Electronic Supplementary Material (ESI) for Materials Advances.

Supplementary Information

Europium-doped Hybrid Nano-complexes: a Potential Strategy for Metastasis Prevention in Osteosarcoma

Yu-Chi Wang[a, *], Fu-I Tung[b, c, *], Zheng-Ying Sung[a], Mei-Hsiu Chen[d, e], Ming-Hong Chen[f], Tse-Ying Liu[a]

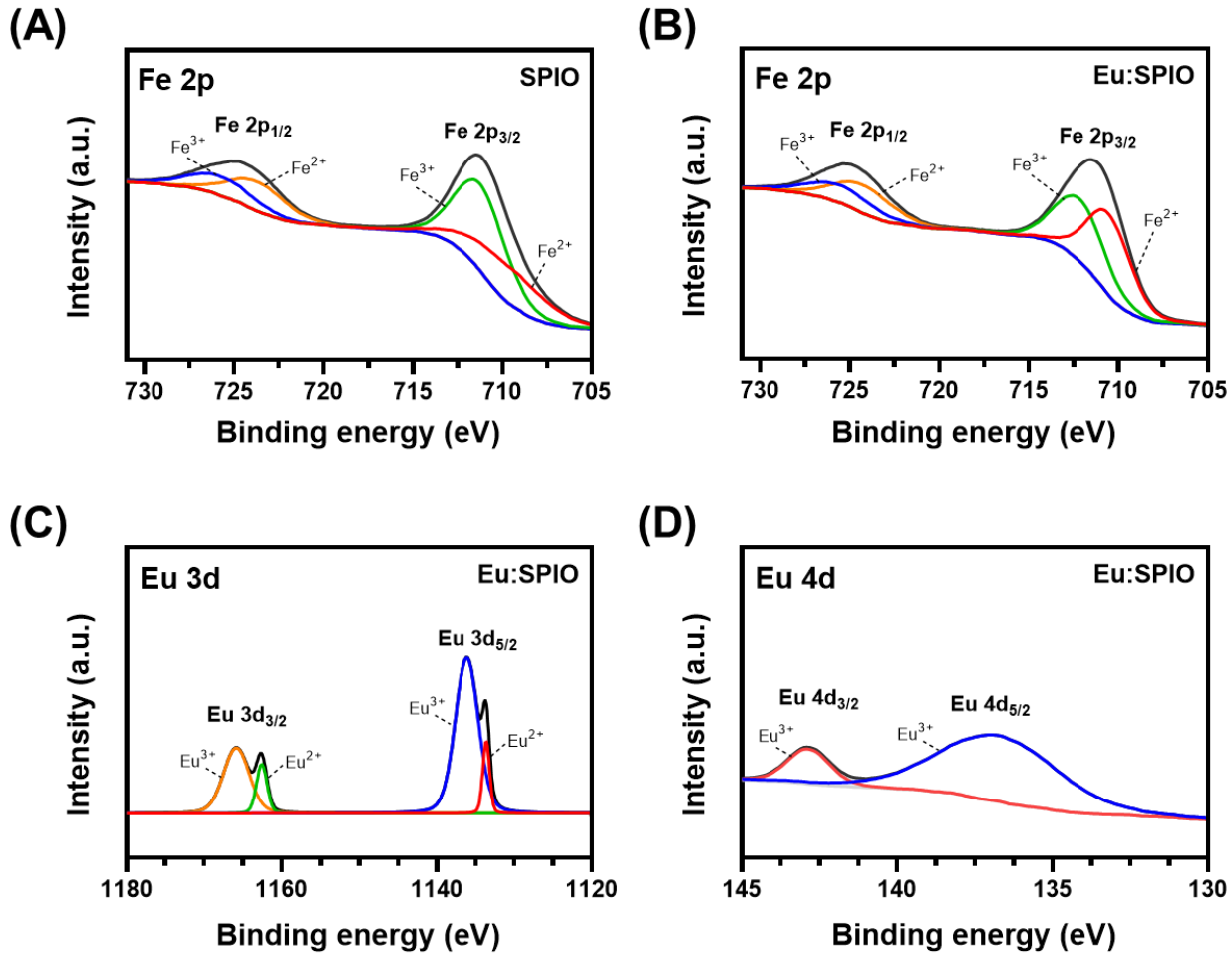

Fig. S1 High-resolution XPS spectra of Fe 2p in (A) SPIO and (B) Eu:SPIO nanocrystals, and (C) Eu 3d and (D) Eu 4d in Eu:SPIO nanocrystals.

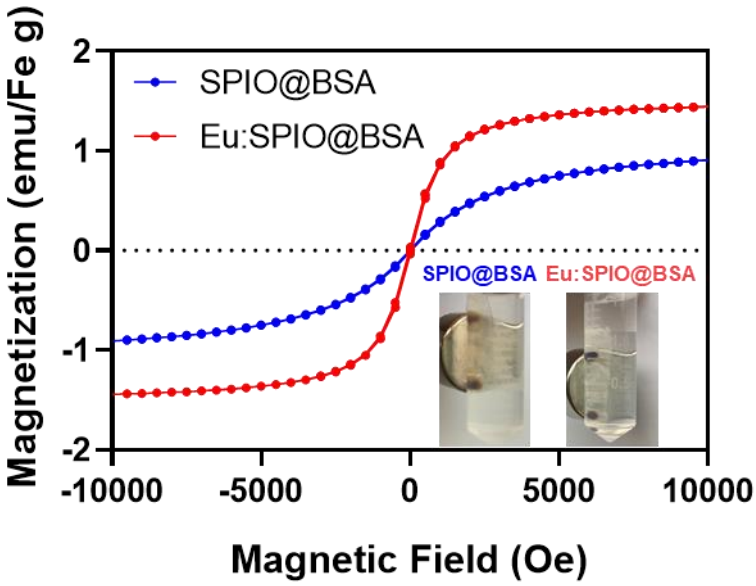

Fig. S2 Magnetization–magnetic field strength (M–H) curves of SPIO@BSA and Eu:SPIO@BSA complexes were normalized based on their Fe content.

Table S1 Atomic percentage (at%) of SPIO nanocrystals, Eu:SPIO nanocrystals, SPIO@BSA NPs, and Eu:SPIO@BSA NPs. The amount of Fe and Eu elements in the SPIO nanocrystals, Eu:SPIO nanocrystals, SPIO@BSA NPs, and Eu:SPIO@BSA NPs were calculated using the data from inductively coupled plasma mass spectrometry (ICP-MS).

	Fe	**Eu**
SPIO	100	0
Eu:SPIO	87.11	12.89
SPIO@BSA	100	0
Eu:SPIO@BSA	90.75	9.25

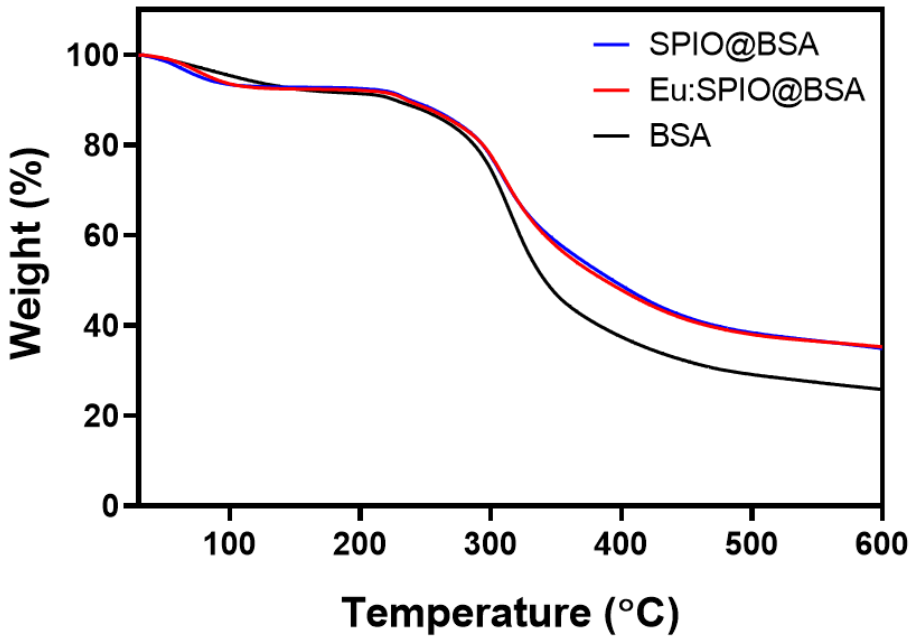

Fig. S3 Thermogravimetric analysis (TGA) curves of SPIO@BSA complexes, Eu:SPIO@BSA complexes, and BSA. The weight loss of nanovehicles and BSA was recorded in the temperature range of 30–600 °C with a heating rate of 10 °C/min under a nitrogen atmosphere.

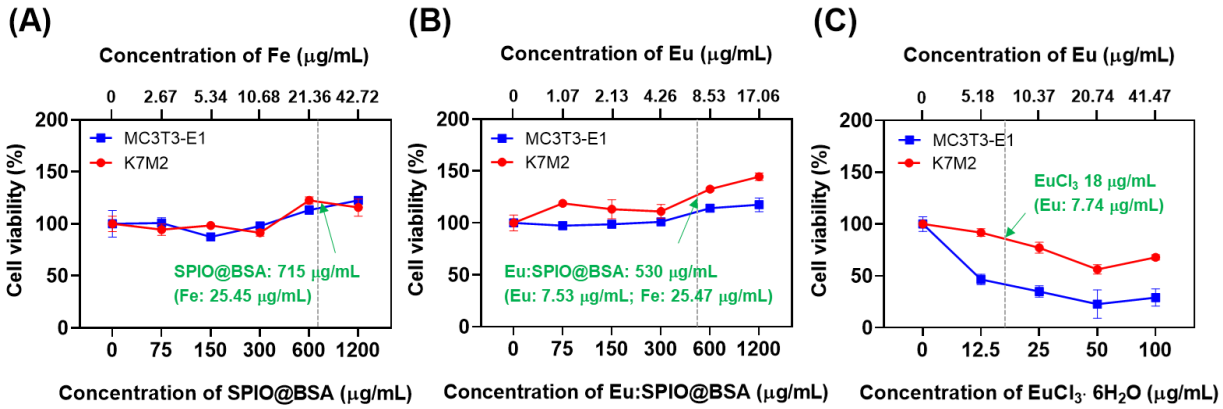

Fig. S4 Effect of (A) SPIO@BSA complexes, (B) Eu:SPIO@BSA complexes, and (C) $EuCl_3 \cdot 6H_2O$ on cell viability of K7M2 and MC3T3-E1 cells at 24 h. Cell viability was evaluated using the PrestoBlue cell viability reagent.

In vitro cell viability assay

The MC3T3-E1 and K7M2 cells (3×10^4 cells per well) were seeded in 24-well plates and incubated for 24 h. Fresh media containing different concentrations of SPIO@BSA complexes (0–1200 μg/mL), Eu:SPIO@BSA complexes (0–1200 μg/mL) and $EuCl_3 \cdot 6H_2O$ (0–100 μg/mL) was incubated with cells for 24 h. After removing the medium, cells were washed twice with PBS and incubated with 5% Presto Blue reagent for 30 min. The resulting solution was detected at 560 nm excitation and 590 nm emission wavelengths using a Tecan Infinite 200 plate reader (Infinite® 200 PRO, TECAN).

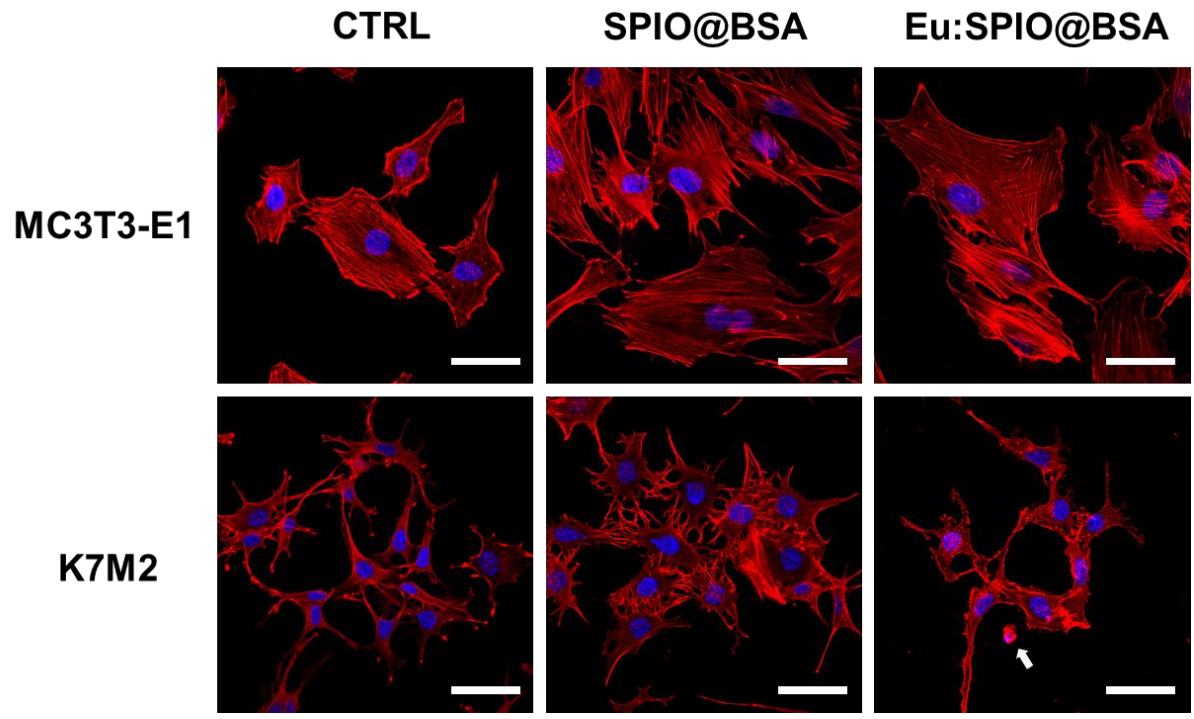

Fig. S5 Confocal images of MC3T3-E1 and K7M2 cells stained with DAPI (blue) and Alexa Fluor™ 568 Phalloidin (red). The main objective of this experiment was to showcase the natural cell morphologies without interference from the signals of SPIO@FITC-BSA and Eu:SPIO@FITC-BSA nano-complexes (depicted as the green signals in **Fig. 2**). Comparing the data in **Fig. 2** and **Fig. S5** reveals a noticeable change in cell morphology, attributed to the uptake of Eu:SPIO@FITC-BSA complexes (indicated by the white arrow). Scale bar = 50 μm.

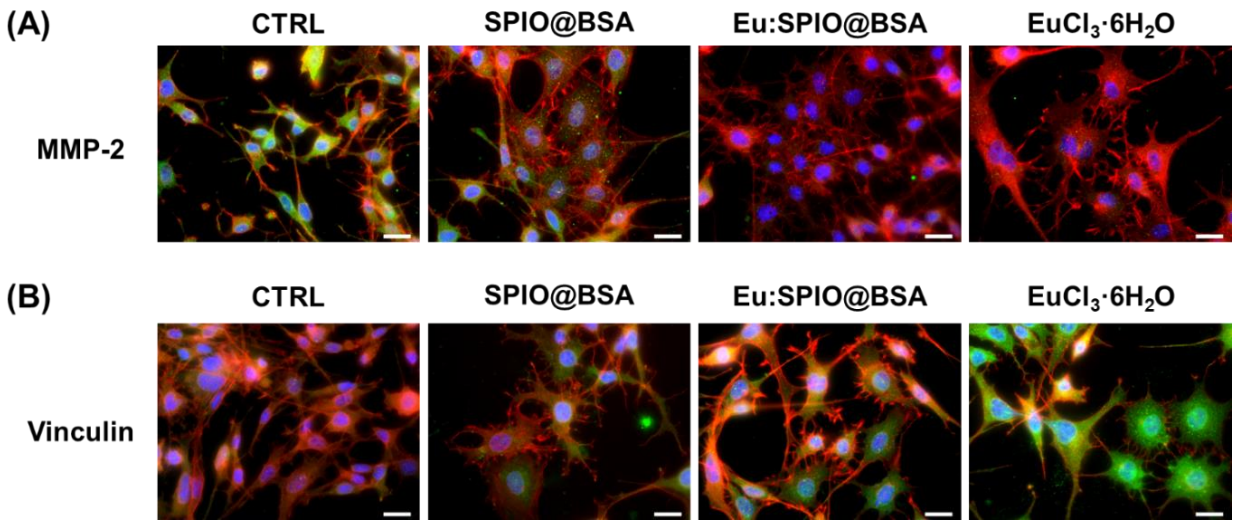

Fig. S6 The expression of (A) matrix metalloproteinase 2 (MMP-2) and (B) vinculin in K7M2 cells. The cells were incubated with SPIO@BSA complexes, Eu:SPIO@BSA complexes, and $EuCl_3 \cdot 6H_2O$ for 24 h, and the expression of MMP-2 and vinculin was analyzed using immunofluorescence staining. Nuclei and actin cytoskeleton were stained with DAPI (blue) and Alexa 594-labeled Phalloidin (red), respectively. MMP-2 and vinculin were stained with Alexa 488-labeled Phalloidin (green). Scale bar = 40 μm. Images of the cells were captured using a digital camera interfaced with a fluorescence microscope.

Immunofluorescence assay

To observe the expression of MMP-2 and vinculin, K7M2 cells were seeded onto 18 mm glass slides in 12-well culture plates at a density of 5×10^4 cells per well. The cells were then incubated with or without samples (i.e., SPIO@BSA complexes, Eu:SPIO@BSA complexes, and $EuCl_3 \cdot 6H_2O$) for 24 h. The concentrations of SPIO@BSA, Eu:SPIO@BSA complexes, and $EuCl_3 \cdot 6H_2O$ were 715 μg/mL, 530 μg/mL, and 18 μg/mL, respectively. The concentrations of SPIO@BSA and Eu:SPIO@BSA complexes were selected for the similar Fe content. In addition, Eu:SPIO@BSA complexes and $EuCl_3 \cdot 6H_2O$ with the similar Eu content were selected. After 24 h, the cells were scratched with a 1 mL pipette tip and cultured for 24 h. Cells were washed twice with PBS, fixed with 4% paraformaldehyde for 15 min, and then permeabilized with Triton X-100 for 5 min. Samples were stained with primary antibodies MMP-2 (GTX104577) and vinculin (GTX113294) (1:500) at 4 ℃ overnight and Alexa Fluor 594 fluorescence secondary antibody (Abcam, ab150080) for 1 h at room temperature. Finally, the samples were stained with Fluoroshield DAPI (GeneTex, GTX30920) and Alexa Fluor 488 conjugated phalloidin (A12379). The distribution and expression of the biomarkers (MMP-2 and vinculin) were observed using a fluorescence microscope.

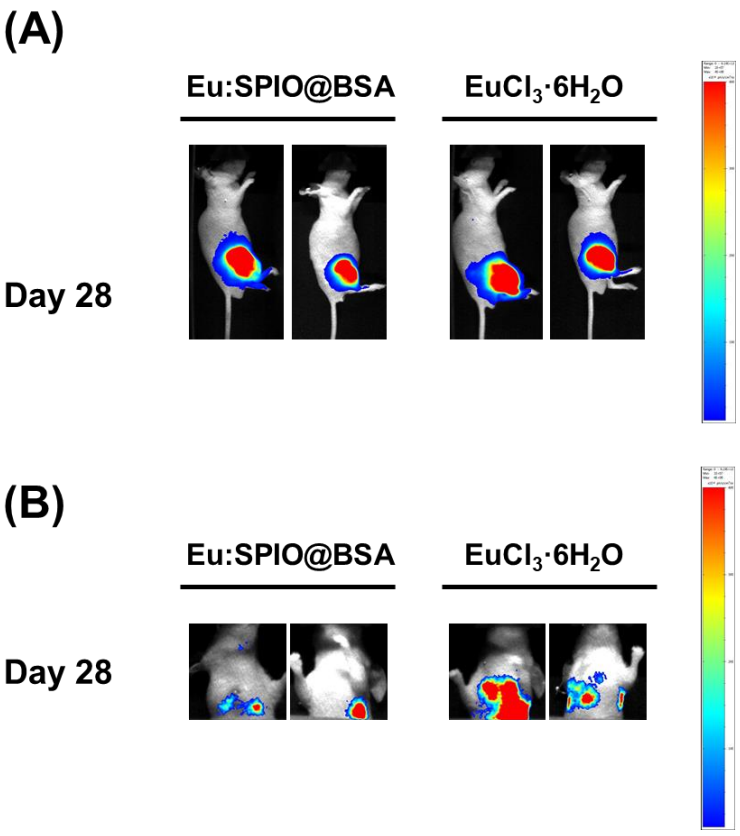

Fig. S7 Therapeutic efficacy of Eu:SPIO@BSA complexes and $EuCl_3 \cdot H_2O$ in mice on day 28. The *in vivo* imaging system (IVIS) images of (A) the leg and (B) the lungs.