Cellular Microbiology (2006) **8**(11), 1826–1839
doi:10.1111/j.1462-5822.2006.00752.x
First published online 4 July 2006

Measles virus induces expression of SIP110, a constitutively membrane clustered lipid phosphatase, which inhibits T cell proliferation

Elita Avota, Harry Harms and Sibylle Schneider-Schaulies*
Institute for Virology and Immunobiology, University of Wuerzburg, Versbacher Str. 7, D-97078 Wuerzburg, Germany.

Summary

Interference of measles virus (MV) with phosphatidyl-inositol-3-kinase (PI3K) activation in response to T cell receptor ligation was identified as important for the induction of T cell paralysis. We now show that MV exposure of unstimulated T cells induces expression of SIP110, an isoform of the lipid phosphatase SHIP145, which is translated from an intron-derived sequences containing mRNA. We found that MV contact can regulate stimulated exon inclusion into pre-mRNAs by targeting PI3K or MAPK-dependent nuclear translocation and activation of splicing regulatory serine–arginine rich (SR) and Sam68 proteins. Induction of SIP110 in resting T cells relied on MV-dependent interference with basal activity of the PI3K. SIP110 was cloned from MV-exposed T cells, and, when transiently expressed in primary or Jurkat T cells, localized into membrane clusters independently of T cell activation. Confirming that SIP110 is a catalytically active lipid phosphatase, its transgenic expression abolished basal and impaired PMA/ionomycin-stimulated phosphorylation of the Akt kinase which is important for T cell proliferation. Thus MV causes induction of SIP110 expression, which constitutively depletes the cellular phosphoinositol-3,4,5-phosphate pool suggesting that thereby the threshold for activation signals necessary for the induction of T cell proliferation is raised.

Introduction

Infections with measles virus (MV) continue to rank among the major causes of vaccine preventable infant deaths worldwide. It is particularly in Third World countries where the transient, yet severe generalized immunosuppression caused by this virus favours the establishment and aggravates the course of secondary infections (Clements and Cutts, 1995). Hallmarks of this immunosuppression are lymphopenia, cytokine imbalance and the inability of peripheral blood lymphocytes to expand in response to polyclonal or antigen-specific stimulation *ex vivo* (Borrow and Oldstone, 1995; Schneider-Schaulies *et al*., 2002). Accumulating evidence mainly from *in vitro*, and more recently also from animal experimentation has placed dendritic cells (DCs) as central to the induction of at least certain aspects of MV interference with immune responses, although their infection *in vivo* has not been substantiated as yet (Schneider-Schaulies *et al*., 2003; Servet-Delprat *et al*., 2003; Hahm *et al*., 2004; Shingai *et al*., 2005; Welstead *et al*., 2005). Infection-dependent deregulations of DC maturation and/or function would, however, be compatible with inadequate cytokine production, transmission of MV or inhibitory signalling to T cells by the viral glycoprotein (gp) complex expressed at high levels on the DC surface (Servet-Delprat *et al*., 2000; 2003; Dubois *et al*., 2001; Schneider-Schaulies *et al*., 2003).

As a consequence of the interaction of the MV gp complex [consisting of the haemagglutinin (H) and the proteolytically activated fusion (F) protein] with their surface, human primary T cells acquire a paralytic state. These remain viable, yet fail to progress into the S phase of the cell cycle in response to mitogens or CD3/CD28 ligation (Schnorr *et al*., 1997; Schneider-Schaulies and ter Meulen, 2002). On a molecular level, interference with T cell receptor (TCR)- or IL-2R-induced PI3/Akt kinase activation and consequently, membrane recruitment of pleckstrin homology (PH) domain containing proteins were found to be central for MV-induced paralysis (Avota *et al*., 2001; 2004). Recruitment of these proteins relies on accumulation of phosphoinositol-poly-phosphates, particularly phosphatidyl-inositol-3,4,5-phosphate (PIP3), whose levels are regulated positively by the phosphatidyl-inositol-3-kinase (PI3K) and negatively by the lipid phosphatases, PTEN and SHIP (Src homology domain 2 containing inositol-5-phosphatase), the latter of which is mainly expressed in cells of haematopoetic origin (Rohr-

Received 14 February, 2006; revised 15 May, 2006; accepted 17 May, 2006. *For correspondence. E-mail s-s-s@vim.uni-wuerzburg.de; Tel. (+49) 931 201 49895; Fax (+49) 931 201 49553.

schneider *et al*., 2000). SHIP has been implicated in regulation of immunoreceptor signalling by its ability to associate with cytoplasmic immunoreceptor tyrosine activation/inhibitory motifs (ITAMs) or ITIMs after tyrosine-phosphorylation and to hydrolyse phospholipids thereby abolishing or terminating stimulatory signals (Rohrschneider *et al*., 2000). Interestingly, SHIP can also associate with the cytoplasmic tail of CD150, the common MV receptor (Tatsuo and Yanagi, 2002; Yanagi *et al*., 2002; Sidorenko and Clark, 2003; Yurchenko *et al*., 2005), yet its relevance for receptor function or MV inhibitory signalling to T cells has not been addressed. Multiple isoforms of SHIP and SHIP-related mRNA and protein species are expressed in developing and differentiating haematopoetic progenitor cells (Geier *et al*., 1997; Rohrschneider *et al*., 2000; Tu *et al*., 2001). In human cells, a splice variant of SHIP (also referred to as SHIP145), SIP110, was described which lacks the N-terminal SH2 domain but retains the central phosphatase and the C-terminal proline rich domains (Kavanaugh *et al*., 1996; Lucas and Rohrschneider, 1999). The biological role of SIP110, as that of its murine orthologue, s-SHIP, which is only expressed in primitive haematopoetic stem cells (Tu *et al*., 2001), is unknown as yet.

When investigating MV-contact-dependent regulation of the PI3K we noted induction of a 110 kDa SHIP-related protein in resting T cells exposed to MV, which is translated from an alternative *SHIP* mRNA containing intron-derived sequences (IDSs). Using signalling-regulated splicing of *CD44* mRNA as a model system we found that by interference with PI3K- or MAPK-dependent activation of splice accessory serine–arginine rich (SR) and Sam68 proteins respectively, MV can regulate inclusion of exons into cellular pre-mRNAs. Remarkably, regulation of SHIP isoform expression by MV in T cells by modulating basal activity of the PI3K provides a beforehand unknown example of autocrine regulation within this pathway. Moreover, transient transgenic expression of SIP110 caused significant inhibition of T cell expansion and thus, induction of this protein by MV signalling may contribute to immunosuppression.

Results

Measles virus induces expression of the SIP110 isoform of SHIP-1

Measles virus exposure impairs IL-2R and TCR ligation-induced activation of the PI3K (Avota *et al*., 2001; 2004) which has also been found to be involved in regulation of cellular pre-mRNA splicing. We have chosen to analyse the potential impact of MV on this process in T cells for *SHIP*, because various isoforms are known to be expressed from this gene (Rohrschneider *et al*., 2000). *SHIP* encodes a lipid phosphatase central to the regulation of the homeostatic and stimulated PIP3 pool and is also involved in CD150 signalling (Sidorenko and Clark, 2003). Human primary T cells were exposed to UV-inactivated wild-type (WTF) or attenuated (ED) MV (Fig. 1A) (each at an amount corresponding to a moi of 1 of live virus) or mock extract in the presence of fusion inhibitory peptide (FIP) and SHIP expression was detected after immunoprecipitation on Western blots. While levels of full-length SHIP protein (SHIP145) were comparable in T cells exposed to either mock or MV for 24 h, MV caused accumulation of an additional SHIP antibody reactive protein of 110 kDa (Fig. 1A). To confirm that SHIP110 is identical to SIP110, a SHIP isoform translated from an alternatively spliced transcript [first isolated from a human placenta cDNA library (Kavanaugh *et al*., 1996; Lucas and Rohrschneider, 1999)] we performed RT-PCR analyses on RNA isolated from T cells exposed to MV or to mock respectively. While transcripts devoid of intronic sequences between exons 5 and 6 (encoding full-length SHIP145) were amplified from both MV- and mock-treated T cells, those including IDS 5′ of exon 6 (containing an in frame stop and an internal translation initiation site) were only detected in cells exposed to MV (Fig. 1B and C). Induction of the SIP110-specific transcript required the MV gps because it did not occur in cells exposed to a recombinant MV expressing vesicular stomatitis virus (VSV) G protein instead (Fig. 1C, upper panel). Furthermore it was produced independently of membrane fusion (which is prevented by FIP inclusion) and CD150 [which is not expressed on resting T cells (Avota *et al*., 2004)] or CD46 ligation (Fig. 1C, bottom panels). As SIP110 transcripts were detected after PI3K but not MAPK inhibition (using LY294002 or U0126 respectively), intron exclusion from *SHIP* primary transcripts in resting T cells depends on PI3K rather than on MAPK activity (Fig. 1C). In summary, IDS are present in the *SHIP* pre-mRNA in T cells exposed to MV or LY294002, indicating that signalling by the MV gp complex targets PI3K activation already in resting T cells in addition to that seen previously after TCR ligation or IL-2 stimulation (Avota *et al*., 2001; 2004). Indeed, MV treatment diminished basal levels of pAkt as did LY294002 in resting T cells (Fig. 1C, right panels).

Measles virus regulates activation of splicing accessory factors and exonic inclusion in CD44 transcripts

Measles virus blocks PI3K activity and thereby regulates splicing of *SHIP* transcripts, and most likely also that of other cellular pre-mRNAs. The PI3K/Akt pathway is important for signalling-regulated alternative mRNA splicing and SR protein activation (Patel *et al*., 2001; 2005; Liu *et al*., 2003). We have chosen to study the effect of MV on these proteins which are essentially involved in constitutive and

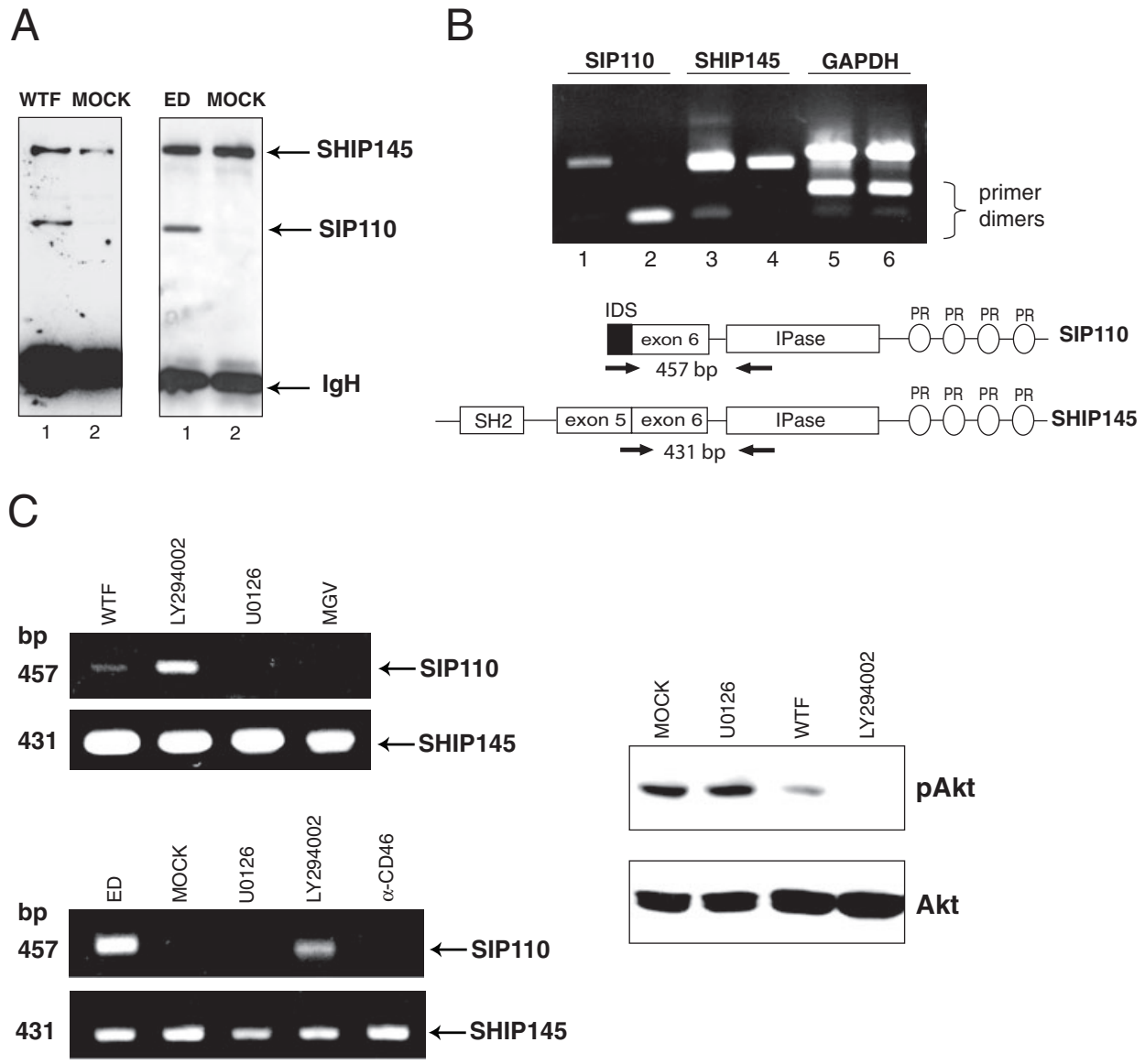


Fig. 1. Measles virus and PI3K inhibitor induce expression of SIP110 in primary T cells.
A. Whole cell extracts isolated from T cells cocultivated with UV-inactivated wild-type virus strain (WTF, left panel), vaccine strain (ED, right panel) or mock extract for 24 h in the presence of FIP were used to analyse expression of SHIP isoforms by immunoprecipitation followed by Western blot detection.
B. RNA was isolated from T cells exposed to WTF (lanes 1, 3 and 5) and mock (lanes 2, 4 and 6) for 24 h. Primers complimentary to the IDS 5′ of exon 6 (lanes 1 and 2) or spanning exon 5/6 boundary (lanes 3 and 4) of SHIP145 were used to detect SIP110 or, respectively, SHIP145 mRNA by RT-PCR. Location of the primers and the length of the amplified products are indicated in the bottom graphs where positions of the SH2, inositolphosphatase (IPase) and proline-rich domains (PR) as well as exons 5 and 6 are shown. Amplification of GAPDH served as control.
C. T cells were exposed to WTF (two upper left panels) and ED (two bottom left panels), LY294002 and U0126 (both panels), MGV (a MV recombinant expressing the VSV G protein instead of the F/H complex) (two upper left panels) or a CD46-specific antibody (two bottom left panels) for 24 h, RNA was isolated and used to analyse expression of SIP110- (upper panels) or SHIP145-encoding mRNA (bottom panels) by RT-PCR. Unstimulated primary T cells were exposed to WTF or MOCK, inhibitors UO126 and LY294002 for 24 h followed by cell lysis. Basal activity of PI3K was determined in cell lysates by Western blot using Ser473 p-Akt specific antibody and detection of Akt protein served as loading control (right panels).

induced pre-mRNA processing, and whose nuclear import and activity depend on phosphorylation of residues in their C-terminal domains (Bourgeois *et al.*, 2004; Graveley, 2005). Consistent with observations made in B cells and fibroblasts (Liu *et al.*, 2003; Patel *et al.*, 2005) we found that T cell activation by PMA/ionomycin for short (Fig. 2A) and prolonged (Fig. 2B) periods caused nuclear transport of phosphorylated SR proteins (pSR) (as detected by the monoclonal antibody mAB104) which was inhibited by LY294002 but not by U0126 (Fig. 2). Indicating a general

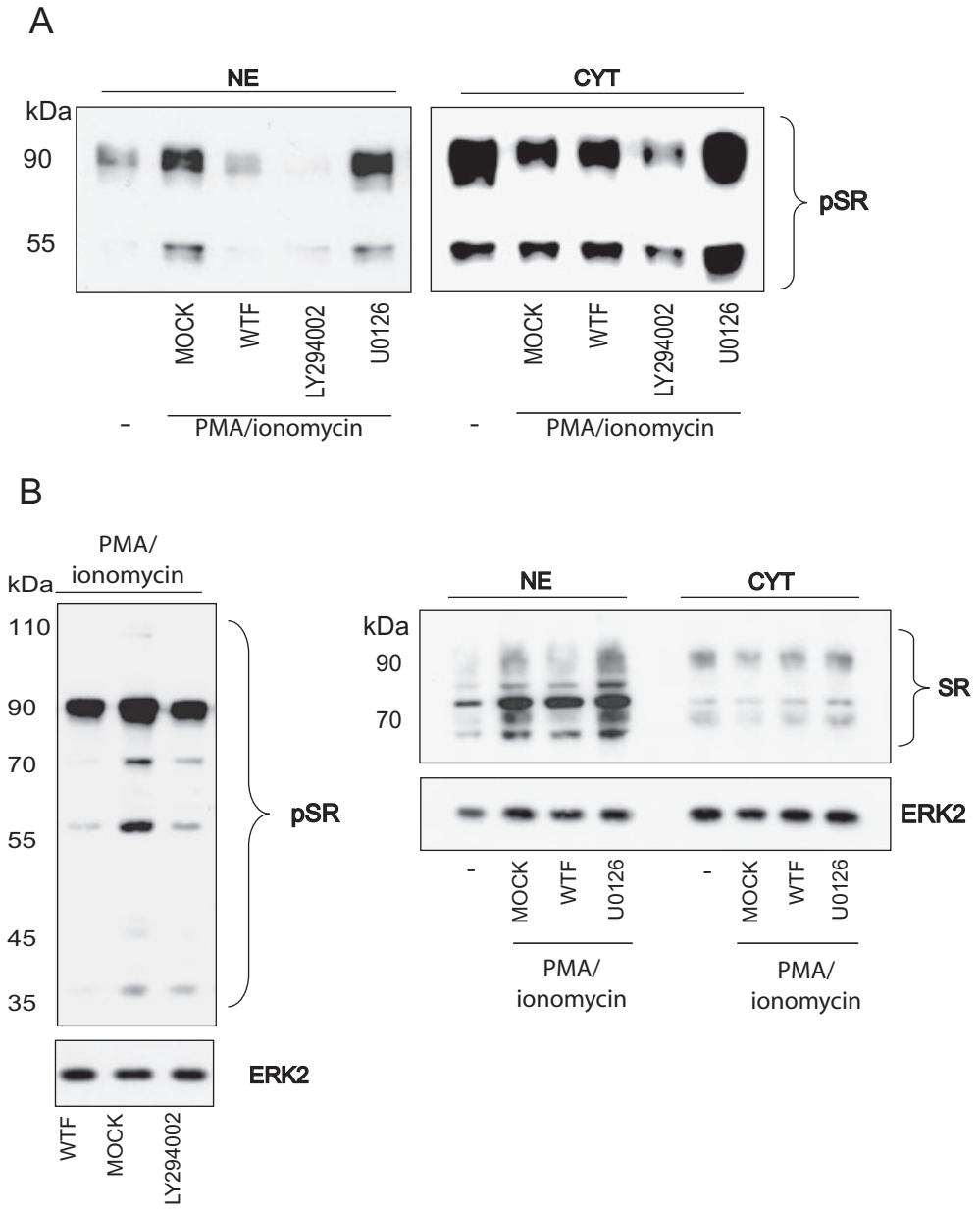


Fig. 2. MV exposure affects SR protein phosphorylation and nuclear accumulation. Primary T cells cocultivated with WTF or mock extract (in the presence of FIP) or treated with LY294002 or UO126 for 24 h were harvested after stimulation by PMA/ionomycin for 1 min (A) or 24 h (B). Nuclear (NE) and cytoplasmic (CYT) extracts were used for detection of pSR proteins (using the phospho-specific mAB104) (A; and B, left panel for nuclear extracts) or accumulation of total SR proteins (B, right panel). ERK2 detection was used as loading control.

effect on induced cellular splicing, MV exposure efficiently abolished PMA/ionomycin-stimulated nuclear accumulation of pSR proteins in T cells (Fig. 2). To address this specifically, we analysed signalling-regulated alternative splicing of *CD44* which has been mechanistically studied in detail in immune cells (Lynch, 2004). We made use of a reporter gene assay established in murine T cells, where expression of a luciferase reporter gene relies on its MAPK stimulation-dependent splicing-mediated fusion to *CD44* variable exon 5 (v5) (Weg-Remers *et al*., 2001). Also in human T cells, luciferase expression was efficiently activated by PMA/ionomycin, and this could be blocked not only by U0126, but also by LY294002 and, dose dependently, by MV (Fig. 3A). In murine T cells, exon v5 inclusion required MAPK-dependent threonine phosphorylation of Sam68, a member of the signal transduction and activation of RNA (STAR) protein family which, as RNA binding proteins, are essentially involved in the regulation of cellular processes also including pre-mRNA splicing (Weg-Remers *et al*., 2001; Lukong and Richard,

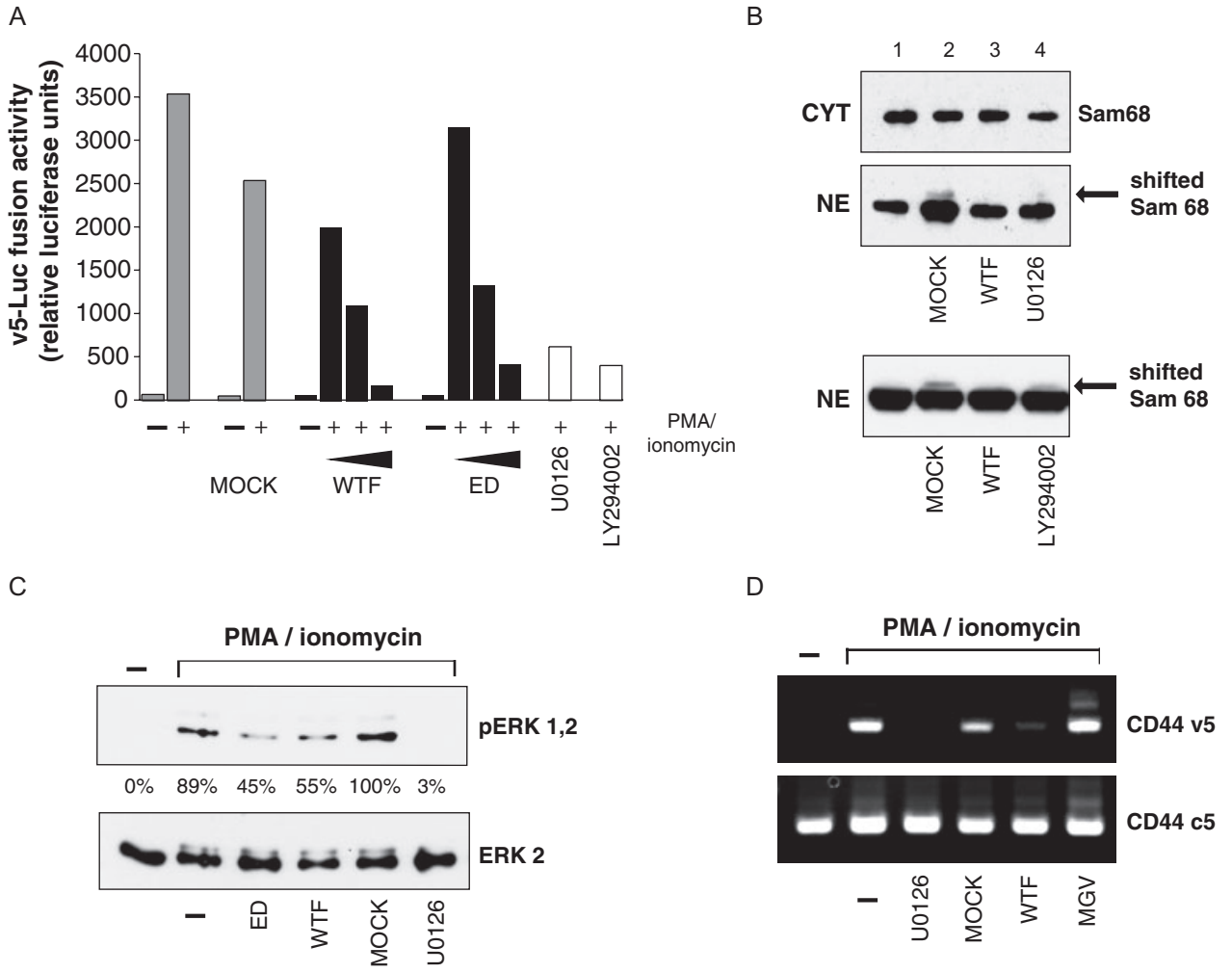


Fig. 3. MV interferes with PMA-stimulated exon v5 inclusion of CD44 in human primary T cells.
A. T cells transfected with pETv5luc were exposed to increasing amounts (corresponding to moi of 0.25, 0.5 and 1 of live virus) of UV-WTF or -ED, mock, U0126 or LY294002 for 24 h and subsequently stimulated with PMA/ionomycin for further 24 h. Luciferase activity in total cell extracts was used to determine the induction efficiency of the v5-luciferase fusion protein.
B. Nuclear (middle and bottom panels) and cytoplasmic (upper panel) extracts prepared from T cells exposed to mock, WTF, LY294002 or U0126 for 24 h or left unstimulated (lane 1) or PMA/ionomycin stimulated for 2 min (lanes 2–4) were used to detect Sam68. The position of the shifted fraction is indicated by arrows.
C. Nuclear extracts of T cells treated as described in (B) were obtained and analysed for the presence of phosphorylated ERK1,2 (upper panel). Relative levels of ERK phosphorylation were analysed by AIDA software (Raytest) [indicated as intensity (%) below the pERK blot]. For loading control, the blot was reprobed using an ERK2-specific antibody (bottom panel).
D. T cells were exposed to WTF (moi 1), mock or U0126 as described in (A), and, in addition, to MGV (moi 1) for 24 h prior to PMA/ionomycin activation and harvested for detection of transcripts containing exon v5 (upper panel) or exon c5 (bottom panel), respectively, by RT-PCR.

2003). We observed that a small fraction of Sam68 with lower electrophoretic mobility possibly indicating phosphorylation was present in nuclear extracts of PMA/ionomycin-stimulated human T cells (Fig. 3B, middle and bottom panels). Interestingly, this was blocked by U0126 and WTF, but not by LY294002 (Fig. 3B, middle and bottom panels respectively). Thus, although both MAPK and PI3K are apparently involved in *CD44* exon v5 inclusion in the reporter construct in human T cells (Fig. 3A), activation of Sam68 occurs independently of PI3K, but rather relies on MAPK activation. The failure of PMA/ionomycin to activate Sam68 in T cells exposed to MV thus indicated that MV contact might target MAPK activation. Indeed, pre-exposure of T cells to MV for 24 h caused a reduction of PMA/ionomycin-induced phosphorylation levels of extracellular signalling regulated kinase (ERK) by approximately 50% as compared with mock-treated controls (Fig. 3C). Consistent with the documented requirement of MAPK-dependent activation of Sam68 for *CD44* exon v5 inclusion and the ability of MV to interfere with MAPK and Sam86 activation, both MV exposure and U0126 treatment efficiently interfered with PMA/ionomycin-induced

CD44 exon v5 (and also that of the variable exons v6 and v9, not shown) but not constitutive exon 5 (c5) inclusion as compared with mock-treated human T cells (Fig. 3D). Regulation of exon inclusion by MV requires the viral gp complex because a recombinant MV expressing VSV G instead had no effect (Fig. 3D). In summary, MV signalling affects activation of SR and STAR proteins, which are involved in *CD44* exon inclusion by its ability to target PI3K and the MAPK pathways.

SIP110 is a SHIP isoform constitutively present in membrane clusters on T cells

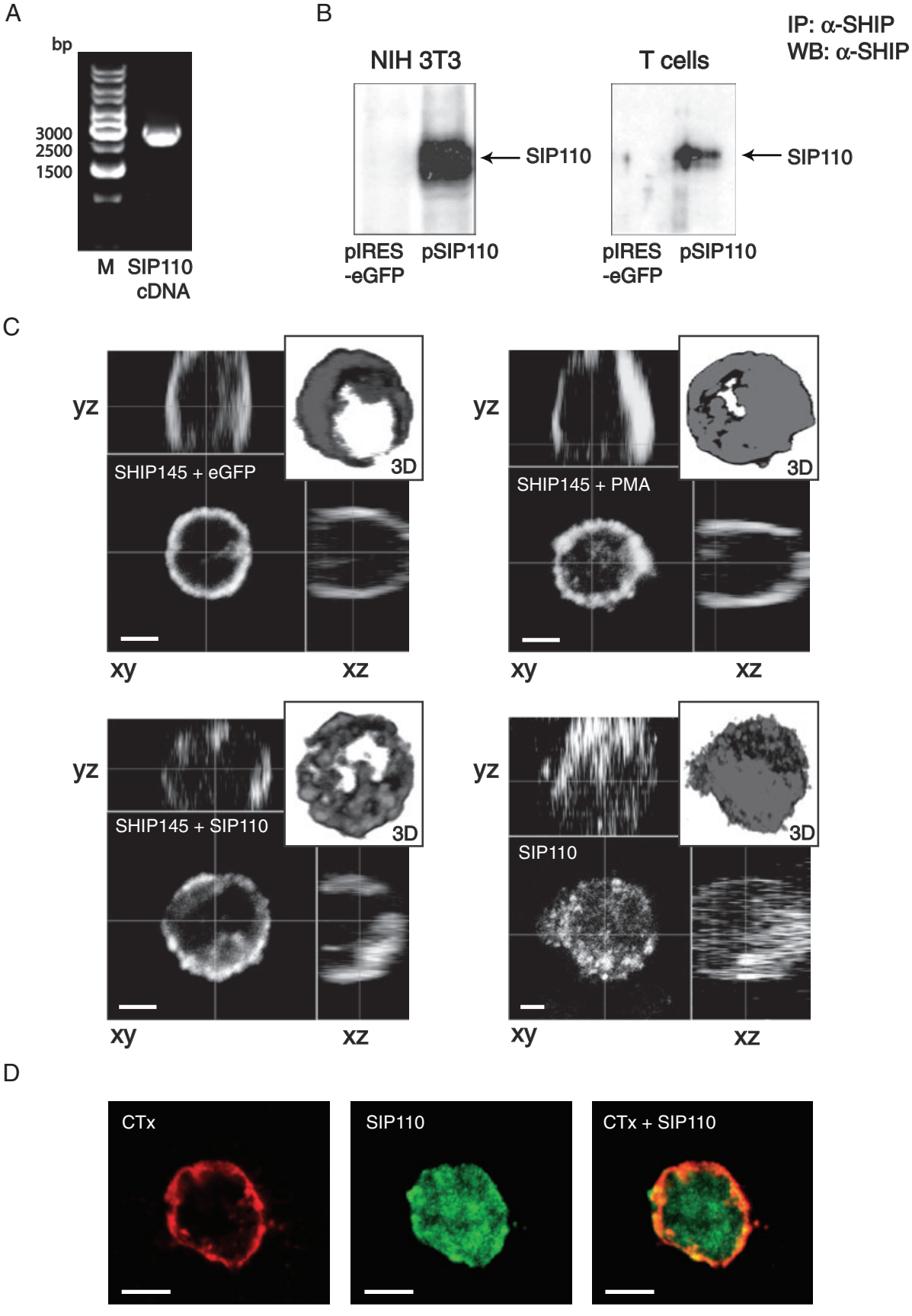
Measles virus contact causes SIP110 accumulation in unstimulated T cells by induction of a *SHIP* transcript containing intronic sequences (Fig. 1). SIP110 shares C-terminal proline-rich and the phosphatase domains with SHIP145, yet lacks an N-terminal SH2 domain (Kavanaugh *et al*., 1996) (see also Fig. 1B) which is essential for activation-dependent tyrosine-phosphorylation and membrane recruitment of SHIP145 (Rohrschneider *et al*., 2000). Functional consequences of SIP110 expression have not yet been studied. To explore these, the SIP110 cDNA was RT-PCR amplified from total RNA isolated from MV-exposed T cells (Fig. 4A) and cloned into pIRES-eGFP (to yield pSIP110). After transfection of pSIP110, but not that of the empty vector into NIH 3T3 or primary human T cells, a SHIP antibody reactive protein of the expected size was detected in immunoprecipitates of whole cell extracts (Fig. 4B) (endogenous SHIP145 was not detected in this particular experiment in T cell extracts). In contrast to what has been described for B cells and NK cells, association of endogenous SHIP with the plasma cell membrane did not require activation in T primary human T cells (Fig. 4C, upper left panels). PMA/ionomycin activation increased SHIP protein accumulation at the cell membrane into clearly discernable clusters (Fig. 4C, upper right panels). Interestingly, primary T cells nucleofected with pSIP110 (but not with the empty control vector) revealed similar clusters of SHIP antibody reactive proteins at the cell membrane in the absence of activation, indicating that transgenic SIP110 expression in resting T cells induces appearance of clusters otherwise confined to stimulated cells (Fig. 4C, bottom left panels). In line with this, SIP110 was detected within membrane clusters when nucleofected into SHIP145-deficient Jurkat cells (Fig. 4C, bottom right panels) part of which, in analogy to what has been described for SHIP145 in B and NK cells, represent choleratoxin-binding lipid rafts (Fig. 4D) (Aman *et al*., 2001; Phee *et al*., 2001; Galandrini *et al*., 2002). As primary or Jurkat T cells transfected to overexpress SHIP145 were of low viability, these were not detected after 24 h (see also Fig. 6C).

SIP110 interferes with both basal and stimulated Akt activation and impairs T cell expansion but not viability

Because SIP110 retains its 5′-phosphatase activity (Kavanaugh *et al*., 1996) and is constitutively organized in membrane clusters (Fig. 4C), it possibly depletes tonic levels of 5′-phosphorylated phosphoinositols and raises the threshold level for their stimulated accumulation in T cells. Indeed, phosphorylated Akt kinase (used as an indicator for accumulation of 5′ phosphoinositols) was detected in unstimulated T cells nucleofected with the vector control (expressing endogenous SHIP145 and a slightly smaller isoform, presumably SHIP135, protein) and this was strongly reduced in T cells expressing SIP110 (where it represented 33% of SHIP145) (Fig. 5A, left and middle panels). Upon PMA/ionomycin stimulation, Akt kinase was efficiently activated in vector- but only moderately in pSIP110 nucleofected cells (Fig. 5A, right two panels). As revealed by confocal microscopy, PMA activation induced formation of pAkt membrane clusters in untransfected and vector-transfected cells, but not in wortmannin-pretreated or SIP110-expressing cells. To address consequences of SIP110 expression on T cell proliferation, pIRES-eGFP, pSIP110 or pSHIP145 (encoding full-length SHIP145) along peGFP were nucleofected into T cells (with an efficiency of 30% for all cultures as controlled by GFP expression). Overexpression of either SIP110 or SHIP145 significantly impaired the ability of the T cell cultures to proliferate in response to CD3/CD28 ligation or PMA/ionomycin stimulation (Fig. 6A). Remarkably, SIP110 or SHIP145 expressing cells failed to enter the G2 phase after CD3/CD28 ligation as revealed by their histone H3 phosphorylation levels which significantly increased only in vector-transfected controls (Fig. 6B). Interestingly, overexpression of SHIP145 but not SIP110 increased the percentage of annexin-binding T cells, indicating that the two isoforms differ in their ability to cause apoptosis in T cells (Fig. 6C).

Discussion

Inhibitory signalling by viral gene products has proven to be central to the induction of T cell unresponsiveness which is a hallmark of MV-induced immunosuppression *in vitro* and *ex vivo* (Niewiesk *et al*., 1997; Schneider-Schaulies *et al*., 2002; Kerdiles *et al*., 2006). Our previous analyses focused on the interference of MV contact with TCR or IL-2R signalling-regulated cascades in order to explain why these fail to induce cytoskeletal rearrangements, polarisation, and proliferation. We found that early after interaction between the MV gp complex and the T cell surface, TCR ligation-dependent activation of the PI3K is efficiently abolished and this involves lack of p85 membrane recruitment. As a consequence, membrane



recruitment of PH domain containing proteins including Vav and Akt as well as activation of their downstream effectors is prevented (Avota *et al*., 2001; 2004).

To study the potential impact of MV on pre-mRNA splicing of cellular transcripts we have first chosen to analyse activation of general splicing accessory proteins such as SR and Sam68 proteins (Lukong and Richard, 2003; Shen and Green, 2004) and exon inclusion into *CD44* transcripts because this is understood in more detail (Lynch, 2004). Exon inclusion into *CD44* transcripts has been directly linked to the activity of SR proteins, the activity of which can be modulated by PI3K (Graveley *et al*., 1999; Graveley, 2000). In addition, SR protein activity is targeted in cells infected by DNA viruses, replication of which involves splicing of viral transcripts, and this can be mediated by virally encoded enzymes or regulation of cellular pathways also including PI3K activation (Chalfant *et al*., 2001; 2002; Cowper *et al*., 2001; Huang *et al*., 2002; Liu *et al*., 2003; Sciabica *et al*., 2003; Patel *et al*., 2005; Kanj *et al*., 2006). Viral regulation of cellular mRNA splicing by receptor contact, as exerted by MV, has not been described as yet. Examples of isoforms produced from alternatively spliced mRNAs in immune cells are numerous (Lynch, 2004), yet the complexity of the exon inclusion process into individual transcripts does not allow to predict which one might be specifically affected by the alterations of SR protein activation induced by MV (Fig. 2).

In addition to SR proteins, Sam68 protein has been implicated into induced inclusion of a model variable exon, *CD44* exon v5, in murine T cells and this relied on MAPK activation (Konig *et al*., 1998; Matter *et al*., 2002). We now show that in addition to that of the PI3K, stimulation-dependent activation of MAPK is also targeted by MV signalling as indicated by the impairment of Sam68 phosphorylation, *CD44* exon v5 inclusion and, directly, ERK activation (Fig. 3). MV interference with ERK activation is, however, observed, late after exposure, indicating it occurs secondary to alterations of other pathways (Fig. 3C). This most likely includes targeting of basal PI3K activity because, interestingly, MAPK inhibition timely correlates with detectable accumulation levels of SIP110 protein (Fig. 1A) to which it may be causatively linked (see below). As shown for MV PI3K interference previously, inhibition of phorbolester-induced MAPK activation relies on the viral gp complex but not on CD150 which is not expressed on resting T cells (Fig. 3) (Schneider-Schaulies and ter Meulen, 2002; Sidorenko and Clark, 2003). As MAPK activation particularly in response to cytokines plays an important role for T cell viability and effector functions (Flavell *et al*., 2002), MV interference with these pathways would be expected to cause alterations of these cells. Similar as described for murine cells (Konig *et al*., 1998; Matter *et al*., 2002), MAPK activation was required for inclusion of *CD44* exon v5 both in the reporter assay and the *CD44* mRNA in human T cells (Fig. 3A and D). The ability of Sam68 to promote *CD44* exon v5 inclusion requires ERK activation, was, however, unaffected by ablation of its putative phosphorylation sites and thus, relevance of threonine phosphorylation (Matter *et al*., 2002), which was only indirectly evidenced by mobility shift of a small fraction in our system, is still unclear (Fig. 3B). Interestingly, also LY294004 prevented *CD44* exon v5 inclusion in the reporter gene assay suggesting that this can also be regulated by PI3K activation (Fig. 3A). In support of this, inclusion of exon v5 into the *CD44* transcript was seen in resting T cells transfected to express either constitutively active PI3K p110 or Akt kinase proteins (not shown), and thus MV interference with PI3K activation possibly also contributes to inhibition of *CD44* exon v5 inclusion. Surprisingly, however, treatment of primary human T cells with LY294004 largely failed to consistently affect PMA/ionomycin-stimulated inclusion of *CD44* exon v5 (not shown), indicating that regulation of this may be more complex. It was, however, beyond the scope of this study to address a potential species or stimulation-dependent regulation of exonic inclusion into *CD44* transcripts used as a model system here.

Although altered expression patterns of CD44 protein isoforms could be of functional importance to T cells as well, we focused our interest on the potential relevance of SHIP isoforms because MV is known to abolish activation of PI3K in response to TCR ligation, indicating that the phospholipids metabolism may be a preferential target in MV-induced T cells paralysis (Avota *et al*., 2001; 2004). SHIP145 is a central regulator of phospho-inositol-

Fig. 4. SIP110 associates with cell membrane clusters of unstimulated T cells.
A. RNA isolated from T cells exposed to WTF in the presence of FIP at moi of 1 for 24 h was used to RT-PCR amplify full-length SIP110 cDNA.
B. After cloning into pIRES-eGFP, pSIP110 or the empty vector were transfected into NIH 3T3 or primary T cells. SIP110 expression was analysed by immunoprecipitation followed by Western Blot analysis.
C. Localization of α-SHIP antibody reactive proteins in unstimulated pIRES-eGFP (upper left panels) or pSIP110-transfected (lower left panels) and non-transfected PMA/Ionomycin-stimulated (upper right panels) primary T cells or pSIP110-transfected Jurkat T cells (which do not express endogenous SHIP) (bottom right panels) was analysed by confocal microscopy. eGFP positive cells were selected for microscopy analysis. x→y, y→z and x→z sections as well as a 3D reconstruction (each panel, upper right picture) are shown. Scale bars represent each 2 μm.
D. SIP110 association with lipid rafts was analysed in pSIP110-transfected Jurkat cells using cholera toxin (CTx-Alexa Fluor 647) as a marker. The fluorescence data are shown in false colours (red for CTx and green for SIP110) to distinguish between dark red Alexa Fluor 647 and bright red Alexa Fluor 594 fluorochromes. Scale bars represent 5 μm.

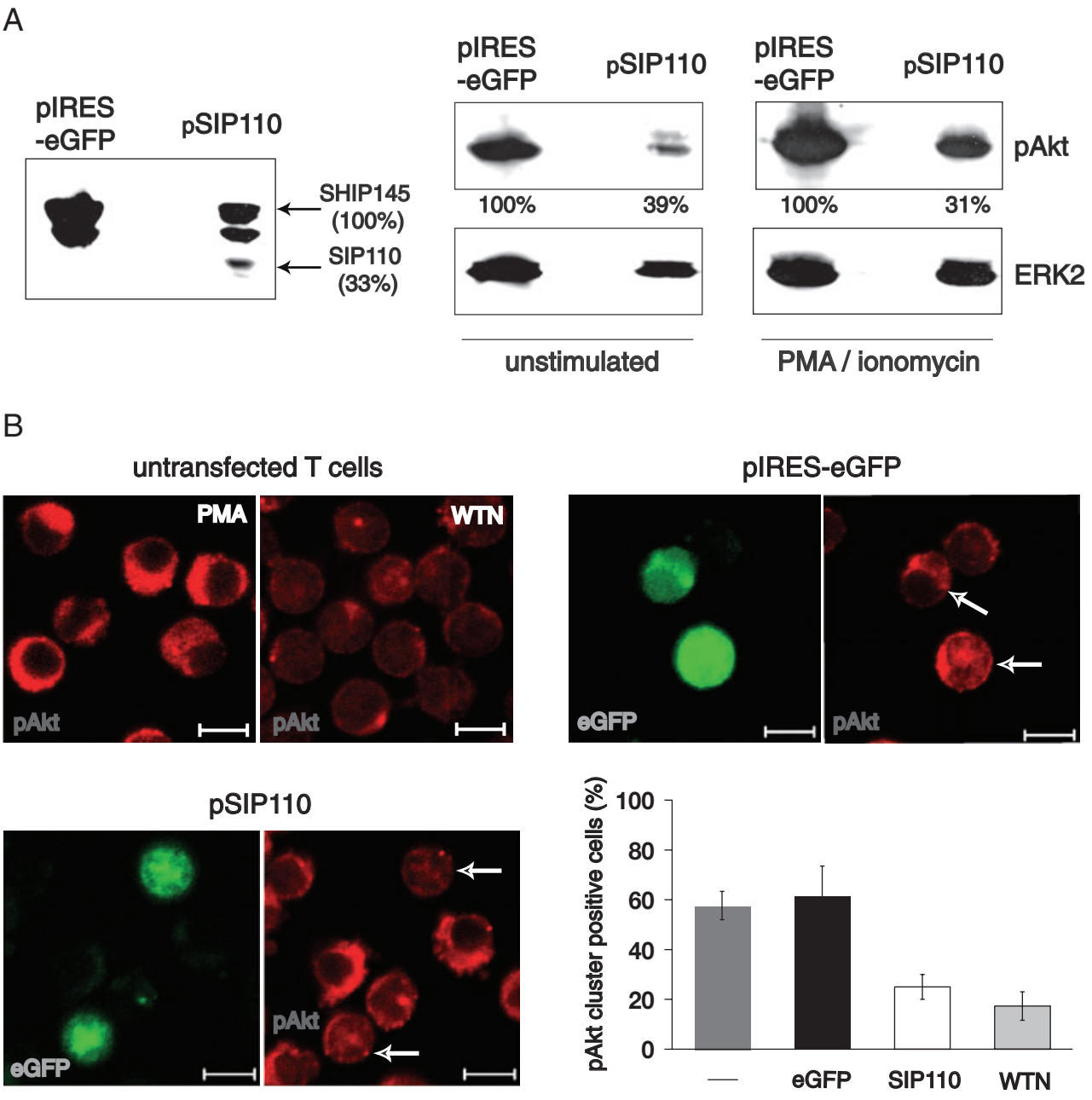
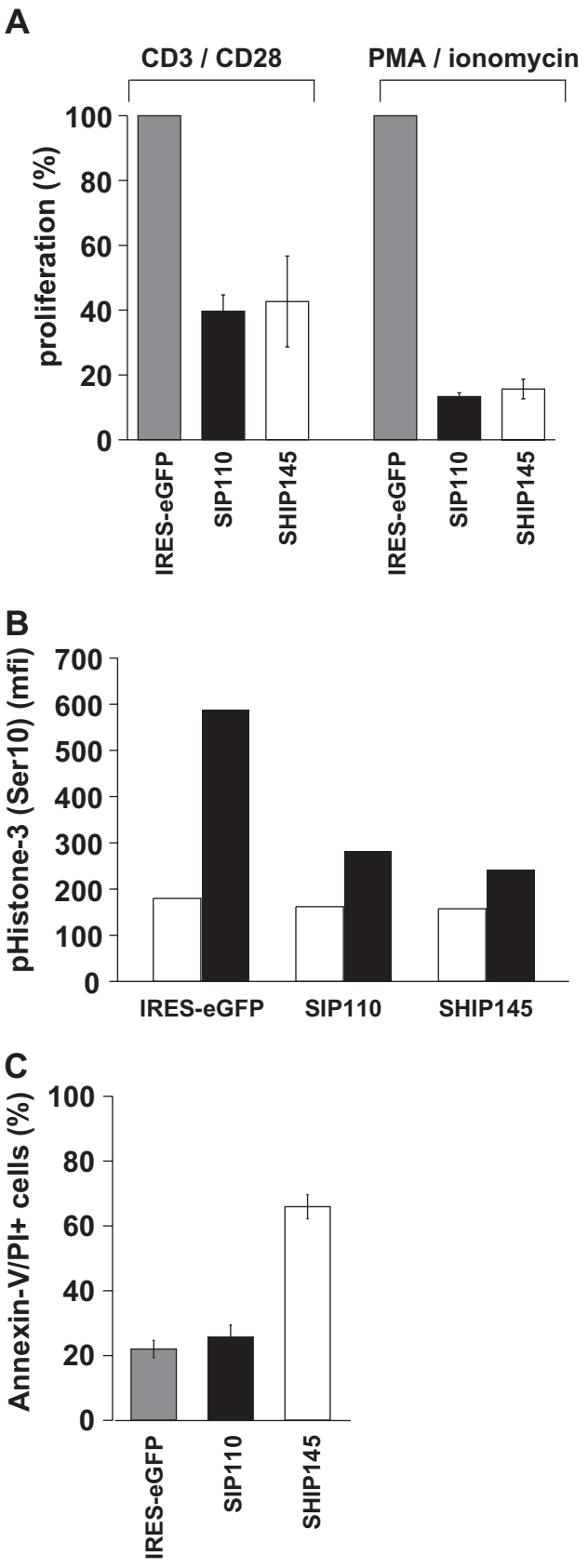


Fig. 5. Expression of SIP110 affects basal and stimulated activation of the Akt kinase.
A. Extracts were prepared from pIRES-eGFP or pSIP110 nucleofected primary T cells prior to (left and middle panels) or after (right panels) PMA/ionomycin activation and analysed for transfection efficiency (eGFP detection by FACS, 40% in pIRES-eGFP and 30% in pSIP110-transfected cultures), expression of SHIP protein isoforms [with SIP110 accumulating to 33% of SHIP135/145 levels as determined on Western blots using AIDA software (Raytest) (left panel)] and pAkt (middle and right panels). Detection of ERK2 served as protein loading control. pAkt levels were quantified using AIDA software and those determined for pSIP110 expressing cultures were expressed as percentage of the vector-transfected cultures (set to 100%) and normalized to ERK protein levels.
B. Primary T cells were left untransfected (upper left panels) or were nucleofected with pIRES-eGFP (upper right panels) or pSIP110 (lower left panels). Cells were PMA stimulated for 20 min [when indicated, pretreated with wortmannin (WTN) for 1 h] and analysed by confocal microscopy for pAkt expression. Scale bars represent 5 μm. Transfected cells were identified by eGFP fluorescence (indicated by arrows) and the percentages of pAkt cluster forming cells were determined (bottom right).



polyphosphate accumulation, and thereby PH domain containing proteins as predominantly involved in cell survival and both malignant and homeostatic proliferation (Rohrschneider *et al*., 2000; March and Ravichandran, 2002; Luo *et al*., 2003). It is generally considered to regulate threshold levels for activation. SHIP145 can induce T cell quiescence by activating Krüppel-like factor 2 (KLF2), but also apoptosis (Buckley *et al*., 2001; Valderrama-Carvajal *et al*., 2002; Wu and Lingrel, 2004; Garcia-Palma *et al*., 2005) and the latter may depend on its accumulation levels (Horn *et al*., 2004). In line with these observations, SHIP145 expression strongly reduced the amount of primary T cells entering mitosis after TCR ligation, yet, dose dependently, also increased the number of apoptotic cells (Fig. 6B and C), implicating that SHIP145 expression needs to be strictly balanced in primary cells. SHIP isoforms are expressed in haematopoetic precursor cells with increasingly larger electrophoretic mobility as differentiation proceeds (Rohrschneider *et al*., 2000). SHIP is recruited to activated receptors including CD150 via its N-terminal SH2 domain where it counteracts PI3K activity (Sidorenko and Clark, 2003). In contrast to what has been reported for B and NK cells (Phee *et al*., 2001; Aman *et al*., 2001; Galandrini *et al*., 2002), membrane association of SHIP in T cells does apparently not require TCR activation which rather induces formation of SHIP clusters, part of which colocalize with lipid rafts (Fig. 4D). As shown for its murine orthologue, s-SHIP, SIP110 membrane recruitment may also occur independently of cell activation and tyrosine phosphorylation, possibly as a result of its interaction with Grb-2 (Kavanaugh *et al*., 1996; Lucas and Rohrschneider, 1999; Rohrschneider *et al*., 2000; Tu *et al*., 2001; March and Ravichandran, 2002). As proposed for splice isoforms of SYK, Fyn and Pyk2, which also lack SH2 yet retain their catalytic domains (Dikic *et al*., 1998; Latour *et al*., 1998; Weil *et al*., 1999), s-SHIP was suggested to modulate the threshold for T cell acti-

Fig. 6. SIP110 expression interferes with T cell proliferation, but not viability.
A. ^{3}H-thymidine incorporation was determined 72 h after CD3/CD28 or PMA/ionomycin stimulation of pIRES-eGFP, pSIP110 and pSHIP145 nucleofected primary T cells. The results of three independent experiments (each performed in triplicates) using T cells from each different healthy donors are shown; values obtained for pIRES-eGFP-transfected cells were set to 100%. In all cultures, on average 30% of cells were GFP-positive.
B. The mean fluorescence intensity (mfi) of phospho-histone3 (Ser10)-expression (a G2 phase marker) was determined in T cells nucleofected with pIRES-eGFP, pSIP110 and pSHIP145 left unstimulated (white bars) or PMA/ionomycin stimulated for 72 h (black bars) by FACS analysis (one out of two experiments is shown).
C. The percentage of Annexin-V-PE positive cells was determined in pIRES-eGFP, pSIP110 or pSHIP145 (cotransfected to express GFP) expressing T cells after 48 h of nucleofection by FACS analysis of the respective GFP expressing populations (results shown were obtained in three independent experiments).

vation (Tu *et al*., 2001), and this may relate to SIP110 as well. First, SIP110 still has a functional 5′-phosphatase activity and thus might, if continually present in membrane clusters, alter the responsiveness of PI3K-driven signals as shown for chemokine-induced chemotaxis and for basal and TCR-dependent activation of Akt kinase phosphorylation (Wain *et al*., 2005 and Fig. 5). Second, its ability to sustain interactions with Grb-2 in resting T cells could interfere with activation-induced formation of Grb-2/SOS complexes and thereby downregulate the signalling via the Ras-ERK pathway. This was observed to occur for other Grb-2 binding proteins as well (Moeller *et al*., 2003; Zhou *et al*., 2004; Ozaki *et al*., 2005), which when overexpressed induced inhibition of cell growth and proliferation as did SIP110 in our system (Fig. 6). Remarkably, MV interference with ERK activation coincides with detection of SIP110 protein (Fig. 3).

In order to provide a paralysing environment in T cells, MV contact by itself would be expected to affect the homeostatic cell metabolism to alter threshold levels for or to entirely abolish activation. We have shown that, in the absence of activation, MV contact induces, by modulating basal PI3K activity and thereby possibly SR protein activity (Figs 2 and 5), SIP110 expression that constitutively associates with membrane clusters. As this requires synthesis and accumulation of SIP110 protein, it is not surprising that ERK activation, which can be targeted by SIP110 competition with Sos for Grb-2 can only be inhibited rather late (24 h) following MV contact (Fig. 3D). Interestingly, an expression of an isoform of the PI3K regulatory subunit p85, p50, is induced after MV contact in T cells (E. Avota and S. Schneider-Schaulies, unpublished) which may correspond to protein expressed from an alternatively spliced mRNA and thus represent another example for MV-regulated splicing in T cells.

Experimental procedures

Cells and viruses

Human peripheral blood mononuclear cells obtained after leukapheresis from healthy donors served as source for T cells which were isolated from sheep-erythrocyte complexes by erythrocyte lysis or were enriched on nylon wool columns. Jurkat clone E6-1, lymphoblastoid BJAB and primary T cells were kept in RPMI 1640 medium containing 10% fetal calf serum (FCS). NIH 3T3 cell were kept in MEM/5% FCS. MV wild-type strain WTF was propagated on BJAB cells, the attenuated Edmonston (ED) strain and MGV, a recombinant MV containing gp G of VSV instead of MV gps H and F on Vero cells (maintained in MEM/5% FCS). Purified viruses and mock preparations were obtained from infected BJAB or Vero cells, or uninfected BJAB or Vero cells, by sucrose gradient ultracentrifugation and were always used inactivated by UV irradiation (1.5 J per cm^2) prior to application onto T cells. Gp content of viruses was determined by Western Blot analysis using monospecific sera directed against the cytoplasmic tails of either F or H proteins (generated in our laboratory), and if not indicated otherwise, amounts of viruses used corresponded to a moi of 1 of live virus. To prevent MV entry and fusion, a FIP (0.2 mM) (Z-D-Phe-L-Phe-Gly-OH; Bachem) was added routinely to all cultures, also including the mock controls.

RT-PCR and cDNA cloning

Cytoplasmic RNA was isolated from 4×107 primary T cells with RNeasy Mini Kit (QIAGEN). Oligo-d(T) primed reverse transcription was performed on 2 μg RNA using Omniscript revertase (QIAGEN). For *CD44* v5 exon inclusion analysis, T cells were pretreated for 24 h with MV (WTF or ED), MGV, 50 μM LY200492 (New England Biolabs, Frankfurt, Germany) or 20 μM UO126 (Sigma) and subsequently stimulated with 40 ng ml^{-1} PMA (Calbiochem) and 0.5 μM ionomycin (Sigma) for further 24 h. For SIP110 mRNA detection and cloning primary T cells were exposed to WTF for 24 h prior to RNA isolation. PuReTaq Ready-To-Go PCR beads (Amersham Biosciences) were used to amplify *CD44* v5 and *SIP110* transcripts. Proofreading Phusions High-Fidelity polymerase (Finnzymes) was used to amplify full-length SIP110 cDNA. Primers used in exon-specific PCRs were as follows: CD44 v5, 5′-CACTGCTTATGAAGGAAAC-TGG-3′ (v5 exon specific) and 5′-TGTTCA-CCAAATGCACCATTTCC-3′ (exon c19 specific); CD44 c5, 5′-TTTACACCTTTTCTACTGTA CACC-3′ and exon c19 specific reverse primer; SIP110, 5′-GGATAATCCTTGATGTTCACCTTG-3′ and 5′-CCACCAAGAT CACCAA-CTTAT-TC-3′; SHIP145, 5′-G-GAGCTCTATG GAGAAGTCATC-3′ and 5′-CCACCAAGAT-CACCAACTTATTC-3′. Primers used in first-round PCR for full-length SIP110 cDNA amplification were: 5′-G-TGCCCACTAATCCTTGATGT-TCACCT-3′ and 5′-CAGC-TCACTGAGGGCTTCACTGCAT-3′. NheI and BamHI restrictions sites flanking 5′ and 3′ the SIP110 cDNA were added by a second-round PCR using following primers: 5′-C-AGTGCTAGCACAACCATGTTCACCTTGTCC-CCTG-3′ and 5′-CAGTGGATCCAGCTCACTGAGGGCTTCACTGCAT-3′. SIP110 cDNA was cloned in pIRES2-eGFP (Clontech) (further referred to as pIRES-eGFP) which allows for simultaneous expression of SIP110 and an IRES driven eGFP protein from a bicistronic mRNA. PCR conditions for exon inclusion studies were 30 s 94°C, 1 min 61°C and 2 min 68°C (35 cycles). SIP110 cDNA was synthesized by two step PCR amplification: 1 min 98°C, 5 s 98°C and 105 s 72°C (35 cycles) followed by 10 min 72°C. For second-round cDNA amplification: 5 s 98°C, 15 s 53°C and 90 s for 5 cycles; 5 s 98°C and 105 s 72°C (30 cycles). The amplified and cloned cDNA and was sequenced using ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Luciferase assays

CD44 variable exon 5 (v5) splice reporter plasmid containing luciferase gene pETv5luc (kindly provided by Harald König) was used to test splicing-dependent exon v5 inclusion in reporter mRNA of MV-treated primary T cells. Four hours following nucleofection with pETv5luc DNA (Matter *et al*., 2002) (kindly provided by H. König) (Amaxa) 1.5×10^7 primary T cells were exposed to UV-inactivated MV (at concentration as indicated) or mock preparations in the presence of FIP for 2 h and subsequently stimulated with PMA/ionomycin for 24 h. When indicated, cells were pretreated with LY200492 or UO126 as indicated above.

Luciferase expression was detected in total cell lysates according to standard procedures (Promega).

Antibodies and protein analysis

For extract preparation, 5×10^7 primary T cells were resuspended in ice cold 10 mM Hepes (pH 7.9), 10 mM KCl and 0.1 mM EDTA, left on ice for further 15 min, followed by passages through a syringe. After centrifugation at 6400 *g* for 15 s, cytoplasmic proteins were acetone precipitated from the supernatant, and sedimented nuclei were lysed in 20 mM Hepes (pH 7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA at 4°C for 30 min by vigorous shaking. When indicated, proteins were immunoprecipitated prior to PAGE separation and transferred onto nitrocellulose membranes and analysed using monoclonal antibodies specific for SR proteins (clone 16H3; Zytomed), pSR proteins (mAb104) [produced from B lymphocyte hybridoma cells (LGC Promochem)], SHIP145 (#P1C1) for both SHIP145 and SIP110 proteins, Sam68 (#C-20), ERK2 (#C-14) and a polyclonal serum (#C-20) for Akt (all: Santa Cruz). Phospho-Akt (Ser473) and phospho ERK1,2 (Thr202/Tyr204)-specific antibodies were obtained from New England Biolabs. Following detection by secondary HRP-conjugated goat anti-rabbit (New England Biolabs) or goat anti-mouse (Dianova) antibodies and ECL (Amersham) development, signals were quantified using AIDA software (Raytest).

T cell proliferation and viability assays

Primary T cells were nucleofected with pIRES2-eGFP, pSIP110-eGFP and pSHIP-myc (kindly provided by M. Tomlinson, University of Birmingham, UK) (Amaxa) and stimulated by PMA/ionomycin or by CD3 and CD28 ligation using anti-CD3 (UCHT1), anti-CD28 (BD Biosciences Pharmingen) 1 μg each per 5×10^6 T cells and plate bound anti-mouse (Dianova) antibodies for 72 h. Proliferation was determined after a 16 h ^{3}H-thymidine pulse in triplicates. Alternatively, cells were fixed in 2% formaldehyde, washed in PBS, chilled on ice for 1 min, resuspended gently in ice-cold methanol (final concentration 90%) and kept at −20°C for at least 24 h before analysing G2 phase cells by using a phospho-histone H3 (pS10) specific Alexa Fluor 647 conjugated antibody (New England Biolabs) by flow cytometry. Apoptosis was analysed in sorted GFP + T cells by propidiumjodid (PI) and 7AAD staining by flow cytometry (FACSCALIBUR).

Confocal microscopy

Jurkat E6-1 cells and primary T cells nucleofected with pSIP110-eGFP (Amaxa) or untransfected primary T cells stimulated with PMA/ionomycin for 20 min were seeded onto poly-L-lysine (Sigma) coated glass slides for 20 min at 4°C. After fixation, cells were permeabilized with 0.33% Saponin and incubated with anti-SHIP antibody (1:50; #P1C1, Santa Cruz), followed by detection with Alexa 594 anti-mouse IgG (Molecular Probes). Each 30 confocal images were acquired per cell treatment from two independent experiments using an inverted microscope (Zeiss LSM META) with an objective plan hypochromat 100×, 1,4 oil and a HENE1 laser, 543 nm. Cells were sliced in 12 sections, each approximately at 0.48 μm intervals. Three-dimensional reconstruction of SHIP145 or SIP110 protein localization in T cells was performed using LSM-510 software (Carl Zeiss MicroImaging). Lipid raft structures were stained by cholera toxin and fluorochrome conjugated CTx-Alexa Fluor 647 (Molecular Probes, 1:20). pAkt (Ser473) was detected in primary T cells exposed to PMA or wortmannin (New England Biolabs) by a rabbit monoclonal antibody (193H12, New England Biolabs, 1:100) followed by an Alexa Fluor 594 conjugated anti-rabbit IgG (Molecular Probes). Twenty-five confocal images each containing two to three transfected cells (detected by eGFP fluorescence) were analysed for pAkt cluster presence.

Acknowledgements

We thank Jürgen Schneider-Schaulies and Paul Duprex for helpful discussions and critical reading of the manuscript, Harald König and Mike Tomlinson for providing pERTv5luc and pSHIP145 respectively, Maren Klett and Beatrix Loth for excellent technical assistance and the Deutsche Forschungsgemeinschaft for funding.

References

Aman, M.J., Tosello-Trampont, A.C., and Ravichandran, K. (2001) Fc gamma RIIB1/SHIP-mediated inhibitory signaling in B cells involves lipid rafts. *J Biol Chem* **276:** 46371–46378.

Avota, E., Avots, A., Niewiesk, S., Kane, L.P., Bommhardt, U., ter Meulen, V., and Schneider-Schaulies, S. (2001) Disruption of Akt kinase activation is important for immunosuppression induced by measles virus. *Nat Med* **7:** 725–731.

Avota, E., Muller, N., Klett, M., and Schneider-Schaulies, S. (2004) Measles virus interacts with and alters signal transduction in T-cell lipid rafts. *J Virol* **78:** 9552–9559.

Borrow, P., and Oldstone, M.B. (1995) Measles virus–mononuclear cell interactions. *Curr Top Microbiol Immunol* **191:** 85–100.

Bourgeois, C.F., Lejeune, F., and Stevenin, J. (2004) Broad specificity of SR (serine/arginine) proteins in the regulation of alternative splicing of pre-messenger RNA. *Prog Nucleic Acid Res Mol Biol* **78:** 37–88.

Buckley, A.F., Kuo, C.T., and Leiden, J.M. (2001) Transcription factor LKLF is sufficient to program T cell quiescence via a c-Myc-dependent pathway. *Nat Immunol* **2:** 698–704.

Chalfant, C.E., Ogretmen, B., Galadari, S., Kroesen, B.J., Pettus, B.J., and Hannun, Y.A. (2001) FAS activation induces dephosphorylation of SR proteins; dependence on the de novo generation of ceramide and activation of protein phosphatase 1. *J Biol Chem* **276:** 44848–44855.

Chalfant, C.E., Rathman, K., Pinkerman, R.L., Wood, R.E., Obeid, L.M., Ogretmen, B., and Hannun, Y.A. (2002) De novo ceramide regulates the alternative splicing of caspase 9 and Bcl-x in A549 lung adenocarcinoma cells. Dependence on protein phosphatase-1. *J Biol Chem* **277:** 12587–12595.

Clements, C.J., and Cutts, F.T. (1995) The epidemiology of measles: thirty years of vaccination. *Curr Top Microbiol Immunol* **191:** 13–33.

Cowper, A.E., Caceres, J.F., Mayeda, A., and Screaton, G.R. (2001) Serine-arginine (SR) protein-like factors that antag-

onize authentic SR proteins and regulate alternative splicing. *J Biol Chem* **276:** 48908–48914.
Dikic, I., Dikic, I., and Schlessinger, J. (1998) Identification of a new Pyk2 isoform implicated in chemokine and antigen receptor signaling. *J Biol Chem* **273:** 14301–14308.
Dubois, B., Lamy, P.J., Chemin, K., Lachaux, A., and Kaiserlian, D. (2001) Measles virus exploits dendritic cells to suppress CD4+ T-cell proliferation via expression of surface viral glycoproteins independently of T-cell trans-infection. *Cell Immunol* **214:** 173–183.
Flavell, R.A., Dong, C., and Davis, R.J. (2002) Signaling and cell death in lymphocytes. *Inflamm Res* **51:** 80–82.
Galandrini, R., Tassi, I., Mattia, G., Lenti, L., Piccoli, M., Frati, L., and Santoni, A. (2002) SH2-containing inositol phosphatase (SHIP-1) transiently translocates to raft domains and modulates CD16-mediated cytotoxicity in human NK cells. *Blood* **100:** 4581–4589.
Garcia-Palma, L., Horn, S., Haag, F., Diessenbacher, P., Streichert, T., Mayr, G.W., and Jucker, M. (2005) Up-regulation of the T cell quiescence factor KLF2 in a leukaemic T-cell line after expression of the inositol 5′-phosphatase SHIP-1. *Br J Haematol* **131:** 628–631.
Geier, S.J., Algate, P.A., Carlberg, K., Flowers, D., Friedman, C., Trask, B., and Rohrschneider, L.R. (1997) The human SHIP gene is differentially expressed in cell lineages of the bone marrow and blood. *Blood* **89:** 1876–1885.
Graveley, B.R. (2000) Sorting out the complexity of SR protein functions. *RNA* **6:** 1197–1211.
Graveley, B.R. (2005) Small molecule control of pre-mRNA splicing. *RNA* **11:** 355–358.
Graveley, B.R., Hertel, K.J., and Maniatis, T. (1999) SR proteins are 'locators' of the RNA splicing machinery. *Curr Biol* **9:** R6–R7.
Hahm, B., Arbour, N., and Oldstone, M.B. (2004) Measles virus interacts with human SLAM receptor on dendritic cells to cause immunosuppression. *Virology* **323:** 292–302.
Horn, S., Endl, E., Fehse, B., Weck, M.M., Mayr, G.W., and Jucker, M. (2004) Restoration of SHIP activity in a human leukemia cell line downregulates constitutively activated phosphatidylinositol 3-kinase/Akt/GSK-3beta signaling and leads to an increased transit time through the G1 phase of the cell cycle. *Leukemia* **18:** 1839–1849.
Huang, T.S., Nilsson, C.E., Punga, T., and Akusjarvi, G. (2002) Functional inactivation of the SR family of splicing factors during a vaccinia virus infection. *EMBO Rep* **3:** 1088–1093.
Kanj, S.S., Dandashi, N., El-Hed, A., Harik, H., Maalouf, M., Kozhaya, L., *et al.* (2006) Ceramide regulates SR protein phosphorylation during adenoviral infection. *Virology* **345:** 280–289.
Kavanaugh, W.M., Pot, D.A., Chin, S.M., Deuter-Reinhard, M., Jefferson, A.B., Norris, F.A., *et al.* (1996) Multiple forms of an inositol polyphosphate 5-phosphatase form signaling complexes with Shc and Grb2. *Curr Biol* **6:** 438–445.
Kerdiles, Y.M., Sellin, C.I., Druelle, J., and Horvat, B. (2006) Immunosuppression caused by measles virus: role of viral proteins. *Rev Med Virol* **16:** 49–63.
Konig, H., Ponta, H., and Herrlich, P. (1998) Coupling of signal transduction to alternative pre-mRNA splicing by a composite splice regulator. *EMBO J* **17:** 2904–2913.
Latour, S., Zhang, J., Siraganian, R.P., and Veillette, A. (1998) A unique insert in the linker domain of Syk is necessary for its function in immunoreceptor signalling. *EMBO J* **17:** 2584–2595.
Liu, X., Mayeda, A., Tao, M., and Zheng, Z.M. (2003) Exonic splicing enhancer-dependent selection of the bovine papillomavirus type 1 nucleotide 3225 3′ splice site can be rescued in a cell lacking splicing factor ASF/SF2 through activation of the phosphatidylinositol 3-kinase/Akt pathway. *J Virol* **77:** 2105–2115.
Lucas, D.M., and Rohrschneider, L.R. (1999) A novel spliced form of SH2-containing inositol phosphatase is expressed during myeloid development. *Blood* **93:** 1922–1933.
Lukong, K.E., and Richard, S. (2003) Sam68, the KH domain-containing superSTAR. *Biochim Biophys Acta* **1653:** 73–86.
Luo, J.M., Yoshida, H., Komura, S., Ohishi, N., Pan, L., Shigeno, K., *et al.* (2003) Possible dominant-negative mutation of the SHIP gene in acute myeloid leukemia. *Leukemia* **17:** 1–8.
Lynch, K.W. (2004) Consequences of regulated pre-mRNA splicing in the immune system. *Nat Rev Immunol* **4:** 931–940.
March, M.E., and Ravichandran, K. (2002) Regulation of the immune response by SHIP. *Semin Immunol* **14:** 37–47.
Matter, N., Herrlich, P., and Konig, H. (2002) Signal-dependent regulation of splicing via phosphorylation of Sam68. *Nature* **420:** 691–695.
Moeller, S.J., Head, E.D., and Sheaff, R.J. (2003) p27Kip1 inhibition of GRB2-SOS formation can regulate Ras activation. *Mol Cell Biol* **23:** 3735–3752.
Niewiesk, S., Eisenhuth, I., Fooks, A., Clegg, J.C., Schnorr, J.J., Schneider-Schaulies, S., and ter Meulen, V. (1997) Measles virus-induced immune suppression in the cotton rat (*Sigmodon hispidus*) model depends on viral glycoproteins. *J Virol* **71:** 7214–7219.
Ozaki, K., Miyazaki, S., Tanimura, S., and Kohno, M. (2005) Efficient suppression of FGF-2-induced ERK activation by the cooperative interaction among mammalian Sprouty isoforms. *J Cell Sci* **118:** 5861–5871.
Patel, N.A., Chalfant, C.E., Watson, J.E., Wyatt, J.R., Dean, N.M., Eichler, D.C., and Cooper, D.R. (2001) Insulin regulates alternative splicing of protein kinase C beta II through a phosphatidylinositol 3-kinase-dependent pathway involving the nuclear serine/arginine-rich splicing factor, SRp40, in skeletal muscle cells. *J Biol Chem* **276:** 22648–22654.
Patel, N.A., Kaneko, S., Apostolatos, H.S., Bae, S.S., Watson, J.E., Davidowitz, K., *et al.* (2005) Molecular and genetic studies imply Akt-mediated signaling promotes protein kinase CbetaII alternative splicing via phosphorylation of serine/arginine-rich splicing factor SRp40. *J Biol Chem* **280:** 14302–14309.
Phee, H., Rodgers, W., and Coggeshall, K.M. (2001) Visualization of negative signaling in B cells by quantitative confocal microscopy. *Mol Cell Biol* **21:** 8615–8625.
Rohrschneider, L.R., Fuller, J.F., Wolf, I., Liu, Y., and Lucas, D.M. (2000) Structure, function, and biology of SHIP proteins. *Genes Dev* **14:** 505–520.
Schneider-Schaulies, S., and ter Meulen, V. (2002) Measles virus and immunomodulation: molecular bases and perspectives. *Expert Rev Mol Med* **2002:** 1–18.
Schneider-Schaulies, S., Schneider-Schaulies, J., Niewiesk, S., and Ter Meulen, V. (2002) Measles virus: immunomodulation and cell tropism as pathogenicity determinants. *Med Microbiol Immunol (Berl)* **191:** 83–87.
Schneider-Schaulies, S., Klagge, I.M., and ter Meulen, V. (2003) Dendritic cells and measles virus infection. *Curr Top Microbiol Immunol* **276:** 77–101.

Schnorr, J.J., Seufert, M., Schlender, J., Borst, J., Johnston, I.C., ter Meulen, V., and Schneider-Schaulies, S. (1997) Cell cycle arrest rather than apoptosis is associated with measles virus contact-mediated immunosuppression in vitro. *J Gen Virol* **78:** 3217–3226.

Sciabica, K.S., Dai, Q.J., and Sandri-Goldin, R.M. (2003) ICP27 interacts with SRPK1 to mediate HSV splicing inhibition by altering SR protein phosphorylation. *EMBO J* **22:** 1608–1619.

Servet-Delprat, C., Vidalain, P.O., Bausinger, H., Manie, S., Le Deist, F., Azocar, O., *et al.* (2000) Measles virus induces abnormal differentiation of CD40 ligand-activated human dendritic cells. *J Immunol* **164:** 1753–1760.

Servet-Delprat, C., Vidalain, P.O., Valentin, H., and Rabourdin-Combe, C. (2003) Measles virus and dendritic cell functions: how specific response cohabits with immunosuppression. *Curr Top Microbiol Immunol* **276:** 103–123.

Shen, H., and Green, M.R. (2004) A pathway of sequential arginine-serine-rich domain-splicing signal interactions during mammalian spliceosome assembly. *Mol Cell* **16:** 363–373.

Shingai, M., Inoue, N., Okuno, T., Okabe, M., Akazawa, T., Miyamoto, Y., *et al.* (2005) Wild-type measles virus infection in human CD46/CD150-transgenic mice: CD11c-positive dendritic cells establish systemic viral infection. *J Immunol* **175:** 3252–3261.

Sidorenko, S.P., and Clark, E.A. (2003) The dual-function CD150 receptor subfamily: the viral attraction. *Nat Immunol* **4:** 19–24.

Tatsuo, H., and Yanagi, Y. (2002) The morbillivirus receptor SLAM (CD150). *Microbiol Immunol* **46:** 135–142.

Tu, Z., Ninos, J.M., Ma, Z., Wang, J.W., Lemos, M.P., Desponts, C., *et al.* (2001) Embryonic and hematopoietic stem cells express a novel SH2-containing inositol 5′-phosphatase isoform that partners with the Grb2 adapter protein. *Blood* **98:** 2028–2038.

Valderrama-Carvajal, H., Cocolakis, E., Lacerte, A., Lee, E.H., Krystal, G., Ali, S., and Lebrun, J.J. (2002) Activin/TGF-beta induce apoptosis through Smad-dependent expression of the lipid phosphatase SHIP. *Nat Cell Biol* **4:** 963–969.

Wain, C.M., Westwick, J., and Ward, S.G. (2005) Heterologous regulation of chemokine receptor signaling by the lipid phosphatase SHIP in lymphocytes. *Cell Signal* **17:** 1194–1202.

Weg-Remers, S., Ponta, H., Herrlich, P., and Konig, H. (2001) Regulation of alternative pre-mRNA splicing by the ERK MAP-kinase pathway. *EMBO J* **20:** 4194–4203.

Weil, R., Levraud, J.P., Dodon, M.D., Bessia, C., Hazan, U., Kourilsky, P., and Israel, A. (1999) Altered expression of tyrosine kinases of the Src and Syk families in human T-cell leukemia virus type 1-infected T-cell lines. *J Virol* **73:** 3709–3717.

Welstead, G.G., Iorio, C., Draker, R., Bayani, J., Squire, J., Vongpunsawad, S., *et al.* (2005) Measles virus replication in lymphatic cells and organs of CD150 (SLAM) transgenic mice. *Proc Natl Acad Sci USA* **102:** 16415–16420.

Wu, J., and Lingrel, J.B. (2004) KLF2 inhibits Jurkat T leukemia cell growth via upregulation of cyclin-dependent kinase inhibitor p21WAF1/CIP1. *Oncogene* **23:** 8088–8096.

Yanagi, Y., Ono, N., Tatsuo, H., Hashimoto, K., and Minagawa, H. (2002) Measles virus receptor SLAM (CD150). *Virology* **299:** 155–161.

Yurchenko, M.Y., Kashuba, E.V., Shlapatska, L.M., Sivkovich, S.A., and Sidorenko, S.P. (2005) The role of CD150–SH2D1A association in CD150 signaling in Hodgkin's lymphoma cell lines. *Exp Oncol* **27:** 24–30.

Zhou, D., Chen, B., Ye, J.J., and Chen, S. (2004) A novel crosstalk mechanism between nuclear receptor-mediated and growth factor/Ras-mediated pathways through PNRC–Grb2 interaction. *Oncogene* **23:** 5394–5404.