Supplementary Data

Whole body physiologically-based pharmacokinetic model for nutlin-3a in mice after intravenous and oral administration

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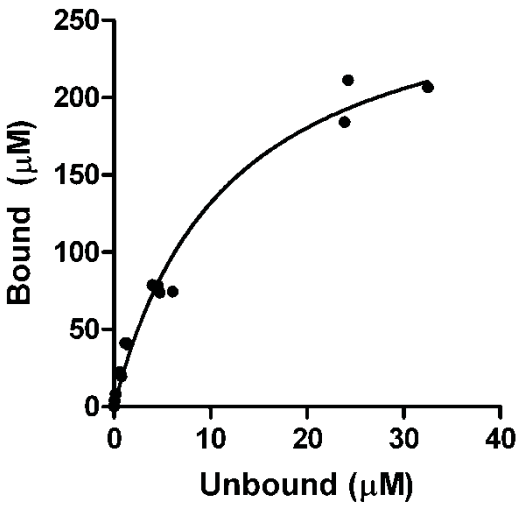
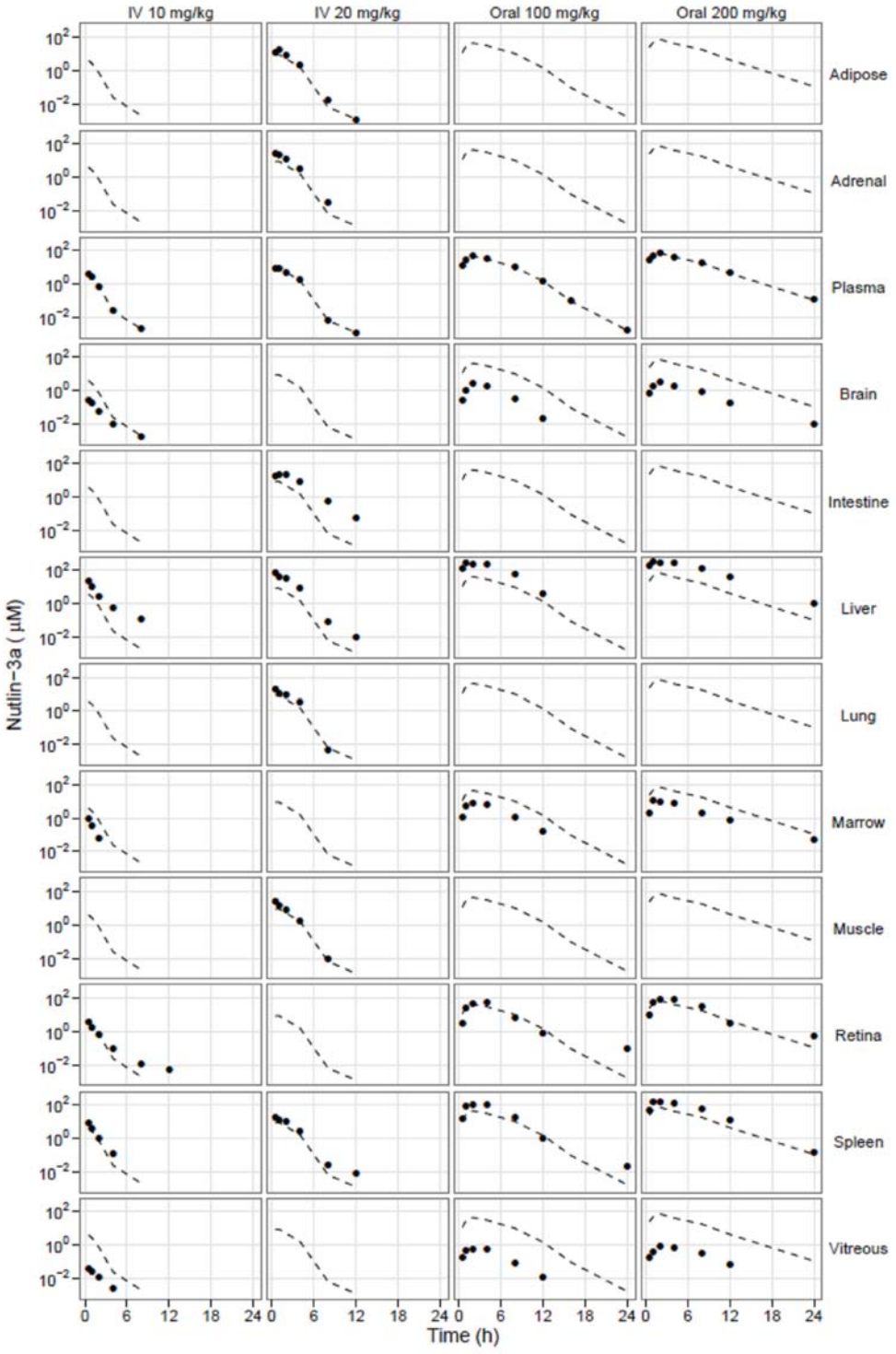
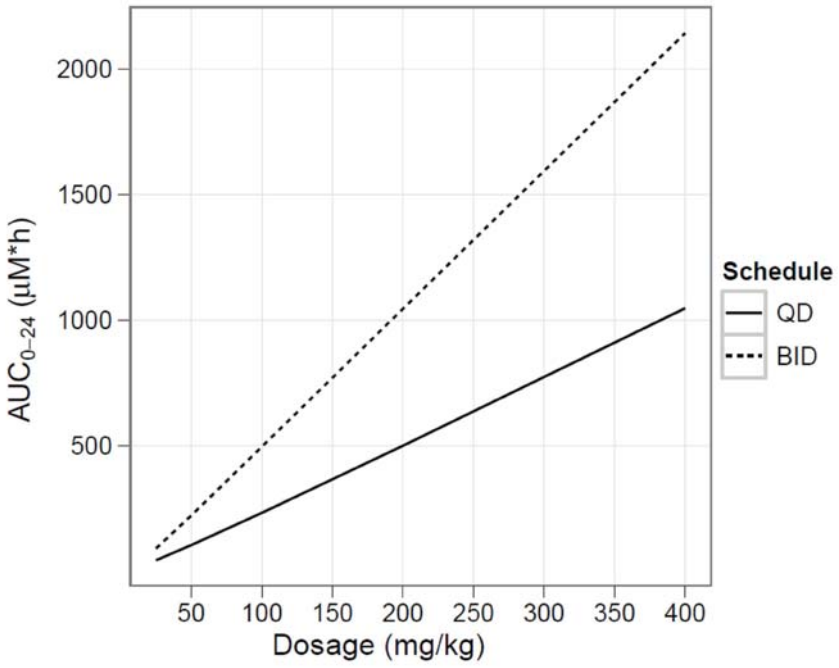
Drug Metabolism and Disposition



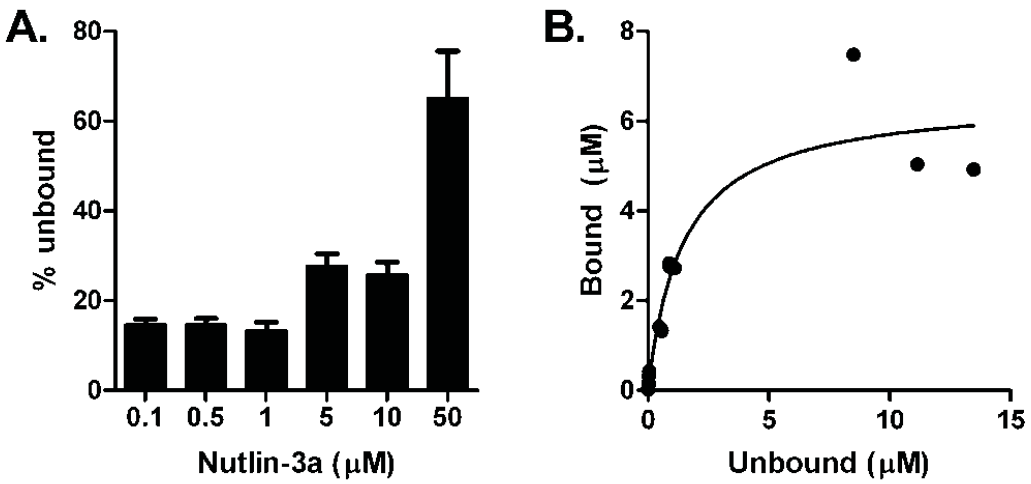
Supplementary Figure S1. Nutlin-3a binding to murine plasma proteins. Bound and unbound nutlin-3a plasma concentrations were determined with equilibrium dialysis and nonlinear regression was performed using the Langmuir equation.



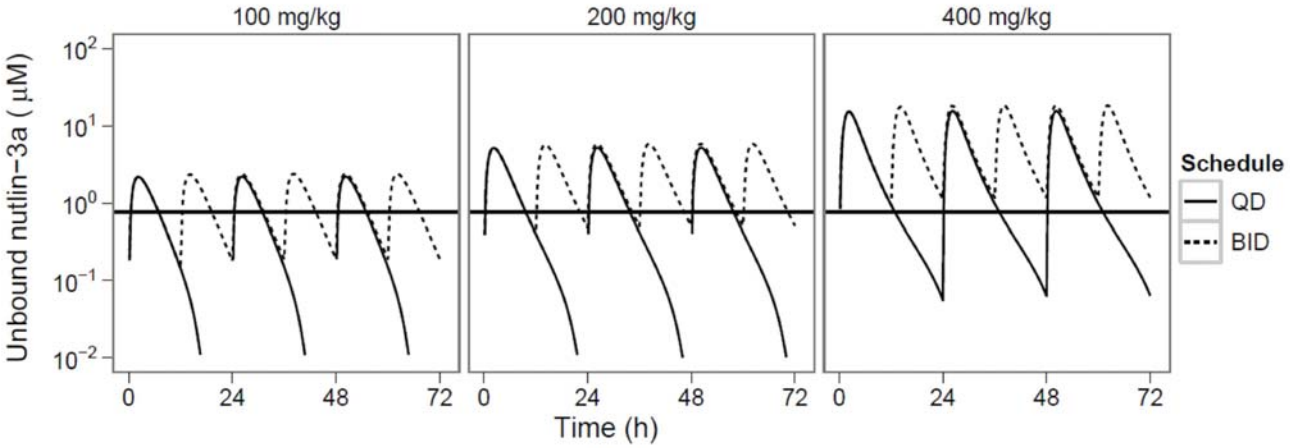
Supplementary Figure S2. Comparison of actual plasma and tissue concentrations of nutlin-3a. The median plasma concentrations are shown in each box as the dashed line and the symbols represent the median tissue concentrations. This plot allows visualization of tissue concentrations relative to plasma.



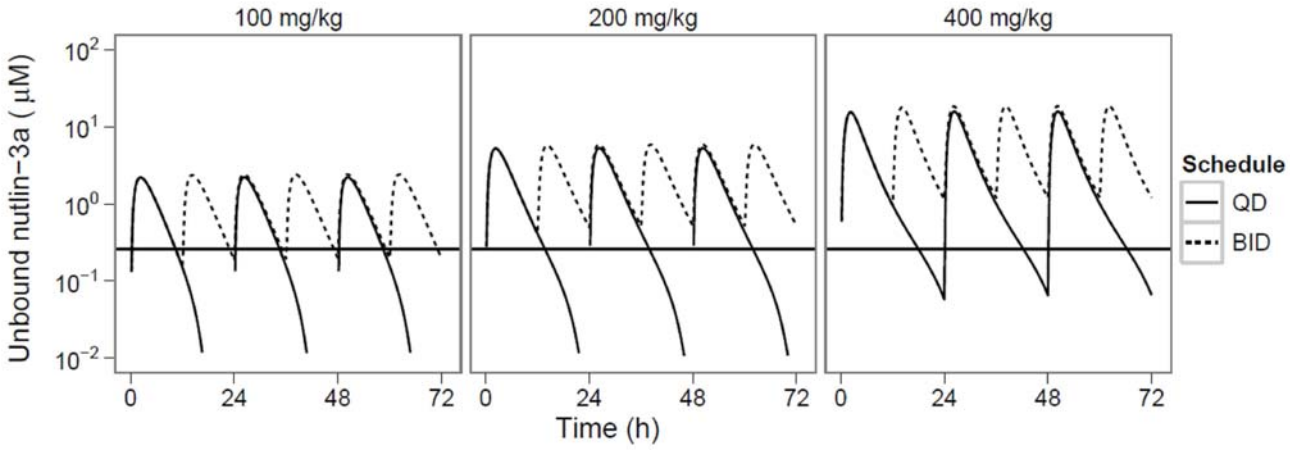
Supplementary Figure S3. Plasma area under the concentration-time curve for 24 h at steady state (AUC_{0-24}) versus nutlin-3a dosage when administered once daily (QD) and twice daily (BID). AUCs were calculated from simulated concentration-time curves based on the final PBPK model.



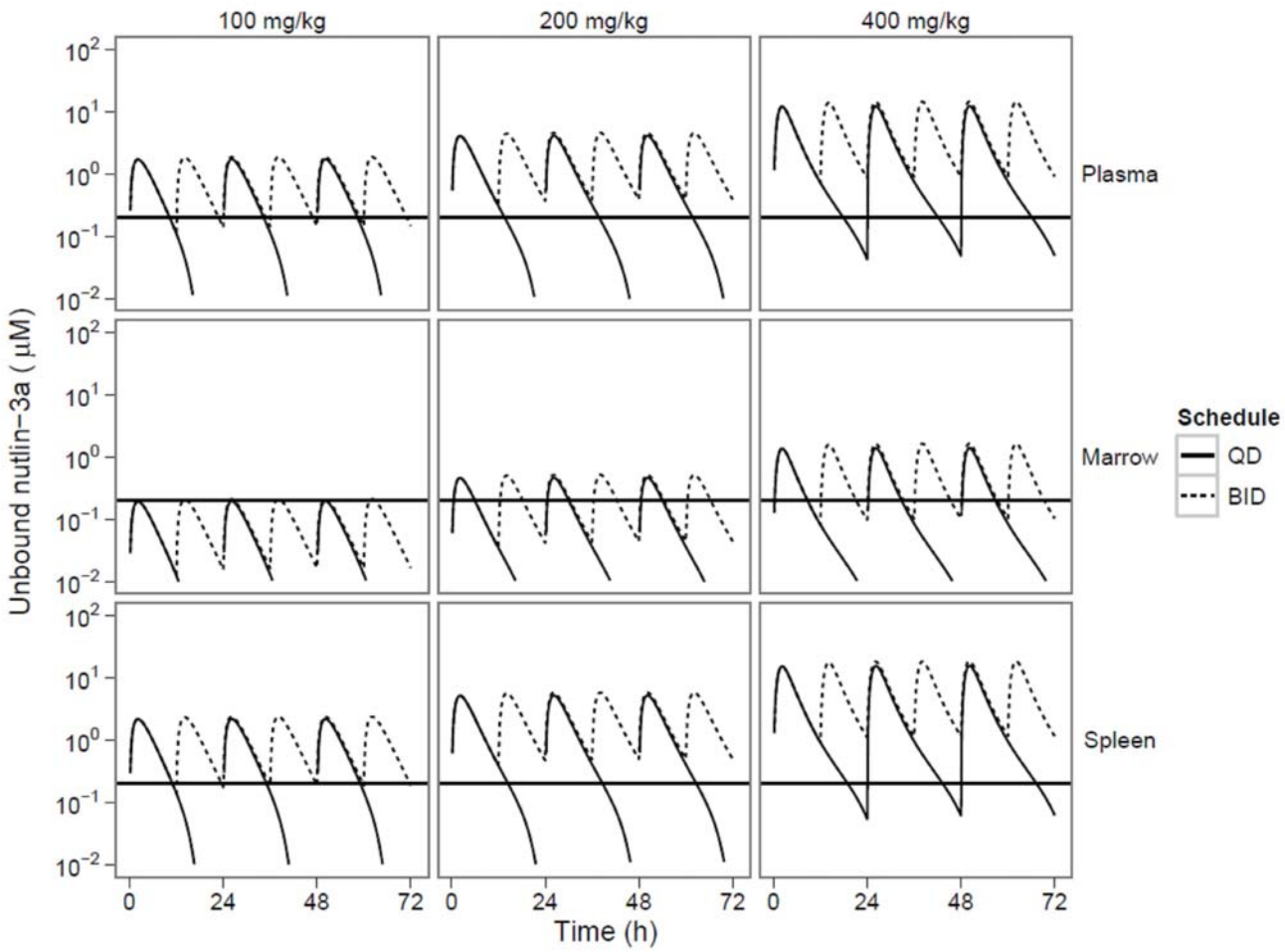
Supplementary Figure S4. Nutlin-3a binding to cell culture media. Bound and unbound nutlin-3a plasma concentrations were determined with equilibrium dialysis. (A) The unbound fraction is shown with increasing total nutlin-3a concentrations. Bars represent the mean and error bars represent the standard deviation for one experiment performed in triplicate. (B) Nonlinear regression was used to fit a binding model (Langmuir equation) to the data.



Supplementary Figure S5. Simulated concentration-time plot of unbound nutlin-3a in the adrenal gland after multiple oral doses given once daily (QD) or twice daily (BID). The horizontal lines represent the unbound IC_{50} of nutlin-3a for IMR-32 p53-wt neuroblastoma cells.



Supplementary Figure S6. Simulated concentration-time plot of unbound nutlin-3a in the muscle after multiple oral doses given once daily (QD) or twice daily (BID). The horizontal lines represent the unbound IC_{50} of nutlin-3a for RMS-YM rhabodmyosarcoma cells.



Supplementary Figure S7. Simulated concentration-time plot of unbound nutlin-3a in the plasma, bone marrow, and spleen after multiple oral doses given once daily (QD) or twice daily (BID). The horizontal lines represent the unbound IC_{50} of nutlin-3a for MDM2-overexpressing primary acute lymphoblastic leukemia cells.

Supplementary Table 1. Nutlin-3a IC_{50} for cell survival in different cell types

Cell type	IC_{50} (μM)	Unbound IC_{50} (μM)	Exposure time	Reference
Weri1 retinoblastoma	1.1	0.21	72 h	Unpublished[a]
IMR-32 neuroblastoma	3.02	0.77	72 h	(Barbieri et al., 2006)
RMS-YM rhabodmyosarcoma	1.25[b]	0.26	48 h	(Miyachi et al., 2009)
Primary MDM2-overexpressing ALL	1.0[b]	0.20	44 h	(Gu et al., 2008)

[a]Mike Dyer, St. Jude Children's Research Hospital, Memphis, TN

[b]Nutlin-3a IC_{50} assumed to be half of racemic nutlin-3 IC_{50}

Supplementary Table 2. Percent time unbound tissue concentration is above unbound IC_{50}

Cell line	Tissue	Time above IC_{50} (%)					
		QD			BID		
		100	200	400	100	200	400
Weri1	Retina	38	53	70	83	100	100
	Vitreous	0	0	5	0	0	17
IMR32	Adrenal	27	39	54	58	85	100
RMS-YM	Muscle	43	58	76	92	100	100
ALL	Plasma	43	57	75	90	100	100
	Spleen	46	61	80	90	100	100
	Marrow	0	23	35	12	48	77