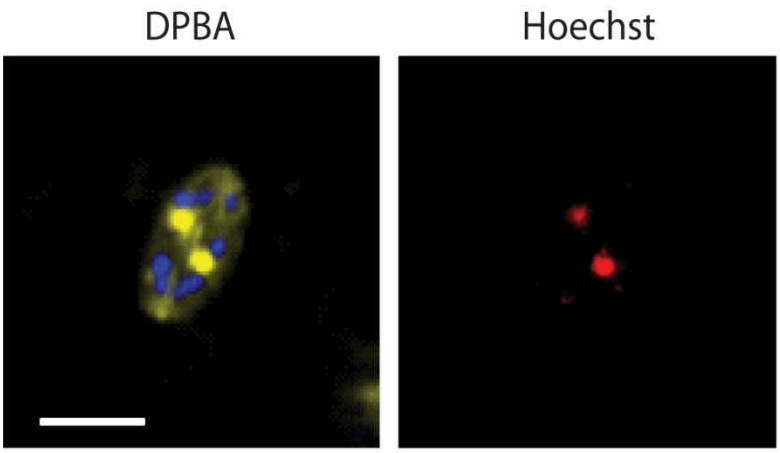
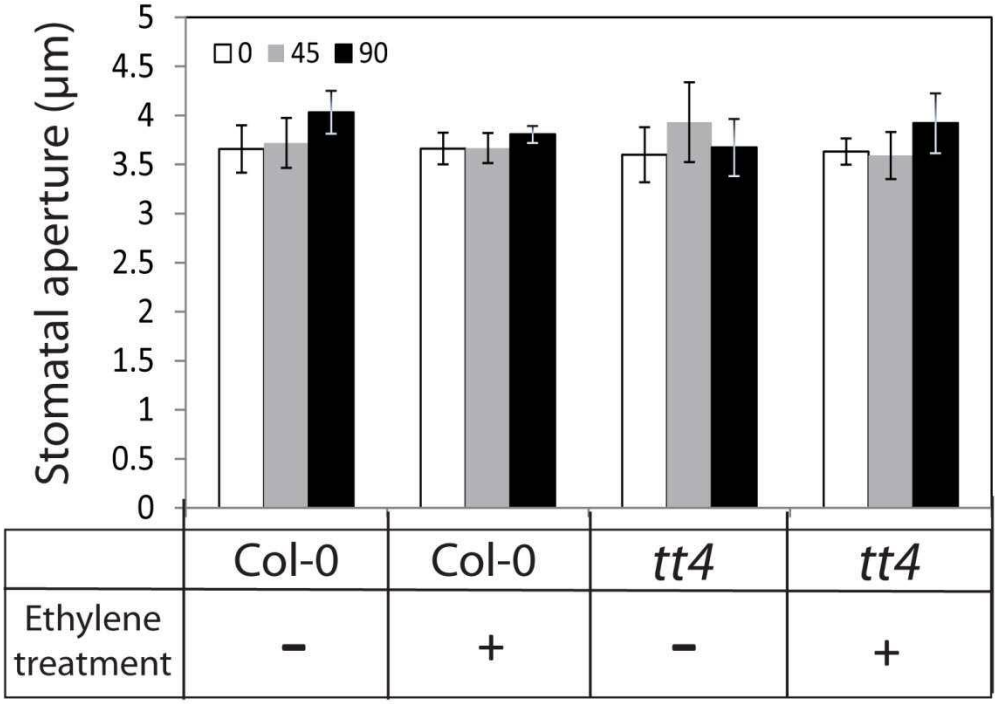
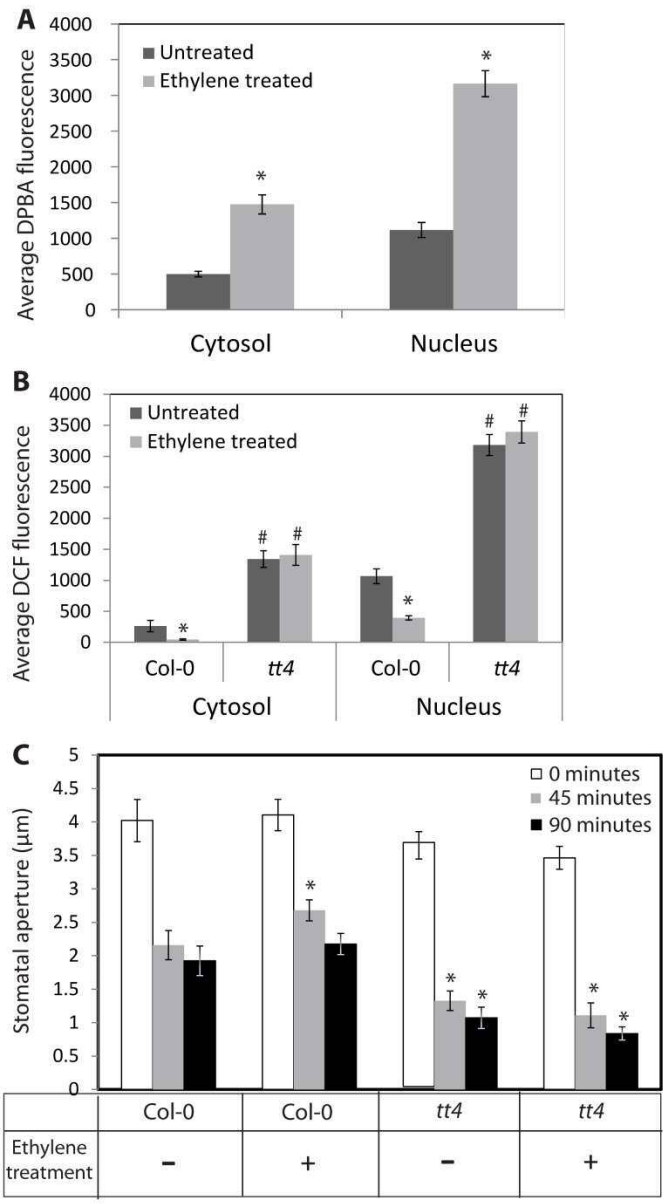


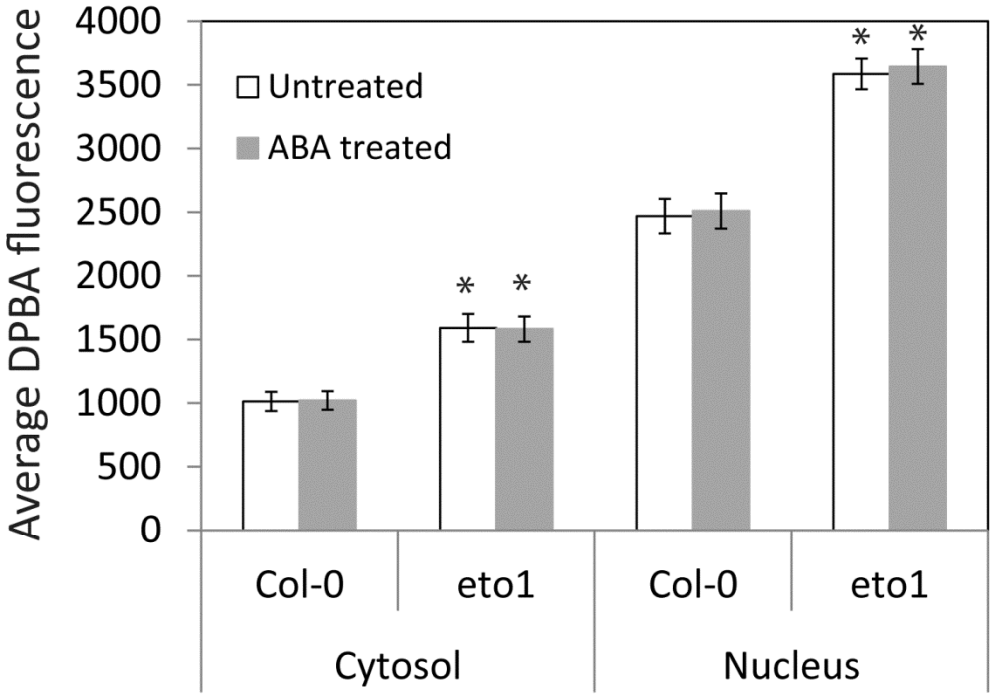
Supplemental Figure 1. Confocal imaging of Col-0 guard cells show enhanced DPBA fluorescence (yellow) in the nuclei of guard cells. Nucleic acid stain, Hoechst 33258, is shown in red. Bar = 15µm..



Supplemental Figure 2: Stomatal aperture widths in opening solution without ABA for 90 minutes. Guard cells were incubated under white light in opening solution for 0, 45, and 90 minutes. Apertures were measured using ImageJ. Error bars represent standard error. n = 30 for each time point.



Supplemental Figure 3. A three hour ethylene treatment induces flavonol accumulation and decreases ROS concentrations the rate of stomatal closure. Intact soil grown plants were incubated in ethylene gas at 5ppm concentration for 3 hours prior to the experiment. A. Subcellular flavonol accumulation in Col-0 with and without ethylene treatment. DPBA fluorescence intensity values were quantified and reported as arbitrary units. * Significant difference between treated and untreated controls within cellular location as determined by Student's t test ($P < 0.02$). Error bars represent standard error. n=30 for each treatment. B. Subcellular ROS accumulation in Col-0 and *tt4* guard cells with and without ethylene treatment. DCF intensity values in the cytosol and nucleus of guard cells were determined and reported as arbitrary units The average ± SE of 30 stomata from three biological replicates are reported. * Significant difference ($P < 0.05$) between mutant and the wild type between treatments. # Significant difference ($P < 0.02$) between mutant and wildtype within cellular location. C. Stomatal aperture widths in response to ABA. ABA sensitivity is indirectly proportional to relative flavonol concentrations. Guard cells were incubated under white light in a 20 µM ABA solution for 0, 45, and 90 minutes. Error bars represent standard error. N=30 for each timepoint. * Significant difference between samples and untreated Col-0 for each time point respectively ($P < 0.05$).



Supplemental Figure 4. Subcellular flavonol accumulation in wild-type guard cells compared to *eto1* after treatment with 20μM ABA. Flavonol accumulation was measured using guard cell DPBA fluorescence intensity values and the average ± SE is reported relative to the levels in the cytosol of Col-0 for 1 experiment n=30 stomata. * Significant difference ($P < 0.005$) between mutant and the wild type within treatment. DPBA fluorescence did not increase in response to 20μM ABA for 2 hours.