The role of Zn^{2+} in shaping intracellular Ca^{2+} dynamics in the heart

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One Sentence Summary: In this review we discuss the role of Zn^{2+} and zinc transporters in regulating cellular Ca^{2+}-dynamics in cardiac muscle.

Abstract

Increasing evidence suggests that Zn^{2+} acts as a second messenger capable of transducing extracellular stimuli into intracellular signalling events. The importance of Zn^{2+} as a signalling molecule in cardiovascular functioning is gaining traction. In the heart, Zn^{2+} plays important roles in excitation-contraction (EC) coupling, excitation-transcription coupling, and cardiac ventricular morphogenesis. Zn^{2+} homeostasis in cardiac tissue is tightly regulated through the action of a combination of transporters, buffers and sensors. Zn^{2+}-mishandling is a common feature of various cardiovascular diseases. However, the precise mechanisms controlling the intracellular distribution of Zn^{2+} and its variations during normal cardiac function and during pathological conditions are not fully understood. In this review we consider the major pathways by which the concentration of intracellular Zn^{2+} is regulated in the heart, the role of Zn^{2+} in EC coupling and discuss how Zn^{2+}-dyshomeostasis resulting from altered expression levels and efficacy of Zn^{2+} regulatory proteins are key drivers in the progression of cardiac dysfunction.

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Introduction

Zinc is an essential trace element which is proposed to interact with more than 10% of the human proteome (Andreini *et al*., 2006). It is essential for processes including cell division (McDonald, 2000), and protein synthesis (Kimball *et al*., 1995). The human body contains approximately 2-3 g of zinc. Of this, ~60% is contained in skeletal muscle, ~30% in bone, ~5% in liver and skin with the remainder distributed in other tissues, with ~0.4% total zinc in the heart (reviewed in Jackson, 1989; Kambe *et al*., 2015). More than 99% of intracellular zinc is bound to proteins, although increasing evidence suggests that exchangeable zinc ions (Zn^{2+}) act as second messengers capable of transducing extracellular stimuli into intracellular signalling events (Yamasaki *et al*., 2007). As more tools become available to study Zn^{2+} the importance and complexity of intracellular Zn^{2+} signalling is beginning to rival that of calcium ions (Ca^{2+}), with key roles for Zn^{2+} evident in regulating many cellular processes. This review will focus on research specific to the cardiovascular system with a focus on the role of intracellular Zn^{2+}.

Zn^{2+} plays an emerging but important role in heart function, including excitation-contraction (EC) coupling (Turan *et al*., 1997; Tuncay *et al*., 2011; Woodier *et al*., 2015; Reilly-O'Donnell *et al*., 2017), excitation-transcription coupling (Atar *et al*., 1995) and cardiac ventricular morphogenesis (Lin *et al.*, 2018). In the heart the $[Zn^{2+}]_i$ is tightly regulated to maintain low labile Zn^{2+} concentrations. Hara *et al* report the total extracellular $[Zn^{2+}]$ to range from high micromolar to 10 μM, while the total intracellular $[Zn^{2+}]$ in mammalian cells is around 200 μM. Intracellular free Zn^{2+} concentrations are much lower than values reported for total Zn^{2+} and are cell-type dependant (Reviewed by Vallee and Falchuk, 1993; Hara *et al.,* 2017). If the exchangeable Zn^{2+} concentration moves outside a narrow range, either in excess or deficiency, this results in cardiac dysfunction, including altered contractile force (for reviews on this topic see Pitt and Stewart, 2015; Stewart and Pitt, 2015; Turan and Tuncay, 2017). This highlights the importance of controlled Zn^{2+}-homeostasis in cardiovascular functioning.

At rest, cardiomyocytes contain a small but measurable pool of free Zn^{2+} in the cytosol reported to be between 2 nM to 100 pM. Certain triggers can lead to the release of Zn^{2+} from proteins and intracellular pools, and this can result in myocardial damage (Turan *et al*, 1997; Chabosseau *et al.*, 2014). Little is known about the precise mechanisms controlling the intracellular distribution of Zn^{2+} and its variations during cardiac functioning. In this review, we consider the major pathways by which $[Zn^{2+}]_i$ is regulated in the heart, the role of Zn^{2+} in EC coupling and how Zn^{2+} dyshomeostasis results in cardiac dysfunction.

Zn^{2+} homeostasis in cardiomyocytes

Zinc binding proteins

Extracellular zinc speciation is a critical factor for Zn^{2+} uptake by all cells, irrespective of the tight control maintained through the action of transporter proteins. This is exemplified by recent work where ^{68}Zn was used to measure zinc flux in immortalised endothelial cells (Coverdale *et al.*, 2022). The concentration of serum albumin in the media was found to impact upon the rate of Zn^{2+} influx. This dynamic is of particular importance as serum albumin is the major carrier of plasma Zn^{2+} in the circulation (Lu *et al*., 2008). In the absence of albumin under the conditions examined (20 μM $^{68}Zn^{2+}$), the cells were unable to control the amount of Zn^{2+} taken up. This was indicated by an increase in total zinc within the cells over time, which was not observed when albumin was present in the media (Coverdale *et al*., 2022). Note that these findings are consistent with an earlier study that found the serum content of the extracellular media to be important for protecting cells of various types from otherwise harmful concentrations of Zn^{2+} (Haase *et al.,* 2015). With relevance to the heart, it is suggested that low serum albumin levels in both males and females are associated with increased risk of myocardial infarction and is linked to adverse outcomes post-myocardial infarction. However, this topic remains controversial (Djoussé *et al*., 2002; Toida *et al*., 2020; Yoshioka *et al*., 2020).

Intracellular Zn^{2+} buffering in cardiomyocytes is tightly controlled by metallothioneins (MTs). MTs are low molecular weight, cysteine-rich proteins that play important roles in metal homeostasis and in the protection against intracellular heavy metal toxicity and oxidative stress at levels sufficient to induce cell damage**.** In humans, there are four main MT isoforms (MT1, MT2, MT3, and MT4) that are encoded by genes located on chromosome 16q13 (Thirumoorthy *et al*., 2011). Each MT protein can bind up to 7 Zn^{2+} ions with high affinity and collectively MTs are thought to gather about 5% to 15% of the cytosolic zinc pool (Coyle *et al*., 2002). MTs work as zinc acceptors and donors to exchange Zn^{2+} with other proteins in the cells via oxidoreduction (Krężel and Maret, 2007). The thiol groups that coordinate zinc in MTs are redox reactive such that oxidation leads to the release of Zn^{2+}. Basal levels of MTs in cells are often low, although they vary across different tissue types and their expression levels can be altered under certain conditions or disease states (Davis and Cousins, 2000). MT2A is the most abundant isoform found in heart, smooth muscle, and endothelial cells, whereas MT1E and MT1X are also significantly expressed in these tissues, suggesting these isoforms collectively play important roles in cardiovascular physiology (Choi *et al*., 2018).

Zinc transporters expressed in the sarco/endoplasmic reticulum (S/ER)

The movement of Zn^{2+} across cell membranes is facilitated by zinc transporters. There are 24 known zinc transporters in humans, which are classified in two groups: Zinc transporters (ZnTs; 1-10) designated to the solute carrier family 30A (SLC30A) and zrt-, irt-related proteins (ZIPs; 1-14), grouped as solute carrier family 39A (SLC39A; Paulsen and Saier, 1997; Grotz *et al*., 1998; Eide, 2004; Palmiter and Huang, 2004; Cousins *et al.,* 2006). ZnTs transport Zn^{2+} from the cytosol into organelles or to the extracellular space, while ZIPs transport Zn^{2+} into the cell from the extracellular matrix or from organelles into the cytosol (Conklin *et al*., 1994; Palmiter and Findley, 1995; Taylor, 2000; Taylor *et al*., 2003). Zn^{2+} can also be transported through Ca^{2+} channels, such as L-type calcium channel (LTCC) in

cardiomyocytes (Atar *et al*., 1995). The expression profile of zinc transporters within the heart are shown in Table 1 (ZIPs) and Table 2 (ZnTs). The localisation of these zinc transporters is illustrated in Figure 1A while Table 3 details the localisation and detection method. Figure 1B shows RNA expression of ZIPs and ZnTs in heart. An increase in intracellular Zn^{2+} leads to metal regulatory transcription factor 1 (MTF-1) binding, resulting in MTF-1 translocation to the nucleus and subsequent activation to bind DNA and initiate MT expression (Bittel *et al*., 1998). It is suggested that Zn^{2+} sequestration into organelles is the first response to Zn^{2+} influx to deal with the potential threat of a harmful increase in cytosolic Zn^{2+} while transcription and translation of zinc transporters and MTs occurs (Kukic *et al*., 2014).

Numerous organelles have been identified as Zn^{2+} stores, as described below. While the S/ER is classically known as a Ca^{2+} store, Zn^{2+} is also stored in this organelle. Using genetically encoded Zn^{2+} sensors the labile Zn^{2+} concentration in the S/ER has been estimated to be between 1 pM and ≥5 nM (Qin *et al*., 2011; Chabosseau *et al*., 2014). There are numerous proteins in the S/ER that bind Zn^{2+}, including calsequestrin 2 (CSQ2) and calreticulin which also bind Ca^{2+} (Baksh *et al*., 1995; Tan *et al*., 2006). The S/ER has Zn^{2+} transporters within its membrane. Localisation of ZnT7 and ZIP7 to the S/ER was first demonstrated in the heart by Tuncay *et al*. (2017). Turan and co-workers also subsequently reported localisation of ZIP8, ZIP14 and ZnT8 to the S/ER in H9C2 cells (embryonic rat myoblasts; Olgar *et al*., 2018a), but ZnT8 has not yet been detected at the gene level (Figure 2).

Zn^{2+} can be sequestered within other cell organelles. Labile Zn^{2+} is undetectable in the nucleus, even though it is estimated that 30-40% of total cellular Zn^{2+} resides in the nucleus (Vallee and Falchuk, 1993, Lu *et al*., 2016). The Golgi is estimated to contain between 0.2 pM and 25.1 nM free Zn^{2+}, while the mitochondria is estimated to contain between 0.14 and 300 pM Zn^{2+} (Qin *et al*., 2011; Park *et al*., 2012; McCranor *et al*., 2012; Chabosseau *et al*.,

2014; Kowada *et al*., 2020). Lysosomes have also been identified as Zn^{2+} stores although the concentration in these organelles has not yet been determined (Roh *et al*., 2012; Kukic *et al*., 2014).

Organelle crosstalk shapes Ca^{2+} and Zn^{2+} signalling

The importance of communication between cellular organelles and exchange of messenger molecules in well established (reviewed by Rossini *et al*., 2020). Membrane-contact sites regulate many cellular functions. In the heart, dysregulation of different organellar cross talk pathways results in pathology (reviewed by Dabravolski *et al*, 2022; Hulsurkar *et al.,* 2022). Some examples of organellar crosstalk between Ca^{2+} and Zn^{2+} are provided below.

Mitochondria and S/ER actively communicate with each other to promote a variety of cellular events. Mitochondria play multiple roles in cardiac cells, including regulation of energy homeostasis, signalling, metabolism, and cell death pathways. Crosstalk between the SR and mitochondria is important in normal cardiomyocyte viability and EC coupling and plays a key role in regulating Ca^{2+}-signalling responses in cardiac muscle (Griffiths and Rutter, 2009; Eisner *et al.,* 2013). While the SR and mitochondria are separate compartments with different functions, the interplay between the SR and mitochondria is essential in supporting cardiomyocyte contraction and relaxation and this organellar crosstalk facilitates adaptation to changing metabolic demands during EC coupling (Dorn II and Maack, 2013; Gorski *et al*., 2015)

Mitochondria have also been identified as intracellular Zn^{2+} stores. Mitochondrial free [Zn^{2+}] is maintained at lower concentrations than found in the cytosol (Ye *et al*., 2001; Kambe *et al*., 2015). Emerging research suggests that in cardiomyocytes the interplay between Zn^{2+} homeostasis and crosstalk between the mitochondria and S/ER is important in cardiovascular diseases (for a recent review see Dabravolski *et al*, 2022). Close contact between the ER and mitochondria was first described by Vance, who through fractionation, identified a pool of phospholipids which were suggested to be involved in the association of

156 the ER and mitochondria (Vance, 1990). These mitochondria associated membranes (MAMs)
157 are the site at which the mitochondria and ER communicate functionally and through
158 structural interaction (Reviewed in Giorgi *et al*., 2009). The role of MAMs in cardiovascular
159 disease is reviewed in detailed by Wang *et al* (Wang, Y. *et al*., 2021). It is thought that
160 intracellular Ca^{2+} machinery including the inositol 1,4,5-trisphosphate receptor (IP3R) may
161 be involved in Ca^{2+} signalling across the mitochondria and ER (Hirota *et al.,* 1999). Emerging
162 evidence suggests that this may also be case with Zn^{2+}.

163 Work from the Turan group illustrates that in aged rats, aged-related increase in intracellular
164 [Zn^{2+}] is reduced using antioxidant MitoTEMPO, while age-related alterations in
165 mitochondrial ZIP7, ZIP8 and ZnT8 are reversed by MitoTEMPO treatment (Olgar *et al*,
166 2019). They also illustrate that key proteins involved in S/ER-mitochondrial coupling
167 including mitofusin-protein (Mfn-1/2), mitochondrial fission protein (Fis-1) and S/ER-
168 mitochondrial bridge protein B-Cell receptor associated protein 31 (Bap31) are significantly
169 altered when ZIP7 was silenced in high glucose and doxorubicin-treated H9C2 cells (Tuncay
170 *et al*, 2019). Protein expression of stromal interaction molecule 1 (STIM1), a S/ER Ca^{2+}
171 sensor that regulates store-operated calcium entry, is also significantly altered in
172 hyperglycaemic and doxorubicin-treated H9C2 cells (Tuncay *et al*, 2019). In cardiomyocytes,
173 it is suggested that STIM1 contributes to the development of cardiac hypertrophy and
174 advancement of heart disease. Although, how STIM1 expression and functionality impacts
175 S/ER Zn^{2+} and Zn^{2+} transporters has not yet been investigated (Bootman and Rietdorf, 2017).
176 Tight coupling between Ca^{2+} and Zn^{2+} dynamics is also important for regulation of cellular
177 functions in the heart. Research by Kamalov and colleagues showed that these ions are
178 intrinsically coupled in aldosterone-treated rat hearts, suggesting their crosstalk contributes
179 to altering the redox state of the cardiomyocytes (Kamalov *et al*., 2009).

180 In the nucleus, Zn^{2+} plays an important role in gene transcription and in maintaining the
181 stability of DNA through zinc-finger proteins, with Zn^{2+} deficiency leading to a reduction in

DNA repair and compromise of integrity due to destabilisation of DNA (Ho, 2004). The effect of nuclear Zn^{2+} dyshomeostasis on the heart/cardiovascular system has to our knowledge not yet been investigated. Zn^{2+} and zinc transporters have also been linked to lysosome function and cellular autophagy in breast tissue and neuronal cell types (Rivera *et al.,* 2018; Kim *et al.,* 2022). In Human Embryonic Kidney (HEK293) cells, Cuajungco and colleagues suggest association of zinc transporter transmembrane protein 163 (TMEM163) and cation channel transient receptor potential mucolipin 1 (TRPML1) is essential for Zn^{2+} homeostasis and disruption to this association may be a mechanism for Zn^{2+}-overload in mucolipidosis type IV disease, a genetic neurodevelopmental disorder (Cuajungco *et al.,* 2014). It is suggested that TRPLM1 agonists lead to cell death through a Zn^{2+}-dependent lysosomal pathway with mitochondrial swelling in metastatic melanoma cells (Du *et al.,* 2021). Interaction of Zn^{2+}/zinc transporters and TRPLM1 have not been investigated in the heart, however Li and Li have reviewed the role of TRPLM1 and Ca^{2+} in cardiovascular disease (Li and Li, 2021).

Coupling of Zn^{2+} and Ca^{2+} homeostasis in the heart

Different divalent cations can often bind to the same or similar binding sites in proteins. In general, Ca^{2+} and Mg^{2+} favour protein binding sites composed of O-ligands (for example aspartic acid or glutamic acid sidechains), whereas Zn^{2+} favours protein binding sites that additionally possess N- and S-ligands (for example histidine and cysteine sidechains, respectively; reviewed by Vallee and Auld, 1990; Alberts *et al.,* 1998; Bindreither and Lackner, 2009; Tang and Yang, 2013). Zn^{2+} sites are typically of a lower coordination number than Ca^{2+} or Mg^{2+} sites (Bock *et al.,* 1995). Whilst a limited degree of overlap does exist (Zn^{2+} also can bind aspartic acid and glutamic acid residues) it is important to point out that Zn^{2+} is typically present (both intracellularly and extracellularly) at a lower concentration than Ca^{2+} and Mg^{2+}. This, together with the respective affinity of a particular site/region for each metal, determines which will bind (or whether competition between different metals

may occur). We have previously shown that the type-2 ryanodine receptor (RyR2) has both high affinity Zn^{2+} activation sites and low affinity Zn^{2+} inhibition sites. Although the inhibitory action of Zn^{2+} is likely a consequence of Zn^{2+} binding to the divalent inhibitory site of the channel, at least some of the activatory sites are distinct from the Ca^{2+} binding sites (Woodier *et al.,* 2015).

As well as ion channels, intracellular proteins are also capable of binding both Ca^{2+} and Zn^{2+}. One example of this is CSQ2, a Ca^{2+}-binding protein located in the S/ER important in Ca^{2+} regulation of RyR2 (Meissner and Henderson, 1987). CSQ2 has been shown to bind both Ca^{2+} and Zn^{2+}, while Zn^{2+} is thought to modulate the function and structure of CSQ2 (Baksh *et al.*, 1995). Baksh and colleagues report that CSQ2 has a large Ca^{2+}-binding capacity (~40-50 moles of Ca^{2+} per mole of protein) with moderate affinity (average $K_d \approx 1$ mM) (Baksh *et al.,* 1995). For Zn^{2+}, the binding capacity is much higher (~200 moles of Zn^{2+} per mole protein) exhibiting and average $K_d \approx 300$ μM (Baksh *et al.,* 1995). It is not known if CSQ2 binds Ca^{2+} and Zn^{2+} at the same sites, however other Ca^{2+} proteins which also bind Zn^{2+}, such as histidine-rich Ca^{2+}-binding protein in skeletal muscle and calmodulin in the brain, possess separate Zn^{2+} and Ca^{2+} binding sites (Baudier *et al*., 1983; Picello *et al*., 1992). Furthermore, Zn^{2+}-binding at Ca^{2+}-effector sites in certain proteins may be unable to induce the same structural changes. For example, in a study by Warren and co-workers, it was shown that when Zn^{2+} bound to the EF-hand motif of calmodulin, the overall structure of the zinc-bound form resembled the apo-form rather than the calcium-bound form (Warren *et al.,* 2007).

The interaction of Ca^{2+} and Zn^{2+} is not a novel concept. Yamasaki and colleagues report that Zn^{2+} release in mast cells from the S/ER, in the form of a Zn^{2+} wave, was Ca^{2+}-dependent (Yamasaki *et al.,* 2007). G-protein coupled receptor 39 (GPR39) was identified to be stimulated by Zn^{2+} by Holst *et al*. (2007) and the receptor is now often referred to as the Zn^{2+} -sensing receptor (ZnR). GPR39 is located on the plasma membrane and is thought to act

as an extracellular Zn^{2+} sensor to trigger activation of several G protein coupled pathways, including the mobilisation of intracellular Ca^{2+} through G_q-coupling (Popovics and Stewart, 2011). The presence of a cellular zinc receptor with the ability to trigger Ca^{2+} release had much earlier been reported by Hershfinkel *et al* (2001). With relevance to G-protein coupled receptors (GPCRs), work by Hojyo and colleagues utilised *Slc39a14*-knock out mice to implicate ZIP14 in GPCR signalling, where it was found that mice that lack the ZIP14 transporter display restricted growth (Hojyo *et al.,* 2011). In the heart, GPCR-signalling can influence intracellular Ca^{2+} signalling, leading to altered cardiac contractility and cardiomyocyte apoptosis (Communal *et al.,* 1999; Nash *et al.,* 2001). While the influence of GPCRs will not be discussed further in this review, Salazar *et al* (2007) and Wang *et al* (2018) have reviewed cardiac GPCRs and the role of GPCRs in cardiovascular disease (Salazar *et al.,* 2007; Wang *et al.,* 2018).

In 1995, Atar and colleagues demonstrated through use of live cell imaging and electrophysiology that Zn^{2+} could enter rat cardiac muscle through the LTCC (Atar *et al*., 1995). While the role of the LTCC in Ca^{2+} handling is well established in EC coupling, little is known about the interaction between LTCCs and Zn^{2+} in the heart (Bodi *et al*., 2005). However, in the brain it was demonstrated that Zn^{2+} accumulation can occur in astrocytes (a sub-type of glial cells in the brain) through LTCC in a manner that is attenuated by ZnT1 (Nolte *et al*., 2004). A subsequent publication by the same group reported that ZnT1 can regulate Zn^{2+} and Ca^{2+} permeation through LTCC in HEK293 cells. In these cells, expression of ZnT1 reduced Ca^{2+} influx by approximately 40% (Segal *et al*., 2004). The Moran laboratory have shown that ZnT1 is also capable of inhibiting LTCC (Beharier *et al*., 2007, 2010; Levy *et al*., 2009). This work shows that crosstalk between ion channels and transporters can influence the cellular movement of ions, which suggests that the interaction of LTCC and ZnT1 can influence cardiac function. Increased ZnT1 protein expression as a result of rapid pacing in culture cardiomyocytes is suggested to lead to reduced Ca^{2+} influx through LTCC and contribute to atrial fibrillation in atrial tachycardia (Beharier *et al*., 2010).

Recent research by Wang *et al*. (Wang, J. *et al*., 2021) has highlighted a link between Ca^{2+} signalling and the expression of Zn^{2+} transporters. Using a cellular model of ischaemia/reperfusion (I/R) involving H9C2 cells and isolated murine cardiomyocytes in combination with Ca^{2+} and Zn^{2+} chelators, the group reported that Ca^{2+}-mobilisation triggers a reduction in ZIP13 protein expression. This reduction of ZIP13 was reported to activate Ca^{2+}/calmodulin-dependent protein kinase II and contribute to I/R injury.

Transient receptor potential kinase ankyrin 1 (TRPA1) is located on the S/ER in cardiac cells, has also been linked to intracellular Ca^{2+} movement and is implicated in atherosclerosis and heart failure (reviewed by Wang *et al.,* 2019). In neurons, TRPA1 has been shown to be Zn^{2+}-activated at [Zn^{2+}] of 300 nM and inhibitory at [Zn^{2+}] >300 μM (Hu *et al.,* 2009). As well as being Ca^{2+} permeable, TRPA1 is also Zn^{2+} permeable. The interaction between Zn^{2+} and Ca^{2+} and its impact on vascular tone regulation has been recently reported by Betrie *et al.* (Betrie *et al.,* 2021). However, this has not been investigated in the heart. TRPML1, transient receptor potential mucolipin 7 (TRPM7) and transient receptor potential cation channel subfamily C member 6 (TRPC6) are also present in the heart, have been linked to cardiac pathologies and are permeable to both Ca^{2+} and Zn^{2+} (reviewed by Bouron *et al.,* 2015).

Actions of Zn^{2+} during excitation-contraction coupling

Cardiac EC coupling is a process which governs contractility of the heart through the carefully controlled release of Ca^{2+} from the S/ER. An action potential travels down the transverse tubule of a cardiomyocyte where depolarisation activates LTCCs, leading to Ca^{2+} influx (Bers, 2002). The resulting [Ca^{2+}] in the dyadic cleft – the intracellular space between the plasma membrane and SR – increases to >10 μM, leading to activation of localised RyR2s on the SR membrane (Bers, 2002). This increase in cytosolic [Ca^{2+}] causes activation of multiple proximal RyR2 channels in a process termed calcium-induced calcium-release (Fabiato, 1983). Recruitment of RyR2 molecules and their synchronous activation is

necessary for a Ca^{2+} release event from the SR to occur (Zima *et al*., 2010). At low micromolar levels intracellular Ca^{2+} binds to troponin C of the troponin complex, causing troponin I inhibition and initiating a conformational change of the troponin-tropomyosin complex (de Tombe, 2003; Fearnley *et al.,* 2011). This allows cross-bridge formation between myosin and actin in the presence of ATP and leads to a power stroke in which ATP is hydrolysed and the contractile machinery activated. This translates into cardiac muscle contraction, termed systole (Bers, 2002; de Tombe, 2003). As such, disruption to Ca^{2+} handling during EC coupling result in impaired cardiac contractility and function.

The effects of Zn^{2+} on cardiomyocyte function are thought to involve a competitive effect of Zn^{2+} on Ca^{2+} regulatory mechanisms. In isolated cardiomyocytes extracellular Zn^{2+} reduces cardiomyocyte contractile functioning (Ciofalo and Thomas, 1965; Yi *et al*., 2012, 2013) and this is thought to be a consequence of extracellular Zn^{2+} being able to act as a charge carrier through LTCC resulting in a 70% reduction in the inward Ca^{2+} current (Atar *et al*., 1995). Studies have shown that cardiomyocytes exposed to extracellular Zn^{2+} display a 50% reduction in S/ER calcium load (Turan 2003; Qin *et al*., 2011; Yi *et al*., 2012) revealing a relationship between intracellular organelles, intracellular Zn^{2+} dynamics and intracellular Ca^{2+} movements.

<u>Zn^{2+}-*induced regulation of RyR2*</u>

RyR2 is the route through which Ca^{2+} is released from the S/ER providing the necessary driving force for cellular contraction. Interestingly, RyR2 discriminates only slightly between divalent cations (Tinker and Williams, 1992), and has been shown to be permeable to Mg^{2+}, Sr^{2+} and Ba^{2+} (Diaz-Sylvester *et al*., 2011), and very recently Zn^{2+} (Gaburjakova and Gaburjakova, 2022). This suggests that Zn^{2+} may contribute to the RyR2 current during EC coupling. Recent work has also suggested that even a very small Zn^{2+} current in the lumen-to-cytosol direction is sufficient to saturate the Zn^{2+} finger motif situated within the C-terminal tail of the four RyR2 subunits, and that binding of Zn^{2+} in this region is essential for RyR2

function (Gaburjakova and Gaburjakova, 2022). At the cellular level, Tuncay and co-workers showed ryanodine-sensitive Zn^{2+} transients with similar kinetics to Ca^{2+} in stimulated rat cardiomyocytes, providing further evidence that the S/ER is an intracellular Zn^{2+} pool and that Zn^{2+} levels are elevated during the cardiac cycle (Tuncay *et al*., 2011). They proposed that the rapid changes in free Zn^{2+} resulted from displacement by Ca^{2+} from intracellular binding sites that are highly sensitive to the redox status of the cardiomyocytes. It is not unreasonable to speculate that RyR2 also contributes to this Zn^{2+} signal.

Zn^{2+} release from the S/ER is unlikely to trigger contraction, but this small release of Zn^{2+} may be sufficient to shape Ca^{2+} dynamics in cardiomyocytes by amplifying the Ca^{2+} response through RyR2. In our own study, it was shown at the single channel level that cytosolic Zn^{2+} can act as a high affinity activator of RyR2 (Woodier *et al*., 2015). Concentrations of free Zn^{2+} ≤1 nM potentiated RyR2 activity but the presence of activating levels of cytosolic Ca^{2+} was a requirement for channel activation. However, at concentrations of Zn^{2+} >1 nM, the main activating ligand switched from Ca^{2+} to Zn^{2+} and the requirement of Ca^{2+} for channel activation was removed. The ability of Zn^{2+} at a concentration of 1 nM to directly activate RyR2 reveals that RyR2 has a much higher affinity for Zn^{2+} than Ca^{2+} (by ~3-orders of magnitude). We also showed that Zn^{2+} modulated both the frequency and amplitude of Ca^{2+}-waves in cardiomyocytes in a concentration-dependent manner and that reduction of the $[Ca^{2+}]_i$ to sub-activating concentrations failed to abolish Ca^{2+}-waves in the presence of 1 nM Zn^{2+}. These data suggest that RyR2-mediated Ca^{2+}-homeostasis is intimately related to intracellular Zn^{2+} levels. In the heart, RyR2 channels operate in closely packed clusters (Baddeley *et al*., 2009; Hayashi *et al*., 2009; Sheard *et al*., 2022). It is conceivable that the Zn^{2+} current mediated through RyR2, although small, is sufficient to sensitise and recruit other RyR2 channels to help shape cellular Ca^{2+} responses. The role of Zn^{2+} as both a high affinity activator of RyR2, modulator of channel function in the absence of Ca^{2+}, and charge carrier that contributes to the RyR2-mediated current is a paradigm shift in our understanding of how RyR2 is activated during EC coupling. The recently identified

role of ZnT1 as a neuronal Ca^{2+}/Zn^{2+} transporter (Gottesman *et al*., 2022) opens the suggestion that Zn^{2+} is delivered to RyR2 by a zinc transporter located in the S/ER or the plasma membrane. However, further work is required to address this question. What is certain is that Zn^{2+} and Ca^{2+} dynamics are intrinsically coupled.

Mitsugumin-23 as a putative Zn^{2+}-regulated Ca^{2+}-permeable ion-channel

RyR2 is not the only Ca^{2+}-permeable ion channel localised to S/ER stores. TMEM109 or Mitsugumin-23 (MG23) is a 23 kDa transmembrane protein found in the S/ER and nuclear membranes of cardiac muscle cells and other tissues including skeletal muscle, epithelial cells, and the brain (Nishi *et al*., 1998). MG23 is a voltage-sensitive non-selective cation channel. MG23 has an unusual morphology as shown by electron microscopy and 3D particle reconstruction. Two types of particles were consistently observed; a small asymmetric particle composed of six homomeric subunits, and a larger bowl-shaped particle forming a hexametric mega structure composed of six asymmetric particles (Venturi *et al*., 2011). The mega pore structure is hypothesised to readily assemble and disassemble, and this is functionally mirrored in the observed gating behaviour of MG23. Recombinant purified MG23 proteins reconstituted into planar lipid bilayers exhibit very unusual gating behaviour characterised by brief 'flickery' opening events and co-ordinated gating of multiple channels (Venturi *et al*., 2011; Reilly O'Donnell *et al.*, 2017). It is likely that both the asymmetric particle and the mega structure both permit ion permeation, and that the unusual gating behaviour reflects the apparent instability of MG23. The MG23 channel has received little attention but given its location and its ability to conduct Ca^{2+}, it is likely that it contributes to the Ca^{2+} leak and/or Ca^{2+} current in cardiac cells. Information regarding modulators of MG23 activity is currently lacking but our recent work has shown that cytosolic Zn^{2+} increases MG23 activity (Reilly O'Donnell *et al.*, 2017). Glutamate, aspartate, histidine and cysteine amino acid residues are commonly associated with Zn^{2+} binding sites. Surprisingly human MG23 does not have any cysteine residues and so lacks the classic C2H2 zinc finger motif.

MG23 does have a common conserved, H-x-x-x-E sequence which is attributed to Zn^{2+} binding in Zn^{2+} transporters including ZIP1, ZIP2 and ZIP3 (Figure 3; Kambe *et al*., 2015). Hydrophobicity plots published by Nishi *et al* (1998) suggests the part of the protein containing this sequence is localised in the SR lumen (Nishi *et al*, 1998). It is not known whether RyR2 and MG23 interact with each other or if MG23 is part of the calcium release unit. One could speculate that the recently described RyR2-mediated Zn^{2+} current might trigger recruitment and initiation of MG23-mediated Ca^{2+} fluxes, as summarised in Figure 4.

Zn^{2+}-induced regulation of IP3Rs

The role of IP_3R in EC coupling is considered of most importance during early cardiac development (Luo *et al*., 2020). As the S/ER matures, the number of RyR2 channels increases and in adult cardiomyocytes RyR2 mRNA levels are ~50-fold higher than IP_3R (Moschella and Marks, 1993). Despite this, IP_3Rs located in the nuclear envelope are involved in excitation–transcription coupling, thereby participating in the control of gene expression (Nakayama *et al*., 2010). In mammalian cardiomyocytes Zn^{2+} plays a key role in excitation-transcription coupling where Zn^{2+} influx through LTCC mediates voltage-dependent gene expression (Atar *et al*., 1995), suggesting a possible link between Zn^{2+} and IP_3R in regulation of gene expression. In dissociated rat hippocampal neuronal cultures relatively small changes in cytosolic Zn^{2+} during stimulation altered expression levels of 931 genes with IP_3R type-2 being markedly upregulated (Sanfold *et al*., 2019). Zn^{2+} can be released from S/ER stores upon IP_3R stimulation. The release of caged inositol 1,4,5-trisphosphate (IP_3) in cultured cortical neurons resulted in the release of Zn^{2+} from thapsigargin-sensitive stores, suggesting that sequestration of Zn^{2+} into the S/ER is important in regulation of intracellular levels and that Zn^{2+} is released following agonist stimulation (Stork and Li, 2010). How Zn^{2+} modulates IP_3 signalling in the heart is an underexplored area of research. Although to date there is no demonstration that IP3Rs are directly modulated by Zn^{2+}, IP_3Rs have a C2H2 zinc finger domain in the C-terminal tail that

plays a critical role in regulation of channel activity (Furuichi *et al*., 1989). Individual or combined cysteine and histidine mutations within this conserved C2H2 domain resulted in the abolition of IP_3R type-1 functioning (Uchida *et al*., 2003; Bhanumathy *et al*., 2012). This C2H2 C-terminal domain region is also highly conserved across the RyR family and is thought to be important in maintenance of the RyR2-mediated Zn^{2+} currents (Gaburjakova and Gaburjakova, 2022), suggesting a fundamental role for Zn^{2+} in intracellular Ca^{2+} channel regulation and cellular Ca^{2+} dynamics.

Dysregulation of cardiac Zn^{2+} homeostasis in disease

Role of Zn^{2+}-binding proteins in disease

The ability of serum albumin in the extracellular environment to bind and buffer Zn^{2+} is known to be compromised by the binding of fatty acids (Stewart *et al*., 2003; Lu *et al*., 2012; Sobczak *et al*., 2021a), which it transports through binding at up to seven different sites (Bhattacharya *et al*., 2000). Total plasma levels of fatty acids are generally quite low (<1 mol eq. relative to albumin; Sobczak *et al*., 2021a; Sobczak *et al*., 2021b) but can be elevated in some disease states. Although high plasma fatty acid levels are known to increase the risk of heart failure and sudden cardiac death (Pilz *et al*., 2007; Djoussé *et al*., 2013), how this dynamic might impact upon cellular Zn^{2+} uptake under physiological conditions has yet to be investigated.

Zn^{2+} supplementation is known to induce cardiac MT expression (Wang *et al*., 2006), emphasising its importance in regulating zinc homeostasis in the heart. Several studies have highlighted a protective role for MTs in helping to prevent/reduce cardiomyopathy and oxidative stress. It has been shown that overexpression of MT in cell and animal models protects cardiomyocytes from diabetic cardiomyopathy (Liang *et al*., 2002; Cai *et al*., 2006; Huang *et al*., 2021). Cardiac-specific overexpression of MT reduces cigarette smoking

418 exposure-induced myocardial contractility and mitochondrial damage (Hu *et al*., 2013). Zinc-
419 induced MT expression has been shown to reduce doxorubicin-induced damage in
420 cardiomyocytes (Kimura *et al*., 2000; Jing *et al*., 2015). In addition, alcohol-induced cardiac
421 hypertrophy and fibrosis were observed in metallothionein-knockout (MT-KO) mice fed an
422 alcohol-containing liquid diet for 2 months but not in wildtype mice fed the same diet (Wang
423 *et al*., 2005). Similarly, doxorubicin-induced cardiomyopathy was found to be more severe in
424 MT-KO mice in than wildtype mice (Kimura *et al*., 2000).

425 The mechanisms by which MTs mediate their cardioprotective effects have been examined.
426 MT protection against doxorubicin-induced cytotoxicity was found to be at least partially
427 mediated via the JAK2/STAT3 pathway in murine cardiomyocytes (Rong *et al*., 2016). MT-
428 induced inhibition of the NF-κB pathway has been linked to prevention of age-associated
429 cardiomyopathy (Cong *et al*., 2016). A recent study suggests that MT2A protects
430 cardiomyocytes from I/R through p38 inhibition (Zhao *et al*., 2021). It has also been shown
431 that MT inhibits doxorubicin-induced mitochondrial cytochrome c release and caspase-3
432 activation in cardiomyocytes (Wang *et al*., 2001). Collectively, these studies demonstrate
433 that MTs act to induce the expression of cardioprotective genes and reduce mitochondrial
434 damage due to oxidative stress in cardiac tissue.

435 *Zinc transporter expression in cardiac dysfunction*

436 In cardiac dysfunction, intracellular Zn^{2+} levels are known to be altered. A role for Zn^{2+} in
437 ischaemia was first established in cerebral ischaemia in rat brain in 1990 (Tønder *et al*.,
438 1990), and later demonstrated in isolated rat cardiomyocytes where an ~30-fold increase in
439 $[Zn^{2+}]_i$ was observed during ischaemia that rapidly decreased upon reoxygenation (Ayaz and
440 Turan, 2006). Hare *et al* observed an accumulation of $[Zn^{2+}]_i$ in the left ventricle of rat cardiac
441 tissue following I/R (Hare *et al*., 2009).

442 Alterations in the expression levels of zinc transporters are associated with several
443 cardiovascular events (Table 4). Hara and colleagues suggest that modulation of ZIP13

expression may be important for inflammatory signalling responses in the heart following *in vitro* treatment with doxorubicin (Hara *et al*., 2022). In S/ER, ZIP7 and ZnT7 expression is reported to be altered in type 2 diabetes and high glucose conditions, which are both considered risk factors for cardiovascular disease. Protein expression of ZIP7 was significantly decreased while expression of ZnT7 was significantly increased in cardiomyocytes cultured in high glucose conditions and in hearts excised from a diabetic rat model (Tuncay *et al*., 2019). Tuncay *et al* also identified significant alterations in ZIP7 and ZnT7 S/ER protein expression in H9C2 cells treated with doxorubicin to simulate heart failure (Tuncay *et al*., 2017). Furthermore, in cardiac tissue from individuals with heart failure the expression of ZIP14 and ZnT8 was significantly increased and ZIP8 levels decreased relative to controls (Olgar *et al*, 2018a). Screening all ZIP and ZnT transporters, Bodiga and colleagues reported alterations in multiple transporters in cardiomyocytes exposed to a hypoxia/reoxygenation protocol, among which were the S/ER-located ZIP7 and ZIP14 transporters (Bodiga *et al*., 2017).

Zn^{2+} dyshomeostasis in EC coupling

The importance of tightly controlled cellular Zn^{2+} homeostasis for the prevention of cardiac dysfunction is beginning to emerge (Alvarez-Collazo *et al*., 2012; Turan and Tuncay, 2017). In animal models, dysregulated levels of intracellular Zn^{2+} are associated with severe cardiac degeneration in Duchenne muscular dystrophy (Crawford and Bhattacharya, 1997). Male mice deficient of ZnT5 have significantly higher frequency of bradyarrhythmias and mortality rate compared with control animals (Inoue *et al*., 2002). Also, Zn^{2+} significantly contributes to oxidant-induced alterations of EC coupling (Turan *et al*., 1997). Defective Zn^{2+} handling contributes to the cellular pathology of certain cardiomyopathies including altered contractility and heart failure (Kleinfeld and Stein, 1968; Kalfakakou *et al*., 1993; Little *et al*., 2010). The underlying mechanism of how Zn^{2+} contributes to these pathologies is still not fully understood. Cytosolic Zn^{2+} has recently been shown to act as a high affinity activator of RyR2, able to activate channels even when $[Ca^{2+}]_i$ is subactivating (Woodier *et al*., 2015;

Reilly O'Donnell *et al*., 2017) providing an important mechanistic explanation for how Zn^{2+} dyshomeostasis can result in altered Ca^{2+} dynamics and cardiac dysfunction. An emerging and important research area is therefore to understand how altered Zn^{2+} levels evoke deleterious effects on cardiac functioning.

Zn^{2+} dyshomeostasis in cardiac morphogenesis

Zinc transporters are of key importance in embryonic development and cardiac morphogenesis. Knock-out of ZnT1 or ZIP7 is embryonically lethal (Andrews *et al.*, 2004; Woodruff *et al*., 2018). Knock-out of ZIP8 is also embryonically lethal in mice with hypertrabeculation and noncompaction of the ventricles observed, while knock-down of ZIP10 in zebrafish results in heart deformities (Taylor *et al.,* 2016; Lin *et al.,* 2018). Additionally, recent research shows primary neonatal cardiomyocytes from ZIP13 knock-out mice display arrhythmic beating (Hara *et al*., 2022).

The findings of Inoue and colleagues are also noteworthy, where ZnT5 knock-out resulted in male-specific sudden death from bradyarrhythmia (Inoue *et al.,* 2002). Loss of function mutation of ZnT5 is reported to result in lethal cardiomyopathy and premature death in case study by Lieberwirth *et al* (2021). This illustrates that zinc transporters as well as calcium channels are necessary in cardiac development and function.

Zn^{2+} dyshomeostasis as a new pharmacological target in cardiovascular disease

Sacubitril/valsartan (formally known as LCZ696) is an active substance in the drug Entersto, which is used to treat chronic heart failure (Khali *et al*., 2018). Sacubitril/valsartan is an angiotensin II type 1 receptor blocker that inhibits neprilysin and is currently being trialled for treatment of patients with chronic systolic heart failure (ClinicalTrials.gov Identifier: NCT01035255; McMurray *et al*., 2013). These trials are of interest as neprilysin is a zinc-dependent plasma membrane type II integral protein metallopeptidase which contains a

Zn^{2+}-binding site on its extracellular C-terminal domain (Fulcher and Kenny, 1983; Nalivaeva *et al*., 2020), linking Zn^{2+} dependent processes with cardiovascular function.

There have also been trials examining the usefulness of Zn^{2+} chelation. The TACT trial (NCT00044213) investigated the effect of chelation therapy using EDTA on the occurrence of subsequent cardiovascular events in participants with previous myocardial infarction (Lamas *et al.*, 2013). EDTA is a chelator of not only Zn^{2+}, but also of Ca^{2+}, Mg^{2+}, Fe^{2+}/Fe^{3+}, Cd^{2+} and Cu^{2+} (Lamas *et al.*, 2013). Reactive binding of EDTA to metals is as follows: Cr^{2+} >Fe^{3+} >Cu^{2+} >Pb^{2+} >Zn^{2+} >Cd^{2+} >Co^{2+} >Fe^{2+} >Mn^{2+} >Ca^{2+} >Mg^{2+}, therefore EDTA will preferentially bind Zn^{2+} (estimated K_d 10^{-16} M) over other divalent metals in plasma including Ca^{2+} (K_d approximately 10^{-11} M) due to the high affinity EDTA has for Zn^{2+} (Waters *et al.,* 2001; commentary by Nyborg and Peersen 2004). The trial concluded that treatment with EDTA modestly reduced the risk of adverse cardiovascular outcomes. However, the evidence was not sufficient to justify the implementation of chelation therapy as a routine post-myocardial infarction treatment (Lamas *et al.*, 2013). The research has been continued in the TACT2 trial, which is focusing on chelation therapy in patients with diabetes who have had a previous myocardial infarction (NCT02733185; U.S. National Library of Medicine, 2022). This trial is due for completion in December 2023 (U.S. National Library of Medicine, 2022). The targeting of Zn^{2+} to improve patient outcome in myocardial infarction and heart failure have not yet resulted in development of new cardiovascular disease treatments. In addition, Zn^{2+} levels cannot be used as a biomarker for cardiovascular disease as several factors including dietary intake and blood glucose levels can alter plasma Zn^{2+} concentration and zinc handling (Fernández-Cao *et al.*, 2019). However, it is possible that chelation of Zn^{2+} in the short term, for example during a myocardial infarction, would help to attenuate the damage observed post-myocardial infarction.

Concluding remarks

The role of ZIPs, ZnTs and Zn^{2+}-binding proteins in the heart provides novel insights into the regulation of cellular Zn^{2+} and its role as a signalling molecule in cardiac tissue. The ability of Zn^{2+} to act as a regulator and/or activator of cellular Ca^{2+} channels suggest a new and important role for Zn^{2+} in cardiac function under both physiological and pathological conditions, raising the suggestion that correction of Zn^{2+} dyshomeostasis may be a novel therapeutic strategy to combat cardiovascular disease.

In comparison to Ca^{2+}, there has been relatively little work investigating the biological function of Zn^{2+} in the heart. Consideration of accurate $[Zn^{2+}]_i$ measurements should be emphasized as failure to acknowledge dynamic Zn^{2+} changes could lead to significant overestimation of $[Ca^{2+}]_i$. Indeed, many of the tools routinely used to measure Ca^{2+} also bind Zn^{2+}, challenging us to consider how many processes driven by Ca^{2+} may also be in part, attributable to Zn^{2+} (Stork and Li, 2006; Figueroa *et al*., 2014; Fujikawa *et al*., 2015). Thanks to the development of appropriate tools enabling us to accurately monitor Zn^{2+} fluxes, and the ability of these methods to distinguish Zn^{2+} from Ca^{2+} in biological systems, the field of zinc biology is currently advancing rapidly (for a comprehensive overview of different Zn^{2+} sensors see Huang and Lippard, 2012; Carpenter *et al*., 2016; Pratt *et al*., 2021;). Much has been learned relating to the intrinsic relationships that exist between Zn^{2+} and Ca^{2+} homeostatic mechanisms and their roles in heart disease. However, more work is needed to fully understand the role of Zn^{2+} in the heart. This includes better understanding of cellular Zn^{2+} dynamics, how Zn^{2+} is regulated and the biological targets of labile Zn^{2+}. This will require a greater appreciation of the spatio-temporal patterning of intracellular Zn^{2+} fluxes in the heart and how these relate to cardiac functioning in health and disease.

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Disclosures

None.

Figure Legends

Figure 1. **Zn^{2+} transporters in the heart. A)** Localisation of zinc transporters in the heart. ZIP transporters are illustrated in blue in the left of the image while ZnT transporters are coloured in red on the right of the image. Transporters with confirmed protein expression through the Human Protein Atlas or reported in published western blot/immunofluorescent in heart tissue homogenates, isolated cardiomyocytes, or cardiac cell lines (such as H9C2 cells) were included. Rough endoplasmic reticulum (rER), sarcoplasmic/endoplasmic reticulum (S/ER), trans-Golgi network (TGN). Created with BioRender.com. **B)** RNA expression of Zn^{2+} transporters in normalized protein-coding transcripts per million (nTPM) in human heart. Figure was created using information available from the Human Protein Atlas, Uhlén et al., 2015 and Choi et al., 2018.

Figure 2. **RNA expression of S/ER-located Zn^{2+} transporters. A)** Mean reads per kilobase of transcript per million reads mapped (RPKM) of Zn^{2+} transporters in human heart (RNA-Seq data from Fagerberg *et al.,* 2014). **B)** Mean RPKM of Zn^{2+} transporters in rat heart (21 weeks; RNA-Seq data from Yu *et al.,* 2014). **C)** Mean RPKM of Zn^{2+} transporters in mouse heart (RNA-Seq data from Yue *et al.,* 2014).

Figure 3. **Possible Zn^{2+} binding sites on MG23.** Partial sequence alignment of human zinc transporters ZIP1, ZIP2 and ZIP3 illustrating the conserved Zn^{2+} binding motif, H-x-x-x-E. This motif is also conserved across human (h), rat (r) and murine (m) MG23.

Figure 4. **Graphical summary of the suggested role of MG23 in cardiovascular function.** MG23 may contribute to the release of Ca^{2+} from S/ER Ca^{2+} stores. In pathophysiological conditions where intracellular Zn^{2+} is elevated, the activity of MG23 will be increased, leading to increased release of Ca^{2+} from the S/ER. Increased $[Zn^{2+}]_i$ will result in activation of RyR2.

573 Dotted lines and question marks suggest putative interactions/functions. Figure created with
574 BioRender.com.

575 **References**

576 Alberts, I.L., K. Nadassy, and S.J. Wodak. 1998. Analysis of zinc binding sites in protein
577 crystal structures. *Protein Sci.* 7(8):1700-1716.

578 Andreini, C., L. Banci, I. Bertini, and A. Rosato. 2006. Counting the zinc-proteins encoded in
579 the human genome. *J. Proteome Res.* 5:196-201.

580 Andrews, G.K., H. Wang, S.K. Dey, and R.D. Palmiter. 2004. Mouse zinc transporter 1 gene
581 provides an essential function during early embryonic development. *Genesis.*
582 40(2):74-81.

583 Alvarez-Collazo, J., C.M. Díaz-García, A.I. López-Medina, G. Vassort, and J.L. Alvarez.
584 2012. Zinc modulation of basal and β-adrenergically stimulated L-type Ca^{2+} current in
585 rat ventricular cardiomyocytes: consequences in cardiac diseases. *Pflugers Arch.*
586 464:459-470.

587 Atar, D., P.H. Backx, M.M. Appel, W.D. Gao, and E. Marban. 1995. Excitation-transcription
588 coupling mediated by zinc influx through voltage-dependent calcium channels. *J. Biol.*
589 *Chem.* 270:2473-2477.

590 Ayaz, M., and B. Turan. 2006. Selenium prevents diabetes-induced alterations in $[Zn2+]_i$ and
591 metallothionein level of rat heart via restoration of cell redox cycle. *Am. J. Physiol.*
592 *Heart Circ. Physiol.* 290:H1071-H1080.

593 Aydemir, T.B., J.P. Liuzzi, S. McClellan, and R.J. Cousins. 2009. Zinc transporter ZIP8
594 (SLC39A8) and zinc influence IFN- γ expression in activated human T cells. *J.*
595 *Leukoc. Bio.* 86(2):337-348.

596 Baddeley, D., I.D. Jayasinghe, L. Lam, S. Rossberger, M.B. Cannell, and C. Soeller. 2009.
597 Optical single-channel resolution imaging of the ryanodine receptor distribution in rat
598 cardiac myocytes. *Proc. Natl. Acad. Sci. USA* 106:22275-22280.

599 Baksh, S. C. Spamer, C. Heilmann, and M. Michalak. 1995. Identification of the Zn^{2+} binding
600 region in calreticulin. *FEBS Lett.* 376:53-57.

601 Baudier, J., K. Haglid, J. Haiech, and D. Gérard. 1983. Zinc ion binding to human brain
602 calcium binding proteins. Calmodulin and S100b protein. *Biochem. Biophys. Res.*
603 *Comm*un. 114(3):1138–1146.

604 Beharier, O., Y. Etzion, A. Katz, H. Friedman, N. Tenbosh, S. Zacharish, S. Bereza, U.
605 Goshen, and A. Moran. 2007. Crosstalk between L-type calcium channels and ZnT-1,
606 a new player in rate-dependent cardiac electrical remodeling. *Cell Calcium* 42:71-82.

607 Beharier, O., Y. Etzion, S. Levi, M. Mor, M. Mor, S. Dror, J. Kahn, A. Katz, and A. Moran.
608 2010. The involvement of ZnT-1, a new modulator of cardiac L-type calcium channels,
609 in atrial tachycardia remodeling. *Ann. N. Y. Acad. Sci.* 1188:87-95.

610 Bers, D. M. 2002. Cardiac excitation–contraction coupling. *Nature.* 415(6868):198– 205.

611 Betrie, A.H., J.A. Brock, O.F. Harraz, A.I. Bush, G.-W. He, M.T. Nelson, J.A. Angus, C.E.
612 Wright, and S. Ayton. 2021. Zinc drives vasorelaxation by acting in sensory nerves,
613 endothelium and smooth muscle. *Nat. Commun.* 12:3296.

614 Bhanumathy, C., P.C.A. Da Fonseca, E.P. Morris, and S.K. Joseph. 2012. Identification of
615 functionally critical residues in the channel domain of inositol trisphosphate receptors.
616 *J. Biol. Chem.* 287:43674-43684

617 Bhattacharya, A.A., T. Grüne, and S. Curry. 2000. Crystallographic analysis reveals common
618 modes of binding of medium and long-chain fatty acids to human serum albumin. *J.*
619 *Mol. Biol.* 303:721-732.

620 Bindreither, D., and P. Lackner. 2009. Structural diversity of calcium binding sites. *Gen.*
621 *Physiol. Biophys.* 28 Spec No Focus:F82-88.

622 Bittel, D., T. Dalton, S.L. Samson, L. Gedamu, and G.K. Andrews. 1998. The DNA binding
623 activity of metal response element-binding transcription factor-1 is activated in vivo
624 and in vitro by zinc, but not by other transition metals. *J. Biol. Chem.* 273:7127-7133.

625 Bock, C.W., A.K. Katz, and J.P. Glusker. 1995. Hydration of zinc ions: a comparison with
626 magnesium and beryllium ions. *J. Am. Chem. Soc*. 117:3754-3765.
627

628 Bodi, I., G. Mikala, S.E. Koch, S.A. Akhter, and A. Schwartz. 2005. The L-type calcium
629 channel in the heart: the beat goes on. *J. Clin. Invest.* 115:3306-3317.

630 Bodiga, V.L., S. Thokala, S.M. Kovur, and S. Bodiga. 2017. Zinc dyshomeostasis in
631 cardiomyocytes after acute hypoxia/reoxygenation. *Biol. Trace Elem Res.* 179:117-
632 129.

633 Bootman. M.D., and K. Rietdorf. 2017. Tissue Specificity: Store-Operated Ca^{2+} Entry in
634 Cardiac Myocytes. In: Groschner, K., Graier, W., Romanin, C. (eds) Store-Operated
635 Ca^{2+} Entry (SOCE) Pathways. Advances in Experimental Medicine and Biology, vol
636 993. Springer, Cham.

637 Bouron, A., K. Kiselyov, and J. Oberwinkler. 2015. Permeation, regulation and control of
638 expression of TRP channels by trace metal ions. *Pflugers Arch.* 467(6):1143-1164.

639 Cai, L., Y. Wang, G. Zhou, T. Chen, Y. Song, X. Li, and J. Kang. 2006. Attenuation by
640 metallothionein of early cardiac cell death via suppression of mitochondrial oxidative
641 stress results in a prevention of diabetic cardiomyopathy. *J. Am. Coll. Cardiol.*
642 48:1688-1697.

643 Carpenter, M.C., M.N. Lo, and A.E. Palmer. 2016. Techniques for measuring cellular zinc.
644 *Arch. Biochem. Biophys.* 611:20-29.

645 Chabosseau, P., E. Tuncay, G. Meur, E.A. Bellomo, A. Hessels, S. Hughes, P.R.V. Johnson,
646 M. Bugliani, P. Marchetti, B. Turan, A.R. Lyon, M. Merkx, and G.A. Rutter. 2014.
647 Mitochondrial and ER-targeted eCALWY probes reveal high levels of free Zn^{2+}. *ACS*
648 *Chem. Biol.* 9:2111-2120.

649 Cho, H.M., J.R. Ryu, Y. Jo, T.W. Seo, Y.N. Choi, J.H. Kim, J.M. Chung, B. Cho, H.C. Kang,
650 S.-W. Yu, S.J. Yoo, H. Kim, and W. Sun. 2019. Drp1-Zip1 interaction regulates
651 mitochondrial quality surveillance system. *Mol. Cell.* 73:364-376.

652 Choi, S., X. Liu, and Z. Pan. 2018. Zinc deficiency and cellular oxidative stress: prognostic
653 implications in cardiovascular diseases. *Acta Pharmacol. Sin.* 39:1120-1132.

654 Ciofalo, F.R., and L.J. Thomas. 1965. The effects of zinc on contractility, membrane
655 potentials, and cation content of rat atria. *J. Gen. Physiol.* 48(5):825-839.

656 Communal, C., K. Singh, D.B. Sawyer, and W.S. Colucci. 1999. Opposing effects of β1- and
657 β2-adrenergic receptors on cardiac myocyte apoptosis. *Circulation.* 100(22):2210-
658 2212.

659 Cong, W., C. Niu, L. Lv, M. Ni, D. Ruan, L. Chi, Y. Wang, Q. Yu, K. Zhan, Y. Xuan, Y. Wang,
660 Y. Tan, T. Wei, L. Cai, and L. Jin. 2016. Metallothionein prevents age-associated
661 cardiomyopathy via inhibiting NF-κB pathway activation and associated nitrative
662 damage to 2-OGD. *Antioxid. Redox Signal.* 25:936-952.

663 Conklin, D.S., M.R. Cuthbertson, and C. Klung. 1994. Interactions between gene products
664 involved in divalent cation transport in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.*
665 244:303-311.

Cousins, R.J., J.P. Liuzzi, and L.A. Lichten. 2006. Mammalian zinc transport, trafficking, and signals. *J. Biol. Chem.* 281:24085-24089.

Coverdale, J.C.P., H.A. van den Burgh, S. Khazaipoul, H.E. Bridgewater, A.J. Stewart, and C.A. Blindauer. 2022. Albumin-mediated extracellular zinc speciation drives cellular zinc uptake. *Chem. Commun.* 58:7384-7387.

Coyle, P., J.C. Philcox, L.C. Carey, and A.M. Rofe. 2002. Metallothionein: the multipurpose protein. *Cell. Mol. Life Sci.* 59:627-647.

Crawford, A.J., and S.K. Bhattacharya. 1987. Excessive intracellular zinc accumulation in cardiac and skeletal muscles of dystrophic hamsters. *Exp. Neurol.* 95:265-276.

Cuajungco, M.P., L.C. Basilio, J. Silva, T. Hart, J. Tringali, C.-C. Chen, M. Biel, and C. Grimm. 2014. Cellular zinc levels are modulated by TRPML1-MEME163 interaction. *Traffic.* 15(11):1247-1265.

Dabravolski, S.A., N.K. Sadykhov, A.G. Kartuesov, E.E. Borisov, V.N. Sukhorukov, and A.N. Orekhov. 2022. Interplay between Zn^{2+} homeostasis and mitochondrial functions in cardiovascular diseases and heart aging. *Int. J. Mol Sci.* 23(13):6890.

Dalton, T.P., L. He, B. Wang, M.L. Miller, L. Jin, K.F. Stringer, X. Chang, C.S. Baxter, and D.W. Nebert. 2005. Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. *PNAS.* 102(9):3401-3406.

Davis, S.R, and R.J. Cousins. 2000. Metallothionein expression in animals: A physiological perspective on function. *J. Nutr.* 130:1085-1088.

de Tombe, P. P. 2003. Cardiac myofilaments: mechanics and regulation. *J. Biomech.* *3*6(5):721–730.

Diaz-Sylvester, P.L; M. Porta, and J.A. Copello. 2011. Modulation of cardiac ryanodine receptor channels by alkaline earth cations. *PLoS ONE* 6:e26693.

690 Djoussé, L., K.J. Rothman, A. Cupples, D. Levy, and R.C. Ellison. 2002. Serum Albumin and
691 risk of myocardial infarction and all-cause mortality in the Framingham Offspring
692 study. *Circulation.* 106:2919-2924.

693 Djoussé, L., D. Benkeser, A. Arnold, J.R. Kizer, S.J. Zieman, R.N. Lemaitre, R.P. Tracey,
694 J.S., Gottdeiner, D. Mozaffarian, D.S. Siscovick, K.J. Mukamal, and J.H. Ix. 2013.
695 Plasma free fatty acids and risk of heart failure. *Circulation* 6:964-969.

696 Dorn II, G.W., and C. Maack. 2013. SR and mitochondria: calcium cross-talk between
697 kissing cousins. *J. Mol. Cell Cardiol.* 55:42-49.

698 Du, L., H. Zhang, H. Zhao, X. Cheng, J. Qin, T. Teng, Q. Yang, and Z. Xu. 2019. The critical
699 role of the zinc transporter Zip2 (SLC39A2) in ischemia/reperfusion injury in mouse
700 hearts. *J. Mol. Cell. Cardiol.* 132:136-145.

701 Du, W., M. Gu, M. Hu, P. Pinchi, W. Chen, M. Ryan, T. Nold, A. Bannaga, and H. Xu. 2021.
702 Lysosomal Zn^{2+} release triggers rapid, mitochondrial-mediated, non-apoptotic cell
703 death in metastatic melanoma cells. *Cell Reports.* 37(3):109848.

704 Eide, D.J. 2004. The SLC39 family of metal ion transporters. *Pflugers Arch.* 447: 796-800.

705 Eisner, V., G. Csordás, and G. Hajnóczky. 2013. Interactions between sarco-endoplasmic
706 reticulum and mitochondria in cardiac and skeletal muscle – pivotal roles in Ca^{2+} and
707 reactive oxygen species signaling. *J. Cell. Sci.* 126(Pt 14):2965-2978.

708 Etzion, Y., A. Ganiel, O. Beharier, A. Shalev, V. Novack, L. Volvich, D. Abrahamov. M.
709 Matsa, G. Sahar, A. Moran, and A. Katz. 2007. Correlation between atrial ZnT-1
710 expression and atrial fibrillation in Humans: a pilot study. *J. Cardiov. Electro.*
711 19(2):157-164.

712 Fagerberg, L., B.M. Hallström, P. Oksvold, C. Kampf, D. Djureinovic, J. Odeberg, M. Habuka,
713 S. Tahmasebpoor, A. Danielsson, K. Edlund, A. Asplund, E. Sjöstedt, E. Lundberg,

C.A-K. Szigyarto, M. Skogs, J.O. Takanen, H. Berling, H. Tegel, J. Mulder, P. Nilsson, J.M. Schwenk, C. Lindskog, F. Danielsson, A. Mardinoglu, A. Sivertsson, K.V. Feilitzen, M. Forsberg, M. Zwahlen, I. Olsson, S. Navani, M. Huss, J. Nielsen, F. Ponten, and M. Uhlén. 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol. Cell Proteomics.* 13(2):397-406.

Fernández-Cao, J.C., M. Warthon-Medina, V.H. Moran, V.H. Arija, C. Doepking, L. Serra-Majem L, N.M. Lowe. 2019. Zinc intake and status and risk of type 2 diabetes mellitus: A systematic review and meta-analysis. *Nutrients* 11:1027.

Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am. J. Physiol.* 245(1):C1–C14.

Fearnley, C.J., Roderick, H.L., and M.D. Bootman. 2011. Calcium signalling in cardiac myocytes. *Cold Spring Harb. Perspect Biol.* 3(11):a004242.

Figueroa, J.A., K.S. Vignesh, G.S. Deepe, Jr., and J. Caruso. 2014. Selectivity and specificity of small molecule fluorescent dyes/probes used for the detection of Zn^{2+} and Ca^{2+} in cells. *Metallomics* 6:301-315.

Fujikawa, K., R. Fukumori, S. Nakamura, T. Katsukake, T. Takarada, and Y. Yoneda. 2015. Potential interactions of calcium-sensitive reagents with zinc ion in different cultured cells. PLoS One 10:e0127421.

Fukada, T., N. Civic, T. Furuichi, S. Shimoda, K. Mishima, H. Higashiyama, Y. Idaira, Y. Asada, H. Kitamura, S. Yamasaki, S. Hojyo, M. Nakayama, O. Ohara, H. Koseki, H.G. dos Santos, L. Bonafe, R. Ha-Vinh, A. Zankl, S. Unger, M.E. Kraenzlin, J.S. Beckmann, I. Saito, C. Rivolta, S. Ikegawa, A. Superti-Furga, and T. Hirano. 2008. The Zinc Transporter SLC39A13/ZIP13 Is Required for Connective Tissue

738 Development; Its Involvement in BMP/TGF-β Signaling Pathways. *PLoS ONE.*
739 3(11):e3642.

740 Fulcher, I.S., and A.J. Kenny. 1983. Proteins of the kidney microvillar membrane. The
741 amphipathic forms of endopeptidase purified from pig kidneys. *Biochem J.* 211:743-
742 53.

743 Furuichi, T., S. Yoshikawa, A. Miyawaki, K. Wada, N. Maeda, and K. Mikoshiba. 1989.
744 Primary structure and functional expression of the inositol 1,4,5-trisphosphate-
745 binding protein P400. *Nature* 342:32-38.

746 Gaburjakova, J., and M. Gaburjakova. 2022. The cardiac ryanodine receptor provides a
747 suitable pathway for the rapid transport of zinc (Zn^{2+}). *Cells* 11:868.

748 Gaither L.A., and D.J. Eide. 2000. Functional expression of the human hZIP2 zinc
749 transporter. *J. Biol. Chem.* 275(8):5560-5564.

750 Gaither L.A., and D.J. Eide. 2001. The human ZIP1 transporter mediates zinc uptake in
751 human K562 erythroleukemia cells. *J. Biol. Chem.* 276(25):22258-22264.

752 Giorgi, C., D.D. Stefani, A. Bononi, R. Rizzuto, and P. Pinton. 2009. Structural and functional
753 link between the mitochondrial network and endoplasmic reticulum. *Int. J. Biochem.*
754 *Cell Biol.* 41(10):1817-1827.

755 Gorski P.A., D.K. Ceholski, and R.J. Hajjar. 2015. Altered myocardial calcium cycling and
756 energetics in heart failure – a rational approach for disease treatment. *Cell Metab.*
757 21(2):183-194.

758 Gottesman, N., H. Asraf, M. Bogdanovic, I. Sekler, T. Tzounopoulos, E. Aizenman, and M.
759 Hershfinkel. 2022. ZnT1 is a neuronal Zn^{2+}/Ca^{2+} exchanger. *Cell Calcium*
760 101:102505.

761 Griffiths, E.J., and G.A. Rutter. 2009. Mitochondrial calcium as a key regulator of
762 mitochondrial ATP production in mammalian cells. *Biochim. Piophys. Acta.*
763 1787(11):1324-1333.

764 Grotz, N., T. Fox, E. Connolly, W. Park, M.L. Guerinot, and D. Eide. 1998. Identification of a
765 family of zinc transporter genes from Arabidopsis that respond to zinc deficiency.
766 *Proc. Natl. Acad. Sci.* 95:7220-7224.

767 Haase, H., S. Hebel, G. Engelhardye, and L. Rink. 2015. The biochemical effects of
768 extracellular Zn^{2+} and other metal ions are severely affected by their speciation in cell
769 culture media. *Metallomics* 7:102-111.

770 Hara, T., T.-A. Takeda, T. Takagishi, K. Fukue, T. Kambe, and T. Fukada. 2017.
771 Physiological roles of zinc transporters: molecular and genetic importance in zinc
772 homeostasis. *J. Physiol. Sci.* 67(2):283-301.

773 Hara, T., I. Yamada, T. Ohashi, M. Tamura, A., Hijikata, T. Watanabe, M. Gao, K. Ito, S.
774 Kawamata, S. Azuma, E. Yoshigai, Y. Sumiyoshi, N. Yasuhiro, O. Ohara, H.G. dos
775 Santos, and T. Fukada. 2022. Role of Scl39a13/ZIP13 in cardiovascular homeostasis.
776 *PLoS One.* 17(10):e0276452.

777 Hare, D., D. Bishop, C. Austin, and P. Doble. 2009. The answer is elemental. *Biochem (Lond)*
778 31:46-49.

779 Hayashi, T., M.E. Martone, Z. Yu, A. Thor, M. Doi, M.J. Holst, M.H. Ellisman, and M.
780 Hoshijima. 2009. Three-dimensional electron microscopy reveals new details of
781 membrane systems for Ca^{2+} signaling in the heart. *J. Cell. Sci.* 122:1005-1013.

782 Hershfinkel, M., A. Moran, N. Grossman, and I Sekler. 2001. A zinc-sensing receptor triggers
783 the release of intracellular Ca^{2+} and regulates ion transport. *Proc. Natl. Acad. Sci.*
784 *USA* 98:11749-11754.

785 Hirota, J., T. Furuichi, and K. Mikoshiba. 1999. Inositol 1,4,5-trisphosphate receptor type 1 is
786 a substrate for caspase-3 and is cleaved during apoptosis in a caspase-3-dependent
787 manner. *J. Biol. Chem.* 274(48):34433-34437.

788 Ho, E. 2004. Zinc deficiency, DNA damage and cancer risk. *J. Nutr. Biochem.* 15(10):572–
789 578.

790 Hojyo, S., T. Fukada, S. Shimoda, W. Ohashi, B.-H. Bin, H. Koseki, and T. Hirano. 2011.
791 The zinc transporter SLC39A14/ZIP14 controls G-protein coupled receptor-mediated
792 signaling required for systemic growth. *PLoS One.* 6(3):e18059.

793 Holst, B., K.L. Egerod, E. Schild, S.P. Vickers, S. Cheetham, L.-O. Gerlach, L. Storjohann,
794 C.E. Stidsen, R. Jones, A.G. Beck-Sickinger, and T.W. Schwartz. 2007. GPR39
795 signaling is stimulated by zinc ions but not by obestation. *Endocrinology.* 148:13-20.

796 Hu, H., M. Bandell, M.J. Petrus, M.X. Zhu, and A. Patapoutian. 2009. Zinc activates
797 damage-sensing TRPA1 ion channels. *Nat. Chem. Biol.* 5(3):183-190.

798 Hu, N., X. Han, E.K. Lane, F. Guo, Y. Zhang, and J. Ren. 2013. Cardiac-specific
799 overexpression of metallothionein rescues against cigarette smoking exposure-
800 induced myocardial contractile and mitochondrial damage. *PLoS One* 8:e57151.

801 Huang, L., C.P. Kirschke, Y. Zhang, and Y.Y. Yu. 2005. The ZIP7 gene (Slc39a7) encodes a
802 zinc transporter involved in zinc homeostasis of the golgi apparatus. *J. Biol. Chem.*
803 280(15):15456-15463.

804 Huang, Z., and S.J. Lippard. 2012. Illuminating mobile zinc with fluorescence from cuvettes
805 to live cells and tissues. *Methods Enzymol.* 505:445-68.

806 Huang, S., J. Wang, H. Men, Y. Tan, Q. Lin, E. Gozal, Y. Zheng, and L. Cai. 2021. Cardiac
807 metallothionein overexpression rescues diabetic cardiomyopathy in Akt2-knockout
808 mice. *J. Cell. Mol. Med.* 25:6828-6840.

809 Hulsurkar, M.M., S.K. Lahrir, J. Karch, M.C. Weng, and X.H.T. Wehrens. 2022. Targeting
810 calcium-mediated inter-organellar crosstalk in cardiac disease. *Expert Opin. Ther.*
811 *Targets.* 26(4):303-317.

812 Human Protein Atlas. Proteinatlas.org. Accessed 29/09/22.

813 Inoue, K., K. Matsuda, M. Itoh, H. Kawaguchi, H. Tomoike, T. Aoyagi, R. Nagai, M. Hori, Y.
814 Nakamura, and T. Tanaka. 2002. Osteopenia and male-specific sudden cardiac
815 death in mice lacking a zinc transporter gene, Znt5. *Hum. Mol. Genet.* 11:1775-1784.

816 Jackson, M.J. 1989. Physiology of Zinc: General Aspects. In: Mills, C.F. (eds) Zinc in Human
817 Biology. ILSI Human Nutrition Reviews. Springer, London.

818 Jing, L., L. Li, J. Zhao, J. Zhao, Z. Sun, and S. Peng. 2015. Zinc-induced metallothionein
819 overexpression prevents doxorubicin toxicity in cardiomyocytes by regulating the
820 peroxiredoxins. *Xenobiotica* 46:715-725.

821 Kalfakakou, V.P., A.M. Evangelou, J. Benveniste, and B. Arnoux. 1993. The effects of Zn^{2+}
822 on guinea pig isolated heart preparations. *Biol. Trace Elem. Res.* 38:289-299.

823 Kamalov, G., P.A. Deshmukh, N.Y. Baburyan, M.S. Gandhi, P.L. Johnson, R.A. Ahokas, S.K.
824 Bhattacharya, Y. Sun, I.C. Gerling, and K.T. Weber. 2009. Coupled calcium and zinc
825 dyshomeostasis and oxidative stress in cardiac myocytes and mitochondria of rats
826 with chronic aldosteronism. *J. Cardiovasc. Pharmacol.* 53(5):414-423.

827 Kambe, T., H. Narita, Y. Yamaguchi-Iwai, J. Hirose, T. Amano, N. Sugiura, R. Sasaki, K.
828 Mori, T. Iwanaga, and M. Nagao. 2002. Cloning and characterization of a novel
829 mammalian zinc transporter, zinc transporter 5, abundantly expressed in pancreatic
830 beta cells. *J. Biol. Chem.* 277(21):19049-19055.

831 Kambe, T., T. Tsuji, A. Hashimoto, and N. Itsumura. 2015. The physiological, biochemical,
832 and molecular roles of zinc transporters on zinc homeostasis and metabolism.
833 *Physiol. Rev.* 95:749-784.

834 Kelleher, S.L., and B. Lönnerdal. 2003. Zn transporter levels and localisation change
835 throughout lactation in rat mammary gland and are regulated by Zn in mammary cells.
836 *J. Nutrition.* 133(11):3378-3385.

837 Kelleher, S.L., V. Veasques, T.P. Croxford, N.H. McCormick, V. Lopez, and J. MacDavid.
838 2012. Mapping the zinc-transporting system in mammary cells: molecular analysis
839 reveals a phenotype-dependent zinc-transporting network during lactation. *J. Cell.*
840 *Physiol.* 227(4):1761-1770.

841 Kim, K.-R., S.E. Park, J.-Y. Hong, J.-Y. Koh, D.-H. Cho, J.J. Hwang, and Y.-H. Kim. 2022.
842 Zinc enhances autophagic flux and lysosomal function through transcription factor EB
843 activation and V-ATPase assembly. *Front. Cell. Neurosci.* 16:895750.

844 Kimball, S.R., S.J. Chen, R. Risca, L.S. Jefferson, and A.E. Leure-duPree. 1995. Effects of
845 zinc deficiency on protein synthesis and expression of specific mRNAs in rat liver.
846 *Metabolism* 44:126-133.

847 Kimura, T., I. Fujita, N. Itoh, N. Muto, T. Nakanishi, K. Takahashi, J. Azuma, and K. Tanaka.
848 2000. Metallothionein acts as a cytoprotectant against doxorubicin toxicity. *J.*
849 *Pharmacol. Exp. Therap.* 292:299-302.

850 Kirschke, C.P., and L. Huang. 2003. ZnT7, a novel mammalian zinc transporter,
851 accumulates zinc in the Golgi apparatus. *J. Biol. Chem.* 278(6):4096-102.

852 Kleinfeld, M., and E. Stein. 1968. Action of divalent cations on membrane potentials and
853 contractility in rat atrium. *Am. J. Physiol.* 215:593-199.

Kowada, T., T. Watanabe, Y. Amagai, R. Liu, M. Yamada, H. Takahashi, T. Matsui, K. Inaba, and S. Mizukami. 2020. Quantitative imaging of labile Zn^{2+} in the Golgi Apparatus using a localized small-molecule fluorescent probe. *Cell chem. Bio.* 27(12): 1521-1531.

Kowalczyk, A., O. Gbadamosi, K. Kolor, J. Sosa, L. Andrzejczuk, G. Gibson, C. St Croix, M. Chikina, E. Aizenman, N. Clark, and K. Kiselyov. 2021. Evolutionary rate covariation identifies SLC30A9 (ZnT9) as a mitochondrial zinc transporter. *Biochem. J.* 478(17):3205-3220.

Krężel, A., and W. Maret. 2007. Different redox states of metallothionein/thionein in biological tissue. *Biochem. J.* 402:551-558.

Kukic, I., S.L. Kelleher, and K. Kiselyov. 2014. Zn^{2+} efflux through lysosomal exocytosis prevents Zn^{2+}-induced toxicity. *J. Cell Sci.* 127:3094-3103.

Lamas, G.A., C. Goertz, R. Boineau, D.B. Mark, T. Rozema, R.L. Nahin, L. Lindblad, E.F. Lewis, J. Drisko, K.L. Lee, and TACT Investigators. 2013. Effect of disodium EDTA chelation regimen on cardiovascular events in patients with previous myocardial infarction: the TACT randomized trial. *JAMA* 309:1241-1250.

Levy, S., O. Beharier, Y. Etzion, M. Mor, L. Buzaglo, L. Shaltiel, L.A. Gheber, J. Kahn, A.J. Muslin, A. Katz, D. Gitler, and A. Moran. 2009. Molecular basis for zinc transporter 1 action as an endogenous inhibitor of L-type calcium channels. *J. Biol. Chem.* 284:32434-32443.

Li, G., and P.-L. Li. 2021. Lysosomal TRPML1 channel: implications in cardiovascular and kidney diseases. *Adv. Exp. Med. Biol.* 1349:275-301.

876 Liang, Q., E.C. Carlson, R.V. Donthi, P.M. Kralik, X. Shen, and P.N. Epstein. 2002.
877 Overexpression of metallothionein reduces diabetic cardiomyopathy. *Diabetes*
878 51:174-181.

879 Lichten, L.A., M.-S. Ryu, L. Guo, J. Embury, and R.J. Cousins. 2011. MTF-1-mediated
880 repression of the zinc transporter Zip10 is alleviated by zinc restriction. *PLoS ONE.*
881 6(6):e21526.

882 Lieberwirth, J.K., P. Joset, A. Heinze, J. Hentschel, A. Stein, A. Iannaccone, K. Steindl, A.
883 Kuechler, and R.A. Jamra. 2021. Bi-allelic loss of function variants in SLC30A5 as
884 cause of perinatal lethal cardiomyopathy. *Eur. J. Hum. Genet.* 29(5):887.

885 Lin, W., D. Li, L. Cheng, L. Li, F. Liu, N.J. Hand, J. A. Epstein, and D.J. Rader. 2018. Zinc
886 transporter Slc39a8 is essential for cardiac ventricular compaction. *J. Clin. Invest.*
887 128:826-833.

888 Little, P.J., R. Bhattacharya, A.E. Moreyra, and I.L. Korichneva. 2010. Zinc and
889 cardiovascular disease. *Nutrition* 26:1050-7.

890 Lu, J., A.J. Stewart, P.J. Sadler, T.J.T. Pinheiro, and C.A. Blindauer. 2008. Albumin as a zinc
891 carrier: Properties of its high-affinity zinc-binding site. *Biochem. Soc. Trans.* 36:1317-
892 1321.

893 Lu, J., A.J. Stewart, D. Sleep, P.J. Sadler, T.J.T. Pinheiro, and C.A. Blindauer. 2012. A
894 molecular mechanism for modulating plasma Zn speciation by fatty acids. *J. Am.*
895 *Chem. Soc.* 134:1454-1457.

896 Lu, Q., H. Haragopal, K.G. Slepchenko, C. Stork, and Y.V. Li. 2016. Intracellular zinc
897 distribution in mitochondria, ER and the Golgi apparatus. *Int. J. Pathophysiol.*
898 *Pharmacol.* 8(1):35-43.

899 Luo, X., W. Li, K. Künzel, S. Henze, L. Cyganek, A. Strano, M.S. Poetsch, M. Schubert, and
900 K. Guan. 2020. IP3R-mediated compensatory mechanism for calcium handling in
901 human induced pluripotent stem cell-derived cardiomyocytes with cardiac ryanodine
902 receptor deficiency. *Front. Cell. Dev. Biol.* 8:722.

903 MacDonald, R.S. 2000. The role of zinc in growth and cell proliferation. *J. Nutr.* 130:1500S-
904 1508S.

905 Martin, A.B., T.B. Aydemir, G.J. Guthrie, D.A. Samuelson, S.-M. Chang, and R.J. Cousins.
906 2013. Gastric and colonic zinc transporter ZIP11 (Slc39a11) in mice responds to
907 dietary zinc and exhibits nuclear localisation. *J. Nutrition.* 143(12):1882-1888.

908 Matsuura, W., T. Yamazaki, Y. Yamaguchi-Iwai, S. Masuda, M. Nagao, G.K. Andrews, and T.
909 Kambe. 2014. Slc29a9 (ZIP9) regulates zinc homeostasis in the secretory pathway:
910 Characterisation of the ZIP subfamily I protein in vertebrate cells. *Biosci. Biotech. &*
911 *Biochem.* 73(5):1142-1148.

912 McCranor, B.J., R.A. Bozym, M.I. Vitolo, C.A. Fierke, L. Bambrick, B.M. Polster, G. Fiskum,
913 and R.B. Thompson. 2012. Quantitative imaging of mitochondrial and cytosolic free
914 zinc levels in an in vitro model of ischemia/reperfusion. *J. Bioenerg. Biomembr.*
915 44(2):253-263.

916 McMurray, J.J., M. Packer, A.S. Desai, J. Gong, M.P. Lefkowitz, A.R. Rizkala, J.L. Rouleau,
917 V.C. Shi, S.D. Solomon, K. Swedberg, M.R. Zile, and PARADIGM-HF Committees
918 and Investigators. 2013. Dual angiotensin receptor and neprilysin inhibition as an
919 alternative to angiotensin-converting enzyme inhibition in patients with chronic
920 systolic heart failure: rationale for and design of the Prospective comparison of ARNI
921 with ACEI to Determine Impact on Global Mortality and morbidity in Heart Failure trial
922 (PARADIGM-HF). *Eur. J. Heart Fail.* 15:1062-73.

923 Meissner, G., and J.S. Henderson. 1987. Rapid Calcium Release from Cardiac
924 Sarcoplasmic Reticulum Vesicles Is Dependent on Ca^{2+} and Is Modulated by Mg^{2+} ,
925 Adenine Nucleotide, and Calmodulin. *J. Biol. Chem.* 262(7):3065–3073.

926 Moschella, M.C., and A.R. Marks. 1993. Inositol 1,4,5-trisphosphate receptor expression in
927 cardiac myocytes. *J. Cell. Biol.* 120:1137-1146.

928 Nakayama, H., I. Bodi, M. Maillet, J. DeSantiago, T.L. Domeier, K. Mikoshiba, J.N. Lorenz,
929 L.A. Blatter, D.M. Bers, and J.D. Molkentin. 2010. The IP3 receptor regulates cardiac
930 hypertrophy in response to select stimuli. *Circ. Res.* 107:659-666.

931 Nalivaeva, N.N., I.A. Zhuravin, and A.J. Turner. 2020. Neprilysin expression and functions in
932 development, ageing and disease. *Mech. Ageing Dev.* 192:111363.

933 Nash, M.S., K.W. Young, R.A. Challiss, and S.R. Nahorski. 2001. Intracellular signalling.
934 Receptor-specific messenger oscillation. *Nature*. 413(6854):381-382.

935 Nishi, M., S. Komazaki, M. Iino, K. Kangawa, and H. Takeshima. 1998. Mitsugumin23, a
936 novel transmembrane protein on endoplasmic reticulum and nuclear membranes.
937 *FEBS Lett.* 432:191-196.

938 Nolte, C., A. Gore, I. Sekler, W. Kreese, M. Hershfinkel, A. Hoffman, K. Kettenmann, and A
939 Moran. 2004. ZnT-1 expression in astroglial cells protects against zinc toxicity and
940 slows the accumulation of intracellular zinc. *Glia* 48:145-155.

941 Nyborg, J.K., and O.B. Peersen. 2004. That zincing feeling: the effects of EDTA on the
942 behaviour of zinc-binding transcriptional factors. *Biochem. J.* 381(Pt3):E3.

943 Olgar, Y., A. Durak, E. Tuncay, C.V. Bitirim, E. Ozcinar, M.B. Inan, Z. Tokcaer-Keskin, K.C.
944 Akcali, A.R. Akar, and B. Turan. 2018a. Increased free Zn^{2+} correlates induction of
945 sarco(endo)plasmic reticulum stress via altered expression levels of Zn^{2+}-
946 transporters in heart failure. *J. Cell. Mol. Med.* 22:1944-1956.

947 Olgar, Y., S. Ozdemir, and B. Turan. 2018b. Induction of endoplasmic reticulum stress and
948 changes in expression levels of Zn^{2+}-transporters in hypertrophic rat heart. *Mol Cell*
949 *Biochem.* 440:209-219.

950 Olgar, Y., E. Tuncay, and B. Turan. 2019. Mitochondria-targeting antioxidant provides
951 cardioprotection through regulation of cytosolic and mitochondrial Zn^{2+} levels with re-
952 distribution of Zn^{2+}-transporters in aged rat cardiomyocytes. *Int. J. Mol. Sci.*
953 20(15):3783.

954 Palmiter, R.D., and S.D. Findley. 1995. Cloning and functional characterization of a
955 mammalian zinc transporter that confers resistance to zinc. *EMBO J.* 14:639-649.

956 Palmiter, R.D., T.B. Cole, and S.D. Findley. 1996. ZnT-2, a mammalian protein that confers
957 resistance to zinc by facilitating vesicular sequestration. *EMBO J.* 15)8):1784-1791.

958 Palmiter, R.D., and L. Huang. 2004. Efflux and compartmentalization of zinc by members of
959 the SLC30 family of solute carriers. *Pflugers Arch.* 447:744-751.

960 Park, J.G., Y. Qin, D.F. Galati, and A.E. Palmer. 2012. New sensors for quantitative
961 measurement of mitochondrial Zn^{2+}. *AACS Chem. Biol.* 7(10):1636-1640.

962 Paulsen, I.T., and M.H. Saier, Jr. 1997. A novel family of ubiquitous heavy metal ion
963 transport proteins. *J. Membrane Biol.* 156:99-103.

964 Picello, E., E. Damiani, and A. Margreth. 1992. Low-affinity Ca^{2+} -binding sites versus Zn^{2+} -
965 binding sites in histidine-rich Ca^{2+} -binding protein of skeletal muscle sarcoplasmic
966 reticulum. *Biochem. Biophys. Res. Commun.* 186(2):659–667.

967 Pilz, S., H. Scharnagl, B. Tiran, B. Wellnitz, U. Seelhorst, B.O. Boehm, and W. März. 2007.
968 Elevated plasma free fatty acids predict sudden cardiac death: a 6.85-year follow-up
969 of 3315 patients after coronary angiography. *Eur. Heart J.* 28:2763-2769.

970 Pitt S.J., and A.J. Stewart. 2015. Examining a new role for zinc in regulating calcium release
971 in cardiac muscle. *Biochem. Soc. Trans.* 43:359-363.

972 Popovics, P., and A.J. Stewart. 2011. GPR39: A Zn^{2+}-activated G protein-coupled receptor
973 that regulates pancreatic, gastrointestinal and neuronal functions. *Cell. Mol. Life Sci.*
974 68: 85-95.

975 Pratt, E.P.S., L.J. Damon, K.J. Anson, and A.E. Palmer. 2021. Tools and techniques for
976 illuminating the cell biology of zinc. *Biochim. Biophys. Acta Mol. Cell. Res.*
977 1868:118865.

978 Qin, Y., P.J. Dittmer, J.G. Park, K.B. Jansen, and A.E. Palmiter. 2011. Measuring steady-
979 state and dynamic endoplasmic reticulum and Golgi Zn^{2+} with genetically encoded
980 sensors. *Proc. Natl. Acad. Sci USA* 108:7351-7356.

981 Reilly-O'Donnell, B., G.B. Robertson, A. Karumbi, C. McIntyre, W. Bal, M. Nishi, H.
982 Takeshima, A.J. Stewart, and S.J. Pitt. (2017) Dysregulated Zn^{2+} homeostasis
983 impairs cardiac type-2 ryanodine receptor and mitsugumin 23 functions, leading to
984 sarcoplasmic reticulum Ca_{2+} leakage. *J. Biol. Chem.* 292: 13361-13373.

985 Rivera, O.C., S.R. Hennigar, and S.L. Kelleher. 2018. ZnT2 is critical for lysosomal
986 acidification and biogenesis during mannary gland involution. *Am. J. Regul. Integr.*
987 *Comp. Physiol.* 315(2):R323-R335.

988 Roh, H.C., S. Collier, J. Guthrie, J.D. Robertson, and K. Kornfeld. 2012. Lysosome-related
989 organelles in intestinal cells are a zinc storage site in C. elegans. *Cell Metab.*
990 15(1):88-99.

991 Rong, J., L. Li, L. Jing, H. Fang, and S. Peng. 2016. JAK2/STAT3 pathway mediates
992 protection of metallothionein against doxorubicin-induced cytotoxicity in mouse
993 cardiomyocytes. *Int. J. Toxicol.* 35:317-326.

994 Salazar, N.C., J. Chen, and H.A. Rockman. 2007. Cardiac GPCRs: GPCR signaling in
995 healthy and failing hearts. *Biochim. Biophys. Acta.* 1768(4):1006-1018.

996 Sanfold, L., M.C. Carpenter, and A.E. Palmer. 2019. Intracellular Zn^{2+} transients modulate
997 global gene expression in dissociated rat hippocampal neurons. *Sci. Rep.* 9:9411.

998 Segal, D., E. Ohana, L. Besser, M. Hershfinkel, A. Moran, and I Sekler. 2004. A role for ZnT-
999 1 in regulating cellular cation influx. *Biochem. Biophys. Res. Commun.* 32:1145-1150.

1000 Sheard, T.M.D., M.E. Hurley, A.J. Smith, J. Colyer, E. White, and I. Jayasinghe. 2022.
1001 Three-dimensional visulization of the cardiac ryanodine receptor clusters and the
1002 molecular-scale fraying of dyads. *Phil. Trans. R. Soc. B.* 377: 20210316.

1003 Sim, D.L., and V.T. Chow. 1999. The novel human HUEL (C4orf1) gene maps to
1004 chromosome 4p12-p13 and encodes a nuclear protein containing the nuclear
1005 receptor interaction motif. *Genomics.* 59(2)224-233.

1006 Sobczak, A.I.S., K.G. Katundu, F. Phoenix, S. Khazaipoul, R. Yu, F. Lampiao, F.
1007 Stefanowicz, C.A. Blindauer, S.J. Pitt, T.K. Smith, R.A. Ajjan, and A.J. Stewart.
1008 2021a. Albumin-mediated alteration of plasma zinc speciation by fatty acids
1009 modulates blood clotting in type-2 diabetes. *Chem. Sci.* 12:4079-4093.

1010 Sobczak, A.I.S., S.J. Pitt, T.K. Smith, R.A. Ajjan, and A.J. Stewart. 2021b. Lipidomic profiling
1011 of plasma free fatty acids in type-1 diabetes highlights specific changes in lipid
1012 metabolism. *Biochim. Biophys. Acta* 1866:158823.

1013 Stewart, A.J., C.A. Blindauer, S. Berezenko, D. Sleep, and P.J. Sadler. 2003. Interdomain
1014 zinc site on human albumin. *Proc. Natl. Acad. Sci. USA* 100:3701-3706.

1015 Stewart A.J., and S.J. Pitt. 2015. Zinc controls RyR2 activity during excitation-contraction
1016 coupling. *Channels* 9:223-225.

1017 Stork, C.J., and Y.V. Li. 2006. Measuring cell viability with membrane impermeable zinc
1018 fluorescent indicator. *J. Neurosci. Methods* 155:180-186.

1019 Stork, C.J., and Y.V. Li. 2010. Zinc release from thapsigargin/IP3-sensitive stores in cultured
1020 cortical neurons. *J. Mol. Signal.* 5:5.

1021 Suzuki, T., K. Ishihara, H. Migaki, K. Ishihara, M. Nagao, Y. Yamaguchi-Iwai, and T. Kambe.
1022 2005. Two different zinc transport complexes of cation diffusion facilitator proteins
1023 localized in the secretory pathway operate to activate alkaline phosphatases in
1024 vertebrate cells. *J. Biol. Chem.* 280(35)30956-30962.

1025 Tan, Y., M. Chen, Z. Li, K. Mabuchi, and M. Bouvier. 2006. The calcium- and zinc-
1026 responsive regions of calreticulin reside strictly in the N-/C-domain. *Biochim. Biophys.*
1027 *Acta.* 1760:745-753.

1028 Tang, S., and J.J. Yang. 2013. Magnesium Binding Sites in Proteins. In: Kretsinger, R.H.,
1029 V.N. Uversky, and E.A. Permyakov (eds). Encycl*opaedia of Metalloproteins.* Springer,
1030 New York, NY.

1031 Taylor K.M. 2000. LIV-1 breast cancer protein belongs to new family of histidine-rich
1032 membrane proteins with potential to control intracellular Zn^{2+} homeostasis. *IUBMB*
1033 *Life* 49:249-253.

1034 Taylor, K.M., and R.I. Nicholson. 2003. The LZT proteins; the LIV-1 subfamily of zinc
1035 transporters. *Biochim. Biophys. Acta.* 1611(1-2):16-30.

1036 Taylor K.M., H.E. Morgan, A. Johnson, L.J. Hadley, and R.I. Nicholson. 2003. Structure-
1037 function analysis of LIV-1, the breast cancer-associated protein that belongs to a new
1038 subfamily of zinc transporters. *Biochem. J.* 375:51-59.

1039 Taylor, K.M., I.A. Muraina, D. Brethour, G. Schmitt-Ulms, T. Nimmanon, S. Ziliotto, P. Kille,
1040 and C. Hogstrand. 2016. Zinc transporter ZIP10 forms a heteromer with ZIP6 which
1041 regulates embryonic development and cell migration. *Biochem. J.* 473(16):2531-2544.

1042 Thirumoorthy, N., S. Shyam Sunder, K.T. Manisenthil Kumar, M. Senthil Kumar, G.N.K.
1043 Ganesh, and M. Chatterjee. 2011. A review of metallothionein isoforms and their role
1044 in pathophysiology. *World J. Surg. Oncol.* 9:54.

1045 Thomas, P., Y. Pang, J. Dong, and A.H. Berg. 2014. Identification and characterisation of
1046 the membrane androgen receptors in the ZIP9 zinc transporter subfamily: II. Role of
1047 Human ZIP9 in testoterone-induced prostate and breast cancer cell apoptosis.
1048 *Endocrinology.* 155:4250-4265.

1049 Tinker A., and A.J. Williams. 1992. Divalent-cation conduction in the ryanodine receptor
1050 channel of sheep cardiac-muscle sarcoplasmic-reticulum. *J. Gen. Physiol* 100: 479-
1051 493.

1052 Toida, T., R. Toida, S. Ebihara, R. Takahashi, H. Komatsu, S. Uezono, Y. Sato, and S.
1053 Fujimoto. 2020. Association between serum zinc levels and clinical index or the body
1054 composition in incident hemodialysis patients. *Nutrients.* 12(10): 3187.

1055 Tønder, N., F.F. Johansen, C.J. Frederickson, J. Zimmer, and N.H. Diemer. 1990. Possible
1056 role of zinc in the selective degeneration of dentate hilar neurons after cerebral
1057 ischemia in the adult rat. *Neurosci. Lett.* 109:247-152.

1058 Tuncay, E., A. Bilginoglu, N.N. Sozmen, E.N. Zeydanli, M. Ugur, G. Vassort, and B. Turan.
1059 2011. Intracellular free zinc during cardiac excitation-contraction cycle: calcium and
1060 redox dependencies. *Cardiovasc. Res.* 2011. 89:634-642.

1061 Tuncay, E., V.C. Bitirim, A. Durak., G.R.J. Carrat, K.M. Taylor, G.A. Rutter, and B. Turan.
1062 2017. Hyperglycemia-induced changes in ZIP7 and ZnT7 expression cause Zn^{2+}

release from the sarco(endo)plasmic reticulum and mediate ER stress in the heart. *Diabetes* 66:1346-1358.

Tuncay E, C.V. Bitirim, Y. Olgar, A. Durak, G.A. Rutter, and B. Turan. 2019. Zn^{2+}-transporters ZIP7 and ZnT7 play important role in progression of cardiac dysfunction via affecting sarco(endo)plasmic reticulum-mitochondria coupling in hyperglycemic cardiomyocytes. *Mitochondrion* 44:41-52.

Turan, B., H. Fliss, and M. Désilets. 1997. Oxidants increase intracellular free Zn^{2+} concentration in rabbit ventricular myocytes. *Am. J. Physiol.* 272:H2095-H2106.

Turan, B. 2003. Zinc-induced changes in ionic currents of cardiomyocytes. *Biol Trace Elem Res*. 94:49-60.

Turan, B., and E. Tuncay. 2017. Impact of labile zinc on heart function: From physiology to pathophysiology. *Int. J. Mol. Sci.* 18:2395.

Uchida, K.; H. Miyauchi, T. Furuichi, T. Michikawa, and K. Mikoshiba. 2003. Critical regions for activation gating of the inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* 278:16551-16560.

Uhlén, M., L. Fagerberg, B.M. Hallström, C. Lindskog, P. Oksvold, A. Mardinoglu, Å. Silvertsson, C. Kampf, E. Sjöstedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C.A. Szigyarto, J. Odeberg, D. Djureinovic, J.O. Takanen, S. Hober, T. Alm, P. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J.M. Schwenk, M. Hamsten, K.V. Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G.V. Heijne, J. Nielsen, and F. Pontén. 2015. Protein expression across human tissue. *Science.* 347(6220): 1260419-1 - 1260419-9.

U.S. National Library of Medicine. 2022. https://www.nlm.nih.gov/. Accessed September/October 2022.

1087 Vallee, B.L., and D.S. Auld. 1990. Zinc coordination, function, and structure of zinc enzymes
1088 and other proteins. *Biochemistry.* 29(24):5647-5659.

1089 Vallee, B.L., and K.H. Falchuk. 1993. The biochemical basic of zinc physiology. *Phys. Rev.*
1090 73(1):79-118.

1091 Vance, J.E. 1990. Phospholipid synthesis in a membrane fraction associated with
1092 mitochondria. *J. Biol. Chem.* 265(13):7248-7256.

1093 Venturi, E., K. Mio, M. Nishi, T. Ogura, T. Moriya, S.J. Pitt, K. Okuda, S. Kakizawa, R.
1094 Sitsapesan, C. Sato, and H. Takeshima. 2011. Mitsugumin 23 forms a massive bowl-
1095 shaped assembly and cation-conducting channel. *Biochemistry* 50:2623-2632.

1096 Wang, G.-W., J.B. Klein, and Y.J. Kang. 2001. Metallothionein inhibits doxorubicin-induced
1097 mitochondrial cytochrome C release and caspase-3 activation in cardiomyocytes. *J.*
1098 *Pharmacol. Exp. Ther.* 298:461-468.

1099 Wang, L., Z. Zhou, J.T. Saari, and J. Kang. 2005. Alcohol-induced myocardial fibrosis in
1100 metallothionein-null mice. *Am. J. Pathol.* 167:P337-344.

1101 Wang, J., Y. Song, L. Elsherif, Z. Song, G. Zhou, S.D. Prabhu, J.T. Saari, and L. Cai. 2006.
1102 Cardiac metallothionein induction plays the major role in the prevention of diabetic
1103 cardiomyopathy by zinc supplementation. *Circulation* 113:544-554.

1104 Wang, J., C. Gareri, and H.A. Rockman. 2018. G-protein-coupled receptors in heart disease.
1105 *Circ. Res.* 123:716-735.

1106 Wang, Z., D. Ye, J. Ye, M. Wang, J. Liu, H. Jiang, Y. Xu, J. Zhang, J. Chen, and J. Wan.
1107 2019. The TRPA1 channel in the cardiovascular system: promising features and
1108 challenges. *Front. Pharmacol.* 10:1253.

1109 Wang, J., X. Cheng, H. Zhao, Q. Yang, and Z. Xu. 2021. Downregulation of the zinc
1110 transporter SLC39A13 (ZIP13) is responsible for the activation of CaMKII at

reperfusion and leads to myocardial ischemia/reperfusion injury in mouse hearts. *J. Mol. Cell. Cardiol.* 152:69-79.

Wang, Y., X. Zhang, Y. Wen, S. Li, X. Lu, XR. Xu, and C. Li. 2021. Endoplasmic reticulum-mitochondria contacts: A potential therapy target for cardiovascular remodeling-associated diseases. *Front. Cell Dev. Biol.* 9:774989.

Woodier, J., R.D. Rainbow, A.J. Stewart, and S.J. Pitt. 2015. Intracellular zinc modulates cardiac ryanodine receptor-mediated calcium release. *J. Biol. Chem.* 290: 17599-17610.

Woodruff G., C.G. Bouwkamp, F.M. de Vrij, T. Lovenberg, P. Bonaventure, S.A. Kushner, and A.W. Harrington. 2018. The zinc transporter SLC39A7 (ZIP7) is essential for regulation of cytosolic zinc levels. *Mol Pharmacol,* 94(3):1092-1100.

Warren, J.T., Guo, Q., and Tang, W.-J. 2007. A 1.3Å structure of zinc-bound N-terminal domain of calmodulin elucidates potential early ion-binding step. *J. Mol. Biol.* 374(2):517-527.

Waters, R.S., N.A. Bryden, K.Y. Patterson, C. Viellon, and R.A. Anderson. 2001. EDTA chelation effects on urinary losses of cadmium, calcium, chromium, cobalt, copper, lead, magnesium, and zinc. *Biol. Trace. Elem. Res.* 83(3):207-221.

Yamasaki, S., K. Sakata-Sogawa, A. Hasegawa, T. Suzuki, K. Kabu, E. Sato, T. Kurosaki, S. Yamashita, M. Tokunaga, K. Nishida, and T. Hirano. 2007. Zinc is a novel intracellular second messenger. *J. Cell. Biol.* 177: 637-645.

Yi, T., Y. Cheema, S.M. Tremble, S.P. Bell, Z. Chen, M. Subramanian, M.M. LeWinter, P. VanBuren, and B.M. Palmer. 2012. Zinc-induced cardiomyocyte relaxation in a rat model of hyperglycemia is independent of myosin isoform. *Cardiovasc. Diabetol.* 11:135.

Yi, T., J.S. Vick, M.J. Vecchio, K.J. Begin, S.P. Bell, R.J. Delay, and B.M. Palmer. 2013. Identifying cellular mechanisms of zinc-induced relaxation in isolated cardiomyocytes. *Am. J. Physiol. Heart Circ. Physiol.* 305:H706-H715.

Yoshioka, G., A. Tanaka, K. Nishihira, Y. Shibata, and K. Node. 2020. Prognostic impact of serum albumin for developing heart failure remotely after acute myocardial infarction. *Nutrients.* 12(9): 2637.

Yu, Y., J.C. Fuscoe, C. Zhao, C. Guo, M. Jia, T. Qing, D.I. Bannon, L. Lancashire, W. Bao, T. Du, H. Luo, Z. Su, W.D. Jones, C.L. Moland, W.S. Branham, F. Qian, B. Ning, Y. Li, H. Hong, L. Guo, N. Mei, T. Shi, K.Y. Wang, R.D. Wolfinger, Y. Nikolsky, S.J. Walker, P. Duerksen-Hughes, C.E. Mason, W. Tong, J. Thierry-Mieg, D. Thierry-Mieg, L. Shi, and C. Wang. 2014. A rat RNA-Seq transcriptomic BodyMap across 11 organs and 4 developmental stages. *Nature comm.* 5:3230.

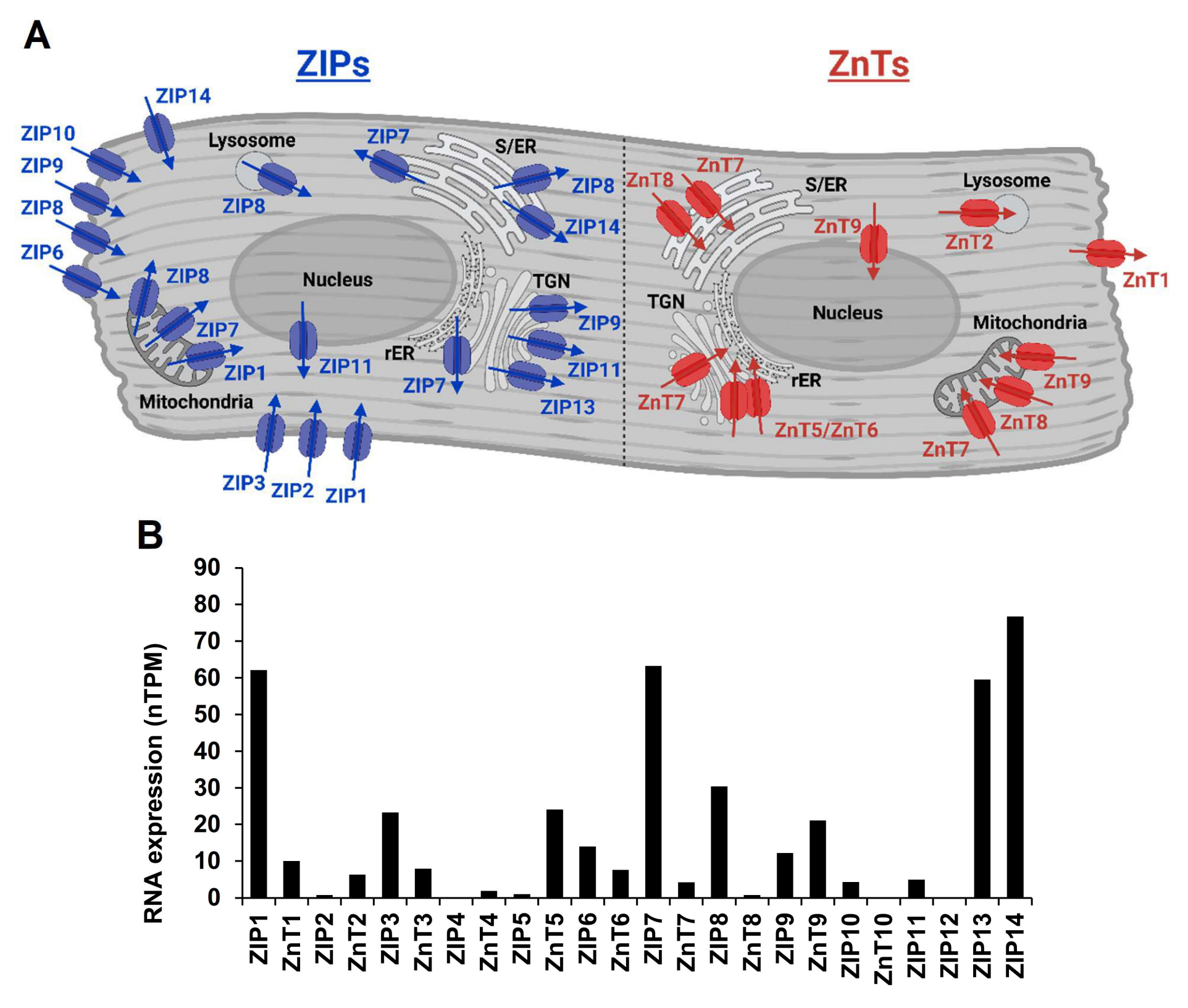
Yue F., Y. Cheng, A. Breschi, J. Vierstra, W. Wu, T. Ryba, R. Sandstrom, Z. Ma, C. Davis, B.D. Pope...and The Mouse ENCODE Consortium. 2014. A comparative encyclopedia of DNA elements in the mouse genome. *Nature.* 515: 355-364.

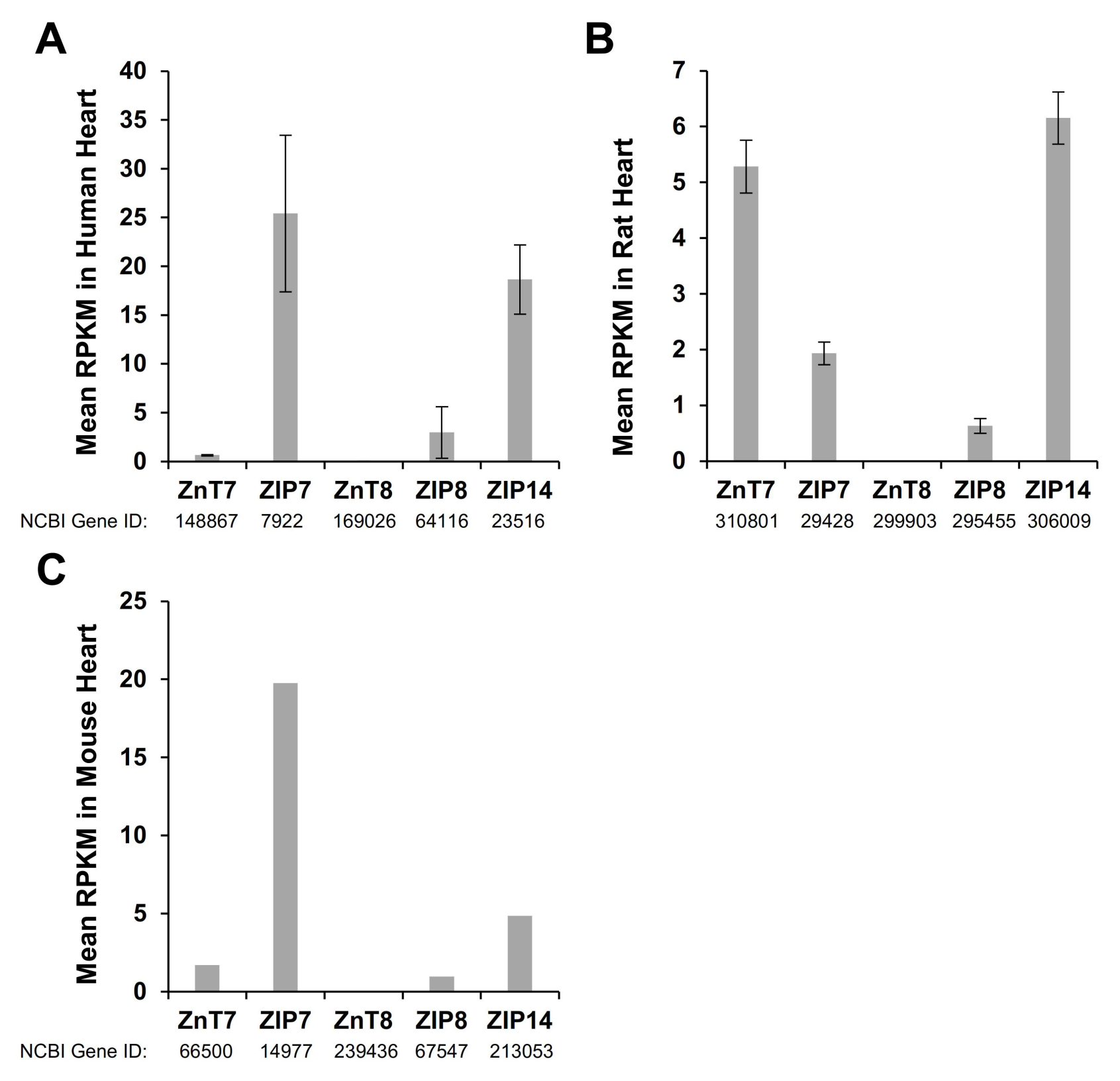
Zhang, H., N. Yang. H. He, J. Chai, X. Cheng, H. Zhao, D. Zhou, T. Teng, X. Kong, Q. Yang, and Z. Xu. 2021. The zinc transporter ZIP7 (*Slc39a7*) controls myocardial reperfusion injury by regulating mitophagy. *Basic Res. Cardiol.* 116:54.

Zhao, L., E. Oliver, K. Maratou, S.S. Atanur, O.D. Dubois, E. Cotroneo, C.-N. Chen, L. Wang, C. Arce, P.L. Chabosseau, J. Ponsa-Cobas, M.G. Frid, B. Moyon, Z. Webster, A. Aldashev, J. Ferrer, G.A. Rutter, K.R. Stenmark, T.J. Aitman, and M.R. Wilkins. 2015. The zinc transporter ZIP12 regulates the pulmonary vascular response to chronic hypoxia. *Nature.* 524:356-360.

Zhao, Y., X. Huang, Y. Lei, and J. Li. 2021. Metallothionein-2A protect cardiomyocytes from ischemia/reperfusion through inhibiting p38. *bioRxiv* doi: 10.1101/2021.09.09.459560. (Preprint posted September 9. 2021).

Zima, A.V., E. Bovo, D.M. Bers, and L.A. Blatter. 2010.Ca^{2+} spark-dependent and – independent sarcoplasmic reticulum Ca^{2+} leak in normal and failing rabbit ventricular myocytes. *J. Physiol.* 588(Pt 23):4743-4757.


A
ZIPs
ZnTs
ZIP14
ZIP10
ZIP9
ZIP8
ZIP6
Lysosome
ZIP8
ZIP7
S/ER
ZIP8
ZIP14
ZIP8
Nucleus
TGN
ZIP9
ZIP7
ZIP1
ZIP11
rER
ZIP11
ZIP7
ZIP13
Mitochondria
ZIP3
ZIP2
ZIP1
ZnT7
ZnT8
S/ER
Lysosome
ZnT9
ZnT2
ZnT1
TGN
Nucleus
Mitochondria
rER
ZnT9
ZnT7
ZnT8
ZnT5/ZnT6
ZnT7
B
RNA expression (nTPM)
90
80
70
60
50
40
30
20
10
0
ZIP1
ZnT1
ZIP2
ZnT2
ZIP3
ZnT3
ZIP4
ZnT4
ZIP5
ZnT5
ZIP6
ZnT6
ZIP7
ZnT7
ZIP8
ZnT8
ZIP9
ZnT9
ZIP10
ZnT10
ZIP11
ZIP12
ZIP13
ZIP14


A
Mean RPKM in Human Heart
40
35
30
25
20
15
10
5
0
ZnT7
ZIP7
ZnT8
ZIP8
ZIP14
NCBI Gene ID:
148867
7922
169026
64116
23516
B
Mean RPKM in Rat Heart
7
6
5
4
3
2
1
0
ZnT7
ZIP7
ZnT8
ZIP8
ZIP14
310801
29428
299903
295455
306009
C
Mean RPKM in Mouse Heart
25
20
15
10
5
0
ZnT7
ZIP7
ZnT8
ZIP8
ZIP14
NCBI Gene ID:
66500
14977
239436
67547
213053


ZIP1 181 CVLVFSLALHSVFEGLAVGLQR 202
ZIP2 166 LVLLLSLSFHSVFEGLAVGLQP 187
ZIP3 171 LSLAFALSAHSVFEGLALGLQE 192
hMG23 66 DAWIGPETMHLVSESSSQVLWA 87
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mMG23 66 DTWLGPETMHVISETLLQVMWA 87

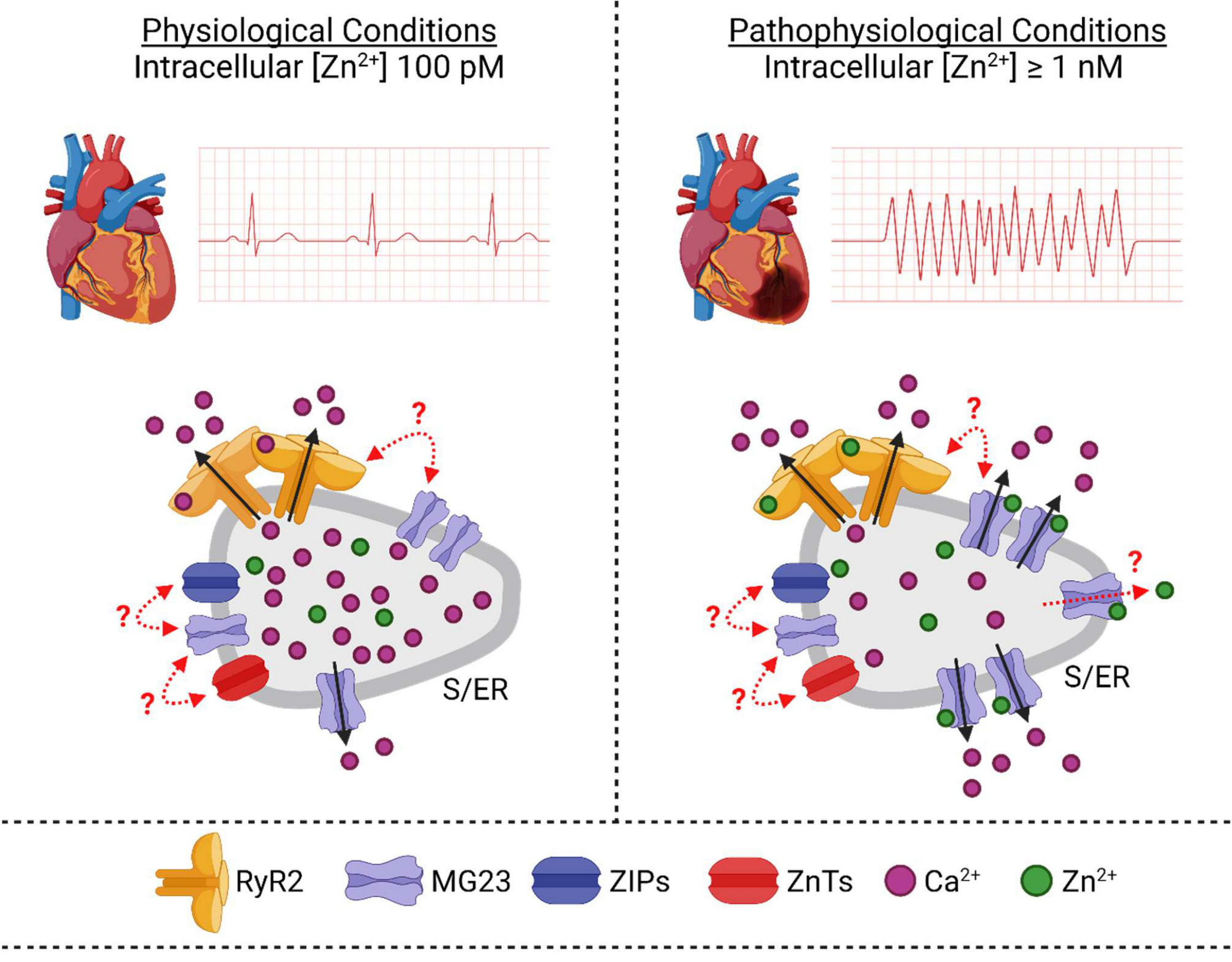
Physiological Conditions
Intracellular [Zn2+] 100 pM
Pathophysiological Conditions
Intracellular [Zn2+] ≥ 1 nM
?
?
?
S/ER
?
?
?
?
S/ER
RyR2
MG23
ZIPs
ZnTs
Ca2+
Zn2+

Table 1.

Protein expression (score) of ZIPs in heart tissue.

	ZIP1	ZIP2	ZIP3	ZIP4	ZIP5	ZIP6	ZIP7	ZIP8	ZIP9	ZIP10	ZIP11	ZIP12	ZIP13	ZIP14
Heart	N/A	Low	High	N/A	N/A	Med	Med	Low	Med	Low	N/A	N/A	ND	Med

Score ranged from high to not detected (ND). N/A illustrates transporters on the atlas which are pending normal tissue analysis. Data obtained from Uhlén M et al., 2015 and Human Protein Atlas.

Table 2.

Protein expression (score) of ZnTs in heart tissue.

	ZnT1	ZnT2	ZnT3	ZnT4	ZnT5	ZnT6	ZnT7	ZnT8	ZnT9	ZnT10
Heart	Low	N/A	ND	N/A	Med	Low	Med	ND	Med	ND

Score ranged from high to not detected (ND). N/A illustrates transporters on the atlas which are pending normal tissue analysis. Data obtained from Uhlén M et al., 2015 and Human Protein Atlas.

Table 3.

Sub-cellular localisation of zinc transporters.

Zinc Transporter	Localisation	Detection Method			Reference
		Immuno-fluorescence	Cell fractionation and immunoblotting	Zn^{2+} influx/efflux assay/measurement of $[Zn^{2+}]$	
ZIP1	PM	✓		✓	Gaither and Eide, 2001.
	Mitochondria	✓	✓		Cho *et al.,* 2019.
ZIP2	PM	✓		✓	Gaither and Eide, 2000.
ZIP3	PM	✓			Kelleher and Lönnerdal, 2003.
ZIP6	PM	✓			Taylor and Nicholson, 2003.
ZIP7	TGN	✓		✓	Huang *et al.,* 2005.
	S/ER	✓	✓	✓	Tuncay *et al.,* 2017.
	Mitochondria	✓	✓	✓	Tuncay *et al.,* 2019.
ZIP8	PM	✓	✓		Dalton *et al.,* 2005.
	Lysosomes	✓			Aydemir *et al.,* 2009.
	Mitochondria S/ER		✓ ✓		Olgar *et al.,* 2019.
ZIP9	PM	✓	✓	✓	Thomas *et al.,* 2014.
	TGN	✓			Matsuura *et al.,* 2014.
ZIP10	PM	✓	✓		Lichten *et al.,* 2011.
ZIP11	TGN	✓			Kelleher *et al.,* 2012.
	Nucleus	✓	✓		Martin *et al.,* 2013.
ZIP13	TGN	✓		✓	Fukada *et al*., 2008.
ZIP14	PM	✓			Taylor *et al.,* 2003.
	S/ER	✓			Olgar *et al.,* 2018a
ZnT1	PM			✓	Palmiter and Findley, 1995.
ZnT2	Lysosomes	✓			Palmiter *et al.,* 1996.
ZnT5	TGN	✓	✓	✓	Kambe *et al.,* 2002.
ZnT6	TGN	✓	✓		Suzuki *et al.,* 2005.
ZnT7	TGN	✓			Kirschke and Huang, 2003.
	S/ER	✓	✓		Tuncay *et al.,* 2017.
	Mitochondria	✓	✓		Tuncay *et al.,* 2019.
ZnT8	Mitochondria S/ER		✓ ✓		Olgar *et al.,* 2019.
ZnT9	Nucleus	✓	✓		Sim and Chow, 1999.
	Mitochondria	✓			Kowalczky *et al.,* 2021.

Sub-cellular localisation of ZIPs and ZnTs as illustrated in figure 1A. PM – Plasma membrane; TGN – Trans-Golgi Network; S/ER – Sarco/Endoplasmic Reticulum.

Table 4.

Studies examining zinc transporters in cardiovascular disease.

Zinc Transporter	Experimental Model	Protocol	Quantification		Expression change	Reference
			Protein Expression	mRNA Expression		
ZIP1	Cardiomyocytes (CMs) isolated from Sprague-Dawley rats (WT, male, 8 weeks)	*In vivo* chronic aldosterone/salt treatment, 4 weeks		✓	↑ ~4.2-fold	Kamalov *et al.,* 2009
	CMs isolated from Wistar Kyoto rats	*In vitro* hypoxia/reoxygenation (H/R)	✓		↑ hypoxia 0.5 to ~1.4 AU ↑ H/R 0.5 to ~0.7 AU	Bodiga *et al.,* 2017
ZIP2	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 1 to ~1.3 AU ↓ H/R 1 to ~0.8 AU (NS)	Bodiga *et al.,* 2017
	Hearts from C57BL/6 mice (WT, male, 8-10 weeks)	*In vivo* ischaemia/reperfusion by left anterior descending coronary artery occlusion.	✓	✓	↑ protein ~150% ↑ mRNA ~4-fold	Du *et al.,* 2019
ZIP3	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 1 to ~1.6 AU ↑ H/R 1 to ~1.6 AU	Bodiga *et al.,* 2017
ZIP6	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 0.8 to ~1 AU (NS) ↓ H/R 0.8 to ~0.7 AU (NS)	Bodiga *et al.,* 2017
ZIP7	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 1 to ~2 AU ↓ H/R 1 to ~0.9 AU (NS)	Bodiga *et al.,* 2017

ZIP7	Hearts from Wistar rats (WT, male, 2 months)	*In vivo* transverse aortic constriction	✓		↑ ~2-fold	Olgar *et al.,* 2018b
	H9C2 cell lysates	*In vitro* doxorubicin (DOX) treatment	✓		↑ ~1.5-fold	Tuncay *et al.,* 2019
	CMs isolated from C57BL/6 mice (WT, male, 8-10 weeks)	*In vitro* H/R	✓		↑ ~0.7 to ~1.2	Zhang *et al.,* 2021
	Hearts from Wistar rats (WT, male, 250-350 g)	*Ex vivo* ischaemia/reperfusion (I/R)	✓		↑ ~0.75 to ~0.9	Zhang *et al.,* 2021
	Hearts from C57BL/6 mice (WT, male, 8-10 weeks)	*In vivo* I/R by left anterior descending coronary artery occlusion	✓	✓	↑ protein ~0.8 to ~1 ↑ mRNA from ~1 to 2	Zhang *et al.,* 2021
ZIP8	H9C2 cell lysates	*In vitro* DOX-treatment	✓		↓ ~0.4-fold	Olgar *et al.,* 2018a
	Human heart failure tissue	Patients with end-stage heart failure	✓		↓ ~0.5-fold	Olgar *et al.,* 2018a
	Hearts from Wistar rats (WT, male, 2 months)	*In vivo* transverse aortic constriction	✓		↓ ~0.5-fold	Olgar *et al.,* 2018b
ZIP9	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 1 to ~2 AU ≈ H/R	Bodiga *et al.,* 2017
ZIP10	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 1 to ~1.5 AU ↑ H/R 1 to ~1.2 (NS)	Bodiga *et al.,* 2017
ZIP11	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 1 to ~2 AU ≈ H/R	Bodiga *et al.,* 2017
ZIP12	Human pulmonary artery smooth muscle cells	*In vitro* hypoxia incubation		✓	↑ ~3-fold	Zhao *et al.,* 2015
ZIP13	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 0.5 to ~2 AU ≈ H/R	Bodiga *et al.,* 2017
ZIP13	Heart tissue from C57BL/6	*In vivo* left anterior	✓	✓	↓ protein	Wang *et al.,* 2021

	mice (WT, male, 8-10 weeks)	descending coronary artery ligation			~0.5-fold ↓ mRNA ~0.6-fold	
	H9C2 cell lysates	*In vitro* H/R	✓		↓ ~0.6-fold	Wang *et al.,* 2021
	Neonatal CMs isolated from new-born c57BL/6N mice	*In vitro* DOX-treatment		✓	↓ ~0.75 to ~0.1	Hara *et al.,* 2022
	Heart tissue from c57BL/6N mice	*In vivo* intraperitoneal DOX injection		✓	↓ ~1 to ~0.6	Hara *et al.,* 2022
ZIP14	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 0.5 to ~2 AU ≈ H/R	Bodiga *et al.,* 2017
	H9C2 cell lysates	*In vitro* DOX-treatment	✓		↑ ~1.5-fold	Olgar *et al.,* 2018a
	Human heart failure tissue	Patients with end-stage heart failure	✓		↑ ~2-fold	Olgar *et al.,* 2018a
	Heart tissue from Wistar rats (WT, male, 2 months)	*In vivo* transverse aortic constriction	✓		↑ ~2.5-fold	Olgar *et al.,* 2018b
ZnT1	Cultures CMs from rats (1 to 2 days old)	*In vitro* rapid pacing	✓		↑ 214.4%	Beharier *et al.,* 2007
	Heart homogenates from Sprague-Dawley rats (WT, male, 250-350 g)	*In vivo* rapid atrial pacing	✓		↑ 148%	Beharier *et al.,* 2007
	Human cardiac tissue	Cardiac tissue obtained from control and atrial fibrillation patients	✓		↑ 0.73 to 1.88	Etzion *et al.,* 2007
	CMs from Sprague-Dawley rats (WT, male, 8 weeks)	*In vivo* chronic aldosterone/salt treatment, 4 weeks		✓	↑ ~2-fold	Kamalov *et al.,* 2009
	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 1 to ~2 AU ↑ 1 to ~1.2 AU (NS)	Bodiga *et al.,* 2017
ZnT2	CMs isolated from Wistar	*In vitro* H/R	✓		↑ hypoxia	Bodiga *et al.,* 2017

	Kyoto rats				0.5 to ~0.6 AU (NS) ↑ H/R 0.4 to ~1.4 AU	
ZnT5	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		≈ hypoxia ↑ H/R 0.8 to 1.2 AU	Bodiga *et al.,* 2017
ZnT7	Hearts from Wistar rats (WT, male, 2 months)	*In vivo* transverse aortic constriction	✓		↓ ~0.6-fold	Olgar *et al.,* 2018b
	H9C2 cell lysates	*In vitro* DOX-treatment	✓		↓ ~0.5-fold	Tuncay *et al.,* 2019
ZnT8	H9C2 cell lysates	*In vitro* DOX-treatment	✓		↑ ~1.6-fold	Olgar *et al.,* 2018a
	Human heart failure tissue	Patients with end-stage heart failure	✓		↑ ~2-fold	Olgar *et al.,* 2018a
	Hearts from Wistar rats (WT, male, 2 months)	*In vivo* transverse aortic constriction	✓		↑ ~1.5-fold	Olgar *et al.,* 2018b
ZnT9	CMs isolated from Wistar Kyoto rats	*In vitro* H/R	✓		↑ hypoxia 0.8 to ~1 AU (NS) ↑ H/R 0.8 to ~1.1 AU (NS)	Bodiga *et al.,* 2017

Changes observed in ZIPs and ZnTs in conditions of cardiovascular disease including experimental model, expression change and study. All expression changes are significant except where NS (not significant) is specified. CMs – cardiomyocytes; DOX – doxorubicin; H/R – hypoxia/reoxygenation; I/R – ischaemia/reperfusion. ↑ denotes increased expression; ↓ illustrates a decrease in expression; ≈ shows no change; NS is not significant.