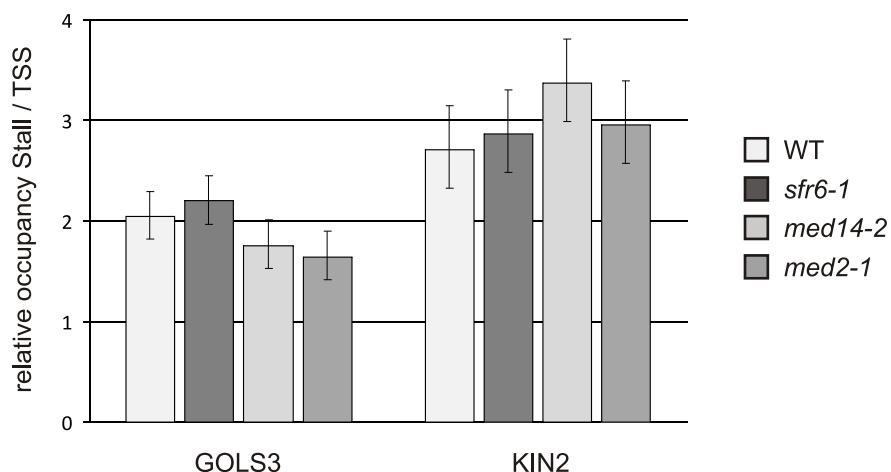
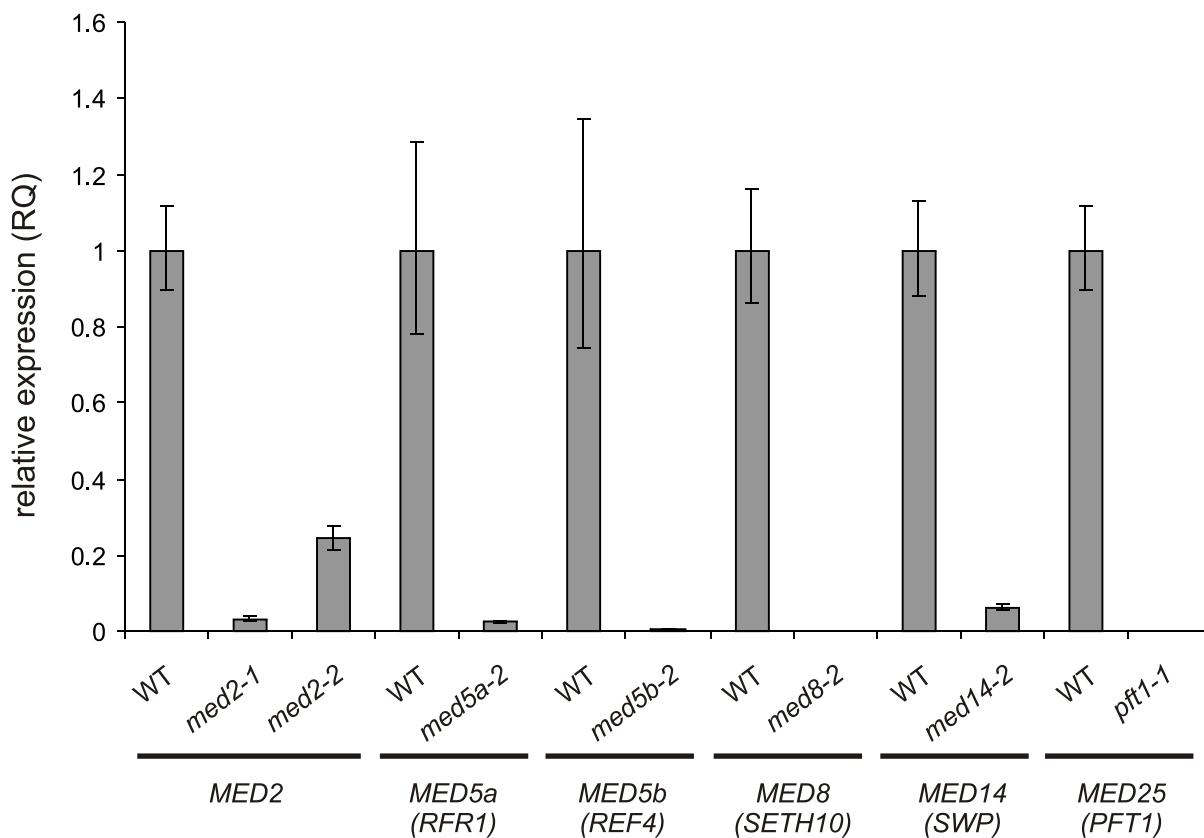


**Supplemental Figure 1. Overexpression of CBF1-YFP elicits COR gene expression in WT but not sfr6-1. H3 occupancy at COR gene promoters alters in response to cold by equivalent amounts in WT and sfr6-1.** (A) to (C) CBF1-YFP overexpression elicits expression of COR genes in wild type but not sfr6-1 plants. qRT-PCR analysis of CBF1 (A), KIN2 (B) and GOLS3 (C) in 8-day-old transgenic *Arabidopsis* seedlings of Col-0 (WT Lines 35 and 40) and sfr6-1 (lines 12 and 20) expressing 35S-CBF1-YFP compared with non-transgenic Col-0 plants (WT) at ambient temperature. Expression is shown after normalisation to PEX4. Each value represents the mean of three technical replicates for each sample normalised to the mean of three technical replicates for PEX4. The error bars represent RQ<sub>MIN</sub> and RQ<sub>MAX</sub> and constitute the acceptable error level for a 95% confidence level according to Student's t-test. (D) to (F) ChIP qRT-PCR analysis of relative levels of histone H3 at KIN2 (D), GOLS3 (E) and LTI78 (F) promoters in 3-week-old plants exposed to 5°C or maintained at 20°C for 4 h indicates that cold treatment reduces Histone H3 occupancy at COR gene promoters and acts independently of SFR6/MED16. Data represent proportion of histone H3 at a given promoter referenced against input and standardised against relative PEX4 histone H3 occupancy. Each value represents the mean of three technical replicates. The error bars represent RQ<sub>MIN</sub> and RQ<sub>MAX</sub> and constitute the acceptable error level for a 95% confidence level according to Student's t-test. Data are representative of 2 biological replicates.

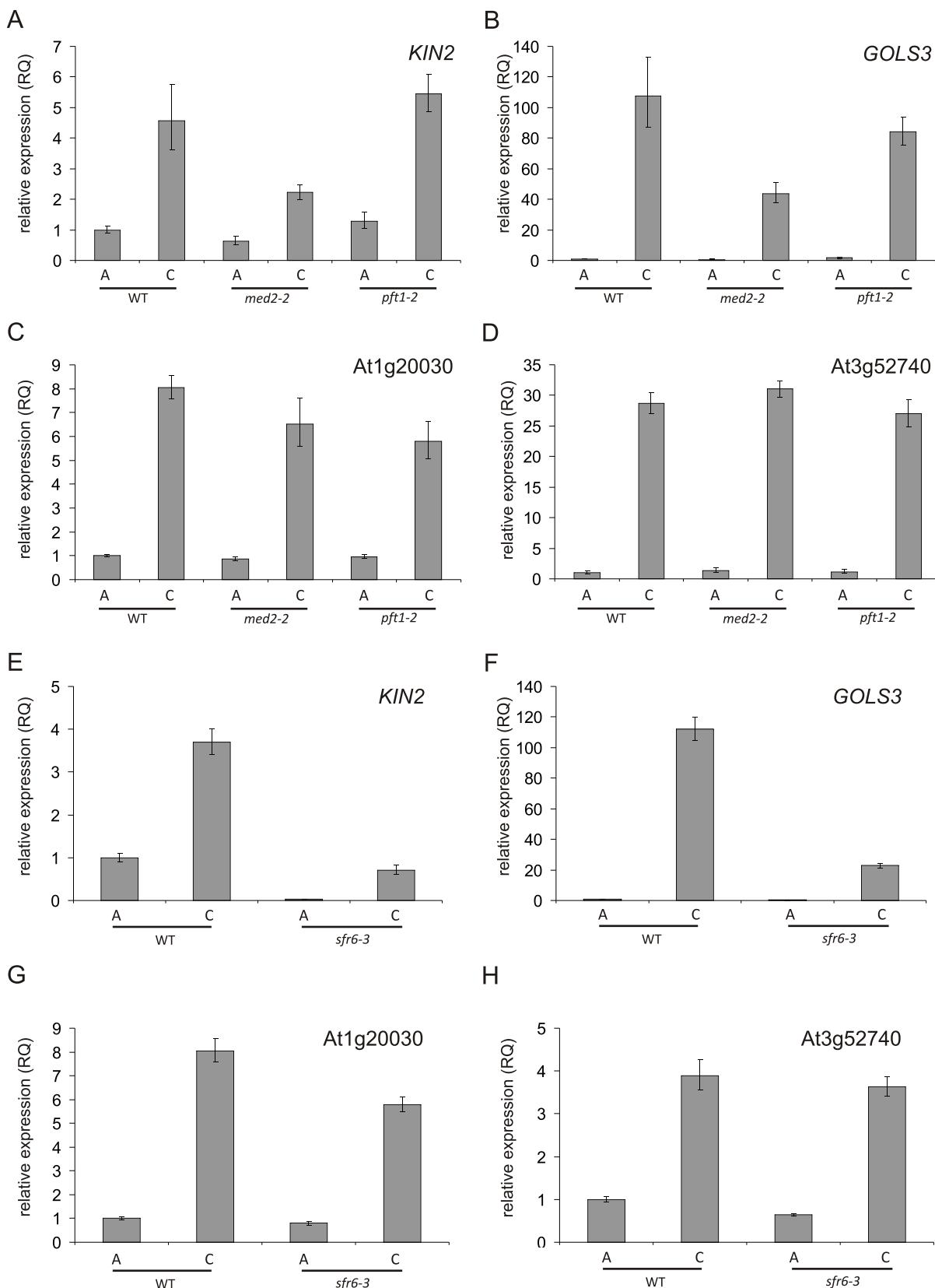


**Supplemental Figure 2. The ratio of RNA polymerase II occupancy at stall sites to occupancy at TSS sites in WT Col-0 and *sfr6-1*, *med2-1* and *med14-2* mutant plants after 4h at 5C is similar.** Data used to calculate ratios are the same as those shown in Figure 2 and Figure 6. Pol II abundance at the stall site is referenced to input and standardised to Pol II abundance at the TSS referenced to input. Each value represents the mean of three technical replicates for each sample. The error bars represent  $RQ_{\text{MIN}}$  and  $RQ_{\text{MAX}}$  and constitute the acceptable error level for a 95% confidence level according to Student's t-test.

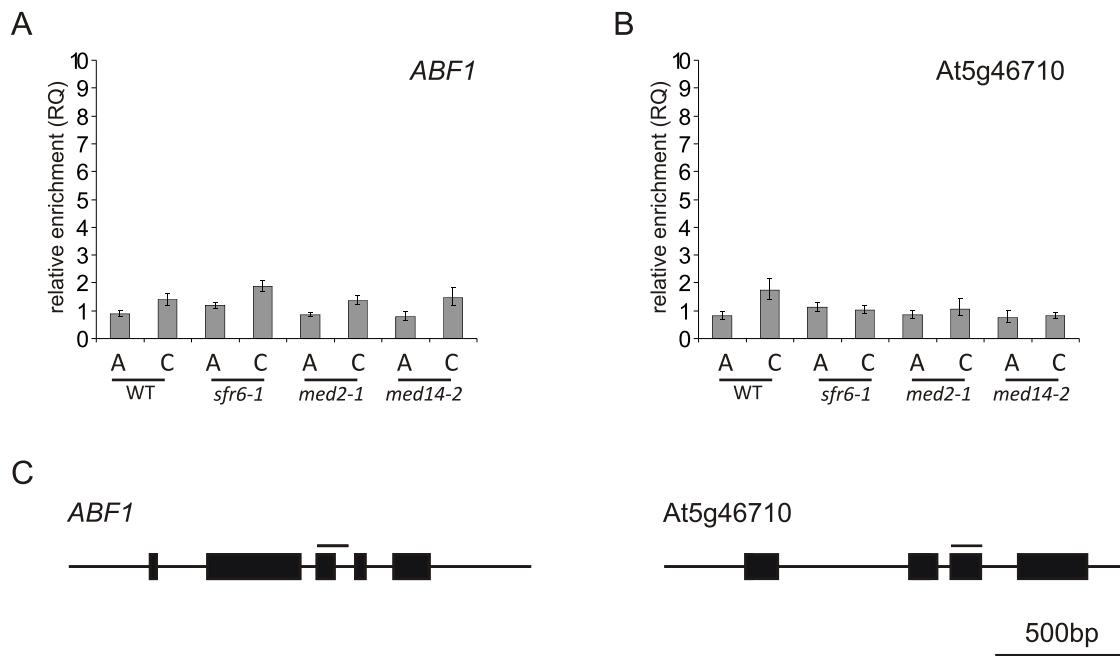




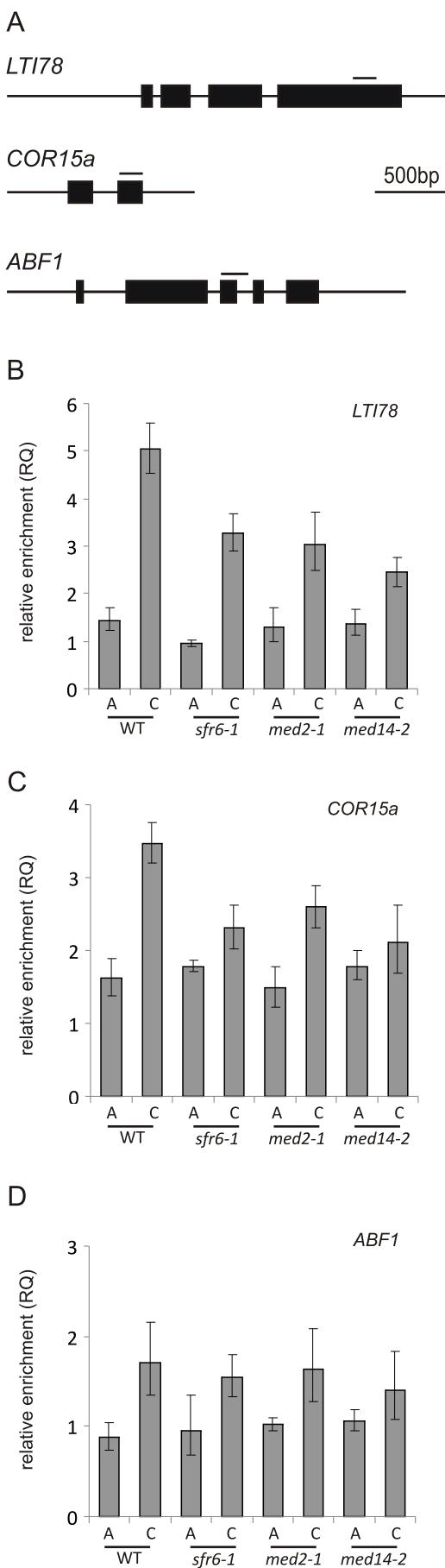
**Supplemental Figure 4. Mediator subunit transcript levels in Mediator subunit mutants.** Expression is shown after normalisation to *PEX4* using qRT-PCR. Relative quantitation of transcript was made from 8-day-old seedlings of WT Col-0 and mutant plants. Expression is shown after normalisation to *PEX4* using qRT-PCR. Each value represents the mean of three technical replicates for each sample normalised to the mean of three technical replicates for *PEX4*. The error bars represent  $RQ_{\text{MIN}}$  and  $RQ_{\text{MAX}}$  and constitute the acceptable error level for a 95% confidence level according to Student's t-test. Results are representative of at least 2 biological replicates.



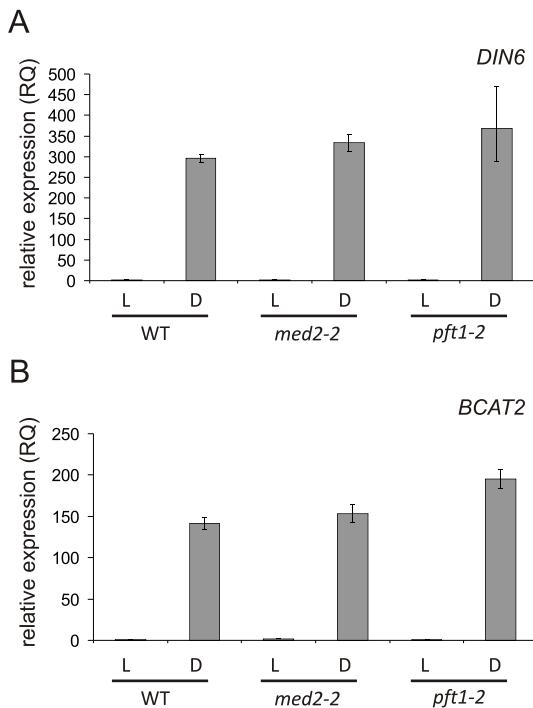
**Supplemental Figure 5. Cold-responsive gene expression in *med2-2*, *pft1-2* and *sfr6-3* compared with wild type is similar to that seen in *med2-1*, *pft1-1* and *sfr6-1* respectively. (A) to (D)** Expression of *KIN2* (A), *GOLS3* (B), At1g20030 (C) and At3g52740 (D) in Col-0 WT, *med2-2* and *pft1-2*. (E) to (H) Expression of *KIN2* (E), *GOLS3* (F), At1g20030 (G) and At3g52740 (H) in Col-0 WT and *sfr6-3*. Measurements were made on 8-day-old seedlings of WT Col-0 and Mediator subunit mutants exposed to cold (C) temperatures (5C) for 6 h or maintained at ambient (A) temperature (20C).



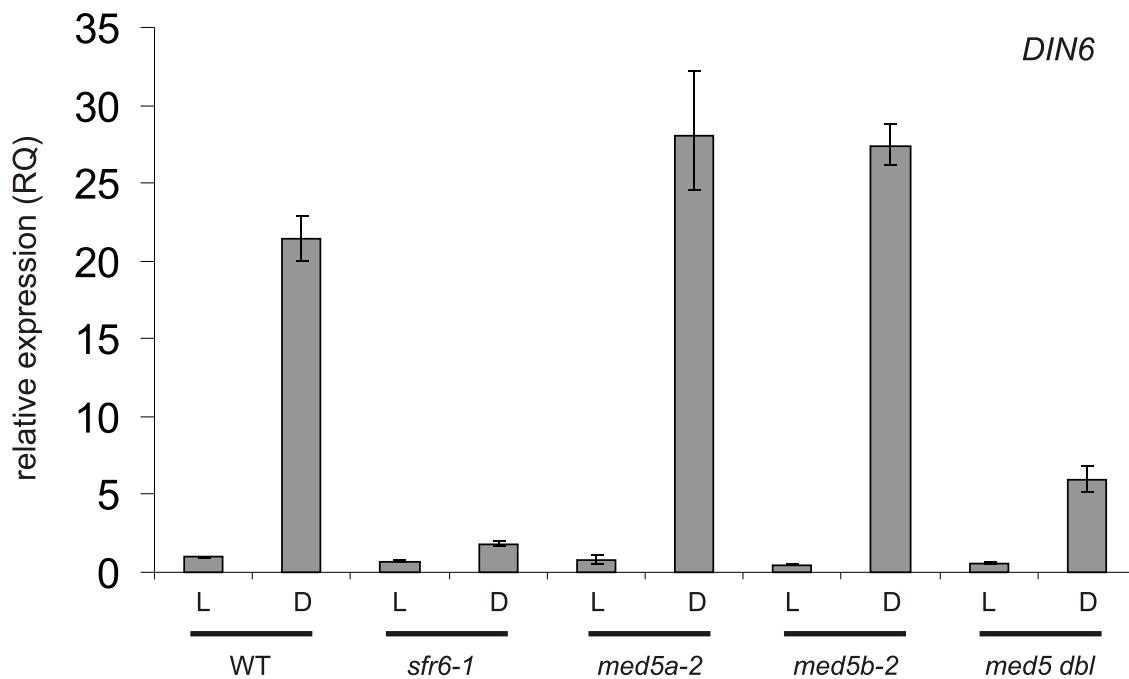
**Supplemental Figure 6. RNA polymerase II recruitment and progression along cold-responsive genes in WT Col-0, *sfr6-1*, *med2-1* and *med14-2* mutants.** (A) to (B) Pol II abundance was measured in 14-day-old seedlings of WT Col-0 and Mediator subunit mutants exposed to cold (C) temperatures (5C) or maintained at ambient (A) temperature (20C) for 6 h. in the middle of the transcribed region of *ABF1* (A) and At5g46710 (B) at the positions shown in (C). Thick black bars indicate the exons of the gene and thin black lines above show the regions amplified by the primers in each ChIP reaction. ChIP enrichment at each location is referenced to input and enrichment at each location was standardised to Pol II abundance at *PEX4* in the same ChIP sample. Each value represents the mean of three technical replicates for each sample normalised to the mean of three technical replicates for *PEX4*. The error bars represent  $RQ_{MIN}$  and  $RQ_{MAX}$  and constitute the acceptable error level for a 95% confidence level according to Student's t-test. Results are representative of 2 biological replicates.



**Supplemental Figure 7. Mediator recruitment to further CRT- and EE-containing genes.** ChIP qRT-PCR analysis of relative abundance of MED6 (a core Mediator subunit) at the transcribed region of genes in 14-day-old seedlings of Col-0 (WT), *sfr6-1*, *med2-1* and *med14-2* mutants. Mediator recruitment was monitored at the positions shown in **(A)**. Thick black bars indicate the exons of the gene and thin black lines above show the regions amplified by the primers in each ChIP reaction. **(B) to (C)** Mediator recruitment to two CBF-controlled CRT-containing genes, *LTI78* **(B)** and *COR15A* **(C)** increases in the cold and, as at *KIN2* and *GOLS3*, is impaired in *sfr6*, *med2* and *med14* mutants. **(D)** Mediator recruitment to one further EE-containing gene, *ABF1* increases slightly in response to cold but is unaffected in *sfr6*, *med2* and *med14* mutants. ChIP enrichment is referenced to input and standardised to MED6 abundance at *PEX4* in the same ChIP sample. Values were calculated using the  $\Delta\Delta C_T$  method and the error bars represent  $RQ_{MIN}$  and  $RQ_{MAX}$  and constitute the acceptable error level for a 95% confidence level according to Student's *t*-test. Data is from one preliminary experiment only and serves as a comparison with other replicated experiments on different genes of the same class. Error bars indicate the level of variation between technical replicates within this experiment.



**Supplemental Figure 8. Dark-responsive gene expression is not reduced in *med2-2* and *pft1-2*, similar to *med2-1* and *pft1-1* respectively.** Expression of *DIN6* (A) and *BCAT2* (B) in *med2-2* and *pft1-2*. Measurements were made on 8-day-old seedlings of WT Col-0 and Mediator subunit mutants exposed to darkness (D) or maintained in the light (L) for 6 h. Expression is shown after normalisation to *PEX4*. Each value represents the mean of three technical replicates for each sample normalised to the mean of three technical replicates for *PEX4*. The error bars represent  $RQ_{MIN}$  and  $RQ_{MAX}$  and constitute the acceptable error level for a 95% confidence level according to Student's t-test. Results are representative of 2 biological replicates.



**Supplemental Figure 9. Expression of dark-induced *DIN6* is reduced in *med5a**med5b* double mutants but not in *med5a* or *med5b* single mutants.** Measurements were made on 8-day-old seedlings of WT Col-0 and Mediator subunit mutants exposed to darkness (D) or maintained in the light (L) for 6 h. Expression is shown after normalisation to *PEX4*. Each value represents the mean of three technical replicates for each sample normalised to the mean of three technical replicates for *PEX4*. The error bars represent  $RQ_{\text{MIN}}$  and  $RQ_{\text{MAX}}$  and constitute the acceptable error level for a 95% confidence level according to Student's t-test. Results are representative of 2 biological replicates.

**Supplemental Table 1.** Table showing frequencies of occurrence of heptamer sequences in the promoters of the 81 genes misregulated in *sfr6* mutants, and probabilities of occurrence relative to whole genome. Ten motifs used to produce consensus for CRT/DRE (Figure 3) are denoted by superscript "a", and single EE-like motif by superscript "b".

Motif	Observed occurrence	Expected occurrence	Occurrence probability
gaccgac <sup>a</sup>	16	1.19	2.5x10 <sup>-13</sup>
atgtcg <sup>a</sup>	19	2.18	2.7x10 <sup>-12</sup>
aaatatc <sup>b</sup>	60	23.68	2.9x10 <sup>-10</sup>
cttcctc	25	5.75	2.6x10 <sup>-9</sup>
agaagaa	42	14.63	4.1x10 <sup>-9</sup>
gccgaca <sup>a</sup>	12	1.22	7.1x10 <sup>-9</sup>
accgaca <sup>a</sup>	16	2.48	9.6x10 <sup>-9</sup>
cgacatc <sup>a</sup>	14	1.85	1.1x10 <sup>-8</sup>
gtcggta <sup>a</sup>	13	1.67	2.7x10 <sup>-8</sup>
gtcggca	10	1.03	1.5x10 <sup>-7</sup>
agccgac	10	1.11	3.0x10 <sup>-7</sup>
acacgtg	15	3.18	1.4x10 <sup>-6</sup>
aataatg	38	16.26	2.9x10 <sup>-6</sup>
gccgacc	7	0.62	4.1x10 <sup>-6</sup>
aagaaga	37	15.87	4.1x10 <sup>-6</sup>
accgact <sup>a</sup>	11	1.87	4.4x10 <sup>-6</sup>
cacgtgg	11	1.88	4.6x10 <sup>-6</sup>
aggtcgg <sup>a</sup>	8	0.95	7.1x10 <sup>-6</sup>
aactgat	17	4.61	7.1x10 <sup>-6</sup>
aagaaaa	82	48.74	8.5x10 <sup>-6</sup>
acgtggc	10	1.66	9.5x10 <sup>-6</sup>
agagggaa	18	5.26	1.1x10 <sup>-5</sup>
acgtcgg <sup>a</sup>	8	1.1	2.0x10 <sup>-5</sup>
cgtggca	9	1.45	2.1x10 <sup>-5</sup>
cacacac	15	4.22	3.6x10 <sup>-5</sup>
attatta <sup>c</sup>	49	26.11	4.1x10 <sup>-5</sup>

**Supplemental Table 2.** Table showing frequencies of occurrence of heptamer sequences in the promoters of the 81 genes not misregulated in *sfr6* mutants, and probabilities of occurrence relative to whole genome.

Motif	Observed occurrences	Expected occurrences	Occurrence probability
aggccca	24	2.27	6.4x10 <sup>-17</sup>
ggcccaa	24	3.66	1.6x10 <sup>-12</sup>
aacccta	24	3.9	5.7x10 <sup>-12</sup>
aagccca	27	5.1	8.8x10 <sup>-12</sup>
cttcttc	26	5.9	9.6x10 <sup>-10</sup>
agcccat	20	3.56	1.5x10 <sup>-9</sup>
accctaa	19	3.72	1.7x10 <sup>-8</sup>
agaagaa	38	13.37	2.8x10 <sup>-8</sup>
atgggcc	15	2.5	6.9x10 <sup>-8</sup>
gggccta	12	1.53	8.3x10 <sup>-8</sup>
aaaccct	23	6.53	4.2x10 <sup>-7</sup>
aaacaaa	82	45.06	4.9x10 <sup>-7</sup>
attgggc	17	4.04	1.3x10 <sup>-6</sup>
ccctaaa	19	5.06	1.6x10 <sup>-6</sup>
acacgcg	9	1.06	1.8x10 <sup>-6</sup>
accctag	10	1.6	7.1x10 <sup>-6</sup>
aatgggc	15	3.81	1.1x10 <sup>-5</sup>
aaagccc	18	5.67	2.8x10 <sup>-5</sup>
agcccaa	17	5.23	3.4x10 <sup>-5</sup>
aagaaga	32	14.75	6.7x10 <sup>-5</sup>
taattaa	41	21.35	1.0x10 <sup>-4</sup>
gcccata	12	3.18	1.2x10 <sup>-4</sup>
aacaaaa	72	45.06	1.3x10 <sup>-4</sup>

**Supplemental Table 3****Oligonucleotides used in this study.****Primers for cloning**

Gene	forward primer	reverse primer
<i>CBF1</i>	CCTCTAGAGGTACCATGAACTCATTTCAGCTT	GGCTCGAGGTAACTCCAAAGCGACACGT

**Primers for real time qRT-PCR detection of transcripts**

Gene	AGI code	forward primer	reverse primer
<i>PEX4</i>	At5g25760	TCATAGCATTGATGGCTCATCCT	ACCCCTCTCACATCACCAGATCTTAG
<i>KIN2</i>	At5g15970	CAACAGGCGGGAAAGAGATAT	CAACAACAAGTACGATGAGTACGA
<i>GOLS3</i>	At1g09350	CAAAGTTGCCCTCCCACAC	GAGCATGGCCAAGACAAGAT
<i>At3g52740</i>	At3g52740	GAAGCATCGGAGAGAGATCG	AGCAGTACGTGCAGACGAGA
<i>At1g20030</i>	At1g20030	TTTCGCTCCCTGAAGAAGAA	CCTTGACATAACTCCGGAGAAG
<i>At1g68500</i>	At1g68500	AGCTCTGTGTTGCCCTT	CGTCCCAAACAACATCATTG
<i>At5g62360</i>	At5g62360	AGCCATGCTCAAATTGGTC	TGGGTACGTGTTGAAAGTGC
<i>At5g46710</i>	At5g46710	CCATGAAACTTGCATTGAGC	CGAGAACCCAAATCGAGAAA
<i>ABF1</i>	At1g49720	AATGAAAACCCATAATAGTGGAGTAA	ATTGTCTTTGGCCAGCAAT
<i>SZF2</i>	At2g40140	TCAAAAAACCCCAACCACTTC	TGCACCGCACATATCTCTTC
<i>LT178</i>	At5g52310	GCACCCAGAAGAAGTTGAACA	TCATGCTCATTGCTTTGTCC
<i>COR15A</i>	At2g42540	CGTTGATCTACGCCGCTAAAG	CTCACCATCTGCTAATGCC
<i>DIN6</i>	At3g47340	GGCCAAGAGAGTTCGTGTT	AGACGTTGATGGGCAAGTA
<i>BCAT2</i>	At1g10070	CGCAAAACTCTGGTCTACCTC	GATGCAGCTTGTGCGTTGA
<i>SFR6</i>	At4g04920	CGCTGAGAGATTCTGGTGA	TGAAACTATCCCACCTCTGC
<i>MED14</i>	At3g04740	TAGCTTGGTTCAAGGCGTT	GGCCATACTGGTCAGGAGA
<i>PFT1</i>	At1g25540	GGAACGGAATGAGAATGGT	ATTGTTGAAGCTGCTTTGG
<i>MED2 (MED32)</i>	At1g11760	GCGACTAGTCTCCCTCCATT	CCATCGAACCGCTACTCATC
<i>MED5a (MED33a; RFR1)</i>	At3g23590	GCCCTGCATGCCATTGTA	GGAAGTCAAACCAACGTTCG
<i>MED5b (MED33b; REF4)</i>	At2g48110	GCCCTGCATGCCTATTG	TCGCTCCAGCGCTTCACT
<i>MED8</i>	At2g03070	AAAGTCAGATACCAGCACTTCAT	CTGTCCTTGAGACATGCTATGC

**Primers for ChIP**

Gene/position	AGI code	forward primer	reverse primer
<i>GOLS3</i> pro	At1g09350	TCGGTACGTCTTTCTTTCA	CTTGTAAAGCAAAATGCTCAA
<i>KIN2</i> pro	At5g15970	AAACGACACGTGATGTCCTGA	TGCCACGTGTAATCTGAAACC
<i>PEX4</i> pro	At5g25760	CTCGCCGGATCAATTCTAG	TAAGGAAACGAAACCGGATG
<i>TSI-A</i>	silent	CTCGTGGGGTTGTGAGGT	AGTATAAGCGCCACCCAGT
<i>PEX4</i> Mid	At5g25760	CTTGGACGCTTCAGTCTGT	GAACGCCCATGGTACATT
<i>GOLS3</i> -500	At1g09350	TGCATATTTTACCAAAACAAAAA	ATTTTCTCTTTGTCTAACGTC
<i>GOLS3</i> TSS	At1g09350	CCATAATCACGGCTCAAAG	TCATCTCAGGTGCCATCTTG
<i>GOLS3</i> Stall	At1g09350	GGAACGGAGACTACGTGAA	GCCTTGGTCCAATAGCTGTC
<i>GOLS3</i> Mid	At1g09350	TGGGGCAGCTACCACTATACA	GAGCATGGCCAAGACAAGAT
<i>GOLS3</i> End	At1g09350	TTCACAGGAAACCGAAAACC	ACAAGAACCTCGCTCGTCAG
<i>GOLS3</i> 3IGR	At1g09350	TGCAATTGGTAAGTTCTCATTT	TTTAGAAGAGATTGTGTTGCAT
<i>KIN2</i> -500	At5g15970	ATGAAAATACGGGAGGTTCG	GTTGTTGCATATGCGTTGG
<i>KIN2</i> TSS	At5g15970	GATTACACGTGGCACACAC	GGAAGGCATTCTGTTGGTC
<i>KIN2</i> Stall	At5g15970	GCTGGCAAAGCTGAGGTA	CCATGGTCAACACAAAATCAA
<i>KIN2</i> End	At5g15970	AGTATATCGGATGCGGCAGT	TCCCCAATTATTATTGAAAATCC
<i>KIN2</i> 3IGR	At5g15970	CGAAATCGTTGTGGTCAATG	ACAATGACGCGAGACAAACA
<i>At3g52740</i> -500	At3g52740	AGTTGAGTTGGTCACACAAAAA	AACCTAAAATTCTATCAAGTGTACAG
<i>At3g52740</i> TSS	At3g52740	CGTCGACGTGTAAGACAACC	TTGCAGGAAACAAAAAGATGAA
<i>At3g52740</i> Stall	At3g52740	TGATGAACATCGACGATACGA	GCTTCGGTTCTCTCAAGC
<i>At3g52740</i> End	At3g52740	TCGTCTGCACGTACTGCTCT	TTGAGGAACAAGAACACATT
<i>At3g52740</i> 3IGR	At3g52740	TGCAATGTGTCGCTCTAAA	TAGGAAAACGGGCAAATGTC
<i>At1g20030</i> TSS2	At1g20030	CATGAGAAAAAGAAAAAGATCTCG	AAGAAAAGAGAGAATAAACATTAGCC
<i>At1g20030</i> Mid	At1g20030	GAATAGCGTCGACGGGAAG	GTAGCTATAGGCGCGTGGAC
<i>At1g20030</i> End	At1g20030	TGTGGTGGTTCTCTGTACCA	CAACGTTGGTCTGTGGAGT
<i>At1g20030</i> -250	At1g20030	AATGGTTCTTAGGACACTTGAA	TTTATGGCTGGAGTTGGA
<i>At1g20030</i> 3IGR	At1g20030	ACATGGGCTATTCCCAACG	TTAGGCCTGGAGGAGTTC
<i>At5g46710</i> MID	At5g46710	TGCCTGCATGGTCTGTATC	CAAGGGAGGTAATGGATGCT
<i>LT178</i> MID	At5g52310	GACTCCGGTCAATGAGAAGG	CCGCCACATAATCTCTACCC
<i>COR15a</i> MID	At2g42540	CAGATGGTGAGAAAGCGAAAG	CTTGTGCGGGCTTCTTTTC
<i>ABF1</i> MID	At1g49720	CAGGCTTACCTGGAACTGG	TTCAGCCTGCAACATAGAGAGA