

## NEWSLETTER

ISSUE 1 - OCTOBER 2015

Dear colleagues, dear friends and supporters of PathCo,

We are pleased to present you the **first issue** of the Newsletter of **Pathogen COinfection: HIV, Tuberculosis, Malaria and Hepatitis C virus - PathCo - Project**.

PathCo Newsletter will be filled with interesting information mainly for all groups external to the PathCo consortium that may have an interest in our research and progress.

Please don't hesitate to forward this mail to anyone who could also be interested in reading it. If they want to receive their own Newsletter in the future they can write at [info@pathco.org](mailto:info@pathco.org). If you're not interested in receiving our Newsletter anymore, you can unsubscribe via mail.

In order to contribute to the contents of the Newsletter, please send news, photos and other material related to PathCo areas of research at [info@pathco.org](mailto:info@pathco.org). We hope you will enjoy reading our latest news.

Best regards,  
The PathCo management team

## The PathCo Project

**PathCo** addresses the challenge of a Seventh Framework Programme 2012 Call project on Co-infection of HIV/AIDS, malaria, tuberculosis and/or hepatitis. The project officially kicked off November 18<sup>th</sup>, 2012. PathCo project will aid to improve our understanding of pathogen co-infection effect(s) on host innate and adaptive immune responses and to develop new approaches to dissect pathogen interactions.

**The PathCo project** brings together powerful multidisciplinary technologies that will improve our understanding of the **complex interactions between infectious agents and the host immune response** that will significantly improve the management of co-infection associated disease.

Recent developments in each of the disease disciplines enable the design of model systems that support pathogen co-infection, highlighting the timeliness of **PathCo's mission to study the biological and immunological consequences of co-infection(s)**.

**The PathCo consortium** consists of ten beneficiaries from five EU countries (The Netherlands, UK, France, Germany and Italy) and one beneficiary from non-EU countries (South Africa).

**PathCo team** is a well-balanced team of immunologists, virologists, clinicians, statisticians, epidemiologists with expertise in HIV/AIDS, TB, malaria and hepatitis C infection research and has the most appropriate scientific and technical background as well as the animal models and instrumentation required to fulfill the goals of this project and to succeed in its mission. PathCo beneficiaries will have access to well-characterised established cohorts of co-infected patients from different geographical locations. Given the widespread global nature of the pathogens under study it is imperative that we are not biased in our selection of patients.

The consortium is co-ordinated by the University of Liverpool (Dr. Bill Paxton, Project Coordinator) and will receive over €5.9 million funding over 5 years from 1<sup>st</sup> November 2012.

The list of PathCo beneficiaries and more information on the project can be found on the website ([www.pathco.org](http://www.pathco.org))

### PROJECT NEWS

#### PathCo Project Second Annual Meeting

On the 18<sup>th</sup>, 19<sup>th</sup> and 20<sup>th</sup> of May 2015, the **Second Annual Meeting of PathCo Project** was held in Rome (Italy). PathCo Second Annual Meeting has been organized only for the partners of the project and members of all beneficiary institutions attended the meeting in Rome.

The meeting was opened by the welcome address of the Project Coordinator (Bill Paxton, University of Liverpool) that also underlined the main aims and expectations of the PathCo Project. Project Coordinator's talk was followed by the presentations of the scientific work packages (WPs) by WP Leaders. WP presentations were focused on the main successes, issues and challenges obtained during the third year of the project as well as on most activities and objectives foreseen for the next period of reference.

The PathCo scientific and financial reporting aspects were also revised and discussed during the meeting. Finally, the dissemination and training activities as well as the strategic tools to be realized to progressively improve the visibility of the project were discussed and agreed by the PathCo Consortium members.



PathCo Consortium in Rome

The second PathCo annual meeting was closed by the report of the **PathCo Scientific and Ethical Advisory Board members** focused on main strengths and weaknesses of the project and giving crucial suggestions to PathCo Consortium members on how optimize resources and efforts during the fourth year of the project.



PathCo-PEACHI joint meeting

During the second day a joint meeting was held between **PathCo** and **PEACHI** projects both focused on HCV, HIV-I and co-infection.

The goal of the **Prevention of Hepatitis C Virus (HCV) and HIV-I Co-Infections - PEACHI - project** ([www.peachi.eu](http://www.peachi.eu)) is to develop simple, affordable and effective vaccine strategies that can be given alone or in combination to prevent hepatitis C virus (HCV), human immunodeficiency virus type I (HIV-I) and co-infection.



The vaccines are based on novel and powerful viral vectors for in vivo delivery of antigens. PEACHI Consortium consists of cutting edge clinical and scientific expertise, partnered with industry. In the last two years PEACHI Consortium members have employed viral vectored technology to develop the most immunogenic HIV-1 and HCV vaccines described to date.

The joint meeting included several lectures on subjects related to HCV/HIV-1 co-infection biology, animal models for HCV/HIV co-infections and new HCV vaccines in HIV-1 infected individuals. More important, lots of discussions amongst PathCo and PEACHI partners have taken place during the whole joint meeting.

### Open Access publications produced by PathCo Project

#### **Innate and adaptive immune responses in HCV infections.**

Heim MH, Thimme R

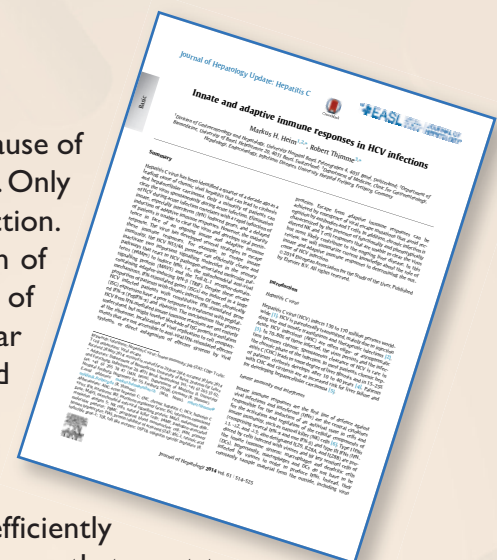
*J Hepatol.* 2014 Nov;61(1 Suppl): S14-25

Hepatitis C virus has been identified a quarter of a decade ago as a leading cause of chronic viral hepatitis that can lead to cirrhosis and hepatocellular carcinoma. Only a minority of patients can clear the virus spontaneously during acute infection. Elimination of HCV during acute infection correlates with a rapid induction of innate, especially interferon (IFN) induced genes, and a delayed induction of adaptive immune responses. However, the majority of patients is unable to clear the virus and develops viral persistence in face of an ongoing innate and adaptive immune response.

The virus has developed several strategies to escape these immune responses.

For example, to escape innate immunity, the HCV NS3/4A protease can efficiently cleave and inactivate two important signalling molecules in the sensory pathways that react to HCV pathogen-associated molecular patterns (PAMPs) to induce IFNs, i.e., the mitochondrial anti-viral signalling protein (MAVS) and the Toll-IL-1 receptor-domain containing adaptor-inducing IFN- $\beta$  (TRIF). Despite these escape mechanisms, IFN-stimulated genes (ISGs) are induced in a large proportion of patients with chronic infection. Of note, chronically HCV infected patients with constitutive IFN-stimulated gene (ISG) expression have a poor response to treatment with pegylated IFN- $\alpha$  (PegIFN- $\alpha$ ) and ribavirin. The mechanisms that protect HCV from IFN-mediated innate immune reactions are not entirely understood, but might involve blockade of ISG protein translation at the ribosome, localization of viral replication to cell compartments that are not accessible to anti-viral IFN-stimulated effector systems, or direct antagonism of effector systems by viral proteins. Escape from adaptive immune responses can be achieved by emergence of viral escape mutations that avoid recognition by antibodies and T cells. In addition, chronic infection is characterized by the presence of functionally and phenotypically altered NK and T cell responses that are unable to clear the virus but most likely contribute to the ongoing liver disease.

In this review, the authors will summarize current knowledge about the role of innate and adaptive immune responses in determining the outcome of HCV infection.



### **Colorectal Mucus Binds DC-SIGN and Inhibits HIV-1 Trans-Infection of CD4+ T-Lymphocytes.**

Stax MJ, Mouser EE, van Montfort T, Sanders RW, de Vries HJ, Dekker HL, Herrera C, Speijer D, Pollakis G, Paxton WA.

*PLoS One.* 2015 Mar 20;10(3):e0122020

Bodily secretions, including breast milk and semen, contain factors that modulate HIV-1 infection.

Since anal intercourse carries one of the highest risks for HIV-1 transmission, our aim was to determine whether colorectal mucus (CM) also contains factors interfering with HIV-1 infection and replication. CM from a number of individuals was collected and tested for the capacity to bind DC-SIGN and inhibit HIV-1 cis- or trans-infection of CD4+ T-lymphocytes.

To this end, a DC-SIGN binding ELISA, a gp140 trimer competition ELISA and HIV-1 capture/transfer assays were utilized. Subsequently the authors of this manuscript aimed to identify the DC-SIGN binding component through biochemical characterization and mass spectrometry analysis. CM was shown to bind DC-SIGN and competes with HIV-1 gp140 trimer for binding. Pre-incubation of Raji-DC-SIGN cells or immature dendritic cells (iDCs) with CM potently inhibits DC-SIGN mediated trans-infection of CD4+ T-lymphocytes with CCR5 and CXCR4 using HIV-1 strains, while no effect on direct infection is observed. Preliminary biochemical characterization demonstrates that the component seems to be large (>100kDa), heat and proteinase K resistant, binds in a  $\alpha$ 1–3 mannose independent manner and is highly variant between individuals. Immunoprecipitation using DC-SIGN-Fc coated agarose beads followed by mass spectrometry indicated lactoferrin (fragments) and its receptor (intelectin-1) as candidates. Using ELISA the authors showed that lactoferrin levels within CM correlate with DCSIGN binding capacity.

In conclusion, CM can bind the C-type lectin DC-SIGN and block HIV-1 trans-infection of both CCR5 and CXCR4 using HIV-1 strains. Furthermore, reported data indicate that lactoferrin is a DC-SIGN binding component of CM. These results indicate that CM has the potential to interfere with pathogen transmission and modulate immune responses at the colorectal mucosa.



### **Obtaining the Plasmodium falciparum full life cycle and observations on the P. ovale liver stages in humanized mice.**

Soulard V, Bosson-Vanga H, Lorthiois A, Roucher C, Franetich JF, Zanghi G, Bordessoulles M, Tefit M, Thellier M, Morosan S, Le Naour G, Capron F, Suemizu H, Snounou G, Moreno-Sabater A, Mazier D.

*Nat Comm* 2015 Jul 24;6:7690.

Experimental studies of Plasmodium parasites that infect humans are restricted by their host specificity. Humanized mice offer a means to overcome this and further provide the opportunity to observe the parasites in vivo. The authors of this manuscript improve on previous protocols to achieve efficient double engraftment of TK-NOG mice by human primary hepatocytes and red blood cells. Thus, the authors obtain the complete hepatic





development of *P. falciparum*, the transition to the erythrocytic stages, their subsequent multiplication, and the appearance of mature gametocytes over an extended period of observation. Furthermore, using sporozoites derived from two *P. ovale*-infected patients, the authors show that human hepatocytes engrafted in TK-NOG mice sustain maturation of the liver stages, and the presence of late-developing schizonts indicate the eventual activation of quiescent parasites. Thus, TK-NOG mice are highly suited for in vivo observations on the *Plasmodium* species of humans.

### **Patterns of HIV, TB, and non-communicable disease multi-morbidity in peri-urban South Africa - a cross sectional study.**

Oni T, Youngblood E, Boule A, McGrath N, Wilkinson RJ, Levitt, NS.  
*BMC Infect Dis.* 2015 Jan 17;15:20.

Many low and middle-income countries are experiencing colliding epidemics of chronic infectious (ID) and non-communicable diseases (NCD). As a result, the prevalence of multiple morbidities (MM) is rising.

The authors of this manuscript conducted a study to describe the epidemiology of MM in a primary care clinic in Khayelitsha. Adults with at least one of HIV, tuberculosis (TB), diabetes (DM), and hypertension (HPT) were identified between Sept 2012-May 2013 on electronic databases. Using unique patient identifiers, drugs prescribed across all facilities in the province were linked to each patient and each drug class assigned a condition.

Results reported in the manuscript show that these 4 diseases accounted for 45% of all prescription visits. Among 14364 chronic disease patients, HPT was the most common morbidity (65%). 22.6% of patients had MM, with an increasing prevalence with age; and a high prevalence among younger antiretroviral therapy (ART) patients (26% and 30% in 18-35 yr and 36-45 year age groups respectively). Among these younger ART patients with MM, HPT and DM prevalence was higher than in those not on ART.

The authors highlight the co-existence of multiple ID and NCD. This presents both challenges (increasing complexity and the impact on health services, providers and patients), and opportunities for chronic diseases screening in a population linked to care. It also necessitates re-thinking of models of health care delivery and requires policy interventions to integrate and coordinate management of co-morbid chronic diseases.



### **Cytotoxic mediators in paradoxical HIV-tuberculosis immune reconstitution inflammatory syndrome.**

Wilkinson KA, Walker NF, Meintjes G, Deffur A, Nicol MP, Skolimowska KH, Matthews K, Tadokera R, Seldon RR, Maartens G, Rangaka MX, Besra G, Wilkinson RJ.

*J Immunol.* 2015 Feb 15;194(4):1748-54. *OI*

Tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) frequently complicates combined antiretroviral therapy and antituberculosis therapy in HIV-1-coinfected tuberculosis patients. The immunopathological mechanisms underlying TB-IRIS are incompletely defined, and improved understanding is required to derive new treatments and to reduce associated morbidity and mortality. The authors of this manuscript performed longitudinal and cross-sectional analyses of human PBMCs from paradoxical TB-IRIS patients and non-IRIS controls (HIV-TB-coinfected patients commencing antiretroviral therapy who did not develop TB-IRIS). Freshly isolated PBMC stimulated with heat-killed *Mycobacterium tuberculosis* H37Rv (hkH37Rv) were used for IFN- $\gamma$  ELISPOT and RNA extraction. Stored RNA was used for microarray and RT-PCR, whereas corresponding stored culture supernatants were used for ELISA. Stored PBMC were used for perforin and granzyme B ELISPOT and flow cytometry. There were significantly increased IFN- $\gamma$  responses to hkH37Rv in TB-IRIS, compared with non-IRIS PBMC ( $p = 0.035$ ). Microarray analysis of hkH37Rv-stimulated PBMC indicated that perforin 1 was the most significantly upregulated gene, with granzyme B among the top five (log2 fold difference 3.587 and 2.828, respectively), in TB-IRIS.



Downstream experiments using RT-PCR, ELISA, and ELISPOT confirmed the increased expression and secretion of perforin and granzyme B. Moreover, granzyme B secretion reduced in PBMC from TB-IRIS patients during corticosteroid treatment. Invariant NKT cell (CD3+Va24+) proportions were higher in TB-IRIS patients ( $p = 0.004$ ) and were a source of perforin. Data reported in this manuscript implicate the granule exocytosis pathway in TB-IRIS pathophysiology. Further understanding of the immunopathogenesis of this condition will facilitate development of specific diagnostic and improved therapeutic options.

### **The impact of HIV exposure and maternal *Mycobacterium tuberculosis* infection on infant immune responses to Bacille Calmette-Guérin vaccination.**

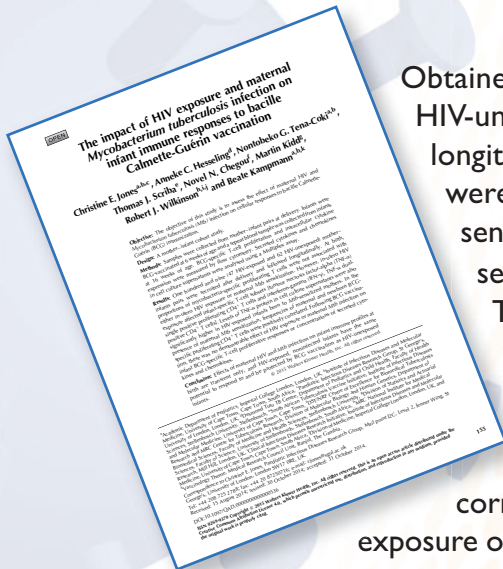
Jones CE, Hesseling A, Tena-Coki NG, Scriba TJ, Chegou, NN, Kidd M, Wilkinson, R.J., Kampmann, B.

*AIDS.* 2015 Jan 14;29(2):155-65.

The objective of this study was to assess the effect of maternal HIV and *Mycobacterium tuberculosis* (Mtb) infection on cellular responses to bacille Calmette-Guérin (BCG) immunization.

To this aim, samples were collected from mother-infant pairs at delivery. Infants were BCG-vaccinated at 6 weeks of age and a repeat blood sample was collected from infants at 16 weeks of age. BCG-specific T-cell proliferation and intracellular cytokine expression were measured by flow cytometry. Secreted cytokines and chemokines in cell culture supernatants were analysed using a Multiplex assay.





Obtained results showed that one hundred and nine (47 HIV-exposed and 62 HIV-unexposed) mother-infants pairs were recruited after delivery and followed longitudinally. At birth, proportions of mycobacteria-specific proliferating T cells were not associated with either in-utero HIV exposure or maternal Mtb sensitization. However, in-utero HIV exposure affected infant-specific T-cell subsets [tumour necrosis factor-alpha (TNF- $\alpha$ ) single positive proliferating CD4+ T cells and interferon-gamma (IFN- $\gamma$ ), TNF- $\alpha$  dual-positive CD4+ T cells]. Levels of TNF- $\alpha$  protein in cell culture supernatants were also significantly higher in HIV-exposed infants born to Mtb-sensitized mothers. In the presence of maternal Mtb sensitization, frequencies of maternal and newborn BCG-specific proliferating CD4+ T cells were positively correlated. Following BCG vaccination, there was no demonstrable effect of HIV exposure or maternal Mtb infection on infant BCG-specific T-cell proliferative responses or concentrations of secreted cytokines and chemokines.

The authors conclude that effects of maternal HIV and Mtb infection on infant immune profiles at birth are transient only, and HIV-exposed, noninfected infants have the same potential to respond to and be protected by BCG vaccination as HIV-unexposed infants.

### **Immune reconstitution inflammatory syndrome in HIV-infected patients.**

Walker NF, Scriven J, Meintjes G, Wilkinson RJ.  
*HIV AIDS (Auckl)*. 2015 Feb 12;7:49-64..

Access to antiretroviral therapy (ART) is improving worldwide. Immune reconstitution inflammatory syndrome (IRIS) is a common complication of ART initiation.

In this review, the authors provide an overview of clinical and epidemiological features of HIV-associated IRIS, current understanding of pathophysiological mechanisms, available therapy, and preventive strategies.

The spectrum of HIV-associated IRIS is described, with a particular focus on three important pathogen-associated forms: tuberculosis-associated IRIS, cryptococcal IRIS, and Kaposi's sarcoma IRIS. While the clinical features and epidemiology are well described, there are major gaps in the understanding of pathophysiology and as a result therapeutic and preventative strategies are suboptimal. Timing of ART initiation is critical to reduce IRIS-associated morbidity. Improved understanding of the pathophysiology of IRIS will hopefully enable improved diagnostic modalities and better targeted treatments to be developed.





### **The road to drug resistance in *Mycobacterium tuberculosis*.**

Koch A and Wilkinson RJ.

*Genome Biol.* 2014 Nov 13;15(11):520.

Sequencing of serial isolates of extensively drug-resistant tuberculosis highlights how drug resistance develops within a single patient and reveals unexpected levels of pathogen diversity.

### **Tricks to Translating TB Transcriptomics.**

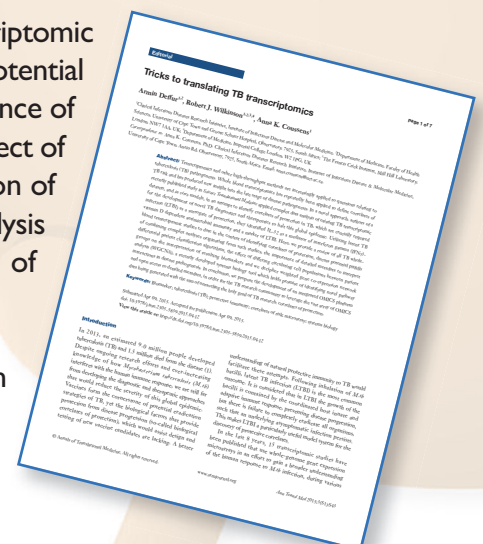
Deffur A, Wilkinson RJ, Coussens AK.

*Ann Transl Med.* 2015 May;3(Suppl 1):S43.

Transcriptomics and other high-throughput methods are increasingly applied to questions relating to tuberculosis (TB) pathogenesis. Whole blood transcriptomics has repeatedly been applied to define correlates of TB risk and has produced new insight into the late stage of disease pathogenesis. In a novel approach, authors of a recently published study in *Science Translational Medicine* applied complex data analysis of existing TB transcriptomic datasets, and *in vitro* models, in an attempt to identify correlates of protection in TB, which are crucially required for the development of novel TB diagnostics and therapeutics to halt this global epidemic. Utilizing latent TB infection (LTBI) as a surrogate of protection, they identified IL-32 as a mediator of interferon gamma (IFN $\gamma$ )-vitamin D dependent antimicrobial immunity and a marker of LTBI.

In this manuscript, the authors provide a review of all TB whole blood transcriptomic studies to date in the context of identifying correlates of protection, discuss potential pitfalls of combining complex analyses originating from such studies, the importance of detailed metadata to interpret differential patient classification algorithms, the effect of differing circulating cell populations between patient groups on the interpretation of resulting biomarkers and we decipher weighted gene co-expression network analysis (WGCNA), a recently developed systems biology tool which holds promise of identifying novel pathway interactions in disease pathogenesis.

In conclusion, the authors of this manuscript propose the development of an integrated OMICS platform and open access to detailed metadata, in order for the TB research community to leverage the vast array of OMICS data being generated with the aim of unraveling the holy grail of TB research: correlates of protection.





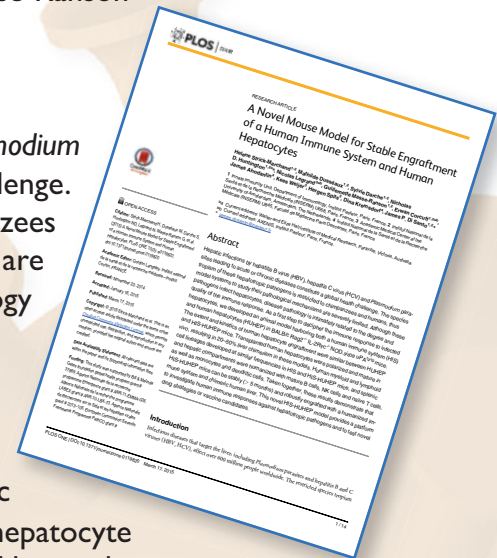
### **A novel mouse model for stable engraftment of a human immune system and human hepatocytes.**

Strick-Marchand H, Dusséaux M, Darche S, Huntington ND, Legrand N, Masse-Ranson G, Corcuff E, Ahodantin J, Weijer K, Spits H, Kremsdorf D, Di Santo JP.  
*PLoS One. 2015 Mar 17;10(3):e0119820.*

Hepatic infections by hepatitis B virus (HBV), hepatitis C virus (HCV) and *Plasmodium* parasites leading to acute or chronic diseases constitute a global health challenge. The species tropism of these hepatotropic pathogens is restricted to chimpanzees and humans, thus model systems to study their pathological mechanisms are severely limited. Although these pathogens infect hepatocytes, disease pathology is intimately related to the degree and quality of the immune response.

As a first step to decipher the immune response to infected hepatocytes, the authors of this manuscript developed an animal model harboring both a human immune system (HIS) and human hepatocytes (HUHEP) in BALB/c Rag2<sup>-/-</sup> IL-2Rγc<sup>-/-</sup> NOD.sirpa uPA<sup>tg/tg</sup> mice. The extent and kinetics of human hepatocyte engraftment were similar between HUHEP and HIS-HUHEP mice. Transplanted human hepatocytes were polarized and mature *in vivo*, resulting in 20-50% liver chimerism in these models. Human myeloid and lymphoid cell lineages developed at similar frequencies in HIS and HIS-HUHEP mice, and splenic and hepatic compartments were humanized with mature B cells, NK cells and naïve T cells, as well as monocytes and dendritic cells.

Taken together, the results reported in the manuscript demonstrate that HIS-HUHEP mice can be stably (> 5 months) and robustly engrafted with a humanized immune system and chimeric human liver. This novel HIS-HUHEP model provides a platform to investigate human immune responses against hepatotropic pathogens and to test novel drug strategies or vaccine candidates.



### **Additional publications generated by PathCo Project**

#### **CD81 is required for rhoptry discharge during host cell invasion by *Plasmodium yoelii* sporozoites.**

Risco-Castillo V, Topçu S, Son O, Briquet S, Manzoni G, Silvie O.  
*Cell Microbiol. 2014 Oct;16(10):1533-48.*

### PathCo additional communication tools

#### PROJECT BROCHURE

Official **PathCo Brochure** has been created and distributed to PathCo partners to disseminate information about the project in any suitable occasion (meetings, congresses, workshops, exhibition, shows or open forum, etc.) to broad audiences.



#### PROJECT WEBSITE

The main source for information on the project is the **PathCo website** ([www.pathco.org](http://www.pathco.org)) where you will find the list of PathCo partners and their contact, more detailed information on the project, recent news on the project, all PathCo publications and press release.