# Red fluorescent CEPIA indicators for visualization of $Ca^{2+}$ dynamics in mitochondria

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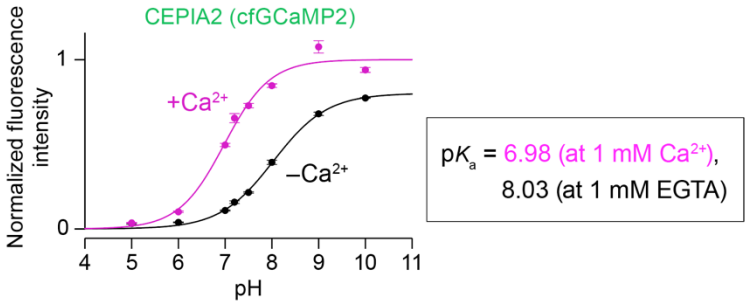
## Supplementary Figures

**A**

| Indicator | Amino acids position in calmodulin sequence | | | | | | | | | | $K_d$ (μM) for $Ca^{2+}$ | Dynamic range | Hill coefficient |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 31 | 36 | 60 | 63 | 77 | 92 | 101 | 111 | 127 | 133 | | | |
| CEPIA2 (cfGCaMP2) | E | L | N | I | K | F | S | N | E | D | 0.67 | 5.1 | 2.5 |
| CEPIA3 | D | L | N | I | K | F | S | N | E | D | 14.5 | 5.0 | 1.5 |
| CEPIA4 | D | L | N | I | K | W | S | N | E | E | 90.2 | 4.9 | 1.7 |
| R-GECO1 | E | M | D | F | N | F | G | D | V | D | 0.19 | 22.2 | 2.2 |
| **R-CEPIA3*** | D | M | D | F | N | F | G | D | V | D | 3.7 | 30.0 | 1.0 |
| **R-CEPIA4*** | D | M | D | F | N | W | G | D | V | E | 26.9 | 23.9 | 1.5 |
| R-GECO1 E31D/F92W/D133E with cfGCaMP2 calmodulin# | D | L | N | I | K | W | S | N | E | E | 16.6 | 21.9 | 2.3 |

*Developed in current study
#Reference 15
CEPIA mutations
Difference between R-GECOs and cfGCaMP2
in vitro properties at pH 7.2

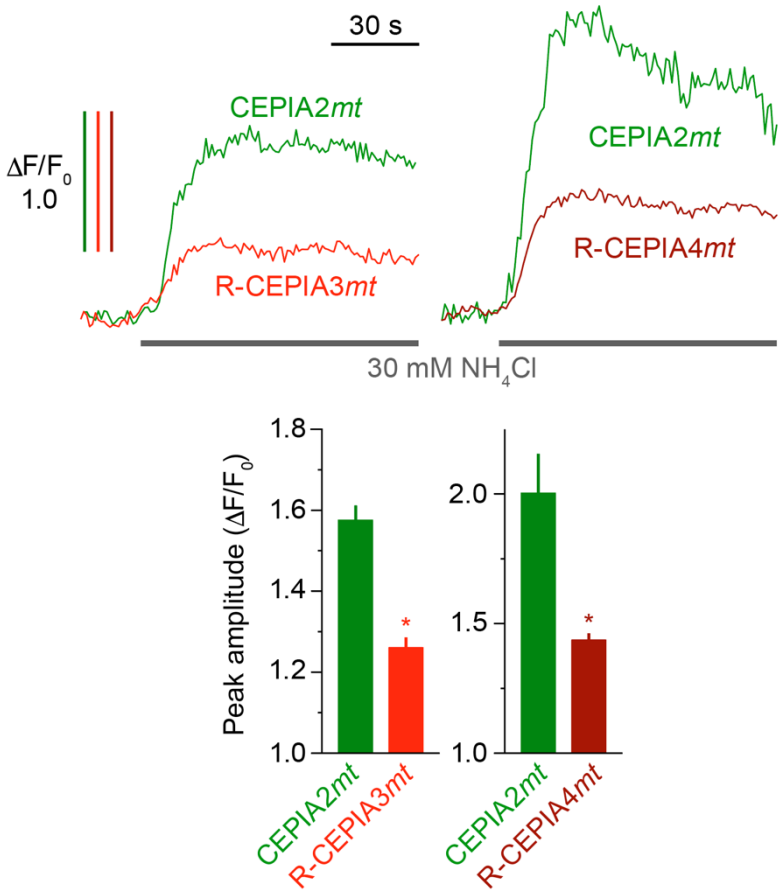
**B**




**Supplementary Figure 1: Comparison of amino acids sequence of calmodulin in CEPIAs and pH titration curve of CEPIA2**

**A**: Comparison of amino acids sequence of calmodulin in CEPIAs.

**B**: pH titration curve of CEPIA2 (cfGCaMP2) in $Ca^{2+}$-containing (1 mM, magenta) or $Ca^{2+}$-free (1 mM EGTA, black) solution. The plots were fitted by a single Hill equation. Mean ± SEM ($n$ = 3).




**Supplementary Figure 2: Effect of alkalization on fluorescence intensity of R-CEPIA3*mt* and R-CEPIA4*mt***

Representative time courses and summarized graphs of the peak amplitude of alkalization-induced changes in the fluorescence intensity of mitochondrial CEPIAs. The data were obtained from HeLa cells expressing R-CEPIA3*mt* and CEPIA2*mt* (left) or R-CEPIA4*mt* and CEPIA2*mt* (right). $n = 6$ and 5 (cells), $*p = 6.64 \times 10^{-8}$ and $1.43 \times 10^{-5}$, for left and right graphs, respectively.

## Supplementary Table

**Supplementary Table 1: The list of primers.**

| Primer number | Sequence |
|---|---|
| 1 (E31D) | GGGCAGAACCCCACAGAAGCAGAGCTCCAG |
| 2 (E31D) | CAGAGACCGCATCACCGTCCCCAGATCCTT |
| 3 (F92W) | GGGATAAGGATGGCAATGGCTACATCGGC |
| 4 (F92W) | ACACACGGAACGCTTCGCGAATTTCCTCT |
| 5 (D133E) | ATCGATGGAGAAGGTCAGGTAAACTACGAA |
| 6 (D133E) | GTCTGCTACCCTGATCATTTCATCAACCTC |

The primers used in the present study were listed. For the primers used for point mutations, the target mutation sites in calmodluin domain of R-GECO1 were indicated in parentheses.