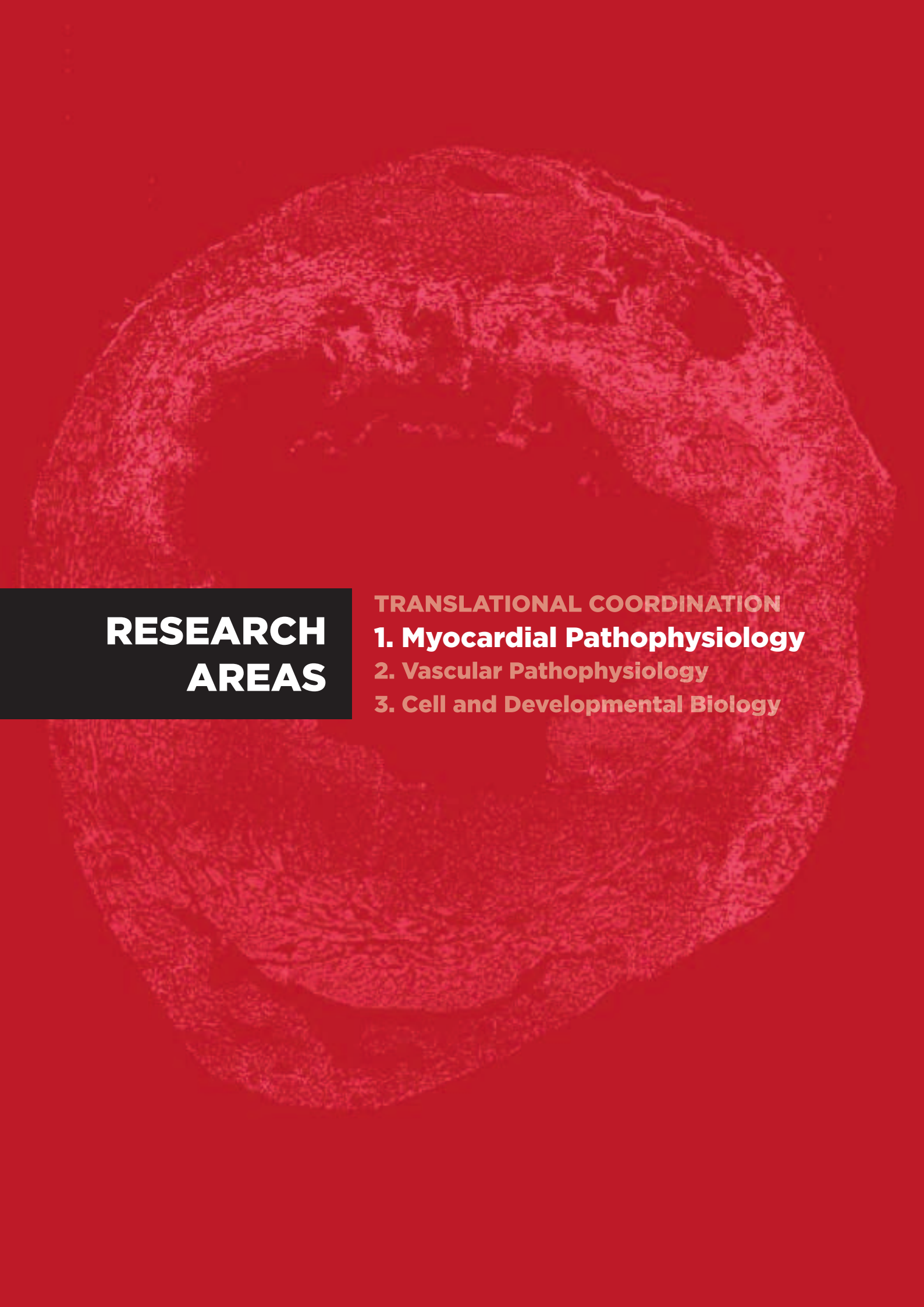


RESEARCH AREAS

TRANSLATIONAL COORDINATION

1. Myocardial Pathophysiology

2. Vascular Pathophysiology

3. Cell and Developmental Biology

cnic | RESEARCH AREAS

1. Myocardial Pathophysiology

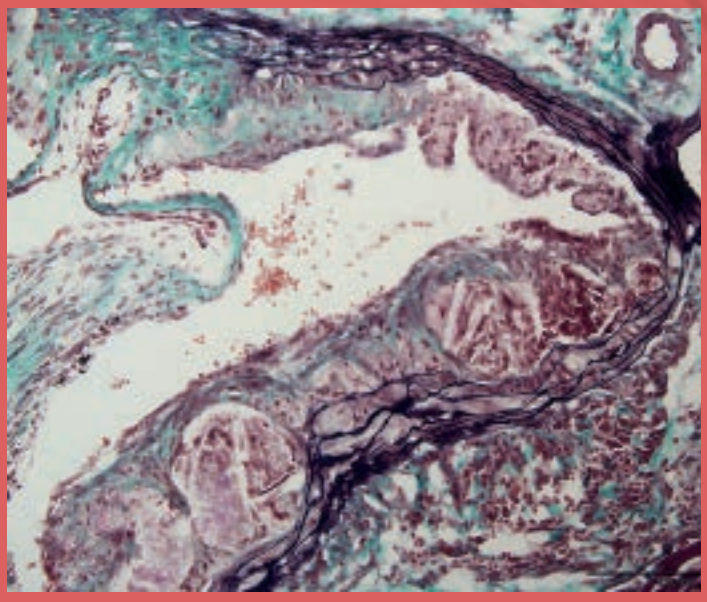
AREA COORDINATORS:

JOSE ANTONIO ENRIQUEZ

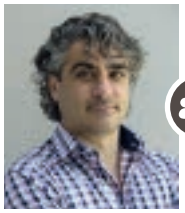
ENRIQUE LARA-PEZZI

RESEARCH INTEREST

The myocardial pathophysiology area integrates scientists from multidisciplinary fields. Basic scientists, cardiologists, and engineers work in a coordinated way to provide invaluable information on the molecular mechanisms that manage the cardiovascular system in homeostasis and disease. Our experimental strategy comprises in vitro and in vivo studies in animal models and humans, an approach that not only provides basic understanding of health and disease, but also improves the translational potential of diagnosis and treatment. Our research focuses on several topics: the oxidative phosphorylation system, role of nuclear receptors in lipid metabolism and inflammatory responses, metabolic syndrome and stress kinases, immunobiology of inflammation, inherited cardiomyopathies, cardiac arrhythmias, electrophysiological characterization of healthy and diseased cardiomyocytes, epigenetic regulation, alternative splicing in cardiac development and heart disease, and cardioprotection during myocardial infarction.



Inherited cardiomyopathies



RESEARCH INTEREST

Our research into cardiovascular disease is based on a simple principle: create to understand, create to treat.

Animal models are essential investigative tools for expanding our understanding of disease; however, the generation and maintenance of genetically modified mouse colonies for research is costly. We have developed an alternative method that uses adeno-associated virus (AAV) vectors, widely used for gene-therapy approaches, to express disease-causing dominant-negative mutants to generate disease models in wild-type mice. Single systemic injection of AAV virus is more versatile, cost-effective, simpler, and time-efficient than transgenic approaches for generating mutant animals.

Our major area of interest is arrhythmogenic right ventricular cardiomyopathy (ARVC). This heart muscle disease is characterized by right ventricular anatomical abnormalities and ventricular arrhythmias that can lead to sudden cardiac death, especially in young athletes. To be able to study the effect of exercise on hearts of mice carrying the most prevalent ARVC-associated mutation in *plakophilin-2 (PKP2)*, we used AAV to express the *R735X* mutant in wild-type mice. Our work shows that injected AAV-*R735X* animals develop an overt ARVC phenotype when subjected to endurance training, supporting the recommendation for exercise cessation in carriers of this mutation.

At the histological level, the right ventricles of endurance-trained R735X-infected mice display connexin 43 delocalization (Cx43) at intercardiomyocyte gap junctions, a change not observed in sedentary mice. To better understand the molecular mechanism underlying the effect of mutant PKP2 expression on Cx43 mislocalization we have developed new molecular reporters and live cell imaging approaches to monitor this important process.

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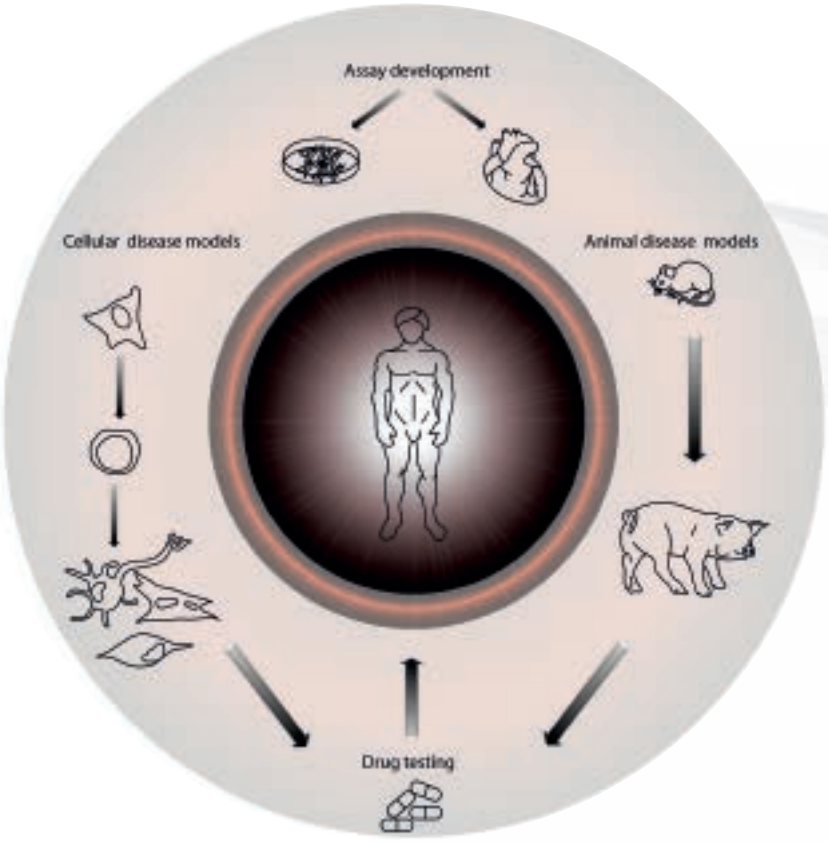
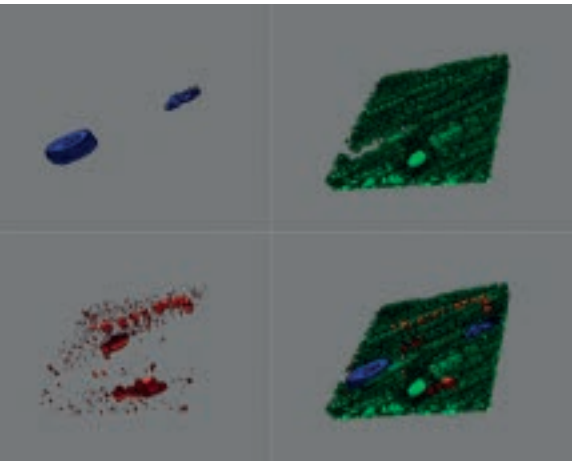
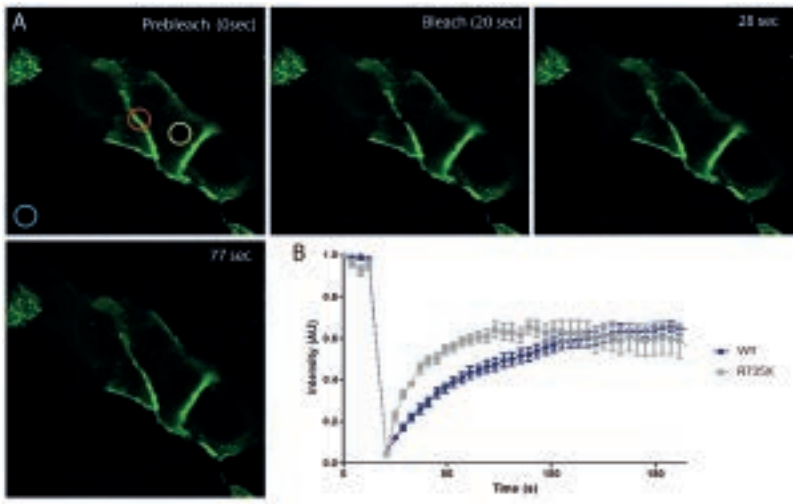


General working-model used in the laboratory to investigate and test compounds in a specific disease. For example, for ARVC we have already developed a cellular model in human induced pluripotent stem cells (iPS) and a mouse model. In the near future we plan to develop a pig model of ARVC, to take advantage of the closer similarity to human physiology.



Super-resolution studies to analyze the effect of the PKP2 mutant protein R735X on its interacting partner connexin 43 (Cx43). Mouse cardiomyocytes from right ventricle expressing human R735X were immunolabeled for gap junctions (Cx43, red) and mitochondria (Tom20, green). Nuclei are visualized by DAPI staining. Z-stacks were acquired at 0.15 µm intervals, and maximum projections of different channels are shown.



Fluorescence recovery after photobleaching (FRAP) measurements to elucidate human PKP2 assembly stability in desmosomes. (A) Typical FRAP experiment using GFP-PKP2 and confocal optical sectioning. The image brightness was adjusted to more clearly depict desmosomes. A reference region in a nonphotobleached area (ROI, blue) was used to correct for unintentional bleaching. A negative control region (ROI, yellow), also outside the photobleached area, was selected to confirm successful correction for unintentional photobleaching, following the correction steps using the reference region. (B) Fluorescence recovery dynamics of GFP-PKP2 (blue) and the mutant GFP-R735X (grey).

MAJOR GRANTS

- Ministerio de Economía y Competitividad (BFU2016-75144-R)

SELECTED PUBLICATIONS

Bárbara González-Terán, Juan Antonio López, Elena Rodríguez, Luis Leiva, Sara Martínez-Martínez, Bernal JA, Luis Jesús Jiménez-Borreguero, Juan Miguel Redondo, Jesus Vazquez, and Guadalupe Sabio. **p38γ and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation**. *Nat Commun* (2016) 7:10477

Cruz FM, Tomé M, Bernal JA*, Bernad A*. **miR-300 mediates Bmi1 function and regulates differentiation in primitive cardiac progenitors**. *Cell Death Dis* (2015) 6:e1953
* Co-corresponding Authors

Cruz FM, Sanz-Rosa D, Roche-Molina M, García-Prieto J, García-Ruiz JM, Pizarro G, Jiménez-Borreguero LJ, Torres M, Bernad A, Ruíz-Cabello J, Fuster V, Ibáñez B, Bernal JA. **Exercise triggers ARVC phenotype in mice expressing a disease-causing mutated version of human plakophilin-2**. *J Am Coll Cardiol* (2015) 65:1438-50

Nakagawa Y, Sedukhina AS, Okamoto N, Nagasawa S, Suzuki N, Ohta T, Hattori H, Roche-Molina M, Narváez AJ, Jeyasekharan AD, Bernal JA, Sato K. **NF-κB signaling mediates acquired resistance after PARP inhibition**. *Oncotarget* (2015) 6: 3825-39

Roche-Molina M, Sanz-Rosa D, Cruz FM, García-Prieto J, López S, Abia R, Muriana FJ, Fuster V, Ibáñez B, Bernal JA. **Induction of sustained hypercholesterolemia by single adeno-associated virus-mediated gene transfer of mutant hPCSK9**. *Arterioscler Thromb Vasc Biol* (2015) 35: 50-9

Functional genetics of the oxidative phosphorylation system

RESEARCH INTEREST

The group researches the mammalian mitochondrial electron transport chain (MtETC) and H+-ATP synthase, which together constitute the oxidative phosphorylation (OXPHOS) system. We view this system as a functional entity, and use a range of approaches aimed at determining its role in health and disease. We are particularly interested in role the OXPHOS system in the development of the cardiovascular system, its relevance to ischemia-reperfusion, and its influence on microvascular blood flow. To better understand the role of mitochondria and their response to metabolic challenges during aging, angiogenesis, and lung performance we use mice with the same nuclear background but carrying different nonpathological variants of mitochondrial DNA throughout the organism (conplastic mice) or a mix of mtDNA variants in the same cell (heteroplasmic mice).

We also study the organization of the respiratory complexes and interacting partners using methods to visualize and quantitatively estimate the supercomplexes (I/III/IV, I/III, and III/IV) in intact cells without the use of detergents that disrupt the mitochondrial inner membrane. This research line includes the use of stimulated emission depletion microscopy to observe different combinations of respiratory complex subunits in mitochondria.

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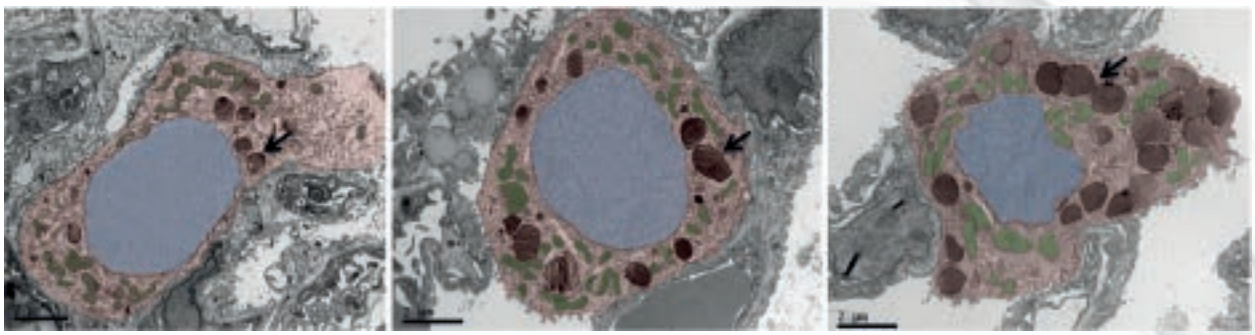
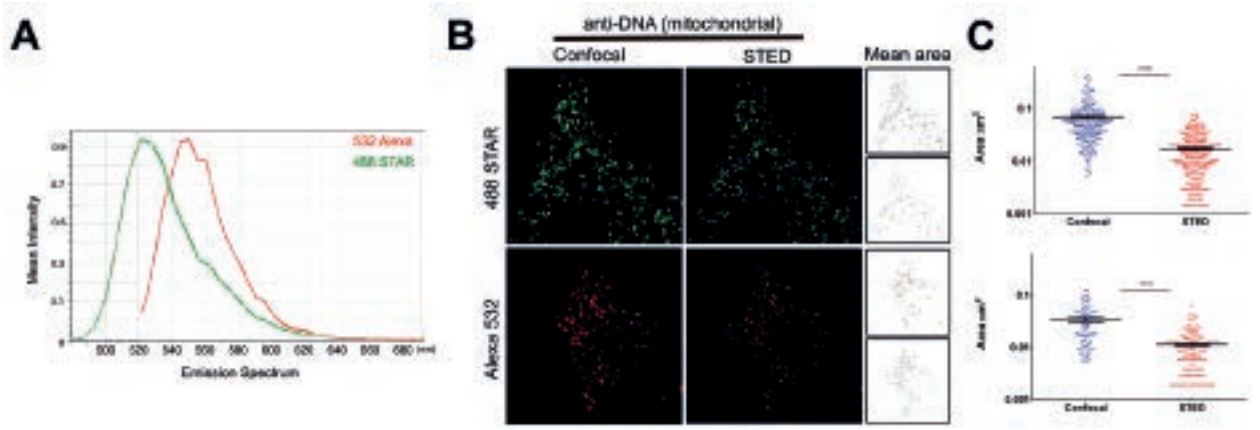


Ultrastructural analysis of type II alveolar epithelial cell (AEC) alterations in heteroplasmic mice. A) Control BL/6^{C57} mice. B) Conplastic BL/6^{NZB} mice. C) Heteroplasmic BL/6$^{C57\text{-}NZB}$ mice. Cytoplasm is shown in red, nuclei in blue, and mitochondria in green. Arrows indicate lamellar bodies (LB), lysosome-related secretory organelles of epithelial cells.



A) Scan of emission wavelengths for Alexa 532 and 488 STAR secondary antibodies bound to the specific IgGs used in this study. (**B**) *Left.* ρ^{0Ctrl} cells were labeled with anti-DNA and 488 STAR (green) or Alexa Fluor 532 (red) secondary antibodies. *Right*. Confocal and STED images were acquired, quantified, and represented as bare particle outlines from ImageJ. (**C**) Data show mean particle area (µm2) ± s.e.m. ***p <0.0001 by Mann Whitney test.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2015-71521-REDC)
- Ministerio de Economía y Competitividad (BFU2013-50448)
- Ministerio de Economía y Competitividad (SAF2012-32776). PI: JA Enríquez
- Marie Curie Initial Training Networks (ITN). Mitochondrial European Educational Training (GA Nº 317433).
- Ministerio de Economía y Competitividad (RyC 2011-07826). PI: Rebeca Acín
- European Commission. Marie Curie Career Integration Grant. PI: Rebeca Acín

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Garaude J*, Acín-Pérez R*, Martínez-Cano S, Enamorado M, Ugolini M, Nistal-Villán E, Hervás-Subbs S, Pelegrín P, Sander LE, Enríquez JA^, Sancho D. **Mitochondrial respiratory-chain adaptations in macrophages contribute to antibacterial host defense**. *Nat Immunol* (2016) 17: 1037-45
*Equal contribution
^Co-corresponding authors

Ana Latorre-Pellicer, Raquel Moreno-Loshuertos, Ana Victoria Lechuga-Vieco, Fátima Sánchez-Cabo, Carlos Torroja, Rebeca Acín-Pérez, Enrique Calvo, Esther Aix, Andrés González-Guerra, Angela Logan, María Luisa Bernad-Miana, Eduardo Romanos, Raquel Cruz, Sara Cogliati, Beatriz Sobrino, Ángel Carracedo, Acisclo Pérez-Martos, Patricio Fernández-Silva, Jesús Ruíz-Cabello, Michael P. Murphy, Ignacio Flores, Jesús Vázquez, José Antonio Enríquez. **mtDNA and nuclear DNA matching shapes metabolism and healthy ageing**. *Nature* (2016) 535: 561-5

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Enriquez, J. A. **Supramolecular Organization of Respiratory Complexes**. *Ann Rev Physiol* (2016) 241: 78:533-61

Advanced development in arrhythmia mechanisms and therapy

RESEARCH INTEREST

The laboratory focuses on the mechanisms underlying complex cardiac arrhythmias found in highly prevalent cardiovascular diseases, as well as in specific population subsets at particular risk of sudden cardiac death. Atrial fibrillation (AF), ventricular fibrillation (VF), and infarct scar-related ventricular tachycardia (VT) are three of the most prevalent cardiac rhythm disorders, and the capacity of current therapeutic strategies to accurately eliminate or prevent the arrhythmogenic substrate in these diseases is limited. Our goal is to achieve in-depth insight into the mechanisms of these complex arrhythmias through the use of appropriate experimental and numerical models, and for this insight to be used to improve patient care and develop new and more specific therapies. We use a translational approach to study infarct scar-related VT in pigs and clinical infarct-related reentrant VT. High-resolution MRI images, both in humans (in vivo) and animals (ex vivo) provide detailed structural information for creating anatomically precise patient and animal-specific 3D reconstructions of the ventricles. Electrophysiologically realistic numerical simulations can be incorporated into the 3D model to induce and characterize reentrant VTs. Computational simulations are validated and compared with electropysiological data and outcomes obtained during the electrophysiological study and ablation procedure, either in animals or in patients.

Sensing and detecting VF with current implantable cardioverter defibrillators (ICDs) is highly reliable in the vast majority of cases. However, an adequate R-wave/electrogram amplitude during VF is crucial to avoid undersensing during spontaneous episodes, which otherwise might lead to a delay or even cessation of ICD therapy. We recently reported that baseline rhythm R-wave amplitudes ≤2.5 mV (interquartile range: 2.3–2.8) can increase the rate of VF R-wave undersensing to the point where detection drops below the minimum nominal sensitivity during spontaneous VF, potentially causing delays in or cessation of VF therapy.

For AF, we aim to develop new computational tools for accurate mapping of the propagation dynamics during fibrillation that will enable clinical electrophysiologists to effectively target the main drivers of the arrhythmia. We use a porcine translational model of different AF stages (paroxysmal, persistent, and long-standing persistent AF) that resembles the human disease. The combination of detailed structural characterization of the atria with *in vivo* and *ex vivo* propagation dynamics will provide the most precise data to date about the propagation dynamics underlying AF maintenance.

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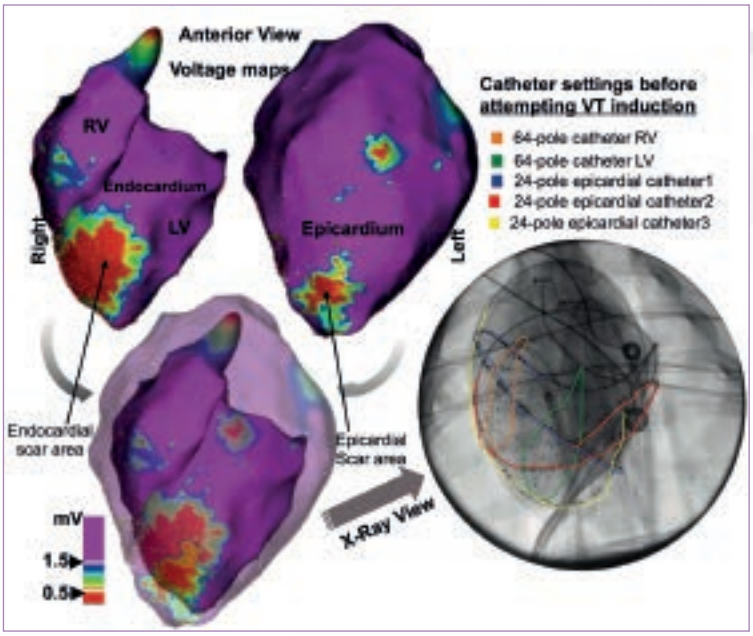
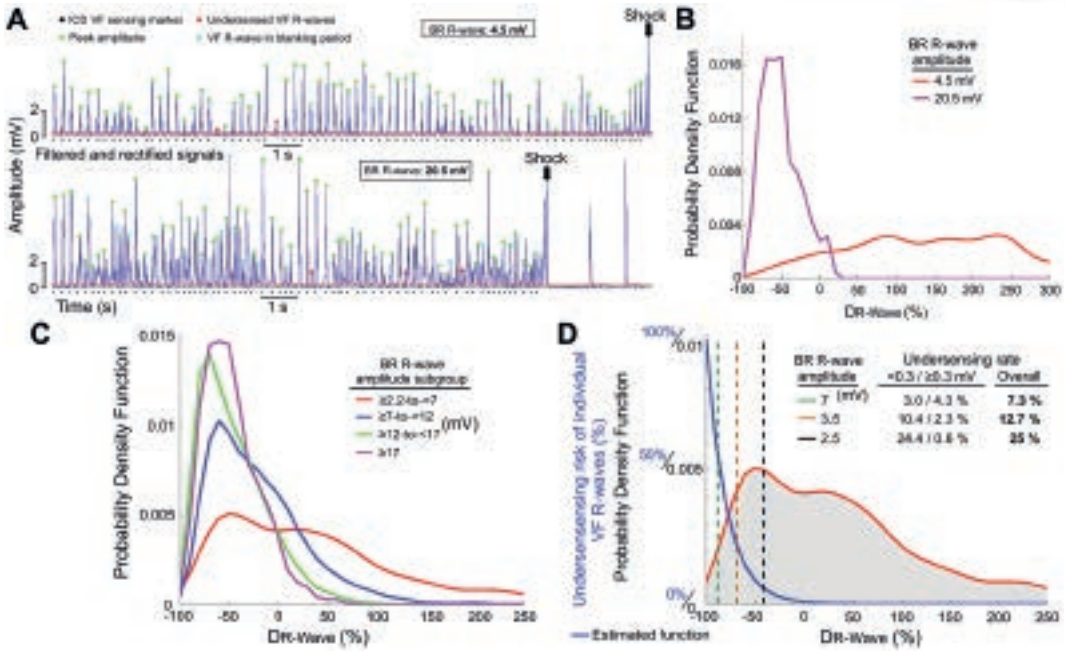


Endocardial and epicardial reconstruction of infarct-related susbtrate.
Voltage maps of the endocardium and epicardium of both ventricles. The structures are superimposed in the lower left panel, and separate in the top panels. The right panel shows an X-ray view of the cardiac silhouette and the multipolar catheters (color coded as indicated) used to charactherize VT activation upon programmed ventricular stimulation and induction.



R-wave amplitude distribution in the four subgroups of BR R-wave amplitude and calculation of the safety threshold.
(A) R-wave amplitude variability during ventricular fibrillation (VF) in two sample episodes of low (upper trace) and high (lower trace) BR R-wave amplitude. (B) Probability density function (PDF) of amplitude differences occurring in the episodes shown in A. (C) PDF of amplitude differences in the four BR R-wave amplitude subgroups. (D) Calculation of the safety threshold for BR R-wave amplitude values using the PDF and the estimated undersensing risk function from the ≥2 to <7 mV subgroup. Three BR R-wave amplitude values are depicted to show a progressive increase in undersensing rates of VF R-waves as the BR R-wave amplitude decreases, mostly due to R-waves <0.3 mV.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2016-80324-R)
- Salud 2000 Foundation.
- Jesús Serra Foundation.
- Pro-CNIC Foundation.

SELECTED PUBLICATIONS

Quintanilla JG, Perez-Villacastin J, Perez-Castellano N, Pandit SV, Berenfeld O, Jalife J, Filgueiras-Rama D **Mechanistic approaches to detect, target, and ablate the drivers of atrial fibrillation**. *Circ Arrhythm Electrophysiol* (2016) 9 e002481

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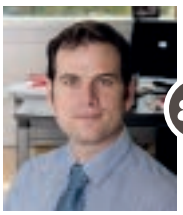
Translational laboratory for cardiovascular imaging and therapy



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Santiago Roura Ferrer
Joaquim Bobi i Gibert
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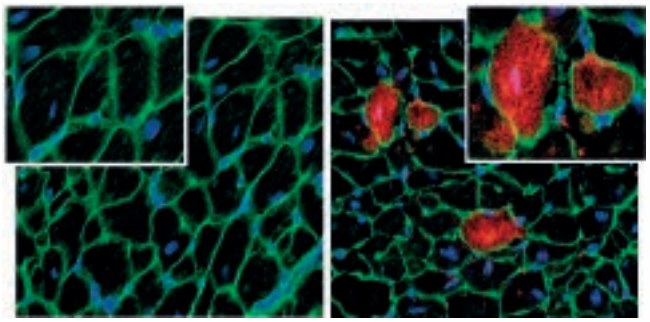
RESEARCH INTEREST

The primary focus of our laboratory is the study of myocardial diseases, from ischemia/reperfusion to heart failure, combining basic and clinical research and including experts in molecular biology, clinical cardiology and neurology, and cardiovascular imaging. We specialize in advanced imaging techniques in animal models that can also be applied to humans, which potentiates the translational nature of our research. Our clinical research is carried out in close collaboration with the Biomedical Research Institute of the Fundación Jiménez Díaz University Hospital.

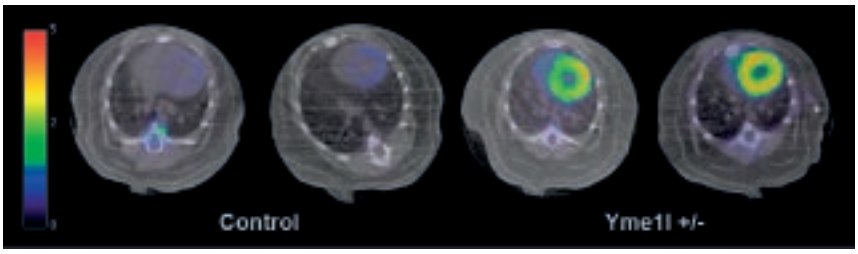
One of our main interests is cardioprotection during myocardial infarction (MI). We study the mechanisms underlying the beneficial effects of several cardioprotective strategies in rodent and large animal models of MI, mainly related to modulation of the beta-adrenergic system. The group is pioneering the use state-of-the-art magnetic resonance imaging (MRI) to better characterize post-infarcted myocardial healing by combining studies in large animal models and human study participants, having also conducted several clinical trials. We have already published the results of the successful randomized METOCARD-CNIC clinical trial, which used MRI to evaluate the effectiveness of early intravenous metoprolol in patients suffering a myocardial infarction, and the study has been continued with the EARLY-BAMI trial, conducted in the Netherlands and Spain. Our most recent trial is the VF-3D-ESSOS study, designed to revolutionize the use of cardiac MRI in the clinical setting. The goal of this study is to clinically validate the use of two ultra-fast sequences that could shorten the duration of cardiac magnetic resonance studies from the current 45 minutes to less than 60 seconds. After validating the sequences in large animals and healthy volunteers, the VF-3D-ESSOS study is now being performed in patients with different types of cardiac injuries.

In parallel with these clinical trials, we study the cellular and molecular mechanisms underlying the observed cardioprotective effect of beta-blockers in in vitro assays and genetically modified small animal models. In addition, following a recent discovery by our group, we are also opening new fields of research focused on the metabolism of heart failure and the study of revolutionary nutritional approaches to treat this condition.



Deletion of Yme1l induces cardiomyocyte necrosis in mice. Immunofluorescence images of 20-week-old Yme1l knockout mice (right) and control mice (left), showing increased cellular necrosis (Evans Blue staining in red) in mutant hearts. Hearts were stained with Evans Blue (red), wheat germ agglutinin (green), and DAPI (blue).

The group is also interested in the myocardial response to pulmonary hypertension. We have developed small and large animal models of pulmonary hypertension and use imaging technology to evaluate the response to different therapies. We have identified beta-3-adrenergic receptor stimulation as a novel therapeutic approach for the treatment of pulmonary hypertension in preclinical studies and have received funding to bring this therapy to a pilot clinical trial (SPHERE-HF) that will start during the coming year.



Ablation of Yme1l induces dilated cardiomyopathy in mice. Positron emission tomography–computed tomography (PET-CT) images of 40-week-old Yme1l knockout and control mice after [^{18}F]FDG injections, showing increased glucose consumption in failing hearts.

MAJOR GRANTS

- Ministerio de Economía y Competitividad - EXPLORA CIENCIA (SAF2013-49663-EXP)
- Ministerio de Economía y Competitividad - Acciones de Dinamización Europa investigación (EUIN2013-50881)
- Ministerio de Economía y Competitividad. ISCIII-FIS (PI13/01979)
- Ministerio de Economía y Competitividad. ISCIII-RETICS (RiC, RD12/0042/0054)
- Marató, Fundación TV3 (REF: 70/C/2012)
- European Commision FP7-PEOPLE-2013-ITN (CARDIONEXT).
- Fundación BBVA. Ayudas a Equipos de Investigación Científica (Proyectos-BBVA-2016)

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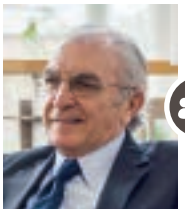
Garcia-Ruiz JM, Fernandez-Jimenez R, Garcia-Alvarez A, Pizarro G, Galan-Arriola C, Fernandez-Friera L, Mateos A, Nuno-Ayala M, Aguero J, Sanchez-Gonzalez J, Garcia-Prieto J, Lopez-Melgar B, Martinez-Tenorio P, Lopez-Martin GJ, Macias A, Perez-Asenjo B, Cabrera JA, Fernandez-Ortiz A, Fuster V, Ibanez B. **Impact of the timing of metoprolol administration during STEMI on infarct size and ventricular function**. *J Am Coll Cardiol* (2016) 67: 2093-104

Cardiac arrhythmia



Head of Laboratory:
José Jalife

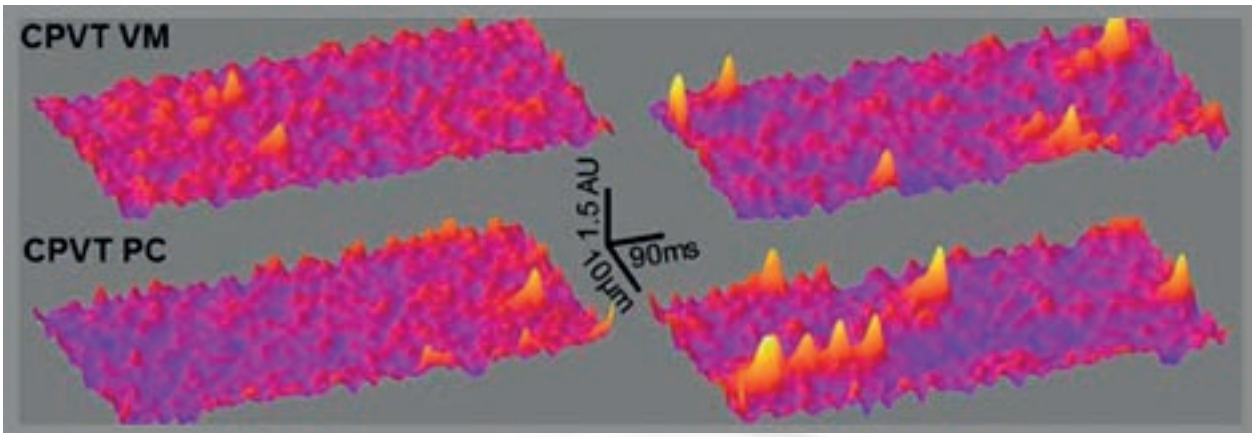
Visiting Student:
Sandeep V. Pandit

RESEARCH INTEREST

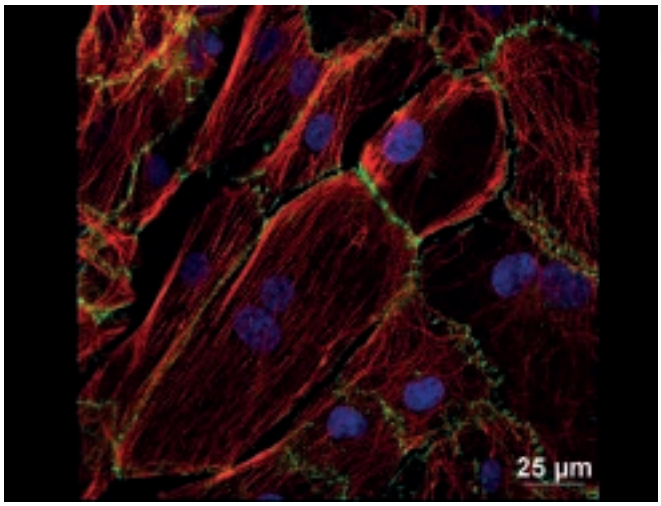
The laboratory investigates the causes of cardiovascular disease and arrhythmias at the molecular, cellular, and electrophysiological levels. Our specific research interests center on 1) the mechanisms of atrial and ventricular fibrillation at the structural and functional level, 2) the molecular genetics of cardiac fibrillation, and 3) the cellular basis of cardiac arrhythmia in genetic and rare diseases that can lead to sudden death, and 4) the use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to investigate molecular mechanisms of arrhythmogenesis.

The laboratory has well-established collaborations with expert engineers, biologists, and clinicians around the world, as well as with other CNIC groups. These partnerships provide a unique research environment in which to generate new and clinically relevant breakthroughs on arrhythmia mechanisms to the benefit of the medical and basic science communities, and ultimately the patient.

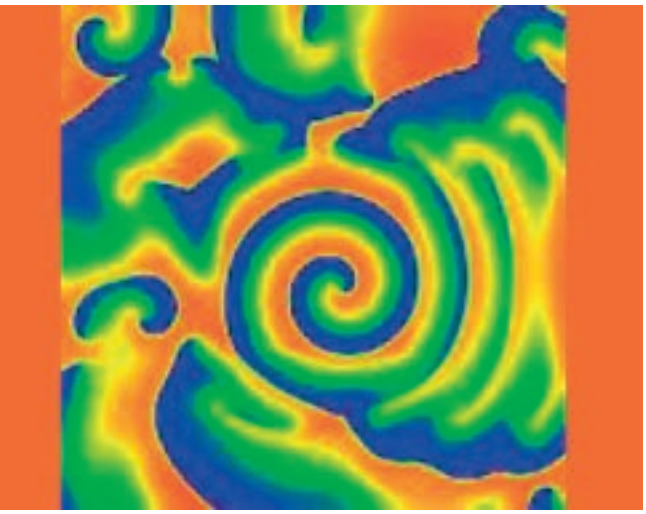
An ongoing multidisciplinary project is the whole genome characterization of large animal models of atrial fibrillation with a clear translational impact. The project aims to define transcriptomic changes in a sheep model of induced atrial fibrillation. Bioinformatic analysis of changes in gene expression and correlation with proteomic data generated by the group will enable mapping of the networks and pathways altered in paroxysmal and persistent states of atrial fibrillation. These results are also being validated in a pig atrial fibrillation model that has recently been established at the CNIC. The use of these models will allow us to better understand the molecular determinants and consequences of atrial fibrillation and to offer new insights into therapeutic targets for this disease.



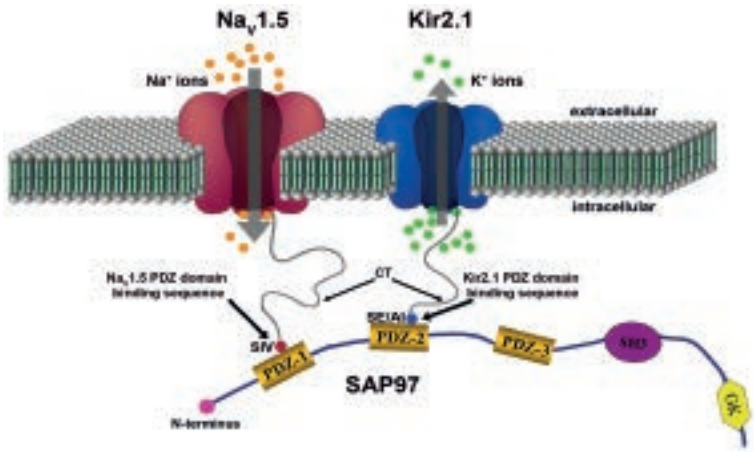
Ca2+ spark frequency (CaSpF) is higher in control and catecholaminergic polymorphic ventricular tachycardia (CPVT) Purkinje cells (PCs) than in ventricular myocytes (VMs). The figure shows 3-dimensional surface plots of representative line scan images for a CPVT VM and CPVT PC for baseline (left) and after treatment with 10 nM/L isoproterenol (right). Willis BC. et al *Circulation* 2016.



Structurally mature human iPSC-derived cardiomyocyte (CM) monolayer cultured on a soft surface. The figure shows CMs with well organized sarcomeres and localization of troponin T (red), N-cadherin (green), and nuclear dapi (blue). Jalife et al, unpublished.



In cardiac fibrillation, the rotor is the driver of reentry located at the center of a spiral wave. The rotational speed determines the degree of turbulence (wave fragmentation) around the rotor; the higher the spin speed the greater the degree of fragmentation. Rotors are not easy to find in the heart. In this computer simulation, the mother rotor occupies less than 0.1% of the total area; the rest is fibrillatory conduction. Samie F, et al. *Circulation Research* 2001.



"Cardiac Channelosome". In the heart, the strong inward rectifier potassium channel (Kir2.1) and the main cardiac sodium channel (Nav1.5) form part of a macromolecular complex ("channelosome") mediated by SAP97 through their respective carboxyl terminus PDZ binding domains.

MAJOR GRANTS

- Leducq Foundation Transatlantic Networks of Excellence Program (not CNIC). Peincipal Investigator
- NIH / NHLBI - R01 (HL122352) (not CNIC). Principal Investigator
- NIH / NHLBI - T32 (HL125242) (not CNIC). Principal Investigator
- The University of Michigan Health Sciences-Peking University Health Science Center Joint Institute. (not CNIC). Principal Investigator.
- Medtronic, Inc. Collaborative Grant to investigate detection of AF sources rom the body surface (non CNIC) Principal Investigator
- Abbott EP. Rotors and AF Reserch Grant (non CNIC) Principal Investigator

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Herron TJ, Rocha AM, Campbell KF, Ponce-Balbuena D, Willis BC, Guerrero-Serna G, Liu Q, Klos M, Musa H, Zarzoso M, Bizy A, Furness J, Anumonwo J, Mironov S, Jalife J. **Extracellular matrix-mediated maturation of human pluripotent stem cell-derived cardiac monolayer structure and electrophysiological function**. *Circulation Arrhythmia & Electrophysiol.* (2016) 9: e003638

Willis C, Pandit SV, Ponce-Balbuena D, Zarzoso M, Guerrero-Serna G, Limbu B, Deo M, Camors E, Ramirez RJ, Mironov S, Herron TJ, Valdivia HH, Jalife J. **Purkinje intracellular sodium surplus drives calcium-linked ventricular arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia**. *Circulation* (2016) 133: 2348-59

Monteiro da Rocha A, Guerrero-Serna G, Helms A, Luzod C, Mironov S, Russell M, Jalife J, Day SM, Smith GD, Herron TJ. **Deficient cMyBP-C protein expression during cardiomyocyte differentiation underlies human hypertrophic cardiomyopathy cellular phenotypes in disease specific human ES cell derived cardiomyocytes**. *J Mol Cell Cardiol.* (2016) 99: 197-206

Rivera-Torres J, Calvo CJ, Llach A, Guzmán-Martínez G, Caballero R, González-Gómez C, Jiménez-Borreguero LJ, Guadix JA, Osorio FG, López-Otín C, Herraiz Martínez A, Cabello N, Vallmitjana A, Benítez R, Gordon LB, Jalife J, Pérez-Pomares JM, Tamargo J, Delpón E, Hove-Madsen L, Filgueiras-Rama D, Andrés V. **Cardiac electrical defects in progeroid mice and Hutchinson-Gilford progeria syndrome patients with nuclear lamina alterations**. *Proc Natl Acad Sci USA* (2016) 15: 113: E7250-9

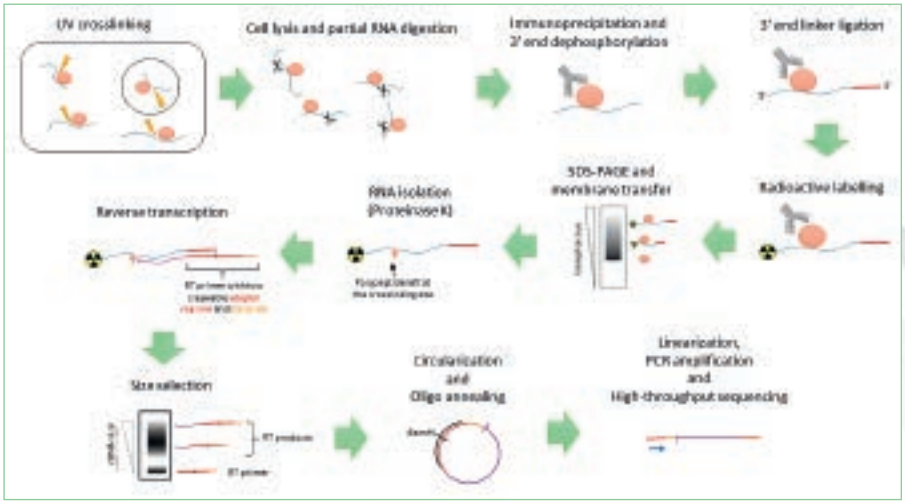
Takemoto Y, Ramirez RJ, Yokokawa M, Kaur K, Ponce-Balbuena D, Sinno MC, Willis BC, Ghanbari H, Ennis SR, Guerrero-Serna G, Henzi BC, Latchamsetty R, Ramos-Mondragon R, Musa H, Martins RP, Pandit SV, Noujaim SF, Crawford T, Jongnarangsin K, Pelosi F, Bogun F, Chugh A, Berenfeld O, Morady F, Oral H and Jalife J. **Inhibition of Galectin-3 Mitigates Atrial Fibrosis and Vulnerability to AF and Increases Rate of Spontaneous Cardioversion to Sinus Rhythm in a model of Persistent Atrial Fibrillation**. *JACC: Basic to Translational Science* (2016) 1: 143–54

Molecular regulation of heart failure

RESEARCH INTEREST

Our laboratory investigates the molecular pathways driving heart remodeling and the development of heart failure, which are still poorly understood. In particular, we focus on the role of RNA binding proteins (RBPs) and alternative splicing in these processes. For this purpose, we developed ATtRACT, an integrated database of RPBs and their associated RNA motifs (Giudice et al., 2016), which is the largest RBP database to date. Using ATtRACT and other bioinformatic tools, we have identified a potential role of some SR-rich splicing factors (SRSF) in post-infarction remodeling. In addition, we have reported that the alternative splicing variant of calcineurin, CnAβ1, has a mechanism of action completely different from other calcineurin isoforms (Gómez-Salinero et al., 2016). Instead of targeting the transcription factor NFAT, CnAβ1 activates the Akt/mTOR signaling pathway to regulate mesodermal differentiation in embryonic stem cells.

Our research also focuses on the study of the pathological mechanisms undelying different cardiomyopathies. In recent years, we have focused on the study of Lafora disease. This disease is characterized by seizures and epilepsy caused by the accumulation of abnormal glycogen deposits in neurons. It was unknown, however, whether these deposits could affect cardiac function. Using two mouse models of Lafora disease, we found that cardiomyocytes accumulate glycogen deposits that result in cardiac hypertrophy and defective contraction. These results suggest that Lafora disease should be considered an inherited metabolic cardiomyopathy like Fabri's or Danon's disease and that Lafora disease patients should be assessed for cardiac abnormalities.



Summary of the cross-linking immunoprecipitation and massive parallel sequencing (CLIP-Seq) protocol. To identify the targets of an RNA-binding protein (RBP), RPBs are cross-linked to their bound RNA, immunoprecipitated, separated on a polyacrylamide gel, and labeled with radioactive ATP. Following reverse transcription, cDNAs of the appropriate size are selected and circularized. The circular DNA molecules are then linearized, amplified by PCR and sequenced by next generation sequencing.

Head of Laboratory:
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Río Hortega Fellow:
Esther González

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Alberto Gatto
Enda Clinton
Girolamo Giudice
Paula Ortiz Sánchez
José Javier Larrasa Alonso
Carlos Martí Gómez-Aldaraví

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Technician:
Marina López Olañeta

Res@CNIC Fellow:
Juan M. Monteagudo Ruiz

Visiting Scientists:
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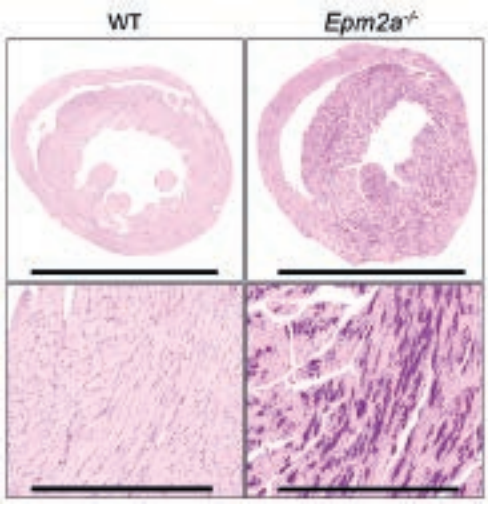
Elísabet Bello Arroyo
Fernando Domínguez Rodríguez
Marta Román Carmena



Glycogen deposits in cardiomyocytes of a Lafora disease mouse model. Heart sections from wild type (WT) mice or mice lacking laforin (Epm2a$^{-/-}$) were analyzed by Periodic acid-Schiff (PAS) staining, which labels glycogen deposits. Lafora knockout mice develop Lafora disease and show abnormal accumulation of glycogen deposits in cardiomyocytes, which is associated with a decline in systolic function. Wild type mice (left) are included as a negative control. Bar, 5 mm (top) or 500 µm (bottom).

MAJOR GRANTS

- European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2013-ITN, "CardioNext" 608027)
- European Commission. Marie Curie Action Initial Training Network (ITN) (FP7-PEOPLE-2011-ITN, "CardioNeT" 289600)
- Comunidad de Madrid (GRUPOSCAM10, "Fibroteam" S2010/BMD-2321)
- Instituto de Salud Carlos III (MSII14/00027)
- Ministerio de Economía y Competitividad (SAF2015-65722-R)

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Lara-Pezzi E, Desco M, Gatto A, Gómez-Gaviro MV. **Neurogenesis: regulation by alternative splicing and related posttranscriptional processes**. *Neuroscientist* pii: 1073858416678604. [Epub ahead of print Nov 10 2016]

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González-Santamaría J, Villalba M, Busnadiego O, López-Olañeta MM, Sandoval P, Snabel J, López-Cabrera M, Erler JT, Hanemaaijer R, Lara-Pezzi E*, Rodríguez-Pascual F*. **Matrix cross-linking lysyl oxidases are induced in response to myocardial infarction and promote cardiac dysfunction**. *Cardiovasc Res* (2016) 109: 67-78
*Co-corresponding authors.

Molecular cardiology

RESEARCH INTEREST

The molecular cardiology laboratory was launched in April 2016, and the period since has been occupied with installing equipment, recruiting expert staff, and establishing knock-in mouse colonies from Professor Priori's laboratory in the CNIC facilities. The patch clamp unit has been set up, and work is progressing on finalizing equipment to simultaneously record intracellular calcium and other ion currents in isolated cardiac cells. Methods are also being established to derive cardiomyocytes from induced pluripotent stem cells (iPSC), with the goal of studying cardiomyocytes differentiated from iPSCs of patients with inherited arrhythmias.

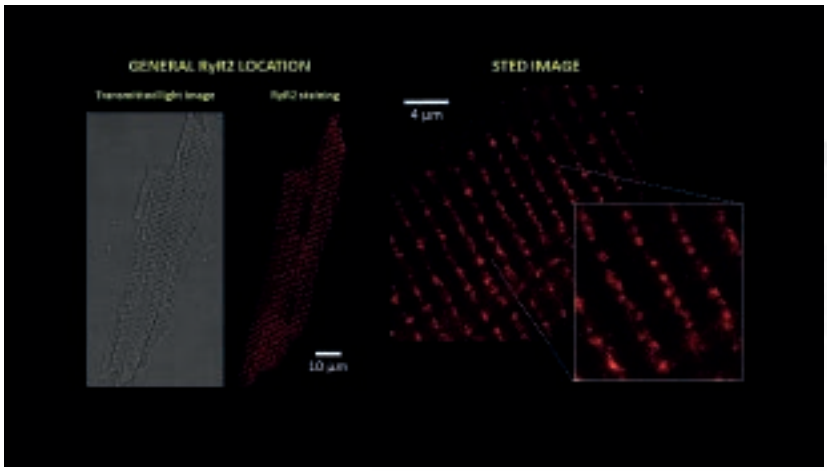
Dr. Priori has dedicated her clinical and research career to understanding the molecular mechanisms underlying inherited arrhythmias, and since 2013 she has focused her attention on the development of molecular therapies for these conditions. A major obstacle in the field is the lack of models for the arrhythmogenic syndromes of interest. The team therefore dedicates part of its effort to developing disease models, ranging from the patient-iPSC-derived cardiomyocytes described above to knock-in and knock-out models in mice and pigs.

The team's current research focuses on 2 severe inherited arrhythmogenic diseases: dominant catecholaminergic polymorphic ventricular tachycardia (CPVT) and Long QT syndrome type 8 (LQT8). The group is currently working with mouse models of the dominant and the recessive forms of CPVT to determine the effects of gene-therapy strategies on intracellular calcium handling and cell electrophysiology. These strategies have been developed by the Molecular Cardiology Laboratory at the ICS Maugeri Institute in Pavia, Italy. The group is also working on an ambitious project to develop a knock-in pig model of LQT8.

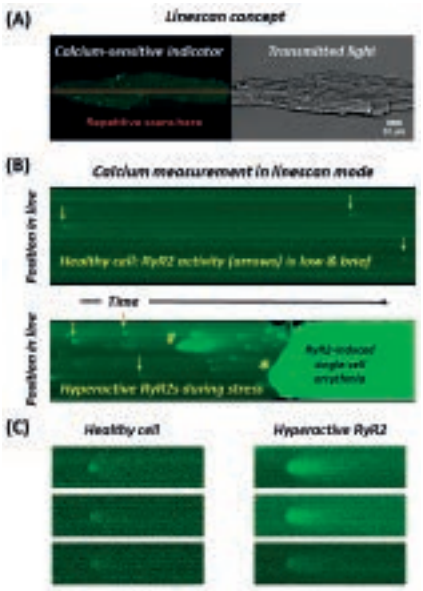
Head of Laboratory:
Silvia Giuliana Priori

Postdoctoral Researchers:
Demetrio Julián Santiago Castillo
Jaroslaw Karol Sochacki

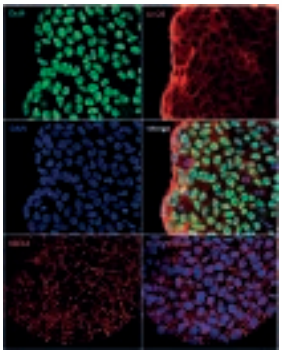
Graduate Technician:
Francesca Romana Antonucci



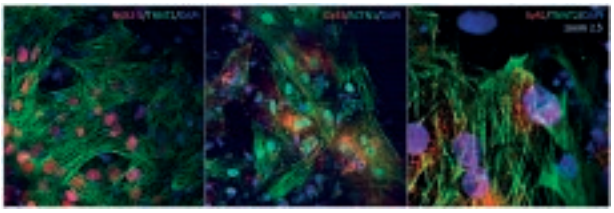
Left: Mouse ventricular myocyte immunostained for the cardiac ryanodine receptor (RyR2), a protein that mobilizes calcium (necessary for cell contraction) during the heartbeat. *Right*: Expanded view of the RyR2 arrangement (super-resolution microscopy). We are investigating whether arrythmogenic cardiovascular disease modifies the diversity of RyR2 cluster shapes, inter-cluster distances, and cluster grouping into "super-clusters".



(A) During a *linescan*, a single line within a cell is repetitively scanned. **(B)** Study of RyR2 activity in *linescan* mode, through calcium movements. In a healthy quiescent cell, RyR2 activition is low, and each calcium release event lasts a few milliseconds (arrows). In unhealthy, stressed, or diseased cells (eg, CPVT), RyR2 activity becomes high (arrows), extremely high and grouped (#), or extremely high, long-lived, and propagated among RyR2 clusters (*). This may interphere with membrane potential, causing arrythmias. **(C)** Details of short-lived calcium release events (*sparks*). Each spark lasts for 20-50 ms (depending on cell status) and extends for about 2 microns.



Correct expression of the pluripotency markers Oct4, Lin28, and SSEA4 in a CPVT-patient-derived iPS cell line. Nuclei are counterstained with DAPI. 40x objective.



Human iPSC-derived cardiomyocytes expressing typical lineage markers: cardiac transcription factor (NKX2-5), cardiac muscle troponin T (TNNT2), gap junction protein connexin 43 (Cx43), cytoskeletal alpha actinin (ACTN1), and cardiac ryanodine receptor (RyR2). 40x objective.

MAJOR GRANTS

- ERC Advanced Grant 2014. Molecular Strategies to Treat Inherited Arrhythmias
- International Postdoctoral Programme, 5th call, CNIC, 2016-19. Shaking the current view: Catecholaminergic polymorphic ventricular tachycardia is a nano-cardiomyopahy

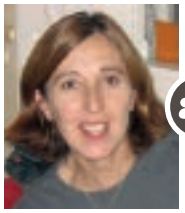
SELECTED PUBLICATIONS

Mazzanti A, Maragna R, Faragli A, Monteforte N, Bloise R, Memmi M, Novelli V, Baiardi P, Bagnardi V, Etheridge SP, Napolitano C, Priori SG. **Gene-Specific Therapy With Mexiletine Reduces Arrhythmic Events in Patients With Long QT Syndrome Type 3.** *J Am Coll Cardiol* (2016) 67: 1053-8

Bezzina CR, Lahrouchi N, Priori SG. **Genetics of sudden cardiac death.** *Circ Res* (2015) 116: 1919-36

Park DS, Cerrone M, Morley G, Vasquez C, Fowler S, Liu N, Bernstein SA, Liu FY, Zhang J, Rogers CS, Priori SG, Chinitz LA, Fishman GI. **Genetically engineered SCN5A mutant pig hearts exhibit conduction defects and arrhythmias.** *J Clin Invest* (2015) 125: 403-12

Nuclear receptor signaling



RESEARCH INTEREST

Macrophages are hematopoietic cells of the myeloid lineage with important functions in development, homeostasis, tissue repair, and immunity. Macrophages can be found in practically all tissues, making important contributions to their homeostasis and protection against injury. Projects in our group focus on elucidating the transcriptional control of macrophages in different tissues, especially in the heart and bone marrow, with special emphasis on their possible medical utility in the treatment of metabolic and cardiovascular diseases.

A special interest of our group is the molecular mechanisms regulating macrophage development and function. Our laboratory has shown that the nuclear receptor retinoid X receptor (RXR) plays a major regulatory role in macrophages, with implications for homeostasis, inflammation, and immunity. Our studies have demonstrated that RXR regulates macrophage transcriptional programs necessary for cell migration, debris clearance, macrophage polarization, cell proliferation and osteoclastogenesis, antiviral response, and lipid metabolism. Our more recent studies suggest that RXR may play important roles in the control of hematopoietic stem cell maintenance and the development and function of different tissue resident macrophages, which might have implications for tissue repair and regeneration. Other studies in our laboratory are aimed at deciphering the role of RXR during heart development, as part of a wider effort to understand the regulatory molecular mechanisms involved in cardiogenesis. To pursue these goals, we are currently conducting complementary loss-of-function and drug-mediated gain-of-function mouse studies and genome-wide transcriptomic (RNA-seq and GRO-seq) and cistromic (ChIP-seq) studies. Using these approaches, we will examine mice lacking RXR in hematopoietic stem cells, macrophages, endothelial cells, and cardiomyoctes, allowing us to examine the specific role of these receptors in tissue homeostasis and injury.

Head of Laboratory:
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Verdiana Trappetti

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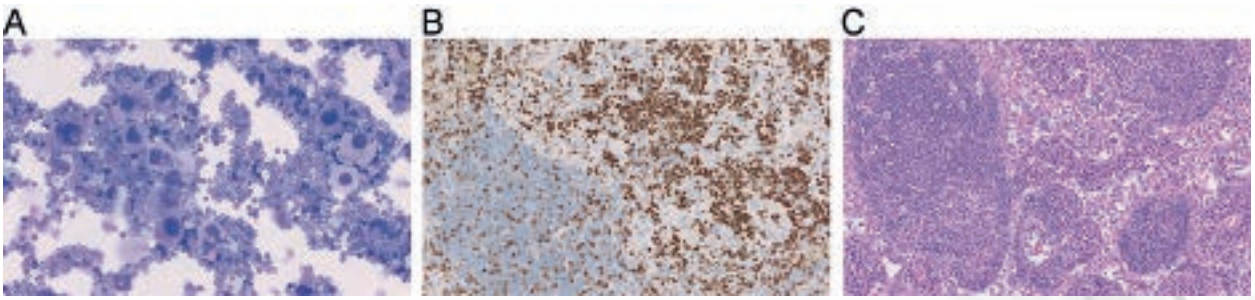
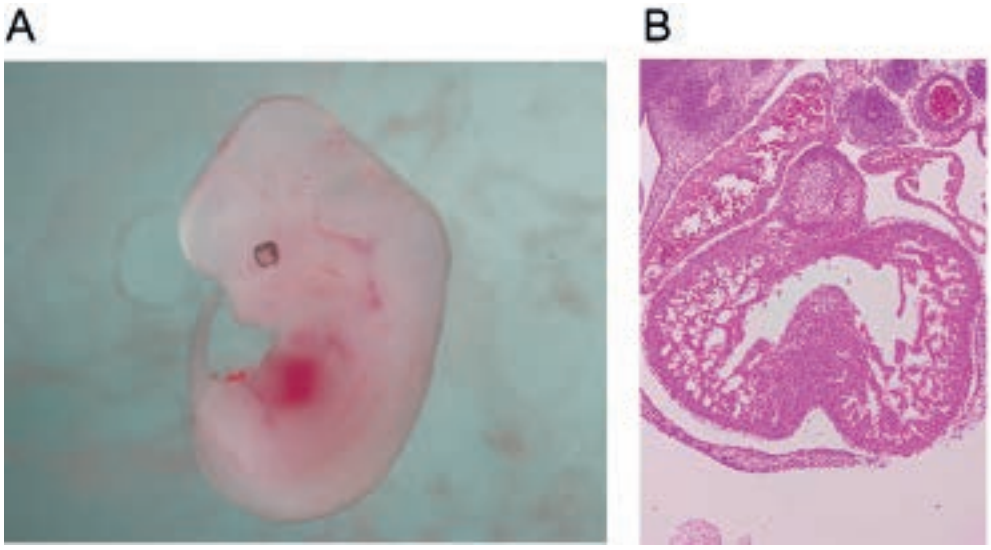
Guadalupe González
Jesús Porcuna

Technician:
Vanessa Núñez González



Role of RXR in hematopoiesis. **(A)** May-Grünwald-Giemsa staining of cytospinned bone marrow cells from RXR-deficient mouse. **(B)** Proliferating cells revealed by Ki67 staining on sections of paraffin-embedded spleen from an RXR-deficient mouse. **(C)** H&E staining of sections of paraffin-embedded lymph node from an RXR-deficient mouse.



Role of RXR during heart development. **(A)** Gross morphological appearance of an RXR-deficient embryo (E12.5). **(B)** H&E staining of the heart of an RXR-deficient embryo (E12.5).

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2015-64287-R)
- Ministerio de Economía y Competitividad (SAF2015-71878-REDT)
- European Commission, 7th Frame Program (FP7-PEOPLE-2013-ITN) (PITN-GA-2013-608027)
- Ministerio de Economía y Competitividad (SAF2012-31483)
- Fundación TV3 Marató 2012 (ref 165/C/12)

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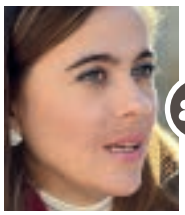
Natrajan MS, de la Fuente AG, Crawford AH, Linehan E, Nuñez V, Johnson KR, Wu T, Fitzgerald DC, Ricote M, Bielekova B, Franklin RJ. **Retinoid X receptor activation reverses age-related deficiencies in myelin debris phagocytosis and remyelination**. *Brain* (2015) 138: 3581-97

Walter W, Sánchez-Cabo F, Ricote M. **GOplot: an R package for visually combining expression data with functional analysis.** *Bioinformatics* (2015) 31: 2912-4

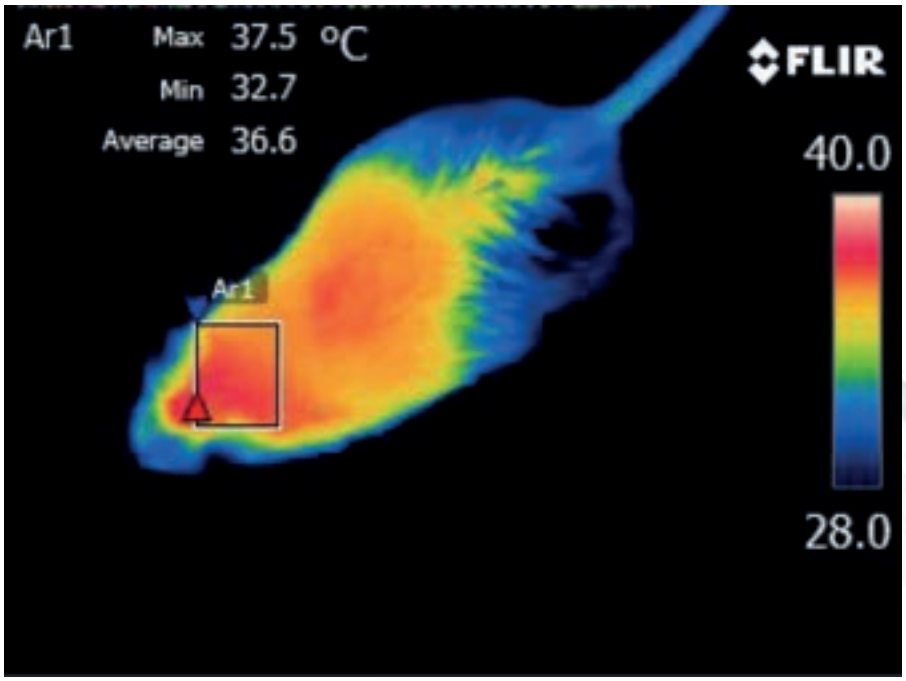
Menéndez-Gutiérrez MP, Rőszer T, Fuentes L, Núñez V, Escolano A, Redondo JM, De Clerck N, Metzger D, Valledor AF, Ricote M. **Retinoid X receptors orchestrate osteoclast differentiation and postnatal bone remodeling.** *J Clin Invest* (2015) 125: 809-23

Stress kinases in diabetes, cancer, and cardiovascular disease



RESEARCH INTEREST

We are working on the role of stress kinases in the development of metabolic diseases such as diabetes, fatty liver disease, and cardiovascular diseases. We have shown that these kinases control TNF production through the phosphorylation of eEF2K and activation of the elongation factor EF2 (*J Clin Invest*, 2013). Our recent work (*EMBO J*, 2016) shows that the lack of p38γ and p38δ in myeloid cells impairs neutrophil migration to the liver and thus protects against diet-induced steatosis and further liver damage. We have also demonstrated that these kinases control postnatal cardiac growth (*Nature Commun*, 2016). Current projects in the lab are continuing our efforts to uncover the role of these kinases in health and disease.



Infrared thermal image of a mouse showing regions surrounded by interscapular brown adipose tissue.

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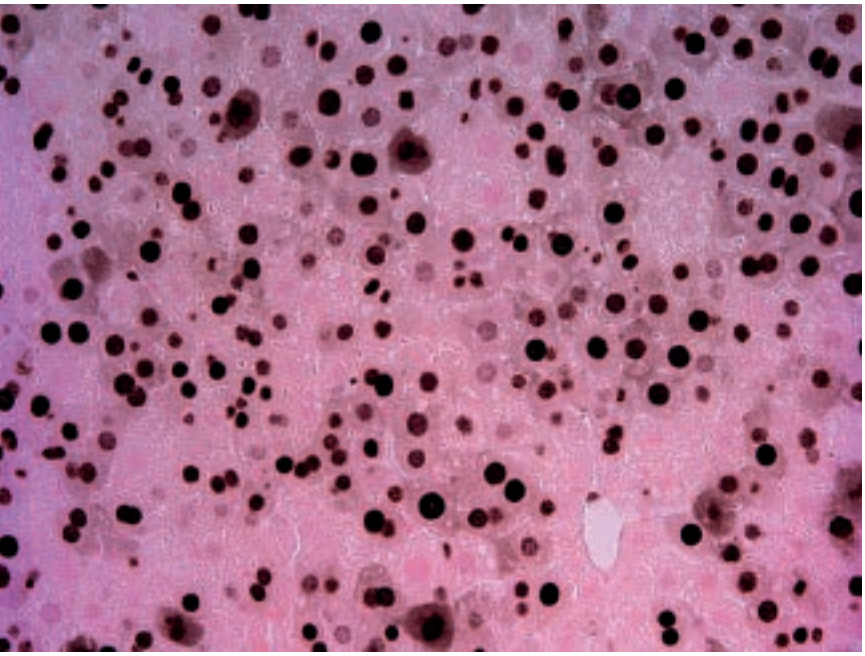
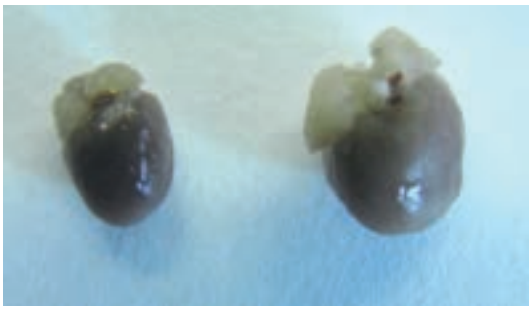


Liver stained with Ki67, showing cell cycle initiation after partial hepatectomy.



Mice lacking p38γ and p38δ have smaller than normal hearts size. Representative images of hearts from a KO mouse (left) and a WT mouse (right).

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2016-79126-R)
- Ministerio de Economía y Competitividad (SAF2013-43506-R)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260464)
- Comunidad de Madrid. INMUNOTHERCAN (S2011/BMD-2326)

SELECTED PUBLICATIONS

González-Terán B, Matesanz N, Nikolic I, Verdugo MA, Sreeramkumar V, Hernández-Cosido L, Mora A, Crainiciuc G, Sáiz ML, Bernardo E, Leiva-Vega L, Rodríguez E, Bondía V, Torres JL, Perez-Sieira S, Ortega L, Cuenda A, Sanchez-Madrid F, Nogueiras R, Hidalgo A, Marcos M, Sabio G. **p38γ and p38δ reprogram liver metabolism by modulating neutrophil infiltration.** *EMBO J* (2016) 35: 536-52

González-Terán B, López JA, Rodríguez E, Leiva L, Martínez Martínez S. Jiménez Borreguero LJ, Redondo JM, Vázquez J, Sabio G. p38 and δ promote heart hypertrophy by targeting the mTOR-inhibitory protein DEPTOR for degradation. *Nat Commun* (2016) 7:10477

Manieri E, Sabio G. **Stress kinases in the modulation of metabolism and energy balance**. *J Mol Endocrinol* (2015) 55: R11-22

Immunobiology

RESEARCH INTEREST

We are interested in the manipulation of dendritic cells (DCs) and macrophages for immunotherapy. The analysis of different DC subsets indicates that they have specific functions and can be selectively targeted to induce specific immune responses. We have investigated the role of DC1s in the generation of $CD8^+$ T cell memory and have found that these cells provide unique signals for the generation of resident memory precursors (Figure 1), which are crucial for surveying and mounting an effective and rapid immune response upon reinfection of skin and mucosae (Iborra et al. 2016a. Immunity).

C-type lectin receptors sense a diversity of endogenous and exogenous ligands that can trigger differential responses. We recently found that Mincle detects a ligand in *Leishmania* (Figure 2) that triggers an inhibitory axis characterized by SHP1 coupling to the FcRγ chain. We conclude that *Leishmania* shifts Mincle to an inhibitory ITAM (ITAMi) configuration that impairs DC activation. Thus, ITAMi can be exploited for immune evasion by a pathogen and may represent a paradigm for self and non-self sensing by ITAM-coupled receptors.

We also explored the mitocondrial adaptations following sensing of bacteria by macrophages (Figure 3) and found that recognition of viable bacteria through TLR- and NLRP3-dependent pathways induces a transient switch in the relative contribution of complexes CI and CII to mitocondrial respiration in macrophages. Notably, pharmacological inhibition of CII during *E. coli* infection decreased IL-1β and increased IL-10 serum-concentrations, resulting in impaired control of bacteria. Our research thus has potential for the development of new vaccines and immunotherapy strategies.

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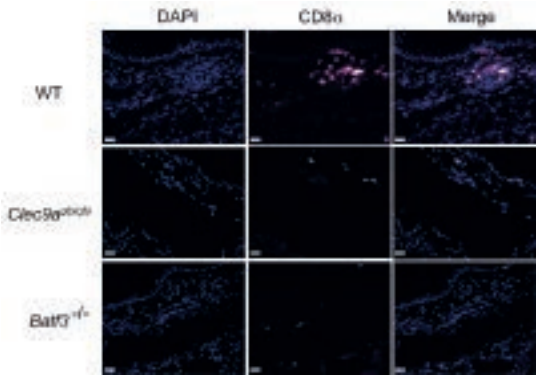
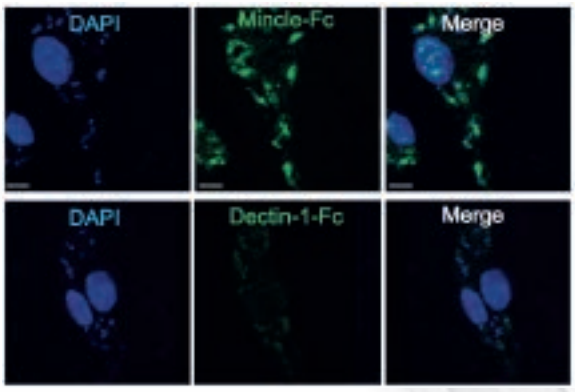
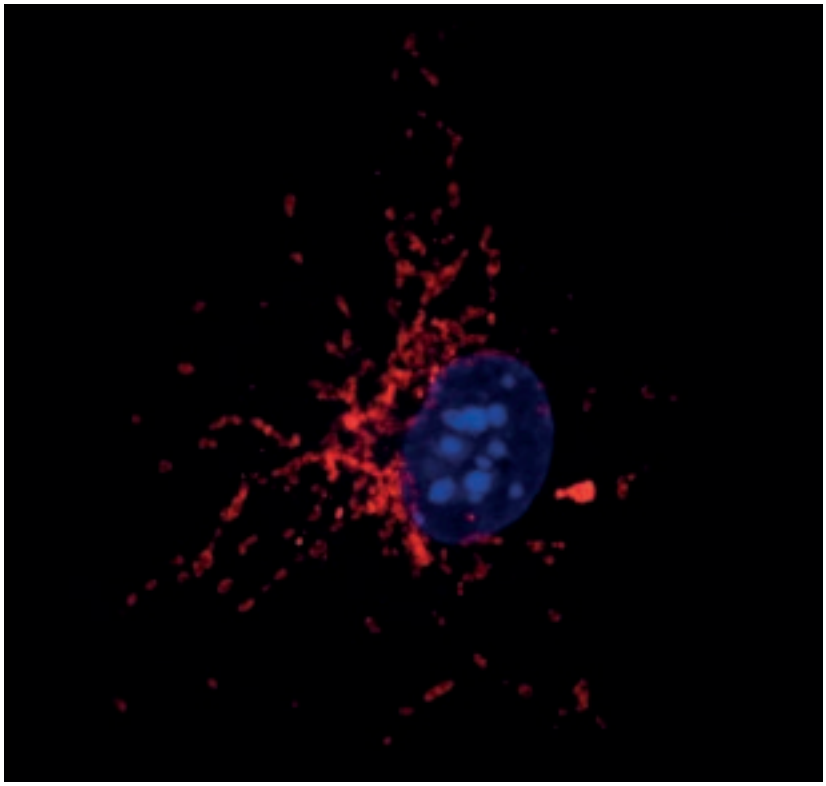


Wild-type mice (WT) or mice deficient in DNGR-1 ($Clec9a^{gfp/gfp}$) or Batf3 ($Batf3^{-/-}$) were infected with vaccinia virus and generation of resident memory CD8+ T cells was tracked for 30 days post-infection in the infected skin by immunofluorescence staining as indicated. Scale bar: 10 µm.



Bone marrow-derived macrophages were preincubated with *Leishmania* promastigotes, fixed, permeabilized and stained with Mincle-Fc or Dectin-1-Fc. Confocal images are shown. Nuclei were counterstained with DAPI. Scale bar: 5 µm.



Bone marrow-derived macrophages were stained for mitochondria using mitotracker (red) and nucleus was counterstained with DAPI.

MAJOR GRANTS

- Ministerio de Economía y Competitividad (SAF2016-79040-R)
- Ministerio de Economía y Competitividad (EUIN2015-62652)
- Ministerio de Economía y Competitividad. Programa Redes de Excelencia 2014. (SAF2014-53563- REDT).
- EU Framework Programme for Research and Innovation H2020. Call: H2020-PERSONALISING HEALTH AND CARE (GA635122-PROCROP).
- Ministerio de Economía y Competitividad (SAF2013-42920-R)
- European Commission. European Research Council Starting Independent Researcher Grant (ERC-StG-260414)
- Research cooperation agreement with MedImmune (Cambridge, UK)
- ERS/EU Marie Curie Post-doctoral Research Fellowships (RESPIRE 2 - 3708-2013).

SELECTED PUBLICATIONS

Iborra S, Martínez-López M, Khouili SC, Enamorado M, Cueto FJ, Conde-Garrosa R, del Fresno C, Sancho D. **Optimal generation of tissue-resident but not circulating memory T cells during viral infection requires crosspriming by DNGR-1+ dendritic cells**. *Immunity (*2016a) 45:847-60

Iborra S, Martínez-López M, Cueto FJ, Conde-Garrosa R, Del Fresno C, Izquierdo HM, Abram CL, Mori D, Campos Martín Y, Reguera RM, Kemp B, Yamasaki S, Robinson MJ, Soto M, Lowell CA Sancho D. **Leishmania uses Mincle to target an inhibitory ITAM pathway in dendritic cells that dampens adaptive immunity to infection**. *Immunity (*2016b) 45:788-801

Garaude J, Acín-Pérez R, Martínez-Cano S, Enamorado M, Ugolini M, Nistal-Villán E, Hervás-Stubbs S, Pelegrín P, Sander LE, Enríquez JA, Sancho D. **Mitochondrial respiratory-chain adaptations in macrophages contribute to antibacterial host defense**. *Nat Immunol* (2016) 17:1037-45

Sanchez-Paulete AR, Cueto FJ, Martinez-Lopez M, Labiano S, Morales-Kastresana A, Rodriguez-Ruiz ME, Jure-Kunkel M, Azpilikueta A, Aznar MA, Quetglas JI, **Sancho D***, Melero I*. **Cancer immunotherapy with immunomodulatory anti-CD137 and anti-PD-1 monoclonal Abs requires Batf3-dependent DCs**. *Cancer Discov* (2016) 6: 71-9 *Co-corresponding authors

Blanco-Menéndez N, Del Fresno C, Fernandes S, Calvo E, Conde-Garrosa R, Kerr WG, Sancho D. **SHIP-1 couples to the dectin-1 hemITAM and selectively modulates reactive oxygen species production in dendritic cells in response to candida albicans**. *J Immunol* (2015) 195: 4466-78