Figure S1

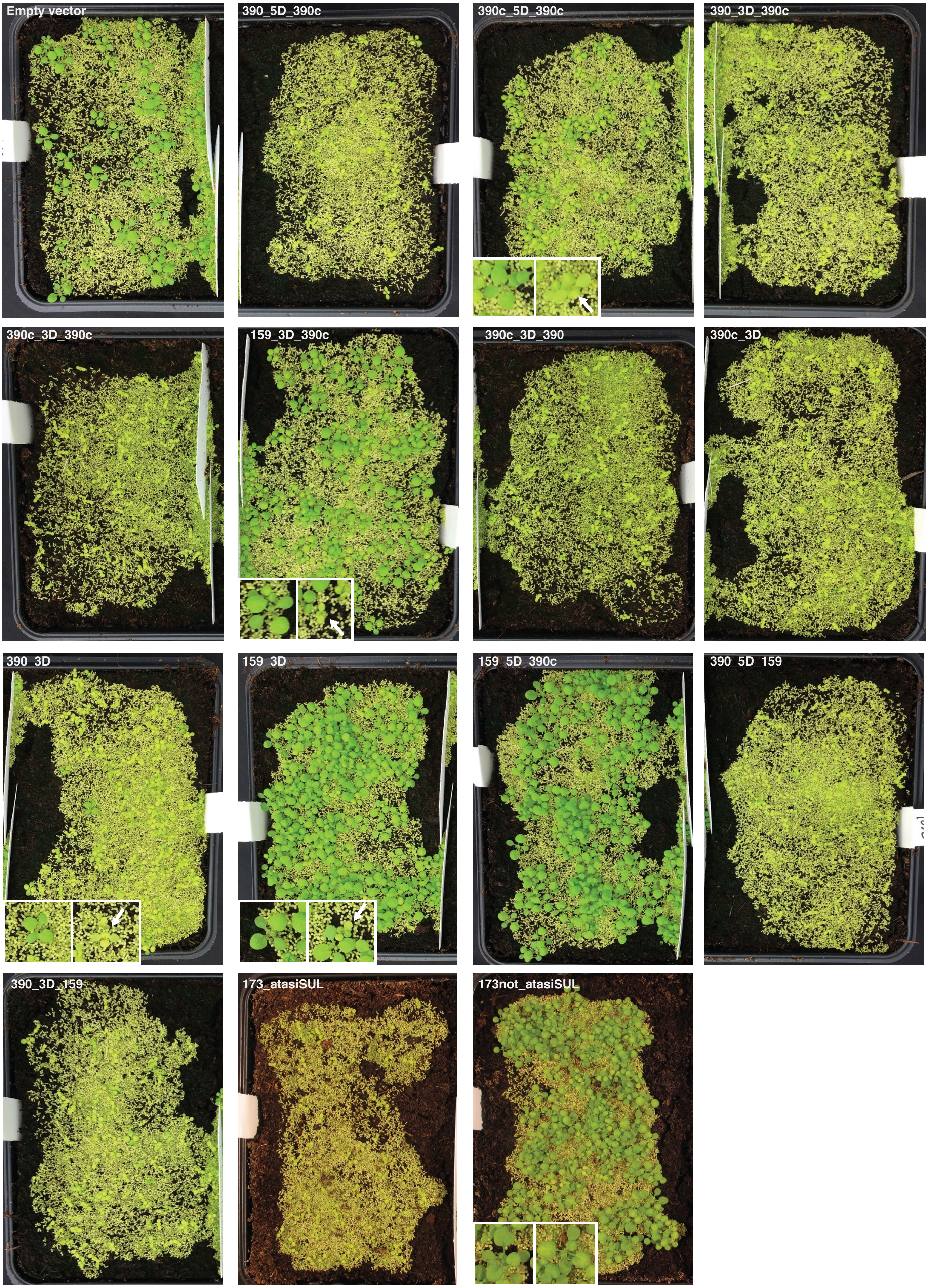

Empty vector
390_5D_390c
390c_5D_390c
390_3D_390c
390c_3D_390c
159_3D_390c
390c_3D_390
390c_3D
390_3D
159_3D
159_5D_390c
390_5D_159
390_3D_159
173_atasiSUL
173not_atasiSUL

Figure S1. T1 populations of the various lines used in this study. 2-weeks old plants were selected on BASTA. In cases where more than one phenotype was observed, magnification is provided, with wild-type-like individuals on the left and bleached individuals on the right. A white arrow points to the referred plant, when necessary.

Figure S2

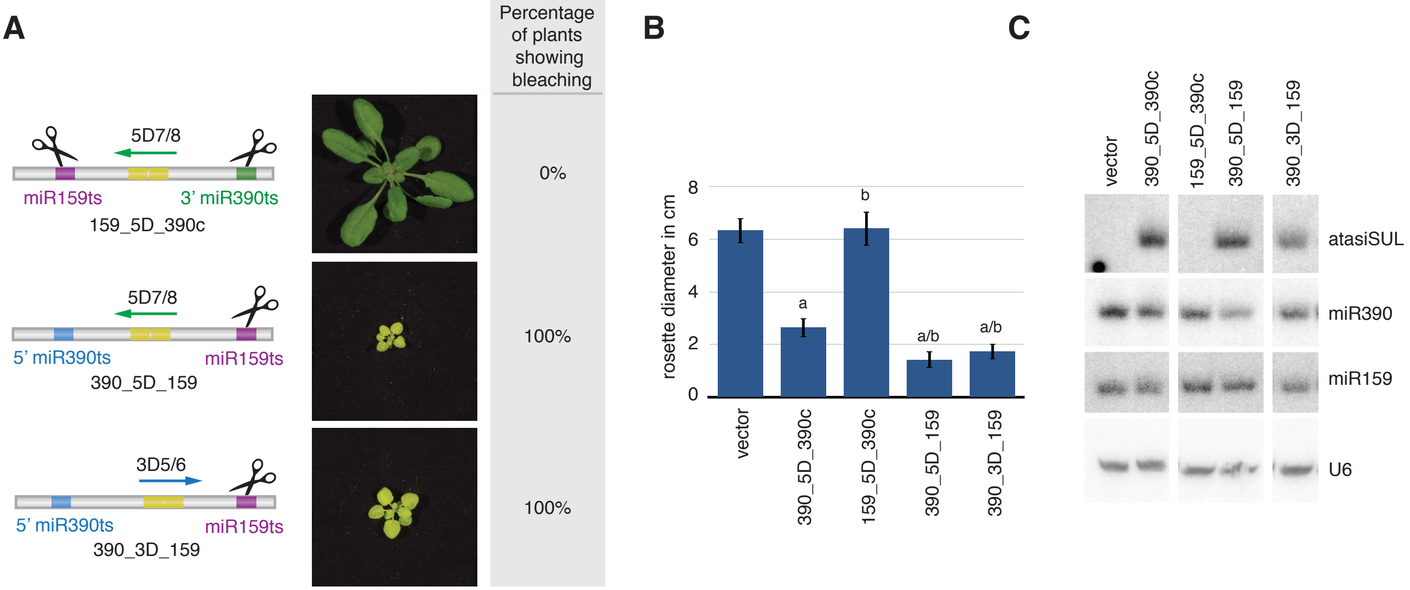

A
Percentage of plants showing bleaching
5D7/8
miR159ts
3' miR390ts
159_5D_390c
0%
5D7/8
5' miR390ts
miR159ts
390_5D_159
100%
3D5/6
5' miR390ts
miR159ts
390_3D_159
100%
B
rosette diameter in cm
0
2
4
6
8
a
b
a/b
a/b
vector
390_5D_390c
159_5D_390c
390_5D_159
390_3D_159
C
vector
390_5D_390c
159_5D_390c
390_5D_159
390_3D_159
atasiSUL
miR390
miR159
U6

Figure S2. Single 3’ cleavage by miR159 allows TAS3 tasiRNA production. A) Schematic of the constructs used, representative phenotypes and silencing frequency as in Figure 1-2. The colour code is as in Figure 1-2. B) Quantification of the bleaching intensity by rosette size was performed as in Figure 1. C) Northern blot and RT-qPCR analyses of small RNAs and SUL mRNA steady state levels, respectively, were performed as described in Figure 1.

Figure S3

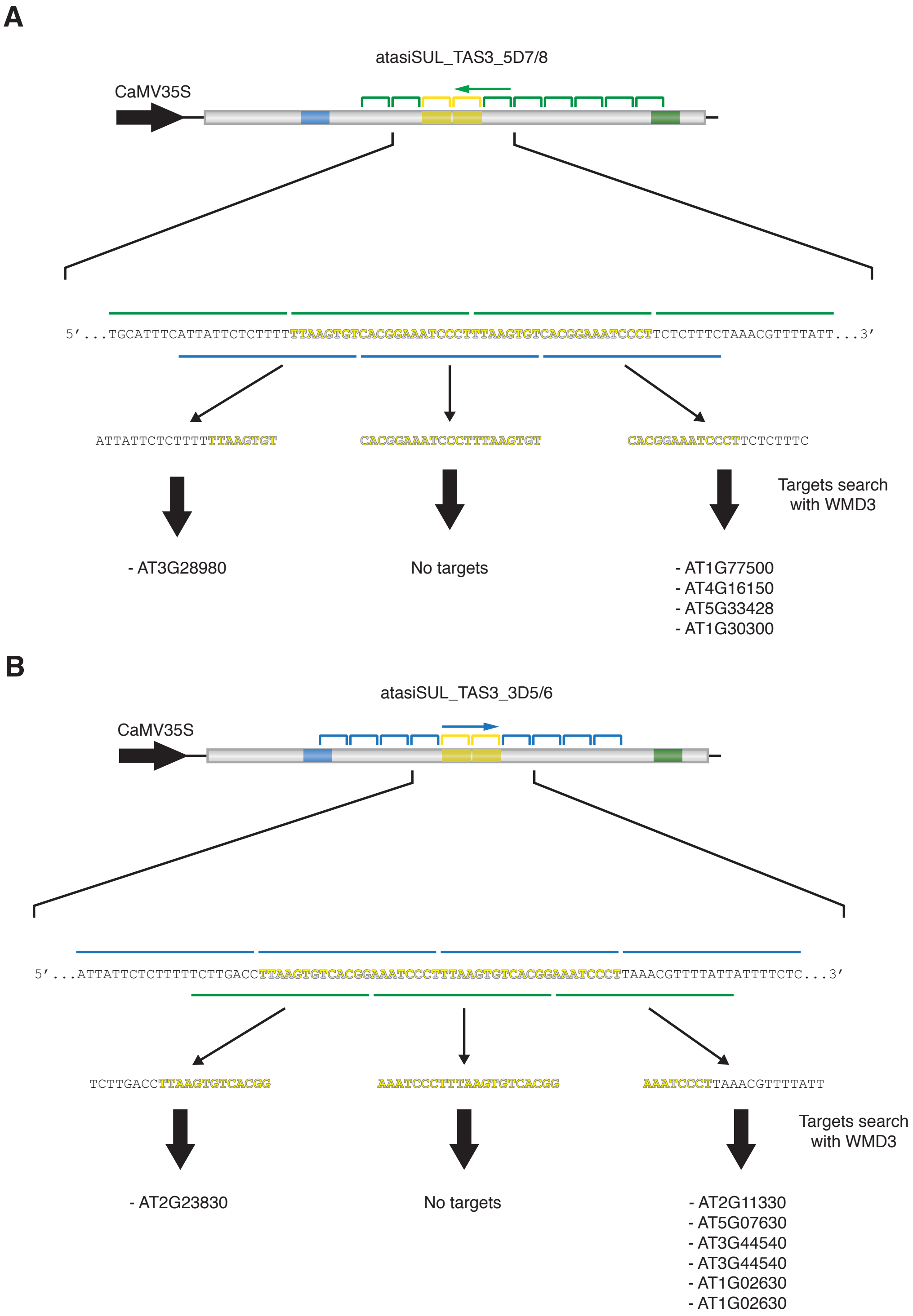

A
atasiSUL_TAS3_5D7/8
CaMV35S
5′...TGCATTTCATTATTCTCTTTTTTAAGTGTCACGGAAATCCCTTTAAGTGTCACGGAAATCCCTTCTCTTTCTAAACGTTTTATT...3′
ATTATTCTCTTTTTTAAGTGT
CACGGAAATCCCTTTAAGTGT
CACGGAAATCCCTTCTCTTTC
Targets search with WMD3
- AT3G28980
No targets
- AT1G77500
- AT4G16150
- AT5G33428
- AT1G30300
B
atasiSUL_TAS3_3D5/6
CaMV35S
5′...ATTATTCTCTTTTTCTTGACCTTAAGTGTCACGGAAATCCCTTTAAGTGTCACGGAAATCCCTTAAACGTTTTATTATTTTCTC...3′
TCTTGACCTTAAGTGTCACGG
AAATCCCTTTAAGTGTCACGG
AAATCCCTTAAACGTTTTATT
Targets search with WMD3
- AT2G23830
No targets
- AT2G11330
- AT5G07630
- AT3G44540
- AT3G44540
- AT1G02630
- AT1G02630

Figure S3. Prediction of offset siRNA sequences produced by opposing phasing, and of their putative mRNA targets. A-B) Magnification of the area encoding the atasiSUL siRNA. The bars on the top of the *TAS3a* sequence indicate the correct register for production of the cognate atasiSUL RNA. The bars on the bottom indicate the predicted register resulting from phasing set by the alternative miRNA target site. The predicted siRNAs resulting from the offset phasing are indicated alongside their respective putative mRNA targets, as predicted by the WMD3 algorithm (wmd3.weigelworld.org – Schwab et al., 2006, Plant Cell 18: 1121-1133).

Figure S4

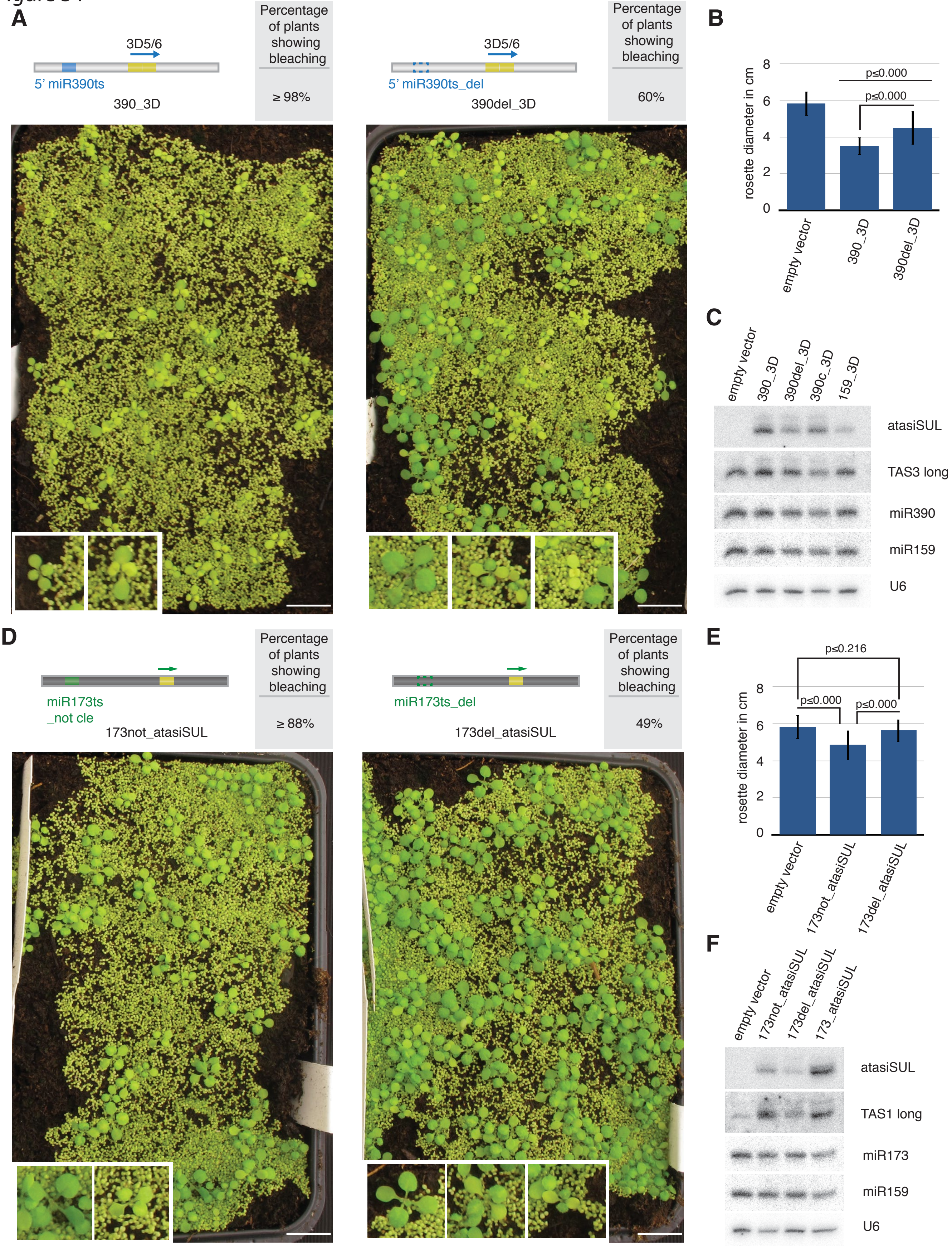

A
3D5/6
5' miR390ts
390_3D
Percentage of plants showing bleaching
≥ 98%
3D5/6
5' miR390ts_del
390del_3D
Percentage of plants showing bleaching
60%
B
rosette diameter in cm
0
2
4
6
8
p≤0.000
p≤0.000
empty vector
390_3D
390del_3D
C
empty vector
390_3D
390del_3D
390c_3D
159_3D
atasiSUL
TAS3 long
miR390
miR159
U6
D
miR173ts
_not cle
173not_atasiSUL
Percentage of plants showing bleaching
≥ 88%
miR173ts_del
173del_atasiSUL
Percentage of plants showing bleaching
49%
E
p≤0.216
p≤0.000
p≤0.000
rosette diameter in cm
0
2
4
6
8
empty vector
173not_atasiSUL
173del_atasiSUL
F
empty vector
173not_atasiSUL
173del_atasiSUL
173_atasiSUL
atasiSUL
TAS1 long
miR173
miR159
U6

Figure S4. Impact of co-suppression on the atasiSUL-based reporter systems. A and D) Populations of T1 plants expressing the TAS3a- (A) or the TAS1-based (D) atasiSUL reporter constructs in which the respective miRNA target sites have been erased are shown on the right. The respective reference T1 populations of plants expressing the cognate non-cleavable sites are shown on the left. The percentage of silencing was calculated based on analysis of at least 50 individual plants in each case. The various phenotypes are depicted in detail in the inlays at the bottom of each panel. B and E) Quantification of bleaching intensity by rosette size measurements of at least 15 individual plants. P-values derived from a t-test (two-sample equal variance; two-tailed distribution) between the different lines and the empty vector control; or between two specific lines are indicated. C and F) Molecular characterization by northern blot was performed as described in Figures 1 and Figure 4.

Figure S5

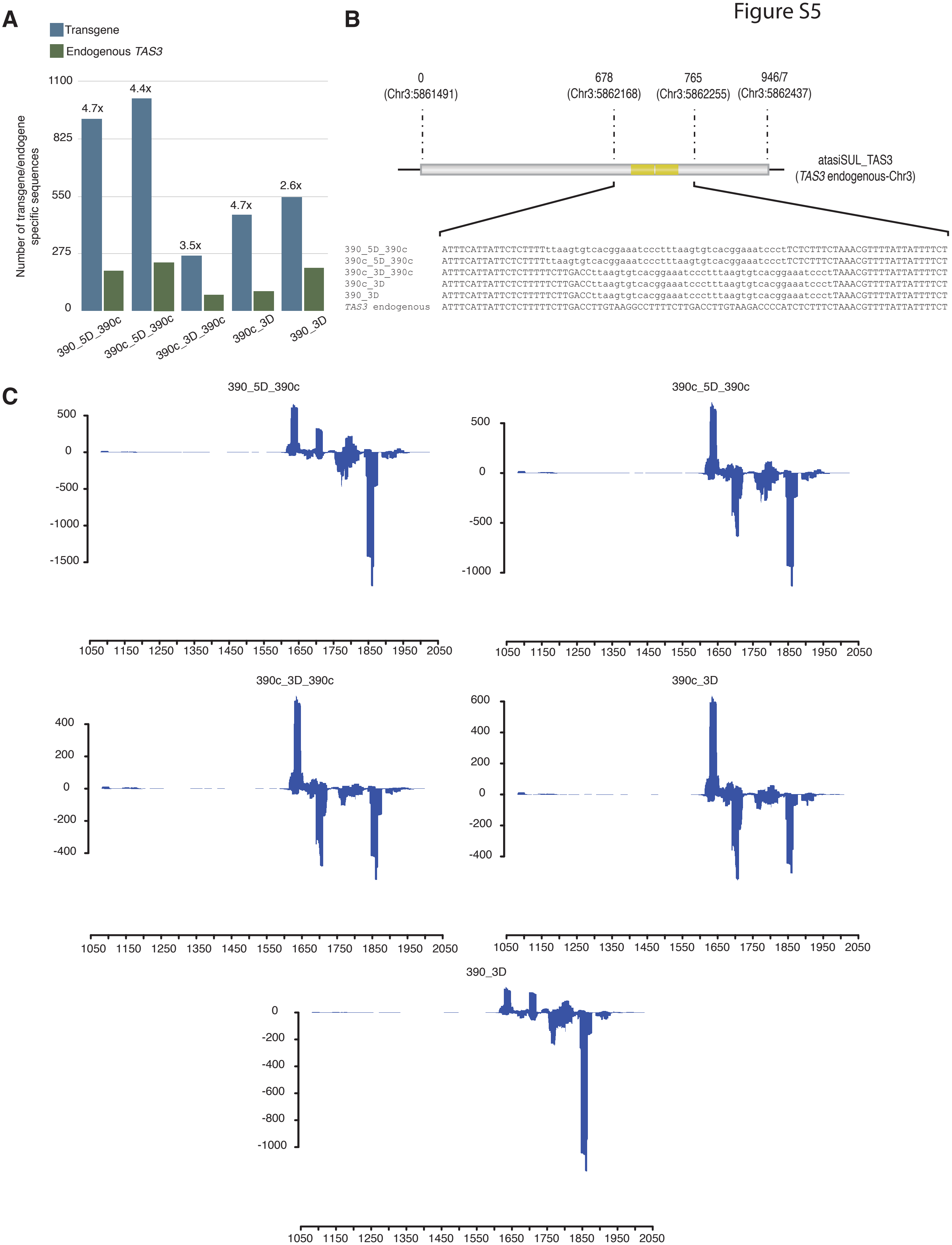
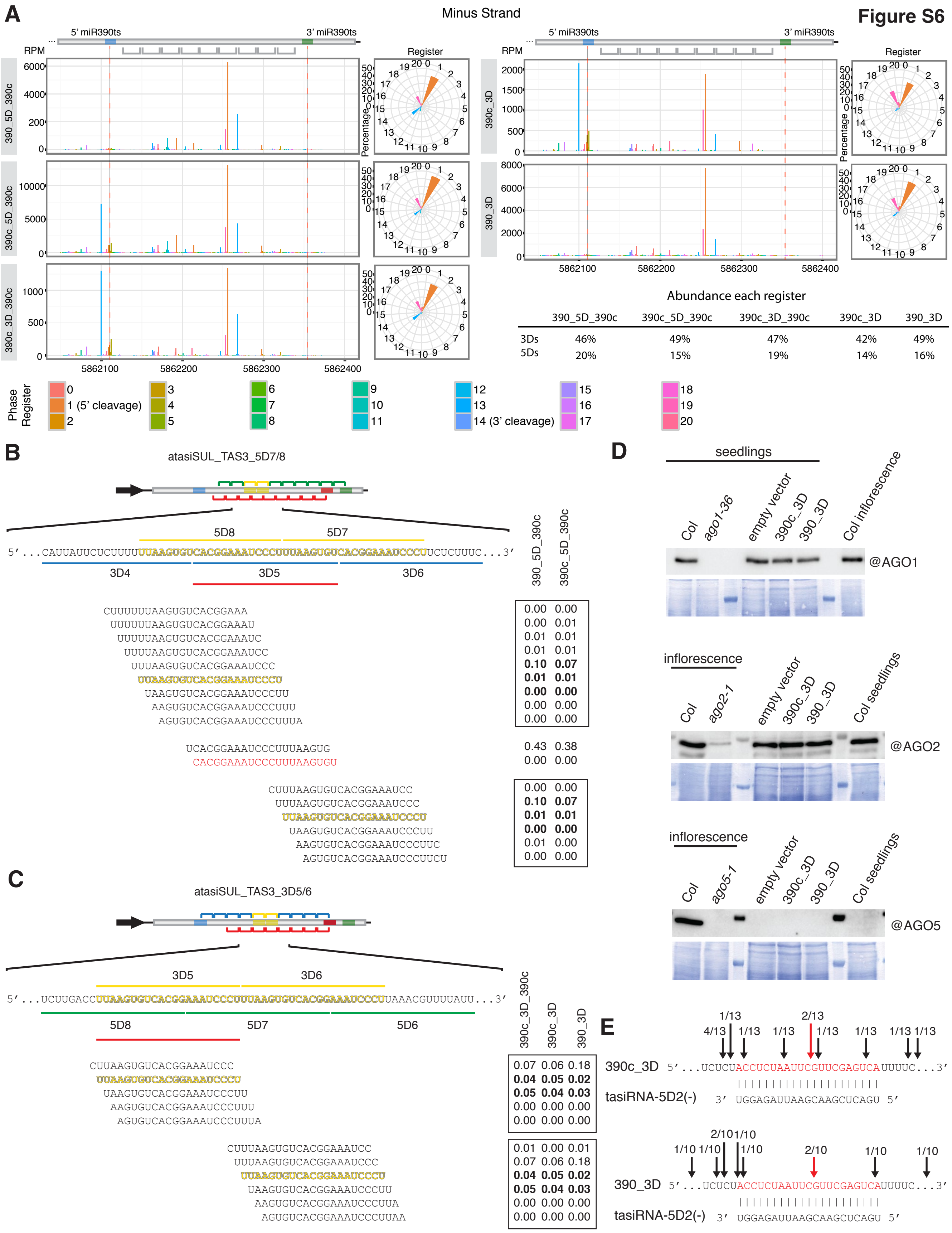

A
Transgene
Endogenous TAS3
Number of transgene/endogene specific sequences
1100
825
550
275
0
4.7x
4.4x
3.5x
4.7x
2.6x
390_5D_390c
390c_5D_390c
390c_3D_390c
390c_3D
390_3D
B
0
(Chr3:5861491)
678
(Chr3:5862168)
765
(Chr3:5862255)
946/7
(Chr3:5862437)
atasiSUL_TAS3
(TAS3 endogenous-Chr3)
390_5D_390c ATTTCATTATTCTCTTTTttaagtgtcacggaaatccctttaagtgtcacggaaatccctTCTCTTTCTAAACGTTTTATTATTTTCT
390c_5D_390c ATTTCATTATTCTCTTTTttaagtgtcacggaaatccctttaagtgtcacggaaatccctTCTCTTTCTAAACGTTTTATTATTTTCT
390c_3D_390c ATTTCATTATTCTCTTTTTCTTGACCttaagtgtcacggaaatccctttaagtgtcacggaaatccctTAAACGTTTTATTATTTTCT
390c_3D ATTTCATTATTCTCTTTTTCTTGACCttaagtgtcacggaaatccctttaagtgtcacggaaatccctTAAACGTTTTATTATTTTCT
390_3D ATTTCATTATTCTCTTTTTCTTGACCttaagtgtcacggaaatccctttaagtgtcacggaaatccctTAAACGTTTTATTATTTTCT
TAS3 endogenous ATTTCATTATTCTCTTTTTCTTGACCTTGTAAGGCCTTTTCTTGACCTTGTAAGACCCCATCTCTTTCTAAACGTTTTATTATTTTCT
C
390_5D_390c
500
0
-500
-1000
-1500
390c_5D_390c
500
0
-500
-1000
390c_3D_390c
400
200
0
-200
-400
390c_3D
600
400
200
0
-200
-400
390_3D
0
-200
-400
-600
-800
-1000
1050
1150
1250
1350
1450
1550
1650
1750
1850
1950
2050

Figure S5. General overview of the deep sequencing of sRNAs. A) Amount of sequences originating exclusively from the transgene or the endogenous copy of *TAS3*. The proportion of contribution of the transgene over the endogenous copy is provided. B) Schematic showing the area and the sequence used for the analysis to determine the respective contributions of the reporter transgene and the endogenous *TAS3* to the total amount of tasiRNA species found in the libraries. Relative and chromosomal (between brackets) positions for the transgene and the endogenous copy of *TAS3,* respectively*,* are indicated. C) Profile of sRNAs mapped to the reporter transgene in the difference lines. Values in the x-axis corresponds to the position on the transgene (bp), y-axis stands for normalized number of reads (in RPM).

Figure S6



Abundance each register

	390_5D_390c	390c_5D_390c	390c_3D_390c	390c_3D	390_3D
3Ds	46%	49%	47%	42%	49%
5Ds	20%	15%	19%	14%	16%

Figure S6: Supporting information for the deep sequencing analysis of sRNA populations. A) Distribution of reads mapping the reporter transgene and their respective phase registers. Note that only the region containing both miR390 target sites and sRNAs mapping the minus strand are shown. The different phase registers are represented as a “clock”. The abundance of sRNAs being produced in phase regarding either the miR390 5’- or 3’- target site (3Ds or 5Ds, respectively) is given. For the abundance relative to the 3D and 5D positions, we have considered the predicted, plus 1nt up- and down-stream shifted sequences as the same register). The expected phase register set by cleavage of each of the miR390 target sites is indicated in the colour legend (5’ cleavage and 3’ cleavage). Black arrows indicate the reads corresponding to the atasiSUL. B-C) Schematic representation of the transgene-specific region containing the atasiSUL. In addition to the miR390 sites, tasiRNA-5D2(-) target site as well the predicted phased siRNAs generated are represented in red. Yellow bars and sequences indicate the atasiSUL in positions 5D7/8 (B) or 3D5/6 (C). Putative tasiRNAs generated within the phase register set by cleavage of the miR390 target site not in phase with the atasiSUL sequence are indicated in blue (B) or green (C). tasiRNAs overlapping with the natural site of siR1778 production are indicated in red. Only selected sequences are displayed in the zoomed area together with their respective abundance (ratio of each specific sequence reads over the total reads mapped in the analysed area). Sequences that could potentially target SUL are shown in the boxes, while bold ratios indicate sequences with two different locations. D) Western blot to detect the expression of different AGO proteins using specific antibodies. E) Schematic of the results of the 5’-RACE analysis conducted for different reporter lines. The region containing the tasiRNA-5D2(-) is depicted (including 5-nt up- and down-stream of the target site). The mapped cDNA ends are indicated with black arrows alongside the number of corresponding clones obtained; the predicted tasiRNA-5D2(-) cleavage site is indicated with a red arrow. Mapped cDNA ends located outside the presented area are depicted as single events.

Supplementary Information for

A single miR390 targeting event is sufficient for triggering TAS3-tasiRNA biogenesis in *Arabidopsis*.

Felipe Fenselau de Felippes, Antonin Marchais, Alexis Sarazin, Stefan Oberlin and Olivier Voinnet

The final sequence of all constructs used in this manuscript is given bellow. The atasiSUL sRNA targeting *CH42* (*SUL*) is shown in **underlined bold letters.** miRNA target sites is represented in *italic underlined.*

390_5D_390c

ATCCCACCGTTTCTTAAGACTCTCTCTCTTTCTGTTTTCTATTTCTCTCTCTCTCAAATGAAAGAGAGAGAAGAGCTCCCATGGATGAAATTAGCGAGACCGAAGTTTCTCCAAGGTGATATGTCTATCTGTATATGTGATACGAAGAGTTAGGGTTTTGTCATTTCGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTAACTAAGATATGTTACTGAACTATATAATACATATGTGTGTTTTTCTGTATCTATTTCTATATATATGTAGATGTAGTGTAAGTCTGTTATATAGACATTATTCATGTGTACATGCATTATACCAACATAAATTTGTATCAATACTACTTTTGATTTACGATGATGGATGTTCTTAGATATCTTCATACGTTTGTTTCCACATGTATTTACAACTACATATATATTTGGAATCACATATATACTTGATTATTATAGTTGTAAAGAGTAACAAGTTCTTTTTTCAGGCATTAAGGAAAACATAACCTCCGTGATGCATAGAGATTATTGGATCCGCTGTGCTGAGACATTGAGTTTTTCTTCGGCATTCCAGTTTCAATGATAAAGC*GGTGTTATCCTATCTGAGCTT*TTAGTCGGATTTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCATTTCATTATTCTCTTTT**TTAAGTGTCACGGAAATCCCTTTAAGTGTCACGGAAATCCCT**TCTCTTTCTAAACGTTTTATTATTTTCTCGTTTTACAGATTCTATTCTATCTCTTCTCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTAC*CTTGTCTATCCCTCCTGAGCTA*ATCTCCACATATATCTTTTGTTTGTTATTGATGTATGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

390c_5D_390c

ATCCCACCGTTTCTTAAGACTCTCTCTCTTTCTGTTTTCTATTTCTCTCTCTCTCAAATGAAAGAGAGAGAAGAGCTCCCATGGATGAAATTAGCGAGACCGAAGTTTCTCCAAGGTGATATGTCTATCTGTATATGTGATACGAAGAGTTAGGGTTTTGTCATTTCGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTAACTAAGATATGTTACTGAACTATATAATACATATGTGTGTTTTTCTGTATCTATTTCTATATATATGTAGATGTAGTGTAAGTCTGTTATATAGACATTATTCATGTGTACATGCATTATACCAACATAAATTTGTATCAATACTACTTTTGATTTACGATGATGGATGTTCTTAGATATCTTCATACGTTTGTTTCCACATGTATTTACAACTACATATATATTTGGAATCACATATATACTTGATTATTATAGTTGTAAAGAGTAACAAGTTCTTTTTTCAGGCATTAAGGAAAACATAACCTCCGTGATGCATAGAGATTATTGGATCCGCTGTGCTGAGACATTGAGTTTTTCTTCGGCATTCCAGTTTCAATGATAAAGC*GGCGCTATCCCTACTGAGCTT*TTAGTCGGATTTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCATTTCATTATTCTCTTTT**TTAAGTGTCACGGAAATCCCTTTAAGTGTCACGGAAATCCCT**TCTCTTTCTAAACGTTTTATTATTTTCTCGTTTTACAGATTCTATTCTATCTCTTCTCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTAC*CTTGTCTATCCCTCCTGAGCTA*ATCTCCACATATATCTTTTGTTTGTTATTGATGTATGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

390_3D_390c

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TCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTA
C*CTTGTCTATCCCTCCTGAGCTA*ATCTCCACATATATCTTTTGTTTGTTATTGATGT
ATGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

390c_3D_390c

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CTCAAATGAAAGAGAGAGAAGAGCTCCCATGGATGAAATTAGCGAGACCGAAGTTTC
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CGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTA
ACTAAGATATGTTACTGAACTATATAATACATATGTGTGTTTTTCTGTATCTATTTC
TATATATATGTAGATGTAGTGTAAGTCTGTTATATAGACATTATTCATGTGTACATG
CATTATACCAACATAAATTTGTATCAATACTACTTTTGATTTACGATGATGGATGTT
CTTAGATATCTTCATACGTTTGTTTCCACATGTATTTACAACTACATATATATTTGG
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*TGAGCTT*TTAGTCGGATTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCAT
TTCATTATTCTCTTTTTCTTGACC**TTAAGTGTCACGGAAATCCCTTTAAGTGTCACG**
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TCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTA
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ATGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

159_3D_390c

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CGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTA
ACTAAGATATGTTACTGAACTATATAATACATATGTGTGTTTTTCTGTATCTATTTC
TATATATATGTAGATGTAGTGTAAGTCTGTTATATAGACATTATTCATGTGTACATG
CATTATACCAACATAAATTTGTATCAATACTACTTTTGATTTACGATGATGGATGTT
CTTAGATATCTTCATACGTTTGTTTCCACATGTATTTACAACTACATATATATTTGG
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390c_3D_390

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390c_3D

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TGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

390_3D

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CGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTA
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TTCATTATTCTCTTTTTCTTGACC**ttaagtgtcacggaaatccctttaagtgtcacg**
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TCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTA
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159_3D

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CTCAAATGAAAGAGAGAGAAGAGCTCCCATGGATGAAATTAGCGAGACCGAAGTTTC

TCCAAGGTGATATGTCTATCTGTATATGTGATACGAAGAGTTAGGGTTTTGTCATTT
CGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTA
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GACATTGAGTTTTTCTTCGGCATTCCAGTTTCAATGATAAAGC*TAGAGCTCCCTTCA*
*ATCCAAA*TTAGTCGGATTTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCAT
TTCATTATTCTCTTTTTCTTGACC**TTAAGTGTCACGGAAATCCCTTTAAGTGTCACG**
GAAATCCCTTAAACGTTTTATTATTTTCTCGTTTTACAGATTCTATTCTATCTCTTC
TCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTA
CACACGATAGGGAGAGATAGAAATCTCCACATATATCTTTTGTTTGTTATTGATGTA
TGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

390del_3D

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CGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTA
ACTAAGATATGTTACTGAACTATATAATACATATGTGTGTTTTTCTGTATCTATTTC
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CATTATACCAACATAAATTTGTATCAATACTACTTTTGATTTACGATGATGGATGTT
CTTAGATATCTTCATACGTTTGTTTCCACATGTATTTACAACTACATATATATTTGG
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CATTAAGGAAAACATAACCTCCGTGATGCATAGAGATTATTGGATCCGCTGTGCTGA
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ACTCGAATTAGTCGGATTTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCAT
TTCATTATTCTCTTTTTCTTGACC**TTAAGTGTCACGGAAATCCCTTTAAGTGTCACG**
GAAATCCCTTAAACGTTTTATTATTTTCTCGTTTTACAGATTCTATTCTATCTCTTC
TCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTA
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TGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

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ATCCCACCGTTTCTTAAGACTCTCTCTCTTTCTGTTTTCTATTTCTCTCTCTCTCAAATGAAAGAGAGAGAAGAGCTCCCATGGATGAAATTAGCGAGACCGAAGTTTCTCCAAGgtgatatgtctatctgtatatgtgatacgaagagttagggttttgtcatttcgaagtcaatttttgtttgtttgtcaataatgatatctgaatgatgaagaacacgtaactaagatatgttactgaactatataatacatatgtgtgtttttctgtatctatttctatatatatgtagatgtagtgtaagtctgttatatagacattattcatgtgtacatgcattataccaacataaatttgtatcaatactacttttgatttacgatgatggatgttcttagatatcttcatacgtttgtttccacatgtatttacaactacatatatatttggaatcacatatatacttgattattatagttgtaaagagtaacaagttctttttttcagGCATTAAGGAAAACATAACCTCCGTGATGCATAGAGATTATTGGATCCGCTGTGCTGAGACATTGAGTTTTTCTTCGGCATTCCAGTTTCAATGATAAAGC*tagagctcccttcaatccaaa*TTAGTCGGATTTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCATTTCATTATTCTCTTTT**ttaagtgtcacggaaatccctttaagtgtcacggaaatccct**TCTCTTTCTAAACGTTTTATTATTTTCTCGTTTTACAGATTCTATTCTATCTCTTCTCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTAC*CTTGTCTATCCCTCCTGAGCTA*ATCTCCACATATATCTTTTGTTTGTTATTGATGTATGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

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*TGAGCTT*TTAGTCGGATTTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCATTTCATTATTCTCTTTT**TTAAGTGTCACGGAAATCCCTTTAAGTGTCACGGAAATCCCT**TCTCTTTCTAAACGTTTTATTATTTTCTCGTTTTACAGATTCTATTCTATCTCTTCTCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTACC*TAGAGCTCCCTTCAATCCAAA*ATCTCCACATATATCTTTTGTTTGTTATTGATGTATGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

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ATCCCACCGTTTCTTAAGACTCTCTCTCTTTCTGTTTTCTATTTCTCTCTCTCTCAAATGAAAGAGAGAGAAGAGCTCCCATGGATGAAATTAGCGAGACCGAAGTTTCTCCAAGGTGATATGTCTATCTGTATATGTGATACGAAGAGTTAGGGTTTTGTCATTTCGAAGTCAATTTTTGTTTGTTTGTCAATAATGATATCTGAATGATGAAGAACACGTAACTAAGATATGTTACTGAACTATATAATACATATGTGTGTTTTTCTGTATCTATTTCTATATATATGTAGATGTAGTGTAAGTCTGTTATATAGACATTATTCATGTGTACATGCATTATACCAACATAAATTTGTATCAATACTACTTTTGATTTACGATGATGGATGTTCTTAGATATCTTCATACGTTTGTTTCCACATGTATTTACAACTACATATATATTTGGAATCACATATATACTTGATTATTATAGTTGTAAAGAGTAACAAGTTCTTTTTTCAGGCATTAAGGAAAACATAACCTCCGTGATGCATAGAGATTATTGGATCCGCTGTGCTGAGACATTGAGTTTTTCTTCGGCATTCCAGTTTCAATGATAAAGC*GGTGTTATCCTATCTGAGCTT*TTAGTCGGATTTTTTCTTTTCAATTATTGTGTTTTATCTAGATGATGCATTTCATTATTCTCTTTTTCTTGACC**TTAAGTGTCACGGAAATCCCTTTAAGTGTCACGGAAATCCCT**TAAACGTTTTATTATTTTCTCGTTTTACAGATTCTATTCTATCTCTTCTCAATATAGAATAGATATCTATCTCTACCTCTAATTCGTTCGAGTCATTTTCTCCTACC*TAGAGCTCCCTTCAATCCAAA*ATCTCCACATATATCTTTTGTTTGTTATTGATGTATGGTTGACATAAATTCAATAAAGAAGTTGACGTTTTTCT

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AAACCTAAACCTAAACGGCTAAGCCCGACGTCAAATACCAAAAAGAGAAAAACAAGAGCGCCGTCAAGCTCTGCAAATACGATCTGTAAGTCCATCTTAACACAAAAGTGAGATGGGTTCTTAGATCATGTTCCGCCGTTAGATCGAGTCATGGTCTTGTCTCATAGAAAGGTACTTTCGTTTACTTCTTTTGAGTATCGAGTAGAGCGTCGTCTATAGTTAGTTTGAGATTGCGTTTGTCAGAAGTTAGGTTCAATGTCCCGGTCCAATTTTCACCAGCCATGTGTCAGTTTCGTTCCTTCCCGTCCTCTTCTTTGATTTCGTTGGGTTACGGATGTTTTCGAGATGAAACAGCATTGTTTTGTT*GTGATTTTTCTCTACAAGCGAA*TAGACC

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TAGAACACCACATCGTAAAGTGAAAT

Supplementary Material and Methods

Western blot analysis

AGO1, AGO2 and AGO5 expression was detected by Western blot using specific antibodies. For AGO1 detection, 30ug of protein was used, while 75ug was utilized for AGO2 and AGO5 experiments. *ago1-36*, *ago2-1* and *ago5-1* were described respectively in Baumberger and Baulcombe, 2005 (PNAS), Lobbes et al, 2006 (EMBO rep) and Katiyar-Agarwal et al, 2007 (Genes and Dev).

tasiRNA-5D2(-) RACE

RACE to detect tasiRNA-5D2(-) cleavage site was done as described in the material and methods section. cDNA was synthetized using a TAS3 specific gene (5'- AGAAAAACGTCAACTTCTTTAT). First round and nested PCR were performed using the adaptor specific primers (described in the main section) and *TAS3*-specific primers (5'- CTTCTTTATTGAATTTATGTC and 5'- AACCATACATCAATAACAAAC).

Contribution of the original atasiSUL reporter transgene and the endogenous TAS3 to the total amount of tasiRNA species found in the libraries.

To determine the contribution of the original atasiSUL reporter transgene and the endogenous *TAS3* to the total amount of tasiRNA species found in the libraries we took in consideration the regions shown in Figure S5B, which in the reporter constructs contain the sequence originating the atasiSUL. We first retrieved all reads that corresponded to these regions. Next we determined how many sequences could be specifically defined as originating from either the transgene or the endogenous *TAS3*.

Table S1. Oligonucleotide sequences.

Purpose	**Sequence**
small RNAs	
atasiSUL	TTA AGT GTC ACG GAA ATC CCT
Probes for sRNA blots	
atasiSUL	AGG GAT TTC CGT GAC ACT TAA
U6 (loading control)	AGG GGC CAT GCT AAT CTT CTC
miR159	TAG AGC TCC CTT CAA TCC AAA
miR173	GTG ATT TCT CTC TGC AAG CGA A
miR390	GGC GCT ATC CCT CCT GAG CTT
TAS1_long, forward	TTC GTT GGG TTA CGG ATG TTT TCG AGA TG
TAS1_long, reverse	ATT TCA CTT TAC GAT GTG GT
TAS3_long, forward	GGA TCC GCT GTG CTG AGA CAT TGA G
TAS3_long, reverse	AAA AGA TAT ATG TGG AGA TTA
RT-PCR and RT-qPCR	
ACT2, forward	GCA CCC TGT TCT TCT TAC CG
ACT2, reverse	AAC CCT CGT AGA TTG GCA CA
GAPC, forward	ACT CAA TCA CTG CTA CTC AG
GAPC, reverse	GTC AAC TTT CCG TTA AGA GC
CH42 (SUL), forward	GCA GGA CAA GCT TCA AGA CC
CH42 (SUL), reverse	CGG TGC CTT AAG CAG TTA GG
TAS3-based reporter, forward	GCA TTA AGG AAA ACA TAA CC
TAS3-based reporter, reverse (atasiSUL-specific)	AGG GAT TTC CGT GAC ACT TAA

5'-RACE	
5'-RNA adaptor	CGA CUG GAG CAC GAG GAC ACU GAC AUG GAC UGA AGG AGU AGA AA
Specifc Oligo for cDNA synthesis	AGG GAT TTC CGT GAC ACT TAA
5'-RNA adaptor first PCR primer	CGA CTG GAG CAC GAG GAC ACT GA
5'-RNA adaptor nested PCR primer	GGA CAC TGA CAT GGA CTG AAG GAG TA
TAS1 PCR primer	AGG GAT TTC CGT GAC ACT TAA GTT C
TAS1 nested PCR primer	TAA TTT TCT AAG ATC CAC CGA TAA ATG GTC
TAS3 PCR primer	AGG GAT TTC CGT GAC ACT TAA GGT C
TAS3 nested PCR primer	CAC AAT AAT TGA AAA GAA AAA ATC CGA C
M13, forward	TGT AAA ACG ACG GCC AGT
pJet1, Forward	ACT ACT CGA TGA GTT TTC GG