COMENIUS UNIVERSITY

Faculty of Medicine

Department of Medical Chemistry, Biochemistry and Clinical Biochemistry

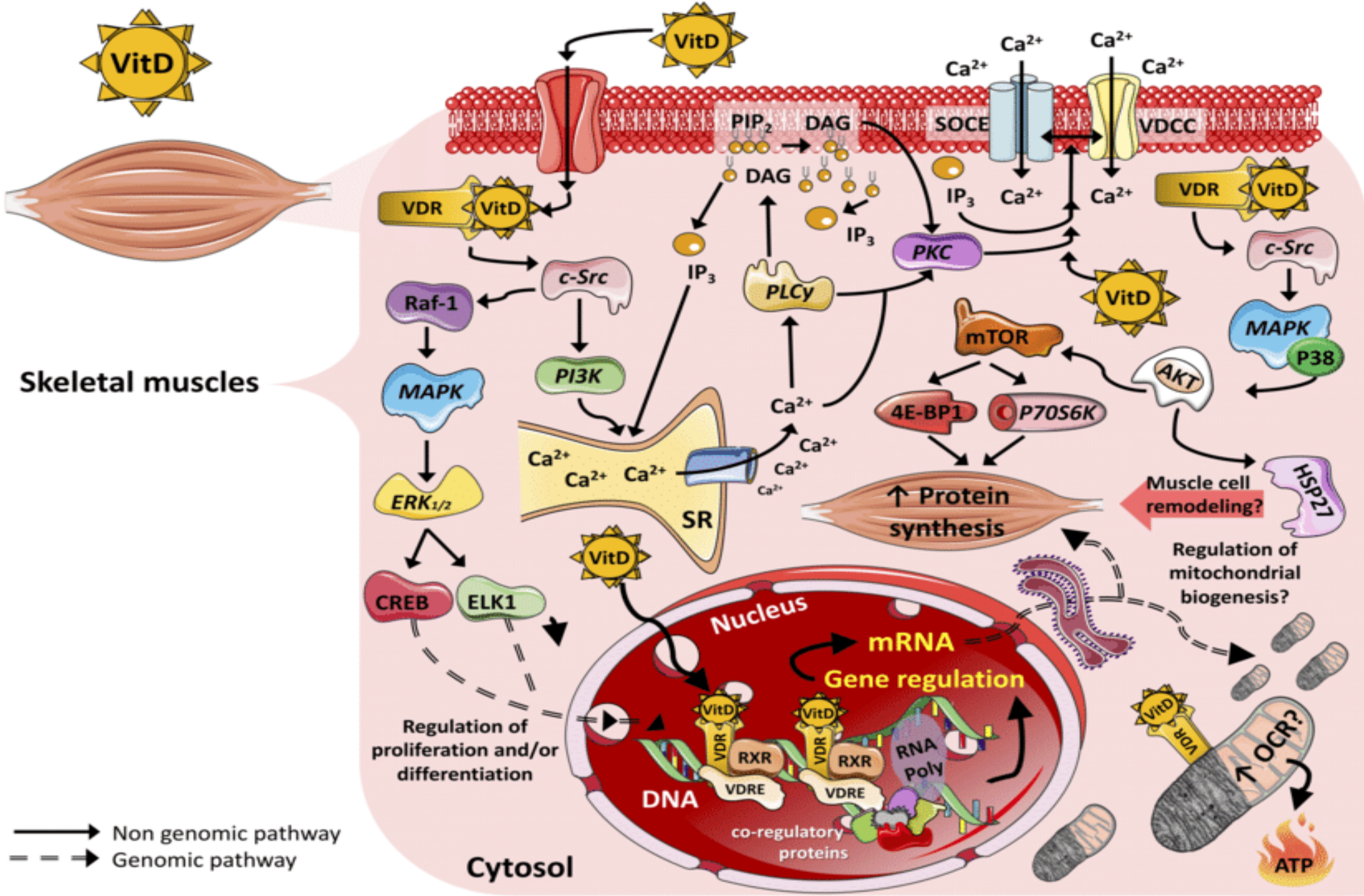
Effect of vitamin D on senescent cells

Ingrid Žitňanová, Mária Janubová, Petra Pazderová, Zuzana Sumbalová

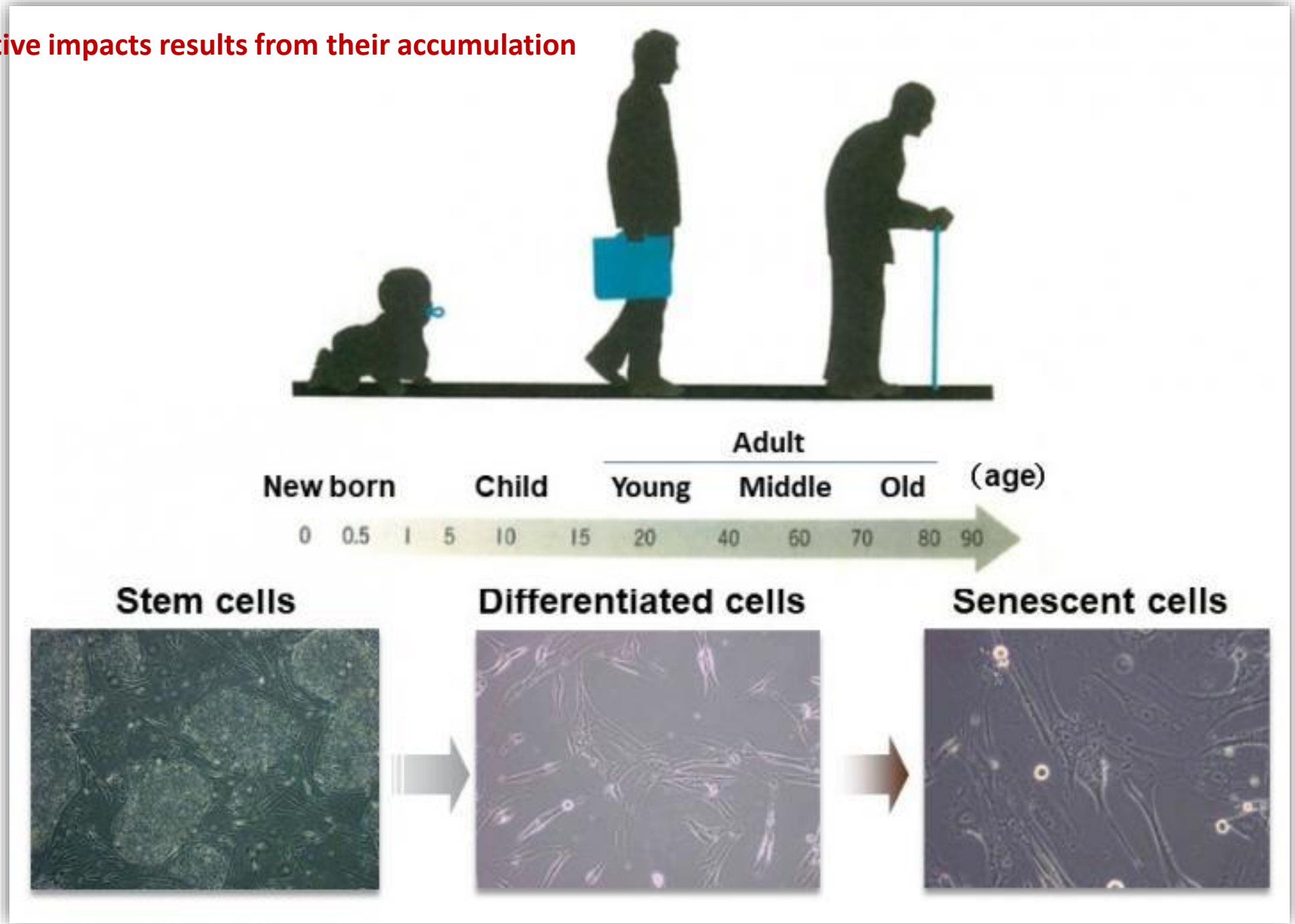
Nutriaging summer school

Ing. Katarína Koňariková, PhD.

2022


VitD
Skeletal muscles
VitD
VDR
VitD
c-Src
Raf-1
MAPK
ERK1/2
CREB
ELK1
PI3K
PIP2
DAG
DAG
IP3
IP3
PLCy
PKC
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
Ca2+
SR
SOCE
VDCC
IP3
Ca2+
Ca2+
VDR
VitD
c-Src
MAPK
P38
VitD
mTOR
AKT
4E-BP1
P70S6K
↑ Protein synthesis
Muscle cell remodeling?
HSP27
Regulation of mitochondrial biogenesis?
VitD
Nucleus
mRNA
Gene regulation
VitD
VitD
VDR
RXR
VDRE
VDR
RXR
VDRE
RNA Poly
DNA
co-regulatory proteins
Regulation of proliferation and/or differentiation
VitD
VDR
↑ OCR?
ATP
Cytosol
Non genomic pathway
Genomic pathway

- **The negative impacts results from their accumulation**



Replicative model of senescence

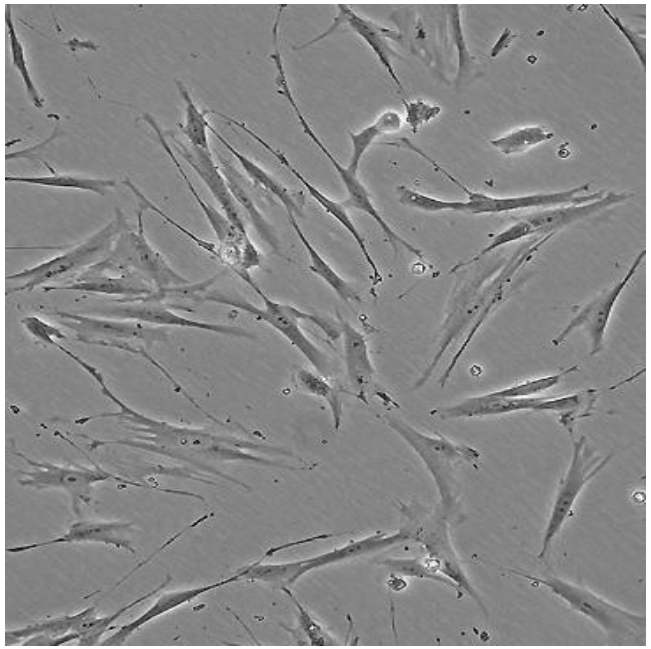
We passaged cells up to the passage 21, when the senescence marker levels were the highest.

Stress induced senescence

Cells were treated with 100 µM hydrogen peroxide for 30 min.

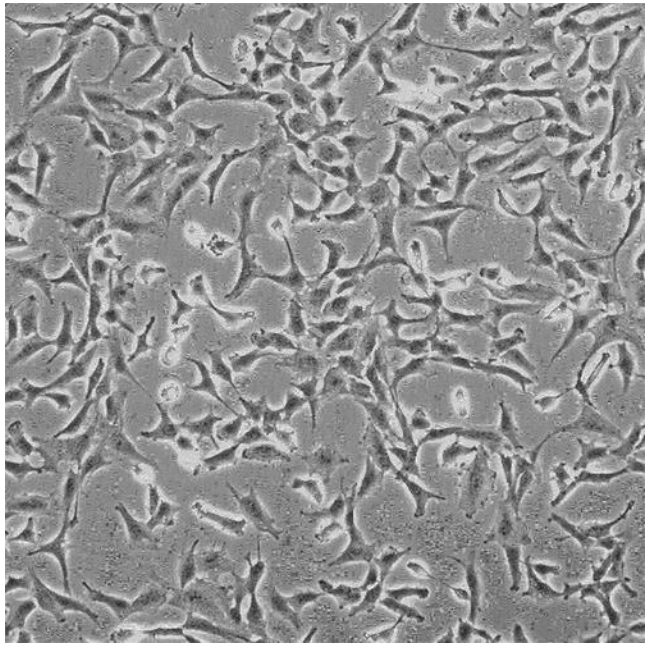
Materials and methods

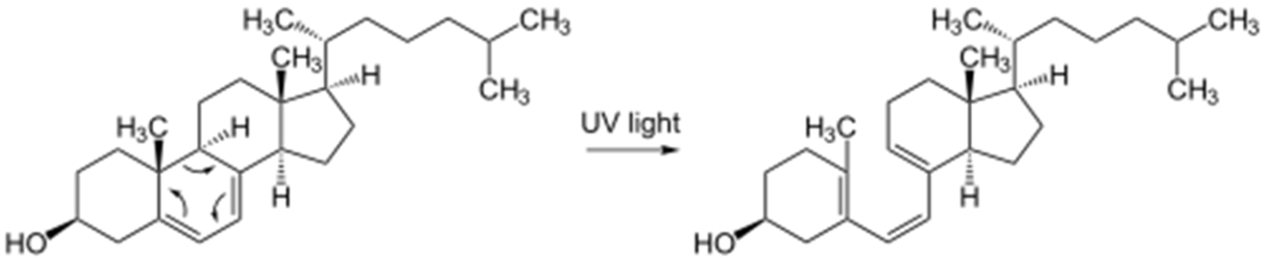
Human lung cells (**MRC-5**)



- in passage 8-10 (p8-10) as normal cells
- in passage 21 (p21) as senescent cells
- senescence induced by 100 μM H_2O_2

Human astrocytes (**NHA5**)





Vitamin D

- 1, 10, 20, 25, 50, 100 nM
- in DMSO
- in EtOH

Methods	Analysis
MTT colorimetric technique	Viability of the cells
Muse® Cell Cycle Assay Kit	Distribution of cells in the individual phases of the cell cycle
Western blot analysis	Molecular mechanism
Quantitative Real-time PCR = qRT-PCR	Gene expression at the level of transcription
Mitochondrial respiration	Measurement of respiration

Methods	Analysis
MTT colorimetric technique	Viability of the cells
Muse® Cell Cycle Assay Kit	Distribution of cells in the individual phases of the cell cycle
Comet assay	Damage of DNA
Western blot analysis	Molecular mechanism
Quantitative Real-time PCR = qRT-PCR	Gene expression level at the level of transcription
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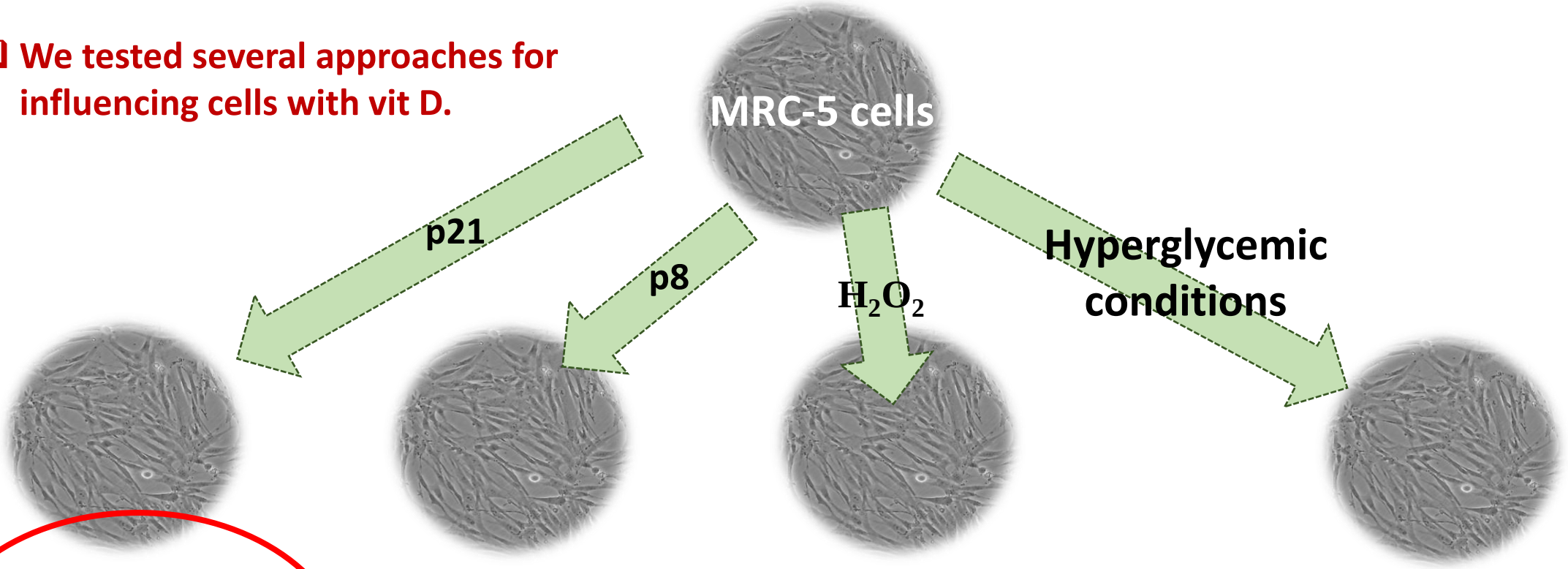
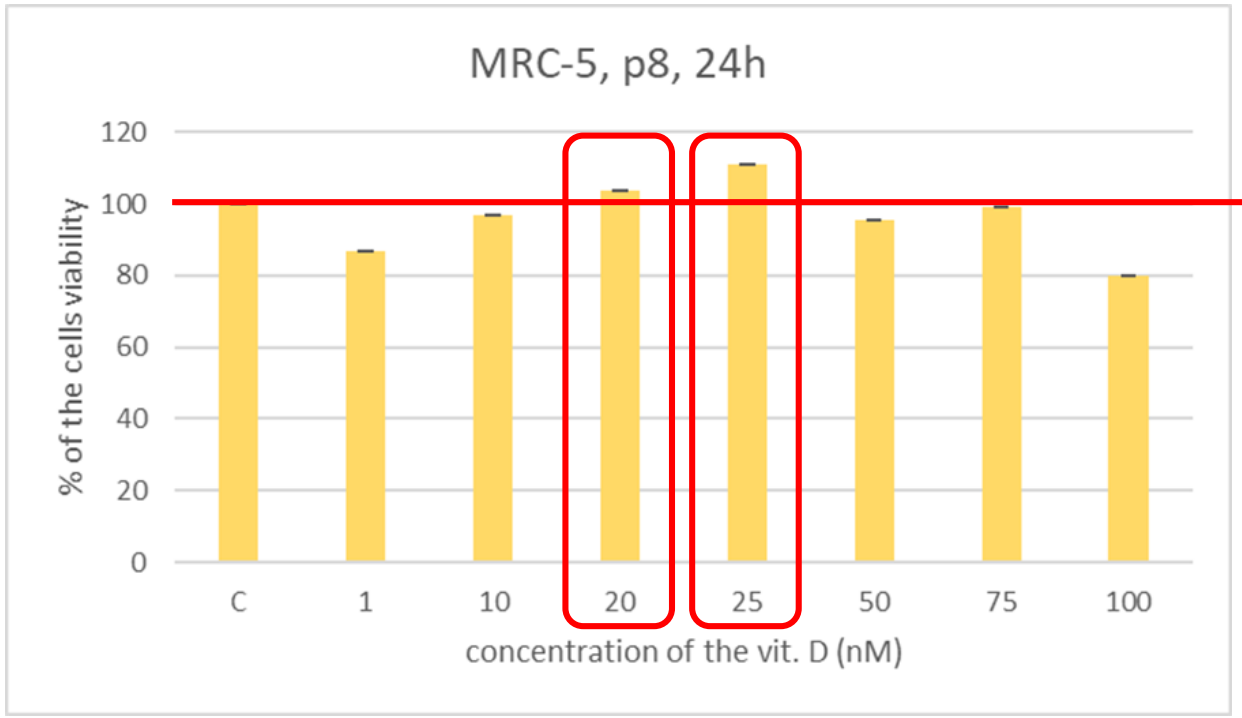
Normoglycemic condition

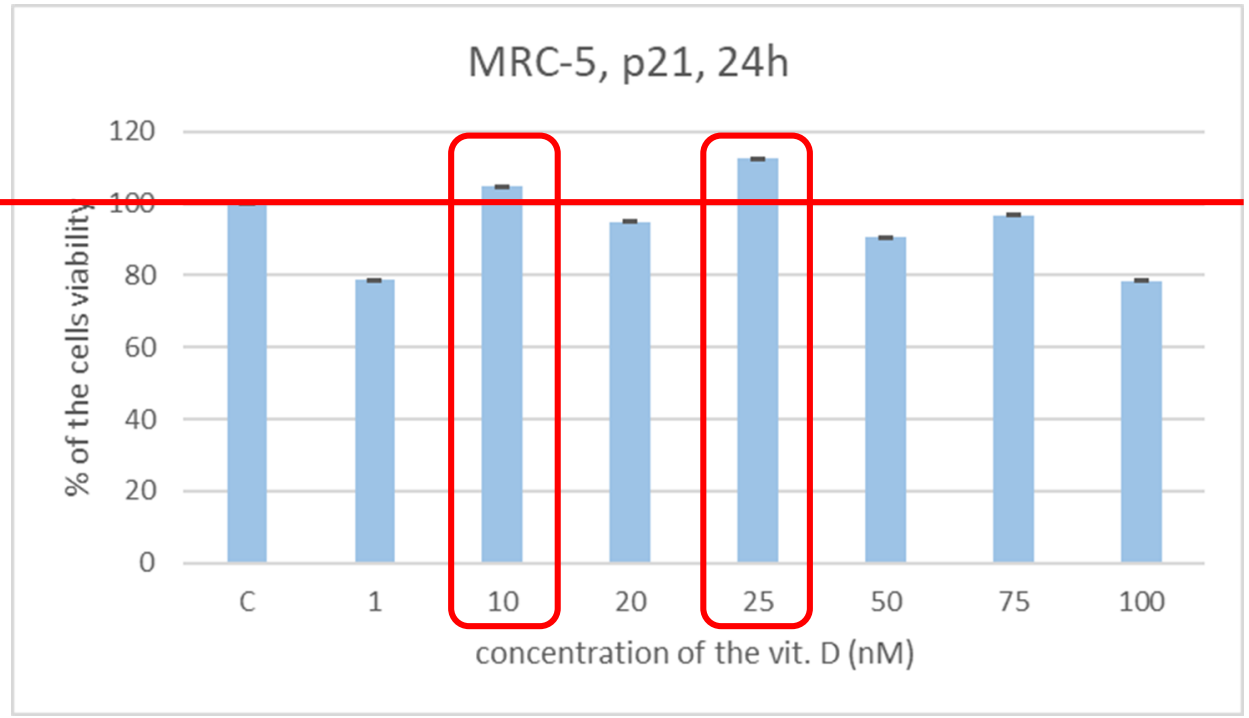
Hyperglycemic condition

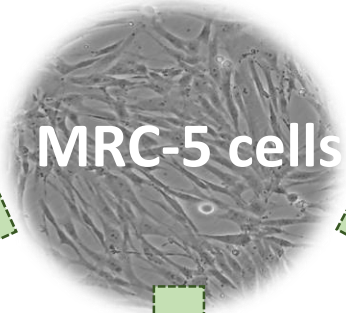
- **We tested several approaches for influencing cells with vit D.**



+ Vitamin D + 24h incubation + medium exchange + incubation until 72h	+ Vitamin D + 24h incubation + medium exchange + incubation until 72h	+ Vitamin D + 24h incubation + medium exchange + incubation until 72h	
+ 24h incubation + vitamin D + medium exchange + incubation until 72h	+ 24h incubation + vitamin D + medium exchange + incubation until 72h	+ 24h incubation + vitamin D + medium exchange + incubation until 72h	+ 24h incubation + vitamin D + medium exchange + incubation until 72h


MRC-5, p8, 24h
% of the cells viability
120
100
80
60
40
20
0
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)


MRC-5, p21, 24h
% of the cells viability
120
100
80
60
40
20
0
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)

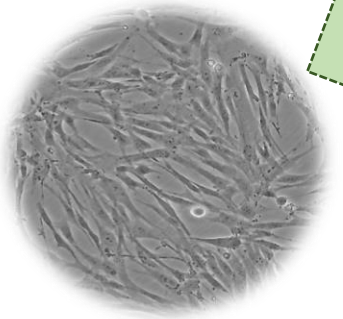


MRC-5 cells

not hyperglycemic

NG

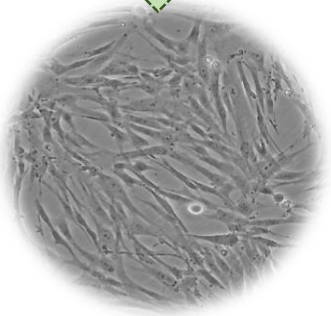
+ seeded the cells under HG conditions
+ incubated for 24 hours
+ affected cells with vitamin D for 24 hours



Vitamin D

Damaged the cells with H_2O_2 and then affected vitamin D

Added vitamin D to the cells and then damaged them with H_2O_2

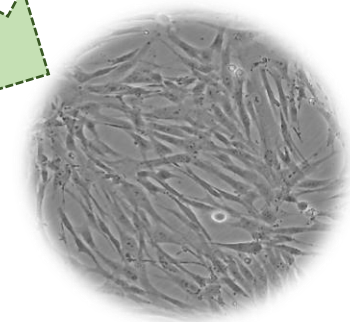


Vitamin D

Damaged the cells with H_2O_2 and then affected vitamin D

Added vitamin D to the cells and then damaged them with H_2O_2

+ seeded the cells under normal conditions
+ incubated for 24 h
+ changed the NG conditions to HG
+ addition of vitamin D



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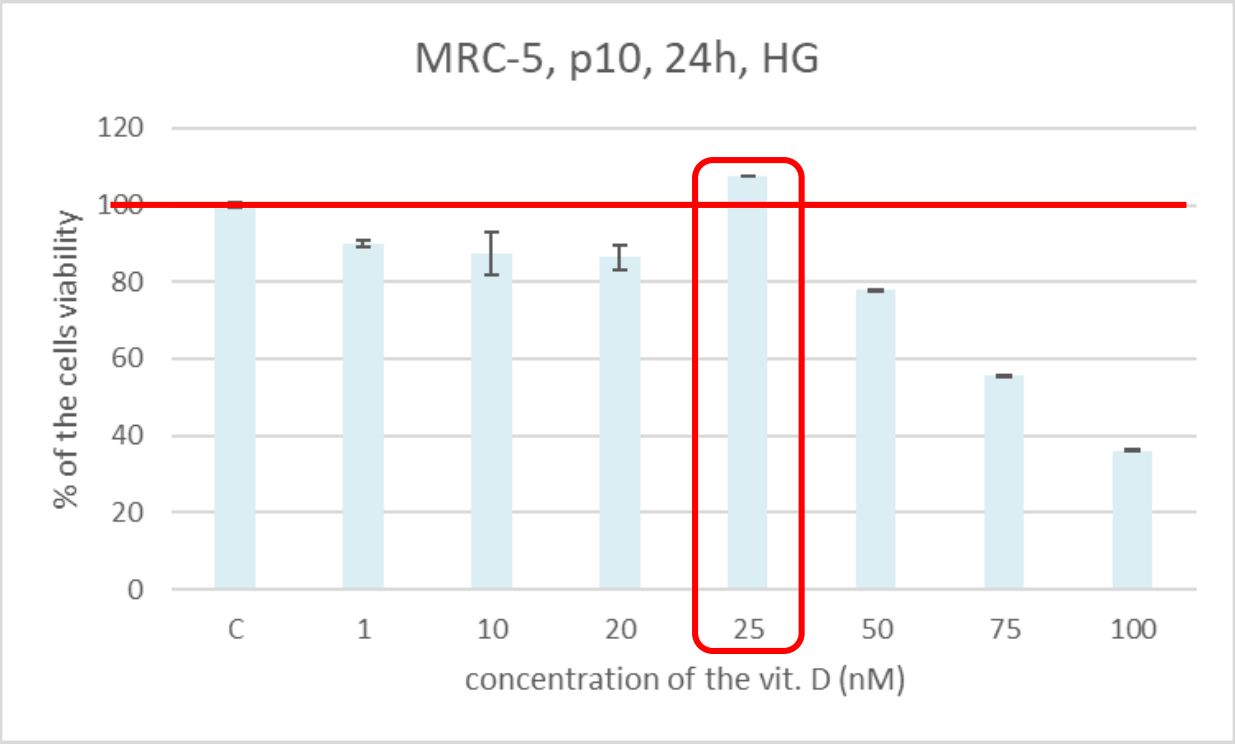
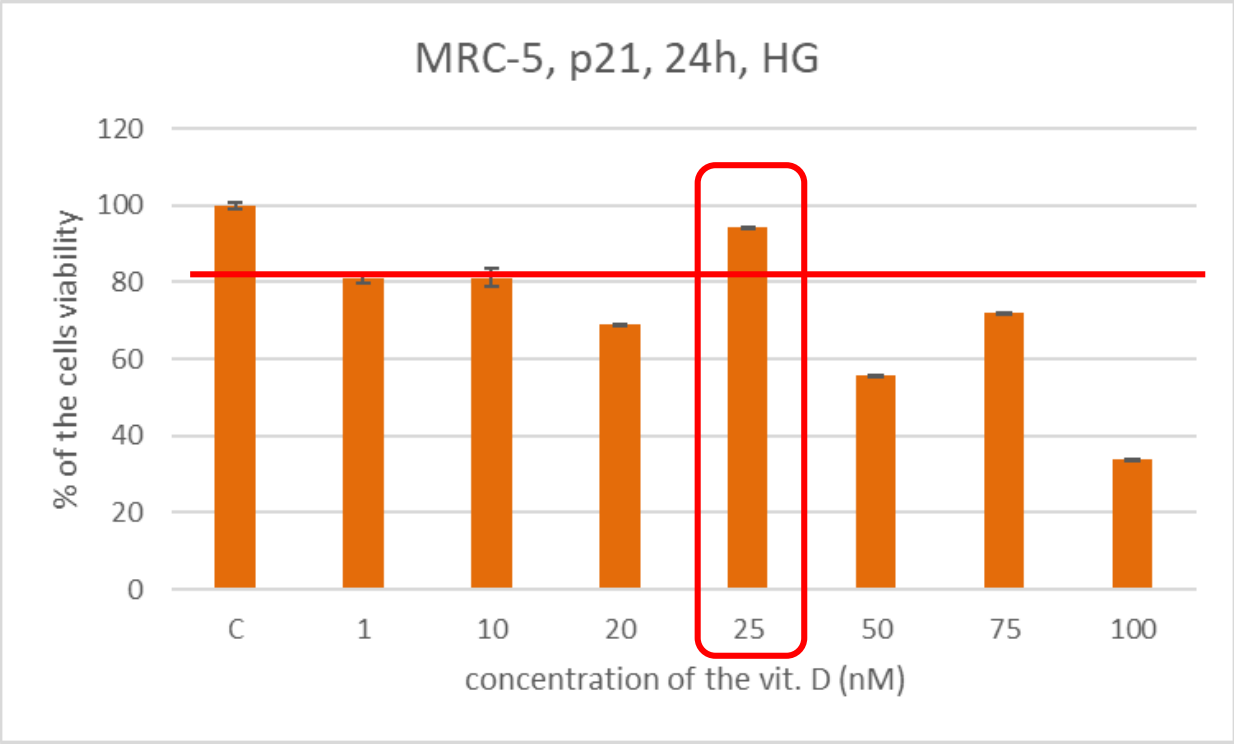

MRC-5, p10, 24h, HG
120
100
80
60
40
20
0
% of the cells viability
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)


MRC-5, p21, 24h, HG
120
100
80
60
40
20
0
% of the cells viability
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)

Methods	Analysis
MTT colorimetric technique	Viability of the cells
Muse® Cell Cycle Assay Kit	Distribution of cells in the individual phases of the cell cycle
Western blot analysis	Molecular mechanism
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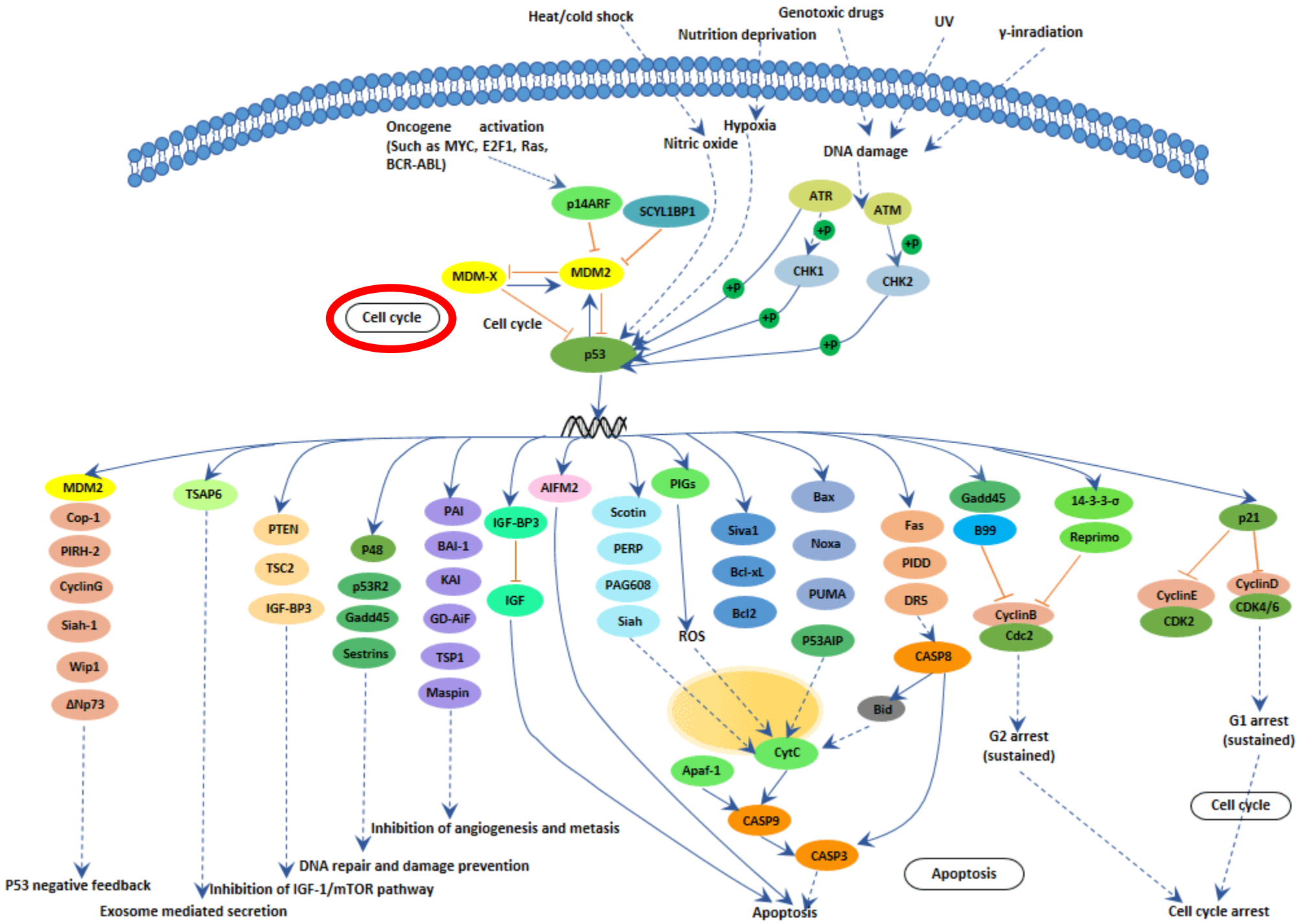
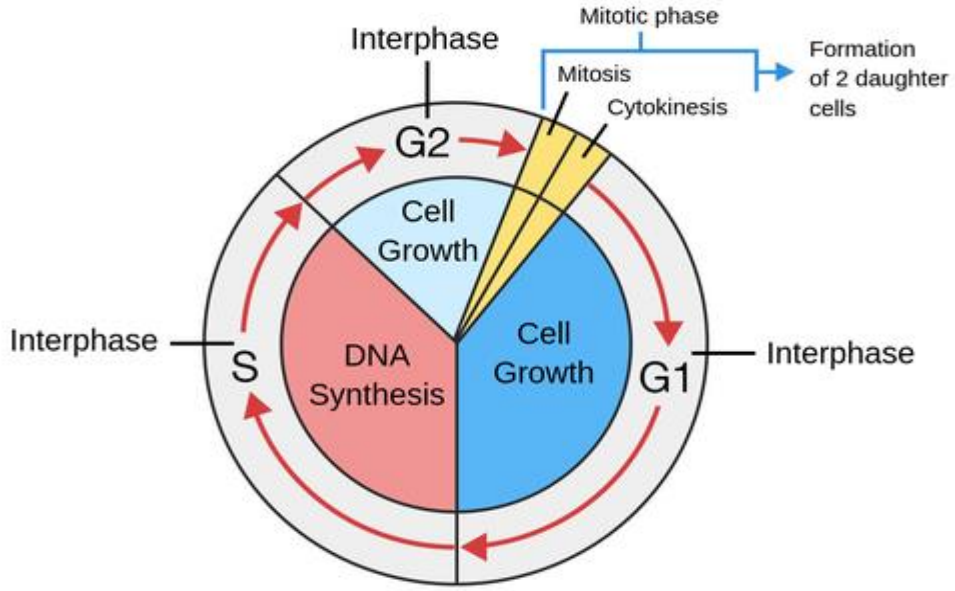
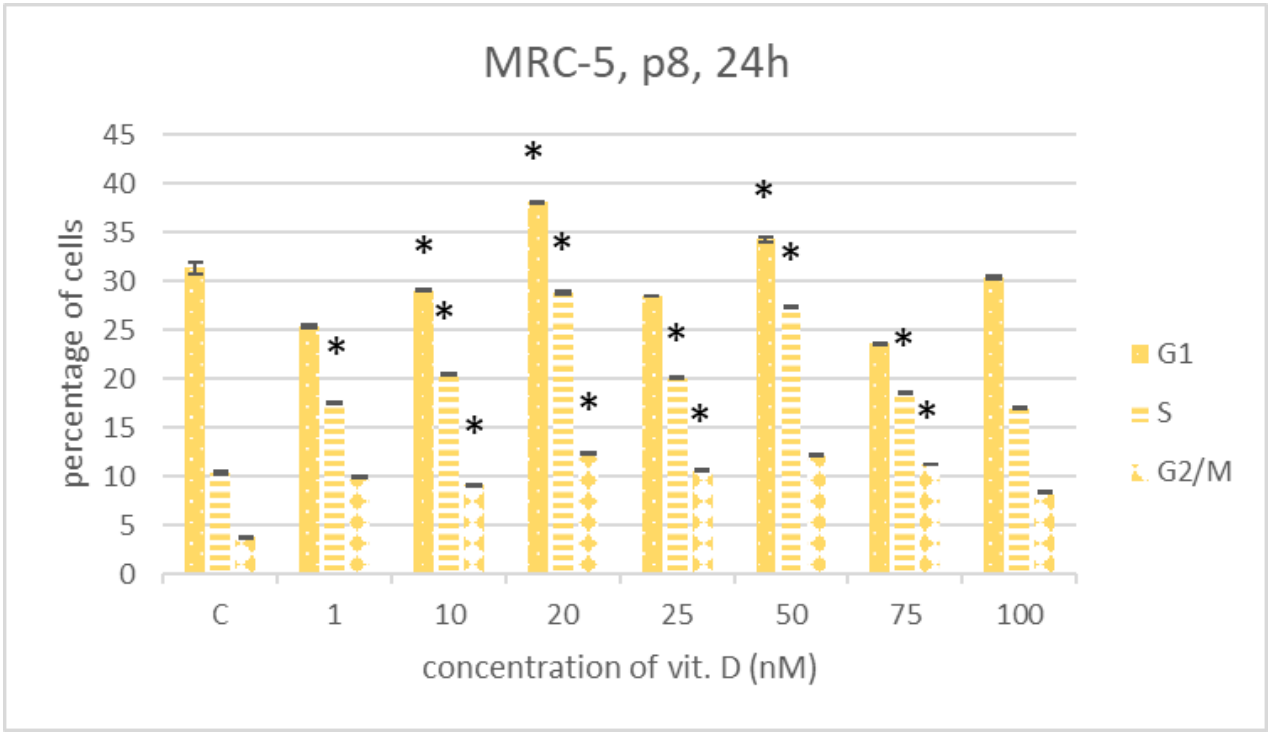
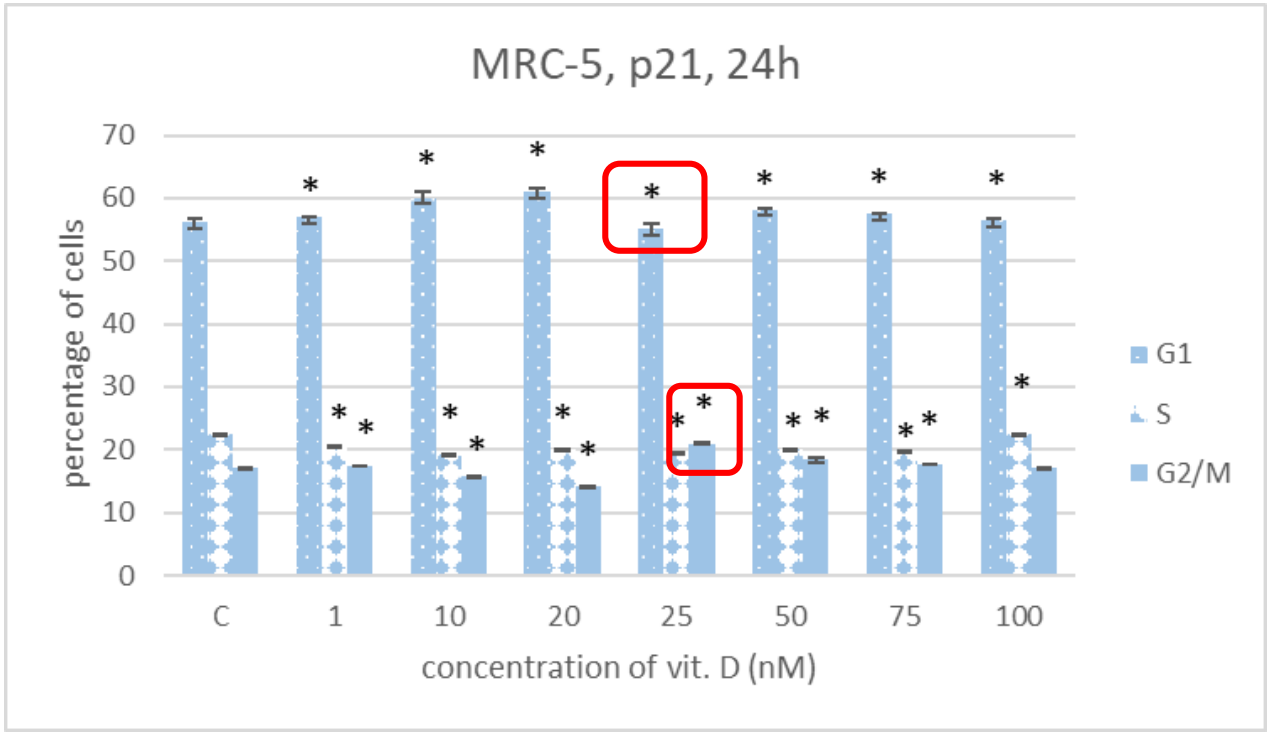
Mitochondrial respiration	Measurement of respiration

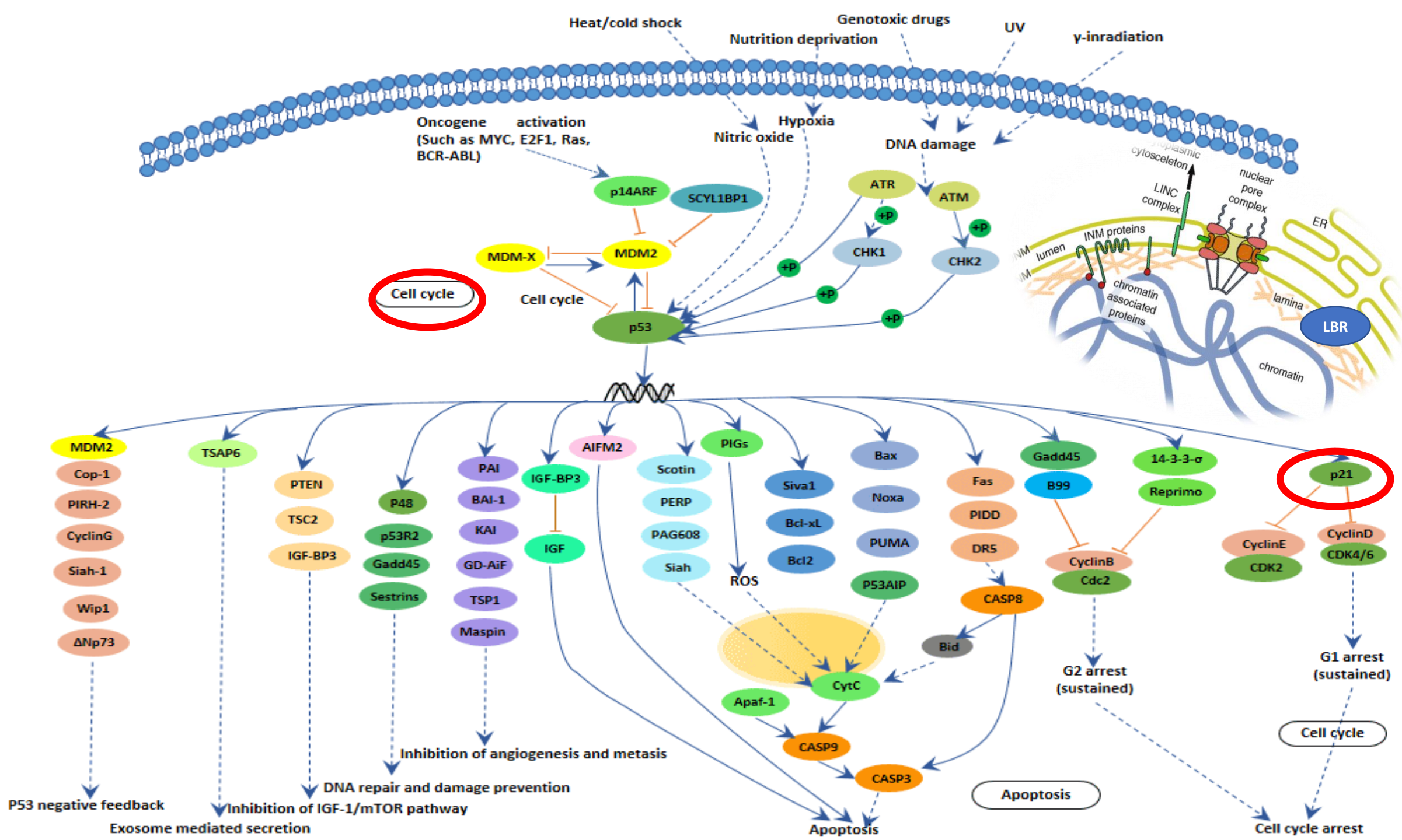

Heat/cold shock
Genotoxic drugs
UV
Nutrition deprivation
γ-inradiation
Hypoxia
Oncogene activation
(Such as MYC, E2F1, Ras,
BCR-ABL)
Nitric oxide
DNA damage
p14ARF
SCYL1BP1
ATR
ATM
+P
MDM-X
MDM2
CHK1
CHK2
Cell cycle
Cell cycle
p53
MDM2
Cop-1
PIRH-2
CyclinG
Siah-1
Wip1
ΔNp73
TSAP6
PTEN
TSC2
IGF-BP3
P48
p53R2
Gadd45
Sestrins
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
IGF-BP3
IGF
AIFM2
Scotin
PERP
PAG608
Siah
PIGs
ROS
Siva1
Bcl-xL
Bcl2
Bax
Noxa
PUMA
P53AIP
Fas
PIDD
DR5
CASP8
Bid
Gadd45
B99
CyclinB
Cdc2
14-3-3-σ
Reprimo
p21
CyclinE
CDK2
CyclinD
CDK4/6
CytC
Apaf-1
CASP9
CASP3
Apoptosis
P53 negative feedback
Exosome mediated secretion
Inhibition of IGF-1/mTOR pathway
DNA repair and damage prevention
Inhibition of angiogenesis and metasis
G2 arrest
(sustained)
G1 arrest
(sustained)
Cell cycle
Apoptosis
Cell cycle arrest

- **Muse® Cell Cycle Assay Kit**
  - **Normoglycemic conditon**








Heat/cold shock
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Nitric oxide
DNA damage
p14ARF
SCYL1BP1
MDM-X
MDM2
Cell cycle
Cell cycle
p53
ATR
ATM
CHK1
CHK2
+P
cytosceleton
LINC complex
nuclear pore complex
ER
INM proteins
lumen
chromatin associated proteins
lamina
LBR
chromatin
MDM2
Cop-1
PIRH-2
CyclinG
Siah-1
Wip1
ΔNp73
TSAP6
PTEN
TSC2
IGF-BP3
P48
p53R2
Gadd45
Sestrins
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
IGF-BP3
IGF
AIFM2
Scotin
PERP
PAG608
Siah
PIGs
ROS
Siva1
Bcl-xL
Bcl2
Bax
Noxa
PUMA
P53AIP
Fas
PIDD
DR5
CASP8
Bid
Gadd45
B99
14-3-3-σ
Reprimo
p21
CyclinE
CDK2
CyclinD
CDK4/6
CytC
Apaf-1
CASP9
CASP3
CyclinB
Cdc2
P53 negative feedback
Exosome mediated secretion
Inhibition of IGF-1/mTOR pathway
DNA repair and damage prevention
Inhibition of angiogenesis and metasis
Apoptosis
Apoptosis
G2 arrest (sustained)
G1 arrest (sustained)
Cell cycle
Cell cycle arrest

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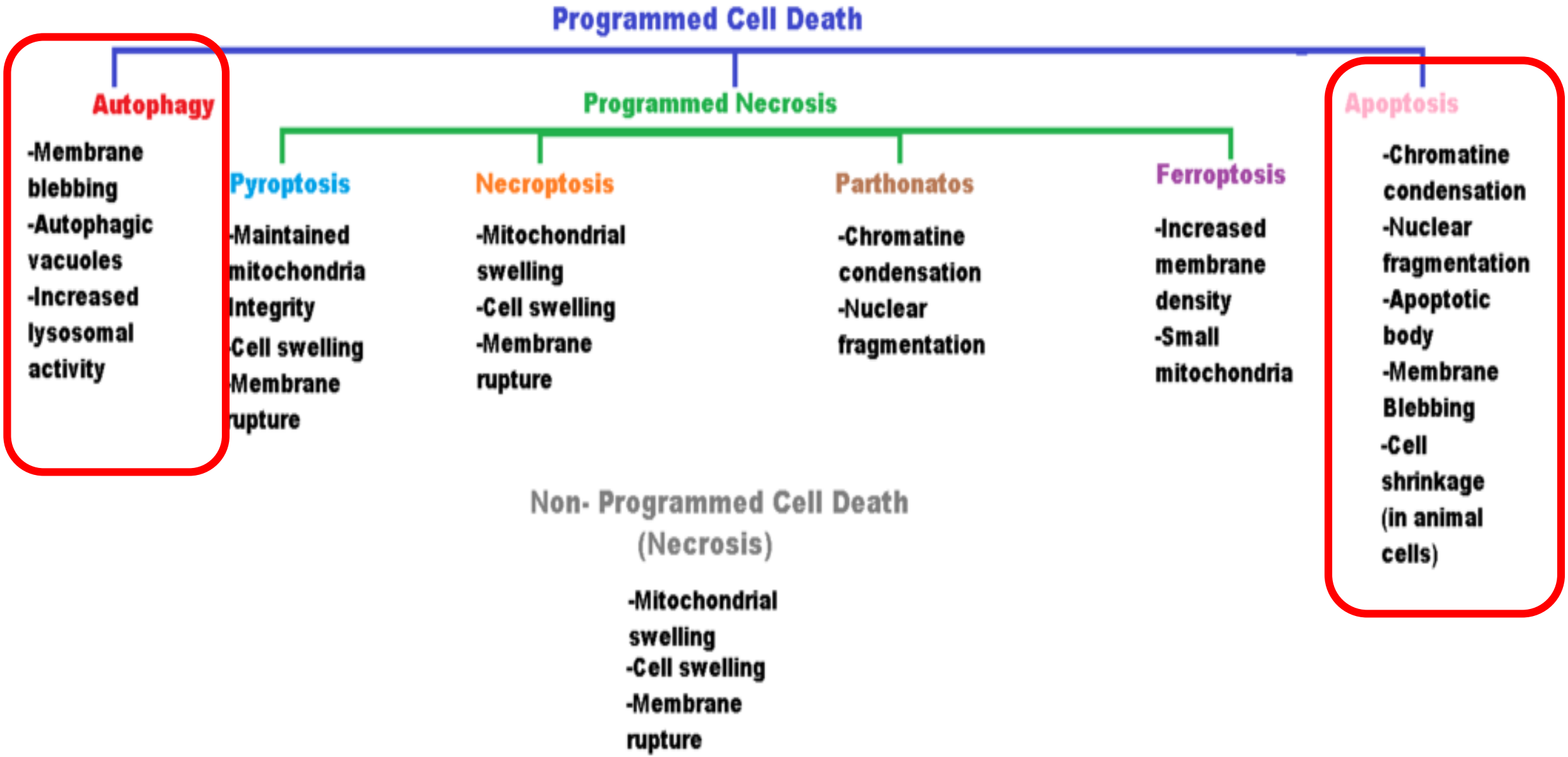
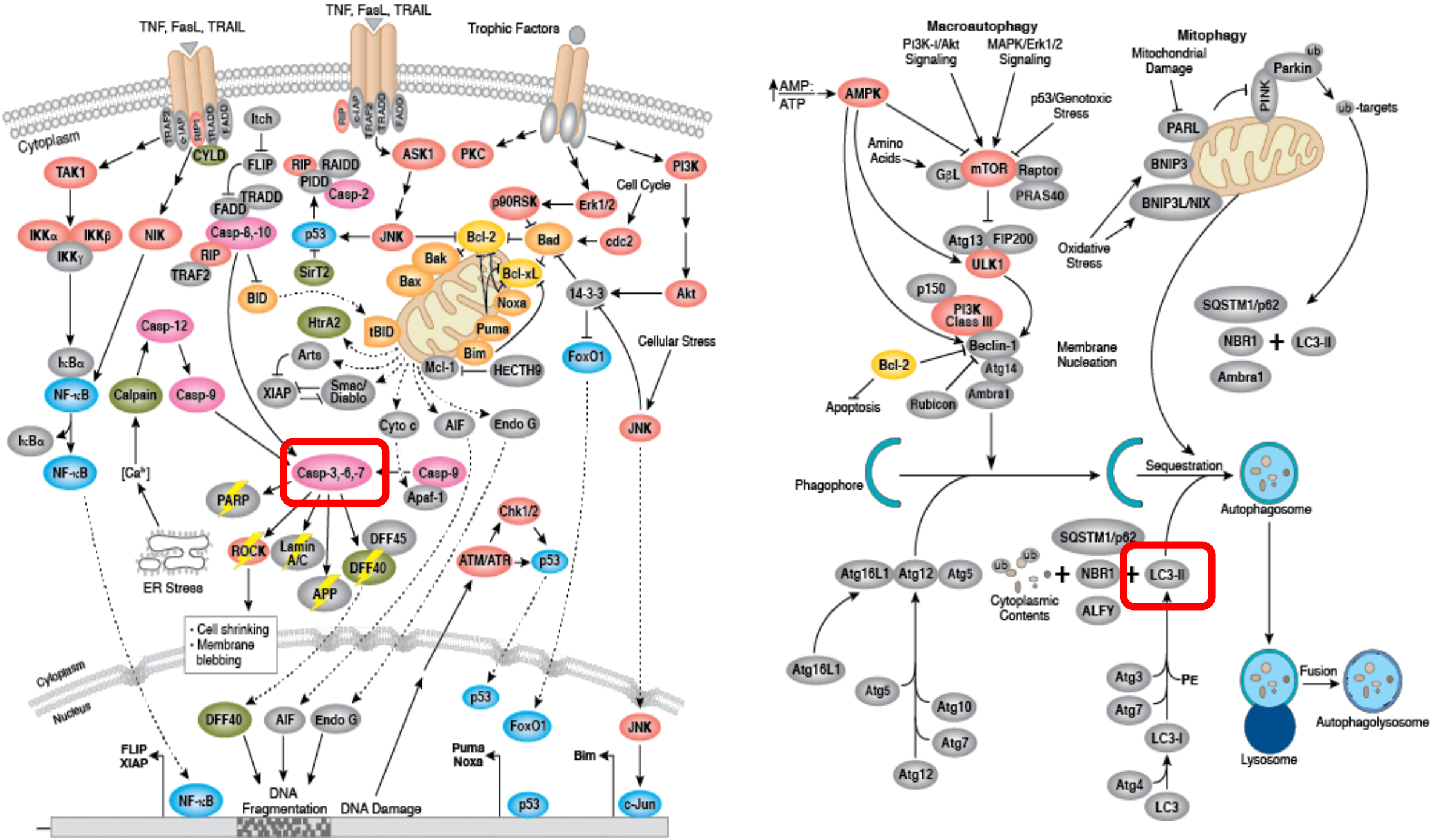
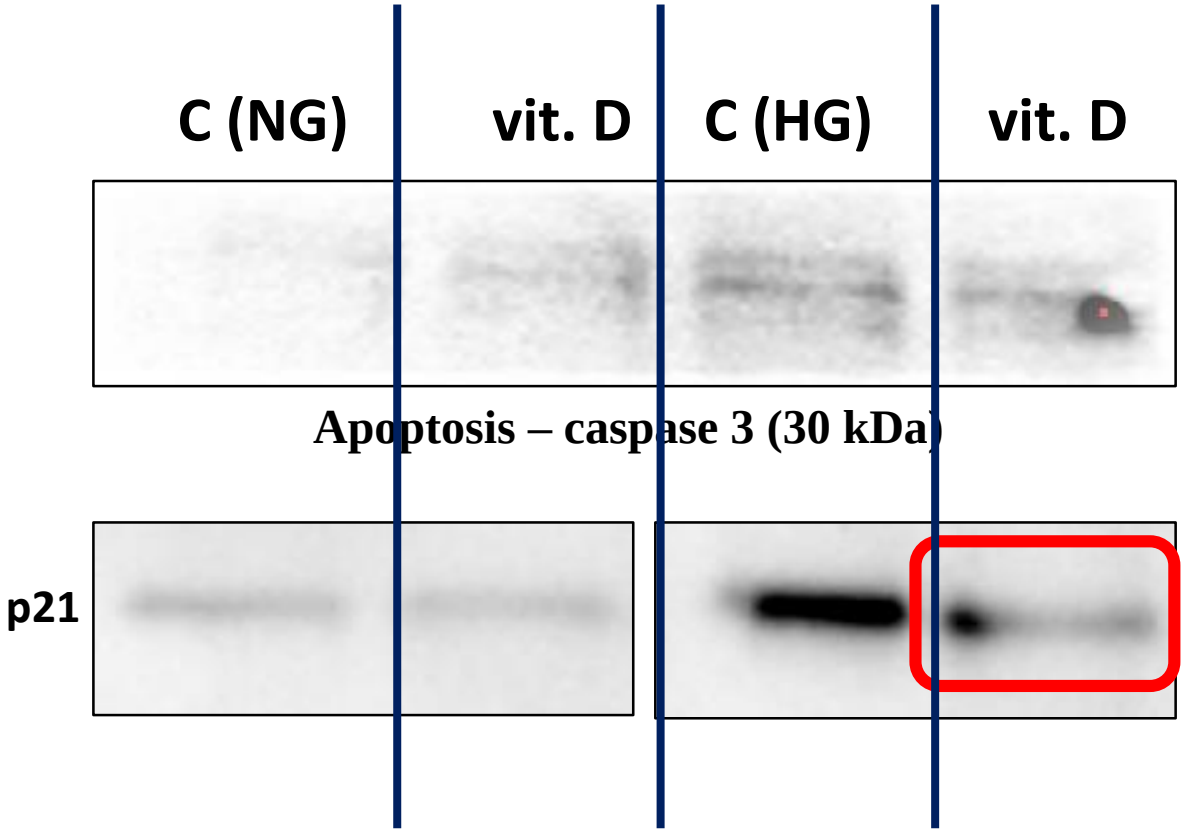

Programmed Cell Death
Autophagy
-Membrane blebbing
-Autophagic vacuoles
-Increased lysosomal activity
Programmed Necrosis
Pyroptosis
-Maintained mitochondria Integrity
-Cell swelling
-Membrane rupture
Necroptosis
-Mitochondrial swelling
-Cell swelling
-Membrane rupture
Parthonatos
-Chromatine condensation
-Nuclear fragmentation
Ferroptosis
-Increased membrane density
-Small mitochondria
Apoptosis
-Chromatine condensation
-Nuclear fragmentation
-Apoptotic body
-Membrane Blebbing
-Cell shrinkage (in animal cells)
Non- Programmed Cell Death (Necrosis)
-Mitochondrial swelling
-Cell swelling
-Membrane rupture

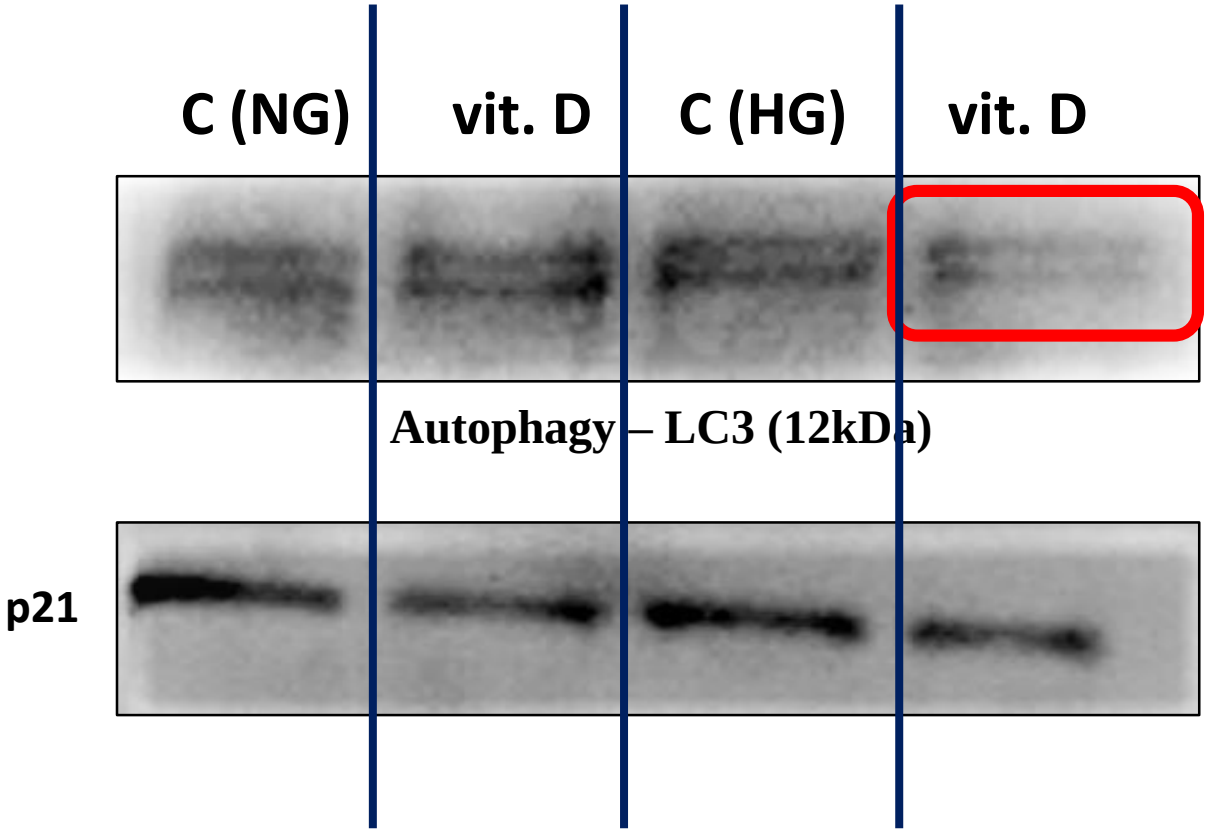

TNF, FasL, TRAIL
TNF, FasL, TRAIL
Trophic Factors
Cytoplasm
TRAF2
c-IAP
RIP1
TRADD
FADD
CYLD
Itch
FLIP
RIP
RAIDD
PIDD
Casp-2
ASK1
PKC
PI3K
Cell Cycle
TAK1
IKKα
IKKβ
IKKγ
NIK
FADD
TRADD
Casp-8,-10
RIP
TRAF2
p53
SirT2
JNK
p90RSK
Erk1/2
cdc2
Bcl-2
Bad
Bak
Bax
Bcl-xL
Noxa
Puma
Bim
Mcl-1
HECTH9
14-3-3
Akt
BID
tBID
HtrA2
Arts
XIAP
Smac/
Diablo
FoxO1
Cellular Stress
JNK
IκBα
NF-κB
Calpain
Casp-12
Casp-9
Cyto c
AIF
Endo G
Casp-3,-6,-7
Casp-9
Apaf-1
PARP
[Ca²⁺]
ER Stress
ROCK
Lamin
A/C
DFF45
DFF40
APP
Chk1/2
ATM/ATR
p53
• Cell shrinking
• Membrane
blebbing
Cytoplasm
Nucleus
DFF40
AIF
Endo G
FLIP
XIAP
NF-κB
DNA
Fragmentation
DNA Damage
p53
FoxO1
Puma
Noxa
p53
Bim
JNK
c-Jun
Macroautophagy
PI3K-I/Akt
Signaling
MAPK/Erk1/2
Signaling
AMP:
ATP
AMPK
p53/Genotoxic
Stress
Amino
Acids
GβL
mTOR
Raptor
PRAS40
Atg13
FIP200
ULK1
p150
PI3K
Class III
Beclin-1
Atg14
Ambra1
Bcl-2
Apoptosis
Rubicon
Membrane
Nucleation
Phagophore
Sequestration
Autophagosome
Atg16L1
Atg12
Atg5
Atg10
Atg7
Atg12
Atg16L1
Atg5
Cytoplasmic
Contents
ub
SQSTM1/p62
NBR1
LC3-II
ALFY
Mitophagy
Mitochondrial
Damage
Parkin
ub
PINK
ub -targets
PARL
BNIP3
BNIP3L/NIX
Oxidative
Stress
SQSTM1/p62
NBR1
+
LC3-II
Ambra1
Atg3
Atg7
PE
LC3-I
Atg4
LC3
Fusion
Lysosome
Autophagolysosome

- **Western blot analysis**
  - **normo and hyperglycemic condition**

- in passage 8 (p8) as normal cells



- in passage 21 (p21) as senescent cells



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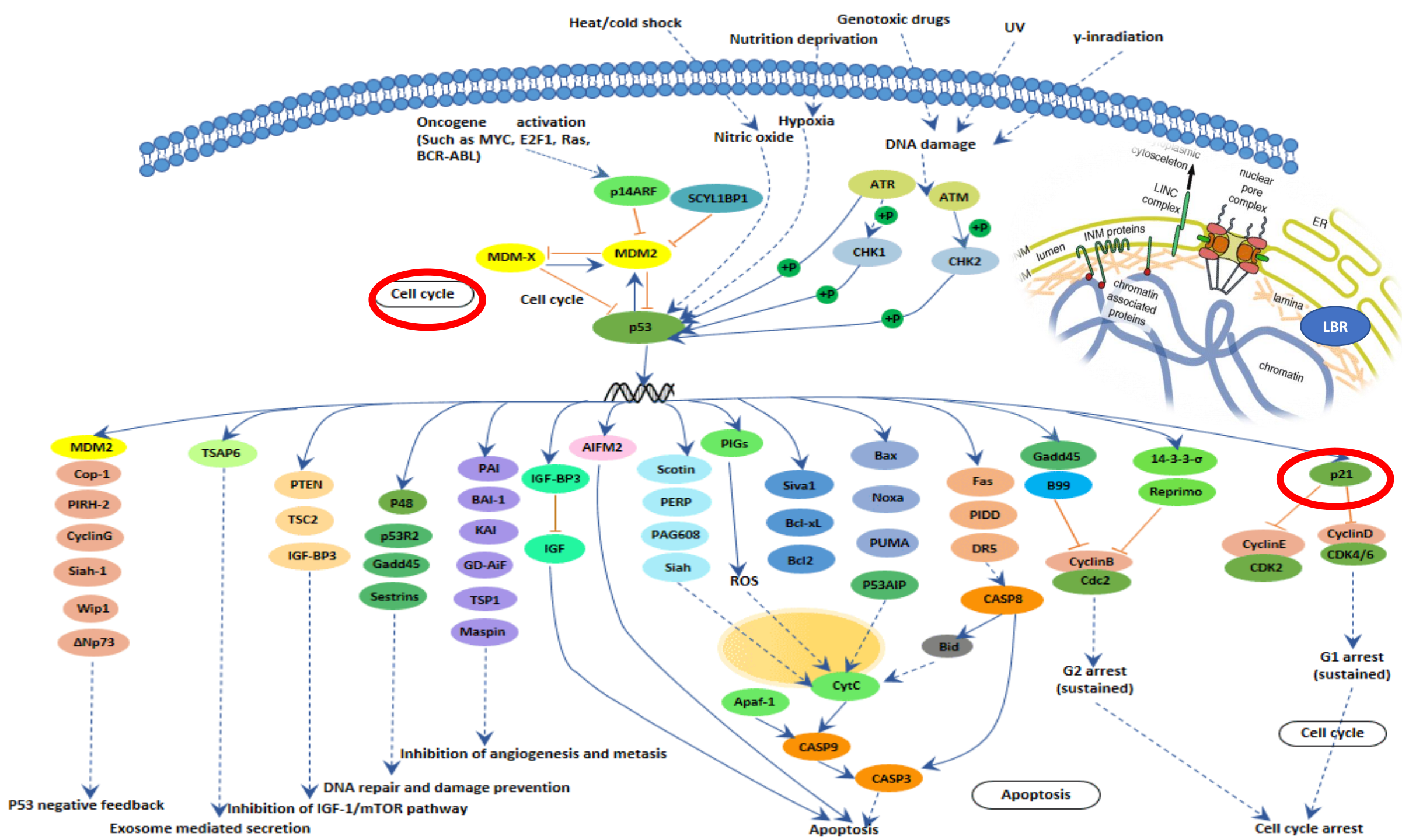
Heat/cold shock
Nutrition deprivation
Genotoxic drugs
UV
γ-inradiation
Oncogene activation (Such as MYC, E2F1, Ras, BCR-ABL)
Hypoxia
Nitric oxide
DNA damage
p14ARF
SCYL1BP1
MDM-X
MDM2
Cell cycle
Cell cycle
p53
ATR
ATM
CHK1
CHK2
+P
cytosceleton
LINC complex
nuclear pore complex
ER
INM proteins
lumen
chromatin associated proteins
lamina
LBR
chromatin
MDM2
Cop-1
PIRH-2
CyclinG
Siah-1
Wip1
ΔNp73
TSAP6
PTEN
TSC2
IGF-BP3
P48
p53R2
Gadd45
Sestrins
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
IGF-BP3
IGF
AIFM2
Scotin
PERP
PAG608
Siah
PIGs
ROS
Siva1
Bcl-xL
Bcl2
Bax
Noxa
PUMA
P53AIP
Fas
PIDD
DR5
CASP8
Bid
Gadd45
B99
14-3-3-σ
Reprimo
p21
CyclinE
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Apoptosis
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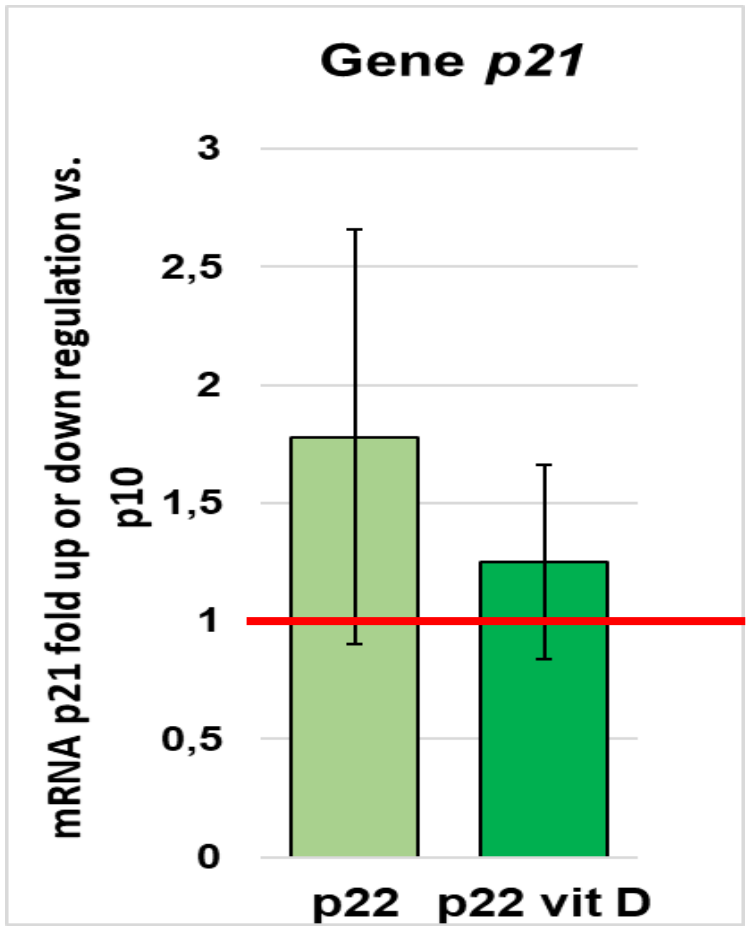
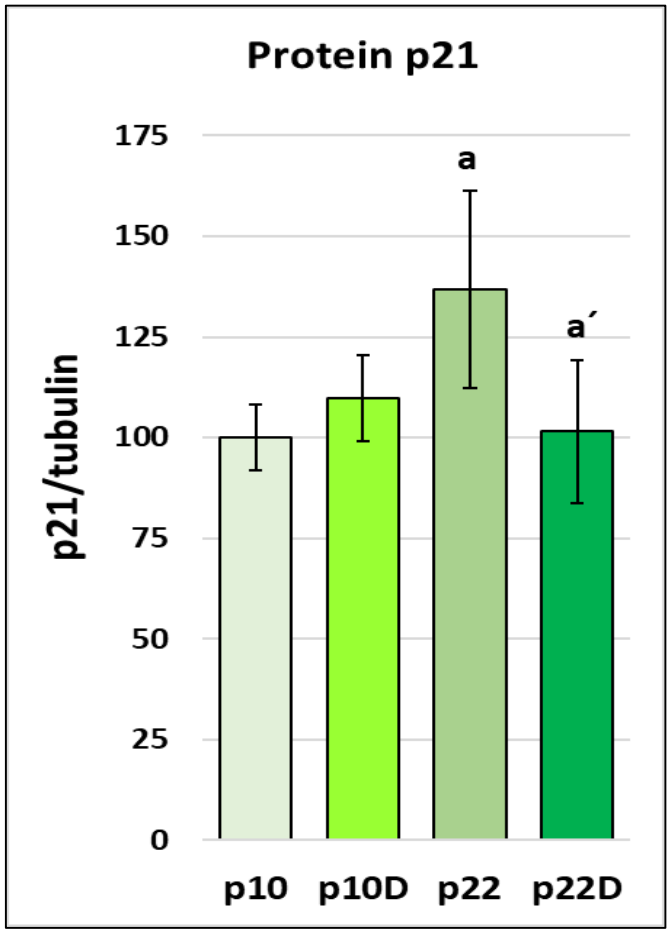
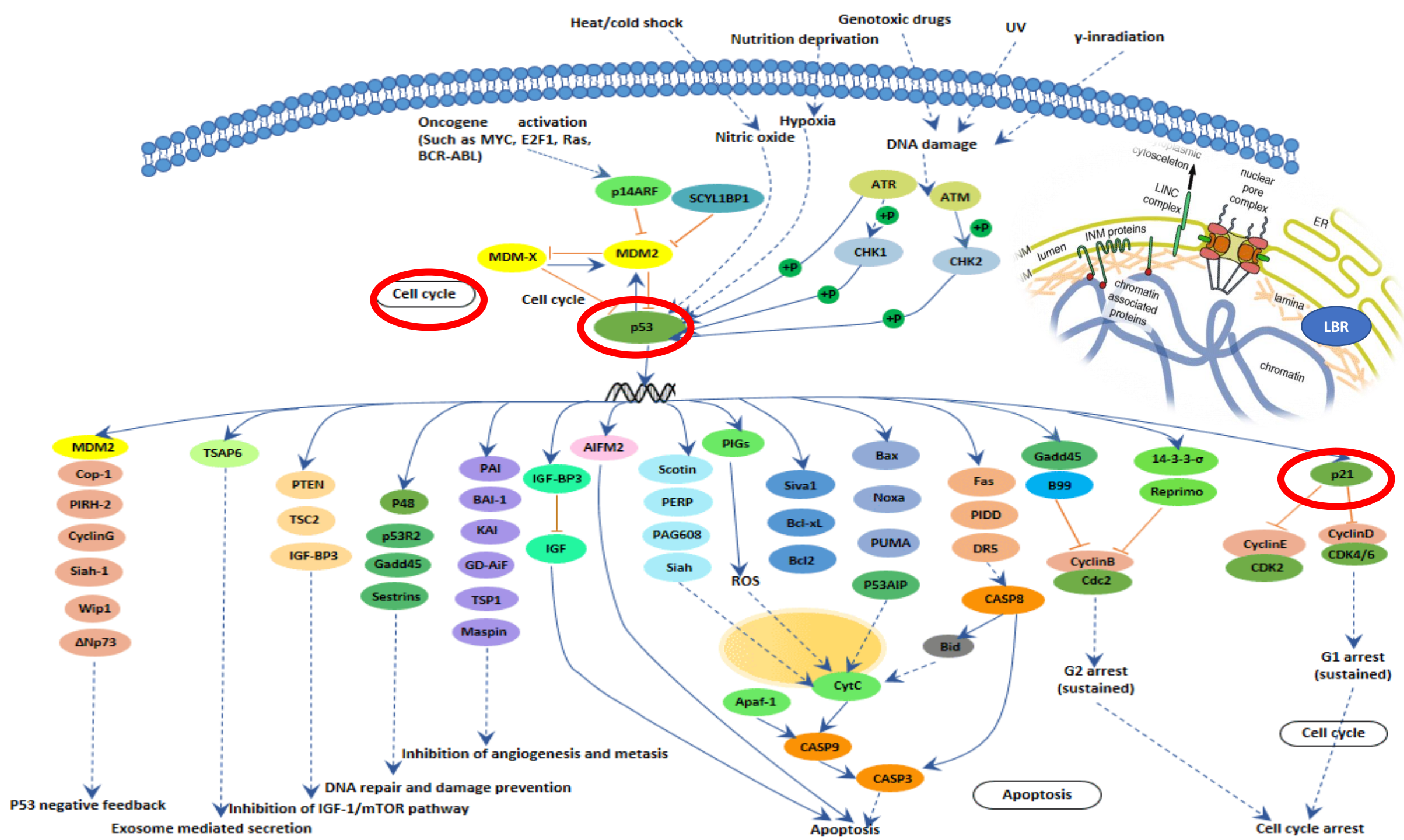
- **quantitative Real-time PCR = qRT-PCR**
  - **Normoglycemic condition**






Heat/cold shock
Genotoxic drugs
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(Such as MYC, E2F1, Ras,
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Hypoxia
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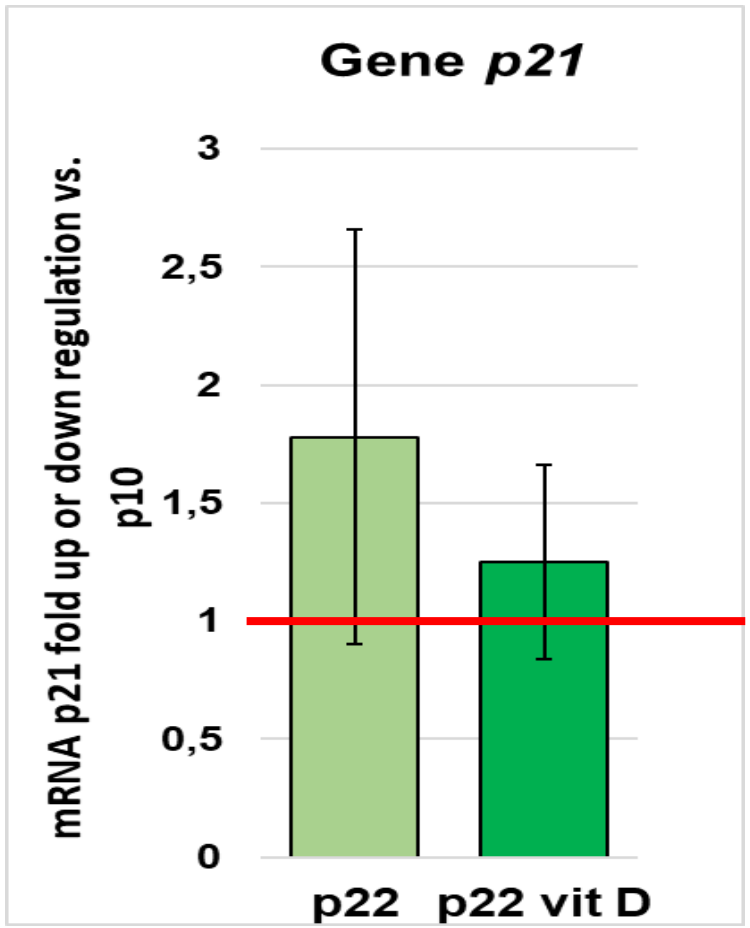
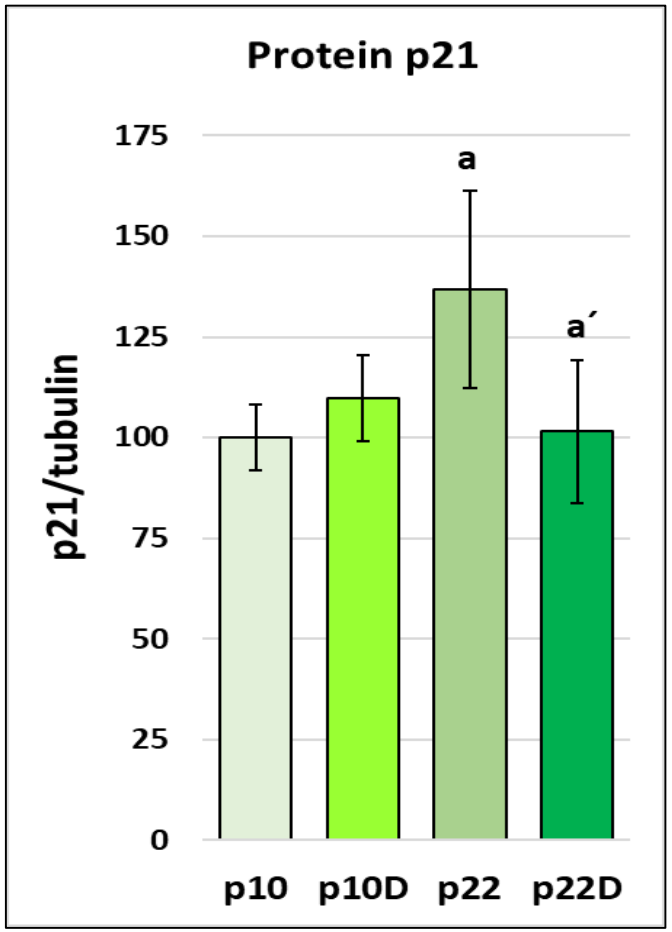
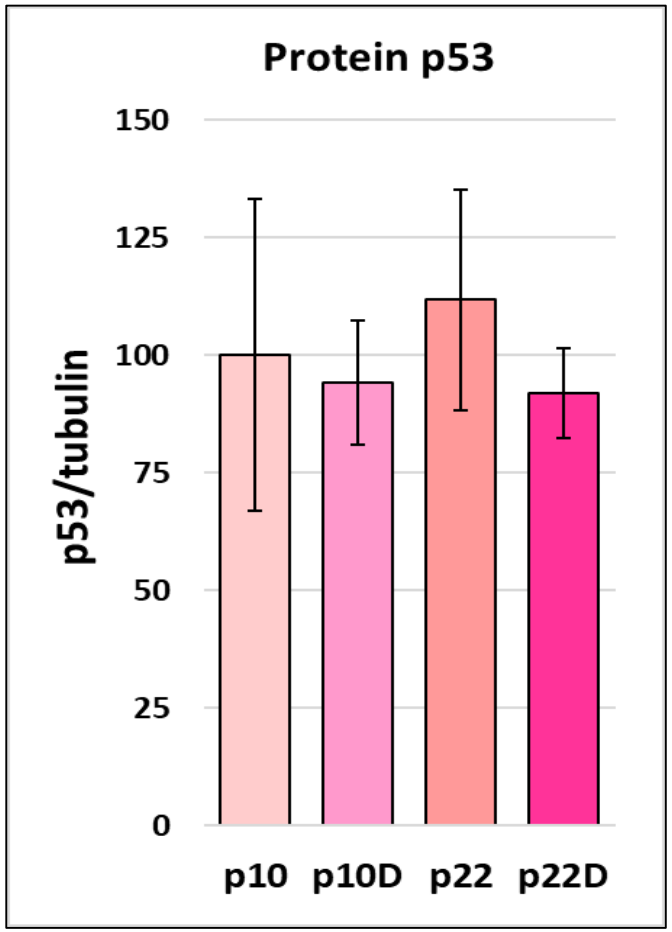
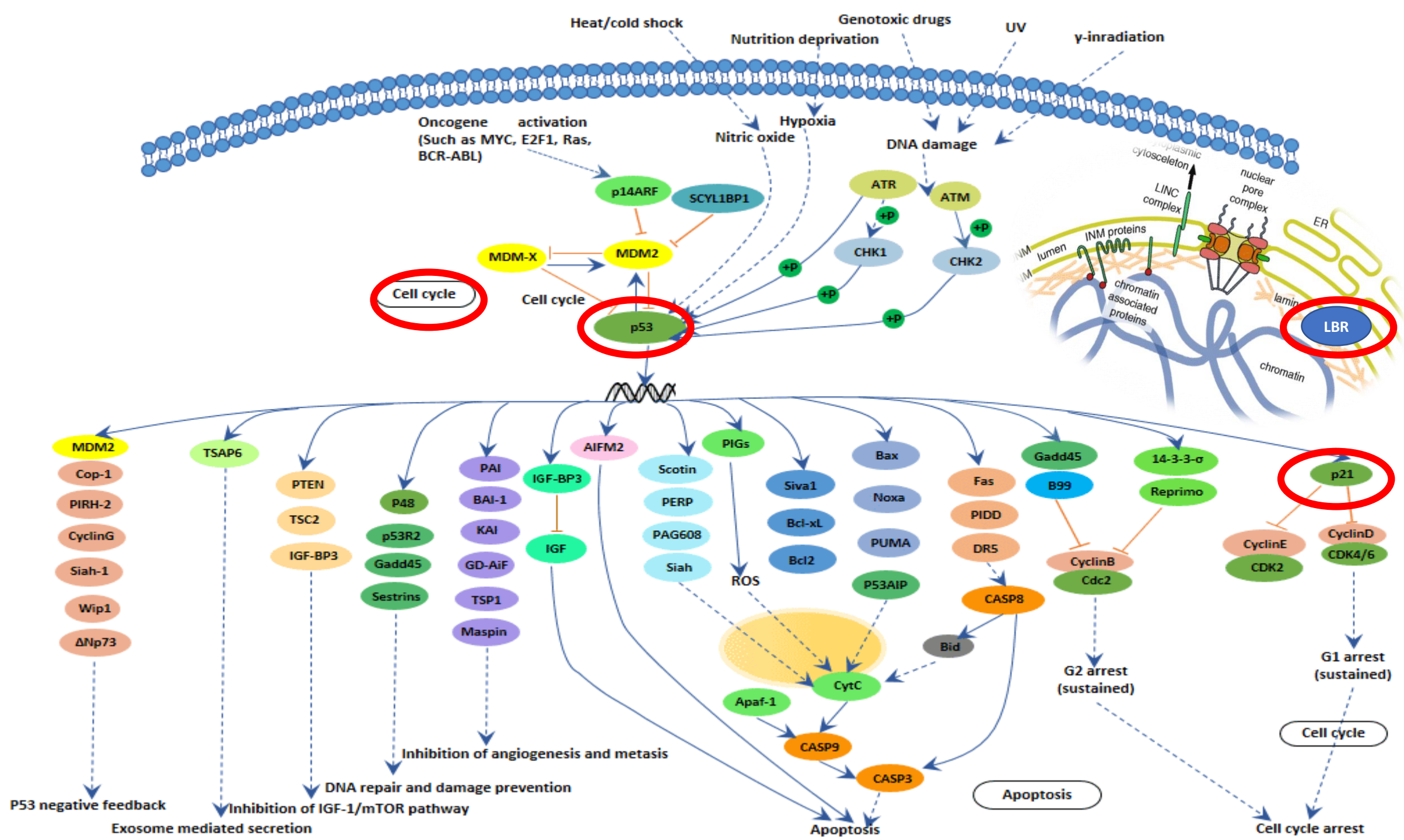
DNA damage
p14ARF
SCYL1BP1
MDM-X
MDM2
Cell cycle
Cell cycle
p53
ATR
ATM
CHK1
CHK2
+P
cytosceleton
LINC complex
nuclear pore complex
ER
INM proteins
lumen
chromatin associated proteins
lamina
LBR
chromatin
MDM2
Cop-1
PIRH-2
CyclinG
Siah-1
Wip1
ΔNp73
TSAP6
PTEN
TSC2
IGF-BP3
P48
p53R2
Gadd45
Sestrins
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
IGF-BP3
IGF
AIFM2
Scotin
PERP
PAG608
Siah
PIGs
ROS
Siva1
Bcl-xL
Bcl2
Bax
Noxa
PUMA
P53AIP
Fas
PIDD
DR5
CASP8
Bid
Gadd45
B99
14-3-3-σ
Reprimo
p21
CyclinE
CDK2
CyclinD
CDK4/6
CyclinB
Cdc2
CytC
Apaf-1
CASP9
CASP3
P53 negative feedback
Exosome mediated secretion
Inhibition of IGF-1/mTOR pathway
DNA repair and damage prevention
Inhibition of angiogenesis and metasis
Apoptosis
G2 arrest
(sustained)
G1 arrest
(sustained)
Cell cycle
Cell cycle arrest
Apoptosis

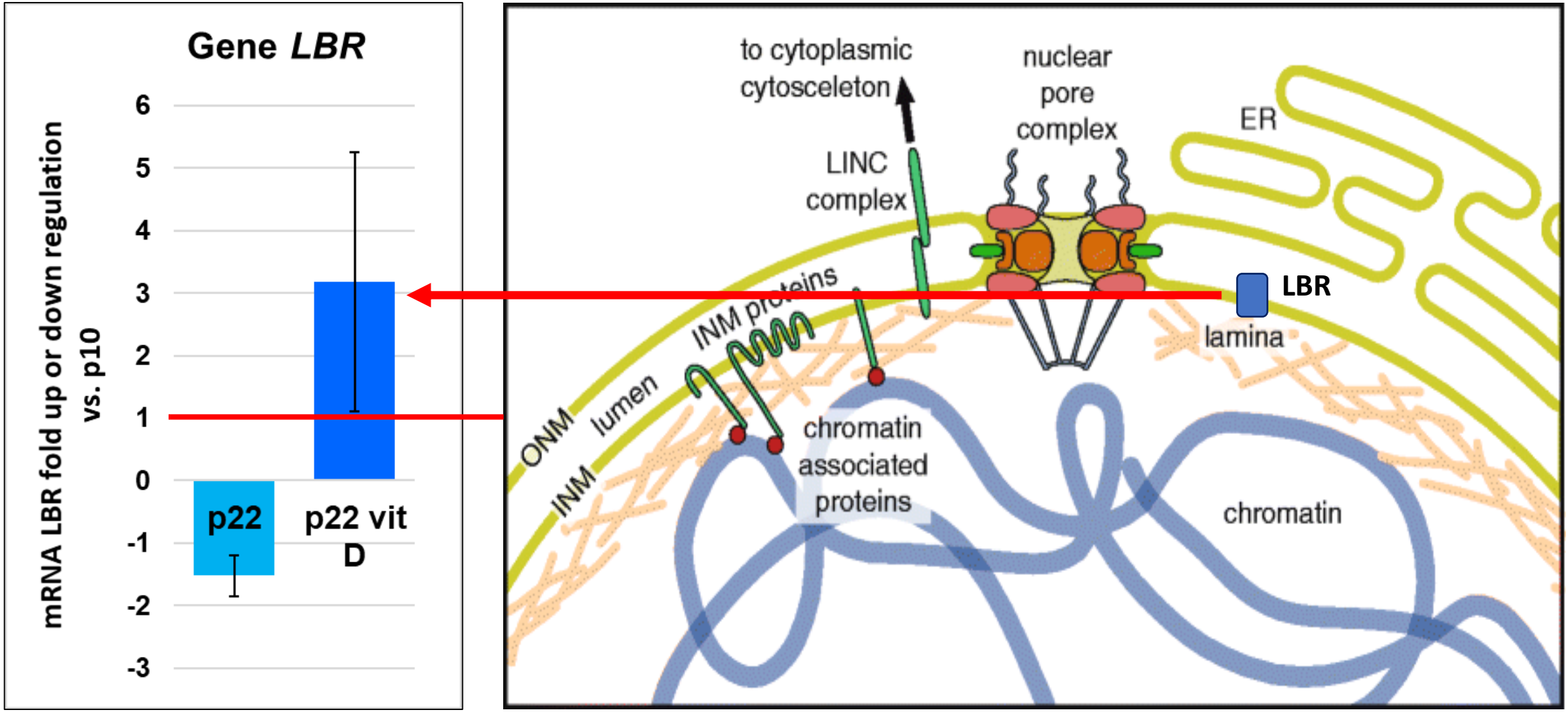
- **quantitative Real-time PCR = qRT-PCR**
  - **Normoglycemic condition**



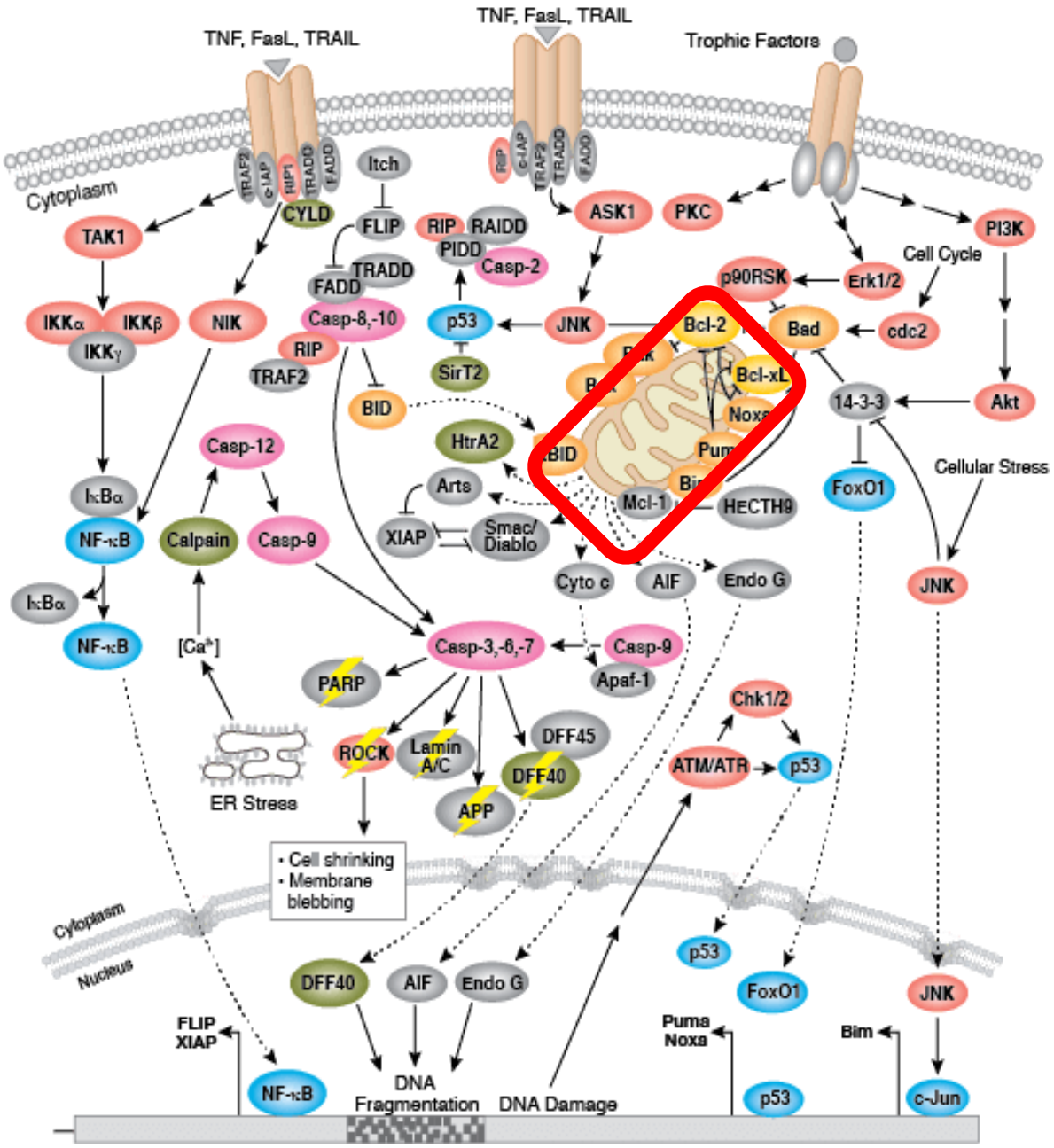


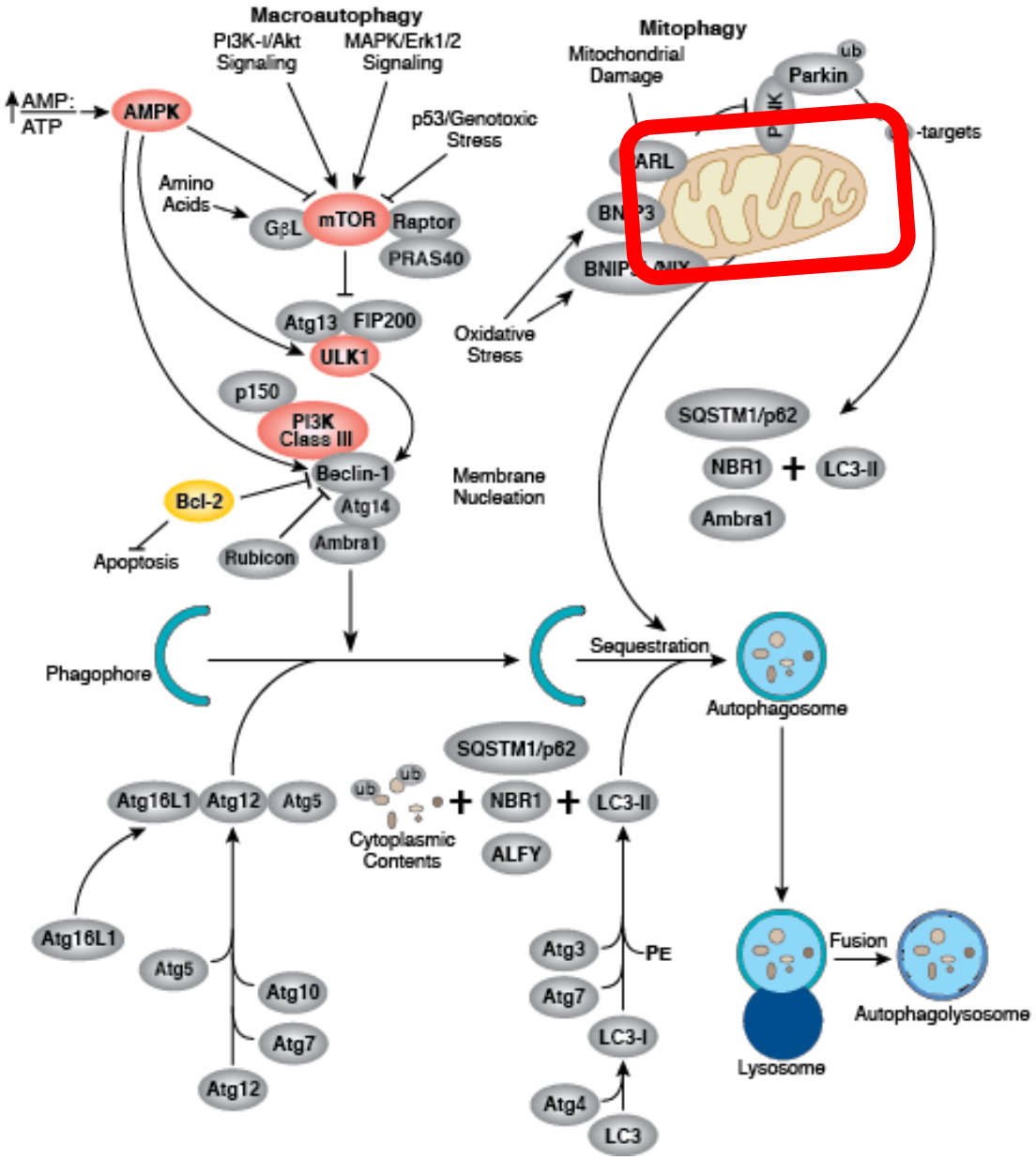



Heat/cold shock
Nutrition deprivation
Genotoxic drugs
UV
γ-inradiation
Oncogene activation
(Such as MYC, E2F1, Ras,
BCR-ABL)
Hypoxia
Nitric oxide
DNA damage
p14ARF
SCYL1BP1
MDM-X
MDM2
Cell cycle
Cell cycle
p53
ATR
ATM
CHK1
CHK2
+P
cytosceleton
LINC complex
nuclear pore complex
ER
lumen
INM proteins
chromatin associated proteins
lamin
LBR
chromatin
MDM2
Cop-1
PIRH-2
CyclinG
Siah-1
Wip1
ΔNp73
TSAP6
PTEN
TSC2
IGF-BP3
P48
p53R2
Gadd45
Sestrins
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
IGF-BP3
IGF
AIFM2
Scotin
PERP
PAG608
Siah
PIGs
ROS
Siva1
Bcl-xL
Bcl2
Bax
Noxa
PUMA
P53AIP
Fas
PIDD
DR5
CASP8
Bid
Gadd45
B99
14-3-3-σ
Reprimo
p21
CyclinE
CDK2
CyclinD
CDK4/6
CyclinB
Cdc2
CytC
Apaf-1
CASP9
CASP3
P53 negative feedback
Exosome mediated secretion
Inhibition of IGF-1/mTOR pathway
DNA repair and damage prevention
Inhibition of angiogenesis and metasis
Apoptosis
G2 arrest
(sustained)
G1 arrest
(sustained)
Cell cycle
Cell cycle arrest
Apoptosis


Gene LBR
mRNA LBR fold up or down regulation vs. p10
6
5
4
3
2
1
0
-1
-2
-3
p22
p22 vit D
to cytoplasmic cytosceleton
nuclear pore complex
ER
LINC complex
LBR
lamina
INM proteins
ONM
lumen
INM
chromatin associated proteins
chromatin

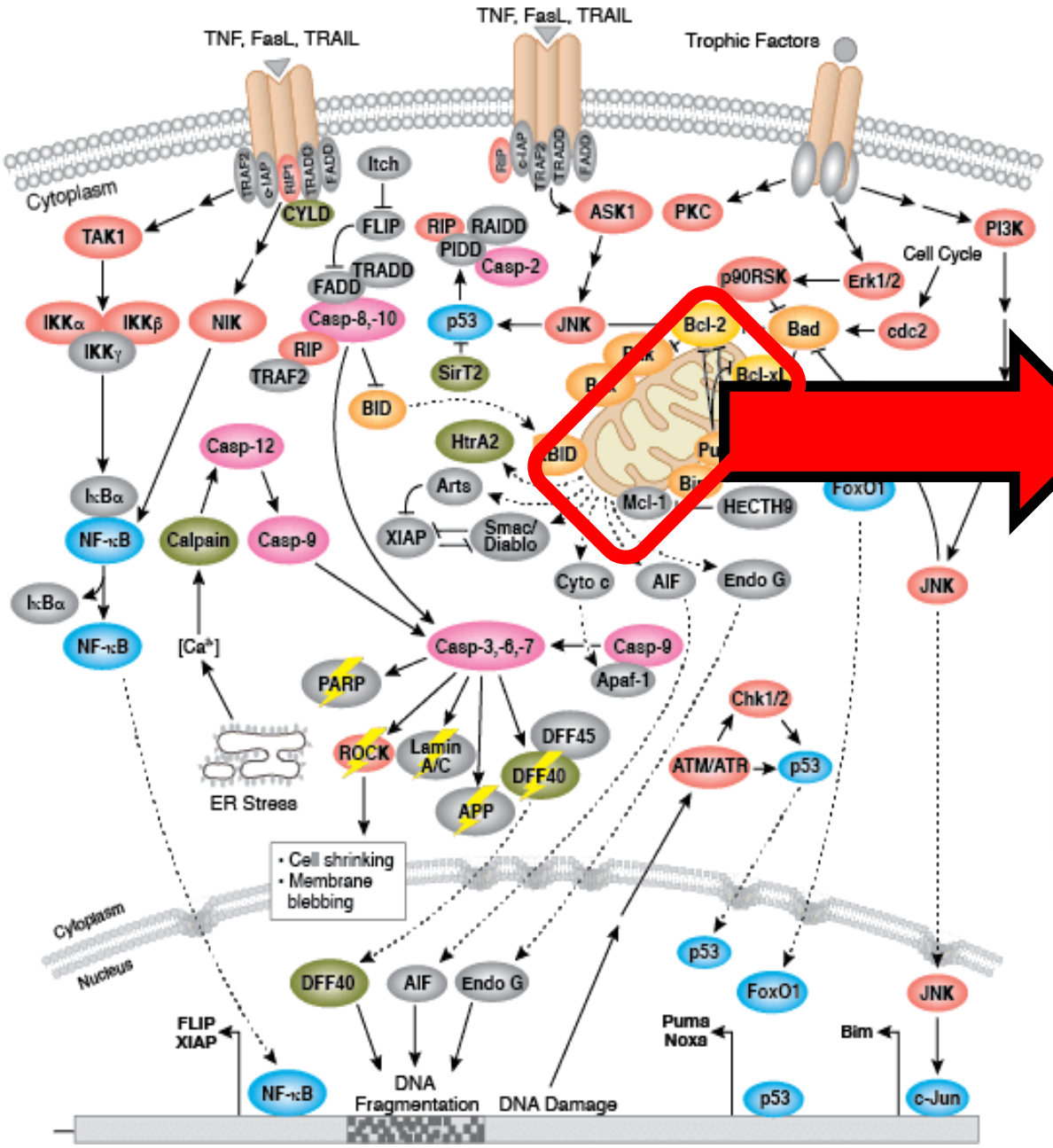
Mitochondrial respiration

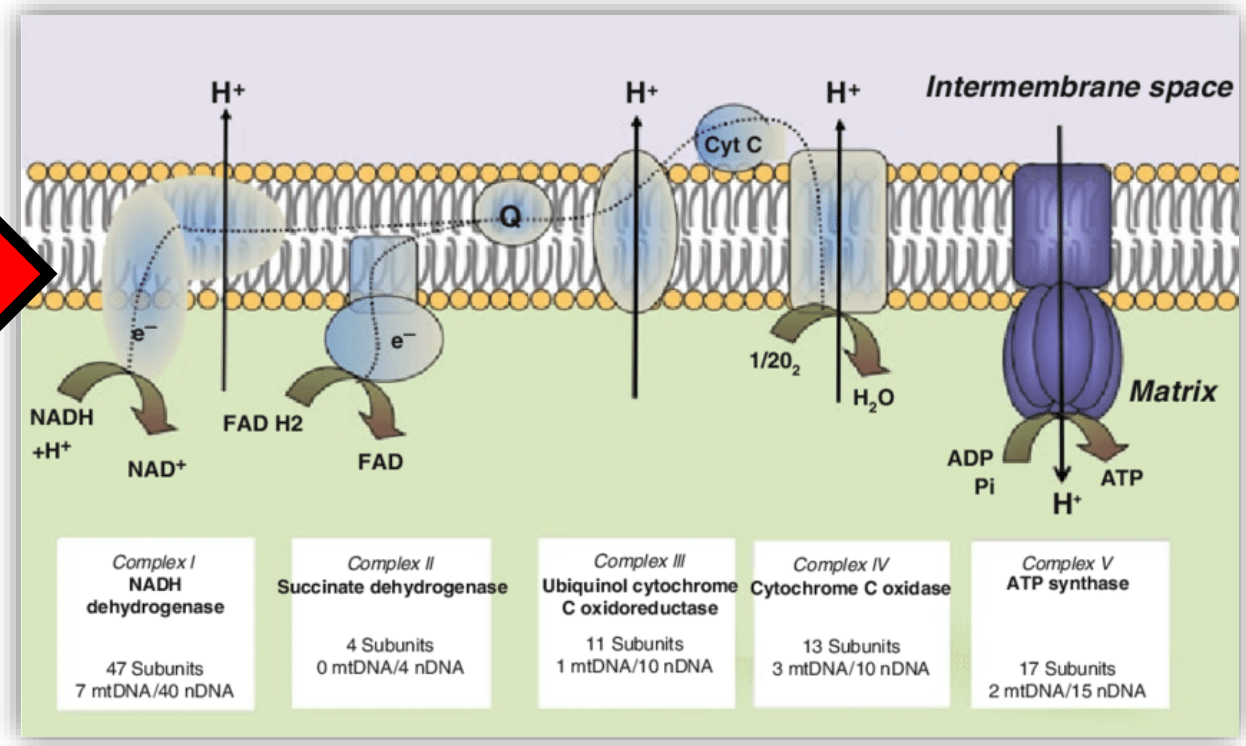


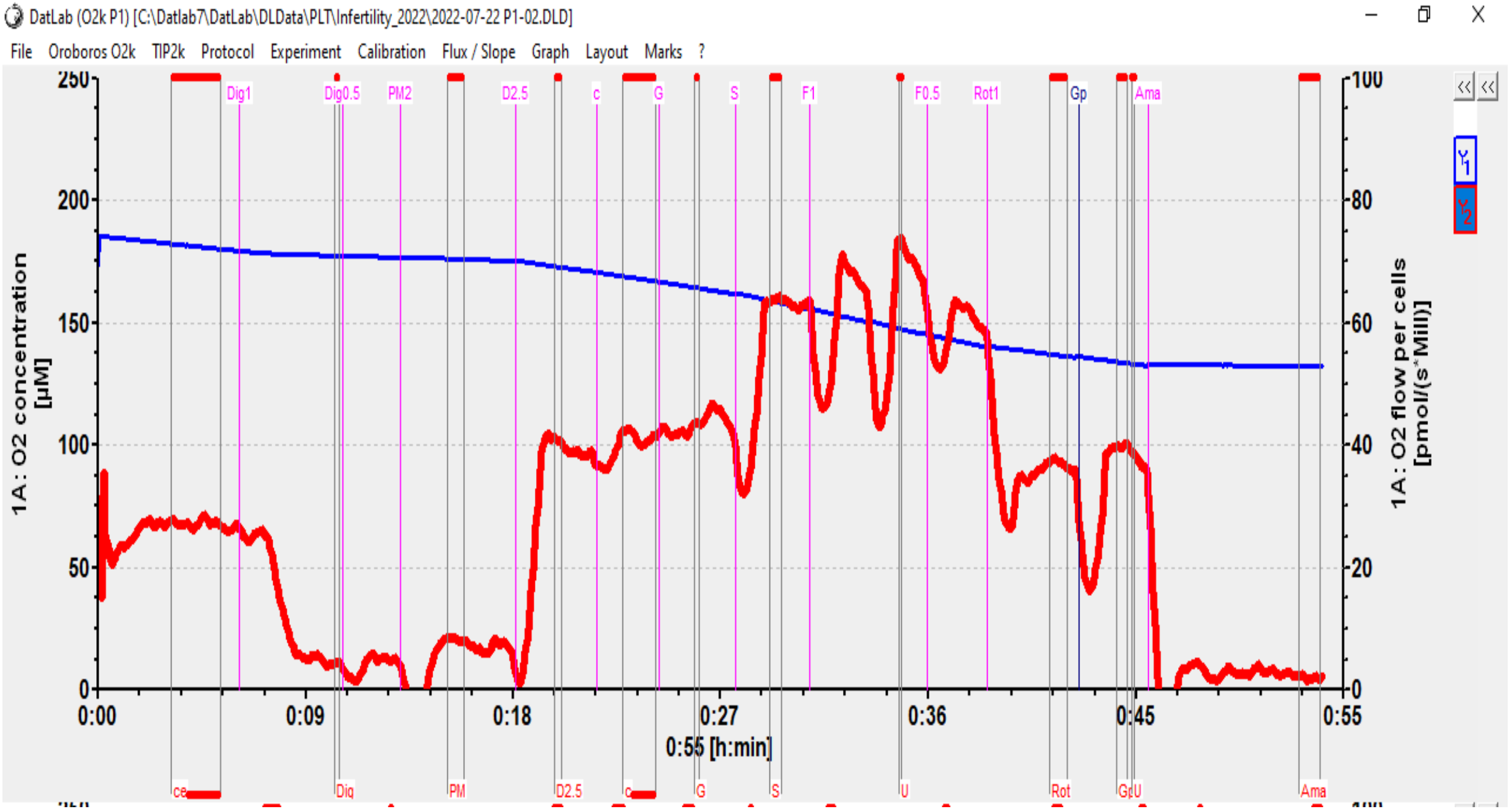


Methods	Analysis
MTT colorimetric technique	Viability of the cells
Muse® Cell Cycle Assay Kit	Distribution of cells in the individual phases of the cell cycle
Western blot analysis	Molecular mechanism
Quantitative Real-time PCR = qRT-PCR	Gene expression at the level of transcription
Mitochondrial respiration	Measurement of respiration

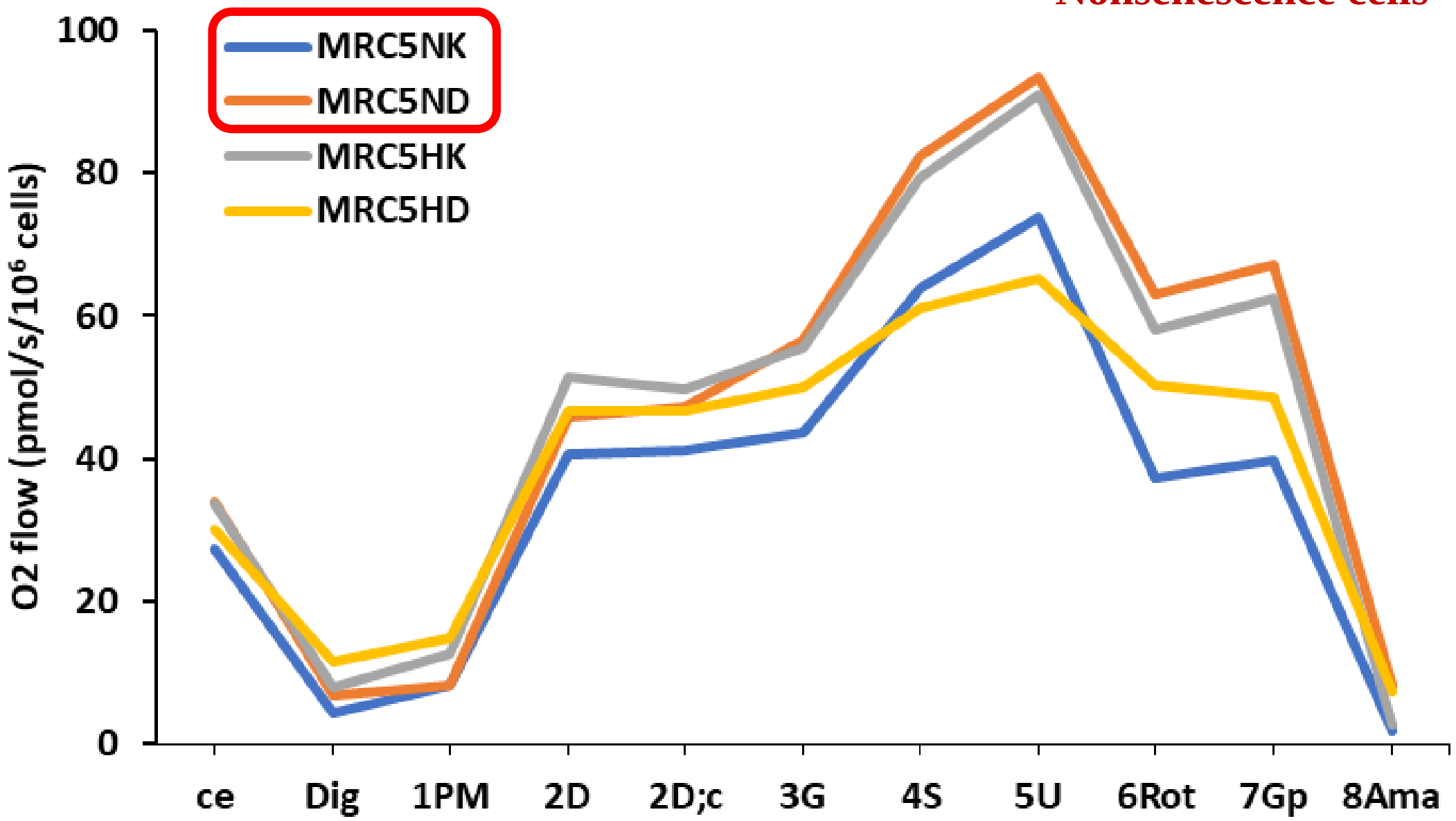
Mitochondrial respiration



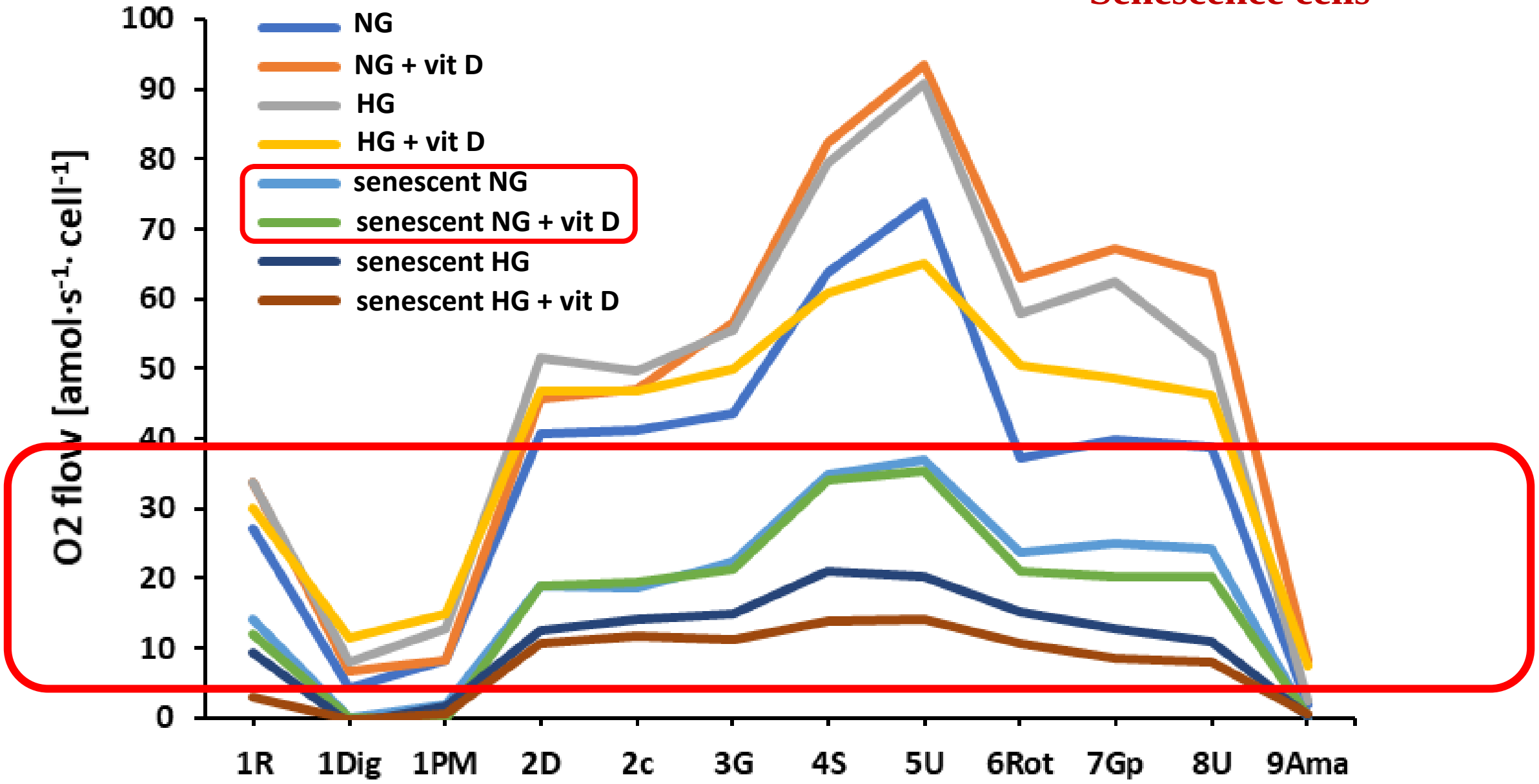



DatLab (O2k P1) [C:\Datlab7\DatLab\DLData\PLT\Infertility_2022\2022-07-22 P1-02.DLD]
File Oroboros O2k TIP2k Protocol Experiment Calibration Flux / Slope Graph Layout Marks ?
Dig1
Dig0.5
PM2
D2.5
c
G
S
F1
F0.5
Rot1
Gp
Ama
1A: O2 concentration [µM]
1A: O2 flow per cells [pmol/(s*Mill)]
Y1
Y2
0:00
0:09
0:18
0:27
0:36
0:45
0:55
0:55 [h:min]
ce
Dig
PM
D2.5
c
G
S
U
Rot
GpU
Ama

- Nonsenescence cells

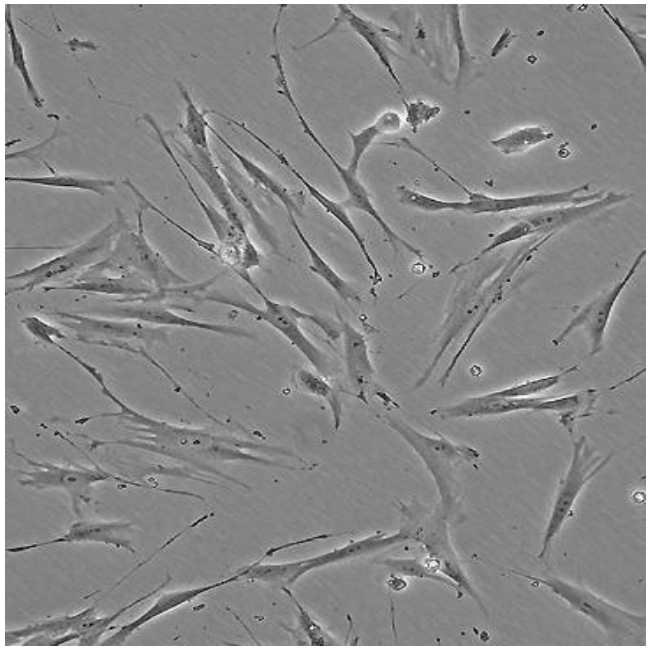


- **Senescence cells**



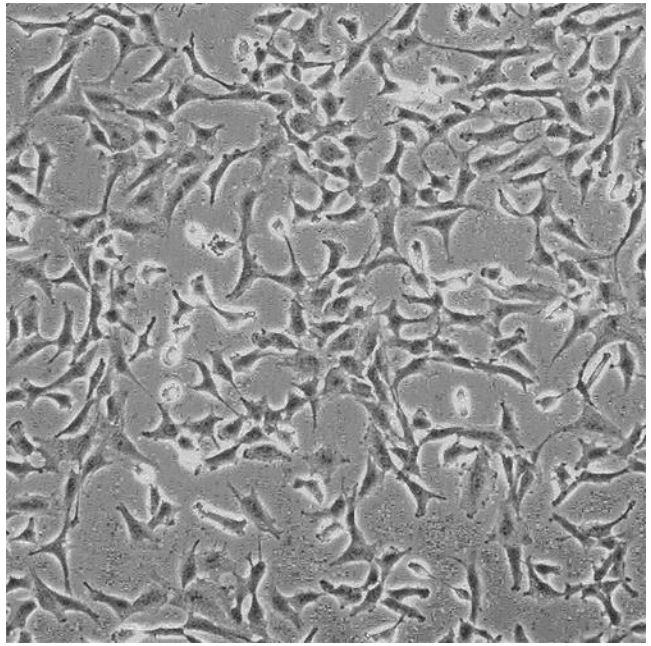
Materials and methods

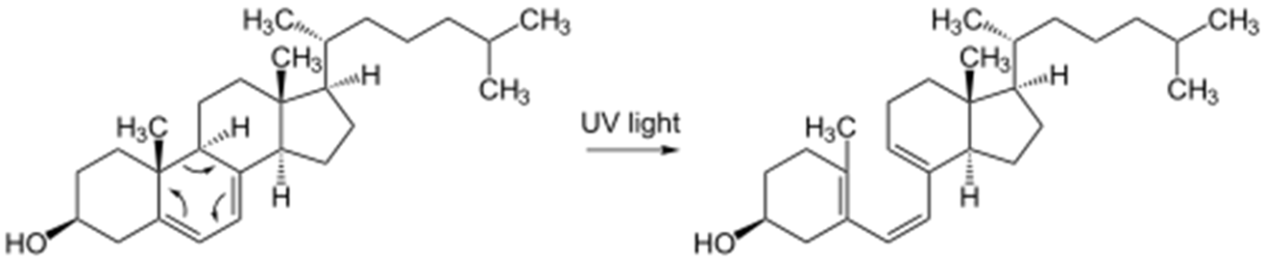
Human lung cells (**MRC-5**)



- in passage 8 (p8) as normal cells
- in passage 21 (p21) as senescent cells
- senescence induced by 100 μM H_2O_2

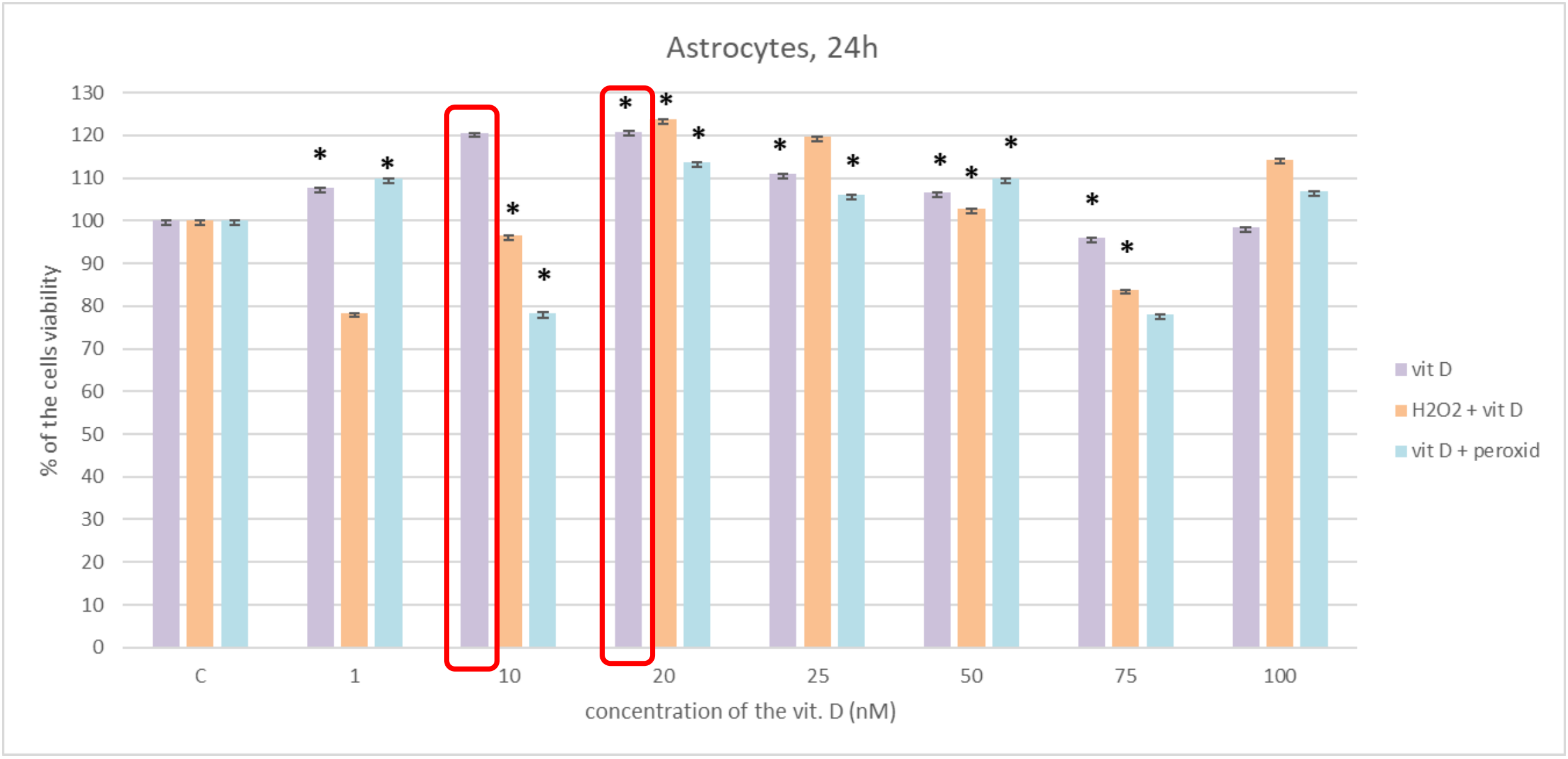
Human astrocytes (**NHA5**)

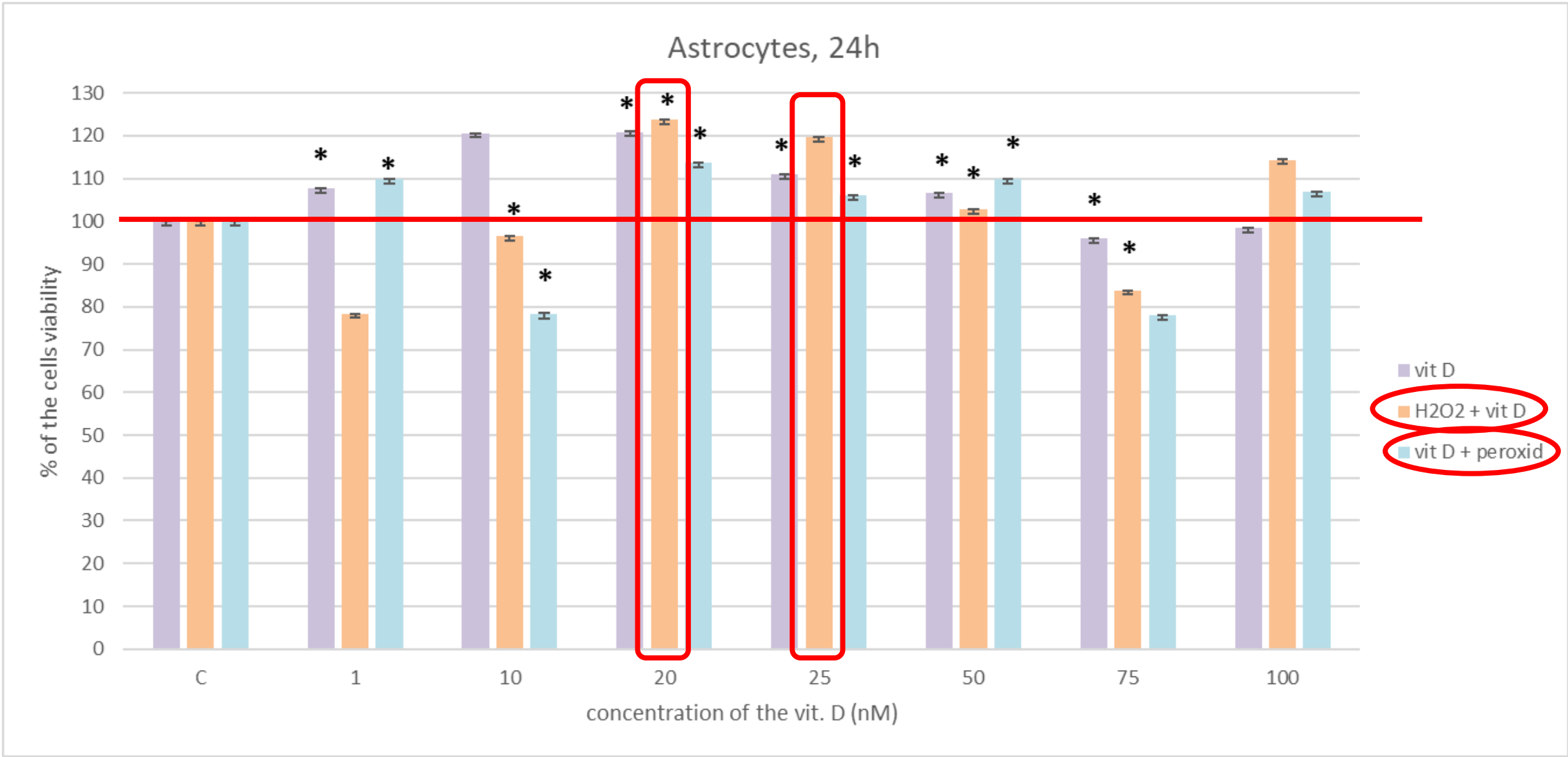


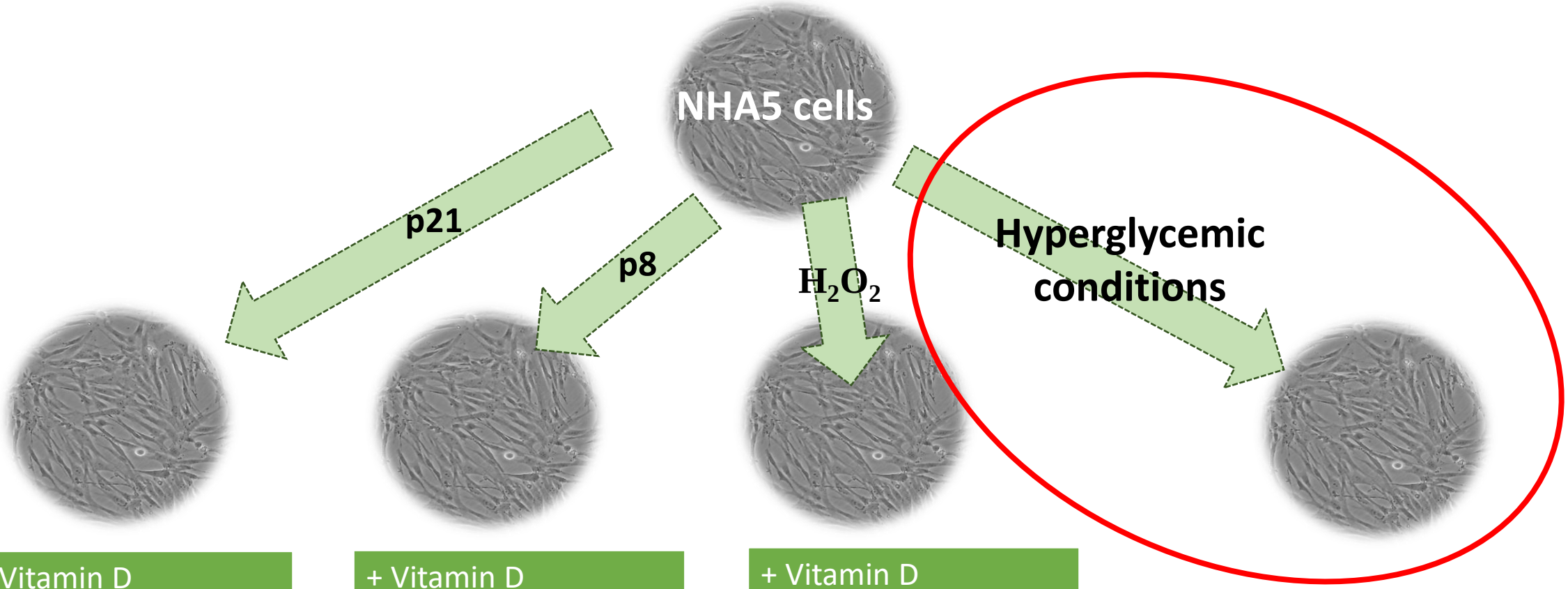


Vitamin D

- 1, 10, 20, 25, 50, 100 nM
- in DMSO
- in EtOH


Astrocytes, 24h
% of the cells viability
130
120
110
100
90
80
70
60
50
40
30
20
10
0
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
vit D
H2O2 + vit D
vit D + peroxid


Astrocytes, 24h
% of the cells viability
130
120
110
100
90
80
70
60
50
40
30
20
10
0
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
vit D
H2O2 + vit D
vit D + peroxid



+ Vitamin D
+ 24h incubation
+ medium exchange
+ incubation until 72h

+ Vitamin D
+ 24h incubation
+ medium exchange
+ incubation until 72h

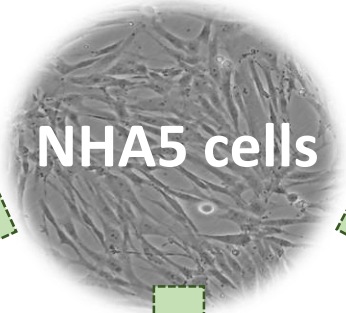
+ Vitamin D
+ 24h incubation
+ medium exchange
+ incubation until 72h

+ 24h incubation
+ vitamin D
+ medium exchange
+ incubation until 72h

+ 24h incubation
+ vitamin D
+ medium exchange
+ incubation until 72h

+ 24h incubation
+ vitamin D
+ medium exchange
+ incubation until 72h

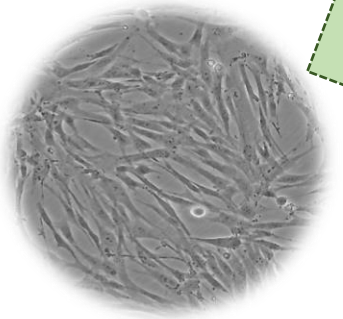
+ 24h incubation
+ vitamin D
+ medium exchange
+ incubation until 72h

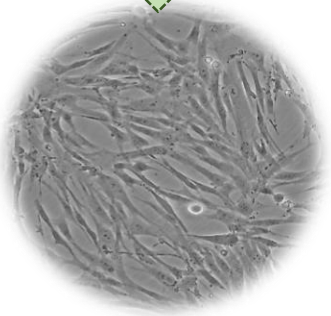


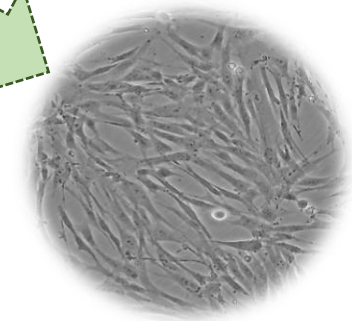
+ seeded the cells under HG conditions
+ incubated for 24 hours
+ affected cells with vitamin D for 24 hours

not hyperglycemic
NG

+ seeded the cells under normal conditions
+ incubated for 24 h
+ changed the NG conditions to HG
+ addition of vitamin D







Vitamin D

Damaged the cells with H_2O_2 and then affected vitamin D

Added vitamin D to the cells and then damaged them with H_2O_2

Vitamin D

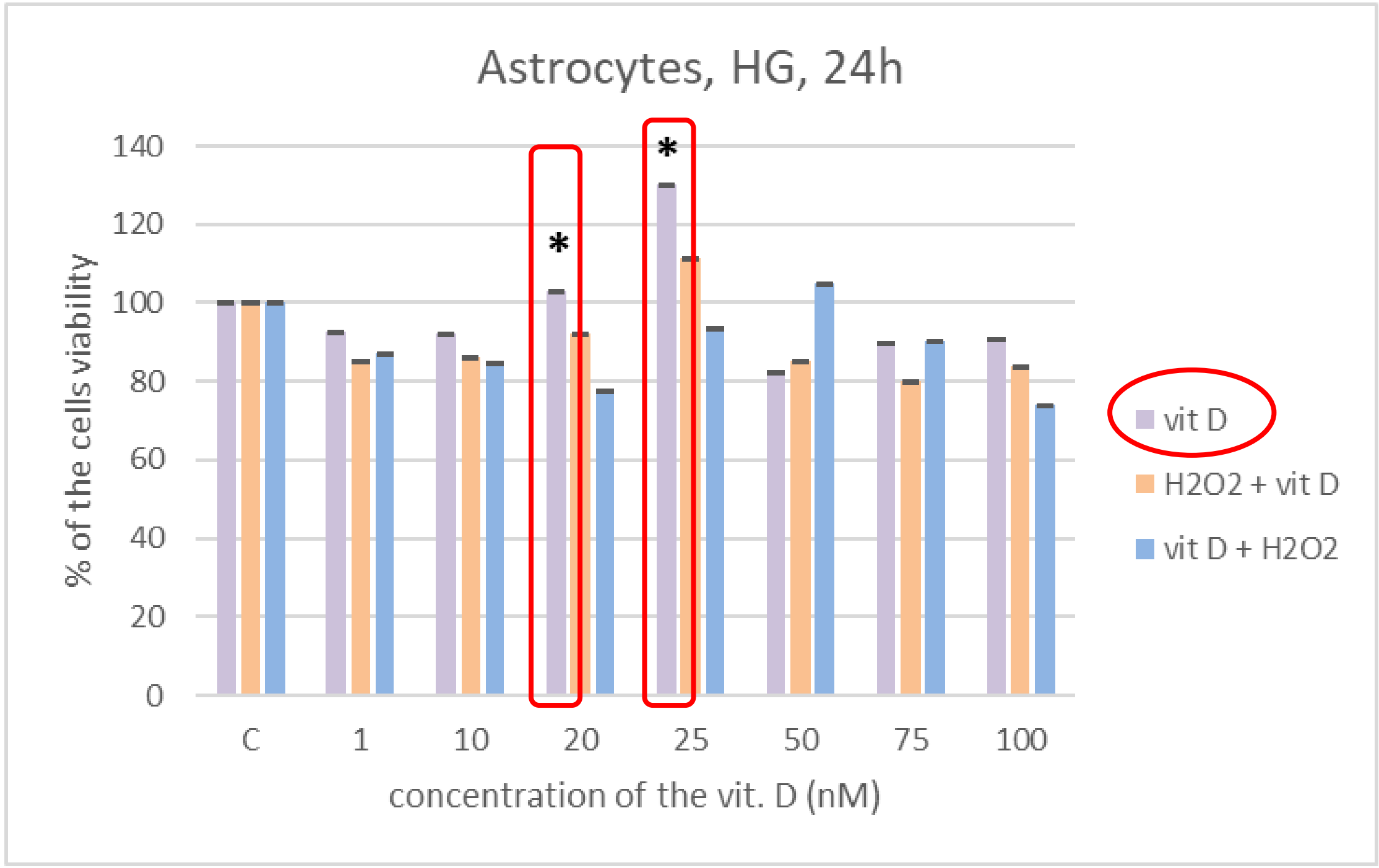
Damaged the cells with H_2O_2 and then affected vitamin D

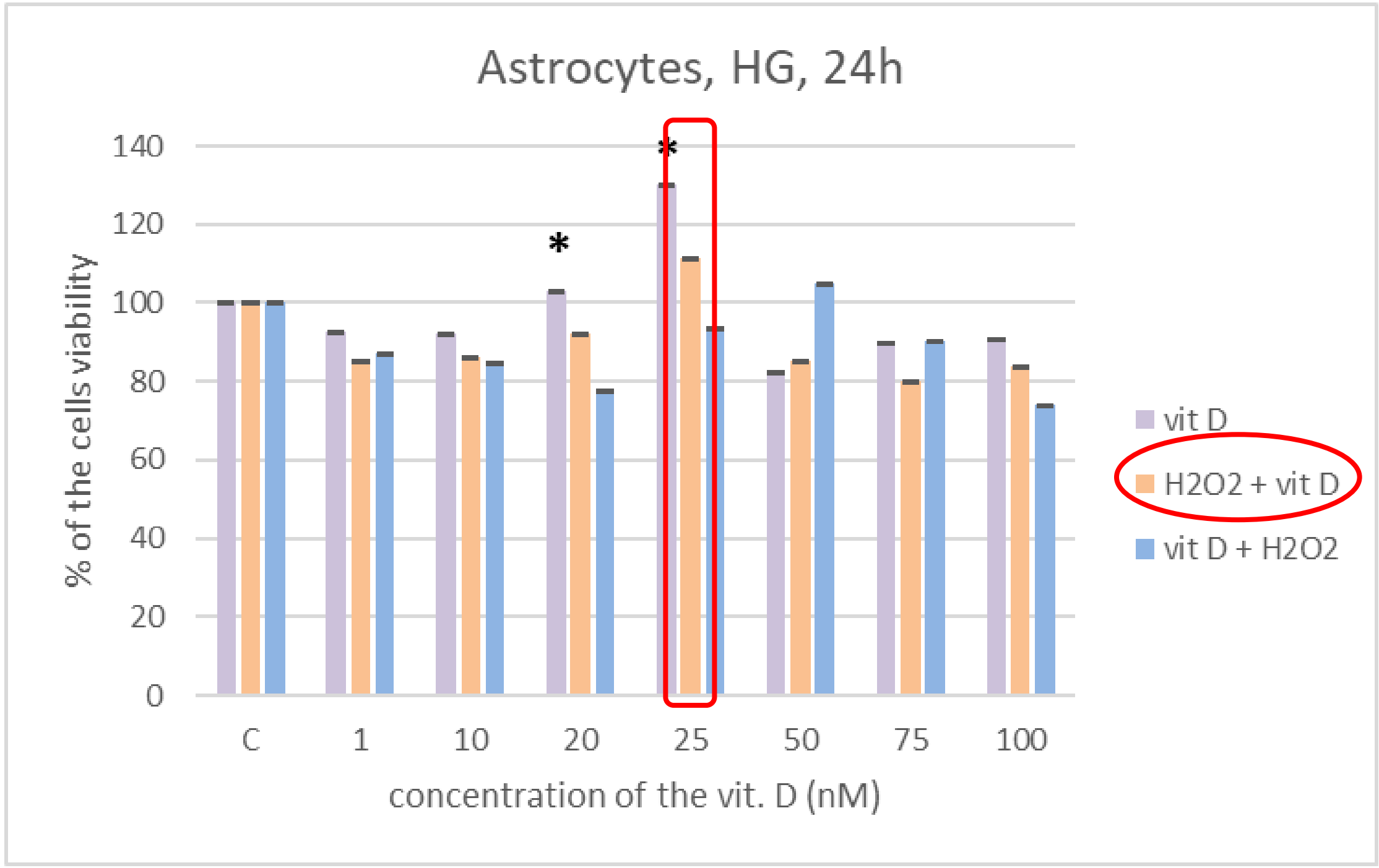
Added vitamin D to the cells and then damaged them with H_2O_2

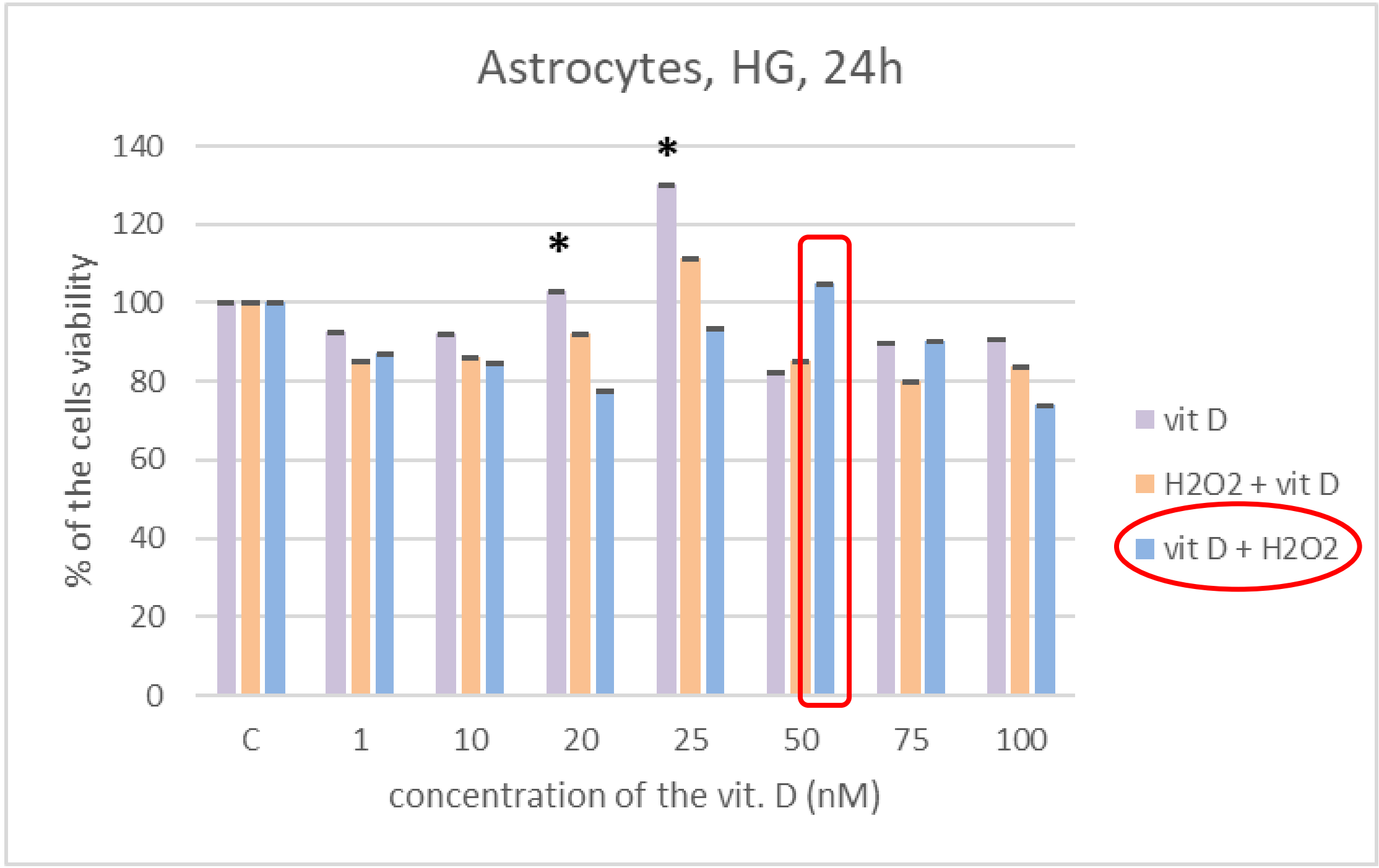
Vitamin D

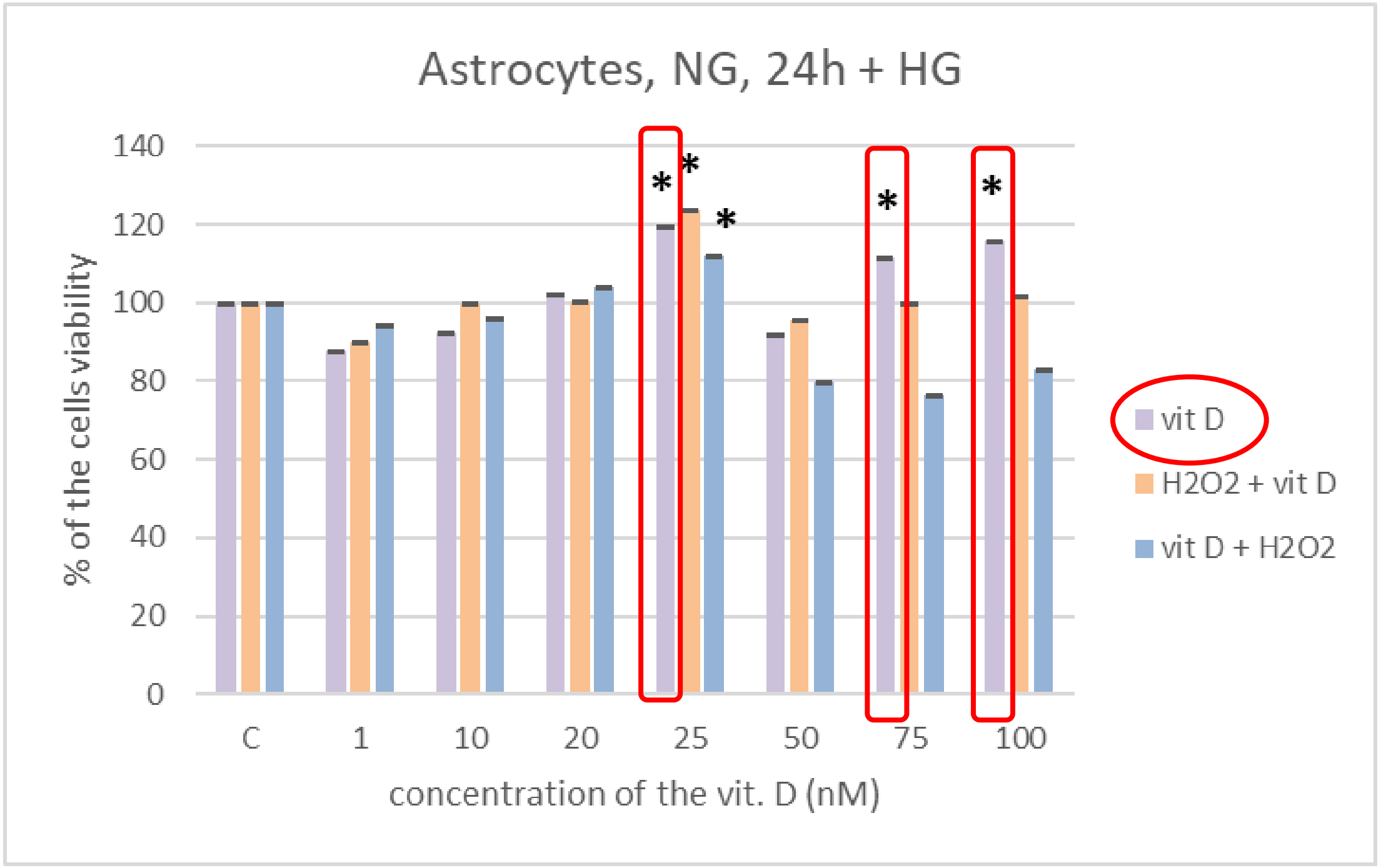
Damaged the cells with H_2O_2 and then affected vitamin D

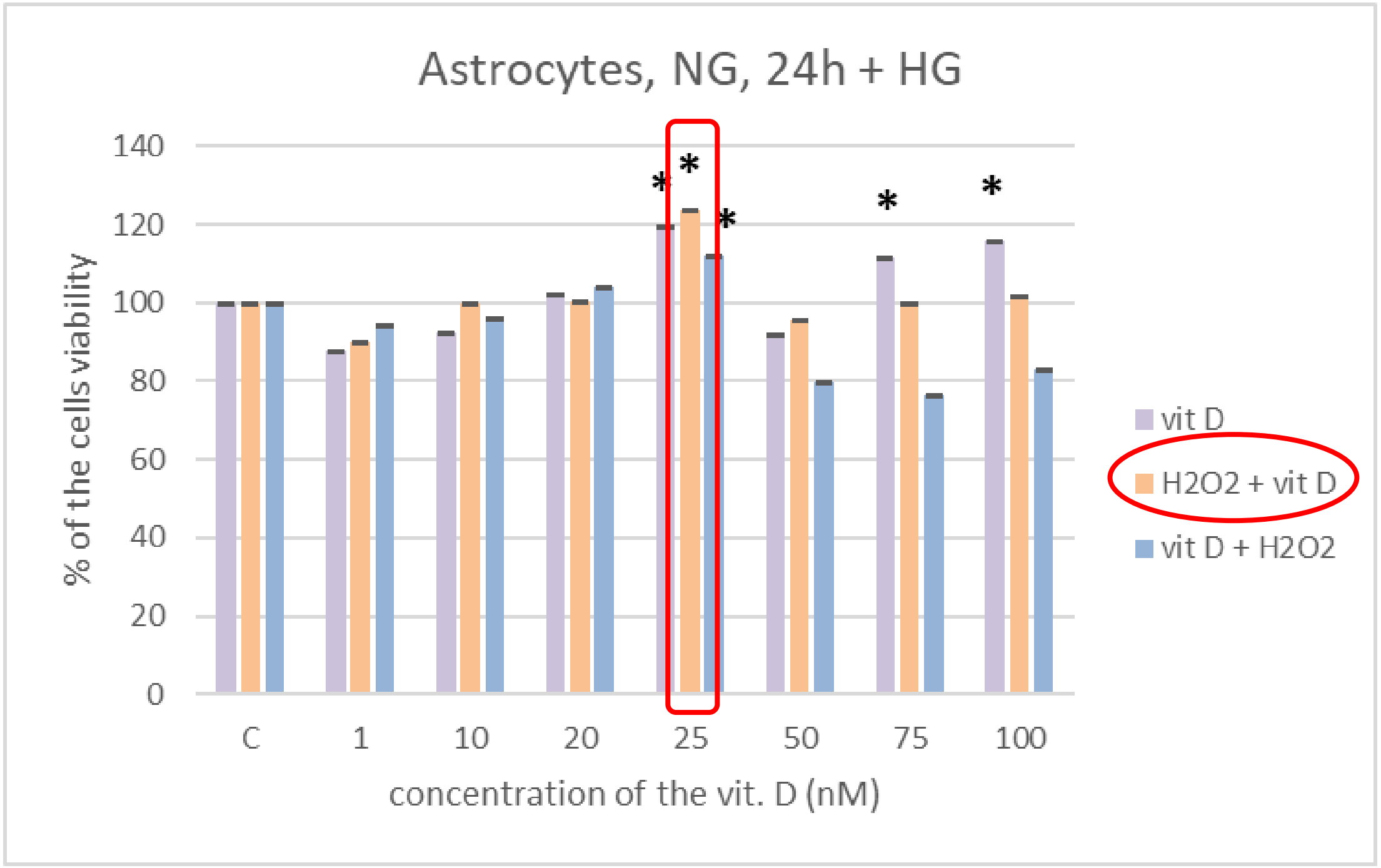
Added vitamin D to the cells and then damaged them with H_2O_2

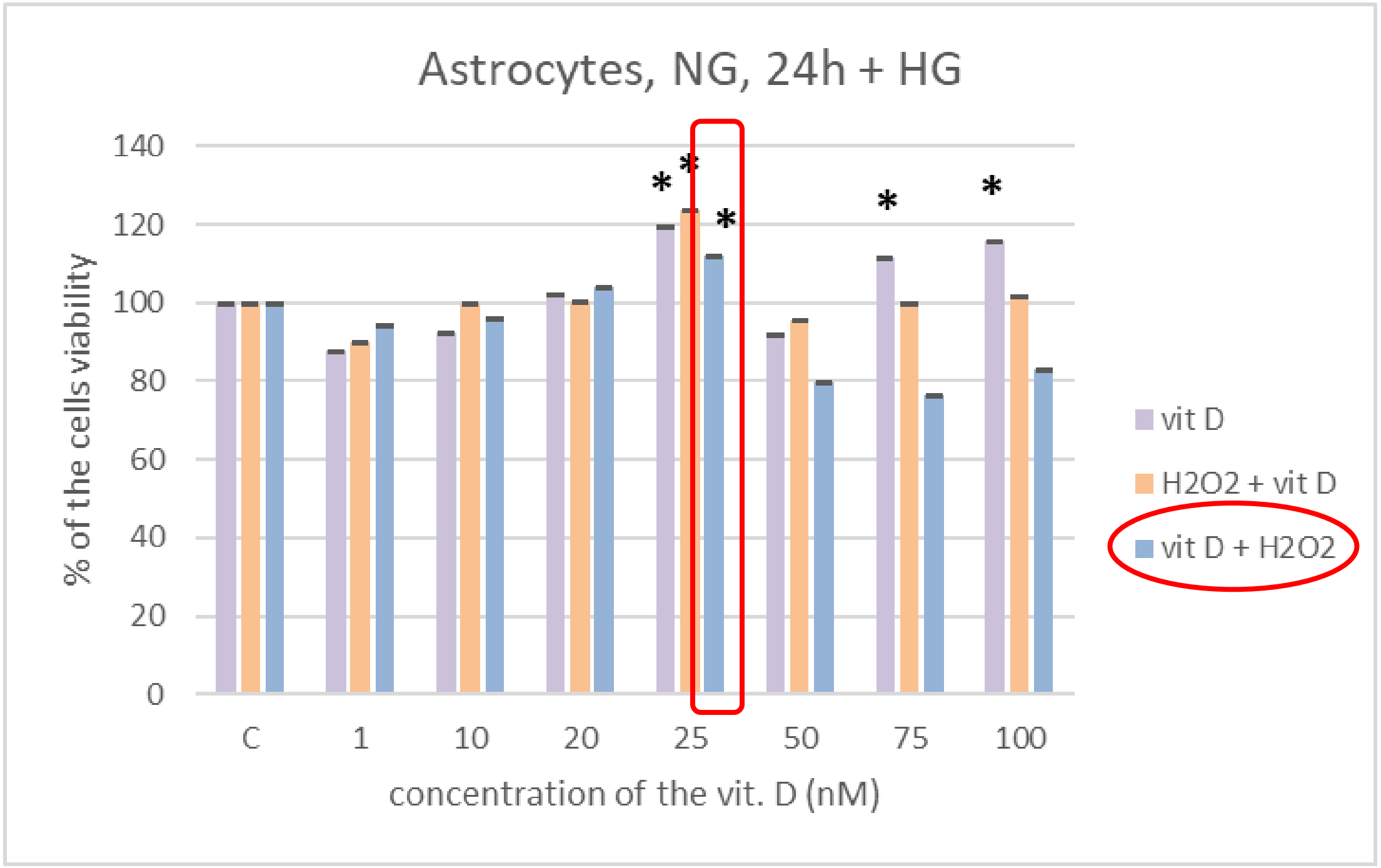

Astrocytes, HG, 24h
% of the cells viability
140
120
100
80
60
40
20
0
*
*
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
vit D
H2O2 + vit D
vit D + H2O2


Astrocytes, HG, 24h
% of the cells viability
140
120
100
80
60
40
20
0
*
*
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
vit D
H2O2 + vit D
vit D + H2O2


Astrocytes, HG, 24h
140
120
100
80
60
40
20
0
% of the cells viability
*
*
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
vit D
H2O2 + vit D
vit D + H2O2


Astrocytes, NG, 24h + HG
% of the cells viability
140
120
100
80
60
40
20
0
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
vit D
H2O2 + vit D
vit D + H2O2


Astrocytes, NG, 24h + HG
% of the cells viability
140
120
100
80
60
40
20
0
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
*
*
*
*
*
vit D
H2O2 + vit D
vit D + H2O2


Astrocytes, NG, 24h + HG
140
120
100
80
60
40
20
0
% of the cells viability
C
1
10
20
25
50
75
100
concentration of the vit. D (nM)
vit D
H2O2 + vit D
vit D + H2O2

Summary

In senescent MRC -5 cells vitamin D in concentration of 25 nM:

- Positively affected cell viability compared to control cells that were not affected by vitamin D
- We observed a slight decrease of cells in the G1 phase compared to the control and an increase in G2/M phase
- It reduced the expression of the p21 gene at the transcriptional and protein levels

Summary

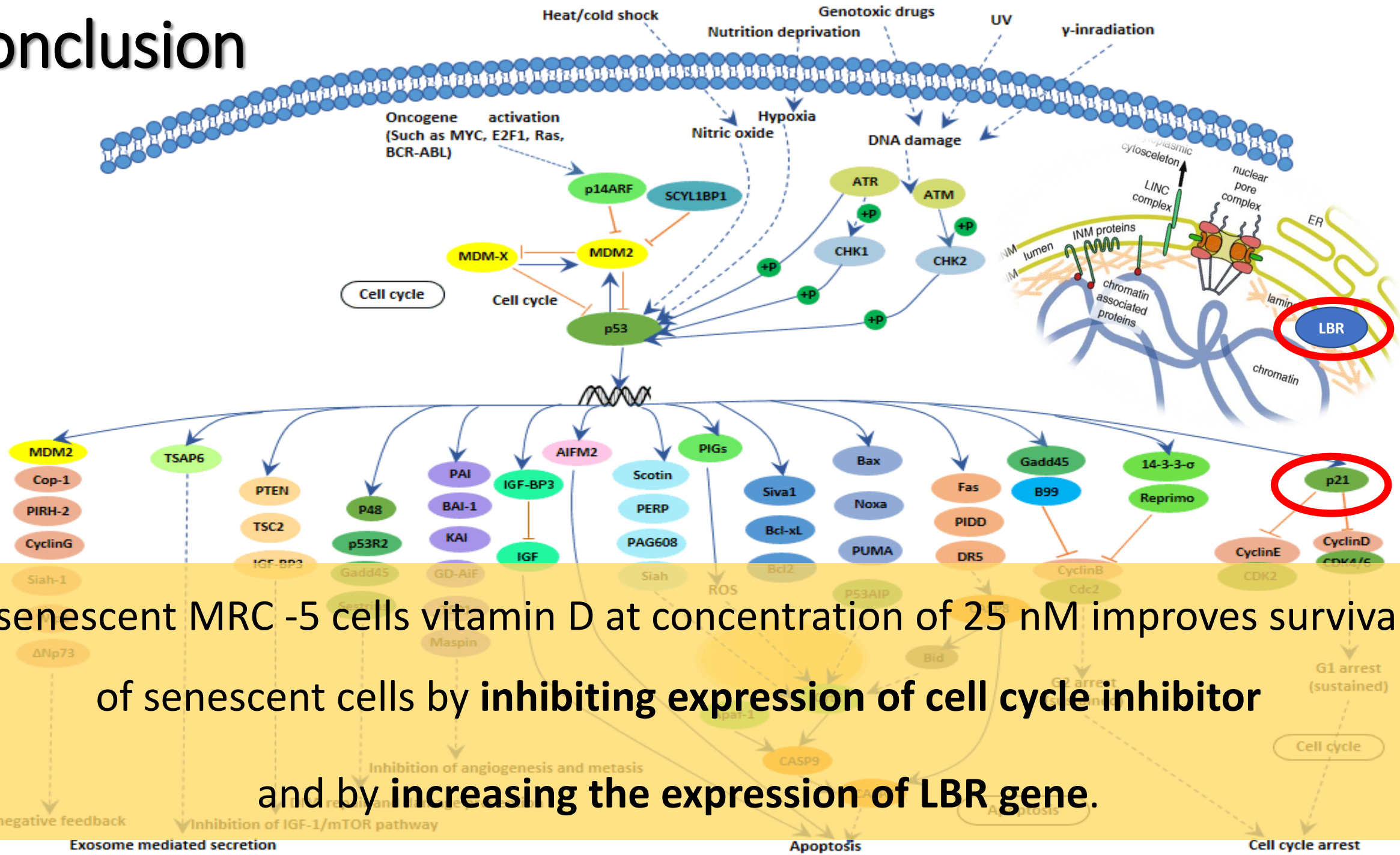
In senescent MRC -5 cells vitamin D in concentration of 25 nM:

- It reduced the expression of the p53 gene at the protein level
- It increased the expression of the LBR gene at the transcriptional level
- Vitamin D reduced LC3 protein levels

In senescence astrocytes vitamin D in concentrations of 25 a 50 nM:

- Positively affected cell viability

Conclusion



In senescent MRC -5 cells vitamin D at concentration of 25 nM improves survival of senescent cells by **inhibiting expression of cell cycle inhibitor** and by **increasing the expression of LBR gene**.

Thank you to my colleagues for their support in carrying out these experiments:

Ingrid Žitňanová, Mária Janubová, Zuzana Sumbalová

Thank you for your attention

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