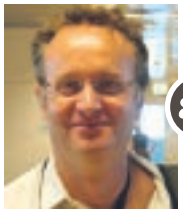
TECHNICAL UNITS

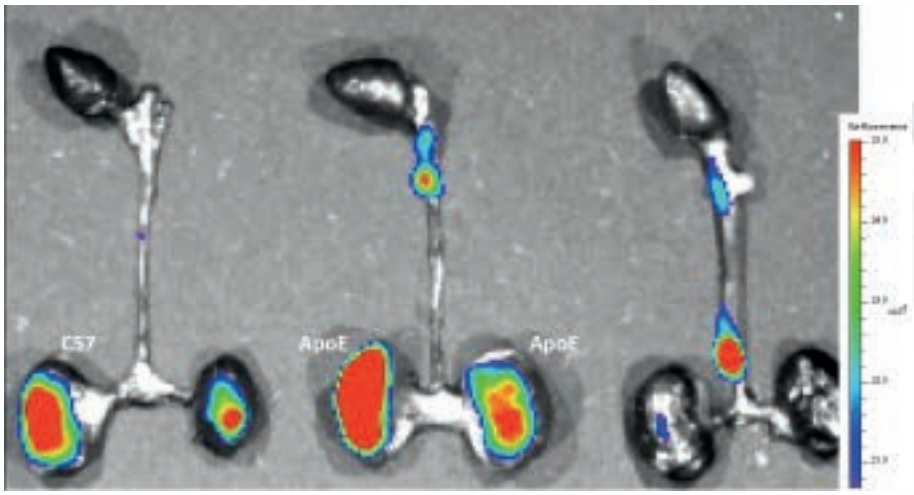
Advanced imaging
Bioinformatics
Cellomics
Comparative medicine
Genomics
Microscopy and dynamic imaging
Pluripotent cell technology
Proteomics/Metabolomics
Transgenesis
Viral vectors



Advanced imaging

RESEARCH INTEREST

The Advanced Imaging Unit (AIU) is a multidisciplinary group offering a range of services to CNIC scientists and carrying out its own research in imaging-related technologies. 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 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 focused on developing new nanotracers, molecular probes, and techniques for site-directed biofunctionalization of biomolecules (peptides, proteins, and antibodies). Currently the unit produces multifunctional nanoparticles for all imaging techniques available at the CNIC, and our research program enables the development of new cardiovascular probes for targeted imaging. 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 experienced in radiolabeling with ^{68}Ga and ^{89}Zr, providing the Center with in-house developed PET radiotracers for cardiovascular nuclear imaging. The CNIC is one of the few centers in Spain with this technology, situating the center at the forefront of radiochemistry research. 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-radiotracers for plaque detection. Plaque detection with a neutrophil-specific fluorescent nano-radiotracer in C57 and ApoE$^{-/-}$ mice.

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Visiting Scientists:
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Ana Fernández
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Sergio Garnica
Juan Miguel Parra

MAJOR GRANTS

- Instituto de Salud Carlos III. Desarrollo tecnológico en Salud (DTS16/00059) PI: Fernando Herranz
- Ministerio de Economía y Competitividad. Plan Nacional de Excelencia (SAF2016-79593-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 (MIT14 - X7118248R) PI: Arnoldo Santos
- Madrid-MIT M+Visión (PRMIT2013) PI: Samuel España
- European Commission FP7-PEOPLE-2013-ITN (CardioNext PITN-GA-2013-608027)
- European Commission FP7-PEOPLE-2010-ITN (П-NET 264864) (NO CNIC).
- Ministerio de Sanidad y Consumo (CIBERES CB06/06/1090)

SELECTED PUBLICATIONS

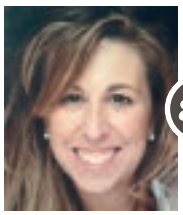
Viswanath P, Najac V, Izquierdo JL, Pankov A, Hong C, Eriksson P, Costello JF, Pieper RO, Ronen SM. **Mutant IDH1 gliomas down-regulate expression of monocarboxylate transporters**. *Oncotarget* (2016) 7: 34942

Marciello M, Pellico J, Fernandez-barahona I, Herranz F, Ruiz-Cabello J, Filice M **Recent advances in the preparation and application of multifunctional iron oxide and liposome-based nanosystems for multimodal diagnosis and therapy** *Interface Focus* (2016) 6: 20160055

Pellico J, Ruiz-Cabello J, Saiz-Alía M, del Rosario G, Caja S, Montoya M, Fernández de Manuel L, Morales M.P., Gutiérrez L., Galiana B., Enríquez J.A., and Herranz F. **Fast synthesis and bioconjugation of 68Ga core-doped extremely small iron oxide nanoparticles for PET/MR imaging** *Contrast Media Mol Imaging* (2016) 11: 203–10

Bujak R, Mateo J, Blanco I, Izquierdo-García JL, Dudzik D, Markuszewski MJ, Peinado VI, Laclaustra M, Barberá JA, Barbas C, Ruiz-Cabello J. **New Biochemical Insights into the Mechanisms of Pulmonary Arterial Hypertension in Humans.** *PLoS One*. (2016) 11: e0160505

Zahraei M, Marciello M, Lazaro-Carrillo A, Villanueva A, Herranz F, Talelli M, Costo R, Monshi A, Shahbazi-Gahrouei D, Amirnasr M, Behdadfar B, and Morales M.P. **Versatile theranostics agents designed by coating ferrite nanoparticles with biocompatible polymers** *Nanotechnology* (2016) 27: 255702



Bioinformatics

RESEARCH INTEREST

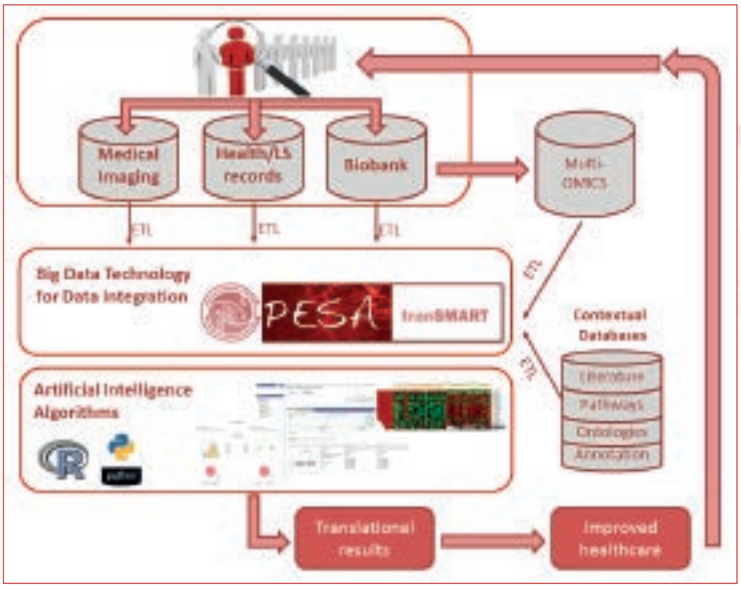
During 2016, the CNIC Bioinformatics Unit implemented new tools and algorithms in three areas of central importance to achieving excellence in biomedical research:

(i) **Big data infrastructure and artificial intelligence methods** to enable **precision medicine** in large cohort studies. The Unit has implemented a web-based warehousing system called tranSMART that enables the integration and analysis of high dimensional data from different sources. Currently, this application supports 3 large cohort studies at the CNIC: **PESA, IM-Joven, and AWHS**.

(ii) Analysis of DNA samples. A pipeline has been established for variant calling from NGS data and a web-based application has been implemented to ease access to results. We are currently working on *ad-hoc* filtering schemes for the prioritization of variants linked to hereditary cardiomyopathies.

(iii) Analysis of single-cell omics data. An analysis pipeline has been established for single-cell data generated with omics technologies and applied to several CNIC projects.

The Unit currently supports 21 CNIC groups and 3 technical units through these and previously established services: downstream analysis and mathematical models for omics technologies, transcriptomics data analysis, data integration, statistical analysis consultancy, administration of HPC infrastructure, modeling of protein structure, and lab automatization (LIMS). The Unit also provides training in bioinformatics through co-supervision of junior bioinformaticians and dedicated training courses in bioinformatics-related fields, such as the BMM9 Masters program and the CNIC Statistics Course).



Data warehousing system implemented by the CNIC Bioinformatics Unit to enable precision medicine through data integration in large cohorts using big data infrastructure and artificial intelligence methodologies.

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Victor Jiménez
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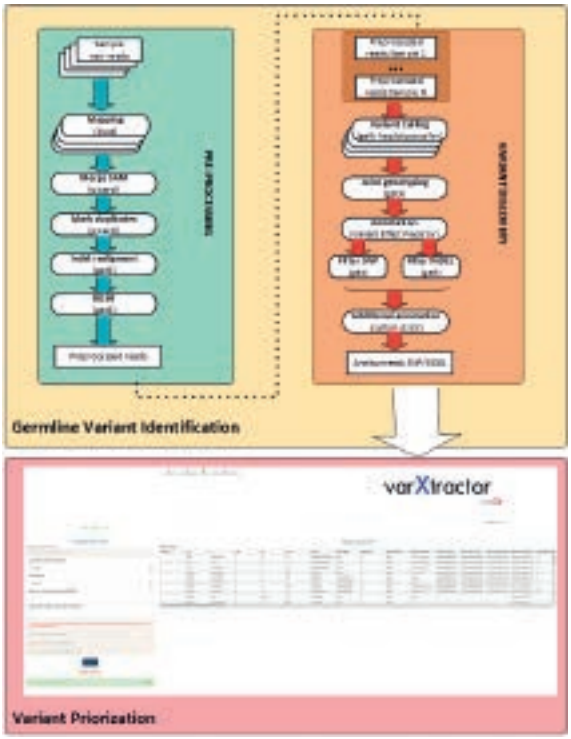
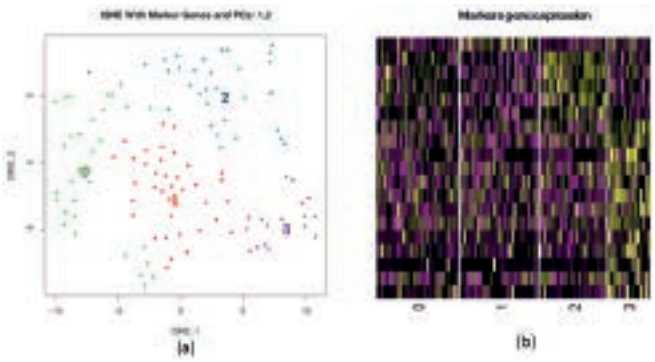
Wencke Walter
(from the Nuclear Receptor Signaling Laboratory, led by Mercedes Ricote)



Analysis pipeline for variant calling from NGS Data. Web-based application for ad-hoc filtering and results visualization.



scRNASeq data: (a) tSNE applied to scRNA-Seq data identified 4 cell clusters according to the expression of a combination of automatically selected markers. (b) Data generated by JA Nicolas (A. Hidalgo Lab) at the CNIC Genomics Unit and SIgN

MAJOR GRANTS

- European Commission. H2020-PERSONALISING HEALTH AND CARE (H2020-PHC-2014-two-stage). APERIM-GA633592.

SELECTED PUBLICATIONS

Menendez-Montes I, Escobar B, Palacios B, Gómez MJ, Izquierdo-Garcia JL, Flores L, Jiménez-Borreguero LJ, Aragones J, Ruiz-Cabello J, Torres M, Martin-Puig S. **Myocardial VHL-HIF signaling controls an embryonic metabolic switch essential for cardiac maturation**. *Dev Cell* (2016) 39: 724-39

Enríquez JA, Sánchez-Cabo F, Vázquez J. **Hypothesis driven versus hypothesis-free: filling the gaps in CoQ biosynthesis.** *Cell Metab* (2016) 24: 525-26

Latorre-Pellicer A, Moreno-Loshuertos R, Lechuga-Vieco AV, Sánchez-Cabo F, Torroja C, Acín-Pérez R, Calvo E, Aix E, González-Guerra A, Logan A, Bernad-Miana ML, Romanos E, Cruz R, Cogliati S, Sobrino B, Carracedo Á, Pérez-Martos A, Fernández-Silva P, Ruíz-Cabello J, Murphy MP, Flores I, Vázquez J, Enríquez JA. **Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing.** *Nature* (2016) 535: 561-5

Giudice G, Sánchez-Cabo F, Torroja C, Lara-Pezzi E. **ATtRACT-a database of RNA-binding proteins and associated motifs**. *Database* (*Oxford*) (2016) *baw035*

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

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* (2016) 18: 7-20

Cellomics

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 2016, we implemented automated analysis of multidimensional cytometry data. We co-organized the **"High-Content Screening Conference"** at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, and also co-organized the CNIC Conference on **"Mechanical forces in phisiology and disease"**. We also established a novel high content analysis (HCA) tool that obtains cytoskeletal rearrangement signatures from the accurate quantification of features, revealing cytoskeletal organization at subcellular resolution (Fig. 2). This tool enabled us to investigate Rab8-induced cytoskeletal reorganizations using siRNA knockdown and drug inhibitors, establishing the role of Rab8 in directional cell migration and delineating the molecular pathways involved in this process. In partnership with the Genetic Control of Organ Development and Regeneration laboratory, we have successfully developed ESC-Track, a computer workflow for 4D segmentation, tracking, lineage tracing, and dynamic context analysis of ESCs. ESC-Track is the only method currently available that enables 4D tracking of cells in the context of both lineage and neighborhood. The Unit has also developed customized image analysis tools for the quantification of macrophages, adipocytes, and collagen or elastin in immunohistological tissue sections and the analysis of lipid droplet subcellular organization and mitochondrial fragmentation in confocal fluorescence images.

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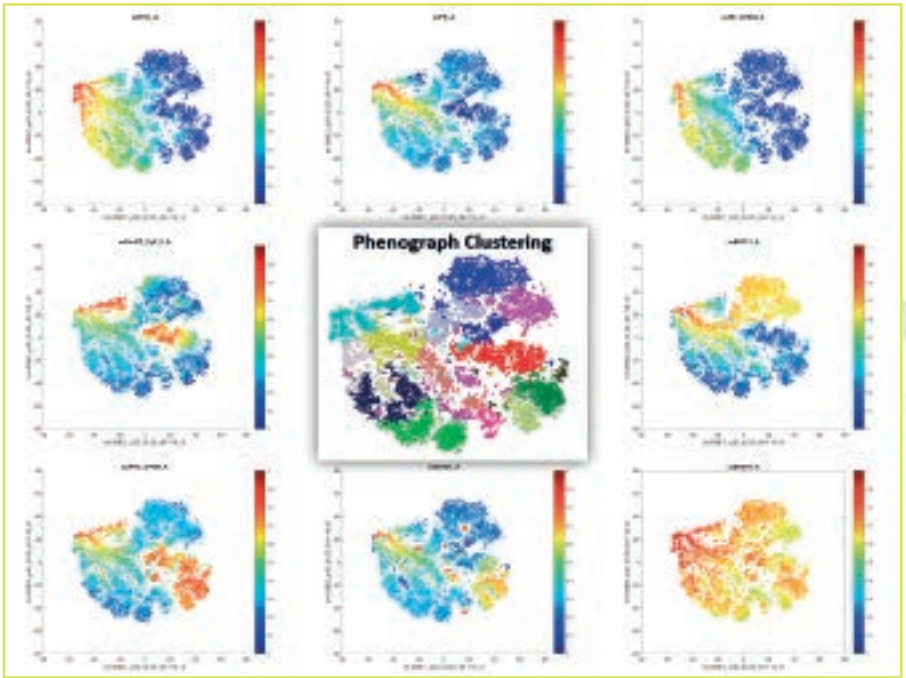
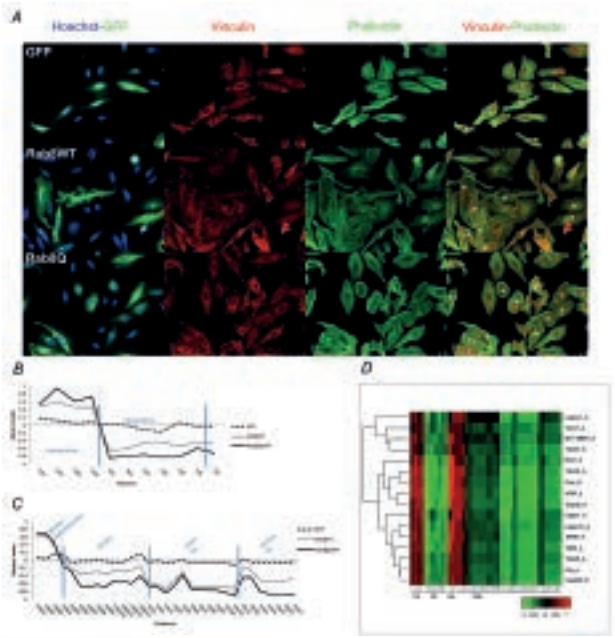
Mariano Vitón
Irene Palacios Doiztua
Elena Prieto

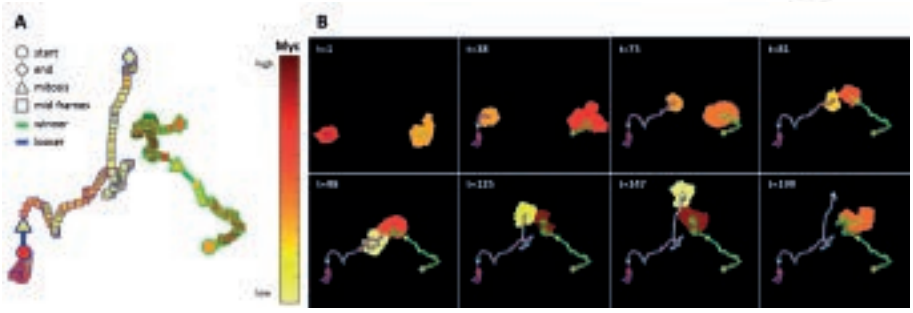
Visiting Scientist:
Marco Cordani



Automated analysis of high dimensional (spectral) cytometry data in mouse lymph node for detection of dendritic cells. Phenograph clustering is used to automatically detect cell populations, represented in a t-SNE map obtained with dimensionality-reduction techniques. Color-coded plots of fluorochrome expression allow identification of populations stained with MHC II (FITC), XCR1 (PE), CD19 (PE-CF59), Ly6C (PerCP_Cy_5.5), CD4 (BV711), CD8 (APC-Fire750), CD103 (BV421), and CD45 (BV570).



HCA analysis of Rab8-promoted cytoskeletal rearrangements: profiling and resulting hierarchical clustering analysis of the effect of siRNAs. HeLa cells expressing GFP (GFP), Rab8Q67L–GFP (Rab8Q), or Rab8WT-GFP (Rab8WT) were stained with phalloidin, anti-vinculin, and Hoechst to reveal actin, focal adhesions (FA), and nuclei. **A)** Representative confocal images. **B,C)** HCA phenotypic profiles were plotted as normalized z values of FA and actin features. **D)** HeLa cells transfected with nontargeting siRNA (Ctrl) or siRNAs targeting the indicated genes (right) were then transfected with Rab8WT-GFP and analyzed by HCA as in B and C. The heatmap shows unsupervised hierarchical clustering of phenotypic profiles.



ESC-T tracking and representation of cell trajectories and contacts and their relationship to GFP expression. mESCs expressing GFP and tdTomato were imaged every 7 minutes by 3D confocal microscopy (Z stacks spaced at 2 µm) and tracked using ESC-T. **a)** Trajectories and color-coded GFP normalized intensity values were obtained at each time-point using ESC-T. Cell coordinates are presented at each time point, with squares color-filled according to the GFP intensity. Mitotic events and starting and final locations are represented by triangles, circles, and diamonds, respectively. Trajectories are highlighted in green and blue. **b)** Video stills obtained at the indicated time points, combining the GFP expression levels and trajectories as in (a).

MAJOR GRANTS

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

SELECTED PUBLICATIONS

Horvath P, Aulner N, Bickle M,Davies AM, Del Nery E, Ebner D, Montoya MC, Östling P, Pietiäinen V, Price LS, Shorte SL, Turcatti G, von Schantz C, Carragher NO. **Screening out irrelevant cell-based models of disease**. *Nature Rev Drug Discov* (2016) 15: 751–69

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

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* (2016) 30: 553-62

Pellico J, Ruiz-Cabello J, Saiz-Alia M, Del Rosario G, Caja S, Montoya MC, Fernandez de Manuel L, Morales MP, Gutierrez L, Galiana B, Enriquez JA, Herranz F. **Fast synthesis and bioconjugation of Ga core-doped extremely small iron oxide nanoparticles for PET/MR imaging.** *Contrast Media Mol Imaging* (2016) 11: 203-10

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

Comparative medicine

RESEARCH INTEREST

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.

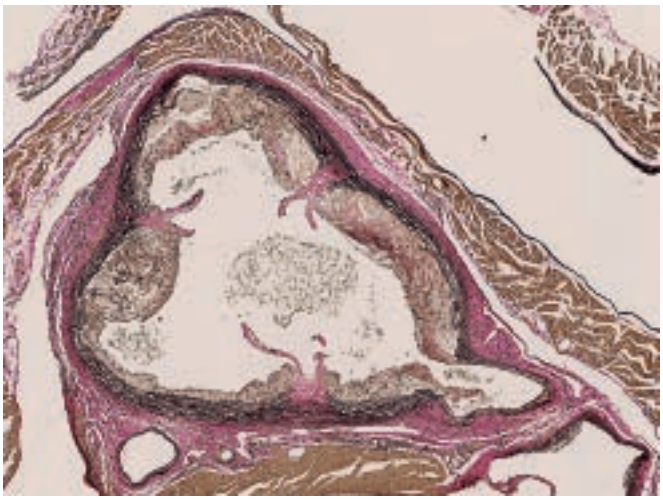
- Pathology Core (PC). The Histopathology Laboratory provides specialized hispathological services including animal necropsy, paraffin and OCT processing and sectioning, histochemical and immunohistochemical staining of tissue sections, digital scanning and image analysis, optical projection tomography 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 continue to provide technical support to the CNIC research groups. We perform analysis of hematology, clinical biochemistry, and coagulation and tests of electrocardiography and blood pressure measurements.

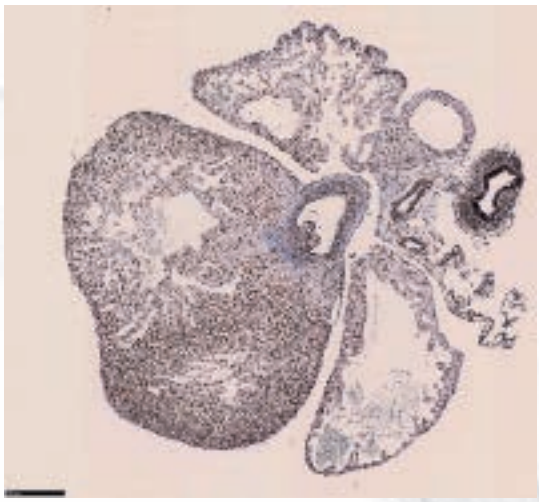
- Veterinary Medicine and Experimental Surgery Core (VMESC). The VMESC provides highly specialized expertise in the surveillance and monitoring of animal health status, disease follow-up, development of surgical animal models with enphasis on minimally invasive procedures, life support, setting up new 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.

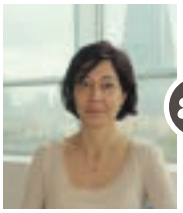


Atheroma plaques in a mouse aortic valve stained with Elastic Van Gieson



Anti Ki67 antibody immunostaining in a mouse neonate heart

Genomics



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Alberto Benguría
Sergio Callejas
Estrella Esquivel

Technicians:
Eduardo Gil
Gema González
Álvaro Merchán

RESEARCH INTEREST

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

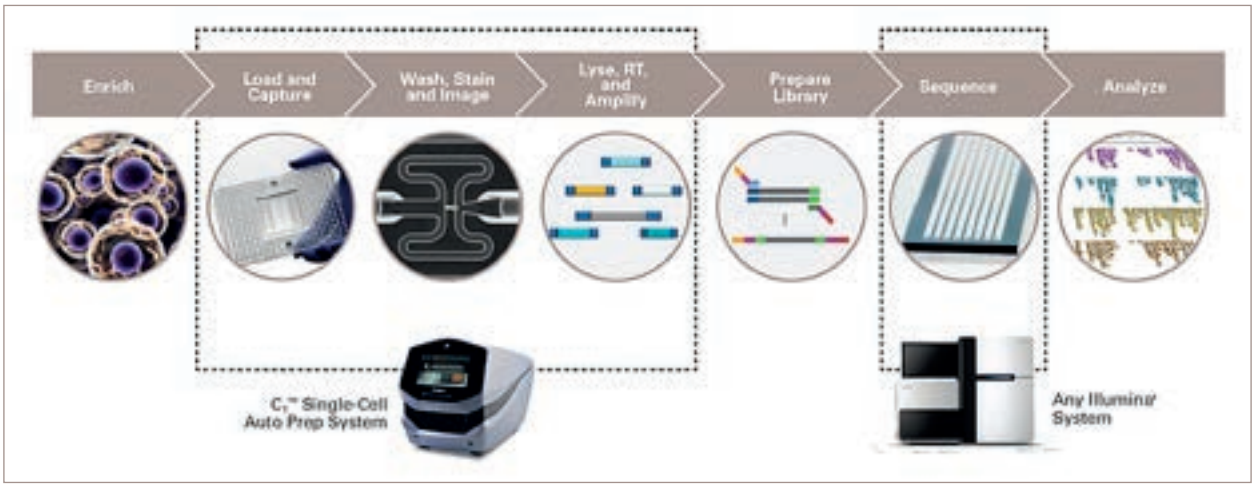
The Unit provides these cutting-edge genomic technologies to the scientific community at the CNIC and beyond, offering a wide variety of NGS applications (RNA Seq, Low input RNA Seq, small RNA-Seq, ChIP Seq, PCR Seq, Exome Sequencing, targeted resequencing, etc.). On each sequencing project the Unit's tasks include project consultation, sample quality control (QC), sample library preparation, and data generation. Several of the top CNIC scientific publications in 2015 and 2016 include NGS experiments performed in the Genomics Unit.

One of the team's scientific and technological research interests focuses on the study of the transcriptome at the single-cell level. The Unit has performed single-cell RNA seq in different cell types using the Fluidigm C1 Single-Cell Auto Prep System. This microfluidic device can isolate up to 96 cells and then process them to produce pre-amplified single-cell cDNA libraries for Illumina mRNA sequencing.

A key development of the Unit in 2016 was full automatization from cell capture to sequence-ready RNA seq libraries. This is essential when working at the single-cell level because automation allows to handle the required number of samples per experiment. By using an open liquid handling platform, the Unit's team has automated the downstream processing of C1 chips, from cDNA QC to the consolidation of samples from multiple chips to standard 96-well plates, followed by library construction, pooling, and QC. Captured cells can be selected for further RNA seq sequencing based on imaging data.

Other services include DNA fragmentation using a Covaris E220 ultrasonicator and the maintenance and management of the CNIC's real-time PCR instruments.

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



Single cell trancriptome analysis workflow using Fluidigm's C1 Single-Cell Auto Prep System

MAJOR GRANTS

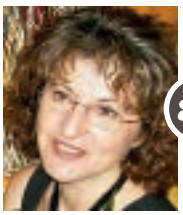
- Ministerio de Economía y Competitividad. FIS (PI14/02120)
- Ministerio de Economía y Competitividad. EXPLORA Tecnología (BFU2014-62250-EXP)

SELECTED PUBLICATIONS

Municio C, Soler Palacios B, Estrada-Capetillo L, Benguria A, Dopazo A, García-Lorenzo E, Fernández-Arroyo S, Joven J, Miranda-Carús ME, González-Álvaro I, Puig-Kröger A. **Methotrexate selectively targets human proinflammatory macrophages through a thymidylate synthase/p53 axis.** *Ann Rheum Dis* (2016) 75: 2157-65

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

Microscopy and dynamic imaging



RESEARCH INTEREST

The Microscopy and Dynamic Imaging Unit is one of the largest light imaging core facilities in Spain. In addition to state-of-art light and confocal microscopes, it maintains and supports advanced technologies in super-resolution, FLIM, single molecule, non-linear, and mesoscopic imaging, linked to customized image analysis. In 2016 the Unit provided more than 20 000 hours of equipment time and supported more than 230 users, including scientists from outside Spain.

The Unit is part of the Advanced Infrastructure for Translational Imaging at the CNIC that has been selected for the Spanish Unique Scientific and Technical Infrastructure (ICTS-ReDib). This facility is accessible to national and international scientists wishing to use the large variety of equipment and high-end technologies in super-solution and FLIM imaging.

Our major scientific achievements of 2016 are related to original applications of super-resolution and FLIM imaging and post-processing image analysis. In collaboration with the CeSI Foundation at the University G. d'Annunzio, Chieti-Pescara, Italy, we used STED-FLIM imaging to demonstrate the direct interaction between CD9 and Trop2 localized in large domains on the plasma membrane of a variety of cancer cell lines. With the CNIC Molecular Cardiology group, we optimized STED imaging to define the organization of RyR2 clusters in wild type and mutant mice. With the Instituto de Ciencia de Materiales and the Hospital Univ. Ramón y Cajal (CSIC, Madrid), we have demonstrated, through a combination of SHG-FLIM imaging approaches in mice, the organ distribution and accumulation of thermal nanoprobes designed for biomedical applications.

Two ongoing super-resolution projects with the Ospedale San Raffaele in Milan, Italy examine cellular stress proteins and molecular markers of chronic lymphatic leukemia, with the aim of resolving the kinetics of assembly of signaling nanoclusters in the endoplasmic reticulum and in leukemia cells from patients.

The first Spanish National School in Super-Resolution Microscopy was organized in partnership with Leica Microsystems, the leading company in STED nanoscopy. Participants from a wide range of scientific backgrounds came from Spain and abroad, and included facilities managers, PhD students, and postdoctoral fellows.

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

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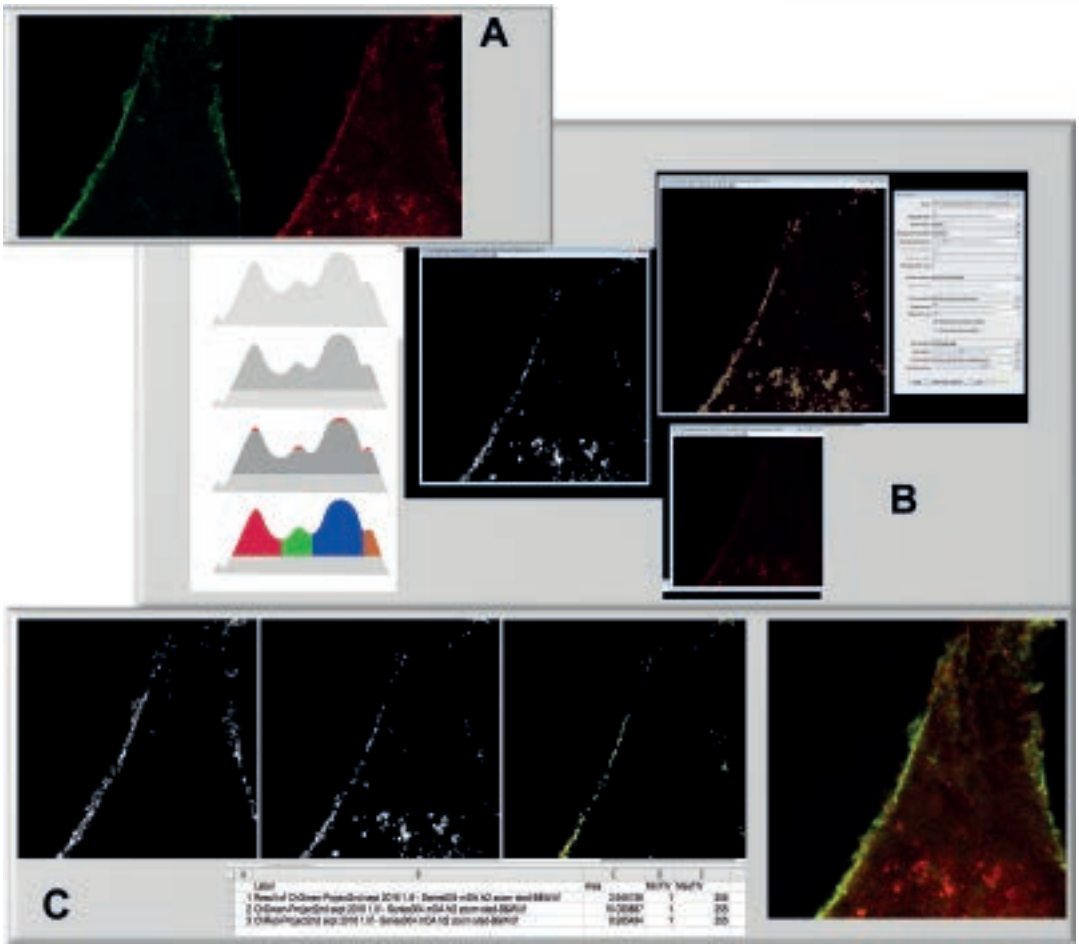
Jorge Ripoll



Quantitative analysis of coincident areas in STED images.
The nanostructured distribution of two membrane proteins in the cell membrane was quantified using recently published algorithms (1) that identify individual foci and clusters of each protein independently and measure the coincident ones.

A- Example of two STED images of proteins in MDA cell plasma membrane stained with 488/561-FAB-I fragments
B- Detection and quantification of clusters
C- Binary masks of the cluster areas detected in each image and quantification of the overlay (in yellow)
D- Composite STED image

(1) *Herbert AD, Carr AM, Hoffmann E (2014) PLoS ONE 9(12): e114749.*

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Garcia-Quintans, N., Sanchez-Ramos, C., Prieto, I., Tierrez, A., Arza, E., Alfranca, A., Redondo, J. M., and Monsalve, M. **Oxidative stress induces loss of pericyte coverage and vascular instability in PGC-1alpha-deficient mice.** *Angiogenesis* (2016) 19, 217-228

Garcia-Quintans, N., Prieto, I., Sanchez-Ramos, C., Luque, A., Arza, E., Olmos, Y., and Monsalve, M. **Regulation of endothelial dynamics by PGC-1alpha relies on ROS control of VEGF-A signaling**. *Free Radic Biol Med* (2016) 93, 41-51

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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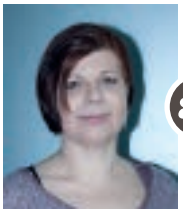
Pluripotent cell technology



RESEARCH INTEREST

The main focus of Pluripotent Cell Tehcnnoly Unit (PCTUnit) is to support CNIC scientists and their direct collaborators in their work with mouse and human stem cells. Our highly qualified staff members offer individualized training in successful stem cell culture, state-of-the-art protocols and expert advice and tecniques for proper maintenance and differentiation of stem cells and somatic cell reprogramming. In order to provide CNIC researchers with a suitable workspace, the PCTUnit houses two culture rooms, each devoted exclusively to mouse or human stem cells. Moreover, by supplying scientists with validated and standardized reagents we ensure experimentally reliability and reproducibility.

In 2016 the Unit continued to facilitate the generation of genetically-modified mice through homologous recombination in mouse embryonic stem cells (mESCs). Procedures for obtaining quality-controlled genetically modified mESCs are an essential requirement for germline transmission and the generation of mutant mice, but are labour-intensive and technically demanding. In this area, our staff takes charge of all the key steps of the gene targeting protocol: electroporation of the targeting vector, selection, karyotyping, and the preparation of cells for appropriate blastocyst microinjection. On request, we also assist researchers in the design of the targeting vector, screening stategy by Southern blot, and qPCR.

The Unit also applies its wide expertise in genetic modification using CRISPR/Cas technology and mESC derivation from mutant mouse lines to create in vitro pluripotent cell models. We use these technologies to supply CNIC researchers with knockout stem cell lines for a wide range of research projects. Our current goal is to improve the efficiency of gene editing using different genome insertion and deletion stategies based on different systems for CRISPR/cas9 complex delivery to stem cells.

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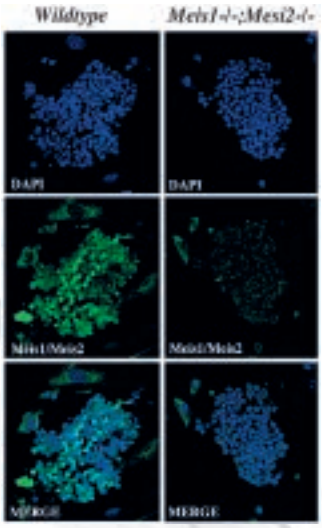
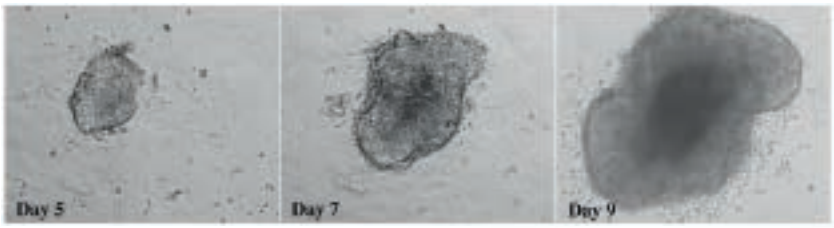


Immunocytochemistry analysis showing lack of Meis1/2 expression in diffretrentaied Meis1-/-; Mesi2-/- double knockout mESCs generated using the CRISPR/Cas9 system.



Phase contrast micrographs showing stages of the inner cell mass (ICM) outgrowth from an isolated blastocyst during the derivation of mouse embryonic stem cells.

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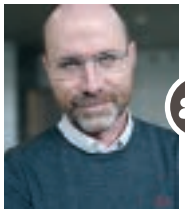
Aguado T, Gutierrez FJ, Aix E, Schneider RP, Giovinazzo G, Blasco MA, Flores I. **Telomere length defines the cardiomyocyte differentiation potency of mouse induced pluripotent stem cells.** doi: 10.1002/stem.2497 (Epub 2016 Sep 26)

Proteomics/Metabolomics



RESEARCH INTEREST

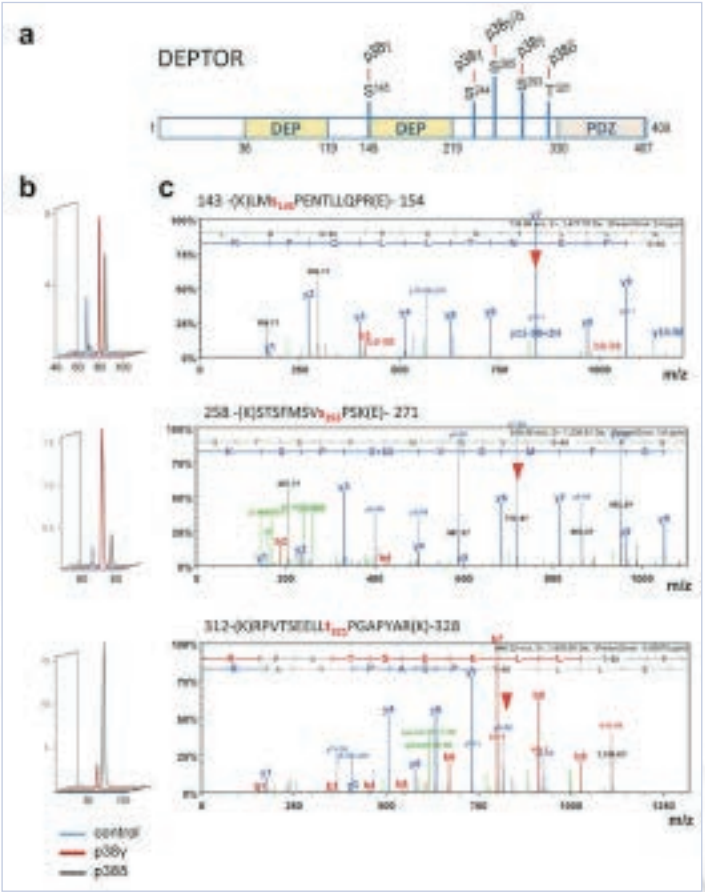
The CNIC Proteomics Unit is dedicated to technological innovation and the development of new methods of interest to the research community. Throughout 2016, the Unit worked on improvements to quantitative analysis of protein expression by shotgun and targeted proteomics using high-throughput technologies based on nanoHPLC coupled to mass spectrometry. The Proteomics Unit houses several nano-HPLC systems coupled to state-of-art mass spectrometers for deep proteome analysis.

During 2016 continuous progress was made in quantitative proteomics approaches, mainly using stable isobaric labeling (iTRAQ and TMT). Particular improvements were made in the development of chromatographic conditions for peptide fractionation, and optimization of the recently incorporated Orbitrap QExactive HF mass spectrometer. We also made progress in the statistical analysis of TMT-derived quantitative data and systems biology interpretation using algorithms developed in house.

These approaches are also being applied to the quantitative analysis of post-translational modifications, including analyses based on database searches and on peptide enrichment. For biomarker discovery in the clinical setting, we are analyzing dozens of plasma samples using depletion protocols of the most-abundant proteins and isobaric labeling. The use of non-depleted samples is under evaluation for the clinical setting.

We are also developing our technological and statistical methodologies for data-independent scanning acquisition mode, which mixes targeted and shotgun approaches, based on signal-independent fragmentation. This experimental approach has been applied to the analysis of the superassembly mechanism of mitochondrial respiratory complexes III and IV.

This robust analytical platform, together with our recognized experience in the field, enables 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. We have improved the quantitative proteomics pipeline at each stage, significantly improving the sensitivity and dynamic range for the analyzed biological systems.



Mass spectrometry analysis of DEPTOR phosphorylation by p38γ and p38δ kinases in vivo by. (a) DEPTOR phosphorylation sites. (b) Quantitative analysis of phosphorylation. (c) MS/MS spectra of each phosphopeptide, showing sequence and assignation of the modified sites.

Modified from González-Teran et al. *Nat Commun* (2016) 7: 10477.

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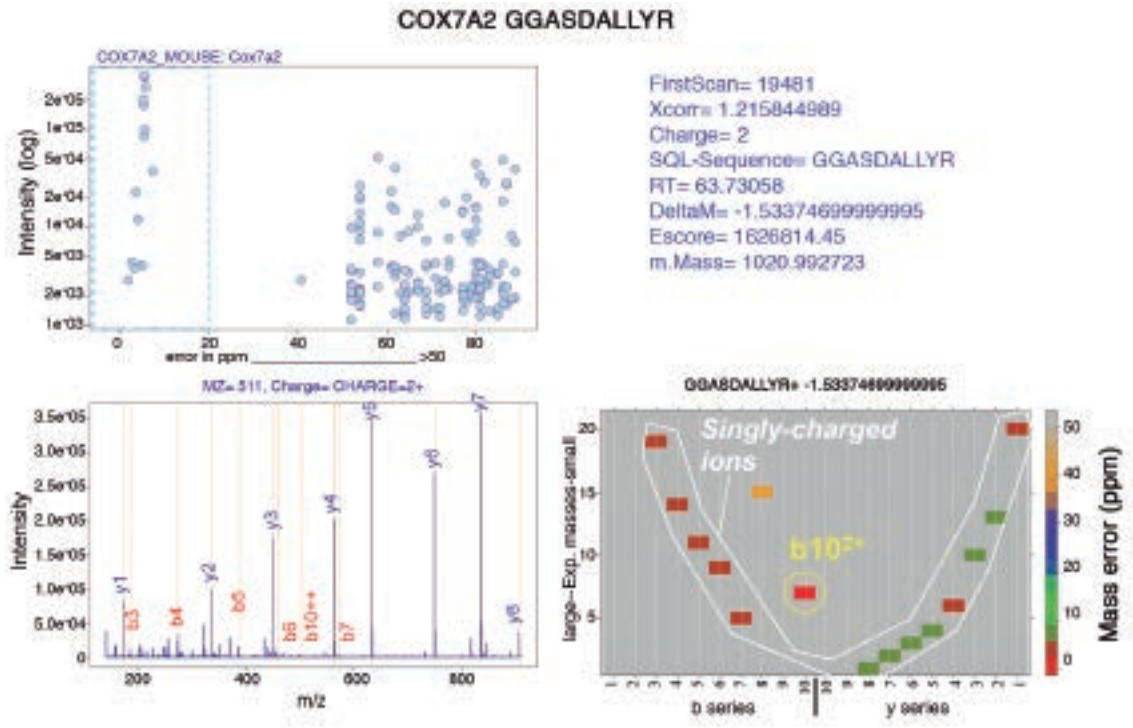

Amino acid sequence of SCAF1. Specificity of MS/MS fragmentation series used to generate quantitative peptide profiles for COX7A2 peptide using Vseq, an in-house program written in R. Top: Intensity vs fragment mass error plot. *Bottom left*: representative MS/MS spectrum of the peptide, indicating the matched fragments. *Bottom right*: Color coded diagram of the mass error of fragments ranked according their m/z values and their correspondence with theoretical fragmentation series. Modified from Cogliati et al. *Nature* (2016) 539: 579–582.

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Blas-Rus N, Bustos-Moran E, Pérez de Castro I, de Carcer G, Borroto A, Camafeita E, Jorge I, Vázquez J, Alarcón B, Malumbres M, Martín-Cofreces NB, Sánchez-Madrid F. Aurora A drives early signalling and vesicle dynamics during T-cell activation. *Nat Commun* (2016) 7:11389

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Latorre-Pellicer A, Moreno-Loshuertos R, Lechuga-Vieco AV, Sánchez-Cabo F, Torroja C, Acín-Pérez R, Calvo E, Aix E, González-Guerra A, Logan A, Bernad-Miana ML, Romanos E, Cruz R, Cogliati S, Sobrino B, Carracedo A, Pérez-Martos A, Fernández-Silva P, Ruiz-Cabello J, Murphy MP, Flores I, Vázquez J, Enríquez JA. **Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing**. *Nature* (2016) 535: 561-5

Transgenesis

RESEARCH INTEREST

The Unit's main activities are rederivation of mouse strains, production of genetically modified mice, and cryopreservation of mouse strains. Rederivation, always done by embryo transfer, cleanses mouse strains of potential infective agents and is used to set up colonies in the SPF zone of the Comparative Medicine Unit. Genetically modified mice are produced according to the requirements of the CNIC's research groups, and are generated using well-established techniques: pronuclear and/or cytoplasmic injection of mouse zygotes, and injection of genetically modified mouse embryonic stem cells (ES-cells) into preimplantation mouse embryos at the 8-cell or blastocyst stages. We also offer gene editing using engineered nucleases (zinc finger nucleases, ZFN) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. Mouse strains are cryopreserved by freezing mouse embryos (2-cell or 8-cell stage) or mouse sperm. The Unit also carries out mouse in vitro fertilization (IVF) using fresh or frozen sperm.

The Unit also cryopreserves sperm from the zebrafish (*Danio rerio*) and offers IVF in this species.

The Unit collaborates with several CNIC groups on specific aspects of their research programs, and participates in the CNIC's training programs by providing theoretical and practical sessions.

Head of Unit:
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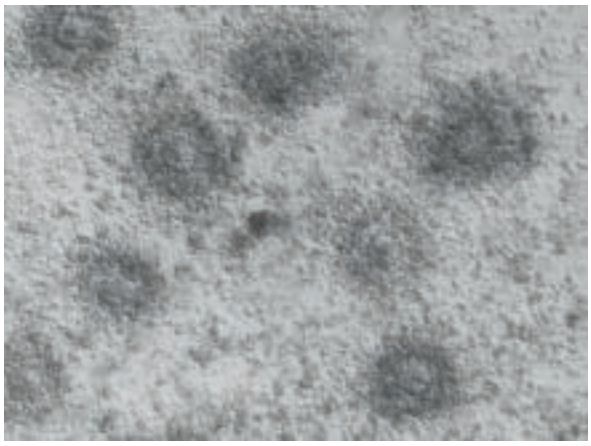
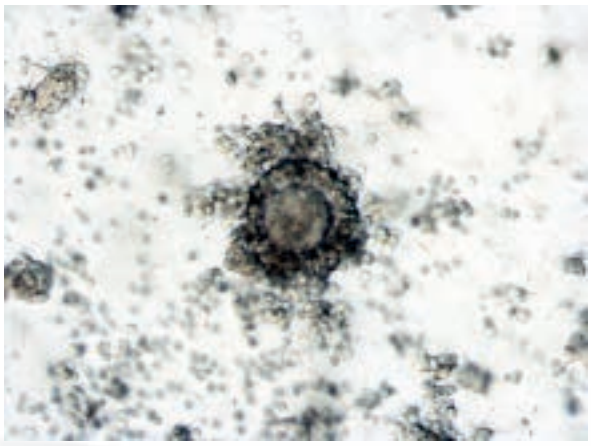
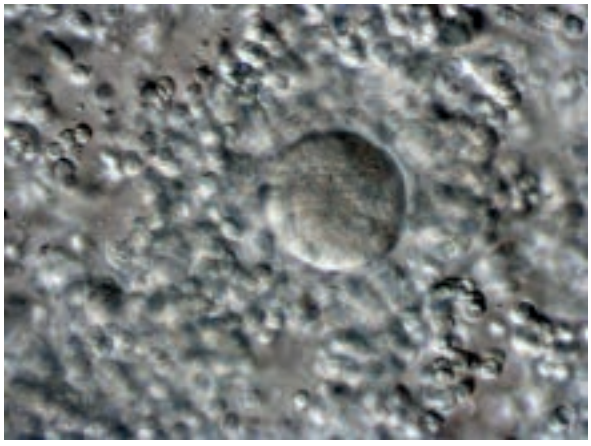
Juan de Dios Hourcade Bueno



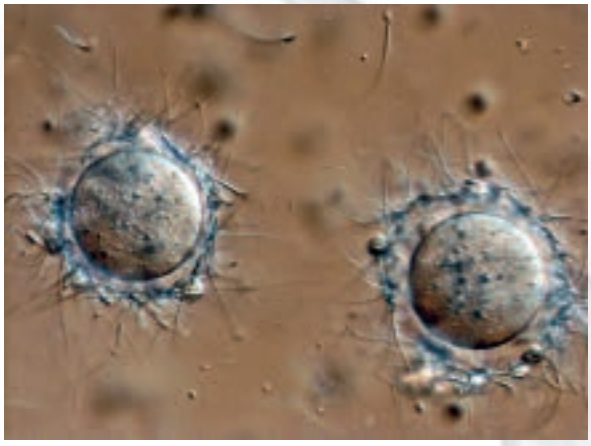
Eight mouse oocytes surrounded by cumulus cells, involved in coordining follicular development and oocyte maturation. Each mass contains a single oocyte surrounded by cumulus cells.



A mouse oocyte surrounded by cumulus cells (low magnification).

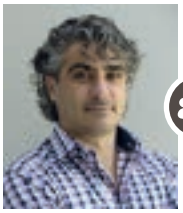


A mouse oocyte surrounded by cumulus cells (high magnification).



Two mouse oocytes surrounded by mouse spermatozoa trying to penetrate de zona pellucida, the glycoprotein layer that surrounds the oocyte (not visible in the picture).

Viral vectors



RESEARCH INTEREST

The main purpose of the Viral Vectors Unit (ViVU) is to provide investigators with the scientific resources needed to produce state-of-the-art recombinant viral vectors for in vivo and in vitro use in gene transfer experiments. The ViVU currently produces 2nd and 3rd generation lentivirus, adenovirus, and adeno-associated virus (AAV) serotypes 6, 8, DJ, and 9. The Unit also maintains a P2 facility with the appropriate expertise, equipment and permissions. We not only offer in-house services to CNIC researchers but also external services and collaborations to researchers from other institutions.

Viral vectors are widely used for gene transfer and gene expression in vitro, and our aim is to boost their use in vivo, in small and large animal models, by developing new tools for innovative applications. The use of viral vectors has several advantages over other methods: they have a high transduction efficiency and can be easily engineered for multiple purposes such as transgene expression, RNA silencing, and tandem CRISPR/Cas9 gRNA constructs, providing spatiotemporal control of any genetic modification and avoiding pitfalls common in 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 stain cellular compartments in vivo. AAV is more versatile, cost-effective, simpler, and time-efficient than transgenic approaches for generating this type of mutant model. These studies set the basis for our future vector development.

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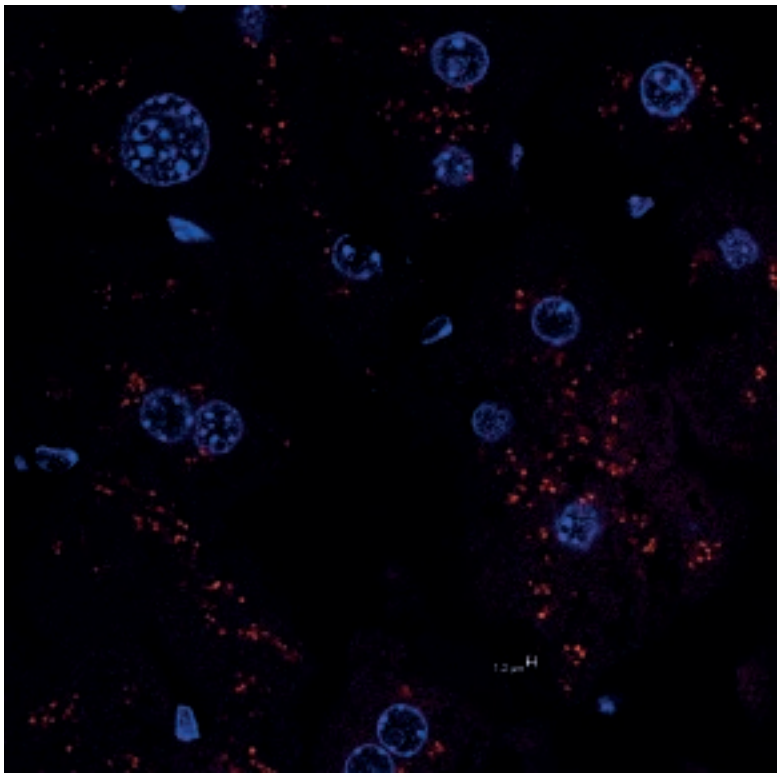
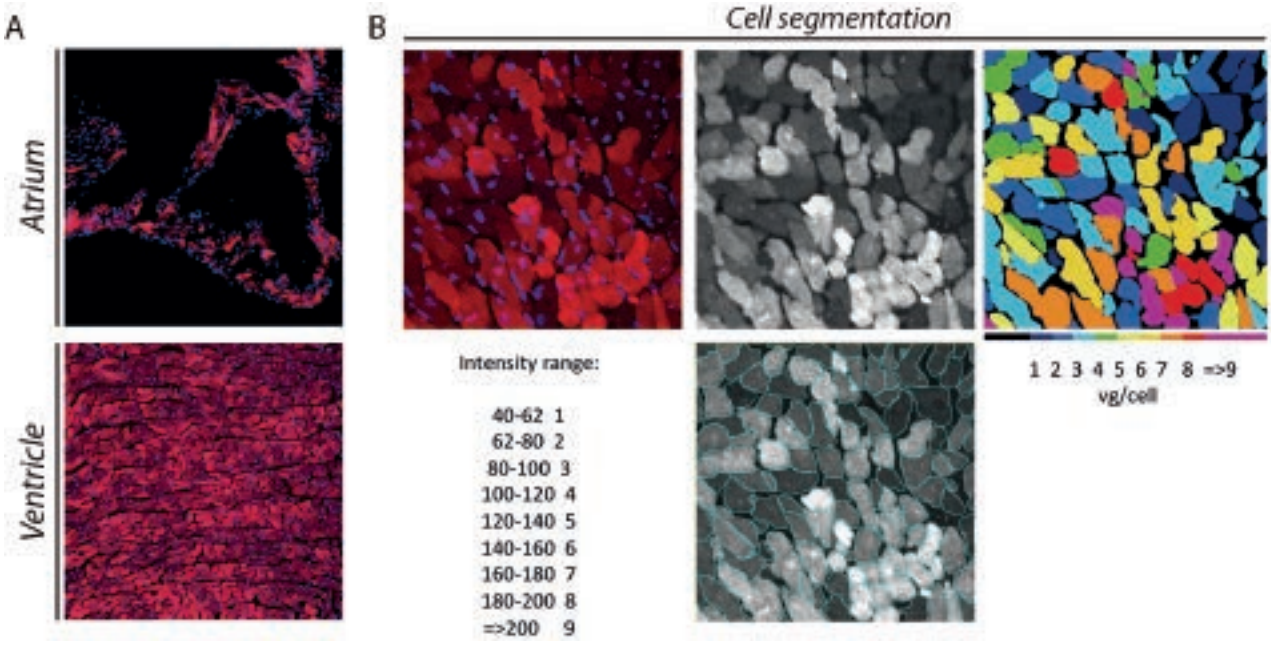
Cristina Márquez



Infection with AAV to identify mitochondria in vivo. Fluorescence imaging of mito-Keima transgene expression in hepatocytes of C57BL6J mice injected intravenously (femoral vein) with AAV2–based vector in packaging serotype 9 with $3.5x10^{10}$ viral genomes (vg). Images were acquired 4 weeks after inoculation.



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

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García-Quintans N, Prieto I, Sánchez-Ramos C, Luque A, Arza E, Olmos Y, Monsalve M. **Regulation of endothelial dynamics by PGC-1α relies on ROS control of VEGF-A signaling.** *Free Radic Biol Med* (2016) 93: 41-51

Ruiz-Andres O, Suarez-Alvarez B, Sánchez-Ramos C, Monsalve M, Sanchez-Niño MD, Ruiz-Ortega M, Egido J, Ortiz A, Sanz AB. **The inflammatory cytokine TWEAK decreases PGC-1α expression and mitochondrial function in acute kidney injury.** *Kidney Int* (2016) 89: 399-410

see also additional publications on page 20