Role of Akt2 and SGK1 in Regulation of Dendritic Cells

Bedeutung von Akt2 und SGK1 bei der Regulation von Dendritischen Zellen

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Abbreviations

$[Ca^{2+}]_i$	intracellular calcium concentration
Ag	antigen
APC	antigen presenting cell
AM	acetylomethyl
BMDC	bone marrow-derived dendritic cell
cDC	conventional dendritic cell
CICR	Ca^{2+}-induced Ca^{2+} release
CRAC	Ca^{2+} release-activated Ca^{2+} channel
CRACM 1/Orai1	calcium release-activated calcium modulator 1
DC	dendritic cell
DCFDA	2',7'-dichlorodihydrofluorescein diacetate
EGTA	ethylene glycol tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
FACS	fluorescence activated cell sorter
FBS	fetal bovine serum
GM-CSF	granulocyte-macrophage colony-stimulating factor

GPCRs	G-protein-coupled receptors
I_{CRAC}	Ca^{2+} release-activated Ca^{2+} currents
IFNs	interferons
IL	interleukin
IP_3	inositol-(1,4,5)-trisphosphate
IP_3R	inositol-(1,4,5)-trisphosphate receptor
RT-PCR	real time polymerase chain reaction
LPS	lipopolysaccharide
MHC	major histocompatibility complex
NEAA	non-essential amino acids
NCKX	K+-dependent Na+/Ca2+ exchangers
NCX	K+-independent Na+/Ca2+ exchangers
NK cell	nature killer cell
P/S	penicillin/streptomycin
PBS	phosphate buffered saline
PDK1	phosphoinositide-dependent kinase 1
PI3K	phosphatidylinositol-3-kinase
pDCs	plasmacytoid dendritic cells

PH	pleckstrin-homology
PKB /Akt	protein kinase B
PLC	phospholipase C
PMCA	plasma membrane Ca^{2+}-ATPases
PtdIns(4,5)P2	phosphatidylinositol-(4,5)-bis-phosphate
RT-PCR	real time polymerase chain reaction
RyR	ryanodine receptor
SERCA	sarco/endoplasmic reticulum Ca2+-ATPases
SGK	serum and glucocorticoid inducible kinase
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOCE	store-operated Ca^{2+} entry
SOCs	store-operated channels
STIM1	stromal interaction molecule 1
TLR	Toll-like receptor
TNF	tumor necrosis factor

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Abstract

The present study investigates the role of Phosphoinositide 3 kinase (PI3K) pathway in the regulation of dendritic cell (DC) functions. DCs are antigen-presenting cells that provide a link between innate and adaptive immunity and are required for initiation of specific T cell-driven immune responses. The present project concentrates on the possible regulation of DC functions by two PI3K-dependent kinases, Akt2/PKBβ and serum- and glucocorticoid-inducible kinase-1 (SGK1), which share 55% identity in their kinase domain regions.

Akt2/PKBβ is known to be required for macrophage and DC migration, though the involved mechanisms remained undefined. On the other hand, DC migration is governed by Ca^{2+} signaling. In the present study, we addressed the mechanisms of Akt2-dependent DC migration by studying the possible involvement of Akt2 in Ca^{2+} signaling. DCs were derived from the bone marrow of Akt2-deficient mice (*akt2*$^{-/-}$) and their wild type littermates (*akt2*$^{+/+}$) and their maturation was induced by lipopolysaccharides (LPS, 1μg/ml). Chemokine CCL21 (25 ng/ml)-induced migration of mature *akt2*$^{-/-}$ DCs was impaired compared to *akt2*$^{+/+}$ DCs. In Ca^{2+} imaging experiments, CCL21 (75 ng/ml)-triggered increase in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) was also significantly diminished in mature *akt2*$^{-/-}$DCs. Moreover, release of Ca^{2+} from intracellular stores induced by thapsigargin (1 μM) and the following store-operated Ca^{2+} entry (SOCE) were reduced in mature *akt2*$^{-/-}$ DCs. Stimulation of P2Y receptors with ATP (100 μM) resulted in IP_3-dependent Ca^{2+} release, which was also significantly decreased in mature *akt2*$^{-/-}$ DCs. IP_3-induced activation of Ca^{2+} release-activated Ca^{2+} (CRAC) channels measured in whole cell patch-clamp experiments was significantly lower in mature *akt2*$^{-/-}$ DCs. Analysis of the expression of IP_3 receptor isoforms in *akt2*$^{+/+}$ DCs and *akt2*$^{-/-}$ DCs by RT-PCR revealed a significantly impaired transcript of IP_3R2 in *akt2*$^{-/-}$DCs. Upon 1h of LPS stimulation mRNA abundance of IP_3R2 was increased in *akt2*$^{+/+}$ but not in *akt2*$^{-/-}$ DCs. Accordingly, protein abundance of IP_3R2, analyzed by western blot, was reduced in mature *akt2*$^{-/-}$DCs. mRNA abundance of transcription factor Ets1, which is known to upregulate the IP_3R2 transcription, was significantly lower in immature *akt2*$^{-/-}$ DCs than in *akt2*$^{+/+}$ DCs. Similarly to IP_3R2, mRNA level of Ets1 was upregulated by LPS (1h) in *akt2*$^{+/+}$ but not in *akt2*$^{-/-}$ DCs. ETS1 protein had lower expression in mature *akt2*$^{-/-}$ DCs compared to *akt2*$^{+/+}$ DCs. DC migration was sensitive to the IP_3R inhibitor Xestospongin C (5 μM), which significantly decreased CCL21-induced migration of *akt2*$^{+/+}$DCs and abrogated the differences between genotypes. Finally, knock-down of ETS1 with siRNA led to impaired RNA abundance of IP_3R2, thapsigargin- and ATP-induced Ca^{2+} release from the stores, SOCE and CRAC channel activation, as well as DC migration. In conclusion, Akt2

upregulates IP_3R2 transcription in DCs, presumably by enhancing the expression of ETS1 and this effect may underlie the stimulating effect of Akt2 on DC migration.

On the other hand, PI3K activation suppresses proinflammatory cytokine production in DCs, which limits excessive T helper (Th1) polarization. Little is known about the mechanisms of PI3K-mediated suppression in DCs. The present study explored whether SGK1 can act as a negative regulator of DC functions downstream from PI3K. To this end, DCs were isolated from the bone marrow or from the spleen of gene targeted mice lacking functional SGK1 (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$). Expression of maturation markers, MHC II, CD86 and CD54, analyzed by flow cytometry, and secretion of interleukin IL-12 upon LPS (100 ng/ml) stimulation, determined by ELISA, were significantly higher and phagocytic capacity, assessed as FITC-dextran uptake, was significantly lower in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ DCs. Moreover, expression of CD86, MHCII and CD54 was also significantly higher in DCs isolated from the spleen of *sgk1*$^{-/-}$ mice than those from *sgk1*$^{+/+}$ mice. Moreover, the nuclear localization of the p65 subunit of the transcription factor NF-κB was significantly enhanced in *sgk1*$^{-/-}$ DCs. N-myc downstream regulated gene 1 (NDRG-1), the physiological substrate of SGK1, which is known to attenuate NF-κB signalling, was similarly expressed in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ DCs, however SGK1-dependent phosphorylation of NDRG-1 was decreased in *sgk1*$^{-/-}$ DCs. Silencing of NDRG1 by siRNA in *sgk1*$^{+/+}$ DCs led to an enhanced nuclear abundance of p65 compared to the control empty vector-treated DCs. These observations point to an important role of SGK1 in the regulation of DC functions and thus disclose a novel element in the cross-talk between innate and adaptive immunity. Impaired phosphorylation of NDRG-1 in SGK1-deficient DCs may underlie enhanced nuclear localization of NF-κB and, accordingly, increased maturation and IL-12 production.

Abstrakt

In der vorliegenden Studie wurde die Rolle des Phosphoinositol 3 Kinase (PI3K) Signalweges in der Regulation der Funktion Dendritischer Zellen untersucht. Dendritische Zellen sind Antigen-präsentierende Zellen, welche einen Link zwischen angeborener und adaptiver Immunität herstellen und für die Aktivierung von spezifischen T-Zellen erforderlich sind. Das gegenwärtige Projekt konzentriert sich auf die mögliche Regulation der Dendritischen Zellfunktion durch zwei PI3K-abhängige Kinasen, Akt2/PKBβ und die Serum- und Glukokortikoid-induzierbare Kinase-1 (SGK1), welche 55% identische Kinasedomänenregion besitzen.

Einerseits ist es bekannt, dass Akt2/PKBβ für Makrophagen und für die Migration Dendritischer Zellen erforderlich ist. Jedoch ist der involvierte Mechanismus noch weitgehend unerforscht. Andererseits wird die Migration Dendritischer Zellen vom Ca^{2+} Signalweg: reguliert. In der vorliegenden Studie wurde der Mechanismus der Akt2-abhängigen Migration in Dendritischen Zellen durch eine mögliche Beteiligung von Akt2 im Ca^{2+} Signalweg untersucht. Dendritische Zellen wurden aus dem Knochenmark von Akt2-defizienten Mäusen (*akt2*$^{-/-}$) und ihren Wildtyp Wurfgeschwistern (*akt2*$^{+/+}$) isoliert und anschließend die Reifung durch das Lipopolysaccharid (LPS, 1µg/ml) induziert. Das Chemokin CCL21 (25ng/ml) induziert die Migration von reifen *akt2*$^{+/+}$ und *akt2*$^{-/-}$ Dendritischen Zellen. Jedoch war die Migration in *akt2*$^{-/-}$ Dendritischen Zellen gegenüber *akt2*$^{+/+}$ Dendritischen Zellen signifikant reduziert. In Ca^{2+} darstellenden Experimenten war die Gabe von CCL21(75 ng/ml) ein Auslöser für den Anstieg der zytosolischen Ca^{2+} Konzentration ($[Ca^{2+}]_i$) in *akt2*$^{+/+}$ Dendritischen Zellen, jedoch auch hier war die ($[Ca^{2+}]_i$) in reifen *akt2*$^{-/-}$ Dendritischen Zellen signifikant vermindert.

Darüber hinaus ist die Freisetzung von Ca^{2+} aus den intrazellulären Speichern durch Thapsigargin (1 µM) induziert. Auf dies folgt ein Speicher-abhängiger Ca^{2+} Einstrom (SOCE), welcher in reifen *akt2*$^{-/-}$ Dendritischen Zellen signifikant reduziert war. Eine Stimulation des P2Y Rezeptors mit ATP (100µM) führte zu einem IP_3-abhängigen Ca^{2+} Ausstrom, welcher in reifen *akt2*$^{-/-}$ Dendritischen Zellen auch signifikant reduziert war.

Eine IP_3-induzierte Aktivierung des CRAC Kanals, gemessen in Ganzzell Patch-Clamp Experimenten, war in reifen *akt2*$^{-/-}$ Dendritischen Zellen signifikant gemindert. Es wurden RT-PCR Analysen durchgeführt, bei diesen die Expression von IP_3 Rezeptor Isoformen in *akt2*$^{+/+}$ und *akt2*$^{-/-}$ Dendritischen Zellen ermittelt werden sollte. Dabei konnte festgestellt werden, dass eine geminderte Transkription von IP_3R2 in *akt2*$^{-/-}$ Dendritischen Zellen vorlag. Eine Inkubation mit LPS von einer Stunde, führte zu einer Stimulation der IP_3R2 mRNA Konzentration in *akt2*$^{+/+}$ Dendritischen

Zellen, jedoch nicht in *akt2*$^{-/-}$ Dendritischen Zellen. Demzufolge war auch die Proteinkonzentration von IP_3R2, analysiert mit Hilfe eines Western Blots, in reifen *akt2*$^{-/-}$ Dendritischen Zellen reduziert.

Die mRNA Konzentration des Transkriptionsfaktors Ets1, welcher bekannt ist, dass er die Transkription von IP_3R2 hochreguliert, war in unreifen *akt2*$^{-/-}$ Dendritischen Zellen gegenüber *akt2*$^{+/+}$ Dendritischen Zellen signifikant erniedrigt. Ähnlich zu IP_3R2, war die mRNA Konzentration von Ets1 durch die Inkubation mit LPS (1 Stunde) in *akt2*$^{+/+}$ Dendritischen Zellen gegenüber *akt2*$^{-/-}$ Dendritischen Zellen signifikant erhöht. Des Weiteren war die Expression des ETS1 Proteins in reifen *akt2*$^{-/-}$ Dendritischen Zellen gegenüber *akt2*$^{+/+}$ Dendritischen Zellen verringert.

Die CCL21-induzierte Migration von *akt2*$^{+/+}$ Dendritischen Zellen wurde durch die Hemmung von IP_3R mit dem Inhibitor Xestospongin C (5µM) signifikant reduziert. Dadurch wurde der Unterschied in der CCL21-induzierten Migration zwischen den beiden Genotypen aufgehoben.

Schlussendlich führt eine Inhibierung von ETS1 mit Hilfe einer siRNA zu einer reduzierten RNA Konzentration von IP_3R2, einer Thapsigargin- und ATP-induzierten Ca^{2+} Freisetzung aus den Speichern, SOCE und CRAC Kanal Aktivierung, sowie zu einer Migration Dendritischer Zellen. Zusammenfassend kann man sagen, dass Akt2 die IP_3R2 Transkription in Dendritischen Zellen hochreguliert. Dies geschieht wahrscheinlich über eine Erhöhung der ETS1 Expression, wobei es wahrscheinlich zu einem stimulierenden Effekt von Akt2 auf die Migration Dendritischer Zellen führt.

Die Aktivität von PI3K hemmt die Produktion von proinflammatorischen Zytokinen in Dendritischen Zellen. Diese Hemmung wirkt limitierend auf die übermäßige T-Helfer (Th1) Polarisation. Wenig ist über den Mechanismus der PI3K-vermittelten Unterdrückung in Dendritischen Zellen bekannt. In der vorliegenden Studie soll untersucht werden, ob SGK1 als ein negativer Regulator in der Funktion Dendritischer Zellen, der PI3K nachgeschaltet, fungiert. Zu diesem Zweck wurden Dendritische Zellen aus dem Knochenmark oder der Milz aus SGK1 defizienten Mäusen (*sgk1*$^{-/-}$) und ihren Wildtyp Wurfgeschwistern (*sgk1*$^{+/+}$) isoliert. Die Expression der Reifungsmarker MHCII, CD86 und CD54, welche mit Hilfe der Durchflusszytometrie analysiert wurden, und die Sekretion von Interleukin-12 (IL-12) nach Stimulation mit LPS (100 ng/ml), gemessen mit Hilfe eines ELISA's, war in *sgk1*$^{-/-}$ gegenüber *sgk1*$^{+/+}$ Dendritischen Zellen signifikant erhöht. Im Gegensatz dazu war die Phagozytose, gemessen mit Hilfe der FITC-Dextran Aufnahme, in *sgk1*$^{-/-}$ gegenüber *sgk1*$^{+/+}$ Dendritischen Zellen signifikant reduziert. Darüber hinaus war die Expression von CD86, MHCII und CD54 in den Dendritischen Zellen signifikant höher, welche aus der Milz von *sgk1*$^{-/-}$ als diejenigen aus *sgk1*$^{+/+}$ Mäusen isoliert wurden.

Des Weiteren war die nukleäre Lokalisation der Untereinheit p65, des Transkriptionsfaktors NF-κB, in *sgk1*$^{-/-}$ Dendritischen Zellen signifikant erhöht. N-myc downstream regulated gene 1 (NDRG-1), das physiologische Substrat von SGK1, welches bekannt ist, dass es den NF-κB Signalweg abmindert, war gleichermaßen in *sgk1*$^{+/+}$ und *sgk1*$^{-/-}$ Dendritischen Zellen exprimiert. Jedoch war die SGK1-abhängige Phosphorylierung von NDRG-1 in *sgk1*$^{-/-}$ Dendritischen Zellen vermindert.

Eine Herunterregulation von NDRG1 durch eine siRNA in *sgk1*$^{+/+}$ Dendritischen Zellen führte gegenüber der Kontroll-siRNA zu einer erhöhten Konzentration an p65 im Kern. Diese Untersuchungen weisen auf eine wichtige Rolle von SGK1 in der Regulation der Funktion Dendritischer Zellen hin. Dies zeigt auf, dass SGK1 ein wichtiges Element in der Vernetzung zwischen angeborener und adaptiver Immunität ist. Eine erhöhte Phosphorylierung von NDRG-1 in SGK1 defizienten Dendritischen Zellen führt wahrscheinlich zu einer nukleären Lokalisation von NF-κB und im Anschluss zu einer erhöhten Reifung und IL-12 Produktion Dendritischer Zellen.

1. Introduction

1.1 Role of Dendritic Cells

The immune system is composed of two major subdivisions, i.e., the antigen (Ag)-nonspecific innate immunity, which was discovered by Elie Metchnikoff in 1891, and the Ag-specific adaptive immunity, discovered by Paul Ehrlich in 1900 (Kaufmann 2008; Paul 2011).

When the pathogen breaks through the physiological barrier in our body, it will encounter the innate immune system, the first line of defense. The innate immune system has two crucial abilities(Janeway and Medzhitov 2002), i.e., to rapidly recognize pathogens and/or tissue injury and to signal the presence of danger to cells of the adaptive immune system, the second line of defense. Phagocytic cells, nature killer (NK) cells, complement, and interferons (IFNs) are the main players in the innate immune system. These players express a variety of pattern recognition receptors to identify molecular patterns that are associated with microbial pathogens such as bacterial lipopolysaccharide (LPS), carbohydrates, and double-stranded viral RNA.

The adaptive immune system, besides being the second line of defense, also affords protection against re-exposure to the same pathogen(Sun, Beilke et al. 2009). The key features of the adaptive immune system are the abilities to rearrange genes of the immunoglobulin family, permitting the creation of a large diversity of Ag-specific clones and immunological memory.

Each of the major subdivisions of the immune system has both cellular and humoral components to carry out its protective function. Although these two subdivisions have distinct functions, there is interplay between them. Dendritic cells (DCs) act as antigen presenting cells (APCs) and become the link between these two parts (**Fig. 1**) (Banchereau, Briere et al. 2000; Lipscomb and Masten 2002).

DCs are the most efficient APCs owing to their unique ability of inducing primary immune responses, therefore permitting the establishment of immunological memory. Besides inducing adaptive immune responses to foreign antigens (Ags), DCs are also crucial for maintaing immune tolerance(Lewis and Reizis 2012). DCs were named for their probing, tree-like shapes (from the Greek ‘dendron’, meaning tree), which were first described by R.Steinman and Z.Cohn in 1973(Steinman and Cohn 1973).

All DCs are ultimately derived from hematopoietic stem and progenitor cells in the bone marrow (Merad and Manz 2009). Hematopoietic stem and progenitor cells give rise to several distinct progenitors that can be differentiated into one or more DC subsets. DCs can be divided into two major populations: the type I interferon-producing plasmacytoid DCs (pDCs) and conventional DCs (cDCs) expressing the surface marker CD11c. Both populations express class II major histocompatibility complex (MHC-II) (LeibundGut-Landmann, Waldburger et al. 2004; Naik, Sathe et al. 2007; Alvarez, Vollmann et al. 2008). The human pDCs express the surface marker CD123, two additional markers, BDCA-2(CD303) and BDCA-4(CD304), and they are restricted to peripheral blood and bone marrow (Barchet, Blasius et al. 2005; McKenna, Beignon et al. 2005). Unlike human pDCs, mouse pDCs express the cell surface antigens B220 and Ly6C. Moreover, mouse pDCs express CD11c, although in low amounts compared with cDCs, and, except in mice treated with the cytokine Flt3 ligand, they do not express CD123 (IL-3α). Mouse pDC express BST-2 (mPDCA) and Siglec-H and are negative for CD11b. cDCs subset is the predominant DC population in the steady state (Colonna, Trinchieri et al. 2004; de Heer, Hammad et al. 2005). Moreover, upon activation, respiratory cDCs produce a range of inflammatory mediators (de Heer, Hammad et al. 2005; Beaty, Rose Jr et al. 2007). Nonlymphoid tissue cDCs emigrate continuously via lymphatics into draining lymph nodes; similar to the resident populations of lymphoid tissue DCs, most migrant cDCs die in lymphoid tissue and do not reenter the blood circulation. Strong inflammatory stimuli can cause an abrupt decrease in the number of lymphoid cDCs, a consequence of maturation-induced apoptosis and migration(Naik, Sathe et al. 2007).

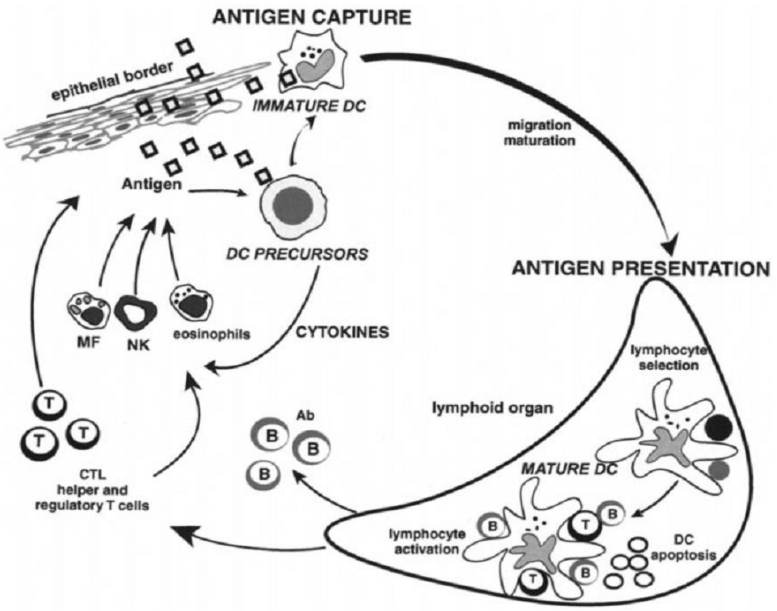

Figure 1. The life cycle of dendritic cells, obtained from J Banchereau Et Al. Annual Review of Immunology, 2000

1.2 Maturation of Dendritic Cells

DC progenitors are not restricted to bone marrow and can be found in multiple locations. These progenitors can differentiate into immature DCs in peripheral tissues (Ocular Periphery and Disorders, Darlene A. Dartt). Immature DCs express only a small amount of MHC II and no co-stimulatory molecules. A major functional characteristic of immature DCs is the capacity for continuous endocytosis in the steady state (Lutz and Schuler 2002; Wilson, El-Sukkari et al. 2004). Thus, immature DCs are able to capture Ags but are still not able to determine T-cell fate and prime immune responses. Once peripheral tissues are inflamed or infiltrated by microbes, immature DCs become activated (Banchereau, Briere et al. 2000; Lutz and Schuler 2002). After capturing and processing Ags, they migrate to second lymph organs, and give rise to mature DCs that downregulate antigen-sampling functions but become exceedingly powerful at priming T cells (Alvarez, Vollmann et al. 2008).

Mature DCs express high cell-surface levels of antigen-presenting MHC molecules and costimulatory molecules CD40, CD80, CD83, and CD86 (Banchereau, Briere et al. 2000). They produce large amounts of proinflammatory cytokines, such as IL-12, IL-18, and TNFα (Lin, Jacek et al. 2006; Schmidt, Nino-Castro et al. 2012). Thus, DCs are capable of inducing TH1- and TH2-cell differentiation, and cytotoxic T lymphocyte response.

Besides the functional changes, as acquisition of high cellular motility, the morphological changes also accompany DC maturation. This includes a loss of adhesive structures, and cytoskeleton reorganization (Banchereau, Briere et al. 2000; Alvarez, Vollmann et al. 2008; Randolph, Ochando et al. 2008).

1.3 Migration of Dendritic Cells

Due to the complex life cycle, the ability of DCs and their progenitors to migrate is a central feature of DCs and indispensable for their functions within the immune system (Alvarez, Vollmann et al. 2008).

The migration of DCs entails 1) the ability of newly formed DCs or their progenitors to exit their place of birth and enter the blood, 2) the recruitment of the circulating cells into target tissues, 3) the extravascular lodging and interstitial motility needed to sample Ags, 4) the capacity to access lymph vessels to travel either to lymph nodes or back to the blood, and 5) the ability to interact with migrating lymphocytes and other immune cells in a manner that allows the exchange of critical information regarding the nature and context of presented Ags (Alvarez, Vollmann et al. 2008).

As mentioned above, many DCs begin their journey with their release from the bone marrow into the blood and subsequent traffic into peripheral lymphoid and nonlymphoid tissues. In nonlymphoid tissues, DCs eventually proceed into lymph nodes through afferent lymphatics and, in some instances, return back to the blood via the thoracic duct (von Andrian and Mempel 2003; Steinman and Hemmi 2006; Alvarez, Vollmann et al. 2008). Compartmental segregation of lymphoid tissues, which facilitates efficient surveillance and targeted cellular response, relies on the precise trafficking of immune cells between the circulation, tissues, and second lymph organs (von Andrian and Mempel 2003; Ricart, John et al. 2010). However, specific responses require directed cell migration that DCs accomplish through selective expression of chemokine receptors, coupled with generation of specific chemokine in immune organs. Specifically, immature DCs utilize specific chemokine receptor-ligand pathways, such as CCR2-CCL2 and, CCR5-CCL5, and CCR6-CCL20, as well as CXCR3-CXCL10 and CXCR4- CXCL12 (SDF1-α) . However, after Ag capture when DCs become mature, they downregulate most of the above mentioned chemokine receptors, with the exception of CXCR4, while upregulating the crucial receptor CCR7. CCR7 has two cognate ligands, CCL19 (ELC, MIP-3 β) and CCL21 (SLC, 6Ckine, Exodus-2), which are highly expressed by peripheral lymphatic endothelial cells as well as LN stroma cells and guide DCs to second lymph organs (Banchereau, Briere et al. 2000; Le, Zhou et al. 2004; Rot and von Andrian 2004). To date 18 human chemokine receptors have been identified, among them four receptors are found on DCs (**Table 1**). Throughout its migration, DCs also require some other assistance to reach their targeted destination, such as highly regulated coordination of protrusion, retraction, adhesion, detachment, and major dynamic rearrangements of the actin cytoskeleton (Saarikangas, Zhao et al. 2009). Therefore DCs express E-cadherin and elastase and release type IV collagenase that may contribute to their migration (de Noronha, Hardy et al. 2005; Alvarez, Vollmann et al. 2008).

Table 1. Details of human chemokine receptors, obtained from C Murdoch, A Finn. Blood, 2000

Receptor	Amino acids	Base pairs	Ligand (high affinity)	Cellular distribution
CXCR1(GPR5)	333	999	Lymphotactin	T, B, NK
CXCR1 (IL BRA)	350	1050	IL8, GCP2	N, M, T, NK, Bs, Ms, En
CXCR2 (IL BRB)	355	1065	IL8, GRO-α, GRO-β, GRO-γ, NAP-2, ENA-	N, M, T, NK, As, Nn, Ms,

			78, GCP-2,	En,
CXCR3	368	1104	IP-10, Mig, I-TAC	Activated T,
CXCR4* (LESTR, FUSIN)	352	1056	SDF-1α, SDF-1β	Myeloid, T, B, Ep, En, DC
CXCR5 (BLR1)	372	1116	BCA-1	B
CX3CR1 (V28)	355	1065	Fractalkline	NK, M, T
CCR1	355	1065	RANTES, MIP-1α, HCC-1, MCP-2, MCP-3, MIP-5, Ckβ8	N, M, T, NK, B, Ms, As, Nn
CCR2A	374	1122	MCP-1, MCP3, MCP4	M
CCR2B↑	360	1080	MCP-1, MCP2, MCP3, MCP4	M, T, B, Bs
CCR3↑	355	1065	Eotaxin, Eotaxin-2, Eotaxin-3, RANTES, MCP2,3,4, MIP-5	Eo, Bs, T
CCR4	360	1080	TARC, MDC	T, P
CCR5↑ (ChemR13)	352	1056	RANTES, MIP-1β, MIP-1α, MCP-2	T, M, MØ, DC
CCR5 (STRL22, DRY-6, GPR-CY4, CKR-L3)	374	1122	MIP-3α	T, B, DC
CCR7 (BRL2, EBI1)	378	1134	MIP-3β, 6-C-kine	T, B, DC
CCR8*↑ (TER1, CKR-L1, ChemR1)	355	1065	I-309	M, Thymus
CCR9 (GPR-9-6)				

D6 (CCR107)	369	1107	TECK	T, Thymus
DARC (Duffy antigen)	384	1152	MCP-1, MCP-3	Placenta, liver
	338	1014	IL8, GRO-α, RANTES, MCP-1, MCP-3, MCP-4, Eoxtaxin	En, RBC, T

In general, migration of any cell involves three components: the formation and maintenance of cell polarity, activation of motility machinery for linear locomotion, and turning or changing direction of movement in response to gradients of environmental cues (Pollard and Borisy 2003; Philipsborn and Bastmeyer 2007; Petrie, Doyle et al. 2009). Besides sensing of signal gradients, chemotaxis of eukaryotes requires the polar differentiation of the cells. A leading edge called the lamellopodia and a trailing edge called the uropod (or rear end) characterize the polarized migrating cells [general about migration, section 1.3] (Small, Stradal et al. 2002; Philipsborn and Bastmeyer 2007; Alvarez, Vollmann et al. 2008; Saarikangas, Zhao et al. 2010).

Calcium release by the PLC-IP_3-IP_3R pathway at the rear was thought to be the major source to counteract the biophysics of calcium diffusion and maintain the calcium gradient which is important for maintaining cell polarity [complicated sentence and you call this part Ca^{2+} signaling in DC migration, so now you should really write about DCs] (Clapham 2007; Bononi, Agnoletto et al. 2011). It has been demonstrated that an extracellular Ca^{2+} influx is required for positive feedback at the leading edge of spontaneously polarized macrophages [you may use it for Discussion but not here] (Evans and Falke 2007). Inhibition of extracellular Ca^{2+} influx leads to loss of leading-edge PI3K activity, disassembly of F-actin, cessation of ruffling, and decay of chemoattractant signals (Evans and Falke 2007). Conversely, increasing cytosolic Ca^{2+} enhances membrane ruffling, PI3K activity, and F-actin accumulation.

In DCs, chemotactic chemokines, such as CXCL12 and CCL21, rapidly increase intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) by triggering Ca^{2+} release from intracellular stores followed by the store-operated Ca^{2+} (SOC) entry (Barbet, Demion et al. 2008; Nurbaeva, Schmid et al. 2012). Inhibition of SOC channels leads to profound changes in DC migration. Blocking of SOC channels impairs CCL21-dependent migration (Matzner, Zemtsova et al. 2008; Xuan, Shumilina et al. 2009). On the other hand, ion channels that control Ca^{2+} flux by setting the membrane potential are also important regulators of DC migration. Ca^{2+} influx brings about a strong local depolarization of the cell membrane, and consequently to sustain Ca^{2+} entry, activity of other channels, which can prevent depolarization, is required. In

mouse DCs ion channels that support Ca^{2+} influx by hyperpolarizing the membrane and thereby providing electrical driving force for Ca^{2+} entry are voltage-gated K+ Kv channels (Matzner, 2008) and Ca^{2+}-activated Cl^- channel ANO6 (Kalina's paper). Accordingly pharmacological or genetical inhibition of Kv or ANO6 channels results in a strong impairment of DC migration (Nici's paper, Kala's paper). On the other hand, transient receptor potential cation channel of melastatin family TRPM4, which functions as a Ca^{2+}-activated non-selective cation channel providing under physiological conditions influx of Na^+ and therefore membrane depolarization, breaks the Ca^{2+} influx and prevents Ca^{2+} overload (Barbet, Demion et al. 2008). Interestingly, migration of TRPM4-deficient mouse DCs is also impaired, suggesting that $[Ca^{2+}]_i$ should be perfectly balanced to enable DC migration.

It has been demonstrated for various cell types that the polar differentiation of migrating cells is established and maintained by generating gradients of Ca^{2+} concentrations through the concerted action of different Ca^{2+} channels and transporters.

1.4 Mechanism of Ca^{2+} Release and Ca^{2+} Entry in Dendritic Cells

Ca^{2+} can enter the cytoplasm from one of the two general sources: it can be released from intracellular stores, or extracellular Ca^{2+} can enter the cell across the plasma membrane (Bootman, Collins et al. 2001; Parekh and Putney 2005; Bootman 2006; Putney 2010). These two processes often occur either simultaneously or sequentially.

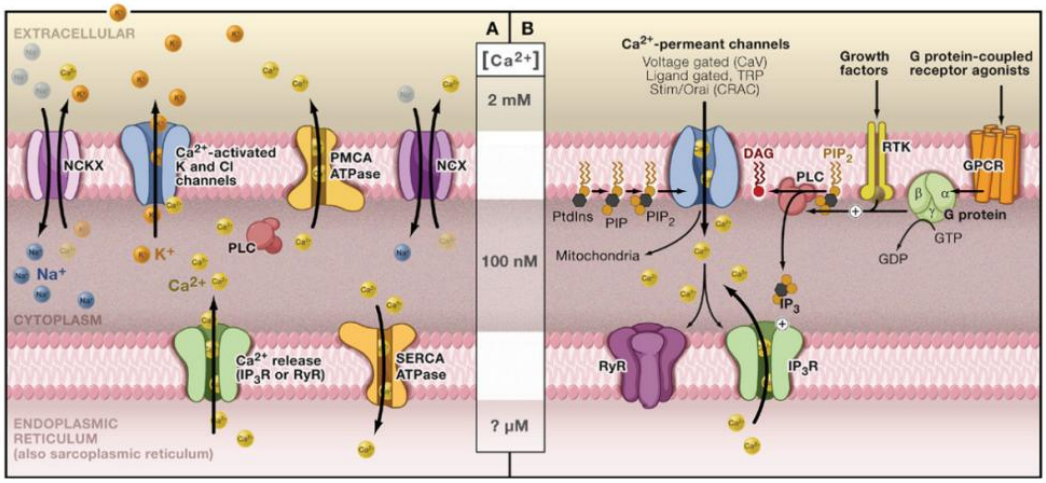

Figure 2. Maintaining and using Ca^{2+} gradients for signaling, obtained from DE Clapham, Cell, 2007

The internal stores are held within the membrane systems of the endoplasmic reticulum (ER) or the equivalent organelle, the sarcoplasmic reticulum of muscle cells (Mazzarello, Calligaro et al. 2003; Rizzuto and Pozzan 2006). Ca^{2+} release from these internal stores is mediated by various channels, of which the inositol-1,4,5-trisphosphate receptor (IP_3R) and ryanodine receptor (RyR) families have been studied extensively (Mikoshiba 2007; Vanderheyden, Devogelaere et al. 2009; Rossi, Tovey et al. 2011). These two channel classes are regulated by several factors, among which the most important activator is Ca^{2+} itself.

Calcium entry secondary to calcium release via IP_3R activation is essential for modulating calcium-dependent processes, particularly in immunity. IP_3 is a second messenger produced through phosphoinositide turnover in response to many extracellular stimuli. The IP_3R is an IP_3-gated Ca^{2+} release channel located on ER. If IP_3 and Ca^{2+} are bound to an IP_3R, it can open and Ca^{2+} will diffuse into the cytosol (Krebs and Michalak 2007; Mikoshiba 2007). IP_3Rs have three isoforms, IP_3R1, 2 and 3. Most work has been performed on the ubiquitously expressed IP_3R1, but it is however clear that various IP_3R isoforms can subtly differ in their properties (Nadif Kasri, Bultynck et al. 2002; Vermassen, Parys et al. 2004). Their affinity for IP_3 displays a rank-order $IP_3R2>IP_3R1>IP_3R3$. The open probability of IP3Rs depends on the IP_3 concentration and the Ca^{2+} concentration in the cytosol (Rahman 2012). It increases with increasing IP_3 concentration. It is low for low Ca^{2+} concentration, increases with increasing Ca^{2+} (100-300nM) and finally decreases again for even higher Ca^{2+} concentrations (above 300nM) (Bootman, Collins et al. 2001; Foskett, White et al. 2007; Vanderheyden, Devogelaere et al. 2009).

RyRs also contain three different isoforms, RyR1, 2 and 3. RyRs are similar to the IP_3R, and when stimulated transport Ca^{2+} into the cytosol (Blaustein, Greger et al. 2001; Wehrens and Marks 2004). It is first demonstrated by O'Connell and his colleagues that immature mouse DCs express functional RyR1 channels, but little or no RyR2 or RyR3, but the expression of RyR1 RNA is reduced dramatically upon maturation (O'Connell, Klyachko et al. 2002). Later, O'Connell has used RyR1 KO mice as a model; their study shows that RyR1 alone is not essential for BMDC functions. Signaling via IP_3Rs appears to be sufficient to mediate Ca^{2+} signaling in mouse DCs (Stolk, Leon-Ponte et al. 2006). In contrast, in human DCs, RyR1 is important for the maturation and activation (Bracci, Vukcevic et al. 2007). Recognizing Ca^{2+} on its cytosolic side establishes a positive feedback mechanism, because a small amount of Ca^{2+} in the cytosol near the receptor will cause it to release even more Ca^{2+} (Fill and Copello 2002). The autocatalytic process of Ca^{2+}-induced Ca^{2+} release (CICR) enables the IP_3Rs and RyRs to communicate with each other to establish coordinated Ca^{2+} signals (Ashby, Craske et al. 2002; Smith and Parker 2009; Taylor and Tovey 2010). The released Ca^{2+} is pumped back either into the ER by Sacro-Endoplasmic Reticulum Calcium ATPases (SERCA pumps) or out of the cell via plasma membrane Ca^{2+} ATPase (PMCA pumps). To maintain low cytoplasmic $[Ca^{2+}]_i$, ATPase exchange protons for two (SERCA) or one (PMCA)

Ca^{2+} per hydrolyzed ATP (Bootman, Collins et al. 2001; Feske 2007; Scharenberg, Humphries et al. 2007). Another extrusion mechanism is the exit of Ca^{2+} out from the cell via the K^+-independent Na^+/Ca^{2+} exchangers (NCX, or SLC8A1-3), and K^+-dependent Na^+/Ca^{2+}- exchangers (NCKX; SLC24A1-5). These transporters exchange one Ca^{2+} ion for three Na^+ ions (NCX) or cotransport one K^+ ion with one Ca^{2+} ion in exchange for four Na^+ ions (NCKX). PMCAs and NC(K)X complement each other: PMCAs are effective at maintaining low internal $[Ca^{2+}]_i$ over long durations, whereas NCX and NCKX can make the rapid adjustments (Parekh and Putney 2005; Feske 2007).

In spite of its importance, the Ca^{2+} release phase is transient, sometimes fully deactivating within a few tens of seconds, as the ER Ca^{2+} stores are necessarily limited. Decreased Ca^{2+} within the ER triggers a process known as store operated calcium (SOC) entry, in which Ca^{2+} channels in the plasma membrane are activated to allow Ca^{2+} to enter from the essentially unlimited extracellular Ca^{2+} pool to the cytosol (Feske 2007; Scharenberg, Humphries et al. 2007). SOC entry does more than simply providing Ca^{2+} for refilling the stores, it itself generates sustained Ca^{2+} signals that control such essential functions as gene expression, cell metabolism and exocytosis. The best characterized SOC channels in DCs are known as 'calcium release-activated calcium' (CRAC) channels. DCs have been shown to possess CRAC channels as a main Ca^{2+} entry pathway (Hsu, O' Connell et al. 2001). Accordingly, Ca^{2+} influx is enhanced by membrane hyperpolarization. CRAC channels are highly Ca^{2+}-selective channels of a low conductance with a characteristic inwardly rectifying current-voltage relationship. The stromal interaction molecule 1 (STIM1) has been identified as the ER-resident Ca^{2+} sensor and calcium release-activated calcium modulator 1 (CRACM 1); also called Orai1, functions as the pore-forming subunit of CRAC channels (Vig, Peinelt et al. 2006). In mammals, several homologs of these proteins exist: STIM1 and STIM2 in the ER and Orai1, Orai2, and Orai3 in the plasma membrane (Parvez, Beck et al. 2008; Vig and Kinet 2009). A strong functional interaction between STIM1 and Orai1 has been demonstrated by many laboratories. STIM1 and Orai1 are necessary and sufficient to generate CRAC-like currents in vitro (Smyth, Hwang et al.; Park, Hoover et al. 2009; Hogan, Lewis et al. 2010; Srikanth and Gwack 2012). After store depeletion, in response to decreased ER Ca^{2+} concentrations, STIM1 aggregates and translocates to form oligomers, moves to the ER-PM junctions, and close to the regions of Ca^{2+} influx from the plasma membrane (Hewavitharana, Deng et al. 2007; Carrasco and Meyer 2011; Cheng, Liu et al. 2011).

Whereas much evidence has been accumulated that Ca^{2+} gradients are indispensable for DC migration, very little is known how ion channels and transporters generating or controlling Ca^{2+} signals are regulated in DCs. The present study concentrates on a signaling pathway that plays an important role in DC biology, which is the phosphatidylinositol-3-kinase (PI3K) pathway.

1.5 PI3K-PDK1-Akt/SGK1 Pathway

1.5.1 Role of PI3K in DCs

Phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases essential for diverse physiological reactions. The lipid products produced by PI3Ks include PI(3)P, PI(3,4)P2 and PI (3,4,5)P3(Rameh and Cantley 1999; Koyasu 2003; Liu, Cheng et al. 2009).

The PI3K family can be divided into three classes—class I, II and III. Each type of PI3K contains a C2 domain and a catalytic domain connected by a helical domain (Walker, Perisic et al. 1999; Bader, Kang et al. 2005). We are interested in the class I

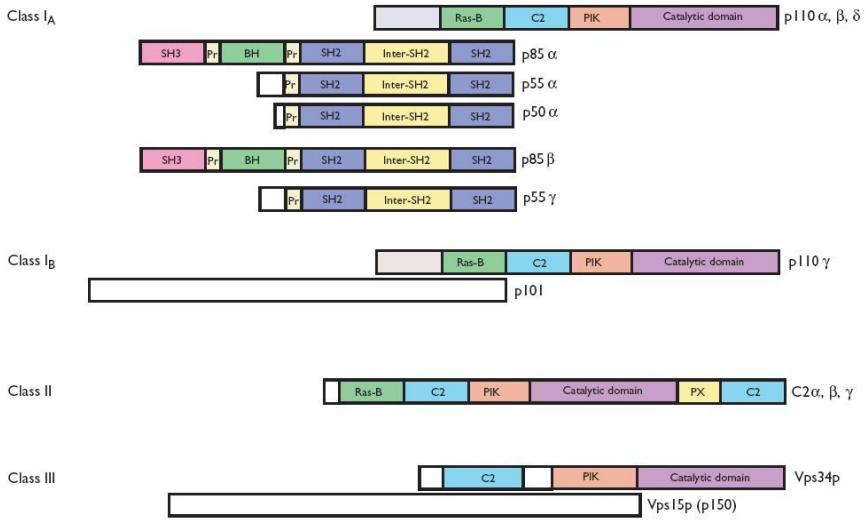

Figure 3. Structural characteristics of the PI3K family, obtained from S Koyasu, Nature Immunology, 2003

PI3Ks, since it is the only class which has been show to activate protein kinase B (PKB; also called Akt) in DCs (Vanhaesebroeck and Alessi 2000; Okkenhaug and Vanhaesebroeck 2003).

The class I PI3Ks is subdivided into class IA and the class IB subclasses based on the differences in their activation mechanisms. The class IA PI3Ks are heterodimeric enzymes having three types of the catalytic subunit: p110α, p110β and p110γ (Vanhaesebroeck and Waterfield 1999; Okkenhaug and Vanhaesebroeck 2003; Hirsch, Costa et al. 2007) and five regulatory subunits: p85α, p85β, p55α, p55β and p55γ. The class IA PI3Ks can be activated by tyrosine-kinase-associated receptors, including antigen, co-stimulatory and cytokine receptors (Koyasu 2003; Okkenhaug and Vanhaesebroeck 2003). For example, interleukin 2 (IL-2), IL-3, IL-6, granulocyte colony-stimulating factor and interferons (IFNs) activate class IA PI3Ks

in many immune cells, including T cells and DCs (Koyasu 2003). The single class IB PI3k enzyme has only one catalytic subunit and one regulatory subunit: p110γ and p101, respectively. P110γ is expressed only in mammals, mainly in leukocytes. The class IB PI3K is activated by G-protein-coupled receptors (GPCRs), including the chemokine receptors (Beer-Hammer, Zebedin et al.; Fruman and Cantley 2002; Saudemont and Colucci 2009).

The preferential substrate of the class I PI3Ks is PI(4,5)P2. The class I PI3Ks can phosphorylate PI(4,5)P2 to generate PI(3,4,5)P3 at the inner leaflet of the plasma membrane. PI(3,4,5)P3 acts as a binding site for pleckstrin-homology (PH) domains (Vanhaesebroeck and Alessi 2000; Tessier and Woodgett 2006). The subset of PH domains bind to PI (3, 4)P2 and PI(3,4,5)P3 with higher affinity than other PIs. Typically, following growth factor activation of PI3K, proteins with PH domains, such as PKB and phosphoinositide-dependent kinase 1 (PDK1), are translocated to the site where PI(3,4,5)P3 is generated at the plasma or endosomal membranes (Scheid, Parsons et al. 2005). There, they interact with other proteins or become activated by PI(3,4)P2 or PI(3,4,5)P3 binding. In fact PKB, which is recruited to the PM by binding PI(3,4)P2 or PI(3,4,5)P3, is considered to be a chief downstream target of PI3K (Vanhaesebroeck and Alessi 2000; Liao and Hung 2010; Yang, Wu et al. 2010).

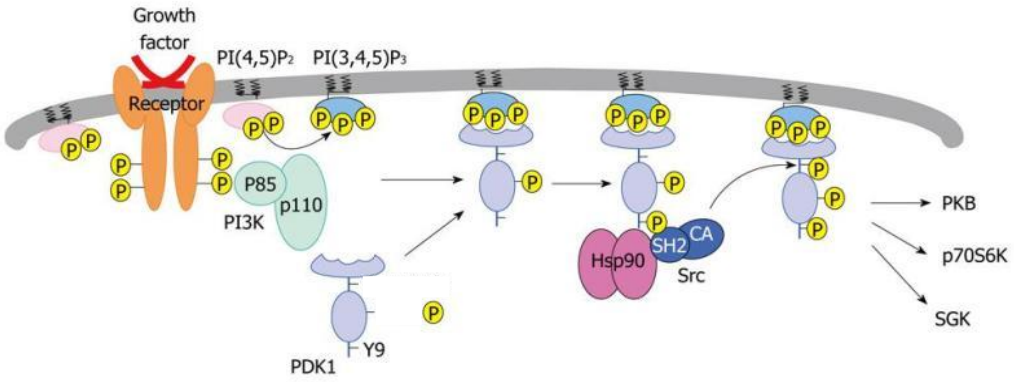

Figure 4. PI3K-PDK1 pathway, obtained from Yang KJ, J Biol Chem, 2008

Many studies indicate that PI3Ks have pleiotropic roles in immune cell activation and function (Koyasu 2003; Okkenhaug and Vanhaesebroeck 2003; Koyasu 2004). In DCs, PI3K is considered to belong to the gate-keeping system, which controls the maturation and survival of DCs, and prevents excessive immune responses (Fukao, Tanabe et al. 2002; Fukao and Koyasu 2003; Shumilina, Zahir et al. 2007; Krawczyk, Holowka et al. 2010). PI3K is activated by LPS, peptidoglycan, CD40 ligand, RANK ligand, and CpG oligonucleotides, all of which induce production of proinflammatory cytokines, such as IL-12, and provide a negative feedback regulation of IL-12 production (Fukao, Tanabe et al. 2002; Fukao and Koyasu 2003).

1.5.2 PDK1 and PDK1 Functions in DCs

One downstream target of PI3K is PDK1, which is a 63 kDa serine/threonine kinase belonging to the AGC superfamily of protein kinases. It phosphorylates a variety of other protein kinases within the AGC subfamily including PKC isoforms, p70 S6K, Rsk, serum and glucocorticoid-inducible kinases (SGKs) and PKB/Akt (Vanhaesebroeck and Alessi 2000; Woodgett 2005; Tessier and Woodgett 2006; Gurevich, Tesmer et al. 2011). In fact, PDK1 was first discovered by its capacity to phosphorylate PKBα/Akt1 at Thr 308 (Alessi, James et al. 1997; Bayascas, Wullschleger et al. 2008; Calleja, Laguerre et al. 2009).

PDK1 has two domains: the N-terminal catalytic domain and the C-terminal PH domain (Mora, Komander et al. 2004; Yang, Shin et al. 2008). The catalytic domain has three ligand binding sites: the substrate binding site, the ATP binding site, and the docking site. The docking site is also known as PKC related kinase 2- interacting factor- pocket (PIF-pocket), it acts as the binding site for the hydrophobic motif of other AGC kinases (Mora, Komander et al. 2004; Woodgett 2005; Li, Yang et al. 2010). PDK1 activates at least 23 AGC kinases by phosphorylating a specific Thr or Ser residue located within the T-loop of the kinase domain(Vanhaesebroeck and Alessi 2000; Mora, Komander et al. 2004; Komander, Kular et al. 2005). Of note, PDK1 has no hydrophobic motif. The catalytic activity of PDK1 is not acutely regulated, thus some targets are constitutively phosphorylated. The PH domain function is mainly to enable the interaction of PDK1 with PI(3,4)P2 and PI(3,4,5)P3 (Vanhaesebroeck and Alessi 2000; Komander, Fairservice et al. 2004; Tessier and Woodgett 2006). PDK1 only encounters some of its kinase substrates, as Akt/PKB at the plasma membrane. However, PDK1 can also translocate to the nucleus in response to mitogens (Roelants, Torrance et al. 2004; Scheid, Parsons et al. 2005; Woodgett 2005).

In the past few years, the crucial role of PDK1 in embryonal development as well as in normal functions of the liver and heart has been well studied using specific tissue deletion in mice (Lawlor, Mora et al. 2002; Mora, Lipina et al. 2005; Mora, Sakamoto et al. 2005; Zaru, Mollahan et al. 2008; Di, Feng et al. 2010). Several studies have addressed the role of PDK1 in T lymphocytes. PDK1 regulates T cell development and controls the production of IL-4 by Th2 cells (Hinton, Alessi et al. 2004; Nirula, Ho et al. 2006). DCs derived from PDK1 deficient mice have several abnormal aspects in vitro, though a normal in vivo development. PDK1-hypomorphic DCs have a reduced TLR-induced macropinocytosis as well as FcR-mediated phagocytosis but enhanced antigen presentation, increased levels of costimulatory molecules and enhanced production of cytokines (IL-12 and IL-10) (Zaru, Mollahan et al. 2008). Thus, similar to PI3K, PDK1 has also a suppressive effect on DC maturation and cytokine production.

1.5.3 Akt and Akt Functions in DCs

PKB/Akt has been identified as a protein kinase with extensive homology with protein kinases A and C, and was therefore termed PKB (Manning and Cantley 2007). On the other hand, PKB/Akt is the cellular homologue of the viral oncoprotein v-Akt, and is therefore referred to Akt. The Akt family has three isoforms: Akt1, 2 and 3 (also known as PKB α, β and γ), which share a high degree of amino acid identity.

Akt is a 57kDa Ser/Thr kinase with three functional domains: an N-terminal PH domain, a kinase domain and a C-terminal hydrophobic motif (Calleja, Laguerre et al. 2009). PH domain preferentially binds PI(3,4,5)P3 and PI(3,4)P2 over other PIs (Huang and Sauer 2010; Dixon, Gray et al. 2011). The catalytic domain of Akt is structurally similar to other protein kinases of AGC family, including PKA and p70S6K, but is most similar to SGKs (Brunet, Park et al. 2001; Scheid and Woodgett 2003; Woodgett 2005; Loffing, Flores et al. 2006). Akt is cytosolic in unstimulated cells, translocates to the plasma membrane upon activation of PI3K. As indicated earlier, PI3K stimulation leads to the production of PI(3,4,5)P3. PDK1 and Akt are recruited to the membrane via binding of their PH domains to PI(3,4,5)P3 (Koyasu 2003; Okkenhaug and Vanhaesebroeck 2003; Mora, Komander et al. 2004; Yoshizaki, Mochizuki et al. 2007). Colocalization of PDK1 and Akt induces the phosphorylation of Ser 473 at the hydrophobic motif site of Akt, and in further causes the phosphorylation of Thr 308 at the T-loop site of Akt (Mora, Komander et

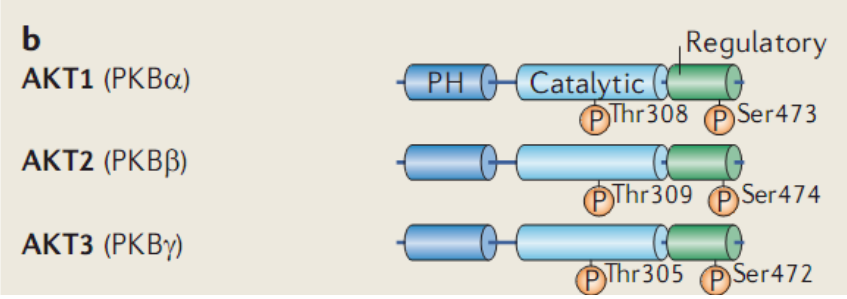

Figure 5. Classification and domain structure of AKT family members, obtained from S Song, L Salmena, PP Pandolfi, Nature Reviews Molecular Cell, 2012

al. 2004; Calleja, Laguerre et al. 2009; Li, Yang et al. 2010). Finally, dually phosphorylated Akt detaches from the plasma membrane and translocates through the cytosol to the nucleus (Manning and Cantley 2007). Activation of Akt and phosphorylation of both these residues are abolished if the cells are incubated with PI3K inhibitors prior to stimulation with agonist. Phosphorylation of both residues, Thr308 in the kinase domain and Ser473 in the C-terminal regulatory domain, is essential for maximal activation of Akt1 (Tessier and Woodgett 2006; Manning and Cantley 2007). The Akt2 and Akt3 isoforms are also activated in response to agonists which activate PI3K, by phosphorylation of the residues equivalent to Thr308and

Ser473 (Crellin, Garcia et al. 2007; Warfel, Niederst et al. 2011). Mutation of either residue of Akt1 to Ala does not prevent the other one from becoming phosphorylated in response to insulin, indicating that the phosphorylation of these residues can occur independently from each other (Vanhaesebroeck and Alessi 2000; Lawlor and Alessi 2001; Richardson, Schalm et al. 2004).

The Akt is a central node in cell signaling downstream of growth factors, cytokines, and other cellular stimuli (Manning and Cantley 2007). Akt plays a key role in multiple cellular processes such as cell survival, growth, proliferation, angiogenesis, metabolism, and migration (Song, Ouyang et al. 2005; Bononi, Agnoletto et al. 2011).

Numerous substrates of Akt have been identified. Thus, Akt may influence plasma membrane and intracellular proteins involved in ionic intracellular homeostasis such as sarco(endo)plasmic reticulum Ca^{2+} ATPase (Rota, Boni et al. 2005) and ryanodine receptors (Barac, Zeevi-Levin et al. 2005). Akt-mediated transcriptional up-regulation of NCX1 and NCX3 has been found in a study on PC12 cells (Formisano et al. 2008). A consensus sequence for Akt phosphorylation (RxRxxS/T) is present in all three types of IP_3Rs that indicate the IP_3R as a target of Akt (Khan, Wagner Ii et al. 2006; Pinton, Giorgi et al. 2008; Hwang, Duncan et al. 2009; Martin, Wang et al. 2012). Akt phosphorylates type-I IP_3R and therefore inhibits Ca^{2+} release from the ER and prevents Ca^{2+} induced apoptosis in non-cardiac cells (Khan et al.2006; Szado et al.2008). Several transcription factors are downstream targets of Akt. Among them are NF-κB, CREB, FoxO2 (Tessier and Woodgett 2006), ETS1 (Lavenburg, Ivey et al. 2003).

DCs express Akt1 and Akt2, but not Akt3 (Li, Syrovets et al. 2009). Dongsu et al. has provided evidences that Akt1 regulates DC survival in innate (LPS-driven) and adaptive (CD40-driven) immune responses. In addition, Akt deficiency leads to defective DC activation and survival (Park, Lapteva et al. 2006). Although Akt1 is a predominant family member in bone marrow-derived DCs (BMDCs), Akt2 also plays an important role in the plasmin-triggered chemotactic response of monocyte-derived DCs (Li, Syrovets et al. 2009). Moreover, in macrophages, it has been shown that Akt2 directly mediates the signal transduction pathway, which regulates actin polymerization and chemotaxis (Zhang, Ma et al. 2009). However, very little is known about the mechanisms of Akt2-dependent regulation of migration. In the present project, the role of Akt2 in DC migration and in other Ca^{2+}-dependent functions was addressed.

1.5.4 Ets-1

One of the transcriptional factors regulated by Akt is ETS1 (Lavenburg, Ivey et al. 2003). ETS proteins are a family of transcription factors that regulate the expression of a myriad of genes in a variety of tissues and cell types (Remy and Baltzinger 2000;

Verger and Duterque-Coquillaud 2002; Hollenhorst, McIntosh et al. 2011). The name "Ets" stems from a sequence that was detected in an avian erythroblastosis virus, E26 carring the v-est oncogene (Sharrocks 2001; Pham, Lawson et al. 2007). The newly discovered sequence was called E26 transformation specific sequence or Ets (Blair and Athanasiou 2000; Dittmer 2003). This family was originally defined by the presence of a conserved DNA binding domain, the Ets domain (Span, Manders et al. 2002; Dwyer, Li et al. 2007). ETS proteins are composed of 85 amino acids, with 4 tryptophan repeats DNA-binding domain that recognizes DNA sequences containing a GGAA/T core motif (Sharrocks 2001; Dittmer 2003). In mouse, the ETS family consists of 28 members and it is known to control important biological processes, including cellular proliferation, differentiation, lymphocyte development and activation, apoptosis and migration in different cell types such as B cells, endothelial cells, fibroblasts and fibroblasts (Koskela and Lassila 2003; Wang, John et al. 2005; Gallant and Gilkeson 2006; Dwyer, Li et al. 2007; Geisinger, Astaiza et al. 2012).

Ets1 is the prototype of the ETS family. The ETS1 protein is closely related to ETS2. In humans, the Ets-1 and Ets2 genes are located on two distinct chromosomes, on chromosome #11 and #21, respectively (Watson, McWilliams-Smith et al. 1985; Dittmer 2003). The Ets-1 protein sequence is highly conserved among species. It can be divided into six domains, A-F. The A- and B- domains are regulatory units, C-domain is the activation domain, the D- (or exon VII) domain and F- domain are regulatory domains that control the activity of E- domain, and the E- domain is the DNA binding Ets domain (Sharrocks 2001; Wasylyk, Schlumberger et al. 2002; Dittmer 2003). Ets1 harbors a conserved 85 amino acid DNA binding domain termed the Ets domain. The Ets1 mRNA is subject to alternate splicing to yield three isoforms of the protein: the full length protein p51 or p54 (based on predicted

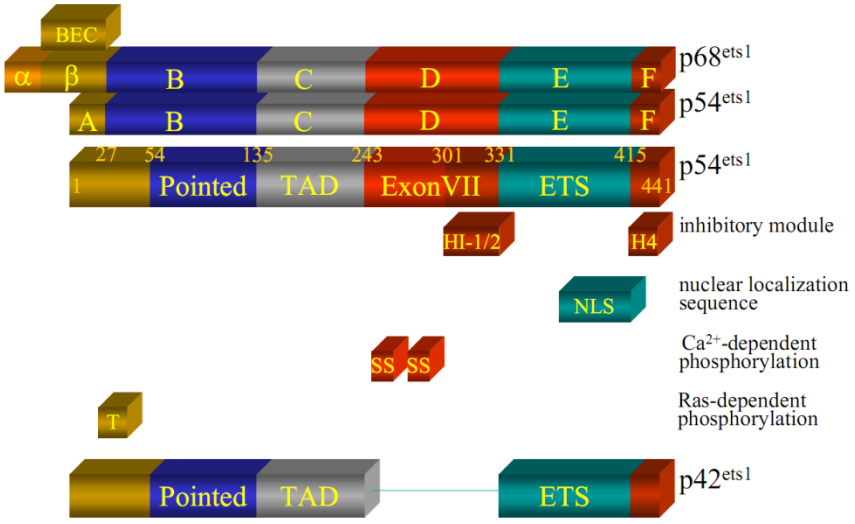

Figure 6. The domains of Ets1 protein, obtained from Rui-Xueleng, Autoimmunityreviews, 2011

molecular weight or on its actual migration in SDS-PAGE gels, respectively), p42, a protein lacking the sequences encoded in exon VII and p27, a protein lacking the sequences encoded in exons III-VI (Dittmer 2003; Lulli, Romania et al. 2010). The D- or exon VII domain comprises two regulatory units. The C-terminal unit is a part of an autoinhibitory module; the N- terminal sequence contains a calcium-responsive phosphorylation site. The two units are functionally connected as phosphorylation within the N- terminal sequence increases the inhibitory effect of the C- terminal unit (Dittmer 2003; Russell and Garrett-Sinha 2010; Hollenhorst, McIntosh et al. 2011). Calcium- dependent phosphorylation of the exon VII domain interferes with the activity of the Ets1 autoinhibitory module and decreases the stability of the ETS protein in T- lymphocytes (Dittmer 2003; Mellstrom, Savignac et al. 2008; Lulli, Romania et al. 2010).

ETS1 is expressed in different cell types and is known to play various roles during both physiological and pathological conditions. ETS1 is a transcription factor that is highly expressed within lymphoid cells, in mice, chickens and humans (Remy and Baltzinger 2000; Lelievre, Lionneton et al. 2001). It is known that ETS1 regulates the function of immune cells including B cells, T cells, NK cells and NK T cells (Colucci, Caligiuri et al. 2003; Eyquem, Chemin et al. 2004; Wang, John et al. 2005; Choi, Geng et al. 2011; Grenningloh, Tai et al. 2011; Ramirez, Chandler et al. 2012). Kevin Barton has demonstrated that ETS1-deficient mice produced by gene targeting develop mature erythrocytes, monocytes, neutrophils, and T and B lymphocytes (Barton, Muthusamy et al. 1998). However, loss of ETS1 partially impairs bone marrow-derived B cell development in that there are fewer bone marrow derived B cells and they are inefficient in the pro-B to pre-B transition (Wang, John et al. 2005). Moreover, ETS1 knockout mice have multiple aberrations in T cell development in the thymus and functional responses in the periphery. Many of these early defects in thymocyte differentiation appear to be due to impaired pre-TCR signaling in the absence of ETS1 (Eyquem, Chemin et al. 2004; Higuchi, Bartel et al. 2007). In addition, studies have demonstrated altered cytokine production by ETS1-deficient CD4 and CD8 T cell subsets (Grenningloh, Kang et al. 2005; Zhu, Yamane et al. 2010).

Many Ets-domain transcription factors are known as nuclear targets of many signaling pathways (Sharrocks 2001). The transcription factor Ets2 is one of several transcription factors that have recently been identified as targets of Akt action in mammalian cells (Oikawa 2004).

1.5.5 SGK1

The serum & glucocorticoid inducible kinase (SGK) family exhibits structural similarity to Akt, though it lacks a PH domain. SGK1 is a member of the AGC family of serine/threonine protein kinases, originally cloned by Firestone laboratory as a

glucocorticoid-responsive gene from mammary tumor cells (Loffing, Flores et al. 2006; Tessier and Woodgett 2006). Like Akt, the SGK family is encoded by three genes in mammalian genomes: SGK1, 2 and 3. SGK1 and 3 are ubiquitously expressed, while SGK2 RNA is only present in liver, kidney and pancreas, and at lower levels in the brain (Lang, Bohmer et al. 2006; Loffing, Flores et al. 2006). SGK2 and SGK3 share 80% amino acid identity with SGK1 and with each other in their catalytic domains. However, only SGK1 is responsive to glucocorticoids at the level of transcription (Arteaga, Alvarez de la Rosa et al. 2007; Pao, Bhargava et al. 2010).

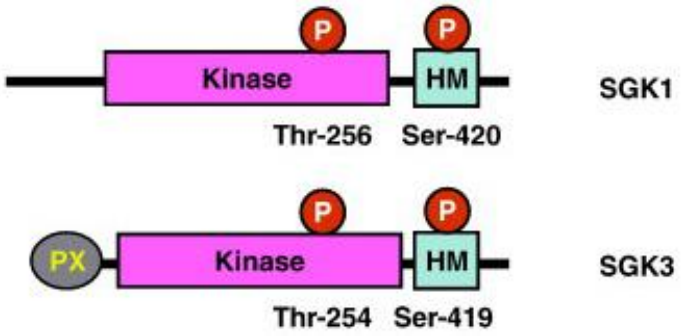

Figure 7. The domain structure of SGK1 and SGK3, obtained from RE Burke - Pharmacology & Therapeutics, 2007

Basically, SGKs have two domains: C-terminal catalytic and N-terminal hydrophobic motif domains (Lang, Bohmer et al. 2006; Tessier and Woodgett 2006). Three enzymes differ in the N-terminal domain. SGK2 contains a short N terminus, with no discernable domain, whereas SGK3 has a longer N terminus comprising a phox homology domain, which is another 3´phosphorylated inositol phospholipid-binding domain (Tessier and Woodgett 2006; He, Lee et al. 2011). The catalytic domain of SGKs shares 55% identity with Akt catalytic domain. Moreover, similar to Akt, SGK1 is activated on phosphorylation by PDK1 activated in their turn by PI3K (Vanhaesebroeck and Alessi 2000; Mora, Komander et al. 2004). All three isoforms of SGKs require PI3K activation for function (Woodgett 2005). A distinguishing feature of SGK1 is its stringent stimulus-dependent regulation of its transcription, intrinsic kinase activity, and subcellular localization (Lang, Bohmer et al. 2006; Bononi, Agnoletto et al. 2011). Many extracellular and intracellular factors can upregulate SGK1 mRNA level, such as FSH, serum, and TGFβ (Stockand 2005; Lang, Bohmer et al. 2006). The SGK1 promoter region contains a glucocorticoid response element, a TATA box, SP-1 elements and putative binding sites for p53, AP-1, NFκB, and Ets-2 (Dittmer 2003; Loffing, Flores et al. 2006).

SGK1 has been shown to foster nuclear translocation and activation of NFκB (Lang, Strutz-Seebohm et al. 2010). SGK1 is also involved in the regulation of many other

transcription factors, e.g. forkhead transcription factor Foxo3a and β-catenin (Brunet, Park et al. 2001; Dehner, Hadjihannas et al. 2008).

Moreover, SGK1 regulates a great variety of ion channels, e.g. Na^+ channels: SCN5A, ENaC, ASIC1; cation/Ca^{2+} channels: TRPV4, TRPV5, TRPV6, Orai1; K^+ channels: ROMK, Kv1.1–5, KCNEx/KCNQ1–5; Cl- channels: VSOAC, ClC2, ClCKa/bartin, CFTR; glutamate receptor GluR6 and transporters, e.g. NHE3, NKCC2, NCC, NaPiIIb, SMIT, GLUT1,4, SGLT1, NaDC, EAAT1–5, SN1, ASCT2, 4F2/LAT, PepT2, and the Na^+/K^+-ATPase (Boehmer, Laufer et al. 2008; Boehmer, Palmada et al. 2008; Schuetz, Kumar et al. 2008; Gehring, Zürn et al. 2009; Krueger, Haerteis et al. 2009; Lang, Artunc et al. 2009; Laufer, Boehmer et al. 2009; Lang, Strutz-Seebohm et al. 2010; Sopjani, Kunert et al. 2010) .

SGK1 can modulate ion channels/transporters via i) direct phosphorylation as have been shown e.g. for ENaC, TRPV4; ii) increasing protein abundance in the plasma membrane via phoshorylation and thereby inhibition of the ubiquitin ligase Nedd4-2, which prepares its targets for clearance from the membrane (shown for e.g. ENaC, SCN5a, ClCKa/bartin, ClC2, Orai1); iii) increasing channel protein abundance in cooperation with NHERF2 ($Na^{+/}H^+$ exchanger regulatory factor 2), a protein mediating trafficking of carriers and channels to the plasma membrane (shown for e.g. ROMK1, TRPV5); iv) upregulation of the transcription factor (NF-κB), which is required for the transcription of channel (Orai1, STIM1) (Lang and Shumilina 2012).

Via the powerful stimulation of Orai1 and STIM1, the components of CRAC (or SOC) channels, which play a pivotal role in immune cell functions, SGK1 has recently been established as an important regulator of immune responses. In mast cells from SGK1-deficient mice, the decreased SOC entry is paralleled by decreased degranulation and a blunted anaphylactic reaction (Sobiesiak, Shumilina et al. 2009; Shumilina, Zemtsova et al. 2010). Along those lines, the decreased Ca^{2+} influx in STIM1-deficient (Baba, Nishida et al. 2007; Vig, DeHaven et al. 2007) or Orai1-deficient (Vig, DeHaven et al. 2007)(149) mast cells similarly compromises degranulation, cytokine production, and anaphylactic reactions. Moreover, the recent study of our group has demonstrated that upon allergen-induced mast cell activation, SGK1-sensitive upregulation of SOC channels is required for Ca^{2+}-dependent depolymerization of actin filaments under the plasma membrane (Schmid, Gu et al. 2012). The depolymerization of F-actin ring provides access of mast cell granules to the plasma memrane (Schmid, Gu et al. 2012) and consequently, decreased degranulation of $sgk1^{-/-}$ mast cells could be rescued by actin-disrupting drug cytochalasin B (Schmid, Gu et al. 2012). Moreover, SGK1 deficiency decreases Ca^{2+} entry, degranulation, adhesion, and aggregation of blood platelets (Borst, Schmidt et al. 2012). However, nothing is known about SGK1 functions in DCs. Therefore, the present study explores the possible SGK1-dependent functions in mouse bone marrow-derived DCs.

2. Materials and Methods

2.1 Materials

2.1.1 Cell Culture

I. Equipment

Name	Firms and Country of Origin
Centrifuge RotiFix 32	Hettich Zentrifugen, Tuttlingen, Germany
Eppendorf cups 1.5 ml	Eppendorf AG, Hamburg, Germany
Eppendorf pipettes 1000 µl, 100 µl, 10 µl	Eppendorf AG, Hamburg, Germany
Heraeus Incubator	Thermo Electron Corporation, Dreieich, Germany
Needles BD Microlance™ 3, 0.55x25 mm	Becton Dickinson Labware, Franklin Lakes, USA
Neubauer counting chamber	Brand, Wertheim, Germany
Pipetus® pipetting aid	Hirschmann Laborgeräte, Eberstadt, Germany
PP-Test tubes 15, 50 ml	Greiner bio-one, Frickenhausen, Germany
Stripette®5, 10, 25 ml	Corning Incorporated, Corning NY, USA

Syringe BD 10 ml Luer-Lok™ Tip	Becton Dickinson Labware, Franklin Lakes, USA
Tissue Culture Dishes 60x15 mm	Becton Dickinson Labware, Franklin Lakes, USA
Vortex	Genie Scientific Industries, Bohemia NY, USA

II. Substance

Name	**Firms and Country of Origin**
Foetal Bovine Serum (FBS) GIBCO	Peprotech/Tebu, Carlsbad, Germany
GMCSF mouse recombinant	GIBCO, Cölbe, Germany
L-Glutamine	Carlsbad, Germany
Lipopolysaccharide E.coli (LPS)	Sigma, Taufkirchen, Germany
MEM Non-Essential Amino Acids	Invitrogen, Karlsruhe, Germany
Penicillin-Streptomycin	Invitrogen, Karlsruhe, Germany
Phosphate buffered saline (PBS)	GIBCO, Carlsbad, Germany
RPMI 1640	GIBCO, Carlsbad, Germany
Trypan blue solution 0,4%	Sigma, Taufkirchen, Germany
β-mercaptoethanol	Invitrogen, Karlsruhe, Germany

2.1.2 Calcium Imaging

I. Equipment

Name	Firms and Country of Origin
Camera Proxitronic	Proxitronic, Bensheim, Germany
Centrifuge RotiFix 32 Hettich	Zentrifugen, Tuttlingen, Germany
Discofix® Stopcock for Infusion Therapy	B. Braun, Melsungen, Germany
Filter Set for Fura-2	AHF Analysentechnik AG, Tübingen, Germany
Filter tips 10, 100, 1000µl	Biozym Scientific, Hess. Oldendorf, Germany
Filter wheel	Sutter Instrument Company, Novato, USA
Infusion Regulator Dosi-Flow 10	Dahlhausen, Köln/Sürth, Germany
Lambda 10-2	Sutter Instrument Company, Novato, USA
Lamp XBO 75	Leistungselektronik Jena GmbH, Jena, Germany
Metafluor software	Universal Imaging, Downingtown, USA
Microscope Axiovert 100	Zeiss, Oberkochen, Germany
Microscope cover glasses round, 30mm diameter, 0.13-0.16 mm	Karl Hecht KG,

	Sondheim, Germany
Neutral densitiy filters 10, 20, 40, 60%	AHF Analysentechnik AG, Tübingen, Germany
Objective fluar 40x/1.3 oil	Carl Zeiss, Oberkochen, Germany
Syringe BD 10 ml, Luer-Lok™ Tip	Becton Dickinson Labware, Franklin Lakes, USA
Syringe BD Perfusion™ 50 ml	Becton Dickinson Labware, Franklin Lakes, USA
Tissue Culture Dishes 60x10 mm	Becton Dickinson Labware, Franklin Lakes, USA
Winged Needle Infusion Set	Butterfly®-Hospira Venisystems, Donegal Town, Ireland

II. Substance

Name	**Firms and Country of Origin**
Ampuwa Fresenius	KABI, Bad Homburg, Germany
CaCl2 x 2 H2O	Carl Roth, Karlsruhe, Germany
Recombinant Murine Exodus-2 (CCL21)	Peprotech, Hamburg, Germany
Ethylene glycol tetraacetic acid (EGTA)	Sigma, Taufkirchen, Germany
Fura-2 AM	Invitrogen, Karlsruhe, Germany
Glucose	Carl Roth, Karlsruhe, Germany
HEPES	Sigma, Taufkirchen, Germany

Immersol 518F	Carl Zeiss, Göttingen, Germany
Ionomycin	Sigma, Taufkirchen, Germany
KCl	Carl Roth, Karlsruhe, Germany
LPS	Enzo Life Sciences GmbH, Lörrach, Germany
MgSO4 x 7 H2O	Sigma, Taufkirchen, Germany
Na2HPO4 x 2 H2O	Sigma, Taufkirchen, Germany
NaCl	Sigma, Taufkirchen, Germany
Thapsigargin	Invitrogen, Karlsruhe Germany

2.1.3 Patch Clamp

I. Equipment

Name	Firms and Country of Origin
Borosilicate glass filaments	Harvard Apparatus, March-Hugstetten, Germany
DMZ puller	Zeitz, Augsburg, Germany
EPC-9 amplifier	Heka, Lambrecht, Germany
ITC-16 Interface	Instrutech, Port Washington, N.Y., USA
Microscope Axiovert 100	Zeiss, Oberkochen, Germany
MS314 electrical micromanipulator	MW, Märzhäuser, Wetzlar, Germany

Pulse software	Heka, Lambrecht, Germany

II. Substance

Name	Firms and Country of Origin
MgSO4 x 7 H2O	Sigma, Taufkirchen, Germany
Na2HPO4 x 2 H2O	Sigma, Taufkirchen, Germany
NaCl	Sigma, Taufkirchen, Germany
Poly-L-Lysine	Sigma, Taufkirchen, Germany
Silicone grease	Carl Roth, Karlsruhe, Germany
Phosphate buffered saline (PBS)	Invitrogen, Karlsruhe Germany
D-myo-inositol-1,4,5-triphosphate (IP_3)	Enzo, Life Sciences, Germany
HCl	Sigma, Taufkirchen, Germany
Immersol 518F	Carl Zeiss, Göttingen, Germany

2.1.4 FACS

I. Equipment

Name	Firms and Country of Origin
FACS Calibur	Becton Dickinson, Heidelberg, Germany
FACS tubes, 1.3 ml, PP, round bottom	Greiner bio-one, Frickenhausen, Germany

II. Reagent

Name	Firms and Country of Origin
APC-conjugated anti-mouse CD11c,clone HL3	BD Pharmingen, Heidelberg, Germany
2',7'-dichlorodihydrofluorescein diacetate (DCFDA)	Sigma, St.Luis, USA

2.1.5 Cytokine Production

I. Equipment

Name	Firms and Country of Origin
Magellan™ software	Tecan Group Ltd., Männedorf, Switzerland
Multi well plates; 96 well	Corning Inc., Corning NY, USA
Sunrise Microplate Reader	Tecan Trading AG, Switzerland

II. Kit

Name	Firms and Country of Origin
Mouse IL-2 ELISA Set	BD Pharmingen, Heidelberg, Germany
Mouse IL-6 ELISA Set	BD Pharmingen, Heidelberg, Germany
Mouse IL-12 ELISA Set	BD Pharmingen, Heidelberg, Germany

2.1.6 Migration

I. Equipment

Name	Firms and Country of Origin
BD Falcon™ Cell culture inserts for 24-well plates. 8.0 µm pores	BD Falcon, Heidelberg, Germany
BD Falcon™ cell culture insert companion plates (24-well, BD cat. no. 353504)	BD Falcon, Heidelberg, Germany
Carl Zeiss Microscopy LSM 700	Carl Zeiss, München, Germany

II. Reagent

Name	Firms and Country of Origin
CCL21	Peprotech/Tebu, Cölbe, Germany
CXCL12	Peprotech/Tebu, Cölbe, Germany
Xestospongin C, Xestospongia sp	Enzo Life Sciences GmbH, Lörrach, Germany
Paraformaldehyde	Sigma, Taufkirchen, Germany
ProLong® Gold Antifade Reagent with DAPI	Invitrogen, Darmstadt Germany

2.1.7 Western Blotting and Nuclear Fractionation

I. Equipment

Name	Firms and Country of Origin
Agarose gel electrophoresis chamber	BioRad, München, Germany
Centrifuge 5415R	Eppendorf, Hamburg, Germany

Densitometer Quantity One	BioRad, München, Germany
GE Healthcare film	GE Healthcare, München, Germany
Nitrocellulose membrane	GE Healthcare, München, Germany

II. Reagent

Name	**Firms and Country of Origin**
Acrylamide/bisacrylamide	Carl Roth, Karlsruhe, Germany
Cell lysis buffer 10x	Cell Signaling Technology, Frankfurt, Germany
Complete Protease Inhibitor Cocktail Tablets	Roche, Mannheim, Germany
Detection reagent	GE Healthcare, München, Germany
Glycine	Sigma, Taufkirchen, Germany
Loading buffer (4x)	Carl Roth, Karlsruhe, Germany
Milk powder	Carl Roth, Karlsruhe, Germany
peqGOLD Protein-Marker IV	Peqlab, Erlangen, Germany
peqGOLD Protein-Marker V	Peqlab, Erlangen, Germany
Sodium dodecyl sulfate (SDS)	Sigma, Hannover, Germany
TEMED	Carl Roth, Karlsruhe, Germany
Tween® 20	Carl Roth, Karlsruhe, Germany

2.1.8 Real Time-PCR

I. Equipment

Name	Firms and Country of Origin
BZO Seal Film	Biozym biotech trading GmbH
CFX96 Real Time System	BioRad, München, Germany
Low Profile 96 Well PCR Plate	PEQLAB Biotechnologie GMBH, Erlangen, Germany
UV Cuvette	Eppendorf AG, Hamburg, Germany

II. Reagent / Kit

Name	Firms and Country of Origin
2-Propanol (Isopropanol)	Sigma, Taufkirchen, Germany
Chloroform	Carl Roth, Karlsruhe, Germany
DEPC water	Promega, Mannheim, Germany
dNTP mix	Promega, Mannheim, Germany
Ethanol 99.7%	VWR, Darmstadt, Germany
GoTaq® qPCR Master Mix	Promega, Mannheim, Germany
peqGOLD TriFast	PEQLAB Biotechnologie GMBH, Erlangen, Germany

Primers	Invitrogen, Darmstadt Germany
Transcriptor High Fidelity cDNA Synthesis Kit	Roche, Mannheim, Germany

2.2 Solutions

2.2.1 Cell Culture Medium

Normal Medium

Substance (RPMI 1640 500ml)

FBS 10%

Penicillin Streptomycin 1%

Glutamine 1%

Nonessential amino acids 1%

β-mercaptoethanol 0.05%

Medium for Silencing

Substance (RPMI 1640 500ml)

Penicillin Streptomycin 1%

Glutamine 1%

Nonessential amino acids 1%

β-mercaptoethanol 0.05%

2.2.2 Intracellular Calcium Imaging

Chemical and Biological Reagents

Standard HEPES Solution

Substance [mmol/L]

NaCl 125

KCl 5

$MgSO_4x7H_2O$ 1.2

HEPES 32.2

$Na_2HPO_4x2H_2O$ 2

$CaCl_2x2H_2O$ 2

Glucose 5

pH 7.4 (NaOH); H_2O (at the 37 ℃)

Ca^{2+} Free Solution

Substance [mmol/L]

NaCl 125

KCl 5

$MgSO_4x7H_2O$ 1.2

HEPES 32.2

$Na_2HPO_4 x2H_2O$ 2

EGTA 0.5

Glucose 5

pH 7.4 (NaOH); H_2O (at the 37 ℃)

2.2.3 Patch Clamp – Pipette Solutions

Pipette Solution [mmol/L]

NaCl 35

CsCl 120

EGTA 10

HEPES 10

IP_3 0.04

pH 7.2 (CsOH); H_2O (at the 20 ℃)

Bath Solution [mmol/L]

NaCl 140

KCl 5

$CaCl_2$ 10

Glucose 20

HEPES 10

pH 7.4 (NaOH); H_2O (at the 20 ℃)

2.2.4 FACS

FACS Buffer

PBS

0.1 % heat-inactivated FBS

Cytokine Production

Coating Buffer

0.2 M sodium phosphate

pH 6.5 (HCl); H_2O (at room temperature)

Assay Diluent

1x PBS

heat-inactivated FBS 10 %

Stop Solution

2 M H_2SO_4

2.2.5 Western Blotting and Nuclear Fractionation

Running Buffer

Tris 25 mM

Glycine 250 mM

SDS 0,1 %

Transfer Buffer

Tris 25 mM

Glycine 192 mM

Methanol 20 %

pH 8.3 (HCl); H_2O (at room temperature)

Wash Buffer (TBS-T)

Tris 50mM

NaCl 150mM

Tween-20 0.05 %

pH 7.4 (HCl); H_2O (at room temperature)

Nuclear Fractionation Buffer A

10 mM HEPES

1.5 mM $MgCl_2$

10 mM KCl

0.5 mM DTT

0.05% NP40

pH 7.9 (HCl); H_2O (at room temperature)

Nuclear Fractionation Buffer B

5 mM HEPES

1.5 mM $MgCl_2$

0.2 mM EDTA

0.5 mM DTT

26% glycerol (v/v)

pH 7.9 (HCl); H_2O (at room temperature)

2.3 Animals

All animal experiments were conducted according to the German law for the welfare of animals and were approved by local authorities.

Akt2-deficient mice (*akt2*$^{-/-}$) and their wild type littermates (*akt2*$^{+/+}$) as well as SGK1- deficient mice (*sgk1*$^{-/-}$) and their wild type littermates (*sgk1*$^{+/+}$) were used in the present study. Male and female mice were studied at the age of 6-12 weeks. Origin of the *akt2*$^{-/-}$ mice, breeding and genotyping were described previously(Cho, Mu et al. 2001). Knockout mice were generated with a targeted disruption in the *Akt2* locus by homologous recombination. The targeting vector was designed to insert *LoxP* sites flanking the sequence containing the coding exons 4 and 5. Mice harboring the targeted allele were identified by Southern blotting and were mated to transgenic mice expressing Cre recombinase driven by a 6-kb 59-flanking sequence from the *Brn/Pou3f4* gene to cause germ-line excision of exons 4 and 5. The progeny carrying both the *Cre* transgene and the targeted allele were mated with wild-type mice to obtain offspring in which the *Cre* transgene was segregated away and the targeted allele was excised, as determined by the PCR and Southern blotting, respectively. These mice were mated inter se to produce offspring with homozygous deletions of *Akt2*.

Origin of the *sgk1*$^{-/-}$ mice, breeding and genotyping were described previously (Wulff, Vallon et al., 2002).

A conditional targeting vector was generated from a 7-kb fragment encompassing the entire transcribed region on 12 exons. The neomycin resistance cassette was flanked

by two loxP sites and inserted into intron 11. Exons 4-11, which code for the *sgk1* domain, were "floxed" by inserting a third loxP site into intron 3. Targeted R1 ES cells were transiently transfected with Cre recombinase. A clone with a recombination between the first and third loxP site (type I recombination) was injected into C57BL/6 blastocytes. Male chimeras were bred to 129/SvJ females. Heterozygous *sgk1*-deficient mice were backcrossed to 129/SvJ wild-type mice for two generations and then intercrossed to generate homozygous $sgk1^{-/-}$ and $sgk1^{+/+}$ littermates (Wulff, Vallon et al., 2002). The animals were genotyped by PCR using standard methods.

2.4 Methods

2.4.1 Culture of Bone Marrow Dendritic Cells

Dendritic cells (DCs) were isolated from the mouse bone marrow as described previously (Matzner, Zemtsova et al. 2008; Xuan, Shumilina et al. 2009). Briefly, bone marrow derived cells were flushed out of the cavities from the femur and tibia with PBS. Cells were then washed twice with RPMI and seeded out at a density of 2 x 10^6 cells per 60 mm dish. Cells were cultured for up to 9 days in RPMI 1640 (GIBCO, Carlsbad) containing: 10% FCS, 1% penicillin/streptomycin, 1% glutamine, 1% non-essential amino acids (NEAA) and 0.05% β-mercaptoethanol. Cultures were supplemented with GM-CSF (35 ng/mL, Immunotools) and fed with fresh medium containing GM-CSF on days 3 and 6. Experiments were performed on days 7-9. Immature and mature DCs were used in the present study. DC maturation was induced by treating the cells with lipopolysaccharides (LPS from *E. coli*, 100 ng/ml, 48h or 1 µg/ml, 24 h, Enzo Life Sciences, Lausen, Switzerland).

2.4.2 Isolation of Splenic DCs

Dendritic cells were isolated from the spleen of 8-12 weeks old mice accoriding to the protocol of Stagg et al. (Stagg, Burke et al., 2001). Briefly, single-cell suspensions were prepared by pressing mouse spleen through a gauze cell strainer (BD Pharmingen, Heidelberg, Germany) and washing in RPMI 1640 (GIBCO, Carlsbad, Germany) containing: 10 % FCS, 1 % penicillin/streptomycin, 1 % glutamine, 1 % non-essential amino acids (NEAA) and 0.05 % β-mercaptoethanol. After overnight incubation nonadherent cells were collected and DCs were purified by centrifugation over a 13.7% (w/v) metrizamide discontinuous gradient.

2.4.3 Intracellular Calcium Imaging

To determine cytosolic Ca^{2+} concentration, the cells were loaded with Fura-2/AM (2 µM, Molecular Probes, Goettingen, Germany) for 15 min at 37°C. Fluorescence measurements were carried out with an inverted phase-contrast microscope (Axiovert 100, Zeiss, Oberkochen, Germany). Cells were excited alternatively at 340 or 380 nm and the light was deflected by a dichroic mirror into either the objective (Fluar 40×/1.30 oil, Zeiss, Oberkochen, Germany) or a camera (Proxitronic, Bensheim, Germany). Emitted fluorescence intensity was recorded at 505 nm and data acquisition was accomplished by using specialized computer software (Metafluor, Universal Imaging Downingtown, USA). As a measure for the increase of cytosolic Ca^{2+} concentration, the slope and peak of the changes in the 340/380 nm ratio were determined for each experiment.

Intracellular Ca^{2+} was measured prior to and following addition of CXCL12 (300 ng/ml) or CCL21 (75 ng/ml) to the Ringer solution (see below). Alternatively, the Ca^{2+} release was measured following addition of MgATP (100 µM, Alfa Aesar GmbH & Co KG, Germany) to the Ca^{2+}-free solution (see below).

In order to measure SOC entry, changes in cytosolic Ca^{2+} were monitored upon depletion of the intracellular Ca^{2+} stores. Experiments were carried out prior to and during exposure of the cells to Ca^{2+}-free solution (see below). In the absence of Ca^{2+}, the intracellular Ca^{2+} stores were depleted by inhibition of the vesicular Ca^{2+} pump by thapsigargin (1 µM, Molecular Probes). Re-addition of Ca^{2+} allowed assessing the store-operated Ca^{2+} entry.

2.4.4 Whole-cell Patch Clamp

Patch clamp experiments were performed at room temperature in voltage clamp, fast-whole-cell mode according to Hamill et al. (Hamill, Marty et al. 1981). The cells were continuously superfused through a flow system inserted into the dish. The bath was grounded via a bridge filled with NaCl Ringer solution. Borosilicate glass pipettes (2-4 MΩ tip resistance; GC 150 TF-10, Harvard Apparatus, UK) heat polished with a microprocessor-driven DMZ puller (Zeitz, Augsburg, Germany) were used in combination with a MS 314 electrical micromanipulator (MW, Märzhäuser, Wetzlar, Germany). The currents were recorded by EPC-9 amplifier (Heka, Lambrecht, Germany) using Pulse software (Heka) and an ITC-16 Interface (Instrutech, Port Washington, N.Y., USA). For I_{CRAC} measurements whole-cell currents were elicited by 200 ms square wave voltage pulses from -50 to +50 mV in 10 mV steps delivered from a holding potential of -30 mV. Alternatively, the currents were recorded with 200 ms voltage ramps from -50 to +50 mV. Leak currents determined as the currents at the very beginning of each experiment

immediately after reaching the whole-cell mode were subtracted. The currents were recorded with an acquisition frequency of 10 kHz and 3 kHz low-pass filtered. The liquid junction potential ΔE between the CsCl-based pipette and the NaCl-based bath solutions estimated according to Barry and Lynch (Barry and Lynch 1991) was 1 mV. The data were not corrected for ΔE.

DCs were superfused with a bath solution containing (in mmol/l): 140 NaCl, 5 KCl, 10 $CaCl_2$, 20 glucose, 10 HEPES/NaOH, pH 7.4. The patch clamp pipettes were filled with an internal solution containing (in mmol/l): 120 CsCl, 35 NaCl, 10 EGTA, 10 HEPES/CsOH, 0.04 inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3, Enzo Life Sciences), pH 7.4.

2.4.5 Migration Assay

For migration assays transwell inserts were used with a pore diameter size of 8 µm. The transwells were placed in a 24-well cell culture plate containing cell culture medium (500 µl) with or without either CXCL12 (50 ng/ml, Peprotech, for immature DCs) or CCL21 (25 ng/ml, Peprotech, for mature DCs) in the lower chamber. The upper chambers were filled with 500 µl cell culture medium containing immature or LPS (1 µg/ml, 24 h) - matured DCs either untreated or treated with IP_3R inhibitor Xestospongin C (5 µM, Enzo Life Sciences, Lausen, Switzerland) for 3h in a concentration of 1 x 10^5 cells/ml. The chamber was placed in a 5% CO_2 37°C incubator for 3h. In the following step, the transwells were placed in 4% PFA for overnight incubation in 4°C, to allow the cells to fix on the membrane. Unattached cells were gently removed with cotton swab, the membrane was then mended on a slide and stained with ProLong® Gold antifade reagent with DAPI (Invitrogen). Cells from five representative areas of each membrane were counted.

2.4.6 Cytokine Production

IL-12, IL-6, IL-2 and TNFα concentrations in culture supernatants from DCs treated with LPS (1 µg/ml, 24 h, for IL-6 measurements), LPS (1 µg/ml, 18 h, for IL-2 measurements), LPS (100 ng/ml, 48 h, for IL-12 measurements), or LPS (1 µg/ml, 4 h, for TNFα measurements) were determined by using OptEIA ELISA kit (BD Pharmingen) according to the manufacturer's protocol.

2.4.7 DC Phagocytosis Assay

DCs (10^6 cells/ml) were suspended in prewarmed serum-free RPMI 1640 medium, pulsed with FITC-conjugated dextran (Sigma-Aldrich, Taufkirchen, Germany) at a final concentration of 1 mg/ml and incubated for 3h at 37°C. Uptake was stopped by

adding ice-cold PBS. Then the cells were washed three times with ice cold PBS supplemented with 5% FCS and 0.01% sodium azide before FACS analysis. DCs were analyzed for the uptake of FITC-dextran.

2.4.8 Western Blotting

Protein lysates were separated by SDS-PAGE 10% gel electrophoresis and transferred onto nitrocellulose membrane. Membrane containing the immobilized proteins was incubated for 1 hour at room temperature with 10% non-fat milk in Tris-buffered saline-0.1% Tween 20 (TBS-T), followed by overnight incubation (4°C) with IP_3R2 primary antibody (1:750; Santa Cruz,), rabbit polyclonal ETS1 (1:200, 56 kDa, Abcam,), NDRG1 (1:1000), NFκBp65(1: 1000), rabbit monoclonal GAPDH (1:1000, 37 kDa, Cell Signaling). The following day membrane was washed 3 times in TBS-T and incubated for 1 h at room temperature with anti-rabbit (1:3000, Cell Signalling) or anti-goat (1:3000, Santa Cruz) secondary antibody and washed again in TBS-T. For detection, membrane was blotted with ECL reagent (GE Healthcare), exposed to X-ray film (GE Healthcare) and developed.

2.4.9 Nuclear Fractionation

Prepare 1 ml of buffer A with added cocktail of usual inhibitors (Roche). Twenty million transfected cells were harvested and washed twice with ice-cold PBS followed by resuspending the cell pellet in 500 µl of buffer A, leave on ice for 10 min. Centrifuge at 4°C at 3000 rpm for 10 min. Remove supernatant and keep it (this will contain everything except large plasma membrane pieces, DNA, nucleoli). On ice resuspend pellet in 374 µl of buffer B and add 26 µl of 4.6 M NaCl. Leave on ice for 30 min. Centrifuge at 24,000 g for 20 min at 4°C. Aliquot supernatant, remove 10 µl for Bradford assay and processed on SDS-PAGE for westernblotting.

2.4.10 Real-Time PCR

Total RNA was extracted from mouse DCs in Tri-Fast (Peqlab, Erlangen, Germany) according to the manufacturer's instructions. After DNAse digestion reverse transcription of total RNA was performed using Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Penzberg, Germany). Real-Time Polymerase chain reaction (RT-PCR) amplification of the respective genes were set up in a total volume of 20 µl using 2 µg of cDNA, 500 nM forward and reverse primer and 2 X GoTaq® Mast Mix (Promega, Mannheim, Germany) according to the manufacturer's protocol. Cycling conditions were as follows: initial denaturation at 95°C for 2 min, followed by 39 cycles of 95°C for 15 s, 58°C for 15 s and 68°C for 20 s. For the amplification the following primers were used (5`->3`orientation):

Table 2. RT-PCR primers sequences

Name	Forward sequence (5´->3´)	Reverse sequence (5´->3´)
Akt1	CAGCACCGGTTCTTTGCC	CAGTCTCAGAGGTGACCTGGG
Akt2	AGTGATGGAGCCACCATGAA	CCACTGCTCGCCCATAGTC
Akt3	CCGCTCGTTGTCCATGC	TTTTGATGAAGAATTACAGCTCAGA
IP3R1	AAGCGGATGGACCTGGTGTTAGAACTG	AATTTGTGCTGTGTGCTTCGCGTAGAACT
IP3R2	CTGTTCTTCTTCATCGTCATCATCATCG	GAAACCAGTCCAAATTCTTCTCCGTGA
IP3R3	CTTCTTTATCGTCATCATCATCGTGTTG	AGGTTCTTGTTCTTGATCATCTGAGCCA
RyR1	TGGGGCACTACAATAACTTC	AGCAGAATGACGATAACGAA
RyR2	CACAGACAATTCCTTCCTCTACCTA	AACACCTCTCTTGGTACATCTTCC
RyR3	AGGTTCCTTGCTCTGTTTGT	TGCTTTGGCCTCTTCTACTG
Ets1	GATATCCTGTGGGAGCATCTAGAGATC	CAGCTGGATCGGCCCACTTCCTGTGTAG
Ndrg1	ACCCTGAGATGGTAGAGGGTCTC	CCAATTTAGAATTGCATTCCACC
Tbp	CACTCCTGCCACACCAGCTT	TGGTCTTTAGGTCAAGTTTACAGCC

Specificity of the PCR product was confirmed by analysis of melting curves.

Real-time PCR amplifications were performed on a CFX96 Real Time System (Bio-Rad). All experiments were done in duplicates. Amplification of housekeeping gene Tbp (TATA binding protein) was performed to standardize the amount of sample RNA. Relative quantification of gene expression was achieved with the Ct method (where Ct is threshold cycle). In addition, PCR products were analysed by agarose gel electroporesis.

2.4.11 Immunoprecipitation

The cells were washed twice in ice-cold phosphate-buffered saline. The cell pellets were transfered into a cell lysis buffer (Cell Signaling) containing complete protease inhibitor mixture (Roche Applied Science). Lysates were washed with 50µl of protein A/G PLUS agarose beads (Santa Cruz) and then the supernatants were incubated overnight with Phospho-Akt Substrate (RXRXXS*/T) antibody. The supernatant and the antibody mixture was incubated with protein A/G PLUS agarose beads that were washed three times in cell lysis buffer, incubated at 37°C for 1 hour, then heated upto 95 °C for 5 min, and processed on SDS-PAGE for immunoblotting.

2.4.12 Gene Silencing

Specific siRNA sequences for Ets1 (Santa Cruz), negative control (Control siRNA, Santa Cruz) and siRNA sequences for Ndrg1 (Invitrogen) and negative control (Invitrogen) were synthesized and annealed by the manufacturer. siRNA transfection was carried out using the GeneSilencer siRNA transfection reagent (Genlantis, San Diego, CA,USA). 4 x 10^6 cells were washed and plated in 6-well plates in 2 ml of serum-free RPMI 1640. The siRNA and the negative control (1000 ng/ml) were incubated with GeneSilencer reagent following the manufacturer's protocol. Transfection mixture was then added to the wells and incubated overnight. The efficiency of silencing was assessed with RT-PCR and western blotting.

2.4.13 Statistical Analysis

Data are provided as means ± SEM, *n* represents the number of independent experiments. Differences were tested for significance using Student's unpaired two-tailed *t*-test or ANOVA. $P<0.05$ was considered statistically significant.

3. Result

Part1. Akt2- and ETS1-dependent Expression of IP_3 Receptor 2 is Required for Dendritic Cell Migration

3.1 Differentiation and Maturation of Bone Marrow Derived DCs

DCs were isolated from gene targeted mice lacking functional Akt2 (*akt2*$^{-/-}$) and their wild type littermates (*akt2*$^{+/+}$).

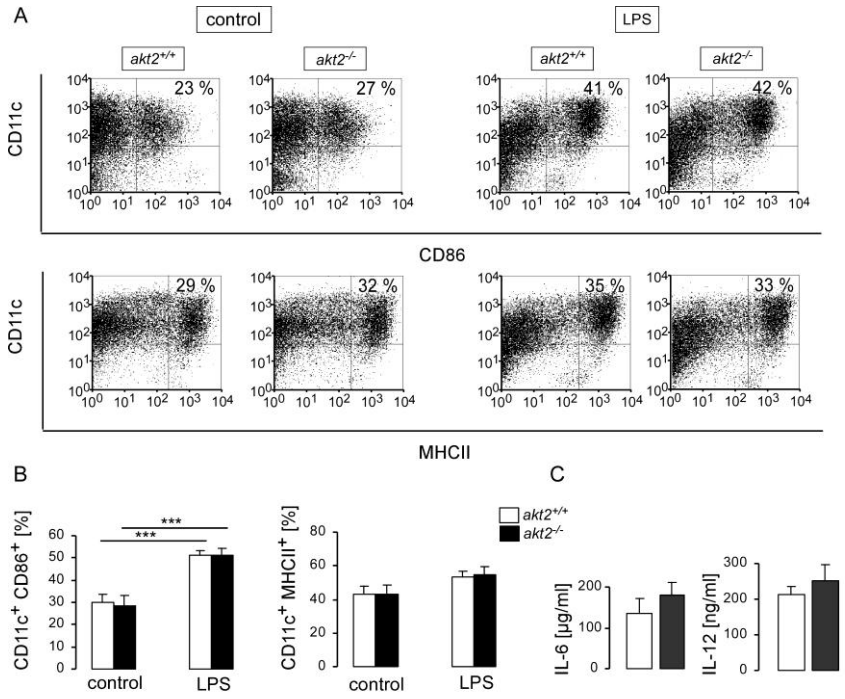

Figure 8 Differentiation, maturation and cytokine production of bone marrow derived dendritic cells (DCs) from *akt2*$^{+/+}$ and *akt2*$^{-/-}$ mice

A. Original dot plots of CD11c$^+$CD86$^+$ (above) and CD11c$^+$MHC II$^+$ (below) DCs at the basal level (control, 1st and 2^d panels) and stimulated with LPS (LPS, 1 µg/ml, 24 h, 3^d and 4th panels) from *akt2*$^{+/+}$ (1st and 3^d panels) and *akt2*$^{-/-}$ (2^d and 4th panels) mice. Numbers depict the percent of cells in the respective quadrants.

B. Arithmetic means ± SEM (n =7) of the percentage of CD11c$^+$CD86$^+$ (left) and CD11c$^+$MHC II$^+$ (right) DCs under control and 24 h after LPS stimulation in primary

cultures from *akt2*$^{+/+}$ (open bars) and *akt2*$^{-/-}$ (closed bars) mice. * (p<0.001), ANOVA.**

As illustrated in **Fig 8**, the surface abundance of both, the costimulatory molecule CD86 and antigen-presenting molecule MHC II, was similar on CD11c^{+} DCs from *akt2*$^{+/+}$ and *akt2*$^{-/-}$ mice. In both genotypes, exposure for 24 hours to bacterial lipopolysaccharides (LPS, 1 µg/ml) resulted in a significant increase of the CD11c^{+}CD86^{+} population. Following LPS treatment, the abundance of CD86 and MHC II was still similar in DCs from *akt2*$^{+/+}$ and *akt2*$^{-/-}$ mice (**Fig. 8A, B**). The release of interleukin 6 (IL-6) and 12 (IL-12) was again similar in LPS-matured DCs from *akt2*$^{+/+}$ and *akt2*$^{-/-}$ mice (**Fig. 8C**).

3.2 Impaired Migration of *akt2*$^{-/-}$ DCs

Migratory capacity of immature *akt2*$^{-/-}$ DCs in response to the CXCL12 chemokine as well as spontaneous migration was not different from *akt2*$^{+/+}$ DCs (**Fig. 9A**). However, in contrast to LPS-matured *akt2*$^{+/+}$ DCs, LPS-matured *akt2*$^{-/-}$ DCs failed to significantly enhance migration in response to the chemokine CCL21 (25 ng/ml) (**Fig. 9B**).

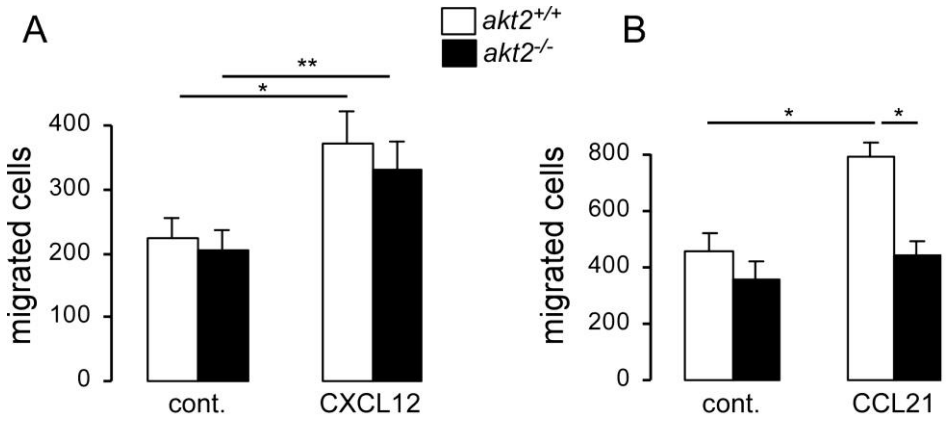

Figure 9. Impaired migration of LPS-matured *akt2*$^{-/-}$ DCs

A.-B. Arithmetic means ± SEM (n =11-18) of spontaneous migration and migration in response to either CXCL12 (50 ng/ml, 3h, immature DCs, A) or CCL21 (25 ng/ml, 3h, LPS (1 µg/ml, 24h)-matured DCs, B) of *akt2*$^{+/+}$ (open bars) and *akt2*$^{-/-}$ (closed bars) DCs. * (p<0.05), ** (p<0.01), ANOVA.

3.3 Reduced CCL21-dependent Increase of $[Ca^{2+}]_i$ in *akt2*$^{-/-}$ DCs

Fura2-fluorescence has been employed to determine, whether the difference in migratory activity of LPS-matured *akt2*$^{-/-}$ DCs was paralleled by the differences in the regulation of cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$). As illustrated in **Fig. 10**, both slope and peak of CCL21 (75 ng/ml)-induced increase of $[Ca^{2+}]_i$ was significantly less pronounced in LPS-matured DCs from from *akt2*$^{-/-}$ mice than in DCs from *akt2*$^{+/+}$ mice. Chemokine-induced increase of $[Ca^{2+}]_i$ is known to be due to the release of Ca^{2+} from intracellular stores followed by the store operated Ca^{2+} entry (SOCE) (Barbet, Demion et al. 2008; Nurbaeva, Schmid et al. 2012).

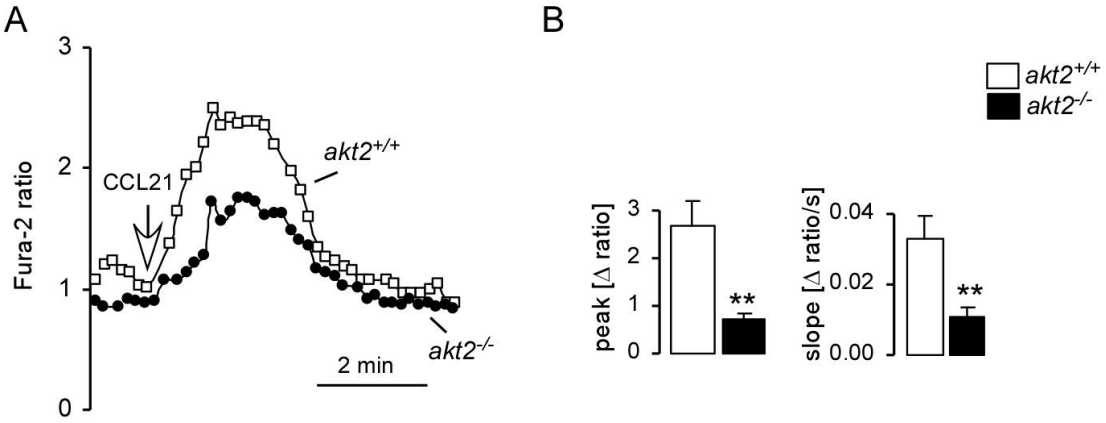

Figure 10. Diminished CCL21-induced increase in intracellular Ca^{2+} concentration in *akt2*$^{-/-}$ DCs

A. Representative original tracings showing the Fura-2 fluorescence ratios (340/380 nm) in LPS (1 µg/ml)-matured *akt2*$^{+/+}$ DCs (open squares) and *akt2*$^{-/-}$ DCs (closed circles) prior to and following acute addition of CCL21 (75 ng/ml; white arrow). For quantification of Ca^{2+} entry the slope (Δ ratio/s) and the peak (Δ ratio) of the fluorescence ratio were calculated by linear regression.

B. Mean (± SEM) of peak (left) and slope (right) of the change in Fura-2 fluorescence following addition of CCL21 (75ng/ml) to mature *akt2*$^{+/+}$ (n = 50, open bars) and *akt2*$^{-/-}$ (n = 40, closed bars) DCs. ** (p<0.01), two-tailed unpaired *t*-test.

3.4 Reduced Ca^{2+} Release and Store-operated Ca^{2+} Entry in LPS-matured *akt2*$^{-/-}$ DCs

Therefore, further experiments addressed the impact of Akt2 on Ca^{2+} release from intracellular stores and on SOCE in LPS-matured DCs. As indicated in **Fig. 11**, the addition of sarcoendoplasmatic reticulum Ca^{2+} ATPase (SERCA) inhibitor thapsigargin (1 µM) in the absence of extracellular Ca^{2+} was followed by an increase of $[Ca^{2+}]_i$, reflecting release of Ca^{2+} from intracellular stores. The slope and the peak of the intracellular Ca^{2+} release were significantly decreased in mature DCs from *akt2-/-* mice (**Fig. 11B**). The readdition of extracellular Ca^{2+} in the continued presence of thapsigargin resulted in a rapid increase of $[Ca^{2+}]_i$, reflecting SOCE. Both, peak and slope of SOCE were significantly higher in DCs from *akt2*$^{+/+}$ mice than in DCs from *akt2*$^{-/-}$ mice. Release of Ca^{2+} induced by physiological IP_3 formation could be assessed through the stimulation of DCs via P2Y receptors with low concentrations of ATP (Pellegatti, Falzoni et al. 2005). Accordingly, ATP(100 µM)-induced release of Ca^{2+} (measured in the absence of extracellular Ca^{2+}) was lower in LPS-matured *akt2*$^{-/-}$ than in *akt2*$^{+/+}$ DCs (**Fig. 11C**).

3.5 Impaired CRAC Channel Activity in LPS-matured *akt2*$^{-/-}$ DCs

Reduced SOCE in mature *akt2*$^{-/-}$ DCs could be confirmed by whole cell patch clamp recordings. As illustrated in **Fig. 12**, the Ca^{2+} release activated Ca^{2+} current I_{CRAC} triggered by IP_3–induced Ca^{2+} store depletion was significantly higher in LPS-matured DCs from *akt2*$^{+/+}$ mice than in *akt2*$^{-/-}$ DCs.

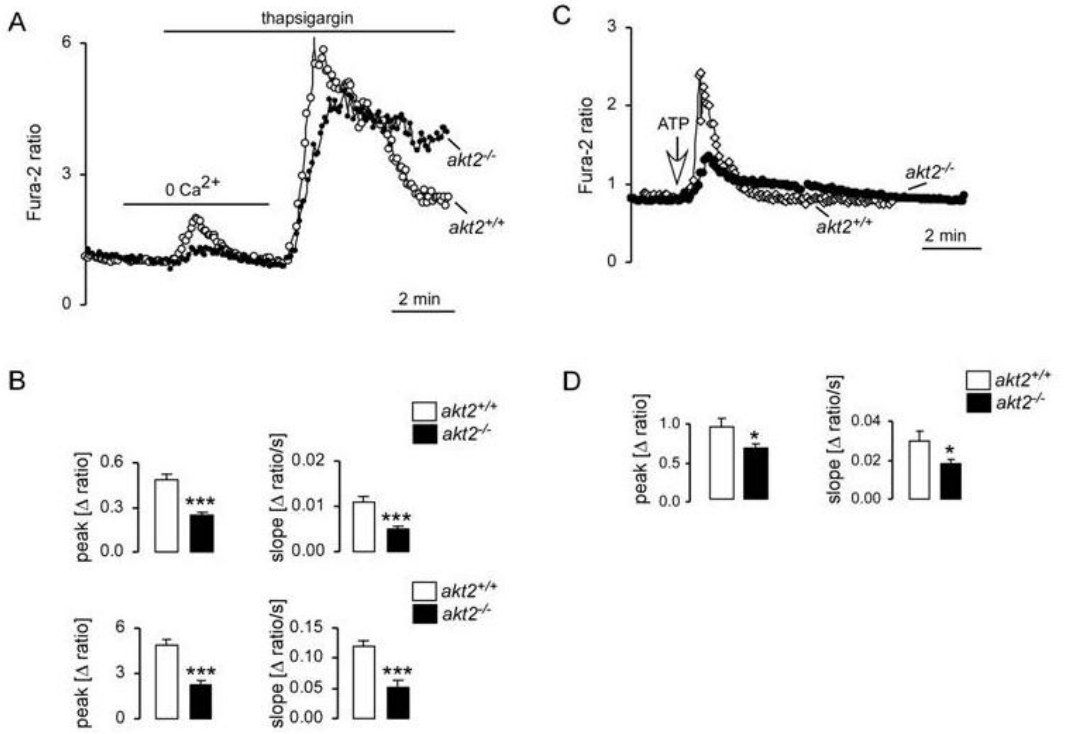

Figure 11. Reduced Ca^{2+} Release and Store-operated Ca^{2+} Entry in *akt2*$^{-/-}$ DCs

A. Representative tracing showing the Fura-2 fluorescence ratio in Fura-2/AM loaded LPS (1 µg/ml)-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs. Experiments were carried out prior to and during exposure to Ca^{2+}-free bath solution. Where indicated, thapsigargin (1 µM) was added to the Ca^{2+}-free bath solution and the release of Ca^{2+} from the stores was assessed. Readdition of extracellular Ca^{2+} in the presence of thapsigargin reflects the entry of Ca^{2+} through the SOC channels.

B. Arithmetic means (± SEM) of the slope (right) and the peak (left) of the change in Fura-2 fluorescence following addition of thapsigargin (1 µM) in the absence (upper bars) and in the presence (lower bars) of extracellular Ca^{2+} to mature *akt2*$^{+/+}$ (n=117, open bars) and *akt2*$^{-/-}$ (n=75, closed bars) DCs. * (p<0.001), two-tailed unpaired *t*-test.**

C. Representative tracing showing the Fura-2 fluorescence ratio in LPS (1 µg/ml)-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs prior to and following addition of ATP (100 µM) to the Ca^{2+}-free bath solution.

D. Arithmetic means (± SEM) of the slope (right) and the peak (left) of the change in Fura-2 fluorescence following addition of ATP (100 µM) in the absence of extracellular Ca^{2+} to LPS (1 µg/ml)-matured *akt2*$^{+/+}$ (n=242, open bars) and *akt2*$^{-/-}$ (n=250, closed bars) DCs. * (p<0.05), two-tailed unpaired *t*-test.

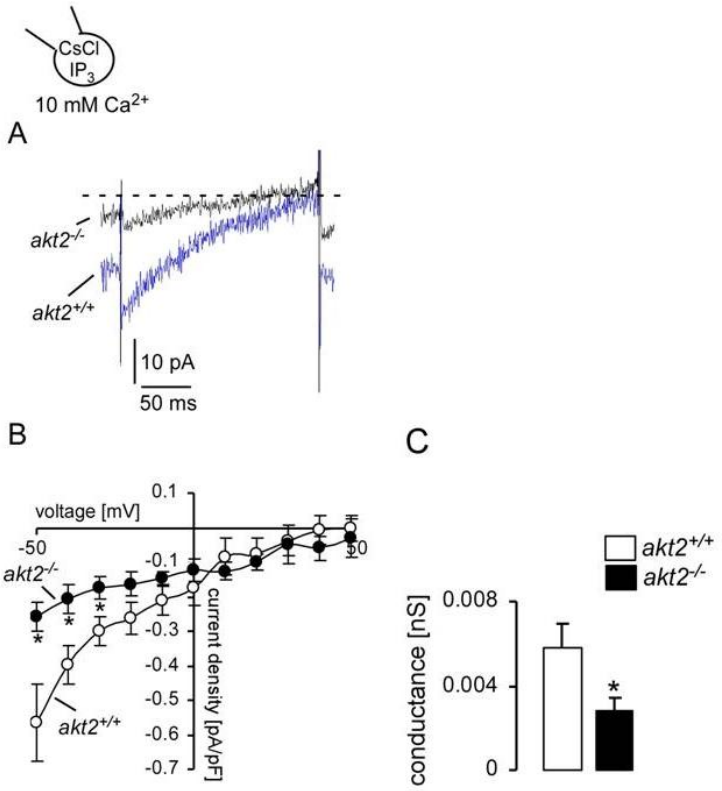

Figure 12. Impaired currents through store-operated Ca^{2+} channels in *akt2*$^{-/-}$ DCs

A. Mean current-voltage (I/V) relationships of currents activated by 40 µM IP_3 normalized to cell capacitance in LPS (1 µg/ml)-matured *akt2*$^{+/+}$ (n=11) and *akt2*$^{-/-}$ (n=15) DCs. * (p<0.05), two-tailed unpaired t-test.

B. Mean whole-cell conductance of inward currents (± SEM) calculated by linear regression of I/V curves shown in (A) between -50 and -10 mV in *akt2*$^{+/+}$ (n = 11, open bars) and *akt2*$^{-/-}$ (n=15, closed bars) DCs. * (p<0.05), two-tailed unpaired t-test.

3.6 Impaired Transcript Level and Protein Abundance of IP_3R2 in Mature *akt2*$^{-/-}$ DCs

Impaired Ca^{2+} release, SOCE and I_{CRAC} in *akt2*$^{-/-}$ DCs could have resulted from Akt2 sensitivity of the Ca^{2+} release pathways such as inositoltrisphosphate receptors IP_3R1-3 or the ryanodine receptor RyR1. As illustrated in **Fig. 13A**, transcript levels of IP_3R1, IP_3R3 and RyR1 were similar in immature *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs, whereas the transcript abundance of IP_3R2 was significantly higher in *akt2*$^{+/+}$ DCs than in *akt2*$^{-/-}$ DCs. Stimulation of *akt2*$^{+/+}$ DCs with LPS (1 µg/ml) resulted in a transient upregulation of IP_3R2 transcript abundance within 1h in *akt2*$^{+/+}$ DCs, an effect significantly impaired in *akt2*$^{-/-}$ DCs (**Fig. 13B**).

Western blot analysis was employed to elucidate whether the differences in transcript levels were paralleled by similar differencs in IP_3R2 protein abundance. Prior to maturation, the IP_3R2 protein abundance was similar in *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs. LPS (1 µg/ml, 24 h)-induced maturation resulted in a significant increase of IP_3R2 protein abundance in *akt2*$^{+/+}$ DCs, but not in *akt2*$^{-/-}$ DCs. Accordingly, following treatment with LPS, the IP_3R2 protein abundance was significantly higher in DCs from *akt2*$^{+/+}$ mice than in DCs from *akt2*$^{-/-}$ mice (**Fig.13C**).

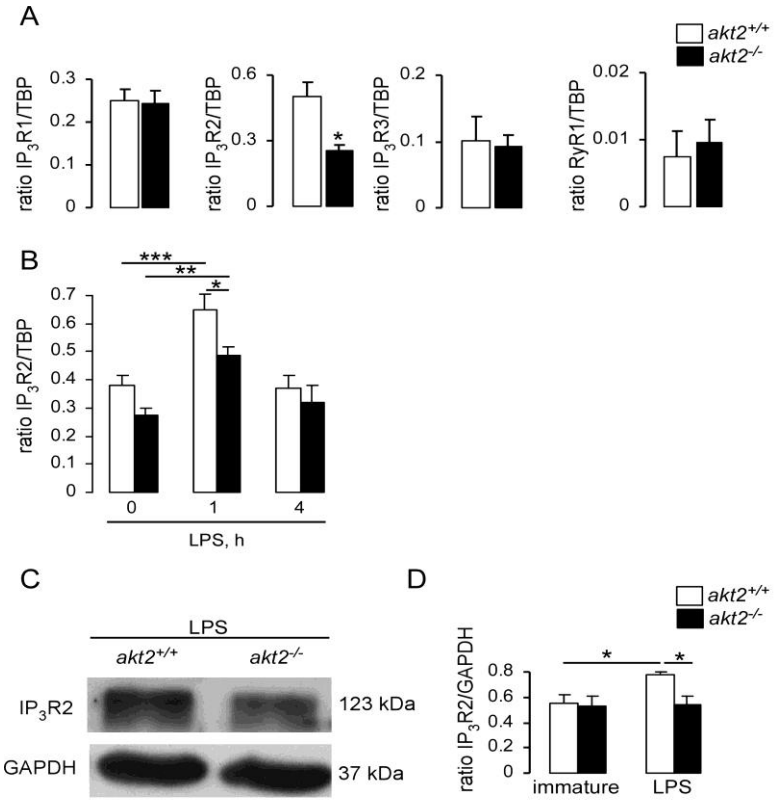

Figure 13. Transcript levels of IP_3R1, IP_3R3 and RyR1 in immature and treated with LPS-1H *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs.

A. Arithmetic means (± SEM, n=3) of the abundance of mRNA encoding the IP_3 receptors: IP_3R1, IP_3R2, and IP_3R3 as well as of the ryanodine receptor RyR1 in immature *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs as assessed by real-time PCR using Tbp mRNA as a reference gene. * (p<0.05), two-tailed unpaired *t*-test.

B. Arithmetic means (± SEM, n=7-13) of the abundance of mRNA encoding IP_3R2 in *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs unstimulated (LPS, 0h) and stimulated with LPS (1 µg/ml, 1h and 4h). * (p<0.05), ** (p<0.01), * (p<0.001), ANOVA.**

C. Original representative Western blot of whole cell lysate protein of IP_3R2 (upper panel) and GAPDH (lower panel) in LPS (1 µg/ml, 24 h)-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs.

D. Arithmetic means ± SEM (n =11-15) of IP_3R2/GAPDH ratio in immature and LPS (1 µg/ml, 24 h)-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs. *(p<0.05), ANOVA.

3.7 Xestospongin C Inhibits the Migration of LPS-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs

To further address the functional significance of Akt2 sensitive IP_3R2 regulation, migratory capacity was tested in LPS-matured DCs from *akt2*$^{+/+}$ and *akt2*$^{-/-}$ mice in the absence and presence of IP_3R inhibitor xestospongin C (5 µM). Xestospongin C significantly decreased the migratory activity of CCL21-treated LPS-matured *akt2*$^{+/+}$ DCs and abrogated the differences in migratory activity between DCs from *akt2*$^{+/+}$ and *akt2*$^{-/-}$ mice. CXCL12-induced migration of immature DCs was also sensitive to xestospongin C, however, no significant difference between *akt2*$^{-/-}$ and *akt2*$^{+/+}$ DCs was observed (**Fig.14**).

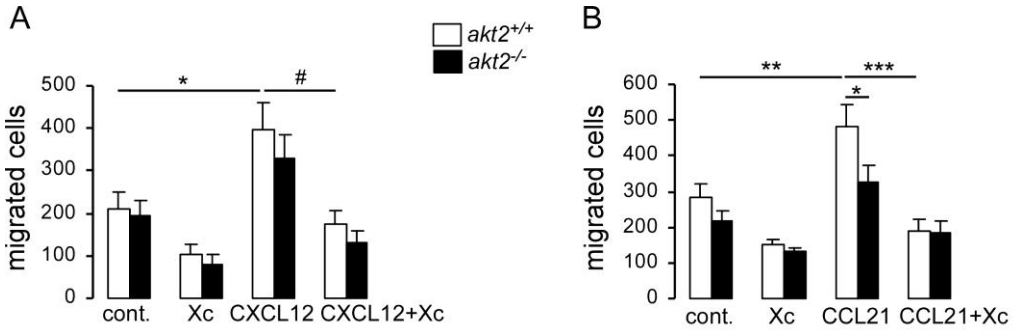

Figure 14. Migration of *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs in the absence and presence of IP_3R iInhibitor Xestospongin C

Arithmetic means ± SEM (n = 5-25) of spontaneous migration and migration in response to either CXCL12 (50 ng/ml, 4h, immature DCs, A) or CCL21 (25 ng/ml, 4h, LPS (1 µg/ml, 24h)-matured DCs, B) of *akt2*$^{+/+}$ (open bars) and *akt2*$^{-/-}$ (closed bars) DCs in the absence or in the presence of the IP_3R inhibitor Xestospongin C (Xc, 5 µM, 3h) *(p<0.05), **(p<0.01), *(p<0.001), ANOVA; #(p<0.05), two-tailed unpaired *t*-test.**

3.8 Impaired Expression of the Transcription Factor ETS1 in *akt2* $^{-/-}$ DCs

PKB/Akt is known to phosphorylate IP_3R, reducing its sensitivity to IP_3 and diminishing Ca^{2+} release (Szado, Vanderheyden et al. 2008). In contrast, reduced IP_3R2 transcript level and protein abundance in *akt2*$^{-/-}$ DCs in the present study suggest that in DCs Akt2 upregulates IP_3R2 on a transcriptional level. However, we examined the phosphorylation status of IP_3R2 by immunoprecipitating the lysates with a phosphospecific antibody that detects the consensus Akt phosphorylation sequence (RXRXX(S/T)) and immunoblotting with IP_3R2-specific antibody (**Fig. 15A**). IP_3R2 was phosphorylated in immature as well as

in LPS-matured DCs, however no significant difference was detected between *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs.

It has been shown that expression of IP_3R3 in CD4 T cells requires the ETS1 transcription factor (Nagaleekar, Diehl et al. 2008). In ETS1-deficient CD4 T cells the level of IP_3R2 protein was also reduced (Nagaleekar, Diehl et al. 2008). Moreover, activated Akt has been shown to lead to increased Ets1 transcription (Lavenburg, Ivey et al. 2003). Therefore we next examined the transcript level of Ets1 in *akt2*$^{-/-}$ and *akt2*$^{+/+}$ DCs. In immature *akt2*$^{-/-}$ DCs the transcript abundance of Ets1 was significantly impaired compared to *akt2*$^{+/+}$ DCs (**Fig. 15B**). Moreover, similar to IP_3R2, LPS (1 µg/ml) induced a transient increase in Ets1 transcript level within 1h in *akt2*$^{+/+}$ DCs, but not in *akt2*$^{-/-}$ DCs. Accordingly, the protein abundance of ETS1 in LPS-matured *akt2*$^{-/-}$ DCs was significantly decreased (**Fig. 15C**).

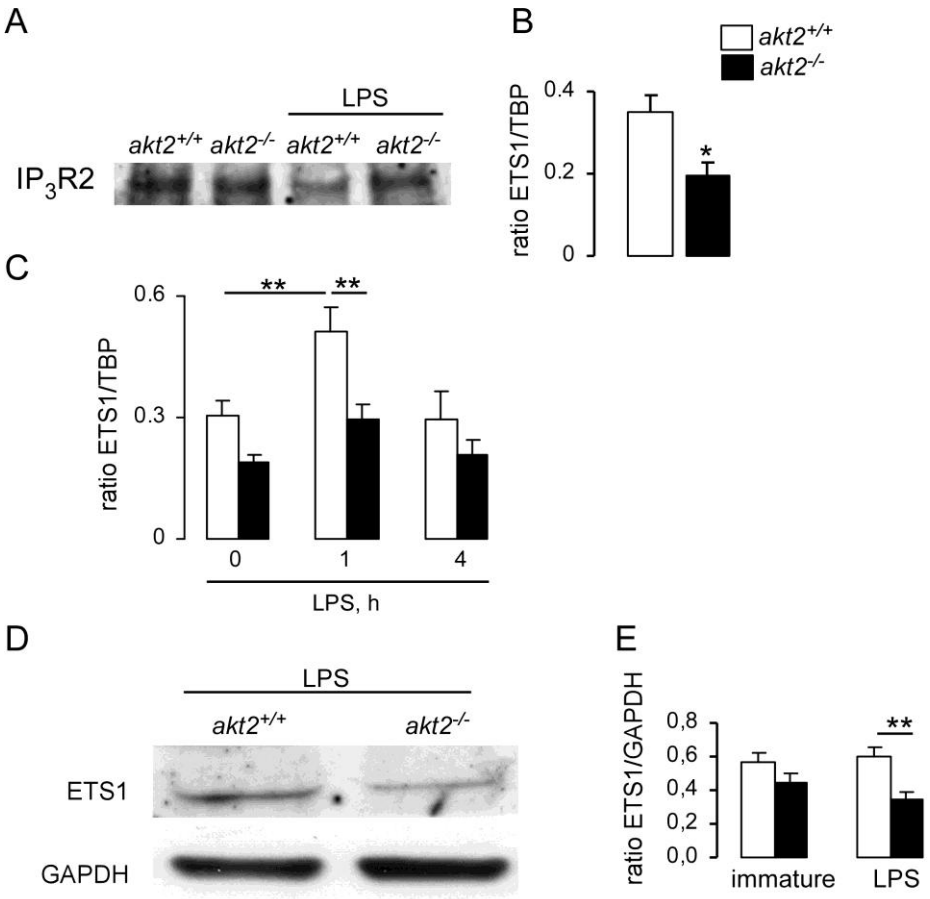

Figure 15. Transcript level and protein abundance of the transcriptional factor Ets1 in *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs.

A. The lysates of immature and LPS (1 μg/ml, 24h)-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs were immunoprecipitated with a phosphospecific antibody that detects the consensus Akt phosphorylation sequence (RXRXX(S/T)) and then immunoblotted with IP_3R2 antibody.

B. Arithmetic means (± SEM, n=6) of the abundance of mRNA encoding Ets1 in immature *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs, as assessed by real-time PCR using Tbp mRNA as a reference gene. *(p<0.05), two-tailed unpaired *t*-test.

C. Arithmetic means (± SEM, n=6-9) of the abundance of mRNA encoding Ets1 in *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs, unstimulated (LPS, 0h) and stimulated with LPS (1 μg/ml, 1h and 4h) as assessed by real-time PCR using TbpP mRNA as a reference gene. **(p<0.01), ANOVA.

D. Original representative Western blot of whole cell lysate protein of ETS1 (upper panel) and GAPDH (lower panel) in LPS (1 μg/ml, 24 h)-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs.

E. Arithmetic means ± SEM (n =6) of ETS1/GAPDH ratio in immature and LPS (1 μg/ml, 24 h)-matured *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs. **(p<0.01), ANOVA.

3.9 Silencing of Ets1 Impaires the IP_3R2 Transcript Abundance upon LPS Stimulation

In order to test whether Akt2-dependent Ets1 expression can underlie decreased IP_3R expression and function in *akt2*$^{-/-}$ DCs, we performed silencing of Ets1 with siRNA (**Fig. 16-19**). The efficiency of silencing assessed with RT-PCR was 47 ± 11% (n=7) and it was confirmed by western blotting (**Fig. 16A**). Upon LPS stimulation (1h), Ets1 and IP_3R2 transcript abundance were reduced in siEts1 DCs (**Fig. 16B**).

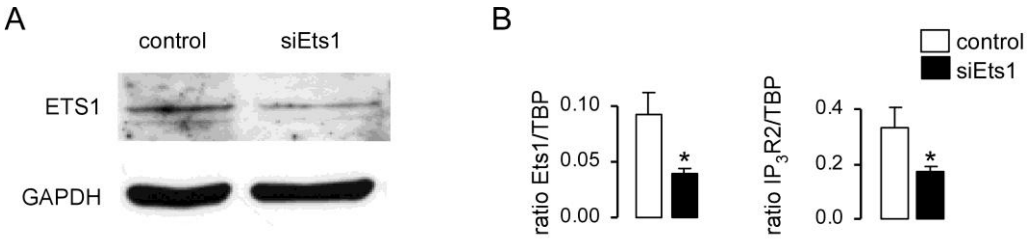

Figure 16. Silencing of the Transcriptional Factor Ets1 Reduces IP3R2 Transcript Abundance upon 1H-LPS Treatment

A. Western blot analysis of whole cell lysate protein of ETS1 in immature control (control siRNA) and siEts1-DCs. Representative experiments showing ETS1 band and GAPDH as loading control (left).

B. Arithmetic means (± SEM) of the abundance of mRNA encoding Ets1 (n=14, left) and IP3R2 (n=7, right) in LPS (1 mg/ml, 24 1 h)-matured control (control siRNA) and siETS1 DCs, as assessed by real-time PCR using Tbp mRNA as a reference gene. * (p<0.05), two-tailed unpaired t-test.

3.10 Silencing of Ets1 in LPS-matured DCs Reduces Ca^{2+} Release and SOCE

Moreover, thapsigargin-induced (**Fig. 17A, B**) and ATP-induced (**Fig. 17C, D**) Ca^{2+} release, thapsigargin-induced SOCE (**Fig. 17A, B**) were significantly impaired in siEts1 LPS (24 h)-matured DC.

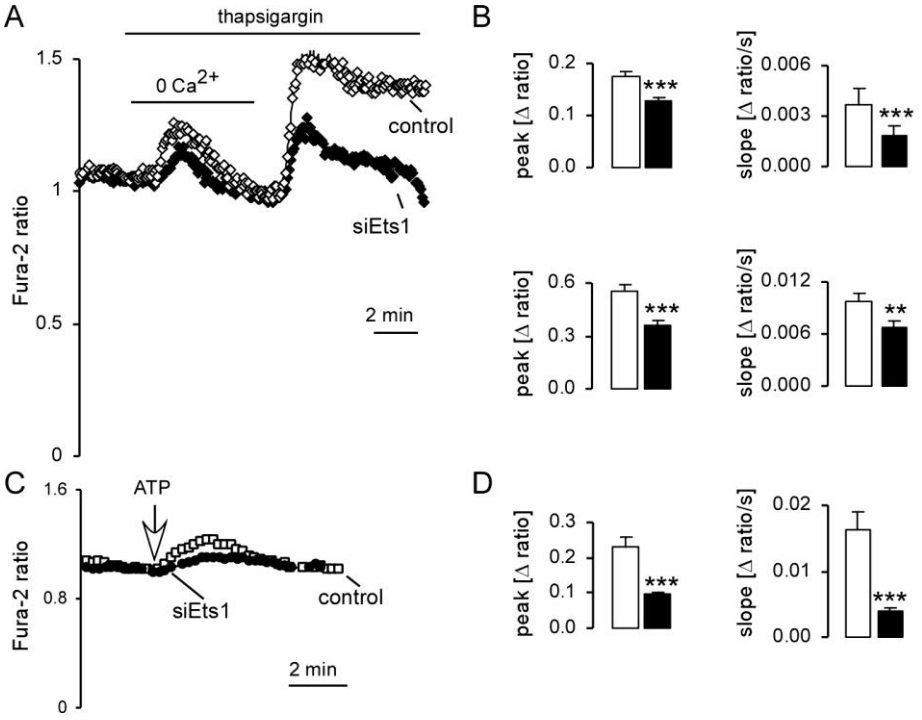

Figure 17. Silencing of the transcriptional factor Ets1 in LPS-matured *akt2*$^{+/+}$ DCs reduces Ca^{2+} release, store-operated Ca^{2+} Entry.

A. Representative tracing showing the Fura-2 fluorescence ratio in LPS (1 μg/ml, 24 h)-matured control (control siRNA) and siEts1-DCs prior to and during exposure to thapsigargin (1 μM) in the Ca^{2+}-free bath solution followed by readdition of extracellular Ca^{2+}.

B. Arithmetic means (± SEM) of the slope (right) and the peak (left) of the change in Fura-2 fluorescence following addition of thapsigargin (1 μM) in the absence (upper bars) and in the presence (lower bars) of extracellular Ca^{2+} to LPS (1 mg/ml, 24 h)-matured control (n=55, open bars) and siEts1 (n=103, closed bars) DCs. **(p<0.01), * (p<0.001), two-tailed unpaired t-test.**

C. Representative tracing showing the Fura-2 fluorescence ratio in LPS (1 μg/ml, 24 h)-matured control (control siRNA) and siEtsTS1-DCs prior to and following addition of ATP (100 μM) to the Ca^{2+}-free bath solution.

D. Arithmetic means (± SEM) of the slope (right) and the peak (left) of the change in Fura-2 fluorescence following addition of ATP (100 μM) in the absence of extracellular Ca^{2+} to LPS (1 μg/ml, 24h)-matured control (n=38, open bars) and siEtsTS1 (n=61, closed bars) DCs. * (p<0.001), two-tailed unpaired t-test.**

3.11 Silencing of Ets1 in LPS-matured DCs Reduces the I_{CRAC} Activity

Similarly, I_{CRAC} (**Fig. 18A, B**) was significantly impaired in siEts1 LPS (24 h)-matured DCs.

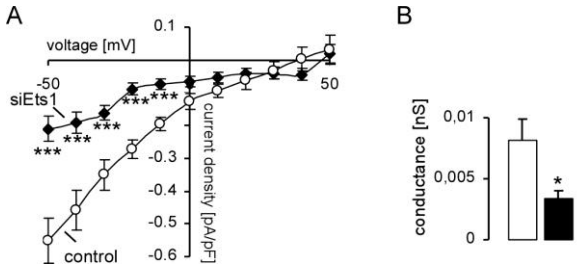

Figure 18. Silencing of the transcriptional factor Ets1 in LPS-matured *akt2*$^{+/+}$ DCs reduces I_{CRAC} activity

A. Mean current-voltage (I/V) relationships of currents activated by 40 µM IP_3 normalized to cell capacitance in LPS (1 µg/ml, 24 h)-matured control (n=20) and siEts1 (n=17) DCs. * (p<0.001), two-tailed unpaired t-test.**

B. Mean whole-cell conductance of inward currents (± SEM) calculated by linear regression of I/V curves shown in (F) between -40 and 0 mV in mature control (n=20) and siEts1 (n=17) DCs. * (p<0.05), two-tailed unpaired t-test.

3.12 Silencing of Ets1 in LPS-matured DCs Impaires Migration

Moreover, CCL21-dependent migration was significantly decreased in siEts1- LPS (24 h)-matured DCs (**Fig. 19**). Those data strongly suggests that Akt2 upregulates Ets1 transcription leading to enhanced expression of IP_3R2. Increased Ca^{2+} release, followed by increased store-operated Ca^{2+} entry underlie, at least partially, stimulating effect of Akt2 on DC migration.

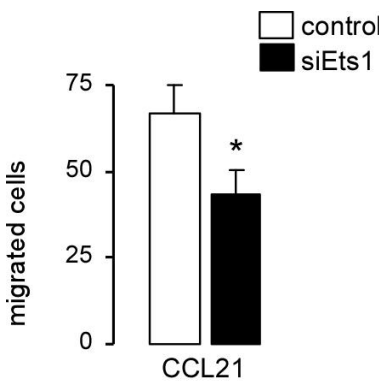

Figure 19. Silencing of the transcriptional factor Ets1 in LPS-matured *akt2*$^{+/+}$ DCs impaires migration

Arithmetic means ± SEM (n =11) of CCL21 (25 ng/ml, 4h)-induced migration in in LPS (1 µg/ml, 24h)-matured control (control siRNA) and siEts1 DCs. * (p<0.05), two-tailed unpaired t-test.

Part2. Serum- and Glucocorticoid-inducible Kinase 1 SGK1 in the Regulation of Dendritic Cell Functions

3.13 SGK1 Deficiency Fosters DC Differentiation and Maturation

For activation and maturation mouse DCs obtained from bone marrow were cultured with or without LPS (100 ng/ml, 48 h). Cells were collected and stained for MHC class II, costimulatory molecule CD86 (**Fig. 20A-D**) and the adhesion molecule CD54 (ICAM-1), which mediates DC-T cell interaction (**Fig. 20E-I**). The basal level of CD86, MHC II and CD54 was higher on $CD11c^+$ gated population obtained from SGK1 knockout mice ($sgk1^{-/-}$), if compared to $CD11c^+$-DCs from the wild-type littermates ($sgk1^{+/+}$) (**Fig. 20C,D,F**). Also stimulation by LPS led to higher receptor abundance in $sgk1^{-/-}$ cells (**Fig. 20C,D,I**), suggesting that SGK1 down-regulates differentiation and maturation of DCs.

Moreover, we isolated DCs from the spleen of $sgk1^{-/-}$ and $sgk1^{+/+}$ mice. Expression of CD86, MHCII and CD54 was higher in splenic $sgk1^{-/-}$ DCs than in splenic $sgk1^{+/+}$ DCs (**Fig. 21**), confirming our observations with bone marrow-derived cells.

3.14 Phagocytic Capacity of $sgk1^{-/-}$ DCs is Impaired

The function of DCs as innate immune effectors involves antigen uptake. PI3K is known to play an important role for phagocytic uptake in DCs (Agrawal, Agrawal et al. 2007). Thus we compared the capacity of $sgk1^{+/+}$ and $sgk1^{-/-}$ DCs to phagocytose antigen by coincubating DCs with FITC-dextran. The phagocytosis of FITC-dextran was found to be significantly reduced in $sgk1^{-/-}$ DCs (**Fig. 22**).

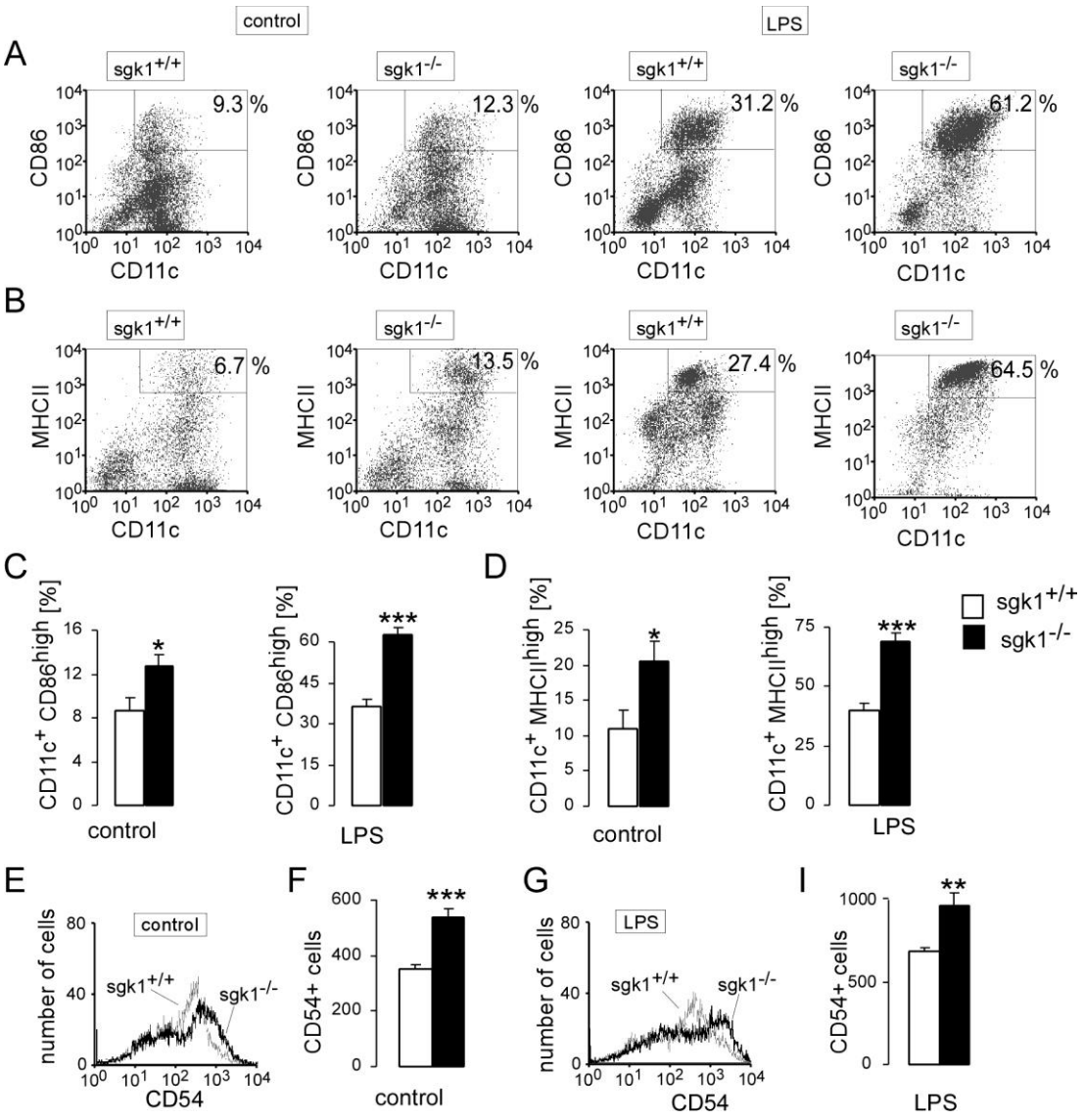

Figure 20. Differentiation and maturation of bone marrow derived DCs from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice

A., B. Original dot plots of CD11c^{+}CD86^{high} (A) and CD11c^{+}MHC II ^{high} (B) DCs at the basal level (control, 1st and 2^{d} panels) and stimulated with LPS (100 ng/ml, 48 h, 3^{d} and 4th panels) from *sgk1*$^{+/+}$ (1st and 3^{d} panels) and *sgk1*$^{-/-}$ (2^{d} and 4th panels) mice. Numbers depict the percent of cells in the respective quadrants.

C., D. Arithmetic means ± SEM (n = 4-5) of the percentage of CD11c^{+}CD86^{+} (C) and CD11c^{+}MHC II^{+} (D) DCs under control and 24 h after LPS stimulation in primary cultures from *sgk1*$^{+/+}$ (closed bars) and *sgk1*$^{-/-}$ (open bars) mice. * (p<0.05) and * (p<0.001) indicate significant difference between *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ cells (two-tailed unpaired *t*-test).**

E., G. Representative FACS histograms depicting the expression of CD54 in control (E) and LPS-stimulated (100 ng/ml, 48 h, G) *sgk1*$^{+/+}$ (grey line) and *sgk1*$^{-/-}$ (black line) cells.

F., I. Arithmetic means ± SEM (n = 9) of fluorescence intensity (MFI) of the CD54 marker in control (F) and LPS-stimulated (100 ng/ml, 48 h, I) *sgk1*$^{+/+}$ (closed bars) and *sgk1*$^{-/-}$ (open bars) cells. ** (p<0.01) and * (p<0.001) indicate significant difference between *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ cells (two-tailed unpaired *t*-test).**

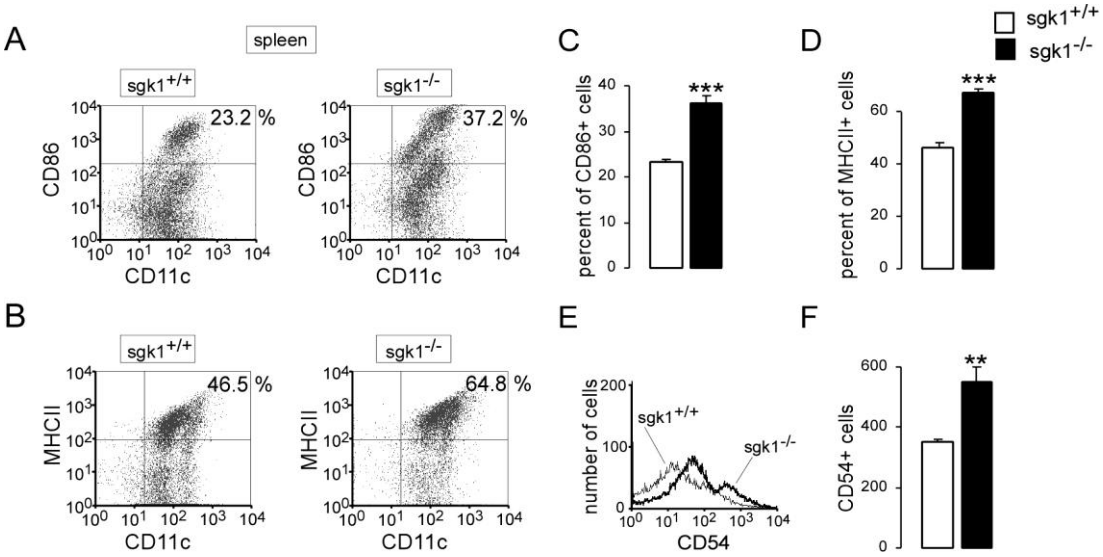

Figure 21. Maturation status of splenic DCs from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice

A., B. Original dot plots of CD11c+CD86+ (A) and CD11c+MHC II+ (B) splenic DCs from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice. Numbers depict the percent of cells in the respective quadrants, acquired within the dead cell gate.

C., D. Arithmetic means ± SEM (n = 3-4) of the percentage of CD11c+CD86+ (C) and CD11c+MHC II+ (D) splenic DCs from *sgk1*$^{+/+}$ (open bars) and *sgk1*$^{-/-}$ (closed bars) mice. * (p<0.001) indicates significant difference between *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ cells (two-tailed unpaired t-test).**

E. Representative FACS histograms depicting the expression of CD54 in *sgk1*$^{+/+}$ (grey line) and *sgk1*$^{-/-}$ (black line) cells.

F. Arithmetic means ± SEM (n = 4) of fluorescence intensity (MFI) of the CD54 marker in *sgk1*$^{+/+}$ (open bars) and *sgk1*$^{-/-}$ (closed bars) cells. ** (p<0.01) indicates significant difference between *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ cells (two-tailed unpaired t-test).

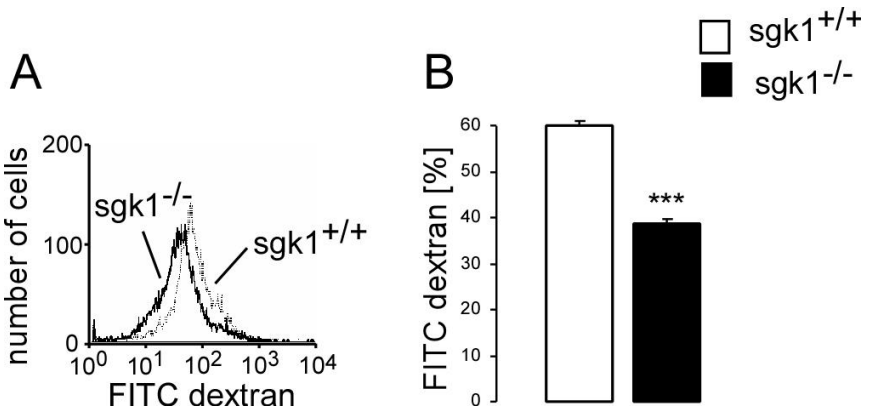

Figure 22. Phagocytic capacity is impaired in *sgk1*$^{-/-}$ DCs

A. Histogram representing the DCs percentage of the uptake of FITC-dextran after 3h by *sgk*$^{+/+}$ and *sgk1*$^{-/-}$ DCs.

B. Arithmetic means ± SEM (n = 6) of FITC-dextran uptake by *sgk*$^{+/+}$ (open bars) and *sgk1*$^{-/-}$ (closed bars) DCs. * (p<0.001) indicates significant difference (two-tailed unpaired t-test).**

3.15 *sgk1*$^{-/-}$ DC Secrete Higher Levels of IL-12 in Response to LPS

We next examined IL-12 production by *sgk1*$^{-/-}$ DCs to determine the involvement of SGK1 signalling in DC cytokine production. DCs were stimulated with LPS (100 ng/ml, 48 h) and IL-12p70 release was analyzed. *sgk1*$^{-/-}$ DCs produced significantly higher amounts of IL-12p70 than *sgk1*$^{+/+}$ DCs (**Fig. 23A**). No significant difference was observed in the production of IL-10, IL-6 and TNF-α (**Fig. 23B-D**).

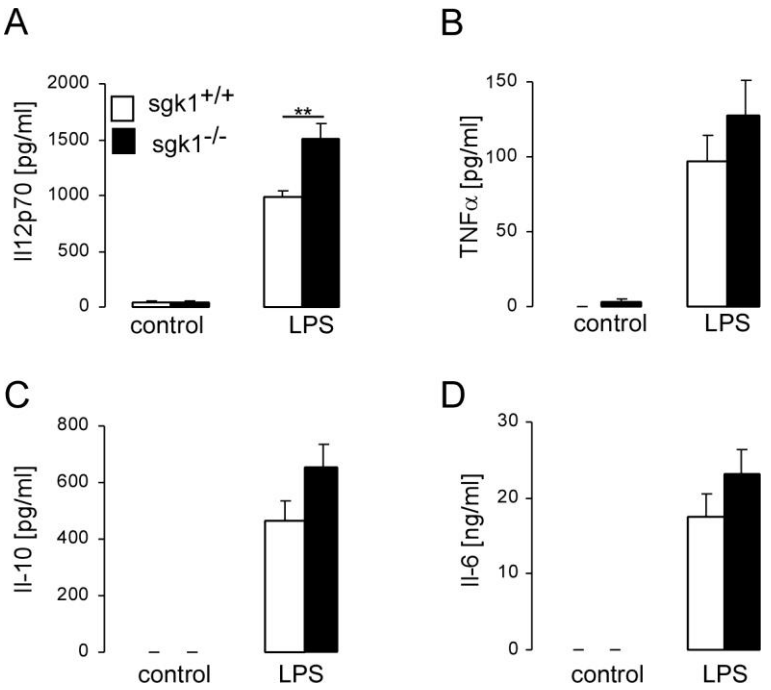

Figure 23. IL-12 secretion is enhanced in *sgk1*$^{-/-}$ DCs

Arithmetic means ± SEM (n = 18) of IL-12, IL-6, TNFα and IL-10 secretion in cultured *sgk1*$^{-/-}$ DCs (closed bars*)* and their wild-type littermates *sgk1*$^{+/+}$ (open bars) under control conditions (control) and after stimulation with LPS (100 ng/ml, 48h). ** indicates significant difference from LPS-stimulated *sgk1*$^{+/+}$ cells (p<0.01; ANOVA).

3.16 Loss of SGK1 Has No Effect on GSK3 Phosphorylation

Glycogen synthase kinase 3 (GSK3) has been demonstrated as a downstream target of PI3K, which mediates its inhibiting action on IL-12 production in DCs (Ohtani, Nagai, Kondo, Mizuno, Nakamura, Tanabe, Takeuchi, Matsuda, and Koyasu, 2008;Martin, Rehani et al., 2005;Zhang, Katz et al., 2009). We, therefore, studied GSK3 expression and LPS-induced phosphorylation in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ DCs. As shown in **Fig. 24**, GSK3 is similarly expressed in DCs of both genotypes and LPS treatment similarly enhanced GSK3 phosphorylation in *sgk1*$^{+/+}$ and in *sgk1*$^{-/-}$ DCs (**Fig. 24**).

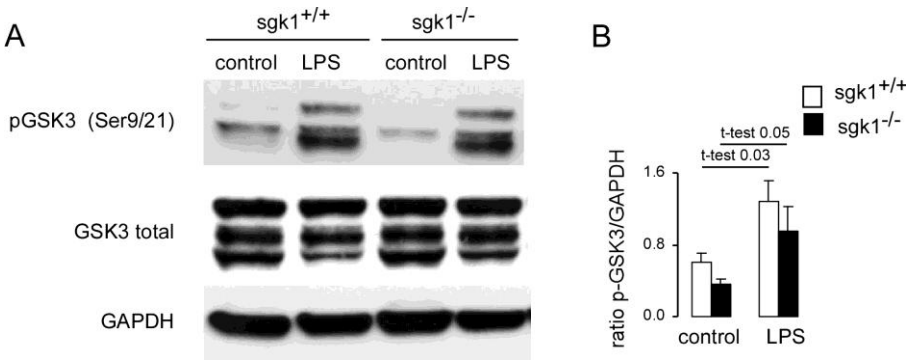

Figure 24. GSK3 phosphorylation is normal in *sgk1*$^{-/-}$ DCs

A. Original western blot of the expression of phosphorylated GSK3 protein (upper panel), of total GSK3 protein (middle panel), and of GAPDH protein (lower panel) in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ DCs prior to (control) and 1h following addition of LPS (1 µg/ml).

B. Arithmetic means ± SEM (n=3-4 independent experiments) of phosphorylated over total GSK3 protein abundance in *sgk1*$^{+/+}$ (open bars) and *sgk1*$^{-/-}$ (closed bars) DCs prior to (control) and 1h following addition of LPS.

3.17 Loss of SGK1 Leads to Upregulation of NF-κB

The NF-κB family of transcription factors plays a major role in regulating DC development, maturation and function (Li and Verma 2002). To gain further insights into the mechanisms responsible for enhanced APC functions of *sgk1*$^{-/-}$ DCs, we analyzed p65 nuclear localization in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ DCs by western blot of nuclear fraction. Already before stimulation, as well as upon stimulation with LPS (1µg/ml, 10 min) the nuclear localization of p65 was strongly enhanced in *sgk1*$^{-/-}$ DCs (**Fig. 25**).

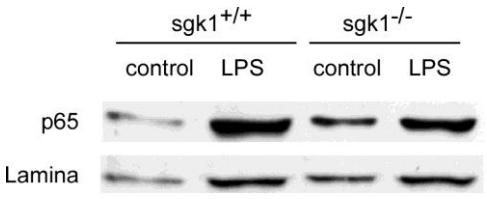

Figure 25. Loss of SGK1 enhances p65 nuclear localization in mouse DCs

Original Western blot of nuclear p65 from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ DCs, which were either stimulated with LPS (1 µg/ml, 10 min) or left untreated (control). Protein loading was controlled by anti-Lamin A antibody.

3.18 Loss of SGK1 Results in Reduced Phosphorylation of NDRG-1

N-myc downstream regulated gene 1 (NDRG1) has been shown to attenuate NF-κB signalling (Hosoi, Izumi et al. 2009). Cells overexpressing NDRG1 demonstrate reduced nuclear translocation of p65 (Hosoi, Izumi et al. 2009). Moreover, NDRG1 is a physiological target of SGK1, phoshorylated by SGK1 at Thr328, Ser330 and Thr346 (Murray, Campbell et al. 2004). On the other hand, phosphorylation of NDRG1 at both Ser330 and Thr346 is required for its suppressive action on the NF-kappaB signaling pathway (Murakami, Hosoi et al. 2010). Accordingly we analyzed NDRG1 expression and phosphorylation in unstimulated and LPS-stimulated *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ DCs. As shown in **Fig.26**, SGK1-dependent phosphorylation of Thr346 and Ser330 of NDRG-1 was decreased in unstimulated as well as in LPS (1h)-stimulated *sgk1*$^{-/-}$ DCs.

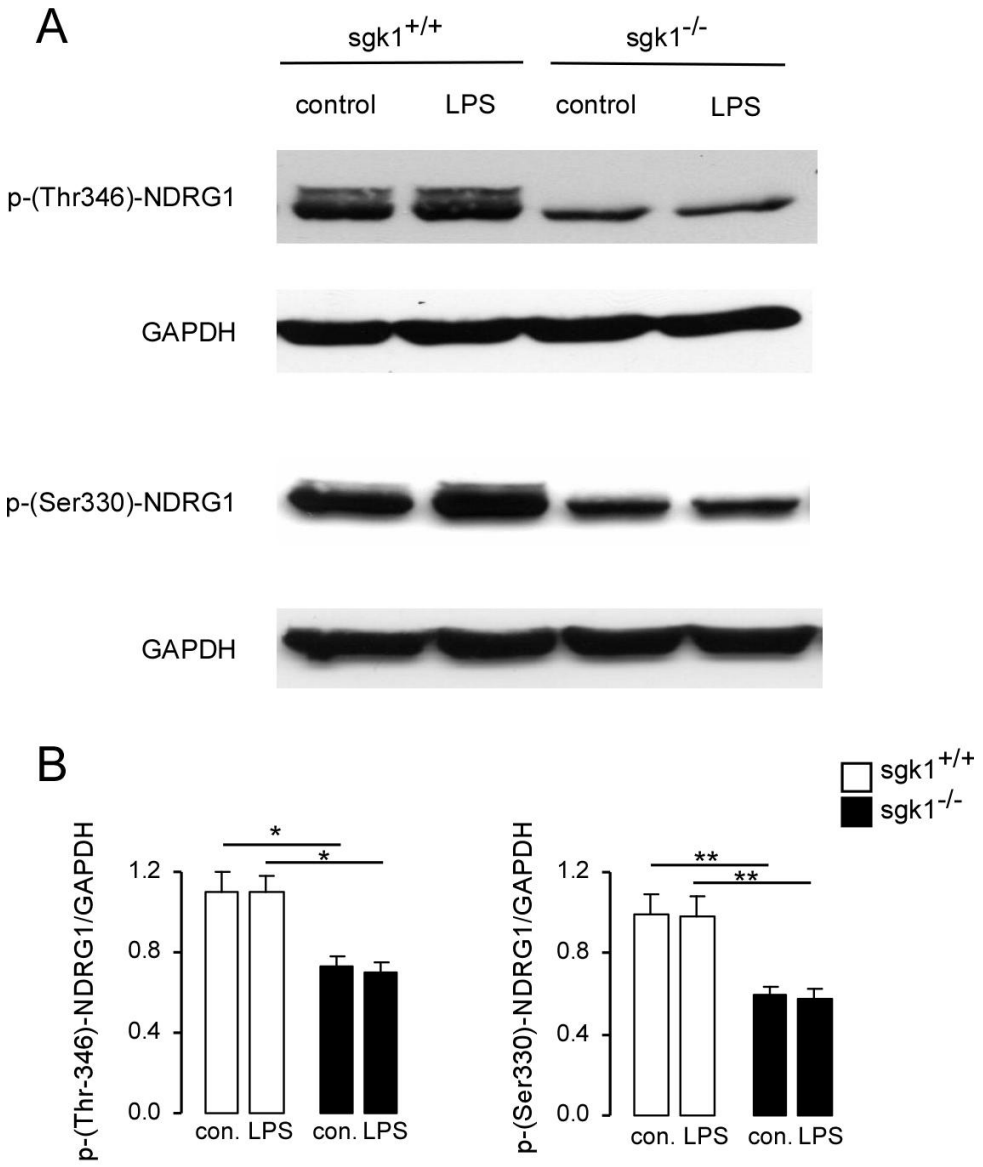

Figure 26. Phosphorylation of NDRG-1 is reduced in *sgk1*$^{-/-}$ DCs

A. Original Western blot of *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ DCs untreated (control) or stimulated with LPS (1 µg/ml, 1h). Protein extracts were analyzed by direct Western blotting using antibodies directed against phospho (p)-NDRG1 at Thr346 (p-(Thr346)-NDRG1) and at Ser330 (p-(Ser330)-NDRG1). Equal protein loading was confirmed with GAPDH antibody.

B. Arithmetic means ± SEM (n =5) of p-(Thr346)-NDRG1/GAPDH and p-(Ser330)-NDRG1/GAPDH ratio in untreated and LPS (1 µg/ml, 1h)-treatedd *sgk1*$^{+/+}$ (open bars) and *sgk1*$^{-/-}$ (closed bars) DCs. * ($p<0.05$), ** ($p<0.01$), ANOVA.

3.19 Knockdown of NDRG1 Results in Enhanced p65 Nuclear Localization

To prove the hypothesis, that diminished phosphorylation of NDRG-1 in *sgk1*$^{-/-}$ DCs may underline enhanced localization of p65 in the nuclei of those cells, we silenced Ndrg1 by siRNA in *sgk1*$^{+/+}$ DCs. This led to an enhanced nuclear abundance of p65 compared to the control empty vector-treated DCs (**Fig. 27**).

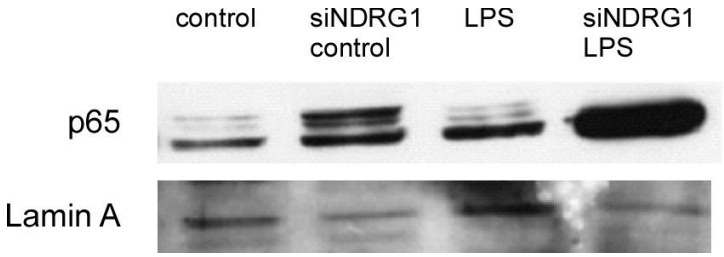

Figure 27. Enhanced nuclear localization of p65 upon NDRG1 knock-down in *sgk1*$^{+/+}$ DCs

Western blot analysis of nuclear p65 in untreated or LPS (1 µg/ml, 1h)-stimulated empty vector-transfected and siNDRG1-DCs. Representative experiments showing p65 band and Lamin-A as loading control.

4. Discussion

In the first part of the present study we demonstrate that Akt2 is required for the expression of IP_3R2 in mouse DCs and accordingly release of Ca^{2+} from intracellular stores and, as a consequence, store-operated Ca^{2+} entry and activation of Ca^{2+} release-activated Ca^{2+} (CRAC) channels are diminished in Akt2 deficient DCs. Moreover, we provide evidence that IP_3R2 expression in DCs is regulated by the Akt-2-dependent transcription factor ETS-1. Finally, we show that reduced IP_3R2 expression in Akt2-deficient DCs may underlie their defective migration.

IP_3R2 protein is similarly expressed in immature nonstimulated *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs. However, upon LPS-induced stimulation, the transcript abundance of IP_3R2 is transiently enhanced in *akt2*$^{+/+}$ but not in *akt2*$^{-/-}$ DCs, and accordingly the expression of IP_3R2 protein is higher in fully mature *akt2*$^{+/+}$ compared to *akt2*$^{-/-}$ DCs. Reduction in IP_3R2 expression is paralleled by impaired function of IP_3Rs in mature *akt2*$^{-/-}$ DCs, as demonstrated by reduced Ca^{2+} release induced by P2Y receptor engagement or by inhibition of sarcoendoplasmatic reticulum Ca^{2+} ATPase (SERCA) with thapsigargin. Consequently, store-operated Ca^{2+} entry as well as IP_3-induced activation of CRAC channels is diminished in Akt2-deficient DCs. Most importantly, increase of cytosolic free Ca^{2+} concentration upon ligation of the CCR7 is strongly reduced in mature *akt2*$^{-/-}$ DCs, which is paralleled by a reduced CCR7-dependent migration of those cells. CCR7-dependent migration was very sensitive to the IP_3R inhibitor Xestospongin C, and no difference in migrating ability could be detected between Xestospongin C-treated *akt2*$^{+/+}$ and *akt2*$^{-/-}$ DCs. This allows us to conclude that reduced expression of IP_3R2 underlies, at least partially, the defective CCR7-dependent migration of Akt2-deficient DCs. Akt2 knockdown has been similarly shown to result in reduced plasmin-triggered migration of human DCs (Li, Syrovets et al. 2010), and CSF-1- and MCP-1-induced chemotaxis of mouse peritoneal macrophages (Zhang, Ma et al. 2009).

Akt2-dependent release of Ca^{2+} through the IP_3R2 could be in line with the well-established PI3K-dependent upregulation of Ca^{2+} mobilization in lymphocytes. Phosporylation of Akt/PKB is compromised and Ca^{2+} flux in response to anti-IgM crosslinking and to anti-CD3 crosslinking was attenuated in p110δ D910A/D910A mice, in accord with an important role for PI3Ks upstream of Tec family kinase–mediated activation of phospholipase Cγ (PLCγ) and Ca^{2+} signalling by antigen receptorsCa^{2+} flux is attenuated in B and T lymphocytes deficient in the catalytic

subunit of PI3K, p110δ, in response to stimulation of B and T cell antigen receptors, respectively .(Okkenhaug, Bilancio et al. 2002). Diminished Ca^{2+} mobilization in B cells with either genetical or pharmacological inhibition of p110δ has been also confirmed in other studies (Inabe and Kurosaki 2002; Jou, Carpino et al. 2002; Bilancio, Okkenhaug et al. 2006). Jou showes in contrast to the absence of detectable Ca^{2+} mobilization in *PLCγ2*$^{-/-}$ B cells, *p110δ*$^{-/-}$ B cells induce an increase in intracellular Ca^{2+} that is approximately 25% of that observed in wild-type cells. In addition, mobilization of intracellular Ca^{2+} upon anti-CD3 stimulation is unimpaired in *p110δ*$^{-/-}$ T cells. The study of Bilancio in primary splenic B cells, demonstrates the PI3K-dependent fraction of early intracellular Ca^{2+} flux is also completely dependent on p110δ catalytic activity. They suggest that most likely, these functions of p110δ derive from the stimulatory effects of its PIP_3 lipid product on the activation of phospholipase C80 (leading to the generation of diacylglycerol and IP_3, which induces the release of intracellular Ca^{2+}) and on PDK1. Again, such a key role for p110 is remarkable given the high level of residual class IA PI3K activity in p110δ D910A/D910A cells. Whether Akt-dependent upregualtion of IP_3R plays a role in those cells needs to be established. However, one study on DT40 B cells and Jurkat cells reports an inhibiting effect of Akt2 on Ca^{2+} mobilization (Martin, Wang et al. 2012). Whereas the initial amplitude of Ca^{2+} mobilization has not been influenced by Akt2, the subsequent clearance of Ca^{2+} is enhanced by Akt2, which results in a reduced duration of Ca^{2+} increase paralleled by a reduced activation of NFAT (Martin, Wang et al. 2012). The mechanism of Akt2-dependent reduction of Ca^{2+} mobilization proposed by Martin et al. involves Akt2 interaction with Bcl-2 proteins, which bind to IP_3R to inhibit Ca^{2+} release (Martin, Wang et al. 2012). Accordingly the cell permeable peptide that blocks the interaction between Bcl-2 and IP_3R abrogates the effect of Akt2 (Martin, Wang et al. 2012). However, enhanced Ca^{2+} clearance rather than modified Ca^{2+} release measured in the presence of Akt2 may also indicate Akt2-dependent upregulation of SERCA or any other extrusion pathways in this model.

Several mechanisms of Akt-dependent regulation of IP_3Rs have been detected so far. A direct interaction and phosphorylation of IP_3R by Akt has been demonstrated (Khan, Wagner et al. 2006; Varga-Szabo, Authi et al. 2008). Szado et al. have shown that Akt-dependent phosphorylation of IP3Rs significantly reduces their Ca^{2+} release activity (Varga-Szabo, Authi et al. 2008), however another study of Khan et al. has not revealed any modification of IP_3R function by Akt phosphorylation (Khan, Wagner Ii et al. 2006). We could detect phosphorylation of IP_3R2 at the Akt specific motif, however no difference in phosphorylated IP_3R2 was observed between *akt2*$^{-/-}$ and *akt2*$^{+/+}$ DCs. Another proposed mechanism involves Akt-dependent regulation of

Bcl-2 anti-apoptotic proteins (Martin, Wang et al. 2012), which are known to interact with IP_3R through their anti-apoptotic BH4 domain and inhibit Ca^{2+} release (Rong, Bultynck et al. 2009). The present study provides another novel mechanism of Akt2- and ETS1-dependent transcription of IP_3R2.

ETS1 is a transcription factor highly conserved throughout evolution, highly expressed in lymphocytes and in tumors (Garrett-Sinha 2013). Ets1 seems to mediate the upregulating effect of Akt2 on IP_3R2 expression in mouse DCs. The kinetics of Ets1 transcription upon LPS stimulation follows the same pattern as IP_3R2 with a rapid and transient upregulation of Ets1 transcript abundance after 1h treatment with LPS, and although the ETS1 protein level is not increased in fully mature compared to immature *akt2*$^{+/+}$ DCs, mature *akt2*$^{-/-}$ DCs expressed significantly less ETS1 protein than mature *akt2*$^{+/+}$ DCs. Moreover, IP_3R2 expression is strongly reduced upon Ets1 knock down. Silencing of Ets1 resulted in a strong impairement of Ca^{2+} release induced either by inhitibition of SERCA with thapsigargin or by ligation of P2Y receptors with ATP. These results are in accordance to the study on ETS1 deficient CD4 T cells in which Ca^{2+} mobilization in response to TCR ligation is impaired (Nagaleekar, Diehl et al. 2008). Moreover, IP_3R3 has clearly been shown to be under transcriptional regulation by Ets1 (Tamura, Hashimoto et al. 2001; Nagaleekar, Diehl et al. 2008).

Ets1 gene expression is known to be stimulated by activated Akt (Lavenburg, Ivey et al. 2003; Ghosh, Basu et al. 2012; Smith, Findlay et al. 2012). In prostate cancer cells elevated Akt (v-akt murine thymoma viral oncogene homolog) activity has been demonstrated to increase ETS1 protein levels and exogenous ETS1 expression is sufficient to rescue invasive potential decreased by inhibition of Akt activity (Smith, Findlay et al. 2012). On the other hand, inhibition of PI3k/Akt pathway blocks transcriptional upregulation of Ets1 by ER stress in human melanoma cells (Dong, Jiang et al. 2011) and by PDGF in human aortic vascular smooth muscle cells (Lang, Artunc et al. 2009). Moreover, knockdown of Akt3 reduced the basal levels of ETS1 in melanoma cells, suggesting that the PI3k/Akt pathway may have a role in regulating constitutive expression of Ets-1 (Dong, Jiang et al. 2011). Expression of ETS1 was reduced in *akt2*$^{-/-}$ DCs only upon stimulation with LPS. LPS has been shown to trigger PI3K stimulation with the consequent Akt phosphorylation in mouse DCs (Fukao, Tanabe et al. 2002; Fukao and Koyasu 2003). Thus, LPS-induced activation of Akt2 is probably required for the continuous expression of ETS1 upon DC maturation.

In conclusion, Akt2 upregulates IP_3R2 transcription in DCs, presumably by enhancing the expression of ETS1 and this effect may underlie the stimulating effect of Akt2 on DC migration.

Similar to PKB/Akt, SGK1 is activated through PI3K and phosphoinositide-dependent kinase PDK1 (Alessi, Andjelkovic et al. 1996; Kobayashi, Deak et al. 1999; Park, Leong et al. 1999). Recent studies indicate that PI3K and PDK1 may function as an endogenous negative feedback that serves to limit excessive innate immune responses (Fukao and Koyasu 2003; Zaru, Mollahan et al. 2008). SGK1 could act downstream of PI3K and PDK1 to mediate this negative feedback in DCs.

The second part of the present study reveals that in contrast to Akt2-deficiency, lack of SGK1 substantially influences maturation and function of DCs. Accordingly, expression of maturation markers and secretion of IL-12 upon LPS stimulation were enhanced and phagocytic capacity was decreased in bone marrow DCs from gene targeted mice lacking functional SGK1 (*sgk1*$^{-/-}$) as compared to DCs from wild type littermates (*sgk1*$^{+/+}$). The differences in genotypes were paralleled by reduced phosphorylation of NDRG-1 and enhanced nuclear translocation of NF-κB in *sgk1*$^{-/-}$ DCs. Silencing of NDRG-1 in *sgk1*$^{+/+}$ DCs increased nuclear NF-κB abundance. The present observations thus disclose SGK1 as a new major player controlling DC maturation and provide the mechanism of SGK1- and NDRG-1-dependent NF-κB regulation.

SGK1 seems to be important for development and maturation of DCs since the expression of CD86, CD54 and MHC class II molecules is higher in *sgk1*$^{-/-}$ cells. Immature DCs are characterized by a high rate of endocytosis, which rapidly decreases during maturation (Banchereau, Briere et al. 2000; Granucci, Zanoni et al. 2003). Thus, in accordance to higher expression of maturation markers, *sgk1*$^{-/-}$ DCs exhibit reduced phagocytic capacity. This is in agreement with a study on human DCs, showing that pharmacological inhibition of PI3K in DCs results in decreased phagocytosis (Agrawal, Agrawal et al. 2007).

In DCs, monocytes and macrophages, PI3K activation suppresses production of inflammatory cytokines, such as IL-12, triggered by TLR signaling (Fukao, Tanabe et al. 2002; Guha and Mackman 2002; Fukao and Koyasu 2003; Goodridge, Harnett et al. 2003; Fang, Pengal et al. 2004; Aksoy, Vanden Berghe et al. 2005; Kuo, Lin et al. 2006; Jackson, Mulcahy et al. 2010). The present study shows that loss of SGK1 results in a higher level of IL-12 secretion by LPS-stimulated DCs, suggesting that SGK1 may be a key player downstream from PI3K and PDK1 preventing excessive IL-12 production.

The nuclear factor-κB (NF-κB)/REL family of transcription factors plays a central role in coordinating the expression of a wide variety of genes that control immune responses (Ardeshna, Pizzey et al. 2000; Li and Verma 2002). PI3K has been shown to negatively

regulate NF-κB in human monocyte-derived DCs (Aksoy, Vanden Berghe et al. 2005), human monocytes (Guha and Mackman 2002; Martin, Schifferle et al. 2003) and mouse macrophages (Fang, Pengal et al. 2004). Thus, PI3K inhibitors increased IκB kinase (IKK)-α/β phosphorylation and IκB-α degradation with a concomitant increase in NF-κB nuclear translocation upon TLR3 or TLR4 engagement in human DCs (Aksoy, Vanden Berghe et al. 2005). Inhibition of the PI3K pathway enhanced LPS-induced TNF-α and TF gene expression via increased activation of transcriptional factors Egr-1, AP-1 and NF-κB in human monocytes (Guha and Mackman 2002). In *Porphyromonas gingivalis* LPS-stimulated human monocytes, inhibition of PI3K resulted in the augmentation of NF-κB p65 activation (Martin, Schifferle et al. 2003). Our data showing enhanced NF-κB nuclear localization in *sgk1*$^{-/-}$ DCs provide evidence that SGK1 is involved in the suppressive effect of PI3K on NF-κB in DCs.

A specific substrate of SGK1, NDRG-1 (N-myc downstream regulated gene 1), which is phosphorylated by SGK1 (and not by Akt) at three different sites (Murray, Campbell et al. 2004), has been shown to attenuate NF-κB signalling (Hosoi, Izumi et al. 2009), when phosphorylated at two SGK1-dependent sites (Ser330 and Thr346) (Murakami, Hosoi et al. 2010). Phopshorylation at Ser330 and Thr346 was strongly reduced in *sgk1*$^{-/-}$ DCs. Moreover, silencing of NDRG-1 in *sgk1*$^{+/+}$ DCs led to enhanced nuclear localization of the NF-κB. Therefore, SGK1 may prevent transport of NF-κB to the nucleus via phosphorylation of NDRG-1.

In contrast to studies showing that the PI3K pathway negatively regulates expression of inflammatory genes in macrophages and DCs, the PI3K pathway positively regulates NF-κB activity and NF-κB-dependent gene expression in other cell types. Thus, in airway epithelial cells the PI3K-Akt positively regulate NF-κB transcriptional activity (Thomas, Monick et al. 2002). In 3T3 fibroblasts, overexpression of a constitutively active form of Akt results in NF-κB-dependent gene expression via the activation of IKK and the p38 MAPK (Madrid, Mayo et al. 2001). SGK1 has been shown to phosphorylate IKKβ (Zhang, Cui et al. 2005) or IKKα (Tai, Su et al. 2009) and thus to promote the degradation of IκB. Moreover, SGK1 increases the acetylation of NF-κB through phosphorylation of p300, which also leads to NF-κB activation(Tai, Su et al. 2009). SGK1 is a positive regulator of NF-κB in mast cells (Eylenstein, Gehring et al. 2012) and megakaryocytes (Borst, Schmidt et al. 2012). Moreover, SGK1 is required for the stimulation and nuclear translocation of NF-κB following mineralocorticoid excess (Vallon, Wyatt et al. 2006; Terada, Ueda et al. 2012) and thrombin (BelAiba, Djordjevic et al. 2006). Thus, it is possible that the effect of PI3K pathway in general and SGK1 in particular on NF-κB is dependent on the presence of one or more cell type-specific co-factors.

Interestingly, DCs lacking SGK1, as well as DCs treated with PI3K inhibitors carry the same functional defects as DCs obtained from elderly humans (Agrawal, Agrawal et al. 2007). DCs from aging individuals are similarly less efficient in phagocytic uptake and have increased secretion of cytokines than the younger counterparts (Agrawal,

Agrawal et al. 2007). In elderly subjects decreased phosphorylation of Akt was observed in LPS-stimulated DCs. Possibly, SGK1 participates in the modification of DCs during ageing.

SGK1 is under strong genomic stimulation by glucocorticoids (Firestone, Giampaolo et al. 2003) and 1,25-dyhydroxyvitamin D_3 (Akutsu, Lin et al. 2001). Given that both hormones have strong inhibitory effect on DC functions (Yamaguchi, Tsumura et al. 1997; Etten and Mathieu 2005; Lyakh, Sanford et al. 2005; Griffin, Dong et al. 2007; Penna, Amuchastegui et al. 2007; Shumilina, Xuan et al. 2010; Heise, Shumilina et al. 2011), it is intriguing to speculate that their mechanism of action in DCs might include SGK1 activation.

In conclusion, genetic knockout of SGK1 leads to profound changes in DC functions, including changes in maturation, phagocytic capacity and cytokine production. Moreover, at least some of these effects seem to be mediated through the reduced phosphorylation of NDRG-1 and enhanced nuclear localization of the transcription factor NF-κB.

References

Agrawal, A., S. Agrawal, et al. (2007). "Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinositide 3-kinase-signaling pathway." The Journal of Immunology **178**(11): 6912-6922.

Aksoy, E., W. Vanden Berghe, et al. (2005). "Inhibition of phosphoinositide 3-kinase enhances TRIF-dependent NF-κB activation and IFN-β synthesis downstream of Toll-like receptor 3 and 4." European journal of immunology **35**(7): 2200-2209.

Akutsu, N., R. Lin, et al. (2001). "Regulation of Gene Expression by 1α,25-Dihydroxyvitamin D3 and Its Analog EB1089 under Growth-Inhibitory Conditions in Squamous Carcinoma Cells " Molecular Endocrinology **15**(7): 1127-1139.

Alessi, D. R., M. Andjelkovic, et al. (1996). "Mechanism of activation of protein kinase B by insulin and IGF-1." The EMBO journal **15**(23): 6541.

Alessi, D. R., S. R. James, et al. (1997). "Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha." Curr Biol **7**(4): 261-9.

Alvarez, D., E. H. Vollmann, et al. (2008). "Mechanisms and consequences of dendritic cell migration." Immunity **29**(3): 325-42.

Ardeshna, K. M., A. R. Pizzey, et al. (2000). "The PI3 kinase, p38 SAP kinase, and NF-κB signal transduction pathways are involved in the survival and maturation of lipopolysaccharide-stimulated human monocyte–derived dendritic cells " Blood **96**(3): 1039-1046.

Arteaga, M. F., D. Alvarez de la Rosa, et al. (2007). "Multiple translational isoforms give functional specificity to serum- and glucocorticoid-induced kinase 1." Mol Biol Cell **18**(6): 2072-80.

Ashby, M. C., M. Craske, et al. (2002). "Localized Ca2+ uncaging reveals polarized distribution of Ca2+-sensitive Ca2+ release sites mechanism of unidirectional Ca2+ waves." The Journal of cell biology **158**(2): 283-292.

Baba, Y., K. Nishida, et al. (2007). "Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses." Nature immunology **9**(1): 81-88.

Bader, A. G., S. Kang, et al. (2005). "Oncogenic PI3K deregulates transcription and translation." Nat Rev Cancer **5**(12): 921-9.

Banchereau, J., F. Briere, et al. (2000). "Immunobiology of dendritic cells." Annu Rev Immunol **18**: 767-811.

Banchereau, J., F. Briere, et al. (2000). "Immunobiology of dendritic cells." Annual review of immunology **18**(1): 767-811.

Barac, Y. D., N. Zeevi-Levin, et al. (2005). "The 1, 4, 5-inositol trisphosphate pathway is a key component in Fas-mediated hypertrophy in neonatal rat ventricular myocytes." Cardiovascular research **68**(1): 75-86.

Barbet, G., M. Demion, et al. (2008). "The calcium-activated nonselective cation channel TRPM4 is essential for the migration but not the maturation of dendritic cells." Nature immunology **9**(10): 1148-1156.

Barchet, W., A. Blasius, et al. (2005). "Plasmacytoid dendritic cells." Immunologic research **32**(1): 75-83.

Barry, P. H. and J. W. Lynch (1991). "Liquid junction potentials and small cell effects in patch-clamp analysis." Journal of Membrane Biology **121**(2): 101-117.

Barton, K., N. Muthusamy, et al. (1998). "The Ets-1 transcription factor is required for the development of natural killer cells in mice." Immunity **9**(4): 555-63.

Bayascas, J. R., S. Wullschleger, et al. (2008). "Mutation of the PDK1 PH domain inhibits protein kinase B/Akt, leading to small size and insulin resistance." Mol Cell Biol **28**(10): 3258-72.

Beaty, S. R., C. E. Rose Jr, et al. (2007). "Diverse and potent chemokine production by lung CD11bhigh dendritic cells in homeostasis and in allergic lung inflammation." The Journal of Immunology **178**(3): 1882-1895.

Beer-Hammer, S., E. Zebedin, et al. "The catalytic PI3K isoforms p110gamma and p110delta contribute to B cell development and maintenance, transformation, and proliferation." J Leukoc Biol **87**(6): 1083-95.

BelAiba, R. S., T. Djordjevic, et al. (2006). "The Serum-and Glucocorticoid-Inducible Kinase Sgk-1 Is Involved in Pulmonary Vascular Remodeling Role in Redox-Sensitive Regulation of Tissue Factor by Thrombin." Circulation research **98**(6): 828-836.

Bilancio, A., K. Okkenhaug, et al. (2006). "Key role of the p110δ isoform of PI3K in B-cell antigen and IL-4 receptor signaling: comparative analysis of genetic and pharmacologic interference with p110δ function in B cells." Blood **107**(2): 642-650.

Blair, D. G. and M. Athanasiou (2000). "Ets and retroviruses - transduction and activation of members of the Ets oncogene family in viral oncogenesis." Oncogene **19**(55): 6472-81.

Blaustein, M. P., R. Greger, et al. (2001). Reviews of Physiology, Biochemistry, and Pharmacology, Springer Verlag.

Boehmer, C., J. Laufer, et al. (2008). "Modulation of the voltage-gated potassium channel Kv1. 5 by the SGK1 protein kinase involves inhibition of channel ubiquitination." Cellular Physiology and Biochemistry **22**(5-6): 591-600.

Boehmer, C., M. Palmada, et al. (2008). "The peptide transporter PEPT2 is targeted by the protein kinase SGK1 and the scaffold protein NHERF2." Cellular Physiology and Biochemistry **22**(5-6): 705-714.

Bononi, A., C. Agnoletto, et al. (2011). "Protein kinases and phosphatases in the control of cell fate." Enzyme Res **2011**: 329098.

Bootman, M. (2006). Calcium Signalling and Regulation of Cell Function.

Bootman, M. D., T. J. Collins, et al. (2001). "Calcium signalling." Semin Cell Dev Biol **12**(1): 3-10.

Borst, O., E. M. Schmidt, et al. (2012). "The serum-and glucocorticoid-inducible kinase 1 (SGK1) influences platelet calcium signaling and function by regulation of Orai1 expression in megakaryocytes." Blood **119**(1): 251-261.

Bracci, L., M. Vukcevic, et al. (2007). "Ca2+ signaling through ryanodine receptor 1 enhances maturation and activation of human dendritic cells." Journal of cell science **120**(13): 2232-2240.

Brunet, A., J. Park, et al. (2001). "Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a)." Molecular and cellular biology **21**(3): 952-965.

Brunet, A., J. Park, et al. (2001). "Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a)." Mol Cell Biol **21**(3): 952-65.

Calleja, V., M. Laguerre, et al. (2009). "3-D structure and dynamics of protein kinase B-new mechanism for the allosteric regulation of an AGC kinase." J Chem Biol **2**(1): 11-25.

Calleja, V., M. Laguerre, et al. (2009). "Role of a novel PH-kinase domain interface in PKB/Akt regulation: structural mechanism for allosteric inhibition." PLoS Biol **7**(1): e17.

Carrasco, S. and T. Meyer (2011). "STIM proteins and the endoplasmic reticulum-plasma membrane junctions." Annual review of biochemistry **80**: 973-1000.

Cheng, K. T., X. Liu, et al. (2011). "Local Ca2+ entry via Orai1 regulates plasma membrane recruitment of TRPC1 and controls cytosolic Ca2+ signals required for specific cell functions." PLoS biology **9**(3): e1001025.

Cho, H., J. Mu, et al. (2001). "Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBbeta)." Science Signaling **292**(5522): 1728.

Choi, H. J., Y. Geng, et al. (2011). "Differential requirements for the Ets transcription factor Elf-1 in the development of NKT cells and NK cells." Blood **117**(6): 1880-7.

Clapham, D. E. (2007). "Calcium signaling." Cell **131**(6): 1047-58.

Colonna, M., G. Trinchieri, et al. (2004). "Plasmacytoid dendritic cells in immunity." Nature immunology **5**(12): 1219-1226.

Colucci, F., M. A. Caligiuri, et al. (2003). "What does it take to make a natural killer?" Nat Rev Immunol **3**(5): 413-25.

Crellin, N. K., R. V. Garcia, et al. (2007). "Altered activation of AKT is required for the suppressive function of human CD4+CD25+ T regulatory cells." Blood **109**(5): 2014-22.

de Heer, H. J., H. Hammad, et al. (2005). Dendritic cell subsets and immune regulation in the lung. Seminars in immunology, Elsevier.

de Noronha, S., S. Hardy, et al. (2005). "Impaired dendritic-cell homing in vivo in the absence of Wiskott-Aldrich syndrome protein." Blood **105**(4): 1590-7.

Dehner, M., M. Hadjihannas, et al. (2008). "Wnt signaling inhibits Forkhead box O3a-induced transcription and apoptosis through up-regulation of serum-and glucocorticoid-inducible kinase 1." Journal of Biological Chemistry **283**(28): 19201-19210.

Di, R. M., Q. T. Feng, et al. (2010). "PDK1 plays a critical role in regulating cardiac function in mice and human." Chin Med J (Engl) **123**(17): 2358-63.

Dittmer, J. (2003). "The biology of the Ets1 proto-oncogene." Mol Cancer **2**: 29.

Dixon, M. J., A. Gray, et al. (2011). "A screen for novel phosphoinositide 3-kinase effector proteins." Mol Cell Proteomics **10**(4): M110 003178.

Dong, L., C. C. Jiang, et al. (2011). "Ets-1 mediates upregulation of Mcl-1 downstream of XBP-1 in human melanoma cells upon ER stress." Oncogene **30**(34): 3716-3726.

Dwyer, J., H. Li, et al. (2007). "Transcriptional regulation of telomerase activity: roles of the the Ets transcription factor family." Ann N Y Acad Sci **1114**: 36-47.

Etten, E. v. and C. Mathieu (2005). "Immunoregulation by 1,25-dihydroxyvitamin D3: Basic concepts." The Journal of steroid biochemistry and molecular biology **97**(1): 93-101.

Evans, J. H. and J. J. Falke (2007). "Ca2+ influx is an essential component of the positive-feedback loop that maintains leading-edge structure and activity in macrophages." Proceedings of the National Academy of Sciences **104**(41): 16176-16181.

Eylenstein, A., E.-M. Gehring, et al. (2012). "Stimulation of Ca2+-channel Orai1/STIM1 by serum-and glucocorticoid-inducible kinase 1 (SGK1)." The FASEB Journal **25**(6): 2012-2021.

Eyquem, S., K. Chemin, et al. (2004). "The Ets-1 transcription factor is required for complete pre-T cell receptor function and allelic exclusion at the T cell receptor beta locus." Proc Natl Acad Sci U S A **101**(44): 15712-7.

Fang, H., R. A. Pengal, et al. (2004). "Lipopolysaccharide-induced macrophage inflammatory response is regulated by SHIP." The Journal of Immunology **173**(1): 360-366.

Feske, S. (2007). "Calcium signalling in lymphocyte activation and disease." Nature Reviews Immunology **7**(9): 690-702.

Fill, M. and J. A. Copello (2002). "Ryanodine receptor calcium release channels." Physiological reviews **82**(4): 893-922.

Firestone, G. L., J. R. Giampaolo, et al. (2003). "Stimulus-dependent regulation of serum and glucocorticoid inducible protein kinase (SGK) transcription, subcellular localization and enzymatic activity." Cellular Physiology and Biochemistry **13**(1): 1-12.

Foskett, J. K., C. White, et al. (2007). "Inositol trisphosphate receptor Ca2+ release channels." Physiological reviews **87**(2): 593-658.

Fruman, D. A. and L. C. Cantley (2002). "Phosphoinositide 3-kinase in immunological systems." Semin Immunol **14**(1): 7-18.

Fukao, T. and S. Koyasu (2003). "PI3K and negative regulation of TLR signaling." Trends Immunol **24**(7): 358-63.

Fukao, T., M. Tanabe, et al. (2002). "PI3K-mediated negative feedback regulation of IL-12 production in DCs." Nat Immunol **3**(9): 875-81.

Gallant, S. and G. Gilkeson (2006). "ETS transcription factors and regulation of immunity." Arch Immunol Ther Exp (Warsz) **54**(3): 149-63.

Garrett-Sinha, L. A. (2013). "Review of Ets1 structure, function, and roles in immunity." Cellular and Molecular Life Sciences: 1-16.

Gehring, E. M., A. Zürn, et al. (2009). "Regulation of the glutamate transporter EAAT2 by PIKfyve." Cellular Physiology and Biochemistry **24**(5-6): 361-368.

Geisinger, M. T., R. Astaiza, et al. (2012). "Ets-1 is essential for connective tissue growth factor (CTGF/CCN2) induction by TGF-beta1 in osteoblasts." PLoS One **7**(4): e35258.

Ghosh, S., M. Basu, et al. (2012). "ETS-1 protein regulates vascular endothelial growth factor-induced matrix metalloproteinase-9 and matrix metalloproteinase-13 expression in human ovarian carcinoma cell line SKOV-3." Journal of Biological Chemistry **287**(18): 15001-15015.

Goodridge, H. S., W. Harnett, et al. (2003). "Differential regulation of interleukin-12 p40 and p35 induction via Erk mitogen-activated protein kinase-dependent and -independent mechanisms and the implications for bioactive IL-12 and IL-23 responses." Immunology **109**(3): 415-425.

Granucci, F., I. Zanoni, et al. (2003). "Dendritic cell regulation of immune responses: a new role for interleukin 2 at the intersection of innate and adaptive immunity." The EMBO journal **22**(11): 2546-2551.

Grenningloh, R., B. Y. Kang, et al. (2005). "Ets-1, a functional cofactor of T-bet, is essential for Th1 inflammatory responses." J Exp Med **201**(4): 615-26.

Grenningloh, R., T. S. Tai, et al. (2011). "Ets-1 maintains IL-7 receptor expression in peripheral T cells." J Immunol **186**(2): 969-76.

Griffin, M. D., X. Dong, et al. (2007). "Vitamin D receptor-mediated suppression of RelB in antigen presenting cells: a paradigm for ligand-augmented negative transcriptional regulation." Archives of biochemistry and biophysics **460**(2): 218-226.

Guha, M. and N. Mackman (2002). "The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory

mediators in human monocytic cells." Journal of Biological Chemistry **277**(35): 32124-32132.

Gurevich, E. V., J. J. Tesmer, et al. (2011). "G protein-coupled receptor kinases: more than just kinases and not only for GPCRs." Pharmacol Ther **133**(1): 40-69.

Hamill, O. P., A. Marty, et al. (1981). "Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches." Pflügers Archiv **391**(2): 85-100.

He, P., S. J. Lee, et al. (2011). "Serum- and glucocorticoid-induced kinase 3 in recycling endosomes mediates acute activation of Na+/H+ exchanger NHE3 by glucocorticoids." Mol Biol Cell **22**(20): 3812-25.

Heise, N., E. Shumilina, et al. (2011). "Effect of dexamethasone on Na+/Ca2+ exchanger in dendritic cells." American Journal of Physiology-Cell Physiology **300**(6): C1306-C1313.

Hewavitharana, T., X. Deng, et al. (2007). "Role of STIM and Orai proteins in the store-operated calcium signaling pathway." Cell calcium **42**(2): 173-182.

Higuchi, T., F. O. Bartel, et al. (2007). "Thymomegaly, microsplenia, and defective homeostatic proliferation of peripheral lymphocytes in p51-Ets1 isoform-specific null mice." Mol Cell Biol **27**(9): 3353-66.

Hinton, H. J., D. R. Alessi, et al. (2004). "The serine kinase phosphoinositide-dependent kinase 1 (PDK1) regulates T cell development." Nat Immunol **5**(5): 539-45.

Hirsch, E., C. Costa, et al. (2007). "Phosphoinositide 3-kinases as a common platform for multi-hormone signaling." J Endocrinol **194**(2): 243-56.

Hogan, P. G., R. S. Lewis, et al. (2010). "Molecular basis of calcium signaling in lymphocytes: STIM and ORAI." Annual review of immunology **28**: 491.

Hollenhorst, P. C., L. P. McIntosh, et al. (2011). "Genomic and biochemical insights into the specificity of ETS transcription factors." Annu Rev Biochem **80**: 437-71.

Hosoi, F., H. Izumi, et al. (2009). "N-myc downstream regulated gene 1/Cap43 suppresses tumor growth and angiogenesis of pancreatic cancer through attenuation of inhibitor of κB Kinase β expression." Cancer research **69**(12): 4983-4991.

Hsu, S., P. J. O' Connell, et al. (2001). "Fundamental Ca2+ signaling mechanisms in mouse dendritic cells: CRAC is the major Ca2+ entry pathway." The Journal of Immunology **166**(10): 6126-6133.

Huang, Y. H. and K. Sauer (2010). "Lipid signaling in T-cell development and function." Cold Spring Harb Perspect Biol **2**(11): a002428.

Hwang, J., R. S. Duncan, et al. (2009). "Progesterone potentiates calcium release through IP3 receptors by an Akt-mediated mechanism in hippocampal neurons." Cell calcium **45**(3): 233-242.

Inabe, K. and T. Kurosaki (2002). "Tyrosine phosphorylation of B-cell adaptor for phosphoinositide 3-kinase is required for Akt activation in response to CD19 engagement." Blood **99**(2): 584-589.

Jackson, A. M., L. A. Mulcahy, et al. (2010). "Role of mitogen-activated protein kinase and PI3K pathways in the regulation of IL-12-family cytokines in dendritic cells and the generation of T H-responses." Eur Cytokine Netw **21**(4): 319-28.

Janeway, C. A., Jr. and R. Medzhitov (2002). "Innate immune recognition." Annu Rev Immunol **20**: 197-216.

Jou, S.-T., N. Carpino, et al. (2002). "Essential, nonredundant role for the phosphoinositide 3-kinase p110δ in signaling by the B-cell receptor complex." Molecular and cellular biology **22**(24): 8580-8591.

Kaufmann, S. H. (2008). "Immunology's foundation: the 100-year anniversary of the Nobel Prize to Paul Ehrlich and Elie Metchnikoff." Nat Immunol **9**(7): 705-12.

Khan, M. T., L. Wagner Ii, et al. (2006). "Akt kinase phosphorylation of inositol 1, 4, 5-trisphosphate receptors." Journal of Biological Chemistry **281**(6): 3731-3737.

Khan, M. T., L. Wagner, et al. (2006). "Akt kinase phosphorylation of inositol 1, 4, 5-trisphosphate receptors." Journal of Biological Chemistry **281**(6): 3731-3737.

Kobayashi, T., M. Deak, et al. (1999). "Characterization of the structure and regulation of two novel isoforms of serum-and glucocorticoid-induced protein kinase." Biochemical Journal **344**(Pt 1): 189.

Komander, D., A. Fairservice, et al. (2004). "Structural insights into the regulation of PDK1 by phosphoinositides and inositol phosphates." EMBO J **23**(20): 3918-28.

Komander, D., G. Kular, et al. (2005). "Role of T-loop phosphorylation in PDK1 activation, stability, and substrate binding." J Biol Chem **280**(19): 18797-802.

Koskela, K. and O. Lassila (2003). "Single-cell analysis of Ets-1 transcription factor expression during lymphocyte activation and apoptosis." Scand J Immunol **57**(1): 56-61.

Koyasu, S. (2003). "The role of PI3K in immune cells." Nat Immunol **4**(4): 313-9.

Koyasu, S. (2004). "Role of class IA phosphoinositide 3-kinase in B lymphocyte development and functions." Biochem Soc Trans **32**(Pt 2): 320-5.

Krawczyk, C. M., T. Holowka, et al. (2010). "Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation." Blood **115**(23): 4742-9.

Krebs, J. and M. Michalak (2007). Calcium: a matter of life or death, Elsevier Science Limited.

Krueger, B., S. Haerteis, et al. (2009). "Cholesterol depletion of the plasma membrane prevents activation of the epithelial sodium channel (ENaC) by SGK1." Cellular Physiology and Biochemistry **24**(5-6): 605-618.

Kuo, C.-C., W.-T. Lin, et al. (2006). "Class I and III phosphatidylinositol 3'-kinase play distinct roles in TLR signaling pathway." The Journal of Immunology **176**(10): 5943-5949.

Lang, F., F. Artunc, et al. (2009). "The Physiological Impact of the Serum-and Glucocorticoid-inducible Kinase SGK1." Current opinion in nephrology and hypertension **18**(5): 439.

Lang, F., C. Bohmer, et al. (2006). "(Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms." Physiol Rev **86**(4): 1151-78.

Lang, F. and E. Shumilina (2012). "Regulation of ion channels by the serum-and glucocorticoid-inducible kinase SGK1." The FASEB Journal.

Lang, F., N. Strutz-Seebohm, et al. (2010). "Significance of SGK1 in the regulation of neuronal function." The Journal of Physiology **588**(18): 3349-3354.

Lang, F., N. Strutz-Seebohm, et al. (2010). "Significance of SGK1 in the regulation of neuronal function." J Physiol **588**(Pt 18): 3349-54.

Laufer, J., C. Boehmer, et al. (2009). "The C-terminal PDZ-binding motif in the Kv1.5 potassium channel governs its modulation by the Na+/H+ exchanger regulatory factor 2." Cellular Physiology and Biochemistry **23**(1-3): 025-036.

Lavenburg, K. R., J. Ivey, et al. (2003). "Coordinated functions of Akt/PKB and ETS1 in tubule formation." FASEB J **17**(15): 2278-80.

Lavenburg, K. R., J. Ivey, et al. (2003). "Coordinated functions of Akt/PKB and ETS1 in tubule formation." The FASEB Journal **17**(15): 2278-2280.

Lawlor, M. A. and D. R. Alessi (2001). "PKB/Akt: a key mediator of cell proliferation, survival and insulin responses?" J Cell Sci **114**(Pt 16): 2903-10.

Lawlor, M. A., A. Mora, et al. (2002). "Essential role of PDK1 in regulating cell size and development in mice." EMBO J **21**(14): 3728-38.

Le, Y., Y. Zhou, et al. (2004). "Chemokines and chemokine receptors: their manifold roles in homeostasis and disease." Cell Mol Immunol **1**(2): 95-104.

LeibundGut-Landmann, S., J. M. Waldburger, et al. (2004). "MHC class II expression is differentially regulated in plasmacytoid and conventional dendritic cells." Nature immunology **5**(9): 899-908.

Lelievre, E., F. Lionneton, et al. (2001). "The Ets family contains transcriptional activators and repressors involved in angiogenesis." Int J Biochem Cell Biol **33**(4): 391-407.

Lewis, K. L. and B. Reizis (2012). "Dendritic cells: arbiters of immunity and immunological tolerance." Cold Spring Harb Perspect Biol **4**(8): a007401.

Li, Q. and I. M. Verma (2002). "NF-κB regulation in the immune system." Nature Reviews Immunology **2**(10): 725-734.

Li, X., T. Syrovets, et al. (2010). "Plasmin triggers chemotaxis of monocyte-derived dendritic cells through an Akt2-dependent pathway and promotes a T-helper type-1 response." Arteriosclerosis, thrombosis, and vascular biology **30**(3): 582-590.

Li, X., T. Syrovets, et al. (2009). "Plasmin triggers chemotaxis of monocyte-derived dendritic cells through an Akt2-dependent pathway and promotes a T-helper type-1 response." Arterioscler Thromb Vasc Biol **30**(3): 582-90.

Li, Y., K. J. Yang, et al. (2010). "Multiple implications of 3-phosphoinositide-dependent protein kinase 1 in human cancer." World J Biol Chem **1**(8): 239-47.

Liao, Y. and M. C. Hung (2010). "Physiological regulation of Akt activity and stability." Am J Transl Res **2**(1): 19-42.

Lin, K. W., T. Jacek, et al. (2006). "Dendritic cells heterogeneity and its role in cancer immunity." J Cancer Res Ther **2**(2): 35-40.

Lipscomb, M. F. and B. J. Masten (2002). "Dendritic cells: immune regulators in health and disease." Physiol Rev **82**(1): 97-130.

Liu, P., H. Cheng, et al. (2009). "Targeting the phosphoinositide 3-kinase pathway in cancer." Nat Rev Drug Discov **8**(8): 627-44.

Loffing, J., S. Y. Flores, et al. (2006). "Sgk kinases and their role in epithelial transport." Annu Rev Physiol **68**: 461-90.

Lulli, V., P. Romania, et al. (2010). "Transcriptional silencing of the ETS1 oncogene contributes to human granulocytic differentiation." Haematologica **95**(10): 1633-41.

Lutz, M. B. and G. Schuler (2002). "Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity?" Trends Immunol **23**(9): 445-9.

Lyakh, L. A., M. Sanford, et al. (2005). "TGF-β and vitamin D3 utilize distinct pathways to suppress IL-12 production and modulate rapid differentiation of human monocytes into CD83+ dendritic cells." The Journal of Immunology **174**(4): 2061-2070.

Madrid, L. V., M. W. Mayo, et al. (2001). "Akt Stimulates the Transactivation Potential of the RelA/p65 Subunit of NF-κB through Utilization of the IκB Kinase and Activation of the Mitogen-activated Protein Kinase p38." Journal of Biological Chemistry **276**(22): 18934-18940.

Manning, B. D. and L. C. Cantley (2007). "AKT/PKB signaling: navigating downstream." Cell **129**(7): 1261-74.

Martin, M., R. E. Schifferle, et al. (2003). "Role of the phosphatidylinositol 3 kinase-Akt pathway in the regulation of IL-10 and IL-12 by Porphyromonas gingivalis lipopolysaccharide." The Journal of Immunology **171**(2): 717-725.

Martin, V. A., W. H. Wang, et al. (2012). "Akt2 inhibits the activation of NFAT in lymphocytes by modulating calcium release from intracellular stores." Cellular Signalling.

Matzner, N., I. M. Zemtsova, et al. (2008). "Ion channels modulating mouse dendritic cell functions." J Immunol **181**(10): 6803-9.

Mazzarello, P., A. Calligaro, et al. (2003). "The sarcoplasmic reticulum: its discovery and rediscovery." Nat Rev Mol Cell Biol **4**(1): 69-74.

McKenna, K., A. S. Beignon, et al. (2005). "Plasmacytoid dendritic cells: linking innate and adaptive immunity." Journal of virology **79**(1): 17-27.

Mellstrom, B., M. Savignac, et al. (2008). "Ca2+-operated transcriptional networks: molecular mechanisms and in vivo models." Physiol Rev **88**(2): 421-49.

Merad, M. and M. G. Manz (2009). "Dendritic cell homeostasis." Blood **113**(15): 3418-27.

Mikoshiba, K. (2007). "The IP3 receptor/Ca2+ channel and its cellular function." Biochem Soc Symp(74): 9-22.

Mikoshiba, K. (2007). "IP3 receptor/Ca2+ channel: from discovery to new signaling concepts." J Neurochem **102**(5): 1426-46.

Mora, A., D. Komander, et al. (2004). "PDK1, the master regulator of AGC kinase signal transduction." Semin Cell Dev Biol **15**(2): 161-70.

Mora, A., C. Lipina, et al. (2005). "Deficiency of PDK1 in liver results in glucose intolerance, impairment of insulin-regulated gene expression and liver failure." Biochem J **385**(Pt 3): 639-48.

Mora, A., K. Sakamoto, et al. (2005). "Role of the PDK1-PKB-GSK3 pathway in regulating glycogen synthase and glucose uptake in the heart." FEBS Lett **579**(17): 3632-8.

Murakami, Y., F. Hosoi, et al. (2010). "Identification of sites subjected to serine/threonine phosphorylation by SGK1 affecting N-myc downstream-regulated gene 1 (NDRG1)/Cap43-dependent suppression of angiogenic CXC chemokine expression in human pancreatic cancer cells." Biochemical and biophysical research communications **396**(2): 376-381.

Murray, J. T., D. G. Campbell, et al. (2004). "Exploitation of KESTREL to identify NDRG family members as physiological substrates for SGK1 and GSK3." Biochemical Journal **384**(Pt 3): 477.

Nadif Kasri, N., G. Bultynck, et al. (2002). "The role of calmodulin for inositol 1,4,5-trisphosphate receptor function." Biochim Biophys Acta **1600**(1-2): 19-31.

Nagaleekar, V. K., S. A. Diehl, et al. (2008). "IP3 receptor-mediated Ca2+ release in naive CD4 T cells dictates their cytokine program." The Journal of Immunology **181**(12): 8315-8322.

Nagaleekar, V. K., S. A. Diehl, et al. (2008). "IP3 receptor-mediated Ca2+ release in naive CD4 T cells dictates their cytokine program." The Journal of Immunology **181**(12): 8315-8322.

Naik, S. H., P. Sathe, et al. (2007). "Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo." Nature immunology **8**(11): 1217-1226.

Nirula, A., M. Ho, et al. (2006). "Phosphoinositide-dependent kinase 1 targets protein kinase A in a pathway that regulates interleukin 4." J Exp Med **203**(7): 1733-44.

Nurbaeva, M. K., E. Schmid, et al. (2012). "Enhanced Ca(2)(+) entry and Na+/Ca(2)(+) exchanger activity in dendritic cells from AMP-activated protein kinase-deficient mice." FASEB J **26**(7): 3049-58.

O'Connell, P. J., V. A. Klyachko, et al. (2002). "Identification of functional type 1 ryanodine receptors in mouse dendritic cells." FEBS letters **512**(1): 67-70.

Oikawa, T. (2004). "ETS transcription factors: possible targets for cancer therapy." Cancer Sci **95**(8): 626-33.

Okkenhaug, K., A. Bilancio, et al. (2002). "Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice." Science Signaling **297**(5583): 1031.

Okkenhaug, K. and B. Vanhaesebroeck (2003). "PI3K in lymphocyte development, differentiation and activation." Nat Rev Immunol **3**(4): 317-30.

Pao, A. C., A. Bhargava, et al. (2010). "Expression and role of serum and glucocorticoid-regulated kinase 2 in the regulation of Na+/H+ exchanger 3 in the mammalian kidney." Am J Physiol Renal Physiol **299**(6): F1496-506.

Parekh, A. B. and J. W. Putney (2005). "Store-operated calcium channels." Physiological reviews **85**(2): 757-810.

Parekh, A. B. and J. W. Putney, Jr. (2005). "Store-operated calcium channels." Physiol Rev **85**(2): 757-810.

Park, C. Y., P. J. Hoover, et al. (2009). "STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1." Cell **136**(5): 876.

Park, D., N. Lapteva, et al. (2006). "An essential role for Akt1 in dendritic cell function and tumor immunotherapy." Nat Biotechnol **24**(12): 1581-90.

Park, J., M. L. L. Leong, et al. (1999). "Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway." The EMBO journal **18**(11): 3024-3033.

Parvez, S., A. Beck, et al. (2008). "STIM2 protein mediates distinct store-dependent and store-independent modes of CRAC channel activation." The FASEB Journal **22**(3): 752-761.

Paul, W. E. (2011). "Bridging innate and adaptive immunity." Cell **147**(6): 1212-5.

Pellegatti, P., S. Falzoni, et al. (2005). "A novel recombinant plasma membrane-targeted luciferase reveals a new pathway for ATP secretion." Molecular biology of the cell **16**(8): 3659-3665.

Penna, G., S. Amuchastegui, et al. (2007). "1, 25-Dihydroxyvitamin D3 selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells." The Journal of Immunology **178**(1): 145-153.

Petrie, R. J., A. D. Doyle, et al. (2009). "Random versus directionally persistent cell migration." Nature Reviews Molecular Cell Biology **10**(8): 538-549.

Pham, V. N., N. D. Lawson, et al. (2007). "Combinatorial function of ETS transcription factors in the developing vasculature." Dev Biol **303**(2): 772-83.

Philipsborn, A. and M. Bastmeyer (2007). "Mechanisms of gradient detection: a comparison of axon pathfinding with eukaryotic cell migration." International review of cytology **263**: 1-62.

Pinton, P., C. Giorgi, et al. (2008). "Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis." Oncogene **27**(50): 6407-6418.

Pollard, T. D. and G. G. Borisy (2003). "Cellular motility driven by assembly and disassembly of actin filaments." Cell **112**(4): 453-465.

Putney, J. W. (2010). "Pharmacology of store-operated calcium channels." Mol Interv **10**(4): 209-18.

Rahman, T. (2012). "Dynamic clustering of IP3 receptors by IP3." Biochem Soc Trans **40**(2): 325-30.

Rameh, L. E. and L. C. Cantley (1999). "The role of phosphoinositide 3-kinase lipid products in cell function." J Biol Chem **274**(13): 8347-50.

Ramirez, K., K. J. Chandler, et al. (2012). "Gene deregulation and chronic activation in natural killer cells deficient in the transcription factor ETS1." Immunity **36**(6): 921-32.

Randolph, G. J., J. Ochando, et al. (2008). "Migration of dendritic cell subsets and their precursors." Annu Rev Immunol **26**: 293-316.

Remy, P. and M. Baltzinger (2000). "The Ets-transcription factor family in embryonic development: lessons from the amphibian and bird." Oncogene **19**(55): 6417-31.

Ricart, B. G., B. John, et al. (2010). "Dendritic cells distinguish individual chemokine signals through CCR7 and CXCR4." J Immunol **186**(1): 53-61.

Richardson, C. J., S. S. Schalm, et al. (2004). "PI3-kinase and TOR: PIKTORing cell growth." Semin Cell Dev Biol **15**(2): 147-59.

Rizzuto, R. and T. Pozzan (2006). "Microdomains of intracellular Ca2+: molecular determinants and functional consequences." Physiol Rev **86**(1): 369-408.

Roelants, F. M., P. D. Torrance, et al. (2004). "Differential roles of PDK1- and PDK2-phosphorylation sites in the yeast AGC kinases Ypk1, Pkc1 and Sch9." Microbiology **150**(Pt 10): 3289-304.

Rong, Y.-P., G. Bultynck, et al. (2009). "The BH4 domain of Bcl-2 inhibits ER calcium release and apoptosis by binding the regulatory and coupling domain of the IP3 receptor." Proceedings of the National Academy of Sciences **106**(34): 14397-14402.

Rossi, A. M., S. C. Tovey, et al. (2011). "Analysis of IP3 receptors in and out of cells." Biochim Biophys Acta **1820**(8): 1214-27.

Rot, A. and U. H. von Andrian (2004). "Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells." Annu Rev Immunol **22**: 891-928.

Rota, M., A. Boni, et al. (2005). "Nuclear targeting of Akt enhances ventricular function and myocyte contractility." Circulation research **97**(12): 1332-1341.

Russell, L. and L. A. Garrett-Sinha (2010). "Transcription factor Ets-1 in cytokine and chemokine gene regulation." Cytokine **51**(3): 217-26.

Saarikangas, J., H. Zhao, et al. (2010). "Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides." Physiological reviews **90**(1): 259-289.

Saarikangas, J., H. Zhao, et al. (2009). "Molecular mechanisms of membrane deformation by I-BAR domain proteins." Curr Biol **19**(2): 95-107.

Saudemont, A. and F. Colucci (2009). "PI3K signaling in lymphocyte migration." Cell Cycle **8**(20): 3307-10.

Scharenberg, A. M., L. A. Humphries, et al. (2007). "Calcium signalling and cell-fate choice in B cells." Nature Reviews Immunology **7**(10): 778-789.

Scheid, M. P., M. Parsons, et al. (2005). "Phosphoinositide-dependent phosphorylation of PDK1 regulates nuclear translocation." Mol Cell Biol **25**(6): 2347-63.

Scheid, M. P. and J. R. Woodgett (2003). "Unravelling the activation mechanisms of protein kinase B/Akt." FEBS Lett **546**(1): 108-12.

Schmid, E., S. Gu, et al. (2012). "Serum-and glucocorticoid-inducible kinase SGK1 regulates reorganization of actin cytoskeleton in mast cells upon degranulation." American Journal of Physiology-Cell Physiology.

Schmidt, S. V., A. C. Nino-Castro, et al. (2012). "Regulatory dendritic cells: there is more than just immune activation." Front Immunol **3**: 274.

Schuetz, F., S. Kumar, et al. (2008). "Regulation of the voltage-gated K+ channels KCNQ2/3 and KCNQ3/5 by serum-and glucocorticoid-regulated kinase-1." American Journal of Physiology-Cell Physiology **295**(1): C73-C80.

Sharrocks, A. D. (2001). "The ETS-domain transcription factor family." Nat Rev Mol Cell Biol **2**(11): 827-37.

Shumilina, E., N. T. Xuan, et al. (2010). "Regulation of calcium signaling in dendritic cells by 1, 25-dihydroxyvitamin D3." The FASEB Journal **24**(6): 1989-1996.

Shumilina, E., N. Zahir, et al. (2007). "Phosphoinositide 3-kinase dependent regulation of Kv channels in dendritic cells." Cell Physiol Biochem **20**(6): 801-8.

Shumilina, E., I. M. Zemtsova, et al. (2010). "Phosphoinositide-dependent Kinase PDK1 in the Regulation of Ca2+ Entry into Mast Cells." Cellular Physiology and Biochemistry **26**(4-5): 699-706.

Small, J. V., T. Stradal, et al. (2002). "The lamellipodium: where motility begins." Trends in cell biology **12**(3): 112-120.

Smith, A. M., V. J. Findlay, et al. (2012). "ETS1 transcriptional activity is increased in advanced prostate cancer and promotes the castrate-resistant phenotype." Carcinogenesis **33**(3): 572-580.

Smith, I. F. and I. Parker (2009). "Imaging the quantal substructure of single IP3R channel activity during Ca2+ puffs in intact mammalian cells." Proceedings of the National Academy of Sciences **106**(15): 6404-6409.

Smyth, J. T., S. Y. Hwang, et al. "Activation and regulation of store 鈥恛 perated calcium entry." Journal of cellular and molecular medicine **14**(10): 2337-2349.

Sobiesiak, M., E. Shumilina, et al. (2009). "Impaired mast cell activation in gene-targeted mice lacking the serum-and glucocorticoid-inducible kinase SGK1." The Journal of Immunology **183**(7): 4395-4402.

Song, G., G. Ouyang, et al. (2005). "The activation of Akt/PKB signaling pathway and cell survival." J Cell Mol Med **9**(1): 59-71.

Sopjani, M., A. Kunert, et al. (2010). "Regulation of the Ca 2+ Channel TRPV6 by the Kinases SGK1, PKB/Akt, and PIKfyve." Journal of Membrane Biology **233**(1): 35-41.

Span, P. N., P. Manders, et al. (2002). "Expression of the transcription factor Ets-1 is an independent prognostic marker for relapse-free survival in breast cancer." Oncogene **21**(55): 8506-9.

Srikanth, S. and Y. Gwack (2012). "Orai1, STIM1, and their associating partners." The Journal of Physiology.

Steinman, R. M. and Z. A. Cohn (1973). "Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution." J Exp Med **137**(5): 1142-62.

Steinman, R. M. and H. Hemmi (2006). "Dendritic cells: translating innate to adaptive immunity." Curr Top Microbiol Immunol **311**: 17-58.

Stockand, J. D. (2005). "Preserving salt: in vivo studies with Sgk1-deficient mice define a modern role for this ancient protein." Am J Physiol Regul Integr Comp Physiol **288**(1): R1-3.

Stolk, M., M. Leon-Ponte, et al. (2006). "IP3Rs are sufficient for dendritic cell Ca2+ signaling in the absence of RyR1." Journal of Leukocyte Biology **80**(3): 651-658.

Sun, J. C., J. N. Beilke, et al. (2009). "Adaptive immune features of natural killer cells." Nature **457**(7229): 557-61.

Szado, T., V. Vanderheyden, et al. (2008). "Phosphorylation of inositol 1, 4, 5-trisphosphate receptors by protein kinase B/Akt inhibits Ca2+ release and apoptosis." Proceedings of the National Academy of Sciences **105**(7): 2427-2432.

Tai, D. J. C., C.-C. Su, et al. (2009). "SGK1 Phosphorylation of IκB Kinase α and p300 Up-regulates NF-κB Activity and Increases N-Methyl-D-aspartate Receptor NR2A and NR2B Expression." Journal of Biological Chemistry **284**(7): 4073-4089.

Tamura, T.-a., M. Hashimoto, et al. (2001). "Promoter structure and gene expression of the mouse inositol 1, 4, 5-trisphosphate receptor type 3 gene." Gene **275**(1): 169-176.

Taylor, C. W. and S. C. Tovey (2010). "IP3 receptors: toward understanding their activation." Cold Spring Harbor Perspectives in Biology **2**(12).

Terada, Y., S. Ueda, et al. (2012). "Aldosterone stimulates nuclear factor-kappa B activity and transcription of intercellular adhesion molecule-1 and connective tissue growth factor in rat mesangial cells via serum-and glucocorticoid-inducible protein kinase-1." Clinical and experimental nephrology **16**(1): 81-88.

Tessier, M. and J. R. Woodgett (2006). "Role of the Phox homology domain and phosphorylation in activation of serum and glucocorticoid-regulated kinase-3." J Biol Chem **281**(33): 23978-89.

Tessier, M. and J. R. Woodgett (2006). "Serum and glucocorticoid-regulated protein kinases: variations on a theme." J Cell Biochem **98**(6): 1391-407.

Thomas, K. W., M. M. Monick, et al. (2002). "Respiratory syncytial virus inhibits apoptosis and induces NF-κB activity through a phosphatidylinositol 3-kinase-dependent pathway." Journal of Biological Chemistry **277**(1): 492-501.

Vallon, V., A. W. Wyatt, et al. (2006). "SGK1-dependent cardiac CTGF formation and fibrosis following DOCA treatment." Journal of molecular medicine **84**(5): 396-404.

Vanderheyden, V., B. Devogelaere, et al. (2009). "Regulation of inositol 1,4,5-trisphosphate-induced Ca2+ release by reversible phosphorylation and dephosphorylation." Biochim Biophys Acta **1793**(6): 959-70.

Vanhaesebroeck, B. and D. R. Alessi (2000). "The PI3K-PDK1 connection: more than just a road to PKB." Biochem J **346 Pt 3**: 561-76.

Vanhaesebroeck, B. and M. D. Waterfield (1999). "Signaling by distinct classes of phosphoinositide 3-kinases." Exp Cell Res **253**(1): 239-54.

Varga-Szabo, D., K. S. Authi, et al. (2008). "Store-operated Ca2+ entry in platelets occurs independently of transient receptor potential (TRP) C1." Pflugers Arch-European Journal of Physiology **457**(2): 377-387.

Verger, A. and M. Duterque-Coquillaud (2002). "When Ets transcription factors meet their partners." Bioessays **24**(4): 362-70.

Vermassen, E., J. B. Parys, et al. (2004). "Subcellular distribution of the inositol 1,4,5-trisphosphate receptors: functional relevance and molecular determinants." Biol Cell **96**(1): 3-17.

Vig, M., W. I. DeHaven, et al. (2007). "Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release 鈥挸 ctivated calcium channels." Nature immunology **9**(1): 89-96.

Vig, M. and J. P. Kinet (2009). "Calcium signaling in immune cells." Nature immunology **10**(1): 21-27.

Vig, M., C. Peinelt, et al. (2006). "CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry." Science Signaling **312**(5777): 1220.

von Andrian, U. H. and T. R. Mempel (2003). "Homing and cellular traffic in lymph nodes." Nat Rev Immunol **3**(11): 867-78.

Walker, E. H., O. Perisic, et al. (1999). "Structural insights into phosphoinositide 3-kinase catalysis and signalling." Nature **402**(6759): 313-20.

Wang, D., S. A. John, et al. (2005). "Ets-1 deficiency leads to altered B cell differentiation, hyperresponsiveness to TLR9 and autoimmune disease." Int Immunol **17**(9): 1179-91.

Warfel, N. A., M. Niederst, et al. (2011). "Disruption of the interface between the pleckstrin homology (PH) and kinase domains of Akt protein is sufficient for hydrophobic motif site phosphorylation in the absence of mTORC2." J Biol Chem **286**(45): 39122-9.

Wasylyk, C., S. E. Schlumberger, et al. (2002). "Sp100 interacts with ETS-1 and stimulates its transcriptional activity." Mol Cell Biol **22**(8): 2687-702.

Watson, D. K., M. J. McWilliams-Smith, et al. (1985). "The ets sequence from the transforming gene of avian erythroblastosis virus, E26, has unique domains on human chromosomes 11 and 21: both loci are transcriptionally active." Proc Natl Acad Sci U S A **82**(21): 7294-8.

Wehrens, X. H. T. and A. R. Marks (2004). Ryanodine Receptors: Structure, function and dysfunction in clinical disease, Springer.

Wilson, N. S., D. El-Sukkari, et al. (2004). "Dendritic cells constitutively present self antigens in their immature state in vivo and regulate antigen presentation by controlling the rates of MHC class II synthesis and endocytosis." Blood **103**(6): 2187-95.

Woodgett, J. R. (2005). "Recent advances in the protein kinase B signaling pathway." Curr Opin Cell Biol **17**(2): 150-7.

Xuan, N. T., E. Shumilina, et al. (2009). "Ca2+-dependent functions in peptidoglycan-stimulated mouse dendritic cells." Cell Physiol Biochem **24**(3-4): 167-76.

Yamaguchi, Y., H. Tsumura, et al. (1997). "Contrasting Effects of TGFβ and TNFβ on the Development of Dendritic Cells from Progenitors in Mouse Bone Marrow." Stem Cells **15**(2): 144-153.

Yang, K. J., S. Shin, et al. (2008). "Regulation of 3-phosphoinositide-dependent protein kinase-1 (PDK1) by Src involves tyrosine phosphorylation of PDK1 and Src homology 2 domain binding." J Biol Chem **283**(3): 1480-91.

Yang, W. L., C. Y. Wu, et al. (2010). "Regulation of Akt signaling activation by ubiquitination." Cell Cycle **9**(3): 487-97.

Yoshizaki, H., N. Mochizuki, et al. (2007). "Akt-PDK1 complex mediates epidermal growth factor-induced membrane protrusion through Ral activation." Mol Biol Cell **18**(1): 119-28.

Zaru, R., P. Mollahan, et al. (2008). "3-phosphoinositide-dependent kinase 1 deficiency perturbs Toll-like receptor signaling events and actin cytoskeleton dynamics in dendritic cells." J Biol Chem **283**(2): 929-39.

Zhang, B., Y. Ma, et al. (2009). "Akt2 is required for macrophage chemotaxis." European journal of immunology **39**(3): 894-901.

Zhang, L., R. Cui, et al. (2005). "Antiapoptotic Effect of Serum and Glucocorticoid-Inducible Protein Kinase Is Mediated by Novel Mechanism Activating IκB Kinase." Cancer research **65**(2): 457-464.

Zhu, J., H. Yamane, et al. (2010). "Differentiation of effector CD4 T cell populations (*)." Annu Rev Immunol **28**: 445-89.

Curriculum Vitae

Education

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Publications

1. **Yang W,** Nurbaeva MK, Schmid E, Russo A, Almilaji A, Szteyn K, Faggio C, Shumilina E, Lang F. Akt2-dependent transcriptional factor ETS1 regulates expression of IP3 receptor 2 in dendritic cells. (in preparation)

2. **Yang W,** Schmid E, Nurbaeva MK, Szteyn K, Leibrock C, Gulbins E, Shumilina E, Lang F. Role of sphingomyelinase in the regulation of mast cell function. *Clinical & Experimental Allergy.* (under review)

3. **Yang W,** Bhandaru M, Pasham V, Bobbala D, Zelenak C, Jilani K, Rotte A, Lang F (2012) Effect of thymoquinone on cytosolic pH and Na^+/H^+ exchanger activity in mouse dendritic cells. *Cell Physiol Biochem.* 29(1-2):21-30.

4. Bhandaru M*, **Yang W*,** Rotte A, Pasham V, Lang F (2012) Regulation of Na^+/H^+ exchanger in dendritic cells by Akt2. *Pflugers Arch.* 463(2):355-63.

5. Sun Q, Dong J, **Yang W,** Jin Y, Yang M, Wang Y, Wang PL, Hu Y, Tsien JZ (2008) Efficient reproduction of cynomolgus monkey using pronuclear embryo transfer technique. *Proc Natl Acad Sci U S A.* 2;105(35):12956-60.

6. Zbidah M, Lupescu A, Herrmann T, Yang W, Foller M, Jilani K, Lang F(2013). Effect of honokiol on erythrocytes. Toxicol In Vitro. (in press)

7. Pasham V, Rotte A, Gu S, **Yang W,** Bhandaru M, Rexhep R, Ganesh P, Michael F, Florian Lang. Upregulation of intestinal NHE3 following saline ingestion. Kidney & Blood Pressure Research. (in press)

8. Schneider S, Schmid S, Schmidt EM, Schmid E, **Yang W,** Mia S, Brucker S, Wallwiener D, Brosens J, Lang F. TGFß1 and SGK1 sensitive store operated Ca^{2+} entry and Orai1 expression in endometrial Ishikawa cells. Molecular Human Reproduction. (in press)

9. Shumilina E, Nurbaeva MK, **Yang W,** Schmid E, Szteyn K, Heise N, Leibrock C, Xuan NT, Kuro-o M, Lang F. Altered regulation of cytosolic Ca^{2+} concentration in dendritic cells from klotho hypomorphic mice. Am J Physiol Cell Physiol.

10. Zbidah M, Lupescu A, **Yang W,** Bosc A, Jilani K, Shaik N, Lang F (2012) Sulindac sulfide--induced stimulation of eryptosis. Cell Physiol Biochem. 30(4):1072-82.

11. Schmid E, Bhandaru M, Nurbaeva MK, **Yang W,** Szteyn K, Russo A, Leibrock C, Tyan L, Pearce D, Shumilina E, Lang F(2012). SGK3 regulates Ca2+ entry and migration of dendritic cells. Cell Physiol Biochem. 30(6):1423-35.

12. Szteyn K, Schmid E, Nurbaeva MK, **Yang W,** Münzer P, Kunzelmann K, Lang F, Shumilina E (2012) Expression and Functional Significance of the Ca^{2+} -Activated Cl^- channel ANO6 in Dendritic Cells. Cell Physiol Biochem. 30(5):1319-32.

13. Schmid E, Gu S, **Yang W,** Münzer P, Schaller M, Lang F, Stournaras C, Shumilina E.(2012) Serum- and glucocorticoid-inducible kinase SGK1 regulates reorganization of actin cytoskeleton in mast cells upon degranulation. Am J Physiol Cell Physiol. 304(1):C49-55

14. Pasham V, Rotte A, **Yang W,** Zelenak C, Bhandaru M, Föller M, Lang F (2012) OSR1-sensitive regulation of Na^+/H^+ exchanger activity in dendritic cells. Am J Physiol Cell Physiol. 303(4):C416-26.

15. Szteyn K, **Yang W,** Schmid E, Lang F, Shumilina E(2012) Lipopolysaccharide-sensitive H+ current in dendritic cells. Am J Physiol Cell Physiol. 303(2):C204-12

16. Bhandaru M, Pasham V, **Yang W,** Bobbala D, Rotte A, Lang F(2012) Effect of azathioprine on Na^+/H^+ exchanger activity in dendritic cells. Cell Physiol Biochem. 29(3-4):533-42

17. Nurbaeva MK, Schmid E, Szteyn K, **Yang W,** Viollet B, Shumilina E, Lang F(2012) Enhanced Ca^{2+} entry and Na^+/ Ca^{2+} exchanger activity in dendritic cells from AMP-activated protein kinase-deficient mice. FASEB J. 26(7):3049-58

18. Eylenstein A, Schmidt S, Gu S, **Yang W,** Schmid E, Schmidt EM, Alesutan I, Szteyn K, Regel I, Shumilina E, Lang F(2012) Transcription factor NF-κB regulates expression of pore-forming Ca^{2+} channel unit, Orai1, and its activator, STIM1, to control Ca^{2+} entry and affect cellular functions. J Biol Chem. 287(4):2719-30.

19. Rotte A, Pasham V, Eichenmüller M, **Yang W,** Bhandaru M, Lang F(2011) Influence of dexamethasone on Na^+/H^+ exchanger activity in dendritic cells. Cell Physiol Biochem. 28(2):305-14

20. Heise N, Shumilina E, Nurbaeva MK, Schmid E, Szteyn K, **Yang W,** Xuan NT, Wang K, Zemtsova IM, Duszenko M, Lang F (2011) Effect of dexamethasone on Na^+/Ca^{2+} exchanger in dendritic cells. Am J Physiol Cell Physiol. 300(6):C1306-13

21. Pasham V, Rotte A, Bhandaru M, Eichenmüller M, Fröhlich H, Mack AF, Bobbala D, **Yang W**, Pearce D, Lang F(2011) Regulation of gastric acid secretion by the serum and glucocorticoid inducible kinase isoform SGK3. J Gastroenterol. 46(3):305-17

22. Tyan L, Sopjani M, Dërmaku-Sopjani M, Schmid E, **Yang W,** Xuan NT, Shumilina E, Lang F(2010) Inhibition of voltage-gated K+ channels in dendritic cells by rapamycin.Am J Physiol Cell Physiol. 299(6):C1379-85

23. Rotte A, Pasham V, **Yang W**, Eichenmüller M, Bhandaru M, Shumilina E, Lang F(2010)Phosphoinositide 3-kinase-dependent regulation of Na^+/H^+ exchanger in dendritic cells. Pflugers Arch. 460(6):1087-96

24. Rotte A, Pasham V, Eichenmüller M, **Yang W**, Qadri SM, Bhandaru M, Lang F(2010) Regulation of basal gastric acid secretion by the glycogen synthase kinase GSK3. J Gastroenterol. 45(10):1022-32.

25. Rotte A, Pasham V, Bhandaru M, Eichenmüller M, **Yang W,** Qadri SM, Kempe DS, Puchchakayala G, Pearce D, Birnbaum MJ, Lang F(2010) Regulation of gastric acid secretion by PKB/Akt2. Cell Physiol Biochem. 25(6):695-704.

Declaration

I hereby declare that this thesis was created with contribution of other laboratory team members.

Contributions

This project describes two cell signaling pathways in dendritic cells, play a crucial role in regulation of physiological functions in those cells. The majority of the data presented in this thesis was collected from the experiments that I performed personally. I received help in calcium imaging from Meerim Nurbaeva and Jing Yan; patch clamp from Kalina szteyn and Ahmad Almilaji; western blotting from Evi Schmid and Antonella Russo, Evi Schmid also performed FACS experiments.