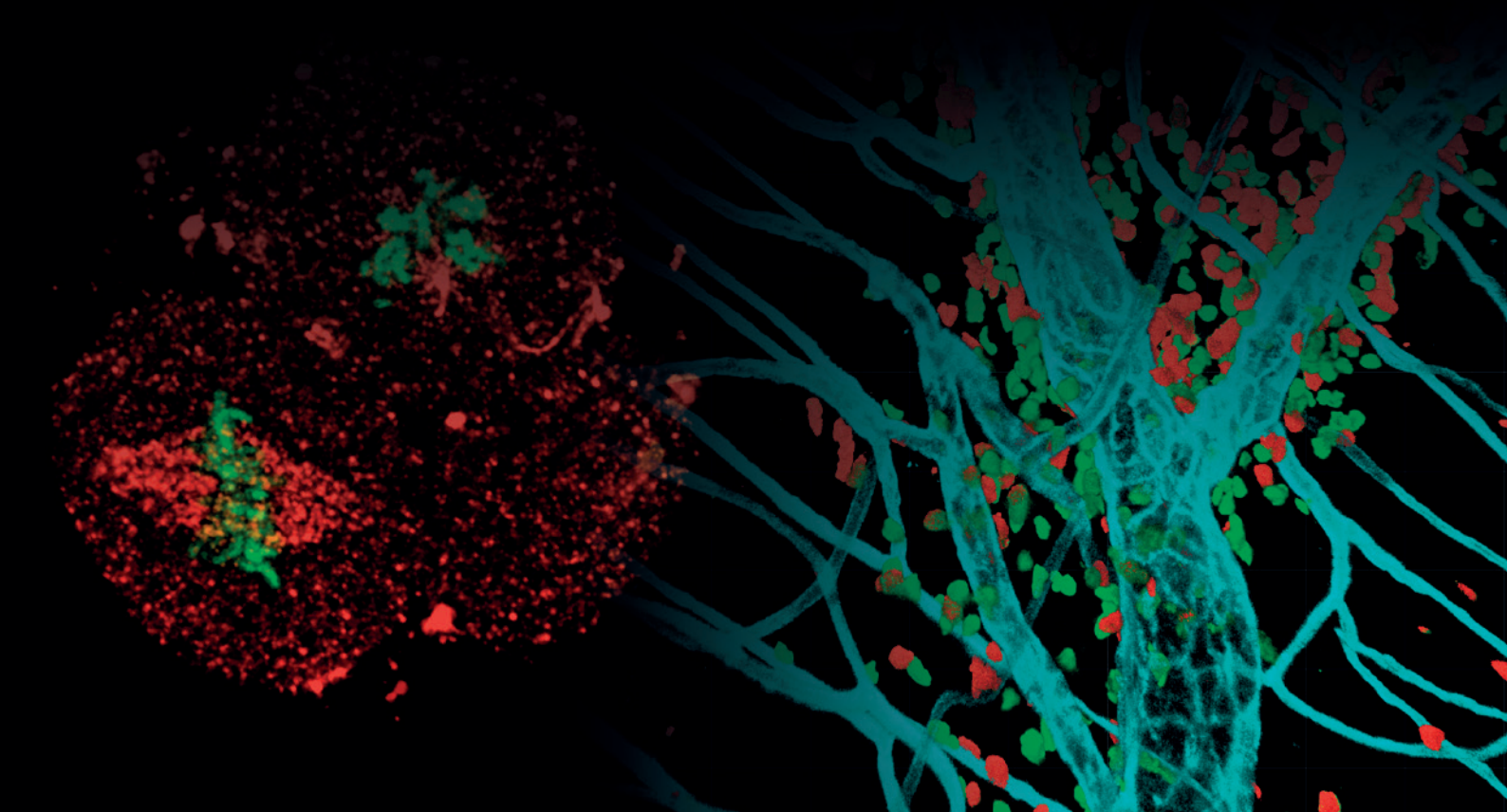
RESEARCH AREAS

1 Myocardial Pathophysiology

2 Vascular Pathophysiology

3 Cell and Developmental Biology



3

RESEARCH AREAS

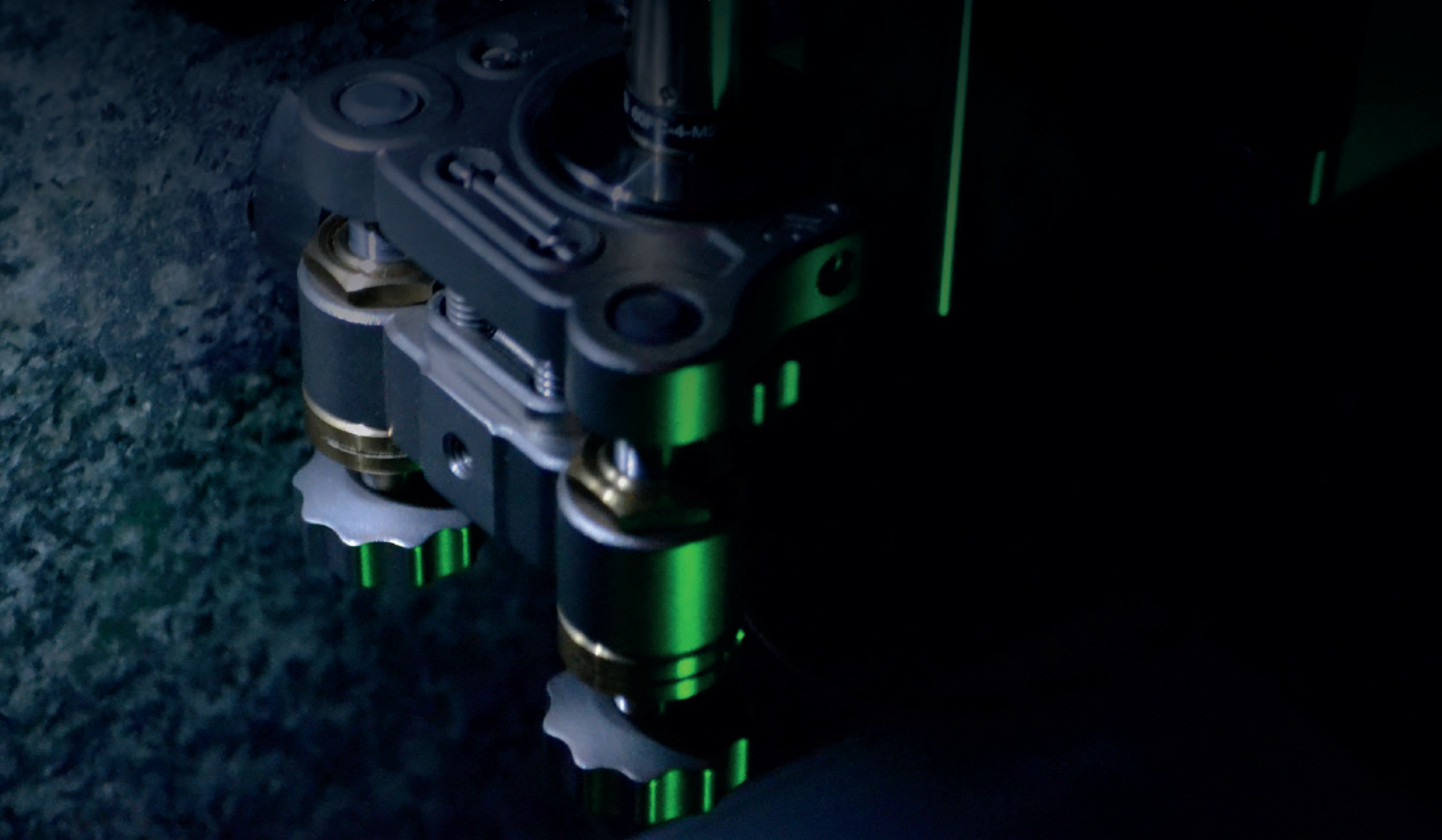
Cell and Developmental Biology

Area coordinators:

Miguel Manzanares *Miguel Ángel del Pozo*

RESEARCH INTEREST

The Cell and Developmental Biology Area comprises 10 laboratories that conduct basic and translational research, ranging from mechanistic aspects of cell signaling and behavior to the principles of cardiovascular development. Research topics include the molecular and cellular embryology of the heart, mechanisms of tissue repair, the underpinnings of heart and vascular homeostasis, and how these aspects relate to disease. Specific research lines are aimed at understanding how temporally and spatially regulated transcriptional networks determine the very first cell fate decisions in the early embryo, as well as the different stages of heart development. Laboratories in the CDB Area also investigate processes important for cardiovascular homeostasis such as angiogenesis, inflammation, and regeneration. Finally, a number of research lines are aimed at elucidating key cell signaling pathways and molecular principles underlying the mechanical properties, function and adaptability of the cardiovascular system, using state-of-the-art cell biophysics and single-molecule techniques.



Single-molecule mechanobiochemistry

Head of Laboratory
Jorge Alegre-Cebollada

RESEARCH INTEREST

The elastic properties of the myocardium determine cardiac performance, and disruptions to these properties underlie diseases such as heart failure and cardiomyopathies, through mechanisms that we are just beginning to understand. In our group, we explore how the mechanics of specific structural proteins determines the macroscopic elasticity of the heart in health and disease. We follow a multidisciplinary approach to measure the mechanical properties of key cardiac proteins such as titin, and investigate how these mechanical properties are affected by posttranslational modifications and mutations that cause cardiomyopathies. During 2015, we set up methods based on mass spectrometry and fluorescent polyacrylamide gels that allow us to monitor redox posttranslational modifications that target titin and other cardiac proteins. We have detected a strong oxidation signal coming from 3 conserved cysteines in the immunoglobulin domains of titin that can engage in disulfide bonds (Figure 1). We also brought the CNIC's first single-molecule atomic force microscope (AFM) into service. This instrument is designed to examine the mechanical properties of proteins, and we have used it to measure the mechanics of protein domains whose mutation gives rise to hypertrophic cardiomyopathy (Figure 2). Also in 2015, we started a new line of research to engineer protein biomaterials with tailored and regulatable mechanical properties that can be predicted from the molecular mechanics of the constituent proteins. These novel materials could find application in tissue engineering and repair.

Postdoctoral Researcher:
Elías Herrero-Galán
Predoctoral Researcher:
Carla Huerta
Masters Students:
Cristina Sánchez-González
Carmen Suay
Technician:
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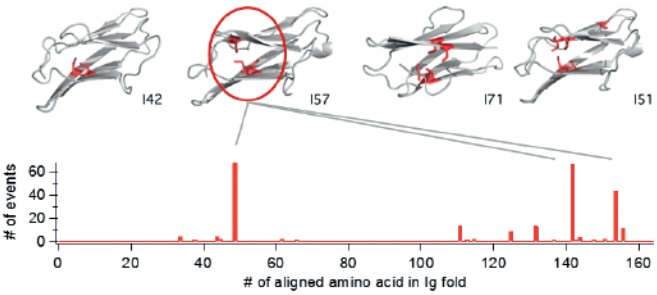
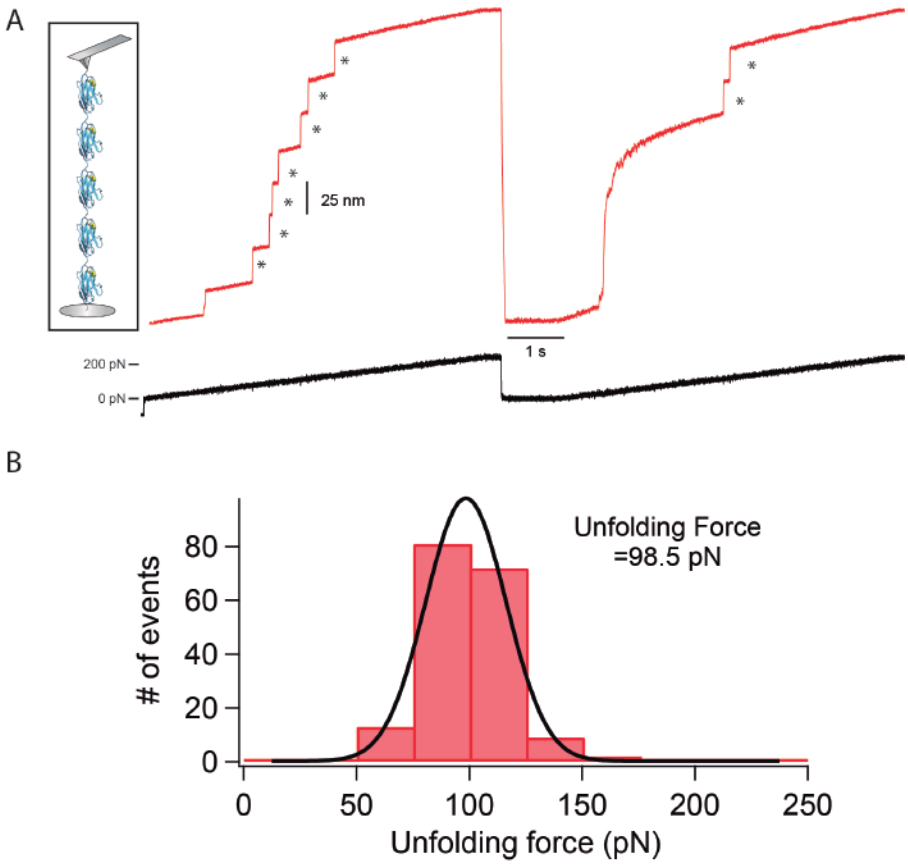


Top: Four different titin Ig domains. Cysteine residues have been highlighted. Domain I57 contains the three most conserved cysteines in titin. ***Bottom***: Frequency of cysteine residues along the sequence of the more than 200 titin Ig domains.



A. A polyprotein made from the repetition of a single domain is pulled using an AFM tip (inset). The force is linearly increased (black) and protein length is monitored (red). We detected the mechanical unfolding of 7 domains (asterisks). Force is then relaxed to 0 pN, allowing refolding of two domains (detected in a second force ramp). **B**. We compiled several traces to determine the mechanical stability of the domain.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (BIO2014-54768-P)*
- *Ministerio de Economía y Competitividad (RYC-2014-16604)*

SELECTED PUBLICATIONS

Saqlain F, Popa I, Fernández JM, Alegre-Cebollada J. **A novel strategy for utilizing voice coil servoactuators in tensile tests of low volume protein hydrogels.** *Macromol Mater Eng* (2015) 300: 369-76

Rivas-Pardo JA, Alegre-Cebollada J, Ramírez-Sarmiento CA, Fernandez JM, Guixé V. **Identifying sequential substrate binding at the single-molecule level by enzyme mechanical stabilization.** *ACS Nano* (2015) 9: 3996-4005

Alegre-Cebollada J, Kosuri P, Giganti D, Eckels E, Rivas-Pardo JA, Hamdani N, Warren CM, Solaro RJ, Linke WA, Fernández JM. **S-glutathionylation of cryptic cysteines enhances titin elasticity by blocking protein folding.** *Cell* (2014) 156: 1235-46

Solsona C, Kahn TB, Badilla CL, Álvarez-Zaldiernas C, Blasi J, Fernandez JM, Alegre-Cebollada J. **Altered thiol chemistry in human amyotrophic lateral sclerosis-linked mutants of superoxide dismutase 1.** *J Biol Chem* (2014) 289: 26722-32

Molecular genetics of angiogenesis

Head of Laboratory
Rui Benedito

RESEARCH INTEREST

Our group is interested in understanding the cellular and molecular mechanisms involved in the formation and homeostasis of blood vessels, which are an important therapeutic target in cardiovascular disease and cancer. Modulation of vascular structure and function in disease is still a major challenge, in part due to our inability to block or induce the exact mechanisms that vessels use to grow under normal physiologic conditions.

We are currently revisiting and challenging some existing concepts in vascular biology by using new genetic tools that enable us to study the function of genes at higher cellular resolution. We aim to identify and characterize new mechanisms involved in vascular differentiation and growth, but also study their importance in the vasculature paracrine function in diverse phisiological or disease situations. Some of these mechanisms are highly conserved, whereas others seem to be active or important only in some vascular beds or pathological contexts.

In the last year we continued to investigate the regulation of endothelial differentiation, proliferation and aging by the Notch, VEGF and FGFR signaling pathways. We began the study of specific genes in the development and homeostasis of the heart coronary vessels, and we also investigated how endothelial cells transdifferentiate into hematopoietic stem cells early in life and how they later modulate their biology in the bone marrow.

Postdoctoral Researchers:
Elena M Doménech
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Briane D Laruy
Carlos López Fernández de Castillejo
Samuel Pontes Querol

Masters Student:
Irene García González

Graduate Technicians:
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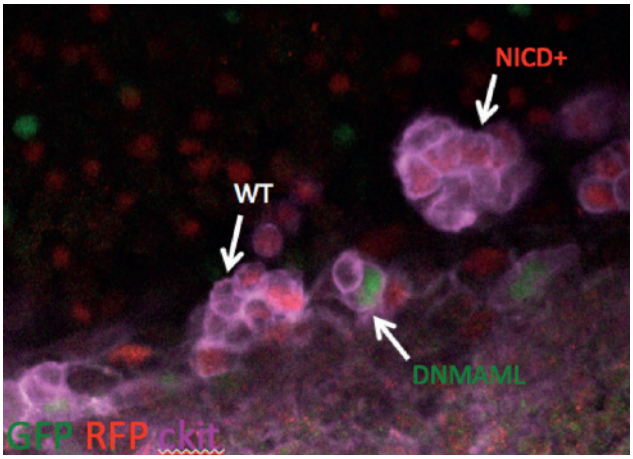
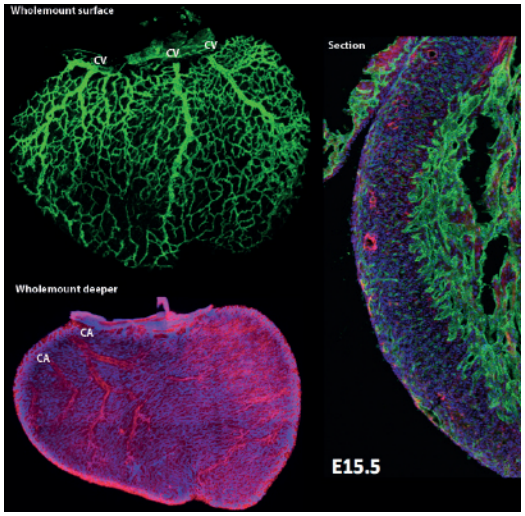
Luis Heredia
Juan Ramón Perea Úbeda-Portugués

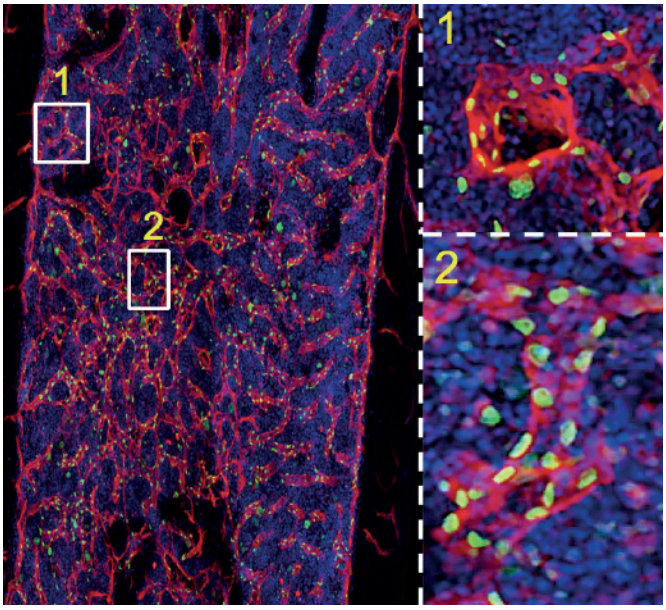
Technician:
Mª Sofía Sánchez Muñoz



Confocal 3D picture showing hematopoietic stem cell clusters (pink/c-Kit+) arising from endothelial cells on the adjacent dorsal floor of the mouse aorta. Notch signaling is higher in cells with red nuclei and lower in cells with green nuclei.



Confocal 3D images of a microdissected embryo heart showing the coronary vasculature. The images show the surface capillaries and veins (green) and the deeper arteries (red). The image to the right shows a heart section.



Confocal 3D images showing intact bone vascular architecture (red) surrounded by blood cells (blue). Cells with green nuclei have high Notch activity.

MAJOR GRANTS

- *European Research Council Starting Grant 2014. (ERC-2014-StG 638028_AngioGenesHD).*
- *Ministerio de Economía y Competitividad (SAF2013-44329-P)*
- *Ministerio de Economía y Competitividad. Contrato Ramón y Cajal (RYC-2013-13209)*
- *Ministerio de Economía y Competitividad. Contrato Posdoctoral PI: Tania Sánchez (FPDI-2013-18049)*
- *Fundación La Caixa CNIC Severo Ochoa. Predoctoral Fellowship. PI: Samuel Pontes*
- *Fundación La Caixa. Predoctoral Fellowship. PI: Macarena Fernández*
- *Boheringer Ingelheim Fons. Predoctoral Fellowship. PI: Carlos López Fernández de Castillejo*
- *European Commission. International IPP. PI: Wen Luo*
- *European Commission. International IPP. PI: Sarita Saraswati*

SELECTED PUBLICATIONS

D'Amato G, Luxán G, Del Monte-Nieto G, Martínez-Poveda B, Torroja C, Walter W, Bochter MS, Benedito R, Cole S, Martinez F, Hadjantonakis AK, Uemura A, Jiménez-Borreguero LJ, de la Pompa JL. **Sequential Notch activation regulates ventricular chamber development (2015)** *Nat Cell Biol* (doi: 10.1038/ncb3280. Epub 2015 Dec 7))

Bernier-Latmani J, Cisarovsky C, Demir CS, Bruand M, Jaquet M, Davanture S, Ragusa S, Siegert S, Dormond O, Benedito R, Radtke F, Luther SA, Petrova TV **DLL4 promotes continuous adult intestinal lacteal regeneration and dietary fat transport** *J Clin Invest* (2015) 125: 4572-86

Rocha SF, Schiller M, Jing D, Li H, Butz S, Vestweber D, Biljes D, Drexler HC, Nieminen-Kelha M, Vajkoczy P, Adams S, Benedito R, Adams RH. **Esm1 Modulates Endothelial tip cell behavior and vascular permeability by enhancing VEGF bioavailability** *Circ Res* (2014) 115: 581-90

Integrin signaling

Head of Laboratory
Miguel Ángel del Pozo

RESEARCH INTEREST

We have shown that crucial cell functions are affected by key mechanoregulatory molecules: integrins (which mediate cell adhesion to the extracellular matrix), Rac/Rho GTPases (which regulate actin cytoskeleton functions & mechanical contractility), and caveolae-resident proteins. Caveolae are actin-linked plasma membrane invaginations abundant in mechanically stressed tissues (including heart, vessels, muscle & fat) and are involved in signaling, viral entry, membrane trafficking & lipid metabolism. The precise functions of caveolae and their main constituent proteins caveolins (Cav 1-3) and cavins (1-4) remain unresolved. Cav-deficient mice show tissue abnormalities, and caveolar disorders are associated with lipodystrophy, muscular dystrophy, osteoporosis, CVD and cancer. We and others have shown that caveolae can sense and transduce mechanical cues. We found that Cav1 can modulate cell shape and responses via force-dependent remodeling of the 3D microenvironment. Elongated cancer associated fibroblasts (CAFs) form stiff, parallel-fiber networks through which cancer cells move rapidly, invading and metastasizing.

Our work shows that stromal-Cav1 drives not only pathological remodeling of the tumor microenvironment, but also physiological remodeling, for example in the mammary gland and the skin. We are now addressing the role of Cav1 in cardiac remodeling after acute myocardial infarction using the LAD (left anterior descending) artery permanent ligation model. Abnormal cardiac remodeling and fibrosis after acute myocardial infarction can lead to heart failure and death.

Rac1 had been detected in the nucleus, and our work has provided insight into the molecular mechanism of Rac1 nucleocytoplasmic shuttling. Rac1-driven nuclear actin polymerization controls nuclear membrane organization and shape. Dysregulation of this mechanism in cancer leads to Rac1 nuclear accumulation, promoting nuclear deformation and cell invasion through narrow spaces.

In 2015 we also established Cav1 as a major checkpoint in the transition from an epithelial to a mesenchymal identity in the peritoneum, through the suppression of MEK-ERK1/2-Snail1 signaling. The efficacy of a MEK pharmacological inhibitor in counteracting the EMT/fibrosis developed in Cav1-/- mice during peritoneal dialysis warrants further translational studies in other chronic inflammatory diseases.

Research Scientists:
Asier Echarri
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Miguel Sánchez Álvarez

Predoctoral Researchers:
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Alberto Díez
Mª del Carmen Aboy
Giulio Fulgoni

Masters Students:
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Olga Boix (since October)

Technicians:
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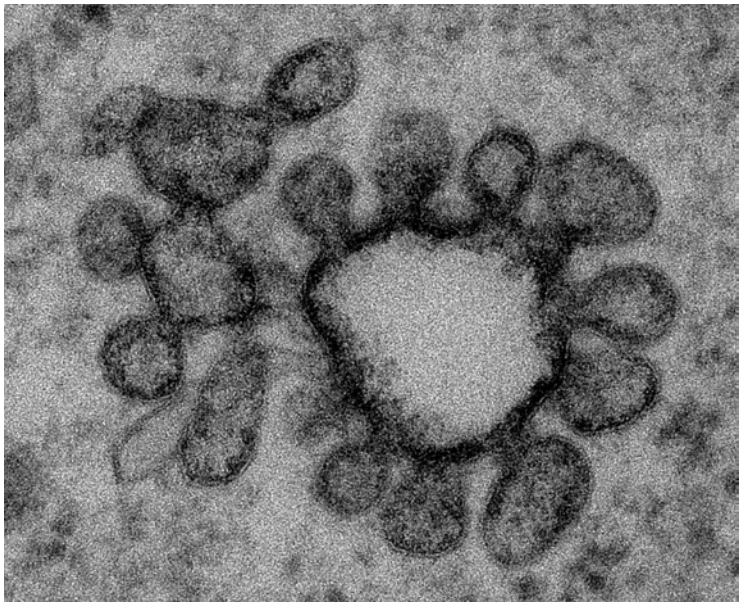
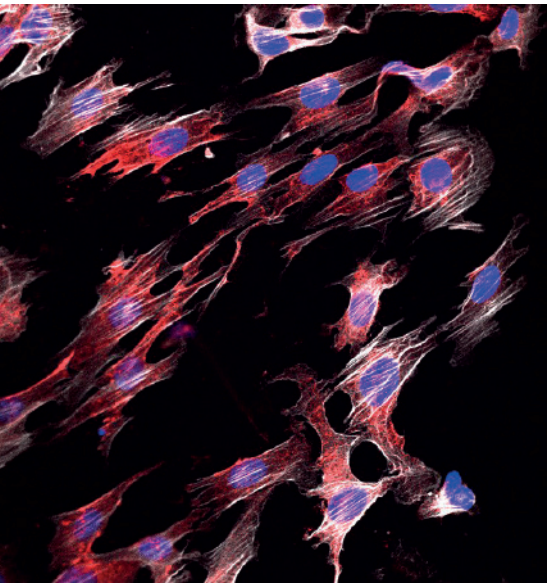
Teresa Osteso Ibáñez
Dácil M. Pavón
Mauro Catalá

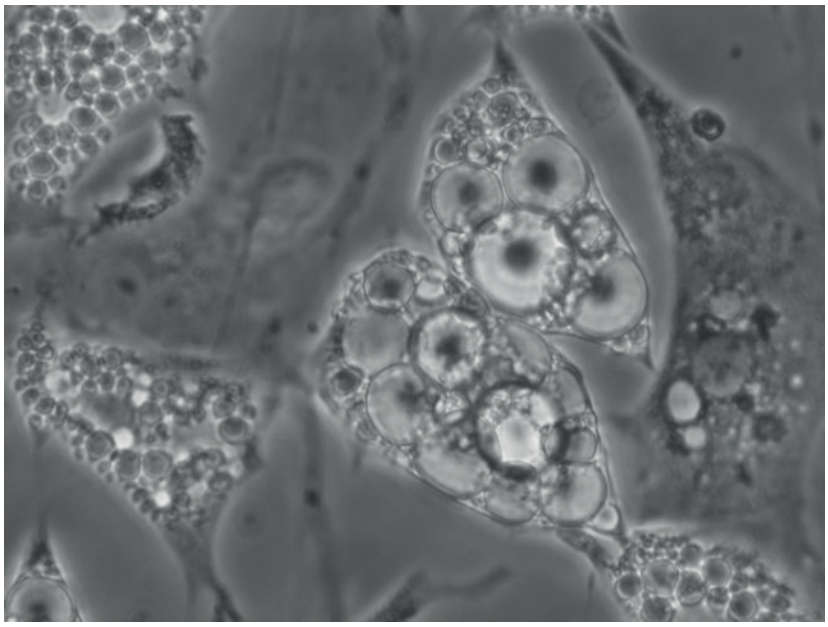
Visiting Scientist:
Raffaele Strippoli



Caveolar Rosettes. A) Surface-connected invaginations of the plasma membrane decorated with caveolae observed by electron microscopy. B) GSD super-resolution image of a cell stained for Cav1. The arrow shows a rosette-like structure decorated with Cav1-positive aggregates.



Cells stretched cyclically over 2 hours, fixed and then stained for Cav1 (red), nuclei (blue) and actin cytoskeleton (gray). Stretching at high amplitude leads to actin fiber alignment.



Adypocytes differentitated in vitro from adipocyte precursors isolated from adult white adipose tissue.

MAJOR GRANTS

- *European Commission. Marie Curie Actions Initial Training Network (ITN) (Horizon 2020, "BIOPOL")*
- *WorldWide Cancer Research (UK) (formerly known as AICR) (AICR 15 – 0404)*
- *Ministerio de Economía y Competitividad (SAF2014-51876-R)*
- *Ministerio de Economía y Competitividad. Consolider COAT (CSD2009-00016)*
- *Ministerio de Economía y Competitividad. Red de Excelencia en Mecanobiología (BFU2014-52586-REDT)*
- *Fundació La Marató TV3 (674/C/2013)*

SELECTED PUBLICATIONS

Echarri A, Del Pozo MA. **Caveolae - mechanosensitive membrane invaginations linked to actin filaments.** *J Cell Sci.* (2015) 128: 2747-58

Kosmalska AJ, Casares L, Elosegui-Artola A, Thottacherry JJ, Moreno-Vicente R, González-Tarragó V, del Pozo MA, Mayor S, Arroyo M, Navajas D, Trepat X, Gauthier NC, Roca-Cusachs P. **Physical principles of membrane remodelling during cell mechanoadaptation.** *Nat Commun* (2015) 6: 7292

Navarro-Lérida I, Pellinen T, Sánchez SA, Guadamillas MC, Wang Y, Mirtti T, Calvo E, Del Pozo M.A. **Rac1 nucleocytoplasmic shuttling drives nuclear shape changes and tumor invasion.** *Dev Cell* (2015) 32: 318-34

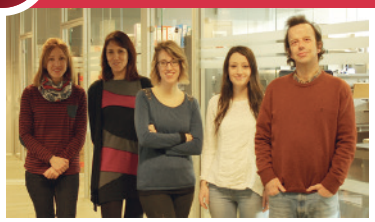
Strippoli R, Loureiro J, Benedicto I, Pérez-Lozano ML, Moreno V, Barreiro O, Pellinen T, Minguet S, Foronda M, Osteso MT, Calvo E, Vázquez J, López-Cabrera M, Del Pozo MA. **Caveolin-1 deficiency induces MEK-ERK1/2-Snail1-dependent epithelial-mesenchymal transition and fibrosis during peritoneal dialysis.** *EMBO Mol Med* (2015) 7: 102-23

Shi Y, Tan SH, Ng S, Zhou J, Yang ND, Khoo GB, McMahon KA, Parton RG, Hill MM, Del Pozo MA, Kim YS, Shen HM. **Critical role of CAV1/caveolin-1 in cell stress responses in human breast cancer cells via modulation of lysosomal function and autophagy**. *Autophagy* (2015) 11: 769-84

Regeneration and aging

Head of Laboratory

Ignacio Flores



Posdoctoral Researcher:
Tania Aguado

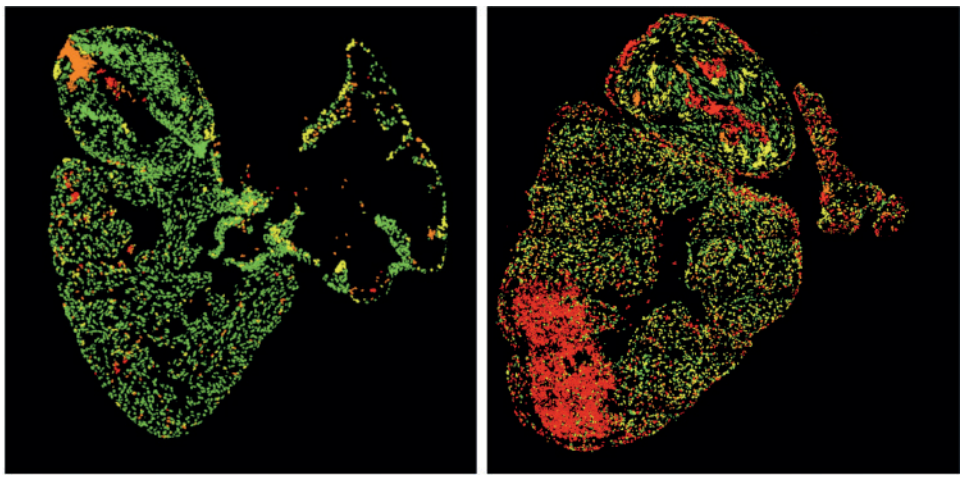
Predoctoral Researchers:
Esther Aix
Dorotha Bednarek
Carlota Sánchez Ferrer

Technician:
Irene de Diego

RESEARCH INTEREST

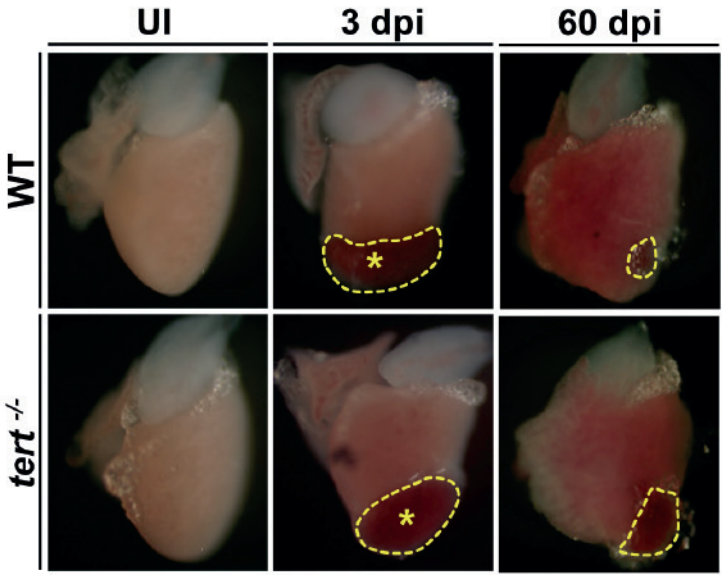
Although recent advances have overturned the old view of the human heart as an inert postmitotic organ, it is clear that the heart's capacity to proliferate, rejuvenate and regenerate is very limited. This presents a problem for strategies to treat damaged hearts after infarction, one of the leading causes of death worldwide.

Our group works on strategies to enhance cardiac regeneration. Toward this goal, we are characterizing the subpopulation of cardiac cells capable of regeneration. Based on this knowledge, we are currently exploring strategies to promote the repair of injured hearts. We have eliminated and reactivated telomerase, an anti-aging enzyme, in adult cardiac cells in order to assess the role of this enzyme in the re-expression of cardiac embryonic genes after infarction and in heart regeneration. A key element of our strategy is the comparison of animal models that differ greatly in their regeneration capacity, from the zebrafish, which can restore up to 20% its heart after injury, through the newborn mouse, whose heart possesses transient regenerative potential, to the adult mouse, in which heart regeneration capacity is very limited. In the zebrafish model, we found that telomerase is essential for zebrafish heart regeneration. The inability to regenerate is mainly due to a strong inhibition of the proliferation response, associated with accumulation of cardiac cells with DNA damage and senescence characteristics. Through these efforts, we hope to achieve a more complete knowledge of the role of endogenous cardiac progenitor cells and telomerase in heart rejuvenation and regeneration, which could eventually lead to the development of improved regeneration therapies.



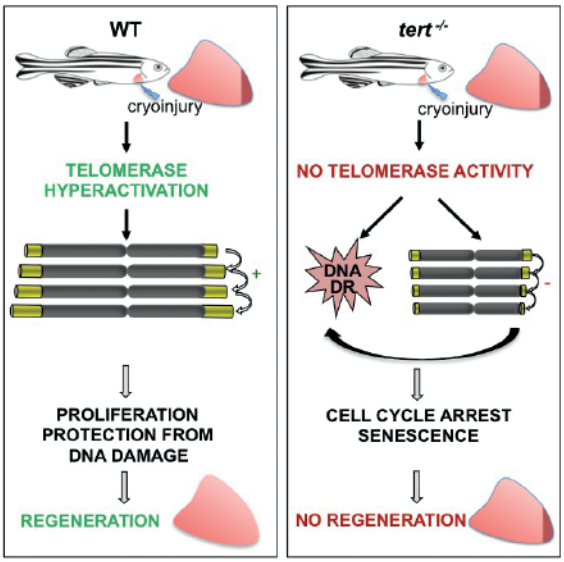
Heart cryoinjury elongates telomeres
Representative telomap images of zebrafish hearts with no injury (left) and 3 days post-injury (right). Nuclei are assigned to a four-color code according to their average telomere fluorescence in arbitrary units. The cells with the longest telomeres are shown in red, and those with the shortest telomeres are shown in green.

Bednarek D et al., Cell Rep, 2015 12(10):1691-703. doi: 10.1016/j.celrep.2015.07.064



Heart regeneration is strongly inhibited in tert$^{-/-}$ animals
Whole-mount views of uninjured and cryoinjured WT and tert$^{-/-}$ zebrafish hearts dissected at the indicated times post-injury. Dotted lines outline the injured area. Asterisks mark the initial injury site.

Bednarek D et al., Cell Rep, 2015 12(10):1691-703. doi: 10.1016/j.celrep.2015.07.064



Model of telomerase function in zebrafish heart regeneration

Bednarek D et al., Cell Rep, 2015 12(10):1691-703. doi: 10.1016/j.celrep.2015.07.064

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2012-38449)*
- *Ministerio de Economía y Competitividad. FIS. RETICS (Red de Investigación Cardiovascular RD12/0042/0045)*
- *Asociación Española contra el Cáncer PI: Tania Aguado*

SELECTED PUBLICATIONS

Bednarek D, Gonzalez-Rosa JM, Guzman-Martinez G, Gutierrez-Gutierrez O, Aguado T, Sanchez-Ferrer C, Marques IJ, Galardi-Castilla M, de Diego I, Gomez MJ, Cortes A, Zapata A, Jimenez-Borreguero LJ, Mercader N*, Flores I*. **Telomerase is essential for zebrafish heart regeneration**. *Cell Rep* (2015) 12: 1691-703
*Co-corresponding authors

Imaging cardiovascular inflammation and the immune response

Head of Laboratory
Andrés Hidalgo Alonso

RESEARCH INTEREST

We are interested in multiple aspects of the biology of myeloid leukocytes and hematopoietic stem and progenitor cells (HSPC), the source of all blood cells. In the past year we have identified the regulatory function of E-selectin ligand 1 (ESL-1) in regulating the hematopoietic microenvironment, or niche, in which these HSPCs reside. We have discovered that through this glycoprotein, HSPCs are able to maintain normal proliferation within the bone marrow. In a completely different organ, the heart, we are working to identify how a group of myeloid cells, cardiac-resident macrophages, prevent fibrosis and maintain normal heart function. We are currently deciphering the mechanisms by which macrophages carry out this important function. Another major interest of the lab lies in understanding the biology of neutrophils. These leukocytes are important because they keep the organism free of pathogenic microorganisms but can also cause major inflammatory injury to organs, for example during sepsis or myocardial infarction. We have found that under healthy conditions these cells recirculate throughout the organism to support basic homeostatic functions, even in distant tissues.

Postdoctoral Researchers:
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Predoctoral Researchers:
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José Ángel Nicolás Ávila

Technicians:
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Georgiana Crainiciuc
Patricia Castro Hernanz

Master Student:
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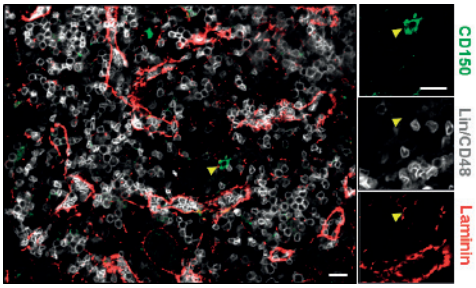
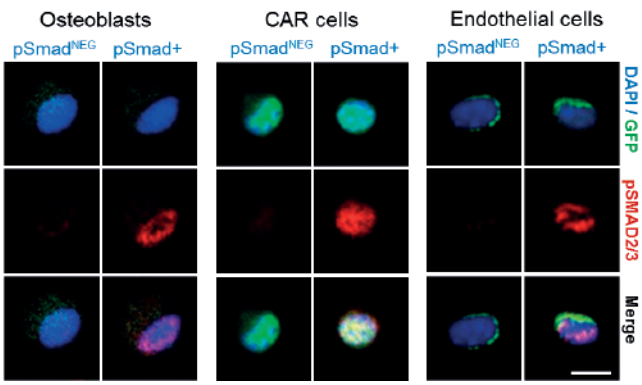
Visiting Scientist:
Linnea A. Weiss



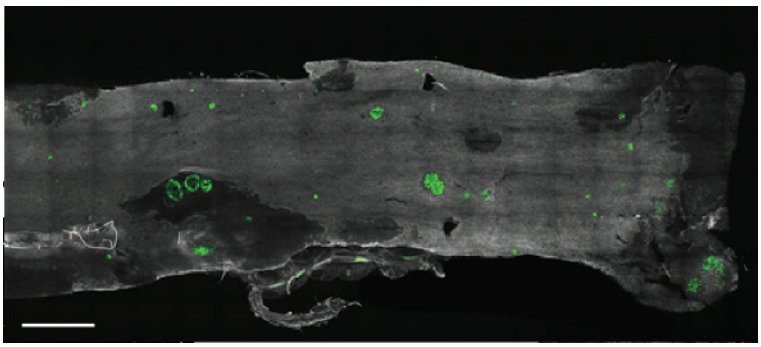
Hematopoietic stem cells in their niche
Confocal image of the bone marrow of a mouse, showing a blood stem cell (green) surrounded by vessels (red) and mature leukocytes (white). Both the proliferation and the status of the niche are controlled by the glycoprotein ESL-1 in stem cells.

Leiva M et al., Nat Commun, 2016 7:10222. doi: 10.1038/ncomms10222



Regulated signaling in bone marrow "niche" cells
Different types of stromal cells that form the hematopietic niche (osteoblasts, CAR or reticular cells, and endothelial cells) expressing varying levels of green fluorescent protein (GFP). These cells display different signailing properties marked by the phosphorylation of Smad2/3 proteins (red) in the nucleus (blue). This signaling pathway is regulated by ESL-1 in the bone marrow.

Leiva M et al., Nat Commun, 2016 7:10222. doi: 10.1038/ncomms10222



Neutrophils cluster in the intestinal mucosa
Whole-mount imaging of the large intestine of a mouse, showing groups, or "clusters", of neutrophils. These intestinal neutrophils can be identified by the expression of a green fluorescent protein (GFP).

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (SAF2013-49662-EXP)*
- *Ministerio de Economía y Competitividad (ERA-NET Infect-ERA 2014 #143 BActInfectERA)*
- *Comunidad de Madrid (P2010-BMD-2314)*
- *Ministerio de Economía y Competitividad (SAF2012-31142)*
- *Fundación La Marató-TV3 (120/C/2015)*

SELECTED PUBLICATIONS

Leiva M, Quintana JA, Ligos JM and Hidalgo A. **Hematopoietic ESL-1 enables stem cell proliferation in the bone marrow by limiting TGFb availability**. *Nat Commun* (accepted)

Martinez-Moreno M*, Leiva M*, Aguilera-Montilla N, Sevilla-Movilla S, Isern de Val S, Arellano-Sánchez N, Gutierrez N, Maldonado R, Martinez-Lopez J, Buño I, Garcia-Marco J, Sánchez-Mateos P, Hidalgo A, Garcia-Pardo A and Teixidó J. **In vivo adhesion of malignant cells to bone marrow microvasculature is regulated by a4b1 cytoplasmic-binding proteins**. *Leukemia* (doi: 10.1038/leu.2015.332 Epub 2015 Dec 10)
* Equal contribution

Lasarte S, Samaniego R, Salinas-Muñoz L, Guía-Gonzalez MA, Weiss LA, Mercader E, Ceballos-Garcia E, Navarro-González T, Moreno-Ochoa L, Perez Millan F, Pion M, Sanchez-Mateos P, Hidalgo A, Muñoz-Fernandez MA and Relloso M. **Sex hormones coordinate neutrophil immunity in the vagina by controlling chemokine gradients**. *J Infect Dis* (doi: 10.1093/infdis/jiv402. Epub 2015 Aug 3)

Gonzalez-Valdes I, Hidalgo I, Bujabarral A, Lara-Pezzi E, Padron L, Garcia-Pavia P, Gomez P, Redondo JM, Ruiz-Cabello JM, Jimenez-Borreguero LJ, Enriquez JA, de la Pompa JL, Hidalgo A and Gonzalez S. **Heart senescence surveillance by Bmi1 limits dilated cardiomyopathy in heart failure**. *Nat Commun* (2015) 6: 6473

Scheiermann C, Frenette PS, Hidalgo A. **Regulation of leukocyte homeostasis in the circulation**. *Cardiovasc Res* (2015) 107: 340

Functional genomics

Head of Laboratory
Miguel Manzanares

RESEARCH INTEREST

In our lab we are interested in the gene regulatory networks that control the early stages of mammalian development and underlie cardiovascular disease. Our research focuses on understanding how cis-regulatory elements located in the non-coding portion of the genome influence the spatial and temporal expression of nearby genes, as well as how their activity is modulated by chromatin structure. We are also exploring how variation in these elements influences disease risk.

With these goals in mind, we have explored how three-dimensional genome structure relates to gene expression in the cardiovascular system. By using high-resolution deep-sequencing-based chromatin conformation techniques, together with CRISP/R genome editing tools, we have described how a gene-specific regulatory loop is established and is essential for proper expression of the ventricle-specific regulatory gene *Irx4*. We further show that this loop is dependent on the architectural chromatin factor CTCF during embryonic development. At present we are using similar approaches to explore the regulatory basis of atrial fibrillation, the most common type of cardiac arrhythmia and a serious health burden worldwide.

We are also exploring novel ways of using genome editing to interrogate the regulatory genome. We have validated the use of the CRISP/R system to generate transient knock-out mouse embryos, without the need to establish mouse lines. And we have used these tools to deliver a reporter cassette to a genomic location at will, in order to "read" the regulatory environment at specific genomic locations. Further development of these tools will allow us to address the nature and role of genomic regions in detail in developmental processes and cardiovascular disease.

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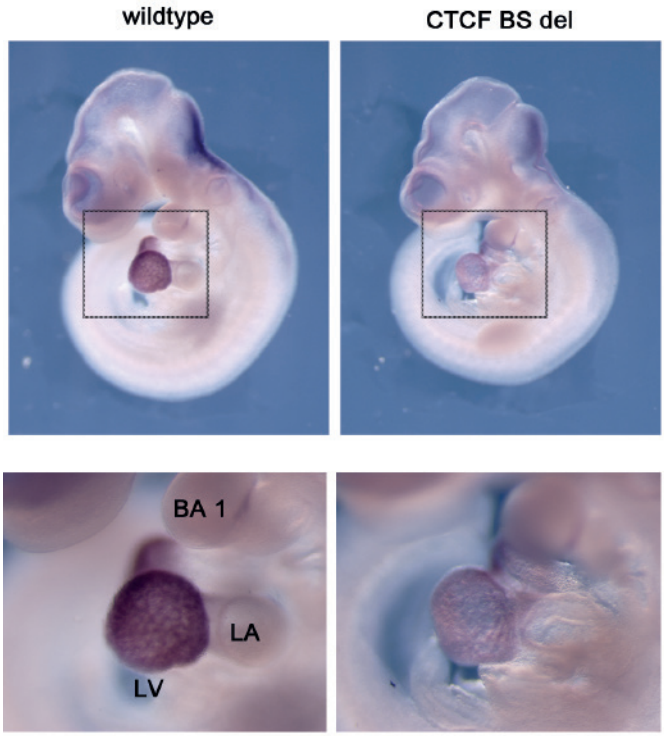
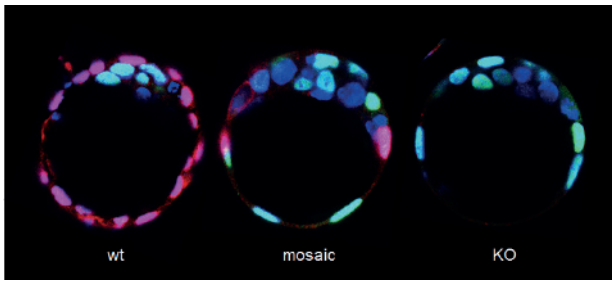
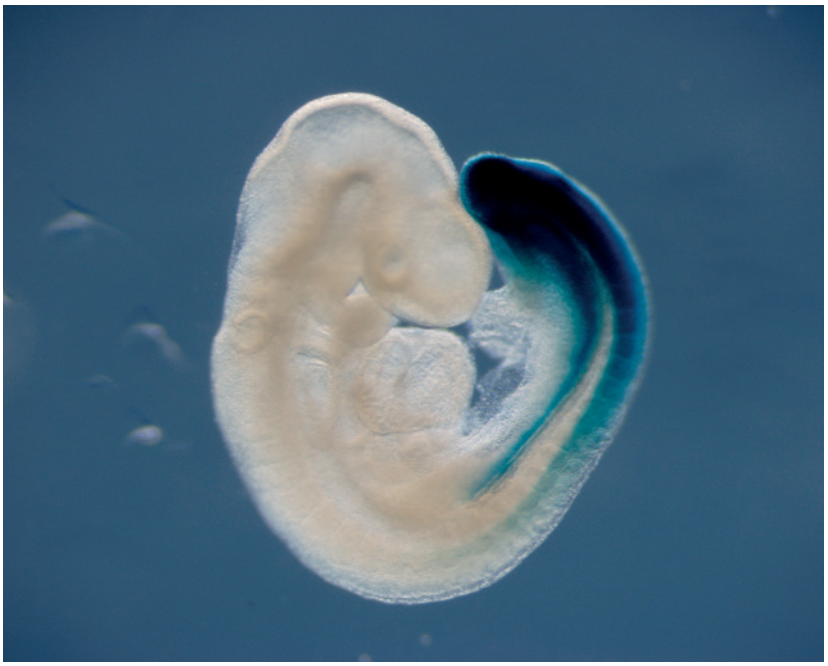
Visiting Scientist:
Gonzalo Carreño Gómez-Tarragona



Deletion by CRISP/R-based genome editing of a specific CTCF binding site in a mouse embryo results in a strong reduction of the expression of the cardiac-specific *Irx4* gene.



Genome-editing of the mouse embryo. Direct injection of Cas9 and specific guide-RNAs targeting the first coding exon of *Cdx2* into the one-cell mouse embryo leads in 3 days to blastocysts in which CDX2 expression (red) is lost partially (mosaic, middle panel) or completely (KO, right panel). Expression of the pluripotency marker NANOG (light blue) is unaffected. Nuclei are shown in dark blue.



Genome-editing of the mouse embryo. CRISP/R-mediated integration of a regulatory reporter cassette in the *Cdx2* locus reproduces the expression of this gene in the posterior part of the E9.5 embryo.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad BFU2014-57703-REDC*
- *Comunidad Autónoma de Madrid. S2010/BMD-2315 (CELLDD-CM).*
- *Fundación Centro Nacional de Investigaciones Cardiovasculares. CNIC Translational Projects (CNIC-08-2009)*

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Manzanares M. **Functional genomics of cardiovascular development and disease.** *Brief Funct Genomics* (2014) 13: 1-2

van Weerd H, Badi I, van den Boogaard M, Stefanovic S, van de Werken HJG, Gomez-Velazquez M, Badia-Careaga C, Manzanares M, de Laat W, Barnett P, Christoffels VM. **A large permissive regulatory domain exclusively controls *Tbx3* expression in the cardiac conduction system.** *Circ Res* (2014) 115: 432-41

Smemo S, Tena JJ, Kim KH, Gamazon ER, Sakabe NJ, Gómez-Marín C, Aneas I, Credidio FL, Sobreira DR, Wasserman NF, Lee JH, Puviindran V, Tam D, Shen M, Son JE, Vakili NA, Sung HK, Naranjo S, Acemel RD, Manzanares M, Nagy A, Cox NJ, Hui CC, Gomez-Skarmeta JL, Nobrega MA. **Obesity-associated variants within *FTO* form long-range functional connections with *IRX3*.** *Nature* (2014) 507: 371-5

Stem cell niche pathophysiology

Head of Laboratory
Simón Méndez Ferrer

RESEARCH INTEREST

Our group studies how the niche maintains and regulates stem cells and how its dysregulation can contribute to disease. Hematopoietic stem cells (HSCs) traffic between bone marrow and circulating blood, which is the basis of for lifesaving clinical transplantation. Our previous work showed that HSC numbers in blood are regulated by the brain, which regulates bone marrow nestin+ mesenchymal stem cells through peripheral nerves. We recently found that HSC-niche mesenchymal stem cells might be different from those that form the skeleton, instead sharing a common origin with peripheral nerves and supporting glial cells (Figure 1). Thus, tight regulation of peripheral stem-cell niches in vertebrates might build upon the developmental relationships among its cellular components. Moreover, we have shown that damage to this regulatory network is essential for the appearance of myeloproliferative neoplasms, diseases that were previously thought to be driven solely by mutated HSCs (Figure 2). Our recent data has also uncovered a selective regulation by sex hormones of the maintenance, survival and proliferation of normal and leukaemic hematopoietic progenitors. These results might explain gender differences in blood cancer incidence and also offer a new way of targeting leukemic stem cells with clinically approved drugs (Figure 3).

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Master Student:
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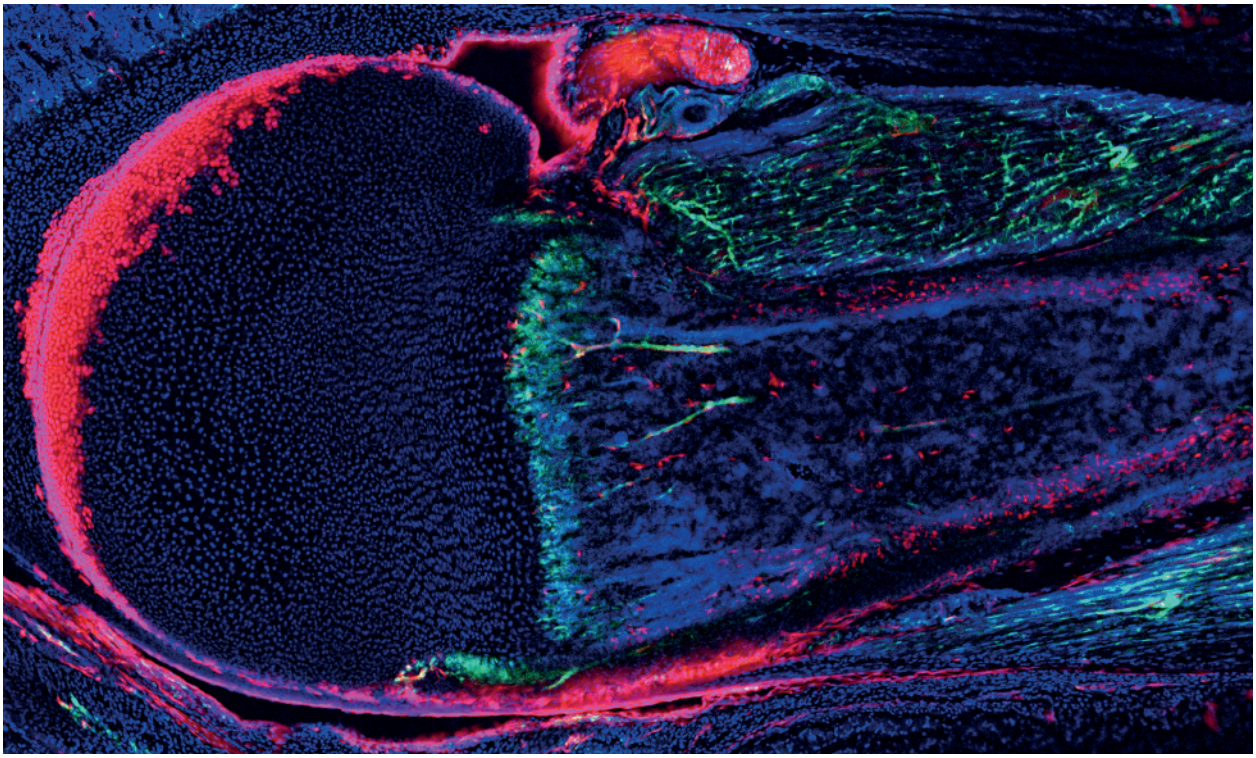
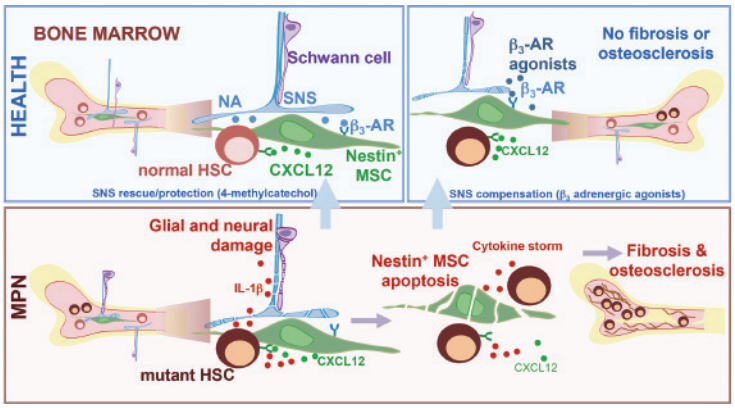
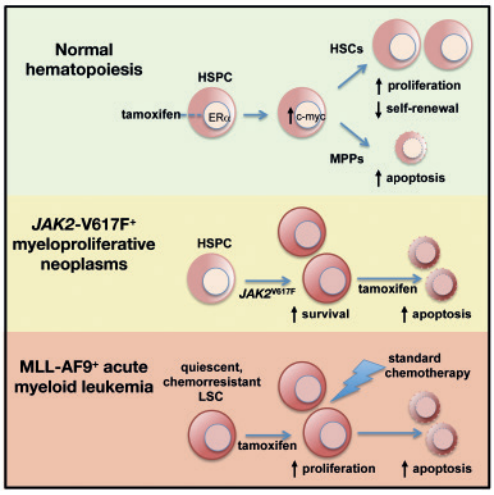


The neural crest is a source of mesenchymal stem cells with specialized functions in the hematopoietic stem cell niche. Neonatal bone marrow section from triple transgenic mouse in which neural crest-derived cells are labeled in red and nestin+ cells in green. Blue signal corresponds to cell nuclei (Isern J et al. eLife 2014).



Neuropathy of the hematopoietic stem-cell niche is essential for myeloproliferative neoplasms. Model illustrating HSC niche alterations and rescue in myeloproliferative neoplasms (MPN). HSC, hematopoietic stem cell; SNS, sympathetic nervous system; MSC, mesenchymal stem cell; NA, noradrenaline; AR, adrenergic receptor; C, control (disease-free mice). (Arranz L et al. Nature 2014)

Graphical Abstract



Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis. Treatment of leukemic mice with the selective estrogen receptor modulator tamoxifen can block the development of myeloproliferative neoplasms and sensitize acute myeloid leukemia to conventional chemotherapy (Sánchez-Aguilera A et al. Cell Stem Cell 2014)

MAJOR GRANTS

- *Howard Hughes Medical Institute. International Early Career Scientist.*
- *Comunidad de Madrid. Convocatoria de Programas de I+D en Biomedicina. (S2011/BMD-2542)*
- *Ministerio de Economía y Competividad (RYC-2011-09209)*
- *Ministerio de Ciencia e Innovación (RYC-2009-04703)*
- *Ministerio de Economia y Competitividad (RYC-2011-09726) PI: Abel Sánchez-Aguilera*
- *Ministerio de Economia y Competitividad (SAF-2011-30308)*
- *European Commission FP7. Marie Curie Career Integration Grant (294262)*
- *European Commission FP7. Marie Curie Career Integration Grant (294096) PI: Abel Sánchez-Aguilera*
- *Ministerio de Economía y Competividad (BFU2012-35892) PI: Joan Isern*

SELECTED PUBLICATIONS

Sánchez-Aguilera A, Arranz L, Martín-Pérez D, García-García A, Stavropoulou V, Kubovcakova L, Isern J, Martín-Salamanca S, Langa X, Skoda RC, Schwaller J and Méndez-Ferrer S. **Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis**. *Cell Stem Cell* (2014) 15:791-80

Arranz L, Sánchez-Aguilera A, Martín-Pérez D, Isern J, Langa X, Tzankov A, Lundberg P, Muntión S, Tzeng YS, Lai DM, Schwaller J, Skoda RC, Méndez-Ferrer S. **Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms.** *Nature* (2014) 512:78-81

Isern J, García-García A, Martín AM, Arranz L, Martín-Pérez D, Torroja C, Sánchez-Cabo F, Méndez-Ferrer S. **The neural crest is a source of mesenchymal stem cells with specialized hematopoietic stem-cell-niche function**. *eLife* (2014) 2014:03696

Zaidi M, Méndez-Ferrer S. **Cell biology: tumour stem cells in bone.** *Nature* (2013) 499:414-6

Isern J, Martín-Antonio B, Ghazanfari R, Martín AM, López JA, Del Toro R, Sánchez-Aguilera A, Arranz L, Martín-Pérez D, Suárez-Lledó M, Marín P, Van Pel M, Fibbe WE, Vázquez J, Scheding S, Urbano-Ispizúa A, Méndez-Ferrer S. **Self-Renewing Human Bone Marrow Mesenspheres Promote Hematopoietic Stem Cell Expansion.** *Cell Rep* (2013) 3:1714-24

Development of the epicardium and its role during regeneration

Head of Laboratory
Nadia Mercader

RESEARCH INTEREST

Unlike adult mammals, zebrafish have the capacity to regenerate their hearts upon several types of injury. In the laboratory, we use cryoinjury to induce cardiac tissue damage, with the aim of mimicking the consequences of tissue loss upon myocardial infarction. Our results show that cardiac fibrosis is reversible and occurs as an intermediate step during regeneration. We aim to unravel the endogenous mechanisms of myofibroblast and extracelular matrix regression, as this might have implications for the design of antifibrotic strategies. We recently examined in detail if the tissue regeneration we observe is accompanied by functional recovery. For this, we set up echocardiography to study ventricular pumping efficiency in the zebrafish. Our results reveal that cryoinjury transiently impairs ventricular fractional volume shortening, but that pumping efficiency recovers completely at later postinjury stages. However, many operated fish show long-term alterations in ventricular wall contraction. Echocardiography thus allows a deeper understanding of the mechanisms of cardiac regeneration. One of the first layers to reestablish during regeneration is the epicardium, the outer layer covering the myocardium. We are interested in how the epicardium forms during embryonic development. Using live imaging in zebrafish embryos we are studying the mechanisms through which the proepicardial cells emerge from the pericardial wall and attach to the myocardium. We found that proepicardium formation is dependent on the beating heart. Our current effort s are dedicated to understanding the underlying mechanosensory pathways.

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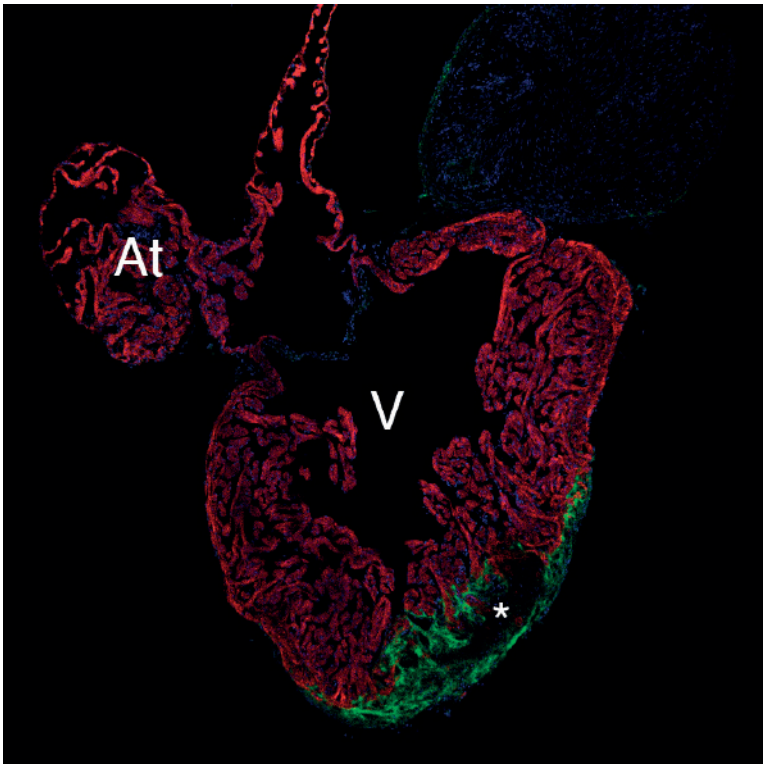
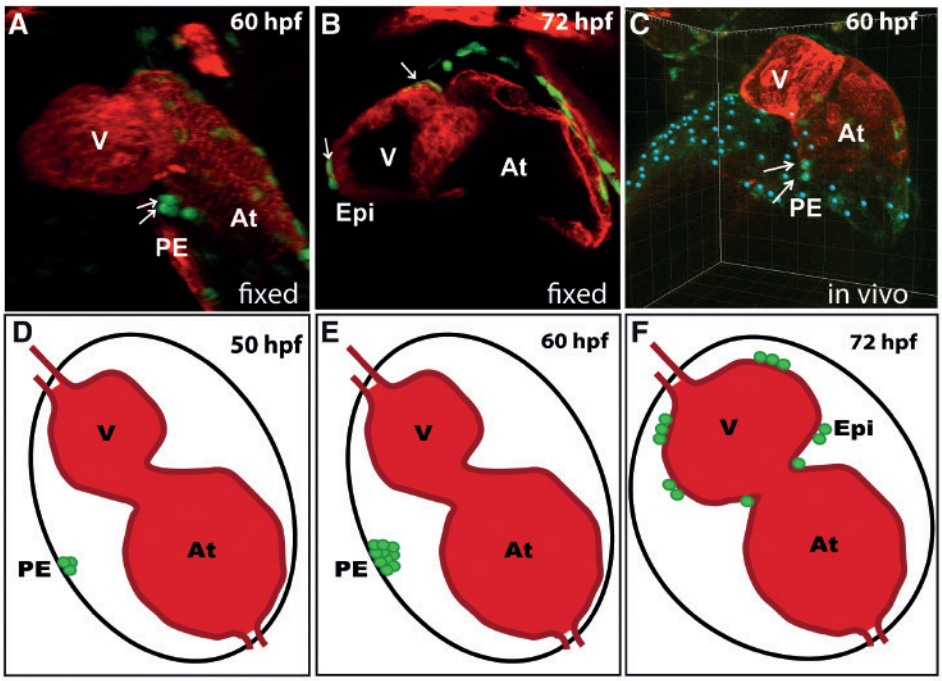


Heart regeneration upon cryoinjury in the zebrafish is preceded by fibrotic tissue deposition. Immunofluorescence with anti-collagen 1 (green) and anti-myosin heavy chain (red) antibodies. The asterisk marks the cryoinjured region. At, atrium; V, ventricle.



In vivo imaging of the developing epicardium in the zebrafish. A and B show whole-mount immunostaining of a transgenic reporter line labeling proepicardial and epicardial cells in green and the myocardium in red. At 60 hours postfertilization (hpf), proepicardial (PE) cells are visible at the inflow tract of the embryonic heart tube. At 72 hpf, some of these cells have attached to the myocardial wall, contributing to epicardium formation. C is a snapshot of a heart in a live embryo of the same transgenic line. This model allows tracking of PE cells and pericardial cells to determine the morphgenetic events leading to epicardium formation. D-F are schematic illustrations of epicardium formation. At, atrium; Epi, epicardium; PE, proepicardium; V, ventricle.

MAJOR GRANTS

- *Ministerio de Economia y Competividad (BFU2011-25297)*
- *Comunidad de Madrid (P2010/BMD-2321)*
- *Tercel (Red de Terapia Celular) (PI: M. Torres)*
- *European Commission. European Research Council Starting Independent Researcher Grant (ERC-337703 2013)*

SELECTED PUBLICATIONS

Bednarek D, González-Rosa JM, Guzmán-Martínez G, Gutiérrez-Gutiérrez Ó, Aguado T, Sánchez-Ferrer C, Marques IJ, Galardi-Castilla M, de Diego I, Gómez MJ, Cortés A, Zapata A, Jiménez-Borreguero LJ, Mercader N*, Flores I*. **Telomerase is essential for Zebrafish Heart Regeneration.** *Cell Rep.* (2015) 12: 1691-703
*Co-corresponding author

Peralta M*, González-Rosa JM*, Marques IJ, Mercader N. **The epicardium in the embryonic and adult zebrafish**. *J Dev Biol* (2014) 2: 101-16
*Equal contribution

Gonzalez-Rosa JM, Guzman-Martinez G, Marques IJ, Sanchez-Iranzo H, Jimenez-Borreguero LJ, Mercader N. **Use of echocardiography reveals reestablishment of ventricular pumping efficiency and partial ventricular wall motion recovery upon ventricular cryoinjury in the zebrafish.** *PLoS One* (2014) 9: e115604

Rodius S, Nazarov PV, Nepomuceno-Chamorro IA, Jeanty C, González-Rosa JM, Ibberson M, da Costa RM, Xenarios I, Mercader N, Azuaje F. **Transcriptional response to cardiac injury in the zebrafish: systematic identification of genes with highly concordant activity across in vivo models.** *BMC Genomics* (2014) 15: 852

Hermann M, Stillhard P, Wildner H, Seruggia D, Kapp V, Sanchez-Iranzo H, Mercader N, Montoliu L, Zeilhofer HU and Pelczar P. **Binary recombinase systems for high-resolution conditional mutagenesis.** *Nucleic Acids Res* (2014) 42: 3894-907

Genetic control of organ development and regeneration

Head of Laboratory

Miguel Torres

RESEARCH INTEREST

We are interested in understanding the cellular basis of developmental processes and how this is contolled by transcription factor networks (TFN). We have developed genetic methods in the mouse that allow us to trace cell lineages in clonal analysis or functional mosaics. Furthermore, we have established culture methods for the live analysis of developmental processes in embryonic stem cells and in the early mouse embryo. Using these new approaches we have described the relevance of cell competition in the early mouse embryo and in the cardiomyocyte lineage of the developing and adult heart. We are currently exploring the molecular and cellular mechanisms underlying cell-cell comparison and loser-cell elimination.

In recent years we have identified the role of *Meis* transcription factors in organogenesis, including limb, eye, cardiovascular and hematopoietic system development. We have formulated new molecular models underlying the activity of the Meis TFN in pattern formation and organ regeneration. Furthermore, we have identified Myc-driven cell competition as a strategy for stimulating the proliferation and replacement of adult cardiomyocyte populations without compromising cardiac function. A current focus of the lab is the transcriptional control of cardiomyocyte proliferation in the adult heart and its impact on cardiac function and repair. Based on evidence from animal models, we hypothesize that the Myc and Meis TFNs play essential roles in controlling adult cardiomyocyte proliferation and cardiac repair. We are currently developing animal models to test these ideas.

Research Scientists:
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Alejandra Cristina López Delgado
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Ester de la Cruz Crespillo
Lin Li

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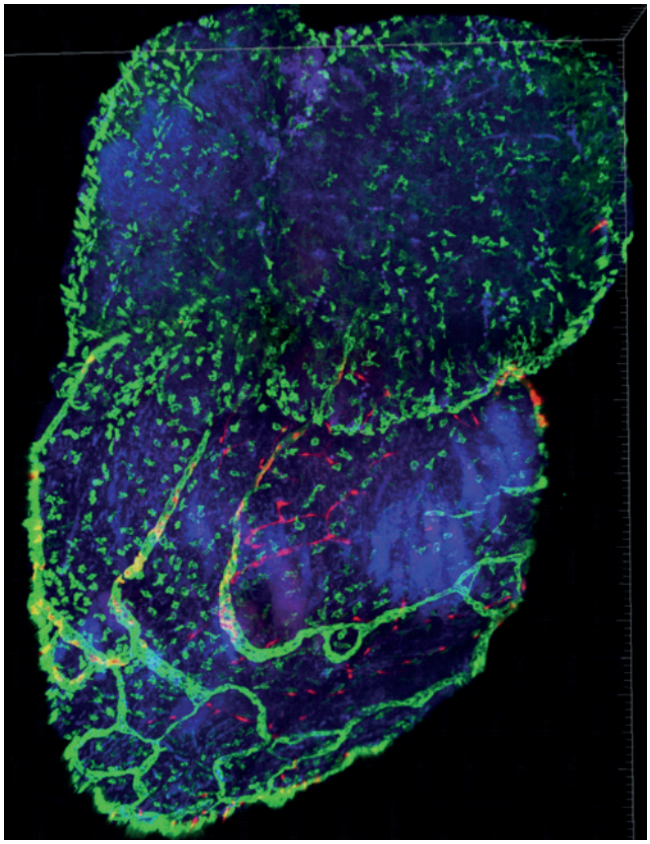
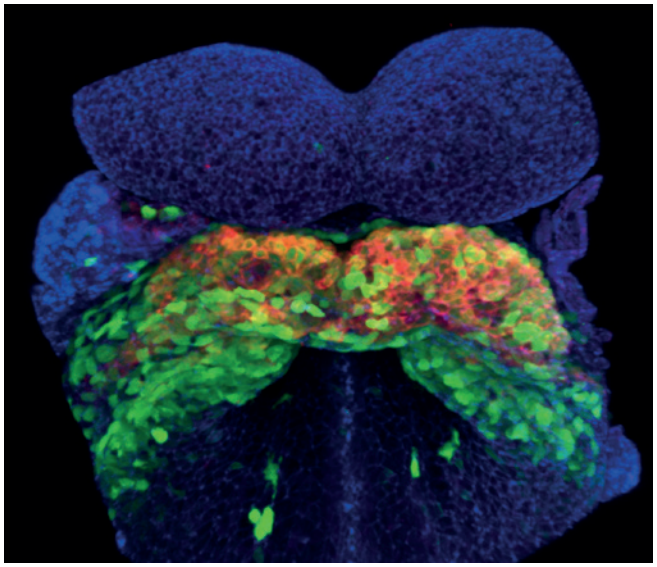
Rocío Sierra Muñoz
Susana Temiño Valbuena

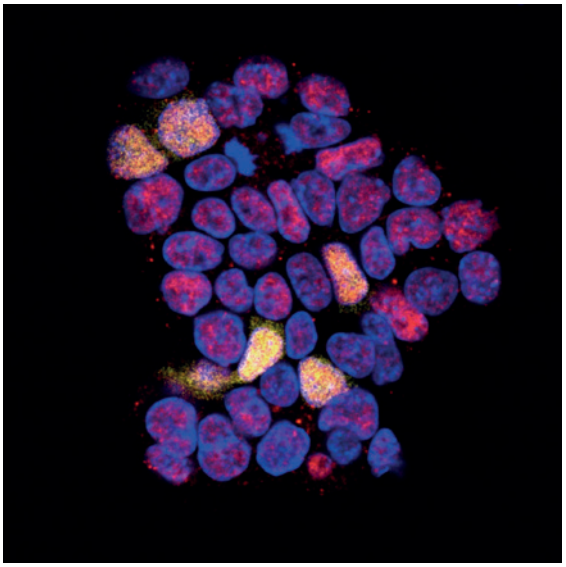
Visiting Scientist:
Juan José Sanz-Ezquerro



Clonal analysis of heart lineages. Image: Ghislaine Lioux
3D confocal reconstruction of a neonatal heart, showing lymphatics (green) and random clones (red) affecting the lymphatic and glial cell lineages.



Early mouse heart development. Image: Kenzo Ivanovitch
3D confocal reconstruction of a neonatal heart, showing all cardiac mesoderm (green) and differentiated primitive cardiomyocytes (green+red).



Myc expression dynamics in ES cells. Image: Covandonga Díaz
An ES cell colony showing nuclei (blue), Myc expression levels (pink) and clonally related cells (yellow).

MAJOR GRANTS

- *Comunidad de Madrid (S2010/BMD-2315)*
- *Ministerio de Economía y Competividad. FIS RETICS (TERCEL: RD12/0019/0005)*
- *Ministerio de Economía y Competividad (BFU2012-310862013-15)*
- *European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2011-ITN, "CardioNeT" 289600) (Coordinador E. Lara)*
- *Ministerio de Economía y Competividad. Red de Excelencia Temática. (BFU2015-70193-REDT)*
- *ERANET-NEURO European Commission and Ministerio de Economía y Competividad. (PCIN-2015-020)*
- *Ministerio de Economía y Competividad. (EUIN2015-62897)*
- *Ministerio de Economía y Competividad. Juan de la Cierva Incorporación. (IJCI-2014-19108). PI: I. Delgado*

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Nat Rev Cancer. (2015) 16: 43-55
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Marcos S, González-Lázaro M, Beccari L, Carramolino L, Martin-Bermejo MJ, Amarie O, Mateos-San Martín D, Torroja C, Bogdanović O, Doohan R, Puk O, Hrabě de Angelis M, Graw J, Gomez-Skarmeta JL, Casares F, Torres M*, Bovolenta P*.
Meis1 coordinates a network of genes implicated in eye development and microphthalmia. *Development* (2015) 142: 3009-20
*Co-corresponding authors

Villa Del Campo C, Claveria C, Sierra R, Torres M **Cell competition promotes phenotypically silent cardiomyocyte replacement in the mammalian heart**. *Cell Rep* (2014) 8: 1741-51

Padron-Barthe L, Temino S, Villa Del Campo C, Carramolino L, Isern J, Torres M **Clonal analysis identifies hemogenic endothelium and not hemangioblasts as the source of the blood-endothelial common lineage in the mouse embryo**. *Blood* (2014) 124: 2523-32

Rosello-Diez A, Arques CG, Delgado I, Giovinazzo G, Torres M. **Diffusible signals and epigenetic timing cooperate in late proximo-distal limb patterning**. *Development* (2014) 141: 1534-43