RESEARCH AREAS

TRANSLATIONAL COORDINATION

1. Myocardial Pathophysiology
2. Vascular Pathophysiology
3. Cell and Developmental Biology

3. Cell and Developmental Biology

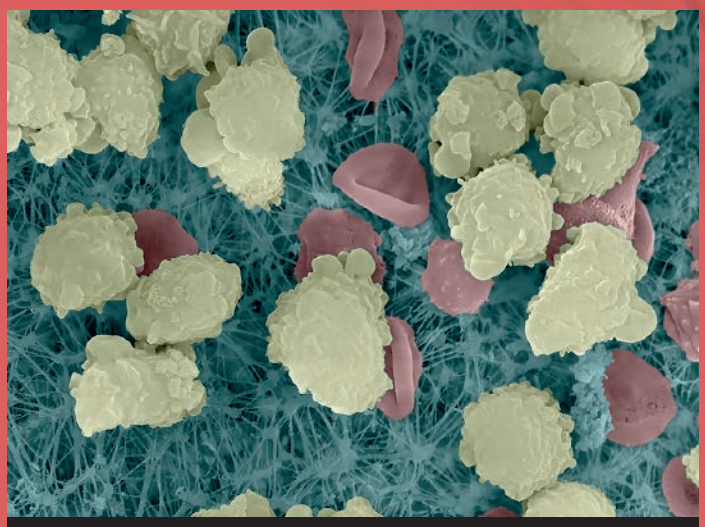
AREA COORDINATORS:

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.



Scanning electron micrograph of aged neutrophils (yellow) and erythrocytes (red) on a synthetic substrate (green).

Molecular mechanics of the cardiovascular system

RESEARCH INTEREST

Our group investigates how the mechanical activity of the heart emerges from the nanomechanical properties of cardiac proteins. We hypothesize that several cardiac diseases result from impaired function of proteins with key mechanical roles or are aggravated by maladaptive modifications that affect protein mechanics. Our group aims define how the mechanical properties of cardiac proteins, as determined by single-molecule atomic force microscopy (AFM), translate into the macroscopic function of the heart. In 2016, we found that several mutant forms of cardiac myosin-binding protein C (cMyBP-C) that cause hypertrophic cardiomyopathy have altered mechanical properties but show no other major structural or functional changes. These mechanical alterations might be the trigger that leads to cardiac hypertrophy, an idea we aim to explore in the future using animal models. Also in 2016, we optimized 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 are now investigating if the levels of these modifications change during different forms of heart disease. As an alternative approach to bringing protein nanomechanics to the macroscopic level, we have produced new biomaterials from proteins whose mechanical properties we determine in the laboratory. For this project, we have set up a customized gel stretcher machine to systematically determine the behavior of our engineered protein hydrogels under a pulling force.

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Technician:
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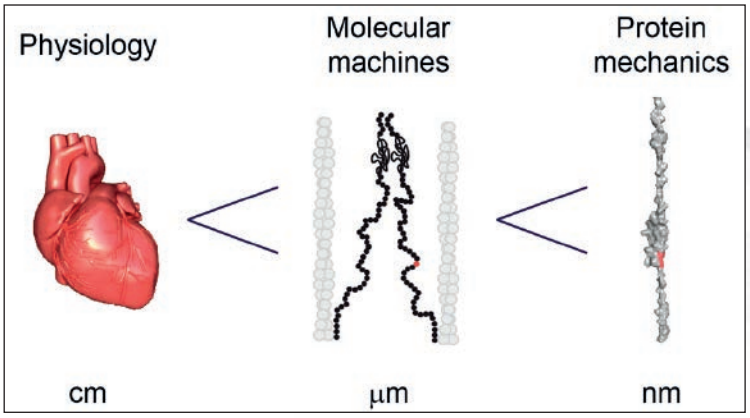
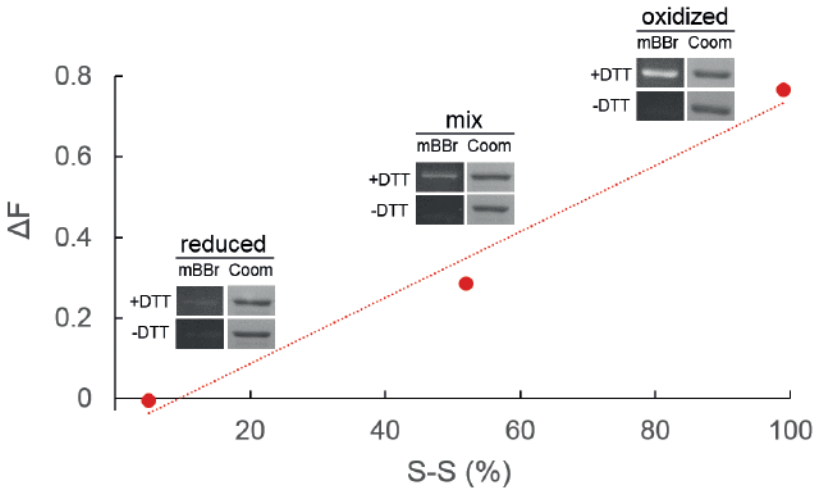


The goal of our lab is to understand how the mechanics of proteins at the nanometer scale determine the mechanical properties of the heart at the macroscopic level.



In-gel fluorescent assay to determine the redox state of cysteines in proteins. The method is based on the reduction of oxidized cysteines by incubation with DTT and subsequent labeling with monobromobimane (mBBr). mBBr reacts with free thiols, generating a flourescent adduct only in samples pretreated with DTT. We use Coomassie staining to normalize the fluorescent signal to the total amount of protein (insets). To test the method, we have used purified proteins of controlled redox state (oxidized or reduced, or mixtures of both).

MAJOR GRANTS

- Comisión Europea and ISCIII (AC16/00045)
- Comunidad de Madrid (PEJ 16/MED/TL-1593)
- Ministerio de Economía y Competitividad (BIO2014-54768-P)
- Ministerio de Economía y Competitividad (RYC-2014-16604)

SELECTED PUBLICATIONS

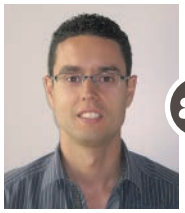
Rivera-de-Torre E, Garcia-Linares S, Alegre-Cebollada J, Lacadena J, Gavilanes JG, Martinez-Del-Pozo A. **Synergistic Action of Actinoporin Isoforms from the Same Sea Anemone Species Assembled Into Functionally Active Heteropores**. *J Biol Chem* (2016) 291: 14109-19

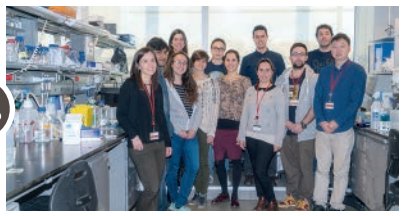
Echelman DJ, Alegre-Cebollada J, Badilla CL, Chang C, Ton-That H, Fernandez JM. **CnaA domains in bacterial pili are efficient dissipaters of large mechanical shocks**. *Proc Natl Acad Sci USA* (2016) 113: 2490-5

Saqlain F, Popa I, Fernandez 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, Ramirez-Sarmiento CA, Fernandez JM, Guixe V. **Identifying sequential substrate binding at the single-molecule level by enzyme mechanical stabilization**. *Acs Nano* (2015) 9: 3996-4005

Molecular genetics of angiogenesis





RESEARCH INTEREST

Our group investigates the cellular and molecular mechanisms involved in the formation and homeostasis of blood vessels in different organs and pathological contexts. Therapeutic modulation of vascular structure and function in disease remains a major challenge, in part due to our inability to induce the exact mechanisms that vessels use to grow under normal physiological conditions.

This past year we revisited and challenged some settled concepts in vascular biology by using new genetic tools that enable us to study the function of vascular genes at higher cellular resolution. We developed new transgenic and gene-targeting strategies to perform conditional mosaic gene function analysis. We also identified a molecular mechanism that prevents excessive and unsustained angiogenesis in the presence of a high mitogenic stimulus. This mechanism is important in the setting of active angiogenesis and high VEGF signaling, as occurs in tumors or after cardiac injury. With this knowledge, we are now working to develop more efficient ways to promote sustained and functional vascular growth.

In addition, we continued to investigate some of the most basic mechanisms involved in the differentiation of endothelial cells into hematopoietic stem and progenitor cells and also how some genes control the development, differentiation, and homeostasis of coronary vessels.

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Sarita Saraswati

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Irene García González
Briane D. Laruy
Carlos López Fernández de Castillejo
Samuel Pontes Querol

Graduate Technicians:
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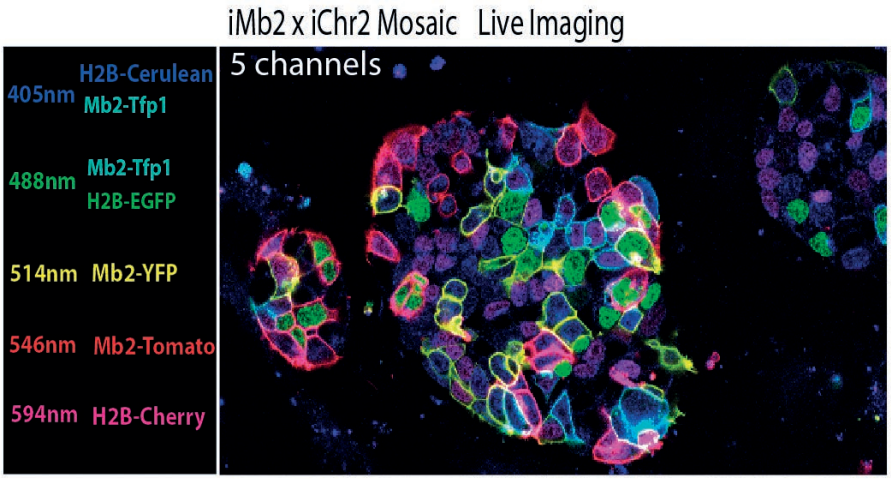
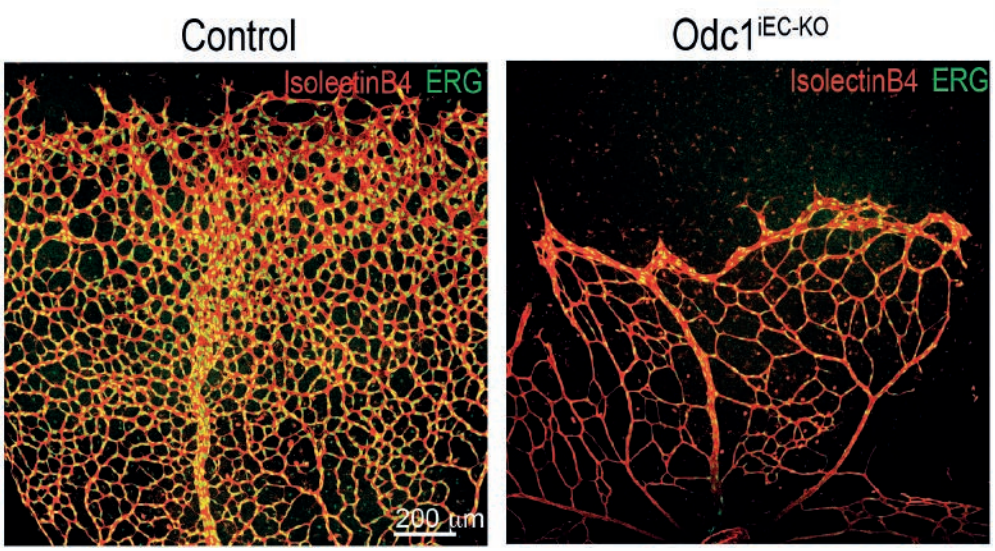
Ana Hermoso Castro

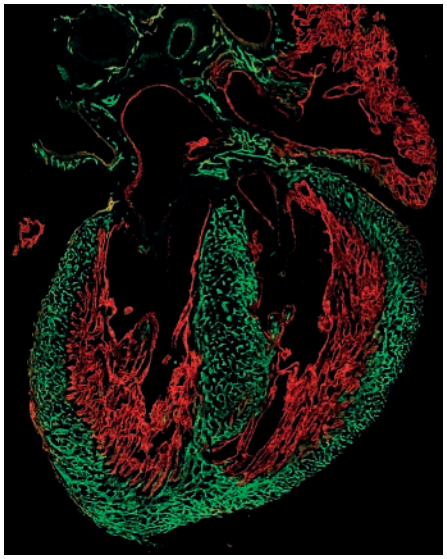
Technician:
M. Sofía Sánchez Muñoz



Multi-channel high-speed confocal imaging of cells expressing different combinations of fluorescent proteins and genes involved in the control of endothelial differentiation and proliferation.



We found Odc1 to be a very important gene for angiogenesis. The formation of new blood vessels is completely blocked in mutant mice specifically lacking Odc1 function in endothelial cells for 4 days.



We used new inducible CreERT2 and reporter mouse lines to genetically target and specifically label the coronary vessels (green) and distinguish them from the endocardium (red). This new methodology will allow us to characterize the role of different genes in coronary vessel development at high molecular and cellular resolution.

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. Posdoctoral contract. PI: Tania Sánchez (FPDI-2013-18049)
- European Commission. International IPP contract. PI: Wen Luo
- European Commission. International IPP contract. PI: Sarita Saraswati
- Fundación La Caixa CNIC Severo Ochoa. Predoctoral Fellowship. PI: Samuel Pontes
- Fundación La Caixa. Predoctoral Fellowship. PI: Macarena Fernández
- Ministerio de Economía y Competitividad. Predoctoral contract (BES-2014-069205) PI: Briane Laruy
- Fundación La Caixa CNIC Severo Ochoa. Predoctoral Fellowship. PI: Irene García
- Boheringer Ingelheim Fons. Predoctoral Fellowship. PI: Carlos López Fernández de Castillejo

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**. *Nat Cell Biol* (2016) 18: 7-20

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

Multidisciplinary translational cardiovascular research

Head of Laboratory:
Héctor Bueno

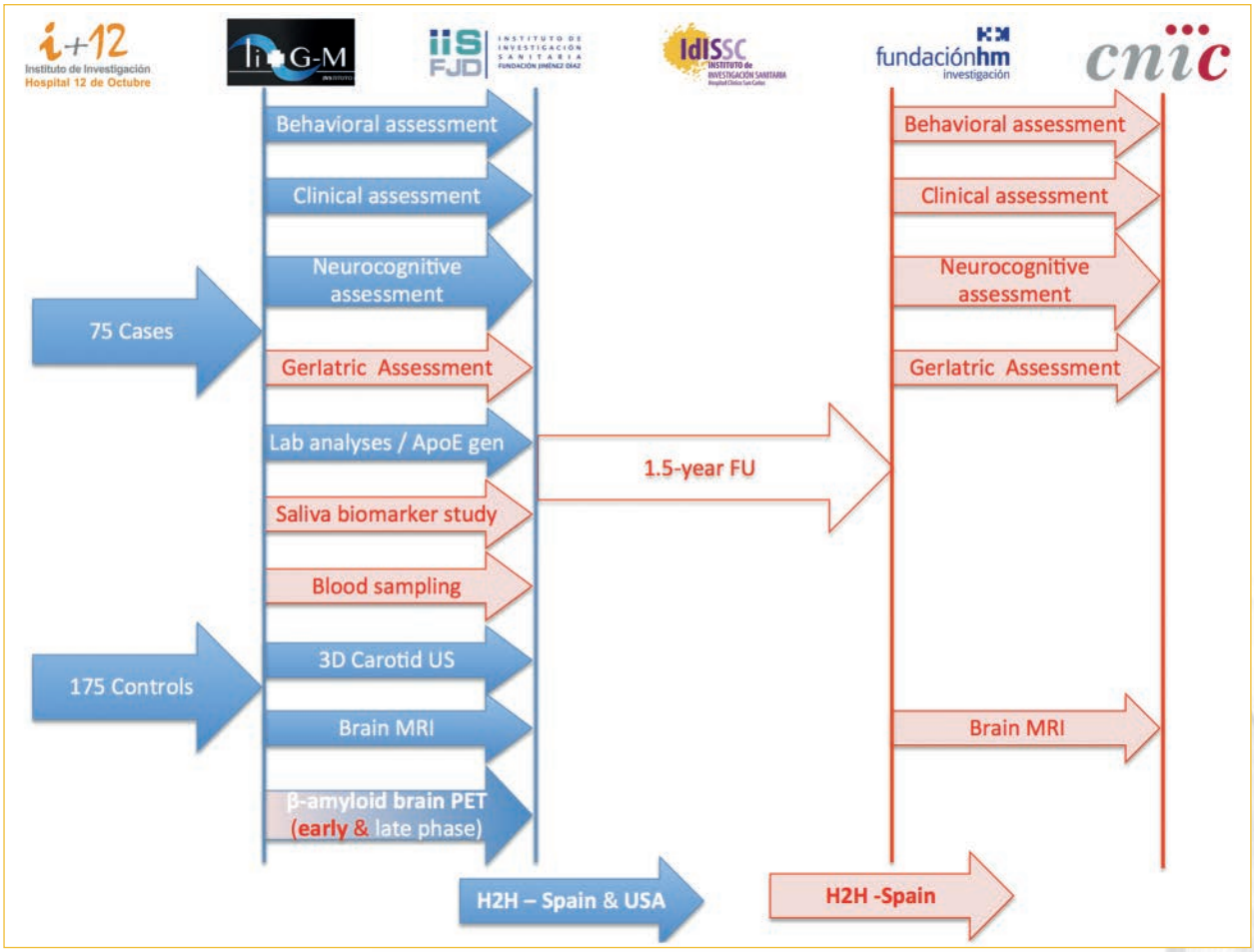
Visiting Scientists:
Alejandro Cortés,
Cardiologist
Juan Górriz,
Cardiology resident
Ana Ramos,
Neuroradilogist
Adolfo Gómez,
Specialist in Nuclear Medicine

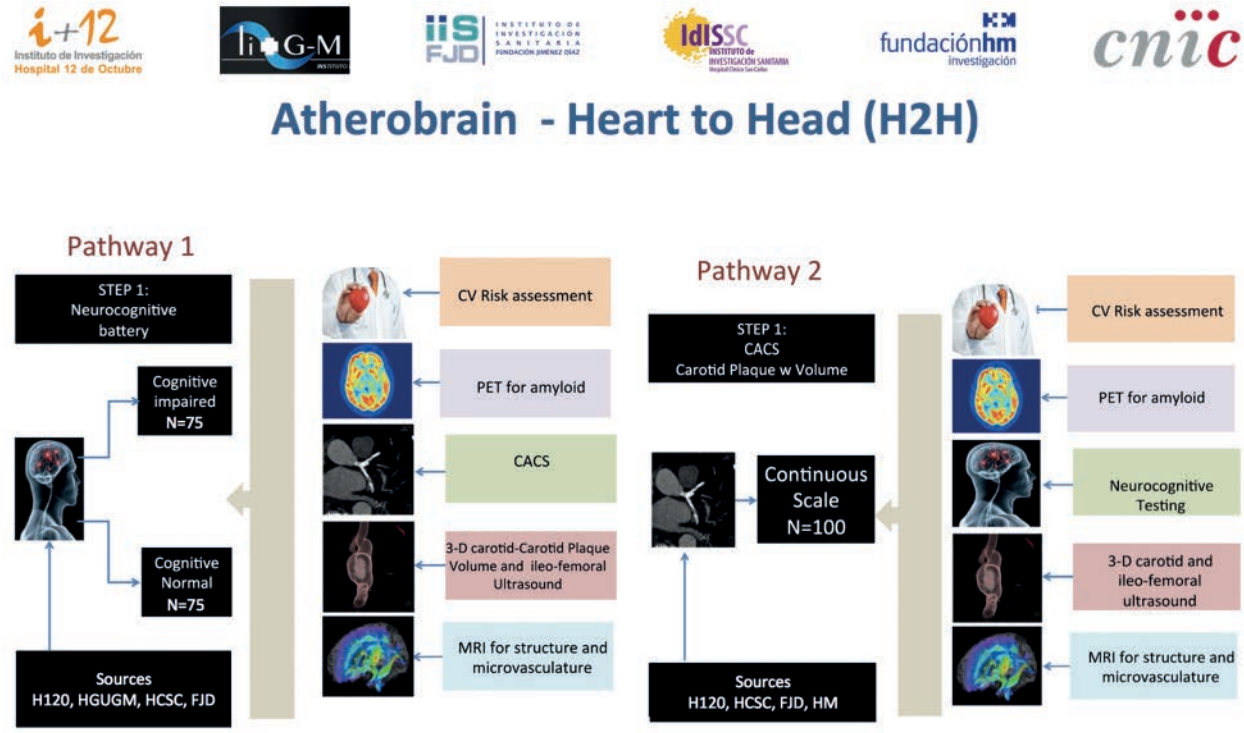
RESEARCH INTEREST

The MTCR group has a strong connection with clinical research in different fields, including atherosclerosis, acute coronary syndromes, acute and chronic heart failure, pulmonary hypertension, cardiovascular ageing and frailty, heart-valve disease, advanced cardiovascular imaging, and genetic and familial cardiovascular diseases. The MTCR group participates actively in the main CNIC translational projects, including the PESA study and the SECURE trial comparing the polypill with standard care for secondary CV prevention.

A major interest of the group is non-standard pathophysiological connections between the cardiovascular system and brain function. A key project in this area is the Atherobrain - Heart to Head (H2H) study. This ISCIII-funded project is run through partnership between the CNIC imaging area, the i+12 institute and *Hospital 12 de Octubre*, and and several hospitals (*12 de Octubre, Gregorio Marañón, Clínico San Carlos, Fundación Jiménez Díaz,* and *Hospitales de Madrid*). H2H examines the relationship between subclinical atherosclerosis, cognitive decline, and Alzheimer´s disease. Related interests include the pathophysiology of stress cardiomyopathy (Tako-Tsubo syndrome) and the role of a positive mental attitude in CV disease patients.

We work in partnership with other CNIC research groups in several research fields, including the basic mechanisms of the early atherosclerosis development (MA Del Pozo and Jacob Benzon), the role of specific microRNAs in cardiovascular disease (Almudena Ramiro and Pilar Martín), the role of progerin and lamin A in aging and atherosclerotic disease (Vicente Andrés), mechanical properties of myocardium and derived translational models (Jorge Alegre), basic mechanisms of the pathophysiology of pulmonary hypertension (Jesus Cabello), and new therapies for pulmonary hypertension (Borja Ibañez).





MAJOR GRANTS

- Ministerio de Economía y Competitividad (PIE16/00021)

SELECTED PUBLICATIONS

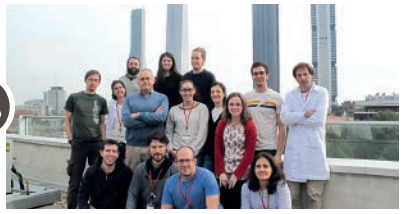
Vidán MT, Blaya-Novakova V, Sánchez E, Ortiz J, Serra-Rexach JA, Bueno H. **Prevalence and prognostic impact of frailty and its components in non-dependent elderly patients with heart failure.** *Eur J Heart Fail* (2016) 18(7):869-75.

Hall M, Dondo TB, Yan AT, Goodman SG, Bueno H, Chew DP, Brieger D, Timmis A, Batin PD, Deanfield JE, Hemingway H, Fox KA, Gale CP. **Association of Clinical Factors and Therapeutic Strategies With Improvements in Survival Following Non-ST-Elevation Myocardial Infarction, 2003-2013.** *JAMA* (2016) 316(10):1073-82.

Peñalvo J, Fernandez-Friera L, Lopez-Melgar B, Uzhova I, Oliva B, Fernández-Alvira JM, Laclaustra M, Pocock S, Mocoroa A, Mendiguren JM, Sanz G, Guallar E, Bansilal S, Vedanthan R, Jiménez-Borreguero J, Ordovas jm, Fernandez-Ortiz A, Bueno H, Fuster V. **Association between a social-business eating pattern and early asymptomatic aterosclerosis**. *J Am Coll Cardiol* (2016) 68(8):805-14.

Ponikowski P, Voors AA, Anker SK, Bueno H, Cleland J, Coats A, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska E, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley J, Rosano G, Ruilope L, Ruschitzka F, Rutten FH, van der Meer, Filippatos G, McMurray JJV. 2016 **ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure.** *Eur Heart J* (2016) 37(27):2129-2200.

Mechanoadaptation and Caveolae Biology



RESEARCH INTEREST

Our long-term aim is to understand reciprocal communication between cells and their environment, with a focus on the biological roles of integrin signaling and caveolae and their components. Caveolae are actin-linked plasma membrane nano-invaginations, abundant in mechanically stressed tissues (heart, vessels, muscle, and fat). Among their many functions, caveolae transduce mechanical cues and communicate tensile stress between cells and the extracellular matrix (ECM), thus driving ECM remodeling; however, understanding is limited about how this happens and how it is coordinated with other cell functions. Ongoing projects address these questions at three levels:

(1) Molecular mechanisms mediating cell-ECM communication and mechanotransduction

We use state-of-the-art cell biology and biophysics methodologies to characterize the contribution of essential caveolar components (such as Cav1 and PTRF) to mechanosensing and the reciprocal interaction between the cell and the ECM. We combine these approaches with high-throughput techniques (HCScreens, quantitative transcriptomics and proteomics, and MS-based interactomics). We recently implemented microfluidics approaches to elucidate whether and how these mechanisms are engaged in the vasculature to counter mechanical challenges derived from blood flow. Reflecting these interests, we co-organized the CNIC conference on Mechanical Forces in Physiology and Disease, which brought together international leaders in the field.

(2) Crosstalk between integrin-associated and caveolar-associated functions and other cell functions

We are studying novel relationships between caveolar components and functions such as organelle trafficking and homeostasis, metabolism, and cell differentation. Our studies support a role for Cav1 in the communication between endoplasmic reticulum and mitochondria, which might specifically enable different steps of fatty acid and cholesterol metabolism. We are also exploring the role of Cav1 in the orchestration of key pleiotropic signaling pathways, such as TGFbeta and Wnt.

(3) Contribution of caveolin-dependent mechanotransduction and signaling regulation to physiology and disease

Our work with different Cav1 KO models reveals a pervasive impact of Cav1 on the regulation of lipid metabolism (fig. 2), ECM remodeling, and mechanotransduction. We are studying the impact of these different contributions to organismal homeostasis and disease.

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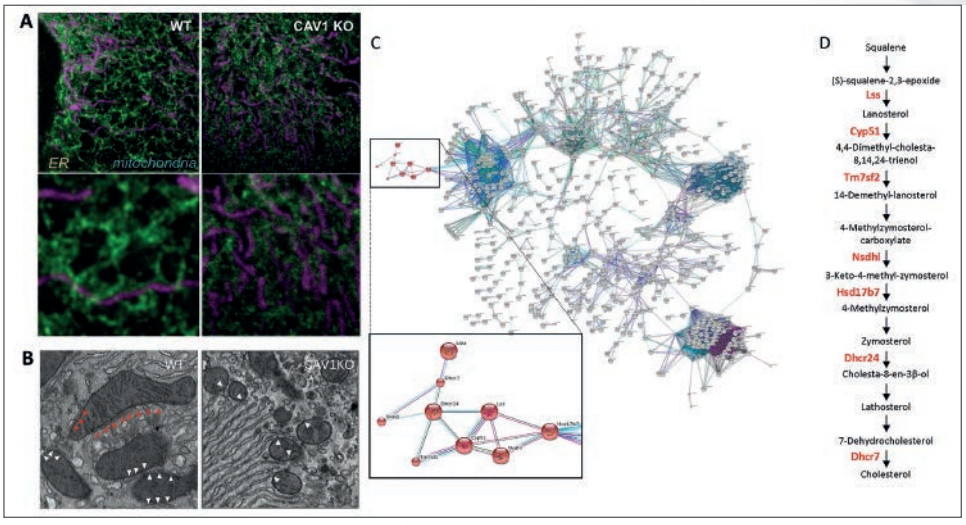
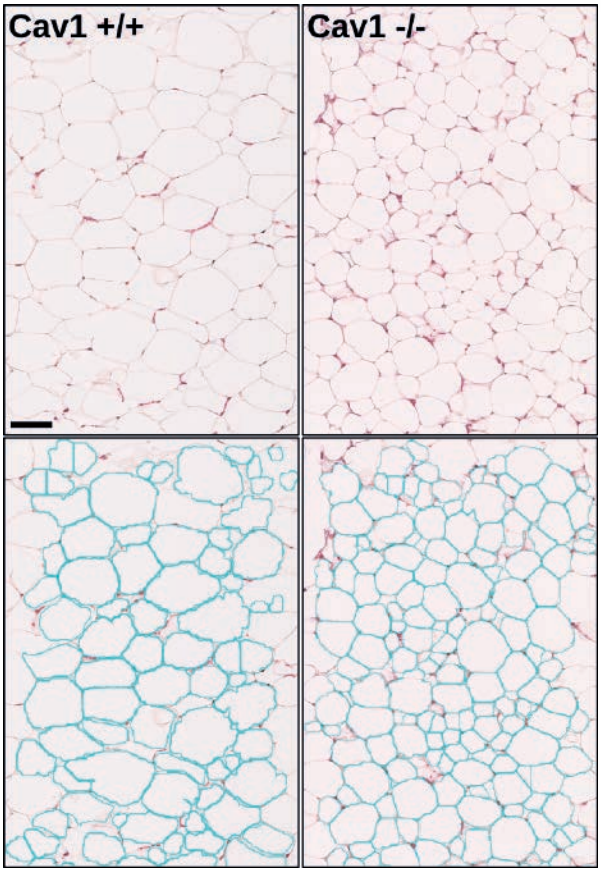


Visualization of the spatial relationship of endoplasmic reticulum (ER) and mitochondrial networks by (A) optical superresolution microscopy and (B) electron microscopy. Cav1 KO cells exhibit disorganized networks of tubular ER, partial fragmentation of mitochondria, and reduced extension of ER-mitochondria contacts. (C and D) Genetic networks established by enriched components of ER-mitochondria contacts include conserved modules for endogenous cholesterol anabolism in hepatocytes, which are profoundly affected upon Cav1 downregulation.



Top. Masson's trichrome staining of visceral Cav1$^{+/+}$ and Cav1$^{-/-}$ adipose tissue sections. Lipodystrophic syndrome is a hallmark of caveolinopathies, and one of its features is the lower volume and altered functioning of adipocytes.

Bottom. Computer vision segmentation allows unbiased quantitation of single-cell level properties (collaboration with Daniel Jiménez, Cellomics Unit, CNIC). Scale bar, 100µm.

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

Sala-Vila A, Navarro-Lérida I, Sánchez-Alvarez M, Bosch M, Calvo C, López JA, Calvo E, Ferguson C, Giacomello M, Serafini A, Scorrano L, Enriquez JA, Balsinde J, Parton RG, Vázquez J, Pol A, Del Pozo MA. Interplay between hepatic mitochondria-associated membranes, lipid metabolism and caveolin-1 in mice. *Sci Rep* (2016) 6: 27351

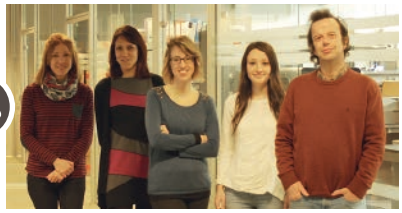
Schönle A, Hartl FA, Mentzel J, Nöltner T, Rauch KS, Prestipino A, Wohlfeil SA, Apostolova P, Hechinger AK, Melchinger W, Fehrenbach K, Guadamillas MC, Follo M, Prinz G, Ruess AK, Pfeifer D, Del Pozo M.A, Schmitt-Graeff A, Duyster J, Hippen KI, Blazar BR, Schachtrup K, Minguet S and Zeiser R. **Caveolin-1 regulates TCR signal strength and regulatory T cell differentiation into alloreactive T cells**. *Blood* (2016) 127: 1930-9

Bravo-Cordero JJ, Cordani M, Díez-Cabezas B, Muñoz-Agudo C, Casanova M, Boullosa C, Guadamillas MC, Ezkurdia I, Soriano SF, González-Pisano D, del Pozo MA and Montoya MC*. **A novel high content analysis tool reveals Rab8-driven actin and FA reorganization through Rho GTPases and calpain/MT1**. *J Cell Sci* (2016) 129: 1734-49

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

Regeneration and aging



RESEARCH INTEREST

Our group studies the molecular mechanisms involved 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% of 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 2015-2016, we found a correlation between the activity of the anti-aging enzyme telomerase and the degree of heart regeneration: relatively high telomerase activity in adult zebrafish and newborn mice, contrasting with low activity in juvenile and adult mice. This prompted us to study in more detail the role of telomerase and telomere length in the process of heart regeneration. In the zebrafish, we found that telomerase is essential for heart regeneration. The inability of zebrafish hearts lacking telomerase to regenerate is mainly due to strong inhibition of the proliferation response, associated with accumulation of cardiac cells with DNA damage and senescence characteristics (Bednarek *et al*. 2015). In the mouse, we found that telomerase is rapidly inactivated during postnatal cardiac maturation and that cardiomyocytes undergo telomere shortening. We also found that telomere shortening activates a DNA damage response, triggers the formation of anaphase bridges, and upregulates the cell-cycle inhibitor p21, leading to the cell-cycle arrest of postnatal cardiomyocytes (Aix *et al*. 2016). We also discovered that telomere length defines the cardiomyocyte differentiation potential of mouse induced pluripotent stem cells (iPSCs). This finding highlights the importance of selecting iPSCs with ample telomere reserves in order to generate high numbers of cardiomyocytes in a fast, reliable, and efficient way (Aguado *et al*. 2016)

Through these efforts, we hope to achieve a more complete knowledge of the role of telomerase and telomere length in cardiomyocyte proliferation and heart regeneration, which could lead to new therapies for heart failure.

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Technician:
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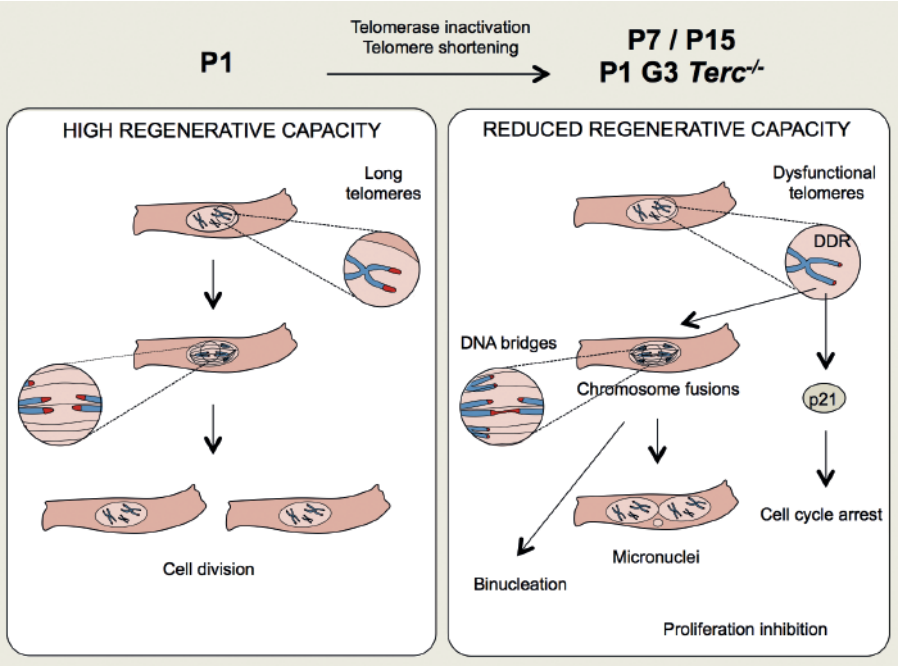
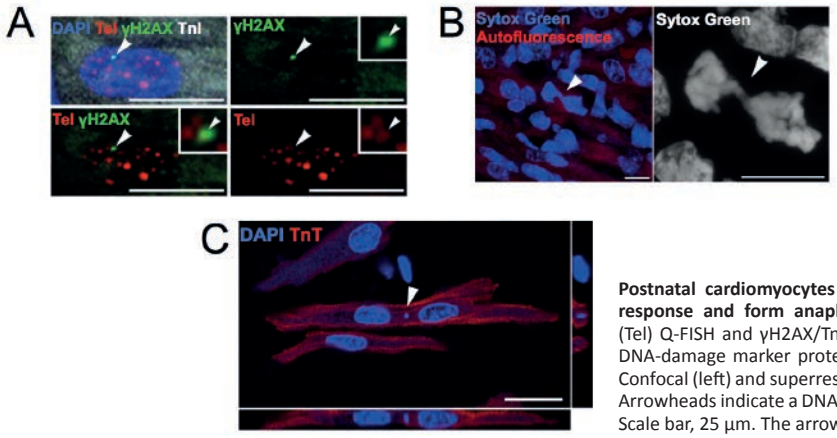
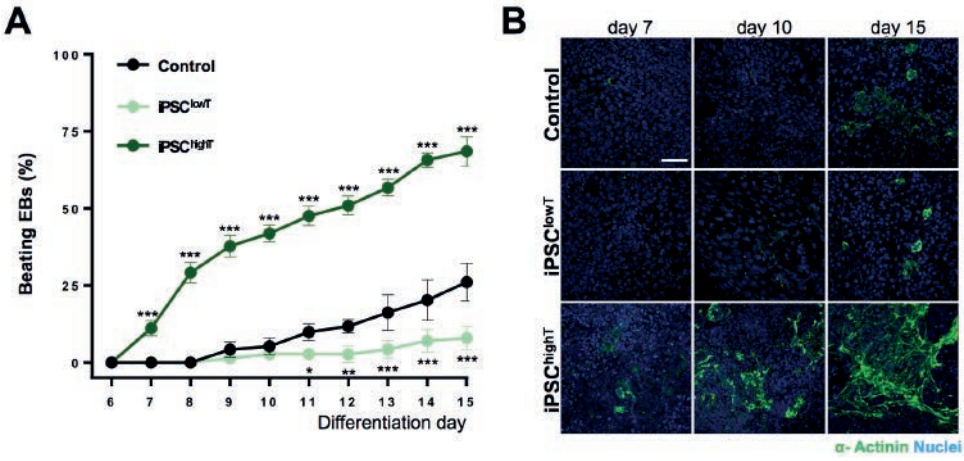


Postnatal telomere dysfunction induces cardiomyocyte cell-cycle arrest through p21 activation: proposed model. At postnatal day P1, a proportion of cardiomyocytes (CM) presents long telomeres, giving them potential to proliferate during postnatal development and in response to cardiac injury. However, during the first two postnatal weeks, most CMs inactivate telomerase and shorten their telomeres. Telomere shortening leads to the appearance of dysfunctional damaged telomeres, chromosome fusions, micronuclei, and binucleation, and at the same time activates p21, ultimately leading to CM cell-cycle arrest. CMs with premature telomere shortening (P1 G3 *Terc*$^{-/-}$ CMs) precociously activate the DNA damage response at telomeres, form anaphase bridges, upregulate p21, and binucleate. These outcomes reinforce the role of telomere shortening in CM cell-cycle withdrawal.



Postnatal cardiomyocytes with telomere shortening activate the DNA damage response and form anaphase bridges and micronuclei. (**A**) Detail of telomere (Tel) Q-FISH and γH2AX/TnI immunofluorescence. Arrowheads indicate foci of the DNA-damage marker protein γH2AX at telomeres in a CM. Scale bars, 10 µm. (**B**) Confocal (left) and superresolution (right) images of DNA bridges in a dividing P8 CM. Arrowheads indicate a DNA bridge. Scale bars, 10 µm. (**C**) P8 CM with a micronucleus. Scale bar, 25 µm. The arrowhead indicates the micronucleus.



Selection of iPSCs with relatively long telomeres improves spontaneous cardiomyocyte differentiation efficiency. (**A**) Percentages of beating EBs during iPSC differentiation. Control, G1 iPSCs; iPSC^{lowT}, G1 iPSCs with relatively short telomeres; iPSC^{highT}, G1 iPSCs with relatively long telomeres. (**B**) Representative images showing α-actinin expression during iPSC differentiation to CMs. Scale bar, 80 µm. Nuclei are counterstained with DAPI.

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

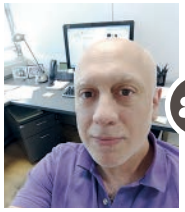
Aix E, Gutiérrez-Gutiérrez Ó, Sánchez-Ferrer C, Aguado T, Flores I. **Postnatal telomere dysfunction induces cardiomyocyte cell-cycle arrest through p21 activation**. *J Cell Biol* (2016) 213: 571-83

Aguado T, Gutiérrez FJ, Aix E, Schneider RP, Giovinnazo G, Blasco MA, Flores I. **Telomere length defines the cardiomyocyte differentiation potency of mouse induced pluripotent stem cells**. *Stem Cells* doi: 10.1002/stem.2497, Sept 26, 2016

Fernández-Alvira JM, Fuster V, Dorado B, Soberón N, Flores I, Gallardo M, Pocock S, Blasco MA, Andrés V. **Short telomere load, telomere length, and subclinical atherosclerosis: the PESA study**. *J Am Coll Cardiol* (2016) 67: 2467-76

Latorre-Pellicer A, Moreno-Loshuertos R, Lechuga-Vieco AV, Sánchez-Cabo F, Torroja C, Acín-Pérez R, Calvo E, Aix E, González-Guerra A, Logan A, Bernad-Miana ML, Romanos E, Cruz R, Cogliati S, Sobrino B, Carracedo Á, Pérez-Martos A, Fernández-Silva P, Ruíz-Cabello J, Murphy MP, Flores I, Vázquez J, Enríquez JA. **Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing**. *Nature* (2016) 525: 561-65

Imaging cardiovascular inflammation and the immune response



RESEARCH INTEREST

Our lab studies immunity, in particular the innate arm of the immune system, which provides continuous support to tissues without undergoing somatic mutations. One of the main cellular components of innate immunity are macrophages, which perform specialized functions in all tissues. We study the mechanisms by which tissue-resident macropahges phagocytose other cells and also investigate the consequences of this activity. We pay special attention to macrophages in the heart, as both the signals that help program their properties and their function in healthy cardiac tissue are unknown. We are working to define both processes by using a model that specifically depletes these cells in adult mice. We also study neutrophils, the most abundant component of the innate immune system. Neutrophils are highly migratory leukocytes that eliminate microbes efficiently but can also inflict severe injury to tissues when they become abnormally activated in vessels. We focus our attention on intrinsic programs within neutrophils that boost immune protection but prevent vascular injury. Neutrophils also participate in homeostatic processes, and we study this activity in the bone marrow, the home of blood stem cells. A population of neutrophils enters the bone marrow each day (with circadian frequency) to regulate hematopoietic niches. We study this regulation and how it influences stem cell maintenance and tissue regeneration.

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Marianna Di Scala
Jackson Li

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Sandra Martín Salamanca

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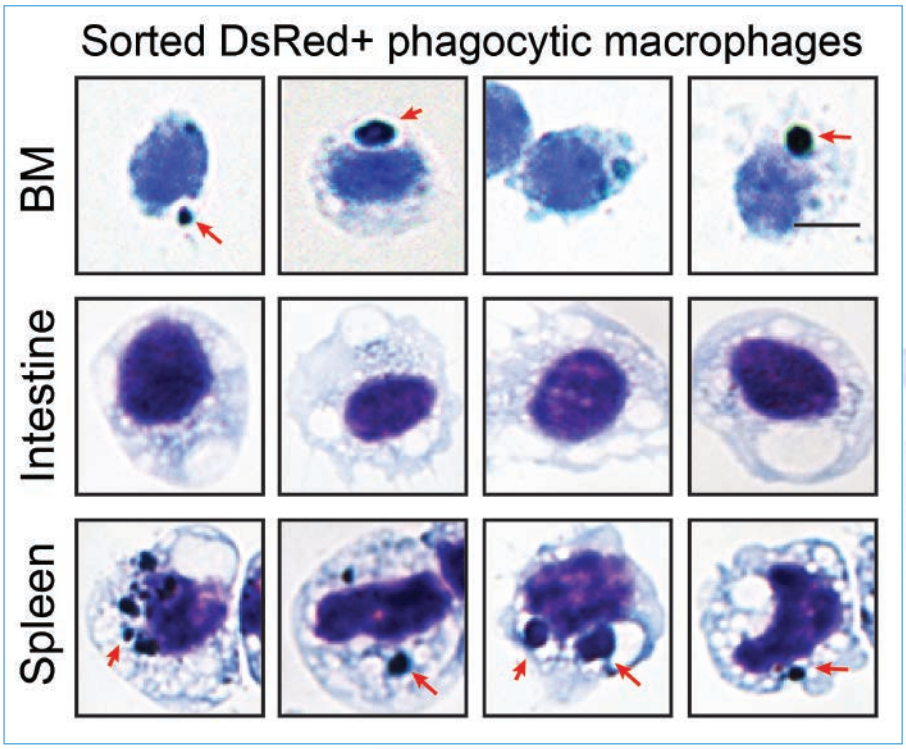
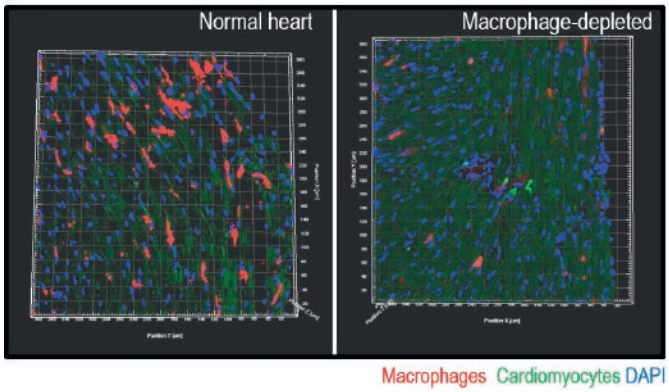
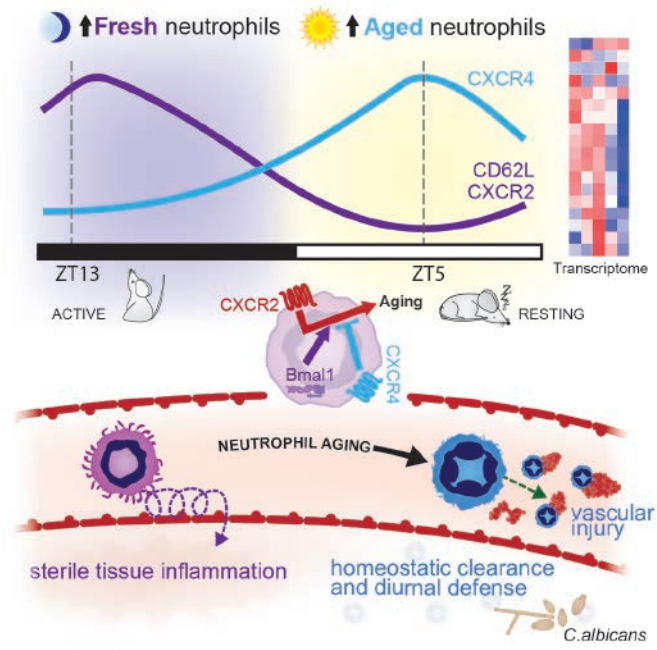
María Casanova Acebes



Phagocytic macrophages in tissues
Images of sorted phagocytic macrophages from the bone marrow (BM), intestine, and spleen, showing evidence of phagocytotic cell uptake and vacuolization (arrowheads).



Tissue-resident macrophages in the heart
3D images of heart sections, showing abundant macrophages (MHCII+, red) interacalated between cardiomyocytes (green) in a normal heart. In a new mouse model, treatment with a drug achieves dramatic depletion of these cells without decreasing other cardiac populations. Blue shows nuclei stained with DAPI.



Dynamics of neutrophils in homeostasis
Neutrophil numbers change with circadian frequency and undergo transcriptomic and phenotypic changes. These circadian changes are regulated by a neutrophil-intrinsic program regulated by the molecular clock and chemokine receptors. This program might be important for neutrophil clearance from tissues, diurnal defense, and prevention of vascular injury.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2013-49662-EXP)
- Ministerio de Economía y Competitividad (ERA-NET Infect-ERA 2014 PCIN-2014-103 / 143 BActInfectERA)
- Ministerio de Economía y Competitividad (SAF2015-65607-R)
- Fundación La Marató-TV3 (120/C/2015)
- Comunidad de Madrid (P2010-BMD-2314)

SELECTED PUBLICATIONS

Leiva M, Quintana JA, Ligos JM and Hidalgo A. **Hematopoietic ESL-1 enables stem cell proliferation in the bone marrow by limiting TGFβ availability**. *Nat Commun* (2016) 7: 10222

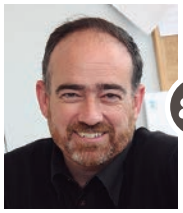
Silvestre-Roig C, Hidalgo A and Soehnlein O. **Neutrophil heterogeneity: implications for homeostasis and pathogenesis**. *Blood* (2016) 127: 2173-81

Adrover JM, Nicolas-Avila JA and Hidalgo A. **Aging, a temporal dimension of neutrophils**. *Trends Immunol* (2016) 37: 334-45

Rossaint J, Kuhne K, Skupski J, Van Aken H, Looney MR, Hidalgo A and Zarbock A. **Directed transport of neutrophil-derived extracellular vesicles enables platelet-mediated innate immune response**. *Nat Commun* (2016) 7: 13464

Di Scala M and Hidalgo A. **Angiogenin defines heterogeneity at the core of the hematopoietic niche**. *Cell Stem Cell* (2016) 19: 284-6

Functional genomics



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 these elements are the target of variation that results in increased risk of human disease.

With these goals in mind, we have explored how 3-dimensional genome structure relates to gene expression in the cardiovascular system. Using high-resolution deep-sequencing-based chromatin conformation techniques in combination 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 showed that this loop is dependent on the architectural chromatin factor CTCF during embryonic development. At present we are applying similar approaches to investigate the regulatory basis of atrial fibrillation, the most common type of cardiac arrhythmia and a serious health burden worldwide.

We are also exploring the role of pluritopiency factors in the transition from the undetermined state to lineage commitment through the use of inducible genetic systems for Oct4 and Nanog. These studies are revealing how these factors control both initial repression and later activation of a critical subset of specification factors during development.

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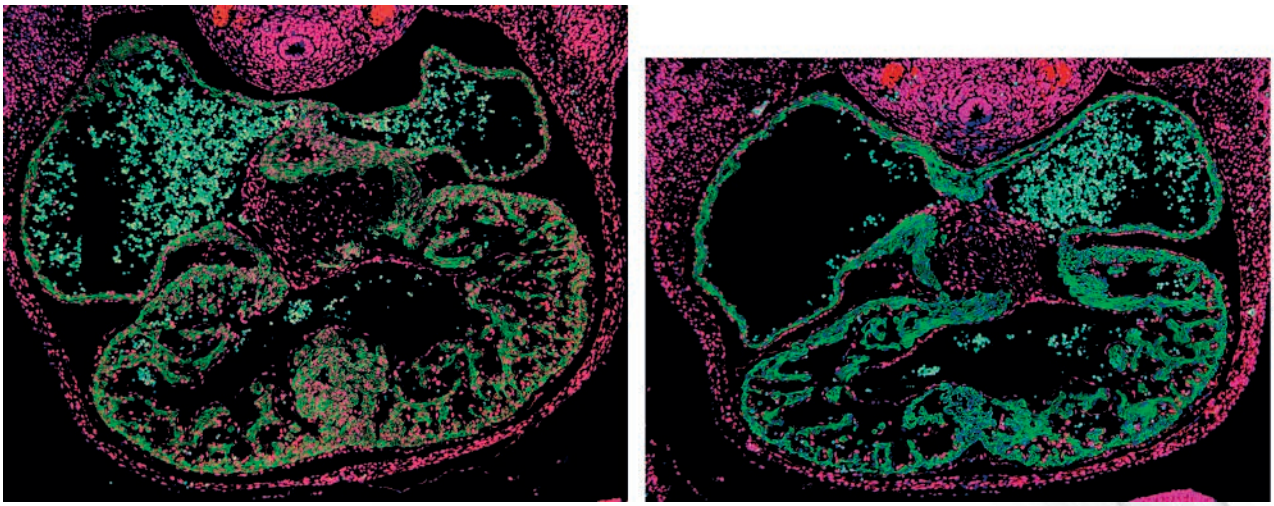
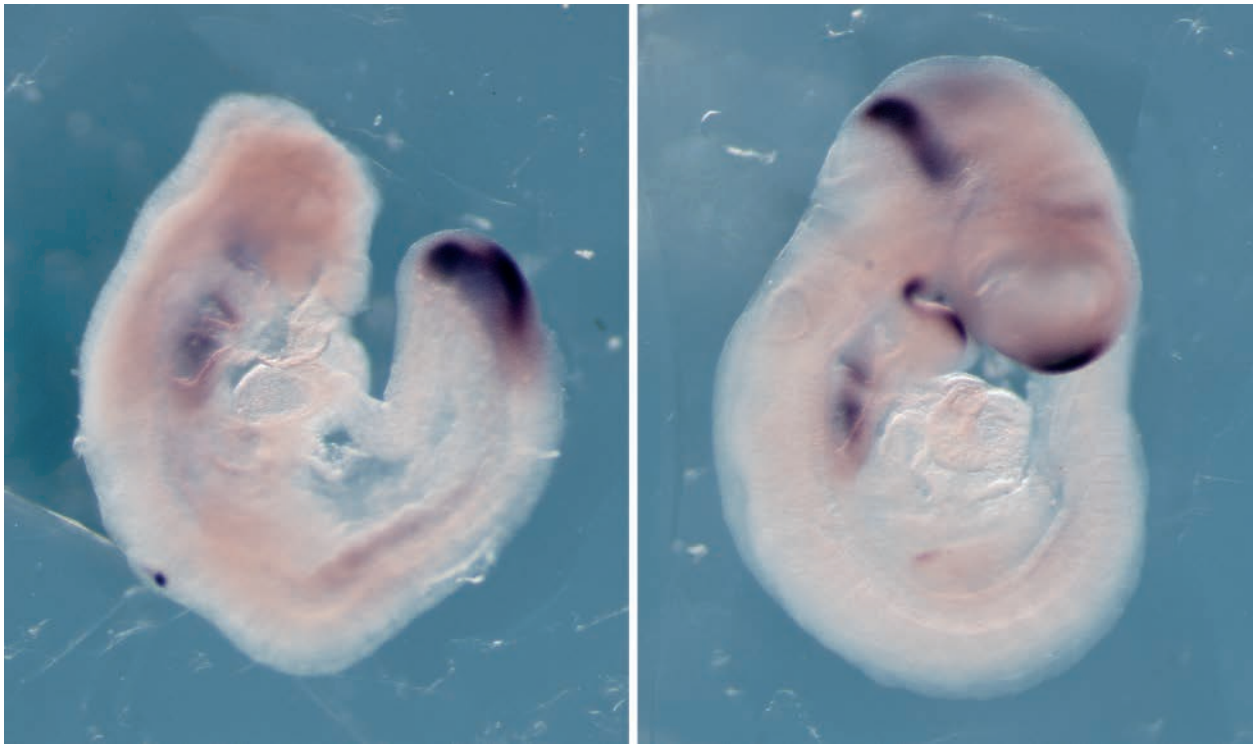


Loss of CTCF in the developing mouse heart leads to cardiac developmental arrest. Comparison of E11.5 control hearts (left) with mutant hearts (right) with conditional homozygous deletion of a Ctcf allele using an Nkx2.5-Cre line. Mutant hearts show a grossly disorganized interventricular septum, as well as thinning of the ventricular myocardial wall. Immunohistochemistry shows CTCF in red and cardiac troponin (CT3) in green. Nuclei are stained with DAPI (blue).



Expression of the developmental marker Fgf8 in control E9.5 embryos (left) and in embryos where expresson of the pluripotency factor Oct4 has been induced from E6.5 to E9.5 (right). While some Fgf8 expression domains are unchanged (such as the tail bud), others are clearly affected (anterior expression in the fronto-nasal mass and expression at the isthmus).

MAJOR GRANTS

- Ministerio de Economía y Competitividad BFU2014-57703-REDC
- Ministerio de Economía y Competitividad BFU2014-54608-P
- Ministerio de Economía y Competitividad BFU2015-72319-EXP

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Menchero S, Rayon T, Andreu MJ, Manzanares M. Signaling pathways in mammalian preimplantation development: Linking cellular phenotypes to lineage decisions. *Dev Dyn* doi:10.1002/dvdy.24471 (Epub Nov 18, 2016)

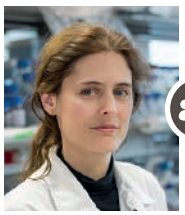
Rayon T, Menchero S, Rollan I, Ors I, Helness A, Crespo M, Nieto A, Azuara V, Rossant J, Manzanares M. **Distinct mechanisms regulate Cdx2 expression in the blastocyst and in trophoblast stem cells**. *Sci Rep* (2016) 6: 27139

Bogdanovic O, Smits AH, de la Calle Mustienes E, Tena JJ, Ford E, Williams R, Senanayake U, Schultz MD, Hontelez S, van Kruijsbergen I, Rayon T, Gnerlich F, Carell T, Veenstra GJ, Manzanares M, Sauka-Spengler T, Ecker JR, Vermeulen M, Gomez-Skarmeta JL, Lister R. **Active DNA demethylation at enhancers during the vertebrate phylotypic period**. *Nat Genet* (2016) 48: 417-26

Aguirre LA, Alonso ME, Badia-Careaga C, Rollan I, Arias C, Fernandez-Minan A, Lopez-Jimenez E, Aranega A, Gomez-Skarmeta JL, Franco D, Manzanares M. **Long-range regulatory interactions at the 4q25 Atrial Fibrillation risk locus involve PITX2c and ENPEP**. *BMC Biol* (2015) 13: 26

CNIC-UNIVERSITY OF BERN COLLABORATIVE PROGRAM

Development of the epicardium and its role during regeneration



RESEARCH INTEREST

In our group we aim to understand the cellular and molecular basis of heart regeneration. Unlike mammals, adult zebrafish have the capacity to regenerate their hearts upon injury to as much as a quarter of the cardiac ventricle with a liquid nitrogen cooled cryoprobe. As an early response, inflammatory cells are recruited to the damaged heart, followed by the expansion of the other layer of the heart, the epicardium, and the endocardium lining the cardiac lumen. This is followed by the formation of a transient scar. This fibrotic tissue is finally replaced by new cardiac muscle, the myocardium. Thus, regeneration occurs in the presence of a scar. We are studying how fibrosis influences heart regeneration. The epicardium is one source of the fibroblasts which contribute to cardiac fibrosis in response to cryoinjury. The epicardium also plays an important trophic role during heart regeneration. We are therefore also interested in understanding the formation of the epicardium during embryogenesis. Due to the small size and transparency of its embryos, the zebrafish offers a unique system for studying heart 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.

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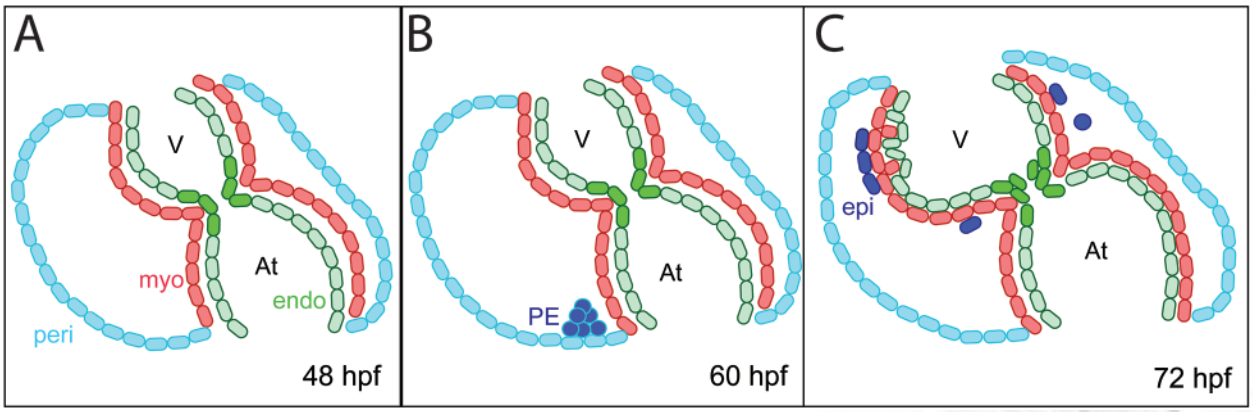
Inês Marques
Carolina García-Poyatos
Marcos Sande Melón
Andrés Sanz Morejón

Visiting Student:
David Bazaga



Epicardium development in the zebrafish. The developing heart tube in the zebrafish. Proepicardial (PE) cells delaminate from the pericardial mesothelium lining the pericardial cavity. PE cells are released into the cavity and attach to the surface of the ventricular myocardium. At, atrium; endo, endocardium, hpf, hours postfertilization; PE, proepicardium; peri, pericardium, v, ventricle.

MAJOR GRANTS

- European Commission. European Research Council Starting Independent Researcher Grant (ERC-337703 2013)
- Ministerio de Educación, Cultura y Deporte. FPU contract (FPU12/3007) PI: H. Sánchez Iranzo
- Ministerio de Economía y Competitividad. Posdoctoral contract (FPDI-2013-16319). PI: L. Andrés Delgado

SELECTED PUBLICATIONS

Cogliati S, Calvo E, Loureiro M, Guaras AM, Nieto-Arellano R, Garcia-Poyatos C, Ezkurdia I, Mercader, N, Vázquez J, Enriquez JA. **Mechanism of super-assembly of respiratory complexes III and IV**. *Nature* (2016) 539: 579-82

Andres-Delgado, L., Mercader, N., **Interplay between cardiac function and heart development**. *Biochim Biophys Acta* (2016)1863: 1707-16

Rodius S, Androsova G, Götz L, Liechti R, Crespo I, Merz S, Nazarov PV, de Klein N, Jeanty C, González-Rosa JM, Muller A, Bernardin F, Niclou SP, Vallar L, Mercader N, Ibberson M, Xenarios I, Azuaje F. **Analysis of the dynamic co-expression network of heart regeneration in the zebrafish**. *Sci Rep* (2016) 6: 26822

Di Donato V, De Santis F, Auer TO, Testa N, Sánchez-Iranzo H, Mercader N, Concordet JP, Del Bene F. **2C-Cas9: a versatile tool for clonal analysis of gene function**. *Genome Res* (2016) 26: 681-92

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 authors

Genetic control of organ development and regeneration

RESEARCH INTEREST

We are interested in understanding the cellular basis of developmental processes and how this is controlled by transcription factor networks (TFN). We have developed genetic methods in the mouse that allow us to trace cell lineages using clonal analysis or functional mosaics. We have also 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 demonstrated the importance 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 of Meis TFN activity underlying 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 are exploring the cardiac regenerative potential of Myc and the role of Meis in maintaining heart function in the adult mouse.

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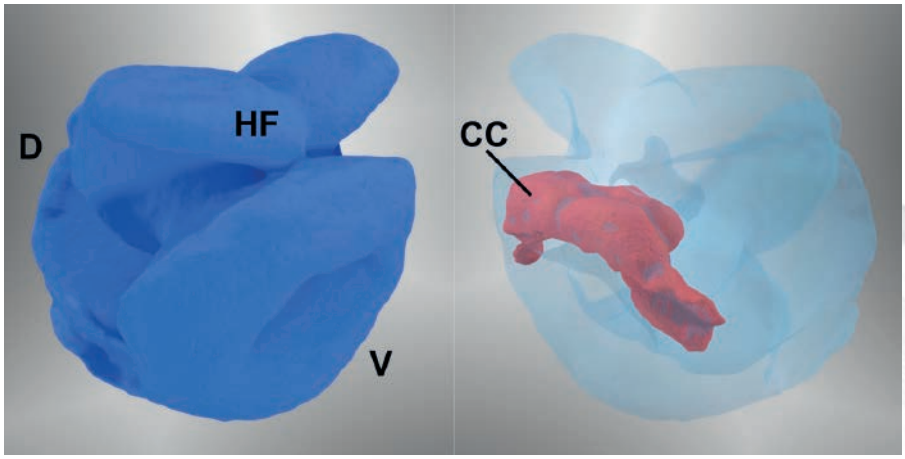
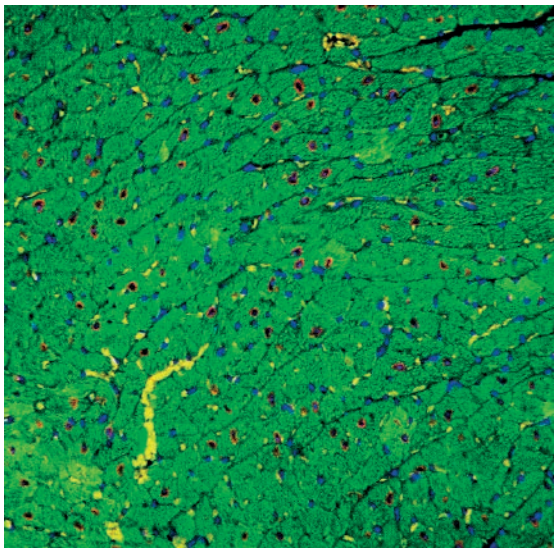
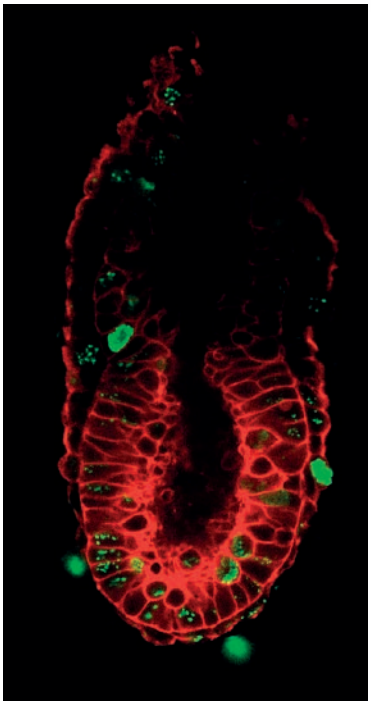
Rocío Sierra Muñoz
Susana Temiño Valbuena



Analyzing heart development by 3D imaging. Surface renderings of complete 3D reconstructions of the mouse embryo (blue) imaged using light-sheet microscopy. On the right, the embryo has been made transparent to show the geometry of the cardiac crescent (red, CC). HF, head fold; V, ventral; D, dorsal. Image: Isaac Esteban-Varela



Characterizing transcription factor function in the adult myocardium. Immunofluorescence analysis of the adult myocardium in a mouse in which *Myc* has been specifically deleted in cardiomyocytes. Troponin-T is shown in green and cell nuclei in blue, and cardiomyocyte nuclei are surrounded by PCM1 signal (red). Image: Noelia Muñoz



Pluripotent cells in the early mouse embryo. Very active cell division is detected in the epiblast of the early mouse embryo, which contains the pluripotent cells able to generate the new organism. At this stage, the pluripotent stem cell pool "cleans" the epiblast of suboptimal cells by cell competition. Cell membranes are shown in red, and chromatin in dividing cells is visualized by Ph3 immunofluorescence (green). Image: Covadonga Díaz-Díaz

MAJOR GRANTS

- Ministerio de Economía y Competividad. FIS RETICS (TERCEL: RD12/0019/0005)
- Ministerio de Economía y Competividad (BFU2015-71519-P)
- Ministerio de Economía y Competividad. Red de Excelencia Temática. (BFU2015-70193-REDT). PI and Network Coordinator: M Torres
- European Commission and Ministerio de Economía y Competividad. (PCIN-2015-020)
- Ministerio de Economía y Competividad. (EUIN2015-62897)
- Marie Sklodowska-Curie Innovative Training Networks (H2020-MSCA-ITN-2016) (GA nº 722427). PI and ITN Coordinator: M Torres
- Ministerio de Economía y Competividad. Juan de la Cierva Incorporación. (IJCI-2014-19108). PI: I. Delgado.
- Human Frontier Science Program. Long-term fellowship 2015. PI: K. Ivanovitch
- Ministerio de Educación, Cultura y Deporte. Predoctoral contract (FPU12/02114). PI: C. Díaz Díaz
- Ministerio de Economía y Competitividad. Predoctoral contract (BES-2013-064374) PI: A.C López
- Fundación La Caixa CNIC Severo Ochoa. Predoctoral Fellowship 2014. PI: N. Muñoz
- Fundación La Caixa CNIC Severo Ochoa. Predoctoral Fellowship 2015. PI: J A. Valvelde
- Ministerio de Educación, Cultura y Deporte. Predoctoral contract (FPU15/02955). PI: M.E. de la Cruz Crespillo

SELECTED PUBLICATIONS

Villa Del Campo C, Lioux G, Carmona R, Sierra R, Muñoz-Chápuli R, Clavería C, Torres M. **Myc overexpression enhances of epicardial contribution to the developing heart and promotes extensive expansion of the cardiomyocyte population**. *Sci Rep* (2016) 6: 35366

Clavería C, Torres M. **Cell Competition: Mechanisms and Physiological Roles**. *Annu Rev Cell Dev Biol* (2016) 32: 411-39

Delgado I, Torres M. **Gradients, waves and timers, an overview of limb patterning models**. *Semin Cell Dev Biol* (2016) 49: 109-15

Fernández LC, Torres M*, Real FX*. **Somatic mosaicism: on the road to cancer**. *Nat Rev Cancer* (2016) 16: 43-55
*Co-corresponding authors

Torres M. **Regeneration: Limb regrowth takes two**. *Nature* (2016) 533: 328-30