

**Supplemental Figure 1.** Photographs of six-week-old rosette leaves from Col, *var2-1*, *var2*(WT), *var2*(267), *var2*(488), and *var2*(267 488).



**Supplemental Figure 2.** Immunoblot analysis of FtsH proteins in mature leaves from *var2*(WT) and *var2*(488).

Green leaves or green portions of variegated leaves from six-week-old seedlings were used in this experiment. Immunoblot analysis was performed exactly as shown in Figure 3C. FtsH2 and FtsH5 antibodies were used for immunodetection; equal loading of lanes was verified by an internal control (CBB-stained LHCII). Numbers after *var2*(WT), *var2*(267), and *var2*(488) denote individual transgenic lines. Experiments were repeated three times.

## Supplemental Data. Zhang et al. Plant Cell. (2010). 10.1105/tpc.110.079202



**Supplemental Figure 3**. *In vivo* D1 degradation assay from independent *var2*(WT) and *var2*(488) transgenic lines.

Protease activity was estimated from immunoblot analysis of the remaining D1 after high-light illumination (1200  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>). Leaf discs harvested from fully expanded mature leaves were used for this study, as described in the *Methods* section. Four representative immunoblots are shown here for different incubation periods (1 h, 2 h, and 3 h with lincomycin, and 2 h without lincomycin as a control). At each time period, proteins were extracted simultaneously from high-light irradiated (denoted as 1, 2, or 3 on the immunoblot) and control leaf discs (not incubated under high light, denoted as 0 on the immunoblot). Samples were loaded side by side on the immunoblot to compare D1 levels. The CBB-stained LHCII was used as an internal control to calculate the remaining D1 level. Estimation of D1 levels was performed similarly, as shown in Figure 5. The ratio at 0 min was adjusted as 1, and relative ratios are shown. D1 levels quantified from five independent experiments (bar indicates SD, *n*=5) are indicated.



**Supplemental Figure 4.** Effect of low temperature on the variegated phenotype of *var1 var2*(WT) and *var1 var2*(488).

Plants were grown on MS agar plates at 17°C for 4 weeks and photographed. As reported previously (Sakamoto et al., 2002), the variegated phenotype of *var2*, but not that of *var1*, was enhanced at low temperature. Images for *var1 var2*(488) show enhanced variegation like that of *var2*, whereas *var1 var2*(WT) does not.



**Supplemental Figure 5.** Estrogen-dependent accumulation of FtsH2 in independent pER8-*var*2(WT) lines.

Transgenic pER8-*var2*(WT) lines were grown on MS plates containing 0, 5, 10, 50, and 100  $\mu$ M 17- $\beta$ -estradiol (indicated on the top). Plants at 14 days after sowing were collected and subjected to protein extract and immunoblot analysis (normalized by fresh weight). Immunoblots from three independent lines #13 (top), #12 (middle), and #3 (bottom) reacted with anti-FtsH2 antisera are shown along with CBB-stained gels. The band corresponding to LHCII is indicated by arrowheads. In each blot, extracts from Col and *var2-1* were included as controls. Experiments were performed with four biological repeats. A similar immunoblot normalized by chlorophyll content was also performed; the result from #12 line is presented in Fig. 9E.

Supplemental Table 1. List of primers used for this study.

Primer	Sequence	Gene/Region
KT204	5'-GTGAGAAACATATTAAAAGCCTGT-3'	VAR2
KT402	5'-CGGTTTGAGTGTTGCAAATCTGGACAAT-3'	VAR2
KT304	5'-TCACGATTGTTCTTTATGTGGCTTAG-3'	VAR2
SQ102F	5'-TATACTGCGTTAGGTGCTA-3'	VARI
SQ110R	5'-GAAATCATTTGATGCTCCGG-3'	VARI
SQ103F	5'-GTAGCCGGAAGAGTTCAGATC-3'	VARI
F2-102	5'-GGTGCATCAAGGGTCCGCGATCTTTTCAAA-3'	FtsH8
F2-101	5'-TTTGATGATATTGCAGTCTTTCCACGGCGG-3'	FtsH8
TMRI-LB2	5'-CCTATTATATCTTCCCAAATTACCAATACA-3'	T-DNA left border
ZnBD-A1	5'-TAGCTTAC <u>GC</u> TGAAGTTGGTCATGCAGTGT-3'	FtsH2(488), site-directed
		mutagenesis
G267D-A1	5'-TTCTTATTG <u>A</u> TCCTCCTGGTACCGGGAA-3'	FtsH2(267), site-directed
		mutagenesis
H417L	5'-AATCGACGGCTTACCTCGAAGCGGGTCATG-3'	E. coli ftsH, site-directed
		mutagenesis
G195D	5'- AAGGCGTCTTGATGGTCG <u>A</u> TCCTCCGGGTA-3'	E. coli ftsH, site-directed
		mutagenesis