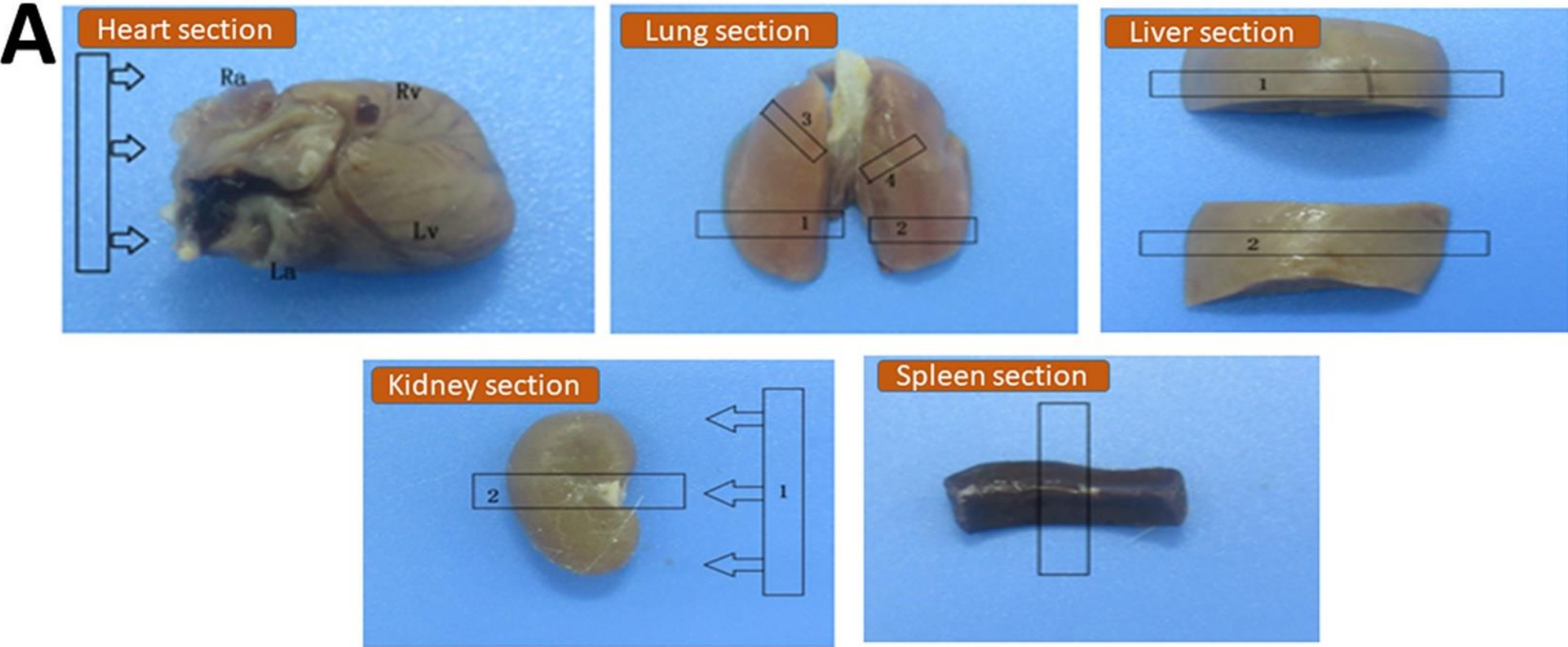


Supplementary Materials

I. Supplementary Figures



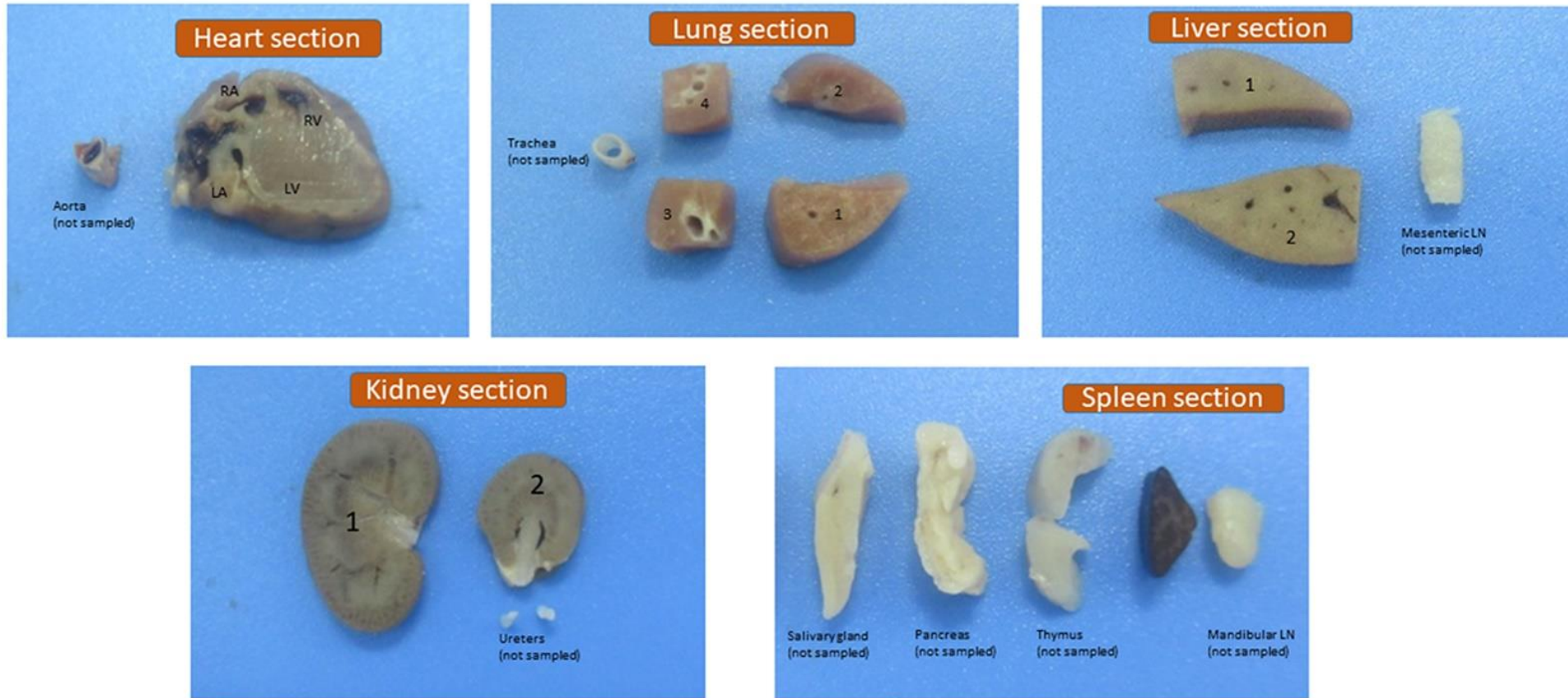
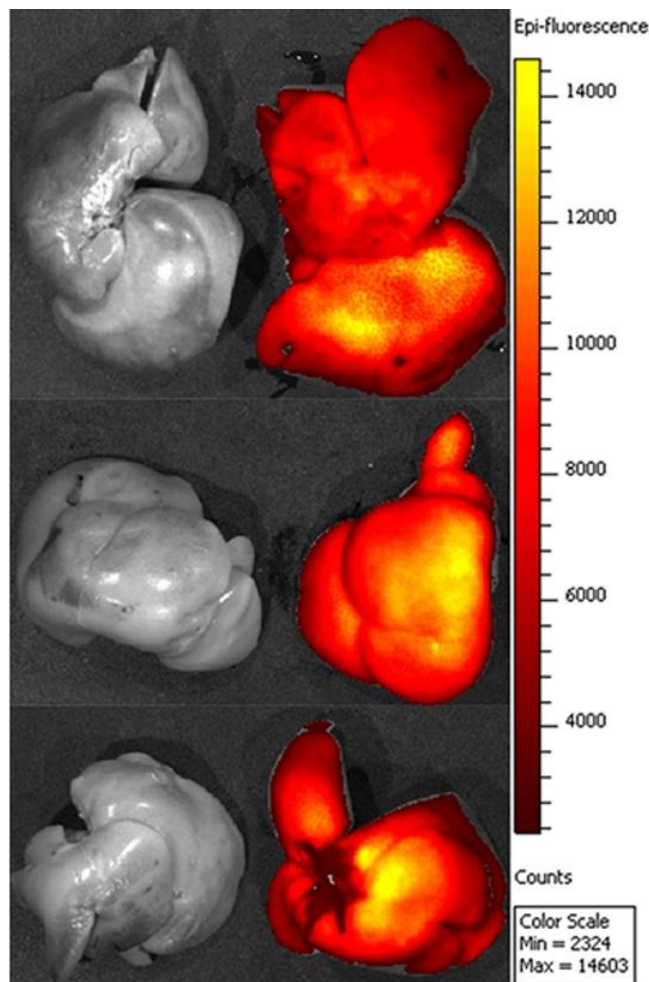
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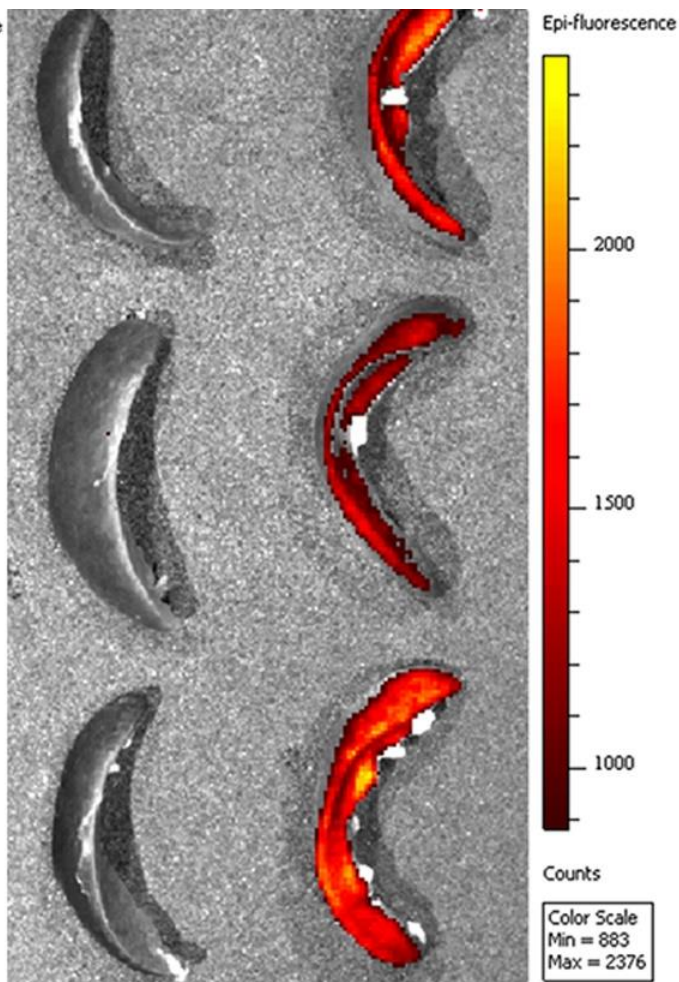
Figure S1. Histopathology processing of rats' tissues.

Note: (A) rectangles through the tissue denote cross sections. Rectangles with arrows indicate longitudinal sections. (B) Tissues were embedded in the mold as viewed from this perspective. At microtomy, tissues on the slide were “mirrored” when compared to these images.

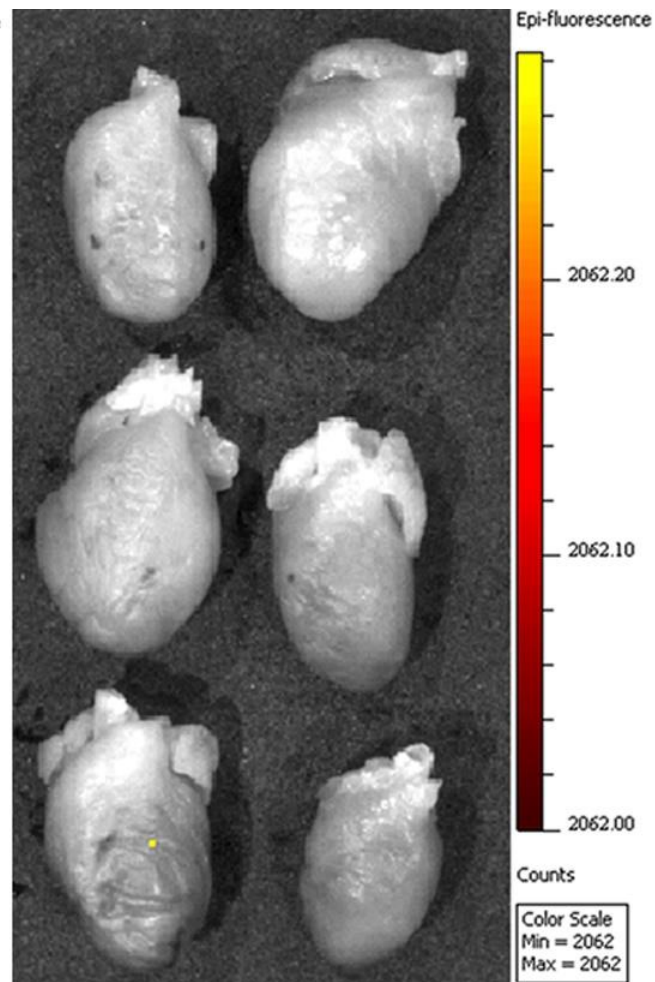
Abbreviations: RA, right atria; LA, left atria; RV, right ventricle; LV, left ventricle; LN, lymph node.



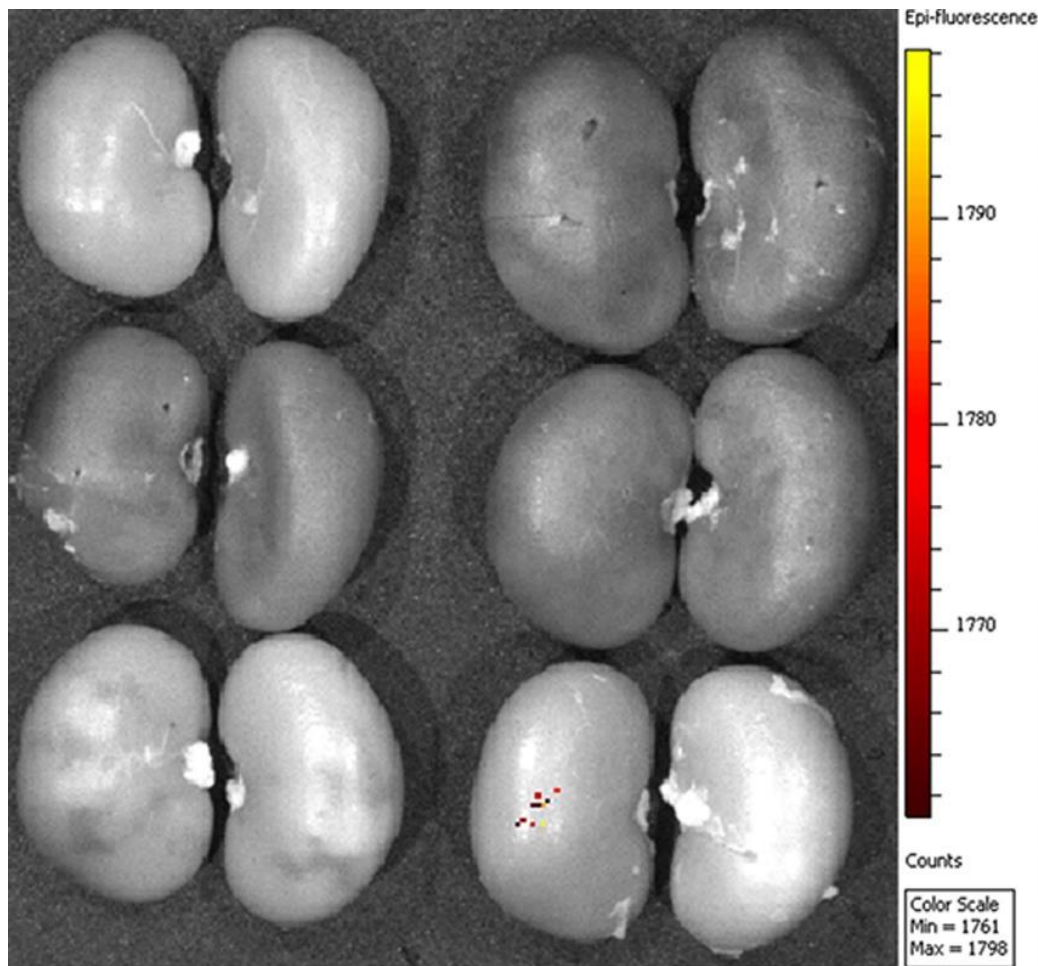
Liver



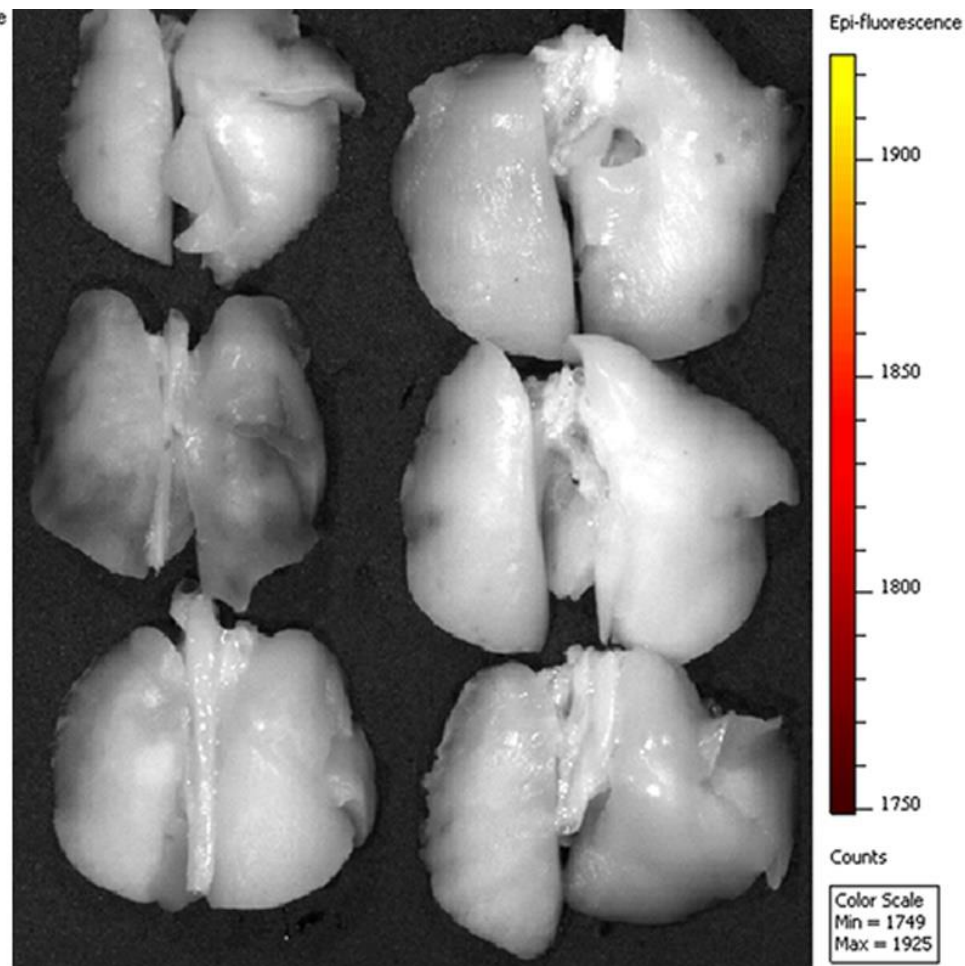
Spleen



Heart



Kidney

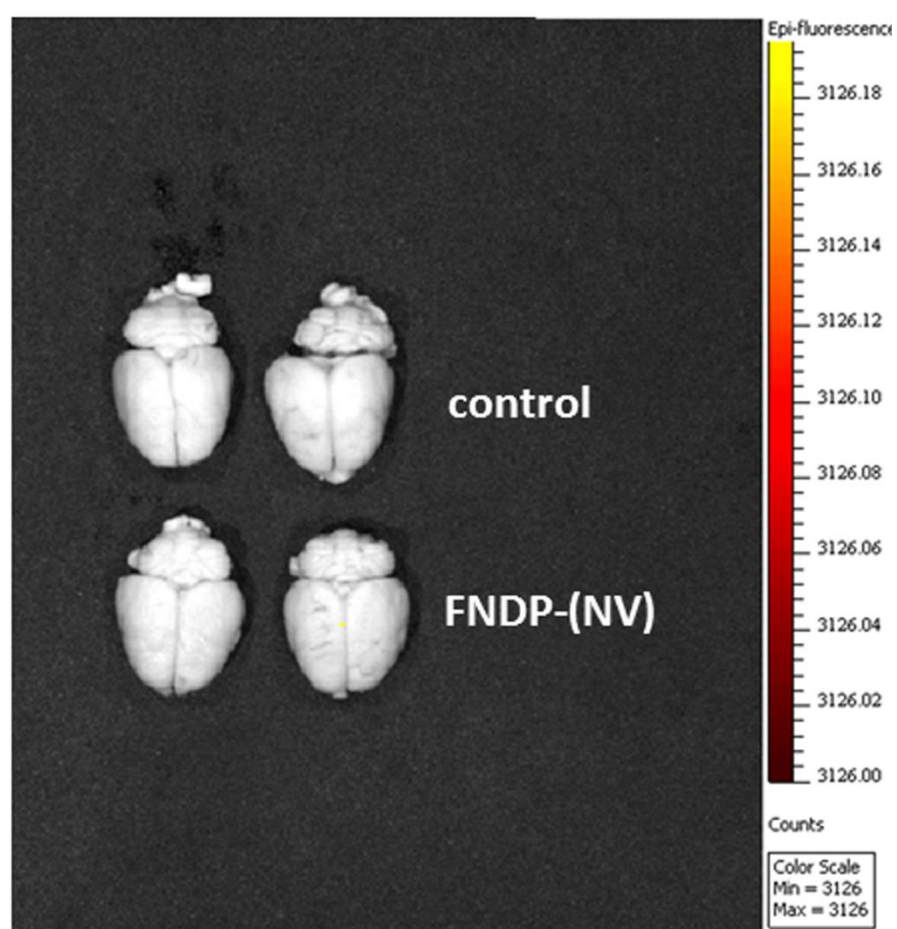
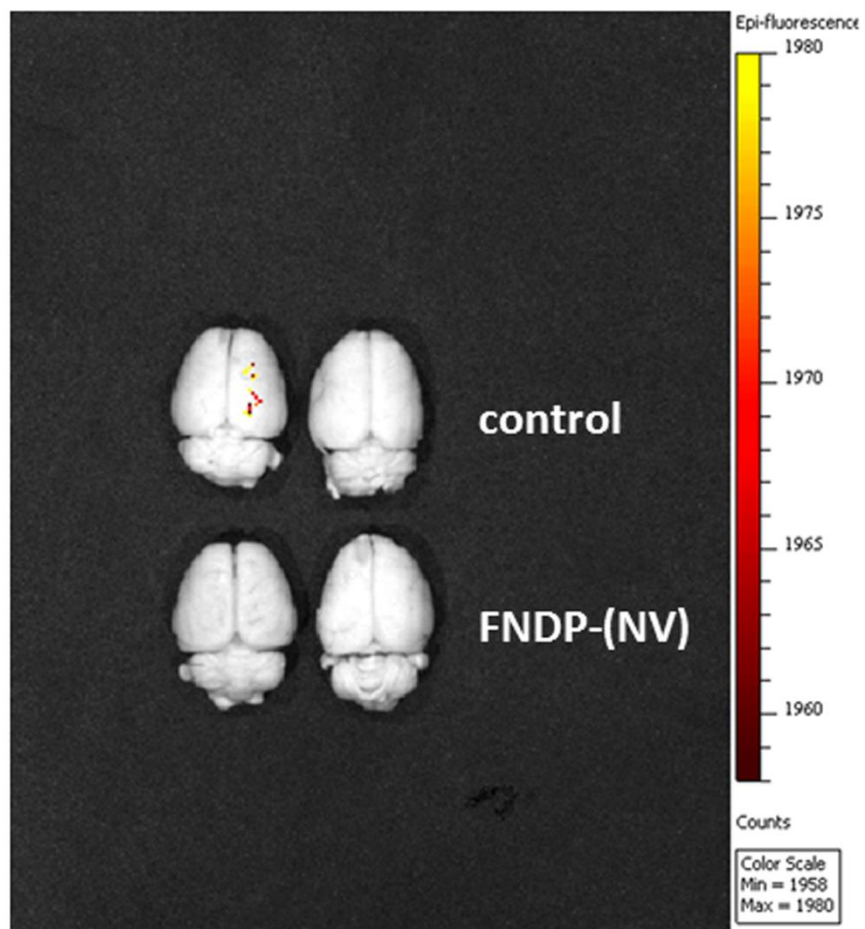


Lung

Figure S2. IVIS images of dissected, isolated intact organs of rats, representing examples of organs.

Note: Organs obtained from three control animals (injected with PBS) are presented on the left column of each image, three FNDP-(NV) treated animals are presented on right column of each image. Intensity of fluorescence is in red (low) to yellow (high) color scale.

Abbreviations: FNDP-(NV), fluorescence nanodiamonds particles with NV active centers; IVIS, in vivo imaging system

A

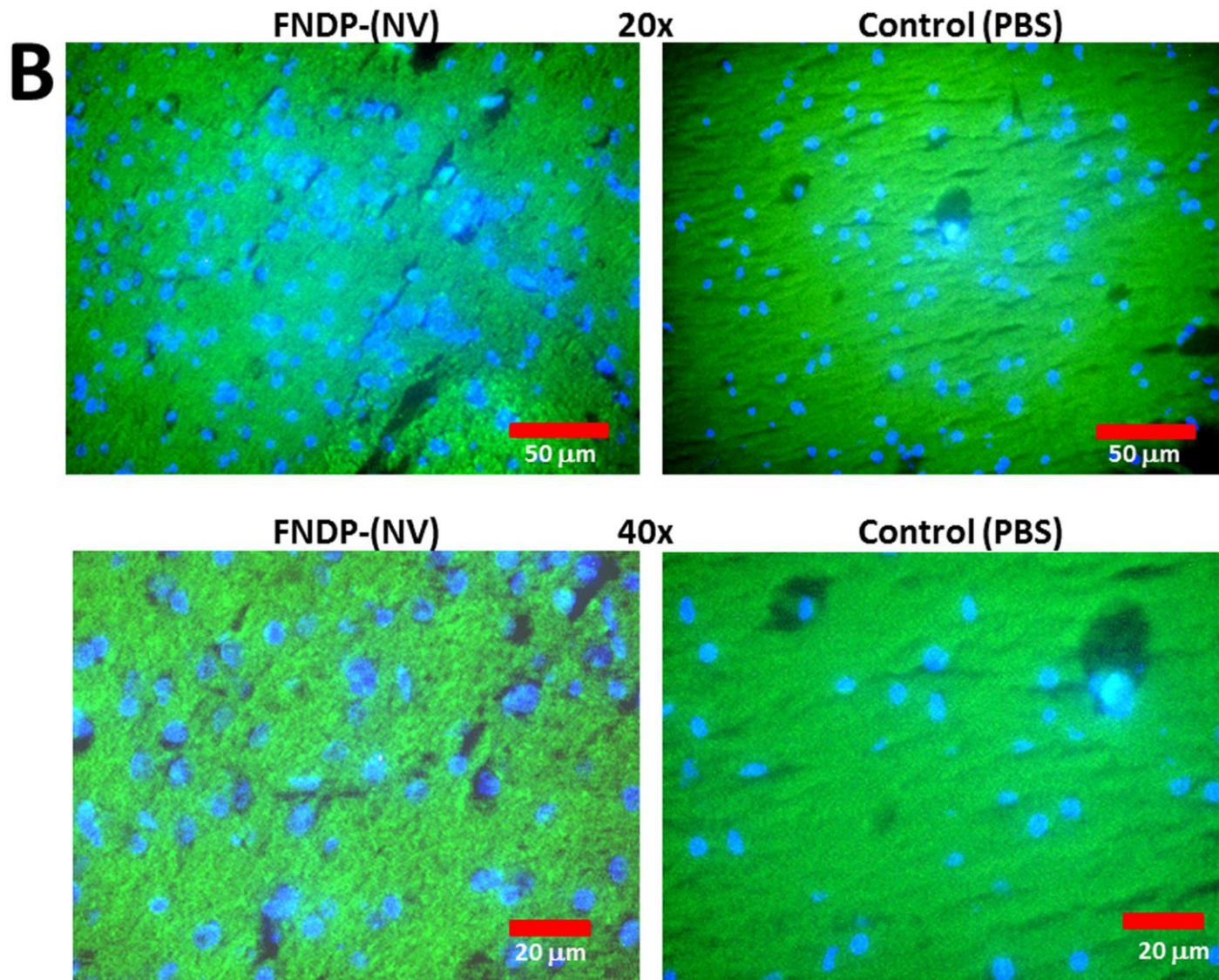


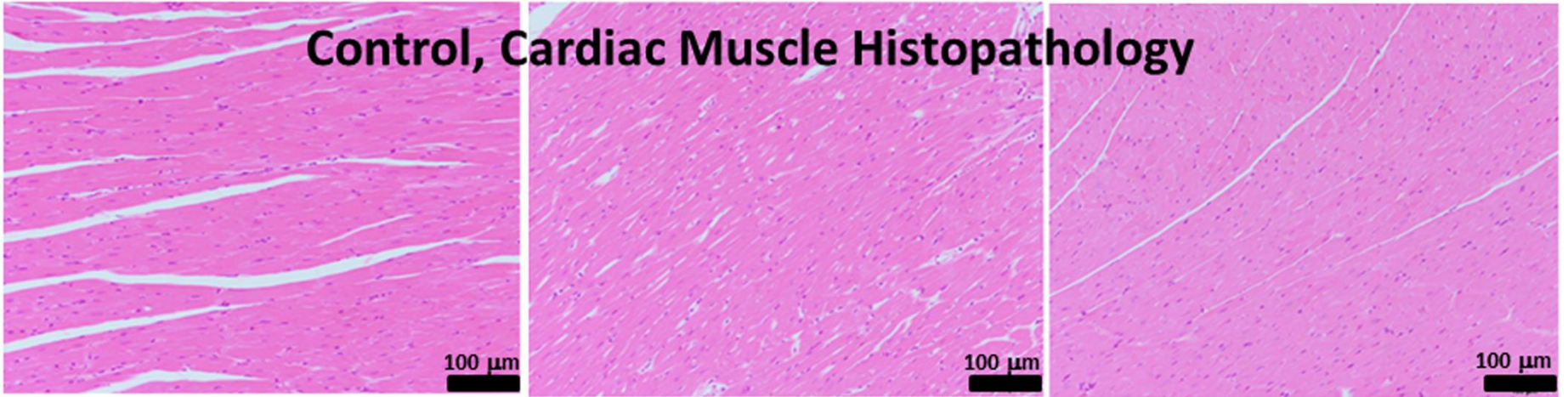
Figure S3. Detection of FNDP-(NV) in the brain of rats

Note: (A) IVIS images of dissected brains isolated in post-injection day 5 (left panel) and day 14 (right panel). The images of brains from control (PBS) animals are presented in top rows, whereas images of brains of animals treated with FNDP-(NV) are presented on the bottom rows. The intensity of fluorescence is in red (low) to yellow (high) color scale. (B) Fluorescence microscope images of paraffin sections of brains from 14 days study. Tissue slides were stained with FITC phalloidin (green) and DAPI (blue). FNDP-(NV) are expected to be marked by red to yellow speckles in red filter (TRITC). Slides were analyzed using fluorescence microscope (Olympus FSX100) with two objectives (20x and 40x).

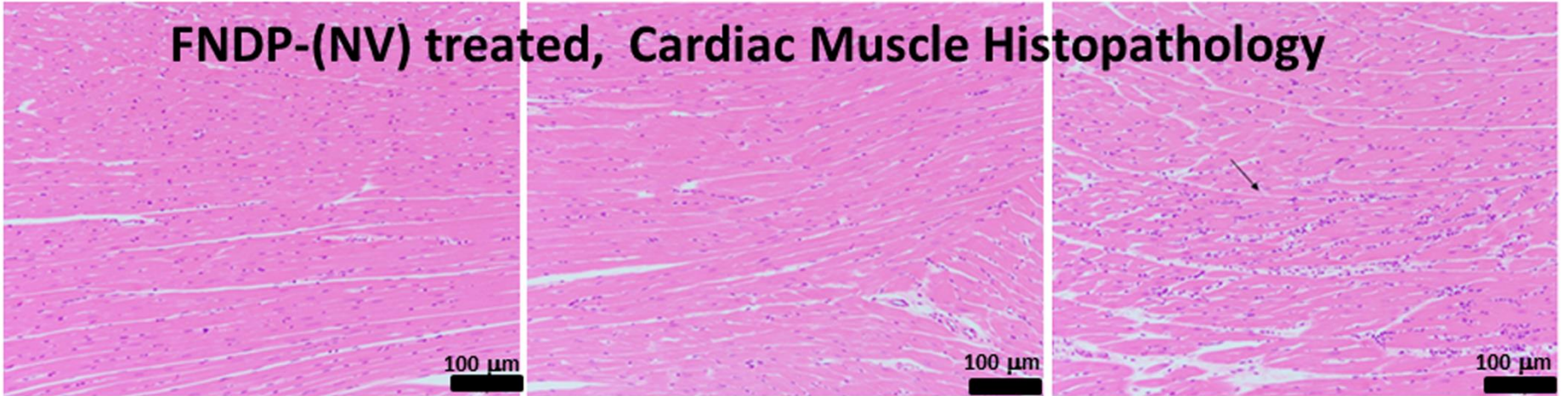
Abbreviations: FNDP-(NV), fluorescence NanoDiamonds particles with NV active centers; IVIS, in vivo imaging system; DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate.

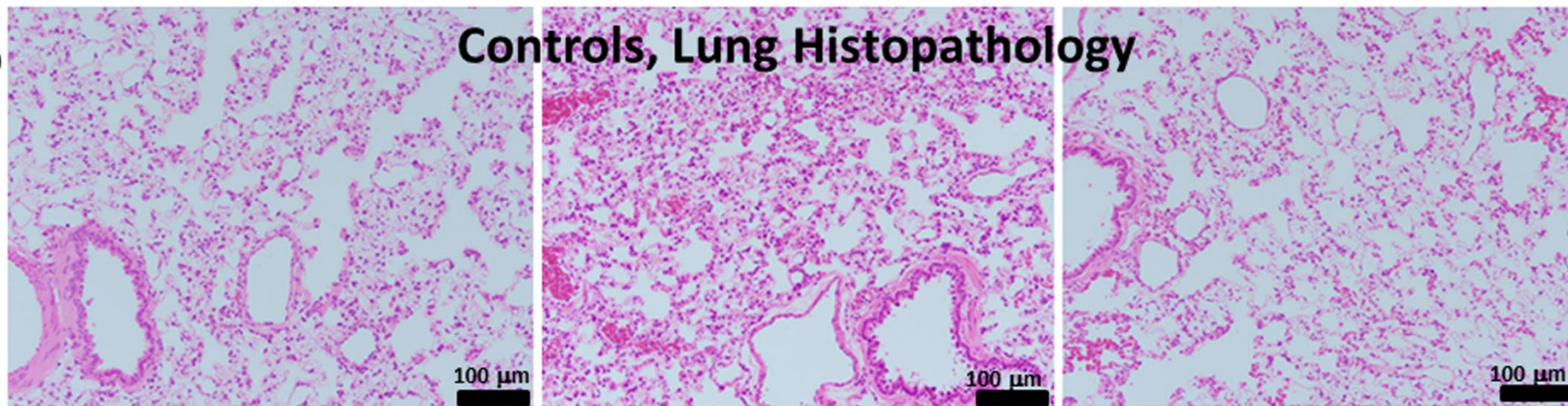
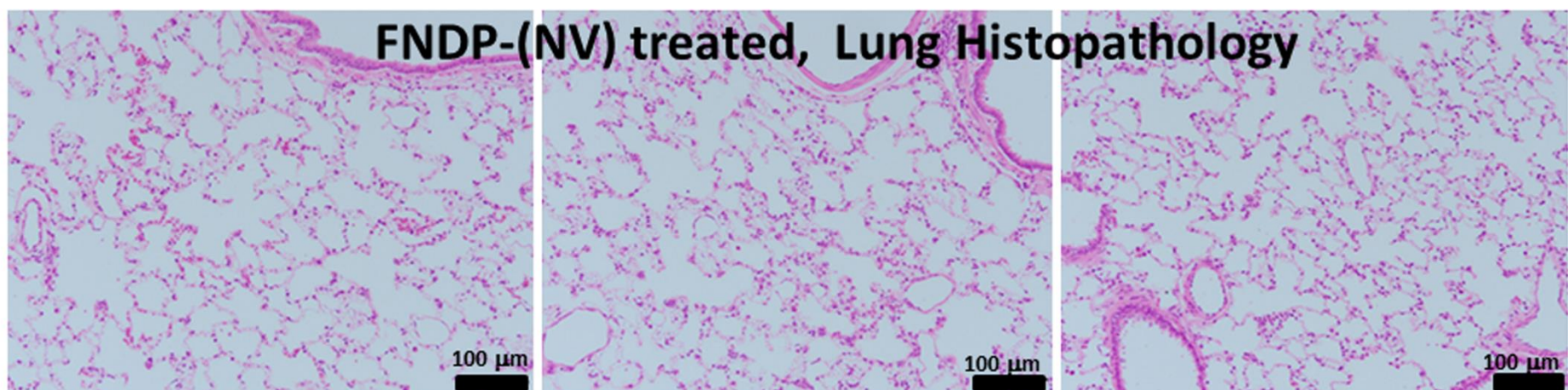
A

Control, Cardiac Muscle Histopathology



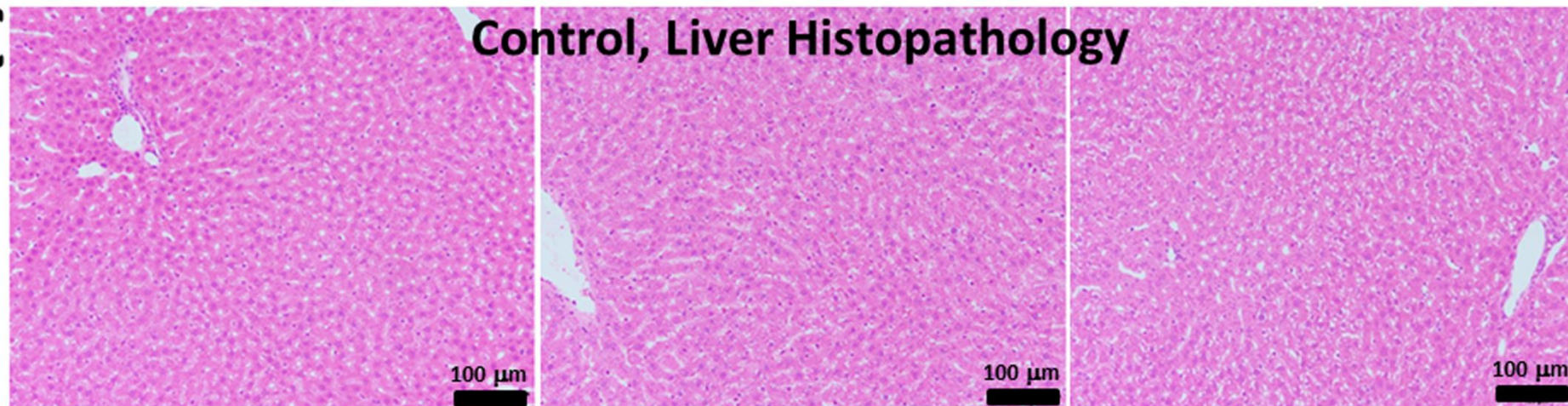
FNDP-(NV) treated, Cardiac Muscle Histopathology



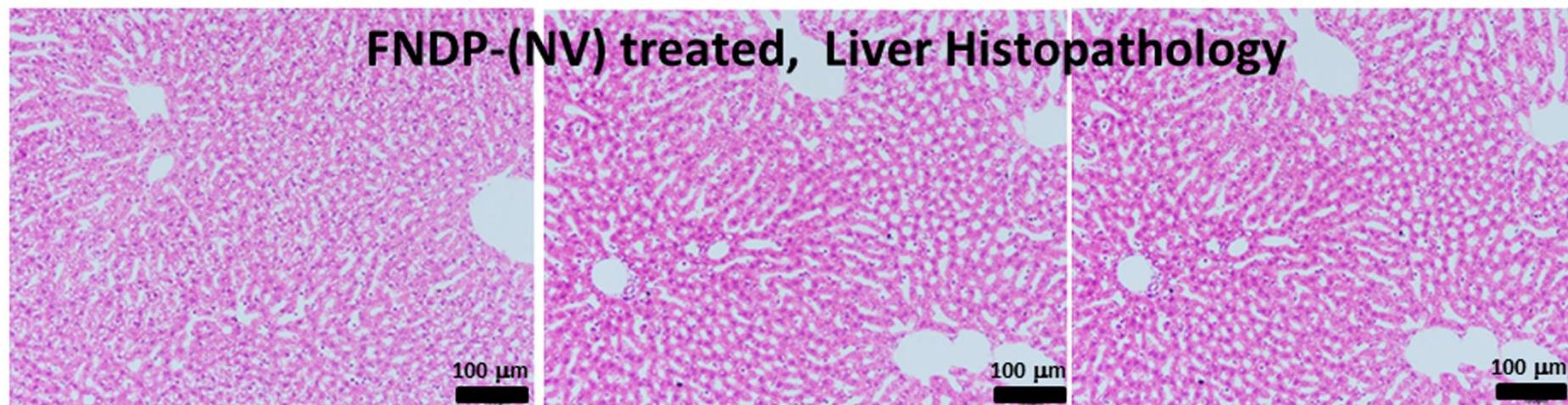
B**Controls, Lung Histopathology****FNDP-(NV) treated, Lung Histopathology**

C

Control, Liver Histopathology

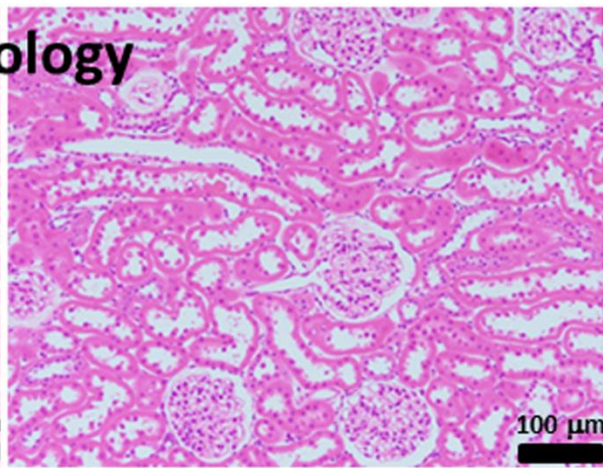
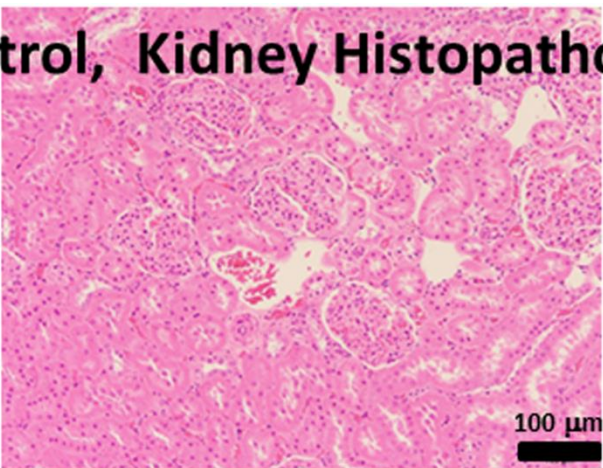
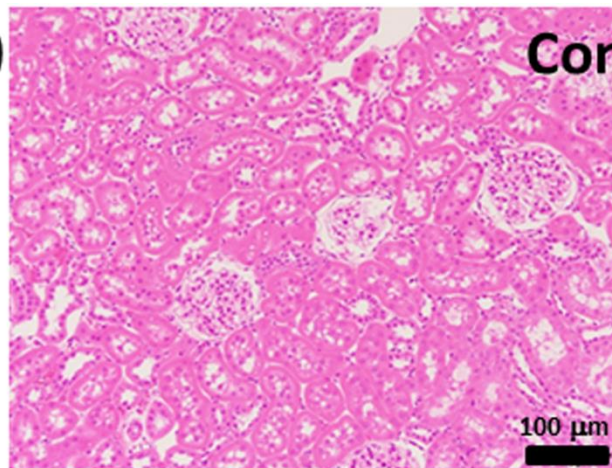


FNDP-(NV) treated, Liver Histopathology

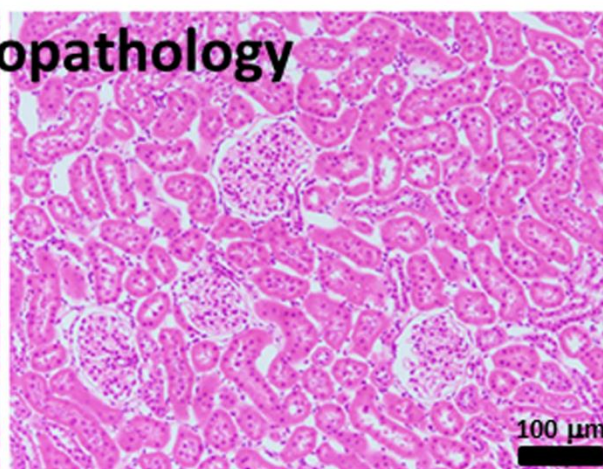
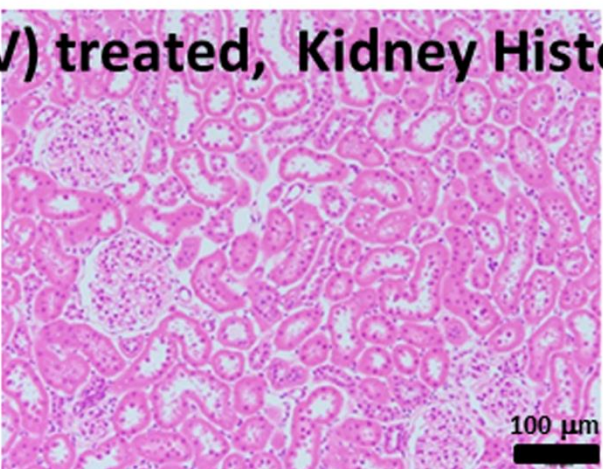
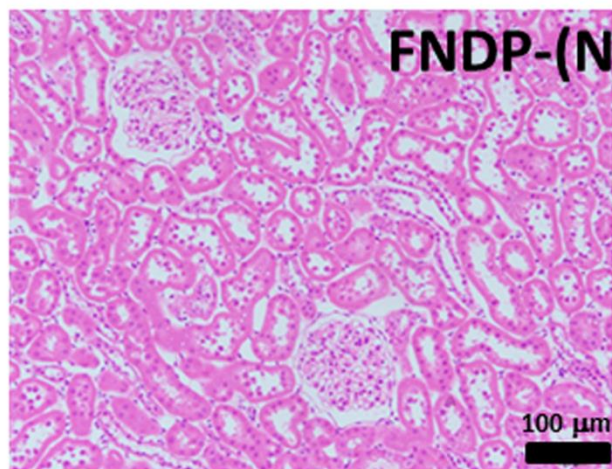


D

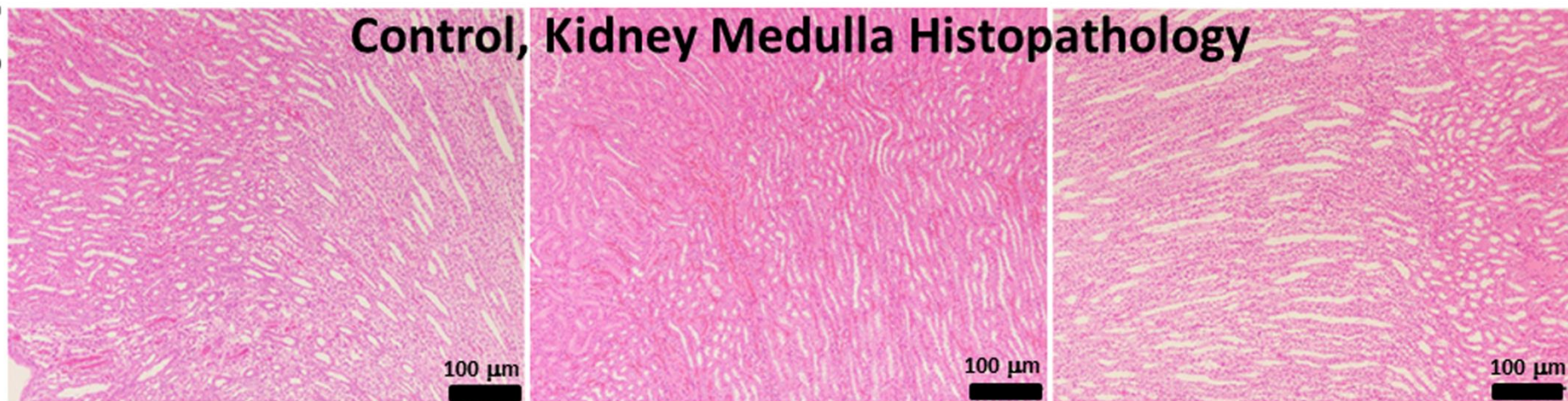
Control, Kidney Histopathology



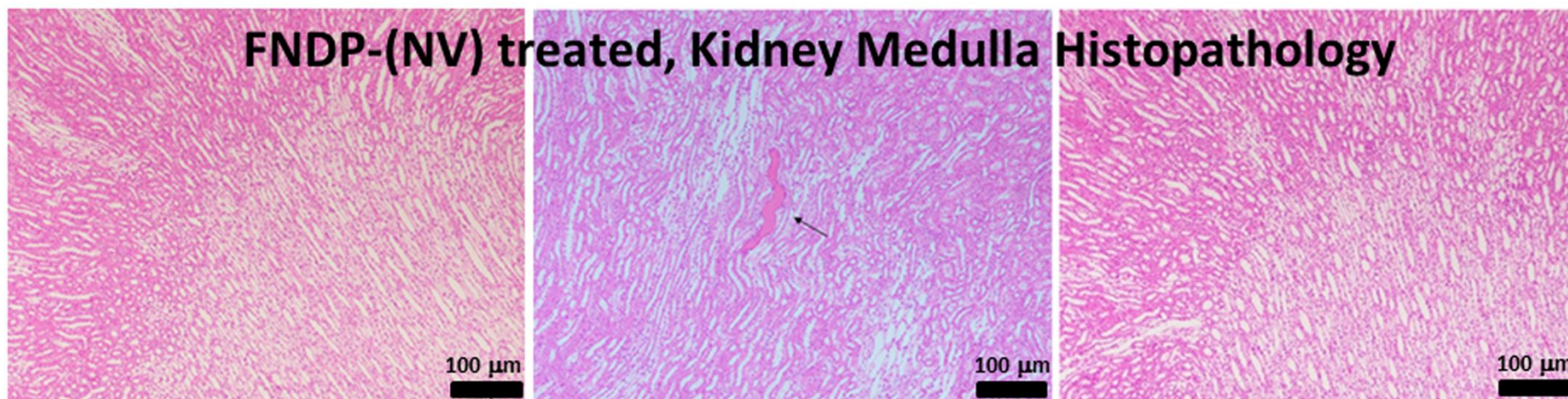
FNDP-(NV) treated, Kidney Histopathology



E Control, Kidney Medulla Histopathology



FNDP-(NV) treated, Kidney Medulla Histopathology



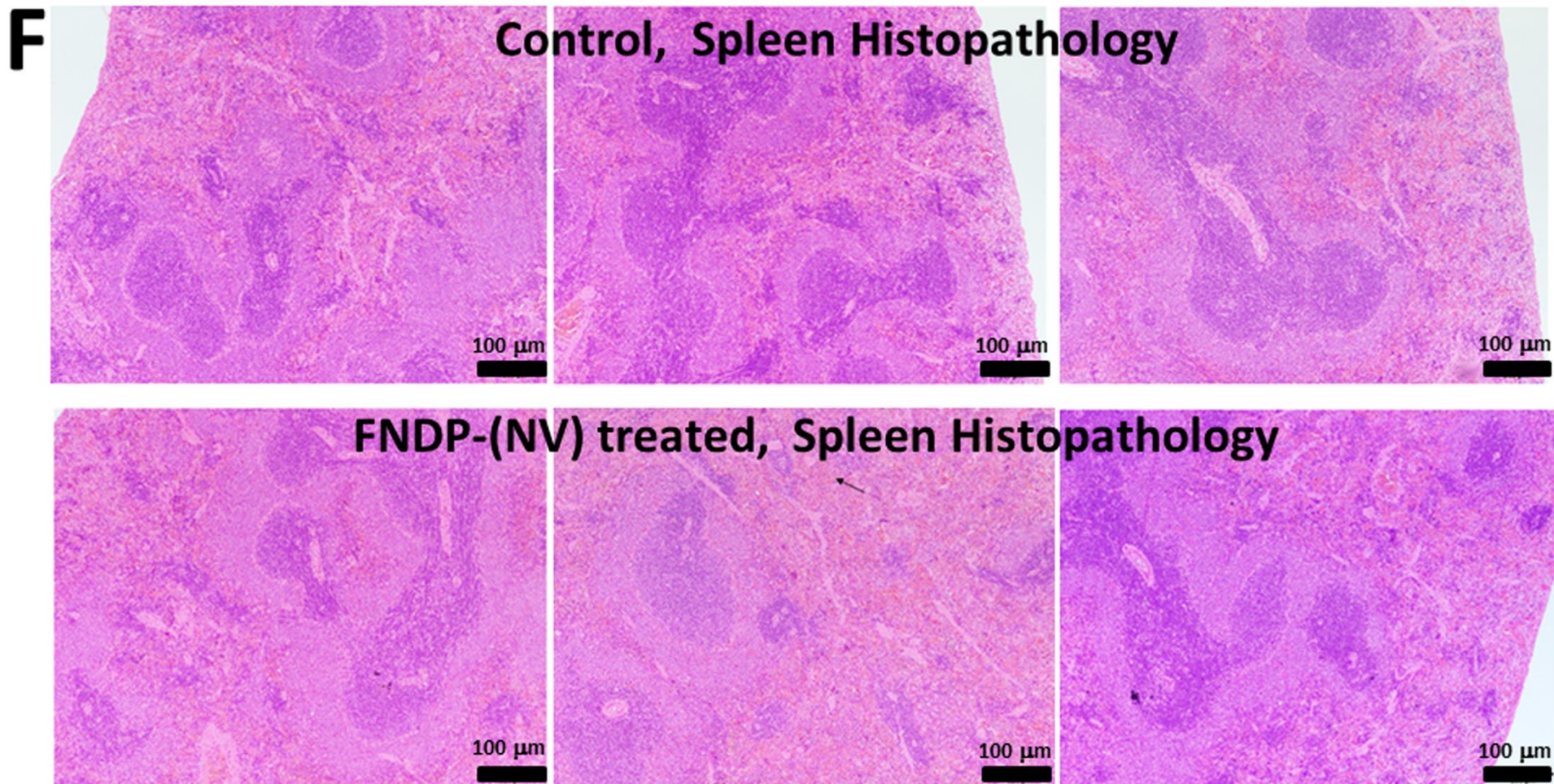


Figure S4. Images of paraffin embedded tissue sections of the organs dissected from the rats treated or not with FNDP-(NV).

Note: (A) Images of heart tissue obtained from control, vehicle (PBS) treated rats (three top images) and FNDP-(NV)-treated rats (three bottom images). Arrow indicates single mononuclear cell. (B) Images of lung tissue obtained from control, vehicle (PBS) treated rats (two top images) and FNDP-(NV)-treated rats (two bottom images). (C) Images of liver tissue obtained from control, vehicle (PBS) treated rats (two top images) and FNDP-(NV)-treated rats (two bottom images). (D) Images of kidney tissue obtained from control, vehicle (PBS) treated rats (two top images) and FNDP-(NV)-treated rats (two bottom images). (E) Images of kidney medulla tissue obtained from control, vehicle (PBS) treated rats (two top images) and FNDP-(NV)-treated rats (two bottom images). Arrow indicates hyaline deposit in one collecting duct. (F) Images of spleen tissue obtained from control, vehicle (PBS) treated rats (two top images) and FNDP-(NV)-treated rats (two bottom images). Arrow indicates slight pigmentation zone suspected as hemosiderin.

Abbreviations: FNDP-(NV), fluorescence nanodiamonds particles with NV active centers

II. Supplementary Tables

Table S1. Dataset of blood analysis of rats treated or not with FNDP-(NV).

Abbreviation: All symbols used for description of blood parameters are described in Figure 4 legend.

Clinical Blood Tests	FNDP(NV)-Z800						Control							
Animal #:	1	2	3	4	5	6	1	2	3	4	5	6	7	8
Hematology														
WBC (k/ul)	9.25	12.66	7.08	6.37	5.55	6.21	2.07	5.52	2.62	--	5.93	5.82	5.82	15.29
RBC (m/ul)	8.32	8.15	8.17	7.72	8.02	7.72	8.05	8.46	8.23	--	8.24	7.53	8.22	7.56
HGB (g/dl)	14.5	14.2	13.9	13.5	14.6	13.9	15.2	15.5	15.7	--	14.7	14.3	14.9	14.8
HCT (%)	45.5	43.1	43.2	42.2	44.3	43.5	45.7	47.2	46.3	--	45.2	43	44.5	43.9
MCV (fl)	54.7	52.8	52.9	54.7	55.3	56.3	56.8	55.8	56.3	--	54.8	57.2	54.1	58.1
MCH (pg)	17.5	17.4	17	17.5	18.2	18.1	18.9	18.4	19.1	--	17.8	18.9	18.2	19.5
MCHC (g/dL)	31.9	32.9	32.1	32	32.8	32.1	33.3	33	33.9	--	32.5	33.1	33.6	33.6
RDW (%)	13.9	13.5	12.5	13.7	12.4	13.3	11.6	12	11.6	--	11.9	11.7	12.1	10.9
PLAT (k/UL)	1083	856	949	1100	776	1000	874	938	745	--	1040	859	912	805
MPV (fl)	7.3	7.4	7.7	8	7.4	7	7.4	7.5	7.8	--	7.6	7.4	7.6	7.1
Coagulation														
APTT(sec)	no	no	16.6	>100	no	17.7	--	--	no	--	no	no	16.6	no
Fibrinogen(mg/dL)	156	136	157	144	155	144	--	--	165	--	152	164	163	154
Prothrombin Time(sec)	10.6	11.2	11.3	11.5	11.8	12.1	--	--	10.7	--	10.9	10.7	11.2	11.2
INR	0.9	1	1	1	1	1	--	--	0.9	--	0.9	0.9	1	1
General														
Sodium (mmol/l)	143	141	142	142	144	143	145	141	140	145	141	143	144	142
Potassium (mmol/L)	7.2	6.7	6.2	6.1	5.9	5.2	6.2	7.4	9.5	5.7	6.8	6.3	6.7	6.1
Chloride (mmol/L)	99	101	101	103	103	103	103	101	100	103	99	101	100	100
CO2 (mmol/L)	24	23	24	24	22	22	23	22	23	19	24	27	26	26
Glucose Random (mg/dL)	133	211	164	196	158	158	142	187	124	161	176	176	171	196
BUN (mg/dL)	20	18	18	15	18	16	16	15	17	18	14	18	18	19
Creatinine (mg/dl)	0.3	0.31	0.36	0.3	0.34	0.32	0.29	0.26	0.32	0.23	0.24	0.3	0.35	0.3
Protein Total (g/dL)	6.8	6.3	6.7	5.8	6.2	5.9	6	6.3	7	5.9	6.1	6.3	6.4	5.6
Albumin (g/dl)	3.31	3.06	3.13	2.93	2.97	2.91	3.25	3.3	3.7	3.13	3.26	3.42	3.28	3.03
Alkaline Phosphatase(U/L)	291	154	212	135	135	150	285	171	185	185	291	187	205	260
AST (u/L)	109	163	123	105	122	113	135	100	200	201	160	92	89	90
ALT (u/L)	42	84	37	51	48	42	41	51	54	47	49	38	48	41
Calcium (mg/dL)	10.3	10.2	10.2	9.6	9.9	9.8	10	9.9	10	9.9	9.8	10.2	10.2	9.9
Total Bilirubin (mg/dl)	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.3	0.6	0.2	0.2	0.1	0.1	0.1

Table S2. Active place avoidance (APA) test results for each individual animal.

Abbreviations: FNDP, fluorescence nanodiamonds particles; Dist, distance; m, meters; s, seconds; #, number.

	Animal	Run#	Week 4							Week 8							Week 12						
			1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
FNDP(NV)-2800	1	Dist (m)	31.07	26.41	32.78	31.42	22.42	29.16	28.32	26.22	28.82	30.06	33.2	31.17	33.22	33.84	28.9	30.33	24.47	30.63	41.63	32.63	32.63
		Shock (#)	34	38	30	43	43	39	31	44	31	18	16	9	7	8	49	30	35	33	13	14	14
		Entrance (#)	16	22	19	10	22	17	11	8	9	11	12	9	7	8	10	10	8	10	13	12	12
		1 st Entry (s)	31	15	20	48	39	23	36	24	29	11	30	31	17	31	23	9	9	47	6	43	14
	2	Dist (m)	34.36	32.33	40.05	39.87	43.44	44.98	43.76	33.86	40.49	36.4	35.76	39.73	39.09	24.81	44.31	39.84	39.69	38.61	37.7	37.34	37.34
		Shock (#)	27	12	7	2	2	0	2	13	6	0	0	1	0	1	3	0	0	0	0	0	1
		Entrance (#)	8	4	6	2	2	0	1	3	1	0	0	1	0	1	3	0	0	0	0	0	1
		1 st Entry (s)	44	20	92	109	41	600	319	1	234	600	600	36	600	38	43	600	600	600	600	600	285
	3	Dist (m)	46.05	41.33	40.38	41.98	32.09	40.38	40.78	46.39	38.96	39.24	38.35	36.28	37.42	37.88	46.68	41.3	36.36	32.4	33.88	37.48	37.48
		Shock (#)	13	1	0	1	0	0	1	7	0	0	3	0	0	2	3	1	1	1	1	0	0
		Entrance (#)	12	1	0	1	0	0	1	3	0	0	2	0	0	2	3	1	1	1	1	0	0
		1 st Entry (s)	29	33	600	9	600	600	108	137	600	600	397	600	600	507	7	430	380	27	101	600	600
	4	Dist (m)	48.32	48.02	39.04	39.18	37.01	38.06	34.19	49.14	34.32	30.99	26.61	30.74	30.36	30.02	44.37	44.73	44.62	43.88	41.88	31.61	36.44
		Shock (#)	31	11	3	17	7	2	4	33	28	1	19	3	0	3	29	14	15	21	16	9	3
		Entrance (#)	16	11	3	12	7	2	4	18	8	1	9	3	0	3	20	14	14	18	14	7	4
		1 st Entry (s)	26	34	26	31	22	246	71	8	64	33	32	39	600	296	35	66	39	41	28	4	93
	5	Dist (m)	33.37	31.88	37.84	33.4	36.61	37	37	45.32	37.12	34.44	33.78	33.82	33.78	33.82	46.85	43.65	37.78	35.71	36.31	35.19	35.19
		Shock (#)	36	46	1	1	0	0	0	23	8	0	0	0	0	0	19	21	12	1	2	0	0
		Entrance (#)	12	16	1	1	0	0	0	14	3	0	0	0	0	0	18	14	9	1	2	0	0
		1 st Entry (s)	3	22	317	232	600	600	600	32	119	600	600	600	600	600	20	37	343	21	31	600	600
	6	Dist (m)	34.07	37.89	49.8	49.33	41.27	41.69	37.07	23.38	38.27	46.04	38.38	37.66	40.76	42.39	43.85	42.44	42.49	41.23	43.22	40.71	27.09
		Shock (#)	44	35	17	13	3	2	3	38	33	19	7	8	9	9	19	16	13	12	3	10	3
		Entrance (#)	14	17	17	13	4	1	2	10	14	13	7	8	9	9	11	16	14	12	3	9	2
		1 st Entry (s)	22	17	10	33	159	283	28	27	4	30	202	37	61	81	39	6	36	39	330	107	291
Control	1	Dist (m)	39.88	61.63	34.68	42.14	33.14	34.39	34.36	44.32	41.32	37.26	43.06	43.73	43.39	41.89							
		Shock (#)	28	21	18	3	0	0	0	14	8	1	2	2	0	0							
		Entrance (#)	17	18	17	3	0	0	0	13	8	1	2	2	0	0							
		1 st Entry (s)	102	12	20	22	600	600	600	1	26	231	21	219	600	600							
	2	Dist (m)	63.83	48.2	43.06	39.98	39.1	37.38	39.12	44.07	49.81	40.97	39.98	41.06	40.79	40.29							
		Shock (#)	38	28	1	1	0	0	1	9	27	3	1	2	1	0							
		Entrance (#)	19	11	1	1	0	0	1	4	8	2	1	1	1	0							
		1 st Entry (s)	7	23	349	179	600	600	21	3	46	23	327	339	416	600							
	3	Dist (m)	30.3	37.29	49.47	40.03	39.67	43.73	40.03	49.05	33.91	30	48.68	42.06	41.95	41.09							
		Shock (#)	24	27	23	3	3	3	1	30	11	22	6	0	0	1							
		Entrance (#)	16	23	18	1	2	3	1	13	3	9	3	0	0	1							
		1 st Entry (s)	3	21	32	4	10	16	333	28	97	18	11	600	600	241							
	4	Dist (m)	37.84	40.05	37.29	33.32	36.81	34.94	34.42	42.76	38.76	39.34	38.01	33.91	38.01	33.91							
		Shock (#)	12	4	2	0	0	1	1	7	1	0	0	0	0	0							
		Entrance (#)	6	4	2	0	0	1	1	6	1	0	0	0	0	0							
		1 st Entry (s)	23	90	69	600	600	164	16	4	363	600	600	600	600	600							
	5	Dist (m)	63.87	69.13	36.93	38.32	66.19	61.36	37.88	69.12	60.43	62.09	31.17	49.93	48.74	48.33							
		Shock (#)	34	28	29	33	27	28	24	26	18	14	16	4	11	4							
		Entrance (#)	20	22	21	20	23	22	17	22	17	14	16	4	11	4							
		1 st Entry (s)	10	23	63	46	7	33	4	6	19	8	10	33	28	63							

III. Supplementary Reports

Histopathology report



DRAFT PATHOLOGY REPORT

HISTOPATHOLOGY EVALUATION OF THE RAT ORGANS

STUDY NO.
608-0001-PT

TESTING FACILITY
WUXI APPTEC (SUZHOU) CO., LTD.
1318 WUZHONG AVENUE, WUZHONG DISTRICT
SUZHOU 215104, CHINA

SPONSOR
DEBINA DIAGNOSTICS INC
NEWTOWN SQUARE, PA 19073

REPORT DATE
2018-MM-DD

1 INTRODUCTION

The purpose of this study was to conduct histopathological evaluation of livers, spleens, kidneys, lungs and hearts obtained from rats that were treated with nanoparticles or PBS (vehicle).

A total of 6 rats (30 organs total) were examined (three treated and three controls rats).

2 REGULATORY COMPLIANCE

The pathology evaluation was not conducted in compliance with international Good Laboratory Practice (GLP) regulations. However, it was conducted in accordance with the study protocol, protocol amendment and Test Facility Standard Operating Procedures (SOPs).

3 MATERIALS AND METHODS

Six male rats were assigned to 2 groups of 3 rats each. The study design is summarized in the Text Table 1.

Text Table 1 Study Design

Group	Single Dose			Numbering of Animals
	Daily Dose (mg/kg)	Volume (mL/kg)	Conc. (mg/mL)	Male
1	PBS	2 mL	NA	1, 2, 3
2	60 mg/kg	2mL	NA	1, 2, 3

When the in-life phase of the study was completed, animals were necropsied and the organs were collected after whole body-perfusion with 10% NBF. The wet tissues were shipped to WuXi (Suzhou), processed, sectioned, stained with hematoxylin and eosin, and examined microscopically. One section per slide was prepared and evaluated for each organ (livers, spleens, kidneys, lungs and hearts) as per test facility SOP.

4 HISTOPATHOLOGY RESULTS

There were no noteworthy findings in the organs evaluated. The findings observed were non-specific common background changes in rats of this age and strain and not considered to be test article (treatment)-related. Mononuclear cell infiltrates in the heart of young rats are common incidental findings and most likely related to the early stages of rodent progressive cardiomyopathy which is more common in males than females (1). Another common aging change in rats is chronic progressive nephropathy, which in young rats may begin with minor histopathologic changes in the kidneys (2). Hemosiderin deposition in the spleen of rats is a common finding related to the normal turnover of erythrocytes in the spleen.

Detailed individual histopathology findings were presented in Text Table 2.

Text Table 2 Individual Histopathology Data

Group Dosage Animal No.	PBS			Nanoparticle 60 mg/kg		
	1	2	3	1	2	3
Heart	-	-	-	-	-	-
Mononuclear cell infiltration, focal						1+
Liver	-	-	-	-	-	-
Spleen						
Pigmentation (resemble hemosiderin)	-	-	-	-	1+	-
Lung	-	-	-	-	-	-
kidney	-	-	-	-	-	-
Cast, hyaline, focal					1+	

Note: -, Not remarkable; 1+, minimal; 2+, mild; 3+, moderate; 4+, marked; 5+, severe;

1 CONCLUSION

There were no pathologically significant findings in the organs evaluated. The findings observed were non-specific common background changes in rats of this age and strain and not considered to be treatment-related.

REFERENCES

- 1 Spontaneous Cardiomyopathy in Young Sprague-Dawley Rats: Evaluation of Biological and Environmental Variability: *Franck Chanut, Carie Kimbrough, Rick Hailey, et al. Toxicologic Pathology Vol 41, Issue 8, pp. 1126 - 1136 , 2013*
- 2 Chronic Progressive Nephropathy in Aging Rats: *Stephen W. Barthold, D.V.M., Ph.D. Toxicologic Pathology ,Vol 7, Issue 1, pp. 1 – 6, First Published January 1, 1979*