Supplementary information

Comparisons of the biodistribution and toxicological examinations after repeated intravenous administration of silver and gold nanoparticles in mice

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Table S1. Classical studies of biodistribution and toxicity of AuNPs/AgNPs in rodents via other exposure routes.

NPs	Size (nm)	Dose	Model	Exposure duration	Tissue accumulation (in order of quantity)	Conclusions	References
AgNPs	20, 15	90 mg kg^{-1}	Rats	Oral, 28 d Re 1, 8, 56 d	St, In, Sp, Te, Li, Br, Ki, Lu, Bl, Bla, He	Oral exposure to AgNPs showed similar distribution pattern with silver salts, and no hepatotoxicity or immunotoxicity were observed	1
AgNPs	10, 25	100, 500 mg kg^{-1}	Rats	Oral, 28 d Re 1, 2, 4 m	Sp, Ov, Li, Ki, Te, Br, Bl,	Tissue clearance of the accumulated NPs over 4 recovery months, which size did not affect the silver distribution.	2
AgNPs	60	30, 300, 1000 mg kg^{-1}	Rats	Oral, 28 d Re 1 d	St, Ki, Li, Lu, Te, Br, Bl,	Gender-related and dose-dependent accumulation of silver content in tissues	3
AgNPs Ag-acetate	14	63, 126, 252 mg kg^{-1}	Rats	Oral, 28 d Re 1 d	NM	No adverse effects were observed in rats after exposure to 14 nm AgNPs at a dose up to 252 mg kg^{-1}	4
AgNPs (PVP)	14 ± 4	252 mg kg^{-1}	Rats	Oral, 28 d Re 1 d	Fe, In, Ki, Li, Br, Lu, Pl, Mu, Ur	Compared to AgNPs, silver acetate show higher concentrations throughout the rat body and lysosomes could be a likely target for side effects of silver.	5
AgNPs	7.9 ± 0.95	1, 10 mg kg^{-1}	Rats	Oral, once Re 1, 4 d	Fe, Li, Bl, Ki, Lu, Ur,	Bioavailability of AgNPs was extreme low after oral exposure, suggesting limited toxicity.	6
AgNPs	20, 110	0.3, 3, 30 mg kg^{-1}	Mice	Oral, 3 d Re 2 d	Fe, GIT, Sp, Li, Ki,	Oral administrational AgNPs at a dose of 30 mg kg^{-1} was well-tolerated in mice and NPs size or coating show minimal effects on fecal elimination	7
AgNPs Ag-acetate	10, 75, 110	9, 18, 36 mg kg^{-1}	Rats	Oral, 13 w Re 0.5 d	MLN, Co, Je, Il, Ki, Sp, Li, Bl, He, Ut	AgNPs predominantly deposited within cells, while Ag-acetate had an affinity for extracellular membranes	8
AgNPs	18	0.7×10^6, 1.4×10^6, 2.9×10^6 particles/cm^3	Rats	Ih, 6 h/d, 90 d	NM	Lung function changes such as the decreases in minute volume and tidal volume as well as lung inflammation were induced by prolonged inhalation exposure to AgNPs	9

AgNPs	18-19	0.6×10^6, 1.4×10^6, 3.0×10^6 particles/cm^3	Rats	Ih, 5 h/d, 5 d/w, 13 w	Lu, OB, Li, Br, Ki, Bl	Inhalation exposure of AgNPs caused the inflammatory responses in the lung and liver.	10
AuNPs (PEGylated)	11, 21, 31	0.07-0.30 mg kg^{-1}	Rats	Ins, 1 h, 1 d	Lu, Li, Sp, Ca, Ki, He, Bl, Br	The PEGylation had no effects on the translocation of AuNPs from the lungs to the circulation.	11
AuNPs	13, 105	12.8 ± 2.42, 13.7 ± 1.32 μg/m^3	Rats	Inh, 6 h/d, 5 d, Re 1, 3, 28 d	Lu, Li, Sp, Ki, Br, Te, Bl	Small NPs had significantly higher concentrations in secondary target organs that translocated from lungs compared to large NPs	12
AgNPs	15, 410	179, 167 μg/m^3	Rats	Inh, 6 h/d, 4 d, Re 1, 7 d	Lu, Li,	Size-related silver nanoparticle distribution in respiratory tract with 410 nm NPs mainly deposited in upper airways and 15 nm NPs showed alveolar accumulation.	13
AgNPs (PVP)	70	0.2, 1 mg kg^{-1}	Rats	Ins, 1 d	NM	Instillation of 1 mg kg^{-1} AgNPs caused mild pulmonary toxicity, while no side effects were observed at a dose of 0.2 mg kg^{-1}	14
AuNPs	1.4, 5, 18, 80, 200, 2.8	0.8 ± 0.1 – 34.5 ± 2.0 μg/rat	Rats	Ins, 1, 3, 24 h	Lu, Ur, Ki, Ca, Bl, Li, Sp, Ut, He, Br,	Small AuNPs with high specific surface area (SSA) was more likely cross the air-blood barrier. While the translocation of NPs to the secondary tissues was independent with the SSA.	15
AuNPs	12.5	0.32, 1.6, 3.2 mg kg^{-1}	Mice	i.p., 8 d	Sp, Li, Ki, Lu, Br, Bl	Dose-dependent accumulation pattern of NPs and no subacute physiological damage.	16

The following abbreviations are used in the Table: NM – not mentioned, Inh – inhalation exposure, Ins – instillation exposure, i.p. – intraperitoneal injection. Re – recovery days, h – hours, d– days, w– weeks, m– months, PVP – polymer polyvinylpyrrolidone, Ov – ovary, Pl – plasma, Br – brain, He – heart, Li – liver, Lu – lung, OB – olfactory bulb, Ki – kidney, In – intestine, MLN – mesenteric lymph nodes, Co – colon, Il – ileum, Je –jejunum, Sp – spleen, GIT – gastro-intestinal tract, Fe – feces, St – stomach, Te – testes, Ut – uterus, Ur – urine, Ca – carcass, Bla – bladder, Bl – blood, Mu – muscle.

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Table S2. The visceral index of organs of Kunming mice following intravenous injection of AgNPs and/or AuNPs. D1 and D28 refer to collection of mice organ on post-injection in day 1 and day 28. These results show mean and standard deviation, n=5. *: $P < 0.05$ versus the control group.

NPs	BW (g)	Liver (mg/g)	Spleen (mg/g)	Kidney (mg/g)	Lung (mg/g)	Heart (mg/g)	Brain (mg/g)	Testis (mg/g)	Seminal vesicle (mg/g)
AgNPs (D1)	38.59±1.81	54.11±3.22	5.67±1.75	14.86±1.44	5.96±0.59	4.87±0.68	11.26±0.52	6.20±1.12*	7.59±0.84
AuNPs (D1)	39.33±3.06	46.59±6.94	4.65±1.25	14.92±1.65	6.25±1.59	5.99±1.08	10.25±0.74	5.90±0.63*	7.71±1.97
AgNPs (D28)	42.65±1.68	50.68±9.70	2.58±0.36*	14.67±3.49	6.68±1.73	5.38±0.72	9.04±0.52*	5.73±0.84*	6.20±2.13*
AuNPs (D28)	42.87±3.18	49.46±5.66	2.95±0.91*	16.18±1.59	6.91±2.61	5.43±0.69	9.62±1.68	6.23±0.94*	5.78±0.63*
Control	41.16±1.07	52.42±4.92	4.77±1.06	18.08±0.61	7.54±2.73	6.44±1.13	11.18±0.25	8.18±1.39	8.87±1.47

Table S3. Biodistribution of Ag and/or Au NPs at 1 d and 28 d after the last injection. These results show mean and standard deviation, n=5.

IOMNs	Heart (μg/g)	Liver (μg/g)	Spleen (μg/g)	Lung (μg/g)	Kidney (μg/g)	Brain (μg/g)	Stomach (μg/g)	Intestine (μg/g)	Testis (μg/g)	Seminal vesicle (μg/g)
AgNPs (D1)	5.00 ± 0.94	35.06±4.78	21.16±11.74	4.64±0.23	3.43±1.15	0.65±0.07	1.14±0.21	1.93±0.37	3.72±0.29	0.38±0.11
AuNPs (D1)	1.18±0.14	61.39 ± 19.99	12.98±1.03	1.06±0.23	5.27±0.72	0.69±0.04	0.60±0.08	0.75±0.18	0.82±0.18	0.18±0.01
AgNPs (D28)	3.85 ± 1.10	29.68±4.50	24.78±6.72	5.03±1.06	0.83±0.14	0.82±0.08	0.65±0.13	2.02±0.83	5.85±1.46	0.30±0.07
AuNPs (D28)	1.03±0.19	47.00±10.62	22.03±2.30	0.90±0.09	1.01±0.14	0.71±0.03	0.55±0.17	0.84±0.16	0.98±0.02	0.20±0.05
Control (AgNPs)	ND	ND	ND	ND	0.01±0.003	ND	ND	ND	ND	ND
Control (AuNPs)	0.04±0.02	0.02±0.03	0.04±0.03	0.031±0.002	0.015±0.0003	0.02±0.0001	0.016±0.0002	0.02±0.0005	0.02±0.005	0.03±0.0017

ND: not detected

Table S4. Recovery ratios of total applied doses of AgNPs and/or AuNPs in both time points. These results show mean and standard deviation, n=5.

	Recovery Ratios of applied doses
AgNPs (D1)	35.87± 9.94%
AuNPs (D1)	31.17± 9.67%
AgNPs (D28)	42.54± 18.87%
AuNPs (D28)	27.78± 6.13%

Table S5. The primes used in qPCR.

Genes	Descriptions	Forward primers (5'—3')	Reverse primes (5'—3')
Bax	Bcl2-associated X protein (Bax)	GATGGCAACTTCAACTGGG	CCGAAGTAGGAGAGGAGGC
Bcl-2	*Bcl-2*	CACTCGACCTTGTTTCTTCCAG	TCCTAACCCCTTGCTCTGCTT
Caspase-8	*Caspase-8*	GGAAGATGACTTGAGCCTGCTTG	CAAGGCTCATTCTTCTCTCTGTGC
Caspase-3	*Caspase-3*	GGAGGCTGACTTCCTGTATGCTT	CCTGTTAACGCGAGTGAGAATG
Caspase-9	*Caspase-9*	AAGAAGACCGGAGTGCAATG	CATGACAGGATTATACAACCGC
p53	*p53*	TACAAGAAGTCACAGCAC	GATACTCGGGATACAAAT
Mt2	Metallothionein 2	TGCATCTGCAAAGAGGCTTC	AAGTTGTGGAGAACGGGTCAG
Mt1	Metallothionein 1	CTGCTGCTCCTGCTGTC	ACTGTATAGGAAGACGCTGG
Zip14	Zrt- and Irt-related protein 14	CATTGAAGTATGGGGGTACGGT	ATGAAGTAGAGCAGGAGCCTCT
Trf	Transferrin	CCGAACAACAAAGAGGAAT	GGTTCTTTCCTTCGGTGTT
Hmox	Heme oxygenase 1	ACCGCCTTCCTGCTCAAC	GAGGAGCGGTGTCTGGGAT
Sod 1	superoxide dismutase 1	TAACTGAAGGCCAGCATGGGT	GGTCTCCAACATGCCTCTCTTC
Sod 2	superoxide dismutase 2	CAGACCTGCCTTACGACTATGG	GCTGAAGAGCGACCTGAGTTGT
Fos	V-fos FBJ murine osteosarcoma viral oncogene homolog	GAAGGGAAAGGAATAAGATGGC	AGTTGGTCTGTCTCCGCTTG
GADPH	Glyceraldehyde-3-phosphate dehydrogenase	ACCCAGAGGACTGTGGATGG	TCAGCTCTGGGATGACCTTG

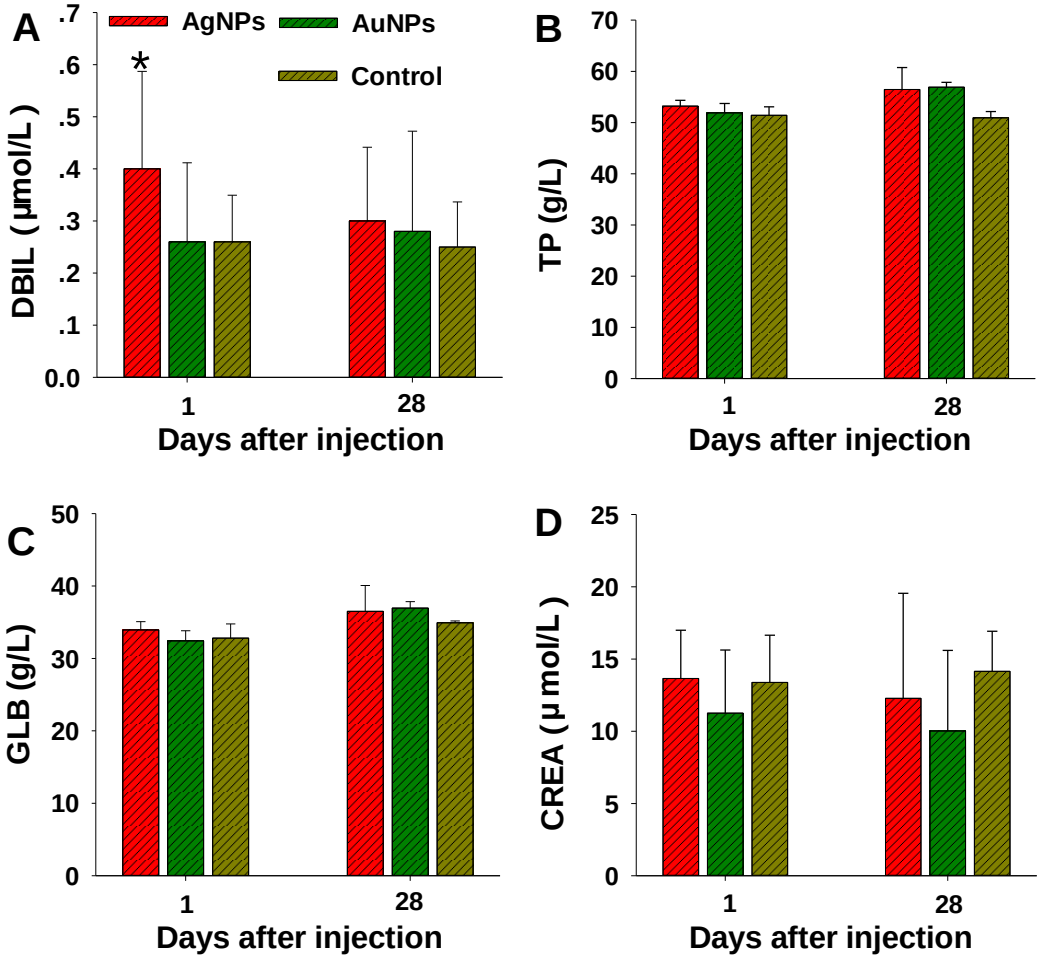


Fig. S1 Serum biochemical analysis from animals treated with AgNPs and/or AuNPs and control. A-D) results exhibit mean and standard deviation of DBIL (A), TP (B), GLB (C), CREA (D). Abbreviations: direct bilirubin, DBIL; total protein, TP; globulin, GLB; creatinine, CRE. n=5. *: $P < 0.05$ versus the control group.

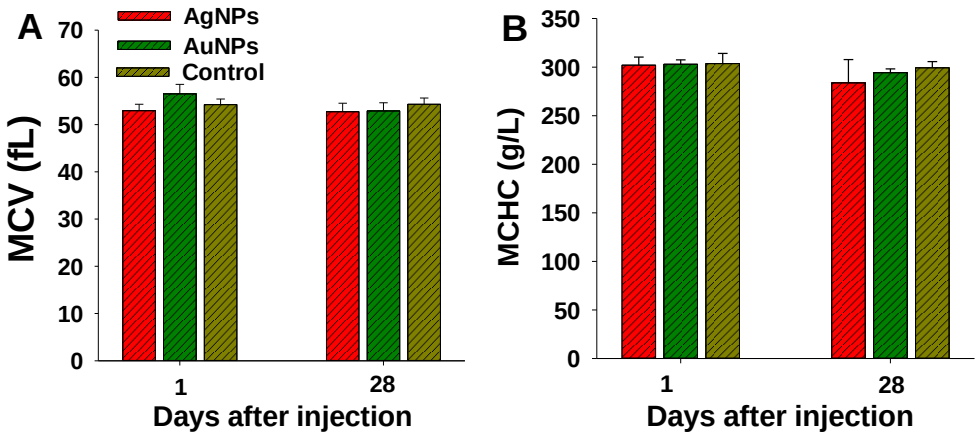
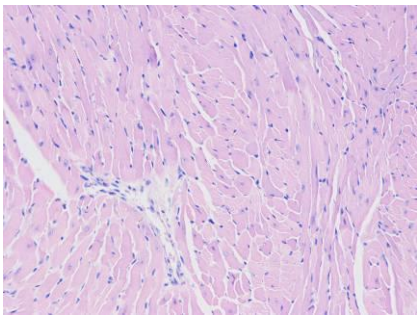
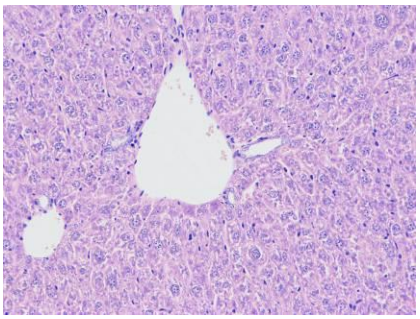


Fig. S2 Whole blood analysis from animals treated with AgNPs and/or AuNPs and control. A,B results are based on mean and standard deviation of mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), respectively. These indicators are not significant changes ($p < 0.05$), n=5.

Heart Liver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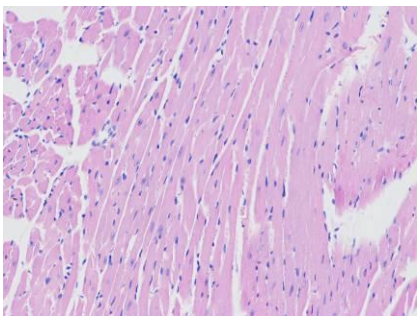
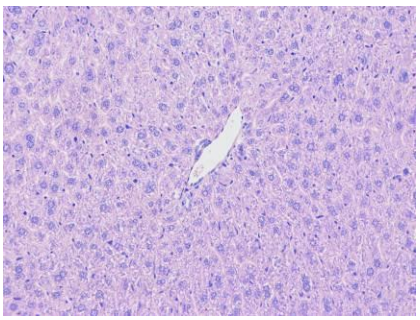
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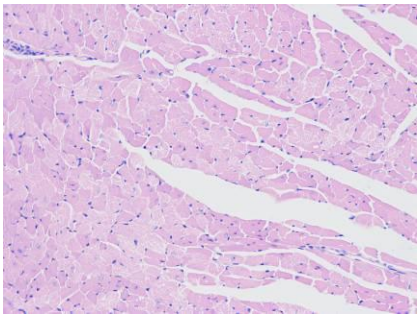
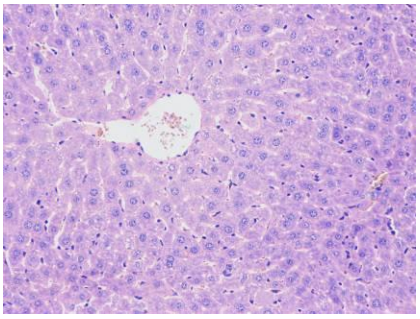
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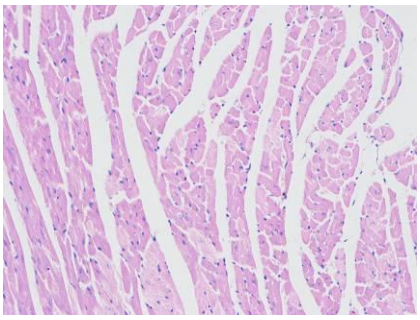
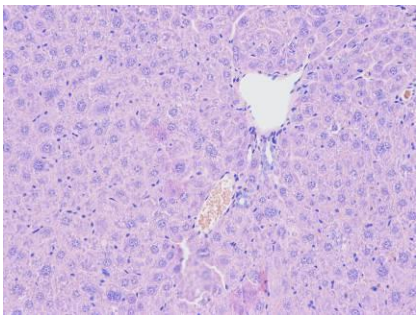
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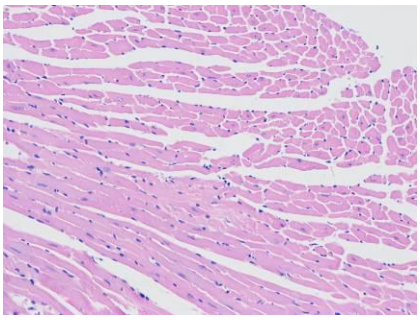
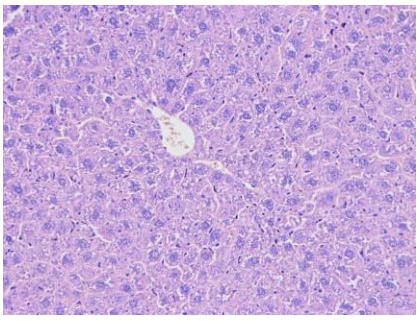
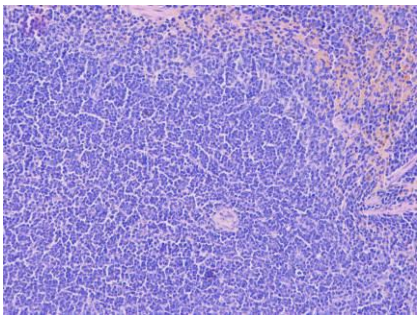
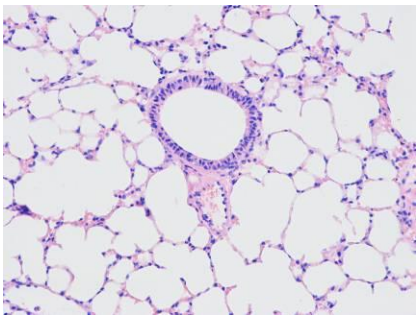


AgNPs (D28)

Spleen Lungs

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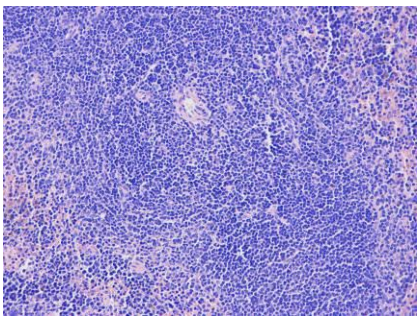
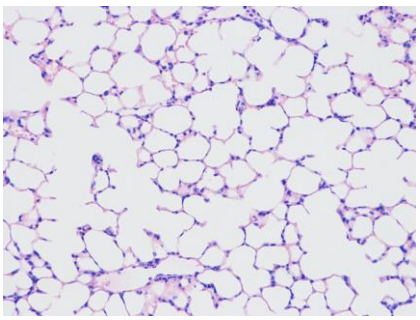

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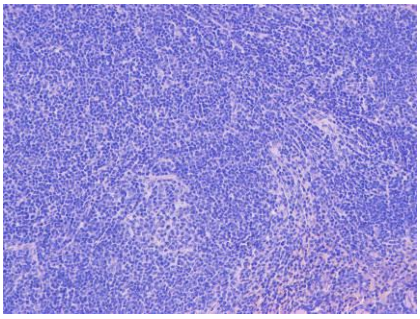
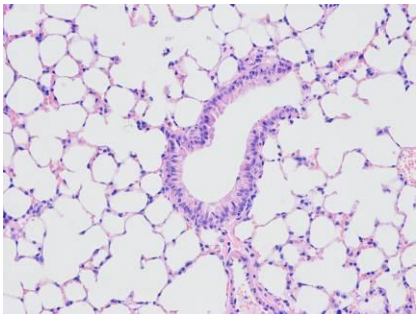

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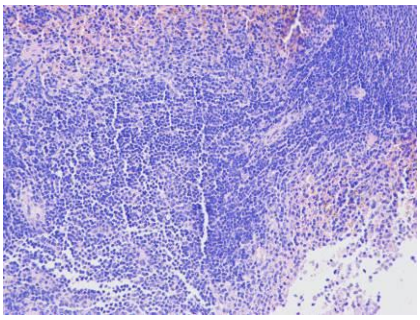
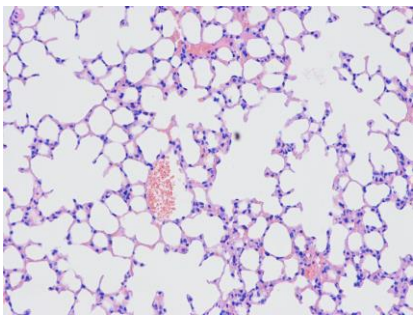

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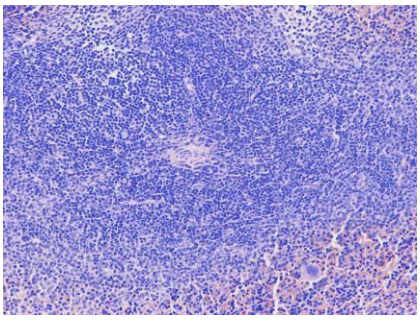
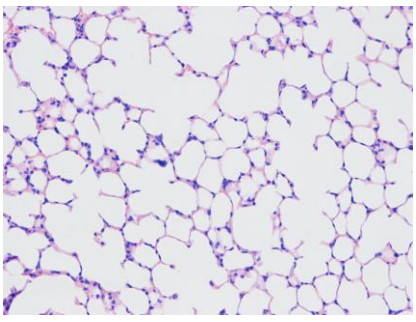
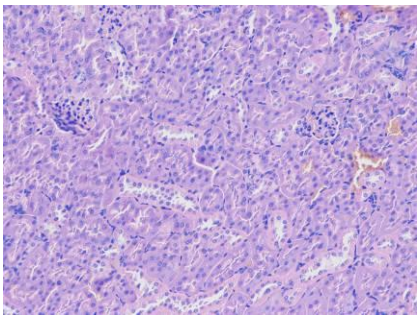
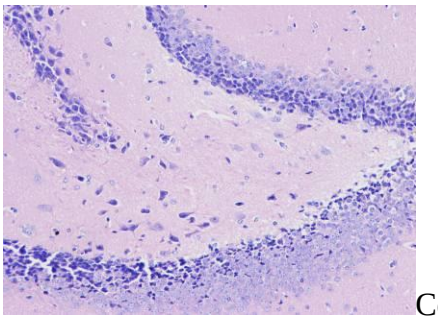



AuNPs (D28)

Kidney Brain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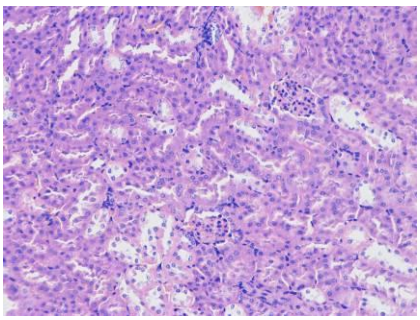
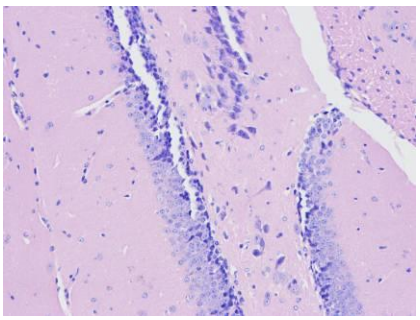
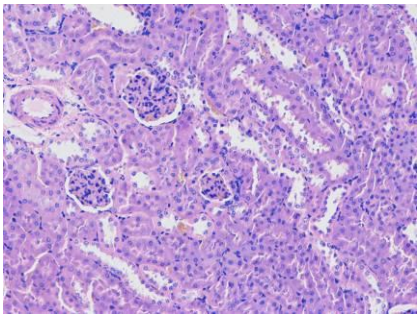
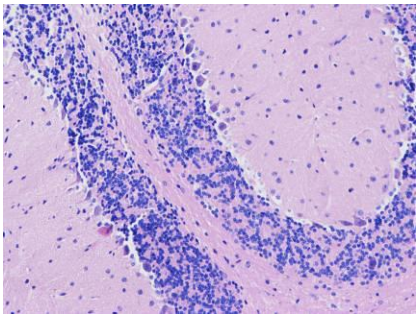
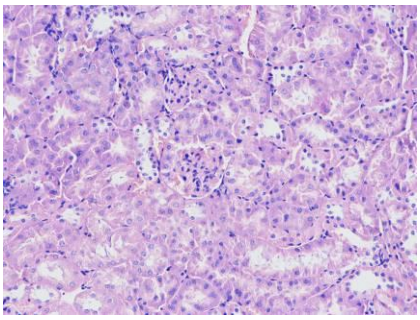
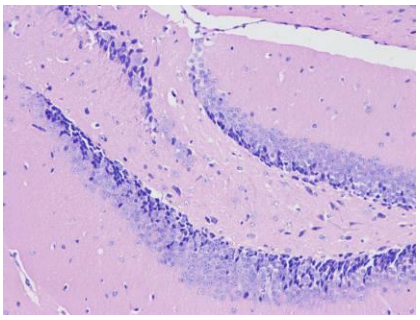
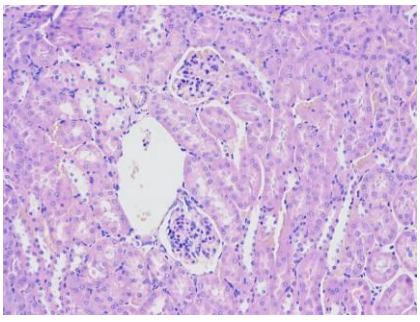
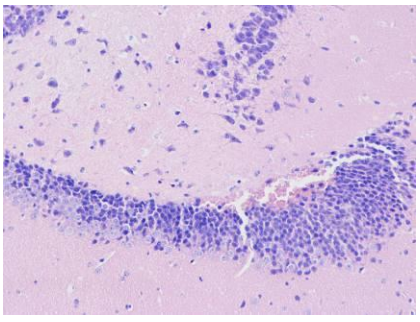


AgNPs (D1)



AuNPs (D1)



AgNPs (D28)



AuNPs (D28)

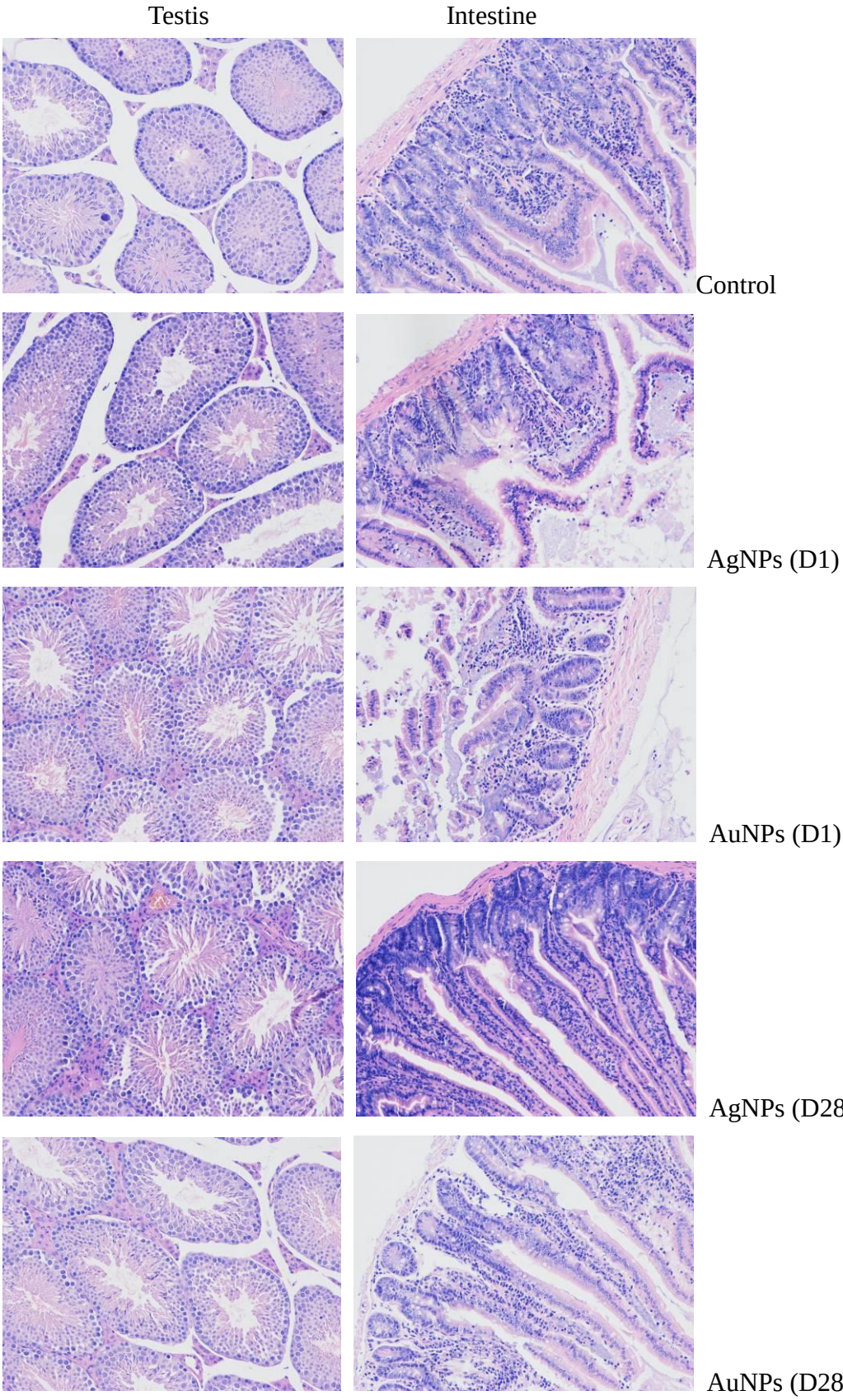


Fig. S3 HE images of various organs of treated AgNPs and/or AuNPs mice. D1 and D28 refer to collection of mice organ on day 1 and day 28 after AgNPs and/or AuNPs administrations, respectively. n=5.