Supplementary Material

Expansion of the strigolactone profluorescent probes repertory: the right probe for the right application

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Supplementary Tables

Compound (no. of replicates) [a]	Concentration	Length of bud at node 3 /branch (mm) [b]	SE	p-value [d]
GC116	10,000 nM	2.43	0.12	0.000000e+00
	1,000 nM	1.67	0.09	0.000000e+00
	1,000 nM	3.13	1.43	0.000000E+00
	1,000 nM	1.60	0.08	0.000000e+00
	1,000 nM	2.22	0.19	0.000000e+00
	1,000 nM	1.97	0.09	0.000000e+00
	1,000 nM	2.21	0.07	0.000000e+00
	100 nM	1.96	0.11	0.000000e+00
	100 nM	1.72	0.11	0.000000e+00
	100 nM	2.66	0.26	2.084775e-10
	100 nM	2.29	0.09	0.000000e+00
	100 nM	2.46	0.15	0.000000e+00
	10 nM	2.47	0.19	3.762324e-12
	10 nM	2.26	0.20	0.000000e+00
	10 nM	5.20	1.48	3.729298e-04
	10 nM	4.34	0.66	0.000000e+00
	10 nM	3.85	0.63	0.000000e+00
	1 nM	6.38	1.09	1.221245e-15
	0.1 nM	11.47	1.40	1.464535e-01

GC155	10,000 nM	2.06	0.10	0.000000e+00
	1,000 nM	1.98	0.23	0.000000e+00
	1,000 nM	1.45	0.10	0.000000e+00
	1,000 nM	1.92	0.11	2.231548e-14
	1,000 nM	1.96	0.11	0.000000e+00
	1,000 nM	2.38	0.14	0.000000e+00
	1,000 nM	2.32	0.11	0.000000e+00
	100 nM	5.98	2.00	2.793725e-03
	100 nM	1.83	0.31	0.000000e+00
	100 nM	3.68	0.87	8.323844e-02
	100 nM	4.11	1.00	0.000000e+00
	100 nM	2.71	0.13	0.000000e+00
	10 nM	5.97	1.73	3.518254e-04
	10 nM	16.85	6.28	1.581675E-02
	10 nM	8.43	1.42	0.000000e+00
GC379	10,000 nM	2.17	0.09	0.000000e+00
	1,000 nM	3.38	0.73	2.260369e-01
	1,000 nM	3.19	0.23	0.000000e+00
	1,000 nM	3.65	0.87	0.000000e+00
	1,000 nM	3.04	0.81	0.000000e+00
	100 nM	2.81	0.54	5.485758e-04
	100 nM	5.02	1.14	0.000000e+00
	100 nM	3.98	0.93	0.000000e+00
	100 nM	14.32	3.98	7.197213e-01

	10 nM	3.54	0.93	0.000000e+00
GC380	10,000 nM	2.15	0.11	0.000000e+00
	1,000 nM	2.33	0.23	9.998758e-01
	1,000 nM	3.47	1.02	0.000000e+00
	1,000 nM	2.08	0.16	0.000000e+00
	100 nM	8.99	1.78	2.013884e-07
	100 nM	11.66	1.93	3.914880e-05
	100 nM	5.90	1.34	0.000000e+00
	10 nM	6.65	1.39	0.000000e+00
GC247	10,000 nM	6.54	1.01	5.605286e-03
	1,000 nM	26.93	2.14	7.244459e-01
	1,000 nM	18.57	1.13	6.735066e-01
	1,000 nM	16.56	1.36	0.000000e+00
	100 nM	15.23	1.46	1.000000e+00
	100 nM	14.78	2.88	2.188517e-01
	100 nM	11.82	1.21	6.683622e-02
	10 nM	12.39	1.20	5.732650e-02
GC93	10,000 nM	3.89	0.55	3.938098e-06
	1,000 nM	21.70	2.48	3.400014e-01
	1,000 nM	10.23	1.34	1.629355e-01
	1,000 nM	11.80	1.30	1.516977e-01
	100 nM	20.83	0.66	1.231200e-02
	100 nM	17.20	1.06	9.999864e-01

	10 nM	17.73	1.10	1.000000e+00
YLG	10,000 nM	2.44	0.43	0.000000e+00
	10,000 nM	4.81	1.02	0.000000e+00
	1,000 nM	7.40	1.87	7.241036e-01
	1,000 nM	15.90	1.79	5.498252e-01
	1,000 nM	3.40	0.68	3.165924e-09
	1,000 nM	5.59	1.10	0.000000e+00
	1,000 nM	14.28	1.97	1.990799e-08
	100 nM	5.27	1.13	4.097332e-02
	100 nM	19.15	1.45	1.000000e+00
	100 nM	8.39	1.68	1.887069e-02
	100 nM	22.73	1.33	9.953965e-01
	100 nM	19.89	2.35	2.426501e-01
	10 nM	24.15	0.98	7.806233e-01
GC240	5,000 nM[c]	2.61	0.23	0.000000e+00
	1,000 nM	14.17	2.02	9.999973e-01
	1,000 nM	6.22	2.01	3.78319e-09
	1,000 nM	5.64	0.85	0.000000e+00
	1,000 nM[c]	2.52	0.25	0.000000e+00
	1,000 nM	6.85	1.05	0.000000e+00
	100 nM	17.88	1.79	9.552628e-01
	100 nM	10.78	1.60	2.039812e-01
	100 nM	17.14	1.35	2.764565e-10
	10 nM	19.68	1.67	4.675064e-01

	10 nM	15.01	1.39	9.998463e-01
	10 nM	22.08	1.91	5.122958e-01
GC486	5,000 nM	14.02	1.80	3.208221e-01
	5,000 nM	7.80	1.61	5.733226E-01
	5,000 nM[c]	17.41	2.10	9.763187e-01
	1,000 nM[c]	9.26	2.37	2.647994e-01
	1,000 nM	23.14	0.94	1.912903e-01
	100 nM	22.10	1.39	7.608111e-02
	10 nM	24.70	1.12	9.932760e-01
GC242	10,000 nM	1.77	0.07	0.000000e+00
	1,000 nM	2.09	0.11	0.000000e+00
	1,000 nM	3.26	0.61	3.554379e-12
	1,000 nM	1.78	0.10	0.000000e+00
	1,000 nM	2.25	0.28	0.000000e+00
	1,000 nM	1.64	0.07	0.000000e+00
	1,000 nM	1.40	0.04	0.000000e+00
	1,000 nM	1.70	0.08	0.000000e+00
	1,000 nM	1.98	0.08	0.000000e+00
	100 nM	2.02	0.08	0.000000e+00
	100 nM	1.80	0.08	0.000000e+00
	100 nM	4.33	1.37	3.508555e-08
	100 nM	4.20	1.73	0.000000e+00
	100 nM	1.75	0.13	0.000000e+00

	100 nM	1.66	0.06	0.000000e+00
	100 nM	2.05	0.10	0.000000e+00
	10 nM	9.36	1.88	2.622541e-01
	10 nM	6.13	1.18	0.000000e+00
7-Acetoxycoumarin	1,000 nM	25.33	1.02	9.998766e-01
	100 nM	22.67	1.91	8.566230e-01
	10 nM	21.81	1.30	6.403749e-02
DIFMUA	1,000 nM	22.17	1.24	1.051261e-01
	100 nM	19.75	1.61	1.669144e-03
	10 nM	23.71	1.70	9.895969e-01
7-hydroxycoumarine	1,000 nM	41.46	9.43	9.991303e-01
DiFMU	10,000 nM	19.07	0.99	7.238675e-01
	10,000 nM	7.67	1.89	6.997665e-01
	5,000 nM	16.25	0.65	9.999711e-01
	5,000 nM	8.44	1.58	6.997665e-01
	1,000 nM	14.76	1.77	7.908909e-01
Resorufin	1,000 nM	14.90	1.13	9.899278e-01
	100 nM	16.50	0.80	9.999784e-01
	10 nM	12.53	1.15	1.447628e-01

Supplementary Table 1. Bud outgrowth inhibition activity assay results for SL probes and derivatives by direct application on *rms1-10* pea mutant plants. [a] All the replicates are presented in Supplemental Table 1. [b] These data were obtained from means ± SE (n = 24), 10 days after treatment. [c] Tested previously (De saint Germain et al 2016). [d] Comparison of the SL treatment to the control treatment (0 nM) using the Kruskal-Wallis rank sum test.

Compound (no. of replicates) [a]	Concentration	Length of bud at node 3 /branch [b]	SE	p-value [c]
GC379	1,000 nM	26.30	0.70	5.353000e-01
GC380	1,000 nM	23.62	1.63	5.353000e-01
GC242	5,000 nM	33.76	2.19	7.156579e-01
	1,000 nM	35.80	1.49	9.991219e-01

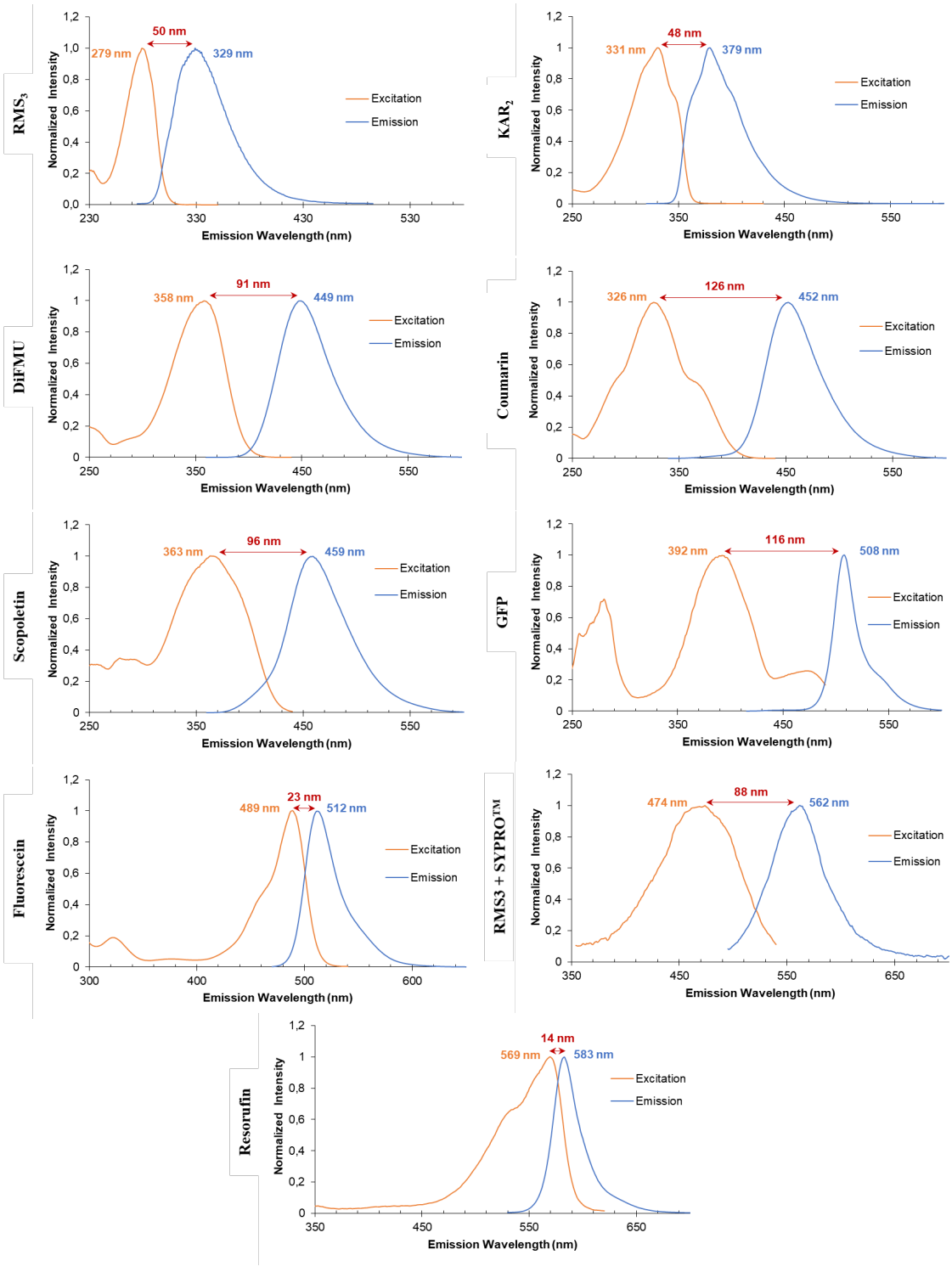
Supplementary Table 2. Bud outgrowth inhibition activity assay results for SL probes by direct application on *rms3-4* pea mutant plants. [a] All the replicates are presented in Supplemental Table 2. [b] These data were obtained from means ± SE (n = 24), 10 days after treatment. [c] Comparison of the SL treatment to the control treatment (0 nM) using the Kruskal-Wallis rank sum test.

Compound (no. of replicates) [a]	Concentration	Length of bud at node 3, 4, 5 /branch (mm) [b]	SE	p-value [f]	p-value [g]	p-value [h]	p-value [i]
GC242	3,000 nM	1.90[c]	0.12	0.000000e+00			
	3,000 nM	2.11[c]	0.14	0.000000e+00		9.999997e-01	0.000000e+00
	3,000 nM	1.26[d]	0.04	0.000000e+00			
	3,000 nM	1.15[d]	0.10	0.000000e+00		6.623814e-03	
	3,000 nM	0.98[d]	0.03	0.000000e+00		6.847110e-06	1.712978e-02
	3,000 nM	1.01[e]	0.05	0.000000e+00			
	3,000 nM	0.71[e]	0.11	0.000000e+00		0.000000e+00	
	3,000 nM	0.76[e]	0.04	0.000000e+00		0.000000e+00	0.000000e+00
YLG	3,000 nM	6.24[c]	2.06	4.097445e-04	3.046445e-02		
	3,000 nM	4.04[c]	0.45	1.469103e-11	6.026631e-01	6.641796e-01	0.000000e+00
	3,000 nM	3.17[d]	1.06	7.253980e-05	4.463705e-03		
	3,000 nM	3.36[d]	1.24	4.647728e-01	1.420836e-04	9.805131e-01	
	3,000 nM	2.07[d]	0.59	1.262800e-03	8.146868e-02	9.902806e-01	9.953035e-01
	3,000 nM	3.25[e]	0.57	5.912796e-05	0.000000e+00		
	3,000 nM	1.91[e]	0.40	4.303795E-01	0.000000e+00	9.996235e-01	
	3,000 nM	1.46[e]	0.14	0.000000e+00	0.000000e+00	9.999605e-01	1.849925e-01
GC93	3,000 nM	11.61[c]	3.82	4.954004e-01		4.909945e-01	
	3,000 nM	3.96[d]	1.14	1.170460e-04		4.122257e-01	

Supplementary Table 3. Bud outgrowth inhibition activity assay results for SL probes and derivatives by vascular supply on *rms1-10* pea mutant plants. [a] All the replicates are presented in Supplemental Table 3. [b] These data were obtained from means ± SE (n = 12), 10

days after treatment. [c] Node 3. [d] Node 4. [e] Node 5. [f] Comparison of the SL treatment to the control treatment (0 nM) using the Kruskal-Wallis rank sum test. [g] Comparison of the SL treatment to (±)-GC242 treatment (3000 nM) using the Kruskal-Wallis rank sum test. [h] Comparison of the SL treatment to (±)-GR24 treatment (3000 nM) using the Kruskal-Wallis rank sum test. [i] Comparison of the SL treatment to wild type Térèse non treated using the Kruskal-Wallis rank sum test.

A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KAR2
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126 nm
Scopoletin
363 nm
459 nm
96 nm
GFP
392 nm
508 nm
116 nm
Fluorescein
489 nm
512 nm
23 nm
RMS3 + SYPRO™
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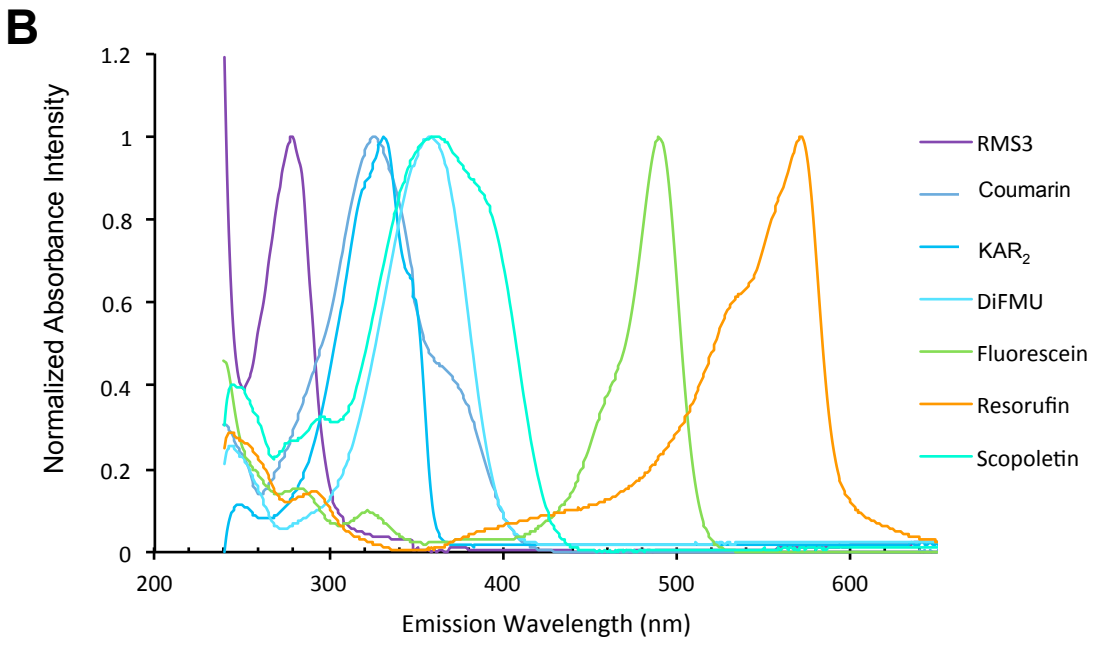
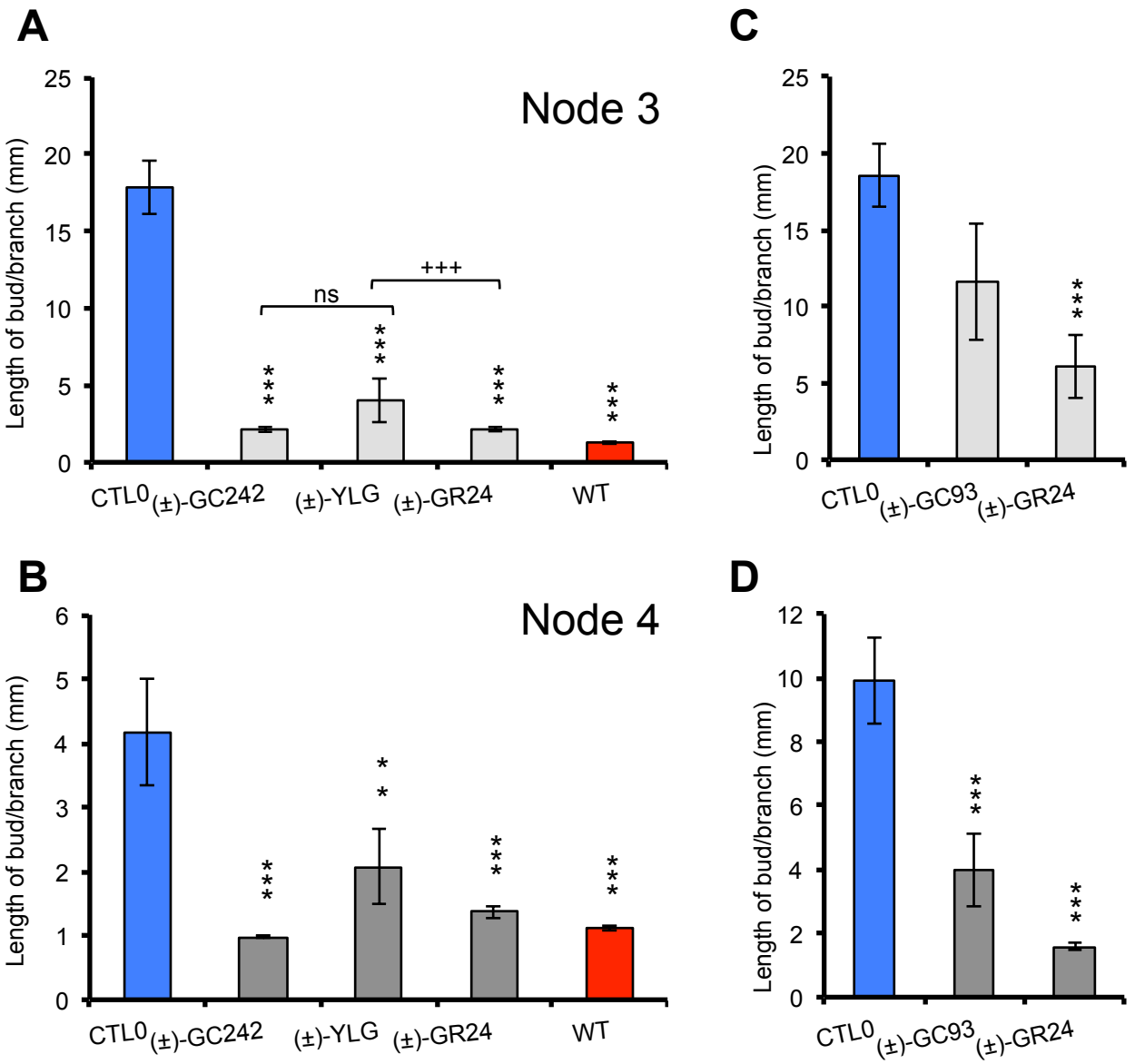
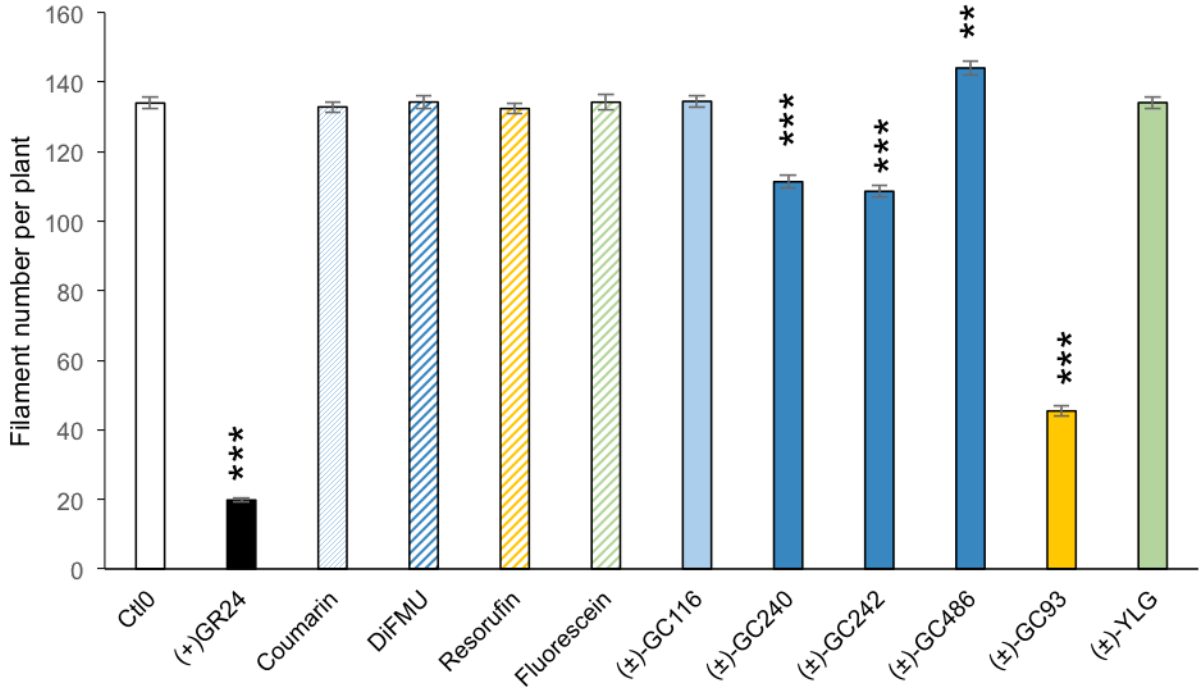


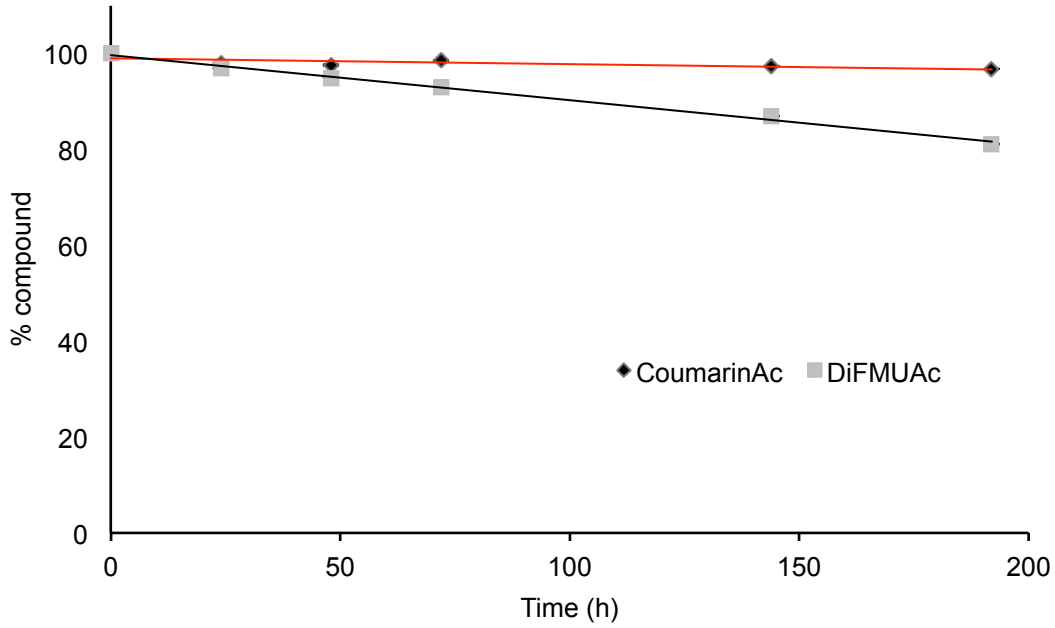
Supplemental Figure 1. Fluorescence emission and excitation spectra recorded in PBS Buffer pH = 7.6 of various fluorophores and RMS3 protein at 25 °C (**A**). Superposition of absorbance spectra recorded in PBS Buffer pH = 7.6 at 25 °C (**B**). The Stokes shift is indicated in red.



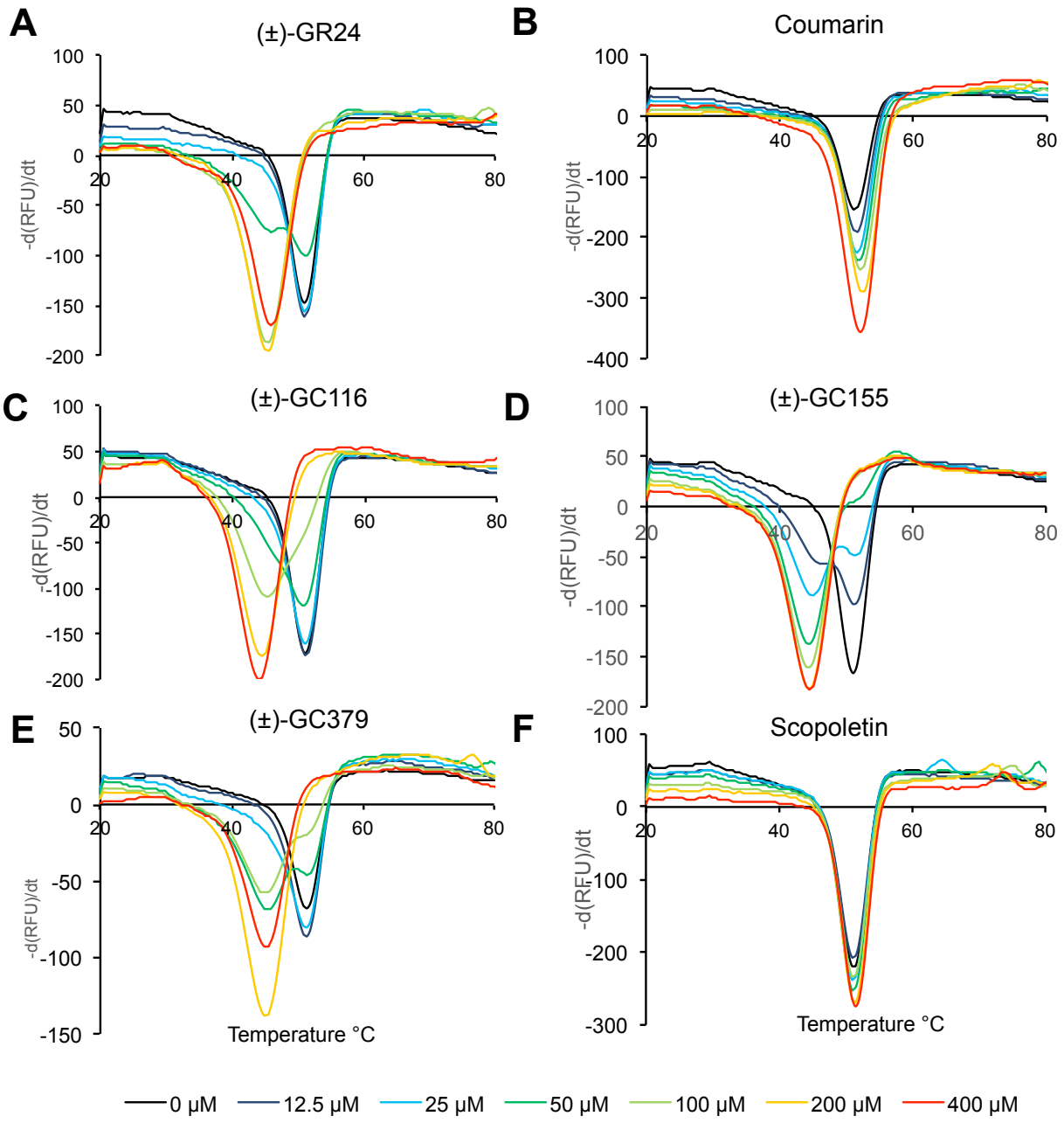
Supplemental Figure 2. Bud outgrowth inhibition activity assay results for SL analogs ((±)-GC242 and (±)-YLG (A,B); (±)-GC93 (C,D)) by vascular supply (3000 nM). [a] All the replicates are presented in Supplemental Table 2 with control 0. [b] These data were obtained from means ± SE (n = 12), 10 days after treatment. . * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant differences with the control treatment (0 nM) (Kruskal–Wallis rank sum test). CTL 0 = control 0. $^{+++}$ $P < 0.001$ indicates significant differences with the (±)-GC242 treatment (3000 nM) (Kruskal–Wallis rank sum test). ns = not significant.



Supplemental Figure 3. Probe bioactivity tested on the moss *P. patens*. Bioactivity of fluorophores and profluorescent probes was assayed by counting the number of filaments grown in the dark following application, and compared to that of DMSO (control 0, white), and (+)-GR24 (in black). Each compound was used at 1 **µ**M. Fluorophore bars are hatched, while pro-fluorescent probe bars are filled. Data are mean ± SE of 24 *Ppccd8* mutant plants (n = 24) grown in 3 different 24-well plates. Significant differences between the control treatment (0 nM) and treated plants are based on ANOVA and Tukey Test as post hoc test; ** $P < 0.01$,*** $P < 0.001$. CTL0 = control 0.



Supplemental Figure 4. Stability of CoumarinAc and DiFMUAc. Chemical hydrolysis of CoumarinAc and DiFMUAc in DMSO at 20 °C. Data are means ± SE (n = 3).



Supplemental Figure 5. DSF assay with SL profluorescent probes. The melting temperature curves of 10 µM RMS3 with (±)-GR24 (**A**), coumarin (**B**), (±)-GC116 (**C**), (±)-GC155 (**D**), (±)-GC379 (**E**) and scopoletin (**F**) at varying concentrations are shown as assessed by DSF. Each line represents