Supplementary information

Dissolution and Degradation of Fmoc-diphenylalanine self-assembled gels results in necrosis at high concentrations *in vitro*

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Statistical Approach used for ANOVA

Two-way ANOVA (repeated measures with both factors) was performed using GraphPad Prism version 6.03 for Windows, GraphPad Software, San Diego California USA, (www.graphpad.com) for the exposure time vs. leaching time experiments. One-way ANOVA was performed using the Analysis ToolPak in Microsoft Excel version 14.0.7109.5000 for Windows for the chemotherapeutic and **Fmoc-FF**.

When an ANOVA (Analysis of Variance) test gave a significant result, to determine *which* group differs, the least significant difference test (LSD) was employed. The LSD calculates the smallest significant difference between two means as if a t-test had been run on those two means. Hence, direct comparisons between two means from two groups are possible. Any difference larger than the LSD calculated is considered a significant result.

$$LSD = t\sqrt{\frac{2MSE/n}{n^*}}$$

Where t is the critical tabled value of the t-distribution with the degrees of freedom (*df*) associated with mean square error (MSE), the denominator of the *F* statistic and *n** is the number of scores used to calculate the means of interest. Further details on the LSD test can be found in the Encyclopedia of Research Design.[1]

Reference:

1. L. J. Williams and H. Abdi, in *Encyclopedia of Research Design*, ed. N. J. Salkind, SAGE Publications, Inc., Thousand Oaks, CA, 2010.

Supplementary Information:

Table S1

Matrix representing the time points sampled and evaluated for Hoechst 33342/propidium iodide (HO/PI) fluorescence microscopy and SEM.

	Exposure time to cells (h)			
Leaching time (h)	24	48	72	96
24	X		X	
48				
72	X		X	
96				

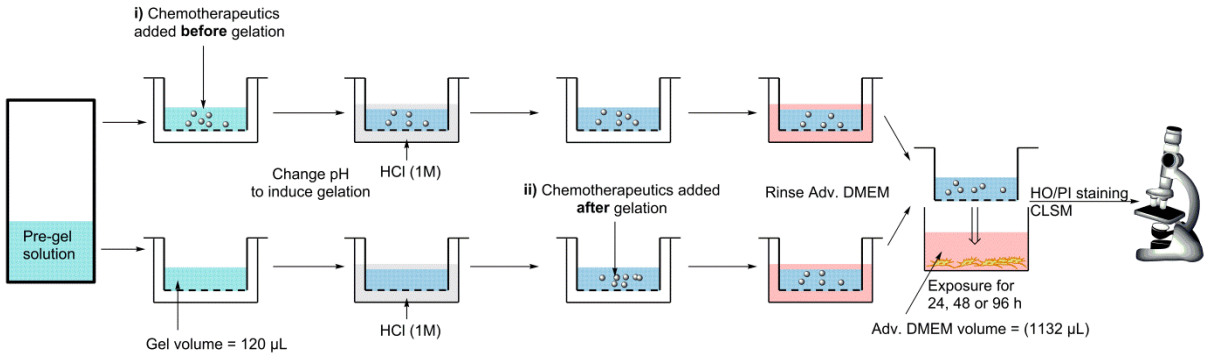

Fig. S1. Schematic showing the protocol used to evaluate the combined effects of **Fmoc-FF** with chemotherapeutics added: (i) before gelation or (ii) after gelation. Stock solutions (12 µL) of 5-Fluorouracil (7.7 mM) or paclitaxel (772 µM) were added to gels (120 µL volume in insert). In the final stage of exposing cells to the gel and drug mixtures, cells were incubated in Adv. DMEM (1.132 mL) to give final concentrations of 82 µM and 8.2 µM for 5-fluorouracil and paclitaxel respectively.

High-performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) Methods for analysis gel lechate

All the samples were analysed using reverse phase liquid chromatography-mass spectrometry (LC-MS) used a Shimadzu Prominence UFLC HPLC system with a LC-20AD pump and associated SIL-20A autosampler. Detection was made using a SPD-M20A PDA detector at 254 nm, the stationary phase used was a XBridge^{TM} C18 (5 μm, 150 × 4.60 mm) analytical column using a 50 μL injection volume on an autosampler. It should be noted that the samples collected were not filtered but just sonicated after 50-fold dilution in water. Additionally, a guard column was not used, however, there was no noticeable pressure building during the course of this analysis (4 time points x n = 4 + blanks and controls > 20 samples). The mobile phase consisted of eluents A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) run at gradient with 5% B from 0-2.5 min and then from 5-90% B from 2.5-20 min and then 90% B from 20-22.5 min. The flow rate was 1 mL/min and the detector wavelength range was set at 200-800 nm. Mass spectra were collected both in positive (+ESI) and negative (-ESI) mode. The analysis on the gel leachate was repeated four times (n = 4) and representative results from these LC-MS measurements shown in Fig. S5-S8.

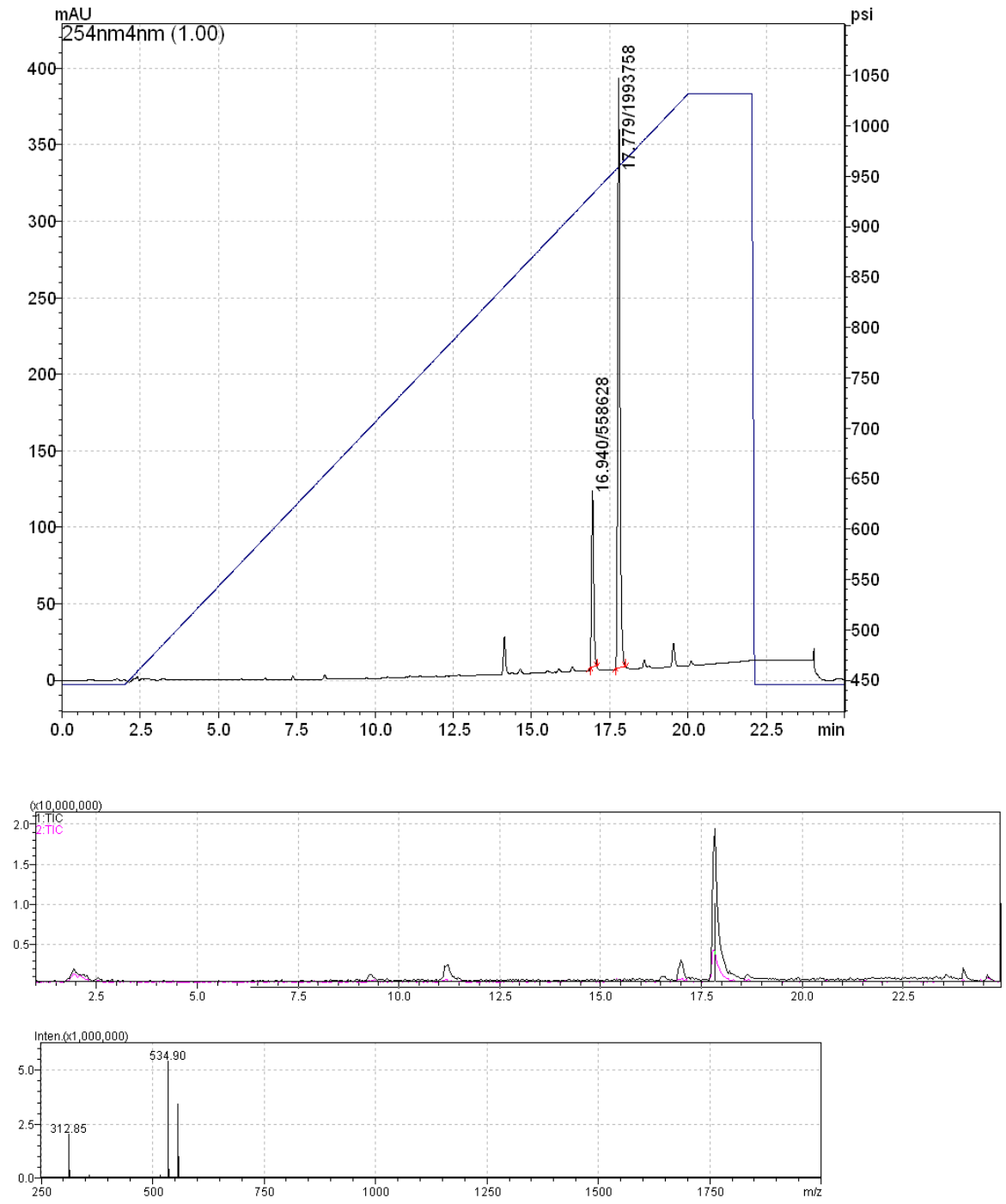

Fig. S2. LC-MS trace of a standard 2.0 mM solution of **Fmoc-FF**, diluted 50 fold prior to injection on the LC-MS column. Top panel: UV-Vis chromatograph (254 nm) with the **Fmoc-FF** peak eluting at R_t = 17.78 min and a minor peak from Fmoc-phenylalanine (**Fmoc-F**) eluting at R_t = 16.93 min (see also Fig. S3). Middle panel: Total Ion-count (TIC) Chromatographs; black = +ESI, pink = -ESI, Bottom panel: Mass spectra (+ESI) of the peak eluting at R_t = 17.78 min.

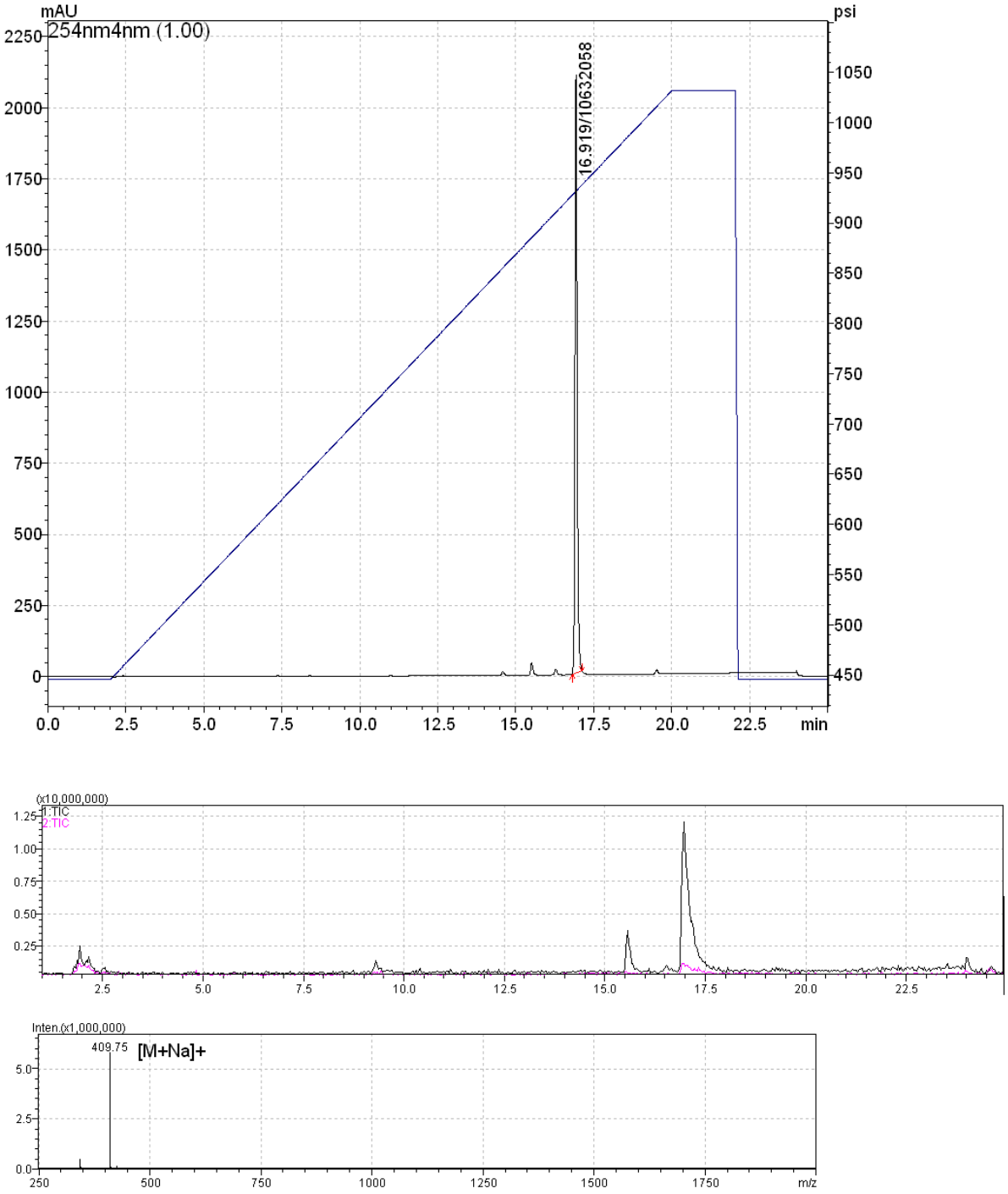

Fig. S3. LC-MS trace of a standard 2.0 mM solution of **Fmoc-F**, diluted 50 fold prior to injection on the LC-MS column. Top panel: UV-Vis chromatograph (254 nm) with the **Fmoc-F** eluting at R_t = 16.93 min. Middle panel: Total Ion-count (TIC) Chromatographs; black = +ESI, pink = -ESI, Bottom panel: Mass spectra (+ESI) of the peak eluting at R_t = 16.93 min.

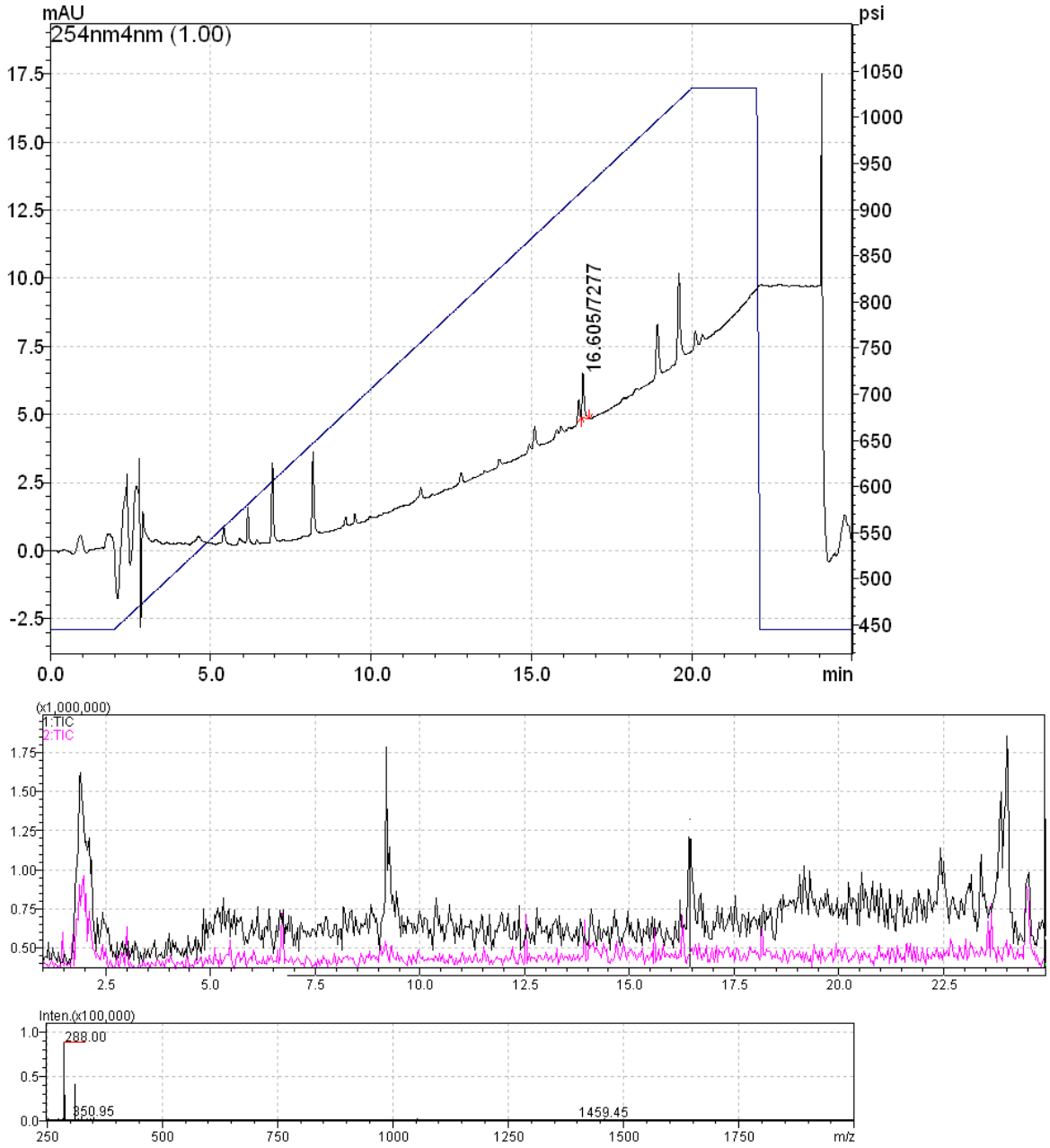

Fig. S4. LC-MS trace of a blank DMEM sample (10 µL) diluted 50 fold prior to injection on the LC-MS column. Top panel: UV-Vis chromatograph (254 nm).). An unidentified peak at R_t = 16.61 min has been highlighted.Middle panel: Total Ion-count (TIC) Chromatographs; black = +ESI, pink = -ESI. Bottom panel: Mass spectra (+ESI) of the peak eluting at R_t = 16.61 min.

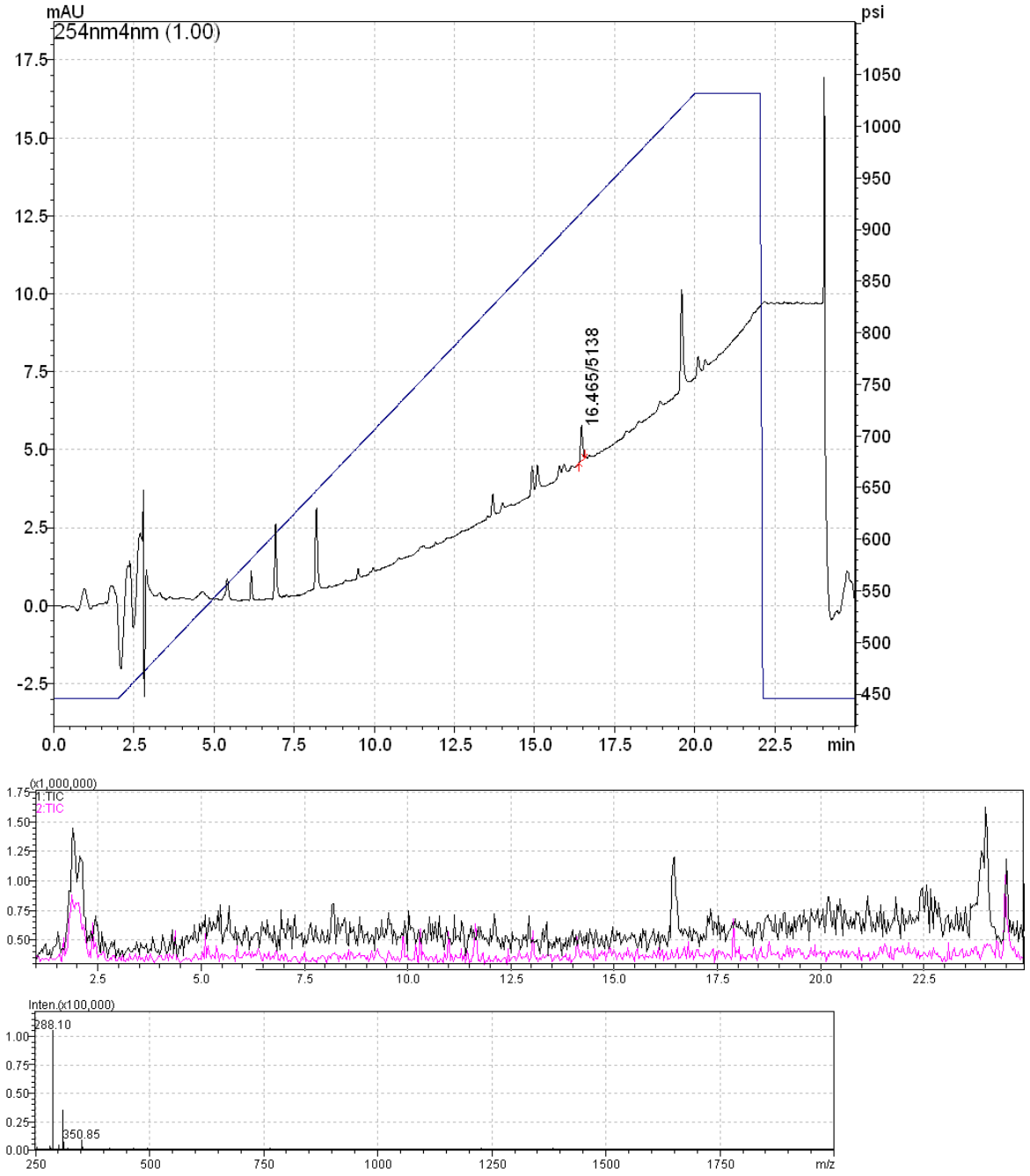

Fig. S5. LC-MS trace of a gel leachate sample (10 µL) taken after 24 h and diluted 50 fold prior to injection on the LC-MS column. Top panel: UV-Vis chromatograph (254 nm). An unidentified peak at R_t = 16.47 min has been highlighted. Middle panel: Total Ion-count (TIC) Chromatographs; black = +ESI, pink = -ESI. Bottom panel: Mass spectra (+ESI) of the peak eluting at R_t = 16.47 min. The ions identified do not match **Fmoc-FF** or any obvious derivatives of **Fmoc-FF**.

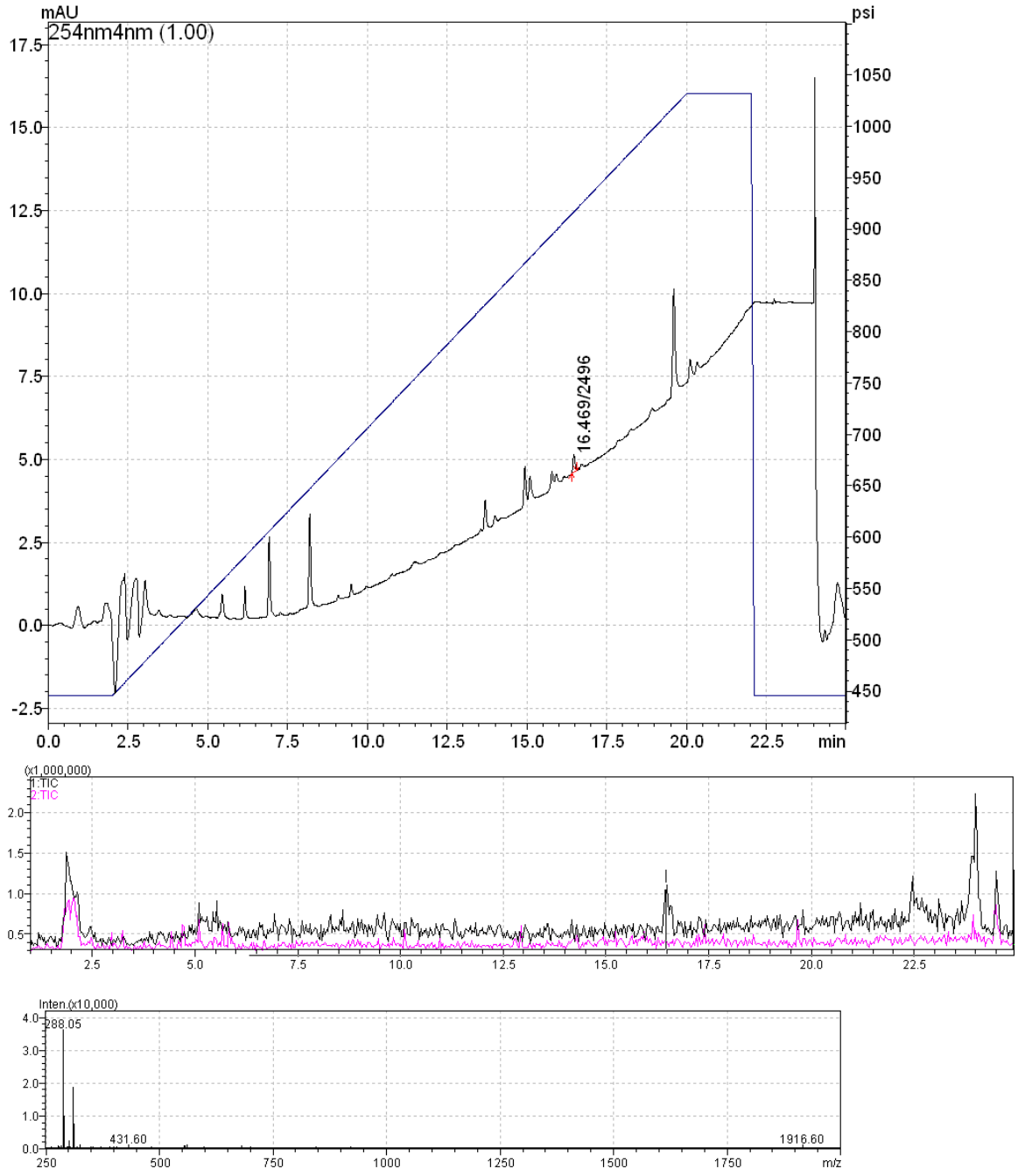

Fig. S6. LC-MS trace of a gel leachate sample (10 µL) taken after 48 h and diluted 50 fold prior to injection on the LC-MS column. Top panel: UV-Vis chromatograph (254 nm). An unidentified peak at R_t = 16.47 min has been highlighted. Middle panel: Total Ion-count (TIC) Chromatographs; black = +ESI, pink = -ESI. Bottom panel: Mass spectra (+ESI) of the peak eluting at R_t = 16.47 min. The ions identified do not match **Fmoc-FF** or any obvious derivatives of **Fmoc-FF**.

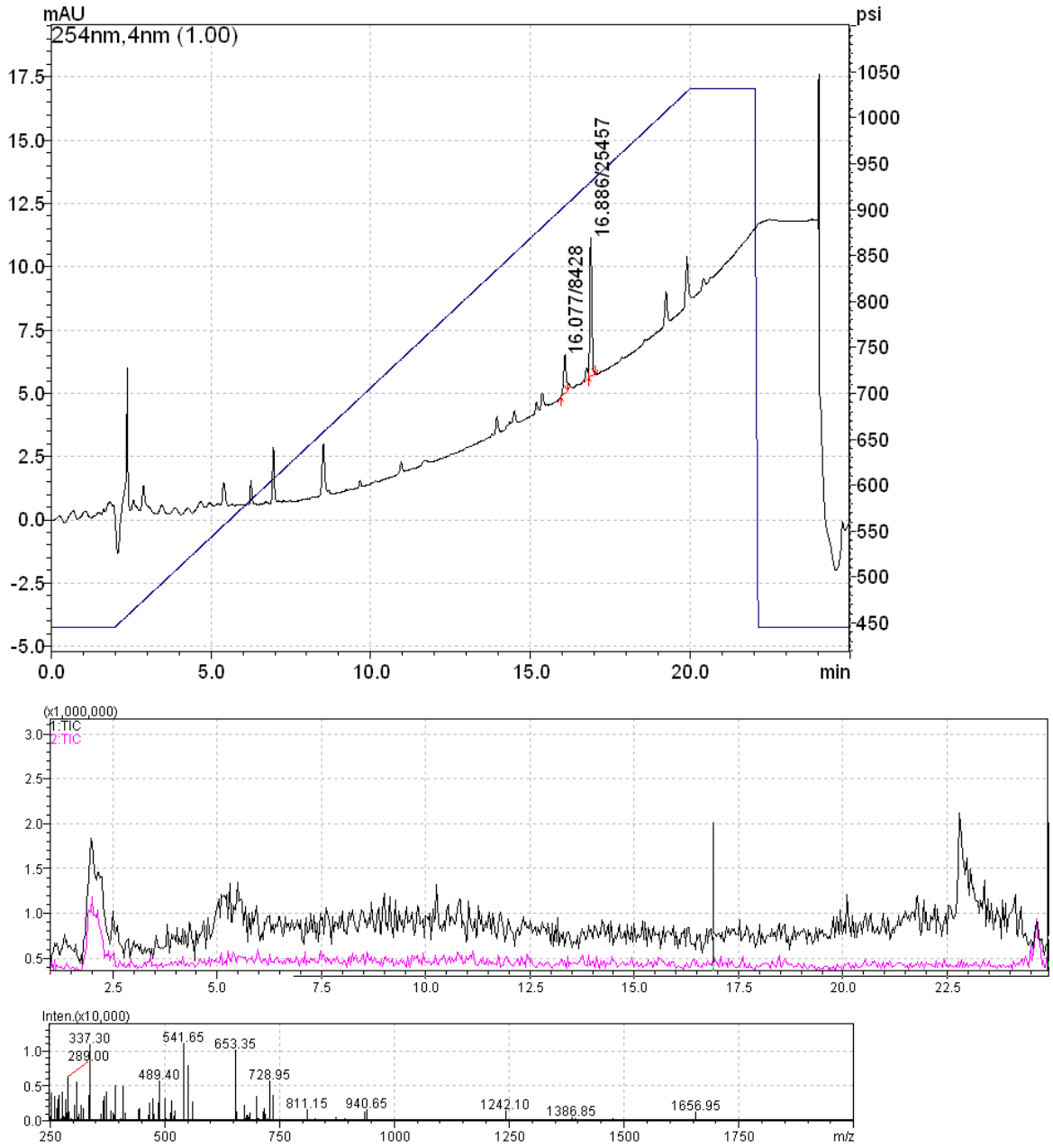

Fig. S7. LC-MS trace of a gel leachate sample (10 µL) taken after 72 h and diluted 50 fold prior to injection on the LC-MS column. Top panel: UV-Vis chromatograph (254 nm). Unidentified peaks at R_t = 16.08 and R_t = 16.89 min have been highlighted. Middle panel: Total Ion-count (TIC) Chromatographs; black = +ESI, pink = -ESI. Bottom panel: Mass spectra (+ESI) of the peak eluting at R_t = 16.89 min. The ions identified do not match **Fmoc-FF** or any obvious derivatives of **Fmoc-FF**.

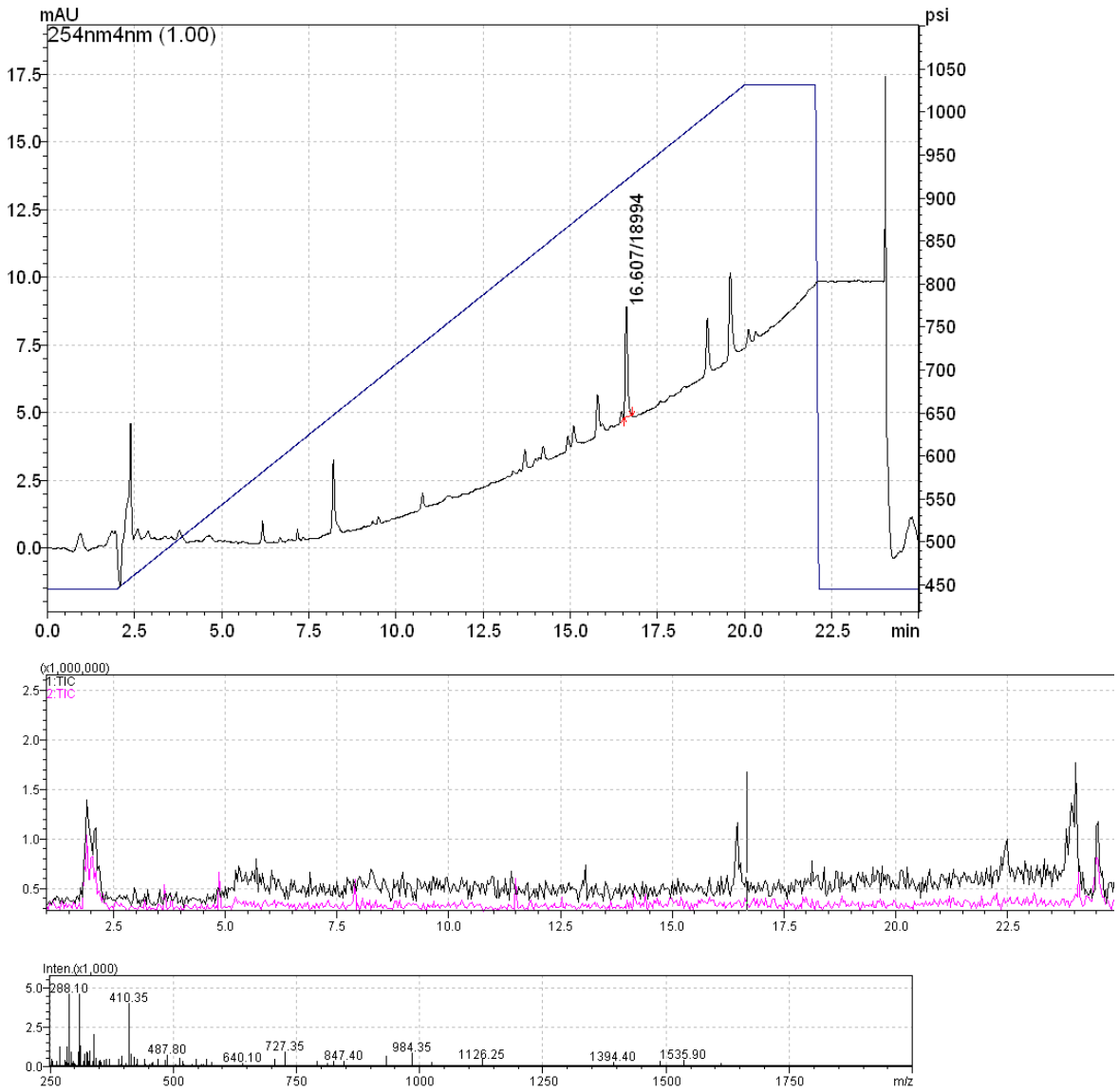

Fig. S8. LC-MS trace of a gel leachate sample (10 μL) taken after 96 h and diluted 50 fold prior to injection on the LC-MS column. Top panel: UV-Vis chromatograph (254 nm). An unidentified peak at R_t = 16.61 min have been highlighted. Middle panel: Total Ion-count (TIC) Chromatographs; black = +ESI, pink = -ESI. Bottom panel: Mass spectra (+ESI) of the peak eluting at R_t = 16.69 min. The ions identified do not match **Fmoc-FF** or any obvious derivatives of **Fmoc-FF**.

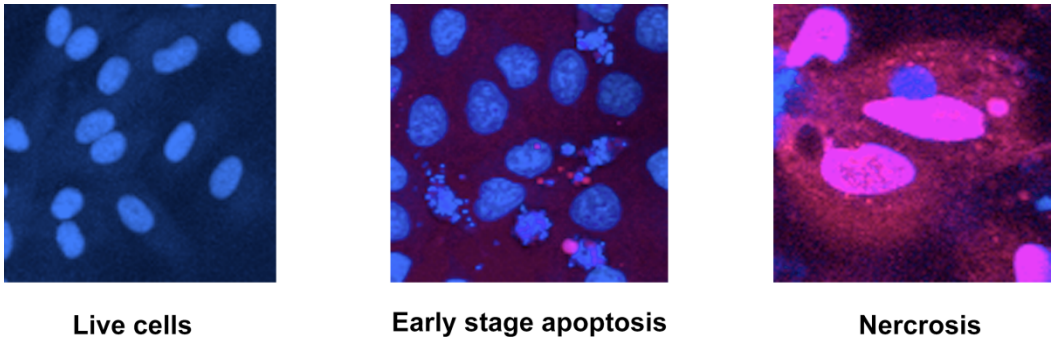

Fig. S9. Stages of cell death as visualised by Hoechst 33342/propidium iodide (HO/PI) staining on a fluorescence microscope.

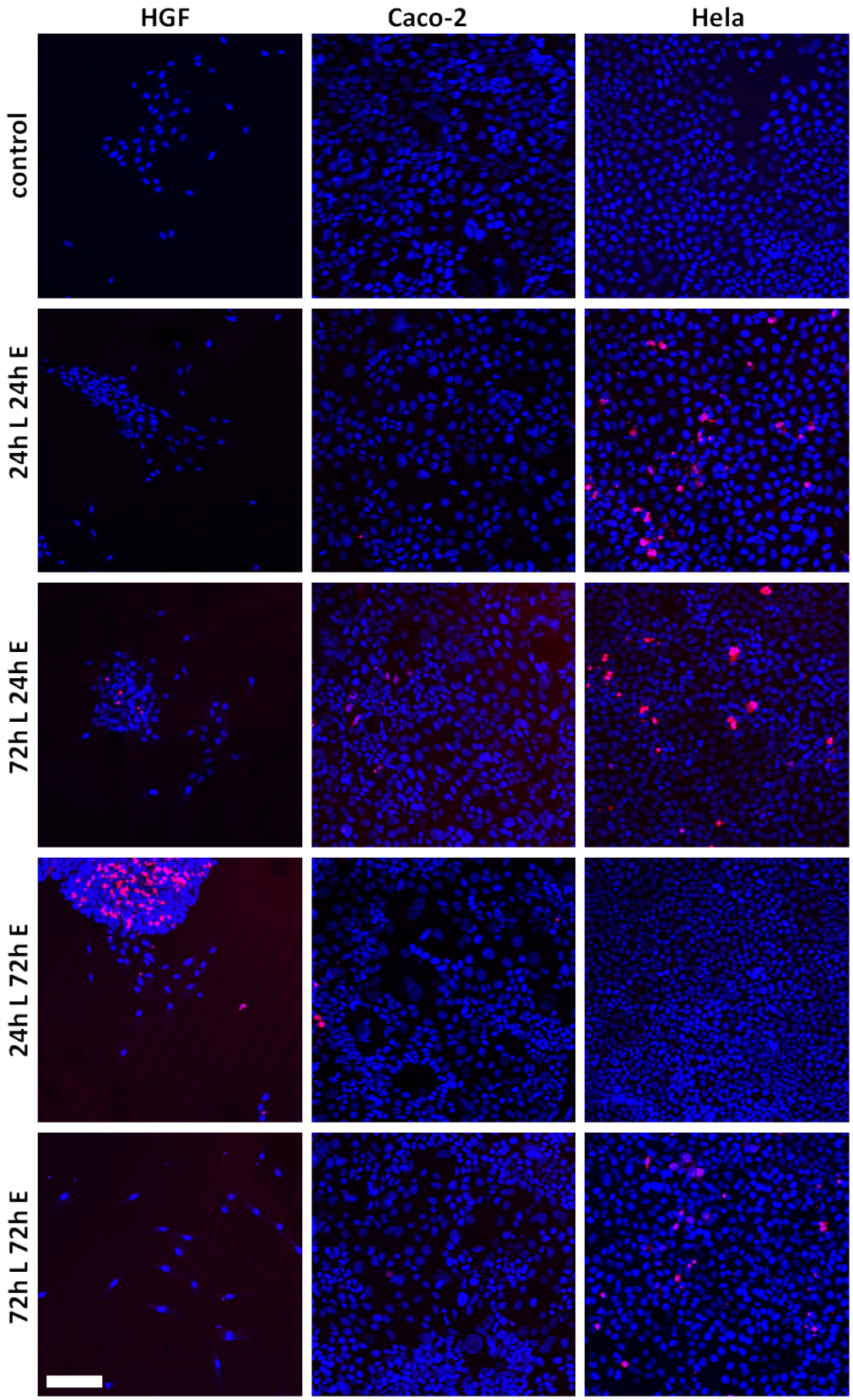

Fig. S10. Fluorescent micrographs of Hoescht/propidium iodide stained HGF-1, Caco-2 and HeLa cell lines exposed to **Fmoc-FF** degradation products. L denotes leaching time. E denotes exposure time. Scale bar, 100 μm. Live cells (blue fluorescence), apoptotic cells (intense bright-blue and blue-violet fluorescence) and necrotic cells (pink fluorescence).

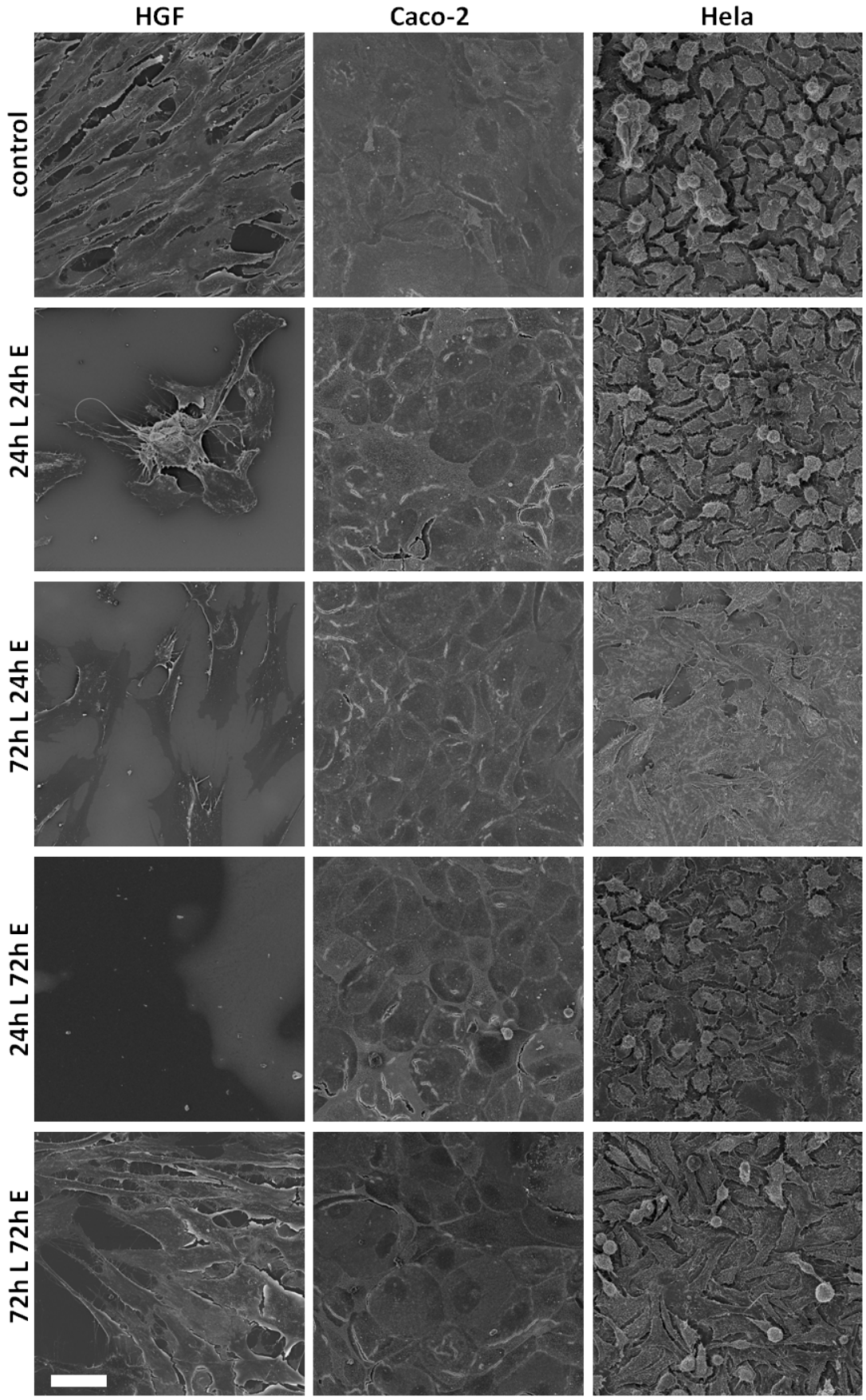

Fig. S11. SEM of cell lines. (L = leaching time), (E = exposure time). Scale bar, 20 μm.

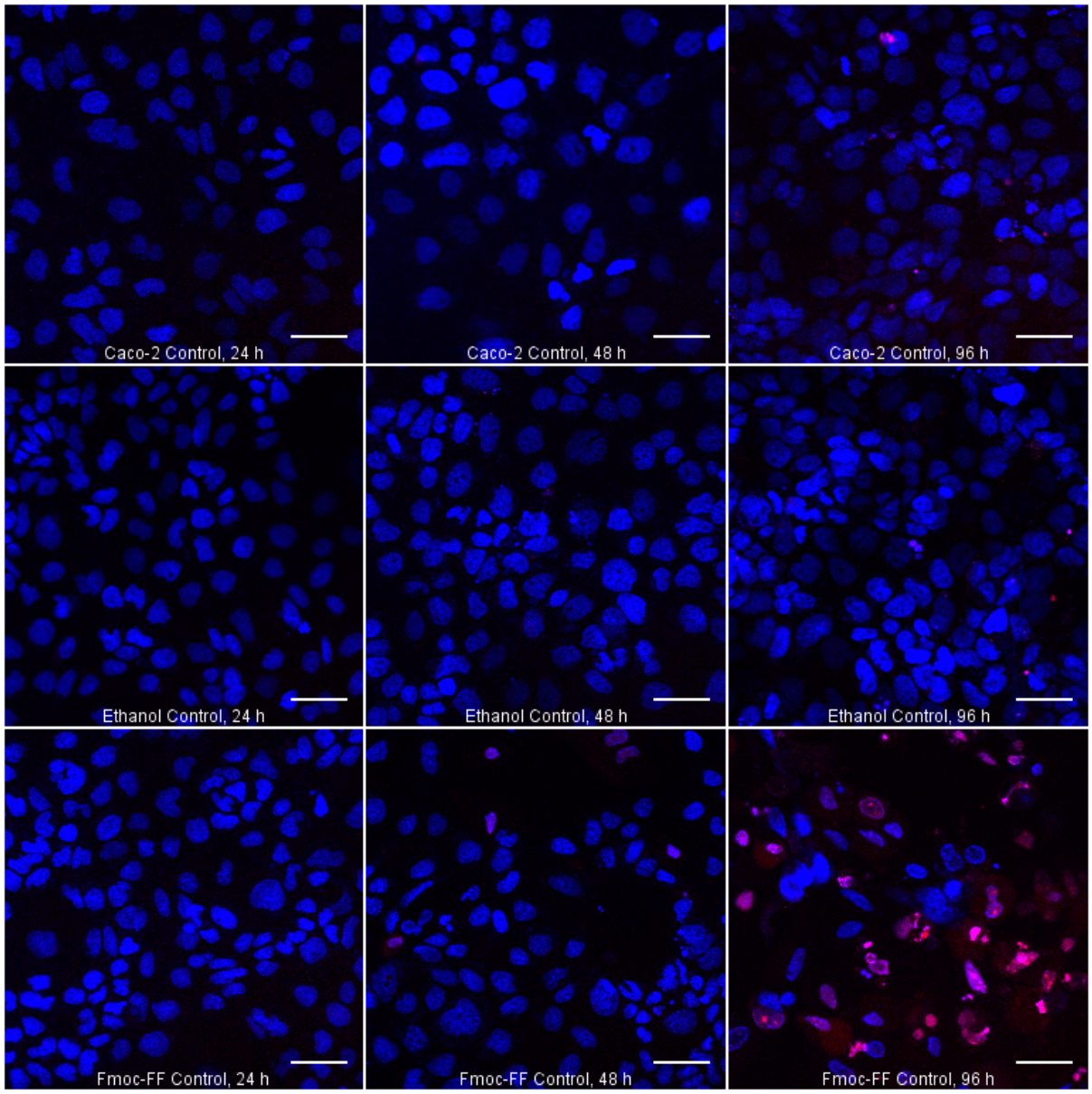

Fig. S12. HO/PI fluorescence micrographs of controls. Scale bar, 50 μm. Live cells (blue fluorescence), apoptotic cells (intense brig ht-blue and blue-violet fluorescence) and necrotic cells (pink fluorescence).

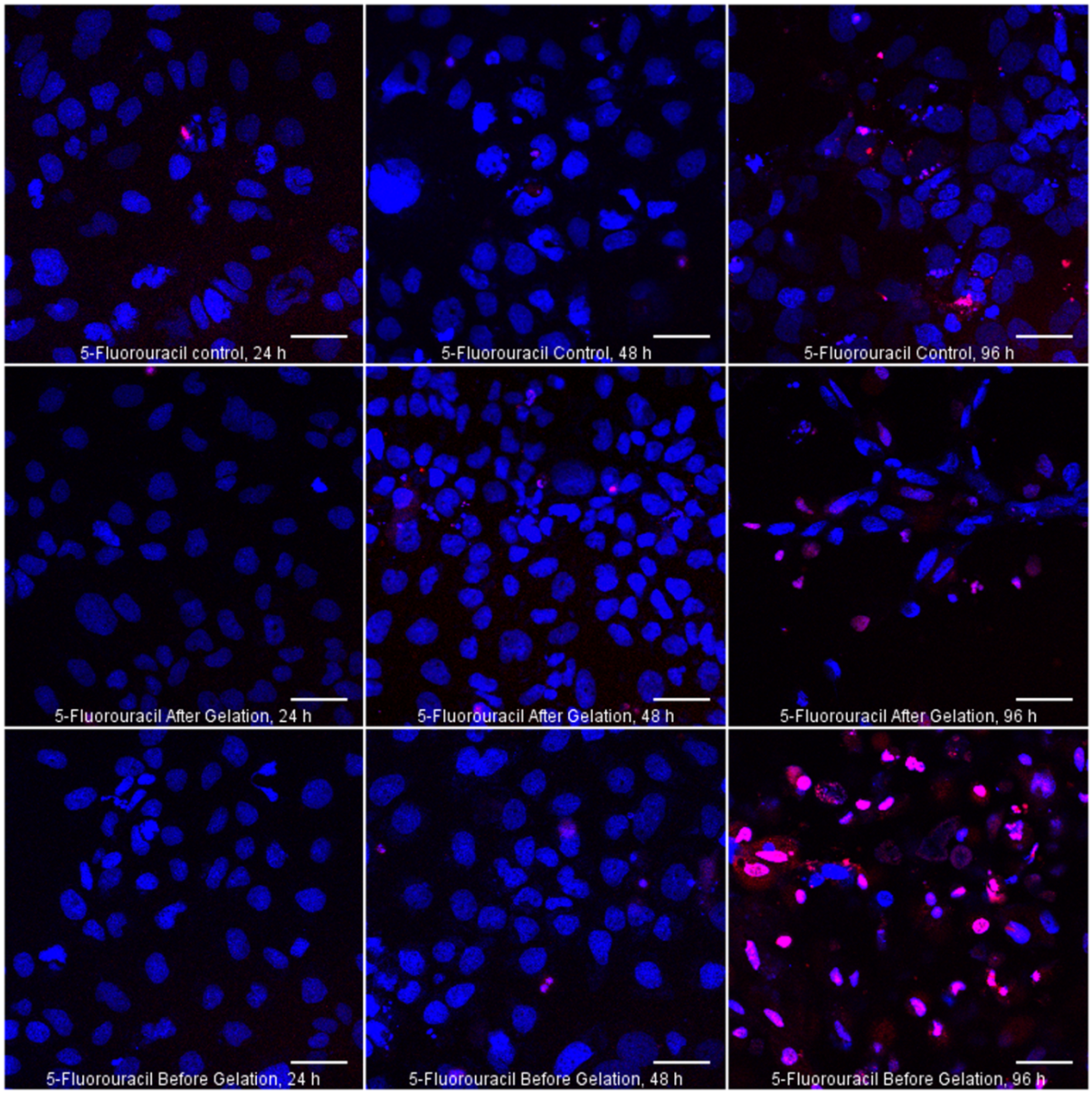

Fig. S13. HO/PI fluorescence micrographs of Caco-2 cells exposed to 5-FU. Scale bar, 50 μm. Live cells (blue fluorescence), early apoptotic cells (intense bright-blue fluorescence), late apoptotic cells (blue-violet fluorescence) and necrotic cells (pink fluorescence).

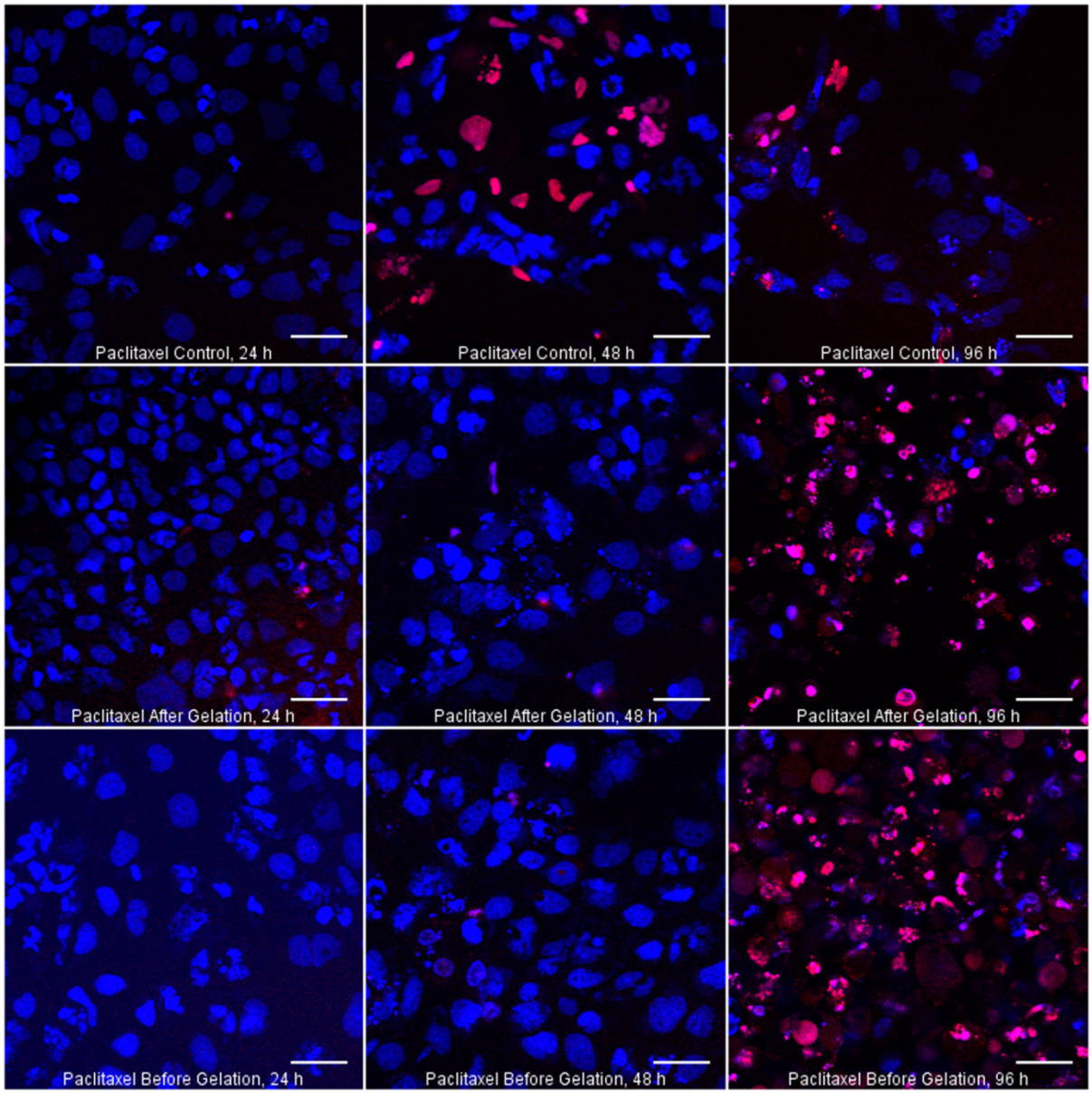

Fig. S14. HO/PI fluorescence micrographs of Caco-2 cells exposed to paclitaxel (Taxol®). Scale bar, 50 μm. Live cells (blue fluorescence), apoptotic cells (intense bright-blue and blue-violet fluorescence) and necrotic cells (pink fluorescence).