Supplemental data:

Supplemental Figure 1. Phenotypes of tomato fruit on the vine at different stages of ripening and over-ripening. For each stage, 8-12 fruit were checked and representative fruit are shown.

B

A

Supplemental Figure 2. The high antioxidant capacity of *AtMYB12* **tomatoes is not maintained as long as for** *Del/Ros1* **fruit.**

(**A**) Total antioxidant capacity of hydrophilic compounds in different tomato lines during ripening. Different letters indicate significantly different values at $p < 0.05$ (one way ANOVA, Tukey post hoc test) within the same genotype.

(**B**) MDA content of different tomato lines during ripening. Error bars show SEM (n=3). Different letters indicate significantly different values at $p < 0.05$ (one way ANOVA, Tukey post hoc test) within the same genotype.

2

Supplemental Figure 3. VIGS of anthocyanin biosynthetic genes in *Del/Ros1* **tomatoes alters the anthocyanin biosynthetic pathway.**

(**A**). From left to right, phenotype of WT, VIGS-*Del/Ros1*, VIGS-*SlF3H*, VIGS-*SlDFR*, VIGS-*SlANS* and *Del/Ros1* MoneyMaker fruit. Pictures were taken at two weeks after breaker. (**B**) Expression of anthocyanin biosynthetic genes in VIGS fruit. Data are represented by comparing the expression of silenced sectors to the non-silenced sectors on the same fruit. Error bars show SEM ($n \geq 2$).

Supplemental Figure 4. Silencing of anthocyanin biosynthetic genes in *Del/Ros1* **tomatoes causes the accumulation of different flavonoid compounds**

(**A**) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 525 nm showing the accumulation of anthocyanins in different fruit. (**B**) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 280 nm showing the accumulation of phenylpropanoid compounds in different fruit. Compounds identified: 1. Cholorgenic acid, 2. Delphinidin 3-(trans-coumaroyl)-rutinoside-5-glucoside (Del-Cou-Rut-Glc), 3. Petunidin 3- (trans-coumaroyl)-rutinoside-5-glucoside (Pet-Cou-Rut-Glc), 4. Rutin, 5. Malvidin 3-(transcoumaroyl)-rutinoside-glucoside (Mal-Cou-Rut-Glc), 6. Kaempfero-3-O-rutinoside, 7. Naringin, 8. Myricetin-coumaroyl-rutinoside (Myr-Cou-Rut), 9. Methyl myricetincoumaroyl-rutinoside (MeMyr-Cou-Rut), 10. Quercetin-coumaroyl-rutinoside (Que-Cou-Rut). 11. Naringenin.

Supplemental Figure 5. Compounds identified by LC-MS. (**A**) List of major compounds identified by LC-MS. Abbreviations were used as described in Figure S4. (**B**) MS and UV spectrum for Myr-Cou-Rut.

Supplemental Figure 6. Crossing anthocyanin mutants with *Del/Ros1* **tomato generates hybrids containing different flavonoid compounds.**

(**A**) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 525 nm showing the accumulation of anthocyanins in fruit of different lines.

(**B**) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 280 nm showing the accumulation of flavonoids and other phenylpropanoid compounds in different fruit. Annotation of compounds is the same as in Supplemental Figure 7.

Supplemental Figure 7. *AtMYB12* **MoneyMaker tomatoes are susceptible to** *B. cinerea***.**

From left to right, WT, *Del/Ros1* and *AtMYB12* MoneyMaker tomatoes infected with *B. cinerea*. White dots represent lesion margins. Fruit were harvested at 7 dpb. Pictures were taken at 3 dpi.

Supplemental Figure 8. Expression of markers for pathogen response in WT and *AtMYB12* **tomatoes following** *B. cinerea* **inoculation.**

(**A**)**-**(**E**) RT-qPCR of pathogen response genes before and after *B. cinerea* infection. (**A**) *Chitinase* (*SlChi*), (**B**) *[β](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2634228/) -1,3-glucanase* (*SlGLU*), (**C**) *Metacaspase 7(SlMCA7),* (**D**) *Pathogenesis-related protein 1*(*SlPR1*) and (**E**) *Hypersensitivity-related gene 203* (*SlHSR203*). (**F**) The total antioxidant capacity of uninfected and infected fruit. Both wound only and wound $+$ infection fruit were analyzed at 3 dpi. Error bars show the SEM, $n=2$. * indicates $p<0.05$ compared to wound only samples.

Supplemental Figure 9. Preparative HPLC to purify anthocyanins from the fruit of delphinidin-producing *Del/Ros***1 tomatoes.**

(**A**) Chromatogram of the preparative HPLC of total anthocyanins (535 nm) and other polyphenols (280 nm) run on the Phenomenex[®] 5 µm Gemini[®] C18 column using a Varian ProStar 210 preparative HPLC.

(**B**) Chromatogram of the preparative HPLC of a single anthocyanin fraction previously separated in (A) and corresponding to delphinidin 3-(coumaroyl)-rutinoside-5-glucoside with a mass of 919 run on the Waters 5 µm XBridge™ C18 prep column using a Waters Prep 2000 HPLC system.

Supplemental Figure 10. Effects of flavonoid derivatives added to the culture medium on the growth of *B. cinerea*. 1 mM of different compounds was added to the standard $\frac{1}{4}$ strength PDB medium containing 5×10^4 spores/mL *B. cinerea* GFP-labeled spores. 64 mg/L Triadiminol was added as a negative control. The fluorescence of each inoculation was measured at t0 and 24 hpi and the ratio (24 hpi v.s. t0) was calculated. Error bars show SEM $(n=4)$. ** ($p<0.01$) indicates significant difference compared to inoculation only with PDB.

1/4 PDB

+1% MeOH

+1 mg/ml Kanamycin

+1 mM Myr-Cou-Rut +1 mM Del-Cou-Rut-Glc +1 mM Pet-Cou-Rut-Glc

+1 mM Rutin

+1 mM Kae-3-O-Rut

Supplemental Figure 11. Effects of flavonoid derivatives added to the culture medium on the germination of *B. cinerea***.** 1 mM of different compounds was added to the standard ¹/4 strength PDB medium containing spores $(5\times10^4$ spores/mL) of *B. cinerea*. Pictures were taken at 16 hpi.

 $\boldsymbol{\mathsf{A}}$

D

 E

Myricetin 3-O-Rhamnoside (Myr-Rha)

1 mM Myr-Rha

Myricetin 3-O-(coumaroyl)-rutinoside (Myr-Cou-Rut)

1 mM Myr-Cou-Rut

Supplemental Figure 12. The structure of B-ring is an important factor determining the scavenging ability of different flavonoids.

(A) Structures of purchased myricetin 3-O-rhammanoside (Myr-Rha) and myricetin 3-O- (coumaroyl)-rutinoside (Myr-Cou-Rut) purified from tomato fruit.

(B) No significant difference was observed in the scavenging ability between Myr-Rha and Myr-Cou-Rut. Error bars show SEM (n=4). The data for Myr-Cou-Rut are drawn from the data shown in **Figure 8D**.

(C) Both Myr-Rha and Myr-Cou-Rut showed no direct inhibition effect on *B. cinerea* growth *in vitro*. Error bars show SEM (n=4). The data for Myr-Cou-Rut are drawn from the data shown in **Figure 8C**.

(D) Both Myr-Rha and Myr-Cou-Rut showed no direct inhibition on *B. cinerea* germination *in vitro*. The data for Myr-Cou-Rut are drawn from the data shown in **Supplemental Figure 9**.

(E) Both Myr-Rha and Myr-Cou-Rut supplements to inocula can significantly reduce pathogen susceptibility on WT tomato fruit. ** indicates significant difference between the tested concentration and the PDB control (p<0.01) in Student's T-test. The data for Myr-Cou-Rut are drawn from the data shown in **Figure 8B.**

Table S1. Levels of major phenylpropanoids in WT, *AtMYB12, Del/Ros1* **and Indigo (***AtMYB12* **x** *Del/Ros1***) MicroTom tomato fruit.** Values are means ±SEM of determinations on two samples.

Table S2. Primers used in this research

