Dysregulated Zn^{2+} homeostasis impairs cardiac type-2 ryanodine receptor and mitsugumin 23 functions, leading to sarcoplasmic reticulum Ca^{2+} leakage

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Supplemental Data

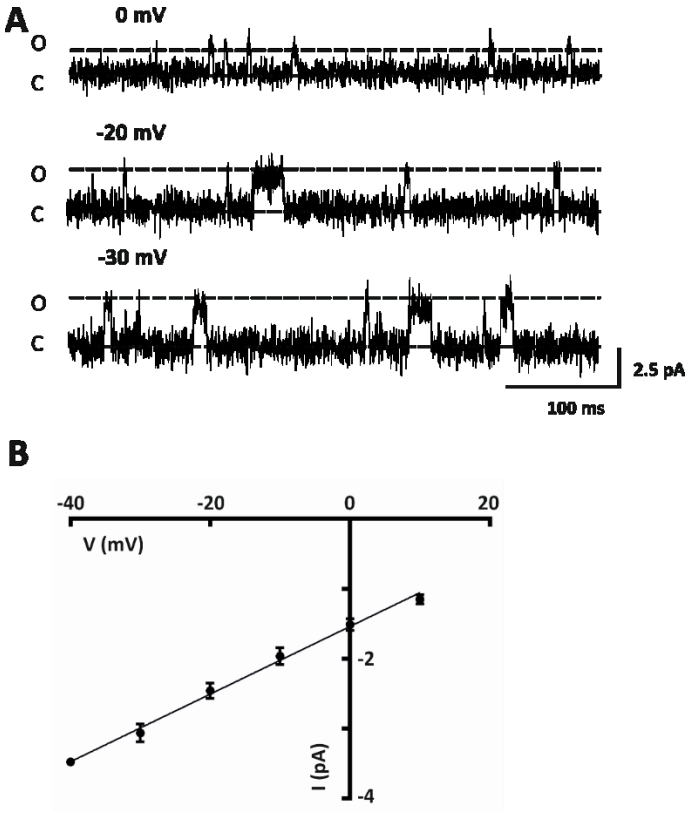

Figure S1. Single-channel properties of purified MG23. **(A)** Purified MG23 single-channel recordings using Ca^{2+} as the permeant ion at the indicated holding potential. The traces in this figure are not representative of Po but were chosen to show current amplitudes clearly. O and C show the fully open and closed states, respectively. **(B)** Current-voltage relationship of MG23 with Ca^{2+} as the permeant ion. Values are mean ± SD (n = 6).

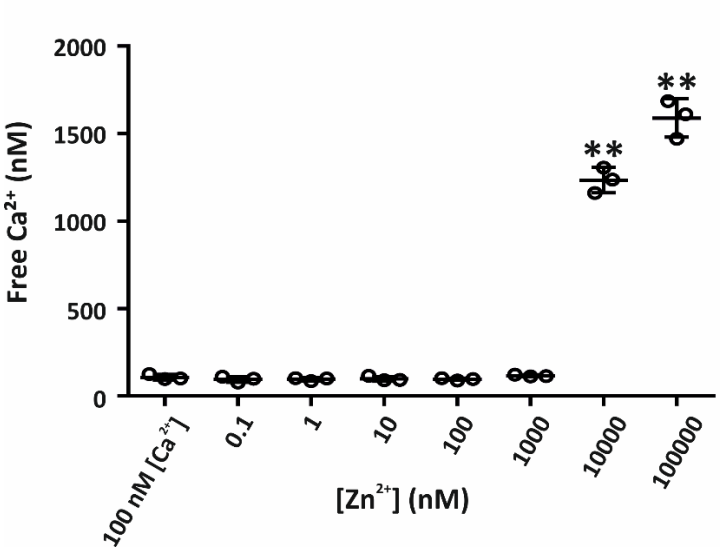

Figure S2. Effects of Zn^{2+} on Ca^{2+} levels in BAPTA treated recording solutions. The free [Ca^{2+}] of our TRIS/HEPES solution prior to the addition of BAPTA was determined using a Ca^{2+}- selective electrode. In order to lower the [Ca^{2+}] of our TRIS/HEPES solution, BAPTA was added until the measured free [Ca^{2+}] was 100 nM. Incremental increase of Zn^{2+} in the range 100 pM - 100 µM was added as $ZnCl_2$ to the BAPTA-treated solution and shown to have no significant effect until the [Zn^{2+}] $\geq$ 10 µM. Data are displayed as mean ± SD, *n*= 3. (**) indicates significance ($p \leq 0.01$) when compared to 100 nM [Ca^{2+}].

Calculated free $[Zn^{2+}]$ and $[Ca^{2+}]$ in BAPTA-treated TRIS/HEPES solutions		
Total $[Zn^{2+}]$ (nM)	Free $[Zn^{2+}]$ (nM)	Free $[Ca^{2+}]$ (nM)
0.109	0.000204	91.3
1.009	0.0019	91.4
10	0.0188	91.6
50	0.0946	92.4
100	0.192	93.5
1000	2.43	118
10000	1290	2600

Table S1: Calculated free $[Zn^{2+}]$ and $[Ca^{2+}]$ in BAPTA-treated TRIS/HEPES solutions. Free Zn^{2+} calculations were performed using the MIQUV program. Calculations were performed using the ionic composition of TRIS/HEPES recording solutions at room temperature (22 ± 2 ^{o}C).

References

1. Bologni, L., Sabatini, A., and Vacca, A. (1983) Complex formation equilibria between 2-amino-2(hydroxymethyl)-1,3,-propanediol (tris, tham) and nickel(II), copper(II), zinc(II) and hydrogen ions in aqueous solutions. *Inorg Chimica Acta* **69**, 71-75