

“Investigating autophagy (re)activation mechanisms to counteract FOP”



Dr. Venturina Stagni

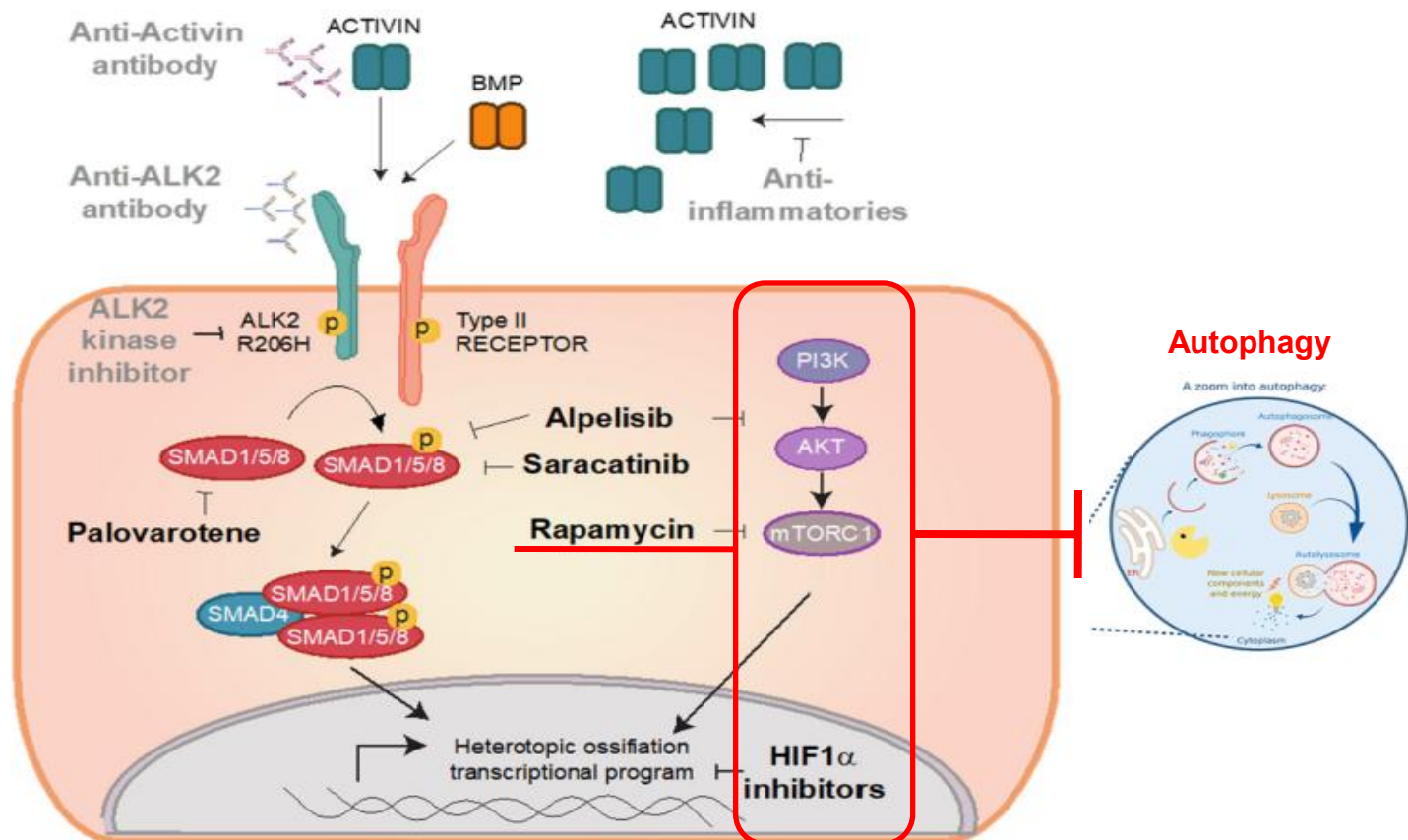


Molecular targeted approaches for FOP

Review

Challenges and Opportunities for Drug Repositioning in Fibrodysplasia Ossificans Progressiva

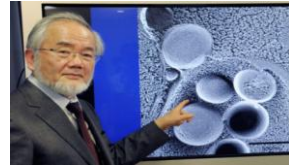
Francesc Ventura ¹, Eleanor Williams ², Makoto Ikeya ³, Alex N. Bullock ², Peter ten Dijke ⁴, Marie-José Goumans ⁵ and Gonzalo Sanchez-Duffhues ^{5,*}



Autophagy: remove, reuse, recycle

Autophagy is the ultimate recycling system of the cell

HOW?

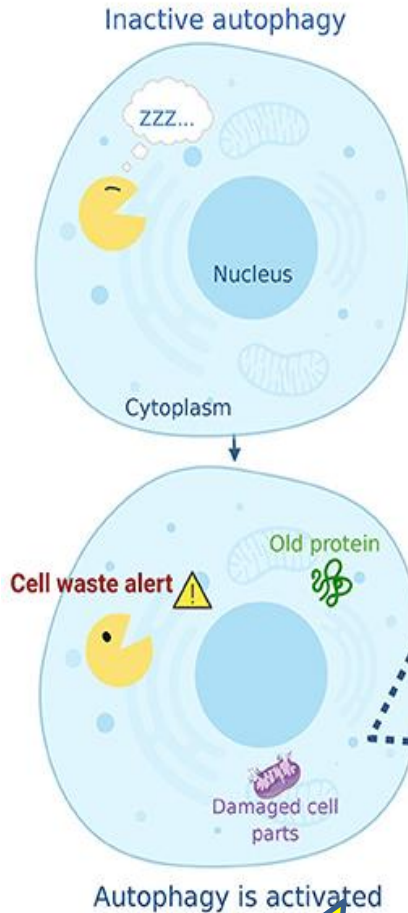


Yoshinori Ohsumi,
Nobel prize in
Medicine 2016

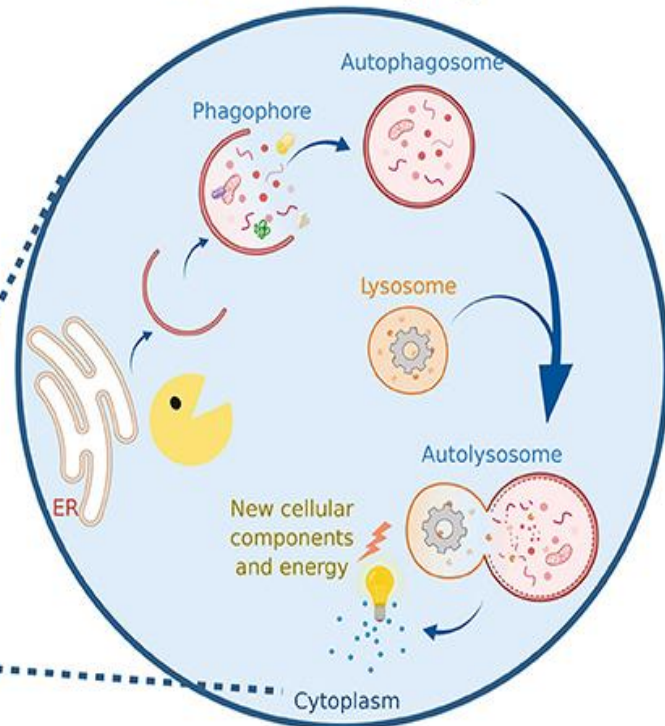


Keep the ^{cell} environment clean!

The word comes from ancient Greek and means "eating of self"



A zoom into autophagy:



Farro et al. 2021 Frontiers for Young Minds

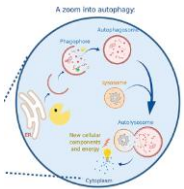
Autophagy is a dynamic and tightly regulated process

Why looking at Autophagy in FOP?

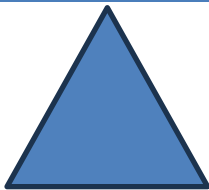
Autophagy dysregulation has been implicated in several muscles and skeletal disorders

(Chen et al J. Of Cachexia 2022 , Moss J et al, 2021)

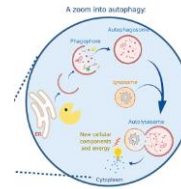
Normal autophagic flux



Muscle regeneration
Chondrocyte differentiation

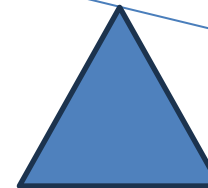


Impaired autophagic flux



muscle atrophy
defects in muscle
regeneration

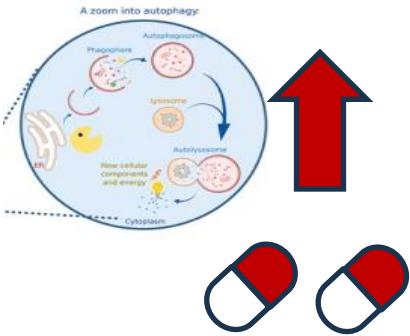
Accelerated chondrocyte
differentiation



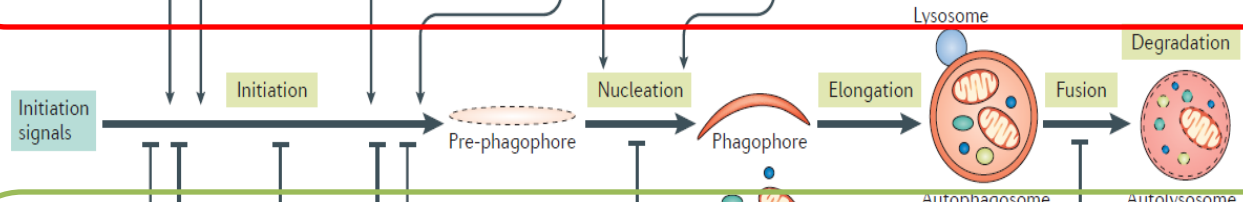
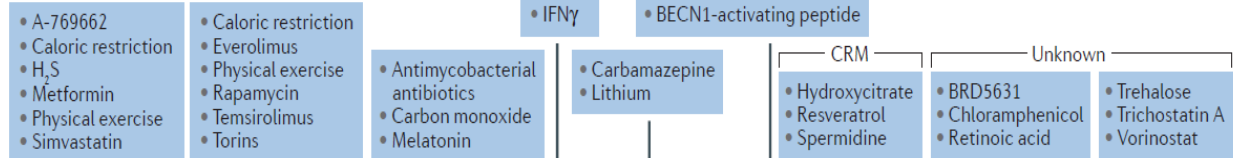
Autophagy modulators drugs

Autophagic processes amenable to therapeutic modulation

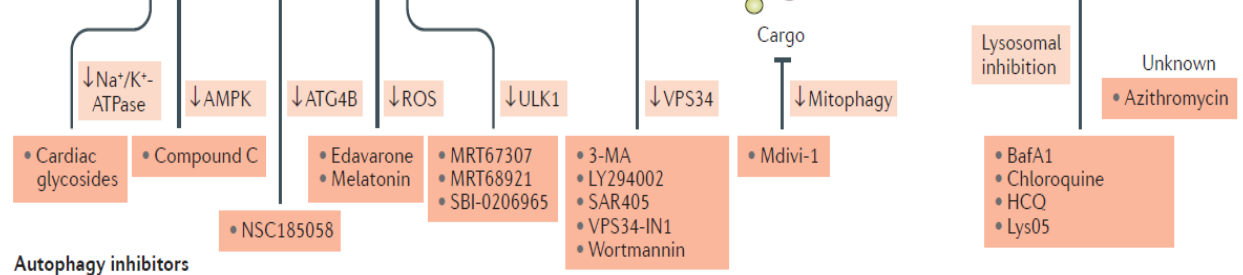
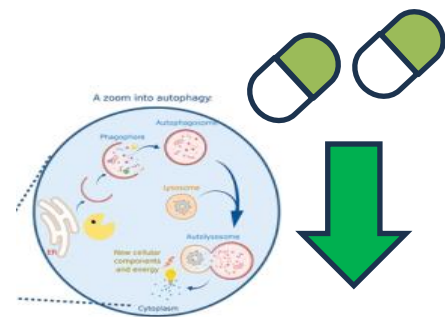
Autophagy Activators



Autophagy activators



Autophagy inhibitors

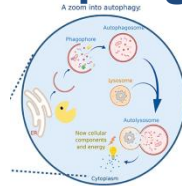


Galluzzi et al. Nature 2017

Autophagic drugs are in trials for several disease

Is Autophagy involved in FOP?

Autophagy



?

ALK2-R206H



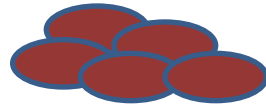
Heterotopic Ossification (HO)

In vitro Cellular Model systems

ALK2^{WT}



ALK2^{R206H}



1-ATDC5 cells

for *in vitro* chondrocyte differentiation

2-U2OS cells

for biochemical and molecular analysis

3-ECFCs cells

derived from FOP patients

ATDC5 cells model system and FOP cells from Prof Sánchez-Duffhues

Hypoxic conditions significantly facilitate the chondrogenic differentiation process *in vitro*, mimic *in vivo* conditions



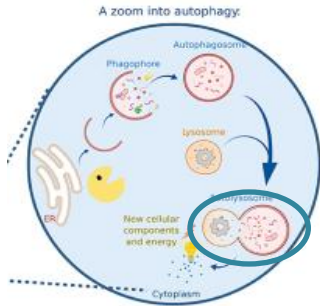
SAPIENZA
UNIVERSITÀ DI ROMA



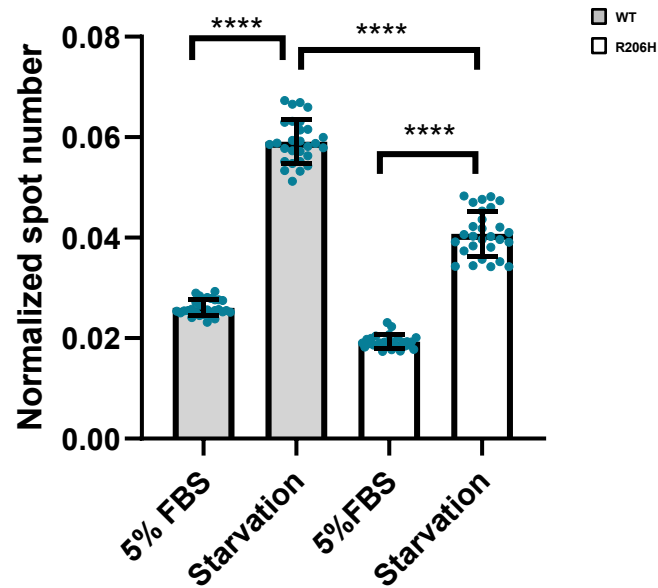
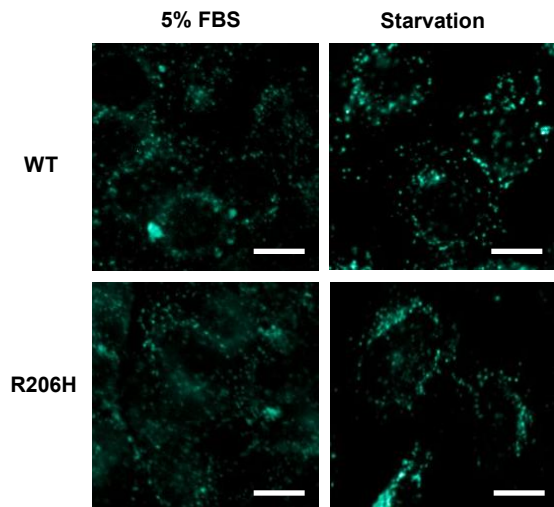
Hypoxic chamber
for cell culture



Evaluation the effect of $ALK2^{R206H}$ expression on the formation of autophagic vacuoles



Monodansylcadaverine (MDC) is a specific marker for autolysosomes



In collaboration with Pedemonte N., Cappato S., Bocciardi R (Gaslini, Genova)

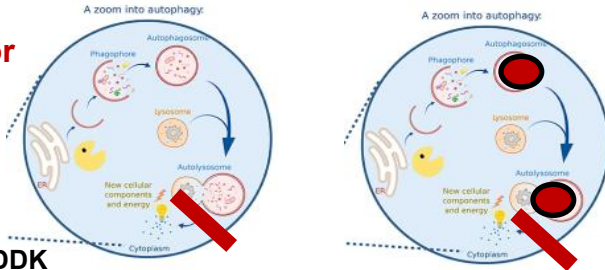
$ALK2^{R206H}$ expressing cells exhibited a significant reduction in the number of autolysosome dots

ALK2^{R206H} mutant receptor localization after inhibition of autophagy with CQ

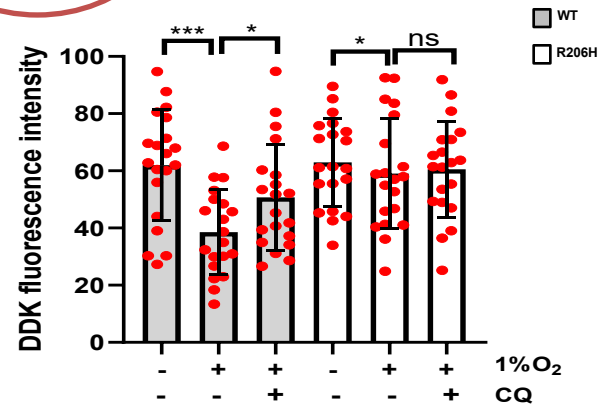
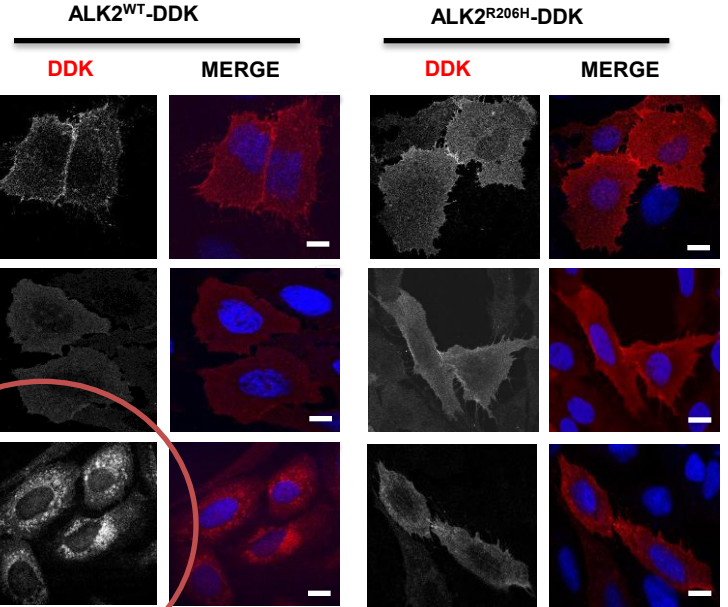
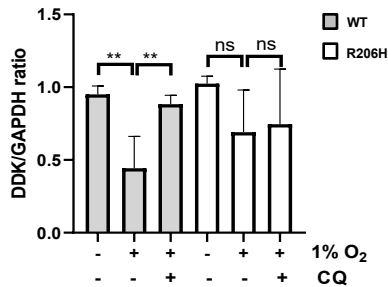
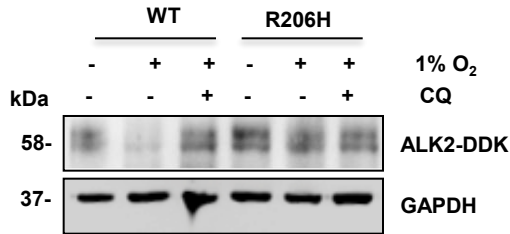
ALK2 receptor



U2OS ALK2-DDK



✓ Autophagic inhibitors
Chloroquine (CQ)



Is the ALK2 receptor localized at autophagosome?

Is the ALK2 receptor localized at autophagosome?

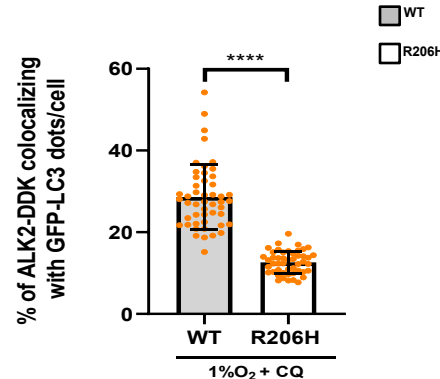
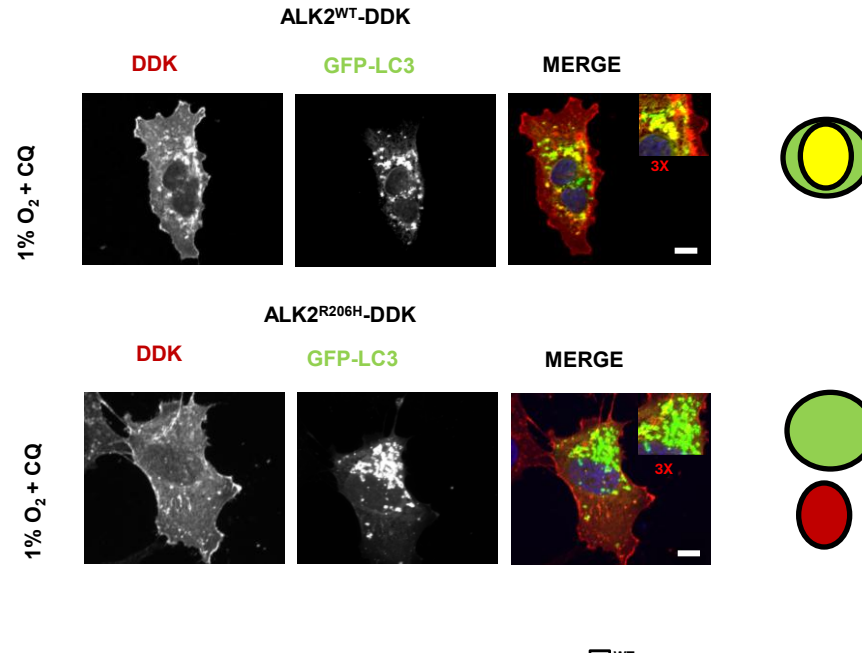
Autophagosome



ALK2 receptor

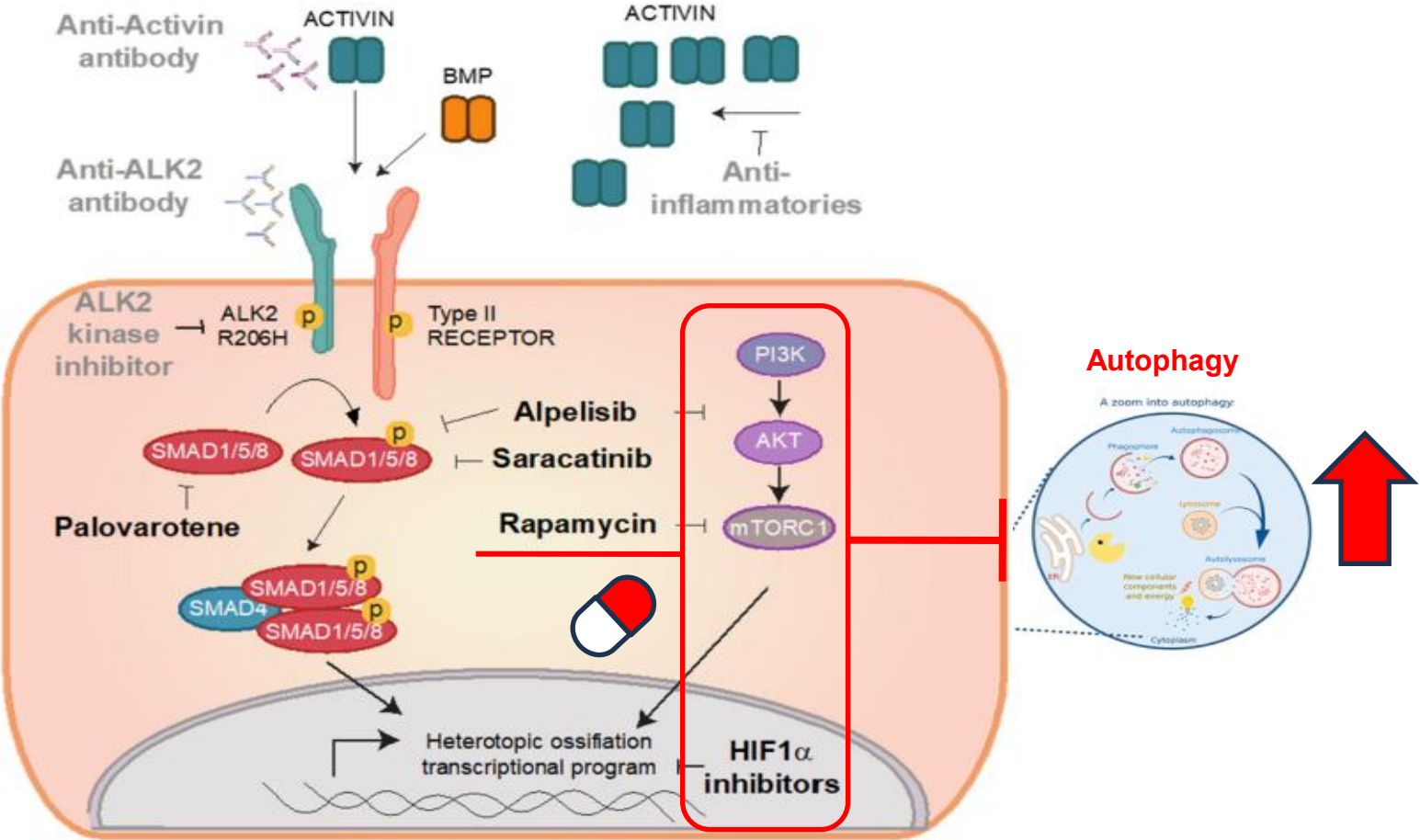


ALK2 receptor
autophagosome
colocalization



This suggest that the p.R206H mutation not only impairs autophagy response but also compromises the degradation of ALK2 by reducing ALK2 association to autophagic vesicles

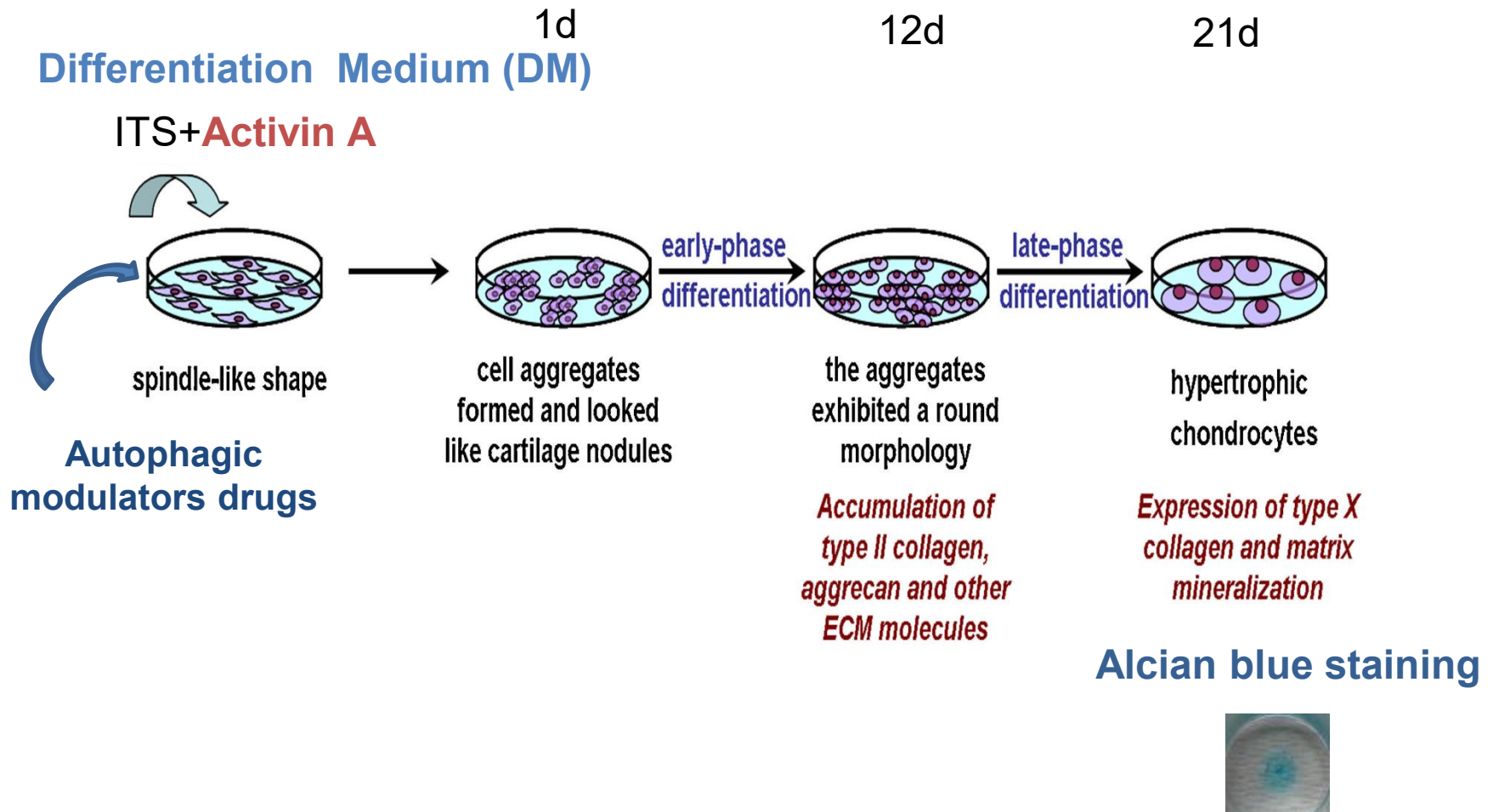
Rapamycin, an mTOR inhibitor, effectively inhibits the Activin-A-induced HO



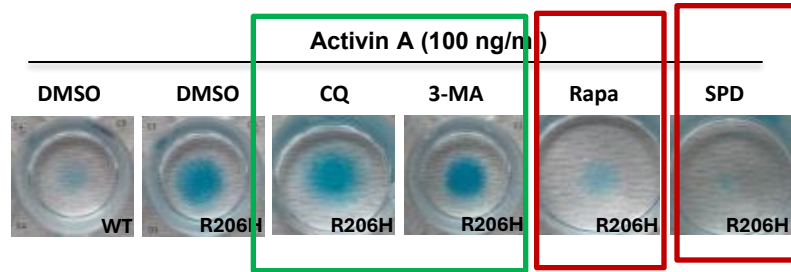
Could Rapamycin regulate HO through its autophagy reactivation function?

Autophagy modulation during Activin A dependent chondrogenesis

Activin A induces in vitro chondrocytes differentiation of ATDC5 **ALK2^{R206H}** expressing cells



Autophagy reactivation inhibits Activin-induced chondrogenesis in FOP cells

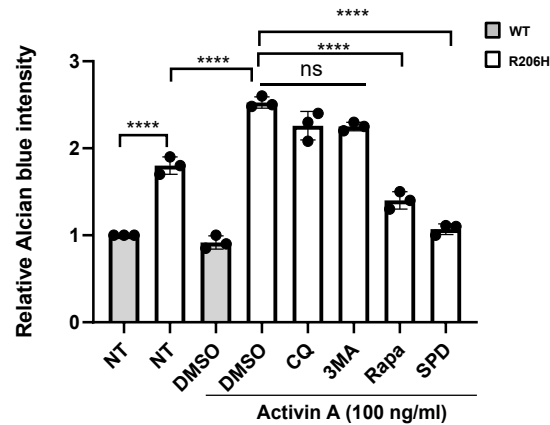


Autophagy inhibitors

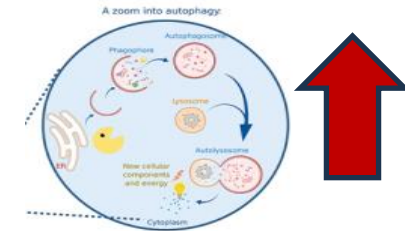


 Chloroquine (CQ)


 3-Methyladenine (3-MA)



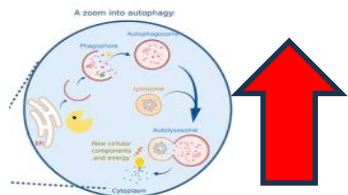
Autophagy Activators



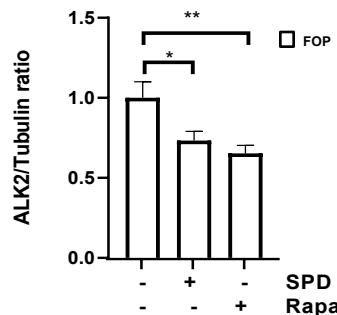
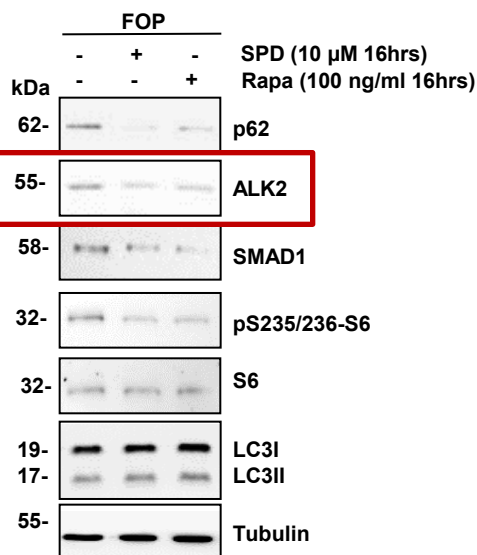
 Rapamycin (Rapa)

 Spermidine Natural compound (SPD)

Induction of Autophagy in primary Endothelial colony forming cells (ECFCs) derived from FOP patients



FOP cells



Rapamicyn

Spermidine
(no toxic natural compound)

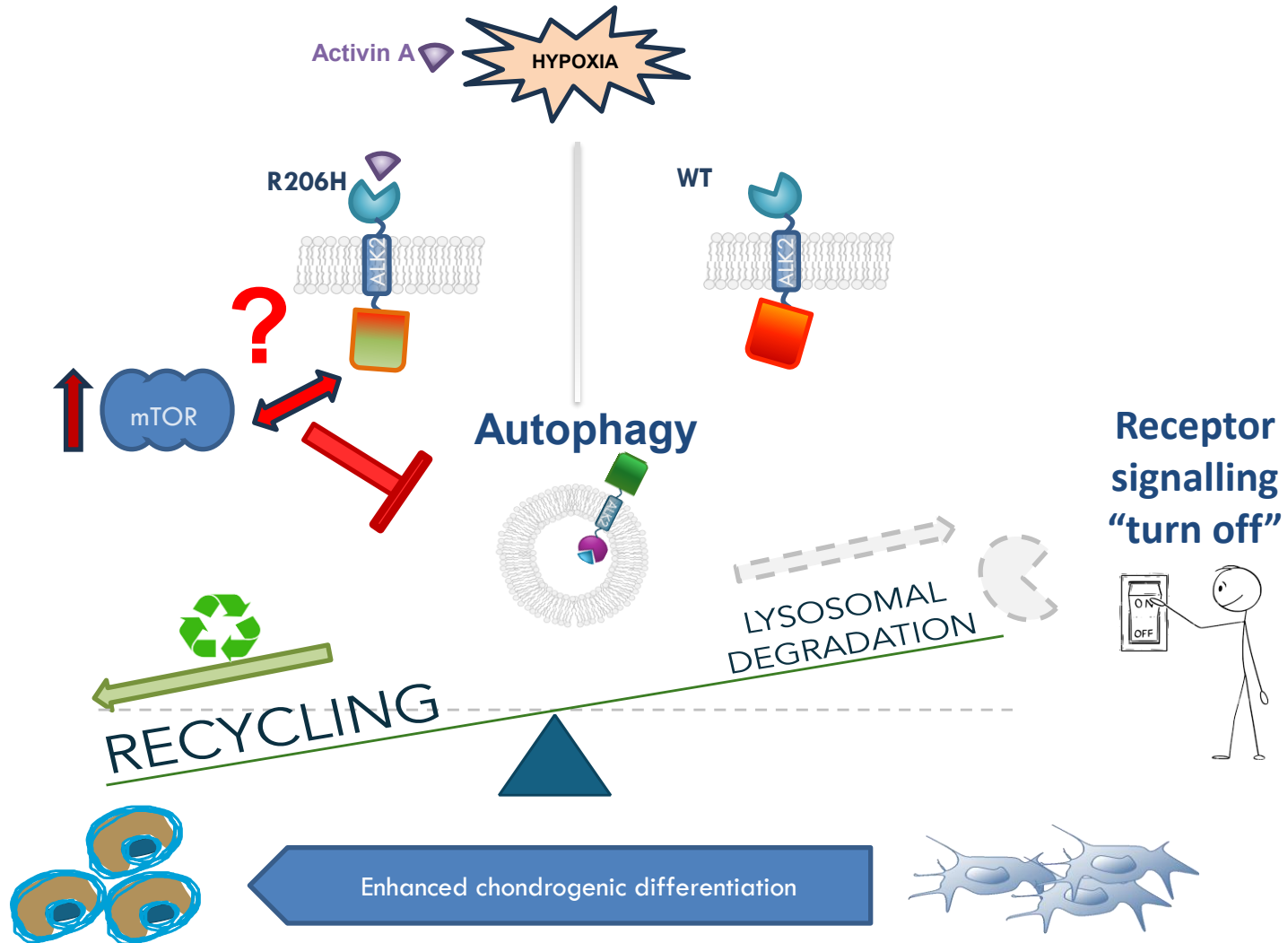
Leiden
(Prof Sánchez-Duffhues 's lab)



These results indicate that pharmacological modulation of autophagy may be an approach for the downregulation of aberrant ALK2^{R206H} receptor signalling in FOP

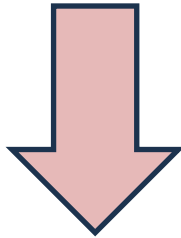
Conclusions

Hypoxic/inflammation induced autophagy could regulate ALK2 receptor degradation. In the presence of mutant receptor autophagy flux is reduced and receptor is not degraded.



Future Perspectives

Our work together supported by the literature highlights two important novelties for the ALK2 signalling that required further investigation



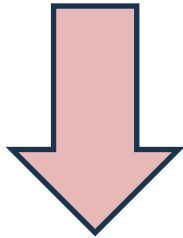
Reactivation of autophagy could be a new therapeutic option for FOP



ALK2 autophagolysosomal degradation could be a new critical mechanism for fine-tuning ALK2 receptor signalling in Hypoxia

Future Perspectives

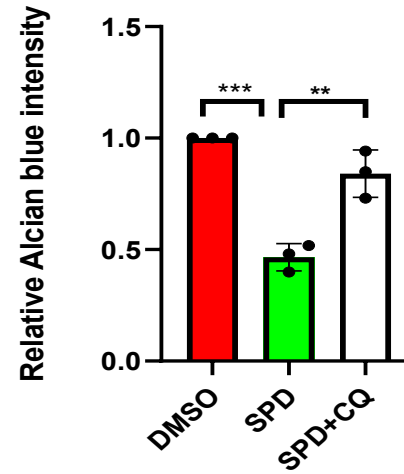
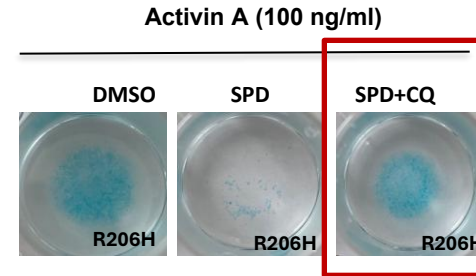
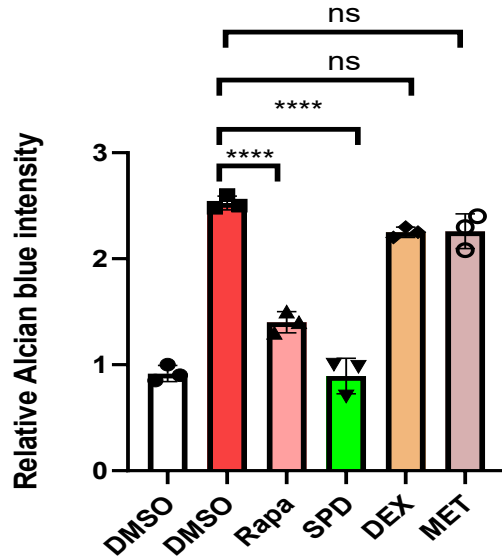
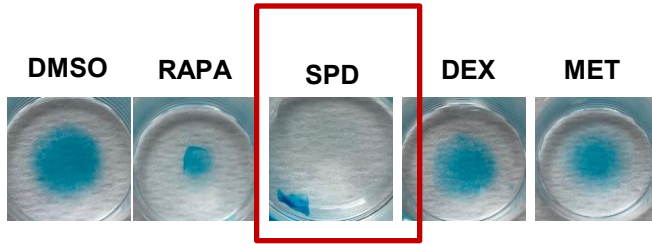
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Reactivation of autophagy could be a new therapeutic option for FOP

Spermidine : a good candidate?

ATDC5 ALK2^{R206H}



Spermidine : a good candidate?

Spermidine

↑
AUTOPHAGIC AND
MITOPHAGIC
BALANCE



↓ Age-related muscle
atrophy (*Sarcopenia*)



↑ Skeletal muscle
metabolic balance

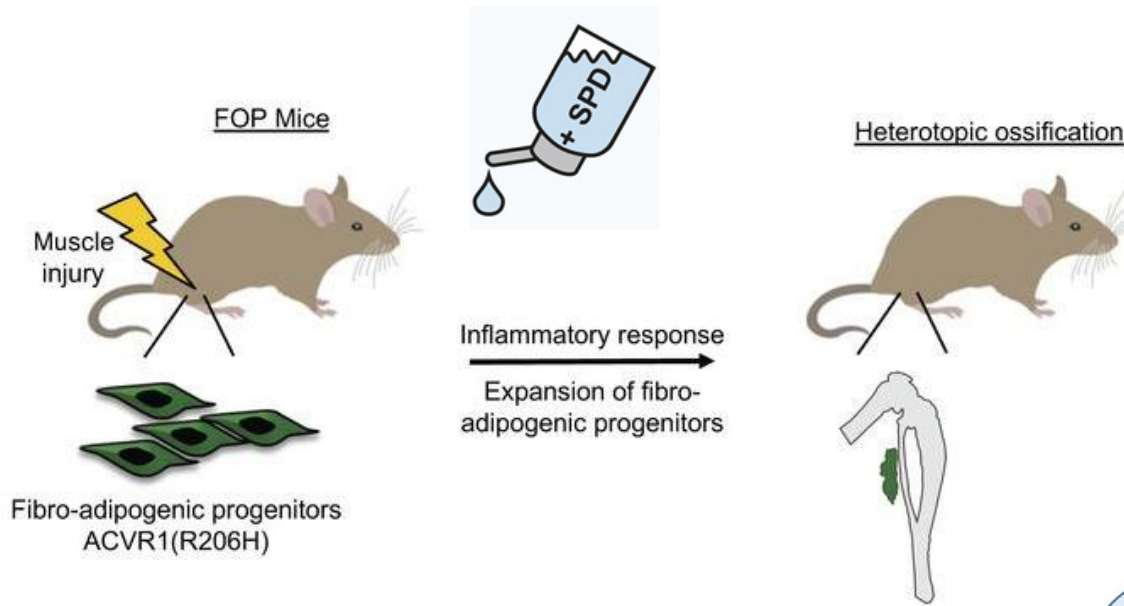
↑ Mitochondrial quality
control



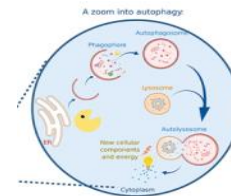
Future Perspectives

Investigating the Role of Spermidine (and other autophagy activator compounds) in a FOP mice model system

Prof. Silvia Brunelli, University of Milan



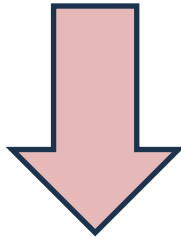
Evaluation of Autophagy in Autophagy Skeletal Muscle Diseases



Prof. Flavie Strappazon (CNRs, ISERM, Lyon, France)

Future Perspectives

Our work together supported by the literature highlights two important novelties for the ALK2 signalling that required further investigation



Reactivation of autophagy could be a new therapeutic option for FOP



ALK2 autophagolysosomal degradation could be a new critical mechanism for fine-tuning ALK2 receptor signalling in Hypoxia

Future Perspectives

Our work together supported by the literature highlights two important novelties for the ALK2 signalling that required further investigation

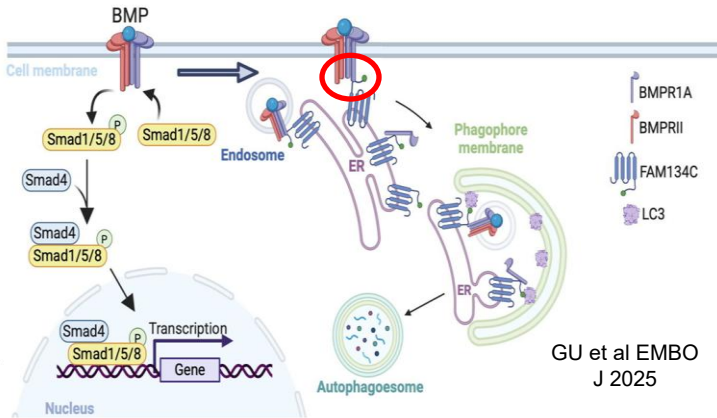


ALK2 autophagolysosomal degradation could be a new critical mechanism for fine-tuning ALK2 receptor signalling in Hypoxia

Work in progress....

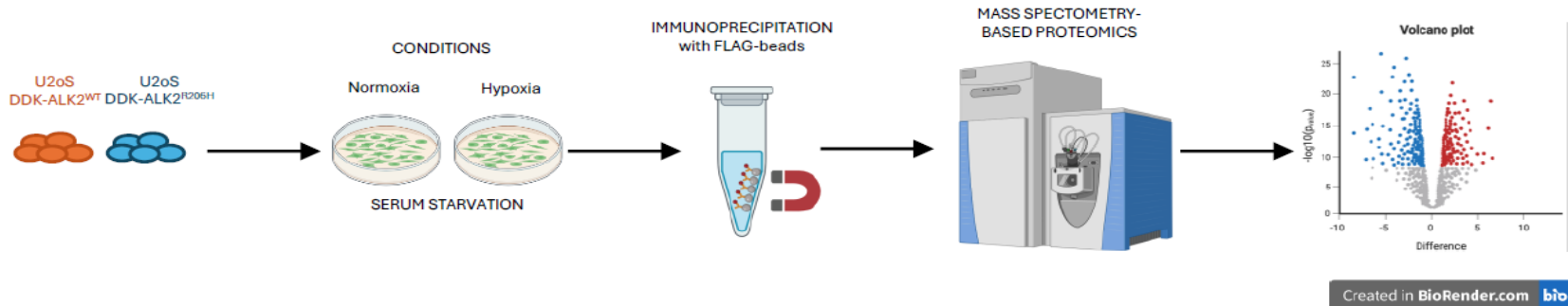
Investigate the molecular mechanism of ALK2 receptor trafficking: interactome analysis

collaboration with Dr Roberto Giambruno (CNR, Palermo)

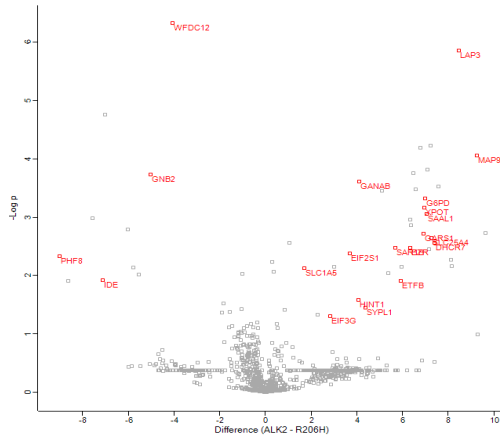


PALERMO

B



Investigate the molecular mechanism of ALK2 receptor trafficking: interactome analysis



ALK2 ^{WT} 21%O ₂	
UNIPROT CODE	PROTEIN NAME
O60701	UGDH
P17931	LGALS3
P25686	DNAJB2
P29317	EPHA2
Q15750	TAB1
Q93100	PHKB
Q9Y2H1	STK38L
O43592	XPOT
O60825	PFKFB2
O75821	EIF3G
O94915	FRYL
P07954	FH
P12235	SLC25A4
P15311	EZR
P49773	HINT1
Q16563	SYPL1
Q49MG5	MAP9
Q9NYG2	ZDHHC3
Q9UBM7	DHCR7

ALK2 ^{R206H} 21%O ₂	
UNIPROT CODE	PROTEIN NAME
O60701	UGDH
P17931	LGALS3
P25686	DNAJB2
P29317	EPHA2
Q13885	TUBB2A
Q93100	PHKB
Q9Y2H1	STK38L
O60869	EDF1
P16152	CBR1
Q15750	TAB1
Q9C0J8	WDR33
Q9Y6Y0	IVNS1ABP

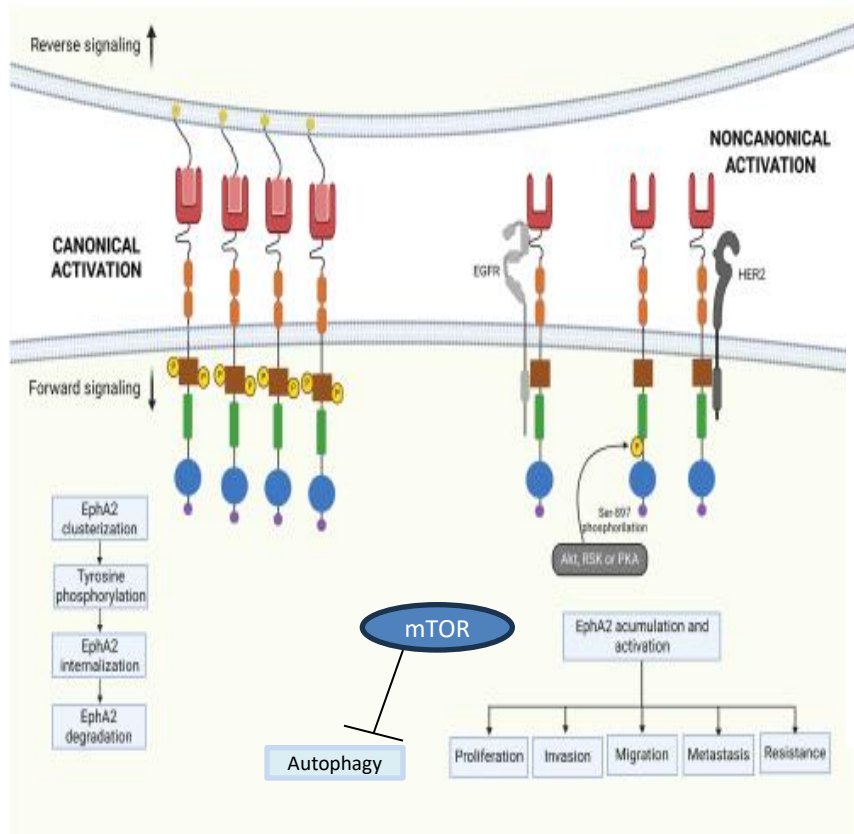


ALK2 ^{WT} 1%O ₂	
UNIPROT CODE	PROTEIN NAME
Q00688	FKBP3
O75635	SERPINB7
O75821	EIF3G
PODME0;Q01105	SET;SETSIP
P36952	SERPINB5
P48594	SERPINB4

ALK2 ^{R206H} 1%O ₂	
UNIPROT CODE	PROTEIN NAME
Q14766	LTBP1
P29317	EPHA2
P24666	ACP1
P25686	DNAJB2
Q5SWX8	ODR4
Q9UPR3	SMG5
Q9Y4Z0	LSM4

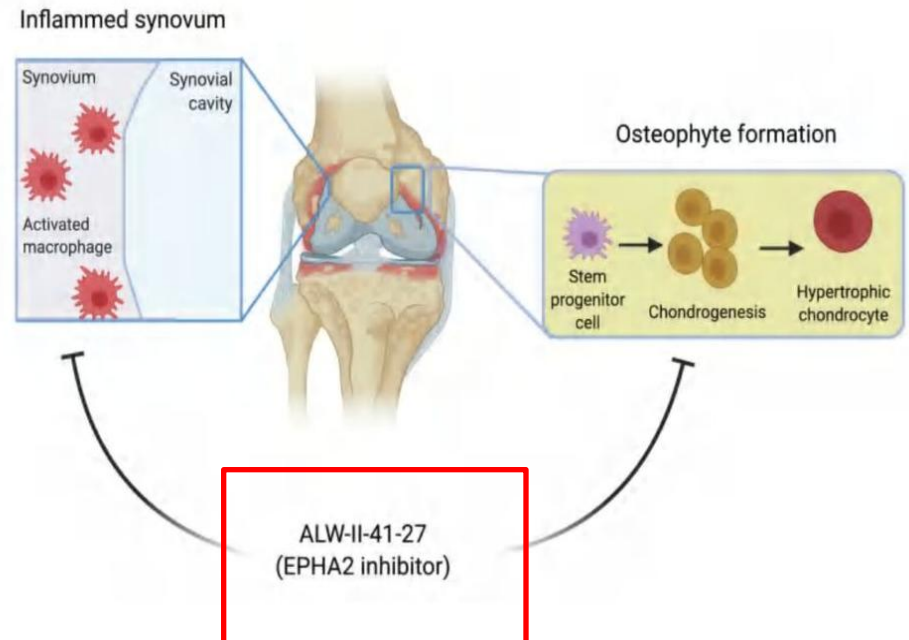
EPHA2 receptor: a good candidate?

Non canonical activation of EPHA2 activates mTOR and impair autophagy



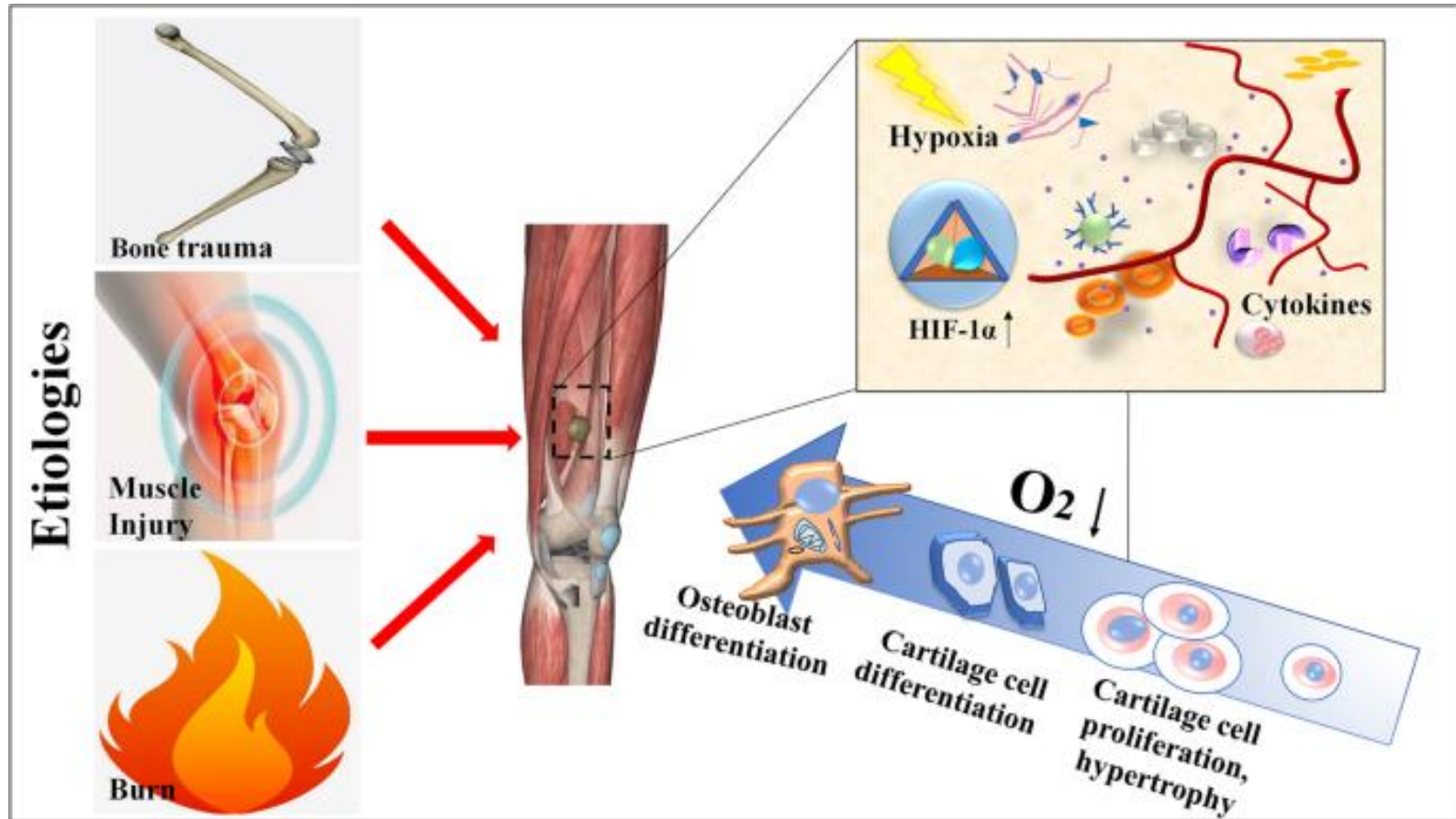
Veiga et al Journal of Molecular medicine 2024

Pharmacologic inhibition of EPHA2 decreases inflammation and pathological endochondral ossification in osteoarthritis

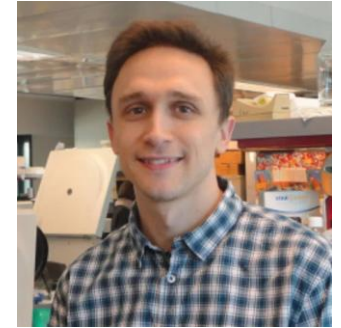
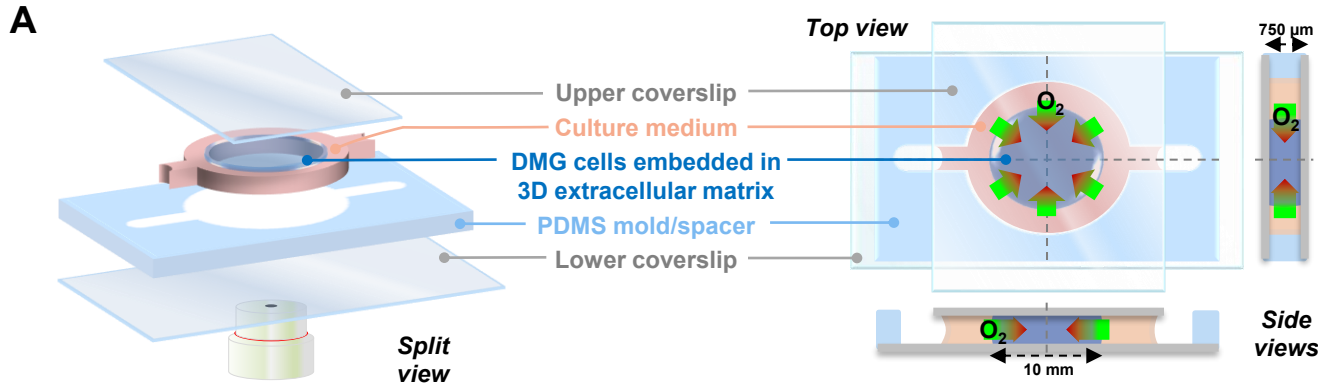


[Ferrao Blanco](#) et al Heliyon 2024

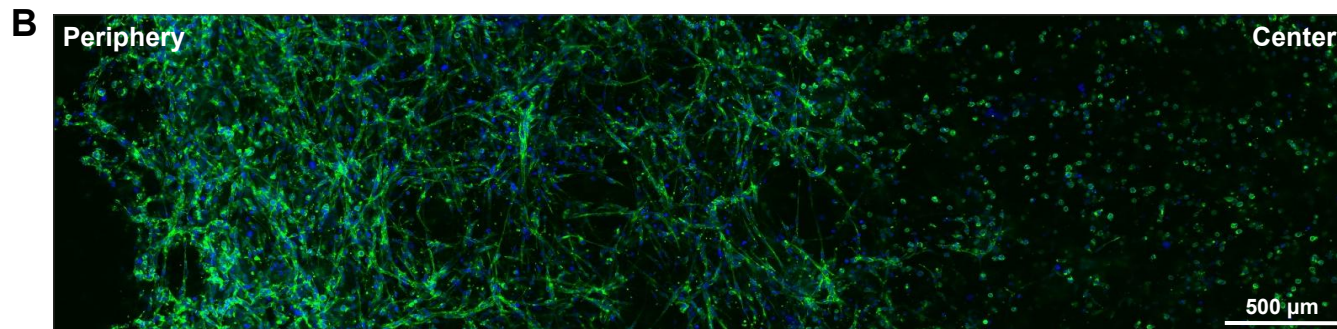
Hypoxia is a critical condition where tissues are deprived of adequate oxygen supply and is crucial during FOP Heterotopic Ossification



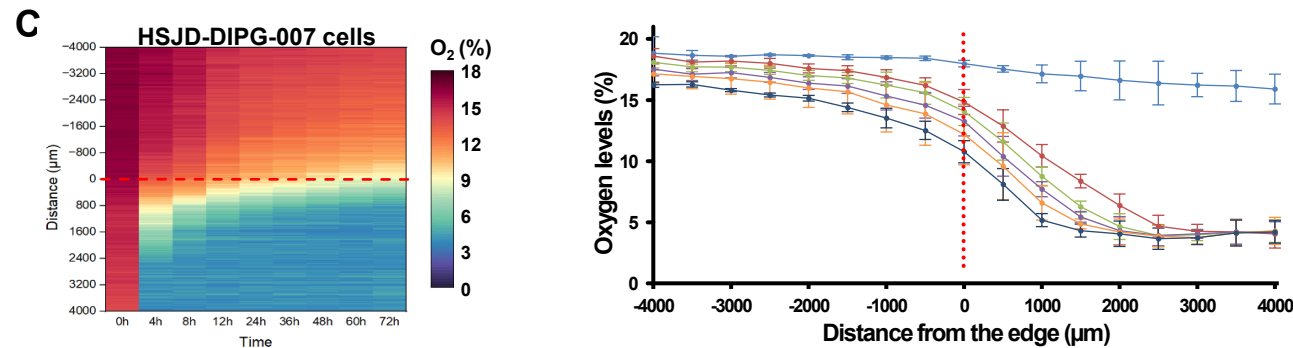
Novel FOP-on-Chip (DoC) device



Prof Alessandro Furlan,
University of Lille,
France



Dr Calypso Hazard
University of Lille



Acknowledgement



CDD *press*

www.nature.com/cddiscovery

ARTICLE OPEN

Interplay between ALK2^{R206H} mutant receptor and autophagy signaling regulates receptor stability and its chondrogenic functions

Check for updates

Laura Coculo^{1,2}, Marius Wits³, Irene Mariani^{1,2}, Giulia Fianco¹, Serena Cappato⁴, Renata Bocciarelli^{4,5}, Nicoletta Pedemonte⁴, Elisabetta Volpe⁶, Serena Ciolfi⁶, Rosario Luigi Sessa^{1,2}, Serena Rinaldo⁶, Francesca Cutruzzola⁶, Daniela Trisciuglio⁶, Marie-José Goumans^{7,8}, Gonzalo Sanchez-Duffhus^{3,9} and Venturina Stagni^{1,2}

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