Supplementary Material for "Physiologically based pharmacokinetic model of digoxin renal drug disposition and application to renal impairment populations" by Scotcher et al.

Table S-1. Input parameters for digoxin PBPK model (without activation of MechKiM (Neuhoff et al., 2013)). Coefficient of variation (%) is given in parentheses where applicable.

Parameter	Value
Phys-chem	
Mol Weight (g/mol)	780.94
log P	1.126
Compound Type	Neutral
B/P	1.07
f_u	0.71
Absorption	
Absorption model	ADAM
Permeability assay	P_{Caco-2}
$P_{app,Caco-2}$ (10E-06 cm/s)	12.7
$P_{app,Caco-2}$ Scalar	1
$P_{eff,man}$ Duodenum (10E-4 cm/s)	0.5
$P_{eff,man}$ Jejunum I (10E-4 cm/s)	4.67
$P_{eff,man}$ Jejunum II (10E-4 cm/s)	4.67
$P_{eff,man}$ Ileum I (10E-4 cm/s)	4.67
$P_{eff,man}$ Ileum II (10E-4 cm/s)	3.67
$P_{eff,man}$ Ileum III (10E-4 cm/s)	2.67
$P_{eff,man}$ Ileum IV (10E-4 cm/s)	1.67
$P_{eff,man}$ Colon (10E-4 cm/s)	0.1
Input Form	Solution
Distribution	
Distribution Model	Full PBPK
V_{ss} mode	Predicted
Prediction method	Method 2
K_p muscle	7.35
K_p adipose	10.8
K_p Scalar	1
Elimination	
Clearance Type	Enzyme Kinetics
In vitro metabolic system	Recombinant
Additional Hep CL_{int} (µL/ min/ million hepatocytes)	0.37 (30%)
Additional Hep $f_{u,mic}$	1
Active Uptake into Hepatocyte	1
CL_R (L/ h)	9.66
Transport	

Parameter	Value
Assume Colon SS	No
Organ/Tissue	Gut
Transporter	ABCB1 (P-gp)
Location	Apical
Function	Efflux
J_{max} (pmol/min/million cells)	434
K_m (µM)	177
A (cm^2)	1
System	User
RAF/REF	2
Organ/Tissue	Liver
CL_{PD} (mL/min/million cells)	0.1
$f_{u,IW}$ Type	Predicted
$f_{u,EW}$ Type	Predicted
Transporter	ABCB1 (P-gp)
Location	Canalicular
Function	Efflux
J_{max} (pmol/min/million cells)	434
K_m (µM)	177
System	User
RAF/REF	1.5

A, area; B/P, blood to plasma partition ratio; CL_{int}, intrinsic clearance; CL_{PD}, passive diffusion clearance; CL_R, renal clearance; f_a, fraction absorbed; f_u fraction unbound in plasma; $f_{u,EW}$, fraction unbound in extracellular water; $f_{u,IW}$, fraction unbound in intracellular water; $f_{u,mic}$, fraction unbound in microsomes; Hep, Hepatocyte HLM, human liver microsomes; J_{max}, Maximum rate of transport; k_a, absorption rate constant; K_m, Michaelis-Menten constant; K_p, tissue to plasma partition coefficient; log P, logarithm of the octanol-water partition coefficient; $P_{app,Caco-2}$, permeability across Caco-2 cell monolayers; $P_{eff,man}$, Human jejunum permeability; pK_a, acid dissociation constant; Q_{gut} hybrid parameter of blood flow and drug permeability; RAF/REF, Relative activity factor/ relative expression factor; V_{max}, maximum rate of metabolism; V_{ss}, volume of distribution at steady state.

Table S-2 Clinical trials used for verification of the digoxin compound file in v14.1 of the SimCYP simulator, prior to simulations using the MechKiM. Basic dosage and demographic information are shown. All subjects were healthy participants.

Dose Information	Subjects Information	Reference
1 mg SD i.v.	8 male, 18-44 years	(Greiner et al., 1999)
1 mg SD Oral	8 male, 18-44 years	(Greiner et al., 1999)
0.01 mg/ kg SD i.v.	12 male, 21-39 years	(Rengelshausen et al., 2003)
0.75 mg SD Oral	12 male, 21-39 years	(Rengelshausen et al., 2003)
0.5 mg SD Oral	12 male, 21-31 years	(Tayrouz et al., 2003)

SD Single dose; i.v. intravenous.

Table S-3. *In vitro* transport kinetics data for digoxin, with respect to the P-gp and OATP4C1 transporters

Transporter	Parameter	Value	Units	System	Reference
P-gp	K_m	177	μM	Caco-2	(Troutman and Thakker, 2003)
	V_{max}	434	pmol/ min/ cm^2	Caco-2	(Troutman and Thakker, 2003)
	K_m	73	μM	Caco-2	(Collett et al., 2004)
	V_{max}	3.4	nmol/ h/ cm^2	Caco-2	(Collett et al., 2004)
	V_{max}	56.7	pmol/ min/ cm^2	Caco-2	(Collett et al., 2004)
	K_m	181	μM	Sf9-MDR1 liposomes	(Kimura et al., 2007)
	V_{max}	578	nmol/ min/ mg	Sf9-MDR1 liposomes	(Kimura et al., 2007)
	K_m	130	μM	Caco-2	(Korjamo et al., 2007)
	V_{max}	2100	fmol/ s/ cm^2	Caco-2	(Korjamo et al., 2007)
	V_{max}	126	pmol/ min/ cm^2	Caco-2	(Korjamo et al., 2007)
	K_m	1000	μM	MDCK-MDR1	(Korjamo et al., 2007)
	V_{max}	594	fmol/ s/ cm^2	MDCK-MDR1	(Korjamo et al., 2007)
	V_{max}	2100	pmol/ min/ cm^2	MDCK-MDR1	(Korjamo et al., 2007)
	K_m	58.2	μM	Caco-2	(Stephens et al., 2001)
	V_{max}	13.0	nmol/ h/ cm^2	Caco-2	(Stephens et al., 2001)
	V_{max}	216.7	pmol/ min/ cm^2	Caco-2	(Stephens et al., 2001)
OATP4C1	K_m	7.8	μM	MDCK-OATP4C1	(Mikkaichi et al., 2004)
	Active uptake rate @ 0.37 μM [a]	0.20	pmol/ 30 min/ mg	MDCK-OATP4C1	(Mikkaichi et al., 2004)
	Active	27	pmol/ 5 min/	CHO-	(Chu et al., 2007)

	uptake rate @ 0.1 µM [a]		200,000 cells	OATP4C1	
	Active uptake clearance [b]	0.234	µL/ min/ million cells	MDCK-OATP4C1	(Mikkaichi et al., 2004)
	Active uptake clearance [b]	270	µL/ min/ million cells	CHO-OATP4C1	(Chu et al., 2007)

[a] Active uptake was uptake in transporter-transfected cells - uptake in mock-transfected cells; [b] Normalised uptake clearance for assay concentration and time, assume 13 million cells per mg protein in MDCK cells (Richardson et al., 1981)

Table S-4. Observed digoxin CL_R values published in literature

Study #	CL_R (mL/ min)	Number of subjects	Reference
1	98.3	12	(Verstuyft et al., 2003)
2	151.0 (Oral) 159.0 (IV)	8 8	(Greiner et al., 1999)
3	131.8	10	(Jalava et al., 1997)
4	120.0	10	(Rengelshausen et al., 2003)
5	197.5	7	(Pedersen et al., 1982)
6	141.0	8	(Koup et al., 1975)
7	169.0	8	(Erik Pedersen et al., 1981)
8	155.7	20	(Schwartz and Migliore, 1984)
9	134.5	7	(Leahey et al., 1981)
10	177.0	8	(Hedman et al., 1990)
11	105.0	10	(Fenster et al., 1985)
12	133.3	12	(Becquemont et al., 2001)
13	125.0	10	(Westphal et al., 2000)
14	194.0	12	(Ding et al., 2004)
15	102.0	24	(Rameis et al., 1984)
16	119.0	4	(Sumner and Russell, 1976)
17	166.0 174.0	6 6	(Hedman et al., 1992)
18	94.2	12	(Penzak et al., 2004)
19	108.3	12	(Kovarik et al., 1999)

Weighted average = 136.7 mL/ min; Range = 92.7 – 197.5 mL/ min

Table S-5. Reported transporter expression of P-gp in Caco-2 and human organs. The calculated kidney: Caco-2 REF used in the mechanistic kidney model was 1.51 (Hilgendorf et al., 2007).

Transporter [a]	Parameter	Method	Published values	"REF" value	Reference
P-gp [b]	Jejunum: Caco-2 ratio	Western Blot	2064 : 1014	2.04	(Troutman and Thakker, 2003)
	Liver: Small intestine ratio	RT-PCR	18.7 : 20.3	0.92	(Miki et al., 2005)
	Kidney: Liver ratio	RT-PCR	50.0 : 20.3	2.463	(Miki et al., 2005)
	Kidney: Intestine ratio	RT-PCR	50.0 : 18.7	2.67	(Miki et al., 2005)
	Liver: Small intestine ratio	RT-PCR	0.023 : 0.0337	0.68	(Nishimura and Naito, 2005)
	Kidney: Liver ratio	RT-PCR	0.0851 : 0.023	3.7	(Nishimura and Naito, 2005)
	Kidney: Intestine ratio	RT-PCR	0.0851 : 0.0337	2.53	(Nishimura and Naito, 2005)
	Jejunum: Caco-2 ratio	RT-PCR	0.830 : 0.216	3.86	(Hilgendorf et al., 2007)
	Liver: Small intestine	RT-PCR	0.734 : 0.830	0.88	(Hilgendorf et al., 2007)
	Kidney: liver	RT-PCR	0.327 : 0.734	0.45	(Hilgendorf et al., 2007)
	Kidney: small intestine	RT-PCR	0.327 : 0.830	0.39	(Hilgendorf et al., 2007)

[a] There were no data available for relative expression of OATP4C1 in kidney compared with transfected cell lines; [b] Three studies reported P-gp relative expression between kidney and intestine. The values were 2.67, 2.53 and 0.39. Multiplying these by the intestine: Caco-2 relative expression factor of 2 used in the SimCYP gave respective kidney: Caco-2 REFs of 5.34, 5.06 and 0.78.

Figure S-1

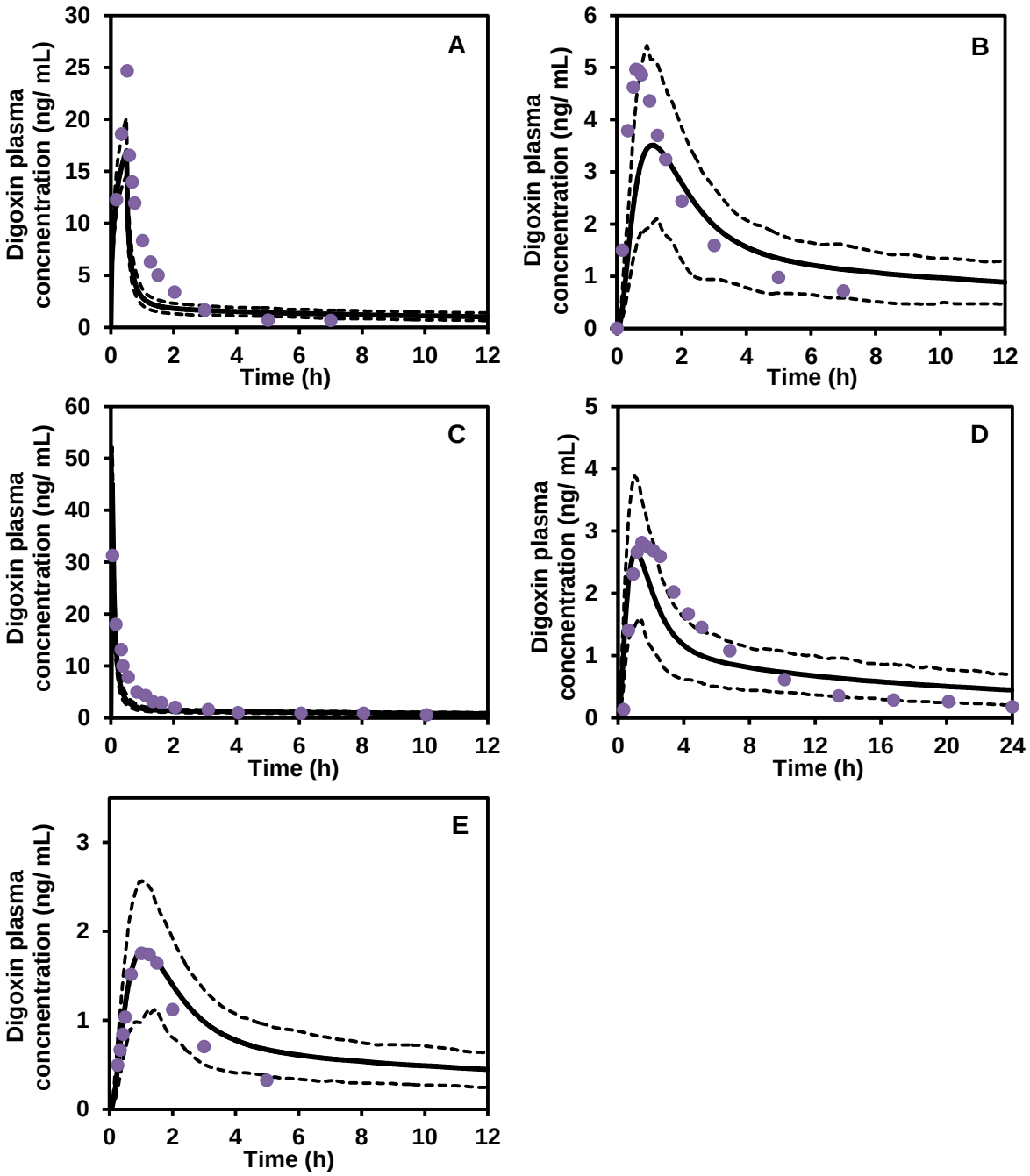

Figure S-1 Simulated plasma concentration-time profiles of digoxin using default full-PBPK model in SimCYP, without activation of MechKiM. A: 1 mg i.v. (Greiner et al., 1999), B: 1 mg Oral (Greiner et al., 1999), C: 0.01 mg/ kg i.v. (Rengelshausen et al., 2003), D: 0.75 mg Oral (Rengelshausen et al., 2003), E: 0.5 mg Oral (Tayrouz et al., 2003), see Table S-II for study details. Mean (solid lines), 5th and 95th percentiles (dashed lines) simulated plasma concentrations of all virtual subjects are overlaid with mean observed data (circles)

Figure S-2

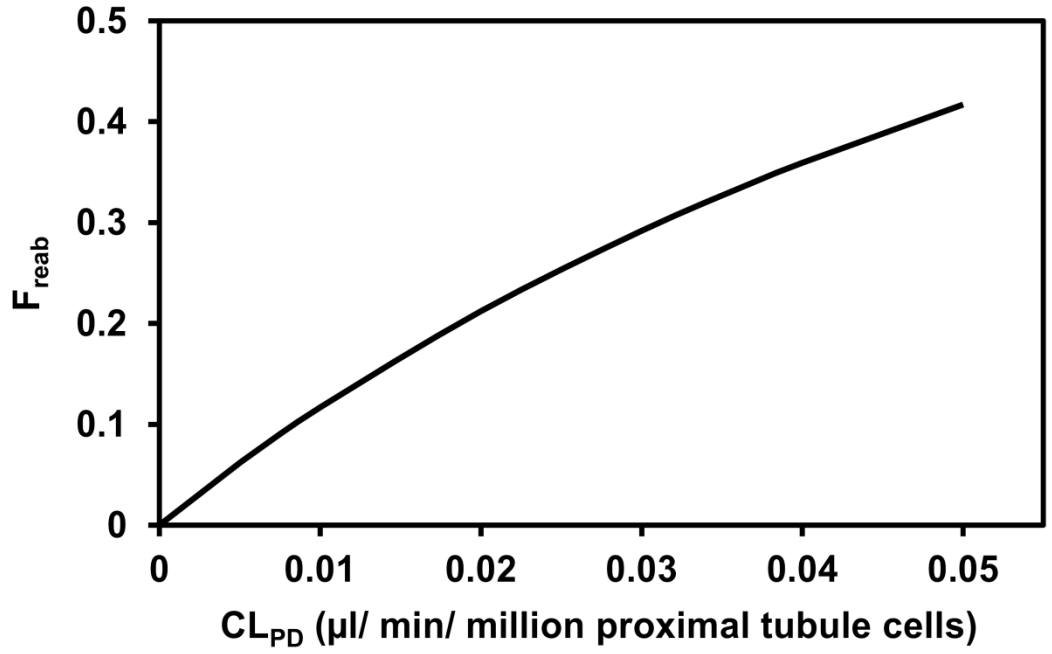

Figure S-2 Sensitivity analysis used to determine CL_{PD} parameter value. F_{reab} was calculated using simulated digoxin CL_R with various values for the CL_{PD} parameter [i.e., filtration and reabsorption] and simulated digoxin CL_R with CL_{PD} = 0 µL/ min/ million PTC [i.e., filtration only]. Digoxin transport in kidney was not considered during these simulations. The optimal range of values for F_{reab} (0.064 – 0.34) was predicted using the static model (Scotcher et al., 2016) and Caco-2 P_{app} data (1.15 – 8.03 × 10^{-6} cm/ s) from the literature (Neuhoff et al., 2003; Zhang and Morris, 2003; Djuv and Nilsen, 2008; Fossati et al., 2008). The CL_{PD} value of 0.01 µL/ min/ million proximal tubule cells was used in the model, and resulted in F_{reab} of 0.12.

Figure S-3

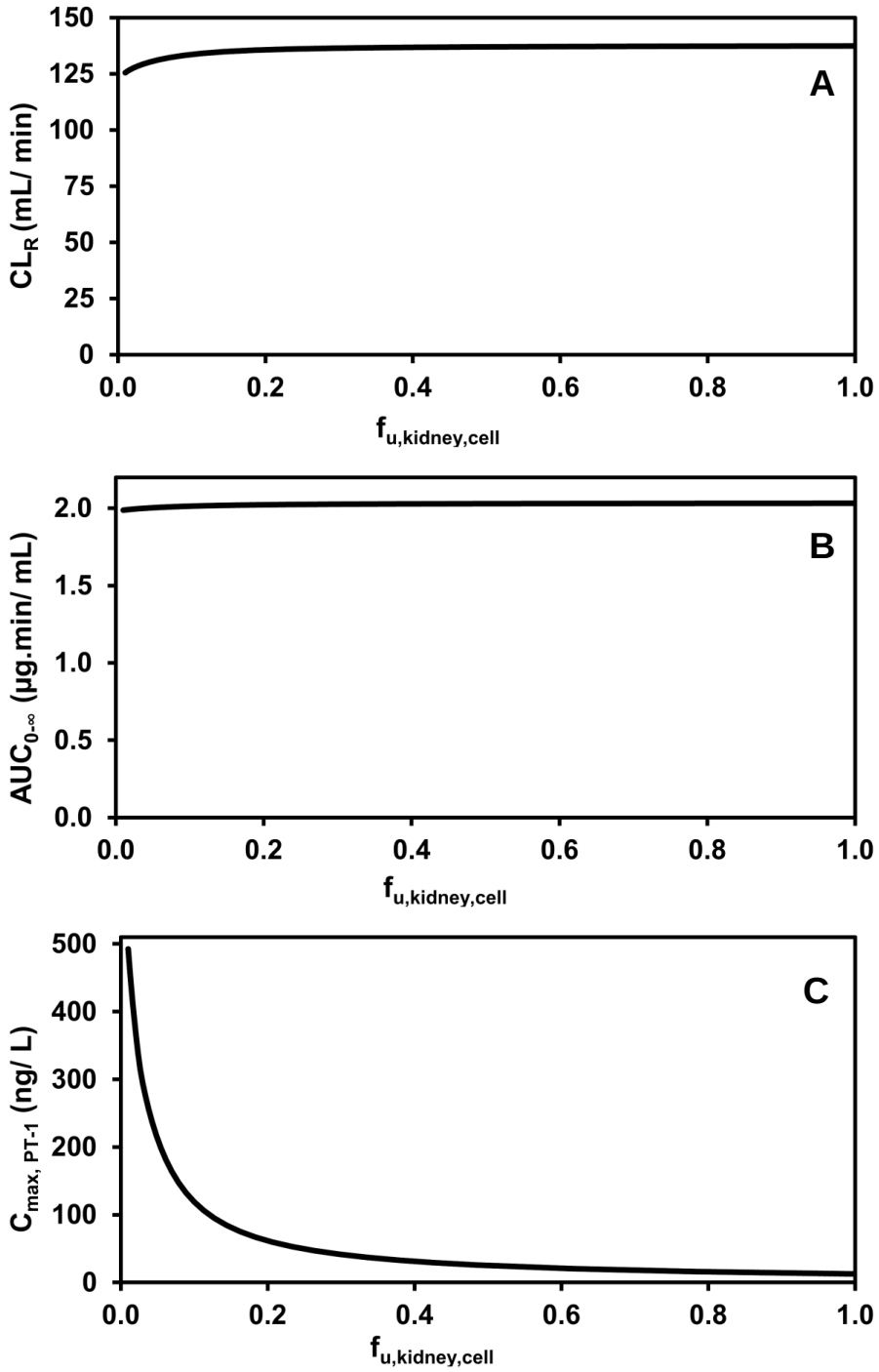

Figure S-3 Simulated digoxin CL_R (A), $AUC_{0-\infty}$ (B) and $C_{max,\ PT-1}$ (C) at different input values for the $f_{u,kidney,cell}$ parameter. $f_{u,kidney,cell}$ was varied using the automated sensitivity analysis tool in the SimCYP simulator in a population representative, following the clinical trial design reported previously (Greiner et al., 1999). $f_{u,kidney,cell}$ predicted using the Rodgers and Rowland method used for simulation of digoxin pharmacokinetics was 0.51 (Rodgers et al., 2005; Rodgers and Rowland, 2006).

Figure S-4

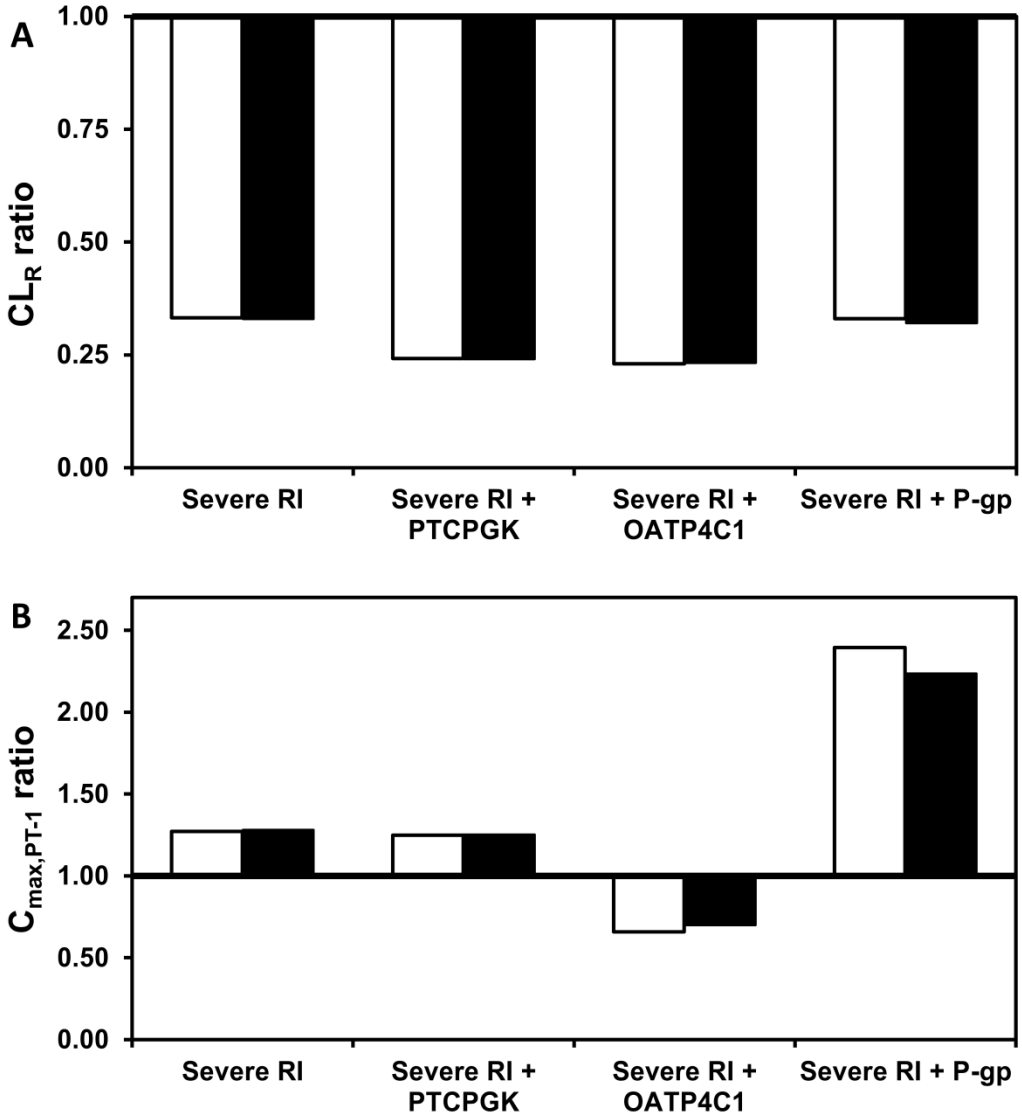

Figure S-4 Impact of a 5-fold increase in CL_{PD} for the proximal tubule compartments (PT-1, PT-2 and PT-3) on simulated CL_R ratio (A) and $C_{max,PT-1}$ ratio for the severe renal impairment (RI) populations. Simulations were performed with the developed digoxin model (CL_{PD} = 0.01 µL/ min/ million cells for all compartments; white bars) or with a modified model (CL_{PD} = 0.05 µL/ min/ million cells for proximal tubule, CL_{PD} = 0.01 µL/ min/ million cells in remaining tubular compartments; black bars). The healthy volunteers (HV) population was used as baseline for calculation of CL_R ratio. The severe renal impairment population was simulated without or with 50% reduction to PTCPGK, OATP4C1 abundance or P-gp abundance parameters, as indicated by horizontal axes labels.

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