

## SUPPORTING INFORMATION

### eZinCh-2: A Versatile, Genetically Encoded FRET Sensor for Cytosolic and Intraorganelle Zn<sup>2+</sup> Imaging

Anne M. Hessel<sup>1</sup>, Pauline Chabosseau<sup>2</sup>, Maarten H. Bakker<sup>1</sup>, Wouter Engelen<sup>1</sup>,  
Guy A. Rutter<sup>2</sup>, Kathryn M. Taylor<sup>3</sup>, and Maarten Merkx<sup>1\*</sup>

<sup>1</sup>Laboratory of Chemical Biology and Institute of Complex Molecular Systems (ICMS), Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands. <sup>2</sup>Section of Cell Biology, Division of Medicine, Imperial College London, London, UK. <sup>3</sup>Breast Cancer Molecular Pharmacology Group, School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK.

\* corresponding author: [m.merkx@tue.nl](mailto:m.merkx@tue.nl)

#### TABLE OF CONTENTS

|                             |  |       |
|-----------------------------|--|-------|
| <b>Supporting methods</b>   | Description of the cloning and mutagenesis of genetic constructs used in this study. Description of protein expression and purification. Description of colocalization studies | p.S2  |
| <b>Supporting table 1</b>   | Overview of sensor properties of eZinCh-2 at pH 7.   | p.S5  |
| <b>Supporting table 2-5</b> | Free Zn <sup>2+</sup> concentrations in various buffer systems at pH 6.0, pH 7.1, pH 7.8 and pH 8.0, respectively  | p.S6  |
| <b>Supporting table 6</b>   | Cytosolic, ER and mitochondrial free Zn <sup>2+</sup> concentrations   | p.S7  |
| <b>Supporting table 7</b>   | Overview of primers used for cloning and mutagenesis   | p.S8  |
| <b>Supporting figure 1</b>  | Zn <sup>2+</sup> titration experiments on three different eZinCh mutants   | p.S9  |
| <b>Supporting figure 2</b>  | ER-eCALWY-4 measurements in MCF-7 and TamR cells   | p.S10 |
| <b>Supporting figure 3</b>  | mito-eCALWY-4 measurements in HeLa cells   | p.S11 |
| <b>Supporting figure 4</b>  | Nucleotide and amino acid sequence of the eZinCh-2 ORF in bacterial expression vector pET28a   | p.S12 |
| <b>Supporting figure 5</b>  | Nucleotide and amino acid sequence of the eZinCh-2 ORF in mammalian expression vector pCMV   | p.S13 |
| <b>Supporting figure 6</b>  | Nucleotide and amino acid sequence of the ER-eZinCh-2 ORF in mammalian expression vector pcDNA3.1  | p.S14 |
| <b>Supporting figure 7</b>  | Nucleotide and amino acid sequence of the mito-eZinCh-2 ORF in mammalian expression vector pShuttle-CMV  | p.S15 |
| <b>Supporting figure 8</b>  | Nucleotide and amino acid sequence of the VAMP2-eZinCh-2 ORF in mammalian expression vector pCMV   | p.S16 |

## Supporting Methods

**Cloning and mutagenesis.** Plasmids encoding for Cerulean and Citrine, connected via a long (GGSGGS)<sub>9</sub> linker, containing different (Cys)<sub>4</sub> binding pockets were synthesized in previous research. A QuikChange Multi Site-Directed mutagenesis (Agilent Technologies) was used to simultaneously introduce different combinations of C144H and C206H mutants in pET28a-eZinCh-4 (primers 1&2 and 3&4 for mutating C144H and C206H, respectively, Supplementary Table 1), generation pET28a-eZinCh-2.1.

Vector pET28a-eZinCh-5 was used to introduce combinations of C206H and C208H mutations by using primer 5&6 and 7&8, respectively, yielding pET28a-eZinCh-2.2, pET28a-eZinCh-2.3 and pET28a-eZinCh-2.4.

To create the mammalian expression vector of one of the eZinCh mutants (pET28a-eZinCh-2), pET28a-eZinCh-2 was digested with *Nde* I and *Not* I to obtain a fragment encoding for eZinCh-2 that could be ligated into a *Nde* I/ *Not* I digested pcCALWY-4 vector to yield the mammalian expression vector peZinCh-2<sup>1</sup>. For ER-targeting a pcDNA3.1 vector containing the Preproinsulin (PPI) signal peptide, cerulean and citrine fused by a nine GGSGGS linker and a four amino acid long retention sequence KDEL at the end of the C-terminus was ordered (Genscript, USA). For mitochondrial targeting a *Not* I restriction site was introduced in the pShuttle-mito-eCALWY-4 by site directed mutagenesis (Agilent Technologies). Next pET28a-eZinCh-2 was digested with *Age* I and *Not* I to obtain a part of the fragment encoding for eZinCh-2 that could be ligated into a *Age* I/ *Not* I digested pShuttle-mito-eCALWY-4 vector, yielding pShuttle-mito-eZinCh-2. To create the vesicle-targeted mammalian expression plasmid, pET28a-eZinCh-2 was digested with *Age* I and *Not* I, followed by ligation into a vesicle-targeted mammalian *Age* I/*Not* I digested expression vector. The correct open reading frame for all expression vectors was confirmed by DNA sequencing (BaseClear, Leiden, The Netherlands).

**Protein expression and purification.** *E. coli* BL21(DE3) transformed with expression plasmid was grown to OD600 in 500 mL Lysogeny Broth (LB) medium containing 50 µg/mL kanamycin in a

shaking incubator at 37 °C. Following induction with 0.1 mM IPTG, the bacterial cultures were grown overnight at 25 °C in a shaking incubator at 250rpm. Cells were harvested by centrifugation and lysed using 10 mL BugBuster Protein Extraction reagent (Novagen) with 10 µL Benzonase. Proteins were purified by Ni-NTA affinity chromatography. Since His-tags are known to readily bind Zn<sup>2+</sup>, they were removed from all constructs using thrombin cleavage. After elution from the Ni-NTA column, the buffer was exchanged to thrombin cleavage buffer (20 mM Tris-HCl (pH 8.4), 150 mM NaCl, 2.5 mM CaCl<sub>2</sub>) using PD10 desalting columns (GE, Healthcare). Cleavage of the thrombin recognition site between the His-tag and the Cerulean N-terminus was initiated by the addition of 0.3 U thrombin protease (Novagen) per mg target protein at a 0.2 mg/mL target protein concentration. Cleavage was carried out at RT for 20 hours, after which 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) was added to inactivate the thrombin enzyme. Proteins were then loaded onto Ni-NTA once again. To separate the cleaved and uncleaved protein and also the His-tags remained behind on the Ni-column. Subsequently the proteins were loaded onto a size exclusion column (Sephacryl S200, GE Healthcare), using a buffer containing 50 mM Tris, 100 mM NaCl, 4 M Urea and 5 mM DTT. The SEC fractions were analyzed by SDS PAGE for correct size and purity, pooled and concentrated using 10 kDa MWCO centrifugation filters.

**Co-localization experiments.** HeLa cells were seeded on coverslips and transfected with plasmids encoding for either ER-eZinCh-2 or mito-eZinCh-2 using Lipofectamine 2000. Proteins were allowed to over express for ~24 h. Cells were washed with HBSS buffer, and stained with 1 µM ER-Tracker Red (Life Technologies) or 300 nM MitoTracker Red (Life Technologies) for 30 min. Cells were imaged on the Leica, TCS SP5X, using the 63x water immersion objective. The expressed eZinCh-2 was excited using the 405 nm laser, followed by recording emission between 515-595 nm. The commercial available ER and Mito Tracker were excited around 587 nm and 581 nm, followed by recording the emission between 605 – 625 nm and 630 – 660 nm, respectively.

For immunocytochemical analysis, INS1(832/13) cells expressing VAMP2-eZinCh2 were fixed in paraformaldehyde 4% for 20 minutes, permeabilized with Triton X-100 (0.5%) for 25 minutes, and

probed with primary antibody against insulin (1:100, DAKO, Cambridgeshire, U.K.), then visualised with Alexa Fluor 568 secondary antibodies (1:200; Life Technologies). Specimens were mounted on glass slides using Vectashield hard set (Vector Laboratories). Image acquisition was performed with a Zeiss Axiovert microscope coupled to a Nipkow spinning-disk head (Yokogawa CSU-10) using a 63x/NA1.4 objective. Two solid-state lasers (CrystaLaser) controlled by a laser-merge module (Spectral Applied Physics) provided wavelengths of 491 nm to excite VAMP2-eZinCh2.2 and 561 nm for insulin. Emitted light was filtered at 525/50 nm and at 630/50nm, respectively. Images were captured with a highly sensitive 16-bit, 512 × 512 pixel back-illuminated EM-CCD camera (ImageEM 9100-13; Hamamatsu).

**Supporting Table 1:** Sensor properties of the eZinCh mutants at pH 7.1.

| Mutants    | Cerulean    | Citrine     | $K_d$<br>(pH 7.1) | Ratiometric<br>change (%)<br>(pH 7.1) |
|------------|-------------|-------------|-------------------|---------------------------------------|
| eZinCh-2.1 | C144H, 206C | 144C, 206C  | 3.7 nM            | 110%                                  |
| eZinCh-2.2 | C206H, 208C | C206H, 208C | 1.0 nM            | 400%                                  |
| eZinCh-2.3 | C206H, 208C | 206C, C208H | 4.8 nM            | 109%                                  |

**Supporting table 2:** Free Zn<sup>2+</sup> concentrations in various buffering systems at pH 6.0, 20 °C.<sup>a</sup>

| Buffering system | 0.1 mM Zn <sup>2+</sup> | 0.2 mM Zn <sup>2+</sup> | 0.3 mM Zn <sup>2+</sup> | 0.4 mM Zn <sup>2+</sup> | 0.5 mM Zn <sup>2+</sup> | 0.6 mM Zn <sup>2+</sup> | 0.7 mM Zn <sup>2+</sup> | 0.8 mM Zn <sup>2+</sup> | 0.9 mM Zn <sup>2+</sup> |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 1 mM EGTA        | 7.77·10 <sup>-8</sup>   | 1.75·10 <sup>-7</sup>   | 3.0·10 <sup>-7</sup>    | 4.66·10 <sup>-7</sup>   | 6.98·10 <sup>-7</sup>   | 1.04·10 <sup>-6</sup>   | 1.62·10 <sup>-6</sup>   | 2.76·10 <sup>-6</sup>   | 5.93·10 <sup>-6</sup>   |
| 5 mM EGTA        | 1.42·10 <sup>-8</sup>   | 2.92·10 <sup>-8</sup>   | 4.47·10 <sup>-8</sup>   | 6.09·10 <sup>-8</sup>   | 7.78·10 <sup>-8</sup>   | 9.54·10 <sup>-8</sup>   | 1.13·10 <sup>-7</sup>   | 1.33·10 <sup>-7</sup>   | 1.54·10 <sup>-7</sup>   |
| 1 mM HEDTA       | 3.31·10 <sup>-12</sup>  | 7.45·10 <sup>-12</sup>  | 1.27·10 <sup>-11</sup>  | 1.99·10 <sup>-11</sup>  | 2.98·10 <sup>-11</sup>  | 4.47·10 <sup>-11</sup>  | 6.96·10 <sup>-11</sup>  | 1.19·10 <sup>-10</sup>  | 2.69·10 <sup>-10</sup>  |

<sup>a</sup>. The free zinc concentrations were calculated using the program MaxChelator using the stability constants present within the program (<http://www.stanford.edu/~cpatton/maxc.html/>)

**Supporting table 3:** Free Zn<sup>2+</sup> concentrations in various buffering systems at pH 7.1, 20 °C.<sup>a</sup>

| Buffering system | 0.1 mM Zn <sup>2+</sup> | 0.2 mM Zn <sup>2+</sup> | 0.3 mM Zn <sup>2+</sup> | 0.4 mM Zn <sup>2+</sup> | 0.5 mM Zn <sup>2+</sup> | 0.6 mM Zn <sup>2+</sup> | 0.7 mM Zn <sup>2+</sup> | 0.8 mM Zn <sup>2+</sup> | 0.9 mM Zn <sup>2+</sup> |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 1 mM EGTA        | 5.5·10 <sup>-10</sup>   | 1.2·10 <sup>-9</sup>    | 2.1·10 <sup>-9</sup>    | 3.3·10 <sup>-9</sup>    | 4.9·10 <sup>-9</sup>    | 7.4·10 <sup>-9</sup>    | 1.2·10 <sup>-8</sup>    | 2.0·10 <sup>-8</sup>    | 4.5·10 <sup>-8</sup>    |
| 5 mM EGTA        | 1.10·10 <sup>-10</sup>  | 2.06·10 <sup>-10</sup>  | 3.16·10 <sup>-10</sup>  | 4.30·10 <sup>-10</sup>  | 5.49·10 <sup>-10</sup>  | 6.74·10 <sup>-10</sup>  | 8.05·10 <sup>-10</sup>  | 9.42·10 <sup>-10</sup>  | 1.08·10 <sup>-9</sup>   |
| 1 mM HEDTA       | 2.02·10 <sup>-13</sup>  | 4.54·10 <sup>-13</sup>  | 7.79·10 <sup>-13</sup>  | 1.21·10 <sup>-12</sup>  | 1.82·10 <sup>-12</sup>  | 2.73·10 <sup>-12</sup>  | 4.25·10 <sup>-12</sup>  | 7.28·10 <sup>-12</sup>  | 1.64·10 <sup>-11</sup>  |

<sup>a</sup>. The free zinc concentrations were calculated using the program MaxChelator using the stability constants present within the program (<http://www.stanford.edu/~cpatton/maxc.html/>)

**Supporting table 4:** Free Zn<sup>2+</sup> concentrations in various buffering systems at pH 7.8, 20 °C.<sup>a</sup>

| Buffering system | 0.1 mM Zn <sup>2+</sup> | 0.2 mM Zn <sup>2+</sup> | 0.3 mM Zn <sup>2+</sup> | 0.4 mM Zn <sup>2+</sup> | 0.5 mM Zn <sup>2+</sup> | 0.6 mM Zn <sup>2+</sup> | 0.7 mM Zn <sup>2+</sup> | 0.8 mM Zn <sup>2+</sup> | 0.9 mM Zn <sup>2+</sup> |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 1 mM EGTA        | 2.32·10 <sup>-11</sup>  | 5.22·10 <sup>-11</sup>  | 8.96·10 <sup>-11</sup>  | 1.39·10 <sup>-10</sup>  | 2.09·10 <sup>-10</sup>  | 3.14·10 <sup>-10</sup>  | 4.88·10 <sup>-10</sup>  | 8.37·10 <sup>-10</sup>  | 1.89·10 <sup>-9</sup>   |
| 1 mM HEDTA       | 3.96·10 <sup>-14</sup>  | 8.92·10 <sup>-14</sup>  | 1.53·10 <sup>-13</sup>  | 2.38·10 <sup>-13</sup>  | 3.57·10 <sup>-13</sup>  | 5.35·10 <sup>-13</sup>  | 8.33·10 <sup>-13</sup>  | 1.42·10 <sup>-12</sup>  | 3.22·10 <sup>-12</sup>  |

<sup>a</sup>. The free zinc concentrations were calculated using the program MaxChelator using the stability constants present within the program (<http://www.stanford.edu/~cpatton/maxc.html/>)

**Supporting table 5:** Free Zn<sup>2+</sup> concentrations in various buffering systems at pH 8.0, 20 °C.<sup>a</sup>

| Buffering system | 0.1 mM Zn <sup>2+</sup> | 0.2 mM Zn <sup>2+</sup> | 0.3 mM Zn <sup>2+</sup> | 0.4 mM Zn <sup>2+</sup> | 0.5 mM Zn <sup>2+</sup> | 0.6 mM Zn <sup>2+</sup> | 0.7 mM Zn <sup>2+</sup> | 0.8 mM Zn <sup>2+</sup> | 0.9 mM Zn <sup>2+</sup> |
|------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| 1 mM EGTA        | 9.61·10 <sup>-12</sup>  | 2.16·10 <sup>-11</sup>  | 3.71·10 <sup>-11</sup>  | 5.77·10 <sup>-11</sup>  | 8.65·10 <sup>-11</sup>  | 1.3·10 <sup>-11</sup>   | 2.02·10 <sup>-10</sup>  | 3.46·10 <sup>-10</sup>  | 7.82·10 <sup>-10</sup>  |
| 1 mM HEDTA       | 2.5·10 <sup>-14</sup>   | 5.63·10 <sup>-14</sup>  | 9.66·10 <sup>-14</sup>  | 1.5·10 <sup>-13</sup>   | 2.25·10 <sup>-13</sup>  | 3.38·10 <sup>-13</sup>  | 5.26·10 <sup>-13</sup>  | 9.03·10 <sup>-13</sup>  | 2.04·10 <sup>-12</sup>  |

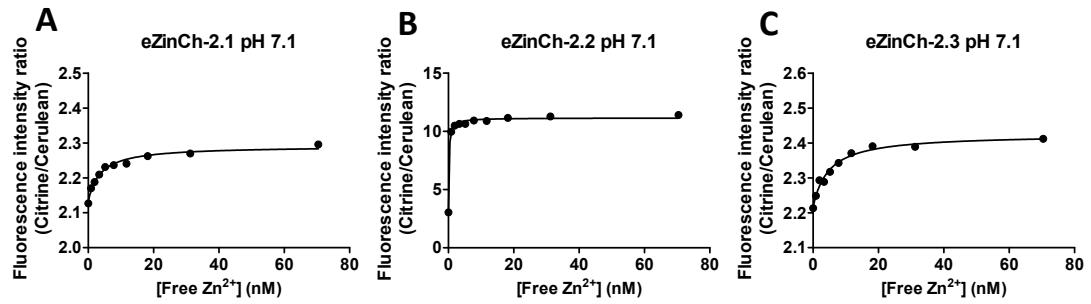
<sup>a</sup>. The free zinc concentrations were calculated using the program MaxChelator using the stability constants present within the program (<http://www.stanford.edu/~cpatton/maxc.html/>)

**Supporting Table 6:** Cytosolic, ER and mitochondrial free Zn<sup>2+</sup> concentrations measured in HeLa cells using different sensor variants

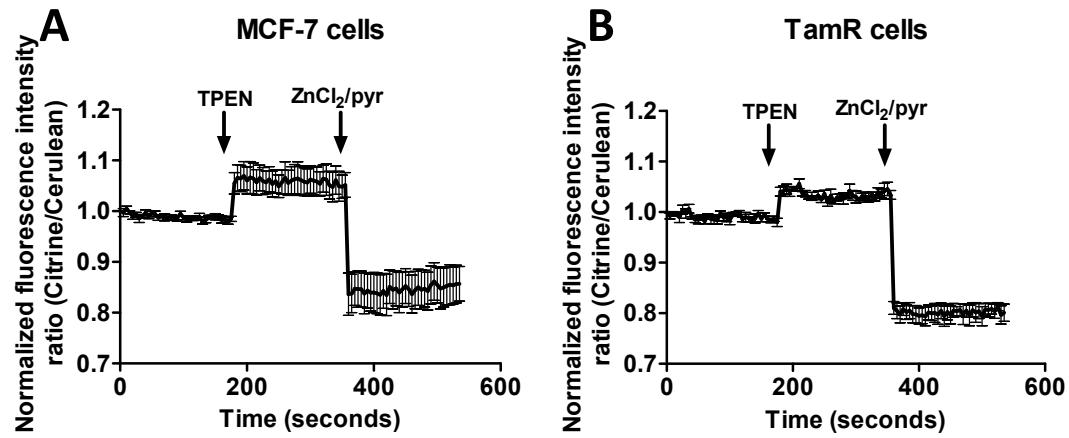
|          | Cytosolic Zn <sup>2+</sup><br>concentration | ER Zn <sup>2+</sup><br>concentration | Mitochondrial Zn <sup>2+</sup><br>concentration |
|----------|---|--------------------------------------|---|
| eCALWY-4 | 0.4 <sup>1</sup>                            | >5 nM <sup>2</sup>                   | 42 ± 28 pM                                      |
| ZapCY-1  | n.d.  | 0.9 ± 0.1 pM <sup>3</sup>            | 0.14 pM <sup>4</sup>                            |
| eZinCh-2 | 0.87 ± 0.1 nM                               | 0.8 ± 0.6 nM                         | 3.3 ± 1.2 pM                                    |

**Supporting Table 7:** Primers used for different cloning different eZinCh mutants

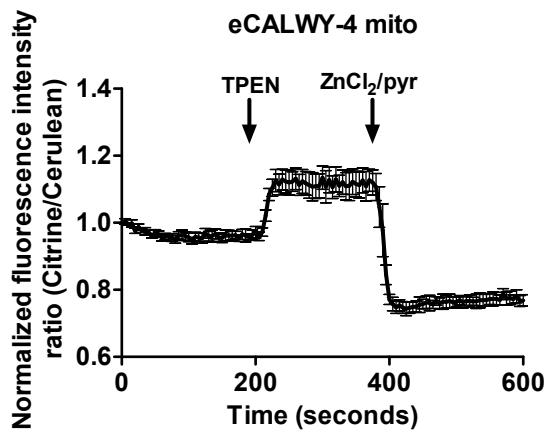
| Primer | Sequence                                      |
|--------|---|
| 1      | 5'-CACAAAGCTGGAGTACCACGCCATCAGCGACAAC-3'      |
| 2      | 5'-CTGGGGCACAGCTTGAGTACCACTACAACAGCCACAAC-3'  |
| 3      | 5'-CTGAGCACCCAGTCCCACCTGAGCAAAGACCCCAAC-3'    |
| 4      | 5'-CTGAGCTACCAGTCCCACCTGAGCAAAGACCCCAAC-3'    |
| 5      | 5'-CTGAGCACCCAGTCCTGCCTGCACAAAGACCCCAACGAG-3' |
| 6      | 5'-CTGAGCTACCAGTCCTGCCTGCACAAAGACCCCAACGAG-3' |
| 7      | 5'-CTGAGCACCCAGTCCCACCTGTGCAAAGACCCCAACGAG-3' |
| 8      | 5'-CTGAGCTACCAGTCCCACCTGTGCAAAGACCCCAACGAG-3' |



**Supporting figure 1:** Zinc binding properties of eZinCh mutants at pH 7.1. Emission ratio of eZinCh mutant as a function of Zn<sup>2+</sup> concentration, EGTA was used as buffering system to obtain the desired free Zn<sup>2+</sup> concentrations. Solid line represents a fit using a 1:1 binding model, yielding a K<sub>d</sub> of ~1 nM at pH 7.1 for eZinCh-2.2. Titration measurements were performed using ~1 μM protein in 150 mM HEPES (pH 7.1), 100 mM NaCl, 10% (vol/vol) glycerol, 0.01% Tween and 1 mM dithiothreitol (DTT), pH 7.1 or 6.0 at 20 °C.



**Supporting figure 2:** Responses of MCF-7 (A), and TamR (B) cells expressing ER-eCALWY-4 to the addition of 50  $\mu\text{M}$  TPEN, followed by the addition of excess 100  $\mu\text{M}$   $\text{Zn}^{2+}$ / 5  $\mu\text{M}$  pyridine. Traces represent the average of at least four cells after normalization of the emission ratio at t=0. Error bars represent SEM.



**Supporting figure 3:** Representative traces of mito-eCALWY-4 expressed in HeLa cells upon addition of 50  $\mu\text{M}$  TPEN, followed by the addition of excess 100  $\mu\text{M}$   $\text{Zn}^{2+}$ /5  $\mu\text{M}$  pyridine. Trace represents the average of four cells after normalization of the emission ratio at t=0. Error bars represent SEM.

|   |   |      |
|---|---|------|
| 1                                       | atgggcagcaggcatcatcatcatcatcagcagcgccatggtgcgcgcggcagccat         | 60   |
| M G S S H H H H H S S G L V P R G S H   |   |      |
| 61                                      | atggtgagcaaggcgaggagctgttaccgggggtggccatcttgcgagctggac            | 120  |
| M V S K G E E L F T G V V P I L V E L D |   |      |
| 121                                     | ggcgacgtaaacggccacaagttcagcgtgtccggcgagggcgatgcacactac            | 180  |
| G D V N G H K F S V S G E G E G D A T Y |   |      |
| 181                                     | ggcaagctgaccctgaagtcatctgcaccaccggtaactgcccgtgccctggccacc         | 240  |
| G K L T L K F I C T T G K L P V P W P T |   |      |
| 241                                     | ctcgtgaccaccctgacctggcgctgcagtgttcgcaccatggccatcta                | 300  |
| L V T T L T W G V Q C F A R Y P D H M K |   |      |
| 301                                     | cagcacgacttcaagtccgcattcggctacgtccaggcgcaccatcttc                 | 360  |
| Q H D F F K S A M P E G Y V Q E R T I F |   |      |
| 361                                     | ttcaaggacacggcaactacaagacccgcgcgaggtgaagttcgaggcgacaccctg         | 420  |
| F K D D G N Y K T R A E V K F E G D T L |   |      |
| 421                                     | gtgaaccgcattcggactcaggcatcactcaaggaggacggcaacatcttggggcac         | 480  |
| V N R I E L K G I D F K E D G N I L G H |   |      |
| 481                                     | aagctggagataacgcattcaggcatacgtctataatcaccgcgacaaggagaac           | 540  |
| K L E Y N A I S D N V Y I T A D K Q K N |   |      |
| 541                                     | ggcatcaaggccaacttcaagatccgcacaacatcgaggacggcagctgcagctcgcc        | 600  |
| G I K A N F K I R H N I E D G S V Q L A |   |      |
| 601                                     | gaccactaccaggcagaacaccccccattcggcgcggccctgtgtccggacaaccac         | 660  |
| D H Y Q Q N T P I G D G P V L L P D N H |   |      |
| 661                                     | tacctgagcaccctgatcactctgcggaaagaccccaacgagaagcgcgatcacatggc       | 720  |
| Y L S T Q S H L C K D P N E K R D H M V |   |      |
| 721                                     | ctgctggagttcgtgaccgcgcggatcactctcgcatggacgactgtacaatgtcc          | 780  |
| L L E F V T A A G I T L G M D E L Y K S |   |      |
| 781                                     | ggaggccggcggacttctgtggcggatccggcggaaagcggcggatccggcggtagcggc      | 840  |
| G G G E L I R G G S G G S G G S G G S G |   |      |
| 841                                     | ggatccggccgtccggcgatccggcgacggccggatccggatccggatccggatcc          | 900  |
| G S G G S G G S G G S G G S G G S G     |   |      |
| 901                                     | ggtgttagccgtgatccggatccggatccggatccggatccggatccggatccggatccggatcc | 960  |
| G G S G G S G G S G G S G G S G G S G   |   |      |
| 961                                     | ccgcggggctcggtacccatggtagtgagcaaggcgaggactgttacccgggtggcc         | 1020 |
| P R G S V P M V S K G E E L F T G V V P |   |      |
| 1021                                    | atccctggctcggactggcgacgtaaacggccacaagttcagcgtgtccggcgaggcc        | 1080 |
| I L V E L D G D V N G H K F S V S G E G |   |      |
| 1081                                    | gaggccgatccgcaccaactcgcaagctggccatctgcggatccggatccggatccggatcc    | 1140 |
| E G D A T Y G K L T L K F I C T T G K L |   |      |
| 1141                                    | cccggtccctggccaccctcggtaccacccctcgatgtgtgtccgcgc                  | 1200 |
| P V P W P T L V T T F G Y G L M C F A R |   |      |
| 1201                                    | taccccgccacacatgaagcagcacttcaactgtccgcattcggcggactacgtc           | 1260 |
| Y P D H M K Q H D F F K S A M P E G Y V |   |      |
| 1261                                    | caggagcgcacccatcttcacggactcggcaactacaagacccgcgcggaggacta          | 1320 |
| Q E R T I F F K D D G N Y K T R A E V K |   |      |
| 1321                                    | ttcgaggcgacaccctgttgcacccatcgactgactggatccggatccggatccggatcc      | 1380 |
| F E G D T L V N R I E L K G I D F K E D |   |      |
| 1381                                    | ggcaacatctggggcacaagctgtggactacaactacaacagccacaacgtctatcatg       | 1440 |
| G N I L G H K L E Y N Y N S H N V Y I M |   |      |
| 1441                                    | ggcgacaaggcacaaggcatcaaggacttcaagatccgcacaacatcgaggac             | 1500 |
| A D K Q K N G I K V N F K I R H N I E D |   |      |
| 1501                                    | ggcagcgtcggactcgccgaccactaccaggcacaaccccccattcggcgcggccctgt       | 1560 |
| G S V Q L A D H Y Q N T P I G D G P V   |   |      |
| 1561                                    | ctgctggccgacaccactacactgtggactaccatgttgcggatccggatccggatccggatcc  | 1620 |
| L L P D N H Y L S Y Q S H L C K D P N E |   |      |
| 1621                                    | aagcgcgatcacatggctctgtggacttcgtgaccgcgcggatccggatccggatccggatcc   | 1680 |
| K R D H M V L L E F V T A A G I T L G M |   |      |
| 1681                                    | gacgagctgtacaagtaagcggccgcact                                     | 1711 |
| D E L Y K -                             |   |      |

**Supporting figure 4: Nucleotide and amino acid sequence of eZinCh-2 for the bacterial expression vector pET28a.**  
The His-tag and thrombin cleavage site are depicted in blue and magenta respectively. Cerulean and citrine are colored cyan, and yellow respectively. The flexible peptide linker is depicted in grey. The binding pocket of the sensor is depicted in red, (206H and 208C).

|      |  |      |
|------|--|------|
| 1    | atataaggcagactggtttagtgaaccgtcagatccgcgtcgcccccaccatggccat   | 60   |
| 61   | I - A E L V - - T V R S A S A A T M G H<br>atggtgagcaaggcgaggagctgttacccgggtggccatcttgcgagctggac       | 120  |
| 121  | M V S K G E E L F T G V V P I L V E L D<br>ggcgacgtaaacggccacaagttcagcgtgtccggcgagggcgatgcccac         | 180  |
| 181  | G D V N G H K F S V S G E G E G D A T Y<br>ggcaagctgaccctgaagttcatctgcaccaccggtaactgcccgtccctggccacc   | 240  |
| 241  | G K L T L K F I C T T G K L P V P W P T<br>ctcgtgaccaccctgacctggcgctgcagtgttcgcaccatggccat             | 300  |
| 301  | L V T T L T W G V Q C F A R P D H M K<br>cagcacgacttcaagttccatgcggccatggccgatctacgtcccgaggacgcaccat    | 360  |
| 361  | Q H D F F K S A M P E G Y V Q E R T I F<br>ttcaaggacacggcaactacaagacccgcgcgaggtgaagttcgaggcgacaccctg   | 420  |
| 421  | F K D D G N Y K T R A E V K F E G D T L<br>gtgaaccgcgtcggactcaaggcatcgactcaaggaggacggcaacatcttgggcac   | 480  |
| 481  | V N R I E L K G I D F K E D G N I L G H<br>aagctggagatacaacgcctacgcgacaaacgtctatataccgcgcacaaggaaac    | 540  |
| 541  | K L E Y N A I S D N V Y I T A D K Q K N<br>ggcatcaaggccaacttcaagatccgcacaacatcgaggacggcagctgcagctcgcc  | 600  |
| 601  | G I K A N F K I R H N I E D G S V Q L A<br>gaccactaccaggcagaacaccccccattcggcgcggccctgtctgtcccgacaaccac | 660  |
| 661  | D H Y Q Q N T P I G D G P V L L P D N H<br>tacctgagcaccctggtccactgtcgaaagaccccaacgagaagcgcgatcacatggc  | 720  |
| 721  | Y L S T Q S H L C K D P N E K R D H M V<br>ctgctggagttcgatccggccggatcaactctcgcatggacgactgtacaatgtcc    | 780  |
| 781  | L L E F V T A A G I T L G M D E L Y K S<br>ggaggccggcggacttctgtggccatccggcggaaagcggcggatccggcgttagccgc | 840  |
| 841  | G G G E L I R G G S G G S G G S G G S<br>ggatccggccggtccggccgatccggccggcagccggatccggatggaaagcggatcc    | 900  |
| 901  | G S G G S G G S G G S G G S G G S<br>ggttgttagccgtggatccggatggaaagcggatccggatccggatccggatccggat        | 960  |
| 961  | G G S G G S G G S G G S G G S G G S<br>cccgccgggtcggtacccatggtgagcaaggcgaggactgttacccgggtggcc          | 1020 |
| 1021 | P R G S V P M V S K G E E L F T G V V P<br>atccctggtcagctggccgcacgtaaacggccacaacgttcagcgtgtccggcgagg   | 1080 |
| 1081 | I L V E L D G D V N G H K F S V S G E G<br>gaggccgatccggccatccggccatggccatccgtggatccggatccggatccggat   | 1140 |
| 1141 | E G D A T Y G K L T L K F I C T T G K L<br>cccgtgccccggccaccctcggtaccacccctcgatgtgtgtccgcgc            | 1200 |
| 1201 | P V P W P T L V T T F G Y G L M C F A R<br>taccggccacacatgaagcagcacttcaagttccgcgttccgcgcggactacgtc     | 1260 |
| 1261 | Y P D H M K Q H D F F K S A M P E G Y V<br>caggagcgcacatcttcttcaaggacgcggcaactacaagacccgcgcgagg        | 1320 |
| 1321 | Q E R T I F F K D D G N Y K T R A E V K<br>ttcgaggcgacaccctgttgcacccgcgtggatccggatccggatccggat         | 1380 |
| 1381 | F E G D T L V N R I E L K G I D F K E D<br>ggcaacatctggggcacaagcttgcgttgcaccaacacagccacaacgttataat     | 1440 |
| 1441 | G N I L G H K L E Y N Y N S H N V Y I M<br>ggcgacaaggcacaaggcatcaaggtaacttcaagatccgcacaacatcgagg       | 1500 |
| 1501 | A D K Q K N G I K V N F K I R H N I E D<br>ggcagcgtcgactccgcggaccatccaggcacaaccccccattcggcgcggccgt     | 1560 |
| 1561 | G S V Q L A D H Y Q N T P I G D G P V<br>ctgctggccgacaaccacttgcgtggatccgttgcgtggatccggatccggat         | 1620 |
| 1621 | L L P D N H Y L S Y Q S H L C K D P N E<br>aagcgcgatcacatggcttgcgtggatccgttgcgtggatccggatccggat        | 1680 |
| 1681 | K R D H M V L L E F V T A A G I T L G M<br>gacgagctgtacaagtaagcggccgcact                               | 1711 |
|      | D E L Y K -  |      |

**Supporting figure 5: Nucleotide and amino acid sequence of eZinCh-2 in a mammalian expression vector.** Cerulean and citrine are colored cyan, and yellow respectively. The flexible peptide linker is depicted in grey. The binding pocket of the sensor is depicted in red, (206H and 208C).

|   |  |      |
|---|--|------|
| 1   | tcgaaattaatacgactcaactatagggagacccaagctggcttagcatggccctgtggatg   | 60   |
| S K L I R L T I G R P K L A S M A L W M   |  |      |
| 61  | ggctcctgcctgtggcgctgtggccctctggggacctgaccaggccagccagcccttc       | 120  |
| R L L P L L A L L A L W G P D P A A A F   |  |      |
| 121                                       | atggtgagcaaggcgaggagctgttaccgggggtggccatctggctcgagctggac         | 180  |
| M V S K G E E L F T T G V V P I L V E L D |  |      |
| 181                                       | ggcacgtaaacggccacaagttcagctgtccggcgaggcgaggccgatgccac            | 240  |
| G D V N G H K F S V S G E G E G D A T Y   |  |      |
| 241                                       | ggcaagctgaccctgaagtcatgtcaccaccggtaactgcccgtccctggccacc          | 300  |
| G K L T L K F I C T T G K L P V P W P T   |  |      |
| 301                                       | ctcgtgaccaccctgaccctggctgcagtctccggctaccaccgaccatagaag           | 360  |
| L V T T L T W G V Q C F A R Y P D H M K   |  |      |
| 361                                       | cagcacgacttcaagtccgcattccggaaaggctacgtccaggagcgcaccatctc         | 420  |
| Q H D F F K S A M P E G Y V Q E R T I F   |  |      |
| 421                                       | ttcaaggacggcaactacaagaccggcgccaggtaactgaggccgacaccctg            | 480  |
| F K D D G N Y K T R A E V K F E G D T L   |  |      |
| 481                                       | gtgaaccgcacatcgactgaaggcatcgactcaaggaggacggcaacatctgggcac        | 540  |
| V N R I E L K G I D F K E D G N I L G H   |  |      |
| 541                                       | aagctggagataacgcacatcagcacaacgtctatccgcgcgacaaggcagaac           | 600  |
| K L E Y N A I S D N V Y I T A D K Q K N   |  |      |
| 601                                       | ggcatcaaggccaaacttcaagatccgcacacaatcgaggacggcgcgtcgcc            | 660  |
| G I K A N F K I R H N I E D G S V Q L A   |  |      |
| 661                                       | gaccactaccaggacaaacacccccatcgcgacggccctgtctgcgcgacaacc           | 720  |
| D H Y Q Q N T P I G D G P V L L P D N H   |  |      |
| 721                                       | tacctgagcacccagtcacactgtggccaaagaccccaacgagaagcgcgatcacatggc     | 780  |
| Y L S T Q S E L K D P N E K R D H M V     |  |      |
| 781                                       | ctcgctggatctgtggccgcggatcacatcgcatggcgactgtacaagtcc              | 840  |
| L L E F V T A A G I T L G M D E L Y K S   |  |      |
| 841                                       | ggaggcggcgagctcattctggccggatccggcggaaagcggcgatccggcgtagccg       | 900  |
| G G G E L I R G G S G G S G G S G G S G   |  |      |
| 901                                       | ggatccggcggtccggatccggcggacggcgccgatccggtgaaagcgggtggatcc        | 960  |
| G S G G S G G S G G S G G S G G S G G S   |  |      |
| 961                                       | ggtgttagccgtggatccggtgaaacgggtgatccggtgatccggatccgggt            | 1020 |
| G G S G G S G G S G G S G G S G G S G G   |  |      |
| 1021                                      | ccgcggggctcgatccctggtagtgagcaaggcgaggactgttacccgggtggcc          | 1080 |
| P R G S V P M V S K G E E L F T G V V P   |  |      |
| 1081                                      | atccctggcgagotggacggcgacgtaaacggccacaacgttcagctgtccggcgagg       | 1140 |
| I L V E L D G D V N G H K F S V S G E G   |  |      |
| 1141                                      | gagggcgatgccacccatcgccaaagctgaccctgaaatctgcaccacccgcaagctg       | 1200 |
| E G D A T Y G K L T L K F I C T T G K L   |  |      |
| 1201                                      | cccgccctggggccaccctctgtgaccacccctcgatccggctacggcgtatgtgtctcgcc   | 1260 |
| P V P W P T L V T T F G Y G L M C F A R   |  |      |
| 1261                                      | taccccgaccacatgaagcagcacacttcaagtcgtccatcgcccaaggctacgtc         | 1320 |
| Y P D H M K Q H D F F K S A M P E G Y V   |  |      |
| 1321                                      | caggagcgcacccatcttcacccatcgccaaacggcgactacaagaccccgccgaggtaag    | 1380 |
| Q E R T I F F K D D G N Y K T R A E V K   |  |      |
| 1381                                      | ttcgaggggcggacaccctgtggacccgcacatcgactgtggaggccatcgactcaaggaggad | 1440 |
| F E G D T L V N R I E L K G I D F K E D   |  |      |
| 1441                                      | ggcaacatctggggcacaagcttggatccactacaacacggccacaacgtctatcatg       | 1500 |
| G N I L G H K L E Y N Y N S H N V Y I M   |  |      |
| 1501                                      | ggcgacaacgagaagaacggccatcaaggtaacttcaagatccggccacaatcgaggac      | 1560 |
| A D K V Q K N G I K V N F K I R H N I E D |  |      |
| 1561                                      | ggcagctgcagtcggccgaccactaccaggacaaacaccccatcgccgacggccctgt       | 1620 |
| G S V Q L A D H Y Q Q N T P I G D G P V   |  |      |
| 1621                                      | ctgctggccgacaaccactaccctggatccatcgacttgcacatcgacttcgtggcc        | 1680 |
| L L P D N H Y L S Y Q S E L C K D P N E   |  |      |
| 1681                                      | aagcgcgatccatgtggctctgtggatccatcgacttgcacatcgacttcgtggcc         | 1740 |
| K R D H M V L L E F V T A A G I T L G M   |  |      |
| 1741                                      | gacgagctgtacaaggcgcacaaaggacgactgttaagcggccgcact                 | 1788 |
| D E L Y K V D K D E I -                   |  |      |

**Supporting figure 6: Nucleotide and amino acid sequence of ER-eZinCh-2.** The N-terminal PPI signal peptide and C-terminal retention sequence KDEL are depicted in green. Cerulean and citrine are colored cyan, and yellow, respectively. The flexible peptide linker is depicted in grey. The binding pocket of the sensor is depicted in red (206H and 208C).

|      |   |      |
|------|---|------|
| 1    | cggcggtcccagtgcgcgcgccaagatccattcggtggggatctcatgtccgtccta     | 60   |
|      | R R L P V P R A K I H S L G D L M S V I                       |      |
| 61   | acggcgctgtgtgcggggcttgcaggctggccggcgctcccaagtgcgcgcgc         | 120  |
|      | T P L L L R G L T G S A R R L P V P R A                       |      |
| 121  | aagatccattcggtgcggggagagatctcaagagctcgagcgcaccatggccat        | 180  |
|      | K I H S L P P E G D L K S S S A T M G H                       |      |
| 181  | atggtgagaacggcgaggagctgttacccgggttgtgcacatctgtcgagctggac      | 240  |
|      | M V S K G E E L F T G V V P I L V E L D                       |      |
| 241  | ggcacgtaaacggccacaagttcagctgtccggcgaggcgaggccatgcacactac      | 300  |
|      | G D V N G H K F S V S G E G E G D A T Y                       |      |
| 301  | ggcaagctgaccctgaaaggcatctgcaccacccgttaagctgcggccctggccacc     | 360  |
|      | G K L T L K F I C T T G K L P V P W P T                       |      |
| 361  | ctcgtgaccacccgtacactggcggtgcagtgttcgcgttgcacatggccatgaag      | 420  |
|      | L V T T L T W G V Q C F A R Y P D H M K                       |      |
| 421  | cagcacacttcataactggccatgcggcaaggctacgtccaggagcgcaccatctc      | 480  |
|      | Q H D F F K S A M P E G Y V Q E R T I F                       |      |
| 481  | ttaaggacacggcaactacaagacccgcggaggtaagttcgaggcgacacccgt        | 540  |
|      | F K D D G N Y K T R A E V K F E G D T L                       |      |
| 541  | gtgaaccgcacatcgagctgaaggcatcactcaaggaggacggcaatctggggcac      | 600  |
|      | V N R I E L K G I D F K E D G N I L G H                       |      |
| 601  | aagctggagataacgcacatcagcgacaaacgtctataatcaccggccacaagaaac     | 660  |
|      | K L E Y N A I S D N V Y I T A D K Q K N                       |      |
| 661  | ggcatcaaggccaaacttcaagatccgcacaacatcgaggacggcagctgcagctgcc    | 720  |
|      | G I K A N F K I R H N I E D G S V Q L A                       |      |
| 721  | ggactactacacgacaaacccccatcgccgacggccctgtgtgcggcacaaccac       | 780  |
|      | D H Y Q N T P I G D G P V L L P D N H                         |      |
| 781  | tacctgagaccacccgtccacccatcgacaaagaccccaacggaaagcggcatacatggc  | 840  |
|      | Y L S T Q S T L K D P N E K R D H M V                         |      |
| 841  | ctgctggagggtcgatccgcgggatcactctcgcatggacgagctgtacaatgtcc      | 900  |
|      | L L E F V T A A G I T L G M D E L Y K S                       |      |
| 901  | ggaggccggcggacttccgtggcgatccggcgaaaggccggatccggcgtagccgc      | 960  |
|      | G G G E L I R G G S G G S G G S G G S G                       |      |
| 961  | ggatccggccggatccggcgatccggcgacggcgatccggatccggatccggatcc      | 1020 |
|      | G S G G S G G S G G S G G S G G S G G S                       |      |
| 1021 | ggtgttagccgtggatccggatccggatccggatccggatccggatccggatccggat    | 1080 |
|      | G G S G G S G G S G G S G G S G G S G G                       |      |
| 1081 | ccggggggctcggttacccatggtgacaaaggccggaggagctgttacccgggttgtggcc | 1140 |
|      | P R G S V P M V S K G E E L F T G V V P                       |      |
| 1141 | atccctggtcagctggacggcgcacgtaaacggccacaagttcagctgtccggcggagggc | 1200 |
|      | I L V E L D G D V N G H K F S V S G E G                       |      |
| 1201 | gagggcgtatccacactacggcaactcgaccctgaaatctgcaccacccggcaagctg    | 1260 |
|      | E G D A T Y G K L T L K F I C T T G K L                       |      |
| 1261 | ccctgtccctggcccacccctcgatccggatccggatccggatccggatccggatcc     | 1320 |
|      | P V P W P T L V T T F G Y G L M C F A R                       |      |
| 1321 | taccccgaccacatgaagcagcacgttcaactggccatcgccatccggatccggatcc    | 1380 |
|      | Y P D H M K Q H D F F K S A M P E G Y V                       |      |
| 1381 | caggagcgcacatcttccatggacggacggcaactacaagacccgcggaggtaag       | 1440 |
|      | Q E R T I F F K D D G N Y K T R A E V K                       |      |
| 1441 | ttcgaggggcgcacccctgttgcacccgttgcacgttgcacgttgcacgttgcac       | 1500 |
|      | F E G D T L V N R I E L K G I D F K E D                       |      |
| 1501 | ggcaacatctggggcacaagctgttgcacactacaacagccacaacgtctatatcatg    | 1560 |
|      | G N I L G H K L E Y N Y N S H N V Y I M                       |      |
| 1561 | gccgacaaggcagaacggcatcaaggtaacttcaagatccggccacaatcgaggac      | 1620 |
|      | A D K Q K N G I K V N F K I R H N I E D                       |      |
| 1621 | ggcagcgtcagctcgccgaccactaccaggcagaacaccccatcgccgacggccctg     | 1680 |
|      | G S V Q L A D H Y Q N T P I G D G P V                         |      |
| 1681 | ctgctggccgacaaaccacttgcgttgcacccatcgatccggatccggatccggat      | 1740 |
|      | L L P D N H Y L S Y Q S H L C K D P N E                       |      |
| 1741 | aagcgcgcatacatggctgttgcggatccgttgcacccggccggatcatctcgccatg    | 1800 |
|      | K R D H M V L L E F V T A A G I T L G M                       |      |
| 1801 | gacgagctgtacaactaaacggccgcact                                 | 1830 |
|      | D E L Y K -   |      |

**Supporting figure 7: Nucleotide and amino acid sequence of mito-eZinCh-2.** The N-terminal signal sequence cytochrome c oxidase subunit VIII depicted in purple. Cerulean and citrine are colored cyan, and yellow, respectively. The flexible peptide linker is depicted in grey. The binding pocket of the sensor is depicted in red (206H and 208C).

|      |   |      |
|------|---|------|
| 1    | gctggtttagtgaccgtcagatccgcctagattaccatgggcatagtcggttaccgcacc        | 60   |
| A    | G L V T V R S A S T M G M S A T A A T                               |      |
| 61   | gtccccctgcgcggccggcggcgggggtggcccccgtgcacccctccaaatcttacc           | 120  |
| V    | P P A A P A G E G G G P P A P P P N L T                             |      |
| 121  | atgttaacaggagactgcgcggcggccggatggatggatggatggatcatcgagg             | 180  |
| S    | N R R L Q Q T Q A Q V D E V V D I M R                               |      |
| 181  | gtgtatgtggacaaggcttcgtggagcgagaccagaagctatcgaaatggatgtcgca          | 240  |
| V    | N V D K V L E R D Q K L S E L D D R A                               |      |
| 241  | atgtccctcaggcaggcccctccagttgaaacaagtgcgcggcagctcaagcgaaa            | 300  |
| D    | A L Q A G A S Q F E T S A A K L K R K                               |      |
| 301  | tactgggtggaaaacctcaagatgtatcatcttggggatgttgcgcctatcc                | 360  |
| Y    | W W K N L K M M I I L G V I C A I I L                               |      |
| 361  | atcatcatcatcgtttacttcgcgcggcggctccgtcgccgcaccatggccat               | 420  |
| I    | I I I I V Y F S T G G S A S A A T M G H                             |      |
| 421  | atgttgagcaaggcgaggagcttgcacccgggtgtgtccatccgtgtcgactggac            | 480  |
| M    | V S K G E E L F T G V P I L V E L D                                 |      |
| 481  | ggcgacgttaacggccacaagttcgcgtgtccggcgaggcgaggcgatgccatcac            | 540  |
| G    | D V N G H K F S V S G E G E G D A T Y                               |      |
| 541  | ggcaagctgaccctgaagtcatctgcaccaccggtaactgcccgtccctggccacc            | 600  |
| G    | K L T L K F I C T T G K L P V P W P T                               |      |
| 601  | ctcgtgaccaccctgaccctggggctgcactgttgcggctaccaccacatgaag              | 660  |
| L    | V T T L T W G V Q C F A R Y P D H M K                               |      |
| 661  | cagcacgacttcaagtccgcacatgcggcggaggctacgtccaggagcgcaccatctc          | 720  |
| Q    | H D F F K S A M P E G Y V Q E R T I F                               |      |
| 721  | ttcaaggacgcggcaactacaagacccgcggcggatgttgcggggcgacaccctg             | 780  |
| F    | K D D G N Y K T R A E V K F E G D T L                               |      |
| 781  | gtgaaccgcatcgagctgaaggcatcgacttaaggaggacggcaacatctggggcac           | 840  |
| V    | N R I E L K G I D F K E D G N I L G H                               |      |
| 841  | aagctggagataacgcacatcgacgacaaacgtctataatcaccgcgcacaaggagaac         | 900  |
| K    | L E Y N A I S D N V Y I T A D K Q K N                               |      |
| 901  | ggcatcaaggccaacttcaagatccgcacacaatcgaggacggcgtcgacgcgc              | 960  |
| G    | I K A N F K I R H N I E D G S V Q L A                               |      |
| 961  | gaccactaccacgacaacacccccatcgccgcggccctgtgtgtcccgacaaaccac           | 1020 |
| D    | H Y Q Q N T P I G D G P V L L P D N H                               |      |
| 1021 | tacctgagcacccatgcacccatcgccaaagaccccaacgagaagcgcgcacatggc           | 1080 |
| Y    | L S T Q S E L K D P N R D H M V                                     |      |
| 1081 | ctgtggaggctgtgaccctggggatactctcgcatggacgactgtacaatgtcc              | 1140 |
| L    | L E F V T A A G I T L G M D E L Y K S                               |      |
| 1141 | ggaggcggcggactcattcggtggcgatccggcggaaacggccggatccggcggttagcggc      | 1200 |
| G    | G G E L I R G G S G G S G G S G G S G                               |      |
| 1201 | ggatccggccgtccggggatccggcggacggccggatccgggtggaaacggcggtatcc         | 1260 |
| G    | S G G S G G S G G S G G S G G S G G S                               |      |
| 1261 | ggtgttagccgtggatccgggtggaaacggccggatccgggtggatccgggtggatccgggggt    | 1320 |
| G    | G S G G S G G S G G S G G S G G S G G                               |      |
| 1321 | ccgcggggctcgatcccatggtagtggcaaggccggaggactgttcccggtggatccgggtggatcc | 1380 |
| P    | R G S V P M V S K G E E L F T G V V P                               |      |
| 1381 | atcttgcgcggactgtggacggccgcgtaaacggccacaacgttcacgtgtccggcgagg        | 1440 |
| I    | L V E L D G D V N G H K F S V S G E G                               |      |
| 1441 | ggggcgatgccacccatcgccaaacgttgcacccatcgacttgcacccacggcaagctg         | 1500 |
| E    | G D A T Y G K L T L K F I C T T G K L                               |      |
| 1501 | cccggtccctggccacccatcgaccacccatcgatgtgtgtgtcgccgc                   | 1560 |
| P    | V P T L V T F G Y G L M C F A R                                     |      |
| 1561 | taccccgaccacatcgacgacgcacttcaatgcgttccgcacatggcgtacgtc              | 1620 |
| Y    | P D H M K Q H D F F K S A M P E G Y V                               |      |
| 1621 | caggacgcaccatcttcaaggacgcacggcaactacaagacccgcggaggtgaag             | 1680 |
| Q    | E R T I F F K D D G N Y K T R A E V K                               |      |
| 1681 | ttcgaggggcggacaccctgtggacccatcgactgtggactcgacttgcaggaggac           | 1740 |
| F    | E G D T L V N R I E L K G I D F K E D                               |      |
| 1741 | ggcaacatctggggcacaagctgtggactacaacacgcccacacgttatcatg               | 1800 |
| G    | N I L G H K L E Y N Y N S H N V Y I M                               |      |
| 1801 | ggccgacaaaggcacaaggacggccatcaaggtaacttcaagatccgcacacatcgaggac       | 1860 |
| A    | D K Q N F K I K V N F K I R H N I E D                               |      |
| 1861 | ggcgcgtgcacgtccgcggaccactaccaggcacaacacccatcgccgcacggccgt           | 1920 |
| G    | S V Q L A D H Y Q Q N T P I G D G P V                               |      |
| 1921 | ctgtgcggcacaaccactacatgtggactaccatcgacttgcgtggatccggat              | 1980 |
| L    | L P D N H Y L S Y Q S E L C K D P N E                               |      |
| 1981 | aaggcgatcatgtggacttgcgtggactaccatcgacttgcgtggatccggat               | 2040 |
| K    | R D H M V L L E F V T A A G I T L G M                               |      |
| 2041 | gacgactgtacaaggtaacggccgcact  | 2070 |
| D    | E L Y K -   |      |

**Supporting figure 8: Nucleotide and amino acid sequence of VAMP2-eZinCh-2.** VAMP2, cerulean, and citrine are colored army green, cyan and yellow respectively. The flexible peptide linker is depicted in grey. The binding pocket for Zn<sup>2+</sup> is depicted in red (206H and 208C).

1. Vinkenborg, J. L., Nicolson, T. J., Bellomo, E. A., Koay, M. S., Rutter, G. A., and Merkx, M. (2009) Genetically encoded FRET sensors to monitor intracellular Zn<sup>2+</sup> homeostasis, *Nat Methods* 6, 737-740.
2. Chabosseau, P., Tuncay, E., Meur, G., Bellomo, E. A., Hessels, A., Hughes, S., Johnson, P. R., Bugliani, M., Marchetti, P., Turan, B., Lyon, A. R., Merkx, M., and Rutter, G. A. (2014) Mitochondrial and ER-targeted eCALWY probes reveal high levels of free Zn<sup>2+</sup>, *ACS Chem Biol* 9, 2111-2120.
3. Qin, Y., Dittmer, P. J., Park, J. G., Jansen, K. B., and Palmer, A. E. Measuring steady-state and dynamic endoplasmic reticulum and Golgi Zn<sup>2+</sup> with genetically encoded sensors, *Proc Natl Acad Sci U S A* 108, 7351-7356.
4. Park, J. G., Qin, Y., Galati, D. F., and Palmer, A. E. New sensors for quantitative measurement of mitochondrial Zn(2+), *ACS Chem Biol* 7, 1636-1640.