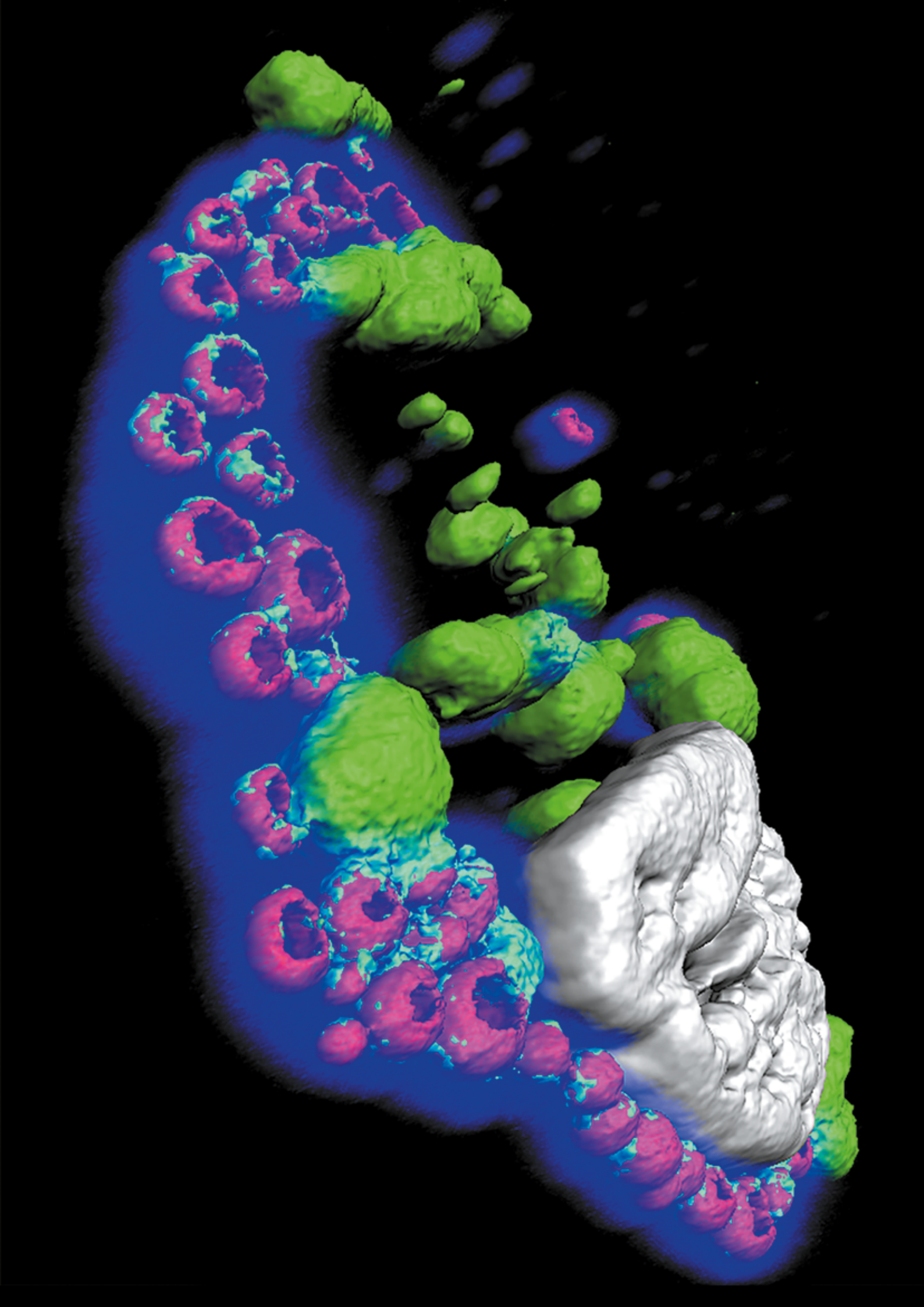

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MOLECULAR AND CELLULAR BIOLOGY

The Department of Molecular and Cellular Biology hosts 14 independent research groups working in two broad, closely interwoven research areas, with the goal of identifying specific therapeutic targets for use in disease prevention and control. The first area focuses on dissecting viral replication mechanisms and on structural studies of key viral proteins, as well as virus-host interactions for important human and veterinary pathogens. The identification of virus and cell elements with key roles in virus replication is essential for the rational design and implementation of new strategies for disease control. Understanding the mechanisms that allow a virus to evade or counteract innate and adaptive host immune responses will allow generation of innovative vaccination strategies and virus-based vaccine vectors. The second area centres on the networks that control mammalian gene expression and on characterising specific genes with critical roles in normal and pathological processes. The aim of this research programme is to identify and exploit molecular targets for diagnostics and therapy. In addition to generating leading edge research, studies in our department help to provide essential scientific background for the development of new biotechnological tools.

Our department also counts with a virus biotechnology platform (VBP) that was created with the aim of providing integral biotechnological solutions to health challenges caused by human and animal viruses. In this regard, in the context of the COVID-19 pandemic several groups have devoted their efforts to fight against SARS-CoV-2 by: i) developing vaccines based on non-replicative SARS-CoV-2 replicons and on poxvirus recombinants; ii) developing a high throughput screening platform to test compound libraries for their antiviral potential against SARS-CoV-2; iii) producing recombinant SARS-CoV-2 proteins as antigens for the development of serological test and potential vaccines; iv) producing monoclonal antibodies for anti-viral therapy; v) controlling viral infection through the modulation of cellular energy metabolism; and vi) using the CRISPR/cas13d technology as a therapeutic tool to target coronavirus RNA genome.

During this period the department has lost one of its research groups due to the retirement of Dr. Amelia Nieto.

HEAD OF DEPARTMENT

Dolores Rodríguez

Image from a 3D reconstruction of IBDV replication complexes. The compact superstructures, exclusively stained with VP2 antibodies, adjacent to viroplasms, correspond to aggregates formed by tightly packaged IBDV virions. Images were captured and processed at the Advanced light microscopy CNB core facility by Daniel Fuentes Martínez, Sylvia Gutiérrez Erlandsson and Ana Oña Blanco. (From José F. Rodríguez's lab).

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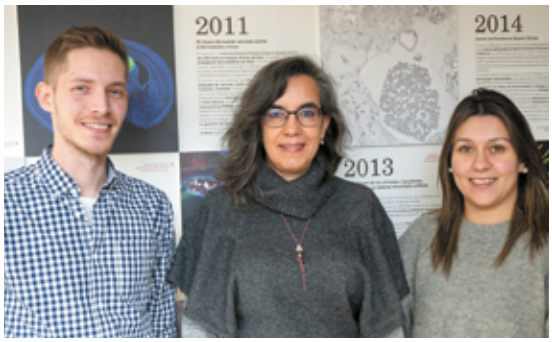

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Escoll M Lastra D, Robledinos-Antón N, Wandosell F, Antón IM, Cuadrado A. WIP modulates oxidative stress through NRF2/KEAP1 in glioblastoma cells. Antioxidants (Basel). 2020; 9 (9): 773-785.

Antón IM, Gómez-Oro C, Rivas S, Wandosell F. Crosstalk between WIP and Rho family GTPases. Small GTPases. 2020; 11: 160-166.

Molecular bases of actin cytoskeleton reorganisation in cell motility, tumour generation and invasiveness

Actin cytoskeleton is an essential contributor to cell motility and invasiveness and therefore understanding the molecules and mechanisms which regulate its temporal and spatial re-organisation is vital to fight tumour invasion and metastasis, the cause of 90% of cancer-associated deaths.

We study the role of actin-related proteins, mainly (N)WASP (neural Wiskott-Aldrich syndrome protein), WIP (WASP interacting protein), TAZ (Transcriptional coactivator with *PDZ*-binding motif) and YAP (Yes-associated kinase), in tumour generation, progression and dissemination, mostly focusing in central nervous system tumours such as deadly gliomas. Through the analysis of glioblastoma samples using biochemical and molecular approaches, in combination with lentivirus-mediated modification of protein expression and advanced imaging analysis, mouse models and proteomics, we have described that WIP acts as a proto-oncogene; WIP overexpression is sufficient to transform primary human astrocytes following pathways which include Pak, formins (mDia) and the GTPase Rac. WIP levels directly correlate with cell proliferation, anchorage-independent growth, stemness and invasive capability and they also control the cellular amount of the co-transcriptional regulators and mechanosensors TAZ and YAP by protecting them from calpain or proteasome-mediated degradation. In contrast, in haematological malignancies (T cell lymphoma), WASP and WIP turn into tumour suppressors as ALK+ lymphomas developed by transgenic NPM-ALK mice are accelerated in WASP- and WIP-deficient mice.

At present we are following a systematic proteomics approach by mass spectrometry to search for common and unique partners in the WIP interactomes identified from glioblastoma versus haematological samples. We hope to identify novel therapeutic targets to find additional and effective cancer treatments. Our ultimate goal is to understand the molecular basis of the mechanism that regulates actin dynamics, a process that underlies numerous essential cellular functions whose deregulation leads to serious human diseases. We thus hope to provide new diagnostic, prognostic and/or therapeutic tools for neurological disorders, tumour initiation and metastasis.

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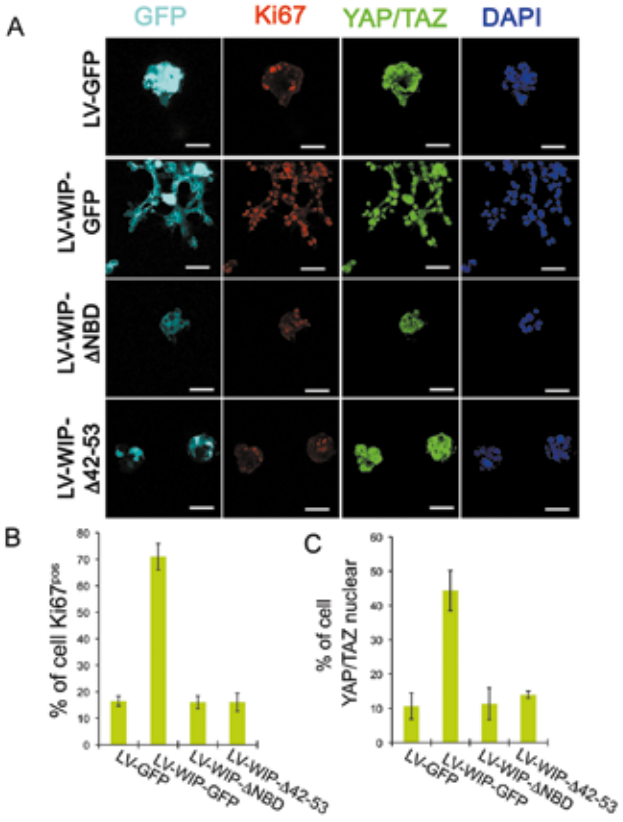

1 *WIP binding to Nck and actin coordinates survival and proliferation to drive cell invasiveness in a YAP/TAZ-dependent manner. MDA-MB-468 cells were transduced with lentivirus encoding GFP, WIP-GFP, WIP-ΔNBD (WIP mutant lacking the Nck binding domain) or WIP-Δ42/53. (WIP mutant lacking the actin binding domain). (A) Confocal images of staining for GFP (cyan), proliferation marker Ki67 (red), YAP/TAZ (green) and nuclei (DAPI, blue) of invasive/non-invasive structures in 3D-Matrigel; bars, 25 µm. (B) Percentage of cells that showed positive staining for Ki67 or (C) nuclear localisation of YAP/TAZ.*

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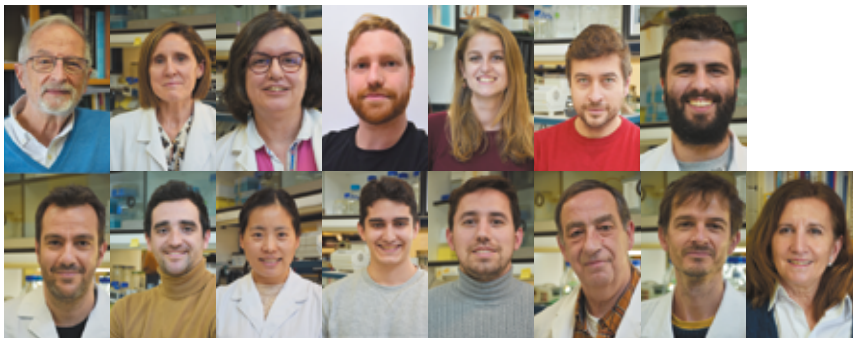


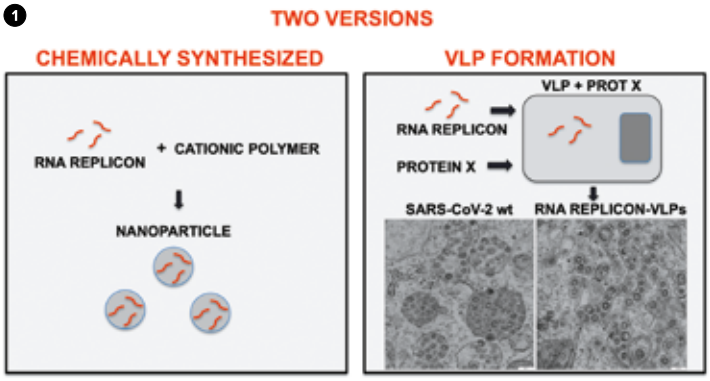
Coronavirus: replication and transcription, virus-host interactions, and protection

Human infections causing pneumonia and acute respiratory distress syndrome (ARDS) are one of the most common cause of death in the EU. The problem is even greater in the elderly population, which responds with lower efficacy to vaccination.

Among the seven known human coronaviruses (CoV), HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1 are the cause of up to 15% of mild respiratory infections. In contrast, SARS-CoV, MERS-CoV, and SARS-CoV-2 cause severe respiratory syndromes. These deadly viruses emerged from animal reservoirs in the 21st century, being SARS-CoV-2 the causative agent of the CoV disease (Covid-19) pandemic. Our laboratory focuses on the study of virus-host interactions, the design of vaccines and the selection of antivirals to protect against severe respiratory CoV infections by modulating the innate immune response in young and elderly populations.

The main aims of our research are:

- **Development of a new generation of SARS-CoV-2 vaccines consisting in replication-competent propagation-deficient RNA replicons, which are safe and promising vaccine candidates, and to determine their efficacy in animal model systems.** Vaccine development includes: (i) Engineering the RNA-replicons by deleting or modifying viral genes responsible for propagation and virulence, using reverse genetics; (ii) Identification of RNA-replicon delivery systems; (iii) Development of packaging cell lines that efficiently complement the generation of virus-like particles (VLPs); (iv) Engineering simplified and safer versions of the replicase complex.
- **To identify cell-signaling pathways involved in CoV replication and pathology** to select antiviral drugs that inhibit these pathways. In particular, we study PBM-PDZ protein-protein interactions involved in the innate immune and inflammatory responses, since overstimulation of these pathways is responsible for increased mortality.
- **To determine the contribution of host miRNAs and virus-derived small RNAs to the inflammatory lung pathology.** These small non-coding RNAs represent antiviral targets. To enhance the efficacy of vaccine candidates in older adults, RNA-replicons delivering immunomodulatory miRNAs will be engineered.



1 *RNA replicon vaccines to protect against highly pathogenic human coronaviruses. Two types of replicon delivery systems have been designed: (i) a chemically synthesised one that includes two components the RNA replicon and a cationic polymer to form nanoparticles. (ii) formation of virus like particles (VLPs) complemented in packaging cells lines with the proteins required for propagation from cell to cell.*

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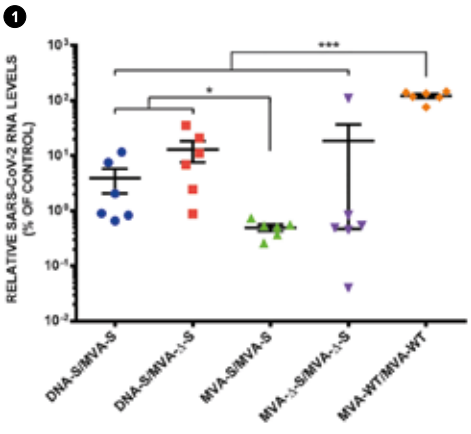
Poxvirus and vaccines

The main objectives of our laboratory are geared towards understanding the molecular basis of the biology of infectious agents and their interaction with the host, as well as to use this knowledge in the development of vaccines that might be effective against emerging viruses, like HIV, chikungunya, ebola, zika, hepatitis C, coronavirus SARS-CoV-2, as well as against cancer. As a model system of an infectious agent and as a delivery vector for expression of genes of interest, we use vaccinia virus (VACV) and the attenuated vaccine strains MVA and NYVAC, members of the poxvirus family. Our goal is to develop the best-in-class immunogens and vaccination protocols to be applied as vaccines against prevalent human diseases.

By studying the behaviour of replication competent and incompetent poxvirus vectors MVA and NYVAC, alone and in combination with other immunogens (DNA, mRNA, alphavirus replicon, VSV vectors, protein), our group has made important contributions in the immune biology of vaccines, the mechanisms of T and B cell immune responses, correlates of protection and the engineering of vaccine candidates against a variety of prevalent human diseases, obtaining in animal models 80-100% efficacy against ebola, chikungunya and zika.

As of January of 2020 the group participates in the fight against the coronavirus SARS-CoV-2 responsible for COVID-19, with the development of a vaccine candidate MVA-CoV2-S that has shown 100% efficacy against SARS-CoV-2 (morbidity, mortality and inhibition of virus replication) in humanised mouse models. Currently with the MVA-CoV2-S vaccine, immunogenicity and efficacy studies are on-going in hamsters and macaque models, as well as there are planned phase I/II clinical trials for 2021. New recombinant vectors and strategies are under development to establish optimal vaccination approaches able to confer wide and long-term protection against different coronavirus variants and strains. This research is supported by national and international grants.



1 *High neutralisation of SARS-CoV-2 by serum from humanised mice immunised with the vaccine candidate MVA-CoV2-S (MVA-S in the graph) in various combinations (from Garcia-Arriaza et al, J Virol 2021).*

2 *The vaccine candidate MVA-CoV2-S administered in one or two doses in humanised mice protects 100% against lethality induced by SARS-CoV-2.*

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Virus-host interactions in hepatitis B virus infection

Our laboratory is interested in understanding virus host interactions that regulate the outcome and pathogenesis of virus infections. Our main objective is to identify vulnerabilities that could be exploited to develop new antiviral therapies. In the last years we have used hepatitis B virus (HBV) and hepatitis C virus (HCV) infection cell culture models. These hepatic viruses are responsible of millions of cases of acute and chronic hepatitis and represent the major etiological agent of liver cancer worldwide.

During the 2019-2020 period, we focused on understanding the role of cellular proteins in the virus life cycle. On one hand, we identified Erlin-1 protein, an endoplasmic reticulum resident protein, as a new host factor required for efficient HCV infection. Gene silencing experiments have demonstrated that Erlin-1 protein regulates early as well as late steps in the HCV life cycle. Interestingly, Erlin-2, a protein with high sequence and functional homology with Erlin-1 protein does not play any important role in HCV infection. Our results provide new insights into functional differences between the two Erlins and identify a new molecular target for therapeutic intervention. On the other hand, we have confirmed and expanded our initial observations that DNA damage response related proteins are key restriction factors for HBV infection. Moreover, we are working on basic aspects of HBV DNA integration, key for cancer development.

Since the SARS-CoV-2 pandemic started our group have teamed up with Dr. Gastaminza's group to establish the CNB Antiviral Screening Platform. The main objective is the identification and characterisation of new antiviral compounds against highly pathogenic human virus infections. We have screened thousands of chemical compounds and identified new families of experimental compounds with antiviral activity against SARS-CoV-2. Moreover, we have identified repurposing drugs that are been considered for clinical testing. Finally, we have signed several research contracts with the pharmaceutical industry.

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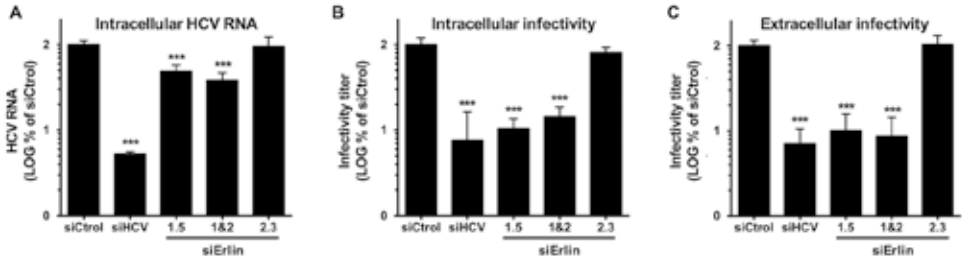

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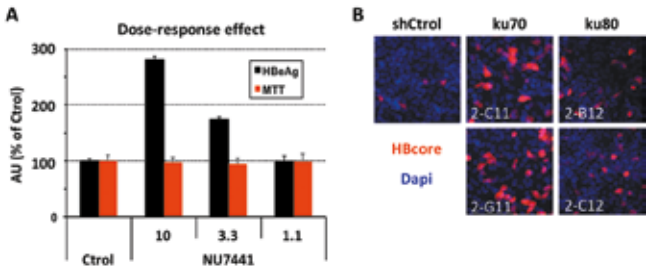

1 *Erlin-1 protein down-regulation interferes with HCV infection, shown by reduced levels of intracellular RNA (A) and infectivity (B) as well as extracellular infectivity (C).*

2 *(A) Treatment with the DNA-PKc inhibitor NU7441 increases HBeAg levels in a de novo HBV infection (B) Silencing of ku70 and ku80 proteins, regulator subunits of DNA-PKc, increases the intracellular accumulation of HBV core protein.*

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Infection by hepatitis C and related viruses

Our laboratory studies pathogenic human viral infections, and focuses on understanding the molecular basis of viral pathogenesis and identifying new molecular targets for antiviral therapy. Our final aim is to propose new therapeutic approaches for antiviral treatment and for reversion of virus-induced pathogenesis. To achieve these general aims, we have implemented cell culture models for infection by hepatitis C virus and other members of the *Flaviviridae* family such as dengue, Zika and West Nile viruses. Given the current health emergency due the COVID-19 pandemic, we have also implemented cell culture models of infection by SARS-CoV-2 coronavirus, including a compound screening platform for antiviral drug discovery.

Although their origin, nature and structure are not identical, a common feature of the aforementioned positive-strand RNA viruses is their ability to subvert host lipids and intracellular membranes to generate replication and assembly complexes. We previously reported that lipin1, a cellular enzyme that converts phosphatidic acid into diacylglycerol, is involved in the formation of the membranous web that hosts hepatitis C virus (HCV) replicase. In the liver, lipin1 cooperates with lipin2 to maintain glycerolipid homeostasis. We extended our previous study of the lipin family on HCV infection, by determining the impact of the lipin2 silencing on viral replication. Our data reveal that lipin2 silencing interferes with HCV virion secretion at late stages of the infection, without significantly affecting viral replication or assembly. Moreover, uninfected lipin2-, but not lipin1-deficient cells display alterations in mitochondrial and Golgi apparatus morphology, suggesting that lipin2 contributes to the maintenance of the overall organelle architecture. Finally, our data suggest a broader function of lipin2 for replication of HCV and other RNA viruses, in contrast with the specific impact of lipin1 silencing on HCV replication. Overall, our studies reveal distinctive functions of lipin1 and lipin2 in cells of hepatic origin, a context in which they are often considered functionally redundant.

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Castro V, Calvo G, Ávila-Pérez G, Dreux M, Gastaminza P. Differential roles of Lipin1 and Lipin2 in the Hepatitis C Virus replication cycle. Cells. 2019; 8 (11): 1456.

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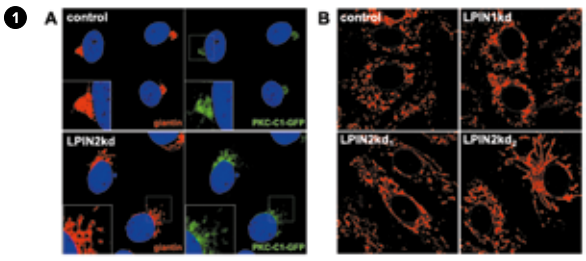
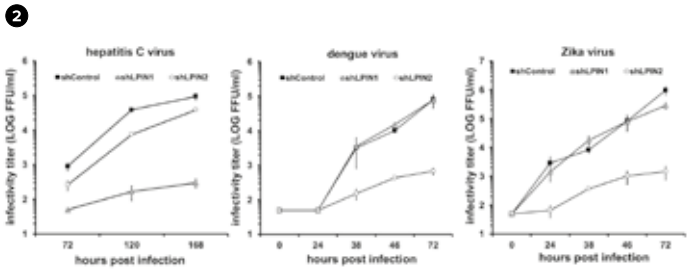
Galindo I, Garaigorta U, Lasala F, Cuesta-Geijo MA, Bueno P, *et al.* Antiviral drugs targeting endosomal membrane proteins inhibit distant animal and human pathogenic viruses. Antiviral Res, 2020; 26: 104990.

Marcos-Villar L, Nistal-Villan E, Zamarreño N, Garaigorta U, Gastaminza P, Nieto A. Interferon-ß stimulation elicited by the Influenza virus is regulated by the histone methylase Dot1L through the RIG-I-TRIM25 signaling axis. Cells 2020; 9 (3): 732.





1 *Lipin2, but not lipin1, silencing causes morphological alterations of the Golgi apparatus and mitochondrial elongation. Huh-7 cells constitutively expressing a DAG sensing probe (PKC-C1-D1-GFP) were transduced with lentiviral vectors expressing non-targeting (control), shRNAs targeting LPIN1 (LPIN1kd) or LPIN2 mRNA (LPIN2kd1 and LPIN2kd2). At day 7 post transduction, control and lipin-deficient cultures expressing the DAG probe were fixed and processed for immunofluorescence microscopy using antibodies against a Golgi Apparatus marker (giantin) or stained with Mitotracker™ red following manufacturer recommendations and imaged in vivo under a confocal microscope at 37oC and 5% CO2 to visualise mitochondria. A-Representative images of the Golgi morphology (red) and subcellular DAG probe (green) PKC-C1-GFP different cell lines. Nuclei were stained with DAPI and are shown in blue. B-Representative images of the mitochondrial morphology in the different cell populations. Cell nucleus is approximately delimited by a dotted white line for reference.*

2 *Lipin2 silencing interferes with hepatitis C, dengue and Zika virus propagation. Huh-7 cells were transduced with lentiviral vectors expressing non-targeting (control), shRNAs targeting LPIN1 (LPIN1kd) or LPIN2 mRNA (LPIN2kd1 and LPIN2kd2). At day 7 post transduction, the different cultures were inoculated with HCV (JFH1-D183v strain), DENV (NGC strain), with ZIKV (BeH819015 strain). Samples of the cell supernatants collected at the indicated time points were used to determine extracellular infectivity titers. Data are shown as average and SD of three biological replicates (n=3).*

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Biological noise and its physiopathological implications

Phenotypic variability of clonal cell populations is mainly due to differential gene expression, in which the mitochondria content is a key factor. This non-genic cellular heterogeneity plays an essential role in many biological processes such as cell differentiation, development, apoptosis, cancer and viral infections. Our laboratory is interested in understanding the origins of this phenotypic variability and its impact on different biological processes to improve our understanding of phenomena like tumour resistance to drugs, virus infection, or cell fate choice.

During years 2019-2020 we have made important contributions in two main areas:

1. Origin of phenotypic variability. We have found that mitochondrial content contributes to heterogeneity in gene products and have a large impact on alternative splicing, which ultimately leads to phenotypic diversity.

2. Physiopathological implications of the variability of mitochondrial content in apoptosis and viral infections. Regarding the apoptosis process, we have described that the cellular mitochondrial content modulates the time to death in response to TRAIL treatment, indicating that this variability could have a great impact on the partial response to chemotherapy observed in the majority of tumours. Regarding viral infections, we have found a correlation between mitochondrial content and virus replication, and that this correlation could be direct or inverse depending of the virus analysed. Due the importance of Zika virus (ZIKV) in human health and the recent COVID-19 pandemic, we have extended these studies to ZIKV and SARS-CoV-2, and we have initiated a new investigation line focused on the study of the molecular bases of the pathogenesis of both viruses. In that sense, we have developed reverse genetic systems for ZIKV and SARS-CoV-2 that has allowed us to identify several virulent factors and several proteins of the cell metabolism important for virus replication. These studies will improve our understanding of ZIKV and SARS-CoV-2 biology and facilitate the development of vaccine and antiviral strategies.

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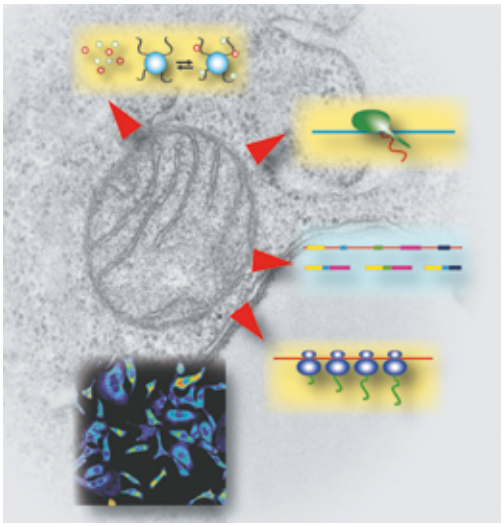

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1 *Mitochondria and gene expression. Electron micrograph of one mitochondrion with radiating arrows to the steps of gene expression where mitochondria play an important role.*

2 *Pathogenicity of rSARS-CoV-2 rescued from a infectious clone. Gross pathological lung lesions of Golden Syrian hamsters infected with rSARS-CoV-2 or the natural isolate (SARS-CoV-2). Presence of congestion and atelectasis (white arrows) and frothy trachea exudates (black arrows) are indicated. Scale bars, 1 cm.*

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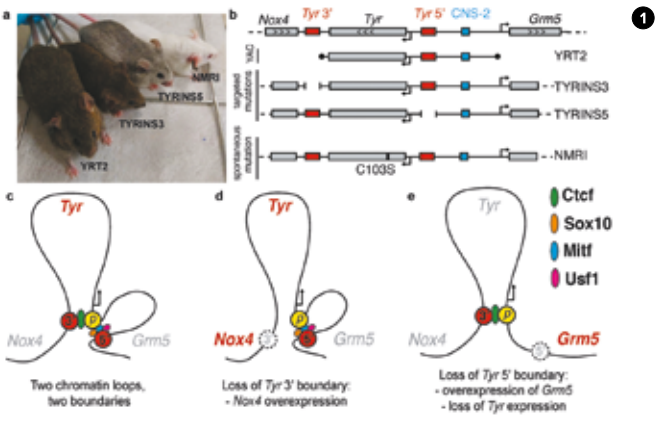
Animal models by genetic manipulation

Our laboratory is interested in understanding the underlying pathological mechanisms of a group of human rare diseases globally known as albinism, a heterogeneous genetic condition associated with mutations in at least 22 genes, characterised by visual impairment and pigmentation alterations. Our work on human rare diseases occurs within our participation in the CIBERER-ISCIII.

Our laboratory has generated and analysed new animal models to study visual abnormalities and different anomalies affecting retina development that are associated with albinism. In collaboration with Angel Carracedo (USC) and Carmen Ayuso (FJD), we have devised, within the CIBERER-ISCIII, a project for the universal genetic diagnostic of all known mutations in albinism. We are already applying this knowledge in cooperation with ALBA, the Spanish association in support of people with albinism and have been able to diagnose more than 120 families.

We are also interested in understanding the function of regulatory elements that are required to define gene expression domains in mammalian genomes. We have used the mouse tyrosinase locus (*Tyr*) as experimental model. This approach has allowed us to identify several key regulatory elements, such as genome boundaries or insulators, which protect the locus from surrounding genes and ensure the faithful gene expression pattern.

As a general strategy, we regularly use transgenic and genome-edited animals, zebrafish and mice to introduce different type of gene constructs in order to investigate the relevance of specific DNA regulatory sequences. The functional analysis of regulatory elements found within the intergenic non-coding genomic sequences can now be addressed more efficiently thanks to the efficient genome editing CRISPR-Cas9 tools. In Spain, where we have pioneered the application of CRISPR technology in mice, we have successfully implemented it in our laboratory and disseminated its use among colleagues by hosting short stays and organising ad-hoc workshops, seminars and courses.



1 *CRISPR-Cas9 genome edited mice used to functionally identify key regulatory elements of the mouse Tyr locus (Seruggia et al. 2020, Sci. Rep.)*

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SELECTED PUBLICATIONS

Momtazi G, Lambrecht BN, Naranjo JR, Schock BC. Regulators of A20 (TNFAIP3): new drug-able targets in inflammation. Am J Physiol Lung Cell Mol Physiol 2019; 316 (3): L456-L469.

Dell'Orco D, Koch KW, Kreutz MR, Naranjo JR, Schwaller B. Neuronal calcium sensors in health and disease. Front Mol Neurosci 2019; 12: 278.

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Gonzalo-Gobernado R, Perucho J, Vallejo-Munoz M, Casarejos MJ, Reimers D, *et al.* Liver growth factor "LGF" as a therapeutic agent for Alzheimer's disease. Int J Mol Sci 2020; 21 (23): 9201.

Functional analysis of transcriptional repressor DREAM

Our major research focus is on the multifunctional protein DREAM and its role in the control of calcium homeostasis in health and disease.

DREAM (downstream regulatory element antagonist modulator), also known as calsenilin or KChIP3, is a Ca2+ binding protein of the neuronal calcium sensors (NCS) superfamily that interacts with specific sites in the DNA to repress transcription of target genes in a Ca2+-dependent manner. In addition, DREAM interacts with specific proteins to exert various specialised functions in different subcellular compartments. Thus, through the control of activity-dependent gene expression and through specific protein-protein interactions, DREAM participates in many physiological processes in and outside the central nervous system. Work reported by us and other groups has shown important regulatory roles for DREAM in learning and memory in the hippocampus, in pain control in the spinal cord as well as in the immune response, in inflammation, in the thyroid gland and in the placenta. Moreover, recent studies have shown the involvement of DREAM in neurodegenerative disorders including Huntington disease (HD), Alzheimer disease (AD) and Amyotrophic Lateral Sclerosis (ALS).

DREAM was originally associated with AD because of its interaction with presenilins, however, altered neuronal calcium and protein homeostasis and early compensatory changes in transcriptional programs are common features of many neurodegenerative disorders which open the opportunity to explore a role for DREAM in these pathologies.

In physiological conditions, binding of calcium or membrane lipids (e.i. arachidonic acid) regulate the interaction with DNA or with other proteins. Newly identified molecules, including glinides, modify DREAM conformation and activity upon binding. In this respect, our interest is to contribute to the definition of more specific DREAM binding molecules, to reveal the molecular mechanisms underlying their effect upon binding to DREAM and to assess their potential therapeutic actions on appropriate cellular and/or mouse models of target pathologies.

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1 *Mouse performing in the Pole test. We use this test to analyse motor coordination in mice expressing the A315T mutation in the TDP-43 gene. In this mouse model of Amyotrophic Lateral Sclerosis (ALS), we assay new DREAM ligands that could ameliorate disease symptoms or delay disease progression. This project is funded by Asahi Kasei Pharma.*

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Mechanisms of interaction between the influenza virus and the infected cell

Influenza A virus (IAV) promotes epigenetic modification in the infected cells. IAV infection increases the methylation of lysine 79 of histone 3 catalyzed by Dot1L enzyme. A decreased antiviral signaling mediated by RIG-I sensor is found in Dot1L-inhibited cells, infected with IAV. Accordingly, Dot1L inhibition decreases the IFN-ß promoter stimulation and RIG-I-MAVS association upon viral infection. Interferon-inducible protein *TRIM25* expression increases in influenza virus infected cells, but Dot1L inhibition reduces both the *TRIM25* expression and *TRIM25* protein levels. *TRIM25* overexpression reverses the defective innate response mediated by Dot1L inhibition elicited upon virus infection or by overexpression of RIG-I signaling intermediates. Thus, TRIM25 is a control point of the RIG-I recognition pathway controlled by Dot1L and may have a general role in RNA viruses recognized by the RIG-I sensor.

Human influenza A virus (hIAV) infection is associated with important cardiovascular complications, although cardiac infection pathophysiology is poorly understood. We evaluated lung and heart viral titers in mice infected with either one of several hIAV strains inoculated intranasally and identified viral replication inside mouse cardiomyocytes, Purkinje cells, and cardiac vessels. In addition, we used human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CMs) to confirm cardiac infection and studied the underlying molecular alterations associated with the *in vivo* electrophysiological phenotype. Both, pathogenic and attenuated hIAV strains infected and replicated in cardiomyocytes, Purkinje cells, hiPSC-CMs and cardiac endothelial cells. Cardiac conduction alterations and high mortality rates were especially pronounced in mice infected with the highly pathogenic strain, compared with mice infected with the attenuated strain. Thus, human IAV can infect the heart and cardiac specific conduction system, which may contribute to cardiac complications and premature death.

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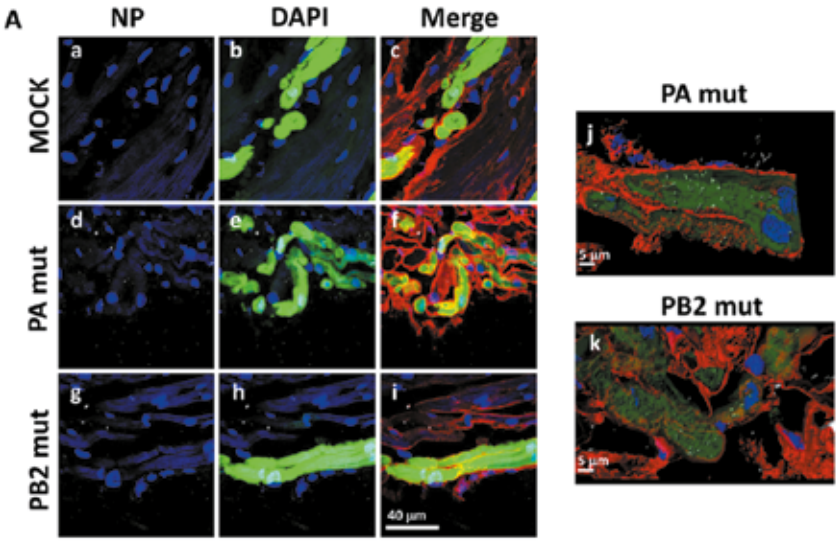

1 *Active replication of influenza A virus in Purkinje cells. (A) Detection of the NP viral protein using immunofluorescence confocal microscopy in heart tissue from Cx40eGFP MOCK-infected mice (upper panels, a–c) and animals infected with 10^6 pfu of either pathogenic strain (PAmut) (middle panels, d–f) or attenuated strain (PB2mut) (lower panels, g–i). Right panels (j–k) show 3D reconstructions of the NP viral protein in Purkinje cells of PAmut- (j) or PB2mut-infected hearts (k) from Cx40eGFP animals. Blue, nuclear staining (DAPI); green, GFP; red, laminin; white, NP viral protein staining*

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Cerebral cortical development

SELECTED PUBLICATIONS

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The cerebral cortex mediates the high functions of the human brain. It contains an extraordinary number and diversity of neurons that after development is completed, form one of the most complex functional networks found in biological systems. Despite their diversity and numbers, cortical neurons wire in following precise and highly stereotyped selective patterns with an apparent invariability. These circuits provide responses to the external world, mediate intellectual processing, and reproduce social behaviors that are optimal and common to all individuals. Still, cortical neurons demonstrate extraordinary plasticity and generate alternative circuits in non-canonical situations, such as the occurrence of genetic mutations or in neurodevelopmental disorders. This includes autism spectrum disorders (ASD), intellectual disabilities, bipolar disorders, schizophrenia, or epilepsy.

Our projects aim to understand the rules of cortical wiring and the emerging of developmental plasticity using the mouse as a model. In recent years, we have focused on the development of corpus callosum connections, which comprise a complex ensemble of interareal circuits responsible for higher-order functions. We aim to understand how neurons encode for the molecular information necessary to build the stereotyped circuits of the CC; how neurons translate this information into selective connectivity while dialoguing with their environment, and how this mechanism of wiring results in non canoncal circuits. We focus on plasticity because of its potential therapeutic implications for treating and managing neurodevelopmental disorders or intervening in others such as the loss of sensory organs or ischemic injury. Our investigation is based on *in vivo* manipulation of circuits by modifying gene expression, sensory input, and circuit activity. We use CRISPR/Cas-mediated knock-in, *in utero* electroporation, electrophysiology, stereotaxic retrotracing injections, pharmacological interventions, and RNA-sequencing among other techniques. In our projects, we collaborate with national and international scientists and clinicians and involve patients and their families.

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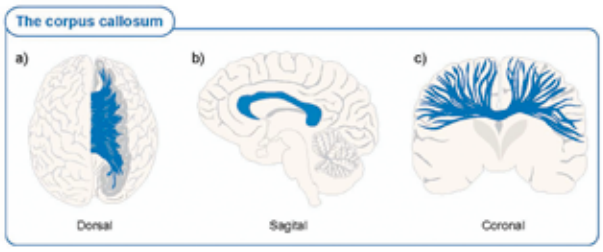

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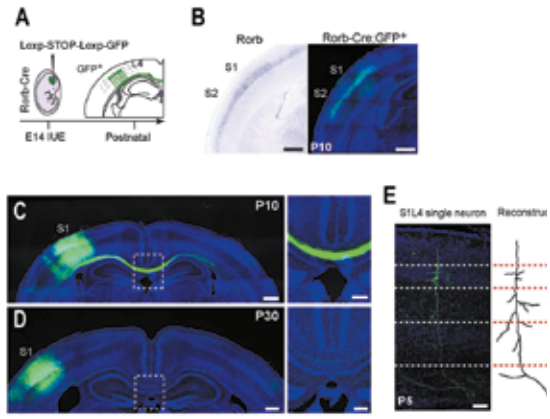

❶ *The general organisation of the corpus callosum (CC) A-C) The CC, shown in blue, is the major commissural tract connecting the cortical hemispheres. A) Dorsal view of the hCC. B) Sagittal view of the hCC. This view is broadly used during clinic diagnosis, as the entire rostro-caudal formation is visible. C) Coronal view of the hCC. Axons of cortical neurons from both hemispheres meet in the midline and cross to the opposite hemisphere to target specific contralateral regions. Most of these projections will connect homotopic regions within the brain, and fewer will connect heterotopic regions. (From De León Reyes et al., Development 2020).*

❷ *Transient callosal projections extend from S1L4 Rorb neurons. A In utero electroporation of Rorb-Cre embryos with a floxed-GFP plasmid at E14. B Left panel, expression of endogenous Rorb expression (Image credit: Allen Institute). Right panel, GFP (green) in a P10 Rorb-Cre animal electroporated at E14 in S1 and S2. DAPI (blue). C –D. Coronal sections of electroporated Rorb-Cre brains. GFP illuminates a subset of L4 neurons and their axons. Right panels show magnification of the CC at the midline. E Single neuron GFP labelling in P5 brains show S1L4 neurons with axons entering the white matter (WM). The reconstruction of that same neuron is shown in the right panel (adapted from De León Reyes et al. Nat Commun 2019).*

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Molecular characterisation and epidemiology of torovirus

Toroviruses are emergent viruses (belonging to *Nidovirales* Order) that cause enteric diseases in different species of domestic animals and could probably represent a zoonotic threat. They are highly distributed worldwide, and yet remain practically ignored. Over the years, our group has developed diagnostic tools allowing us to carry out epidemiological studies that revealed a high prevalence of porcine torovirus in Spanish farms. As it has been evidenced by the COVID-19 pandemic, it is of utmost importance studying new potential zoonotic pathogens like toroviruses. The knowledge acquired from these studies could contribute to adopt therapeutic or preventive measurements in the eventuality of a disease outbreak.

One of the main focuses of our research is the study of the virus-host interaction that would determine the outcome of the disease. During this period we demonstrated that the equine torovirus Berne virus (BEV), the prototype member of the Torovirus genus, induces autophagy at late times post-infection. We have observed that BEV replication also induces ER stress at the time when selective autophagy is taking place, suggesting that the autophagy pathway is activated in response to the hefty accumulation of virus-encoded polypeptides during the late phase of BEV infection.

We maintain a collaboration with Dr. José F. Rodríguez (CNB) to characterise the potential relationship between the innate immune response and pathogenesis caused by infectious bursal disease virus (IBDV). Specifically, we have studied the role of the interferon pathway in acute and persistent IBDV infections. At present, we are involved in a project aimed at elucidating the relevance of defective viral genomes (DVGs) in the establishment of persistent infections by both, IBDV and torovirus.

In the context of the COVID-19 pandemic we have also collaborated with Drs. Lluís Montoliu and Almudena Fernández from CNB, and Dr. Miguel Angel Moreno from the CABD (CSIC/UPO) in a project aimed at using the CRISPR-Cas technology to target coronavirus RNA genome.

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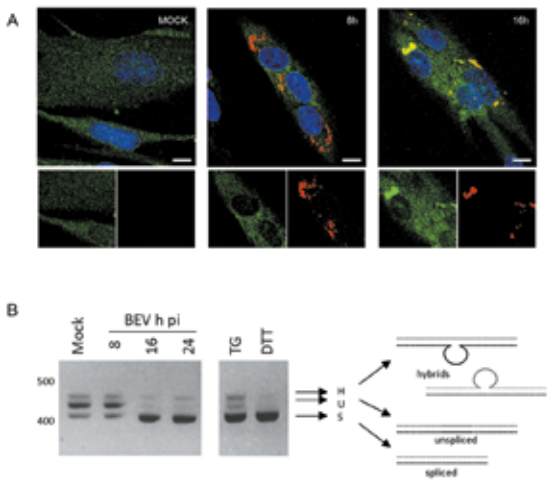

1 *BEV induces selective autophagy and ER stress. (A) Immunofluorescence analysis of mock- and BEV-infected cells showing the colocalization of the viral protease M^{pro} (red) and the cargo protein p62 (green) at late times pi (16h). Cell nuclei were stained with DAPI (blue). Enlarged representative areas showing the fluorescence corresponding to M^{pro} and p62 are shown in the lower panels. Scale bars, 10 µm. (B) Splicing of XBP-1 mRNA, analysed by RT-PCR, was observed in BEV-infected cells at 16 and 24hpi, as well as in cells treated thapsigargin (TG) or dithiothreitol (DTT) used as controls.*

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SELECTED PUBLICATIONS

Broto L, Romero N, Méndez F, Diaz-Beneitez E, Candelas-Rivera O, *et al.* Type I Interferon acts as a major barrier to the establishment of infectious bursal disease virus (IBDV) persistent infections. J Virol 2020; JVI.02017-20.

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Birnavirus molecular biology

During the last few years, our work has been mainly devoted to understanding the interaction between IBDV and host cells and the impact of the innate antiviral response on both virus-induced pathogenesis and the establishment of persistent IBDV infections. Our studies have unveiled the chief role of type I interferons on both phenomena, showing that the activation of the JAK-STAT pathway early after IBDV infection leads to a massive apoptotic response contributing to the deadly cytokine storm that destroys the bursal tissue and eventually finish off infected birds. Conversely, we have shown that the genetic inactivation of the JAK-STAT pathway significantly reduces IBDV cytopathogenicity and largely enhances the susceptibility of infected cells to sustaining long-term, productive, persistent infections.

Currently, in collaboration with Dr. Soubies's group (OIE Reference Laboratory for Gumboro Disease, French Agency for Food, Environmental and Occupational Heath Safety, Ploufragan, France) we are further dissecting mechanisms responsible for the establishment of persistent infections in infected chickens, a major IBDV biological trend likely playing a major role on IBDV dissemination and reemergence. We are particularly interested in deciphering the role of cell/virus genetic elements (e.g. micro-RNAs and defective virus genomes) within this phenomenon. Additionally, we focus on the characterisation of used by IBDV-encoded polypeptides, namely VP3 and VP5, involved in the evasion of innate antiviral cell responses. Finally, we are committed to further knowledge about the IBDV replication process, specifically on the morphogenesis of replicative complexes and progeny assembly and virus egress mechanisms.

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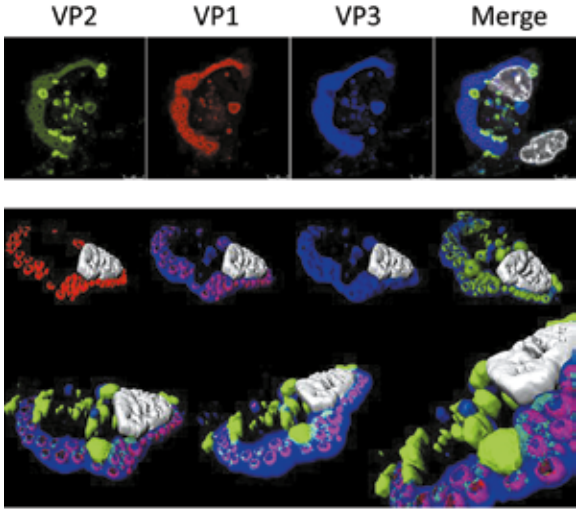

1 *Characterisation of IBDV replication complexes. Upper panels correspond to a single confocal plane captured from an IBDV-infected QM7 cell immuno-stained with antibodies specifically recognising the three structural virus polypeptides, i.e. VP1 (red), VP2 (green) and VP3 (blue). The cell nucleus, stained with DAPI, is shown in white. Lower panel show images from a 3D reconstruction, generated with confocal planes captured along the z-axis, showing the presence of quasi-spherical nucleation domains embedded within large viroplasms. The compact superstructures, exclusively stained with VP2 antibodies, adjacent to viroplasms, correspond to aggregates formed by tightly packaged IBDV virions.*

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Development, differentiation and regeneration in vertebrates

Our group is interested in understanding the molecular and cellular basis of organ formation during embryonic development. Signalling pathways involved in embryogenesis are also required for homeostasis of adult tissues and for repair of damaged organs. Moreover, malfunction of these pathways can lead to disease. Studying developmental genes and signals can therefore offer new avenues for treatment of prevalent diseases such as inflammatory diseases and cancer and also to improve the regenerative ability of tissues in the adult.

We are analysing the relationship between inflammation, regeneration and disease. The role of inflammation in regenerative processes is controversial. In some cases it has been shown to improve tissue healing but in other instances it has been shown to be detrimental for regeneration. In collaboration with the group of Ana Cuenda (Department of Immunology and Oncology, CNB) we are analysing the functions of p38MAPKs in this context, using conditional KO mouse lines. We are using a model of cancer associated to inflammation (colon cancer associated to colitis, CAC) in mice to address this problem. By chemically inducing damage to the colon, which triggers an inflammatory response, we are investigating the role of p38MAPK signalling in the regeneration of the epithelium, the control of inflammation and the activity of different immune cells during tumour initiation and progression.

We are also interested in the functions of p38MAPKs during infections and their role in triggering inflammatory responses. Using a model of candidiasis in mice we have uncovered important regulatory activities for p38γ and p38δ in controlling the extent of inflammation and thus in development of sepsis. The identification of new pharmacological inhibitors of these kinases is very important for research and for novel therapeutic treatments and we are involved in their discovery and characterisation.

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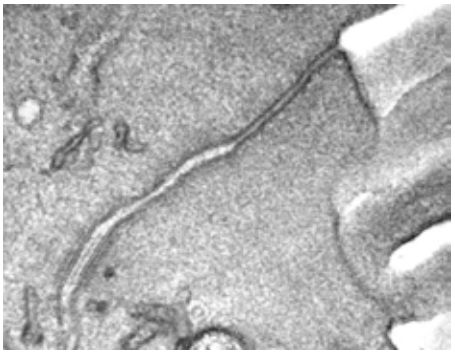

❶ *Electron microscopy image of the intercellular apical junction in colonic epithelial cells.*

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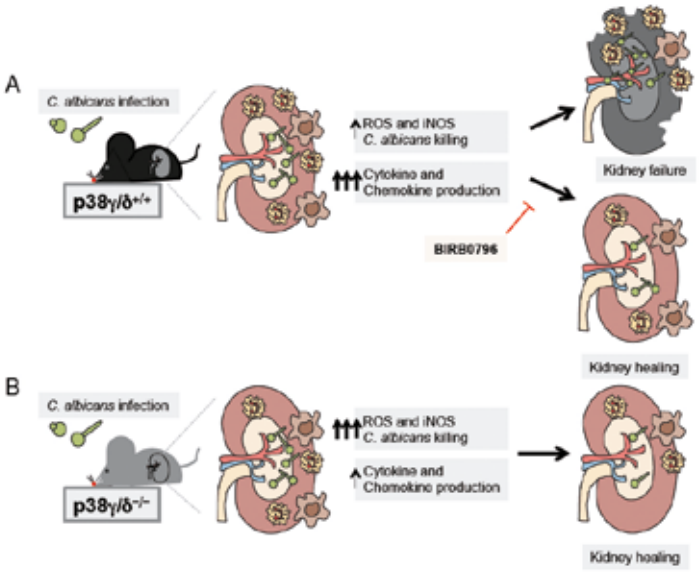

❷ *Schematic diagram showing that p38γ/p38δ regulate both host acute inflammatory response and the killing of C. albicans. The inhibitory action of BIRB0796 ameliorates inflammation in the kidney. (from Alsina-Beauchamp et al. EMBO Molecular Medicine 2018, e8485).*

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SELECTED PUBLICATIONS

Osuna-Pérez J, García-Ferreras R, Veiga E. From cellular microbiology to bacteria-based next generations of cancer immunotherapies. Cell Microbiol 2020; 22: e13187.

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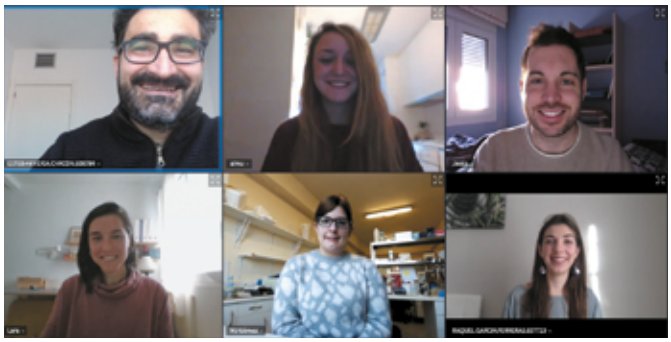

Cellular immunobiology and microbiology

We are focused in generating novel immunotherapies using the ability of bacteria to modify the immune responses. We have discovered that $CD4^+$ T cells contributes to the early immune response capturing bacteria by transfagocytosis. Surprisingly, the transfagocytic $CD4^+$ T cells destroy bacteria and become hyperinflammatory (Cruz-Adalia *et al*. 2014). Moreover, we have discovered that bacteria exposure "trains" conventional $CD4^+$ T cells. Trained $CD4^+$ T cells (bacT), contrary to the role separation dogma in immunology, became potent antigen presenting cells able to (1) cross-present antigens from captured bacteria, activating naïve $CD8^+$ T cells that became effective cytotoxic cells and (2) generating central memory; activities involved in the removal of tumours.

Note that actually there exist huge efforts to generate central memory $CD8^+$ T cells from tumour infiltrating lymphocytes. These effects, together with (3) the localised secretion of inflammatory cytokines by bacT cells, which could block the immunosuppressive environment generated by solid tumors, prompted us to hypothesised that bacT cells could be useful in antitumour therapies. This hypothesis was tested in proof-of-concept model of aggressive mouse tumours. Mice treated with bacT cells that have captured/killed bacteria expressing tumour antigen were protected against tumour development (Cruz-Adalia *et al*. 2017). These discoveries challenged the dogma of adaptive/innate immunity role separation and guided our current research to find novel tumour immunotherapies.

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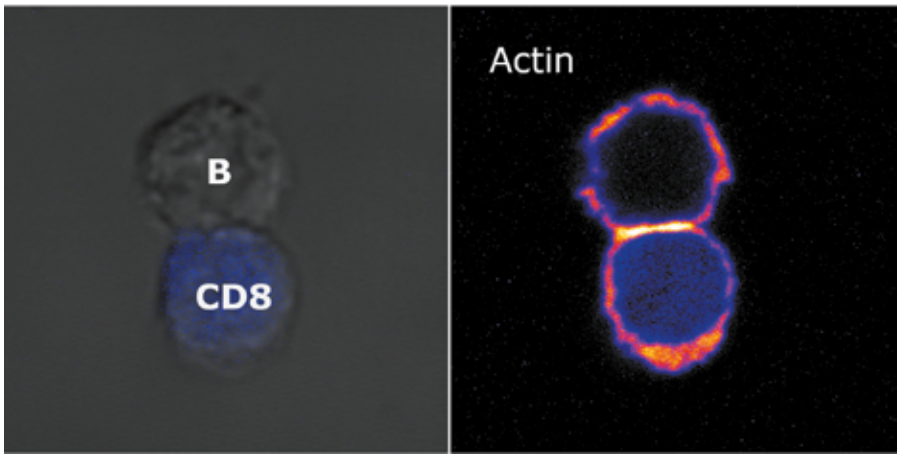

❶ *Immune synapse formed by a bacteria trained lymphocyte (B) and a naïve CD8+ T cell.*