APPENDIX PDF

Adaptation to plant shade relies on rebalancing the transcriptional activity of the PIF7-HFR1 regulatory module.

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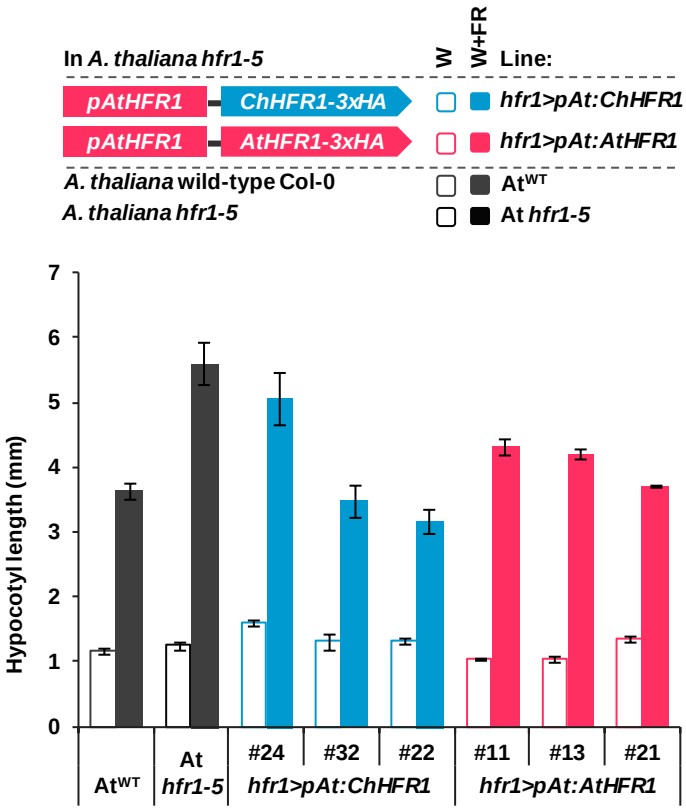
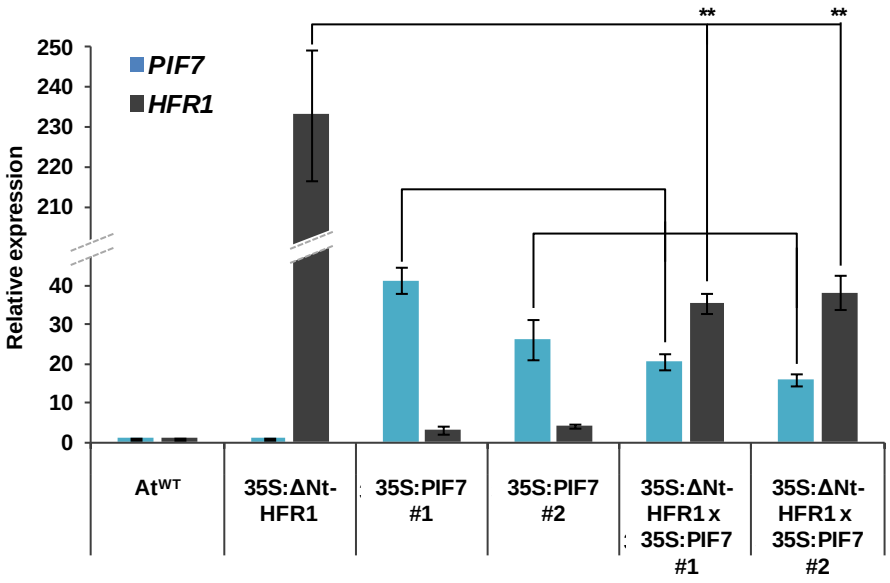
1. APPENDIX FIGURE S1.



Appendix Figure S1. ChHFR1 and AtHFR1 complement the *A. thaliana hfr1-5* mutant long hypocotyl phenotype. Hypocotyl length of the shown lines grown as indicated in Fig 3C. Values were used to generate data on Fig 3C.

2. APPENDIX FIGURE S2.



Appendix Figure S2. Relative expression levels of *AtHFR1* and *AtPIF7* genes in transgenic lines overexpressing *GFP-ΔNt-HFR1* and/or *PIF7-CFP*. Relative expression, normalized to *UBQ10*, was estimated in seedlings grown for 7 days in W. Expression values are the mean ± SE of three independent biological replicates relative to At^{WT}. Asterisks mark significant differences in expression (Student *t*-test: ** p-value <0.01; * p-value <0.05) relative to 35S:GFP-ΔNt-HFR1-GFP or 35S:PIF7-CFP values.

3. APPENDIX SUPPLEMENTARY METHODS.

Generation of RNAi-HFR1 plants of *C. hirsuta*

To generate an RNAi construct for silencing of the endogenous *ChHFR1*, a fragment of 222 bp was PCR amplified using primers CTO35 + CTO36 (Appendix Table S2) and cDNA of 7-day old *C. hirsuta* seedlings grown 1 h under W+FR. This partial fragment of *ChHFR1* (ptChHFR1) was cloned into pCRII-TOPO (Invitrogen, www.thermofisher.com) to generate pCT17, which was confirmed by sequencing. An *Eco*RI fragment of pCT17 was subcloned into pENTR3C vector (Invitrogen), to create the Gateway entry clone pCT19 (to have ptChHFR1 flanked with attL1 and attL2, attL1<ptChHFR1<attL2). Recombination of pCT19 with the destination vector pB7GWIWG2(I), which contains attR1 and attR2 sites, using Gateway LR Clonase II (Invitrogen), gave pCT33 (35S:attB1<RNAi-ChHFR1<attB2). This plasmid is a binary vector conferring resistance to the herbicide phosphinothricin (PPT) in plants and the antibiotic spectinomycin in bacteria. *Agrobacterium tumefaciens* strain $C_{58}C_1$ (pGV2260) was transformed with pCT33 by electroporation and colonies were selected on solid YEB medium with rifampicin (100 µg/mL), kanamycin (25 µg/mL) and spectinomycin (100 µg/mL). Wild-type *C. hirsuta* (Ox, Ch^{WT}) plants were transformed by floral dipping and transgenic seedlings were selected on 0.5xGM- medium (Roig-Villanova *et al*, 2006) containing 50 µg/mL PPT. Transgene in seedlings of T1 generation was verified by PCR genotyping using specific primers. Plants homozygous for the transgene were finally used for experiments.

Isolation of *HFR1* mutants of *C. hirsuta*

To obtain loss-of-function mutants of *ChHFR1* in *C. hirsuta* (named as *chfr1*) we employed the CRISPR-Cas9 gene editing system (Morineau *et al*, 2017). The guide RNA targeting ChHFR1 ($gRNA_{ChHFR1}$, 5'-GTT-GAA-GAC-TGC-AGA-TTT-GT-3') was synthesized to be under the control of the *A. thaliana U6* promoter (pU6) sequence and flanked by the Gateway attB1 and attB2 recombination sites (IDT, https://eu.idtdna.com/pages) (attB1<pU6:$gRNA_{ChHFR1}$<attB2). This sequence was recombined with the vector pDONR207 using Gateway BP Clonase II (Invitrogen) to generate the entry vector pSP101 (attL1<pU6:$gRNA_{ChHFR1}$<attL2). In a recombination reaction of pSP101 with pDE-Cas9 (Fauser *et al*, 2014) using Gateway LR Clonase II, a binary vector pSP102 was created (attB1<pU6:$gRNA_{ChHFR1}$<attB2, Cas9). This vector, that contains the information to target ChHFR1, confers resistance to PPT in plants and spectinomycin in bacteria. *A. tumefaciens* strain $C_{58}C_1$ (pGV2260) was transformed with pSP102 by electroporation and colonies were selected on solid YEB medium with antibiotics, as indicated before for pCT33. Wild-type *C. hirsuta* (Ox, Ch^{WT}) plants were transformed by floral dipping and resistant transgenic seedlings were selected on 0.5xGM- medium containing PPT (30 µg/mL). These T1 seedlings were PCR genotyped using primers MJO27 and MJO28 (Appendix Table S2) to detect the presence of the transgene. In the following T2 generation, a total of six seedlings with a *sis* phenotype from 1 independent transgenic line were selected and grown to maturity. An *HFR1* fragment of 664 bp around the $gRNA_{ChHFR1}$ target sequence was amplified by PCR from gDNA of each plant using primers CTO29 + CTO36 (Appendix Table S2). Sequencing of these fragments indicated the presence of

mutations in the *ChHFR1* gene. Descendants of these plants (T3 generation) were reselected in shade and sequenced to confirm the unambiguous presence of the mutated *chfr1* alleles. In the T4 generation, seedlings sensitive to PPT (indicating the loss of T-DNA insertion) were selected, which resulted in the isolation of the *chfr1-1* and *chfr1-2* mutant allele lines (Fig EV1). The wild-type and these mutant alleles were genotyped by PCR using primers SPO104 + SPO107 (for *ChPIF7*), SPO105 + SPO107 (for *chfr1-1*) and SPO106 + SPO107 (for *chfr1-2*) (Appendix Table S2).

Generation of *A. thaliana hfr1-5* transgenic lines expressing *AtHFR1* or *ChHFR1* under the control of different promoters

We amplified a 2 kbp fragment of *AtHFR1* promoter starting immediately before the ATG of *AtHFR1* gene using gDNA of *A. thaliana* wild-type Col-0 (At^{WT}) as a template and primers SPO26 + SPO27 (Appendix Table S2). This fragment was subcloned into pCRII-TOPO to generate pSP51. From the different clones analyzed, the best one was pSP51.10, with three 1 bp-deletions in the amplified region, none affecting the G-boxes, known to be necessary for PIF binding.

AtHFR1 coding sequence was amplified from pJB30 (Galstyan *et al*, 2011) using primers RO25 + SPO30 (Appendix Table S2), which removed the stop codon and introduced a *Xho*I site at the N-terminal site. After subcloning this fragment into pCRII-TOPO, which gave pSP54 (*AtHFR1*), the insert was sequenced to confirm its identity. The 3xHA fragment was amplified from plasmid pEN-R2-3xHA-L3 (Karimi *et al*, 2007) and primers SPO31 (which added a *Sal*I site) + SPO32 (which added a *Xho*I site, Appendix Table S2). This fragment was subcloned into pCRII-

TOPO to generate pSP55 (*3xHA*), whose insert was sequenced to confirm its identity. A *BamH*I-*Xho*I fragment of pSP54 was subcloned into pSP55 digested with *BamH*I and *Sal*I to generate pSP57 (*AtHFR1-3xHA*). A *BamH*I-*Xho*I fragment of pSP57 was subcloned into the same sites of pENTR3C vector which gave pSP59. This plasmid contained *AtHFR1-3xHA*, with an extra *Xba*I site in the C-terminus end, flanked with attL1 and attL2 sites (attL1<*AtHFR1-3xHA*^{XbaI}<attL2). *Xba*I restriction site in pSP59 was removed by filling the site with Klenow enzyme after digestion, and religation to generate pSP84 (attL1<*AtHFR1-3xHA*<attL2). Recombination of pSP84 with the binary vector pIR101 (attR1<*ccdB*<attR2) (Molina-Contreras *et al*, 2019) (using Gateway LR Clonase II) resulted in pSP88 (attB1<*AtHFR1-3xHA*<attB2). An *Xba*I fragment of pSP51 was subcloned into the same site of pSP88 which gave pSP90 (*pAtHFR1*:attB1<*AtHFR1-3xHA*<attB2). This binary vector confers resistance to spectinomycin in bacteria and PPT in plants.

ChHFR1 CDS was amplified using cDNA from wild-type *C. hirsuta* (Ox, Ch^{WT}) seedlings and primers SPO28 + SPO29 (Appendix Table S2), which removed the stop codon and introduced a *Xho*I site. This PCR product was subcloned into pCRII-TOPO to generate pSP53 (*ChHFR1*). Selected colonies were sequenced to confirm their identity. A *BamH*I-*Xho*I fragment of pSP53 was subcloned into pSP55 digested with *BamH*I-*Sal*I to generate pSP56 (*ChHFR1-3xHA*). A *BamH*I-*Xho*I fragment of pSP56 was subcloned into the same site of pENTR3C vector, which gave pSP58. This plasmid contained *ChHFR1-3xHA*, with an *Xba*I site in the C-terminus end, flanked with attL1 and attL2 sites (attL1<*ChHFR1-3xHA*^{XbaI}<attL2). *Xba*I restriction site in pSP58 was removed by

filling the site with Klenow enzyme after digestion, and religation to generate pSP83 (attL1<*ChHFR1-3xHA*<attL2). Recombination of pSP83 with the binary vector pIR101 using Gateway LR Clonase II resulted in pSP87 (attB1<*ChHFR1-3xHA*<attB2). An *Xba*I fragment of pSP51 was subcloned into the same site of pSP87 which gave pSP89 (*pAtHFR1*:attB1<*ChHFR1-3xHA*<attB2). This binary vector confers resistance to spectinomycin in bacteria and PPT in plants.

To overexpress *ChHFR1*, a *BamH*I-*Xho*I fragment of pSP58 was subcloned into the *BamH*I-*Sal*I digested pCAMBIA1300 based pCS14 (Sorin *et al*, 2009) to generate pSP81 (*35S:ChHFR1-3xHA*). This binary vector confers resistance to kanamycin in bacteria and hygromycin in plants.

A. thaliana hfr1-5 plants were transformed with pSP81, pSP89 and pSP90, as previously described. Transgenic seedlings were selected on 0.5xGM- medium with PPT (15 µg/mL) or hygromycin (30 µg/mL), verified by PCR genotyping using specific primers. Homozygous transgenic plants with 1 T-DNA insertion were finally used for experiments.

Generation of constructs for transient expression in *N. benthamiana* leaves

To overexpress *ChHFR1* and *AtHFR1* in *N. benthamiana*, a Gateway vector was created using pCAMBIA1302 (*35S:mGFP5*) as a backbone. An *Nsi*I-*Hind*III fragment of pEarlyGate 100 (*35S*:attR1<*ccdB*<attR2) (Earley *et al*, 2006) was subcloned into pCAMBIA1302 digested with *Pst*I-*Hind*III, which gave pSP135 (*35S*:attR1<*ccdB*<attR2, *35S:mGFP5*). Recombination of pSP58 and pSP59 (both linearized with *Nhe*I) with the binary vector pSP135 using Gateway LR Clonase II gave pSP141 (*35S*:attB1<*ChHFR1-3xHA*<attB2, *35S:mGFP5*) and pSP142

(*35S*:attB1<At*HFR1-3xHA*<attB2, *35S:mGFP5*), respectively. These two binary vectors also overexpress mGFP5 and confer resistance to kanamycin in bacteria.

To generate constructs overexpressing *ChHFR1* and *AtHFR1* with the COP1 binding domains exchanged (Fig 5C), we employed a PCR-based mutagenesis. Using pSP90 as a template, a fragment of 205 bp was amplified with RO25 and SPO126 primers, and a larger fragment of 821 bp was amplified using SPO127 and SPO32 primers. Both PCR fragments were used to amplify *AtHFR1* with the COP1 binding domain from *ChHFR1* (named in here as *AtHFR1**). The resulting fragment was subcloned into pCR8/GW/TOPO (Invitrogen) to generate pSP130, which was confirmed by sequencing. Using pSP89 as a template, a fragment of 291 bp was amplified with SPO28 and SPO128 primers, and a fragment of 800 bp was amplified using SPO129 and SPO32. Both PCR fragments were used to amplify *ChHFR1* with the COP1 binding domain from *AtHFR1* (named in here as *ChHFR1**). The resulting fragment was subcloned into pCR8/GW/TOPO to generate pSP131, which was confirmed by sequencing. Recombination of pSP130 and pSP131 with the binary vector pSP135 using Gateway LR Clonase II gave pSP132 (*35S*:attB1<*AtHFR1*-3xHA*<attB2, *35S:mGFP5*) and pSP133 (*35S*:attB1<*ChHFR1*-3xHA*<attB2, *35S:mGFP5*), respectively. Both vectors also overexpress mGFP5 and confer resistance to kanamycin in bacteria.

N. benthamiana plants were agroinfiltrated with the *A. tumefaciens* (strain GV3101) transformed with pSP141, pSP142, pSP132 or pSP133, and the same strain expressing the HcPro protein (Vilela *et al*, 2013) and kept in the greenhouse under long-day photoperiods. Samples were taken 3 days after agroinfiltration.

Generation of constructs for the Yeast 2 Hybrid (Y2H) assays

AtPIF7 CDS was amplified using cDNA of *A. thaliana* wild-type Col-0 (At^{WT}) seedlings and primers JO414 + JO415 (Appendix Table S2), which removed the STOP codon and introduced a *Xho*I site. This PCR product was subcloned into pCRII-TOPO to generate pRA1 (*AtPIF7*). The insert was sequenced to confirm its identity. A *Xho*I fragment of pRA1 was subcloned into pSP55 digested with *Sal*I to generate pRA2 (*AtPIF7-3xHA*). An *Eco*RI fragment of pRA2 was subcloned into the same site of pENTR3C entry vector which gave pRA3 (attL1<*AtPIF7-3xHA*<attL2). This PIF7-3xHA had a stop codon immediately before the ATG, which prevented from cloning it in frame with the yeast derived proteins. Therefore, the *PIF7-3xHA* gene was PCR amplified using pRA3 as a DNA template and primers BAO4 + BAO5 (Appendix Table S2) to add attB1 and attB2 sequences (attB1<*AtPIF7-3xHA*<attB2). This fragment was recombined with pDONR207 using Gateway BP Clonase II to obtain pBA7 (attL1<*AtPIF7-3xHA*<attL2). The insert was sequenced to confirm its identity. In a recombination reaction of pBA7 and pGBKT7-GW (Chini *et al*, 2009) which contained the Gal4 DNA-binding domain (BD, attR1<ccdB<attL2; it confers Trp auxtrophy), and pBA7 and pGADT7-GW (Chini *et al*, 2009) which contained the Gal4 activation domain (AD, attR1<ccdB<attL2; it confers Leu auxtrophy), using Gateway LR Clonase II, pBA10 (BD-attB1<*AtPIF7-3xHA*<attB2) and pBA11 (AD-attB1<*AtPIF7-3xHA*<attB2) were obtained. These plasmids allowed expressing the fusion BD-PIF7-3xHA or AD-PIF7-3xHA proteins under the *ADH1* promoter in yeast, respectively.

Protein expression and purification for the MST experiments

Sf9 cells were cultured in HyClone SFX-Insect Cell Culture Media. The codon optimized COP1 gene (residues 349-765 corresponding to the WD40 domain) for expression in Sf9 cells, was PCR amplified and cloned into a modified pFastBac (Geneva Biotech) insect cell expression vector using Gibson assembly (Gibson *et al*, 2009). The final construct contained a tandem N-terminal His10-Twin-Strep-tags, a TEV (tobacco etch virus protease) cleavage site prior to COP1 WD40 coding sequence in the pFastBac vector. This construct was transformed into DH10MultiBac cells (Geneva Biotech). White colonies, implying successful recombination, were selected and bacmids were purified by the alkaline lysis method. Sf9 cells were transfected with the bacmid using Profectin (AB Vector). eYFP-positive cells (P0) were observed after 1 week and subjected to two rounds of viral amplification. Sf9 cells at a density of 1–2 x 10^6 cells·ml^{-1} were infected with amplified P2 virus at a Multiplicity of infection (MOI) between 2 to 3. Infected Sf9 cells were grown for 72 h at 28°C and 110 rpm. The cell pellet was then harvested by centrifugation at 2000 x g for 15 min, pellets were flash frozen and stored at –20°C.

Pellets from one liter of Sf9 cell culture were dissolved in 25 ml of buffer A (20 mM HEPES pH 7.5, 300 mM NaCl, 2 mM β-ME), supplemented with 10% [v/v] glycerol, a pinch of DNase, and 1 Roche cOmplete^{TM} protease inhibitor tablet. Dissolved pellets were lysed by sonication and centrifuged at 60,000 x g for 45 minutes at 4°C. The supernatant was consecutively filtered through 2-µm 1-µm and 0.45-µm filters prior to loading onto Ni^{2+}-affinity column (HisTrap excel, GE Healthcare). After the loading, Ni^{2+}-affinity column was washed with buffer A and

eluted directly onto a coupled Strep-Tactin Superflow XT column (IBA) using buffer B (20 mM HEPES pH 7.5, 500 mM NaCl, 500 mM imidazole, 2 mM β-ME). The Strep-Tactin column was washed with buffer A and COP1 was eluted with 1x Buffer BXT (IBA) supplemented with 2 mM β-ME. It was cleaved overnight at 4°C with TEV protease and subsequently purified from the protease and affinity tag by a second Ni^{2+} affinity column. COP1 WD40 was concentrated to 10 µM and was labeled immediately.

GUS lines

Transgenic lines expressing GUS were based on a modified pIR101 plasmid (Molina-Contreras *et al*, 2019) which contains the reporter *GUS* gene in a promoterless context (attB1<*GUS*<attB2). *Xba*I fragment of pSP51 was subcloned into the same site of modified pIR101 to give pSP86 (*pAtHFR1*:attB1<*GUS*<attB2). This binary vector confers resistance to spectinomycin in bacteria and PPT in plants. *A. thaliana* wild-type Col-0 (At^{WT}) plants were transformed with this construct as described previously.

GUS staining

Histochemical GUS assays were done as described (Roig-Villanova *et al*, 2006), incubating seedlings at 37ºC without ferricyanide/ferrocyanide.

4. APPENDIX TABLE S1. Primers used for gene expression analyses. Primers BO40 and BO41 for amplifying *UBQ10* (Sorin *et al*, 2009), SPO102 and SPO103 (*AtEF1α* and *ChEF1α*), SPO113 and SPO114 (*AtSPC25* and *ChSPC25*), and SPO115 and SPO116 (*AtYLS8* and *ChYLS8*) have been described before (Molina-Contreras *et al*, 2019).

Gene	Primer name	Sequence (5' – 3')
ChEF1α	CTO9 (F)	GGCCGATTGTGCTGTCCTTA
	CTO10 (R)	TCACGGGTCTGACCATCCTTA
ChHFR1	CTO13 (F)	CGGCGTCGTGTCCAGATC
	CTO14 (R)	TGAACCTTTTCGCGTCAGTG
ChPIL1	CTO17 (F)	GAAGACCCCAAAACAACGGTT
	CTO18 (R)	CCCTCATCGTACTCGGTCTCA
ChYUC8	CTO51 (F)	TTACGCCGGGAAAAAAGTTCT
	CTO52 (R)	GCGAAATGGTTGGCTAGGTC
ChXTR7	CTO69 (F)	TGGTGTTCCTTTCCCAAAAAA
	CTO70 (R)	CCACCTCTCGTAGCCCAATC
AtHFR1, ChHFR1	SPO88 (F)	CCAGCTTCTTCTCCTCA
	SPO89 (R)	CATCGCATGGGAAGAAAAATC
AtPIF4, ChPIF4	SPO108 (F)	CCAATACCCTCCAGATGAAGAC
	SPO109 (R)	TCTCTGAGGTTGGTCTCTGG
AtPIF5, ChPIF5	SPO110 (F)	CATTAATCAGATGGCTATGCA
	SPO111 (R)	AACTGTACCGGGTTTTGACA
AtPIF7, ChPIF7	SPO112 (F)	TCCGCTCTGGATCGGAAACTC
	SPO64 (R)	TGCTCGTCCCCGTCGTCCAT
	SPO142 (R)	TCTCATCCTCTGGTTTATCC

5. APPENDIX TABLE S2. Primers used for cloning or/and genotyping. Primer RO25 (Roig-Villanova *et al*, 2007) has been described before.

Gene	Primer name	Sequence (5' – 3')
ChHFR1 WT	SPO104 (F)	CTGTTGAAGACTGCAGATTTG
	SPO107 (R)	CCTAAGGCAAGATTCTTTGAA
chfr1-1 *chfr1-2*	SPO105 (F)	CTGTTGAAGACTGCAGATTA
	SPO106 (F)	CTGTTGAAGACTGCAGATTTT
attB1 attB2	MJO27 (F)	GGGGACAAGTTTGTACAAAAAAGCAGGCT
	MJO28 (R)	GGGGACCACTTTGTACAAGAAAGCTGGGT
pAtHFR1	SPO26 (F)	GCTCTAGAGTAAAGATAACGTTCT
	SPO27 (R)	GCTCTAGAGTTAGTTAAAGAGATA
ChHFR1	SPO28 (F)	CCATGGGTTTTCCATTTTCTCG
	SPO29 (R)	GGCTCGAGGAGTCTTCCCATCGCA
ChHFR1	CTO29 (F)	ATGATCATCATCAAATTGTTC
AtHFR1	RO25 (F)	AACATGTCGAATAATCAAGCTTTCATG
	SPO30 (R)	GGCTCGAGTAGTCTTCTCATCGCA
3xHA	SPO31 (F)	CCGTCGACGGTGGAGGCGGTTCAG
	SPO32 (R)	GGCTCGAGTCAAGCGTAATCTGGA
RNAi-ChHFR1	CTO35 (F)	CAAACACATAATGATCATCATC
	CTO36 (R)	ATCACTCCAGATCTGGACACGA
*ChHFR1**	SPO128 (R)	CTTCTTTATGAATCTCTGGAACAATCTGAAGA TAATTATCTGTTTGATCATGACCAAAA
	SPO129 (F)	GTTCCAGAGATTCATAAAGAAGTAGAAAATGC GAAGGAGGATTTGTTGGTTGTTGTC
*AtHFR1**	SPO126 (R)	CTTTCTGAATCTCTGGAACAATTTGATGATGA TCATTATGAGTTTGATCATGATCAAAG
	SPO127 (F)	GTTCCAGAGATTCAGAAAGAAGAACGACTGTT GAAGACTGCAGATTTATTGGTTGTTGTC
AtPIF7	JO414 (F)	TAACACATGTCGAATTATGGAG
	JO415 (R)	GGCTCGAGATCTCTTTTCTCATGATTC
AtPIF7 + attB1	BAO4 (F)	GGGGACAAGTTTGTACAAAAAAGCAGGCTAC ATGTCGAATTATGGAGTTAAAG
AtPIF7 + attB2	BAO5 (R)	GGGGACCACTTTGTACAAGAAAGCTGGGTGT CAAGCGTAATCTGGAACGTC

6. APPENDIX TABLE S3. Synthetic peptides used for microscale thermophoresis (MST) experiments.

The peptides were acetylated (Ac) at the N-terminal and aminated (-NH2) at the C-terminal. The C-terminal tyrosine (Y) residue was added to quantify peptide concentrations via absorbance at 280 nm.

Name	Sequence	Company
AtHFR1 VP	Ac-YLQIVPEI-NH2	Genescript
ChHFR1 VP	Ac-HHQIVPEIY-NH2	Genescript
At/ChHFR1 VP	Ac-LLVVVPDEY-NH2	Genescript
AtCRY1	Ac-EDQMVPSITY-NH2	Peptide Synthesis Laboratory
HsTRIB1	Ac-SDQIVPEY-NH2	Peptide Synthesis Laboratory

7. APPENDIX REFERENCES

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