SCHOOL OF MEDICINE
HERMAN B WELLS CENTER FOR PEDIATRIC RESEARCH

FIRULLI PPG RETREAT
BIDDLE HOTEL
BLOOMINGTON, INDIANA

Functional studies on ventricular chamber development, CHD & cardiomyopathy

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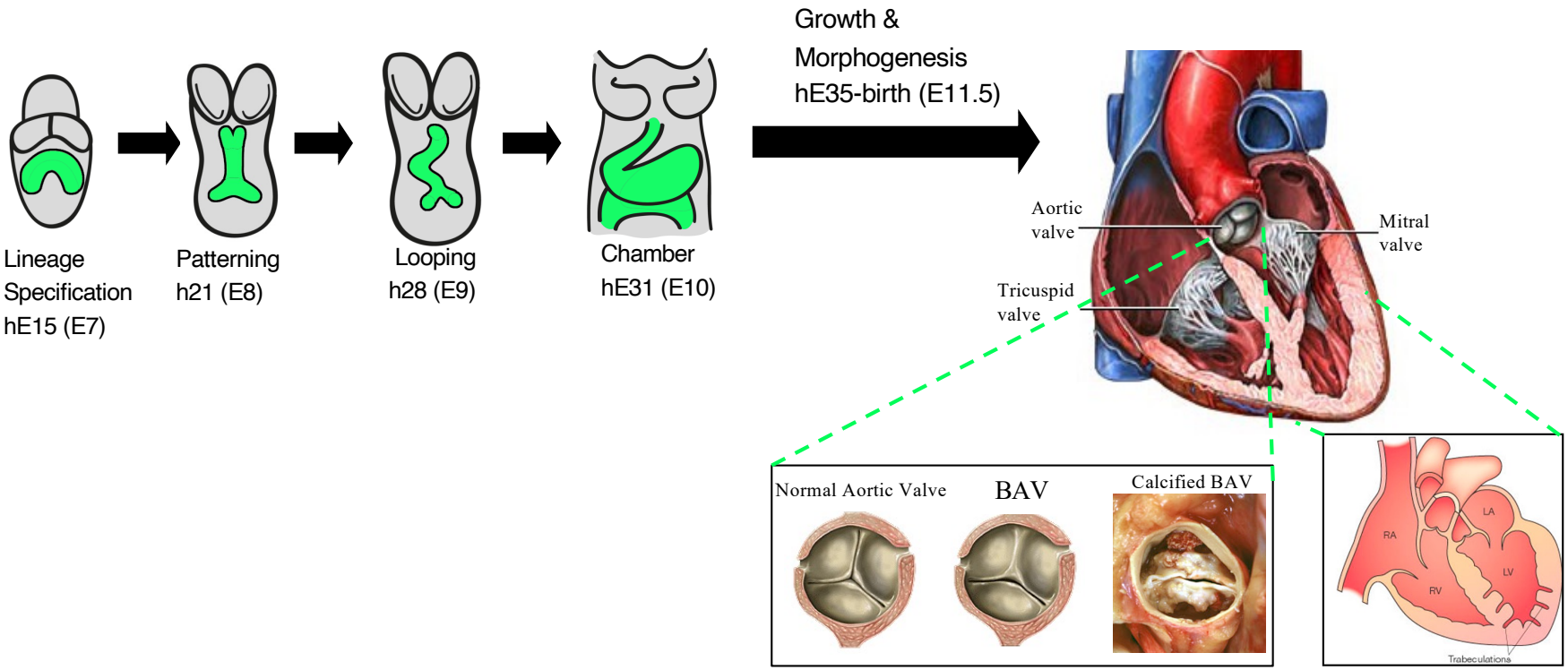
Instituto de Salud Carlos III
FundaciónProcnic
cnic
EXCELENCIA SEVERO OCHOA 2022-2025
AGENCIA ESTATAL DE INVESTIGACIÓN



Cardiac Development → Mature Ventricular chambers, Valves & Coronaries

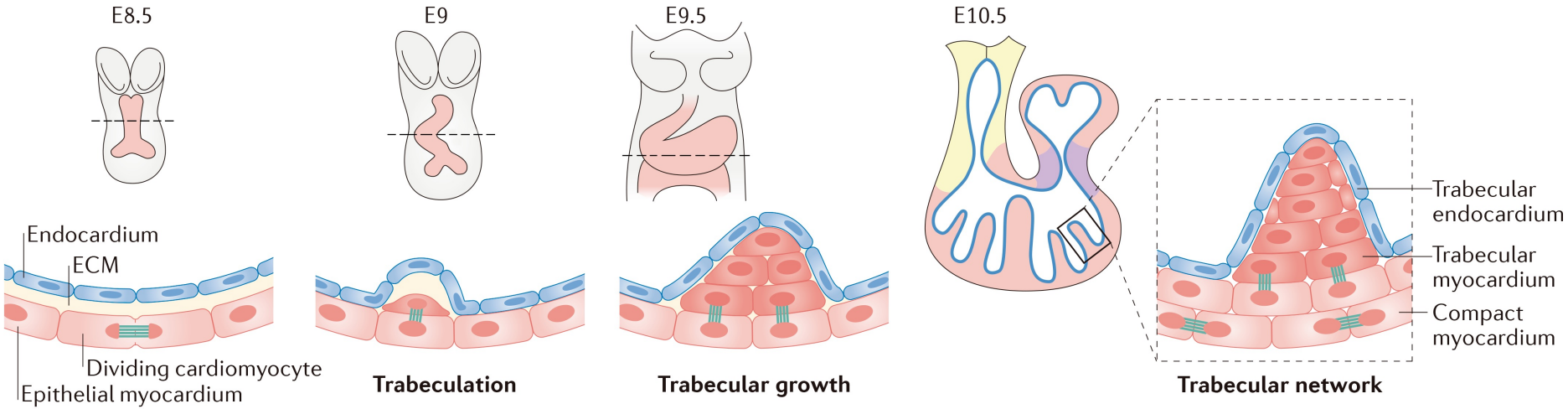
Cellular behaviors & mechanisms ↕

Signals interplay

Cardiac Disease: CHD, Cardiomyopathy & Valve Disease (BAV & CAVD)

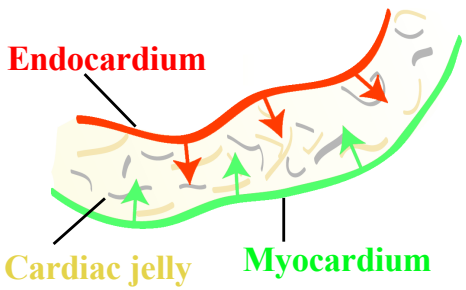
GOAL: Identification of novel developmental/pathological mechanisms, disease biomarkers and/or therapeutical targets for CVD

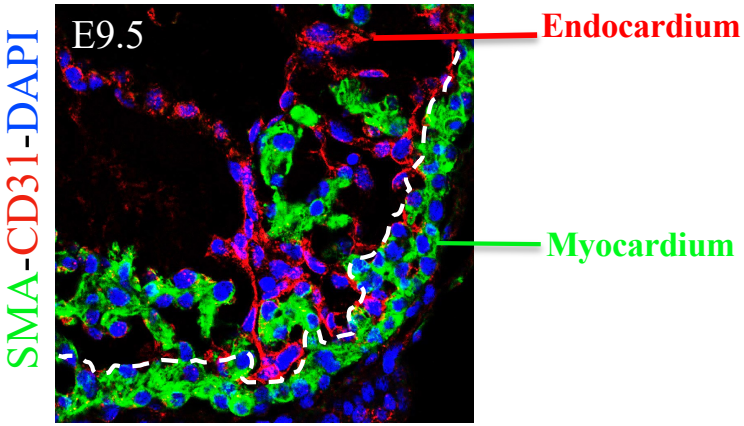
Early ventricular chamber development: Trabeculation



MacGrogan et al. (2018) *Nat Rev Cardiol*

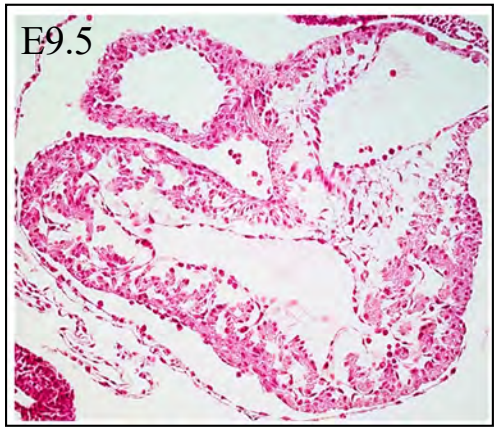
- Endocardium-myocardium crosstalk

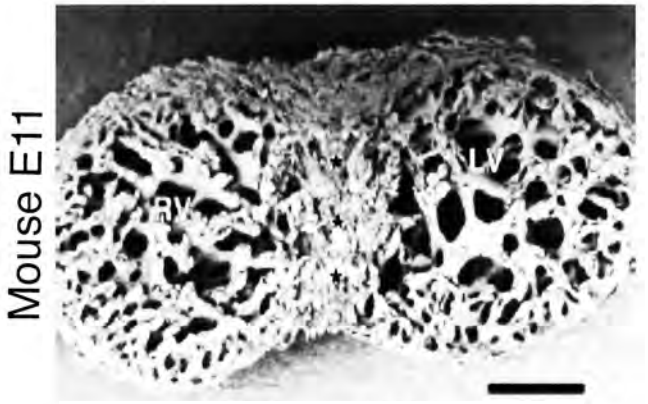




Trabeculae

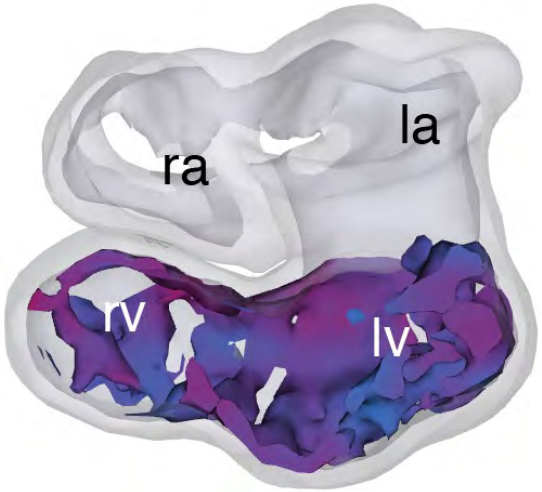
- Contractive force
- Essential for embryonic viability
- Myocardial nourishment: Oxygenation and nutrient exchange
- Increase in ventricular mass
- Purkinje fibers (VCS)



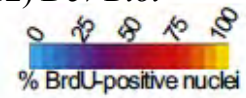


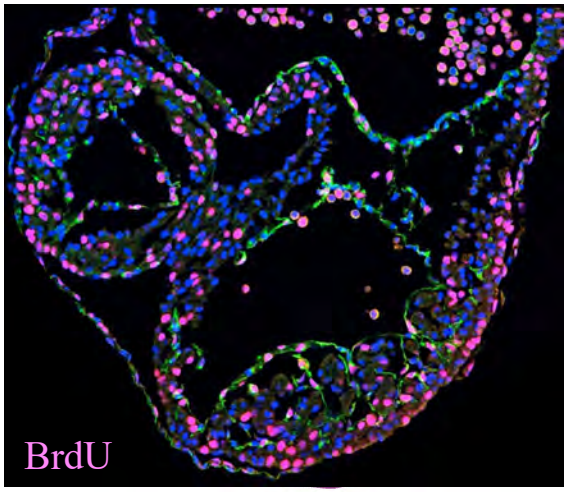
Sedmera et al. (2000) *Anat Record*

Proliferation

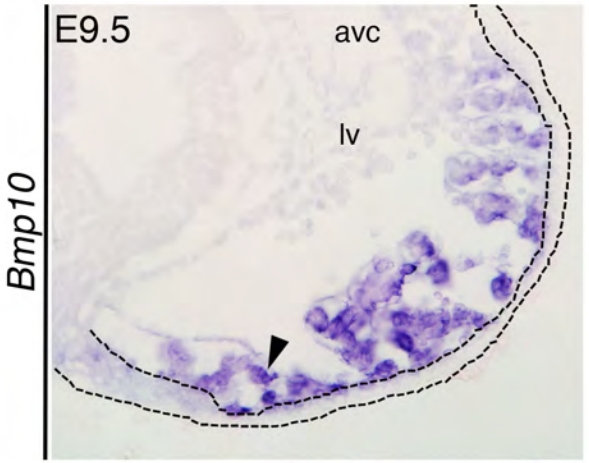


de Boer et al. (2012) *Dev Biol*

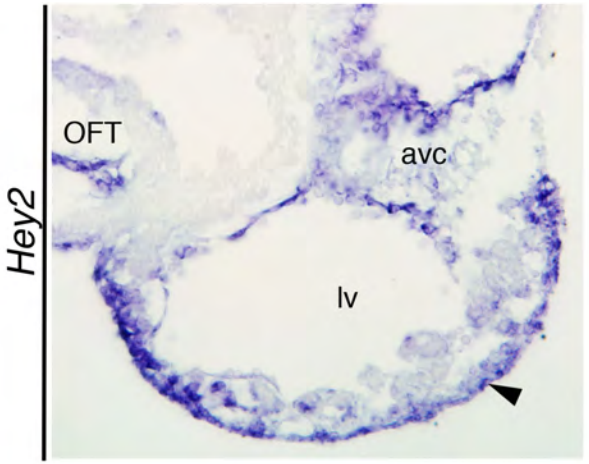




Differentiation

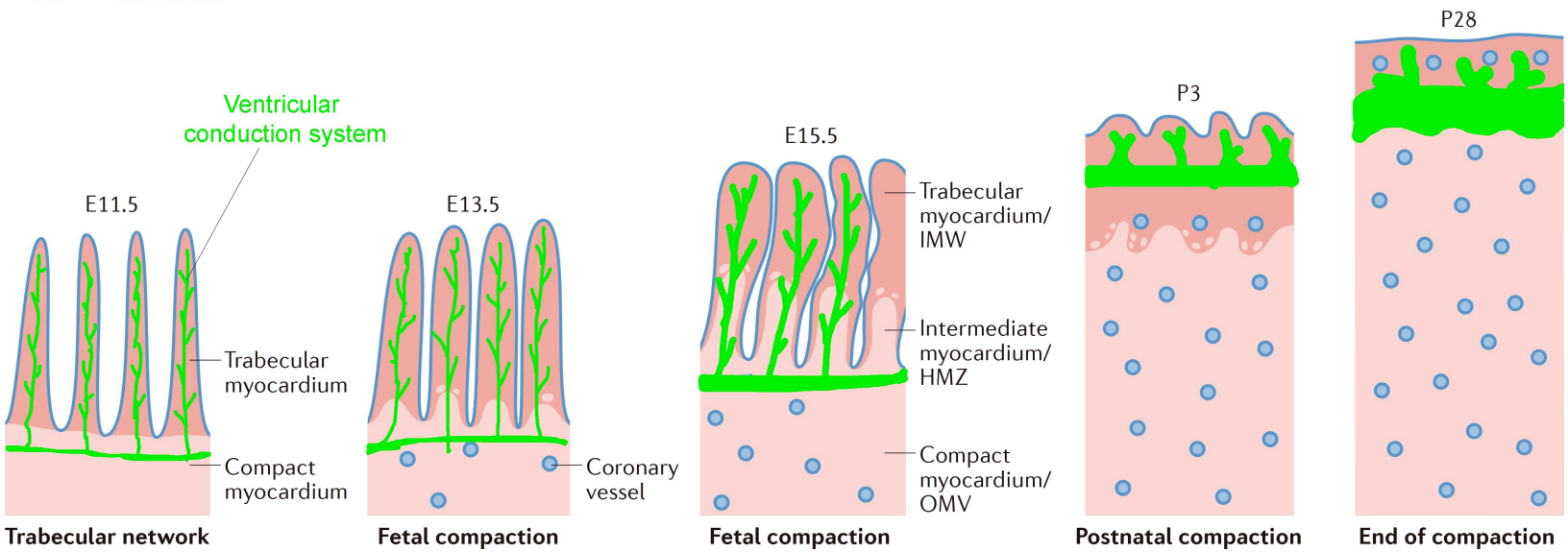


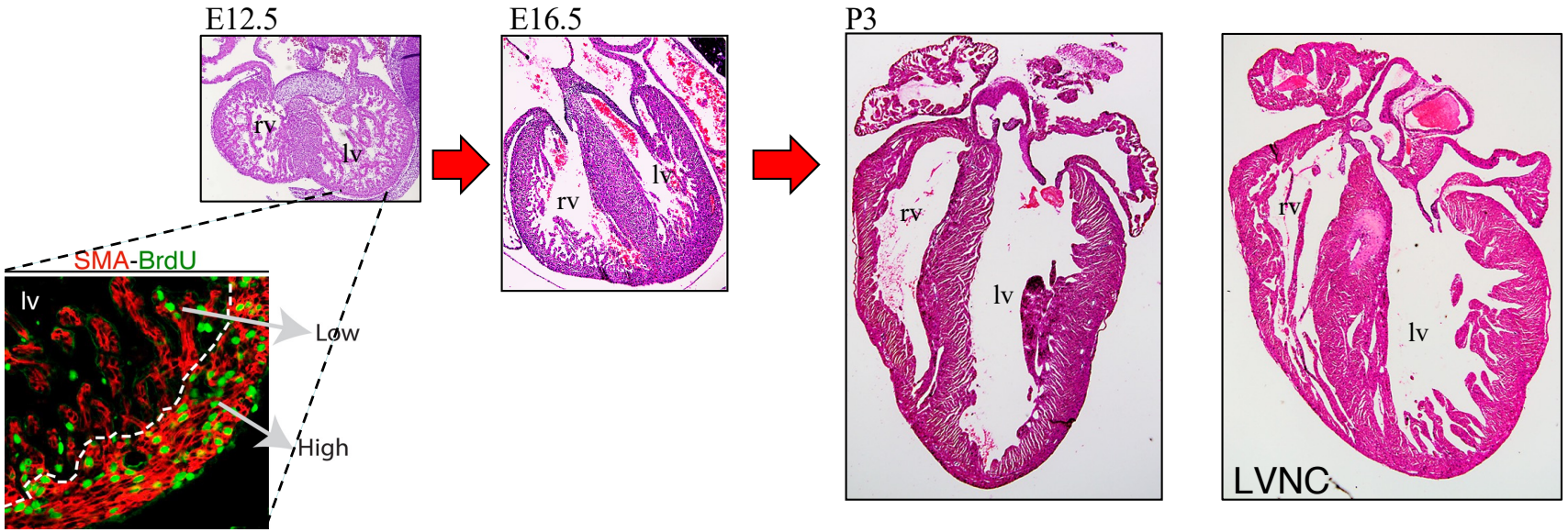
Trabecular myocardium



Compact myocardium

Late ventricular chamber development: Compaction





Endocardium-myocardium dependent

Questions:

- Mechanism of trabeculation? How do trabeculae grow?
  - Cell polarity---Oriented cell division?
  - EMT-like process?

- Mechanism of compaction?

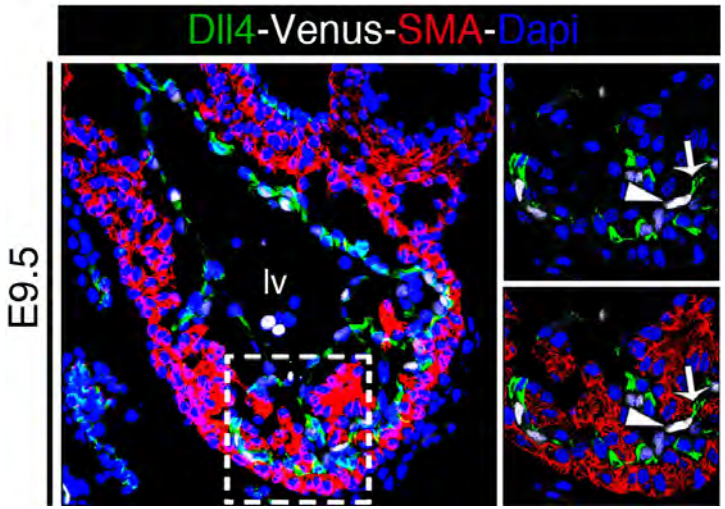
- Compaction and coronary vessel formation

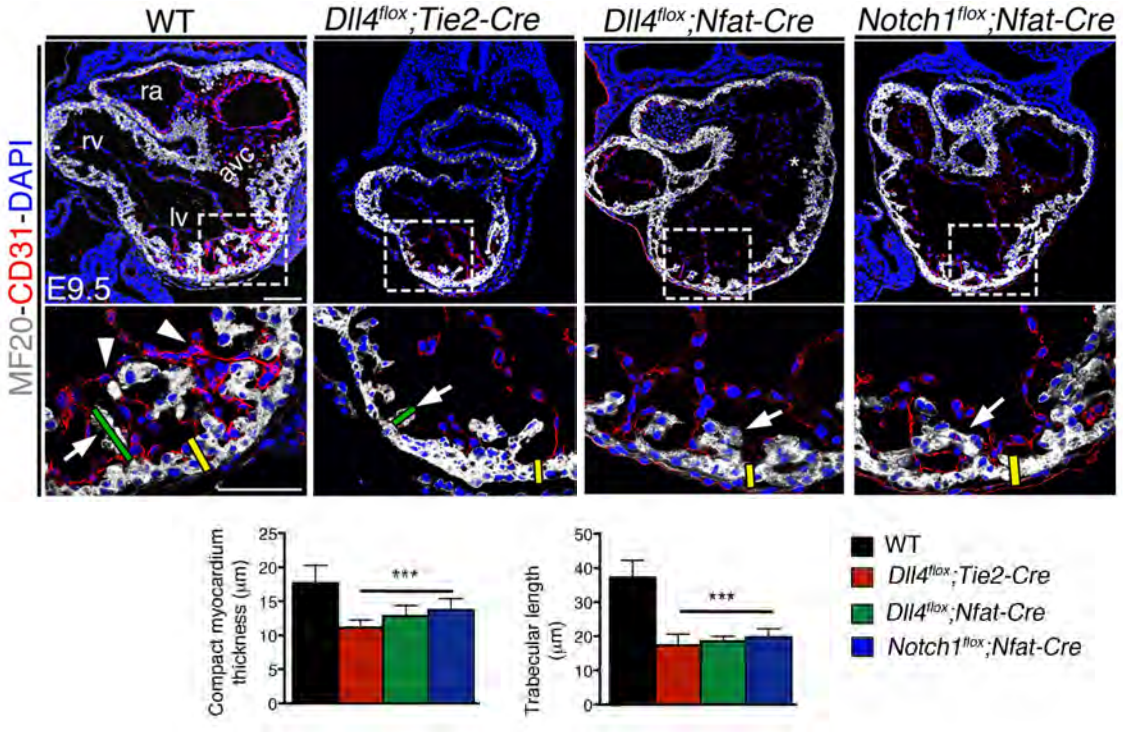
- Ventricular maturation & cardiomyopathy: Patterning, heterogeneity, differentiation

- Signaling pathways…

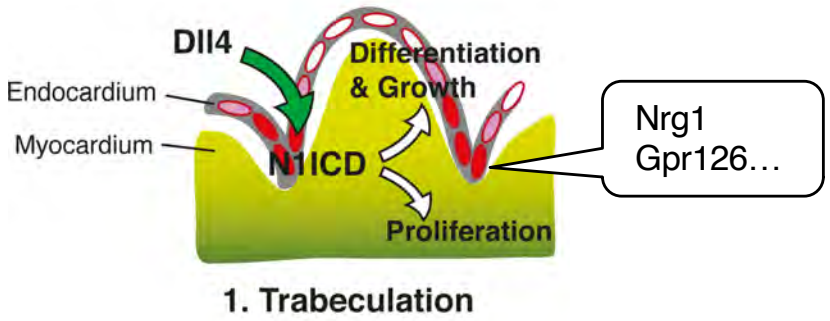
NOTCH signalling in trabeculation

Gaetano D'Amato





- Endocardial Dll4-Notch signalling is essential for trabeculation
- Loss of Dll4-Notch1 signalling impairs cardiomyocyte proliferation and differentiation



D'Amato et al. (2016) *Nat Cell Biol*

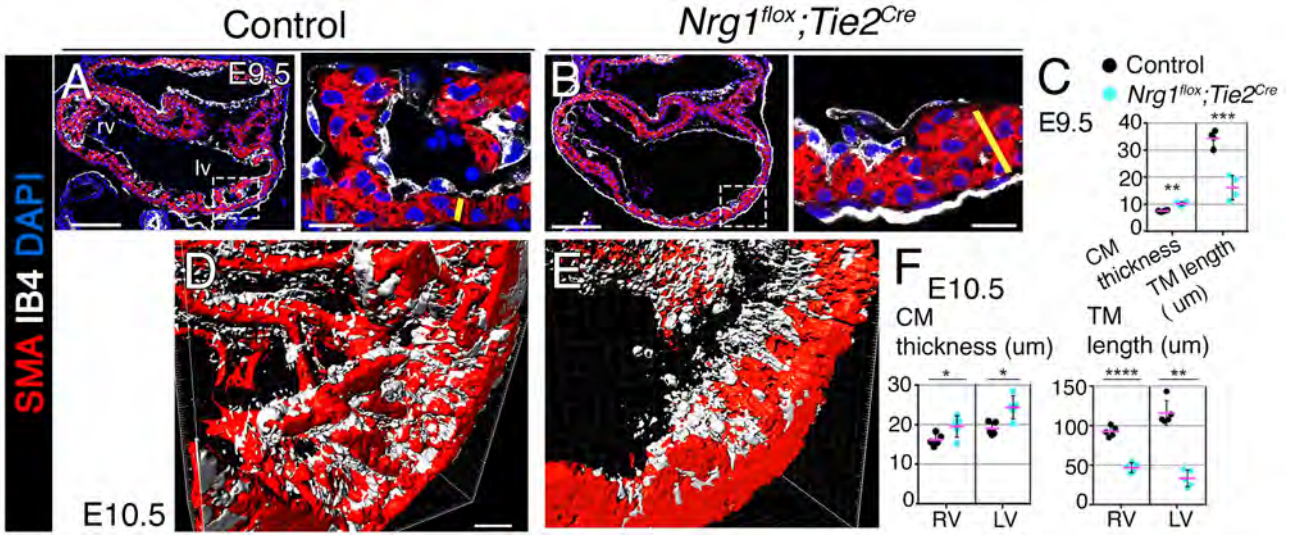
MacGrogan et al. (2018) *Nat Rev Cardiol*

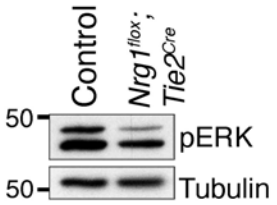
Torregrosa-Carrión et al. (2021) *Sci Advances*

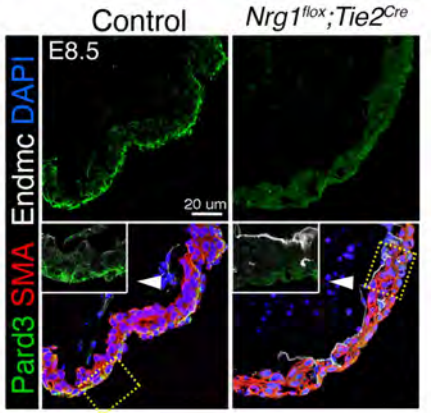
Nrg1-ErbB2,4 signalling in trabeculation

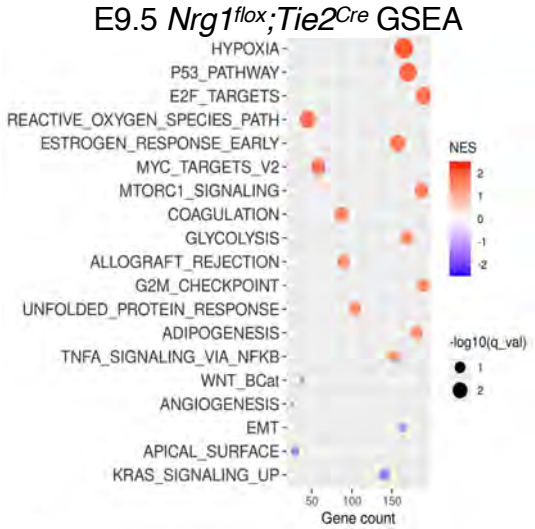
- Standard Nrg1 or ErbB2/4 KOs do not trabeculate [Meyer & Birchmeier (1995) *Nature*; Gassman et al. (1995) *Nature*; Lee et al. (1995) *Nature*]
- NOTCH functions upstream of Nrg1/ErbB2 during trabeculation [Grego-Bessa et al. (2007) *Dev Cell*]
- ECM dynamics during trabeculation are regulated by a Notch-Nrg1 interplay [del Monte-Nieto et al. (2018) *Nature*]

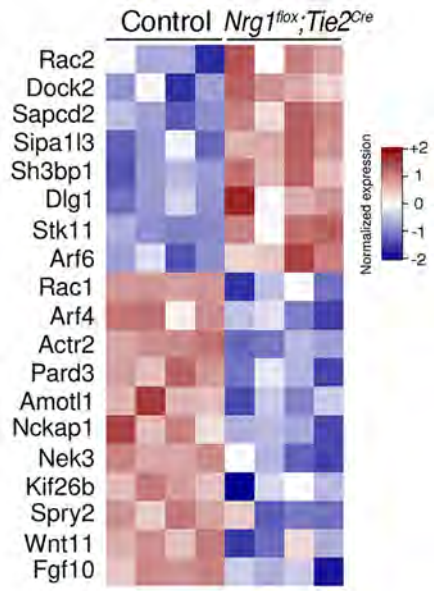
Paula Gómez-Apiñaniz

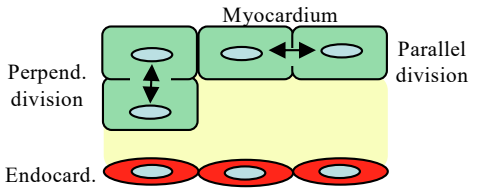






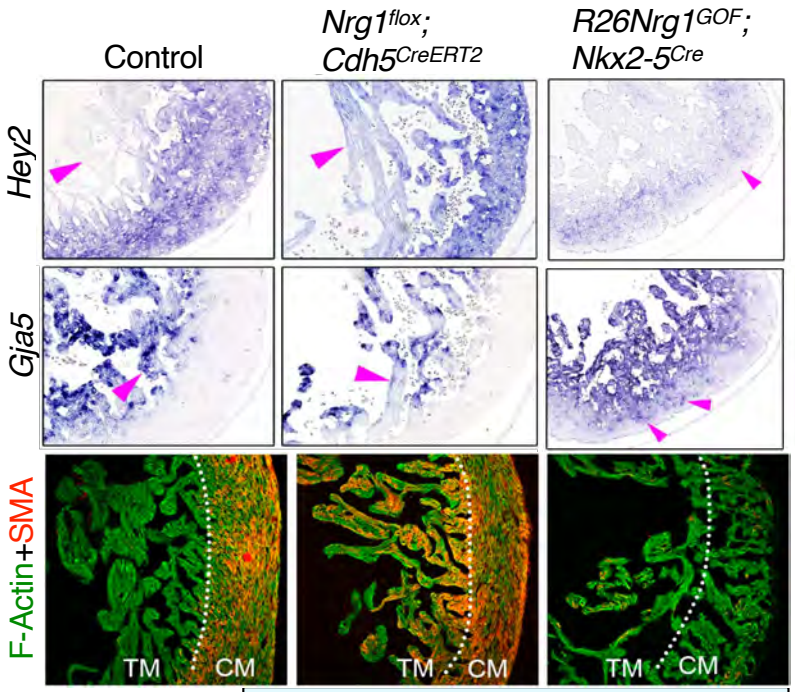




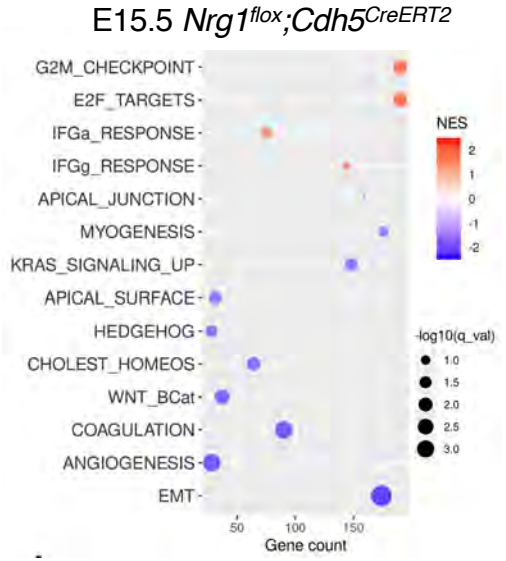


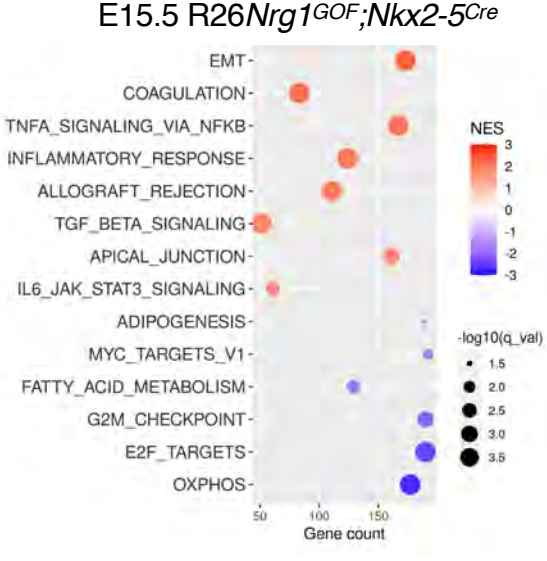
Defective OCD: Horizontal bias

Nrg1-ErbB2,4 signalling in ventricular wall maturation/compaction



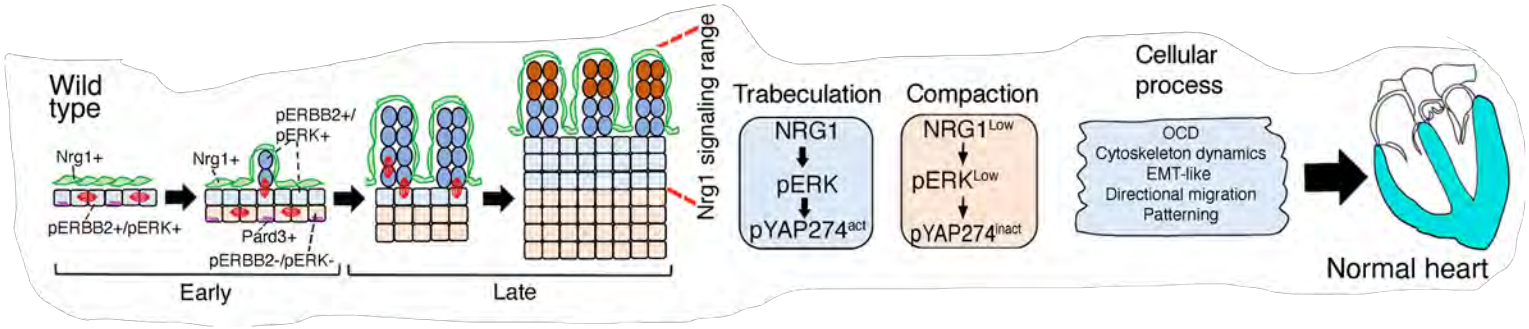
Altered patterning & VCS differentiation





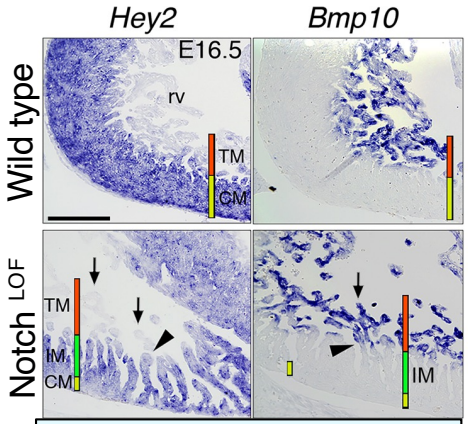
Joaquim Grego-Bessa

Donal MacGrogan

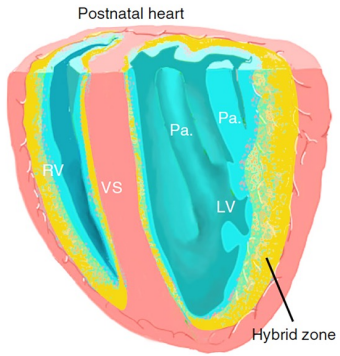


Nrg1-ErbB2/B4 regulate cardiomyocyte maturation during chamber development. Grego-Bessa et al. (2023) *Circ Res*

Mouse heart

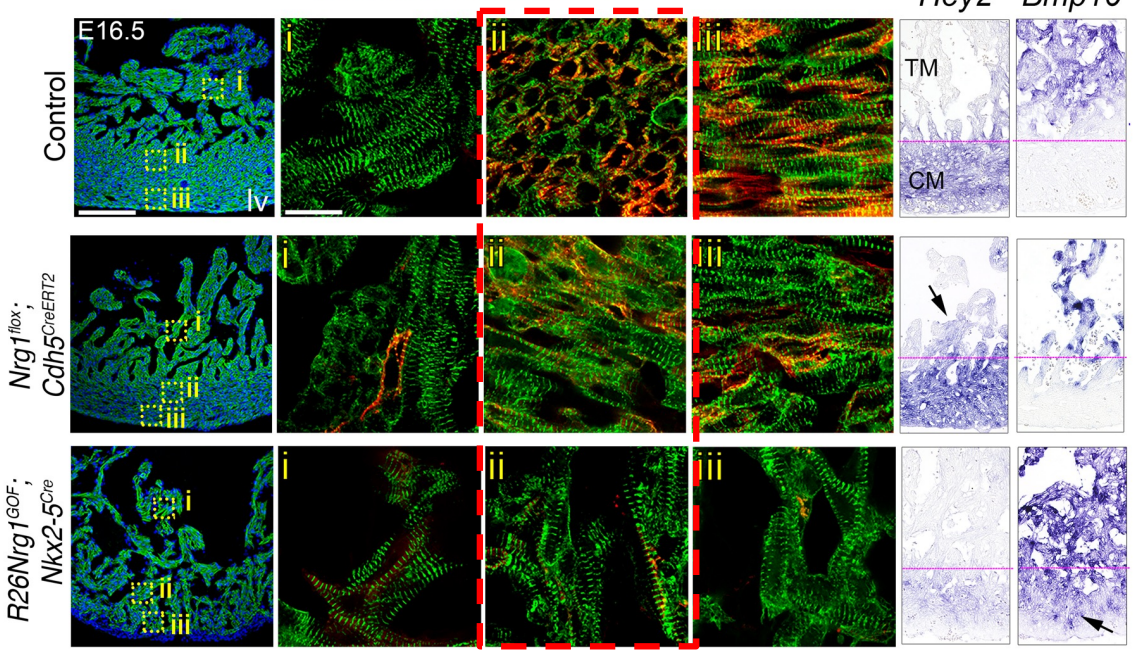


D'Amato et al. (2016) *Nat Cel Biol*



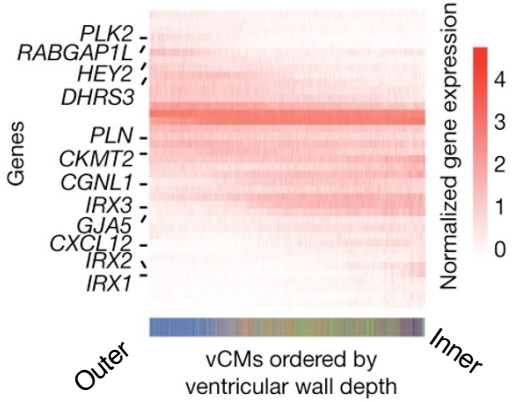
Tian et al. (2017) *Nat Comm*

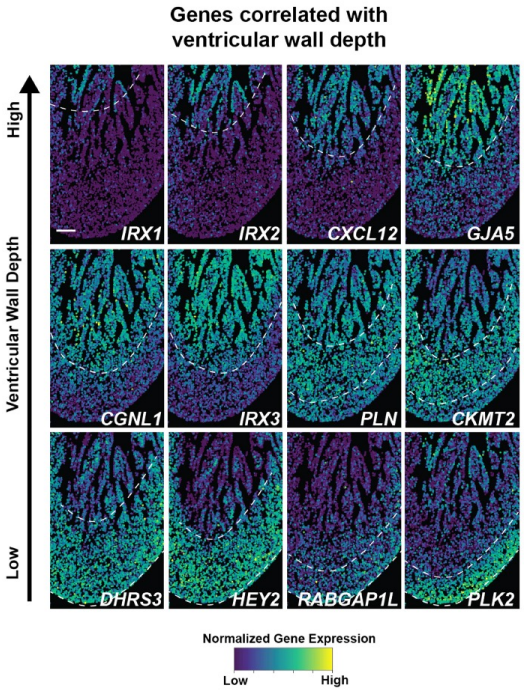
Intermediate (IM) CM - hybrid myocardial zone



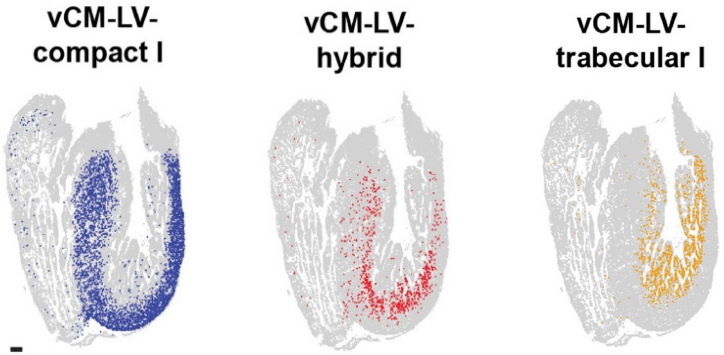
Intermediate CM layer (ii) missing in Nrg1^{LOF} or ^{GOF} models: Patterning defect

Farah et al. (2024) Spatially organized cellular communities form the developing human heart. *Nature*

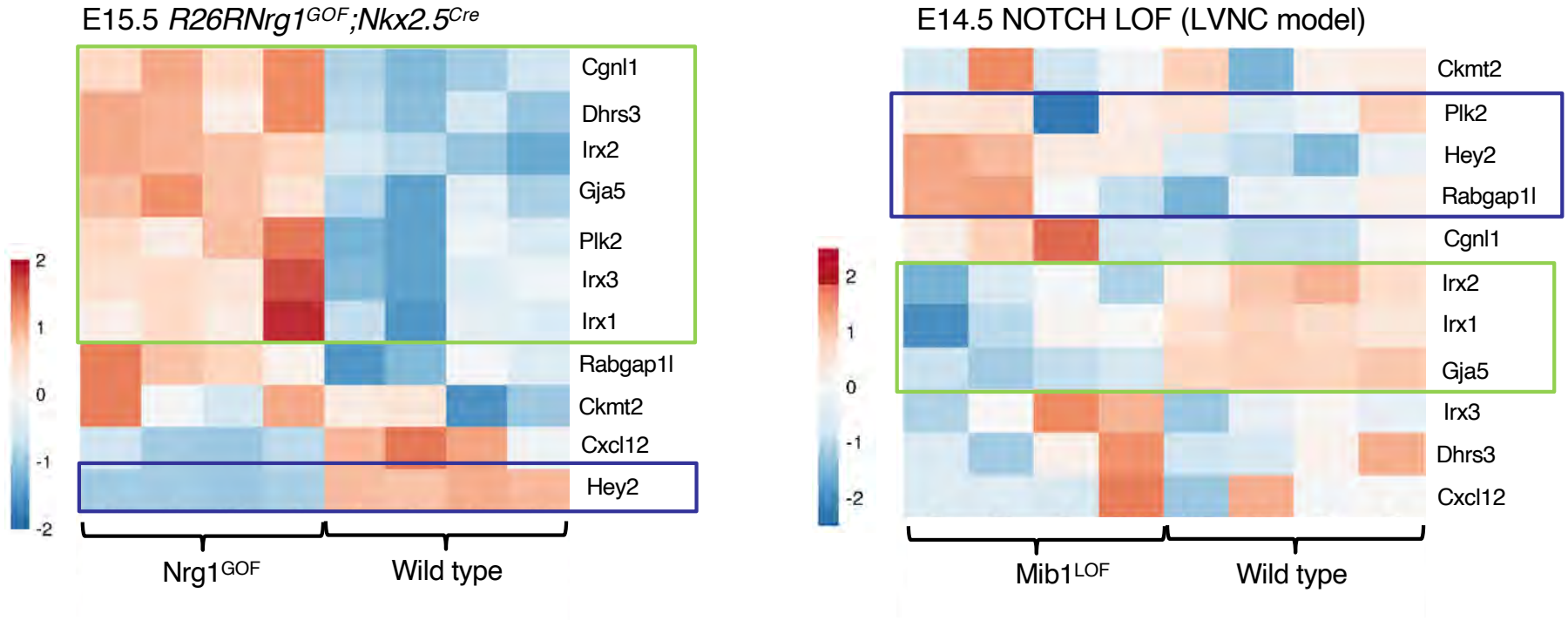




Human heart

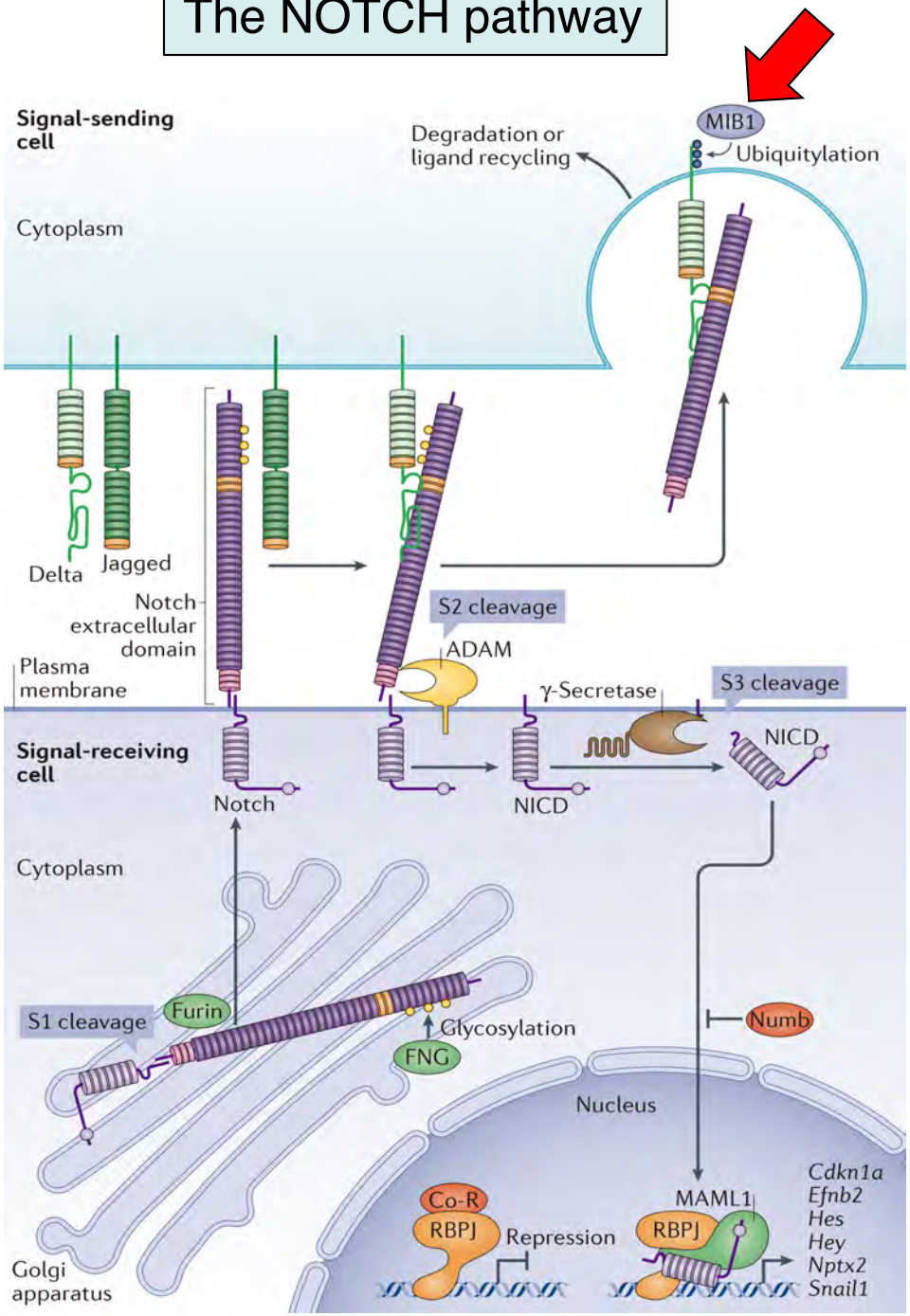


This hybrid ventricular cardiomyocyte population transiently co-expresses compact and trabecular myocardium genes



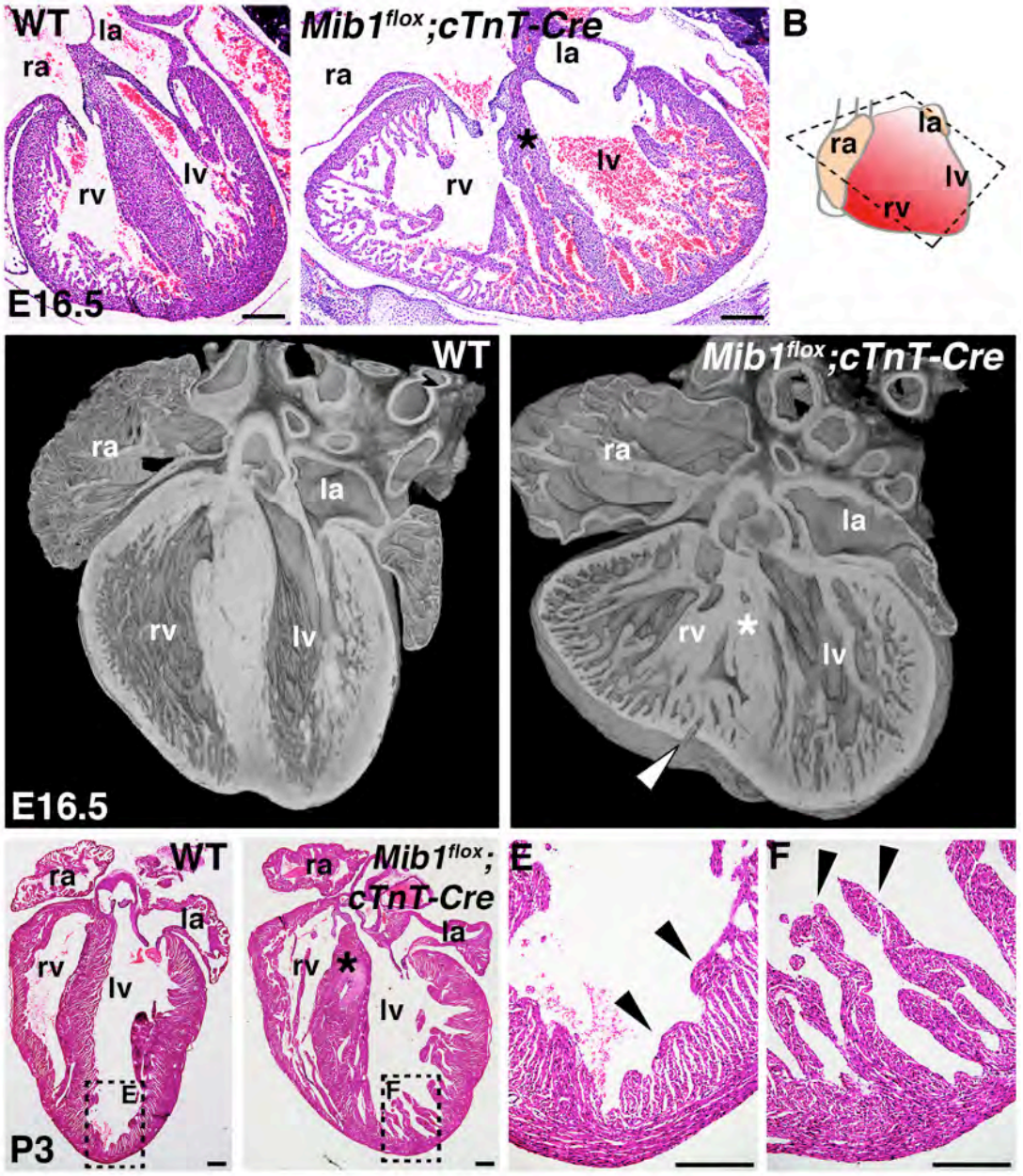
- Nrg1 signaling manipulation disrupts ventricular wall gene expression: Patterning defect. Nrg1^{GOF}: TM genes up (VCS).
- Mib1-Notch signaling abrogation disrupts ventricular maturation: Patterning defect.
- Mouse scRNA-seq data do not suggest a human heart-like cardiomyocyte cell population complexity across the developing ventricular wall.
- Relevance in cardiomyopathy?
- Ongoing…

The NOTCH pathway



Mib1 disruption in the embryonic myocardium impairs compaction

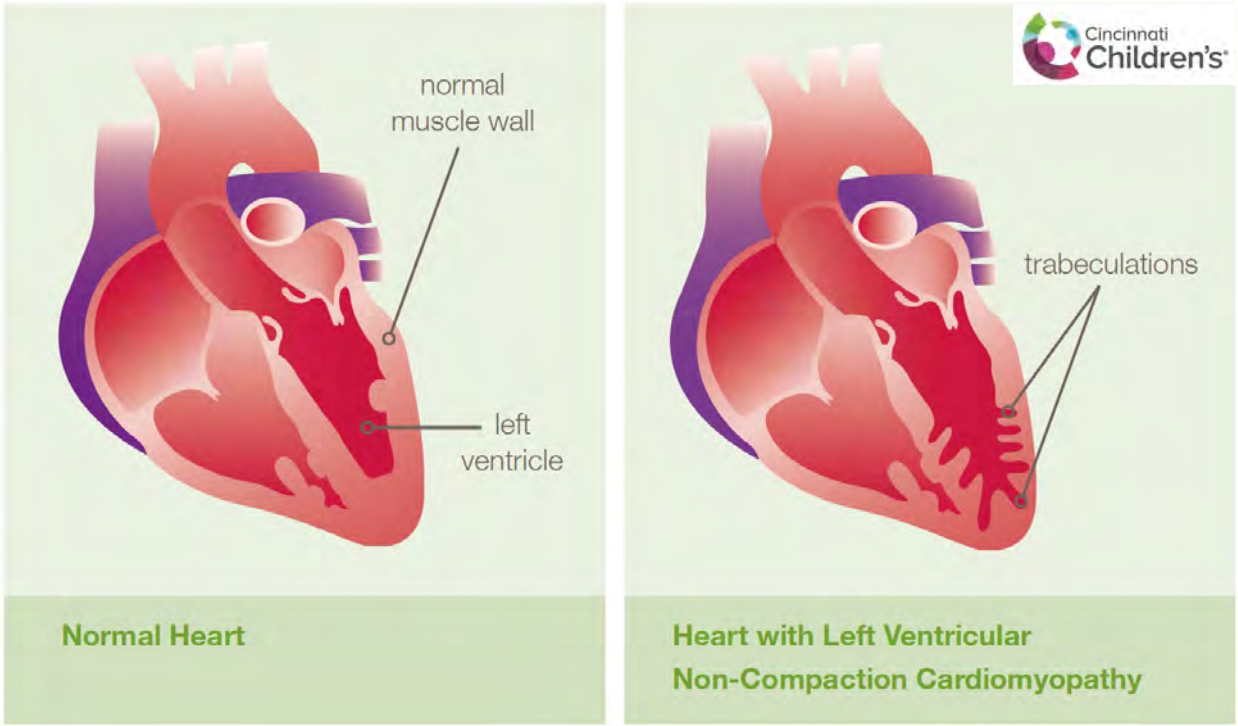
Guillermo Luxán



- Dilated Heart
- Thinner compact myocardium
- Large non-compacted trabeculae
- Impaired heart function
- LVNC?

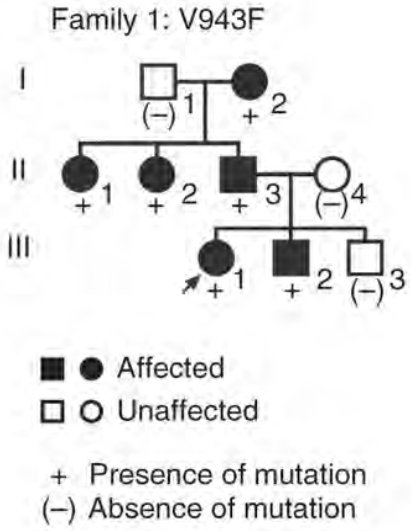
Left Ventricular Non Compaction (LVNC)

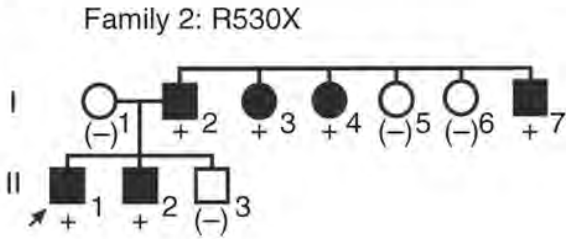
- Genetic cardiomyopathy: 0.05–0.3% of the population. Pathogenesis poorly understood.
- Arrest of compaction of the trabecular embryonic myocardium? Developmental basis?
- Asymptomatic course to reduced ventricular function with rapid deterioration requiring heart transplantation.
- Complications: Systemic embolism, malignant arrhythmias, heart failure and sudden death.
- Caused by mutations in genes encoding sarcomere, cytoskeletal, nuclear membrane and chaperone proteins…
- Mixed phenotypes: LVNC and HCM, or LVNC and DCM may coexist in the same patient.



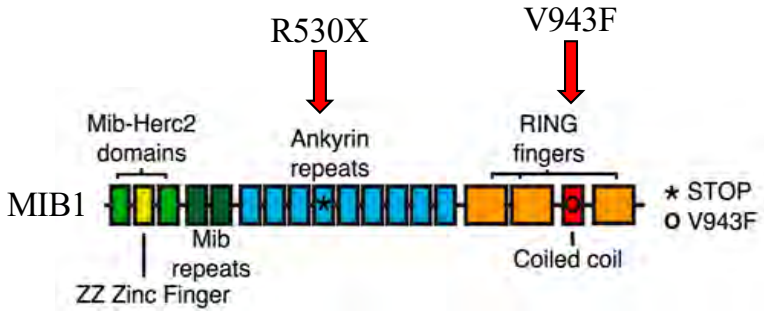
Mutations in *MIB1* cause LVNC

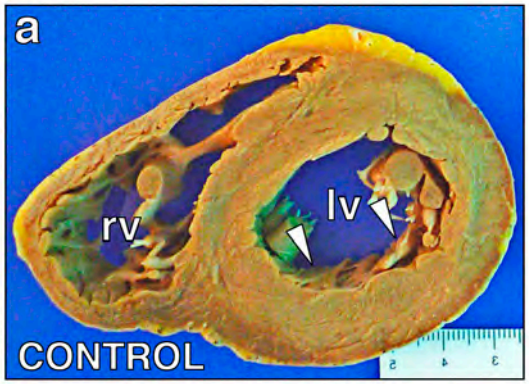
LVNC cohort with
100 cases: 50% familial

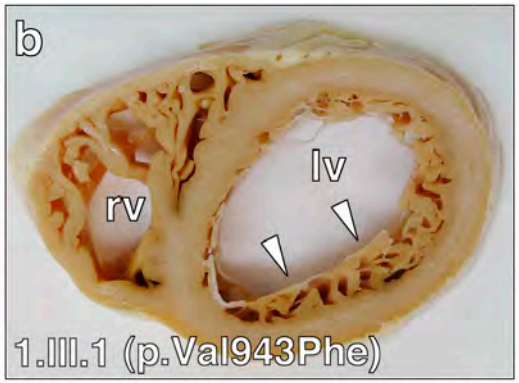


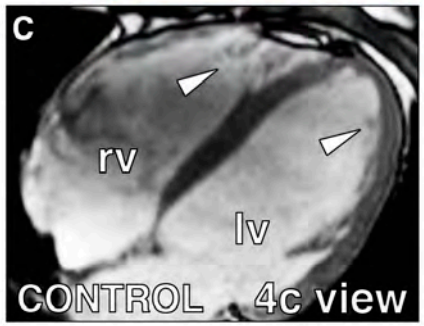


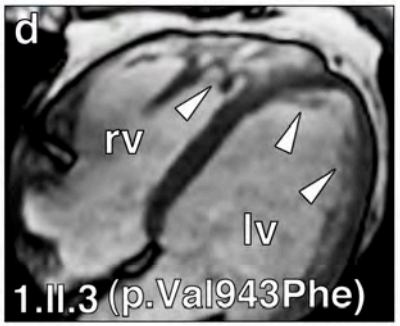
Heterozygous mutations,
autosomal dominant inheritance

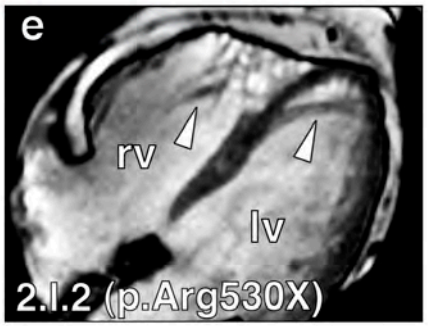












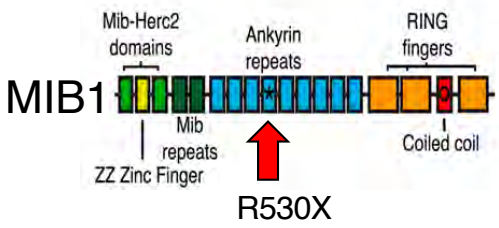
Luxán et al. (2013) *Nat Med*

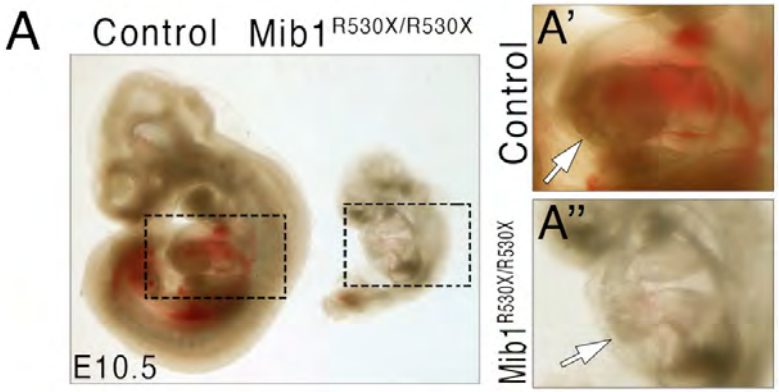
D'Amato et al. (2016) *Nat Cell Biol*

MacGrogan et al. (2018) *Nat Rev Cardiol*

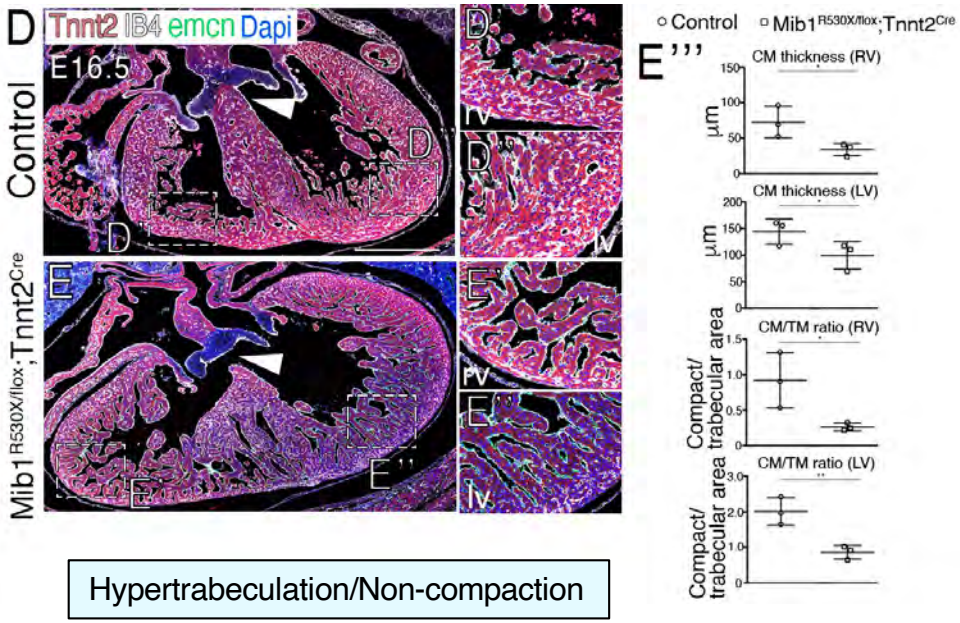
Generation & analysis of humanized mouse models harboring *Mib1* mutations

Marcos Siguero-Álvarez

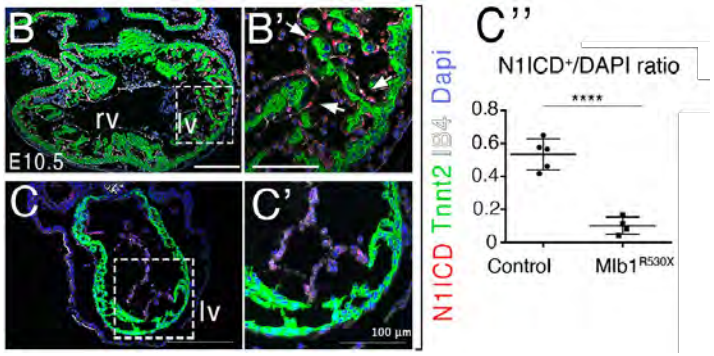




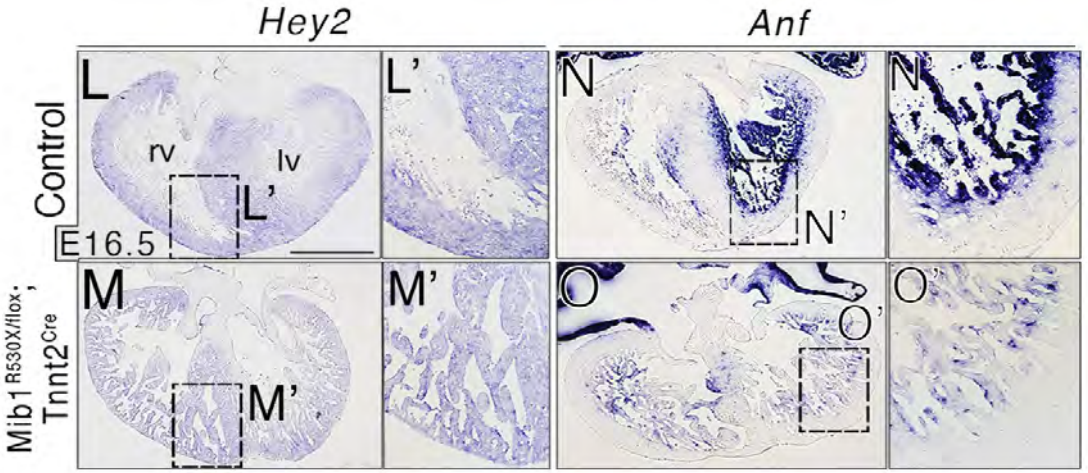
Embryonic lethality



Hypertrabeculation/Non-compaction

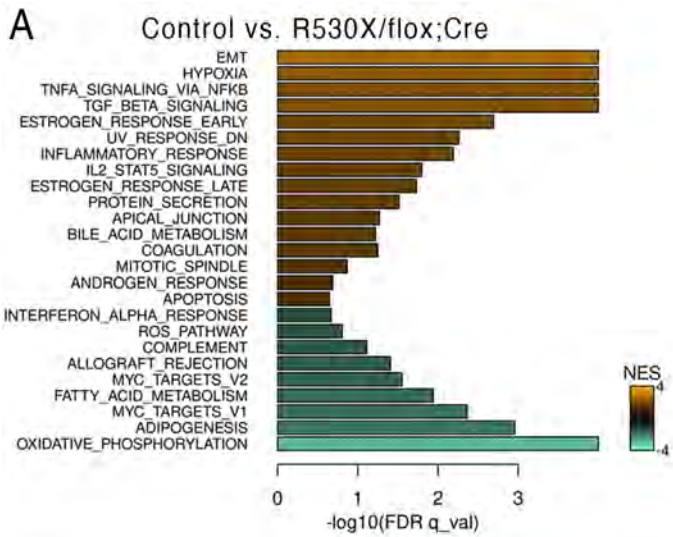


NOTCH signaling reduction



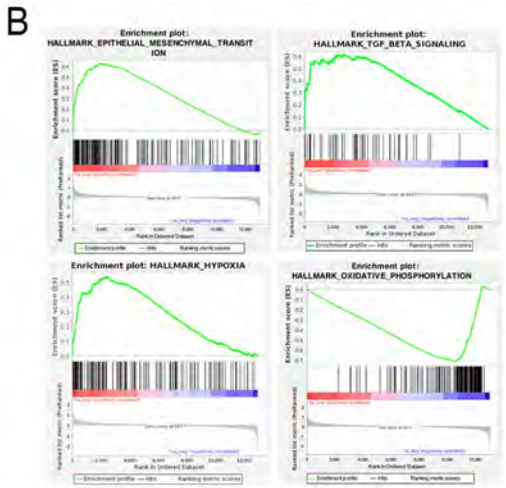
Chamber patterning disrupted

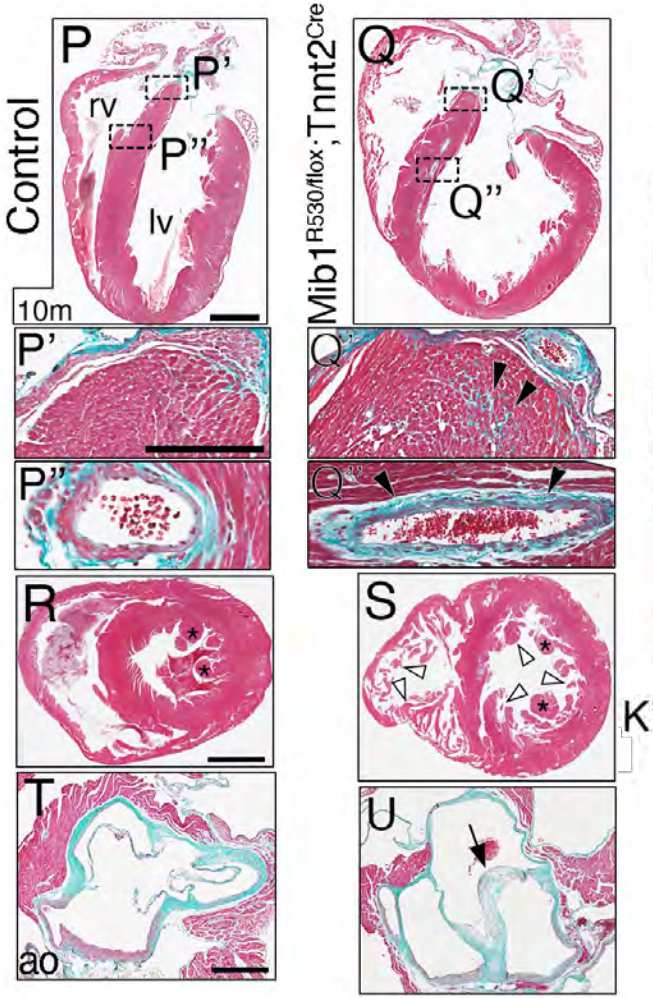
Mouse E15.5



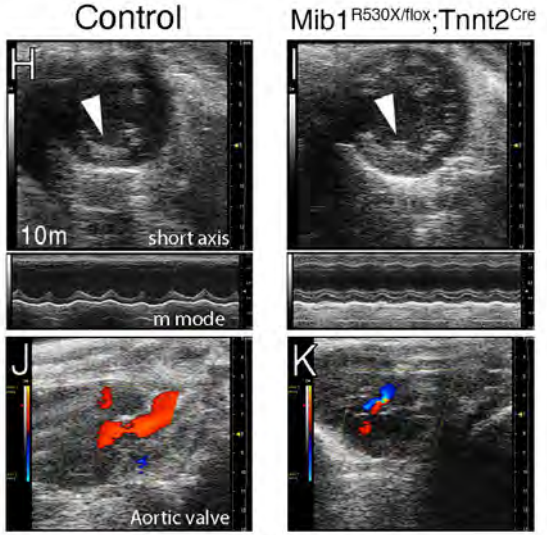
-Activation of cellular dynamics (EMT), Hypoxia and TGFB signaling

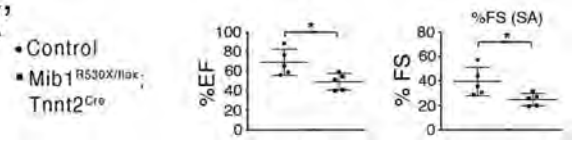
-Impaired metabolic maturation



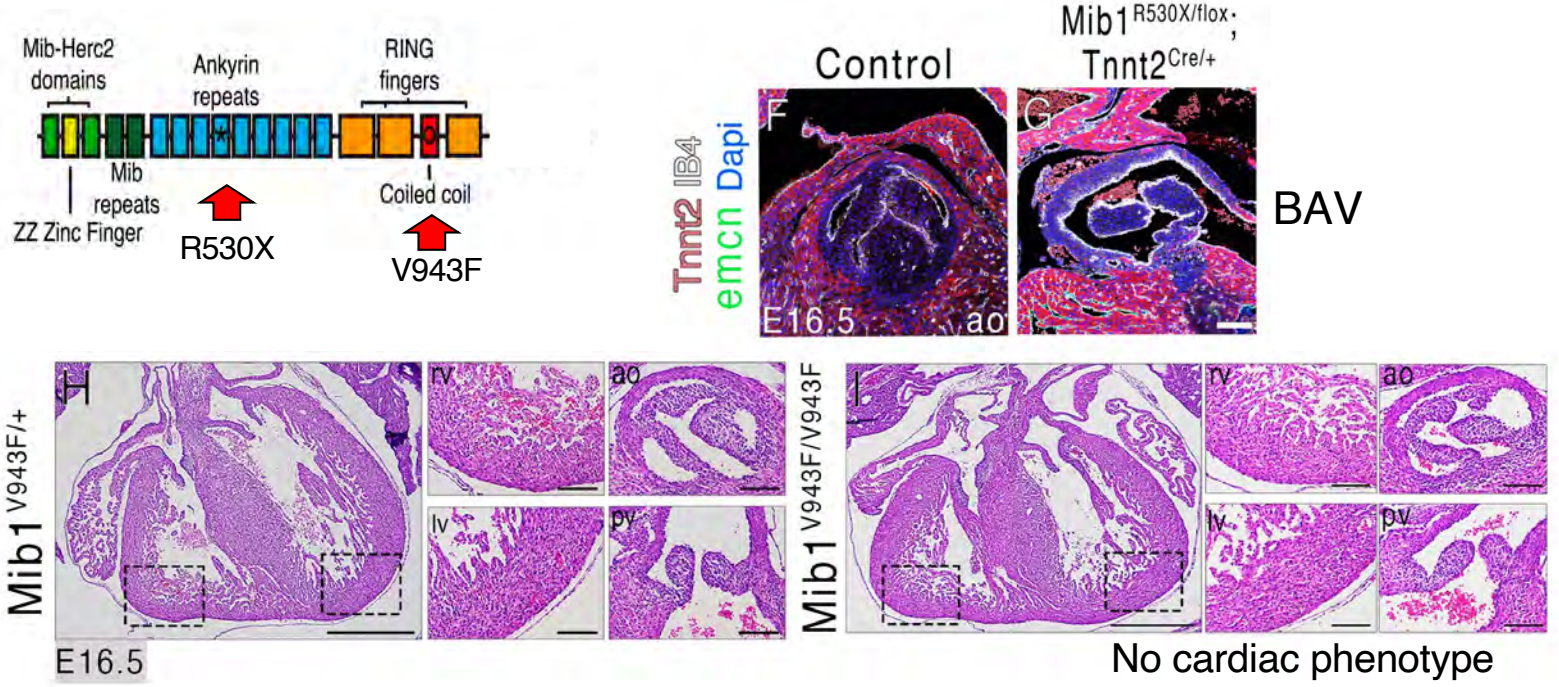


Adult: Hypertrabeculation, fibrosis, cardiac dilation, aortic valve dysmorphology



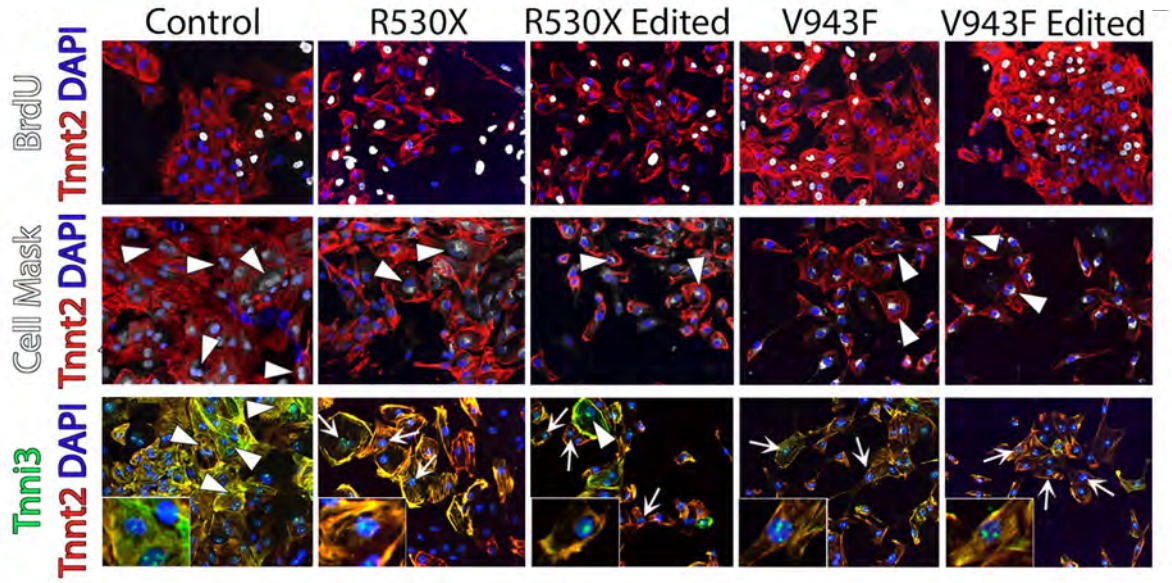


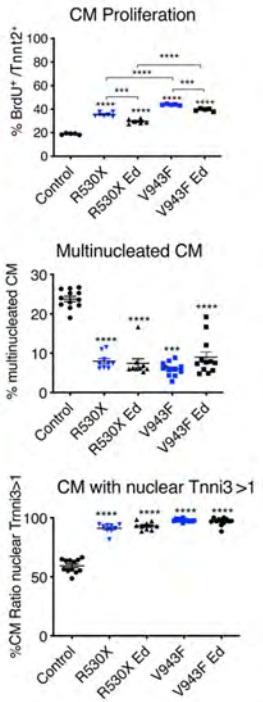
Impaired ventricular function, Valve regurgitation

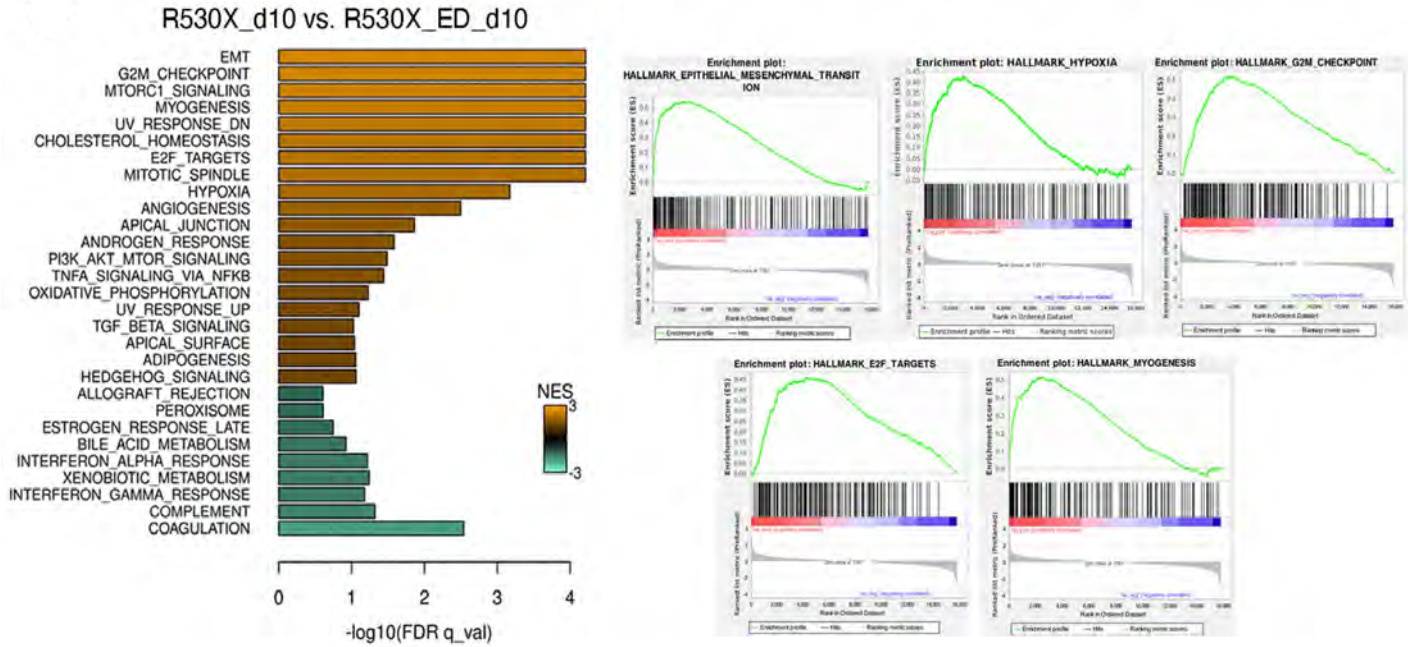


The $Mib1^{V943F}$ and $Mib1^{R530X}$ mutations cause BAV in a NOTCH-sensitized genetic background

MIB1^{R530X} and MIB1^{V943F} hiPSC-CM







Collaboration with:

Dr. J. R. Gimeno, HU Virgen Arrixaca, Murcia, SPAIN

Dr. A. Raya, IDIBELL, BCN SPAIN

Activation of a promigratory gene program (EMT), increased cellular proliferation, and impaired cardiomyocyte maturation

WES of LVNC families

Paradox:

-Recessive Mib1-LVNC inheritance in mice …

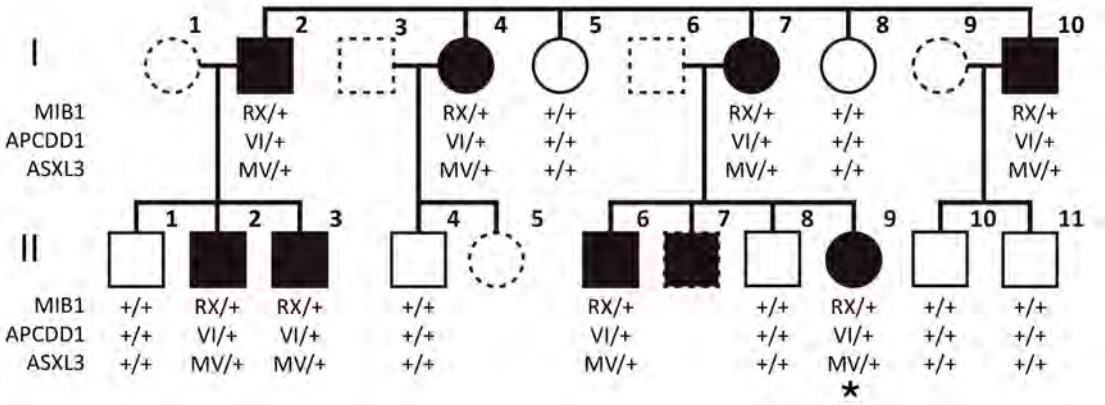
-Modifier genes?

-WES of LVNC families

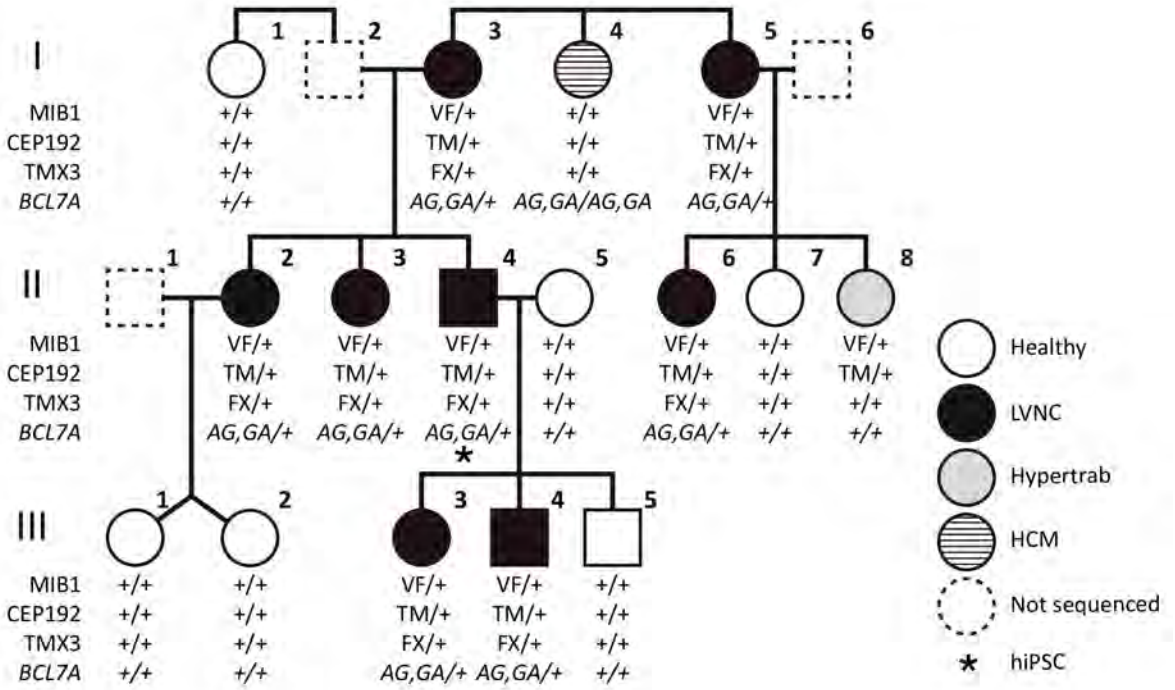
MIB1^{R530X}
- APCDD1^{V150I} (rs3748415)
- ASXL3^{M1415V} (rs181303838)

MIB1^{V943F}
- CEP192^{T1547M} (rs143331552)
- TMX3^{F191X} (rs143627864)
- *BCL7A*^{AG,GA} (rs884785) (rs884786)

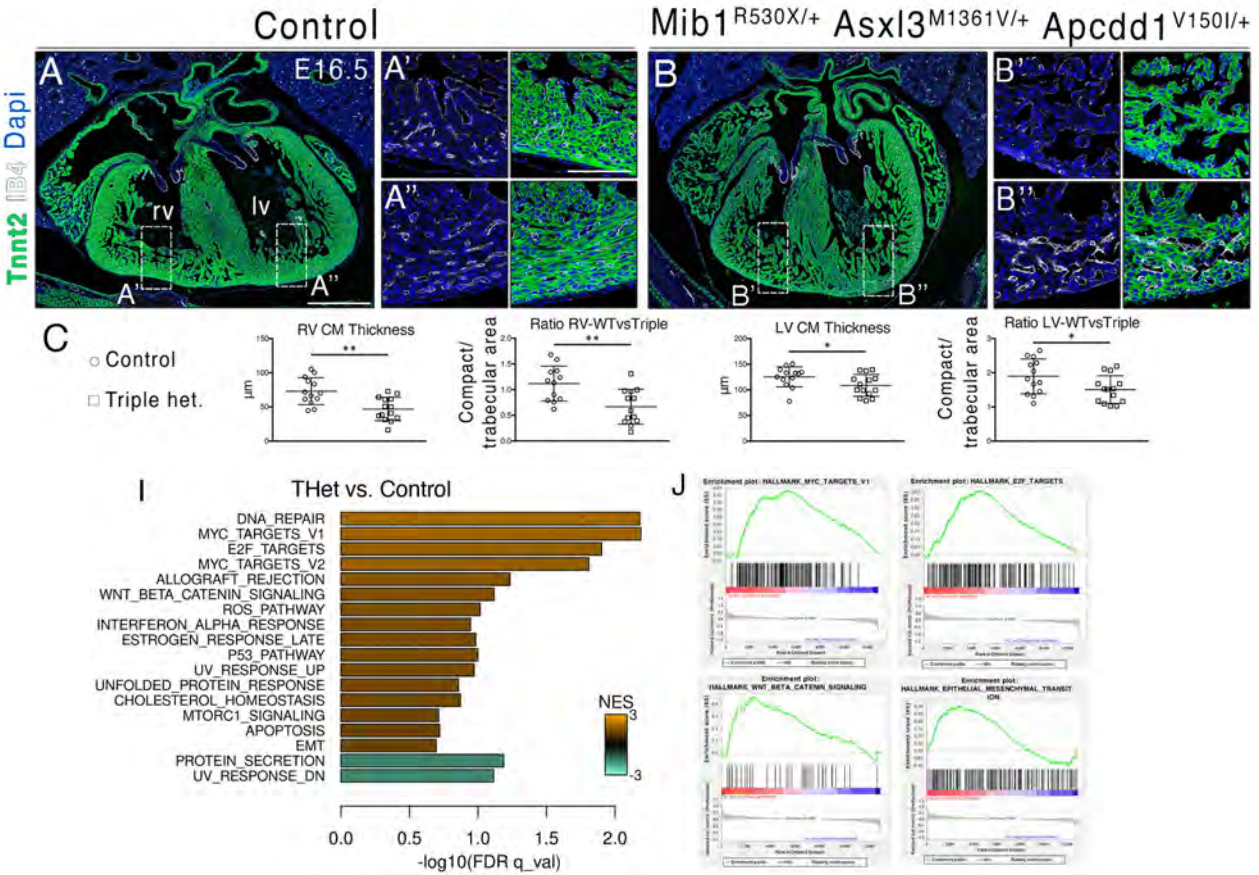
MIB1^{R530X}



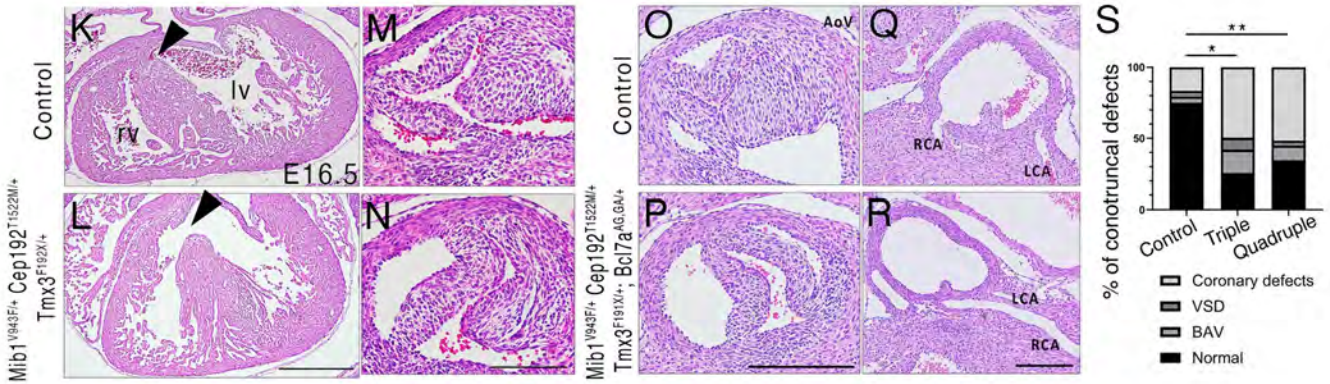
MIB1^{V943F}

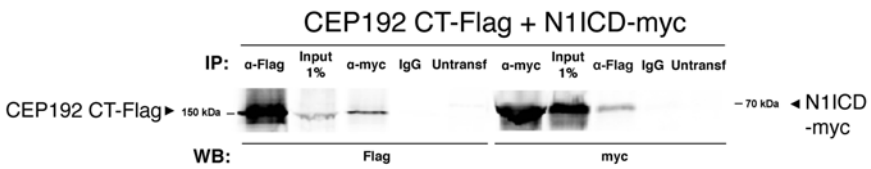


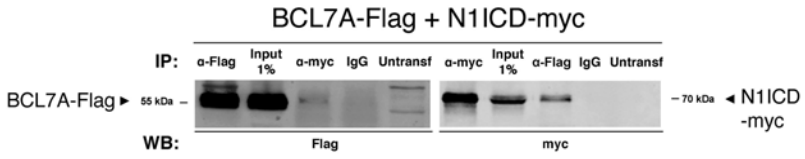
Mib1$^{R530X/+}$ triple mutant combinations: LVNC



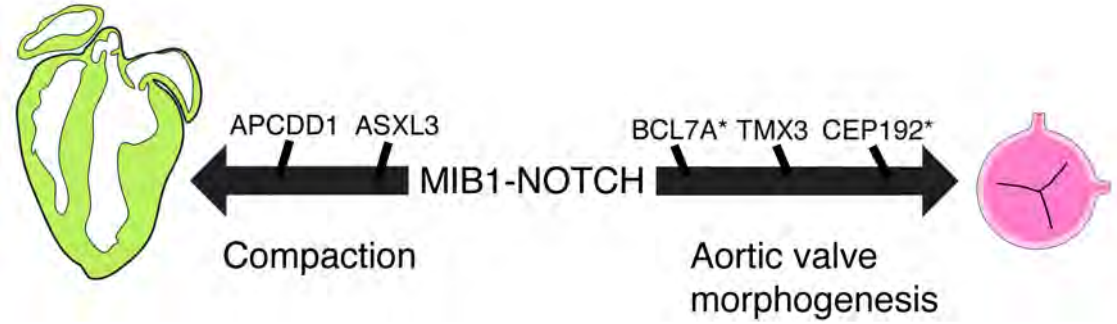
Mib1$^{V943F/+}$ triple or quadruple mutant combinations: BAV and associated defects





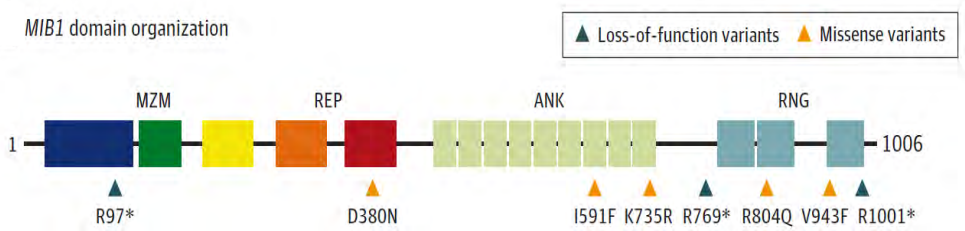


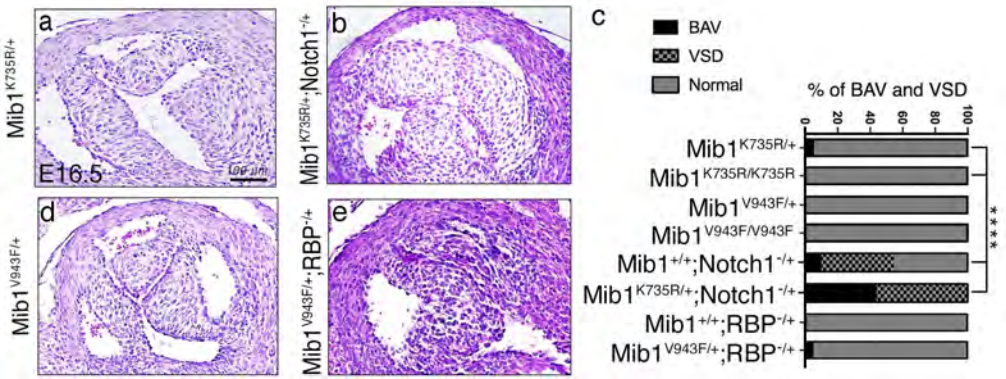
Direct interaction between CEP192, BCL7A and N1ICD



Siguero-Álvarez et al (2023). A Human Hereditary Cardiomyopathy Shares a Genetic Substrate With Bicuspid Aortic Valve. *Circulation*

Mutations in *MIB1* cause human BAV

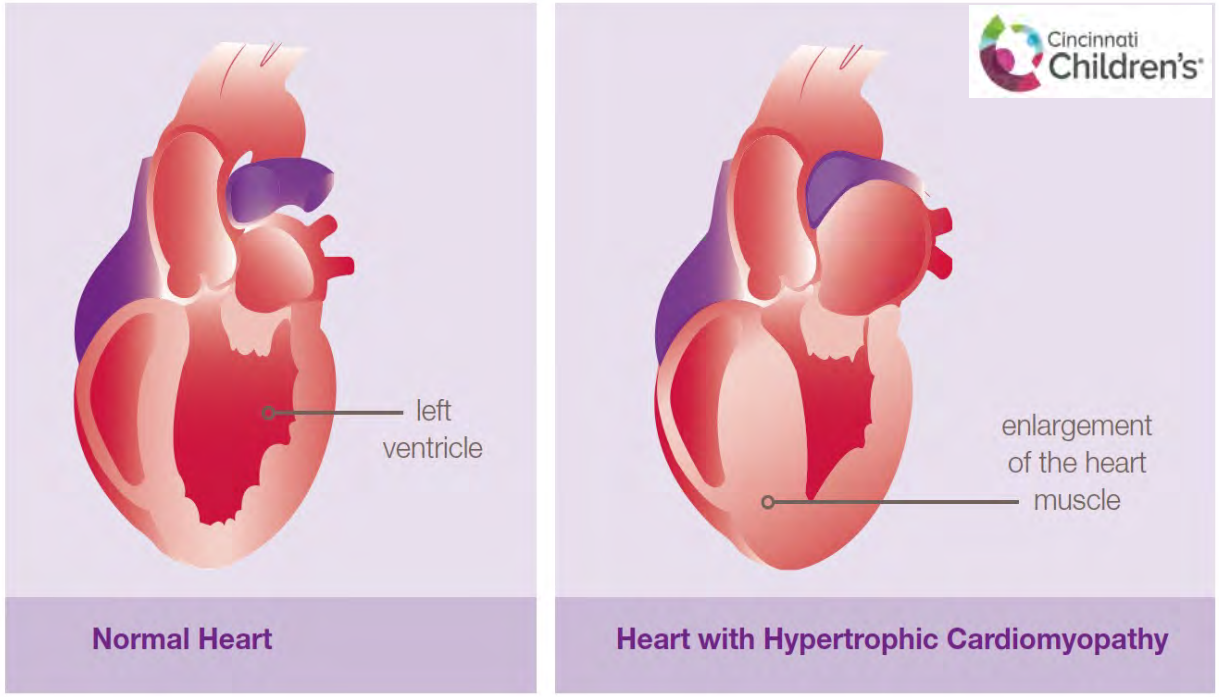




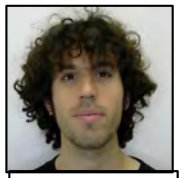
Collaboration with R. Durst (Hadassah Medical School, Jerusalem, ISRAEL)

Tessler et al (2023). Novel Association of the NOTCH Pathway Regulator *MIB1* With the Development of Bicuspid Aortic Valve. *JAMA Cardiol*

Hypertrophic Cardiomyopathy (HCM)



- "Sarcomere" disease characterized by thickened ventricles and septum.
- Cardiomyocyte hypertrophy, sarcomere disarray and interstitial fibrosis.
- Ventricular myocardium stiffness.
- Disease can range from asymptomatic to heart failure and sudden cardiac arrest.
- Caused by mutations in sarcomere genes: *MYH7,* ***MYBPC3****, TNNT2, TNNI3, ACTC1*.



Alejandro Salguero

Sarcomere genes (*MYBPC3*) in HCM and LVNC:

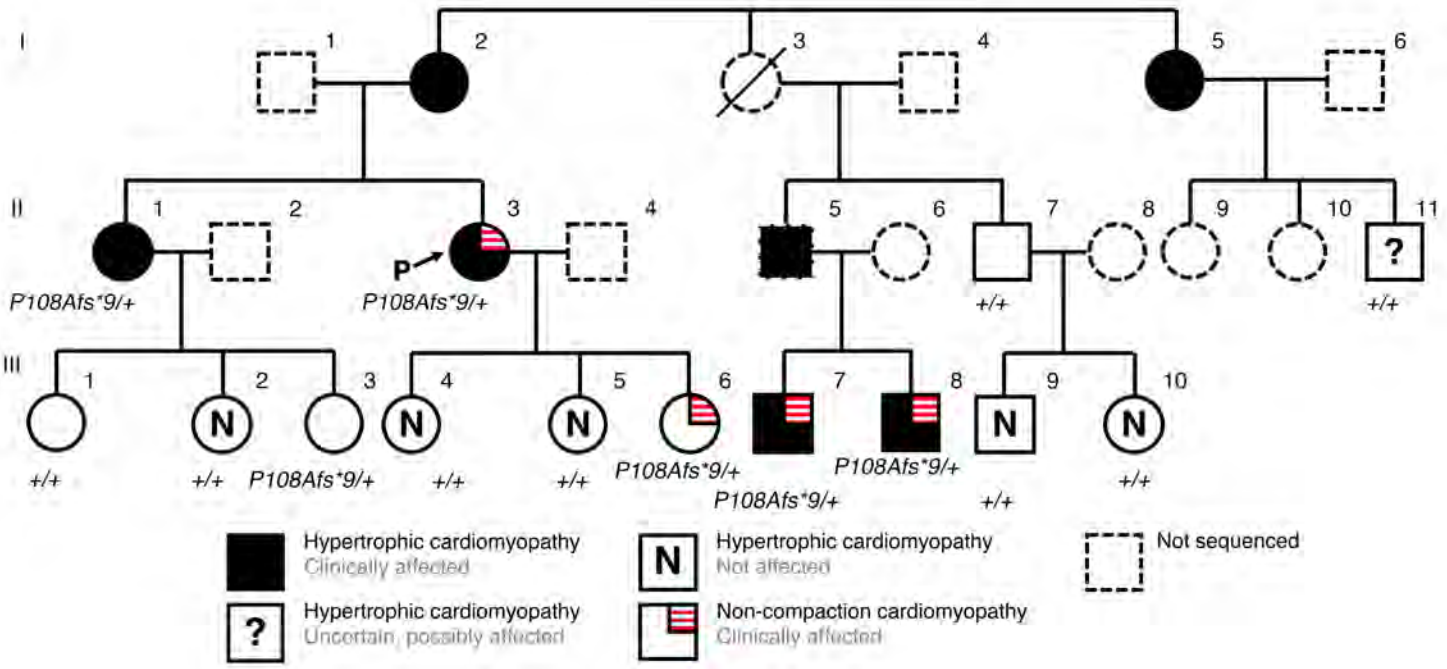
Is there a common genetic & developmental basis?

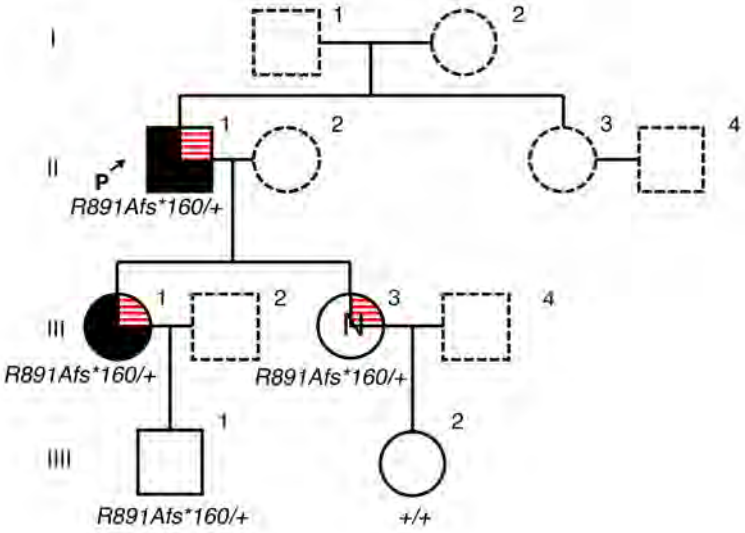
Collaboration with

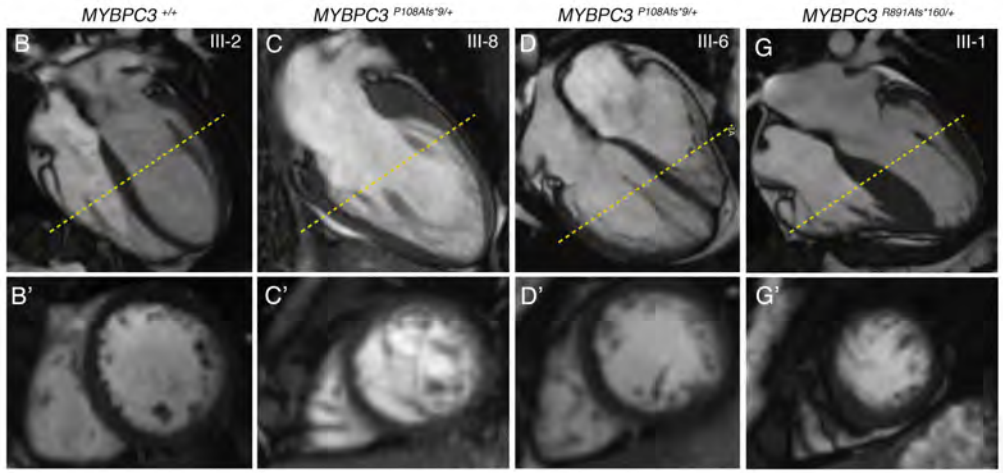
Dr. J R Gimeno-Blanes

(HU Virgen Arrixaca,

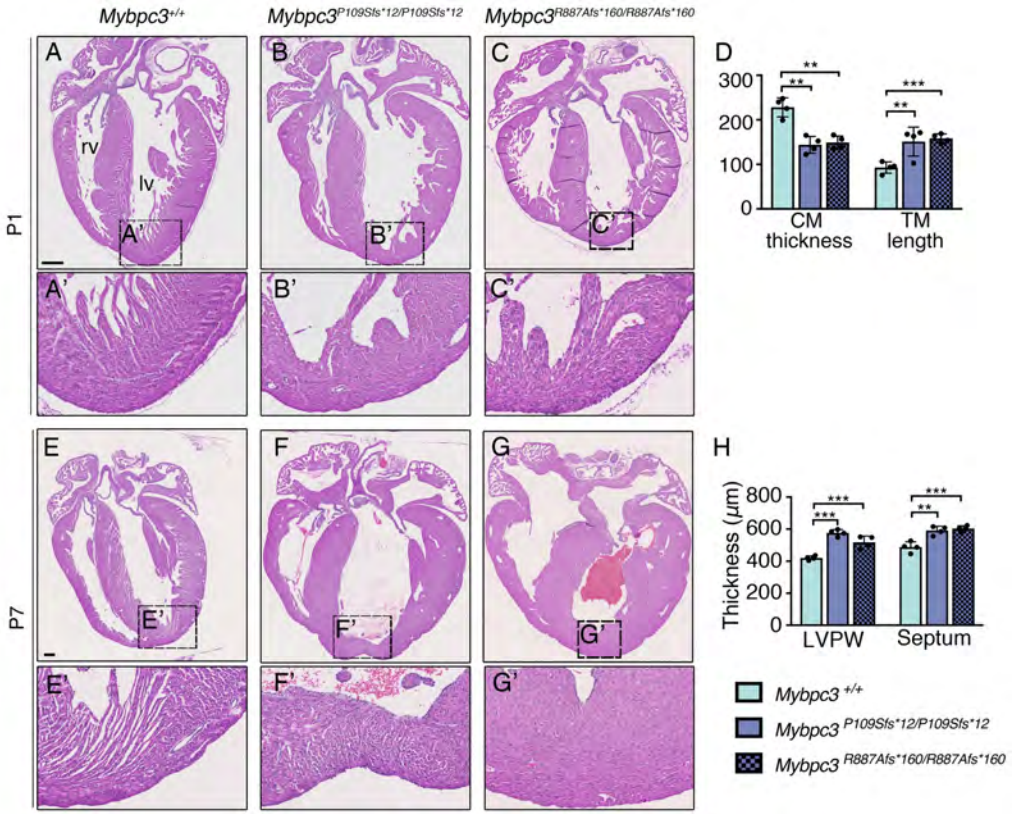
Murcia, Spain



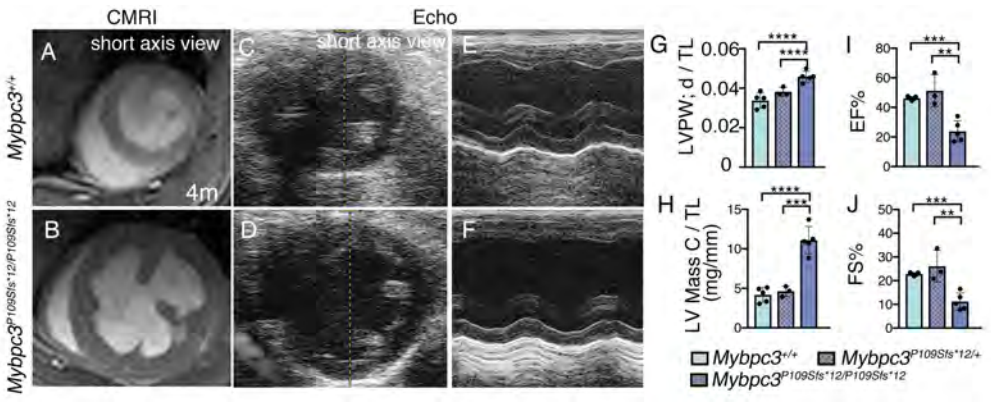




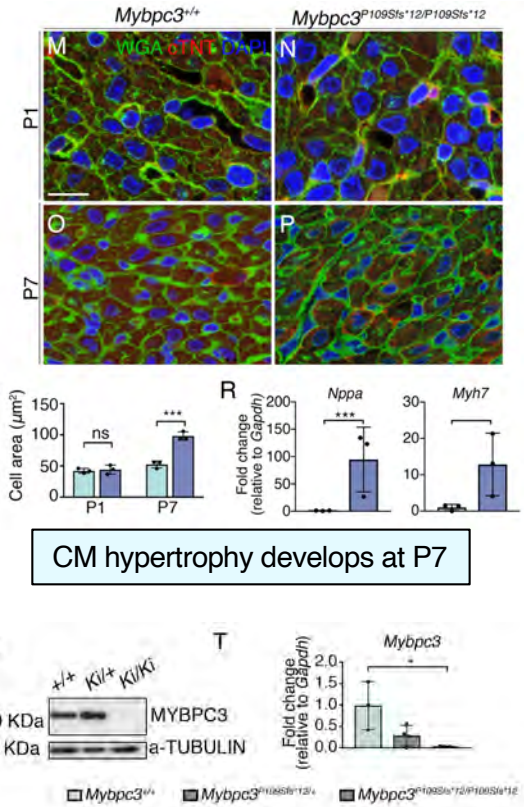
Humanized mouse models: Hypertrabeculation at P1 “evolves” into hypertrophy at P7



Mutants show a transient (E16.5-P6) hypertrabeculation phenotype

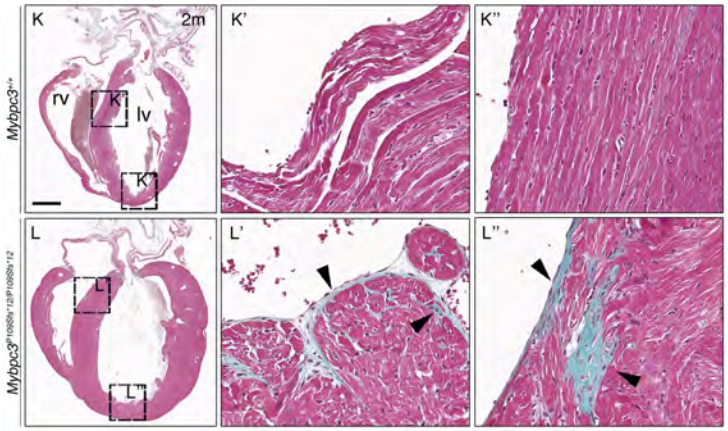


Functional hypertrophy



CM hypertrophy develops at P7

Null alleles



Fibrosis

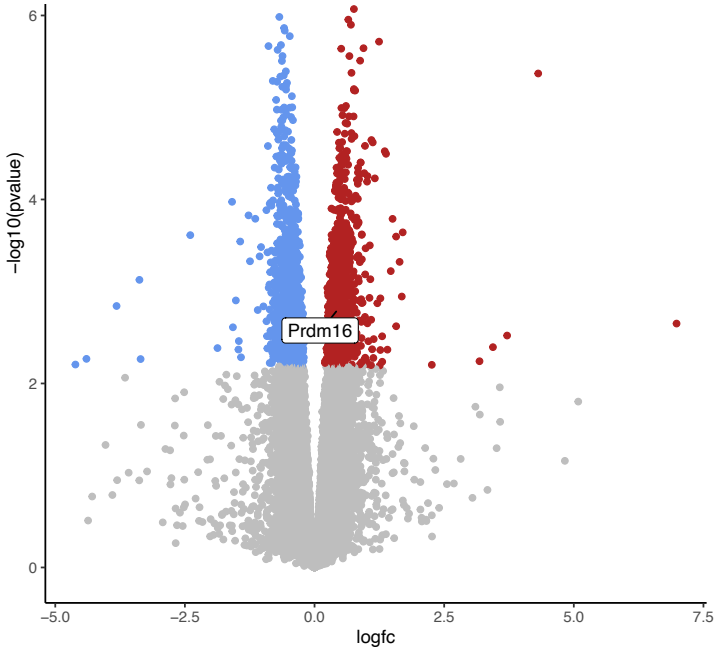
P1

EMT, Cytoskeleton dynamics, TGFβ signalling

G2M, RICTOR, YAP, WNT1

OXPHOS, FA metabolism

MYC

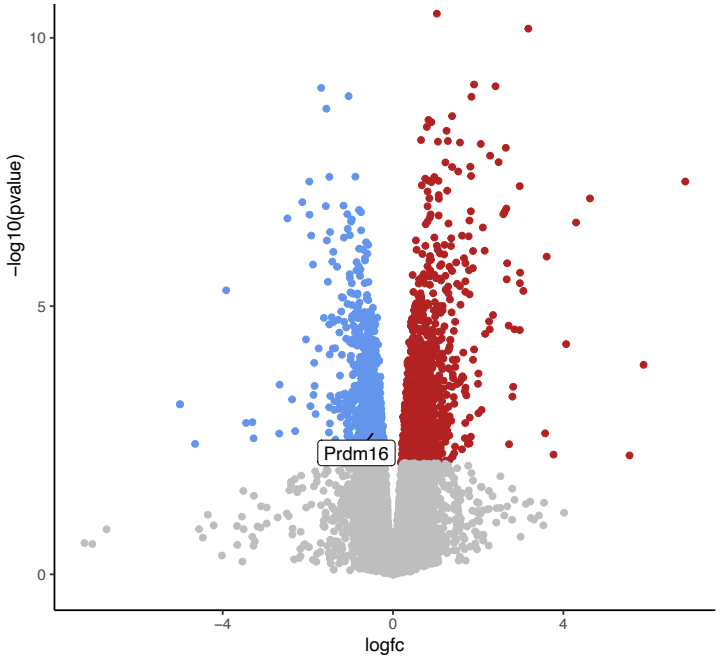


P7

OXPHOS, ROS, EIF2 signalling

G2M, DNA repair, P53

MYC

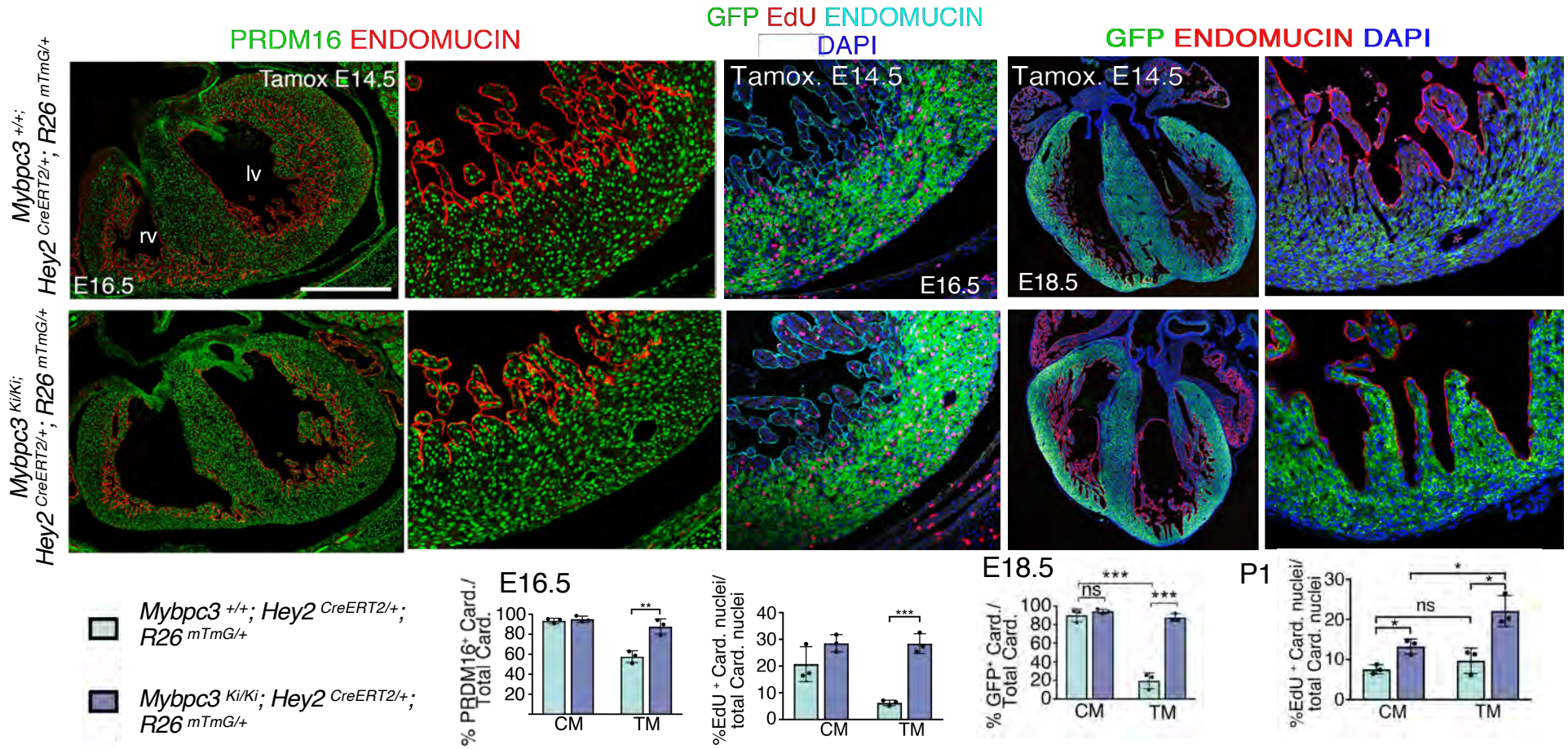


Circulation. 2022;145:586–602. DOI: 10.1161/CIRCULATIONAHA.121.056666

ORIGINAL RESEARCH ARTICLE

PRDM16 Is a Compact Myocardium-Enriched Transcription Factor Required to Maintain Compact Myocardial Cardiomyocyte Identity in Left Ventricle

Tongbin Wu, PhD*; Zhengyu Liang, PhD*; Zengming Zhang, PhD; Canzhao Liu, PhD; Lunfeng Zhang, PhD; Yusu Gu, MS; Kirk L. Peterson, MD; Sylvia M. Evans, PhD; Xiang-Dong Fu, PhD; Ju Chen, PhD



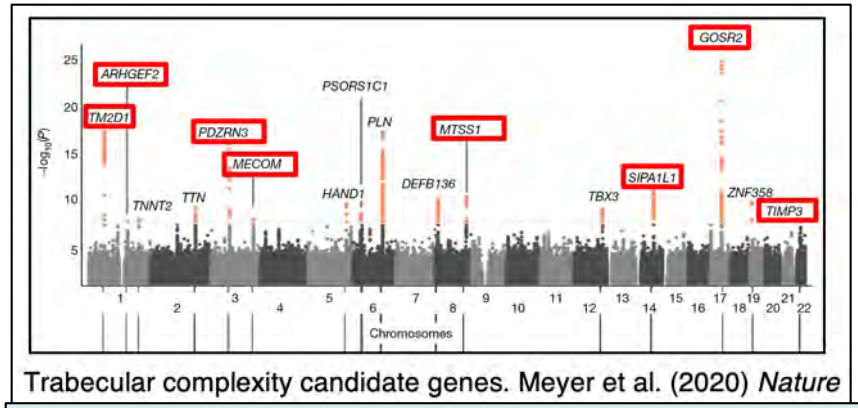
- Prdm16 expansion to trabeculae
- Increased trabecular proliferation (E16.5-P1)
- Abnormal Hey2+ lineage contribution to trabeculae
- Transient hypertrabeculation prior to HCM?
- Common disease mechanism? LVNC?
- Ongoing

HUMAN GENETICS OF TRABECULAR ARCHITECTURE

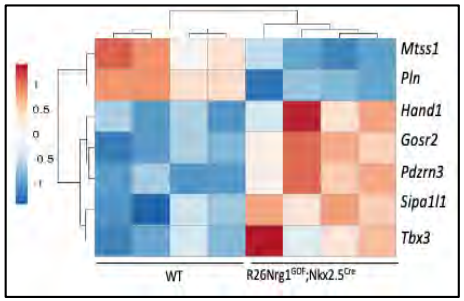
Collaboration with Declan P. O'Regan (MRC, London)

Meyer… and O'Regan (2020) *Nature*.

GWAS of 18,096 UK Biobank samples with MRI imaged trabecular complexity phenotype found several loci associated to CVD.



Trabecular complexity candidate genes. Meyer et al. (2020) *Nature*

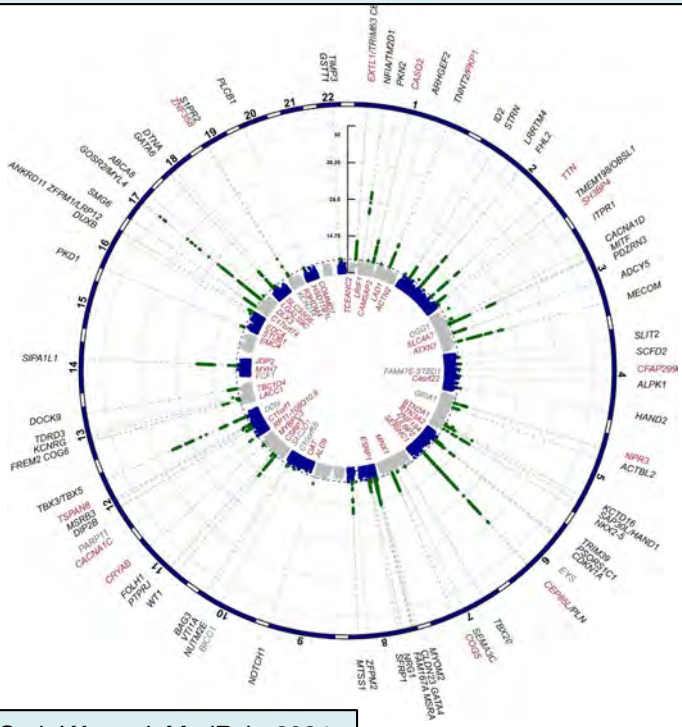


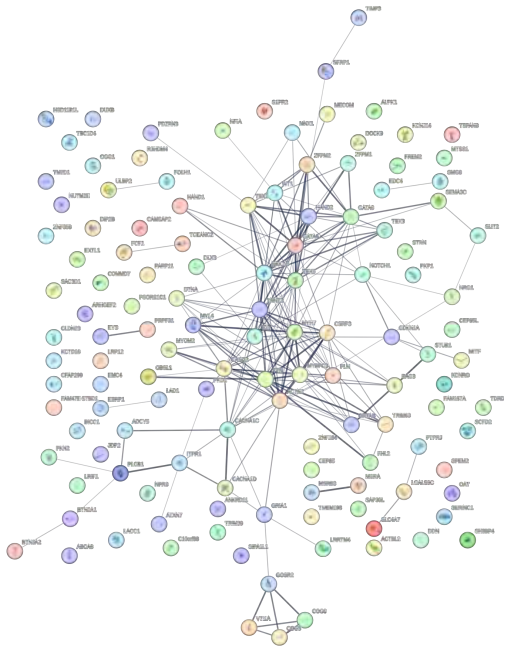
Grego-Bessa et al. (2023) *Circ Res*

Marcos Siguero

Deregulated gene expression in mice with premature VCS differentiation

GWAS + RVAS of 47,803 UK Biobank samples found 56 genes regulating ventricular and VCS development, extending the previously identified candidate loci to 68.

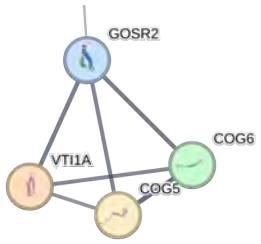




Cristina Roy

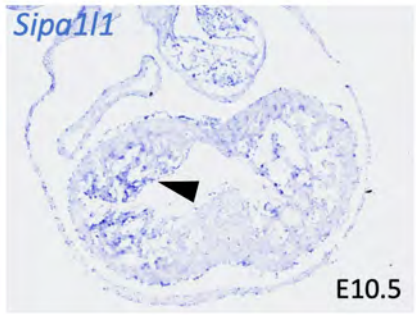
Javier Santos

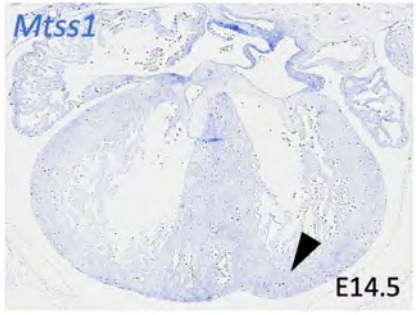
Violeta Sebastián

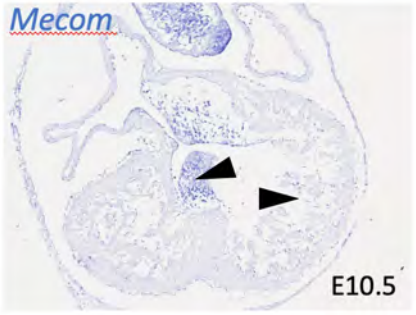


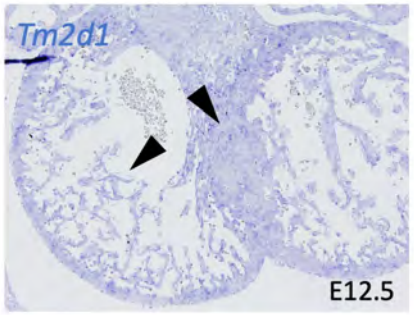
McGurk KA et *al. MedRxiv.* 2024

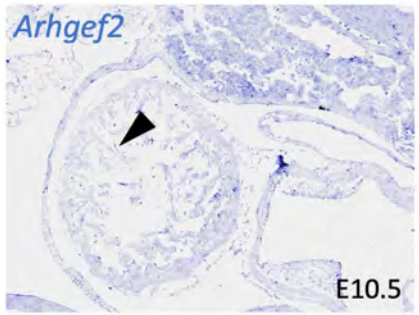
Expression pattern suggests involvement in cardiac development

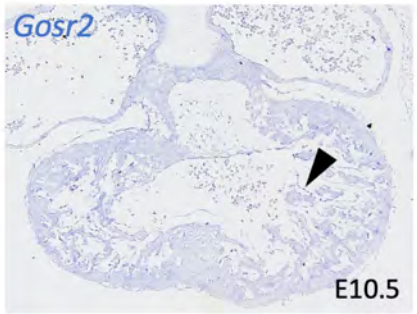


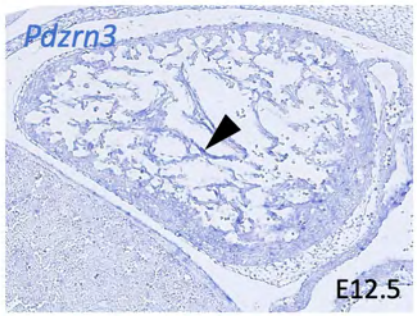












New mouse lines

Gene	Strategy
Pdzrn3	Standard KO
	Conditional KO
Mecom	Standard KO
	Conditional KO
Mtss1	Standard KO
Tm2d1	Standard KO
	Conditional KO
Arhgef2	Standard KO
Sipa1l1	Standard KO
Gosr2	Standard KO

Ongoing work…

Summary:

- Nrg1-ErbB2/4 signaling regulates CM cytoskeletal dynamics (EMT-like), proliferation and maturation during ventricular wall development, acting in part via YAP regulation.
- MIB1-NOTCH signaling is required for both human and mouse chamber and valve development.
- During compaction MIB1-NOTCH signaling is required for cardiomyocyte maturation.
- Ventricular chamber and valve development share a genetic basis (*MIB1-NOTCH*) influenced by modifier genes (ie: *ASXL3, APCDD1, CEP192, TMX3* and *BCL7A*). Combined heterozygous disruption of these genes causes cardiomyopathy and/or BAV. Dominant phenotypic manifestation.
- Defective NOTCH signaling leads to CHD: BAV and LVNC.
- HCM caused by *Mybpc3* nonsense and missense mutations in mice occurs through a transient postnatal hypertrabeculation phase and impaired CM maturation involving Prdm16. Ongoing…

Open questions:

- Does the embryonic mouse heart show the same complexity than the embryonic human heart?
- Is this relevant for cardiomyopathy or CHD?
- Are the newly identified genes (GWAS+RVAS) relevant for ventricular development and/or cardiomyopathy?

Thanks to…

Juan Ramón Gimeno-Blanes
María Sabater Molina
IMIB-HU Arrixaca, Murcia, Spain

Ronen Durst
Idit Tessler
Hadassah Med. School
Jerusalem, Israel

Declan P. O'Regan
MRC London, UK

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Fátima Sánchez-Cabo
Carlos Torroja
Manuel J. Gómez
Jorge de la Barrera
Bioinformatics Unit
CNIC

Other CNIC Units:
Transgenesis,
Genomics,
Pluripotent Cells
Technology,
Advanced Imaging,
Microscopy

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Cofinanciado por la Unión Europea

AGENCIA ESTATAL DE INVESTIGACIÓN

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Enfermedades Cardiovasculares