CLAIMS

1. A method of detecting changes in Ca^{2+} levels in a cell, the method comprising:
    a. obtaining a sample comprising cells engineered to express one or more low affinity Ca^{2+} indicator selected from the group consisting of:SEQ ID Nos 12, 14, 16 and 18, or a polypeptide that has at least 90% sequence identity to any one of the foregoing, which is fluorescent and has an affinity for Ca^{2+} with a Kd of greater than 20 µM, but excluding SEQ ID NO. 2;
    b. exposing the cells to excitation light; and
    c. detecting changes in endoplastic reticulum (ER), sarcoplastic reticulum (SR) and/or mitochondria Ca^{2+} levels by visualizing or imaging the cells.
2. The method in claim 1, wherein the sample comprises a cell culture or stem cells.
3. The method of claim 2 wherein the sample comprises a cell culture of stable immortalized cell line.
4. The method of claim 3 wherein the cell culture comprises a HL1 cell line.
5. The method in claim 1, wherein the indicator is ratiometric.
6. The method in claim 1, wherein the indicator is intensiometric.
7. The method in any one of claims 1-6, wherein the indicator is used in combination with another fluorescent indicator.
8. The method of claim 7 which uses a single wavelength two-colour imaging method.
9. The method of claim 7 wherein the other fluorescent indicator is a cytoplasmic calcium indicator.
10. The method of any one of claims 1-9, wherein the indicator is targeted to an organelle with an organelle-specific targeting sequence.
11. A low affinity fluorescent Ca^{2+} polypeptide selected from the group consisting of: SEQ ID Nos 12, 14, 16 and 18, or a polypeptide that has at least 90% sequence identity to any one of the foregoing, which is fluorescent and has an affinity for Ca^{2+} with a Kd of greater than 20 µM, but excluding SEQ ID NO. 2.

12. The polypeptide of claim 11 which has the amino acid sequence of one of SEQ ID NOs. 12, 14, 16 or 18.

13. The polypeptide of claim 11 or 12 further comprising an organelle-specific targeting sequence.

14. The polypeptide of claim 13 comprising the targeting sequence of SEQ ID NO. 19.

15. The polypeptide of claim 11 or 12 which comprises a mutation in SEQ ID NO 4. selected from the group consisting of: I54A, I330M, and D327N/I330M/D363N.

16. The polypeptide of claim 11 having a K_d for Ca^{2+} greater than 60 μM.

17. A polynucleotide encoding a polypeptide of one of claims 11 -16.

18. The polynucleotide of claim 17 comprising a nucleic acid sequence selected from the group consisting of:

    a. SEQ ID NO. 11, 13, 15, or 17;

    b. a nucleic acid sequence having at least 90% sequence identity to one of SEQ ID NO. 11, 13, 15, or 17, and encoding a fluorescent Ca^{2+} indicator, having a K_d for Ca^{2+} greater than 20 μM, or optionally 60 μM, but excluding SEQ ID NO. 1;

    c. a nucleic acid sequence encoding a fluorescent Ca^{2+} indicator comprising an amino acid sequence of SEQ ID No. 12, 14, 16 or 18; and

    d. a nucleic acid sequence encoding a fluorescent Ca^{2+} indicator, having a K_d for Ca^{2+} greater than 20 μM, and having at least 90% sequence identity to an amino acid sequence of SEQ ID No. 12, 14, 16 or 18, but excluding SEQ ID NO. 2.

19. The polynucleotide of claim 17 or 18, further comprising a sequence which encodes an organelle-specific targeting sequence.

20. The polynucleotide of claim 19 wherein the organelle-specific targeting sequence encodes SEQ ID NO. 19.

21. The polynucleotide of claim 17 or 18 which encodes a mutation in SEQ ID NO. 4 selected from the group consisting of: I54A, I330M, and D327N/I330M/D363N.

22. A vector comprising the polynucleotide of one of claims 17-21.

23. A host cell comprising the polynucleotide sequence of one of claims 17-21, or a vector of claim 22.

24. The host cell of claim 23 which is a cardiomyocyte.

25. The host cell of claim 23 which co-expresses another fluorescent calcium indicator.