

Supplemental Figure 1. Cold and salt treatments modulate hydrogen peroxide levels.

(Á, B) Amplex red assay showing relative H₂O₂ levels in wild-type Landsberg (Wt) seedlings. (A) Cold and (B) Salt stress (120 min) treatments were performed as described in Fig. 1A and Fig. 2A respectively. Treatment with DCMU was performed as described in Fig. 3B,C and sampling was performed after 120 min of stress treatments. Error bars represent standard deviation of at least 7 biological replicates. Welch's t-test P-value *<0.05, ***<0.0005, ****<0.0001.
(C, D) Representative images of Wt seedling leaves stained with the ROS sensitive dye H₂DCFDA after 120 min of (C) cold and (D) salt stress treatments as descibed in Fig. 1A, B respectively. Shown are the superimposed images with oxidized H₂DCFDA represented in green and chlorophyll in red. Scale bars are 10 µm.



Supplemental Figure 2. Loss of *GCN2* renders increased sensitivity towards cold stress in the Columbia ecotype.

(A) Top - Representative images of 3-days-old wild-type Columbia (Wt (Col)) and *gcn2-2* mutant (*gcn2-2*) seedlings grown under 16 h light and 8 h dark long day (LD) cycle at 22°C. Seedlings were grown on media with 0.1% sucrose for 3-days and transferred to no sucrose (Day 0).

Bottom - Same seedlings after 30 days of LD cycle at 4°C. Scale bars are 10 mm.

(B) Primary root length of Wt and *gcn2-2* mutant seedlings from panel A. Error bars indicate standard error of the mean from four biological replicates with n>80 per experiment. (Welch's t-test *P-value <0.05)



Supplemental Figure 3 . Effect of cold stress on photosynthetic efficiency of wild-type and gcn2 mutants. Time course analysis of photosystem II maximal quantum yield (Qymax (Fv/Fm)) in rosette stage plants of (A) wild-type Landsberg (Wt (Ler)) and gcn2-1 mutant , (B) wild-type Columbia (Wt(CoI)) and gcn2-2 mutants under cold stress at 4°C and recovery at 22°C. Error bars represent standard error of the mean from three biological replicates. Welch's t-test P-value >0.5.



Supplemental Figure 4. Loss of GCN2 renders higher sensitivity towards salt stress in Columbia ecotype

(A) Representative images of wild-type Columbia (Wt (Col)) and gcn2-2 mutant (gcn2-2) seedlings grown under 16 h light and 8 h dark period. After 3-days on 0.1% sucrose plant media, seedlings were transferred to a fresh plate (Day 0) on the same medium (Mock), or supplemented with 150mM NaCl (salt treatment), or 300mM mannitol (osmotic control). Scale bar is 10mm. Bleached seedlings are marked with an asterisk and were excluded from root length measurements. (B) Primary root length of Wt and gcn2-2 mutants from panel (A). Error bars indicate standard error of the mean of four biological replicates with n>36 per experiment (Welch's t-test ** P-value <0.005; *** P-value <0.0005).

Mock

Mannitol

NaCl



Supplemental Figure 5 . Effect of salt stress on photosynthetic efficiency of wild-type and gcn2. Time course analysis (Day) of PSII maximal quantum yield (Qymax (Fv/Fm)) of wild-type Landsberg (Wt (Ler)) and gcn2-1 mutant (gcn2-1) seedlings after transfer to 0.1% sucrose (Mock) or 150mM NaCI (Salt) containing media. Error bars represent standard error of the mean from five biological replicates. Welch's t-test P-value >0.5.