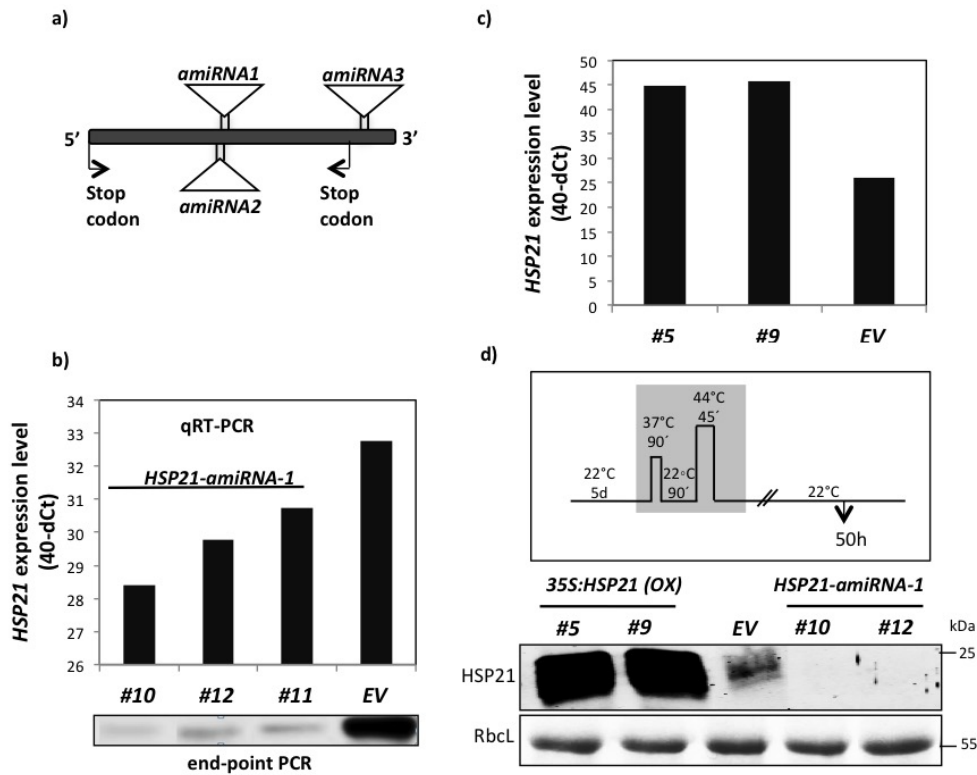
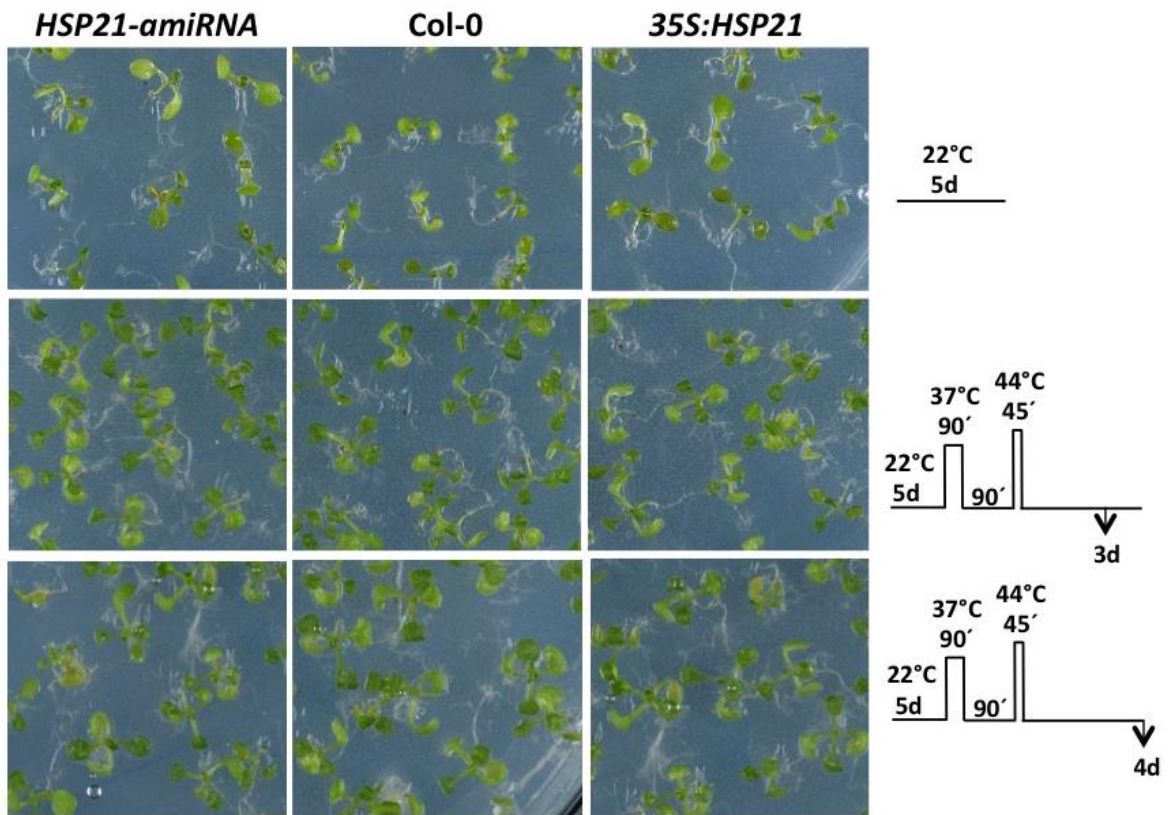


## Supplementary Figure 1



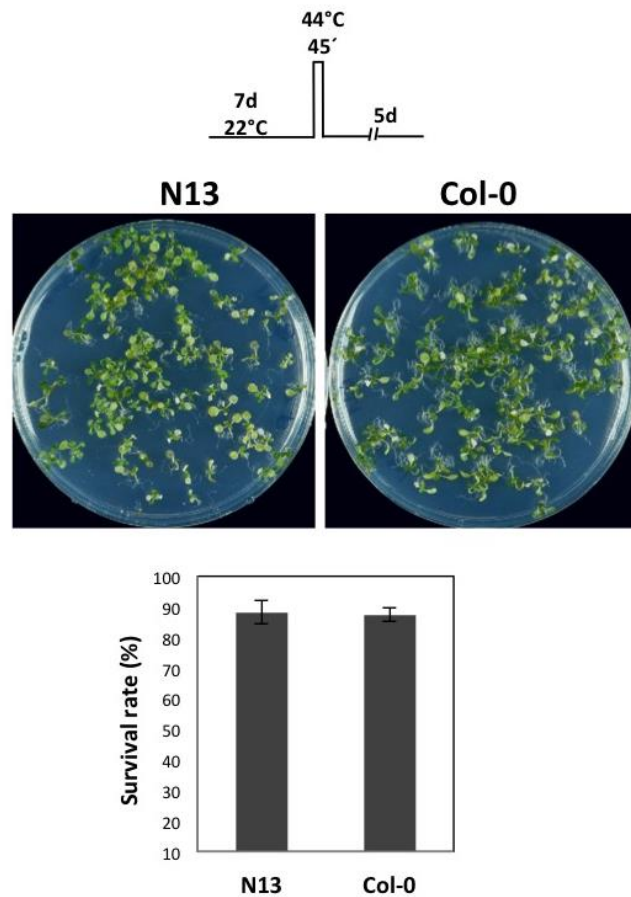
**Supplementary Figure 1. *HSP21* expression in *35S:HSP21* and *hsp21* knockdown plants. (a)** Since no T-DNA insertion line for *HSP21* is available in the publicly available T-DNA collections, we employed an artificial microRNA (amiRNA)-mediated knockdown strategy to generate plants with reduced *HSP21* gene activity (see Methods). Schematic presentation of the *HSP21* coding sequence showing the positions of different sites targeted by the designed amiRNAs. Of the three amiRNA constructs transformed, only amiRNA1 caused a strong reduction in *HSP21* transcript levels (~4- to 16-fold; determined by qRT-PCR in different lines). **(b)** Reduced *HSP21* transcript abundance in all three lines of *amiRNA-1* seedlings (#10, 11 and 12) confirmed by qRT-PCR (n = 2) and end-point PCR. For end-point PCR, primers annealing to the start and stop regions of *HSP21* were used. *cDNA* was prepared from RNA of leaves of five-day-old heat-treated (37°C for 90 min, then 22°C for 90 min, then 44°C for 45 min) seedlings. **(c)** Elevated expression of *HSP21* in *35S:HSP21* plants (lines #5 and #9) compared to empty-vector (EV) control plants. Expression was analysed by qRT-PCR (n = 2). Values were expressed as the difference between an arbitrary value of 40 and dCt, so that high 40 - dCt value indicates high gene expression level. **(d)** Immunodetection of *HSP21* protein in *HSP21* transgenics and EV plants exposed to the HS regime shown schematically, using anti-*HSP21* antibody (top panel). Reduction and increase in *HSP21* protein was confirmed in amiRNA and overexpression lines, respectively. RbcL, ribulose-1,5-bisphosphate carboxylase/ oxygenase large subunit (loading control; bottom panel). kDa, kilo Dalton.

Supplementary Figure 2.



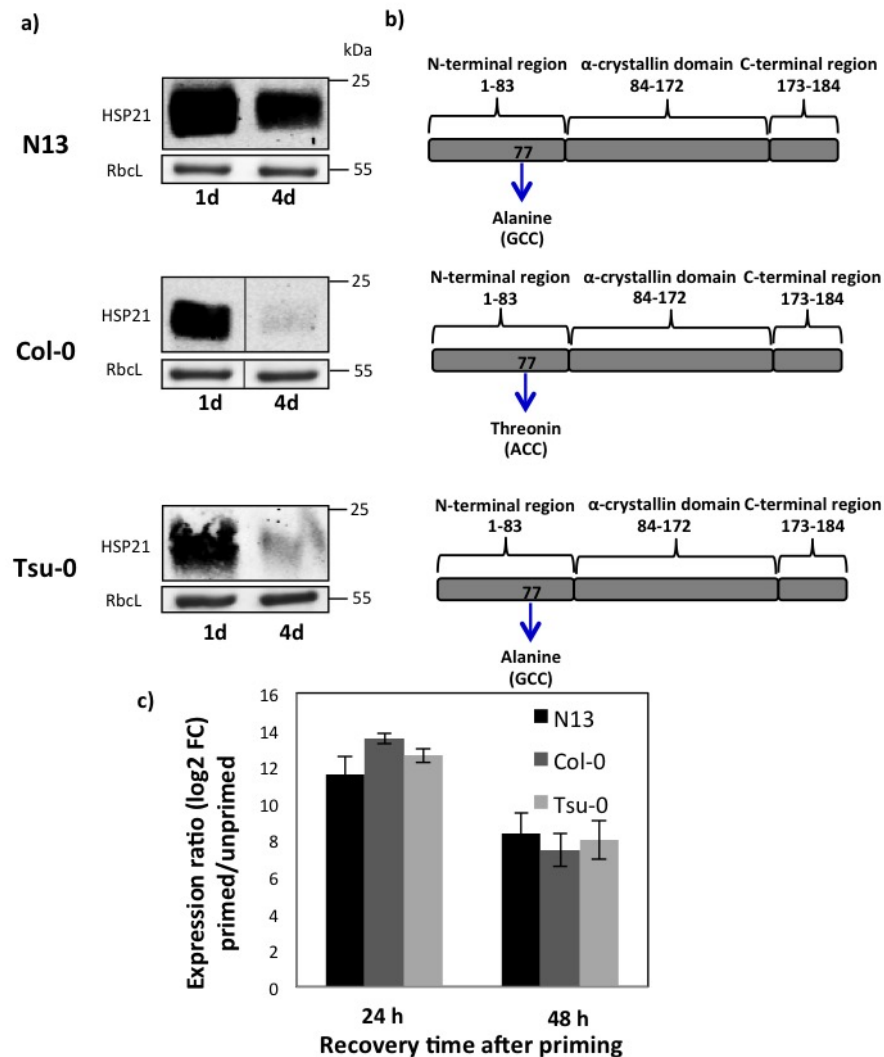
**Supplementary Figure 2. Phenotype of *HSP21* transgenic and wild-type (Col-0) plants before and after priming.** The phenotype of *HSP21*-amiRNA, *35S:HSP21* and Col-0 plants before heat stress priming (upper row of panels), and after 3 d (middle panels) and 4 d (lower panels) of recovery. Growth condition and heat stress regimes are schematically shown on the right of each row of panels.

Supplementary Figure 3.



**Supplementary Figure 3. Assessment of basal heat tolerance in N13 and Col-0.** (a) Seven-day-old N13 and Col-0 seedlings were subjected to 44°C for 45 min. The seedlings were then transferred to normal growth condition and photographed seven days later. (b) Seedling survival was scored seven days after the recovery period; data are represented as percentage of the initial viable seedlings. Means  $\pm$  SD are given (n = 7 plates with ~ 50 seedlings each).

Supplementary Figure 4.



**Supplementary Figure 4. Protein level and sequence comparison of HSP21 in *Arabidopsis* accessions N13, Col-0 and Tsu-0.** (a) Immunoblot analyses of HSP21 during the memory phase (one and three days after priming) shown on the right of each section. Note the much higher abundance of HSP21 protein level in N13 compared to Col-0 and Tsu-0 at day 4 into the memory phase. kDa, kilo Dalton. Bands shown for Col-0 seedlings have been rearranged for presentation purpose. The original blot image is shown in Supplementary Fig. 10. (b) Comparison of the HSP21 protein sequences (deduced from cDNA sequences) in accessions N13, Col-0 and Tsu-0. The threonine 77 in Col-0 is changed to alanine in N13 and Tsu-0 due to an ACC to GCC transition. (c) HSP21 expression in N13, Col-0 and Tsu-0 seedlings during the memory phase (24 h and 48 h) compared to unprimed controls. FC, fold change. Error bars indicate means  $\pm$  SD of three independent biological replicates each containing a pool of  $\sim$ 100 seedlings.

**Supplementary Figure 5. Nucleotide Alignment of *FtsH6* Coding Sequences from *Arabidopsis* Accessions.** (a) PCR-amplified *FtsH6* coding sequences of accessions Col-0 and N13 were sequenced and compared. Sequence polymorphisms are shown in yellow. A nucleotide deletion (G) at position 379 of the N13 *FtsH6* open reading frame leads to a premature translation stop codon (red). (b) Alignment of *FtsH6* amino acid sequences from *Arabidopsis* accessions.

**a) *FtsH6* sequences from accessions Col-0 and N13.**

Col-0	1	ATGGCATCATCATCATCAGCTTTGCTTTCCCTCTCTCCAACATCCCAAC	50
N13	1	ATGGCATCATCATCATCAGCTTTGCTTTCCCTCTCTCCAACATCCCAAC	50
Col-0	51	CTGTAGTAAGAAGTCTCAACAATTTCAAAAACCGGCTTCTTTATCCAAAT	100
N13	51	CTGTAGTAAGAAGTCTCAACAATTTCAAAAACCGGCTTCTTTATCCAAAT	100
Col-0	101	CCAGTCACACA CATAAACCTAGTCTCAAACCCAAATTTTACACCACAAA	150
N13	101	CCAGTCACACC CATAAACCTAGTCTCAAACCCAAATTTTACACCACAAA	150
Col-0	151	TTCACTAAGAGAAATTTACTGAGTTTGACGACTGCCTTGGGATTTACGTC	200
N13	151	CTCACTAAGAGAAATTTACTGAGTTTGACGACTGCCTTGGGATTTACGTC	200
Col-0	201	AGCGCTAGGAACTGTTCTTGCTCACCCCGCAAAGCTGAACCAGAAGCTC	250
N13	201	AGCGCTAGGAACTGTTCTTGCTCACCCCGCAAAGCTGAACCAGAAGCTC	250
Col-0	251	CCATCGAAGCCACTTCCAATAGAATGTCGTATTCGAGATTCCTGCAGCAT	300
N13	251	CCATCGAAGCCACTTCCAATAGAATGTCGTATTCGAGATTCCTGCAGCAT	300
Col-0	301	CTGAAAGAGAATGAAGTGAAGAAAGTTGACTTGATCGAGAACGGAACGGT	350
N13	301	CTGAAAGAGAATGAAGTGAAGAAAGTTGACTTGATCGAGAACGGAACGGT	350
Col-0	351	TGCGATCGTAGAGATCTCTAATCCAGTAGTAGGAAAGATCCAGAGGGTTA	400
N13	351	TGCGATCGTAGAGATCTCTAATCCAGTA-TAGGAAAGATCCAGAGGGTTA	399
Col-0	401	GGGTAAACCTTCCCGGTTTACCAGTCGATCTGGTGAGGGAGATGAAGGAG	450
N13	400	GGGTAAACCTTCCCGGTTTACCAGTCGATCTGGTGAGGGAGATGAAGGAG	449
Col-0	451	AAGAACGTCGATTCGCTGCTCATCCCATGAATGTGAACTGGGGAGCTTT	500
N13	450	AAGAACGTCGATTCGCTGCTCATCCCATGAATGTGAACTGGGGAGCTTT	499
Col-0	501	CTTGCTCAACTTCTTGGGGAATTTAGGGTTTCCTTTGATCTTGCTTGTCT	550
N13	500	CTTGCTCAACTTCTTGGGGAATTTAGGGTTTCCTTTGATCTTGCTTGTCT	549
Col-0	551	CTCTGCTTTTAAACATCTTCTTCAAGAAGAAACCCTGCTGGACCTAACTTG	600
N13	550	CTCTGCTTTTAAACATCTTCTTCAAGAAGAAACCCTGCTGGACCTAACTTG	599
Col-0	601	CCTTTTGGTCTTGGAAGAAGCAAAGCTAAGTTTCAGATGGAGCCTAACAC	650
N13	600	CCTTTTGGTCTTGGAAGAAGCAAAGCTAAGTTTCAGATGGAGCCTAACAC	649
Col-0	651	AGGGATAACGTTTCGAGGATGTAGCAGGAGTAGACGAAGCCAAGCAAGACT	700
N13	650	AGGGATAACGTTTCGAGGATGTAGCAGGAGTAGACGAAGCCAAGCAAGACT	699
Col-0	701	TTGAAGAGATCGTTGAATTTTTGAAAACCCAGAGAAATTCTCAGCTTTG	750
N13	700	TTGAAGAGATCGTTGAATTTTTGAAAACCCAGAGAAATTCTCAGCTTTG	749
Col-0	751	GGGGCTAAAATCCCAAAGGCGTCTTGTTGACCGACCGCCAGGAACCGG	800
N13	750	GGGGCTAAAATCCCAAAGGCGTCTTGTTGACCGACCGCCAGGAACCGG	799
Col-0	801	AAAGACACTCTTGCCAAGGCTATAGCCGGAGAAGCCGGTGTTCCTTTTT	850
N13	800	AAAGACACTCTTGCCAAGGCTATAGCCGGAGAAGCCGGTGTTCCTTTTT	849
Col-0	851	TTTCATTGCTGGTTCGGAGTTCATAGAGATGTTTGTGGTGTAGGAGCA	900

N13	850	TTTCATTGCTGGTTCGGAGTTCATAGAGATGTTTGTGGTGTAGGAGCA	899
Col-0	901	TCTAGAGCTAGAGACTTGTTTAAACAAGGCAAAGGCTAATTCACCTTGTAT	950
N13	900	TCTAGGGCTAGAGACTTGTTTAAACAAGGCAAAGGCTAATTCACCTTGTAT	949
Col-0	951	AGTGTTTCATTGATGAGATTGATGCTGTTGGGAGAATGAGAGGAACCGGTA	1000
N13	950	AGTGTTTCATTGATGAGATTGATGCTGTTGGGAGAATGAGAGGAACCGGTA	999
Col-0	1001	TAGGAGGAGGAAACGACGAACGTGAGCAGACGCTAAACCAGATATTAACC	1050
N13	1000	TAGGAGGAGGAAACGACGAACGTGAGCAGACGCTAAACCAGATATTAACC	1049
Col-0	1051	GAAATGGATGGGTTTGCGGGAATACCGGAGTAATTGTGATCGCTGCAAC	1100
N13	1050	GAAATGGATGGGTTTGCGGGAATACCGGAGTAATTGTGATCGCTGCAAC	1099
Col-0	1101	GAACCGGCCAGAGATTCTAGACTCTGCTTTGCTCCGACCAGGAAGATTCG	1150
N13	1100	GAACCGGCCAGAGATTCTAGACTCTGCTTTGCTCCGACCAGGAAGATTCG	1149
Col-0	1151	ATAGGCAGGTATGTTGGTTAATCCTTAAACCGAATAAATCGAACAGGTTT	1200
N13	1150	ATAGGCAGGTATGTTGGTTAATCCTTAAACCGAATAAATCGAACAGGTTT	1199
Col-0	1201	GGTATAATGTCAACATGTTTTAAACAGGTTTCTGTTGGTTTACCGGATAT	1250
N13	1200	GGTATAATGTCAACATGTTTTAAACAGGTTTCTGTTGGTTTACCGGATAT	1249
Col-0	1251	AAGAGGAAGAGAGGAGATATTAAGTTACAGCAGAAGCAAGAAACTCG	1300
N13	1250	AAGAGGAAGAGAGGAGATATTAAGTTACAGCAGAAGCAAGAAACTCG	1299
Col-0	1301	ACAAAGATGTATCTTTGAGCGTAATTGCCATGAGAACTCCAGGTTTTAGT	1350
N13	1300	ACAAAGATGTATCTTCGAGCGTAATTGCCATGAGAACTCCAGGTTTTAGT	1349
Col-0	1351	GGAGCTGACTTGGCCAATCTTATGAACGAAGCAGCGATTCTCGCCGGAAG	1400
N13	1350	GGAGCTGACTAGGCCAATCTTATGAACGAAGCAGCGATTCTCGCCGGAAG	1399
Col-0	1401	AAGAGGAAAAGACAAGATTACCCTTACAGAGATCGACGACTCTATCGATC	1450
N13	1400	AAGAGGAAAAGACAAGATTACCCTTACAGAGATCGACGACTCTATCGATC	1449
Col-0	1451	GGATAGTCGCCGGAATGGAAGGGACAAAGATGATCGACGGTAAAAGTAAA	1500
N13	1450	GGATAGTCGCCGGAATGGAAGGGACAAAGATGATCGACGGTAAAAGTAAA	1449
Col-0	1501	GCGATTGTAGCGTACCATGAAGTAGGACATGCAATTTGTGCGACTTTGAC	1550
N13	1500	GCGATTGTAGCGTACCATGAAGTAGGACATGCAATTTGTGCGACTTTGAC	1549
Col-0	1551	GGAGGGTCATGATCCGGTTCAGAAAGTTACGTTGGTTCCTAGAGGTCAAG	1600
N13	1550	GGAGGGTCATGATCCGGTTCAGAAAGTTACGTTGGTTCCTAGAGGTCAAG	1559
Col-0	1601	CACGTGGTCTCACGTGGTTCTTACCGGGAGAAGATCCGACATTGGTGTCT	1650
N13	1600	CACGTGGTCTCACGTGGTTCTTACCGGGAGAAGATCCGACATTGGTGTCT	1649
Col-0	1651	AAACAGCAATTGTTTCGCTAGAATCGTCGGAGGACTCGGAGGCAGAGCCGC	1700
N13	1650	AAACAGCAATTGTTTCGCTAGAATCGTCGGAGGACTCGGAGGCAGAGCCGC	1699
Col-0	1701	CGAAGATGTAATTTTTGGAGAACCGGAGATAACCACCGGCGCTGCCGGTG	1750
N13	1700	CGAAGATGTAATTTTTGGAGAACCGGAGATAACCACCGGCGCTGCCGGTG	1749
Col-0	1751	ATCTCCAGCAAGTCACTGAGATAGCAAGACAGATGGTGACGATGTTTGGT	1800
N13	1750	ATCTCCAGCAAGTCACTGAGATAGCAAGACAGATGGTGACGATGTTTGGT	1799
Col-0	1801	ATGTCGGAAATCGGTCCATGGGCACTAACCGACCCAGCGGTTAAGCAAAA	1850
N13	1800	ATGTCGGAAATCGGTCCATGGGCACTAACCGACCCAGCGGTTAAGCAAAA	1849
Col-0	1851	CGATGTGGTTCTACGGATGCTAGCGAGGAACTCAATGTCCGAGAACTTG	1900
N13	1850	CGATGTGGTTCTACGGATGCTAGCGAGGAACTCAATGTCCGAGAACTTG	1899
Col-0	1901	CGGAGGATATTGATTCTTGCGTGAAGAAAATAATCGGTGACGCTTACGAG	1950
N13	1900	CGGAGGATATTGATTCTTGCGTGAAGAAAATAATCGGTGACGCTTACGAG	1949

Col-0 N13	1951 1950	GTTGCGAAAAAGCACGTGAGGAATAATAGAGAAGCTATAGACAAGCTTGT GTTGCGAAAAAGCACGTGAGGAATAATAGAGAAGCTATAGACAAGCTTGT	2000 1999
Col-0 N13	2001 2000	TGATGTTTTGTTGGAGAAAGAAACCTTAACGGGAGACGAGTTTCGAGCAA TGATGTTTTGTTGGAGAAAGAAACCTTAACGGGAGACGAGTTTCGAGCAA	2050 2049
Col-0 N13	2051 2050	TTCTGTCTGAGTATACTGATCAACCGTTAAATACCGATGGCGATGT <b>A</b> AAGA TTCTGTCTGAGTATACTGATCAACCGTTAAATACCGATGGCGATGT <b>T</b> AGA	2100 2099
Col-0 N13	2101 2100	ATTCGAATCAATGACTTGATTAGTGTCTAA ATTCGAATCAATGACTTGATTAGTGTCTAA	2130 2129

### FtsH6 protein from Col-0

Met ASSSSALSFP LSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQ**I**LHHK**F**TKRNL  
 LSLTTALGFTSALGTVLAHPAKAEPEAPIEATSNRMetSYSRFLQHLKENEVKKVDLI  
 ENGTVAIVEISNPVVGKIQRVRVNLPLGPDVLRVREMetKEKNVDFAAHPMetNVNW  
 GAFLNFLGNLGFPLILLVSLLLTSSSRNPAGPNLPFGLGRSKAKFQMetEPNTGIT  
 FEDVAGVDEAKQDFEEIVEFLKTPEKFSALGAKIPKGVLLTGPPGTGKTLLAKAIAG  
 EAGVPFFSLSGSEFIEMetFVGVGASRARDLFNKAKANSPCIVFIDEIDAVGRMetRG  
 TGIGGGNDEREQTLNQILTEMetDGFAGNTGVIVIAATNRPEILDSALLRPGRFDRQ  
 VCWLILKPNKSNRFGIMetSTCFKQVSVGLPDIRGREEILKVHSRSKLDKDVLSVI  
 AMetRTPGFSGADLANLMetNEAAILAGRRGKDKITLTEIDDSIDRIVAGMetEGTK  
 MetIDGKSKAIVAYHEVGHAICATLTEGHDPVQKVTLVPRGQARGLTWFLPGEDPT  
 LVSKQQLFARIVGGLGGRAAEDVIFGEPEITGAAGDLQQVTEIARQMetVTMetFG  
 MetSEIGPWALTDPAVKQNDVVLRMetLARNSMetSEKLAEDIDSCVKKIIGDAYEVA  
 KKHVRNNREAIKLDVLLKETLTGDEFRAILSEYTDQPLNTDGDVIRINDLISV  
**Stop**

### FtsH6 protein from N13

Met ASSSSALSFP LSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQ**T**LHHK**L**TKRNL  
 LSLTTALGFTSALGTVLAHPAKAEPEAPIEATSNRMetSYSRFLQHLKENEVKKVDLI  
 ENGTVAIVEISNPV **Stop**

### b) Alignment of FtsH6 amino acid sequences from *Arabidopsis* accessions.

The following *Arabidopsis* accessions were included in our preliminary screen for differences in thermomemory: Col-0, N13, Cvi-0, C24, Ct, Can-0, Lip-0, Mdn-1, Mh-0, Mt-0, Aitba-1, Abd-0, Bl-1, Bur-0, Dog-4, Tsu-0, St-0, Kas-1, Yo-0, Xan-1, Kondara, Ler-0, Ko-2, Ri-0, Fei-0, Leo-1, Yeg-1, Ste-0, ICE1, Kin-0, Le-0, Oy-0, N14, Sah-0, Berk, Hiro, Ita, HEK2, Stepn, Shign, and Borsk2.

The FtsH6 amino acid sequence alignment shown below includes sequences from all above accessions with the exception of sequences that were identical to those of Col-0 (namely Lip-0, Mh-0, Bl-1, Tsu-0,

Ler-0, Ko-2, Fei-0, Ste-0, and Oy-0), or those for which no sequence information was available (N14, Sah-0, Berk, Hiro, Ita, HEK2, Stepn, Shign, and Borsk2). Also not included in the alignment is the sequence of N13, which has a premature stop codon (see above).

FtsH6 sequences were extracted from the Arabidopsis 1001 Genomes project database (<http://signal.salk.edu/atg1001/3.0/gebrowser.php>). For the multi-sequence alignment the MUSCLE tool (<http://www.ebi.ac.uk/Tools/msa/muscle>) was used.

Amino acids divergent from Col-0 are highlighted in red. Conservative substitutions are indicated as ‘:’ in the Col-0 sequence, while non-conservative exchanges are indicated by a free space. Asterisks (\*) denote amino acids identical to Col-0.

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Can-0      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Cvi-0      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Bur-0      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Leo-1      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Le-0       MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
ICE1       MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Yeg-1      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Kondara    MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Yo-0       MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Dog-4      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Aitba-1    MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Kin-0      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Ri-0       MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
St-0       MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Abd-0      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Mdn-1      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Xan-1      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Mt-0       MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Ct         MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
C24        MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Kas-1      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
Col-0      MASSSSALSFPLSNIPTCSKKSQQFQKPASLSKSSHTHKPSLKTQILHHKFTKRNLSSLT
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Can-0      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Cvi-0      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Bur-0      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Leo-1      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Le-0       TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
ICE1       TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Yeg-1      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Kondara    TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Yo-0       TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Dog-4      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Aitba-1    TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Kin-0      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Ri-0       TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
St-0       TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Abd-0      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Mdn-1      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Xan-1      TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Mt-0       TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
Ct         TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV
C24        TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV

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Kas-1 TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV  
Col-0 TALGFTSALGTVLAHPAKAEPEAPIEATSNRMSYSRFLQHLKENEVKKVDLIENGTVAIV  
\*\*\*\*\*:\*\*\*\*\*

Can-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Cvi-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Bur-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Leo-1 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Le-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
ICE1 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Yeg-1 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Kondara EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Yo-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Dog-4 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Aitba-1 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Kin-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Ri-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
St-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Abd-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Mdn-1 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Xan-1 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Mt-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Ct EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
C24 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Kas-1 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
Col-0 EISNPVVGKIQRVRVNLGPLPVDLVREMKEKNVDFAAHPMNVNWGAFLNLFNLGNLGFPLI  
\*\*\*\*\*:\*\*\*\*\*

Can-0 LLVSLLLTSSSRNPAGPNLPGFLGRSKAKFQMEPNTGITTFEDVAGVDEAKQDFEEIVEF  
Cvi-0 LLVSLLLTSSSRNPAGPNLPGFLGRSKAKFQMEPNTGITTFEDVAGVDEAKQDFEEIVEF  
Bur-0 LLVSLLLTSSSRNPAGPNLPGFLGRSKAKFQMEPNTGITTFEDVAGVDEAKQDFEEIVEF  
Leo-1 LLVSLLLTSSSRNPAGPNLPGFLGRSKAKFQMEPNTGITTFEDVAGVDEAKQDFEEIVEF  
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Kin-0 LLVSLLLTSSSRNPAGPNLPGFLGRSKAKFQMEPNTGITTFEDVAGVDEAKQDFEEIVEF  
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Col-0 LKTPEKFSALGAKIPKGVLLTGPPGTGKTLAKAIAGEAGVPPFFSLSGSEFIEMFVGVGA  
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Yo-0 SRARDLFNKAKANSPCIVFIDEIDAVGRMRGTGIGGGNDEREQTLNQILTEMDGFAGNTG  
Dog-4 SRARDLFNKAKANSPCIVFIDEIDAVGRMRGTGIGGGNDEREQTLNQILTEMDGFAGNTG  
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Xan-1 SRARDLFNKAKANSPCIVFIDEIDAVGRMRGTGIGGGNDEREQTLNQILTEMDGFAGNTG  
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Bur-0 VIVIAATNRPEILDSALLRPGFRDQVCWLIILKPNKSNRFGIMSTCFKQVSVGLPDIRGR  
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Aitba-1 VIVIAATNRPEILDSALLRPGFRDQVCWLIILKPNKSNRFGIMSTCFKQVSVGLPDIRGR  
Kin-0 VIVIAATNRPEILDSALLRPGFRDQVCWLIILKPNKSNRFGIMSTCFKQVSVGLPDIRGR  
Ri-0 VIVIAATNRPEILDSALLRPGFRDQVCWLIILKPNKSNRFGIMSTCFKQVSVGLPDIRGR  
St-0 VIVIAATNRPEILDSALLRPGFRDQVCWLIILKPNKSNRFGIMSTCFKQVSVGLPDIRGR  
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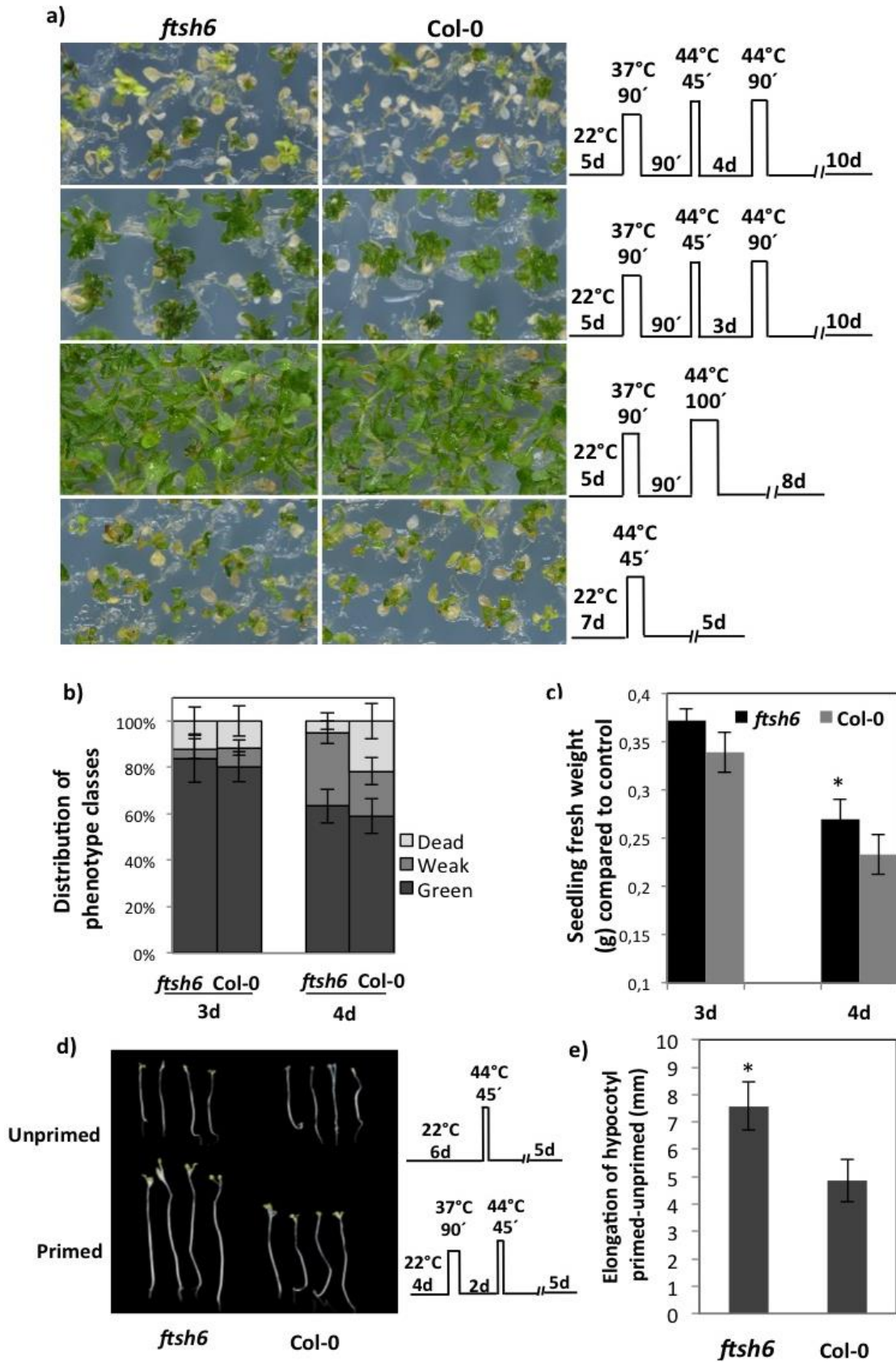
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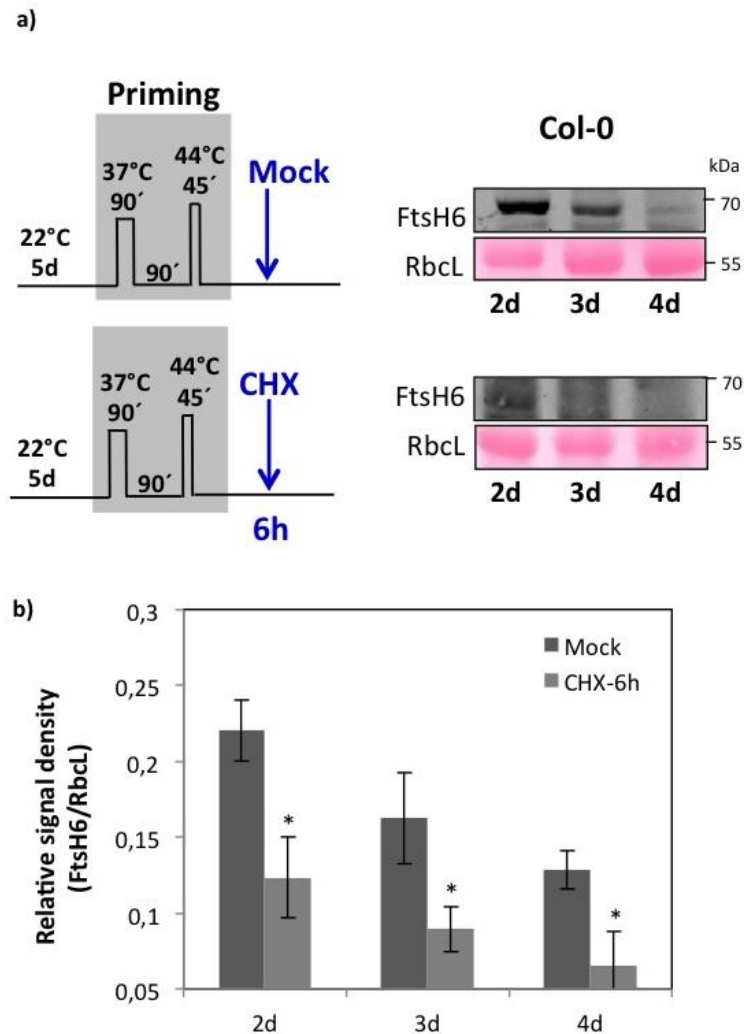
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Supplementary Figure 6.



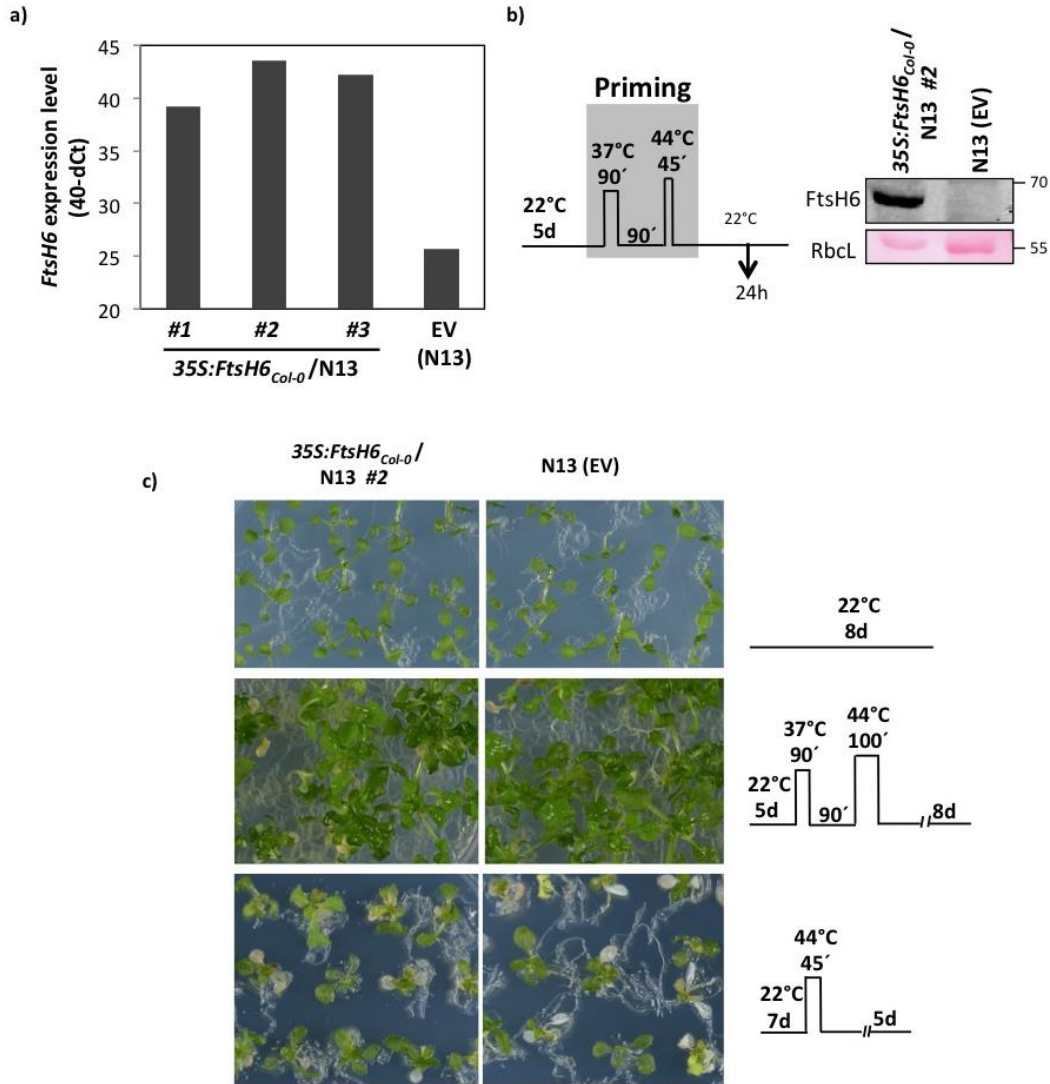
**Supplementary Figure 6. Basal and acquired HS tolerance, and thermomemory of *ftsh6* mutants.** Seedlings of *ftsh6* mutant and wild-type (Col-0) plants were exposed to different HS regimes (schematically shown on the right of each section). **(a)** Upper middle and top panels: HS triggering stimulus after extended recovery, i.e., 4 and 3 days, respectively, following the priming treatment. Lower middle panel: acquired HS tolerance. Bottom panel: basal HS tolerance; HS applied to 7-day-old seedlings. Following HS, the seedlings were transferred to normal growth condition and photographed after 10 days (upper middle and top panels), 8 days (lower middle panel), or 5 days (bottom panel). **(b)** and **(c)** Quantification of results shown in **a**. **(b)** The percentage of seedlings in different phenotype classes. At 3 d recovery, data for *ftsh6* mutants are not significantly different from Col-0. At 4 d recovery all data for *ftsh6* mutants are different from Col-0 ( $p < 0.05$ ; Student's *t*-test). **(c)** Seedling fresh weight compared to control plants. Means  $\pm$  SD are given ( $n = 5$  plates with  $\sim 25$  seedlings each). **(d)** Hypocotyl elongation assay for *ftsh6* mutants and Col-0 under HS. Six-day-old seedlings were subjected to the HS triggering stimulus without (unprimed) or with (primed) pre-treatment with moderate temperature stress as shown schematically. Photos were taken 5 days after triggering. **(e)** Hypocotyl lengths measured 5 days after recovery from the HS triggering stimulus and compared between primed and unprimed seedlings. Means  $\pm$  SD are given ( $n = 7$  plates with  $\sim 20$  seedlings each). Asterisks indicate statistically significant difference ( $p < 0.05$ ; Student's *t*-test) from the Col-0 control.

Supplementary Figure 7.



**Supplementary Figure 7. The effect of cycloheximide on the accumulation of FtsH6 protein in *Arabidopsis* accession Col-0.** (a) Immunoblot analyses of FtsH6 protein in Col-0 at days 2, 3 and 4 of the memory phase upon cycloheximide (CHX) and mock (0.1% DMSO) treatment. CHX and mock treatment was applied to seedlings at 6 h into the memory phase, as shown schematically. The seedlings were harvested for immunoblotting at days 2, 3 or 4. kDa, kilo Dalton. (b) Signals of the immunoblot analysis were quantified using ImageJ and normalized to the amount of RbcL in the same samples. Mean  $\pm$  SD are given ( $n = 3$ ; independent biological replicates each representing a pool of  $\sim 120$  seedlings grown on 6 plates; gel blots used for the quantification are shown in Supplementary Fig. 10). Asterisks indicate significant difference in the level of FtsH6 between CHX- and mock-treated samples at each indicated time point ( $p < 0.05$ ; Student's  $t$ -test).

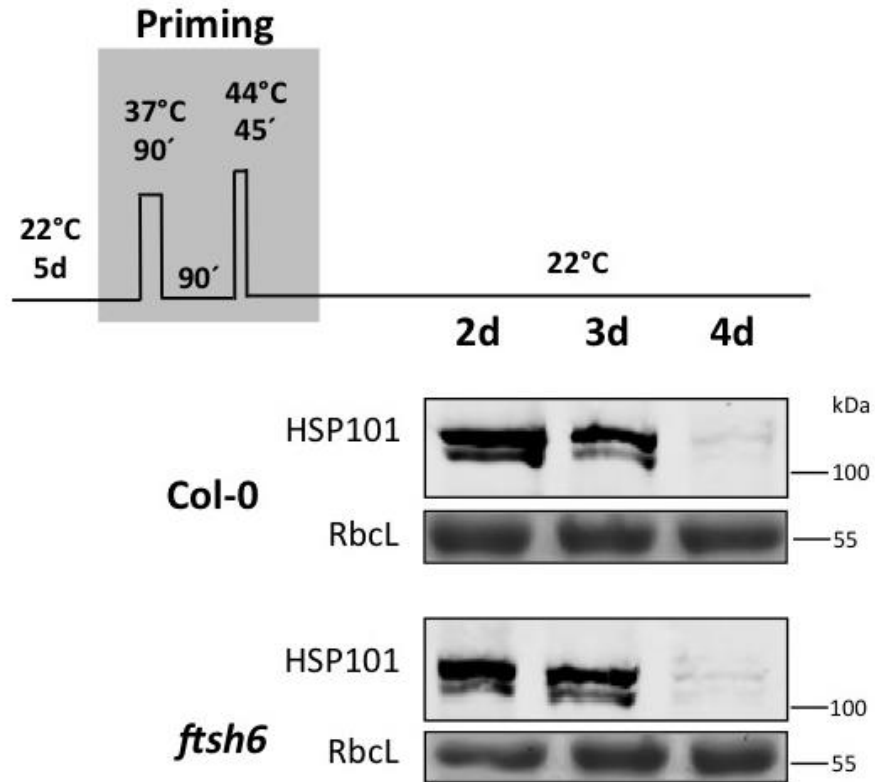
Supplementary Figure 8.



**Supplementary Figure 8. *FtsH6* expression in  $35S:FtsH6_{Col-0}/N13$  plants. (a)** Elevated expression of *FtsH6* in  $35S:FtsH6_{Col-0}/N13$  plants (lines #1, #2 and #3) compared to N13 empty-vector (EV) control plants. Expression was analysed by qRT-PCR (n = 2). Values are expressed as the difference between an arbitrary value of 40 and dCt, so that high 40-dCt value indicates high gene expression level. **(b)** Immunodetection of *FtsH6* protein in  $35S:FtsH6_{Col-0}/N13$  and N13 EV seedlings exposed to the HS regime shown schematically, using anti-*FtsH6* antibody. Note the presence of *FtsH6* protein in  $35S:FtsH6_{Col-0}/N13$  plants. Rbcl, ribulose-1,5-bis-phosphate carboxylase/oxygenase large subunit (loading control; bottom panel). kDa, kilo Dalton. **(c)** The phenotype of  $35S:FtsH6_{Col-0}/N13$  and N13 empty-vector (EV) seedlings exposed to different HS regimes (schematically shown on the right of each section in panels).

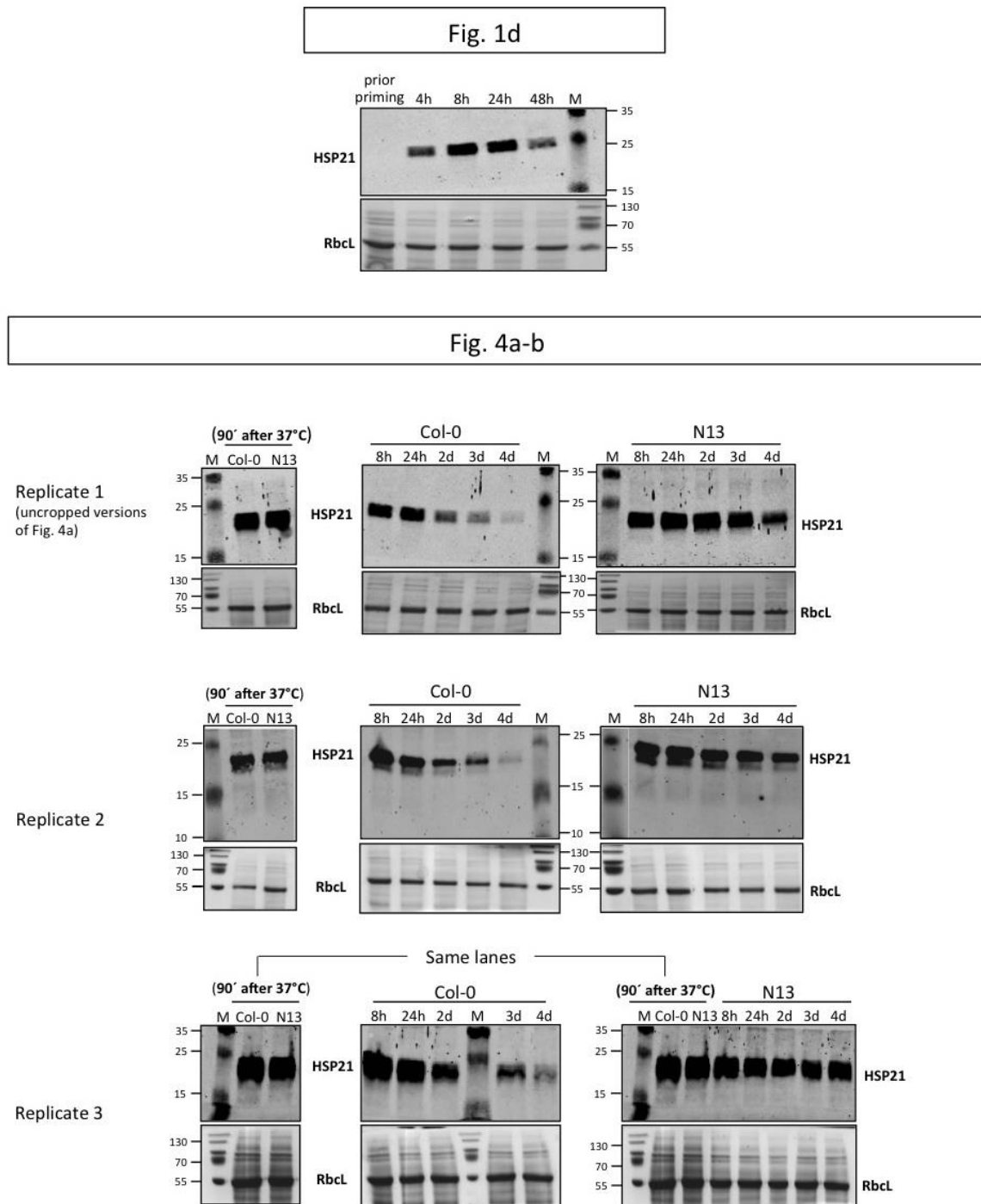


Supplementary Figure 9.



**Supplementary Figure 9. Immunodetection of HSP101 protein in Col-0 and *ftsh6*.** Immunoblot analysis of HSP101 protein was performed in Col-0 and *ftsh6* seedlings at days 2, 3 and 4 of the memory phase, using anti-HSP101 antibody (Abcam). Note the decline of HSP101 protein level at day 4 of the memory phase with no difference between Col-0 and *ftsh6*. RbcL, ribulose-1,5-bis-phosphate carboxylase/oxygenase large subunit (loading control). kDa, kilo Dalton.

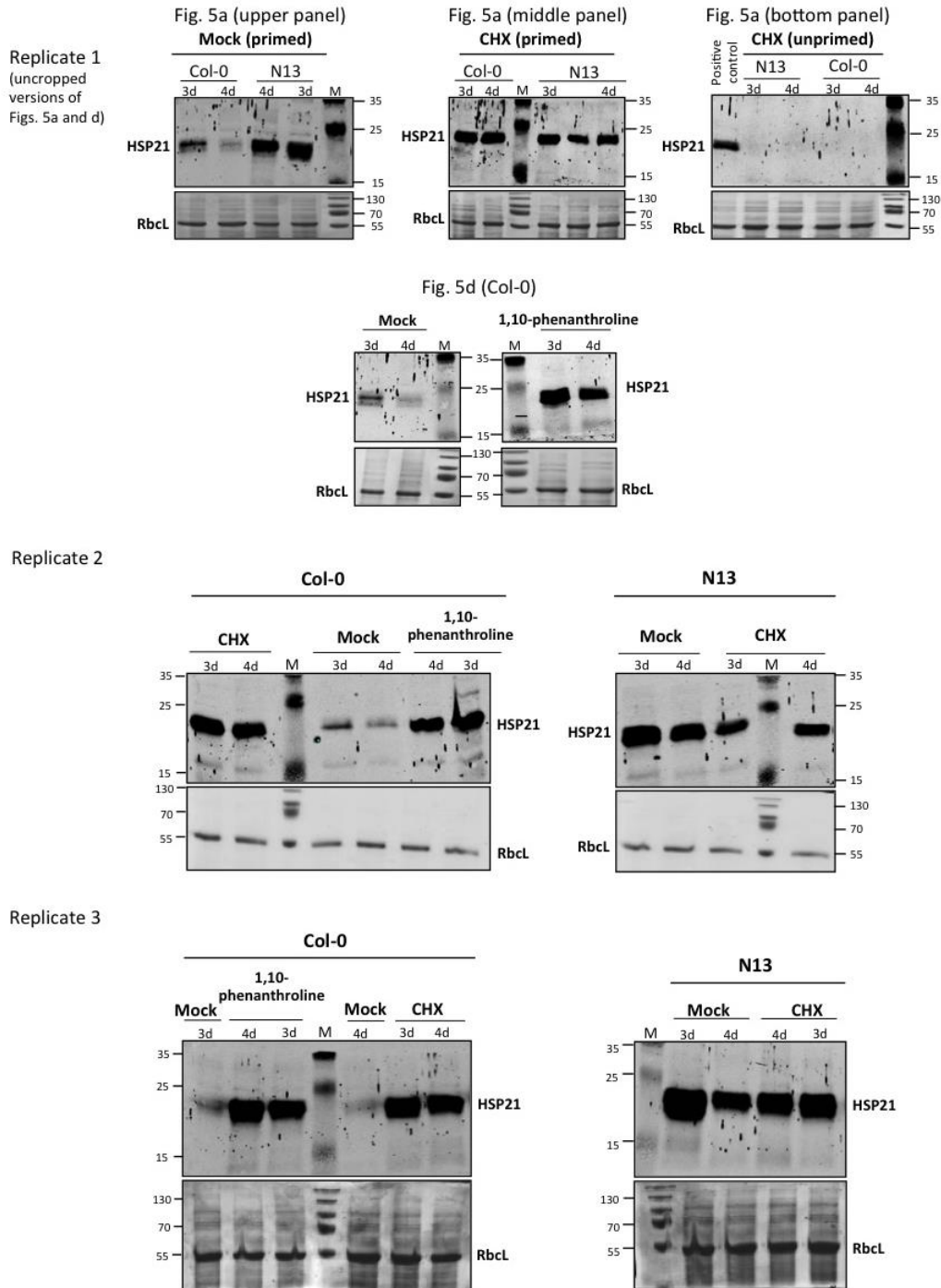
Supplementary Figure 10.



**Supplementary Figure 10. Uncropped gel and western blot images presented in this manuscript.** Labelling above each image indicates the corresponding figure in the main manuscript or the supplement. M, molecular weight marker. Numbers left and right of images indicate protein molecular weights in kilo Dalton.

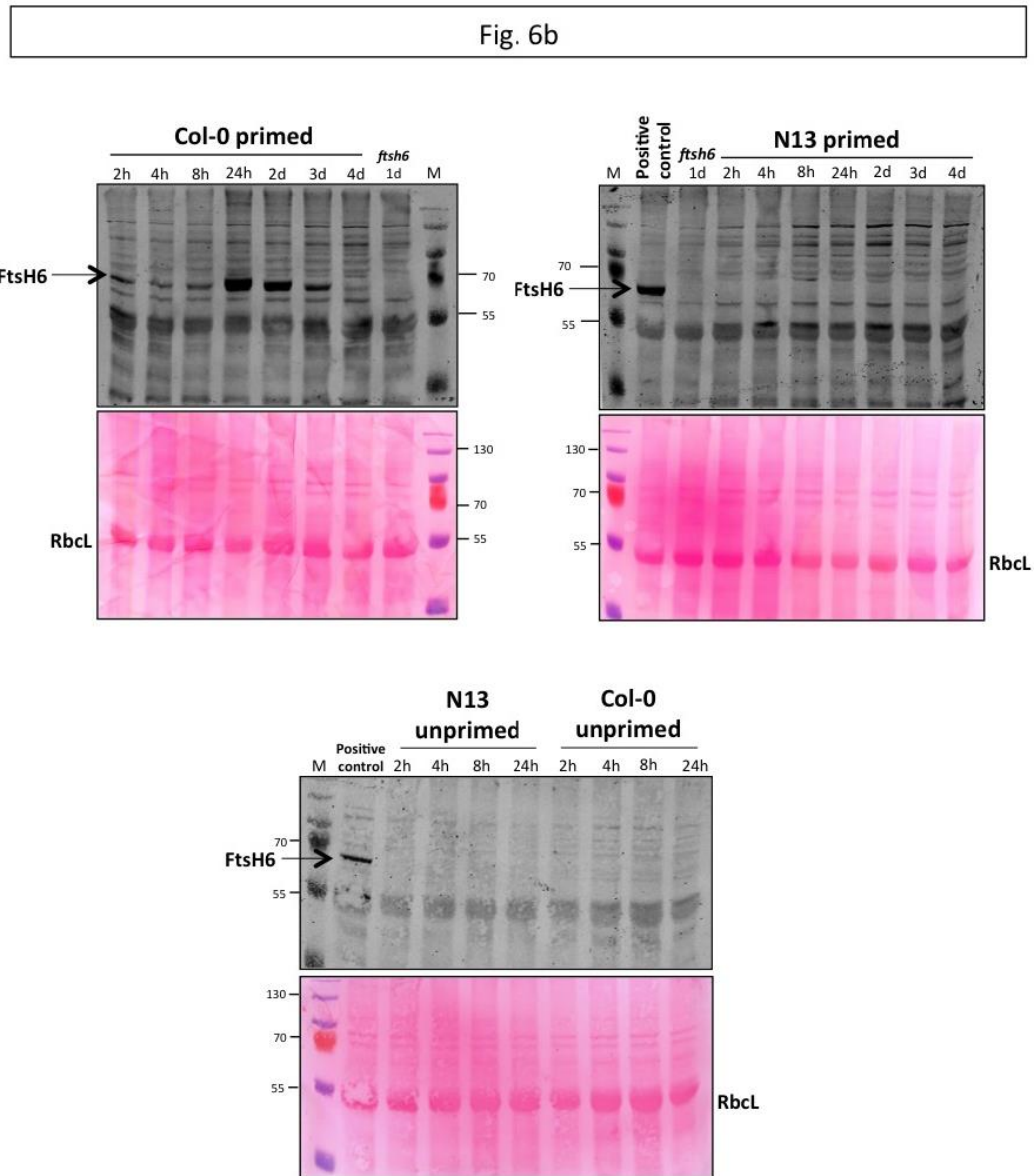
Supplementary Figure 10 (continued).

Fig. 5a-e



**Supplementary Figure 10. Uncropped gel and western blot images presented in this manuscript.** Labelling above each image indicates the corresponding figure in the main manuscript or the supplement. M, molecular weight marker. Numbers left and right of images indicate protein molecular weights in kilo Dalton.

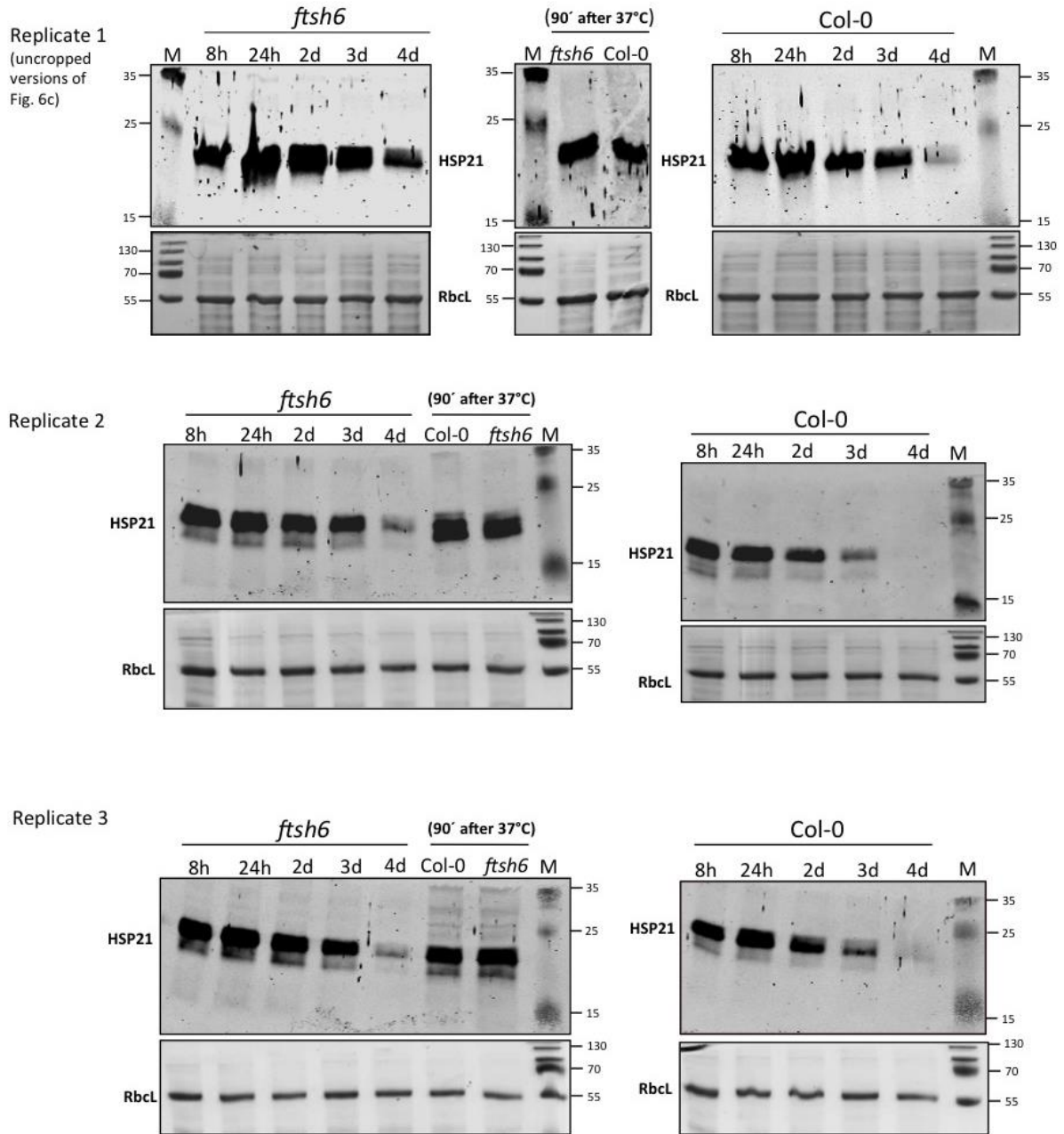
Supplementary Figure 10 (continued).



**Supplementary Figure 10. Uncropped gel and western blot images presented in this manuscript.** Labelling above each image indicates the corresponding figure in the main manuscript or the supplement. M, molecular weight marker. Numbers left and right of images indicate protein molecular weights in kilo Dalton.

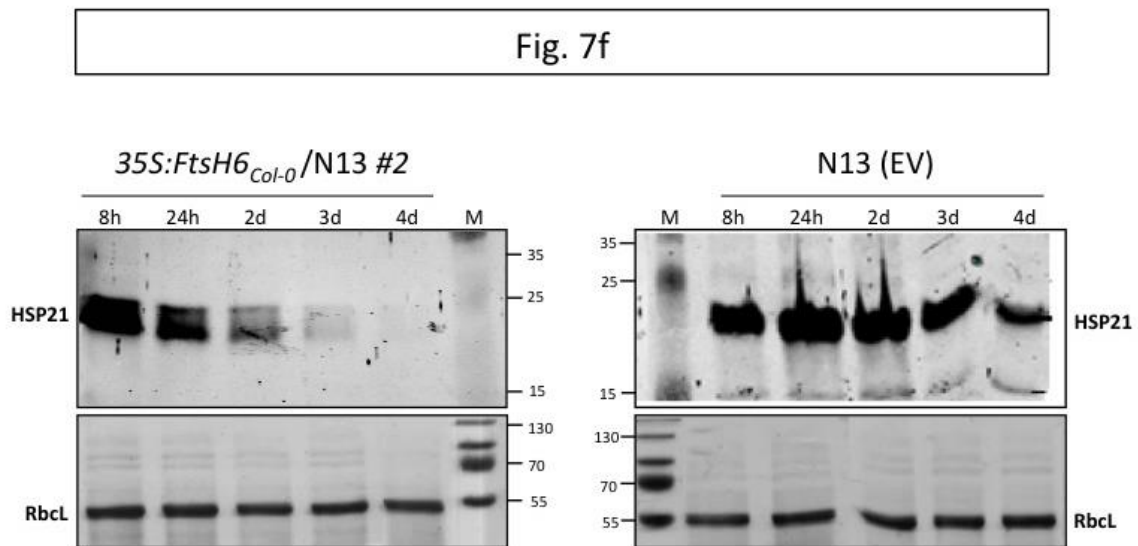
Supplementary Figure 10 (continued).

Fig. 6c-d



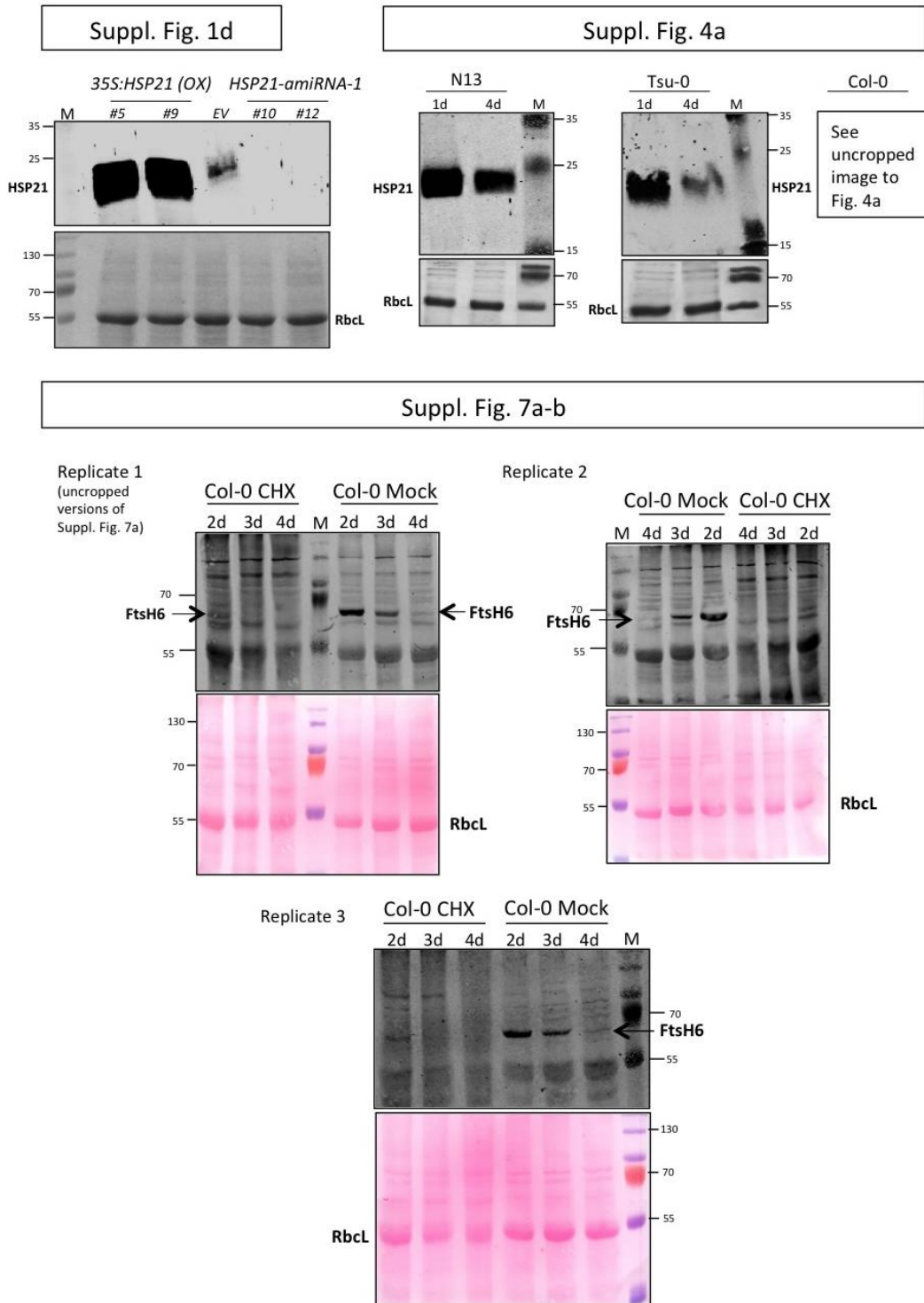
Supplementary Figure 10. Uncropped gel and western blot images presented in this manuscript. Labelling above each image indicates the corresponding figure in the main manuscript or the supplement. M, molecular weight marker. Numbers left and right of images indicate protein molecular weights in kilo Dalton.

Supplementary Figure 10 (continued).



**Supplementary Figure 10. Uncropped gel and western blot images presented in this manuscript.** Labelling above each image indicates the corresponding figure in the main manuscript or the supplement. M, molecular weight marker. Numbers left and right of images indicate protein molecular weights in kilo Dalton.

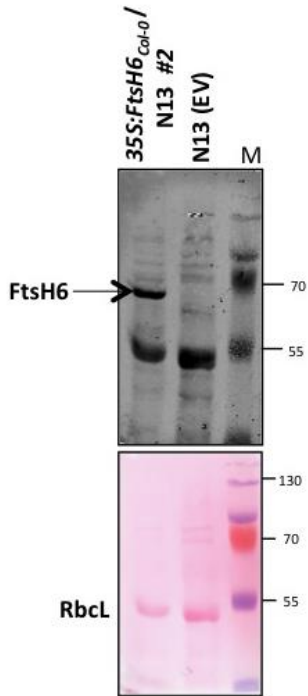
Supplementary Figure 10 (continued).



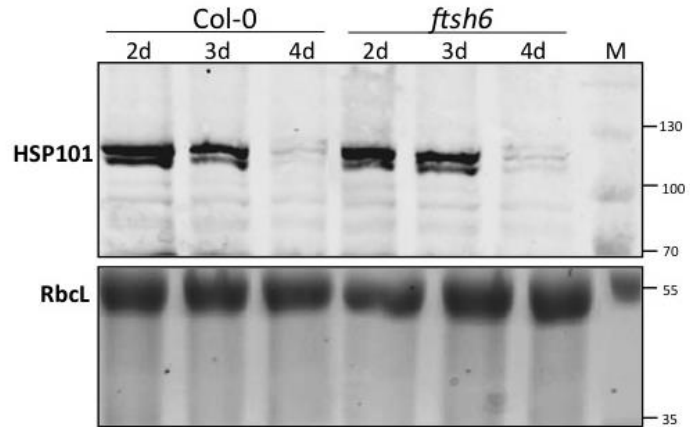
**Supplementary Figure 10. Uncropped gel and western blot images presented in this manuscript.** Labelling above each image indicates the corresponding figure in the main manuscript or the supplement. M, molecular weight marker. Numbers left and right of images indicate protein molecular weights in kilo Dalton.

Supplementary Figure 10 (continued).

Suppl. Fig. 8b



Suppl. Fig. 9



**Supplementary Figure 10. Uncropped gel and western blot images presented in this manuscript.** Labelling above each image indicates the corresponding figure in the main manuscript or the supplement. M, molecular weight marker. Numbers left and right of images indicate protein molecular weights in kilo Dalton.



**Supplementary Table 1.** Primer sequences.

Primers for cloning	Sequence	Sites added
<b>35S:HSP21</b>		
HSP21-Forward	<u>GTTTAAAC</u> ATGGCTTCTACACTCTCATTGCG	added <i>PmeI</i> cloning site underlined
HSP21-Reverse	TTAATTA <u>AACTACTGAATCTGGACATCGATGA</u>	added <i>PacI</i> cloning site underlined
<b>HSP21-amiRNA</b>		
<b>amiRNA-1</b>		
I miR-s-1	GATTAGTATCTAACATTTGTCGCCTCTCTTTTGTATTCCA	
II miR-a-1	AGGCGACAAATGTTAGATACTAATCAAAGAGAATCAATGA	
III miR*s-1	AGGCAACAAATGTTACATACTATTACAGGTCGTGATATG	
IV miR*a-1	GAATAGTATGTAACATTTGTTGCCTACATATATATTCCCTA	
<b>amiRNA-II</b>		
I miR-s-2	GATGTATCTAACATTTGTCGCATCTCTCTTTTGTATTCCA	
II miR-a-2	AGATGCGACAAATGTTAGATACTAATCAAAGAGAATCAATGA	
III miR*s-2	AGATACGACAAATGTAAGATACTTACAGGTCGTGATATG	
IV miR*a-2	GAAGTATCTTACATTTGTCGTATCTACATATATATTCCCTA	
<b>amiRNA-III</b>		
I miR-s-3	GATATAATGTTGATCGAGTCCTATCTCTCTTTTGTATTCC	
II miR-a-3	GATAGGACTCGATCAACATTATATCAAAGAGAATCAATGA	
III miR*s-3	GATAAGACTCGATCATCATTATTTACAGGTCGTGATATG	
IV miR*a-3	GAAATAATGATGATCGAGTCTTATCTACATATATATTCCCT	
A Primer (universal for making amiRNA)	<u>GTTTAAAC</u> CTGCAAGGCGATTAAGTTGGGTAAC	added <i>PmeI</i> cloning site underlined
B primer (universal for making amiRNA)	TTAATTA <u>AAGCGGATAACAATTTACACAGGAAACAG</u>	added <i>PacI</i> cloning site underlined
<b>qRT-PCR primers</b>		
<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
<i>Actin2</i>	TCCCTCAGCACATTCCAGCAGAT	AACGATTCTGGACCTGCCTCATC
<i>HSP21</i>	TGGACGTCTCTCTTTTCGGATTG	TTTGTGCGCATCGTCTCATTGG
<i>FtsH6</i>	GCCGGAATGGAAGGGACAAAGATG	ATCATGACCCTCCGTCAAAGTCG