Supporting Information for

In Vivo Anticancer Activity of a Rhenium(I) Tricarbonyl Complex

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Experimental.

Physical Measurements.

Titrations were carried out using a Metrohm Titrando 888 titrator equipped with a Ross Orion combination electrode (8103BN, ThermoFisher Scientific), a Metrohm 806 exchange unit with an automatic burette, and a Thermomix 1442D circulating water bath. A gentle flow of argon gas was passed through a solution of 30% KOH, prepared by dissolving KOH pellets in freshly boiled MilliQ water, in order to exclude CO_2. UV–vis spectra were obtained using an Agilent Cary 8454 UV–vis spectrophotometer. Luminescence spectra were acquired using a Varian Eclipse fluorometer. ICP-MS was performed using an ELAN DRC-E ICP-MS equipped with an S-10 autosampler. For ex vivo tissue damage analysis, animal organs were trimmed manually and processed on a Sakura Tissue-Tek VIP 6 tissue processor. Tissues were then embedded in paraffin on a Sakura Tissue-Tek TEC 5 embedding station followed by sectioning and mounting on glass slides using a Microm microtome (5 µm thickness). The glass slides were stained with H&E using an automatic Thermo Scientific Varistain Gemini ES and coverslipped on a Thermo Scientific ClearVue. H&E stained slide review was conducted using an Olympus BX51 optical microscope equipped with an Olympus DP20 camera. Tissue images were obtained and processed using the Olympus DP2-BSW software.

Materials and Reagents. All reagents were purchased from commercial vendors. Solvents used were of ACS grade or higher. H&E stains were made using Harris hematoxylin (Anatech LTD.) and eosin Y (1% alcoholic solution, Harleco). Compound **1** was synthesized using previously described methods.[1] Its purity was verified to be >95% by HPLC and ^{1}H NMR spectroscopy.

Spectrophotometric Titration of 1.

The pK_a of the axial water of **1** was determined using a modified literature method.[2] A solution of **1** (40 µM) was prepared in 5 mM HNO_3 and 95 mM KNO_3. To this solution was added a solution of KOH (100 mM) in KNO_3 (100 mM) in 5–25 µL increments, and the pH was monitored after each addition. The electronic absorption spectrum of **1** at each pH was obtained (Figure S1a). A plot of pH versus absorbance change was generated (Figure S1b), and the data were fit using the following equation, where absorbance difference is the ratio of the absorbance at 260 nm and 280 nm minus the maximal absorbance ratio, and the maximal absorbance difference is the maximal change in the absorbance ratio at 260 and 280 nm:

$$Absorbance\ Difference\ =\ (Maximal\ Absorbance\ Difference)(\frac{10^{(pH-pK_a)}}{1+10^{(pH-pK_a)}})$$

Emission Spectra of 1.

The emission spectrum of a 300 µM solution of **1** was obtained in 100 mM MOPS buffer at varying pH values between 4 and 11. These spectra are shown in Figure S2 Notably, the emission spectra each contain two peaks, with maxima at approximately 420 and 560 nm. Similar emission spectra have been reported previously for related $Re(CO)_3$ complexes,[3,4] and the higher energy transition is assigned to an intraligand fluorescent transition, whereas the lower energy transition is due to the characteristic ^{3}MLCT of $Re(CO)_3$ polypyridyl complexes. The luminescence of the complex was also investigated in the presence of varying concentrations of sodium phosphate at a constant pH. Compound **1** was incubated at 37 °C for 4 h with 0, 10, or

100 mM phosphate in MOPS buffer at pH 7.4. The emission spectra upon excitation at 350 nm in the presence of phosphate are shown Figure S3.

Cellular Uptake and Localization Analysis of 1. A2780 or A2780CP70 ovarian cancer cells were plated in 100 mm × 20 mm tissue culture dishes and allowed to grow to 80% confluency. Growth media was then replaced with 6 mL of 10 µM **1** in media or with 2% MilliQ water in media as a negative control. After 24 h, the cells were washed with 3 mL of Dulbecco's phosphate-buffered saline solution (DPBS) and harvested using trypsin. Cell lysates were then obtained for whole cell, nuclear, and mitochondrial samples using modified versions of previously described methods shown below.[5] The bicinchoninic acid (BCA) assay was used to determine protein content for lysate samples.[6] The procedure for measuring protein content was previously described by the manufacturer. In a 96-well plate, 25 µL of cell lysate was combined with 200 µL of working reagent, incubated for 30 min, and then the absorbance was measured at 562 nm. Protein concentrations were then extrapolated from a calibration curve using bovine serum albumin (BSA) standard dilutions. Samples were measured in triplicates.

Whole cell lysates were obtained by centrifuging harvested cells, washing with 1 mL of DPBS, lysing with 1 mL of 4% sodium dodecyl sulfate (SDS) lysis buffer (4% w/v SDS, 150 mM NaCl, 50 mM triethanolamine), and vortexing on the highest setting for 10 s. The supernatant was transferred to a clean tube. 750 µL of the lysate was diluted with an additional 750 µL MilliQ water and 5 mL 70% HNO_3 followed by sonication for 30 min. After digestion, 3 mL of the resulting solution was diluted with 8 mL MilliQ water. The rhenium content in each sample was determined using ICP-MS. Results were reported as the mass ratio of metal to protein (ng/µg) in each sample, in which protein content was determine using a BCA assay.

Nuclear lysate samples for ICP-MS were prepared from one 100 mm × 20 mm tissue culture dish. The harvested cells were centrifuged and washed with 1 mL DPBS then resuspended in 1 mL hypotonic buffer (20 mM Tris-HCl, 10 mM NaCl, 3 mM $MgCl_2$, pH 7.4). After incubating on ice for 20 min, 50 µL of 10% w/v NP-40 was added, and the solution was vortexed on the highest setting for 10 s. After centrifuging at 3000 g for 10 min, the pellet was resuspended in 1 mL MilliQ water and sonicated for 10 min. After lysing, 750 µL of the lysate was transferred to a clean tube. Samples were further digested with 750 µL of 2% HNO_3. The rhenium content in each sample was determined using ICP-MS. Results were reported as the mass ratio of metal to protein (ng/µg) in each sample.

Mitochondrial lysate samples for ICP-MS were prepared from one 100 mm × 20 mm tissue culture dish. The harvested cells were centrifuged and washed with 1 mL DPBS then resuspended in 500 µL mitochondria extraction buffer (200 mM mannitol, 68 mM sucrose, 50 mM piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), 50 mM KCl, 5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 2 mM $MgCl_2$, 1 mM dithiothreitol, 1:500 v/v protease inhibitor cocktail) and incubated over ice for 20 min. The suspension was then homogenized by 35 passes through a 25 gauge needle and a 1 mL syringe. After centrifuging at 150 g for 5 min, the supernatant was transferred to a new microcentrifuge tube and centrifuged at 10,000 g for 10 min. The resulting pellet was resuspended in 1 mL of MilliQ water and sonicated for 10 min. To a 1.5 mL cryovial, 750 µL of the lysate was transferred to a clean tube. Samples were further digested with 750 µL of 2% HNO_3. The rhenium content in each sample was determined using ICP-MS. Results were reported as the mass ratio of metal to protein (ng/µg) in each sample.

Animals. All animal procedures were approved by the Northwestern University IACUC No. IS00000556 and were in accordance with the Northwestern University's policies on the care, welfare, and treatment of laboratory animals. Blinding occurred during body weight and tumor volume analyses. For all experiments, NSG mice (strain NOD.Cg-Prkdc^{scid}Il2rgtm1Wjl/SzJl) were used at ~7 weeks old for evaluation in PDX models. Each cage contained up to five mice and offered Certified Rodent Diet (Harlan Tekland) and water. The animal room was set to maintain between 68–75 °F, a relative humidity of 30–70%, a minimum of 15 room air changes per hour, and a 12 h light/dark cycle, which was interrupted for study-related activities. Information on tumor inoculation is described under the In Vivo Tumor Growth Inhibition section. Mice were euthanized under either carbon dioxide or using isoflurane followed by bilateral thoracotomy or cervical dislocation.

MTD Studies. NSG mice were acclimated for 5 days. Animals were weighed then injected intravenously with a solution of **1** at concentrations of 10, 20, 40, 60, or 80 mg per kg of mouse. The mice were observed for adverse clinical side effects for 24 h. Surviving animals were euthanized, and major organs were harvested and weighed.

In Vivo Tumor Growth Inhibition. Ovarian PDXs[7] were resected from propagation mice and implanted subcutaneously in the right flank of twenty mice. The animals were then separated into four groups of five, and tumors were allowed to grow over 100 mm^3. Mice were then injected intravenously with either vehicle (85% aqueous dextrose (5% in water), 10% DMSO, 5% Kolliphor HS15) or solutions of **1** (10, 20 or 40 mg/kg). Their body weights and tumor volumes were measured twice per week over 31 days. Tumor volumes were estimated by measuring the width (W) and length (L) of the tumor using a caliper and calculated using the formula:

$$Volume = \frac{W^2 L}{2}$$

After treatment completion, mice were euthanized, and tumors were dissected and weighed. Major organs (kidneys, liver, spleen, heart, lung, brain, and tumor) were harvested and fixed in 10% formalin.

Ex Vivo Tissue Processing for H&E Stained Slide Review. Fixed tissues were trimmed, placed on cassettes, and loaded onto a tissue processor for embedding with paraffin wax. The embedded tissues were then sectioned, mounted onto glass slides, and dried. These slides were then stained with H&E using an automated H&E stainer. Slide review was conducted at the Section of Anatomic Pathology within the Animal Health Diagnostic Center at Cornell University. Necrotic tissue percentages were estimated visually.

Ex Vivo Tissue Digestion and Analysis for Metal Content. Formalin-fixed tissues were patted dry and weighed. To each tissue was added 4 mL 30% H_2O_2 (trace grade) and 8 mL 70% HNO_3 (ARISTAR grade) and heated at 130 °C until tissues were completely digested. Solutions were cooled down, and 1 mL of each solution was diluted with 2.5 mL MilliQ water. Samples were then analyzed to determine rhenium content using ICP-MS. Results were reported as the mass ratio of rhenium to tissue (pg/mg) in each sample.

Table S1. Pre-treatment body weights and post-treatment organ weights of mice treated with 10, 20, 40, 60, and 80 mg/kg compound **1** for determining MTD.

Treatment (mg/kg)	Organ	Weight (g)
10[a]	Body Weight	23.07 ± 0.96
	Left Kidney	0.15 ± 0.01
	Right Kidney	0.16 ± 0.01
	Liver	0.93 ± 0.17
	Spleen	0.05 ± 0.04
	Heart	0.11 ± 0.01
	Lung	0.14 ± 0.01
	Brain	0.46 ± 0.02
20[a]	Body Weight	22.23 ± 0.40
	Left Kidney	0.13 ± 0.01
	Right Kidney	0.12 ± 0.01
	Liver	0.90 ± 0.07
	Spleen	0.02 ± 0.01
	Heart	0.10 ± 0.01
	Lung	0.16 ± 0.02
	Brain	0.45 ± 0.01
40[b]	Body Weight	22.95 ± 0.49
	Left Kidney	0.14 ± 0.01
	Right Kidney	0.15 ± 0.02
	Liver	0.86 ± 0.04
	Spleen	0.03 ± 0.01
	Heart	0.11 ± 0.01
	Lung	0.17 ± 0.05
	Brain	0.45 ± 0.02
60[b]	Body Weight	23.30 ± 1.84
	Left Kidney	0.15 ± 0.01
	Right Kidney	0.14 ± 0.01
	Liver	0.85 ± 0.05
	Spleen	0.05 ± 0.02
	Heart	0.10 ± 0.01
	Lung	0.16 ± 0.06
	Brain	0.46 ± 0.04
80[bc]	Body Weight	21.05 ± 3.04

[a] = data represents three independent animals.
[b] = data represents two independent animals.
[c] = mice died within 30 s of treatment, therefore, major organs were not harvested after 24 h.

Table S2. Percent necrosis of PDX tumors determined through histological analysis of H&E stained tissues for mice treated with vehicle or compound **1** at 10, 20, or 40 mg/kg.

Treatment (mg/kg)	Percent Necrotic Tissue
Vehicle	40
10	25
20	10
40	5

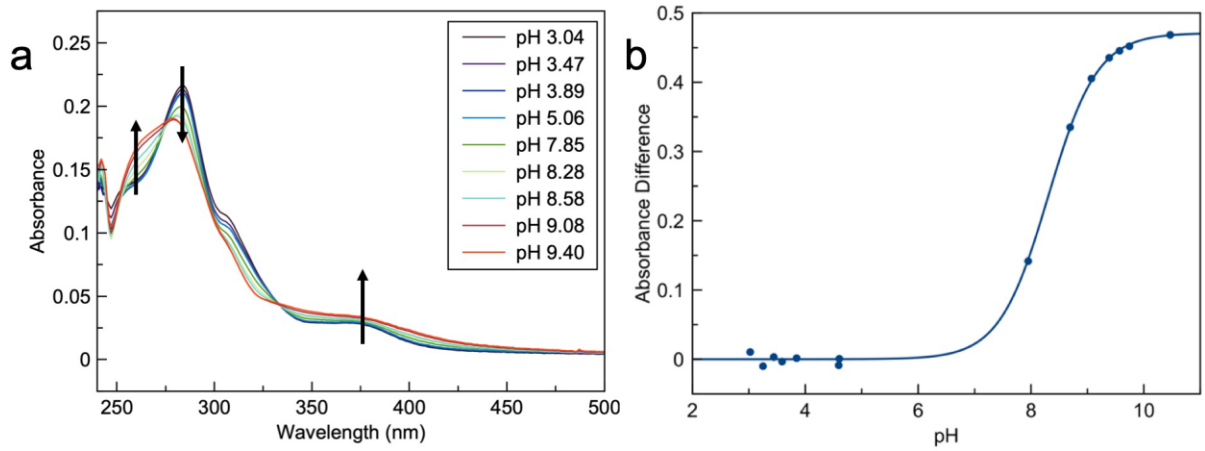

Figure S1. (a) UV–vis spectra and (b) plot of absorbance difference (A_{280}/A_{260}) from titrating **1** in a HNO_3/KOH solution containing 0.1 M KNO_3.

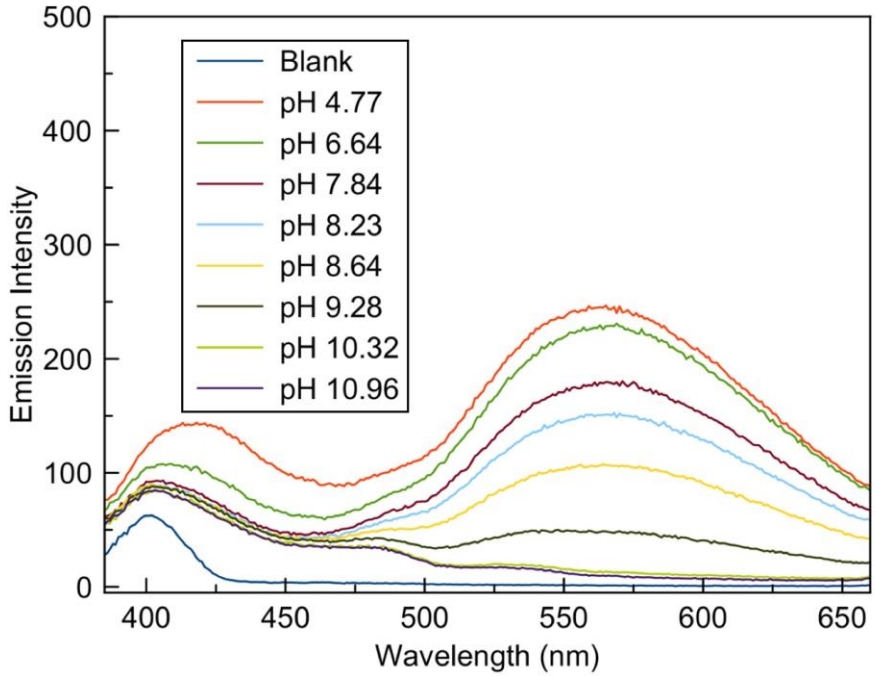

Figure S2. Luminescence spectrum of **1** in MOPS buffer at varying pH values.

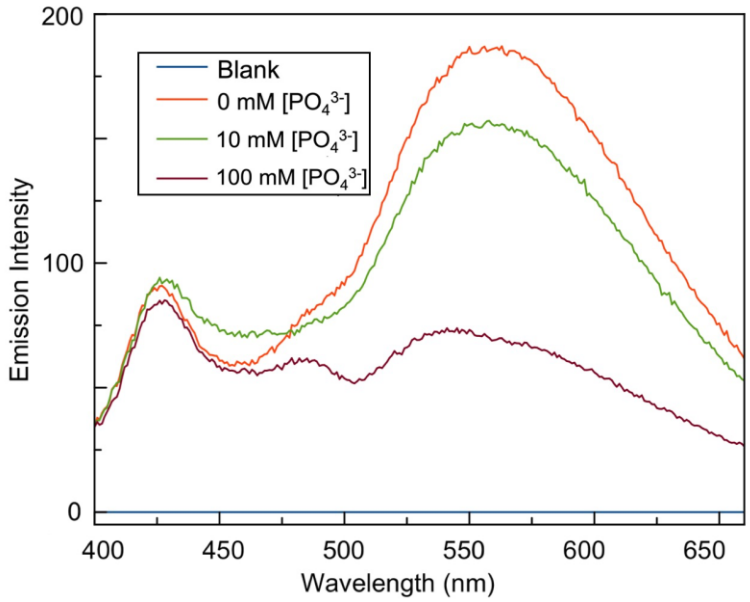

Figure S3. Luminescence spectra of **1** in MOPS buffer (pH 7.4) after incubation with varying concentrations of inorganic phosphate.

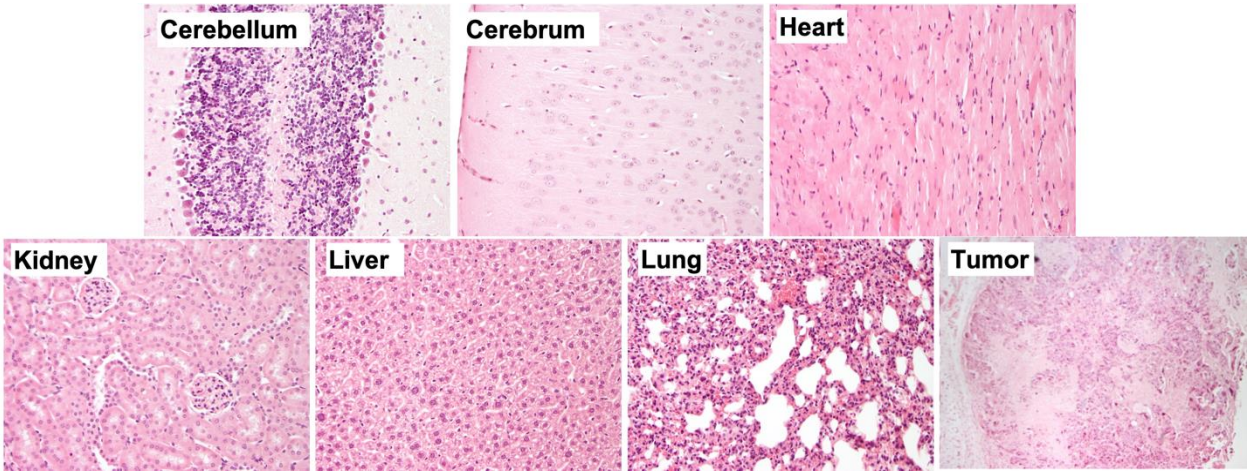

Figure S4. H&E stained slides of mice tissue treated with vehicle (10% DMSO, 5% Kolliphor HS15, 4.25% dextrose in water).

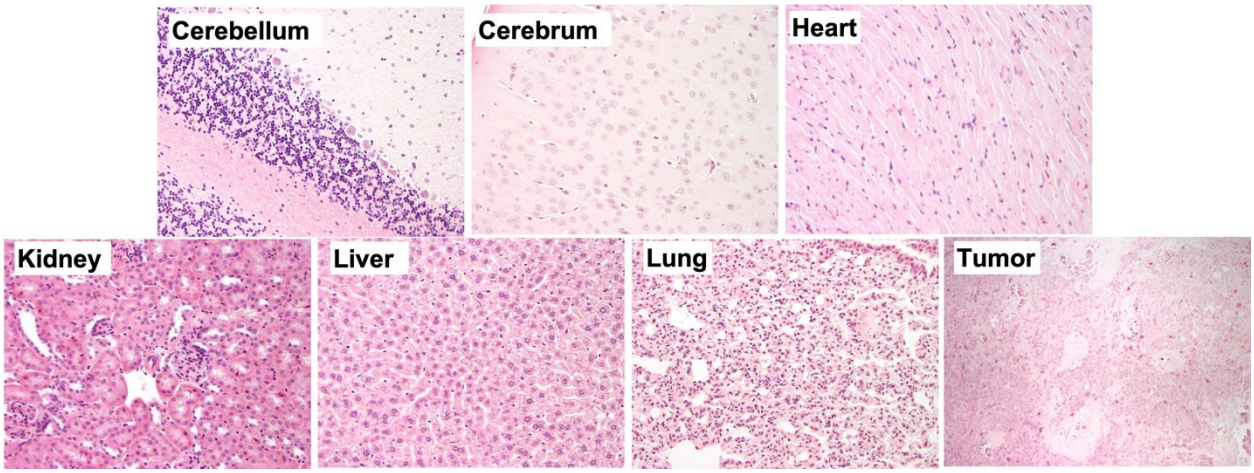

Figure S5. H&E stained slides of mice tissue treated with 10 mg/kg **1**.

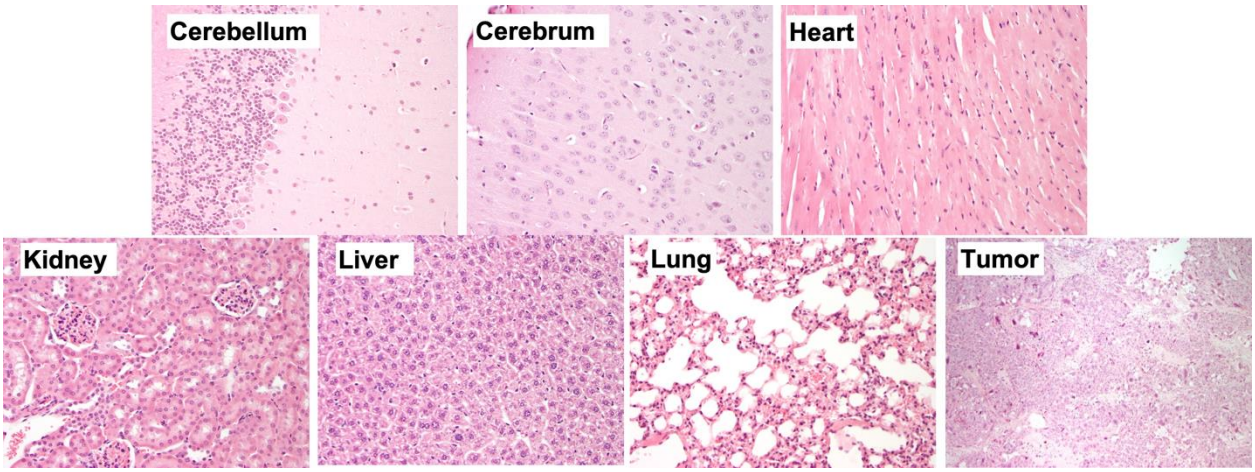

Figure S6. H&E stained slides of mice tissue treated with 20 mg/kg **1**.

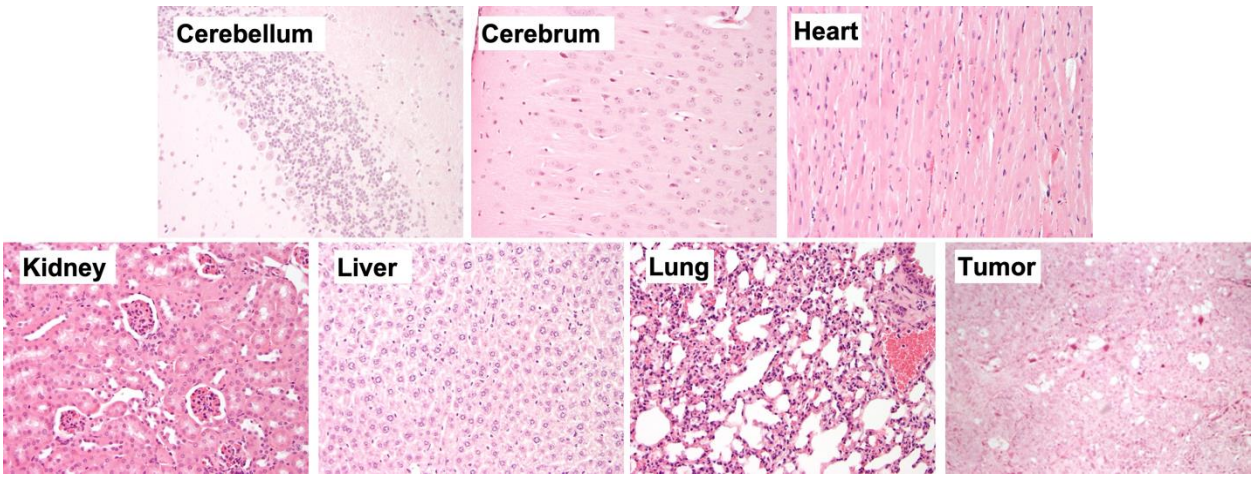

Figure S7. H&E stained slides of mice tissue treated with 40 mg/kg **1**.

Notes and References.

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