

Supplemental Experimental Procedures

2D-DIGE analysis—Total proteins were extracted from CA or DA-treated samples using TCA-Acetone extraction methods (Amme *et al.* 2006) and were dissolved with lysis-buffer [30 mM Tris-HCl (pH 8.5), 8 M urea, 2% (w/v) CHAPS]. 2D-DIGE analysis was performed with CyDye DIGE Fluors (minimal dyes, GE Healthcare) and experimental details were previously described (Minami *et al.* 2009). 25 µg of total proteins were minimally labeled with Cy3 or Cy5 (GE Healthcare). Equal amounts of proteins for all time point of samples were mixed and labeled with Cy2 and analyzed as internal standard. Two independent samples and standards (Cy3, Cy5 and Cy2 labeled samples) were mixed and the volume was adjusted to a total of 350 µl with rehydration buffer [2× sample buffer containing 40 mM dithiothreitol and 1% (v/v) IPG buffer]. These diluted samples were then loaded onto isoelectrofocusing gel strips with an immobilized linear pH gradient (Immobiline™ DryStrip pH 4-7 NL, 18 cm). The strips were rehydrated at 20°C for 12 h at 100 V with the samples and were then isoelectrofocused at 20°C using the IPGphor system with progressive increases in voltage [500V (1h), 1,000V (1h), 2,000V (1h), 4,000V (1h), 5,000V (1h), 6,000V (2h) and 8,000V (7h)]. Gel strips were subjected to 10% SDS-PAGE analysis after equilibration

(Laemmli 1970). Three CyDye images were scanned with a Molecular Imager FX (Bio-Rad, Hercules, CA) and data were analyzed using the PDQuest V 8.0 software (Bio-Rad).

Amme, S., Matros, A., Schlesier, B., and Mock, H. P. (2006) Proteome analysis of cold stress response in *Arabidopsis thaliana* using DIGE-technology. *J Exp Bot* 57, 1537-1546

Minami, A., Fujiwara, M., Furuto, A., Fukao, Y., Yamashita, T., Kamo, M., Kawamura, Y., and Uemura, M. (2009) Alterations in detergent-resistant plasma membrane microdomains in *Arabidopsis thaliana* during cold acclimation. *Plant Cell Physiol* 50, 341-359

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685