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Supplementary Information for

Flavonols control pollen tube growth and integrity by regulating ROS homeostasis during high temperature stress

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This PDF file includes:

Figs. S1 to S3
Captions for databases S1 to S3

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Supplementary Figures

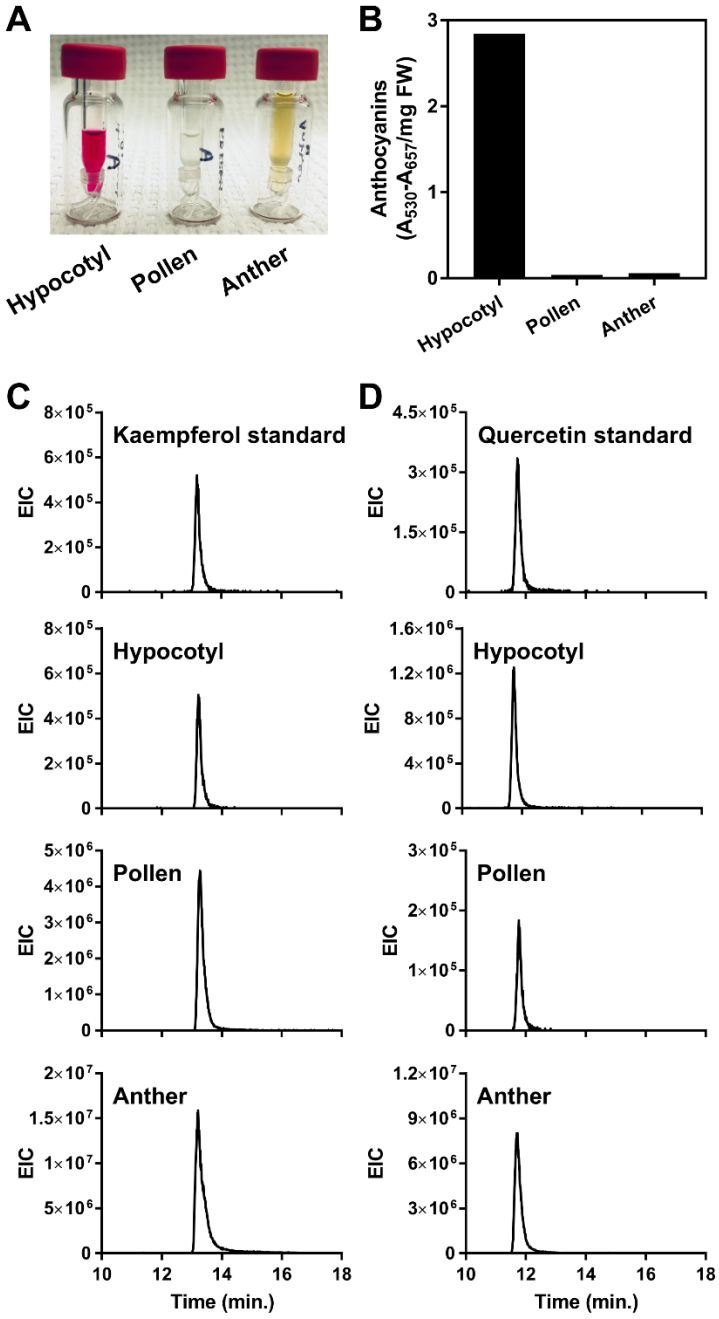

Figure S1. Tomato pollen and anthers accumulate flavonols, but not anthocyanins. (A) Photograph of methanolic extracts of wildtype hypocotyls, pollen grains, and anthers. Pink coloration in extracts indicates presence of anthocyanins in the tissue. (B) Quantification of anthocyanins by spectrophotometry in hypocotyls, pollen grains and anthers. (C) Extracted ion chromatograms for the flavonol kaempferol in hypocotyls, pollen grains, and anthers. (D) Extracted ion chromatograms for the flavonol quercetin in hypocotyls, pollen grains and anthers. (C and D) The x-axis represents retention time, while the y-axis shows the extracted ion current (EIC).

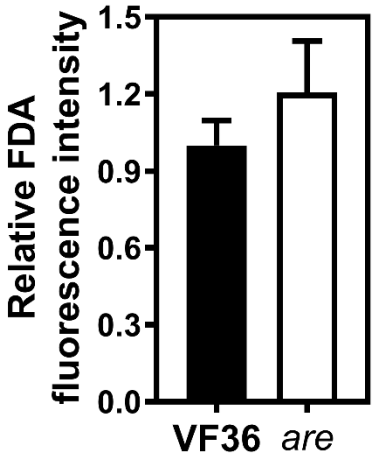

Figure S2. VF36 and *are* pollen tubes exhibit the same fluorescence levels derived from the ROS-insensitive dye fluorescein diacetate (FDA). Quantification of ROS-insensitive FDA-derived fluorescence in pollen tubes of VF36 and *are*. Data are mean ± SEM. n = 30 tubes measured across three independent experiments. FDA signal in VF36 and the *are* mutant was not significantly different (p-value = 0.35).

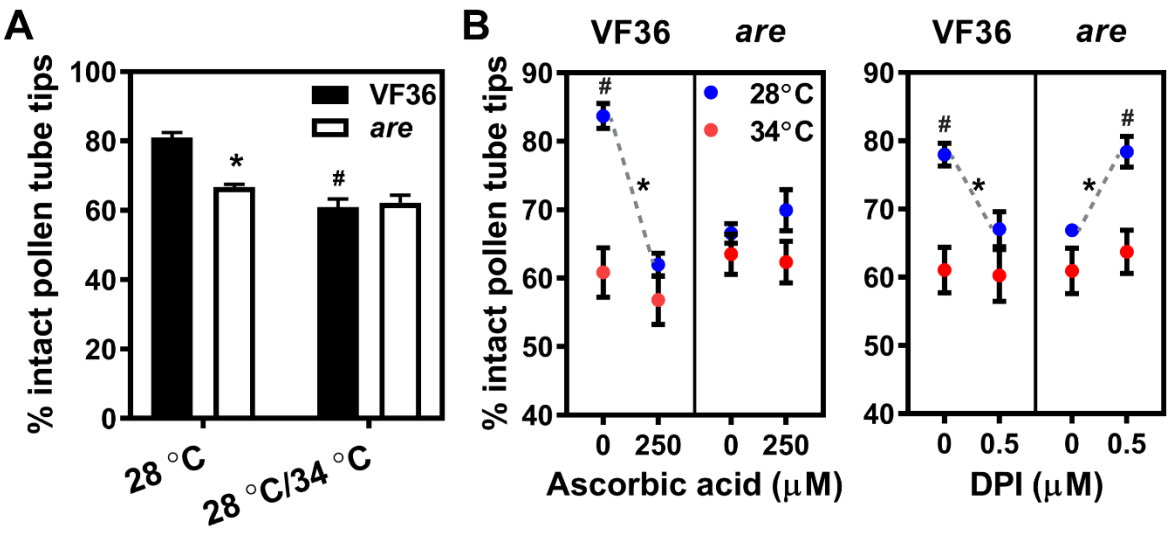

Figure S3. Heat stress impairs pollen tube integrity in a ROS-independent manner. Quantification of pollen tube integrity in VF36 and the *are* mutant at two different temperature regimes. Pollen tubes were grown in germination medium for 90 minutes at 28°C and then for 150 minutes at 34°C or kept at 28°C for another 150 minutes. Integrity was assessed for n > 300 tubes across two independent experiments. Data are shown as the mean ± SEM. Asterisks indicate significant differences between VF36 and *are* and pound signs differences between temperature treatments, according to a two-way ANOVA followed by a Fisher's LSD test with $p<0.05$. (B) Quantification of pollen tube integrity in VF36 and the *are* mutant. Pollen tubes of VF36 and the are mutant were grown for 90 minutes at 28°C and then transferred for 150 minutes to 34°C or kept for another 150 minutes at 28°C. Supplementation of the pollen germination medium with the non-enzymatic antioxidant ascorbic acid or the NADPH oxidase inhibitor diphenylene iodinium (DPI) occurred after 90 minutes of growth at 28°C. Data represent mean ± SEM. n > 300 tubes assessed for integrity across two independent experiments. Grey, dotted lines with asterisks indicate significant differences between control and treatment within the same genotype and temperature regime. Pound signs indicate significant differences in pollen tube integrity between 28°C and 34°C within the same genotype and treatment.

Additional data table S1-S3 (separate files)

Dataset S1. Best fit values for the one phase decay model describing the tip-to-shank gradient in FDA and DCF fluorescence observed along pollen tubes of the wildtype VF36.
Dataset S2. Test statistics for the effects of antioxidant treatments and heat stress on pollen tube length in VF36 and the *are* mutant.
Dataset S3. Test statistics for the effects of antioxidant treatments and heat stress on pollen tube integrity in VF36 and the *are* mutant.