Centre INRAE		Centre Val de Loire (Nouzilly)
Unité	**Codique**	UMR 1282
	Intitulé	Infectiologie et Santé Publique
Titre de la thèse[1]		Flagellin treatment: an immunomodulation strategy for controlling swine influenza infection

Summary *(10 – 15 lignes)*

The pig industry is facing an increasing number of challenges with regard to the public's demand for a sustainable, profitable and safe agrifood production, while being confronted with the steadily growing threat posed by animal infectious diseases. Swine influenza A virus (SIAV) predisposes to the porcine respiratory disease complex, causing tremendous economic losses worldwide. Diminishing the disease threat by means of prevention has proven difficult, because current SIAV vaccines fail to provide sterilizing and cross-protective immunity. Stimulation of innate immunity with flagellin, a TLR5 agonist and modulator of the respiratory innate immune system, has emerged as a promising alternative strategy to increase resistance to diseases. We have previously shown that flagellin administration triggers the expression of antiviral genes in the pig and protects against influenza infection in mice, suggesting that flagellin may also boost the immune defense against SIAV in pigs. We hypothesize that this beneficial effect can be attributed to a transient pro-inflammatory activity and its positive effect on resident immune cells maturation. The aims of this PhD project are to: **(i) provide a comprehensive understanding of the molecular and cellular host-virus interactions that are modulated by flagellin. (ii) provide the proof-of-concept for the protective activity of flagellin against SIAV infection in pigs.** We will combine state-of-the-art confocal imaging, single cell RNA sequencing (scRNA-seq), and gene editing approaches to decipher the complexity of the pig's responses to SIAV infections and the protective mechanisms induced by flagellin. We expect to identify host genes patterns and pathways that are critical drivers of the antiviral host response to SIAV. Functional assays (using CRISPR-Cas9 knockout cell culture systems) will be used to validate these candidates. Ultimately, this project will help to develop better and safer prevention and control strategies to fight viral infections in swine.

1 Thesis Directors

Ignacio Caballero Posadas (HDR)

Sascha Trapp

List of 5 recent publications related to the thesis project

1. Isabelle Fleurot, Raquel López-Gálvez, Pascal Barbry, Antoine Guillon, Mustapha Si-Tahar, Andrea Bähr, Nikolai Klymiuk, Jean-Claude Sirard, **Ignacio Caballero**. TLR5 signalling is hyper-responsive in porcine cystic fibrosis airways epithelium. J Cyst Fibros 2022 Mar;21(2):e117-e121. doi: 10.1016/j.jcf.2021.08.002. **IF: 5.52**

2. Raquel López-Gálvez, Isabelle Fleurot, Pablo Chamero, Sascha Trapp, Michel Olivier, Claire Chevaleyre, Céline Barc, Mickael Riou, Christelle Rossignol, Antoine Guillon, Mustapha Si-Tahar, Tobias May, Pascal Barbry, Andrea Bähr, Nikolai Klymiuk, Jean-Claude Sirard, **Ignacio Caballero**. Airway administration of flagellin regulates the inflammatory response to Pseudomonas aeruginosa. Am J Respir Cell Mol Biol 2021. Oct;65(4):378-389. doi: 10.1165/rcmb.2021-0125OC **IF: 7.74**
3. Pascal Barbry, Brice Marcet, **Ignacio Caballero**. Where is the Cystic Fibrosis Transmembrane Conductance Regulator? Am J Respir Crit Care Med 2021. May 15;203(10):1214-1216 doi: 10.1164/rccm.202012-4434ED. **IF: 30.5**
4. Bryson KJ, Garrido D, Esposito M, McLachlan G, Digard P, Schouler C, Guabiraba R, **Trapp S**, Vervelde L. Precision cut lung slices: a novel versatile tool to examine host-pathogen interaction in the chicken lung. Vet Res. 2020 Jan 10;51(1):2. doi: 10.1186/s13567-019-0733-0.
5. **Trapp S**, Soubieux D, Lidove A, Esnault E, Lion A, Guillory V, Wacquiez A, Kut E, Quéré P, Larcher T, Ledevin M, Nadan V, Camus-Bouclainville C, Marc D. Major contribution of the RNA-binding domain of NS1 in the pathogenicity and replication potential of an avian H7N1 influenza virus in chickens. Virol J. 2018 Mar 27;15(1):55. doi: 10.1186/s12985-018-0960-4.

Université d'inscription de l'étudiant en thèse	Université François Rabelais de Tours
École doctorale	Santé, Sciences Biologiques et Chimie du Vivant (SSBCV)

2 Project description

Enjeux scientifiques et socio-économiques auxquels répond le projet

Swine influenza A virus (SIAV) is one of the leading viral pathogens in the porcine respiratory disease complex (PRDC). SIAV is highly contagious and infects most of the affected herd. In addition, SIAV can predispose the pig to secondary respiratory infections with pathogenic bacteria (such as *Actinobacillus pleuropneumoniae*), which in turn can trigger severe respiratory illness[1], thereby increasing the economic burden (more than 10$ reduced return/pig[2]). SIAV is also of significant concern for human health due to its zoonotic potential since pigs can serve as an intermediate host ("mixing vessel") for different avian and mammalian influenza A viruses[2,3]. Novel Research and Innovation approaches are urgently needed to address the threats posed by SIAV since the currently available vaccines fail to provide protective immunity against all present virus clades. Development of new solutions that target this pathogen is part of a global "One Health" strategy. However, first and foremost, it is the pig sector that will directly benefit from any successful development in this field. In France this sector produces >2 million tons of pork, the third largest production in Europe. Confronted with this challenge, immune stimulation protocols in pigs are considered a promising strategy[4]. Stimulation of innate immunity mobilizes multiple host defense mechanisms that accelerate clearance of microorganisms. Mucosal pre-treatment with the TLR5 ligand flagellin (a bacterial pathogen-associated molecular pattern or PAMP) has demonstrated protective activity in bacterial and viral disease models in pig and mice. This effect was attributed to its pro-inflammatory activity and to a positive effect on resident immune cells maturation, and concomitantly promoted the full resolution of the inflammatory process[5].

This Thesis project aims to develop an innovative intervention strategy to improve the innate immune defenses and pathogen resilience of pig herds. The positive effect of boosting innate immunity will provide a better defense not only against SIAV, but against a variety of other pathogens affecting pigs, notably those involved in the PRDC. In addition, the project will lead to a better understanding of the mechanisms surrounding innate antiviral immunity and efficient "clearance" of SIAV infection. Thus, we will provide the Veterinary Pharma and Diagnostics industry with new therapeutic targets and biomarkers for developing novel prevention and control therapies and diagnostics tools. The project results could be used to benchmark the efficacy of the immune response in pigs following immunostimulatory treatments or vaccination. Finally, flagellin treatment can prove useful as an adjunct therapy to complement present-day vaccination strategies in swine due to its positive effect on DCs maturation. This solution would benefit the swine breeding and selection sector, Veterinary Pharma, the Animal Feed and Nutrition industry, not to mention the consumer and society (in line with DSA GOS 2 and 5).

State of the art - Originality

Mucosal response to swine influenza in the lungs. Swine influenza A virus (SIAV) infects the upper and lower airways of pigs, replicating mainly in the tracheobronchial epithelium. Although SIAV targets mostly epithelial cells, recent studies show that SIAV has the ability to infect other cell types, including immune cells[6]. Epithelial and dendritic cells (DCs) will recognize specific viral motifs through pattern recognition receptors (PRRs) such as RIG-I, MDA-5, TLR3 or TLR7. Signaling by these receptors will lead to the activation of the NF-κB and IRF3/7 transcription factors and the production of type I interferons (IFN-I) and pro-inflammatory cytokines[7,8]. This pro-inflammatory response will lead to the recruitment of neutrophils and monocyte-derived DCs (moDCs)[9-11]. Single cell confocal imaging showed that the dynamics of NF-κB and IRF3/7 activation are a key factor used by the cells to decode an inflammatory stimulus sensed by PRRs and fine-tune the cell's decision making[12]. Interestingly, **influenza A viruses (IAV) subvert the cellular signaling response, notably through the action of the non-structural protein NS1, which inhibits the type I IFN responses by blocking IRF3 activation**[13]. On the other hand, the role of NF-κB in the cellular response to IAV infection is controversial, as it regulates both anti- and pro-viral pathways[8]. Despite the use of NF-κB inhibitors as anti-influenza drugs in pre-clinical trials [14], **the**

mechanisms that dictate the anti- or pro-viral roles of NF-κB during IAV infection are still poorly understood. Hence, further research is needed to decipher the NF-κB and IRF3/7 interactions with SIAV and its viral factors and the role that they may have in viral replication as well as the host response to secondary infections.

Studies defining the host response to IAV infection are mostly based on bulk cell population analyses, failing to capture the complexity of the infected airways as well as the heterogeneity of the cell response to IAV[6]. Single-cell sequencing technologies make it now possible to explore the heterogeneity of the cellular response to virus infection in combination with an in-depth analysis of the expression of viral genes in each individual cell. This allows for a better understanding of the mechanisms used by SIAV to disrupt or pirate the cell machinery and to favor viral replication. In this regard, single cell studies show that IAV infection and the cell responses to the virus are highly heterogeneous, with the presence of both "infected" as well as "bystander" cells[6,15,16]. These studies are shedding new light on the pathogenesis of IAV by showing a direct link between viral gene expression and the host response[16] and pointed to the important role of mitochondria during infection[6].

Flagellin as a candidate molecule for stimulation of innate immunity. Toll-like receptors (TLRs) are part of a major family of PRRs and are expressed in most cell types, including airway epithelial and hematopoietic cells[7]. Flagellin, the TLR5 ligand, is the structural protein of the bacterial flagellum. Apical localization of TLR5 in the respiratory mucosa allows airway epithelial cells to be amongst the first responders to flagellated bacteria[17]. Flagellin interacts with TLR5 to activate NF-κB, a key regulator of innate immunity and inflammation[18]. The flagellin-mediated response is short-lived due to the strong feedback regulatory mechanisms involving transcriptional and post-transcriptional regulators such as TNFAIP3 (also known as A20), or the NF-κB inhibitors IκBα and IκBε[19]. Mucosal exposure to flagellin has demonstrated protective activities in bacterial[20] and viral disease models, including influenza and post-influenza pneumococcal infection[5]. We already obtained preliminary results demonstrating that animals infected with *Actinobacillus pleuropneumoniae* (a major bacterial pathogen in the PRDC) and treated with flagellin have reduced lesions in the lungs and reduced bacterial load. This protective effect could be attributed to the pro-inflammatory activity on airway epithelium and modulation of resident immune cells maturation (such as DCs)[5,20]. Indeed, we have also observed that flagellin nebulization in pig lungs induces an early and transitory release of pro-inflammatory cytokines, followed by the recruitment of neutrophils, moDC and cDC2 Importantly, this protective effect of flagellin seems not to be limited to bacterial infections. **The laboratory of our collaboration partner JC Sirard showed that both systemic and intranasal administration of flagellin decreased the levels of viral RNA and infectious virus in the lungs of H3N2 IAV-infected mice[21]. Furthermore, our own results showed that flagellin nebulization in the pig lungs triggers the expression of several antiviral genes (i.e. OAS1, Mx1), which, combined with the observed recruitment and maturation of DCs into the lungs, suggests that flagellin can help to control SIAV infection**. We have also shown that flagellin treatment can modulate the NF-κB response to a second inflammatory stimulus, which could have a direct impact in the interactions between SIAV and the NF-κB signaling pathway. Recent reports also evidenced an important role of mitochondrial Ca^{2+} signaling as a modulator of flagellin-induced TLR responses[22] and as a critical factor for influenza virus entry in mammalian cells[23]. Moreover, mitochondria-derived succinate is reported to decrease viral load and inflammation in IAV-infected mice[24]. Similarly, we have already produced data showing that **treatment of airway epithelial cells with flagellin is able to modulate mitochondrial Ca^{2+} signaling.** Establishing a link between the immunomodulatory activities of flagellin, mitochondrial function, and virus entry into porcine cells would allow us to devise new strategies to improve the antiviral host response to SIAV.

Originality. This is a highly competitive interdisciplinary project that will allow 1) delivering the proof-of-concept that flagellin administration protects against SIAV in pigs; 2) improving our understanding of the mechanisms used by SIAV during lung infection and; 3) deciphering the mechanisms of flagellin-mediated protection against SIAV. To tackle the complex mechanistic events that govern the outcome of SIAV infection, we will combine state-of-the-art single cell technologies (scRNA-seq and single cell confocal imaging) with advanced computational analyses. The results will be confirmed using CRISPR-Cas9 knockout cells. The use of such cutting-edge techniques

will allow the PhD student to unveil the determinants of SIAV infection and flagellin-mediated protection in the swine respiratory tract.

Research Question

The overall goal of this thesis project is to identify the **cellular and molecular determinants involved in SIAV pathogenesis and the mechanisms used by flagellin to modulate viral-host crosstalk.** The research questions addressed by the PhD student are: (i) How does SIAV infection modulate the dynamics of NF-κB and IRF3 activation and the cell inflammatory response? (ii) Does flagellin stimulation protect against SIAV infection and how?

Working hypothesis

Our working hypothesis is that SIAV disrupts the kinetics of NF-κB and IRF3 activation to evade the immune response and favor its replication. Subversion of cellular signaling will impact the cellular response to a secondary inflammatory stimulus, favoring secondary infections. We hypothesize that flagellin treatment will boost protection against SIAV through several routes: (i) modulating cell signaling dynamics; (ii) expression of antiviral genes; and (iii) recruitment and maturation of inflammatory cells to the site of infection.

Material and Methods

Materials: They key materials required for the project are already present in our laboratory or are accessible through our network of collaborators. SIAV (A/Swine/Bissendorf/IDT1864/2003, H3N2)stocks and cell lines, including several clones of functionally immortalized porcine bronchial epithelial cells, are available in our laboratory. The H3N2-Bissendorf strain is a clinical isolate from a pig with respiratory distress and was previously used by our team for in vitro and in vivo infection studies. Other SIAV strains, e.g. isolates of currently circulating H1N1 or H1N2 strains, can be obtained from the Pig Virology and Immunology (VIP) Unit of the Ploufragan-Plouzané-Niort Laboratory (ANSES). Confocal microscope, 10X Chromium for scRNA-seq, Tecan Spark plate reader for high throughput fluorescence and luminescence screening experiments, real-time qPCR machines, microfluidic qPCR (Fluidigm / Biomark HD) machine, and multi-laser flow cytometers are all accessible at our unit and our staff has been trained to operate this equipment. The PFIE is present on-site to perform *in vivo* SIAV infections. Cost-free Transnational Access (TNA) to a high containment facility of the VetBioNet network, e.g. at WBVR (NL), CReSA (ES), APHA (UK) or IVI (CH), would be subject to an application for a TNA grant from the ISIDORe infrastructure project (HE 2022-25). We have longstanding and ongoing collaborations with JC Sirard, who provides the flagellin, and P Barbry to perform scRNA-seq analysis.

Methods: All of the methods used in this project are already optimized and routinely carried out in the lab. Medium-throughput RT-qPCR protocols (Fluidigm arrays and bio-informatics pipelines for data analysis) have been developed to follow the expression of cellular and viral genes in avian and porcine cells[22]. Confocal microscopy methods tracking NF-κB and IRF3 translocation at the single cell level using ImageJ and CellProfiler were recently published by our lab[22]. All virological techniques applied in the project (virus titration, viral load analysis by RT-qPCR) are standard protocols that are well established in the laboratory.

Research programme

1. Effect of flagellin on the cellular signaling dynamics of SIAV infected cells.

The unique T3 porcine bronchial epithelial cell line[20], which we previously demonstrated to be susceptible to SIAV infection, and to present a similar phenotype to primary bronchial epithelial cells, will be used for the experiments. We will: **(i)** characterize the dynamics of NF-κB and IRF3 activation as well as alterations in mitochondrial function after SIAV infection; **(ii)** evaluate whether SIAV-infected cells have an altered innate immune response; and **(iii)** determine the effect of flagellin treatment on SIAV-induced alterations. For this, T3 cells will be treated with flagellin (100 ng/ml) or left untreated and infected or not with different SIAV strains (H3N2-Bissendorf or

H1N1/H1N2 strains). We will quantify the **NF-κB and IRF3 translocation dynamics** upon stimulation with different concentrations of known NF-κB and IRF3 activators (flagellin, CpG, poly(I:C), TNF-α) using an anti-P65 and an anti-IRF3 antibody[20]. An anti-NS1 antibody will be used to identify infected cells and to quantify the levels of infection. This approach will allow us to better evaluate changes in cellular responses related to infection and flagellin treatment in a heterogeneous population where not all of the cells may be infected. **To determine the alterations in the immune response induced by SIAV and the effect of flagellin on infection,** cells will be infected or not with H3N2-Bissendorf or H1N1/H1N2 viruses for 0, 2, 6, 10 and 24 and 48h. The dynamics of viral replication and host immune gene expression will be evaluated using a 48x48 high-throughput microfluidic RT-qPCR array already optimized in the lab. **Alterations in mitochondrial functions** will be monitored with the mitochondrial calcium reporter plasmid mito-CMV-GCaMP6f using live-cell calcium imaging in a fluorescent videomicroscopy system at the level of the single cell. Flow cytometry analysis of mitochondrial ROS will be assessed by MitoSox staining. Mitochondrial morphology will be evaluated in individual cells by confocal microscopy using a specific mitochondrion targeted GFP. Gene expression changes will be evaluated using a 96x96 microfluidic medium-throughput RT-qPCR approach (Biomark HD, Fluidigm). Cytotoxic and antiviral effects of flagellin treatment will be tested using the commercial Viral ToxGlo assay. Viral load will be evaluated using conventional plaque assay.

2. **Evaluation of the protective effect of flagellin against SIAV infection *in vivo* and single cell RNA "signatures" in response to SIAV and flagellin.**

The aim of this task is to determine whether flagellin has a beneficial protective effect against SIAV infection and to characterize the underlying mechanisms associated with protection. Five-week old pigs will be inoculated intranasally with 2 x10^6 TCID50 of the SIAV H3N2-Bissendorf strain (isolated from a clinical PRDC case) using a MAD Nasal™ Intranasal Mucosal Atomization Device (Teleflex®). Twenty-four hours after infection animals will be anesthetized and flagellin (1.5 mg/pig) will be delivered or not (mock treatment) by nebulization. Clinical assessment (weight gain, respiratory performance, pig survival) will be performed twice a day. Blood samples will be collected daily to perform a white blood cell count. Cytokine analysis in the plasma/serum will be performed using a multiplex ELISA assay (Millipore Porcine cytokine/chemokine magnetic bead panel kit). Nasal swabs will be used to evaluate viral load using conventional plaque assay as a proxy of viral secretion by the infected pigs. Animals will be sacrificed at 3 dpi. SIAV genomic loads will be evaluated in the nasal swabs by RT-qPCR targeting the M gene. Bronchoalveolar lavages will be collected for cytokine evaluation by ELISA. Tracheal and lung tissue from the different lung lobes will be collected for evaluation of mucosal immunity, inflammatory markers by RT-qPCR, quantification of SIAV genomic loads and histological analysis of lung lesions. Lung lobes and tracheal tissue will be fixed and OCT frozen at -80°C for immuno-histochemistry as well as *in situ* hybridization of selected inflammatory and viral markers. Samples will be collected at the level of the primary bronchi and cells will be dissociated to produce scRNA-seq libraries, which will be analyzed at IPMC (using a protocol validated in our collaboration with P. Barbry). Ad hoc bioinformatics pipelines, including machine learning and artificial intelligence algorithms developed in house, will be used to identify and interpret single-cell transcriptomic measurements from viral and host origins and integrate single-cell data. These set of experiments will provide information regarding the different cell types and cell states (infected or bystander cells) and the differential gene expression in each cell type between control and experimental conditions, providing insights into flagellin protective mechanisms and relevant immune pathways. It should be noted that, because of the heavy workload in this task, the PhD student will be substantially supported by the scientific and technical staff in the 3IMo team, notably with regard to the treatment and infection protocol, the post-mortems and tissue/blood sampling and the sample processing.

3. **Validation of identified gene patterns using an *in vitro* model of SIAV infection.**

We will select a subset of genes (up to 10) based on the key determinants of infection and immune protection suggested by our data. For that, sgRNA lentiviral plasmids targeting our genes of interest will be created using up

to 3 individual sgRNAs from a published porcine CRISPR KO library[25,26]. Ribonucleoprotein nucleofections will be performed in T3 cells using a Lonza nucleofector system. Then, cells will be plated in a 96-well plate and the efficiency of gene editing evaluated by Sanger sequencing using Inference of CRISPR Edits (ICE) analysis (Synthego). Once we have validated a high efficiency of gene editing, T3 KO cells will be treated (or not) with flagellin and infected with SIAV for 48h. Infected cells will be stained using an anti-NS1 antibody and evaluated by flow cytometry and confocal microscopy. Cell viability, cell proliferation and the expression of viral and host (inflammatory response) genes will be evaluated as described above (Fluidigm/Biomark HD). In case of difficulties generating the T3 KO cells, alternative siRNA approaches can be applied.

Calendar

Year 1: Initiation of *in vitro* experiments, confocal microscopy and gene expression studies, evaluation of mitochondrial function. **Year 2:** *in vivo* evaluation of the protective effect of flagellin and scRNA-seq analysis. **Year 2-3:** functional testing of differentially-expressed host genes, writing of the publications and PhD dissertation.

Expected publications

Publication 1: Dynamics of NF-κB and IRF3 translocation and immune response in bronchial epithelial cells.
Publication 2: Evaluation of the protective effect of flagellin against SIAV.

Acquired skills

Cognitive skills: Advanced knowledge in virology and viral immunity in the context of a complex pathosystem. Advanced knowledge in cell biology and cell signaling. Experimental design, data analysis (from bulk to single cell level), and presentation of results both to scientific audiences (lab meeting setting, conferences) and to the public (outreach events). Writing scientific articles.

Technical skills: Large animal experimentation (possibility to obtain a diploma "Formation à l'expérimentation animale de niveau 1"). Cell culture using cell lines. Classic virology skills (virus production, titration, infection, etc.). Confocal microscopy and image analysis. Analysis of gene expression dynamics by traditional and microfluidic (Fluidigm) RT-qPCR. Perform single cell RNA-seq experiments. Analysis of transcriptomic data. R programming for advanced statistical analysis.

Feasability and funding of the project

This project takes advantage of a firmly-established collaboration network that the thesis (co-)supervisors have built, allowing to address the project's subject in a multidisciplinary approach combining veterinary science, virology, molecular biology, single cell approaches and mathematics, which will greatly benefit the student.

Ignacio Caballero (Thesis director and direct supervisor, CRCN, HDR, UMR ISP) is the coordinator of the ANR project PIGIMMUNITY that focuses on the modulation of innate immunity in pigs by flagellin treatment. He has an extensive experience in pig models of lung infection, confocal microscopy and single cell analysis of the NF-κB pathway. **Sascha Trapp** (Thesis co-supervisor, CRCN, ISP) is the head of the 3IMo team, coordinator of the EU H2020 infrastructure project VetBioNet and expert in viral epizootic diseases, notably animal influenza infections. **Ferdinand Roesch** (CRCN, ISP) is a researcher in the 3IMo team and an expert in virology and the use of CRISPR-Cas9 screening to identify host factors related to viral infection. *In vivo* experiments will be performed at the **PFIE experimental unit.** Alternatively, the *in vivo* experiments can be performed at one of the high- containment facilities of the VetBioNet network (where possible via cost-free Transnational Access supported by the ISIDORe infrastructure project).

The PHASE department will be involved through collaboration with **Pablo Chamero**, head of the INERC team at the UMR PRC. He is an expert in high-resolution, live-cell Ca^{2+} imaging, and *in situ* hybridization. He has established new imaging methods in the context of Ca^{2+} measure in organelles, including mitochondria in pig cells with Dr Caballero. Other collaboration partners include: **Pascal Barbry** (Research Director CNRS, Sophia

Antipolis); he is the creator (1999) and head of the group "Physiological Genomics of the Eukaryotes", of the UCAGenomiX platform, and one of the founders of the national infrastructure France Génomique. His group has developed an outstanding expertise in single cell RNA seq and bioinformatics. He already validated with I. Caballero the protocols to perform single cell RNAseq on pig lung tissue. **Jean-Claude Sirard** (CIIL) is leader and principal investigator of the Team "Bacteria, Antibiotics & Immunity" within the Center for Infection and Immunity of Lille on the Campus of Institut Pasteur de Lille. He is an expert in the development of immune-interventions using flagellin to induce protection against infection. He will provide flagellin and its recombinant optimized variants for the study, and will help paving the path for a potential translation of the results to the market. He has already developed 7 patents for the use of flagellin and is currently the coordinator of the FAIR project (EU H2020) that is producing clinical trials to use flagellin as a therapy against human lung diseases.

We have received funding for FFE Institut Carnot that allows the successful development of the project. The student will have the opportunity to work with our collaborators **Pascal Barbry** (IPMC, Nice), an expert in scRNA-seq analysis; and with the **Pablo Chamero** (UMR PRC), expert in high-resolution, live-cell Ca^{2+} imaging, and *in situ* hybridization.

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3 Candidate

We are looking for a curious and motivated student with strong critical thinking skills, enjoying teamwork but also capable to work inautonomy. Training in computer programming (Python, R) would be greatly appreciated. Past experience of laboratory internship