pharmaceutics

Supplementary Materials: Undercover Toxic Ménage à Trois of Amylin, Copper (II) and Metformin in Human Embryonic Kidney Cells

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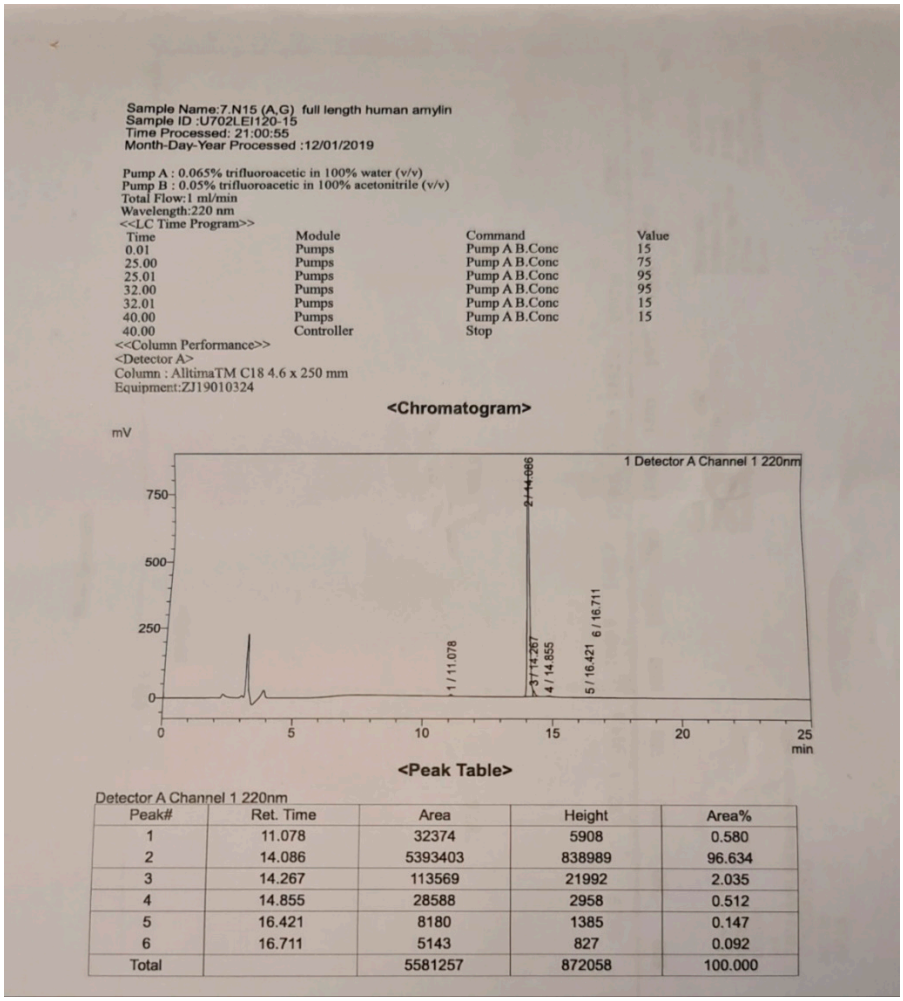


Scheme S1. Chromatogram of hIAPP sample delivered by GenScript.

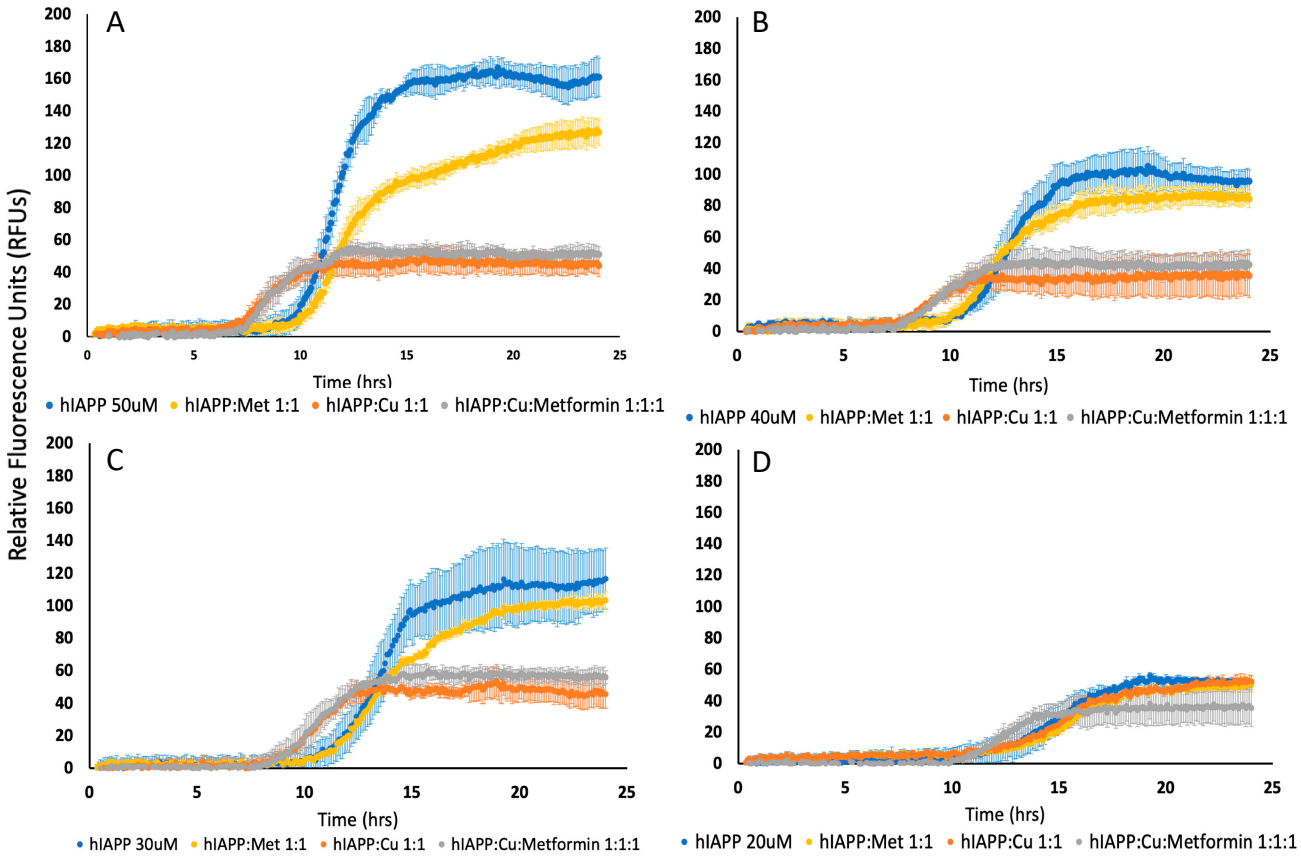


Figure S1. Effect of Cu (II) and Cu (II)/Metformin on hIAPP (in a 50 mM HEPES-buffered solution pH 7.4) fluorescence with ThT (20 μM) at 25 °C in different concentration conditions: (**A**) 50 μM; (**B**) 40 μM; (**C**) 30 μM and (**D**) 20 μM.

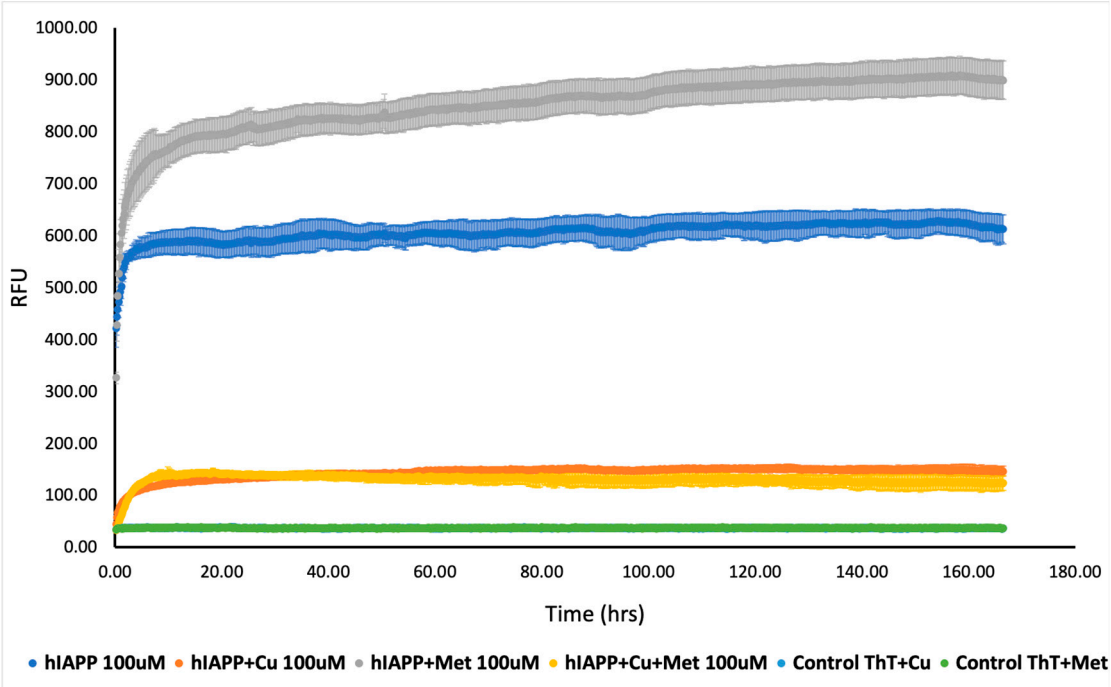


Figure S2. Effect of Cu (II) (100 μM) and Cu (II)/Metformin (100 μM; 1:1 molar ratio) on hIAPP (100 μM; in a 50 mM HEPES-buffered solution pH 7.4) fluorescence with ThT (20 μM) at 25 °C.

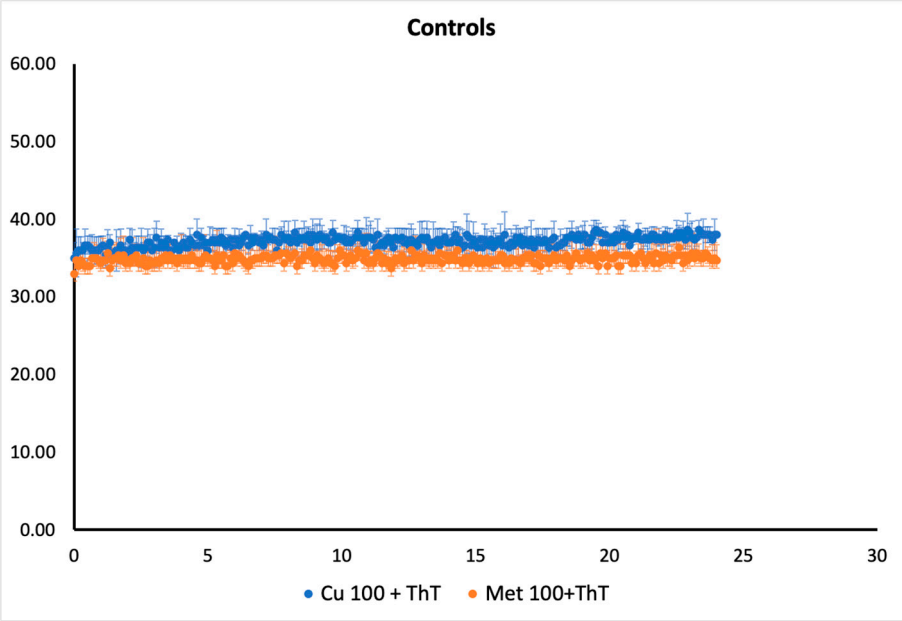
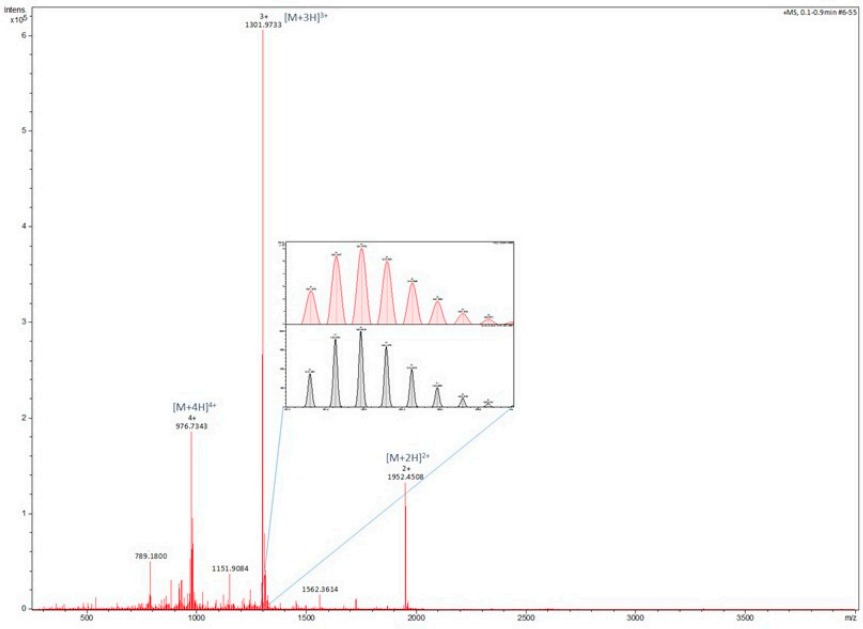


Figure S3. Effect of Cu (II) (100 μM) and Metformin (100 μM) on ThT (20 μM) fluorescence at 25 °C.

A



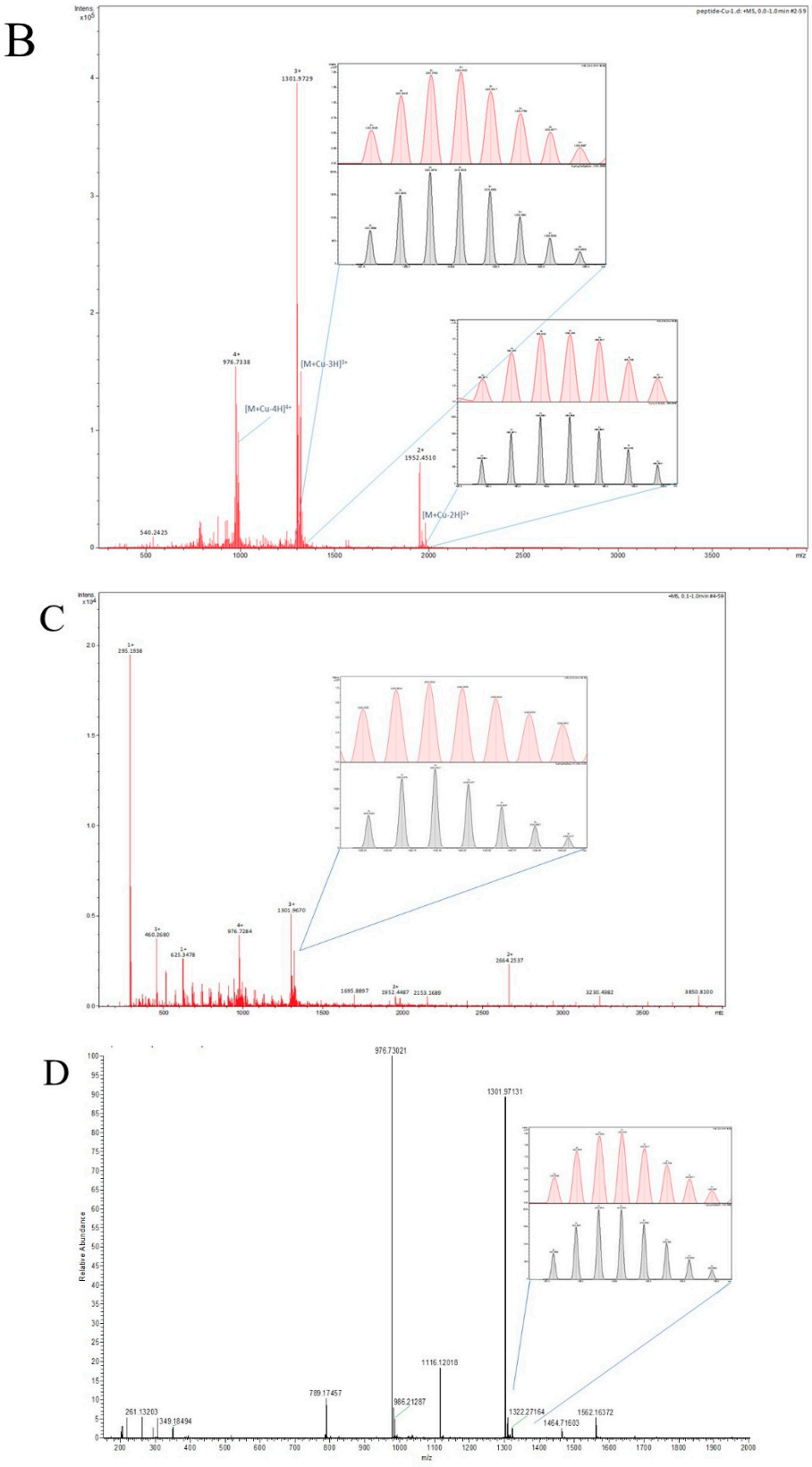


Figure S4. Full Scan Mass spectra acquired using the ESI-(+)-Micro-TOF-MS instrument. (**A**) hlAPPI solution. Signals at 976.7343; 1301.9733 and 1952.4508 *m*/*z* are assigned to hIAPP (M) at different protonation states: $[M+4H]^{4+}$; $[M+3H]^{3+}$; $[M+2H]^{2+}$; respectively. (**B**) hlAPPI/Cu(II) solution. Signals at 1322.6102 and 1983.4039 *m*/*z* are assigned to copper complexes: $[M+Cu(II)+H]^{3+}$ and $[M+Cu(II)]^{2+}$, respectively. (**C**) Metformin/hIAPP solution. Signal at 1342.9244 *m*/*z* is assigned to metformin (Met) adduct: $[M+Met-3H]^{3+}$. (**D**) Cu(II)/hIAPP/Metformin solution. Signal 1322.6102 is assigned to copper complexes: $[M+Cu(II)+H]^{3+}$. Insets: comparison of experimental (up) and simulated (down) isotopic distributions.

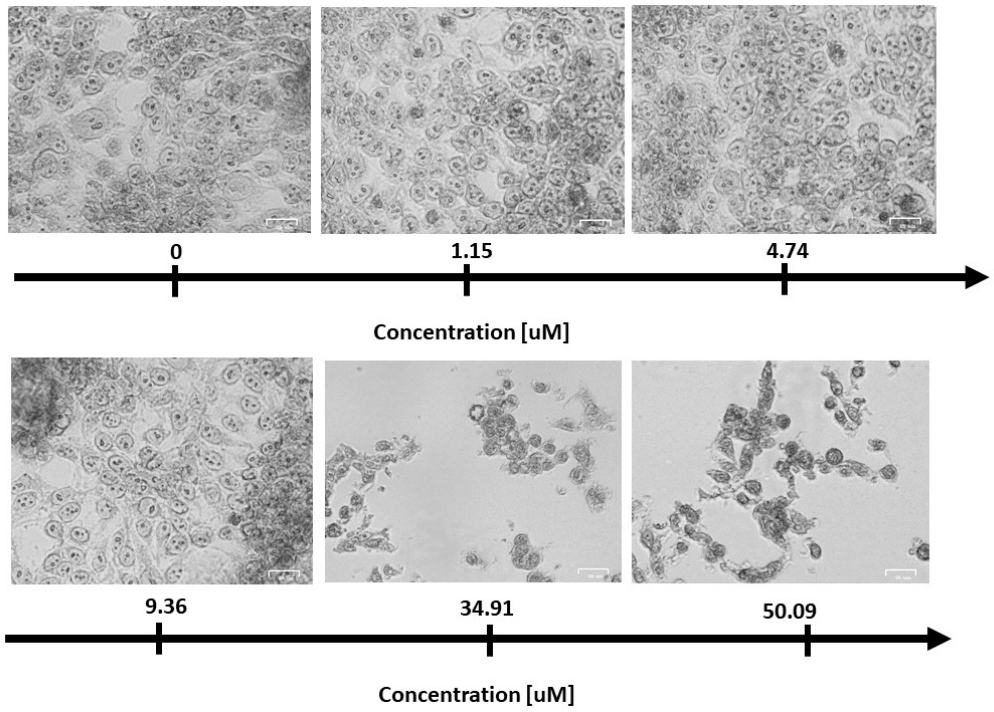


Figure S5. Live cell images of 293T cell cultures incubated 24h with growing concentration of hIAPP.

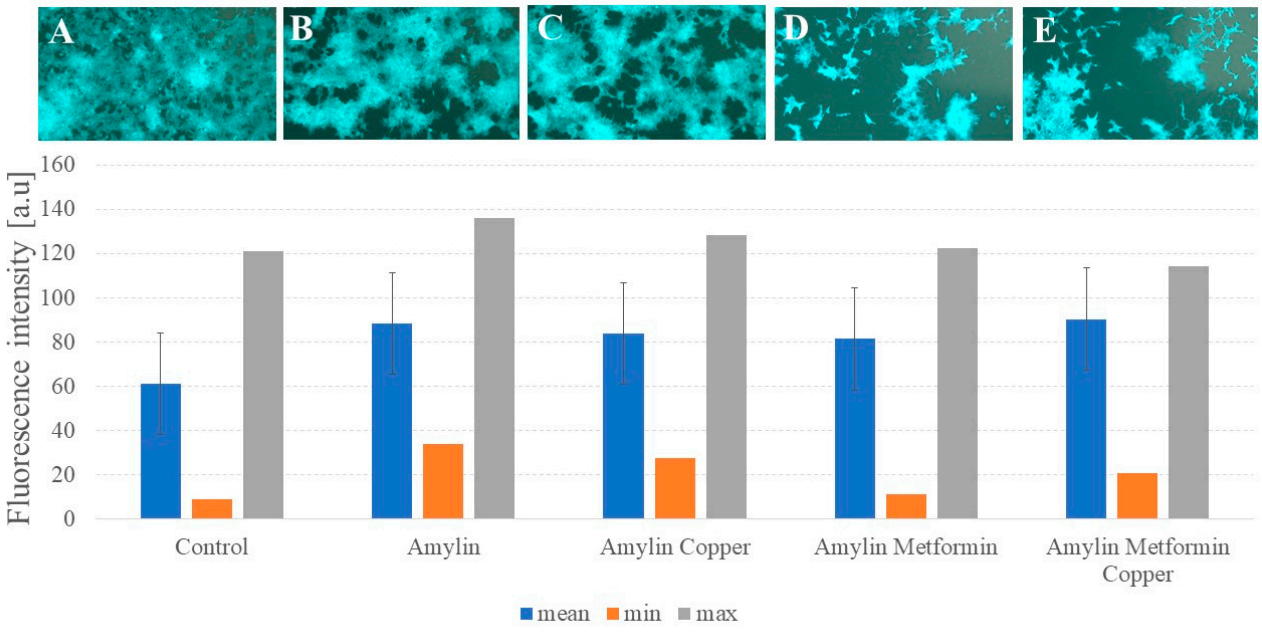


Figure S6. ThT live-cell fluorescence staining of 293T cells growing (**A**) in normal conditions; (**B**) with hIAPP (4.74 μM); (**C**) with hIAPP (4.74 μM) and copper ions (4.74 μM); (**D**) with hIAPP (4.74 μM) and metformin (4.74 μM); (**E**) with hIAPP (4.74 μM), copper ions (4.74 μM) and metformin (4.74 μM) for 24 h. The mean, min and max fluorescence intensity of each experimental sample (down) was calculated with ImageJ software.

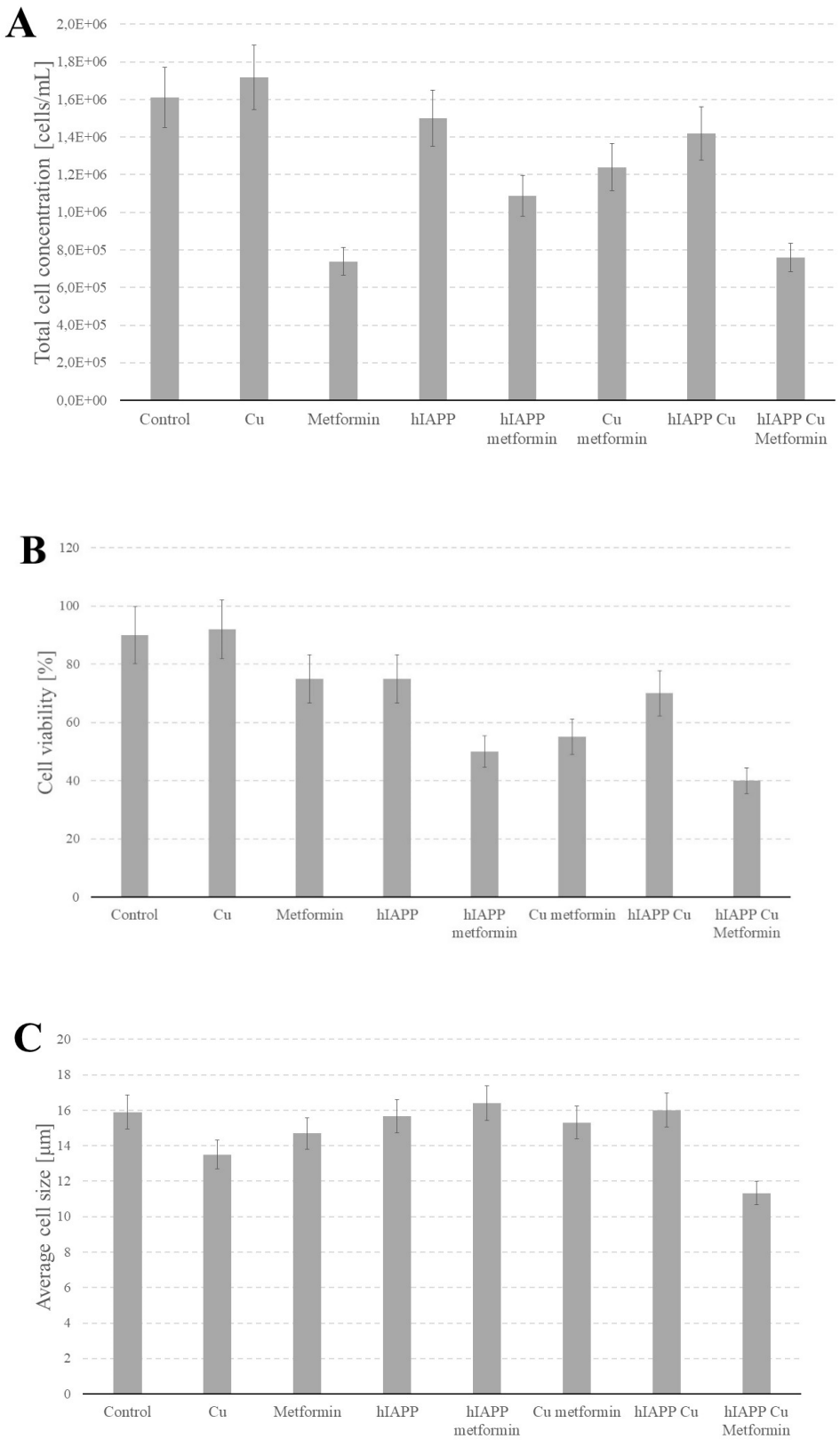


Figure S7. The graphical representation of LunaFL Cell Counter results of cell concentration (**A**), cell viability (**B**) and cell size (**C**).