*Supplementary Material*

# Iron Oxide Incorporated Conjugated Polymer Nanoparticles for Simultaneous Use in Magnetic Resonance and Fluorescent Imaging of Brain Tumors

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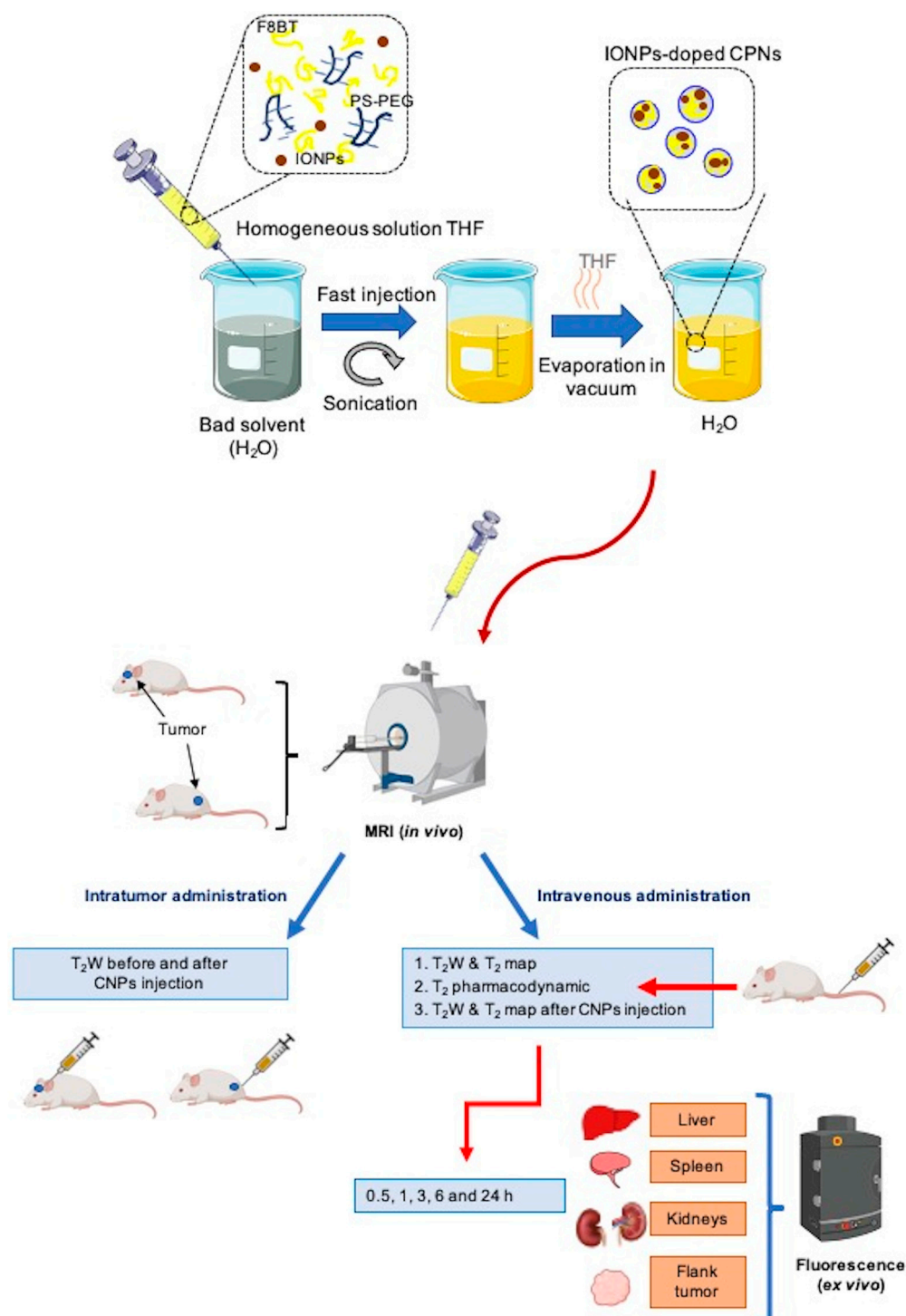
**Figure S1.** Overview of the experimental workflow followed in the preparation, characterization, in vitro and in vivo evaluation of the IONP-doped CPNs. The scheme shows the steps to synthesize and purified the IONP-doped-CNPs in the upper panels and the preclinical assessment of the nanoparticles in the lower.
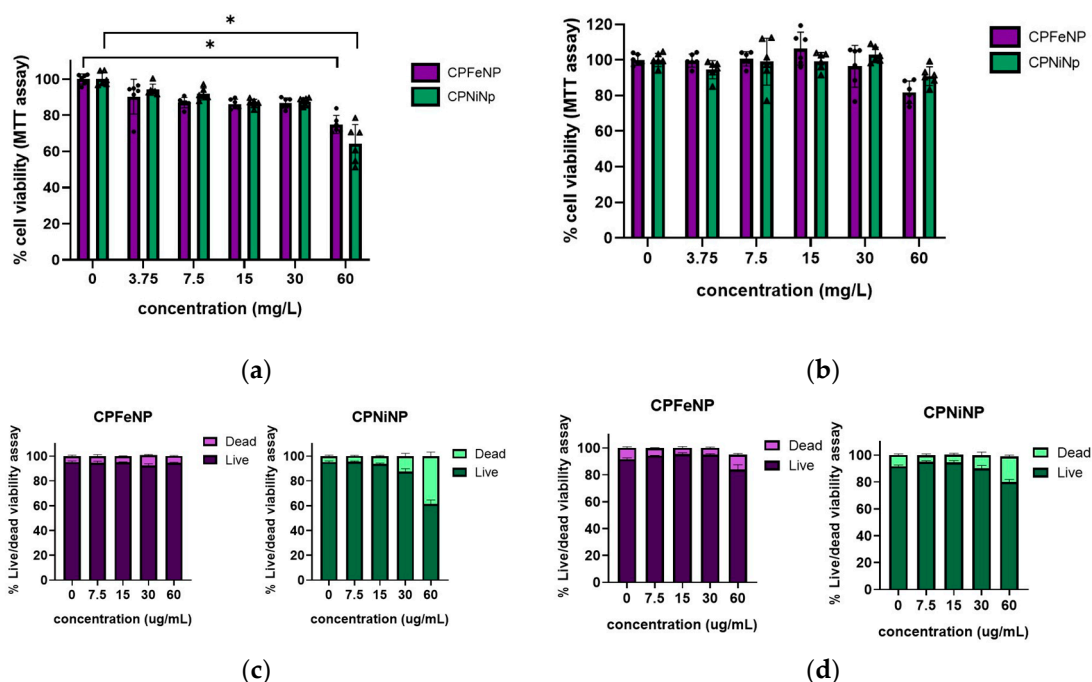


**Figure S2.** Biocompatibility evaluation of IONP-doped CPNs in GBM human cell lines. MTT viability quantification for (**a**) U-87 MG and (**b**) T98G. Cell viability quantified by flow cytometry using LIVE/DEAD cell viability assays for (**c**) U-87 MG and (**d**) T98G. (*$p < 0.05$).
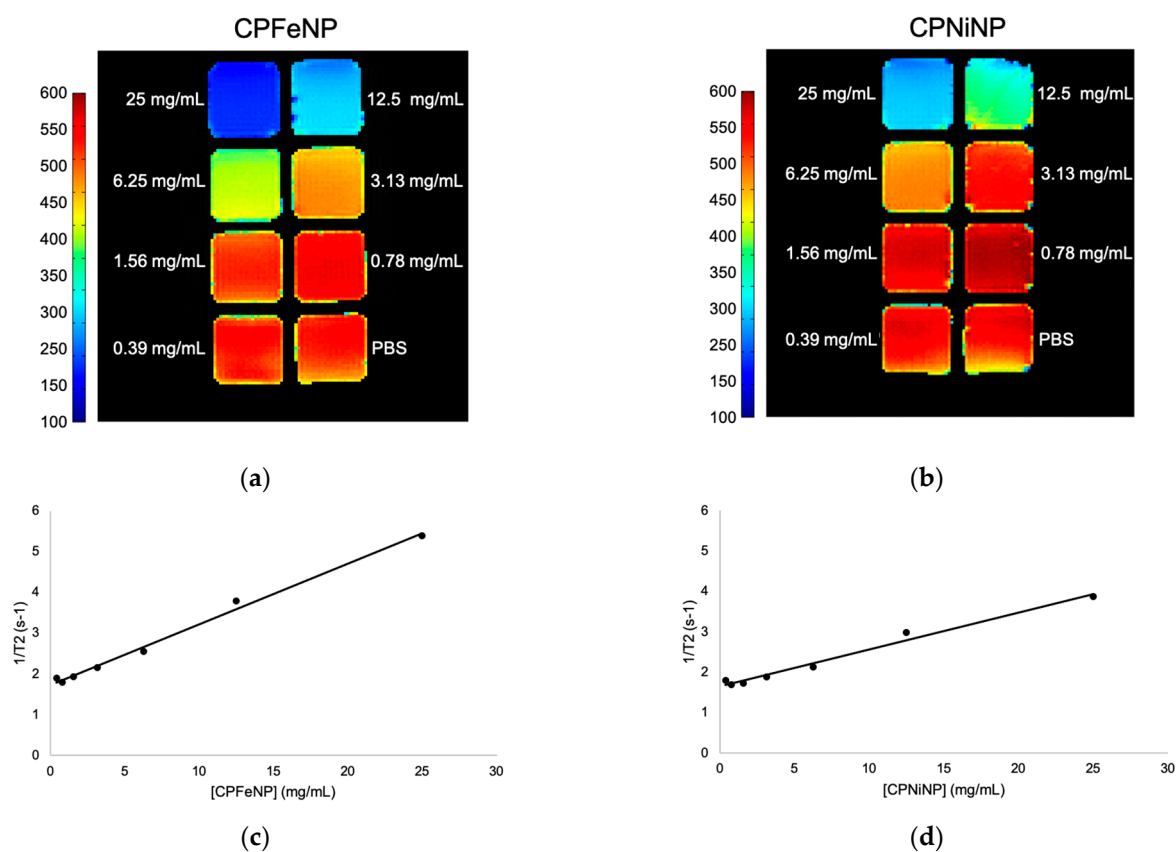
**Figure S3.** Relaxivities determination of IONP-doped CPNs. $T_2$ maps of nanoparticles at decreasing concentrations: (**a**) CPFeNP and (**b**) CPNiNP. Graphical linear regression of relaxation rates ($1/T_2$) versus increasing nanoparticle concentration: (**c**) CPFeNP and (**d**) CPNiNP.
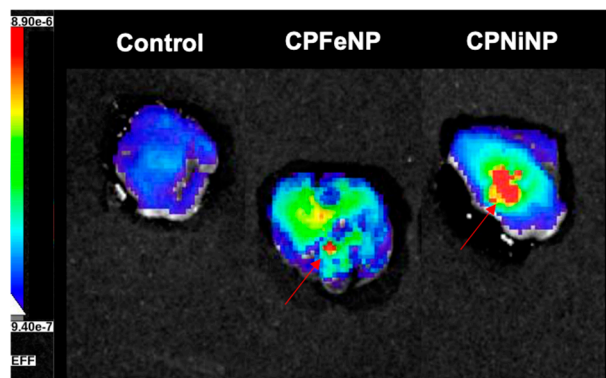


**Figure S4.** Fluorescence images of tumors after i.t. IONP-doped CPN administration. Images show excised tumors from a control mouse without particle administration (left), a flank tumor after CPFeNP i.t. injection (middle) and a tumor after CPNiNP i.t. injection. Red arrows point to the high fluorescence point due to IONP-doped-CPNs injection.
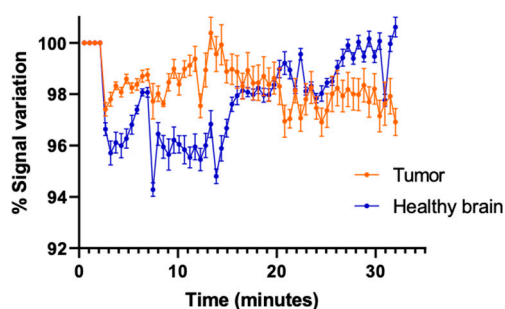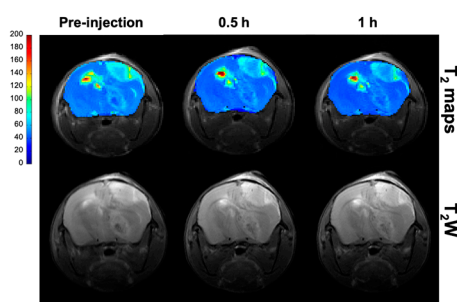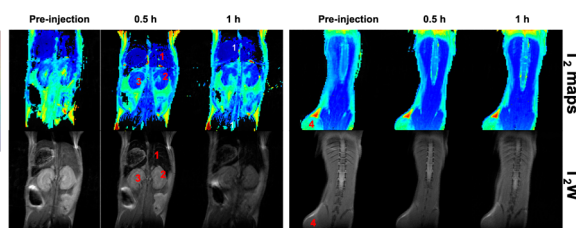


(**a**)
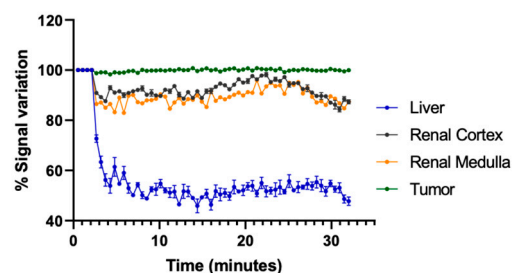


(**b**)
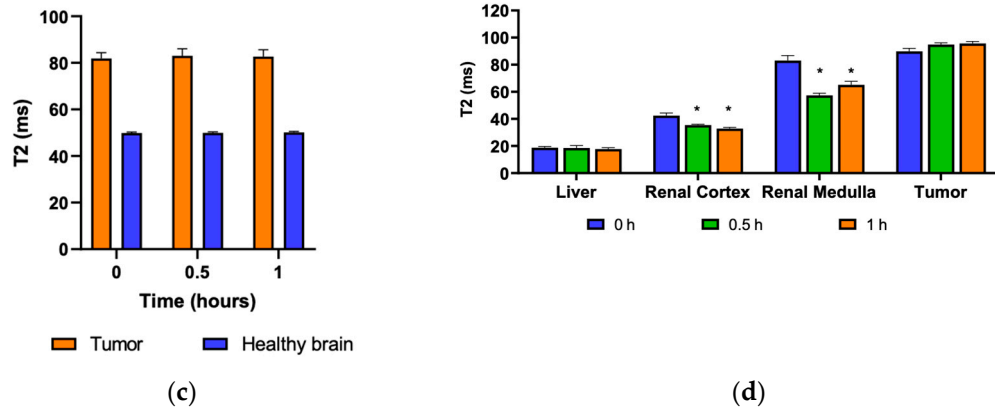
**Figure S5.** Biodistribution MRI studies of Endorem®. Graphics show the variation in MRI signal intensity (mean ± SEM) in the different organs evaluated: tumor and contralateral healthy brain in the orthotopic glioma model (**a**) and liver, renal cortex, renal medulla and tumor in the heterotopic GBM model (**b**).



(**a**)



(**b**)

(**c**)



(**d**)

**Figure S6.** T2 maps and T$_2$W images of GBM models injected with Endorem®. T2 color-code based maps and T$_2$W images acquired before the i.v. administration of Endorem®, and at 0.5 and 1 h after the injection, in the of the orthotopic (**a**) and the heterotopic GBM model (**b**), numbers indicate the tissues where the ROIs were selected to do the measurements: 1, liver; 2, renal cortex; 3, renal medulla; 4, flank-tumor. Graphics show the variation in T$_2$ values (mean ± SEM) of the ROIs along the temporal evaluation in the orthotopic (**c**), and heterotopic tumor bearing mice (**d**). Statistical symbols correspond to the comparison of the data to the pre-injection value. (*$p < 0.05$).