SYSTEMS BIOLOGY OF T-CELL ACTIVATION

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1. EXECUTIVE SUMMARY

Introduction to SYBILLA

The immune system is responsible for fighting off invaders, such as bacteria or viruses. Following recognition by the immune system these disease-causing microbes are destroyed. This is done in a precise way in which healthy uninfected tissues are not attacked by the immune system. The main cells of the immune system that cause these effects are called T cells. However, in certain situations, the T cells can attack and damage normal tissue. This leads to autoimmune diseases. Although 5% of the population suffers from these diseases, no efficient therapeutic treatment is currently available. It is not known in detail how a T cell makes the decision to attack or not, and why it sometimes gets it wrong. T cell activation is a complex process relying on multiple layers of tightly controlled intracellular signalling modules that form an intricate network. And a quantitative understanding of this network is required to understand how T cells react and how manipulate T cells behaviour with novel drugs.

SYBILLA "Systems Biology of T-cell activation in health and disease" is a multidisciplinary consortium of 17 scientific and industrial partners that was funded by the European Union from from April 2008 until September 2013 and will continue working after EU funding has ceased. Each partner contributes particular expertise, including molecular biology, genetics, biochemistry, mathematical modelling and medicine. Joining them in a common effort, SYBILLA unravelled the complex molecular mechanisms that determine whether, and how, T cells become activated by a pathogen, or as in autoimmune disease, by cells from our own tissues.

Main achievements of SYBILLA

SYBILLA has investigated this complex molecular network with unprecedented quantitative precision. This has led to the generation of a number of logical and mechanistic models that describe the behavior of this network, cumulating in the "Virtual T cell". The "Virtual T cell" is a computer program with which the user can simulate the behaviour of this network and thus predict the activation outcome of T cells under different stimulation and perturbation conditions. The virtual T cell is now available to the public via the SYBILLA homepage: www.sybilla-t-cell.de. It will aid in the identification of biological biomarkers, intracellular drug targets and elaborated systems to predict the impact of any kind of drugs on T-cell activation, and thus will help in the treatment of autoimmune diseases.

To reach its goal, SYBILLA optimized state-of-the-art data collection techniques and developed novel reagents, in order to generate robust and quantitative transcriptome and proteome data on the network. These standardized protocols and reagents are available to the public via the SYBILLA homepage and the company EXBIO, respectively. Remarkably, a number of knock-in mice have been generated to quantitatively and comprehensively monitor the molecular dynamics of the early modules of the network in primary T cells which opened up a new avenue for dissecting the mechanism of T cell signaling. Many data have been stored on the SYBILLA data base repository and were thus used for bioinformatics and mathematical modelling efforts.

SYBILLA has advanced two complementary modelling strategies: large-scale modelling of information flow through the network following Boolean ("on-off") rules and dynamic modelling of modules of T-cell signalling that incorporate mechanistic detail. These data-driven approaches have led to predictions on hitherto unknown functional connections and dynamics in the network – in particular on the discrimination between foreign antigen and self-peptides – that were confirmed experimentally.

Furthermore, the differentiation of naïve, primary human T helper cells to either Th1 or Th2 subsets has been studied at the phospho-proteome, transcriptome and epigenome level. This was combined with siRNA-mediated screens for kinase and phosphatase involvements. Using bioinformatics tools signatures of different T cell activation states have been identified. In fact, several novel key regulatory proteins were identified and the pharmacological inhibition of PKCθ and PKCα proved to be a good treatment of autoimmune diseases. Thus, Sotrastaurin was developed as a first-in-class immuno-suppressive PKC antagonist that potently inhibits immune cell activation in the clinic.

Finally, young researchers were trained in interdisciplinary work, more than 100 articles were published, and scientific conferences as well as events for the general public were organized. Ethical and gender issues were handeled professionally.

In order to minimize person-to-person or lab-to-lab variations in quantitative data generation, partner ALU-FR will get an automated robotic platform that will conducts those experiments in the future.

For more information please look at: www.sybilla-t-cell.de

2. SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES

WP01

The main objective in WP01 has been the development of state of the art data collection techniques that allow for the robust quantitative profiling of T cell signaling dynamics in healthy and diseased situations by SYBILLA members. During the course of the SYBILLA project the WP01 team (ETHZ, UOXFa, EXBIO, MPI-IB, MUI) managed to accomplish all major goals and thus was able to provide timely access to state of the art data collection techinques. For example, the methodology developed in WP01 allowed identifying the interactomes of the TCR, Lck, THEMIS and LAT-SLP-76 modules. Moreover, phosphoproteomics of TCR activation revealed the complex network activated by the TCR-controlled kinome and the role of the LAT-SLP76 complex. Also, and importantly, the above methodologies allowed for the first time to quantitatively and comprehensively monitor the molecular dynamics of the T cell signalosome in primary T cells at unprecedented sensitivity which opened up a whole new avenue for dissecting the mechanism of T cell signaling. Furthermore, nine novel monoclonal antibodies have been generated by EXBIO and have been extensively characterized by other SYBILLA partners yielding to a unique new analytical toolbox for ongoing and future Tcell signaling research. Taken together, techniques developed in WP01 turned out to be essential for the discovery of novel signaling components, posttranslational modifications and highly informative signaling patterns that yielded promising new research direction for SYBILLA members and other scientists interested in understanding the molecular basis of immunodeficiencies and autoimmune diseases from a systems level perspective.

WP02

WP02 was crucial for the success of the whole SYBILLA approach since its main objective was the development of reagents and robust, standardized protocols that were mandatory for fulfilling most of the SYBILLA milestones. WP02 objectives consisted in the development of (i) peptide-MHC tetramer reagents allowing to monitor T cell responses, (ii) standardized protocols for mouse and human T cell-based assays, (iii) a panel of vectors coding for selected tagged proteins, (iv) Jurkat T cells expressing selected tagged proteins and (v) a series of knock-in mice expressing OneStrep-Tagged (OST) version of several key components of the TCR signalling pathway. The items corresponding to those deliverables have been already extensively used in SYBILLA WPs (WP03-WP09). For instance the knock-in mice allowed CNRS and ETH to obtain key informations on the interactome of the ZAP-70, LAT, SLP-76 molecules in primary CD4 T cells.

Updated and robust version of 22 of the standardized methological protocols developed in WP02 were made available via the SYBILLA homepage to the open public. Even after the EU funding of SYBILLA, we will keep the SYBILLA homepage active and upload new standardized protocols when appropriate. Although the standardized protocols were crucial for many of the SYBILLA projects, we still felt that person-to-person or lab-to-lab variations should be minimized. This can best be achieved by an automated robotic platform that conducts experiments to deliver accurate biochemical data, that will be used for mechanistic, data-based models. Therefore, partner ALU-FR successfully applied for such a robotic system at the German government. Thus, in the future the robot will perform many of the quantitative experiments.

WP03

In WP03 SYBILLA partners generated quantitative data on TCR-proximal outside-in signalling events using the standardized protcols and reagents developed in WP01 and WP02. WP03 started with generating dose response data of different affinity pMHC tetramers binding to the TCR and correlating them with functional outputs. Next, SYBILLA determined how the affinity of the pMHC ligand influences the CD3 conformational change, characterized the TCR interactome and studied signalling modules directly downstream of the TCR (the kinases Lck/Fyn) or one step down (LAT-SLP76 signalosome). To this end, CSIC, MPI-IB, ALU-FR, UOXFa, UNIBAS, UNIMAG and ETHZ have generated kinetic data in a quantitative fashion of TCR and LAT-SLP76 interactomics, phosphoproteome data and had perturbed signalling by using mutants of the pMHC ligand, TCR mutants unable to undergo the conformational change, TCR mutants unable to recruit the adaptor protein Nck, and mutants of Lck, LAT and SLP76. A large collection of datasets has been generated that has allowed to build mathematical models in WP07 on the multimeric nature of the pMHC ligand as important for quality discrimation and to reach important conclusions such as the necessity to regulate substrate exposure (CD3 tails) rather than kinase activity (Lck) to initiate TCR signalling.

sMoreover, the discovery of Kidins220 as a novel TCR binding partner and THEMIS as a novel LAT binding partner, led work aimed to define their roles in TCR signaling and T cell activation.

WP04
It has been well established that members of the protein kinase C (PKC) family have important roles in T lymphocytes. SYBILLA focused in WP04 on the physiological involvement of distinct PKC isotypes in immune cell functions. This included a detailed cellular pathway analysis of selected PKC family members and their essential downstream signalling effectors. Consequently, MUI performed in depth functional analysis of the physiological functions of the direct PKC signalling intermediates and/or substrates. MUI thereby resolved these PKC effector targets in the molecular context of T cell biology. Finally and as a translational aspect, the pharmacological inhibition of PKCtheta and PKCalpha appears to provide a successful approach for the treatment of autoimmune diseases in mice. This target validation led to the development of Sotrastaurin as a first-in-class immunosuppressive PKC antagonist that potently inhibits immune cell activation in the clinic. Thus, during the SYBILLA funding period, WP04 has defined the particularly critical PKC gene products, PKCtheta and PKCalpha, as the “flavor of PKC” in T cells and resolved key downstream effector mechanisms that are necessary for full antigen receptor-mediated T cell activation ex vivo and T cell-mediated immunity in vivo. Further, UOXFa and ALU-FR showed that Kidins220 promotes sustained Erk signalling in T cells, by linking B-Raf to the TCR.

WP05
In WP05, the main goals were to extract a T-cell activation induced and Th1 or Th2 cell fate-specific gene expression signatures by carrying out high-quality quantitative measurements of the dynamic behavior of the TCR/CD3 + CD28 +/- cytokine (IL4 or IL12)-induced signalling in primary human CD4+ T-cells and to identify the critical signalling molecules and epigenetic regulatory elements determining the early phases of T-helper cell differentiation into the Th1 or Th2 cell subtypes. To reach these goals, several approaches and study platforms were used: (i) By using the standardised protocols (WP02), UTU employed siRNA library screening to study the role of all known human kinases and phosphatases (about 1000 genes in total) during Th cell activation and differentiation into Th1 and Th2. (ii) By using three different gene expression analysis platforms: microarray, next generation sequencing and ChIP-sequencing, UTU studied the gene transcriptional profile and the status of the enhancer regulatory elements during the early stages after TCR-activation of Th cells cultured in the absence (Th0) or presence of IL12 (Th1) or IL4 (Th2). (iii) UTU the phosphoproteome of human CD4+ T-cells in response to TCR/CD3 stimulation in the absence or presence of Th1 or Th2 polarizing cytokines and in response to siRNA-mediated perturbations of key signalling components. UTU also studied the kinetics of intracellular distribution of some identified important signalling components in human primary human T cells. The studies are likely to provide novel insights into signals and networks that determine the fate of human primary CD4+ and provide valuable basis for developing new therapies for immune mediated diseases.

WP06
WP6 had broadly four key goals: (i) data, information and knowledge gathering, (ii) experimental data acquisition and storage into a SYBILLA database, (iii) identification of signatures of different T-cell activation networks, and (iv) online presentation of a Virtual T-Cell. During the early phase of SYBILLA, data and knowledge of T cell activation was collected, stored in a computer readable format, and distributed to project partners. A large part of the work carried out in WP06 revolved around storage of high-throughput data from experimental partners into the T cell specific database which TUT set up for SYBILLA needs. During the project, the amount of data stored into the database has acculated, and now SybillaDB also includes a large collection of publicly available data from various T cell related diseases. Another key incredient of WP06 was identification of signatures of different T-cell activation states and networks. This work was carried out at multiple levels of biologogical information and molecular regulation. Activation network signatures have been characterized at transcriptome, phosphoproteome and epigenome level by TUT. Finally, detailed dynamic models of TCR or T cell activation developed in WP07 together with visualizations, have been made available for the consortium members in an interactive way to the SybillaDB web page.

WP07
The central goal of WP07 was to establish a productive interplay between the mechanistically-based mathematical modelling of signalling in T cells and experimental work that leads to quantitative insights into the complex signalling dynamics and their functional implications. To achieve this, we

have followed two strategies: On the one hand, we have developed a comprehensive Boolean network model that provides an effective overview of the large T-cell signalling network, including signals from the TCR, coreceptors and cytokine/chemokine receptors. This model has been used to dissect the flow of information through the network. These computational analyses have led to several novel predictions that have subsequently been confirmed experimentally, including the dependence of Erk activation on Src kinase, PI3 kinase and novel PKCs. On the other hand, we have developed dynamic models of modules of T-cell signalling that incorporate mechanistic detail and increasingly linked them to a dynamic model of information flow from the plasma membrane to the cytoplasmic signalling pathways. This data-driven dynamic modelling approach has shed new light on a key question of T cell immunology, namely how accurate discrimination between foreign antigens and self-peptides can be achieved that defies conventional mass action. We have shown that ligand discrimination is not solely a function of the interaction of the TCR and the MHC-presented peptide but requires the TCR proximal signalling machinery to read off the ligand dwell time. Taken together, these findings provide a quantitative basis for pharmacological interference and engineering of the T-cell signalling network.

WP08

The goals of WP08 were to validate our previously generated models and to characterize the dynamic properties of the TCR signaling network in primary T cells. To do this we took several approaches. An optimized protocol to transfect siRNAs into T cells with the aim of decreasing expression of several key, signaling molecules. Such modified T cells were analyzed for perturbations in the signaling networks. T cells expressing mutant molecules were also studied to determine the local and distal effects of deranged TCR signaling. Examples of this were the study of T cells expressing a mutant CD3ε protein, which is unable to undergo a conformational change and T cells expressing a mutant CXCR4 gene. A detailed analysis of protein phosphorylation was undertaken in T cells expressing various signaling molecules with a genetically encoded purification tag. The idea behind these experiments was to follow tyrosine and serine phosphorylation with precise kinetics. The role of Kidins220 in modulating ERK signaling was studied by reconstituting mice with Kidins220 deficient bone marrow. ERK signaling plays a key role in the response of thymocytes and T cells. Finally the role of PAG in regulating Src kinase activity was studied by reducing the PAG expression and examining the effect on proximal signaling. These experiments generated some surprising results leading us to re-evaluate our ideas about baseline signaling in unstimulated T cells.

WP09

T cells play a critical role in many autoimmune diseases: a significant proportion of the European population suffers from these diseases, many of which are not treated satisfactorily. Organ-specific and systemic autoimmune diseases as well as allergies are caused by T cell-dependent, unbalanced immune responses. A better understanding of the intracellular signalling cascades involved in the onset of such conditions is required for the development of novel therapeutic approaches. Mice with mutations in the adaptor protein LAT, that acts downstream of the TCR, were used by CNRS to assess how changes in the TCR signalling network promote Th1 and Th2 autoimmune pathologies, with this work leading to the delineation of the LAT/SLP76 signalosome. In addition to the action of molecules directly downstream of the TCR, other signalling pathways, at least some of which show crosstalk with TCR signalling, are implicated in the development of autoimmune diseases, such as cytokine signalling in multiple sclerosis (MS). The IL-7 and IL-2 signalling pathways have been genetically associated with MS and thus genotype-dependent differences in the pathways were assessed using primary human cells by UOXFb. Subsequent to these analyses, the effects observed were studied in humanized mouse models to investigate the functional consequences of the interaction between cytokine signalling and MS-relevant HLA-TCR signalling. Lastly, T cell activation in diabetic NOD mice, MS patients and immunodeficient patients with autoimmune diseases has been studied by partners DKFZb, IDIBAPS and ALU-FR.

3. DESCRIPTION OF MAIN S&T RESULTS / FOREGROUNDS

WP01

A systems level description and modelling of the molecular processes that underly Tcell activation in health and disease requires state of the art technology platforms for systematic and robust collection of biochemical data. Since data for the systematic analysis of RNA and DNA have has been largely established the involved SYBILLA teams focussed their efforts on the development of techniques that allow for a comprehensive analysis of proteins, their posttranslational modifications and interactions with other proteins. This includes mass spectrometry based proteomics techniques (UOXFa, ETHZ) but also the development of antibodies and antibody based techniques (EXBIO, MPI-IB/ALU-FR, MUI, CNRS, CSIC). We succeeded in the development of robust methods for the isolation and quantitative analysis signaling complexes. These methods set the ground for the systematic quantitative analysis of signaling complexes that form a large signaling network for the control of Tcell activation. The high sensitivities achieved along with development of robust label free techniques allowed the analysis of signaling complexes and networks not only from Tcell lines but also from relatively small amounts of primary CD4+ Tcells. Several workpackages could profit from the developed techniques and several manuscripts have been published or are in preparation for publication.

Besides the analysis of protein interactions WP01 also concentrated on the development of label free quantitative method for the systematic profiling of phosphopeptides following Tcell activation. This was needed as several SYBILLA tasks included experimental systems that are not accessible to isotope labelling for quantitative MS analysis. Using the established protocols we managed to profile changes in phosphopeptide abundance following the stimulation of primary Tcells isolated from human chord blood in the context of WP05 in a close collaboration with the SYBILLA member UTU.

The second large effort in WP01 focussed on the development of new highly specific antibody reagents for SYBILLA by partner EXBIO. High quality antibody reagents in particular for proteins and phosphosites discovered by SYBILLA members provide a multitude of important new research applications necessary to dissect the molecular mechanisms that control signaling in activated Tcells. Partner EXBIO managed to generate a set of nine highly specific monoclonal antibody reagents. These reagents have been carefully and extensively tested by SYBILLA members (MUI, MPI-IB/ALU-FR, CNRS, CSIC and UOXFa).

In WP01 we have developed and evaluated several existing methods for efficient purification of protein complexes that are compatible for subsequent quantitative LC-MS analysis. These efforts yielded a streamlined integrated procedure for the quantitative analysis of signaling complexes. We have optimized these methods for membrane proximal complexes as well as soluble intracellular signaling complexes. Besides the improvement of the biochemical part of the analysis we also aimed at a new method for label free quantitative MS analysis suited for relative as well as absolute quantitatiave analysis. We have documented our results in a proof of concept study and further developed the method for increased sensitivity. These advances immediately were implemented in a collaborative study between partners ETHZ and CNRS. The results clearly demonstrate for the first time the feasibility of studying dynamic protein interaction networks underlying Tcell signaling in a physiological context by combining mouse genetics with systems biology approaches. These efforts resulted in the dynamic representation of LAT-SLP76 signalosome dynamics in primary CD4+ Tcells (Roncagalli et al., 2013, under review). The results uncovered novel components of early Tcell signaling and suggests a new mechanism for the previously observed LAT independent phosphorylation involving the membrane protein CD6. In addition these technical advances have been used to address the mechanisms for the recruitment of signaling proteins to the TCR in a collaborative project between partners ETHZ and MPI-IB/ALU-FR. The second area of research in WP01 focussed on the development of techniques for sensitive phospho-proteome analysis. By implementing a method that combines the advantages of TiO2 with immobilized metal affinity chromatography (IMAC) we increased the coverage of standard methods. Combining these techniques with precursor ion intensity based MS quantitation enabled systematic phosphopeptide profiling from limited amounts of human chord blood samples in a collaborative project between UTU and ETHZ (described in WP05). A related method was used for systematic profiling of the tyrosine phospho-proteome, which uncovered THEMIS as a novel signalosome component (Brockemeyer et al., JBC, 2010).

Moreover, phosphoproteomics of TCR activation revealed the complex network activated by the TCR-controlled kinome and the role of the LAT-SLP-76 complex (UOXFa: Salek et al. 2013, PloSOne). Importantly, THEMIS interactome analysis carried out using methods developed in WP1, has revelead its interaction with SHP protein family members. The THEMIS-SHP complex explains its role as TCR

signal attenuator after recruitement onto LAT and in the establishment of the threshold between positive and negative selection during T cell development (UOXFa: Fu et al. Nature 2013 and Paster et al. Submitted, 2103).
Finally the work in WP01 resulted in a set of high quality monoclonal antibodies. These antibody reagents allow detection and quantitation of proteins and phosphoprotein using classical Western blotting, immunofluorescence microscopy or FACS analysis. These reagents were invaluable for studying the mechanisms by which novel signaling proteins coordinate Tcell activation and provided unique research opportunities for SYBILLA research groups. The value of these antibodies is reflected in a list of recently published reports. This includes work using antibodies against the lymphoid lineage-specific actin-uncapping protein Rltpr by the CNRS group, which lead to a recent publication in Nat. Immunology or the work by the partners CSIC and MPI-IB/ALU-FR on the recruitment of NCK to the TCR using the SYBILLA antibody directed against NCK. The panel of anti-phospho CD3zeta antibodies will enable further experimental work for modelling of early events of T cell activation.

WP02
WP2 was in charge of developing a key tool box, the availability of which has permitted to reach several SYBILLA milestones and led to several publications in highly visible journals.
More specifically, WP2 resulted in particular in the generation of robust and standardized assays for the purification and stimulation of MHC class I-restricted OT-I primary T-cells and of for primary human $CD4^+$ T-cells. For primary human T cells, electroporation protocols were achieved using a square pulse machine (Xcell, BioRad) that performs better than the Amaxa system for siRNA delivery. Protocols have been established for cytokine secretion (BioPlex, BioRad), proliferation (either CFSE dilution or thymidine incorporation), upregulation of activation markers (CD69, CD25), as well as induction of apoptosis (Annexin V/propidium iodide (PI) staining, caspase 3 activity, and Fas/FasL expression).
Spectacular achievements have also permitted primary T cell transfection with siRNA library in a 96-well mode, using a shuttle nucleofector high throughput sytem (Amaxa).
Moreover, the generation of large batches of peptide-MHC tetramer ligands allowed to stimulate T cells under physiological conditions.
One goal of SYBILLA was to generate experiment-based interactomes of several modules of the TCR signalling network. To achieve this goal, each individual protein of a module has to be appropriately tagged and expressed individually in a T-cell. Using an ORFeome collection and expression of HA-Strept III-tagged proteins and the Jurkat FLP line, several partners involved in WP2 have contributed to generate a panel of over 30 different stable Jurkat transfectants that encompasses key molecules of the TCR-signalling cassette. Those transfectants were stimulated according to a standardized protocol. Protein purification and MS analysis were subsequently performed allowing to derive publishable results for most of the engineered Jurkat T-cell cell lines.
Finally, three lines of knock-in mice expressing a One-STrEP-tag at the C-terminus of endogenous Zap70, Lat and SLP76 proteins were constructed and validated in the frame of WP2. These lines were also archived in the form of frozen embryos. They were used to analyse in a quantitative and time-resolved manner the signalling events that occur following engagement of the T cell antigen receptor in conventional T cells.

WP03
WP03 had 4 major objectives that were: 1) To decipher the role of the conformational change at CD3 in discriminating ligand quality; 2) To understand how signals of different quality are transmitted through the different TCR/CD3 forms; 3) To define the role of Lck and Fyn in ligand discrimination; 4) Kinetics of phosphorylation and assembly of the LAT-SLP76 signalosome. The goal of these objectives was to generate quantitative data in a time-dependent fashion of the effect of ligand quality on activation of very early TCR-proximal pathways. The earliest change in the TCR upon engagement by its pMHC ligand is the induction of an intramolecular change in the conformation. The involvement of the conformational change (CC) in ligand discrimination was studied using H-2Kb MHC-I tetramers loaded with antigen peptides of different affinity for the OT-I TCR. The generated results show a correlation between pMHC affinity for the TCR and the capacity to induce the CC. Unexpectedly, two key findings were generated that may help to shed light on how the TCR distinguishes self from non-self pMHC ligands: 1) the response to increasing concentrations of ligand is bell-shaped, indicating that there is concentration below and above which the response of the TCR worseness; 2) the CC in the TCR has an optimum time of ~6 min. The optimal concentration is dependent of the quality of the

ligand in a way that low-affinity pMHC would never reach such optimum. The temporal window for induction of the CC also introduces a discriminator for ligand recognition since an infinity engagement of the TCR by abundant low-quality self pMHC would not be activatory due to a countdown effect. These phenomena have been modelled in mathematical terms to provide mechanistic solutions. The requirement for the CC to activate T cells has also been studied by perturbing the outside-in transmission of signals in the TCR using a point mutant in the TCR that prevents such transmission. This point mutant (C80G) in the CD3ε subunit of the TCR in the germ-line of mice completely prevents the development of αβ T cells, thus indicating that the CC is essential for TCR signaling during development. Another aim of WP03 related to immediate TCR signaling was to generate dense datasets by phosphoproteomics that allow to follow the phosphorylation of the TCR itself as well as to generate new interactomics data to identify novel direct effectors of the TCR. We have identified Tyr111 and Tyr123 of CD3ζ, and Tyr181 of CD3ε as sites that become differentially phosphorylated by pMHC ligands of affinities closely sit at both sides of the threshold for full T cell activation. Proteomics analysis has allowed us to identify ~400 proteins many of them with differential affinity for phosphorylated vs. non-phosphorylated forms of the TCR ITAMs. Two of these two new interactors of the TCR have been studied in full, such as the known GPCR effector β-arrestin-1 and the B-Raf-associated protein Kidins220.

The phosphorylation and interactome of the immediate downstream TCR effectors Lck/Fyn has also been approached, yielding important information on the residues that become phosphorylated in these kinases. The most relevant finding of these studies is that the amount of Lck in its active form does not increase upon TCR triggering, suggesting that it is not the change in the activity of the kinase but its localization in the membrane or the accessibility of the TCR tails to phosphorylation what changes upon TCR triggering, resulting in the phosphorylation of the ITAMs. The interactome of the Lck/Fyn module has also been studied in a timely fashion allowing the construction of a hierarchy of interactions and phosphorylations in a 30 s-20 min interval.

The LAT/SLP76 signalosome has been studied using a novel technique (two-plexed IP-FCM) and a more conventional SILAC method. These studies have allowed to identify new interactors of LAT and SLP76 and to define the existence of common and isolated pools of proteins that interact with both adaptor proteins. The major conclusion from these experiments is that LAT and SLP76 do not interact exactly with the same proteins and therefore it is not correct to talk about a LAT/SLP76 signalosome since SLP76 might play roles independent of LAT and LAT plays roles not associated to SLP76. Similar data were reported by UOXFa when analyzing by MS Lat and SLP-76 intreractomes separately (unpublished data). To integrate all interactors in the at least 3 types of complexes and to understand the hierarchy of formation and their relation to the most immediate TCR effectors is something that will be pursued in the near future. Importantly, work in WP3 lead to the discovery of THEMIS and novel effector LAT-borne signaling that regulates by a negative feedback mechanism TCR signal input and, in so doing, prevents cell death in positively selecting double-positive thymocytes. These data, combined with a study of the global dynamics of TCR-proximal and distal signal propagation (e.g., TCR-induced pTyr, pSer, pThr phosphoproteome) assigned to LAT an key regulatory function to favour double-positive thymocytes and mature T cells survival after TCR stimulation.

In conclusion, our degree of knowledge on how signals are initiated and transmitted downstream the TCR has improved considerably in the 5 years of Sybilla thanks to the integration of groups with different expertise but common interests.

WP04

To induce and maintain the complete effector cytokine-producing capacity of a T cell after TCR stimulation and activation of CD28 PKC-mediated signalling for NFAT/AP-1 transactivation is essential. Despite the significant progress in assembling the PKC puzzle in T lymphocytes, defining downstream PKC substrates, including their effector functions, triggered by this phosphorylation step remained to be investigated in physiological settings. From these investigations, innovative possibilities are likely to emerge for the manipulation of T cell pathways in treating immunological diseases.

The underlying goal that has been successfully completed in WP4/SYBILLA was to understand and define PKC isotype selective signal transduction networks in T lymphocytes. This included the identification of key signalling elements of the PKC signalling intermediates and validation of innovative pathway concepts, i.e. phospho-protein signatures for different stimulation conditions or response outcomes. MUI has focused on the physiological involvement of distinct PKC isotypes in immune cell functions. Consequently, MUI performed in depth functional analysis of the physiological

effector functions of the direct PKC substrates, i.e. NR2F6, Coronin1a, CYLD, TGFbetaRI and Cbl-b and resolved these PKC effector targets in the molecular context of T cell biology. MUI has particularly defined new candidate effector pathways mediated by PKC in T cells:
(i) We provide evidence that PKC-induced signalling critically involves a pathway of the orphan nuclear receptor NR2F6, presumably by stimulating the release of NR2F6 from DNA-binding sites. This inactivation facilitates NFAT/AP-1 binding to its enhancers in the IL-2 and IL-17A promoters. In agreement, PKCalpha/theta double-knockout T cells show almost no TCR/NFAT/AP-1 transactivation signalling (Gruber et al., Mol Immunol. 2009. 46(10):2071-9), whereas NR2F6-knockout T cells show markedly upregulated TCR/NFAT/AP-1 transactivation (Hermann-Kleiter et al., J Autoimmun. 2012. 39(4):428-40).
(ii) We defined both PKCalpha and PKCtheta as redundant target PKC isotypes for the development of immunosuppressive therapeutics. For the first time, we generated PKCalpha/theta double knockout mice and examined their T cell dependent immune response phenotypes in direct comparison to mice carrying single deficiencies as well as wild type controls (Gruber et al., Sci Signal. 2009. 2(76):ra30). In short, our analysis revealed that PKCalpha and PKCtheta have complementary roles in modulating T cell immunoreactivity. A pronounced NFAT DNA-binding defect in double-knockout cells was the result of a severely reduced nuclear translocation of NFAT due to GSK3beta deregulation.
(iii) We defined the role of PKCtheta/Cbl-b axis as critical mechanism in peripheral tolerance induction. Cbl-b was established to play a key role in antigen-specific immunotolerance, nevertheless, the molecular mechanism of Cbl-b activity regulation remained unknown. We identify PKCtheta, an established activator of T cell signalling, as the critical intermediary for CD28 costimulation-induced Cbl-b inactivation. Our results reveal a nonredundant antagonism between PKCtheta and Cbl-b and PKCtheta-mediated targeting of Cbl-b for ubiquitination appears rate limiting for T cell activation responses in vitro and in vivo (Gruber et al., Mol Immunol. 2009. 46(10):2071-9). Mechansitically, Cbl-b physically interacts with and ubiquitinates SMAD7, suggesting a PTM regulation of SMAD7 by Cbl-b. Concomitant genetic loss of Smad7 abrogated both TGFbeta resistance of cblb-/- T cell cytokine responses and enhanced tumor rejection of cblb-/- mice. These results demonstrate an essential and non-redundant role of Cbl-b in controlling TGFbetaR signalling thresholds and, subsequently, T cell response output behaviour by directly targeting SMAD7 for degradation (Hinterleitner et al., Journal of molecular cell biology 2013 [Epub ahead of print].).
(iv) We defined the PKC interacting protein Coronin1a as an essential regulator of the TGFbeta receptor/SMAD3 signalling pathway in Th17 cells that plays a central role in maintaining immune homeostasis by regulating the initiation and termination of immune responses. We provide experimental evidence of a non-redundant role of Coronin1a in the regulation of Th17 cell effector functions and, subsequently, in the development of immune disease (Kaminski et al., J Autoimmun. 2011;37(3):198-208).
(v) We have been examining PKCtheta/beta single and double knockout mice and observed a redundant involvement of PKCtheta and PKCbeta in the canonical NFkappaB signalling pathway. Mechanistically, we define a PKCtheta-CYLD protein complex and an interaction between the positive PKCtheta/beta and the negative CYLD signalling pathways that both converge at the level of TAK1/IKK/IkappaBalpha/NFkappaB and NFAT transactivation. In T cells, CYLD is endoproteolytically processed upon stimulation by the paracaspase MALT1 in a PKCdependent fashion, which is required for robust IL-2 transcription. The formation of a direct PKCtheta/CYLD complex appears to regulate the short-term spatial distribution of CYLD, subsequently affecting NFkappaB and NFAT repressional activity of CYLD prior to its MALT1-dependent inactivation. This study establishes CYLD as a new and critical PKC interactor in T cells and reveals that the antagonistic PKCtheta/beta-CYLD crosstalk is crucial for the adjustment of immune thresholds in primary mouse CD3+ T cells (Thuille et al., PloS one. 2013;8(1):e53709).
Finally and (vi), we describe PKCalpha, a Ca^{2+}/phospholipid-dependent protein serine/threonine kinase, as positive regulator of the TGFbetaR/SMAD pathway. While PKCtheta has been the most studied PKC isotype in T cells due to its key role in antigen receptor signalling, PKCalpha has only more recently been implicated in T cell mediated immune functions. We revealed an essential and nonredundant immune-modulatory function for PKCalpha in adaptive immunity (Pfeifhofer et al., J. Immunol. 2006;176:6004–6011). Despite of its potential importance in T cell mediated immunity, however, the molecular aspects of PKCalpha's function in T cells and its physiological effector substrates have remained biochemically undefined. Here, we identified PKCalpha as a Signalling intermediate specific to the Th17 cell subset in the activation of TGFbetaRI. We have shown that PKCalpha physically interacts and functionally cooperates with TGFbetaRI to promote robust SMAD2-3 activation. Furthermore, PKCalpha-deficient cells demonstrated a defect in SMAD-dependent IL-2 suppression, as well as decreased STAT3 DNA binding within the Il17a promoter. Consistently,

PKCalpha-deficient cells failed to mount appropriate IL-17A responses in vitro and were resistant to induction of Th17-cell-dependent expérimental immune encephalomyelitis in vivo (Meisel et al., Immunity. 2013;38(1):41-52).
In addition, UOXFa and ALU-FR identified Kidins220 as an interaction partner of the TCR and of the Ser/Thr kinase B-Raf. IN WP04 ALU-FR could show that Kidins220 is a positive regulator of sustained Erk activation (Deswal et al., J Immunol 2013: 190(5):1927-35). This is of high relevance, since sustained Erk is required for efficient T cell activation.

WP05

The major goal of this WP was to generate kinetic data on the TCR (+/- Th1/Th2 differentiating cytokines) signalling network in human CD4+ cells and to identify transcriptional and phosphoprotein signatures that characterise the cellular response phenotype.
By using Affymetrix GeneChip Expression Analysis System, UTU completed genom-wide transcriptional profiling of 23 different activation states. Results clearly indicated that the majority of the differentially regulated genes were due to TCR activation alone. Very few changes were obtained at the early time points under the Th1 culturing conditions. However, about 1% of the analysed genome was differentially regulated (compared to only activated cells) at these early time points of Th2 culturing conditions. UTU constructed a kinetic profile of the genome-wide transcriptional response to IL-4 stimulation combined with TCR activation. In total, 640 genes were upregulated and 460 genes were downregulated by IL-4 at one or more time points. The overall expression kinetics showed that the early IL-4-mediated signalling can be roughly split into two phases; the rapid wave of up-regulation from 0.5hr to 4hr is followed by downregulation starting at 6hr after polarization. This study provides a detailed picture of the gene regulation induced by TCR activation in the presence or absence the Th1 and Th2 driving cytokines. In addition to many known genes, the lists of the regulated genes contain many novel and un-annotated genes with unknown functions.
ETHZ and UTU optimized experiments for the recovery of phosphopeptides from primary human $CD4_+$ T-cells. Consequently, UTU applied TiO_2 enrichment strategy and analyzed the global phosphopeptide content of triplicate biological samples of cord blood $CD4_+$ T cells TCR-activated and cultured for 4, 12, and 48 hrs in the absence or presence of Th1 or Th2 polarizing cytokines. The results revealed relative quantification of 753 phosphorylation sites (623 Serine, 108 Threonine, and 16 Tyrosine). The results of the canonical pathway enrichment analysis indicated that both T cell receptor signaling and CD28 signaling, as well as IL-4 signaling were significantly enriched. Furthermore, the SYBILLA partner TUT used an own currently developed novel probability-based algorithm LIGAP to analyse the temporal kinetics of the detected phosphosites. The results suggest that in each Th subset, more than 150 phosphosites are regulated in a lineage-specific manner with more than 90% probability. These lineage-specific patterns provide clues for potential novel regulatory mechanisms driving Th1 and Th2 differentiation.
UTU used an siRNA library screening approach to identify new kinases and phosphates important for both activation and differentiation of human primary CD4+ T-cells. To achieve this, UTU used the siGENOME SMARTpool siRNA libraries targeting 720 and 256 kinases and phosphatases, respectively. By using the standardized protocols (WP02), UTU studied the influences of target knockdowns on IFNγ production (in Th1 cells), Gata3 and CRTH2 expression (in Th2 cells) and CD69 expression/Annexin-PI staining (in Th0 cells). The results from this primary siRNA screening showed that among the 720 analysed kinase knockdowns, there were 5.5 % hits influencing IFNγ production either up or down. Among these, 3.8% were affecting only IFNγ whereas 1.6% were influencing IFNγ together with CD69 and/or cell viability. The corresponding values for the 257 phosphatase knockdowns were 10%, 7.7% and 2.3%, respectively. Furthermore, 5% of the kinases and 4.2% of the phosphatases influenced expression of either CD69 alone or CD69 together with cell viability. Finally, the hit frequency influencing only the cell viability was 7% and 5.8% among the kinases and phosphatases, respectively. siRNA screen in the Th2 cells showed that 8-14% of the 1000 kinase/phosphatase siRNAs behaved as hits influencing either Gata3 and/or CRTH2 expression. In a secondary siRNA screening process, re-analysis and validation of the identified siRNA hits showed that, in Th1 cells, 26 of the kinase and phosphatase hits had a reproducible influence on IFNγ production in 1-3 biological replicates. Of these, 6 siRNA hits influenced also either CD69 or cell viability. Among the Th2 siRNA hits, 7 were verified as hits affecting Gata3 and/or CRTH2, in at least 1 or 2 biological replicates. Based on the results presented here, a set of the kinase and phosphatase primary hits, influencing either Th1 or Th2, were reproduced during the secondary siRNA screening.
UTU focused on the PIM family kinase genes (PIM1, PIM2 and PIM3) which were identified as regulators of human Th1 cell differentiation. To study the target genes of these kinases, UTU used

siRNA-mediated knockdown approach combined with microarray-based platform to study how PIM knockdowns influence the global transcriptome of Th0, Th1 and Th2 targeted Th cells. Altogether, 288, 245 and 211 differentially expressed genes were found in cells polarized in Th0, Th1 or Th2 direction, respectively. Subsequent analysis identified NFATc1,NFκB, STAT5 and ATF2 whose target genes were enriched among the differentially regulated genes and supporting the idea that the effects of PIM kinases are mediated at least partly through enhanced activation of NFAT and /or NF-κB. Also as part of his task, ChIP-on-chip and ChIP-seq platforms were used to study the regulatory role of SATB1 and STAT6 and enhancers during early human Th1/Th2 cell differentiation. The results revealed that SATB1 is involved in the regulation of more than 300 genes. Furthermore, STAT6-regulated genes were identified using RNAi technology and the primary target genes of STAT6 were dissected from the downstream mediators of the signaling cascade by ChIP-sequencing. By integrating these data on a comprehensive map of detailed transcriptional kinetics of undisturbed differentiation of naive human CD4+ T cells enabled UTU to draw an experimentally validated pathway of molecular events that mediates IL-4-STAT6 signaling toward Th2 cell phenotype. In addition, enhancer-specific histone modification was used to generate the first global maps of regulatory elements during the early cell-fate specification of human T helper cells. By examining Th cells 72 hr after polarization toward the Th1 and Th2 cell lineages, UTU identified over 30,000 Th cell enhancers (including enhancers unique for Th1 and Th2 cells) and which were enriched for TF binding sites, providing insight into early gene regulation and lineage specification. The data also determined that many associated SNPs with these TFBS are regulatory SNPs and that these lineage-specific enhancers overlap a great number of SNPs from genome-wide association studies (GWAS) for various autoimmune disorders, including type 1 diabetes, rheumatoid arthritis, Crohn's disease, and asthma. Several SNPs altered TF binding-site motifs, and a subset of such SNPs within these predicted sites influenced TF binding. This provides insight into how SNPs located at enhancers may contribute to early human T cell lineage specification and disease pathogenesis and is a valuable basis for additional investigation on the role of TF binding in human disease. These chromatin-based enhancer maps provide a view of lineage-specific gene regulation during early human T cell specification. These unique enhancers and the bound TFs may be critical for driving gene expression essential for each lineage commitment.
UTU extended the PIM kinase knockdown studies to investigate the influence of PIM downregulations on the serine/threonine phosphorylation profile of a panel of signaling molecules involved in downstream TCR signalling pathways. This was done by combining siRNA-mediated knockdown of all the PIM kinases with intracellular phosphoflow cytometry analysis in human cord blood primary CD4+ T-cells which were TCR-activated and polarized towards Th1 or Th2. By using some 40 serine/threonine phospho-epitope specific antibodies, the data showed that as a result of the PIM knockdowns, the phosphorylation profile of three protein substrates (pAKTsubstrate, pPKAsubstrtae & S6rP) were changed.
Moreover, UTU studied the cellular distribution of two important proteins, SPINT2 and HNRNPK, which were identified as STAT6-interacting and STAT6-regulated proteins, respectively. By using immunofluorescence staining, SPINT2 was shown to be specifically expressed only on Th2 cells and forms pearl-like structures in the cell membrane. Furthermore, the results showed that prior to cell activation, HNRNPK is mainly in the cytoplasm. However, upon cell activation HRNPK enters the nucleus in a time-dependent manner, and co-localizes with phosphorylated STAT6 in the nucleus of the Th2 cells.

WP06

WP6 started with a data and information gathering task in order to bring state-of-the-art into a computer readable format. As was already known at the beginning of the project, much was/is known about T cell specific networks but information was unfortunately scattered around the scientific literature as well as databases. MEDICEL collected all the relevant data and information and stored them into their data warehouse system where it was accessible for the project partners. Unfortunately, MEDICEL went unexpectedly in a state of insolvency. Data collected until that point were then lost. Thinking retrospectively, however, the initial data and information collection phase was not a waste of time as it has directly or indirectly helped e.g. in constructing the large scale qualitative signalling network models (by UNIMAG).
TUT established a comprehensive database, which consists of various types of high-throughput data from T cells in different conditions. Overall, the database helps making at least three significant contributions. First, the databse serves as a unified access point to access high-throughput data from the SYBILLA project. Second, because all the data in the SybillaDB is carefully integrated and

curated, a database user does not need to go through the often very tedious work of integrating and annotating the samples individually. Third, the standard data analysis pipelines and statistical methods for high-throughput data provide a very convenient framework to analyse and integrate various types of high-throughput data, even with only little experience in the statistical data analysis. We have already demonstrated in several deliverable reports that how easily a user can carry out non-trivial integrative data analysis tasks. Analysis tasks which one meets often in practise, include e.g. integrative analysis of (1) transcriptome data and known gene function annotations and pathways, (2) phosphoprotein data and signalling pathway annotations, (3) phosphopoteome and interactome analysis, (4) and transcriptome and epigenetic data analysis. While our database system does not attempt to provide all possible data analysis options for all possible integrative data analysis tasks, it does provide the basic functionality that a non-expect bioinformatician can also copy with. The SybillaDB itself has not yet resulted in a separate publication although we are working towards such a manuscript. However, the biggest impact of the database has had on our daily systems biology work where we have used the integrated data in SybillaDB to browse through the data and, importantly, to create new biological hypothesis about T cell function.

SYBILLA has generated a large collection of highly valuable measurements of T cell function. Detailed analysis of such data can help revealing networks, which are responsible for driving the T cell activation and differentiation. Signatures of T cell activation networks have in particular been characterized using large-scale transcriptome data generated in the SYBILLA project. These include e.g. comprehensive networks associated to Th2 differentation (Elo et al., 2010), Th1 and Th2 differentiation (Äijö et al., 2012) and the role of selected regulators, such as STAT6 (Elo et al., 2010) and PIM family (Tahvanainen et al, in press). Moreover, during the SYBILLA project, we have made good progress in moving to the direction of detecting changes in dynamics. These have been reported for the Th1 and Th2 cells in (Äijö et al., 2012) and for Th17 cells in (Tuomela et al., 2012). Similarly, preliminary results on detecting human T cell activation and differentiation related dynamic phosphoproteome changes have been reported in (Deliverable D06.4).

More recently, the work on detecting signature networks has been geared from transcriptome and phosphoproteome towards epigenome. One of our key results in the SYBILLA project has the characterization of distal regulatory elements during the early phase of human T cell differentiation (Hawkins et al., 2013). Epigenomic changes are interesting for several reasons. First, epigenome is known to be responsive to environment. Thus, we can start correlating activation induced epigenomic changes with those coming from the environment. While this research direction is still in its infancy, our genome-wide enhancer maps for T cell activation provide one early milestone in that direction. Secondly, as also reported in (Hawkins et al., 2013), a large majority of autoimmune disease associated SNPs are located in intergenic regions and that these regulatory SNPs are found to overlap significantly with early T cell lineage specific enhancers. In (Hawkins et al., 2013), we demonstrated both computationally and experimentally that these SNPs at T cell specific enhancers can affect transcription factor binding. Consequently, regulatory SNPs are likely to fine-tune, either to bad or good direction, the balance between T cell subsets. These key findings of overlapping histone modifications and putative regulatory SNPs, although not experimentally validated, form an interesting set of targets for validation.

SYBILLA has made available some of the detailed T cell receptor signalling models developed in WP7. Online access to this easy-to-use simulator provides a possibiility for non-computer science experts to carry out e.g. comparative network or sensitivity analysis by varying initial concentrations or some other parameters of the model. Without the possibility to run these simulation online on our SybillaDB server, many simulation tests would remain untested. Moreover, online presentation of these models provides a simple way to test different hypothesis concerning e.g. the T cell receptor mechanisms.

As another alternative to characterize all of the mechanisms of T cell activation and differentiation, we have set up the SybillaDB together with standard data analysis methods. While detailed modelling of molecular mechanisms at whole cell level is impractical at the moment, our large collections of the experimental data provide means to better understand regulatory processes involved in T cell activation. A combination of (1) integrated and curated database, (2) easy to use statistical and explanatory data analysis, as well as (3) integration of multiple levels of measurement data (transcriptome, phosphoproteome, interactome, epigenome) provide means to explore the molecular profiles of T cell activation and differentiation in order to build a global view of the T cell function.

WP07

The mathematical modeling in WP07 followed a dual and complementary strategy by developing large-scale models of information flow through the network following Boolean ("on-off") rules and dynamic models of modules of T-cell signalling that incorporate mechanistic detail. The research of WP07 was divided into five interconnected tasks.

Task 1 has been concerned with dissecting the combinatorial complexity of TCR signalling. Following a data-driven approach, MPI-IB, ALU-FR, UNIBAS, CSIC and DKFZa developed the first experimentally validated mathematical model of multivalent ligand binding to the TCR and the CD8 coreceptor, including the formation of TCR and CD8 clusters. This model showed that multivalent ligand binding provides, in addition to the previously hypothesized kinetic proofreading, a further mechanism for achieving a hallmark of T-cell activation: the inability to compensate poor ligand affinity with high concentration. Intriguingly, the model predicts non-monotonic dose-response curves of T-cell activation with respect to ligand concentration that have subsequently been confirmed experimentally. A second key aspect of combinatorial complexity at the level of the TCR is multi-site phosphorylation and recruitment of interaction partners to the phosphorylated TCR. We achieved the determination of the kinetic parameters of the reversible phosphorylation and protein recruitment processes that immediately follow TCR triggering from experimental data (MPI-IB, ALU-FR, using reagents from EXBIO WP01), thus providing an important resource for further quantitative studies.

Task 2 on systems analysis of ligand discrimination by the TCR complex built directly on the modelling results of the first task. In this area, already many mathematical models exist; DKFZa progressed beyond the state of the art by strictly adhering to a data-based modelling strategy. Rather than investigating what mechanisms could potentially explain the observed accurate ligand discrimination by T cells, we asked which mechanisms were implied by, and consistent with, quantitative data obtained for this purpose within the SYBILLA consortium. This work showed that a definite answer on how self-nonself discrimination is achieved mechanistically cannot yet be given but it generated a number of specific and quantitative hypotheses, several of which could already be proven experimentally by consortium partners. Most importantly, kinetic proofreading of TCR ligand dwell time by TCR proximal signalling events is compatible with time-resolved data on these processes but appears to require synergy between several mechanisms of TCR triggering, including regulated kinase access and phosphatase displacement.

Task 3 was devoted to the qualitative modelling of the signalling network using Boolean modelling approaches (UNIMAG). Starting from an initial model that connected the TCR and its coreceptors via signal transduction to the activation of key transcription factors and mediators of cell growth, the model has been continuously expanded to include chemokine and cytokine receptors and thus developed into a unique comprehensive resource for studying the T-cell signalling network that is available online. Importantly, by analysis signal flow solely based on Boolean rules, hitherto unknown dependencies in the signalling network were predicted and subsequently verified experimentally, including the dependence of Erk activation on Src kinase, PI3 kinase and novel PKCs and the role of the Src kinase Fyn. Moreover, a technique for a direct dynamization of this large-scale network was developed.

The mechanistically-based kinetic modelling of the signalling network in T cells was subject of Task 4. A key result of this modeling approach linked with parallel experimental work has been the discovery that antigen signalling gates cytokine signals that drive T-cell differentiation. Cyclosporine A-sensitive antigen signaling through the NFAT pathway exerts both activating and inhibitory effects on CD4+ T cell differentiation and thus orchestrates the initial polarization of cytokine producers upon antigen challenge but delays definite differentiation into cytokine-specialized memory cells until the immune response proves effective.

Task 5 sought to exploit the results of the foregoing tasks to identify sensitive nodes in the T-cell signalling network that are potential pharmacological targets for therapeutic intervention. We developed and experimentally validated a computational framework that links dynamic network models to cell proliferation. These results provide a complementary approach to the druggable-target concept for pharmacology by identifying (cell type-specific) signalling nodes that have particularly high control on a proliferation or other cell functions. Moreover, we have provided the first mathematical model to statistically infer the program of T-cell differentiation into memory and effector cells from experimental data, showing that memory precursors develop early during an immune response and give rise to rapidly proliferating, short-lived effector cells both during primary and recall immune responses.

Taken together, WP07 has established lasting and synergistic interactions between the mathematical modelling and the experimental investigation of T cell signalling that have led to unprecedented quantitative insights and form a fruitful basis for further systems-medicine approaches in immunology.

Finally, one goal of SYBILLA was to generate an on-line interactive version of the T-cell signalling network (called virtual T cell) to the scientific community. This is now possible via the world-wide web using the SYBILLA homepage (www.sybilla-t-cell.de) that will be active for the next years. The virtual T cell includes 403 elements (proteins or other signalling molecules) covering 15 inputs (receptors such as the TCR, its co-receptor CD4/CD8, IL2 receptor, etc), and 52 outputs (representing transcription factors, cell cycle regulators, etc.). We believe that the virtual T cell provides an optimal platform to analyze how subtle differences in individual nodes might have a global influence on the activation state of T cells. Additionally, our virtual T cell provides predictions which indicate the signaling component(s) whose function may be altered, thereby allowing researchers to focus their experimental efforts.

WP08

The goals of WP08 were to characterize the dynamic properties of the TCR-CD3 signaling network in primary T cells. We wanted to do this in order to validate the models we had generated in previous WPs. To do this we took several approaches.

We first developed an optimized protocol to transfect siRNAs with the aim of decreasing expression of several key, signaling molecules. By reducing the expression of a central signaling molecule, we were able to evaluate the effects on TCR signaling; this allowed us to validate some of the key properties of our models. Another key validation step came from the analysis of a CD3ε mutant, which fails to transduce a conformational change. The key finding was that the Ca2+ dependent signaling pathways were deleterioiusly affected in the absence of a conformational change while PI3K mediated signaling pathways were unperturbed. This implies that there is a very proximal divergence in TCR mediated signaling. An additional project focused on the role of Kidins220 in mediating sustained ERK signaling. Reconstituting irradiated mice with Kidins220 deficient bone marrow resulted in a block of T cell development at the DP stage. This argues for a role of sustained ERK signaling in positive selection and adds additional experimental support for the idea, which was initially suggested by another Sybilla member. Experiments in this aim used a genetically encoded purification tag on SLP-76 or ZAP-70 to allow isolation of signaling complexes developing over a defined time period. A detailed of ζ chain phosphorylation showed that the individual tyrosine and serine residues are phosphorylated with different kinetics. siRNA treatment was used to decrease the levels of the PAG protein which regulates src kinase activity by recruiting Csk. Partial depletion of PAG led to increased src kinase activity resulting in increased activity of proximal TCR signaling. However surprisingly these T cells became anergic rather than hypersensititve. Maintaing a proper level of src kinase activity in unstimulated cells seems to be important in maintaining their ability to response to TCR engagement. Finally, a mutant CXCR4 was introduced into murine T cells. This provides a model system to study the signaling defect observed in patients suffering from WHIM syndrome. T cells expressing the mutant CXCR4 failed to form long lasting interactions with APCs resulting in defective T cell priming. This eventually led to a IgG response to antigen. Overall, WP08 made significant headway into establishing the role of several key, signaling proteins in maintaining the T cell in a “state of readiness” to efficiently respond to antigen

WP09

The overall objective of WP09 has been to understand how the TCR/CD3 signalling network is perturbed in T cell-mediated diseases. This objective has been approached from several different directions. In the first instance systemic changes in this signalling network were explored by partner CNRS, using mice with Th1- and Th2-driven autoimmune pathologies. More specifically, mice carrying particular genetic changes in genes encoding components of the TCR transduction machinery have been used to better comprehend the regulation of TCR signalling events. For example, the LAT adaptor protein is a critical node of the TCR signalling machinery, and *in vivo* modelling studies have shown that defective LAT signalosomes lead to a pathological condition that indicates the importance of LAT in regulating T cell homeostasis and terminal differentiation (Archambaud *et al*. 2009 *J. Immunol*.; Mingueneau *et al*. 2009 *Immunity*; Roncagalli *et al*. 2010 *Semin. Immunopathol*.; Roncagalli *et al.* 2010 *Trends Immunol*.; Liang *et al*. 2013 *Nat. Immunol*.). In addition, a more holistic insight into the signalling network that causes Th1- and Th2-driven autoimmune pathologies has been obtained using the LAT mouse models in conjunction with phospho-proteomic and transcriptomic analyses (along with partners UOXFa, ETHZ and UTU) that has further contributed to the interpretation of these models. Key aspects of this network are the phosphorylation kinetics and the assembly of the LAT-SLP76 signalosome. Quantitative meaurements have been used (along with partners UOXFa and

ETHZ) to investigate these aspects and the resulting data have been integrated into the kinetic models of the LAT-SLP76 signalosome.
This work performed as part of Tasks 09.1 and 09.2 has allowed the initially planned objectives to be met. In achieving these goals a number of state-of-the-art methodologies have been employed: partner CNRS have used a “fast-track assembly line” platform to engineer innovative knock-in mouse models, in-depth signalling analyses have been performed using high-throughput, global phospho-proteomic and transcriptomic analyses, and phospho-specific antibodies have been used combinatorially in multiplexed biochemical assays.
The perturbation of TCR/CD3 signalling in T cell-mediated diseases has also been investigated by partner UOXFb in the context of the T cell-driven autoimmune condition, multiple sclerosis (MS), through an analysis of how this signalling interacts with disease-associated cytokine-induced signal transduction cascades. The objectives pertaining to this investigation were approved following amendments in a prior reporting period. In order to be able to assess the role of cytokine signal transduction in conjunction with TCR/CD3-mediated signalling, the functional effect of MS-associated SNPs on the IL-7R pathway were assessed with primary human cell samples using a genotype-selectable donor cohort and a bioinformatics analysis of the components shared between MS-associated candidate gene pathways and the TCR signalosome were assessed (with partner TUT). Using these results together with partner UNIMAG’s Boolean modelling, primary human immune cells were further used to investigate the crosstalk between the TCR and the IL-7 signalling pathways. It was demonstrated that there was a level of synergism between these signalling cascades, as indicated by the enhanced effects observed on IL-7RA downregulation and IL-2RA and CD4 co-receptor upregulation when cells were co-cultured with TCR-targeting stimuli and IL-7, but a certain degree of antagonism between the pathways was also observed. Overall, these findings demonstrate a complex crosstalk between the TCR/CD3 and the IL-2/7 signalling pathways that may have varying roles under homeostatic and activatory conditions to promote autoreactivity. This complexity was further dissected using TCR-MHC class I and II humanized mouse model systems and showed that disease-relevant TCR/CD3 signalling occurring in the context of transgene-induced perturbed cytokine signalling that promotes disease risk ultimately leads to changes in T helper cell differentiation (in particular Th1, Th17 and Th1/17 cells) and in the regulation conferred by the CD8+ T cell compartment.
The described work and findings have allowed the new deliverables D09.3-D09.7 to be met. The use of genotype-selectable volunteer cohorts is a recent approach that has had a significant impact on the experiments performed as part of Tasks 09.7 and 09.8. In addition, study design aspects of this task have contributed to proof-of-principle work in this field performed by UOXFb (Gregory *et al.* 2012 *Nature*; Fugger *et al*. 2012 *N. Engl. J. Med*.; Dendrou *et al*. 2013 *Nat. Med*.). In addition, performing phosflow and other functional experiments on the basis of Boolean modelling is a hitherto unused methodology in the context of assessing disease-relevant pathways and next generation transgenic technologies were also used for the *in vivo* modelling (Attfield *et al*. 2012 *Immunol. Rev*.; Attfield & Fugger 2013 *J. Immunol*.), such that a state-of-the-art approach has been employed to address the collective objectives.

4. POTENTIAL IMPACT AND MAIN DISSEMINATION ACTIVITIES AND EXPLOITATION RESULTS

SYBILLA impact achieved in comparison to what was documented in Annex I of the grant agreement:

T-cells are a type of white blood cell that circulate around our bodies, scanning for infections. Sometimes the detecting process goes awry, and the T-cells mistakenly attack the body's own cells, as in autoimmune diseases, or harmless substances as in allergies. These diseases are increasing in the Western world and good cures are often missing. In order, to develop new therapeutic treatments for these diseases, one needs to better understand how T-cells become activated and how they can distinguish infected cells from healthy cells or inoffensive substances.
SYBILLA has developed and made public a large number of standardized protocols to study the activation of T-cells. This is important, so that experiments done by different groups can be directly compared. Together with new reagents and mouse models that SYBILLA has developed, this allowed gaining detailed insight into the molecules that govern T-cell activation. SYBILLA has found a large number of new genes that are involved in T-cell activation and has done experiments to discover their function. Some of them might turn out to be ideal drug targets or biomarkers to refine prognosis of autoimmune diseases in the future.
In addition, SYBILLA has uncovered molecular mechanisms with which the immune system discriminates between the healthy cells of the own body and infected cells. Importantly, SYBILLA has produced an interactive online platform to show what happens when T-cells decide whether to trigger an immune response against foreign substances. The system allows researchers to test different combinations of factors and inhibitors, shining a light onto the workings of the T-cell. This so called "virtual T cell" is now publicly-available allowing researchers to click through the signalling network of the T-cells. Users can switch on 12 receptors, including the T-cell receptor, identify the signals on the surface of other cells, or bind messengers. Taking account of the 403 elements in the system, the model then computes the behaviour of the network. The result is a combination of the activity of 52 proteins that predict what will happen with the cell.

A detailed description fo the potential impact for each WP is given below :

WP01
It can be expected that the technical advances developed in WP01 will impact on systems level analysis of Tcell signaling. Specifically the developed MS based techniques for unbiased characterization of dynamic protein interaction networks and signaling complexes in truely physiological systems will open entire new analytical possibility for the Tcell research community. This represents a significant step forward in the field and is likely to provide new mechanistic insights into the coordination of concurrent signaling processes. The achieved comprehensiveness and quantitative accuracy of MS based protein measurements will facilitate model development to better predict the outcome of molecular perturbations often associated with autoimmune diseases. In addition the sensitive profiling of phosphopeptide abundance from small amount of human samples will hopefully allow for a systematic monitoring of complex signaling states associated with immunopathological conditions. Finally we expect that panel of monoclonal SYBILLA antibodies besides facilitating basic research goals, have commercial potential for subsequent exploitation by SYBILLA partner EXBIO.
The findings and key experimental approaches of WP01 have been published: Borroto et al. 2013 *Journal of immunology* 190, 1103; Dopfer et al. 2010 *Immunology letters* 130, 43; Liang et al. 2013 *Nature immunology* 14, 858; Salek & Acuto 2012 *Analytical chemistry* 84, 8431; Wepf et al. 2009 *Nature methods* 6, 203; Brockmeyer et al. 2011 *J biol chem* 286, 7535; Salek et al. 2013, *PloS One* 8 (10) e77423.

WP02
The panel of knockin mice generated in the frame of SYBILLA WP02 permits to analyse in a quantitative and time-resolved manner the signalling events that occur following engagement of the T cell antigen receptor. They also permit to analyse those events in conventional T cells, regulatory T cells as well as developing T cells. The unprecedented level of resolution reached with those mice

Mis en forme : Anglais (États Unis)

permit to assess with a high resolution the impact of immunomodulatory compounds and also help defining the signalling nodes that are the most vulnerable to pharmacological manipulation.
The findings and key experimental approaches of WP02 have been published: Archambaud et al. 2009, *J Immunol.* 182(5):2680-9; Mingueneau et al. 2009 *Immunity* 31(2):197-208 ; Roncagalli et al. 2010 *Semin Immunopathol*. 32(2):117-25 ; Roncagalli et al. 2010 *Trends Immunol*. 31(7):253-9 ; Chevrier et al. 2012 *Front Immunol*. 3:27 ; Liang et al. 2013 *Nat Immunol*. 14(8):858-66

WP03
The work developed in WP03 along the 5 years of Sybilla has allowed and will allow the publication of joint scientific papers in journals of high impact. Most data is still to be published, for instance phosphoproteomics of T cells stimulated with ligands of varied affinity, models derived of WP03 indicating that a dimeric pMHC is required to trigger the TCR and facilitates ligand quality discrimination. The effect of perturbation of signal transduction with TCR, Lck and LAT mutants is also to be published. All this data will provide the scientific community a better understanding of the early TCR signaling events. Although many data generated in WP03 is still to be published, the results generated jointly in WP03 have been disseminated in congresses, symposia and individual talks all around the world. In addition to the scientific interest of the main findings in WP03, there are findings that are susceptible for patenting like the two-color IP-FCM technique and hundreds of data showing new interactions and new phosphorylation sites that open the door to the exploitation of these findings for the development of T cell activation inhbitors of use in the treatment of transplant rejection and autoimmune diseases.
The findings and key experimental approaches of WP03 published so far: Stirnweiss A et al. (2013) *Sci. Signal. 6:ra13*; Martínez-Martín, N et al. (2009) *Science Signaling* 11;2(83):ra43; Arechaga, I et al. (2010). *Int. Immunol*. 22: 897-903; Kumar, R et al. (2011). *Immunity* 35: 375-387; Martínez-Martin, N et al. (2011). *Immunity* 35: 208-222; Blanco, R & Alarcón, B. (2012). *Frontiers Immunol* 3:115; Borroto, A. et al. (2013). *J. Immunol*. 190: 1103-1112; Schamel, WWA & Alarcón, B. (2013). *Immunol. Rev.* 251: 13-20.; Deswal S et al., *J Immunol*. 2013 Mar 1;190:1927-35; Nika Ket al. Immunity. 2010. 25;32:766-777; Brockmeyer C, et al. *J. Biol. Chem*. 2011. 286:7535-47; Reinherz EL et al. *Front Immunol.* 2011;2:3; Salek M, Acuto O. *Anal Chem*. 2012 Oct 16;84(20):8431-8446; Paster W., et al. J. Immunol. 2013 190:3749-3756; Salek, Met al. 2013. PloS One. Oct 30;8(10):e77423.

WP04
In view of the urge to develop pharmaceutical drugs for immunosuppression, our academic results served to foster a compound finding program by Novartis Basel leading to the potent PKC selective inhibitor Sotrastaurin as a new class of immunosuppressive agents affecting T cell activation. In addition, our work also paved the way to develop international cooperations within SYBILLA and its leading scientists from the translational research fields of autoimmunity and will foster extensive efforts towards developing immunosuppressive therapeutics targeting these PKC pathways even beyond the SYBILLA program time frame.
The findings and key experimental approaches of WP04 have been published in several scientific articles to date (Key original publications: Gruber et al. 2013 *Journal of molecular cell biology*; von Essen et al. 2013 *European journal of immunology* 43(6):1659-66; Meisel et al. 2013 *Immunity* 38(1):41-52; Thuille et al. 2013 *PloS one* 8(1):e53709; Hermann-Kleiter et al. 2012 *J Autoimmun*. 39(4):428-40; Hinterleitner et al. 2012 *PLoS One* 7(9):e44295; Lutz-Nicoladoni et al. 2012 *Immunol Cell Biol* 90(1):130-4; Kaminski et al. 2011 *J Autoimmun* 37(3):198-208; Gruber et al. 2010 *Immunol Lett* 132(1-2):6-11; Evenou et al. 2009 *J Pharmacol Exp Ther* 330(3):792-801; Gruber et al. 2009 *Sci Signal* 2(76):ra30; Gruber et al. 2009 *Mol Immunol* 46(10):2071-9. Reviews: Gruber et al. 2013 *Oncoimmunology* 2(2):e22893; Wallner et al. 2012 *Clin Dev Immunol* 2012:692639; Pfeifhofer-Obermair et al. 2012 *Front Immunol* 3:220; Hermann-Kleiter N, Baier G. 2010 *Blood* 115(15):2989-97; Baier G, Wagner J. 2009 *Curr Opin Cell Biol* 21(2):262-7). One SYBILLA-related patent was filed: (WO/2011/119062) Cbl-b AS PKCtheta MARKER.

WP05
A complex interactions of gene signaling and transcriptional regulatory networks and chromatin structure that can act as a gatekeeper, control the cell-fate decisions. A profound and integrated understanding of the intracellular signalling networks (which determine the fate of T-helper cells during their differentiation) will allow to develope new biological models and biomarkers and identify intracellular drug targets to treat immune-mediated diseases such as autoimmune diseases, allergy and asthma. These diseases constitute largest category illnesses dominating in the industrialised world and costing society enormous amounts of resources and money. The data generated within

WP05 (including: identification of the major players in the IL-4-STAT6-mediated regulation of gene expression during the early human Th2 cell differentiation process; generation of a high confidence Th cell lineage specific genes; and characterizing the lineage-specific enhancer repertoire of activated and early differentiating Th1 and Th2 cells) provide a unique resource for studies on Th cell differentiation and, in particular, for rational design of therapy for pathogenic human immune responses. As en example, it was found in T05.3 that the lineage-specific enhancers overlap a great number of SNPs from genome-wide association studies (GWAS) for various autoimmune disorders, including type 1 diabetes, rheumatoid arthritis, Crohn's disease, and asthma. Several SNPs altered transcription factor (TF) binding-site motifs, and a subset of such SNPs within these predicted sites influenced TF binding. This provides insight into how SNPs located at enhancers may contribute to early human T cell lineage specification and disease pathogenesis and is a valuable basis for additional investigation on the role of TF binding in human disease. Hopefully these data will accelerate and pave the way to develope better diagnostic tools and successful treatment of the immune mediated diseases and lead to an improved health and a better quality of life.
The findings and key experimental approaches of WP05 have been published: Ahlfors et al. 2010 *Blood* 116: 1443-1453; Elo et al. 2010 *Immunity* 32: 852-862; Edelman et al. 2012 *BMC Genomics* 13, 572; Hawkins et al. 2013 *Immunity* 38: 1271-1284; Tahvanainen et al. 2013 *J Biol Chem*. 1;288(5):3048-58.

WP06

High-throughput measurement technologies procude immense amounts of quantitative information about molecular state of a cell population. The use of these technologies in biolocal research creates two kinds of needs: data storage, and data analysis. During the SYBILLA project we have worked to provide useful solutions to both of these needs.
While a number of biomolecular databases exist in the world, they are typically general purpose databases aiming at serving all subfields of biosciences. The use of such technology platforms can be cumbersome, or at least far from optimal, if one is interested in only one targeted biological question. Having this in mind, we have set up a T cell specific database, which consists of highly curated and integrated information and data from T cells. We believe that our approach can have socio-economical impact. First, an open-source resource on T cell data will definitely raise interest in research groups in universities and industry, both in EU countries and elsewhere. Second, our sincerest hope is that our database, as well as the results from the whole SYBILLA consortium, will at the end contribute (directly/indirectly) to help improving diagnostics and/or treatment of various autoimmune/T-cell related diseases. Third, given the increasing amount of quantitative data produced in the systems biological field, the needs for storing massive data collections are constantly increasing. Correspondingly, solutions to store, retrieve and analyze large data collections will come even more common in the future, and commercial solutions for that purpose are expected to gain a larger market share. This is where our T cell specific database may have potential for commercialization.
Similar conclusions as for the database apply also to computational and statistical tools used and developed in the SYBILLA project. During the project, we have developed several novel strategies to analyze temporal data (both WP6 and WP7), which is essential in order to correctly interpret complex time-course data for T cell activation and differentiation. Majority of the implemented computational tools are publicly available for research community thus helping to solve similar problems in projects.
Finally, the key results from WP6, identification of T-cell activation network signatures, are all disseminated via peer-reviewed publications.
WP06 led to the following publications: Äijö T et al., *BMC Genomics*, 13:572, 2012; Deswal S. et al. *PLoS ONE* 6:e22928 ; Elo, L. L. et al. *Immunity*, Vol. 32, No. 6, pp. 727-862, 2010; Hawkins RD et al. *Immunity* 38(6):1271-1284; He, H.H et al. 2010 *Nat. Genet*. 42, 343–347; Heintzman, N.D. et al. (2007). *Nat. Genet*. 39, 311–318; Kong, L et al. (2013) *Nature Methods*, 10, 814-815; Tahvanainen J, et al. *The Journal of Biological Chemistry*, in press; Tuomela S. et al. *Blood*, Vol. 119, No. 23, pp. e151-160, 2012.

WP07

The work in WP07 has strongly advanced the development of quantitative and systems immunology by establishing true synergies between modelers and experimentalists that will actively continue beyond SYBILLA. Establishing data-driven modelling as a pivotal tool in this field, unprecedented quantitative insights have been gained at multiple levels, including the fundamental mechanisms of self-nonself discrimination by T cells, the wiring of the signalling network, and the interplay of antigen

signalling with cytokine production and memory differentiation. A novel application of mathematical modelling to the statistical inference of differentiation programs of T cells from *in-vivo* data has been devised and successfully applied to define the program of CD8 T cell diversification into effector and memory cells. All these insights have very considerable application potential to systems medicine, including T cell pharmacology, vaccination research and cell therapy. Because of the novelty of using mathematics to infer biological mechanism from experimental data, it is expected that further broadening of basic systems-medicine research in immunology will precede major efforts by the pharmaceutical industry. A key step to disseminate SYBILLA findings has been the online presentation of its models (Virtual T cell).
WP07 led to the following publications: Schulz E et al. *Immunity* 30, 678-688 (2009); Salazar C et al. *FEBS J.* 276, 3177-3198 (2009); Salazar C et al. *Trends Cell Biol* 20, 634-641 (2010); Beyer T et al. *PLoS Comput Biol* 7: e1002121 (2011); Deswal et al. *PLoS One* 6:e22928 (2011); Ballerstein K et al *Front Biosci* 5:149-166 (2013); Buchholz V et al. *Science* 340, 630-635 (2013) and contributed to WP03 publication Nika K et al. *Immunity* 32: 766-777 (2010).

WP08
The work in WP08 has increased our understanding of several different foci of T cell signaling. Both proximal and distal TCR signaling pathways were investigated. Some of the work has been published and other studies will be published in the near future. The publications are listed separately. Much of this work has also been presented at scientific congresses where it has reached a wide scientific audience. Among other things, the exploitable findings include the role of PAG in maintaining T cell responsiveness. While it will take some time to know whether these findings can be translated into medicines or therapies, this kind of ground breaking basic science is always the first step towards treating disease and improving our health.
WP08 led to the following publications: Smida M et al. 2013 *Signal*. 11:28; Cammann C et al; 2012 *Journal of Clinical & Cellular Immunology* S12: 012; manuscripts describing the work as part of two doctoral theses are in preparation.

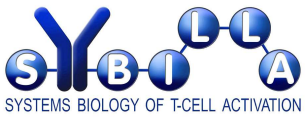
WP09
A significant proportion of the European population suffers from T cell-driven diseases, many of which are not treated satisfactorily; for example in the case of MS some 2.5 million people are affected by this condition worldwide and there is no cure. Organ-specific and systemic autoimmune diseases as well as allergies are caused by T cell-dependent, unbalanced immune responses. The work performed through this workpackage has: (i) contributed to the understanding of TCR/CD3 signalling under pathophysiological conditions, (ii) enabled the generation of a panel of knockout mice that may have a therapeutic impact by allowing the identification of novel drugable targets in the context of Th1 and Th2-driven conditions, (iii) provided initial evidence suggesting that genotype-dependent patient stratification may be beneficial for the more efficacious treatment of patients with complex diseases, and (iv) raised the possibility of cell-based therapeutic strategies in treating these diseases. The potential for more appropriate treatment of patients with T cell-driven diseases may have an impact not just on the patients themselves, but also on their family, friends and healthcare providers and would therefore have an a broader socio-economic impact.
The findings and key experimental approaches of WP09 have been published in several scientific articles to date (Archambaud *et al*. 2009 *J. Immunol*.; Mingueneau *et al*. 2009 *Immunity*; Roncagalli *et al*. 2010 *Semin. Immunopathol*.; Roncagalli *et al.* 2010 *Trends Immunol*.; Liang *et al.* 2013 *Nat. Immunol.*; Harkiolaki *et al*. 2009 *Immunity*; Fugger *et al*. 2009 *Nat. Rev. Immunol*.; Attfield *et al*. 2012 *Immunol. Rev*.; Fugger *et al.* 2012 *N. Engl. J. Med*.; Gregory *et al.* 2012 *Nature*; Dendrou *et al*. 2013 *Nat. Med*.; Attfield & Fugger 2013 *J. Immunol*.; Haghikia *et al.* 2013 *Trends Mol. Med*.). In addition, the work performed as part of WP09 has been disseminated through international conferences, symposia and workshops to the wider scientific community, but has also been disseminated to the general public through presentations and online news websites.
The exploitable foreground resulting from this WP includes: (i) a panel of proprietary knockout mice that may allow the identification of novel drugable molecules involved in T cell activation that will be used for the general advancement of knowledge (partner CNRS, forseen embargo date: end of 2013), and (ii) a proprietary transgenic mouse model with perturbed IL-7 signalling to be used for the general advancement of knowledge (partner UOXFb).

5. ADDRESS OF PUBLIC WEBSITE AND RELEVANT CONTACT DETAILS

Project website address:

www.sybilla-t-cell.de

Project Logo



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