Hybrid peptides inspired by the RWQWRWQWR sequence inhibit cervical cancer cells growth in vitro

Natalia Ardila-Chantré[1], Claudia Marcela Parra-Giraldo[2], Yerly Vargas-Casanova[2], Andrea Carolina Barragán-Cardenas[3], Ricardo Fierro-Medina[4], Zuly Jenny Rivera-Monroy[4], Jhon Erick Rivera-Monroy[5], Javier Eduardo García-Castañeda[1*]
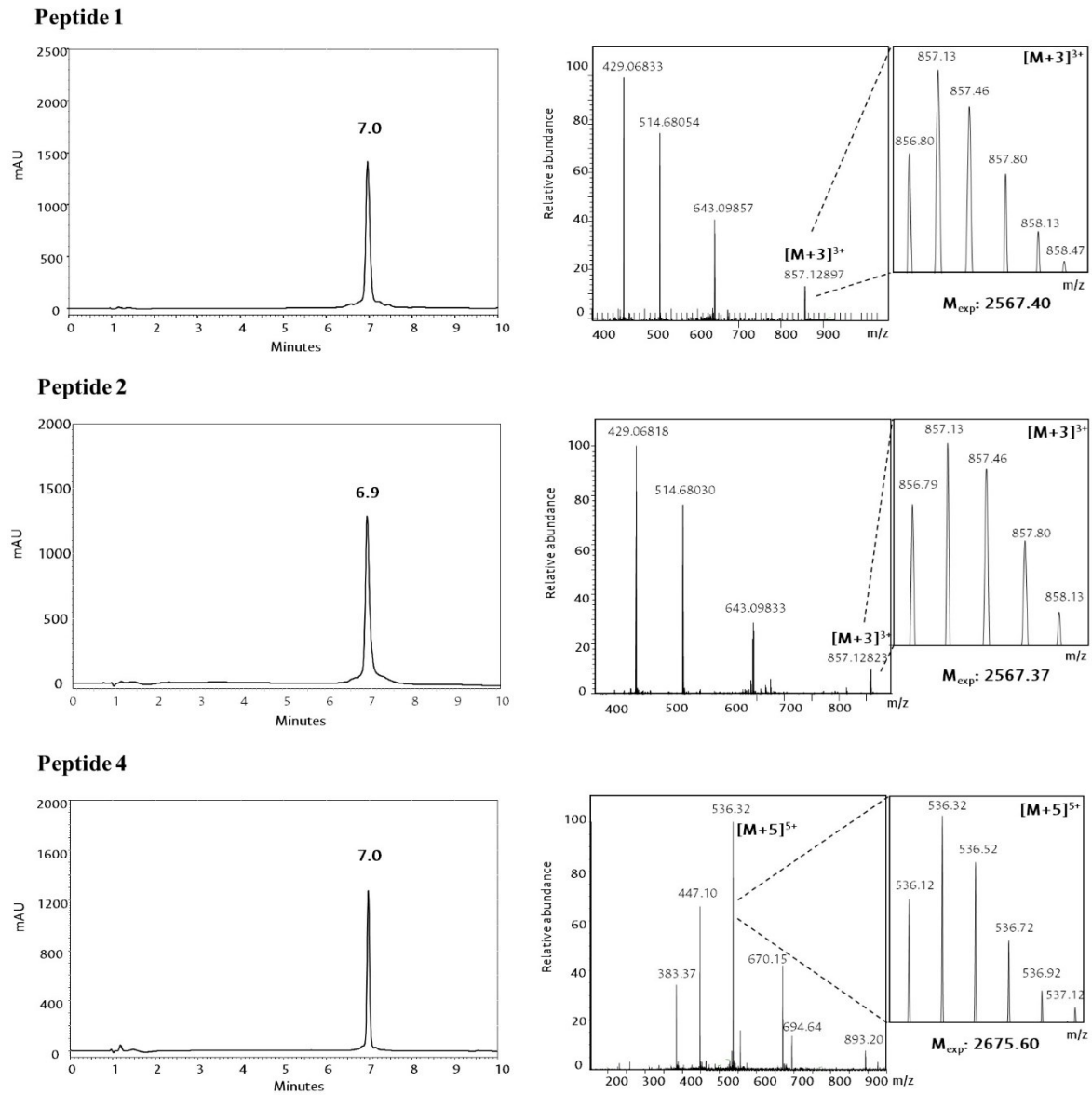
Supplementary materials 1	
Table S1	Table S1
Figure S1–12	Figure S1–12

In this supplement there are the figures corresponding to the characterization of the hybrid peptides, the cell viability curves and hemolysis curves, as well as the microphotographs of the treated cells in this study.
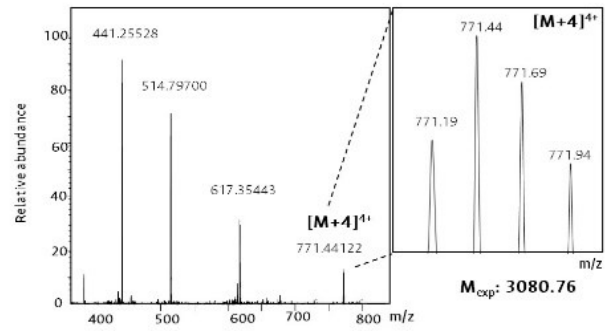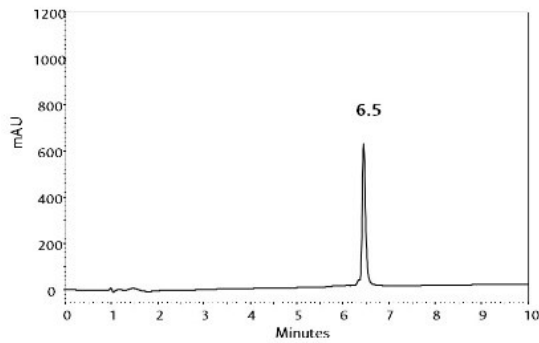

Figure S1. Characterization of hybrid peptides designed with anticancer peptides, include chromatographic profile (RP-HPLC) and ESI-mass spectrum

Peptide 5

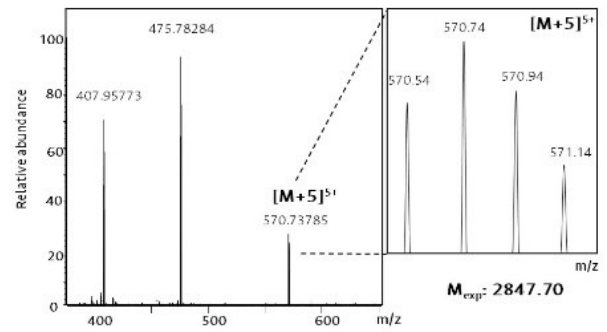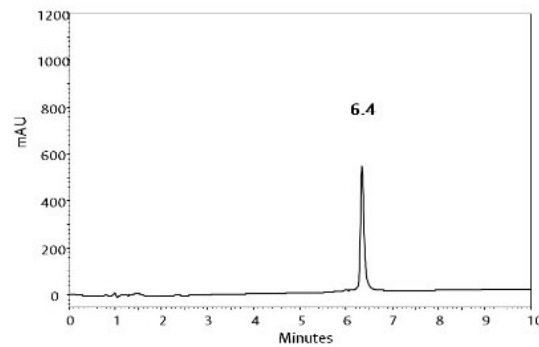**Peptide 6**
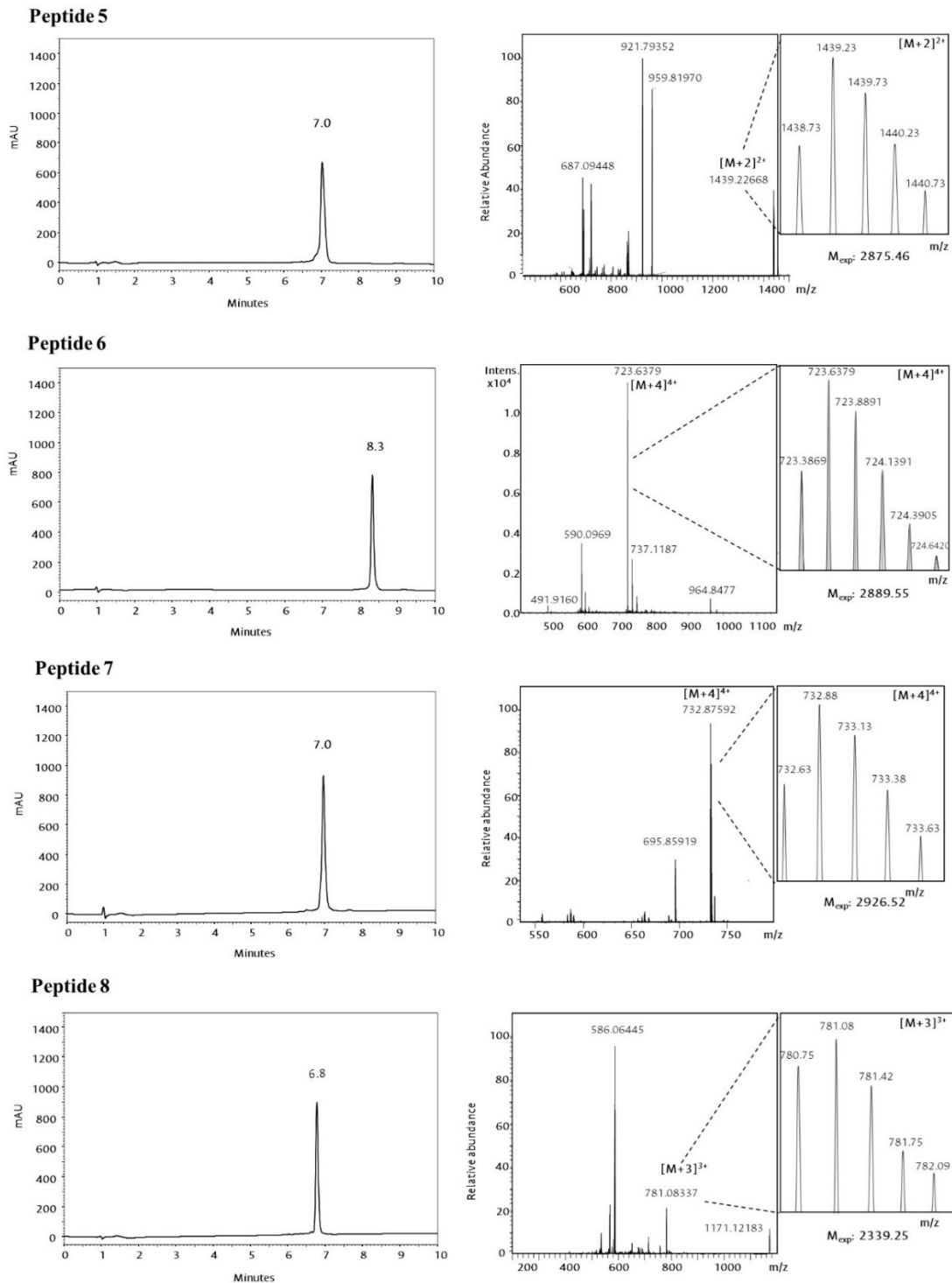
Peptide 7

Peptide 8

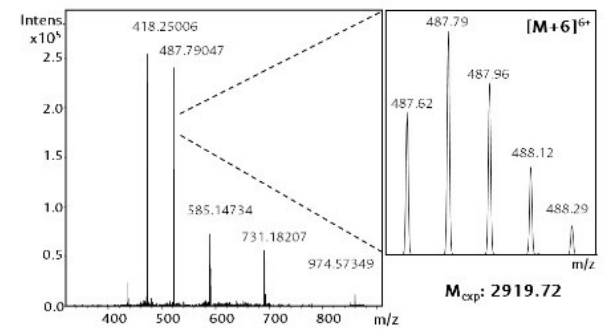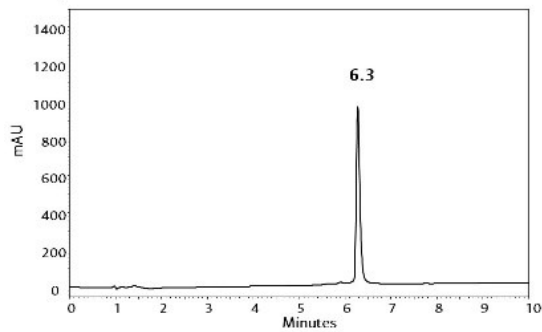**Figure S2.** Characterization of hybrid peptides designed with cell-targeting peptides, include chromatographic profile (RP-HPLC) and ESI-mass spectrum
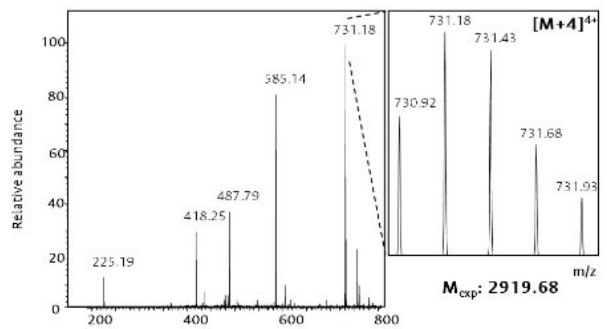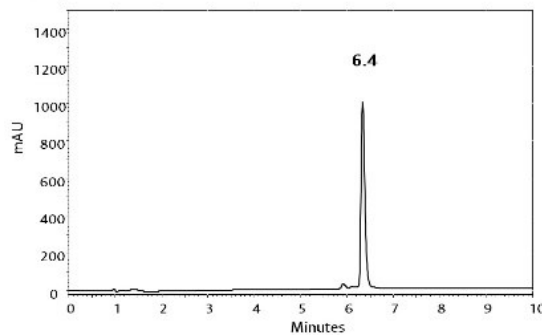
Peptide 9

Peptide 10

Peptide 11
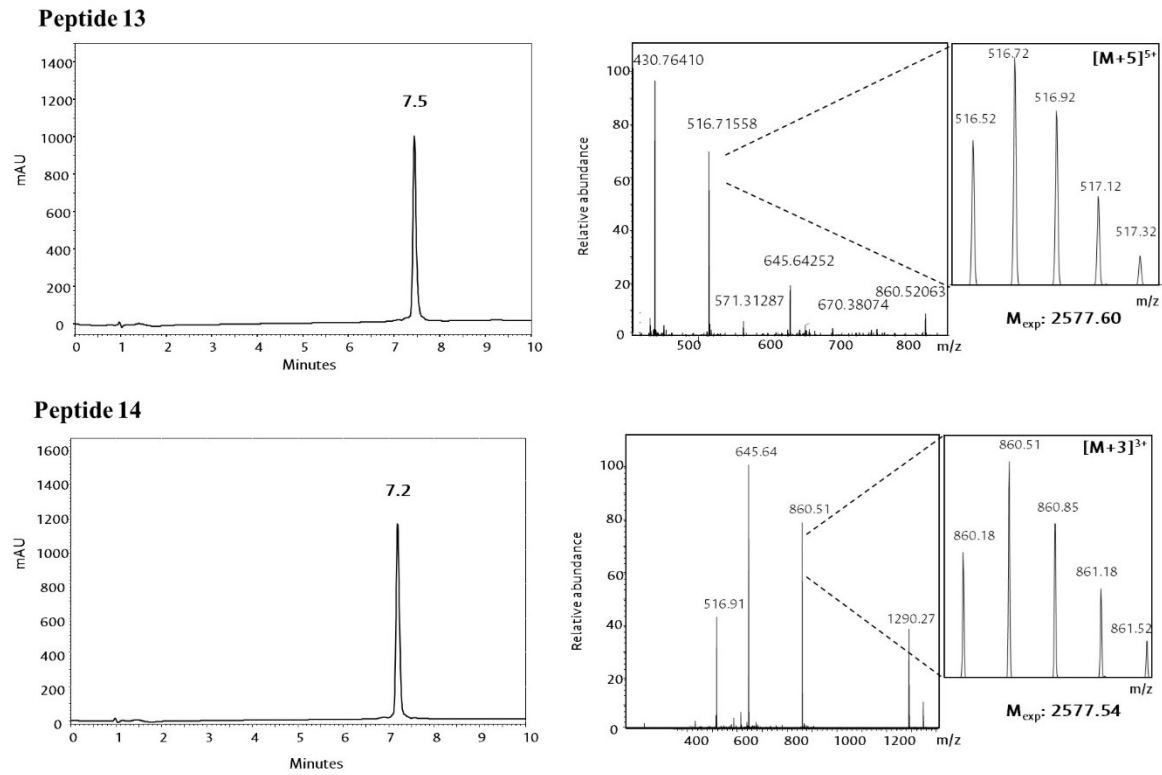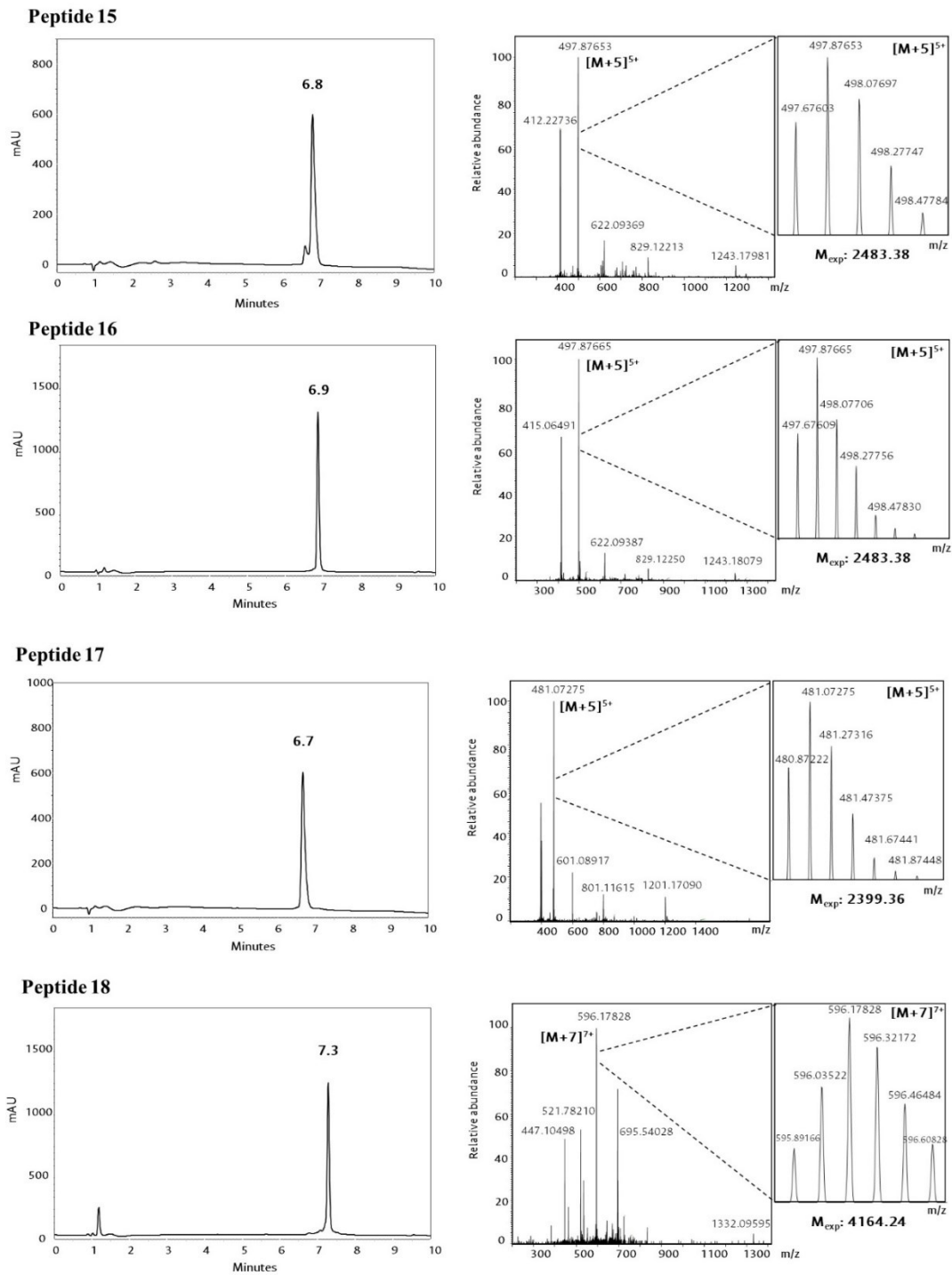

Peptide 12

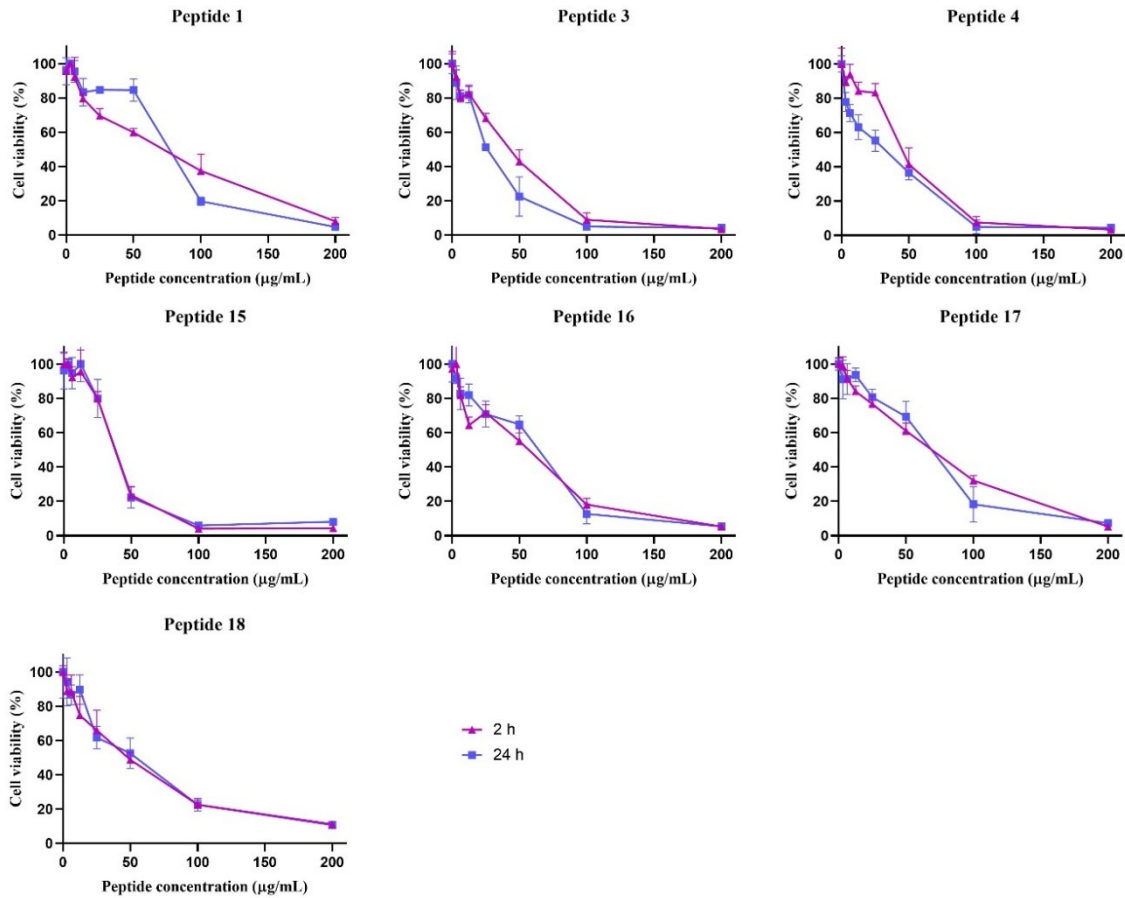**Peptide 13**

Peptide 14

Figure S3. Characterization of hybrid peptides designed with cell-targeting peptides, include chromatographic profile (RP-HPLC) and ESI-mass spectrum

Figure S4. Characterization of hybrid peptides designed with replacement of Arg by Lys and dimeric hybrid peptide, include chromatographic profile (RP-HPLC) and ESI-mass spectrum

Figure S5. Cytotoxic effect of hybrid peptides against HeLa cancer cell lines after 2 h and 24 h of treatment at 37 °C. The data are expressed as the mean ± SE (n = 3)
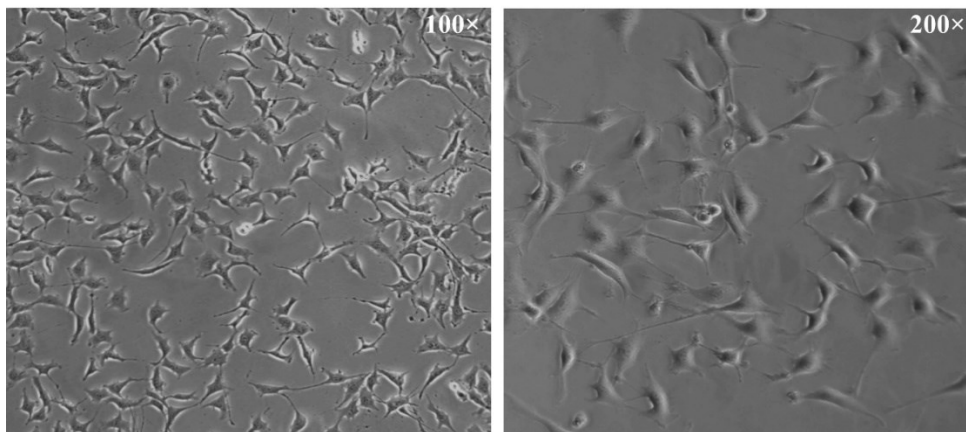
Figure S6. Sub confluent (50%) HeLa cells monolayer visualized at a total magnification of 100× and 200×
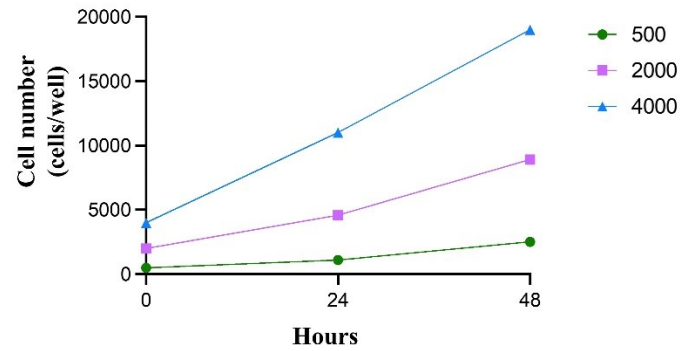
Figure S7. Comparison of cell growth profiles measured with trypan blue exclusion using three different initial densities: (green) 500 (t= 0), 1097 (24 h) and 2500 cells/well (48 h); (Pink) 2000 (t= 0), 4583 (24 h) and 8900 cells/well (48 h); (Blue) 4000 (t= 0), 11000 (24 h) and 19000 cells/well (48 h) (n = 3)
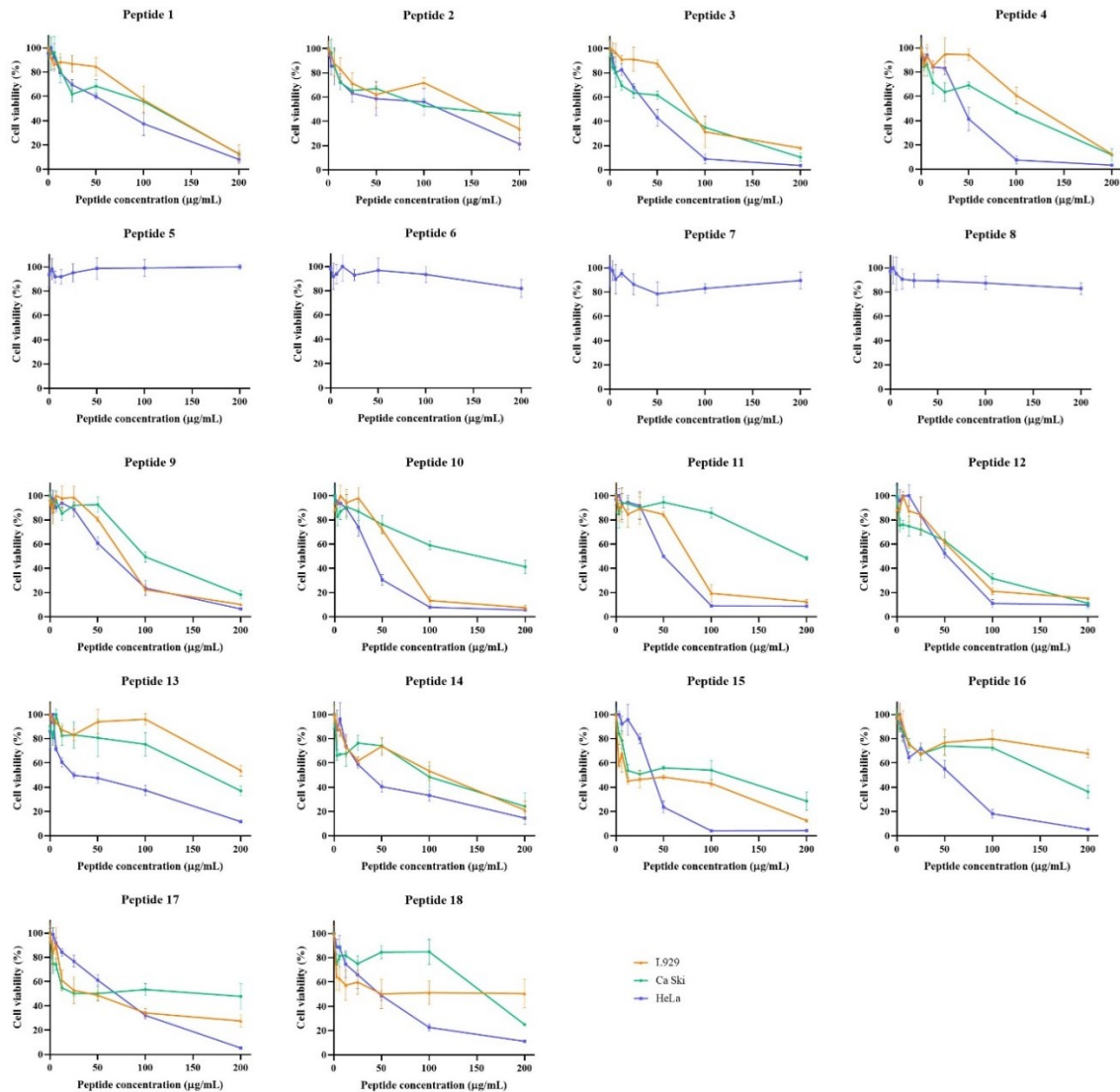
Figure S8. Cytotoxic effect of hybrid peptides against cervical cancer cell lines and fibroblast cells, after 2 h treatment at 37 °C. The data are expressed as the mean ± SE (n = 3)

Peptide 2   Peptide 9   Peptide 10   Peptide 11   Peptide 12   Peptide 13

Peptide 14   Peptide 15   Peptide 16   Peptide 17   Peptide 18
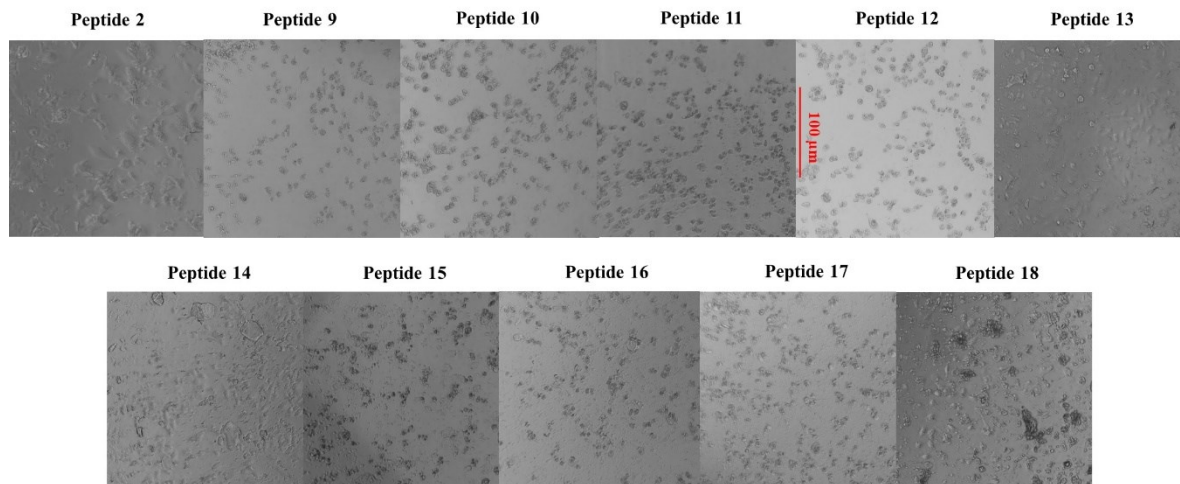
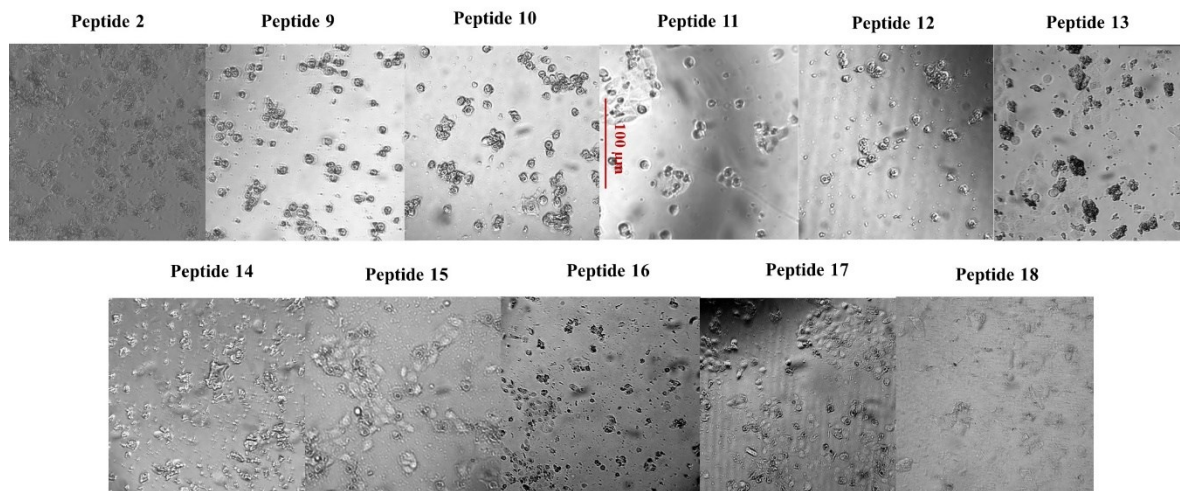**Figure S9.** Microphotographs by contrast microscopy (200×) of the HeLa cells treated with hybrid peptides designed with anticancer peptides and cell-penetrating peptides, at 200 µg/mL, after 2 h treatment at 37°C

Peptide 2   Peptide 9   Peptide 10   Peptide 11   Peptide 12   Peptide 13

Peptide 14   Peptide 15   Peptide 16   Peptide 17   Peptide 18

Figure S10. Microphotographs by contrast microscopy (200×) of the Ca Ski cells treated with hybrid peptides designed with anticancer peptides and cell-penetrating peptides, at 200 µg/mL, after 2 h treatment at 37°C
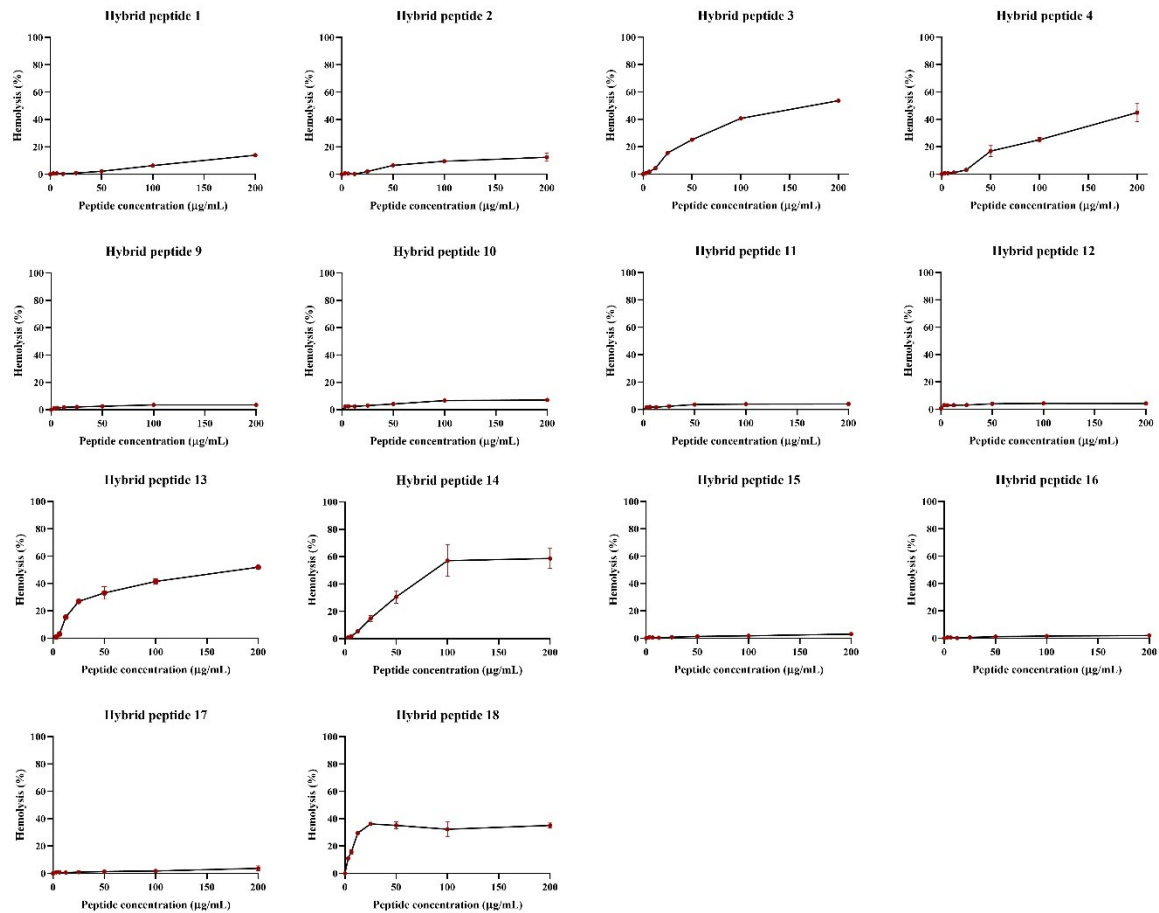
Figure S11. Hemolytic effect of hybrid peptides designed with anticancer peptides and cell-penetrating peptides, after 2 h treatment at 37 °C. The data are expressed as the mean ± SE (n = 3)
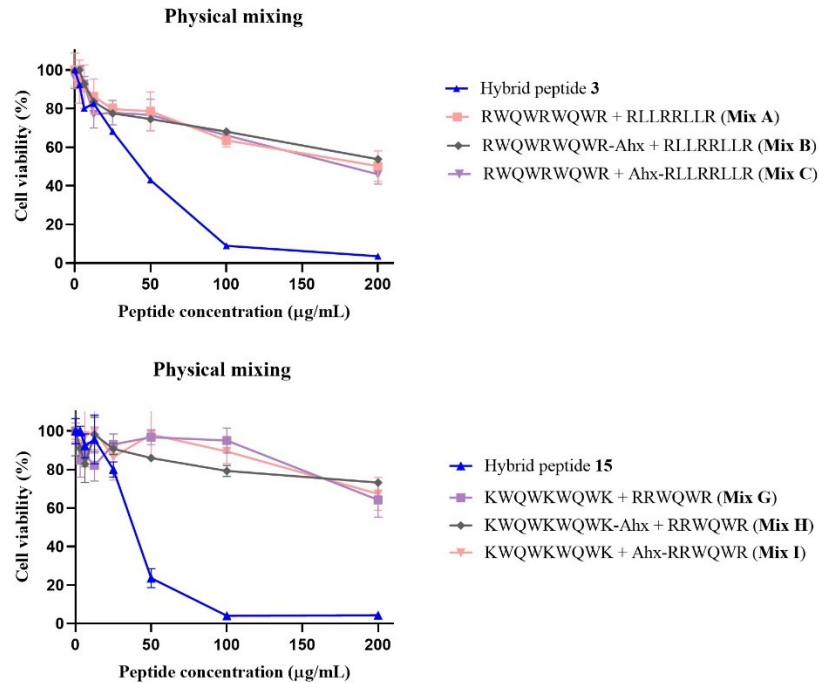
Figure S12. Cytotoxic effect of the physical mixing of the precursor peptides that make up the hybrid peptide **3** and **15** against HeLa cell line, after 2 h treatment at 37 °C. The data are expressed as the mean ± SE (*n* = 3)

Table S1. Cytotoxic effect of hybrid peptides in HeLa cells after 2 h and 24 h of treatment at 37°C

Code	HeLa IC$_{50}$ (µM)	
	2 h	**24 h**
1	21.6	28.2
3	13.3	9.2
4	16.0	7.7
15	14.6	14.6
16	15.7	19.0
17	23.9	25.3
18	9.6	10.6