Supplemental Material

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Role of CYP2A5 in the Bioactivation of the Lung Carcinogen 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone in Mice

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Legends for Supplemental Figures

Supplemental Fig. 1. LC-MS detection of NNK metabolites formed by mouse liver and lung microsomes. Liver (A-C) and lung (D-F) microsomal incubations were performed without (A, D; negative controls) or with NADPH (B, C, E, F). NNK concentration was at 10 μM, and sodium bisulfite was added to some samples (C, F) to trap OPB. Inclusion of bisulfite in the negative control incubations (A, D) did not change the chromatograms (not shown). Metabolites were detected using the MIM-EPI acquisition mode, as described in *Methods*; the MS/MS product-ion spectra of the detected metabolites are shown in Figure S2. The *m/z* values for the detected compounds were: NNK, 208; NNAL, 210; HPB, 166; NNK-N-oxide, 224; OPB-bisulfite, 246; OPBA, 180.

Supplemental Fig. 2. MS/MS product-ion spectra of detected NNK metabolites. The mass spectra were obtained in the MIM-EPI acquisition mode for NNK (A), NNAL (B), HPB (C), NNK-N-oxide (D), OPB-bisulfite (E), and OPBA (F).

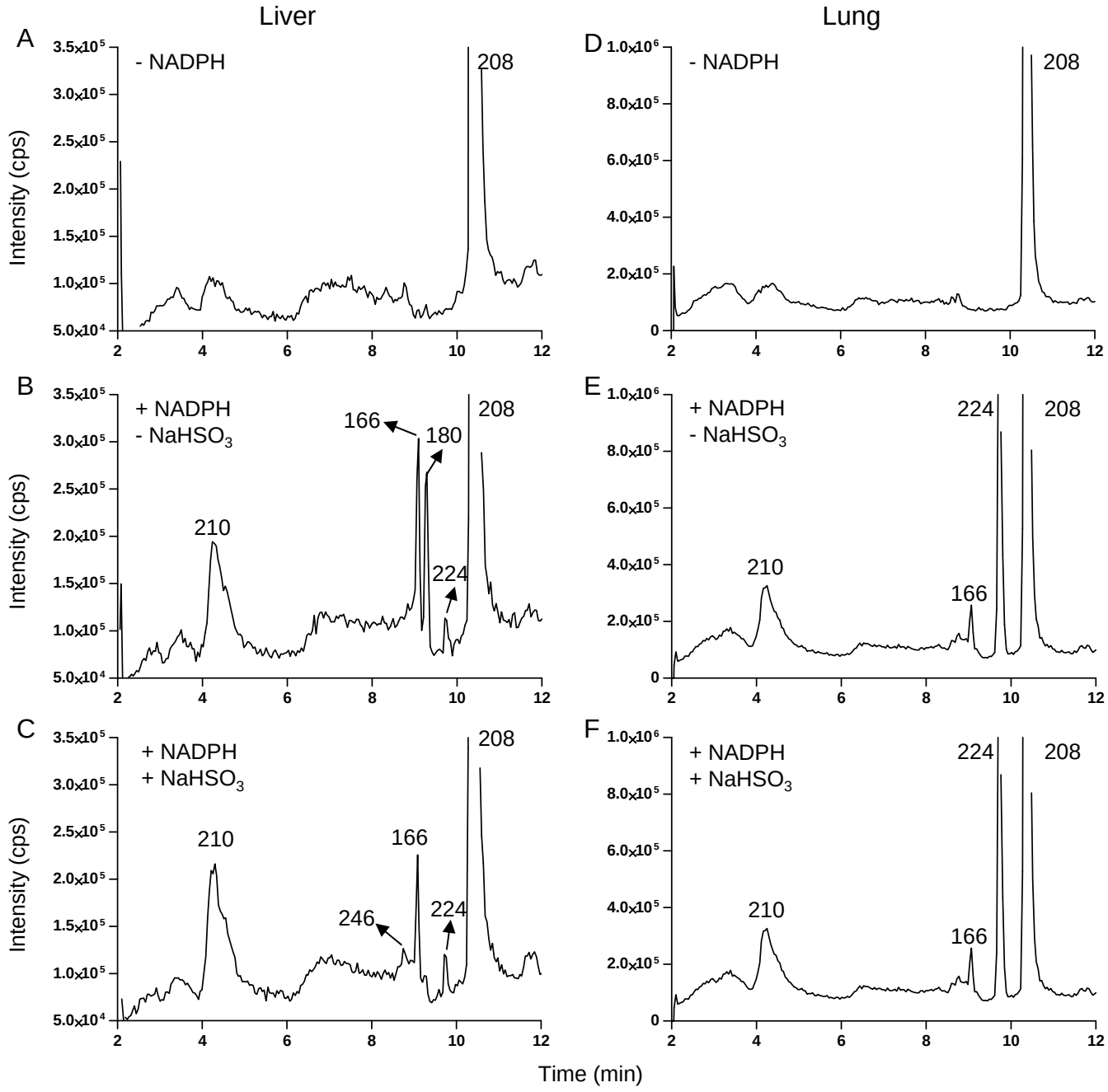
Supplemental Table 1

Kinetic parameters for the formation of NNK metabolites by WT A/J and B6 mouse lung and liver microsomes

Apparent Km and Vmax values for the microsomal formation of three major NNK metabolites (HPB, NNK-N-oxide, and sodium bisulfite-trapped OPB) were determined as described in *Methods*. Values represent means ± S.D. of values determined for three separate microsomal samples, each prepared from tissues pooled from five 2-month-old female mice. Values for WT B6 mice are from Table 1.

Metabolite	Strain	Lung			Liver		
		Km (μM)	*Vmax* (*pmol/min/mg*)	*Vmax/Km*	*Km (μM)*	*Vmax* (*pmol/min/mg*)	*Vmax/Km*
OPB	WT (A/J)	27.8 ± 2.2	58.1 ± 2.3	2.1	27 ± 2	220 ± 10	8.0
	WT (B6)	28.0 ± 1.9	57.3 ± 1.8	2.0	29 ± 4	220 ± 10	7.6
HPB	WT (A/J)	2.6 ± 0.6	24.5 ± 1.0	9.3	16 ± 2	102 ± 3	6.3
	WT (B6)	2.8 ± 0.3	24.3 ± 0.5	8.7	18 ± 3	105 ± 5	5.9
NNK-N-oxide	WT (A/J)	1.6 ± 0.2	37.1 ± 1.8	23.2	81 ± 16	19 ± 2	0.2
	WT (B6)	1.6 ± 0.2	35.8 ± 3.1	22.4	84 ± 18	19 ± 2	0.2

Supplemental Figure 1



Supplemental Figure 2