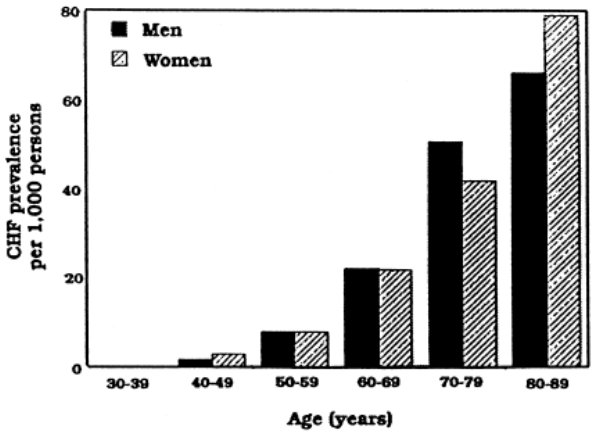
MicroRNA-34a is a pivotal age-induced regulator of cardiac apoptosis, telomere maintenance and contractile function: Implications for therapeutic inhibition

Reinier Boon
Institute for Cardiovascular Regeneration
Center for Molecular Medicine
Goethe University, Frankfurt

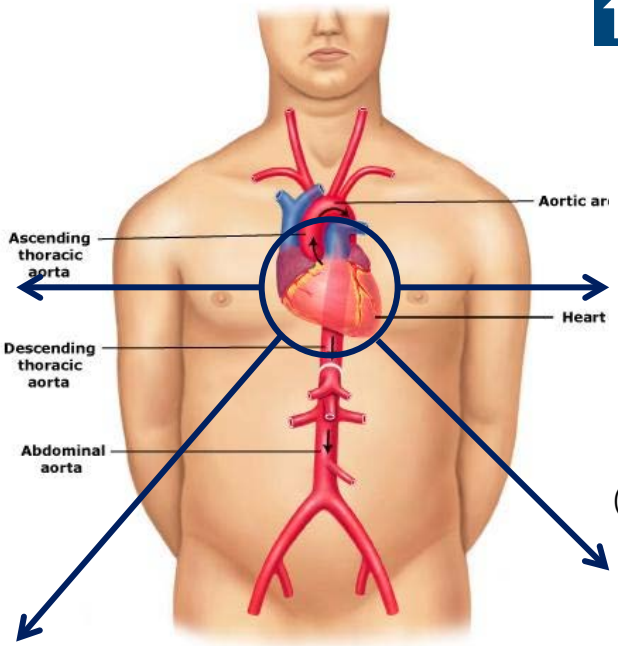
I have no conflict of interest

Age is the major risk factor for cardiovascular disease

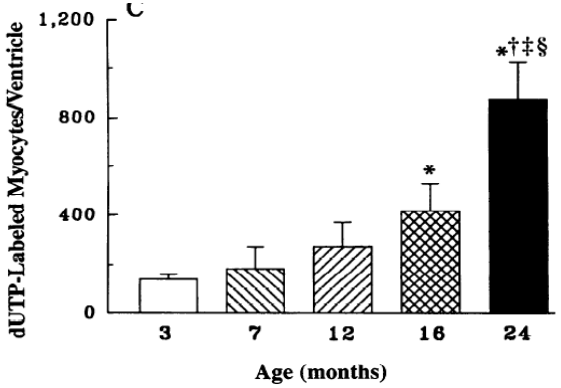
Heart failure



(Lakatta, Heart Failure Rev 2002)



Cardiomyocyte Apoptosis



(Kaystura *et al*., Am. J. Physiol. 1996)

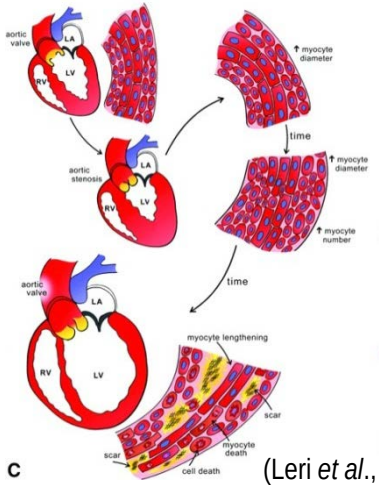
Cardiac fibrosis

8 weeks 60 weeks

(Swinnen *et al*., Circulation 2009)

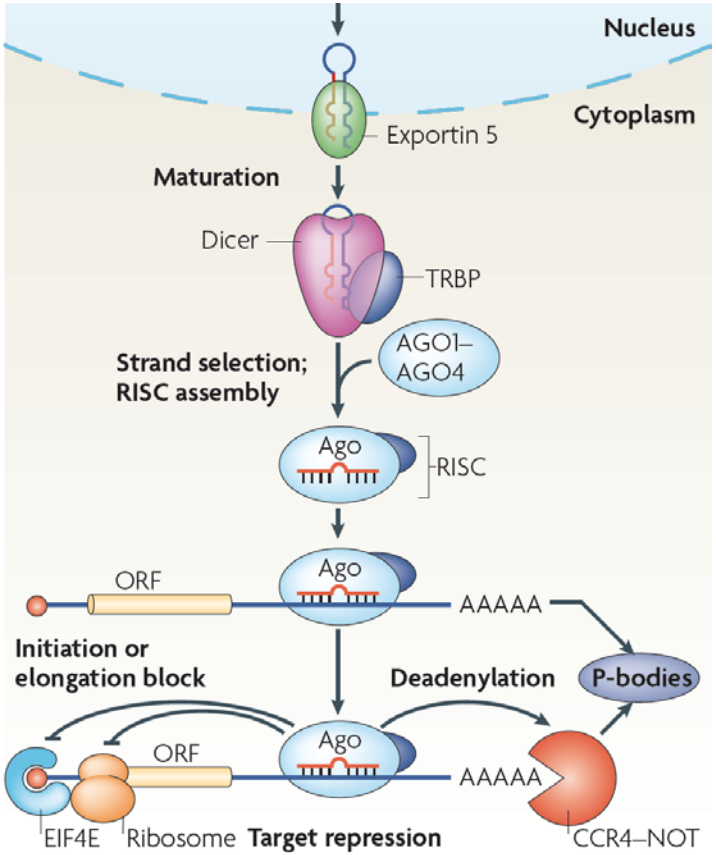
A role for microRNAs?

Hypertrophy

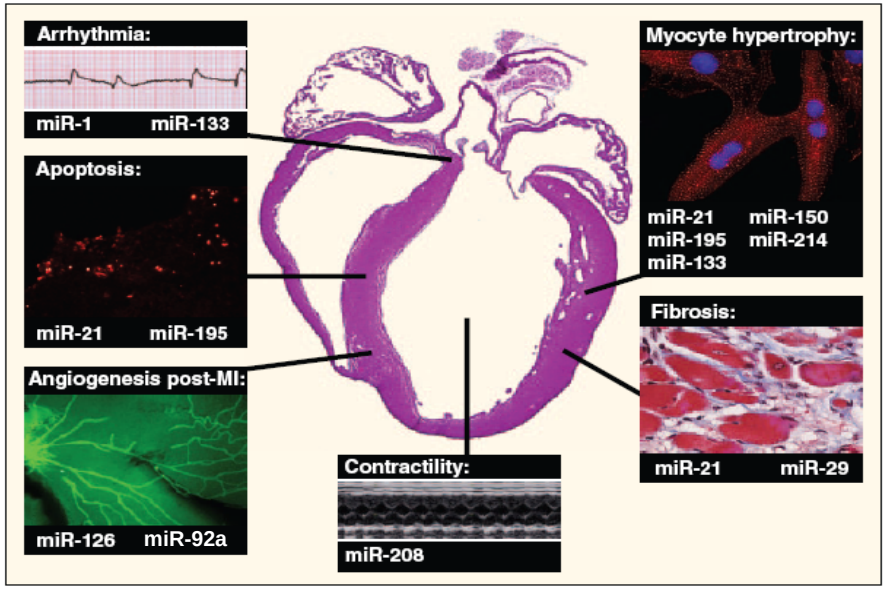


(Leri *et al*., Circ Res 2011)

miRNAs play a role in cardiovascular biology



(Inui *et al.*, Nat Rev Mol Cell Biol 2010)



(van Rooij *et al.*, Circ Res 2008 (modified))

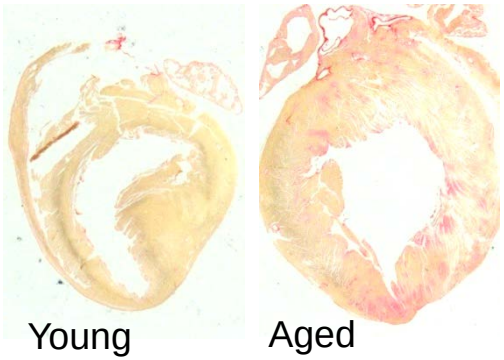
- miRNAs bind partially complimentary to target mRNAs
- One miRNA can have >100 target genes

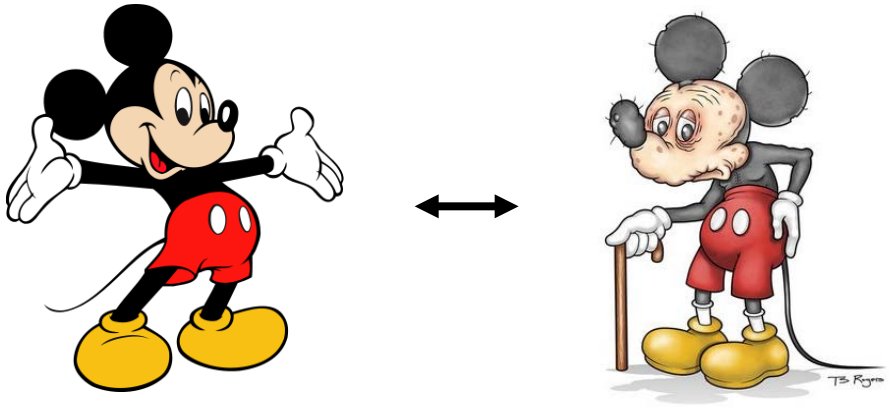
Aim: Identification of miRNAs that are dysregulated by age in the heart

Aging heart: Profiling set-up

- Isolate RNA from the heart
- MicroRNA profiles and mRNA profiles (micro-arrays)

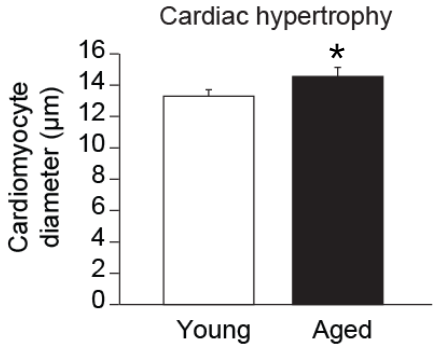
Fibrosis in the heart

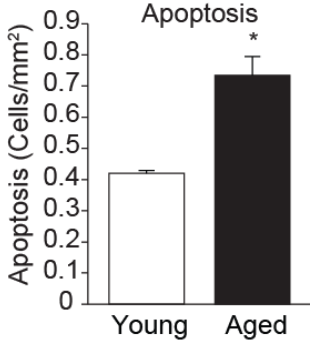




6 weeks

18 months





MicroRNA Profiling Results

Upregulated by age

GeneName	fold	FDR-p
mmu-miR-574-5p	2.00	0.00001
mmu-miR-29b	1.98	0.00001
mmu-miR-669c	1.96	0.00000
mmu-miR-21	1.96	0.00001
mmu-miR-34b-5p	1.80	0.00003
mmu-miR-34c	1.76	0.00001
mmu-miR-146a	1.70	0.00002
mmu-miR-468	1.63	0.00000
mmu-miR-1224	1.62	0.00031
mmu-miR-142-3p	1.60	0.00174
mmu-miR-24-1*	1.56	0.00040
mmu-miR-221	1.56	0.00002
mmu-miR-34a	1.55	0.00003
mmu-miR-29a	1.53	0.00011

Downregulated by age

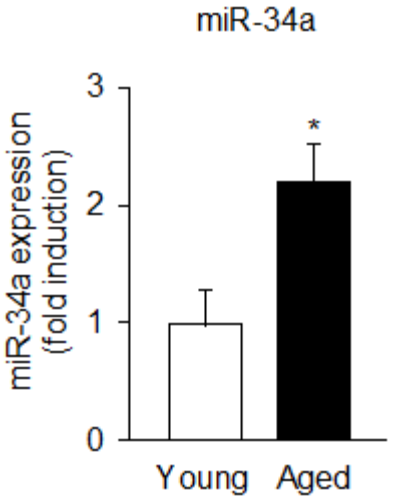
GeneName	fold	FDR-p
mmu-miR-181c	-1.61	0.00009
mmu-miR-130a	-1.58	0.00004
mmu-miR-181b	-1.53	0.00001
mmu-miR-127	-1.51	0.00008

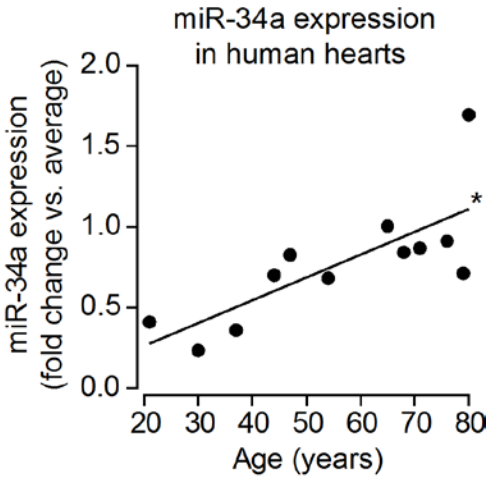
- Myocardial infarction
- Aortic Banding
- Calcineurin-transgenic mice
- Chronic Angiotensin II infusion

→ miR-21 ↑

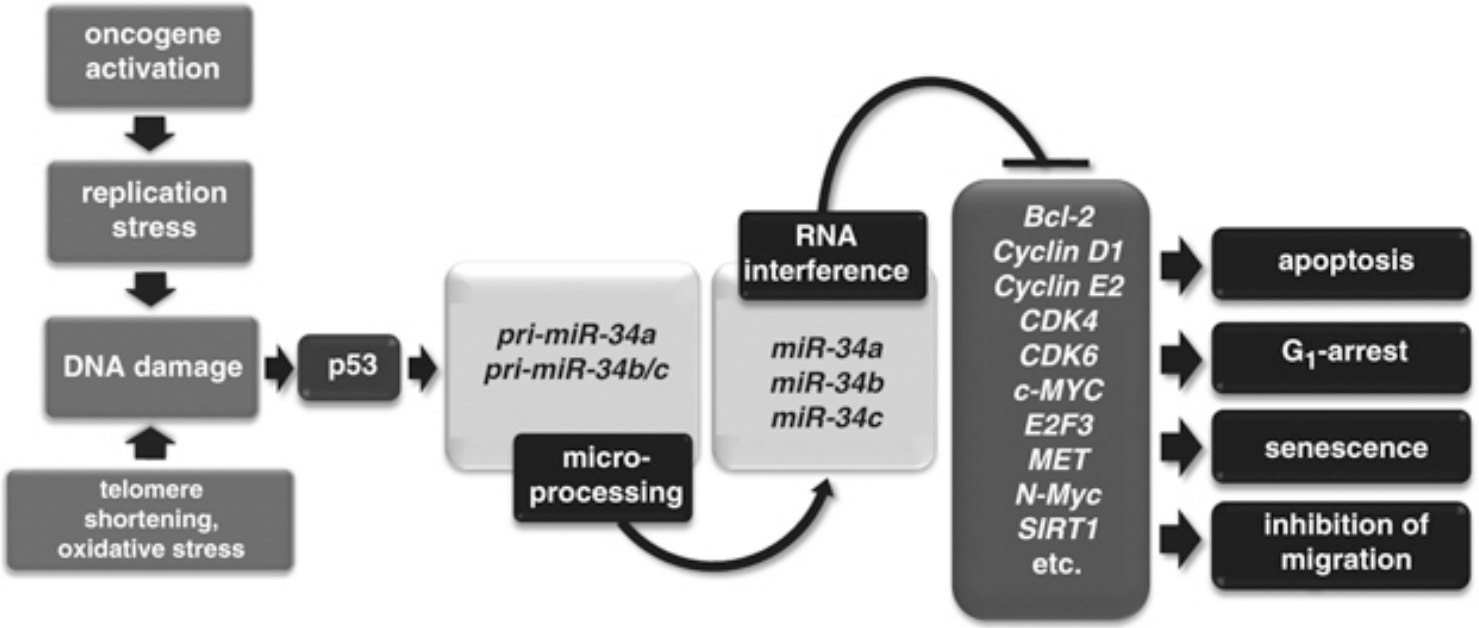
(van Rooij *et al.*, PNAS 2008)
(Thum *et al.*, Nature 2008)
(Patrick *et al.*, JCI 2010)

miR-34a u**ggcagug**ucuuagcugguugu → Alone
miR-34b ua**ggcagug**ucauuagcugauug
miR-34c a**ggcagug**uaguuagcugauugc → Together in a cluster



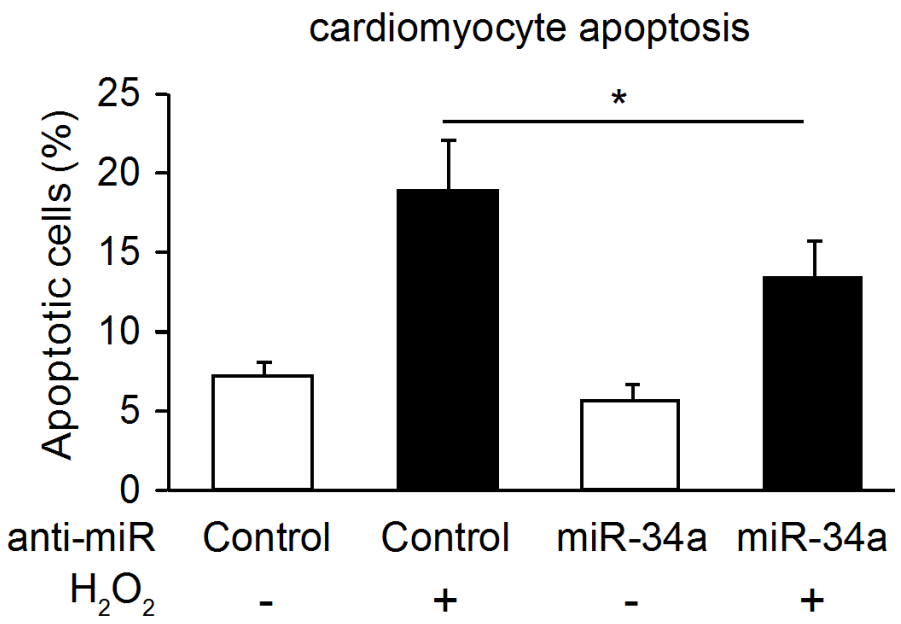


MiR-34a is known to play a role in apoptosis and senescence

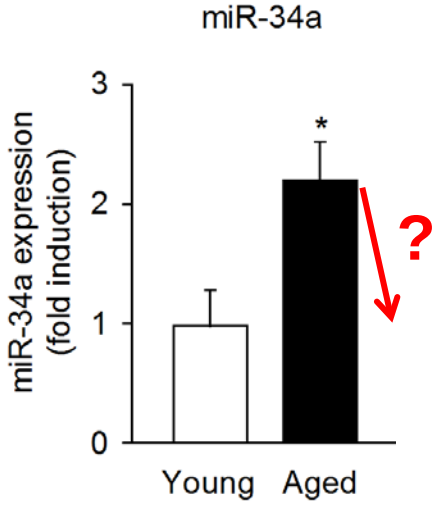


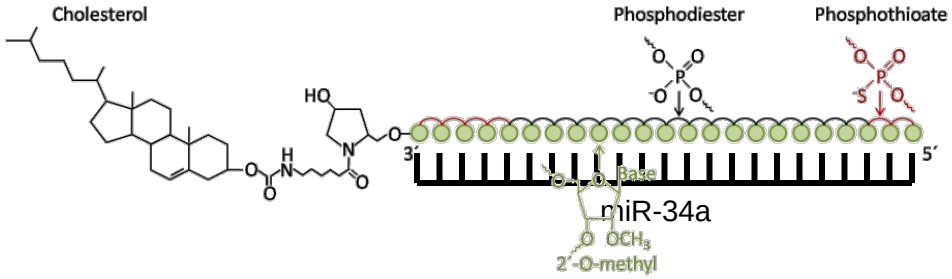
(Hermeking, Cell Death and Differentiation 2010)

MiR-34a inhibition reduces cardiomyocyte apoptosis *in vitro*

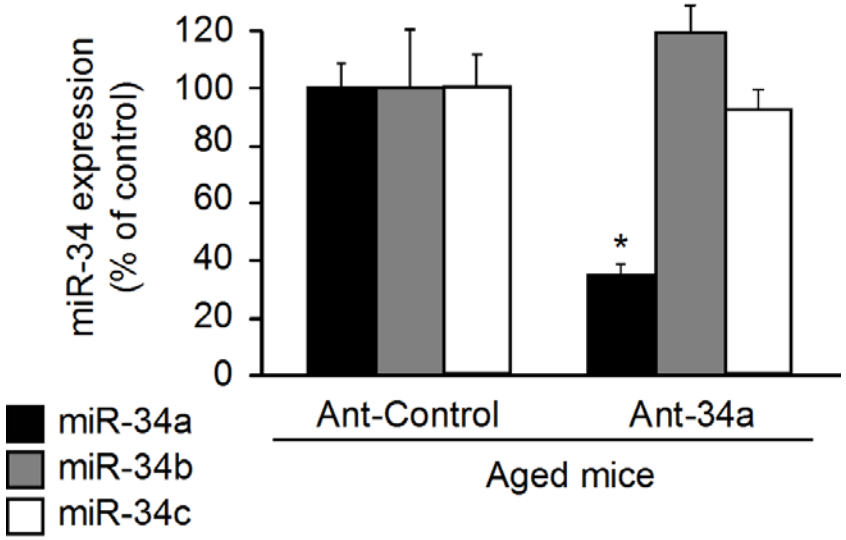


AntagomiR-34a treatment efficiently knocks down miR-34a and inhibits apoptosis *in vivo*

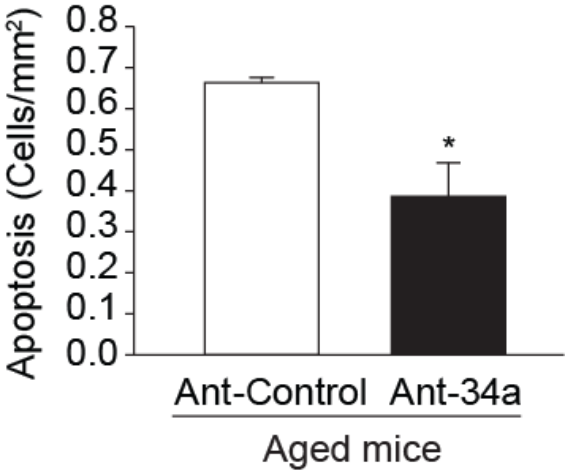




Cardiac miR-34 levels, 2 days after IV injection

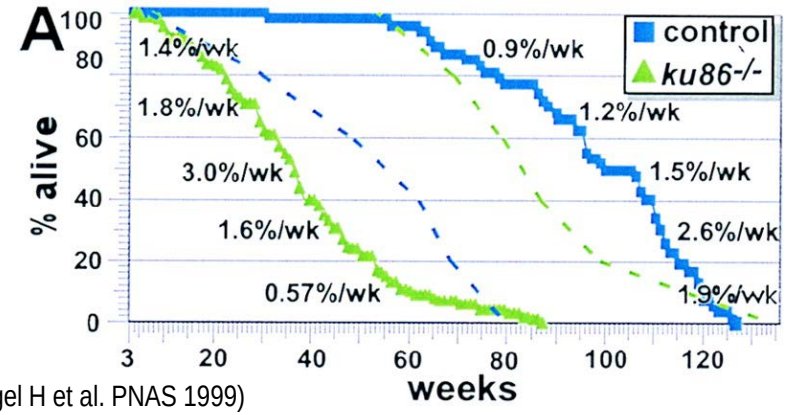


Cardiomyocyte apoptosis in vivo

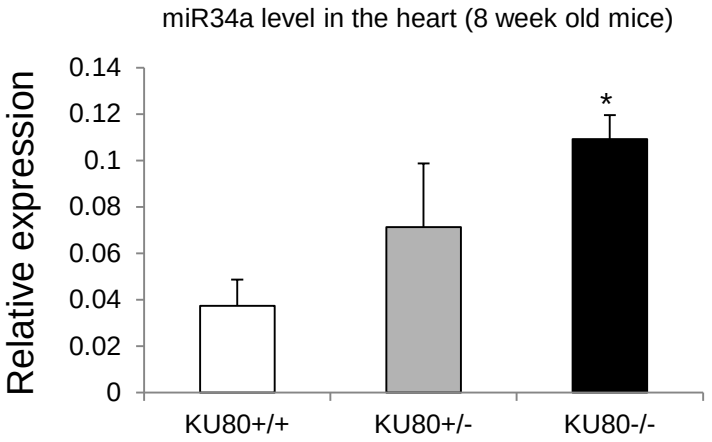


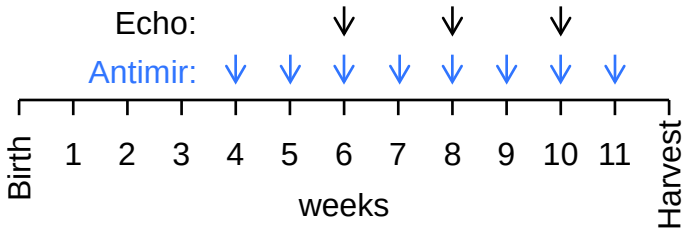
7 days after IV injection

Inhibition of miR-34a in progeria mice rescues cardiac function

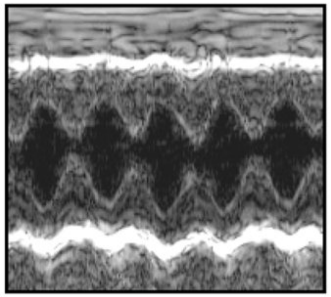


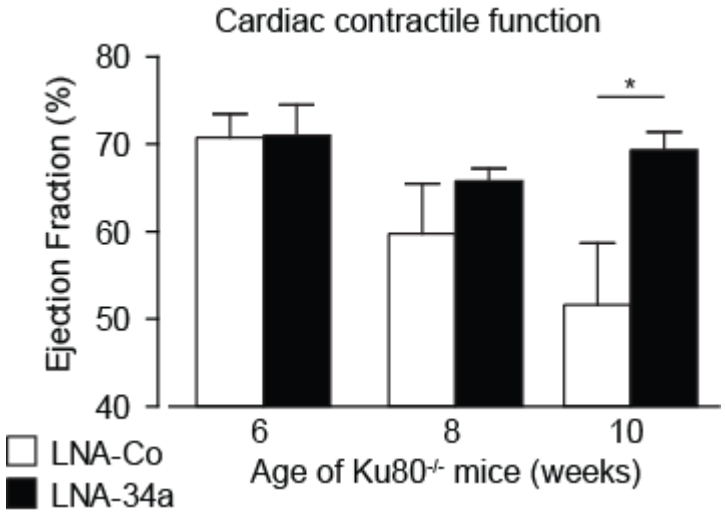
(Vogel H et al. PNAS 1999)





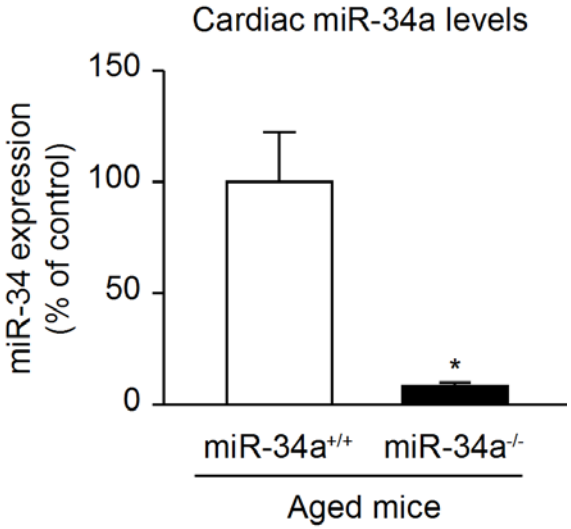
Monitoring of heart function by echo

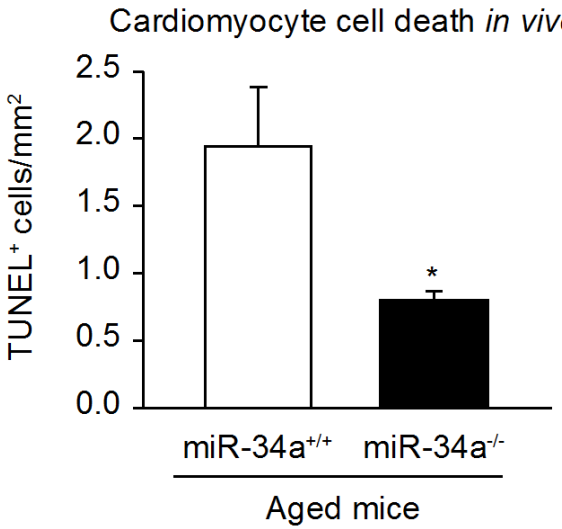


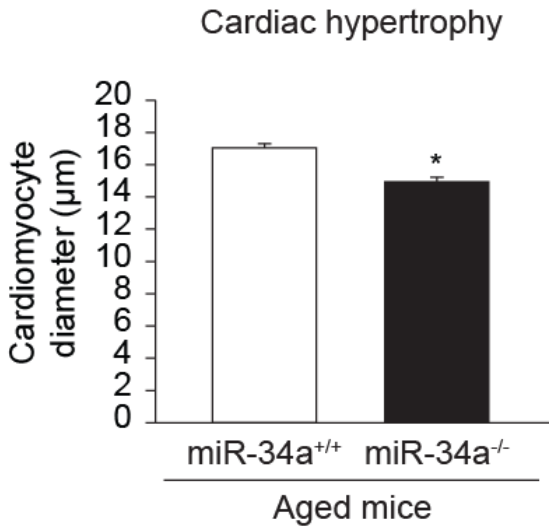


Ku80$^{-/-}$ mice provided by Prof. Dr. Sassoon, Paris

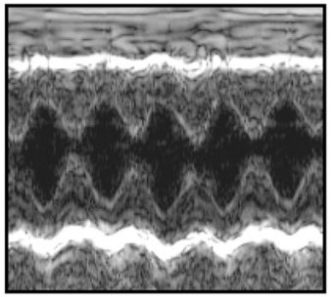
Aged miR-34a$^{-/-}$ mice have maintained cardiac function

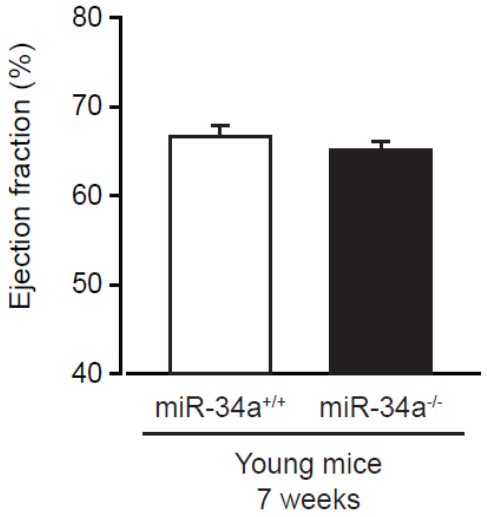


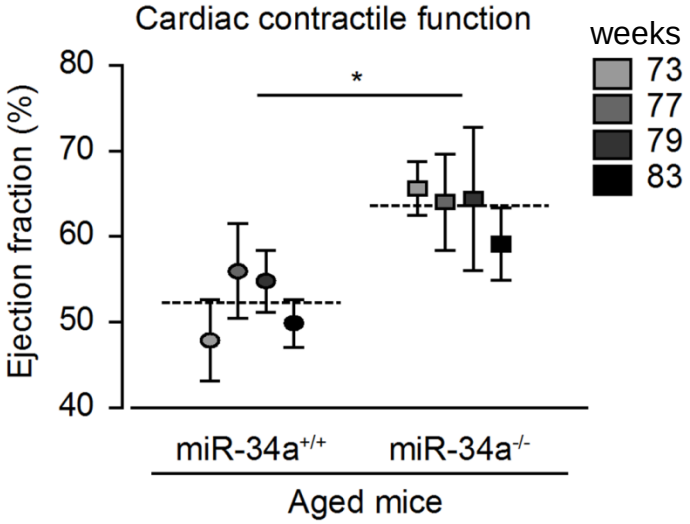




Monitoring of heart function by echo

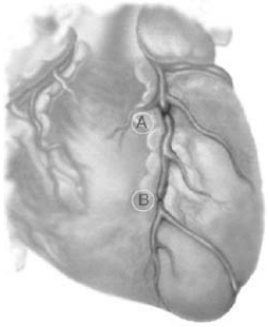




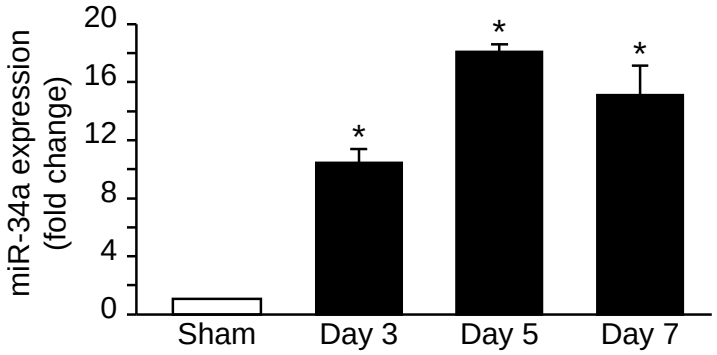


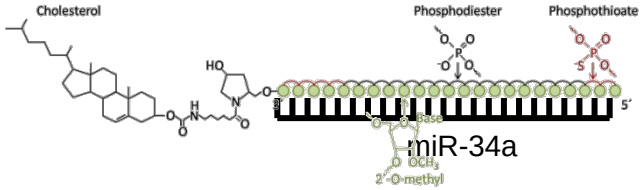
miR-34a$^{-/-}$ mice provided by Prof. Dr. Hermeking, Munich

Antagomir-34a treatment improves cardiac function after acute myocardial infarction

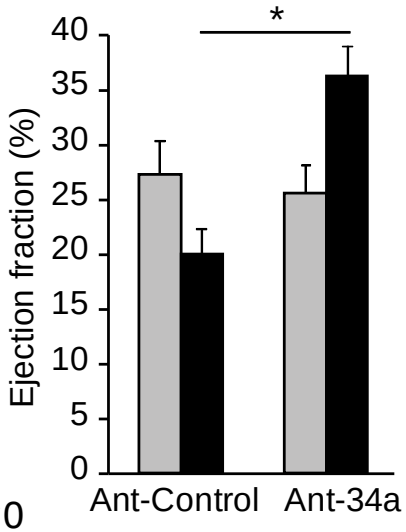


miR-34a levels in the infarct zone

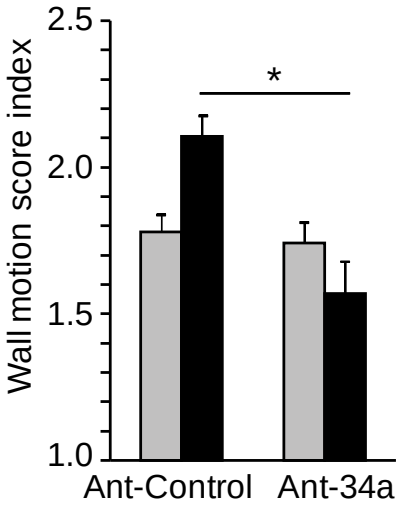




Ejection fraction

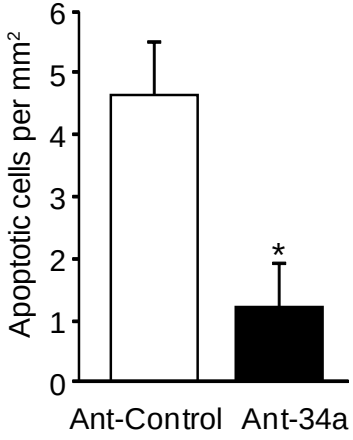


Wall motion score index

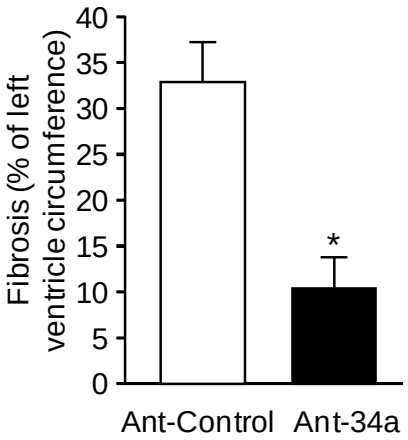


Histology:

Apoptosis (infarct)

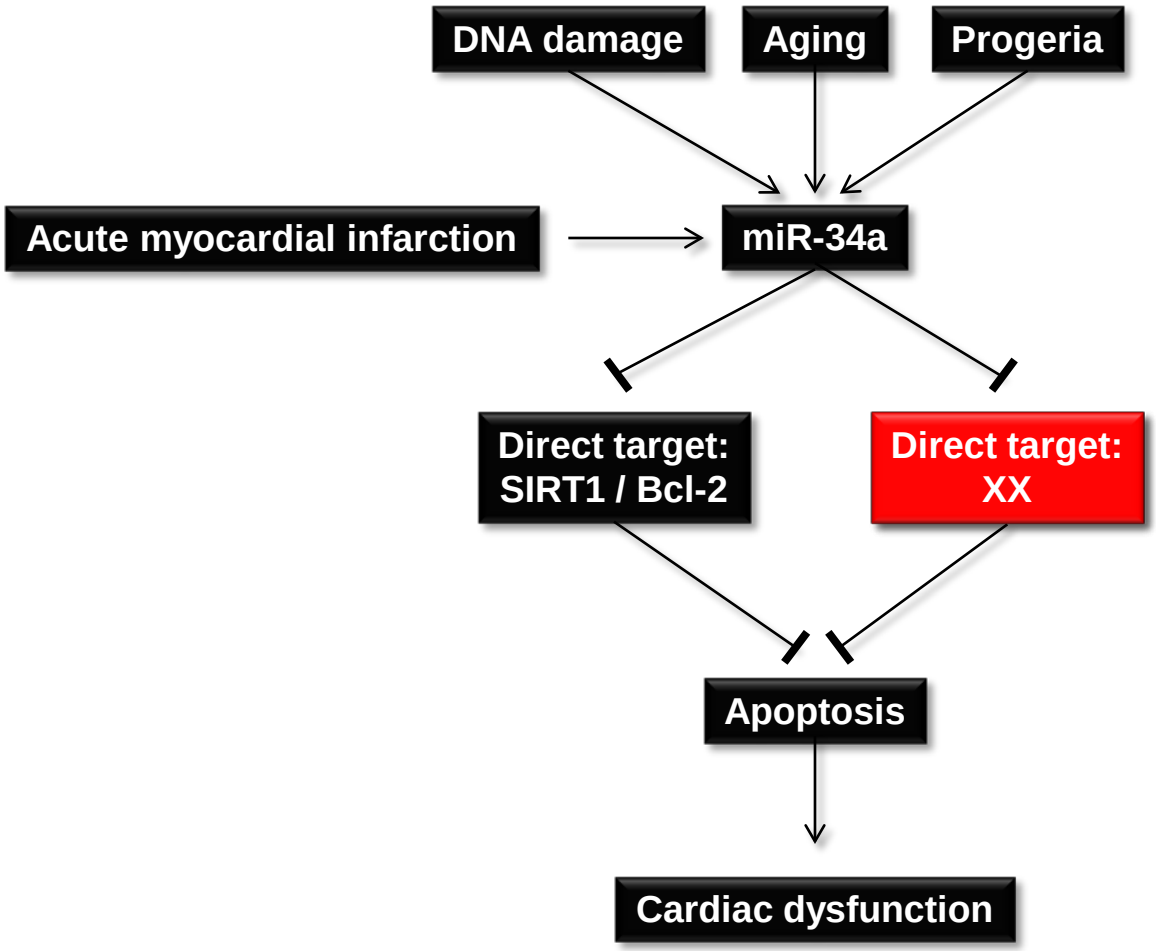


Fibrosis

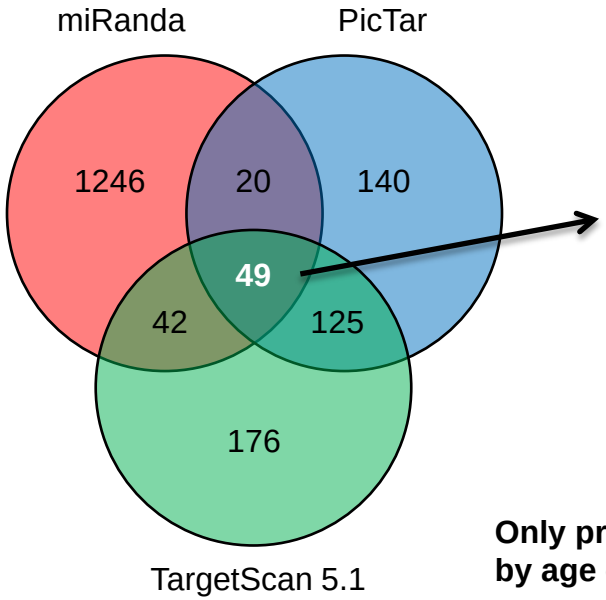


day0
day14

How does miR-34a augment cardiac apoptosis?



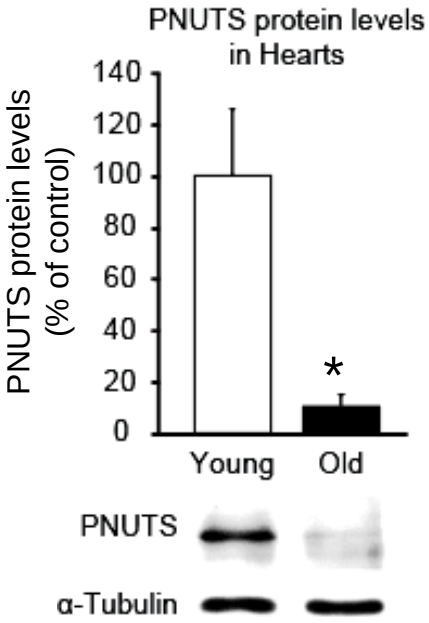
In silico predicted targets of miR-34a: PNUTS



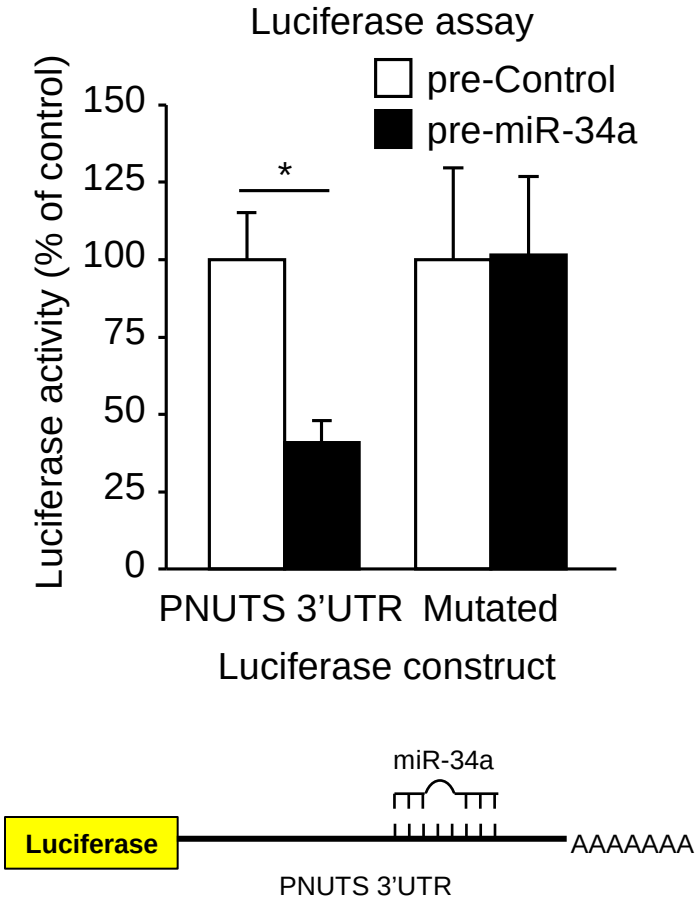
ABR	DPYSL4	MYRIP	PPP1R11	STRN3
ACCN1	E2F5	NAV3	PTPRM	SYVN1
ALDOA	EEF2K	NFE2L1	PURB	TAF5
AXL	ELMOD1	NRIP3	RPS6KA4	TCF12
BTBD11	FOXP1	NUMBL	RRAS	TNRC4
CACNB3	FUT8	PACS1	SEMA4C	TTC19
CNTNAP1	GALNT7	PHF15	SIDT1	UBP1
COL12A1	JAG1	PKP4	SLC30A3	UHRF2
CRHR1	LEF1	PLCG1	SNX15	ZDHHC23
DBC1	MLLT3	PPP1R10	SRPR	

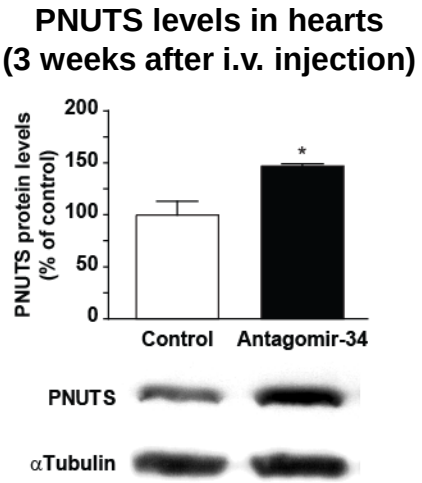
Only predicted target that is downregulated (<-1.5 fold) by age on the mRNA level (micro-array) (-2.0 fold)

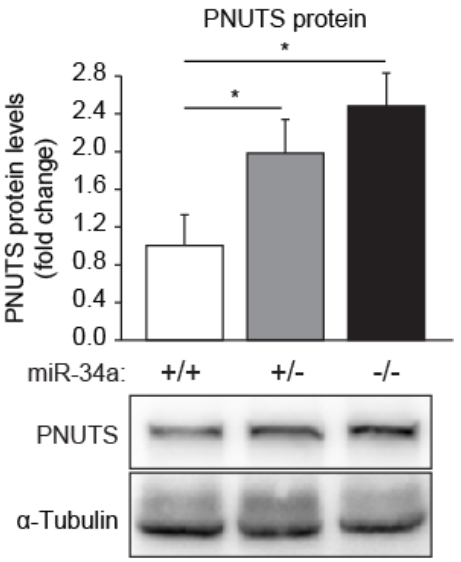
Also known as: Protein Phosphatase 1 Nuclear Targeting Subunit (PNUTS)



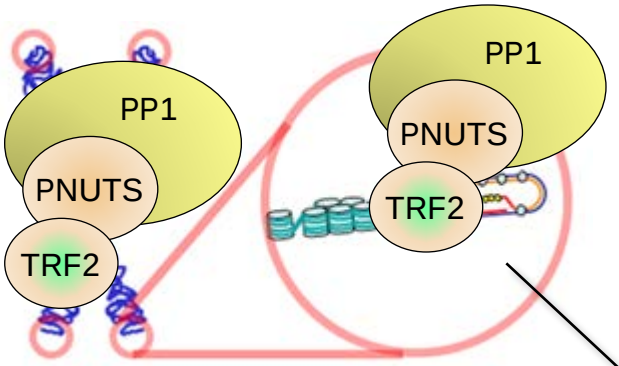
PNUTS is a direct target of miR-34a







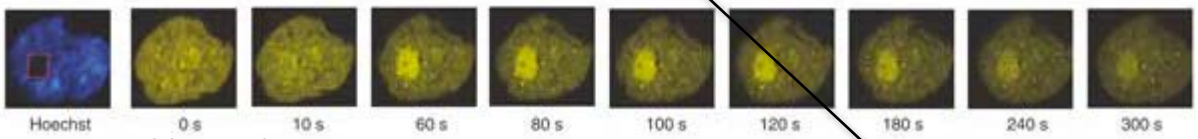
PNUTS interacts with telomere regulator TRF2



- PNUTS interacts with TRF2 at telomeres (Kim *et al.* Nat Struct Mol Biol. 2009)
- TRF2 protects telomeres from degradation and prevents apoptosis (Karlseder *et al. Science* 1999)

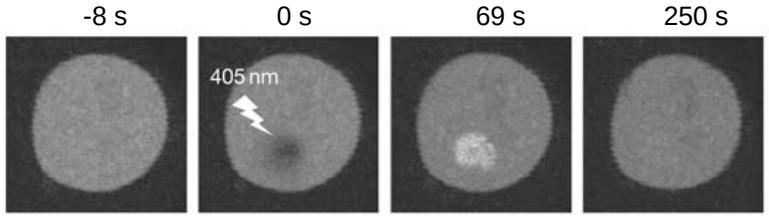
- TRF2 loss-of-function is linked to human heart failure (Oh H *et al.* PNAS 2003)

TRF2 localizes to DNA Damage



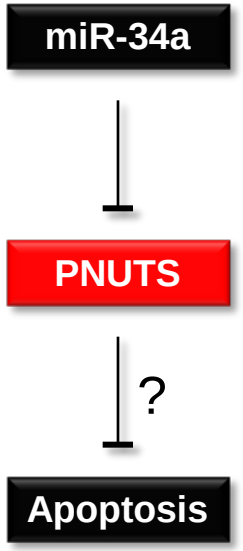
Bradshaw *et al.* Nat. Genet. 2005

PNUTS localizes to DNA Damage

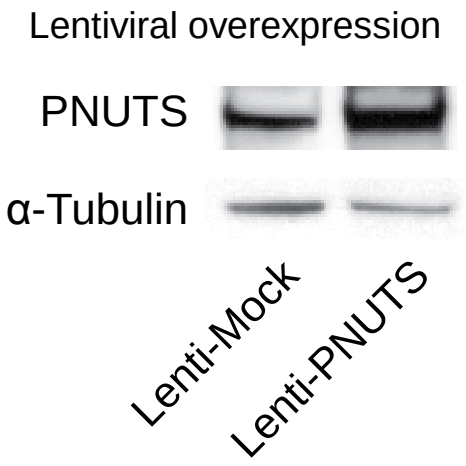


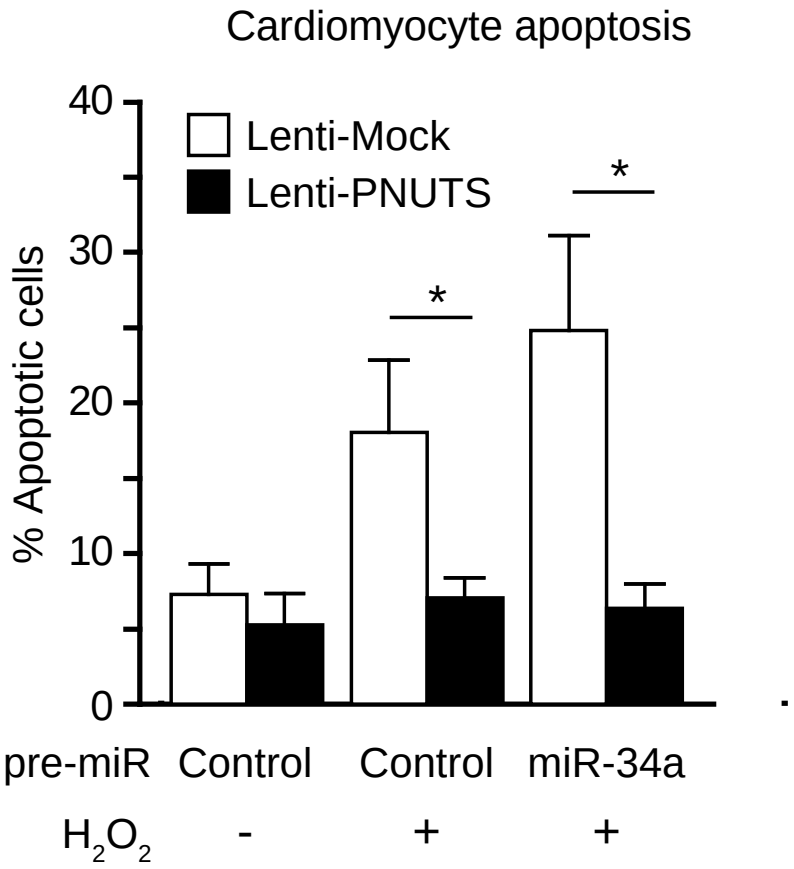
Landsverk *et al.* EMBO Rep. 2010

Apoptosis



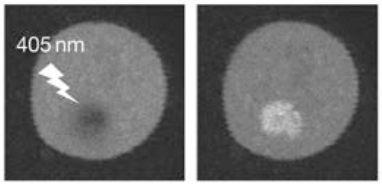
PNUTS overexpression rescues miR-34a-induced apoptosis in cardiomyocytes *in vitro*





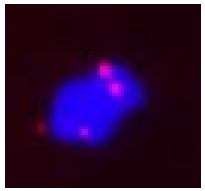
PNUTS reduces Chk2 activation

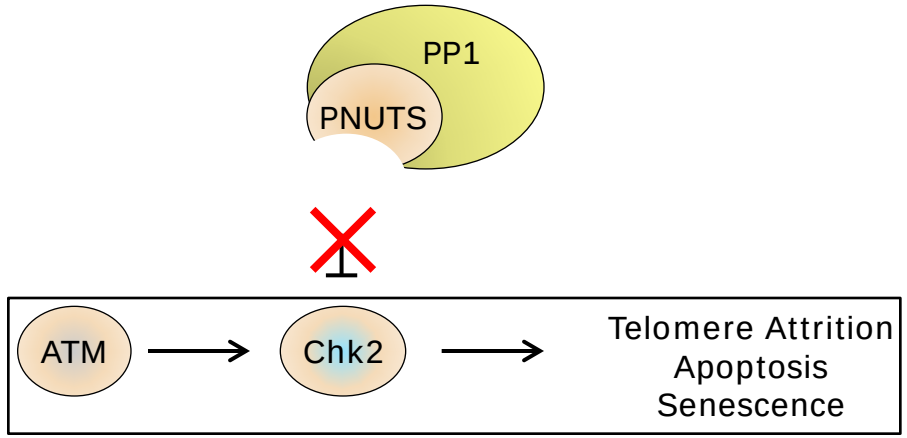
DNA Damage

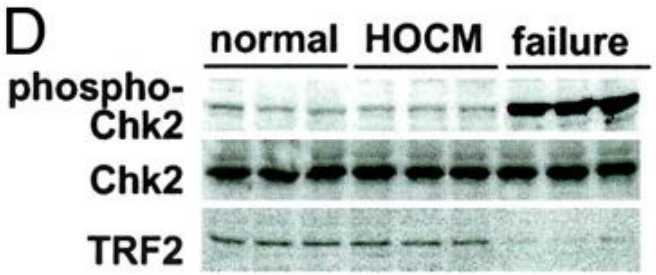


Landsverk *et al.* EMBO Rep. 2010

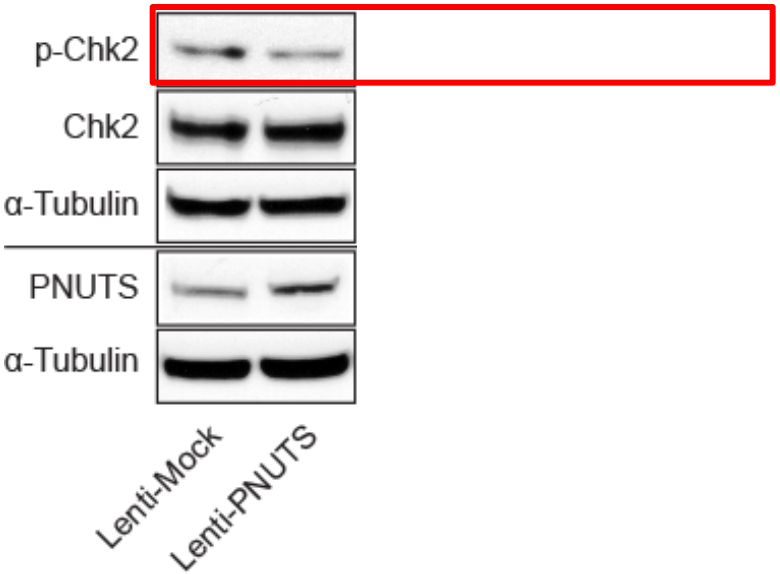
Telomere dysfunction



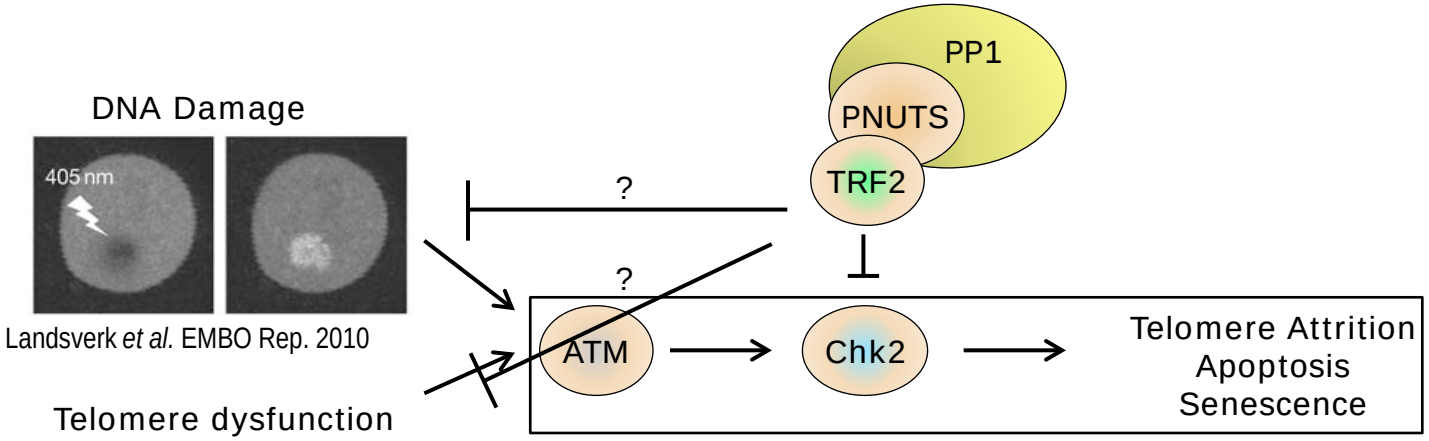




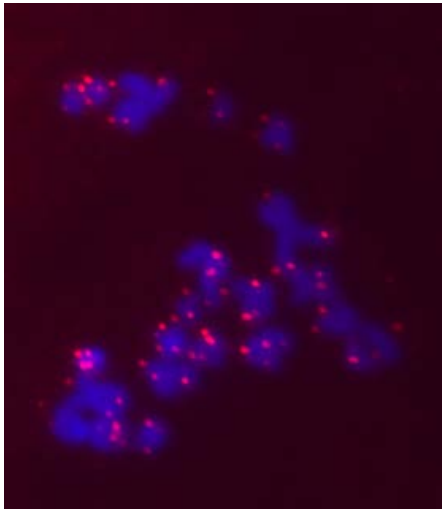
(Oh H *et al.* PNAS 2003)

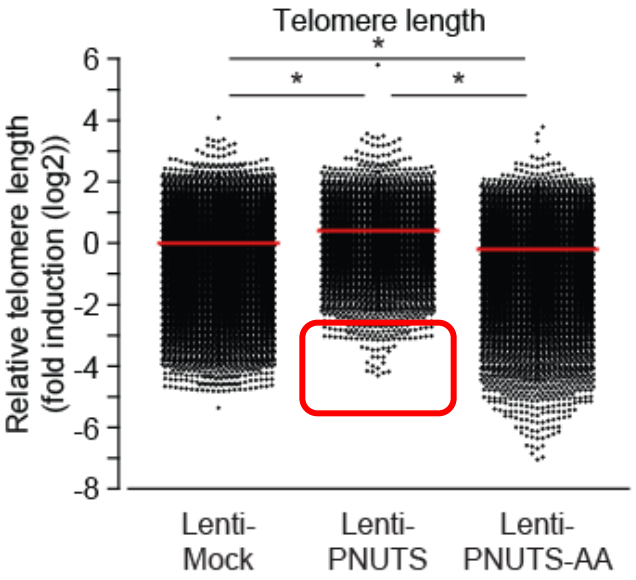


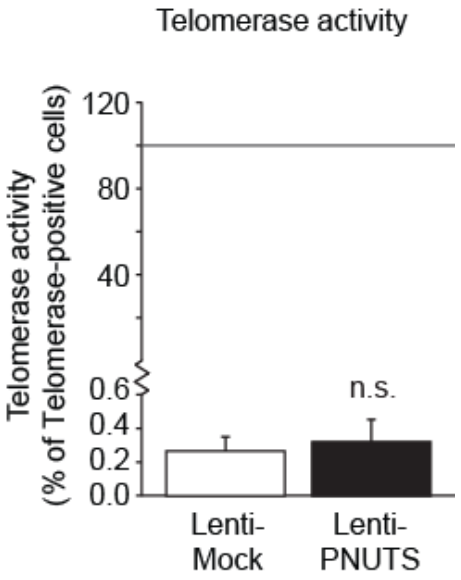
PNUTS induces telomere maintenance



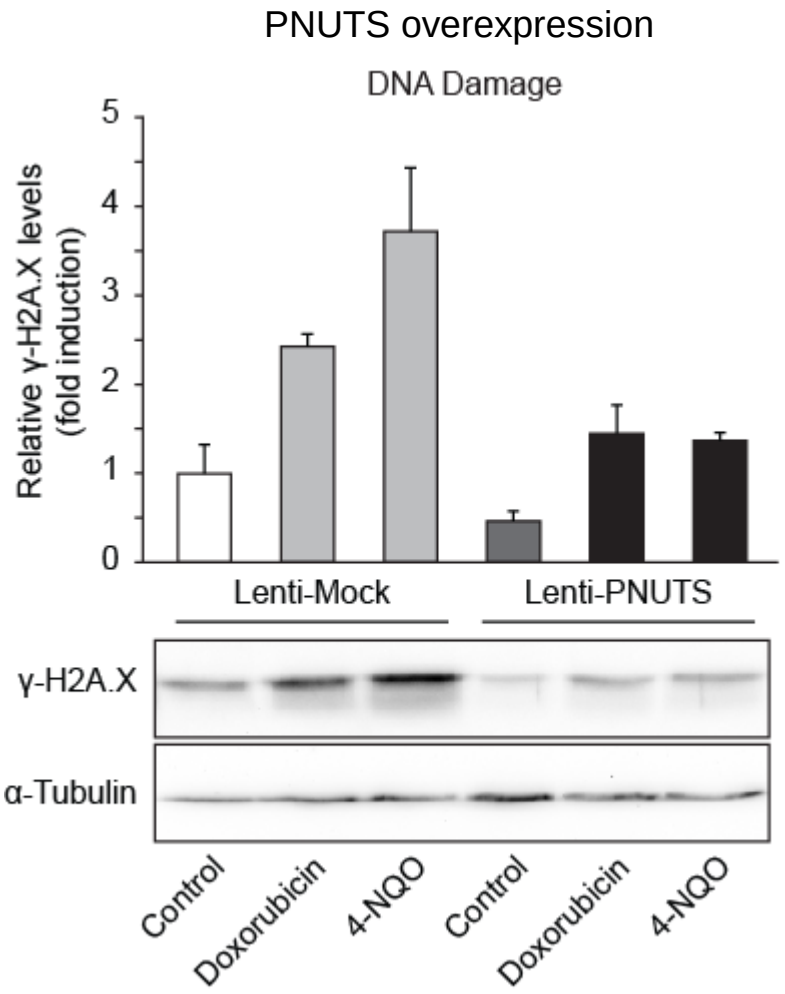
Telomere Q-FISH



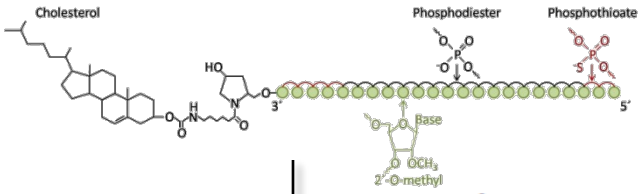




PNUTS inhibits DNA damage



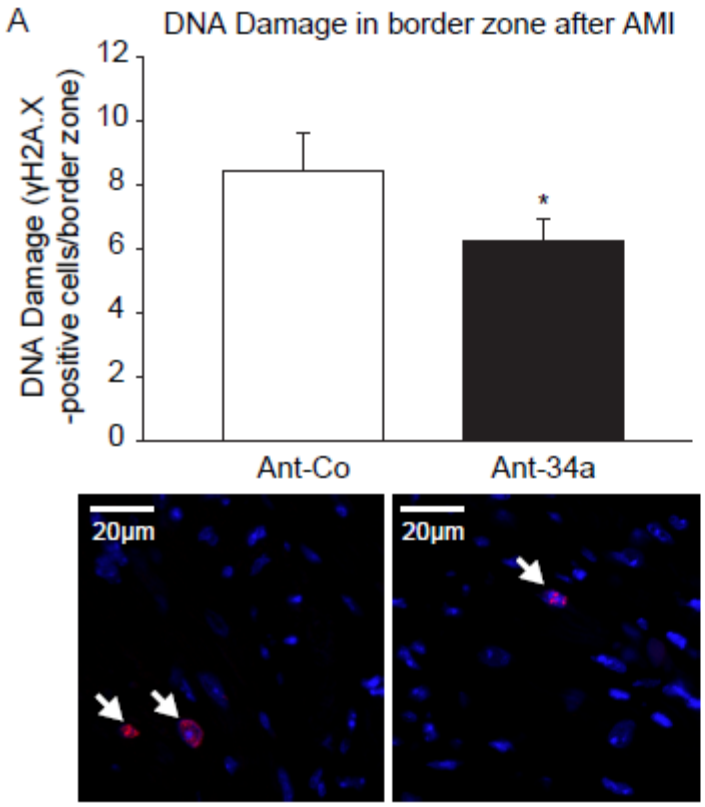
miR-34a inhibition reduces DNA Damage after AMI

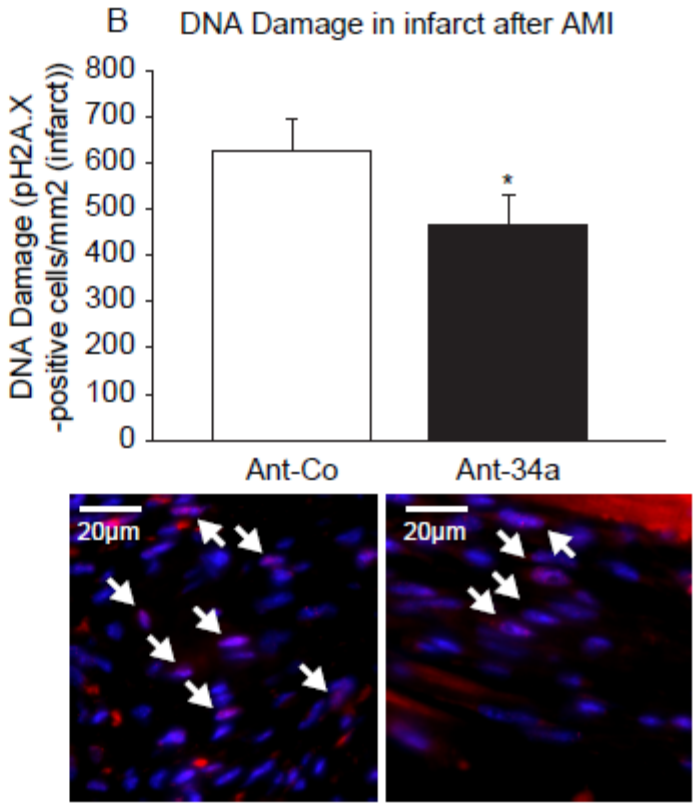


miR-34a

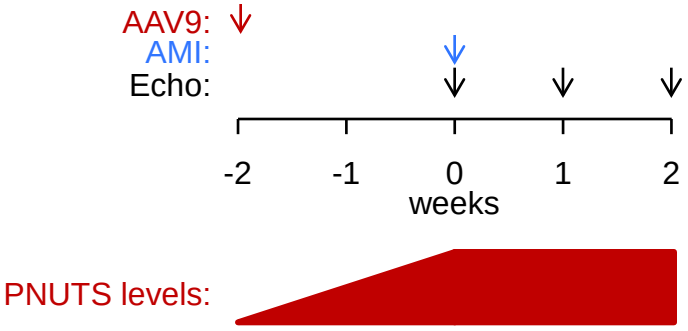
PNUTS

DNA Damage

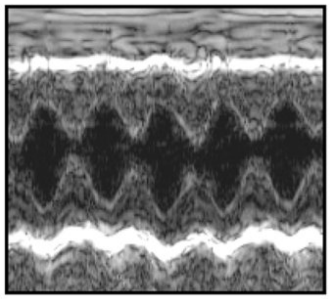




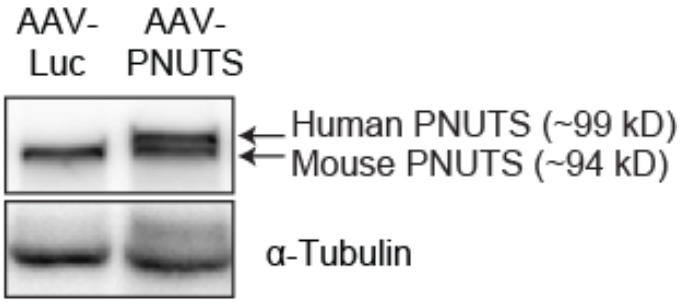
Cardiac PNUTS overexpression preserves cardiac function after AMI



Monitoring of heart function by echo

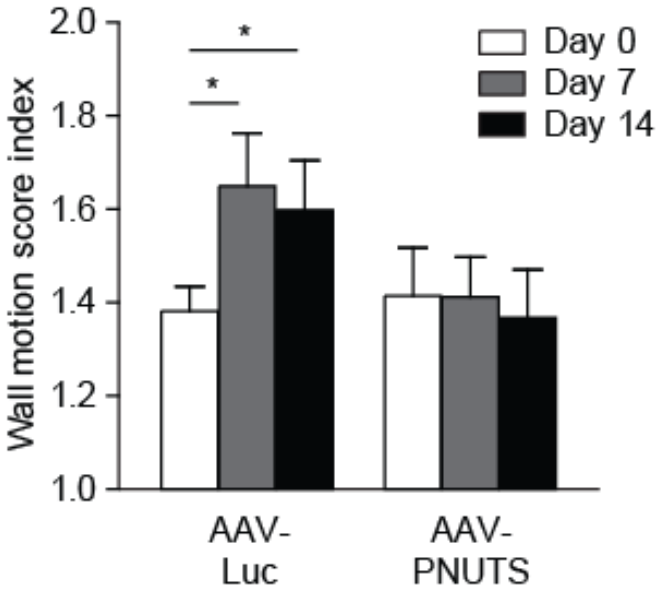


Cardiac PNUTS protein levels



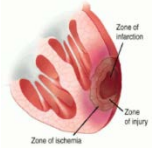
AAV9 with cardiac-specific CMV-enhanced myosin light chain promoter

Cardiac contractile function



AAV Vectors provided by Dr. Müller, Heidelberg

Graphical Summary



AMI

Aging

Ku80$^{-/-}$

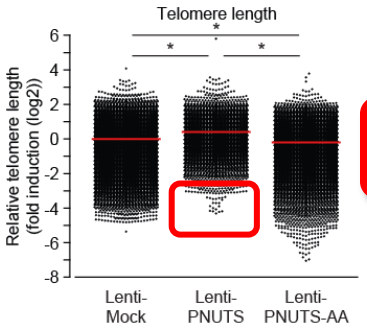
miR-34a

Other targets

PNUTS

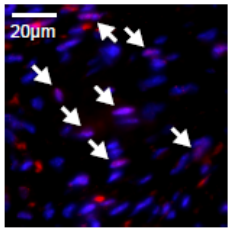
AAAAAA

TRF2



Telomere Dysfunction

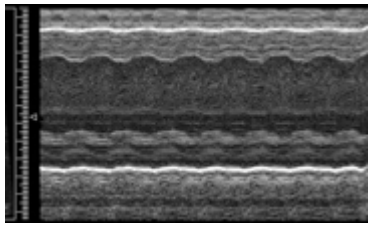
DDR



Apoptosis

Fibrosis

Hypertrophy



Contractile dysfunction

Acknowledgements

Institute for Cardiovascular Regeneration, Goethe University, Frankfurt
Kazuma Iekushi
Timon Seeger
Susanne Heydt
Franziska Gehring
Natalja Reinfeld
Ariane Fischer
Marion Muhly-Reinholz
Michael Potente
Stefanie Dimmeler

Goethe University, Frankfurt
Joachim Ehrlich
Ralf Brandes
Andreas Zeiher

Heidelberg University Clinic
Oliver Müller

Ludwig-Maximilians-University, Munich
Heiko Hermeking