Table SI. Total anthocyanin levels in hypocotyl extracts, as quantified by absorbance at 540 nm.

Genotype	VF36	*are*
Normalized anthocyanin content	1 ± 0.033	0.134 ± 0.014*

Data are expressed as fraction of wild-type. Average and standard error are reported for n=9. Asterisk indicates significant difference from wild-type with $p \leq 0.05$, determined by Student's t-test.

Table SII. Flavonoid levels in 6-day old seedlings of p*35S:CHR-CHS* and its wild-type.

	Moneymaker (ng/gfw)	p*35S:CHR-CHS* (ng/gfw)	Ratio[a]
Naringenin	15.6 ± 0.7	758.8 ± 16.4*	48.64
Kaempferol	2394.2 ± 82.3	2979.6 ± 49.6	1.24
Quercetin	1351.4 ± 603.9	1317.4 ± 72.3	0.97
Myricetin	1478.1 ± 247.2	1148.7 ± 60.3	0.77

Average and standard error are reported for n=3. Asterisk represents significant difference between genotypes with $p \leq 0.05$, determined by Student's t-test. [a] Ratio represents values from p*35S:CHR-CHS* divided by Moneymaker.

Table SIII. Two-way ANOVA results from transcript abundance analysis in *are* and wild-type in both hypocotyl and root tissues.

	Genotype		Tissue		Interaction	
	F ratio	P value	F ratio	P value	F ratio	P value
CHS	10.68	0.0114	919.13	<0.0001	11.72	0.009
CHI	0.83	0.3892	283.23	<0.0001	0.75	0.411
F3H	4.38	0.0696	241.18	<0.0001	3.68	0.0915
FLS	36.36	0.0003	1493.3	<0.0001	34.18	0.0004
F3'H1	5.92	0.041	13.73	0.006	1.73	0.2252
F3'H2	1.17	0.3117	438.24	<0.0001	0.45	0.5213
F3'H3	17.29	0.0032	136.82	<0.0001	16.69	0.0035
F3'5'H	14.77	0.0049	1081.44	<0.0001	15.9	0.004
DFR	41.07	0.0002	1086.2	<0.0001	41.35	0.0002
ANS	0.68	0.432	179.45	<0.0001	1.87	0.2085

Table SIV. Quantification of flavonoids in 6-day old transgenic seedlings.

	Hypocotyls (ng/gfw)			
	VF36	VFOE3	VFOE4	VFOE5
Naringenin	12.3 ± 0.6	6.3 ± 0.8	12.2 ± 0.7	8.9 ± 1.8
Kaempferol	205.9 ± 27.0	26.9 ± 11.4	136.2 ± 18.5	55.2 ± 1.6
Quercetin	17.5 ± 7.1	1.4 ± 0.1	5.3 ± 1.6	1.1 ± 0.7
Myricetin	681.0 ± 45.5	543.9 ± 78.1	839.1 ± 78.1	704.8 ± 162.2
	are	*are*OE5	*are*OE13	*are*OE11
Naringenin	81.9 ± 2.0	12.4 ± 0.7	12.6 ± 1.4	14.7 ± 0.5
Kaempferol	5.5 ± 1.0	78.4 ± 12.6	209.4 ± 17.8	193.8 ± 47.0
Quercetin	3.2 ± 0.6	0.1 ± 0.0	0.7 ±0.3	0.1 ± 0.01
Myricetin	289.5 ± 22.0	758.5 ± 15.1	795.6 ± 72.7	783.2 ± 41.6

Averages and standard error are report for n=3-10.

Table SV. Results from a general linear model with a Poisson distribution results for lateral root formation in wild-type and *are* with and without NPA treatment.

	Estimate	Std. Error	z value	Pr (>|z|)
Intercept	2.14	0.09	24.26	2e-16
Genotype effect	0.66	0.11	6.16	7.23e-10
Treatment effect	-1.31	0.19	-7.00	2.65e-12
Genotype-treatment interaction	0.73	0.21	3.42	0.00062

Table SVI. Oligonucleotide primers used in this study.

Target	5' primer	3' primer
SlCHS-2 qRT-PCR	GGCCGGCGATTCTAGATCA	TTTCGGGCTTTAGGCTCAGTT
SlCHI-1 qRT-PCR	CAGGTCCATTTGAGAAATTCACT	GCATCTCTGCATCACTGTAGG
SlF3H qRT-PCR	TGAAAAGACCCTTGAAACAA	CGATTCTCTCACATATTTCA
SlFLS qRT-PCR	TAAGATTTGGCCTCCTCCTG	ACCAAGCCCAAGTGATAAGC
SlF3'H-1 qRT-PCR	CACCTTACGGACCACGG	GTGTTCTGACTTCTTCCTGT
SlF3'H-2 qRT-PCR	GTGATGGAATTGTTCAGCG	TTAAACTCAGAGTCGTCAAATAA
SlF3'H-3 qRT-PCR	TTGCATCTCGTCCTGAATTA	ATCAAGCCTTTTAGGACTAAATATC
SlF3'5'H qRT-PCR	CCACGTTGGAAGTTGCTAAG	TGGCTTGCATCGAACATC
SlDFR qRT-PCR	GAAGGCTGCAATGGAAGAAG	GATTAAGCTTGGTGGGAACG
SlANS qRT-PCR	AGGGGTTTTAGGCCAGATG	ATGTCCAAGGCTATGGAAGC
SlActin qRT-PCR	TCTCTGTTGGCCTTGGGATT	CTTCGAGTTGCTCCTGAGGAA
SlF3H pDONR-221	AAAAAGCAGGCTCCATGGCTCC AGGAAC	AGAAAGCTGGGTCCTTCTAAGCGA AGATTTGG
attB1 and attB2	GGGGACAAGTTTGTACAAAAAA GCAGGCT	GGGGACCACTTTGTACAAGAAAGC TGGGT
nptII	TGAATGAACTGCAGGACGAG	AGCCAACGTATGTCCTGAT

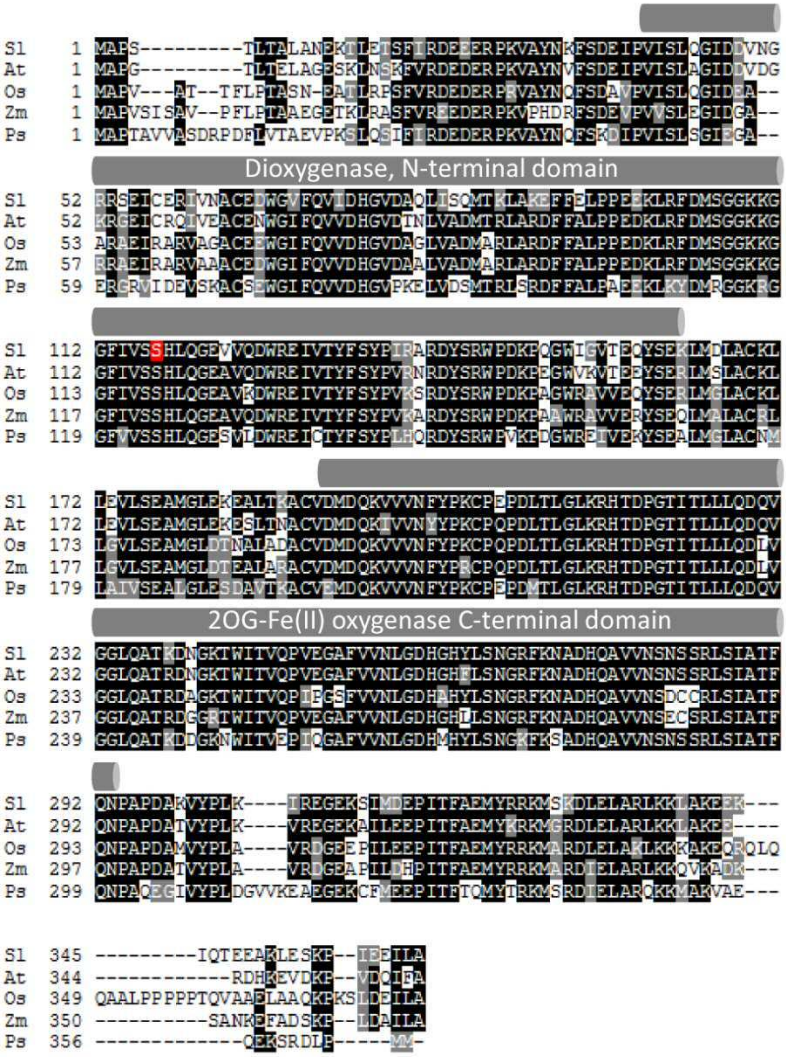

Figure S1. Alignment of F3H peptide sequences from multiple distantly-related plant species shows high conservation of this gene. Both N- and C-terminal activity domains are marked by gray bars over sequences. Black residues indicate high conservation, white residues indicate low conservation, and gray residues indicate differing amino acid structure but with similar functional groups. Red shading indicates the location of the S>N mutation in the *are* mutant *F3H*. Sl, *Solanum lycopersicum*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Zm, *Zea mays*; Ps, *Pisum sativum*.

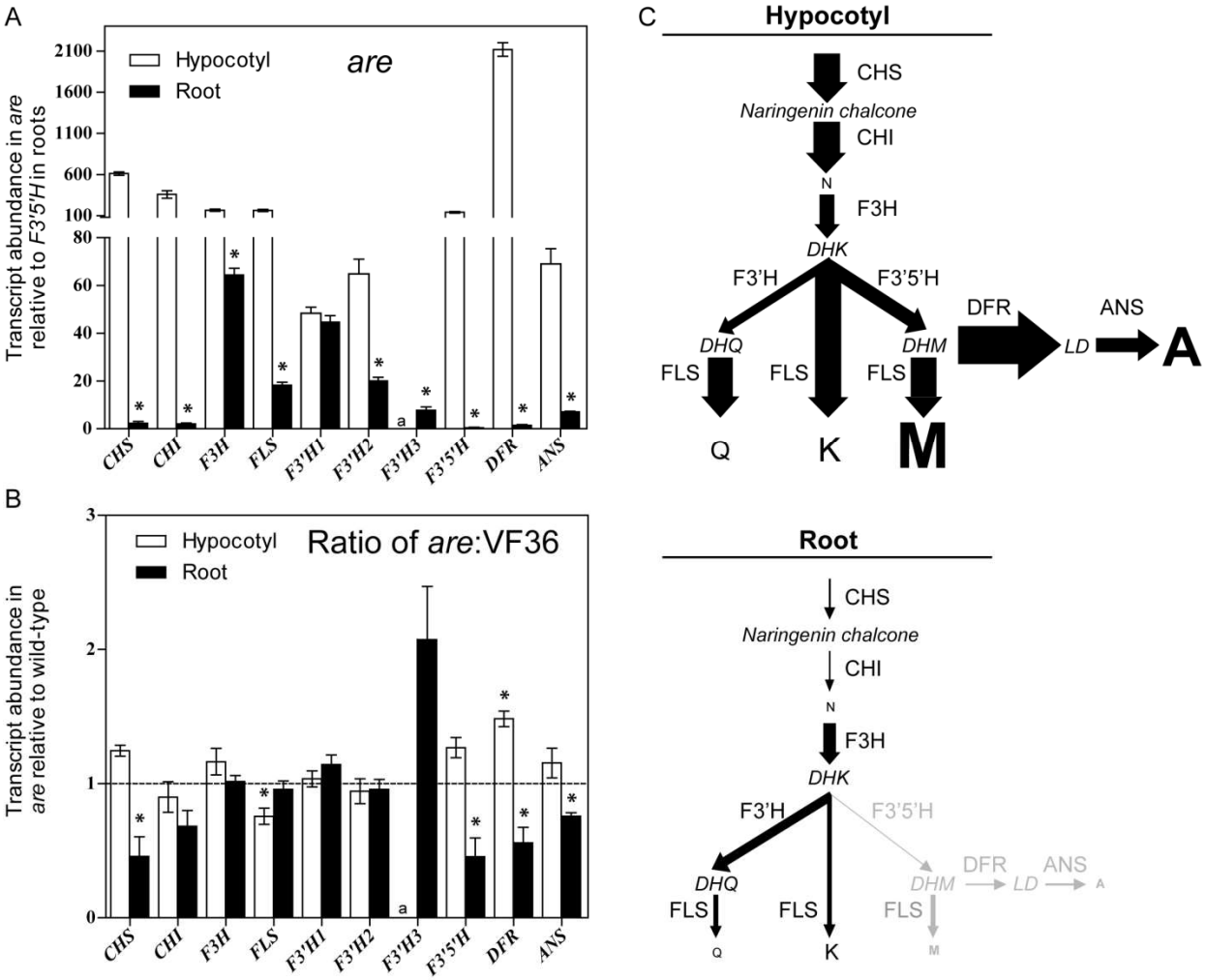

Figure S2. Transcript abundance of flavonoid biosynthetic genes in *are,* and biosynthetic pathways showing relative levels of transcripts and metabolites in wild-type. A, Transcript abundance of flavonoid biosynthetic genes in hypocotyls and roots of the *are* mutant. Quantities are reported normalized to *F3'5'H* in roots. Scales differ above and below the y-axis break, to clearly show levels of low abundance transcripts. B, Transcript abundance of flavonoid biosynthetic genes in hypocotyls and roots of the *are* mutant relative to VF36, illustrating differences between the two genotypes. Transcript levels in wild-type are normalized to 1, as denoted by the dashed line. Averages and standard error are reported for 9 biological replicates each with three technical replicates. In A and asterisk indicates difference between tissue types. In B, asterisk indicates difference between genotypes. Significance was determined by Student's t-test with P≤0.05. *a* indicates that transcript levels were below the threshold of detection. C, Illustration of flavonoid biosynthetic gene transcript levels and flavonoid metabolite levels in hypocotyls and roots of wild-type tomato seedlings. Transcripts levels are indicated by thickness of arrows, while metabolite levels are indicated by boldness and size of fonts. Intermediate metabolites not measured in this study are in italics, and no indication of their levels are given. Arrows and text in gray represent transcripts and metabolites at negligable levels. N, naringenin; Q, quercetin; K, kaempferol; M, myricetin; LD, leucodelphinidin; A, anthocyanins.

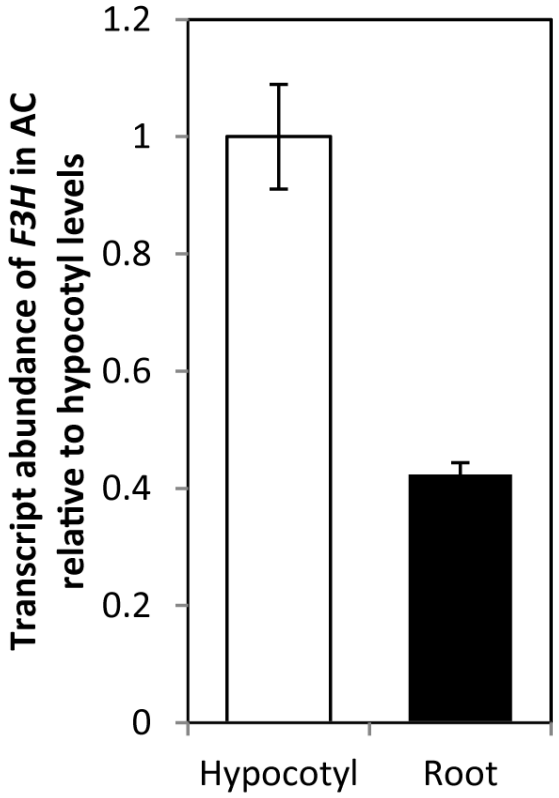

Figure S3. Transcript abundance in the *F3H* gene in Ailsa Craig hypocotyls and roots. Data are presented relative to levels in hypocotyls. Averages and standard error are reported for 3 biological replicates each with 3 technical replicates. Asterisk represents significant difference determined by Student's t-test with $p \leq 0.05$.

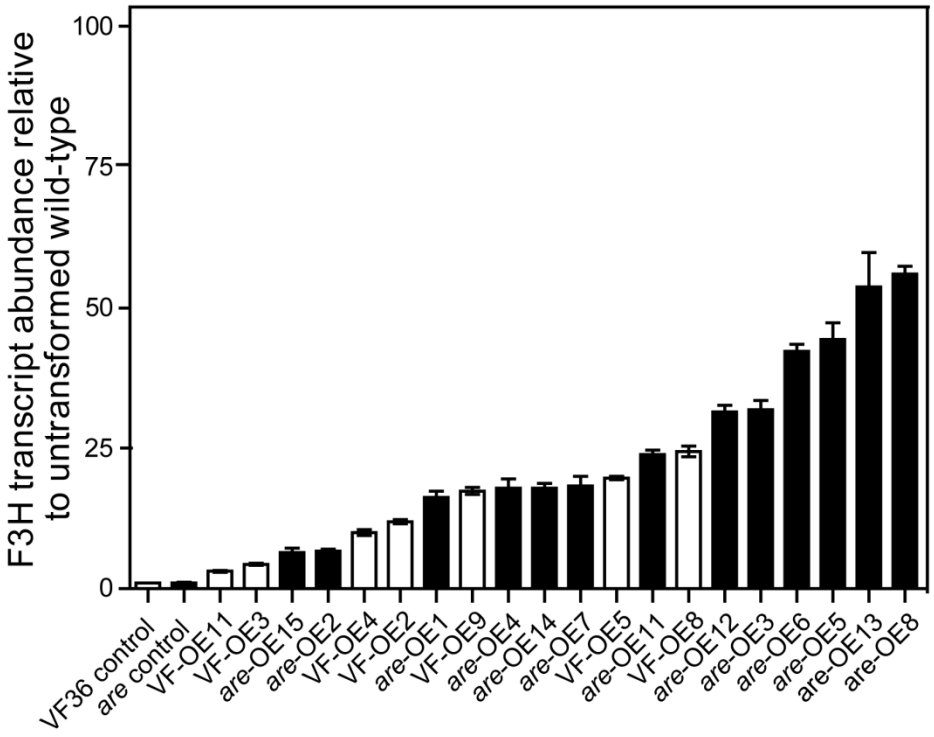

Figure S4. *F3H* transcript abundance in young expanding leaves of T_0 generation transgenic VF36 and *are* tomato plants over-expressing the wild-type F3H gene. All data are reported relative to transcript abundance in untransformed VF36, which is normalized to 1. Average and SE are reported for 3 biological replicates with 3 technical replicates each. All transgenic lines are significantly different from their respective controls with $p \leq 0.01$. Lines in the *are* background are shown with black bars.

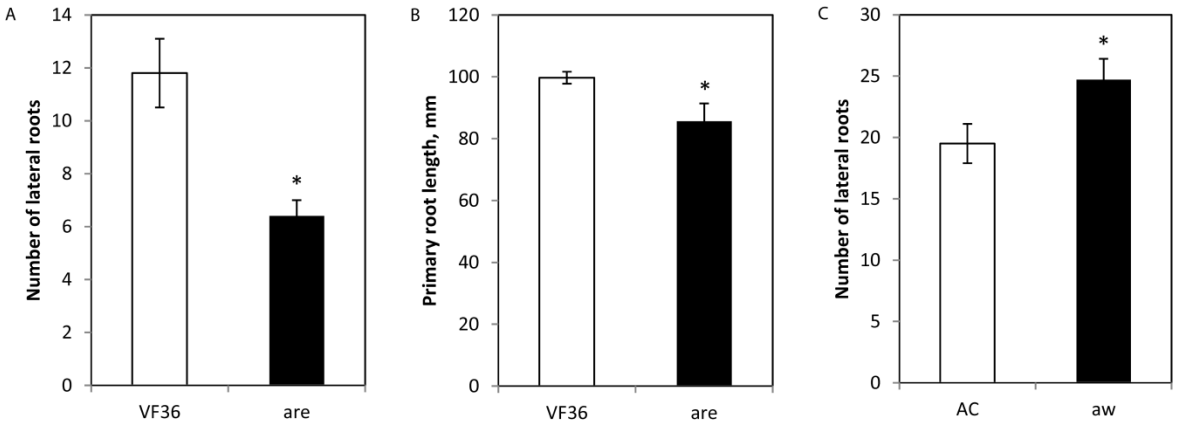

Figure S5. A, Lateral root numbers are greater in the VF36 wild type than in *are* with seedlings grown in Turface media. Roots were quantified 14 days after seed planting. Average and standard error are presented for n=9 samples. Asterisk represents significant difference between genotypes with $p<0.005$. B, The primary root length of *are* seedlings is slightly shorter than the VF36 wild type. Root lengths were measured on seedlings grown for 5 days on standard media. Average and standard error are presented for n=21 samples. Asterisk represents significant difference between genotypes with $p<0.05$. C, Lateral root numbers are greater in *aw* seedlings than in the Ailsa Craig wild type. Roots were quantified 5 days after growth on standard media. Average and standard error are presented for n=14 samples. Asterisk represents significant difference between genotypes with $p<0.05$.

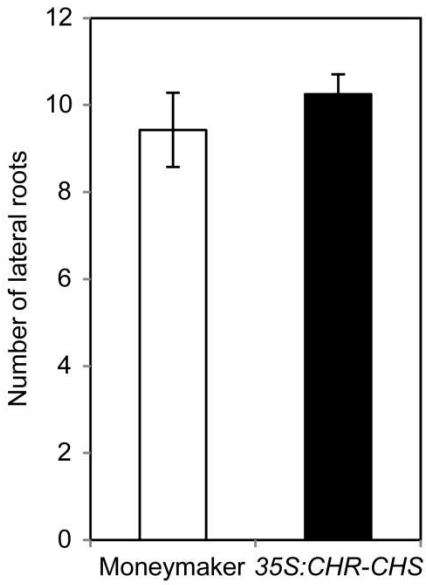

Figure S6. Numbers of lateral roots in 6 day old seedlings of *35S:CHR-CHS* are not significantly different than in wild-type. Average of n=16 samples and SE is presented. Lack of significance determined by Student's t-test.

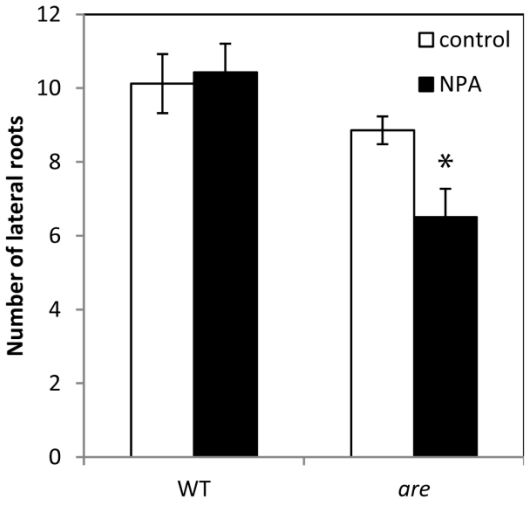

Figure S7. Number of emerged lateral roots above the site of NPA or control application. NPA or control droplets were placed 2 cm below root shoot junction. Average and standard error are reported for 9 seedlings. Asterisk indicates significant difference from control treatment with $p \leq 0.05$

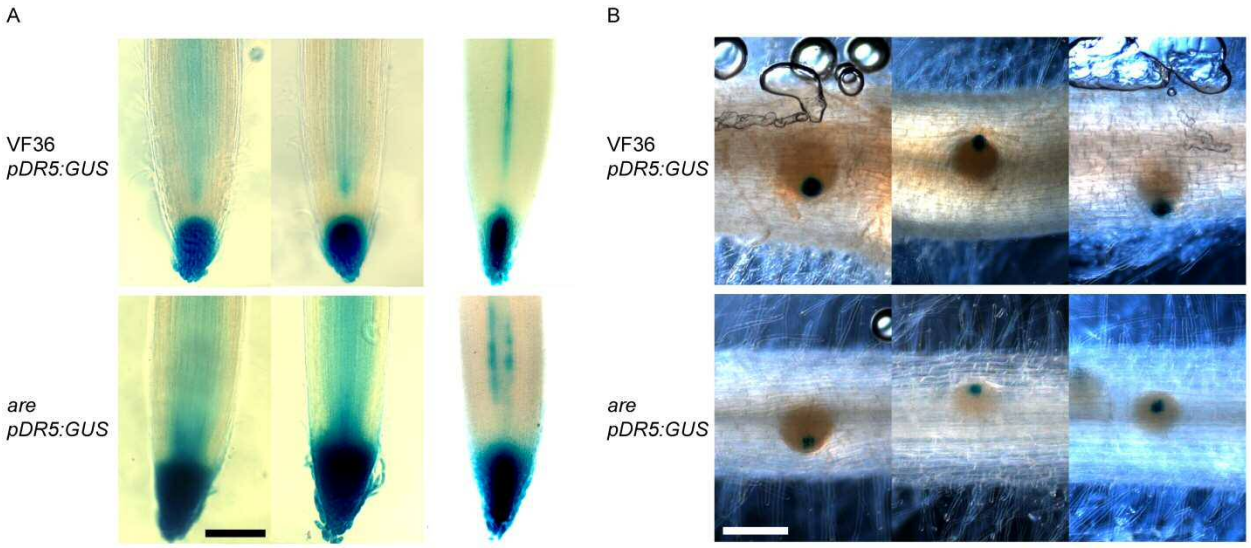

Figure S8. Montage of root tips and lateral root primordia stained to reveal GUS expression. A, GUS-stained primary root tips of wild-type or *are* seedlings containing the p*DR5:GUS* transgene. Scale bar = 200 µm. B, Lateral root primordia with GUS staining in wild-type or *are* seedlings. Scale bar = 100 µm. Seedlings are 3 days old, the stage at which lateral root primordia start forming and before emergence of lateral roots.

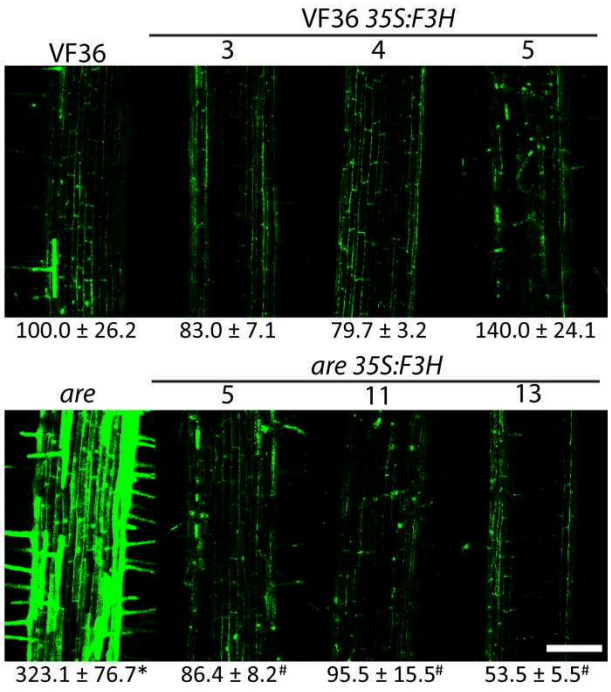

Figure S9. Fluorescent DCF staining is reduced in transgenic roots overexpressing the wild-type *F3H* gene. Images show representative roots from 3 seedlings tested for each genotype. Images were taken in the mature region of the primary root. The first column shows unstransformed lines. The remaining three columns show transformed lines with numbers indicating individual transgenic events. Scale bar = 200 µm. Numbers below each image represent average and standard error of quantified DCF fluorescence relative to untransformed VF36. Asterisk indicates significant difference from untransformed VF36 and number symbol indicates significant difference from untransformed are with n=6 and $p<0.01$.