

# Identification of microRNA-mRNA functional interactions in UVB-induced senescence of human diploid fibroblasts

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*BMC Genomics* 2013, 14:224.

- Einführung
  - Begriffsklärung: Seneszenze, mRNA, miRNA, UVB, p53
  - Ziele dieses Manuskripts
- Methoden
- Ergebnisse
- Interpretation / Diskussion

Seneszenz (lat. *senescere* = „alt werden“)

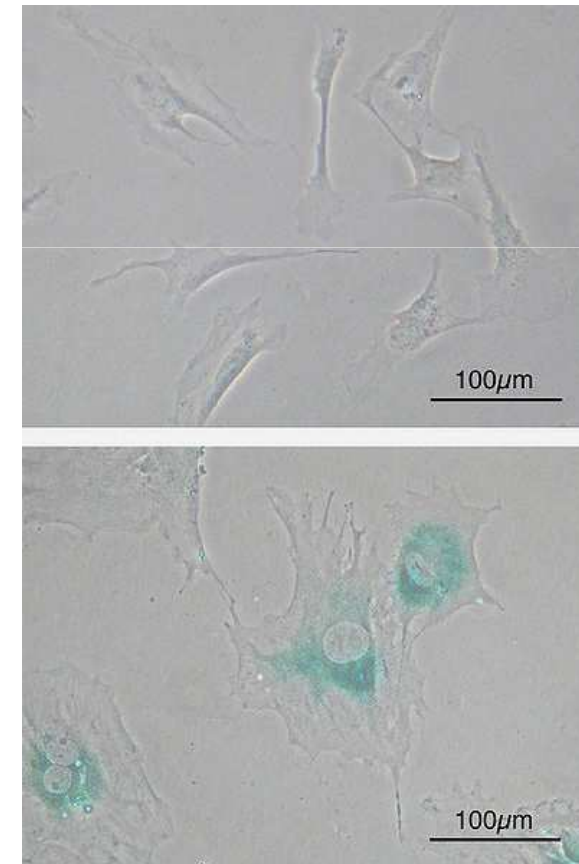
Hayflick'sche Gesetz:

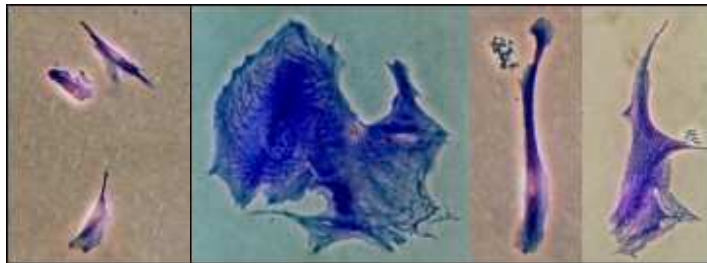
diploide Zellen teilen sich ~50 mal

Verhindert überschießendes Wachstum

**Murine Embryonale Zellen:**

oberes Bild bevor Seneszenz,  
Bild unten seneszente Zellen nach mehreren  
Passagen,  $\beta$ -galactosidase (blau) als Marker  
Für Seneszenz

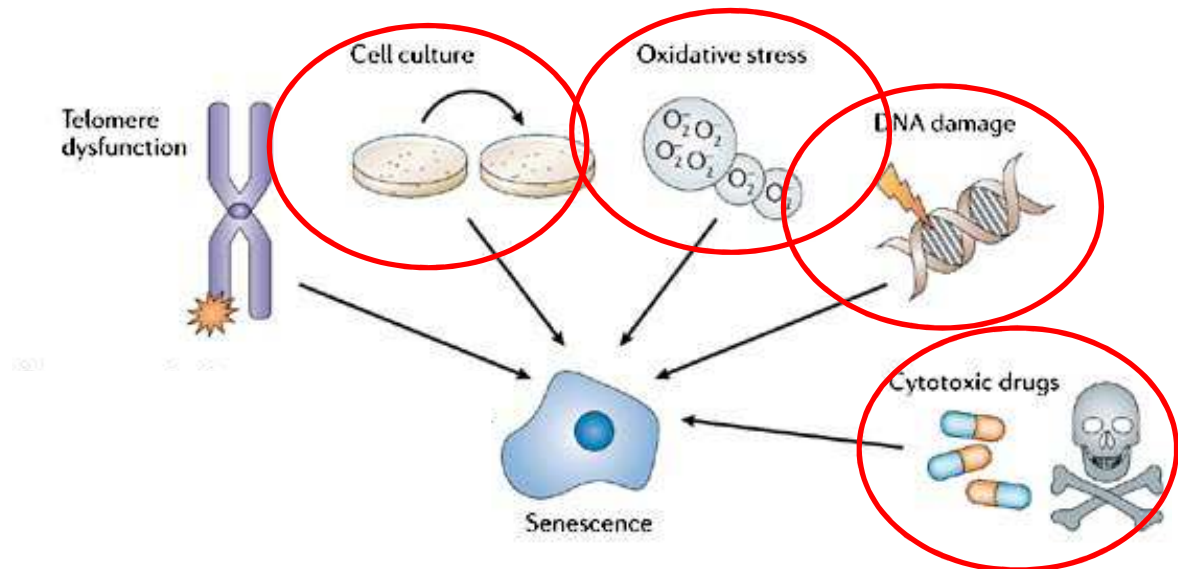




normal Fibroblasten      senescente Fibroblasten



## SIPS (stress-induced premature senescence)



Hass et al. Cell Communication and Signaling 2011 9:12

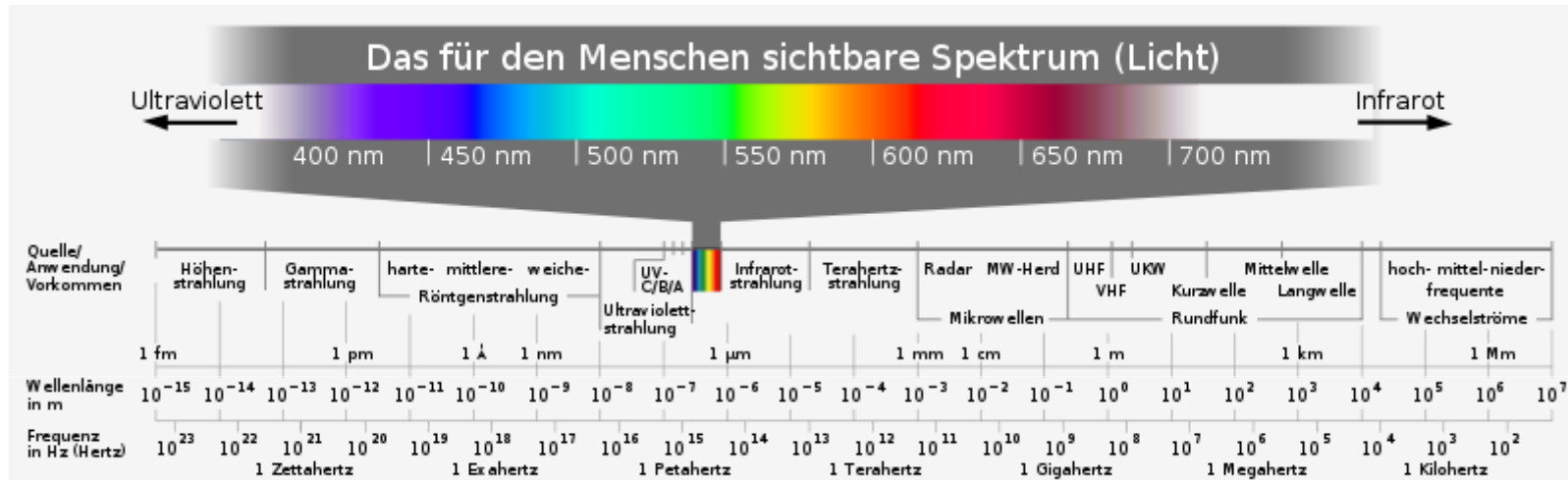
[http://www.senescence.info/cell\\_aging.html](http://www.senescence.info/cell_aging.html)

Collado et al. Nature Reviews Cancer 6, 472–476 (June 2006)

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 Nature Reviews | Cancer

Vienna 2013

# UVB



	Wellenlänge	
UVA	315-380nm	hohes Melanomrisiko (freie Radikale)
UVB	280-315nm	obere Hautschichten, Sonnenbrand, Hautbräunung
UVC	100-280nm	sehr kurzwellig, gelangt nicht bis zur Erdoberfläche

UVB Strahlen sind ein Trigger für SIPS

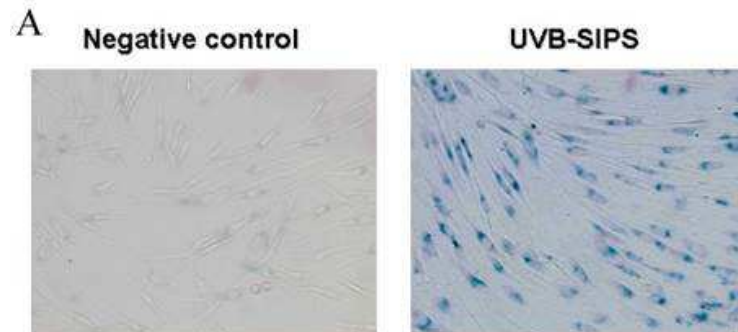
UVB aktiviert:

**p53** → „klassischer“ Seneszenz Signalweg

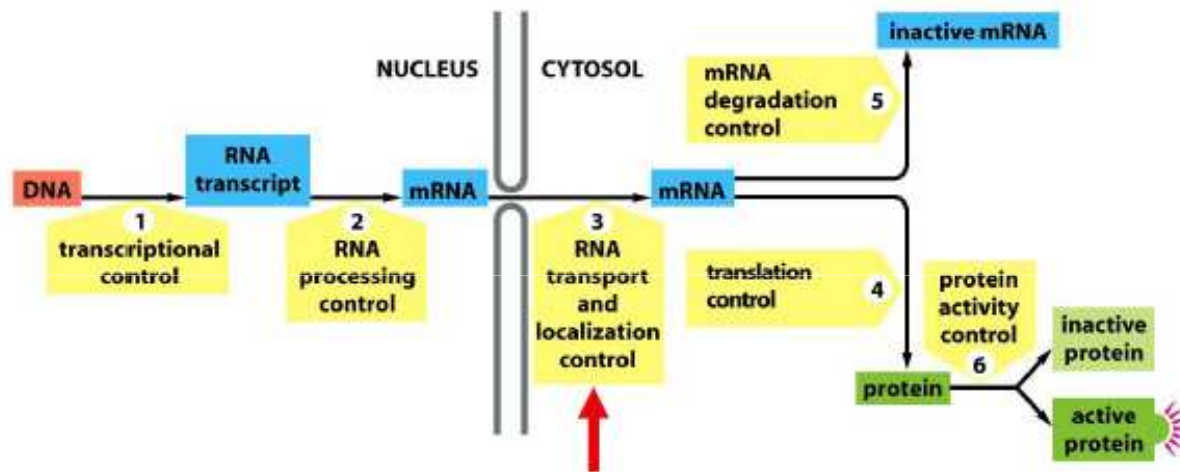
zusätzlich p53 **unabhängige** Signalwege

Bildung von freien Radikalen

→ multiple zelluläre Effekte



# mRNA



Control of eukaryotic gene expression

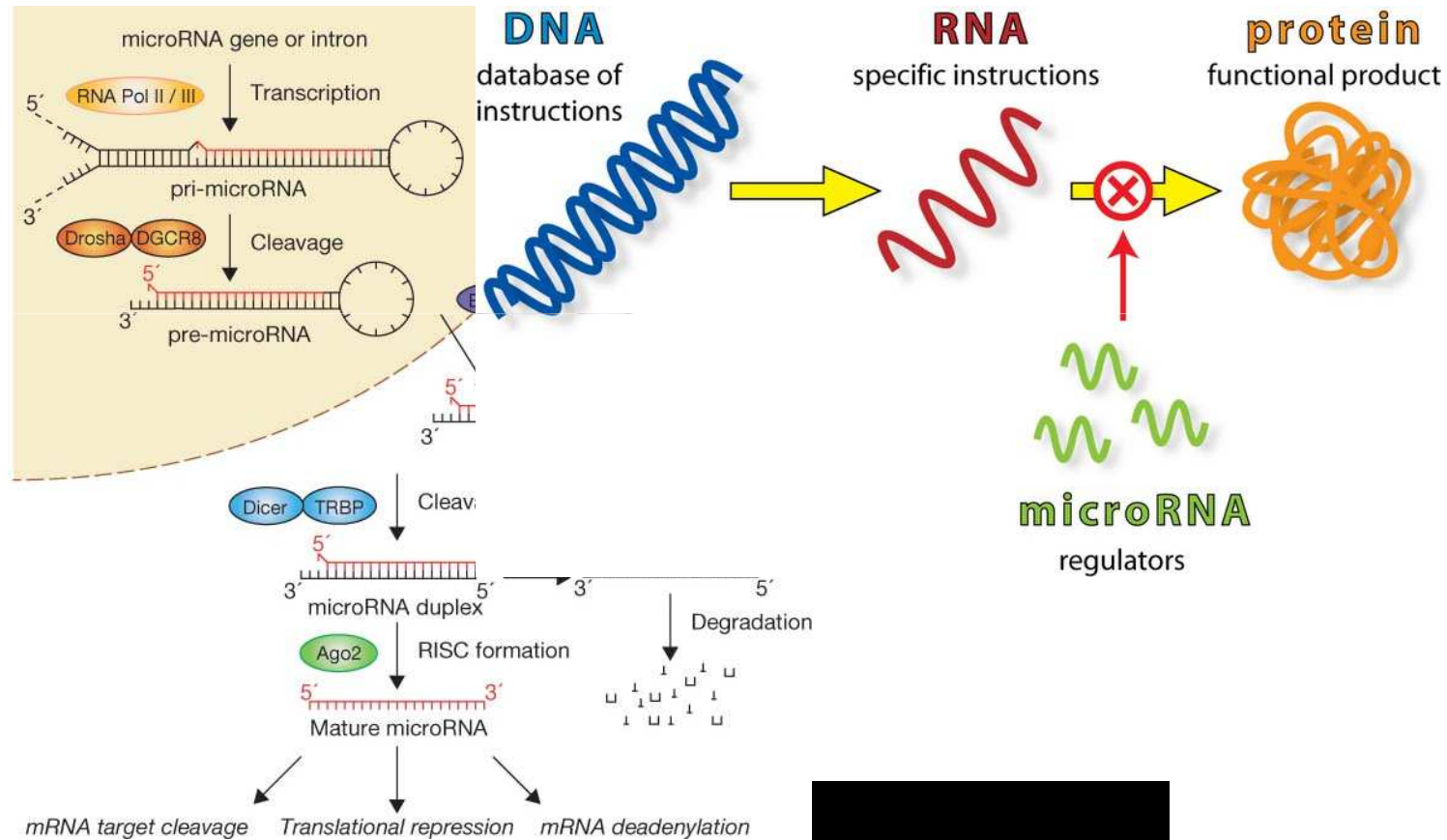


<http://www.youtube.com/watch?v=SMtWvDbfHLo>

Alberts, 7th edition 2008,

Vienna 2013

# miRNA



<http://www.youtube.com/watch?v=-9pROnSD-A>

Winter et al Nature Cell Biology 2009

Vienna 2013



# Was wir bereits wissen

UVB → Seneszenz

via p53 abhängige und unabhängige Wege

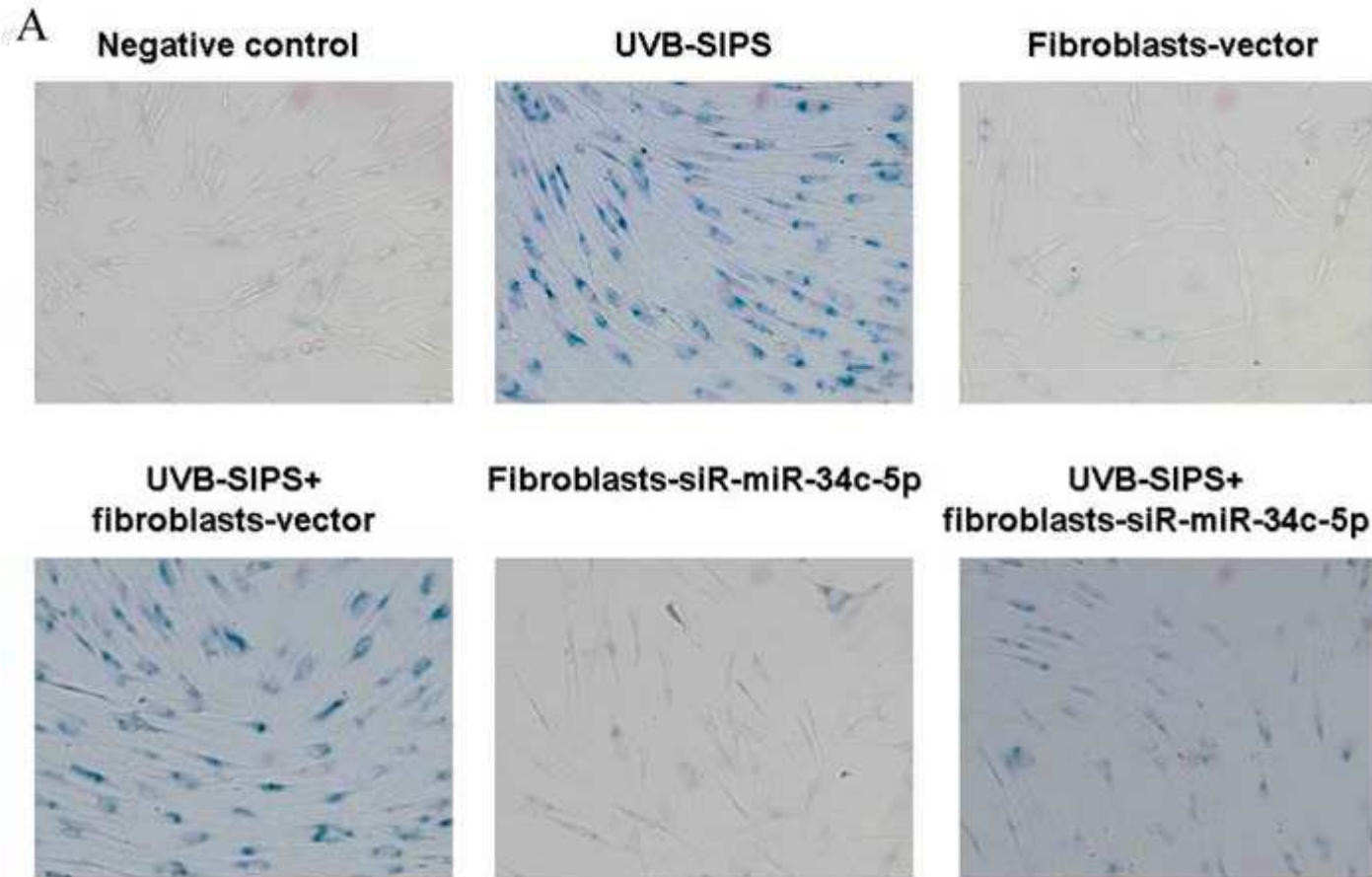
miRNAs → regulieren Proteinsynthese

mRNAs → sind Targets von miRNAs

Deduktive Fragestellung:

Welchen **Einfluss** haben **miRNAs** in der  
**UVB** meditierten **Seneszenz** - Induktion

# Vorstudien



# Studienziel Studienplan

Ziel: Beschreibung von **UVB induzierten**  
molekularen **Seneszenz-Mechanismen** in  
humanen diploiden Fibroblasen (**HDF**)

Methoden:

in-vitro Studie

HDF von Neugeborenen gewonnen (for sure)

Osteosarkom-Zelllinie

UVB Behandlung

3,000 bis 5,000 J/m<sup>2</sup> von Tag 1 bis 4

Zellernte am Tag 1; 4; 7 und 9

## Seneszenz Nachweis:

SA- $\beta$ -galactosidase Färbung

Protein, mRNA & miRNA Isolierung

Microarray Analysis

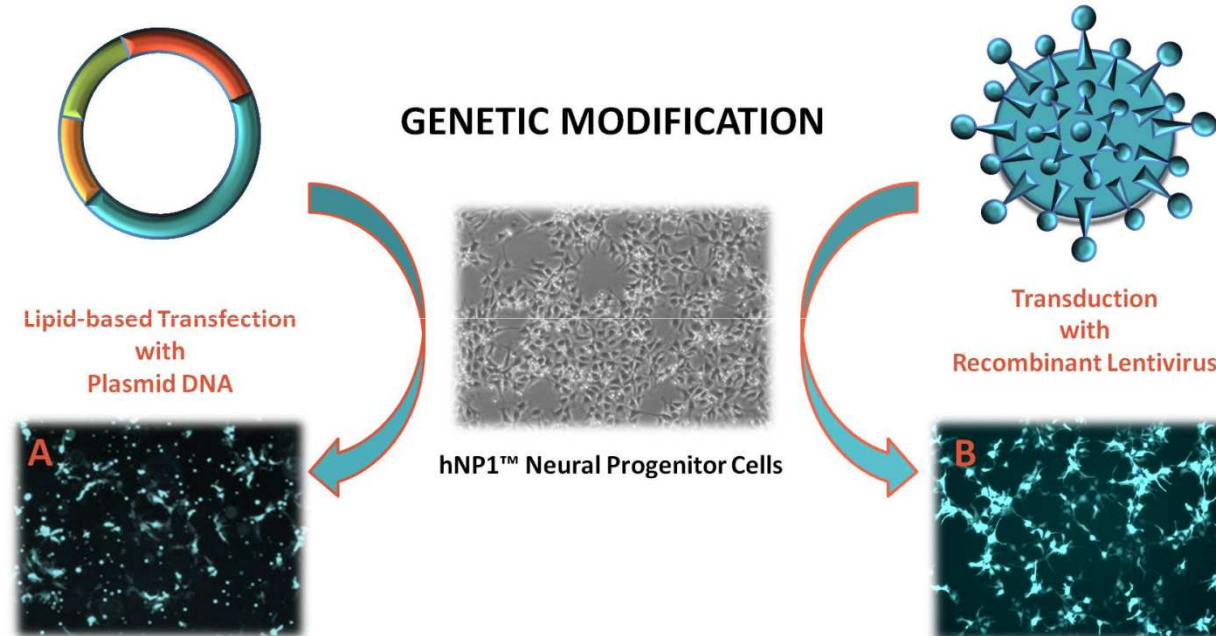
RT-PCR

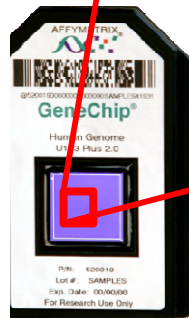
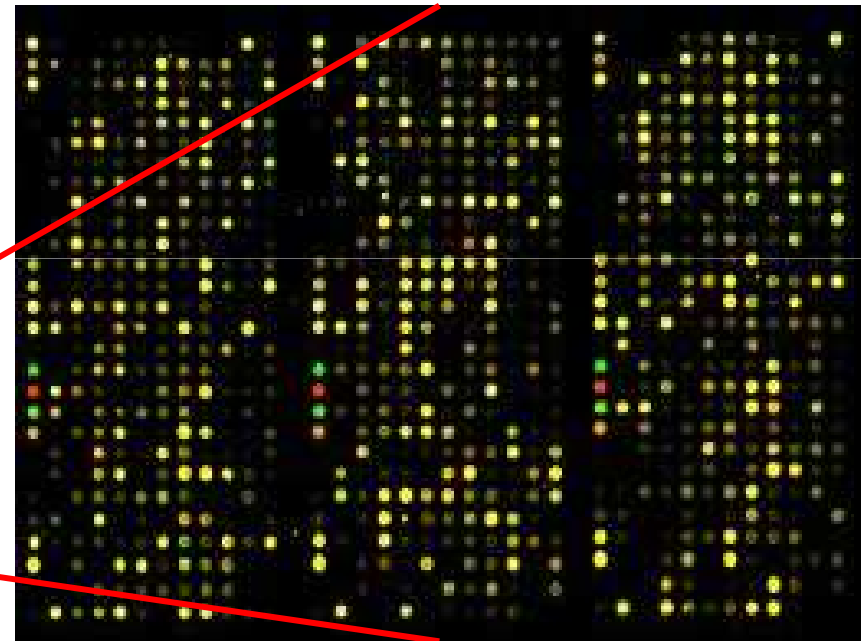
Locked nucleic acid (LNA)-miRNA

Immunoblotting

SDS Gel Elektrophorese

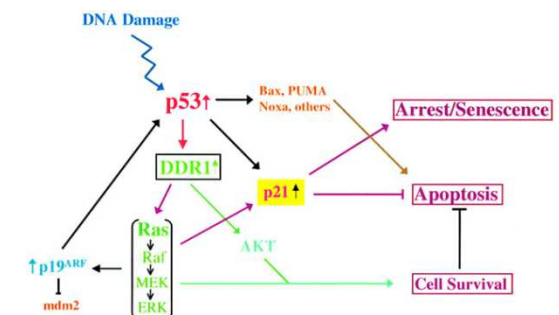
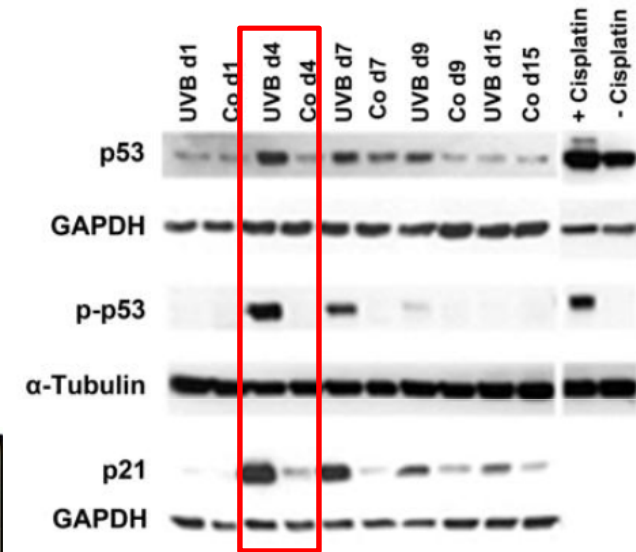
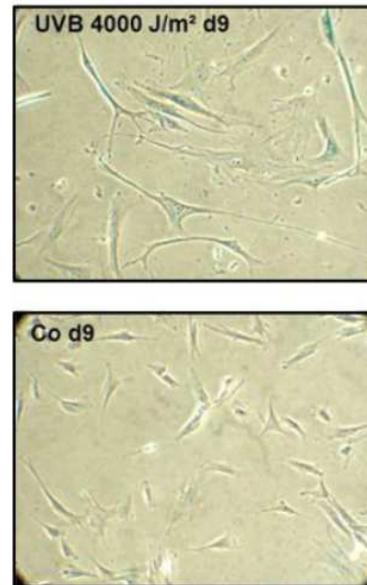
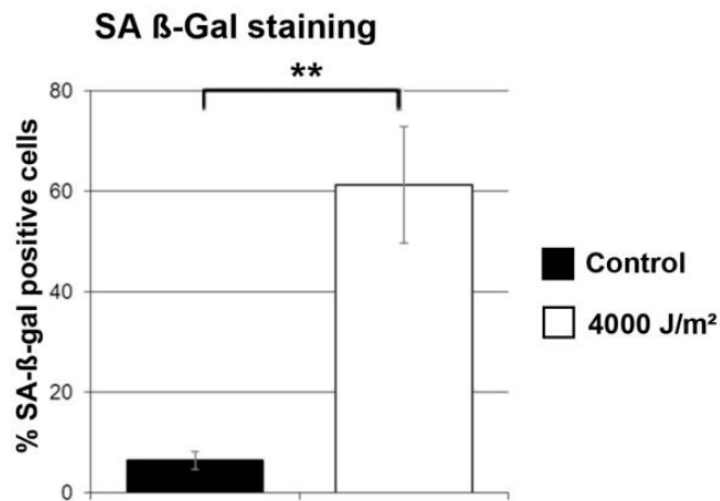
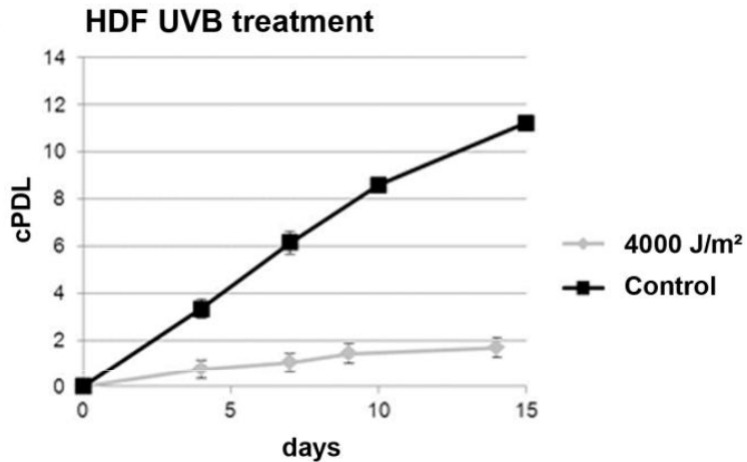
# Virale Transduction





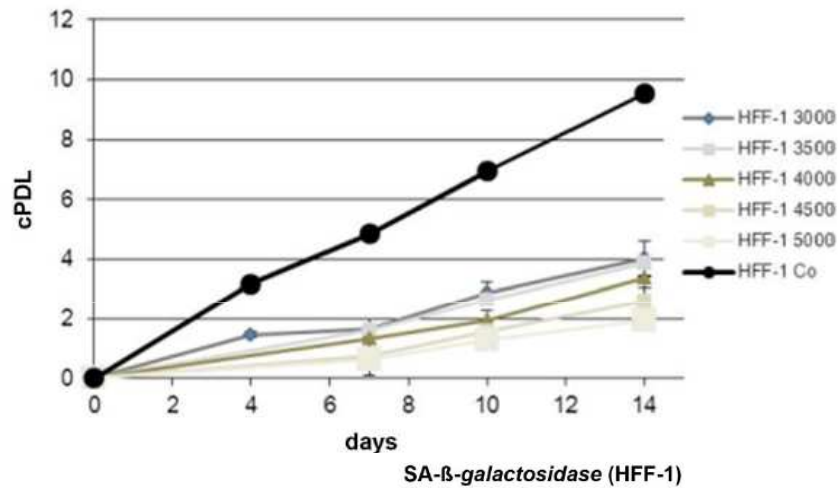
# Ergebnisse

PDL = population doublings

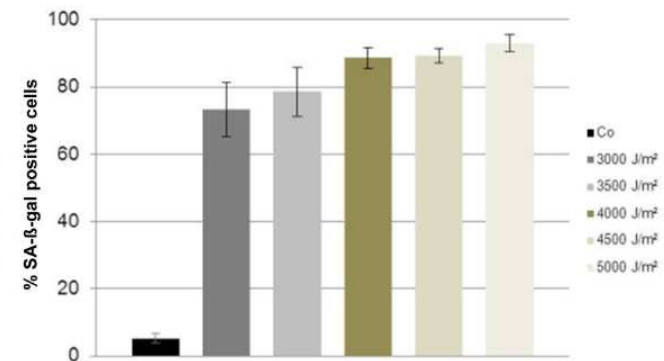
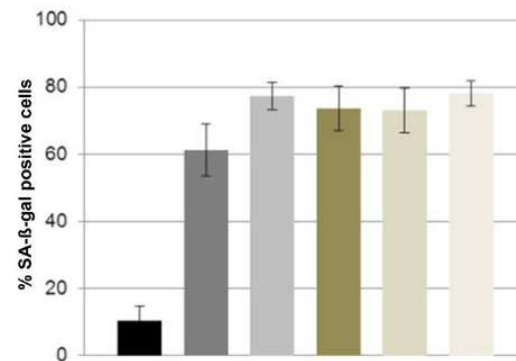
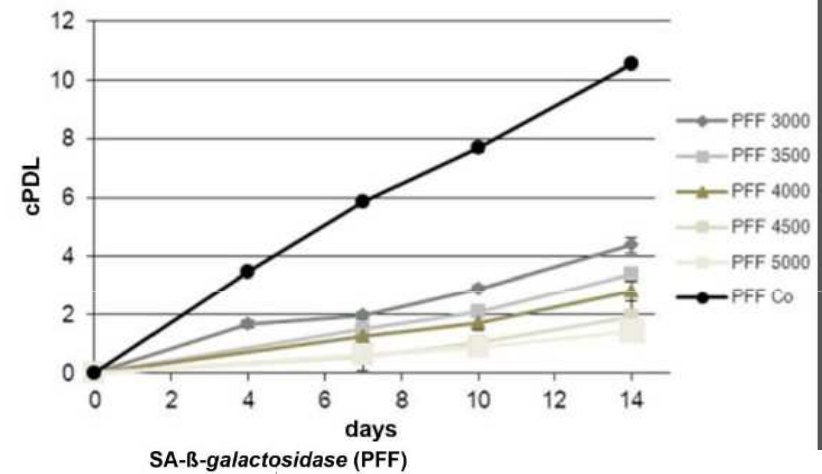


HDF = human diploid fibroblasts

HFF1 UVB treatment



PFF UVB treatment



PFF = isolated fibroblasts from newborn foreskin



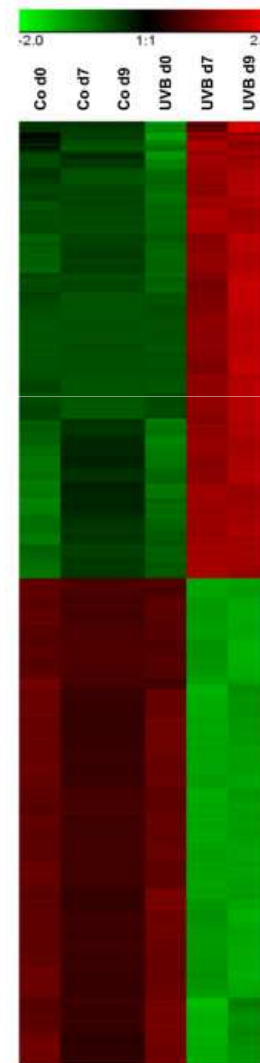
## Transcriptome Analysis:

1219 up regulated / 1077 down regulated

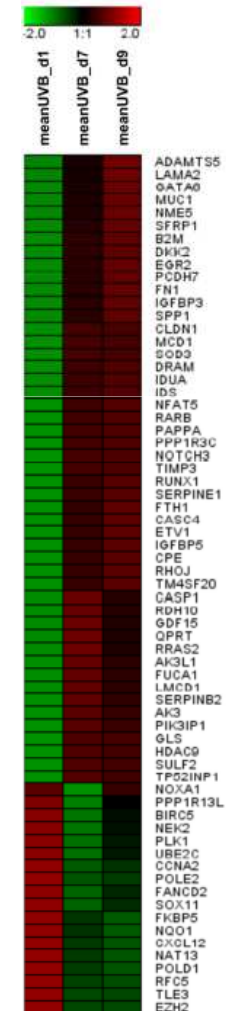
## Representative pathways

	Up-regulated	Down-regulated
→ Cell Cycle: G2/M DNA Damage Checkpoint Regulation	1	10
→ Role of BRCA1 in DNA Damage Response	2	18
→ Cell Cycle: G1/S Checkpoint Regulation	4	8
→ p53 Signalling	8	5
Aryl Hydrocarbon Receptor Signalling	7	11
Pyrimidine Metabolism	4	16
Nicotinate and Nicotinamide Metabolism	3	6
Hepatic Fibrosis / Hepatic Stellate Cell Activation	12	1

Microarray expression



qPCR expression



miRNA expression:

806 miRNAs were analyzed

mRNA – miRNA anti-correlation-pairs

8 miRNAs showed anti-correlation → 5 were chosen for further analysis

## Representative miRNA expression

miRNA expression (fold expression)					target mRNA
miRNA	d1	d4	d7	d9	
hsa-miR-20a	-1,067	-1,987	-1,598	-2,629	VEGFA, E2F1, RUNX1, CCND1
hsa-miR-20b	-1,046	-1,702	-1,288	-2,073	CDKN1A, VEGFA, E2F1, CCND1, RUNX1
hsa-miR-15a	1,037	-1,661	-1,666	-2,274	BCL2, VEGFA
hsa-miR-93	-1,034	-1,567	-1,613	-2,722	E2F1, VEGFA, CDKN1A
hsa-miR-101	1,024	-1,212	2,465	1,244	MYCN, EZH2

Figure 3

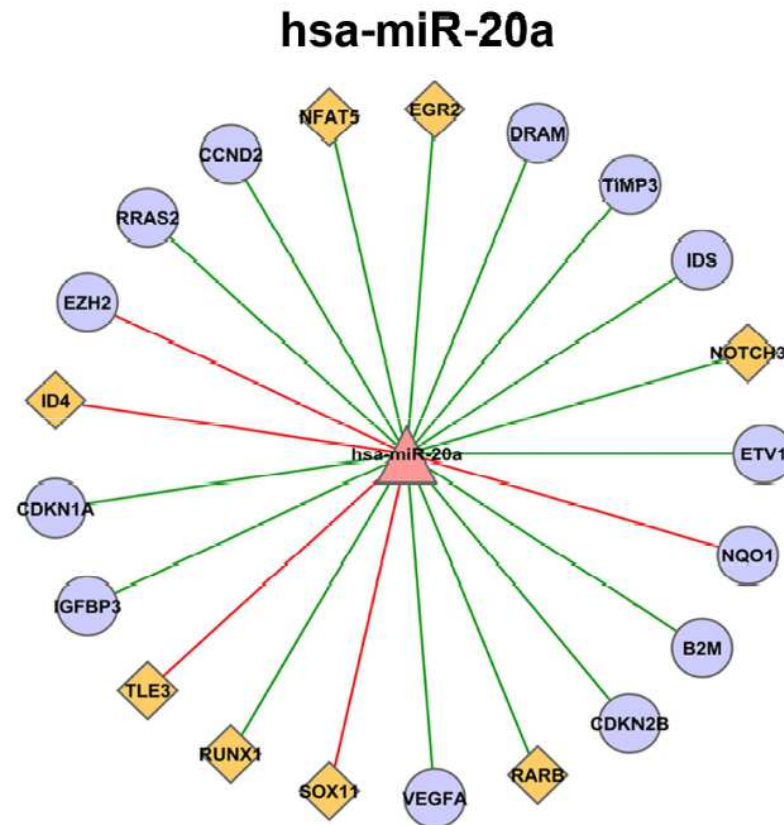
red = upregulated mRNA

blue = downregulated mRNA

black = not regulated mRNA

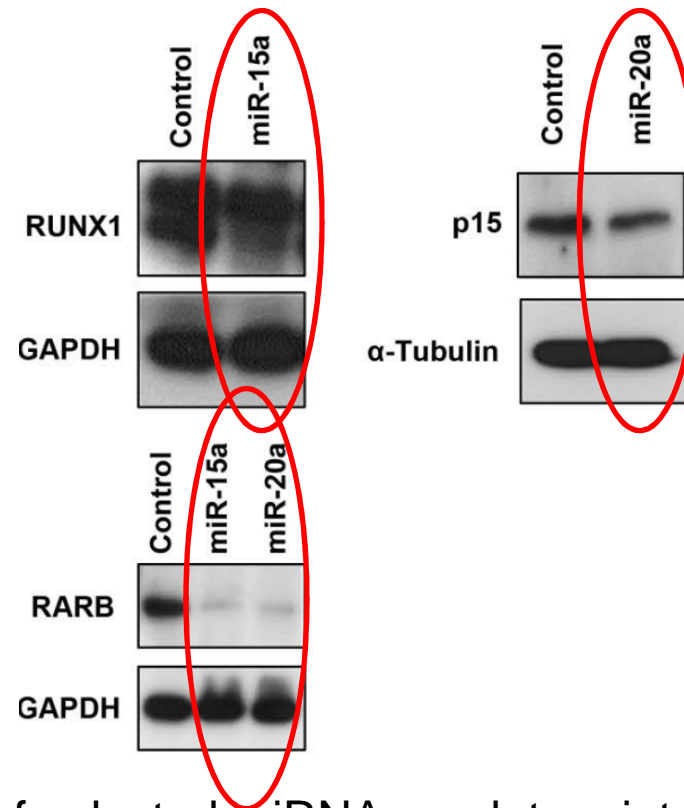
# Ergebnisse

z.B. miR-20a geht runter und CDKN1A geht hoch

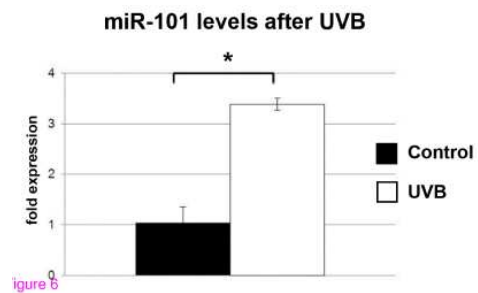
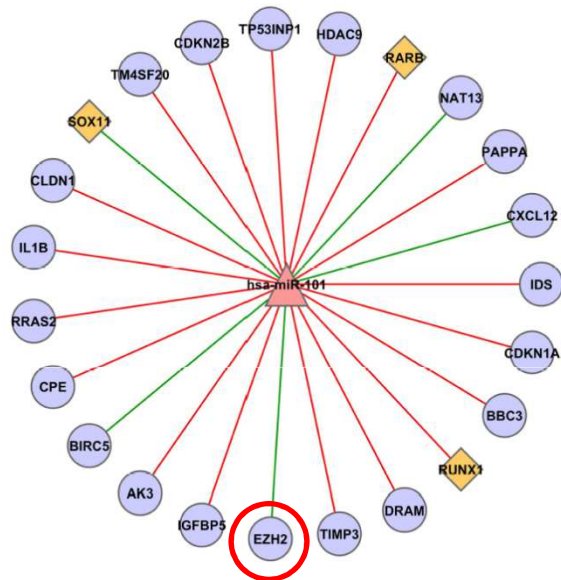


red = positive correlation between miRNA und mRNA  
 green = negative correlation

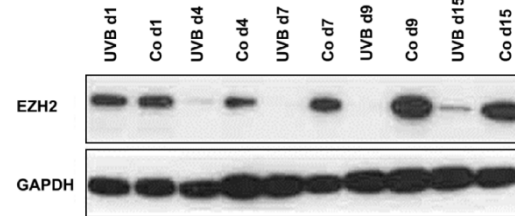
# Ergebnisse



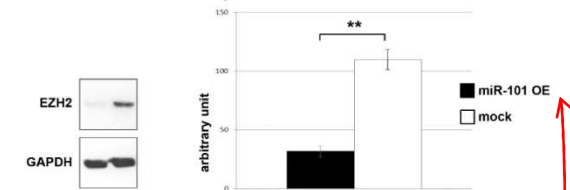
Validation of selected miRNA regulatory interactions. miR-15a and miR-20a were overexpressed in HDF as indicated. Extracts



## EZH2 expression levels

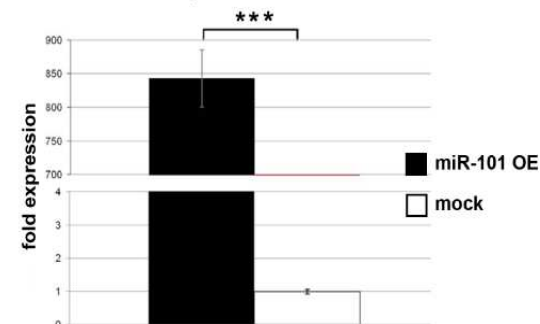


## EZH2 protein level

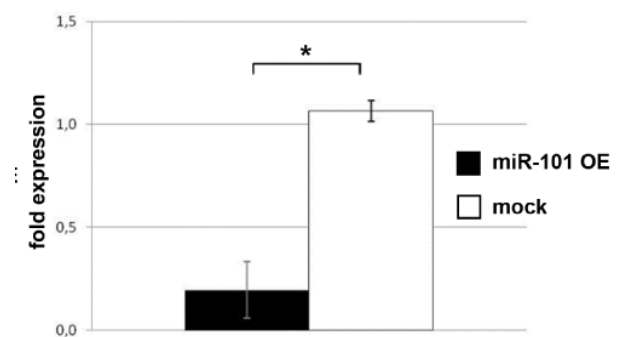


## miR-101 overexpression

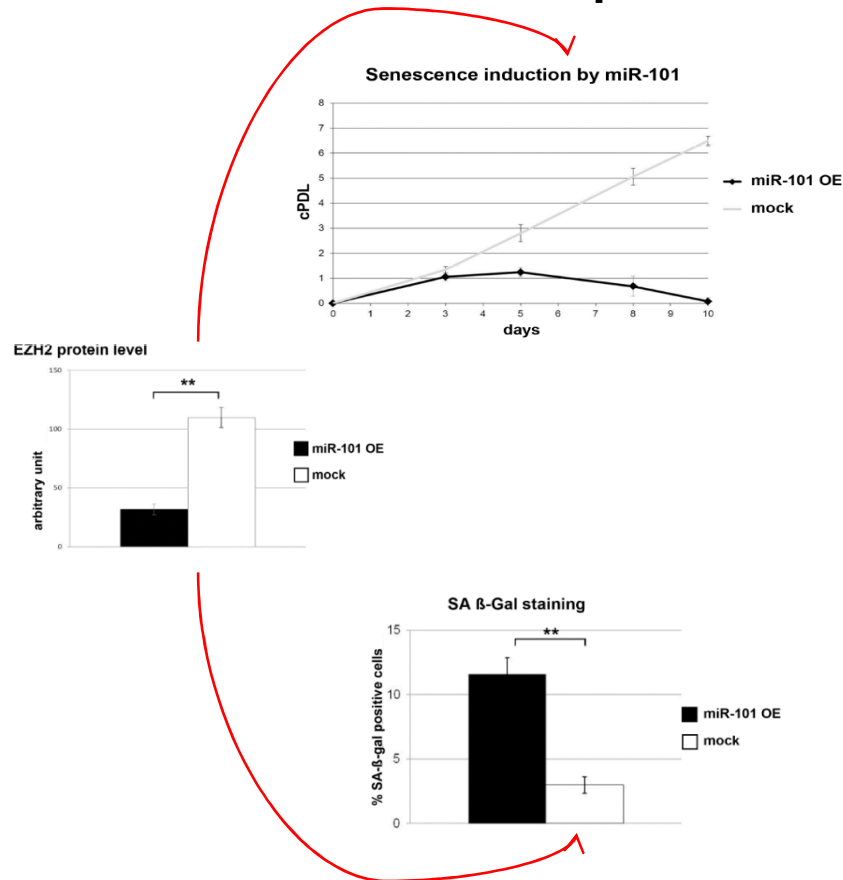
### A miR-101 expression levels



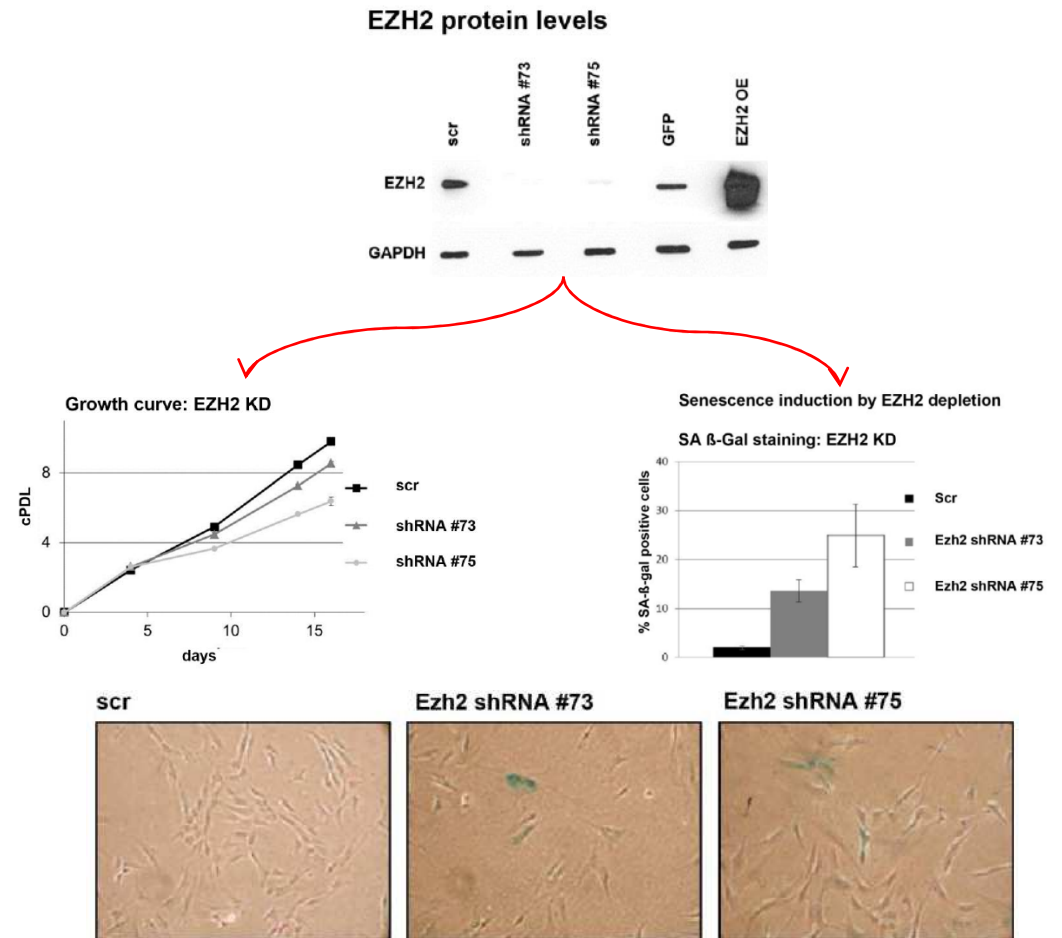
### B EZH2 mRNA levels



## miR-101 overexpression

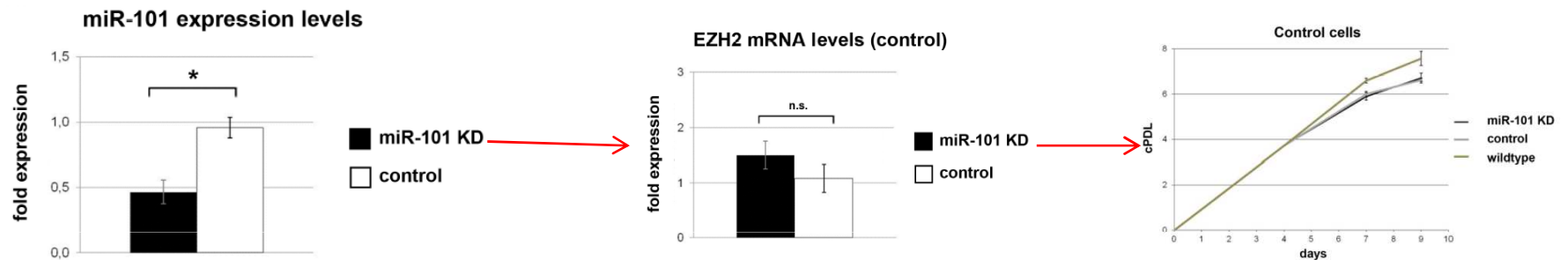


## EZH2 knockdown

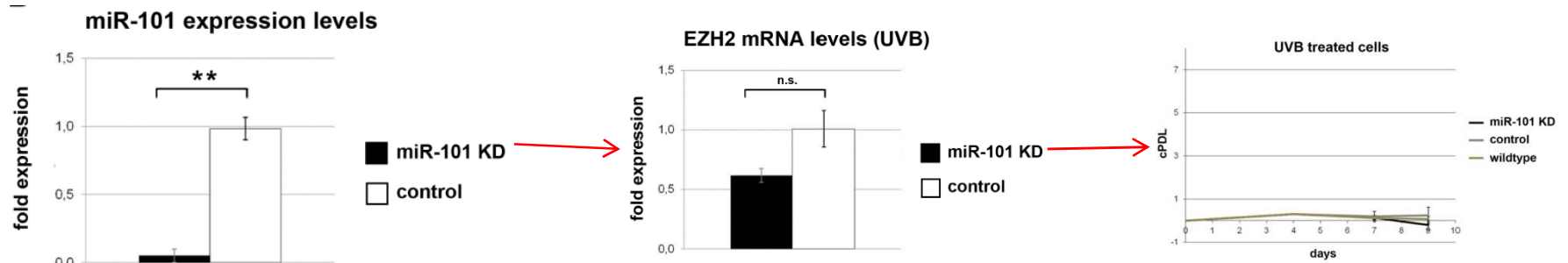


**Fragestellung:** *Verhindert die Depletion von miR-101 die Seneszenz Induktion?*

Non-irradiated

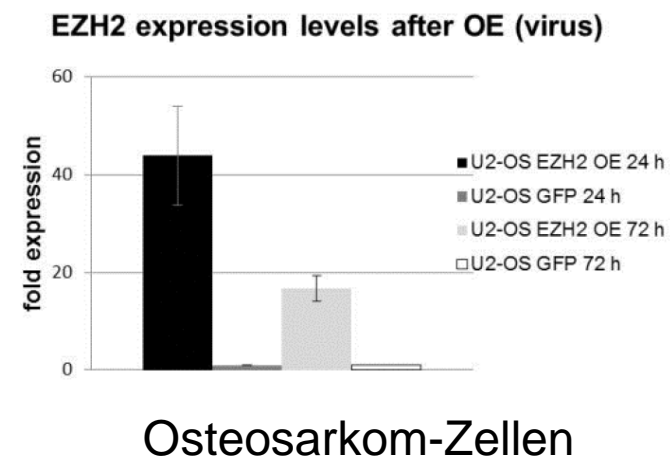
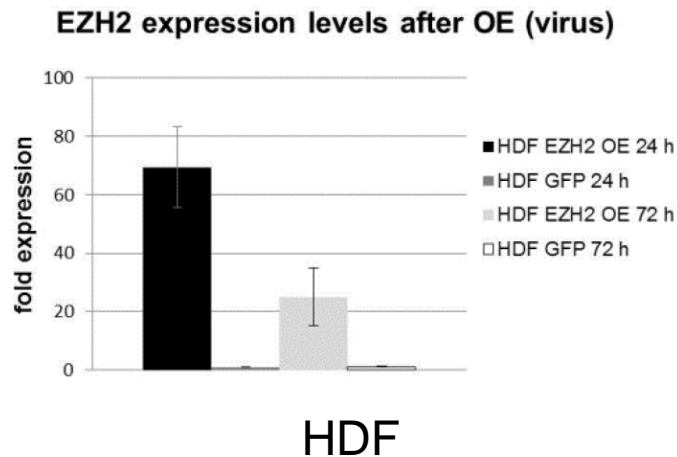


UVB-irradiated

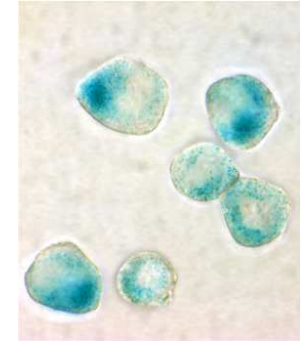


Eine **Reduktion** von **miR-101** führt weder zu einer vermehrten Expression von Ezh2 noch zu einer Änderung des Phänotyps.

**Fragestellung:** *Führt die direkte **Induktion** der **Ezh2** Expression zu einer Änderung auf **Proteinebene** und / oder zu einer Änderung des **Zell-Phänotyps**?*







- **UVB-Strahlen** induzieren **Seneszenz** in HDF
- Bioinformatische Analysen beschreiben, dass **5 miRNAs** in diesen Prozess involviert sind.
- Für diese **miRNAs** wurden zahlreiche neue Targets **prädiziert**.
- Die **Überexpression** von **miR-101** oder die Hemmung von **Ezh2** führt zu einer Seneszenz Induktion, auch in Abwesenheit von UVB Bestrahlung
- Jedoch die **Hemmung** von miR-101 oder Überexpression von Ezh2 können die Seneszenz Induktion von UVB-Strahlen nicht verhindern
  - Die Autoren schließen daraus, dass **mehrere Signalwege** zu einer Aktivierung des Seneszenz Phänotyps führen.

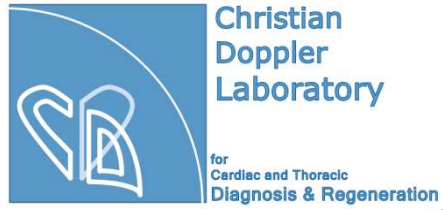
Interessante Fragestellungen:  
Was würde passieren wenn wir den Schritt der Seneszenz  
zeitlich nach hinten verschieben könnten? Welche  
Weiterentwicklung würde uns erwarten?  
Hier einige **Modellorganismen** für weitere Forschung



Rougheye rockfish  
Lebenserwartung:  
225 Jahre



Aldabra-Riesenschildkröte  
Lebenserwartung:  
>250 Jahre



Vielen Dank