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Supplementary Information for Part 1

Three mutations repurpose a plant karrikin receptor to a strigolactone receptor

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Legends for Movies S1 to S3

Other supplementary materials for this manuscript include the following:
Movies S1 to S3

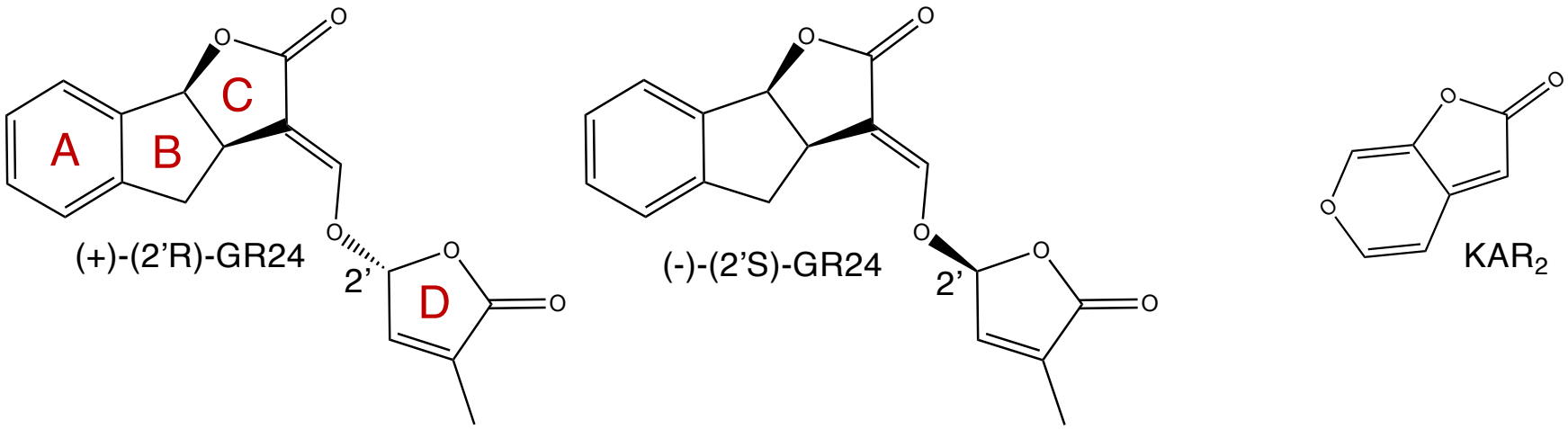
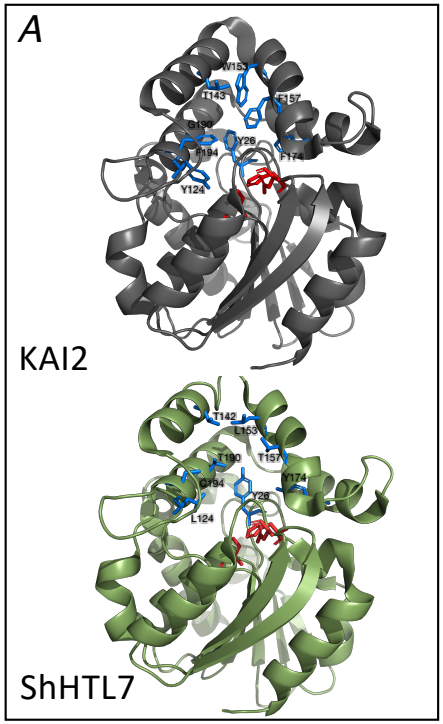
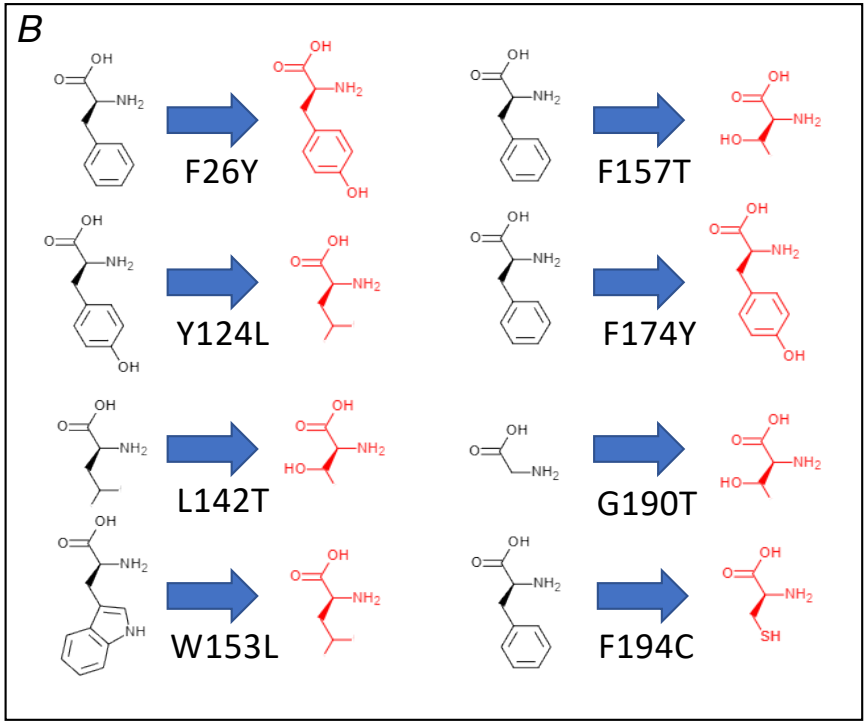


Fig. S1. Structures of 2'R- and 2S'-enantiomeric forms of strigolactones (SLs) and karrikin (KAR_2). For SLs the synthetic GR24 is shown and the 2' chirality is at the enol-ether bridge between the C and D-ring. For karrikin KAR_2 is shown.





C

Residue	KAI2	ShHTL7
26	Phe	Tyr
95	Ser	Ser
121	Ser	Thr
124	Tyr	Leu
134	Phe	Phe
139	Leu	Met
142	Leu	Thr
143	Phe	Leu
146	Ile	Leu
153	Trp	Leu
154	Cys	Ser
157	Phe	Thr
161	Ala	Leu
174	Phe	Tyr
190	Gly	Thr
193	Ile	Ile
194	Phe	Cys
196	Ser	Leu
217	Asp	Asp
219	Ala	Met
246	His	His

Fig. S2. Selection of eight ShHTL7 residues for substitution in KAI2. (*A*) Crystal structures representing the eight residues highlighted in blue, The three catalytic residues highlighted in red. Structures are visualized using PYMOL version 2.3.2. (*B*) Chemical structures of the amino acids at the positions substituted in KAI2 to generate JGI variants. Structures in black represent the amino acids present at these positions in KAI2, structures in red represent their counterparts in ShHTL7. Letter codes and numbers below the arrows denote the substitutions that were made to produce the JGI variants. (*C*) The choice of the eight substitutions (pink boxes) were made based on increased polarity and decreased size (blue amino acids) in ShHTL7 versus the KAI2 equivalent. Red boxes represent the three catalytic residues.

Catalytic triad
Substitution residues
Polar side chain
Non-Polar side chain
Electrically charged side chain

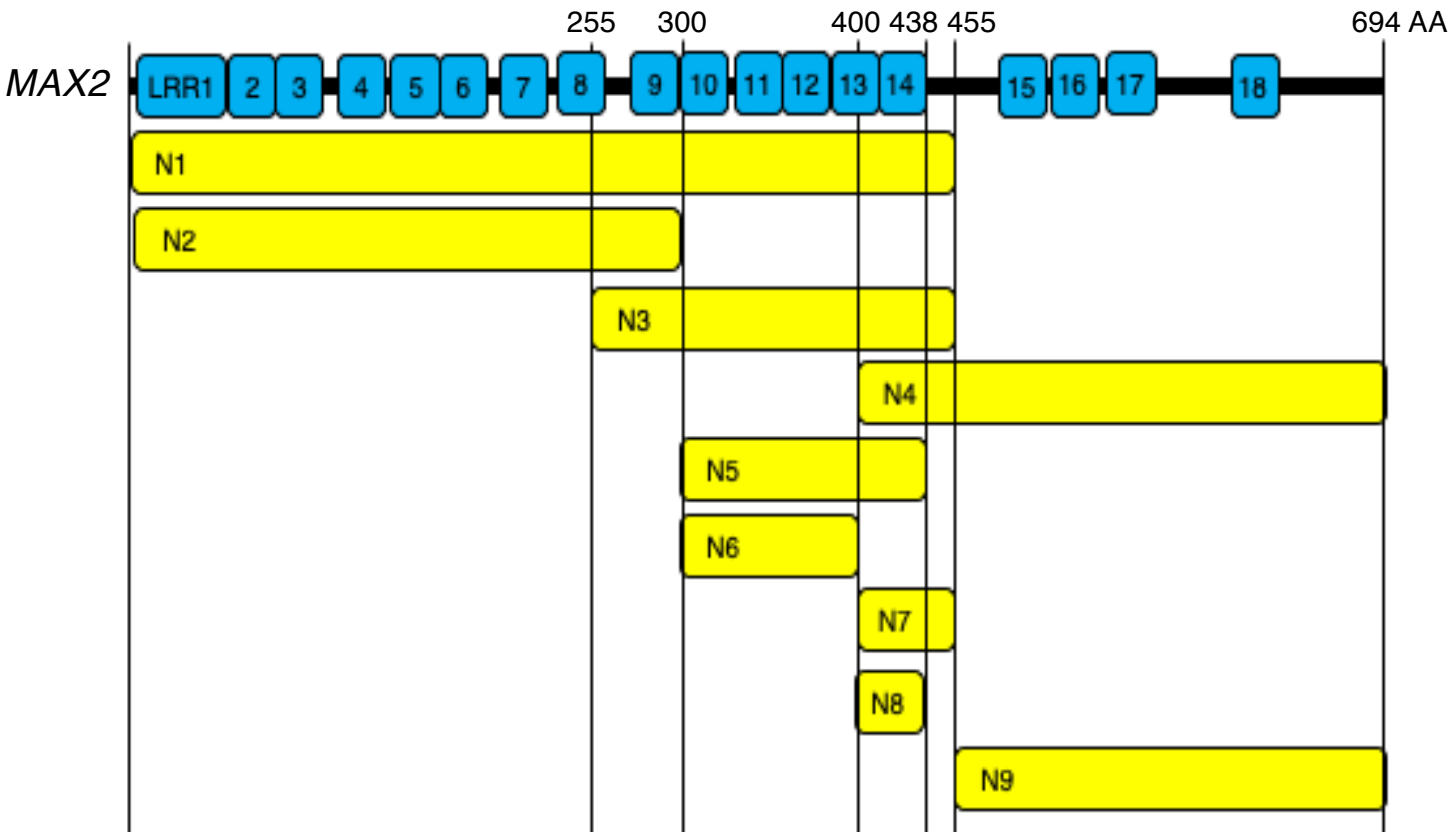


Fig. S3. Map of MAX2 fragments used in yeast-two-hybrid (Y2H) experiments.

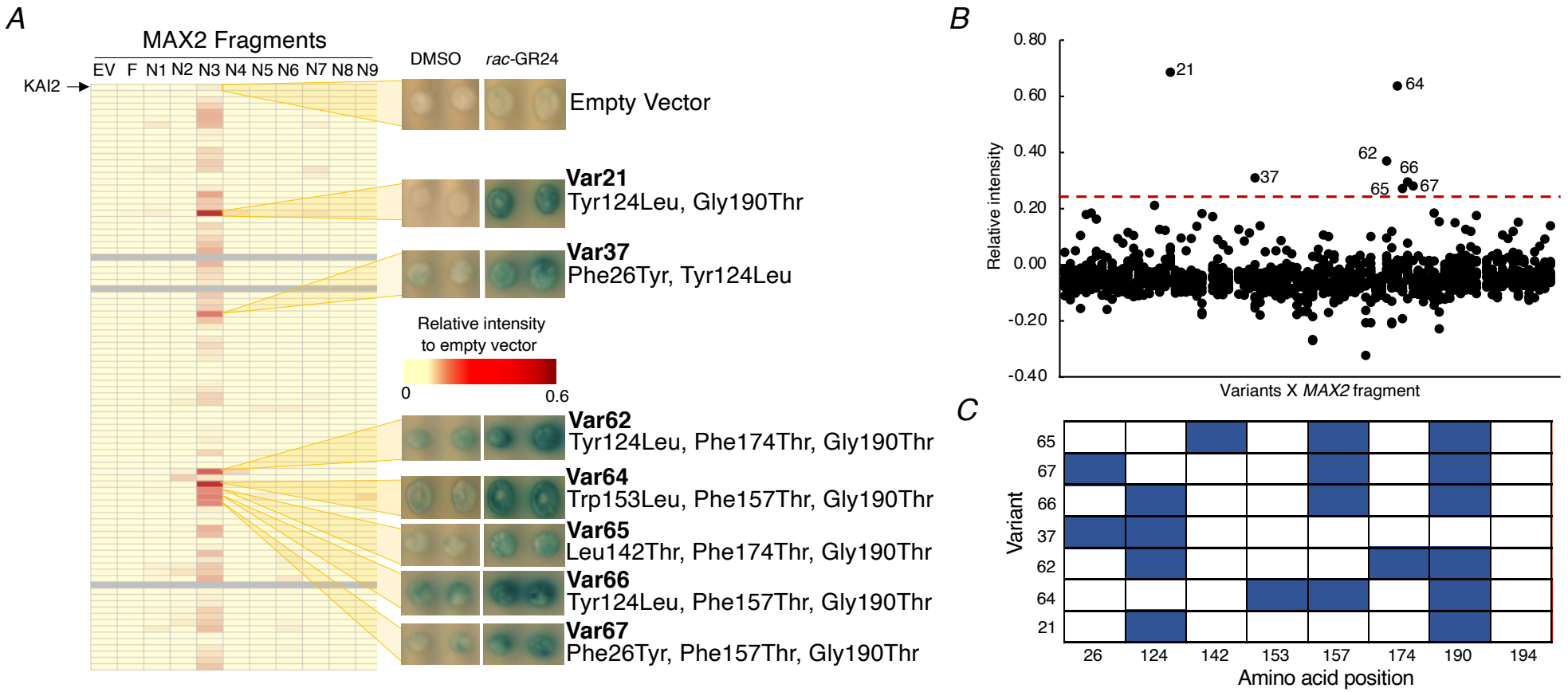
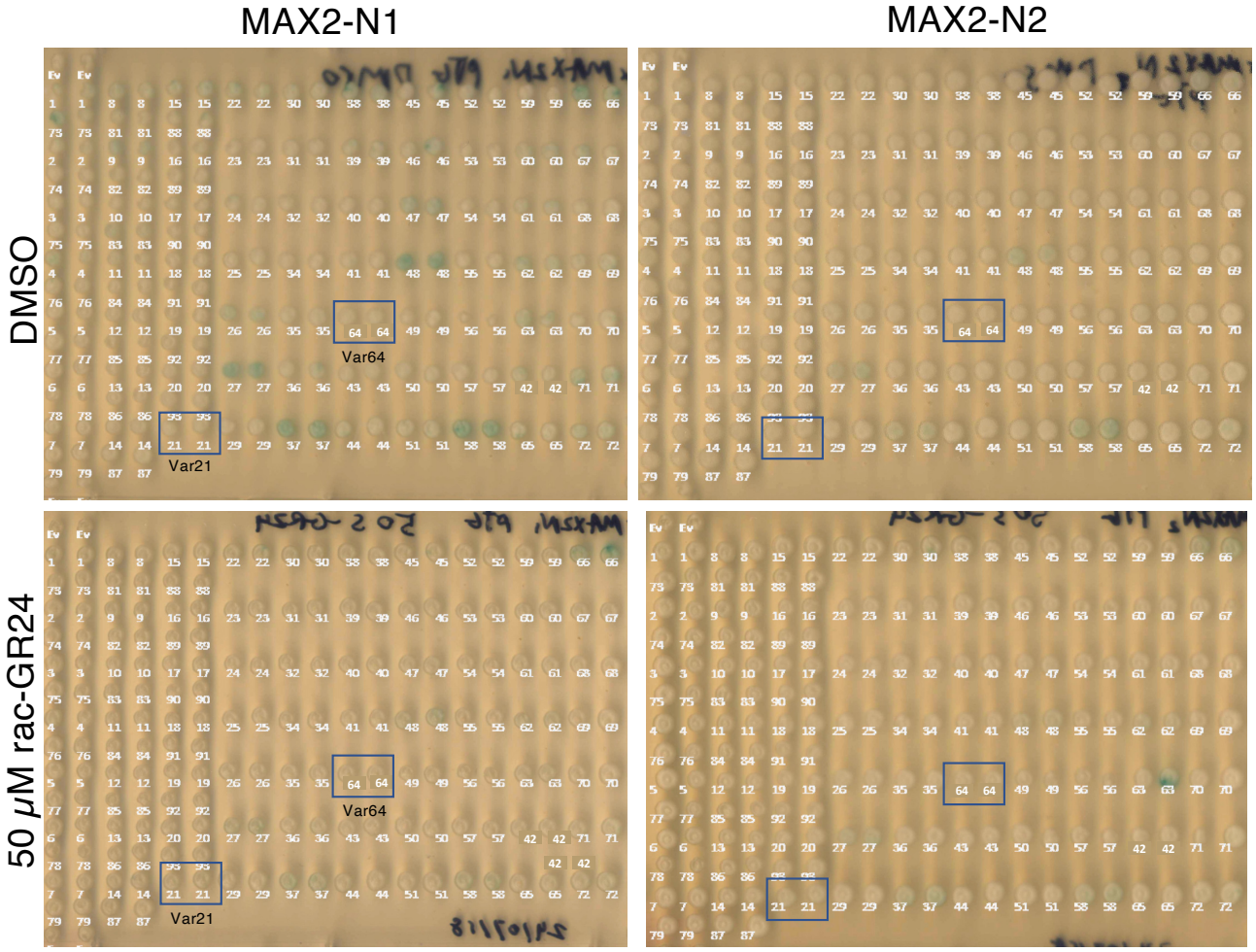


Fig. S4. Yeast-two hybrid interactions of KAI2 variants with MAX2 (*A*) Heatmap of log-fold change scores based on interactions relative to DMSO conditions queried against all MAX2 fragments (**see Fig. S3**). EV, empty vector, F, full-length protein, N, fragments shown in **Fig. S3**. Red shading indicates increased interaction, with the seven strongest interactions (Var21, 37, 62, 64, 65, 66, 67) shown. Grey lines, construct that could not be cloned. (*B*) Scatterplot showing the log-fold change scores calculated for each combination of an individual mis-expressed *KAI2* chimeric variant with full-length, MAX2 fragments and empty vector control based on Y2H interaction intensities. (*C*) Table of statistically significant hits in Y2H screen.

Fig. S5. Images of Yeast 2-hybrid (Y2H) interactions used to generate the Y2H heat map shown in **Fig. 1**. Ev is an empty vector colony. Each duplicate variant colony has its number beneath it. The two blue boxes represent Var21 and Var64.



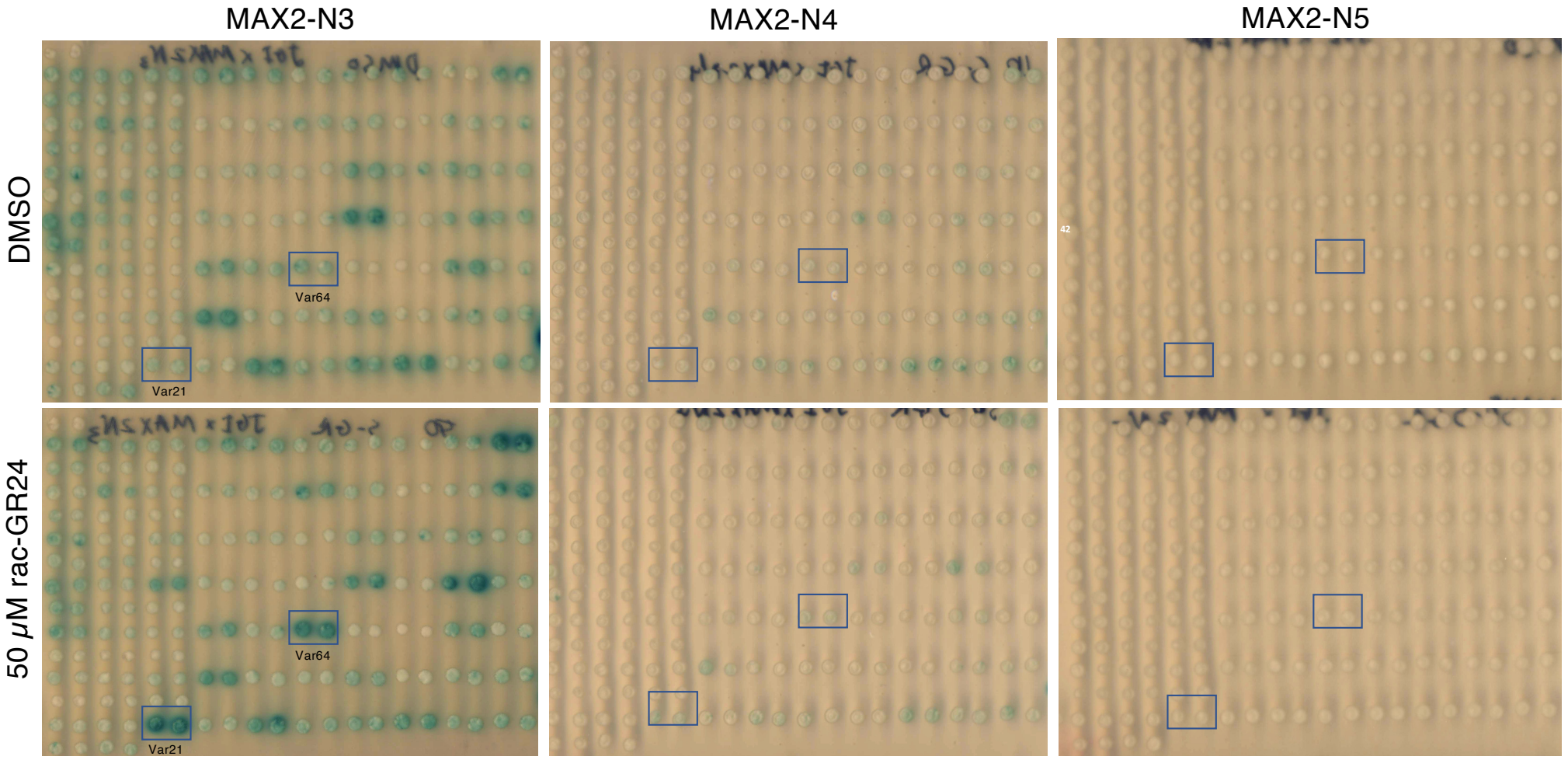

MAX2-N3
MAX2-N4
MAX2-N5
DMSO
50 μM rac-GR24
Var64
Var21
Var64
Var21

Fig. S5. Cont'd

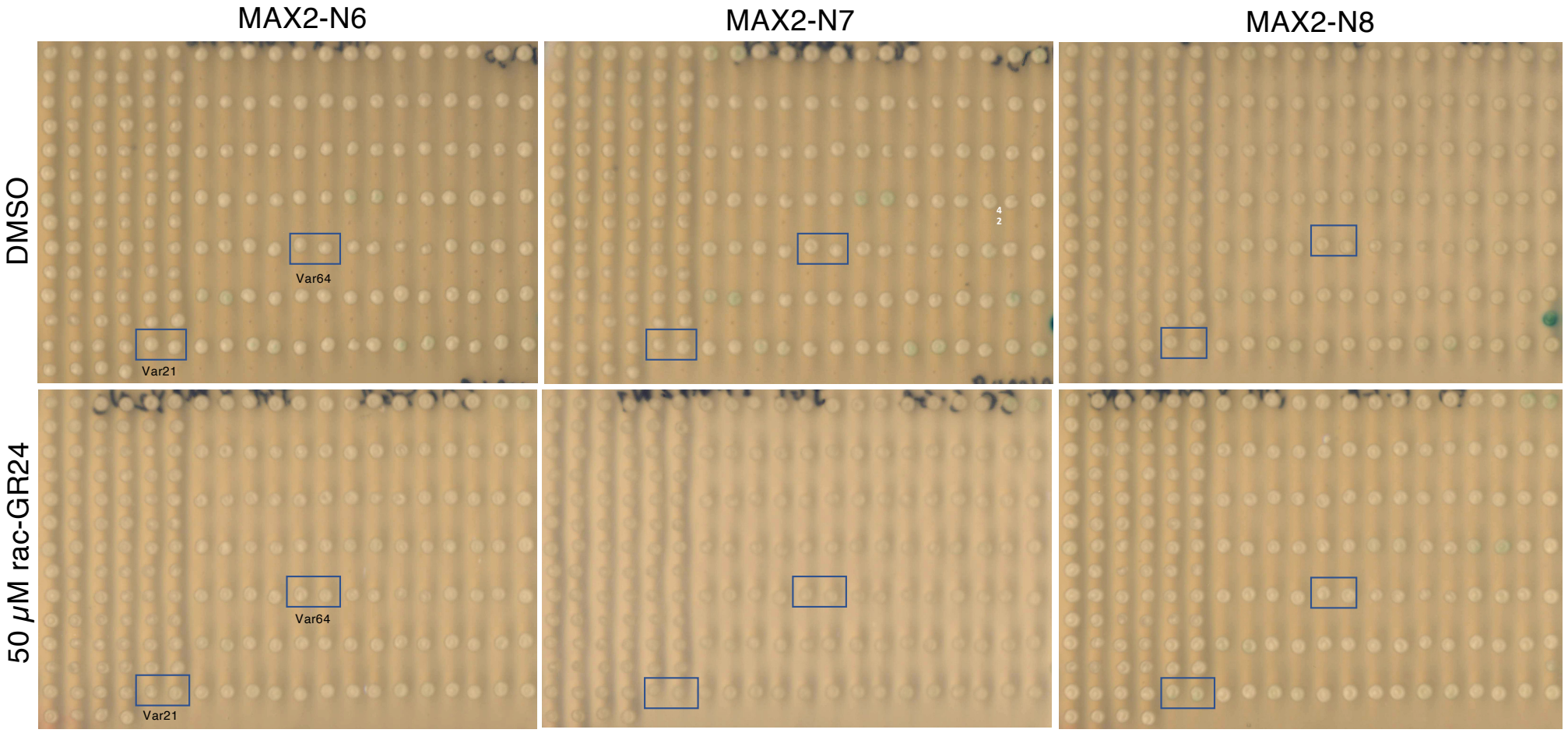

MAX2-N6
MAX2-N7
MAX2-N8
DMSO
50 μM rac-GR24
Var64
Var21
Var64
Var21

Fig. S5. Cont'd

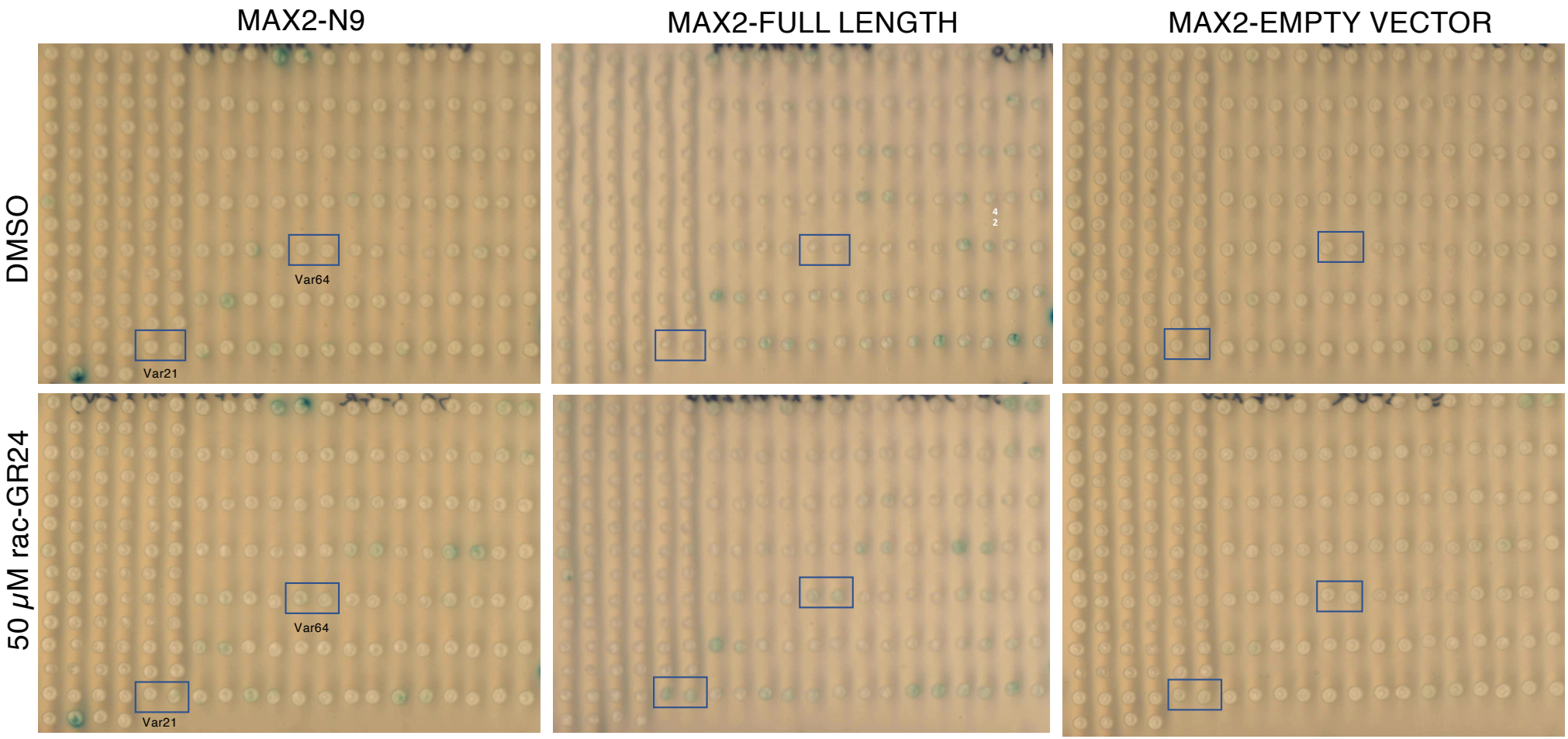

MAX2-N9
MAX2-FULL LENGTH
MAX2-EMPTY VECTOR
DMSO
50 μM rac-GR24
Var64
Var21
Var64
Var21

Fig. S5. Cont'd

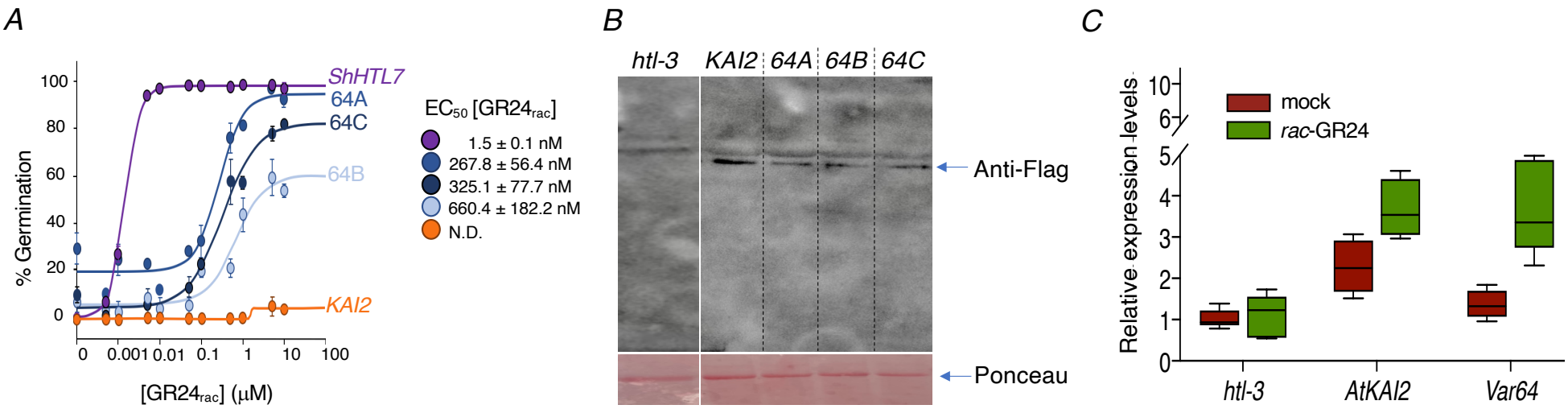


Fig. S6. Characterization of three independent Var64 transgenic lines. (*A*) Percent germination of mis-expressed *ShHTL7*, *KAI2* and three independent Var64 seed (64A, 64B, 64C) on PAC (20 *µ*M) and increasing *rac*-GR24 concentrations. Four-parameter logistic curves were fitted to the data, and EC_{50} values, ± standard error, of *rac*-GR24 concentrations have been included for those lines showing a response on SL. Based on these curves, the effective concentration that germinates 50% of seed (EC_{50}) was calculated. (*B*) Western blot analysis of expression of mis-expressed *KAI2* and three independent Var64 seed (64A, 64B, 64C). Mis-expressed genes were Flag tagged and probed with an Anti-flag antibody. Ponceau staining indicates equal loading. (*C*) Quantitative PCR (qPCR) of *KUF1* transcripts of germinated (24 hrs) seeds in the light on PAC in the absence or presence of *rac*-GR24. Each point represents a biological replicate and each sample represents three replicates. qPCR primers *AtKUF1F* (ATTGTTGTGGTGGATGTTG), *AtKUF1R* (ATTCGGTTGACTCATTCTTG*),* *AtACT8F* (CCACGAGACAACTTACAAC), *AtACT8R* (TGCCACGACCTTAATCTT). Expression values are relative to the At2g20000 reference gene. Bar=SD.

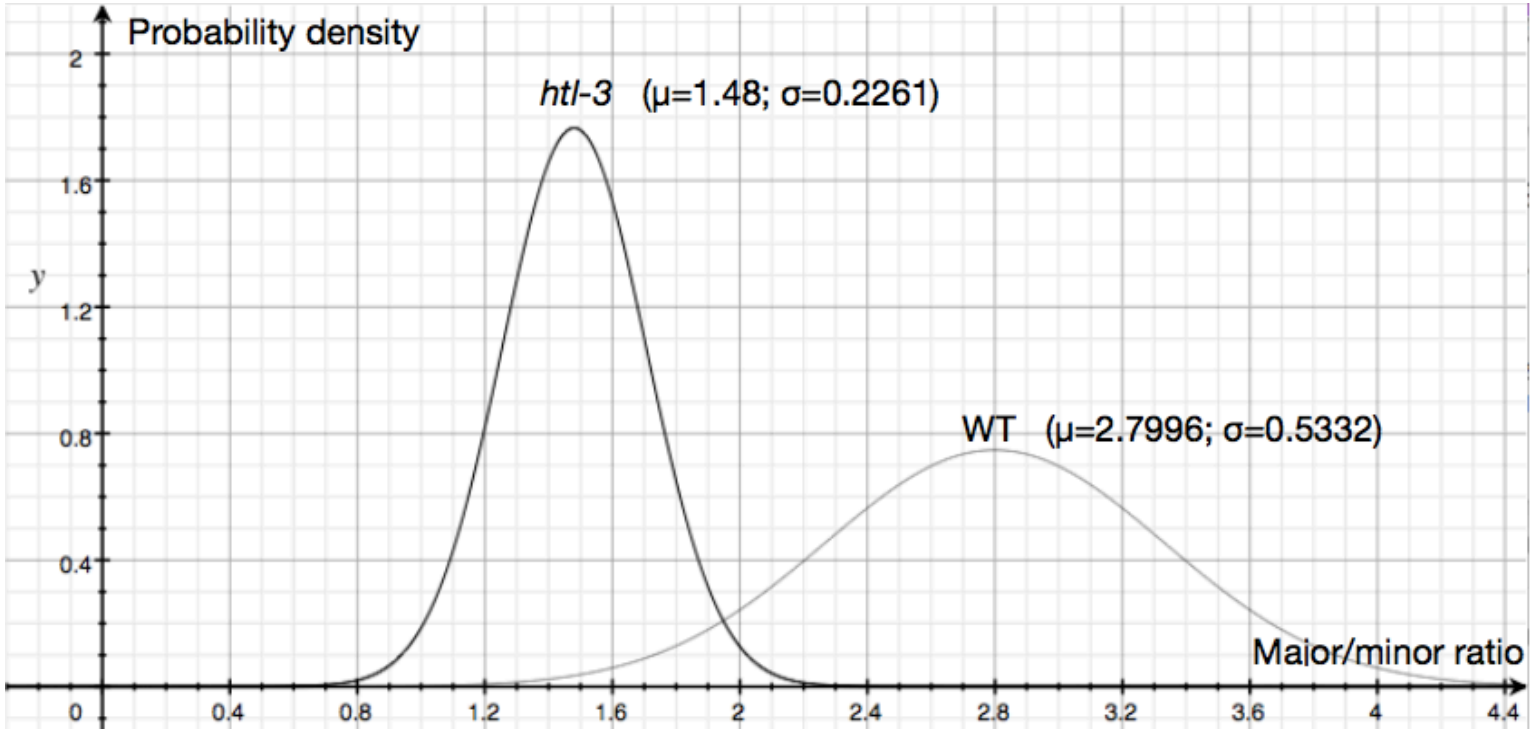


Fig. S7. Standard distribution of length/width (major/minor) ratio *of htl-3* and wild type Columbia (WT) leaves. Each distribution was a normal distribution based on the population mean (μ) and standard deviation (σ) approximated by that of the 26 control samples. WT: y=(1/(0.53332•√(2π)))•*e*-(x-2.7996)^2/(2•(0.53332)^2); *htl-3*: y=(1/(0.2261•√(2π)))•*e*-(x-1.48)^2/(2•(0.2261)^2). Three categories given by 5% cutoff: *htl-3*: x<1.85; WT: x>1.92; ambiguous: 1.85<x<1.92. Based on the two distributions, in order to meet the 0.05 significance level, any sample data smaller than 1.85 would be considered as *htl-3*, while sample data larger than 1.92 would be considered as wild type. Note that there is an ambiguous region in between (1.82-1.92) where the phenotype can not be statistically determined in this case.

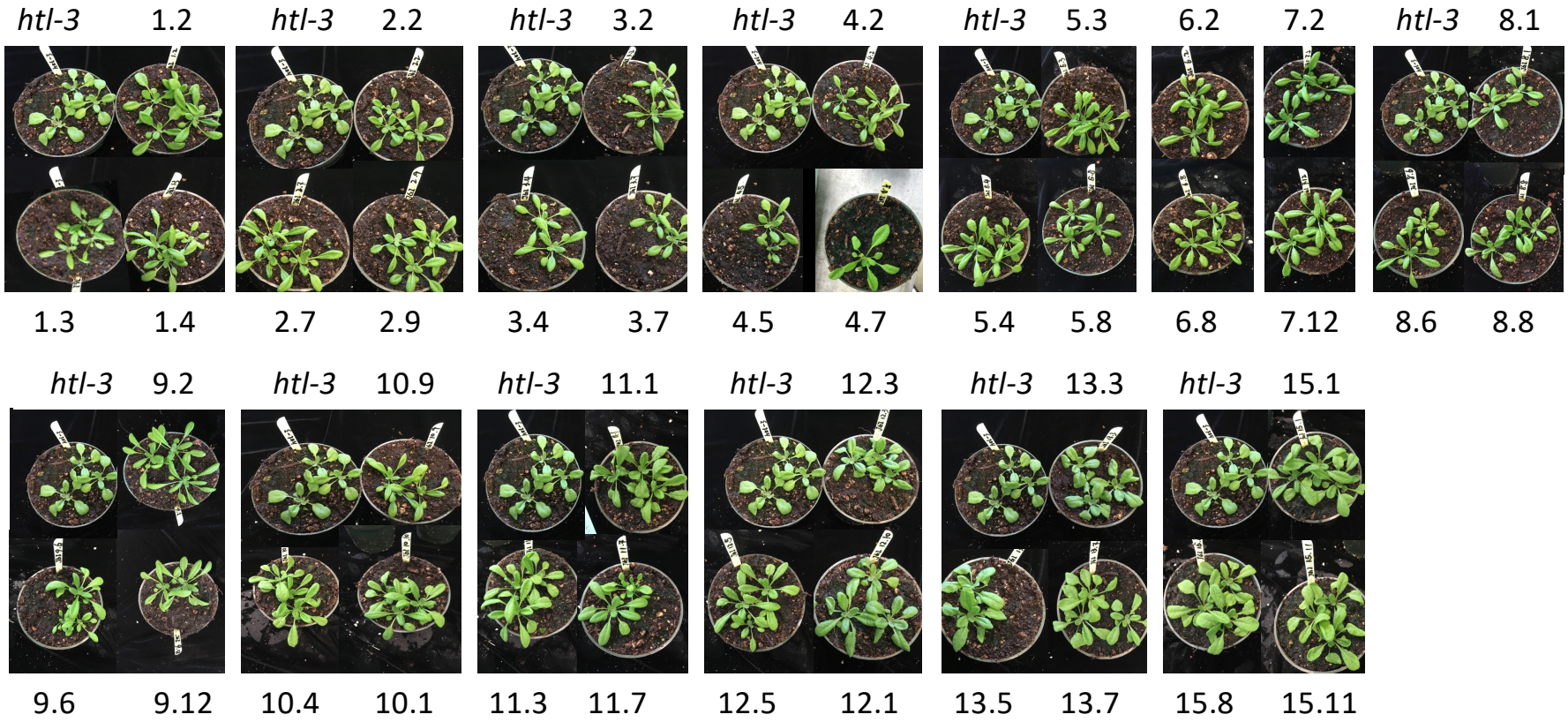
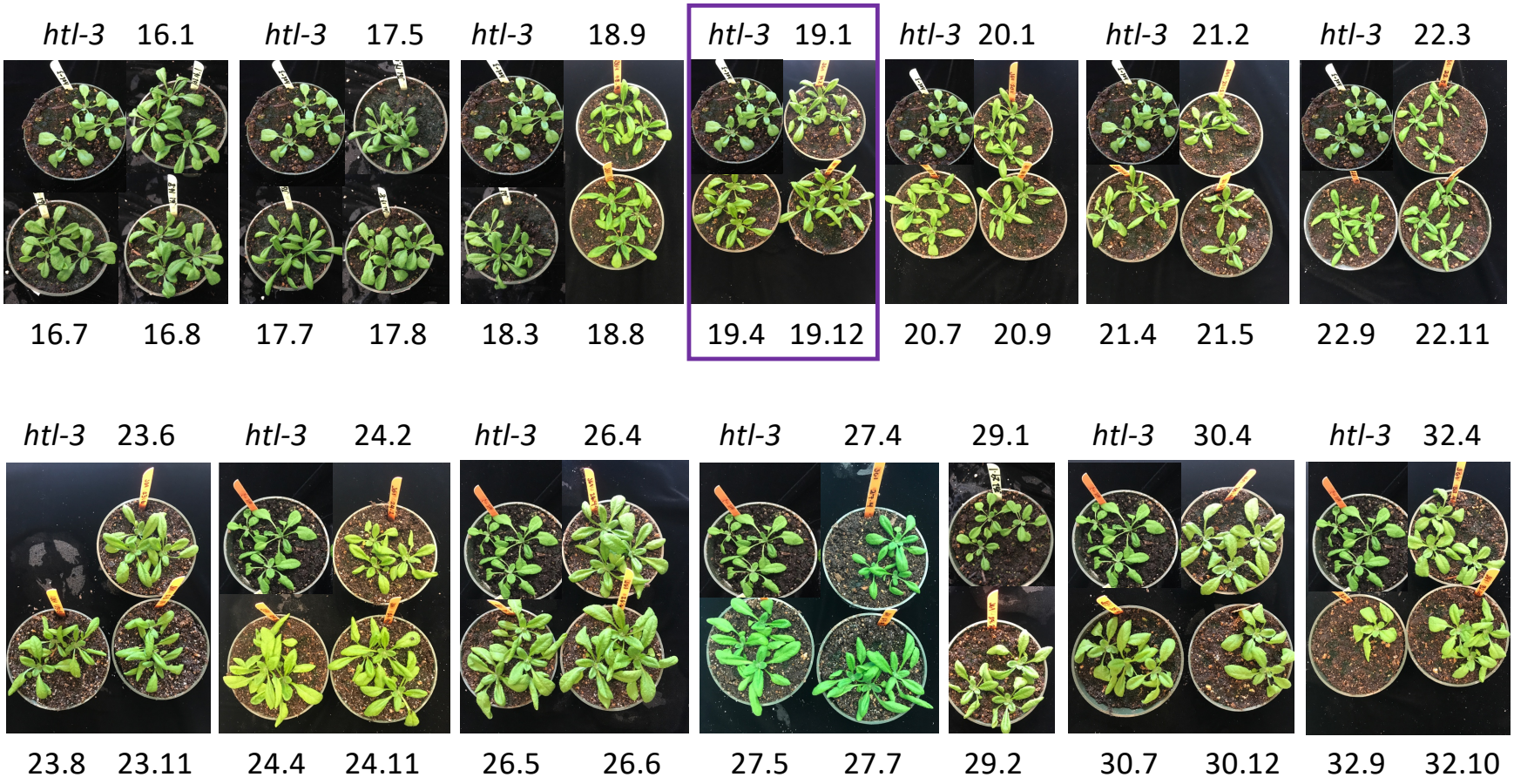


Fig. S8. Images and quantification of transgenic variant lines used to generate **Fig. 1*C*.** Independent lines of Var19 and Var64 are highlighted.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htl-3 17.5
htl-3 18.9
htl-3 19.1
htl-3 20.1
htl-3 21.2
htl-3 22.3
16.7 16.8 17.7 17.8 18.3 18.8 19.4 19.12 20.7 20.9 21.4 21.5 22.9 22.11
htl-3 23.6
htl-3 24.2
htl-3 26.4
htl-3 27.4
29.1
htl-3 30.4
htl-3 32.4
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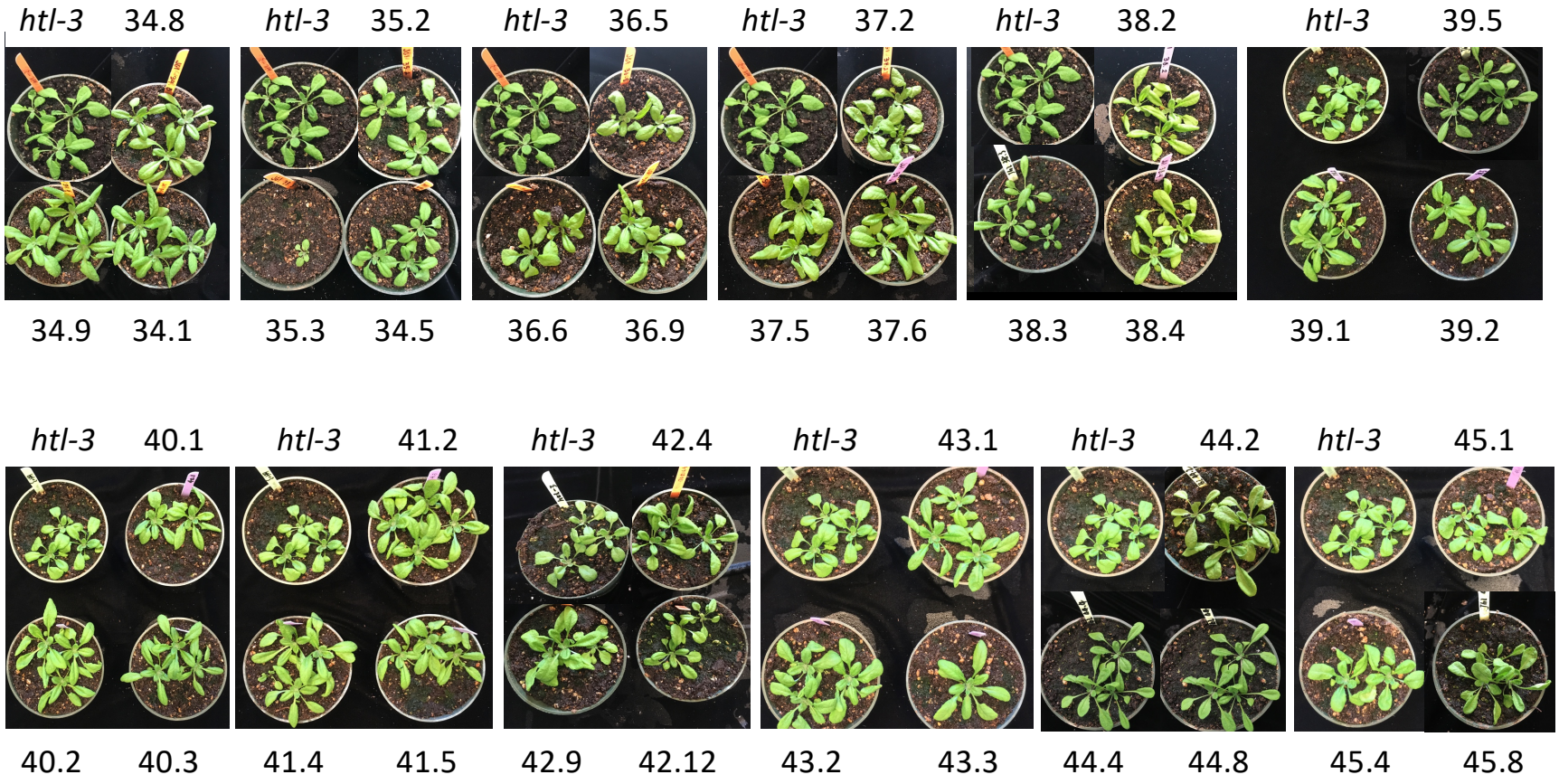
Fig. S8. Con't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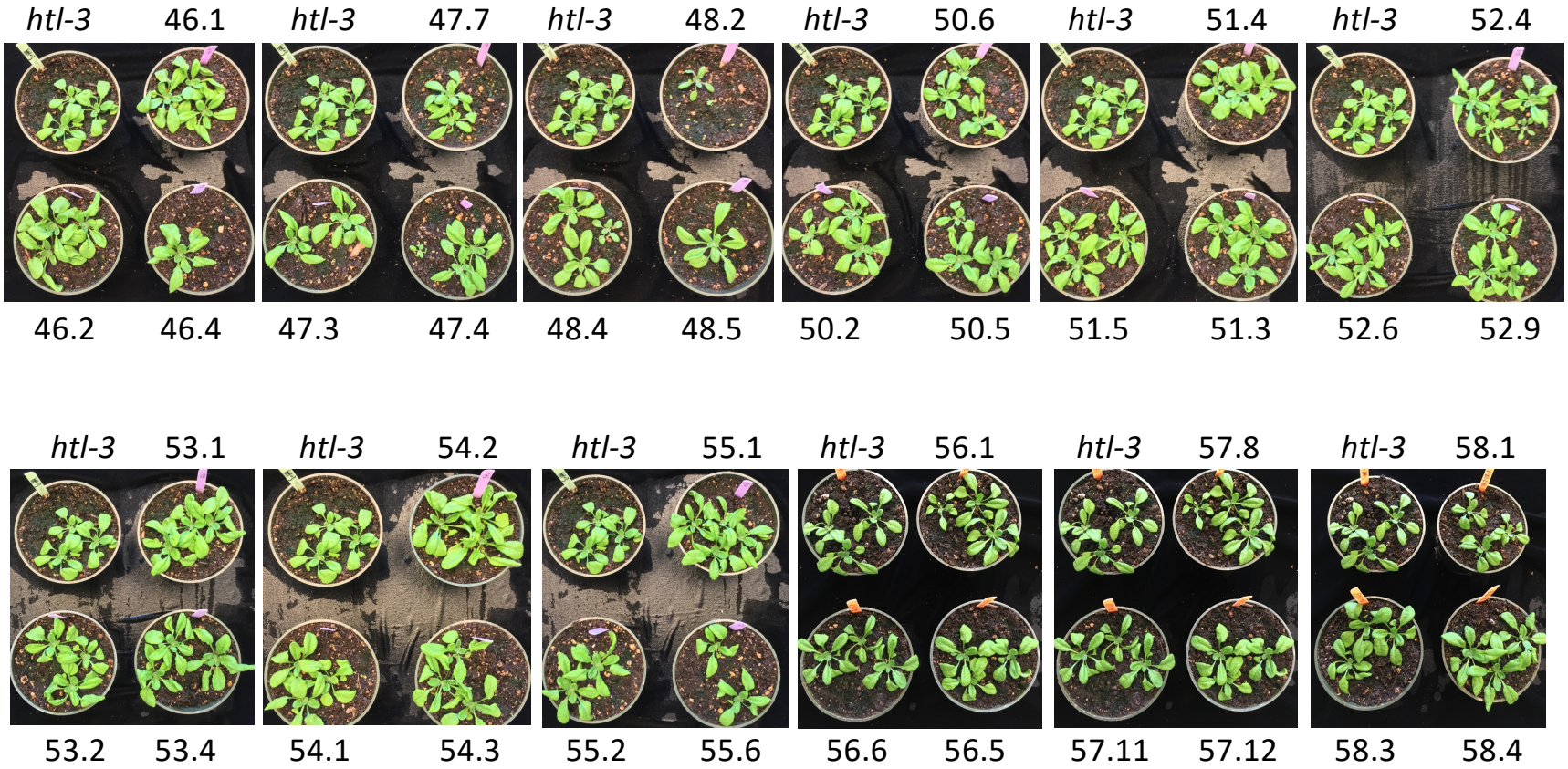
htl-3 35.2
htl-3 36.5
htl-3 37.2
htl-3 38.2
htl-3 39.5
34.9 34.1
35.3 34.5
36.6 36.9
37.5 37.6
38.3 38.4
39.1 39.2
htl-3 40.1
htl-3 41.2
htl-3 42.4
htl-3 43.1
htl-3 44.2
htl-3 45.1
40.2 40.3
41.4 41.5
42.9 42.12
43.2 43.3
44.4 44.8
45.4 45.8

Fig. S8. Cont'd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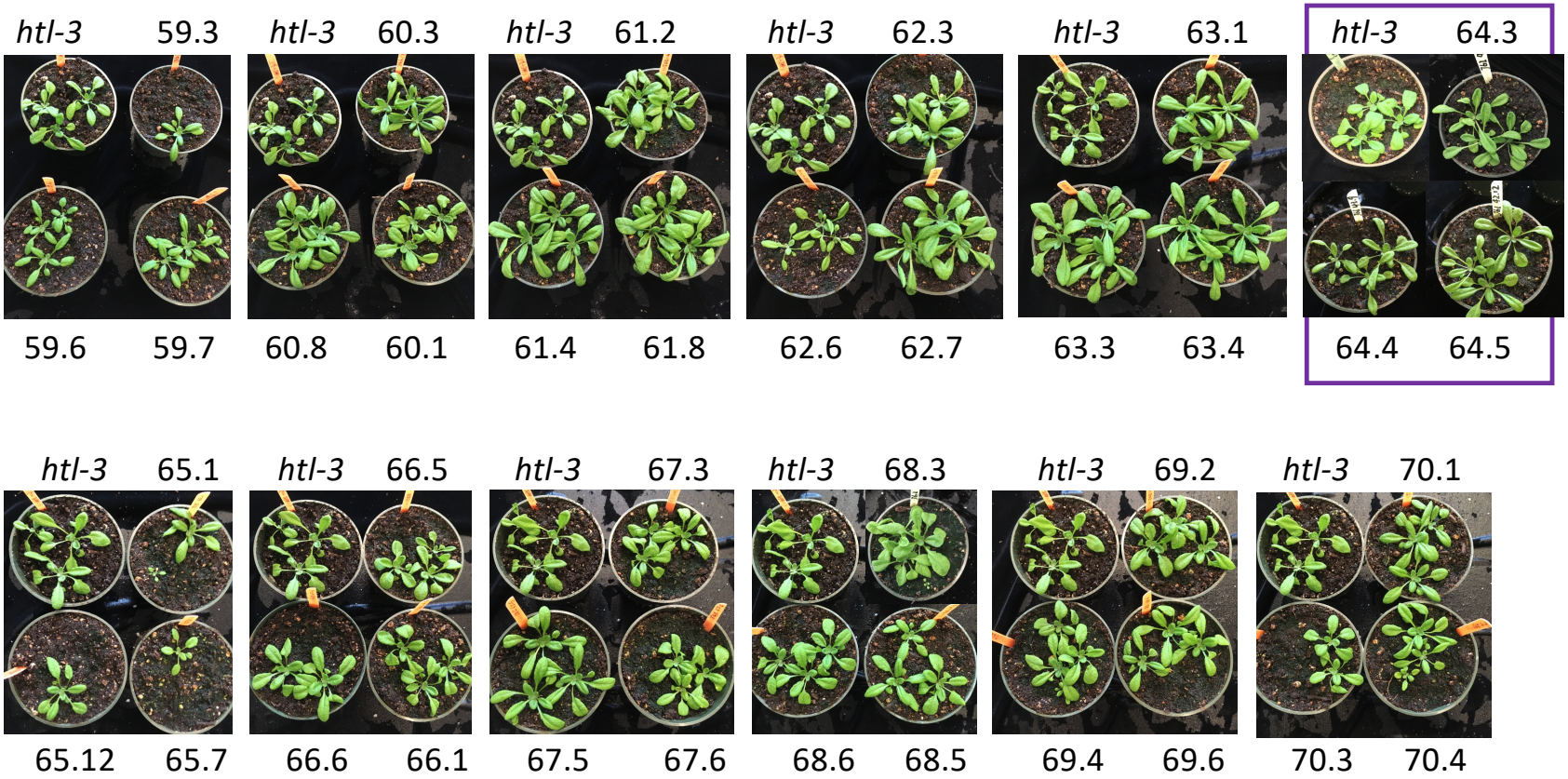
htl-3 52.4
46.2 46.4 47.3 47.4 48.4 48.5 50.2 50.5 51.5 51.3 52.6 52.9
htl-3 53.1
htl-3 54.2
htl-3 55.1
htl-3 56.1
htl-3 57.8
htl-3 58.1
53.2 53.4 54.1 54.3 55.2 55.6 56.6 56.5 57.11 57.12 58.3 58.4

Fig. S8. Cont'd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htl-3 61.2
htl-3 62.3
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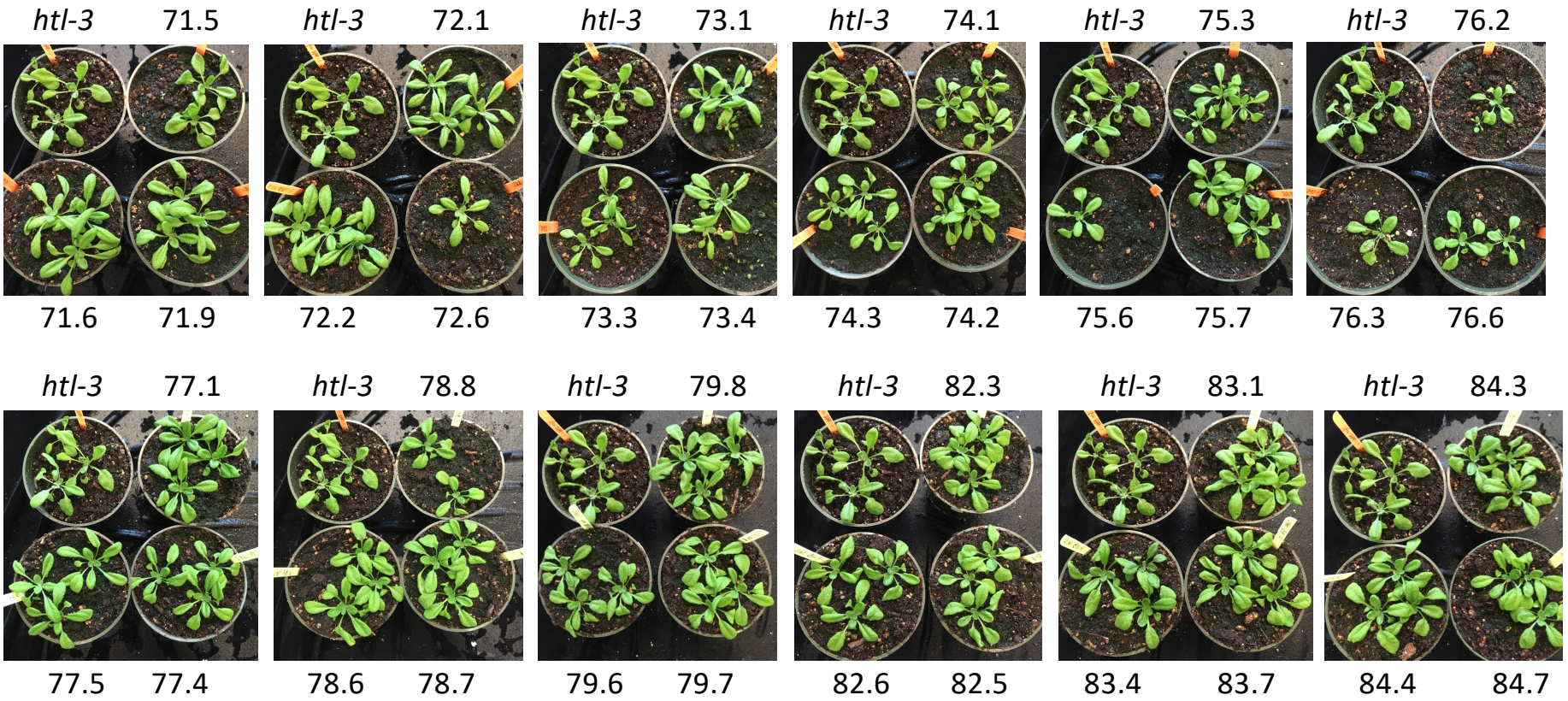
htl-3 64.3
59.6 59.7
60.8 60.1
61.4 61.8
62.6 62.7
63.3 63.4
64.4 64.5
htl-3 65.1
htl-3 66.5
htl-3 67.3
htl-3 68.3
htl-3 69.2
htl-3 70.1
65.12 65.7
66.6 66.1
67.5 67.6
68.6 68.5
69.4 69.6
70.3 70.4

Fig. S8. Cont'd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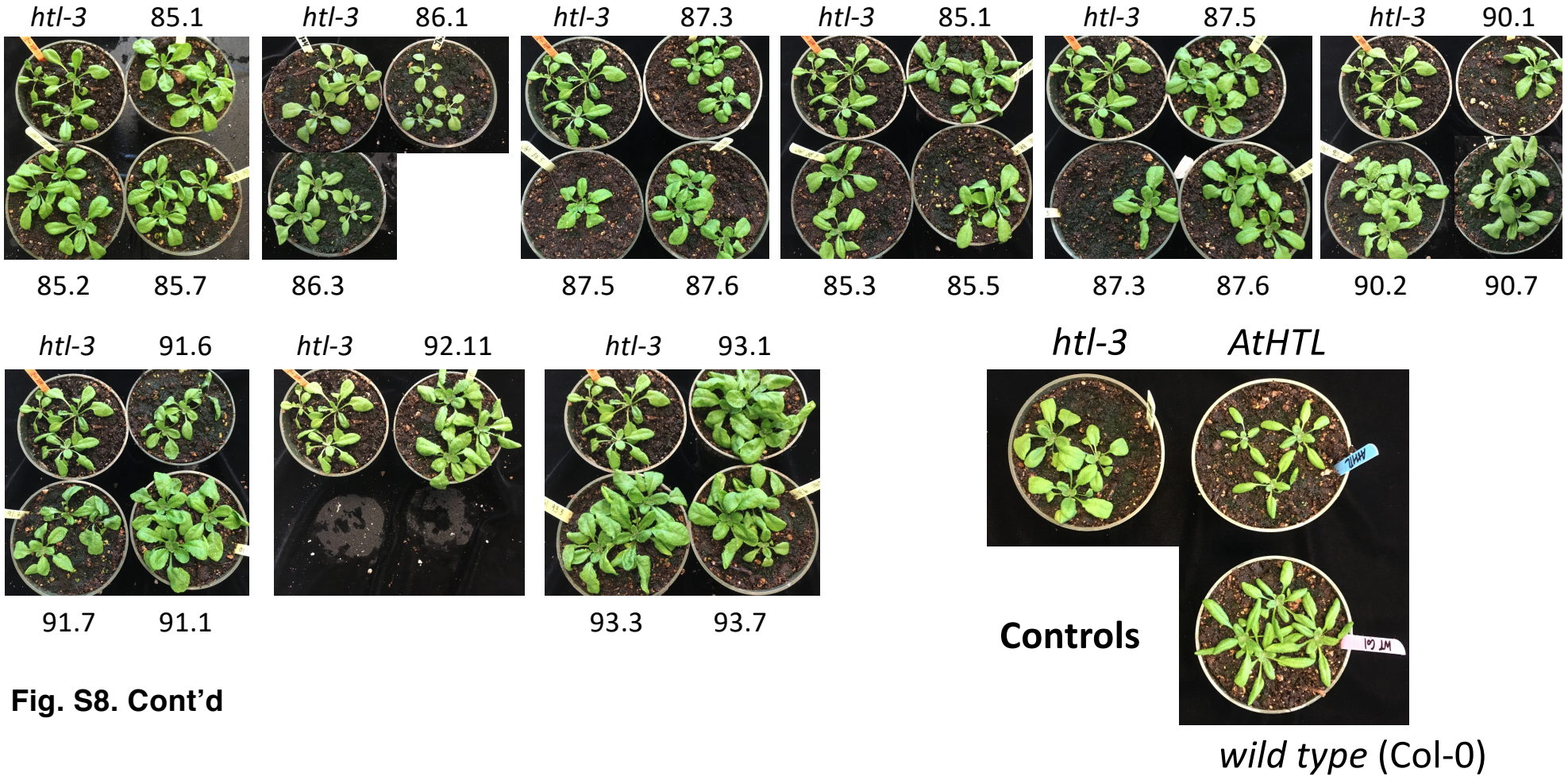
71.6 71.9
72.2 72.6
73.3 73.4
74.3 74.2
75.6 75.7
76.3 76.6
htl-3 77.1
htl-3 78.8
htl-3 79.8
htl-3 82.3
htl-3 83.1
htl-3 84.3
77.5 77.4
78.6 78.7
79.6 79.7
82.6 82.5
83.4 83.7
84.4 84.7

Fig. S8. Cont'd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htl-3 90.1
85.2 85.7
86.3
87.5 87.6
85.3 85.5
87.3 87.6
90.2 90.7
htl-3 91.6
htl-3 92.11
htl-3 93.1
91.7 91.1
93.3 93.7
htl-3
AtHTL
Controls
wild type (Col-0)

Fig. S8. Cont'd

Variant	leaf	major	minor	leaf ratio	rescued or not
1.2	6	151.29	93.86	1.61	Not Rescued
1.3	6	120.06	57.82	2.08	Rescued
1.4	6	127.98	60.27	2.12	Rescued
2.2	6	80.51	53.29	1.51	Not Rescued
2.7	6	157.87	49.61	3.18	Rescued
2.9	6	152.95	57.16	2.68	Rescued
3.2	6	159.34	56.40	2.83	Rescued
3.4	6	173.11	60.50	2.86	Rescued
3.7	6	140.15	65.73	2.13	Rescued
4.2	6	91.59	46.53	1.97	Rescued
4.5	6	191.86	75.73	2.53	Rescued
4.7	6	585.45	245.01	2.39	Rescued
5.3	6	270.44	90.37	2.99	Rescued
5.4	6	331.23	131.18	2.53	Rescued
5.8	6	235.30	92.08	2.56	Rescued
6.2	6	255.09	124.43	2.05	Rescued
6.8	6	198.58	83.57	2.38	Rescued
7.2	6	369.82	130.11	2.84	Rescued
7.3	6	239.60	123.78	1.94	Rescued
8.1	6	231.35	86.68	2.67	Rescued
8.6	6	277.89	104.80	2.65	Rescued
8.8	6	276.38	110.82	2.49	Rescued
9.2	6	320.29	110.41	2.90	Rescued
9.6	6	344.90	134.85	2.56	Rescued
9.12	6	240.84	108.80	2.21	Rescued
10.4	6	176.69	67.30	2.63	Rescued
10.9	6	176.31	68.47	2.57	Rescued
10.1	6	213.63	102.61	2.08	Rescued
11.1	6	256.27	119.03	2.15	Rescued
11.3	6	323.36	168.78	1.92	Ambiguous
11.7	6	289.00	159.95	1.81	Not Rescued
12.3	6	143.75	73.39	1.96	Rescued
12.5	6	298.23	116.27	2.56	Rescued
12.1	6	157.21	82.94	1.90	Ambiguous
13.3	6	158.32	150.03	1.06	Not Rescued
13.5	6	186.09	94.70	1.97	Rescued
13.7	6	241.63	125.51	1.93	Rescued
15.1	6	350.98	190.80	1.84	Not Rescued
15.8	6	309.69	158.11	1.96	Rescued
15.11	6	284.55	172.78	1.65	Not Rescued
16.1	6	365.44	162.60	2.25	Rescued
16.7	6	279.24	132.34	2.11	Rescued
16.8	6	290.51	126.73	2.29	Rescued
17.5	6	222.18	112.30	1.98	Rescued
17.7	6	361.51	123.66	2.92	Rescued
17.8	6	268.74	151.20	1.78	Not Rescued
18.3	6	307.57	159.01	1.93	Rescued
18.4	6	308.68	106.90	2.89	Rescued

Variant	leaf	major	minor	leaf ratio	rescued or not
18.9	6	285.36	125.80	2.27	Rescued
19.1	6	284.52	112.04	2.54	Rescued
19.9	6	326.96	131.35	2.49	Rescued
19.12	6	373.55	142.19	2.63	Rescued
20.1	6	306.30	100.89	3.04	Rescued
20.7	6	320.34	143.28	2.24	Rescued
20.9	6	367.40	155.85	2.36	Rescued
21.2	6	310.96	102.57	3.03	Rescued
21.4	6	223.50	97.04	2.30	Rescued
21.8	6	276.09	128.11	2.16	Rescued
22.3	6	588.86	295.42	1.99	Rescued
22.9	6	275.02	110.95	2.48	Rescued
22.11	6	313.52	113.70	2.76	Rescued
23.8	6	294.26	105.05	2.80	Rescued
23.11	6	305.96	165.42	1.85	Not Rescued
24.3	6	307.60	145.31	2.12	Rescued
24.11	6	347.18	141.23	2.46	Rescued
26.4	6	301.99	150.07	2.01	Rescued
26.5	6	349.29	159.31	2.19	Rescued
26.6	6	275.01	144.96	1.90	Ambiguous
27.5	6	342.70	187.24	1.83	Not Rescued
27.4	6	251.57	119.47	2.11	Rescued
27.7	6	374.68	139.79	2.68	Rescued
29.1	6	228.13	118.73	1.92	Ambiguous
29.2	6	401.95	168.35	2.39	Rescued
30.4	6	371.84	168.60	2.21	Rescued
30.7	6	359.57	191.60	1.88	Ambiguous
30.12	6	194.83	133.40	1.46	Not Rescued
32.9	6	544.43	300.12	1.81	Not Rescued
32.1	6	295.36	168.53	1.75	Not Rescued
34.8	6	344.48	161.47	2.13	Rescued
34.9	6	396.41	166.19	2.39	Rescued
34.1	6	482.38	197.52	2.44	Rescued
35.2	6	302.50	192.87	1.57	Not Rescued
35.3	6	512.32	279.41	1.83	Not Rescued
35.5	6	251.36	162.64	1.55	Not Rescued
36.5	6	309.46	198.22	1.56	Not Rescued
36.6	6	289.73	179.84	1.61	Not Rescued
36.9	6	375.76	166.34	2.26	Rescued
37.2	6	250.28	202.37	1.24	Not Rescued
37.5	6	315.15	180.48	1.75	Not Rescued
37.6	6	304.96	150.23	2.03	Rescued
38.2	6	300.28	116.27	2.58	Rescued
38.3	6	345.72	144.03	2.40	Rescued
38.4	6	228.22	101.10	2.26	Rescued
39.1	6	100.00	245.03	2.45	Rescued
39.2	6	100.00	225.89	2.26	Rescued
39.5	6	246.92	100.71	2.45	Rescued
40.1	6	192.05	109.21	1.76	Not Rescued

Variant	leaf	major	minor	leaf ratio	rescued or not
40.2	6	259.39	116.12	2.23	Rescued
40.3	6	240.09	130.69	1.84	Not Rescued
41.3	6	249.72	103.84	2.40	Rescued
41.5	6	207.29	121.19	1.71	Not Rescued
41.8	6	223.14	114.98	1.94	Rescued
42.4	6	269.30	102.31	2.63	Rescued
42.9	6	212.62	108.89	1.95	Rescued
42.12	6	235.03	92.97	2.53	Rescued
43.1	6	174.64	116.97	1.49	Not Rescued
43.2	6	229.41	127.71	1.80	Not Rescued
43.3	6	269.48	134.79	2.00	Rescued
44.2	6	212.86	101.91	2.09	Rescued
44.4	6	273.75	143.72	1.90	Ambiguous
44.8	6	353.47	141.17	2.50	Rescued
45.1	6	220.25	162.28	1.36	Not Rescued
45.4	6	721.03	378.41	1.91	Ambiguous
45.8	6	277.88	141.70	1.96	Rescued
46.1	6	250.72	140.79	1.78	Not Rescued
46.2	6	176.07	143.48	1.23	Not Rescued
46.4	6	249.54	174.62	1.43	Not Rescued
47.3	6	187.19	89.03	2.10	Rescued
47.4	6	133.09	93.94	1.42	Not Rescued
47.7	6	206.54	111.35	1.85	Ambiguous
48.2	6	222.04	132.42	1.68	Not Rescued
48.4	6	342.15	161.78	2.11	Rescued
48.5	6	402.12	182.29	2.21	Rescued
50.2	6	180.49	133.43	1.35	Not Rescued
50.5	6	216.71	133.54	1.62	Not Rescued
50.6	6	273.91	130.07	2.11	Rescued
51.3	1	204.82	122.48	1.67	Not Rescued
51.4	1	220.85	109.18	2.02	Rescued
51.5	1	190.98	127.66	1.50	Not Rescued
52.4	1	289.44	143.55	2.02	Rescued
52.6	1	202.23	104.56	1.93	Rescued
52.9	1	206.88	108.07	1.91	Ambiguous
53.1	1	224.61	132.80	1.69	Not Rescued
53.2	1	217.26	159.25	1.36	Not Rescued
53.3	1	209.40	92.34	2.27	Rescued
54.1	1	224.05	98.16	2.28	Rescued
54.2	1	213.27	124.32	1.72	Not Rescued
54.3	1	202.89	110.23	1.84	Not Rescued
55.1	1	267.71	158.14	1.69	Not Rescued
55.2	1	234.35	117.48	1.99	Rescued
55.5	1	288.62	160.17	1.80	Not Rescued
56.5	1	294.12	149.77	1.96	Rescued
56.9	1	293.03	136.90	2.14	Rescued
56.1	1	278.30	171.20	1.63	Not Rescued
57.8	1	256.94	137.30	1.87	Ambiguous
57.11	1	288.93	140.17	2.06	Rescued
57.12	1	298.83	170.53	1.75	Not Rescued

Variant	leaf	major	minor	leaf ratio	rescued or not
58.1	1	182.73	119.89	1.52	Not Rescued
58.3	1	249.74	159.86	1.56	Not Rescued
58.4	1	324.66	156.31	2.08	Rescued
59.3	1	393.29	180.84	2.17	Rescued
59.6	1	NA	NA	NA	NA
59.7	1	216.74	88.99	2.44	Rescued
60.3	1	256.77	105.30	2.44	Rescued
60.8	1	285.81	171.20	1.67	Not Rescued
60.1	1	310.17	191.00	1.62	Not Rescued
61.2	6	279.59	145.40	1.92	Rescued
61.4	6	397.98	165.39	2.41	Rescued
61.8	6	349.63	172.66	2.03	Rescued
62.3	6	464.72	192.55	2.41	Rescued
62.6	6	462.73	291.33	1.59	Not Rescued
62.7	6	333.11	148.04	2.25	Rescued
63.1	6	314.09	128.54	2.44	Rescued
63.3	6	296.54	123.35	2.40	Rescued
63.4	6	323.02	133.77	2.41	Rescued
64.3	6	363.03	166.99	2.17	Rescued
64.4	6	406.32	186.79	2.18	Rescued
64.5	6	299.39	153.94	1.94	Rescued
65.1	6	314.90	144.24	2.18	Rescued
65.12	6	384.74	197.80	1.95	Rescued
66.5	6	264.68	177.56	1.49	Not Rescued
66.6	6	341.74	210.20	1.63	Not Rescued
66.1	6	257.45	172.22	1.49	Not Rescued
67.3	6	274.44	118.00	2.33	Rescued
67.5	6	340.05	135.02	2.52	Rescued
67.6	6	261.40	141.48	1.85	Not Rescued
68.3	6	279.21	150.82	1.85	Ambiguous
68.5	6	279.21	150.82	1.85	Ambiguous
68.6	6	260.66	161.32	1.62	Not Rescued
69.2	6	327.92	155.41	2.11	Rescued
69.4	6	261.51	153.20	1.71	Not Rescued
69.6	6	270.46	107.54	2.51	Rescued
70.1	6	294.85	146.40	2.01	Rescued
70.2	6	534.00	286.20	1.87	Ambiguous
70.4	6	229.41	106.15	2.16	Rescued
71.5	6	284.59	145.96	1.95	Rescued
71.6	6	365.91	153.77	2.38	Rescued
71.9	6	341.86	165.76	2.06	Rescued
72.1	6	263.94	117.98	2.24	Rescued
72.2	6	327.65	154.97	2.11	Rescued
72.6	6	589.05	245.37	2.40	Rescued
73.1	6	300.45	138.49	2.17	Rescued
73.3	6	267.41	127.70	2.09	Rescued
73.4	6	553.92	226.70	2.44	Rescued
74.1	6	250.55	133.06	1.88	Ambiguous
74.2	6	259.06	140.90	1.84	Not Rescued
74.3	6	306.79	172.35	1.78	Not Rescued
75.3	6	232.14	128.83	1.80	Not Rescued

Variant	leaf	major	minor	leaf ratio	rescued or not
75.6	6	388.54	255.55	1.52	Not Rescued
75.7	6	255.16	148.59	1.72	Not Rescued
76.2	6	543.07	282.74	1.92	Rescued
76.3	6	179.38	109.87	1.63	Not Rescued
76.6	6	192.81	105.21	1.83	Not Rescued
77.1	6	318.00	141.80	2.24	Rescued
77.4	6	276.73	145.94	1.90	Ambiguous
77.5	6	313.89	152.10	2.06	Rescued
78.6	6	244.28	162.02	1.51	Not Rescued
78.7	6	274.17	159.10	1.72	Not Rescued
78.8	6	201.72	120.37	1.68	Not Rescued
79.6	6	273.57	152.91	1.79	Not Rescued
79.7	6	298.10	153.25	1.95	Rescued
79.8	6	276.22	134.32	2.06	Rescued
81.1	6	276.88	141.06	1.96	Rescued
81.6	6	249.38	149.22	1.67	Not Rescued
81.12	6	264.66	147.28	1.80	Not Rescued
82.3	6	277.15	185.60	1.49	Not Rescued
82.5	6	302.05	171.51	1.76	Not Rescued
82.6	6	282.50	174.01	1.62	Not Rescued
83.1	6	313.88	173.87	1.81	Not Rescued
83.4	6	358.48	166.94	2.15	Rescued
83.7	6	291.89	179.88	1.62	Not Rescued
84.3	6	317.35	166.62	1.90	Ambiguous
84.4	6	323.50	180.05	1.80	Not Rescued
84.7	6	274.82	151.54	1.81	Not Rescued
85.1	6	281.35	209.44	1.34	Not Rescued
85.2	6	310.01	188.47	1.64	Not Rescued
85.7	6	261.45	177.54	1.47	Not Rescued
86.1	6	110.45	91.38	1.21	Not Rescued
86.3	6	184.42	94.80	1.95	Rescued
86.4	6	NA	NA	NA	NA
87.3	6	233.66	156.69	1.49	Not Rescued
87.5	6	162.63	98.34	1.65	Not Rescued
87.6	6	313.23	207.41	1.51	Not Rescued
88.1	6	279.19	162.78	1.72	Not Rescued
88.3	6	156.49	80.20	1.95	Rescued
88.4	6	220.25	123.05	1.79	Not Rescued
89.1	6	282.59	182.94	1.54	Not Rescued
89.3	6	221.67	113.10	1.96	Rescued
89.7	6	314.53	179.34	1.75	Not Rescued
90.1	6	188.96	119.16	1.59	Not Rescued
90.2	6	262.65	168.12	1.56	Not Rescued
90.7	6	291.46	135.09	2.16	Rescued
91.6	6	251.83	135.57	1.86	Ambiguous
91.7	6	251.07	166.97	1.50	Not Rescued
91.1	6	281.53	176.63	1.59	Not Rescued
92.11	6	330.16	220.29	1.50	Not Rescued
93.1	6	387.18	214.33	1.81	Not Rescued
93.3	6	283.49	201.29	1.41	Not Rescued
93.7	6	695.50	233.48	2.98	Rescued

Fig. S8. Cont'd

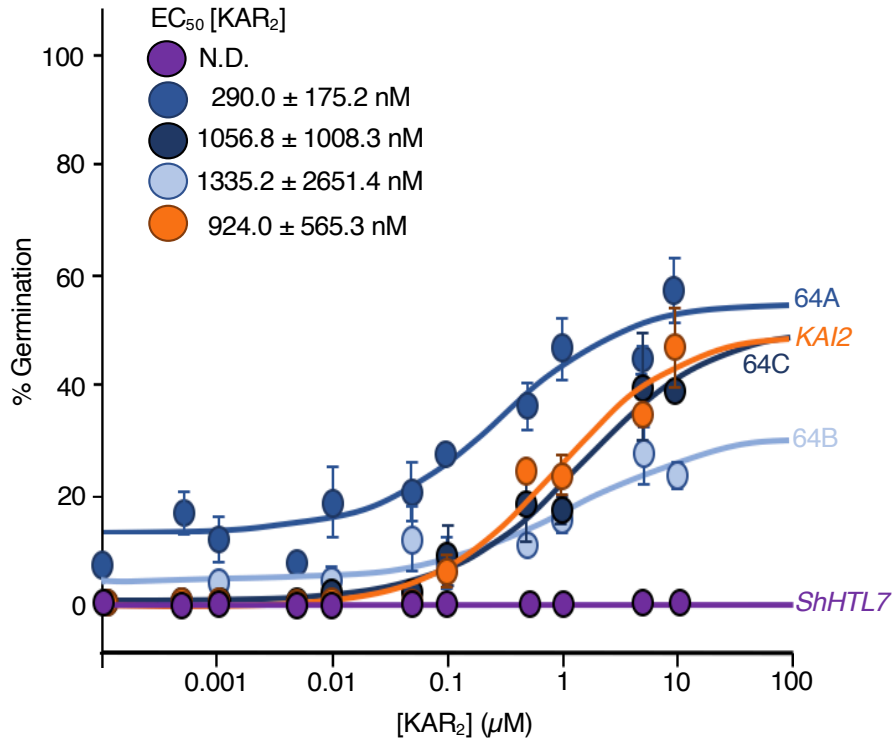

Fig. S9. Germination response of *ShHTL7*, *KAI2*, and Var64 seed to karrikin. Misexpressed *ShHTL7*, *KAI2* and three biological independent Var64 seed (64A, 64B, 64C) germinated on PAC (20 μM) and increasing KAR_2 concentrations. Four-parameter logistic curves were fitted to the data, and EC_{50} values, ± standard error, of KAR_2 concentrations have been included for those lines showing response to karrikin. Based on these curves, the effective concentrations that germinates 50% of seed (EC_{50}) are indicated.

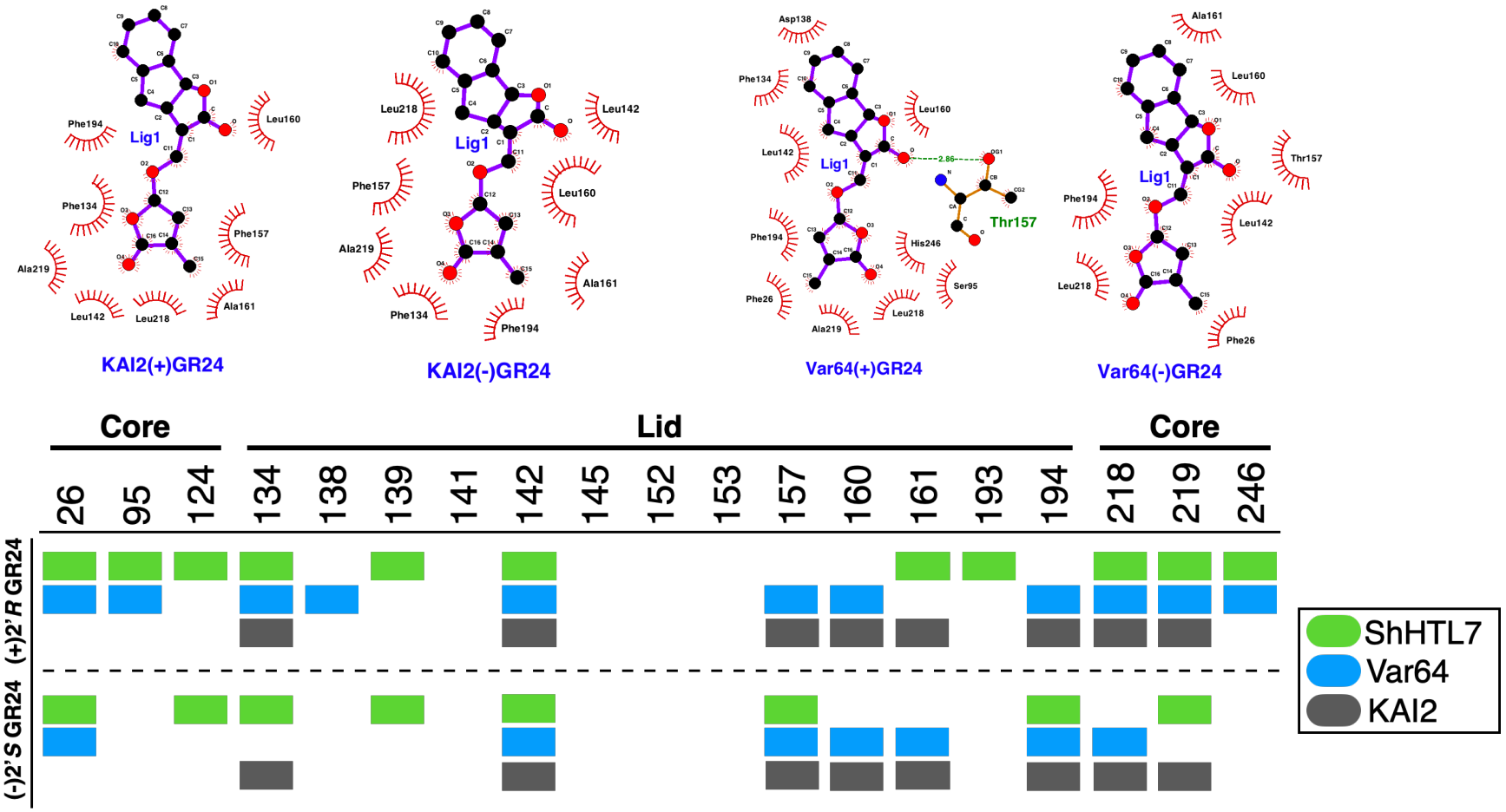

Fig. S10. *In silico* docking of 2'*R*-GR24 and 2'*S*-GR24. The two GR24 enantiomers were docked into ShHTL7, Var64, and KAI2. As expected, ShHTL7 displays the most interactions with 2'*R*-GR24. KAI2 has the most contacts with 2'*S*-GR24.

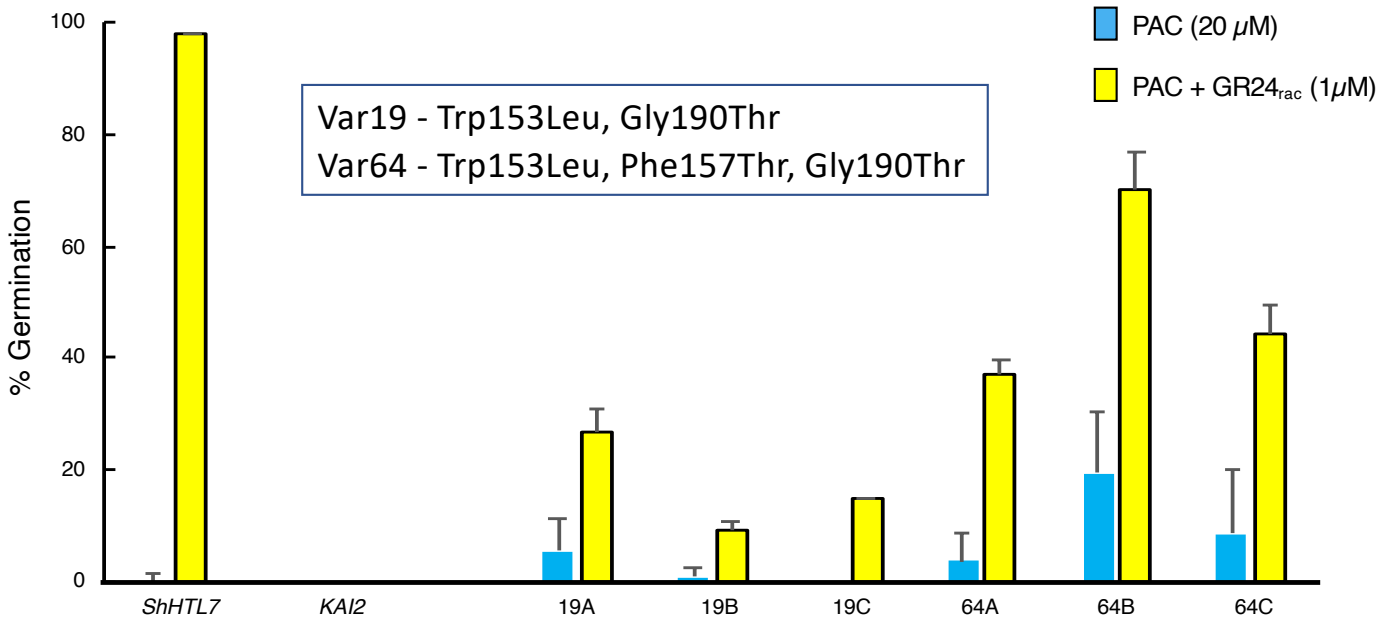

Fig. S11. SL dependent germination of mis-expressed *ShHTL7*, *KAI2,* three biologically independent Var19 lines (19A, 19B, 19C) and three biologically independent Var64 lines (64A, 64B, 64C). Each line was tested three times Bar = S.D

Table S1. Protein-ligand docking statistics

Protein	Ligand	SwissDock	AutoDock	RMSD vs 5DJ5
Var64	*(2'R)*-GR24	-7.02 kcal mol^{-1}	-6.97 kcal mol^{-1}	3.025 Å
Var64	*(2'S)*-GR24	-6.33 kcal mol^{-1}	-6.39 kcal mol^{-1}	3.100 Å
KAI2	*(2'R)*-GR24	-5.50 kcal mol^{-1}	-5.41 kcal mol^{-1}	2.901 Å
KAI2	*(2'S)*-GR24	-5.92 kcal mol^{-1}	-5.84 kcal mol^{-1}	3.148 Å

Table S2. Molecular dynamics simulations statistics

System	Convergence timepoint	Average RMSD after convergence (ligand)	Average Potential Energy
Var64 + (*2'R*)-GR24 #1	214 ns	0.4175 Å	-3.679 kJ/mol
Var64 + (*2'R*)-GR24 #2	198 ns	0.4093 Å	-3.128 kJ/mol
Var64 + (*2'R*)-GR24 #3	223 ns	0.4419 Å	-3.709 kJ/mol
KAI2 + (*2'R*)-GR24 #1	270 ns	0.9592 Å	-3.721 kJ/mol
KAI2 + (*2'R*)-GR24 #2	291 ns	0.9817 Å	-3.875 kJ/mol
KAI2 + (*2'R*)-GR24 #3	284 ns	0.9799 Å	-3.814 kJ/mol
Var64 #1	59 ns	N/A	-1.395 kJ/mol
Var19 #1	43 ns	N/A	-1.118 kJ/mol
KAI2 #1	28 ns	N/A	-1.204 kJ/mol

Table S3 Protein-Ligand Interaction Profile

Structure	Residue ID	Distance to ligand (Å)
DWARF14-GR24 (5DJ5)	Phe28	3.77 + 3.62
	Ser97	2.40
	Val98	3.47
	Phe126	3.72
	Phe136	3.81
	Val144	3.29
	Tyr159	3.48 + 4.02
	Val194	3.94
	Phe195	3.84
	Val219	3.26
	Ser220	3.62
	His247	4.52 + 3.88
DWARF14-CLIM (4IHA)	Phe28	3.07
	His96	5.41
	Val98	3.94 + 2.74
	Phe126	3.84
	Val194	3.20
	His247	3.31 + 3.83 + 5.01
OsD14-7'carba-4BD product (5YZ7)	Phe78	3.53
	Val148	3.85
	Phe186	3.6
	Val194	3.99
	Tyr209	3.91 + 4.38
	Val244	3.49
	Phe245	3.73
Var64-(+)GR24	Phe26	3.80
	Ser95	2.32
	Phe134	3.71
	Asp138	3.88
	Leu142	3.54 + 3.83
	Thr157	3.65
	Leu160	3.52
	Phe194	3.93
	Leu218	3.96
	Ala219	3.99
	His246	4.64

Movie S.1. The Var64 receptor displays increased flexibility and a larger binding pocket. A 1 μs molecular dynamics simulation of KAI2 (grey) and Var64 (blue) reveals that the αE loop of Var64 presents increased motility, and its three mutations (shown in red) result in improved flexibility along the X-axis of the protein. These two factors would produce a more efficient perception of SLs as the active site opening area of Var64 is increased by 11.5 Å.

Movie S.2. Enhanced interaction between Var64 and 2*'R*-(+)GR24. 1 μs molecular dynamics simulation reveals that the polar Thr190 in Var64 (blue) does a better job stabilizing the ligand than its WT counterpart. Upon binding, the αD helix of Var64 increases its movement, possibly resulting in enhanced interactions with its downstream protein partner.

Movie S.3. The WT Phe157 in Var19 directly affects the flexibility of the receptor. 0.5 μs molecular dynamics simulation comparing the flexibility of Var19 (grey) and Var64 (blue) reveals that the presence of the WT Phe157 (grey sticks) from KAI2 produces stronger interactions between the opposite sides of the lid domain; this results in a decreased flexibility and a reduced binding pocket area, making the interaction with SL more challenging that in Var64 (variant residues shown in red sticks).