Inhibition of p38 translocation to the nucleus may serve as a therapeutic tool for treating cancer

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30.3.20

Signal transduction

- Signal transduction is the process by which a chemical or physical signal is transmitted from the cellular environment all the way to the target molecules inside the cell.
- Proteins responsible for detecting stimuli are generally termed receptors. The changes elicited by ligand binding (or signal sensing) in a receptor give rise to a biochemical cascade, which is a chain of biochemical events known as a signaling pathway.

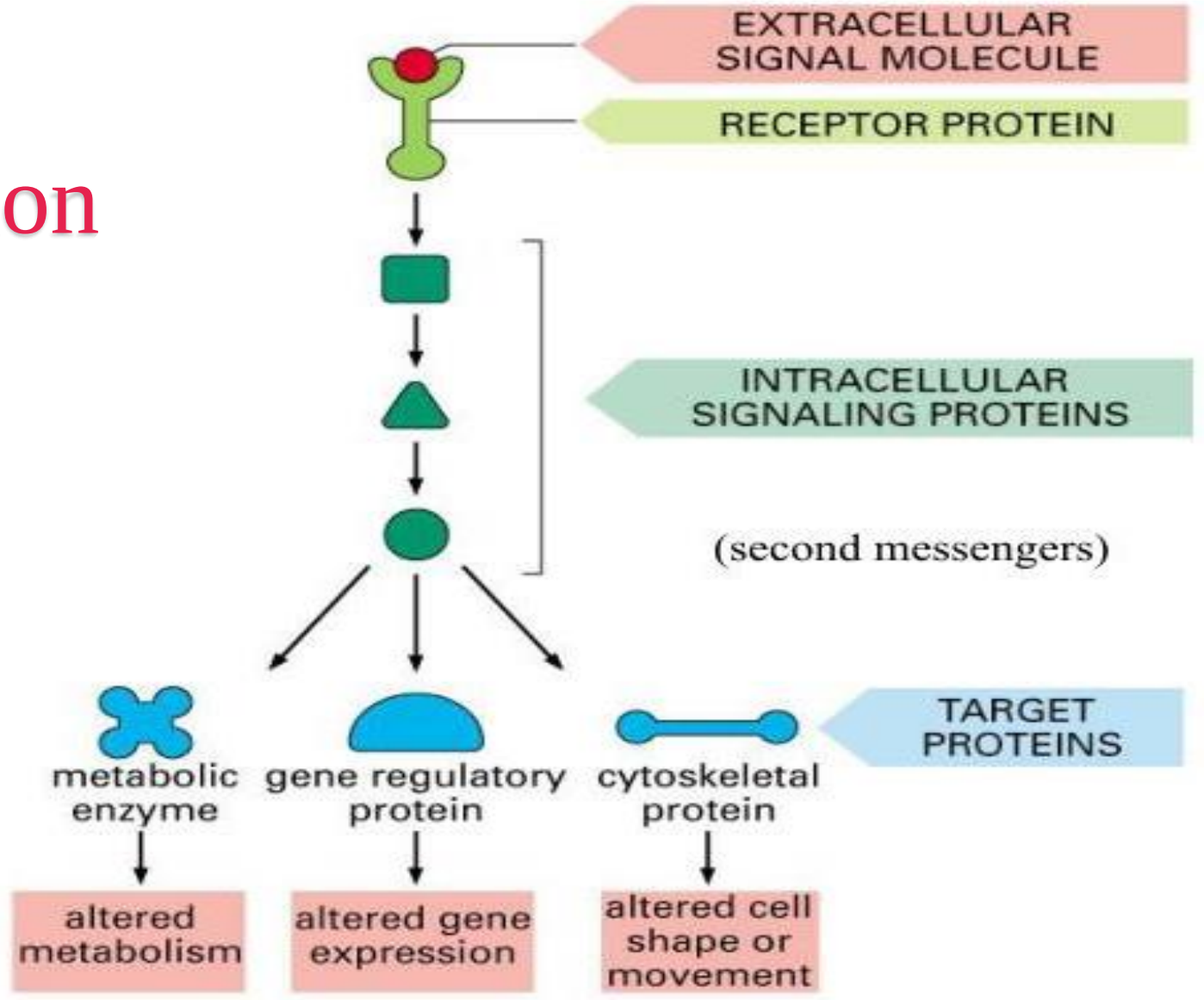
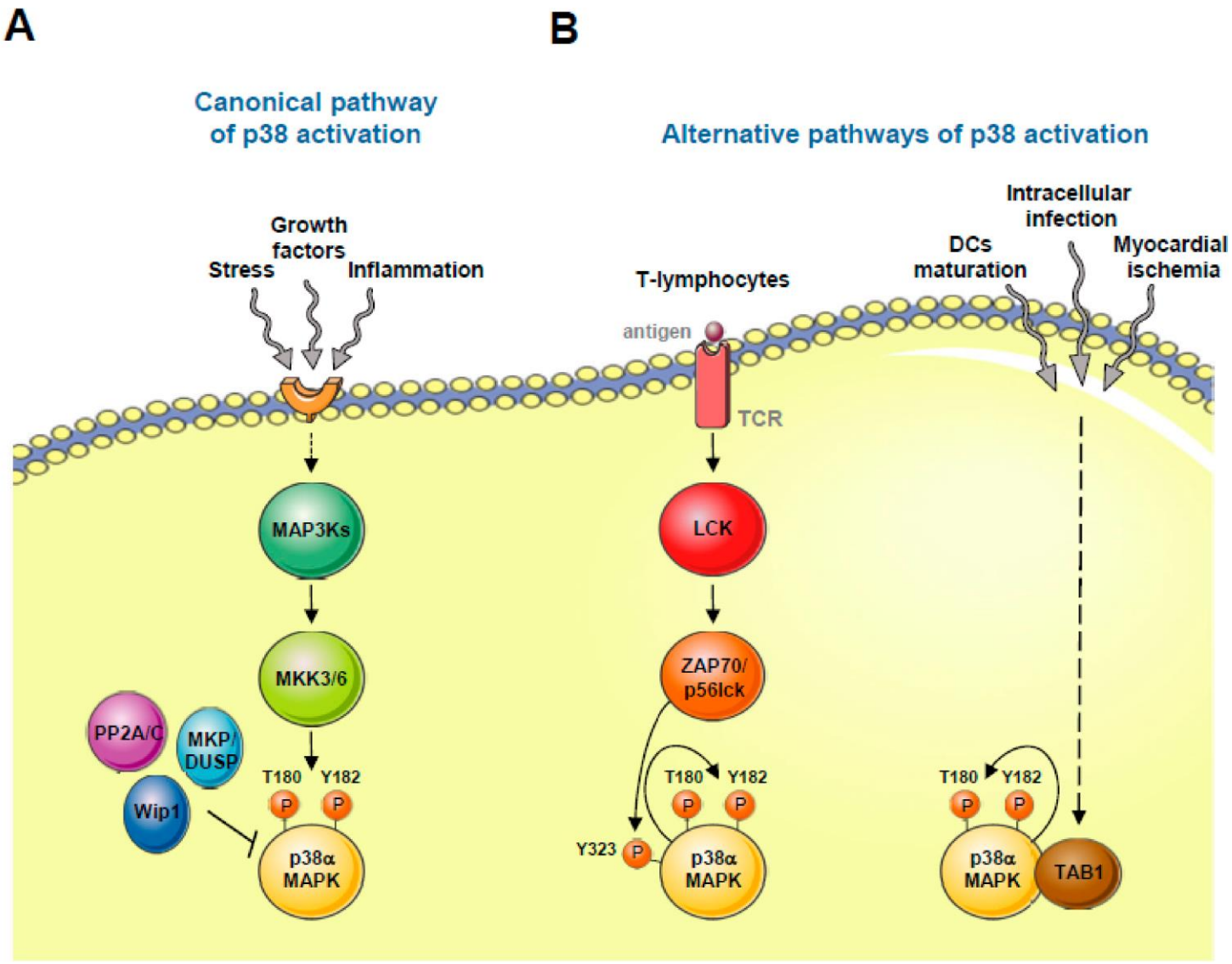


Figure 15–1. Molecular Biology of the Cell, 4th Edition.

MAPK-P38 pathway

P38 mitogen-activated protein kinases are a class of mitogen-activated protein kinases (MAPKs) that are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation, apoptosis and autophagy.



https://www.ncbi.nlm.nih.gov/pubmed/32168915

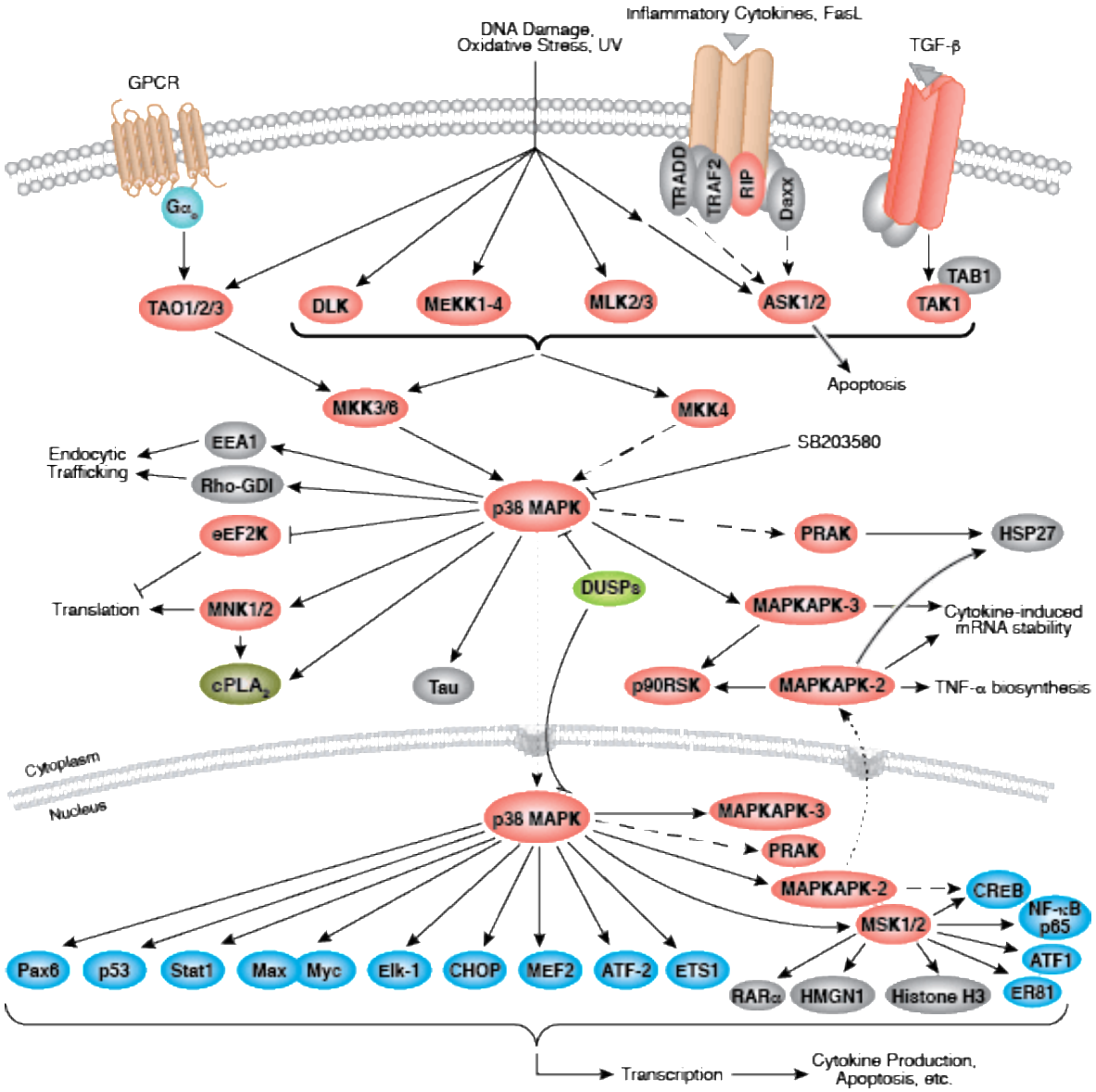
P38α

p38α is a ubiquitous protein kinase strongly activated by **stress signals** (such as inflammatory cytokines).

However, recent reports have also illustrated **pro tumorigenic** functions for p38α. Thus, p38α signaling may facilitate the survival and proliferation of tumor cells, contributing to the progression of some tumor types. In addition, p38α activation helps tumor cells to survive chemotherapeutic treatments.

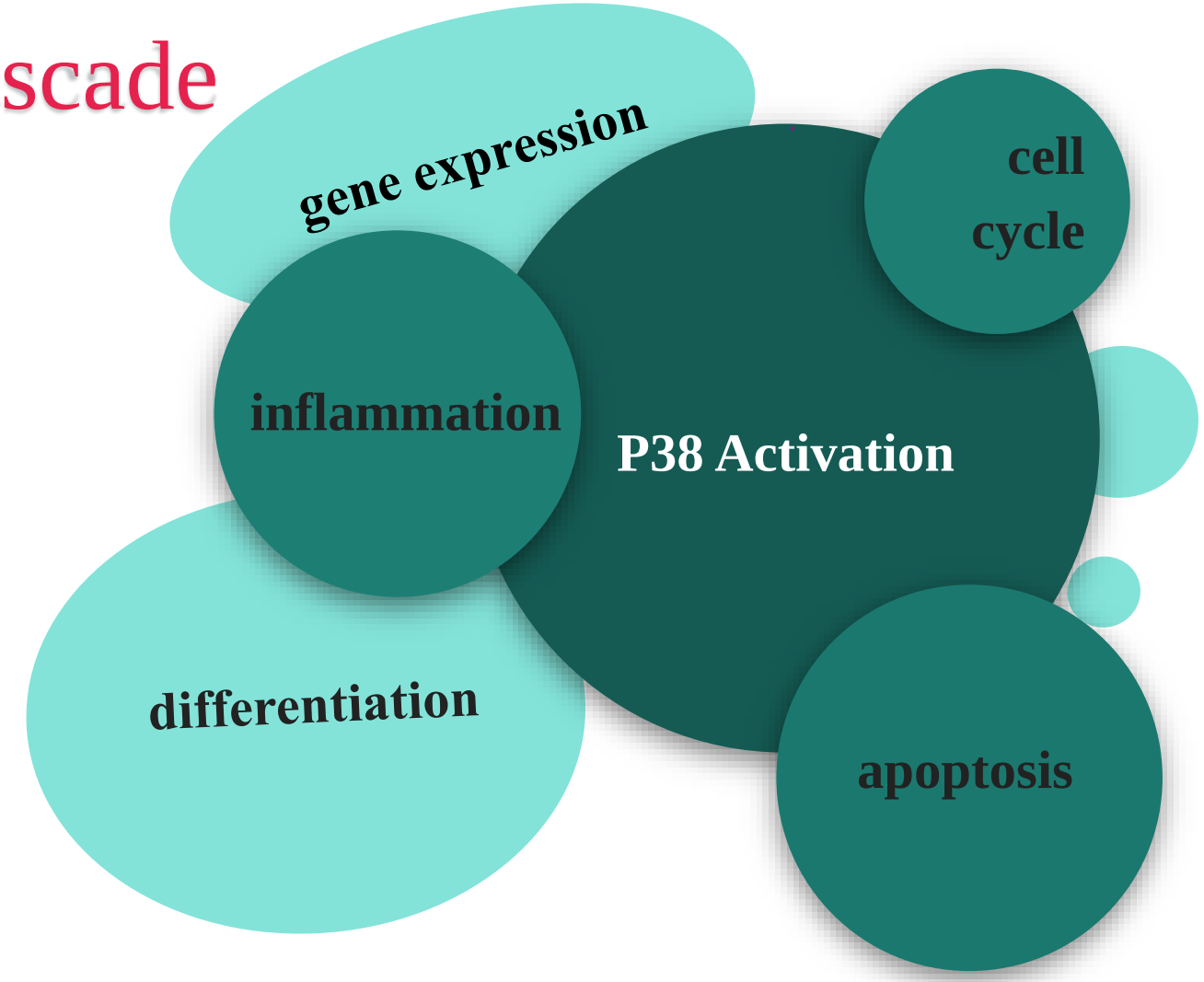
p38 cascade

- There are four isoforms of p38 : p38α, p38β, p38γ and p38δ.
- They have also splice variants (total of ten)
- Activation mechanisms
- Dual phosphorylation motif **Thr-Gly-Tyr** (TGY)
- Substrates and functions



https://www.cellsignal.co.uk/contents/science-cst-pathways-pi3k-akt-mapk-signaling/p38-mapk-signaling/pathways-mapk-p38

p38 cascade



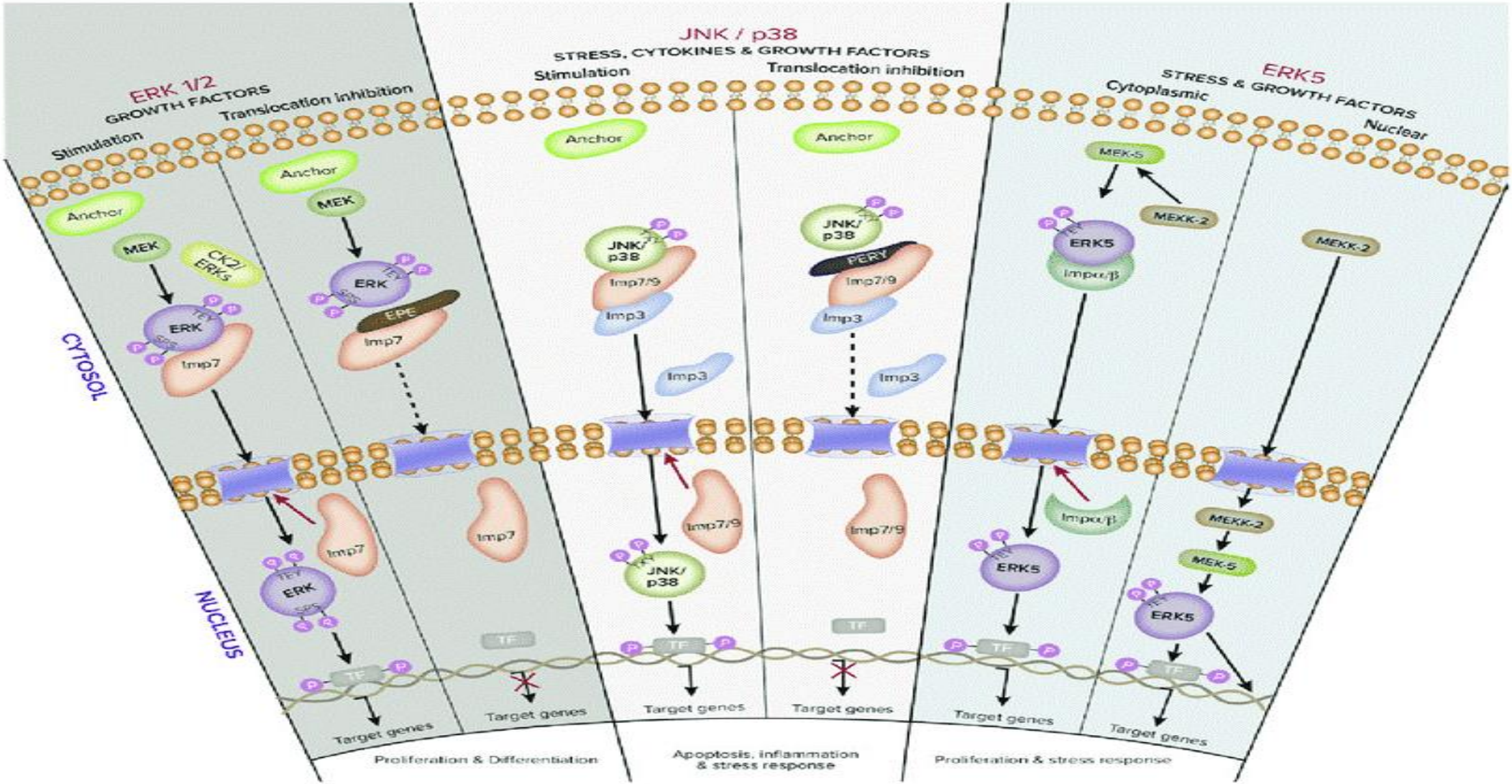
- **Activation of p38α is not only dependent on stimulus, its can be different by the condition of cell type .**

PERY peptide

PERY is a peptide that inhibits entry of P38 into the nucleus.

P38 usually binds to Imp9/Imp7 with Imp3 and translocate into the nucleus, PERY addition prevents P38 binding to Imp9/Imp7 .

The peptide prevented the development of inflammation-associated colon cancer in mice. These findings suggest that this peptide might be used to treat colitis in patients and prevent the development of colitis-induced colon cancer.



https://www.karger.com/Article/FullText/494085

PERY is a peptide, therefore, it is not stable, and because of this, there is a need to find small molecules which have activity and specificity like that of PERY in inhibiting p38 pathway.

Objectives

- Finding suitable small molecules, which mimic PERY’s effects on Imp9 interaction.
- Characterizing small molecules effects on p38a translocation upon stimulation.
- Examine small molecules effects on cells viability.
- Checking the efficacy and the specificity of the small molecules.

Experiments flow

- Small molecules were designed and prepared by Dr. Zlatko jebena from the Institute of Organic Chemistry and Biochemistry CASPrague 6, Czech Republic. These molecules were tested for :

1. Effect on p38a translocation- Immunofluorescence imaging.

2. Cell viability.

3. Signaling response to small molecules using – Immunoblotting with antibodies to other signaling components (western blot) without/with Anisomycin.

4. Examine the effect of the small molecules on the interaction of IMP9/3 with p38a- Co Immunoprecipitation (CoIP).

1.Immunofluorescence imaging

P38αβ HeLa

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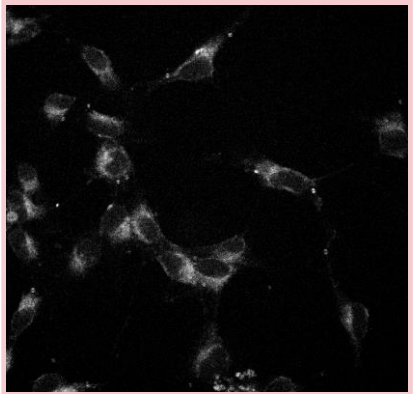
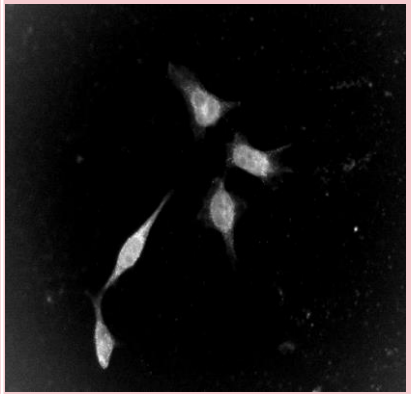
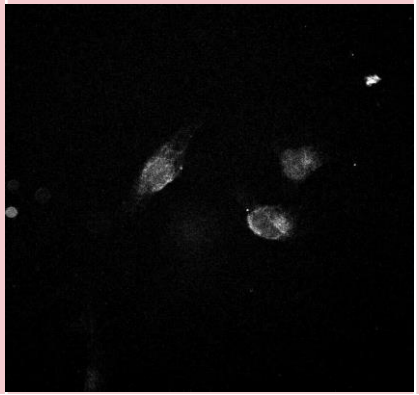
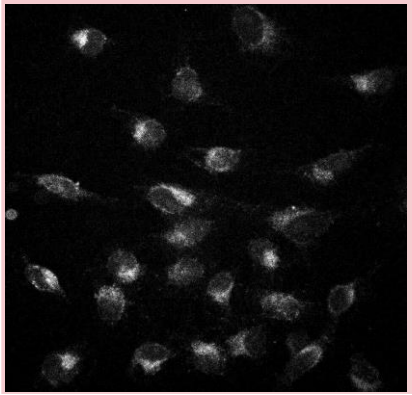
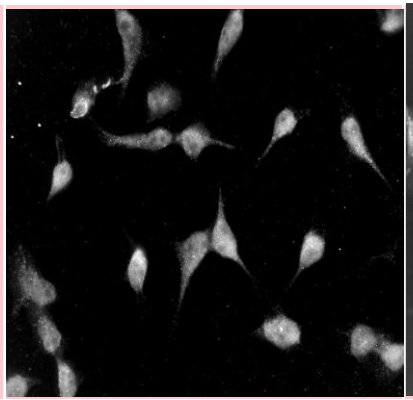
P38						
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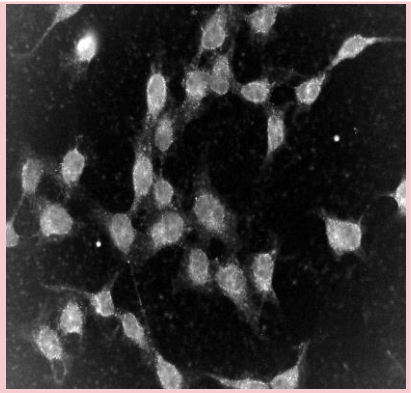
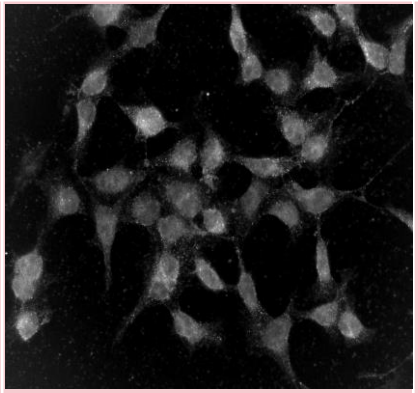
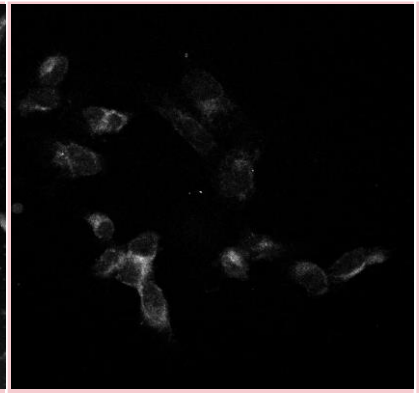
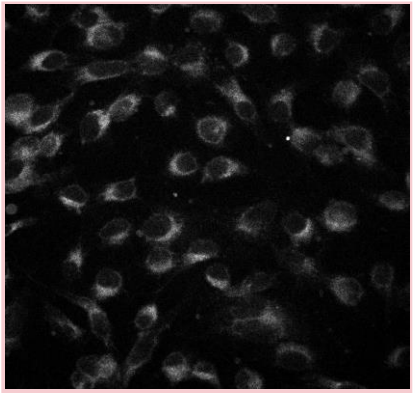
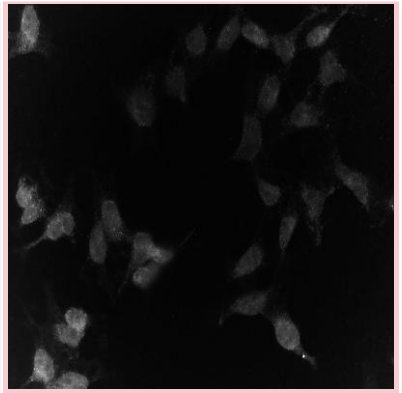
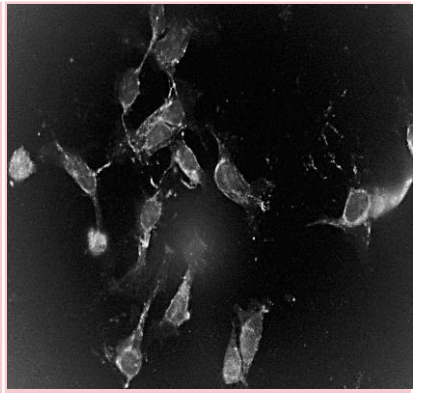
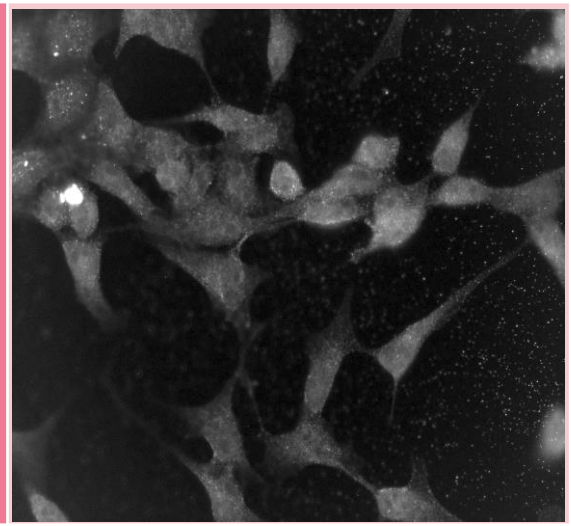
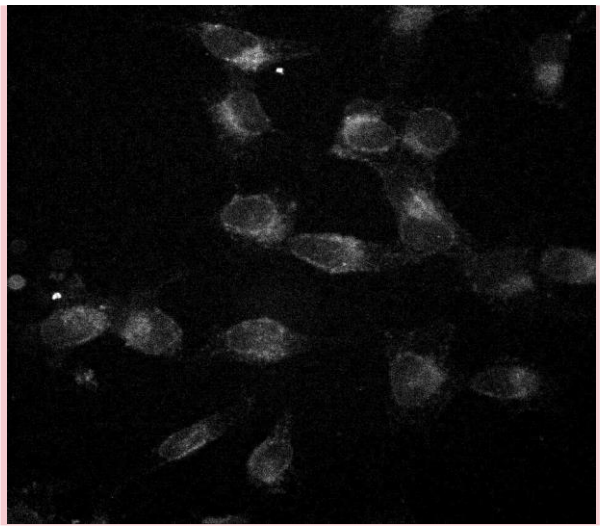
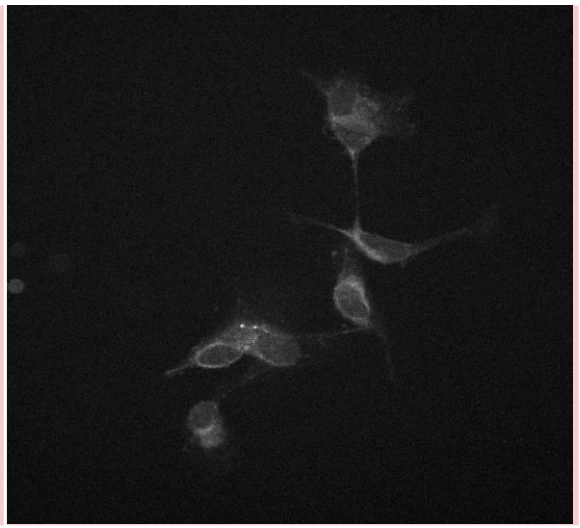
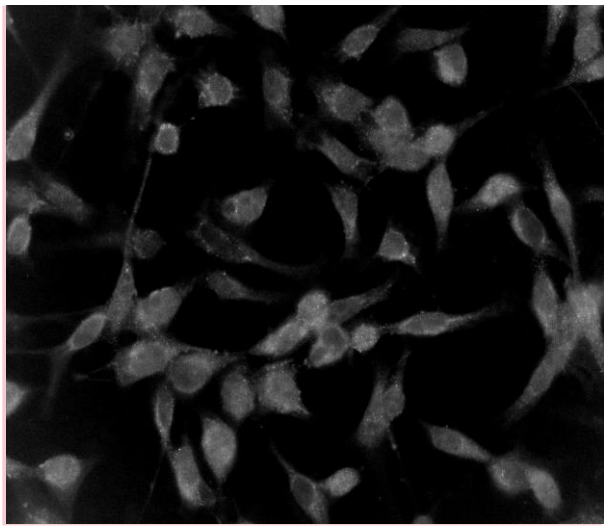
P38						

- Without stimulation /+ with stimulation

P38αβ HELA

	B6-	B7-	B8-	A1-
P38				
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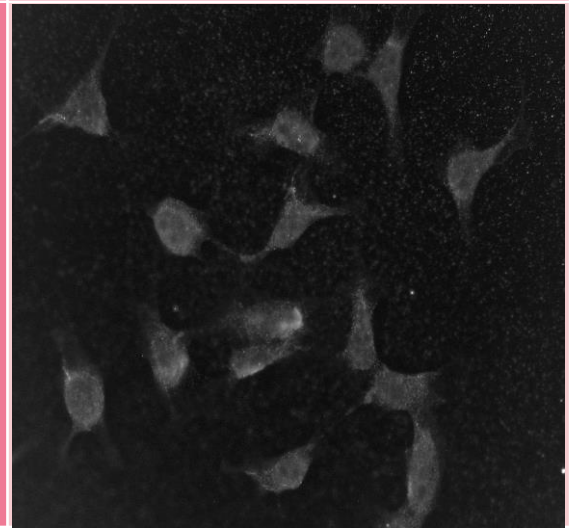
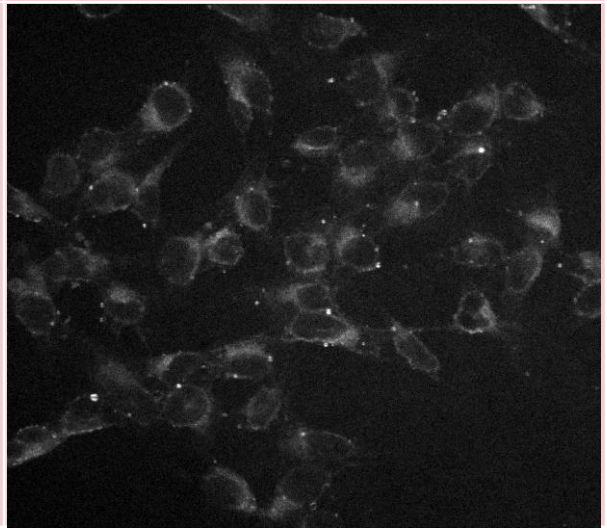
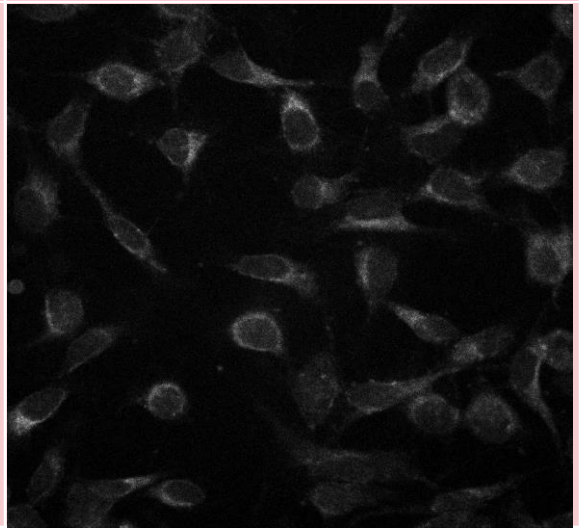
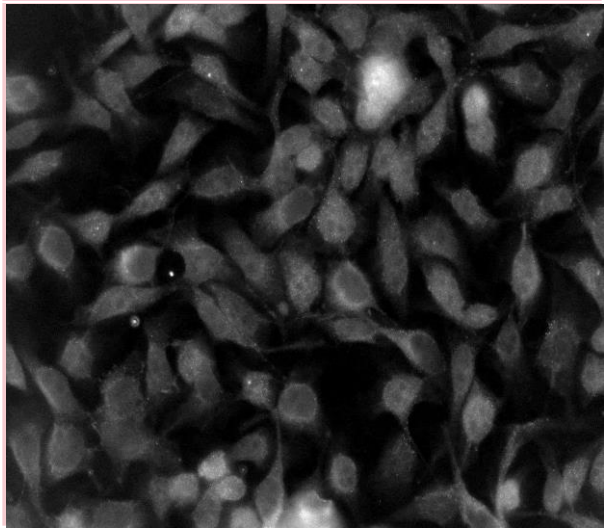
P38				

- Without stimulation /+ with stimulation

P38αβ MDA-MB231

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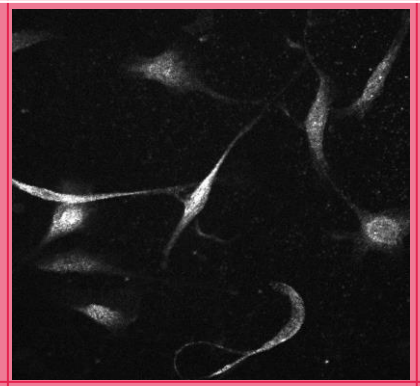
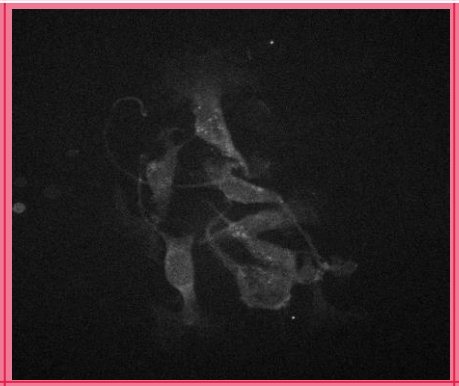
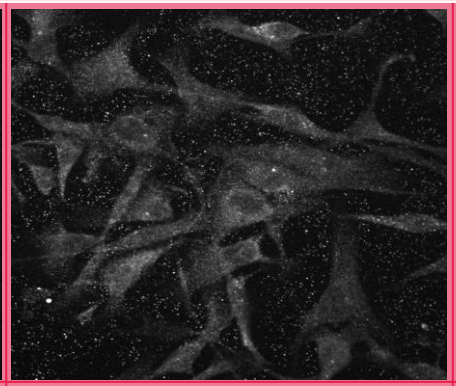
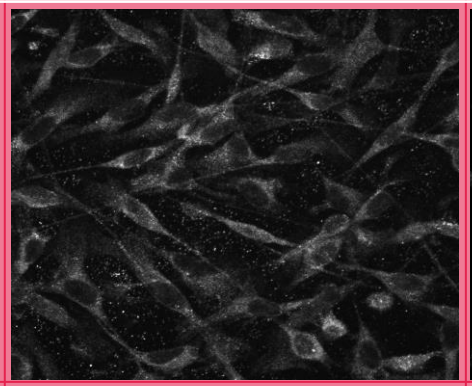
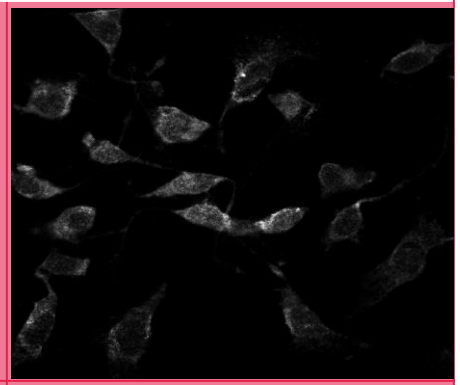
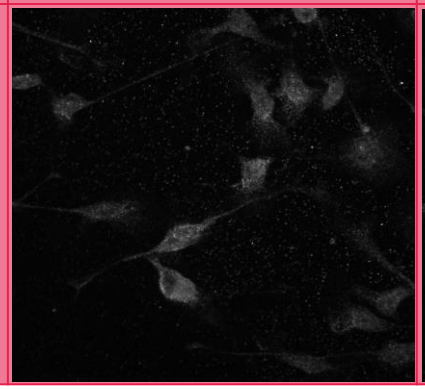
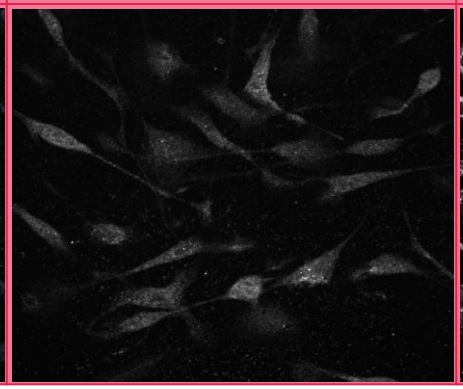
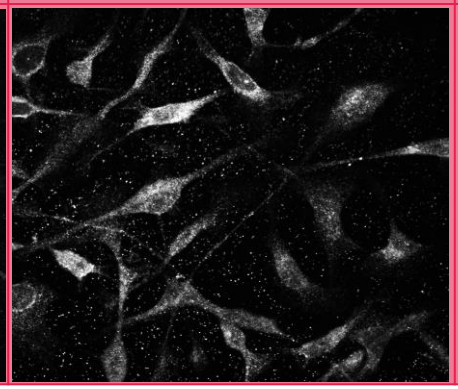
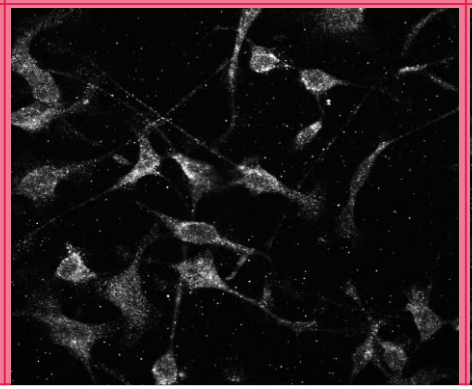
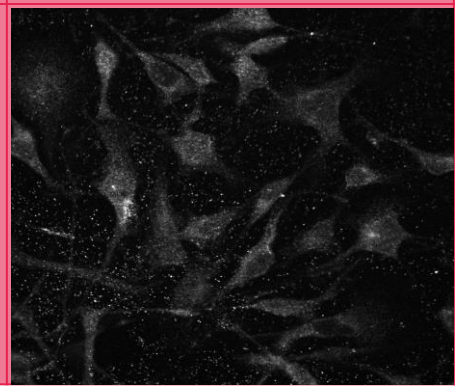
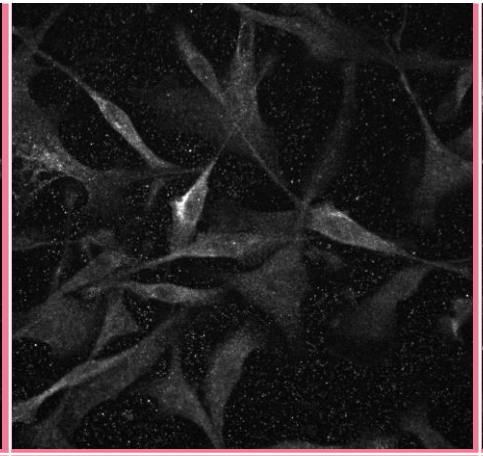
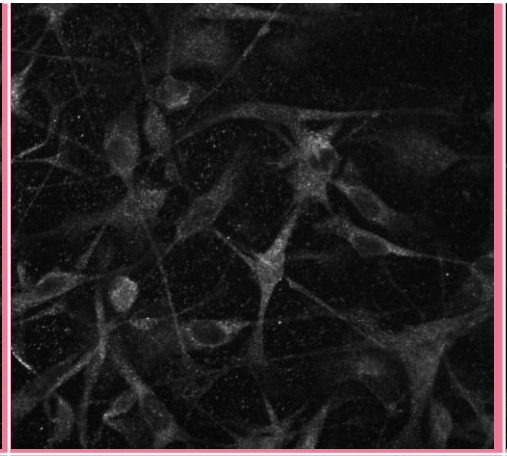
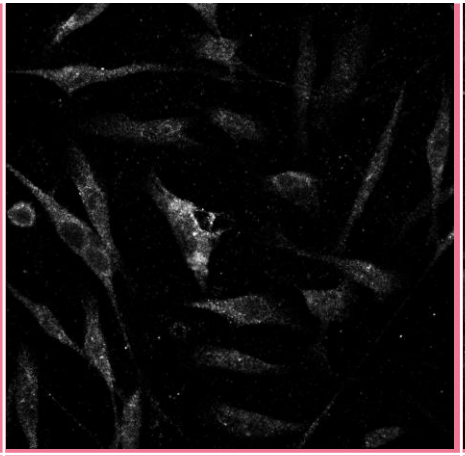
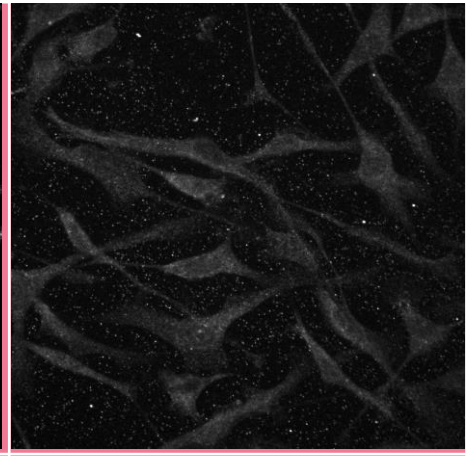
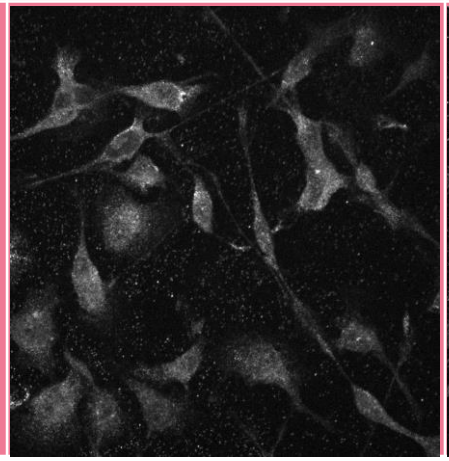
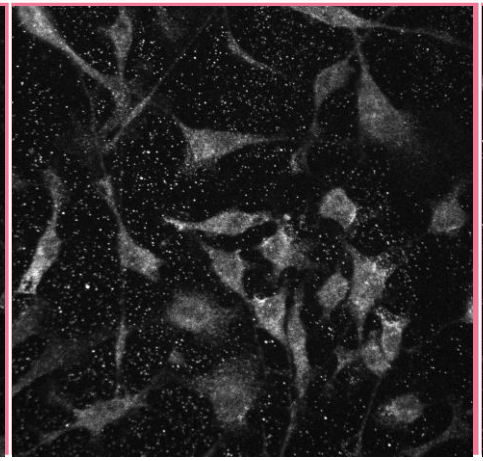
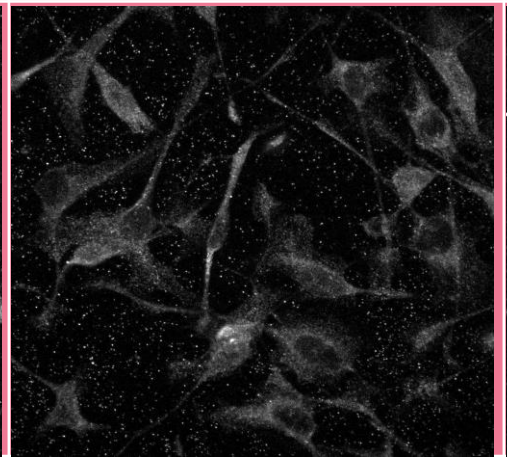
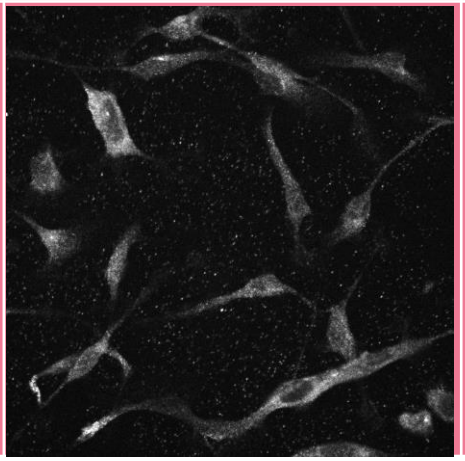
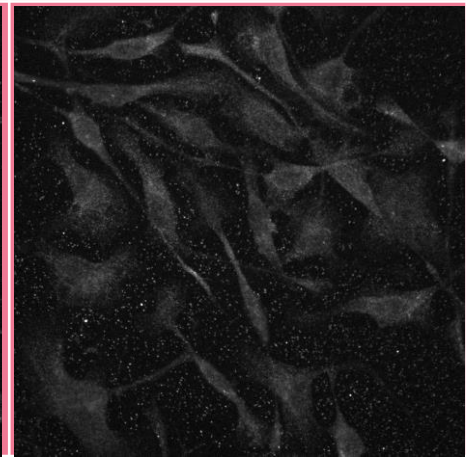
P38					
	Stimulated	B1+	B2+	B3+	B4+
P38					

- Without stimulation /+ with stimulation

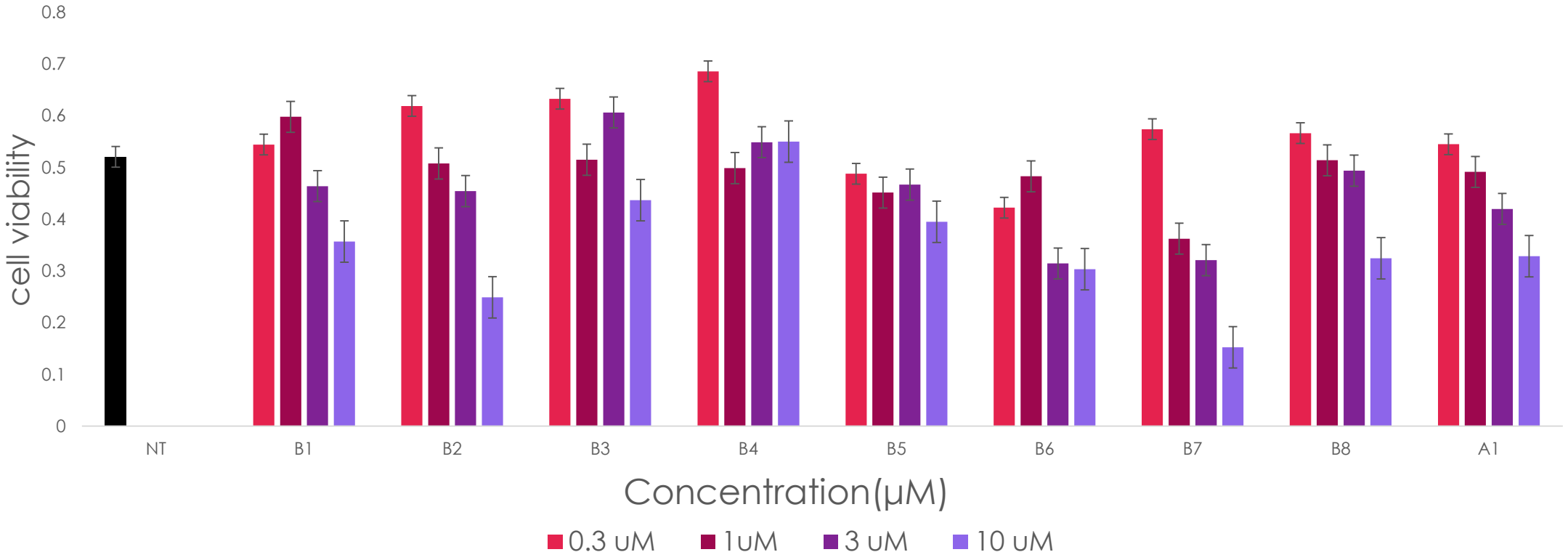
P38αβ MDA231

	B5-	B6-	B7-	B8-	A1-
P38					
	B5+	B6+	B7+	B8+	A1+
P38					

- Without stimulation /+ with stimulation

2.Cell viability

HeLa Cells

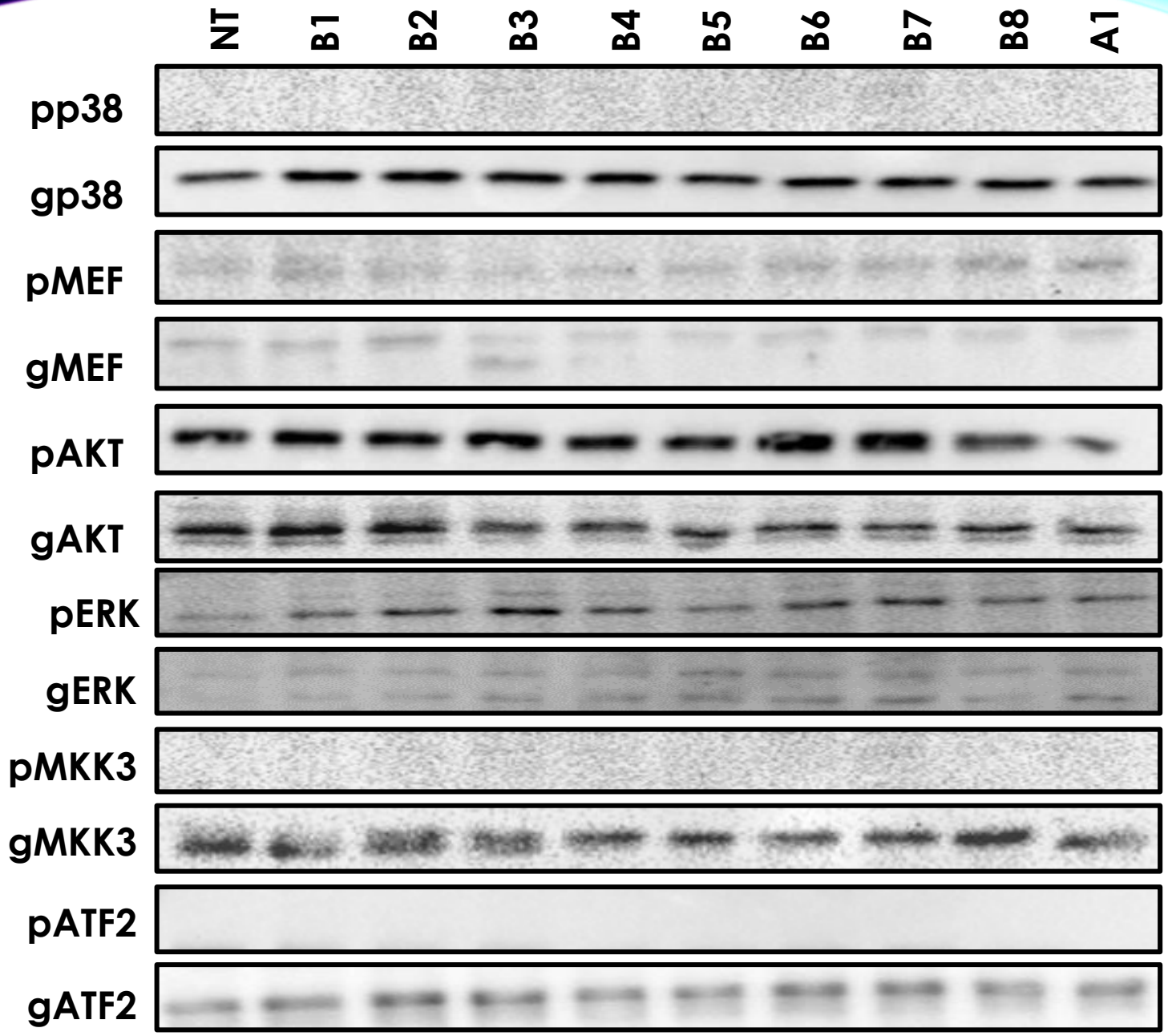


Conclusions

- The substances B6,B7 decrease cell proliferation in HeLa cells
- The small molecules being tested do not change the signaling (relative to the NT cells)

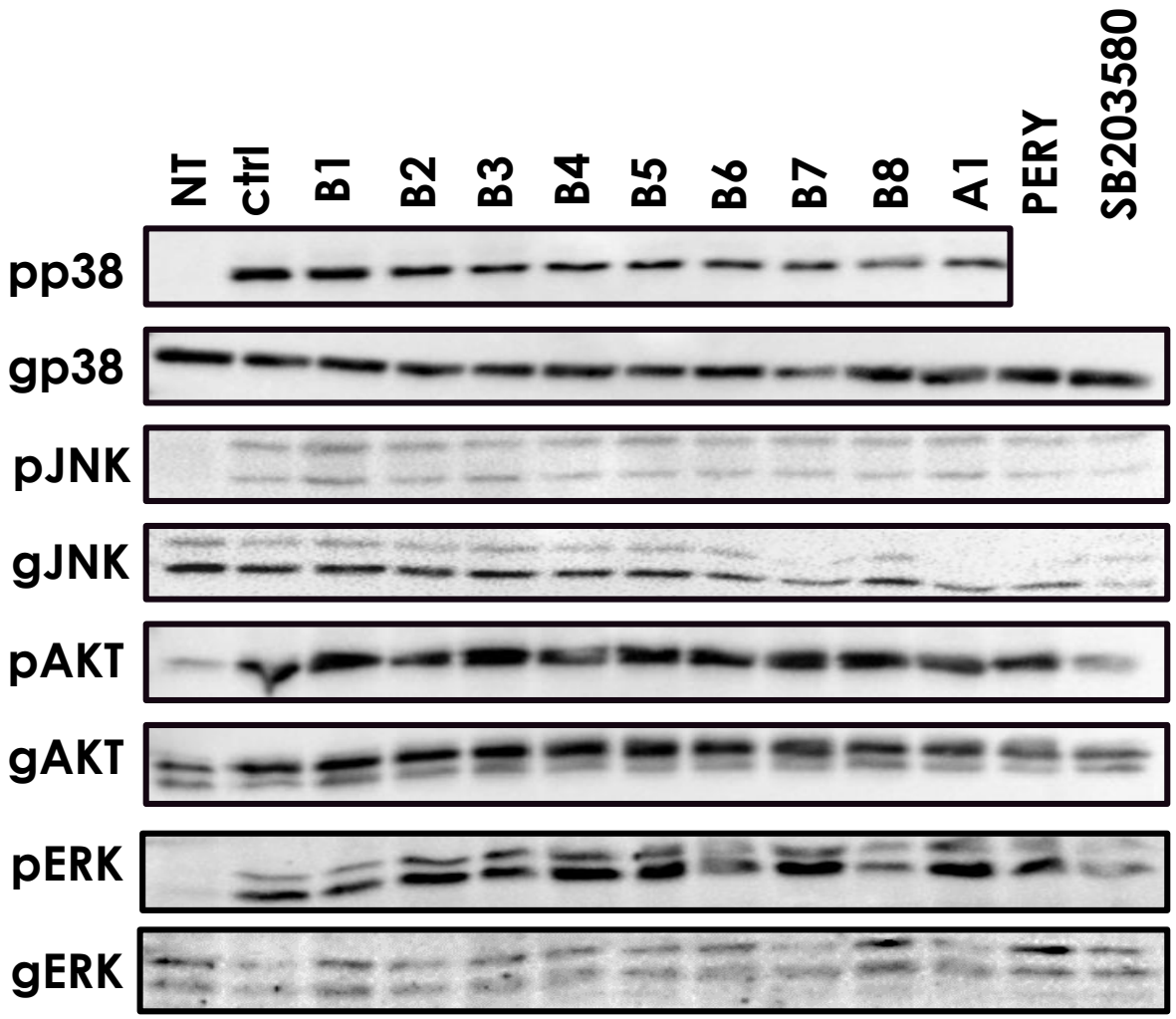
3.Immunoblotting (western blot) without Anisomycin

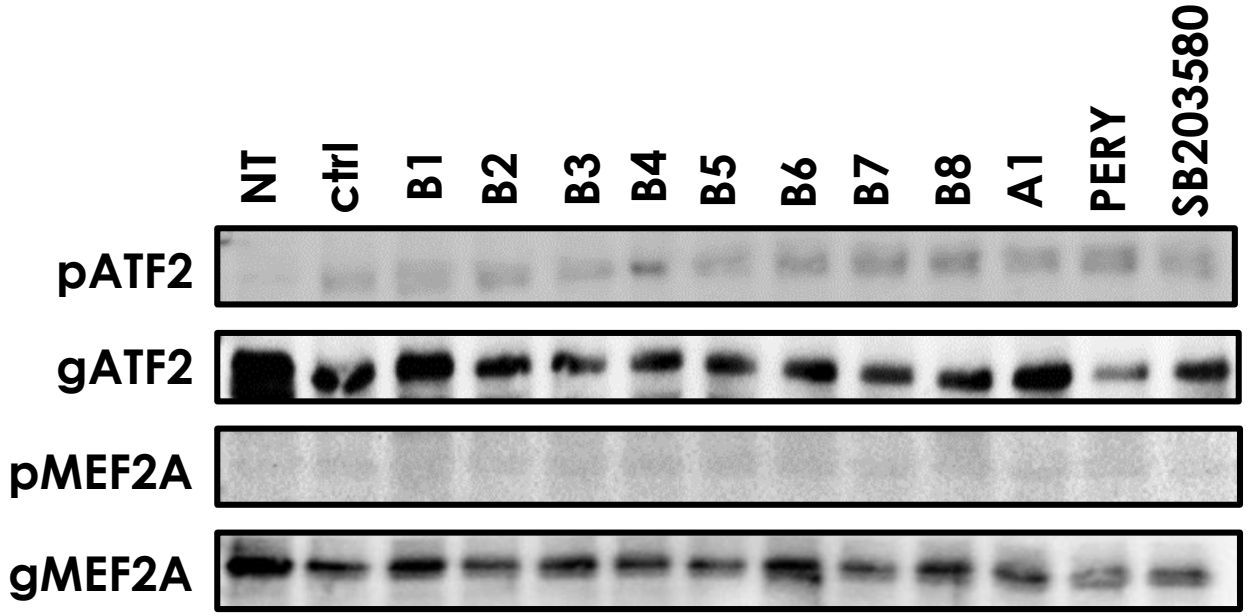
HeLa cells starved overnight (16-18 hrs), then incubated for 2 hrs. with small molecules in the final conc. of 10mM



3. Immunoblotting (western blot) with Anisomycin

HeLa cells starved overnight (16-18 hrs), incubated for 2 hrs with small molecules in the final conc. of 10mM then stimulated with Anisomycin for 30 min.

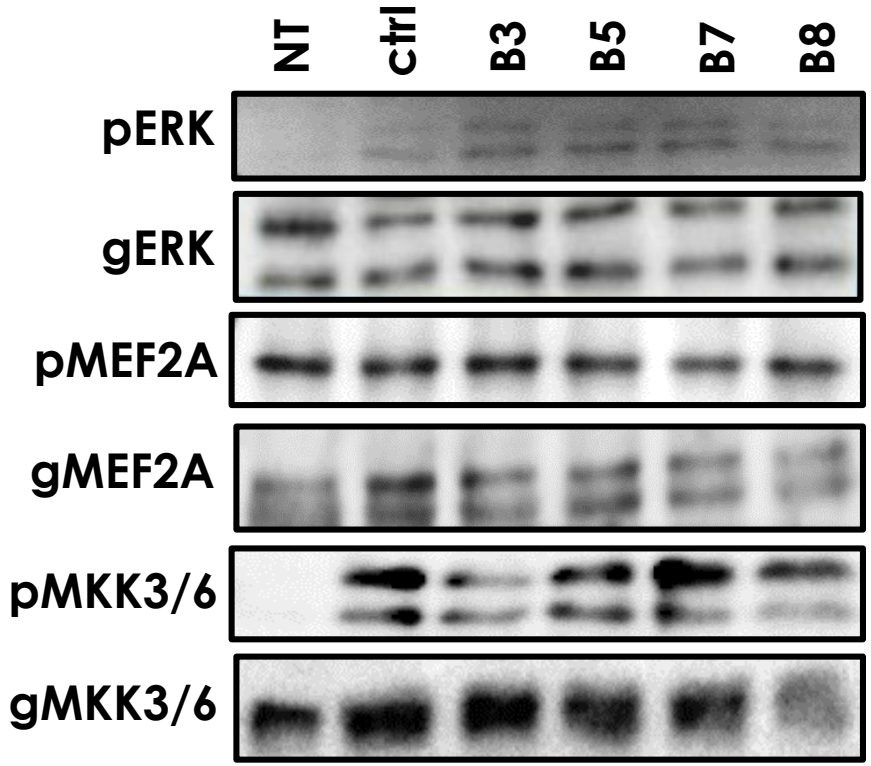



NT
ctrl
B1
B2
B3
B4
B5
B6
B7
B8
A1
PERY
SB203580
pATF2
gATF2
pMEF2A
gMEF2A

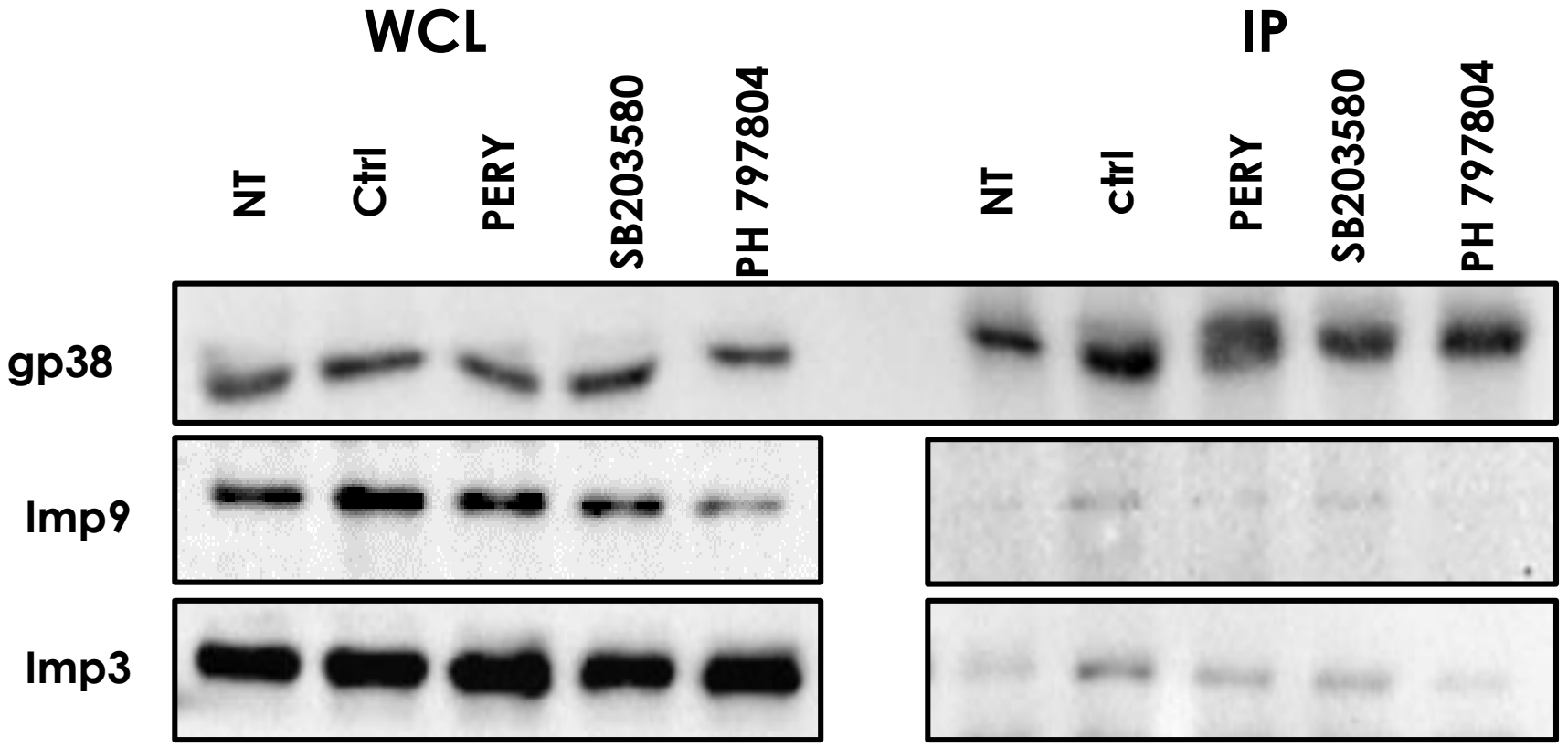
Conclusions

- The best substances that inhibited the entry of p38 into the nucleus are B3,B5,B7,B8. therefore, in the following experiments we have decided to continue with those inhibitors.

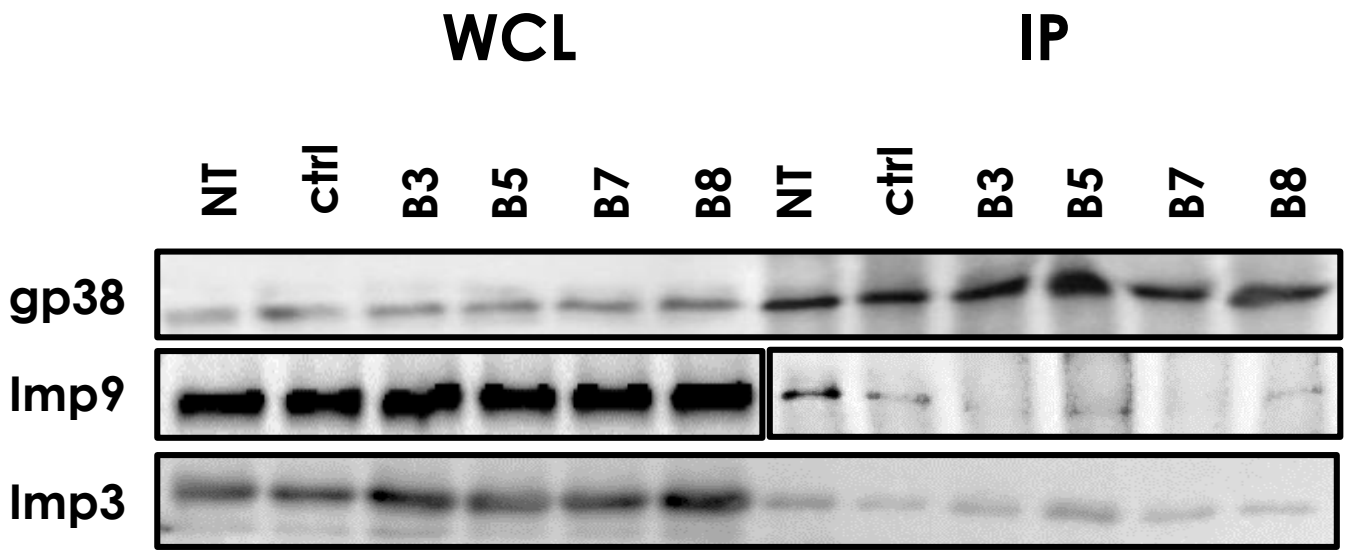
Immunoblotting (western blot) with Anisomycin



4. Co Immunoprecipitation (CoIP)



4. Co Immunoprecipitation (CoIP)



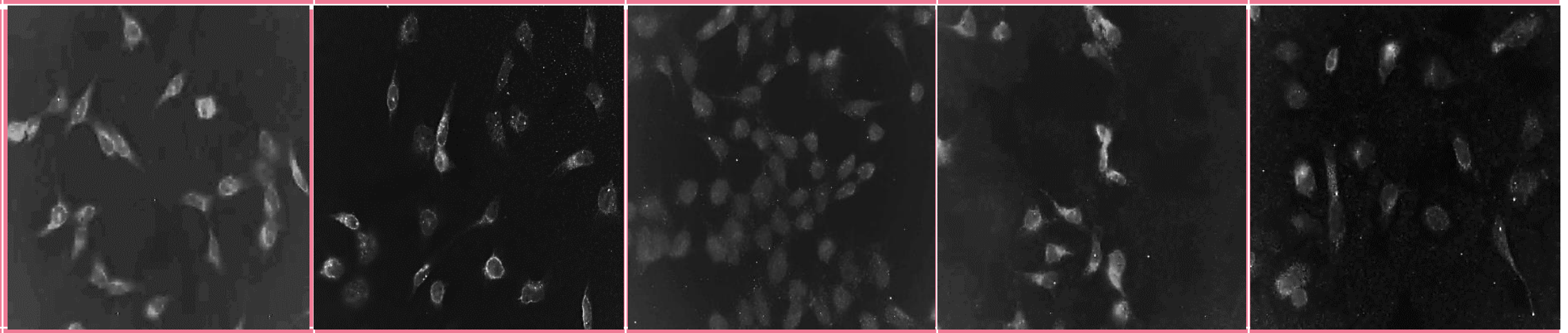
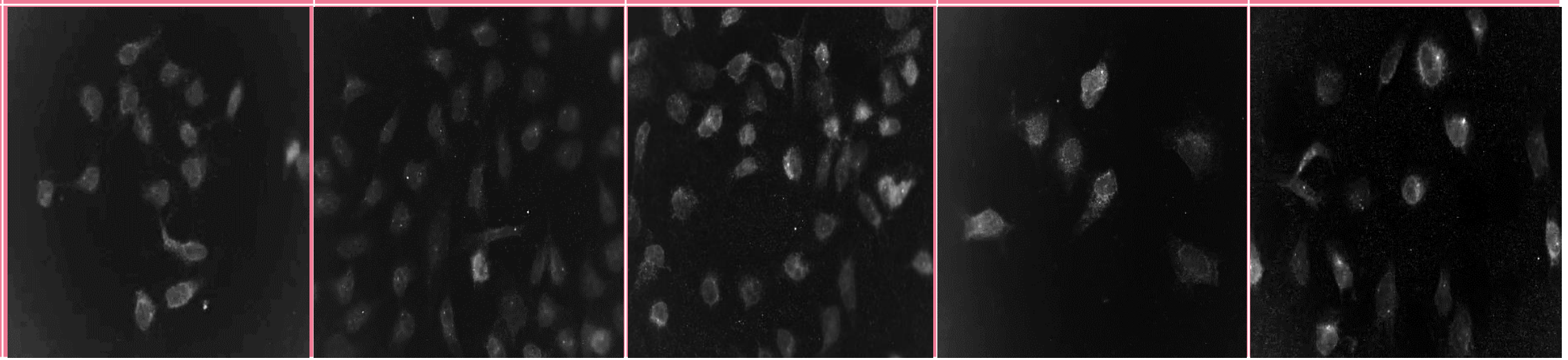
Conclusions

- The small molecules B3,5,7,8 reduced interactions with Imp9

6 other small molecules→ the same experiments

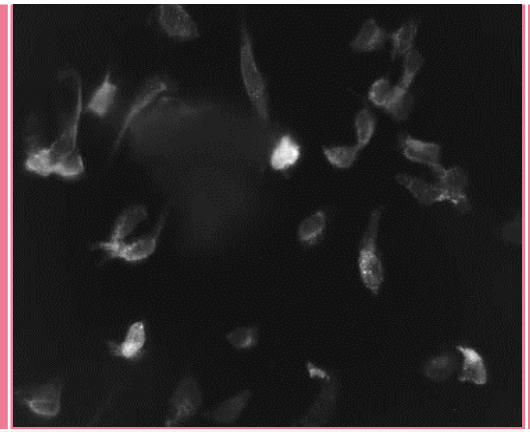
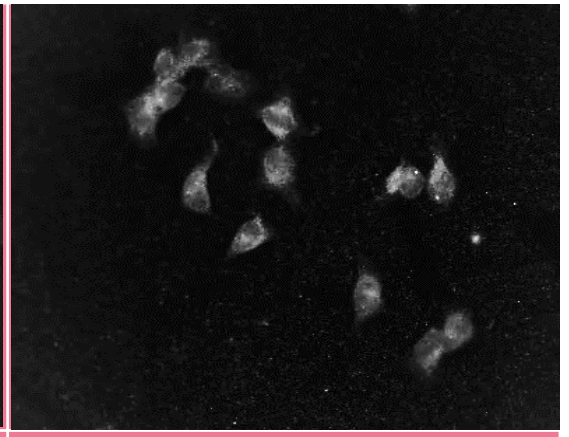
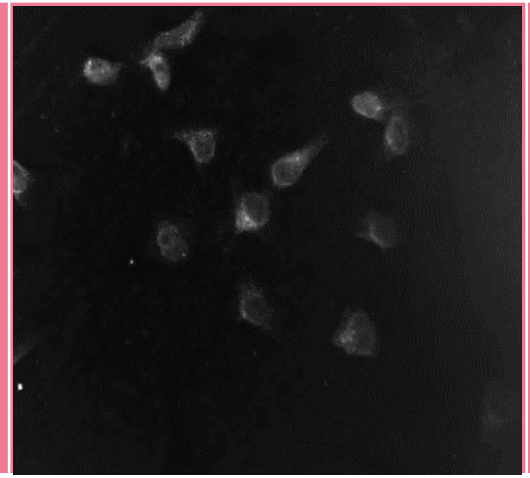
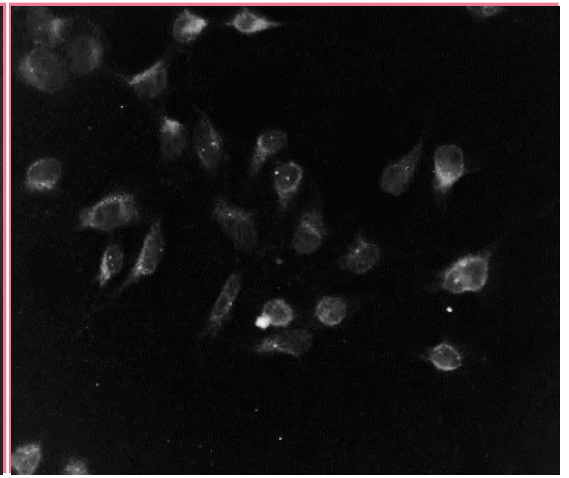
Immunofluorescence imaging

P38αβ HELA

	NT	C1-	C2-	C3-	C4-
P38					
	Stimulated	C1+	C2+	C3+	C4+
P38					

- Without stimulation /+ with stimulation

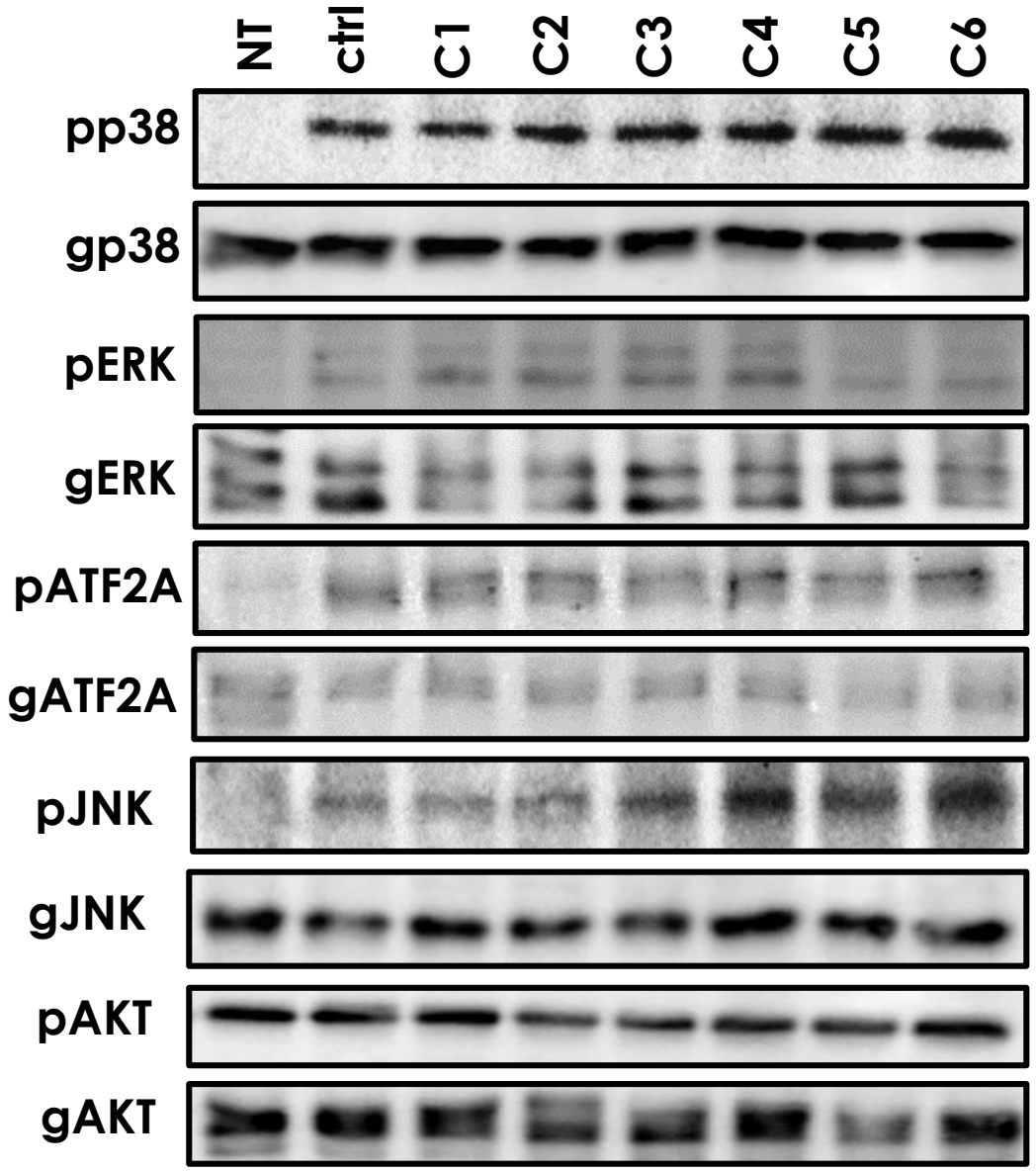
P38αβ HELA

	C5-	C6-
P38		
	C5+	C6+
P38		

- Without stimulation /+ with stimulation

Immunoblotting (western blot) with Anisomycin

HeLa cells starved overnight (16-18 hrs), incubated for 2 hrs with small molecules in the final conc. of 10mM then stimulated with Anisomycin for 30 min.



Conclusions

- The small molecules C1,2,4,5,6 showed reduced translocation of p38a/b to the nucleus.

Summarizing table

	Transloca-tion Inhibition	Effect on viability	Effect on ERK	Effect on signaling	Comments signaling	Comments
B1	0%	15%	NO	NO	P38/AKT/ JNK/ ATF/MEF2	Trans-Anis
B2	100%	25%	NO	NO		Trans-Anis/death
B3	100%	0%	NO	NO		-
B4	30%	0%	NO	NO		Trans-Anis
B5	100%	0%	NO	NO		Trans-Anis/morph
B6	0%	42%	NO	NO		-
B7	100%	40%	NO	NO		-
B8	100%	0%	NO	NO		-
A1	82%	28%	NO	NO		
C1	100%		NO	NO	P38/AKT/ JNK/ATF	
C2	50%		NO	NO		Morph
C3	30%		NO	NO		Death
C4	80%		NO	YES	JNK affected AKT not	
C5	100%		YES	YES		morph
C6	100%		YES	YES		

Comments signaling: The signaling molecules tested (+/- Anisomycin).
Trans- Anis: The compound itself caused p38 translocation
Death: the compound caused apoptosis.
Morph: Morphological changes

Future experiments

- To check the phosphorylation of other proteins upon the addition of the 6 small molecules C1-C6
- To do co immunoprecipitation of p38 after treatment with the molecules C1-C6
- To check the phosphorylation of proteins after the addition of the molecules C1-C6 and without the addition of Anisomycin
- To check localization of p38 in cells after stimulation and the addition of the small molecules (C1-C6) by checking the protein in different cell fractions
- To improve the specificity and efficacy of some of the small molecules.
- The JNK protein also binds to importin 7/9 (like P38), so the effect of the small molecules on its nuclear translocation should be investigated.
- To test the physiological/pathological effect of B3 and B8 in cell lines and animals.

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