Supplementary Information

Comparing Semiconductor Nanocrystal Toxicity in Pregnant Mice and Non-Human Primates

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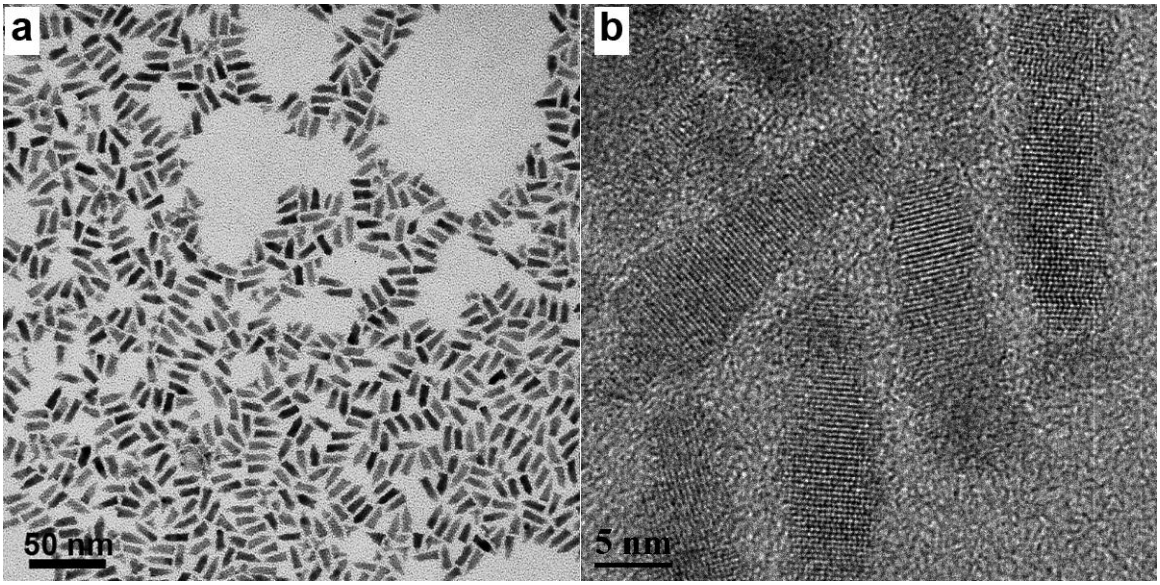

Figure S1 TEM images of the quantum rod nanocrystals. **(a)** Rod shaped semiconductor nanocrystal at a low resolution and **(b)** high resolution TEM image of the nanocrystals showing fringes.

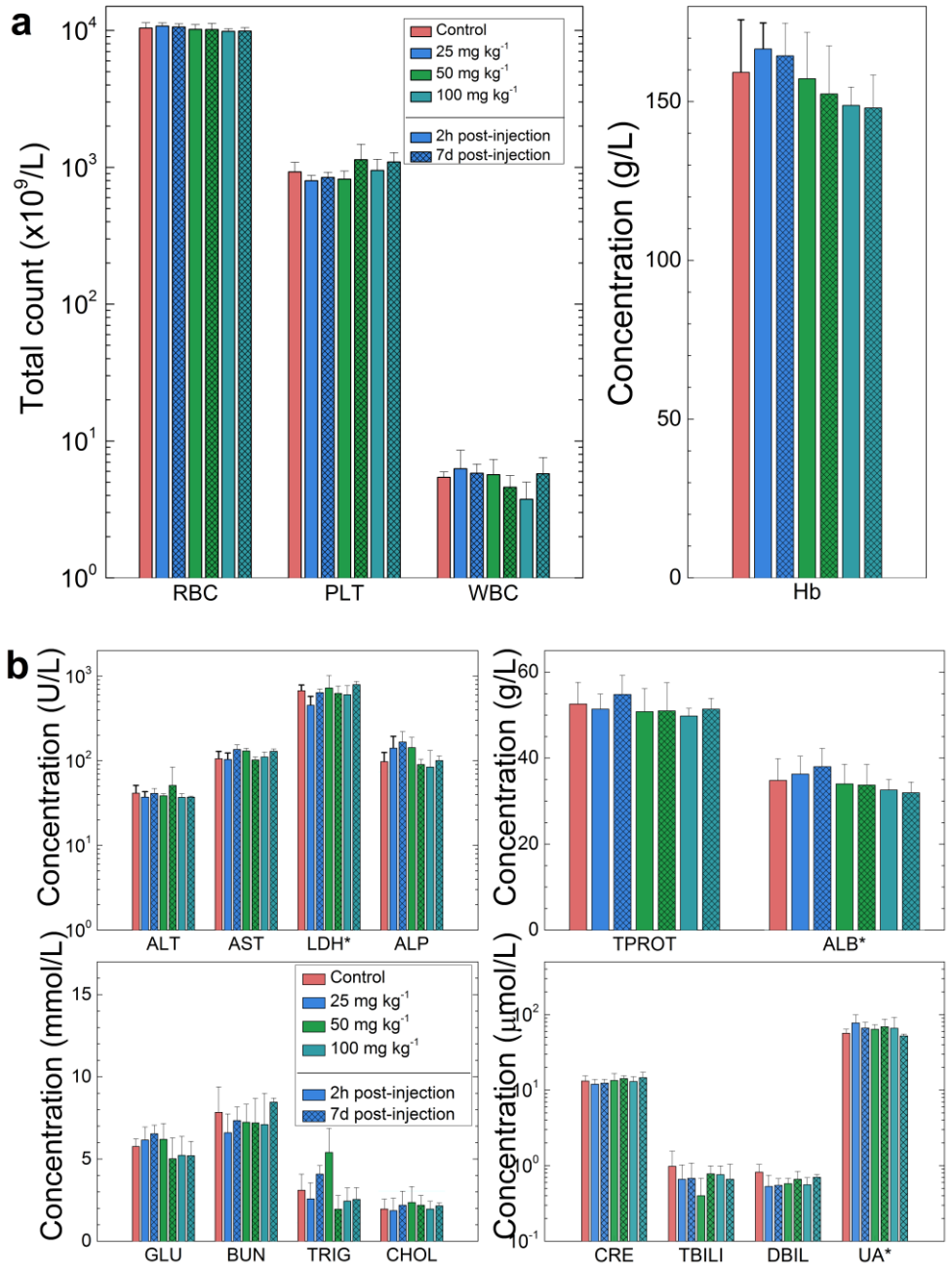

Figure S2. Characterization of the maternal health of the semiconductor nanoparticles treated mice. Routine blood count **(a)** and serum biochemistry assays **(b)** of the mice 2 h and 7 days after semiconductor nanoparticle injection at dosages of 25, 50 and 100 mg kg^{-1} on E12 (n = 5), in comparison with control group (n = 5). Comparable results were observed between the semiconductor nanoparticles treated and non-treated mice. Abbreviations: red blood cell count, RBC; platelet count, PLT; white blood cell count, WBC; neutrophil granulocyte, NE; lymphocyte, LY; monocyte, MO; alanine transaminase, ALT; aspartate transaminase, AST; lactate dehydrogenase, LDH; alkaline phosphatase, ALP; total protein, TPROT; albumin, ALB; blood glucose, GLU; blood urea nitrogen, BUN; triglyceride, TRIG; total cholesterol, CHOL; creatinine, CRE; total bilirubin, TBILI; direct bilirubin, DBIL; uric acid, UA. Bars indicate ±SD, no statistically significant differences were recorded ($P < 0.05$) between the control and QD-treated groups, n=5.

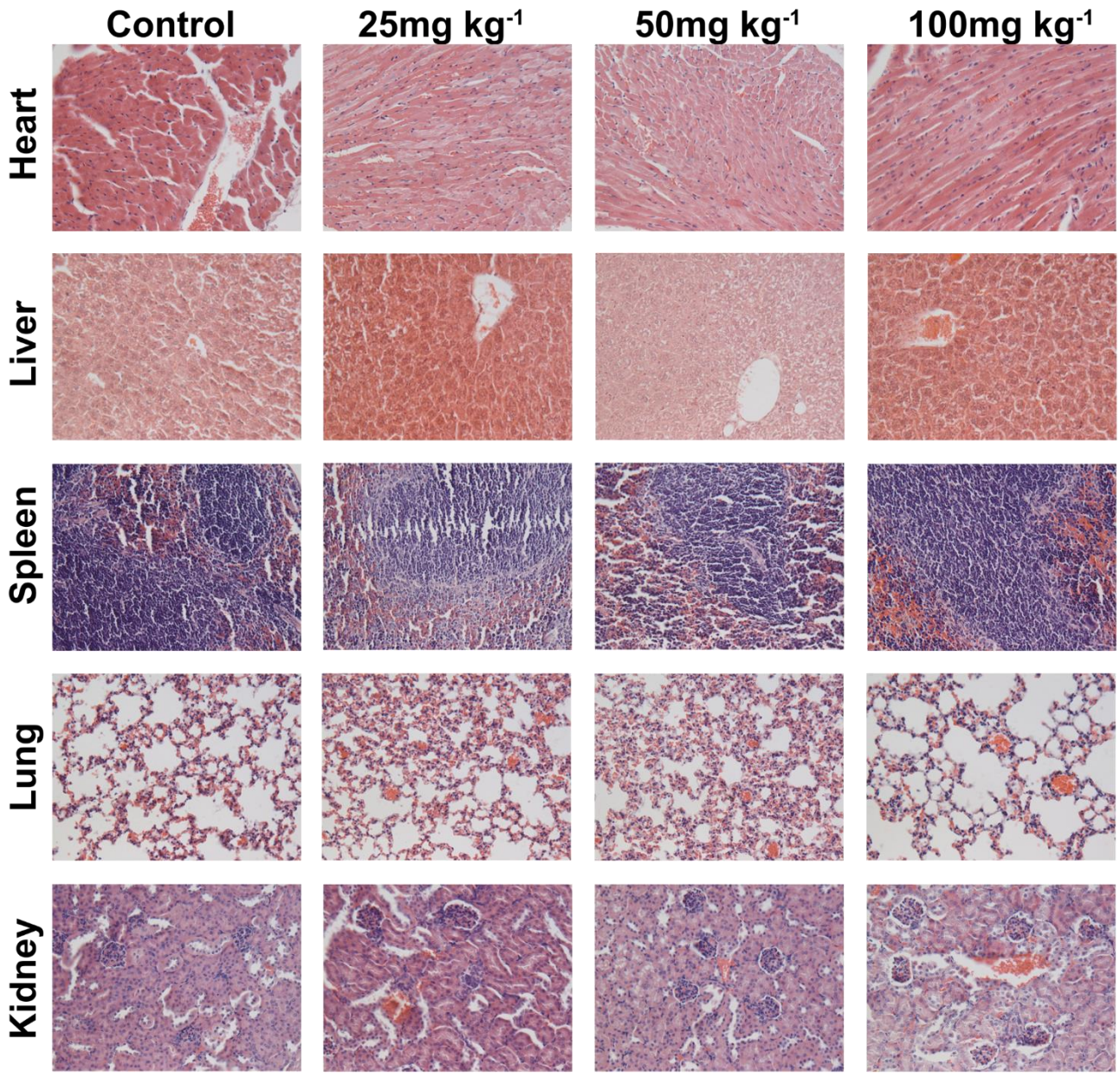

Figure S3. Histological sections of the major organs (heart, liver, spleen, lung and kidney) of mice 7 days after semiconductor nanoparticle injection at dosages of 25, 50 and 100 mg kg^{-1} on E12, in comparison with the control.

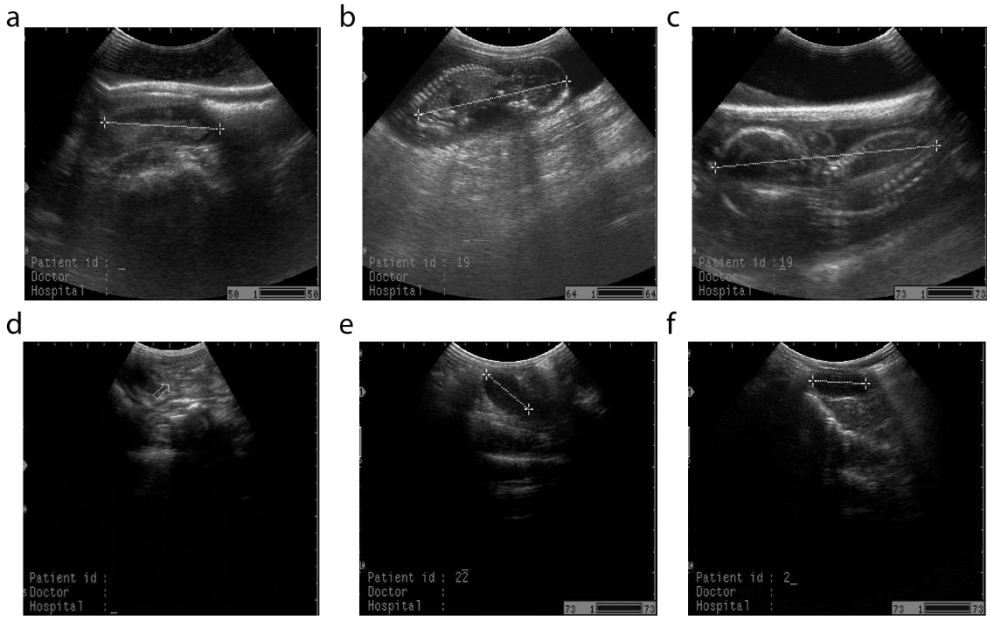

Figure S4. Ultrasound images of treated macaca fascicularis. **(a)** Gestational sac at 4 weeks after conception with a lateral length of 5.56 mm. **(b)** Fetus at 8 weeks with a crown-rump length of 7.32 cm. **(c)** Fetus at 14 weeks with a crown-rump length of 10.7 cm (the week of nanoparticle injection). **(d)** Image of intrauterine fetal demise at 8 weeks after nanoparticle injection (22 weeks after conception). **(e)** Image of uterus with lateral length of 2.60 cm for control animal after delivery of offspring. **(f)** Image of uterus with lateral length of 2.53 cm for treated animal after the occurrence of miscarriage.

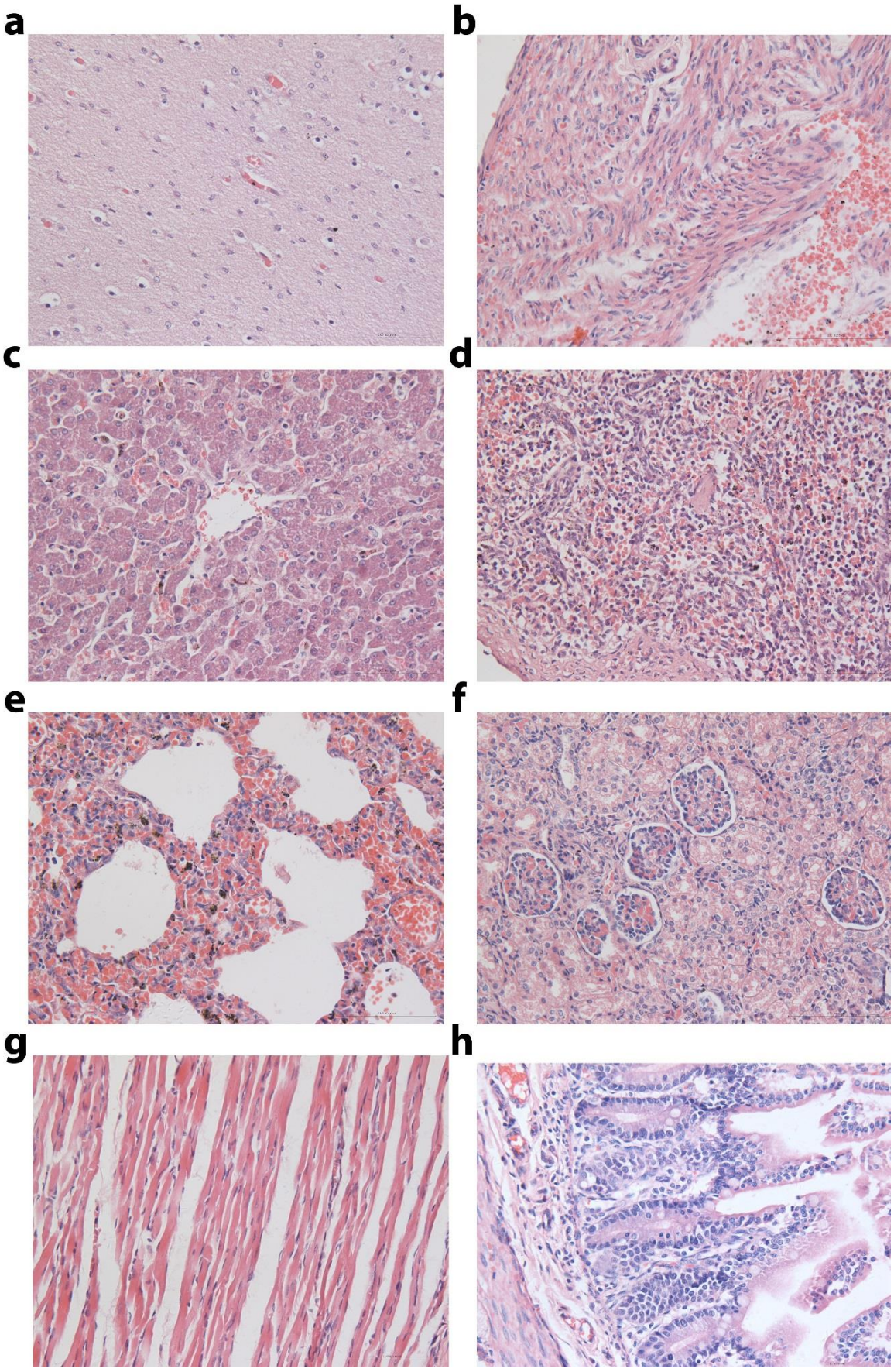

Figure S5. Histological sections of the major organs of miscarriage fetus of nanoparticles treated

animal #2. Tissues were harvested from **(a)** brain, **(b)** heart, **(c)** liver, **(d)** spleen, **(e)** lung, **(f)** kidney, **(g)** muscle and **(h)** intestine.

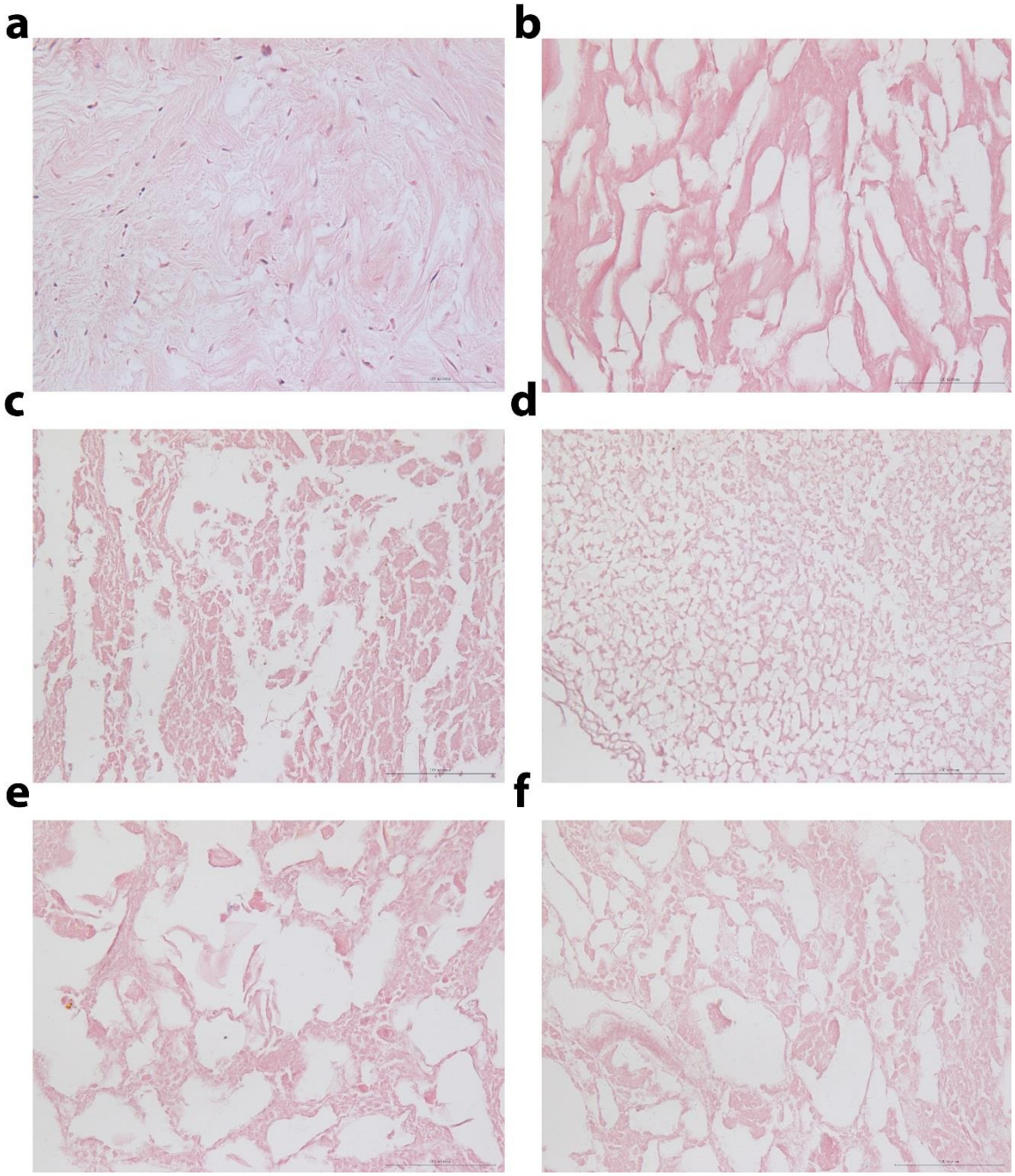

Figure S6. Histological sections of the major organs of the stillbirth fetus from the nanoparticles treated animal #15. Intrauterine fetal demise was identified from ultrasound scan 8 weeks after nanoparticles injection. Cellular self-destruction was identified for the tissue samples, which was due to the intrauterine fetal demise. Tissues were harvested from **(a)** brain, **(b)** heart, **(c)** liver, **(d)** spleen, **(e)** lung and **(f)** kidney.

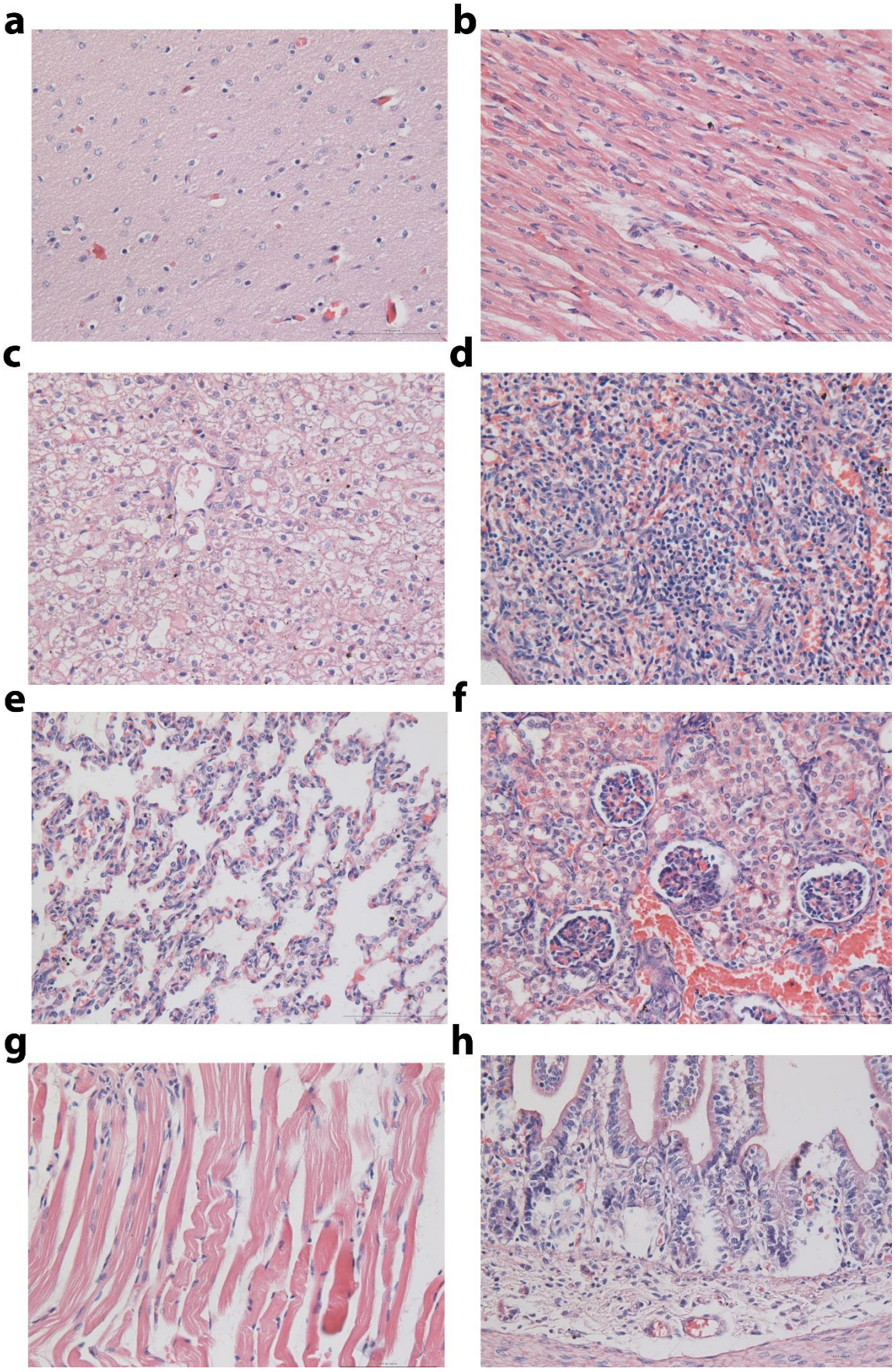

Figure S7. Histological sections of the major organs of a fetus from accidental miscarriage, collected

from the general population in the animal breeding center. Tissues were harvested from **(a)** brain, **(b)** heart, **(c)** liver, **(d)** spleen, **(e)** lung, **(f)** kidney, **(g)** muscle and **(h)** intestine.

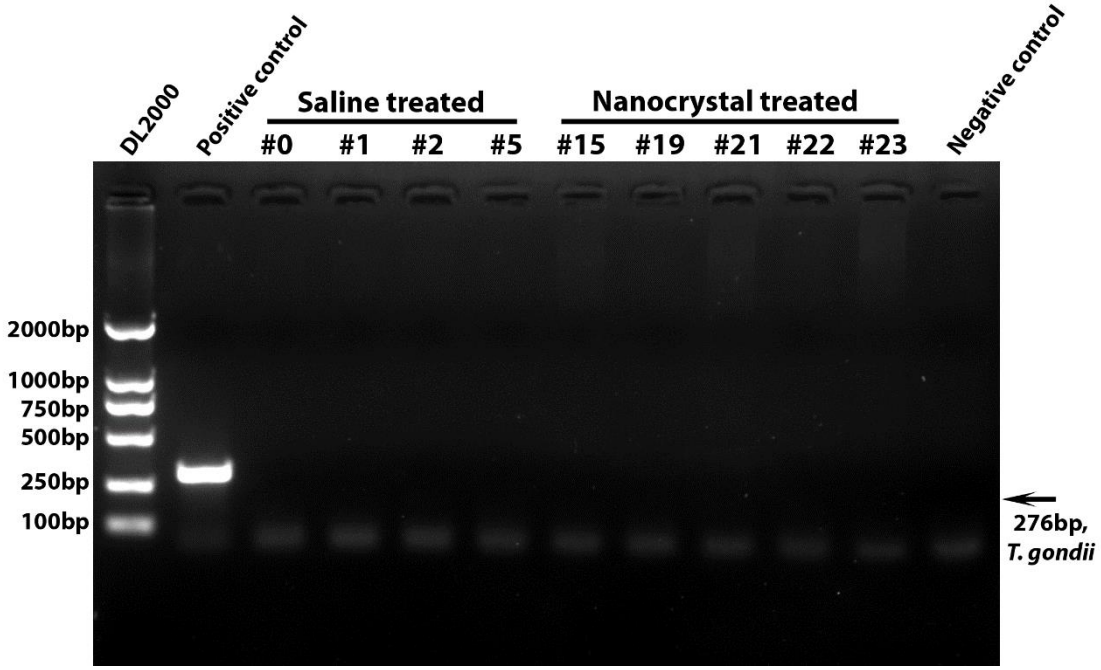

Figure S8. Agarose gel electrophoresis result shows no signal at the *Toxoplasma gondii* position of 276bp for all the monkeys in the experiments, including four saline treated and five nanocrystal treated animals, as indicated by the arrow. DL2000 represents a size marker of 2000bp. The positive control is a fragment of *Toxoplasma gondii* cloned in a plasmid. Both the positive and negative control samples are from the vendor detection kit.

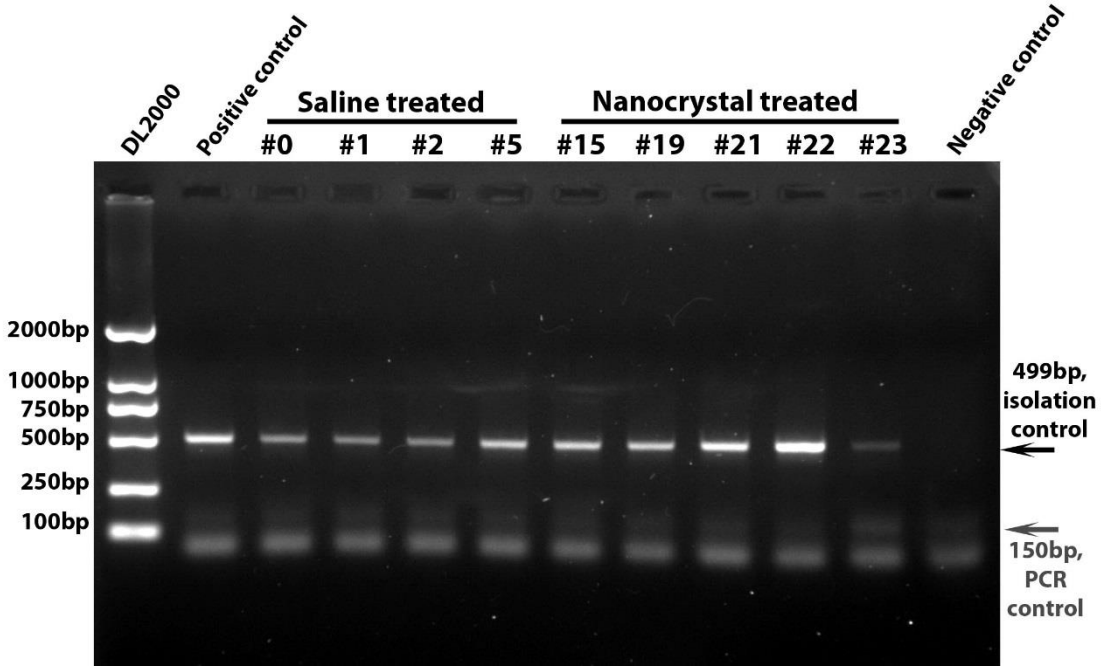

Figure S9. A control experiment for Toxoplasma gondii detection as instructed by the vendor of the detection kit. Agarose gel electrophoresis result shows clear bands at the isolation control of 499bp

and the PCR control of 150bp for all monkeys in the saline treated and nanocrystal treated groups. The band positions were indicated by the arrows and referenced by a DNA size marker DL2000. The positive control is a fragment of *Toxoplasma gondii* cloned in a plasmid. Both the positive and negative control samples are from the vendor detection kit.

Toxoplasma gondii (*T. gondii*) is a widespread intracellular protozoan parasite that can infect all warm-blooded animals[1]. It is reported that toxoplasmosis during early pregnancy can cause miscarriage, spontaneous abortion, fetal death or congenital infection in human and macaque[2-5]. In this study, blood samples from all the animals during early pregnancy were collected for toxoplasmosis analysis using a polymerase chain reaction (PCR) detection kit. The *T. gondii* DNA was isolated from the blood samples using spin-column chromatography and used as template in a PCR process for amplification and detection. As shown in Figure S8, no signals were detected at the target position of *T. gondii* (276 base-pair, indicated by the arrow). This indicates that all the animals, including four in the saline treated control group and five in the nanocrystal treated experimental group, were not infected by *T. gondii* during pregnancy. In addition, a control experiment was also carried out to identify possible PCR inhibition or inadequate isolation as instructed by the kit vendor, where the results showed successful DNA isolation and PCR process (Figure S9).

Method

Toxoplasmosis analysis. Maternal venous blood samples from all nine monkeys, including four sailing treated control group and five in the nanocrystal treated experimental group, were collected in evacuated tubes containing EDTA. Toxoplasmosis analysis were carried out using a *Toxoplasma gondii* PCR detection kit from Norgen Biotek Corporation (Canada, Product #44700) following the vendor protocol. Briefly, *Toxoplasma gondii* DNA from the venous blood samples were isolated using spin-column chromatography. The DNA samples were then used as template in a polymerase chain reaction (PCR) for amplification on a T-Gradient ThermoBlock thermocycler (Biometra GmbH, Germany). The PCR products were electrophoresed on 2% ethidium bromide-stained 1X TAE 1.7% agarose gel and observed under a Gel Doc™ XR+ imaging system (Bio-Rad).

References

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