

## SUPPLEMENTAL FIGURE LEGENDS

Suppl. Figure 1. **Comparison of the deduced amino acid sequences of six P450 clones from the *petunia stigma*.** The most conserved motifs are underlined with the dotted line for oxygen binding and activation motif, the dashed line for ERR triad, and the solid line for heme binding motif, respectively. The absolutely conserved residues are shaded in dark and the highly conserved are in grey.

Suppl. Figure 2. **Phylogenetic relationships of fatty acid  $\omega$ -hydroxylases (FA OHases) and other cytochrome P450 proteins.** The unrooted tree is generated by the TreeView program based on the sequence alignment using Clustal X (see Materials and Methods). Amino acid sequences of P450s were obtained from the Cytochrome P450 Homepage (<http://drnelson.utmem.edu/CytochromeP450.html>). These include those for FA OHases from plants such as CYP86A2 and 86A8 from *A. thaliana*, and CYP94A1 and 94A2 from *Vicia sativa*; from bacteria and fungi such as CYP102 from *Bacillus megaterium*, CYP52 B1 from *Candida tropicalis*, CYP52D1 from *Candida maltosa* and CYP52E1 from *Candida apicola*; and from mammals such as CYP4A1 and 4A11 from *Rattus norvegicus* and CYP4F2 from *Homo sapiens*. The other P450s include those for FA oxidases (epoxygenase or in-chain hydroxylase) from plants such as CYP726A1 from *Euphorbia lagascae* and CYP81B1 from *Helianthus tuberosus*; and those for plant hormone synthesis such as CYP88A3 and CYP714A1 from *A. thaliana*. The scale bar indicates the number of amino acid substitutions per site.

Suppl. Figure 3. **Preincubation assays of CYP86A22.** Microsomes from insect cells infected with the CYP86A22:PR1 dual recombinant baculovirus alone were preincubated for various times ranging from 0 min to 30 min, followed by adding [1-<sup>14</sup>C]-labeled 16:0-CoA, NADPH and the regeneration system, and incubated at 30°C for an additional 15 minutes. Methylated products from these reactions were separated on TLC plate, and radioactive signals were detected by an instant imager.

## Suppl. FIGURE 1

CYP86A22 (1) -----MEVSTTMMI~~V~~AI~~V~~AA~~Y~~LL~~W~~FK~~S~~IT~~K~~SM~~K~~G-P~~K~~G---P~~T~~M~~W~~P~~V~~V~~C~~SL~~P~~GL~~I~~EN~~G~~NR~~M~~HE~~W~~IAD~~N~~L  
 CYP94A13 (1) MSLDLQLSTSLFFI~~V~~PL~~L~~FF~~L~~IK~~F~~K~~T~~I~~I~~AH~~T~~LC~~S~~PK~~P~~SK~~I~~PT~~S~~Y~~P~~I~~I~~C~~S~~Y~~F~~S~~I~~L~~A~~N~~K~~D~~H~~R~~L~~Q~~W~~I~~S~~D~~I~~I  
 CYP714G3 (1) MEIN~~Y~~FF~~K~~V~~L~~LS~~V~~ML~~I~~W~~L~~FL~~S~~FL~~V~~V~~K~~Y~~N~~T~~L~~V~~A~~K~~P~~L~~K~~R~~I~~A~~L~~R---K~~Q~~G~~I~~O~~C~~PP~~G~~T~~L~~L~~G~~N~~L~~L~~E~~I~~H~~R~~A~~F~~S~~H~~A~~  
 CYP749B1 (1) -----M~~M~~T~~A~~I~~C~~F~~V~~F~~L~~V~~G~~L~~A~~L~~V~~L~~A~~R~~F~~L~~Y~~K~~S~~W~~W~~Y~~P~~V~~S~~L~~Q~~L~~L~~M~~K~~---S~~Q~~G~~I~~K~~C~~PP~~Y~~K~~A~~P~~N~~W~~N~~----Y~~A~~K~~G~~V~~L~~D  
 CYP88C1 (1) --MEYDSMFLYTALAV~~G~~IL~~T~~I~~W~~S~~I~~L~~K~~NG~~N~~G~~W~~F~~Y~~T~~F~~K~~F~~S~~S~~N~~K~~C~~R~~L~~P~~P~~G~~D~~M~~C~~W~~P~~F~~F~~G~~N~~M~~L~~H~~F~~V~~K~~C~~L~~S~~N~~Y~~D~~L~~A  
 CYP704A14 (1) -----M~~T~~T~~T~~I~~L~~S~~L~~L~~F~~C~~G~~F~~I~~I~~H~~L~~V~~T~~R~~K~~L~~L~~G~~R~~Y~~G~~K~~K-----R~~Y~~H~~P~~I~~G~~C~~T~~M~~F~~N~~Q~~L~~L~~N-----F~~H~~R~~L~~H~~D~~Y~~M~~

CYP86A22 (61) RACSGTYQ~~T~~C~~I~~C~~A~~I~~P~~F~~L~~ARK~~Q~~-----L~~V~~T~~V~~T~~C~~D~~P~~K~~N~~L~~E~~H~~I~~L~~K~~V~~R~~F~~D~~N~~Y~~P~~K~~G~~P~~T~~W~~  
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 CYP714G3 (68) SIVSGTNNGLP~~I~~SH~~D~~I~~Q~~S~~N~~L~~F~~P~~F~~V~~D~~R~~W~~L~~K~~Y~~G~~E~~L~~F~~T~~I~~S~~I~~G~~S~~T~~Q~~I~~L~~V~~V~~A~~R~~P~~E~~M~~V~~R~~E~~I~~M~~N~~T~~S~~Q~~N~~F~~G~~-K~~P~~P~~Y~~H  
 CYP749B1 (59) MEV~~K~~S~~T~~S~~A~~P~~M~~E~~I~~S~~H~~D~~I~~P~~R~~L~~F~~P~~V~~Y~~S~~W~~I~~N~~L~~Y~~G~~K~~N~~F~~L~~H~~W~~I~~D~~T~~Q~~P~~L~~V~~V~~T~~D~~I~~N~~L~~I~~K~~E~~L~~L~~S~~D~~K~~E~~G~~S~~F~~D~~K~~V~~Q~~L~~E  
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 CYP704A14 (53) TDLAGK~~H~~K~~T~~Y~~R~~L~~I~~S~~P~~F~~R~~N-----E~~I~~Y~~S~~S~~D~~P~~A~~N~~V~~E~~Y~~I~~L~~K~~T~~N~~F~~D~~N~~Y~~C~~K~~G~~W~~Y~~S

CYP86A22 (110) QAVFH~~L~~L~~G~~E~~G~~I~~F~~N~~S~~D~~C~~D~~T~~W~~L~~F~~Q~~R~~K~~T~~A~~A~~E~~F~~T~~T~~R~~T~~L~~R~~Q~~A~~M~~G~~R~~W~~N~~R~~A~~I~~K~~N~~R~~F~~C~~P~~L~~E~~M~~A~~Q~~V~~Q~~G--K~~P~~V~~D~~L  
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 CYP714G3 (137) MESL~~K~~P~~L~~F~~G~~D~~G~~I~~I~~T~~S~~S~~C~~V~~T~~W~~N~~S~~Q~~R~~K~~V~~L~~A~~P~~E~~F~~R~~L~~E~~K~~L~~K~~G~~M~~F~~K~~L~~I~~Q~~E~~A~~A~~L~~M~~I~~V~~D~~E~~W~~K~~A~~E~~I~~E~~E~~A~~Q~~G~~G~~I~~V~~E~~I~~H~~I  
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 CYP88C1 (118) QYI~~L~~E~~L~~L~~R~~K~~E~~---P~~I~~C~~G~~T~~T~~N~~Q~~E~~D~~K~~L~~A~~R~~R~~L~~T~~T~~P~~I~~K~~S~~H~~G~~L~~V~~S~~F~~F~~F~~D~~F~~L~~S~~E~~N~~V~~K~~T~~S~~F~~E~~K~~W~~A~~S~~E~~K~~---P~~I~~E~~L~~  
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CYP86A22 (178) QD~~L~~L~~R~~L~~R~~L~~T~~F~~D~~N~~I~~C~~G~~L~~A~~F~~G~~K~~D~~P~~E~~T~~L~~S~~P~~E~~L~~P~~E~~N-N~~F~~A~~T~~S~~F~~D~~R~~A~~T~~E~~A~~T~~L~~H~~R~~F~~I~~M~~P~~-E~~F~~V~~W~~K~~L~~K~~M~~L~~G~~L~~G~~E~~V~~S  
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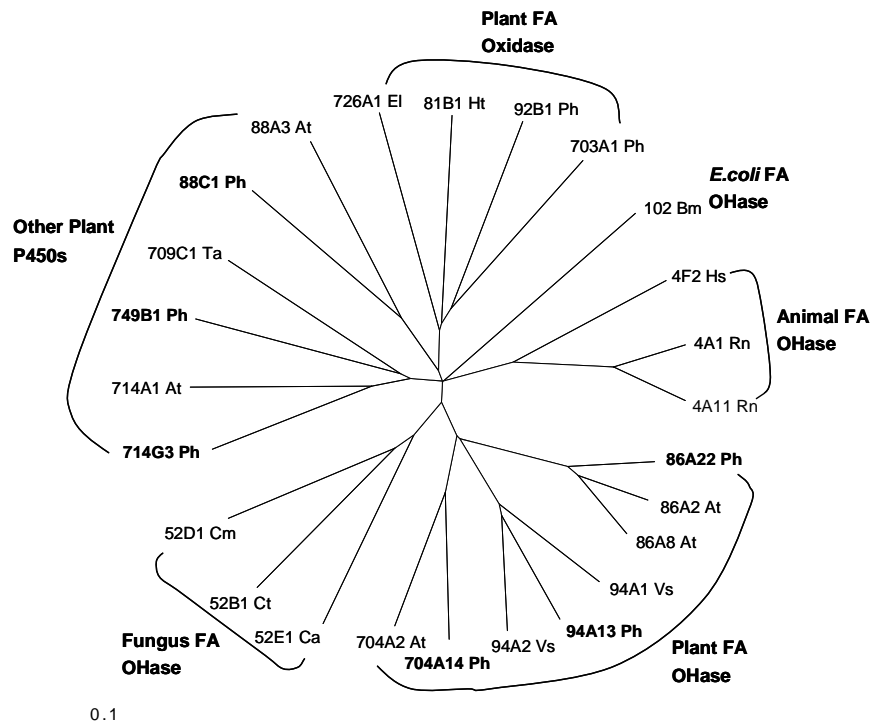
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 CYP88C1 (306) A~~K~~V~~A~~T~~Q~~T~~M~~L~~L~~Q~~N~~H~~P~~E~~C~~L~~K~~K~~A~~K~~E~~Q~~E~~E~~I~~V~~K~~R~~R~~T~~S~~---P--N~~E~~G~~L~~N~~F~~S~~E~~I~~G~~Q~~M~~K~~Y~~V~~T~~N~~V~~I~~N~~E~~T~~L~~R~~L~~G~~S~~T~~E  
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CYP86A22 (383) P~~E~~D~~S~~K~~H~~V~~I~~S~~D~~D~~Y~~L~~P~~D~~G~~T~~F~~V~~P~~A~~C~~S~~N~~I~~T~~Y~~S~~I~~Y~~S~~T~~G~~R~~M~~K~~F~~I~~W~~G~~E~~D~~C~~L~~E~~F~~K~~P~~E~~R~~W~~M~~S~~Q~~D~~G~~D~~K~~----F~~Q~~V~~Q~~D~~T~~F~~R~~  
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 CYP714G3 (391) P~~L~~H~~P~~R~~E~~A~~F~~A~~D~~M~~K~~F~~G~~D-I~~Y~~I~~P~~K~~L~~N~~I~~W~~L~~L~~A~~T~~M~~L~~H~~T~~D~~K~~K~~I~~W~~G~~P~~D~~A~~N~~E~~F~~N~~P~~D~~R~~F~~E~~N~~G~~V~~A~~G~~A-----C~~T~~H~~P~~Y~~L~~  
 CYP749B1 (378) V~~F~~L~~H~~R~~K~~V~~K~~R~~N~~I~~K~~L~~G~~E-L~~R~~L~~P~~A~~C~~M~~E~~V~~Y~~I~~A~~S~~L~~A~~V~~H~~H~~N~~S~~E~~I~~W~~G~~E~~D~~H~~L~~F~~K~~P~~E~~R~~F~~A~~E~~G~~V~~A~~K~~A~~T~~R-----D~~Q~~L~~M~~A  
 CYP88C1 (370) T~~V~~L~~F~~R~~D~~A~~R~~T~~D~~V~~N~~L~~N~~G-Y~~T~~I~~P~~E~~G~~W~~K~~C~~L~~A~~L~~L~~G~~N~~F~~Y~~K~~D~~P~~D~~T~~Y~~V~~K-P~~N~~E~~F~~I~~P~~S~~R~~W~~D~~D~~L~~E~~V~~K~~P~~-----A~~S~~---  
 CYP704A14 (371) P~~V~~D~~A~~K~~I~~C~~L~~S~~D~~D~~T~~L~~P~~D~~G~~F~~S~~V~~N~~K~~G~~D~~M~~V~~S~~Y~~Q~~P~~Y~~A~~M~~G~~R~~M~~K~~F~~I~~W~~G~~D~~D~~A~~E~~Y~~K~~P~~E~~R~~W~~L~~D~~G~~D~~G~~F~~F~~R~~-----Q~~E~~N~~P~~F~~K~~

CYP86A22 (449) F~~V~~A~~F~~N~~A~~G~~P~~R~~I~~C~~L~~G~~K~~D~~L~~A~~Y~~L~~Q~~M~~K~~S~~I~~A~~A~~A~~V~~L~~R~~H~~R~~L~~A~~V~~A~~P~~G~~H~~K~~V~~E~~Q~~K~~M~~S~~--L~~T~~L~~F~~M~~K~~D~~G~~L~~V~~M~~N~~V~~T~~P~~R~~D~~L~~T~~P~~I  
 CYP94A13 (444) Y~~P~~V~~F~~Q~~A~~G~~P~~R~~I~~C~~L~~G~~K~~E~~M~~A~~F~~L~~Q~~M~~K~~R~~V~~V~~A~~G~~V~~L~~Q~~R~~F~~K~~V~~V~~P~~A~~V~~E~~Q~~V~~G~~V~~E~~F~~E~~F~~I~~S~~Y~~L~~T~~A~~K~~M~~K~~G~~G~~F~~P~~V~~T~~I~~E~~E~~R~~I----  
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 CYP749B1 (442) F~~L~~S~~F~~G~~F~~L~~R~~K~~C~~V~~G~~F~~N~~F~~A~~Q~~M~~E~~V~~K~~I~~A~~L~~C~~M~~I~~L~~Q~~R~~Y~~R~~F~~T~~V~~S~~P~~N~~Y~~R~~H~~F~~P~~T~~L~~V~~--M~~G~~L~~W~~P~~K~~H~~G~~I~~Q~~I~~M~~L~~H~~P~~L~~----  
 CYP88C1 (428) F~~L~~P~~F~~E~~V~~G~~L~~R~~M~~C~~P~~C~~A~~N~~L~~V~~R~~L~~E~~V~~A~~V~~L~~H~~Y~~F~~L~~N~~Y~~R~~L~~E~~M~~L~~D~~P~~D~~S~~T~~P~~E~~K~~C~~L~~A~~R~~F~~K~~K~~L~~S~~A-----  
 CYP704A14 (436) F~~T~~A~~F~~Q~~A~~G~~P~~R~~I~~C~~L~~G~~K~~E~~F~~A~~Y~~R~~Q~~M~~K~~-I~~F~~S~~A~~V~~L~~L~~R~~Y~~F~~G~~F~~R~~L~~S~~D~~D~~K~~K~~N~~V~~N~~Y~~R~~T~~M~~I~~N~~L~~H~~I~~D~~G~~L~~H~~I~~R~~I~~F~~H~~R-----

CYP86A22 (517) LAKIEK~~F~~G~~K~~V~~E~~S~~C~~A~~G~~E~~H~~L~~I~~N~~N~~G~~I~~H~~Q~~P~~G~~S~~I~~A~~V~~N~~G~~I~~A~~A  
 CYP94A13 (510) -----  
 CYP714G3 (517) -----  
 CYP749B1 (505) -----  
 CYP88C1 (483) -----  
 CYP704A14 (500) -----

Suppl. FIGURE 2



Suppl. FIGURE 3

