Supporting Information for

From Cow Manure to Bioactive Carbon Dots: A Light-up Probe for Bioimaging Investigations, Glucose Detection and Potential Immunotherapy Agent for Melanoma Skin Cancer

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EXPERIMENTAL SECTION

Synthesis of PBAC-dots

15 g of fresh cow manure were dried at 120 °C and grinded into fine powder. The powder was calcined at 300 °C for 3 h and cooled to room temperature. The resulting black powder was further refluxed in nitric acid solution (5.0 M, 250 mL) for 72 h. The suspension was then cooled, filtered off to remove undissolved particles and centrifuged at 6000 rpms for 20 min. The supernatant containing the C-dots was filtered through a 0.22 μm membrane to remove the large particles. Water was then removed (by evaporation) and the C-dots (8.00 g) were obtained. For the surface functionalization (amidation), the C-dots (4.00 g) were treated with freshly distilled thionyl chloride (10 mL) at reflux temperature for 4 h. After cooling to room temperature, the excess thionyl chloride was removed under vacuum and anhydrous ethylenediamine (30 mL) was added. The mixture was allowed to react for 4 h at 115 °C. After cooling and removing the excess of diamine under vacuum, the crude solid was dissolved in water, centrifuged at 6000 rpm for 20 min and filtered through a 0.22 μm membrane affording the modified C-dots in nearly quantitative yields. 1.00 g of the amine-passivated C-dots and 5.00 g of 4-Formylphenylboronic acid (Sigma-Aldrich, San Luis, USA) were dissolved in 500 mL of methanolic solution (70%). The solution was refluxed for 24 h, using a Dean-Stark apparatus. The imine bonds were reduced with gradual addition of sodium borohydride (~10 g) and vigorous stirring. The solvent was removed under vacuum and 250 mg of the **PBAC-dots** were washed with 200 mL of dioxane (Sigma-Aldrich, San Luis, USA), redispersed in water, centrifuged at 6000 rpm for 20 min and then filtered through a 0.22 μm membrane.

Characterization

The FTIR spectra were obtained using a Jasco FT/IR-4100 spectrometer with resolution of 4 cm^{-1} and 100 cumulative scans. The transmission KBr technique was used. The pellets were prepared using 2 mg of each material and 98 mg of KBr previously dried for 4 h at 150 ^{o}C. The HRTEM images were acquired in a JEOL JEM 2010 electron microscope, operating at an acceleration voltage of 200 kV. A diluted ethanolic solution of the nanoparticles was deposited onto a 400-mesh C-coated cooper grids (Ted Pella Inc, Redding, USA), and dried in air. The XPS spectra were measured at LNLS Campinas/Brazil. The spectra were collected using an InSb (111)

double crystal monochromator at fixed photon energies of 1840 and 3000 eV. The hemispherical electron analyzer was set at a passing energy of 30 eV, and the energy step was 0.1 eV, with an acquisition time of 100 ms/point. The overall resolution was around 0.3 eV. The monochromator photon energy calibration was done at the Si K edge (1839 eV). An additional calibration of the analyzer's energy was performed using a standard Au foil (Au $4f_{7/2}$ peak at 83.8 eV). Photoluminescence spectra were performed using a Fluorolog 3 ISA/Jobin-Yvon spectrofluorometer (Horiba, Kyoto, Japan) equipped with Hamamatsu R928P photomultiplier and a 450 W Xe arc lamp. All spectra were corrected for the instrumental function.

Cell Culture

Murine melanoma (B16F10) and fibroblast (NIH3T3) cells were obtained from American Type Culture Collection (ATCC, Rockville, USA). NIH3T3 and B16F10 cells were cultured in DMEM (Gibco, New York, USA), supplemented with 10% (v:v) fetal bovine serum (Gibco, New York, USA), 1% (v:v) PenStrep antibiotic (100 μg/ml penicillin and 100 μg/ml streptomycin; Gibco, New York, USA) and buffered with sodium bicarbonate (Sigma-Aldrich, San Luis, USA). Both cells lineages were maintained in a 5% CO_2 humidified atmosphere at 37ºC.

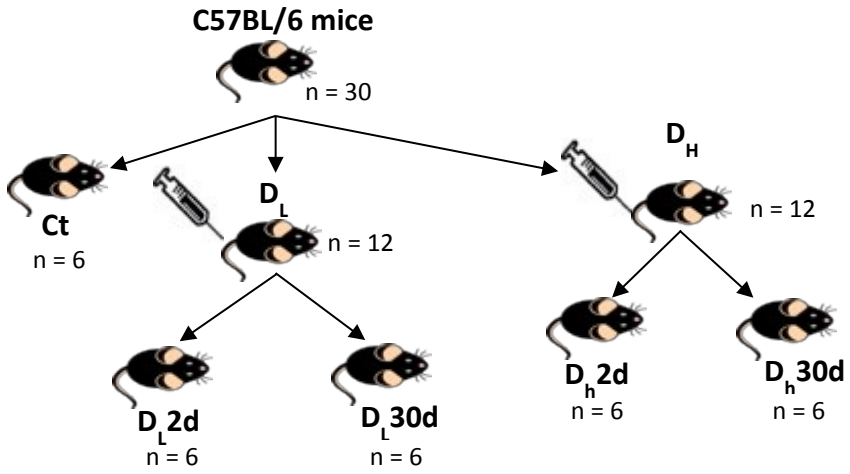
***In Vitro* Cytotoxicity Studies**

In order to evaluate cytotoxicity of **PBAC-dots**, MTT (3,4,5-dimethylthiazol-2,5 biphenyl tetrazolium bromide) assays were performed. Tests were done using **PBAC-dots** at different concentrations (0.01, 0.05 and 0.1 mg mL^{-1}) and control (PBS) for up to 24 h. Each experiment was repeated three times with a minimum of five replicates.

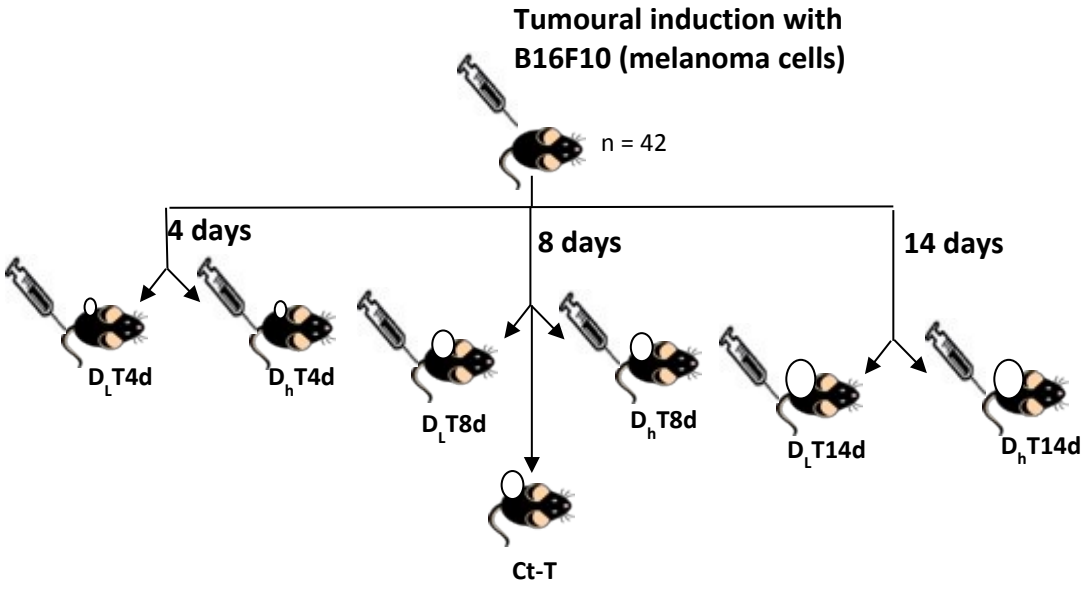
***In Vivo* Experimental Design**

All procedures with animals were performed according to the Ethics Committee in Animal Use of the University of Brasilia. Female C57BL/6 mice with 12 weeks old were divided into two major groups: healthy mice (n=30) and mice with melanoma (n=42). Animals without melanoma were divided into 5 groups (each with n=6, Scheme S1): Control group (Ct) with untreated mice euthanized after 30 days, and experimental groups with mice that received **PBAC-dots** at 0.16 mg mL^{-1} (D_L) or 0.31 mg mL^{-1} (D_h) and observed for 2 days (D_L2d and D_h2d) or 30 days (D_L30d and D_h30d).

Animals with melanoma were divided into 7 groups (each with n=6, Scheme S2): Control group (Ct-T), with untreated mice observed for 2 days; and experimental groups with mice that received **PBAC-dots** at 0.16 mg mL^{-1} (D_L) or 0.31 mg mL^{-1} (D_h) after different times of tumour growth: 4 days (D_LT4d and D_hT4d), 8 days (D_LT8d and D_hT8d) and 14 days (D_LT14d and D_hT14d). The injection of the PBS solution of **PBAC-dots** (0.16 or 0.31 mg mL^{-1}, 100µL) was intravenous in tail vein.



Scheme S1. Experimental design of animals without melanoma. D_L = 0.16 mg mL^{-1}, D_h = 0.31 mg mL^{-1}. Ct: control group; D_L2d and D_h2d: animals euthanized after 2 days; D_L30d and D_h30d: animals euthanized after 30 days.



Scheme S2. Experimental design of animals with melanoma. Ct-T: control group; D_LT4d: animals that received **PBAC-dots** (0.16 mg mL^{-1}) after 4 days of melanoma growth; D_hT4d: animals that received **PBAC-dots** (0.31 mg mL^{-1}) after 4 days of melanoma growth; D_LT8d: animals that received **PBAC-dots** (0.16 mg mL^{-1}) after 8 days of melanoma growth; D_hT8d: animals that received **PBAC-dots** (0.31 mg mL^{-1}) after 8 days of melanoma growth; D_LT14d: animals that received **PBAC-dots** (0.16 mg mL^{-1}) after 14 days of melanoma growth; D_hT14d: animals that received **PBAC-dots** (0.31 mg mL^{-1}) after 14 days of melanoma growth.

Tumour Induction and Imaging

Flank tumours were prepared by subcutaneous injection of $3.25x10^5$ B16F10 cells suspended in serum-free medium into 4 – 6-week-old C57BL/6 female mice. After 4, 8 and 14 days of tumour inoculation, **PBAC-dots** solutions (0.16 and 0.31 mg/mL) were injected by intravenous route. Biodistribution *in vivo* and *ex vivo* of nanoparticles were visualized by Lumina XR Series III® (Lumina- Perkin Elmer, Waltham, USA), with the most appropriated filter set for **PBAC-dots** (excitation at 420 nm and emission at 520 nm). Images were made right after injection, 2, 4, 6, 24, 48 h (D_L2d, D_h2d, Ct-T, D_LT4d, D_hT4d, D_LT8d, D_hT8d, D_LT14d and D_hT14d groups) and 30 days (Ct, D_L30d, D_h30d groups) after injection of the **PBAC-dots**. *Ex vivo* images were made after the last *in vivo* image was performed (2 or 30 days) of the tissues collected. The tissues were placed in 4% paraformaldehyde (Sigma-Aldrich, San Luis, USA).

Evaluation of *In Vivo* Clinical Parameters for Both Concentrations of PBAC-dots

We studied the variation of body weight, food and water intake of Ct, D_L30d and D_h30d groups at days 1, 4, 7, 11, 14, 18, 21, 24, 27 and 30 after **PBAC-dot** injection. In addition, blood and serum samples were analysed for the same groups and for D_L2d and D_h2d groups, after the observing time. Analyses of blood samples were performed by veterinary hematological analyser Sysmex pocH-100iV Diff TM (Sysmex, Kobe, Japan) for the following parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, W-SCR, W-MCR, W-LCR, W-SCC, W-MCC, and W-LCC. For serum specimens, analyses were done by ChemWell-T equipment (LabTest, Lagoa Santa Brazil) for ALT (ALT/GTP Liquiform), AST (AST/GOT Liquiform), creatinine (K Creatinine), LDH (LDH Liquiform), alkaline phosphatase (Alkaline Phosphatase Liquiform) and urea (Urea UV Liquiform) with support from the Sabin Institute.

Analysis of PBAC-dot toxicity in collected tissue

After injection of **PBAC-dots** and the observing time, different tissues were collected (brain, blood, kidney, liver, lung, spleen and tumour) to evaluate the toxicity of the nanoparticles. Briefly, the tissues were fixated in 4% paraformaldehyde for 3 h at room temperature and processed for classical histology. Sections of 3μm were obtained from the tissues in a microtome (Leica, Wetzlar, Germany) and stained with Hematoxylin and Eosin to be analysed at a AxiosKop 2 (Zeiss, Oberkochen, Germany) light microscope.

Statistical Analysis

Anova one-way test with Tukey *post-hoc* and Kruskal-Wallis test with Dunn *post-hoc* were used to evaluate the significance of the data. To be considered significant, it was used level of significance $p < 0.005$. Data are presented as mean ± SE.

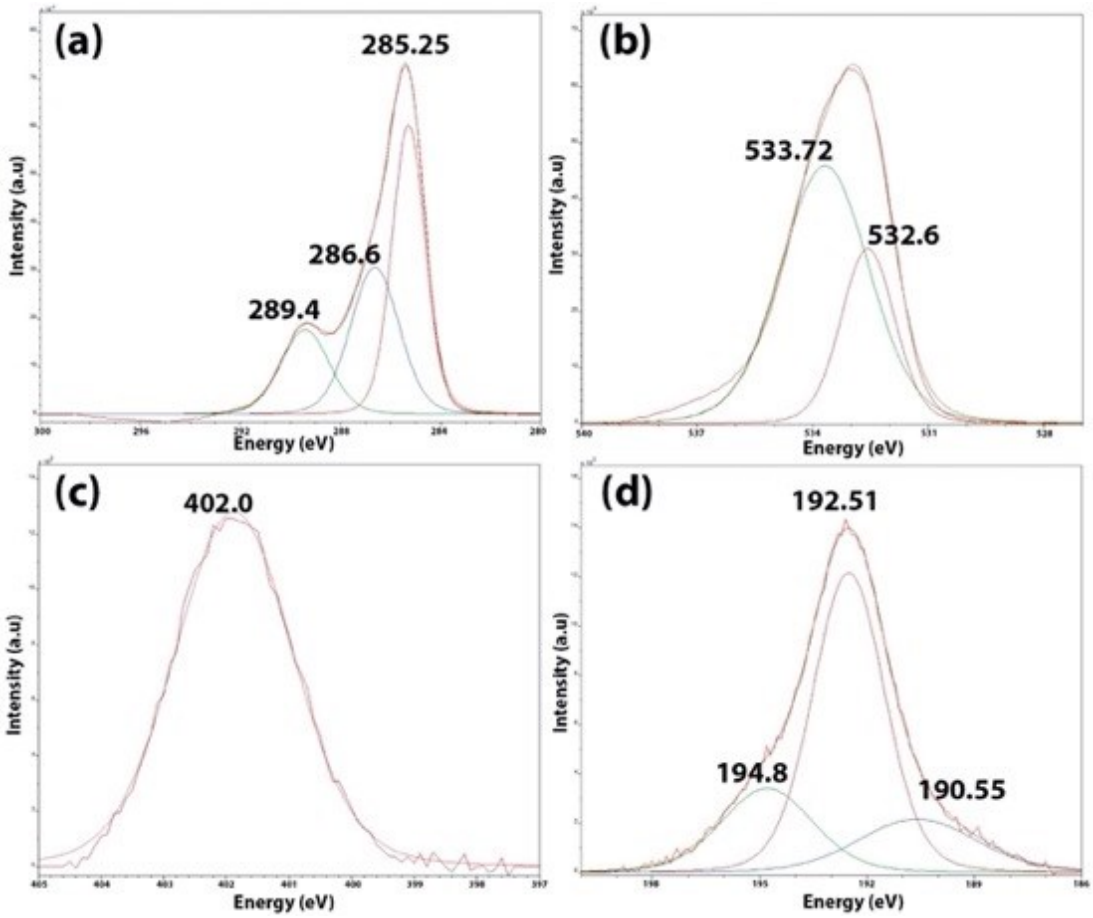


Figure S1. High-resolution XPS spectra of **PBAC-dots** (a) C1s; (b) O1s; (c) N1s and (d) B1s.

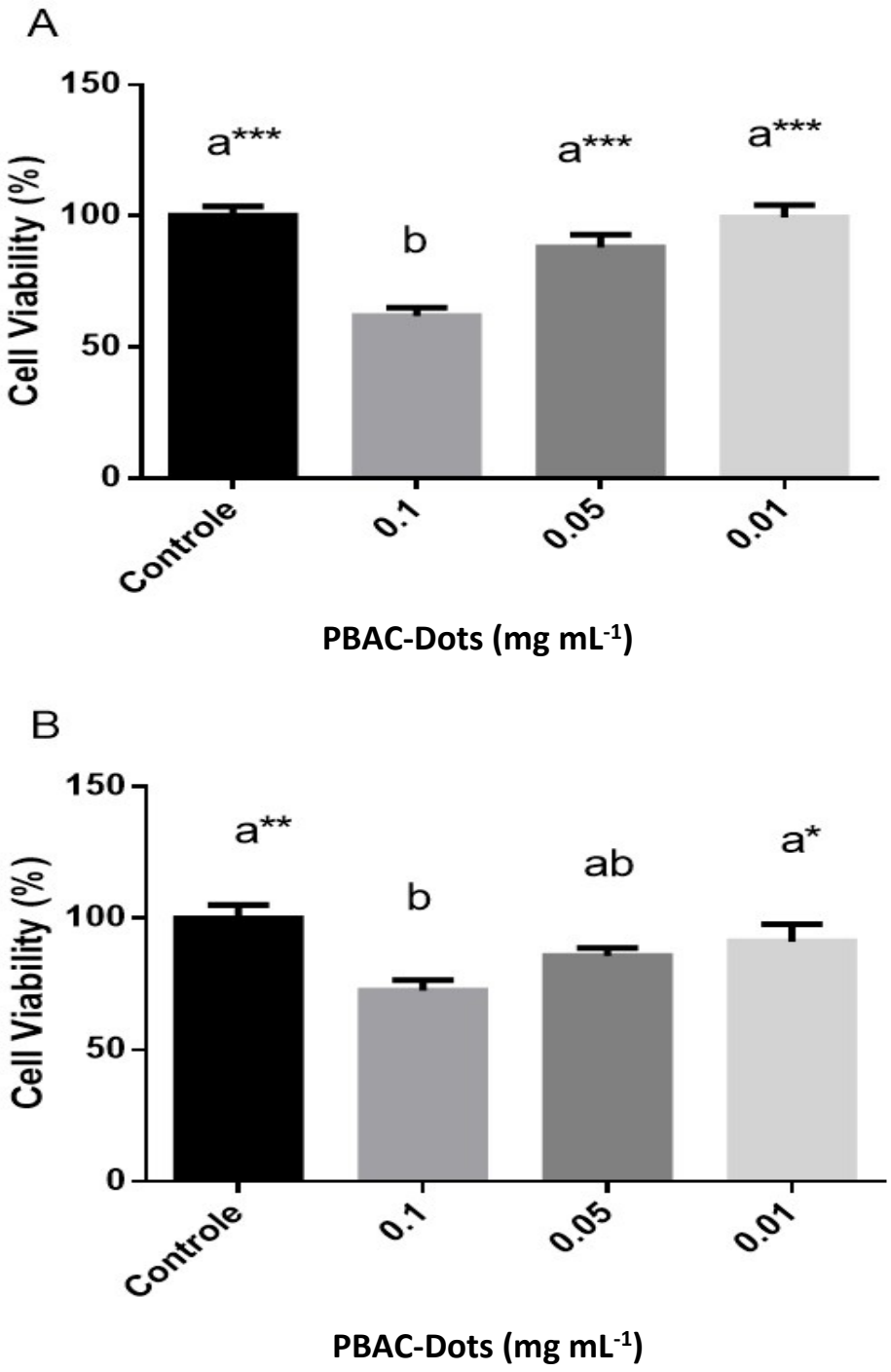


Figure S2. Cell viability of murine fibroblast - NIH/3T3 (**A**) and murine melanoma cells - B16F10 (**B**) after 24 h of exposure to 3 concentrations of **PBAC-dots**: 0.01; 0.05 and 0.1 mg mL^{-1}, evaluated by MTT method. Different letters mean different statistics. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.005$.

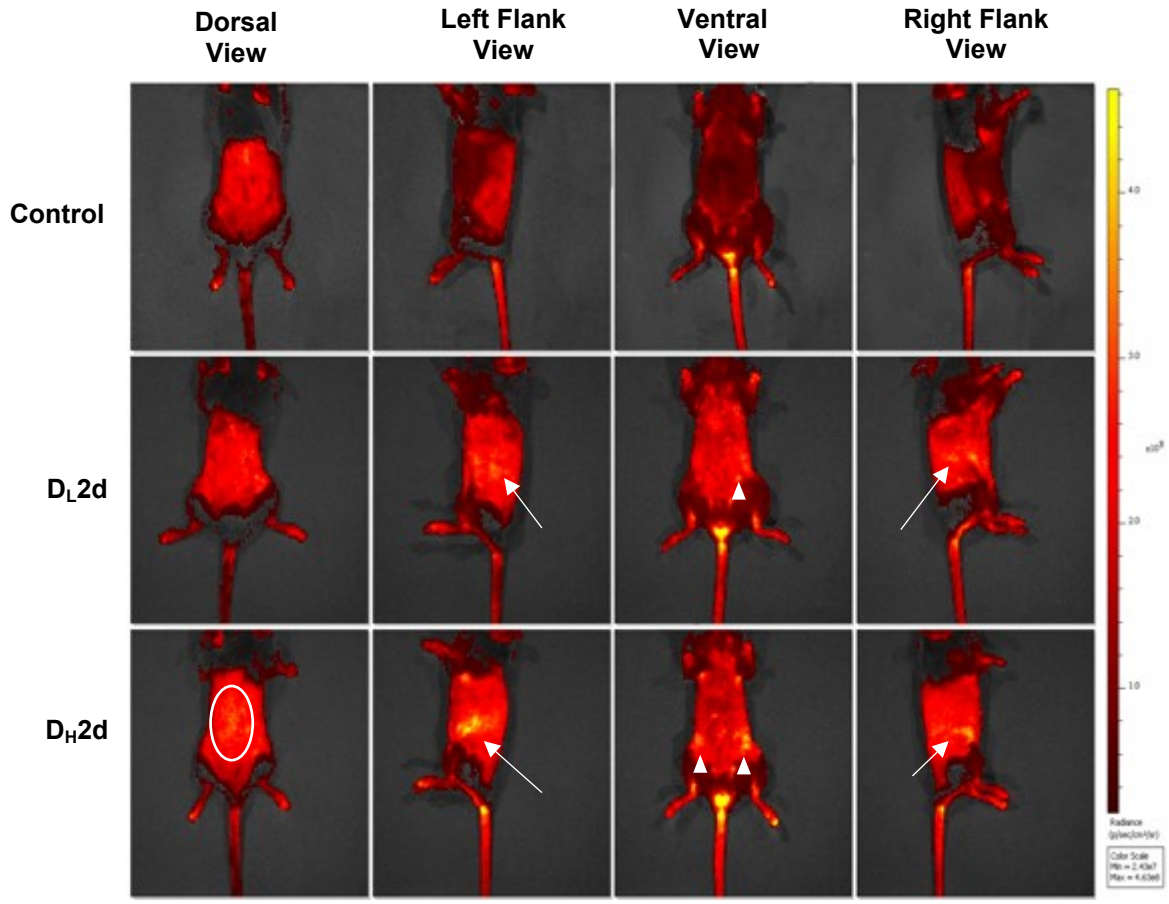


Figure S3. Fluorescence bioimages of C57BL/6 mice right after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_L2d:** non-tumour mice that were injected with 0.16 mg mL^{-1}solution of **PBAC-dots** and euthanized after 2 days. **D_H2d:** non-tumour that were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

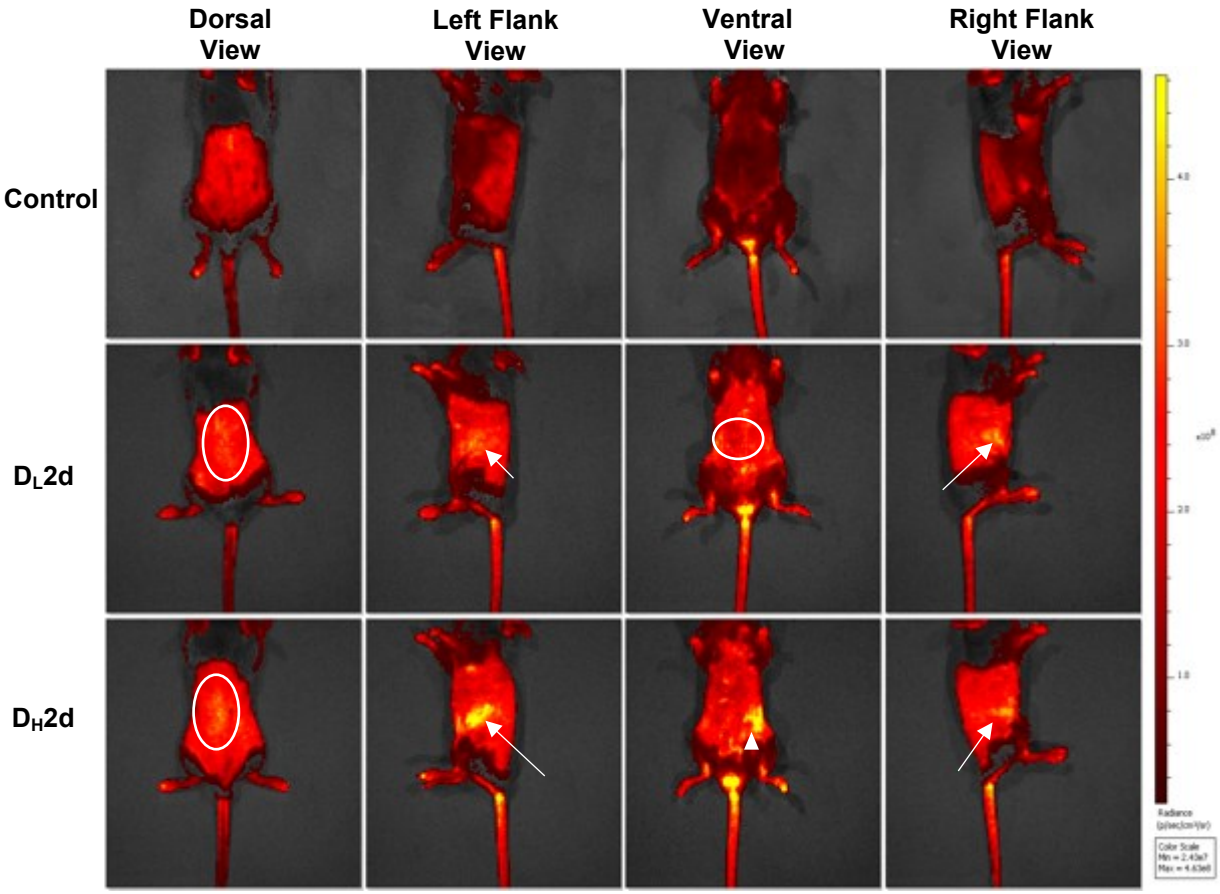


Figure S4. Fluorescence bioimages of C57BL/6 mice 2 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_L2d:** non-tumour mice that were injected with 0.16 mg mL^{-1}solution of **PBAC-dots** and euthanized after 2 days. **D_H2d:** non-tumour mice that were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on ventral and dorsal regions.

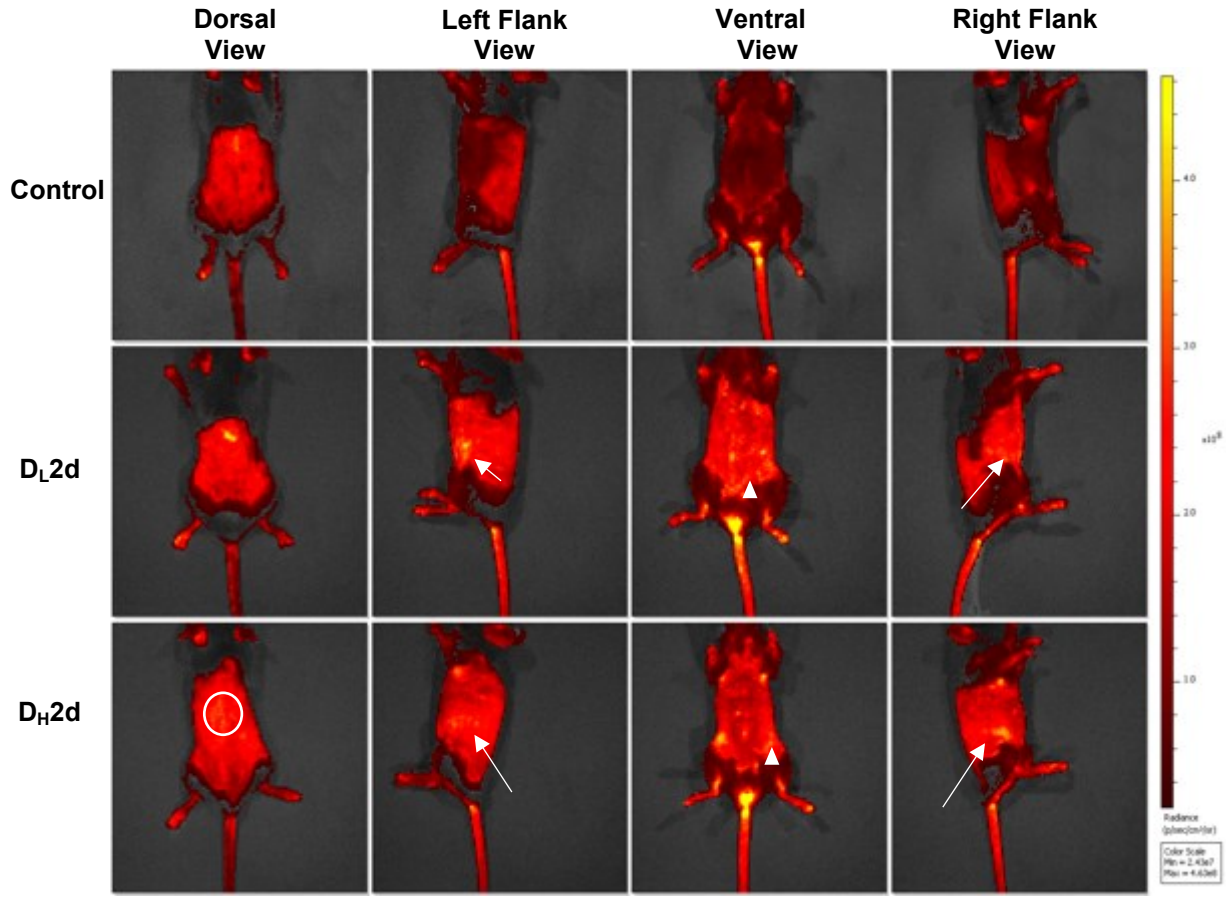


Figure S5. Fluorescence bioimages of C57BL/6 mice 6 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_L2d:** non-tumour mice that were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. **D_H2d:** non-tumour mice that were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

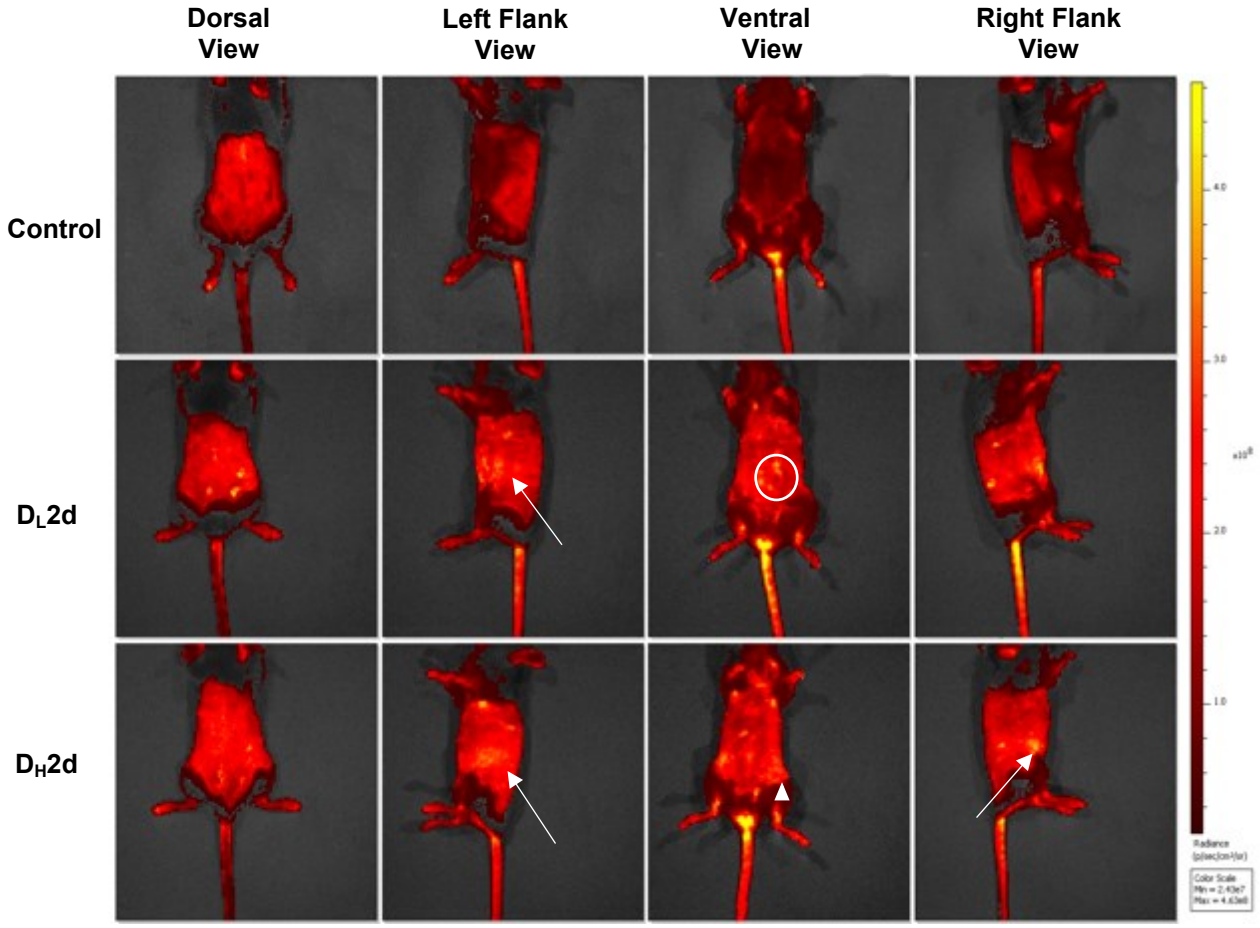


Figure S6. Fluorescence bioimages of C57BL/6 mice 24 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_L2d:** non-tumour that were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. **D_H2d:** non-tumour mice that were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on ventral region.

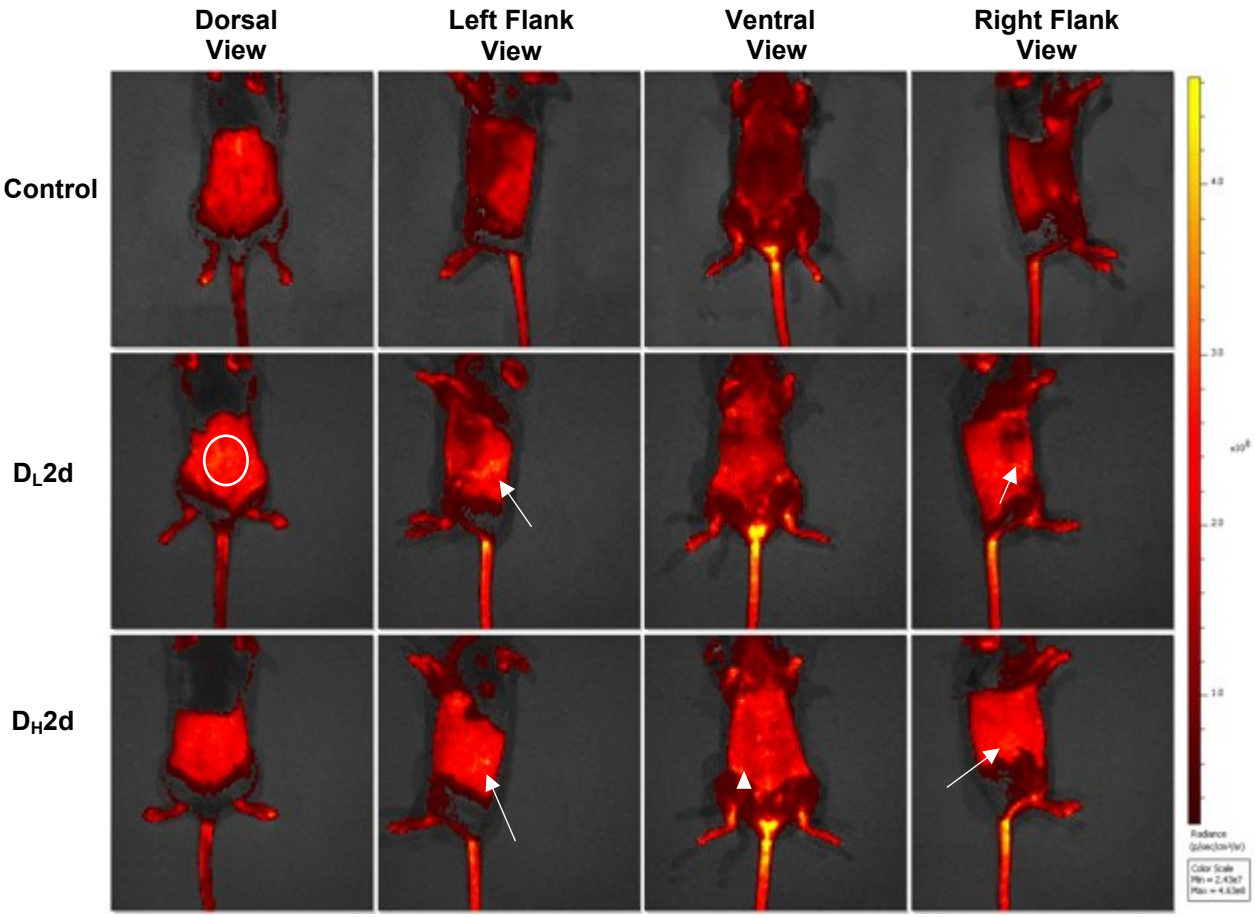


Figure S7. Fluorescence bioimages of C57BL/6 mice 48 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_L2d:** non-tumour mice that were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. **D_H2d:** non-tumour mice that were injected with 0.31 mg mL^{-1}solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

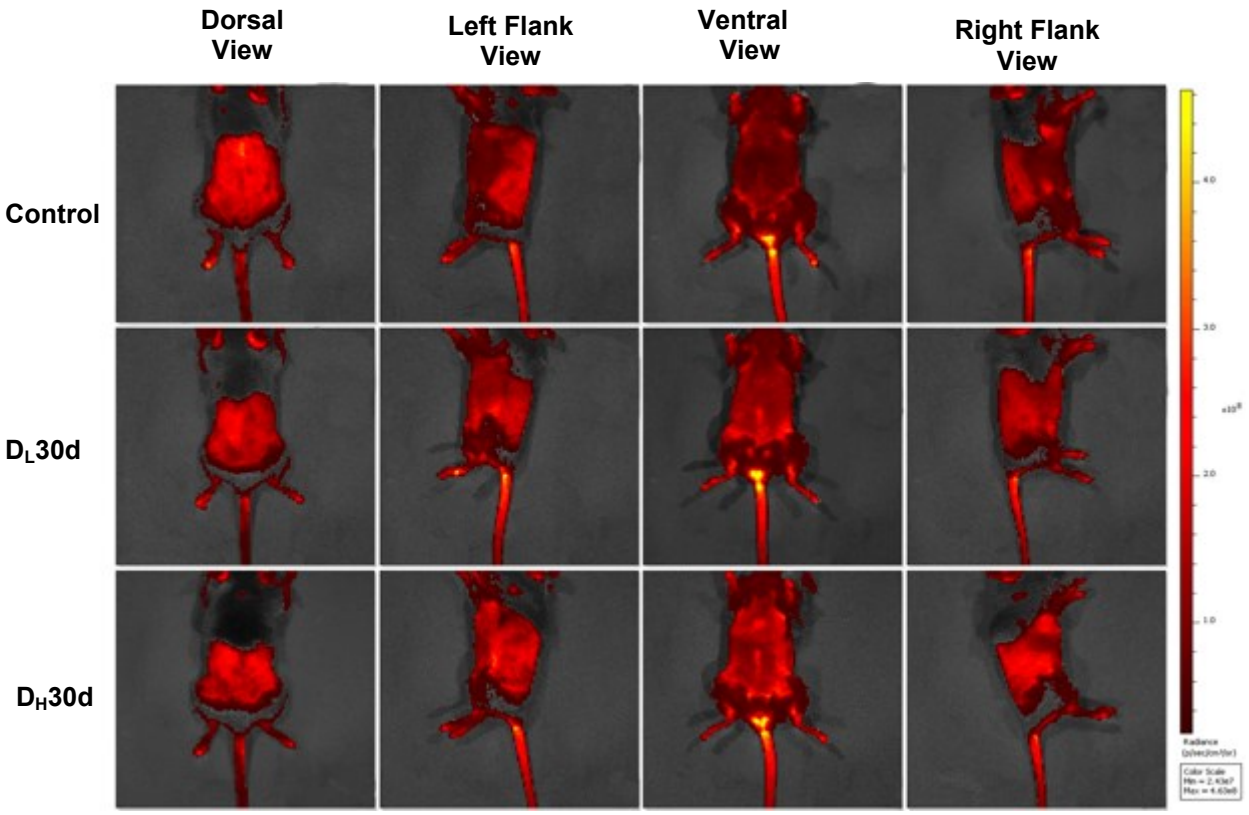


Figure S8. Fluorescence bioimages of C57BL/6 mice 30 days after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_L30d:** non-tumour mice injected with 0.16 mg mL^{-1}solution of **PBAC-dots** and euthanized after 30 days. **D_H30d:** non-tumour mice injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized after 30 days. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

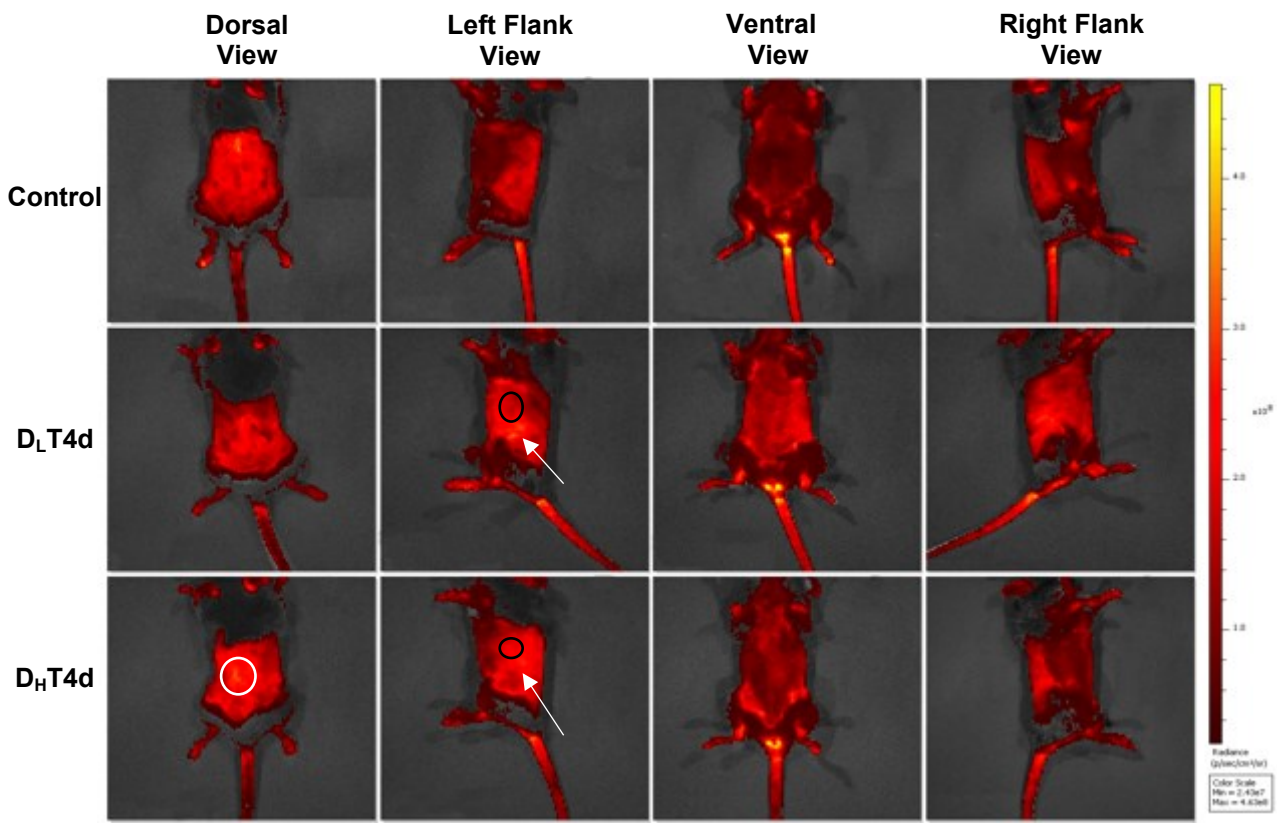


Figure S9. Fluorescence bioimages of C57BL/6 mice 4 days after tumour induction and right after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT4d:** mice 4 days after tumour induction , injected with 0.16 mg mL^{-1}solution of **PBAC-dots** and euthanized after 2 days. **D_HT4d:** mice 4 days after tumour induction, injected with 0.31 mg mL^{-1} solution of PBAC-dots and euthanized after 2 days. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White circles indicate fluorescence on dorsal region.

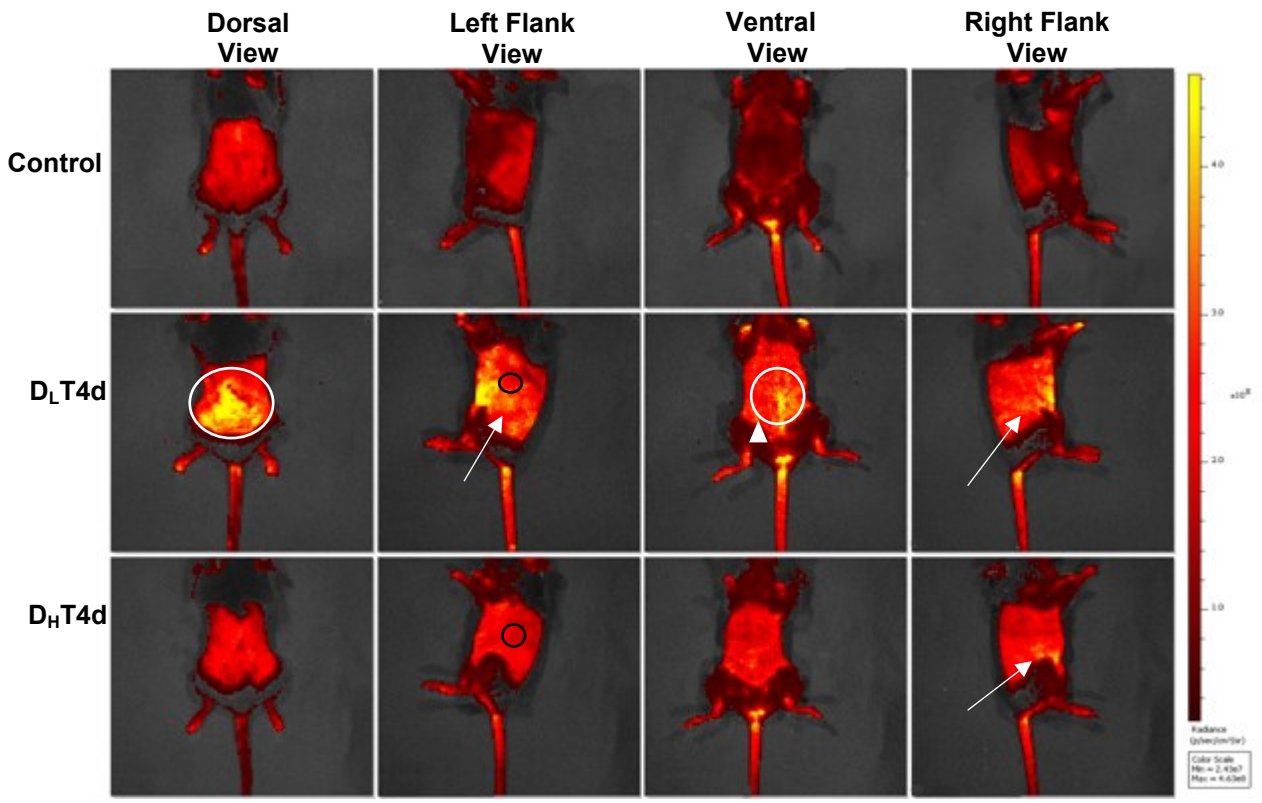


Figure S10. Fluorescence bioimages of C57BL/6 mice after 4 days of tumour induction and 2 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT4d:** mice 4 days after tumour induction, which received the 0.16 mg mL^{-1} solution of **PBAC-dots** and were euthanized after 2 days. **D_HT4d:** mice 4 days after tumour induction, which received the 0.31 mg mL^{-1} solution of **PBAC-dots** and were euthanized after 2 days. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal and ventral region.

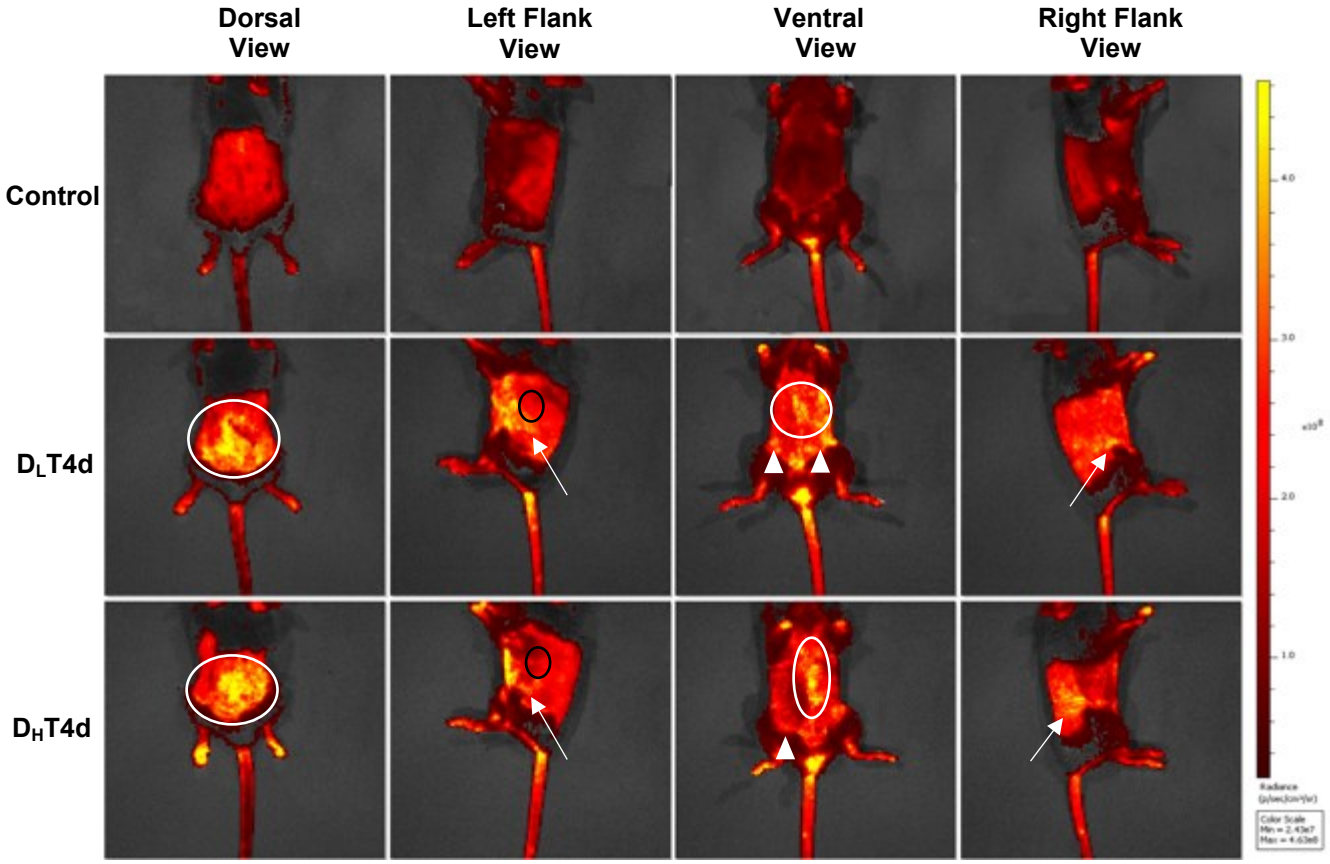


Figure S11. Fluorescence bioimages of C57BL/6 mice after 4 days of tumour induction and 4 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT4d:** mice 4 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. **D_HT4d:** mice 4 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal and ventral region.

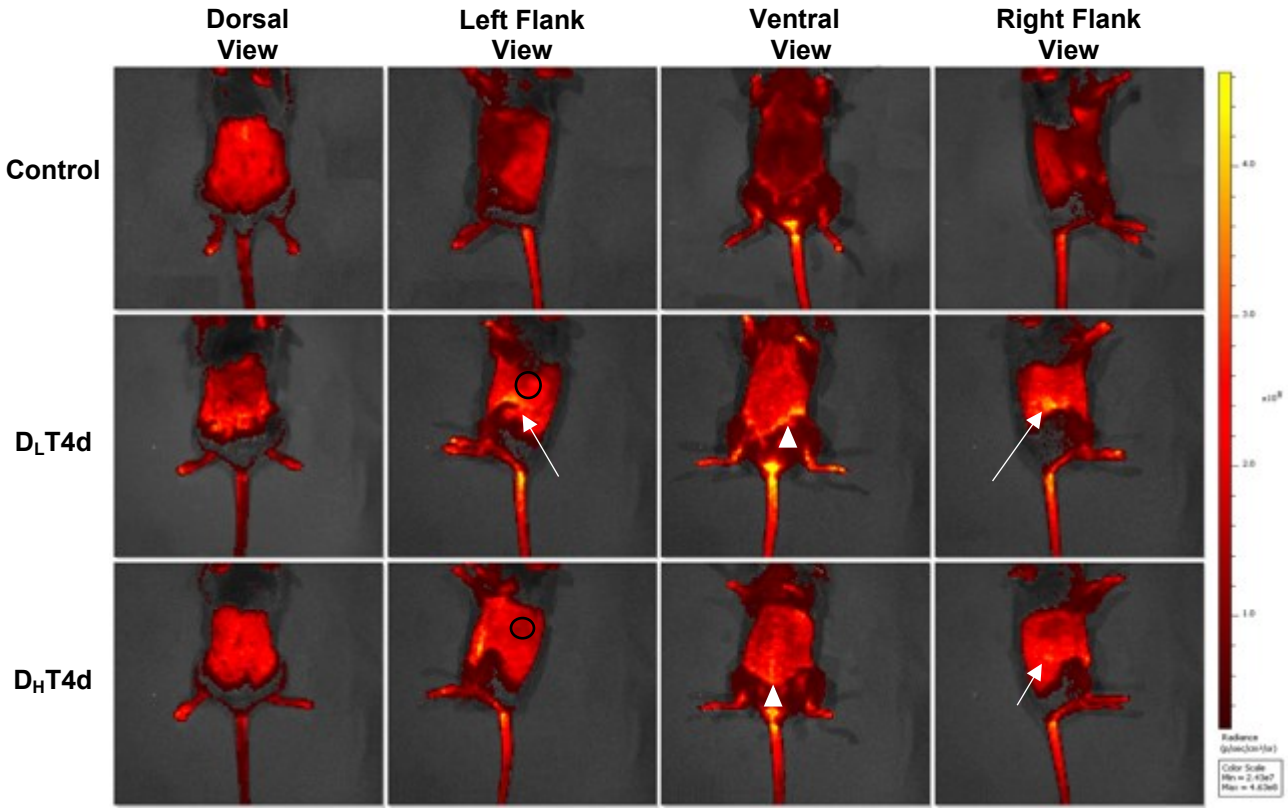


Figure S12. Fluorescence bioimages of C57BL/6 mice after 4 days of tumour induction and 6 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT4d:** mice 4 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. **D_HT4d:** mice 4 days after tumour induction, which were injected with 0.31 mg mL^{-1}solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions.

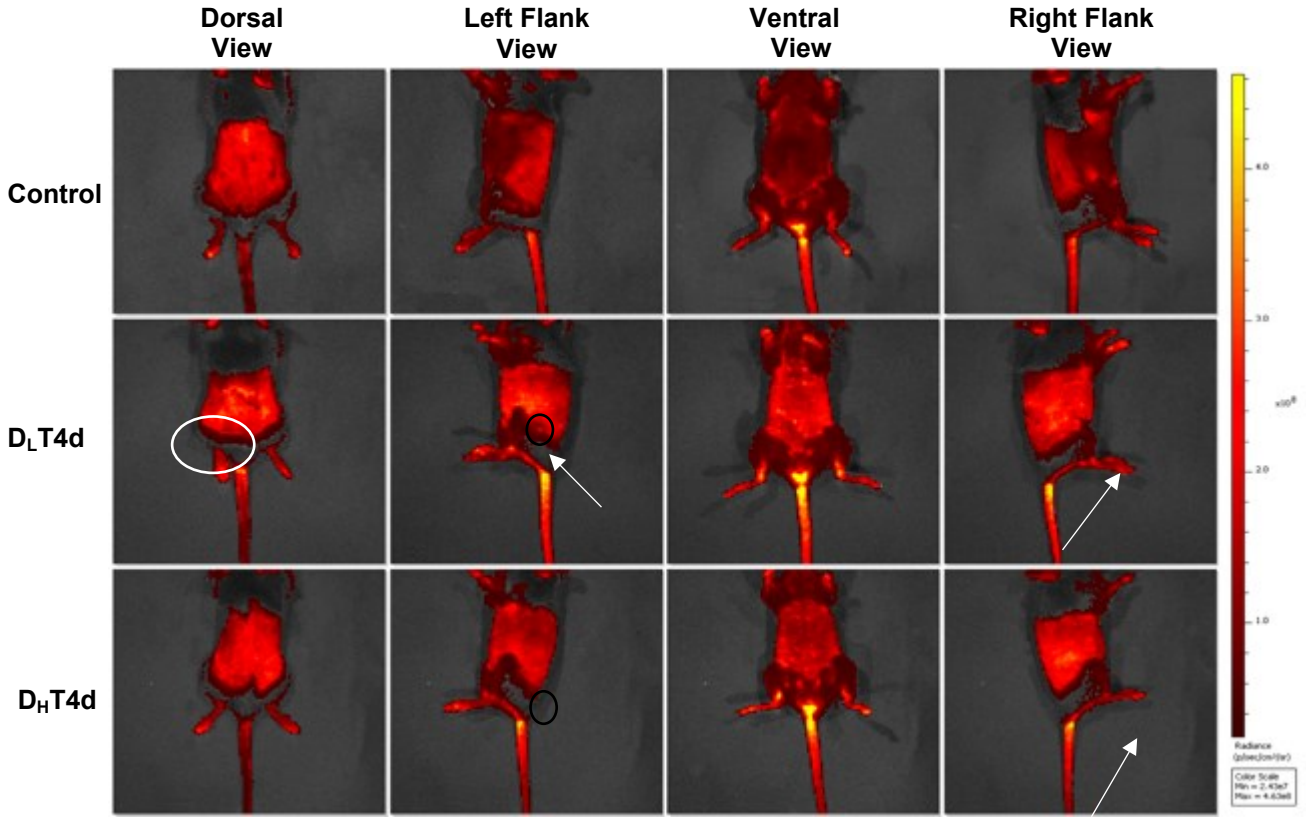


Figure S13. Fluorescence bioimages of C57BL/6 mice after 4 days of tumour induction and 24 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT4d:** mice 4 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. **D_HT4d:** mice 4 days of tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized after 2 days. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White circles indicate fluorescence on dorsal region.

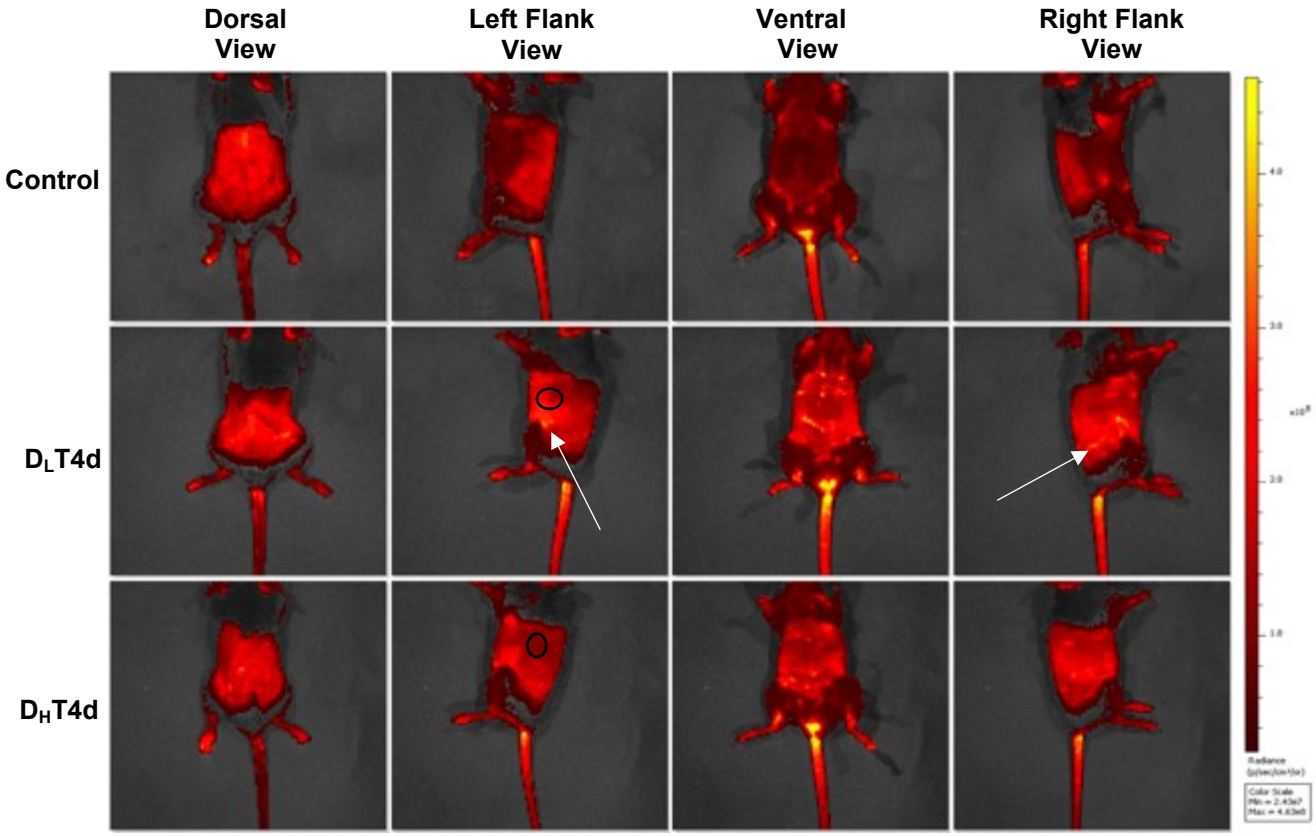


Figure S14. Fluorescence bioimages of C57BL/6 mice after 4 days of tumour induction 48 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT4d:** mice 4 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT4d:** mice 4 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. Black circles indicate tumour regions.

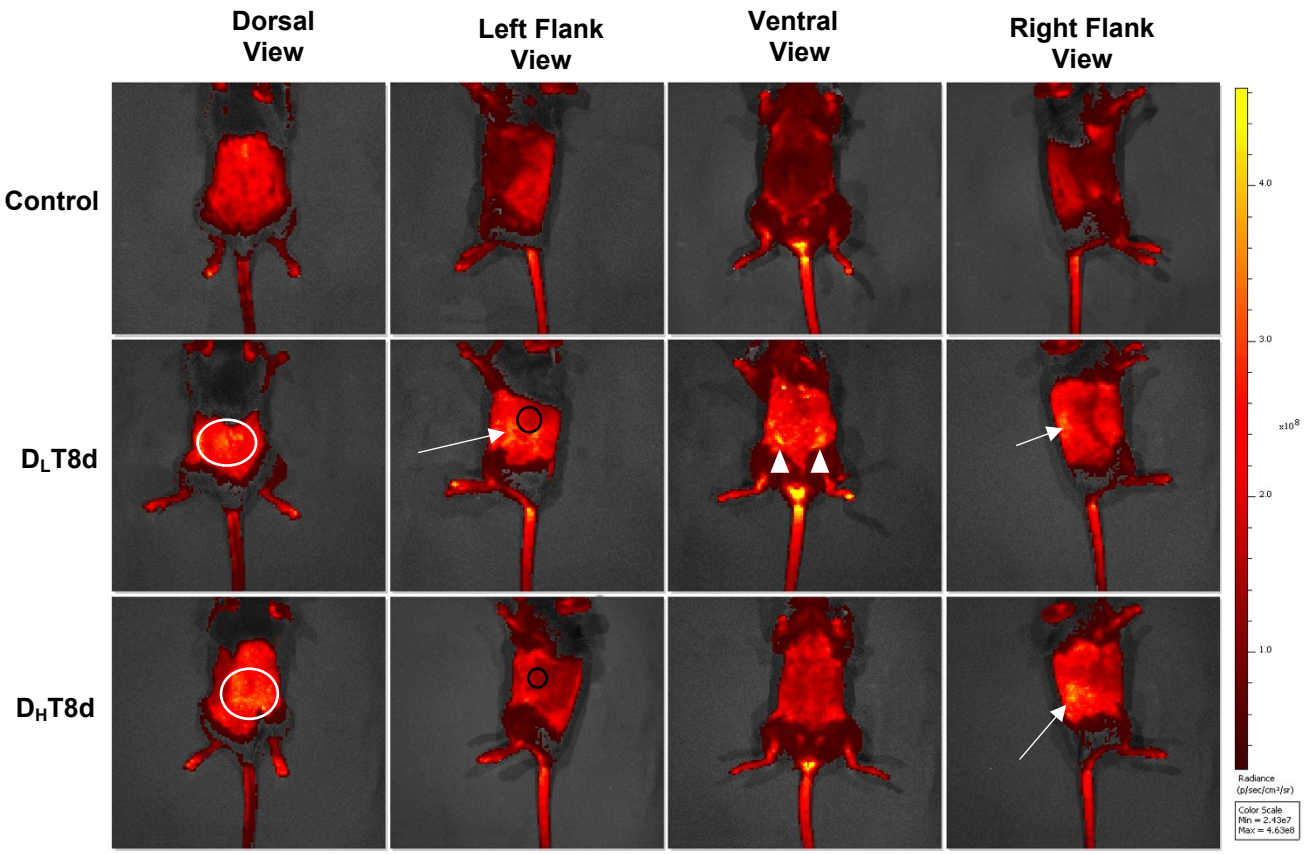


Figure S15. Fluorescence bioimages of C57BL/6 mice after 8 days of tumour induction right after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT8d:** mice 8 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT8d:** mice 8 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

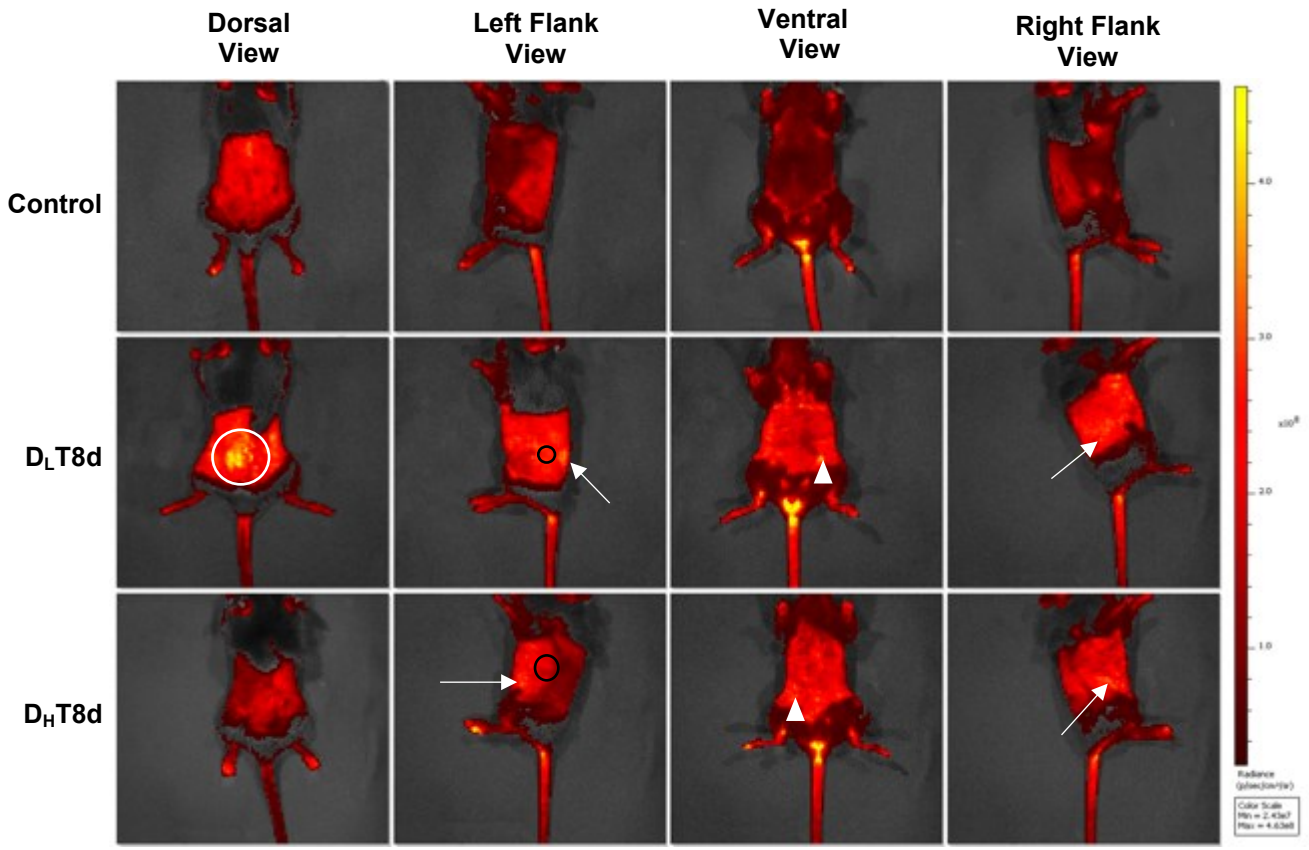


Figure S16. Fluorescence bioimages of C57BL/6 mice after 8 days of tumour induction and 2 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT8d:** mice 8 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT8d:** mice 8 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

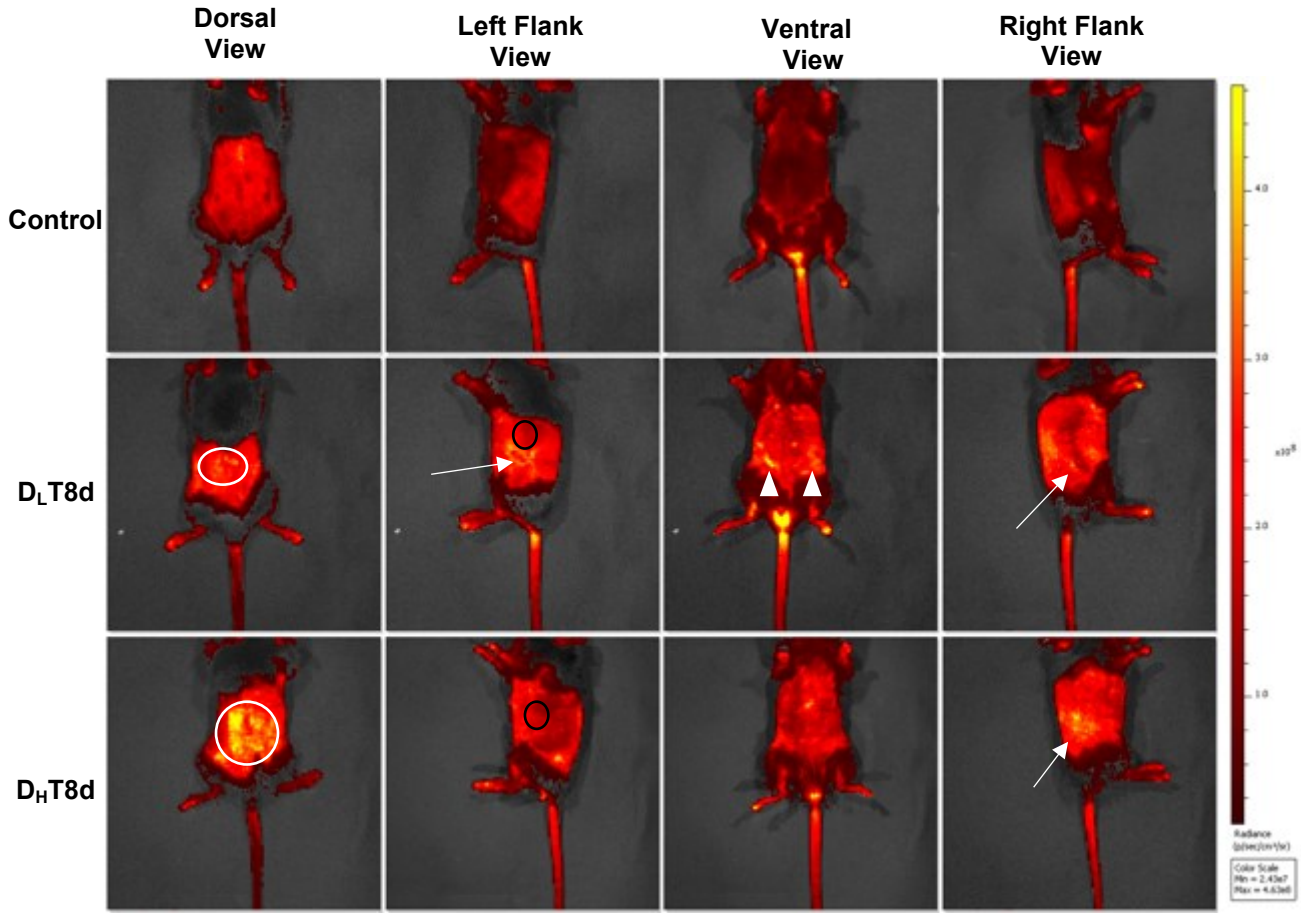


Figure S17. Fluorescence bioimages of C57BL/6 mice after 8 days of tumour induction and 4 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT8d:** mice 8 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT8d:** mice 8 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

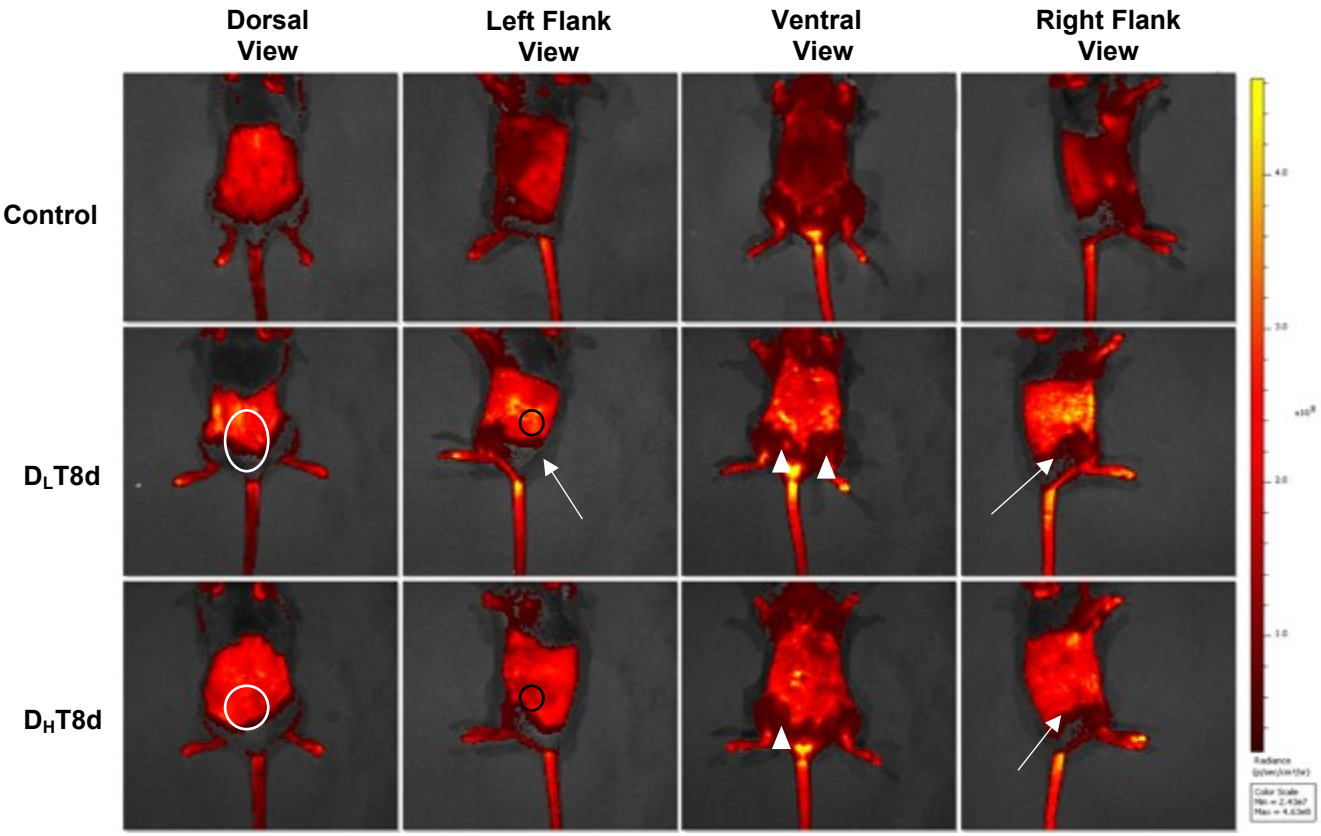


Figure S18. Fluorescence bioimages of C57BL/6 mice after 8 days of tumour induction and 6 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT8d:** mice 8 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT8d:** mice 8 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

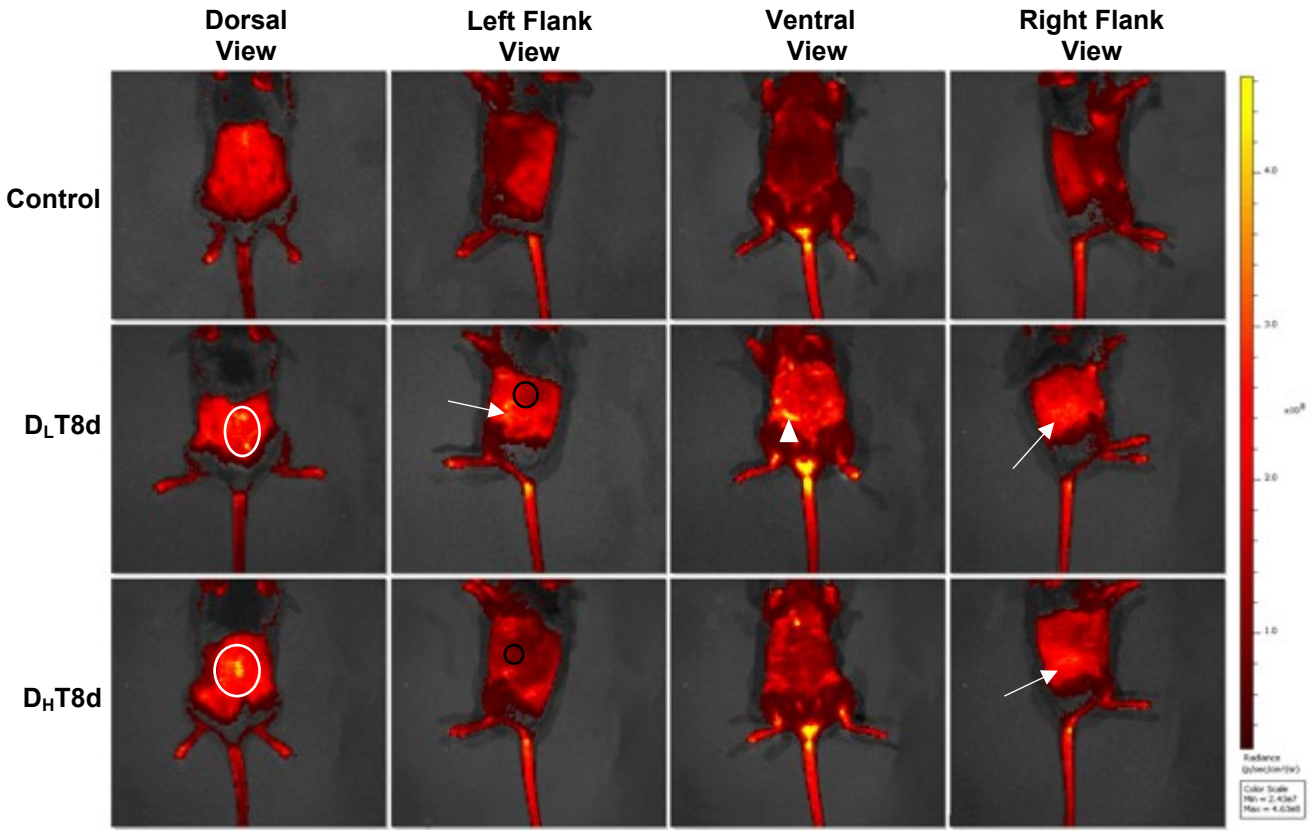


Figure S19. Fluorescence bioimages of C57BL/6 mice after 8 days of tumour induction 24 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT8d:** mice 8 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT8d:** mice 8 days after tumour induction, which were injected with 0.31 mg mL^{-1}solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

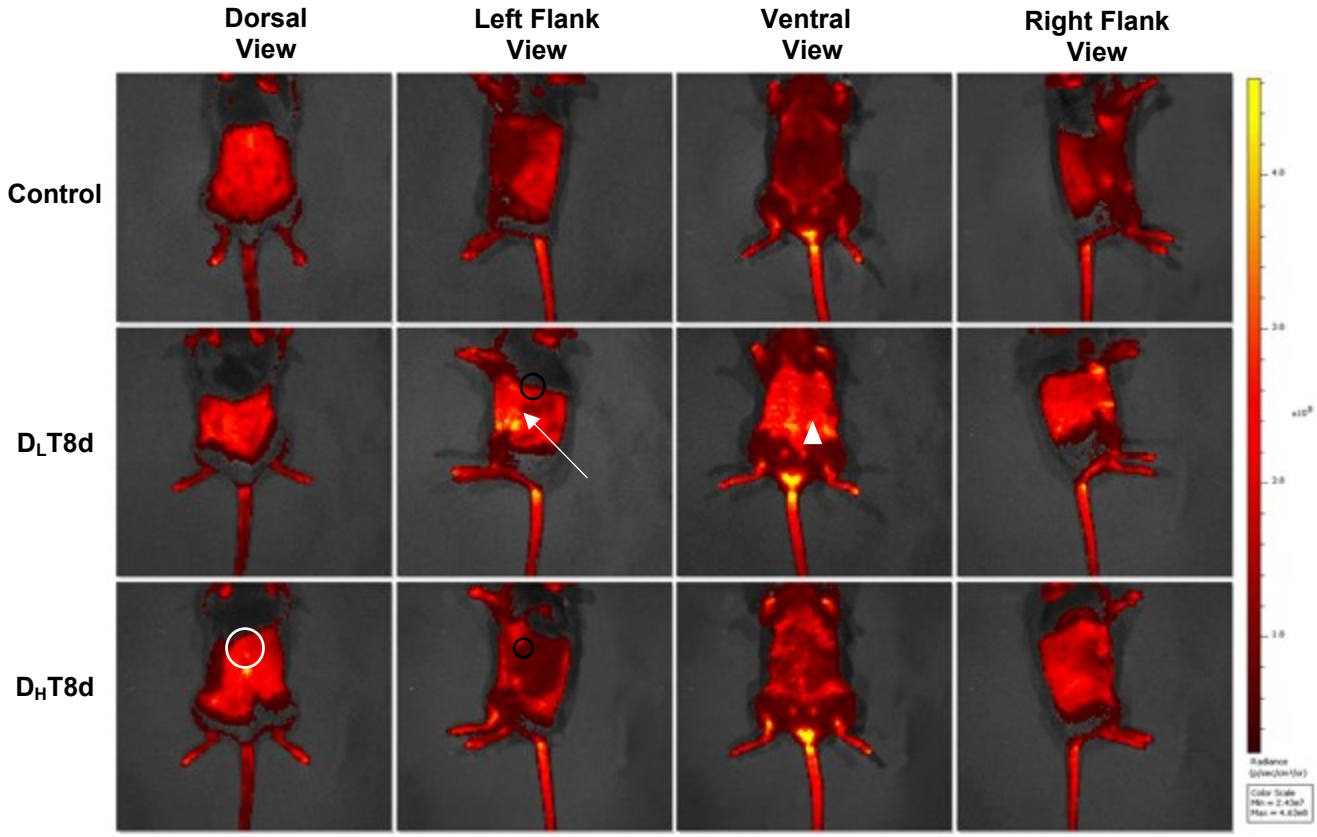


Figure S20. Fluorescence bioimages of C57BL/6 mice after 8 days of tumour induction and 48 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT8d:** mice 8 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT8d:** mice 8 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. Black circles indicate tumour regions. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

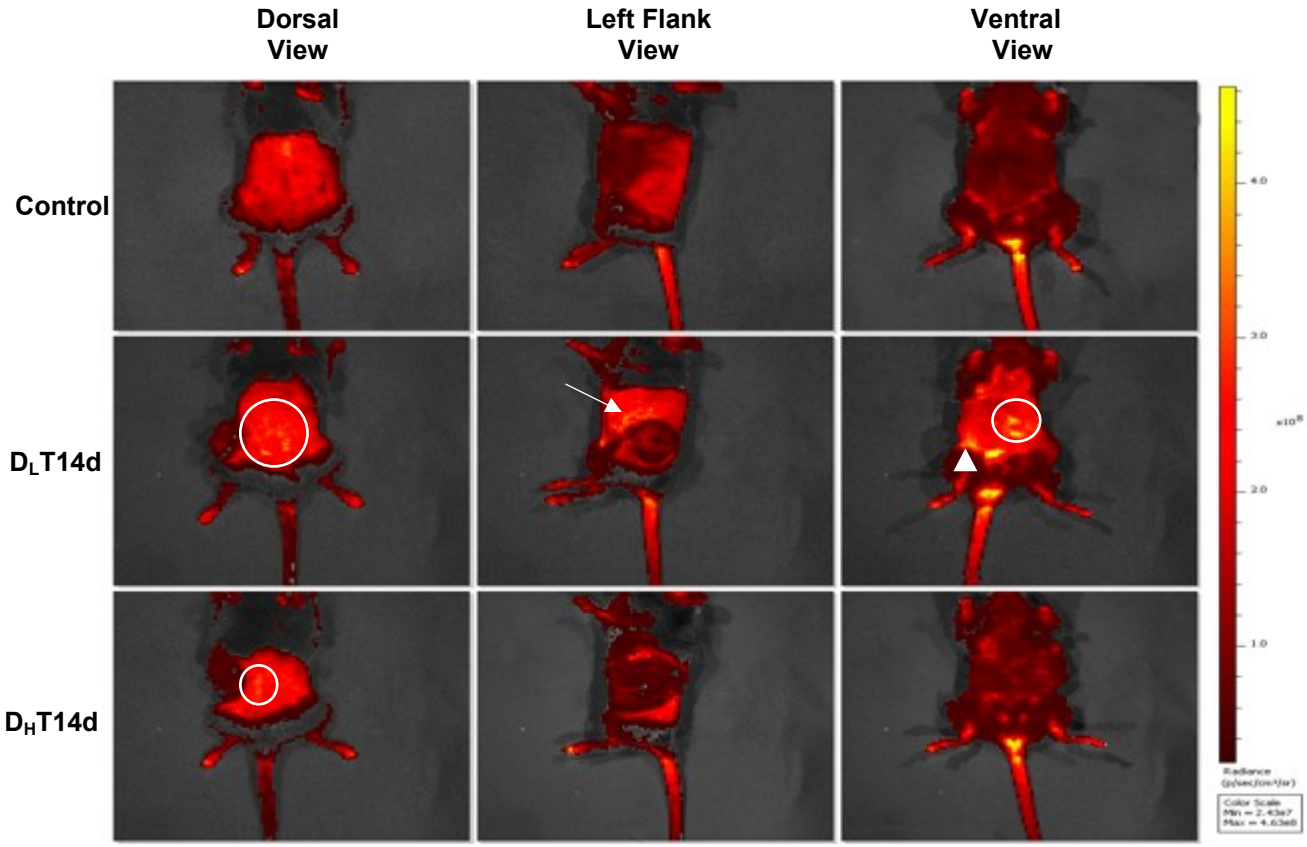


Figure S21. Fluorescence bioimages of C57BL/6 mice after 14 days of tumour induction right after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT14d:** mice 14 days after tumour induction, which were injected with 0.16 mg mL^{-1}solution of **PBAC-dots** and euthanized 2 days later. **D_HT14d:** mice 14 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal and ventral region.

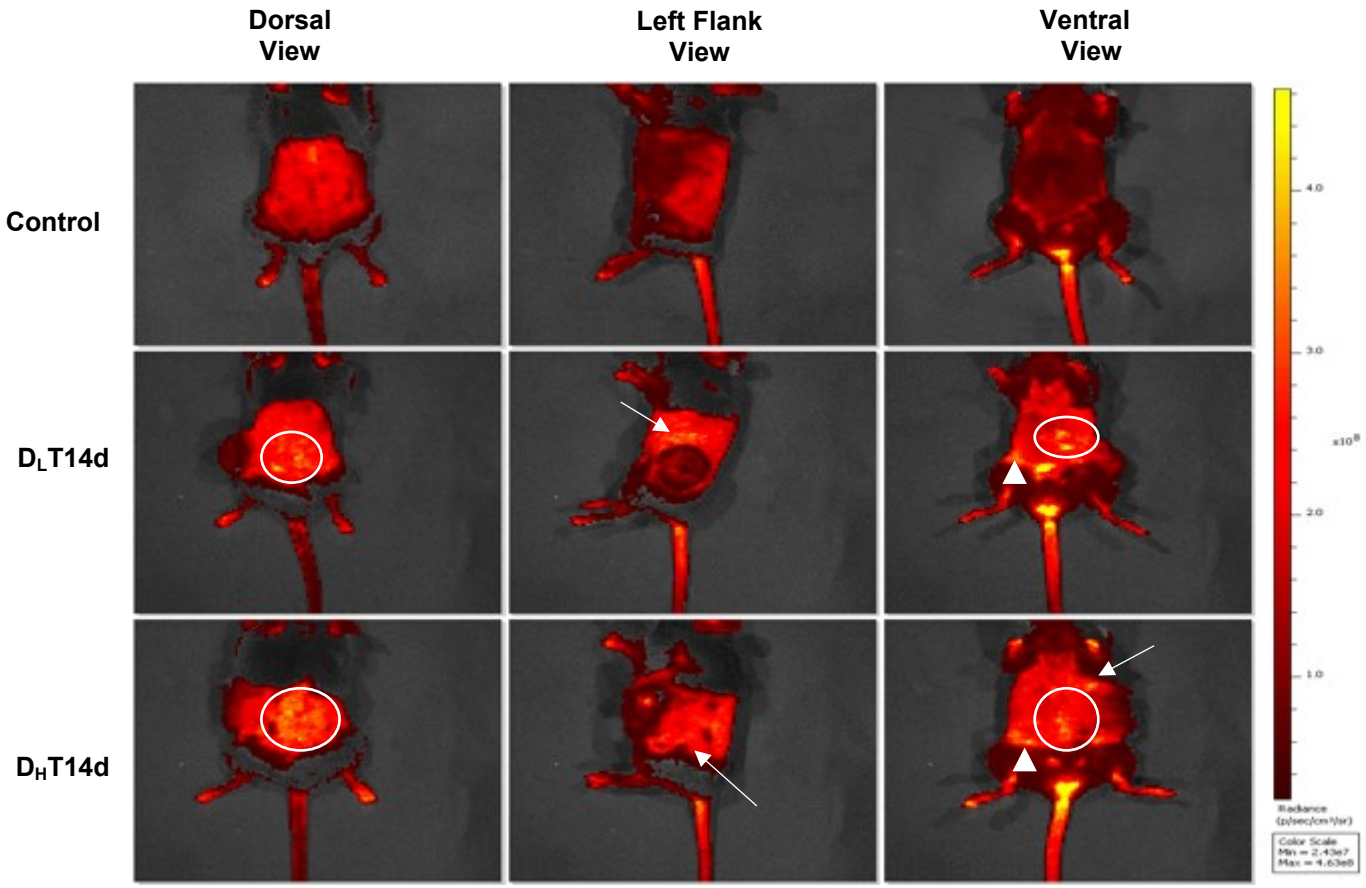


Figure S22. Fluorescence bioimages of C57BL/6 mice after 14 days of tumour induction and 2 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT14d:** mice 14 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT14d:** mice 14 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal and ventral region.

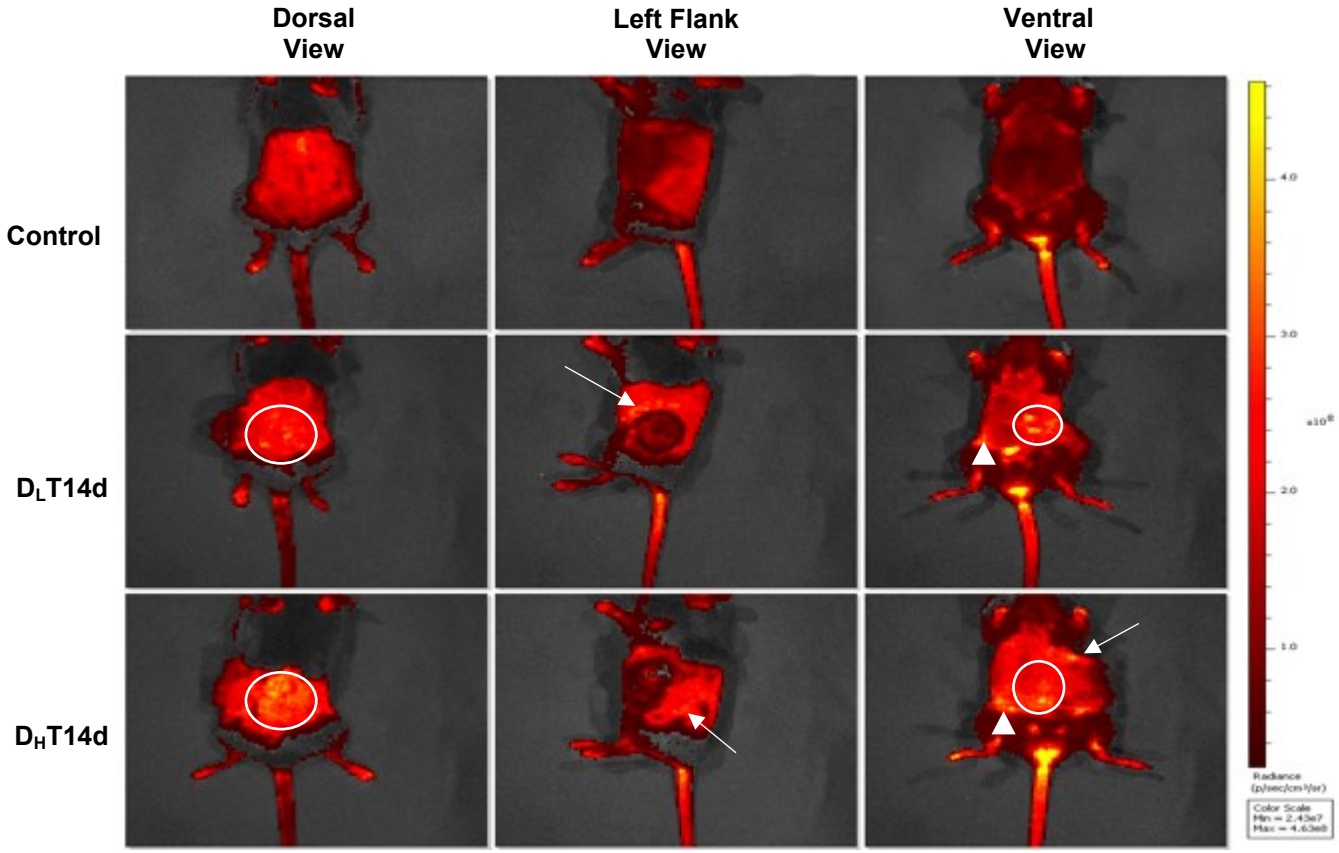


Figure S23. Fluorescence bioimages of C57BL/6 mice after 14 days of tumour induction and 4 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT14d:** mice 14 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT14d:** mice 14 days after tumour induction, which were injected with 0.31 mg mL^{-1}solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal and ventral region.

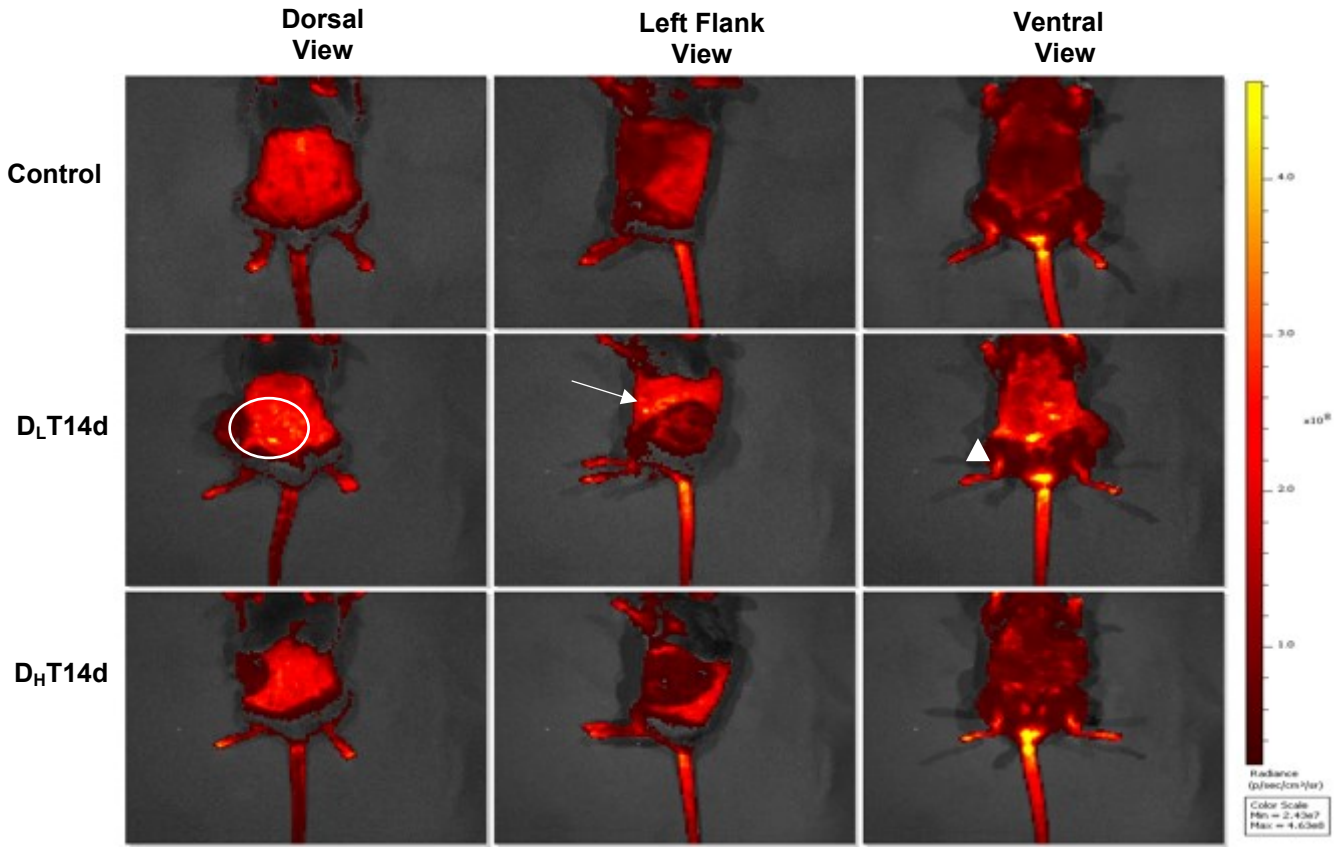


Figure S24. Fluorescence bioimages of C57BL/6 mice after 14 days of tumour induction and 6 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT14d:** mice 14 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT14d:** mice 14 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

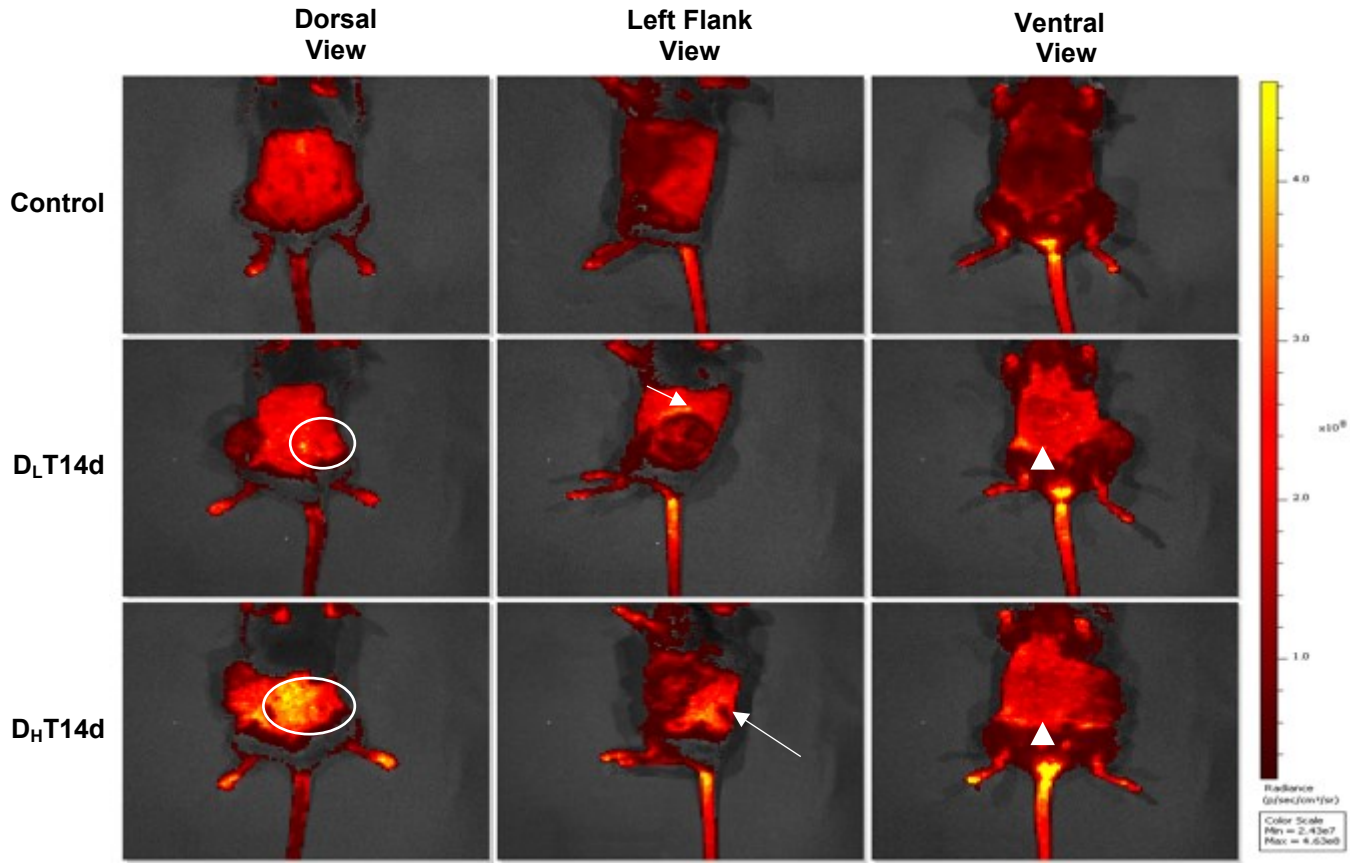


Figure S25. Fluorescence bioimages of C57BL/6 mice after 14 days of tumour induction and 24 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT14d:** mice 14 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT14d:** mice 14 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

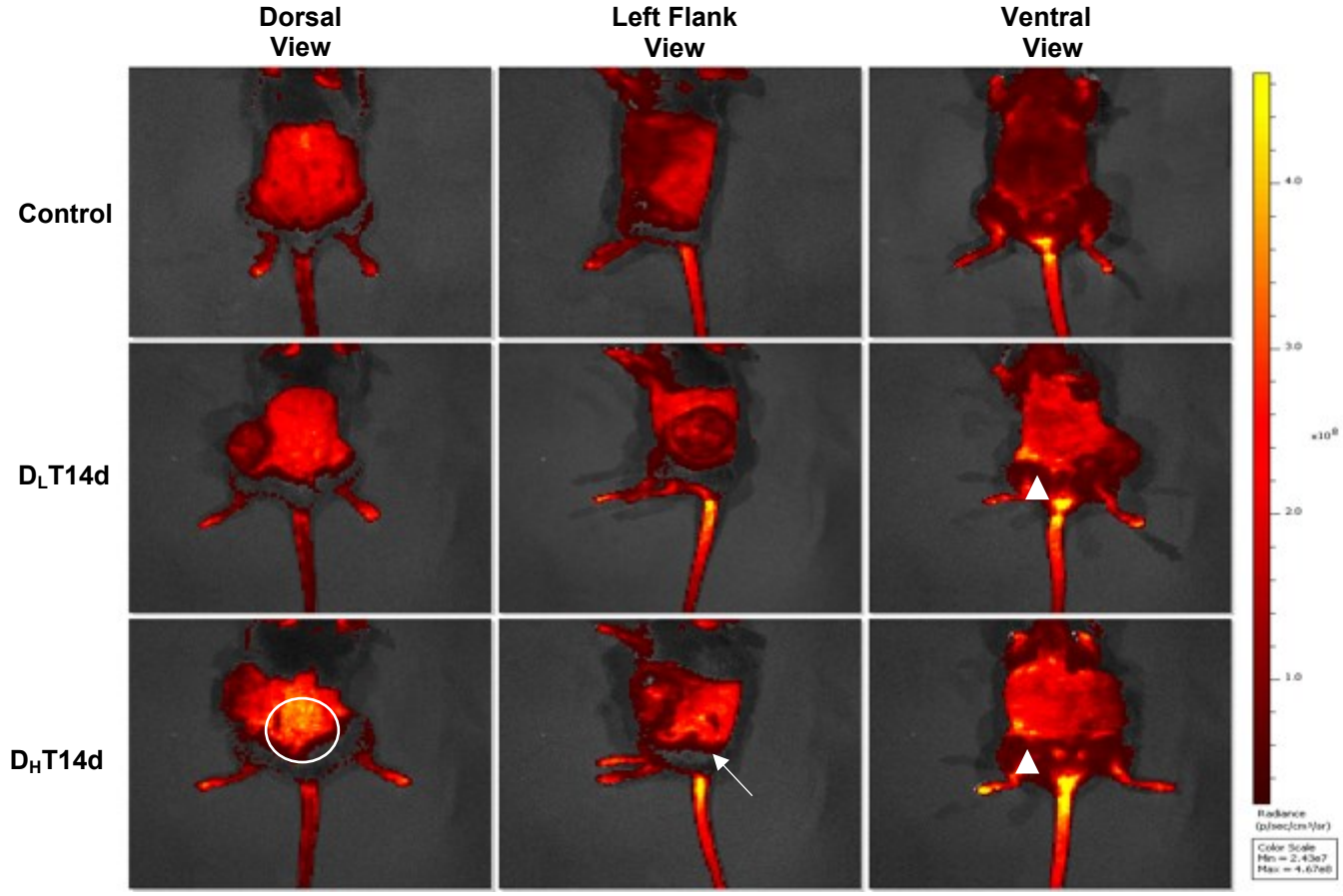


Figure S26. Fluorescence bioimages of C57BL/6 mice after 14 days of tumour induction 48 h after **PBAC-dots** intravenous injection (0.16 and 0.31 mg mL^{-1}). **D_LT14d:** mice 14 days after tumour induction, which were injected with 0.16 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. **D_HT14d:** mice 14 days after tumour induction, which were injected with 0.31 mg mL^{-1} solution of **PBAC-dots** and euthanized 2 days later. White arrows show fluorescence on kidney region. White triangles indicate fluorescence on ureter regions. White circles indicate fluorescence on dorsal region.

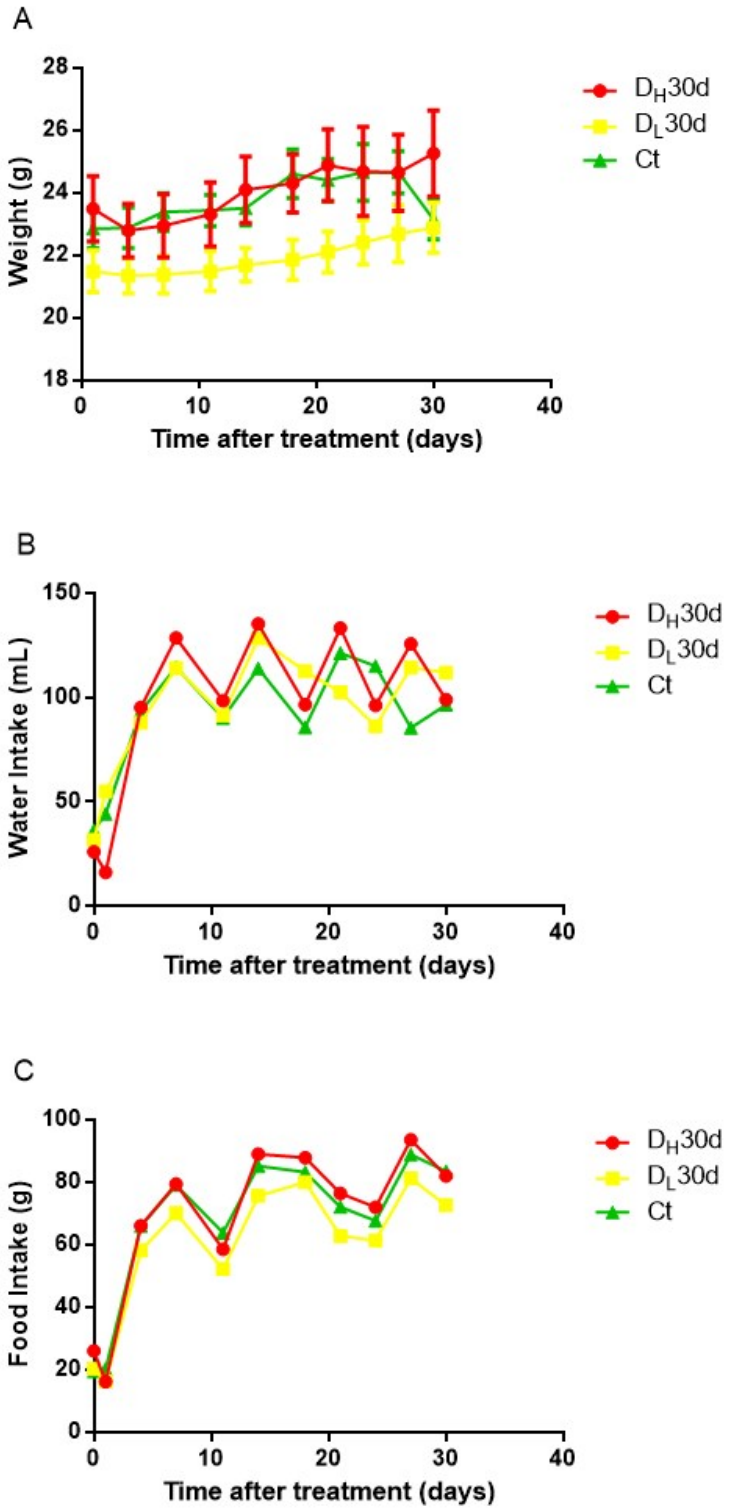


Figure S27. Variation of body weight (**A**), water intake (**B**) and food intake (**C**) by C57BL/6 mice after **PBAC-dot** in two concentrations (0.16 mg mL^{-1}, yellow line; 0.31 mg mL^{-1}, red line) or PBS injection (control, green line) over 30 days.

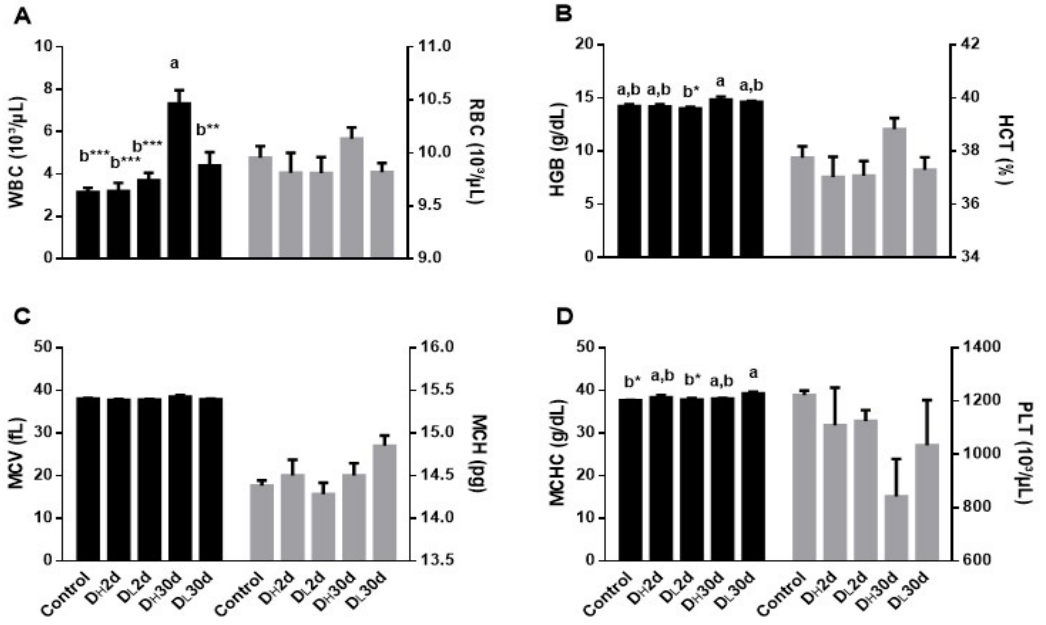


Figure S28. Blood count of mice C56BL/6 after 2 and 30 days of **PBAC-dot** endovenous application (0.16 and 0.31 mg mL^{-1}). **A**: Total number of white cells (WBC) and red cells (RBC). **B**: haemoglobin (HGB) and haematocrit (HTC). **C**: mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). **D**: mean corpuscular haemoglobin concentration (MCHC) and platelets number (PLT). Different letters mean different statistics. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.005$.

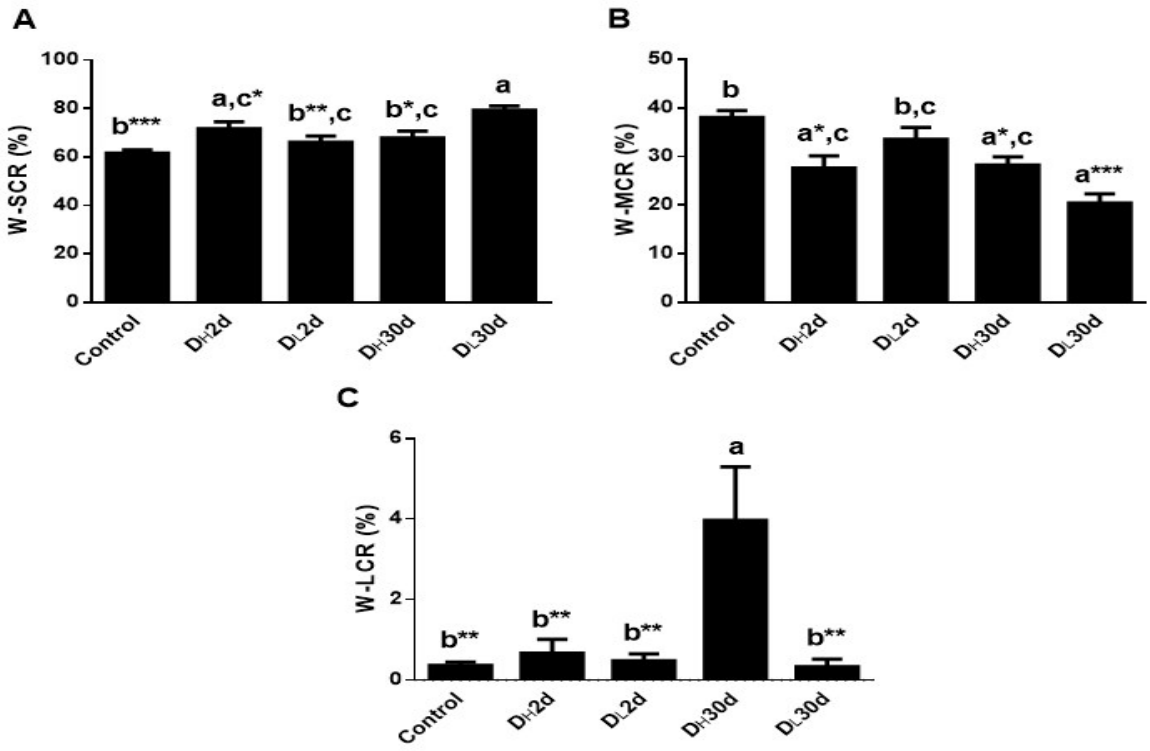


Figure S29. C57BL/6 mice lymphogram after 2 and 30 days of **PBAC-dot** endovenous applicaton (0.16 and 0.31 mg mL^{-1}). **A**: percent ratio of small blood cell (W-SCR). **B**: percent ratio of medium blood cell (W-MCR). **C**: percent ratio of large blood cell (W-LCR). Different letters mean different statistics. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.005$.

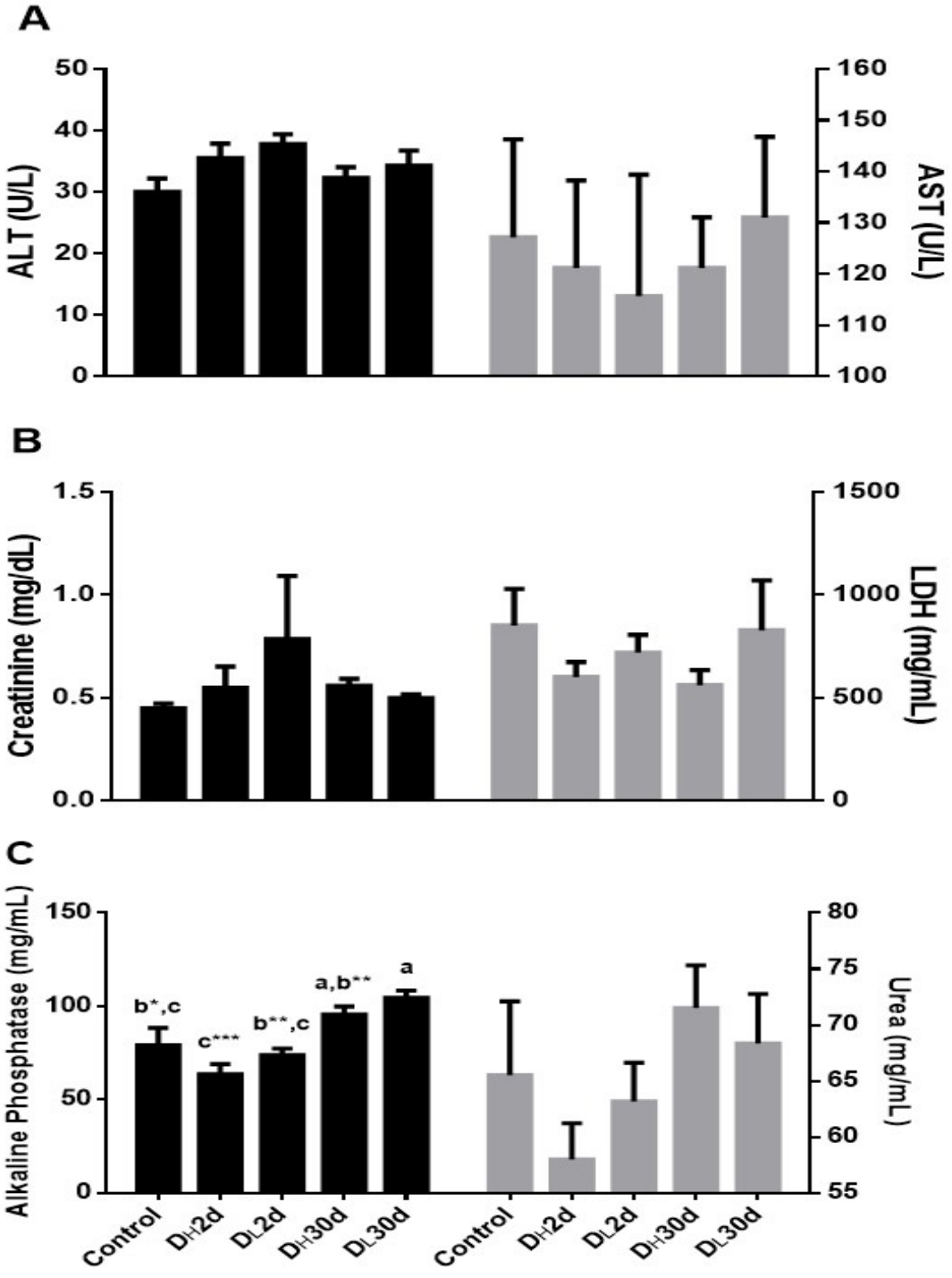


Figure S30. C57BL/6 mice serum levels of ALT and AST (A), Creatinine and LDH (B), Alkaline Phosphatase and Urea(C) after 2 and 30 days of **PBAC-dot** endovenous application (0.16 and 0.31 mg mL^{-1}). Different letters mean different statistics. * = $p<0.05$; ** = $p<0.01$; *** = $p<0.005$