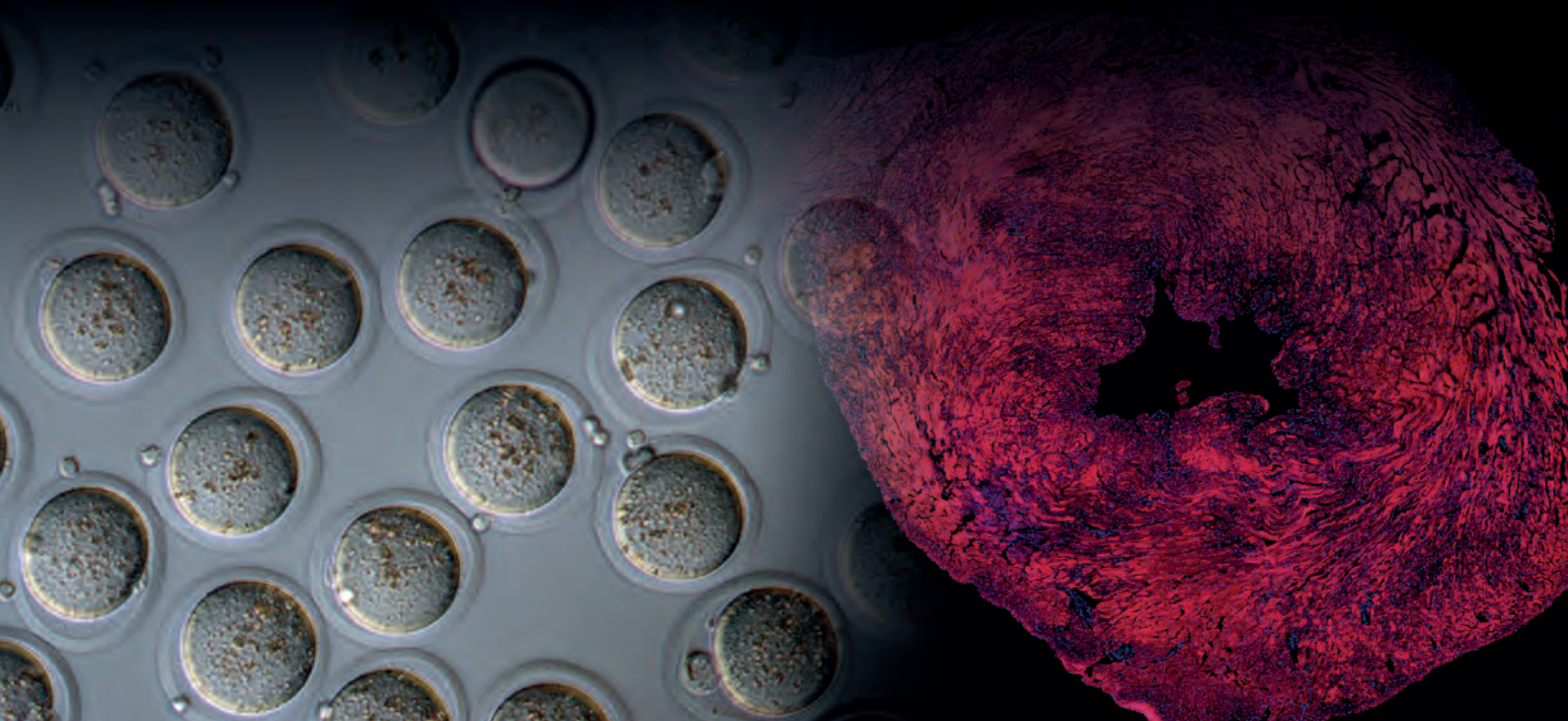
TECHNICAL UNITS

- Advanced Imaging
- Bioinformatics
- Cellomics
- Comparative Medicine
- Genomics
- Microscopy and Dynamic Imaging
- Pluripotent Cell Technology
- Proteomics/Metabolomics
- Transgenesis
- Viral Vectors



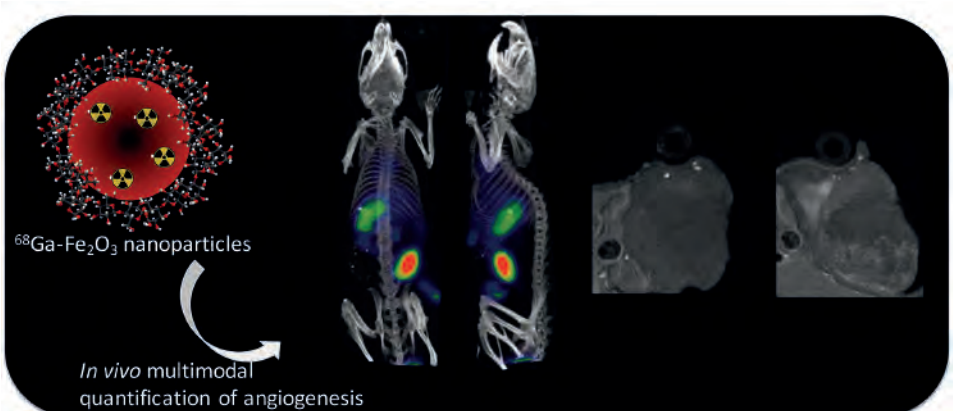
Advanced Imaging

Head of Unit

Jesús Ruiz-Cabello Osuna

RESEARCH INTEREST

The Advanced Imaging Unit (AIU) is a multidisciplinary group working on the development of new imaging applications and molecular imaging tools that will expand knowledge of the molecular and cellular events underlying cardiovascular disease. The three core areas of the AIU's research and service are 1) cardiovascular imaging, 2) nanomedicine and radiochemistry, and 3) metabolomics (research only). The AIU offers the CNIC and the wider scientific community support and expertise in cardiovascular imaging using five state-of-the-art modalities: MRI, X-ray CT, nuclear imaging (PET), ultrasound (echocardiography) and optical (2- and 3-dimensional luminescence and fluorescence). For its nanomedicine and radiochemistry program, the AIU has a dedicated nanotechnology and bioorganic chemistry laboratory dedicated to developing new nanoparticles, molecular probes, techniques for site-directed biofunctionalization of biomacromolecules (peptides, proteins and antibodies), and tools for the oriented immbolization of these molecules for the diagnosis and treatment of cardiovascular diseases. Currently the unit produces multifunctional nanoparticles for all imaging techniques available at the CNIC. The range of nanoparticles includes iron oxide, liposomes, carbon dots and gold nanoparticles, and all of them are functionalized with specifc cardiovascular biomarkers. The Unit's radiochemistry laboratory is now fully operative for ^{68}Ga and ^{89}Zr, providing the Center with specific PET radiotracers for cardiovascular nuclear imaging. On a daily basis, the imaging unit works with conventional (cyclotron obtained) radiotracers (^{18}F-FDG, ^{18}F-FMISO, ^{18}F-NaF, etc.) for the noninvasive assessment of different cardiovascular diseases. The Unit also has long experience in metabolic data analysis using ^{18}F-FDG PET, magnetic resonance spectroscopy (^{13}C, ^{31}P, ^{1}H) and mass spectrometry, as well as statistical and image and spectroscopic processing tools developed in-house. The Unit is also engaged in developing new techniques for cardiovascular imaging (PET, CT and MRI), which are tested and validated on small and large animal models and finally transferred to human applications. Our research in these areas ranges from technical developments and chemistry advances to *in vitro* studies and tracking of biological processes *in vivo*.



Nano-radiochemistry applications. *Left*: ^{68}Ga core-doped iron oxide nanoparticles for angiogenesis quantification. *Right*: PET/CT imaging of tumor-bearing mice 1 hour after injection of ^{68}Ga-C-IONP-RGD, showing strong activity in the tumor. *Right*: Axial T1-weighted spin echo MRI of the tumor area in a mouse before injection of ^{68}Ga-C-IONP-RGD and 24 hours post-injection.

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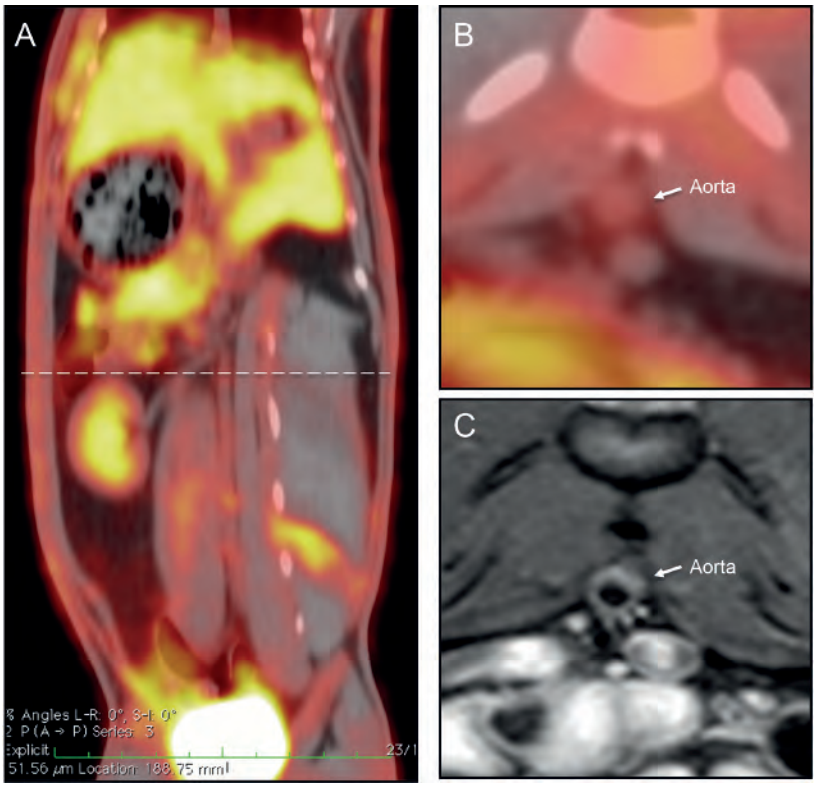


In vivo **^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) of metabolic rate/ inflammation, and magnetic resonance imaging (MRI) monitoring of atherosclerotic plaque progression**. A, Combined PET/computed tomography (CT) coronal view of the abdominal aorta, illustrating the ^{18}F-FDG uptake of an atherosclerotic rabbit after 8 months on an atherogenic diet. B, Axial view of the slice indicated with a dotted line in the coronal view, showing increased FDG uptake in the aorta. C, Corresponding T1-weighted MRI showing development of an aortic atherosclerosis lesion.

MAJOR GRANTS

- *Ministerio de Sanidad y Consumo (CIBERES CB06/06/1090)*
- *European Commission FP7-PEOPLE-2010-ITN (П-NET 264864) (NO CNIC).*
- *European Commission FP7-PEOPLE-2013-ITN (CardioNext PITN-GA-2013-608027)*
- *Ministerio de Economía y Competitividad. FIS RETICS (Terapia Celular: RD12/0019/0005) PI: Miguel Torres, Colaborador Jesus Ruiz Cabello*
- *Ministerio de Economía y Competitividad. Modalidad Generación Conocimiento (MAT2013-47303-P) PI: Fernando Herranz*
- *Instituto de la Salud Carlos III. FIS-FEDER (PI14/01427) PI: Jesús Mateo*
- *Ministerio de Economía y Competitividad. SAF2014-58920-R PI: Samuel España*
- *Ministerio de Economía y Competitividad. SAF2014-59118-JIN. PI: Marco Filice*
- *Madrid-MIT M+Visión (PRMIT2013) PI: Samuel España*
- *Madrid-MIT M+Visión (MIT14 - AAE867002) PI: Teresa Arias*
- *Madrid-MIT M+Visión (MIT14 - X7118248R) PI: Arnoldo Santos*

SELECTED PUBLICATIONS

Mateo J, Benito M, España S, Sanz J, Jiménez-Borreguero J, Fuster V, Ruiz-Cabello J. **Magnetic resonance imaging of the atherosclerotic mouse aorta.** *Methods Mol Biol* (2015) 1339: 387-94

Mateo J, Bilbao I, Vaquero JJ, Ruiz-Cabello J, España S. **In vivo ^{18}F-FDG-PET imaging in mouse atherosclerosis.** *Methods Mol Biol* (2015) 1339: 377-86

Salinas B, Ruiz-Cabello J, Lechuga-Vieco AV, Benito M, Herranz F. **Surface-functionalized nanoparticles by olefin metathesis: A chemoselective approach for in vivo characterization of atherosclerosis plaque.** *Chemistry - A European Journal* (2015) 21: 10450–6

Pellico J, Lechuga-Vieco AV, Benito M, García-Segura JM, Fuster V, Ruiz-Cabello J, Herranz F. **Microwave-driven synthesis of bisphosphonate nanoparticles allows in vivo visualisation of atherosclerotic plaque**. *RSC Advances* (2015) 5: 1661-5

Groult H, Ruiz-Cabello J, Pellico J, Lechuga-Vieco AV, Bhavesh R, Zamai M, Almarza E, Martín-Padura I, Cantelar E, Martínez-Alcázar MP, Herranz F. **Parallel multifunctionalization of nanoparticles: a one-step modular approach for in vivo imaging.** *Bioconjug Chem* (2015) 26: 153-60

Bioinformatics

Head of Unit

Fernando Martínez

Support Scientists:

Fátima Sánchez Cabo
Carlos Torroja
Manuel José Gómez Rodríguez

Predoctoral Researchers:

Wencke Walter
(from the Nuclear Receptor Signaling Laboratory, led by Mercedes Ricote)
Alberto Gatto
(from the Molecular Regulation of Heart Development and Disease Laboratory, led by Enrique Lara-Pezzi)
Girolamo Giudice
(from the Molecular Regulation of Heart Development and Disease Laboratory, led by Enrique Lara-Pezzi)

RESEARCH INTEREST

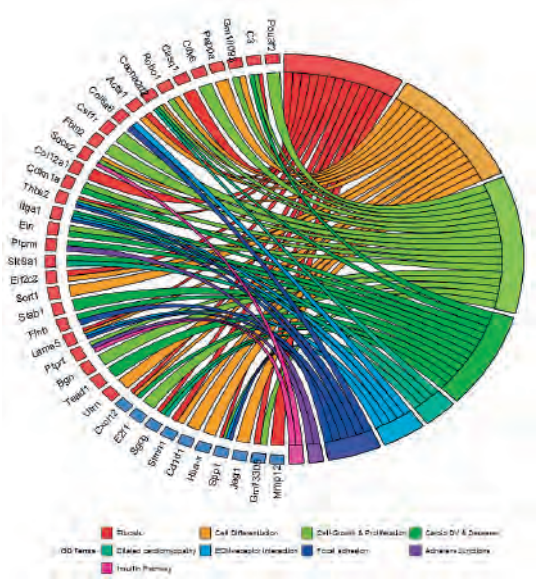
The Bioinformatics Unit provides *ad-hoc*, state-of-the-art bioinformatic, data analysis and computational biology solutions to support and enhance CNIC research projects in a collaborative environment.

During 2015the Unit worked with several research groups to has developed two new bioinformatics tools to visualize and gain insight into the biology of complex systems explored with omics technologies. (i) GOplot (Walter W et al, *Bioinformatics* 2015) is a Bioconductor package to ease the integration of omics data with functional analysis results (Figure 1). (ii) ATtRACT (Giudice G et al, *Database* 2016) is a database of RNA binding protein motifs that integrates resources disseminated in various repositories to facilitate the identification of enriched motifs in the sequence of sets of alternatively spliced genes (Figure 2).

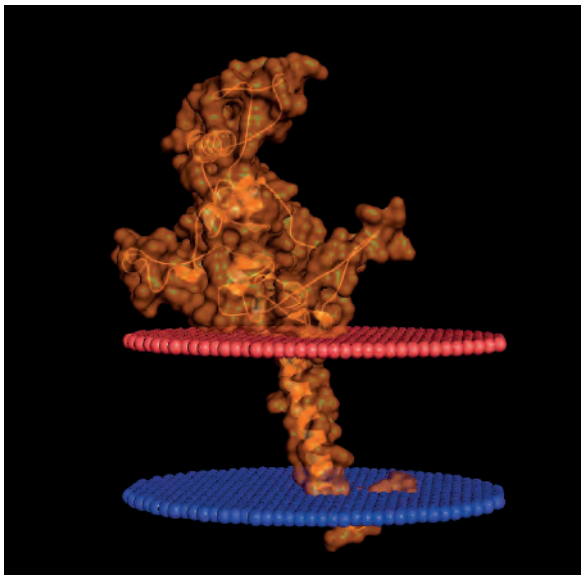
Another main focus during 2015 was the implementation of state-of-the-art software suites, tools and pipelines for structural bioinformatic analysis, modeling and protein docking (Figure 3). These solutions give CNIC researchers a new view of the 3D and protein interaction environments to help them in their research.

In addition, the Unit is moving towards translational bioinformatics by automating the already implemented pipelines to allow the analysis of hundreds of samples of large human cohorts. We are also involved in the implementation of tools and methodology to integrate heterogeneous *omics* data, as well as in the development of pipelines for the analysis of single-cell data, in collaboration with the Genomics Unit and members of the EU consortia funded by the H2020 APERIM program (http://aperim.eu/).

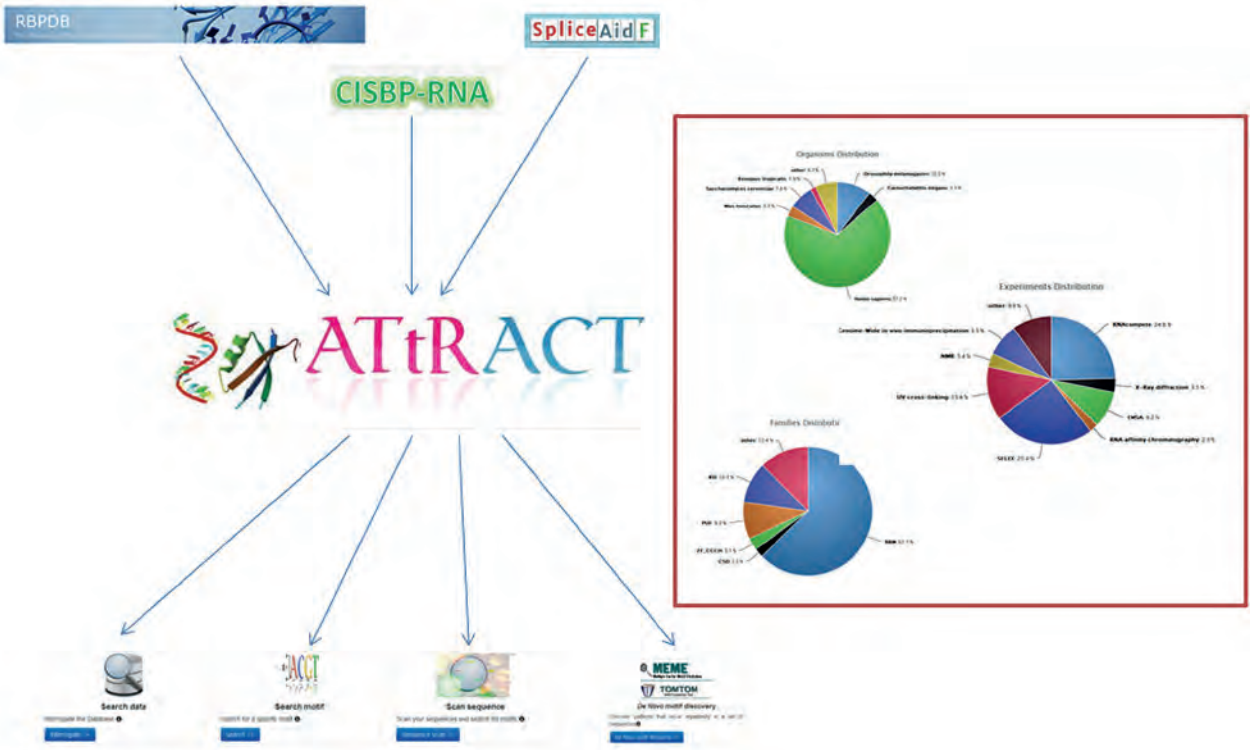
The Unit has also continued to provide customized advice and training to CNIC researchers on the analysis and interpretation of their experimental data. In this regard, we have completed the implementation of the Bioinformatics Unit Galaxy Platform, which will be soon opened to CNIC researchers, allowing them to analyze their own *omics* data.



Example of a circos plot produced with the GOplot package (Walter et al, 2015) to display the association between differentially expressed genes and the corresponding enriched pathways (D'Amato et al, 2015)



3D view of the emerin protein embedded in the nuclear membrane



Summary of the Attract database (Giudice G, Sánchez-Cabo F, Torroja C*, Lara-Pezzi E*, ATtRACT - A database of RNA binding proteins and associated motifs. Accepted for publication in *Database*)

SELECTED PUBLICATIONS

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

Martin-Alonso M, Garcia-Redondo AB, Guo D, Camafeita E, Martinez F, Alfranca A, Mendez-Barbero N, Pollan A, Sanchez-Camacho C, Denhardt DT, Seiki M, Vazquez J, Salaices M, Redondo JM, Milewicz DM, Arroyo AG. **Deficiency of MMP17/MT4-MMP proteolytic activity predisposes to aortic aneurysm in mice**. *Circ Res* (2015) 117: e13-26

Marcos S, Gonzalez-Lazaro M, Beccari L, Carramolino L, Martin-Bermejo MJ, Amarie O, Martin DM, Torroja C, Bogdanovic O, Doohan R, Puk O, de Angelis MH, Graw J, Gomez-Skarmeta JL, Casares F, Torres M, Bovolenta P. **Meis1 coordinates a network of genes implicated in eye development and microphthalmia**. *Development* (2015) 142: 3009-20

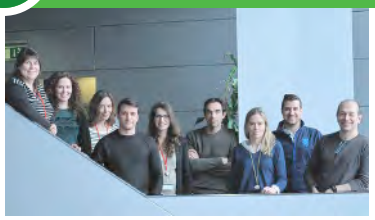
D'Amato G, Luxan G, Del Monte-Nieto G, Martinez-Poveda B, Torroja C, Walter W, Bochter MS, Benedito R, Cole S, Martinez F, Hadjantonakis AK, Uemura A, Jimenez-Borreguero LJ, de la Pompa JL. **Sequential Notch activation regulates ventricular chamber development**. *Nat Cell Biol* (doi: 10.1038/ncb3280.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

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

Cellomics

Head of Unit

María Montoya



RESEARCH INTEREST

The Cellomics Unit provides the CNIC with the two principal cell analytical techniques, flow cytometry and high content screening (HCS), and supports quantitative image-based research.

In 2015, we implemented spectral flow cytometry technology with a newly acquired Sony SP6800 Spectral analyzer (Fig. 1). We also organized the High Content Screening Workshop at the CNIC, which brought together researchers from academia and industry to discuss state-of-the-art methodology and the latest computing solutions for high content screening technology. In partnership with the Integrin Signaling group, we successfully developed an HCS assay for analyzing extracellular matrix remodeling based on multiparametric image analysis of a combination of texture and intensity parameters obtained from fibronectin signals. In control experiments Y27 induced a chaotic phenotype (Z' = 0.5) whereas TGFβ induced an organized phenotype (Z' = 0.28) (Fig. 2). The Unit has also programmed an automatic segmentation and tracking computational pipeline for confocal 4D time-lapse videos of cell membrane signals recorded in cultured cells. This work has been done in partnership with the Genetic control of organ development and regeneration laboratory, and allows the automated extraction of phenotypic parameters. An additional data analysis and cell lineage tree tracing pipeline was developed in MATLAB (Fig.3B). The Unit also developed customized image analysis tools for a variety of purposes, for example, quantification of collagen deposition or blood vessels in heart and tumor immunohistological tissue sections.

Research Scientists:

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Daniel Jiménez

Predoctoral Researcher:

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(since 1 December)

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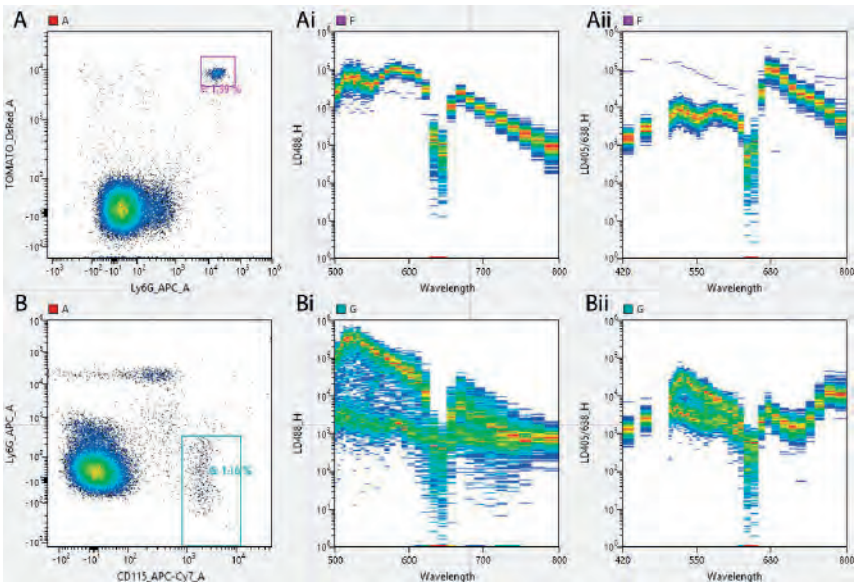
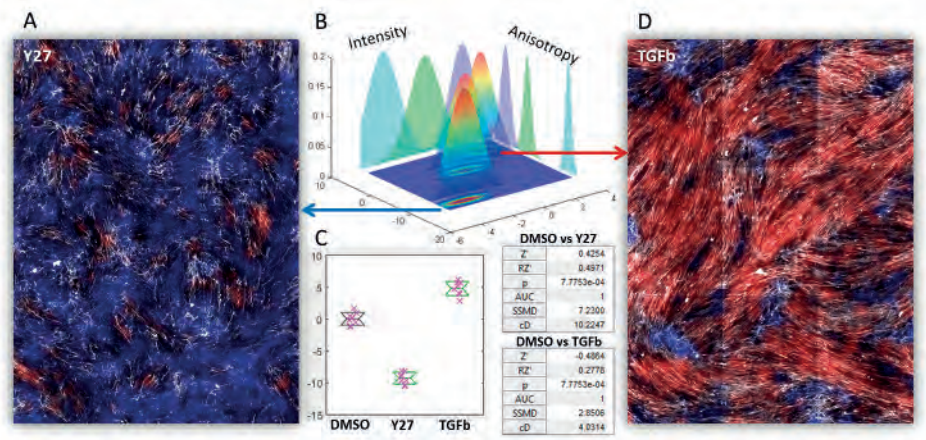
Mariano Vitón
Irene Palacios Doiztua
Elena Prieto
(since 1 December)

Visiting Scientist:

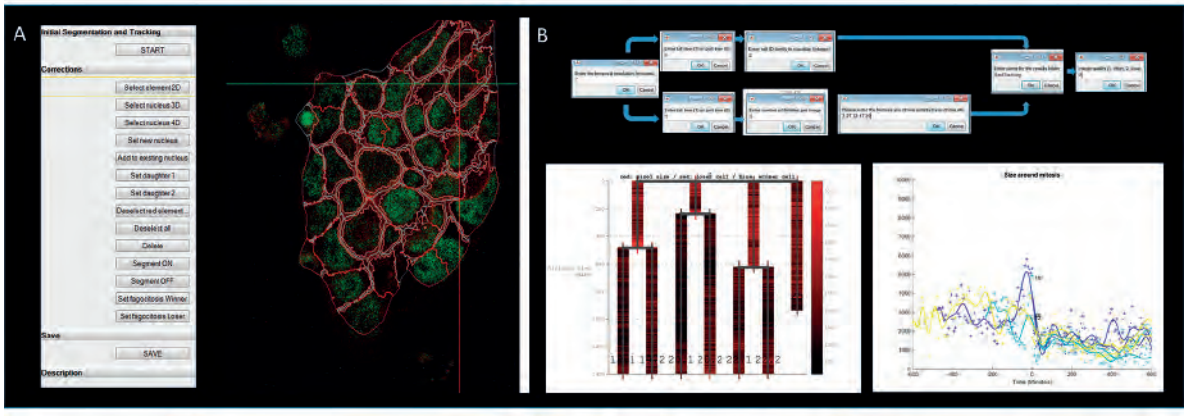
Marco Cordani



Detection of myeloid subpopulations by spectral flow cytometry. Mouse peripheral blood cells labeled with Ly6C FITC, Ly6G APC, and CD115 APC-CY7. **A,B)** Dot plot representations of subpopulations of interest TdTomato+/Ly6G+ (F) and CD115+/Ly6G- (G). Spectra are shown for analysis of the F **(Ai, Aii)** and G **(Bi, Bii)** subpopulations, and were obtained by exciting with lasers at 488 nm **(Ai, Bi)** and 638 nm **(Aii, Bii)**.



Quantification of the organization of the extracellular matrix of using multiparametric image analysis. **A, D)** Fn staining of Hela cells treated with Y27 (left) or TGFβ (right) displaying chaotic (blue) and organized (red) phenotypes. **B)** Gaussian mixture model of texture and intensity parameters. **C)** The resulting value of texture and intensity combination for eight independent wells of the indicated treatments.



Cell segmentation, tracking and data analysis tool. **A)** Segmentation and tracking computational pipeline with edition interface developed in Definiens. **B)** Data analysis and cell lineage tree tracing developed in MATLAB, showing visualization options and an example of the resulting graphics.

MAJOR GRANTS

- *Ministerio de Economía y Competitividad (BIO2014-62200-EXP)*
- *European Union (641639) (H2020 ITN-BIOPOL)*

SELECTED PUBLICATIONS

Pellico J, Ruiz-Cabello J, Saiz-Alía M, del Rosario G, Caja S, Montoya MC, Laura Fernández de Manuel L, Puerto Morales M, Gutiérrez L, Galiana B, Enríquez JA, Herranz F. **Fast synthesis and bioconjugation of 68Ga core-doped extremely small iron oxide nanoparticles for PET/MR imaging**. *Contrast Media Mol Imaging* (accepted)

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

Rallon NI, Mothe B, Lopez Bernaldo DE Quiros JC, Plana M, Ligos JM, Montoya MC, Muñoz MA, Esteban M, Garcia F, Brander C, Benito JM; RISVAC03 Study Group. **Balance between activation and regulation of HIV-specific CD8 T cells response after MVA-B therapeutic vaccination**. *AIDS* (doi: 10.1097/QAD.0000000000000966. Epub 2015 Nov 19)

Jimenez-Carretero D, González G., Rodríguez-López S., Kumamaru KK, George E, San José ER, Ledesma-Carbayo MJ (2015). **Automated axial right ventricle to left ventricle diameter ratio computation in computed tomography pulmonary angiography**. *PloS One* (2015) 10: e0127797

Petitjean C, Zuluaga MA, Bai W, Dacher JN, Grosgeorge D, Caudron J, Ruan S, Ayed IB, Cardoso MJ, Chen HC, Jimenez-Carretero D, Ledesma-Carbayo MJ, Davatzikos C, Doshi J, Erus G, Maier OM, Nambakhsh CM, Ou Y, Ourselin S, Peng CW, Peters NS, Peters TM, Rajchl M, Rueckert D, Santos A, Shi W, Wang CW, Wang H, Yuan J. **Right ventricle segmentation from cardiac MRI: A collation study**. *Med Image Anal* (2015) 19:187-202

Comparative Medicine

The Unit develops and manages laboratory animal models to reproduce the principal human cardiovascular diseases, working closely with the CNIC research teams and apllying the 3 Rs. The Unit tries to refine these animal models by identifying factors that could interfere with research project aims, be a source of non-representative data, or have a major impact on animal welfare.

The Comparative Medicine Unit's support for in vivo work at the CNIC is organized into five core work areas.

- *Animal Husbandry*. The Unit's technicians, managers and veterinarians are trained to work under the facility's SPF conditions and take charge of the daily husbandry of the animal colonies. The Unit enacts an environmental enrichment program to support species-specific behaviors to maximize animal welfare and wellbeing.

- *Pathology Core* (PC). The Histopathology Laboratory provides specialized hispathological services including animal necropsy, paraffin and OCT processing and sectioning, histochemical and immunohistochemical staining of tissue sections, digital scanning and image analysis, optical projection tomography with an OPT 3001 scanner and general support to CNIC researchers with phenotyping and histopathological evaluation of their animal models.

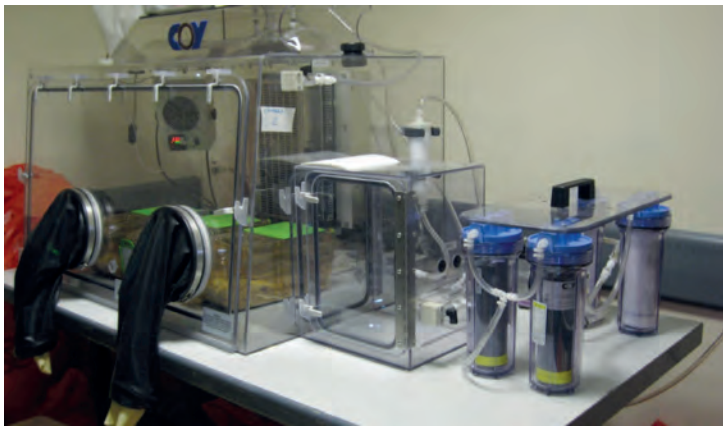
- *Phenotyping Core* (PhC). In this area, we have added new equipment to meet the needs of the CNIC research groups, including a coagulation analyzer and a metabolic cages system.

- *Veterinary Medicine and Experimental Surgery Core* (VMESC). The VMESC provides highly specialized expertise in the surveillance and monitoring of animal health status, disease follow-up, development of surgical animal models with enphasis on minimally invasive procedures, life support, setting up new experimental strategies that reproduce human cardiovascular diseases, and acquisition of pathophysiological data. The VMESC team is run by two clinical veterinarians with extensive expertise in laboratory animal science and four specialist veterinary technicians.

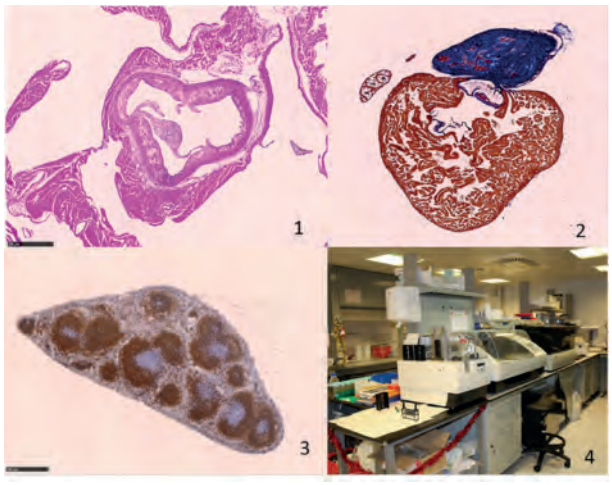
- *Quality Control Core* (QCC). The QCC follows the recommendations of the latest FELASA report (*Laboratory Animals* 2014, 48(3):178-192).

The Comparative Medicine Unit maintains ISO 9001 accreditation for all five core work areas.

PRIMUS anesthesia equipment



Hypoxic chamber



1. Atheroma plaques in a mouse aortic valve stained with H&E. **2**. Zebrafish heart stained with Acid Fuchsin Orange G to visualize collagen (blue). **3** Immunohistochemistry staining with anti-PAX5 antibody in a mouse spleen highlighting the B lymphocytes of the white pulp. **4**. Histopathology laboratory.

Genomics

Head of Unit

Ana Dopazo

Support Scientists:

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Alberto Benguría

Technician:

Rebeca Álvarez

RESEARCH INTEREST

The Genomics Unit currently focuses on second generation sequencing (NGS) technologies for genome analysis using the Illumina HiSeq 2500 and MiSeq sequencers.

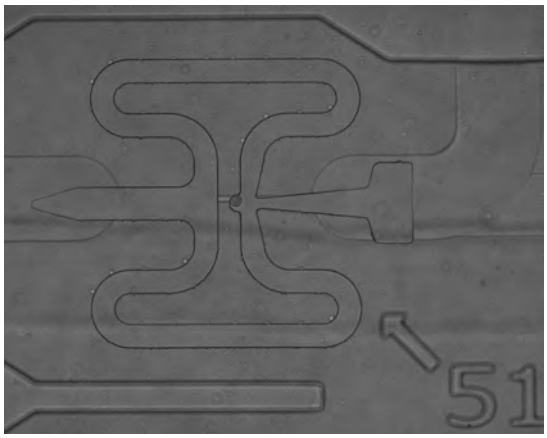
The Unit provides these cutting-edge genomic technologies to the scientific community at the CNIC and beyond, offering a wide variety of NGS applications (RNA Seq, Low input RNA Seq, small RNA-Seq, ChIP Seq, PCR Seq, Exome Sequencing, targeted resequencing, etc). On each sequencing project the Unit's tasks include project consultation, sample quality check, sample library preparation, and data generation. Several of the top CNIC scientific publications in 2014 and 2015 include NGS experiments performed in the Genomics Unit.

One of the team´s scientific and technological research interests focuses on low-input OMICS, including genomics, transcriptomics and miRNomics, with special emphasis on the study of the transcriptome at the single-cell level. The Unit has started to perform single-cell RNA seq using the Fluidigm C1 Single-Cell Auto Prep System. This microfluidic device captures individual cells, and facilitates the generation of single-cell cDNA libraries for Illumina mRNA sequencing.

The Unit continues to automate the newly incorporated NGS library preparation protocols by using an open liquid handling platform. This is especially essential when working at the single-cell level since automation allows handling the processing of the needed higher number of samples per experiment. Additionally this step avoids the bottleneck created by the high sample number typically sequenced in the Unit, and also reduces the risk of human error.

Other services include DNA fragmentation using a Covaris E220 ultrasonicator, the maintenance and management of real-time PCR instruments (one AB 7000 and two ABI 7900HT machines) and a TaqMan array processing service.

In addition to providing these high-quality genomics services, the Unit performs its own research.



Individual human liver tumor cell isolated using the C1 Single-Cell Auto Prep System.

MAJOR GRANTS

-Ministerio de Economía y Competitividad. FIS (PI14/02120)
-Ministerio de Economía y Competitividad. EXPLORA Tecnología (BFU2014-62250-EXP)

SELECTED PUBLICATIONS

Blanco FJ, Ojeda-Fernandez L, Aristorena M, Gallardo-Vara E, Benguria A, Dopazo A, Langa C, Botella LM, Bernabeu C. **Genome-wide transcriptional and functional analysis of endoglin isoforms in the human promonocytic cell line U937**. *J Cell Physiol* (2015) 230: 947-58

Stateva SR, Salas V, Benguría A, Cossío I, Anguita E, Martín-Nieto J, Benaim G, Villalobo A. **The activating role of phospho-(Tyr)-calmodulin on the epidermal growth factor receptor.** *Biochem J* (2015) 472: 195-204

Hill R, Kalathur R, Callejas S, Colaco L, Brandao R, Serelde B, Cebria A, Blanco-Aparicio C, Pastor J, Futschik M, Dopazo A, Link W. **A novel Phosphatidylinositol 3-Kinase (PI3K) inhibitor directs a potent FOXO-dependent, p53-independent cell cycle arrest phenotype characterized by the differential induction of a subset of FOXO-regulated genes.** *Breast Cancer Res* (2014) 16: 482

Microscopy and Dynamic Imaging

Head of Unit

Valeria R Caiolfa

Staff Scientists:

Moreno Zamai
Antonio Manuel Santos Beneit
Elvira Arza
Verónica Labrador Cantarero

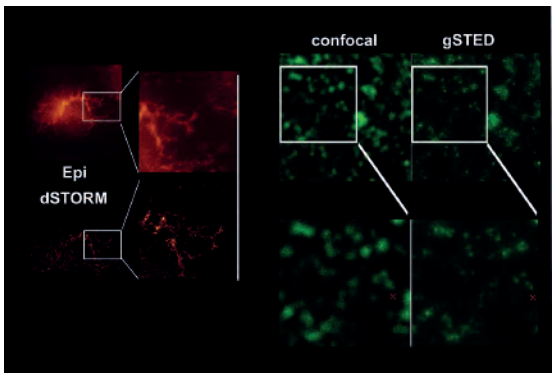
Visiting Scientists:

AntonioTrullo
Mª Eugenia Pérez-Ojeda Rodríguez

RESEARCH INTEREST

In 2015 we have continued the development of the super-resolution platform and Single Plane Illumination Imaging (SPIM). We have introduced 2-color 3D STED imaging for three-dimensional imaging of cells at resolution higher than 80 nm in the x,y and z planes, providing critical capacity for colocalization studies of complex cellular organelles and subcellular structures. Super-resolution image analysis was improved by applying newly developed deconvolution approaches.

We have exploited the flexible configuration of our multiphoton microscopes to investigate novel meso-scale bone-mimicking models that bridge the gap between microfluidic, macro-scale studies and high-throughput screening of the effects of multiple variables on the vascularization of bone-mimicking tissues. We have studied the influence of endothelial cell (EC) density and the relative proportions of ECs, mesenchymal stem cells and osteo-differentiated MSCs cultured in hydrogel-type matrices. In close collaboration with the Galeazzi Hospital in Milan, these studies allowed the identification of synergistic correlations among critical parameters driving microvascular network development within a bone-mimicking environment, and could be translated to any vascularized tissue.



Comparison of the detection of calreticulin in the endoplasmic reticulum by (left) epifluorescence and single molecule localization microscopy in direct STORM (dSTORM) and (right) confocal imaging and gated stimulated emission depletion (gSTED).

The Unit continued its training activities through one-to-one programs and workshops, and and contributed to the CNIC-JOVEN training plan (ACERCATE, CICERONE and the Master Program) through theoretical and practical sessions.

MAJOR GRANTS

- INFRA-MINECO-2013 - Plataforma Biomédica Avanzada CNIC en Nanoscopía multimodal

SELECTED PUBLICATIONS

Bersini S, Gilardi M, Arrigoni C, Talo G, Zamai M, Zagra L, Caiolfa V, Moretti M. Human in vitro 3D co-culture model to engineer vascularized bone-mimicking tissues combining computational tools and statistical experimental approach. *Biomaterials* (2015) 76: 157-72

Groult H, Ruiz-Cabello J, Pellico J, Lechuga-Vieco AV, Bhavesh R, Zamai M, Almarza E, Martin-Padura I, Cantelar E, Martinez-Alcazar MP, Herranz F, **Parallel multifunctionalization of nanoparticles: a one-step modular approach for in vivo imaging.** *Bioconj Chem* (2015) 26: 153-60

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Pluripotent Cell Technology

Head of Unit

Giovanna Giovinazzo

Support Scientists:

Francisco Gutiérrez
Elisa Santos

Technicians:

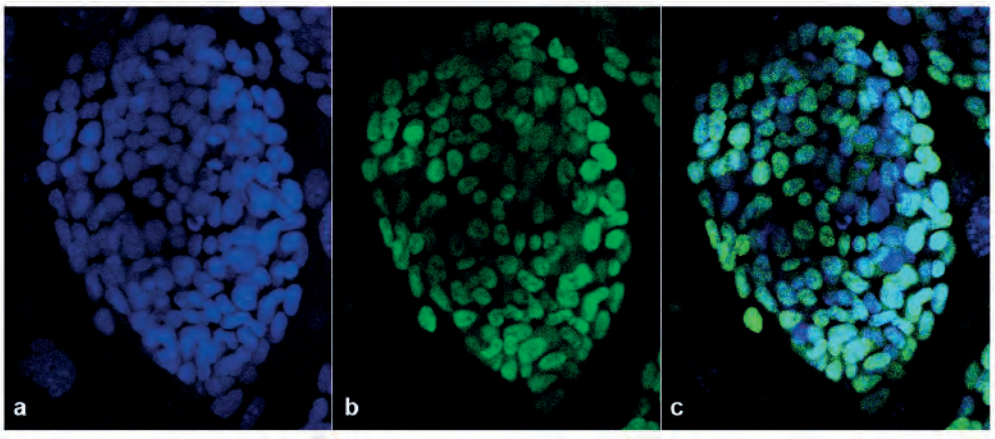
María Ángeles Sanguino
Carles Moreno Soriano

RESEARCH INTEREST

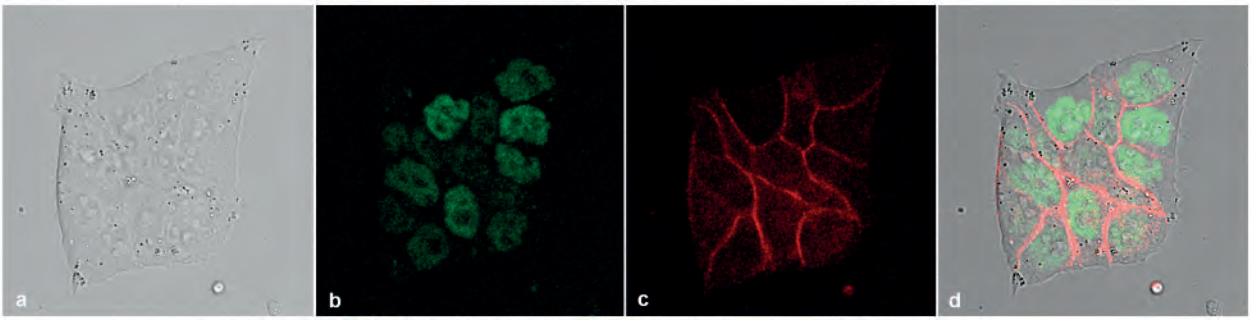
The Pluripotent Stem Cell Technology Unit supports CNIC researchers whose research involves the culture and manipulation of mouse and human pluripotent stem cells. Our staff offer expertise and comprehensive training in successful stem cell culture, supply protocols and validated reagents, and give expert advice on the maintenance and differentiation of stem cells.

One of the Unit's core functions is to facilitate gene-targeting experiments to produce quality-controlled genetically-modified mESCs, an essential requirement for germline transmission and the generation of knockout, knockin and conditional mutant mice. The Unit undertakes all key steps in the gene-targeting protocol: electroporation of the targeting vector, selection, karyotyping, culture, and the preparation of cells for blastocyst injection. The Unit's service portfolio also includes Neo removal, random insertion and screening by Southern blot. On request, we can also assist CNIC researchers with the design of targeting vectors and screening strategies. The Unit also applies its wide expertise in genetic modification to create *in vitro* models of pluripotent cells using the CRISPR/cas system, and we supply knockout stem-cell lines for diverse research projects. On request, we can also assist CNIC researchers in fine-tuning differentiation protocols for a specific lineage.

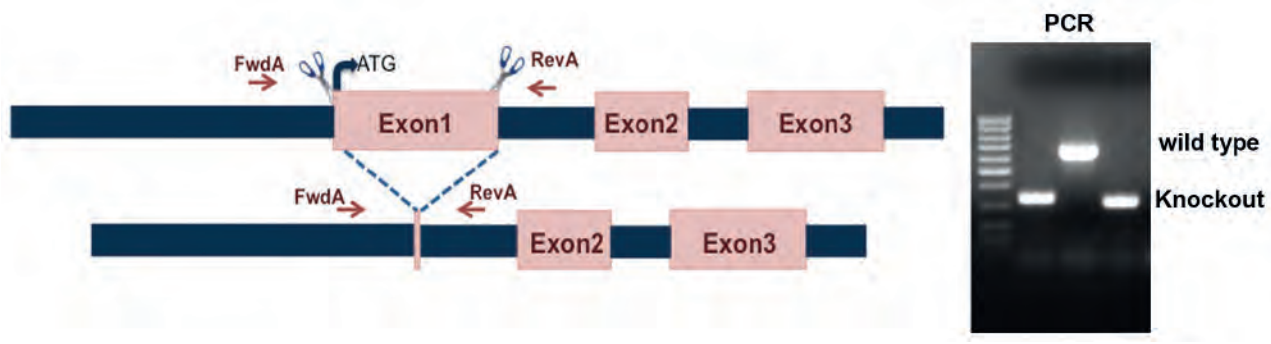
Human induced pluripotent stem cells (hiPSC) are an extraordinarily valuable source of cells for basic and translational research, including drug development and disease modeling. The Unit is also able to provide expert advice on the design of experiments involving hPSCs and provide the latest cutting-edge technology for genome editing for generating in vitro models of cardiovascular disease.



Immunocytochemistry detection of pluripotent cell markers in mESCs. a) DAPI. b) Nanog. c) Merged view.



Confocal image of mycEGFP mESCs a) Brightfield image of an undifferentiated colony. **b)** Nuclear localization of mycEGFP protein (green). c) Membrane marker expression linked to dtTomato fluorescent protein. d) Merged view



Generation of knockout mESC lines using the CRISPr/Cas9 strategy. Strategy for creating a knockout stem cell line and PCR screening to detect genetically modified clones.

SELECTED PUBLICATIONS

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

Gonzalez-Lazaro M, Rosello-Diez A, Delgado I, Carramolino L, Sanguino MA, Giovinazzo G*, Torres M*. **Two new Targeted alleles for the comprehensive analysis of Meis1 functions in the mouse** *Genesis* (2014) 52: 967-75
*Co-corresponding authors

Proteomics/Metabolomics

Head of Unit

Juan Antonio López

Support Scientists:

Enrique Calvo
Emilio Camafeita
Iakes Ezkurdia

Technicians:

Raquel Mesa
Rocío Campo (since 1 December)
Ricardo Magni (since 1 December)

Visiting Scientist:

María Gómez Serrano (IIB-CSIC)

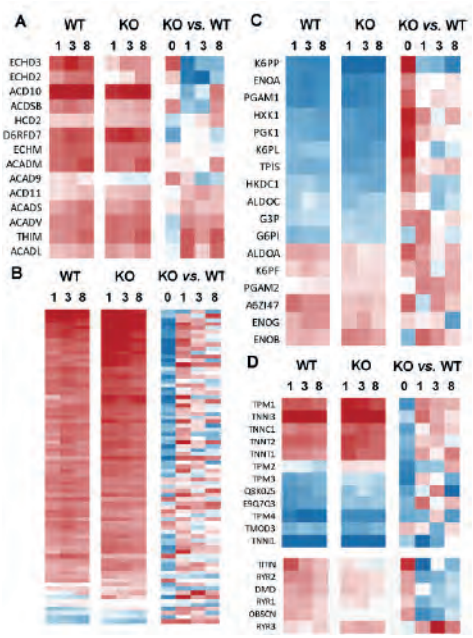
RESEARCH INTEREST

The CNIC Proteomics Unit devotes considerable effort to technological innovation, through the continuous development of new methods of interest to the research community. Throughout 2015, the Unit worked on improvements to the quantitative analysis of protein abundance by shotgun and targeted proteomics using high-throughput technologies based on nano-liquid chromatography coupled to mass spectrometry.

Progress was also made on quantitative proteomics using multiplexed stable isobaric labeling (iTRAQ and TMT). Particular improvements were made in the development of chromatographic conditions for HPLC-based peptide fractionation, and optimization of the recently incorporated Orbitrap Trybrid Fusion mass spectrometer. We also made progress in the statistical analysis of TMT-derived quantitative data and systems biology interpretation of the results using algorithms developed in house.

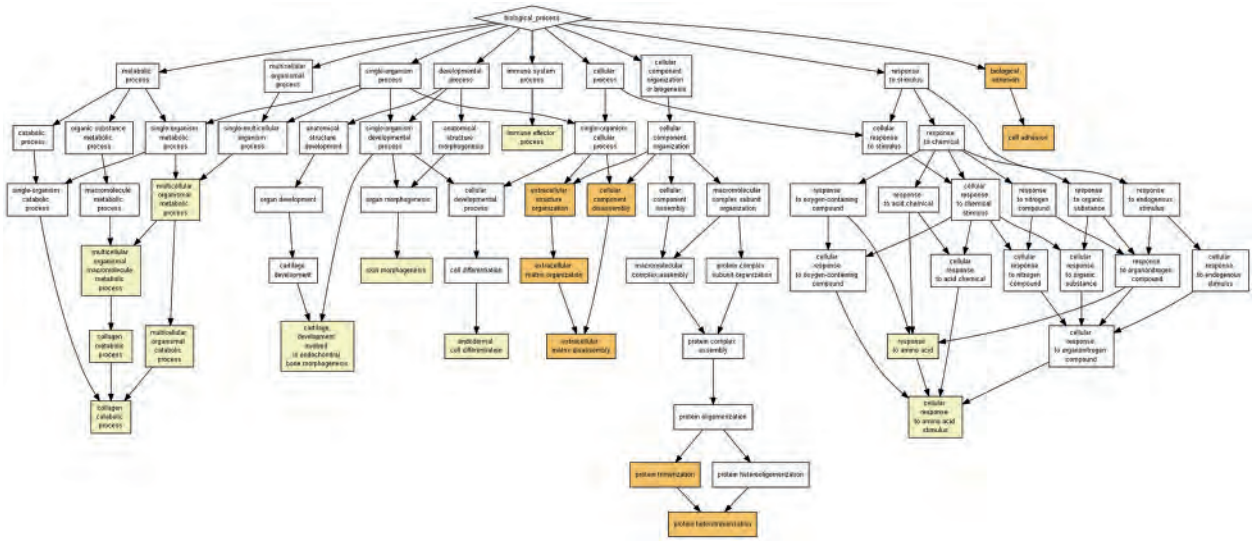
These approaches are also being applied to the quantitative analysis of posttranslationally modified peptides, directly identified by database searches or using enrichment protocols. For biomarker discovery in the clinical setting we are analyzing dozens of plasma samples using depletion protocols of the most-abundant proteins and multiplexed quantitation.

We are developing technological and statistical methodologies for data-independent scanning approaches, which mix targeted and shotgun approaches and produce complete fragment profiles of all peptide species. This robust analytical platform is allowing us to manage large research projects that require qualitative and quantitative proteomic approaches to measure differential protein expression, characterize posttranslational modifications, and map protein-protein interactions in different biological systems.



Quantitative proteomics analysis showing metabolic changes due to deficiency in executioner caspases 3 and 7. (A-D) Relative abundance profiles of selected proteins during mouse development (0, 1, 3 and 8-month-old). The changes are expressed separately for KO and WT animals in relation to the abundances at t = 0, while in the rightmost column the abundance of proteins in the KO are compared with that of the WT animals at each time point. Selected proteins include those related to beta-oxidation (A), oxidative phosphorylation complexes (B), glycolysis (C), and structural and contractile proteins (D).

Modified from Cardona *et al*. 2015. doi:10.1371/journal.pone.0131411.g003.



Proteomics analysis of the adipose-derived mesenchymal stem cell (ADMSC) secretome. White matter injury restoration after ADMSC cell administration in an experimental model of subcortical ischemic stroke. Gene onthology enrichment (GO) analysis of the proteins identified reveals important biological functions.
Modified from Otero-Ortega *et al*. Stem Cell Research & Therapy 2015. doi:10.1186/s13287-015-0111-4

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Burillo E, Lindholt JS, Molina-Sánchez P, Jorge I, Martinez-Pinna R, Blanco-Colio LM, Tarin C, Torres-Fonseca MM, Esteban M, Laustsen J, Ramos-Mozo P, Calvo E, Lopez JA, Vega de Ceniga M, Michel JB, Egido J, Andrés V, Vazquéz J, Meilhac O, Martin-Ventura JL. **ApoA-I/HDL-C levels are inversely associated with abdominal aortic aneurysm progression.** *Thromb Haemost.* (2015) 113: 1335-46

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Martin-Rojas T, Mourino-Alvarez L, Alonso-Orgaz S, Rosello-Lleti E, Calvo E, Lopez-Almodovar LF, Rivera M, Padial LR, López JA, de la Cuesta F & Barderas MG. **iTRAQ proteomic analysis of extracellular matrix remodeling in aortic valve disease**. *Sci Rep* (2015) 5:17290.

Transgenesis

Head of Unit

Luis-Miguel Criado Rodríguez

Support Scientists:

José Mª Fernández Toro

Juan de Dios Hourcade Bueno

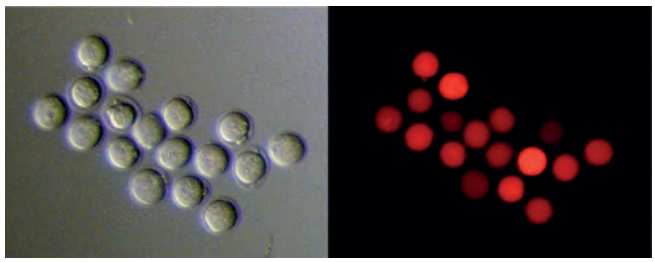
RESEARCH INTEREST

The main goal of the Unit is to provide genetically modified mouse strains, including knockout and knockin strains, to the CNIC research groups. This is achieved using well-established techniques, mainly the direct injection of DNA molecules into zygote pronuclei or the injection of genetically modified mouse embryonic stem cells (mESC) into 8-cell and blastocyst mouse embryos to obtain chimeric mice. Following the global trend toward the production of transgenic mice by gene edition with engineered nucleases, the Unit has successfully produced genetically modified mice using zinc finger nucleases (ZFN) and the CRISPR/Cas9 system, and in 2015 this methodology has been definitively established.

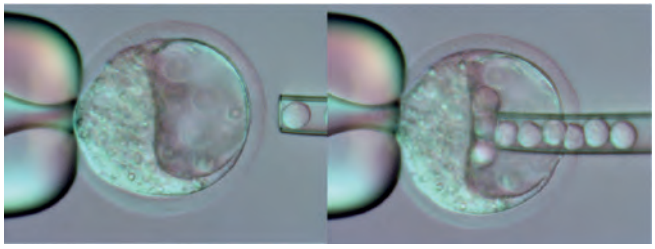
Other impontant activities include the rederivation of mouse strains by embryo transfer, cryopreservation of mouse strains by freezing embryos and sperm, mouse in vitro fertilization (mIVF), and intracytoplasmic sperm injection (ICSI). Moreover, the Unit also cryopreserves sperm from zebrafish (*Danio rerio*) and carries out in vitro fertilization with fresh and frozen sperm from this important vertebrate model organism.

In addition to its routine work, the Unit collaborates with several CNIC groups on specific aspects of their research programs, and Unit members participate in the CNIC's training programs by providing theoretical and practical sessions.

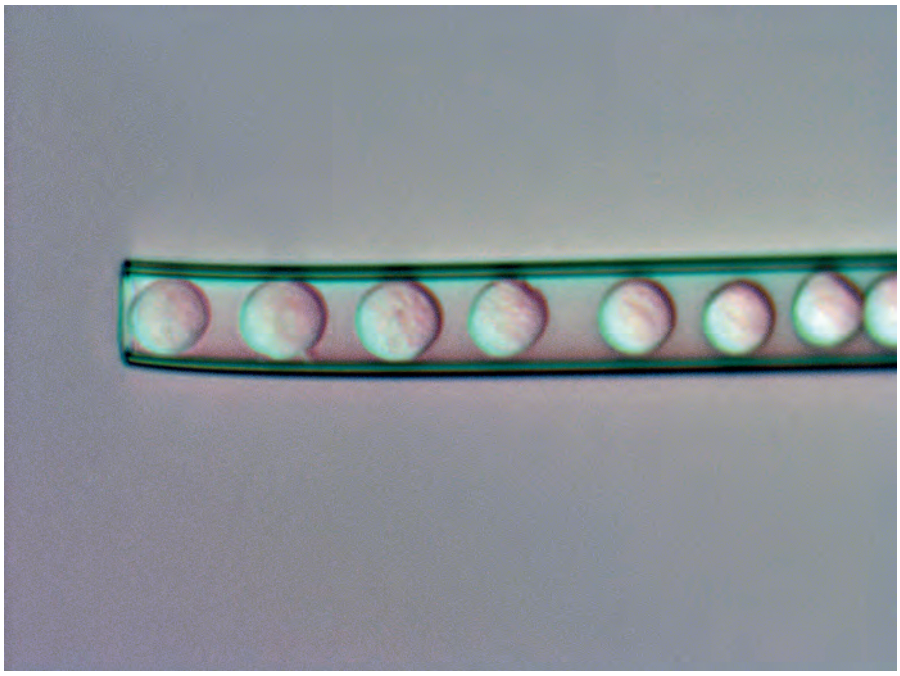
B6CBAF1/J mouse zygote showing the *zona pellucida* (glycoprotein structure surrounding the plasma membrane) and the two pronuclei containing nucleoli.



B6CBAF1/JxC57BL/6J mouse zygotes after electroporation in the presence of tetramethylrhodamine dextran (TMR-D): visible light (left) and fluorescent light (right).



Microinjection of a C57BL/6J mouse blastocyst with genetically modified mESCs for the production of chimeric mice: before (left) and after (right) injection.



Blunt injection needle containing genetically modified mESCs ready for microinjection into mouse blastocysts.

C57BL/6J mouse oocytes produced by ultra-superovulation of sexually immature females.

Viral Vectors

Head of Unit

Juan Antonio Bernal

Support Scientists:

Cristina Sánchez-Ramos
Raúl Torres

Technicians:

Joan García
Cristina Márquez
Aida García

Visiting Scientist:

Catarina Reis
(CNIO)

RESEARCH INTEREST

The main purpose of the Viral Vectors Unit (ViVU) is to provide investigators with the scientific resources necessary to produce state-of-the-art recombinant viral vectors for in vivo and in vitro use in gene transfer experiments. The ViVU currently produces lentivirus, adenovirus and adeno-associated virus (AAV) serotypes 8 and 9, and maintains a P2 facility with the appropriate expertise, equipment and permissions.

Viral vectors are widely used for gene transfer and gene expression in vitro, and our aim is to develop these tools for new applications. Viral vectors are an attractive choice because of their high transduction efficiency and their ease and flexibility of application; these vectors can be used to genetically express or inhibit one gene or a combination of genes in specific areas and periods of time, while avoiding compensation phenomena or other drawbacks associated with traditional animal models.

We have developed an alternative to transgenic animals, in which AAV vectors, widely used for gene-therapy approaches, express disease-causing mutated genes to generate disease models in wild-type mice. We have also used AAV vectors to compensate genetic defects by knocking down specific genes in vivo.

Our work demonstrates that single systemic injection of AAV is more versatile, cost-effective, simpler, and time-efficient than transgenic approaches for generating this types of mutant model. These studies set the basis for our future vector development.

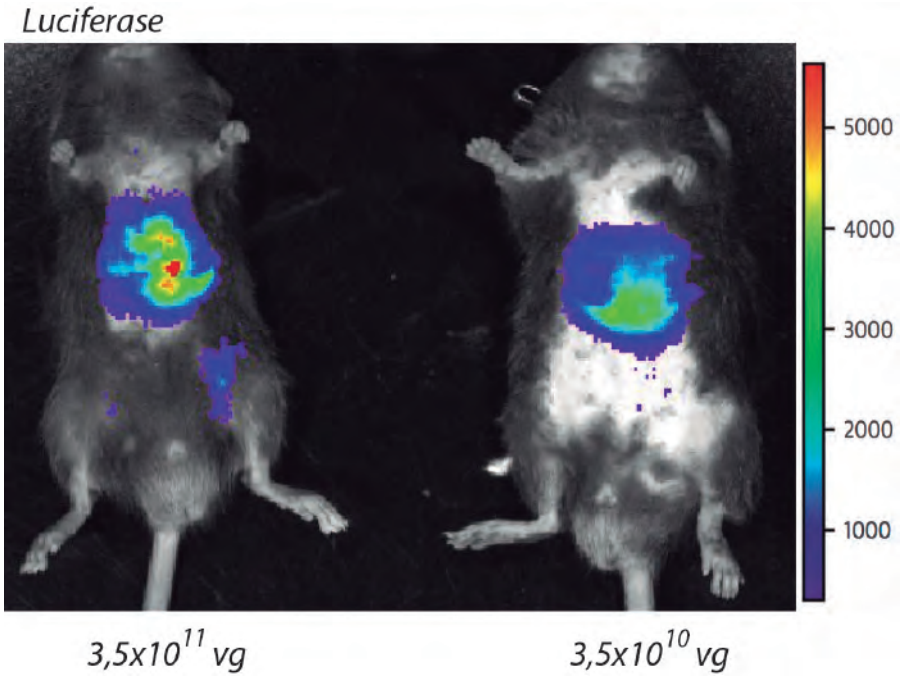


Figure 1. Infection with adeno-asociated virus serotype 9 *in vivo*. (A) Representative live-animal bioluminescence imaging of luciferase (Luc) transgene expression in C57BL6J mice injected intravenously (femoral vein) with AAV2–based vector in packaging serotype 9 at doses of $3.5x10^{10}$ and $3.5x10^{11}$ viral genomes (vg). Images were acquired 4 weeks after inoculation.

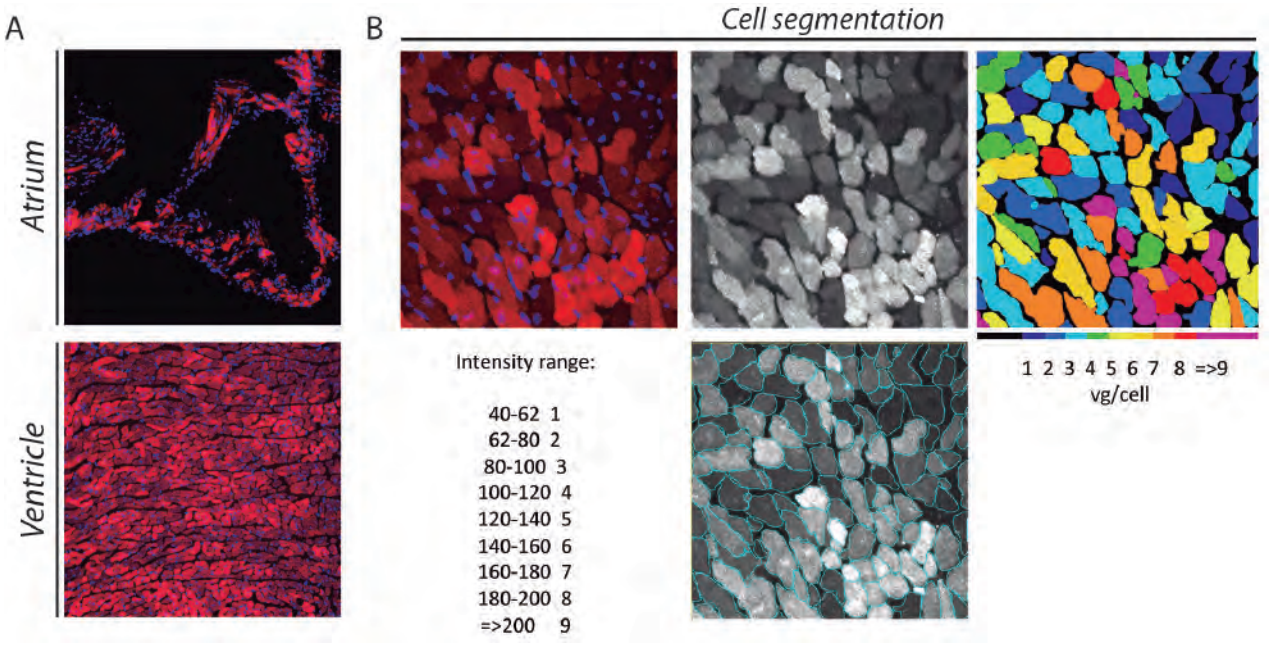


Figure 2. Cardiac expression driven by the specific cardiac promoter TnT. **(A)** Representative fluorescence microscopy images of cross sections of AAV-transduced hearts, illustrate showing expression of EGFP throughout the left atrium and ventricle. **(B)** Magnified images show the mosaic cellular distribution of wild-type cardiac PKP2 expression in the heart. **(B)** Fluorescence intensity segmentation and quantification of transduced protein expression, used to assign the number of integrated viral genomes per cardiomyocyte cardiomyocyte.

SELECTED PUBLICATIONS

Enrique Gallego-Colon, Maria Villalba, Joanne Tonkin, Francisco Cruz, Juan Antonio Bernal, Luis Jesús Jiménez-Borreguero, Michael Schneider, Enrique Lara-Pezzi, and Nadia Rosenthal. Intravenous delivery of adeno-associated virus 9-encoded IGF-1Ea propeptide improves post-infarct cardiac remodeling. *npj Regenerative Medicine* (accepted).

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Torres R, Garcia A, Jimenez M, Rodriguez S, Ramirez JC. **An integration-defective lentivirus-based resource for site-specific targeting of an edited safe-harbour locus in the human genome.** *Gene Ther* (2014) 21: 343-52

Ramirez JC, Torres R.
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