Supplemental Table 1 – P450 and P420 Content of Purified Enzyme Preparations*

Variant	P450, μM	P420, μM
***1**	40	3
***34**	96	14
***17-2**	17	5.5
***17-3**	19	11
***53**	75	14
Thr309Ala mutant	40	4

*Calculated by the method of Omura and Sato (Omura and Sato, 1964b).

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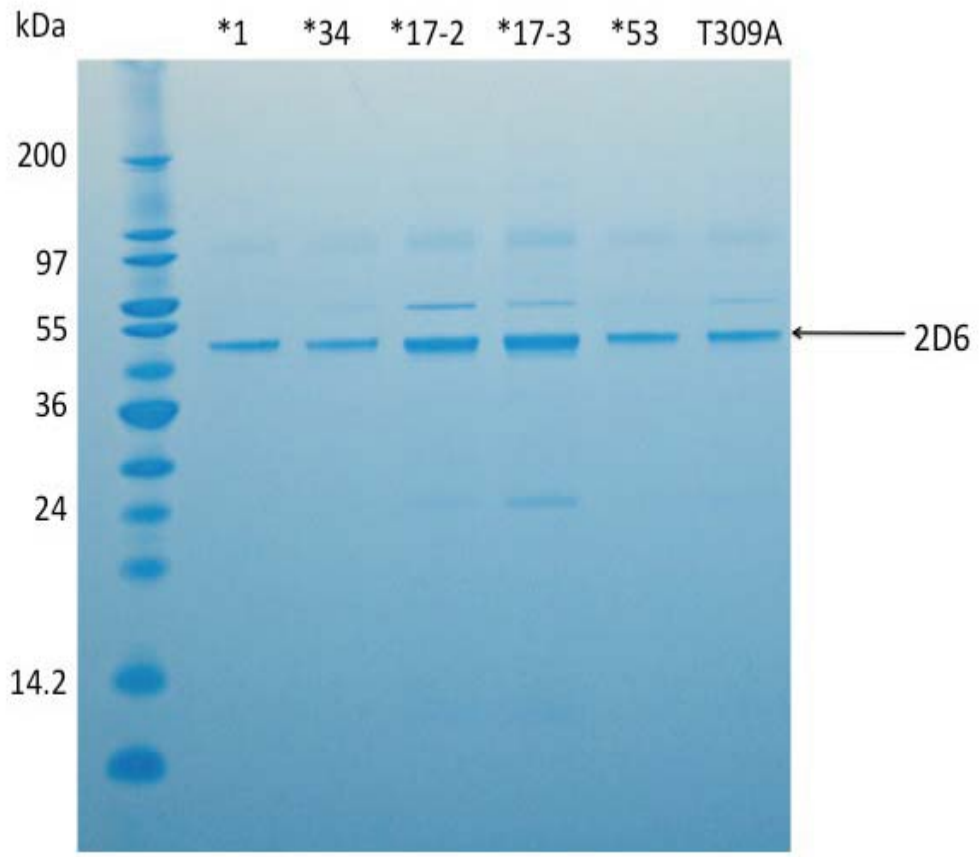

Supplemental Fig. 1. CYP2D6 enzymes were purified to greater than 90% homogeneity by Coomassie blue staining.

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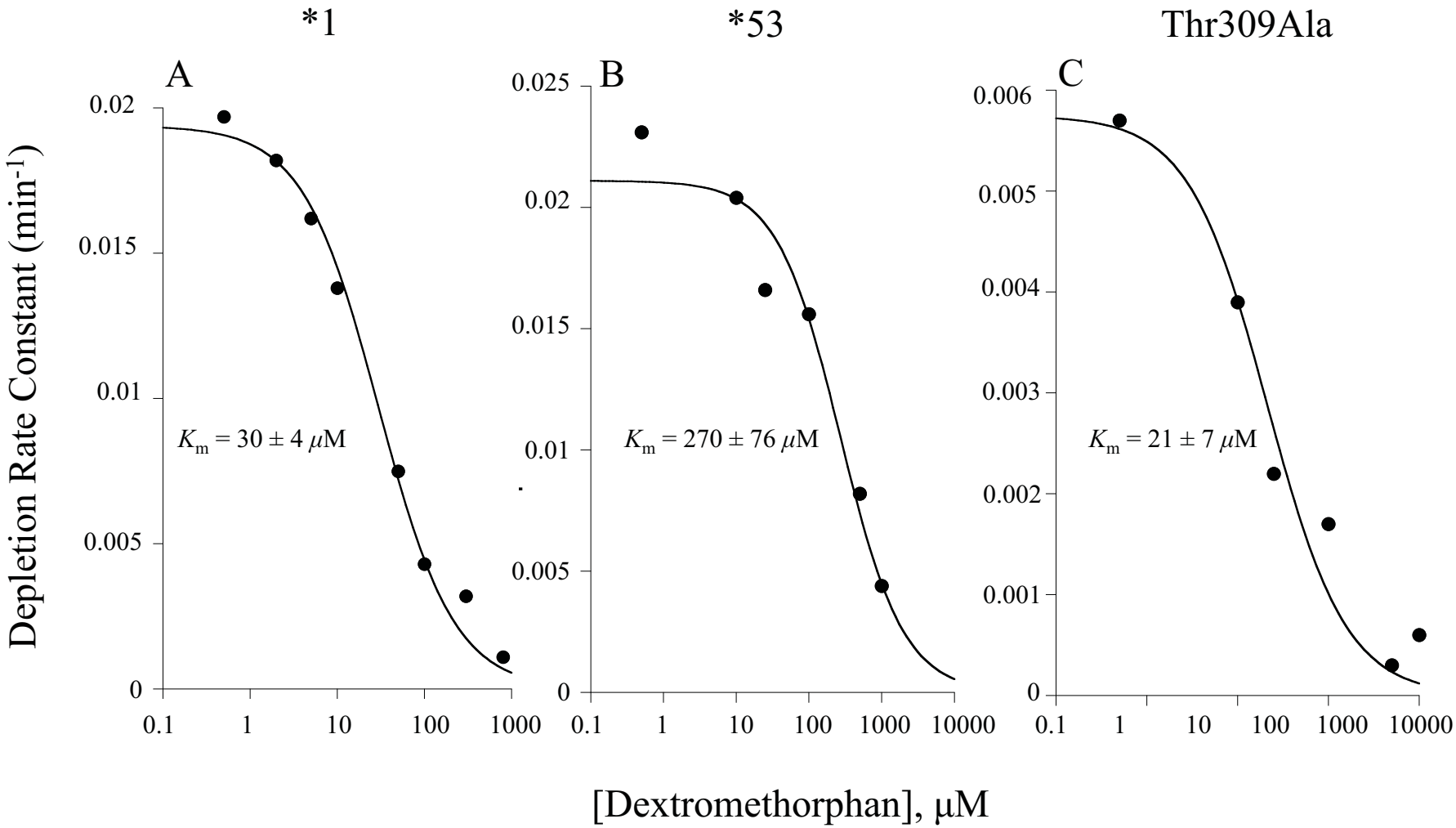

Supplemental Fig. 2. Depletion rate constants versus dextromethorphan concentration for indicated purified CYP2D6 enzymes. The K_M was determined at the inflection point as described in the Materials and Methods. (A) CYP2D6*1, K_m = 30 ± 4 μM; (B) CYP2D6*53, K_m = 270 ± 76 μ; and (C) Thr309Ala mutant, K_m = 21 ± 7 μM.

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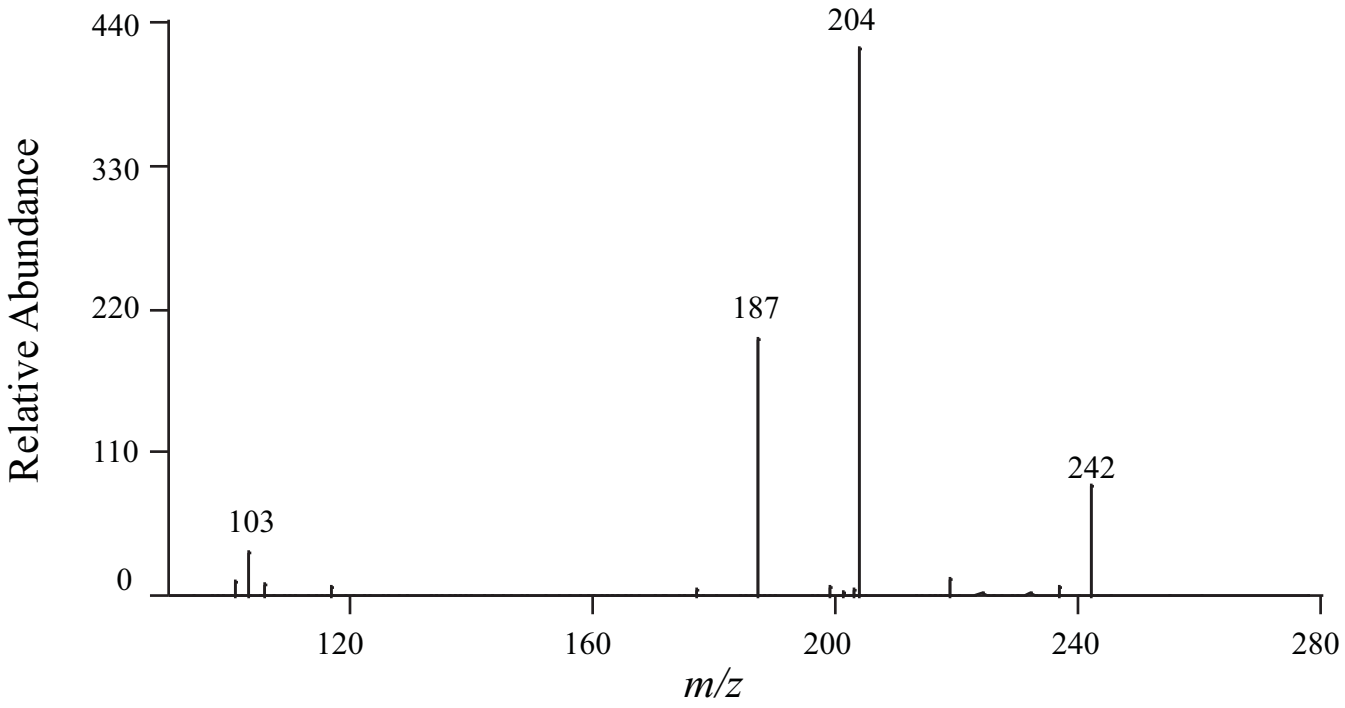

Supplemental Fig. 3. MS^2 fragmentation of bufuralol metabolite (260 *m/z*) eluting at 28 min and formed predominantly by the Thr309Ala mutant (labeled *e* in Fig. 3B).

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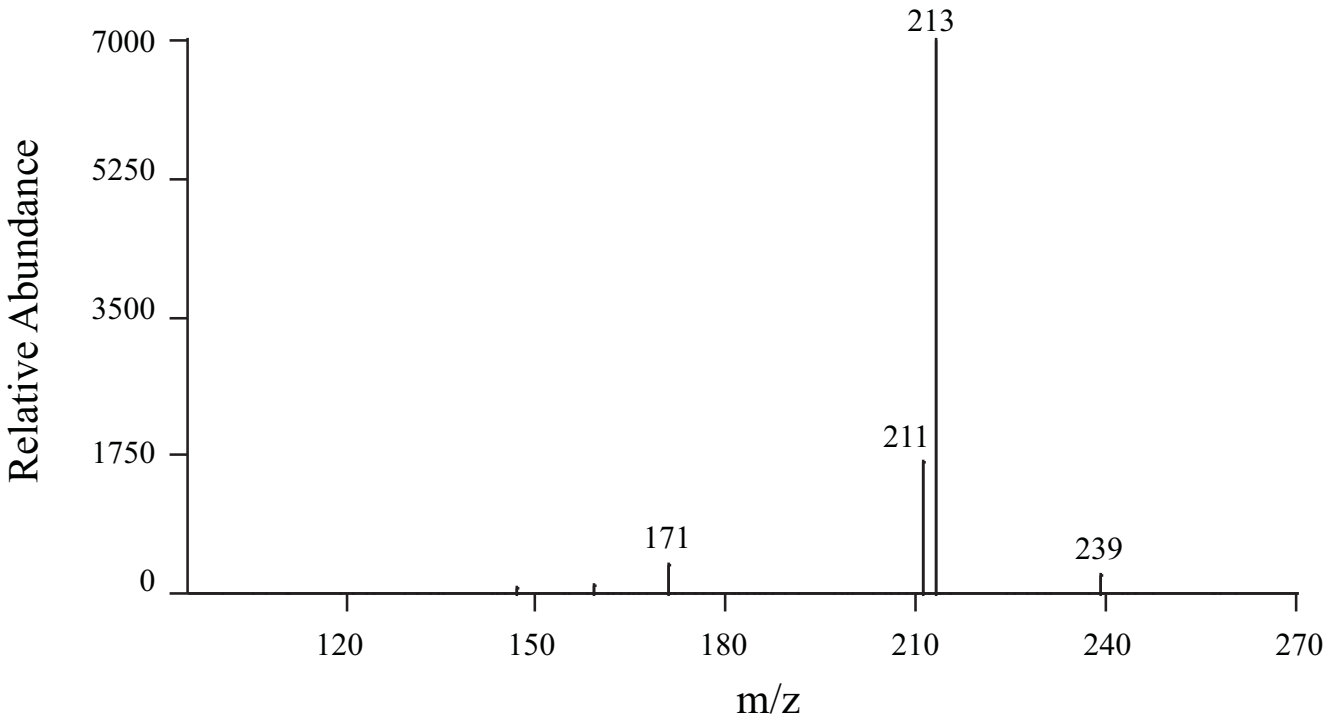

Supplemental Fig. 4. MS^2 fragmentation of dextromethorphan mono-oxygenation metabolite (288 *m/z*) eluting at ~6 min formed only by the *53 variant (labeled *d* in Fig. 4C and Supplemental Fig. 6). The MS^2 fragmentation was identical for the peaks at 6 and 7 min (labeled *d* and *e* in Fig. 4C).

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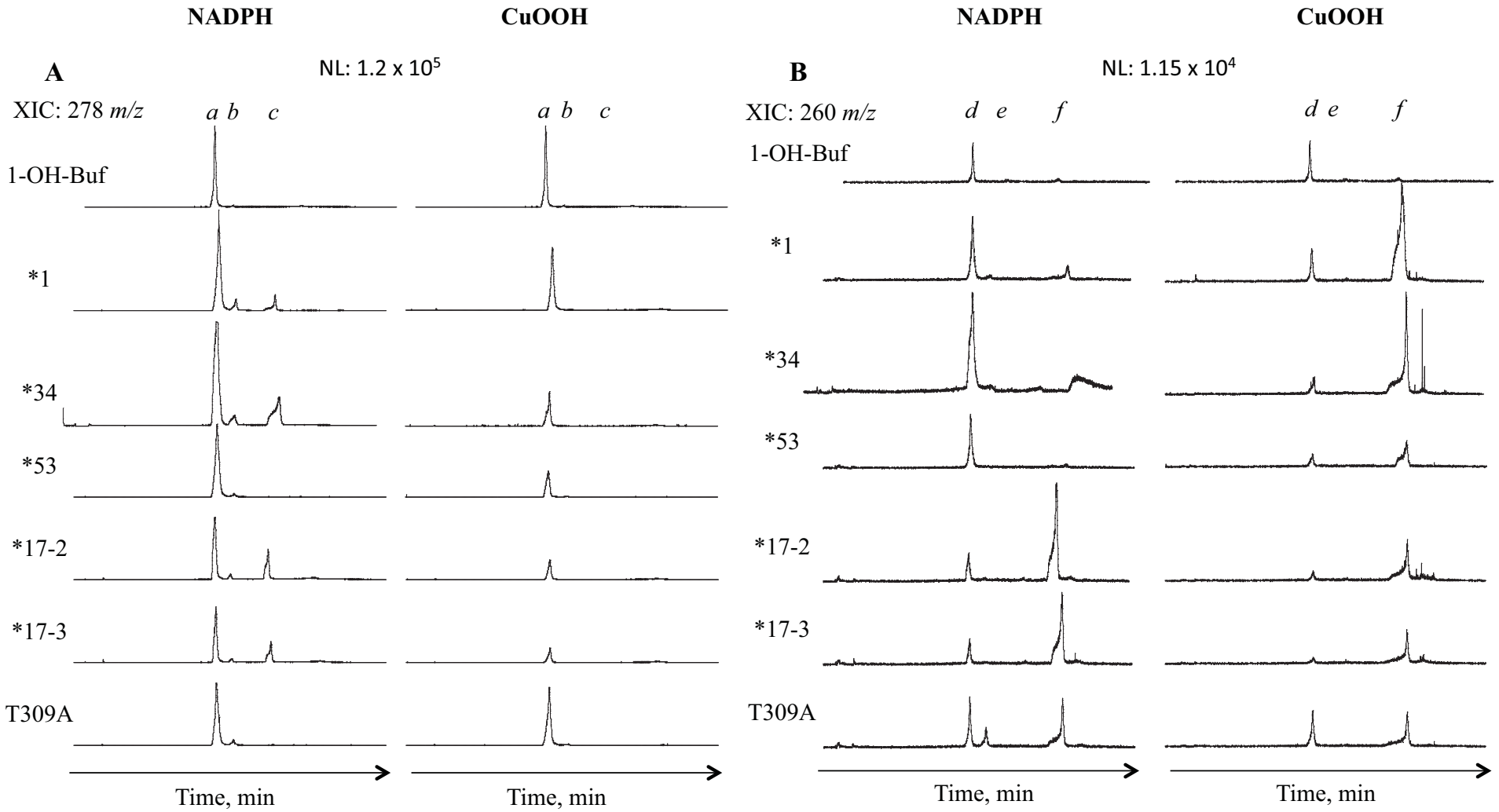

Sup**plemental Fig. 5.** Extracted ion chromatograms of bufuralol metabolites formed by purified CYP2D6 enzymes in presence of NADPH generating system or CuOOH. (A) Extracted ion chromatograms for hydroxybufuralol (278 *m/z*, M+H). 1'-Hydroxy-, 4-hydroxy- and 6-hydroxybufuralol eluted at 25, 28, and 35 min (*a*, *b*, and *c*), respectively, with 1'-hydroxybufuralol (*a*) as the major metabolite in reactions with NADPH and the only metabolite observed in reactions with CuOOH. The chromatograms were converted to the same scale with a normalized level of 1.2 x 10^5. (B) Extracted ion chromatograms for dehydrogenated ($\Delta^{1',2'}$) bufuralol (260 *m/z*, M+H) eluting at 41 min (*f*). The peak at 25 min (*d*) was a contaminant present in the standard as well as all in the reactions with both NADPH and CuOOH. Also, an additional metabolite was formed by the Thr309Ala mutant and eluted at 28 min (*e*) (see Supplemental Fig. 3 for MS). The chromatograms were converted to the same scale with a normalized level of 1.15 x 10^4, an order of magnitude less than the 278 *m/z* XIC in panel A.

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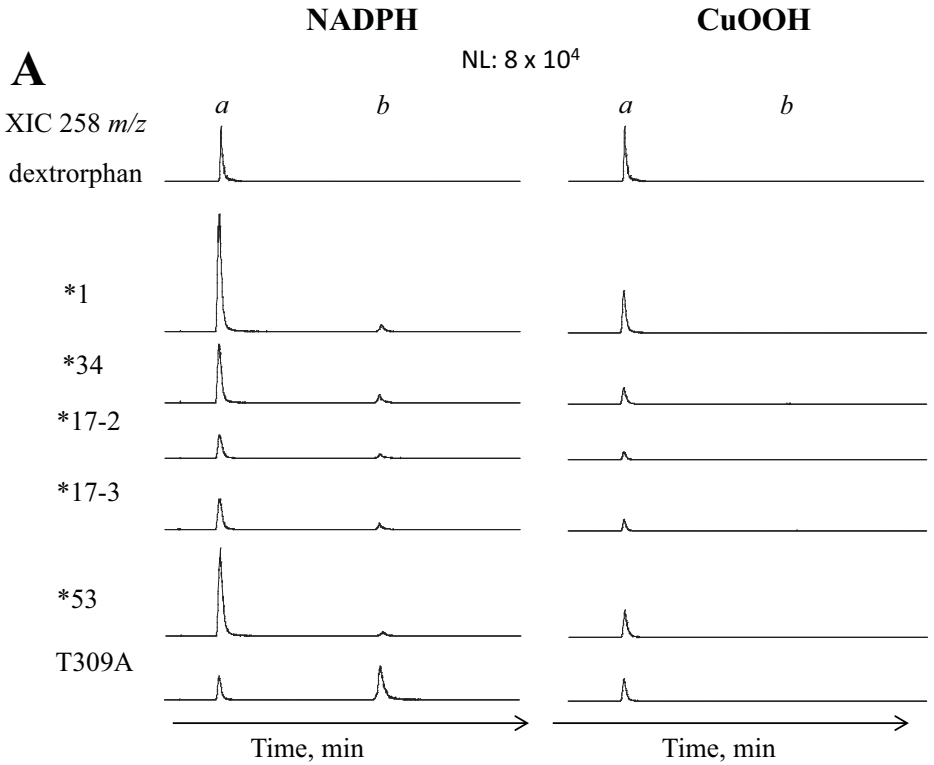

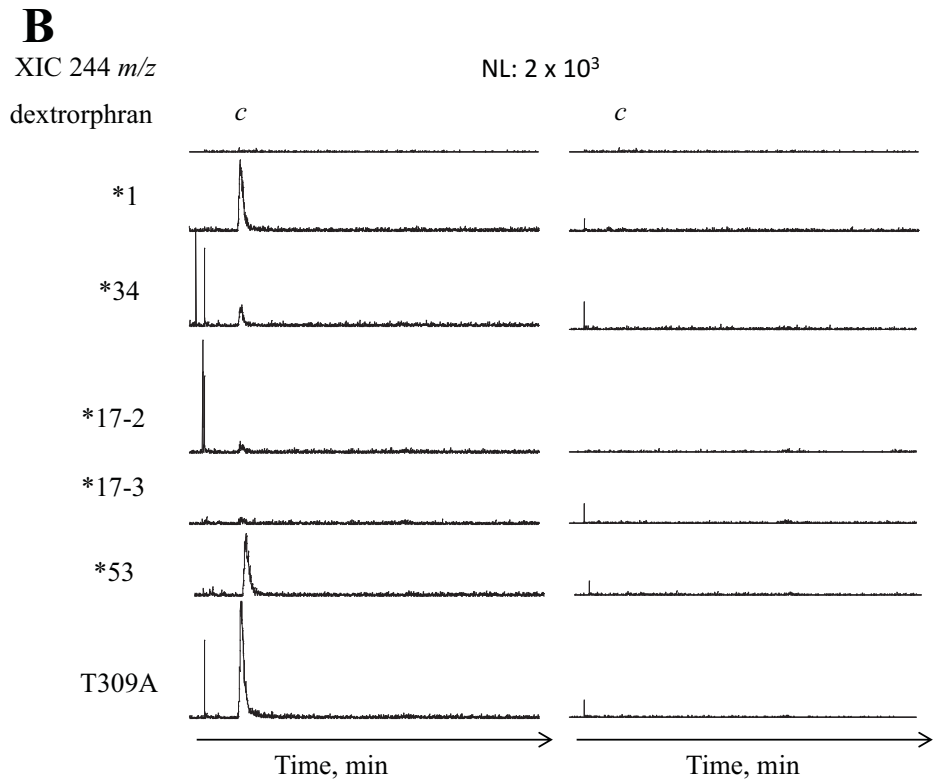

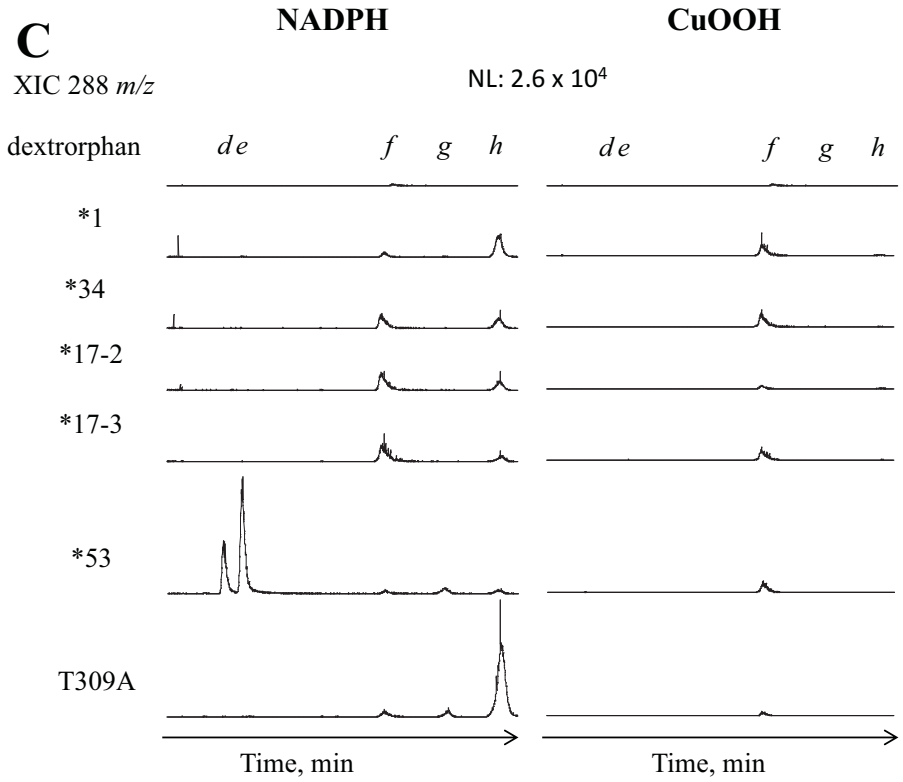

Supplemental Fig. 6. Extracted ion chromatograms of dextromethorphan metabolites formed by purified CYP2D6 enzymes in presence of NADPH generating system or CuOOH. (A) Extracted ion chromatograms for dextrorphan eluting at 6 min and 3-methoxymorphinan at 22 min (258 *m/z*, M+H) (*a* and *b*, respectively). In the presence of CuOOH, 3-methoxymorphinan (*b*) was not observed. Chromatograms were converted to the same scale with a normalized level of 8 x 10^4. (B) Extracted ion chromatograms for 3-hydroxymorphinan (*c*) (244 *m/z*, M+H). 3-Hydroxymorphinan (*c*) was not formed in reactions with CuOOH as oxygen surrogate. Chromatograms were converted to the same scale with a normalized level of 2 x 10^3. (C) Extracted ion chromatograms for mono-oxygenated dextromethorphan (288 *m/z*, M+H). CYP2D6*53 formed hydroxylated dextromethorphan products eluting at 5.6 and 7.5 min (*d* and *e*) (see Supplemental Fig. 4 for MS). All CYP2D6 enzymes formed some *N*-oxide at 33 min (*h*), with Thr309Ala producing the highest levels. CuOOH did not support formation of hydroxylated dextromethorphan (*d,e*) or the *N*-oxide (*h*). All chromatograms were converted to the same scale with a normalized level of 2.6 x 10^4.

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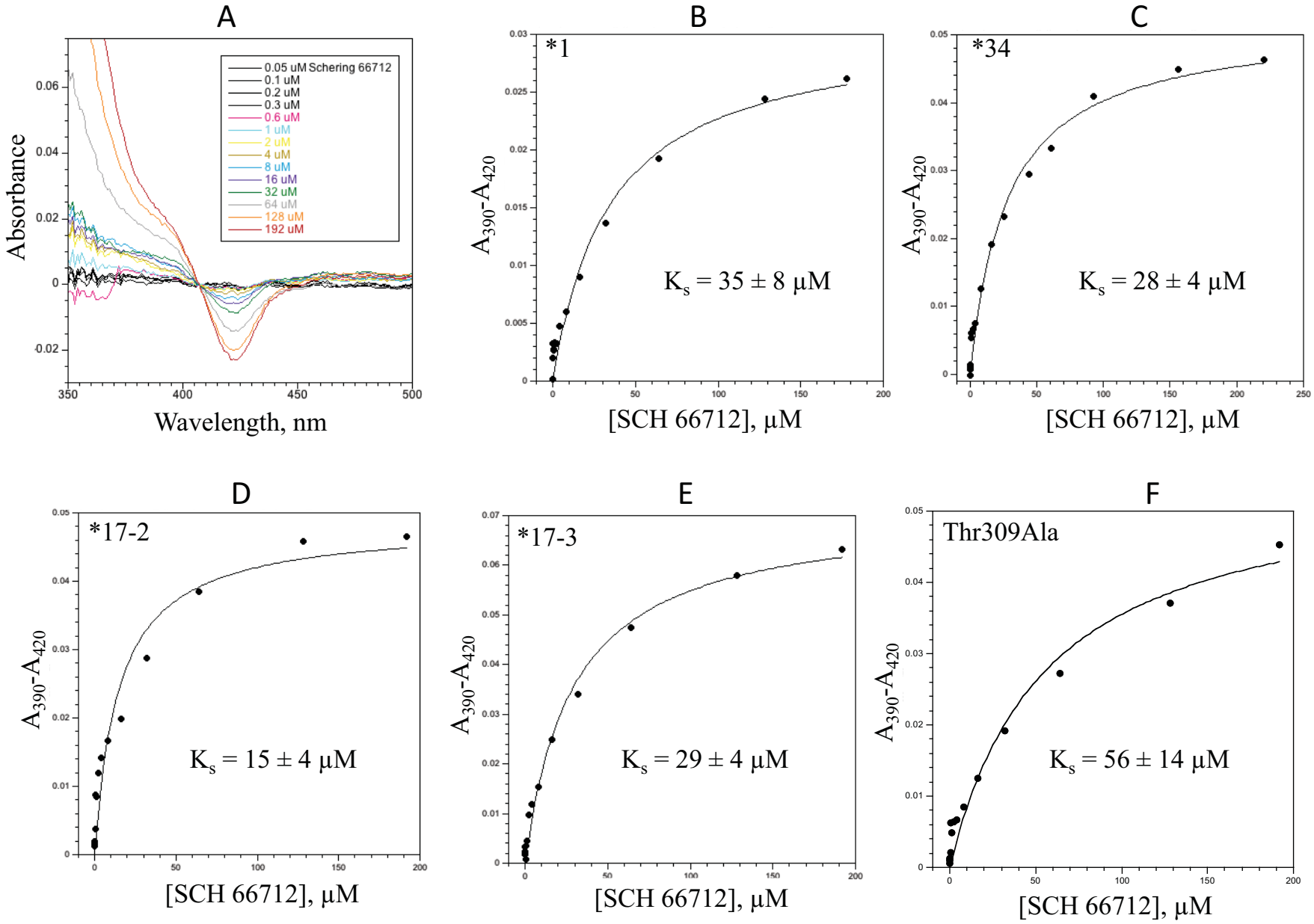

Supplemental Fig. 7. Titration of purified CYP2D6 enzymes with SCH 66712 resulted in Type I binding spectra. Purified CYP2D6 enzymes (1 µM in each case) were divided into each of two cuvettes and a baseline was set. Aliquots (0 – 200 µM, final) of SCH 66712 in H_2O were added to the sample cuvette and equal volumes of H_2O were added to the reference cuvette. (A) Type I spectral binding for *1. The increase in absorbance at lower wavelengths is due to addition of SCH 66712 that has a λ_{max} of 270 nm. (B-F) Plot of $\Delta A_{430-395}$ vs. concentration of SCH 66712 for each CYP2D6 enzyme. K_s was determined using: $[CYP2D6 \bullet substrate] = 0.5\ (K_s + E_t + S_t) - [0.25\ (K_s + E_t + S_t)^2 - E_t S_t]^{1/2}$.

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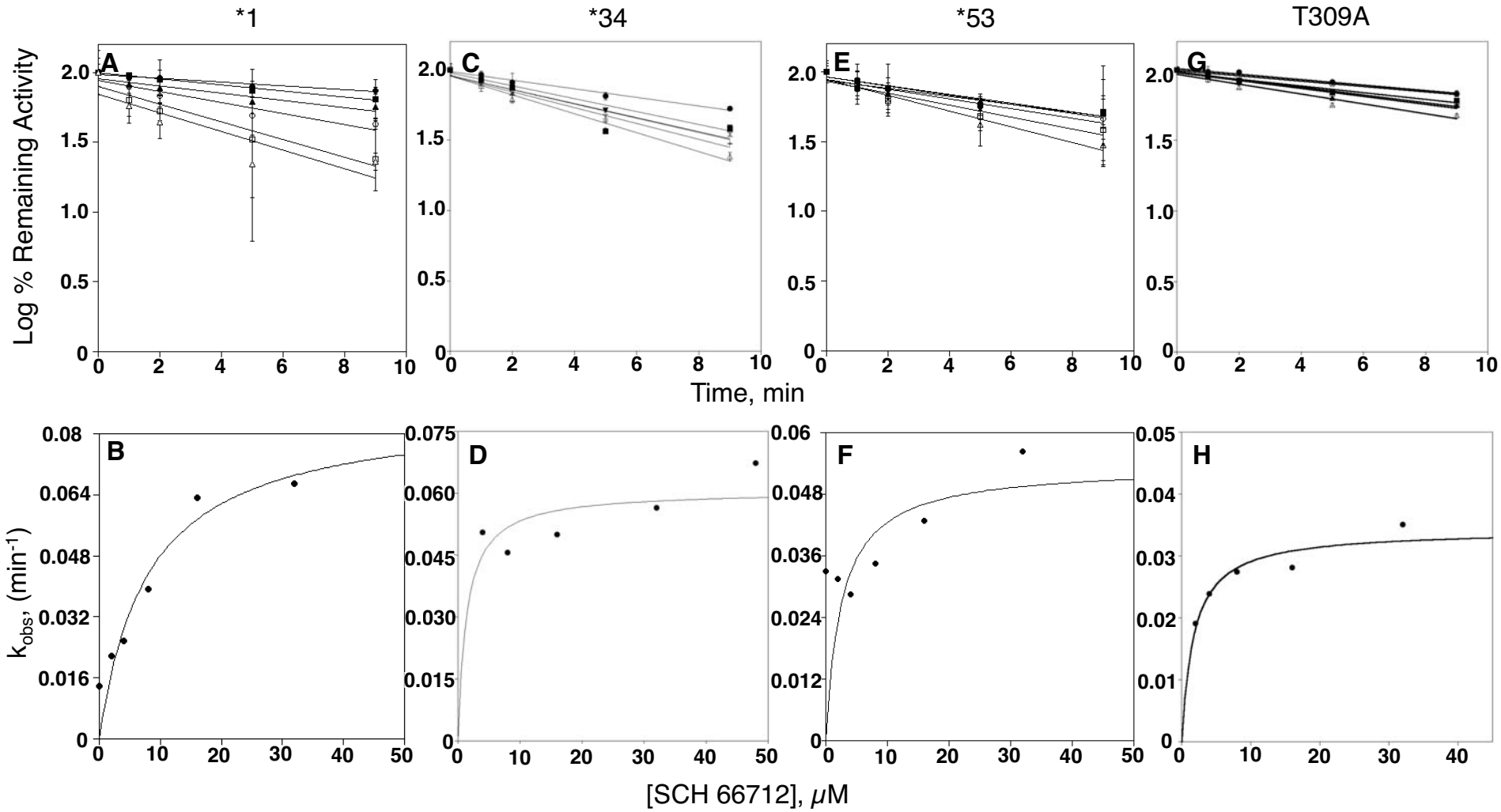

Supplemental Fig. 8. Kitz-Wilson analysis of inactivation of purified CYP2D6 enzymes *1, *34, *53, and Thr309Ala by SCH 66712 (0, 4, 8, 16, 32, and 48 μM). Dextromethorphan was used as the reporter substrate. (A) Inactivation of *1. (B) K_I and k_{inact} for the inactivation of *1 by SCH 66712 were 8 ± 4 μM and 0.09 ± 0.02 min^{-1}, respectively, as determined using Kitz-Wilson analysis and non-linear regression. (C) Inactivation of *34. (D) K_I and k_{inact} for the inactivation of *34 by SCH 66712 were 1 ± 1 μM and 0.061 ± 0.006 min^{-1}, respectively. (E) Inactivation of *53. (F) K_I and k_{inact} for the inactivation of *53 by SCH 66712 were ~3 μM and 0.05 ± 0.02 min^{-1}, respectively. (G) Inactivation of Thr309Ala by SCH 66712. (H) K_I and k_{inact} for the inactivation of Thr309Ala by SCH 66712 were 1.7 ± 0.5 μM and 0.034 ± 0.002 min^{-1}, respectively. Results are summarized in Table 5.

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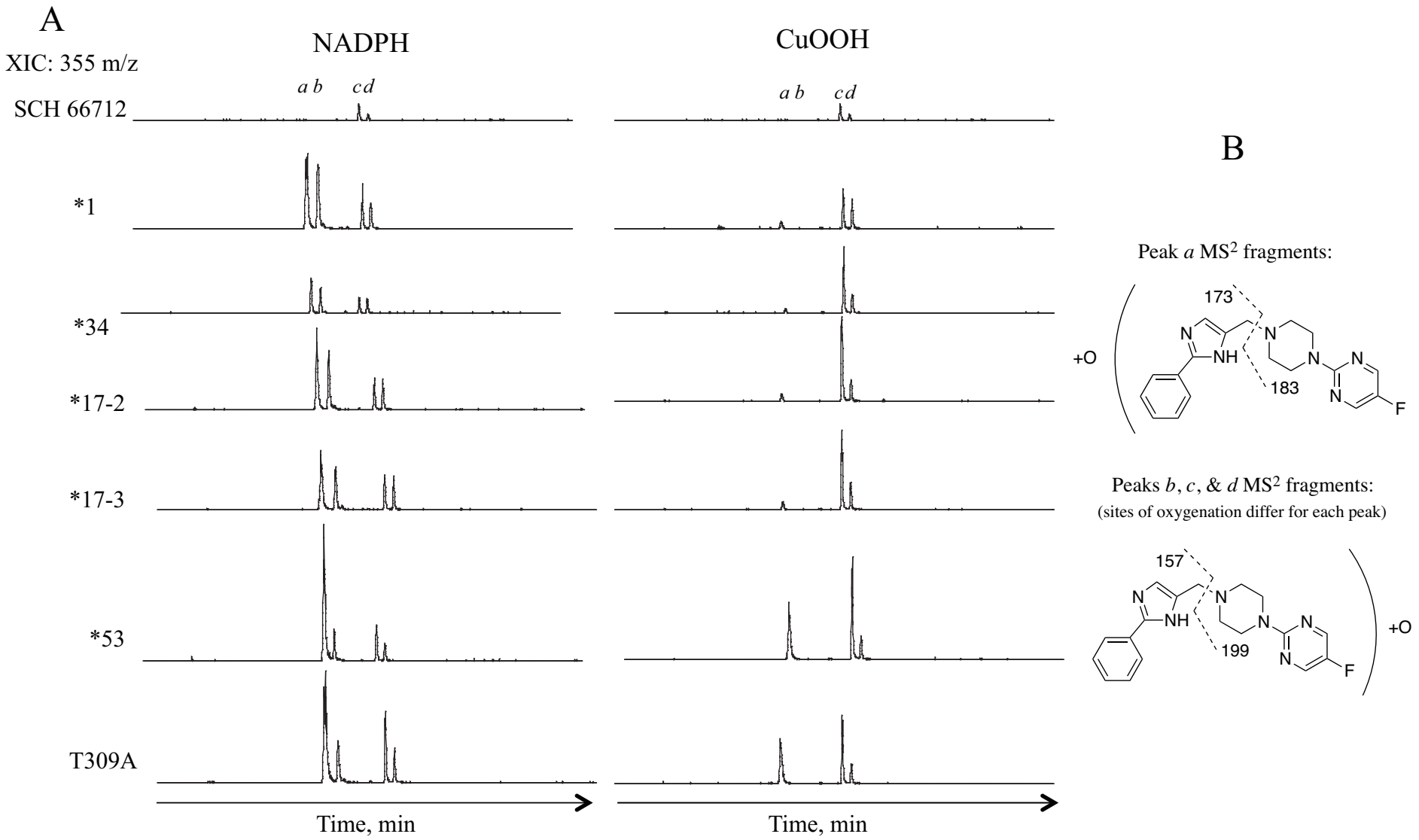

Supplemental Fig. 9. A. Extracted ion chromatogram (355 *m/z*, M+H) for hydroxylated SCH 66712 metabolites formed by purified CYP2D6 enzymes in presence of NADPH generating system or CuOOH. Mono-oxygenated metabolites eluted at 17 (*a*), 18 (*b*), 23 (*c*), and 24 (*d*) min, as previously observed (Nagy, 2011; Bolles, 2014). The later eluting peaks at 23 (*c*) and 24 (*d*) min are also present in low amounts in the control reaction. The metabolite eluting at 17 (*a*) min was the most prominent, especially in reactions with CYP2D6*53 and Thr309Ala mutant. CuOOH supported formation of all the metabolites, except the one eluting at 18 (*b*) min, with substantial increase in the metabolite at 23 (*c*) min. Metabolite *a* represents hydroxylation on the phenyl ring of SCH 66712. The other three metabolites are oxygenation on the other end of the molecule as previously described (Nagy, 2011). The chromatograms were converted to the same scale with a normalized level of 1.2 x 10^5. **B**. MS^2 fragmentation sites for peaks *a, b, c*, and *d* that represent mono-oxygenation of SCH 66712 by CYP2D6. More information in (Nagy, 2011).

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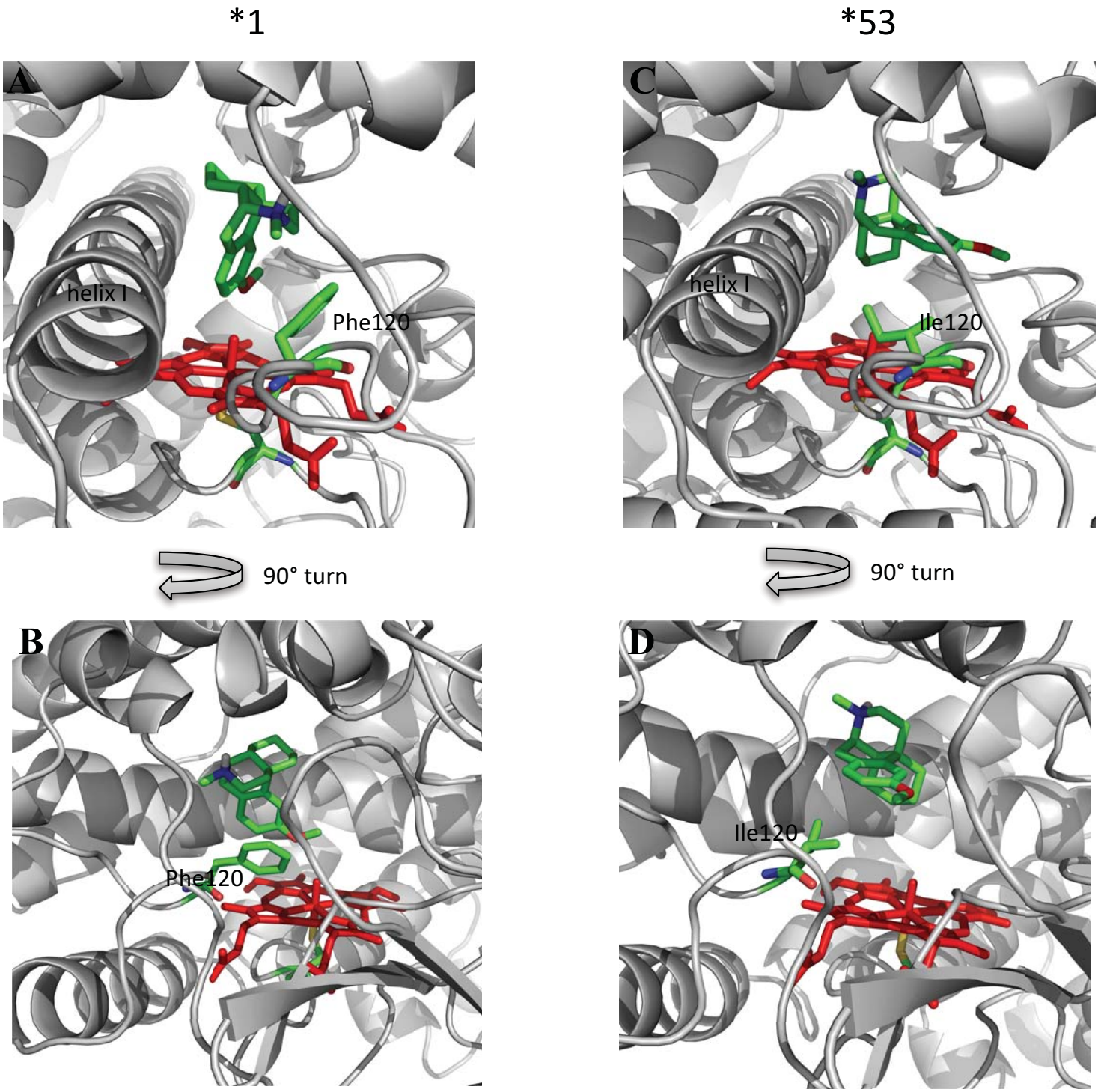

Supplemental Fig. 10. Docking of dextromethorphan in the active site of *1 (A,B) and *53 (C,D). PDB ID 3QM4, Chain A, was used for docking for *1. The *53 model, with amino acid changes of Ala122Ser and Phe120Ile, was prepared in molecular dynamics simulations with 3QM4 as previously described (de Waal, 2014). Dextromethorphan is shown in green with nitrogen and oxygen atoms in blue and red, respectively. The heme is shown in red. Phe120 in *1 and Ile120 in *53 are shown as sticks and labeled. The distance between the center of the dextromethorphan aromatic ring and Phe120 in *1 is 3.5 Å. The distance between the oxygen of dextromethorphan and the activated oxygen of the heme iron (compound 1) is 3.4 Å. In *53, dextromethorphan is situated differently and is ~4.5 Å from both the activated oxygen of the heme and Ile120.

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