

This is a repository copy of *Arabidopsis ELF3 sub-nuclear localization responds to changes in ambient temperature*.

White Rose Research Online URL for this paper:  
<https://eprints.whiterose.ac.uk/177983/>

Version: Accepted Version

---

**Article:**

Ronald, James, Wilkinson, Anthony J [orcid.org/0000-0003-4577-9479](https://orcid.org/0000-0003-4577-9479) and Davis, Seth Jon [orcid.org/0000-0001-5928-9046](https://orcid.org/0000-0001-5928-9046) (Accepted: 2021) *Arabidopsis ELF3 sub-nuclear localization responds to changes in ambient temperature*. *Plant Physiology*. ISSN 0032-0889 (In Press)

---

**Reuse**

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

**Arabidopsis ELF3 sub-nuclear localization responds to changes in ambient temperature.**

James Ronald<sup>1</sup>, Anthony J. Wilkinson<sup>2</sup>, Seth J. Davis<sup>1,3,\*</sup>.

<sup>1</sup>University of York, Department of Biology, Heslington, York YO10 5DD, UK.

<sup>2</sup>University of York, Department of Chemistry, Heslington, York YO10 5DD, UK.

<sup>3</sup>State Key Laboratory of Crop Stress Biology, School of Life Sciences, Henan University, Kaifeng 475004, China.

\*Corresponding author: [seth.davis@york.ac.uk](mailto:seth.davis@york.ac.uk)

JR, AJW, and SJD conceived the original research plans; AJW and SJD supervised the experiments; JR performed the experiments; JR wrote the article with contributions of all the authors; SJD agrees to serve as the author responsible for contact and ensures communication.

Acknowledgements

## Reduction in ELF3 localization to foci in response to warming temperature

We thank York Biology Imaging and Cytometry for their assistance and technical advice in this work.

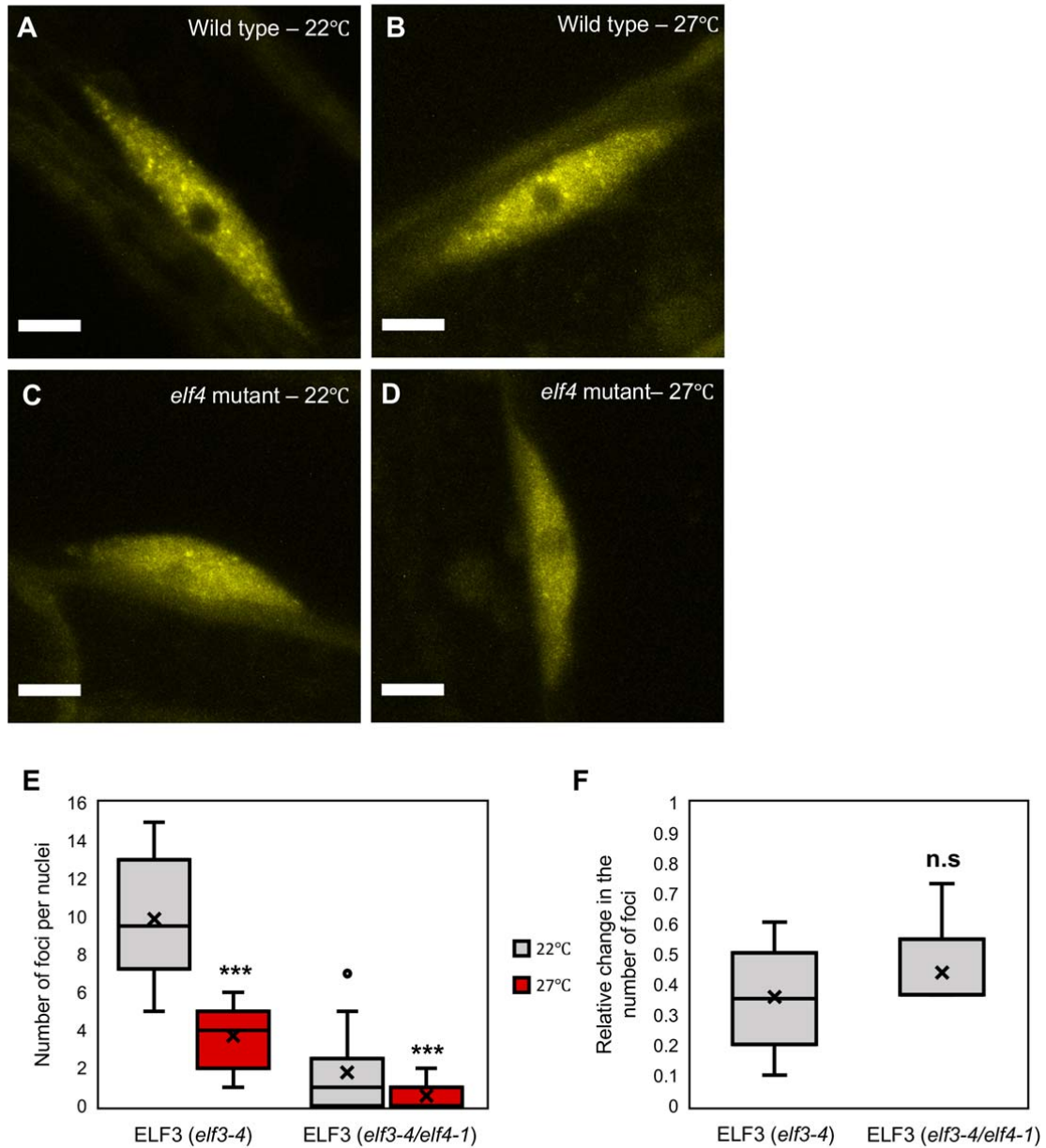
J.R is funded by a BBSRC studentship award (ref 1792522). Work on this project was also supported by a BBSRC awards BB/N018540/1 and BB/V006665/1, and the 111 Project grant D16014.

1 **Dear Editor,**

2 Plants adapt their development to daily and seasonal ambient temperature  
3 fluctuations. Warming triggers a suite of molecular responses that leads to pronounced  
4 changes in plant development and architecture (Quint et al., 2016). Collectively, this response  
5 is called thermomorphogenesis. The evening complex (EC) is a transcriptional regulatory  
6 complex composed of EARLY FLOWERING3 (ELF3), ELF4, and LUX ARRHYTHMO  
7 (LUX) that has emerged as a hub in the circadian clock and plant development (Nusinow et  
8 al., 2011, Herrero et al., 2012, Ezer et al., 2017). The ability of the EC to bind to DNA is  
9 temperature-dependent, with warm temperature reducing the association of the EC to DNA  
10 (Raschke et al., 2015, Press et al., 2016, Ezer et al., 2017, Silva et al., 2020). However, it is  
11 unclear how warm temperature inhibits the DNA binding ability of the EC. Previously, we  
12 observed that ELF3 localizes to sub-nuclear structures called foci (Herrero et al., 2012).  
13 Impaired localization of ELF3 to foci correlated with elevated expression of EC targets  
14 (Anwer et al., 2014), suggesting that foci could be sites where the EC binds to DNA and  
15 represses gene expression. Therefore, we hypothesized that warm temperatures inhibits EC  
16 function by reducing the localization of ELF3 to foci.

17 To test this, first we investigated whether warm temperature influenced the sub-  
18 nuclear localization of ELF3 in Arabidopsis. Using the previously described *35S::YFP:ELF3*  
19 (*elf3-4*) line (Herrero et al., 2012), we observed that a two-hour 27°C temperature pulse  
20 resulted in fewer and smaller foci in hypocotyl nuclei (**Figure 1A, 1C, 1E**). ELF4 was found  
21 to be required for ELF3 to these localize to foci (Kolmos et al., 2011, Herrero et al., 2012,  
22 Anwer et al., 2014) and was also proposed to have a warm temperature specific function in  
23 the EC (Silva et al., 2020, Jung et al., 2020). Therefore, we investigated whether ELF4  
24 regulated the sensitivity of ELF3 foci to warm temperature. We introgressed the  
25 *35S::YFP:ELF3* line into the *elf3-4/elf4-1* mutant. This line will be referred to as ELF4 (4-),

Reduction in ELF3 localization to foci in response to warming temperature



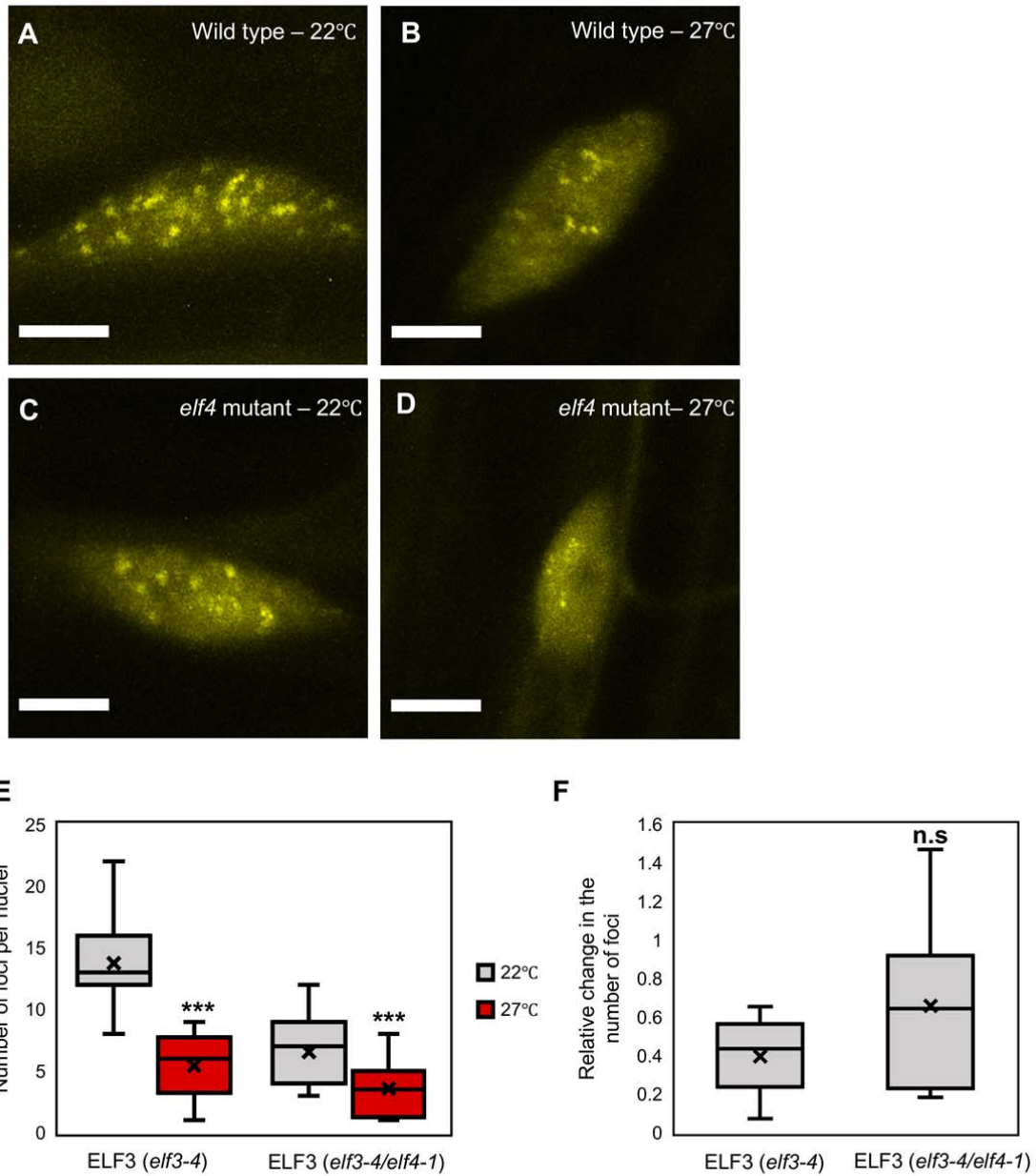
26 while the original line will be called ELF3 (4+). The localization of ELF3 (4-) to foci was  
27 impeded at 22°C (Figure 1B) and this was further reduced by a 27°C pulse (Figure 1D-E).  
28 However, the relative change in the number of foci for ELF3 (4-) in response to the 27°C  
29 pulse was similar to ELF3 (4+) (Figure 1F). Together, warm temperatures suppress the  
30 localization of ELF3 to foci and ELF4 has a limited role within this.

31 To understand if the response of ELF3 foci to temperature was tissue-dependent, we  
32 investigated the effect of a 27°C pulse on foci formation in root nuclei. As in hypocotyl  
33 nuclei, a 27°C pulse suppressed the localization of ELF3 (4+) to foci in root nuclei and these  
34 foci were smaller and less bright than at 22°C (**Figure 2A-B**). There was no significant  
35 change in the magnitude of effect caused by the 27°C pulse between the tissue types, with  
36 ELF3 (4+) foci reduced by 62% and 60% in hypocotyl and root nuclei, respectively.  
37 Therefore, ELF3 foci do not have a tissue-dependent response to warm temperature.

38 As ELF4 protein moves from shoot to root tissue, and this movement is temperature  
39 sensitive (Chen et al., 2020), we examined the requirement of ELF4 in regulating the thermal  
40 responsiveness of ELF3 foci in root nuclei. As with ELF3 (4+), ELF3 (4-) localized to foci in  
41 root nuclei and these foci were larger and brighter than those in hypocotyl nuclei (**Figure 1C,**  
42 **2C**). The phenotypic effect of the *elf4-1* mutation on ELF3 foci abundance was weaker in  
43 root nuclei, with foci only reduced by 52% compared to an 81% reduction in hypocotyl  
44 nuclei at 22°C. ELF3 (4-) foci in root nuclei were also reduced by a 27°C temperature pulse  
45 (**Figure 2D-E**). However, this effect was more variable and on average weaker than the  
46 response of ELF3 (4+) foci to the 27°C pulse (**Figure 2F**). As with ELF3 (4+), ELF3 (4-) foci  
47 appeared smaller and less bright after a 27°C pulse in root nuclei (**Figure 2C-D**). Combined,  
48 ELF4 does not have a critical role in buffering ELF3 foci against warming temperatures in  
49 either hypocotyl or root nuclei.

50 The decrease in foci number following a 27°C pulse could reflect changes in the  
51 nuclear accumulation of ELF3. To investigate this, we measured ELF3 (4+) and ELF3 (4-)  
52 nuclear signal at 22°C and 27°C in hypocotyl and root nuclei. As we have reported previously  
53 (Herrero et al., 2012), ELF4 is required for proper nuclear accumulation of ELF3  
54 (**Supplementary Figure 1**). Regardless of the temperature, ELF3 (4-) had a lower nuclear  
55 accumulation than ELF3 (4+) in hypocotyl and root nuclei. The 27°C pulse also strongly

Reduction in ELF3 localization to foci in response to warming temperature



56 reduced the nuclear accumulation of ELF3 (4+) in hypocotyl nuclei (**Supplementary Figure**  
57 **1A**). Furthermore, there was an additive effect of the 27°C pulse and the *elf4-1* mutation on  
58 the nuclear accumulation of ELF3 in hypocotyl nuclei. A similar response to the 27°C pulse  
59 was seen in root nuclei for both ELF3 (4+) and ELF3 (4-) (**Supplementary Figure 1B**). As  
60 with hypocotyl nuclei, the 27°C pulse and the *elf4-1* mutation had an additive effect on ELF3  
61 nuclear accumulation (**Supplementary Figure 1B**). The reduced nuclear accumulation of

## Reduction in ELF3 localization to foci in response to warming temperature

62 ELF3 at 27°C is consistent with a recent report that ELF3 is degraded by BBX18 and  
63 XBAT31 at warm temperatures (Zhang, et al. 2021).

64 In summary, the localization of ELF3 to foci is suppressed by warm temperature and  
65 ELF4 does not seem to regulate this process. Thus, ELF4 must stabilize the function of the  
66 EC at warm temperatures through a separate mechanism (Silva et al., 2020). As the  
67 localization of ELF3 to foci was associated with increased transcriptional activity of ELF3, a  
68 reduction in foci may contribute to the weaker EC function at warm temperatures (Kolmos et  
69 al., 2011, Ezer et al., 2017). We also highlight a recent report that observed ELF3 localizing  
70 to sub-nuclear structures called speckles in response to warming (Jung et al., 2020). Direct  
71 comparisons are complicated because of the different genetic resources and experimental  
72 conditions used, but in the supplementary text we discuss why our results may diverge from  
73 the work of Jung *et al.*, (2020).

74



## Reduction in ELF3 localization to foci in response to warming temperature

75 **Figure 1 – Elevated temperature reduces the association of ELF3 to foci in hypocotyl nuclei.** The  
76 localization of ELF3 at dusk in hypocotyl nuclei of **(A-B)** *35S::YFP:ELF3 elf3-4* or **(C-D)**  
77 *35S::YFP:ELF3 elf3-4/elf4-1* plants. Images were taken at **(A, C)** 22°C or **(B, D)** after a two-hour  
78 27°C pulse started at ZT6 (short-day 8/16 photoperiods). **(E)** Number of foci per nucleus under the  
79 respective treatment. **(F)** Relative change in the number of ELF3 foci following a temperature pulse in  
80 the *elf3-4* or *elf3-4/elf4-1* mutant. Data was made relative to the respective genotype at 22°C. For *elf3-*  
81 *4/elf4-1*, nuclei with no focus were removed this from calculation. Images were collected on two  
82 occasions. Significance was determined by a T-test: n.s = no significance, \*\*\* =  $p < 0.001$ . Scale bars  
83 are 5  $\mu\text{m}$ .

84

85 **Figure 2 – A 27°C pulse inhibits the association of ELF3 to foci in root nuclei.** The localization of  
86 ELF3 at dusk in root nuclei of **(A-B)** *35S::YFP:ELF3 elf3-4* or **(C-D)** *35S::YFP:ELF3 elf3-4/elf4-1*.  
87 Images were taken at **(A, C)** 22°C or **(B, D)** after a two-hour 27°C pulse started at ZT6 (short-day 8/16  
88 photoperiods). **(E)** Number of foci per nuclei under the respective treatment. **(F)** Relative change in  
89 the number of ELF3 foci following a temperature pulse in the *elf3-4* or *elf3-4/elf4-1* background. Data  
90 was made relative to the respective genotype at 22°C. Images were collected on two occasions.  
91 Significance was determined by a T-test: n.s = no significance, \*\*\* =  $p < 0.001$ . Scale bars are 5  $\mu\text{m}$ .

92

93 **Supplementary Figure 1 – Elevated temperature reduces the nuclear accumulation of ELF3.**

94 The relative nuclear signal of ELF3 in **(A)** hypocotyl or **(B)** root nuclei. Nuclear signal was quantified  
95 using the images collected for the foci counts of figure 1 and figure 2, respectively. The nuclear signal  
96 was made relative to the nuclear signal of ELF3 in the *elf3-4* background at 22°C for each respective  
97 tissue type. Images were collected on two separate occasions with a combined n of 12 or more images  
98 analyzed for each respective genotype and temperature treatment. Significance was calculated using a  
99 one-way ANOVA with a tukey-HSD post-hoc test. Different letters signify a significance of  $p < 0.05$ .

**Figure 1**

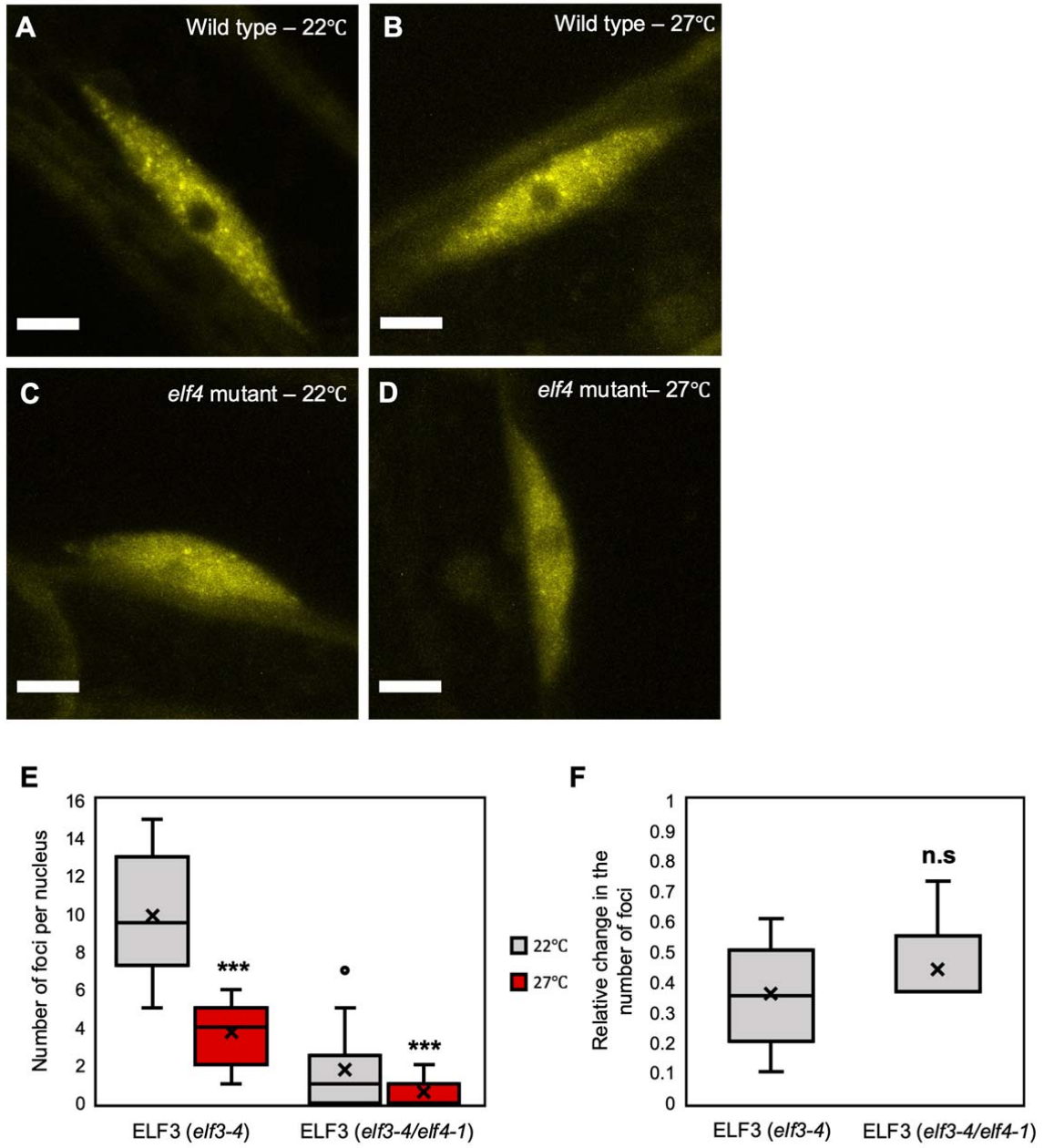
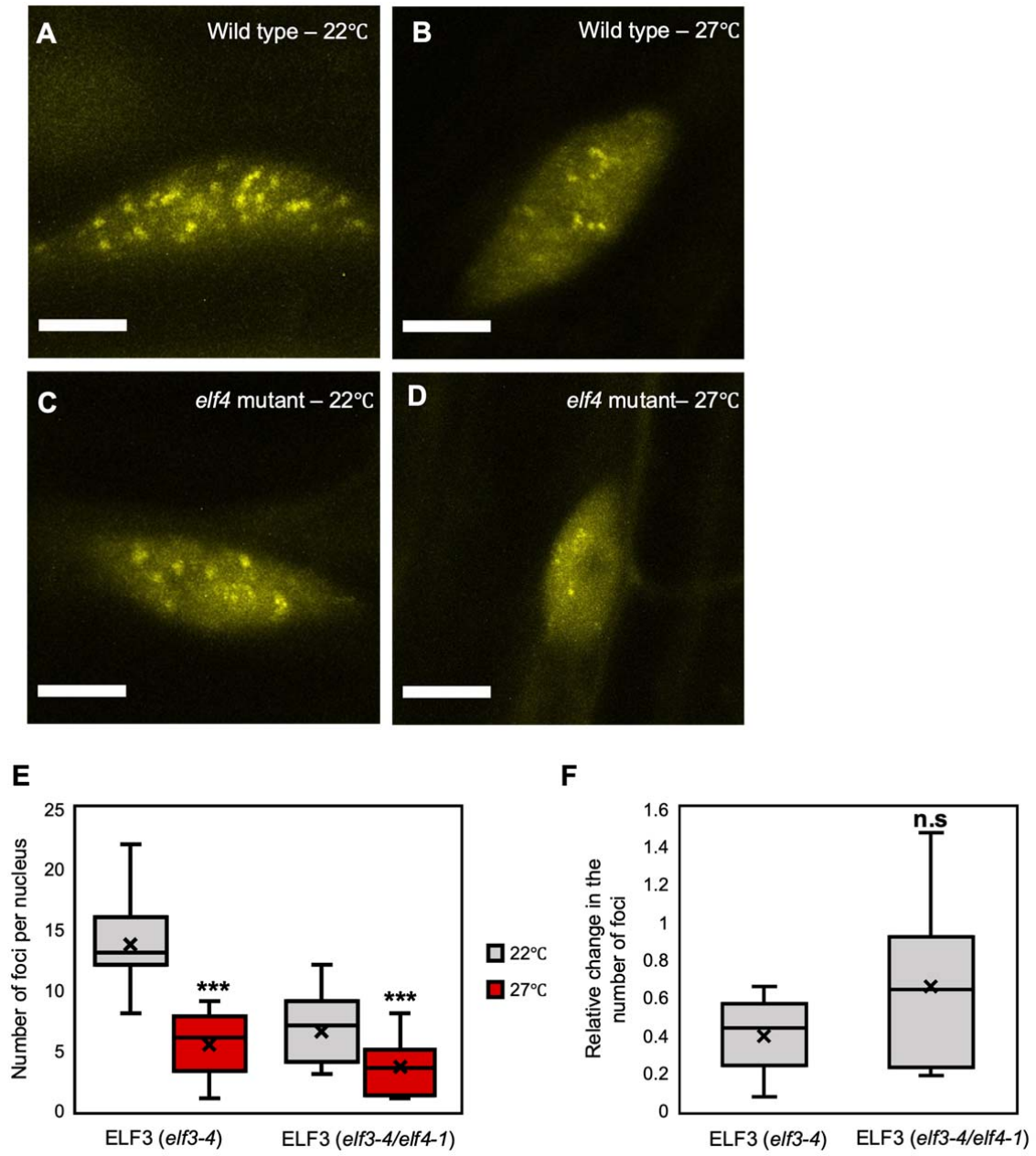
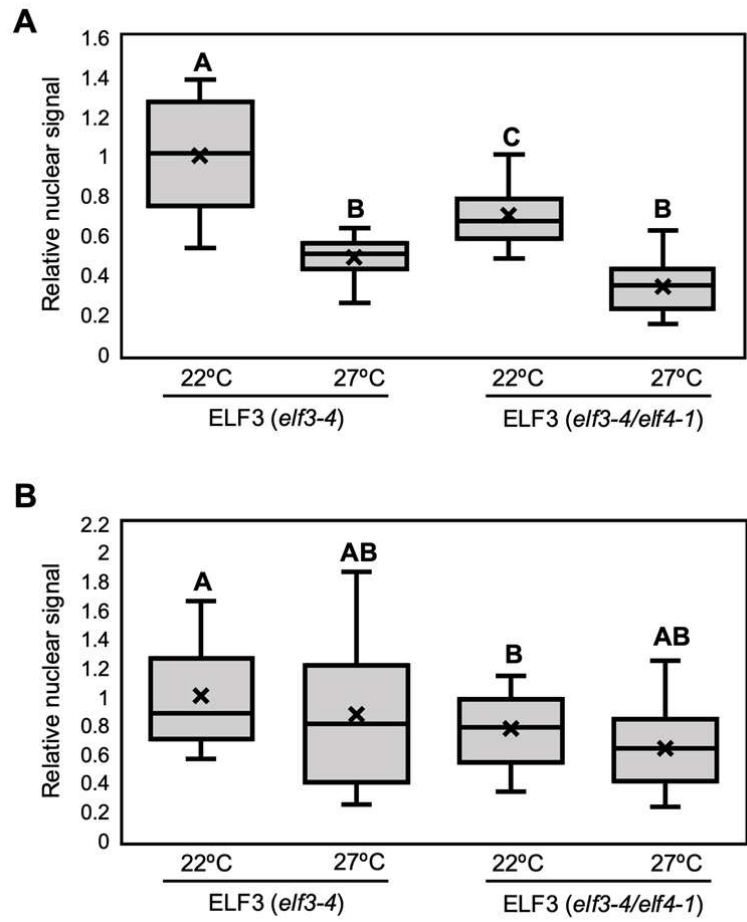


Figure 2



100

101 **Supplementary Figure 1**



- 102 ANWER, M. U., BOIKOGLU, E., HERRERO, E., HALLSTEIN, M., DAVIS, A. M.,  
 103 VELIKKAKAM JAMES, G., NAGY, F. & DAVIS, S. J. 2014. Natural variation  
 104 reveals that intracellular distribution of ELF3 protein is associated with function in  
 105 the circadian clock. *eLife*, 3, e02206.
- 106 CHEN, W. W., TAKAHASHI, N., HIRATA, Y., RONALD, J., PORCO, S., DAVIS, S. J.,  
 107 NUSINOW, D. A., KAY, S. A. & MAS, P. 2020. A mobile ELF4 delivers circadian  
 108 temperature information from shoots to roots. *Nature Plants*, 6, 416-426.
- 109 EZER, D., JUNG, J.-H., LAN, H., BISWAS, S., GREGOIRE, L., BOX, M. S.,  
 110 CHAROENSAWAN, V., CORTIJO, S., LAI, X., STÖCKLE, D., ZUBIETA, C.,  
 111 JAEGER, K. E. & WIGGE, P. A. 2017. The evening complex coordinates  
 112 environmental and endogenous signals in Arabidopsis. *Nature plants*, 3, 17087-  
 113 17087.
- 114 HERRERO, E., KOLMOS, E., BUJDOSO, N., YUAN, Y., WANG, M., BERNS, M. C.,  
 115 UHLWORM, H., COUPLAND, G., SAINI, R., JASKOLSKI, M., WEBB, A.,  
 116 GONÇALVES, J. & DAVIS, S. J. 2012. EARLY FLOWERING4 recruitment of  
 117 EARLY FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock.  
 118 *Plant Cell*, 24, 428-43.
- 119 JUNG, J.-H., BARBOSA, A. D., HUTIN, S., KUMITA, J. R., GAO, M., DERWORT, D.,  
 120 SILVA, C. S., LAI, X., PIERRE, E., GENG, F., KIM, S.-B., BAEK, S., ZUBIETA,  
 121 C., JAEGER, K. E. & WIGGE, P. A. 2020. A prion-like domain in ELF3 functions as  
 122 a thermosensor in Arabidopsis. *Nature*, 585, 256-260.
- 123 KOLMOS, E., HERRERO, E., BUJDOSO, N., MILLAR, A. J., TÓTH, R., GYULA, P.,  
 124 NAGY, F. & DAVIS, S. J. 2011. A Reduced-Function Allele Reveals That  
 125 *EARLY FLOWERING3* Repressive Action on the Circadian Clock Is  
 126 Modulated by Phytochrome Signals in *Arabidopsis*. *The Plant Cell*, 23,  
 127 3230-3246.
- 128 NUSINOW, D. A., HELFER, A., HAMILTON, E. E., KING, J. J., IMAIZUMI, T.,  
 129 SCHULTZ, T. F., FARRÉ, E. M. & KAY, S. A. 2011. The ELF4-ELF3-LUX  
 130 complex links the circadian clock to diurnal control of hypocotyl growth. *Nature*, 475,  
 131 398-402.
- 132 PRESS, M. O., LANCTOT, A. & QUEITSCH, C. 2016. PIF4 and ELF3 Act Independently  
 133 in Arabidopsis thaliana Thermoresponsive Flowering. *PloS one*, 11, e0161791-  
 134 e0161791.
- 135 QUINT, M., DELKER, C., FRANKLIN, K. A., WIGGE, P. A., HALLIDAY, K. J. & VAN  
 136 ZANTEN, M. 2016. Molecular and genetic control of plant thermomorphogenesis.  
 137 *Nature Plants*, 2, 15190.
- 138 RASCHKE, A., IBAÑEZ, C., ULLRICH, K. K., ANWER, M. U., BECKER, S.,  
 139 GLÖCKNER, A., TRENNER, J., DENK, K., SAAL, B., SUN, X., NI, M., DAVIS, S.  
 140 J., DELKER, C. & QUINT, M. 2015. Natural variants of ELF3 affect  
 141 thermomorphogenesis by transcriptionally modulating PIF4-dependent auxin  
 142 response genes. *BMC Plant Biology*, 15, 197.
- 143 SILVA, C. S., NAYAK, A., LAI, X., HUTIN, S., HUGOUVIEUX, V., JUNG, J.-H.,  
 144 LÓPEZ-VIDRIERO, I., FRANCO-ZORRILLA, J. M., PANIGRAHI, K. C. S.,  
 145 NANAO, M. H., WIGGE, P. A. & ZUBIETA, C. 2020. Molecular mechanisms of  
 146 Evening Complex activity in Arabidopsis. *Proceedings of the National Academy of*  
 147 *Sciences*, 117, 6901-6909.
- 148 ZHANG, L. L. SHAO, Y. J. DING, L. WANG, M. J., DAVIS, S. J. & LIU, J. X. (IN PRESS)  
 149 XBAT31 regulates thermoresponsive hypocotyl growth through mediating  
 150 degradation of the thermosensor ELF3 in Arabidopsis. *SCIENCE Advances*

## Parsed Citations