

**Supplemental Figure S1.** NCED5 has 9'-*cis*-epoxycarotenoid dioxygenase activity in vitro.

HPLC profile of the reaction products from 9'-*cis*-neoxanthin incubated with GST-NCED5 (A) or GST-NCED3 fusion protein (C). Putative C<sub>25</sub> product was eluted at the retention time of 6.2 min. cN, 9'-*cis*-neoxanthin; C<sub>25</sub>, predicted C<sub>25</sub> product.

Full-scan mass spectrum of predicted *cis*-xanthoxin produced from the reaction of 9'-*cis*-neoxanthin incubated with GST-NCED5 (B) or GST-NCED3 fusion protein (D).

**Supplemental Figure S2.** Thermoinhibition resistant germination of seeds from ABA-deficient mutants and lines with T-DNA insertion in *NCED* genes.

(A) T-DNA insertion sites in *NCED2*, *NCED5*, *NCED6* and *NCED9* genes. Arrow indicates 5' to 3' direction of the gene.

(B) Effect of imbibition temperature on germination of *aba1-1*, alleles of *nced* single mutants, *aba2-1* and *aa3-4* seeds. After-ripened seeds were imbibed at 22 °C (open bars), 33 °C (gray bars) and 34 °C (black bars) under continuous illumination for 5 days. Error bars show SD (n=3).

**Supplemental Figure S3.** Evaluation of embryo and endosperm separation by qRT-PCR of *EPR1* and *GA3ox2* transcripts.

Total RNA was prepared from embryo (open bars) and endosperm/testa (gray bars) separated from Columbia seeds imbibed at 22 °C under continuous illumination for 24 h. Transcript levels of endosperm marker gene, *EPR1* and embryo marker gene, *GA3ox2* were quantified by qRT-PCR with CYBR Green. Primer sequences of *EPR1* used were described previously (Penfield et al., 2004). Primer sequences of *GA3ox2* were

5'-GTTTCCAAGTGAGAGATGGGTAG-3' and 5'-TCTCCACTTCCCAAACACTGGT-3'. A single amplification product in each reaction was confirmed by a dissociation curve.