ELECTRONIC SUPPLEMENTARY MATERIAL

Determination and localization of specific proteins in individual ARPE-19 cells by single cell and laser ablation ICP-MS using iridium nanoclusters as label

Paula Menero-Valdés[1], Ana Lores-Padín[1], Beatriz Fernández[1*], C. Derrick Quarles Jr.[2], Montserrat García[3, 4], Héctor González-Iglesias[5], Rosario Pereiro[1*]

[1]*Department of Physical and Analytical Chemistry, University of Oviedo, Julian Clavería 8, 33006 Oviedo, Spain.*

[2]*Elemental Scientific, Inc., 7277 World Communications Drive, Omaha, NE 68122, USA.*

[3]*Instituto Oftalmológico Fernández-Vega, Avda. Dres. Fernández-Vega, 34, 33012 Oviedo, Spain*

[4]*Department of Cellular Morphology and Biology, Faculty of Medicine, Julian Clavería, 33006 Oviedo, Spain.*

[5]*Department of Technology and Biotechnology of Dairy Products, Instituto de Productos Lácteos de Asturias, Consejo Superior de Investigaciones Científicas (IPLA-CSIC), Villaviciosa, Spain.*

*Corresponding authors email addresses: fernandezbeatriz@uniovi.es & mrpereiro@uniovi.es

DESCRIPTION OF THE SUPPLEMENTARY MATERIAL

This Electronic Supporting Material contains details about the Experimental section as well as some additional comments about Results and Discussion section.

The Experimental section describes the reagents employed for the synthesis and characterization of the IrNCs and IrNCs-immunoprobes, the culture and incubation of ARPE-19 cells, the immunocytochemistry (ICC) assays for the detection of target proteins by MS and fluorescence, the cellular preparation for sc-ICP-MS and LA-ICP-MS, as well as the ELISA analyses. Furthermore, protocols for the synthesis and characterization of IrNCs and IrNCs-immunoprobes are also included.

In the Results and Discussion section, results regarding the optimization of the glucose-induced oxidative stress treatment, the determination of proteins in individual ARPE-19 cells by sc-ICP-MS, as well as the bioimaging of APOE and claudin-1 in individual ARPE-19 cells by LA-ICP-MS are also collected.

EXPERIMENTAL

Reagents and Materials

(1) Synthesis of IrNCs and labelling of immunoprobes

For the synthesis of IrNCs, $IrCl_3 \cdot xH_2O$ (99.9% crystals, Sigma-Aldrich) was employed as metal precursor, $NaBH_4$ (98% powder, Sigma-Aldrich) as reducing agent, and lipoic acid (98% powder, Acros Organics) and tri-sodium citrate dihydrate (99% crystalline powder, Merck) as surface ligands. Ultrapure deionized water (18.2 MΩ cm, Purelab Flex 3 & 4) was used and pH was adjusted with NaOH (97%, Merck). Anti-h-APOE antibody (PA1-26902, produced in goat, Invitrogen, Thermo-Fischer Scientific) and Anti-h-claudin-1 antibody (H00009076-M02, produced in mouse, Abnova) were selected for the preparation of IrNCs immunoprobes following the carbodiimide strategy with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (98% powder, Acros Organics) and N-hydroxysuccinimide (NHS) (>98% powder, Sigma-Aldrich). IrNCs and labelled immunoprobes were purified with Amicon ultracentrifugal filter units (Merck) of 3 and 100 kDa pore size respectively.

(2) ARPE-19 cell line and culture conditions

Immortalized human retinal pigment epithelial cells ARPE-19 (ATCC) were cultured with Dulbecco's modified eagle medium/nutrient mixture F-12 (DMEMF12) (Sigma-Aldrich) supplemented with 10% (v/v) inactivated fetal bovine serum (Gibco, Thermo-Fischer Scientific) and 1% (v/v) P/S (penicillin/streptomycin) (Gibco, Thermo-Fischer Scientific) or in hybridoma serum free medium (Gibco, Thermo-Fischer Scientific) supplemented with 5% (v/v) L-glutamine (Gibco, Thermo-Fischer Scientific) and 1% (v/v) P/S. Cells subjected to stress conditions were treated with D-(+)-glucose (99.5%, Sigma-Aldrich) diluted in supplemented hybridoma. The viability of the cells was studied with a CyQuant™ Direct cell proliferation assay (C35011, Thermo-Fischer Scientific). For evaluation of the production of reactive oxygen species (ROS), cells were treated with H_2DCFDA (Invitrogen, Thermo-Fischer Scientific) dissolved in dimethyl sulfoxide. Cells treated with 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) (Sigma-Aldrich) were employed as positive control.

Cells were cultured in 96-well polystyrene microplates, Corning® cell culture flasks, or cultured in Nunc™ Lab-Tek™ chambers (0.8 cm^2/well, Sigma-Aldrich). These later ones were previously treated with poly-L-lysine (Sigma-Aldrich). Trypsin EDTA (Gibco, Thermo-Fischer Scientific) was employed to remove adherent cells. Cell fixation was done with paraformaldehyde (PFA) (VWR Chemicals). After fixation, cells were stored in all cases in 10 mM phosphate buffered saline (PBS) with pH 7.4 at 4 °C. All materials and reagents employed for cell culture were autoclaved.

(3) Immunoassays for the detection of proteins by MS and fluorescence

The immunoassays with IrNCs labelled immunoprobes were performed employing PBS as washing solution, Triton X-100 (Sigma-Aldrich) to permeabilize cellular membranes, donkey or goat serum (both from Sigma-Aldrich) (donkey serum for APOE and goat serum for claudin-1) and bovine serum albumin (BSA) (99% powder, Merck) to block non-specific interactions. For validation purposes, Alexa® fluor goat Anti-mouse-594 and Alexa® fluor donkey Anti-goat-594 (both from Thermo-Fischer Scientific) were employed as tags for immunofluorescence assays.

(4) Cellular preparation for sc-ICP-MS and LA-ICP-MS

After the immunoassay protocol, cells suspensions were diluted to 10^5 cells mL^{-1} in 50 mM Trizma® hydrochloride (99.9% crystals, Sigma-Aldrich) adjusting pH to 7.4 with hydrochloric acid (VWR Chemicals) for sc-ICP-MS analysis. Transport efficiency of the sample introduction system was daily checked with a citrate stabilized PtNPs standard (46 ± 3 nm, NanoComposix). Pt, Fe and Ir calibration was performed with ICP standards (1000 μg mL^{-1}, Sigma-Aldrich). In the case of LA-ICP-MS measurements, the samples did not require more preparation steps after performing the immunoassay.

(5) ELISA kits for APOE and claudin-1 determination in cells lysates

The average concentration of the proteins in control and GL-treated ARPE-19 cells was also determined by commercial ELISA kits from Cloud-Clone Corp. for APOE (SEA704Hu) and MyBioSource for claudin-1 (MBS2704401). ARPE-19 cells were centrifuged, re-suspended in 10 mM Tris-HCl pH 7.4 (Sigma Aldrich) and lysed to extract the cytosolic and membrane proteins content by ultrasonication (Bandelin sonoplus HD2070 probe). Supernatants obtained by centrifugation (15,700 g-force, 30 min at 4 °C) were stored at -80 °C until the ELISA assays.

Experimental Methods

(1) Synthesis of IrNCs and IrNCs immunoprobes

The protocol for the synthesis of IrNCs has been previously reported [1]. Briefly, IrNCs were synthesized employing sodium citrate as stabilizing agent; 20 mL of 0.03% (m/v) $IrCl_3 \cdot xH_2O$ were mixed with 3 mL 1% (m/v) trisodium citrate dihydrate solution, and pH was adjusted to 8 with NaOH while refluxing for 20 min with mechanical stirring. Then 1 mL of 0.1 M $NaBH_4$ was added, and the reaction mixture was allowed to cool to room temperature while stirring for 30 min. In a second step, 10 mL of the IrNCs were placed in a glass vial with 28 μmol of lipoic acid and 4 μL of 2 M NaOH, and stirring was maintained for 15 h to modify the surface of the IrNCs (exchange of citrate by lipoic acid). The resulting IrNCs were purified with 3 kDa Amicon filters by ultracentrifugation. The average diameter of the IrNCs was 1.89 ± 0.03 nm.

The two immunoprobes were prepared by bioconjugation of the specific antibodies (Anti-h-APOE and Anti-h-claudin-1) to the IrNCs following carbodiimide chemistry. The corresponding antibody (Ab) was diluted in PBS to 100 μg mL^{-1} and the required volume of IrNCs was added to have a molar ratio NCs:Ab of 10:1. Then, a solution containing EDC and NHS was added (molar ratio Ab:EDC:NHS equal to 1:1500:1500) and mixed in the vortex for 2 h. The IrNCs immunoprobes were purified by ultracentrifugation with 100 kDa Amicon filters and the Ir-to-Ab stoichiometry (amplification factor) in the immunoprobes was determined. For such purpose, a similar procedure to that previously described by Menero-Valdés *et al.* [1] was carried out both for the IrNCs:Anti-h-APOE and IrNCs:Anti-h-claudin-1 immunoprobes.

RESULTS AND DISCUSSION

Optimization of the glucose-induced oxidative stress treatment

ARPE-19 cells were subjected to GL treatment with different concentrations (0, 5, 10, 30, 60 and 100 mM) for 48 h and the cell viability and cellular production of ROS were investigated. **Fig. S1** shows the experimental results obtained for the optimization of the glucose induced oxidative stress treatment with ARPE-19 cells.

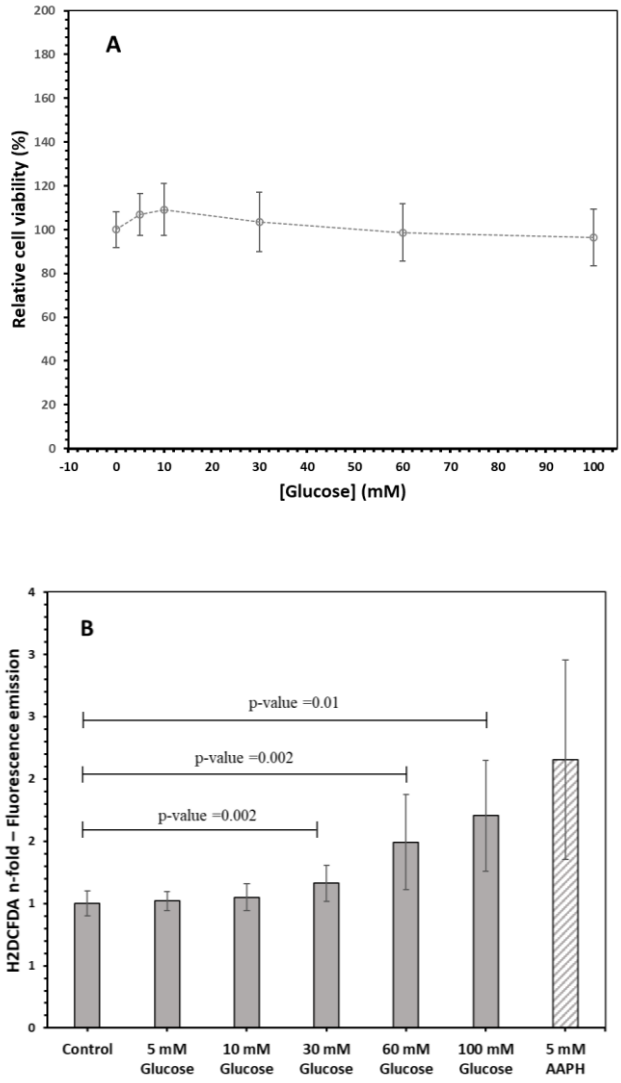

Figure S1. Optimization of the glucose induced oxidative stress treatment with ARPE-19 cells. A) Relative cell viability (expressed as %) obtained by CyQuant viability assay at different glucose concentrations (5-100 mM) for 48 h. The absorbance of the CT cells (non-treated) was considered as 100% cell viability. Uncertainties represent the standard deviation of the mean of six different biological replicates; B) n-fold change of fluorescence emission for the production of ROS species by ARPE-19 cells treated with different concentrations of glucose. AAPH was employed as positive control. Uncertainties given represent the standard deviation of the mean of ten biological replicates.

 # Determination of proteins in individual ARPE-19 cells by sc-ICP-MS

Optimization of the cell concentration. **- Fig. S2** depicts the time-resolved profiles of $^{56}Fe^{+}$ obtained by sc-ICP-MS for the analysis of ARPE-19 cells (CT without IrNCs immunoprobe) at different cellular concentrations from $2 \cdot 10^{4}$ cells mL^{-1} up to $1 \cdot 10^{6}$ cells mL^{-1}.

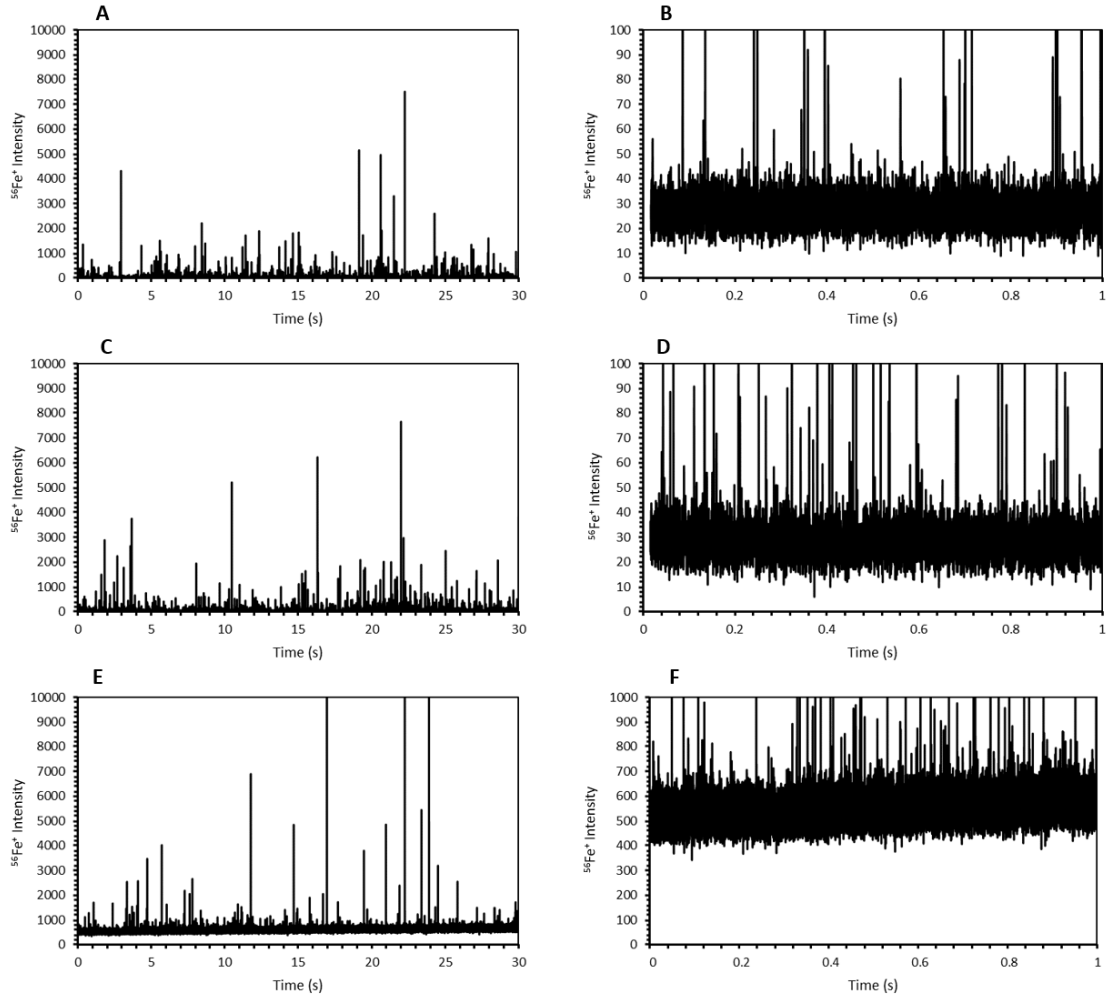

Figure S2. Time-resolved profiles of $^{56}Fe^{+}$ obtained by sc-ICP-MS for the analysis of ARPE-19 cells (CT without IrNCs immunoprobe) at different cellular concentrations. A & B) $2 \cdot 10^{4}$ cells mL^{-1}; C & D) $1 \cdot 10^{5}$ cells mL^{-1}; and E & F) $1 \cdot 10^{6}$ cells mL^{-1}.

Optimization of the immunoassay using IrNCs as label. - For the experiments, cells were diluted to $1 \cdot 10^5$ cells mL^{-1}, and $^{56}Fe^+$ and $^{193}Ir^+$ signals were sequentially detected by sc-ICP-MS in the same suspension. $^{56}Fe^+$ was measured to check the integrity of the cells and to confirm the number of cell events detected with $^{193}Ir^+$ (from the IrNCs immunoprobe). The data was fitted to a Poisson distribution in the case of $^{56}Fe^+$ and to a Gaussian filter in the case of $^{193}Ir^+$. The suitability of the filter depends on the background signal; higher counting rates work better with Gaussian filters, while Poisson filters work better with low background levels [2].

Fe is an intrinsic element of ARPE-19 cells, therefore the background can be attributed to any disrupted cell or to metal impurities from reagents. The $^{193}Ir^+$ background signal can be mainly attributed to the IrNCs immunoprobe that was not successfully removed with the washing step or to immunoprobes specifically bound to proteins from disrupted cells. If applying a Poisson filter to the $^{193}Ir^+$ signal, the L_d was underestimated and the events coming from the free immunoprobes were detected as cellular events, overestimating the number of cells in the suspension (see in **Fig. S3A** the events marked with red arrows; L_d Poisson = 40 counts and L_d Gaussian = 400 counts). Nevertheless, when applying a Gaussian filter, events from IrNCs immunoprobe not specifically labelled to the cells were successfully discriminated. The number of Fe events was calculated using Poisson filter (**Fig. S3B**, L_d Poisson = 100 counts) and used to corroborate the number of events in the suspension (comparable number of events were obtained for $^{56}Fe^+$ and $^{193}Ir^+$).

Additionally, **Fig. S4** displays the experimental results obtained by sc-ICP-MS for the analysis of CT ARPE-19 cells labelled with IrNCs:Anti-h-APOE immunoprobe using different Ab concentrations (2, 5, 10, 20, and 40 μg mL^{-1}) and washing steps (1, 2 or 3).

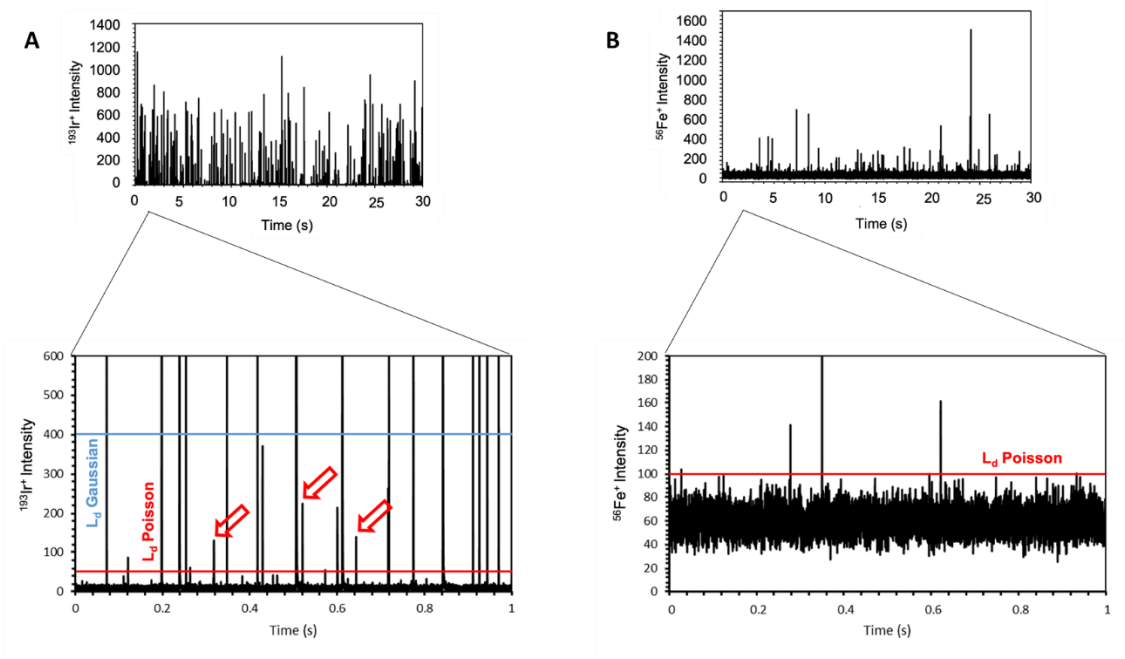

Figure S3. Time-resolved profiles of A) $^{193}Ir^{+}$ and B) $^{56}Fe^{+}$ obtained by sc-ICP-MS analysis in ARPE-19 cells (CT cells; 10 mg mL^{-1} IrNCs:Anti-h-APOE immunoprobe). The red lines indicate the event limit of detection (L_d) when applying a Poisson distribution and the blue line indicates L_d for a Gaussian filter.

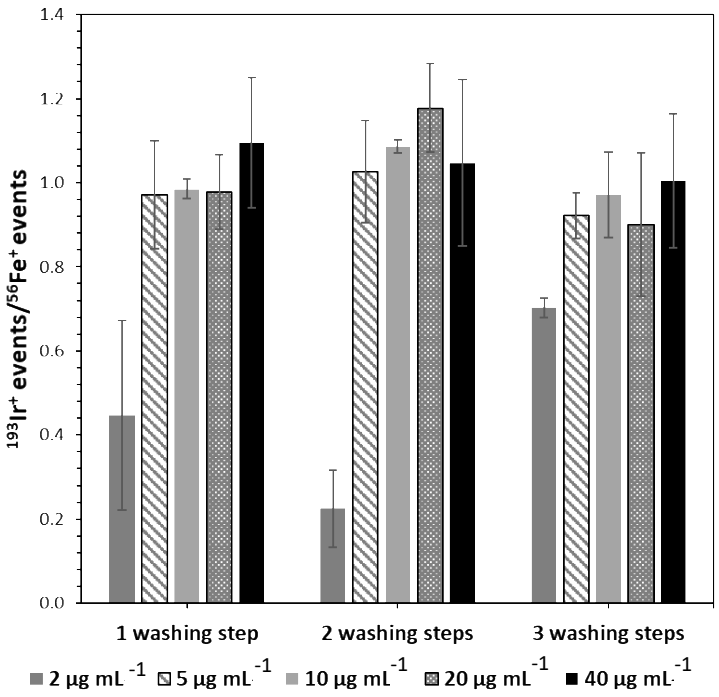

Figure S4. Immunoassay optimization for the determination of APOE by sc-ICP-MS in CT ARPE-19 cells using IrNCs:Anti-h-APOE immunoprobe. Different Ab concentrations were evaluated in the immunoprobe as well as different washing steps with PBS (after incubation of the immunoprobe). The graph depicts the ratio of detected events using $^{193}Ir^{+}$ intensity over detected events with $^{56}Fe^{+}$ intensity.

Determination of APOE in CT and GL-treated ARPE-19 cells.- The effect of oxidative stress induced by glucose in the expression of APOE in ARPE-19 cells was studied in individual CT and GL-treated cells by sc-ICP-MS. On one hand, **Fig. S5A** and **S5B** depict the time-resolved profiles obtained for $^{193}Ir^{+}$ for CT and GL-treated ARPE-19 cells, respectively. Furthermore, **Table S1** contains the results obtained for the determination of APOE in ARPE-19 cells by sc-ICP-MS (five biological replicates per condition were analyzed for CT and GL-treated cells, with three analytical replicates per biological sample). On the other hand, **Fig. S6** shows the mass frequency histograms obtained, representing the percentage of cells that contain a certain amount of the protein, for all the biological replicates in CT and GL-treated cells.

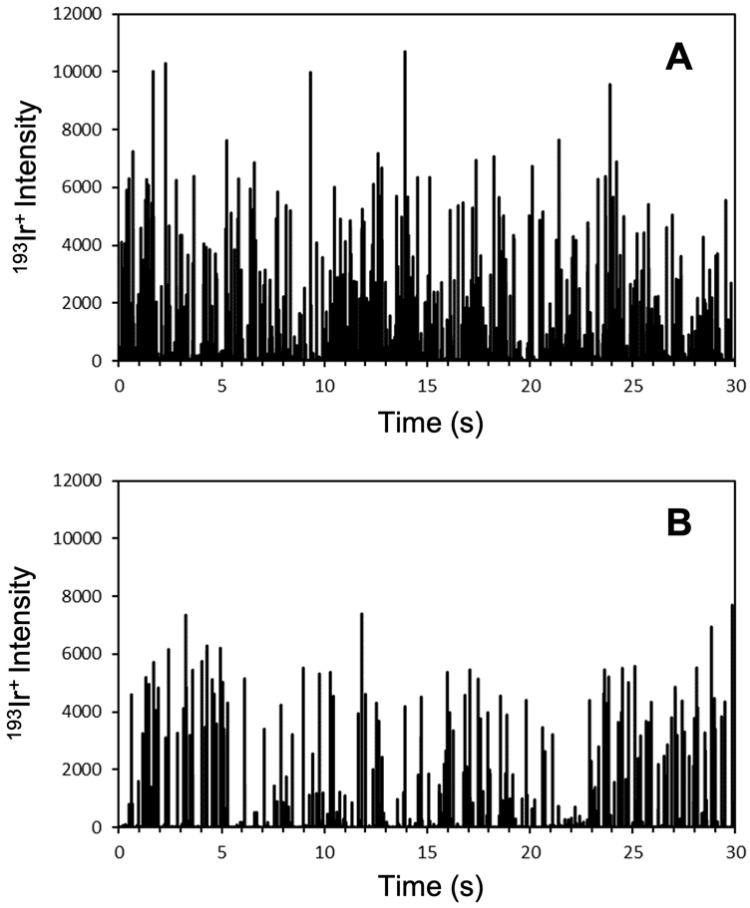

Figure S5. $^{193}Ir^{+}$ time-resolved profile obtained for the analysis of APOE in ARPE-19 cells employing IrNCs:Anti-h-APOE immunoprobe. A) CT cells (CT1; R2); and B) GL-treated cells (GL5; R2- 100 mM; 48 h).

Table S1: Experimental results obtained for the determination of APOE in ARPE-19 cells by sc-ICP-MS using IrNCs immunoprobe as label.

							Range (fg APOE/cell)			
		Ir events (30 s)	Ir events/Fe events	fg Ir/cell	fg APOE/cell	LOD (fg APOE/cell)	Minimum Value	Maximum Value	fg APOE/cell	fg APOE/cell
CT1	R1	351	1.2	4.9	0.45	0.001	0.001	2.99	0.40 ± 0.05	0.38 ± 0.05
	R2	273	0.9	4.0	0.37	0.025	0.026	1.63		
	R3	294	1.0	4.0	0.37	0.038	0.041	2.02		
CT2	R1	189	0.9	3.6	0.34	0.020	0.021	2.17	0.337 ± 0.003	
	R2	302	1.0	3.4	0.34	0.042	0.042	1.48		
	R3	274	0.8	3.6	0.33	0.042	0.042	1.78		
CT3	R1	359	1.2	4.8	0.45	0.057	0.058	2.89	0.40 ± 0.05	
	R2	326	1.2	3.9	0.37	0.081	0.082	1.76		
	R3	327	1.1	4.0	0.38	0.028	0.099	2.30		
CT4	R1	307	1.1	5.5	0.52	0.060	0.099	2.30	0.45 ± 0.06	
	R2	280	1.0	4.7	0.44	0.026	0.164	1.98		
	R3	282	1.1	4.3	0.40	0.031	0.121	1.66		
CT5	R1	180	1.1	3.2	0.30	0.032	0.032	1.94	0.33 ± 0.06	
	R2	201	1.0	3.0	0.28	0.031	0.032	1.36		
	R3	204	1.1	4.3	0.40	0.040	0.041	2.80		
GL-treated 1	R1	555	1.0	3.1	0.29	0.002	0.003	2.50	0.30 ± 0.01	0.28 ± 0.04
	R2	463	1.0	3.3	0.30	0.002	0.003	1.62		
	R3	411	1.0	3.3	0.31	0.003	0.003	1.96		
GL-treated 2	R1	395	1.0	3.4	0.32	0.002	0.002	2.28	0.29 ± 0.03	
	R2	366	1.0	3.0	0.28	0.002	0.002	1.78		
	R3	394	1.0	2.9	0.27	0.002	0.002	1.97		
GL-treated 3	R1	325	1.0	2.3	0.21	0.002	0.002	1.82	0.22 ± 0.01	
	R2	320	1.0	2.4	0.23	0.002	0.002	1.60		
	R3	299	1.1	2.4	0.22	0.002	0.002	1.82		
GL-treated 4	R1	261	1.0	3.2	0.30	0.004	0.009	2.00	0.32 ± 0.02	
	R2	245	1.0	3.3	0.31	0.004	0.010	2.19		
	R3	288	1.0	3.6	0.34	0.002	0.014	1.62		
GL-treated 5	R1	404	1.1	3.3	0.31	0.001	0.001	2.18	0.28 ± 0.03	
	R2	202	1.1	2.8	0.26	0.001	0.002	1.42		
	R3	335	0.9	2.8	0.26	0.001	0.001	1.67		

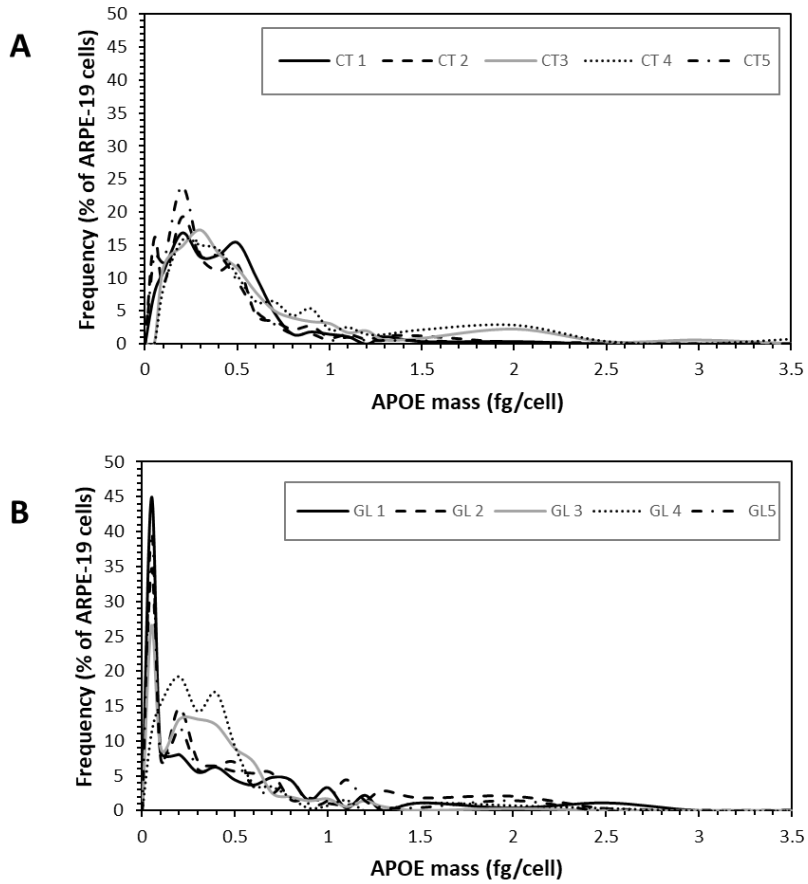

Figure S6. Mass frequency histogram (in percentage) for APOE in ARPE-19 cells obtained by sc-ICP-MS using Anti-h-APOE immunoprobe. Both graphs include the histograms for the five biological replicates measured (each as the average of three analytical replicates). A) CT cells and B) GL-treated cells.

Determination of claudin-1 in CT and GL-treated ARPE-19 cells.- **Fig. S7** depicts the time-resolved profiles obtained for $^{193}Ir^{+}$ for CT and GL-treated ARPE-19 cells and **Table S2** contains the results obtained for the determination of claudin-1 in ARPE-19 cells by sc-ICP-MS (five biological replicates per condition were analyzed for CT and GL-treated cells, with three analytical replicates per biological sample). Additionally, **Fig. S8** shows the mass frequency histograms obtained, representing the percentage of cells that contain a certain amount of the protein, for all the biological replicates in CT and GL-treated cells.

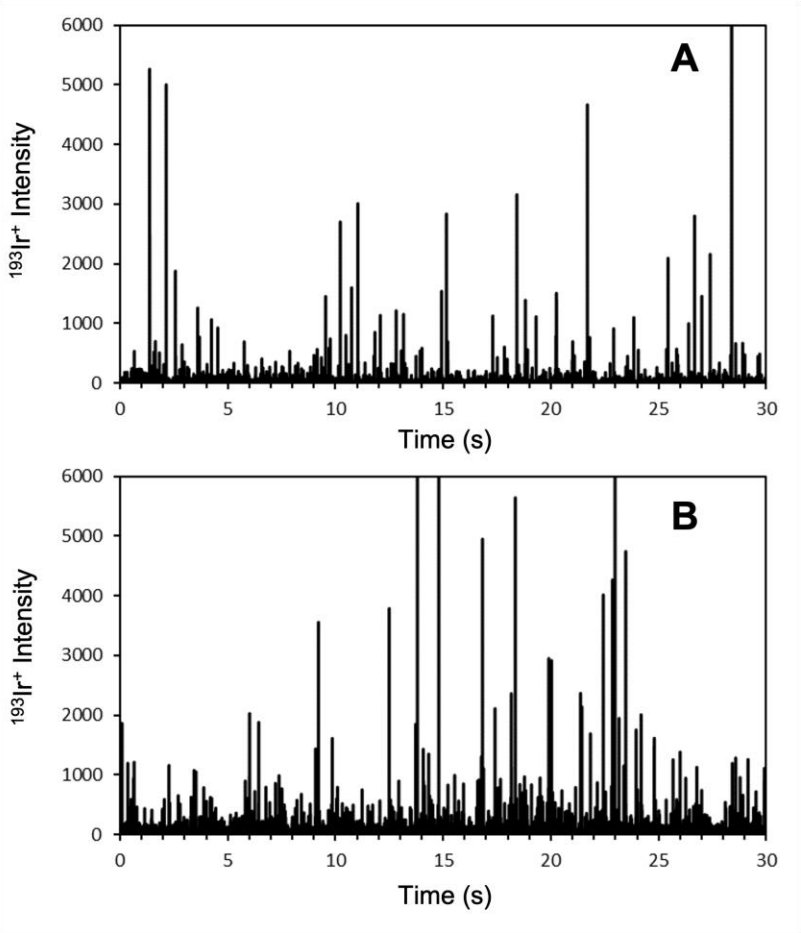

Figure S7. $^{193}Ir^+$ time-resolved profile obtained for the analysis of claudin-1 in ARPE-19 cells employing IrNCs:Anti-h-claudin-1 immunoprobe. A) CT cells (CT1; R1) and B) GL-treated cells (GL1; R1 - 100 mM; 48 h).

Table S2. Experimental results obtained for the determination of claudin-1 (CLD) in ARPE-19 cells by sc-ICP-MS using IrNCs immunoprobe as the elemental label. Five biological replicates per condition were analysed (CT and GL-treated) with three analytical replicates per biological sample.

							Range (ag claudin-1/cell)			
		Ir events (30s)	Ir events/Fe events	fg Ir/cell	ag CLD/cell	LOD (ag CLD/cell)	Minimum Value	Maximum Value	ag CLD/cell	ag CLD/cell
CT1	R1	180	1.0	0.35	49	1.8	2.1	931	43 ± 5	42 ± 3
	R2	185	1.2	0.28	39	1.0	1.7	1093		
	R3	171	1.2	0.29	41	4.8	7.5	632		
CT2	R1	211	1.0	0.28	40	4.8	7.3	853	40.0 ± 0.6	
	R2	249	0.9	0.29	41	1.6	2.8	902		
	R3	269	0.8	0.28	40	1.3	2.7	795		
CT3	R1	276	1.0	0.31	43	1.8	3.0	825	42 ± 4	
	R2	298	1.2	0.33	46	1.6	2.5	1499		
	R3	279	1.2	0.26	37	1.9	2.5	1501		
CT4	R1	152	0.9	0.29	41	1.4	2.3	1551	46 ± 5	
	R2	232	0.8	0.33	46	1.9	3.2	522		
	R3	205	1.1	0.36	50	0.6	3.6	855		
CT5	R1	187	0.8	0.27	38	0.6	3.0	795	38 ± 1	
	R2	232	1.2	0.28	39	1.2	4.2	807		
	R3	153	0.9	0.26	37	3.0	4.2	1212		
GL-treated 1	R1	279	1.1	0.35	49	2.5	9.0	1695	52 ± 4	63 ± 7
	R2	234	0.9	0.36	50	5.8	4.9	971		
	R3	304	1.1	0.40	56	3.0	5.6	1117		
GL-treated 2	R1	273	1.0	0.45	62	3.3	6.0	1537	66 ± 5	
	R2	268	0.8	0.51	72	3.8	11.0	1104		
	R3	205	0.8	0.46	64	7.1	3.6	860		
GL-treated 3	R1	166	0.9	0.47	65	2.2	3.0	1455	68 ± 14	
	R2	185	1.1	0.60	84	1.8	3.5	756		
	R3	172	1.0	0.40	56	10.5	16.0	667		
GL-treated 4	R1	148	0.9	0.49	69	9.7	15.0	508	68 ± 12	
	R2	192	1.2	0.40	56	10.3	16.2	850		
	R3	171	1.3	0.57	80	2.1	2.4	1287		
GL-treated 5	R1	246	0.9	0.46	64	1.5	2.6	509	61 ± 5	
	R2	156	1.0	0.45	63	1.5	1.1	437		
	R3	201	1.0	0.40	36	0.7	1.7	970		

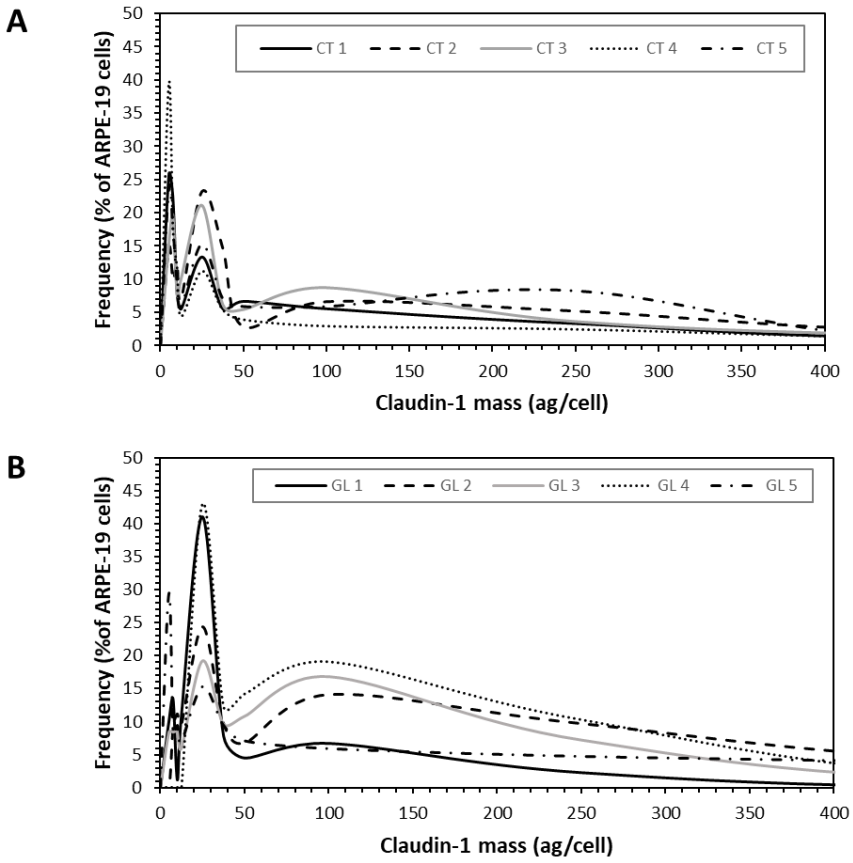

Figure S8. Mass frequency histogram (in percentage) for claudin-1 in ARPE-19 cells obtained by sc-ICP-MS using Anti-h-claudin-1 immunoprobe. Both graphs include the histograms for the five biological replicates measured (each as the average of three analytical replicates). A) CT cells and B) GL-treated cells.

Bioimaging of proteins in individual ARPE-19 cells by LA-ICP-MS

Concerning the analysis of APOE in ARPE-19 cell, the optical images of the ablated areas within the chamber well (taken with the laser camera prior to ablation) are shown in **Fig. S9. Fig. S10 and S11** show respectively amplifications of the 2D-images obtained for APOE and claudin-1 distribution by LA-ICP-MS in CT and GL-treated cells where each individual cell has the scale bar adjusted to its particular case. Thus, it is possible to identify the subcellular distribution of the proteins and identify the regions where APOE (**Fig. S10**) and claudin-1 (**Fig. S11**) was at higher or lower levels. Furthermore, **Fig. S12 and S13** collect respectively the single line profile obtained for $^{193}Ir^{+}$ intensity across individual cells for APOE and claudin-1; it is possible to see the variation of the protein distribution (in counts) along the cell diameter. **Fig. S14** shows the histograms corresponding to CT and GL-treated cells labelled with the IrNCs:Anti-h-claudin-1 immunoprobe. Finally, **Fig. S15** depicts the distribution of APOE and claudin-1 observed by conventional immunofluorescence.

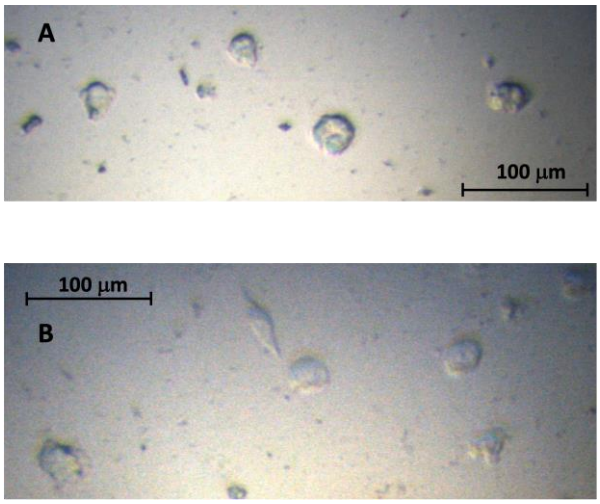

Figure S9. Optical images of the CT and GL ARPE-19 cells before LA sampling. A) CT cells and B) GL-treated cells. Qualitative 2D-images obtained by LA-ICP-MS are shown in Fig.3A and 3B, respectively.

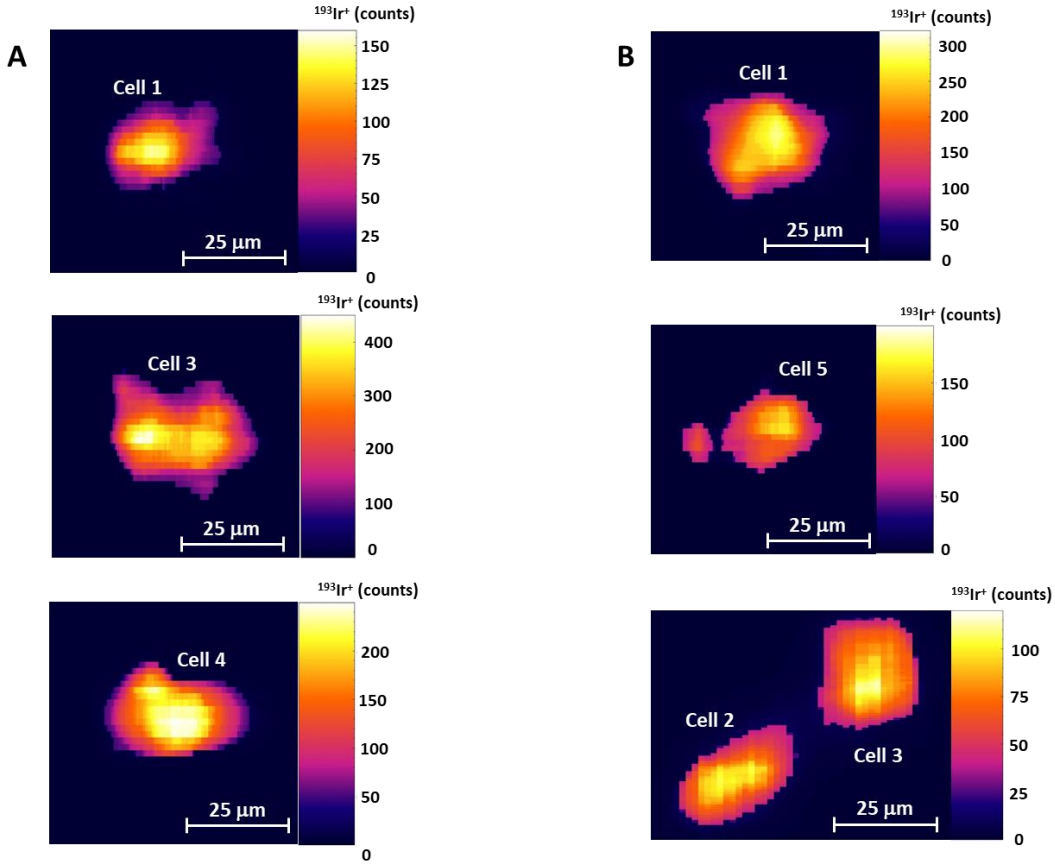

Figure S10. Qualitative 2D-images obtained by LA-ICP-MS for APOE distribution in CT and GL-treated ARPE-19 cells (IrNCs:Anti-h-APOE immunoprobe). Scale of $^{193}Ir^{+}$ intensity was individually adjusted for each cell to study the distribution of the protein. A) APOE distribution in CT cells (cells #1, #3, and #4 of Fig. 4A), and B) APOE distribution in GL-treated cells (cells #1, #2, #3, and #5 of Fig. 4B)

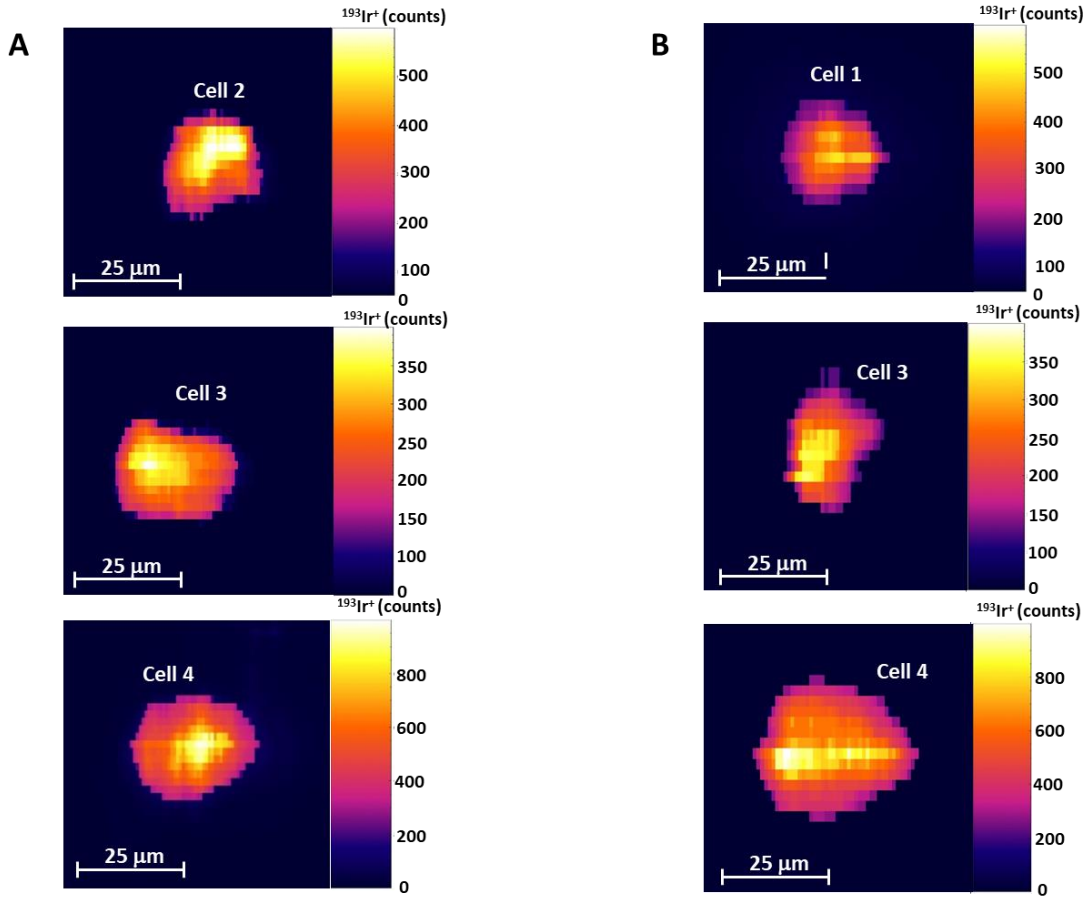

Figure S11. Qualitative 2D-images obtained by LA-ICP-MS for claudin-1 distribution in CT and GL-treated ARPE-19 cells (IrNCs:Anti-h-claudin-1 immunoprobe). Scale of $^{193}Ir^{+}$ intensity signal was individually adjusted for each cell to highlight the distribution of the protein. A) Claudin-1 in CT cells (cells #2, #3, and #4 of Fig. 5A), and B) Claudin-1 in GL-treated cells (cells #1, #3, and #4 of Fig. 5B)

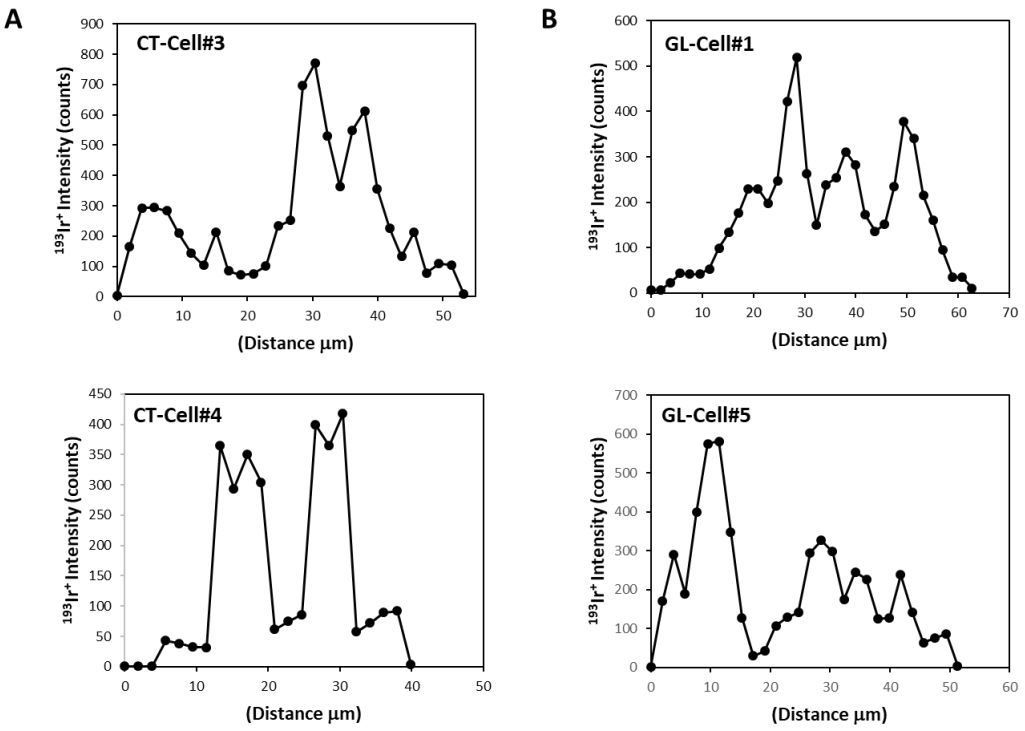

Figure S12. Single line profile indicating the variation of $^{193}Ir^+$ signal (APOE distribution) across individual ARPE-19 cells analyzed by LA-ICP-MS using IrNCs:Anti-h-APOE immunoprobe. A) CT cells; and B) GL-treated cells. The number of the cell corresponds to the 2D-images showed at Fig. 4.

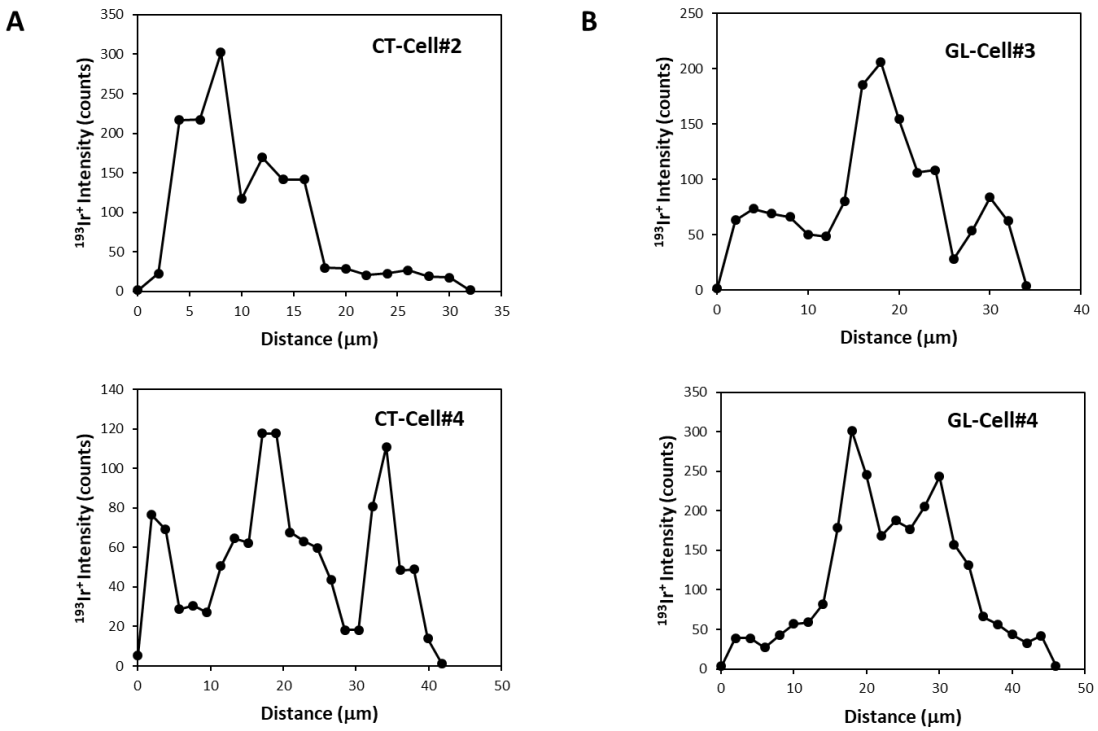

Figure S13. Single line profile indicating the variation of $^{193}Ir^+$ intensity (claudin-1 distribution) across individual ARPE-19 cells analyzed by LA-ICP-MS using IrNCs:Anti-h-claudin-1 immunoprobe. A) CT cells and B) GL-treated cells. The number of the cell corresponds to the 2D-images shown in Fig. 5.

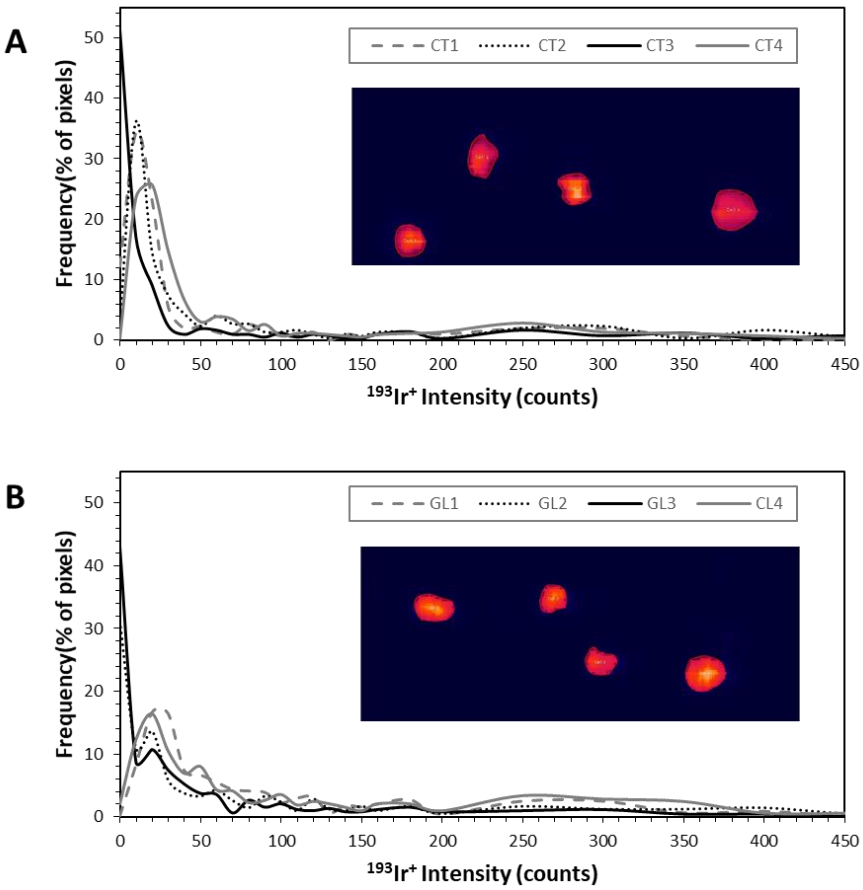

Figure S14. Counts frequency histogram (in percentage of pixels) for claudin-1 protein in ARPE-19 cells obtained by LA-ICP-MS analysis using Anti-h-claudin-1 immunoprobe. The insets show the 2D-image obtained by LA-ICP-MS indicating the area of each individual cell. A) CT cells; and B) GL-treated cells.

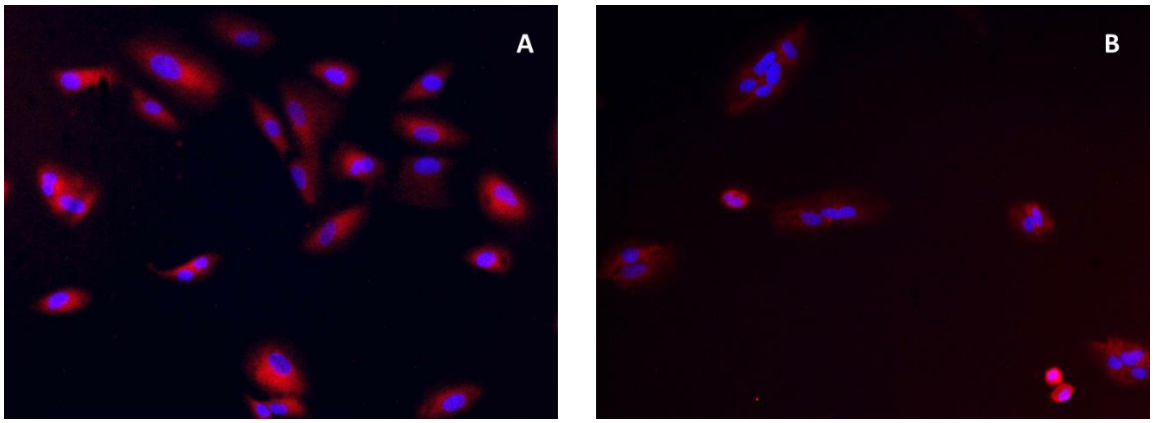

Figure S15. Images obtained by fluorescence microscopy for (A) APOE and (B) claudin-1 distribution in CT ARPE-19 cells employing in the immunocytochemistry protocol a secondary Ab marked with Alexa®594 to label both proteins (in red) and DAPI to visualize the cell nuclei (in blue).

REFERENCES

[1] P. Menero-Valdés, A. Lores-Padín, B. Fernández, H. González-Iglesias, R. Pereiro, Iridium nanoclusters as high sensitive-tunable elemental labels for immunoassays: Determination of IgE and APOE in aqueous humor by inductively coupled plasma-mass spectrometry, Talanta. 244 (2022) 123424. https://doi.org/10.1016/j.talanta.2022.123424.

[2] T.E. Lockwood, R. Gonzalez De Vega, D. Clases, An interactive Python-based data processing platform for single particle and single cell ICP-MS, J. Anal. At. Spectrom. 36 (2021) 2536–2544. https://doi.org/10.1039/d1ja00297j.