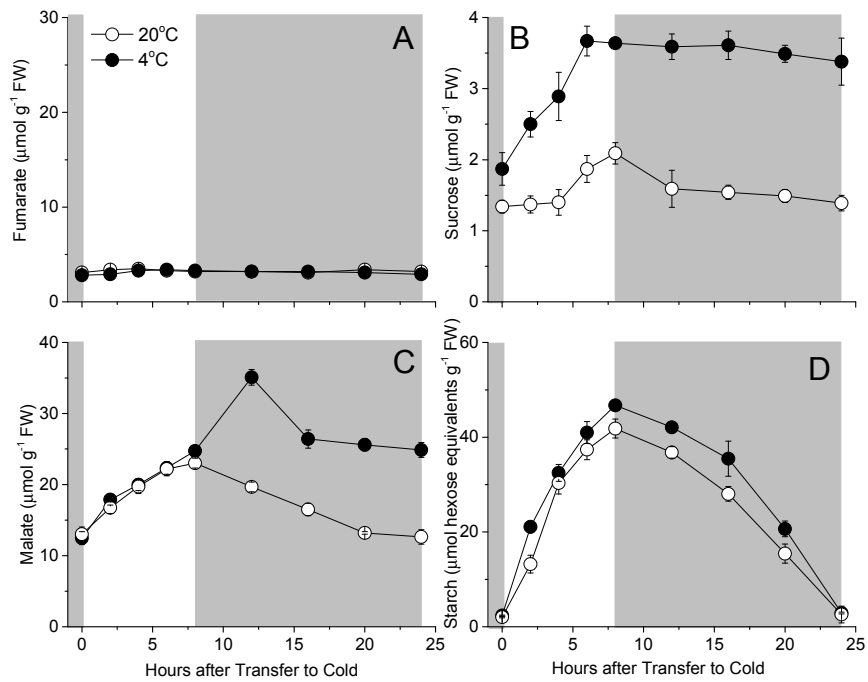
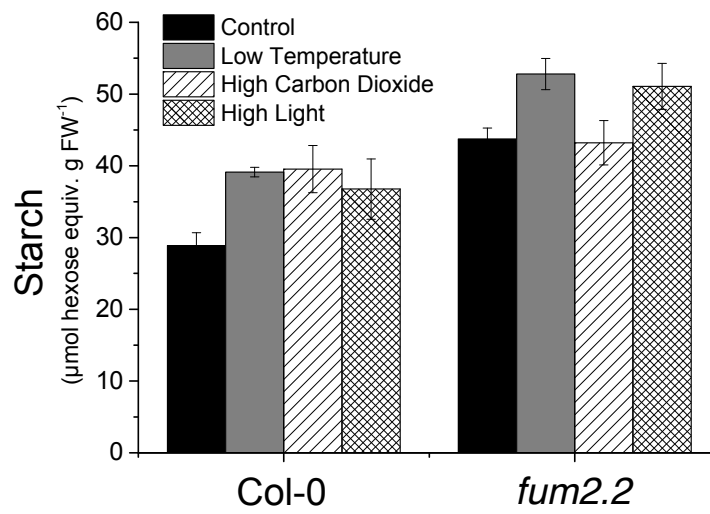


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2 **Figure S1 Principal Component Analysis of gas chromatography electron ionization time-of-flight**  
 3 **mass spectrometry (GC-EI-TOF/MS) data from leaf material of *fum2.2* at 20 or 4 °C.** Plants of  
 4 *fum2.2* were grown under  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  light, 8 h light/16 h dark,  $500 \mu\text{L L}^{-1} \text{CO}_2$ , 20 °C light/18 °C  
 5 dark conditions for 8 w, before being transferred to 4 °C for a single photoperiod. Fully expanded  
 6 leaves were flash-frozen and excised at 0, 4 and 8 h after the photoperiod began. Circles are  
 7 indicative only and have no statistical significance. (A) PCA constructed from the GC-EI-TOF/MS data  
 8 from *fum2.2* line during the first photoperiod after transfer to 4 °C. Ten PCs were used in the  
 9 calculation, accounting for 98.9% of the total variance. PC1 explained 65.9% of the total variance,  
 10 with PC2 explaining a further 27.1% of the total variance. (B) Loadings plot of PCA data, indicating  
 11 metabolites responsible for the variance in the data. In (B) named metabolites are those judged not  
 12 to cluster with the majority of metabolites: organic acids are represented by filled circles, sugars by  
 13 grey circles, amino acids by open circles, sugar alcohols by grey squares and unknown metabolites by  
 14 striped circles.



**Figure S2. Diurnal metabolite levels under low temperature conditions in *fum2.1*.** Fumarate (A), sucrose (B), malate (C) and starch (D) levels were determined throughout a 24 h period in plants exposed to low temperature. Plants of *fum2.1* were grown under  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  light, 8 h light/16 h dark,  $500 \mu\text{L L}^{-1} \text{CO}_2$ , 20 °C light/18 °C dark conditions for 8 w, then transferred to low temperature (4 °C) for a 24 h period. Fully expanded leaves were excised and flash-frozen from individual plants at 2 h intervals in the light, and 4 h intervals in the dark.

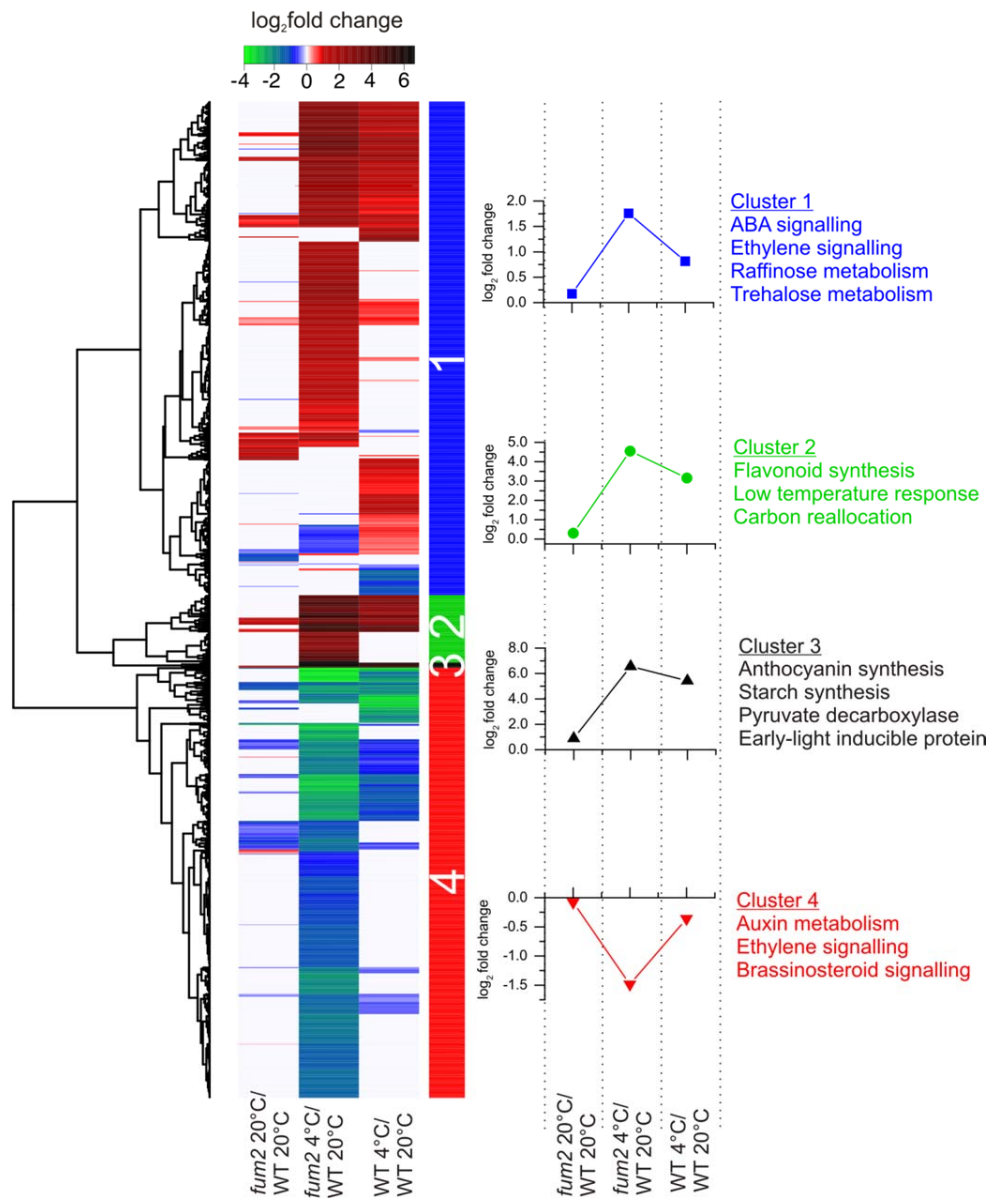


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3 **Figure S3. Starch levels determined by enzyme linked assays under different environmental**  
 4 **conditions.** Plants of Col-0 and *fum2.2* were grown under  $100 \mu\text{mol m}^2 \text{s}^{-1}$  light, 8 h light/16 h dark,  
 5  $500 \mu\text{L L}^{-1}$   $\text{CO}_2$ ,  $20 \text{ }^\circ\text{C}$  light/ $18 \text{ }^\circ\text{C}$  dark conditions for 8 w. Plants were then transferred to control  
 6 (identical) conditions, low temperature ( $4 \text{ }^\circ\text{C}$ ), high carbon dioxide ( $1500 \mu\text{L L}^{-1}$ ) or high light ( $400$   
 7  $\mu\text{mol m}^2 \text{s}^{-1}$  light) for one photoperiod. Measurements were taken at the end of the photoperiod (8  
 8 h) from liquid  $\text{N}_2$  flash-frozen, fully-expanded leaves, as in Fig. 2.

9

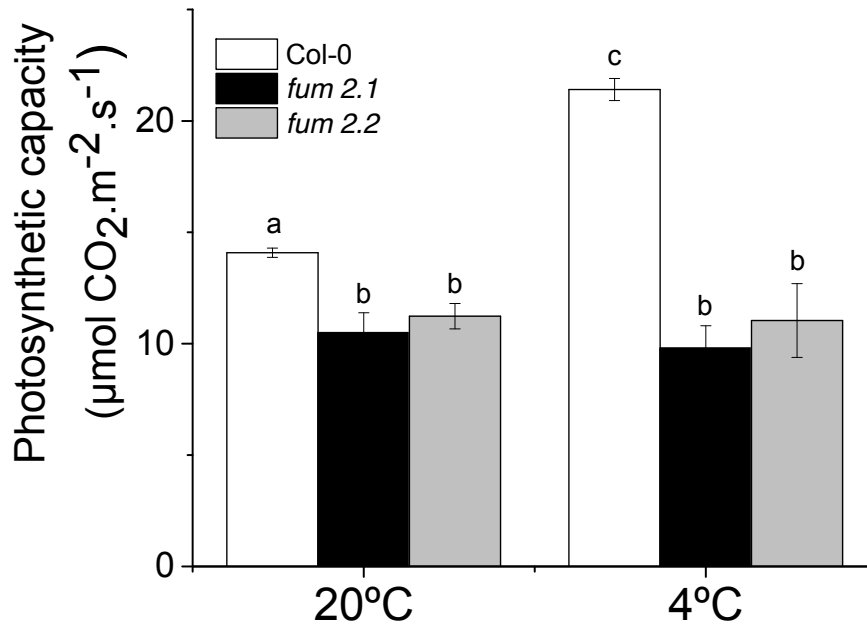


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3 **Figure S4. Cluster analysis of changes in gene expression during acclimation to low temperature in**  
 4 **WT and *fum2*.** A heat map was constructed from the results of the cluster analysis after 8 hrs at 4°C  
 5 comparing fold changes in genes relative to the WT at 20°C. Only genes that were differentially  
 6 expressed ( $P < 0.05$ , Fold change  $> 2$ ) between the 2 genotypes at 20°C or 4°C were included. Clusters  
 7 were assigned according to the colored bar in the center of the figure, and average log<sub>2</sub> fold change  
 8 graphs are shown for each cluster. Clustering was done using the Euclidean distance method using  
 9 complete linkages. Ontological groups over-represented in each cluster were defined using DAVID  
 10 (17)

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2 **Figure S5.** Photosynthetic capacity in plants of Col-0, *fum2.1* and *fum2.2* grown at 20 °C for 8 w and  
 3 in plants then acclimated to 4 °C for 7 d. Each bar represents the mean +/- standard error of at least  
 4 3 replicate plants, letters indicate significantly different values (ANOVA  $p < 0.05$ ). Photosynthetic  
 5 capacity is taken as the rate of photosynthesis measured on attached leaves at 20°C, following 20  
 6 mins illumination at  $2000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , in an atmosphere of  $2000 \mu\text{L L}^{-1} \text{CO}_2$

7