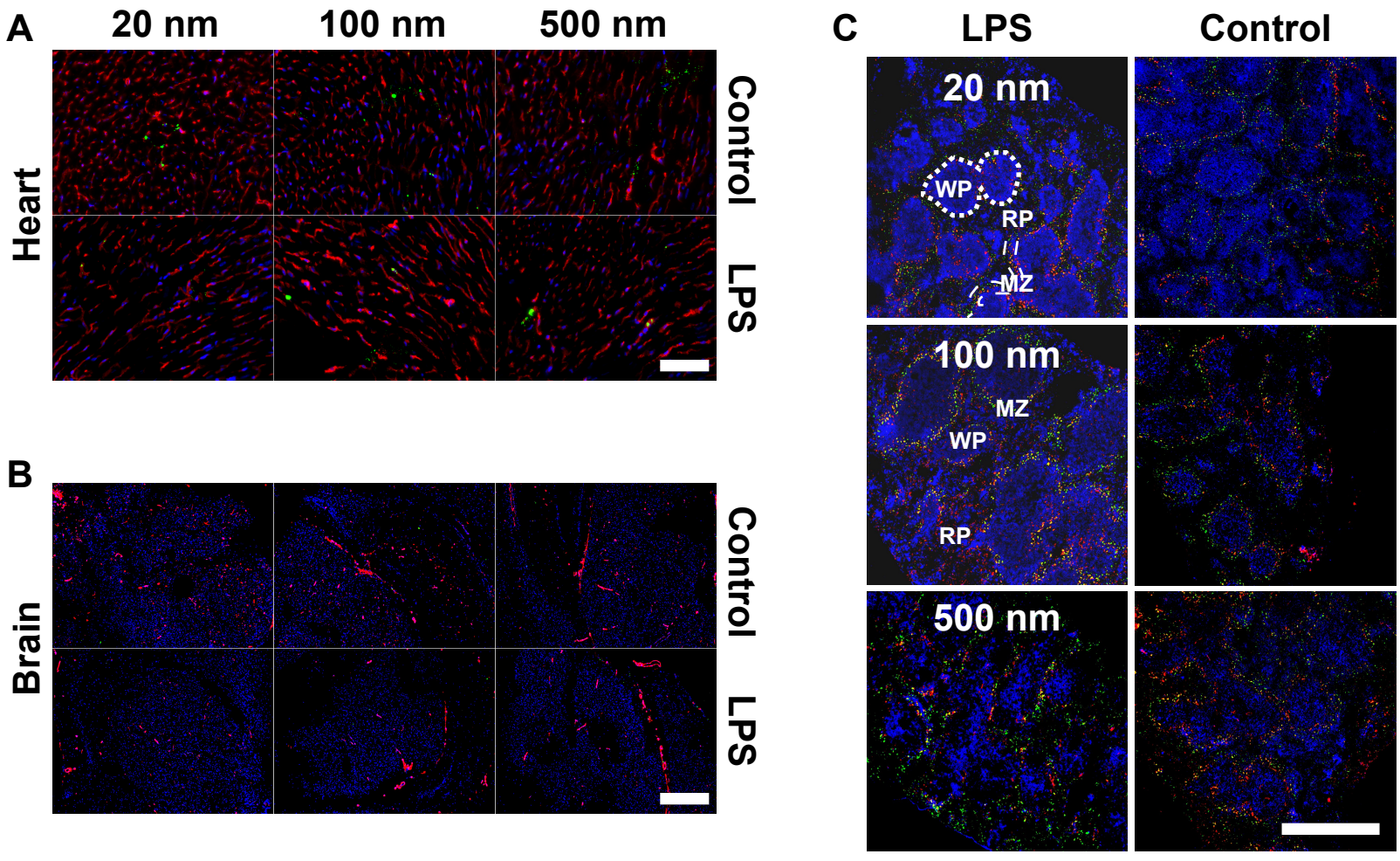
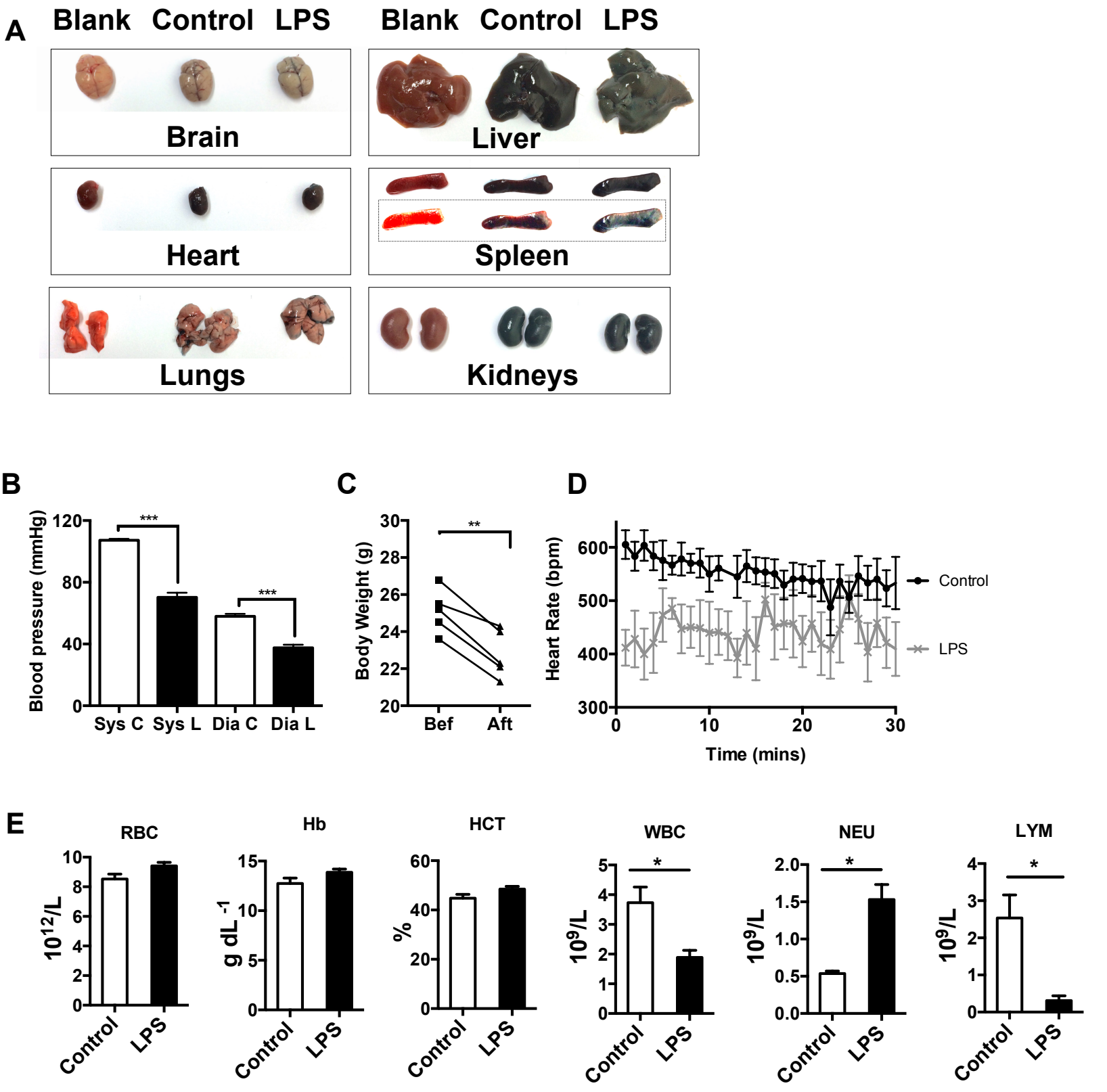
Electronic Supplementary Material (ESI) for Nanoscale.

Chen & Lundy et al., NR-ART-06-2015-003626
Electronic Supplemental Information



ESI Fig. S1.
(A) shows immunofluorescence staining of isolectin (red) and nanoparticles (green), for 20 nm, 100 nm and 500 nm nanoparticles in control and LPS-treated mice, as per Fig. 3. Very few nanoparticles are observed in the heart, with no clear difference between any treatment groups, in agreement with HPLC and IVIS analyses. Scale bar = 50 μm. **(B)** shows the brain, where almost no nanoparticles are visualised, as expected. Scale bar = 500 μm. **(C)** shows lower magnification images of the spleen, as per Fig. 3. It is clear that the majority of nanoparticles, of all sizes, accumulate in the marginal zones (MZ, marked by dashed lines) surrounding the white pulp (WP, marked by dotted line), with few nanoparticles present in the red pulp (RP). Scale bar = 10 μm



ESI Fig. S2.

(A) shows representative images of vital organs after Evans blue extravasation assay, as per Figure 6. The spleen and kidneys both present as darker blue in LPS-inflamed mice, compared to control mice. Blank organs, without Evans blue administration, are shown for comparison. For the spleen, an image with enhanced brightness, but no colour adjustment, is shown, to more clearly show the increased blue staining after LPS treatment. **(B)** LPS-treated (L) mice show significantly lower systolic (Sys) and diastolic (Dia) blood pressures than control (C) animals. **(C)** Mouse weight reduced following LPS administration. **(D)** Mouse heart rate, measured continuously for 30 minutes, is significantly reduced in LPS-treated animals. **(E)** Complete blood count analysis reveals no change in total erythrocyte count (RBC), haemoglobin (Hb) or haematocrit/packed cell volume (HCT). However, LPS-treated animals show a reduced total white blood cell count (WBC), increased neutrophils (NEU) and lymphocytopaenia (LYM). n = 5 for all groups. * = $P < 0.05$, ** = $P = P < 0.01$, *** = $P < 0.001$.

Nanoparticle	Core Diameter, TEM (nm)	Hydrodynamic Diameter, DLS (nm)	Zeta potential (mV)
20 nm	22.1 ± 2.7	32.4 ± 0.7	-31.8 ± 0.5
100 nm	96.6 ± 6.1	121.8 ± 0.5	-37.0 ± 1.5
500 nm	454.1 ± 15.8	500.7 ± 4.4	-42.7 ± 1.3

ESI Fig. S3. Sizing of polystyrene nanoparticles utilised in this study. Core diameter of nanoparticles was verified by transmission electron microscopy (TEM) and hydrodynamic sizing was measured by dynamic light scattering (DLS).

Antibody	Clone	Conjugate	Manufacturer
CD45/LCA/Ly-5	30-F11	PE-cy7	eBioscience
F4/80	CI:A3-1	APC	ABD Serotec
Ly-6g/Gr-1	1A8	Brilliant Violet 432	eBioscience
CD206	MR5D3	RPE	ABD Serotec
CD11b	M1/70	PERCP-cy5.5	eBioscience
FVD eFluor 780	-	-	eBioscience

ESI Fig. S4. Table showing antibodies used for flow cytometry.