Replacing animal testing by genotoxicity and carcinogenic cell transformation assessment using a tissue regenerating cell line from fish with GFP-labelled histones

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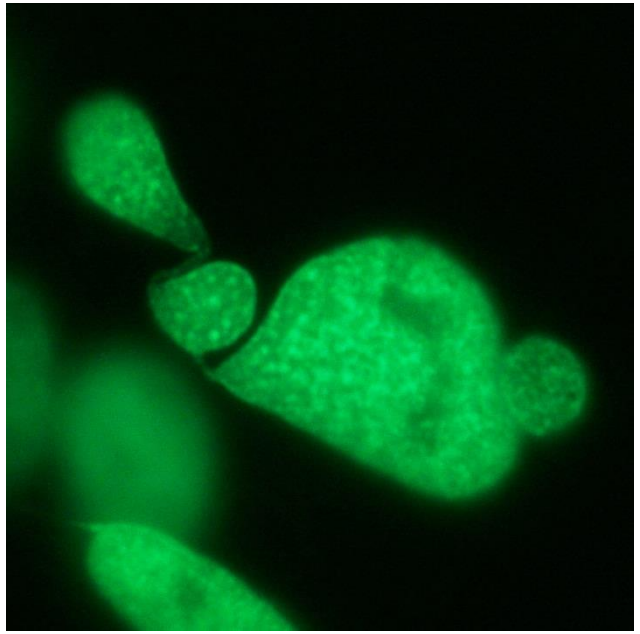
D Derichsweiler, K Pfannkuche, B Allner

UNIKLINIK KÖLN



GOBIO

Outline

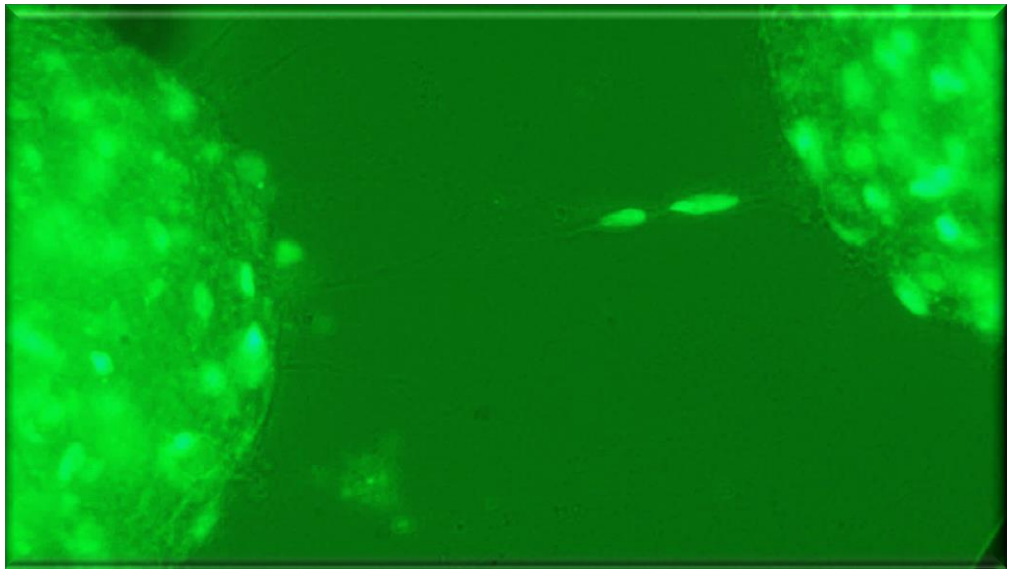
Background Research Approach

Biology of the of the presented cell line

Standard method versus H2BGFP Cells

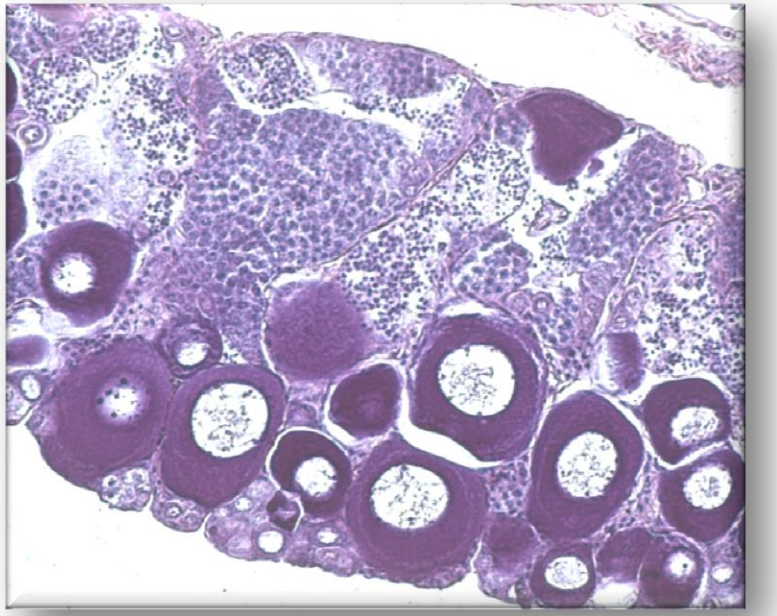
Downstream analyses of cell transformation

What is stemness - what is cancer?



Background Research Approach

Ambisexual fish



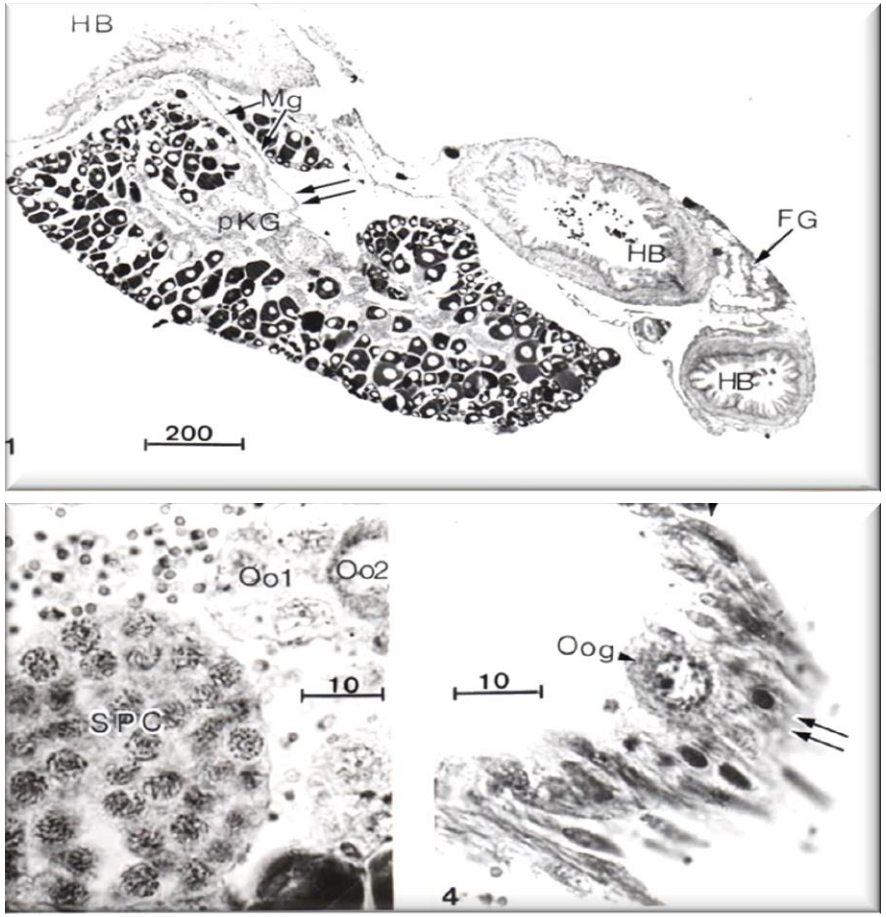
Regeneration

Gonadal recrudescence

Pluripotent cells livelong available: seasonal sexual maturation from primordial germ cells to mature gonads

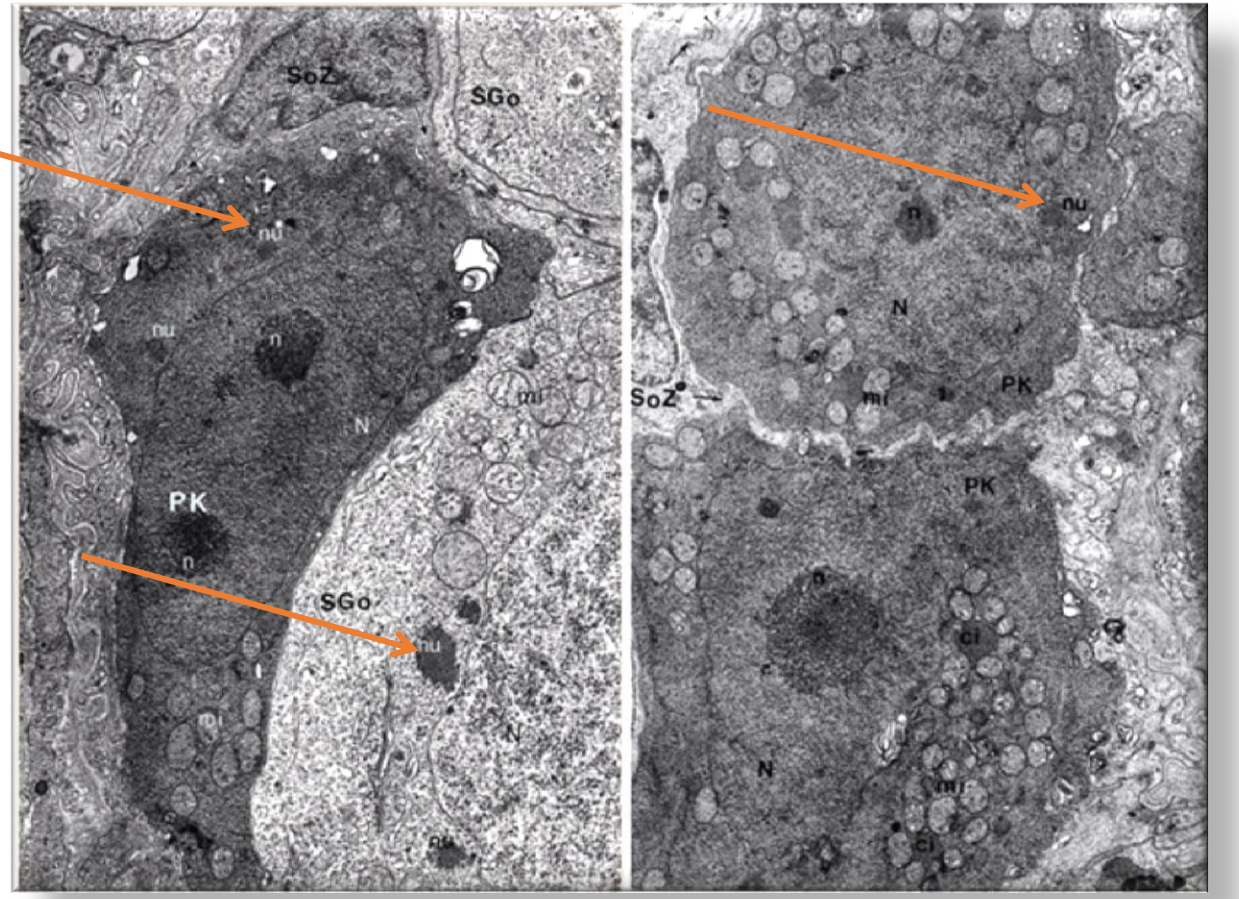
Background Research Approach

Anatomy / Histology

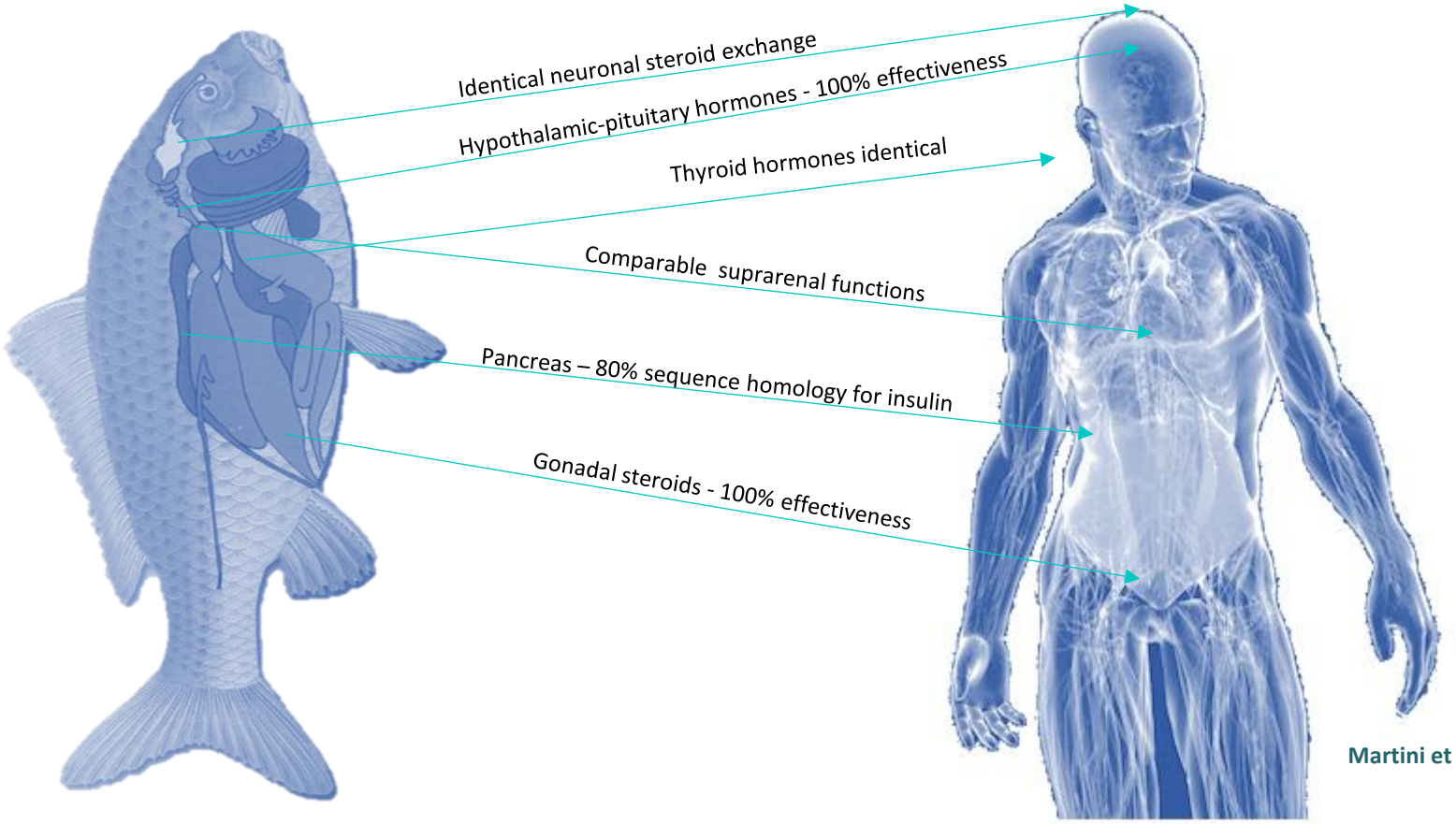


Ultrastructure

vasa



Background Research Approach



Martini et al., 2011. Human Anatomy Pearson Ed. Lim..
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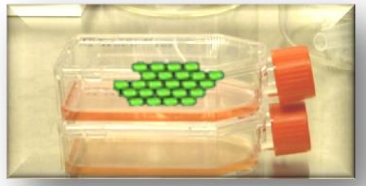
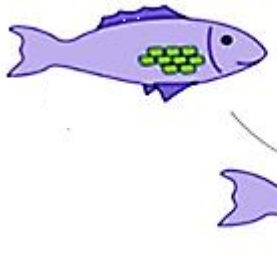
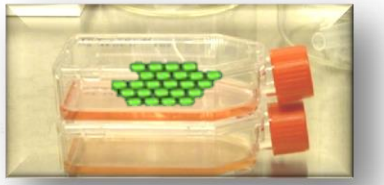
Biology of the Presented Cell Line

KCB-GFP = DSM ACC3285 Budapest treaty
26°C growth temperature / no CO_2

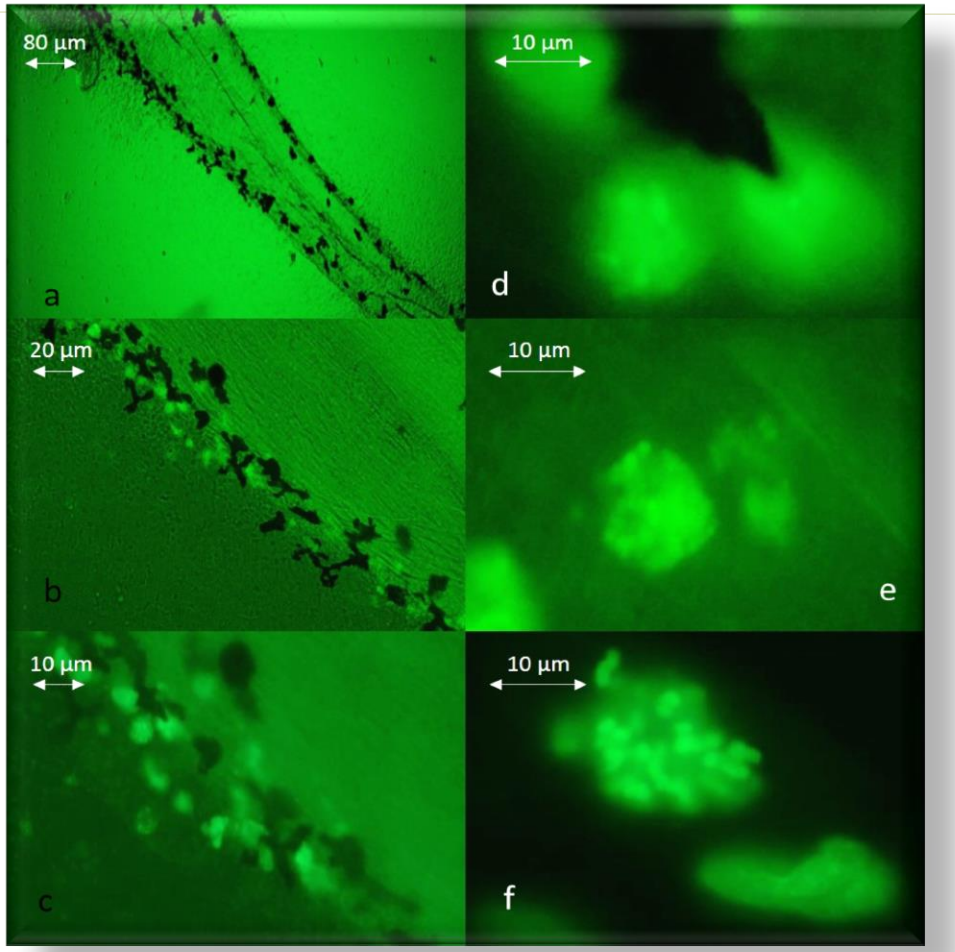




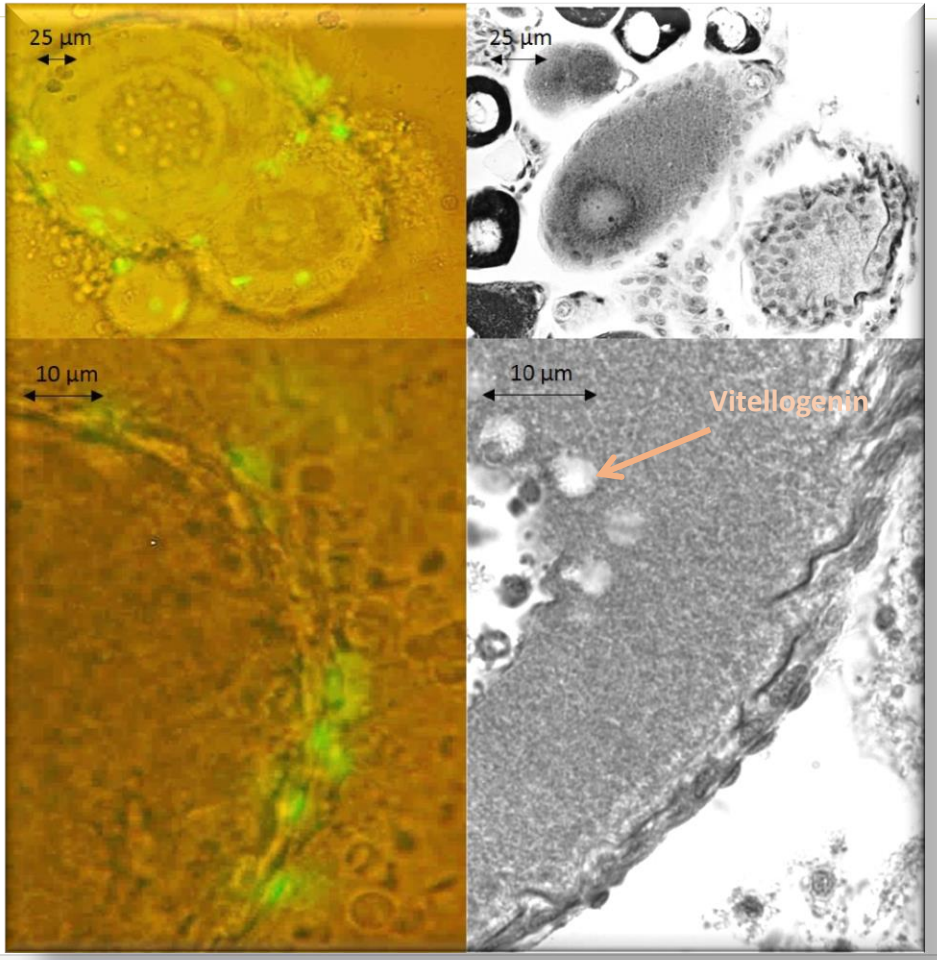


Biology of the Presented Cell Line

Functional after transplantation



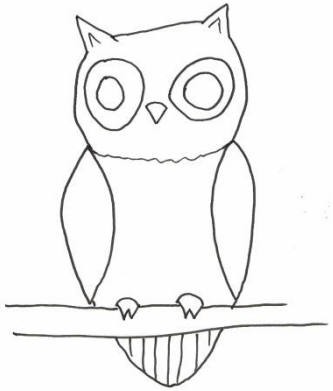
Co-culture differentiation



Background Research Approach

The GOBIO approach:

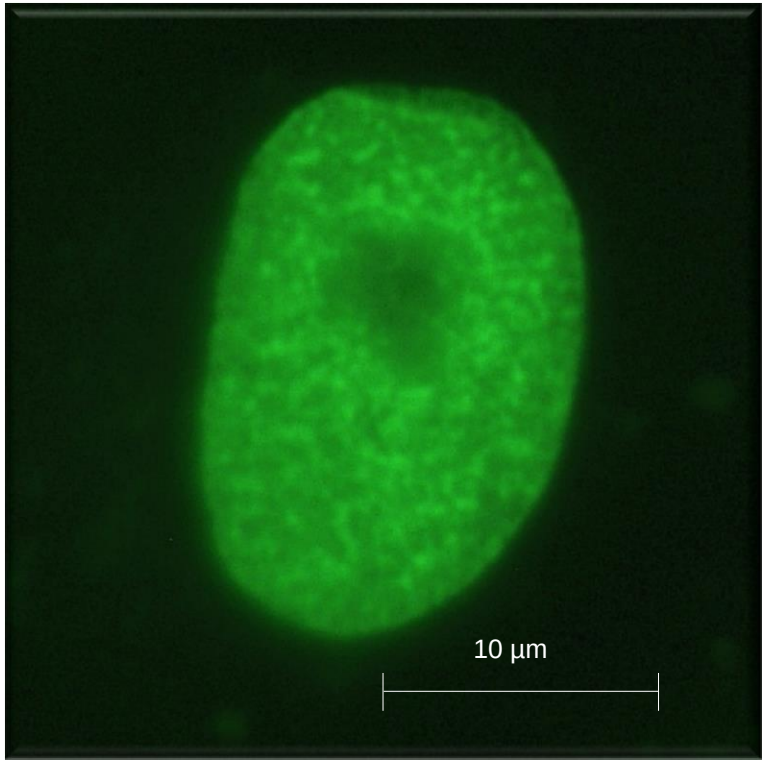
„CNS-derived motile pluripotent stem cells regenerate and sustain normal function of peripheral organs livelong in vertebrates

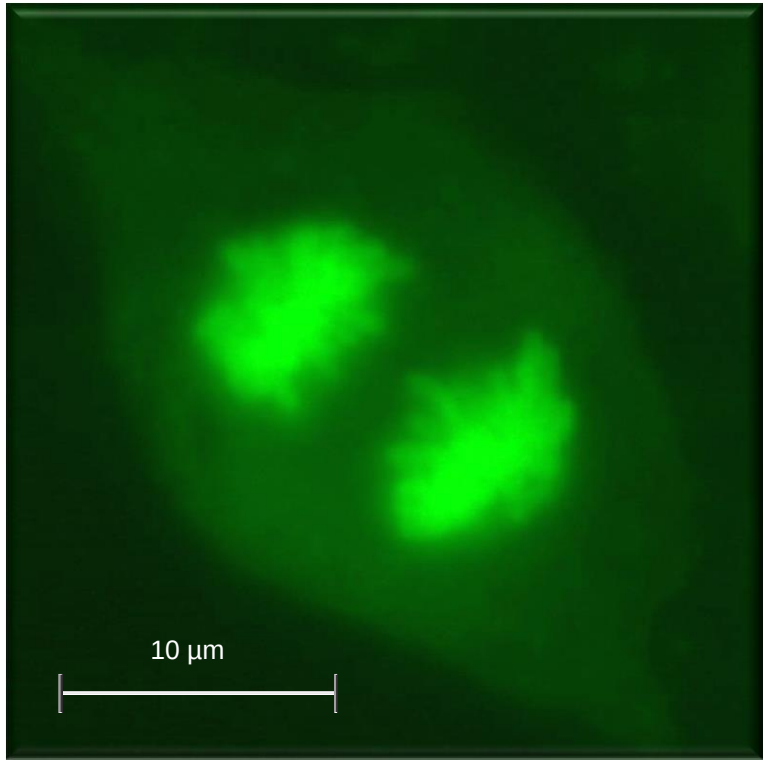


Athene concept

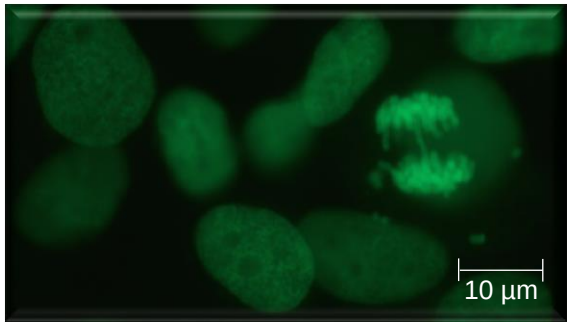
Investigation of driving factors sustaining health instead of simulation of disease “

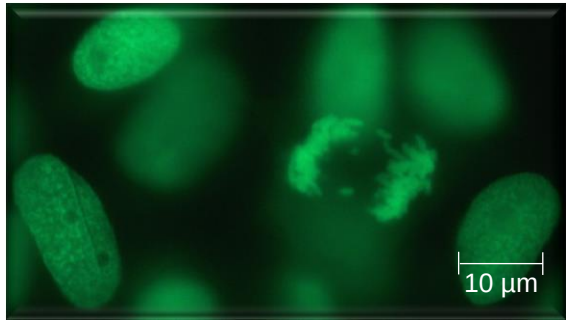
Visualization of Nuclear Structures

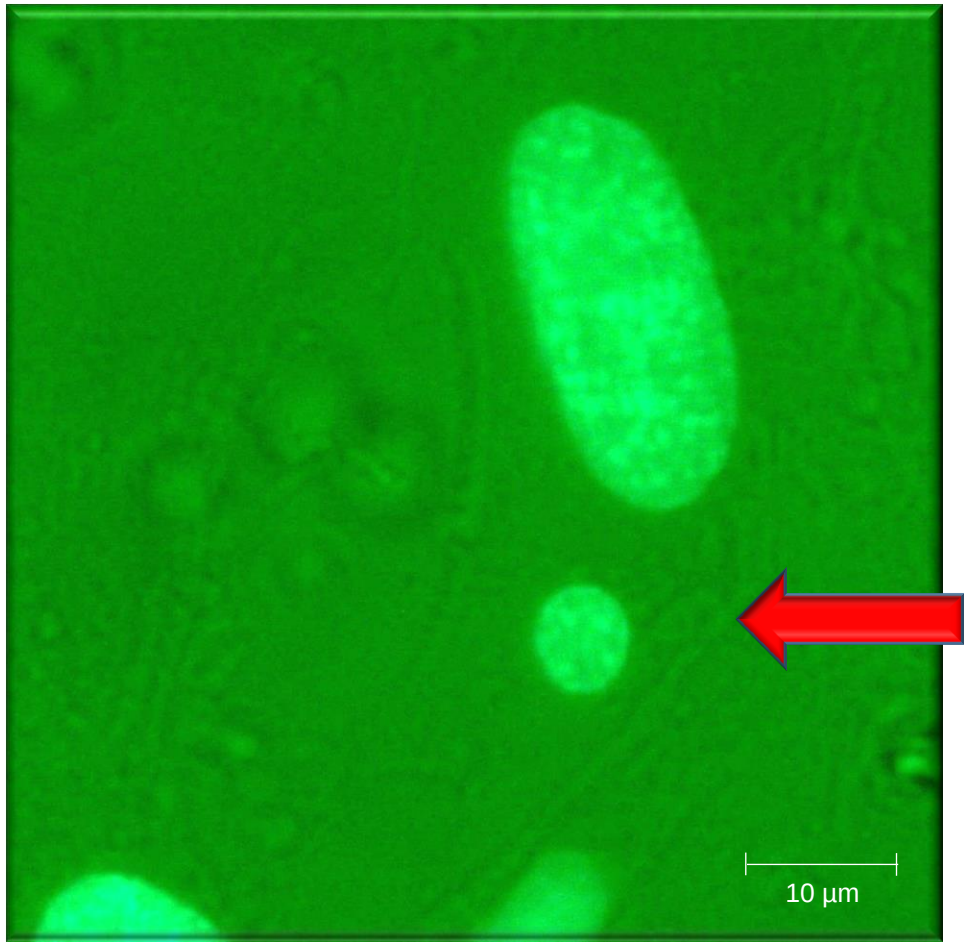




Visualization of Genotoxic Damages





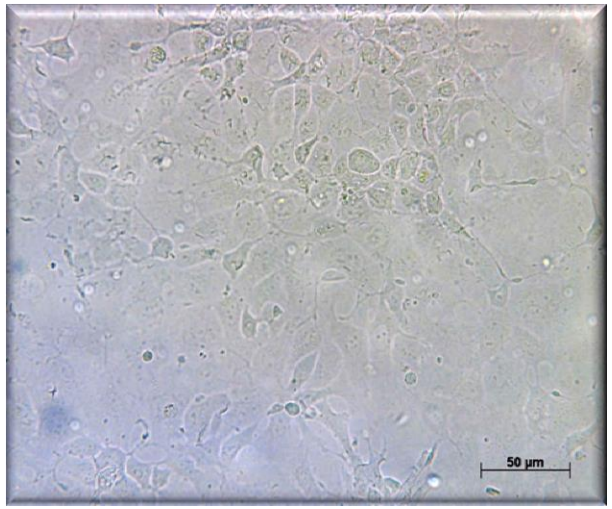


Standard Method versus H2BGFP Cells

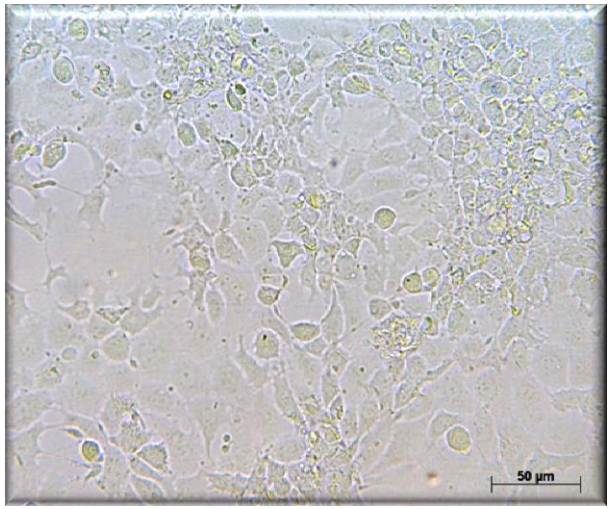
Genotoxicity is not detectable in living cells
mammalian cells used in standard methods are immortal (tumor derived or hybridomas)

KCB H2B Cells can be transformed, cell death takes place

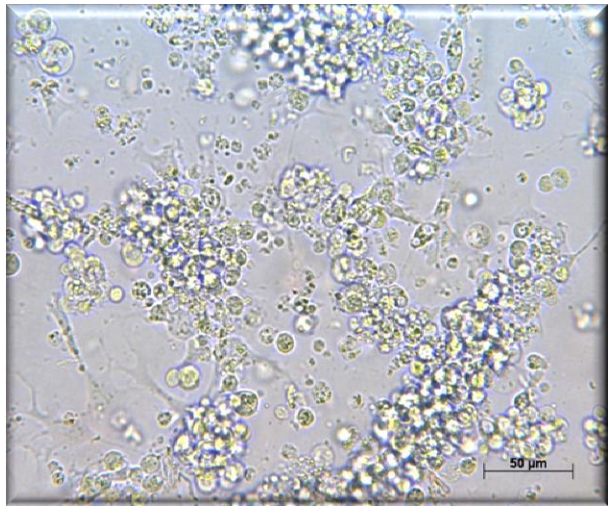
normal



genotoxic affected ?

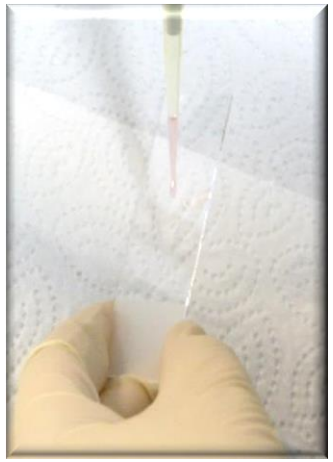


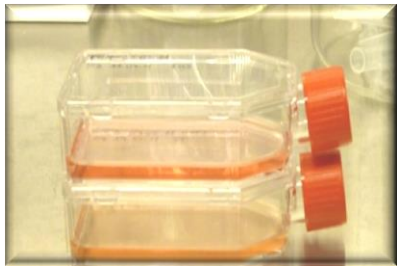
cytotoxic

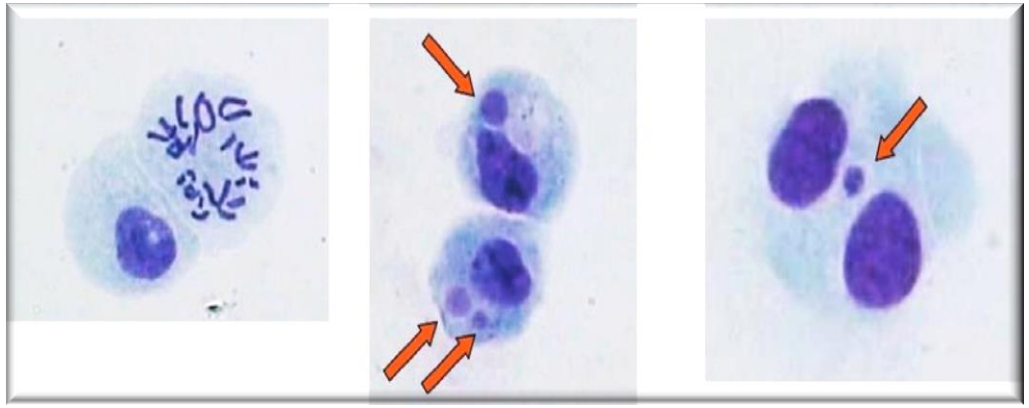


Standard Method versus H2BGFP Cells

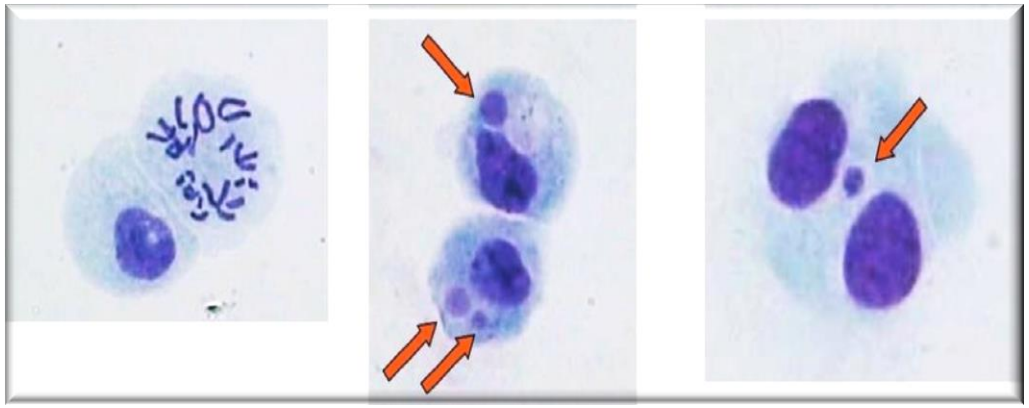
- Stock culture of the cells / Storage of cells
- Pre-culture in flasks
- Transfer of cells to plates and exposure to test compounds
- Transfer of cells onto microscope slides
- Cytological staining
- Microscopic scoring of MN-frequency





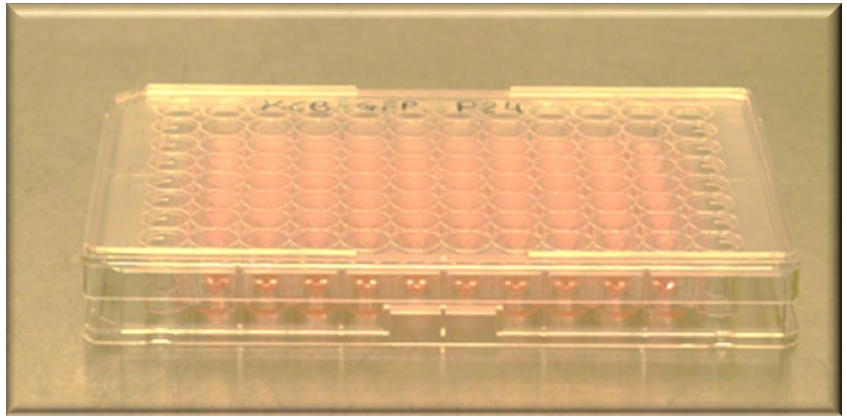


Standard Method versus H2BGFP Cells



Standard Method versus H2BGFP Cells

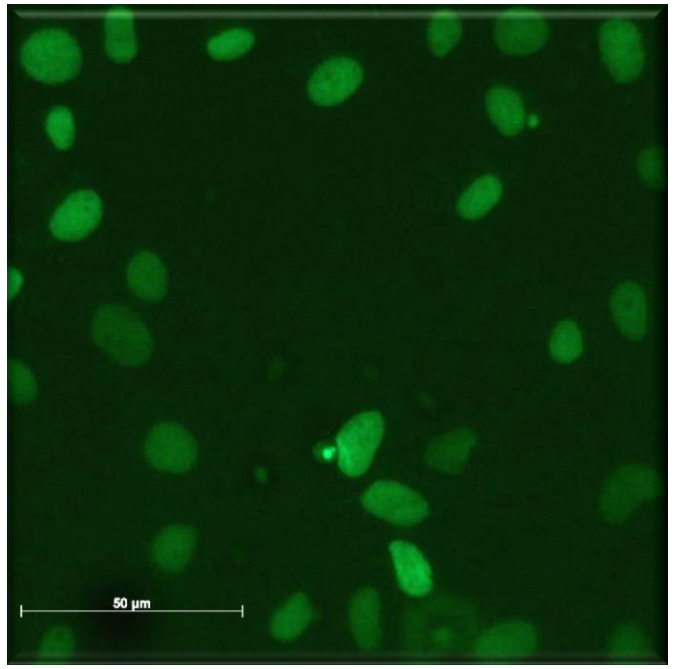
A Novel Approach

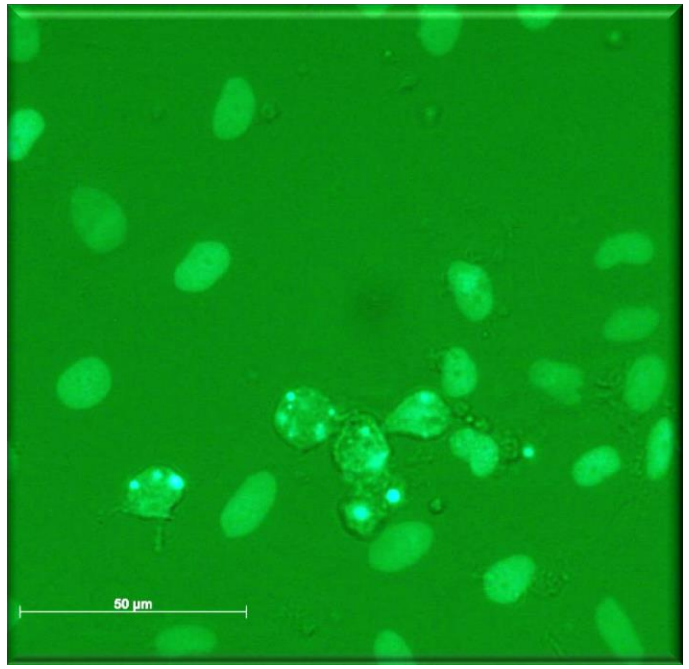


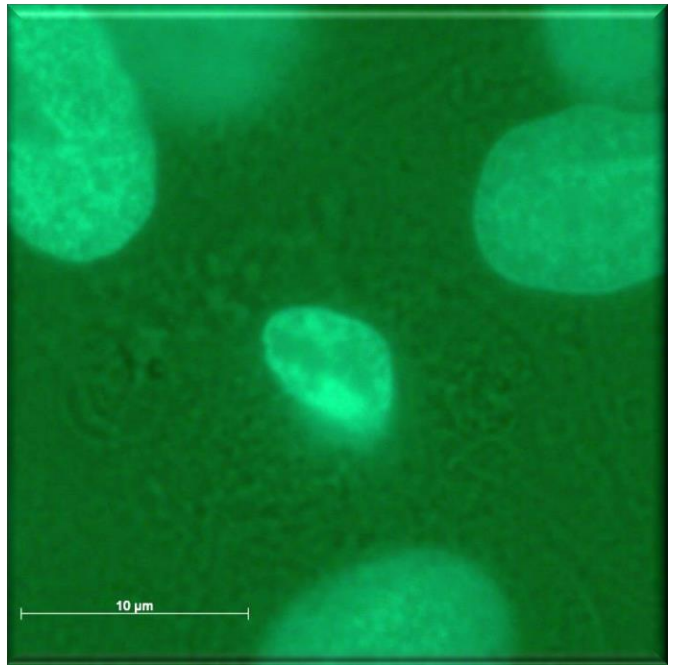
Cell coated plates stored at 4° C

- Replace storage medium through recovery medium
- Let the cells recover and grow to 60% confluence
- Expose cells to test compounds
- Replace exposure media through growth medium
- Score MN frequency
- Passage cells for downstream analyses

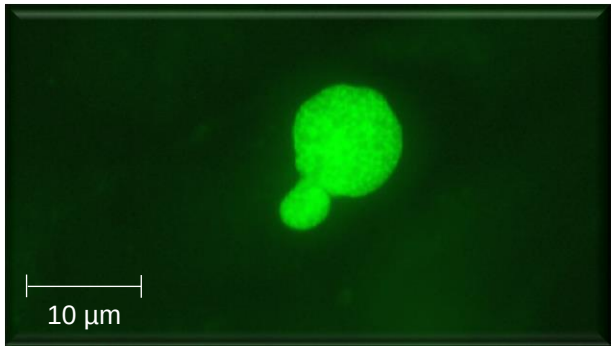
Standard Method versus H2BGFP Cells





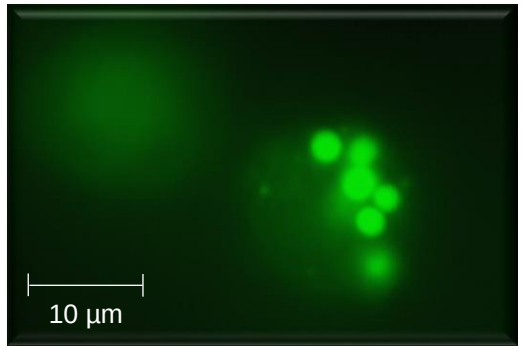


Standard Method versus H2BGFP Cells

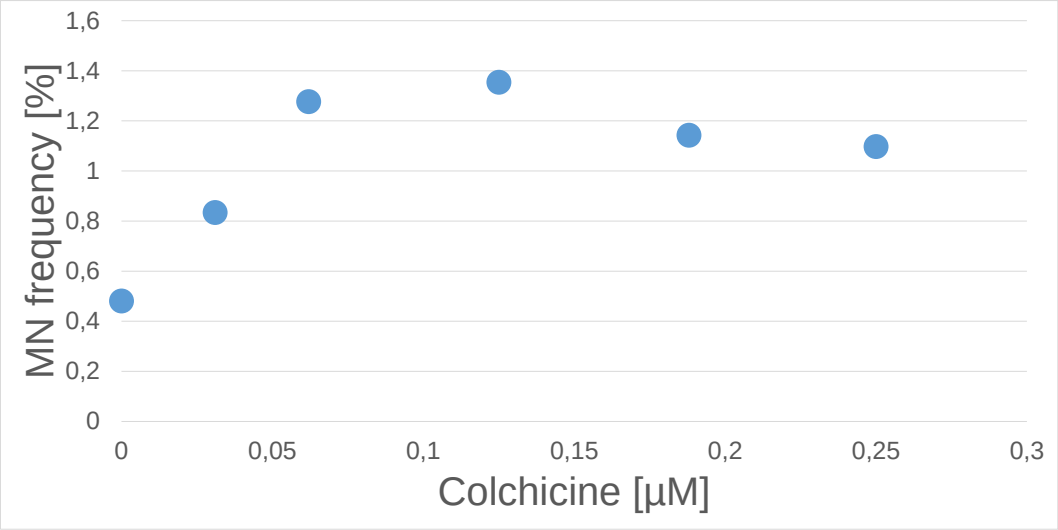


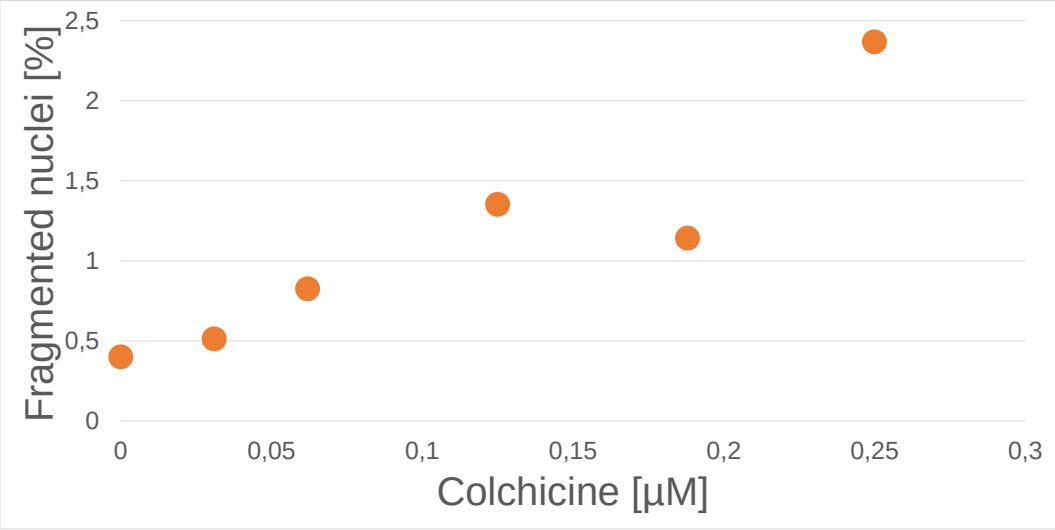
Colchicine
(Aneugen)

Micronucleus frequency



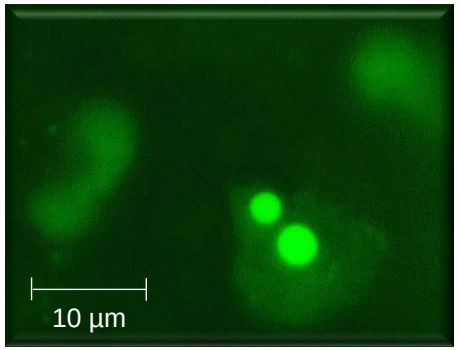
Frequency of fragmented nuclei



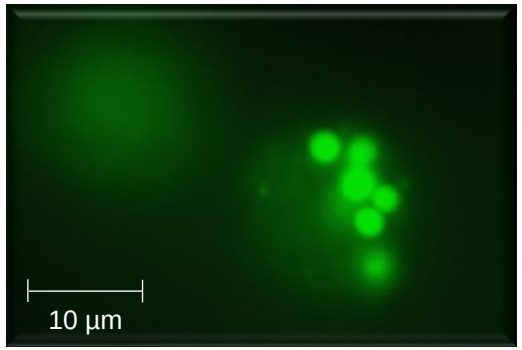


Standard Method versus H2BGFP Cells

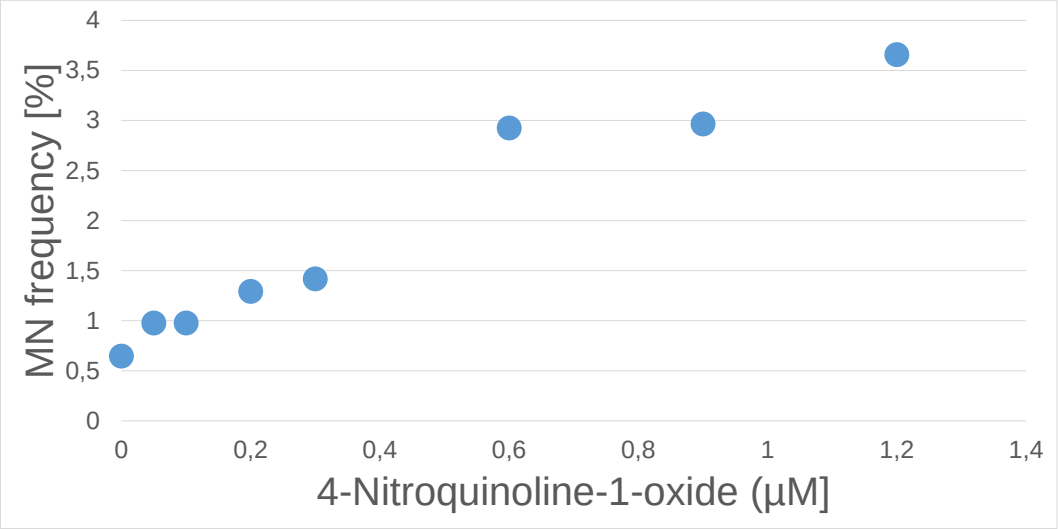
4-Nitroquinoline-1-oxide
(Clastogen)

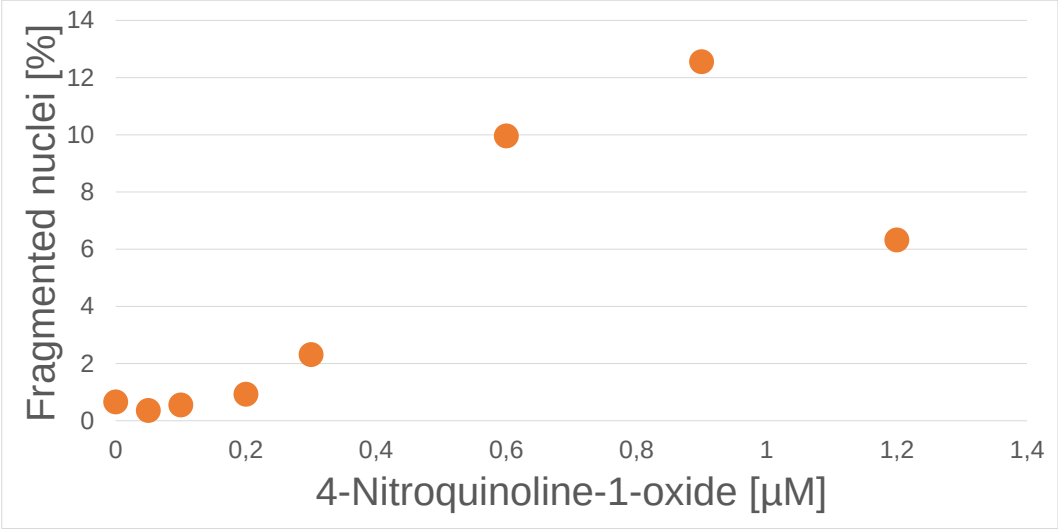


Micronucleus frequency

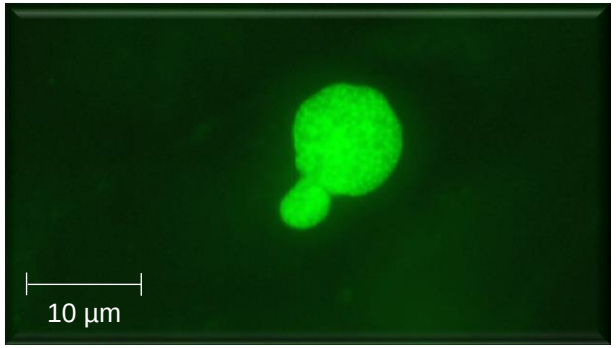


Frequency of fragmented nuclei

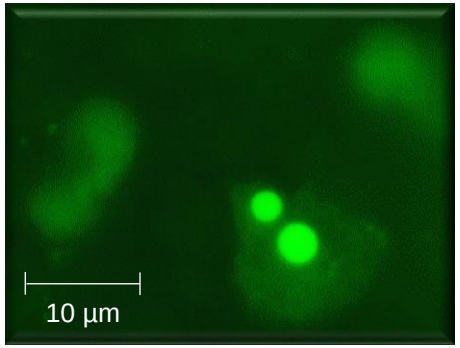




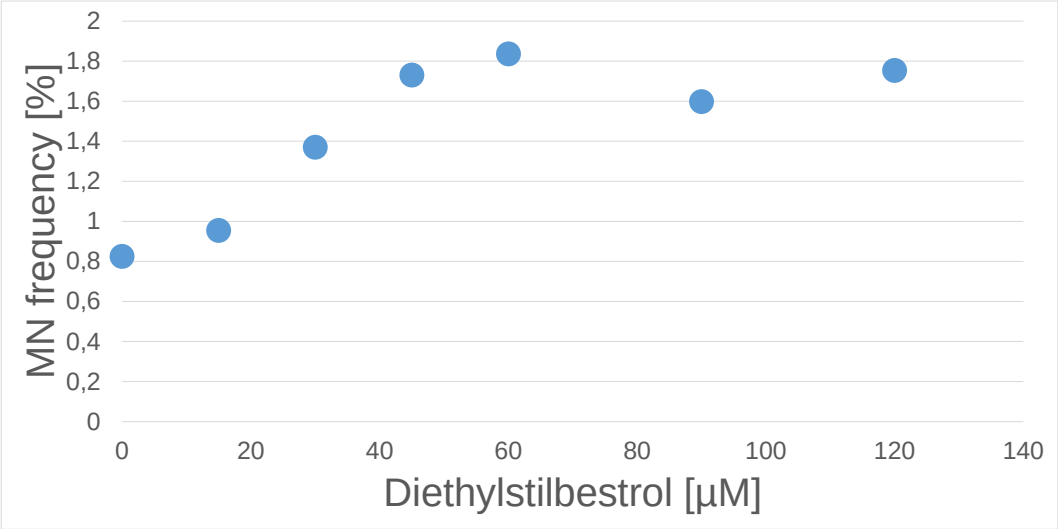
Standard Method versus H2BGFP Cells

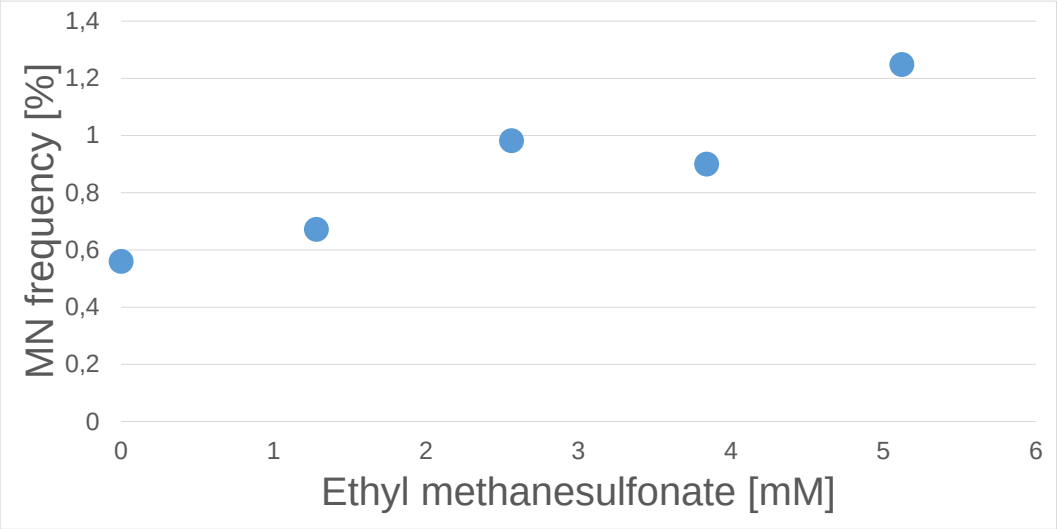


Diethylstilbestrol
(Aneugen)

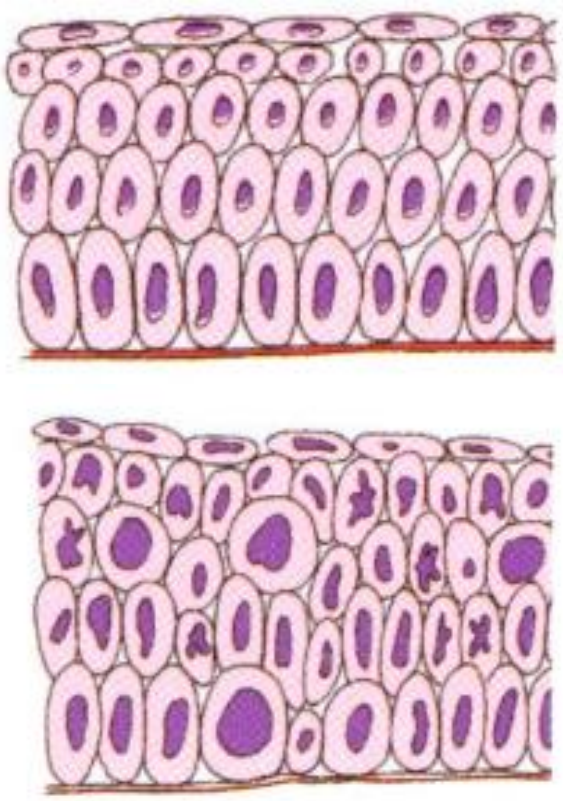


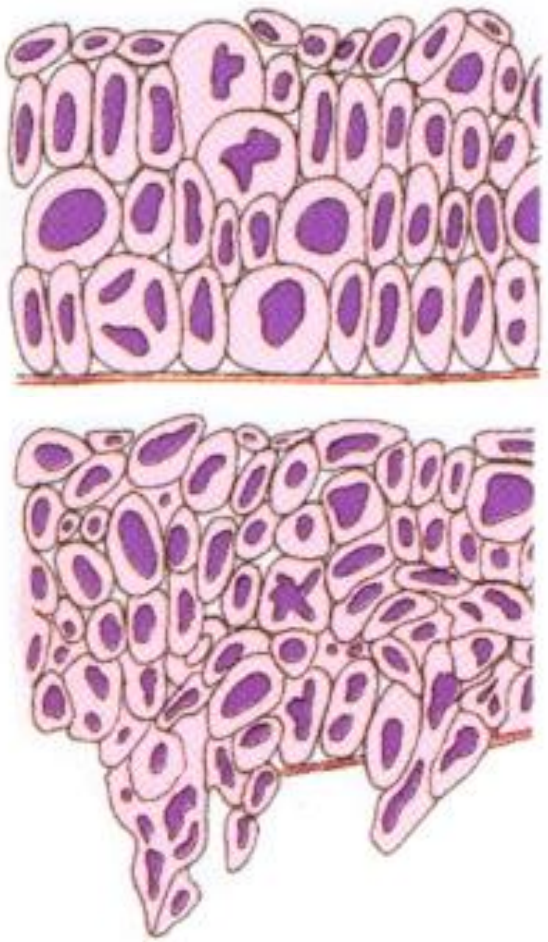
Ethyl methanesulfonate
(Clastogen)





Downstream Analysis Criteria for Carcinogenic Transformation



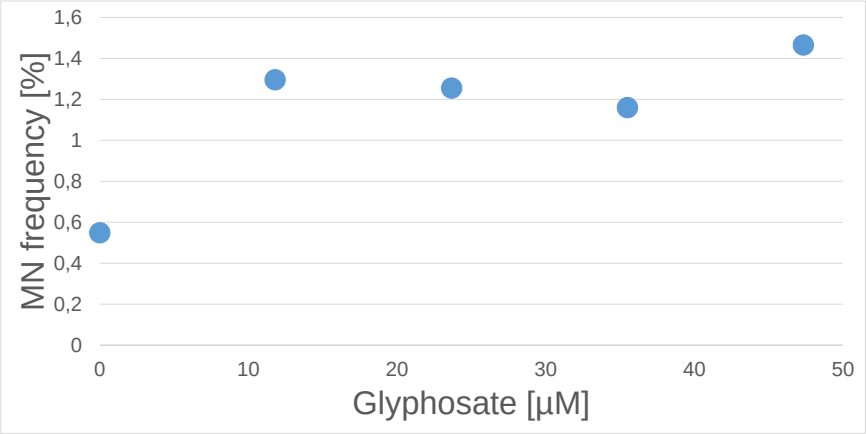


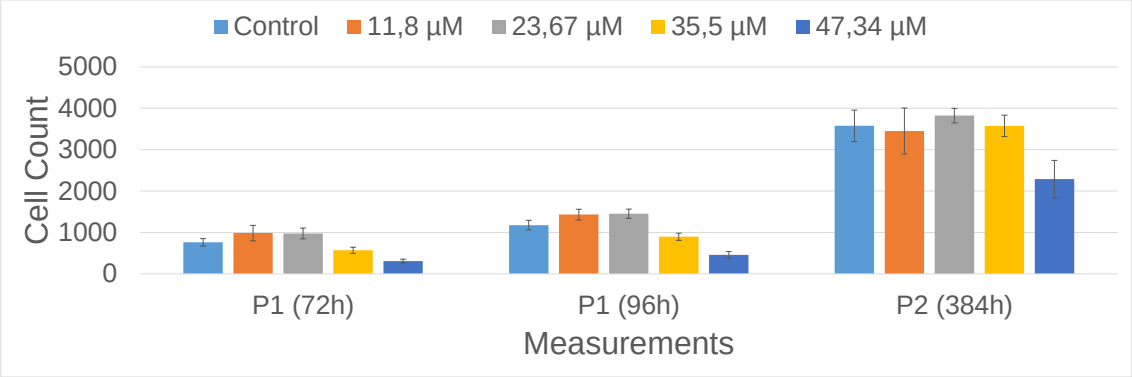
Onkogene

Gen	Genfunktion
PDGF	kodiert für den von Plättchen abgeleiteten Wachstumsfaktor *(platelet derived growth factor);* verändert in bestimmten Hirntumoren (Gliomen)
erb-B	kodiert für den Rezeptor des epidermalen Wachstumsfaktors (EGF); verändert in bestimmten Hirntumoren (Glioblastomen) und Mammakarzinomen
erb-B2	auch *HER-2* oder *neu* genannt; kodiert für einen Wachstumsfaktor-Rezeptor; verändert in Karzinomen der Mamma, der Speicheldrüsen und Eierstöcke
RET	kodiert für einen Wachstumsfaktor-Rezeptor; beteiligt an Schilddrüsenkarzinomen
Ki-ras	kodieren für kleine GTP-bindende Proteine, welche an der inneren Zellmem- bran sitzen und sind an der Signalübermittlung von Rezeptor-Tyrosin-Kinasen, wie z.B. dem EGF Rezeptor, beteiligt; beteiligt am Karzinom der Lunge, Eierstöcke, des Dickdarms und der Bauchspeicheldrüse
N-ras	kodieren für kleine GTP-bindende Proteine; beteiligt an Leukämien
c-myc	das *c-myc* -Protein bindet direkt an regulatorische Bereiche der aktivierten Gene; beteiligt an Leukämien sowie Karzinom von Mamma, Magen und Lunge
N-myc	Menge des *N-myc*-Proteins spielt eine entscheidende Rolle für den geregelten Ablauf der Apoptose; beteiligt am Neuroblastom und Glioblastom
L-myc	Involviert in Kontrolle der Zellproliferation und Differenzierung; beteiligt am Lungenkarzinom
Bcl-2	kodiert für ein Protein, das den programmierten Zelltod (die Apoptose) verhindert; beteiligt am follikulären B-Zell-Lymphom
Bcl-1	auch *PRAD* 1 genannt; kodiert für Cyclin D 1, eine stimulatorische Komponente der Zellzyklusuhr; beteiligt an Mammakarzinom und an Tumoren im Kopf und Halsbereich
MDM 2	kodiert für einen Gegenspieler des Tumorsuppressor-Proteins *p53*; beteiligt an Bindegewebstumoren (Sarkomen) und anderen Tumoren

https://www.ccc.ac.at/fileadmin/ccc/Uploads_Webssite/Die_Tumorzelle_Regele.pdf

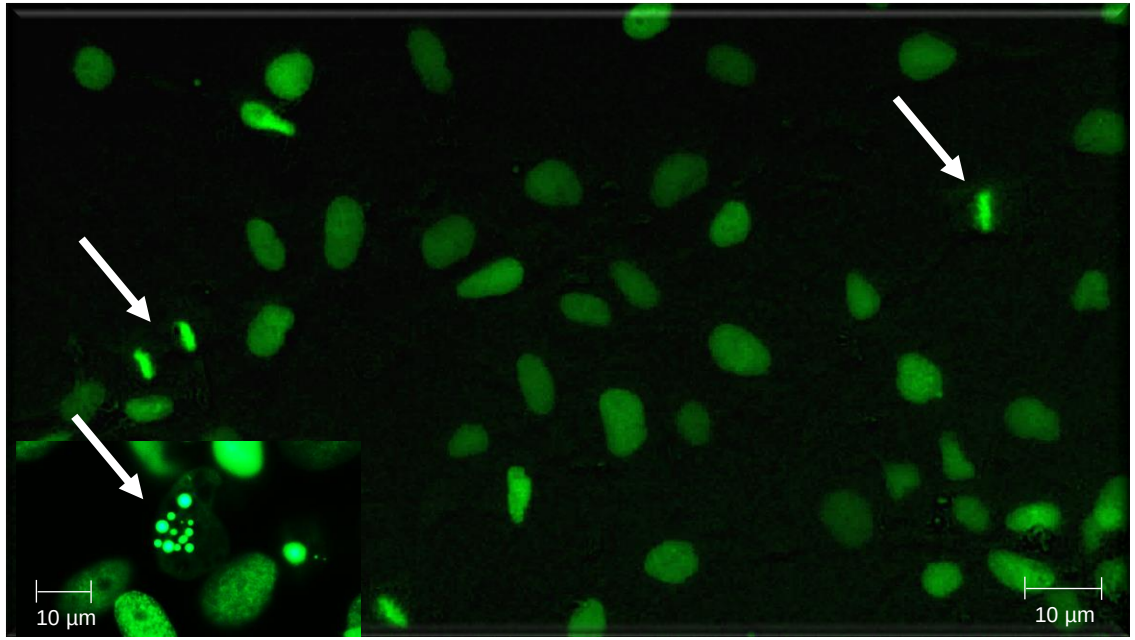
Downstream Analysis of Cell Transformation



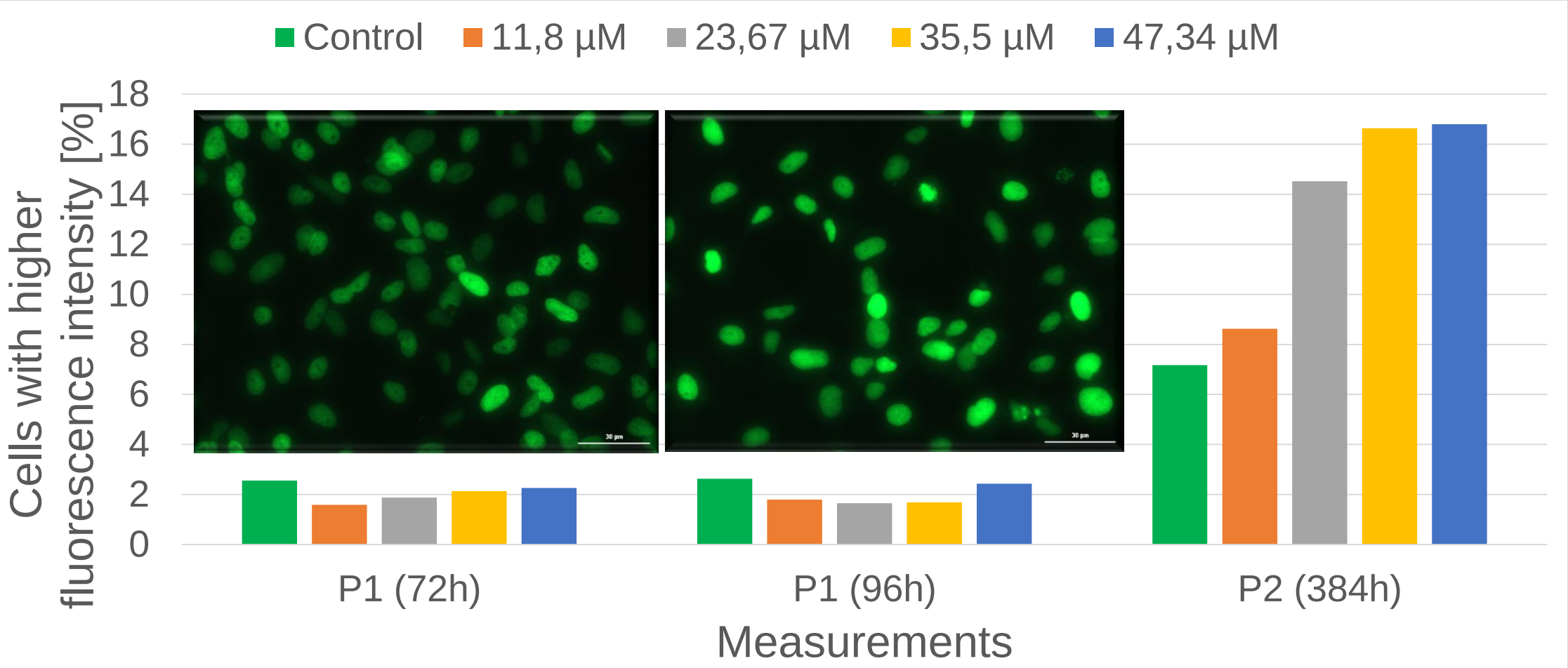


Improved analyses of cell proliferation

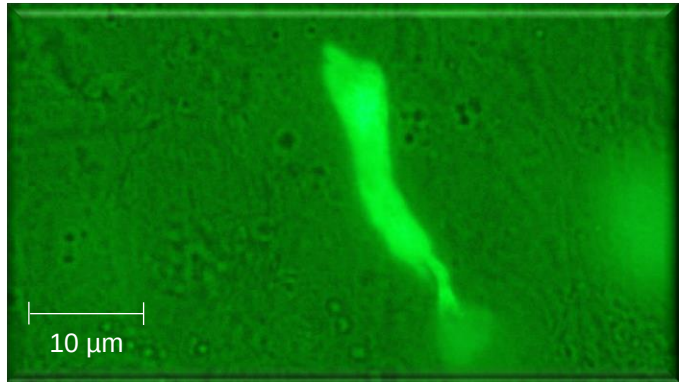
- Number of cells
- Number of mitoses
- Number of dying cells

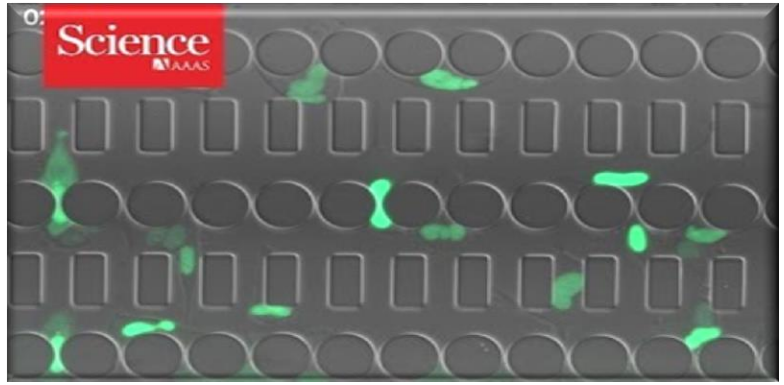


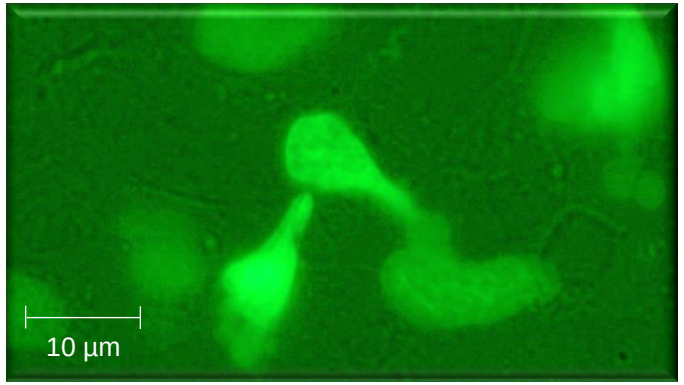
Downstream Analysis of Cell Transformation

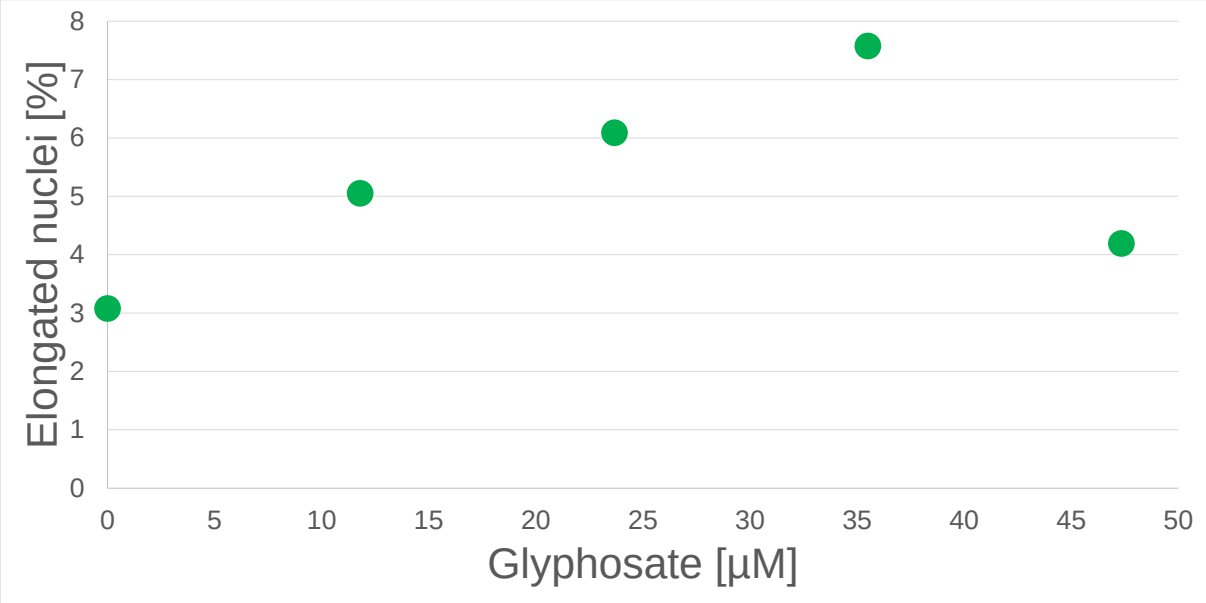


Downstream Analysis of Cell Transformation



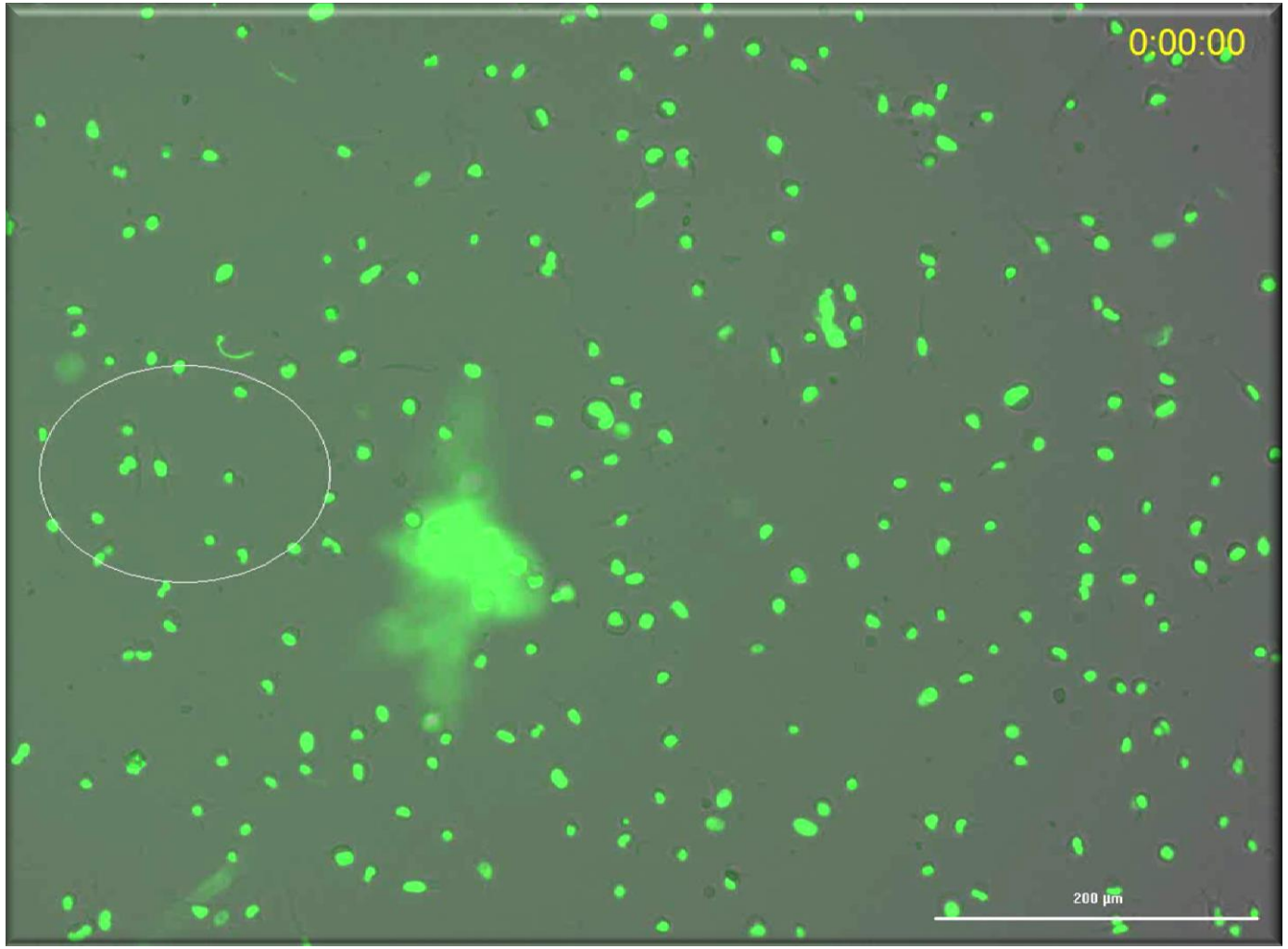


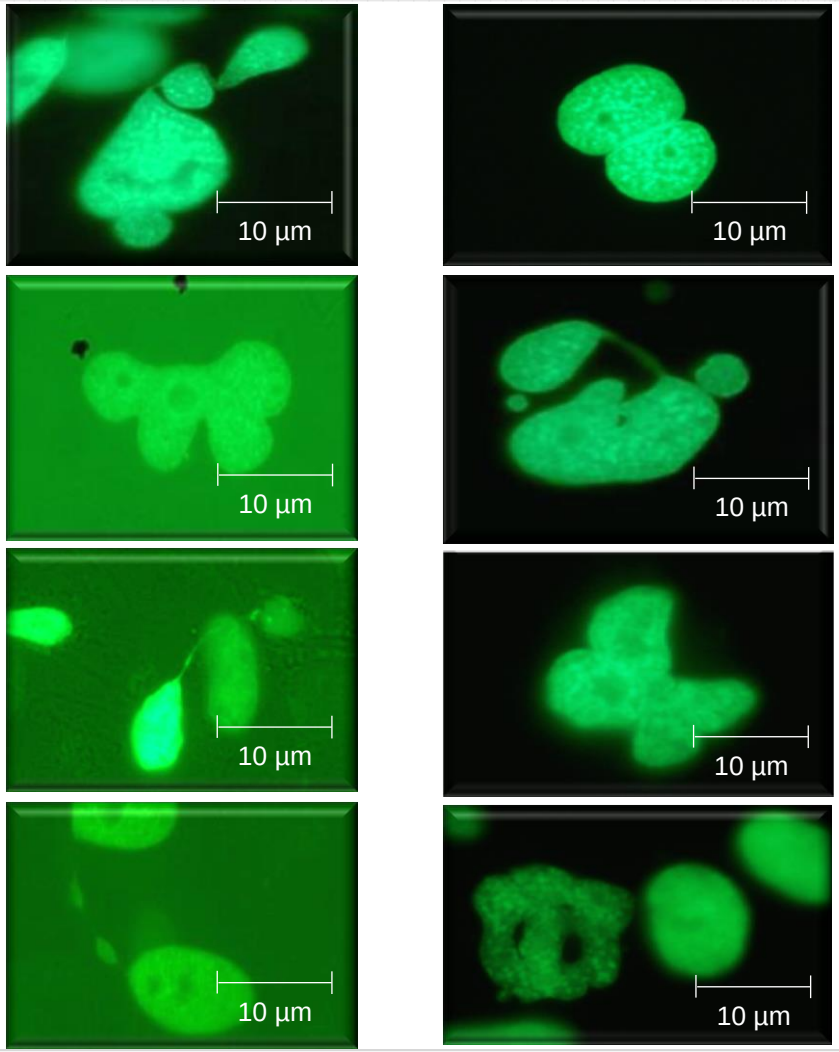




Cell motility/invasiveness

What is Stemness - what is Cancer?

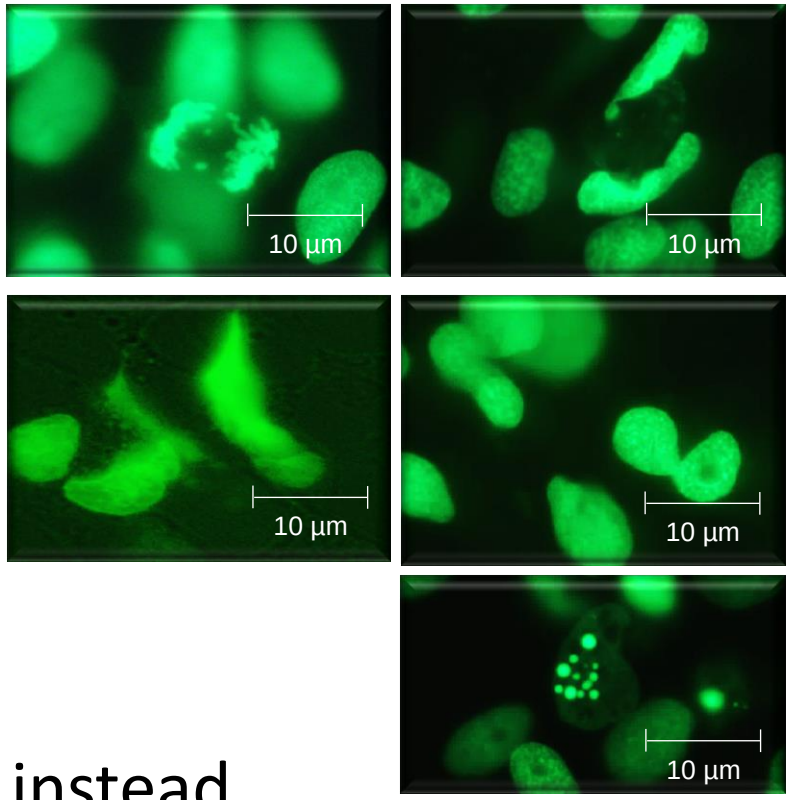




Conclusion

Stemness to cancer:

- chromosomal/DNA Damages causing
- nuclear abnormalities pyknosis apoptosis?
- promotion of cell motility leading to invasiveness
- promotion of horizontal gene transfer in polynucleated cells
- the related gene expression

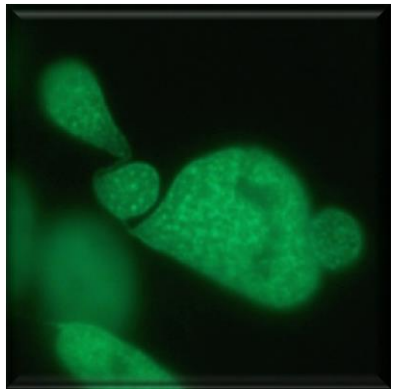


call for sound in vitro studies instead of animal testing

CF Lerche, BN Hölzel, T Allner, B Allner

J Hescheler,

D Derichsweiler, K Pfannkuche,



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