Expanded View Figures

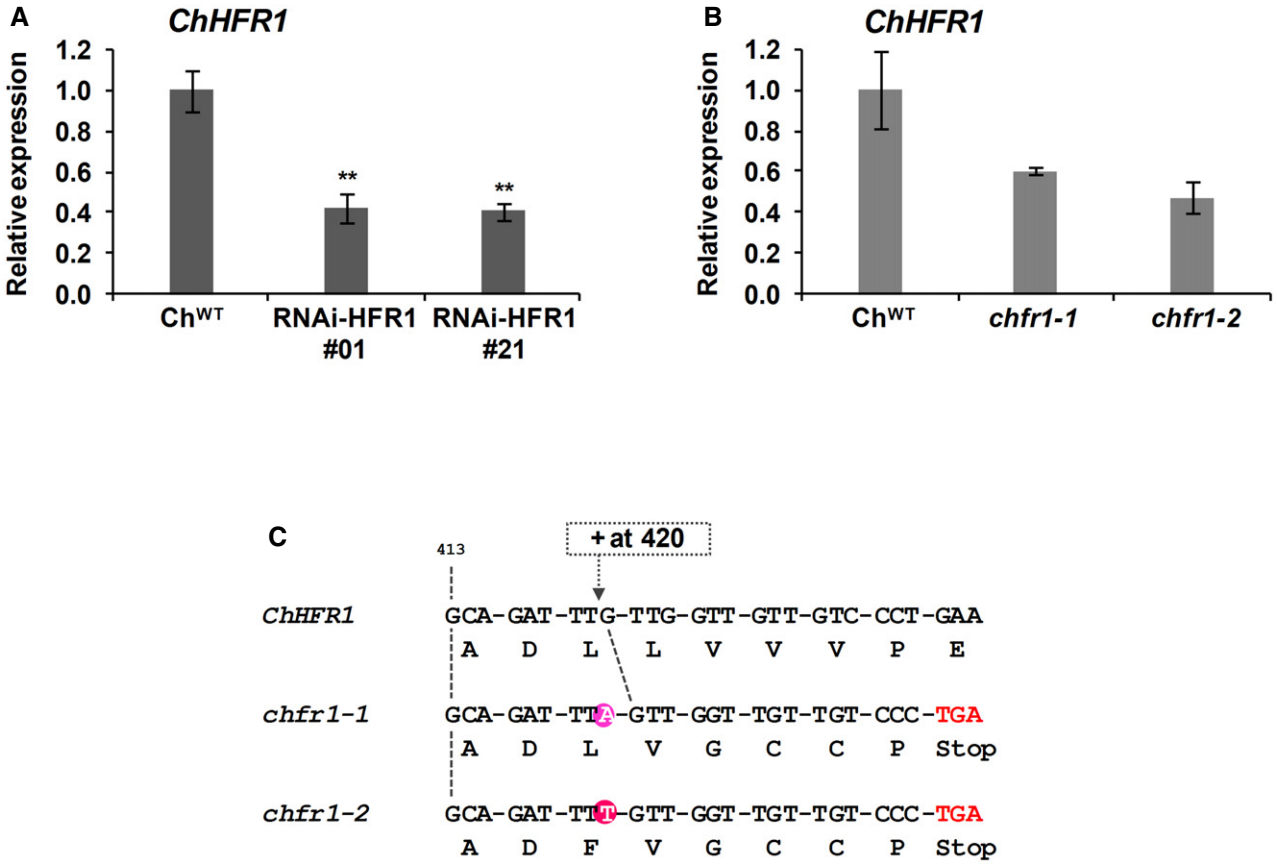

Figure EV1. Characterization of RNAi-HFR1 and *chfr1* mutants in *Cardamine hirsuta*.

A, B Relative expression levels of *ChHFR1* gene, normalized to *EF1α* in Ch^{WT}, (A) two RNAi-HFR1 lines (#01 and #21) and (B) the two *chfr1* mutants of *C. hirsuta*. Seedlings were grown for 7 days in W. Expression values are the mean ± SE of three independent biological replicates relative to Ch^{WT}. Asterisks mark significant differences (Student's *t*-test: $^{**}P$-value < 0.01) relative to Ch^{WT}.

C The two identified *chfr1-1* and *chfr1-2* mutants have one nucleotide insertion at position 420 of the *ChHFR1* ORF, which leads to a frame shift and a premature stop codon.

Figure EV2. Alignments of *HFR1*, *PIF4*, *PIF5*, and *PIF7* partial DNA sequences in *Arabidopsis thaliana* and *Cardamine hirsuta*.

A Location of shared primers and amplicons used for comparison of expression levels by RT-qPCR between species.

B Transcript abundance of *PIF4* and *PIF5*, normalized to *YLS8*, *SPC25*, and *EF1α* in Ch^{WT} and At^{WT} grown as in Fig 2. Expression values are the means ± SE of three independent biological replicates relative to the data of At^{WT} grown in continuous W at day 3. Asterisks mark significant differences (2-way ANOVA: $^{**}P$-value < 0.01, $^{***}P$-value < 0.001) between Ch^{WT} and At^{WT} when grown under W (black asterisks) or W + FR (red asterisks).

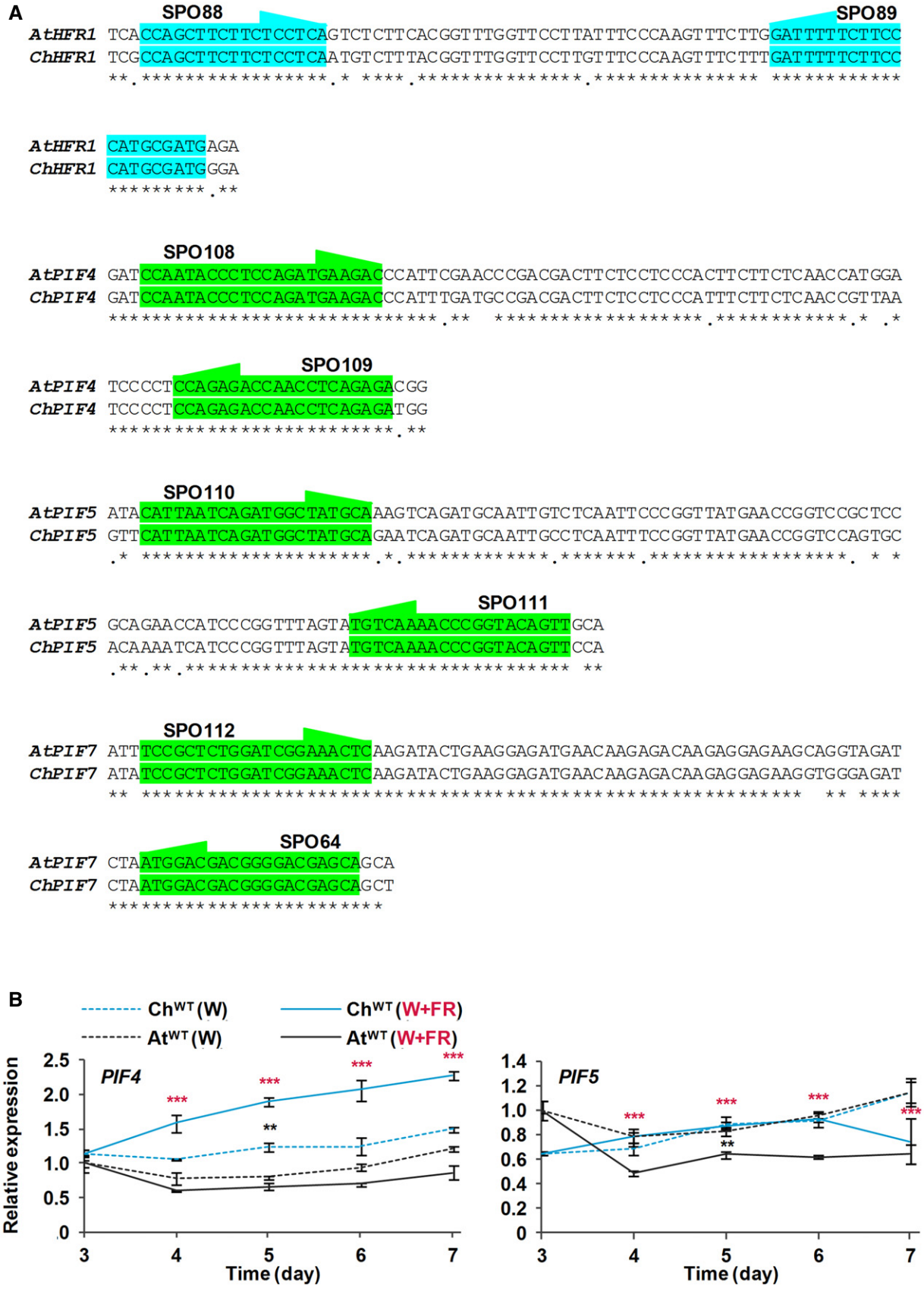

Figure EV2.

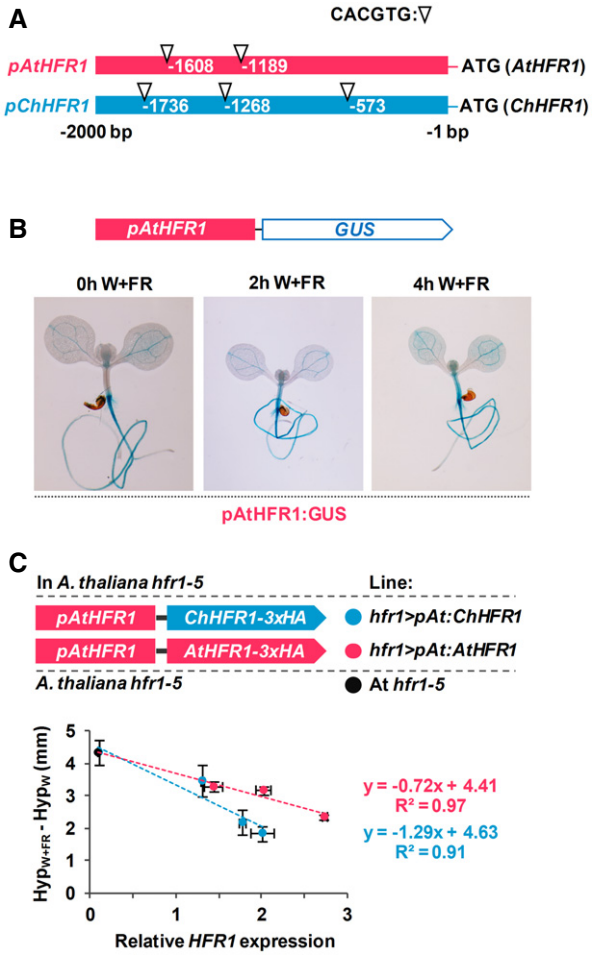

Figure EV3. *ChHFR1* and *AtHFR1* complement the *Arabidopsis thaliana hfr1-5* mutant long hypocotyl phenotype.

A Cartoon of *HFR1* promoters from *A. thaliana* (*pAtHFR1*) and *C. hirsuta* (*pChHFR1*). These promoters cover 2,000 bp from the beginning of the translation start of the two *HFR1* genes. The positions of G-boxes (CACGTG) are indicated with arrows.

B GUS staining of representative *A. thaliana* seedlings expressing *GUS* under the *pAtHFR1* (line #03). Seven-day-old W-grown seedlings were treated with W + FR for the indicated amount of time.

C Correlation between Hyp_{W+FR}-Hyp_W (means ± SE of at least four biological replicates, data shown in Fig 3C) and relative levels of *ChHFR1* or *AtHFR1* expression (means ± SE of three biological replicates, data shown in Fig 3B). The estimated regression equations and the R^2 values are shown for each plot.

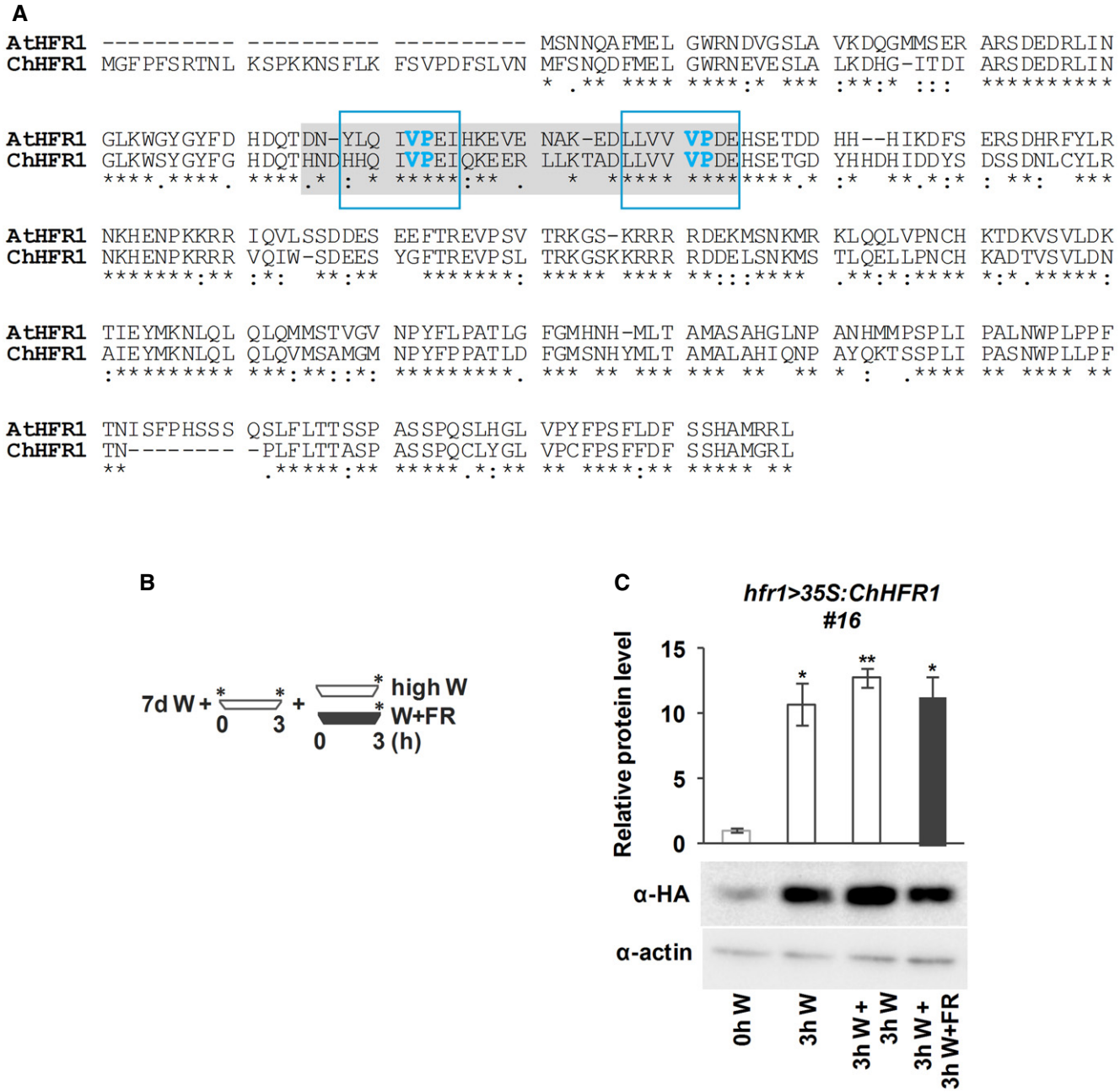

Figure EV4. ChHFR1 protein accumulates in high W.

A Alignment of AtHFR1 and ChHFR1 protein sequences. Putative COP1 interacting motifs, defined in AtHFR1, are highlighted with a light gray box. VP motifs are highlighted with blue letters. Amino acid sequences inside the blue line rectangles correspond to the synthetic AtHFR1, ChHFR1, and At/ChHFR1 VP peptides used in the microscale thermophoresis assays (Appendix Table S3).

B Cartoon representing the light treatments given to seedlings to estimate relative HFR1-3xHA levels. Seedlings grown for 7 days in low W (~ 20 $\mu mol/m^2 \cdot s^1$, R:FR $\approx$ 6.4) were first moved to high W (~ 100 $\mu mol/m^2 \cdot s^1$, R:FR $\approx$ 3.9) for 3 h and then either transferred to high W (control) or high W + FR (R:FR $\approx$ 0.06) for 3 h. Seedling samples were collected at the time points indicated with asterisks.

C Relative HFR1-3xHA protein levels of *hfr1>35S:ChHFR1* seedlings (line #16) grown as indicated in B, with a representative immunoblot in a lower panel. Relative protein levels are the mean ± SE of three independent biological replicates relative to the data point of 0 h in high W (0 h W). Asterisks mark significant differences in protein levels (Student's *t*-test: ***P*-value < 0.01; **P*-value < 0.05) relative to the 0 h W value.

Source data are available online for this figure.

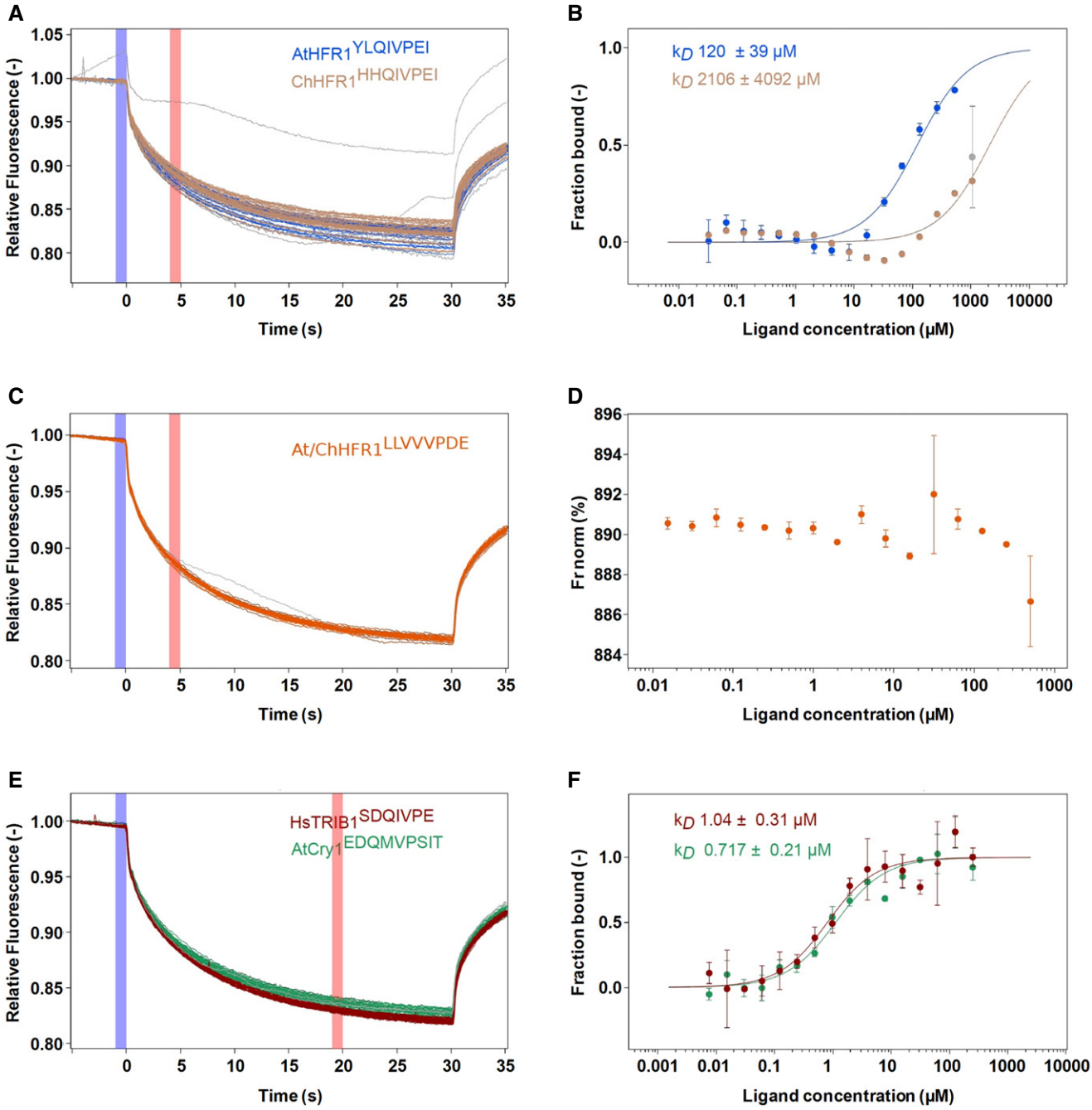

Figure EV5. Microscale thermophoresis (MST) experimental traces and analysis.

A–F Raw MST traces and analysis of AtCOP1 WD40 with different peptides in triplicates (duplicates for At/ChHFR1 VP). The concentration of AtCOP1 WD40 is fixed at 0.15 µM mixed with 16 serially diluted peptide concentrations at 1:1 ratio. Panels A, C, and E show the normalized MST traces. The blue box area illustrates the fluorescence before activation of the infrared (IR) laser and red box area illustrates average fluorescence after activation of the IR laser. Average values ± SD (error bars) were subsequently used for fluorescence normalization. k_D fit displaying fraction bound as a function of ligand concentration is shown in adjacent right panels B, D, and F. (A) Raw MST traces for AtHFR1 (in blue) and ChHFR1 (in light-brown) VP peptides. Individual concentrations that showed slight aggregation or precipitation are shown in gray and were excluded from the k_D fit calculation. (B) Fitted data over a concentration range from 0.032 to 500 µM for AtHFR1 VP (blue dots) and 0.032 to 1,000 µM for ChHFR1 VP (light-brown dots) were used to derive the corresponding dissociation constant k_D. (C) Raw MST traces for At/ChHFR1 VP peptide (in orange). One concentration that showed slight precipitation or aggregation is shown in gray. A concentration range of 0.0154 to 506 µM was used for the At/ChHFR1 VP. (D) No k_D was determined, as no binding between COP1 WD40 and the At/ChHFR1 VP peptide (orange dots) was detected. (E) A concentration range from 0.0076 to 250 µM for HsTRIB1 (in red) and AtCRY1 (in green) peptides was used. Raw MST traces show no aggregation or precipitation effects during this binding. One AtCRY1 VP outlier is shown in gray. (F) The k_D for HsTRIB1 (brown dots) and AtCRY1 (green dots) VP peptides was calculated using the normalized traces.