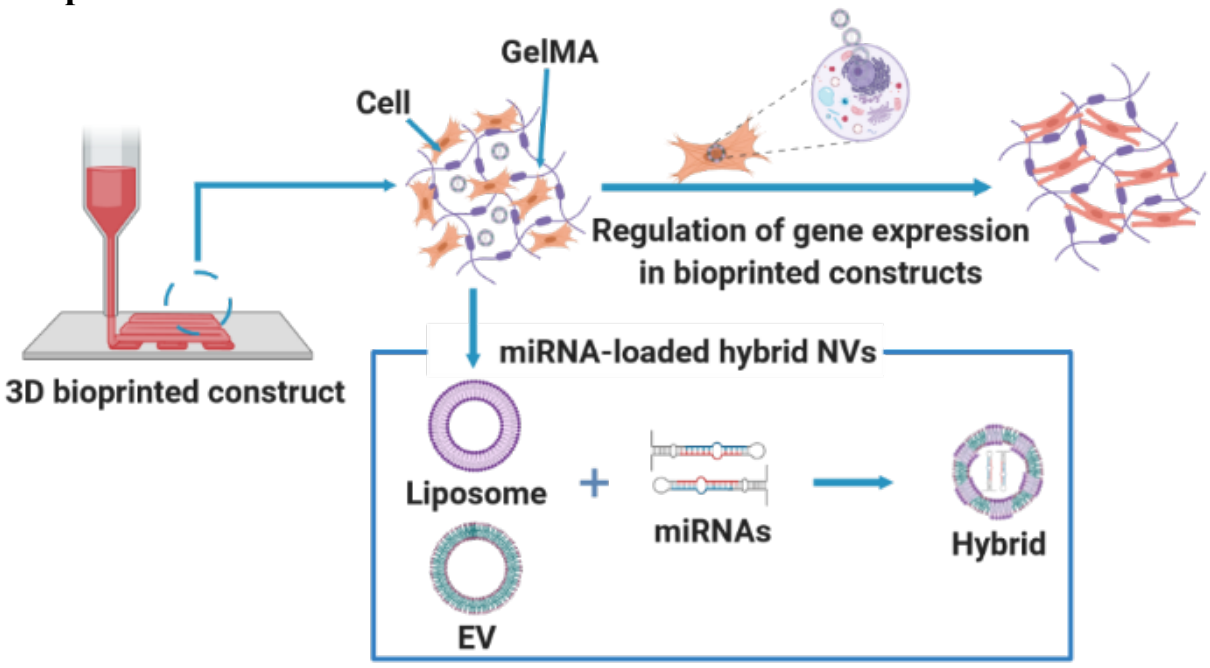
Hybrid extracellular vesicles-liposome incorporated advanced bioink to deliver microRNA

Supporting Information

Graphical Abstract



To regulate gene expression in 3D bioprinted constructs, hybrid extracellular vesicles-liposomes nanovesicles encapsulating microRNAs are embedded in gelatin-based bioinks. The targeting hybrid nanovesicles improved the native bioink's mechanical properties, biological functions, and printing ability without hampering its desirable biodegradability and biocompatibility. This unique bioink is a great candidate for bioprinting tissue grafts to treat injuries or diseases.

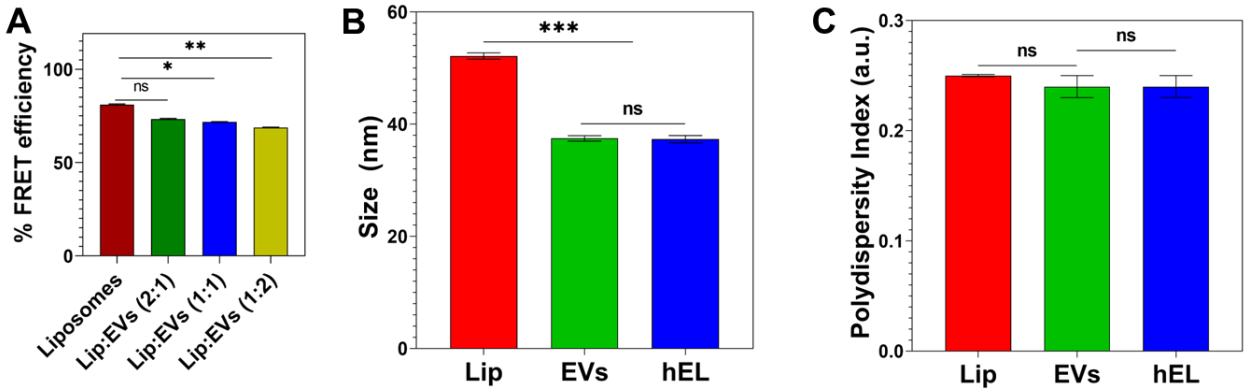
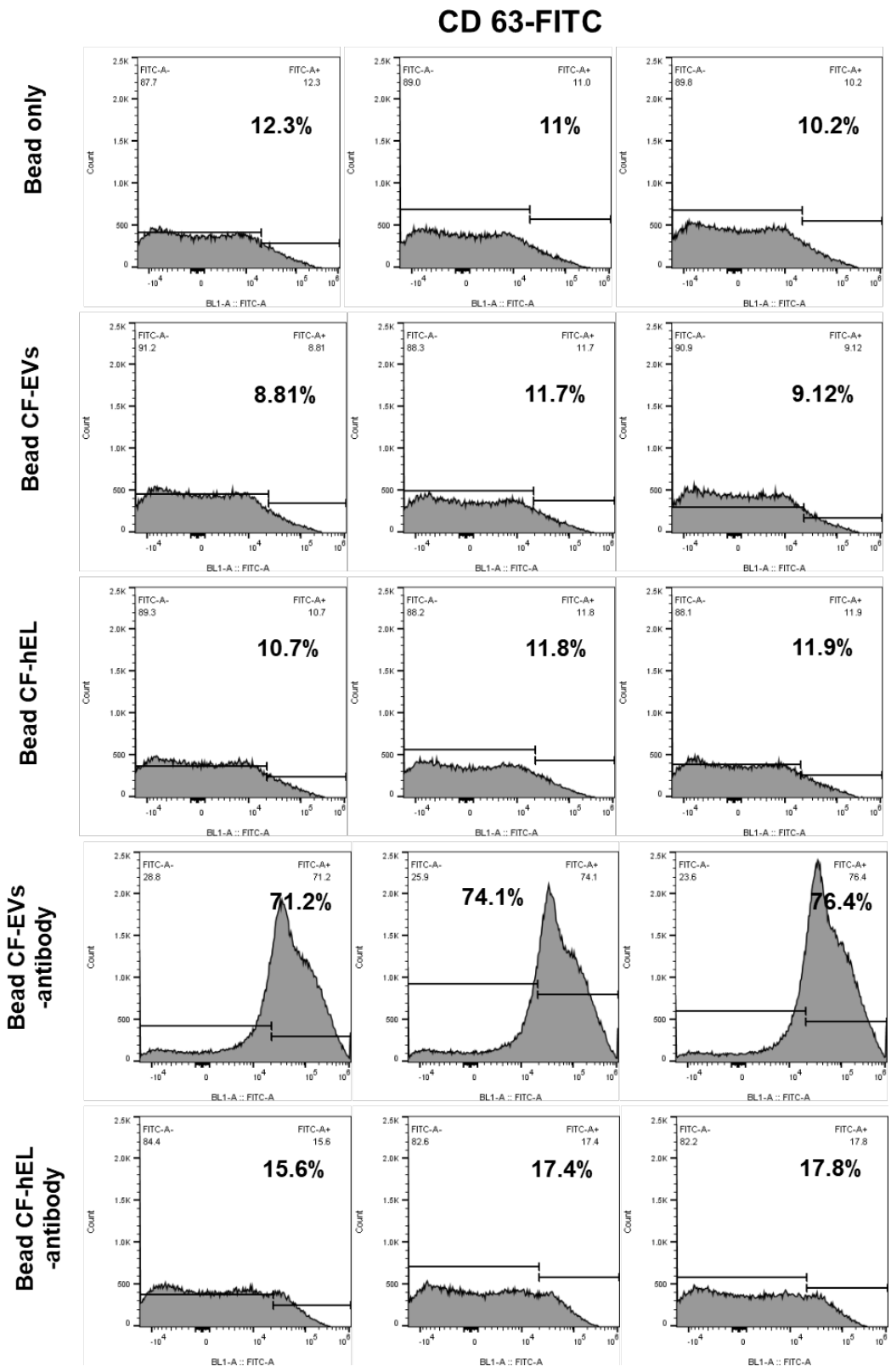
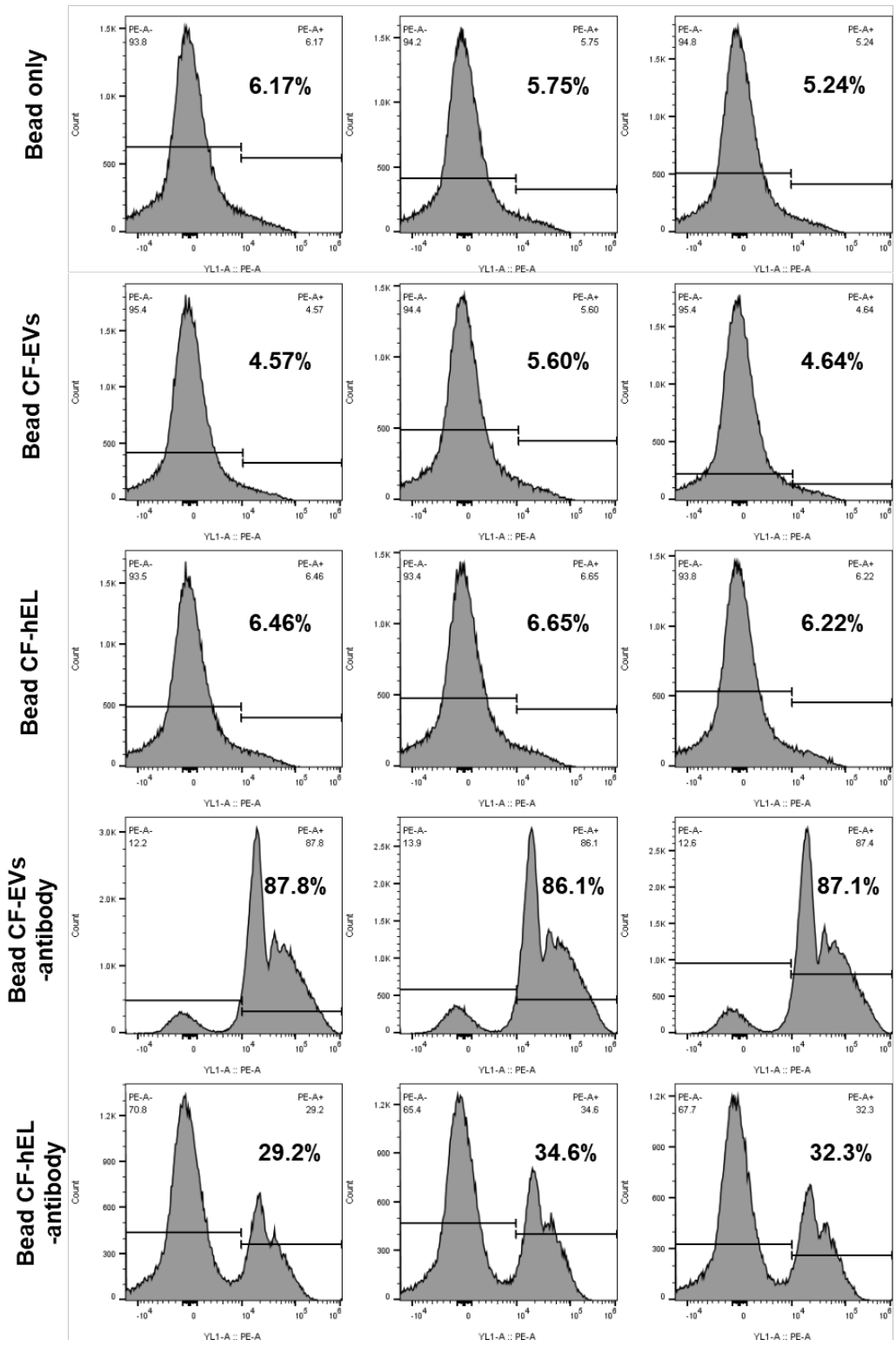


Figure S1. (A) The quantification of percentage FRET efficiency of different ratios of liposomes (Lip) to extracellular vesicles (EVs) (n=3). (B) Average size of NVs (n=3). (C) Average polydispersity index (PdI) of NVs (n=3). All data are expressed as mean ± standard deviation. Significance is indicated as *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$).


CD 63-FITC
Bead only
Bead CF-EVs
Bead CF-hEL
Bead CF-EVs -antibody
Bead CF-hEL -antibody
12.3%
11%
10.2%
8.81%
11.7%
9.12%
10.7%
11.8%
11.9%
71.2%
74.1%
76.4%
15.6%
17.4%
17.8%
FITC-A-
FITC-A+
Count
BL1-A :: FITC-A


CD 9-PE
Bead only
Bead CF-EVs
Bead CF-hEL
Bead CF-EVs -antibody
Bead CF-hEL -antibody
6.17%
5.75%
5.24%
4.57%
5.60%
4.64%
6.46%
6.65%
6.22%
87.8%
86.1%
87.1%
29.2%
34.6%
32.3%
Count
YL1-A :: PE-A

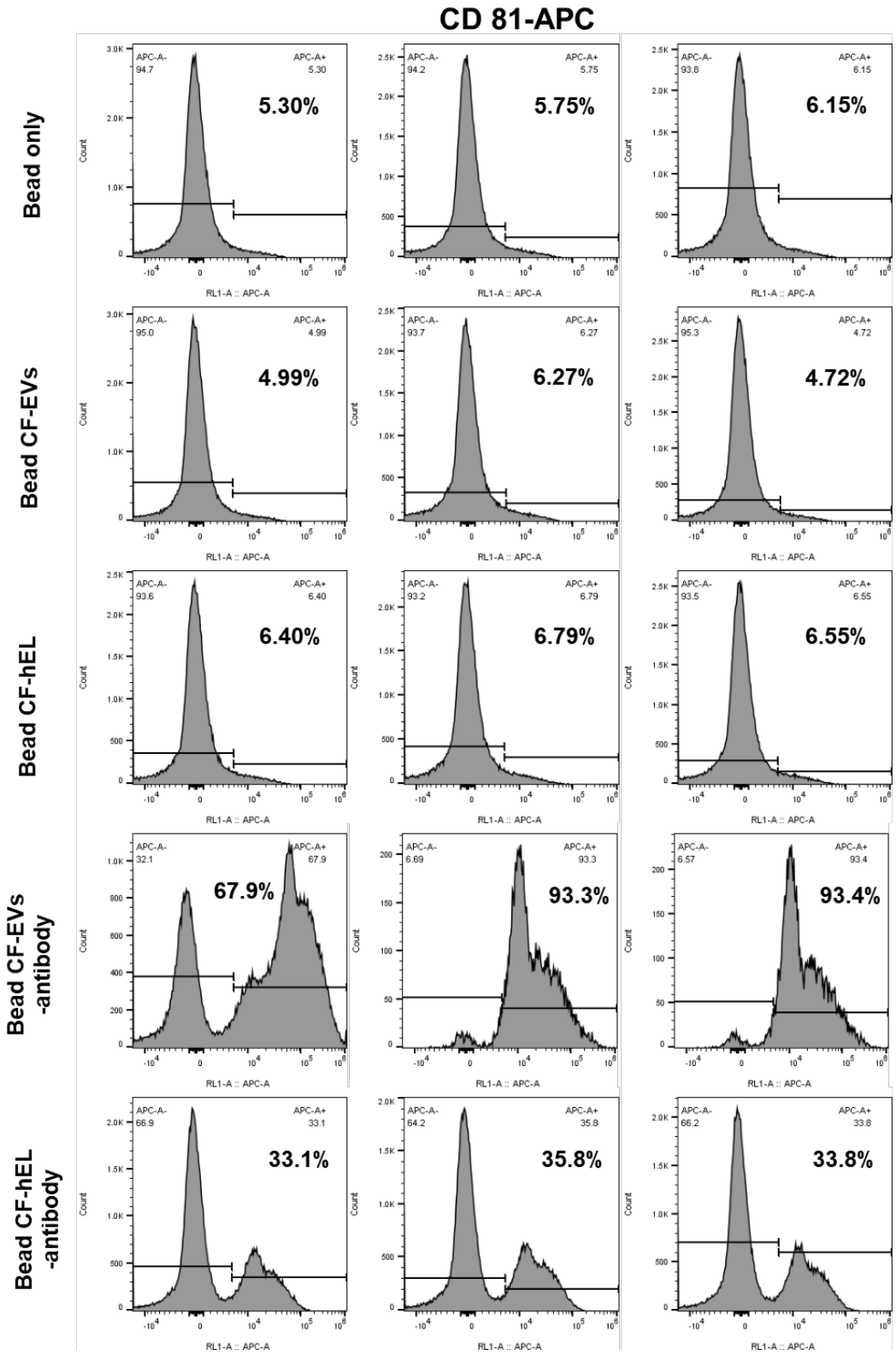


Figure S2. FACS analysis of markers CD9, CD63, and CD81 on EVs and hELs.

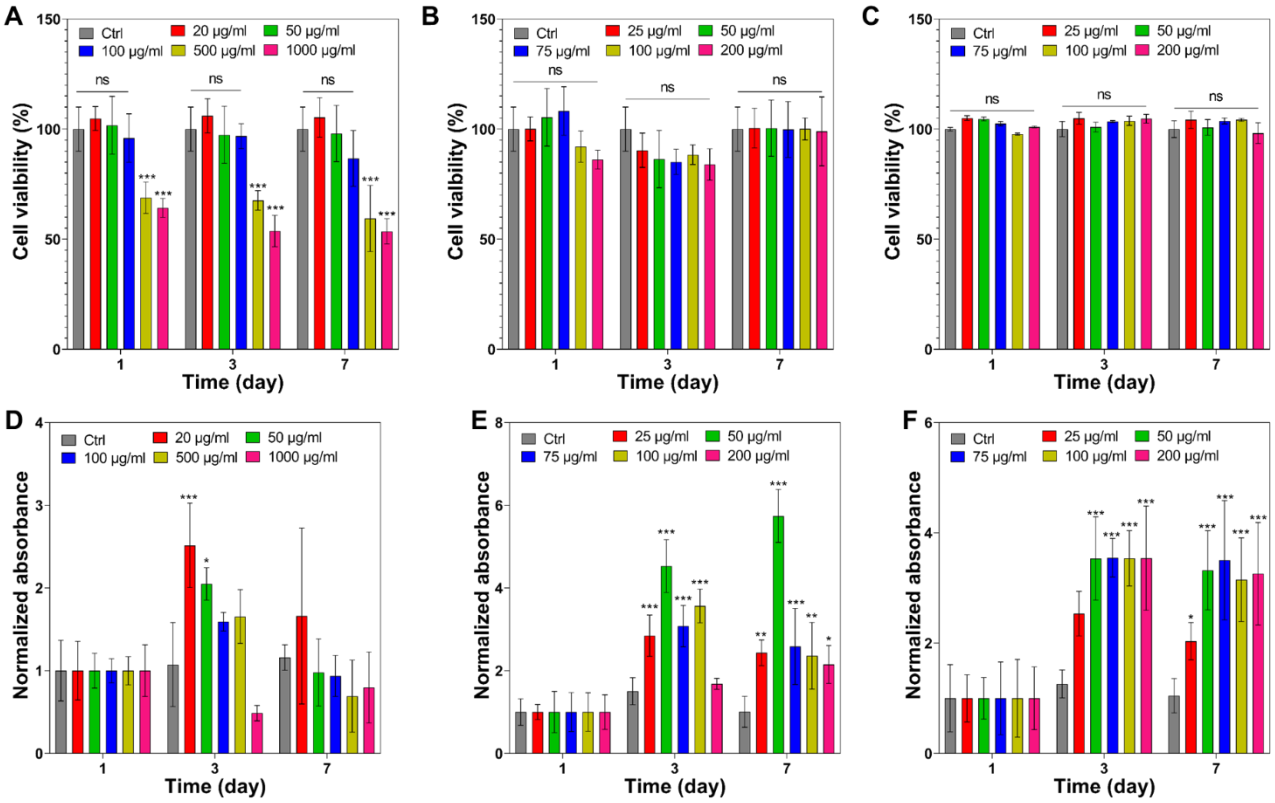


Figure S3. (A-C) Quantified viability of CFs cultured with different concentrations of (A) Lip, (B) EVs, and (C) hybrid NVs for 7 days (n=3). (D-F) PrestoBlue results showing the cell proliferation of CFs with different concentrations of (D) Lip, (E) EVs, and (F) hybrid NVs for 7 days (n=3). All data are expressed as mean ± standard deviation. Significance is indicated as *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$).

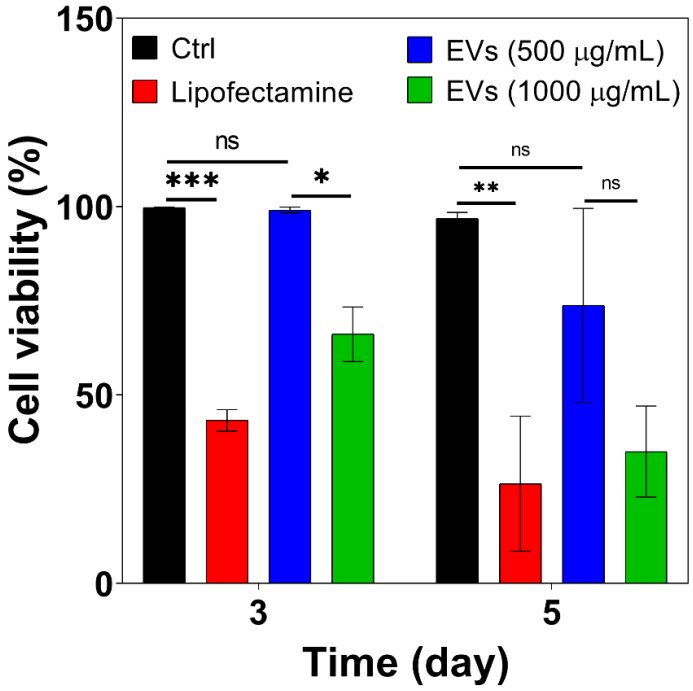


Figure S4. Quantified viability of CFs cultured with high concentrations of EVs and lipofectamine (n=3). All data are expressed as mean ± standard deviation relative to the control. Significance is indicated as *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$).

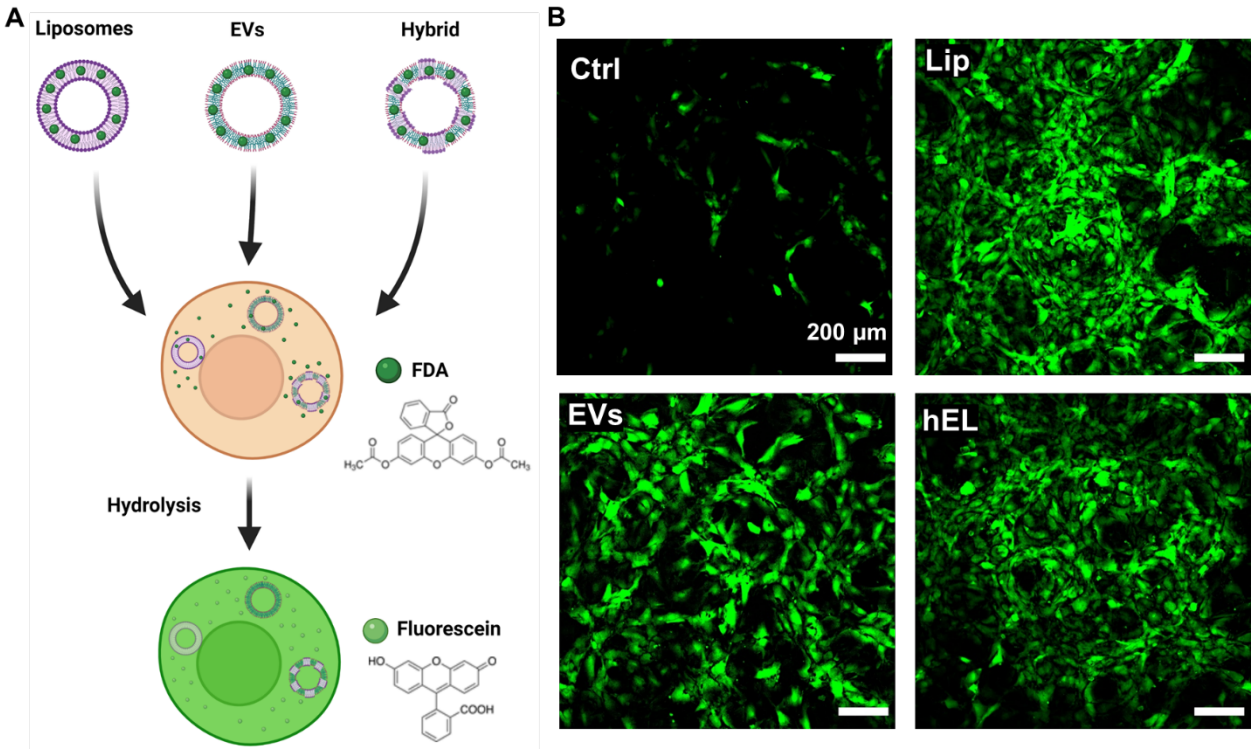


Figure S5. (A) Schematic representation of FDA-delivery *via* NVs. Created with BioRender.com. (B) Fluorescence images of FDA-loaded NVs uptake by CFs following incubation for 20 minutes.

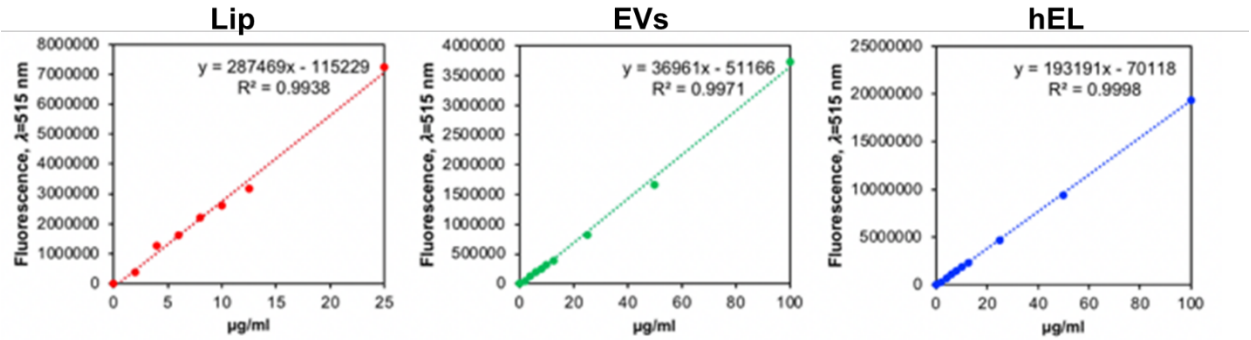


Figure S6. Standard curves of PKH67-labeled NVs with various concentrations. The fluorescence intensity of PKH67-labeled NVs was measured at λ = 515 nm.

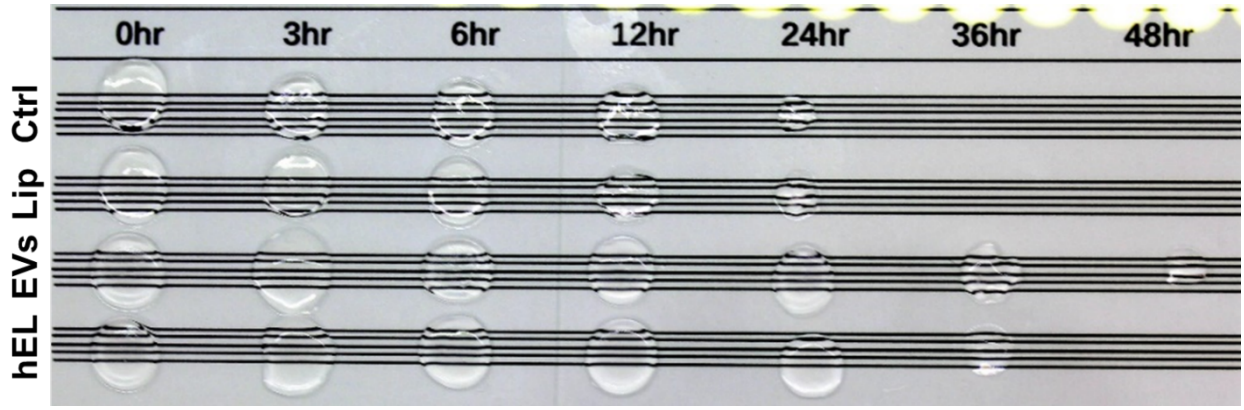


Figure S7. Images of 7.5% (w/v) Gel-NVs hydrogels degradation test performed in solutions of 0.1 U/mL of collagenase Type II inside an incubator operating at 37 °C for 0, 3, 6, 12, 24, 36, and 48 hours.