

## **SUPPLEMENTARY MATERIAL**

### **SI-IAA3, a tomato Aux/IAA at the crossroads of auxin and ethylene signalling involved in differential growth**

Salma Chaabouni<sup>1</sup>, Brian Jones<sup>2</sup>, Corinne Delalande<sup>1</sup>, Hua Wang<sup>1</sup>, Zhengguo Li<sup>1</sup>, Isabelle Mila<sup>1</sup>, Pierre Frasse<sup>1</sup>, Alain Latché<sup>1</sup>, Jean-Claude Pech<sup>1</sup>, and Mondher Bouzayen<sup>1\*</sup>

## Supplementary Tables

Table S1. Percentage identity of the antisense region relative to the other members of tomato Aux/IAAs family.

Target genes	% of identity
SI-AA3	100
IAA1	79
IAA4	74
IAA17	67
IAA14	65
IAA8	65
IAA16	64
IAA27	64
IAA7	63
IAA9	63
IAA6	62
IAA12	59
IAA26	56
IAA13	56
IAA29	54

Table S2. Auxin- and ethylene-response genes. The accession numbers and primers-sequences of the genes described in this article are listed below.

Category	SGN Number	Primers sequences
<b>Auxin-response genes</b>		
<b>Aux/IAAs</b>		
<i>IAA1</i>	SGN-U323670	<b>F</b> 5'-TGAATCTAAGTCAGTTCTGATCATGTC-3' <b>R</b> 5'-ATGATGTTTTCTGTAGATCTCACTG-3'
<i>IAA4</i>	SGN-U316052	<b>F</b> 5'-AACAAAGAGGGCTTGCGCTGAG-3' <b>R</b> 5'-GTGTCTGGCACAGGTGGA-3'
<i>IAA6</i>	SGN-U320280	<b>F</b> 5'-CCAAAAGAGGGAATGGAGGTT-3' <b>R</b> 5'-TGTTCTCCCTTCATCATCATTTC-3'
<i>IAA7</i>	SGN-U318191	<b>F</b> 5'-ACTAACCTCCATCATAATGATAATATTCC-3' <b>R</b> 5'-ACCCCACCACTTGAGCCTTA-3'
<i>IAA8</i>	SGN-U317606	<b>F</b> 5'-CCTAACAAATCTGTAATTCTCAAAGTGAAA-3' <b>R</b> 5'-GCATCCAGTCTCCATCTTATCTTC-3'
<i>IAA9</i>	SGN-U313802	<b>F</b> 5'-CCCTTGCACCCTCCA-3' <b>R</b> 5'-AGCGTCTGAAAATCCTCGTTG-3'

<i>IAA12</i>	SGN-U322787	<b>F</b> 5'-CCACCGCGATCTTCAGCATAA-3' <b>R</b> 5'-TCTGTTTCAGGGAGCGGC-3'
<i>IAA13</i>	SGN-U322644	<b>F</b> 5'-AGTCTTTAACGCTCTGGATGGATCA-3' <b>R</b> 5'-AAACATCCCGAATGGAACATCT-3'
<i>IAA14</i>	SGN-U318434	<b>F</b> 5'-GTTTACGCATAATGAAAGGATCAGAAG-3' <b>R</b> 5'-TTATCTATGGAGCTTGCACACCA-3'
<i>IAA16</i>	SGN-U332300	<b>F</b> 5'-GCGTGTTGGGTGCGGA-3' <b>R</b> 5'-CGATTCCAGTTCATCATTCCCATTAG-3'
<i>IAA17</i>	SGN-U323974	<b>F</b> 5'-CAAGAATTATTGATGCCCTAACCAA-3' <b>R</b> 5'-ACTATTCAAAAGGTCCATCAGTTCC-3'
<i>IAA26</i>	SGN-U320412	<b>F</b> 5'-AAAGGCTGCGTGTGTTGAAA-3' <b>R</b> 5'-CAAGATCTGTTGGCTACATCTTGT-3'
<i>IAA27</i>	SGN-U322175	<b>F</b> 5'-ATTCTGCTACTTGATAATCTTGACA-3' <b>R</b> 5'-TGTCCATTGATGAAACACAGCTCT-3'
<i>IAA29</i>	SGN-U320261	<b>F</b> 5'-GGTTTGATGATAGCTCTCCGATA-3' <b>R</b> 5'-ACGTCTTACGTTCAACTACTCCTTCA-3'
<b>ARFs</b>		
<i>ARF1</i>	SGN-U317693	<b>F</b> 5'-TCTCCTTCATCATTCTCATACTG-3' <b>R</b> 5'-GAACCATTCTCACCACTAAC-3'
<i>ARF2</i>	SGN-U314233	<b>F</b> 5'-GCAAGGTCAAGAGTTATCGA-3' <b>R</b> 5'-CATTGGTTCTCAGACAAGTC-3'
<i>ARF3</i>	SGN-U320946	<b>F</b> 5'-AATTGCAGTATCAGACTTGG-3' <b>R</b> 5'-TCTAGATATCCCAGAACTAGGA-3'
<i>ARF4</i>	SGN-U319581	<b>F</b> 5'-CATTATTGTTGGTGACTTTGTG-3' <b>R</b> 5'-GACCTTTGAAACCTATTGG-3'
<i>ARF5</i>	SGN-U324457	<b>F</b> 5'-CCTTCAGAGTTGTCATTCCCT-3' <b>R</b> 5'-AACATCATTCAAATCTCATACC-3'
<i>ARF6</i>	SGN-U319318	<b>F</b> 5'-CCAACATATCCCTAGTACTTCAG-3' <b>R</b> 5'-GTGCCTGAGATAATTAGTTGGT-3'
<i>ARF7</i>	SGN-U327712	<b>F</b> 5'-TCAACTCCTCAAACATACCT-3' <b>R</b> 5'-TGAACATCCAAATAATCCATCTG-3'
<i>ARF8</i>	SGN-U327976	<b>F</b> 5'-TGACATCGAATGGAAATTCAAG-3' <b>R</b> 5'-GTCTCTTAGCACTAACAAACAC-3'
<i>ARF11</i>	SGN-U327759	<b>F</b> 5'-TCATTGTTGGATGTTCAAAGG-3' <b>R</b> 5'-GAAGTCTTGAAAGTAGTATACTC-3'
<i>ARF18</i>	SGN-U321591	<b>F</b> 5'-AATCTACACTCGGCATTGTC-3' <b>R</b> 5'-AAGCTTCCTATCTTATCATTGGA-3'
<i>SAUR</i>	SGN-U318031	<b>F</b> 5'-TCCAATGGGCGGTGTCA-3' <b>R</b> 5'-GCCTCAGATCCTATTCAAGCGA-3'
<i>GH3</i>	SGN-U319351	<b>F</b> 5'-CATCACAAACATACGCCGGTTATA-3' <b>R</b> 5'-GCTGAGTTGAAACCCGGT-3'
<b>Ethylene-response genes</b>		
<b>ERFs</b>		
<i>Pti6</i>	SGN-U314347	<b>F</b> 5'-CGGTGGAGATAAAAGCGAAAAC-3' <b>R</b> 5'-CCACTTCGCAGAACCTAGATT-3'
<i>JERF1</i>	SGN-U313488	<b>F</b> 5'-ACTTCGTGAGGAAACCCCTGAAC-3' <b>R</b> 5'-GTTACTAATATAAGTCATGTTGGGCTGAA-3'
<i>JERF2</i>	SGN-U320682	<b>F</b> 5'-TTCTTCGTGTCGAAAATACTAAGTTCAAG-3' <b>R</b> 5'-ACTCTAAATTCTCAAGAAATCCAGAAC-3'

---

	SGN-U329134	<b>F</b> 5'-GAAGAAAGCGATCGATTGAAGA-3' <b>R</b> 5'-TTTTCCCCATGGCCTCTGT-3'
	SGN-U319706	<b>F</b> 5'-ATCATTACCATGGAATGATCAACATT-3' <b>R</b> 5'-CCGTCTATAACTTCTTCGAGGTTAA-3'
<i>Le ERF2</i>	SGN-U315233	<b>F</b> 5'-GTT CCTCTCAACCCCAAACG-3' <b>R</b> 5'-TTCATCTGCTCACCACTGTAGA-3'
	SGN-U315595	<b>F</b> 5'-GAATGATGACGGAATTGTAATGAAGA-3' <b>R</b> 5'-TTCCACAATCCCAAATTGAAGA-3'
	SGN-U317007	<b>F</b> 5'-GAGCTAATGGCTGATTTTGATATAAGTT-3' <b>R</b> 5'-AAATGGTAGAACAGCACGAGAAAG-3'
	SGN-U321808	<b>F</b> 5'-CGGAGATAAGAGATCCAAGTCGAA-3' <b>R</b> 5'-CTTAAACGCTGCACAATCATAAGC-3'
<i>Le ERF3</i>	SGN-U315194	<b>F</b> 5'-TGGAGCGAAAGCGAAAAC TAA-3' <b>R</b> 5'-GTCTGACTCGGACTCCGATTG-3'
	SGN-U327266	<b>F</b> 5'-CACCA GTAGTAAGGTGACCCGGATGAAG-3' <b>R</b> 5'-CACCGATCATCCACCACAGA-3'
<i>Pti4</i>	SGN-U317071	<b>F</b> 5'-GCGAAATGGATCAACAGTTACCA-3' <b>R</b> 5'-ATTAGACGACTGAAGCTTGAATTCC-3'

#### **SI-HLS**

**F** 5'-CCTATACCGCCGCCGATACT-3'  
**R** 5'-ACC GAGATTGAGAGGGTTGTTG-3'

#### **E8**

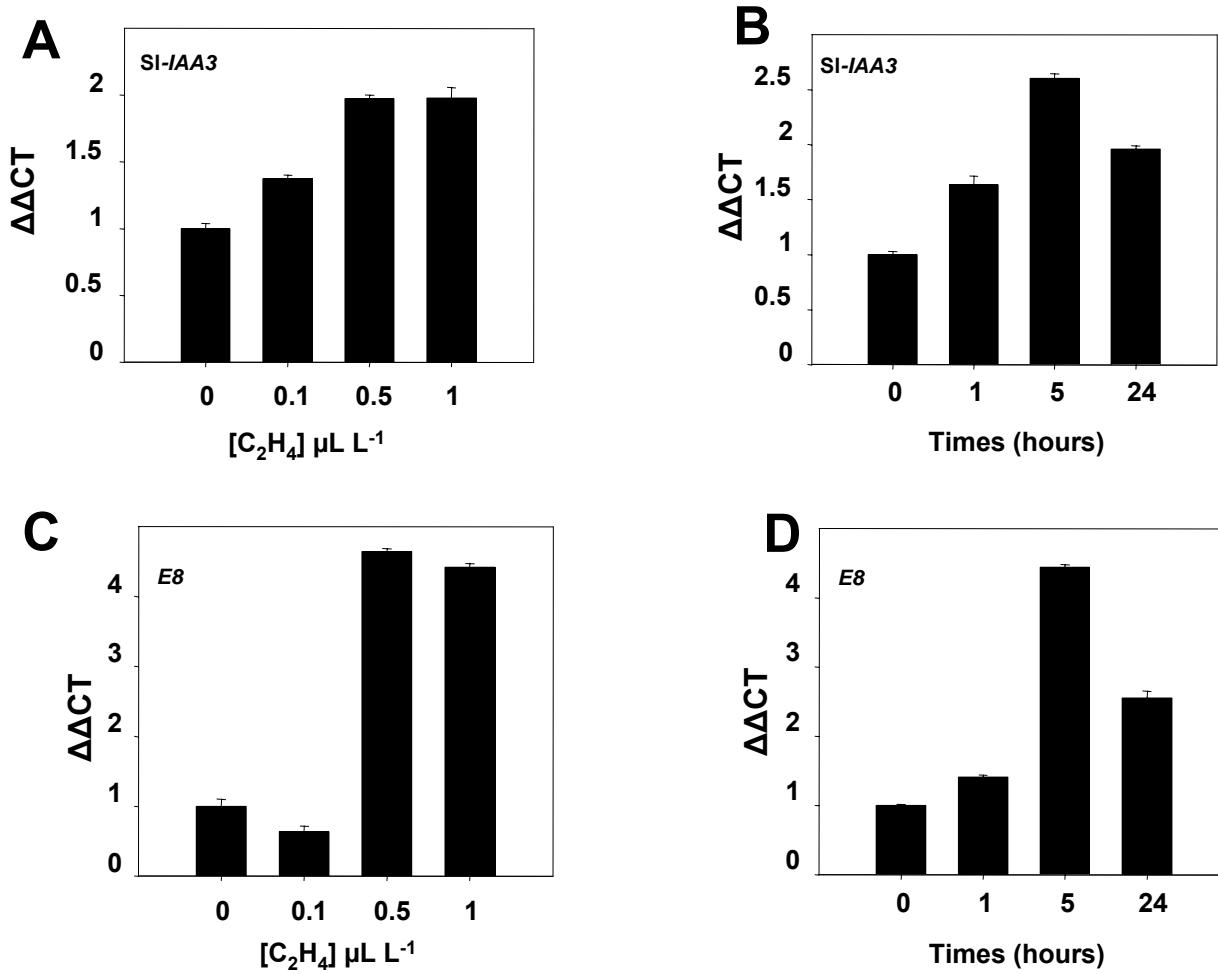
SGN-U314505      **F** 5'-CTACTACCCACCATGTCCTCAGC-3'  
**R** 5'-AAGGATCGTTACAAACCCAATATCG-3'

#### **Reference gene**

##### **Actin**

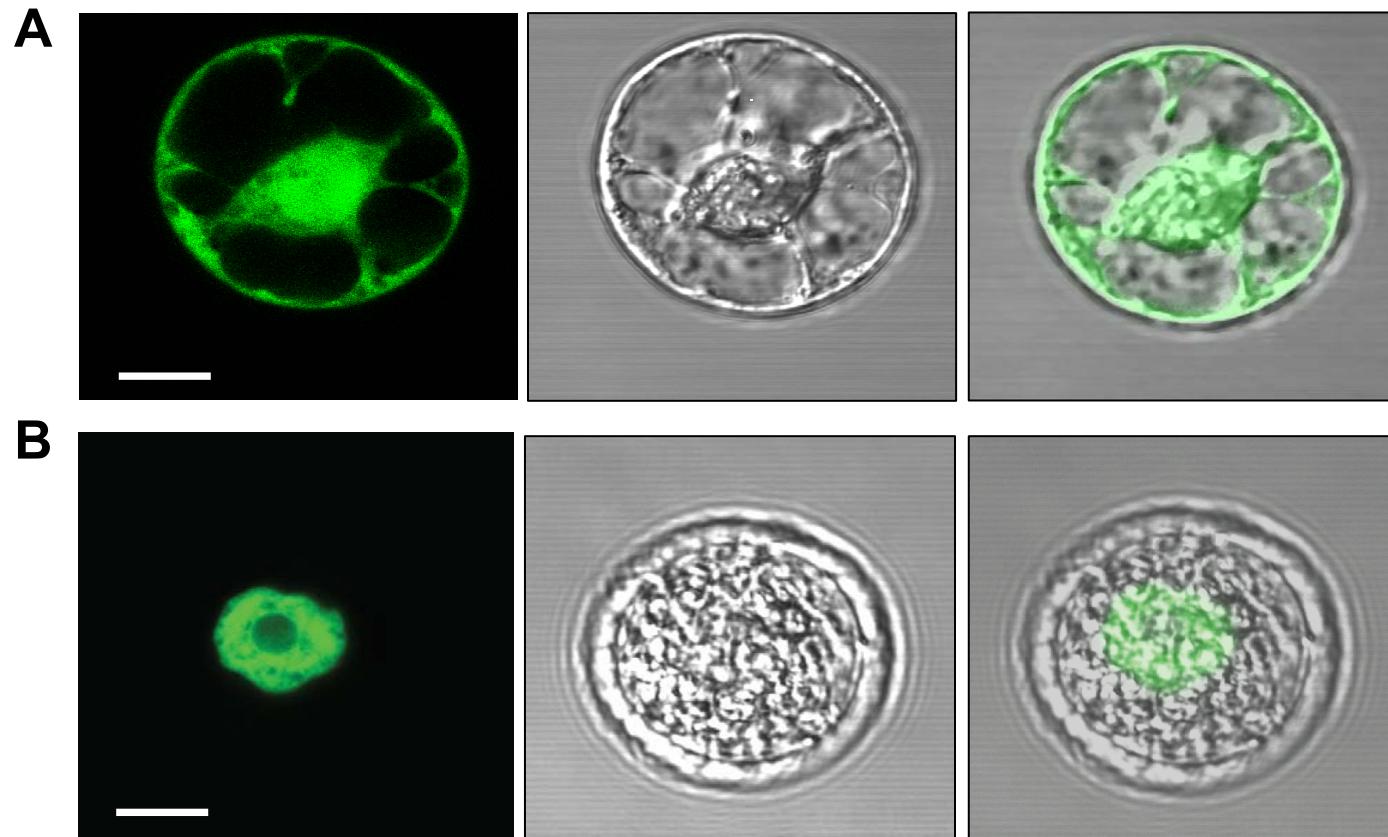
Q96483      **F** 5'-TGTCCCTATTACGAGGGTTATGC-3'  
**R** 5'-AGTTAAATCACGACCAGCAAGAT-3'

---

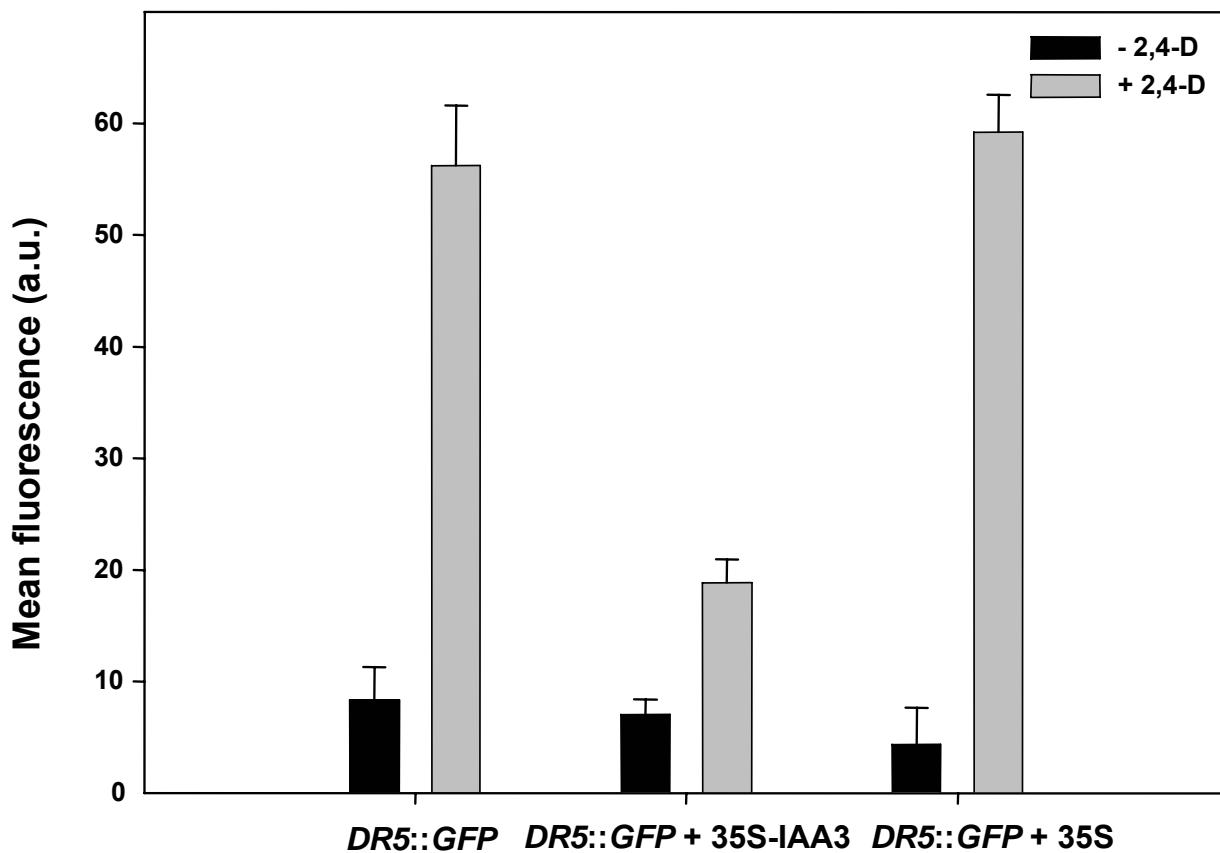


**Fig. S1.** Ethylene regulation of SI-IAA3. Ethylene dose response (**A**) and time course (**B**) of SI-IAA3 induction in 5-day-old dark-grown WT seedlings. Expression of the ethylene-responsive gene *E8* was used as control for hormone treatment (**C,D**).  $\Delta\Delta\text{CT}$  on the y axis refers to the fold difference in SI-IAA3 expression relative to untreated seedlings. The data are mean values of 3 replicates  $\pm\text{SE}$ .

Chaabouni et al., Fig. S1



**Fig. S2.** Subcellular localization of SI-IAA3 protein. SI-IAA3::GFP fusion protein was transiently expressed in BY-2 tobacco protoplasts and sub-cellular localization was analyzed by confocal laser scanning microscopy. The merged pictures of the green fluorescence channel (left panels) and the corresponding bright field (middle panels) are shown (right panels). **A.** Control cells expressing GFP alone. **B.** Cells expressing the SI-IAA3::GFP fusion protein. The scale bar indicates 10  $\mu$ m.



**Fig. S3.** SI-IAA3 protein represses *in vivo* the activity of *DR5*. Tobacco protoplasts were transformed either with the reporter construct (*DR5::GFP*) alone or with both the reporter and effector constructs (35S-IAA3) and incubated in the presence or absence of 50  $\mu$ M 2,4-D. GFP fluorescence was measured 16 h after transfection. A mock effector construct lacking SI-IAA3 was used as a control for the co-transfection experiments. Transformations were performed in triplicate. Mean fluorescence is indicated in arbitrary unit (a.u.)  $\pm$  SE.

Chaabouni et al., Fig. S3