

Supplemental Data. Tamada et al. (2009). *ARABIDOPSIS TRITHORAX-RELATED 7* is required for methylation of lysine 4 of histone H3 and for transcriptional activation of *FLOWERING LOCUS C*

Supplemental Table 1. *atx* Mutant Lines Analyzed in the Manuscript

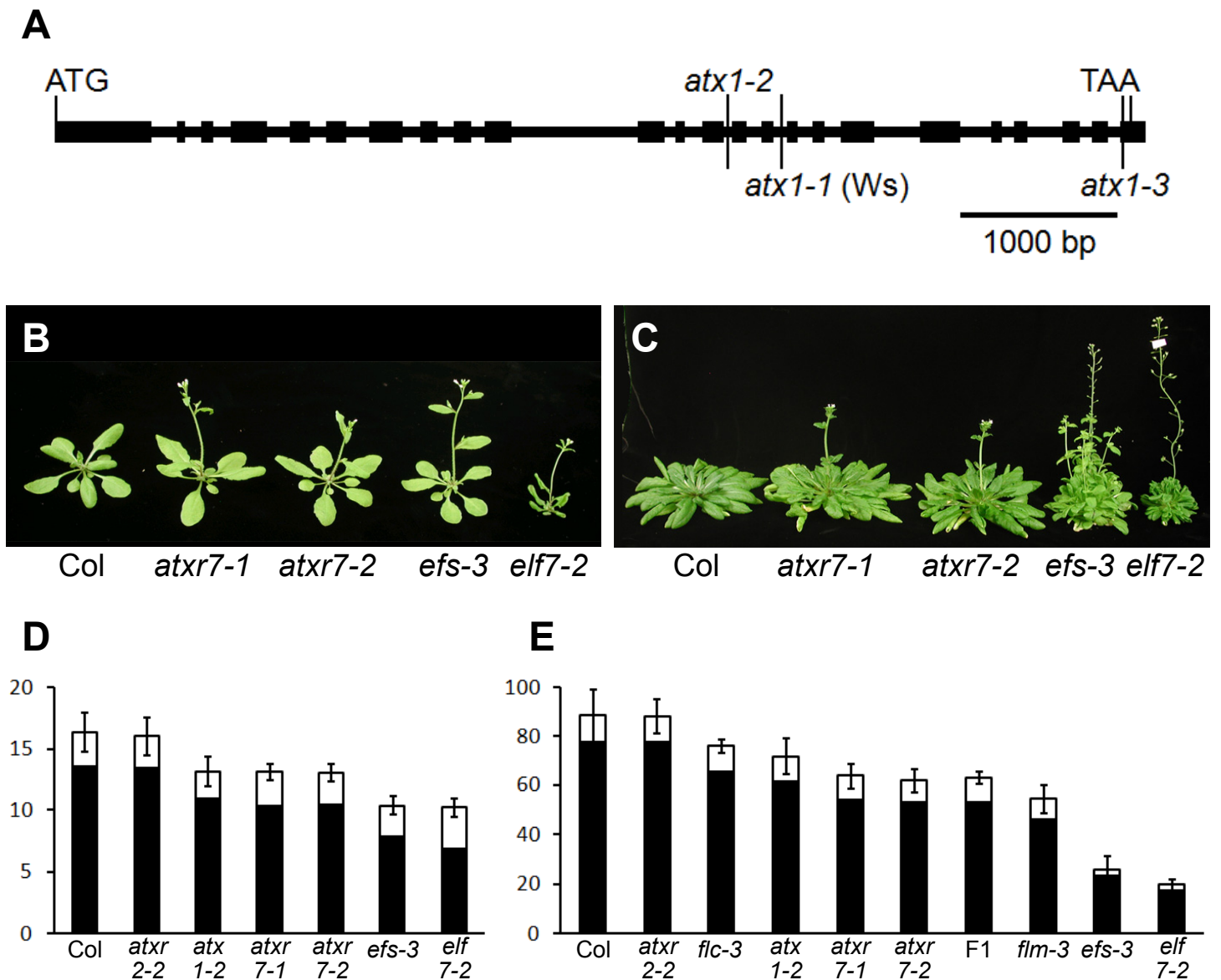
Gene	Locus	Mutant name ^a	Line name	miscellaneous ^b
<i>ATX1</i>	At2g31650	<i>atx1-2</i> *	SALK_149002	Pien et al., 2008
		<i>atx1-3</i> *	SALK_119016C	
<i>ATX2</i>	At1g05830	<i>atx2-1</i>	SALK_074806	Pien et al., 2008
		<i>atx2-2</i>	SALK_117262	Saleh et al., 2008
		<i>atx2-3</i> *	GABI_057B09	
<i>ATX3</i>	At3g61740	<i>atx3-1</i>	SAIL_255_F11	
		<i>atx3-2</i> *	SAIL_582_H12	
<i>ATX4</i>	At4g27910	<i>atx4-1</i>	SAIL_1247_E09	
		<i>atx4-2</i> *	SALK_060156	
<i>ATX5</i>	At5g53430	<i>atx5-1</i>	WiscDsLox461-46418	
		<i>atx5-2</i> *	SAIL_705_H05	
<i>ATXR1</i>	At1g26760	<i>atxr1-1</i> *	GABI_721A04	
		<i>atxr1-2</i>	GABI_355F02	
		<i>atxr1-3</i>	SALK_117606	
		<i>atxr1-4</i>	SALK_028842	
<i>ATXR2</i>	At3g21820	<i>atxr2-1</i>	SAIL_600_E07	
		<i>atxr2-2</i> *	SALK_026154	
		<i>atxr2-3</i>	WiscDsLox471C3	
<i>ATXR3</i>	At4g15180	<i>atxr3-1</i> *	WiscDsLox361D10	
		<i>atxr3-2</i>	GABI_489F05	
		<i>atxr3-3</i>	SALK_021008	
<i>ATXR4</i>	At5g06620	<i>atxr4-1</i> *	SALK_065711C	
		<i>atxr4-2</i>	SALK_117779	
<i>ATXR7</i>	At5g42400	<i>atxr7-1</i> *	SALK_149692	
		<i>atxr7-2</i> *	SAIL_446_F12	
		<i>atxr7-3</i>	FLAG_370G04	Ws background

^aAsterisks beside the mutant name indicate the lines into which active *FRI* locus was introgressed.

^b*atx1-2*, *atx2-1* and *atx2-2* were previously described in the noted papers. Except for *atxr7-3* in the Ws background, all of the mutants are in the Col background.

Supplemental Table 2. Primers for Realtime PCR

Gene	Locus	Forward primer (5' to 3')	Reverse primer (5' to 3')
1, RT-PCR			
<i>FLC</i>		CCGAACTCATGTTGAAGCTTGTTGAG	CGGAGATTTGTCCAGCAGGTG
<i>FLM</i>		GGAAAGAATACGTTGCTGGCAACA	CCGTTGATGATGGTGGCTAATTGA
<i>MAF2</i>		CGAAATACATCATGCTGATGAACTTG	GCTTTGGACTATTTCTAGTAACTCTTGA
<i>MAF3</i>		GGAAATAAAGGTAAAACAAAACGAAGCTCTT	GAACTCTGATATTTGTCTACTAAGGTACA
<i>MAF4</i>		GATGGGGAAGATGAAGAAGTCTGT	AGTCTCCGGTGGCTTGTGT
<i>MAF5</i>		GAAACAGGGGATGAAAGAGCAGTA	TGGGCTGTGGCCAGAGCTAT
<i>CO</i>		GCTCCTCAGGGACTCACTAC	CTCCGGCACAACACCAGTTTC
<i>ACT2</i>		GGTAACATTGTGCTCAGTGGTGG	AACGACCTTAATCTTCATGCTGC
2, ChIP			
<i>FLC</i>	1	AGCAGTGGCGGATCCAAGAA	CCCCACATCAATCCAAGTTCA
	2	GCTGATACAAGCATTTCACCAAA	CTTAAATGTCCACACATATGGCAAT
	3	GTATCGTAGGGGAGGAAAGA	GAAGACAAGATTGCCACGTGTA
	4	GAAAGAAATAAAGCGAGAAAAGGAA	GGCTTTGTGCCCTAATTTGAT
	5	GTTGTTTCTCGGTTCTGTGT	GCTAAAAAGCTTCTTCACGACAT
	6	GTGGACCTATTACTTGGTGATT	GCTGAGTTTTTTGAAGCTACCAA
	7	CAATCTTTTGTGTGAAAATCGACAA	ACAAGGCTGTGTGAATGACAA
	8	GAGGCTTATGTTTAGGGTTCTT	GAAAAGTCATACAAAGGCATACAGAT
	9	CGGTTGTTGGACATAACTAGGTT	CAAACCCAGACTTAACCAGACT
	10	CTTAAGTAGACGGAATAAGTCAA	TACTACTCAAGATCTCGATGCAA
<i>Ta3</i>		TGGGGCTCTCCAAATGTAAC	GGCTTTTCCACTCACAAAACCTT



Supplemental Figure 1. Flowering Phenotype of H3K4 Methylase Mutants

(A) *ATX1* gene structure and T-DNA insertion sites. Thick and thin lines indicate exons and introns, respectively. *atx1-2* and *atx1-3* are in the Col background.

(B) and **(C)** Representative plants of Col, *atxr7-1*, *atxr7-2*, *efs-3* and *elf7-2* grown in long days (**[B]**, 23 days old) or short days (**[C]**, 10 weeks old).

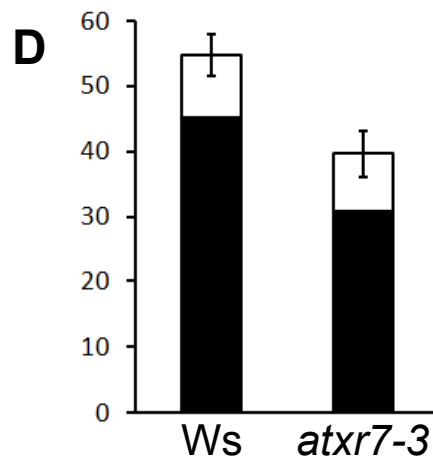
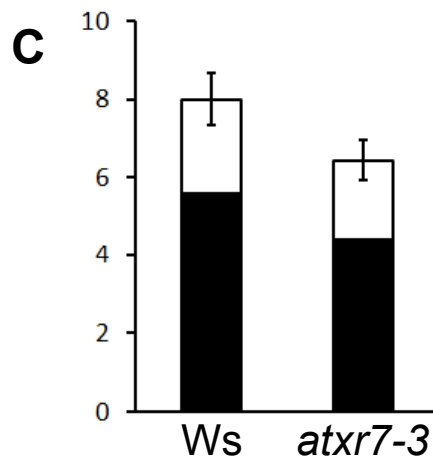
(D) and **(E)** Primary leaf number at flowering of *atx* and typical early flowering mutants grown in long days (**(D)**) or short days (**(E)**). *atxr2-2* is a negative control which did not show any flowering phenotype. F1 indicates F1 plants whose parents are *atxr7-1* and *atxr7-2* mutants. Closed and open bars indicate rosette and cauline leaves, respectively. Bars indicate standard deviation.



Ws *atxr7-3*



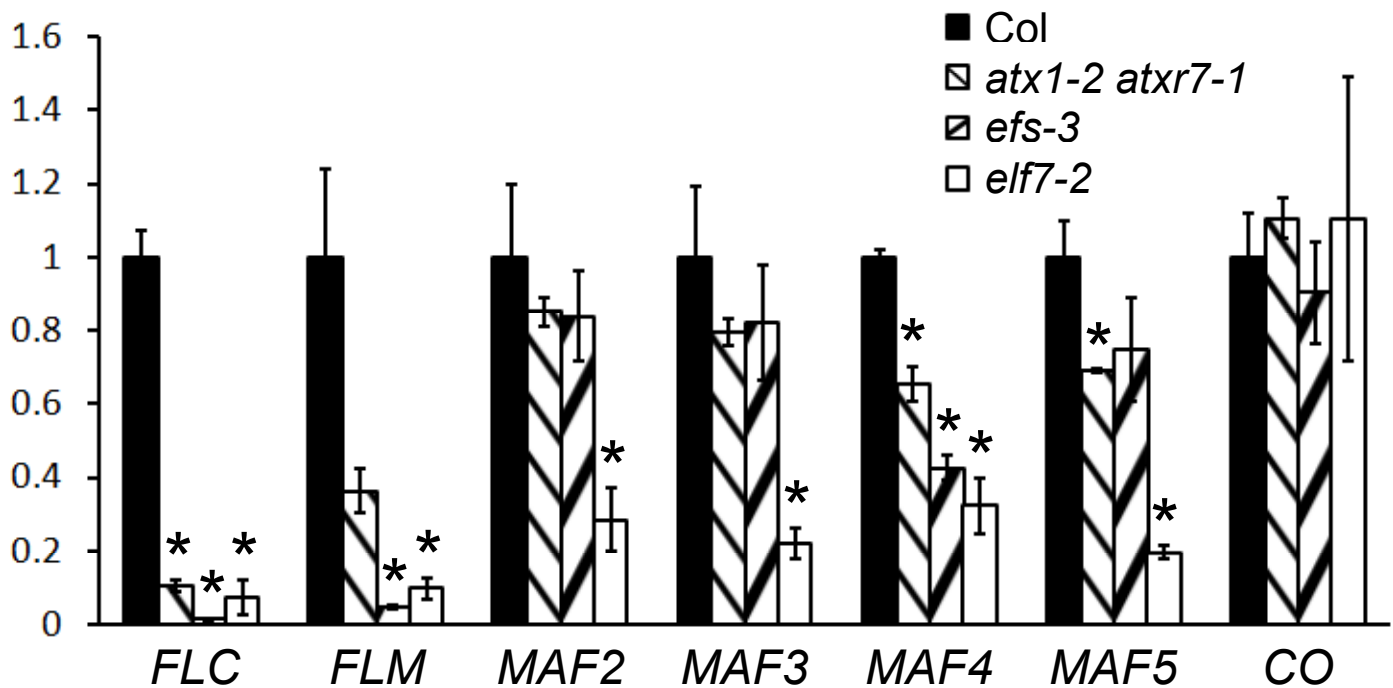
Ws *atxr7-3*



Supplemental Figure 2. Flowering Phenotype of *atxr7* in the Ws Background

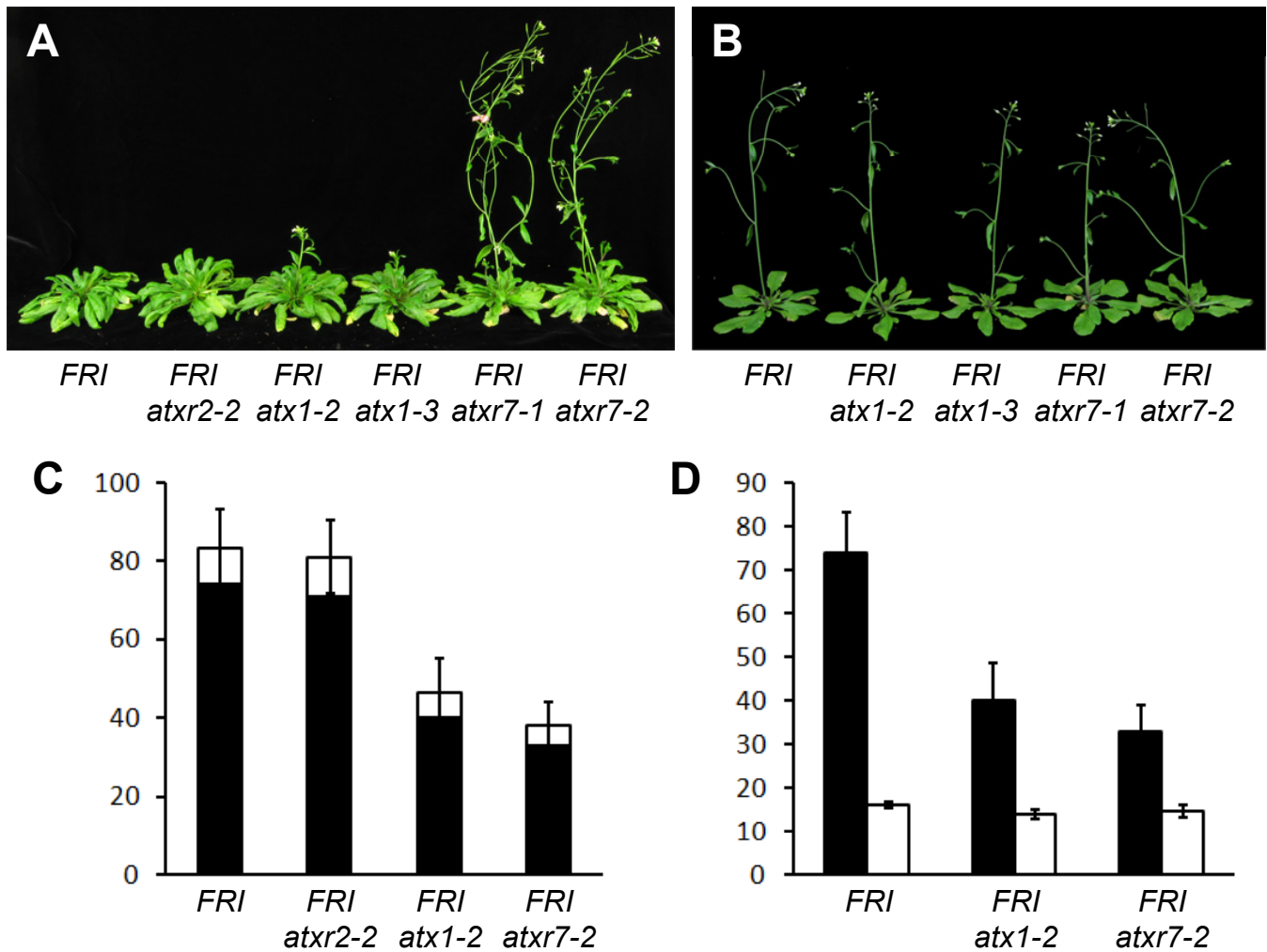
(A) and (B) Representative plants of Ws and *atxr7-3* grown in long days ([A], 3 weeks old) or in short days ([B], 10 weeks old).

(C) and (D) Primary leaf number at flowering of Ws and *atxr7-3* grown in long days (C) or in short days (D). Closed and open bars indicate rosette and cauline leaves, respectively. Bars indicate standard deviation.



Supplemental Figure 3. mRNA Levels of *FLC* and *FLC*-Related Family Genes in *atx1-2 atxr7-1*, *efs-3* and *elf7-2* Mutants

Real-time PCR was performed to analyze the expression level. The averages of the results from 3 different biological replicates are shown. Each experiment was normalized to *ACTIN2* expression. Bars indicate the standard error. Asterisks beyond the bars indicates the significant difference in the expression of the genes between Col and mutants ($p < 0.05$).



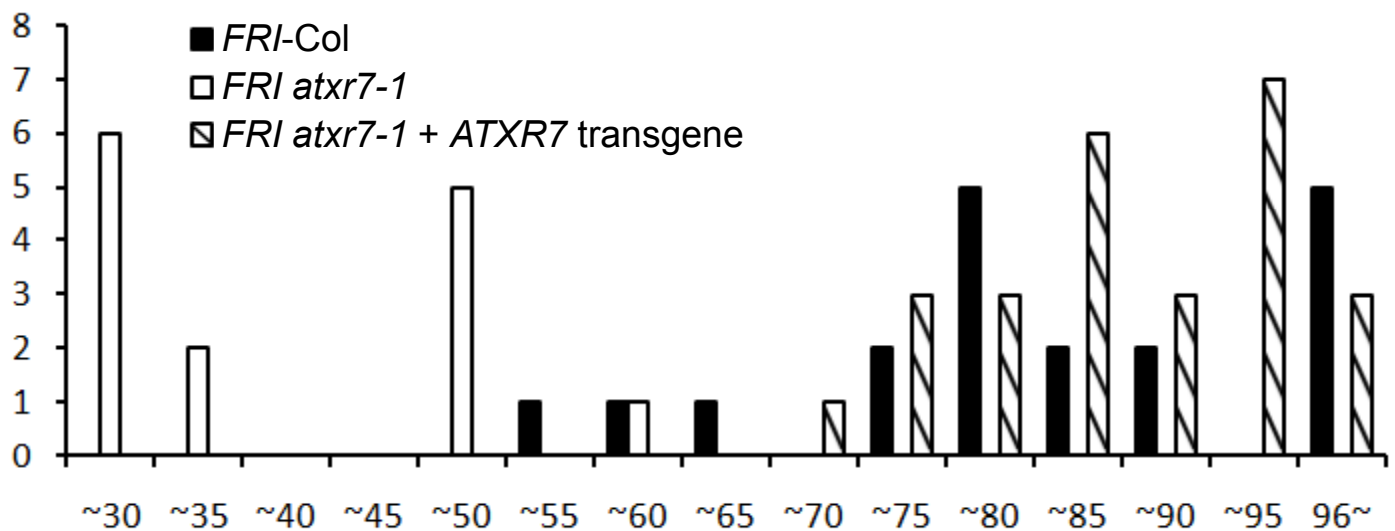
Supplemental Figure 4. Effect of Vernalization on H3K4 Methylase Mutants

(A) Representative 7-week-old plants of *FRI*-Col, *FRI atxr2-2*, *FRI atx1-2*, *FRI atx1-3*, *FRI atxr7-1* and *FRI atxr7-2* grown in long days. Same plants in Figure 3A (*FRI*-Col, *FRI atx1-2* and *FRI atxr7-2*) are presented. *atxr2-2* is a negative control which did not suppress or accelerate the delayed-flowering phenotype caused by *FRI*.

(B) Representative 7-week-old plants of *FRI*-Col, *FRI atx1-2*, *FRI atx1-3*, *FRI atxr7-1* and *FRI atxr7-2* grown in long days with vernalization (40 days of 4 °C exposure).

(C) Primary leaf number at flowering of *FRI*-Col, *FRI atxr2-2*, *FRI atx1-2* and *FRI atxr7-2* grown in long days. Closed and open bars indicate rosette and cauline leaves, respectively. Bars indicate standard deviation.

(D) Primary rosette leaf number at flowering of *FRI*-Col, *FRI atx1-2* and *FRI atxr7-2* grown in long days with (open bar) or without (closed bar) vernalization. Bars indicate standard deviation.



Supplemental Figure 5. Rapid Flowering Phenotype Caused by the *atxr7* Lesion Is Rescued by an *ATXR7* Transgene

The X- and Y-axis indicate rosette leaf number and the number of the plants of *FRI-Col* (closed bar, n = 19), *FRI atxr7-1* (open bar, n = 14) and different T1 transformants of *FRI atxr7-1* *ATXR7pro:ATXR7-FLAG* (hatched bar, n = 26).