

Figure S6

FIGURE S6 Sensitivity of hab1 and pp2ca mutant plants to cold stress. (a) Physical interaction of RCAR5 with HAB1 and PP2CA in yeast. Interaction was indicated by growth on selection medium (SC-Ade-His-Leu-Trp); growth on SC-Leu-Trp was used as a control (right). (b) BiFC assay of interactions of RCAR5 with HAB1 and PP2CA. RCAR5:VYNE was co-expressed with HAB1:CYCE or PP2CA:CYCE in the leaves of tobacco. Scale bar = 20 mm. (c, d) Relative expression levels of PP2C genes during germination. Data were obtained from the Arabidopsis eFP Browser with 'Germination' (c) (Winter et al., 2007) and 'Klepikova Atlas' (d) (Klepikova et al., 2016) as data sources in the Bio-Analytic Resource for Plant Biology (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi). H, seeds from the silque; S, stratification; L, light. (e, f) Relative expression levels of PP2C genes in Arabidopsis shoots and roots in response to cold stress. Data were obtained from the Arabidopsis eFP Browser with 'Abiotic stress' (Winter et al., 2007) as the data source. (g) qRT-PCR analysis of HAB1 and PP2CA gene expression in Arabidopsis leaves in response to cold stress (4°C). Actin8 was used as an internal control for normalization. The expression level of each gene at 0 h was set to 1.0. (h) Vertical growth of Pro35S:RCAR5, hab1, pp2ca-1, and WT plants exposed to ABA or LT. Seeds of each plant were plated on 0.5× MS medium supplemented with 0 µM or 0.75 µM ABA and germinated under normal or cold stress conditions. Scale bar = 1 cm. Representative images were taken at 8 DAI (for ABA) and 21 DAI (for LT). (i) Germination rates of Pro35S:RCAR5, hab1, pp2ca-1, and WT plants. Seeds of these plants (n=100) were plated on $0.5 \times$ MS medium and germinated in the dark at 4°C. All data represent mean ± SD of three independent experiments. Different letters indicate significant differences between WT and transgenic plants (ANOVA; P < 0.05).