Electronic Supplementary Information (ESI)

Europium–Tannic acid Nanocomplexes Devised for Bone Regeneration under Oxidative or Inflammatory Environments

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Supplementary Tables

Supplementary Table S1. Identification of the most relevant ATR-FTIR frequency assignments (cm^{-1}) of TA and EuTA NCs.

Frequency Assignment	Wavenumber (cm^{-1})				
	TA	**EuTA 25%**	**EuTA 50%**	**EuTA 75%**	**EuTA 100%**
O–H stretching	3000-3500				
C=O stretching Carbonyl	1700	1705	1705	1704	1704
C=C stretching Aromatic	1606	1606	1606	1605	1605
C=C stretching H-symmetric Aromatic	1528	1505	1505	1504	1504
C–H bending Aromatic Asymmetric, and C–C stretching	1446	1445	1445	1444	1444
C−O stretching ring gallic	1319	1345	1345	1341	1344
Phenyl acetate, C−O bending, and C–H bending Aromatic Symmetric	1183	1199	1199	1195	1198
H–C=C–C bending and C–H deformation; Eu–O stretching	1086	1085	1083	1080	Overlap
C=C stretching deformation	1010	1037	1037	1032	1037
C–H bending Aromatic Out-plane	869	874	877	874	875
C–H bending Aromatic Out-plane Asymmetric	830	830	831	830	830
C–H bending Aromatic Out-plane Symmetric	746	755	756	755	756
Eu–O bending	-	583	586	615	616
Eu–O bending	-	539	536	537	539

Supplementary Table S2. Regression model and parameters calculated for Figure 5A.

[Agonist] vs. response -- Variable slope (four parameters)	TA	EuTA 25%	EuTA 50%	EuTA 75%	EuTA 100%
Bottom	17.62	12.48	12.34	13.25	13.23
Hillslope	3.066	5.844	3.219	3.439	3.629
Top	93.05	81.76	84.67	86.43	91.77
EC50	4.396	4.589	4.305	3.685	5.17
logEC50	0.6431	0.6617	0.634	0.5664	0.7135
Span	75.42	69.28	72.33	73.18	78.54
Degrees of Freedom	16	16	16	16	16
R squared	0.9979	0.9969	0.9943	0.9933	0.997

Supplementary Table S3. Regression model and parameters calculated for Figure 5B.

[Agonist] vs. response (three parameters)	TA	EuTA 25%	EuTA 50%	EuTA 75%	EuTA 100%
Bottom	0.1165	-0.4773	0.453	-0.1045	-0.03062
Top	67.3	56.34	62.69	72.92	54.37
EC50	0.7577	0.8763	1.014	1.264	1.134
logEC50	-0.1205	-0.05734	0.005842	0.1018	0.05456
Span	67.18	56.82	62.23	73.02	54.4
Degrees of Freedom	42	42	42	42	42
R squared	0.9874	0.9625	0.964	0.9846	0.9812

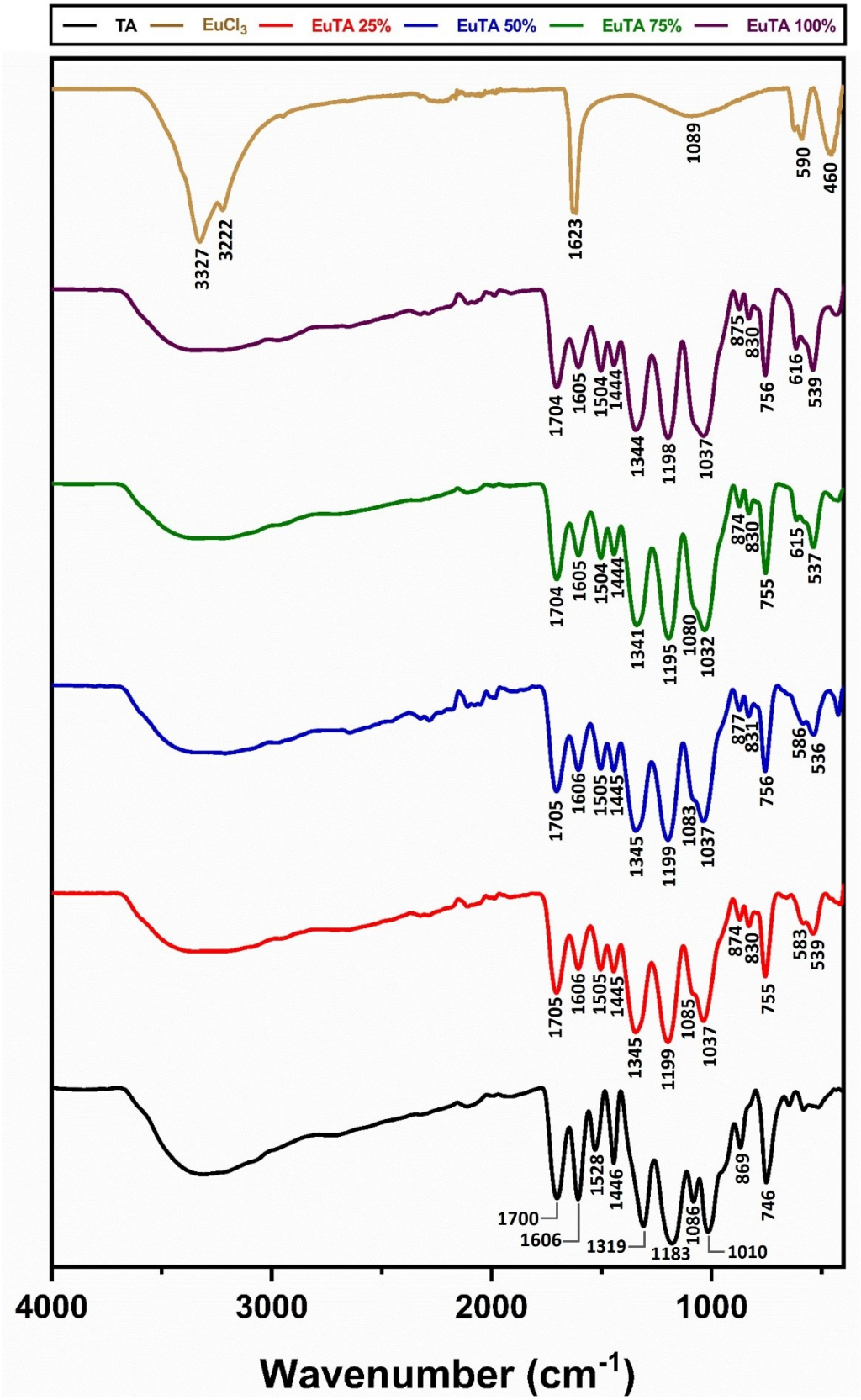
Supplementary Table S4. Regression model and parameters calculated for Figure 5D.

[Agonist] vs. normalized response -- Variable slope	TA	EuTA 25%	EuTA 50%	EuTA 75%	EuTA 100%
Hillslope	10.74	12.89	13.41	22.09	20.31
EC50	65.32	47.86	46.52	46.23	46.18
logEC50	1.815	1.68	1.668	1.665	1.664
Degrees of Freedom	14	17	14	15	15
R squared	0.9854	0.9926	0.9909	0.9953	0.9771

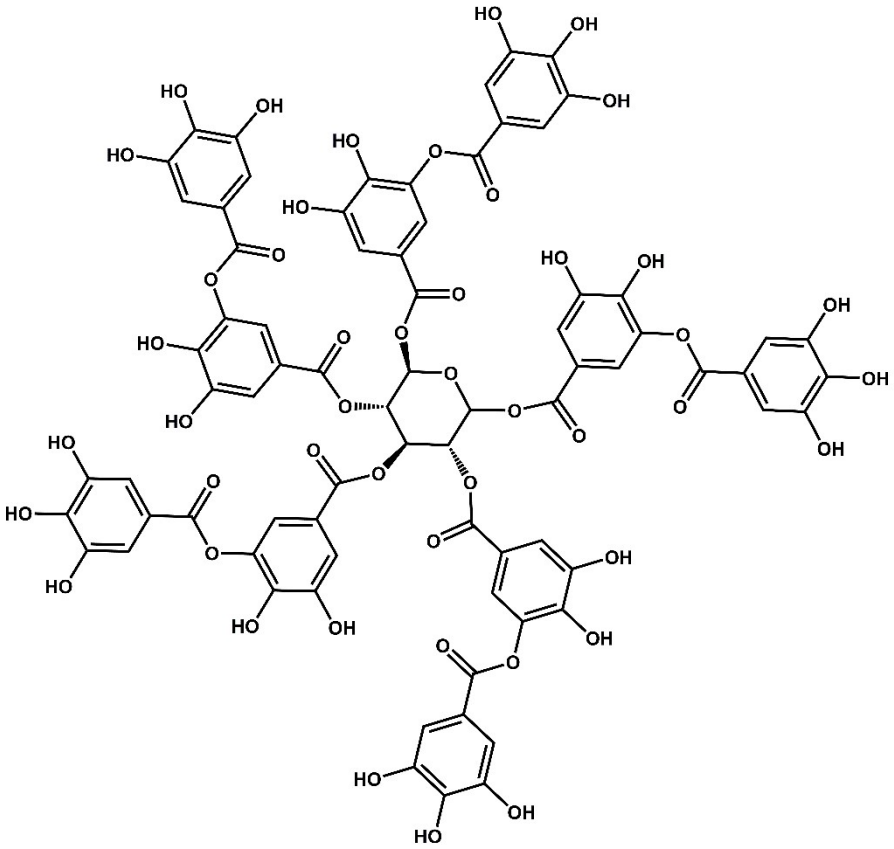
Supplementary Table S5. Regression model and parameters calculated for Figure 5E.

[Agonist] vs. response -- Variable slope (four parameters)	TA	EuTA 25%	EuTA 50%	EuTA 75%	EuTA 100%
Bottom	-1.419	0.09469	-0.9997	0.1277	0.06043
Hillslope	1.125	2.053	2.357	3.654	3.154
Top	87.82	83.35	76.57	77.21	77.72
EC50	864.8	239.8	182.2	174.8	150.2
logEC50	2.937	2.38	2.261	2.243	2.177
Span	89.24	83.26	77.57	77.09	77.66
Degrees of Freedom	17	17	17	17	17
R squared	0.9842	0.974	0.9893	0.9953	0.9908

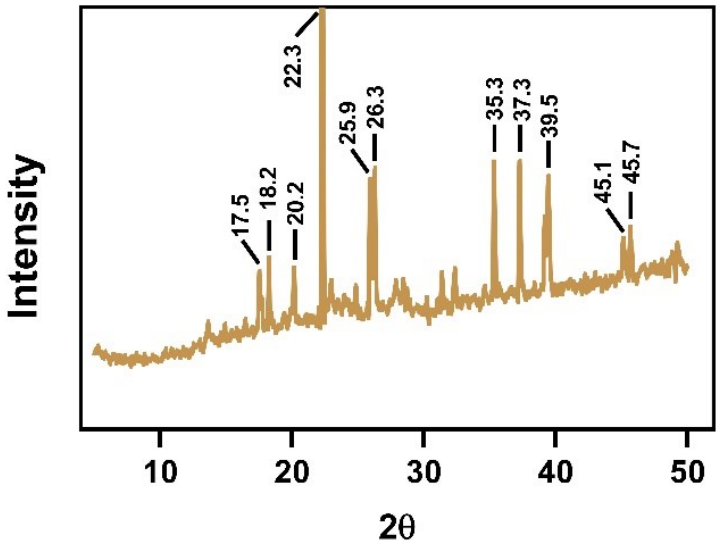
Supplementary Figures



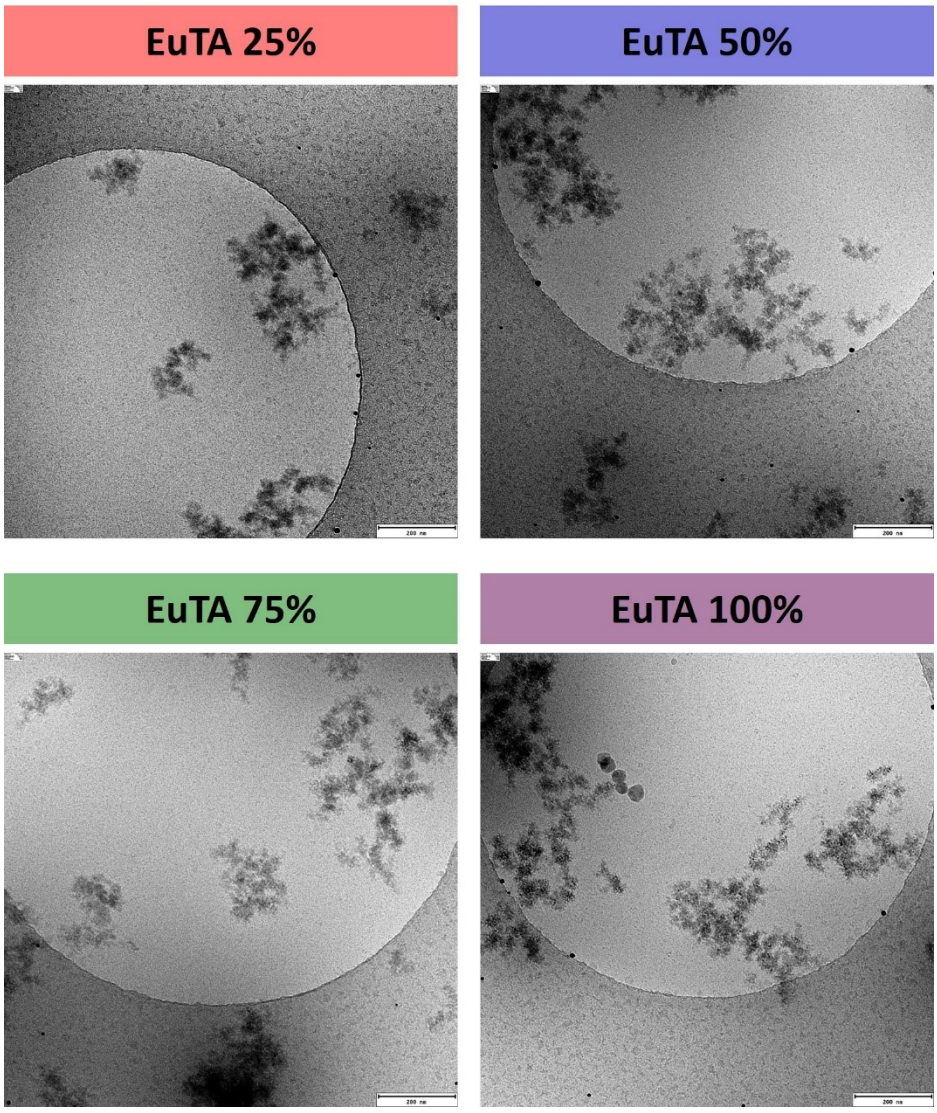
Supplementary Figure S1. ATR-FTIR spectroscopical characterization of TA, $EuCl_3$, and EuTA NCs (4000 to 400 cm^{-1}).



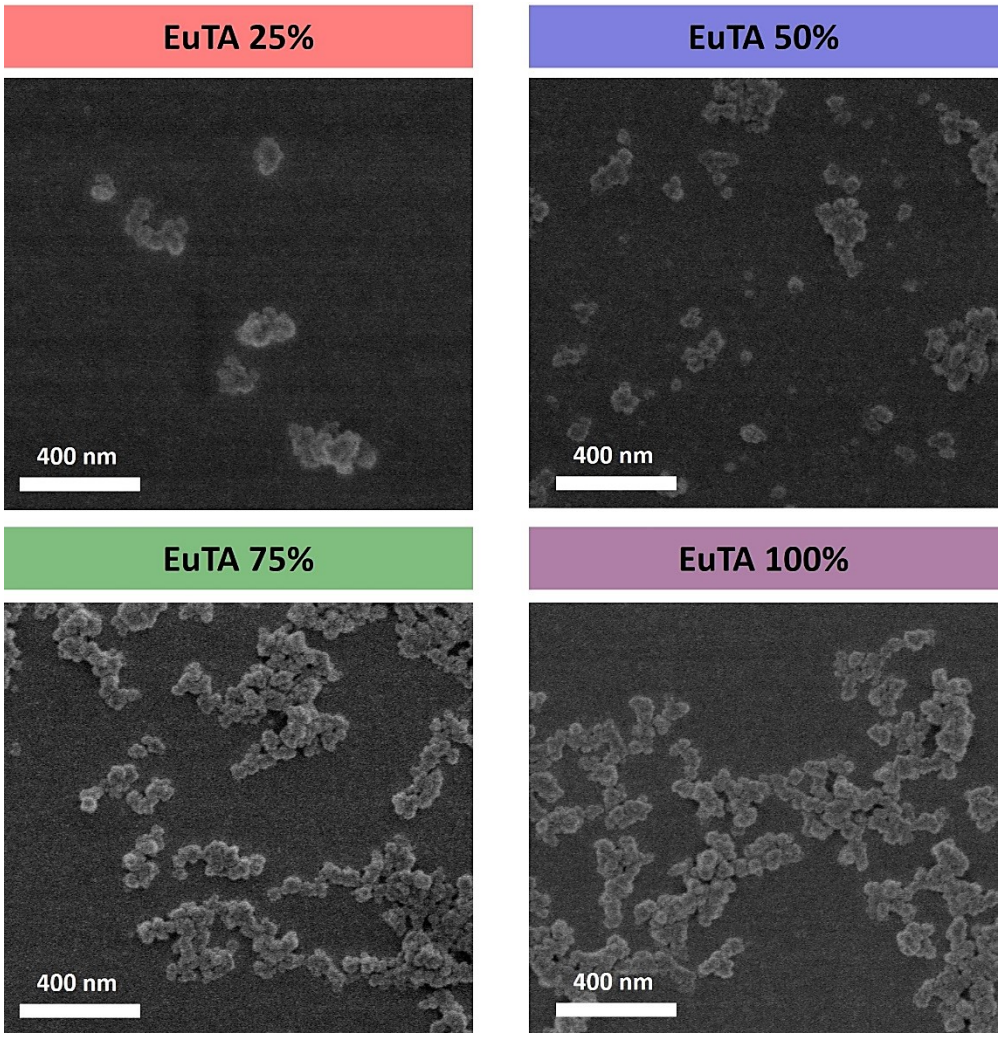
Supplementary Figure S2. Chemical structure of tannic acid.



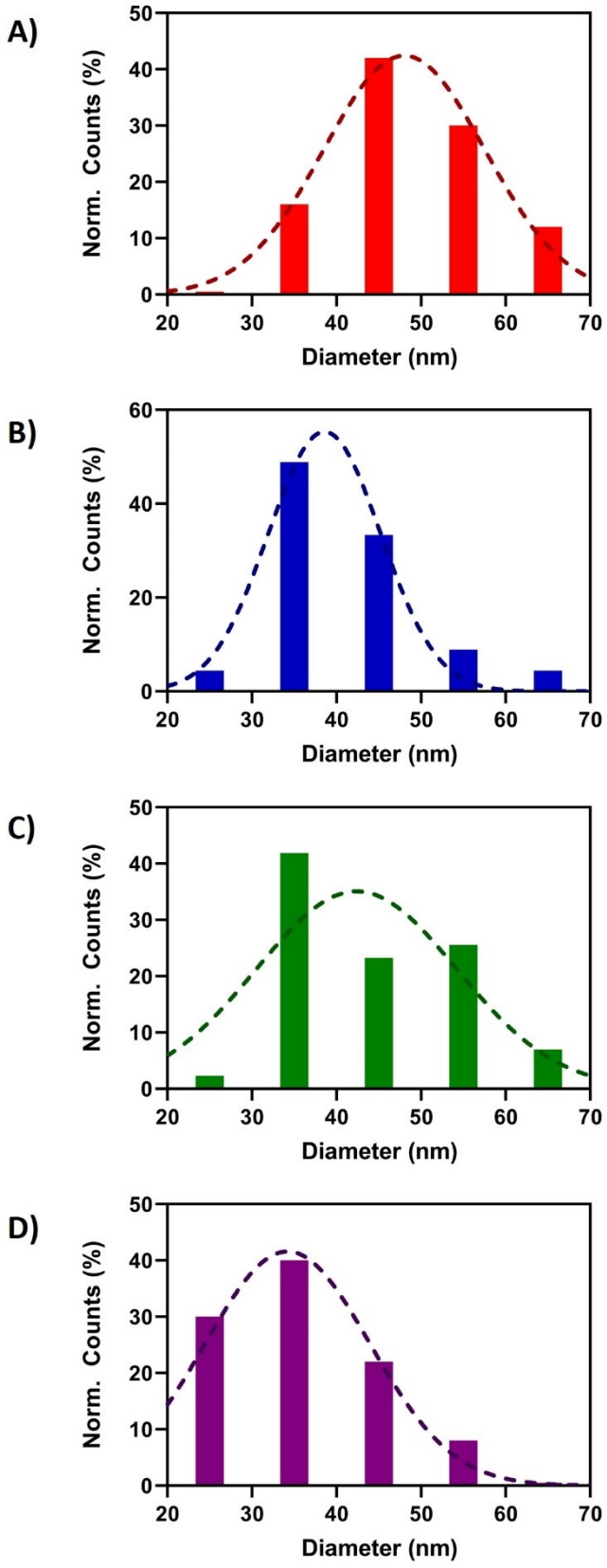
Supplementary Figure S3. X-ray diffractogram of $EuCl_3$.



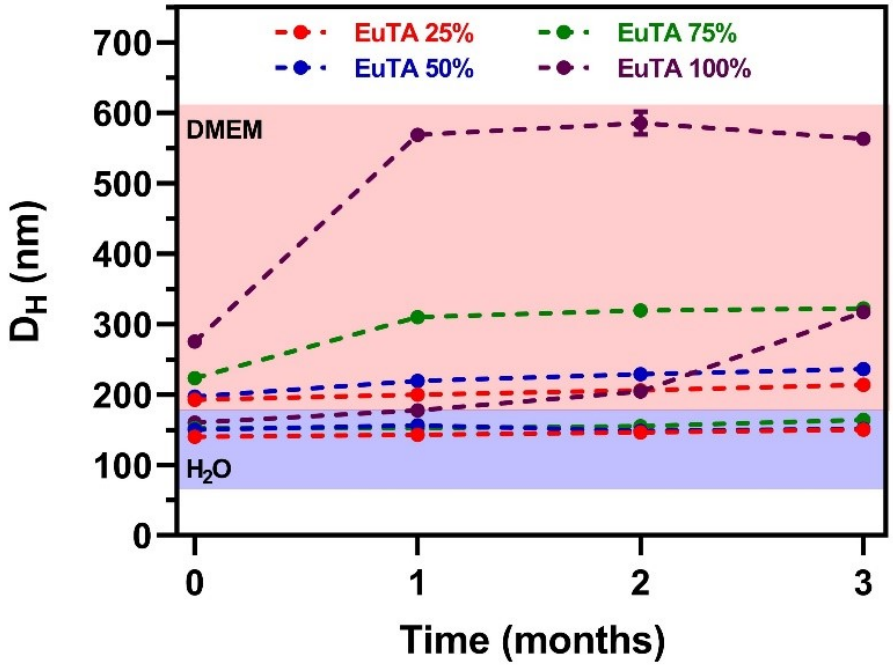
Supplementary Figure S4. Cryo-TEM images of EuTA NCs with different C. R. values. Black bars correspond to 200 nm.



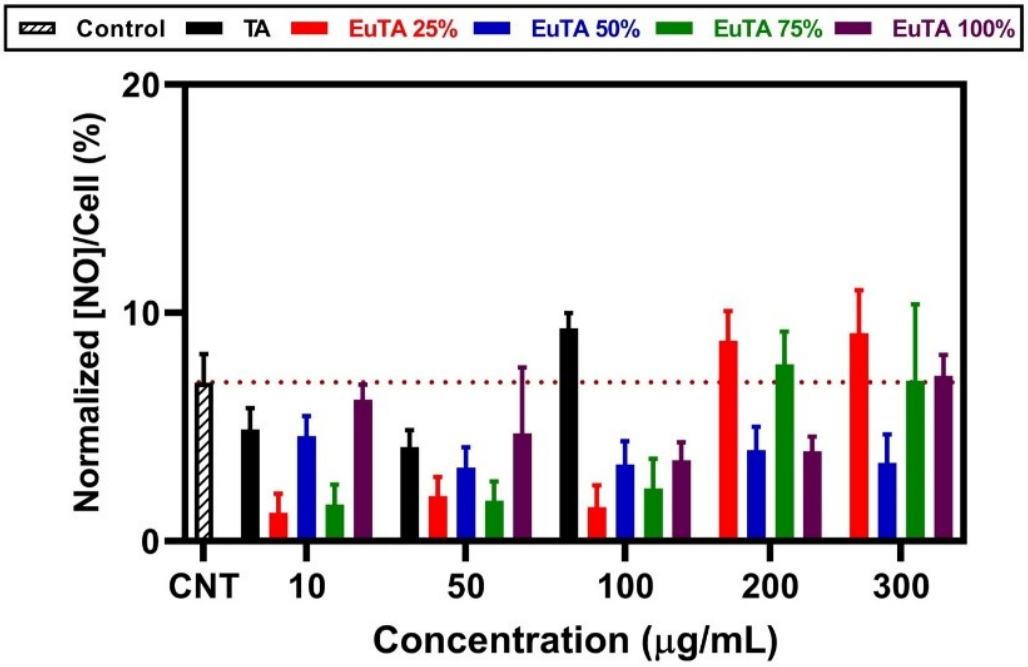
Supplementary Figure S5. FE-SEM micrographs of EuTA NCs with different C. R. values.



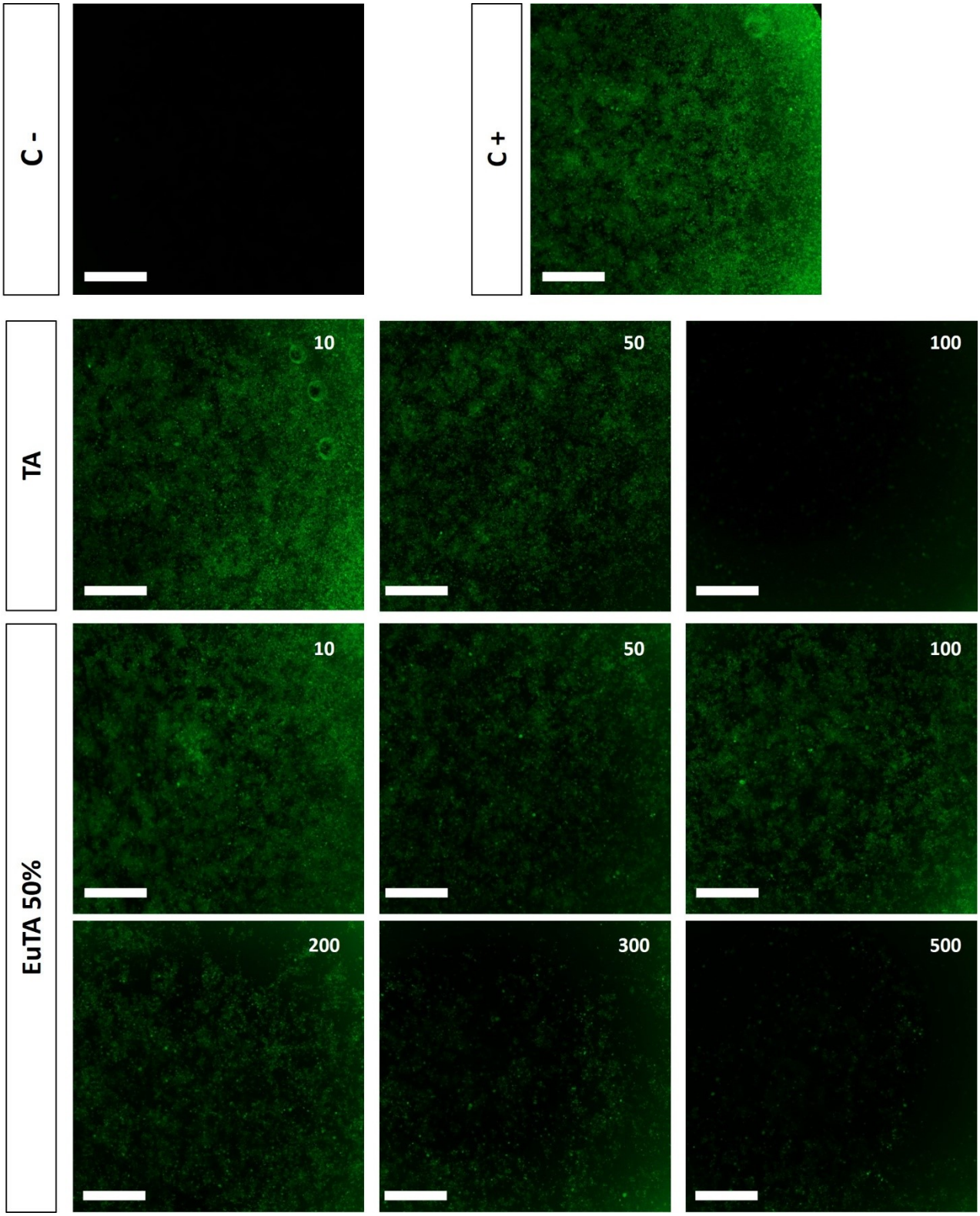
Supplementary Figure S6. Histograms depicting the size distribution of the nanoparticles that conform the EuTA NCs with different C. R. values: A) 25%, B) 50%, C) 75%, and D) 100%.



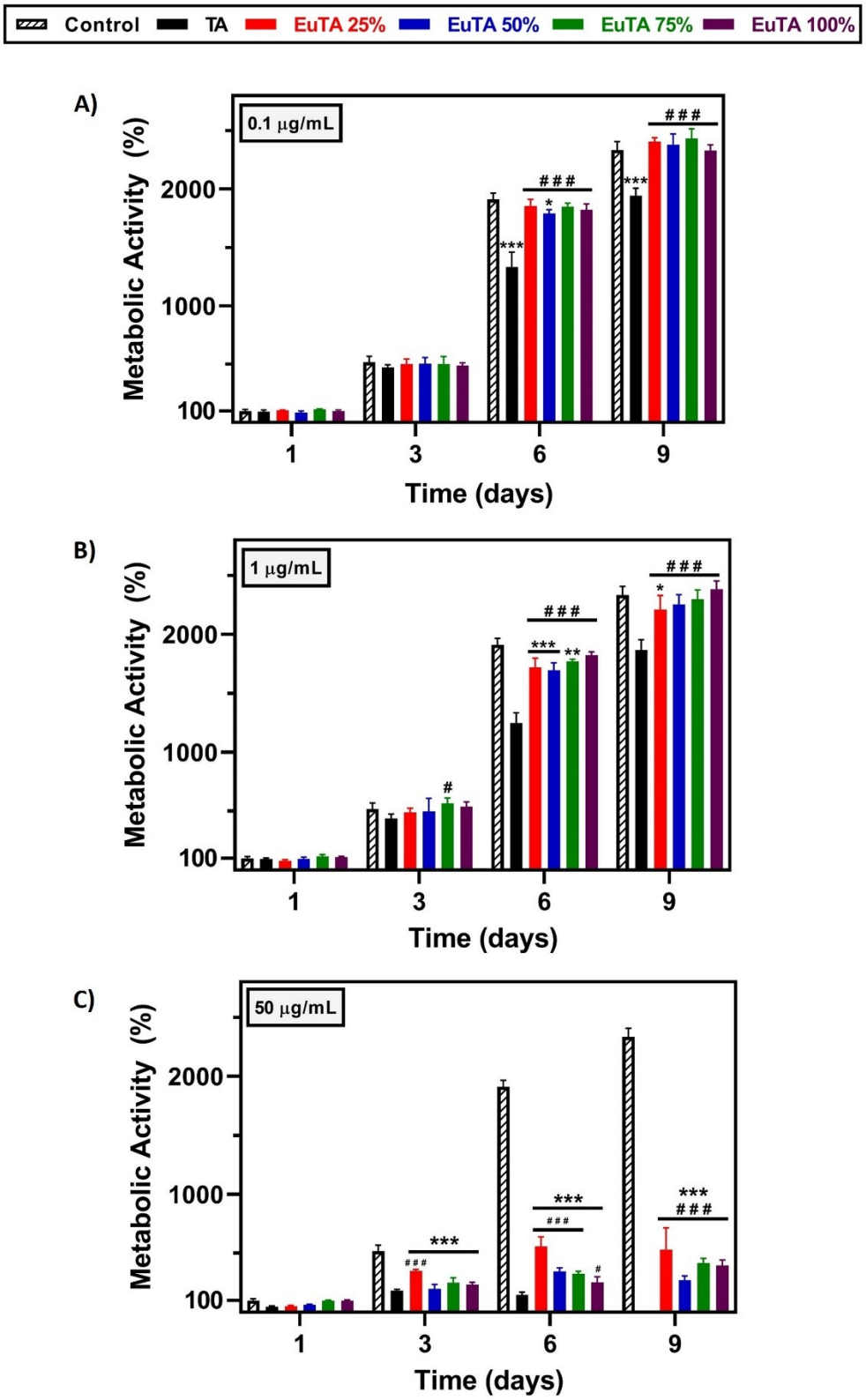
Supplementary Figure S7. Long-term stability study of EuTA NCs conducted in distilled water and DMEM for up to 3 months.



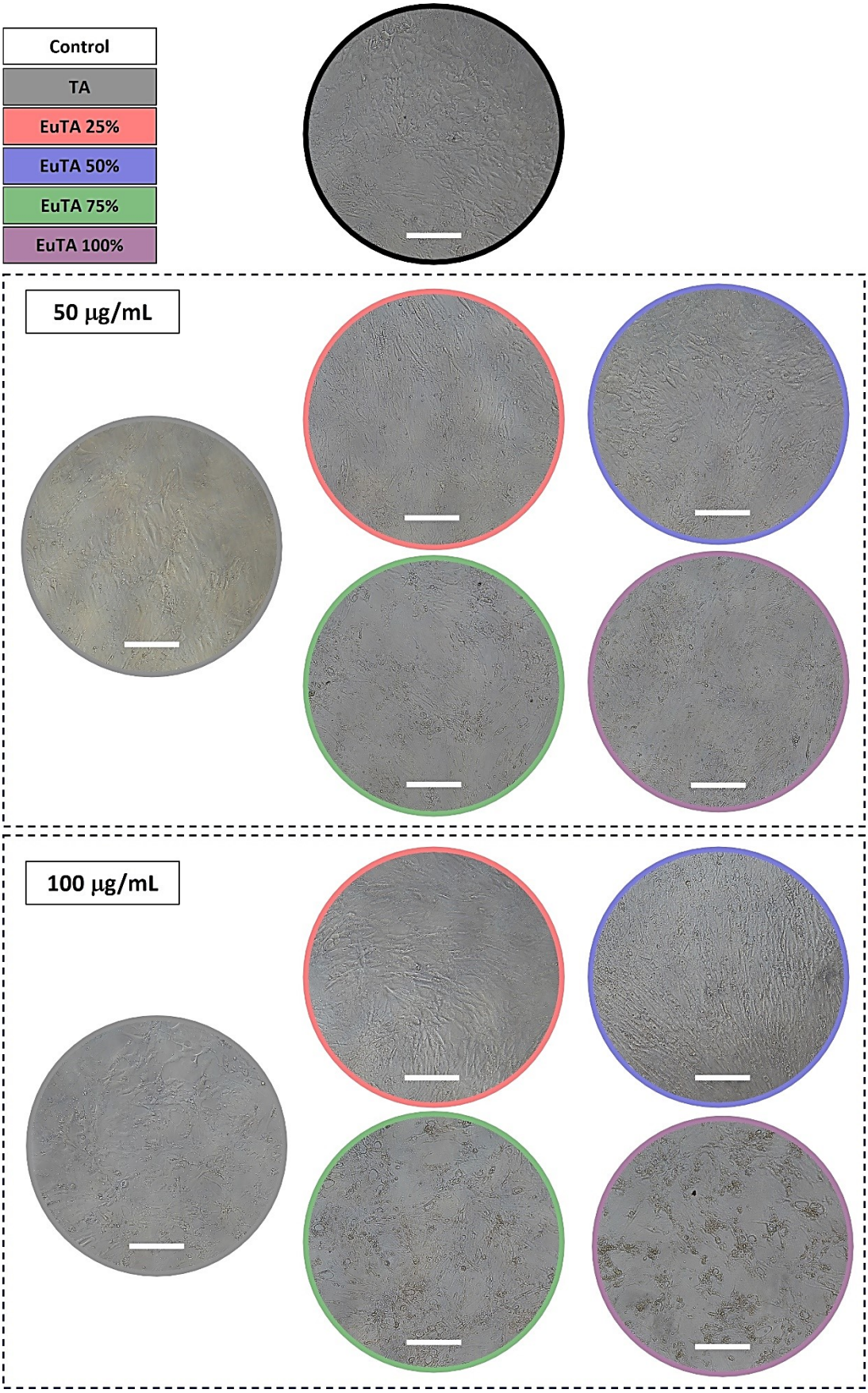
Supplementary Figure S8. Production of NO by resting macrophages treated with different concentrations of TA or EuTA NCs, and corrected attending to the metabolic activity of the cultures. Normalization was performed assuming a 100% release of NO by LPS-stimulated macrophages in the absence of any treatment.



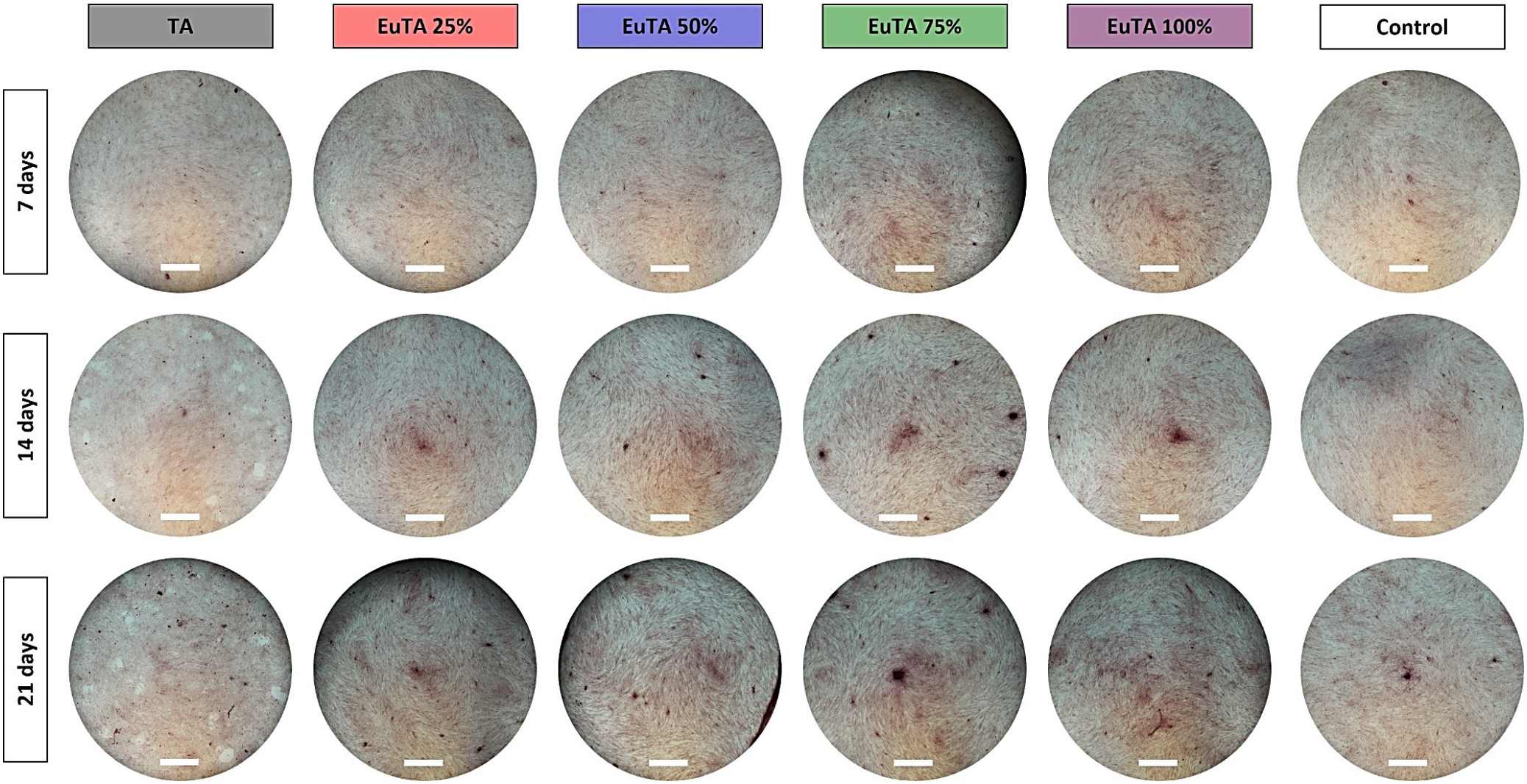
Supplementary Figure S9. Evaluation of the intracellular ROS scavenging activity of a 24-h pre-treatment with TA and EuTA NCs before a 15-minute exposure to 100 mM H_2O_2 by qualitatively assessing the fluorescence of DCFH-DA dye by fluorescence microscopy imaging. White bars correspond to 250 μm.



Supplementary Figure S10. Cell proliferation assay of independent cultures determined by monitoring the metabolic activity via alamarBlue measurements at different incubation times with 0.1, 1, and 5 μg/mL of TA or EuTA NCs. ANOVA: * and # symbols are used to compare conditions at the same experimental times with control and TA-treated cultures, respectively. Statistical significance: *,# $p < 0.05$; **,## $p < 0.01$; ***,### $p < 0.001$



Supplementary Figure S11. Phase contrast optical microscopy images of fHOB cultures following a three-day treatment with 50 or 100 μg/mL TA or EuTA NCs. White bars correspond to 250 μm.



Supplementary Figure S12. Phase contrast optical microscopy images of fHOB cultures treated with 10 μg/mL TA or EuTA NCs for different times and stained with AzR. White bars correspond to 250 μm.