

**Supporting Information for**

**In vivo Anticancer Activity of a Rhodium Metalloinsertor  
in the HCT116 Xenograft Tumor Model**

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## Supporting Experimental Section

**Purification of Rhodium Metalloinsertor Compounds.** The purification of Rh-PPO and Rh-PPE was modified from the literature preparations as follows: the crude reaction mixture of Rh-PPO or Rh-PPE was loaded onto a C18 SPE cartridge equilibrated with 15% acetonitrile in 0.1% TFA(aq). The concentration of acetonitrile was gradually increased and the complex eluted from the SPE cartridge with 25% acetonitrile in 0.1% TFA(aq) then dried *in vacuo*. The SPE purified complex was then dissolved in minimal acetonitrile before HPLC purification and filtered. Rh-PPO was purified by HPLC using an isocratic method of 75:25 MeCN:H<sub>2</sub>O + 0.1% TFA over 60 min. Rh-PPE was purified by HPLC using a gradient elution from 85:15 to 5:95 ACN:H<sub>2</sub>O + 0.1% TFA over 30 minutes. Peaks corresponding to the desired products were verified using ESI-MS and were collected using an automatic fraction collector or by hand. The chloride salts were obtained from a Sephadex QAE anion exchange column equilibrated with 0.1 M MgCl<sub>2</sub> and complex structure was verified using NMR.

**Concentration Determination of Rhodium Complexes.** A stock solution of each rhodium metalloinsertor was made in MilliQ water and a UV-Vis trace was recorded. The concentration of stock solution was then determined by using the Cary UV-Vis instrument, as well as molar absorptivity values from the literature.<sup>21</sup> The following molar absorptivity values were used to estimate the concentration of Rh-PPO in water: UV-vis (H<sub>2</sub>O, pH 7.0): 270 nm (122,400 M<sup>-1</sup> cm<sup>-1</sup>), 300 nm (41,600 M<sup>-1</sup> cm<sup>-1</sup>), 430 nm (12,300 M<sup>-1</sup> cm<sup>-1</sup>). The following molar absorptivity values were used to estimate the concentration of Rh-PPE in water: UV-vis (H<sub>2</sub>O, pH 7.0): 270 nm (165,800 M<sup>-1</sup> cm<sup>-1</sup>), 300 nm (56,300 M<sup>-1</sup> cm<sup>-1</sup>), 430 nm (16,100 M<sup>-1</sup> cm<sup>-1</sup>).

### Tumor Volume and Final Tumor Weights Analysis for Intratumoral Efficacy

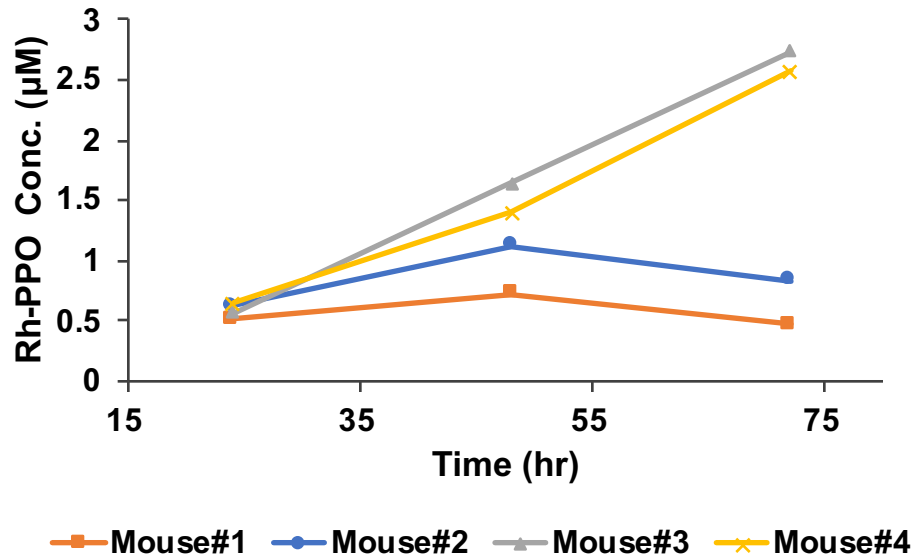
**Experiment.** Female NSG mice (28-37 g) were injected subcutaneously with HCT116 cells (2.5 x 10<sup>6</sup>) suspended in McCoy's media; 100 µL injections into the right flank. Tumors were allowed to grow until they reached ~90 mm<sup>3</sup> (11 days). Mice were then randomly assigned to each

treatment group with 5 or 10 mice in each, so each group had an average tumor volume of 80-90 mm<sup>3</sup>. Mice were assigned to the following treatment groups: saline (n=5) and Rh-PPO at 1 mpk (n=10). Rh-PPO was dissolved in saline at the MTD (1 mpk) and administered intratumorally based on the schedule outlined in Figure 6A. Saline was administered intratumorally to the control group based on the same schedule. Mouse body weights and tumor volumes were measured twice per week over the course of the study, and tumors were excised and weighed on day 18 of the study. Tumor volumes (TV) were estimated by measuring the width (W) and length (L) of the tumor using a digital caliper and calculated based on the following formula:  $TV = W^2L/2$ .

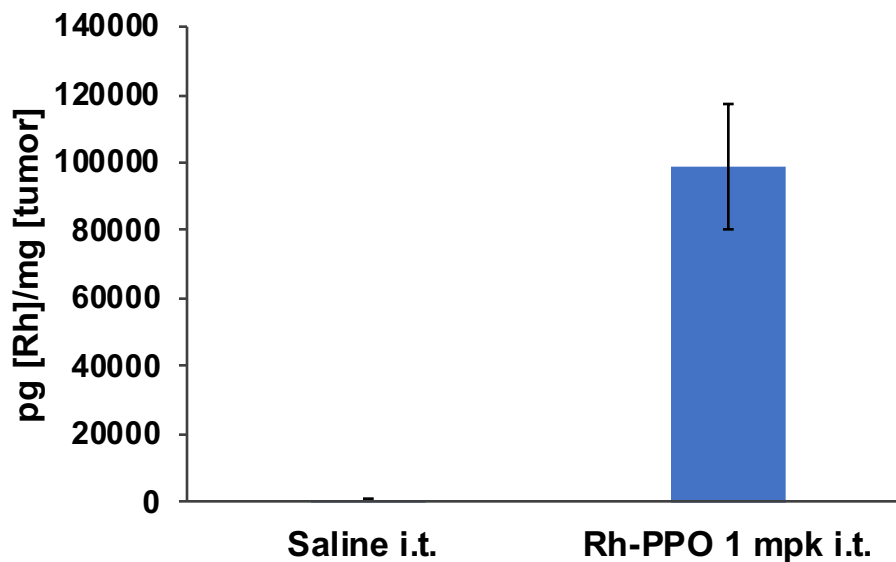
**Rh-PPO Continuous Infusion Pharmacokinetic Studies.** Initial in vivo studies with Rh-PPO utilized Alzet osmotic pumps (Cupertino, CA) to achieve continuous infusion of the drug. In these studies, osmotic pumps were filled with Rh-PPO at 1 mpk and implanted subcutaneously in four mice. Importantly, Mouse#1 and Mouse#2 received 2 mL, daily, subcutaneous infusions of saline for hydration. Mice were assessed for Rh-PPO plasma concentration by performing tail vein blood draws every 24 hr. After blood was collected, it was immediately transferred to heparinized blood collection vials on ice at time intervals of 24, 48, and 72 hr. The blood samples were centrifuged at 15,000 g at RT for 5 min, and the plasma supernatant was transferred to 1.5 mL microcentrifuge tubes and maintained at -80°C until analysis. Plasma samples were analyzed for Rh-PPO concentration using LC-MS/MS and results are displayed in Figure S1.

**LC-MS/MS Analysis of Plasma Samples.** Standard Rh-PPO and internal standard (INS) Rh-PPE solutions were made as needed and concentration determined via UV-Vis. Mouse plasma for preparation of standards and quality controls (QC) were obtained from BioChemed Services. LC-MS/MS analysis was performed using a Waters Acquity UPLC system (Milford, MA, USA) interfaced with a Waters Quattro Premier XE Mass Spectrometer. HPLC separation was achieved using a Luna 3µ Phenyl-Hexyl 100 x 2 mm column (Phenomenex,

Torrance, CA, USA) proceeded by a Phenomenex Phenyl guard column (Torrance, CA, USA). The column temperature was maintained at 40°C. The mobile phase consisted of A (0.1% formic acid, 10% ACN in water) and B (0.1% formic acid in acetonitrile). The following gradient program was used: 0% B (0min, 0.3 ml/min), 8% B (3.2 min, 0.3ml/min), 60% B (4.0 min, 0.3ml/min), 85% B (5.5 min, 0.3 ml/min), 0% B (5.6 min, 0.3ml/min), 0% B (8.5 min, 0.3ml/min). The Total run time was 8.5 minutes. The auto-injector temperature was maintained at 5°C. The strong needle wash solution was 0.1% formic acid in 50% ACN and 50% water and the weak needle wash solution was 0.1 % formic acid and 10% ACN in water. The electrospray ionization source of the mass spectrometer was operated in positive ion mode with a cone gas flow of 25 L/hr and a desolvation gas flow of 900 L/hr. The capillary voltage was set to 0.7 kV for both Rh-PPO and INS (Rh-PPE), and the cone voltages were optimized to 45 V for Rh-PPO and 51 V for INS, respectively. The collision voltages were set to 48 V for both Rh-PPO and Rh-PPE. The source temperature was 125°C and the desolvation temperature was 480°C. A solvent delay program was used from 0 to 3.8 minutes and from 5.2 to 8.5 minutes for Rh-PPO and INS, to minimize mobile phase to flow to the source. MassLynx version 4.1 software was used for data acquiring and processing.



**Figure S1. Plasma accumulation of Rh-PPO administered via continuous infusion is dependent on mouse hydration.** Concentration of Rh-PPO in mouse plasma of NSG mice given continuous infusion doses of Rh-PPO at 1 mpk for 72 hr. Importantly, Mouse#1 and Mouse#2 received 2 mL, daily, subcutaneous infusions of saline. Amount of intact Rh-PPO detected in mouse plasma was analyzed via LC-MS/MS every 24 hrs for 72 hrs total. Continuous infusion was accomplished using an osmotic pump.



**Figure S2. Significant accumulation of rhodium in tumors occurs after intratumoral Rh-PPO treatment.** Rhodium uptake in HCT116 xenograft tumors from Efficacy Experiment # 3 mice receiving intratumoral injections. Rhodium accumulation in tumors was determined using ICP-MS analysis of tumors digested in nitric acid and normalized to initial tumor weight. The rhodium concentration of tumors from saline (n=5) and Rh-PPO at 1 mpk (n=10) treated mice was averaged with error shown as the standard error of the mean. Statistically significant difference between Rh-PPO and saline treatment group tumor rhodium content was found using Anova test; #P<0.005.

## Datasets S1. In Vivo Tumor Weight Measurements

### Intraperitoneal Tumor Weights – Raw Data

Saline i.p. - Tumor weight (g)	Rh-PPO 0.5 mpk i.p. - Tumor weight (g)	Rh-PPO 1 mpk i.p. - Tumor weight (g)	Oxaliplatin 7.5 mpk i.p. - Tumor weight (g)
0.91	0.805	0.872	0.642
0.783	0.574	0.701	0.868
0.905	0.525	0.686	1.032
1.071	0.76		1.009
	1.041	0.621	0.58
1.025	0.702		0.55
1.424	0.673	0.72	0.706
0.711	0.722	0.631	0.638
0.835	0.538	0.873	0.387
0.677	0.732	0.501	0.675

Note, blank cells indicate mouse died and tumor weight measurements were not collected.



## Intratumoral Tumor Weights – Raw Data

<b>Rh-PPO 1 mpk i.t. - Tumor weight (g)</b>
0.111
0.334
0.197
0.228
0.274
0.272
0.236
0.278
0.11
<b>Saline i.t. - Tumor weight (g)</b>
0.356
0.537
0.45
0.465
0.428

# Datasets S2. In Vivo Tumor Volume Measurements

## Intraperitoneal Tumor Volumes – Raw Data

	Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	Day 24	Day 28
	Tumor Vol (mm3)	Tumor Vol (mm3)	Tumor Vol (mm3)	Tumor Vol (mm3)	Tumor Vol (mm3)	Tumor Vol (mm3)	Tumor Vol (mm3)	Tumor Vol (mm3)	Tumor Vol (mm3)
<b>Saline</b>									
cage 1- #1	110.2824			918.4794795	1123.406007	1671.755261			
cage 1- #2	129.264536			697.4147	1007.128	1007.128	2405.949741		
cage 1- #3	123.226774								
cage 9- #2	267.67424								
cage 15- #2	57.4749705	404.1005	623.379718						
cage 16- #4	35.2915965	36.96	385.2814875						
cage 2- #2	114.825852	206.245771	525.430611	609.162014	768.4198515	1045.606887	1839.850112		
cage 3- #1	158.468778	246.116082	340.796862	547.8027455	658.148645	927.446409	1558.034322		
cage 3- #2	57.286368	97.20375	246.652672	412.990711	659.328646	837.714312	1424.357456	2111.229056	
cage 3- #3	70.2058475	133.6088765	251.60845	320.533624	545.7179	902.4836445	1560.116064		
cage 6- #2	92.5615125	174.609375	386.1423865	559.377158	737.116875	1002.11629	1453.178606		
cage 15- #3	70.9632	163.0153485	371.4984	537.103412	715.96305	1275.183063	2259.189296		
cage 16- #1	84.26048	144.6658765	269.01	369.133888	652.599497	1067.17435	1519.300139		
<b>Rh-PPO 0.5 mpk</b>									
cage 4- #1	63.963375	132.786572	230.399225	401.6982375	533.77881	1022.7178	1051.764525		
cage 4- #2	119.34	208.9359375	332.616375	521.235	866.3152905	1010.673054	1534.730488		
cage 4- #3	171.036504	262.6230375	393.7298255	456.715271	772.66875	554.340956	1320.578213	1869.482189	
cage 5- #1	56.5636135	78.7152	153.437372	278.7883385	390.3622285	940.253184	1001.214006	1215.516098	1930.529388
cage 5- #2	82.79712	153.2681235	268.485678	308.646134					
cage 6- #1	93.5631705	248.652468	349.652601	498.331368	740.6794035	940.253184	1358.057955	1774.37925	
cage 6- #3	70.2462	168.114804	407.1794	533.71045	611.17425	907.352234	1220.961375	1843.510914	
cage 14- #1	138.742128	210.933008	561.701412	654.112102	785.383424	1067.127206	1512.158472		
cage 15- #1	109.6021875	251.787692	375.581864	518.942225	1033.888544	1190.394612	1768.945647		
cage 16- #2	104.5766915	180.0914	274.341888	447.800024	473.326084	636.383728	989.4576125	1126.824368	1944.6348
<b>Rh-PPO 1 mpk</b>									
cage 7- #1	96.612388	149.4548955	325.408266	488.96	566.1199485	839.838888	1051.823225	1569.767586	1624.139032
cage 7- #2	146.4564	220.651488	288.5301715	613.7856	880.81047	1171.307592	1710.446634		
cage 8- #1	80.454951	186.015744	269.086776	439.000598	578.601184	874.271554	1164.701741	1548.532202	
cage 8- #3	72.3341025	179.5158855	270.5122575	425.85102	562.005368	779.414162	967.612928	1230.11133	1845.883728
cage 8- #4	87.214414	213.69205	381.85605	554.32656	610.364331	800.89438	931.974331		
cage 9- #3	159.930581	299.060562	610.236792	1039.20021	1555.295011	2092.695552			
cage 9- #4	40.886937	120.089728	176.644566	348.2781875	600.5593125	719.114888	1361.073526	1607.395626	
cage 13- #1	67.96449	203.35785	421.4250755	491.489856	641.9000705	922.716	1249.985839	1867.5	
cage 13- #2	158.92155	341.565112	591.8520125	954.236095	1325.0952	1599.251769			
cage 13- #4	122.47937	243.791832	432.088664	648.807432	960.585808	1296.166413	1823.91875		
<b>Oxaliplatin 7.5 mpk</b>									
cage 10- #1	93.35584	219.7876	311.539688	441.0954815	644.76405	866.578176	1145.321566		
cage 10- #2	22.071168	68.152581	156.683868	214.908512	412.100822	491.720352	817.634176	1111.062668	1505.012943
cage 10- #3	112.071168	216.8656	421.30543	541.8934125	652.404326	894.819528	1214.259688		
cage 10- #4	68.4176375	125.375186	300.7280185	386.9015625	656.1429255	767.629306			
cage 11- #1	124.621893	233.081289	371.0314825	474.871875	775.60875	956.122398	1396.399516		
cage 11- #2	47.08125	126.5625	239.357025	331.236499	385.2185005	586.8945355	375.417504	1028.823894	1613.091659
cage 11- #3	119.1285225	296.74645	509.6257105	632.6331885	921.243785	1038.370094			
cage 11- #4	171.6890175	421.062496	509.096875	748.963138	1050.732585	1476.595908			
cage 12- #1	78.597307	103.586784	168.92326	317.76541	493.893486	747.070192	969.9802565	1136.091575	
cage 12- #2	150	280.09995	375.10705	443.467086	562.856	784.0717605	1113.073684	1291.821336	
cage 12- #3	78.502823	111.912426	193.6440025	273.805872	289.40625	511.6488	543.71583	484.893448	





## Datasets S4. ICP-MS Metal Content in Tissues and Tumors Data

### Rhodium and Platinum Content in Intraperitoneal Treated Tumors – ICP-MS Data

	Concentration Rh (ppb) * dilution factor/ mg tumor	Concentration Pt (ppb) * dilution factor/mg tumor
<b>Vehicle (saline) control i.p.</b>		
cage 1- #1	0.052482784	0.174745509
cage 1- #3	0.022531228	0.042625976
cage 2- #1	0.093664191	0.142277228
cage 2- #3	0.037153341	0.065453461
cage 4- #2	0.109776952	0.089832714
cage 4- #3	0.07568267	0.080070258
cage 1- #2	0.004738574	0.067641682
cage 2- #4	0.006072531	0.024917695
cage 4- #1	0.002875635	0.024974619
<b>Rh-PPO at 0.5 mg/kg i.p.</b>		
cage 5- #3	0.200547143	0.226314286
cage 6- #1	0.322616484	0.056967033
cage 6- #2	0.094322674	0.044488372
cage 6- #3	0.171555707	0.043967391
cage 24- #2	0.208127395	0.062203065
cage 7- #2	0.290863118	0.035437262
cage 8- #2	0.197181122	0.008826531
cage 17- #1	0.079676829	0.016707317
cage 5- #1	0.15257176	0.016688588
cage 5- #2	0.088366085	0.011910224
<b>Rh-PPO at 1 mg/kg i.p.</b>		
cage 8- #3	0.134970226	0.07550308
cage 9- #2	0.234481092	0.075420168
cage 9- #3	0.269531504	0.048313008
cage 11- #1	1.094359375	0.9578125
cage 11- #2	0.244751572	0.058836478
cage 11- #3,	0.406635922	-0.044368932
cage 10- #1	0.301413093	0.043182844
cage 12- #1	0.255026462	0.079610028
cage 23- #2	0.257204013	0.04
<b>Oxaliplatin at 7.5 mg/kg i.p.</b>		
cage 12- #2	0.043661877	3.105823755
cage 12- #3	0.048279592	4.303673469
cage 13- #1	0.03365097	2.330083102
cage 13- #3	0.039495704	3.459725086
cage 14- #4	0.072209424	4.384267016
cage 16- #3	0.075027941	2.091
cage 23- #1	0.048582105	3.251136842
cage 14- #1	0.000952141	5.536246851
cage 14- #2	-0.004772727	2.755787879
cage 14- #3	0.000786585	2.245243902

## Rhodium and Platinum Content in Intratumoral Treated Tumors – ICP-MS Data

	Concentration Rh (ppb) * dilution factor/ mg tumor	Concentration Pt (ppb) * dilution factor/mg tumor
<b>Rh-PPO at 1 mg/kg intratumoral</b>		
cage 9- #1	5.922840426	-0.234893617
cage 15- #2	40.83617448	-0.025364583
cage 16- #2	26.32727273	-0.111931818
cage 16- #1	20.53647423	-0.007835052
cage 19- #1	11.42046333	-0.045666667
cage 19- #3	6.354634328	-0.152686567
cage 24- #1	23.76146947	-0.088549618
cage 18- #3	17.11177959	0.020081633
cage 20- #1	26.1112129	0.026064516
<b>Vehicle (saline) control intratumoral</b>		
cage 21- #2	0.158122356	0.016767372
cage 22- #1	0.10492284	0.002098765
cage 22- #2	0.12198419	0.031620553
cage 7- #1	0.033510843	0.018184739
cage 21- #1	0.050166906	0.025956835

### Rhodium and Platinum Content in Intraperitoneal Treated Tissues – ICP-MS Data

	Concentration Rh (ppb) * dilution factor/ mg tumor	Concentration Pt (ppb) * dilution factor/ mg tumor
<b>Oxaliplatin at 7.5 mg/kg i.p.</b>		
10#3 A1	0.17829945	242.919607
10#3 A3	0.094879348	443.0708903
10#3 B	0.008816889	342.7291784
10#3 D1	0.004114044	124.3464885
10#3 D2	-0.025683651	167.3286638
10#3 E1	-0.043280032	224.335784
10#3 E2	0.095076839	78.74368501
10#3 H	-0.003543336	12.44292709
10#1 A1	0.126506317	359.0415544
10#1 B	0.101263501	305.633639
10#1 D1	0.017931769	156.7277576
10#1 E1	0.010757841	113.858287
10#1 H	0.014587965	9.335616896
11#4 A1	-0.062400483	601.1345934
11#4 A3	-0.005571233	311.0945959
11#4 B	-0.004463519	390.7801062
11#4 D1	-0.031065713	374.4779474
11#4 E1	0.043973111	51.30132232
11#4 E2	-0.004523583	117.5325547
11#4 H	0.083906749	8.812573372
11#2 A1	0.169437977	106.6160663
11#2 A2	0.340750585	118.2702385
11#2 A3	0.062338557	113.899206
11#2 B	0.021722813	141.9773533
11#2 D1	0.015740738	43.02392895
11#2 D2	0.04528978	36.40353381
11#2 E1	0.020061231	21.83394386
11#2 E2	0.023902919	10.90924139
11#2 H	0.034607283	2.221601463

	Concentration Rh (ppb) * dilution factor/ mg tumor	Concentration Pt (ppb) * dilution factor/ mg tumor
<b>Vehicle (saline) control i.p.</b>		
3#1 A1	1.856486514	19.23619273
3#1 A3	4.799438355	6.295367395
3#1 B	11.36125269	1.995471773
3#1 C	3.013897888	48.94880637
3#1 D	1.447793281	10.59307808
3#1 E	2.120759553	3.704958679
3#1 H	0.065882936	10.77105334
3#1 HAIR	6.354828323	550.5596687
15#3 A1	0.023858391	4.145966125
15#3 A3	0.005993033	12.13183995
15#3 B	0.007557964	1.380781403
15#3 D1	0.25615475	10.46665026
15#3 D2	0.329158836	20.05541704
15#3 E	0.114416615	5.621444823
15#3 H	-0.003846825	3.882656789
9#2 A1	1.426893229	17.37924609
9#2 A3	0.137333992	6.08201788
9#2 B	0.051006264	5.492320148
9#2 D1	0.737777879	29.24023824
9#2 D2	0.643142941	23.52012595
9#2 E1	0.805770483	4.413523233
9#2 E2	0.248857236	2.701947997
9#2 H	0.02478345	3.953170803
<b>Rh-PPO at 1 mg/kg i.p.</b>		
7#2 A1	38.0914265	5.804048038
7#2 A3	32.73457745	36.58183788
7#2 B	173.9853362	4.028755221
7#2 D	16.15486724	5.166148974
7#2 E	11.00520615	3.098016679
7#2 H	2.153127739	13.35097418
13#2 A1	10.35623497	14.36942475
13#2 A3	38.96588945	4.495299769
13#2 B	176.4218406	2.223283403
13#2 D1	15.02168215	6.548777844
13#2 D2	9.26298776	11.71885114
13#2 E1	13.39236568	7.171561355
13#2 E2	10.2622706	7.635733478
13#2 H	0.296102457	4.256441418
7#1 A1	10.77906918	<0.00
7#1 A3	23.01540005	<0.00
7#1 B	112.8607282	<0.00
7#1 D	18.43725262	<0.00
7#1 E	2.272573512	<0.00
7#1 H	0.127447346	<0.00



Letters represent the following tissue code:  
A1/A2/A3. Spleen, pancreas, kidneys  
B. Liver  
C. Heart, skeletal muscle, lungs  
D1/D2. Small intestine, colon  
E1E2. Stomach, cecum  
H. Brain  
I. Tibia/Femur

**Datasets S5. LC-MS/MS Intraperitoneal Rh-PPO Plasma Concentration Data**

<b>Rh-PPO in Mouse Plasma (Dose 1mg/kg )</b>	
<b>Sample ID</b>	<b>Concentration (uM Rh-PPO)</b>
<i>0.5h-#1</i>	0.188
<i>0.5h-#2</i>	1.56
<i>0.5h-#3</i>	0.191
<i>1.0h-#1</i>	0.129
<i>1.0h-#2</i>	0.35
<i>2h-#1</i>	0.285
<i>2h-#2</i>	0.469
<i>2h-#3</i>	0.226
<i>4h-#1</i>	0.171
<i>4h-#2</i>	0.228
<i>4h-#3</i>	0.123
<i>8h-#1</i>	0.0312
<i>8h-#2</i>	0.0255
<i>8h-#3</i>	0.0415
<b>Rh-PPO in Mouse Plasma (Dose 0.5 mg/kg )</b>	
<b>Sample ID</b>	<b>Concentration (uM Rh-PPO)</b>
<i>0.5h-#1</i>	1.069
<i>0.5h-#2</i>	0.267
<i>4h-#1</i>	0.0556
<i>4h-#2</i>	0.127
<i>8h-#1</i>	0.0031
<i>8h-#2</i>	0.0053