

**Fig. S1. Mass spectra on carpels:** Mass spectra taken from a single pixel in the middle of the carpels of Fig. 1. Peaks corresponding to kaempferol (K), quercetin (Q), and isorhamnetin (I) are labeled, as are their rhamnose (-R) and hexose (-H) glycosides.



Fig. S2. LDI-mass spectra of flavonoid standards without matrix: quercetin (a), quercetin rhamnoside (b), and rutin (quercetin-3-(glucoside-rhamnoside)) (c). Significant fragmentation to the aglycone is observed for the mono- and di-glycosides. Quercetin fragments ( $\bigcirc$ ) and contaminations ( $\bigtriangledown$ ) are marked.



Fig. S3. LDI-mass spectra of flavonoid standards with graphite matrix: quercetin (a), quercetin rhamnoside (b), and rutin (quercetin-3-(glucoside-rhamnoside)) (c). Significant fragmentation to the aglycone is observed for the mono- and di-glycosides. Quercetin fragments
(●) and contaminations (▼) are marked.



**Fig. S4. Flavonoid image on petals without matrix:** Optical and MS images of wild-type (top series) and tt7 mutant (bottom series) *Arabidopsis thaliana* petals obtained without matrix showing distributions of kaempferol (K), quercetin (Q) and isorhamnetin (I), along with their rhamnose (R) and hexose (H) glycosides. All plotted m/z represent the deprotonated pseudomolecules, [M-H]<sup>-</sup>. The irregular shape at the top corners of the wild-type images is a result of folding of the petal during sample drying. Apparent differences in intensities between the distal and proximal regions in the *tt7* petal are an artifact of lower overall ion yields from the proximal region and can be largely corrected by normalization to the total ion current (TIC). However, without matrix background, normalization with TIC introduces another artifact because of no signal outside the tissue, so they are presented without normalization.



Fig. S5. Semi-quantification of flavonoids in distal and proximal region of petals without matrix: Relative ion signals of flavonoids in two regions of wild-type (WT) and tt7 mutant *Arabidopsis* petals. The values are obtained from 25 pixels in each region of MS images in four replicates. Error bars represent one standard deviation. All signals are normalized to TIC. Data was collected without the use of a matrix. Inset shows a comparison of summed kaempferol, quercetin, and isorhamnetin aglycone and glycoside signals from WT petals to kaempferol aglycone and glycoside signals from tt7 petals. In the calculation of inset data, m/z 447 (kaempferol hexoside/quercetin rhamnoside) was assumed to be entirely due to quercetin rhamnoside in WT and entirely due to kaempferol hexoside in tt7.