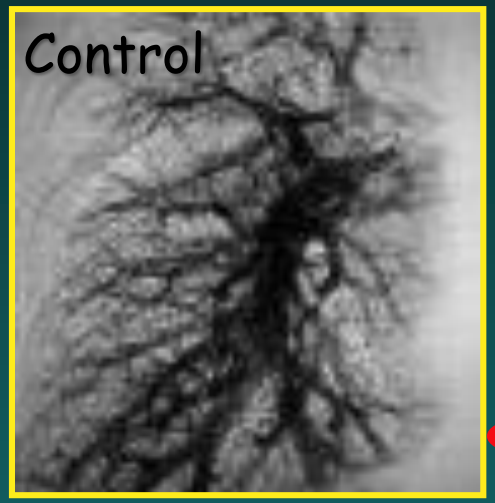
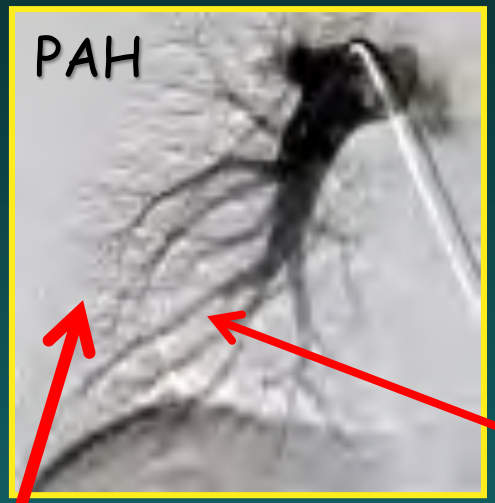
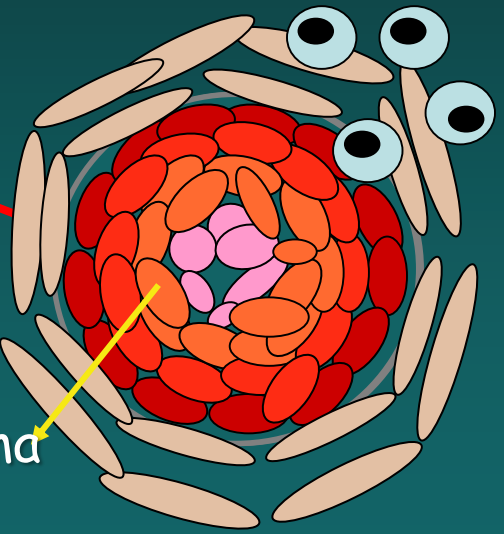
Molecular Mechanisms Inform New Therapies for Pulmonary Arterial Hypertension

PA Hypertension is Characterized by Vessel Loss, Occlusive Proliferation of Vascular Cells, and Inflammation

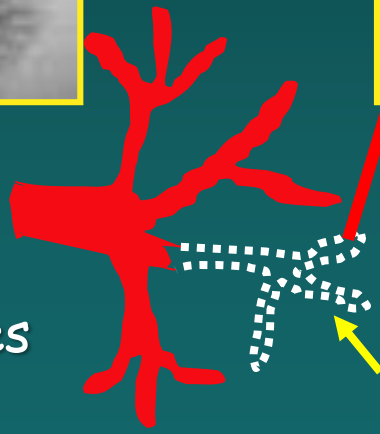




Narrowing of arteries



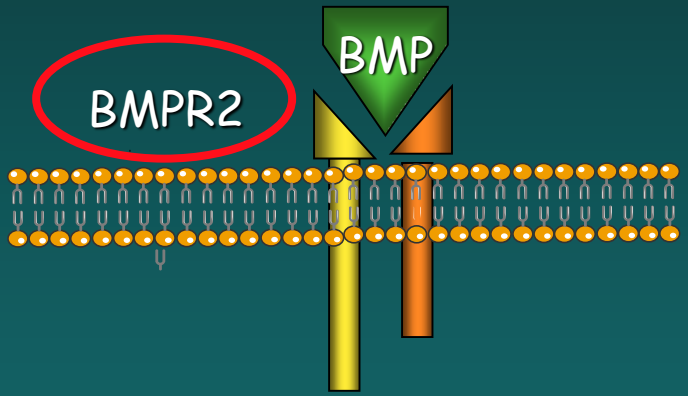
Neointima



Loss of arteries

Endothelial apoptosis

Mutation or Reduced Function of BMPR2 is Observed in Pulmonary Arterial Hypertension (PAH):



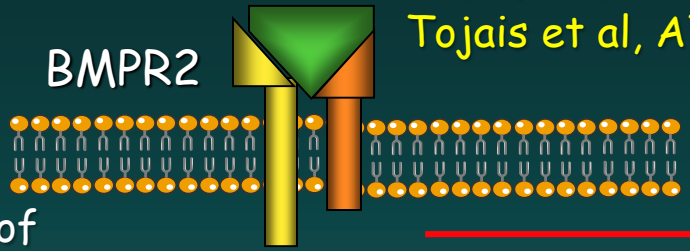
Mutation in BMPR2
in 70% familial PAH and
20% of sporadic IPAH:

Reduced BMPR2 function in all forms of PAH

BMPR2 Maintains Vascular Homeostasis

Assembles elastin fibers
Tojais et al, ATVB 2017

BMPR2

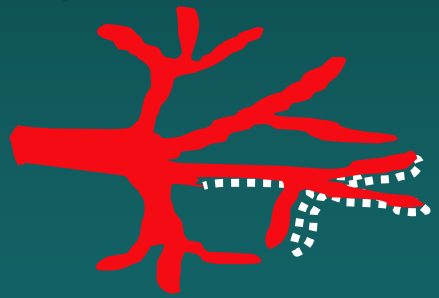


PPARγ

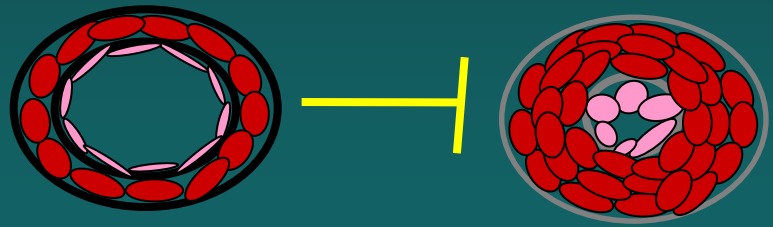
Suppresses inflammation

Sawada et al,
J Exp Med, 2014

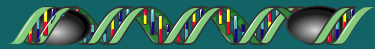
Promotes regeneration of endothelium after injury
Alastalo, JCI, 2011,
Miyagawa, Circ Res 2019



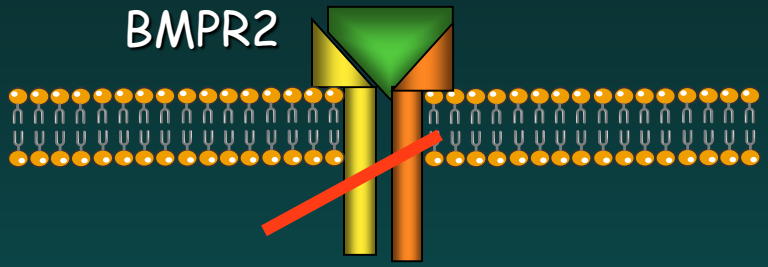
Blocks proliferation of SMC
Hansmann, JCI, 2008



Induces DNA repair
Diebold Cell Metabolism 2017,
Li Cell Reports, 2019



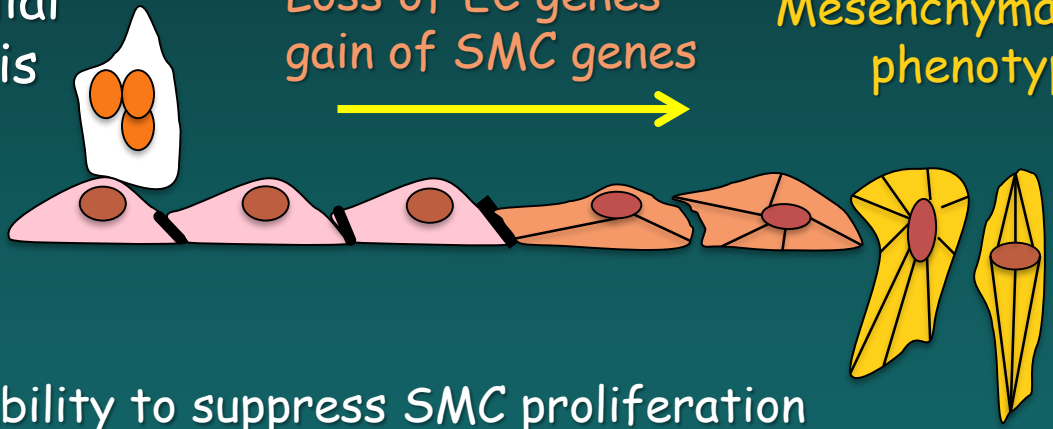
Loss of BMPR2 Promotes Endothelial-Mesenchymal Transition



Endothelial apoptosis

Loss of EC genes
gain of SMC genes

Mesenchymal-SMC phenotype

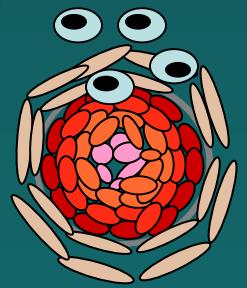


Rachel Hopper
Circulation, 2016

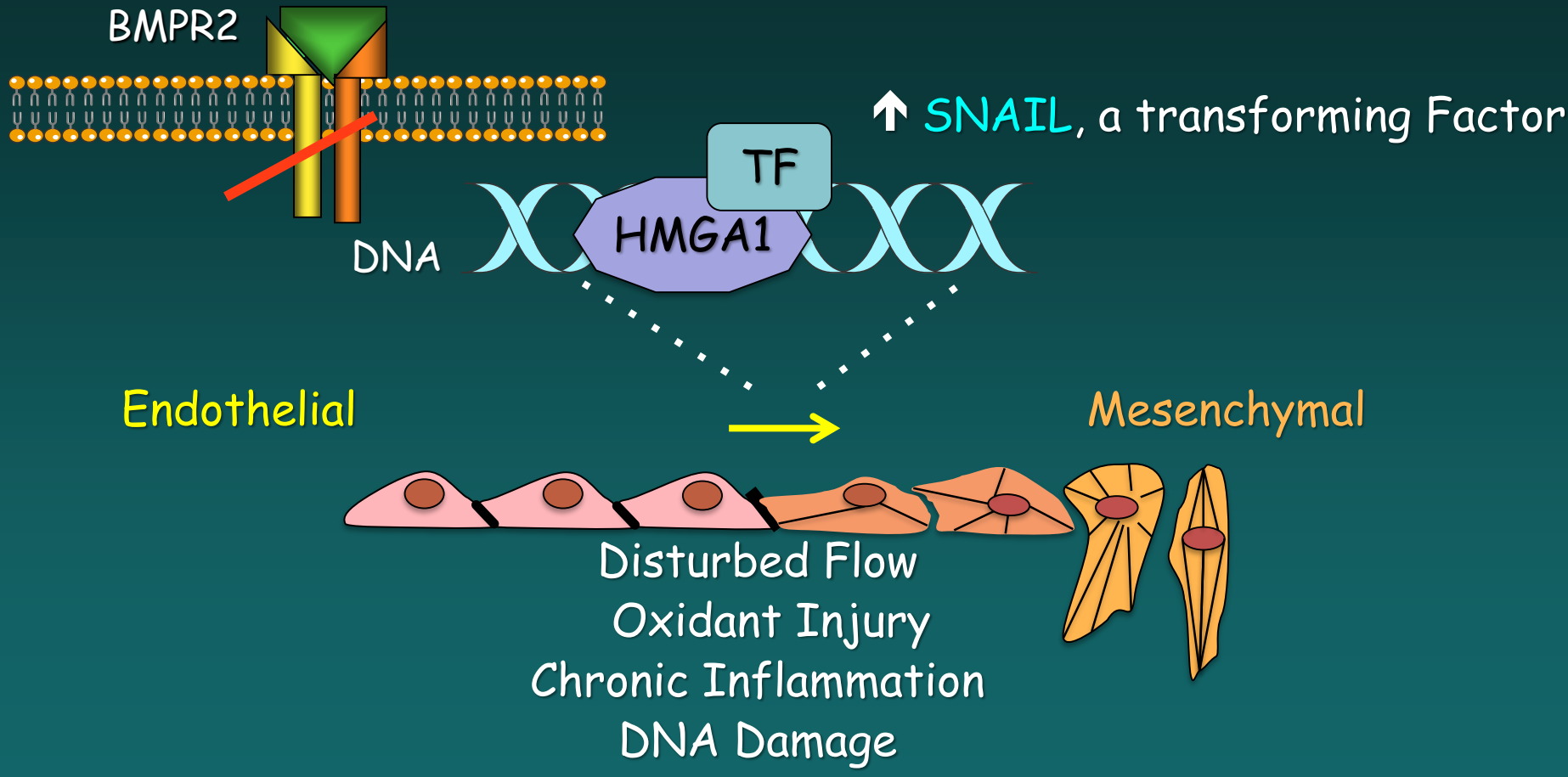
Inability to suppress SMC proliferation

Reduced barrier function

Compromised anti-inflammatory properties

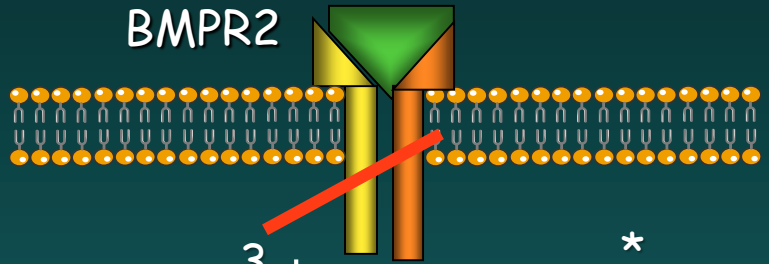


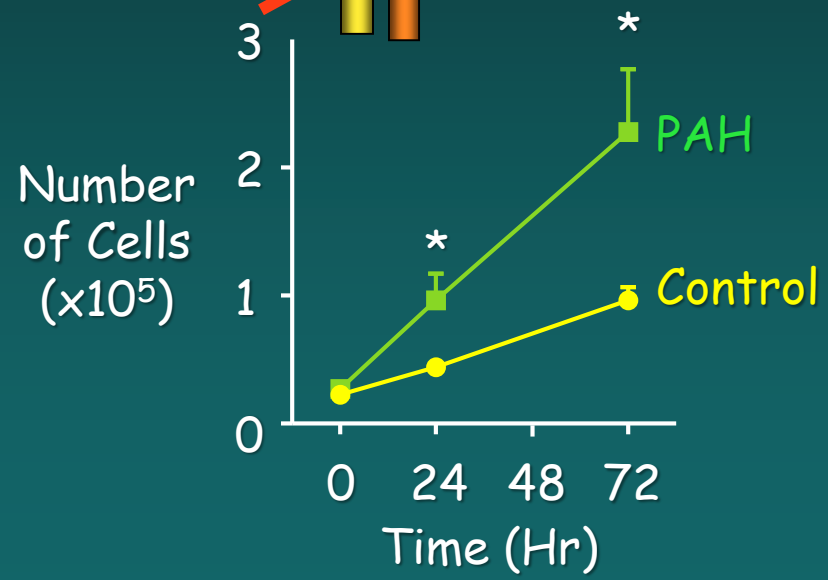
Mechanism of Endothelial Mesenchymal Transition in PAH

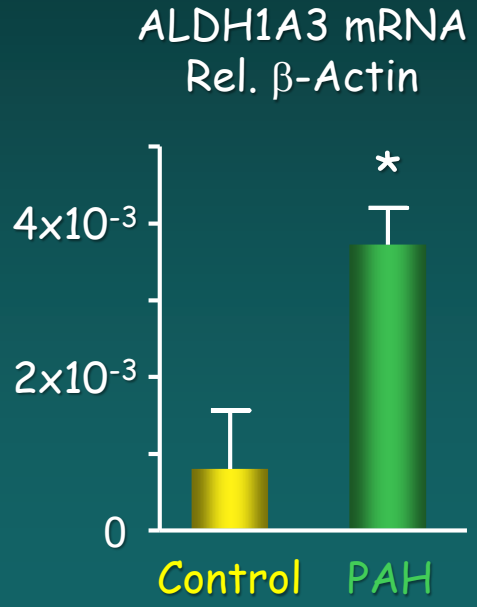


Loss of BMPR2 in Smooth Muscle Cells from PAH Patients Increases Proliferation via Aldehyde Dehydrogenase ALDH1A3

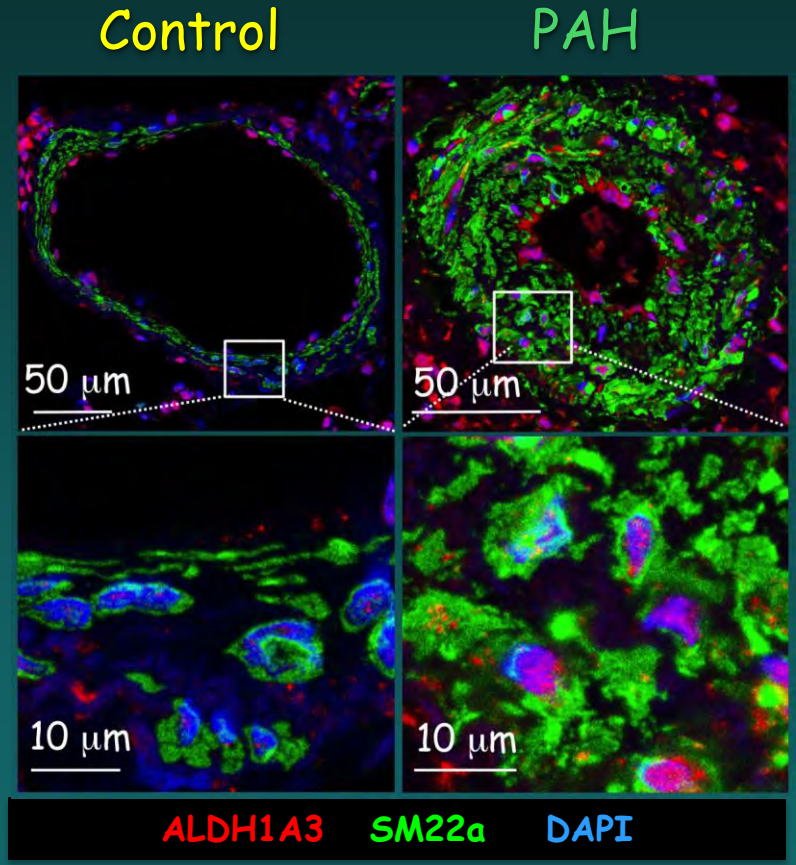
Dan Li



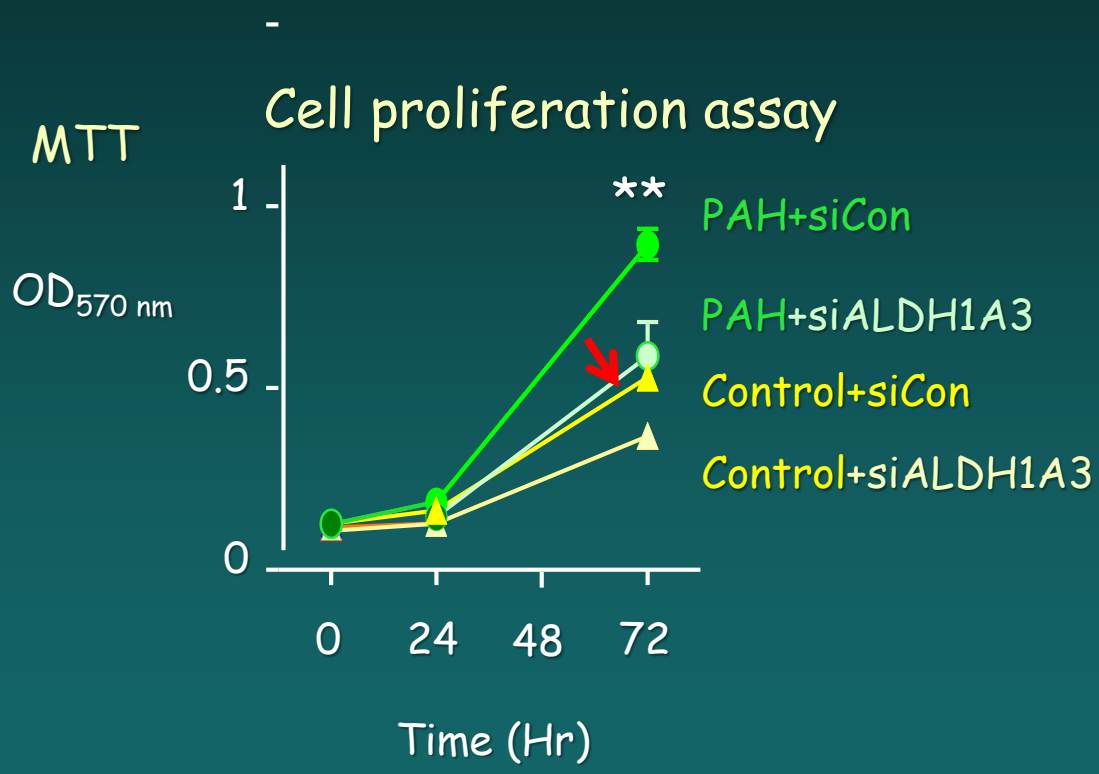




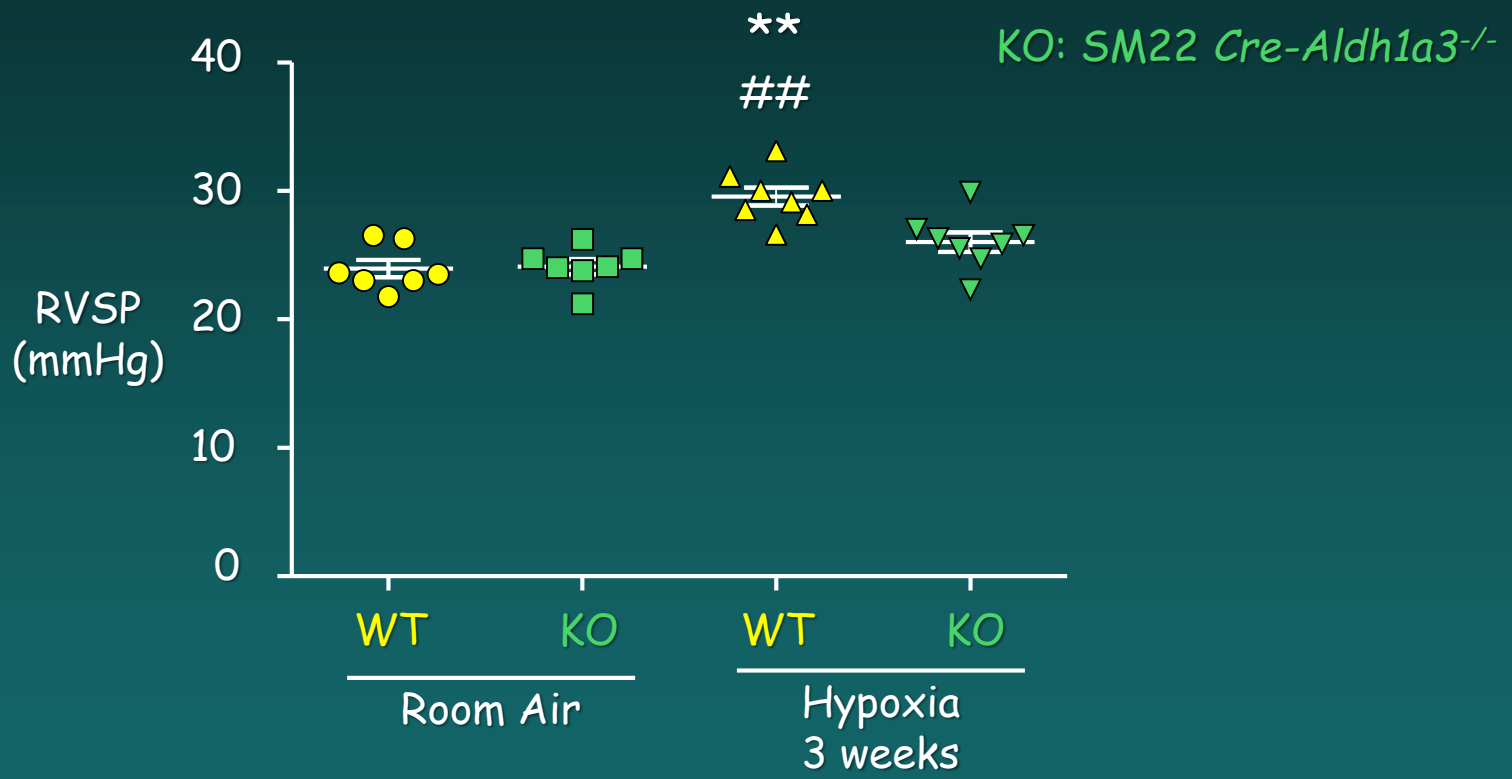
In PAH, Nuclear ALDH1A3 is Increased in PA SMC



Reducing ALDH1A3 (si) Suppresses PAH Smooth Muscle Cell Proliferation

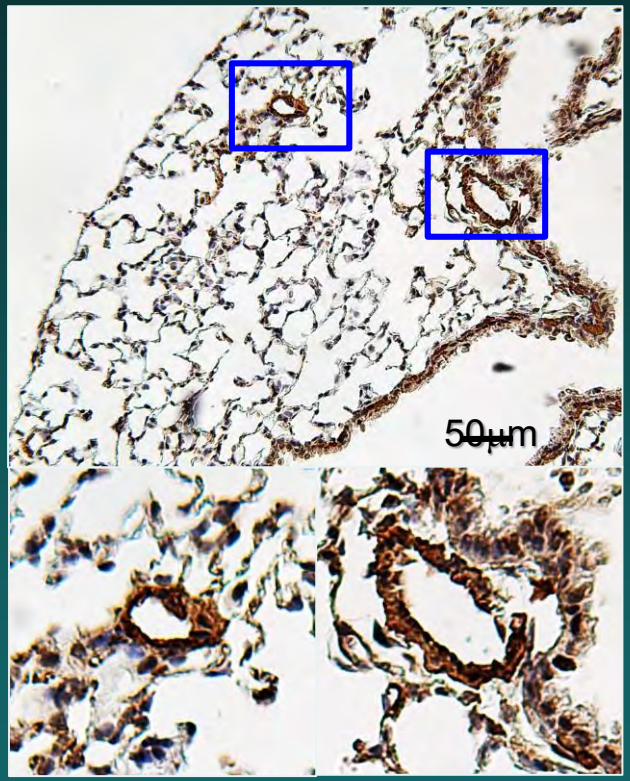


SMC-*Aldh1a3*$^{-/-}$ KO Mice Do Not Develop Hypoxic PH

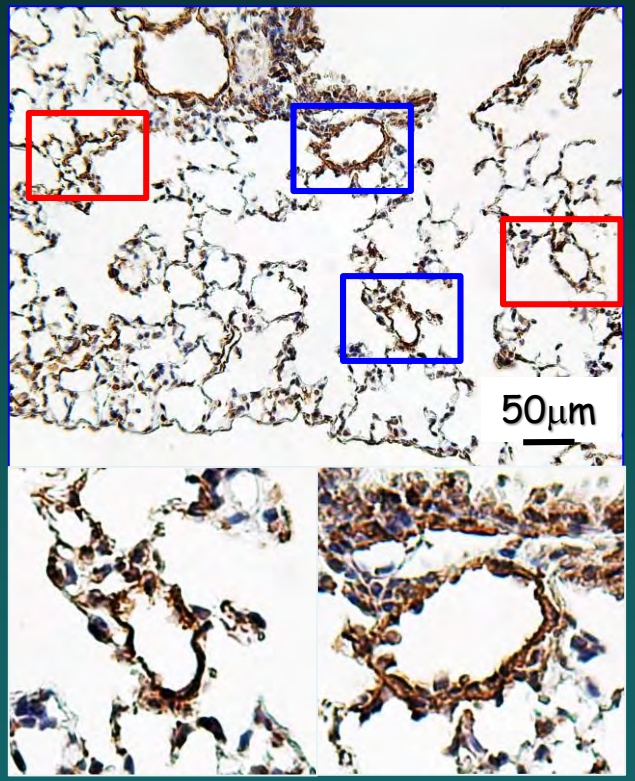


*SM22 Cre-Aldh1a3$^{-/-}$ male mice. N=8; **P<0.01 vs. KO in Hx, and ##P<0.01 vs. WT, Room Air, one-way ANOVA and Bonferroni's*

Deleting Aldh1a3 in SMC Eliminates PH and Abnormal Muscularization of PAs in Hypoxia

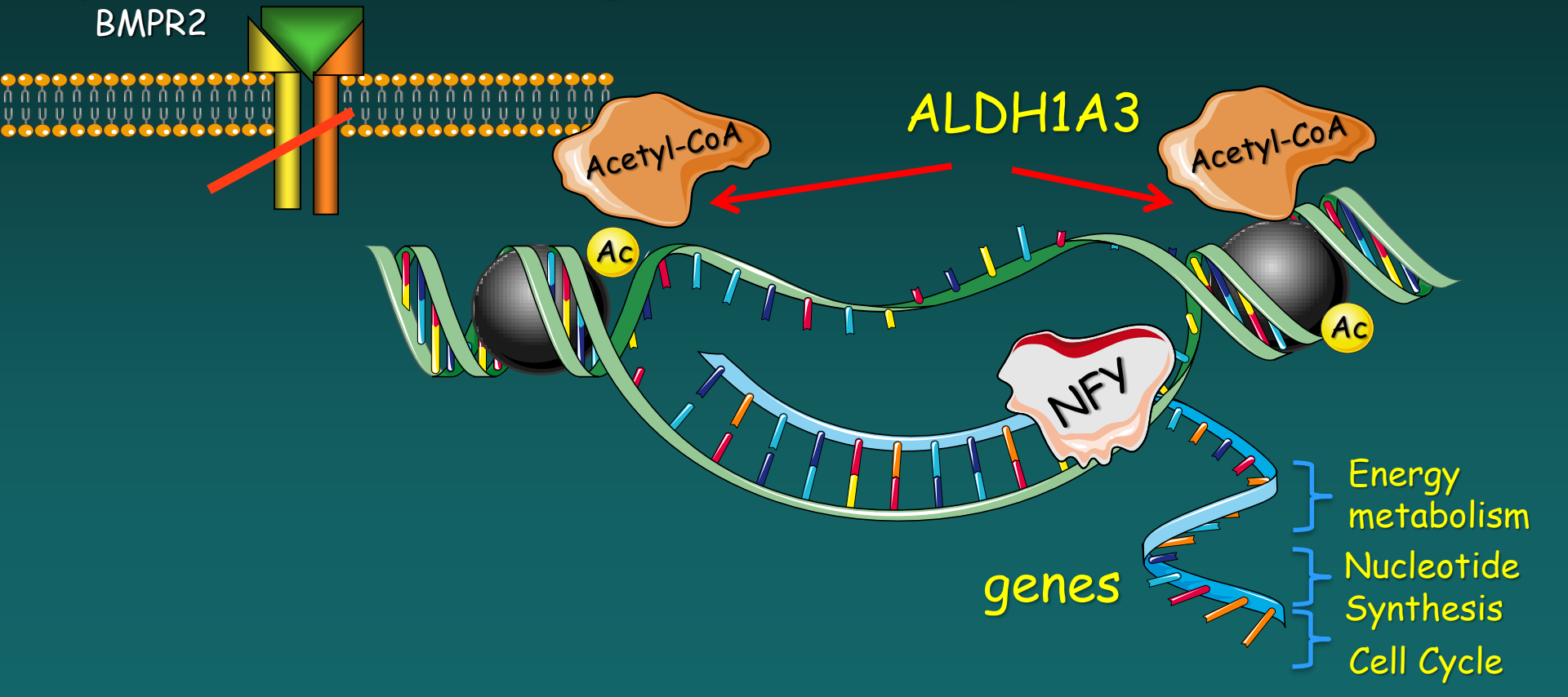


WT



SM22 *Aldh1a3*$^{-/-}$

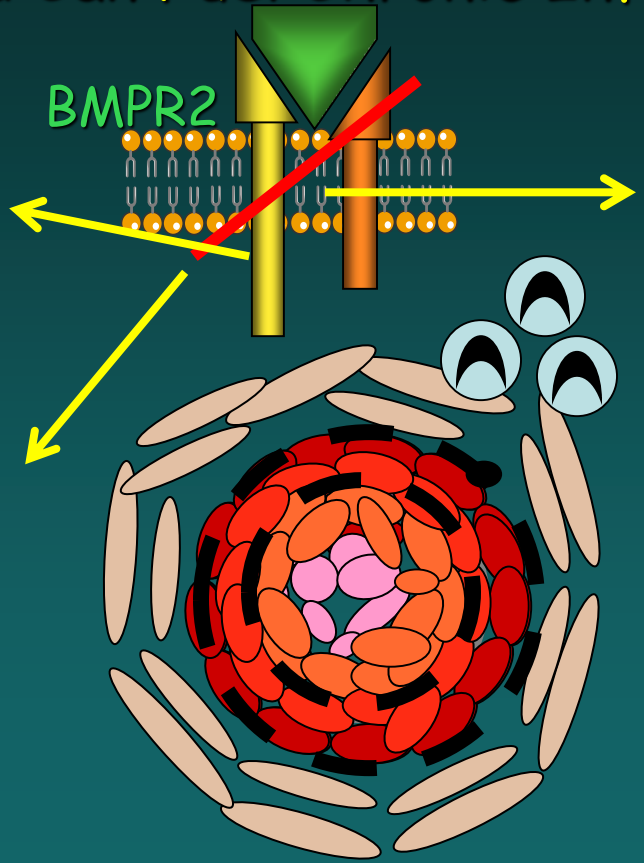
Loss of BMPR2 Increases ALDH1A3 to Acetylate Histones, Open Chromatin and Regulate Genes Required for Proliferation



Loss of BMPR2 Underlies EC Dysfunction, SMC Proliferation and Can Fuel Chronic Inflammation

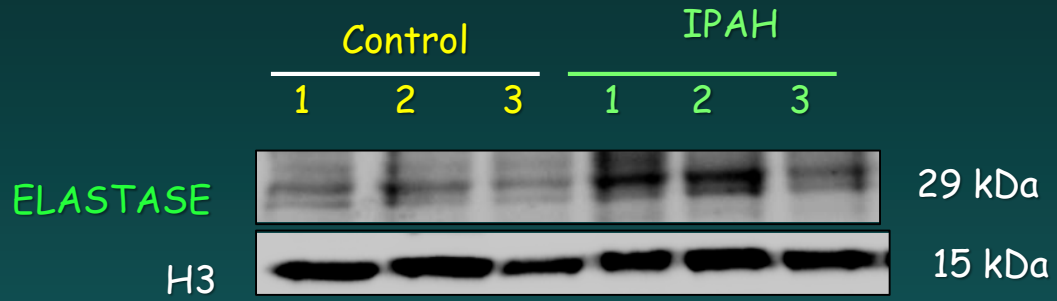
Impairs arterial regeneration of and causes EC transformation to SM-like cells

Enhances SMC proliferation via ALDH1A3



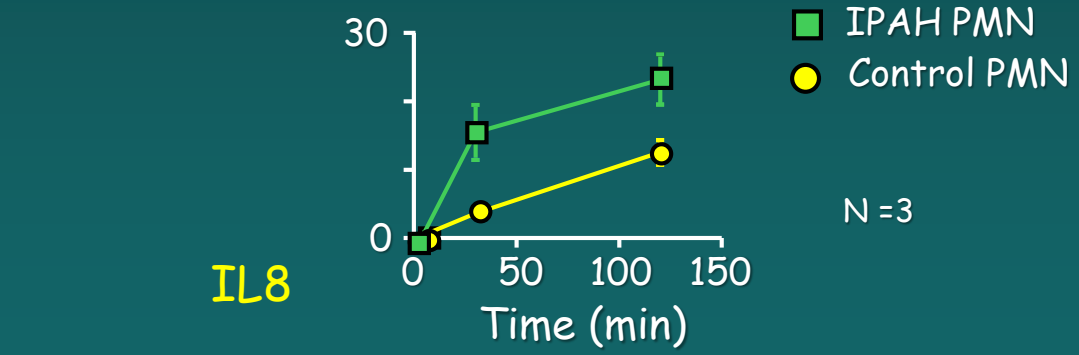
Enhances recruitment of ABNORMAL inflammatory cells to injured PAs causing further structural damage

IPAH Neutrophils (PMN) Have Increased Elastase and Release More Active Elastase in Response to Stimulation

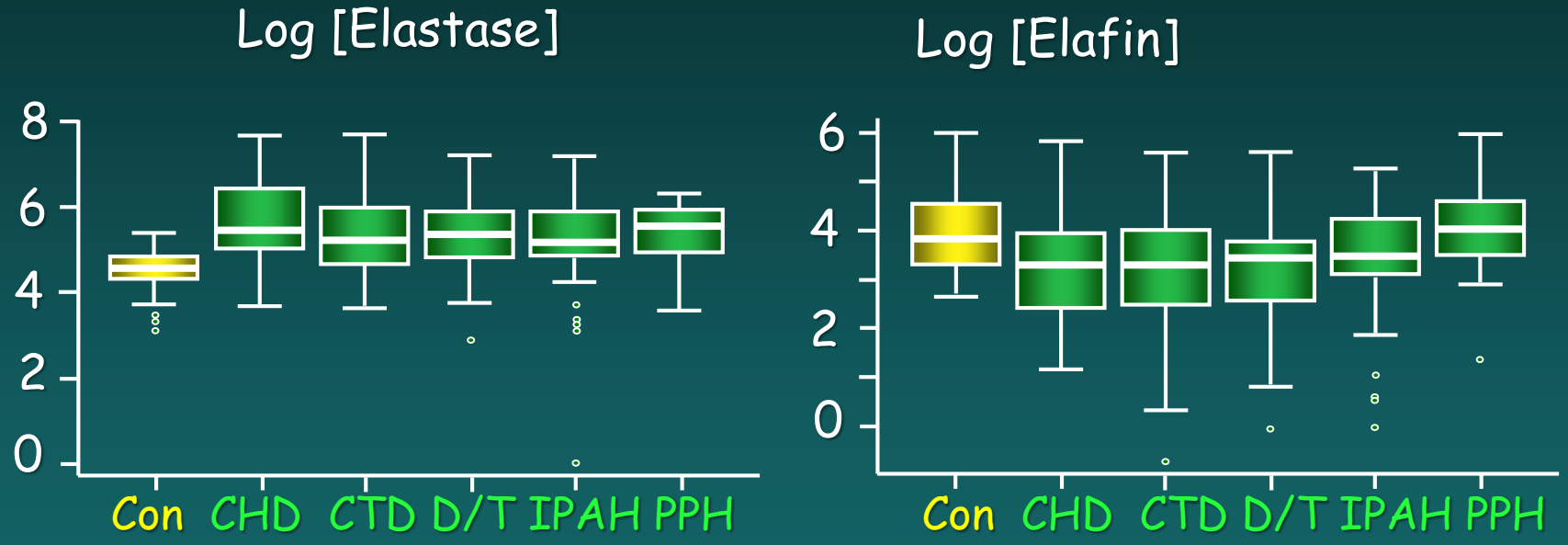


Shalina Taylor

ELASTASE Activity in Response to IL8 (% Total)



Circulating Elastase is Increased & Inhibitor Elafin is Reduced in Patients with All Forms of PA Hypertension (Zamanian et al)

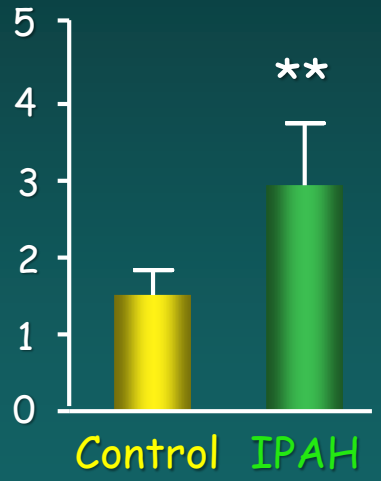


CHD Congenital Heart CTD Connective Tissue D/T Drugs and Toxins
IPAH Idiopathic PAH PPH Portopulmonary PAH

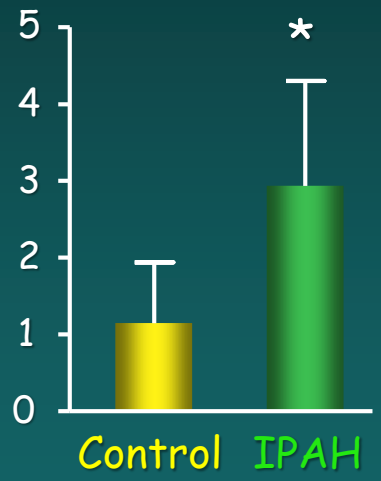
PAH Neutrophils Show a type I Interferon Response Related to Elevated Endogenous Retroviral Elements (HERVs)

Gene Name	Log 2 Fold change
IFIT1	
EIF2AK2 (PKR)	
DDX58 (RIG-I)	
UBE2E1	
IFNAR1	
IFNGR2	
EIF4E3	
IFNAR2	

HERV-K envelope



HERV-K dUTPase



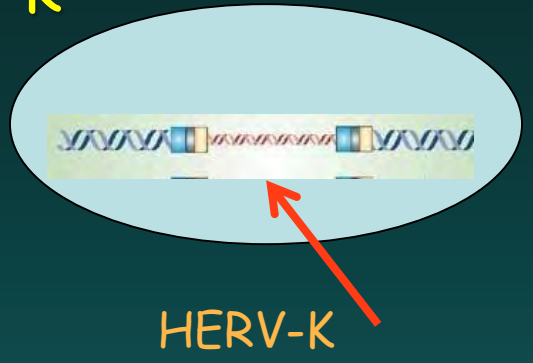
Receptors and effectors of interferon type 1 response

N=6; *p<0.05, **p<0.01

Endogenous Retrovirus HERV-K

- Endogenous retrovirus sequences are stored in our genome as remnants of ancestral retroviral infections.

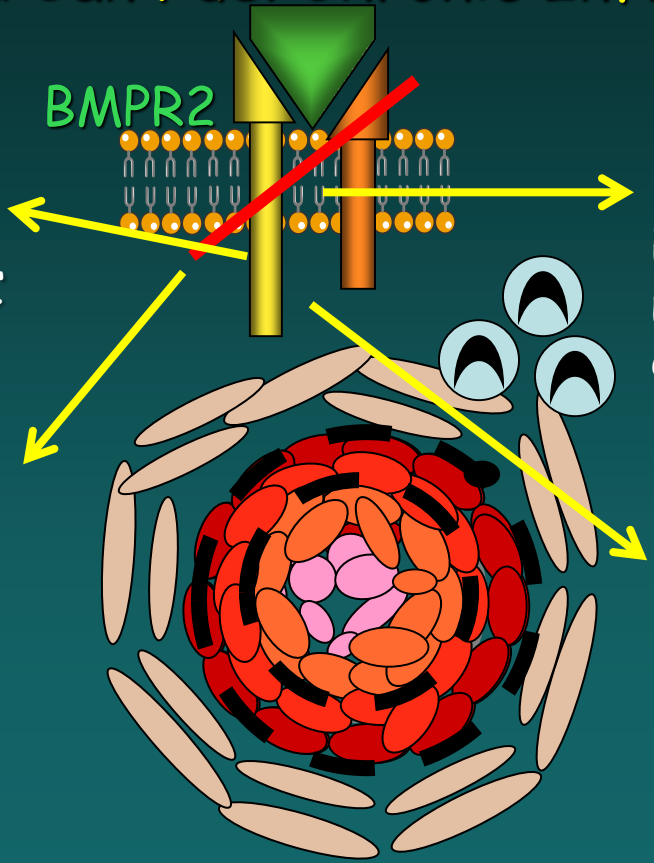
  They are not infectious but their products dUTPase and envelope proteins can induce a chronic innate immune response.



- Abnormal amplification of HERV-K has been linked to autoimmunity and cancer, and PAH (Saito et al, Circulation, 2016)

HERV-K dUTPase Can Induce Elastase in Neutrophil Line

Loss of BMPR2 Underlies EC Dysfunction, SMC Proliferation and Can Fuel Chronic Inflammation



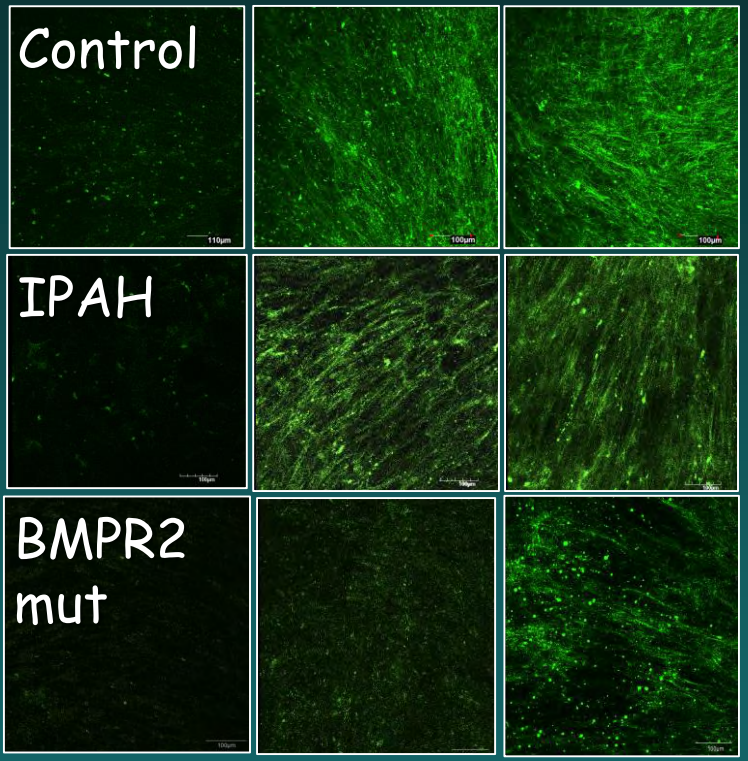
Impairs regeneration of small vessels after injury and causes EC transformation to SM-like cells

Enhances SMC proliferation via ALDH1A3

Enhances recruitment of neutrophils with elevated elastase to PAs

Impairs assembly of elastic fibers

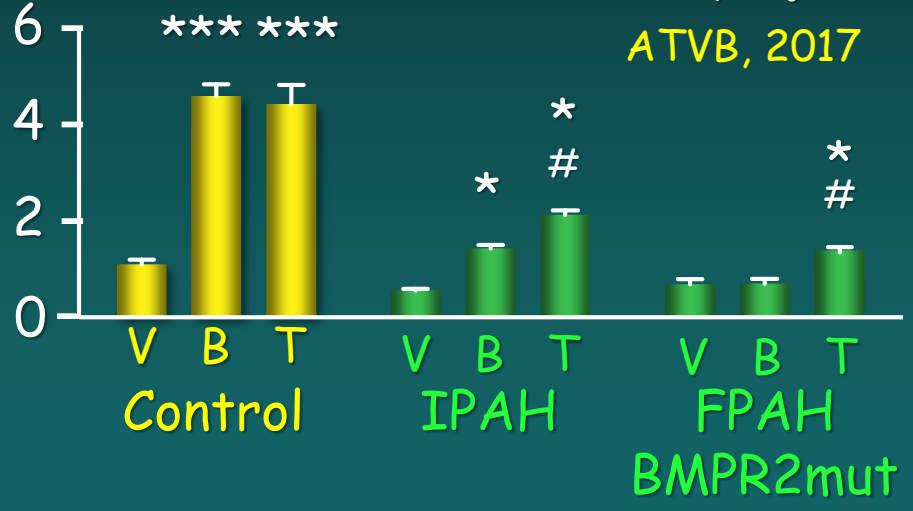
Loss or Mutation of BMPR2 Reduces Elastic Fiber Deposition in Cells from PAH Patients



Nancy Tojais

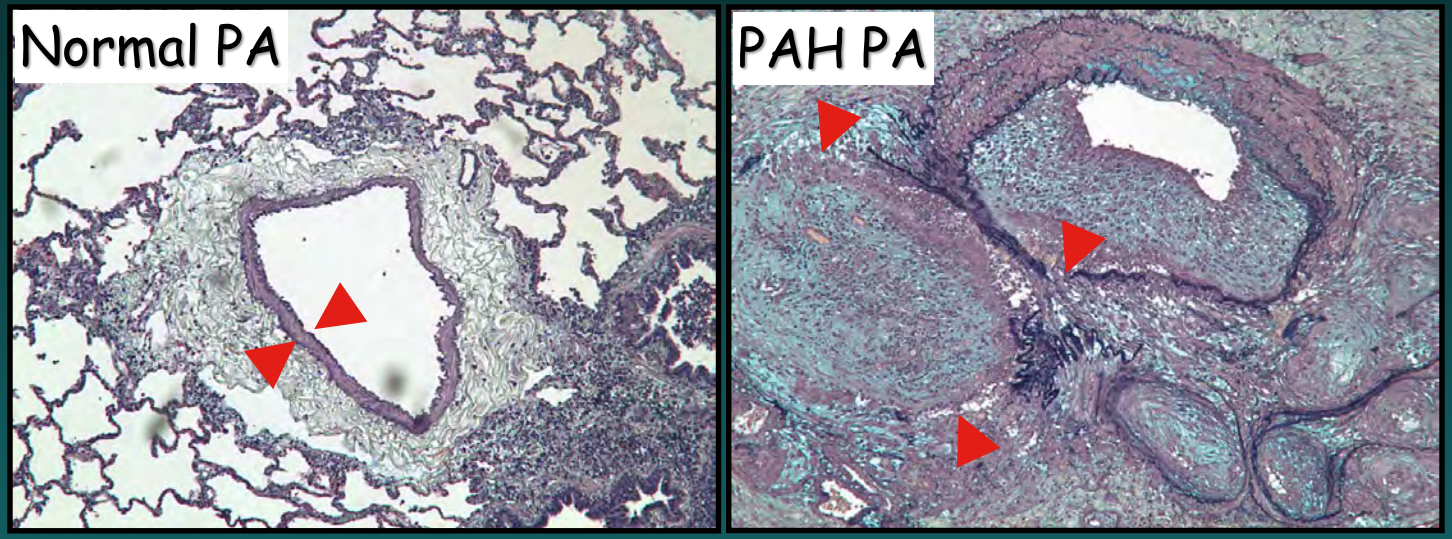
ATVB, 2017

Elastin
% Fluorescence



* vs. Vehicle; # vs. Donor

Degradation of Elastin Precedes & Accompanies Progressive Pulmonary Vascular Pathology



Loss of elastin:

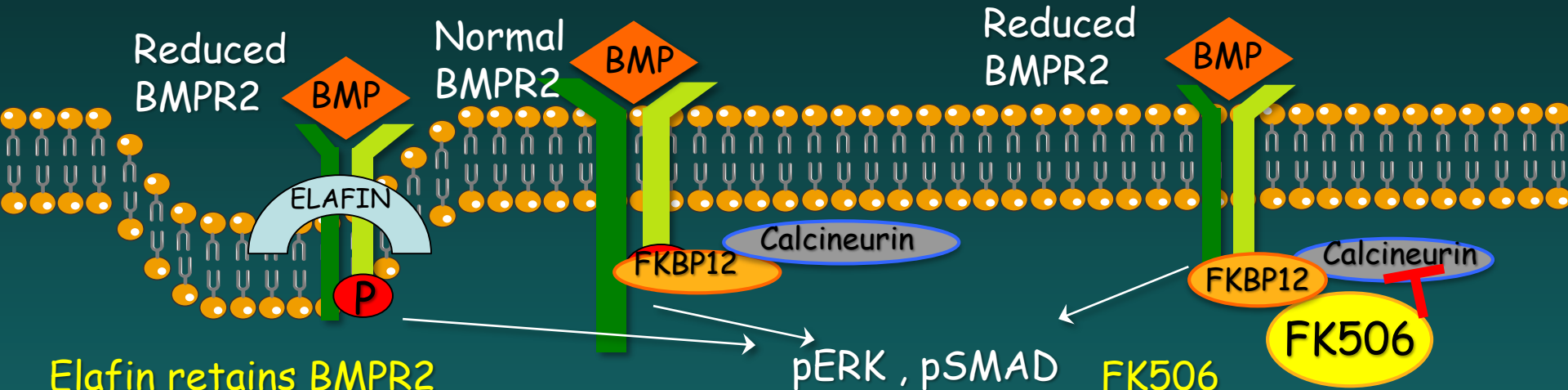
- Increases vascular stiffness
- Promotes SMC proliferation & obliterative changes
- Leads to loss of the distal microcirculation
- Breakdown products of elastin are pro-inflammatory

Ideal PAH Therapies Activate BMPR2 and Suppress Inflammation

FK506: Identified on a high throughput screen of 4500 compounds, as top activator of BMPR2 *(Spiekerkoetter, JCI, 2013)*

Elafin: A recombinant endogenous elastase inhibitor found by serendipity to activate BMPR2 *(Nickel, AJRCCM, 2015)*

Immunosuppressant Tacrolimus (FK506) and Elastase Inhibitor Elafin Amplify a Weak BMPR2 Signal

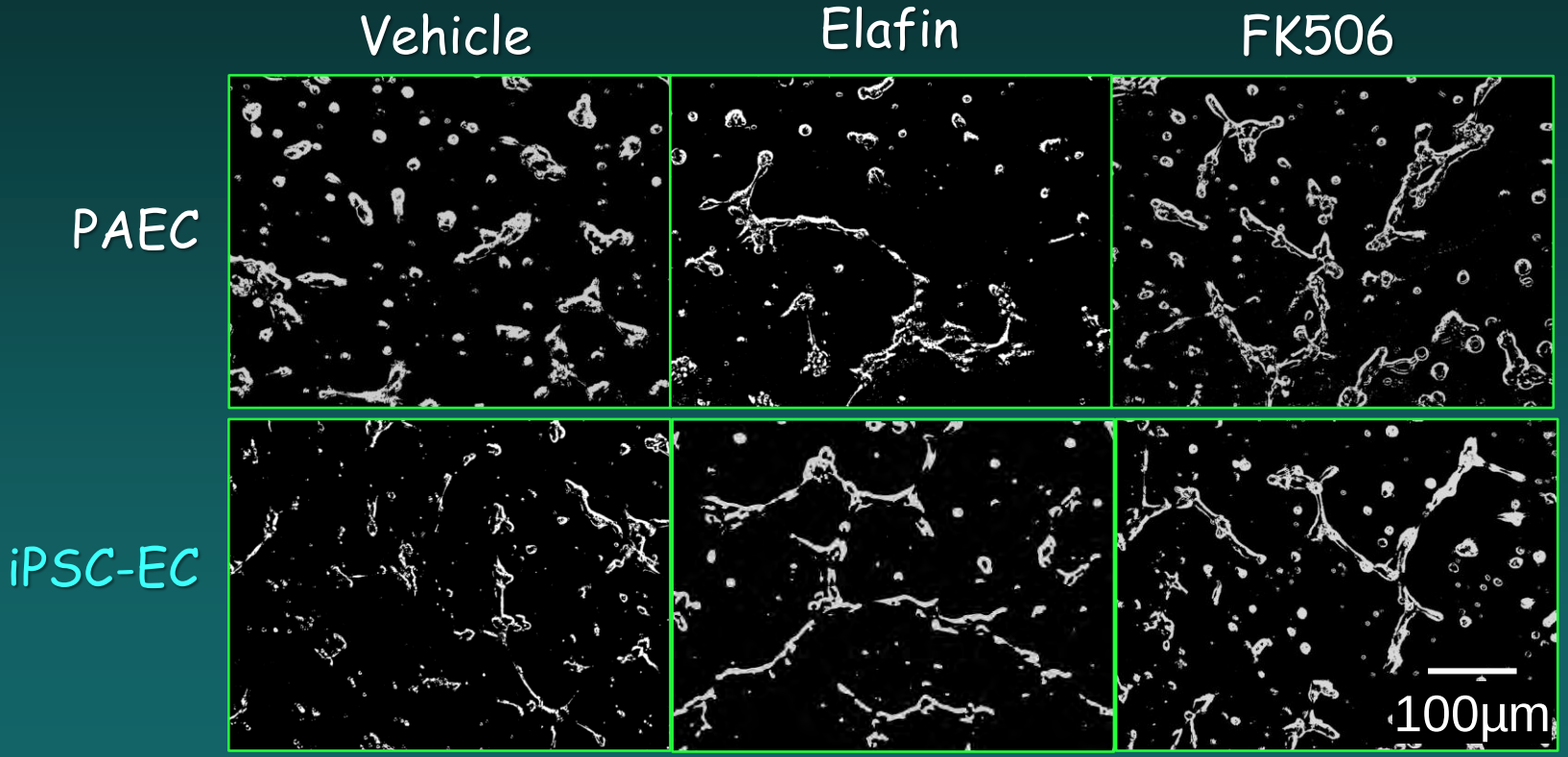


Elafin retains BMPR2 in caveoli to amplify a weak signal

pERK , pSMAD signals and gene expression

FK506 removes FKBP12 & blocks calcineurin to amplify a weak signal

IPAH PAEC and iPSC-EC Respond to Elafin and FK506 by Similar Improvement in Angiogenesis



High-Throughput Drug Screen in to Find a Drug to Repurpose to Reverse iPSC Derived Vascular Cell Dysfunction

PAH Patients

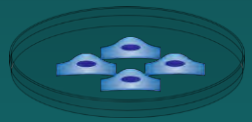
Skin Fibroblasts

Sendai

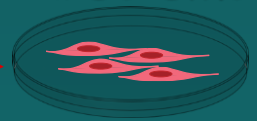
O K S M

iPSCs

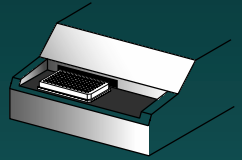
iPSC-EC



iPSC-SMC



4,500 compounds
8 doses (0.156 - 20μM)

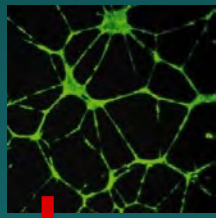


Luminometer

↑EC Survival >50%
(Caspase activity)

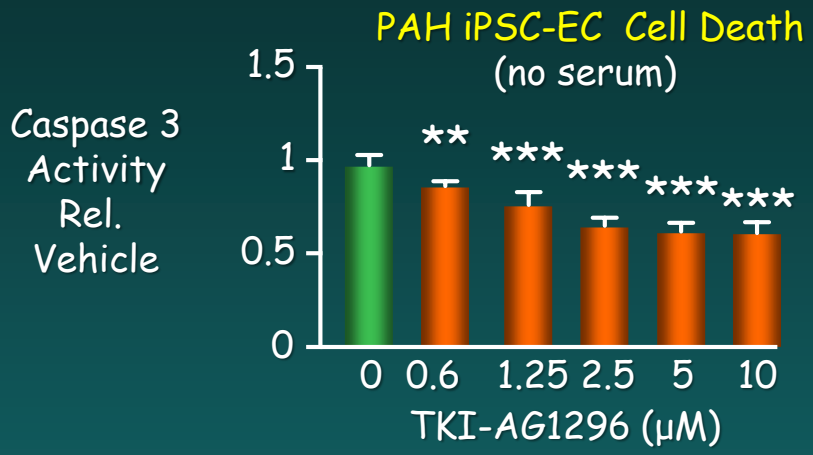
CellTiter-Glo®

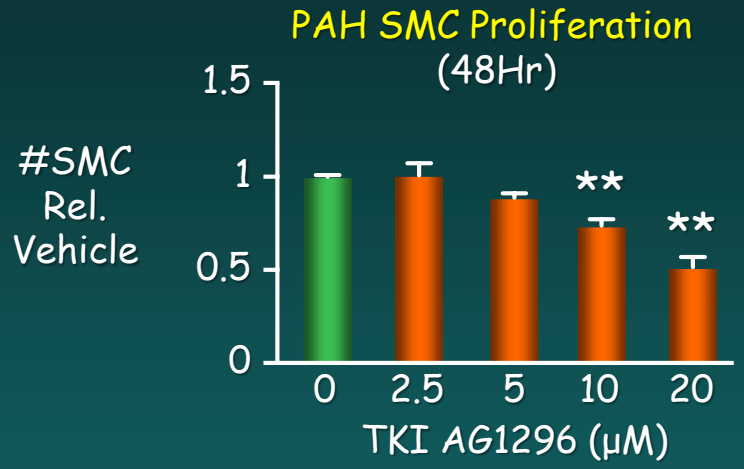
Mingxia Gu



SMC proliferation

Drug Screening: Tyrosine Kinase Inhibitor SG1296 Improves PAH EC Survival and Suppresses PA SMC Proliferation





iPSC-EC Survival Improved

SMC Proliferation Reduced

*n=6 PAH cell lines; **p<0.01, ***p<0.001 vs. Veh, by one-way ANOVA with Bonferroni*

Using Bioinformatics to Find Additional Rx

Identify bait compound
TKI AG1296

→

LINCS DATA BASE
AG1296
expression profile
across multiple cells

→

Bait signature

Gene X	Gene Y

+

PAH gene signature
GEO database
Lung: 32 PAH, 28 Healthy
PBMC: 52 PAH, 57 Healthy

PAH signature	Gene X	Gene Y
Anti signature	Gene X	Gene Y

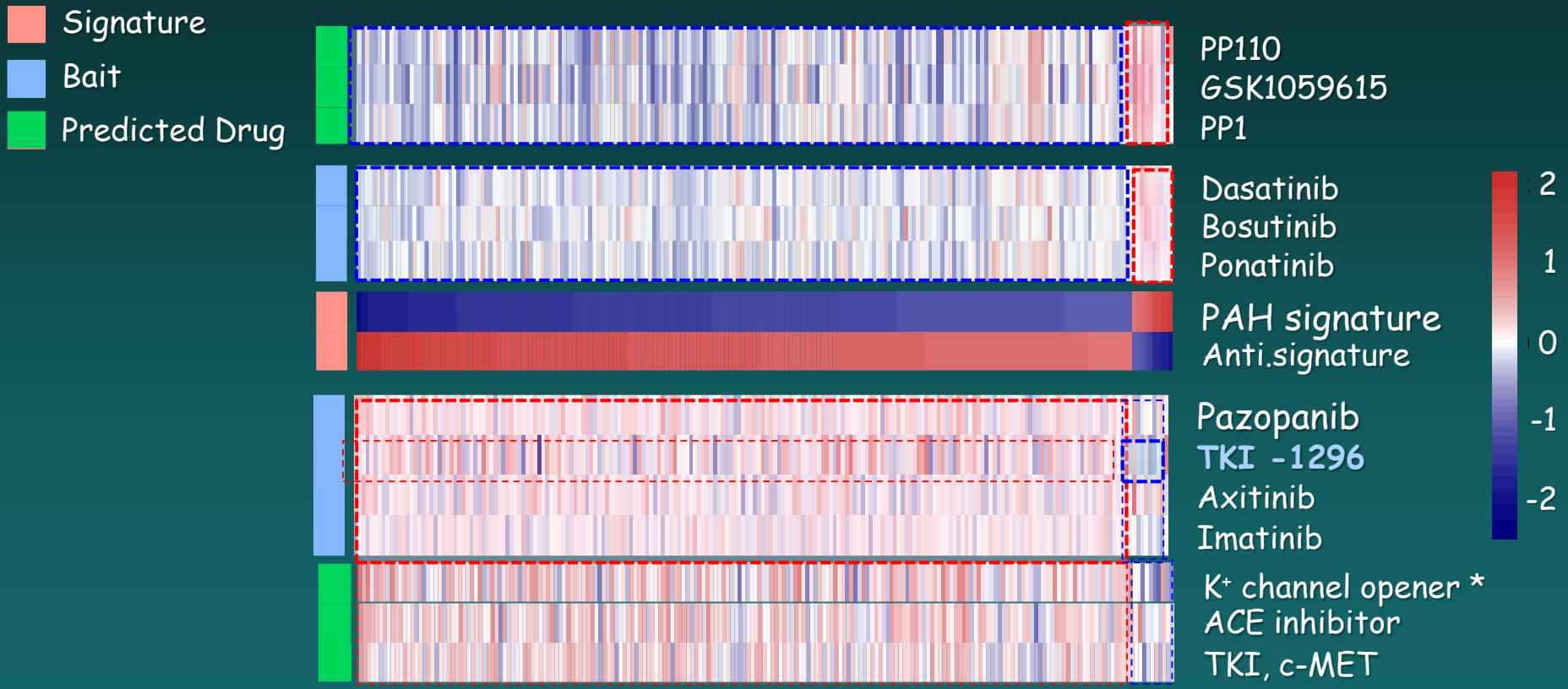
←

Specific pathway
targeted by the TKI

Bait-signature	Gene X	Gene Y
Anti-PAH signature	Gene X	Gene Y

Predict new drug with
similar signature

Bioinformatics Informs Drugs that Can Prevent or Cause PAH

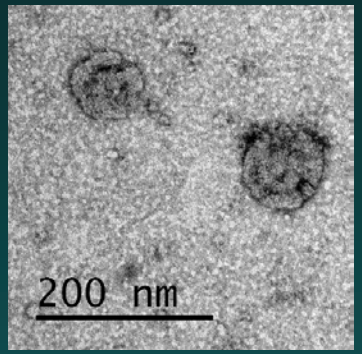


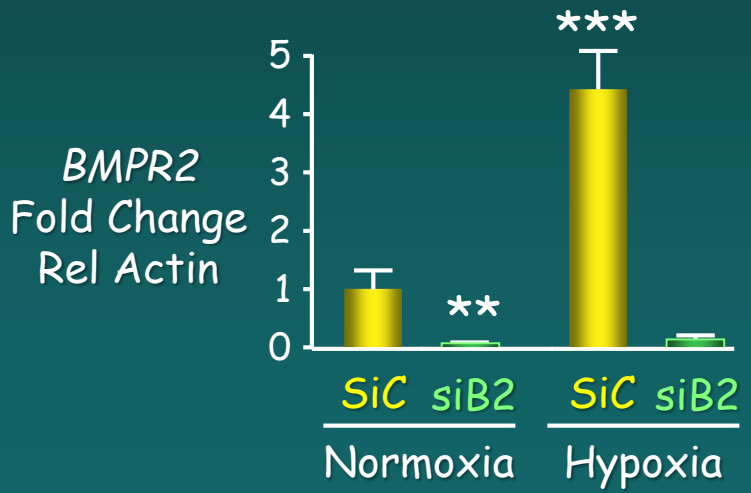
BMPR2 is Elevated and Packaged in Exosomes and Released by Cells Under Stress

David Marciano

Exosomes as Therapy for Pulmonary Hypertension

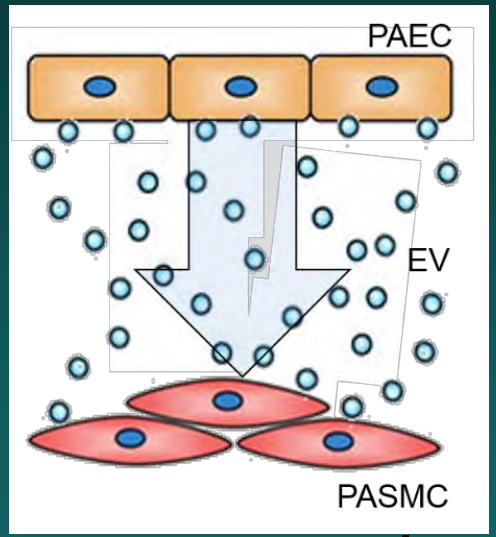
Make exosomes cell particles





**p<0.01, ***p<0.001, vs. siC, Normoxia

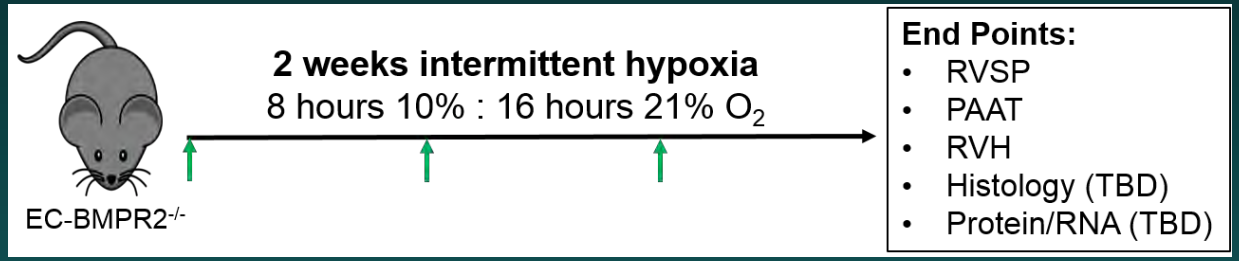
BMPR2 enriched exosomes reverse PAH EC and SMC dysfunction

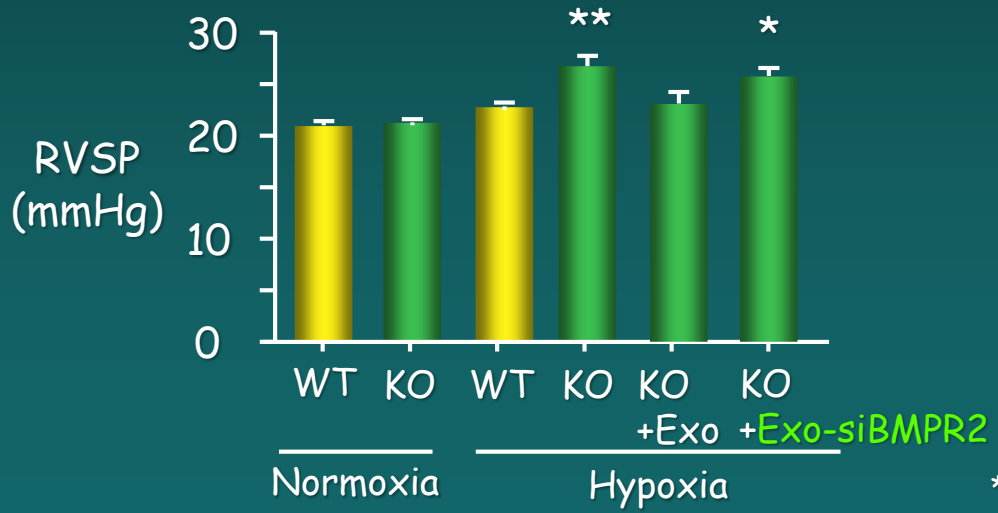


Endo Cells

Smooth Muscle Cells

Administration of BMPR2 via Endothelial Exosomes Prevents PH in Transgenic Mice with EC Lacking BMPR2





*p<0.05, **p<0.01 vs. KO, Hypoxia

Conclusions

- Reduced BMPR2 induces adverse effects related to PAH

  (i) transformation of endothelial to smooth muscle like cells
  (i) proliferation of smooth muscle cells,
  (iii) recruitment of elastin degrading neutrophils
  (iv) impaired elastic fiber assembly

- Therapies that restore BMPR2 function have promise in reversing PAH.

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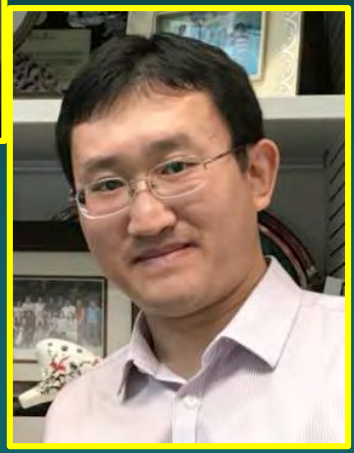

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