The Effects of Enzymatic Activation on the Distribution of Fluorescently Tagged MMP-2 Cleavable Peptides in Cancer Cells and Spheroids

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Supplementary Data

Figure S1
Figure S2
Figure S3
Figure S4
Figure S5
Figure S6
Figure S7
Figure S8

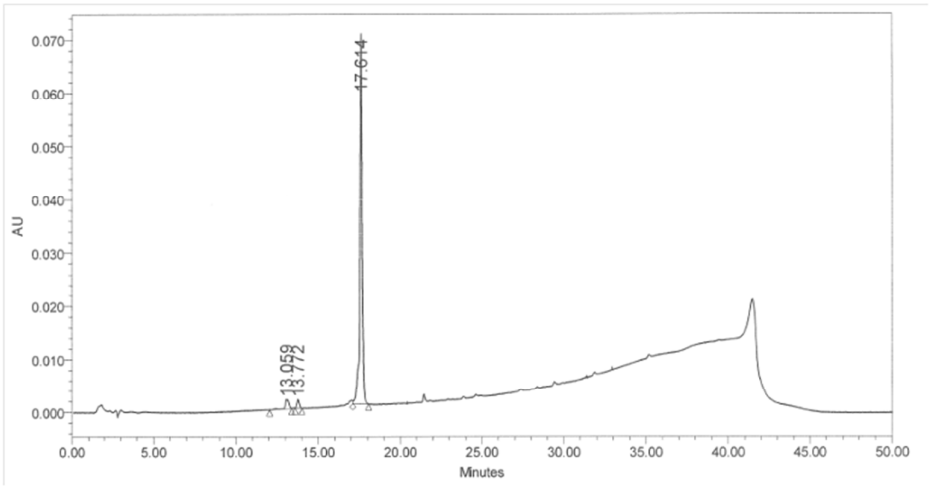

Figure S1. Analytical HPLC trace of K(FITC)IPVSLRSK(c343)-OH (**1**).

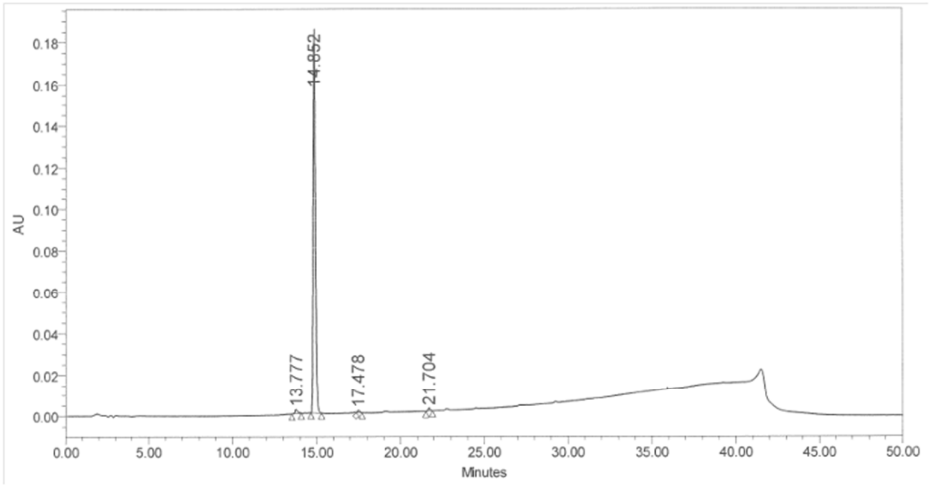

Figure S2. Analytical HPLC trace of K(FITC)IPVS-OH (**2**).

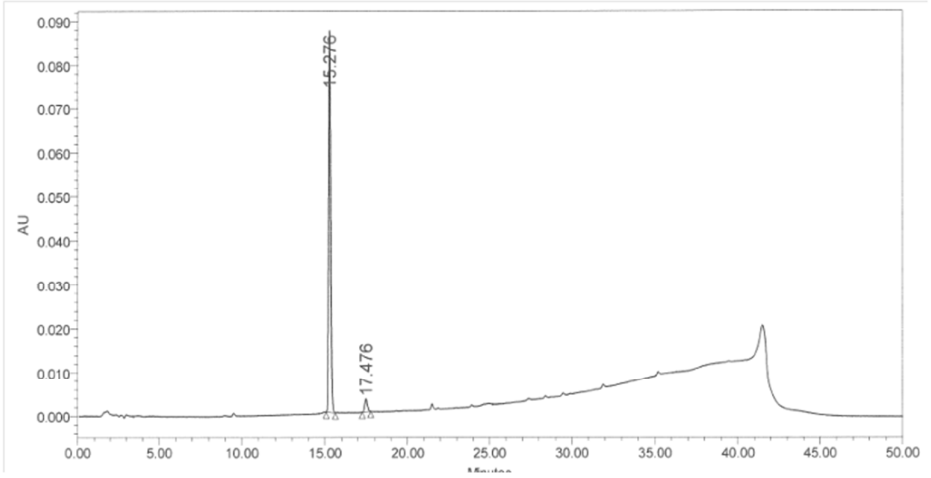

Figure S3. Analytical HPLC trace of LRSK(c343)-OH (**3**).

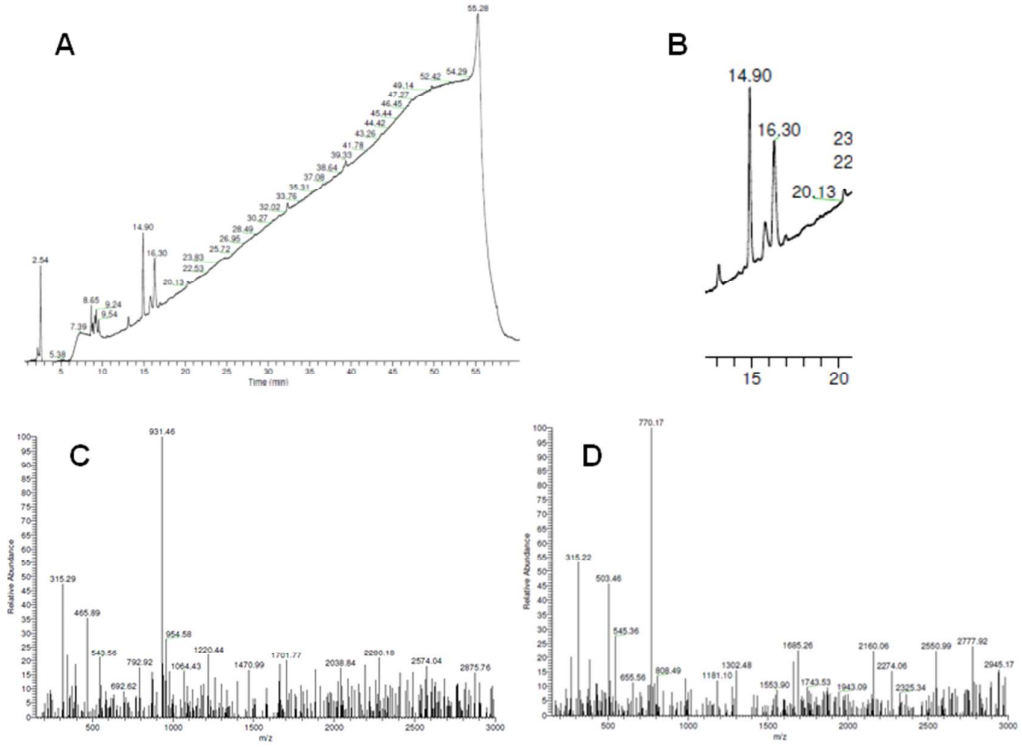

Figure S4. Cleavage assay of K(FITC)IPVSLRSK(c343)-OH (**1**) monitored using LC-MS. **A** is the chromatogram, **B** is the expansion of the chromatogram at the relevant region, **C** is the mass spectrum of the peak at 14.90 mins indicating the presence of K(FITC)IPVS-OH (**2**)and **D** is the mass spectrum of the peak at 16.30 mins indicating the presence of LRSK(c343)-OH (**3**).

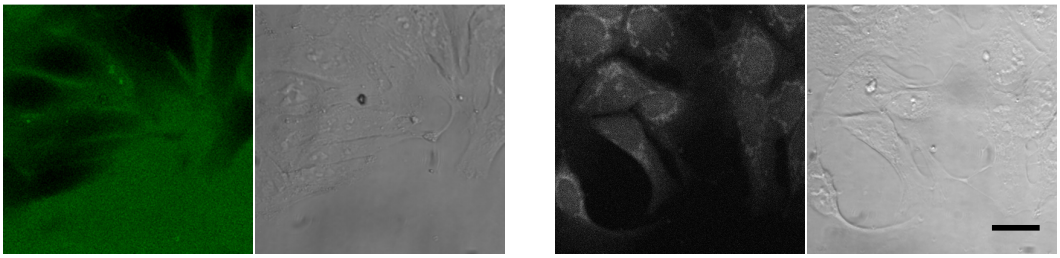

Figure S5. Confocal microscopy images of DLD-1 cells treated with **A**: FITC, **B**:c343, with their respective bright field images. Incubation time was 4 hrs. Images of FITC were obtained in the green channel. Images of c343 were obtained in the blue channel and are shown in black and white for clarity. Scale bar represents 20 μm.

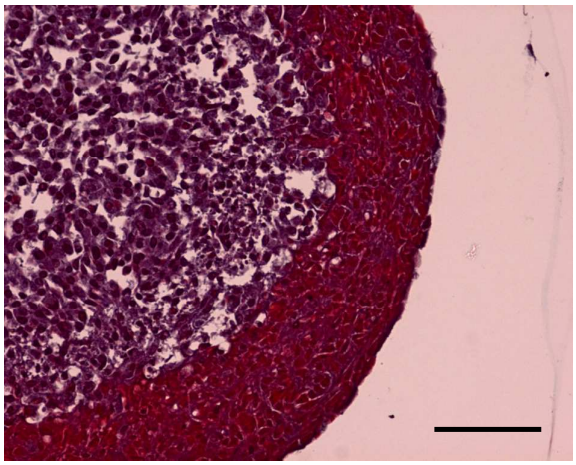

Figure S6. Decreased cell density and increased collagen staining in the centre of spheroids. Large spheroids (5.5 x 10^5 cells) were grown as previously described, fixed in 4% paraformaldehyde for 1 hr, paraffin embedded and 5 µm sections cut through the central region of the spheroid. Masson's trichrome stain was used to detect collagen fibres. Scale bar represents 100 µm.

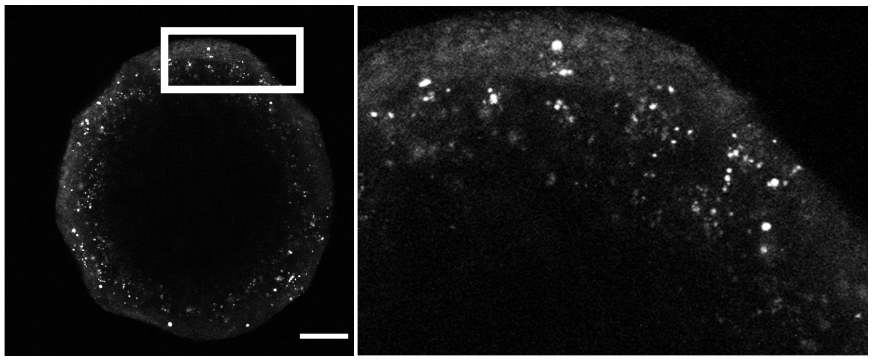

Figure S7. Confocal image of the Draq5 nuclear stain in spheroids. Due to poor penetration of the stain into the live spheroid, only the small apoptotic nuclei are easily visible. Scale bar 200 µm.

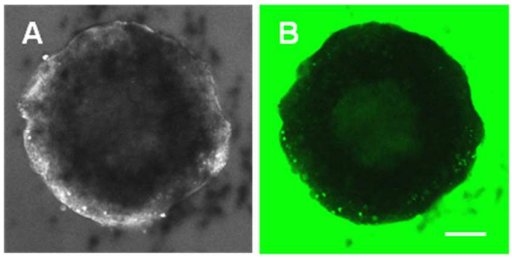

Figure S8. Confocal microscopy images of DLD-1 spheroids treated with K(FITC)IPVSLRSK(c343)-OH (**1**) and imaged once after 4 hrs incubation in parallel to the experiment shown in Figure 7. **A** is in the blue channel (image shown in black and white for clarity) and **B** is in the green channel. Scale bar represents 200 μm.