples was measured at 265 nm . Next, 4 μL of ascorbate oxidase (0.5 e.a./μl) was added and the samples were vortexed (30 s). After 5 min incubation, the optical density (A_2) was measured again at 265 nm. To assess the total ascorbate contents, 3.3 μL of dithiothreithol (DTT) were added to 250 μL of supernatant, vortexed (30 s) and incubated (15 min, 25°C). Then, 1.8 μL of sodium phosphate buffer (0.1 mol/L, pH 5.6) was added to 200 μL of each reduced sample, vortexed (30 s) and the optical density (A_3) at 265 nm was assessed. The contents of oxidized ascorbate (dehydroascorbic acid) were calculated as the difference between the values obtained for the total and reduced ascorbate. The statistical calculations were done as described previously [4]. The length of the optical way – 1 cm.

Protocol S1-6. Analysis of Primary Metabolites.

Analysis of the temperature-stable polar primary metabolites relied on water-methanol extraction of frozen ground cucumber roots (45 ± 10 mg) as described by Chantseva, Bilova, Smolikova, Frolov and Medvedev [1] with minor modifications. Aliquots (100 μL) of the extracts (total extract volume was 1100 μL) were freeze-dried overnight. The aliquot volumes were adjusted in a series of preliminary optimization experiments to 0.5, 1, 2, 4, 8, 16, 31, 63 and 125 μL of a pooled root extract (n = 3). The residues were sequentially derivatized with methoxyamine hydrochloride (MOA) and N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) according to the earlier established procedure [1]. The samples (1 μL) were analyzed by GC-EI-Q-MS using a GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan), equipped with an auto sampler AOC-5000 Plus (Shimadzu) under the instrumental settings summarized in Table S1-8. Prior to the analysis, the samples were randomized with quality controls (QCs –aliquots of the pool prepared from equal volumes of all extracts) injected after each four samples.

Analysis of temperature-labile anionic primary metabolites was accomplished according to n integrated protocol including isolation of both polar primary and semi-polar secondary compounds. The applied procedure relied on the three-step extraction procedure described by Leonova, *et al.* [5] with minor modifications. Approximately 100 ± 5 mg of frozen ground root material were placed in 2 mL microtubes filled with 200 mg glass beads (0.75–1 mm diameter), 3 and 1 stainless steel beads of 3 mm and 5 mm diameter, respectively, and supplemented with 900 μL of cold (-80°C) dichloromethane (DCM)/ethanol mixture (2:1, v/v) with addition of 100 μL of ice-cold 1:200 HCl:water. After intensive homogenization (Mixer Mill MM 400 ball mill, Retsch, Haan, Germany, 30 Hz, 2x 30 s) and centrifugation (4°C, 10 000 g, 5 min), 300 μL of the polar supernatant fraction were

transferred into new pre-cooled 1.5 mL polypropylene microtubes. The residues were supplemented with 50 μL of ice-cold 1:200 HCl:water (v/v) mixture. The samples were mixed and centrifuged as described above, before 120 μL of the polar supernatant fractions were combined with the first portions and lyophilized. Then 180 μL of cold EtOH:water (3:1, v/v) solution were added into each sample and shaken at 1900 rpm (i.e., the maximum intensity) for 5 min at 4°C. In the next step, the samples were sonicated (3 min), and again shaken at 1900 rpm for 5 min at 4°C. This treatment was repeated twice more. Then the samples were centrifuged for 5 min at 12 000 x g, 4°C. The samples were analyzed with the above described randomization/standardization strategy by RP-IP-UHPLC-ESI-QqQ-MS/MS using Waters ACQUITY H-Class UPLC System (Waters GmbH, Eschborn, Germany), coupled on-line to an AB Sciex QTRAP 6500 LC-MS/MS System (AB Sciex, Darmstadt, Germany) under the chromatographic and mass spectrometric settings summarized in Supplementary information 1 (Table S1-9).

Protocol S1-7. Analysis of Semi-Polar Secondary Metabolites.

To the non-polar supernatant fractions (700 μL) were added 500 μL cold (-80°C) tetrahydrofuran. After mixing (1900 rpm, 5 min, 4°C) and centrifugation (4°C, 10 000 g, 5 min) the non-polar supernatant fraction (500 μL) was combined with the first portion and gently dried under reduced pressure at 4°C in a Labconco CentriVap vacuum concentrator (Labconco, Kansas City, Missouri, USA). The residues were reconstituted in 180 μL of a water-methanol mixture (1:3, v/v), vortexed (3000 g, 15 s), centrifuged (4°C, 10000 g, 10 min), and the supernatants were filtered as described above. The samples were analyzed with the above described randomization/standardization strategy by reversed phase-high performance liquid chromatography-electrospray ionization-quadrupole-time-of-flight mass spectrometry (RP-HPLC-ESI-QqTOF-MS) using liquid chromatograph-mass spectrometer S LCMS-9030 System (Shimadzu), operated in positive or negative ion mode. Data acquisition relied on the sequential window acquisition of all theoretical mass spectra (SWATH) mode.

Protocol S1-8. Protein isolation and determination.

The protein extraction was accomplished with approximately 250 mg of milled frozen root material by relied on the method of Frolov, *et al.* [6] with some modifications. The 700 μL of cold (4°C) phenol extraction buffer (0.7 mol/L sucrose, 0.1 mol/L KCl, 5 mmol/L ethylenediaminetetraacetic acid, 2% (v/v) β-mercaptoethanol and 1 mmol/L phenylmethylsulfonyl fluoride in 0.5 mol/L Tris-HCl buffer, pH 7.5) was added to the plant material. The suspensions were vortexed for 30 s. Then 700 μL of cold phenol (4°C) preliminarily saturated with 0.5 mol/L Tris-HCl buffer (pH 7.5) were added. After further mixing for 30 s, the samples were shaken (30 min, 900 rpm, 4°C) and centrifuged (5000x g, 15 min, 4°C). Afterwards, the phenolic (upper) phase was washed two times with equal volumes of the phenol extraction buffer (after each buffer addition: vortexing 30 s, shaking for 30 min at 900 rpm at 4°C, and centrifugation at 5000x g for 15 min at 4°C). Then, the proteins were precipitated by addition of 1 mL of ice-cold (–20°C) ammonium acetate in methanol (0.1 mol/L), followed by storage overnight at −20°C. Next morning, the protein fraction was collected by centrifugation (5000x g, 10 min, 4°C), and the supernatants were discarded. The pellets were washed twice with two volumes of methanol (relative to the volume of the phenol phase), and twice with the same volume of acetone (both at 4°C). Each time after re-suspending, the samples were centrifuged (5000 x g, 10 min, 4°C) and supernatants were discarded. Finally, the cleaned pellets were dried under air flow in a fume hood for 1 h and then re-constituted in 70 μL of 4% sodium dodecyl sulphate (SDS). The subsequent determination of protein concentrations was done by Bicinchoninic Acid Kit for Protein Determination (Sigma-Aldrich).

Protocol S1-9. Solid Phase Extraction.

The proteolytic hydrolysates were pre-cleaned by reversed phase solid phase extraction (RP-SPE) using the elution scheme of Spiller, *et al.* [7] with minor modifications. Stage-Tips with six layers of C-18 Extraction Disks (Sigma-Aldrich, 66883-U) were prepared in 200 μL polypropylene pipette tips and inserted in 2 mL tubes via plastic tube adaptors. The Stage-Tips were conditioned with 100 μL of MeOH (2000x g, 5 min, 25°C) and equilibrated with two portions of 200 μL of 0.1% (v/v) formic acid (FA, 2000x g, 5 min, 25°C), before the individual tryptic digests were applied and the stage tips were centrifuged (2000x g, 5 min, 25°C). After the washing (2 x 200 μL of 0.1% (v/v) FA) with centrifugation after each step (2000x g, 5 min, 25°C), the peptides were eluted by sequential application of 150 μL of 60% (v/v) acetonitrile and 150 μL of 80% (v/v) acetonitrile, 0.1% (v/v) FA. Finally, the pooled eluate was

transferred to 0.5 mL polypropylene tubes and dried (4°C) under the reduced pressure in the vacuum concentrator CentriVap Vacuum Concentrator (Labconco).

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