Supplementary Figures

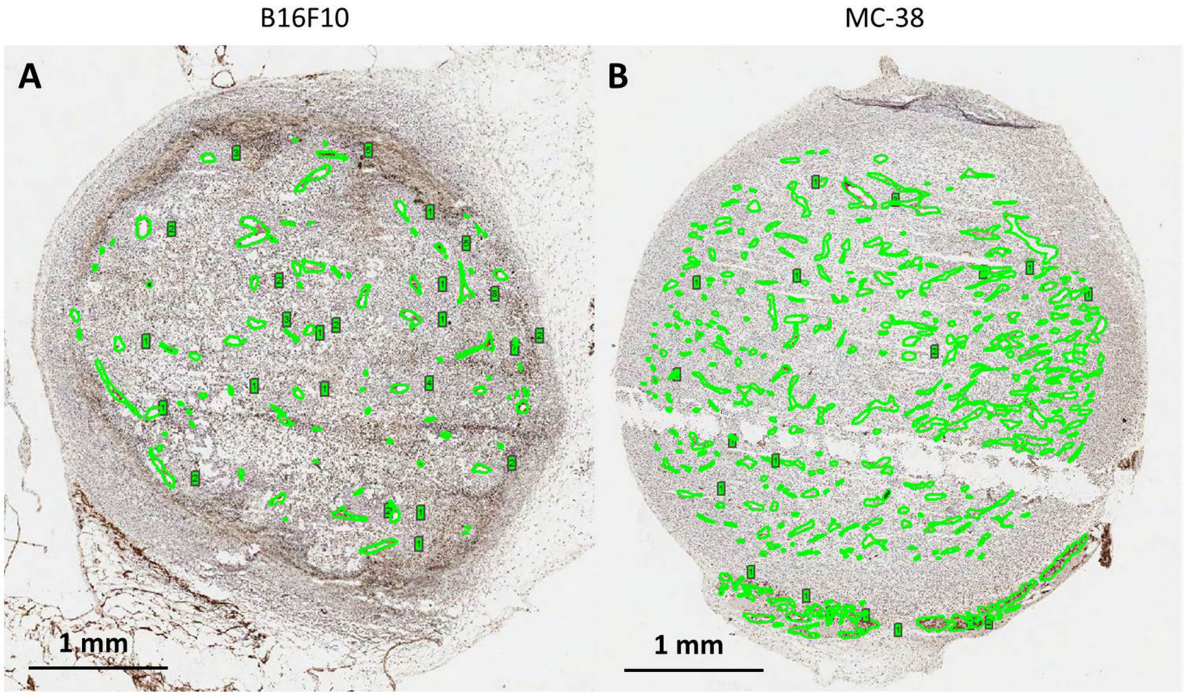

Figure S1. Representative melanoma (A) and colorectal (B) derived tumour sections stained for CD31, from mice injected with c(RGDyK)-MPIO. CD31 positive vessels are segmented (green curved lines) and the number of counted c(RGDyK)-MPIO indicated (green square tags). In both subcutaneous tumours there is a rim of poorly vascularised tissue at the edge of the tumours, in which neither vessels nor MPIO are found. The pattern of vessel distribution appears to be more homogeneous in the melanoma-derived tumours (A) than in the colorectal-derived tumours (B), with some increase in vessel density and c(RGDyK)-MPIO retention towards the right hand edge of the central region. In (B) there is also a thin region of highly vascularised tissue at the very bottom edge of the tumour.

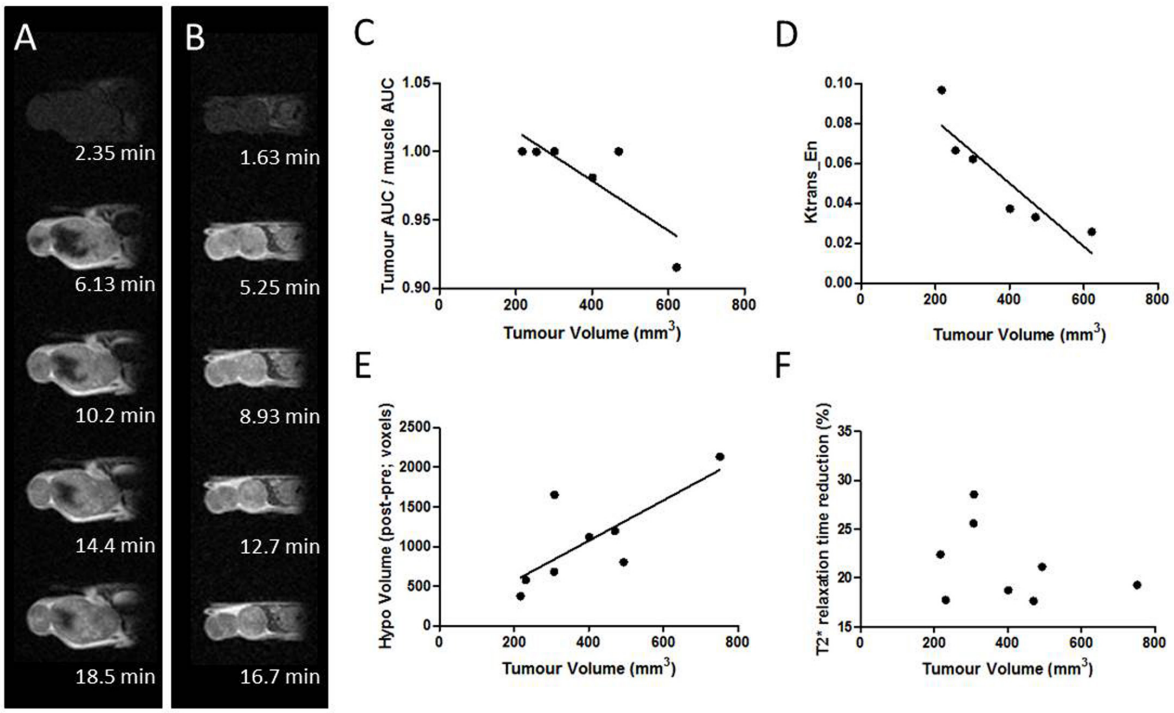

Figure S2. (A,B) Representative slice from 3D DCE dataset, showing gadolinium enhancement over time, from (A) the largest colorectal tumour (621 mm^3) and (B) the smallest colorectal tumour (219 mm^3). (C-F) Linear regression analysis of DCE and microparticle data with respect the size of the tumour. (C) Ratios of AUC of tumour over muscle plotted against tumour volume, demonstrating a negative correlation with increasing tumour volume ($R^2 = 0.6716$; $P < 0.05$). (D) K_{trans} of the enhanced voxels against tumour volume, demonstrating a negative correlation with increasing tumour volume ($R^2 = 0.8097$; $P < 0.05$). (E, F) Correlation data from the c(RGDyK)-MPIO injected cohort of colorectal tumour animals (n = 8); (E) difference (post – pre) of hypointensity volumes *vs.* tumour volume, and (F) T_2* relaxation time *vs.* tumour volume. Whilst the c(RGDyK)-MPIO hypointense volumes showed a significant positive correlation with tumour volume (E; $R^2 = 0.8478$, $P < 0.05$), no such correlation was found between the % reduction in T_2* relaxation times indicating that this measurement is independent of tumour size. This finding indicates that tumour vascularity remained largely constant across the range of tumour sizes studied.

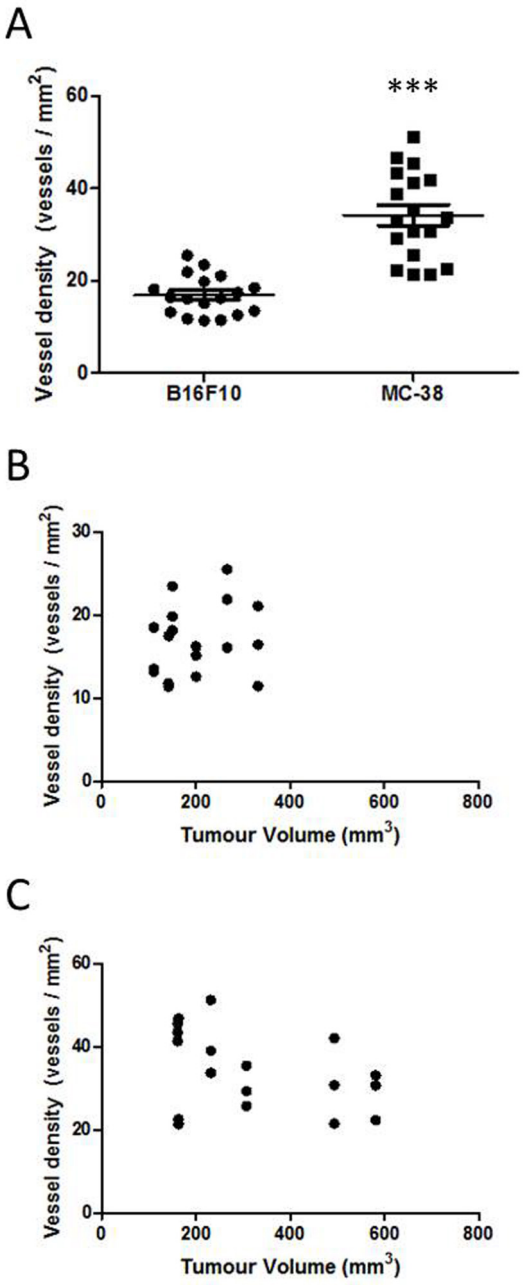

Figure S3. (A) Vertical scatter plot represent the number of vessels per mm^2 tumour area for both B16F10 and MC38 tumours, assessed immunohistochemically (n=6 per group, 3X field of view). Vessel density was significantly greater in the MC38 tumours than in the B16F10 tumours (***$P < 0.0001$). Some spread in the vessel density was apparent, particularly within the MC38 cohort, and so the relationship with tumour volume was assessed (B, C). However, no significant correlations were found between tumour vessel density and increasing tumour volume, again indicating that tumour vascularity per unit tumour area remained largely constant across the range of tumour sizes studied (in accord with the T_2* analysis in Figure S2, F).

Supplementary Methods

Synthesis of dextran coated nanoparticles

A 1 litre 3 neck round flask was charged with 6.75 g of $FeCl_3 \cdot 6H_2O$ (Sigma Aldrich, UK), 75 g of dextran 10 Pharmacological grade (Pharmacosmos, batch Nr. HR210) and 100 ml of water. The sample was stirred using an overhead stirrer at 300 rpm for 15 min until complete dissolution of the solids, and deoxygenated thoroughly by repeated cycles of vacuum assisted by sonication and argon flushing. After the first deoxygenation cycle, 3.0 g of $FeCl_2 \cdot 4H_2O$ (Sigma Aldrich, UK) in 25 ml of water was added and the solution was deoxygenated by the above procedure (4 times). While being stirred at 600 rpm, 20 ml of 30 % NH_4OH (Sigma Aldrich, UK) was added at a rate of 168 ml/h. The reaction was heated to 80 ºC and then stirred at this temperature for 1 hour. The solution was cooled and dialyzed against water in a Spectra/Por 2 membrane (MWCO 6-8000) for 21 hours against 5 litter of water with water changes after 2 and 4 h.

Particle size was measured by dynamic light scattering in a Zetasizer Nano ZS (Malvern, UK). Briefly, 20 µl of particles were dispersed in 700 µl of water, the sample was shaken for 15 min and placed in polystyrene cuvette. Size measurement was performed in triplicate at 25 °C and results were analysed using the general purpose analysis model provided by the instrument software. The product particle size was 20.5 ± 8.9 nm with a PdI of 0.188.