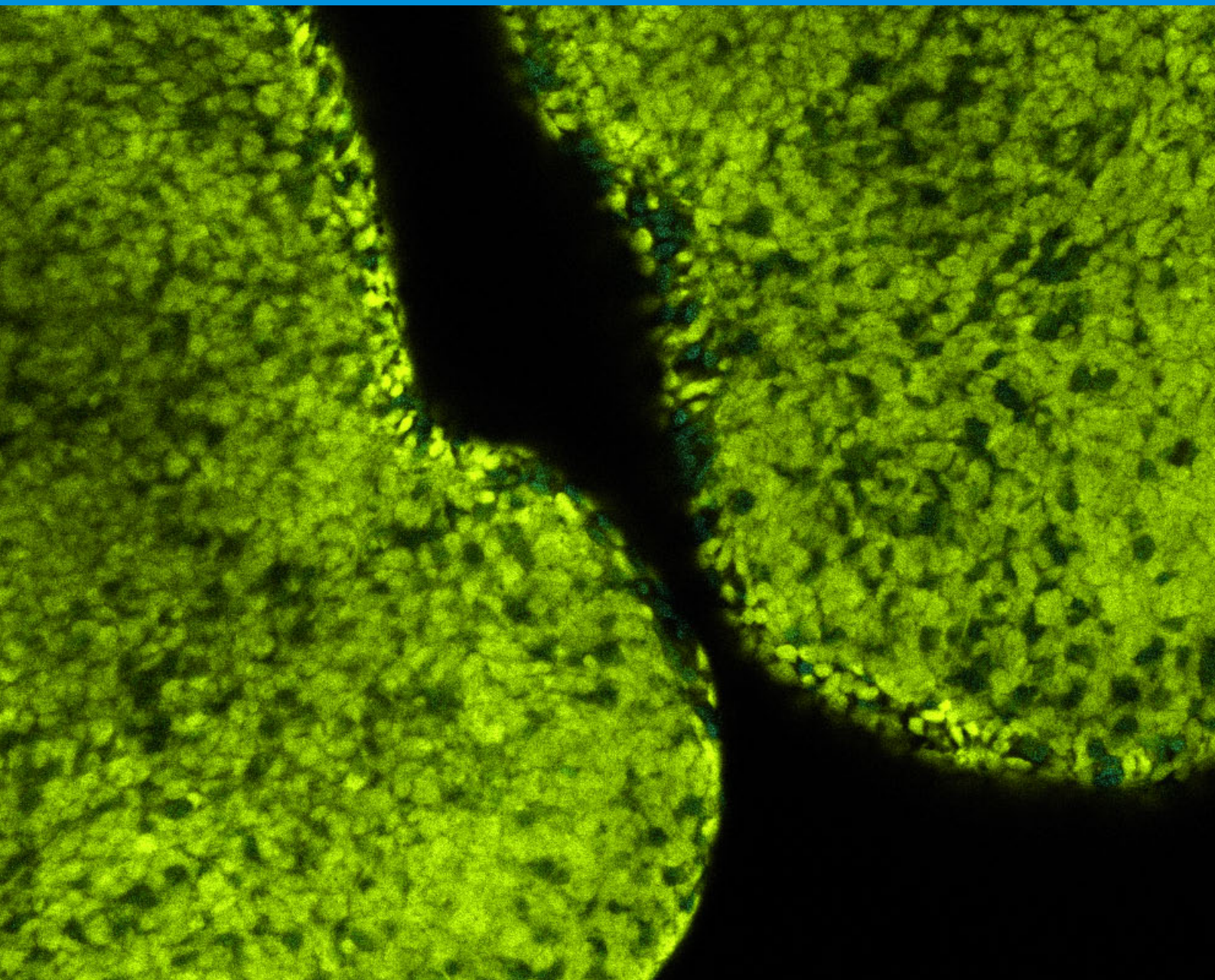


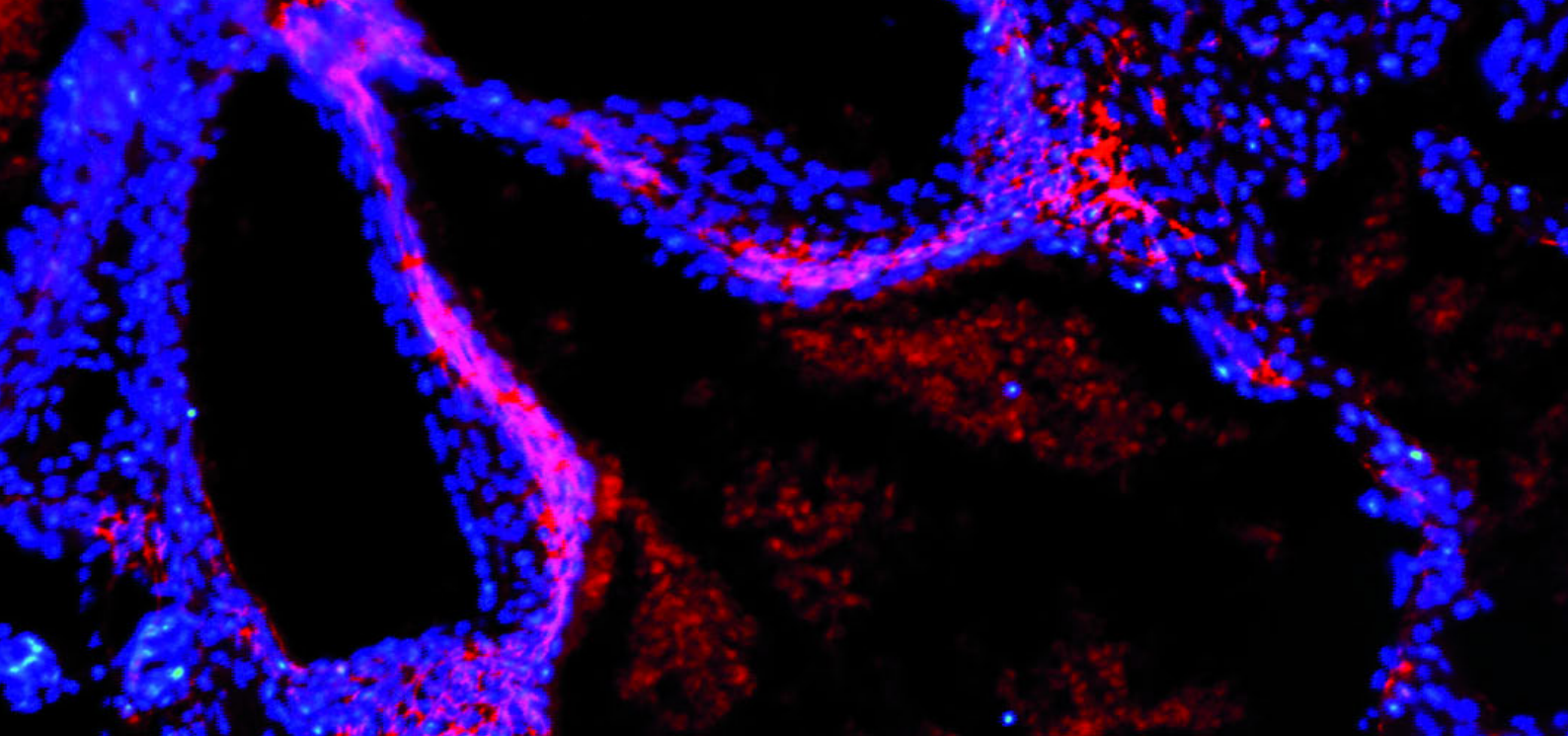
Basic Research Departments

Basic Research Departments



Cardiovascular Developmental Biology





Basic Research Departments

1 Cardiovascular Developmental Biology

Research in the CDB department is structured into three strategic areas:

- the early steps in the establishment of the embryonic lineages,
- the origin, differentiation and patterning of cardiovascular cell lineages,
- the integration of signaling pathways in cardiac development, homeostasis and disease.

These processes are studied in chick, mouse and zebrafish animal models through a variety of complementary experimental approaches including cell biology, imaging, global gene expression analysis and biochemistry.

DEPARTMENT DIRECTOR: *Miguel Torres*

DEPARTMENT MANAGEMENT: *Beatriz Ferreiro*
Ángel Ciprés

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ADMINISTRATIVE SUPPORT: *Sandra Cillero*

*Genetic control of organ
development and regeneration*

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Research Scientists: *Nadia Mercader
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Postdoctoral Researchers: *Cristina Clavería
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Predocctoral Researchers: *Juan Manuel González-Rosa
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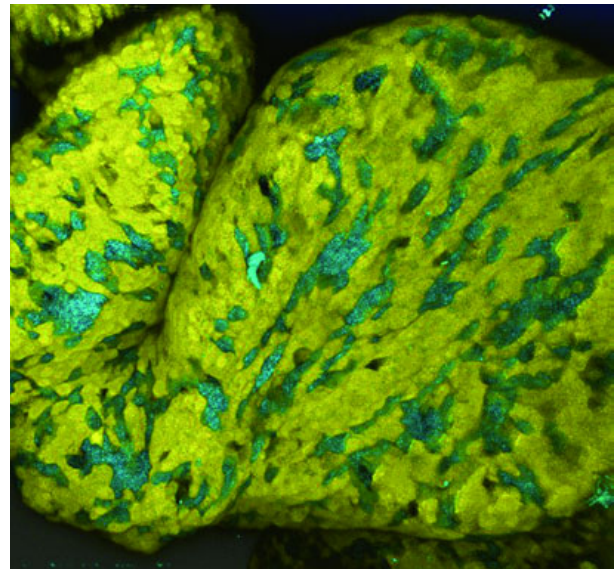
Technicians: *Joana Fuentes
Rocío Sierra
Susana Temiño*

Visiting Scientist: *Clara García-Andrés*

**RESEARCH INTEREST**

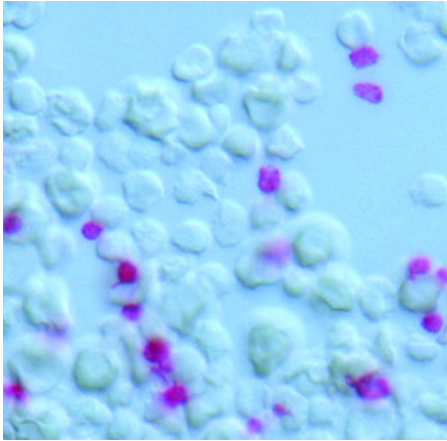
We aim to understand the cellular basis of cardiovascular development, homeostasis and regeneration. A main focus of the laboratory is the role of regionally-expressed transcription factors in cardiovascular development. In this area, we have generated gain- and loss-of-function mouse models of the homeodomain transcription factors Meis and Pbx. This work has revealed new roles for these factors in cardiovascular development, and also demonstrated the involvement of platelets in lymphangiogenesis, which suggests a general role of this blood lineage in vascular morphogenesis and remodeling that might be relevant to vascular disease. We have also developed new genetic mosaic mouse models that allow in vivo clonal analysis and random mosaic gene manipulation. These approaches are being used to investigate cell lineage relationships and topology during cardiovascular development and to explore the role of cell competition in the mouse embryo.

Our work on heart regeneration concentrates on the epicardium, which is the outermost layer of the vertebrate heart and plays an important role during cardiac development as a source of progenitor cells and signals controlling myocardial proliferation. Recently a role for the epicardium has been suggested during regeneration, but its exact function in this setting is still unknown. Using the zebrafish model we are analyzing the formation of the epicardium in vivo and studying the fate of epicardially derived cells and their role during cardiac regeneration.

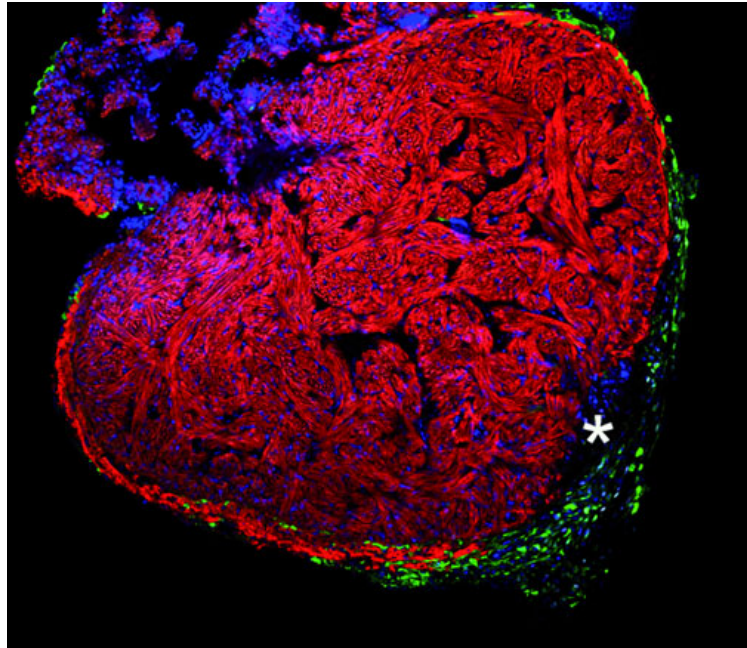


Amira 3D reconstruction compiled from a confocal Z-stack of a random mosaic E9.5 heart. The image shows a ventral view of the heart tube, encompassing the outflow tract and the right and left ventricles. The cell distribution in the mosaic reveals the regional tissue deformation occurring during heart morphogenesis

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Circulating platelets in the mid-gestation mouse embryo revealed by staining for CD41



Regenerating adult zebrafish heart, with a newly-formed epicardial layer (green cells) covering the injured area (asterisk)



MAJOR GRANTS

- COST – European Cooperation in the field of Scientific and Technical Research (EU RTD FP7, Ref. BM0805) PI and Action Chair: M.Torres
- Ministerio de Ciencia e Innovación. FIS. RETICS (TERCEL: RD06/0010/0008). PI: M.Torres
- Ministerio de Ciencia e Innovación (BFU2009-08331). PI: M.Torres
- Ministerio de Ciencia e Innovación (BFU2008-00212/BMC). PI: N.Mercader
- Ministerio de Ciencia e Innovación. (RYC-2006-001694). PI: N.Mercader
- Ministerio de Ciencia e Innovación. FIS (CP09/00100). PI S. Martin Puig
- Comunidad de Madrid (CM S-SAL0190-2006). PI: M.Torres
- EU Marie Curie (FP7-IEF-GA-2009-251226). PI: R. Costa.



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García-Andrés C, Torres M. Comparative expression pattern analysis of the highly conserved chemokines SDF1 and CXCL14 during amniote embryonic development. *Dev Dyn* (2010) 239: 2769-77

Rosello CA, Torres M. Gene transfer by electroporation into hemogenic endothelium in the avian embryo. *Dev Dyn* (2010) 239: 1748-54

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González-Rosa JM, Padrón-Barthe L, Torres M, Mercader N. Lineage tracing of epicardial cells during development and regeneration. *Rev Esp Cardiol* (2010) 63: 36-48

*Intercellular signaling
in cardiovascular development and disease*

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Postdoctoral Researchers: *Jesús Chamorro
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Donal Macgrogan
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Álvaro González
Guillermo Luxán
Juliane Münch*

Technicians: *Vanesa Bou
Ana Cabrero
Eva García
Patricia Martínez*

Visiting Scientist: *José María Pérez-Pomares*

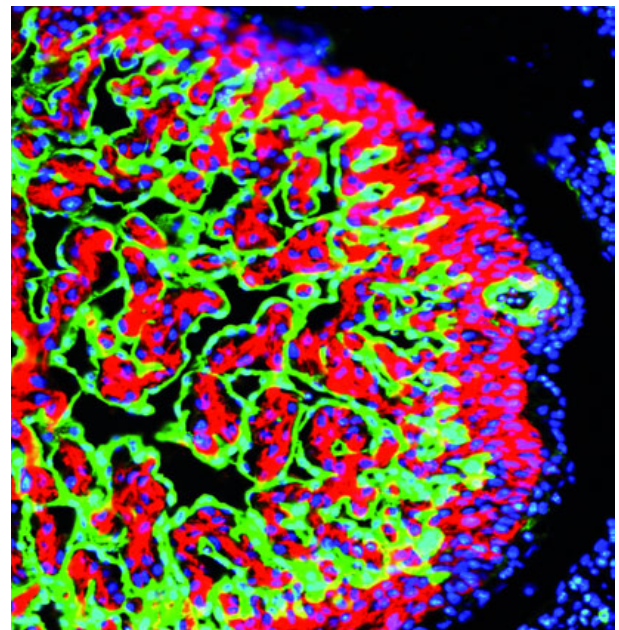
**RESEARCH INTEREST**

We are interested in the signaling mechanisms that regulate cardiac development and homeostasis and how these may be altered in the diseased heart. During the last year our efforts have centered on the role of Notch signaling in the development of the epicardium and coronary vasculature, the development and function of the heart valves and chambers, and the implication of Notch in zebrafish heart and fin regeneration.

The epicardium, the epithelial covering of the heart, is involved in coronary vessel and cardiac interstitium development and in myocardial growth and maturation. We have found that Notch inactivation in the epicardium impairs coronary artery differentiation and severely reduces ventricular myocardium thickness. We also study *mind bomb1 (Mib1)*, which encodes a ubiquitin ligase essential for Notch signaling. *Mib1* inactivation in cardiac endothelium and myocardium causes valve prolapse and impairs ventricle development. We are currently generating new mouse models to manipulate the expression of other molecules that interact with Notch in the embryonic or adult heart.

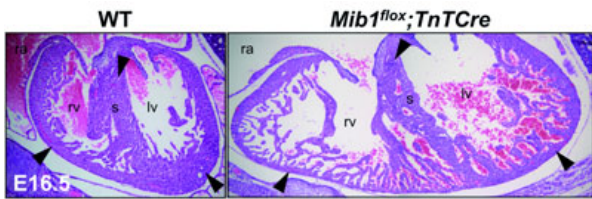
We have established an *in vitro* model of aortic valve disease using porcine valve cells, in which we can inhibit Notch signaling. Notch inhibition downregulates the Notch target *Hey1* and concomitantly upregulates the osteogenic signal *Bmp2*, suggesting that Notch represses osteoblast differentiation in the healthy valve. This approach is complemented by work with double *ApoE*- and *Notch*-deficient mice, in which we study the combined action of endothelial dysfunction and Notch deficiency in valve disease. We are also analyzing the correlation between NOTCH pathway alterations and disease severity in samples from valve disease patients.

The role of Notch in heart regeneration is being studied in the zebrafish ventricular ablation model. Notch is reactivated after cardiac damage and its activity is sustained throughout the repair process. Ectopic Notch activation impairs cardiac repair, and we are studying whether defective epicardial signaling is involved.

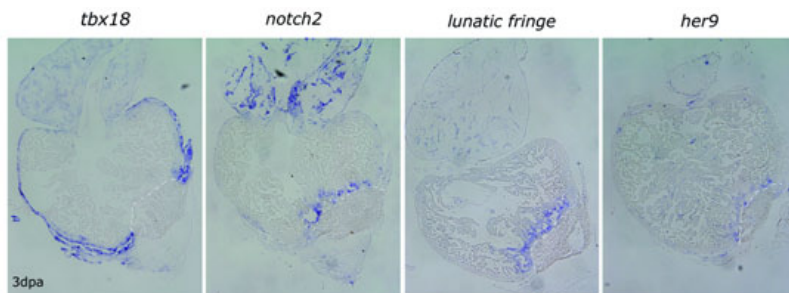


CD31/α-SMA staining in a transverse section of the left ventricle of an E13.5 WT1;N1^{lox} mutant mouse embryo, showing a fistula formed by endothelium surrounded by SMCs. CD31 (green), α-SMA (red), DAPI (blue).

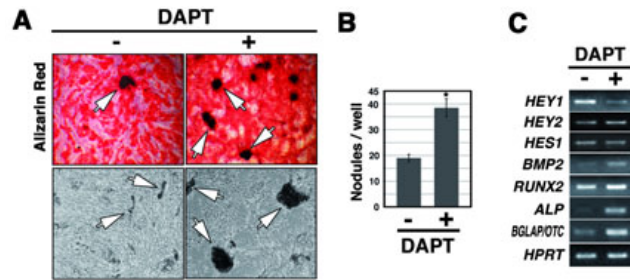
1 Cardiovascular Developmental Biology



Cross-section of the heart at E16.5. Left, wild type; right, Mib1; cTnT-Cre mutant. Note the reduction in compact zone myocardium (arrowheads) and the increased ventricular trabeculation. lv, left ventricle; s, septum; rv, right ventricle; ra, right atrium.



Expression of Notch signaling elements (blue) in the regenerating zebrafish heart. The dotted line delineates the amputation plane.



Notch inactivation leads to decreased Hey1 expression in porcine aortic valve cells, activation of osteoblast-specific genes and increased calcification in vitro. (A) Alizarin Red staining and DIC images of cultured porcine aortic valve interstitial cells showing calcification foci induced by the Notch inhibitor DAPT (arrows). (B) Quantification shows a two-fold increase in calcification foci in Notch-inhibited cells. (C) RT-PCR analysis showing reduced Hey1 expression and increased osteogenic gene expression in Notch-inhibited cells.



MAJOR GRANTS

- European Commission FP6 (LSHM-CT-2005-018630)
- European Commission FP7, Initial Training Network (215761)
- Ministerio de Ciencia e Innovación (SAF 2007-62445)
- Ministerio de Ciencia e Innovación. FIS RETICS (Recava II: RD06/0014/0038)
- Ministerio de Ciencia e Innovación. FIS RETICS (TERCEL: RD06/0010/1013)
- Ministerio de Ciencia e Innovación. FIS (CD08/00257). PI: B. Prados
- Ministerio de Ciencia e Innovación. FIS (CD09/00452). PI: M. Nus
- Comunidad de Madrid (P-2006/BIO-194). PI and coordinator of the five groups of the Network: J.L. de la Pompa
- Centro Nacional de Investigaciones Cardiovasculares (FPIT CNIC-09)
- Fundació La Marató de TV3 (081731)
- Junta de Castilla y León, Grupos de Excelencia (GR-176)



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MacGrogan D, Nus M, de la Pompa JL. **Notch signaling in cardiac development and disease.** *Curr Top Dev Biol* (2010) 92: 333-365.

Grego-Bessa J, Pérez-Pomares JM, de la Pompa JL. **Signalling pathways in valve formation - origins of congenital defects.** In: Rosenthal N. and Harvey R. (Eds) *Heart Development and Regeneration* (2010) 2nd Ed. New York: Academic Press, 389-413.

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Martínez-Poveda B, Verotta L, Bombardelli E, Quesada AR, Medina MA. **Tetrahydrohyperforin and octahydrohyperforin are two new potent inhibitors of angiogenesis.** *PLoS One* (2010) 5: e9558

*Stem cells in organ generation,
regeneration and aging*

Head of Laboratory: *Ignacio Flores*

Postdoctoral Researcher: *Tania Aguado*

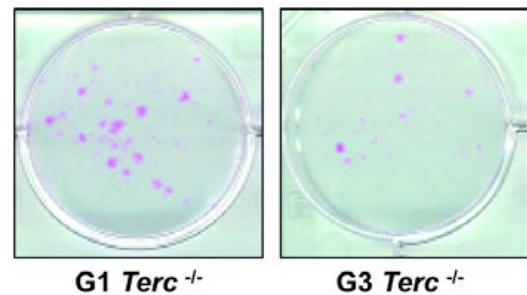
Predoctoral Researchers: *Esther Aix*
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Technician: *Irene de Diego*

**RESEARCH INTEREST**

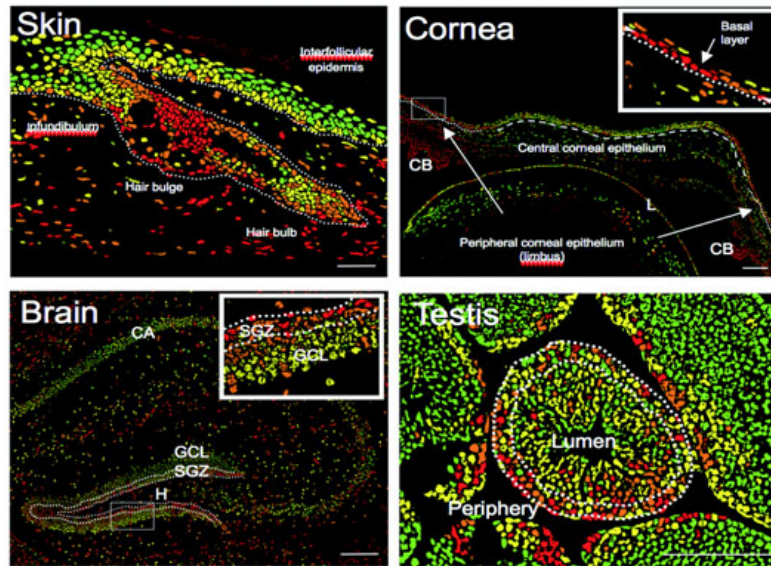
How an organ develops and persists during adult life is a fundamental question in biology. One hypothesis of organ maintenance is that stem cell functionality determines the ability of tissues to replace worn-out or injured parts. However, for most tissues the nature of the organ-forming cells and stem cells is poorly defined. We recently showed that within tissues there is a gradient in the length of telomeres, the physical chromosome ends. Given that telomeres shorten with each cell division, we hypothesize that the most primitive cells will be those cells harboring the longest telomeres.

We are currently conducting a high-content telomere length analysis to study the location, prevalence and status of putative cardiac stem cells and their progeny during organogenesis and aging. We are also examining the relationship between telomere length and the ability of cardiac cells to generate new cardiac tissue. Finally, we are investigating the factors that regulate telomere length, with the aim of defining their contribution to cell differentiation. Through these approaches, we hope to obtain a more complete picture of the role of stem cells in organ formation and maintenance, which could lead to the development of improved regeneration therapies.



Telomere attrition diminishes the proliferation potential of stem cells ex vivo. Representative images showing the number and size of macroscopic colonies formed by keratinocytes isolated from telomerase-deficient $G1Terc^{-/-}$ mice (relatively long telomeres) and $G3Terc^{-/-}$ mice (relatively short telomeres).

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Cells with the longest telomeres locate to stem cell compartments in mouse tissues. Representative topographic telomere length maps generated from confocal telomere Q-FISH images. Nuclei are pseudo-colored according to their average telomere fluorescence, from the longest telomeres (red) to the shortest (green).



MAJOR GRANTS

- Ministerio de Ciencia e Innovación (SAF2009-10480)
- Ministerio de Ciencia e Innovación (RYC-2006-3067)
- Asociación Española contra el Cáncer. AECC (2009). PI: T. Aguado



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Ferrón SR, Marqués-Torrejón MA, Mira H, Flores I, Taylor K, Blasco MA, Fariñas I. Telomere shortening in neural stem cells disrupts neuronal differentiation and neuritogenesis. *J Neurosci* (2009) 29: 14394-407

Flores I, Blasco MA. A p53-dependent response limits epidermal stem cell functionality and organismal size in mice with short telomeres. *PLoS One* (2009) 4: e4934

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Molecular regulation of heart development and disease



Head of Laboratory:

Enrique Lara-Pezzi

Technician:

Marina Mercedes López Oñaleta

Master Student:

Jesús María López Salinero

Visiting Scientist:

María Villalba Otero

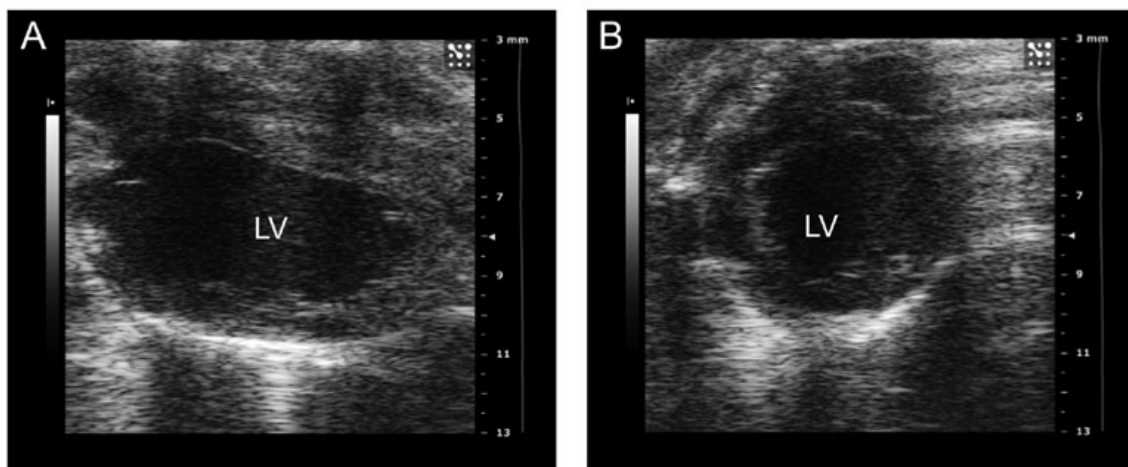


RESEARCH INTEREST

Our lab studies the molecular mechanisms that regulate cardiac development and heart disease. One of our major interests is the role of alternative splicing (AS) in these processes. AS is the molecular process that removes introns from immature pre-mRNAs and links exons together in different combinations. AS affects 86% of all human genes and is in part responsible for the great diversity of proteins that are generated from the relatively small number of genes found in the human genome.

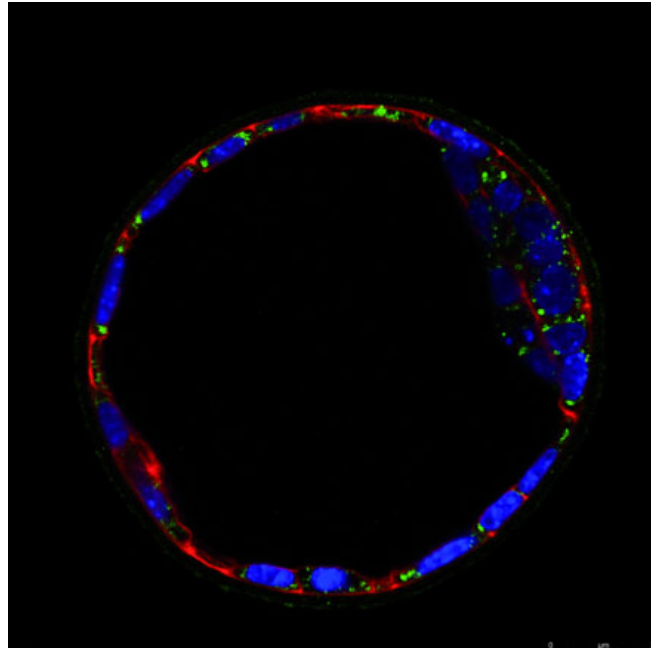
Together with the Genomics and Bioinformatics Units at the CNIC, we have used high density exon microarrays and RNA-Seq to create a global map of AS isoforms expressed during heart failure. This map has enabled us to identify cis-regulatory sequences and trans-regulatory splicing factors associated with AS, and we are analyzing the roles of these factors in the heart through knockdown and knockout strategies.

A prime example of how alternative splicing can dramatically change protein function is the calcineurin variant CnA β 1. Calcineurin regulates a wide variety of physiological and pathological processes, including cardiac development and hypertrophy. CnA β 1 is a naturally occurring splice variant of the calcineurin A β gene which contains a unique C-terminal region, different from the autoinhibitory domain present in all other CnA isoforms. We previously showed that CnA β 1 regulates cell proliferation and enhances skeletal muscle regeneration. Our recent results show that CnA β 1 protects the heart from the effects of myocardial infarction by improving cardiac function and reducing inflammation and scar formation. We are now exploring the role of CnA β 1 in stem cells and in the developing embryo, where it is strongly expressed.



Ultrasound analysis of an infarcted mouse heart. The figure shows short-axis (A) and long-axis (B) views of the heart of a CnA β 1 transgenic mouse 28 days after the induction of myocardial infarction by permanent ligation of the left coronary artery. LV, left ventricle. Images were obtained in diastole.

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CnA β 1 distribution in the early embryo. The image shows a confocal immunofluorescence image of a mouse blastocyst stained with an anti-CnA β 1 antibody (green). Cell membrane is stained with rhodamine-labeled phalloidin (red) and nuclei are counterstained with DAPI (blue).



MAJOR GRANTS

- European Commission FP7. Marie Curie European Reintegration Grant (239158)
- Ministerio de Ciencia e Innovación (BFU2009-20016)
- Ministerio de Ciencia e Innovación. FIS (CP08/00144)
- British Heart Foundation (PG/08/084/25827). co-PI, E. Lara-Pezzi. Funds held at Imperial College London, UK
- British Heart Foundation (PG/07/020/22503). co-PI, E. Lara-Pezzi. Funds held at Imperial College London, UK



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Functional genomics of embryonic pluripotency and heart development

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Teresa Rayón

Masters Student: Melisa Gómez

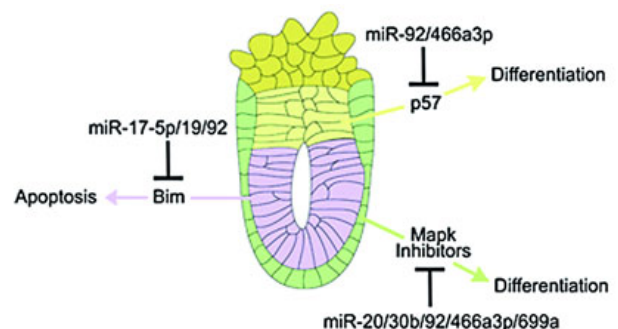


RESEARCH INTEREST

The central aim of our research is to understand how genome activity is regulated during development, and how this can contribute to human disease. For our approach, we screen for and identify distal acting cis-regulatory sequences, and study how they act on their target genes and how these targets are organized into gene regulatory networks underlying a specific biological state. This work is conducted through a combination of bioinformatics, comparative genomics, genome-wide analysis, and functional assays in transgenic mouse embryos, chick embryos, and stem cells.

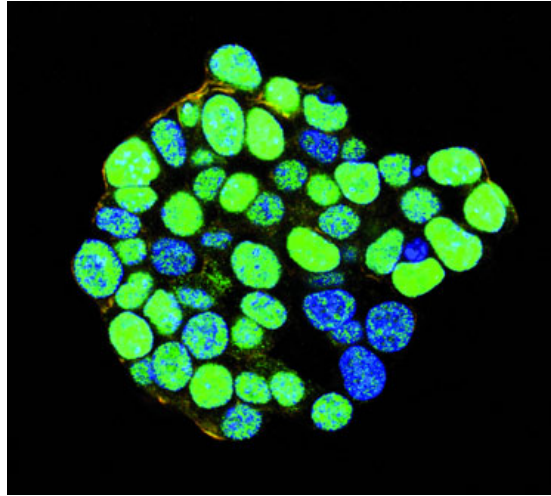
We recently showed that the sustained pluripotency of embryonic cells is an evolutionary novelty in mammals. The core transcription factors that establish embryonic pluripotency in mammals are not expressed in the early chick embryo in a manner compatible with them having this role. Using bioinformatics tools we found that the regulatory elements through which these core factors control their downstream targets are not present in the chick genome, and thus appeared de novo in the mammalian lineage. We have also analyzed the role of miRNAs in the early mouse embryo by using a Dicer loss-of-function model. Using extraembryonic stem cells to characterize phenotypes in detail, we find that the three blastocyst-derived stem cell populations have different requirements for miRNAs. Our study highlights how miRNAs do not have critical patterning or lineage-specification roles in the early embryo, but rather act as modulators of signaling pathways that ensure proper growth and proliferation.

Another area of interest is the role of genomic regions associated with increased risk of human diseases such as diabetes or colorectal cancer. Genome-wide association studies show that many of these associations fall in intergenic regions, and, through a combination of comparative genomics, transgenic assays and studies of chromatin structure, we have found that many of these the risk-associated regions contain cis-regulatory elements. These studies highlight the gene-regulatory basis for many human diseases, and open an important area of research that we will be actively pursuing in the future.



Distinct roles of miRNAs in the early mouse embryo. In the epiblast (purple) miRNAs inhibit the pro-apoptotic factor Bim, thus preventing cell death. In contrast, miRNAs in extraembryonic tissues act to prevent differentiation. In the trophoblast (yellow), this is achieved by inhibition of the cell cycle regulator p57, while in the extraembryonic endoderm (green) miRNAs inhibit negative regulators of the Mapk signaling pathway.

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A colony of mouse ES cells showing the expression of the pluripotency factor Oct4 (green). Nuclei are counterstained in blue.



MAJOR GRANTS

- European Commission FP7. EuroSyStems (200720)
- Ministerio de Ciencia e Innovación (BFU2008-00838)
- Ministerio de Ciencia e Innovación. CONSOLIDER Project (CSD2007-0008)
- Ministerio de Ciencia e Innovación (JCI-2008-2980). PI: C. Arias
- Centro Nacional de Investigaciones Cardiovasculares. CNIC-08-2009. PI and coordinator of four groups: M. Manzanares



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Ragvin A, Moro E, Fredman D, Navratilova P, Drivenes O, Engström PG, [Alonso ME](#), de la Calle Mustienes E, Gómez-Skarmeta JL, J Tavares MJ, Casares F, [Manzanares M](#), van Heyningen V, Molven A, Njølstad PR, Argenton F, Lenhard B, Becker TS. **Long-range gene regulation links genomic type 2 diabetes and obesity risk regions to *HHEX*, *SOX4* and *IRX3*.** *Proc Natl Acad Sci U S A* (2010) 107:775-80

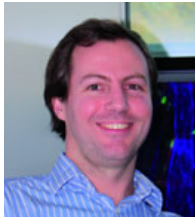
[Pernaute B](#), [Cañon S](#), Crespo M, [Fernandez-Tresguerres B](#), Rayon T, [Manzanares M](#). **Comparison of extraembryonic expression of *Eomes* and *Cdx2* in pre-gastrulation chick and mouse embryo unveil regulatory changes along evolution.** *Dev Dyn* (2010) 239: 620-9

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#Joint 1st authors; *Corresponding authors

Pittman AM, Naranjo S, Jalava SE, Twiss P, Ma Y, Olver B, Lloyd A, Vijaykrishnan J, Qureshi M, Broderick P, van Wezel T, Morreau H, Tuupanen S, Aaltonen LA, [Alonso ME](#), [Manzanares M](#), Gavilán A, Visakorpi T, Gómez-Skarmeta JL, Houlston RS. **Allelic variation at the 8q23.3 colorectal cancer risk locus functions as a cis-acting regulator of *EIF3H*.** *PLoS Genetics* (2010) 6: e1001126. (Comment in *Cell* 2010: 143, 179)

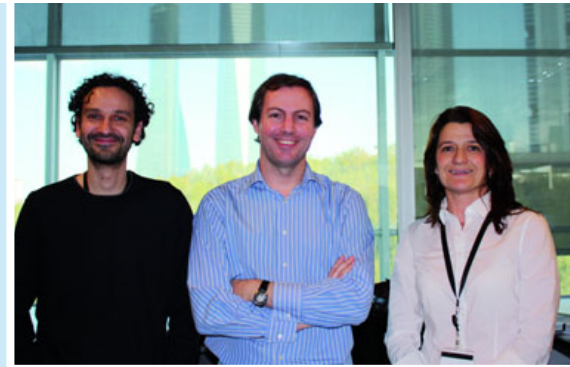
[Fernandez-Tresguerres B](#), [Cañon S](#), [Pernaute B](#), Rayon T, Crespo M, Torroja C, [Manzanares M](#). **Evolution of the mammalian embryonic pluripotency gene regulatory network.** *Proc Natl Acad Sci U S A* (2010) 107: 19955-60. (Comments in *PNAS* 2010: 107, 19606; *Nat Rev Genet* 2010: 11, 818)

Stem cell niche pathophysiology

Head of Laboratory: *Simón Méndez Ferrer*

Postdoctoral Researcher: *Joan Isern Marín*

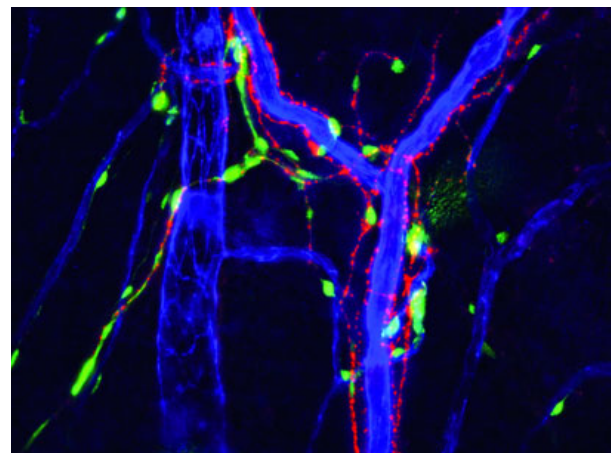
Technician: *Ana María Martín de Ana*

**RESEARCH INTEREST**

Stem cells reside in specialized niches that allow them to self-renew, proliferate, differentiate and migrate according to the organism's requirements. Our recently created group studies the mechanisms by which the stem cell niche fulfils these complex functions and how its deregulation contributes to disease.

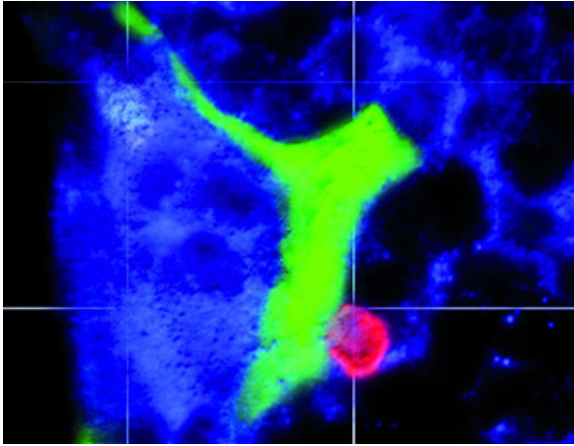
Our earlier work described a tight regulation of the bone marrow stem cell niche by circadian oscillations of sympathetic activity. Light onset induces noradrenaline release from bone marrow varicosities, leading to rapid downregulation of CXCL12/SDF-1, the only chemokine known to direct hematopoietic stem cell (HSC) migration. Our recent studies indicate that the stromal cells targeted by the sympathetic nervous system and that regulate this HSC traffic are peri-vascular nestin⁺ cells. These cells are true niche cells: they colocalize with HSCs, express high levels of core HSC maintenance genes, selectively downregulate these genes during HSC mobilization by granulocyte colony-stimulating factor (G-CSF) or β_3 -adrenergic stimulation, and their deletion triggers significant alterations in bone marrow HSC homing and content. Interestingly, peri-vascular nestin⁺ cells are also functional mesenchymal stem cells (MSC): they account for all mesenchymal activity (fibroblastic colony-forming units), show clonal multilineage differentiation toward the three major mesenchymal lineages, display robust self-renewal in serial transplantations and contribute to osteochondral lineages in vivo. These findings suggest that the bone marrow stem cell niche is composed of a unique pairing of MSCs and HSCs, tightly regulated by local input from the microenvironment and by long-distance cues from hormones and the autonomic nervous system.

Our investigation of this sympathetic regulation has revealed that β_2 - and β_3 -adrenergic receptors have different functions in bone marrow, but cooperate during G-CSF-induced HSC mobilization. This process is not the mirror image of that which triggers HSC homing to the bone marrow. We have gained insight into differential homing pathways—some of which are shared with leukocytes—by showing redundant and nonredundant roles for the adhesion molecules ICAM-1, ICAM-2, and VCAM-1 in lymphocyte homing. We have also uncovered some of the mechanisms by which the niche regulates progenitor cell differentiation during development, showing that the transcription factor Eklf critically regulates the formation of primitive erythroid cells and their maturation in a dose-dependent manner.

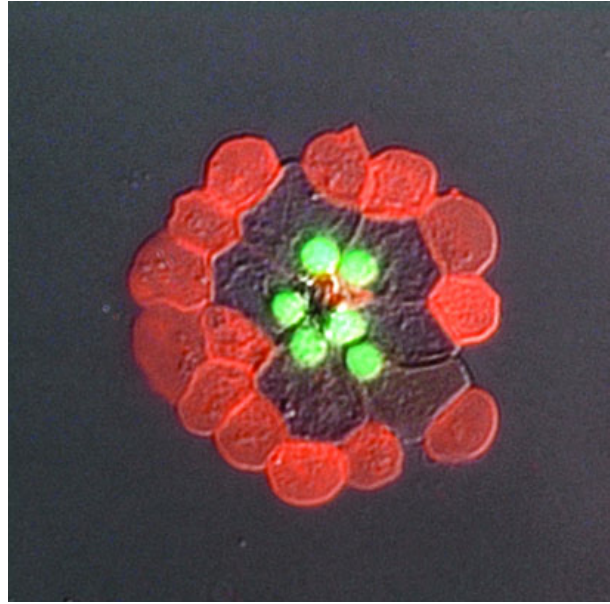


Peri-vascular nestin⁺ mesenchymal stem cells are innervated by sympathetic fibers in the bone marrow. Projection stack (~100 μ m) of fluorescent images showing the distribution of Nestin-GFP⁺ cells (green), CD31/PECAM⁺ vascular endothelial cells (blue) and tyrosine hydroxylase⁺ sympathetic nerve fibers (red) after whole mount staining of the skull bone marrow.

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A bone marrow stem cell niche made for two. Projection stack (~15 μm) of fluorescent images showing a CD150^+ (red) CD48^- , CD3e^- , Ter119^- , Gr1^- , B220^- and CD11b^- (antigens labeled in blue) hematopoietic stem cell adjacent to a nestin-GFP^+ mesenchymal stem cell (green) in the bone marrow (from *Nature* 466: 829-34). Grid, 50 μm



The image shows a fluorescence overlay of Ter119 -stained cytocentrifuged embryonic blood cells from $\text{E13.5 } \epsilon\text{-globin::H2B-GFP;Eklf}^{\pm}$ transgenic mouse embryos. Ter119 (red) is expressed on the enucleated definitive erythrocytes at the periphery of the cluster but not on the larger, nucleated primitive erythroblasts (EryP) in the center (green nuclei). From: *Blood*, 116:3972-80 (Front cover)



MAJOR GRANTS

- ASH Scholar Award. American Society of Hematology
- Ministerio de Ciencia e Innovación. (RYC-2009-04703)



SELECTED PUBLICATIONS

Méndez-Ferrer S*, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS* **Mesenchymal and haematopoietic stem cells form a unique niche in the bone marrow.** *Nature* (2010) 466: 829-34. (Full Article)
*Corresponding authors

Méndez-Ferrer S, Battista M, Frenette PS. **Cooperation of β_2 - and β_3 -adrenergic receptors in hematopoietic progenitor cell mobilization.** *Ann N Y Acad Sci* (2010) 1192: 139-44

Méndez-Ferrer S, Frenette PS. **$\text{G}\alpha_s$ uncouples haematopoietic stem cell homing and mobilisation.** *Cell Stem Cell* (2009) 4: 379-80

Boscacci RT, Pfeiffer F, Gollmer K, Sevilla AI, Martín AM, Soriano SF, Natale D, Henrickson S, von Andrian UH, Fukui Y, Mellado M, Deutsch U, Engelhardt B, Stein JV. **Comprehensive analysis of lymph node stroma-expressed Ig superfamily members reveals redundant and nonredundant roles for ICAM-1, ICAM-2, and VCAM-1 in lymphocyte homing.** *Blood* (2010) 116: 915-25

Isern J, Fraser ST, He Z, Baron MH. **Dose-dependent regulation of primitive erythroid maturation and identity by the transcription factor Eklf .** *Blood* (2010) 116: 3972-80 (Front cover)

*Role of new genes
in cardiovascular development***Head of Laboratory:** *Juan José Sanz Ezquerro***Predocctoral Researchers:** *Verónica Uribe Sokolov***Masters Student:** *Laura González Calero***Technician:** *Claudio Badía Careaga***RESEARCH INTEREST**

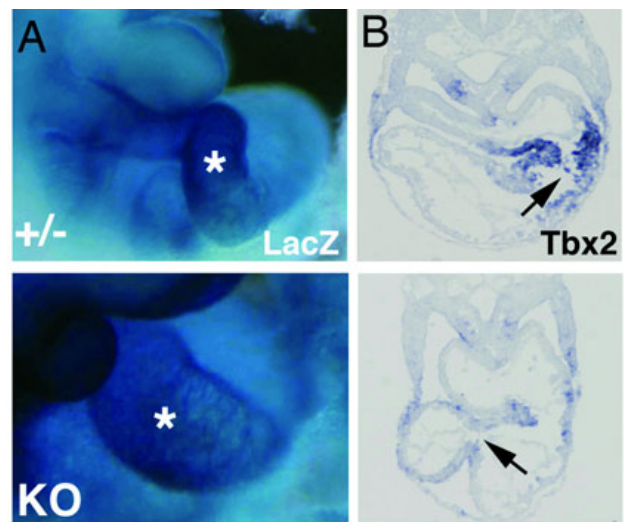
Our group investigates the molecular and cellular basis of organogenesis during embryonic development. Our approach combines studies in chick and mouse embryos with in vitro cell culture models to dissect the role of new genes in the morphogenesis of the heart and other aspects of cardiovascular development.

Much of our recent work focuses on the role during embryogenesis of *Arid3b*, a transcription factor of the highly conserved ARID family. *Arid3b*-null embryos die early in development and present with severe heart defects, but the exact roles of *Arid3b* in development remain unclear.

During the last year we advanced our analysis of these heart defects. The most severe alterations occur at the poles of the heart, especially a noticeable shortening of the outflow tract, a reduction in the size of the inflow region, and abnormal patterning and maturation of the AV canal. Mutant embryos show abnormal expression of several molecular markers of both the secondary heart field and chambers, pointing to important roles for *Arid3b* in several aspects of heart development. We also observed defects in the cytoskeleton and motility of mutant cells in vitro. Based on these findings, we believe that *Arid3b* might control the regulated addition and differentiation of heart precursor cells from the second heart field to the heart.

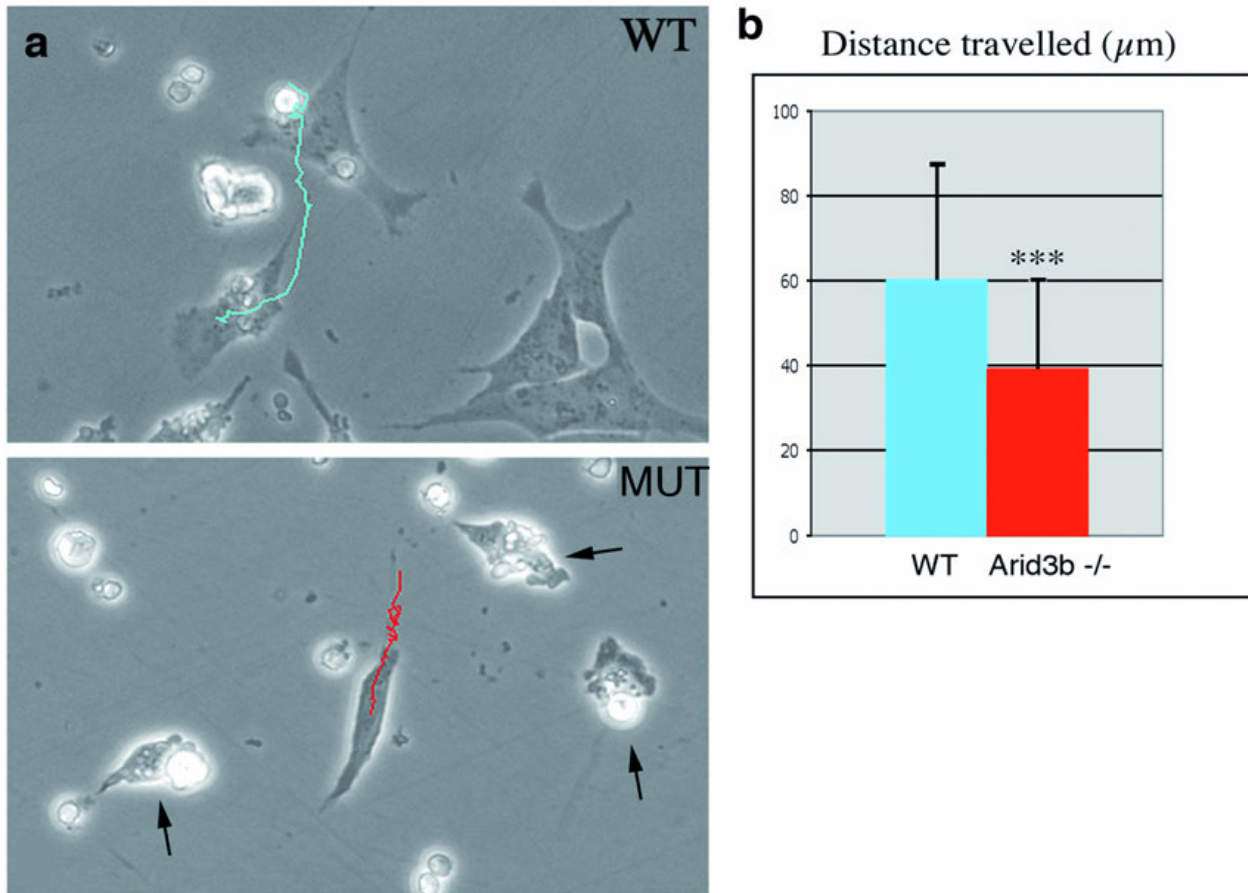
Our plans include an analysis of the cellular basis of *Arid3b* functions (for example its involvement in cell migration and epithelial to mesenchymal transition) and the identification of its molecular targets by microarray analysis.

Another area of interest is the role of *norrin* during chick embryo development. Mutations in *norrin* cause Norrie disease in humans, a retinal dysplasia characterized by abnormal vascularization of the retina. We have found that chicken *norrin* is expressed in tissues besides the eye during embryogenesis and we are characterising its expression pattern and biological functions.



*Heart development defects in *Arid3b* knockout (KO) embryos. A, LacZ staining of heterozygous and KO embryos at E9.5. Note the shortening and widening of the outflow tract (asterisks). B, In situ hybridisation for *Tbx2* at E9.5 shows absence of expression in the atrioventricular canal (arrows) of KO hearts.*

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Arid3b mutant embryonic fibroblasts show defective motility in vitro. **A**, Example of the path traveled by cells after 3 hours in culture (blue and red lines mark trajectories and images correspond to the last frames of the time-lapse sequence). **B**, Quantification of the data in **A**, showing that *Arid3b* cells travel significantly shorter distances than wild type cells.



MAJOR GRANTS

- Fundació La Marató TV3 (082031)
- Ministerio de Ciencia e Innovación. FIS (CP07/251)



SELECTED PUBLICATIONS

Casanova JC, Uribe V, Badia-Careaga C, Giovinazzo G, Torres M, Sanz-Ezquerro JJ. Apical Ectodermal Ridge morphogenesis in limb development is controlled by *Arid3b*-mediated regulation of cell movements. *Development* (accepted)