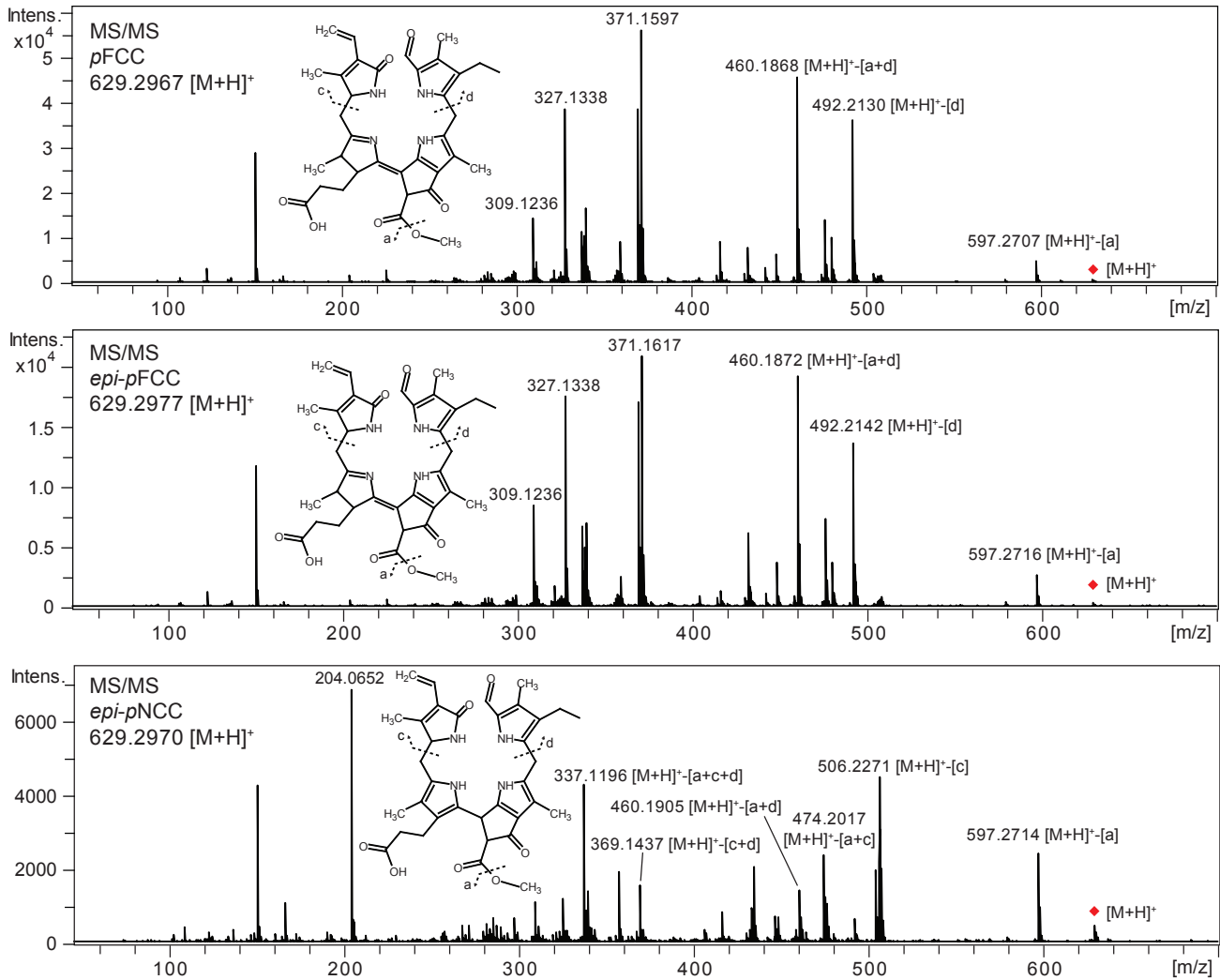
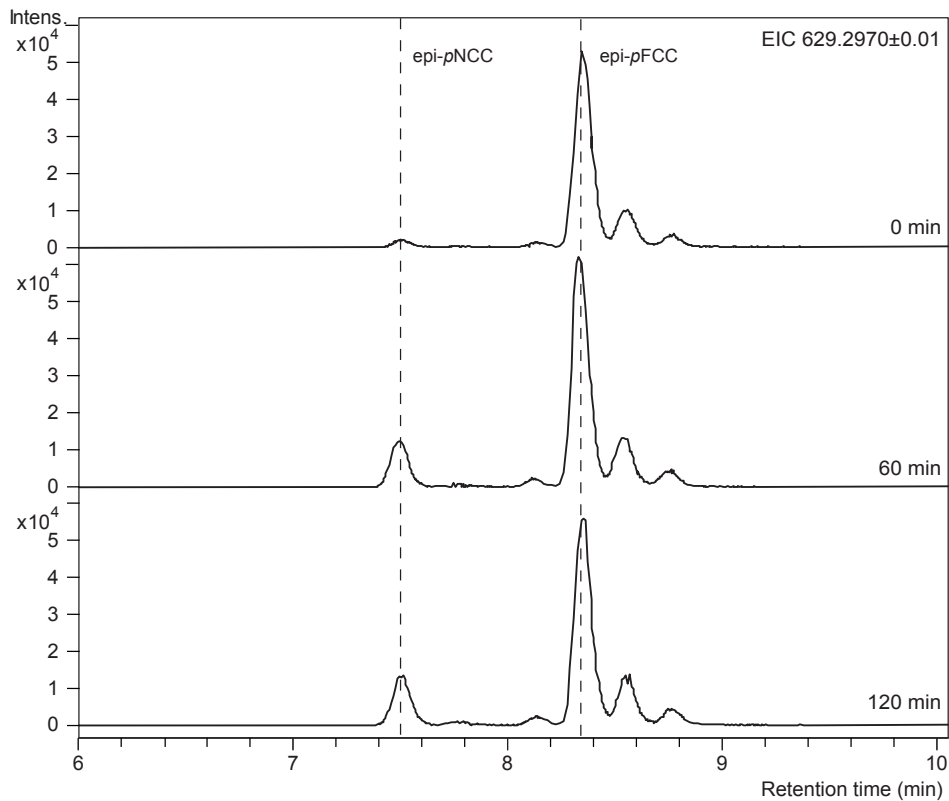


Supplemental Figure 1

A



B

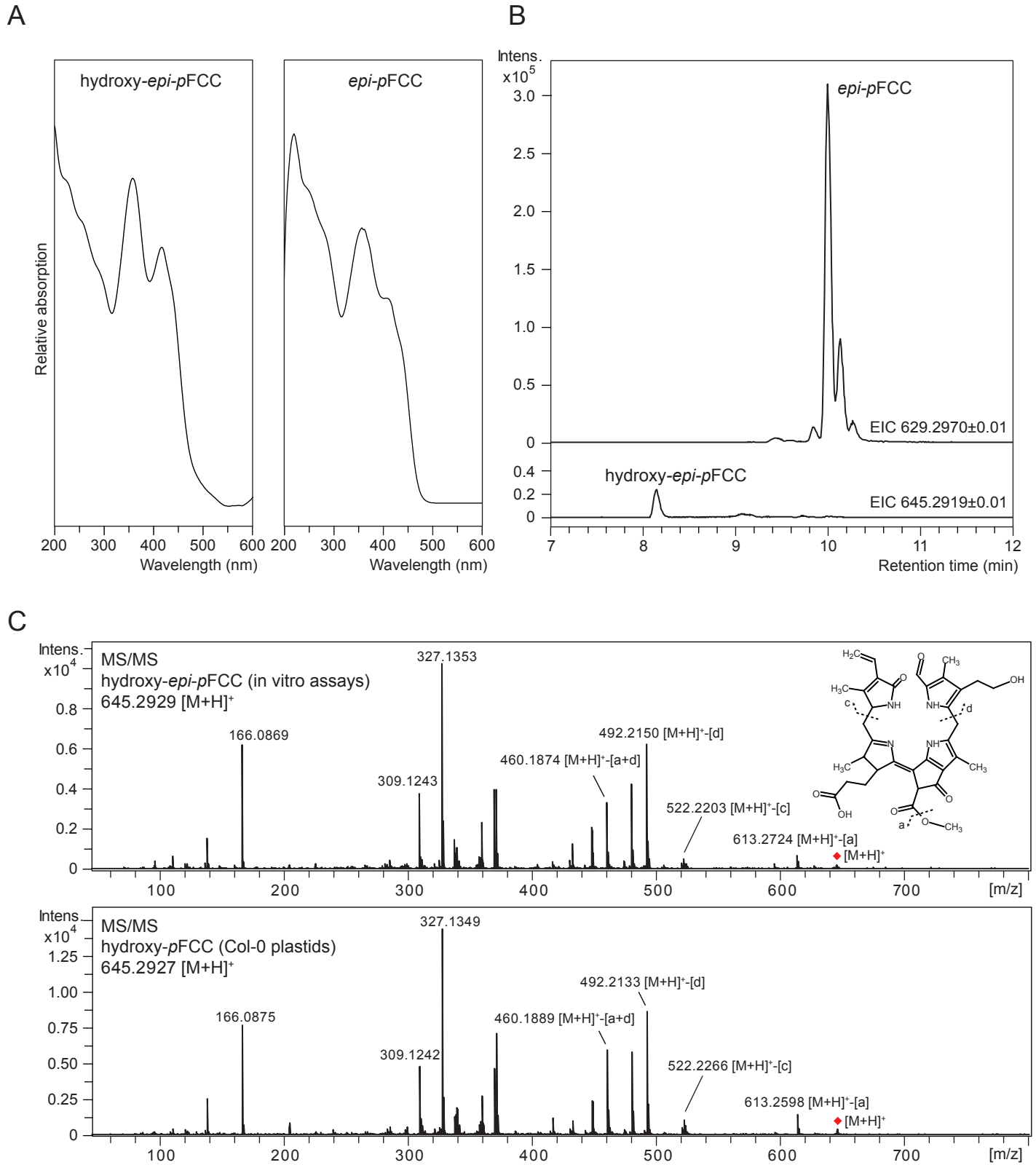


Supplemental Figure 1. Confirmation of *epi-pFCC* and *pFCC* Identity and *epi-pFCC*-to-*epi-pNCC* Isomerization.

(A) MS/MS fragmentation spectra of *pFCC* (top panel; obtained from extracts of *acd2-2*-*At-RCCR* chloroplasts; see Figure 2C) and *epi-pFCC* [middle panel; used for acid-catalyzed isomerization; see panel (B) 0 min] are identical, but distinct from *epi-pNCC* [bottom panel; product of acid-catalyzed isomerization; see panel (B) 120 min]. Constitutional formulae and MS/MS fragmentation sites are shown. [M+H]⁺ indicates the precursor ion.

(B) *epi-pFCC*-to-*epi-pNCC* isomerization assay. Purified *epi-pFCC* obtained from PAO/RCCR assays was incubated at pH 5 for up to 120 min and analyzed by LC-MS. Extracted ion chromatograms at 629.2970 \pm 0.01 are shown.

Supplemental Figure 2

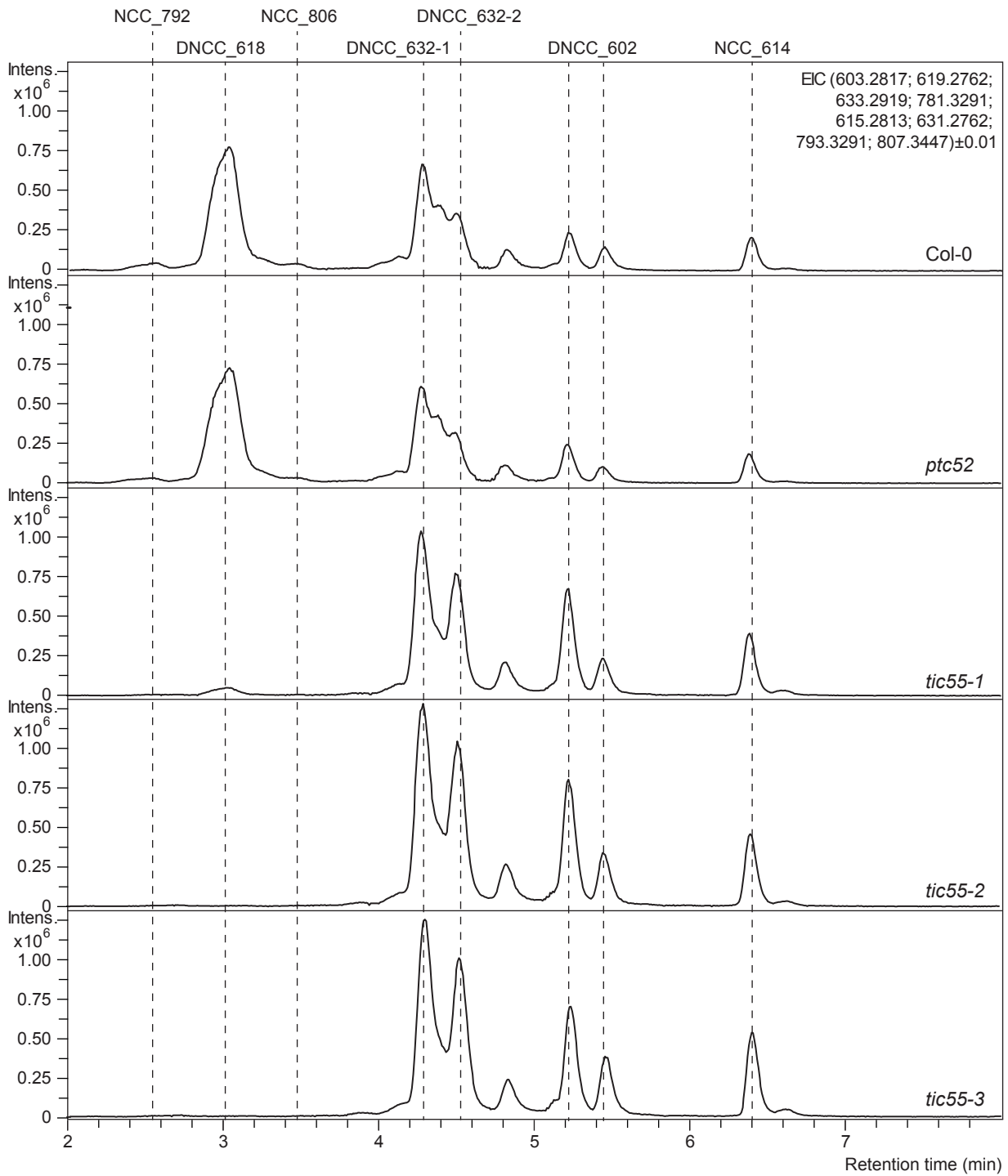
Supplemental Figure 2. LC-MS Confirmation of Hydroxy-*epi-pFCC*.

(A) Absorption spectra of hydroxy-*epi-pFCC* (left) and *epi-pFCC* (right) obtained from PAO/RCCR assays (see Figure 2A).

(B) A hydroxylation assay after 40 min incubation (see Figure 2B) was analyzed by LC-MS/MS. Extracted ion chromatograms (EIC) for *epi-pFCC* (629.2977 \pm 0.01) and hydroxy-*epi-pFCC* (645.2929 \pm 0.01) are shown.

(C) MS/MS spectra of hydroxy-*epi-pFCC* [obtained from the in vitro assay shown in panel (B)] and hydroxy-*pFCC* (obtained from wild type Arabidopsis gerontoplasts; see Figure 1A) are shown. Note that the MS/MS spectra are highly similar.

Supplemental Figure 3



Supplemental Figure 3. LC-MS Analysis of Phyllobilins from Col-0 and Rieske-type Oxygenase Mutants.

Leaves of short-day-grown plants after 5 d of dark-induced senescence were extracted and analyzed by LC-MS. Extracted ion chromatograms (EICs) for masses of phyllobilins known to occur in Col-0 (Christ et al., 2016) are shown. Note that the phyllobilin patterns are highly similar between wild type and *ptc52*, but distinct in all three *tic55* mutants. Thus, the most abundant hydroxylated phyllobilin (DNCC_618) is absent in *tic55-2* and *tic55-3*, and highly reduced in *tic55-1*.

Supplemental Table 1. List of Primers Used in This Study.

Purpose	Name	Sequence (5'→3')
T-DNA confirmation primer		
<i>tic55-1</i>	RP	TCTTGACACATTTGCCCTCTC
	LP	CAAAAGAGCTACGCGTTAACG
<i>tic55-2</i>	RP	AACATGAGCAGGATCCATCAG
	LP	GACGTCGTTTAGCCAAAGTTTC
<i>tic55-3</i>	RP	AGCAGAGAGAGCTGCAATGAG
	LP	TTGTTACGAAGATCGGTGTCC
<i>ptc52-1</i>	RP	AATGGAATGTGTGTAGGAGCG
	LP	TAGTCCAGTGGTCCAGCATTC
<i>tic62</i>	GK-439H04_LP	TTGATCAGATTTTCGATCTCCTG
	GK-439H04_RP	AGAGCTTGCTCTAATGGGACC
<i>tic32</i>	GK-117H08_LP	TTTCTGGACATGTTTGGTTCC
	GK-117H08_RP	TATTTAGCCACTGCACCAACC
T-DNA primers	Salk LBb1.3	ATTTTGCCGATTTTCGGAAC
	Sail LB2	GCTTCCTATTATATCTTCCCAAATTACCAATACA
Cloning of GFP fusions		
TIC55-GFP	XmaI_withTP_FP	CCCCCGGGATGGCTGTTCCATTTCTAAG
	SpeI_w/oSTOP_RP	GGACTAGTTAGTCTTCTGTGTGTTCTAATG
PTC52-GFP	BamHI_withTP_FP	CGGGATCCATGGAAGCTGCTCTTGC
	BamHI_w/oSTOP_RP	CGGGATCCAACAACAGCATGGTTGTAG
Sequencing primers		
TIC55	400_F_TIC55	CATCGTTGGCTAAGTTGTC
	500_R_TIC55	GGAATCTTAGCACTTGCAGG
	700_F_TIC55	GAGAATCTGATGGATCCTGC
	1100_F_TIC55	CGTCTCAAATGAGGTTCTG
PTC52	400_F_PTC52	GTCTGATGGAAGGATTGATCAATG
	500_R_PTC52	GGACCGGAGGACCATCAG
	700_F_PTC52	CTTACGGGTACGATGTCTTG
	1000_F_PTC52	CGGTCAGTCCAGGTCGTAG