Figure S1. Sucrose-regulated genes make coordinate contributions to the separation of experiments by principal component 2.
Following principal component analysis (PCA) (Figure 1) to identify the major differences between independent experiments to identify cold-responsive genes, we plotted the loadings for the first two principal components against each other. To visualize the contribution of sugar-regulation, we then overplotted these to indicate genes reported to be induced (blue) or repressed (red) 6 h after sucrose addition to seedlings (Solfanelli et al., 2005).

Figure S2. Relationship between diurnal, circadian and sucrose regulated genes overlapping with those contributing to variance between cold experiments.
Following principal component analysis (PCA) we extracted the top 500 genes contributing to principal components (PC) 1-5 and compared them to diagnostic sets of diurnal, circadian and sucrose-regulated genes (Table II), which are represented by red, green and blue, respectively. Proportional area Venn diagrams (http://www.venndiagram.tk/) show the relationship between the three diagnostic sets for each PC. For example, diurnal/circadian-regulated genes have a large contribution to PC 1 and 4, whilst diurnal/sugar regulation have most influence for PC 2.

Figure S3. The oscillations of circadian clock components are dampened in light-dark cycles in the cold.
Experimental repeat of Figure 3 in which plants were grown under low light, rather than normal light. Targeted expression analysis for several circadian clock (black text), circadian output (dark red text) and cold-regulated (blue text) genes was performed using quantitative RT-PCR. Plants were grown under warm diurnal conditions under low light in long-days (16 h) before transfer to 4°C 8 h after dawn. Whole rosettes were sampled from individual plants every 4h across the first day in warm conditions and for days 1, 2, 7 and 14 in the cold. The y axis shows raw expression (Ct – log2 scale) values normalized by subtracting the mean of three control genes. The x axis shows time after dawn with night shown in dark grey. Data are means from 3 biological replicate plants. Standard deviations are not shown for clarity but averaged 0.5 Ct.

Figure S4. The oscillations of circadian clock components are stopped in continuous light in the cold.

Experimental repeat of Figure 4 in which plants were grown under low light and transferred at ZT8, rather than ZT14. Targeted expression analysis for several circadian clock (black text), circadian output (dark red text) and cold-regulated (blue text) genes was performed using quantitative RT-PCR. Plants were grown under warm diurnal conditions under low light in long-days (16 h) before transfer to continuous light at 20°C or 4°C 8 h after dawn. Whole rosettes were sampled from individual plants every 4 h across until 64 h. The y axis shows raw expression (Ct – log2 scale) values normalized by subtracting the mean of three control genes. The x axis shows time after subjective dawn with subjective night shown in light grey. Data are means from 3 biological replicate plants. Standard deviations are not shown for clarity but averaged 0.8 Ct.

Figure S5. Experiment and replicate-specific bias in the cold-response of circadian-regulated genes that peak at different phases of the day.

As for Figure 6, but with replicates plotted separately. The overlap between circadian-regulated genes that peak at different phases (Edwards et al., 2006) of the day (ZT – time after subjective dawn) and those responding to cold in independent studies (Table I) were compared. For direct comparability we selected the 1000 most induced (blue) and 1000 most repressed (red) genes in each experiment and made the comparison using Fisher exact tests. Experiments are lettered as in Table I and labeled as in Figure 1; lowercase letters denote soil grown plants; colors indicate the light-regime; red, continuous light for control and cold; blue, diurnal for control and continuous light for cold; green, diurnal for control and cold. Plotted are bars showing the log odds ratios which show whether the genes at a specific phase are more or less likely to be cold-responsive than expected by chance. Significance (fdr corrected p-value < 0.05) is denoted by solid bars whilst non-significant log odd ratios are hatched.

Table S1. The cold-responsive transcriptome shows significant correlation between independent experiments.

We investigated the correlation between cold-responsive genes from several independent studies investigating gene expression after one day of cold treatment (Table I). GCRMA expression estimates (Wu et al., 2004) were used to calculate the cold minus control log2 differences for each replicate for the 16640 probesets that were detected in at least one experiment. Pearson correlation (r) values are shown and values greater than 0.6, 0.7 and 0.8 are highlighted in yellow, orange and red, respectively. Experiments are lettered as in Table I and labeled as in Figure 1; lowercase letters denote soil grown plants; colors indicate the light-regime; red, continuous light for control and cold; blue, diurnal for control and continuous light for cold; green, diurnal for control and cold.

Table S2. There are massive amounts of differences in cold-responsive genes between independent experiments.

We investigated the disagreement in the identity of cold-responsive genes between several independent studies investigating gene expression after one day of cold treatment (Table I). It is common for thousands of differences between different experiments, often in excess of 50% of all identified changes. GCRMA expression estimates (Wu et al., 2004) were used to calculate the cold minus control log2 differences for each replicate for the 16640 probesets that were detected in at least one experiment. Genes that were changed two-fold in one experiment but not the other were counted and divided by the total number of two-fold changed. Values greater than 0.4, 0.5 and 0.6 are highlighted in yellow, orange and red, respectively. Experiments are lettered as in Table I and labeled as in Figure 1; lowercase letters denote soil grown plants; colors indicate the light-regime; red, continuous light for control and cold; blue, diurnal for control and continuous light for cold; green, diurnal for control and cold.

Table S3. Primers used in this study.