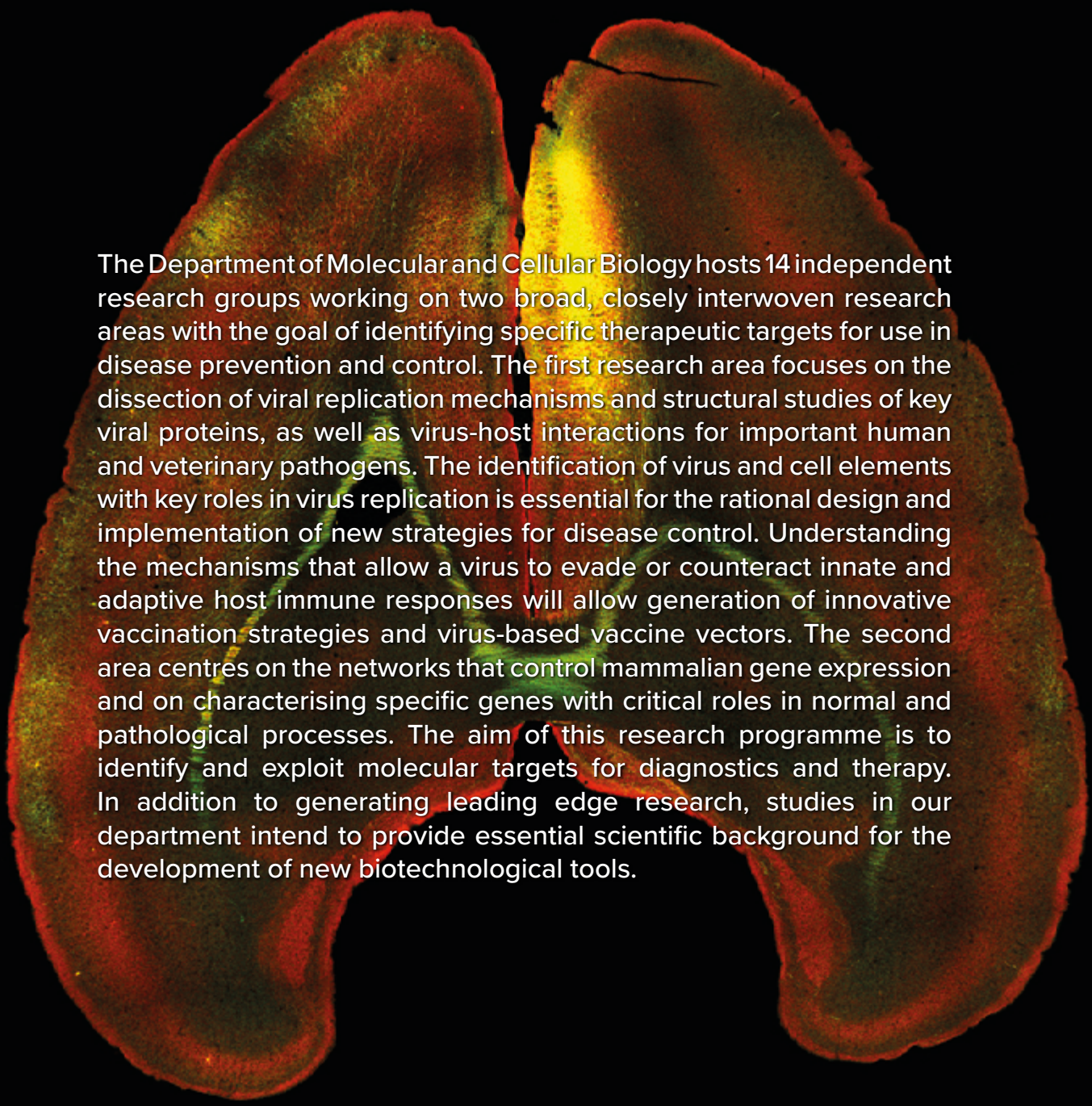


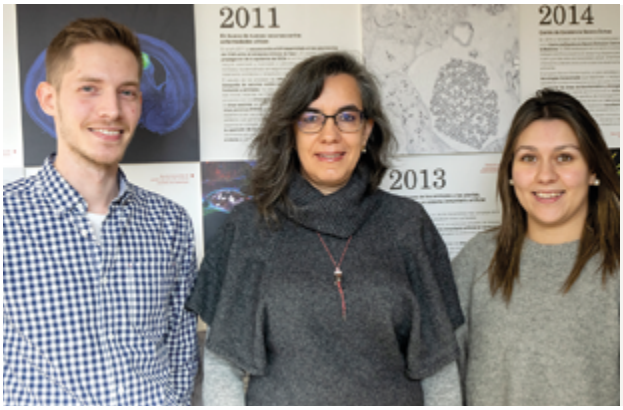
The Department of Molecular and Cellular Biology hosts 14 independent research groups working on two broad, closely interwoven research areas with the goal of identifying specific therapeutic targets for use in disease prevention and control. The first research area focuses on the dissection of viral replication mechanisms and 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 intend to provide essential scientific background for the development of new biotechnological tools.

Molecular and Cellular Biology

HEAD OF DEPARTMENT
Francisco J. Iborra

RESEARCH GROUPS

1. **Molecular bases of actin cytoskeleton reorganisation in cell motility, tumour generation and invasiveness**
   Inés M. Antón
2. **Coronavirus: replication, virus-host interactions, and protection**
   Luis Enjuanes & Isabel Sola
3. **Poxvirus and vaccines**
   Mariano Esteban
4. **Virus-host interactions in hepatitis B virus infection**
   Urtzi Garaigorta
5. **Hepatitis C virus infection**
   Pablo Gastaminza
6. **Biological noise and its physiopathological implications**
   Francisco J. Iborra & Fernando Almazán
7. **Animal models by genetic manipulation**
   Lluís Montoliu
8. **Functional analysis of the transcriptional repressor DREAM**
   José Ramón Naranjo
9. **Mechanisms of interaction between the influenza virus and the infected cell**
   Amelia Nieto
10. **Cerebral cortical development**
    Marta Nieto
11. **Molecular characterisation and epidemiology of torovirus**
    Dolores Rodríguez
12. **Molecular biology of birnaviruses**
    José F. Rodríguez
13. **Embryonic development and differentiation in vertebrates**
    Juan José Sanz-Ezquerro
14. **Cellular immunobiology and microbiology**
    Esteban Veiga Chacón



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

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PhD STUDENT
Sergio Rivas

SELECTED PUBLICATIONS

Pfajfer L, Seidel MG, Houmadi R, Rey-Barroso J, Hirschmugl T, Salzer E, Antón IM, Urban C, Schwinger W, Boztug K, Dupré L. WIP deficiency severely affects human lymphocyte architecture during migration and synapse assembly. Blood 2017; 130: 1949-1953.

Escoll M, Gargini R, Cuadrado A, Anton IM, Wandosell F. Mutant p53 oncogenic functions in cancer stem cells are regulated by WIP through YAP/TAZ. Oncogene 2017; 36: 3515-3527.

Rivas S, Antón IM, Wandosell F. WIP-YAP/TAZ as a new pro-oncogenic pathway in glioma. Cancers (Basel) 2018; 10: E191.

Rivas S, Gómez-Oro C, Antón IM, Wandosell F. Role of Akt isoforms controlling cancer stem cell survival, phenotype and self-renewal. Biomedicines 2018; 6: E29.

Antón IM, Gómez-Oro C, Rivas S, Wandosell F. Crosstalk between WIP and Rho family GTPases. Small GTPases 2018; 29: 1-7.

Alterations in the PI3K-Akt-GSK3/mTORC pathways are the basis of ageing-related pathologies like neurodegenerative disorders (Alzheimer's disease) or cancer. Our research interest focuses on the role of actin-binding elements in the regulation of Akt-mediated signalling routes that control motility, invasiveness and survival and how they contribute to cell differentiation/degeneration or tumourigenesis. Our model actin-binding proteins are (N)WASP (neural Wiskott-Aldrich Syndrome Protein), WIP (WASP Interacting Protein) and WIRE (WIP Related).

Using animal models, recombinant lentivirus and advanced imaging techniques (two and three dimensional cultures) in combination with biochemical and proteomic approaches, we have described a relevant role for WIP in neuronal differentiation through the regulation of survival pathways (PI3K-Akt and mTORC1). Most tumours are initiated and maintained from a subpopulation of migratory and invasive mesenchymal cancer stem cells (CSC) which are responsible for the acquisition of aggressive tumour phenotypes being more resistant to many therapeutic approaches and responsible for tumour recurrence, hence they are attractive targets for novel treatments.

Our work showed that WIP is preferentially expressed in invasive tumour samples (glioblastoma and breast cancer) and affect tumour phenotype at two levels: 1) **initiation**, as it participates in the establishment and maintenance of CSC preventing their apoptotic caspase-dependent cell death; 2) **progression**, as it promotes CSC capacity to degrade the extracellular matrix contributing to invasiveness and metastasis. WIP expression leads to the sequestration of the destruction complex in multivesicular bodies and thus promotes the stability of the transcriptional co-activators YAP/TAZ. WIP is also an essential part of a p53-mediated oncogenic cascade that maintain tumour growth capacity and the stem phenotype. Our findings demonstrate an oncogenic role for WIP through an Akt-related developmental/oncogenic axis and hopefully they will contribute to find new biomarkers and therapeutic targets to fight neurodegenerative disorders and metastases.

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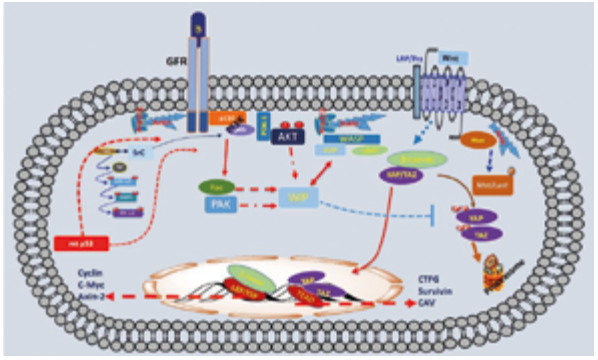

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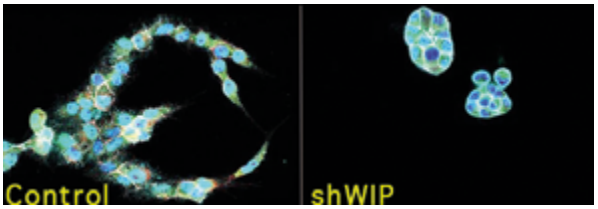

❶ *Schematic of several routes that control glioma progression, including the novel WIP-YAP/TAZ pathway that connects mutant p53 and growth-factor-receptor signals via Akt with oncogenesis and cell invasiveness.*

❷ *WIP expression increases cell invasiveness. Control (left) or shWIP interfered (right) MDA-MB-231 cells were seeded on Matrigel and stained for YAP/TAZ in green, actin in red, integrin in cyan and nuclei in blue.*

Coronavirus: replication, virus-host interactions, and protection

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SELECTED PUBLICATIONS

Morales L, Oliveros JC, Fernandez-Delgado R, tenOever BR, Enjuanes L, Sola I. SARS-CoV-encoded small RNAs contribute to infection-associated lung pathology. Cell Host Microbe 2017; 21: 44-355.

Canton J, ..., Enjuanes L, Sola I. MERS-CoV 4b protein interferes with the NF-kappaB-dependent innate immune response during infection. PLoS Pathog 2018; 14: e1006838.

Castaño-Rodriguez C, Honrubia JM, Gutierrez-Álvarez J, ..., Sola I, Enjuanes, L. Role of severe acute respiratory syndrome coronavirus viroporins E, 3a and 8a in replication and pathogenesis. mBio 2018; 9: e02325-17

Stalin Raj V, Okba NMA, Gutierrez-Álvarez J, Fernandez-Delgado R, Sola I,..., Enjuanes L, Haagmans BL. Chimeric camel/human heavy-chain antibodies protect against MERS-CoV infection. Sci Adv 2018; 4: eaas9667.

Letko M, Miazgowicz K, McMinn R, Seifert SN, Sola I, Enjuanes L, Carmody A, van Doremalen N, Munster V. Adaptive evolution of MERS-CoV to species variation in DPP4. Cell Rep 2018; 24: 1730-1737.

Human infections causing pneumonia and acute respiratory distress syndrome (ARDS) are a growing health problem. In 2015, respiratory diseases were the third most common cause of death in the EU. The problem is even greater in the elderly population, which responds with significant lower efficacy to vaccination.

Viruses are responsible for most respiratory infections. Among them, human coronaviruses (CoV) are the cause of up to 15% of all respiratory problems. Six human CoVs have been described, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS-CoV, the last two leading to deadly infections. Our laboratory focuses on the design of vaccines and selection of antivirals to protect against human respiratory CoV infections by modulating the innate immune response in young and elderly populations.

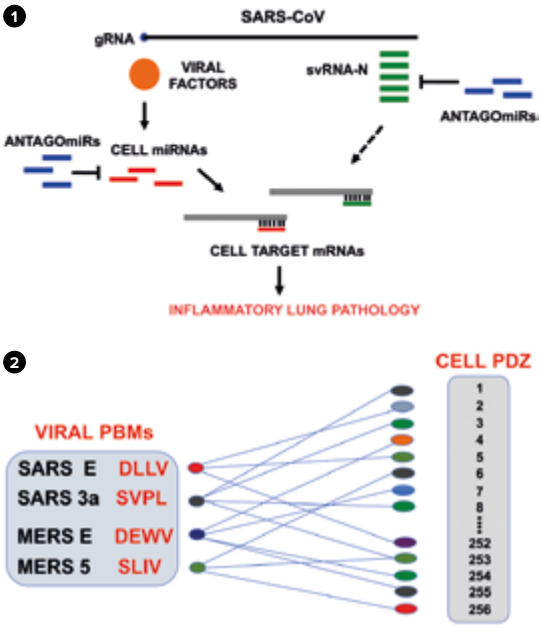
The main aims of our research are to:

• **Identify CoV genes responsible for virus virulence**, to delete or modify these genes using reverse genetics in order to develop new generation vaccines such as replication-competent propagation-deficient RNA replicons, which are safe and promising vaccine candidates, and to determine their effectiveness in animal model systems. The expression of micro-RNAs with immunomodulating capacities will provide enhanced efficiency in older adults.

• **Identify cell-signalling pathways involved in CoV replication and pathology**, and to select antiviral drugs that inhibit these pathways interfering with virus replication or pathology. In particular, we study PBM-PDZ protein-protein interactions involved in the innate immune and inflammatory responses, since overstimulation of these pathways seems responsible for an increase in fatalities during SARS-CoV and MERS-CoV epidemics.

• **Determine the contribution of host miRNAs and virus-derived small RNAs to the inflammatory lung pathology** induced by CoV infection. These small non-coding RNAs represent targets for antivirals.

• **Study the effect of specific IFN-stimulated genes on the replication and innate immune responses of respiratory viruses**, such as influenza and CoVs, which induce diseases associated with excessive immune signalling.



1 *Interactions of viral protein-PBMs with cellular PDZ-containing proteins. Highly virulent human coronaviruses, such as SARS-CoV and MERS-CoV, include at least two proteins each containing PDZ binding motifs (PBMs) (left) that may interact with 256 unique PDZ motifs present in more than 400 cellular protein isoforms (right).*

2 *Contribution of host and viral small non-coding RNAs to SARS-CoV lung pathology. Infection by a virulent SARS-CoV induces the differential expression of host microRNAs (miRNAs), which are mainly associated with the regulation of cell target mRNAs related to cytokine-mediated inflammatory pathways, suggesting that the viral-induced inflammatory pathology is in part coordinated by miRNAs.*

Poxvirus and vaccines

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Beatriz Isla
Sara Moreno

The research interest of the Poxvirus and Vaccines laboratory focuses on the control of human pathogens by developing safe and effective vaccines. This is accomplished by studying the biology of poxviruses and their application as vaccine candidates, using cultured cells as well as various animal models (mice and monkeys) together with human clinical trials. By studying the behaviour of replication competent and incompetent poxvirus vectors MVA and NYVAC, our group has made important contributions to the immune biology of vaccinia virus, the mechanisms of T cell and B cell humoral immune responses, and engineering vaccine candidates against diseases like HIV/Aids, hepatitis C, chikungunya, ebola, zika, malaria and leishmaniasis, some of which have advanced to phase I/II clinical trials (HIV).

In the 2017-2018 period, in collaboration with other groups, we have made the following achievements:

• We identified immune mechanisms and established T and B cell immune responses relevant to protection against HIV-1 in preclinical studies (mice and monkeys).

• We defined, in a phase I clinical trial, the immunogenicity of a prophylactic clade C vaccine (MVA-C) against HIV-1 developed by our group, as well as the long-term immune responses to the other clade B vaccine candidate (MVA-B).

• We generated novel vaccine candidates and established protocols of immunisation that are safe and efficiently protect animals against pathogens: chikungunya, ebola, zika, malaria and leishmaniasis.

Our current research aims to optimise the immunogenicity of the poxvirus vectors MVA and NYVAC as recombinant vaccine candidates, alone and in combination with other immunogens (DNA, mRNA, alphavirus replicon, protein), to identify immune mechanisms in preclinical and clinical trials, and to establish correlates of protection. Our goal is to develop the best-in-class immunogens and vaccination protocols to be applied against prevalent human diseases.

Our research is supported by national and international grants, resulted in the publication of over 350 papers in international journals, 11 patents and the direction of 32 PhD theses. We maintain a fruitful collaboration with HIV vaccine experts in the USA and Europe through CAVD projects financed by the Bill and Melinda Gates Foundation and EU H2020 program, as well as with other EU colleagues developing HIV, ebola, chikungunya and zika vaccines.

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Di Pilato M, Mejías-Pérez E, Sorzano COS, Esteban M. distinct roles of vaccinia virus NF-κB inhibitor proteins A52, B15, and K7 in the immune response. J Virol. 2017; 91: e00575-17.

Enamorado M, Iborra S, *et al.* Enhanced anti-tumour immunity requires the interplay between resident and circulating memory CD8+ T cells. Nat Commun 2017; 8: 16073.

Lázaro-Frías A, Gómez-Medina S, *et al.* Distinct immunogenicity and efficacy of poxvirus-based vaccine candidates against ebola virus expressing GP and VP40 proteins. J Virol 2018; 92: e00363-18.

Pérez P, Q Marín M, *et al.* A vaccine based on a modified vaccinia virus ankara vector expressing zika virus structural proteins controls zika virus replication in mice. Sci Rep 2018; 8: 17385.

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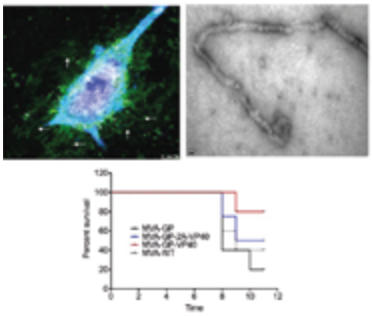

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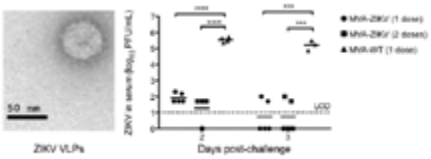
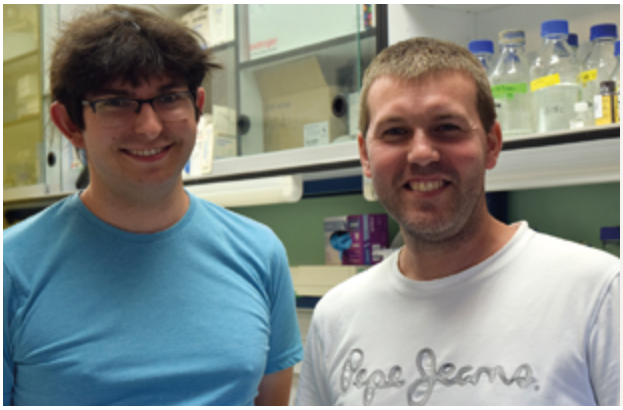


❶ *Ebola vaccine MVA-EBOV, showing by confocal microscopy the cell destruction, by electron microscopy the VLPs and in mice the efficacy of a single dose against ebola virus (line in red).*

❷ *Zika vaccine MVA-ZIKA, showing the VLP formation and efficacy in mice.*



Virus-host interactions in hepatitis B virus infection

GROUP LEADER
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PhD STUDENT
Andoni Gómez Moreno

MASTER'S STUDENT
Pilar Gomollón Zueco

SELECTED PUBLICATIONS

Gómez-Moreno A, Garaigorta U. Hepatitis B virus and DNA damage response: Interactions and consequences for the infection. Viruses 2017; 9: E304.

Kruse RL, Shum T, Tashiro H, Barzi M, Yi Z, Whitten-Bauer C, Legras X, Bissig-Choisat B, Garaigorta U, Gottschalk S, Bissig KD. HBsAg-redirected T cells exhibit antiviral activity in HBV-infected human liver chimeric mice. Cytotherapy 2018; 20: 697-705.

Hepatitis B virus (HBV) represents an important human pathogen causing acute and chronic hepatitis. Over 250 million people are chronically infected, and more than 780,000 people die every year due to complications of HBV, including liver cirrhosis and hepatocellular carcinoma. Currently, approved therapies suppress very effectively virus replication and viremia, but they are not curative because they do not eliminate the nuclear viral episomal DNA (cccDNA), a hallmark of HBV persistence. Therefore, there is an imminent need to develop novel cccDNA-targeting therapies that eradicate HBV from chronically infected patients.

We believe that, by understanding basic aspects of cccDNA biology, we will unravel new aspects of viral pathogenesis and identify vulnerabilities in the virus life cycle that could be exploited for the development of novel and safe curative antiviral strategies.

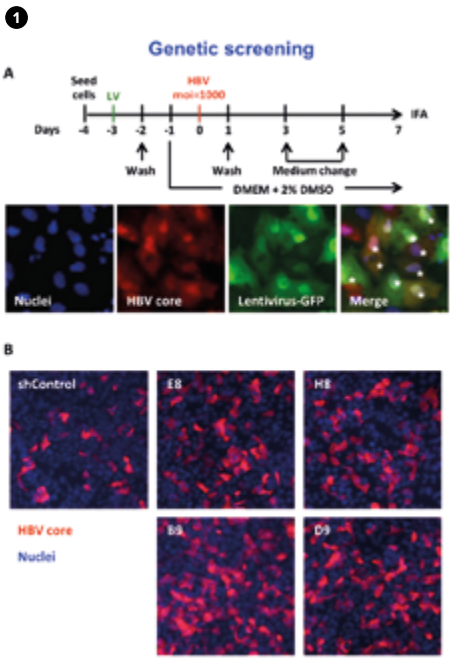
Thus, our group is interested in deciphering the cellular factors, pathways and mechanisms that regulate cccDNA levels in infected cells. To this end, we make use of HBV infection cell culture models and apply genetic, molecular, biochemical and virological approaches to understand cccDNA formation and homeostasis.

The specific aims of our research are to:

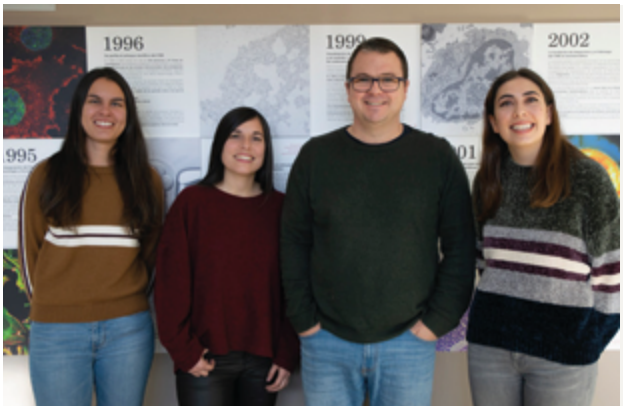
Identify and characterise cellular proteins and pathways that regulate cccDNA formation. We have performed loss-of-function genetic and pharmacological screenings that identified three DNA damage response proteins, namely Aquarius, Senataxin and DNA-PK, as factors restricting the accumulation of HBV cccDNA during infection. Experiments to understand the mechanism by which these cellular factors regulate the HBV life cycle are underway.

• Develop an in-house cell-based ELISA for antiviral small molecules screening.

• Visualise cccDNA formation in infected cells. We have recently started a project aimed to chemically label cccDNA while it is being formed in the infected cells. This technique will allow us to perform cccDNA localisation studies that are difficult with current technologies.



1 *A) Genetic screening set up performed to identify cellular proteins that regulate HBV infection. B) Increased HBV infection in Aquarius (AQR:E8 & H8) and Senataxin (SETX: B9 and D9) deficient cells.*



Hepatitis C virus infection

GROUP LEADER
Pablo Gastaminza Landart

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Ginés Ávila Pérez

TECHNICIAN
Gema Calvo Gutiérrez

PhD STUDENT
Victoria Castro Illana

UNDERGRADUATE STUDENTS
Alba Escalera
Nicolás Aguillón

SELECTED PUBLICATIONS

Coto-Llerena M, Koutsoudakis G, Boix L, López-Oliva JM, Caro-Pérez N, Fernández-Carrillo C, González P, Gastaminza P, Bruix J, Forns X, Pérez-Del-Pulgar S. Permissiveness of human hepatocellular carcinoma cell lines for hepatitis C virus entry and replication. Virus Res 2017; 240: 35-46.

Moreno E, Gallego I, Gregori J, Lucía-Sanz A, Soria ME, Castro V, Beach NM, Manrubia S, Quer J, Esteban JI, Rice CM, Gómez J, Gastaminza P, Domingo E, Perales C. internal disequilibria and phenotypic diversification during replication of Hepatitis C Virus in a noncoevolving cellular environment. J Virol 2017; 91: e02505-16.

Sepúlveda-Crespo D, Jiménez JL, Gómez R, De La Mata FJ, Majano PL, Muñoz-Fernández MÁ, Gastaminza P. Polyanionic carbosilane dendrimers prevent hepatitis C virus infection in cell culture. Nanomedicine 2017; 13: 49-58.

Perez-Berna AJ, Valcarcel R, Rodríguez MJ, Chichón FJ, Sorrentino A, Carrascosa JL, Gastaminza P, Pereiro E. The dual-axes for soft X-ray cryo-tomography reveals ultrastructural alterations of the host cell during hepatitis C infection by increasing the isotropic axial resolution. Microsc Microanal 2017; 23(S1), 976-77.

Mingorance L, Castro V, Ávila-Pérez G, Calvo G, Rodriguez MJ, Carrascosa JL, Pérez-Del-Pulgar S, Forns X, Gastaminza P. Host phosphatidic acid phosphatase lipin1 is rate limiting for functional hepatitis C virus replicase complex formation. PLoS Pathog 2018; 14: e1007284.

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. We consider that determining the cellular and molecular mechanisms by which the virus replicates provides new opportunities in the fight against clinically relevant human pathogens. To achieve these general aims, we use cell culture models of infection, in which pharmacological and genetic manipulation enables the study of fundamental aspects of virus-host interactions.

During the 2017-2018 period, we have been focused on the role of lipins, a family of lipid-modifying enzymes, in hepatitis C virus infection. Lipins control glycerophospholipid homeostasis, including triglyceride and phospholipid biosynthesis. They also control the levels of very important lipid second messengers such as phosphatidic acid and diacylglycerol (DAG). We have recently demonstrated that the best characterised member of the lipin family, lipin1, is rate-limiting for the production of HCV replicase complexes, probably because it provides an optimal lipid environment for recruitment of host and viral factors that assemble the viral replicase. We have verified that lipin1 dependency is exquisitely specific for hepatitis C virus infection; infection by flaviviruses dengue and Zika or by respiratory viruses such as coronavirus hCoV-229E or influenza virus is not limited in lipin1-deficient cells. Thus, we have defined a new HCV-specific molecular target for HCV-specific antiviral intervention. Future studies will involve the study of other members of the family, including lipin2. Lipin2-deficient cells display a completely different phenotype, as they are refractory to infection by all the aforementioned viruses. Overall, our results provide not only new antiviral targets for therapeutic intervention but also molecular insights into the different functions of host genes involved in lipid homeostasis.

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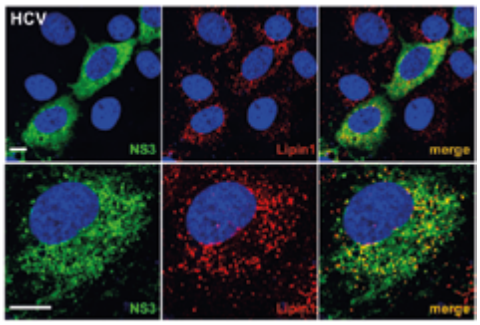

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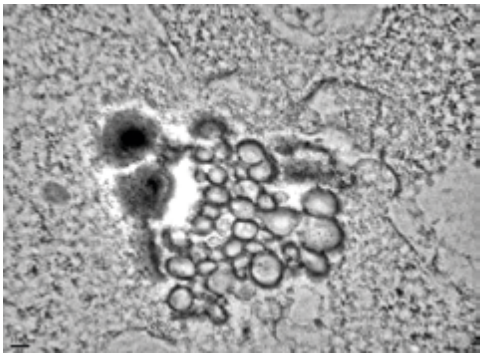
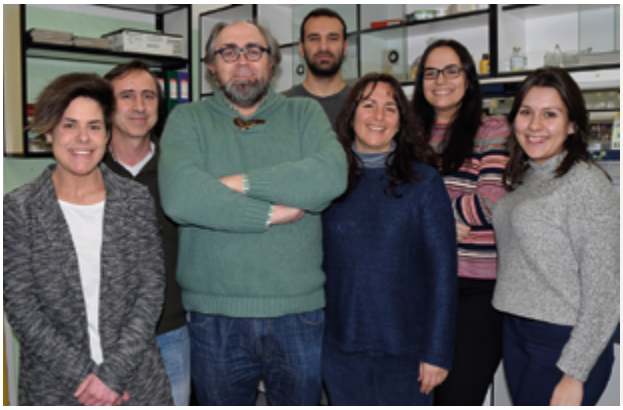


1 *Immunofluorescence microscopy images showing lipin1 (red) colocalisation with viral protein NS3 (green) in HCV-infected human hepatoma cells (Huh-7).*

2 *Transmission electron microscopy of human hepatoma (Huh-7) cells expressing HCV replicase components and forming characteristic double and multiple membrane vesicles (DMV and MMV). The number of HCV-induced membranous structures are significantly reduced in lipin1-deficient cells (PLoS Pathog 2018; 14: e1007284).*



Biological noise and its physiopathological implications

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VISITING SCIENTIST
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(Hospital Clínico San Carlos, Madrid, Spain)

The non-genic heterogeneity of cell populations (phenotypic variability), which is due mainly to differential gene expression, plays an important 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 physiological processes. The knowledge of this variability will improve our understanding of phenomena like tumour resistance to drugs, virus infection, or cell fate choice.

During the years 2017-2018, we have made important achievements in two main areas:

1. Origin of phenotypic variability.

We have found that mitochondrial content is a main factor of phenotypic variability in a clonal cell population. The mitochondrial content of each cell contributes to heterogeneity in gene products and has a large impact on alternative splicing, thus modulating both the abundance and type of mRNAs, which ultimately leads to phenotypic diversity.

2. Physiopathological implications of the variability of mitochondrial content in biological processes.

2.1. Effect of mitochondria variability on apoptosis in response to chemotherapy. We have found that the cellular mitochondrial content determines the apoptotic fate and modulates the time to death in response to TRAIL treatment. This finding could have a great impact on the understanding of tumour relapse and partial response to chemotherapy.

2.2. Effect of mitochondrial variability on viral infections. Using vaccinia virus and transmissible gastroenteritis coronavirus as experimental models, we have determined that there is an inverse correlation between the mitochondrial content and viral replication. Due the importance of Zika virus (ZIKV) in human health, we have extended these studies to ZIKV and we have initiated a new investigation line focused on the study of the molecular bases of ZIKV pathogenesis. In that sense, we have developed a ZIKV reverse genetic system that has allowed us to generate an attenuated recombinant virus providing fully protection against ZIKV in a mouse model. These studies will improve our understanding of ZIKV biology and facilitate the development of vaccine and antiviral strategies.

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Márquez-Jurado S, Díaz-Colunga J, das Neves RP, Martinez-Lorente A, Almazán F, Guantes R, Iborra FJ. Mitochondrial levels determine variability in cell death by modulating apoptotic gene expression. Nat Commun 2018; 9: 389.

Lima AF, May G, Díaz-Colunga J, Pedreiro S, Paiva A, Ferreira L, Enver T, Iborra FJ, das Neves RP. Osmotic modulation of chromatin impacts on efficiency and kinetics of cell fate modulation. Sci Rep 2018; 8: 7210.

Diot A, Agnew T, Sanderson J, Liao C, Carver J, das Neves, RP, Gupta R, Guo Y, Waters C, Seto S, Daniels MJ, Dombi E, Lodge T, Morten K, Williams SA, Enver T, Iborra FJ, Votruba M. Poulton J. Validating the RedMIT/GFP-LC3 mouse model by studying mitophagy in autosomal dominant optic atrophy due to the OPA1Q285STOP mutation. Front Cell Dev Biol 2018; 6: 103.

Márquez-Jurado S, Nogales A, Ávila-Pérez G, Iborra F., Martínez-Sobrido L, Almazán, F. An alanine-to-valine substitution in the residue 175 of Zika virus NS2A protein affects viral RNA synthesis and attenuates the virus *in vivo*. Viruses 2018; 10: E547.

Ávila-Pérez G, Nogales A, Martín V, Almazán F, Martínez-Sobrido L. Reverse genetic approaches for the generation of recombinant Zika virus. Viruses 2018; 10: E597.

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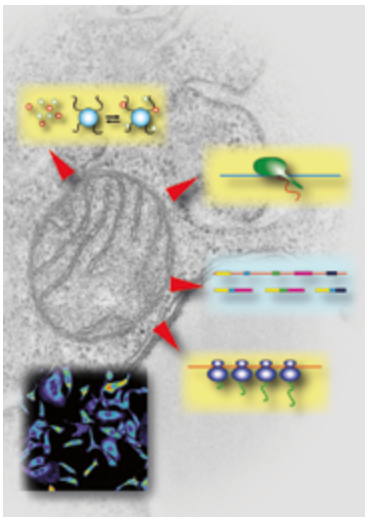

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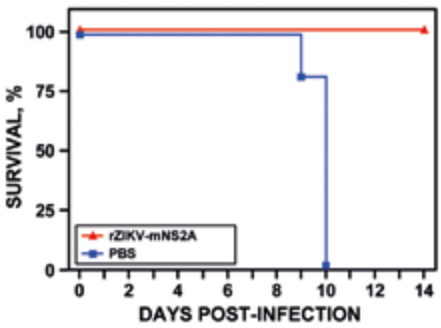

❶ *Mitochondria and gene expression. Electron micrograph of one mitochondrion with radiating arrows to the steps of gene expression where mitochondria play an important role.*

❷ *Protection efficacy of mutant virus rZIKV-mNS2A. Female 4-to-6-week old A129 mice (IFNR-/-) were mock-vaccinated (PBS) or vaccinated with rZIKV-mNS2A. At 21 days after vaccination, mice were challenged with a lethal dose of ZIKV and survival were monitored daily for 14 days.*

Animal models by genetic manipulation

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Juan Manuel Rosa

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 20 genes, characterised by visual impairment and, often, pigmentation alterations. These research projects on human rare diseases are performed 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 and other retinopathies such as achromatopsia. Furthermore, using mouse models, we have explored the use of small molecules as potential therapeutic candidates for albinism. In collaboration with Angel Carracedo (USC) and Carmen Ayuso (FJD), we have devised, within the CIBERER-ISCIII, a proposal for the universal genetic diagnose 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. Within CIBERER, we are actively contributing to the genetic diagnosis of many people with albinism in Spain.

We are also interested in understanding the function of regulatory elements that are required to define gene expression domains in mammalian genomes and contribute to specify its expression pattern in space and time. The mouse tyrosinase locus, used as experimental model, has allowed us to identify several genome boundaries or insulators, which protect the locus from surrounding genes. We use transgenic animals, zebrafish and mice to introduce different type of gene constructs in order to investigate the relevance of specific sequences. The functional analysis of regulatory elements found within the intergenic sequences can now be addressed more efficiently thanks to the new genome editing system, CRISPR-Cas9. In Spain, we pioneered the application of this technology in mice, successfully implemented it in our laboratory and disseminated its use among colleagues by hosting short stays and organising *ad-hoc* workshops, seminars and courses.

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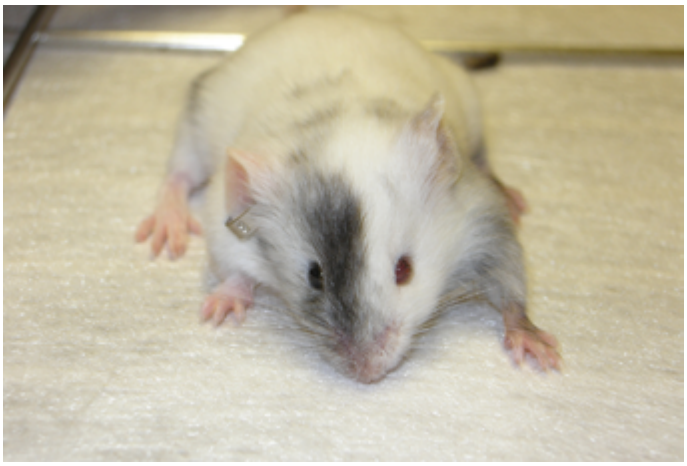

1 *Genome-edited mouse generated with CRISPR tools displaying the classical mosaicism characteristic of these animal models.*

SELECTED PUBLICATIONS

Montoliu L, Marks MS. A new type of syndromic albinism associated with mutations in AP3D1. Pigment Cell Melanoma Res 2017; 30: 5-7.

Mato-Berciano A,... Montoliu L, Fillat C. A NOTCH-sensitive uPAR-regulated oncolytic adenovirus effectively suppresses pancreatic tumor growth and triggers synergistic anticancer effects with gemcitabine and nab-paclitaxel. Oncotarget 2017; 8: 22700-22715.

Raspa M, Guan M, Paoletti R, Montoliu L, *et al*. Dry ice is a reliable substrate for the distribution of frozen mouse spermatozoa: A multi-centric study. Theriogenology 2017; 96: 49-57.

Fernández A, Josa S, Montoliu L. A history of genome editing in mammals. Mamm Genome 2017; 28: 237-246.

Rubio-Fernández M ..., Montoliu L, de la Villa P, Martín-Nieto J, Cruces J. Impairment of photoreceptor ribbon synapses in a novel Pomt1 conditional knockout mouse model of dystroglycanopathy. Sci Rep 2018; 8:8543.

Functional analysis of the transcriptional repressor DREAM

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SELECTED PUBLICATIONS

Benedet T, Gonzalez P, Oliveros JC, Dopazo JM, Ghimire K, Palczewska M, Mellstrom B, Naranjo JR. Transcriptional repressor DREAM regulates trigeminal noxious perception. J Neurochem 2017; 141: 544-552.

López-Hurtado A, Burgos DF, González P, Dopazo XM, González V, Rábano A, Mellström B, Naranjo JR. Inhibition of DREAM-ATF6 interaction delays the onset of cognition deficits in a mouse model of Huntington's disease. Mol Brain 2018; 11: 13.

Cantero-Recasens G, Butnaru CM, Valverde MA, Naranjo JR, Brouwers N, Malhotra V. KChIP3 coupled to Ca^{2+} oscillations exerts a tonic brake on baseline mucin release in the colon. eLife 2018; 7: e39729.

Naranjo R, González P, López-Hurtado A, Dopazo JM, MellströmB, Naranjo JR. Inhibition of the Neuronal Calcium Sensor DREAM modulates Presenilin-2 Endoproteolysis. Front Mol Neurosci 2018; 11: 449.

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.

Our major research focus is on the multifunctional protein DREAM (downstream regulatory element antagonist modulator) and its role in the control of calcium homeostasis in health and disease.

DREAM, also known as calsenilin or KChIP3, is a Ca^{2+} 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 Ca^{2+}-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 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, inflammation, thyroid gland and placenta. Moreover, recent studies have shown the involvement of DREAM in several neurodegenerative disorders including Huntington´s disease (HD) and Alzheimer´s disease (AD).

DREAM was originally associated with AD because of its interaction with presenilins. However, altered neuronal calcium 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 arachidonic acid regulate the interaction with DNA or with potassium channels, respectively. 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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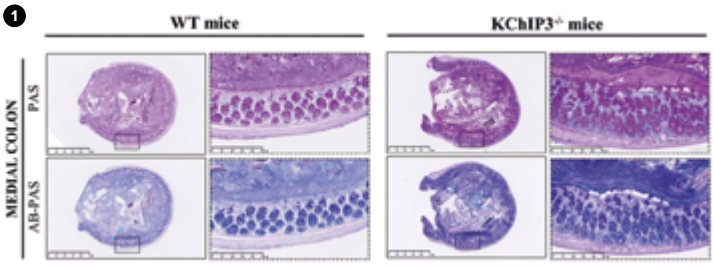

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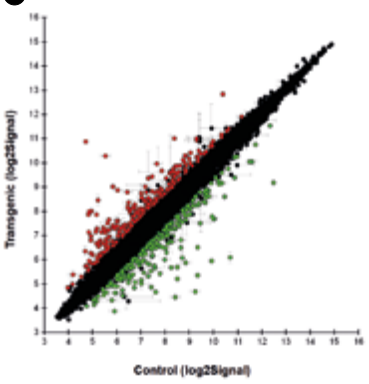

1 *DREAM/Kcnip3-/- mice show increased mucus layer at the medial colon (Elife 2018; 7: e39729).*

2 *Scatter Plot. Transcriptomic analysis of trigeminal ganglia in daDREAM transgenic mice*

Mechanisms of interaction between the influenza virus and the infected cell

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SELECTED PUBLICATIONS

Martínez I, Oliveros JC, Cuesta I, de la Barrera J, Ausina V, Casals C, de Lorenzo A, García E, García-Fojeda B, Garmendia J, González-Nicolau M, Lacoma A, Menéndez M, Moranta D, Nieto A, Ortín J, Pérez-González A, Prat C, Ramos-Sevillano E, Regueiro V, Rodriguez-Frandsen A, Solís D, Yuste J, Bengoechea JA, Melero JA. Apoptosis, Toll-like, RIG-I-like and NOD-like receptors are pathways jointly induced by diverse respiratory bacterial and viral pathogens. Front Microbiol 2017; 8: 276.

Nieto A, Pozo F, Vidal-García M, Omeñaca M, Casas I, Falcón A. Identification of rare PB2-D701N mutation from a patient with severe influenza: contribution of the PB2-D701N mutation to the pathogenicity of human Influenza. Front Microbiol 2017; 8: 575.

Vasilijevic J, Zamarreño N, Oliveros JC, Rodriguez-Frandsen A, Gómez G, Rodriguez G, Pérez-Ruiz M, Rey S, Barba I, Pozo F, Casas I, Nieto A, Falcón A. Reduced accumulation of defective viral genomes contributes to severe outcome in influenza virus infected patients. PLoS Pathog 2017; 13: e1006650.

Marcos-Villar L, Díaz-Colunga J, Sandoval J, Zamarreño N, Landeras-Bueno S, Esteller M, Falcón A, Nieto A. Epigenetic control of influenza virus: role of H3K79 methylation in interferon-induced antiviral response. Sci Rep 2018; 8: 1230.

Influenza A virus (IAV) polymerase plays a crucial role controlling the expression of the viral and the host genome as well as viral pathogenicity. IAV uses a variety of mechanisms to control the antiviral response, such as the induction of epigenetic changes in specific histone residues that control the interferon signalling pathway, possibly through alterations in cellular sensors of viral RNAs, including methylation of lysine 79 of histone 3 together with a general decrease of histone acetylation.

Defective viral genome RNAs (DVGs) have been detected in virus particles. They have the 3' and 5' ends of the parental RNA segments, and most have a single large central deletion that generates viral RNAs of 180-1,000 nucleotides. DVGs potentiate the host response possibly through recognition of double-stranded RNA by receptors that activate antiviral signalling cascades. Specific point mutations at the viral polymerase control DVGs production, and decreased levels of DVGs correlate with increased pathogenesis in mice; conversely increased levels diminish *in vivo* pathogenesis.

Performing genomic analysis of viruses isolated from a cohort of previously healthy IAV infected patients with highly severe/fatal outcome, we showed that these viruses accumulated fewer DVGs than viruses isolated from a cohort of mildly infected patients. suggesting that low DVGs abundance constitutes a new virulence pathogenic marker in humans and that reduced accumulation of DVGs constitutes a virulent factor itself. Further characterisation of a recombinant virus with a point mutation in the PA subunit that produces low amount of DVGs, showed that the virus replicates efficiently in the heart, causes cardiac disorders and induces sudden death in infected animals. Influenza virus uses a plethora of mechanisms to increase its pathogenicity; viral proteins and viral RNAs work coordinately to accomplish an efficient infection mediated by this virus that possesses low genomic information.

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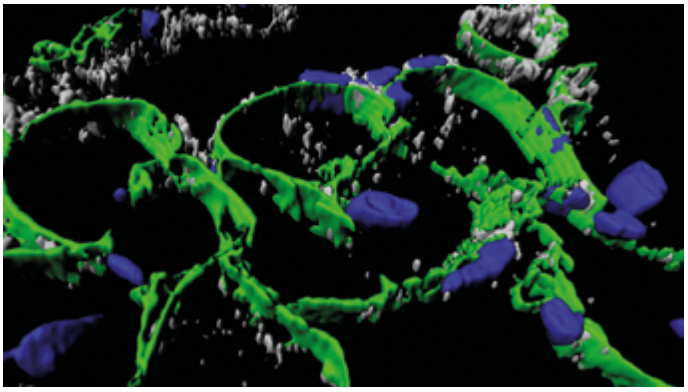

1 *Presence of influenza virus protein in cardiomycytes of infected mice. White: viral nucleoprotein; blue: nuclei; green: lamina.*

Cerebral cortical development

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SELECTED PUBLICATIONS

Nieto Guil AF, Oksdath M, Weiss LA, Grassi DJ, Sosa LJ, Nieto M, Quiroga S. IGF-1 receptor regulates dynamic changes in neuronal polarity during cerebral cortical migration. Sci Rep 2017; 7: 7703.

Briz CG, Navarrete M, Esteban JA, Nieto M. In utero electroporation approaches to study the excitability of neuronal subpopulations and single-cell connectivity. J Vis Exp 2017; 120: e55139.

Wang CF, Hsing HW, Zhuang ZH, Wen MH, Chang WJ, Briz CG, Nieto M, Shyu BC, Chou SJ. Lhx2 expression in postmitotic cortical neurons initiates assembly of the thalamocortical somatosensory circuit. Cell Rep 2017; 18: 849-856.

Weiss LA and Nieto M. The crux of Cux genes in neuronal function and plasticity. Brain Res 2019; 1705: 32-42.

Our brain contains an extraordinary number and diversity of neurons, which together form one of the most complex functional networks found in biological systems. These circuits wire during development in manners that guarantees the brain's optimal responses to the external world and its capacity for intellectual processing and common social behaviours.

Using the mouse as a model, we aim to understand how neurons encode for the molecular information necessary to reproduce and build precisely the stereotyped circuits of the cortex; and how neurons translate this information into selective connectivity while dialoging with their environment. We focused on the extreme plasticity of this process for its potential therapeutic implications. While cortical neurons seem predetermined to follow strict rules of wiring, they also show a remarkable rewiring capacity that ensures optimal functional connectivity in abnormal scenarios, such as the loss of sensory organs, developmental defects or ischemic injury. Understanding and manipulating these intrinsic properties of developing neuronal networks (highly reproducible selective connectivity and plasticity) is key to understand normal brain functions and to manage and treat neurodevelopmental disorders, such as autism spectrum disorders (ASD), intellectual disabilities, bipolar, schizophrenia or epilepsy; it has also implications for the treatment of neurodegeneration and brain injury.

Our investigation is based on *in vivo* manipulation of wild type circuits by modifying gene expression, sensory input and circuit activity. For this purpose, we use transgenic approaches, including CRISPR/Cas-mediated knock-in, *in utero* electroporation, electrophysiology, stereotaxic retrotracing injections, pharmacological interventions and RNA-sequencing.

The main projects are:

i. Investigating the action and downstream programs of Cux1 and Cux2 transcription factors in the specification of the cortical upper layer neurons.

ii. Understanding the development of interhemispheric connections of the *corpus callosum*, its plasticity and its involvement in neurodevelopmental disorders.

iii. The role of sensory circuits in the coordinated wiring of cortical networks; pharmacological interventions.

iv. The action of interneurons.

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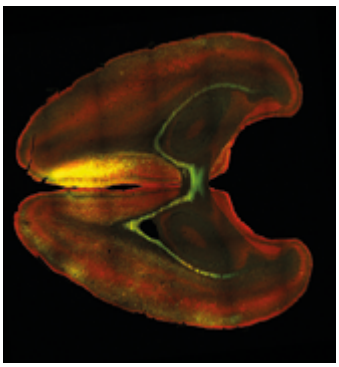

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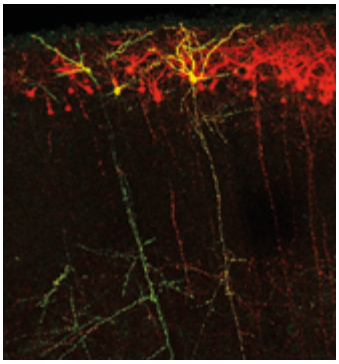

1 *Tracing of corpus callosum connections in the mouse using in utero electroporation. Confocal image of a section of a mouse brain electroporated at embryonic day 15. Yellow and green label the electroporated neurons and their axonal ramifications through the corpus callosum.*

2 *A Cre recombinase-diluted strategy enables sparse labeling of cortical neurons. Images obtained after vectors were delivered to layer II-III neurons by in utero electroporation at embryonic day 15.5 and coronal sections were made at postnatal day 16. Low amounts of CAG-CRE were electroporated. The CAG-DsRed vector was co-transfected as control (red). GFP (green) is expressed only in those neurons that also incorporated Cre, allowing the recombination of the LoxP sites in the CALNL-GFP vector. The sparse labelling allows distinguishing individual neurons and to trace their branching.*

Molecular characterisation and epidemiology of torovirus

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SELECTED PUBLICATIONS

Méndez F, Romero N, Cubas LL, Delgui L, Rodríguez D, Rodríguez JF. Non-lytic egression of infectious bursal disease virus (IBDV) particles from infected cells. PLoS One 2017; 12: e0170080.

Cubas-Gaona LL, Diaz-Beneitez E, Ciscar M, Rodríguez JF, Rodríguez D. Exacerbated apoptosis of cells infected with infectious bursal disease virus (IBDV) upon exposure to Interferon alpha (IFN-α). J Virol 2018; 92: e00364-18.

Toroviruses are positive-sense single-stranded RNA viruses belonging to the *Nidovirales* order that cause gastrointestinal disease in different domestic animal species and in humans. Toroviruses are distributed worldwide, and high prevalence has been reported in porcine and bovine livestock in different countries, including ours. Our research during this period has been focussed on the virus-host interaction to uncover the signalling pathways induced by torovirus infection that may contribute to either benefit or control virus infection. In this regard, we have shown that BEV, the prototype member of the *Torovirus* genus, activates the autophagy machinery as a cellular defence mechanism.

On the other hand, we have recently started a collaboration with the group of José F. Rodríguez at the CNB aimed at deciphering the molecular bases of infectious bursal disease virus (IBDV) pathogenesis. IBDV infection is responsible for the immunosuppression and/or death of infected birds, causing heavy losses to the poultry industry worldwide. We are focussed on the study of the effect of IFN-α/β and IFN-γ secreted by infected macrophages on IBDV-mediated cell death, a phenomenon that appears to be critical in the depletion of B-cell populations and the destruction of the *bursa of Fabricius* (BF), the major lymphoid organ in chickens, and also the main IBDV target organ. An intriguing hypothesis that arises from our results, to explain the destruction of the BF in IBDV infected chicken, is that IFN secreted by infected macrophages and lymphocytes may contribute to exacerbate cell death by apoptosis, leading to a chicken state of immunosuppression. Therefore, our objective is to characterise in depth the process leading to apoptosis of infected cells upon IFN treatment, both in cultured cells but also in bursal cells from IBDV-infected animals.

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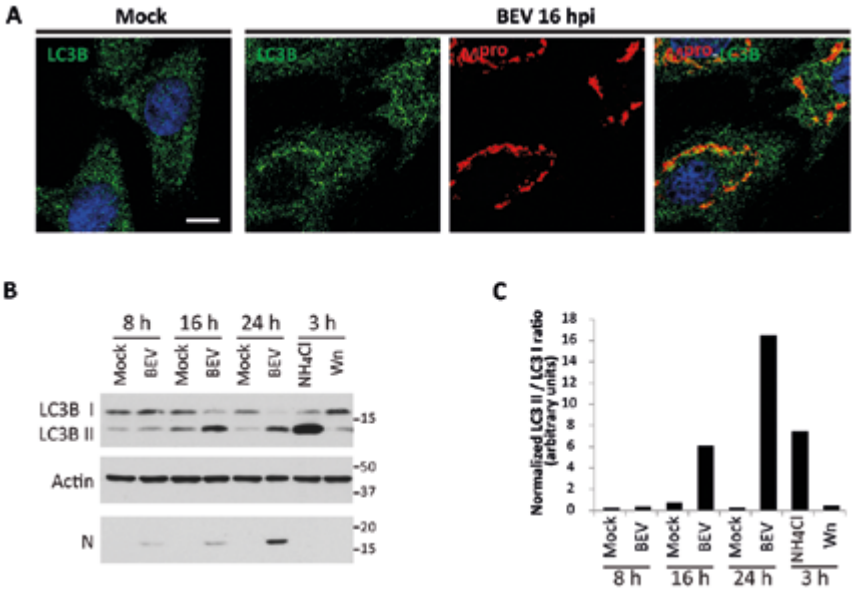

1 *BEV infection activates the autophagy pathway.*
(A) Inmunofluorescence analysis of LC3 (green) and BEV Mpro (red) proteins in BEV-infected E. Derm cells. The nuclei were stained with DAPI. Scale bar, 10 µm.
(B) Western blot analysis of the conversion of LC3B I to LC3B II in extracts of E. Derm cells, mock-infected or infected with BEV, collected at 8, 16 and 24 hpi. Extracts of E. Derm cells treated with ammonium chloride (20 mM, NH4CL) or wortmannin (5 µm, Wn) during 3 h were included as control. Anti-BEV-N and anti-actin antibodies were used as infection and load control respectively.
(C) Quantification of the ratio LC3B II / LC3B I by densitometry.

Molecular biology of birnaviruses

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SELECTED PUBLICATIONS

Méndez F, Romero N, Cubas LL, Delgui L, Rodríguez D, Rodríguez JF. Non-lytic egression of infectious bursal disease virus (IBDV) particles from infected cells. PLoS One 2017; 12: e0170080.

Cubas-Gaona LL, Diaz-Beneitez E, Ciscar M, Rodríguez JF, Rodríguez D. Exacerbated apoptosis of cells infected with infectious bursal disease virus (IBDV) upon exposure to Interferon alpha (IFN-α). J Virol 2018; 92: e00364-18.

Birnaviruses are unconventional double-stranded (dsRNA) RNA viruses displaying unique structural features and molecular strategies to ensure genome expression/replication whilst controlling the onset of cellular innate antiviral responses. Our main birnavirus model is the infectious bursal disease virus (IBDV), the etiological agent of a devastating immunosuppressive avian disease, causing major losses to the poultry industry world-wide. Our work is currently focussed on two major topics, namely i) the mechanism underlying the non-lytic release of infectious virions, and ii) the molecular basis of IBDV-induced pathogenesis and virulence. We are currently closely collaborating with the group led by Dr. Dolores Rodríguez at the CNB to unravel the molecular mechanisms used by IBDV to counteract the antiviral cellular immune responses.

In addition to our IBDV work, we maintain collaborative links with academic and industrial research groups for vaccine development.

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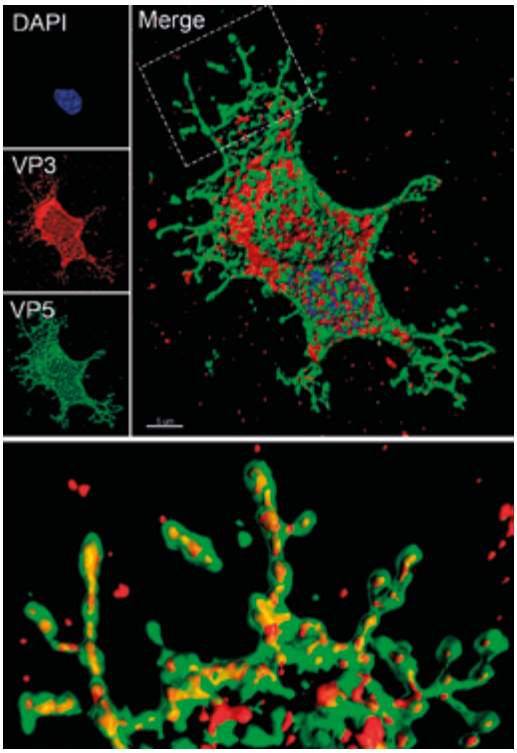

1 *Non-lytic IBDV release. Upper left panels correspond to immunofluorescence images from an IBDV-infected QM7 cell stained with antibodies against the VP5 (green) and VP3 (red) IBDV polypeptides. Cell nuclei were stained with DAPI (blue). The right panel shows a 3D volume rendering of the image shown in left panels. The lower panel shows an isosurface 3D volume rendering detail (2.5x) from the upper right image. Images were acquired with a confocal multispectral Leica TCS SP8 system and processed using the Imaris software at the CNB Advanced Light Microscopy Core facility.*

Embryonic development and differentiation in vertebrates

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SELECTED PUBLICATIONS

Cuenda A. and Sanz-Ezquerro JJ. p38γ and p38δ: from spectators to key physiological players. Trends Biochem Sci 2017; 42: 431-442.

Sanz-Ezquerro JJ, Musterberg AE, Stricker S. Signalling pathways in embryonic development. Front Cell Dev Biol 2017; 5: 76.

Alsina-Beauchamp D, Escós A, Fajardo P, González-Romero D, Díaz-Mora E, Risco A, Martín-Serrano MA, Del Fresno C, Dominguez-Andrés J, Aparicio N, Zur R, Shpiro N, Brown GD, Ardavín C, Netea MG, Alemany S, Sanz-Ezquerro JJ, Cuenda A. Myeloid cell deficiency of p38γ/p38δ protects against candidiasis and regulates antifungal immunity. EMBO Mol Med 2018; 10: 8485.

Our group is interested in understanding the molecular and cellular basis of organ formation during embryonic development. This knowledge is important for identifying the origin of congenital malformations and to understand the basis of morphological evolution. We use animal models (mouse and chicken embryos) to address several biological questions related to development, such as morphogenesis and cell differentiation. We also study the role of developmental pathways in adult tissue homeostasis and regeneration.

We have previously shown that Fgf signalling controls the formation of phalanges in digits. Moreover, we provided evidence that the last phalange has special features, including the presence of a specific molecular program that could be related to nail formation and the regenerative potential of the digit tips. We have continued the analysis of the Fgf signalling pathway during digit development, investigating how the maintenance of Fgf signals can induce the production of extra phalanges (hyperphalangy). We have also studied the expression and regulation in digits of MKP3 (a phosphatase that negatively regulates Fgf signalling) and the possible role of a MKP3 negative feedback loop in producing truncation of avian digits.

We are also interested in the relationship between inflammation and regeneration. 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 to regeneration. In collaboration with the group of Ana Cuenda (Department of Immunology and Oncology, CNB) we are using a model of cancer associated to inflammation (colon cancer associated to colitis, CAC) 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 in the induction of tumour formation.

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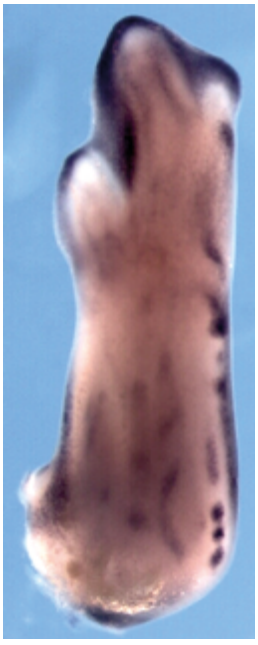

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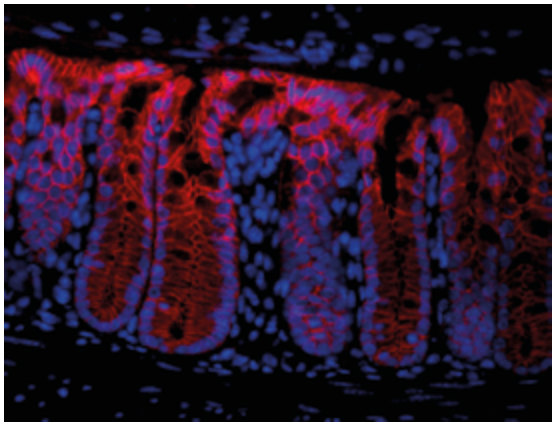
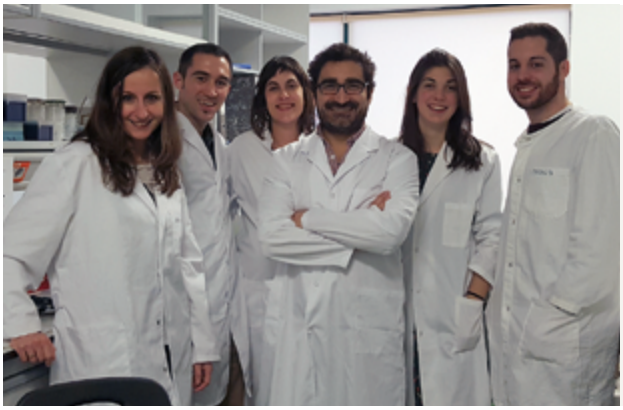


❶ *Expression of MKP3 in a chicken embryo wing detected by RNA in situ hybridisation.*

❷ *Immunohistochemistry staining of mouse colon crypts showing expression of E-Cadherin (red). Cell nuclei are visualised in blue by DAPI staining.*



Cellular immunobiology and microbiology

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SELECTED PUBLICATIONS

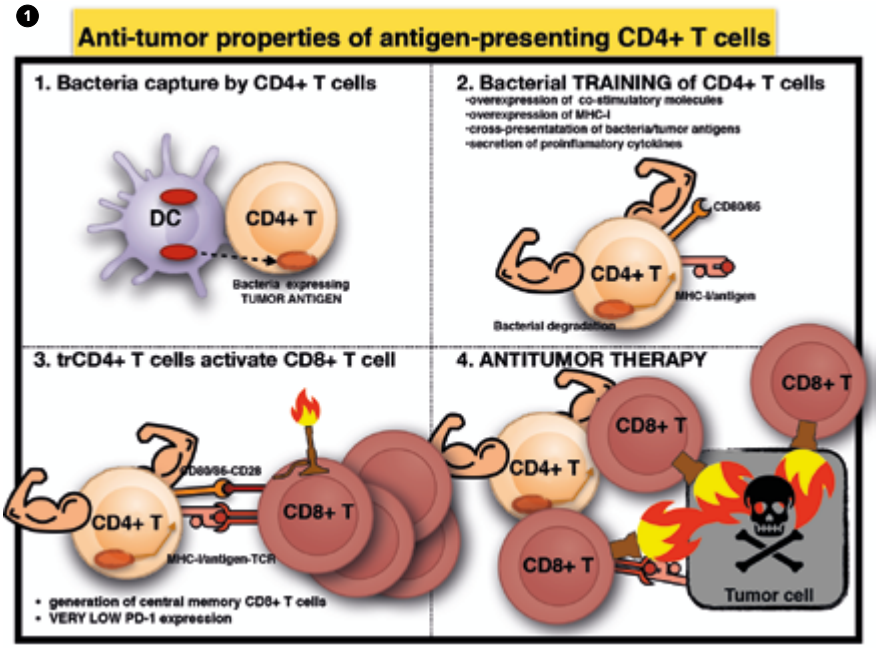
Cruz-Adalia A, Ramirez-Santiago G, Osuna-Pérez J, Torres-Torresano M, Zorita V, Martínez-Riaño A, Boccasavia V, Borroto A, Martínez Del Hoyo G, González-Granado JM, Alarcón B, Sánchez-Madrid F, Veiga E. Conventional CD4+ T cells present bacterial antigens to induce cytotoxic and memory CD8+ T cell responses. Nat Commun 2017; 8: 1591.

Antitumour therapies potentiating the immune response (mainly antibodies anti check point inhibitors) have emerged in recent years, allowing to treat previously intractable tumours. The response rates, however, remain low and these treatments are associated in many cases to undesirable side effects. It is therefore necessary to find novel therapies with increased response rates, minimising side effects and ideally leading to complete and lasting removal of the tumour.

We have discovered that conventional CD4+ T cells can be "trained" after bacteria capture. Trained (tr), CD4+ T cells 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 trCD4+ T cells, which could block the immunosuppressive environment generated by solid tumours, prompted us to hypothesised that trCD4+ T cells could be useful in antitumour therapies.

This hypothesis was tested in a proof-of-concept model of aggressive mouse melanoma. Mice treated with trCD4+ T cells that have captured/killed bacteria expressing tumour antigens (we generated bacteria expressing neoantigens from known tumour models) were protected against tumour development.

Now we are studying the antitumour potential of trCD4+ T cells (using different models of solid and liquid tumours) and the molecular mechanisms of trCD4+ T cells-driven antitumour responses.



1 *CD4+ T cell capture and destroy bacteria by transphagocytosis (1). Exposure to bacteria "trains" CD4+ T cells that overexpress MHC-I and coestimulatory molecules and secrete locally inflammatory cytokines (2). Moreover, bacteria-trained (tr) CD4+ T cells differentiate into antigen cross-presenting cells, potently activating naïve CD8+ T cells, and generate central memory CD8+ T cells expressing very low levels of PD-1(3). These activities are all involved in the removal of tumours. We are testing the antitumour activity of trCD4+ (4).*