Supplemental Data, Achard et al., 2008 The cold-inducible CBF1 factor-dependent signalling pathway modulates the accumulation of the growth repressing DELLA proteins via its effect on gibberellin metabolism.



Supplemental Figure 1. pRGA:GFP-RGA transgenic line expresses a GFP-RGA fusion protein that is detectable by western-blot using an anti-GFP antibody. Immunodetection of GFP-RGA (by an antibody to GFP) in untreated (Mock) or GA₃-treated (10 mM for 1 h) in 7 day-old pRGA:GFP-RGA seedling. WT sample serves as negative control. PSTAIRE serves as loading control.



Supplemental Figure 2. Expression of *CBF1* in T3 transgenic *CBF1*-ox lines. Each lane was loaded with 15 μ g of total RNA prepared from 2 week-old T3 transgenic *CBF1*-ox lines and WT (2 h cold-treated [CT] and control). The northern-blot was hybridized with a specific probe for *CBF1*. The transgenic *CBF1*-ox line #9 expresses *CBF1* to similar level to WT plants that have been cold-treated for 2 h. This line was therefore selected for further studies.



Supplemental Figure 3. *Arabidopsis* DELLAs contribute to freezing tolerance. 2 week-old WT, *gai, gai-t6 rga-24, CBF1*-ox and *CBF1*-ox *gai-t6 rga-24* plants grown in a growth room (22°C; 16 h photoperiod) were placed at -10°C for 24 h. Plants were then placed at 4°C for 6 h for recovery before being returned to the original growth room. Photographs were taken after 4 days of recovery under normal growth conditions. Plant survival rate is summarized in Figure 6.



Supplemental Figure 4. Cold enhances both CBF-regulon and sugar levels via a DELLAindependent mechanism. (A) Relative levels of *RD29a*, *COR15a*, *COR47* and *P5CS2* gene transcripts (determined by real-time RT-PCR) in 2 week-old WT, gai and gai-t6 rga-24 seedlings that had been subjected to 4 h cold (light grey) and controls non cold-treated (black). Data are means \pm SE. (B) Mean (\pm SE) total soluble sugars (glucose, fructose and sucrose) were determined from leaf tissue of 2 week-old WT, gai and gai-t6 rga-24 coldacclimated (dark grey) and non-acclimated (light grey) plants. The levels of individual sugars were determined by enzymatic assays.



Supplemental Figure 5. Cold-treatment slows at the same level the vegetative growth of WT and quadruple-DELLA mutant plants. Flowering time (time at which 50% of plants had bolted; \pm SD, n>20) of WT (dark grey) and quadruple-DELLA mutant (light grey) plants grown in soil in long days (16-h photoperiod, 22°C). At 14 day-old, plants were cold-treated (4°C) for 3 or 7 days (and controls, untreated) as indicated, then replaced at 22°C.

Primer list.

GA20ox1: TTTCACCGGACGCTTCTCC and CGCAAAACCGGAAAGAAAGG GA20ox2: CGATCTCTCAAGCCAAGACTCG and TCGCTGACGCCATGATTG GA20ox3: CAACCTCTCCAAGTCCCACTCA and AGTAGCCTCCGATGCCAAGC GA3ox1: TCCCGGATTCTTACAAGTGGAC and GCCGGAGGAGAAGGAGCA GA3ox2: CCCCTCCACGATTTCCGTA and TGCGAACCACATCAACTTGG GA2ox1: CTTCGCTGGACCTTCATTGAC and ACAACCTCTCGTCCTCATTGTCT GA2ox2: GGACCAAACGGTGACGTTG and GTACTCCTCCACCGACTCACG GA2ox3: GGCACACCCCTGCAATTTT and CCAGAAATTTGCTCGACATTCTC GA2ox4: GATGGCATGTGGGTTTCTGTC and TCTCCCGTTCGTCATCACCT GA2ox6: CCACGCAAATCCGACAGC and GCCAAATCTCTAACCGTGCGTA RD29a: GGGTAGAGATTATGTGGCGGAGA and TTCCTTTGTCGTCGTTTCCTTC COR15a: CTCTGCCGCCTTGTTTGC and CTGAGAAAGCTGCGGCGTA COR47: CCGAGCACGAGACACCAAC and TCCACGATCCGTAACCTCTGTT P5CS2: CGAAAATCCCAGTGCTAGGC and TGCCATGTCCAGTTTACCAGACT GAPDH: TTGGTGACAACAGGTCAAGCA and AAACTTGTCGCTCAATGCAATC At4g26410: GAGCTGAAGTGGCTTCAATGAC and GGTCCGACATACCCATGATCC RGA: GATGTTGGGTTGGCACACTC and GACCTACCAAAACGATATATATA GAI: GCTCTGTTCAACGGCGGTG and GAAATTTTTCAATTACCTAATATAATG RGL1: GTTGCTTGGATGGCAAACGC and ATCATTTCATTGGCCTGACCCTG RGL2: TGGCAGACGCGACCACTCAT and TACTCGTTCTCTTAACTCTCAA RGL3: ACCTCTAATCGCTGCATCGG and CTGTTGTTACATACACACATG ELF1α: TTGCTCCCACAGGATTGACCACTG and TCACTTCGCACCCTTCTTGACG CBF1: GCTGAGATGGCAGCTCGTGC and CTATCGAATATTAGTAACTCC