Cu-doped extremely small iron oxide nanoparticles with large longitudinal relaxivity; one-pot synthesis and *in vivo* targeted molecular imaging

*Irene Fernández-Barahona[†], Lucía Gutiérrez[‡], Sabino Veintemillas-Verdaguer[¨], Juan Pellico[†], María del Puerto Morales[¨], Mauro Catala[§], Miguel A. del Pozo[§], Jesús Ruiz-Cabello[¦], Fernando Herranz[†]****

[†] Instituto de Química Médica, CSIC, Juan de la Cierva 3, 28006 Madrid; Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) and CIBERES. Spain.
[‡] Departamento de Química Analítica, Instituto de Nanociencia de Aragón, Universidad de Zaragoza, Instituto de Ciencia de Materiales de Aragón (ICMA/CSIC) y CIBER-BBN. 50018 Zaragoza, Spain
[¨] Instituto de Ciencia de Materiales de Madrid, CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco, 28049 Madrid, Spain
[§] Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)
[¦] CIC biomaGUNE, Paseo de Miramón 182, 20014 Donostia - San Sebastián, Spain; Ikerbasque, Basque Foundation for Science, 48013 Bilbao, Spain; Universidad Complutense de Madrid and Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES). 28029 Madrid. Spain

SUPPORTING INFORMATION

Mice tumor allografts model

Tumor allografts. We have used a murine breast adenocarcinoma cell line (EO771) cultured in DMEM+ 10% Newborn calf serum (Gibco, Life Technologies). Female C57BL6 mice, 8 weeks old, were bred and housed under pathogen-free conditions in our animal facilities at CNIC. Prior to injection, tumor cells were trypsin detached, washed twice, and resuspended in PBS to a final concentration of 106 cells/13 μL. The cell suspension was then mixed with 5-μL growth factor–reduced Matrigel (BD Biocoat) and 2-μL trypan blue solution (Sigma Aldrich) and maintained on ice until injection. Mice were anesthetized with 5% Isofluorane (Abbott), laid on their backs, and injected with 20-μL cell suspension in Matrigel directly in the fourth mammary fad pad through the nipple with a Hamilton syringe. Tumor growth was monitored weekly using digital callipers, and tumor volume was calculated according to the formula: L × W2/2 = mm3. Imaging studies were performed after 5 weeks after implant when tumors reached.

Table S1. Physicochemical data of prepared nanoparticles

sample	Core size (nm)	H_D (nm)	Saturation magnetization (emu g^{-1} Fe+Cu)	r1 ($mM^{-1}s^{-1}$) [Fe+Cu]	r2 ($mM^{-1}s^{-1}$) [Fe+Cu]	r2/r1	RGD/NP	Zeta potential (mV)
IONP	4.2 ± 1.0	15.0 ± 1.0	82.3 ± 0.3	11.9 ± 0.3	22.9 ± 1.3	1.9	-	36.1 ± 1.0
Cu1.7-NP	3.2 ± 0.9	7.7 ± 0.6	76.6 ± 0.3	13.6 ± 0.3	32.1 ± 1.4	2.4	-	-34. 1 ± 0.7
Cu4-NP	3.5 ± 0.8	16.1 ± 0.7	56.2 ± 0.3	15.7 ± 0.6	32.8 ± 1.0	2.1	-	-33. 2 ± 0.4
Cu28-NP	4.4 ± 2.9	16.0 ± 1.5	43.8 ± 0.4	8.5 ± 1.3	20.1 ± 0.9	2.4	-	-33. 6 ± 0.5
IONP-RGD	-	37.3 ± 2.5	-	-	-	-	16.1	-12.0 ± 0.2
Cu4-NP-RGD	-	31.9 ± 2.9	-	-	-	-	15.9	-12.9 ± 0.3

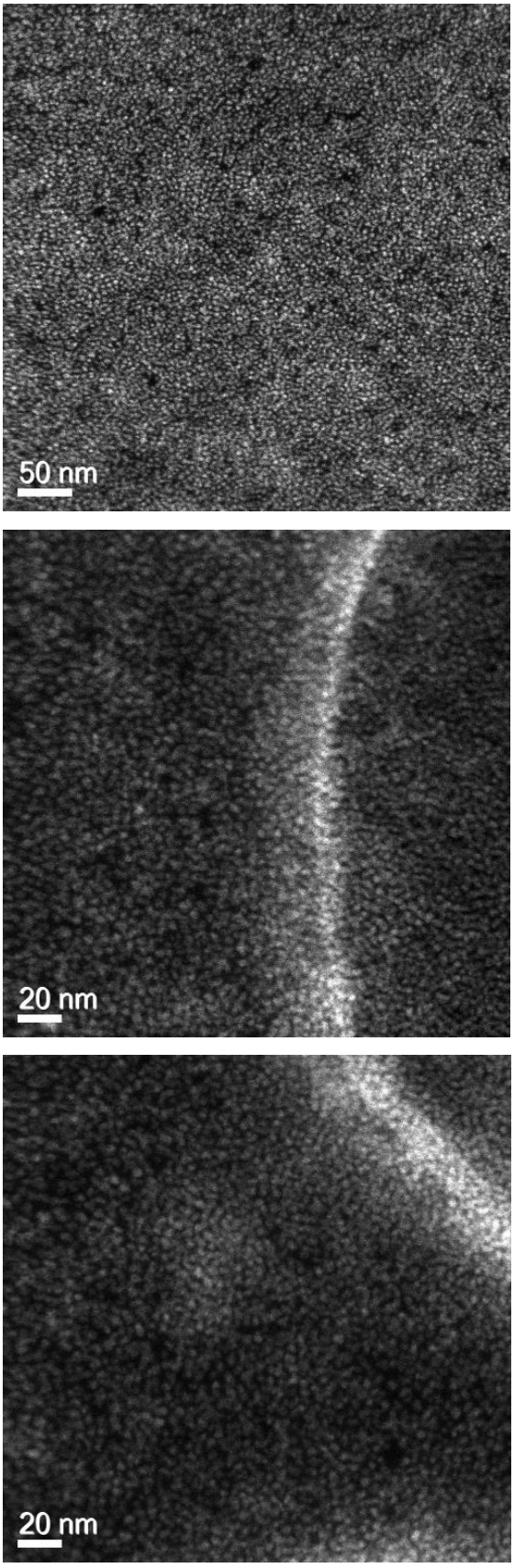

Figure S1. STEM-HAADF images of **Cu1.7-NP.**

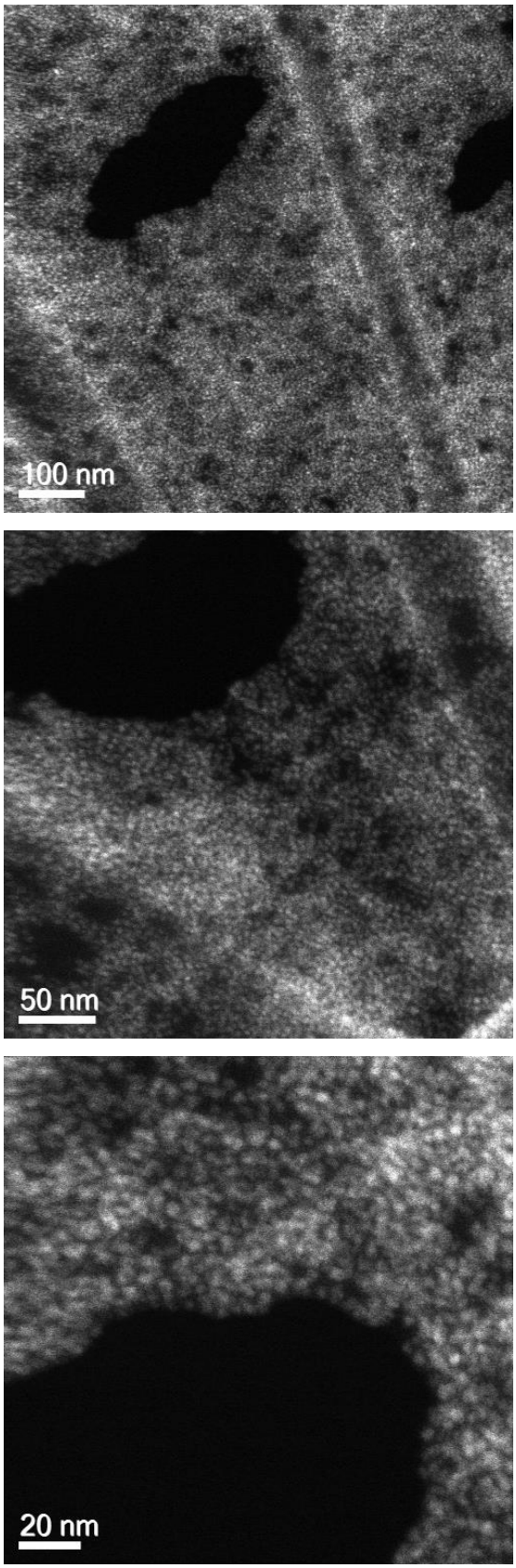

Figure S2. STEM-HAADF images of **Cu4-NP.**

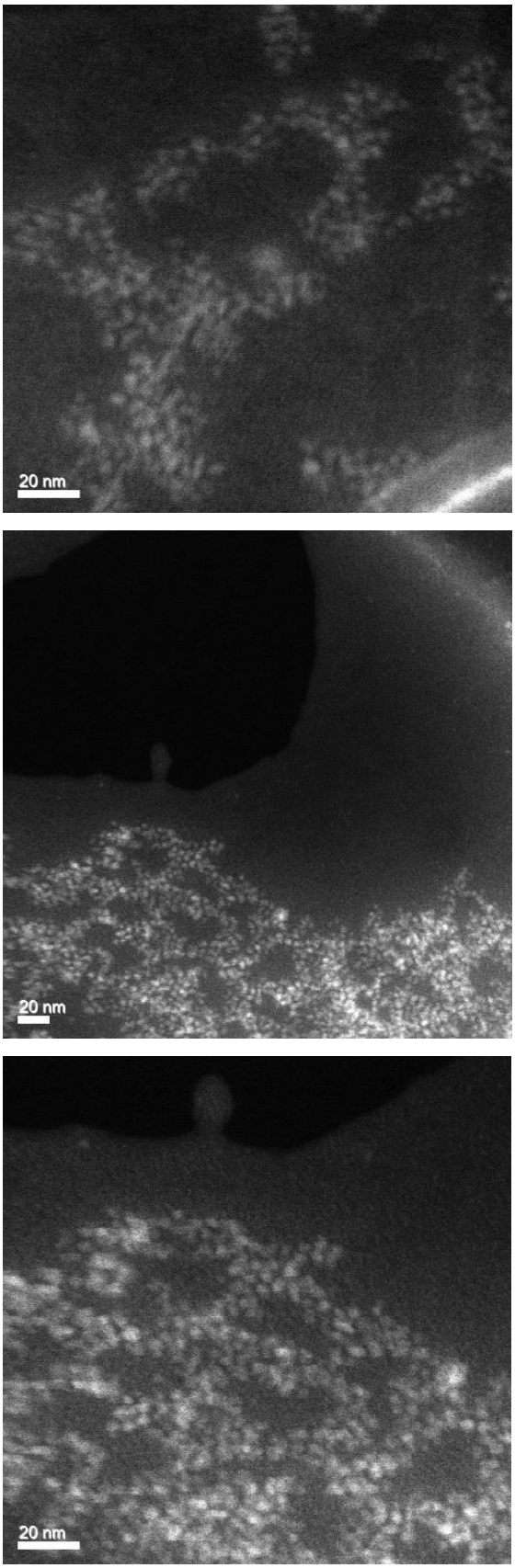

Figure S3. STEM-HAADF images of **Cu28-NP.**

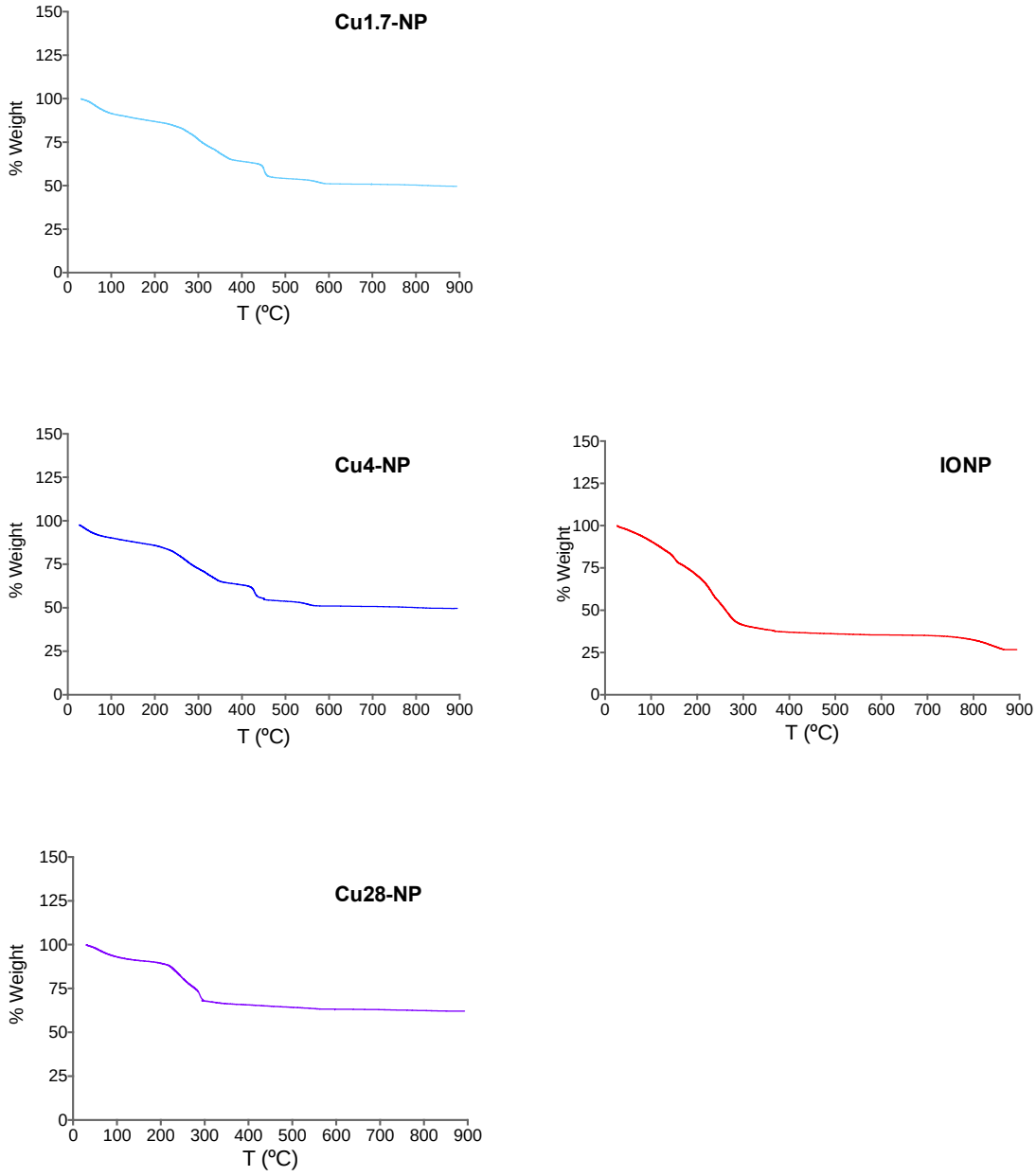

Figure S4. TGA curves for **Cu1.7-NP**, **Cu4-NP**, **Cu28-NP** and **IONP**.

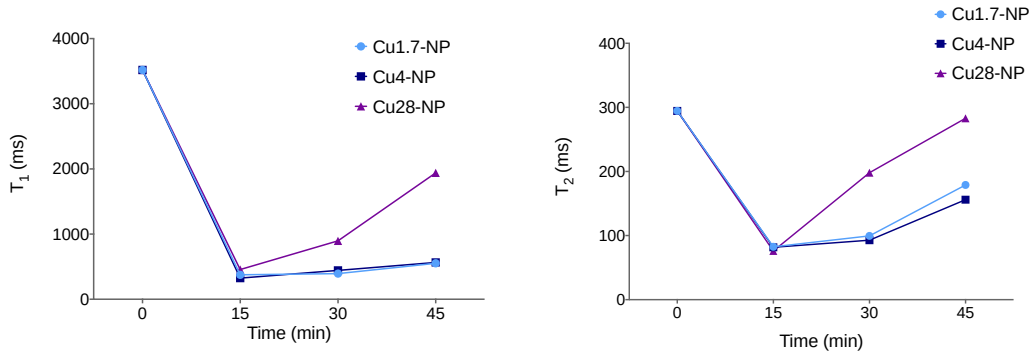

Figure S5. T1 and T2 values measured by relaxometry in mice blood samples after the injection of **Cu1.7-NP**, **Cu4-NP** and **Cu28-NP**.

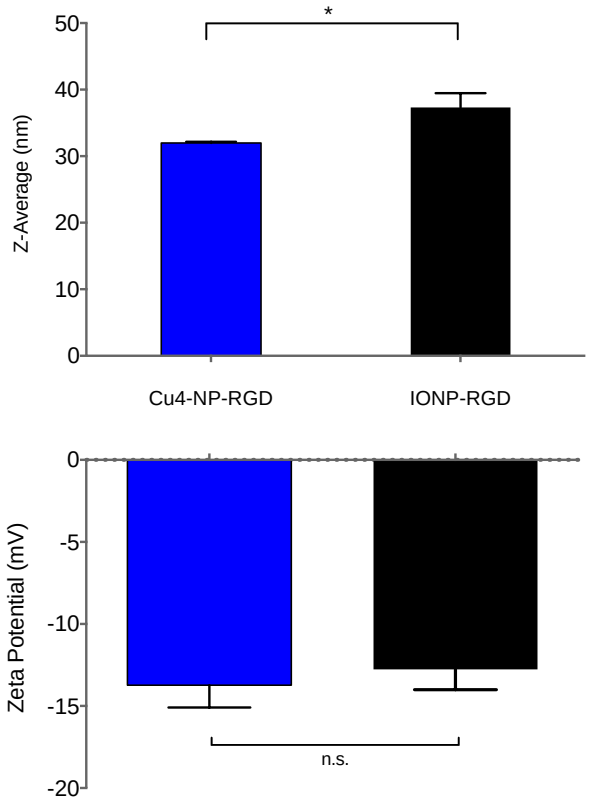

Figure S6. Z-average and zeta potential values for Cu4-NP-RGD and IONP-RGD.

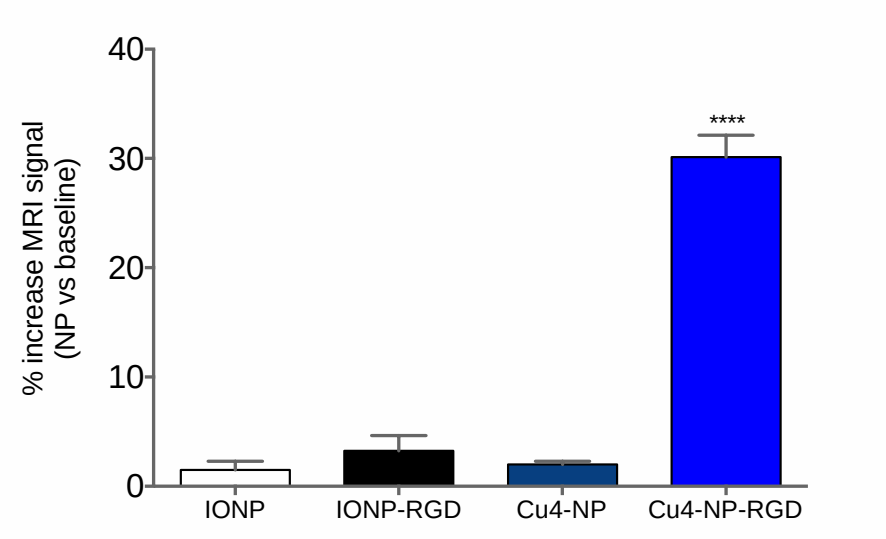

Figure S7.

Percentage of increase in MRI signal intensity, before and after, the injection of **IONP**, **IONP-RGD**, **Cu4-NP** and **Cu4-NP-RGD**.