

## SUPPLEMENTAL DATA

### Functional Analysis of Cytochrome P450 21A2 Variants Involved in Congenital Adrenal Hyperplasia

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FIGURE S1. CD spectra of wild-type P450 21A2 and mutants plotted as a function of temperature.

FIGURE S2. Fe<sup>2+</sup>-CO v. Fe<sup>2+</sup> difference spectra of several variants of P450 21A2.

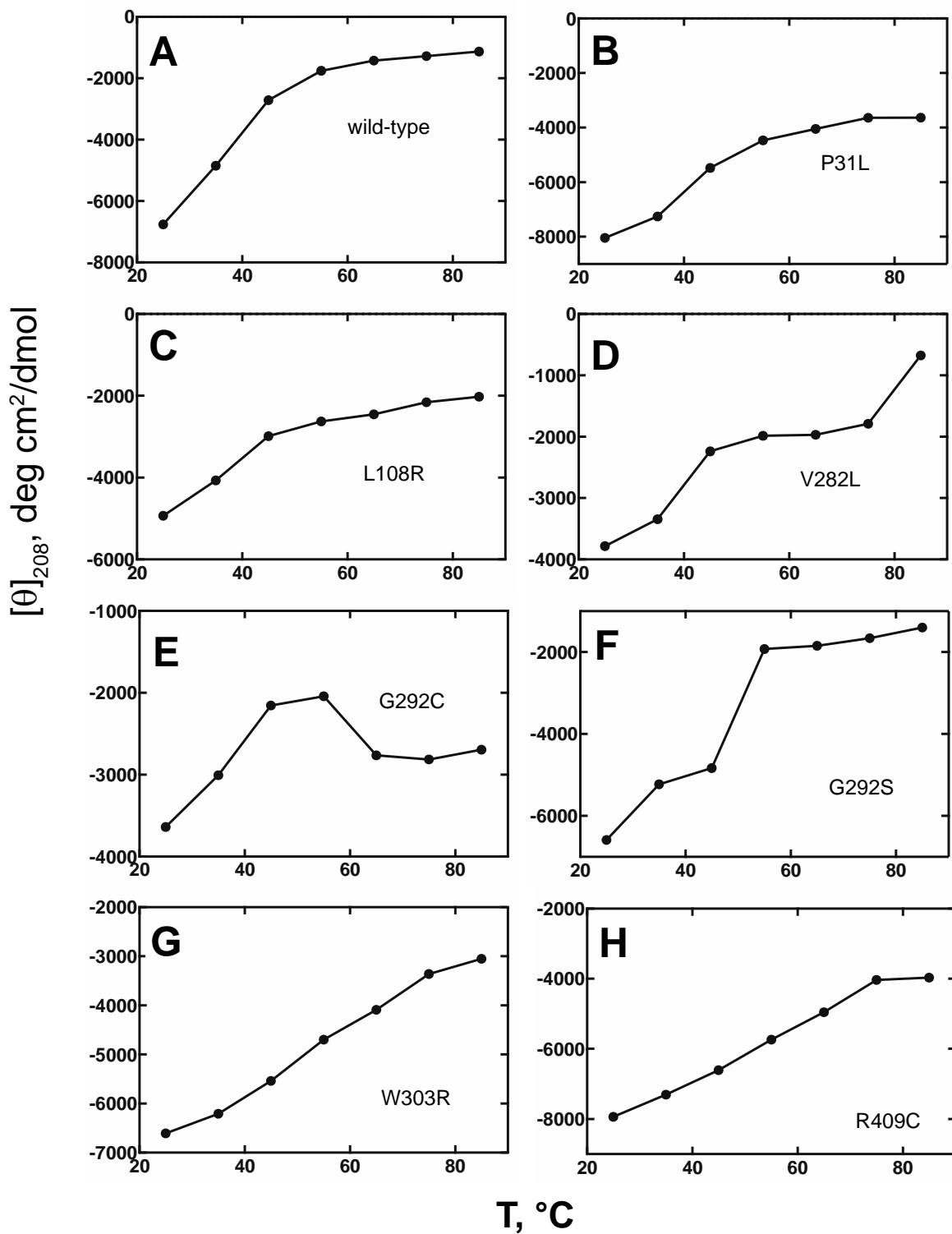


FIGURE S1. CD spectra of wild-type P450 21A2 and mutants plotted as a function of temperature. A, wild-type P450 21A2; B, P31L; C, L108R; D, V282L; E, G292C; F, G292S; G, W303R; H, R409C. See Fig. 5 for CD spectra at each temperature.

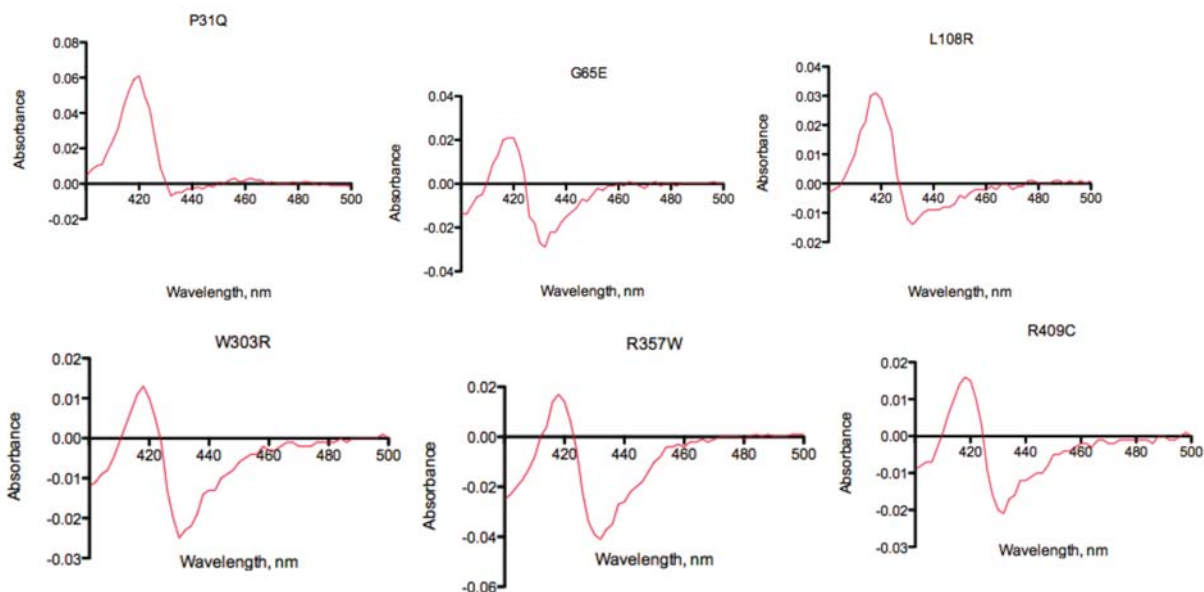


FIGURE S2.  $\text{Fe}^{2+}$ -CO v.  $\text{Fe}^{2+}$  difference spectra of several variants of P450 21A2 (in bacterial cells, not normalized for protein concentration). See text and Fig. 6 for methodology. Reduced P450-CO complex spectra of P450 variants were acquired in bacterial cells (Fig. 6). The presence of the 450 nm band is indicative of structurally-intact protein, and the band at 420 nm is indicative of cytochrome P420, a denatured form.