Mitochondrial calcium: mechanisms, regulation and functions in physiology and disease

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20 **Abstract**

21 Calcium ions (Ca^{2+}) constitute one of the most versatile signalling molecules with many
22 physiological functions, prominently including muscle contraction, neuronal excitability, cell
23 migration and growth. By uptaking and releasing Ca^{2+} mitochondria serve as important
24 regulators of cellular Ca^{2+}. Mitochondrial Ca^{2+} has also other important functions, such as
25 regulation of mitochondrial metabolism and ATP production and cell death. In recent years,
26 the identification of the molecular machinery regulating mitochondrial Ca^{2+} accumulation and
27 efflux has expanded the number of conditions that are strongly associated with the functions
28 of mitochondrial Ca^{2+} channels or transporters. Indeed, several physiological and pathological
29 processes have now been linked to alterations in mitochondrial Ca^{2+} levels. Thus,
30 understanding the mechanisms of mitochondrial Ca^{2+} regulation and function in different cell
31 types is an important task in biomedical research with the mitochondrial Ca^{2+} machinery
32 emerging as a novel target for the treatment of several disorders.

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35 **Keywords:** Calcium (Ca^{2+}) signaling, homeostasis, MCU complex, cell death, inflammation,
36 cancer, ion channels, heart, metabolism

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41 **[H1] Introduction**

42 The two adjectives most commonly used to define calcium (Ca^{2+}) signaling are "universal" and
43 "versatile"[1]. The universality of Ca^{2+} as a signalling molecule derives from its ubiquity as an
44 intracellular second messenger that controls a wide range of critical processes, whereas the
45 versatility of Ca^{2+} depends on its ability to generate signals with largely different spatial and
46 temporal forms (BOX 1). Among the many organelles, mitochondria play major roles as both
47 regulators and decoders of Ca^{2+} inputs. Due to their intrinsically dynamic nature,
48 mitochondria can localize at specific positions throughout the cell, shaping the Ca^{2+} response
49 in a strategic fashion and acting as Ca^{2+}-dependent effectors of a vast range of processes, such
50 as energy production and cell death.

51 As described by Ernesto Carafoli in a fascinating historical review[2], the first indirect evidence
52 of Ca^{2+} transport inside mitochondria dates back to 1953 (REF [3]), but the concept that isolated
53 mitochondria could take up high levels of Ca^{2+} by using ATP-derived energy emerged only in
54 the 1960s with observations made by two independent groups[4,5]. Since then, mitochondria
55 have been thought of as well-defined structures capable of accumulating large amounts of
56 Ca^{2+} ions inside their matrix. Over the past sixty years, intense research in the Ca^{2+} field
57 defined the basic features of mitochondrial Ca^{2+} handling and clearly established the role of
58 mitochondria in the regulation of cellular Ca^{2+} homeostasis, as well as specific functions of
59 mitochondrial Ca^{2+} uptake. At resting conditions, the Ca^{2+} concentration ($[Ca^{2+}]$) inside
60 mitochondria approaches the values measured in the bulk cytoplasm (100-200 nM), but
61 during stimulation with $[Ca^{2+}]$-increasing agents, mitochondria accumulate 10–20-fold more
62 Ca^{2+} than the cytosolic compartment. The sources of Ca^{2+} required for such $[Ca^{2+}]$ rises are
63 external, represented by the extracellular milieu ($[Ca^{2+}]$ of ~1 mM) from where Ca^{2+} is taken
64 up by plasma membrane channels (the prevailing mechanism in neurons and other excitable
65 cells) or internal via the release of Ca^{2+} from internal sources — endoplasmic reticulum (ER)
66 or sarcoplasmic reticulum (SR) in muscle cells and the Golgi apparatus — via different classes
67 of intracellular channels (e.g., the inositol 1,4,5 trisphosphate receptor (IP3Rs) or ryanodine
68 receptors (RyRs). These intracellular Ca^{2+} stores are loaded with Ca^{2+} ($[Ca^{2+}]$: >500 μM) at the
69 expense of ATP hydrolysis via the activity of a Ca^{2+} pumps (sarco/endoplasmic Ca^{2+} ATPase,
70 or SERCA, and the secretory pathway Ca^{2+}-transport ATPases, or SPCAs)**.** The reduction of ER
71 intraluminal Ca^{2+} results in a massive Ca^{2+} entry from the extracellular space, a mechanism
72 known as capacitative Ca^{2+} influx or SOCE (store-operated Ca^{2+} entry), aimed to provide Ca^{2+}

for the refilling of the ER and regulate a wide number of signaling functions by increasing the cytosolic [Ca^{2+}][6] (Fig.1).
The close proximity of mitochondria to such Ca^{2+} pools, in particular the ER, and the presence of a highly Ca^{2+}-selective channel located at the inner mitochondrial membrane (IMM), explain how large amounts of Ca^{2+} could enter these organelles. Ca^{2+} uptake is driven by a **membrane potential** difference ($\Delta\Psi$), generated by the **respiratory chain**, which provides the thermodynamic force required for positively charged ions to enter the matrix. However, Ca^{2+} does not remain inside mitochondria but rather is rapidly extruded into the cytoplasm through a complex system of Ca^{2+} antiporters, restoring the basal state level. Thus, the coordination of this highly sophisticated Ca^{2+} machinery, which consists of different pumps, channels and auxiliary proteins, is crucial for the maintenance of mitochondrial Ca^{2+} homeostasis, which in turn further demonstrates the impact of the mitochondrial compartment in the regulation of cellular Ca^{2+} signaling.
In this review, we will focus on the physiological role of mitochondrial Ca^{2+} and its deregulation in several pathological contexts. Moreover, we will describe the molecular details of the different Ca^{2+} transporters and provide mechanistic insight into the related regulatory pathways of mitochondrial Ca^{2+} exchange, discussing the most recent discoveries and the many unanswered questions and conflicting interpretations.

[H1] Mitochondrial Ca^{2+} entry

The continuous development of methods for measuring [Ca^{2+}], based on either luminescent or fluorescent probes (BOX 2), has enabled the characterization of the intrinsic mechanisms regulating mitochondrial Ca^{2+} handling. To reach the internal matrix region, cytosolic Ca^{2+} has to cross two membranous systems, the outer mitochondrial membrane (OMM) and the IMM, which both harbour protein pores enabling regulated Ca^{2+} uptake (Fig. 2).

[H3] The mitochondrial calcium channels

The first barrier, the OMM, is considered highly permeable to Ca^{2+} ions, and this permeability is ensured by high expression of the OMM protein VDAC (voltage-dependent anion channel), which forms membrane pores and represents the first molecular interface between mitochondria and Ca^{2+} stores (the ER/SR and the extracellular space). VDAC porins exist in three subtypes (from 1 to 3), which are expressed more or less ubiquitously but vary in their isoform ratios and sub-mitochondrial distribution among tissues[7]. Recent findings have

106 confirmed the key role of VDACs in mitochondrial Ca^{2+} transfer across the OMM in different
107 contexts[8,9], leading to a high [Ca^{2+}] in the intermembrane space (IMS) (Fig. 2a).
108 The channel responsible for Ca^{2+} passing through the IMM is termed the mitochondrial
109 calcium uniporter (MCU). It is now firmly established that the MCU is a macromolecular
110 complex composed of pore-forming subunits and regulatory proteins[10-12] (Fig. 2b). The
111 molecular characterization of the entire complex was made possible by studies, which
112 simultaneously identified the *bona fide* channel component MCU (previously known as
113 CCDC109a)[13,14]. The other elements of the holocomplex are the MCU regulator MCUb (also
114 known as CCDC109b)[15] and the IMS-resident protein MICU1 (REF [16]), which binds to its
115 paralogue MICU2 (REF [17]) to form heterodimeric structures[18,19] associated with MCU through
116 the single-pass membrane protein SMDT1 (later renamed EMRE, or essential MCU
117 regulator)[20]. Notably, additional components of the complex have been described, including
118 MICU3 (REF [17]) and MICU1.1, a MICU1 splicing variant with higher Ca^{2+}-binding affinity than
119 MICU1[21], which are tissue-specific members of the uniporter and are expressed in the central
120 nervous system and skeletal muscle, respectively.
121 The working model of the uniporter is the product of extensive research: after passing
122 through VDACs, incoming calcium is first handled by MICU1-2 dimers, due to their strategic
123 IMS localization and the presence of two Ca^{2+}-binding EF-hand domains in both the MICU1
124 and MICU2 amino acid sequences. Loss-of-function studies have definitively demonstrated
125 that MICU1-2 dimers are the gatekeepers of the uniporter, setting the Ca^{2+} threshold for MCU
126 activation and limiting the detrimental accumulation of Ca^{2+} inside the matrix under basal
127 (unstimulated) conditions[22-24]. Thus, by sensing the IMS Ca^{2+} levels, MICU1/2 proteins gate
128 the activity of the pore, allowing mitochondrial Ca^{2+} uptake exclusively at high [Ca^{2+}].
129 However, while MICU2 is *per se* a genuine inhibitor of the uniporter, reducing mitochondrial
130 Ca^{2+} entry at both low and high cytosolic [Ca^{2+}][18,19], the mechanistic role of MICU1 is currently
131 debated, since it has been suggested to be a purely stimulatory subunit[18] or to play a dual role,
132 inhibiting entry at low [Ca^{2+}] and cooperating with MCU when the [Ca^{2+}] rises[22].
133 The pore-forming subunit MCU forms pentameric structures in vitro[25], and its activity is
134 strictly dependent on EMRE, since mammalian MCU does not transport Ca^{2+} in an EMRE
135 knockout (KO) background[20]. Although it was originally proposed that EMRE might control
136 MCU by sensing the [Ca^{2+}] in the matrix through its C-terminal domain[26], subsequent, and in
137 our biased opinion more convincing, observations revealed that the C-terminus of EMRE is
138 located in the IMS, rather than the matrix[27,28], connecting the MICU1-2 sensors to MCU and
139 thus regulating Ca^{2+} entry[28]. MCU is negatively regulated by its paralogue MCUb[15], though

140 overexpression of MCUb in *Trypanosoma cruzi* does not have a dominant negative effect on
141 MCU[29].
142 This characterization of the function of the MCU complex fulfills all the properties that were
143 attributed to the uniporter several years before its molecular discovery, such as sensitivity to
144 Ruthenium red (RR) inhibition, a high Ca^{2+} selectivity[30], induction of Ca^{2+} uptake only in
145 energized mitochondria, and a low Ca^{2+} affinity (apparent K_D of 20-30 μM)[31]. This implies that
146 the function of the MCU complex completely relies on two main parameters, the
147 mitochondrial membrane potential and the [Ca^{2+}] in the area surrounding the channel.
148
149 ***[H3] Mitochondrial membrane potential as a driving force for calcium uptake***
150 After the acceptance of the **chemiosmotic theory**, it was postulated that the driving force for
151 Ca^{2+} entry is the proton electrochemical gradient potential, generated by the serial reduction
152 of electrons through the respiratory electron transport chain (ETC). The reductive transfer of
153 electrons through respiratory complexes I–IV produces the energy required to pump H^+ ions
154 against their concentration gradient in the IMS, resulting in a $\Delta\Psi$ of 150-180 mV (negative
155 inside, thus favoring cation entrance) (Fig. 2c). As proof of this concept, dinitrophenol and
156 carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), two uncouplers of oxidative
157 phosphorylation, dissipate the membrane potential across the IMM, thereby almost resetting
158 the $\Delta\Psi$ and abolishing Ca^{2+} entry via the uniporter[32,33].
159
160 ***[H3] The role of ER-mitochondria tethering in mitochondrial calcium uptake***
161 The participation of mitochondria in many Ca^{2+} signaling pathways depends on close
162 interactions with the ER calcium store, and the ensuing formation of ER-mitochondria contact
163 sites. The distance between the ER and the mitochondrion at these sites varies between 10
164 and 60 nm[34], and the ER associates more frequently with mitochondria than with other
165 organelles[35,36]. This allows mitochondria to be exposed, upon the opening IP3R to
166 microdomains of high [Ca^{2+}] that are necessary to induce Ca^{2+} accumulation through the low-
167 affinity MCU complex.
168 These synaptic-like associations, called mitochondria-associated membranes (MAMs)[37], are
169 small enough to allow contact between proteins on the surface of both organelles and ensures
170 that upon agonist Ca^{2+} mobilization, the [Ca^{2+}] on the cytosolic surface of the OMM reaches
171 levels 10-fold higher than those in the bulk cytosol[38] (Fig. 2a). Conversely, the [Ca^{2+}] to which
172 the OMM is exposed during SOCE is similar between mitochondria located near the plasma
173 membrane and those located in other intracellular areas. However, mitochondria can also

174 form associations with the plasma membrane called plasma membrane-associated
175 mitochondria (PAM)[39], where mitochondria are exposed to a 3-fold higher [Ca^{2+}] upon
176 activation of voltage-gated Ca^{2+} channels in the plasma membrane[38].
177

178 **[H1] Mitochondrial Ca^{2+} efflux**

179 Historically, two major systems have been postulated to extrude Ca^{2+} from the matrix: the
180 sodium (Na^+)/Ca^{2+} exchanger (mNCX) and the H^+/Ca^{2+} (mHCX) exchanger. The first appears
181 to be the predominant antiporter in excitable tissues (heart, brain), whereas the latter mainly
182 leads to Ca^{2+} release in non-excitable tissues (liver, kidney). The stoichiometry of mNCX-
183 driven transport is defined as electrogenic, with 3 (or 4) Na^+ for 1 Ca^{2+} (REF [40]), whereas the
184 exchange ratio of mHCX is electroneutral (2 H^+ for 1 Ca^{2+})[41] (Fig. 2d). Thus, the two Ca^{2+} efflux
185 systems mediate the extrusion of matricial Ca^{2+} toward the IMS, reaching the cytosolic
186 compartment by the VDAC channels or additional Ca^{2+} extruding mechanisms located at OMM,
187 such as the NCX family member NCX3 (REF [42]). Although Na^+-dependent and Na^+-independent
188 Ca^{2+} exit mechanisms have been described since the 1970s, the molecular identities of the
189 different components of mitochondrial Ca^{2+} efflux were revealed only a few years ago.

190 In 2010 it was shown that mNCX is a gene product of mammalian *SLC8B1* (REF [43]), most
191 frequently known as NCLX, since it catalyzes not only Na^+/Ca^{2+} exchange but also lithium
192 (Li^+)-dependent Ca^{2+} transport. This unique property, which was originally ascribed to the
193 mNCX[44], together with i) NCLX mitochondrial localization, ii) sensitivity to the classical mNCX
194 inhibitor CGP-37157, and iii) alteration of mitochondrial Ca^{2+} efflux in multiple cell types
195 lacking NCLX activity[43,45-47], definitively demonstrates that NCLX is the mitochondrial
196 Na^+/Ca^{2+} exchanger.

197 While the molecular nature of the mNCX is generally accepted today, the identity of the
198 H^+/Ca^{2+} antiporter is more controversial. In 2009 it was demonstrated that the IMM protein
199 LETM1 (leucine zipper EF-hand containing transmembrane protein 1) acts as a H^+/Ca^{2+}
200 exchanger in both Drosophila and mammalian cells, as well as in proteoliposomes[48].
201 Moreover, the authors suggested that LETM1 might promote mitochondrial Ca^{2+} entry under
202 certain conditions, functioning as a high-affinity Ca^{2+} uptake system alternative to the MCU
203 complex[48]. This concept has been confirmed by others[49,50], but the huge advances in the
204 understanding of the mechanisms of the uniporter have decreased the interest in such
205 observations.

206 The role of LETM1 in matricial Ca^{2+} release has also been questioned[51]; indeed, LETM1 was
207 first reported as a K^+/H^+ exchanger[52-54], and some LETM1-related features described in initial

studies, such as a stoichiometry of 1 H^{+} for 1 Ca^{2+} and sensitivity to the MCU inhibitor RR[48], conflict with those originally described for the mHCX. Novel findings obtained with a highly purified LETM1-containing liposome system partially clarified these issues, suggesting electroneutral transport of Ca^{2+} and insensitivity to RR and CGP-37157, thereby reaffirming that LETM1 is the mHCX[55]. Moreover, LETM1 might oligomerize in hexameric structures, thus acting as a transporter, even though it only contains a single transmembrane helix[56]. However, due to the numerous conflicting results, including opposite mitochondrial Ca^{2+} levels observed in LETM1-silenced cells[45,48,50,56,57], more experimental evidence is required to firmly establish the functional role of LETM1 as a component of the mitochondrial Ca^{2+} efflux machinery.

Importantly, mHCX and mNCX could not be the unique molecular pathways aimed to extrude Ca^{2+} from the mitochondrial matrix. It has also been proposed that, under certain conditions, the transient opening of the mPTP (mitochondrial permeability transition pore) might represent an alternative Ca^{2+} efflux pathway[58], although other observations question this hypothesis[59].

[H1] Regulation of Mitochondrial Ca^{2+}

Molecular studies have revealed that the heterogeneity of the mitochondrial Ca^{2+} machinery is associated with an equally variable regulatory system, which operates at multiple levels to maintain physiological Ca^{2+} homeostasis. Indeed, many proteins have been reported to be genuine regulators of Ca^{2+} uptake by acting on specific molecular targets or controlling essential mitochondrial functions (Table 1).

Due to its strategic position, VDAC is the preferential target for endogenous proteins located at the interface between mitochondria and the cytoplasm, including the **Bcl-2 family** members[60]. For example, Bcl-xL interacts with VDAC1 and VDAC3, but not VDAC2, shaping mitochondrial Ca^{2+} entry by favoring Ca^{2+} transfer across the OMM[61]. VDAC gating requires a voltage-dependent transition between the closed and open states, but the closed VDAC conformation shows higher cation-selectivity and Ca^{2+} permeability[62]. Thus, Bcl-xL should promote VDAC closure, as previously suggested[63], thereby enhancing mitochondrial Ca^{2+} accumulation. However, this aspect remains largely unclear, since it has been reported that i) Bcl-xL promotes the open VDAC state, rather than the closed configuration[64]; ii) VDAC could assume a cation-selective open conformation[65]; and iii) it has been reported that Bcl-xL inhibits VDAC1 activity, thus lowering, rather than increasing, the mitochondrial $[Ca^{2+}]$[66].

Several intra-mitochondrial proteins have been suggested to regulate Ca^{2+} signaling by altering the MCU complex functions. The first in chronological order is MCUR1 (MCU regulator 1, also known as CCDC90a). It has been shown that MCUR1 binds MCU at the matrix interface and that MCUR1 knockdown abolishes Ca^{2+} uptake in intact cells[67]. The interaction between MCU and MCUR1 has been reported in other studies[68-70], and MCUR1 was recently proposed to have a role as a scaffold factor in the assembly of the uniporter complex[71]. Conversely, when a proteomic assay was used to identify components of the MCU complex, MCUR1 was not recognized[20], and the yeast *Saccharomyces cerevisiae*, which lacks any uniporter activity, possesses an MCUR1 ortholog, named fmp32, suggesting a function for MCUR1 outside the MCU complex. Moreover, MCUR1/CCDC90a has been described as a co-factor in the assembly of the respiratory chain, rather than the uniporter, indicating that the reduction of Ca^{2+} uptake observed in MCUR1-deleted cells may be due to alteration of the mitochondrial membrane potential[72]. Therefore, the idea that the MCUR1-related Ca^{2+} defect, ascribed to modulation of MCU function, is secondary to mitochondrial membrane depolarization caused by respiratory chain disorders cannot be ruled out. Notably, in hepatocarcinoma cells, MCUR1 appears to regulate Ca^{2+} entry in an uniporter-dependent manner, although forced MCUR1 silencing induces a decrease in $\Delta\Psi$[73]. This mitochondrial potential drop has been attributed to a concomitant trigger of apoptosis[73], but, in our opinion, it is inconsistent with exclusive inhibition of MCU activity, since genetic manipulation of the pore-forming subunit has never been linked to cell death induction.

Similarly to MCUR1, SLC25A23 has been proposed as a novel regulator of the uniporter[74], although it has a homolog in yeast (Sal1) and was originally described as a Ca^{2+}-regulated mitochondrial ATP-Mg/Pi carrier[75,76]. Other proteins, including canonical transient receptor potential 3 (TRPC3)[77] and uncoupling protein 2-3 (UCP2-3)[78], are involved in mitochondrial Ca^{2+} accumulation, most likely through MCU complex-independent mechanisms.

Several regulatory factors, shown in Table 1, have been reported to maintain the correct distance between the ER and mitochondria, thereby ensuring proper Ca^{2+} transfer. Particularly worthy of mention are two OMM proteins, mitofusin-2 (MFN2) and PDZD8. MFN2 was originally characterized as an ER-mitochondria tether[79], but its role was strongly contested[80], and it is still unclear whether MFN2 promotes or inhibits ER-mitochondria contacts. PDZD8, previously known as a regulator of retroviral infection[81], has recently been described as the long-sought mammalian ortholog of the yeast Mmm1 protein, which is a component of the ER-mitochondria encounter structure at ER-mitochondria contact sites and coordinates Ca^{2+} exchange exclusively via its ER-mitochondria tethering role[82].

Post-translational modifications represent additional layers in the regulation of mitochondrial Ca^{2+} handling. The first example of phosphorylation events capable of finely tuning mitochondrial Ca^{2+} homeostasis is attributed to the PKC family[83]. Recently, a regulatory pathway for Ca^{2+} efflux has been identified[84]: both PINK1 and PKA positively regulate NCLX, and inhibition of Ca^{2+} release in PINK1 KO cells could be reverted by PKA-mediated NCLX phosphorylation[84]. Intriguingly, PINK1 is also able to boost mitochondrial Ca^{2+} extrusion by phosphorylating LETM1, the putative mHCX, at Thr192 (REF [85]).

Both VDAC function and interactions with other molecular partners are affected by multiple phosphorylation events. In high-fat conditions, GSK3-mediated VDAC phosphorylation at a threonine residue is drastically reduced, and its permeability to Ca^{2+} is increased, leading to disruption of the VDAC–Bcl-xL complex[86].

The uniporter complex is also subjected to phosphorylation events. The proline-rich tyrosine kinase 2 (Pyk2) can target MCU, promoting the formation of tetrameric channels[87]. In addition, during heart disease, a matricial pool of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylates MCU at two sites (Ser57 and Ser92), resulting in a higher Ca^{2+} response[88]. Interestingly, phosphorylation of the N-terminal residue 92 seems to be critical for MCU activity[68]. However, subsequent electrophysiological studies failed to confirm the regulatory effect of CaMKII on MCU[89].

No phosphorylation events have been associated with the other uniporter complex components, although MICU1 can be methylated by protein arginine methyl transferase 1 (PRMT1)[90]. Moreover, the m-AAA protease (AFG3L2/SPG7) has been shown to degrade unassembled EMRE, thus ensuring the correct stoichiometry between the different complex subunits and preserving uniporter activity[91,92]. Overall, these observations demonstrate that mitochondrial Ca^{2+} uptake and efflux are regulated on multiple levels. An aberration in a single regulatory mechanism could result in the harmful remodeling of Ca^{2+} mitochondrial Ca^{2+} fluxes, which in turn can lead to changes in cellular Ca^{2+} homeostasis and specific pathological phenotypes (see next sections). Therefore, it is not surprising that multiple disease-associated micro-RNAs are emerging as crucial players in the control of mitochondrial [Ca^{2+}]. Indeed, decreases in the gene expression of both *VDAC*, by miR-7 (REF [93]) and miR-29a[94], and *MCU*, by miR-25 (REF [95-97]), miR-138 (REF [97]), miR-1 (REF [98]), and miR-340 (REF [99]), are thought to be key molecular events underlying different pathological contexts.

Mitochondria in Ca^{2+} Homeostasis

309 Three main functional roles have been attributed to mitochondrial Ca^{2+} uptake: i) stimulation
310 of ATP production by activation of **mitochondrial dehydrogenases**, ii) regulation of cell
311 death and iii) spatiotemporal remodeling of intracellular Ca^{2+}. The first two aspects have
312 obvious implications in multiple physio-pathological processes and will be discussed in the
313 section below.
314 Numerous correlative studies performed in different cellular types have suggested that
315 mitochondria shape the intracellular Ca^{2+} response both locally and in the bulk cytoplasm.
316 However, these observations have been obtained using chemical compounds with low
317 specificity or having a strong impact on mitochondrial functions and thus on the whole
318 cellular metabolism, producing some spurious and controversial results[100]. The molecular
319 characterization of Ca^{2+} influx and efflux pathways has provided new evidence supporting the
320 concept that mitochondria can act as cytosolic Ca^{2+}-buffering systems. During IP3-mediated
321 ER Ca^{2+} release, the peak amplitude of the cytosolic Ca^{2+} response is significantly lower in
322 cells where the mitochondrial Ca^{2+} uptake capacity was increased by MCU over-expression[14].
323 Notably, the elevated cytoplasmic Ca^{2+} levels observed in PDZD8-depleted neurons, where
324 ER-mitochondria tethering is disrupted, could be related to reduced mitochondrial Ca^{2+}
325 buffering activity[82]. Similar findings have been obtained in NCLX-silenced **astrocytes**[101],
326 although this effect is more pronounced upon capacitative Ca^{2+} influx, rather than after ER
327 Ca^{2+} depletion. Thus, mitochondria promptly take up cytoplasmic Ca^{2+}, regardless of whether
328 it derives from internal stores (ER) or the extracellular space.
329 The knockdown of both UCP2 and MCU, with consequent reduction of mitochondrial Ca^{2+}
330 uptake, strongly inhibits SOCE by limiting aggregation of STIM1 and activation of ORAI1, the
331 pore-forming subunit of the CRAC (Ca^{2+} release-activated Ca^{2+} channels) at the plasma
332 membrane[102]. These findings reveal that the Ca^{2+} buffering capacity of mitochondria
333 positioned at the opening of IP3Rs could represent a pivotal mechanism in the modulation of
334 SOCE, as previously suggested[103].
335 The cytosolic [Ca^{2+}] could also affect the function of both ER-resident proteins (IP3R)[104] and
336 store-operated Ca^{2+} channels[105], thus implying a regulatory role for mitochondria due to the
337 buffering of local Ca^{2+} rises. High cytosolic Ca^{2+} levels in the area surrounding the channels
338 inhibit their activity, further reducing Ca^{2+} release through the IP3R or Ca^{2+} entry by ORAI1.
339 The strategic positioning of mitochondria lowers the [Ca^{2+}], preventing negative feedback and
340 sustaining channel activity. Indeed, MCU loss induces inefficient mitochondrial Ca^{2+} buffering
341 capacity, which in turn limits CRAC function by enhancing Ca^{2+}-mediated slow inactivation of
342 the channels[106]. Accordingly, the number of cytosolic Ca^{2+} oscillations, generated by discharge

343 of Ca^{2+} from IP3-sensitive stores, is significantly lower in MCU knockdown cells, which reflects
344 IP3R inhibition due to impaired mitochondrial Ca^{2+} uptake[106]. Overall, by controlling Ca^{2+}
345 channel functions and collecting large amounts of Ca^{2+} in specific subcellular areas,
346 mitochondria have the ability to preclude the propagation of Ca^{2+} waves, thereby regulating
347 specific cellular processes that depend (or that are regulated) by Ca^{2+} (REF [107]). For example,
348 in neonatal cardiomyocytes, reducing mitochondrial Ca^{2+} uptake results in a prominent
349 increase in the amplitude of beat-to-beat cytosolic Ca^{2+} oscillations, which in turn contributes
350 to extend the contraction[108].
351 However, several aspects require more clarification, including the complex relationship
352 between mitochondrial Ca^{2+} uptake and SOCE. For example, in breast cancer cell lines, it has
353 been observed that abolition of mitochondrial Ca^{2+} accumulation by MCU depletion could
354 reduce[109] or marginally increase[110] SOCE. Although different experimental conditions might
355 explain some contradictory results, other important factors should be taken in account,
356 including the number of mitochondria and their subcellular distribution, which could vary
357 substantially depending on cell type or condition[38,111], or the impact of other organelles in the
358 local buffering of cytoplasmic Ca^{2+} (REF [112,113]). Moreover, NCLX could also mediate redox
359 control of SOCE that is independent from mitochondrial Ca^{2+} buffering capacity. Upon ER Ca^{2+}
360 depletion, a rapid rise in cytosolic Na^{+} occurs, promoting NCLX activity and mitochondrial
361 Ca^{2+} efflux, which in turn results in a reduction of matrix [Ca^{2+}] and ROS generation. This
362 pathway is essential for sustaining capacitative Ca^{2+} influx, since prolonged ROS production is
363 associated to SOCE inhibition by oxidation of Orai1 at Cysteine 195 (REF [114]).
364

365 **[H1] Pathophysiology of Mitochondrial Ca^{2+}**

366 The concept that mitochondrial Ca^{2+} is a crucial regulator of cell life and death originates i)
367 from its ability to activate oxidative metabolism, mitochondrial respiration and ATP synthesis
368 by stimulating three Ca^{2+}-sensitive matrix dehydrogenases[115] and ii) from the fact that
369 intracellular Ca^{2+} deregulation can trigger either necrosis, apoptosis or autophagy[116-118]. This
370 dual role explains how mitochondrial Ca^{2+} has prominent implications in multiple physio-
371 pathological processes. Among them, here we will focus on i) insulin secretion, ii) heart
372 functionality, and iii) inflammation to highlight as, in different tissues, highly differentiated
373 and specialized, mitochondrial Ca^{2+} homeostasis acts as a master regulator of cellular
374 functions. Nevertheless, recent preclinical and clinical data have indicated that mitochondrial
375 Ca^{2+} derangement is a novel feature of cancer pathology (BOX 3).

376 ***[H3] Insulin secretion and links to diabetes***

377 Pancreatic beta cells (β-cells) are the body's sole source of circulating insulin. β-cells are
378 specifically designed to synthesize and store large amounts of insulin[119], which is secreted
379 based on the demand of target tissues. In healthy individuals, β-cells sense changes in plasma
380 glucose concentration and respond by releasing corresponding amounts of insulin into the
381 bloodstream. Despite decades of research, the molecular mechanisms underlying the
382 activation of β-cells are not yet clearly defined.

383 Nutrient secretagogues, especially glucose, initiate downstream signals enabling β-cells to
384 break down sugar and release insulin by stimulating mitochondrial energy metabolism[120]. It is
385 assumed that glucose mobilizes Ca^{2+} from the ER store. The consequent formation of Ca^{2+}
386 microdomains at nearby mitochondria permits efficient mitochondrial Ca^{2+} uptake via the
387 MCU complex, followed by Ca^{2+}-triggered ATP production. The resulting shift in the cytosolic
388 ATP:ADP ratio leads to the closure of ATP-sensitive K^{+} (K_{ATP}) channels on the plasma
389 membrane of β-cells, eliciting plasma membrane depolarization. Once a threshold potential is
390 reached, voltage-gated Ca^{2+} channels in the plasma membranes of β-cells open, generating
391 individual Ca^{2+} microdomains beneath the plasma membrane[121].

392 The main role of the increase in sub-plasma membrane Ca^{2+} is to permit insulin release[122],
393 possibly through the activation and migration of protein kinase C (PKC) β-II to the surface of
394 secretory vesicles localized in that area, although the details of the mechanism are still not
395 fully understood[123]. Moreover, mitochondria that are strategically situated close to plasma
396 membrane Ca^{2+} channels are able to sense microdomains of high Ca^{2+} concentration in their
397 proximity, take up Ca^{2+} through the MCU complex and fuel the exocytotic process by
398 producing ATP.

399 Thus, the mitochondrial Ca^{2+} machinery plays a fundamental physiological role in glucose-
400 mediated insulin secretion[124], both by initiating and sustaining the process. In this scenario,
401 the generation of two different types of microdomains with high Ca^{2+} concentrations — at the
402 interface with ER and the plasma membrane — that are associated with two pools of
403 mitochondria allows acceleration of Ca^{2+}-dependent mitochondrial metabolism and the
404 regulation of the two secretion stages independently: mitochondria forming MAMs are
405 fundamental in initiating the secretion process upon the release of Ca^{2+} from ER channels,
406 including RyRs[125], whereas mitochondria forming PAMs are tightly associated with the cell
407 surface and seem to be more important in sustaining and amplifying the phase of insulin
408 secretion[124], upon Ca^{2+} entry from the extracellular space (Fig. 3a).

Defects in mitochondrial Ca^{2+} homeostasis within pancreatic β-cells, with a consequent reduction of mitochondrial ATP production[126] and thus impaired insulin secretion[127], are considered one of the causal factors in the etiology of both type 1 and type 2 diabetes[128].
Chronic ER Ca^{2+} depletion due to leaky RyR channels is responsible for decreased mitochondrial Ca^{2+} uptake and β-cell failure, representing a potent risk factor for the development of diabetes[125]. Therefore, strategies to rejuvenate or replace these cells, as well as those to restore an efficient mitochondrial Ca^{2+} response, represent promising therapeutic approaches for these diseases and their complications. Both the MCU channel[57] and its regulatory partner MICU1 (REF [129]) from the MCU complex have been indicated to play a fundamental role in the β-cell feed-forward mechanism of Ca^{2+} entry into mitochondria, which guarantees insulin secretion and thus represents a promising therapeutic target for diabetes.
Recently, it has been demonstrated how the enhancement of K^+ flux across the ER membrane through ER-localized TALK-1 channels facilitates Ca^{2+} release from the ER, improving mitochondrial ATP production. These observations revealed TALK-1 as a possible therapeutic target to reduce ER Ca^{2+} handling defects and thus restore correct mitochondrial Ca^{2+} homeostasis in β-cells during the pathogenesis of diabetes[130].

[H3] Cardiac cell functions and heart failure

Calcium is of vital importance in maintaining cardiac cell function during the cardiac cycle (excitation, contraction or diastole, and relaxation or systole). Meanwhile, it also plays a key role in the pathology of heart failure, being responsible for cardiac cell death via apoptotic and necrotic pathways[131].
Under physiological conditions, Ca^{2+} signaling in the heart exerts three main functions controlling the so-called excitation-contraction coupling (*EC coupling*), excitation-transcription coupling (*ET coupling*) and excitation-metabolism coupling (*EM coupling*) mechanisms. While EC and ET coupling are governed essentially by cytosolic Ca^{2+} transients that drive contraction and cardiac muscle genes activation or inactivation, mitochondrial Ca^{2+} contributes to local control of oxidative metabolism (EM coupling), generating the ATP needed to power cardiac excitation and contraction during every heartbeat[132].
In mammalian cardiac cells, the cardiac cycle starts with the generation of an automatic **action potential** (AP) in a group of specialized cells, named sinoatrial (SA) nodal cells, responsible for initiating the electrical cardiac impulse needed for contraction. The AP begins with a change in the voltage of the cell membranes of heart cells that becomes more positive, mainly due to the opening of sodium channels, which allow Na^+ to flow into the cell. This

443 depolarization, also called the “funny” current (for its property of being activated by very
444 negative membrane potentials), permits a threshold potential of about −50 mV to be reached,
445 inducing a progressive opening of L-T Ca^{2+} channels (LTCCs) in the sarcolemmal membrane.
446 The consequent cytosolic Ca^{2+} influx through LTCCs is sufficient to regulate and activate
447 mitochondrial functions and thus ATP production[133], through the generation of Ca^{2+}
448 microdomains around nearby mitochondria. Calcium influx through LTCCs also triggers Ca^{2+}
449 release from the nearby junctional SR via IP3Rs and RyRs in a process known as Ca^{2+}-induced
450 Ca^{2+} release (CICR), a crucial process for cardiac muscle contraction[134]. The high local
451 cytosolic [Ca^{2+}] generated during the CICR process initiates contraction by binding **troponin
452 C** on **myofilaments** and contributes to mitochondrial metabolism, permitting Ca^{2+} uptake into
453 mitochondria[135]. A rapid increase in mitochondrial Ca^{2+} is essential for telegraphing the
454 enhanced metabolic demand for ATP to promote oxidative phosphorylation. Thus,
455 mitochondrial Ca^{2+} is fundamental to providing the necessary link between ATP supply and
456 demand during cardiomyocyte contraction (Fig. 3b).
457 In this context, the MCU is critical for boosting heartbeat frequency by favoring rapid Ca^{2+}
458 mitochondrial uptake during the cardiac cycle[108]. Only the generation of well-defined
459 mitochondrial Ca^{2+} microdomains, either at the entrance of voltage-gated Ca^{2+} channels at the
460 plasma membrane (sarcolemma) after LTCC opening or in close proximity to RyRs in the SR
461 during CICR events, can explain mitochondrial Ca^{2+} uptake in cardiac myocytes. Importantly,
462 MCU-enhanced oxidative phosphorylation is also required for reloading SR Ca^{2+} stores and
463 sustaining fight or flight heart rate increases in response to energy demand[136].
464 To date, it remains controversial whether variations in the mitochondrial [Ca^{2+}] occur quickly
465 in a beat-to-beat fashion, taking place synchronously with cytosolic Ca^{2+} fluctuations, or
466 whether Ca^{2+} uptake could occur slowly[137]. Undoubtedly, mitochondria sense cytosolic Ca^{2+}
467 signals and transform them into mitochondrial energy production, and they are far from being
468 only passive Ca^{2+} sinks. Thus, there is an emerging consensus regarding the existence of
469 subpopulations of mitochondria exposed to high levels of [Ca^{2+}] with the ability to take up
470 Ca^{2+} on a beat-to-beat basis[138]. It is possible that the new mechanistic understanding of the
471 MCU functions and advancements in Ca^{2+} measurement technologies (BOX 2) could help solve
472 this disagreement.
473 As mitochondrial Ca^{2+} is fundamental to maintaining the ATP supply in myocardial cells,
474 disrupted cardiomyocyte Ca^{2+} homeostasis is recognized as a major contributor to the heart
475 failure phenotype[139]. Acute heart diseases, i.e., ischemia/reperfusion (I/R) injury, are mainly
476 attributed to mitochondrial Ca^{2+} overload together with increased production of reactive

477 oxygen species (ROS) caused by an overshoot of the mitochondrial respiratory chain activity
478 that, driving the activation of the mitochondrial permeability transition pore (mPTP), lead to
479 necrotic and apoptotic cardiac cell death. Thus, targeting the regulatory systems of
480 mitochondrial Ca^{2+} homeostasis, such as the MCU complex and NCLX, as well as limiting ROS
481 generation and mPTP activation could represent a potential therapeutic strategy to combat
482 these pathologies.
483 The use of mPTP inhibitors, such as cyclosporin A, has been reported in experimental studies
484 to reduce myocardial infarction (MI) size and preserve cardiac function. However, in clinical
485 trials, opposite effects were observed by the same group upon administration of cyclosporin
486 A, thus failing to demonstrate a final and conclusive benefit for clinical outcomes[140,141].
487 Therefore, more specific and novel mPTP inhibitors, based on new findings regarding the
488 molecular composition of mPTP[142] are required to translate mPTP inhibition as a
489 cardioprotective strategy into clinical practice.
490 In parallel, other strategies are being explored. Cardiac-specific MCU-KO or dominant
491 negative MCU-expressing mice showed a drastic reduction of mitochondrial Ca^{2+} uptake and
492 were strongly protected against I/R injury[143-145], revealing MCU as a potential new target for
493 cardioprotective drug design. In agreement with this view, the increased MCU current
494 induced by activation of CaMKII during I/R promotes mPTP opening and myocardial death[88].
495 Thus, although this pathway is debated (see section above), mitochondrial-targeted CaMKII
496 inhibition might be another strategy to prevent or reduce myocardial death and heart failure.
497 In addition to MCU, it has been recently identified how Ca^{2+} efflux is essential for maintaining
498 cardiac cellular function. Targeting NCLX by increasing its activity is sufficient to prevent
499 mitochondrial Ca^{2+} overload and to limit mPTP opening, with a consequent reduction in MI
500 size and cell death after I/R injury[47]. These data suggest that augmenting Ca^{2+} efflux is another
501 promising therapeutic strategy in this type of disease.
502 Furthermore, ROS-targeted therapies, although they have yielded mixed results in attenuating
503 I/R-induced damage[146], could also be considered an available alternative, possibly in
504 combination with the other aforementioned treatments.
505 Importantly, the regulation of contact sites between the SR and mitochondria also has a role
506 in the cardiac I/R process. In particular, down-regulation of the tyrosine phosphatase
507 PTPIP51 (protein tyrosine phosphatase-interacting protein 51), a crucial regulator of ER/SR-
508 mitochondria contacts (Table 1), protects cardiomyocytes from mitochondrial Ca^{2+} overload
509 and death, highlighting its role as a new therapeutic target during I/R injury[147].

In chronic heart failure, a major myocyte dysfunction is related to stasis and accumulation of cytoplasmic Ca^{2+}. This defect in failing cells largely results from decreased expression and activity of the SR Ca^{2+}-ATPase[148] and increased RyR Ca^{2+} leakage (in particular the type 2 receptor RyR2), due to their redox modifications[149] or PKA hyperphosphorylation[150,151].

The consequent amount of Ca^{2+} that accumulates in the cytoplasm results in continuous and persistent asynchronous mitochondrial Ca^{2+} overload and dysfunction, contributing to heart failure and myocyte cell death. In these conditions, less Ca^{2+} can be released from the SR upon LTCC activation, resulting in a decreased magnitude of Ca^{2+} transients, as well as reduced ATP supply, with a consequent contractile dysfunction[152]. At the same time, SR Ca^{2+} leakage is sufficient to induce spontaneous APs and is therefore considered an important trigger for cardiac arrhythmias[153]. Overall, these findings further support that targeting both MCU and NCLX to prevent mitochondrial Ca^{2+} overload in combination with systems to reduce SR Ca^{2+} mishandling could represent good strategies for intervention in these types of heart dysfunctions.

[H3]Inflammatory responses and pathological inflammation

The first evidence that mitochondrial Ca^{2+} is important during inflammation dates back more than 30 years[154], but increasing interest in this area has only become evident since the molecular identity of the MCU complex and localization of the **NLRP3 inflammasome** at mitochondria[155] were elucidated.

Studies have highlighted how chronic stress enhances mitochondrial Ca^{2+} accumulation, which in turn induces excessive and sustained inflammation. Mitochondrial Ca^{2+} homeostasis is reported to be disrupted in infectious diseases, where MCU seems to be the main player in the regulation of bacteria- and virus-induced activation of inflammation.

Pseudomonas aeruginosa infection of airway epithelial cells from patients with cystic fibrosis (CF) drives excessive MCU-mediated mitochondrial Ca^{2+} accumulation, which is critical for the activation of a heightened NLRP3-dependent inflammatory response. The relationship between the *P. aeruginosa*-dependent inflammatory response and mitochondrial Ca^{2+} perturbation exacerbates the CF pathological phenotype[156]. Loss of MCU *in vitro* has been shown to reduce mitochondrial Ca^{2+} uptake and to blunt activation of the NLRP3 inflammasome induced by *Pseudomonas*[156].

MCU-mediated Ca^{2+} overload was also found to be essential for a virus-induced inflammatory response. Indeed, MCU specifically interact with MAVS (mitochondria anti-viral signaling) complexes localized on mitochondria and positively regulates the release of the pro-

544 inflammatory cytokine IFN-β upon viral infection[157]. Chronic viral infection is accompanied by
545 ER stress, inducing mitochondrial Ca^{2+} overload through a MCU/MAVS-dependent pathway,
546 with subsequent sustained IFN-β production that contribute to autoimmune diseases.
547 Knockdown or silencing of MCU (or MAVS) reduces mitochondrial Ca^{2+} uptake capacity and
548 significantly decreases virus-induced IFN-β levels[157] and thus the inflammatory status.
549 The importance of regulating MCU under stress conditions, when the risk of Ca^{2+} overload
550 is elevated, has also been reported in a model of the inflammatory response induced after
551 tissue injury/loss in the liver. During liver regeneration after partial hepatectomy, loss of
552 MICU1, the Ca^{2+}-sensing regulator of MCU, leads to an enhanced and sustained pro-
553 inflammatory response**,** associated with mitochondrial Ca^{2+} overload that switches the fate of
554 the cell from proliferation to death[158]. The mitochondrial Ca^{2+} overload response is followed
555 by mPTP opening, sensitizing MICU1-deficient hepatocytes to death instead of permitting
556 cell proliferation and regeneration[158].
557 These findings all support the notion that increased Ca^{2+} flux through the uniporter complex
558 fuels important pathogenic pathways related to an inflammatory state, identifying this
559 channel as a potential target in the treatment of inflammation-associated diseases.
560
561 **Future Perspectives**
562 A large body of evidence has accumulated regarding the molecular basis of mitochondrial Ca^{2+}
563 homeostasis, a pivotal regulator of many cellular functions: it plays a crucial role in regulating
564 mitochondrial functions and adapting mitochondrial activity to cellular needs but it is also
565 tightly coupled to the cytosolic Ca^{2+} changes induced by a variety of stimuli. Importantly,
566 genetic or environmental alterations in intracellular Ca^{2+} signaling are linked to many human
567 diseases, including common disorders. Thus, a complete understanding of the pathways
568 allowing mitochondrial Ca^{2+} entry and release is crucial for the characterization of the
569 molecular routes that are activated on the basis of different cellular functions of Ca^{2+}.
570 Future research will uncover many other aspects of mitochondrial Ca^{2+} homeostasis. In
571 particular, we will attempt to gain a complete understanding of mitochondrial Ca^{2+} regulation.
572 This includes i) the identification of the related functions of the newly discovered accessory
573 proteins and post-translational modifications of mitochondrial Ca^{2+} channels and
574 transporters; ii) a broader investigation of the involvement of mitochondrial Ca^{2+} homeostasis
575 in genomics, proteomics, and metabolomics for a definition of the “omic” signature of
576 mitochondrial Ca^{2+} signaling; and iii) the development of new drugs targeting mitochondrial
577 Ca^{2+} pathways.

578 Moreover, the role of mitochondrial Ca^{2+} signaling in many human disorders will be further
579 clarified through the identification of specific mutations in the proteins responsible for the
580 maintenance of mitochondrial Ca^{2+} homeostasis. For example, considering the role of Ca^{2+}
581 signaling and mitochondria in neurons, the mechanisms of diseases such as multiple sclerosis,
582 Alzheimer's disease, and Parkinson's disease as well as those with more complex phenotypes,
583 such as psychiatric disorders, will be better understood and new therapeutic approaches
584 proposed. Moreover, the contribution of mitochondrial Ca^{2+} in cancer-specific environmental
585 settings will be clarified opening the possibility to modulate mitochondrial Ca^{2+} homeostasis
586 to enhance the efficacy of cytotoxic agents.
587 Finally, the participation of mitochondrial Ca^{2+} in the process of aging[159] and its importance in
588 stem cell biology[160] will produce new and exciting achievements in the future and will attract
589 scientists from other fields to this fascinating and still innovative area of mitochondrial
590 biology.
591
592 **BOX 1**
593 **Calcium as a second messenger**
594 Ca^{2+} ions are ubiquitous second messengers that allow the information conveyed by
595 extracellular and intracellular signals to be translated into an intracellular effect. The
596 versatility and complexity of Ca^{2+}-mediated signaling can be inferred by the simple
597 observation that a rise in cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) is elicited by a wide variety of molecules
598 and decoded into very different, sometimes opposite effects. To fulfill such a complex
599 signaling role, the $[Ca^{2+}]_c$ rises follow precise spatiotemporal patterns. Indeed, the study of
600 Ca^{2+} signaling at the single-cell level has revealed the occurrence of spatially controlled rises.
601 The localized rises can remain confined, thereby preventing the inappropriate stimulation of
602 different cellular domains, or gradually diffuse, as in the case of the "Ca^{2+} waves", i.e., the
603 orderly propagations of $[Ca^{2+}]_c$ rises throughout the cell. The complexity is not limited to
604 spatial organization. Indeed, it is now well known that agonists, such as histamine, ATP or
605 carbachol, can induce, in many cell types, a series of repetitive $[Ca^{2+}]_c$ increases, commonly
606 referred to as "Ca^{2+} oscillations". Each of these signaling patterns, through the specific
607 recruitment of downstream effectors, is decoded into the appropriate cellular effect.
608 A wide range of mammalian proteins are regulated by Ca^{2+}, classified as buffer or trigger
609 proteins[161]. Ca^{2+} buffers encompass Calsequestrin and Calreticulin, located at sarcoplasmatic
610 and endoplasmatic reticulum, respectively, and the cytosolic proteins Calbindin and
611 Calretinin, as well as the relatively slow buffer Parvalbumin. This class of molecules not only

612 cooperate with Ca^{2+} channels, transporters and pumps for shaping the intracellular Ca^{2+}
613 signals, but also display multiple functions and physiological roles[162]. Trigger proteins include
614 modulators of muscle contraction (Troponin C), proteases (Calpain), kinases (Protein Kinase
615 C, PKC) phosphatases (Calcineurin B), or key mediators of different enzymes (Calmodulin).
616 Most of them possess one or multiple EF-hand calcium-binding motifs and change their
617 conformation upon binding Ca^{2+}, two exceptional features that have been employed for the
618 creation of intra-organellar Ca^{2+} fluorescent indicators (see Box 2). Thus, Ca^{2+} binds to a huge
619 number of proteins, affecting their localizations, molecular associations and functions, and it
620 provides an active regulation of vast array of biological processes, such as contraction, gene
621 transcription, and differentiation. The role of Ca^{2+} signals in the intracellular transcriptional
622 activity, by regulating key factors as NFAT and CREB, strengthens its impact on cellular
623 plasticity and long-term changes. In this view, the crucial function of Ca^{2+} in coordinating
624 different cellular events is not limited to variations in its cytosolic levels, but could be
625 extended to changes in Ca^{2+} signaling inside the organelles. For example, lysosomal Ca^{2+}
626 activates Calcineurin, which in turn promotes TFEB (Transcription Factor EB) translocation
627 into the nucleus and transactivation of target genes[112]. After fertilization, mitochondrial Ca^{2+}
628 entry sustains ROS production and cell cycle progression in early *Xenopus* embryos[163]. These
629 findings reveal as spatial micro-rearrangements of Ca^{2+} homeostasis have important
630 biological consequences and that aberrations in the intracellular Ca^{2+} machinery could
631 represent the crucial aspect in many pathological scenarios.
632

633 **BOX 2**

634 **Methods for measuring mitochondrial Ca^{2+}**

635 There are two main genetically encoded strategies currently used to design functional probes
636 that measure mitochondrial [Ca^{2+}]: those based on the calcium-activated photoprotein
637 aequorin and those based on fluorescent protein (FP). Using appropriate mitochondria-
638 targeting signals, aequorin has been directed to both the OMM and IMS, although the most
639 commonly used version is the aequorin chimera targeted to the mitochondrial matrix by the
640 pre-sequence of subunit VIII of cytocrome c oxidase[164]. Aequorin provides important benefits
641 over GFP-based indicators, such as a wide dynamic range, a high signal-to-noise ratio, and the
642 ability to emit light upon Ca^{2+} binding rather than requiring potentially damaging light
643 excitation. However, the use of aequorin displays some pitfalls, including low light emission
644 by the photoprotein that renders it inappropriate for imaging Ca^{2+} waves at the single-cell
645 level. These disadvantages have led to the extensive employment of alternative methods, such

646 as FP-based approaches, which combine bright fluorescence with efficient targeting, or
647 synthetic fluorescent dyes, which do not require transfection and are very user-friendly.
648 Rhod-2 AM is the most commonly used chemical probe, although it cannot be precisely
649 targeted to mitochondria, and its measurement exhibits multiple drawbacks[165].
650 Mitochondrial fluorescent Ca^{2+}-indicators are based on a calmodulin/Ca^{2+}-sensing protein,
651 and they are classified into two families, the first represented by the Förster resonance energy
652 transfer (FRET)–based cameleon type, and the second by an engineered single FP type, such
653 as GCaMP and pericam. Mitochondrial-targeted cameleons (2mt-D2cpv and its variants),
654 consisting of cyan and yellow FPs as the donor and acceptor, linked by calmodulin and the
655 M13 peptide from the myosin light-chain kinase, and mito-pericam, built up by combining a
656 circularly permutated FP and a Ca^{2+}-responsive element, allow ratiometric Ca^{2+}
657 measurements; the development of mito-GCaMP chimeras, and their derivatives mito-CEPIAs
658 and mito-GECOs, has expanded the spectra for the analysis of mitochondrial [Ca^{2+}].
659 Intriguingly, by fusing GFP and aequorin, a new class of ratiometric Ca^{2+} probes, termed GAPs,
660 has been generated[166]. GAP indicators have been targeted to various organelles, including
661 mitochondria, but the performance of mito-GAP constructs has not been tested yet by other
662 groups.
663
664 **BOX 3**
665 **Mitochondrial Ca^{2+} in cell death and cancer**
666 There is no doubt that cell death belongs to the numerous cell functions on which Ca^{2+} exerts
667 a complex regulatory role. It has long been known that in neurons and other cell types an
668 unchecked increase in $[Ca^{2+}]_c$ can trigger apoptosis[167-169], and likewise, agents able to release
669 Ca^{2+} from intracellular stores have been shown to be pro-apoptotic[170]. On the other hand, the
670 inhibition of Ca^{2+} signalling removes a crucial trophic effect and agents, such as ethanol, that
671 block NMDA-dependent Ca^{2+} signalling cause massive neuronal apoptosis during brain
672 development[171].
673 As to the site of action of the "apoptotic" Ca^{2+} signal, mitochondria again emerge as a critical
674 site. While transient mitochondrial Ca^{2+} oscillations stimulate metabolism and constitute a
675 pro-survival signal, prolonged mitochondrial Ca^{2+} overload is a fundamental trigger to initiate
676 apoptosis through the opening of mPTP[116,118]. Indeed, treatment with apoptotic stimuli causes
677 a release of Ca^{2+} from the ER and induces dramatic changes of mitochondrial morphology and
678 release of caspase co-factors. If Ca^{2+} changes are prevented, mitochondrial morphology is
679 preserved, and the cells are protected from apoptosis[118].

680 The mitochondrial Ca^{2+} machinery represents thus a key decoding station for cell fate
681 decisions. Several proto-oncogenes and tumor suppressors critically control these decisions
682 by modulating mitochondrial Ca^{2+} homeostasis.
683 By either controlling Ca^{2+} signals arising from the ER (e.g., PML, PTEN, p53, Akt) or directly
684 modifying the activity of mitochondrial proteins involved in Ca^{2+} homeostasis (e.g., FHIT,
685 STAT, Fus1), they are able to modulate anti- or pro-apoptotic signals, preventing or
686 facilitating mitochondrial Ca^{2+} overload.
687 In response to different stress signals, tumor suppressors and proto-oncogenes act at the ER
688 level by modulating Ca^{2+} store content and/or Ca^{2+} dynamics (e.g., Ca^{2+} leakage, Ca^{2+} uptake
689 and Ca^{2+} release)[172], while at mitochondria, by affecting the MCU complex or LETM1 activities,
690 they regulate the mitochondrial Ca^{2+} threshold[173,174].
691 During the past few years, it has become evident that the failure of this tuned mitochondrial
692 Ca^{2+} signaling is a hallmark of cancer cells that favors their survival and augments their
693 resistance to cell death.
694 Inactivating mutations in tumor suppressor genes or hyper-activation of oncogenes lead, in
695 many types of cancer, to diminished mitochondrial Ca^{2+} uptake and therefore increased pro-
696 survival potential, with a consequent inhibition of apoptosis and uncontrolled cell
697 proliferation.
698 Restoring proper mitochondrial Ca^{2+} signaling could therefore be a promising avenue for re-
699 sensitizing cancer cells to their intrinsic pro-apoptotic signaling mechanisms and to
700 anticancer therapeutic interventions. Indeed, many chemotherapeutic agents, as well as
701 photodynamic therapy[175,176], exert their cytotoxic effects via ER-mitochondrial Ca^{2+} signaling,
702 and therefore their action is completely abolished in cancer cells displaying altered Ca^{2+}
703 kinetics.
704

705 **Figure Legends**

706 **Figure 1: Intracellular Ca^{2+} signaling**

707 In the cartoon is reported a basic overview of calcium signaling.
708 The ER is the major intracellular Ca^{2+} storage organelle. The sarco/endoplasmic-
709 reticulum Ca^{2+}-ATPase SERCAs actively pump Ca^{2+} into the store. The dynamic release of Ca^{2+}
710 from the ER is mediated by the ryanodine receptor (RyR) and the inositol 1,4,5-triphosphate
711 receptor (IP3R). Ca^{2+} released from the ER is captured by the nearby mitochondria located in
712 close contact with the ER through the voltage-dependent anion channel VDAC and the MCU
713 complex, activating cell metabolism.

The depletion of the ER results in the translocation of the transmembrane Ca^{2+} sensor protein, stromal interaction molecule 1 (STIM1) to the junctions between ER and the plasma membrane where it binds and activates the Ca^{2+} channel protein Orai1 for store-operated Ca^{2+} entry (SOCE). The intracellular Ca^{2+} influx is also mediated by TRP channels, most of them are activated by the depletion of Ca^{2+} from the ER, whereas the plasma membrane Ca^{2+} ATPase (PMCAs) function to export Ca^{2+} from the cytosol and maintain the intracellular Ca^{2+} concentration at the basal value for proper cell signaling. Beside ER, lysosomes have been recently recognized as the second largest store of intracellular Ca^{2+} that are able to release Ca^{2+} through the TRPML1 channel, crucial for maintaining a correct lysosomal membrane trafficking.

Figure 2: The Mitochondrial Ca^{2+} Uptake Pathway

The formation of high [Ca^{2+}] microdomains between the endoplasmic reticulum (ER) and mitochondria is critical for ensuring proper Ca^{2+} entry into the mitochondrial matrix. **a)** The correct distance between the ER and mitochondria (~50 nm) is preserved by different regulators, including mitofusin-2 (MFN2), PDZD8 and the complex VAPB (vesicle-associated membrane protein-associated protein B)-PTPIP51 (protein tyrosine phosphatase-interacting protein 51). Once released by inositol 1,4,5 trisphosphate receptors (IP3Rs), the ER Ca^{2+} enters into mitochondria through the OMM (outer mitochondrial membrane) protein VDAC (voltage-dependent anion channel) and thus reach the matrix side via the MCU (mitochondrial Ca^{2+} uniporter) complex, located at the IMM (inner mitochondrial membrane). **b)** The MCU complex consists of the pore-forming subunit MCU and the transmembrane proteins MCUb (MCU regulatory subunit b) and EMRE (essential MCU regulator) in association with the intermembrane space (IMS) proteins MICU1 (mitochondrial calcium uptake protein 1) and MICU2. MCUR1 (MCU regulator 1) regulates Ca^{2+} entry from the matrix, but its role as a specific MCU complex component is highly controversial. **c)** The activity of the electron transport chain (ETC) results in the pumping of H^+ ions outside the mitochondrial matrix, thereby generating an electrochemical proton gradient. This gradient consists of two components: the difference between the cytosolic and matrix pH and the membrane potential difference ($\Delta\Psi$), which is maintained at approximately -180 mV and represents the driving force for mitochondrial Ca^{2+} uptake. **d)** Mitochondria contain both Na^+-dependent and Na^+-independent mechanisms for Ca^{2+} extrusion toward the cytoplasm. The molecular nature of the Na^+/Ca^{2+} exchanger (mNCX) has been identified in NCLX, an IMM protein containing 13 transmembrane domains and catalyzing K^+-independent electrogenic transport. LETM1

748 (leucine zipper EF-hand containing transmembrane protein 1) has been proposed as the
749 H^+/Ca^{2+} exchanger (mHCX). It acts as a transporter by forming hexameric structures and
750 pumps Ca^{2+} ions in an electroneutral manner. However, it has been suggested that LETM1
751 works as a K^+/H^+ exchanger, rather than an mHCX. Very recent findings have shown that
752 LETM1 contributes to Na^+ cycling, thus modulating Ca^{2+} fluxes in an indirect way[177].
753 PKA: protein kinase A; PINK1: PTEN-induced putative kinase 1.
754
755 **Figure 3: The role of mitochondrial Ca^{2+} in physio-pathological processes.**
756 The generation of high Ca^{2+} microdomains (MAMs: mitochondria associates membranes (with
757 ER) or PAMs: plasma membrane-associated membranes) is fundamental to permit
758 mitochondrial Ca^{2+} uptake through the MCU complex. This figure proposes a schematic
759 representation of the signals that happen inside the cells in response to **a)** glucose stimulation
760 (pancreatic β cell), **b)** action potential (AP) (cardiac cell) and **c)** infections or tissue injury
761 (epithelial cell). **a)** Glucose [1] induces the release of Ca^{2+} from the ER through inositol 1,4,5
762 trisphosphate receptors (IP3Rs) towards mitochondria [2] that start producing ATP [3]. The
763 increased metabolisms permit the opening of Ca^{2+} channels on the plasma membrane [4] and
764 in turn insulin release [5]. **b)** In the cardiac cell, the AP induces the opening of Na^+ channels on
765 the plasma membrane [1] followed by the opening of L-T Ca^{2+} channels [2]. The cytosolic
766 increased of Ca^{2+} is captured by mitochondria to produce ATP [3] and induces the Ca^{2+}-
767 induced Ca^{2+} release (CICR) with a consequent release of Ca^{2+} from the ER through ryanodine
768 receptors (RyRs) that permits contraction [4] and sustains mitochondrial metabolism and
769 ATP production [5]. **c)** Different chronic stress situations, including tissue damage or infection
770 [1] induce ER stress [2] with a consequent prolonged Ca^{2+} transfer towards mitochondria and
771 mitochondrial Ca^{2+} overload [3].
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782 **Table 1: Regulatory pathways of mitochondrial Ca^{2+}**

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Regulator	Ca^{2+} Regulation	Molecular Mechanism	Disease links and reference(s)
VDAC channels			
*** Bcl-xL**	Positive	Interacts with VDAC1-3, probably promoting VDAC closure and calcium permeability	- [61]
*** Bcl-xL**	Negative	Inhibits VDAC1 through its BH4 domain	- [66]
GSK3	Negative	Phosphorylates VDAC at a Thr residue	Liver steatosis [86]
miR-7	Negative	Reduces *VDAC1* expression and inhibits mPTP opening	Parkinson's disease [93]
miR-29a	- (Probably negative)	Reduces *VDAC1* expression and improves survival upon ischaemia	Cerebral ischemia [94]
Uniporter complex			
*** MCUR1**	Positive	Interacts with MCU, promoting Ca^{2+} entry	Hepatocellular carcinoma [67-70,73]
*** SLC25A23**	Positive	Interacts with MCU, promoting Ca^{2+} entry	- [74]
PyK2	Positive	Activated PyK2 translocates to mitochondria, phosphorylating MCU and favoring tetrameric channel pore formation	Myocardial death [87]
CaMK-II	Positive	Phosphorylates MCU at Ser57 and Ser92	Myocardial death and heart failure [88]
miR-25	Negative	Reduces the expression of the pore-forming subunit *MCU*	Colon and prostate cancer; pulmonary arterial hypertension (PAH) [95-97]
miR-138	Negative	Reduces the expression of the pore-forming subunit *MCU*	Pulmonary arterial hypertension (PAH) [97]
miR-1	Negative	Reduces the expression of the pore-forming subunit *MCU*	Cardiac hypertrophy [98]
miR-340	Negative	Reduces *MCU* expression and inhibits breast cancer cell migration	Breast cancer [99]
PRMT1	Negative	Methylates MICU1, allowing the engagement of UCP2/3 in Ca^{2+} entry	- [90]
AFG3L2/SPG7	Negative	Loss of AFG3L2/SPG7 induces the formation of constitutively active MCU-EMRE complexes	Neuro-degeneration [91,92]
Mia40	Negative	Mia40 ensures the association of MICU1 with the inhibitory subunit MICU2	- [19]
ER-mitochondria tethering			
*** MFN2**	Positive	MFN2 loss detaches the ER from mitochondria	Charcot-Marie-Tooth neuropathy type 2A (CMT2A); obesity, insulin resistance [79,178]
*** MFN2**	Negative	MFN2 loss increases the ER-mitochondria association	Charcot-Marie-Tooth neuropathy type 2A (CMT2A); obesity, insulin resistance

			80,179
PDZD8	Positive	PDZD8 is a structural and functional ortholog of the yeast ERMES protein MMM1	- [82]
PTPIP51	Positive	Interacts with the ER protein VAPB to regulate ER-mitochondria tethering	I/R injury; amyotrophic lateral sclerosis (ALS); Parkinson's disease [147,180-182]
GRP75	Positive	Forms a molecular complex with IP3R and VDAC	- [183]
FATE1	Negative	Regulates Ca^{2+} transfer and steroid hormone production	Adrenocortical carcinoma [184]
Presenilin-2	Positive	Increases the frequency of Ca^{2+} hot spots at MAMs	Alzheimer's disease [185]
Fis1	Positive	Interacts with Bap31 at the ER, forming a complex to activate the initiator procaspase-8	- [186]
PACS-2	Positive	PACS-2 loss detaches the ER from mitochondria	Obesity, insulin resistance [187,188]

787
788

Mitochondrial Ca^{2+} efflux			
PINK1	Negative	PINK1 KO cells display reduced mitochondrial Ca^{2+} efflux. PINK1 increases Ca^{2+} release by phosphorylating LETM1 at Thr192	Parkinson's disease [84,85]
PKA	Negative	Phosphorylates NCLX at Ser258, increasing Ca^{2+} efflux	Parkinson's disease [84]

789

Mitochondrial membrane potential			
*** MCUR1 (CCDC90a)**	Positive	Acts as a cytochrome c oxidase (COX) assembly factor	- [72]
SK2 channel	Negative	Activation of mitochondrial SK2 reduces respiration and ROS formation	- [189]

790
791 The * indicates proteins with a controversial role
792 Abbreviations: VDAC (Voltage Dependent Anion Channel), Bcl-xL (B-cell Lymphoma-extra
793 large), GSK3 (Glycogen Synthase Kinase 3), mPTP (mitochondrial Permeability Transition
794 Pore), MCUR1 (Mitochondrial Calcium Uniporter Regulator 1), MCU (Mitochondrial Calcium
795 Uniporter), SLC25A23 (Solute Carrier Family 25 Member 23), Pyk2 (proline-rich tyrosine
796 kinase 2), CaMK-II (Ca2+ /calmodulin-dependent protein kinase II), PRMT1 (Protein arginine
797 N-methyltransferase 1), MICU1 (Mitochondrial Calcium Uptake 1), UCP2/3 (Mitochondrial
798 uncoupling protein 2/3), AFG3L2 (AFG3 Like Matrix AAA Peptidase Subunit 2), SPG7 (Spastic
799 paraplegia 7), Mia40 (Mitochondrial intermembrane space import and assembly protein 40),

800 MICU2 (Mitochondrial Calcium Uptake 2), MFN2 (Mitofusin 2), ER (Endoplasmic Reticulum),
801 PDZD8 (PDZ domain-containing protein 8), PTPIP51 (Protein tyrosine phosphatase
802 interacting protein 51), VAPB (Vesicle-associated membrane protein-associated protein B/C),
803 GRP75 (Glucose Regulated Protein 75), IP3R (Inositol 1,4,5 Trisphosphate Receptor), FATE1
804 (Fetal and adult testis-expressed transcript protein 1), MAMs (Mitochondria Associated
805 Membranes), Fis1 (Mitochondrial fission 1 protein), Bap31 (B-cell receptor-associated
806 protein 31), PACS-2 (Phosphofurin acidic cluster sorting protein 2), PINK1 (PTEN-induced
807 putative kinase 1), LETM1 (Leucine zipper-EF-hand containing transmembrane protein 1),
808 PKA (Protein Kinase A), NCLX (Sodium/Calcium/Lithium Exchanger), CCDC90a (Coiled- Coil
809 Domain-Containing Protein 90A), COX (Cytochrome c oxidase), SK2 (Potassium
810 intermediate/small conductance calcium-activated channel).
811

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820

821 **Glossary**
822

823 **Membrane potential**

824 The difference in electric potential (measured in mV) between the interior and exterior of a
825 biological membrane, which generates from different concentrations of ions, such as H^+, Na^+,
826 K^+ and Cl^-.
827

828 **Respiratory chain**

829 The Electron Transport Chain (ETC) consists of four complexes that transfer electrons from
830 NADH and FADH2 to oxygen, which is reduced to water. Electron flow within these
831 transmembrane complexes leads to the transport of H^+ across the inner mitochondrial
832 membrane (IMM), generating an electrochemical proton gradient (negative inside the matrix).
833

834 **Chemiosmotic theory**

835 The energy stored in the form of transmembrane electrochemical gradient is used to produce
836 ATP inside the mitochondrial matrix. The protons move back across the IMM through the F_1
837 F_o ATPase enzyme, coupling the electrochemical gradient to ATP production by combining
838 ADP with inorganic phosphate.

839

840 **Bcl-2 family**

841 A large group of evolutionarily-conserved proteins that shares Bcl-2 homology (BH) domains.
842 Bcl-2 family members are deeply involved in cell death regulation, consisting of both anti-
843 (Bcl-2, Bcl-xL) and pro-apoptotic (Bax, Bak) factors.

844

845 **Mitochondrial dehydrogenases**

846 A subclass of oxidoreductase enzymes that oxidizes substrates by transferring H^+ to electron
847 acceptors, such as NAD^+, $NADP^+$ or FAD. Four dehydrogenases are regulated by Ca^{2+} ions,
848 increasing ATP supply during energy demand, which include: i) the FAD-glycerol phosphate
849 dehydrogenase, which faces the intermembrane space (IMS) of mitochondria; ii) the pyruvate
850 dehydrogenase, iii) the NAD-isocitrate dehydrogenase and iv) the oxoglutarate
851 dehydrogenase, all found in the mitochondrial matrix.

852

853 **Astrocytes**

854 The most numerous and heterogeneous neuroglial cells in the central nervous system,
855 distinguished by a star-like morphology with multiple primary processes originating from the
856 soma. The cytoskeleton of astrocytes is formed by intermediate filaments, commonly used as
857 specific markers for astrocytes identification, such vimentin and GFAP (glial fibrillary acidic
858 protein).

859

860 **Action potential**

861 Consists in a movement of charge sufficient to generate a large and brief deviation in the
862 membrane potential. It is used to communicate information between neurons and from
863 neurons to muscle fibers.

864

865 **Troponin C**

866 A component of the Troponin complex, together with Troponin I and T, which regulates
867 muscle contraction by Ca^{2+} binding. Through its multiple EF-hand domains, Troponin C acts as

868 the Ca^{2+} sensor inside the complex, initiating the cascade of events that leads to contraction of
869 striated muscle by interacting with its partner Troponin I after Ca^{2+} binding.
870

871 **Myofilaments**

872 The principal molecular regulators of contraction in cardiac and skeletal muscles, responsible
873 for force generation and motion. The myofilaments consist primarily of thick filament myosin
874 and thin filament actin proteins, as well as additional components, including Troponin, Titin
875 and Nebulin.
876

877 **NLRP3 inflammasome**

878 Upon activation, the nucleotide-binding oligomerization domain-like receptors family pyrin
879 domain containing 3 (NLRP3) sensor associates with the adaptor molecular apoptosis-
880 associated speck-like protein containing a caspase-recruitment domain (ASC) and the
881 precursor pro-caspase 1, to form the NLRP3 inflammasome complex, leading to activation of
882 caspase-1, secretion of mature pro-inflammatory cytokines and induction of inflammatory cell
883 death (or pyroptosis).
884

885 **Key points**

886

887 - **Mitochondria are the master regulators of intracellular Ca^{2+} homeostasis.**
888 - **Mitochondrial Ca^{2+} entry and extrusion are regulated by a sophisticated**
889 **molecular toolkit.**
890 - **Mitochondria are strictly associated with the Endoplasmic Reticulum (ER), and**
891 **accumulate Ca^{2+} via a multimeric complex, termed Mitochondrial Calcium**
892 **Uniporter, using the potential generated across the inner membrane, reaching**
893 **Ca^{2+} values several fold higher than those of bulk cytosol.**
894 - **Mitochondria release Ca^{2+} back to the cytosol via Na^{+}-dependent or -independent**
895 **mechanisms**
896 - **Different molecular pathways converge on mitochondria, exerting their**
897 **physiopathological functions through modulation of Ca^{2+} homeostasis.**
898 - **Mitochondrial Ca^{2+} channels and transporters are now considered new**
899 **pharmacological targets in different pathological scenarios.**

900

901 **References**
902

903 1 Berridge, M. J., Lipp, P. & Bootman, M. D. The versatility and universality of calcium
904 signalling. *Nat Rev Mol Cell Biol* **1**, 11-21, doi:10.1038/35036035
905 35036035 [pii] (2000).
906 2 Carafoli, E. Historical review: mitochondria and calcium: ups and downs of an unusual
907 relationship. *Trends Biochem Sci* **28**, 175-181, doi:S0968-0004(03)00053-7 [pii]
908 10.1016/S0968-0004(03)00053-7 (2003).
909 3 Slater, E. C. & Cleland, K. W. The calcium content of isolated heart-muscle sarcosomes.
910 *Biochem J* **54**, xxii (1953).
911 **Demonstrates, together with references 4 and 5, that mitochondria bound Ca^{2+} actively.**
912 4 Deluca, H. F. & Engstrom, G. W. Calcium uptake by rat kidney mitochondria. *Proc Natl*
913 *Acad Sci U S A* **47**, 1744-1750 (1961).
914 5 Vasington, F. D. & Murphy, J. V. Ca ion uptake by rat kidney mitochondria and its
915 dependence on respiration and phosphorylation. *J Biol Chem* **237**, 2670-2677 (1962).
916 6 Prakriya, M. & Lewis, R. S. Store-Operated Calcium Channels. *Physiol Rev* **95**, 1383-
917 1436, doi:95/4/1383 [pii]
918 10.1152/physrev.00020.2014 (2015).
919 7 Shoshan-Barmatz, V. & Mizrachi, D. VDAC1: from structure to cancer therapy. *Front*
920 *Oncol* **2**, 164 (2012).
921 8 De Stefani, D. *et al.* VDAC1 selectively transfers apoptotic Ca2+ signals to mitochondria.
922 *Cell Death Differ* **19**, 267-273, doi:cdd201192 [pii]
923 10.1038/cdd.2011.92 (2012).
924 9 Shimizu, H. *et al.* Mitochondrial Ca(2+) uptake by the voltage-dependent anion channel
925 2 regulates cardiac rhythmicity. *Elife* **4**, doi:10.7554/eLife.04801 (2015).
926 10 Kamer, K. J. & Mootha, V. K. The molecular era of the mitochondrial calcium uniporter.
927 *Nat Rev Mol Cell Biol* **16**, 545-553, doi:nrm4039 [pii]
928 10.1038/nrm4039 (2015).
929 11 Marchi, S. & Pinton, P. The mitochondrial calcium uniporter complex: molecular
930 components, structure and physiopathological implications. *J Physiol* **592**, 829-839,
931 doi:jphysiol.2013.268235 [pii]
932 10.1113/jphysiol.2013.268235 (2014).
933 12 Foskett, J. K. & Philipson, B. The mitochondrial Ca(2+) uniporter complex. *J Mol Cell*
934 *Cardiol* **78**, 3-8, doi:S0022-2828(14)00375-7 [pii]
935 10.1016/j.yjmcc.2014.11.015 (2015).
936 13 Baughman, J. M. *et al.* Integrative genomics identifies MCU as an essential component
937 of the mitochondrial calcium uniporter. *Nature* **476**, 341-345, doi:nature10234 [pii]
938 10.1038/nature10234 (2011).
939 14 De Stefani, D., Raffaello, A., Teardo, E., Szabo, I. & Rizzuto, R. A forty-kilodalton protein
940 of the inner membrane is the mitochondrial calcium uniporter. *Nature* **476**, 336-340,
941 doi:nature10230 [pii]
942 10.1038/nature10230 (2011).
943 **Demonstrates, together with reference 13, that MCU/CCDC109a is the pore-forming**
944 **subunit of the uniporter complex.**
945 15 Raffaello, A. *et al.* The mitochondrial calcium uniporter is a multimer that can include a
946 dominant-negative pore-forming subunit. *EMBO J* **32**, 2362-2376, doi:emboj2013157
947 [pii]
948 10.1038/emboj.2013.157 (2013).
949 16 Perocchi, F. *et al.* MICU1 encodes a mitochondrial EF hand protein required for Ca(2+)
950 uptake. *Nature* **467**, 291-296 (2010).

951 17 Plovanich, M. *et al.* MICU2, a paralog of MICU1, resides within the mitochondrial
952 uniporter complex to regulate calcium handling. *PLoS One* **8**, e55785,
953 doi:10.1371/journal.pone.0055785
954 PONE-D-12-28515 [pii] (2013).
955 18 Patron, M. *et al.* MICU1 and MICU2 finely tune the mitochondrial Ca2+ uniporter by
956 exerting opposite effects on MCU activity. *Mol Cell* **53**, 726-737, doi:S1097-
957 2765(14)00074-4 [pii]
958 10.1016/j.molcel.2014.01.013 (2014).
959 19 Petrungaro, C. *et al.* The Ca(2+)-Dependent Release of the Mia40-Induced MICU1-
960 MICU2 Dimer from MCU Regulates Mitochondrial Ca(2+) Uptake. *Cell Metab* **22**, 721-
961 733, doi:S1550-4131(15)00410-6 [pii]
962 10.1016/j.cmet.2015.08.019 (2015).
963 20 Sancak, Y. *et al.* EMRE is an essential component of the mitochondrial calcium
964 uniporter complex. *Science* **342**, 1379-1382, doi:science.1242993 [pii]
965 10.1126/science.1242993 (2013).
966 21 Vecellio Reane, D. *et al.* A MICU1 Splice Variant Confers High Sensitivity to the
967 Mitochondrial Ca2+ Uptake Machinery of Skeletal Muscle. *Mol Cell* **64**, 760-773,
968 doi:S1097-2765(16)30623-2 [pii]
969 10.1016/j.molcel.2016.10.001 (2016).
970 22 Csordas, G. *et al.* MICU1 controls both the threshold and cooperative activation of the
971 mitochondrial Ca(2)(+) uniporter. *Cell Metab* **17**, 976-987, doi:S1550-4131(13)00193-
972 9 [pii]
973 10.1016/j.cmet.2013.04.020 (2013).
974 23 Mallilankaraman, K. *et al.* MICU1 is an essential gatekeeper for MCU-mediated
975 mitochondrial Ca(2+) uptake that regulates cell survival. *Cell* **151**, 630-644, doi:S0092-
976 8674(12)01225-1 [pii]
977 10.1016/j.cell.2012.10.011 (2012).
978 **Demonstrates, together with reference 22, as MICU1 controls the threshold for MCU**
979 **activation.**
980 24 Liu, J. C. *et al.* MICU1 Serves as a Molecular Gatekeeper to Prevent In Vivo
981 Mitochondrial Calcium Overload. *Cell Rep* **16**, 1561-1573, doi:S2211-1247(16)30897-X
982 [pii]
983 10.1016/j.celrep.2016.07.011 (2016).
984 25 Oxenoid, K. *et al.* Architecture of the mitochondrial calcium uniporter. *Nature* **533**,
985 269-273, doi:nature17656 [pii]
986 10.1038/nature17656 (2016).
987 26 Vais, H. *et al.* EMRE Is a Matrix Ca(2+) Sensor that Governs Gatekeeping of the
988 Mitochondrial Ca(2+) Uniporter. *Cell Rep* **14**, 403-410, doi:S2211-1247(15)01495-3
989 [pii]
990 10.1016/j.celrep.2015.12.054 (2016).
991 27 Yamamoto, T. *et al.* Analysis of the structure and function of EMRE in a yeast
992 expression system. *Biochim Biophys Acta* **1857**, 831-839, doi:S0005-2728(16)30066-4
993 [pii]
994 10.1016/j.bbabio.2016.03.019 (2016).
995 28 Tsai, M. F. *et al.* Dual functions of a small regulatory subunit in the mitochondrial
996 calcium uniporter complex. *Elife* **5**, doi:10.7554/eLife.15545 (2016).
997 29 Chiurillo, M. A. *et al.* Different Roles of Mitochondrial Calcium Uniporter Complex
998 Subunits in Growth and Infectivity of Trypanosoma cruzi. *MBio* **8**, doi:mBio.00574-17
999 [pii]
1000 10.1128/mBio.00574-17 (2017).

1001 30 Kirichok, Y., Krapivinsky, G. & Clapham, D. E. The mitochondrial calcium uniporter is a
1002 highly selective ion channel. *Nature* **427**, 360-364, doi:10.1038/nature02246
1003 nature02246 [pii] (2004).
1004 31 Bragadin, M., Pozzan, T. & Azzone, G. F. Kinetics of Ca2+ carrier in rat liver
1005 mitochondria. *Biochemistry* **18**, 5972-5978 (1979).
1006 32 Babcock, D. F., Herrington, J., Goodwin, P. C., Park, Y. B. & Hille, B. Mitochondrial
1007 participation in the intracellular Ca2+ network. *J Cell Biol* **136**, 833-844 (1997).
1008 33 Friel, D. D. & Tsien, R. W. An FCCP-sensitive Ca2+ store in bullfrog sympathetic neurons
1009 and its participation in stimulus-evoked changes in [Ca2+]i. *J Neurosci* **14**, 4007-4024
1010 (1994).
1011 34 Rizzuto, R. *et al.* Close contacts with the endoplasmic reticulum as determinants of
1012 mitochondrial Ca2+ responses. *Science* **280**, 1763-1766 (1998).
1013 **Demonstrates the importance of ER-mitochondria contacts in prompt mitochondrial**
1014 **Ca^{2+} accumulation.**
1015 35 Gaigg, B., Simbeni, R., Hrastnik, C., Paltauf, F. & Daum, G. Characterization of a
1016 microsomal subfraction associated with mitochondria of the yeast, Saccharomyces
1017 cerevisiae. Involvement in synthesis and import of phospholipids into mitochondria.
1018 *Biochim Biophys Acta* **1234**, 214-220, doi:0005-2736(94)00287-Y [pii] (1995).
1019 36 Willson, V. J. & Tipton, K. F. Purification and characterization of ox brain NAD+-
1020 dependent isocitrate dehydrogenase. *J Neurochem* **33**, 1239-1247 (1979).
1021 37 Giorgi, C. *et al.* Mitochondria-associated membranes: composition, molecular
1022 mechanisms, and physiopathological implications. *Antioxid Redox Signal* **22**, 995-1019,
1023 doi:10.1089/ars.2014.6223 (2015).
1024 38 Giacomello, M. *et al.* Ca2+ hot spots on the mitochondrial surface are generated by
1025 Ca2+ mobilization from stores, but not by activation of store-operated Ca2+ channels.
1026 *Mol Cell* **38**, 280-290, doi:S1097-2765(10)00280-7 [pii]
1027 10.1016/j.molcel.2010.04.003 (2010).
1028 39 Suski, J. M. *et al.* Isolation of plasma membrane-associated membranes from rat liver.
1029 *Nat Protoc* **9**, 312-322, doi:nprot.2014.016 [pii]
1030 10.1038/nprot.2014.016 (2014).
1031 40 Jung, D. W., Baysal, K. & Brierley, G. P. The sodium-calcium antiport of heart
1032 mitochondria is not electroneutral. *J Biol Chem* **270**, 672-678 (1995).
1033 41 Gunter, K. K., Zuscik, M. J. & Gunter, T. E. The Na(+)-independent Ca2+ efflux
1034 mechanism of liver mitochondria is not a passive Ca2+/2H+ exchanger. *J Biol Chem*
1035 **266**, 21640-21648 (1991).
1036 42 Scorziello, A. *et al.* NCX3 regulates mitochondrial Ca(2+) handling through the
1037 AKAP121-anchored signaling complex and prevents hypoxia-induced neuronal death. *J*
1038 *Cell Sci* **126**, 5566-5577, doi:jcs.129668 [pii]
1039 10.1242/jcs.129668 (2013).
1040 43 Palty, R. *et al.* NCLX is an essential component of mitochondrial Na+/Ca2+ exchange.
1041 *Proc Natl Acad Sci U S A* **107**, 436-441, doi:0908099107 [pii]
1042 10.1073/pnas.0908099107 (2010).
1043 **Demonstrates as NCLX/SLC8B1 is the mitochondrial Na^{+}/Ca^{2+} exchanger.**
1044 44 Crompton, M., Kunzi, M. & Carafoli, E. The calcium-induced and sodium-induced
1045 effluxes of calcium from heart mitochondria. Evidence for a sodium-calcium carrier.
1046 *Eur J Biochem* **79**, 549-558 (1977).
1047 45 De Marchi, U. *et al.* NCLX protein, but not LETM1, mediates mitochondrial Ca2+
1048 extrusion, thereby limiting Ca2+-induced NAD(P)H production and modulating matrix
1049 redox state. *J Biol Chem* **289**, 20377-20385, doi:M113.540898 [pii]
1050 10.1074/jbc.M113.540898 (2014).

1051 46 Kim, B., Takeuchi, A., Hikida, M. & Matsuoka, S. Roles of the mitochondrial Na(+)-
1052 Ca(2+) exchanger, NCLX, in B lymphocyte chemotaxis. *Sci Rep* **6**, 28378, doi:srep28378
1053 [pii]
1054 10.1038/srep28378 (2016).
1055 47 Luongo, T. S. *et al.* The mitochondrial Na(+)/Ca(2+) exchanger is essential for Ca(2+)
1056 homeostasis and viability. *Nature* **545**, 93-97, doi:nature22082 [pii]
1057 10.1038/nature22082 (2017).
1058 **Demonstrates the crucial role of NCLX in heart failure.**
1059 48 Jiang, D., Zhao, L. & Clapham, D. E. Genome-wide RNAi screen identifies Letm1 as a
1060 mitochondrial Ca2+/H+ antiporter. *Science* **326**, 144-147, doi:326/5949/144 [pii]
1061 10.1126/science.1175145 (2009).
1062 49 Waldeck-Weiermair, M. *et al.* Leucine zipper EF hand-containing transmembrane
1063 protein 1 (Letm1) and uncoupling proteins 2 and 3 (UCP2/3) contribute to two
1064 distinct mitochondrial Ca2+ uptake pathways. *J Biol Chem* **286**, 28444-28455,
1065 doi:M111.244517 [pii]
1066 10.1074/jbc.M111.244517 (2011).
1067 50 Doonan, P. J. *et al.* LETM1-dependent mitochondrial Ca2+ flux modulates cellular
1068 bioenergetics and proliferation. *FASEB J* **28**, 4936-4949, doi:fj.14-256453 [pii]
1069 10.1096/fj.14-256453 (2014).
1070 51 Nowikovsky, K., Pozzan, T., Rizzuto, R., Scorrano, L. & Bernardi, P. Perspectives on: SGP
1071 symposium on mitochondrial physiology and medicine: the pathophysiology of LETM1.
1072 *J Gen Physiol* **139**, 445-454, doi:jgp.201110757 [pii]
1073 10.1085/jgp.201110757 (2012).
1074 52 Froschauer, E., Nowikovsky, K. & Schweyen, R. J. Electroneutral K+/H+ exchange in
1075 mitochondrial membrane vesicles involves Yol027/Letm1 proteins. *Biochim Biophys*
1076 *Acta* **1711**, 41-48, doi:S0005-2736(05)00062-3 [pii]
1077 10.1016/j.bbamem.2005.02.018 (2005).
1078 53 Nowikovsky, K. *et al.* The LETM1/YOL027 gene family encodes a factor of the
1079 mitochondrial K+ homeostasis with a potential role in the Wolf-Hirschhorn syndrome.
1080 *J Biol Chem* **279**, 30307-30315, doi:10.1074/jbc.M403607200
1081 M403607200 [pii] (2004).
1082 54 Hashimi, H., McDonald, L., Stribrna, E. & Lukes, J. Trypanosome Letm1 protein is
1083 essential for mitochondrial potassium homeostasis. *J Biol Chem* **288**, 26914-26925,
1084 doi:M113.495119 [pii]
1085 10.1074/jbc.M113.495119 (2013).
1086 55 Tsai, M. F., Jiang, D., Zhao, L., Clapham, D. & Miller, C. Functional reconstitution of the
1087 mitochondrial Ca2+/H+ antiporter Letm1. *J Gen Physiol* **143**, 67-73, doi:jgp.201311096
1088 [pii]
1089 10.1085/jgp.201311096 (2014).
1090 56 Shao, J. *et al.* Leucine zipper-EF-hand containing transmembrane protein 1 (LETM1)
1091 forms a Ca2+/H+ antiporter. *Sci Rep* **6**, 34174, doi:srep34174 [pii]
1092 10.1038/srep34174 (2016).
1093 57 Quan, X. *et al.* Essential role of mitochondrial Ca2+ uniporter in the generation of
1094 mitochondrial pH gradient and metabolism-secretion coupling in insulin-releasing
1095 cells. *J Biol Chem* **290**, 4086-4096, doi:M114.632547 [pii]
1096 10.1074/jbc.M114.632547 (2015).
1097 58 Elrod, J. W. *et al.* Cyclophilin D controls mitochondrial pore-dependent Ca(2+)
1098 exchange, metabolic flexibility, and propensity for heart failure in mice. *J Clin Invest*
1099 **120**, 3680-3687, doi:43171 [pii]
1100 10.1172/JCI43171 (2010).

1101 59 De Marchi, E., Bonora, M., Giorgi, C. & Pinton, P. The mitochondrial permeability
1102 transition pore is a dispensable element for mitochondrial calcium efflux. *Cell Calcium*
1103 **56**, 1-13, doi:S0143-4160(14)00053-0 [pii]
1104 10.1016/j.ceca.2014.03.004 (2014).
1105 60 Rostovtseva, T. K. & Bezrukov, S. M. VDAC regulation: role of cytosolic proteins and
1106 mitochondrial lipids. *J Bioenerg Biomembr* **40**, 163-170, doi:10.1007/s10863-008-
1107 9145-y (2008).
1108 61 Huang, H. *et al.* An interaction between Bcl-xL and the voltage-dependent anion
1109 channel (VDAC) promotes mitochondrial Ca2+ uptake. *J Biol Chem* **288**, 19870-19881,
1110 doi:M112.448290 [pii]
1111 10.1074/jbc.M112.448290 (2013).
1112 62 Tan, W. & Colombini, M. VDAC closure increases calcium ion flux. *Biochim Biophys Acta*
1113 **1768**, 2510-2515, doi:S0005-2736(07)00213-1 [pii]
1114 10.1016/j.bbamem.2007.06.002 (2007).
1115 63 Arbel, N., Ben-Hail, D. & Shoshan-Barmatz, V. Mediation of the antiapoptotic activity of
1116 Bcl-xL protein upon interaction with VDAC1 protein. *J Biol Chem* **287**, 23152-23161,
1117 doi:M112.345918 [pii]
1118 10.1074/jbc.M112.345918 (2012).
1119 64 Vander Heiden, M. G. *et al.* Bcl-xL promotes the open configuration of the voltage-
1120 dependent anion channel and metabolite passage through the outer mitochondrial
1121 membrane. *J Biol Chem* **276**, 19414-19419, doi:10.1074/jbc.M101590200
1122 M101590200 [pii] (2001).
1123 65 Pavlov, E. *et al.* The mitochondrial channel VDAC has a cation-selective open state.
1124 *Biochim Biophys Acta* **1710**, 96-102, doi:S0005-2728(05)00231-8 [pii]
1125 10.1016/j.bbabio.2005.09.006 (2005).
1126 66 Monaco, G. *et al.* The BH4 domain of anti-apoptotic Bcl-XL, but not that of the related
1127 Bcl-2, limits the voltage-dependent anion channel 1 (VDAC1)-mediated transfer of pro-
1128 apoptotic Ca2+ signals to mitochondria. *J Biol Chem* **290**, 9150-9161, doi:M114.622514
1129 [pii]
1130 10.1074/jbc.M114.622514 (2015).
1131 67 Mallilankaraman, K. *et al.* MCUR1 is an essential component of mitochondrial Ca2+
1132 uptake that regulates cellular metabolism. *Nat Cell Biol* **14**, 1336-1343, doi:ncb2622
1133 [pii]
1134 10.1038/ncb2622 (2012).
1135 68 Lee, Y. *et al.* Structure and function of the N-terminal domain of the human
1136 mitochondrial calcium uniporter. *EMBO Rep* **16**, 1318-1333, doi:embr.201540436 [pii]
1137 10.15252/embr.201540436 (2015).
1138 69 Vais, H. *et al.* MCUR1, CCDC90A, Is a Regulator of the Mitochondrial Calcium Uniporter.
1139 *Cell Metab* **22**, 533-535, doi:S1550-4131(15)00472-6 [pii]
1140 10.1016/j.cmet.2015.09.015 (2015).
1141 70 Chaudhuri, D., Artiga, D. J., Abiria, S. A. & Clapham, D. E. Mitochondrial calcium
1142 uniporter regulator 1 (MCUR1) regulates the calcium threshold for the mitochondrial
1143 permeability transition. *Proc Natl Acad Sci U S A* **113**, E1872-1880, doi:1602264113
1144 [pii]
1145 10.1073/pnas.1602264113 (2016).
1146 71 Tomar, D. *et al.* MCUR1 Is a Scaffold Factor for the MCU Complex Function and
1147 Promotes Mitochondrial Bioenergetics. *Cell Rep* **15**, 1673-1685, doi:S2211-
1148 1247(16)30477-6 [pii]
1149 10.1016/j.celrep.2016.04.050 (2016).

1150 72 Paupe, V., Prudent, J., Dassa, E. P., Rendon, O. Z. & Shoubridge, E. A. CCDC90A (MCUR1) is a cytochrome c oxidase assembly factor and not a regulator of the mitochondrial calcium uniporter. *Cell Metab* **21**, 109-116, doi:S1550-4131(14)00555-5 [pii]
1153 10.1016/j.cmet.2014.12.004 (2015).
1154 73 Ren, T. *et al.* MCUR1-Mediated Mitochondrial Calcium Signaling Facilitates Cell Survival of Hepatocellular Carcinoma via Reactive Oxygen Species-Dependent P53 Degradation. *Antioxid Redox Signal*, doi:10.1089/ars.2017.6990 (2017).
1157 74 Hoffman, N. E. *et al.* SLC25A23 augments mitochondrial Ca(2)(+) uptake, interacts with MCU, and induces oxidative stress-mediated cell death. *Mol Biol Cell* **25**, 936-947, doi:mbc.E13-08-0502 [pii]
1160 10.1091/mbc.E13-08-0502 (2014).
1161 75 del Arco, A. & Satrustegui, J. Identification of a novel human subfamily of mitochondrial carriers with calcium-binding domains. *J Biol Chem* **279**, 24701-24713, doi:10.1074/jbc.M401417200
1164 M401417200 [pii] (2004).
1165 76 Fiermonte, G. *et al.* Identification of the mitochondrial ATP-Mg/Pi transporter. Bacterial expression, reconstitution, functional characterization, and tissue distribution. *J Biol Chem* **279**, 30722-30730, doi:10.1074/jbc.M400445200
1168 M400445200 [pii] (2004).
1169 77 Feng, S. *et al.* Canonical transient receptor potential 3 channels regulate mitochondrial calcium uptake. *Proc Natl Acad Sci U S A* **110**, 11011-11016, doi:1309531110 [pii]
1171 10.1073/pnas.1309531110 (2013).
1172 78 Trenker, M., Malli, R., Fertschai, I., Levak-Frank, S. & Graier, W. F. Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca2+ uniport. *Nat Cell Biol* **9**, 445-452, doi:10.1038/ncb1556 (2007).
1175 79 de Brito, O. M. & Scorrano, L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456**, 605-610, doi:nature07534 [pii]
1177 10.1038/nature07534 (2008).
1178 80 Filadi, R. *et al.* Mitofusin 2 ablation increases endoplasmic reticulum-mitochondria coupling. *Proc Natl Acad Sci U S A* **112**, E2174-2181, doi:1504880112 [pii]
1180 10.1073/pnas.1504880112 (2015).
1181 81 Zhang, S. & Sodroski, J. Efficient human immunodeficiency virus (HIV-1) infection of cells lacking PDZD8. *Virology* **481**, 73-78, doi:S0042-6822(15)00057-4 [pii]
1183 10.1016/j.virol.2015.01.034 (2015).
1184 82 Hirabayashi, Y. *et al.* ER-mitochondria tethering by PDZD8 regulates Ca(2+) dynamics in mammalian neurons. *Science* **358**, 623-630, doi:358/6363/623 [pii]
1186 10.1126/science.aan6009 (2017).
1187 **Shows as mammalian PDZD8 is functionally equivalent to MMM1, a member of the yeast ER-mitochondria encounter structure (ERMES), which is the most well-defined ER-mitochondrial tethering complex.**
1190 83 Pinton, P., Leo, S., Wieckowski, M. R., Di Benedetto, G. & Rizzuto, R. Long-term modulation of mitochondrial Ca2+ signals by protein kinase C isozymes. *J Cell Biol* **165**, 223-232, doi:10.1083/jcb.200311061
1193 jcb.200311061 [pii] (2004).
1194 84 Kostic, M. *et al.* PKA Phosphorylation of NCLX Reverses Mitochondrial Calcium Overload and Depolarization, Promoting Survival of PINK1-Deficient Dopaminergic Neurons. *Cell Rep* **13**, 376-386, doi:S2211-1247(15)00992-4 [pii]
1197 10.1016/j.celrep.2015.08.079 (2015).

1198 85 Huang, E. *et al.* PINK1-mediated phosphorylation of LETM1 regulates mitochondrial
1199 calcium transport and protects neurons against mitochondrial stress. *Nat Commun* **8**,
1200 1399, doi:10.1038/s41467-017-01435-1
1201 10.1038/s41467-017-01435-1 [pii] (2017).
1202 86 Martel, C. *et al.* Glycogen synthase kinase 3-mediated voltage-dependent anion channel
1203 phosphorylation controls outer mitochondrial membrane permeability during lipid
1204 accumulation. *Hepatology* **57**, 93-102, doi:10.1002/hep.25967 (2013).
1205 87 O-Uchi, J. *et al.* Adrenergic signaling regulates mitochondrial Ca2+ uptake through
1206 Pyk2-dependent tyrosine phosphorylation of the mitochondrial Ca2+ uniporter.
1207 *Antioxid Redox Signal* **21**, 863-879, doi:10.1089/ars.2013.5394 (2014).
1208 88 Joiner, M. L. *et al.* CaMKII determines mitochondrial stress responses in heart. *Nature*
1209 **491**, 269-273, doi:nature11444 [pii]
1210 10.1038/nature11444 (2012).
1211 89 Fieni, F., Johnson, D. E., Hudmon, A. & Kirichok, Y. Mitochondrial Ca2+ uniporter and
1212 CaMKII in heart. *Nature* **513**, E1-2, doi:nature13626 [pii]
1213 10.1038/nature13626 (2014).
1214 90 Madreiter-Sokolowski, C. T. *et al.* PRMT1-mediated methylation of MICU1 determines
1215 the UCP2/3 dependency of mitochondrial Ca(2+) uptake in immortalized cells. *Nat*
1216 *Commun* **7**, 12897, doi:ncomms12897 [pii]
1217 10.1038/ncomms12897 (2016).
1218 91 Konig, T. *et al.* The m-AAA Protease Associated with Neurodegeneration Limits MCU
1219 Activity in Mitochondria. *Mol Cell* **64**, 148-162, doi:S1097-2765(16)30462-2 [pii]
1220 10.1016/j.molcel.2016.08.020 (2016).
1221 92 Tsai, C. W. *et al.* Proteolytic control of the mitochondrial calcium uniporter complex.
1222 *Proc Natl Acad Sci U S A* **114**, 4388-4393, doi:1702938114 [pii]
1223 10.1073/pnas.1702938114 (2017).
1224 93 Chaudhuri, A. D., Choi, D. C., Kabaria, S., Tran, A. & Junn, E. MicroRNA-7 Regulates the
1225 Function of Mitochondrial Permeability Transition Pore by Targeting VDAC1
1226 Expression. *J Biol Chem* **291**, 6483-6493, doi:M115.691352 [pii]
1227 10.1074/jbc.M115.691352 (2016).
1228 94 Stary, C. M., Sun, X., Ouyang, Y., Li, L. & Giffard, R. G. miR-29a differentially regulates cell
1229 survival in astrocytes from cornu ammonis 1 and dentate gyrus by targeting VDAC1.
1230 *Mitochondrion* **30**, 248-254, doi:S1567-7249(16)30159-3 [pii]
1231 10.1016/j.mito.2016.08.013 (2016).
1232 95 Marchi, S. *et al.* Downregulation of the mitochondrial calcium uniporter by cancer-
1233 related miR-25. *Curr Biol* **23**, 58-63, doi:S0960-9822(12)01370-X [pii]
1234 10.1016/j.cub.2012.11.026 (2013).
1235 96 Pan, L. *et al.* MiR-25 protects cardiomyocytes against oxidative damage by targeting
1236 the mitochondrial calcium uniporter. *Int J Mol Sci* **16**, 5420-5433, doi:ijms16035420
1237 [pii]
1238 10.3390/ijms16035420 (2015).
1239 97 Hong, Z. *et al.* MicroRNA-138 and MicroRNA-25 Down-regulate Mitochondrial Calcium
1240 Uniporter, Causing the Pulmonary Arterial Hypertension Cancer Phenotype. *Am J*
1241 *Respir Crit Care Med* **195**, 515-529, doi:10.1164/rccm.201604-0814OC (2017).
1242 98 Zaglia, T. *et al.* Content of mitochondrial calcium uniporter (MCU) in cardiomyocytes is
1243 regulated by microRNA-1 in physiologic and pathologic hypertrophy. *Proc Natl Acad*
1244 *Sci U S A* **114**, E9006-E9015, doi:1708772114 [pii]
1245 10.1073/pnas.1708772114 (2017).

99 Yu, C. *et al.* Mitochondrial calcium uniporter as a target of microRNA-340 and promoter of metastasis via enhancing the Warburg effect. *Oncotarget* **8**, 83831-83844, doi:10.18632/oncotarget.19747
19747 [pii] (2017).
100 Pizzo, P., Drago, I., Filadi, R. & Pozzan, T. Mitochondrial Ca(2)(+) homeostasis: mechanism, role, and tissue specificities. *Pflugers Arch* **464**, 3-17, doi:10.1007/s00424-012-1122-y (2012).
101 Parnis, J. *et al.* Mitochondrial exchanger NCLX plays a major role in the intracellular Ca2+ signaling, gliotransmission, and proliferation of astrocytes. *J Neurosci* **33**, 7206-7219, doi:33/17/7206 [pii]
10.1523/JNEUROSCI.5721-12.2013 (2013).
102 Deak, A. T. *et al.* IP3-mediated STIM1 oligomerization requires intact mitochondrial Ca2+ uptake. *J Cell Sci* **127**, 2944-2955, doi:jcs.149807 [pii]
10.1242/jcs.149807 (2014).
103 Gilabert, J. A., Bakowski, D. & Parekh, A. B. Energized mitochondria increase the dynamic range over which inositol 1,4,5-trisphosphate activates store-operated calcium influx. *EMBO J* **20**, 2672-2679, doi:10.1093/emboj/20.11.2672 (2001).
Shows the key role played by mitochondrial Ca^{2+} buffering in modulating SOCE (Store-Operated Ca^{2+} entry)
104 Rizzuto, R., De Stefani, D., Raffaello, A. & Mammucari, C. Mitochondria as sensors and regulators of calcium signalling. *Nat Rev Mol Cell Biol* **13**, 566-578, doi:nrm3412 [pii]
10.1038/nrm3412 (2012).
105 Parekh, A. B. Regulation of CRAC channels by Ca2+-dependent inactivation. *Cell Calcium* **63**, 20-23, doi:S0143-4160(16)30204-4 [pii]
10.1016/j.ceca.2016.12.003 (2017).
106 Samanta, K., Douglas, S. & Parekh, A. B. Mitochondrial calcium uniporter MCU supports cytoplasmic Ca2+ oscillations, store-operated Ca2+ entry and Ca2+-dependent gene expression in response to receptor stimulation. *PLoS One* **9**, e101188, doi:10.1371/journal.pone.0101188
PONE-D-14-20224 [pii] (2014).
107 Wacquier, B., Combettes, L., Van Nhieu, G. T. & Dupont, G. Interplay Between Intracellular Ca(2+) Oscillations and Ca(2+)-stimulated Mitochondrial Metabolism. *Sci Rep* **6**, 19316, doi:srep19316 [pii]
10.1038/srep19316 (2016).
108 Drago, I., De Stefani, D., Rizzuto, R. & Pozzan, T. Mitochondrial Ca2+ uptake contributes to buffering cytoplasmic Ca2+ peaks in cardiomyocytes. *Proc Natl Acad Sci U S A* **109**, 12986-12991, doi:1210718109 [pii]
10.1073/pnas.1210718109 (2012).
109 Tang, S. *et al.* Mitochondrial Ca(2)(+) uniporter is critical for store-operated Ca(2)(+) entry-dependent breast cancer cell migration. *Biochem Biophys Res Commun* **458**, 186-193, doi:S0006-291X(15)00131-X [pii]
10.1016/j.bbrc.2015.01.092 (2015).
110 Tosatto, A. *et al.* The mitochondrial calcium uniporter regulates breast cancer progression via HIF-1alpha. *EMBO Mol Med* **8**, 569-585, doi:emmm.201606255 [pii]
10.15252/emmm.201606255 (2016).
111 Quintana, A. *et al.* T cell activation requires mitochondrial translocation to the immunological synapse. *Proc Natl Acad Sci U S A* **104**, 14418-14423, doi:0703126104 [pii]
10.1073/pnas.0703126104 (2007).

1295 112 Medina, D. L. *et al.* Lysosomal calcium signalling regulates autophagy through
1296 calcineurin and TFEB. *Nat Cell Biol* **17**, 288-299, doi:ncb3114 [pii]
1297 10.1038/ncb3114 (2015).
1298 **Identifies lysosomal Ca^{2+} homeostasis as a crucial factor for signalling pathways that**
1299 **regulate cellular physiology.**
1300 113 Sbano, L. *et al.* TFEB-mediated increase in peripheral lysosomes regulates store-
1301 operated calcium entry. *Sci Rep* **7**, 40797, doi:srep40797 [pii]
1302 10.1038/srep40797 (2017).
1303 114 Ben-Kasus Nissim, T. *et al.* Mitochondria control store-operated Ca2+ entry through
1304 Na+ and redox signals. *EMBO J* **36**, 797-815, doi:embj.201592481 [pii]
1305 10.15252/embj.201592481 (2017).
1306 115 McCormack, J. G., Halestrap, A. P. & Denton, R. M. Role of calcium ions in regulation of
1307 mammalian intramitochondrial metabolism. *Physiol Rev* **70**, 391-425 (1990).
1308 116 Giorgi, C. *et al.* Mitochondrial Ca(2+) and apoptosis. *Cell Calcium* **52**, 36-43, doi:S0143-
1309 4160(12)00037-1 [pii]
1310 10.1016/j.ceca.2012.02.008 (2012).
1311 117 Rimessi, A. *et al.* Perturbed mitochondrial Ca signals as causes or consequences of
1312 mitophagy induction. *Autophagy* **9**, doi:24795 [pii] (2013).
1313 118 Marchi, S. *et al.* Mitochondrial and endoplasmic reticulum calcium homeostasis and cell
1314 death. *Cell Calcium* **69**, 62-72, doi:S0143-4160(17)30062-3 [pii]
1315 10.1016/j.ceca.2017.05.003 (2018).
1316 119 Schuit, F. C., In't Veld, P. A. & Pipeleers, D. G. Glucose stimulates proinsulin biosynthesis
1317 by a dose-dependent recruitment of pancreatic beta cells. *Proc Natl Acad Sci U S A* **85**,
1318 3865-3869 (1988).
1319 120 Wiederkehr, A. & Wollheim, C. B. Mitochondrial signals drive insulin secretion in the
1320 pancreatic beta-cell. *Mol Cell Endocrinol* **353**, 128-137, doi:S0303-7207(11)00396-0
1321 [pii]
1322 10.1016/j.mce.2011.07.016 (2012).
1323 121 Hoppa, M. B. *et al.* Chronic palmitate exposure inhibits insulin secretion by dissociation
1324 of Ca(2+) channels from secretory granules. *Cell Metab* **10**, 455-465, doi:S1550-
1325 4131(09)00307-6 [pii]
1326 10.1016/j.cmet.2009.09.011 (2009).
1327 122 Rutter, G. A., Tsuboi, T. & Ravier, M. A. Ca2+ microdomains and the control of insulin
1328 secretion. *Cell Calcium* **40**, 539-551, doi:S0143-4160(06)00180-1 [pii]
1329 10.1016/j.ceca.2006.08.015 (2006).
1330 123 Pinton, P. *et al.* Dynamics of glucose-induced membrane recruitment of protein kinase
1331 C beta II in living pancreatic islet beta-cells. *J Biol Chem* **277**, 37702-37710,
1332 doi:10.1074/jbc.M204478200
1333 M204478200 [pii] (2002).
1334 124 Wiederkehr, A. *et al.* Mitochondrial matrix calcium is an activating signal for hormone
1335 secretion. *Cell Metab* **13**, 601-611, doi:S1550-4131(11)00136-7 [pii]
1336 10.1016/j.cmet.2011.03.015 (2011).
1337 **Demonstrates as mitochondrial Ca^{2+}/ATP coupling is required for required for signal-**
1338 **dependent hormone secretion.**
1339 125 Santulli, G. *et al.* Calcium release channel RyR2 regulates insulin release and glucose
1340 homeostasis. *J Clin Invest* **125**, 1968-1978, doi:79273 [pii]
1341 10.1172/JCI79273 (2015).
1342 126 Tarasov, A. I. *et al.* The mitochondrial Ca2+ uniporter MCU is essential for glucose-
1343 induced ATP increases in pancreatic beta-cells. *PLoS One* **7**, e39722,
1344 doi:10.1371/journal.pone.0039722

1345 PONE-D-12-06521 [pii] (2012).
1346 127 Tarasov, A. I. *et al.* Frequency-dependent mitochondrial Ca(2+) accumulation regulates
1347 ATP synthesis in pancreatic beta cells. *Pflugers Arch* **465**, 543-554,
1348 doi:10.1007/s00424-012-1177-9 (2013).
1349 128 Wright, L. E. *et al.* Increased mitochondrial calcium uniporter in adipocytes underlies
1350 mitochondrial alterations associated with insulin resistance. *Am J Physiol Endocrinol*
1351 *Metab* **313**, E641-E650, doi:ajpendo.00143.2016 [pii]
1352 10.1152/ajpendo.00143.2016 (2017).
1353 129 Alam, M. R. *et al.* Mitochondrial Ca2+ uptake 1 (MICU1) and mitochondrial ca2+
1354 uniporter (MCU) contribute to metabolism-secretion coupling in clonal pancreatic
1355 beta-cells. *J Biol Chem* **287**, 34445-34454, doi:M112.392084 [pii]
1356 10.1074/jbc.M112.392084 (2012).
1357 130 Vierra, N. C. *et al.* TALK-1 channels control beta cell endoplasmic reticulum Ca(2+)
1358 homeostasis. *Sci Signal* **10**, doi:10/497/eaan2883 [pii]
1359 10.1126/scisignal.aan2883 (2017).
1360 131 Murgia, M., Giorgi, C., Pinton, P. & Rizzuto, R. Controlling metabolism and cell death: at
1361 the heart of mitochondrial calcium signalling. *J Mol Cell Cardiol* **46**, 781-788,
1362 doi:S0022-2828(09)00109-6 [pii]
1363 10.1016/j.yjmcc.2009.03.003 (2009).
1364 132 Bers, D. M. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol* **70**, 23-
1365 49, doi:10.1146/annurev.physiol.70.113006.100455 (2008).
1366 133 Viola, H. M., Arthur, P. G. & Hool, L. C. Evidence for regulation of mitochondrial function
1367 by the L-type Ca2+ channel in ventricular myocytes. *J Mol Cell Cardiol* **46**, 1016-1026,
1368 doi:S0022-2828(08)01472-7 [pii]
1369 10.1016/j.yjmcc.2008.12.015 (2009).
1370 134 Cannell, M. B., Cheng, H. & Lederer, W. J. The control of calcium release in heart muscle.
1371 *Science* **268**, 1045-1049 (1995).
1372 135 Bers, D. M. Cardiac excitation-contraction coupling. *Nature* **415**, 198-205,
1373 doi:10.1038/415198a
1374 415198a [pii] (2002).
1375 136 Wu, Y. *et al.* The mitochondrial uniporter controls fight or flight heart rate increases.
1376 *Nat Commun* **6**, 6081, doi:ncomms7081 [pii]
1377 10.1038/ncomms7081 (2015).
1378 137 O'Rourke, B. & Blatter, L. A. Mitochondrial Ca2+ uptake: tortoise or hare? *J Mol Cell*
1379 *Cardiol* **46**, 767-774, doi:S0022-2828(08)01443-0 [pii]
1380 10.1016/j.yjmcc.2008.12.011 (2009).
1381 138 Williams, G. S., Boyman, L., Chikando, A. C., Khairallah, R. J. & Lederer, W. J.
1382 Mitochondrial calcium uptake. *Proc Natl Acad Sci U S A* **110**, 10479-10486,
1383 doi:1300410110 [pii]
1384 10.1073/pnas.1300410110 (2013).
1385 139 Griffiths, E. J. Mitochondrial calcium transport in the heart: physiological and
1386 pathological roles. *J Mol Cell Cardiol* **46**, 789-803, doi:S0022-2828(09)00107-2 [pii]
1387 10.1016/j.yjmcc.2009.03.001 (2009).
1388 140 Piot, C. *et al.* Effect of cyclosporine on reperfusion injury in acute myocardial infarction.
1389 *N Engl J Med* **359**, 473-481, doi:359/5/473 [pii]
1390 10.1056/NEJMoa071142 (2008).
1391 141 Cung, T. T. *et al.* Cyclosporine before PCI in Patients with Acute Myocardial Infarction.
1392 *N Engl J Med* **373**, 1021-1031, doi:10.1056/NEJMoa1505489 (2015).

1393 142 Bonora, M. *et al.* Mitochondrial permeability transition involves dissociation of F1FO
1394 ATP synthase dimers and C-ring conformation. *EMBO Rep* **18**, 1077-1089,
1395 doi:embr.201643602 [pii]
1396 10.15252/embr.201643602 (2017).
1397 143 Luongo, T. S. *et al.* The Mitochondrial Calcium Uniporter Matches Energetic Supply
1398 with Cardiac Workload during Stress and Modulates Permeability Transition. *Cell Rep*
1399 **12**, 23-34, doi:S2211-1247(15)00615-4 [pii]
1400 10.1016/j.celrep.2015.06.017 (2015).
1401 144 Kwong, J. Q. *et al.* The Mitochondrial Calcium Uniporter Selectively Matches Metabolic
1402 Output to Acute Contractile Stress in the Heart. *Cell Rep* **12**, 15-22,
1403 doi:10.1016/j.celrep.2015.06.002
1404 S2211-1247(15)00585-9 [pii] (2015).
1405 145 Rasmussen, T. P. *et al.* Inhibition of MCU forces extramitochondrial adaptations
1406 governing physiological and pathological stress responses in heart. *Proc Natl Acad Sci*
1407 *U S A* **112**, 9129-9134, doi:1504705112 [pii]
1408 10.1073/pnas.1504705112 (2015).
1409 146 Becker, L. B. New concepts in reactive oxygen species and cardiovascular reperfusion
1410 physiology. *Cardiovasc Res* **61**, 461-470, doi:10.1016/j.cardiores.2003.10.025
1411 S0008636303007077 [pii] (2004).
1412 147 Qiao, X. *et al.* PTPIP51 regulates mouse cardiac ischemia/reperfusion through
1413 mediating the mitochondria-SR junction. *Sci Rep* **7**, 45379, doi:srep45379 [pii]
1414 10.1038/srep45379 (2017).
1415 148 Mercadier, J. J. *et al.* Altered sarcoplasmic reticulum Ca2(+)-ATPase gene expression in
1416 the human ventricle during end-stage heart failure. *J Clin Invest* **85**, 305-309,
1417 doi:10.1172/JCI114429 (1990).
1418 149 Terentyev, D. *et al.* Redox modification of ryanodine receptors contributes to
1419 sarcoplasmic reticulum Ca2+ leak in chronic heart failure. *Circ Res* **103**, 1466-1472,
1420 doi:CIRCRESAHA.108.184457 [pii]
1421 10.1161/CIRCRESAHA.108.184457 (2008).
1422 150 Marx, S. O. *et al.* PKA phosphorylation dissociates FKBP12.6 from the calcium release
1423 channel (ryanodine receptor): defective regulation in failing hearts. *Cell* **101**, 365-376,
1424 doi:S0092-8674(00)80847-8 [pii] (2000).
1425 151 Bellinger, A. M. *et al.* Remodeling of ryanodine receptor complex causes "leaky"
1426 channels: a molecular mechanism for decreased exercise capacity. *Proc Natl Acad Sci U*
1427 *S A* **105**, 2198-2202, doi:0711074105 [pii]
1428 10.1073/pnas.0711074105 (2008).
1429 152 Gomez, A. M. *et al.* Defective excitation-contraction coupling in experimental cardiac
1430 hypertrophy and heart failure. *Science* **276**, 800-806 (1997).
1431 153 Shiferaw, Y., Aistrup, G. L. & Wasserstrom, J. A. Intracellular Ca2+ waves,
1432 afterdepolarizations, and triggered arrhythmias. *Cardiovasc Res* **95**, 265-268,
1433 doi:cvs155 [pii]
1434 10.1093/cvr/cvs155 (2012).
1435 154 Somasundaram, S. & Sadique, J. The role of mitochondrial calcium transport during
1436 inflammation and the effect of anti-inflammatory drugs. *Biochem Med Metab Biol* **36**,
1437 220-230, doi:0885-4505(86)90129-5 [pii] (1986).
1438 155 Zhou, R., Yazdi, A. S., Menu, P. & Tschopp, J. A role for mitochondria in NLRP3
1439 inflammasome activation. *Nature* **469**, 221-225, doi:nature09663 [pii]
1440 10.1038/nature09663 (2011).
1441 **Demonstrates as NLRP3 inflammasome activates at Mitochondria-associated**
1442 **Membranes (MAMs).**

1443 156 Rimessi, A. *et al.* Mitochondrial Ca2+-dependent NLRP3 activation exacerbates the
1444 Pseudomonas aeruginosa-driven inflammatory response in cystic fibrosis. *Nat*
1445 *Commun* **6**, 6201, doi:ncomms7201 [pii]
1446 10.1038/ncomms7201 (2015).
1447 **Shows the implication of mitochondrial Ca^{2+} and MCU complex in Cystic Fibrosis.**
1448 157 Cheng, J. *et al.* Amplified RLR signaling activation through an interferon-stimulated
1449 gene-endoplasmic reticulum stress-mitochondrial calcium uniporter protein loop. *Sci*
1450 *Rep* **6**, 20158, doi:srep20158 [pii]
1451 10.1038/srep20158 (2016).
1452 158 Antony, A. N. *et al.* MICU1 regulation of mitochondrial Ca(2+) uptake dictates survival
1453 and tissue regeneration. *Nat Commun* **7**, 10955, doi:ncomms10955 [pii]
1454 10.1038/ncomms10955 (2016).
1455 159 Uzhachenko, R. *et al.* Mitochondrial protein Fus1/Tusc2 in premature aging and age-
1456 related pathologies: critical roles of calcium and energy homeostasis. *Aging (Albany*
1457 *NY)* **9**, 627-649, doi:101213 [pii]
1458 10.18632/aging.101213 (2017).
1459 160 Lee, S. *et al.* Polo Kinase Phosphorylates Miro to Control ER-Mitochondria Contact Sites
1460 and Mitochondrial Ca(2+) Homeostasis in Neural Stem Cell Development. *Dev Cell* **37**,
1461 174-189, doi:10.1016/j.devcel.2016.03.023 (2016).
1462 161 Clapham, D. E. Calcium signaling. *Cell* **131**, 1047-1058, doi:S0092-8674(07)01531-0
1463 [pii]
1464 10.1016/j.cell.2007.11.028 (2007).
1465 162 Schwaller, B. Cytosolic Ca2+ buffers. *Cold Spring Harb Perspect Biol* **2**, a004051,
1466 doi:cshperspect.a004051 [pii]
1467 10.1101/cshperspect.a004051 (2010).
1468 163 Han, Y. *et al.* Ca(2+)-Induced Mitochondrial ROS Regulate the Early Embryonic Cell
1469 Cycle. *Cell Rep* **22**, 218-231, doi:S2211-1247(17)31851-X [pii]
1470 10.1016/j.celrep.2017.12.042 (2018).
1471 164 Bonora, M. *et al.* Subcellular calcium measurements in mammalian cells using jellyfish
1472 photoprotein aequorin-based probes. *Nat Protoc* **8**, 2105-2118, doi:nprot.2013.127
1473 [pii]
1474 10.1038/nprot.2013.127 (2013).
1475 165 Fonteriz, R. I. *et al.* Monitoring mitochondrial [Ca(2+)] dynamics with rhod-2,
1476 ratiometric pericam and aequorin. *Cell Calcium* **48**, 61-69, doi:S0143-4160(10)00101-
1477 6 [pii]
1478 10.1016/j.ceca.2010.07.001 (2010).
1479 166 Rodriguez-Garcia, A. *et al.* GAP, an aequorin-based fluorescent indicator for imaging
1480 Ca2+ in organelles. *Proc Natl Acad Sci U S A* **111**, 2584-2589, doi:1316539111 [pii]
1481 10.1073/pnas.1316539111 (2014).
1482 167 Sattler, R. & Tymianski, M. Molecular mechanisms of calcium-dependent excitotoxicity.
1483 *J Mol Med (Berl)* **78**, 3-13 (2000).
1484 168 Hardingham, G. E. & Bading, H. The Yin and Yang of NMDA receptor signalling. *Trends*
1485 *Neurosci* **26**, 81-89, doi:S0166-2236(02)00040-1 [pii]
1486 10.1016/S0166-2236(02)00040-1 (2003).
1487 169 Danese, A. *et al.* Calcium regulates cell death in cancer: Roles of the mitochondria and
1488 mitochondria-associated membranes (MAMs). *Biochim Biophys Acta* **1858**, 615-627,
1489 doi:S0005-2728(17)30004-X [pii]
1490 10.1016/j.bbabio.2017.01.003 (2017).
1491 170 Mattson, M. P. & Chan, S. L. Neuronal and glial calcium signaling in Alzheimer's disease.
1492 *Cell Calcium* **34**, 385-397, doi:S0143416003001283 [pii] (2003).

1493 171 Ikonomidou, C. *et al.* Ethanol-induced apoptotic neurodegeneration and fetal alcohol
1494 syndrome. *Science* **287**, 1056-1060, doi:8262 [pii] (2000).
1495 172 Bittremieux, M., Parys, J. B., Pinton, P. & Bultynck, G. ER functions of oncogenes and
1496 tumor suppressors: Modulators of intracellular Ca(2+) signaling. *Biochim Biophys Acta*
1497 **1863**, 1364-1378, doi:S0167-4889(16)00003-3 [pii]
1498 10.1016/j.bbamcr.2016.01.002 (2016).
1499 173 Chakraborty, P. K. *et al.* MICU1 drives glycolysis and chemoresistance in ovarian
1500 cancer. *Nat Commun* **8**, 14634, doi:ncomms14634 [pii]
1501 10.1038/ncomms14634 (2017).
1502 174 Huang, B. *et al.* Suppression of LETM1 by siRNA inhibits cell proliferation and invasion
1503 of bladder cancer cells. *Oncol Rep* **38**, 2935-2940, doi:10.3892/or.2017.5959 (2017).
1504 175 Giorgi, C. *et al.* Intravital imaging reveals p53-dependent cancer cell death induced by
1505 phototherapy via calcium signaling. *Oncotarget* **6**, 1435-1445, doi:2935 [pii]
1506 10.18632/oncotarget.2935 (2015).
1507 176 Kuchay, S. *et al.* PTEN counteracts FBXL2 to promote IP3R3- and Ca(2+)-mediated
1508 apoptosis limiting tumour growth. *Nature* **546**, 554-558, doi:nature22965 [pii]
1509 10.1038/nature22965 (2017).
1510 177 Austin, S. *et al.* LETM1-Mediated K(+) and Na(+) Homeostasis Regulates Mitochondrial
1511 Ca(2+) Efflux. *Front Physiol* **8**, 839, doi:10.3389/fphys.2017.00839 (2017).
1512 178 Schneeberger, M. *et al.* Mitofusin 2 in POMC neurons connects ER stress with leptin
1513 resistance and energy imbalance. *Cell* **155**, 172-187, doi:S0092-8674(13)01094-5 [pii]
1514 10.1016/j.cell.2013.09.003 (2013).
1515 179 Wang, P. T. *et al.* Distinct mechanisms controlling rough and smooth endoplasmic
1516 reticulum contacts with mitochondria. *J Cell Sci* **128**, 2759-2765, doi:jcs.171132 [pii]
1517 10.1242/jcs.171132 (2015).
1518 180 De Vos, K. J. *et al.* VAPB interacts with the mitochondrial protein PTPIP51 to regulate
1519 calcium homeostasis. *Hum Mol Genet* **21**, 1299-1311, doi:ddr559 [pii]
1520 10.1093/hmg/ddr559 (2012).
1521 181 Stoica, R. *et al.* ER-mitochondria associations are regulated by the VAPB-PTPIP51
1522 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nat Commun* **5**, 3996,
1523 doi:ncomms4996 [pii]
1524 10.1038/ncomms4996 (2014).
1525 182 Paillusson, S. *et al.* alpha-Synuclein binds to the ER-mitochondria tethering protein
1526 VAPB to disrupt Ca(2+) homeostasis and mitochondrial ATP production. *Acta*
1527 *Neuropathol* **134**, 129-149, doi:10.1007/s00401-017-1704-z
1528 10.1007/s00401-017-1704-z [pii] (2017).
1529 183 Szabadkai, G. *et al.* Chaperone-mediated coupling of endoplasmic reticulum and
1530 mitochondrial Ca2+ channels. *J Cell Biol* **175**, 901-911, doi:jcb.200608073 [pii]
1531 10.1083/jcb.200608073 (2006).
1532 184 Doghman-Bouguerra, M. *et al.* FATE1 antagonizes calcium- and drug-induced apoptosis
1533 by uncoupling ER and mitochondria. *EMBO Rep* **17**, 1264-1280, doi:embr.201541504
1534 [pii]
1535 10.15252/embr.201541504 (2016).
1536 185 Zampese, E. *et al.* Presenilin 2 modulates endoplasmic reticulum (ER)-mitochondria
1537 interactions and Ca2+ cross-talk. *Proc Natl Acad Sci U S A* **108**, 2777-2782,
1538 doi:1100735108 [pii]
1539 10.1073/pnas.1100735108 (2011).
1540 186 Iwasawa, R., Mahul-Mellier, A. L., Datler, C., Pazarentzos, E. & Grimm, S. Fis1 and Bap31
1541 bridge the mitochondria-ER interface to establish a platform for apoptosis induction.
1542 *EMBO J* **30**, 556-568, doi:emboj2010346 [pii]

1543 10.1038/emboj.2010.346 (2011).
1544 187 Simmen, T. *et al.* PACS-2 controls endoplasmic reticulum-mitochondria communication
1545 and Bid-mediated apoptosis. *EMBO J* **24**, 717-729, doi:7600559 [pii]
1546 10.1038/sj.emboj.7600559 (2005).
1547 188 Arruda, A. P. *et al.* Chronic enrichment of hepatic endoplasmic reticulum-mitochondria
1548 contact leads to mitochondrial dysfunction in obesity. *Nat Med* **20**, 1427-1435,
1549 doi:nm.3735 [pii]
1550 10.1038/nm.3735 (2014).
1551 189 Honrath, B. *et al.* SK2 channels regulate mitochondrial respiration and mitochondrial
1552 Ca(2+) uptake. *Cell Death Differ* **24**, 761-773, doi:cdd20172 [pii]
1553 10.1038/cdd.2017.2 (2017).
1554
1555