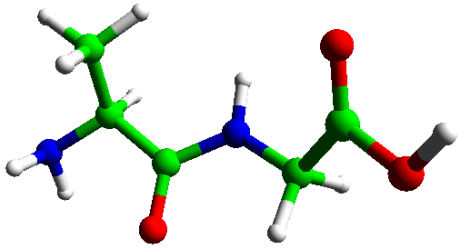
CHARACTERIZATION of HYALURONAN-MIMETIC PEPTIDES as ANTI-CANCER AGENTS



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London Regional Cancer Program

Western

Problem:

Most currently used anti-cancer therapeutics are not so cancer-cell-specific traits to exert their actions.

Unfortunately, normal healthy cells that divide rapidly are also killed, and conversely slow-growing or dormant cancer cells are left unaffected.

Another drawback of current chemotherapeutic agents is their lack of efficacy against multidrug-resistant tumors.

Thus, the emergence of multidrug-resistant cancers and the lack of targeted therapies for many cancers underscore an unmet need for new therapeutics with novel modes of action towards cancer cells.

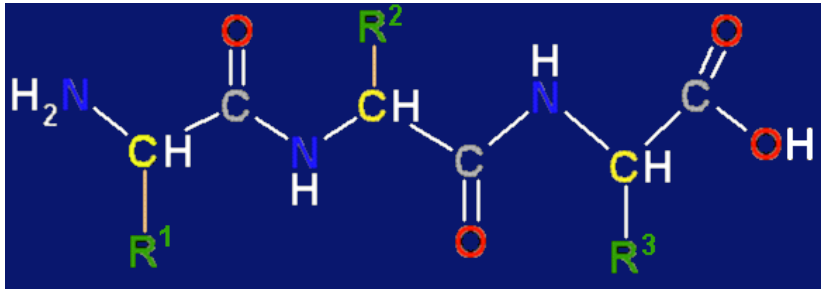
Introduction

Cancer-selective peptides offer great promise because their main mode of action is specific target of cancer cells, resulting in cell death.

Natural or synthetic peptides act as therapeutic agents capable of inducing death or increase the apoptotic effect of chemotherapeutic agents.

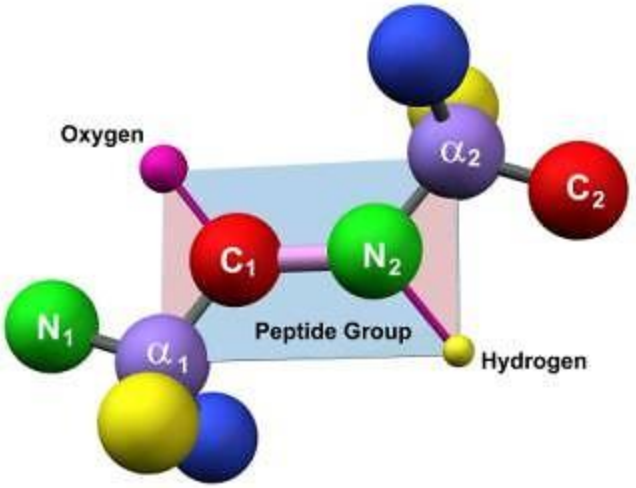
Cancer-selective membrane-lytic peptides such as cationic antimicrobial peptides physically disrupt of cancer cell membranes or cancer cell mitochondrial membranes, resulting in cell death.

Necrotic peptides isolated from Australian frogs and toads, insect cecropins and various defensins, as well as apoptotic peptides from various sources also possess moderate anti-cancer activity.

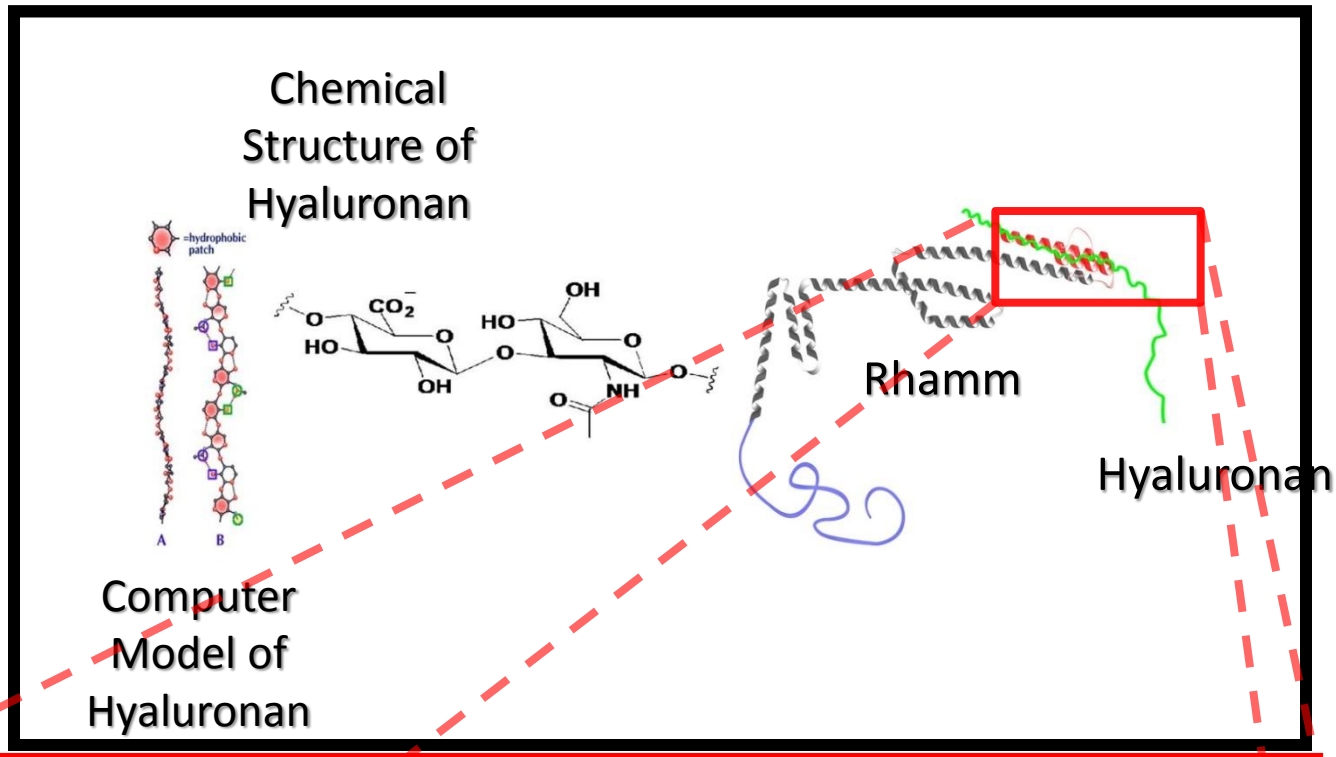


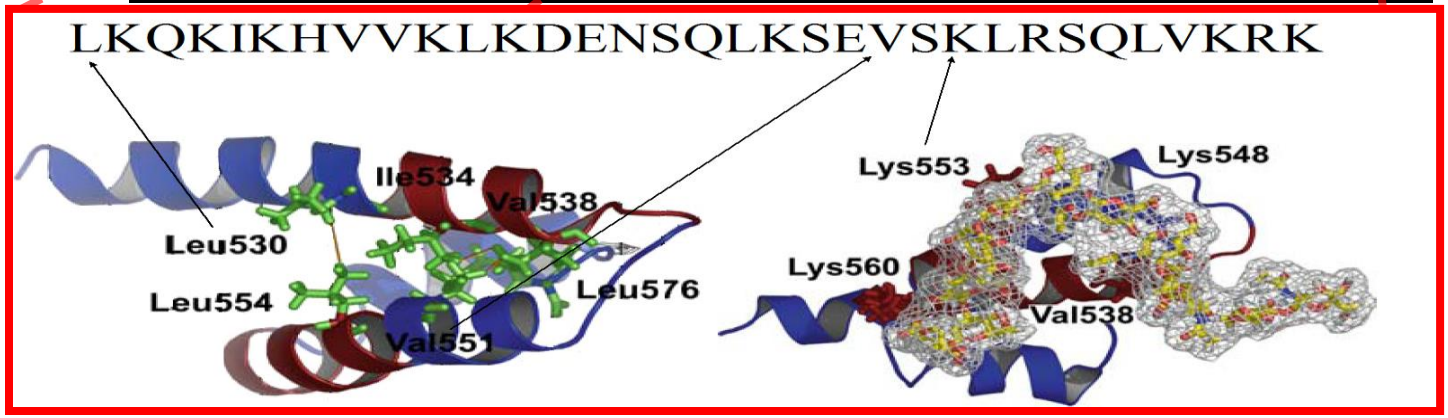
The goal of research:

Investigation of therapeutic potential of HA-mimetic peptides for treatment cancer disease.



Rhamm-HA Binding Model





HA-mimetic peptides were design and synthesized*

Peptide #35

Peptide #37

Peptide #40

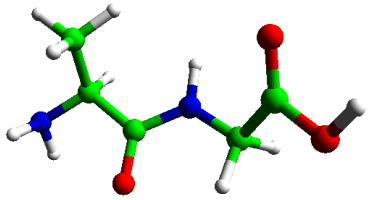
Peptide#135 (peptide D)

HA-mimetic peptides are specific and selective ligands for the RHAMM.

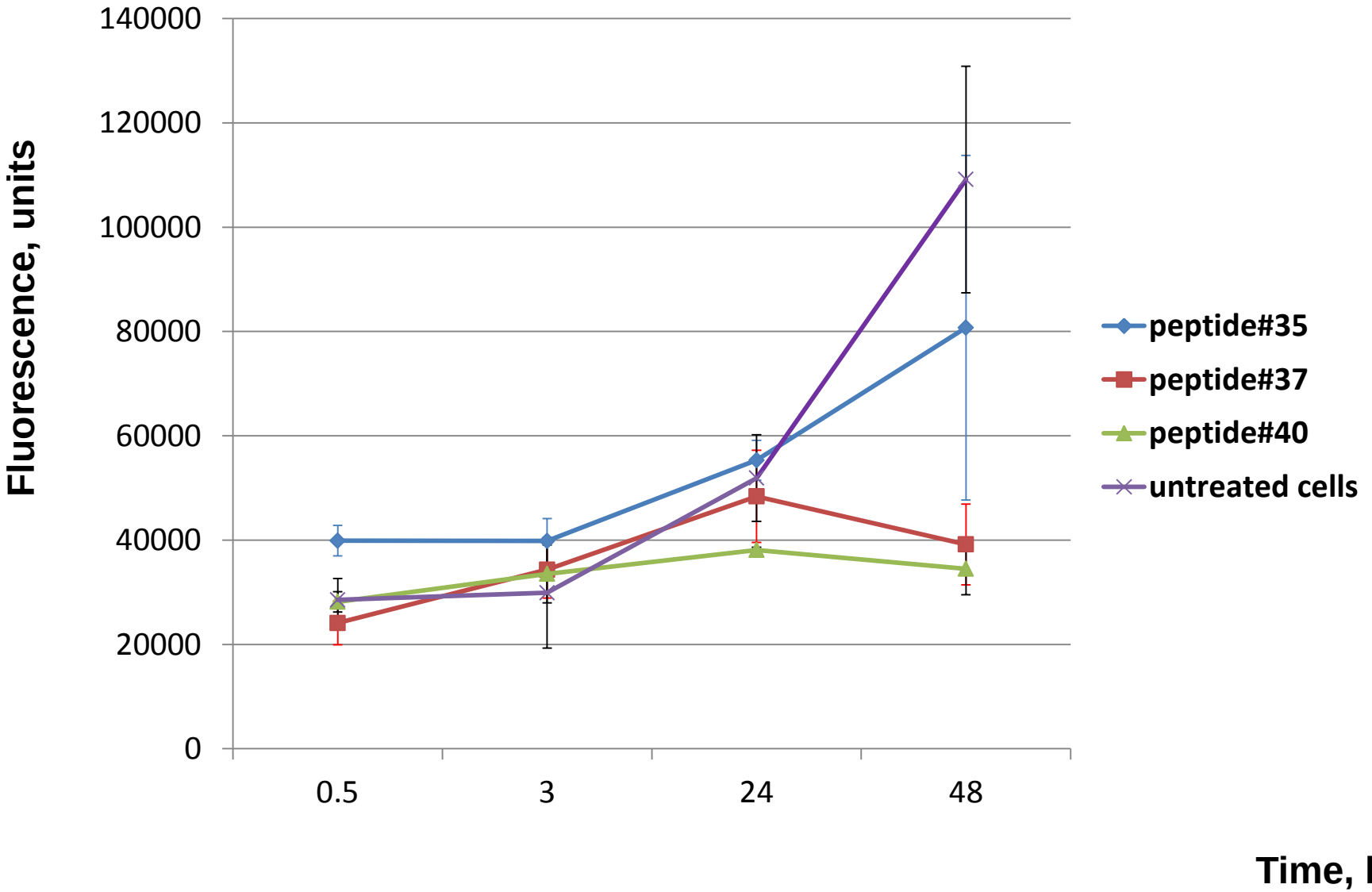
*** In collaboration with Kenneth Virgel N. Esguerra (Luyt lab)**

OUTLINE:

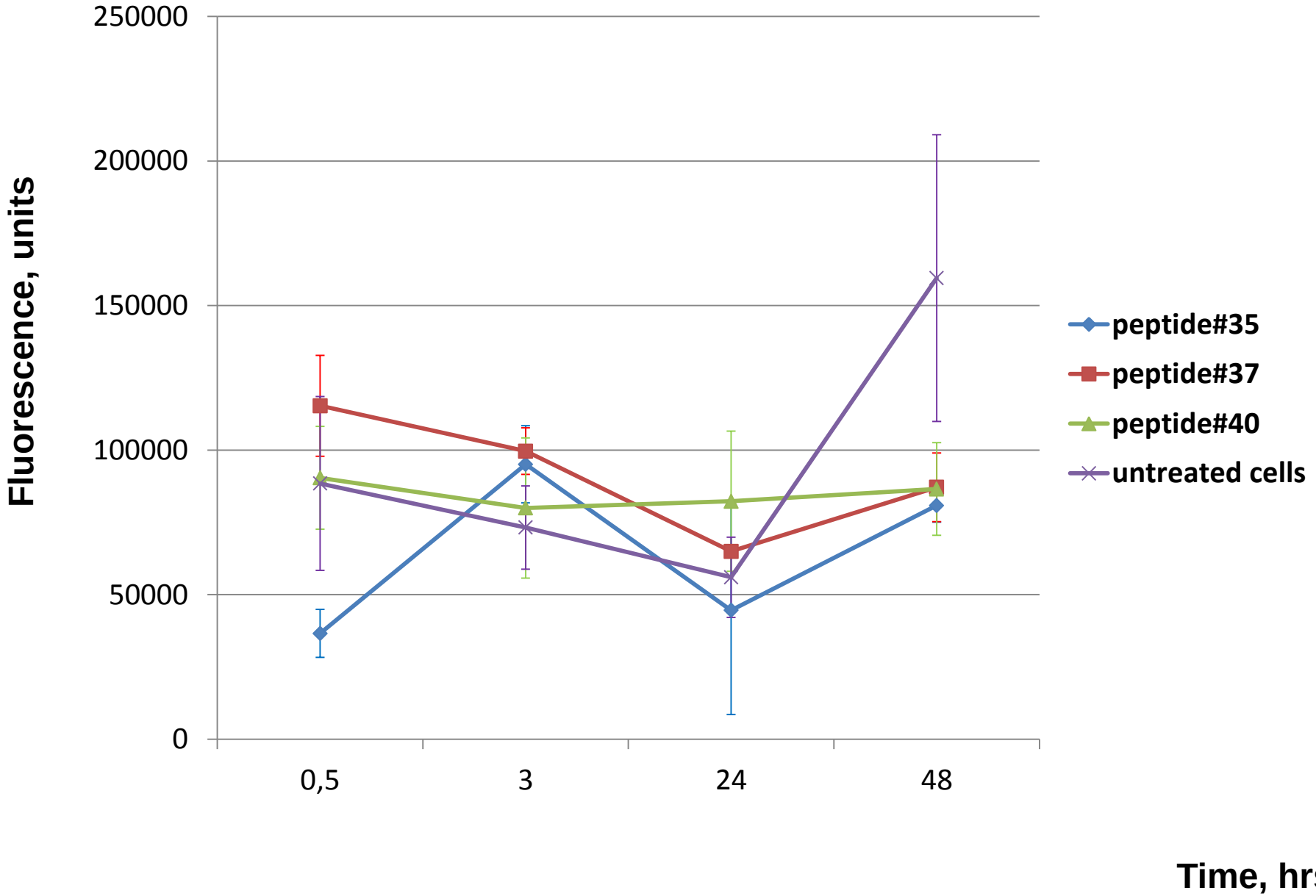
1. Effect HA-mimetic peptides on metabolic activity, cell viability, proliferation, apoptosis and necrosis of cancer cells.
2. Effect HA-mimetic peptides on invasion cancer cells.
3. Study effect of HA-mimetic peptides on development of tumor in mice.



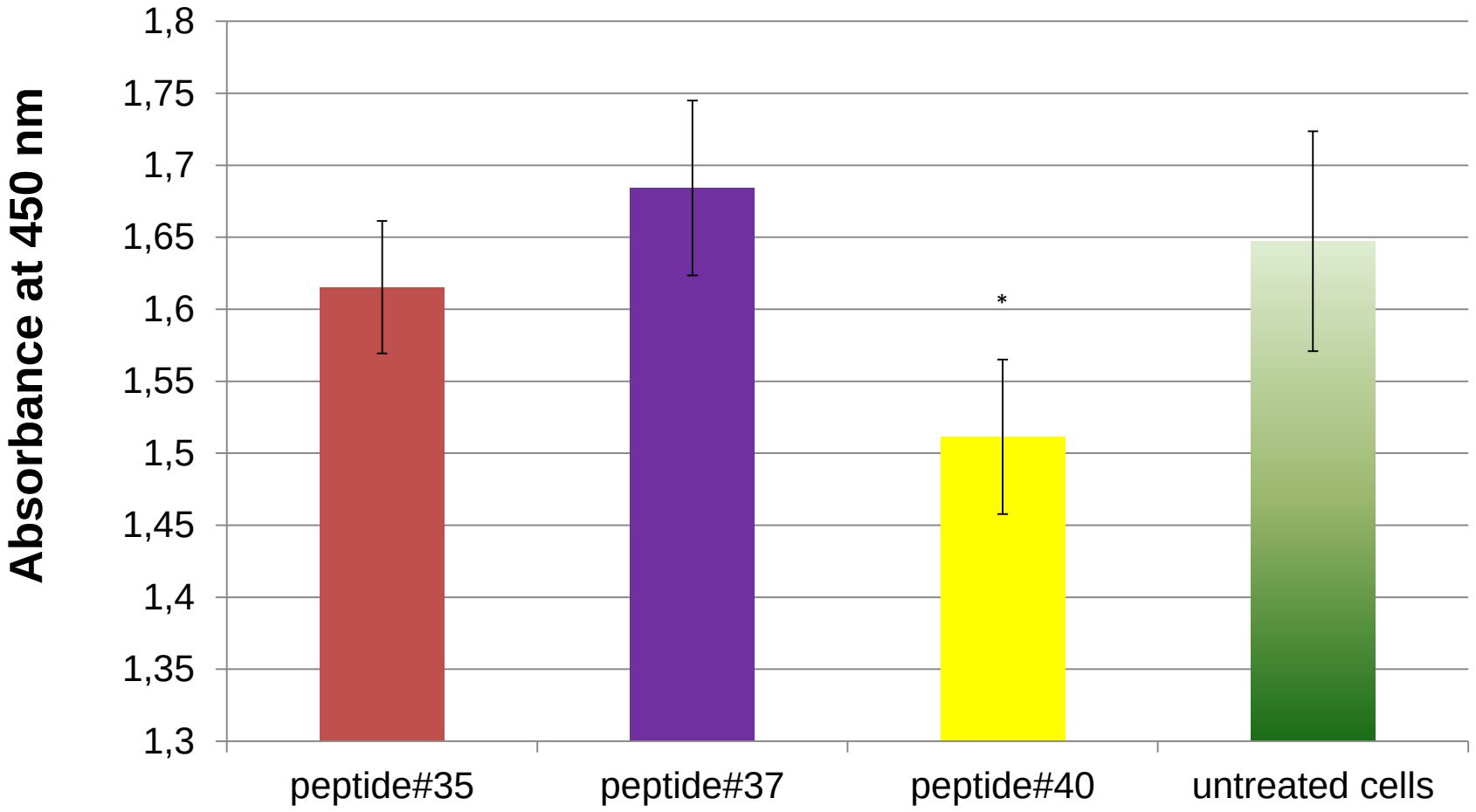
Cell viability of HA-mimetic peptides-treated prostate cancer cells (PC3mLN4).



Cell viability of HA-mimetic peptides-treated breast cancer cells (MDA-MB-231).

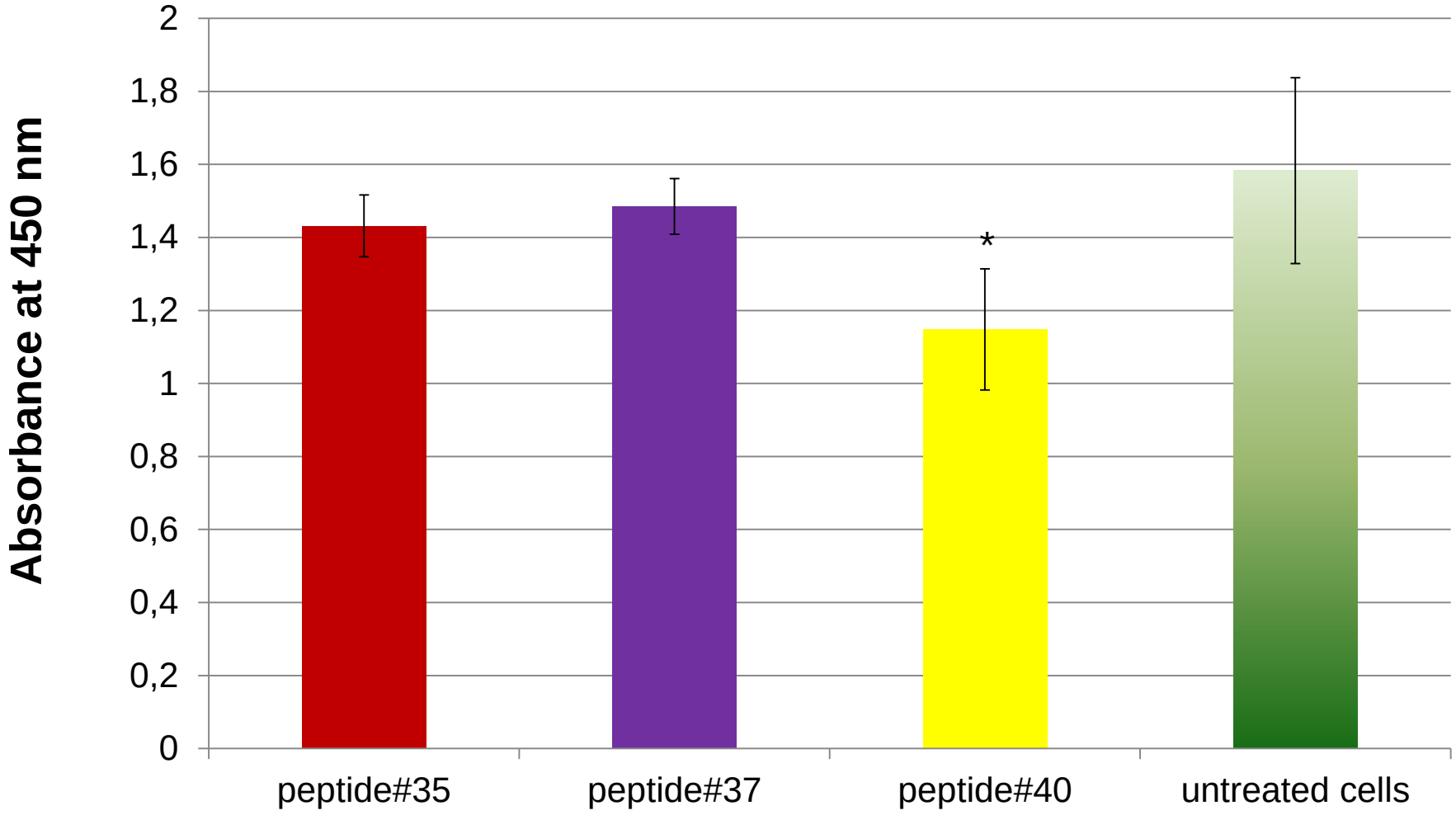


Effect HA-mimetic peptides on proliferation prostate cancer cells (PC3M-LN4), using BrdU assay.



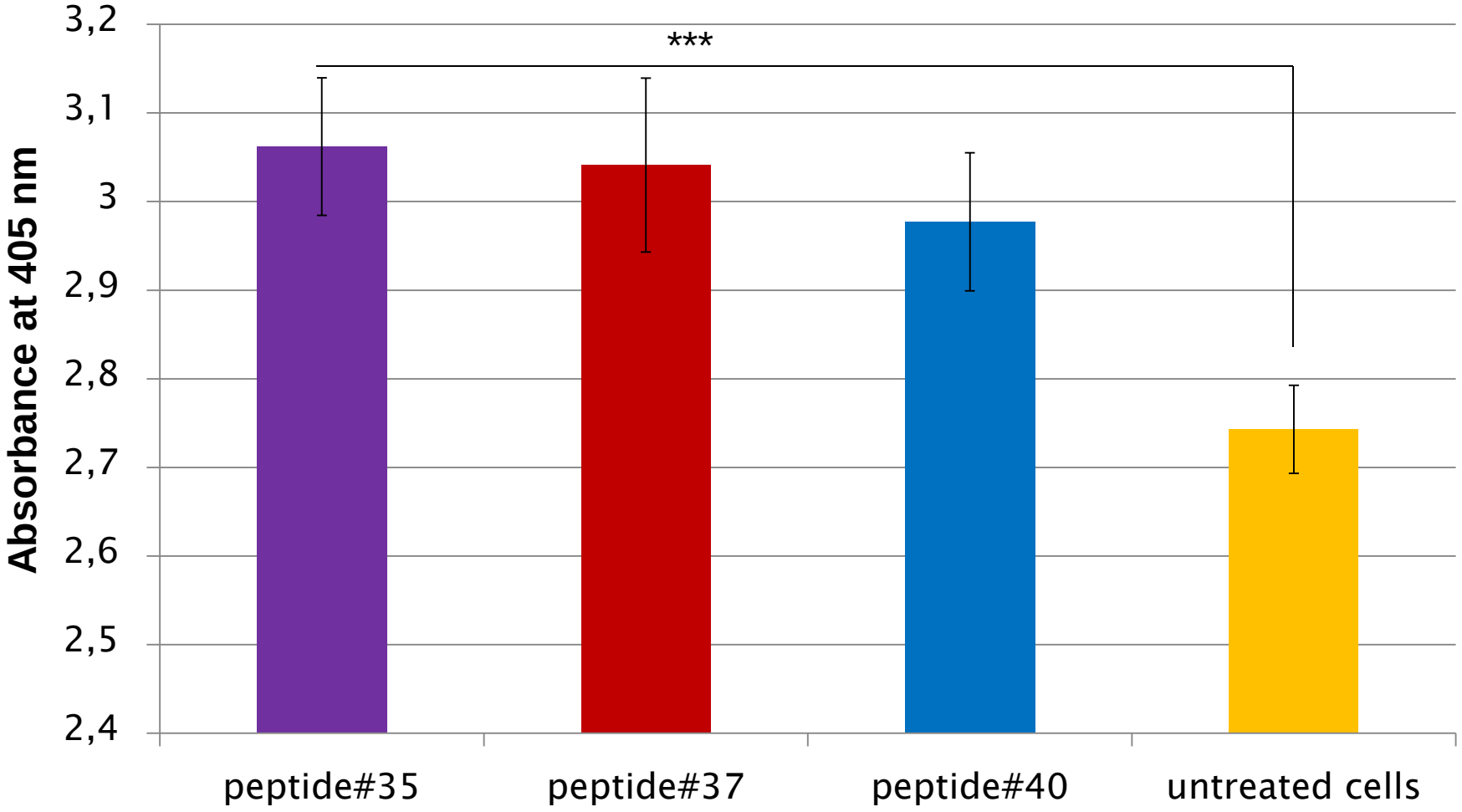
Data were analyzed using one-way ANOVA, means are significant different at * $p<0.05$.

Effect HA-mimetic peptides on proliferation breast cancer cells (MDA231), using BrdU assay.



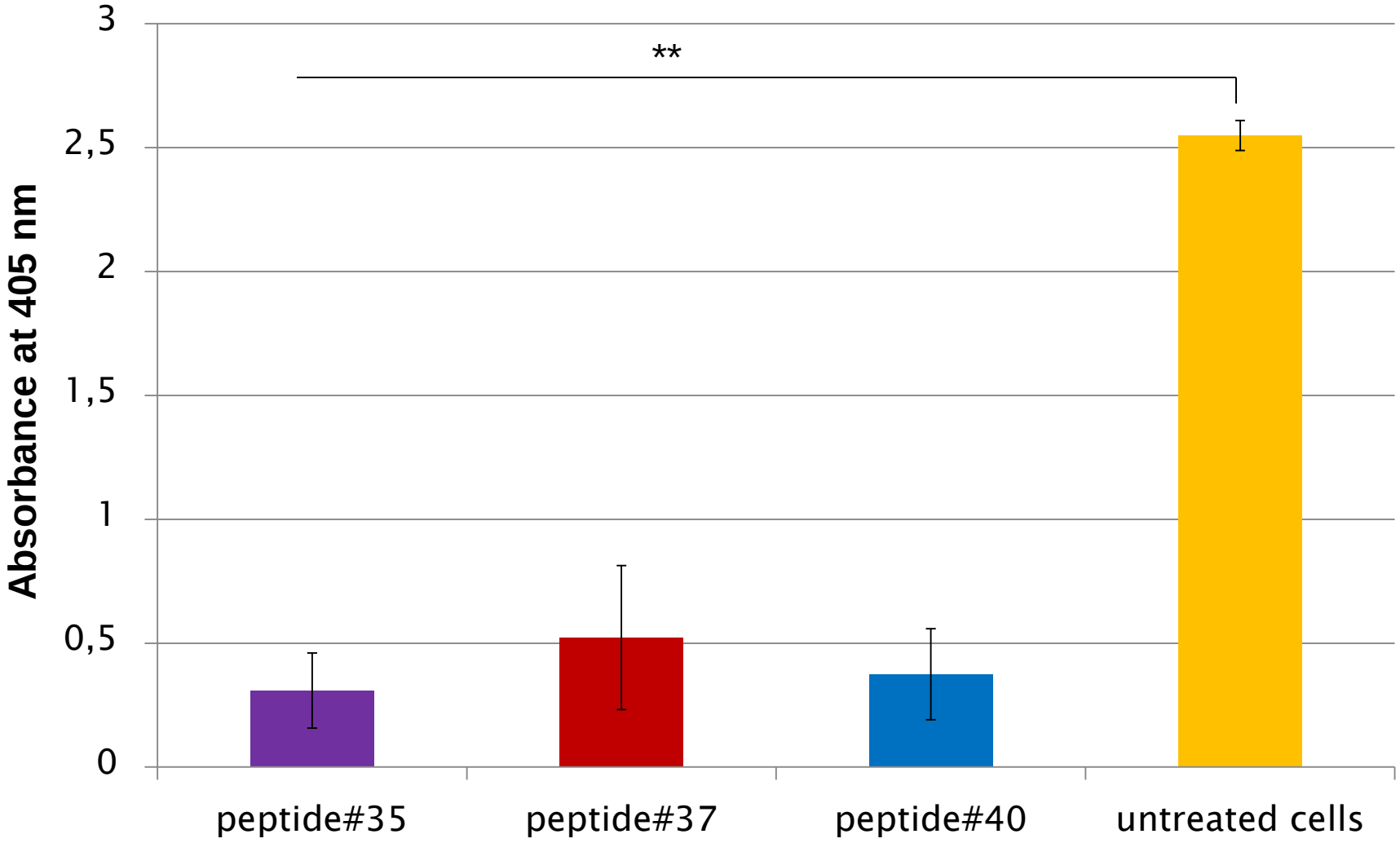
Data were analyzed using one-way ANOVA, means are significant different at * $p<0.01$.

Induction of apoptosis of PC3mLN4 cells by HA-mimetic peptides.



Data were analyzed using one-way ANOVA, means are significant different at (***- $p<0.05$).

Inhibition of necrosis of PC3mLN4 cells by HA-mimetic peptides.



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MCF 7 cells

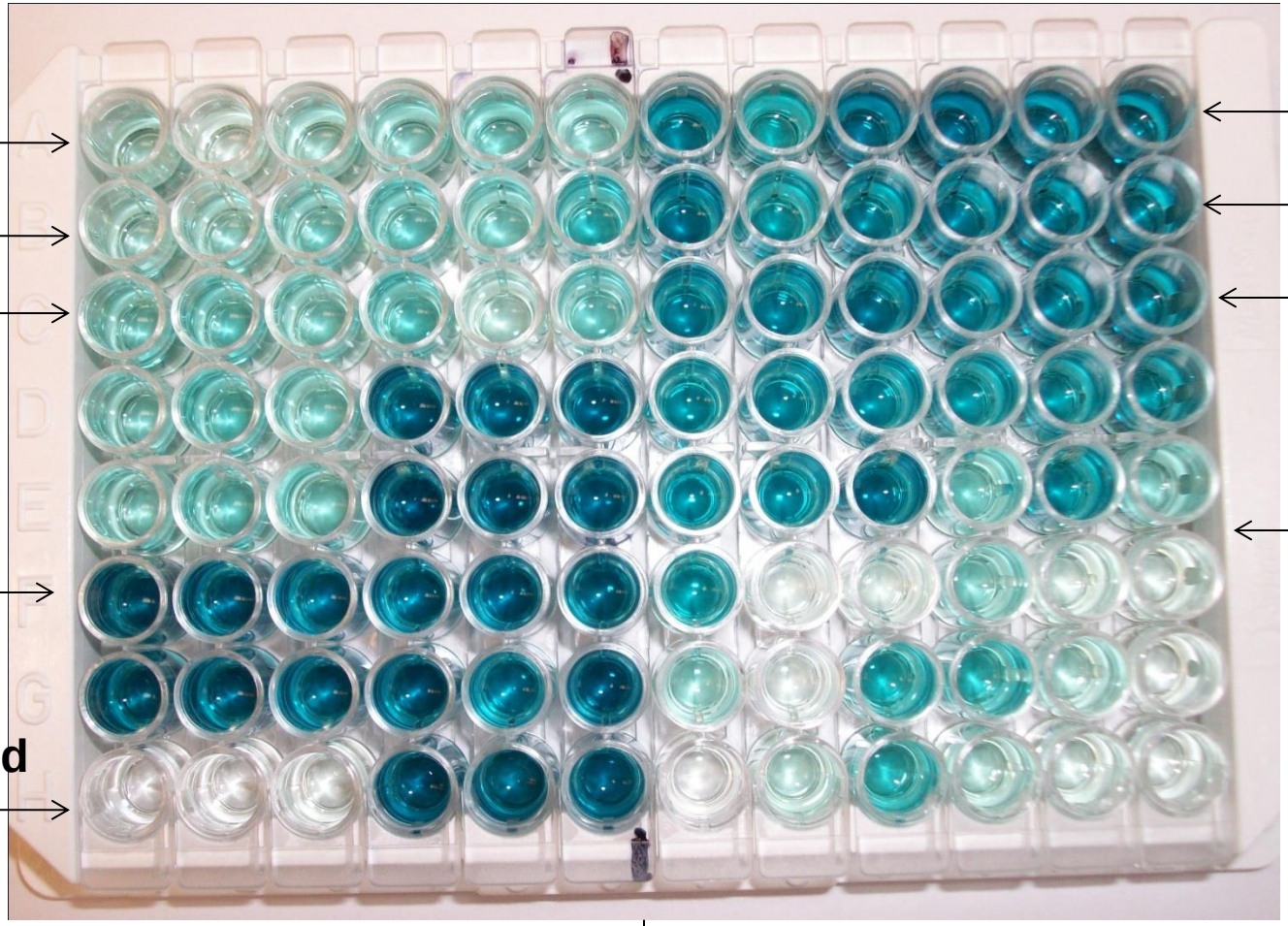
PC3mLN4 cells

apoptosis

P#35

P#37

P#40



necrosis

background

apoptosis

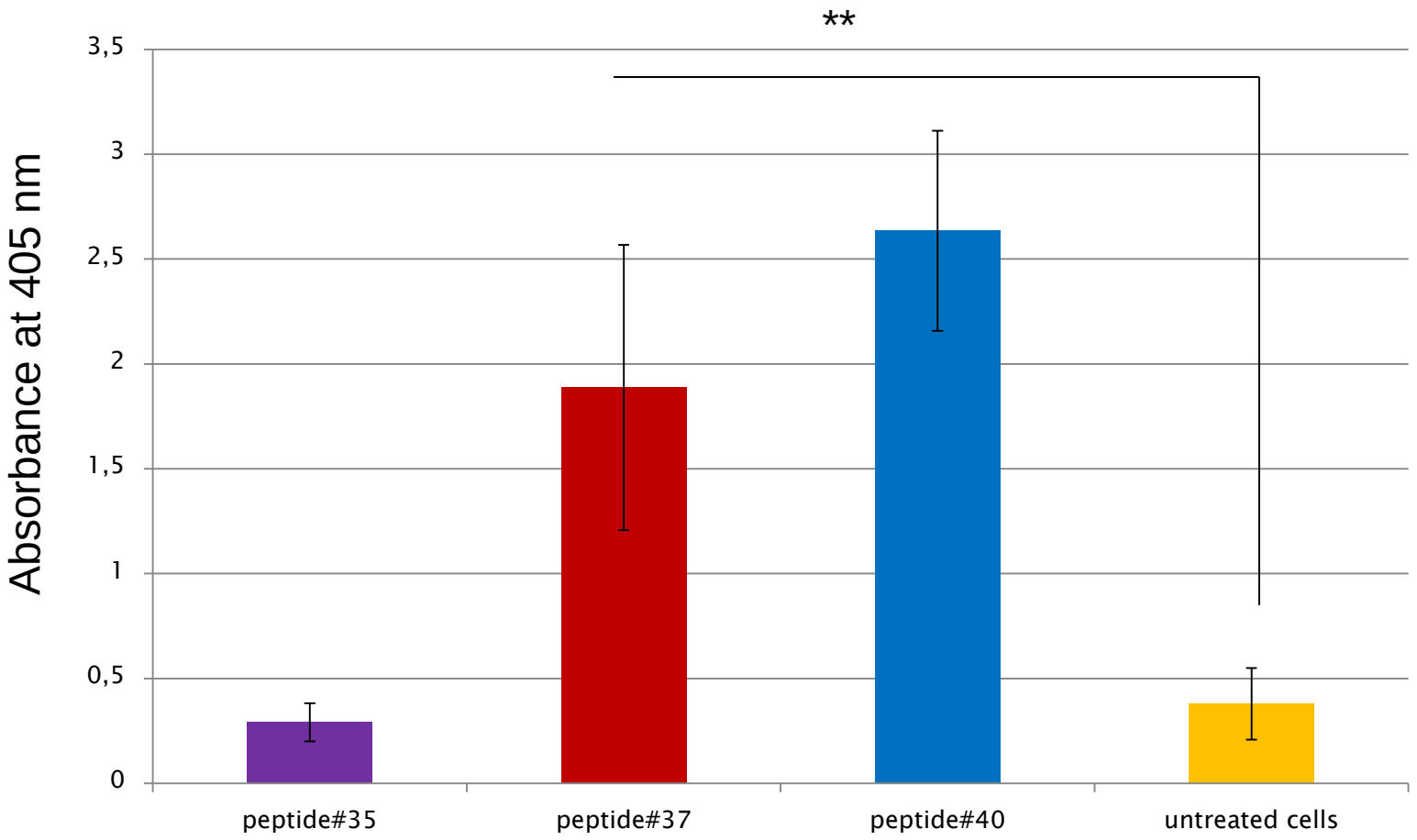
P#35

P#37

P#40

necrosis

Induction of apoptosis of MDA231 cells by HA-mimetic peptides.



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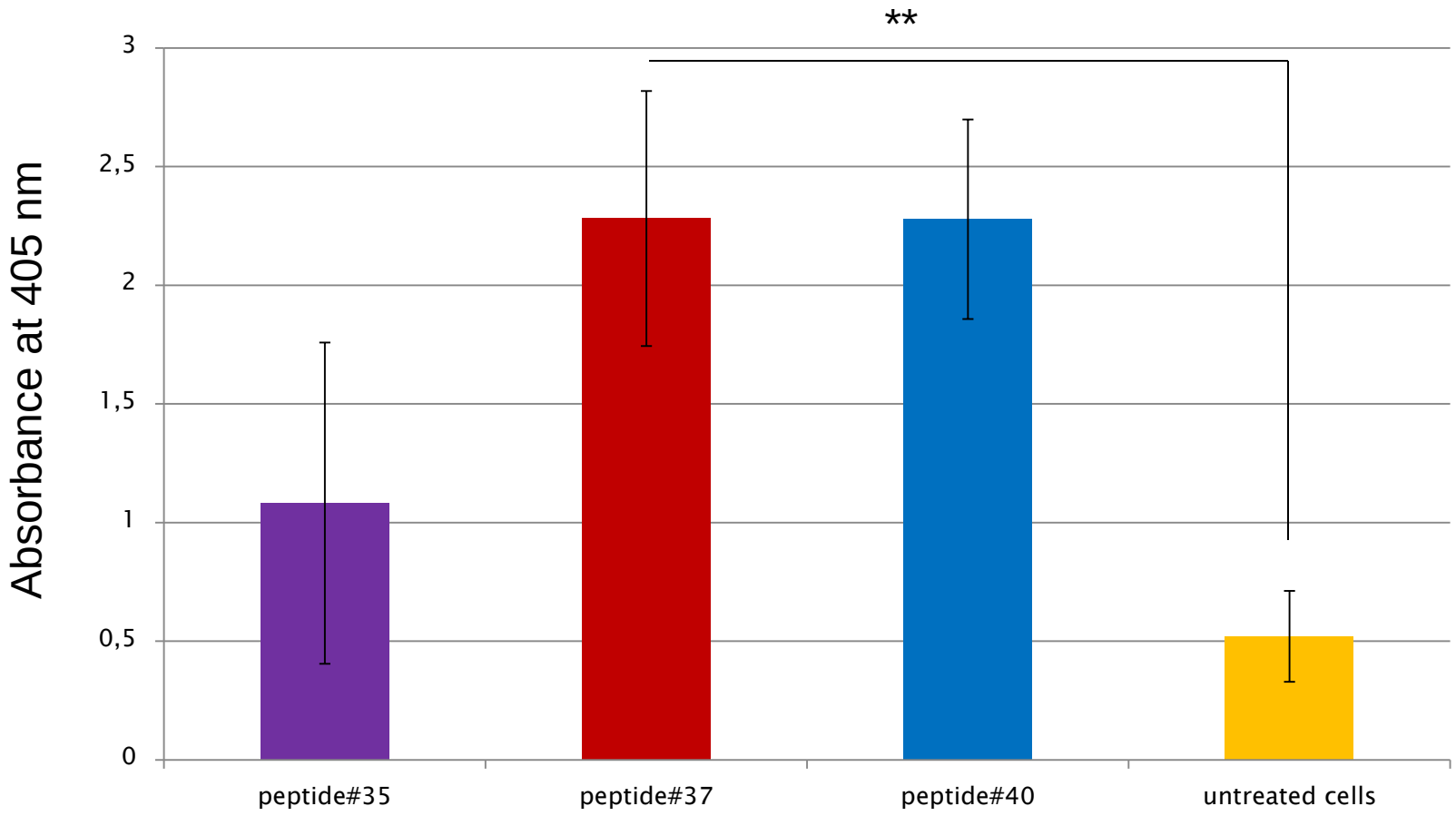
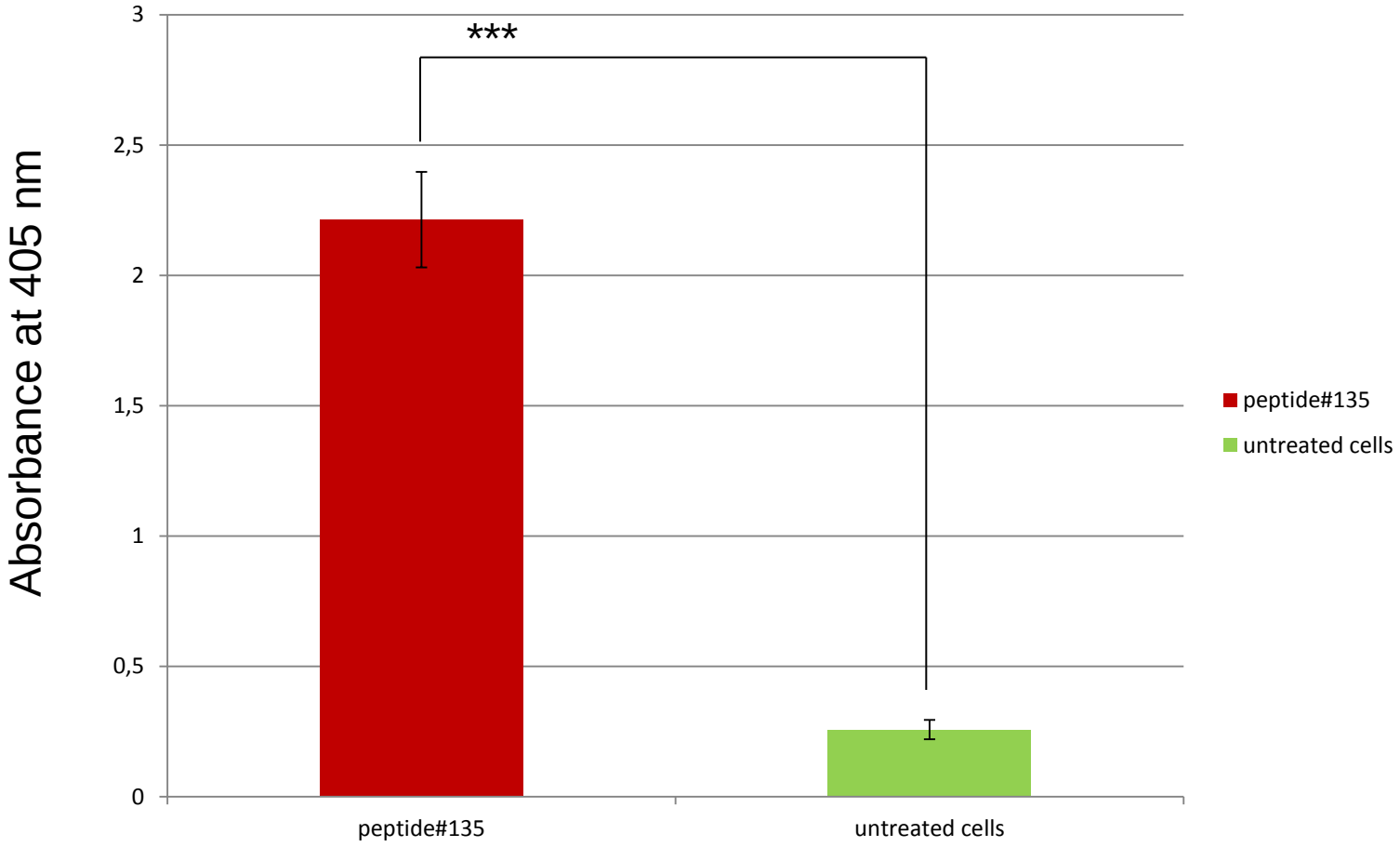
Induction of necrosis of MDA231 cells by HA-mimetic peptides.



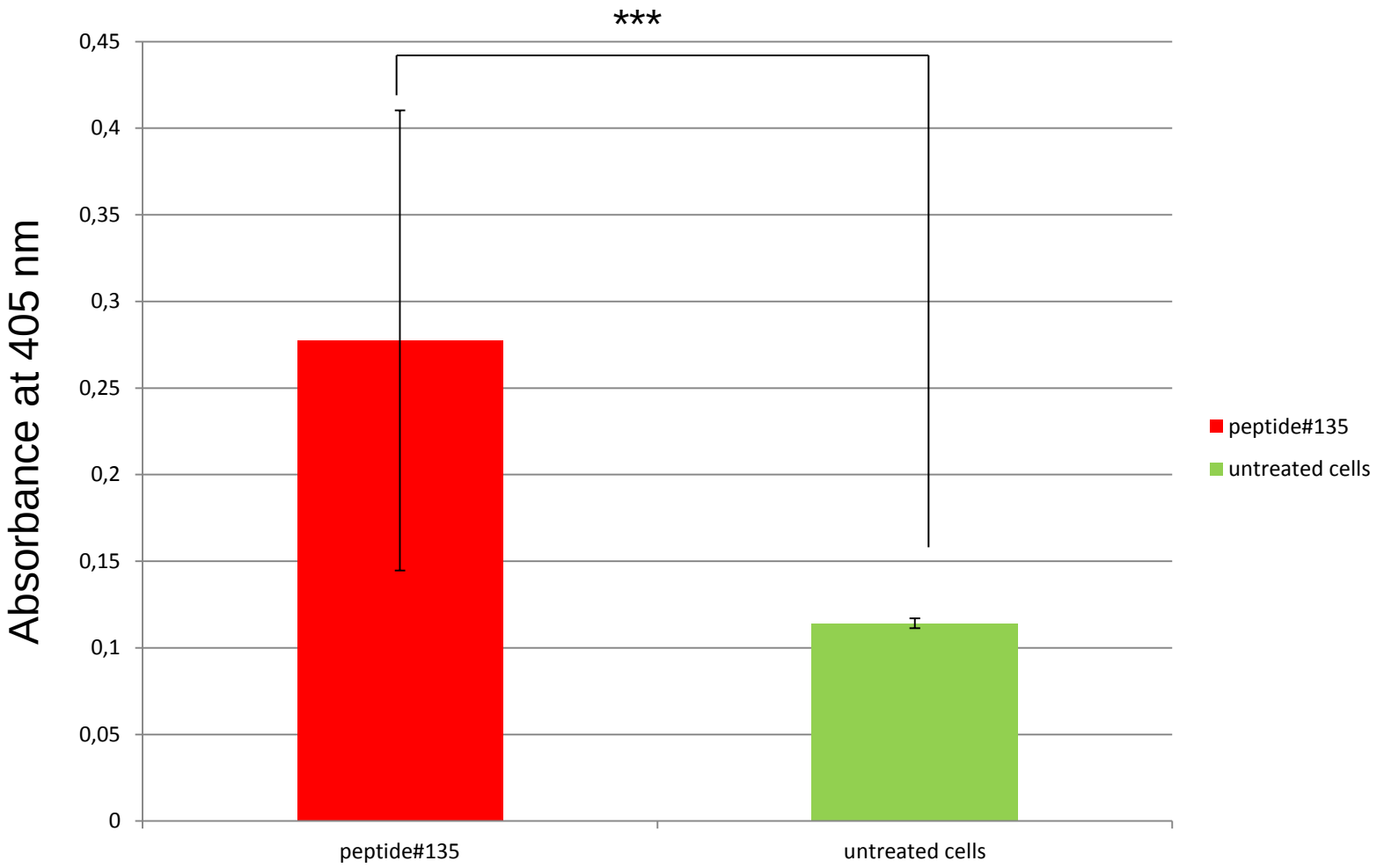
Data were analyzed using one-way ANOVA, means are significant different at (**- $p<0.05$).

Effect of Peptide D (#135) on apoptosis of PC3M-LN4 cells, using cell death detection ELISA.

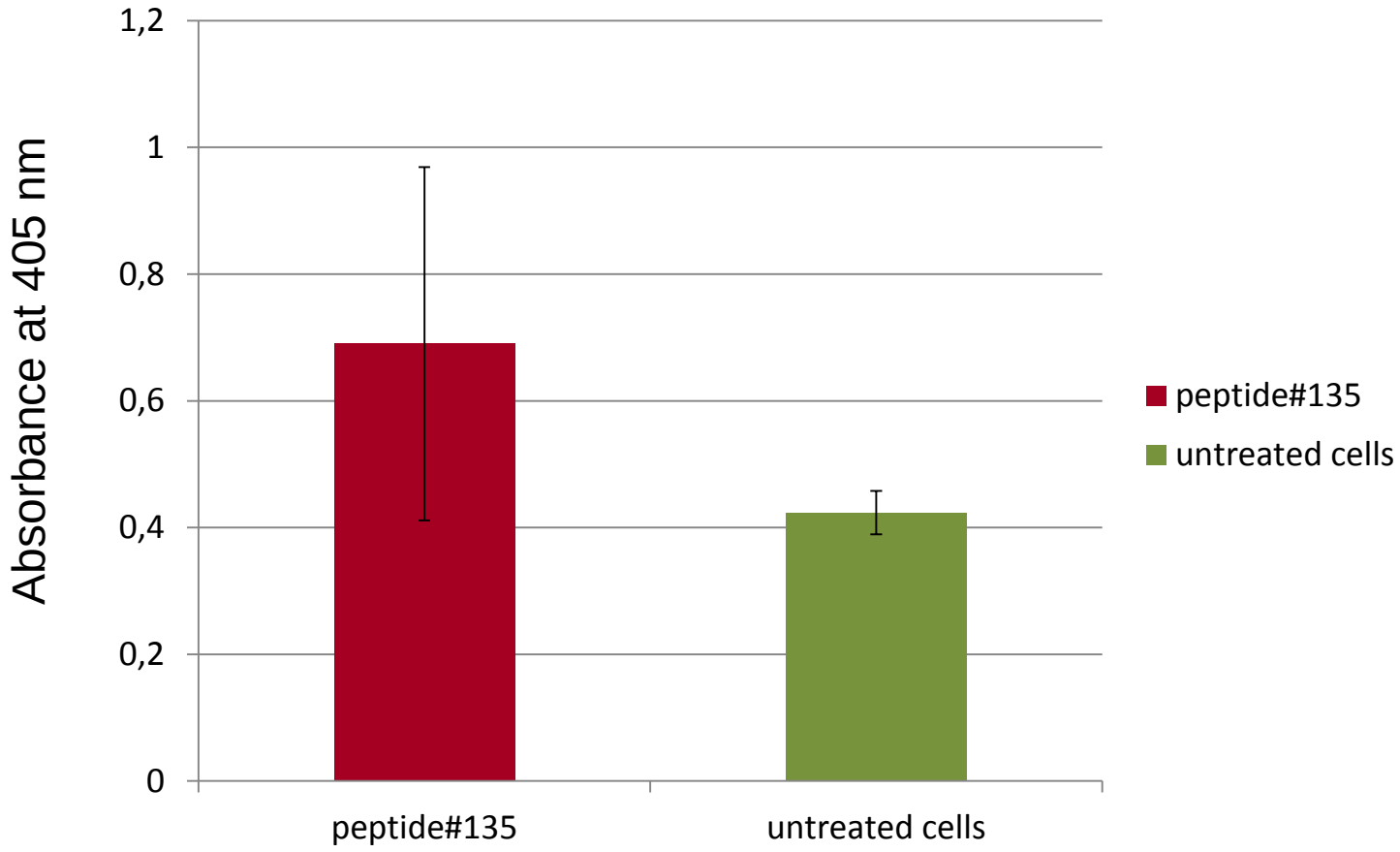


Data were analyzed using t-test, means are significant different at (***- p<0.0001)

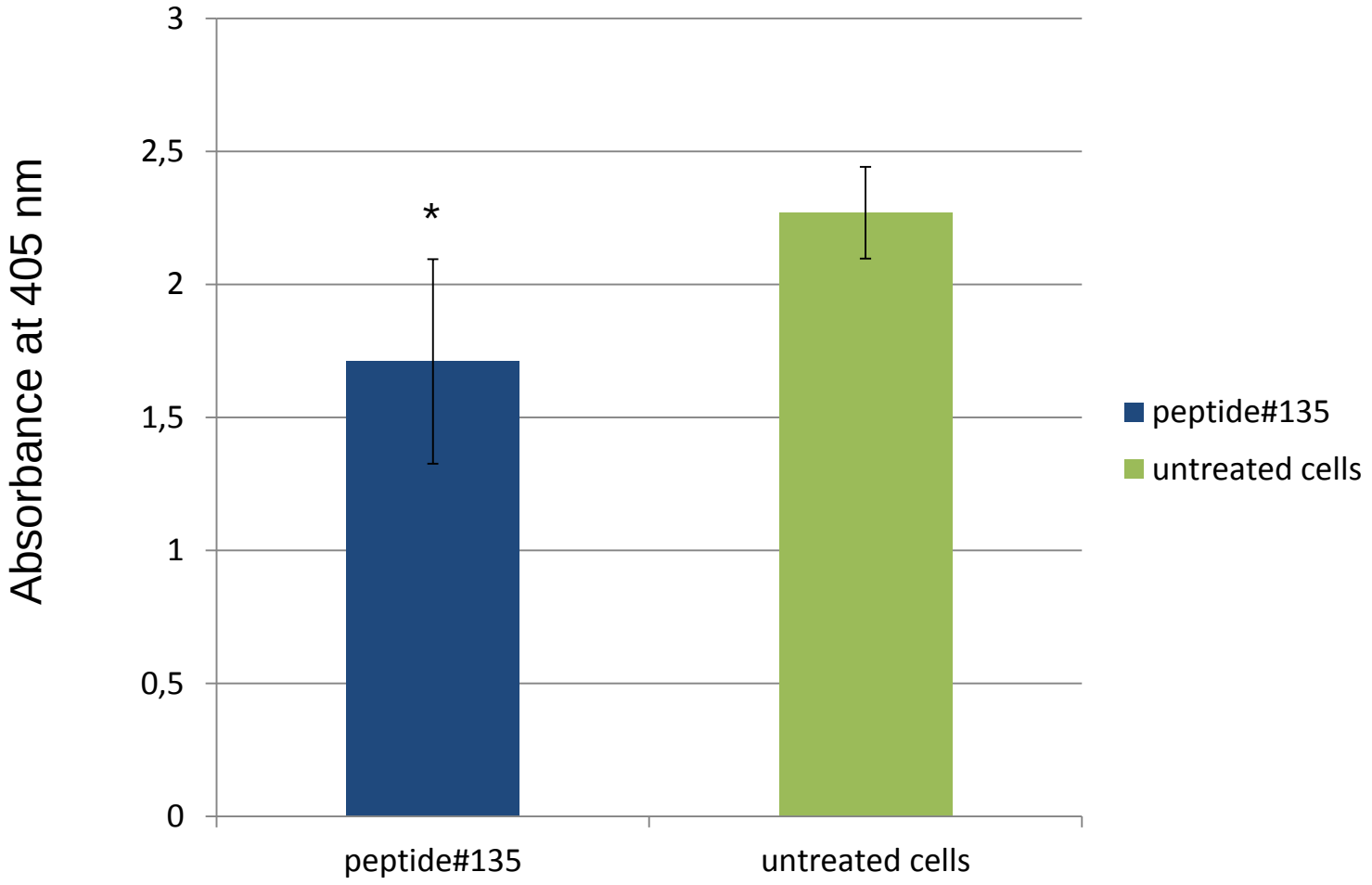
Effect of peptide D (#135) on necrosis of PC3M-LN4 cells, using cell death detection ELISA.



Effect of peptide D (#135) on apoptosis of Rhamm (-/-) cells, using cell death detection ELISA kit.

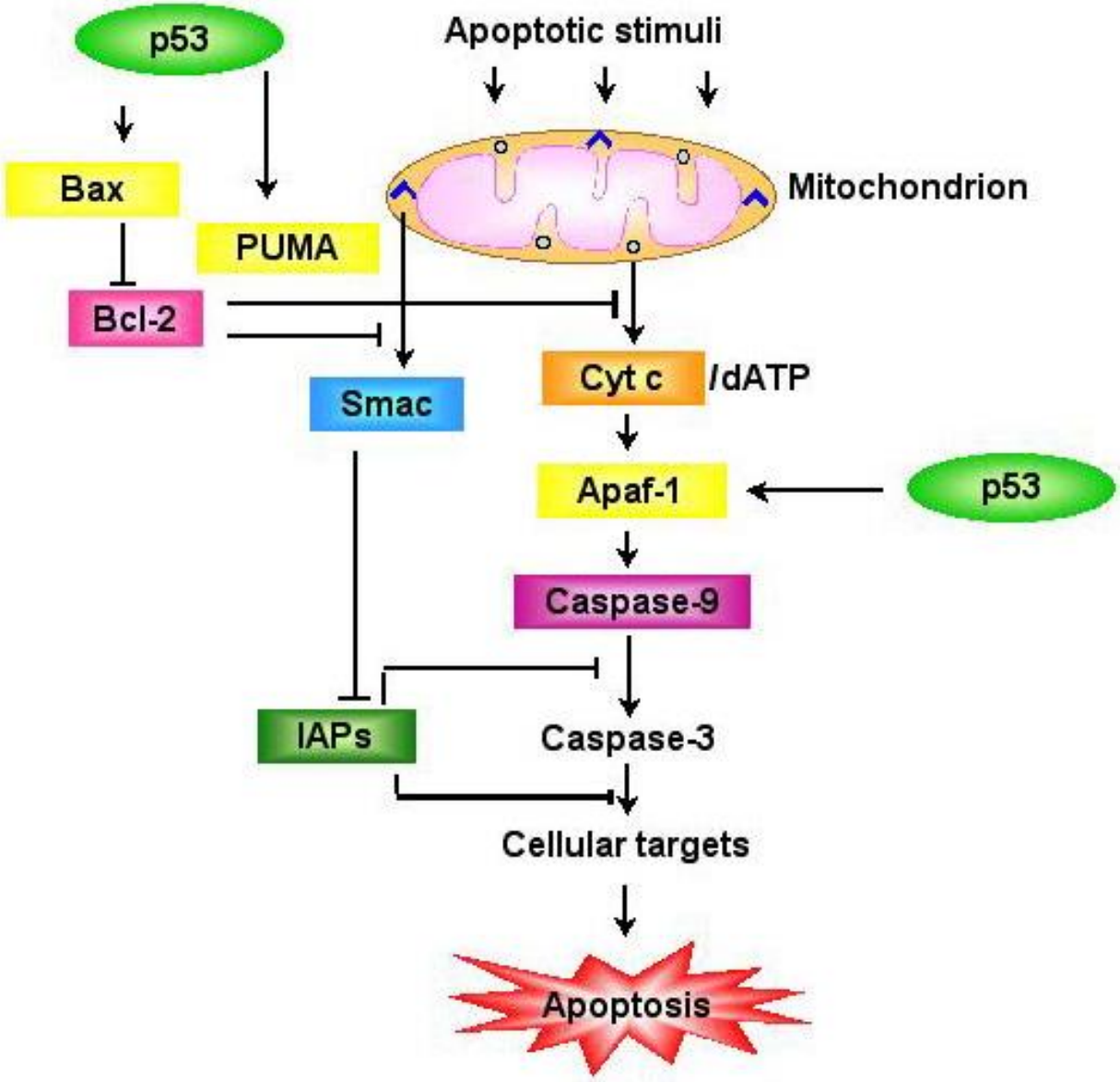


Effect of peptide D (#135) on necrosis of Rhamm (-/-) cells, using cell death detection ELISA.

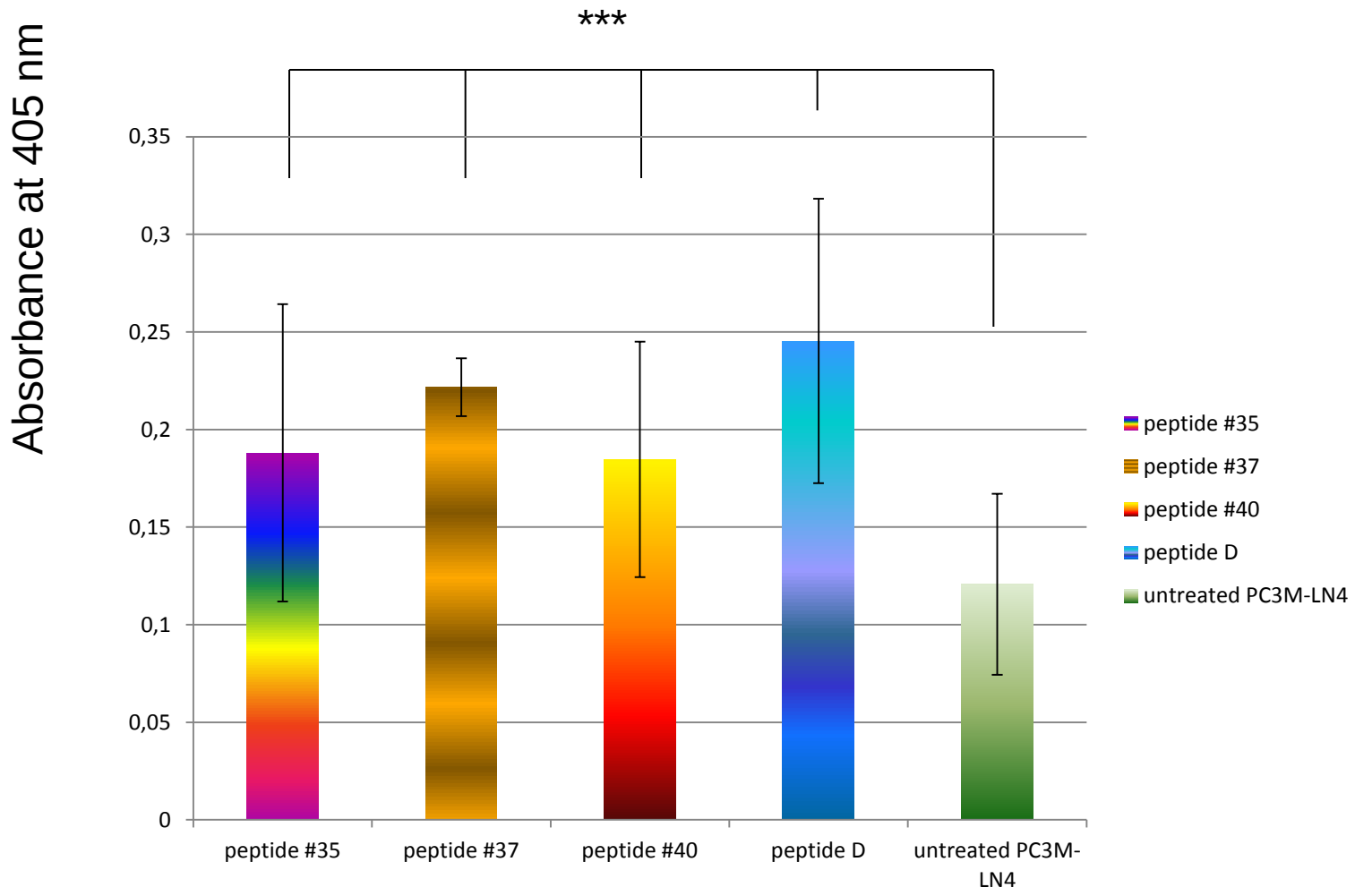


Mechanism of apoptosis:

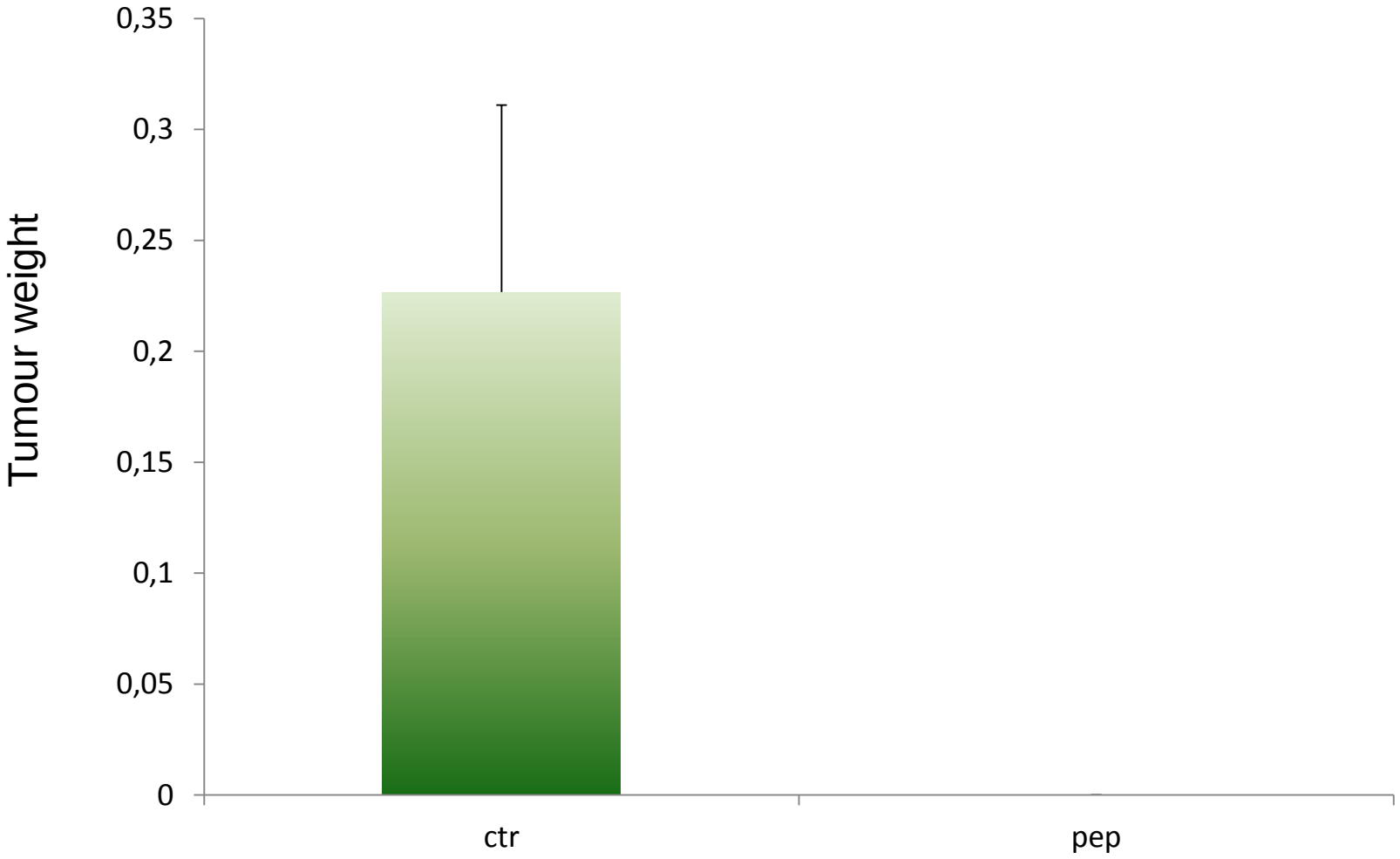
1. The canonic apoptotic process triggers extrinsic or intrinsic caspase-dependent pathways. In the extrinsic pathway, membrane-bound death receptors face the exterior of plasma membranes to receive stimuli of proapoptotic ligands and transmit signals by activating downstream initiators, caspases-8/10.
2. In the intrinsic pathway, the transduction of signals is due to B-cell lymphoma (BCL)-2 protein family-induced cytochrome c release from mitochondrial intermembrane spaces by proapoptotic stimuli, such as oxidative stress and DNA damage. Cytosolic Cyt c recruits apoptotic protease-activating factor and procaspase-9 to form apoptosomes. Activated caspases-8, -9, and -10 promote apoptosis by cleaving effector caspases, including caspases-3 and -7, into the active forms which causes proteolysis of their substrates and inhibits the normal physiological functioning of unhealthy cells.


p53
Apoptotic stimuli
Bax
PUMA
Mitochondrion
Bcl-2
Cyt c
/dATP
Smac
Apaf-1
p53
Caspase-9
IAPs
Caspase-3
Cellular targets
Apoptosis

Effect of HA-mimetic peptides on caspase-3 activity in PC3M-LN4 cells.



Effect peptide D (#135) on development prostate tumor in mice.



Study the influence of HA-mimetic peptides on invasion of breast and prostate cancer cells.

The invasion of cells through tissue and associated cellular matrix is a critical activity in embryological development and cancer metastasis.

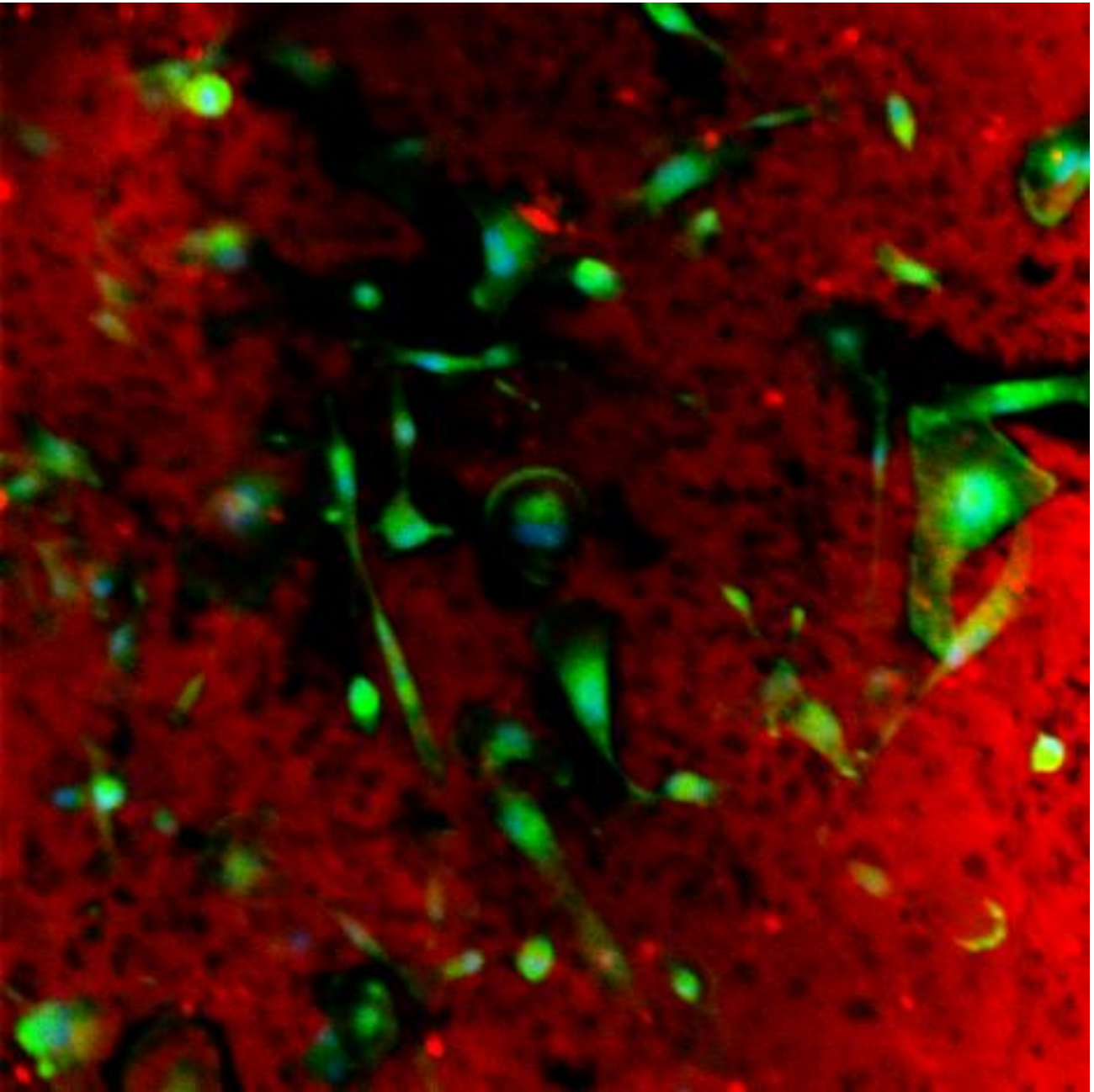
A key cellular feature involved in matrix degradation is the formation of protrusions of localized protease activity, termed invadopodia or prodosomes.

An effective method for visualising invadopodia formation involves the plating cells onto thin layer of fluorescently-labeled matrix. Areas of degradation are associated with a loss of fluorescence, and these regions may be microscopically imaged and colocolized with molecules of interest in the proteolytic pathway.

Potential activators or inhibitors of invadopodia formation may be investigated for their influence on the degree and frequency of matrix degradation.

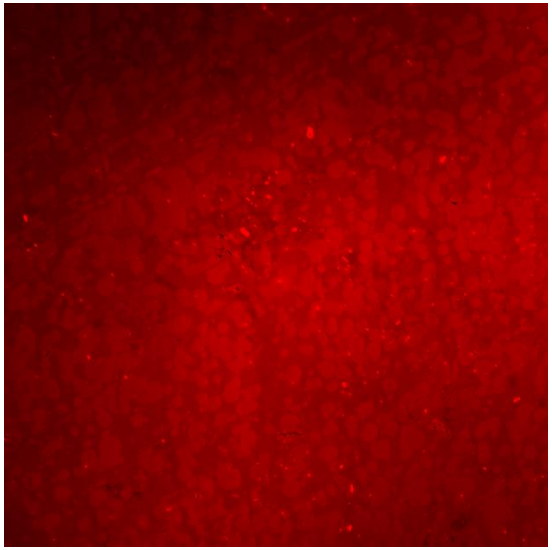
Degradation may be quantified by image analysis and used to characterize proteolytic time-courses and modulator effects on invadopodia formation.

Analysis invadopodia in invasive tumor cells. Fluorescent gelatin (Cy3) degradation and phalloidin/DAPI staining of MDA–MB-231 cells. Magnification x20.



Blank

Cy3-labeled gelatin, no cells



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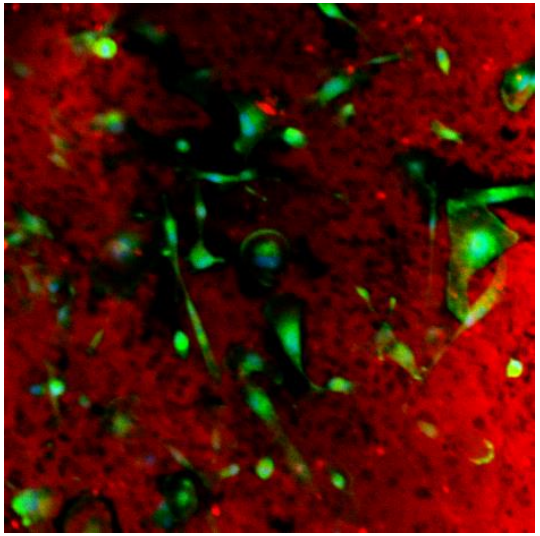
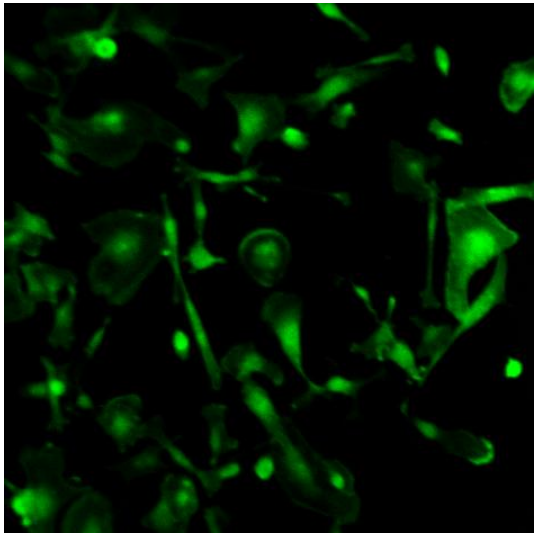
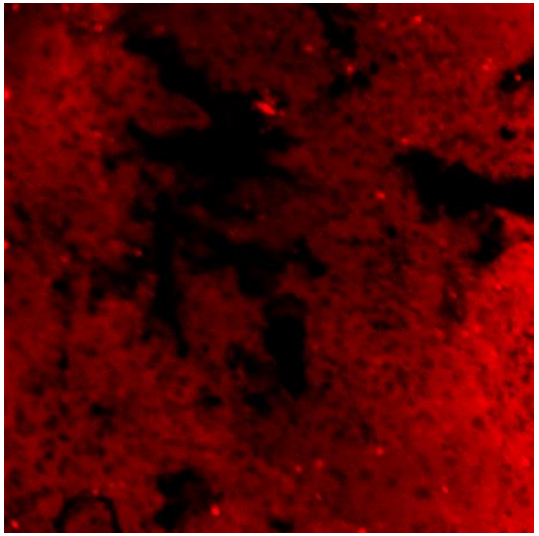


MERGE

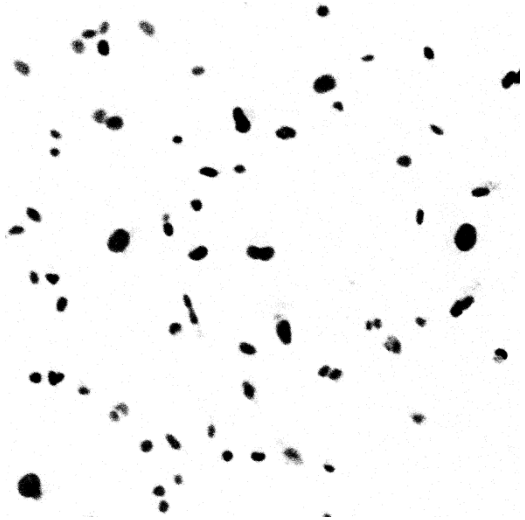


FITC



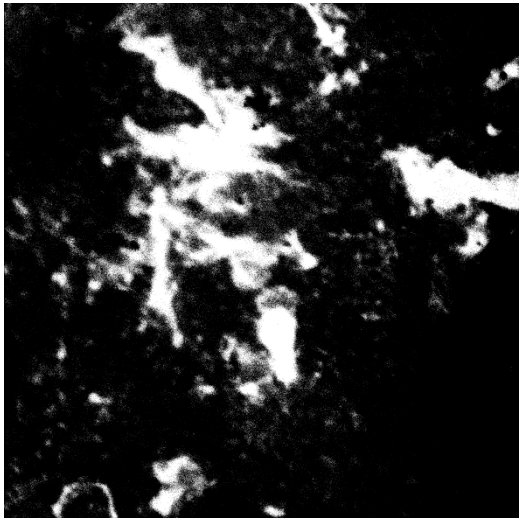
Cy3

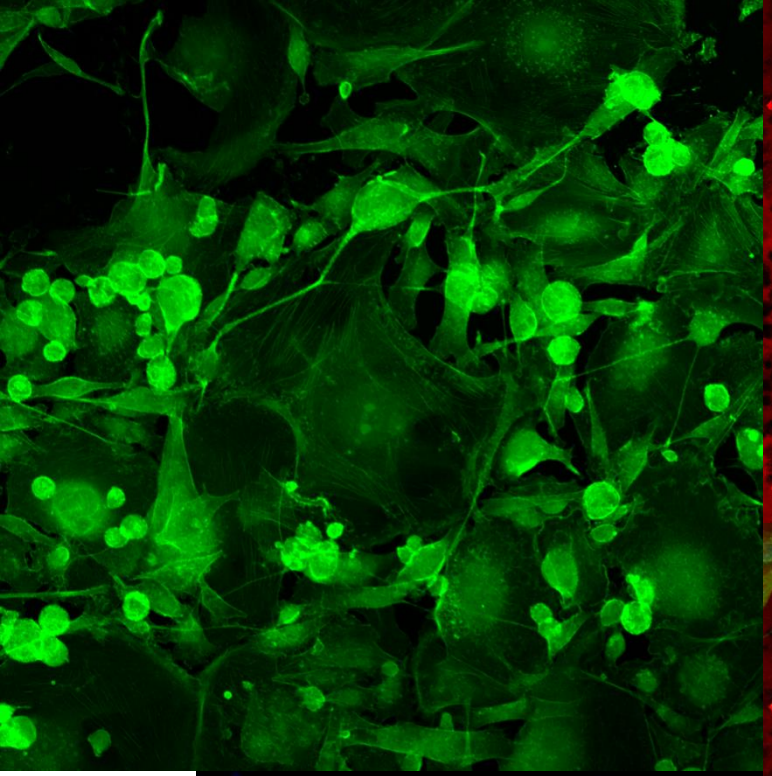
Number of cells

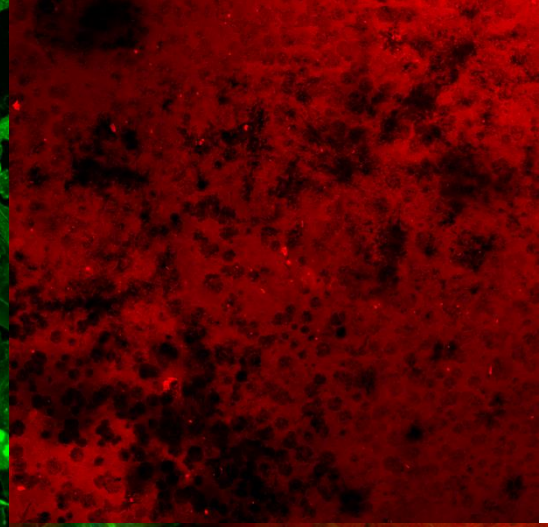


Cell area

Degraded area-white

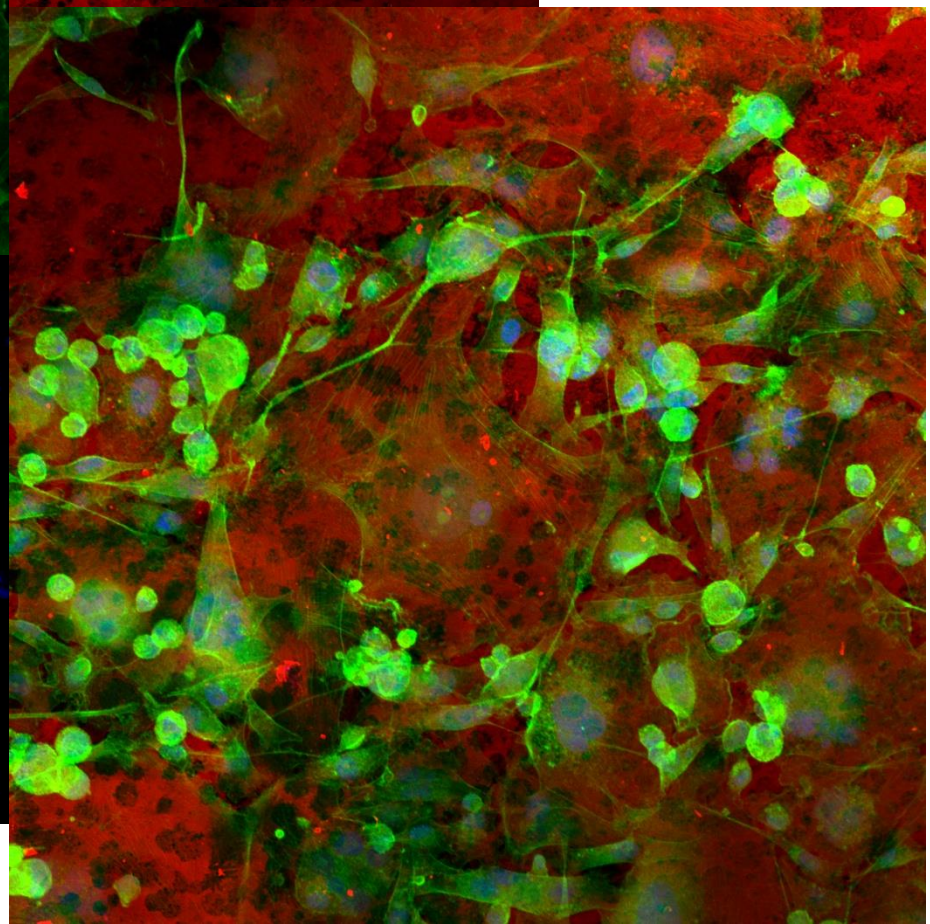


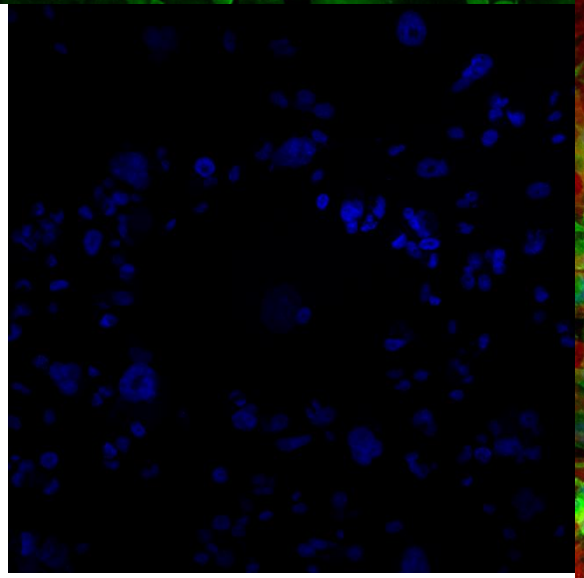




Fluorescent gelatin (Cy3 labeled) degradation and phalloidin/DAPI staining of MDA–MB-231 cells. Magnification x20.

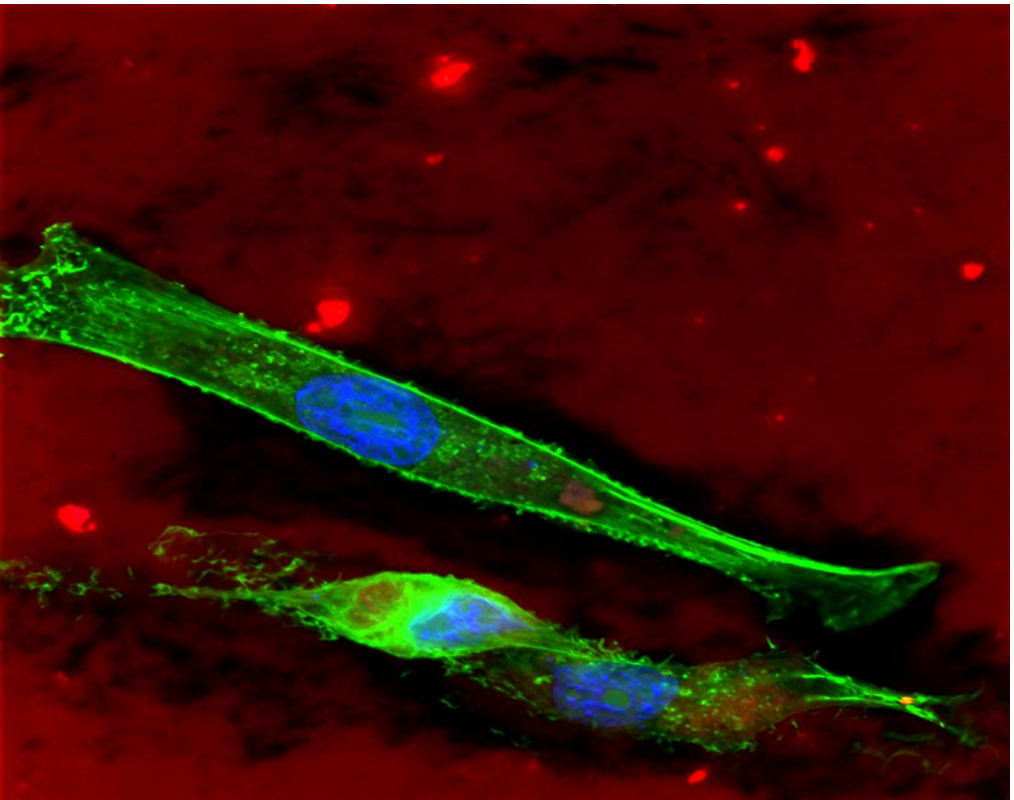
MERGE

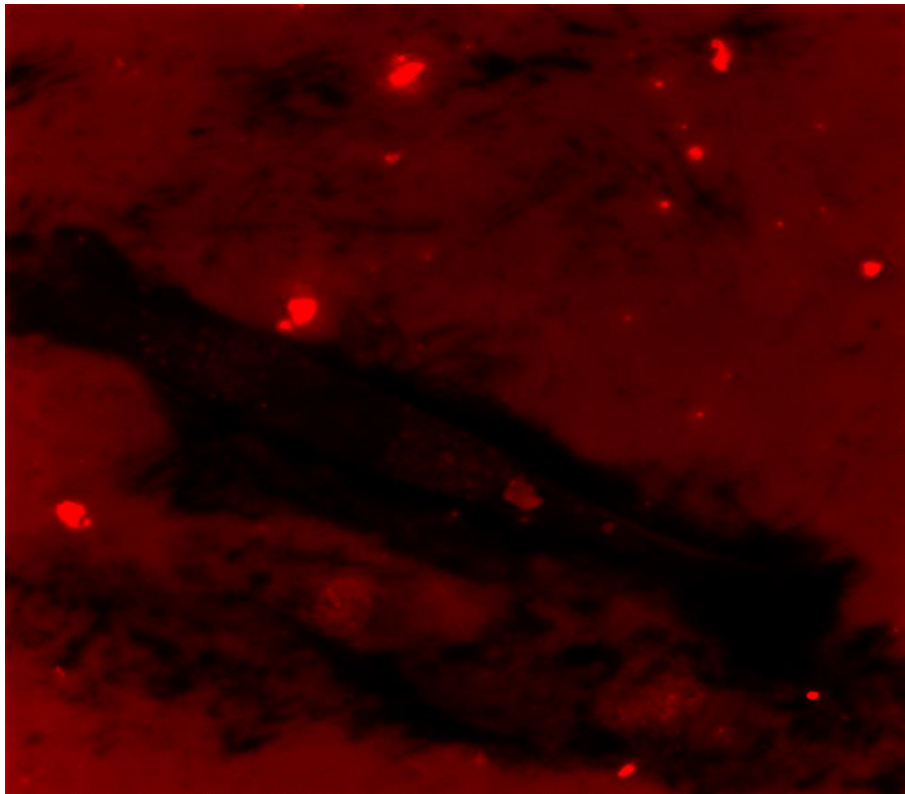




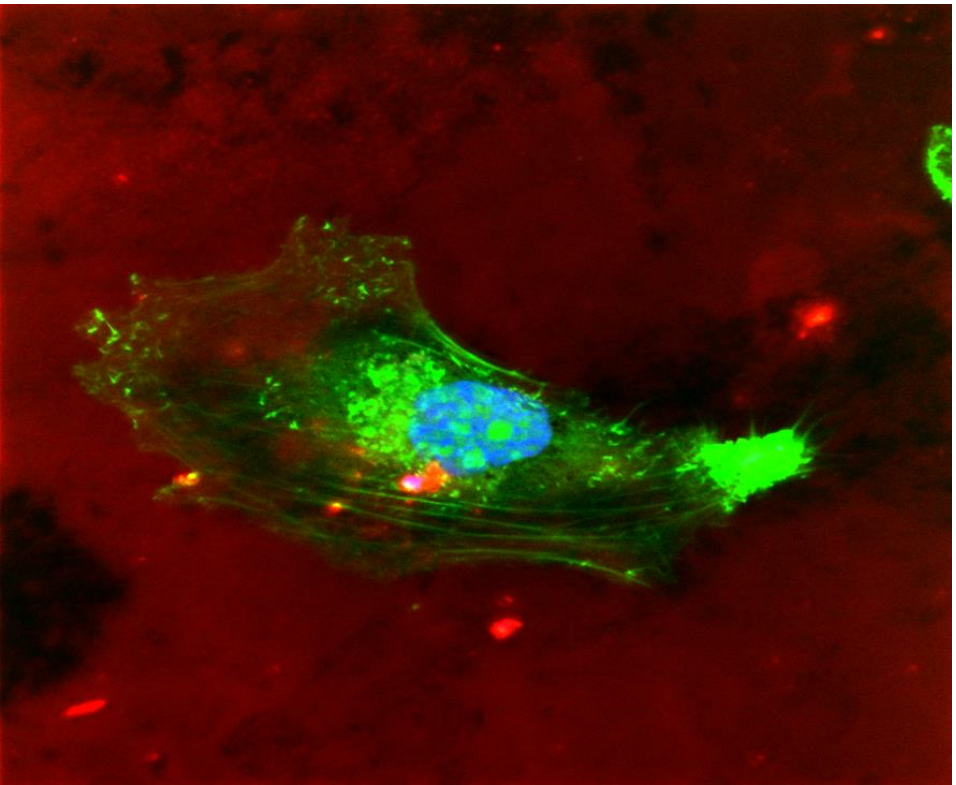
DAPI

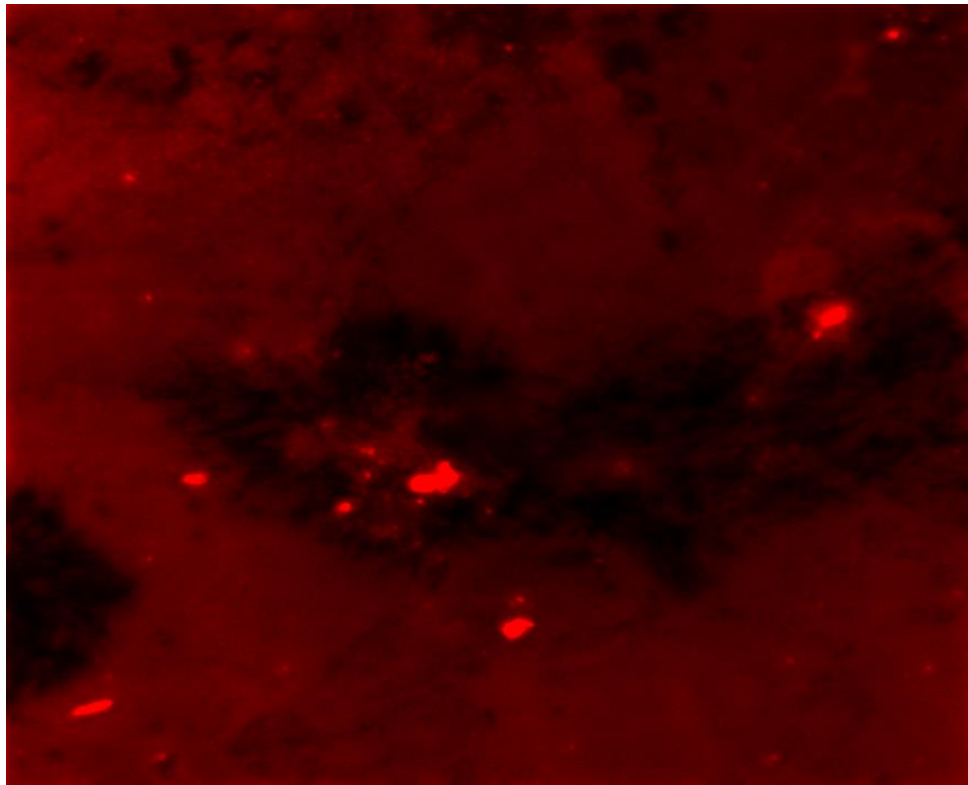
Fluorescent gelatin (Cy3 labeled) degradation and phalloidin/DAPI staining of MDA–MB-231. Magnification x60 cells.



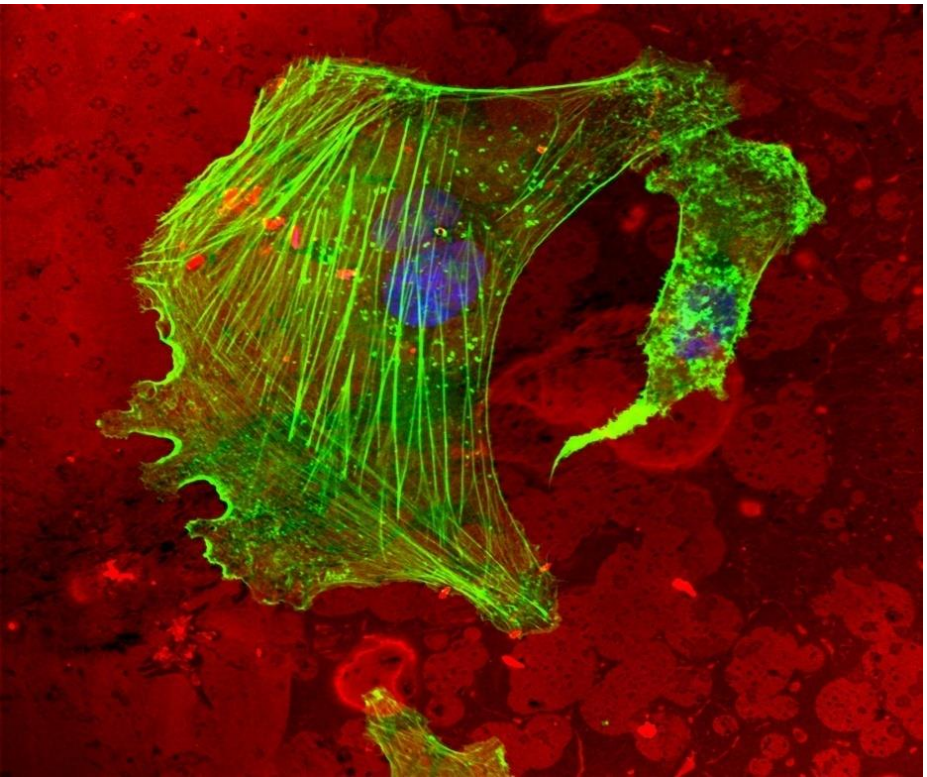


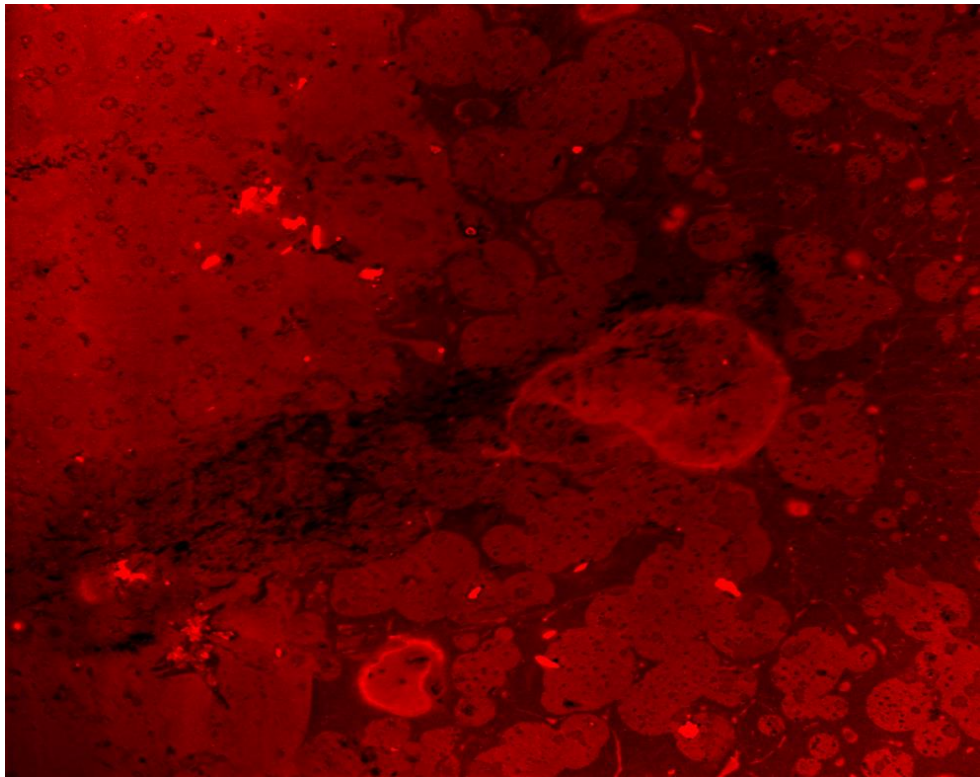
Fluorescent gelatin (Cy3 labeled) degradation and phalloidin/DAPI staining of MDA–MB-231. Magnification x60 cells.



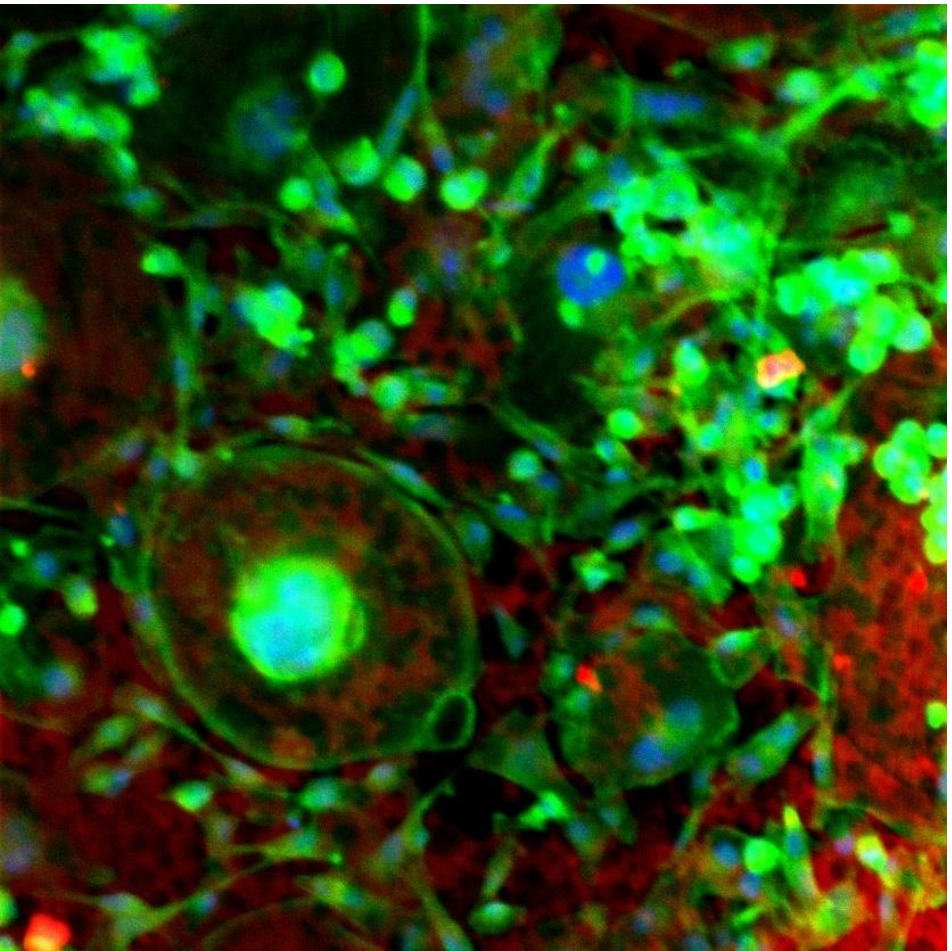


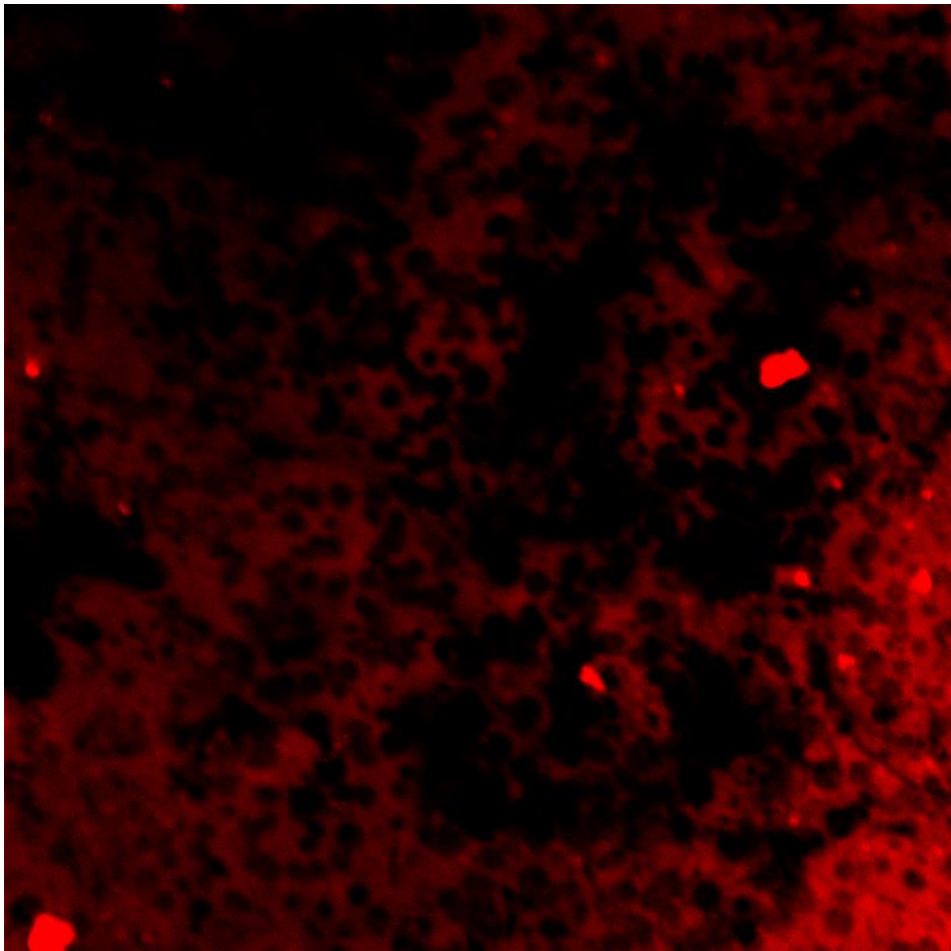
Fluorescent gelatin (Cy3 labeled) degradation and phalloidin/DAPI staining of MDA–MB-231. Magnification x60 cells.





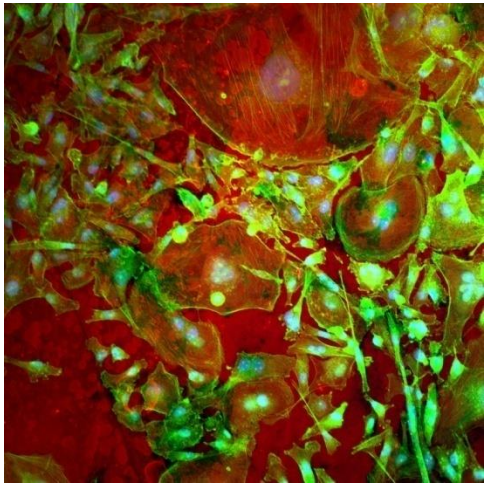
Fluorescent gelatin (Cy3 labeled) degradation and phalloidin/DAPI staining of MDA–MB-231. Magnification x20 cells.



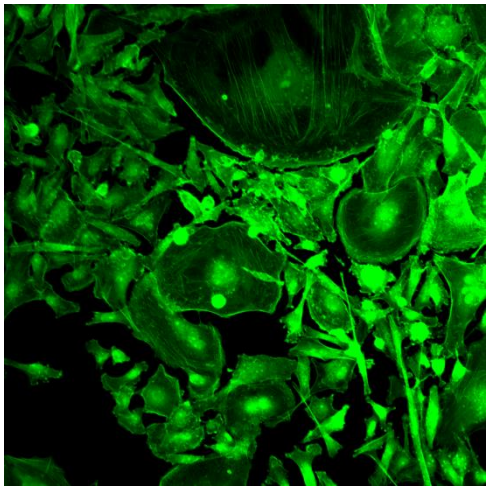


Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #35 on invadopodia formation. Magnification x20.

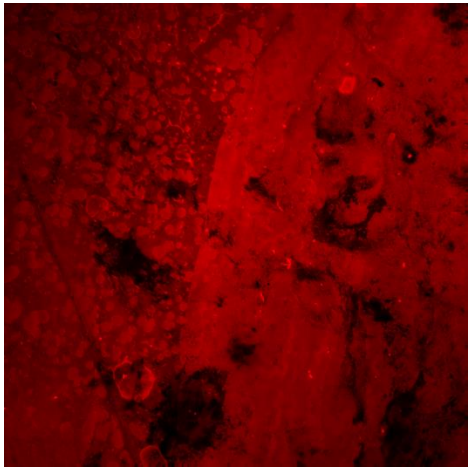
Merge



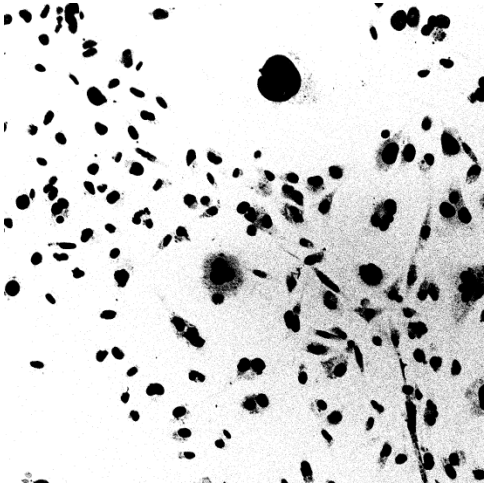
FITC



Cy3

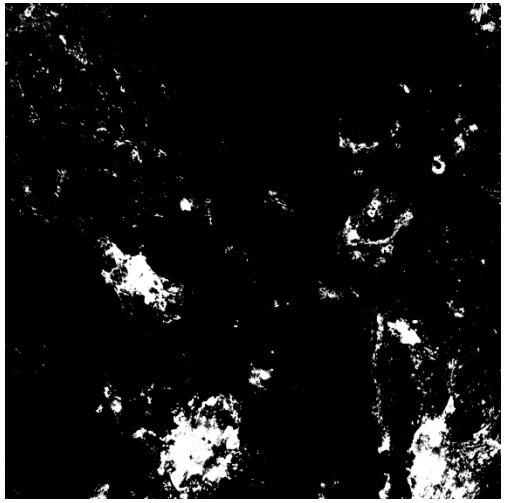


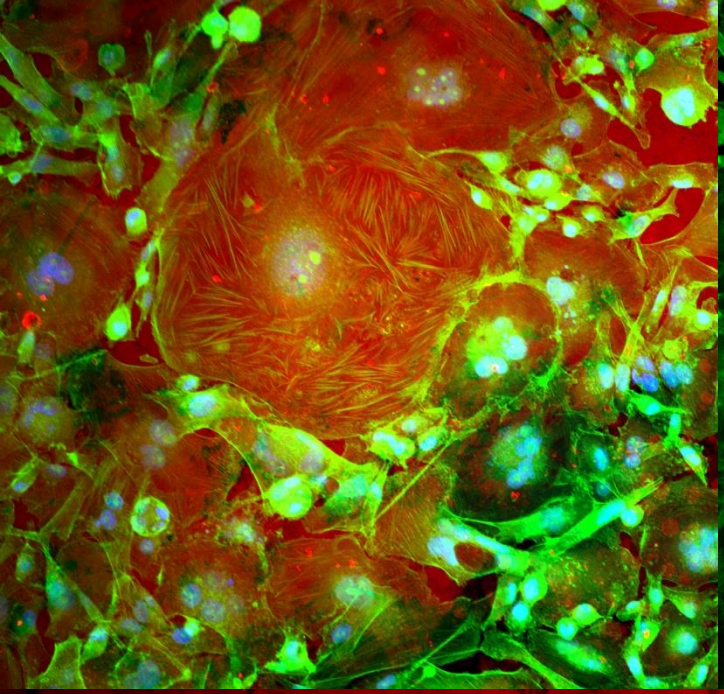
Numbers of cells

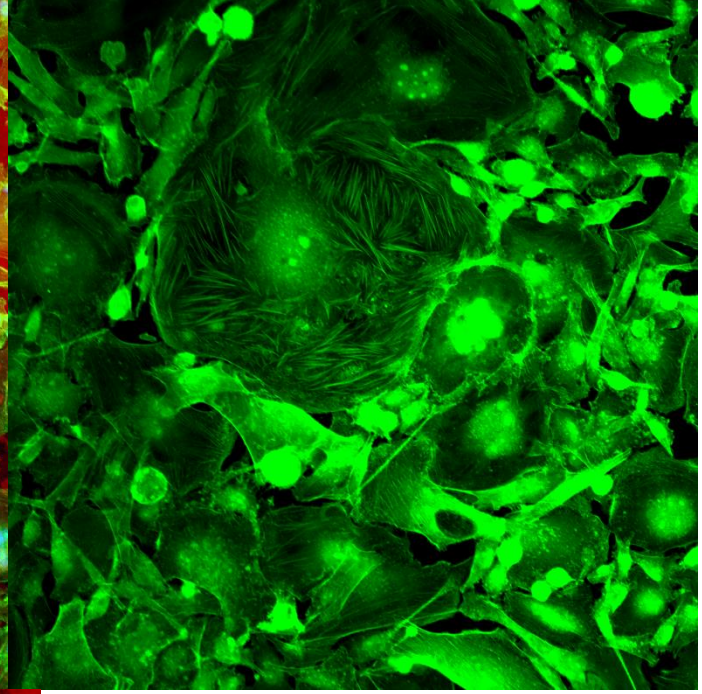


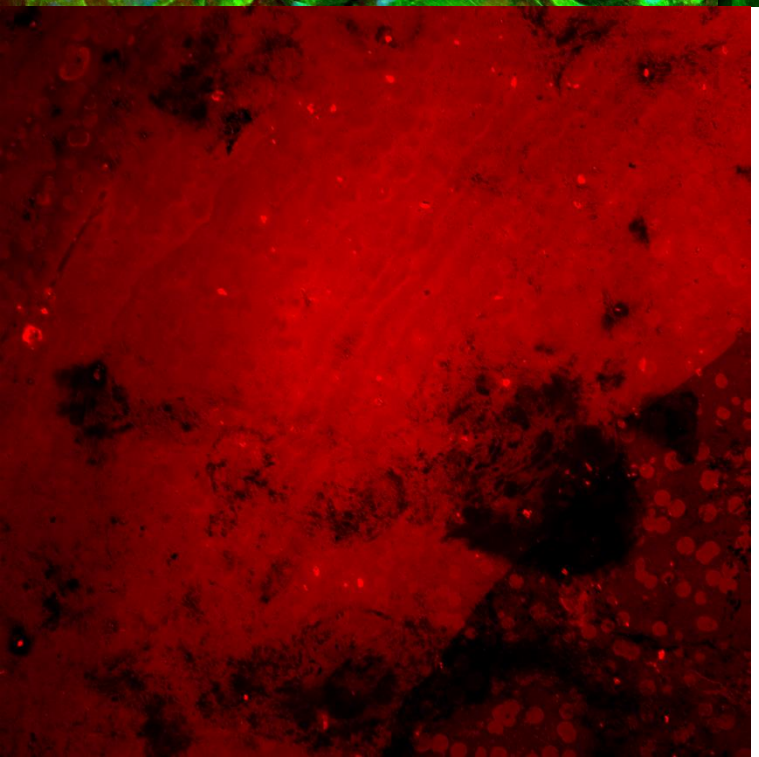
Cell area-black

Degraded area-white

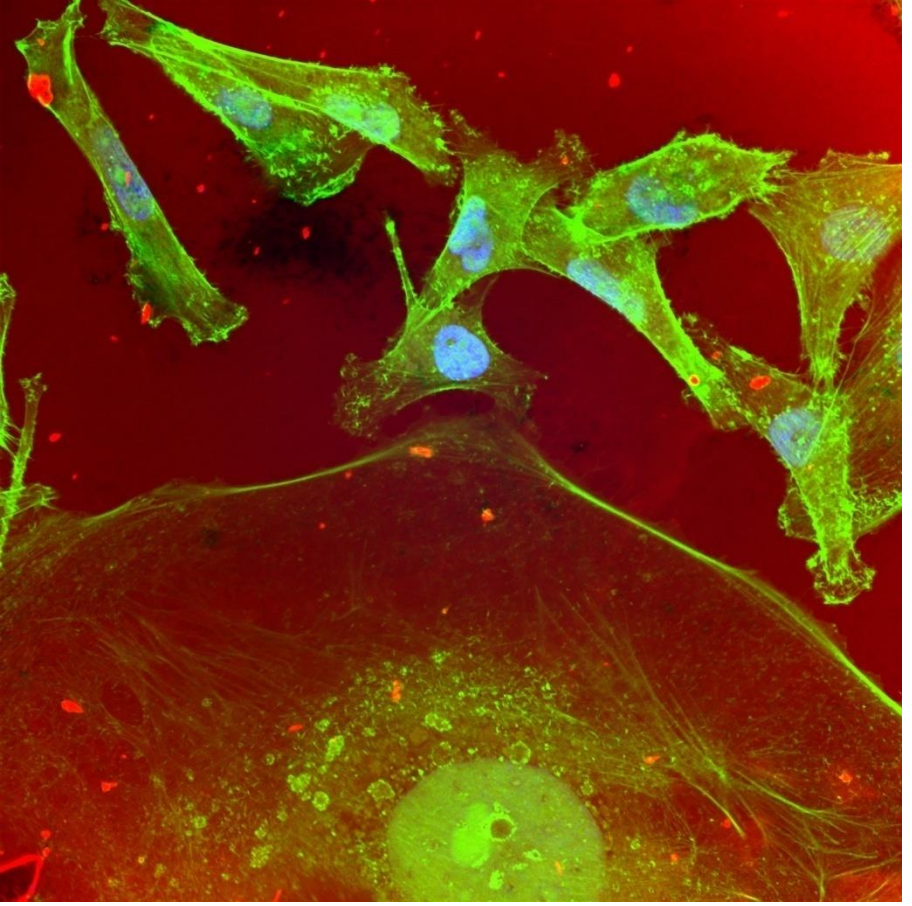




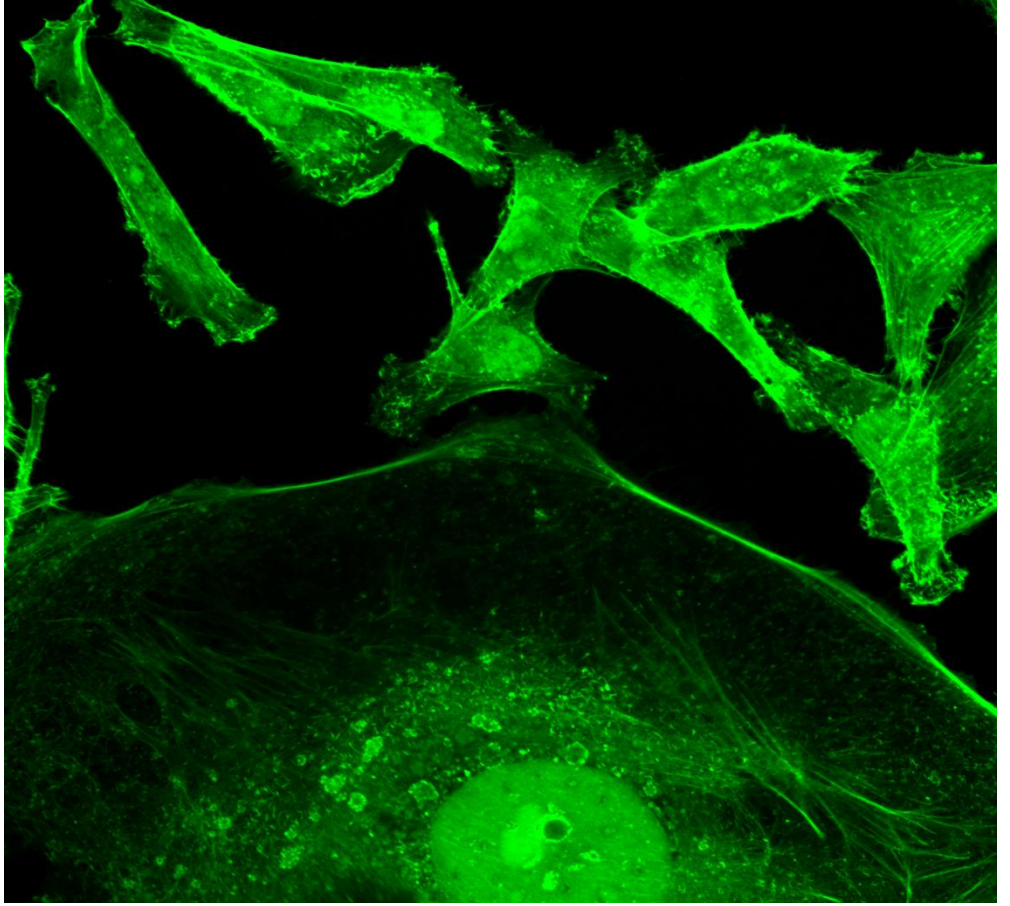




Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #35 on invadopodia formation. Magnification x20.



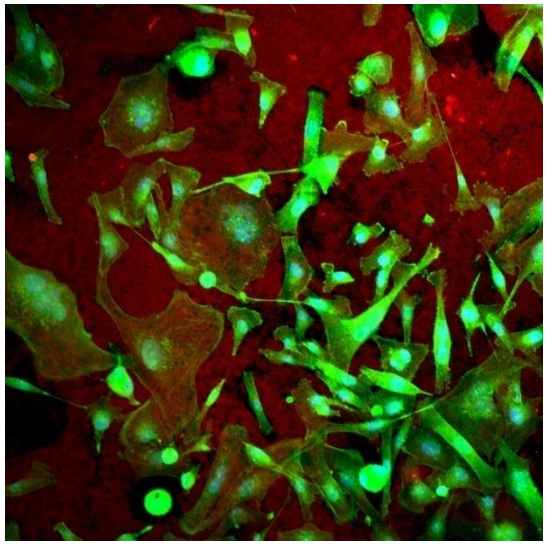
MERGE



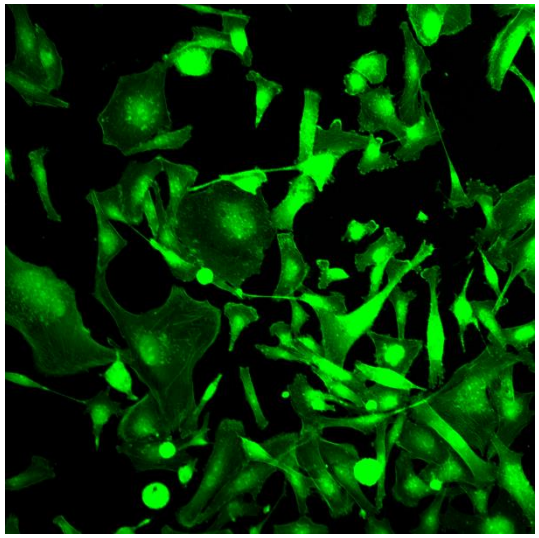
FITC

Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #35 on invadopodia formation MDA-MB 231 cells. Magnification x60.

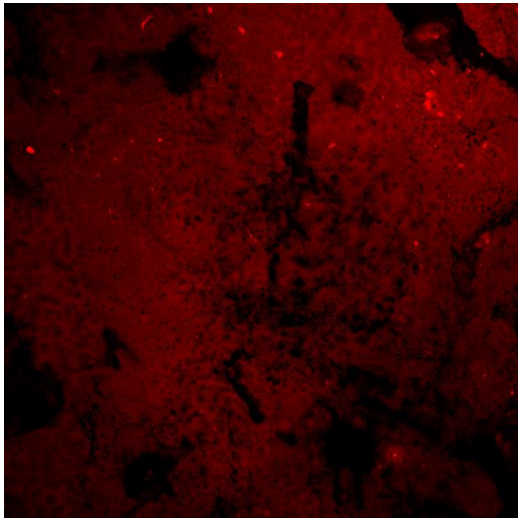
Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #37 on invadopodia formation. Magnification x20.



MERGE



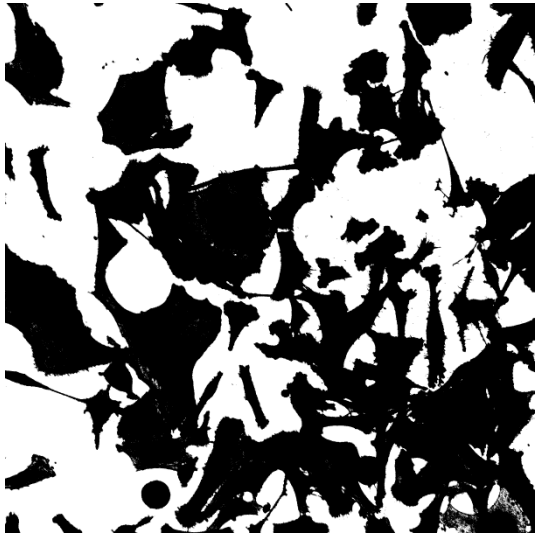
FITC



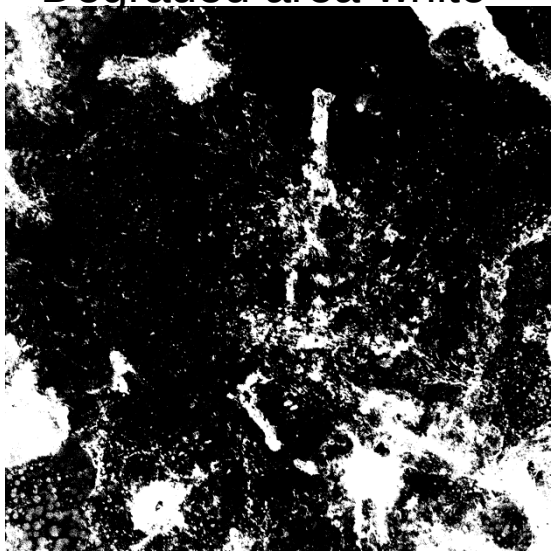
Cy3

Number of cells

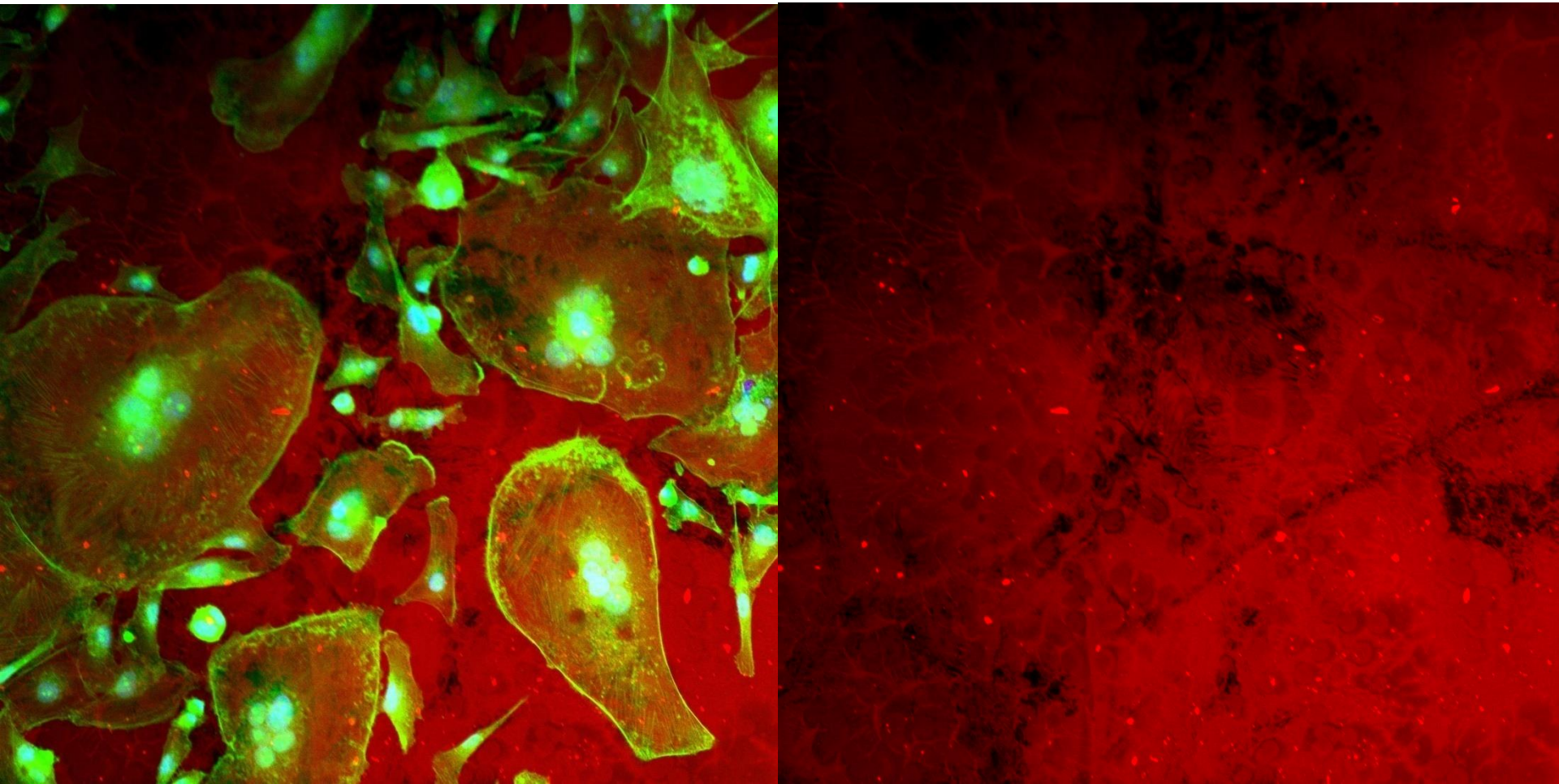
Cell area

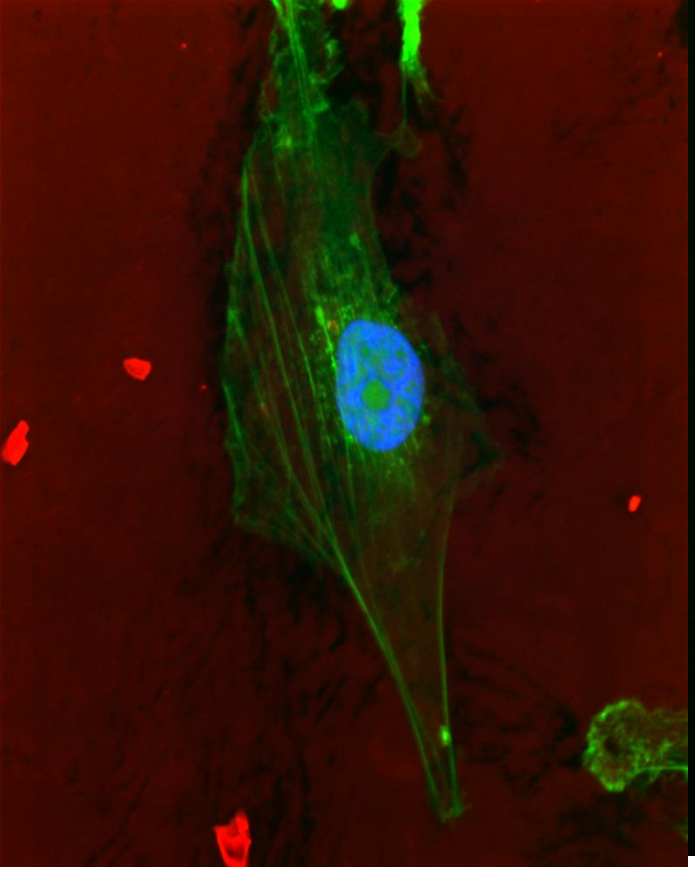


Degraded area-white

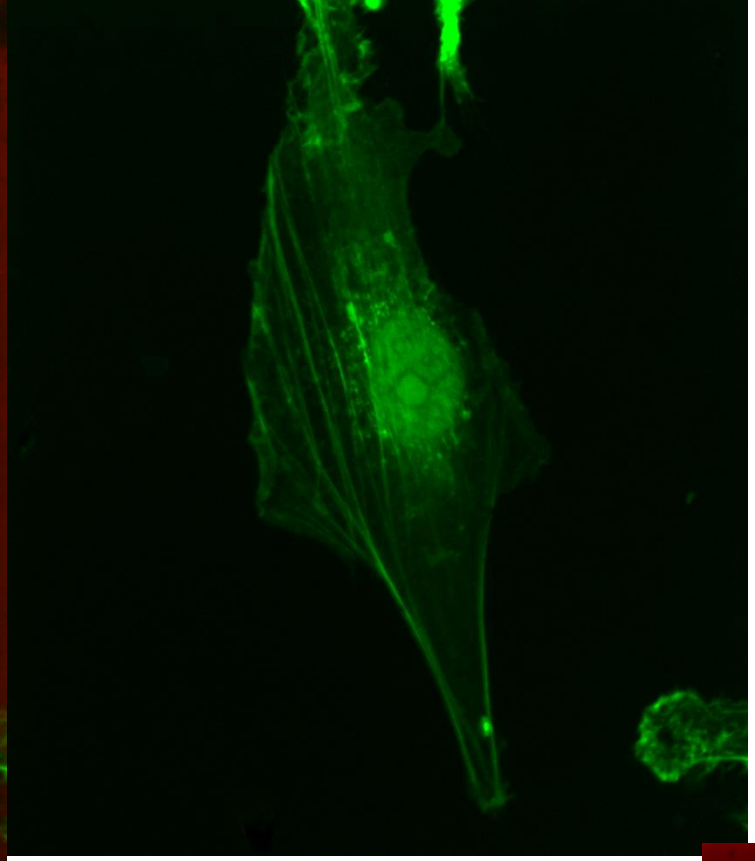


Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #37 on invadopodia formation. Magnification x20.



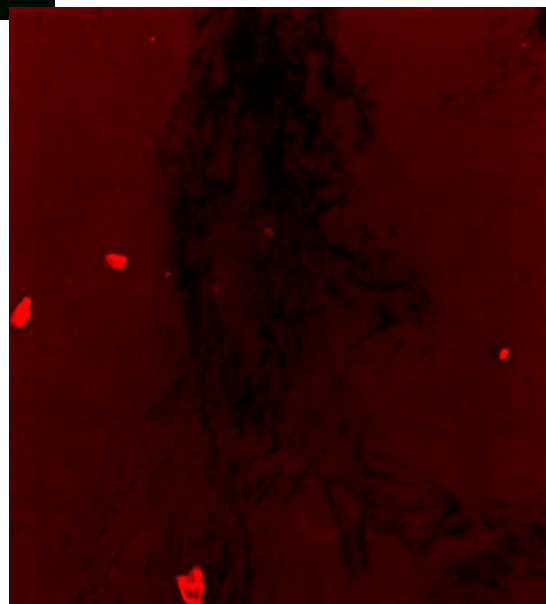


MERGE

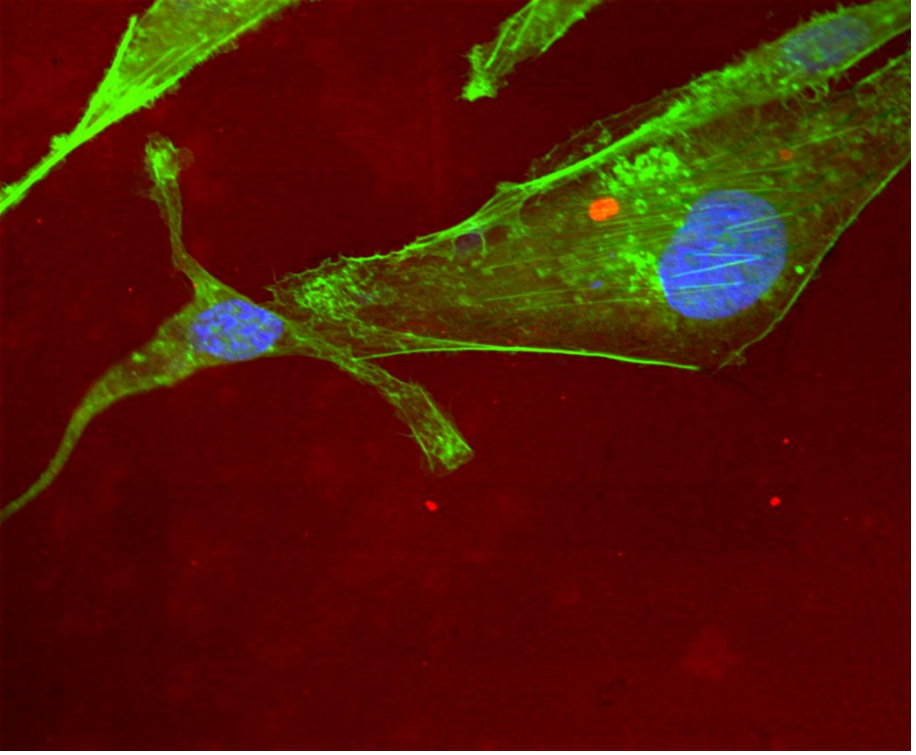


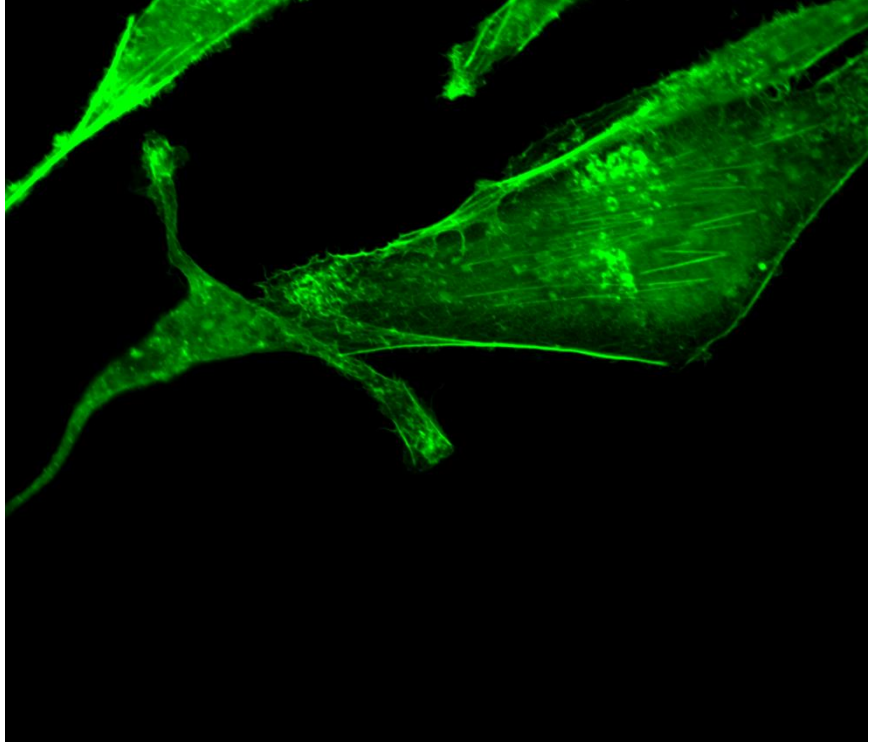
FITC

Cy3

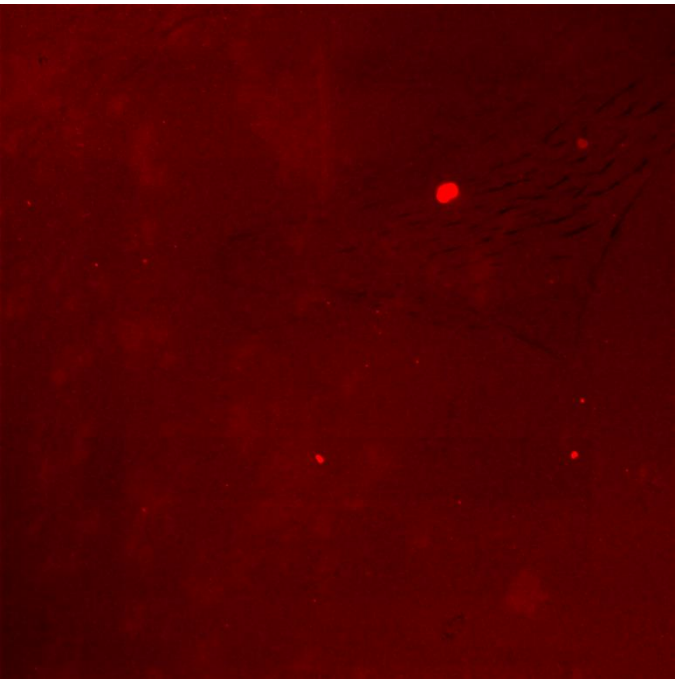


Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #37 on invadopodia formation MDA-MB-231 cells. Magnification x60.





FITC

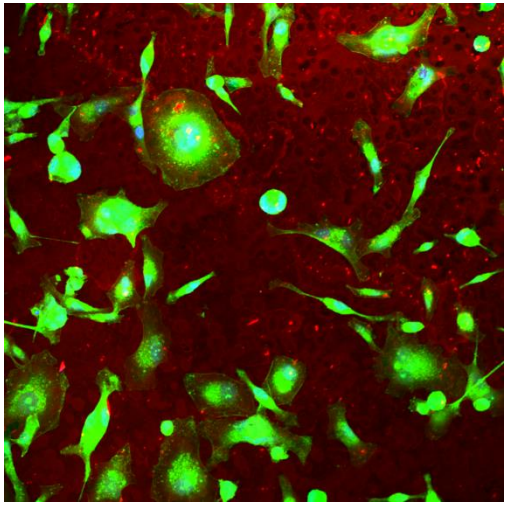


Cy3

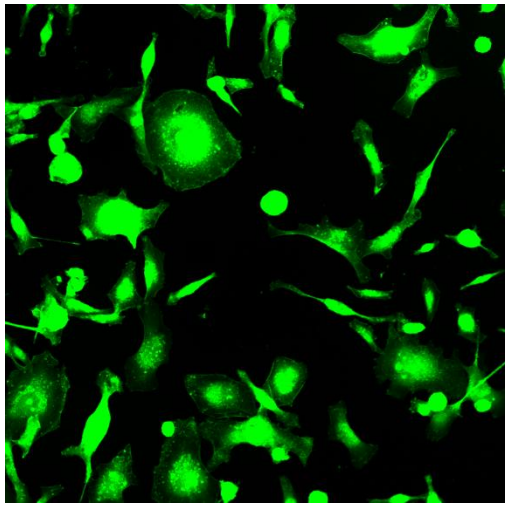
Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #40 on invadopodia formation MDA-MB-231 cells. Magnification x60.

Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #40 on invadopodia formation. Magnification x20.

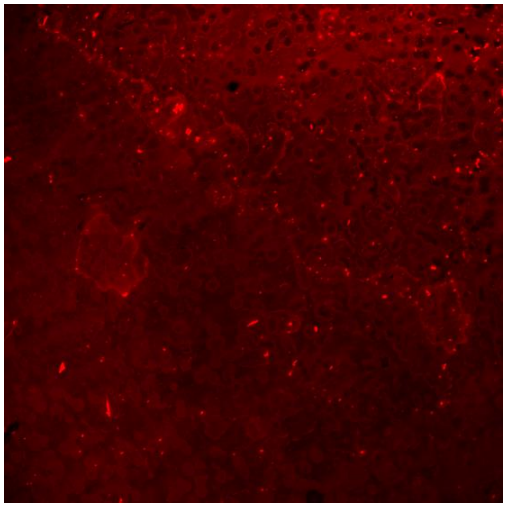
MERGE



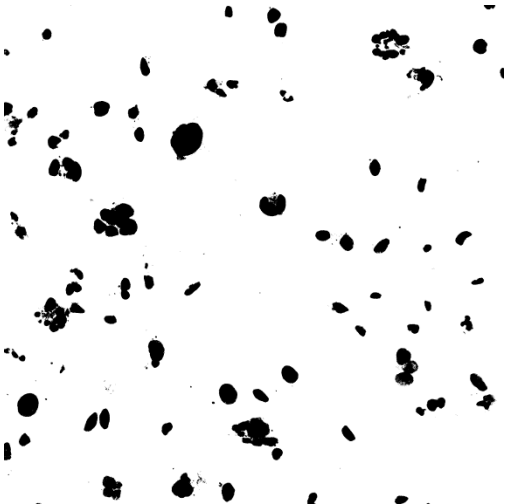
FITC



Cy3

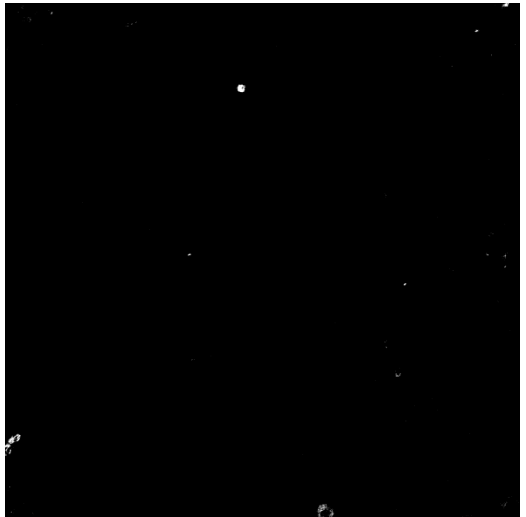


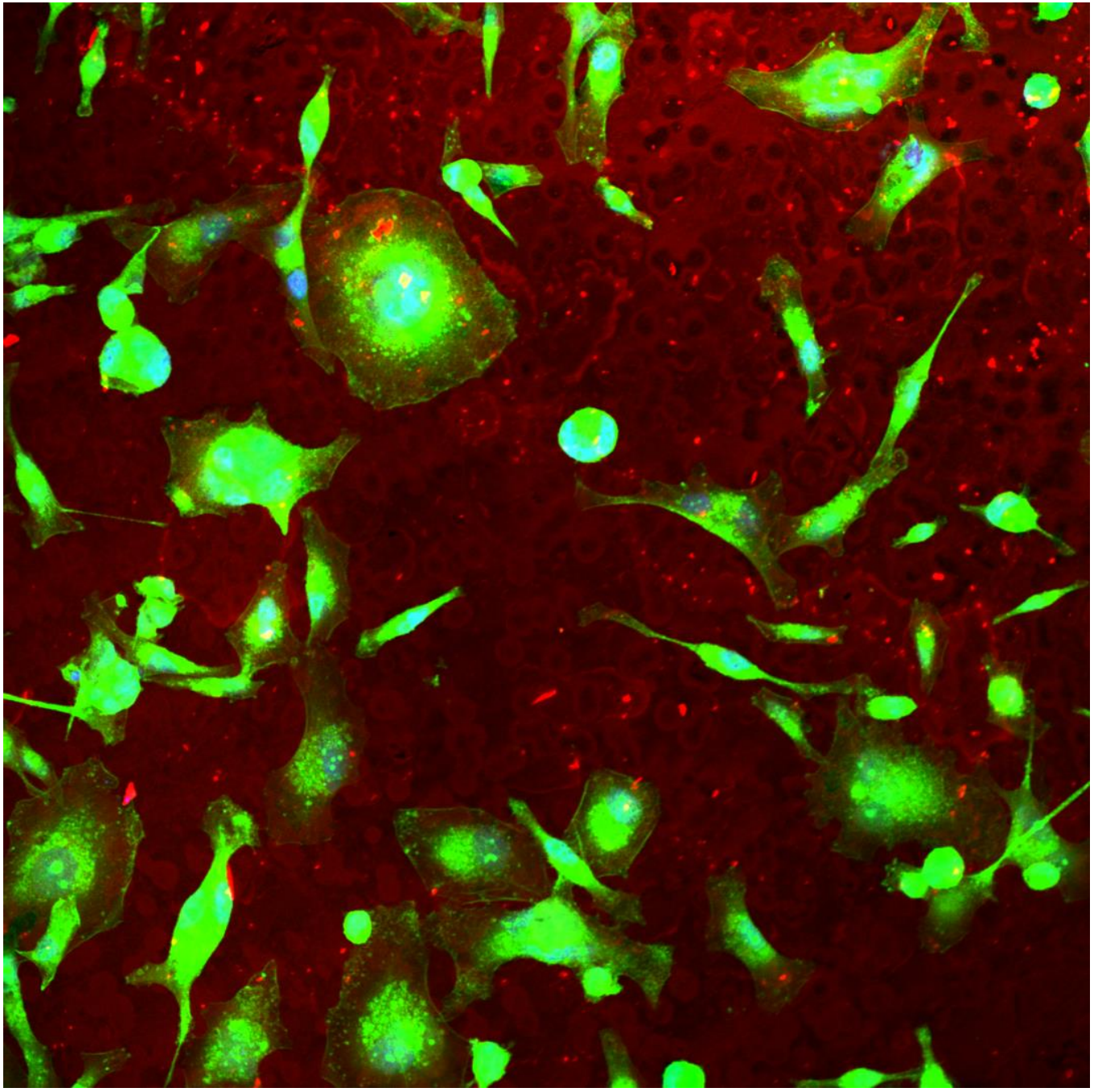
Number cells



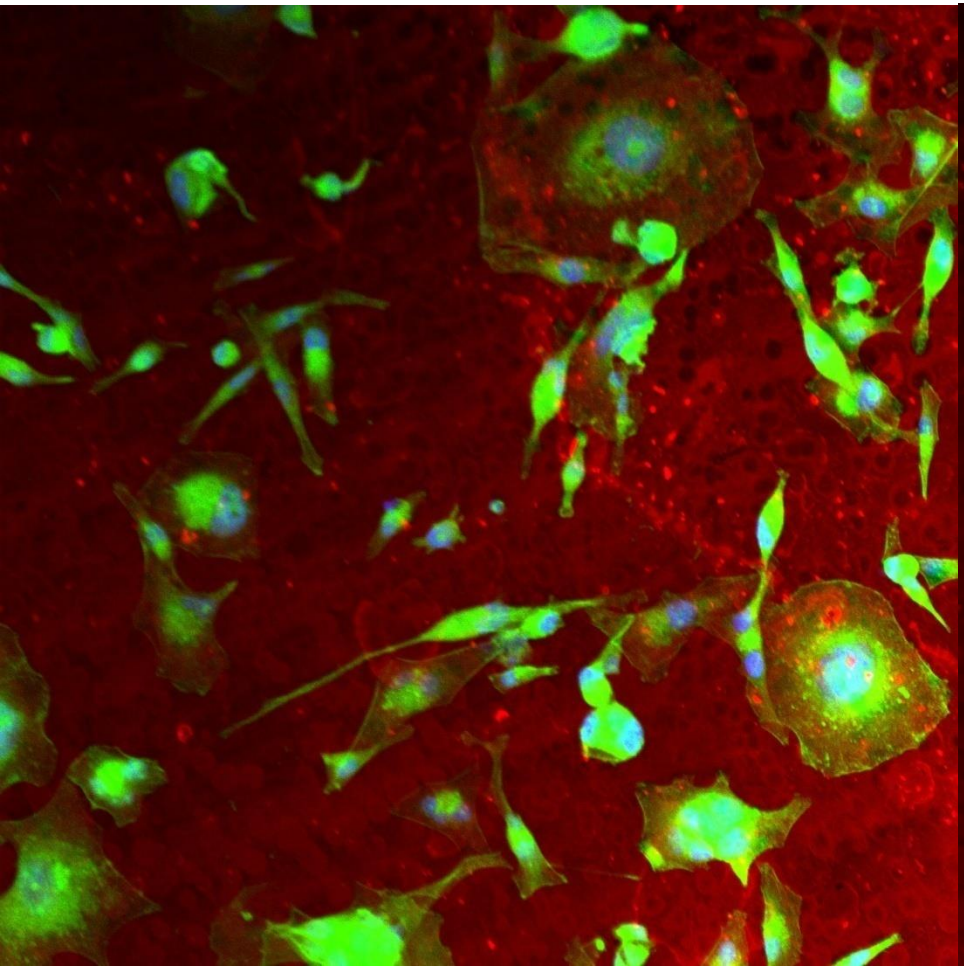
Cell area

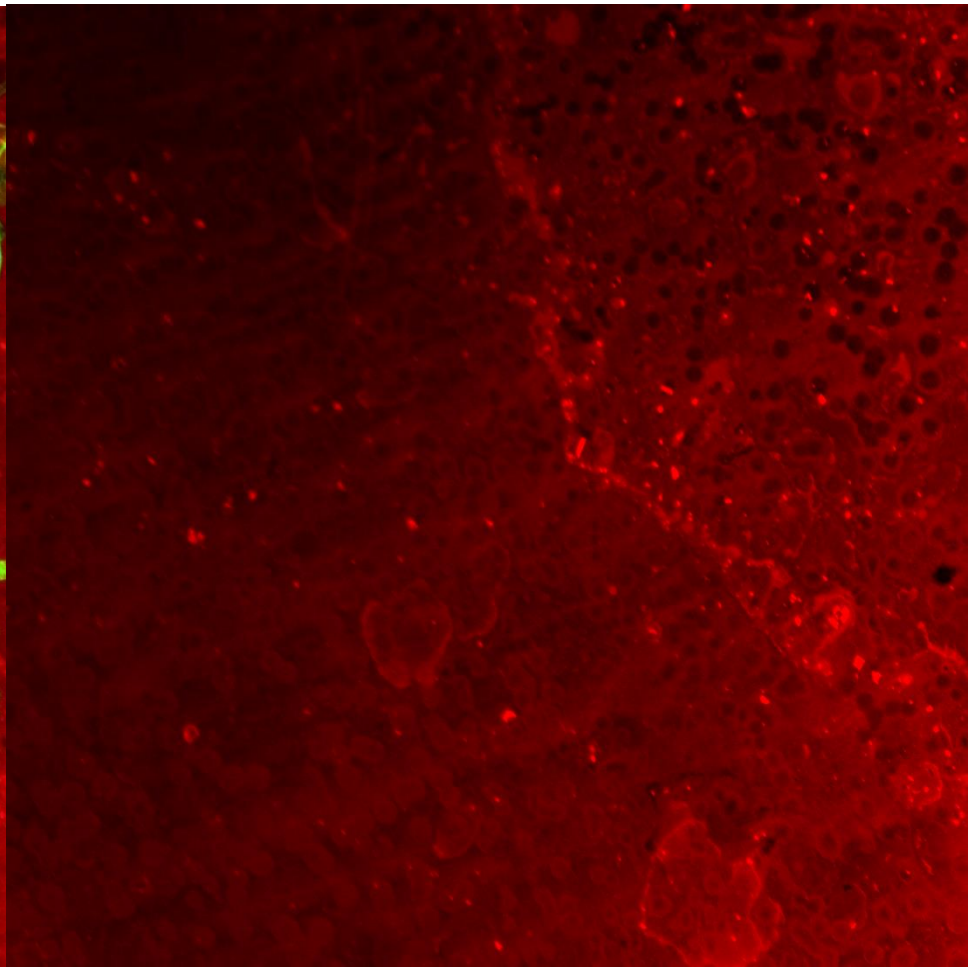
Degraded area-white





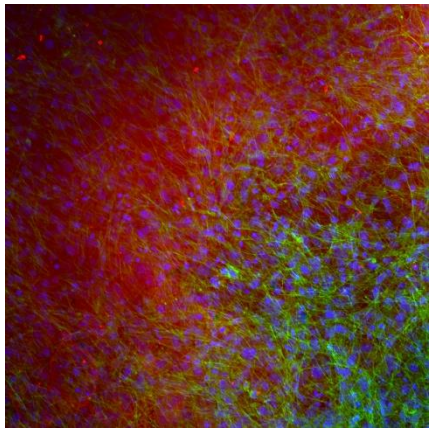
Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #40 on invadopodia formation. Magnification x20.



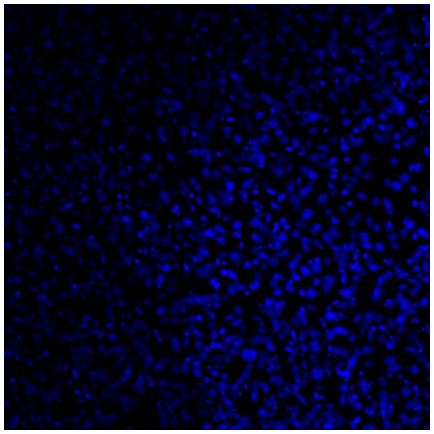


Analysis invasion in normal 10 T 1/2 cells. Fluorescent gelatin (Cy3) degradation and phalloidin/DAPI staining of 10 T 1/2 cells. Magnification x20.

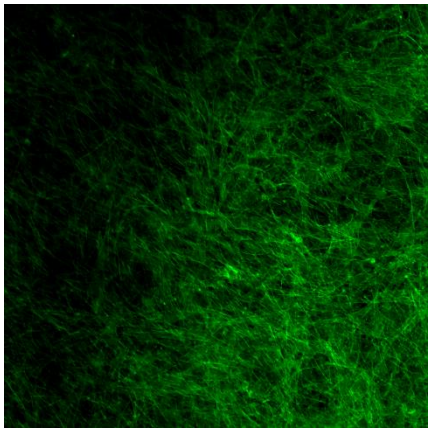
MERGE



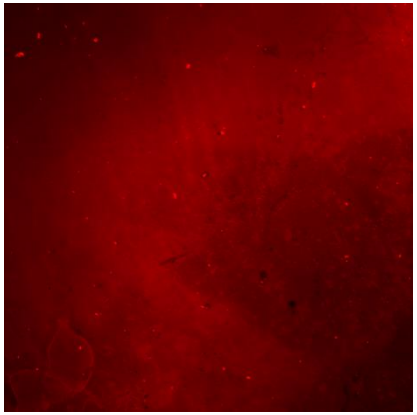
DAPI



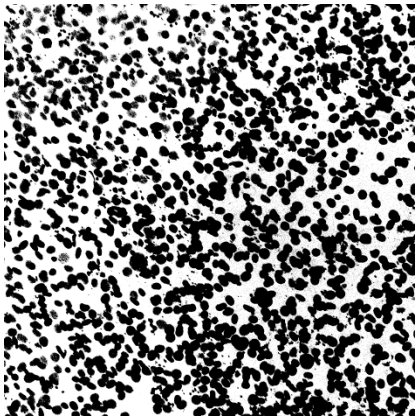
FITC



Cy3

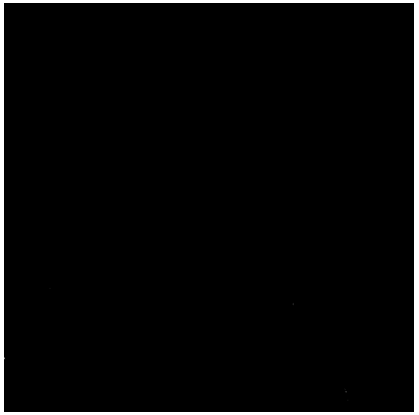


Number of cells



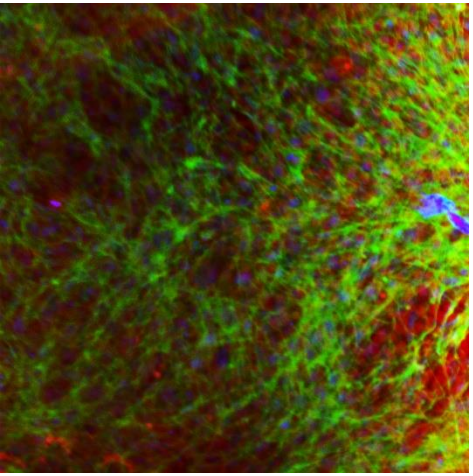
Cell area

Degraded area-white

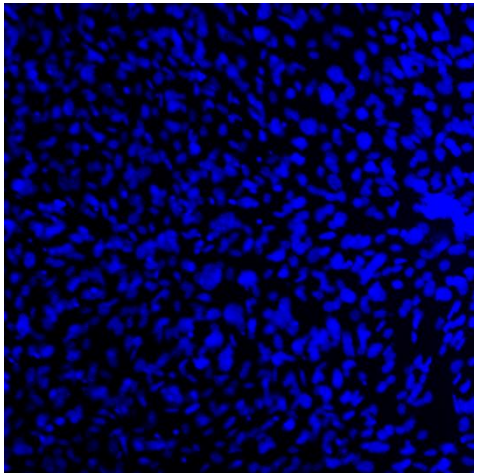


Analysis invasion in Ko (Rhamm -/-) cells. Fluorescent gelatin (Cy3) degradation and phalloidin/DAPI staining of Ko (Rhamm-/-) cells. Magnification x20.

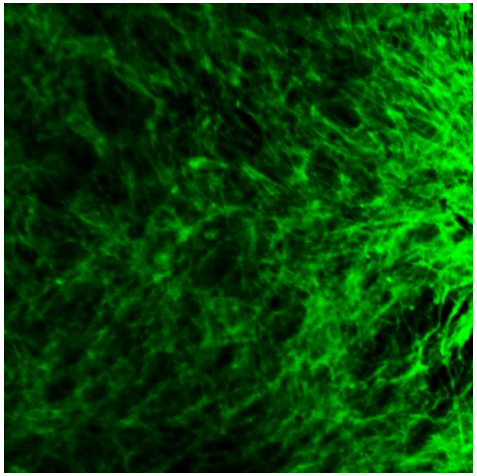
MERGE



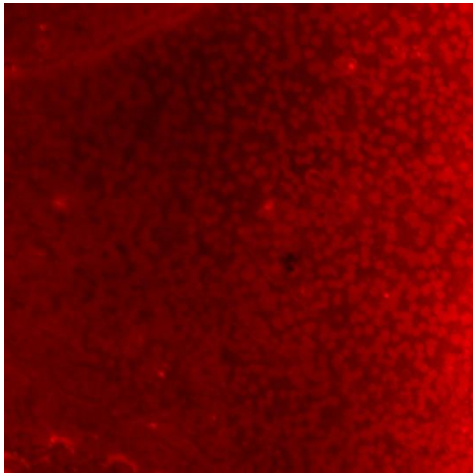
DAPI



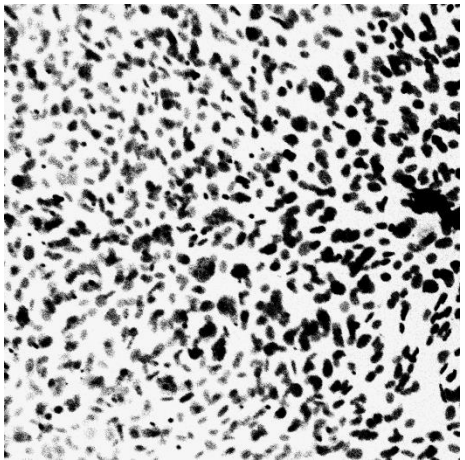
FITC



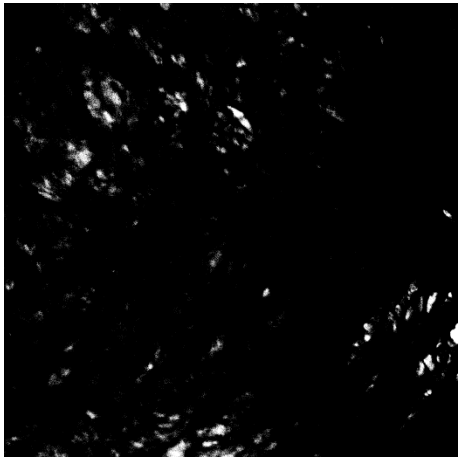
Cy3



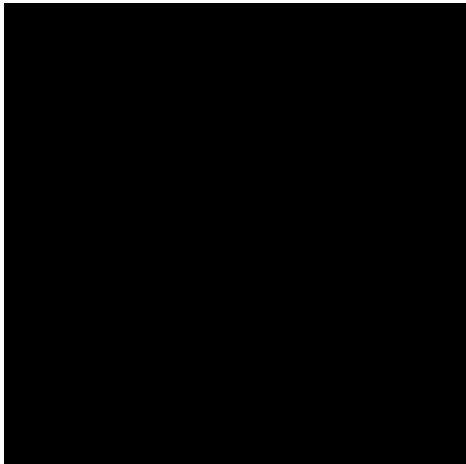
Number of cells



Cell area

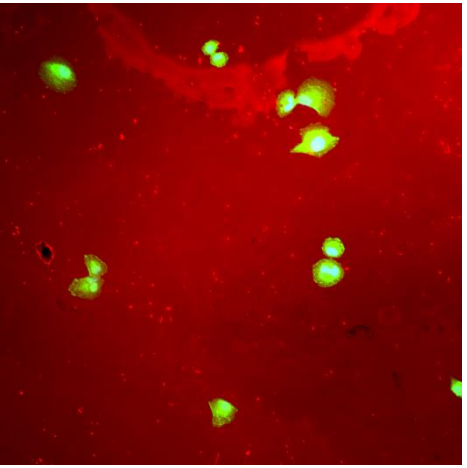


Degraded area-white

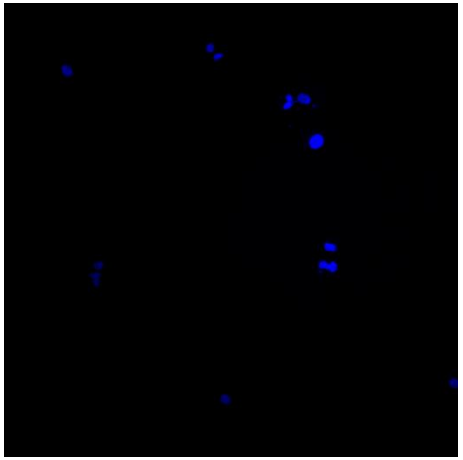


Analysis invasion in human skin melanoma (WM1552c) cells. Fluorescent gelatin (Cy3) degradation and phalloidin/DAPI staining of WM1552c cells. Magnification x20.

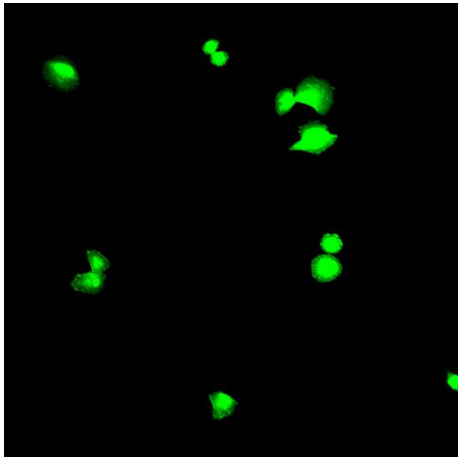
MERGE



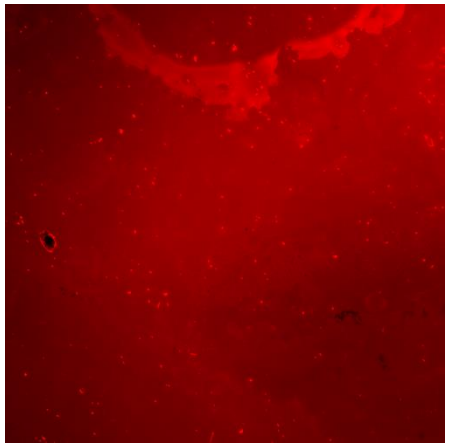
DAPI



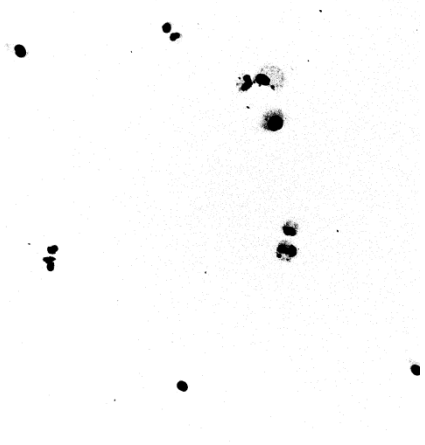
FITC



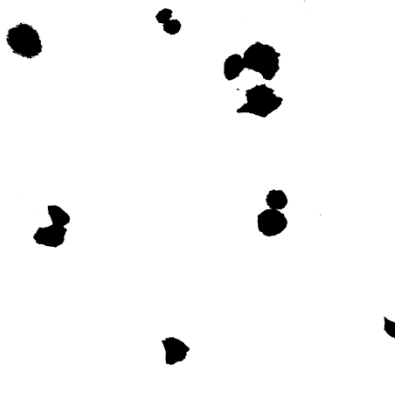
Cy3



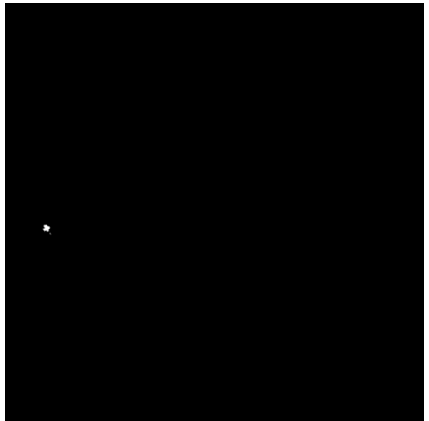
Number cells



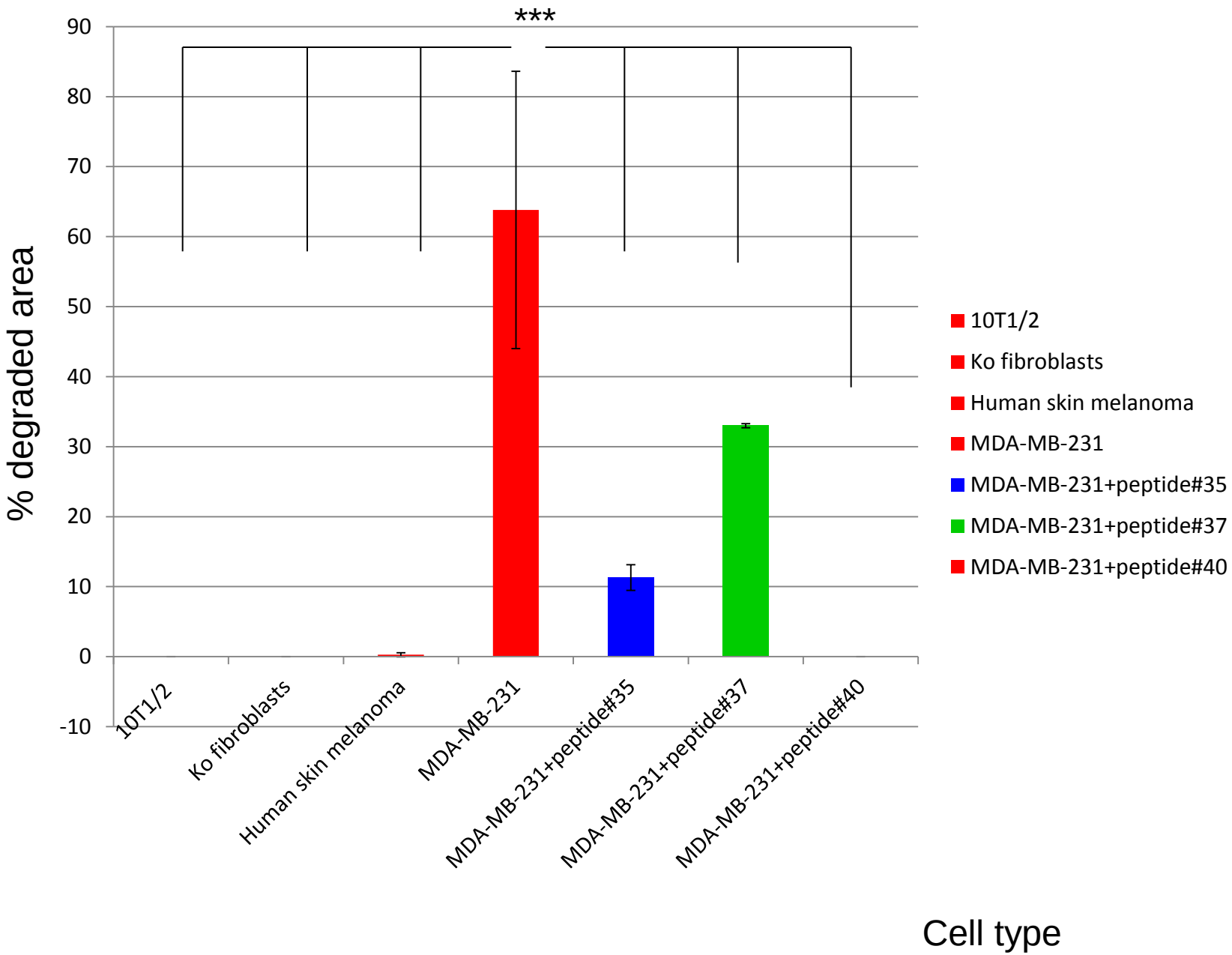
Area cells



Degraded area-white

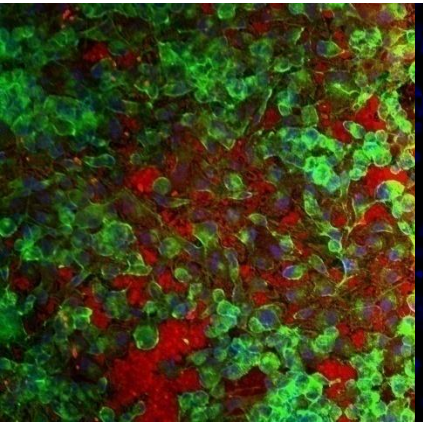


Cy3-Gelatin: % Degradation area of total cell area.

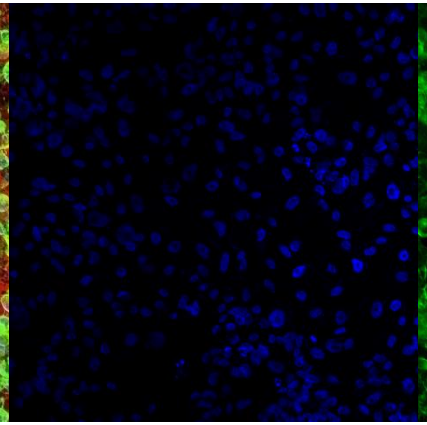


Analysis invasion in PC3M-LN4 cells. Fluorescent gelatin (Cy3) degradation and phalloidin/DAPI staining of PC3M-LN4 cells. Magnification x20.

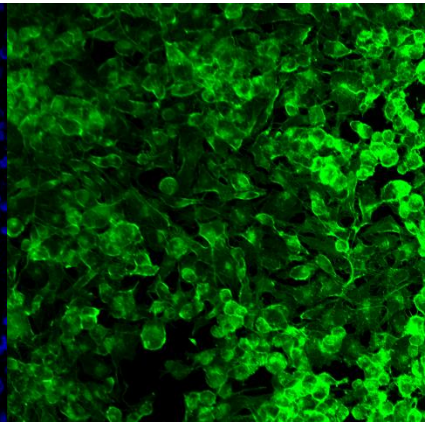
MERGE



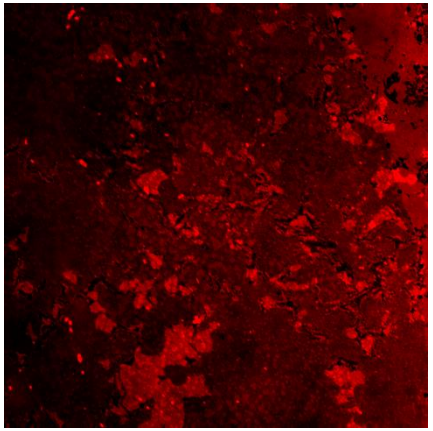
DAPI



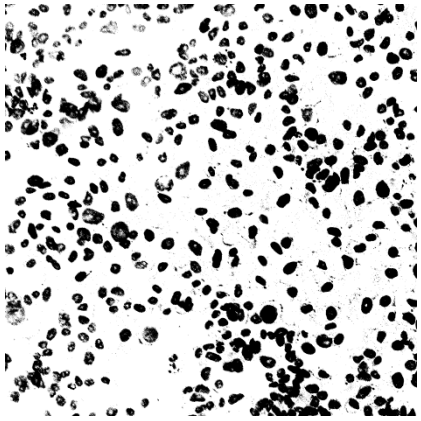
FITC



Cy3

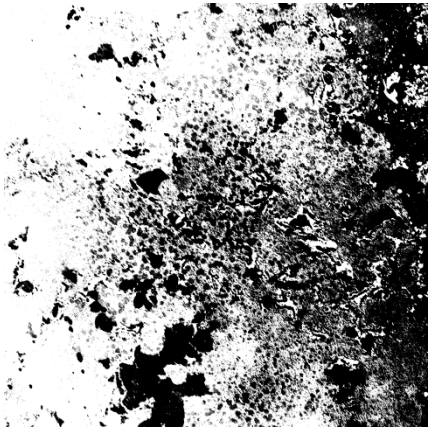


Numbers of cells

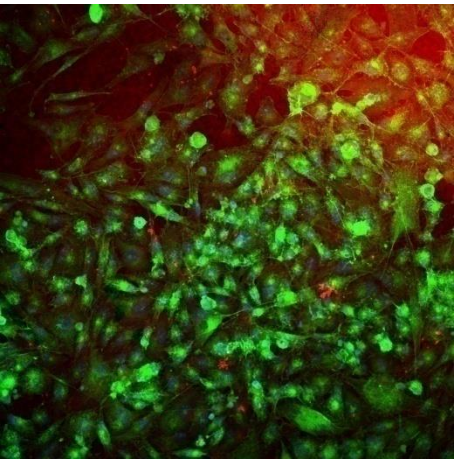


Cell area-black

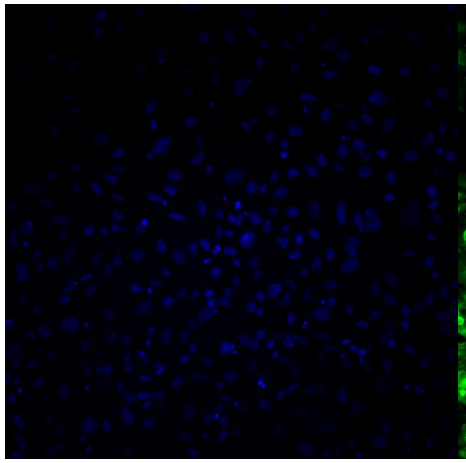
Degraded area-white



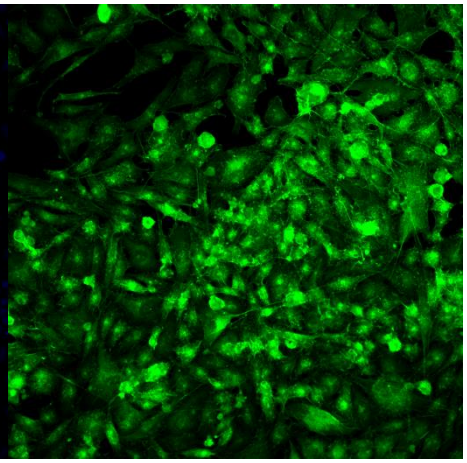
Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of HA-peptide #37 on invadopodia formation of PC3M-LN4 cells. Magnification x20.



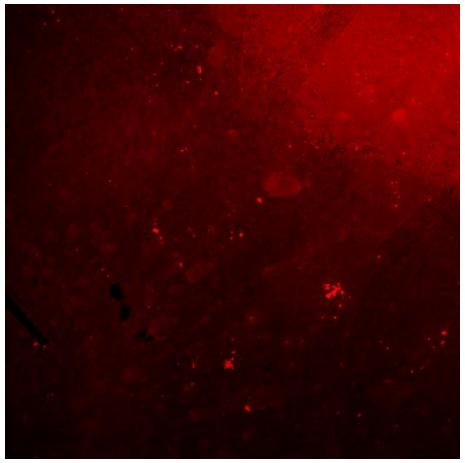
MERGE



DAPI

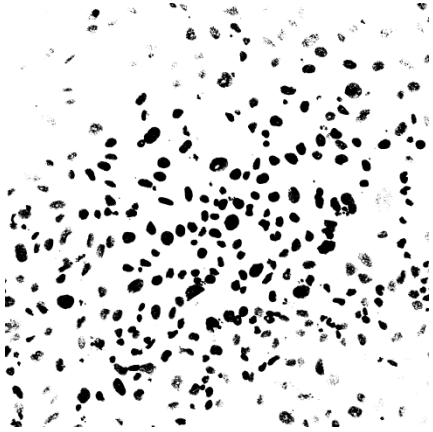


FITC

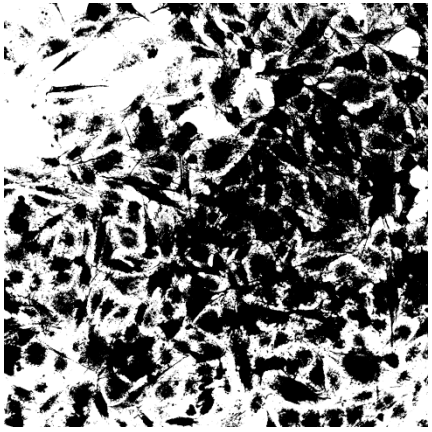


Cy3

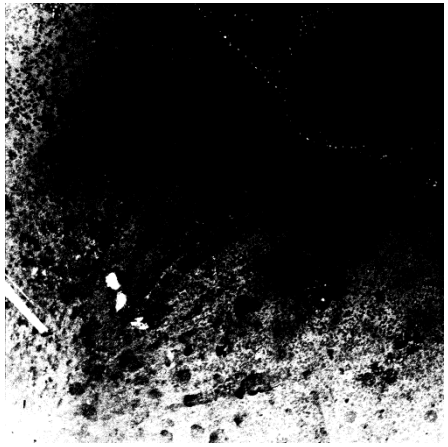
Numbers of cells



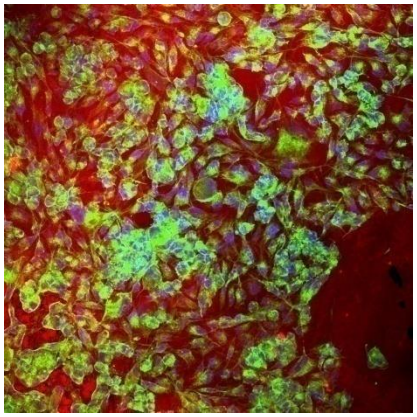
Cell area-black



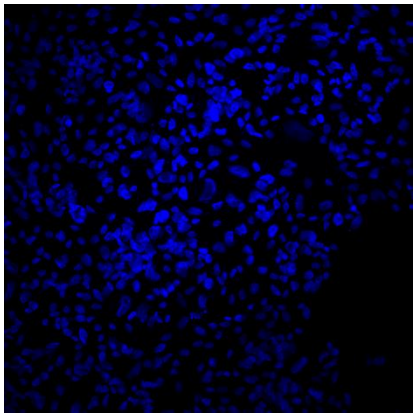
Degraded area-white



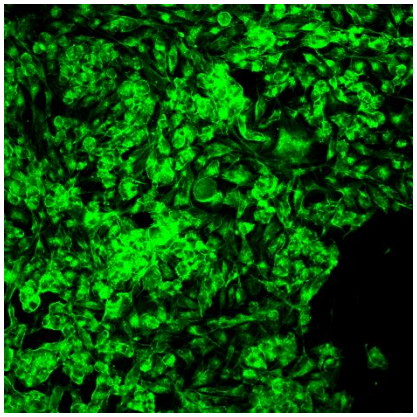
Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of peptide D on invadopodia formation of PC3M-LN4 cells. Magnification x20.



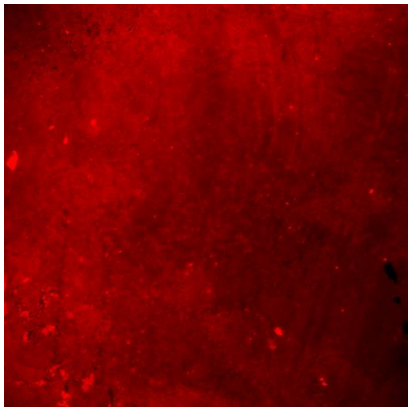
MERGE



DAPI

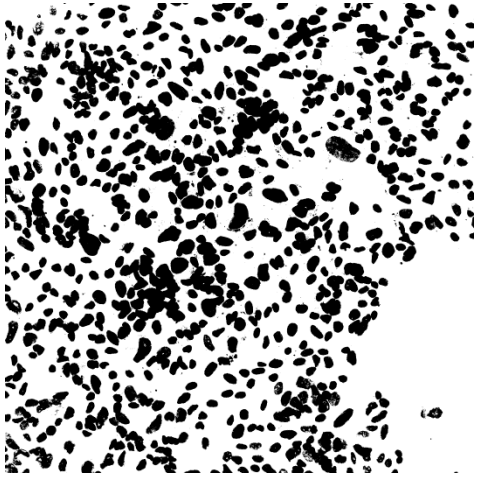


FITC

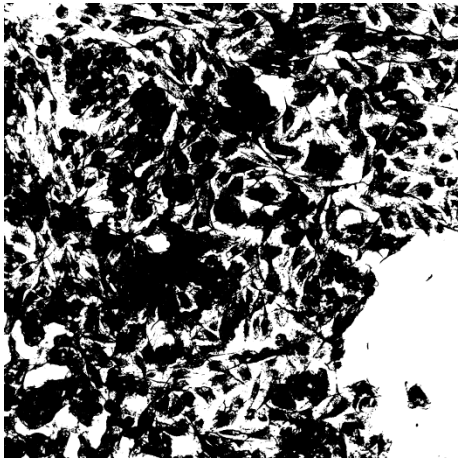


Cy3

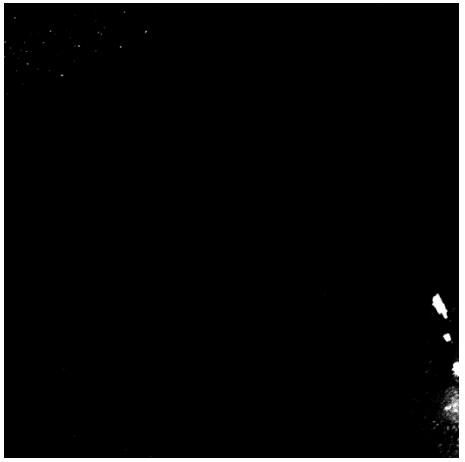
Numbers of cells



Cell area-black



Degraded area-white



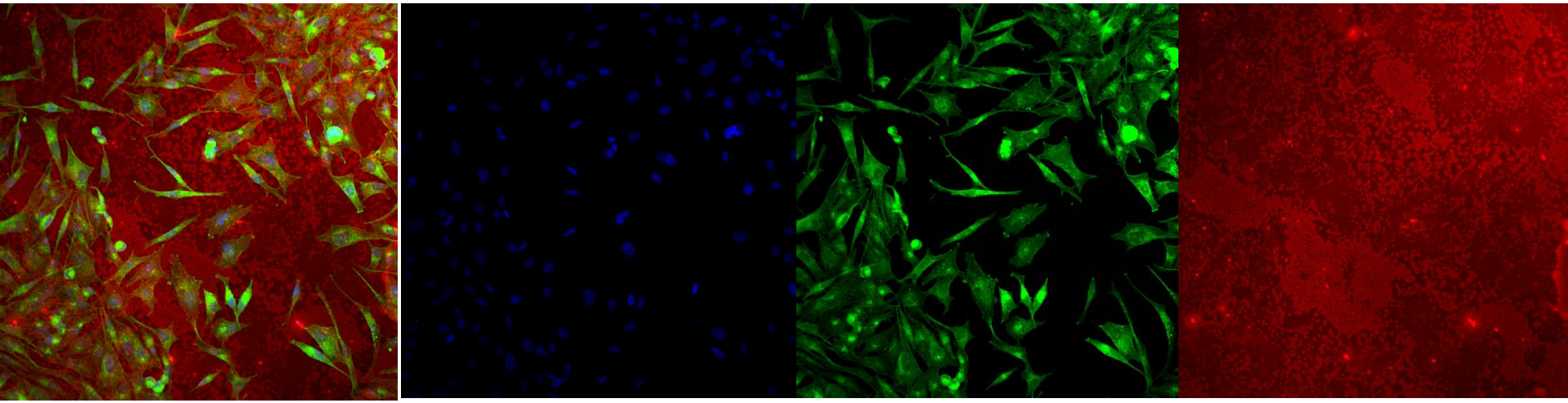
Modulation of gelatin degradation by HA-mimetic peptides. Assess activity of peptide D on invadopodia formation of PC3M-LN4 cells. Magnification x20.

MERGE

DAPI

FITC

Cy3

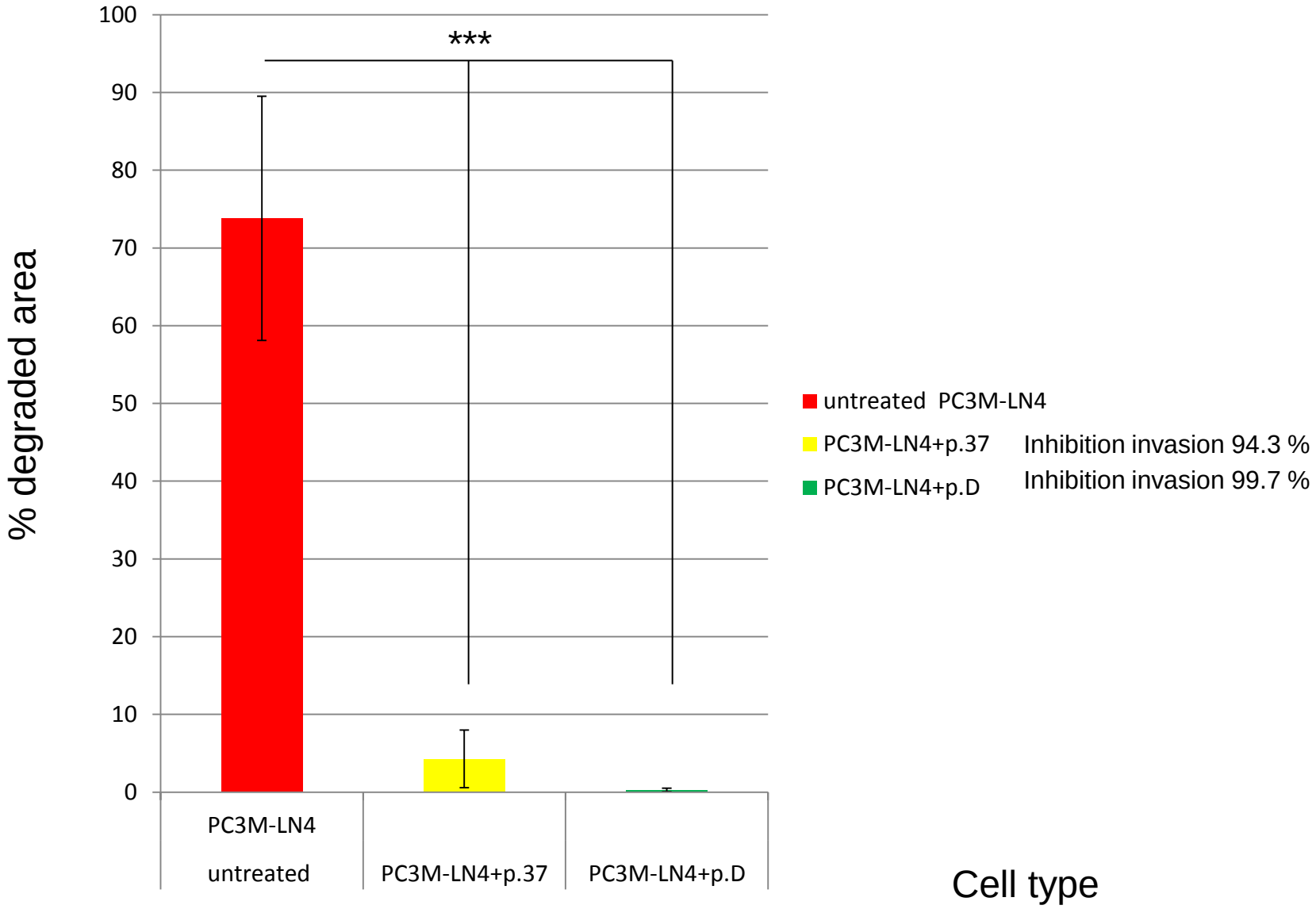


Numbers of cells

Cell area-black

Degraded area-white

Cy3-Gelatin:% Degradation area of total PC3M-LN4 cells area.



Summary:

1. HA-mimetic peptides inhibited the cell viability breast and prostate cancer cells.
2. Peptide#40 inhibited cell proliferation in breast and prostate cancer cells.

3. HA-mimetic peptides induced apoptotic and modulated necrotic death in breast and prostate cancer cells.

4. HA-mimetic peptides inhibited the invasion of breast and prostate cancer cells.

CONCLUSIONS

1. These results suggest that HA-mimetic peptides are potential anti-cancer agents for treatment cancer disease.
3. These findings suggest that HA-mimetic peptides can be used as peptide drugs, which are target-specific, cost-effective, and easily designed for cancer therapy.

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Russian found D.Zimina “Dynastia”

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TBCRU Pamela Greenaway-Kohlmeier Translational Breast Cancer Research Unit

Department of Defense
Congressionally Directed Medical Research Programs

Thanks for your ATTENTION!

Q&A