Figure 1

A

Stratification

No stratification

B

Germination rate

C

DOG1 expression level

D

Percentage of isoform

E

Percentage of affected splicing events

WT [●] atrn1-1 [●]

intron retained

downstream 5'SS

upstream 5'SS

Ex2 Ex3

intron spliced 11% 89% intron retained 28/45

exon included 10% 90% exon skipped 20/31

upstr. 5'SS 30% 70% downstr. 3'SS 10/21

upstr. 3'SS 12% 88% downstr. 3'SS 16/47
Figure 2

A

<table>
<thead>
<tr>
<th></th>
<th>RT+</th>
<th>IP</th>
<th>Input</th>
</tr>
</thead>
<tbody>
<tr>
<td>U6</td>
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</tr>
<tr>
<td>U5</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>U2</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>U3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>18s rRNA</td>
<td></td>
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</tr>
</tbody>
</table>

B

SC35  PolII  PolIIA  PolII0

AtNTR1  
Overlap  
DAPI  
Overlap
Figure 3

At5g45830 (DOG1)

0.5 Kb

AtNTR1 enrichment in WT normalized for atntr1-1

DOG1-p  DOG1-ex2  DOG1-ex3  IGR

WT  atntr1-1

negative control
Figure 4

<table>
<thead>
<tr>
<th>isoform</th>
<th>AS type</th>
<th>Atnr1-f vs. WT</th>
<th>Herboxidiene vs. WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>upstr. 5'SS</td>
<td>-21%</td>
<td>-9%</td>
</tr>
<tr>
<td>II</td>
<td>downstr. 5'SS</td>
<td>21%</td>
<td>-13%</td>
</tr>
<tr>
<td>III</td>
<td>IR</td>
<td>0%</td>
<td>23%</td>
</tr>
</tbody>
</table>

At5g45830 (DOG1)

At2g32330

At5g04430
Figure 5

![Graph showing PolII ChIP% of input across different conditions.

**Table:**

<table>
<thead>
<tr>
<th>Alternative Splice Sites</th>
<th>PolII Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTAAGT 5'SS strong - strong, consensus like alternative splice sites</td>
<td><strong>High</strong></td>
</tr>
<tr>
<td>GTGACT 5'SS wt - weak alternative splice sites</td>
<td><strong>Low</strong></td>
</tr>
</tbody>
</table>
Figure 6

A

WT  TFIIISmut  DOG1

intron retained

exon included

intron spliced

exon skipped

upstr. 5'SS

downstr. 5'SS

upstr. 3'SS

downstr. 3'SS

Percentage of affected splicing events

0%  25%  50%  75%  100%

B

affected/analyzed

intron spliced  79%  21%  intron retained  14/71

exon included  78%  22%  exon skipped  18/44

upstr. 5'SS  82%  18%  downstr. 5'SS  11/56

upstr. 3'SS  58%  42%  downstr. 3'SS  19/113

Percentage of affected splicing events

0%  25%  50%  75%  100%

C

Percentage of affected splicing events

0%  25%  50%  75%  100%

WT  TFIISmut  atnrt1-1

Percentage of affected splicing events

0%  25%  50%  75%  100%

DOG1

Ex2  Ex0

Ex1  Ex2  Ex3  Ex4  Ex5

Ex1  Ex2  Ex3  Ex4  Ex5

Ex11  Ex12

Ex8  Ex9  Ex10

Ex1  Ex2  Ex3  Ex4  Ex5

Ex1  Ex2  Ex3  Ex4  Ex5
Supplementary Figure S1

A

Germination rate

0%  25%  50%  75%  100%
0  1  2  3  4  5  6  7  8  9  10  11
Days after sowing

B

DOG1 relative expression

0%  20%  40%  60%  80%  100%  150%

C

DOG1

DOG1α  DOG1β  DOG1γ  DOG1δ

D

Percentage of isoform

0%  20%  40%  60%  80%  100%

E

At5g43910

upstr. 3'SS

downstr. 3'SS

At4g38510

upstr. 5'SS

downstr. 5'SS

exon included  exon skipped

intron retained  intron spliced

0%  25%  50%  75%  100%

F

WT atntr1-1

G

PRC relative expression

0%  100%  200%  300%  400%  500%  600%

H

WT atntr1-1

I

WT atntr1-1 AINTR1 AINTR1-GFP

22°C

30°C

J

GFP WF Overlap

Root tip
Supplementary Figure S2

A

<table>
<thead>
<tr>
<th>Arabidopsis</th>
<th>AtNTR1 dependent</th>
<th>AtNTR1 independent</th>
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</thead>
<tbody>
<tr>
<td>-2</td>
<td>-1</td>
<td>1</td>
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</tbody>
</table>

B

<table>
<thead>
<tr>
<th>Arabidopsis</th>
<th>AtNTR1 dependent</th>
<th>AtNTR1 independent</th>
</tr>
</thead>
<tbody>
<tr>
<td>-6</td>
<td>-5</td>
<td>-4</td>
</tr>
</tbody>
</table>

C

At1g23970

5' SS wt

promoter

consensus

5' SS strong

D

D

upstr. 5' SS

downstr. 5' SS

Percentage of isoform

5' SS wt (Col-0)

5' SS wt (atntr1-1)

5' SS strong (Col-0)

5' SS strong (atntr1-1)

18% 62%

19% 81%

41% 57%

32% 68%
Supplementary Figure S3

A

@22°C

@30°C

B

YFP

Chl

merged

WF

YFPc

ILP1-YFPn

AtNTR1-YFPc

ILP1-YFPn

yfp

AtNTR1-AD + BD

AtNTR1-AD + ILP1-AD

AD + ILP1-BD

BD + ILP1-AD

C

AtNTR1-BD + AD

AtNTR1-AD + BD

AtNTR1-AD + ILP1-BD

AtNTR1-BD + ILP1-AD

AD + ILP1-BD

BD + ILP1-AD

D

WT

atntr1-1

ilp1-1

DOG1

intron retained

downstr. 5' SS

upstr. 5' SS

0% 25% 50% 75% 100%

upstr. 3' SS

downstr. 3' SS

0% 25% 50% 75% 100%

exon included

exon skipped

0% 25% 50% 75% 100%

intron retained

intron spliced

0% 25% 50% 75% 100%

Percentage of isoform

Percentage of isoform
Supplementary Figure S4

**At2g32330**

0.5 Kb

**At4g36690**

0.5 Kb

**At3g23280**

0.5 Kb

**At5g04430**

0.5 Kb

**At2g16940**

0.5 Kb

**AtNTR1 enrichment in WT**

**AtNTR1 enrichment in WT**

**AtNTR1 enrichment in WT**

**AtNTR1 enrichment in WT**
Supplementary Figure S7

A

WT

TFIISmut

B

WT
tflis-1
TFIISmut

C

Exon included
Exon skipped

D

all analyzed

0 10 52 284

WT

tflis-1
TFIISmut

Percentage of isoform

0% 25% 50% 75% 100%

upstr. 3'SS
downstr. 3'SS

upstr. 5'SS
downstr. 5'SS

exon included
exon skipped

intron retained
intron spliced

At4g36690
Ex11 Ex12

At5g57630
Ex2 Ex3 Ex4 Ex5

At2g15530
Ex1 Ex2 Ex3 Ex4

At2g46790
Ex3 Ex4 Ex5

0% 25% 50% 75% 100%

percentage of isoform
Supplementary Figure S8

<table>
<thead>
<tr>
<th>Isoform</th>
<th>AS type</th>
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<th>TFIIISmut</th>
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</tr>
<tr>
<td>II</td>
<td>upstr. 3'SS</td>
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</tr>
<tr>
<td>III</td>
<td>IR</td>
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<td>0%</td>
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</table>

**Supplementary Figure S8**
Supplementary Figure S8

<table>
<thead>
<tr>
<th>Isoform</th>
<th>AS type</th>
<th>atrn1-1 vs. WT</th>
<th>TFIIISmut vs. WT</th>
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<td>II</td>
<td>upstr. 3'SS</td>
<td>-3%</td>
<td>14%</td>
</tr>
<tr>
<td>III</td>
<td>IR</td>
<td>3%</td>
<td>0%</td>
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<table>
<thead>
<tr>
<th>Isoform</th>
<th>AS type</th>
<th>atrn1-1 vs. WT</th>
<th>TFIIISmut vs. WT</th>
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<table>
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<th>TFIIISmut vs. WT</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>upstr. 5'SS</td>
<td>14%</td>
<td>-16%</td>
</tr>
<tr>
<td>II</td>
<td>downstr. 5'SS</td>
<td>6%</td>
<td>9%</td>
</tr>
<tr>
<td>III</td>
<td>IR</td>
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<td>-7%</td>
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</table>

At5g04430 0.5 Kb

At2g16940 0.5 Kb

At5g45830 (DOG1) 0.5 Kb

At5g45830 (DOG1)
Supplementary Figure S10

A

Percentage of isoforms for particular event after normalization for WT or atntr1-1

0% 25% 50% 75% 100%

B

Percentage of isoforms for particular event after normalization for WT or atntr1-1

0% 25% 50% 75% 100%
Supplementary Figure S11

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>atntr1-1</th>
<th>tflls</th>
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<td><img src="image2.png" alt="image" /></td>
<td><img src="image3.png" alt="image" /></td>
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<tr>
<td>MS +6AU</td>
<td><img src="image4.png" alt="image" /></td>
<td><img src="image5.png" alt="image" /></td>
<td><img src="image6.png" alt="image" /></td>
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14 days old plants

<table>
<thead>
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<th>WT</th>
<th>atntr1-1</th>
<th>atntr1-2</th>
<th>tflls</th>
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<tbody>
<tr>
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<td><img src="image8.png" alt="image" /></td>
<td><img src="image9.png" alt="image" /></td>
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</tr>
<tr>
<td>MS +6AU</td>
<td><img src="image11.png" alt="image" /></td>
<td><img src="image12.png" alt="image" /></td>
<td><img src="image13.png" alt="image" /></td>
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</table>

20 days old plants