1 **Modulation of IP₃ receptor stability and its implication in**
2 **cell death and disease**

3 *Bruno Seitaj, Geert Bultynck[#], Hristina Ivanova*

4 KU Leuven, Laboratory of Molecular & Cellular Signaling, Dep. Cellular and Molecular Medicine,
5 Campus Gasthuisberg O/N-I bus 802, Herestraat 49, B-3000 Leuven, Belgium

6 [#] correspondence to: Geert Bultynck; tel: +32-16-330215; email: geert.bultynck@kuleuven.be

Abstract

Ca^{2+} is a ubiquitous and versatile second messenger, which is important for processes like cell death and survival. Intracellular Ca^{2+} dynamics are tightly controlled by several Ca^{2+} -transport systems. The main intracellular Ca^{2+} -release channel is inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R), which is responsible for the Ca^{2+} flux from the endoplasmic reticulum. IP_3R dysfunction has been implicated in various pathological conditions including cancer and neurodegenerative diseases. Hence, IP_3Rs function at the common cross-point of different pro-survival and cell-death signaling pathways and are targeted by several proteins. A number of protein modifications directly impact IP_3R gating and thus Ca^{2+} -flux properties. Yet, several regulating mechanisms emerged to regulate total IP_3R levels by altering IP_3R stability and turnover through proteasomal degradation and protease cleavage. In the present review, we will highlight the mechanisms and the impact of (i) IP_3R ubiquitination and the following proteasomal degradation and (ii) IP_3R cleavage by two major classes of proteases, namely caspases and calpains.

Intracellular Ca²⁺ signaling: IP₃R as a key player

Ca²⁺ plays a fundamental role in a plethora of physiological processes, including secretion, muscle contraction, mitochondrial bioenergetics, cell proliferation and cell death in virtually all cell types (Berridge et al., 2000; Clapham, 2007). These divergent functions of Ca²⁺ signals are governed by the complex spatiotemporal organization. Ca²⁺ signals can appear as transient Ca²⁺ elevations (such as pro-survival Ca²⁺ oscillations, which boost mitochondrial energetics) or as sustained Ca²⁺ elevations (such as pro-apoptotic Ca²⁺ events) (Berridge, 2007; Berridge et al., 2003). Furthermore, Ca²⁺ rises can propagate throughout the cell or they can occur in localized microdomains (La Rovere et al., 2016). Ca²⁺ microdomains result from the apposition of different intracellular Ca²⁺ stores, like endoplasmic reticulum (ER), mitochondria, lysosomes, etc or of these organelles with the plasma membrane (Raffaello et al., 2016).

The intracellular Ca²⁺ homeostasis is tightly modulated by the interplay between a variety of Ca²⁺ transport systems, Ca²⁺ binding proteins and their regulators. The majority of intracellular Ca²⁺ is accumulated in the ER, where it is buffered by Ca²⁺-binding proteins like calreticulin, calnexin and calsequestrin (Prins and Michalak, 2011). Ca²⁺ is transported into the ER by the sarco/endoplasmic reticulum Ca²⁺ ATPase (Vandecaetsbeek et al., 2011). This ER Ca²⁺ uptake is counteracted by Ca²⁺-leak and -release mechanisms. Upon cell stimulation with hormones, neurotransmitters or antibodies, Ca²⁺ is mobilized and released into the cytosol through inositol 1,4,5-trisphosphate (IP₃) receptors (IP₃Rs) (Fedorenko et al., 2014). The ubiquitously expressed IP₃Rs function as tetrameric channels that mediate Ca²⁺ fluxes from the ER upon activation by IP₃ (Foskett et al., 2007; Mikoshiba, 2015). In addition to the ER, IP₃Rs are also present in the nuclear envelope, Golgi apparatus, secretory vesicles and even the plasma membrane (Taylor et al., 1999; Thillaiappan et al., 2017). At the level of the ER, IP₃Rs are not equally distributed and depending on their exact localization and the strength of their stimulation, different events might be observed. IP₃Rs can form clusters, where the activation of a single channel (blips) (Parker et al., 1996) can result in Ca²⁺ induced Ca²⁺ release by activating the neighboring channels (puffs) (Callamaras et al., 1998; Yao et al., 1995) or in Ca²⁺ waves propagating through the cell between the different IP₃R clusters (Dawson et al., 1999).

Cytosolic Ca²⁺ rises can promote: i) cell survival and proliferation by modulating gene transcription controlled by the calcineurin/nuclear factor of activated T cells (NFAT) signaling (Crabtree and Schreiber, 2009; Roderick and Cook, 2008), ii) autophagy by activating CAMKK β (Woods et al., 2005) or iii) cell death by activating the cell-death executioners caspases and calpains (Blomgren et al., 2001; Harwood et al., 2005; Squier et al., 1994). Ca²⁺ can also accumulate in different organelles, including mitochondria and lysosomes. In fact, the ER, mitochondria and lysosomes represent Ca²⁺-signaling networks through microdomains formed by contact sites (La Rovere et al., 2016; Raffaello et al., 2016). As such, Ca²⁺ release of the ER leads in a rapid increase in mitochondrial Ca²⁺ levels, on the one hand dampening cytosolic Ca²⁺ rises and on the other hand fueling the mitochondria with an essential co-factor for several mitochondrial enzymes involved in the TCA cycle and oxidative phosphorylation (Rizzuto et al., 2012). Yet, Ca²⁺ overloading in the mitochondria results in loss of mitochondrial membrane integrity, involving the opening of the mitochondrial permeability transition pore either indirectly via ROS produced by disintegration of the complex II (Hwang et al., 2014) or directly by Ca²⁺ binding to the F₀F₁-type ATPase dimers, the molecular identity of the pore (Giorgio et al., 2017). Also, lysosomes emerged as important Ca²⁺-signaling organelles. Lysosomes can sequester Ca²⁺ released from the ER (Lopez-Sanjurjo et al., 2013), whereas Ca²⁺ released from lysosomes (and endosomes) can trigger global Ca²⁺ mobilization from ER Ca²⁺ stores (Kilpatrick et al., 2016).

A fraction of the IP₃R population localizes at the contact sites between the ER and mitochondria where together with the voltage-dependent anion channels (VDACs) at the outer mitochondrial membrane and the mitochondrial Ca²⁺ uniporter (MCU) at the inner mitochondrial membrane, facilitate the “quasi-synaptic” ER to mitochondria Ca²⁺ transfer (Csordas et al., 1999; Raffaello et al., 2016; Rizzuto et al., 1993). Basal IP₃R activity results in Ca²⁺ oscillations, which promote mitochondrial functioning and drive NADH and ATP production (Cardenas et al., 2010; Cardenas et al., 2016; Foskett et al., 2007). Inhibition of IP₃R activity results in decreased Ca²⁺ supply to the mitochondria, reduced ATP levels and activation of AMPK which triggers autophagy (Cardenas et al., 2010). IP₃Rs not only sustain cells’ mitochondrial bio-energetics but also establish cells’ susceptibility to engage apoptosis upon cell damage and cell stress. Furthermore, the action of several cell-death inducing agents, including H₂O₂, ceramide and several anti-cancer drugs, is mediated through Ca²⁺ mobilization from the ER (Bittremieux et al., 2016; Kerkhofs et al., 2017; Marchi et al., 2017a; Parra et al., 2013; Pinton et al., 2001).

IP₃R isoforms: structure and regulation

IP₃Rs are encoded by three different genes, ITPR1, ITPR2 and ITPR3, resulting in the expression of three isoforms, named IP₃R1, IP₃R2 and IP₃R3. They share 60-80% homology in the amino acid sequence. In addition, alternative splicing variants exist, in particular with IP₃R1 having three splice sites (Foskett; Mikoshiba, 2007). IP₃R isoforms can assemble into homo- or hetero-tetrameric channel complexes and most of the cells express more than one isoform. Nevertheless, very often a particular isoform is predominantly present (Monkawa et al., 1995). This is the case with IP₃R1 in neuronal cells, IP₃R2 in liver and muscle cells and IP₃R3 in most cultured cell types (Ivanova et al., 2014).

IP₃R1, IP₃R2 and IP₃R3 share similar structure with each monomer consisting of N-terminal ligand-binding domain (LBD), which consists of a suppressor domain (a.a. 1-225 according to the mouse IP₃R1 sequence) and an IP₃-binding core (a.a. 226-604 according to the mouse IP₃R1 sequence), followed by a central, modulatory domain where multiple regulatory factors bind and regulate the channel activity and finally a C-terminal domain containing 6 trans-membrane segments and a cytosolic tail. The channel pore is formed by the intervening luminal loops located between the 5th and the 6th trans-membrane segments (Baker et al., 2017; Fan et al., 2015; Michikawa et al., 1994; Sudhof et al., 1991; Yoshikawa et al., 1996; Yule et al., 2010). Important structural insights were gained by studying IP₃R1, the most abundant and thereby the best characterized isoform. Recently, the crystal structure of this isoform in absence of IP₃ was determined by a high-resolution cryo-electron microscopy (4.7 Å) (Fan et al., 2015; Hamada et al., 2017). This was a critical step in studying the functioning of IP₃R, since it revealed that the cytosolic C-terminus directly interacts with the N-terminal LBD of adjacent subunits (Fan et al., 2015), which interaction is disturbed by conformational change in response to IP₃ binding to the LBD. Recently, this model has been refined and it was proposed that the conformational changes that occur upon IP₃ binding are transmitted to the channel pore through a unique and distally located in the modulatory domain leaflet structure (Hamada et al., 2017).

Among the key functional regulators of IP₃Rs are its ligand IP₃, Ca²⁺ itself, ATP, various proteins as well as different post-translational modifications, including phosphorylation, glycosylation, ubiquitination, thiol-modifications, etc. (Booth et al., 2016; Bultynck et al., 2004; Foskett et al., 2007; Ivanova et al., 2014; Khan et al., 2013; Vanderheyden et al., 2009).

Binding of IP₃ to the IP₃-binding core is required for channel activation and IP₃Rs deficient for IP₃ binding failed to mediate Ca²⁺ release. The suppressor domain also plays a significant role, since its removal resulted in uncoupling of IP₃ binding from channel opening (Uchida et al., 2003). The suppressor domain was proposed to mediate the transfer of the conformational changes, which result from IP₃ binding, to the pore opening (Rossi et al., 2009). Furthermore

the suppressor domain was shown to be part of the region interacting with residues close to the channel pore (Boehning and Joseph, 2000). The three IP₃R isoforms are characterized by different sensitivity towards IP₃ with IP₃R2 being the most sensitive, followed by IP₃R1 and finally IP₃R3 being the least sensitive isoform (Tu et al., 2005b). Additional layer of diversity in the Ca²⁺ signals is achieved by the formation of IP₃R heterotetramers. Very recently, by using concatenated IP₃Rs, it was shown that IP₃-deficient monomer-subunits acted in a dominant-negative manner over wild-type monomers. In that way the stoichiometry of IP₃ binding required for initiation of Ca²⁺ release was defined and it was demonstrated that all 4 monomers need to be occupied by IP₃ in order to achieve proper channel activation (Alzayady et al., 2016; Taylor and Konieczny, 2016). A previous study aimed to examine the contribution of individual isoforms within an IP₃R heterotetramer toward shaping the Ca²⁺ signals and it demonstrated that the IP₃ sensitivity of IP₃R heterotetramers is much more complex than a simple sum of the sensitivity of the individual monomers (Chandrasekhar et al., 2016). IP₃R heterotetramers displayed “blended” sensitivity towards IP₃, where the individual monomers contributed equally to the IP₃ sensitivity (Alzayady et al., 2013b; Chandrasekhar et al., 2016). Recently, more insights on the spatiotemporal characteristics of initial Ca²⁺ signals in response to agonists have been obtained, indicating that Ca²⁺ signals are initiated by immobile IP₃Rs that are located close to the plasma membrane (Thillaiappan et al., 2017).

Cytosolic Ca²⁺ was found to exert both, stimulatory and inhibitory effect on IP₃R function. Such a bell-shaped dependence with low cytosolic Ca²⁺ levels having stimulatory and high cytosolic Ca²⁺ levels having inhibitory effect (Bezprozvanny et al., 1991; Finch et al., 1991; Iino, 1990) was described in all three IP₃R isoforms, but with isoform-specific characteristics (Mak et al., 2001; Miyakawa et al., 1999; Tu et al., 2005a). In contrast to IP₃, multiple Ca²⁺-binding sites were described, some of which appeared conserved among the different isoforms (Sienaert et al., 1996; Sienaert et al., 1997). The exact mechanism and mechanistic role of these sites remains elusive, since different groups described different properties: either inhibition or activation of the receptor upon Ca²⁺ binding was observed (Mak et al., 1998; Marchant and Taylor, 1997). The ER Ca²⁺ content also modulates IP₃R function. Lower ER Ca²⁺ level results in lower sensitivity of the channel to IP₃ (Missiaen et al., 1992; Parys et al., 1993).

Although ATP was not sufficient by itself to activate IP₃R, it displayed stimulatory effect when present in submillimolar concentrations, increasing the frequency of the channel opening triggered by IP₃ (Bezprozvanny and Ehrlich, 1993; Missiaen et al., 1997). The binding occur in the modulatory domain of the three different isoforms. In contrast, when present at higher concentrations, ATP inhibited the channel activity (Bezprozvanny and Ehrlich, 1993; Missiaen et al., 1997). The inhibition was proposed to occur due to inhibition of IP₃ binding by the high concentrations of ATP (Yule et al., 2010). Similarly to the sensitivity to IP₃, the 3 isolated isoforms have different sensitivity to ATP with IP₃R2 being again the most sensitive isoform (Betzenhauser et al., 2008). Interestingly, the same isoform is affected by ATP only at submaximal concentration of IP₃, while the activity of IP₃R1 and IP₃R3 is modulated by ATP in the presence of different concentrations of IP₃ (Yule et al., 2010). Furthermore, the mode of ATP regulation appeared to be determined by IP₃R2 irrespectively of the presence of another isoform (Alzayady et al., 2013b; Chandrasekhar et al., 2016).

IP₃R ubiquitination and proteasomal degradation

Ubiquitin–proteasome pathway (UPP)

Two major pathways for degradation of proteins exist. While autophagy is responsible for the degradation of the long living or aggregated proteins, the UPP is involved in the degradation of the majority of cellular proteins. The proteins that undergo UPP are a subject of a post-translational modification, during which the 8-kDa ubiquitin molecule is added to the targeted protein. This process might occur as a monoubiquitination at a single or multiple Lys residues

or as a polyubiquitination, where ubiquitin chains are formed (Dittmar and Selbach, 2017; Komander, 2015). The ubiquitin molecule contains 7 Lys, where the chains can be built (Akutsu et al., 2016). Initially it was proposed that ubiquitination proteins are targeted for degradation by 26S proteasome (Hershko and Ciechanover, 1998). By studying the effect of the different chains, it was found that ubiquitination can serve not only as a trigger for proteasomal degradation, but also for other cellular processes, including histone modification, recruitment of proteins involved in DNA damage repair, regulation of protein trafficking (Clague and Urbe, 2010; Komander and Rape, 2012; Kulathu and Komander, 2012; Spence et al., 1995).

IP₃R down-regulation by ubiquitination

As stated above, IP₃R function is regulated by various post-translational modifications, including ubiquitination. Initially, it was observed that over-production of IP₃ and thus over-stimulation of IP₃Rs results in a decline in IP₃R-protein levels, (typically about 50% loss of the total IP₃R pool) (Wojcikiewicz and Nahorski, 1991). Such an “IP₃Rs down-regulation” appeared to be a protective mechanism against G protein-coupled receptor activation, which cells employ to avoid chronic Ca²⁺ mobilization in the cytosol (Wojcikiewicz, 2004). Further studies revealed that the down-regulation of IP₃Rs is a result of their proteasomal degradation (Wojcikiewicz, 1995; Wojcikiewicz et al., 1994), which is a highly selective process, since other ER proteins were not affected (Pearce et al., 2007; Wang et al., 2009). Further work revealed that (i) IP₃R degradation occurs in several cell types and models (Oberdorf et al., 1999; Sliter et al., 2011; Wojcikiewicz et al., 1999; Wojcikiewicz et al., 2003) and (ii) all three isoforms serve as substrates for ubiquitination (Oberdorf et al., 1999), although IP₃R2 appeared to be more resistant to proteasomal degradation (Wojcikiewicz, 1995). The proteasome-mediated IP₃Rs regulation was proposed to occur in response to prolonged exposure of cells to agonists, resulting in sustained IP₃ levels, and to require Ca²⁺ elevations (Alzayady and Wojcikiewicz, 2005; Wojcikiewicz et al., 1994). Interestingly, not all subunits in a tetramer are simultaneously ubiquitinated, allowing fine-tuning through the selective degradation of individual monomers (Sliter et al., 2008). Using IP₃R1 as a model, it was revealed that potential targets for ubiquitination are at least 11 surface-exposed Lys residues, located in the central, modulatory domain (Sliter et al., 2008). Later it was demonstrated that the other isoforms, IP₃R2 and IP₃R3, are also ubiquitinated at approximately the same sites (Sliter et al., 2011). Of note, there is a discrepancy between the ubiquitination of endogenous *versus* exogenous IP₃Rs. The exogenous IP₃Rs did not require any stimulus for their ubiquitination and a stimulus-dependent ubiquitination was observed only at very low levels of expression (Wojcikiewicz et al., 2003). Recently, F-box protein FBXL2, one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), was identified to bind to and ubiquitinate IP₃R3, thereby targeting it for proteasomal degradation (Bultynck and Campanella, 2017; Kuchay et al., 2017). Such downregulation resulted in a reduced Ca²⁺ transfer to the mitochondria and to cell resistance to Ca²⁺-dependent pro-apoptotic stimuli like H₂O₂. The interaction between FBXL2 and IP₃R3 required the C-terminal CaaX domain of FBXL2, which is responsible for FBXL2 membrane localization and a region in the ligand-binding domain of IP₃R3, more particularly in the IP₃-binding core. In agreement with previous studies, the proposed mechanism of IP₃R3 ubiquitination by FBXL2, involves IP₃ binding to the IP₃-binding core. This causes conformational changes, displacing the suppressor domain and allowing FBXL2 to reach its binding site (Kuchay et al., 2017).

The ubiquitination of IP₃R is a complex process, which is regulated by various factors. As stated above, this modification requires IP₃ binding to the LBD and occurs in condition of chronic IP₃ production. In addition, cytosolic Ca²⁺ elevations are also needed. Surprisingly, phosphorylation, which was shown to trigger the polyubiquitination of many proteins (Nguyen

et al., 2013), does not contribute to the modification of IP₃R (Wojcikiewicz et al., 2003). IP₃R ubiquitination appeared to be modified in cancer cells as discussed below.

IP₃R degradation and cancer

Cancer cells are characterized by uncontrolled proliferation and thereby they often display dysregulated Ca²⁺ signalling (Monteith et al., 2017). The altered Ca²⁺ homeostasis results from dysfunction of the main intracellular Ca²⁺ transport systems due to mutations, aberrant expression, altered stability or regulation (Monteith et al., 2012). As the main intracellular Ca²⁺ release channel, it is well established that IP₃R plays a critical role in cancer development and survival (Ivanova et al., 2017). Recently it was demonstrated that cancer cells are addicted to constitutive ER-mitochondria Ca²⁺ transfer and while in both normal and cancer cells the pharmacological or genetic inhibition of IP₃R triggers autophagy, only the normal cells take advantage and exploit autophagy as a survival mechanism (Bultynck, 2016; Cardenas et al., 2016). This critical ER-mitochondrial Ca²⁺ transfer occurs at the contact sites between the two organelles and it is highly dependent on the proper functioning of IP₃R. Many oncogenes and oncosuppressors reside at the MAM interface (Danese et al., 2017; Giorgi et al., 2015; Marchi et al., 2014). These proteins exert precise control of the Ca²⁺ flux from the ER to the mitochondria either directly by interacting with IP₃R or indirectly by interacting with other Ca²⁺-transport systems, thereby affecting steady state ER Ca²⁺ loading (Akl and Bultynck, 2013; Bittremieux et al., 2016). IP₃R3 is the isoform that is most abundant at the MAMs and as such it is an important target for regulating the Ca²⁺ release in these microdomains (Bultynck and Campanella, 2017; De Stefani et al., 2012; Marchi et al., 2017b; Pedriali et al., 2017). However, it is clear that IP₃R function in cells will be influenced also by its stability, which will control the total Ca²⁺ flux from the ER to the mitochondria and thereby cell death and survival. The IP₃R stability has recently attracted attention with the identification of different survival proteins as modulators of IP₃R degradation in cell death and survival with an impact for health and disease.

PTEN and IP₃R phosphorylation status

The tumor suppressor PTEN is an evolutionary conserved phosphatase with dual specificity, which is able to remove a phosphate from lipids (Maehama and Dixon, 1998) and proteins (Dey et al., 2008). PTEN contains catalytic and C2 domains and a C-terminal PDZ-binding motif (Waite and Eng, 2002). Its function as a lipid phosphatase is involved in the inhibition of the phosphoinositide 3-kinase/protein kinase B (PKB/Akt) pathway. PTEN dephosphorylates PIP₃ to PIP₂, preventing PKB/Akt activation (Bos, 1995). Inactivation of PTEN, which is observed in various cancers, including breast, colorectal, prostate cancer, glioblastoma and endometrial carcinoma (Leslie and Downes, 2004), results in increased activity of PKB/Akt promoting cell growth, proliferation and survival (Chalhoub and Baker, 2009). Initially, PTEN was considered to localize exclusively in the cytoplasm and to transiently associate with the plasma membrane, where it dephosphorylates PIP₃ (Vazquez et al., 2006). Later studies demonstrated that PTEN can translocate to various organelles including i) nucleus, where it controls DNA reparation and sensitivity to genotoxic stress (Bassi et al., 2013; Trotman et al., 2007), ii) mitochondria where it associates with Bax and promotes apoptotic cell death (Zhu et al., 2006). Recently, PTEN was found to localize at the ER part of the MAMs, where it acts as a protein phosphatase. PTEN co-immunoprecipitated with IP₃R3 and counteracted its phosphorylation by PKB/Akt. By shifting the phosphorylation state of IP₃R3 towards desphosphorylation, PTEN promotes IP₃R3-mediated Ca²⁺ release from the ER and subsequent Ca²⁺ flux into mitochondria and cell death (**Fig. 1**) (Bononi et al., 2013).

PTEN and IP₃R ubiquitination status

1 Recently, a phosphatase-independent role for PTEN has been found in the regulation of IP₃R
2 activity (Kuchay et al., 2017). In fact, PTEN was reported to compete with FBXL2 for the
3 ligand-binding domain of IP₃R3, thereby preventing the ubiquitination of the channel. Similarly
4 to FBXL2, PTEN bound more stably to the fragments lacking the suppressor domain.
5 Importantly catalytically-dead PTEN mutants that lack phosphatase activity remained capable
6 of opposing the FBXL2-mediated IP₃R3 degradation. Given the important role of IP₃R3 in the
7 regulation of Ca²⁺ release from the ER and subsequent influx in the mitochondria, the
8 degradation of the channel appears as an important control mechanism of cell death. PTEN loss
9 increased FBXL2 binding to IP₃R3, thereby promoting IP₃R3 degradation *via* FBXL2 and cell
10 resistance towards pro-apoptotic stimuli. Such a mechanism seems to be exploited by cancer
11 cells, which very often display deficiencies in PTEN expression (**Fig. 1 & 2**). In human prostate
12 tumors and cancer cell lines the PTEN and IP₃R3 levels correlated. The role of IP₃R3
13 degradation is also evident from experiments showing that an IP₃R3 mutant, which is resistant
14 to degradation, was able to sensitize tumor cells with low or no PTEN expression to Ca²⁺-
15 dependent cell death (Kuchay et al., 2017).

16 Hence, ER-resident PTEN has been emerging as a critical regulator of IP₃R3 function and
17 thereby of Ca²⁺-dependent cell death. PTEN exploits different mechanisms to reduce pro-
18 apoptotic ER Ca²⁺ release and subsequent mitochondrial flux. On one side, PTEN
19 dephosphorylates and suppresses IP₃R3 (Bononi et al., 2013) and on the other side, it inhibits
20 IP₃R3 degradation (Kuchay et al., 2017). In both cases, PTEN presence might result in toxic
21 Ca²⁺ release, while its reduced levels or mutations, which are common for cancer cells, result
22 in reduced Ca²⁺ release and cell survival.

23 **BAP1 and IP₃R ubiquitination status**

24 BRCA1-associated protein 1 (BAP1) is a potent tumor-suppressor, which is a member of the
25 ubiquitin C-terminal hydrolase subfamily of deubiquitylating enzymes (Carbone et al., 2013).
26 BAP1 mutations and allelic deletions are often observed in human cancers, including
27 mesothelioma, uveal melanoma, intrahepatic cholangiocarcinomas (Jiao et al., 2013), renal cell
28 carcinoma (Farley et al., 2013). BAP1 was described to localize in the nucleus, where it
29 maintains the genome integrity, since cells with reduced BAP1 levels were significantly
30 impaired in DNA repair (Yu et al., 2014). Nevertheless, in response to multi-
31 monoubiquitylation of its nuclear localization signal, the protein can translocate to the cytosol,
32 although its function there remained largely unknown until recently. Indeed, an ER-resident
33 fraction of BAP1 was demonstrated to exist. Such ER-located BAP1 targets the IP₃R3, where
34 it deubiquitinates and thereby stabilizes the channel (Bononi et al., 2017). This allowed
35 extensive Ca²⁺ release upon stimulation, which resulted in cell death. This is particularly
36 relevant for cancer cells displaying reduced levels of BAP1, as evident from the fact that
37 fibroblast cell cultures from different BAP1^{+/-} carriers appeared to be resistant to apoptosis
38 triggered by various stimuli. In response to toxic agents, BAP1^{+/-} cells displayed reduced Ca²⁺
39 rises in the cytosol and mitochondria compared to normal fibroblasts. This difference was
40 attributed to a difference in the expression of IP₃R3, since the mRNA levels of IP₃R3 gene were
41 not affected by the presence of BAP1, while the protein levels were significantly decreased in
42 the absence of BAP1. The binding determinants of the interaction were also investigated and it
43 appeared that BAP1 targets the N-terminus of IP₃R3 (**Fig. 2**). The most prominent interaction
44 occurred *via* the middle domain of BAP1. Although its N-terminal domain was not sufficient
45 by itself to bind to the N-terminus of IP₃R3, it increased the efficiency of the interaction with
46 the middle domain (Bononi et al., 2017).

Thus, this study proposes a potential mechanism of cell transformation and apoptosis escape by cancer cells, which includes i) failure in DNA repair due to reduced nuclear BAP1 on one side and ii) decreased Ca^{2+} release and transfer to the mitochondria due to reduced extra-nuclear BAP1 levels and thereby enhanced $\text{IP}_3\text{R3}$ degradation on another.

IP_3R and proteolysis

Proteolysis through specific proteases is another common mechanism for regulation of protein functions. IP_3Rs appeared to be substrates of two major classes of proteolytic enzymes, caspases and calpains. Caspases are cysteine aspartic endoproteases, which play the role of apoptotic executioners. Calpains are a class of cysteine proteases, directly activated by intracellular Ca^{2+} , which were also implicated in apoptosis (Momeni, 2011).

Caspase 3-mediated proteolysis of IP_3R and apoptosis

Caspase 3 is a major executioner in apoptosis. Multiple proteins were identified as targets of caspase 3, among which critical players in apoptosis, like Bcl-2 family members (Fischer et al., 2003). $\text{IP}_3\text{R1}$, but not $\text{IP}_3\text{R2}$ and $\text{IP}_3\text{R3}$, was found to be a substrate of caspase 3 in cells undergoing apoptosis. It was demonstrated that, during staurosporin (STS) induced apoptosis, caspase 3 targets downstream of the 1888 DEVD consensus site in the central, modulatory domain (domain 4) of $\text{IP}_3\text{R1}$ (**Fig. 2**) (Hirota et al., 1999). The cleavage results in the generation of two fragments: an N-terminal (containing the IP_3 binding site) and an ER associated C-terminal fragment that contains the channel-pore domain. The C-terminal fragment has been proposed to form a Ca^{2+} -leak channel (Assefa et al., 2004; Haug et al., 2000; Hirota et al., 1999; Nakayama et al., 2004; Verbert et al., 2008). Yet, expression of this fragment by itself only mildly increased unidirectional Ca^{2+} -flux from the ER and mildly affected steady-state ER Ca^{2+} content, indicating that this fragment by itself cannot deplete the ER. Furthermore, recent work indicated that the N- and C-terminal fragments that arise upon caspase 3-cleavage remain associated with each other and constitute a functional $\text{IP}_3\text{R1}$ channel that is able to respond to IP_3 (Alzayady et al., 2013a). In fact, even recombinant expression of both fragments results in the *de novo* assembly of a functional $\text{IP}_3\text{R1}$ channel (Alzayady et al., 2013a). Thus, it is clear that caspase 3 mediated cleavage of IP_3Rs per se is not sufficient to lead to IP_3 independent Ca^{2+} leak from ER. The contribution of caspase 3-mediated $\text{IP}_3\text{R1}$ cleavage to the apoptotic process remains debated. On the one hand, it was shown that triple IP_3R -knockout DT40 cells expressing wild-type $\text{IP}_3\text{R1}$ were susceptible to apoptotic stimuli, while cells expressing a non-cleavable IP_3R mutant displayed slower apoptotic rate and were as resistant to STS as the cells knock out for all IP_3Rs (Assefa et al., 2004). Also, the Ca^{2+} rises brought about by STS in $\text{IP}_3\text{R1}$ -expressing triple knock out (TKO) cells were annihilated by mutating the caspase 3-cleavage site in $\text{IP}_3\text{R1}$, resembling the lack of Ca^{2+} rises observed in the TKO cells. Hence, it was proposed that the pro-apoptotic Ca^{2+} signals and apoptosis sensitivity/progression are mediated by the cleavage of $\text{IP}_3\text{R1}$ by caspase 3 (Assefa et al., 2004). On the other hand, it was shown that Ca^{2+} elevations, which occur in the early stages of apoptosis are not necessarily due to IP_3R cleavage by caspase 3, since inhibition of the enzyme did not affect STS induced Ca^{2+} rises (Akimzhanov et al.). The cleavage of IP_3R occurs when cell death is induced by STS and the caspase pool is widely activated while it was not possible to detect the cleaved fragment in cells treated with other apoptotic stimuli like Trail and UV (Elkoreh et al., 2012), suggesting that this event is more likely to happen in the late stage of apoptosis.

Bok, which is a member of the Bcl-2 family of proteins, can prevent IP₃R proteolysis during apoptosis (Schulman et al., 2016; Schulman et al., 2013). In contrast to most of the other Bcl-2 proteins, which emerged as regulators of IP₃R by targeting and directly modulating the channel function (Vervliet et al., 2016), the pro-apoptotic member Bok failed to impact the IP₃R's ability to release ER calcium stores (Schulman et al., 2013). Instead, via its N-terminus, Bok binds in the proximity of the caspase 3 binding site (a.a. 1895-1903 of IP₃R1) and thereby antagonizes the proteolysis of the channel (**Fig. 2**). Bok interacts mainly with IP₃R1 and IP₃R2, while IP₃R3 which hasn't been identified as a target of caspase 3 does not bind prominently to Bok. Bok affects IP₃R stability and *vice versa*, IP₃R's are actually critical for maintaining Bok stability (Schulman et al., 2016). Recent work indicated that Bok is constitutively active and is not antagonized by anti-apoptotic Bcl-2-family members (Llambi et al., 2016). Thus, its action appears to be controlled at the level of its protein stability, where the majority of Bok is bound to IP₃R's and thereby is stabilized and protected from undergoing ER-associated degradation pathway. Bok remains associated with IP₃R's in response to agonists, apoptotic stimuli, ER-stress-inducing stimuli, indicating that this mechanism mainly operates to degrade newly synthesized Bok (Schulman et al., 2016). The contribution of IP₃R's in this process is not fully understood. On the one hand, IP₃R's stabilize Bok, thus making Bok available for mitochondrial outer membrane permeabilization (MOMP). On the other hand, IP₃R's may scaffold Bok and act as an ER sink for Bok, limiting its availability at the mitochondria and preventing its participation in MOMP. Indeed, the pro-apoptotic function of overexpressed Bok was counteracted by its binding to IP₃R (Schulman et al., 2016).

The role of Bok in human cancer is still under investigation. Although Bok is deleted in different cancer types (Beroukhi et al., 2010), its deletion in mice did not have any significant effect (Ke et al., 2012). Nevertheless, Bok was shown to facilitate apoptosis by promoting MOMP in a Bax/Bak-independent way and that its function is regulated by the regulation of its proteasomal degradation. Thus, it can be speculated that in addition to its role in MOMP, Bok also prevents IP₃R cleavage and thereby increases the Ca²⁺ release, supporting an ongoing apoptosis in a Ca²⁺ dependent manner.

Calpain-mediated proteolysis of IP₃R and apoptosis

The other major class of proteases, calpains also target IP₃R's. Initially, only IP₃R3 was considered as a substrate (Diaz and Bourguignon, 2000), but later IP₃R1 was also shown to be cleaved by calpains (Kopil et al.). Calpain targeted the modulatory domain of IP₃R, downstream of caspase 3 (**Fig. 2**) and calpain-mediated degradation of IP₃R resulted in an accumulation of a stable proteolytic fragment of ~ 95 kDa. Such fragments were able to form channels, which although displayed similar conductance as the wild type IP₃R, had significantly higher open probability at resting Ca²⁺ and were not activated by IP₃ (Kopil et al.). Furthermore, overexpression of this cleaved product led to increased Ca²⁺ leak and lower ER content, thereby increasing cell death. The authors proposed that the calpain-mediated IP₃R cleavage is an early event and that the proteolyzed channel may act as a feed-forward mechanism to enhance cell death (Kopil et al.). The same conclusion was made also in another study where calpain-mediated cleavage of IP₃R1 resulted in impaired ER Ca²⁺ buffering and thereby in neurodegeneration in primary cortical neurons (Kopil et al., 2012).

1 **Conclusion**

2 Dysregulated Ca^{2+} signaling is emerging as an important feature of different pathological
3 conditions. The function of IP_3Rs as a major regulator of intracellular Ca^{2+} signals and in
4 particular the $\text{IP}_3\text{R3}$ as the main isoform located at the MAMs, is often altered in cancer cells,
5 while $\text{IP}_3\text{R1}$ is mostly affected in neurodegenerative diseases. In addition to the direct
6 modulation of IP_3Rs by various oncogenes and oncosuppressors, some of them affect also the
7 degradation or the proteolysis of the protein. Major oncosuppressors like PTEN and BAP1
8 facilitate the degradation of $\text{IP}_3\text{R3}$, thereby preventing mitochondrial Ca^{2+} overload and cell
9 death. In addition, IP_3R can be subjected to proteolytic cleavage by caspases and calpains,
10 resulting in the generation of fragments with Ca^{2+} release activity, thereby facilitating ongoing
11 apoptosis.
12

13 **Acknowledgements**

14 The work in the authors' laboratory has been supported by grants from the KU Leuven
15 (OT14/101) and from the Research Foundation - Flanders (FWO) (G.0C91.14N, G.0A34.16N,
16 G090118N) and the Research Council - KU Leuven (OT14/101). HI is a holder of a Ph.D.
17 Fellowship from the FWO.

References

- Akimzhanov, A.M., Barral, J.M., and Boehning, D. Caspase 3 cleavage of the inositol 1,4,5-trisphosphate receptor does not contribute to apoptotic calcium release. *Cell Calcium*. 53, 152-158.
- Akl, H., and Bultynck, G. (2013). Altered Ca^{2+} signaling in cancer cells: proto-oncogenes and tumor suppressors targeting IP_3 receptors. *Biochim Biophys Acta*. 1835, 180-193.
- Akutsu, M., Dikic, I., and Bremm, A. (2016). Ubiquitin chain diversity at a glance. *J Cell Sci*. 129, 875-880.
- Alzayady, K.J., Chandrasekhar, R., and Yule, D.I. (2013a). Fragmented inositol 1,4,5-trisphosphate receptors retain tetrameric architecture and form functional Ca^{2+} release channels. *J Biol Chem*. 288, 11122-11134.
- Alzayady, K.J., Wagner, L.E., 2nd, Chandrasekhar, R., Monteagudo, A.M., Godiska, R., Tall, G.G., Joseph, S.K., and Yule, D.I. (2013b). Functional inositol 1,4,5-trisphosphate receptors assembled from concatenated homo- and heteromeric subunits. *J Biol Chem*.
- Alzayady, K.J., Wang, L., Chandrasekhar, R., Wagner, L.E., 2nd, Van Petegem, F., and Yule, D.I. (2016). Defining the stoichiometry of inositol 1,4,5-trisphosphate binding required to initiate Ca^{2+} release. *Sci Signal*. 9, ra35.
- Alzayady, K.J., and Wojcikiewicz, R.J. (2005). The role of Ca^{2+} in triggering inositol 1,4,5-trisphosphate receptor ubiquitination. *Biochem J*. 392, 601-606.
- Assefa, Z., Bultynck, G., Szlufcik, K., Nadif Kasri, N., Vermassen, E., Goris, J., Missiaen, L., Callewaert, G., Parys, J.B., and De Smedt, H. (2004). Caspase-3-induced truncation of type 1 inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis. *J Biol Chem*. 279, 43227-43236.
- Baker, M.R., Fan, G., and Serysheva, II. (2017). Structure of IP_3R channel: high-resolution insights from cryo-EM. *Curr Opin Struct Biol*. 46, 38-47.
- Bassi, C., Ho, J., Srikumar, T., Dowling, R.J., Gorrini, C., Miller, S.J., Mak, T.W., Neel, B.G., Raught, B., and Stambolic, V. (2013). Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. *Science*. 341, 395-399.
- Beroukhi, R., Mermel, C.H., Porter, D., Wei, G., Raychaudhuri, S., Donovan, J., Barretina, J., Boehm, J.S., Dobson, J., Urashima, M., Mc Henry, K.T., Pinchback, R.M., Ligon, A.H., Cho, Y.J., Haery, L., Greulich, H., Reich, M., Winckler, W., Lawrence, M.S., Weir, B.A., Tanaka, K.E., Chiang, D.Y., Bass, A.J., Loo, A., Hoffman, C., Prensner, J., Liefeld, T., Gao, Q., Yecies, D., Signoretti, S., Maher, E., Kaye, F.J., Sasaki, H., Tepper, J.E., Fletcher, J.A., Tabernero, J., Baselga, J., Tsao, M.S., Demichelis, F., Rubin, M.A., Janne, P.A., Daly, M.J., Nucera, C., Levine, R.L., Ebert, B.L., Gabriel, S., Rustgi, A.K., Antonescu, C.R., Ladanyi, M., Letai, A., Garraway, L.A., Loda, M., Beer, D.G., True, L.D., Okamoto, A., Pomeroy, S.L., Singer, S., Golub, T.R., Lander, E.S., Getz, G., Sellers, W.R., and Meyerson, M. (2010). The landscape of somatic copy-number alteration across human cancers. *Nature*. 463, 899-905.
- Berridge, M.J. (2007). Inositol trisphosphate and calcium oscillations. *Biochem Soc Symp*, 1-7.
- Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*. 4, 517-529.
- Berridge, M.J., Lipp, P., and Bootman, M.D. (2000). The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol*. 1, 11-21.
- Betzenhauser, M.J., Wagner, L.E., 2nd, Iwai, M., Michikawa, T., Mikoshiba, K., and Yule, D.I. (2008). ATP modulation of Ca^{2+} release by type-2 and type-3 inositol (1, 4, 5)-trisphosphate receptors. Differing ATP sensitivities and molecular determinants of action. *J Biol Chem*. 283, 21579-21587.

- 1 Bezprozvanny, I., and Ehrlich, B.E. (1993). ATP modulates the function of inositol 1,4,5-
2 trisphosphate-gated channels at two sites. *Neuron*. 10, 1175-1184.
- 3 Bezprozvanny, I., Watras, J., and Ehrlich, B.E. (1991). Bell-shaped calcium-response curves of
4 Ins(1,4,5)P₃- and calcium-gated channels from endoplasmic reticulum of cerebellum.
5 *Nature*. 351, 751-754.
- 6 Bittremieux, M., Parys, J.B., Pinton, P., and Bultynck, G. (2016). ER functions of oncogenes
7 and tumor suppressors: Modulators of intracellular Ca²⁺ signaling. *Biochim Biophys*
8 *Acta*. 1863, 1364-1378.
- 9 Blomgren, K., Zhu, C., Wang, X., Karlsson, J.O., Leverin, A.L., Bahr, B.A., Mallard, C., and
10 Hagberg, H. (2001). Synergistic activation of caspase-3 by m-calpain after neonatal
11 hypoxia-ischemia: a mechanism of "pathological apoptosis"? *J Biol Chem*. 276, 10191-
12 10198.
- 13 Boehning, D., and Joseph, S.K. (2000). Direct association of ligand-binding and pore domains
14 in homo- and heterotetrameric inositol 1,4,5-trisphosphate receptors. *EMBO J*. 19,
15 5450-5459.
- 16 Bononi, A., Bonora, M., Marchi, S., Missiroli, S., Poletti, F., Giorgi, C., Pandolfi, P.P., and
17 Pinton, P. (2013). Identification of PTEN at the ER and MAMs and its regulation of Ca
18 signaling and apoptosis in a protein phosphatase-dependent manner. *Cell Death Differ*.
- 19 Bononi, A., Giorgi, C., Patergnani, S., Larson, D., Verbruggen, K., Tanji, M., Pellegrini, L.,
20 Signorato, V., Olivetto, F., Pastorino, S., Nasu, M., Napolitano, A., Gaudino, G., Morris,
21 P., Sakamoto, G., Ferris, L.K., Danese, A., Raimondi, A., Tacchetti, C., Kuchay, S.,
22 Pass, H.I., Affar, E.B., Yang, H., Pinton, P., and Carbone, M. (2017). BAP1 regulates
23 IP3R3-mediated Ca²⁺ flux to mitochondria suppressing cell transformation. *Nature*.
24 546, 549-553.
- 25 Booth, D.M., Enyedi, B., Geiszt, M., Varnai, P., and Hajnoczky, G. (2016). Redox
26 Nanodomains Are Induced by and Control Calcium Signaling at the ER-Mitochondrial
27 Interface. *Molecular cell*. 63, 240-248.
- 28 Bos, J.L. (1995). A target for phosphoinositide 3-kinase: Akt/PKB. *Trends Biochem Sci*. 20,
29 441-442.
- 30 Bultynck, G. (2016). Onco-IP3Rs Feed Cancerous Cravings for Mitochondrial Ca²⁺. *Trends*
31 *Biochem Sci*. 41, 390-393.
- 32 Bultynck, G., and Campanella, M. (2017). Tumor suppressive Ca²⁺ signaling is driven by IP₃
33 receptor fitness. *Cell Stress*. 1, 73-78.
- 34 Bultynck, G., Szlufcik, K., Kasri, N.N., Assefa, Z., Callewaert, G., Missiaen, L., Parys, J.B.,
35 and De Smedt, H. (2004). Thimerosal stimulates Ca²⁺ flux through inositol 1,4,5-
36 trisphosphate receptor type 1, but not type 3, via modulation of an isoform-specific
37 Ca²⁺-dependent intramolecular interaction. *Biochem J*. 381, 87-96.
- 38 Callamaras, N., Marchant, J.S., Sun, X.P., and Parker, I. (1998). Activation and co-ordination
39 of InsP₃-mediated elementary Ca²⁺ events during global Ca²⁺ signals in *Xenopus*
40 oocytes. *J Physiol*. 509 (Pt 1), 81-91.
- 41 Carbone, M., Yang, H., Pass, H.I., Krausz, T., Testa, J.R., and Gaudino, G. (2013). BAP1 and
42 cancer. *Nat Rev Cancer*. 13, 153-159.
- 43 Cardenas, C., Miller, R.A., Smith, I., Bui, T., Molgo, J., Muller, M., Vais, H., Cheung, K.H.,
44 Yang, J., Parker, I., Thompson, C.B., Birnbaum, M.J., Hallows, K.R., and Foscett, J.K.
45 (2010). Essential regulation of cell bioenergetics by constitutive InsP₃ receptor Ca²⁺
46 transfer to mitochondria. *Cell*. 142, 270-283.
- 47 Cardenas, C., Muller, M., McNeal, A., Lovy, A., Jana, F., Bustos, G., Urrea, F., Smith, N.,
48 Molgo, J., Diehl, J.A., Ridky, T.W., and Foscett, J.K. (2016). Selective Vulnerability of
49 Cancer Cells by Inhibition of Ca²⁺ Transfer from Endoplasmic Reticulum to
50 Mitochondria. *Cell Rep*. 15, 219-220.

- 1 Chalhoub, N., and Baker, S.J. (2009). PTEN and the PI3-kinase pathway in cancer. *Annu Rev*
2 *Pathol.* 4, 127-150.
- 3 Chandrasekhar, R., Alzayady, K.J., Wagner, L.E., 2nd, and Yule, D.I. (2016). Unique
4 Regulatory Properties of Heterotetrameric Inositol 1,4,5-Trisphosphate Receptors
5 Revealed by Studying Concatenated Receptor Constructs. *J Biol Chem.* 291, 4846-
6 4860.
- 7 Clague, M.J., and Urbe, S. (2010). Ubiquitin: same molecule, different degradation pathways.
8 *Cell.* 143, 682-685.
- 9 Clapham, D.E. (2007). Calcium signaling. *Cell.* 131, 1047-1058.
- 10 Crabtree, G.R., and Schreiber, S.L. (2009). SnapShot: Ca²⁺-calcineurin-NFAT signaling. *Cell.*
11 138, 210, 210 e211.
- 12 Csordas, G., Thomas, A.P., and Hajnoczky, G. (1999). Quasi-synaptic calcium signal
13 transmission between endoplasmic reticulum and mitochondria. *EMBO J.* 18, 96-108.
- 14 Danese, A., Patergnani, S., Bonora, M., Wieckowski, M.R., Previati, M., Giorgi, C., and Pinton,
15 P. (2017). Calcium regulates cell death in cancer: Roles of the mitochondria and
16 mitochondria-associated membranes (MAMs). *Biochim Biophys Acta.* 1858, 615-627.
- 17 Dawson, S.P., Keizer, J., and Pearson, J.E. (1999). Fire-diffuse-fire model of dynamics of
18 intracellular calcium waves. *Proc Natl Acad Sci U S A.* 96, 6060-6063.
- 19 De Stefani, D., Bononi, A., Romagnoli, A., Messina, A., De Pinto, V., Pinton, P., and Rizzuto,
20 R. (2012). VDAC1 selectively transfers apoptotic Ca²⁺ signals to mitochondria. *Cell*
21 *Death Differ.* 19, 267-273.
- 22 Dey, N., Crosswell, H.E., De, P., Parsons, R., Peng, Q., Su, J.D., and Durden, D.L. (2008). The
23 protein phosphatase activity of PTEN regulates SRC family kinases and controls glioma
24 migration. *Cancer Res.* 68, 1862-1871.
- 25 Diaz, F., and Bourguignon, L.Y. (2000). Selective down-regulation of IP₃ receptor subtypes by
26 caspases and calpain during TNF alpha -induced apoptosis of human T-lymphoma cells.
27 *Cell Calcium.* 27, 315-328.
- 28 Dittmar, G., and Selbach, M. (2017). Deciphering the Ubiquitin Code. *Molecular cell.* 65, 779-
29 780.
- 30 Elkoreh, G., Blais, V., Beliveau, E., Guillemette, G., and Denault, J.B. (2012). Type 1 inositol-
31 1,4,5-trisphosphate receptor is a late substrate of caspases during apoptosis. *J Cell*
32 *Biochem.* 113, 2775-2784.
- 33 Fan, G., Baker, M.L., Wang, Z., Baker, M.R., Sinyagovskiy, P.A., Chiu, W., Ludtke, S.J., and
34 Serysheva, II. (2015). Gating machinery of InsP3R channels revealed by electron
35 cryomicroscopy. *Nature.* 527, 336-341.
- 36 Farley, M.N., Schmidt, L.S., Mester, J.L., Pena-Llopis, S., Pavia-Jimenez, A., Christie, A.,
37 Vocke, C.D., Ricketts, C.J., Peterson, J., Middleton, L., Kinch, L., Grishin, N., Merino,
38 M.J., Metwalli, A.R., Xing, C., Xie, X.J., Dahia, P.L.M., Eng, C., Linehan, W.M., and
39 Brugarolas, J. (2013). A novel germline mutation in BAP1 predisposes to familial clear-
40 cell renal cell carcinoma. *Mol Cancer Res.* 11, 1061-1071.
- 41 Fedorenko, O.A., Popugaeva, E., Enomoto, M., Stathopoulos, P.B., Ikura, M., and
42 Bezprozvanny, I. (2014). Intracellular calcium channels: inositol-1,4,5-trisphosphate
43 receptors. *Eur J Pharmacol.* 739, 39-48.
- 44 Finch, E.A., Turner, T.J., and Goldin, S.M. (1991). Calcium as a coagonist of inositol 1,4,5-
45 trisphosphate-induced calcium release. *Science.* 252, 443-446.
- 46 Fischer, U., Janicke, R.U., and Schulze-Osthoff, K. (2003). Many cuts to ruin: a comprehensive
47 update of caspase substrates. *Cell Death Differ.* 10, 76-100.
- 48 Foscett, J.K. Inositol trisphosphate receptor Ca²⁺ release channels in neurological diseases.
49 *Pflugers Arch.* 460, 481-494.

- 1 Foskett, J.K., White, C., Cheung, K.H., and Mak, D.O. (2007). Inositol trisphosphate receptor
2 Ca^{2+} release channels. *Physiol Rev.* 87, 593-658.
- 3 Giorgi, C., Missiroli, S., Patergnani, S., Duszynski, J., Wieckowski, M.R., and Pinton, P.
4 (2015). Mitochondria-associated membranes: composition, molecular mechanisms, and
5 physiopathological implications. *Antioxid Redox Signal.* 22, 995-1019.
- 6 Giorgio, V., Burchell, V., Schiavone, M., Bassot, C., Minervini, G., Petronilli, V., Argenton,
7 F., Forte, M., Tosatto, S., Lippe, G., and Bernardi, P. (2017). Ca^{2+} binding to F-ATP
8 synthase beta subunit triggers the mitochondrial permeability transition. *EMBO Rep.*
9 18, 1065-1076.
- 10 Hamada, K., Miyatake, H., Terauchi, A., and Mikoshiba, K. (2017). IP_3 -mediated gating
11 mechanism of the IP_3 receptor revealed by mutagenesis and X-ray crystallography. *Proc*
12 *Natl Acad Sci U S A.* 114, 4661-4666.
- 13 Harwood, S.M., Yaqoob, M.M., and Allen, D.A. (2005). Caspase and calpain function in cell
14 death: bridging the gap between apoptosis and necrosis. *Ann Clin Biochem.* 42, 415-
15 431.
- 16 Haug, L.S., Walaas, S.I., and Ostvold, A.C. (2000). Degradation of the type I inositol 1,4,5-
17 trisphosphate receptor by caspase-3 in SH-SY5Y neuroblastoma cells undergoing
18 apoptosis. *J Neurochem.* 75, 1852-1861.
- 19 Hershko, A., and Ciechanover, A. (1998). The ubiquitin system. *Annu Rev Biochem.* 67, 425-
20 479.
- 21 Hirota, J., Furuichi, T., and Mikoshiba, K. (1999). Inositol 1,4,5-trisphosphate receptor type 1
22 is a substrate for caspase-3 and is cleaved during apoptosis in a caspase-3-dependent
23 manner. *J Biol Chem.* 274, 34433-34437.
- 24 Hwang, M.S., Schwall, C.T., Pazarentzos, E., Datler, C., Alder, N.N., and Grimm, S. (2014).
25 Mitochondrial Ca^{2+} influx targets cardiolipin to disintegrate respiratory chain complex
26 II for cell death induction. *Cell Death Differ.* 21, 1733-1745.
- 27 Iino, M. (1990). Biphasic Ca^{2+} dependence of inositol 1,4,5-trisphosphate-induced Ca release
28 in smooth muscle cells of the guinea pig taenia caeci. *J Gen Physiol.* 95, 1103-1122.
- 29 Ivanova, H., Kerkhofs, M., La Rovere, R.M., and Bultynck, G. (2017). Endoplasmic Reticulum-
30 Mitochondrial Ca^{2+} Fluxes Underlying Cancer Cell Survival. *Front Oncol.* 7, 70.
- 31 Ivanova, H., Vervliet, T., Missiaen, L., Parys, J.B., De Smedt, H., and Bultynck, G. (2014).
32 Inositol 1,4,5-trisphosphate receptor-isoform diversity in cell death and survival.
33 *Biochim Biophys Acta.* 1843, 2164-2183.
- 34 Jiao, Y., Pawlik, T.M., Anders, R.A., Selaru, F.M., Streppel, M.M., Lucas, D.J., Niknafs, N.,
35 Guthrie, V.B., Maitra, A., Argani, P., Offerhaus, G.J.A., Roa, J.C., Roberts, L.R., Gores,
36 G.J., Popescu, I., Alexandrescu, S.T., Dima, S., Fassan, M., Simbolo, M., Mafficini, A.,
37 Capelli, P., Lawlor, R.T., Ruzzenente, A., Guglielmi, A., Tortora, G., de Braud, F.,
38 Scarpa, A., Jarnagin, W., Klimstra, D., Karchin, R., Velculescu, V.E., Hruban, R.H.,
39 Vogelstein, B., Kinzler, K.W., Papadopoulos, N., and Wood, L.D. (2013). Exome
40 sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1
41 in intrahepatic cholangiocarcinomas. *Nat Genet.* 45, 1470-1473.
- 42 Ke, F., Voss, A., Kerr, J.B., O'Reilly, L.A., Tai, L., Echeverry, N., Bouillet, P., Strasser, A.,
43 and Kaufmann, T. (2012). BCL-2 family member BOK is widely expressed but its loss
44 has only minimal impact in mice. *Cell Death Differ.* 19, 915-925.
- 45 Kerkhofs, M., Giorgi, C., Marchi, S., Seitaj, B., Parys, J.B., Pinton, P., Bultynck, G., and
46 Bittremieux, M. (2017). Alterations in Ca^{2+} Signalling via ER-Mitochondria Contact
47 Site Remodelling in Cancer. *Adv Exp Med Biol.* 997, 225-254.
- 48 Khan, S.A., Rossi, A.M., Riley, A.M., Potter, B.V., and Taylor, C.W. (2013). Subtype-selective
49 regulation of $\text{IP}(3)$ receptors by thimerosal via cysteine residues within the $\text{IP}(3)$ -
50 binding core and suppressor domain. *Biochem J.* 451, 177-184.

- 1 Kilpatrick, B.S., Yates, E., Grimm, C., Schapira, A.H., and Patel, S. (2016). Endo-lysosomal
2 TRP mucolipin-1 channels trigger global ER Ca^{2+} release and Ca^{2+} influx. *J Cell Sci.*
3 129, 3859-3867.
- 4 Komander, D. (2015). Biochemistry. Details of destruction, one molecule at a time. *Science.*
5 348, 183-184.
- 6 Komander, D., and Rape, M. (2012). The ubiquitin code. *Annu Rev Biochem.* 81, 203-229.
- 7 Kopil, C.M., Siebert, A.P., Foskett, J.K., and Neumar, R.W. (2012). Calpain-cleaved type 1
8 inositol 1,4,5-trisphosphate receptor impairs ER Ca^{2+} buffering and causes
9 neurodegeneration in primary cortical neurons. *J Neurochem.* 123, 147-158.
- 10 Kopil, C.M., Vais, H., Cheung, K.H., Siebert, A.P., Mak, D.O., Foskett, J.K., and Neumar,
11 R.W. Calpain-cleaved type 1 inositol 1,4,5-trisphosphate receptor (InsP₃R1) has InsP₃-
12 independent gating and disrupts intracellular Ca^{2+} homeostasis. *J Biol Chem.* 286,
13 35998-36010.
- 14 Kuchay, S., Giorgi, C., Simoneschi, D., Pagan, J., Missiroli, S., Saraf, A., Florens, L.,
15 Washburn, M.P., Collazo-Lorduy, A., Castillo-Martin, M., Cordon-Cardo, C., Sebt,
16 S.M., Pinton, P., and Pagano, M. (2017). PTEN counteracts FBXL2 to promote IP3R3-
17 and Ca^{2+} -mediated apoptosis limiting tumour growth. *Nature.* 546, 554-558.
- 18 Kulathu, Y., and Komander, D. (2012). Atypical ubiquitylation - the unexplored world of
19 polyubiquitin beyond Lys48 and Lys63 linkages. *Nat Rev Mol Cell Biol.* 13, 508-523.
- 20 La Rovere, R.M., Roest, G., Bultynck, G., and Parys, J.B. (2016). Intracellular Ca^{2+} signaling
21 and Ca^{2+} microdomains in the control of cell survival, apoptosis and autophagy. *Cell*
22 *Calcium.* 60, 74-87.
- 23 Leslie, N.R., and Downes, C.P. (2004). PTEN function: how normal cells control it and tumour
24 cells lose it. *Biochem J.* 382, 1-11.
- 25 Llambi, F., Wang, Y.M., Victor, B., Yang, M., Schneider, D.M., Gingras, S., Parsons, M.J.,
26 Zheng, J.H., Brown, S.A., Pelletier, S., Moldoveanu, T., Chen, T., and Green, D.R.
27 (2016). BOK Is a Non-canonical BCL-2 Family Effector of Apoptosis Regulated by
28 ER-Associated Degradation. *Cell.* 165, 421-433.
- 29 Lopez-Sanjurjo, C.I., Tovey, S.C., Prole, D.L., and Taylor, C.W. (2013). Lysosomes shape
30 Ins(1,4,5)P₃-evoked Ca^{2+} signals by selectively sequestering Ca^{2+} released from the
31 endoplasmic reticulum. *J Cell Sci.* 126, 289-300.
- 32 Maehama, T., and Dixon, J.E. (1998). The tumor suppressor, PTEN/MMAC1,
33 dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate.
34 *J Biol Chem.* 273, 13375-13378.
- 35 Mak, D.O., McBride, S., and Foskett, J.K. (1998). Inositol 1,4,5-trisphosphate [correction of
36 tris-phosphate] activation of inositol trisphosphate [correction of tris-phosphate]
37 receptor Ca^{2+} channel by ligand tuning of Ca^{2+} inhibition. *Proc Natl Acad Sci U S A.*
38 95, 15821-15825.
- 39 Mak, D.O., McBride, S., and Foskett, J.K. (2001). Regulation by Ca^{2+} and inositol 1,4,5-
40 trisphosphate (InsP₃) of single recombinant type 3 InsP₃ receptor channels. Ca^{2+}
41 activation uniquely distinguishes types 1 and 3 InsP₃ receptors. *J Gen Physiol.* 117, 435-
42 446.
- 43 Marchant, J.S., and Taylor, C.W. (1997). Cooperative activation of IP₃ receptors by sequential
44 binding of IP₃ and Ca^{2+} safeguards against spontaneous activity. *Curr Biol.* 7, 510-518.
- 45 Marchi, S., Bittremieux, M., Missiroli, S., Morganti, C., Patergnani, S., Sbano, L., Rimessi, A.,
46 Kerkhofs, M., Parys, J.B., Bultynck, G., Giorgi, C., and Pinton, P. (2017a). Endoplasmic
47 Reticulum-Mitochondria Communication Through Ca^{2+} Signaling: The Importance of
48 Mitochondria-Associated Membranes (MAMs). *Adv Exp Med Biol.* 997, 49-67.

- 1 Marchi, S., Giorgi, C., Oparka, M., Duszynski, J., Wieckowski, M.R., and Pinton, P. (2014).
2 Oncogenic and oncosuppressive signal transduction at mitochondria-associated
3 endoplasmic reticulum membranes. *Mol Cell Oncol.* 1, e956469.
- 4 Marchi, S., Patergnani, S., Missiroli, S., Morciano, G., Rimessi, A., Wieckowski, M.R., Giorgi,
5 C., and Pinton, P. (2017b). Mitochondrial and endoplasmic reticulum calcium
6 homeostasis and cell death. *Cell Calcium*.
- 7 Michikawa, T., Hamanaka, H., Otsu, H., Yamamoto, A., Miyawaki, A., Furuichi, T., Tashiro,
8 Y., and Mikoshiba, K. (1994). Transmembrane topology and sites of N-glycosylation
9 of inositol 1,4,5-trisphosphate receptor. *J Biol Chem.* 269, 9184-9189.
- 10 Mikoshiba, K. (2007). The IP₃ receptor/Ca²⁺ channel and its cellular function. *Biochem Soc*
11 *Symp.* 9-22.
- 12 Mikoshiba, K. (2015). Role of IP₃ receptor signaling in cell functions and diseases. *Adv Biol*
13 *Regul.* 57, 217-227.
- 14 Missiaen, L., De Smedt, H., Droogmans, G., and Casteels, R. (1992). Luminal Ca²⁺ controls the
15 activation of the inositol 1,4,5-trisphosphate receptor by cytosolic Ca²⁺. *J Biol Chem.*
16 267, 22961-22966.
- 17 Missiaen, L., Parys, J.B., Smedt, H.D., Sienaert, I., Sipma, H., Vanlingen, S., Maes, K., and
18 Casteels, R. (1997). Effect of adenine nucleotides on myo-inositol-1,4,5-trisphosphate-
19 induced calcium release. *Biochem J.* 325 (Pt 3), 661-666.
- 20 Miyakawa, T., Maeda, A., Yamazawa, T., Hirose, K., Kurosaki, T., and Iino, M. (1999).
21 Encoding of Ca²⁺ signals by differential expression of IP₃ receptor subtypes. *Embo J.*
22 18, 1303-1308.
- 23 Momeni, H.R. (2011). Role of calpain in apoptosis. *Cell J.* 13, 65-72.
- 24 Monkawa, T., Miyawaki, A., Sugiyama, T., Yoneshima, H., Yamamoto-Hino, M., Furuichi, T.,
25 Saruta, T., Hasegawa, M., and Mikoshiba, K. (1995). Heterotetrameric complex
26 formation of inositol 1,4,5-trisphosphate receptor subunits. *J Biol Chem.* 270, 14700-
27 14704.
- 28 Monteith, G.R., Davis, F.M., and Roberts-Thomson, S.J. (2012). Calcium channels and pumps
29 in cancer: changes and consequences. *J Biol Chem.* 287, 31666-31673.
- 30 Monteith, G.R., Prevarskaya, N., and Roberts-Thomson, S.J. (2017). The calcium-cancer
31 signalling nexus. *Nat Rev Cancer.* 17, 367-380.
- 32 Nakayama, T., Hattori, M., Uchida, K., Nakamura, T., Tateishi, Y., Bannai, H., Iwai, M.,
33 Michikawa, T., Inoue, T., and Mikoshiba, K. (2004). The regulatory domain of the
34 inositol 1,4,5-trisphosphate receptor is necessary to keep the channel domain closed:
35 possible physiological significance of specific cleavage by caspase 3. *Biochem J.* 377,
36 299-307.
- 37 Nguyen, L.K., Kolch, W., and Kholodenko, B.N. (2013). When ubiquitination meets
38 phosphorylation: a systems biology perspective of EGFR/MAPK signalling. *Cell*
39 *Commun Signal.* 11, 52.
- 40 Oberdorf, J., Webster, J.M., Zhu, C.C., Luo, S.G., and Wojcikiewicz, R.J. (1999). Down-
41 regulation of types I, II and III inositol 1,4,5-trisphosphate receptors is mediated by the
42 ubiquitin/proteasome pathway. *Biochem J.* 339 (Pt 2), 453-461.
- 43 Parker, I., Choi, J., and Yao, Y. (1996). Elementary events of InsP₃-induced Ca²⁺ liberation in
44 *Xenopus* oocytes: hot spots, puffs and blips. *Cell Calcium.* 20, 105-121.
- 45 Parra, V., Moraga, F., Kuzmicic, J., Lopez-Crisosto, C., Troncoso, R., Torrealba, N., Criollo,
46 A., Diaz-Elizondo, J., Rothermel, B.A., Quest, A.F., and Lavandero, S. (2013). Calcium
47 and mitochondrial metabolism in ceramide-induced cardiomyocyte death. *Biochim*
48 *Biophys Acta.* 1832, 1334-1344.
- 49 Parys, J.B., Missiaen, L., De Smedt, H., and Casteels, R. (1993). Loading dependence of
50 inositol 1,4,5-trisphosphate-induced Ca²⁺ release in the clonal cell line A7r5.

- Implications for the mechanism of quantal Ca^{2+} release. *J Biol Chem.* 268, 25206-25212.
- Pearce, M.M., Wang, Y., Kelley, G.G., and Wojcikiewicz, R.J. (2007). SPFH2 mediates the endoplasmic reticulum-associated degradation of inositol 1,4,5-trisphosphate receptors and other substrates in mammalian cells. *J Biol Chem.* 282, 20104-20115.
- Pedriali, G., Rimessi, A., Sbrano, L., Giorgi, C., Wieckowski, M.R., Previati, M., and Pinton, P. (2017). Regulation of Endoplasmic Reticulum-Mitochondria Ca^{2+} Transfer and Its Importance for Anti-Cancer Therapies. *Front Oncol.* 7, 180.
- Pinton, P., Ferrari, D., Rapizzi, E., Di Virgilio, F., Pozzan, T., and Rizzuto, R. (2001). The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. *EMBO J.* 20, 2690-2701.
- Prins, D., and Michalak, M. (2011). Organellar calcium buffers. *Cold Spring Harb Perspect Biol.* 3.
- Raffaello, A., Mammucari, C., Gherardi, G., and Rizzuto, R. (2016). Calcium at the Center of Cell Signaling: Interplay between Endoplasmic Reticulum, Mitochondria, and Lysosomes. *Trends Biochem Sci.* 41, 1035-1049.
- Rizzuto, R., Brini, M., Murgia, M., and Pozzan, T. (1993). Microdomains with high Ca^{2+} close to IP_3 -sensitive channels that are sensed by neighboring mitochondria. *Science.* 262, 744-747.
- Rizzuto, R., De Stefani, D., Raffaello, A., and Mammucari, C. (2012). Mitochondria as sensors and regulators of calcium signalling. *Nat Rev Mol Cell Biol.* 13, 566-578.
- Roderick, H.L., and Cook, S.J. (2008). Ca^{2+} signalling checkpoints in cancer: remodelling Ca^{2+} for cancer cell proliferation and survival. *Nat Rev Cancer.* 8, 361-375.
- Rossi, A.M., Riley, A.M., Tovey, S.C., Rahman, T., Dellis, O., Taylor, E.J., Veresov, V.G., Potter, B.V., and Taylor, C.W. (2009). Synthetic partial agonists reveal key steps in IP_3 receptor activation. *Nat Chem Biol.* 5, 631-639.
- Schulman, J.J., Wright, F.A., Han, X., Zluhan, E.J., Szczesniak, L.M., and Wojcikiewicz, R.J. (2016). The Stability and Expression Level of Bok Are Governed by Binding to Inositol 1,4,5-Trisphosphate Receptors. *J Biol Chem.* 291, 11820-11828.
- Schulman, J.J., Wright, F.A., Kaufmann, T., and Wojcikiewicz, R.J. (2013). The Bcl-2 protein family member Bok binds to the coupling domain of inositol 1,4,5-trisphosphate receptors and protects them from proteolytic cleavage. *J Biol Chem.* 288, 25340-25349.
- Sienaert, I., De Smedt, H., Parys, J.B., Missiaen, L., Vanlingen, S., Sipma, H., and Casteels, R. (1996). Characterization of a cytosolic and a luminal Ca^{2+} binding site in the type I inositol 1,4,5-trisphosphate receptor. *J Biol Chem.* 271, 27005-27012.
- Sienaert, I., Missiaen, L., De Smedt, H., Parys, J.B., Sipma, H., and Casteels, R. (1997). Molecular and functional evidence for multiple Ca^{2+} -binding domains in the type 1 inositol 1,4,5-trisphosphate receptor. *J Biol Chem.* 272, 25899-25906.
- Sliter, D.A., Aguiar, M., Gygi, S.P., and Wojcikiewicz, R.J. (2011). Activated inositol 1,4,5-trisphosphate receptors are modified by homogeneous Lys-48- and Lys-63-linked ubiquitin chains, but only Lys-48-linked chains are required for degradation. *J Biol Chem.* 286, 1074-1082.
- Sliter, D.A., Kubota, K., Kirkpatrick, D.S., Alzayady, K.J., Gygi, S.P., and Wojcikiewicz, R.J. (2008). Mass spectrometric analysis of type 1 inositol 1,4,5-trisphosphate receptor ubiquitination. *J Biol Chem.* 283, 35319-35328.
- Spence, J., Sadis, S., Haas, A.L., and Finley, D. (1995). A ubiquitin mutant with specific defects in DNA repair and multiubiquitination. *Mol Cell Biol.* 15, 1265-1273.
- Squier, M.K., Miller, A.C., Malkinson, A.M., and Cohen, J.J. (1994). Calpain activation in apoptosis. *J Cell Physiol.* 159, 229-237.

- 1 Sudhof, T.C., Newton, C.L., Archer, B.T., 3rd, Ushkaryov, Y.A., and Mignery, G.A. (1991).
2 Structure of a novel InsP3 receptor. *Embo J.* 10, 3199-3206.
- 3 Taylor, C.W., Genazzani, A.A., and Morris, S.A. (1999). Expression of inositol trisphosphate
4 receptors. *Cell Calcium.* 26, 237-251.
- 5 Taylor, C.W., and Konieczny, V. (2016). IP₃ receptors: Take four IP₃ to open. *Sci Signal.* 9,
6 pe1.
- 7 Thillaiappan, N.B., Chavda, A.P., Tovey, S.C., Prole, D.L., and Taylor, C.W. (2017). Ca²⁺
8 signals initiate at immobile IP3 receptors adjacent to ER-plasma membrane junctions.
9 *Nat Commun.* 8, 1505.
- 10 Trotman, L.C., Wang, X., Alimonti, A., Chen, Z., Teruya-Feldstein, J., Yang, H., Pavletich,
11 N.P., Carver, B.S., Cordon-Cardo, C., Erdjument-Bromage, H., Tempst, P., Chi, S.G.,
12 Kim, H.J., Misteli, T., Jiang, X., and Pandolfi, P.P. (2007). Ubiquitination regulates
13 PTEN nuclear import and tumor suppression. *Cell.* 128, 141-156.
- 14 Tu, H., Wang, Z., and Bezprozvanny, I. (2005a). Modulation of mammalian inositol 1,4,5-
15 trisphosphate receptor isoforms by calcium: a role of calcium sensor region. *Biophys J.*
16 88, 1056-1069.
- 17 Tu, H., Wang, Z., Nosyreva, E., De Smedt, H., and Bezprozvanny, I. (2005b). Functional
18 characterization of mammalian inositol 1,4,5-trisphosphate receptor isoforms. *Biophys*
19 *J.* 88, 1046-1055.
- 20 Uchida, K., Miyauchi, H., Furuichi, T., Michikawa, T., and Mikoshiba, K. (2003). Critical
21 regions for activation gating of the inositol 1,4,5-trisphosphate receptor. *J Biol Chem.*
22 278, 16551-16560.
- 23 Vandecaetsbeek, I., Vangheluwe, P., Raeymaekers, L., Wuytack, F., and Vanoevelen, J. (2011).
24 The Ca²⁺ pumps of the endoplasmic reticulum and Golgi apparatus. *Cold Spring Harb*
25 *Perspect Biol.* 3.
- 26 Vanderheyden, V., Devogelaere, B., Missiaen, L., De Smedt, H., Bultynck, G., and Parys, J.B.
27 (2009). Regulation of inositol 1,4,5-trisphosphate-induced Ca²⁺ release by reversible
28 phosphorylation and dephosphorylation. *Biochim Biophys Acta.* 1793, 959-970.
- 29 Vazquez, F., Matsuoka, S., Sellers, W.R., Yanagida, T., Ueda, M., and Devreotes, P.N. (2006).
30 Tumor suppressor PTEN acts through dynamic interaction with the plasma membrane.
31 *Proc Natl Acad Sci U S A.* 103, 3633-3638.
- 32 Verbert, L., Lee, B., Kocks, S.L., Assefa, Z., Parys, J.B., Missiaen, L., Callewaert, G., Fissore,
33 R.A., De Smedt, H., and Bultynck, G. (2008). Caspase-3-truncated type 1 inositol 1,4,5-
34 trisphosphate receptor enhances intracellular Ca²⁺ leak and disturbs Ca²⁺ signalling. *Biol*
35 *Cell.* 100, 39-49.
- 36 Vervliet, T., Parys, J.B., and Bultynck, G. (2016). Bcl-2 proteins and calcium signaling:
37 complexity beneath the surface. *Oncogene.* 35, 5079-5092.
- 38 Waite, K.A., and Eng, C. (2002). Protean PTEN: form and function. *Am J Hum Genet.* 70, 829-
39 844.
- 40 Wang, Y., Pearce, M.M., Sliter, D.A., Olzmann, J.A., Christianson, J.C., Kopito, R.R.,
41 Boeckmann, S., Gagen, C., Lechner, G.S., Roitelman, J., and Wojcikiewicz, R.J.
42 (2009). SPFH1 and SPFH2 mediate the ubiquitination and degradation of inositol 1,4,5-
43 trisphosphate receptors in muscarinic receptor-expressing HeLa cells. *Biochim Biophys*
44 *Acta.* 1793, 1710-1718.
- 45 Wojcikiewicz, R.J. (1995). Type I, II, and III inositol 1,4,5-trisphosphate receptors are
46 unequally susceptible to down-regulation and are expressed in markedly different
47 proportions in different cell types. *J Biol Chem.* 270, 11678-11683.
- 48 Wojcikiewicz, R.J. (2004). Regulated ubiquitination of proteins in GPCR-initiated signaling
49 pathways. *Trends Pharmacol Sci.* 25, 35-41.

- 1 Wojcikiewicz, R.J., Ernst, S.A., and Yule, D.I. (1999). Secretagogues cause ubiquitination and
2 down-regulation of inositol 1, 4,5-trisphosphate receptors in rat pancreatic acinar cells.
3 *Gastroenterology*. 116, 1194-1201.
- 4 Wojcikiewicz, R.J., Furuichi, T., Nakade, S., Mikoshiba, K., and Nahorski, S.R. (1994).
5 Muscarinic receptor activation down-regulates the type I inositol 1,4,5-trisphosphate
6 receptor by accelerating its degradation. *J Biol Chem*. 269, 7963-7969.
- 7 Wojcikiewicz, R.J., and Nahorski, S.R. (1991). Chronic muscarinic stimulation of SH-SY5Y
8 neuroblastoma cells suppresses inositol 1,4,5-trisphosphate action. Parallel inhibition of
9 inositol 1,4,5-trisphosphate-induced Ca^{2+} mobilization and inositol 1,4,5-trisphosphate
10 binding. *J Biol Chem*. 266, 22234-22241.
- 11 Wojcikiewicz, R.J., Xu, Q., Webster, J.M., Alzayady, K., and Gao, C. (2003). Ubiquitination
12 and proteasomal degradation of endogenous and exogenous inositol 1,4,5-trisphosphate
13 receptors in alpha T3-1 anterior pituitary cells. *J Biol Chem*. 278, 940-947.
- 14 Woods, A., Dickerson, K., Heath, R., Hong, S.P., Momcilovic, M., Johnstone, S.R., Carlson,
15 M., and Carling, D. (2005). Ca^{2+} /calmodulin-dependent protein kinase kinase-beta acts
16 upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab*. 2, 21-33.
- 17 Yao, Y., Choi, J., and Parker, I. (1995). Quantal puffs of intracellular Ca^{2+} evoked by inositol
18 trisphosphate in *Xenopus* oocytes. *J Physiol*. 482 (Pt 3), 533-553.
- 19 Yoshikawa, F., Morita, M., Monkawa, T., Michikawa, T., Furuichi, T., and Mikoshiba, K.
20 (1996). Mutational analysis of the ligand binding site of the inositol 1,4,5-trisphosphate
21 receptor. *J Biol Chem*. 271, 18277-18284.
- 22 Yu, H., Pak, H., Hammond-Martel, I., Ghram, M., Rodrigue, A., Daou, S., Barbour, H., Corbeil,
23 L., Hebert, J., Drobetsky, E., Masson, J.Y., Di Noia, J.M., and Affar el, B. (2014).
24 Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair.
25 *Proc Natl Acad Sci U S A*. 111, 285-290.
- 26 Yule, D.I., Betzenhauser, M.J., and Joseph, S.K. (2010). Linking structure to function: Recent
27 lessons from inositol 1,4,5-trisphosphate receptor mutagenesis. *Cell Calcium*. 47, 469-
28 479.
- 29 Zhu, Y., Hoell, P., Ahlemeyer, B., and Krieglstein, J. (2006). PTEN: a crucial mediator of
30 mitochondria-dependent apoptosis. *Apoptosis*. 11, 197-207.

Figures and legends

Fig. 1 Modulation of IP₃R3 channels by ubiquitination and phosphorylation is a mechanism, exploited by cancer cells to avoid cell death. (A) IP₃R3 is the most abundant isoform at the MAMs where it facilitates ER-mitochondrial Ca²⁺ fluxes. The tight regulation of this process enables adequate cell death sensitivity of healthy cells undergoing genotoxic stress and cell damage, preventing oncogenesis. IP₃R3 function is modulated by different factors and modifications, among which are ubiquitination (grey, u) and phosphorylation (green, p). FBXL2 is a protein with a ubiquitin ligase activity, which targets IP₃R3 and mediates its ubiquitination and subsequent proteasomal degradation. PKB is a kinase which by phosphorylating IP₃R3 decreases its activity. The tumour suppressor PTEN can compete with and antagonize both, FBXL2 and PKB, thereby blocking the ubiquitination or the phosphorylation of IP₃R3. In normal cell exposed to oncogenic stress, deubiquitination and dephosphorylation of IP₃R3 will result in stable and active channel, which will sustain adequate ER-mitochondrial Ca²⁺ transfer and thereby engaging cell death mechanism. (B) Many cancer types display loss of PTEN expression, enabling FBXL2 and PKB to bind to and modulate the receptor. The ubiquitinated and/or phosphorylated IP₃R3 will be degraded in the proteasomes or will be inhibited, resulting in impaired ER-mitochondrial Ca²⁺ fluxes. Exploiting this mechanism cancer cells will avoid cell death in response to genotoxic stress and cell damage. This phenomenon is an early event in oncogenesis, enabling malignant cell formation.

Fig. 2 Linear representation of IP₃R and the proteins involved in the regulation of its stability. The linear representation of the IP₃R is divided in three functional domains: (i) N-terminal ligand-binding domain, (ii) the modulatory domain and (iii) the C-terminal domain. **A) Ubiquitination of IP₃R.** FBXL2 (red) competes with PTEN (blue) for the ligand-binding domain of IP₃R. While FBXL2 interaction with the receptor results in IP₃R ubiquitination and subsequent degradation, PTEN, independently of its lipid/protein phosphatase activity, prevents this process. BAP1 (green) also targets the ligand-binding domain and the binding leads to deubiquitination and thereby stabilization of the receptor. The impact of FBXL2 and BAP1 on IP₃Rs has been shown for the type 3 IP₃R isoform. **B) Protease-mediated cleavage of IP₃R.** IP₃Rs are targets of caspase 3 (light blue) and calpain (dark blue), which cleave the receptor within the modulatory domain. Caspase 3-mediated cleavage is counteracted by the binding of Bok to the IP₃R. The impact of caspase 3 and calpain on IP₃R integrity has been shown for type 1 IP₃R isoform.