Supplementary information.

Optimization of Nanoparticle Uptake Protocol applied to Amniotic-Derived Cells: Unlocking the Therapeutic Potential

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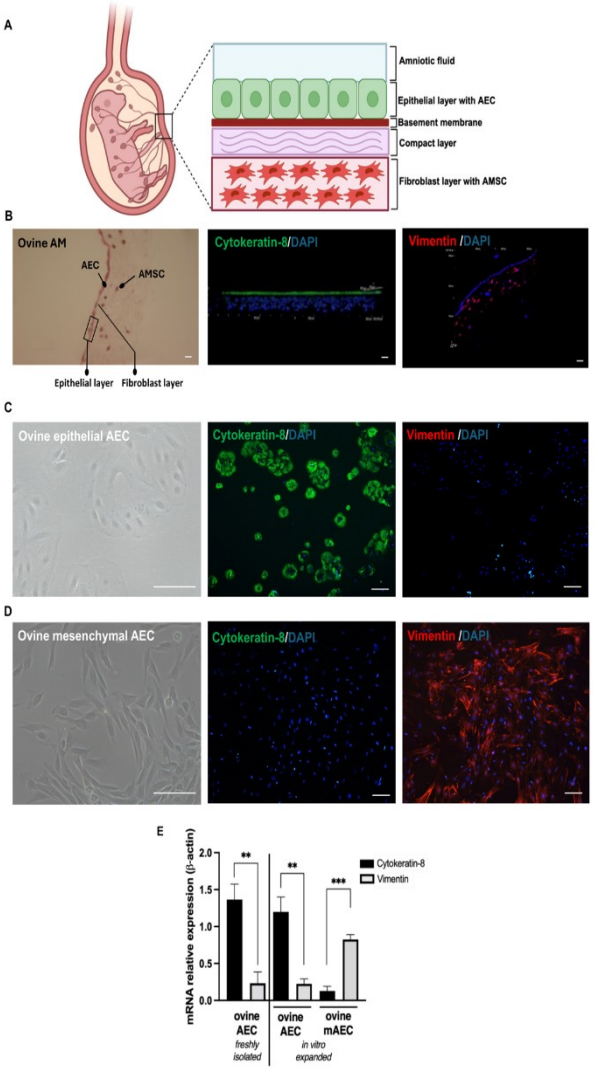
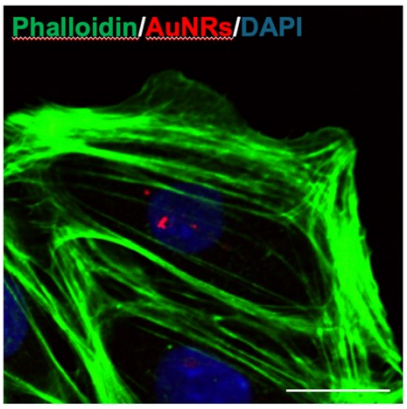
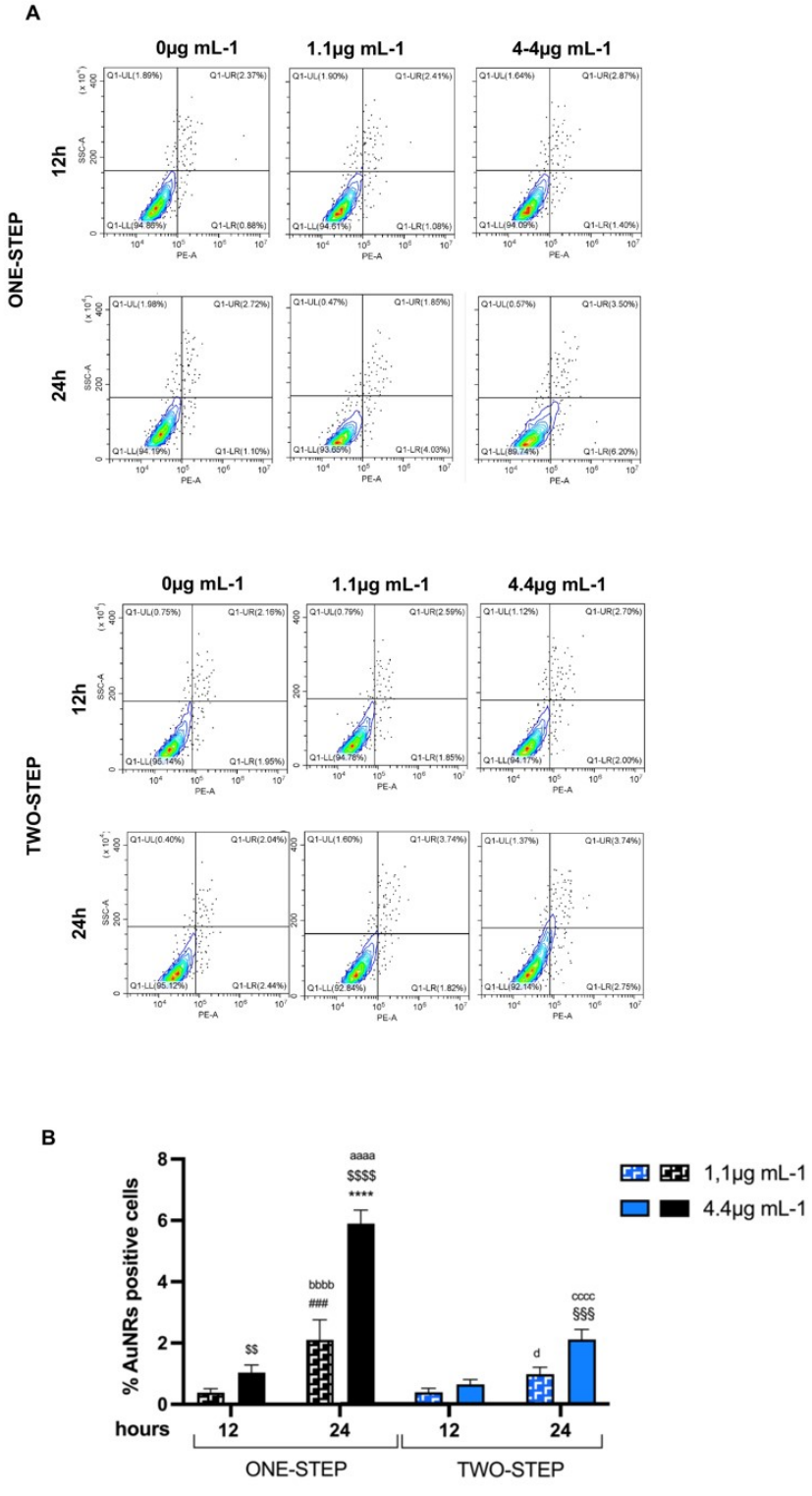


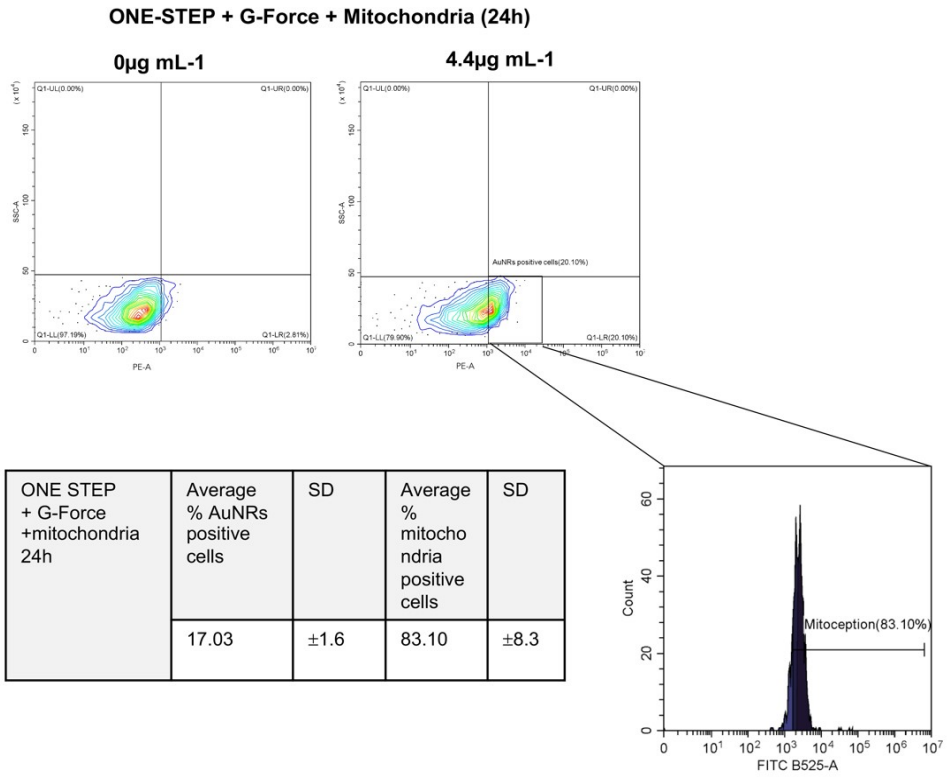
Supplementary Figure 1. Phenotypic characterization of Amniotic-derived cells. *Immunofluorescence analysis for Cytokeratin-8 and Vimentin in AM and expanded AECs.* ***(A)*** *Schematic representation of fetal membrane epithelial and mesenchymal layers.* ***(B)*** *Freshly isolated ovine AM, processed through immunofluorescence analysis using Cytokeratin-8 (an epithelial marker) and Vimentin (a mesenchymal marker), and imaged with phase-contrast microscopy, demonstrated the specificity of the two markers for the cells located in the fibroblast layer (Vimentin: red fluorescence) where mesenchymal stromal cells are located and for the cells located in the epithelial layer (Cytokeratin-8: green). Scale Bar: 50 µm. (**C**) Phenotypic characterization of isolated and in vitro amplified ovine AECs after three passages of expansion carried out with P_4 supplementation (AECs) or* ***(D)*** *without P_4 to obtain the mesenchymal phenotype (mAECs). Scale Bars for both: 50 µm.* ***(E)*** *Real-time qPCR related to Cytokeratin-8 and Vimentin mRNA expression in ovine AECs and mAECs. Significant changes were represented with ** for $p < 0.01$ or *** for $p < 0.0001$.*



Supplementary Figure 2. Evaluation of AuNRs Internalization into AECs through Confocal Laser Scanning. *A representative CLSM at 100x magnification (with oil) of mAECs after 24 hours of 4.4 µg mL-1 AuNRs uptake with one-step cultural strategy was selected. Positive cells are shown in red due to the functionalization of the AuNRs with rhodamine dye, phalloidin which topologically identifies cell boundaries was stained in green, nuclei were stained with DAPI (blu). The identical scenario is evident across various time points when employing the two-step culture strategy. Scale bar:10 µm.*

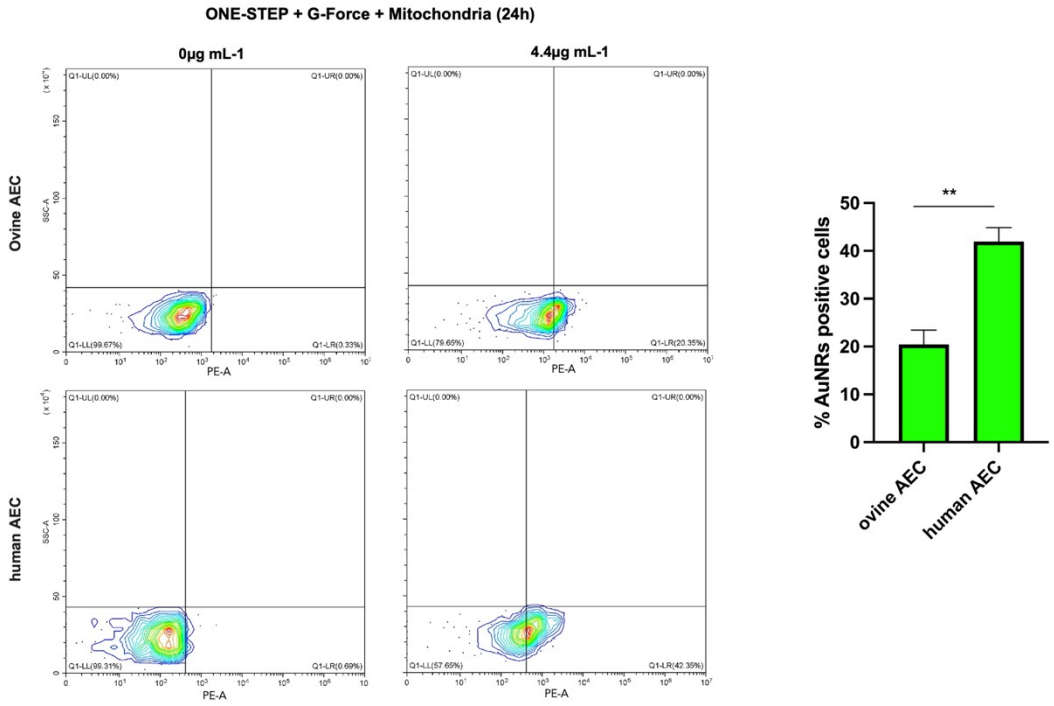


Supplementary Figure 3. Percentage of AuNRs uptake with different incubation concentration of AuNRs (4.4 µg mL-1 and 1.1 µg mL-1), incubation time window (12h-24h) and cultural strategies (one-step and two-step). (A) *Percentage of mAECs enclosing AuNRs were measured by PE intensity (x-axis) and side scattering (SSC; y-axis) flow cytometry dot plot.* ***(B)*** *Average % values related to the positive cells detected by flow cytometry. The significance of data was statistically analyzed as follow. One-step 4.4 µg mL-1 vs. two-step 4.4 µg mL-1 was indicated with **** for $p < 0.0001$; One-step 1.1 µg mL-1 vs. two-step 1.1 µg mL-1 was indicated with $^{###}$ for $p < 0.001$; One-step 4.4 µg mL-1 vs. One-step 1.1 µg mL-1 was indicated with $^{\$\$}$ or $^{\$\$\$\$}$ for $p < 0.001$ and $p < 0.0001$; Two-step 4.4 µg mL-1 vs. Two-step 1.1 µg mL-1 was indicated with §§§ for $p < 0.001$. One-step 24h with 4.4 µg mL-1 vs. One-step 12h with 4.4 µg mL-1 was indicated with ^{aaaa} for $p < 0.0001$; One-step 24h with 1.1 µg mL-1 vs. One-step 12h with 1.1 µg mL-1 was indicated with ^{bbbb} for $p < 0.0001$; Two-step 24h with 4.4 µg mL-1 vs. Two-step 12h with 4.4 µg mL-1 was indicated with ^{cccc} for $p < 0.0001$; Two-step 24h with 1.1 µg mL-1 vs. Two-step 12h with 1.1 µg mL-1 was indicated with ^{d} for $p < 0.05$*



ONE STEP + G-Force +mitochondria 24h	Average % AuNRs positive cells	SD	Average % mitochondria positive cells	SD
	17.03	±1.6	83.10	±8.3

Supplementary Figure 4. Mitochondria incorporation into AuNRs positive mAECs following one-step protocol with G-force and mitochondria supplementation. *Panels show representative flow cytometer plots for mAECs exposed 24h to 0 µg mL-1 or 4.4 µg mL-1 with one-step + G-force + mitochondria. The table reports the average % values ± SD related to the AuNRs and mitochondria positive mAECs detected by flow cytometry.*



Supplementary Figure 5. AuNRs labeling stability over time in ovine and human AECs. *Representative flow cytometer plots showing the % ±SD of positive AuNRs AECs of ovine and human origin. Statistic related to AuNRs positive cell percentage was indicated with * for p<0.05*