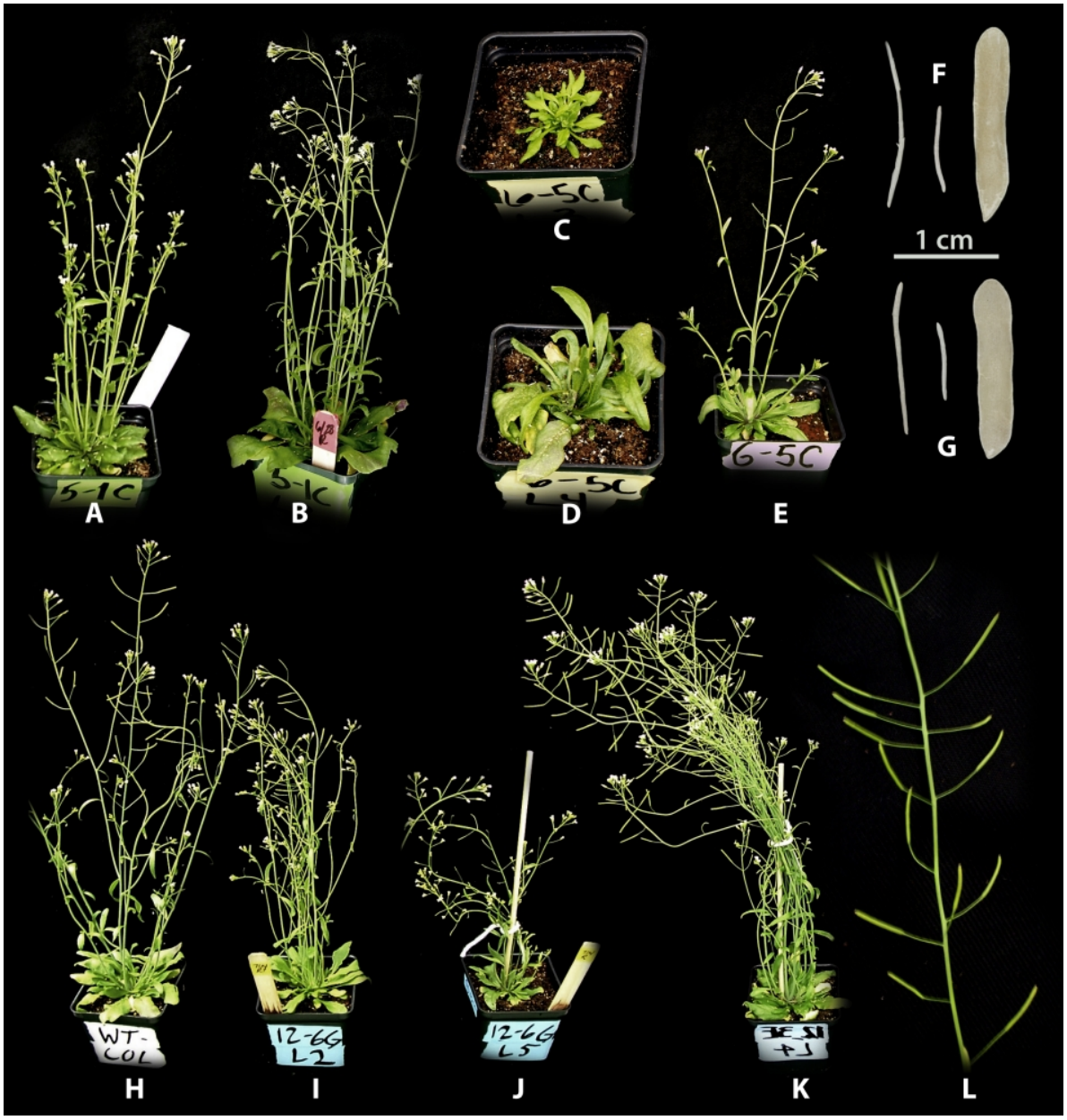
Supporting Figures and Tables

Figure S1.



Additional examples of repeatable phenotypes from independent T1 plants transformed with clones 05_01C, 06_05C, 12_06G and 12_03E.

(A-B) Short fruit on T1 plants from clone 05_01C plants. These T1 plants additionally shared stunted tertiary branches with slight loss of apical dominance (see wildtype plant in H for

comparison).

(C-E) T1 plants from clone 06_05C showed abnormal vegetative development (C, D) and produced few seeds. T1 plant from clone 06_05C with short fruit (E).

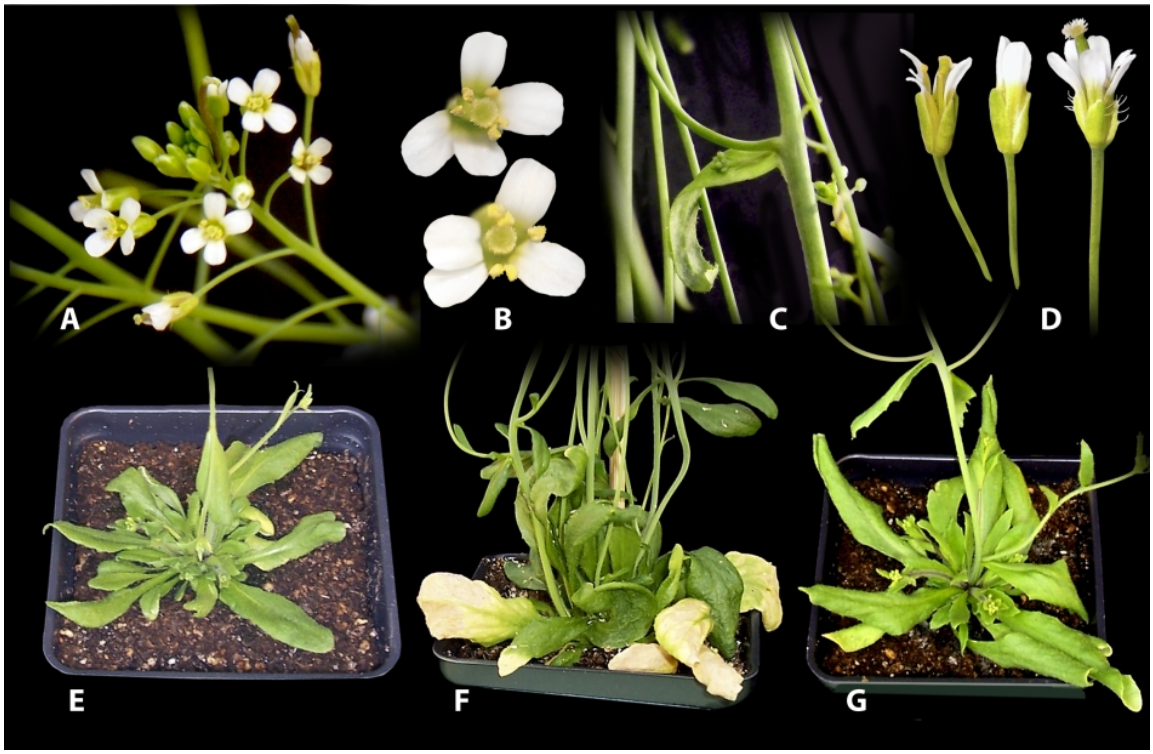
(F-G) Short valves from T1 plants 05_01C (F, center) and 06_05C (G, center). On the sides of each transformant valve is a wildtype valve from *A. thaliana* (left) and *L. alabamica* (right).

(H) *Arabidopsis thaliana* (Columbia).

(I-J) Two dwarf T1 plants from clone 12_06G. The wildtype plant (H) was sowed at the same time as the dwarf plants.

(K-L) Clustered fruit of a T1 plant (K) and its kanamycin-resistant offspring (L) from clone 12_03E.

Figure S2.



Additional examples of repeatable phenotypes from independent T1s transformed with clones 09_09A, 12_05A, and 11_01D.

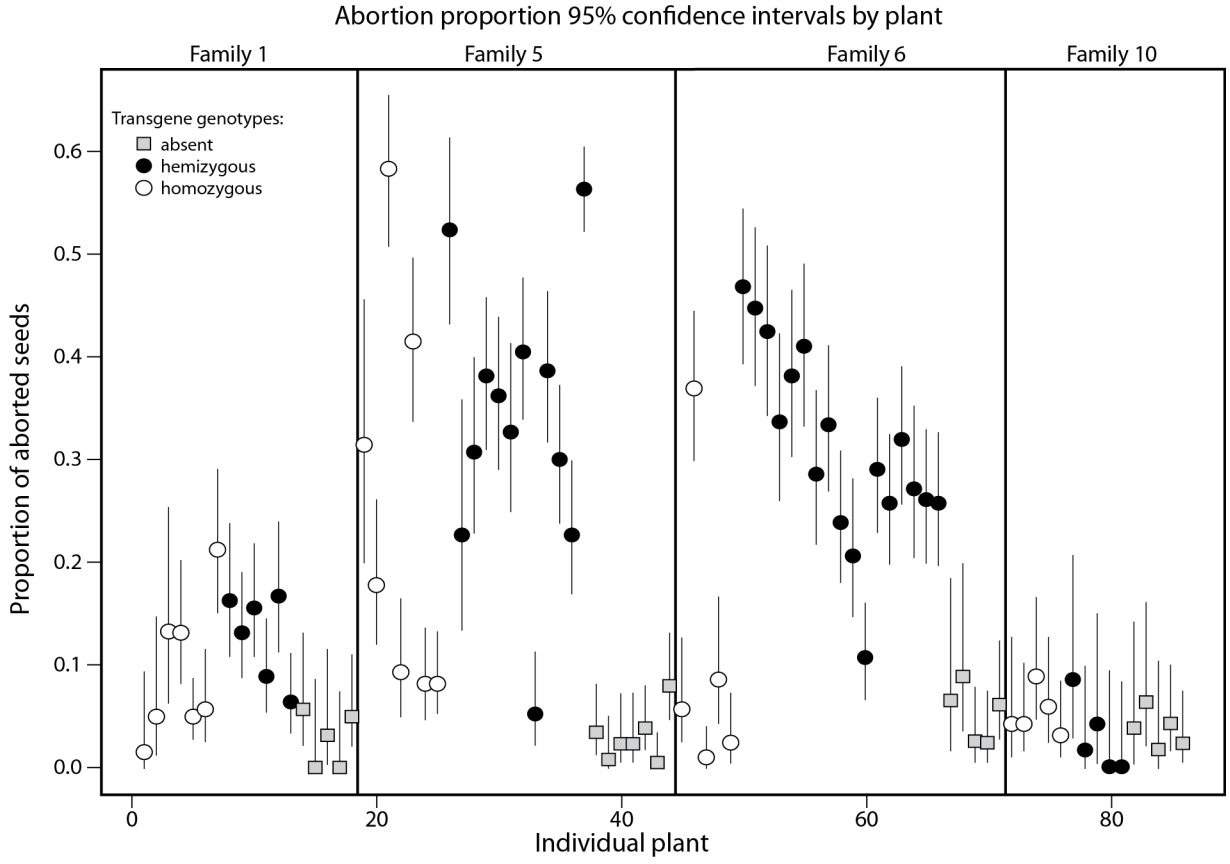
(A-B) Unevenly spaced petals on a T1 plant from clone 09_09A (A), its kanamycin-resistant offspring (B, top), and a kanamycin-resistant T2 plant from a different T1 line (B, bottom).

(C) A decurrent cauline leaf in a T1 plant generated with clone 12_05A.

(D) Splayed flower petals of two T1 plants from clone 11_01D (E, sides), flanking a flower from wildtype *A. thaliana* (center). Photo taken in late afternoon when normal flowers are closed.

(E-G) Abnormal rosette leaf development of T1 plants from clone 11_01D. Rosette leaf examples show some lobing (E), and severe (F) or moderate (G) contortion and twisting.

Figure S3.



Seed abortion rates in four T2 families (lines 1, 5, 6, and 10) segregating for clone 11_11B. For each family the seed abortion rates are plotted for the wildtype homozygotes (squares), transgene homozygotes (open circles), and hemizygotes (closed circles) with 95% confidence intervals.

Examination of the transgene-containing lines suggests a bimodal distribution, with a subset of lines having low levels of seed abortion (similar to wildtype lines) and a second subset having elevated abortion levels.

Table S1. Observed kanamycin resistant:sensitive ratio (KRS) in screened T1 lines.

Clone	Line	Observed T2 segregation			Interpretation[a]
		resistant	*sensitive*	KRS(:1) [99% CI]	
05_01C	1	271	66	4.1 [2.88, 5.85]	1
05_01C	2	322	170	1.9 [1.48, 2.42]	<1
05_01C	4	466	154	3.0 [2.38, 3.84]	1
05_01C	12	372	163	2.3 [1.79, 2.91]	<1
05_01C	13	307	68	4.5 [3.20, 6.38]	1-2
05_01C	27	440	93	4.7 [3.53, 6.35]	1-2
06_05C	1	454	19	23.9 [13.07, 43.68]	2
06_05C	3	343	320	1.1 [0.88, 1.31]	<1
06_05C	4	376	19	19.8 [10.80, 36.27]	2
06_05C	7	165	110	1.5 [1.09, 2.06]	<1
09_09A	1	198	57	3.5 [2.36, 5.12]	1
09_09A	2	341	88	3.9 [2.85, 5.27]	1
09_09A	4	350	59	5.9 [4.13, 8.52]	1-2
09_09A	5	315	103	3.1 [2.28, 4.10]	1
11_01D	1	260	145	1.8 [1.37, 2.34]	<1
11_01D	3	365	100	3.7 [2.73, 4.88]	1
11_01D	4	231	115	2.0 [1.50, 2.70]	<1
11_01D	5	309	190	1.6 [1.28, 2.06]	<1
11_01D	6	360	97	3.7 [2.76, 4.98]	1
11_01D	7	465	180	2.6 [2.06, 3.24]	1
11_01D	25	434	133	3.3 [2.53, 4.21]	1
11_01D	30	430	72	6.0 [4.30, 8.29]	1-2
11_11B	1	433	109	4.0 [3.01, 5.24]	1-2
11_11B	2	54	401	0.1 [0.09, 0.20]	<1

11_11B	5	462	109	4.2 [3.22, 5.58]	1-2
11_11B	6	37	9	4.1 [1.58, 10.71]	1
11_11B	10	481	79	6.1 [4.45, 8.32]	1-2
11_11B	15	88	262	0.3 [0.24, 0.46]	<1
12_03E	1	69	665	0.1 [0.07, 0.14]	<1
12_03E	4	435	171	2.5 [2.02, 3.21]	1
12_03E	5	404	153	2.6 [2.07, 3.37]	1
12_03E	13	293	57	5.1 [3.54, 7.46]	1-2
12_03E	14	502	161	3.1 [2.47, 3.94]	1
12_05A	4	354	92	3.8 [2.85, 5.20]	1
12_05A	5	304	84	3.6 [2.63, 4.97]	1
12_05A	6	1497	491	3.0 [2.67, 3.49]	1
12_05A	7	440	184	2.4 [1.91, 3.00]	1
12_06G	1	32	114	0.3 [0.17, 0.47]	<1
12_06G	2	112	75	1.5 [1.02, 2.19]	<1
12_06G	8	379	122	3.1 [2.38, 4.06]	1
12_06G	38	413	134	3.1 [2.39, 3.98]	1
12_06G	39	356	116	3.1 [2.33, 4.04]	1
12_06G	41	523	103	5.1 [3.85, 6.70]	1-2
12_06G	42	413	138	3.0 [2.32, 3.86]	1

[a] If the 99% confidence interval of the observed KRS odds estimate contains the null expectation for a single locus (1) or two, unlinked loci (2) this is indicated. If the 99% confidence interval is below the null expectation for a single locus (<1), silencing of the selectable marker is likely. If the 99% confidence interval is between the null expectations for one and two loci (1-2), then this could indicate two linked loci or two (or more) loci with some silencing.

Table S2. Tests for gene silencing among lines with KRS ratios significantly less than 3.

Clone	Line	Observed T2 segregation		Interpretation
		KRS(:1) [99% CI]	*NPTII* (:1) [99% CI]*	
05_01C	2	1.9 [1.48, 2.42]	6.3 [1.56, 25.02]	a
05_01C	12	2.3 [1.79, 2.91]	2.6 [0.93, 7.04]	a
06_05C	3	1.1 [0.88, 1.31]	2.2 [0.82, 5.88]	a
06_05C	7	1.5 [1.09, 2.06]	1.3 [0.50, 3.57]	a
11_01D	1	1.8 [1.37, 2.34]	1.6 [0.52, 4.68]	a
11_01D	4	2.0 [1.50, 2.70]	1.5 [0.58, 3.69]	a
11_01D	5	1.6 [1.28, 2.06]	3.0 [1.05, 8.59]	a
11_11B	2	0.1 [0.09, 0.20]	1.4 [0.54, 3.74]	b
11_11B	15	0.3 [0.24, 0.46]	2.3 [0.84, 6.51]	b
12_03E	1	0.1 [0.07, 0.14]	0.3 [0.12, 0.95]	c
12_06G	1	0.3 [0.17, 0.47]	1.0 [0.44, 2.26]	c
12_06G	2	1.5 [1.02, 2.19]	4.7 [1.66, 13.77]	b

* Between 23-40 T2 offspring were genotyped for presence/absence of *NPTII*.

[a] Unclear whether the deficient KRS ratio reflects a deficit of the *NPTII* genotype in the T2 generation. 99% confidence interval of the *NPTII* ratio includes the deficient KRS ratio and at least one Mendelian segregation ratio (3 and/or 15).

[b] Gene silencing accounts for the deficient KRS ratio. 99% confidence interval of the *NPTII* ratio lacks the KRS ratio and contains the Mendelian ratio (3) expected for a single transgene locus.

[c] Lines show a significant deficit of the *NPTII* genotype in the T2 generation. A more complex genetic mechanism (e.g. Ray *et al.*, 1997) may account for this effect. The 99% confidence interval of the *NPTII* ratio is beneath the lowest Mendelian ratio 3.