Dual-FRET imaging of IP_3 and Ca^{2+} revealed Ca^{2+}-induced IP_3 production maintains long lasting Ca^{2+} oscillations in fertilized mouse eggs

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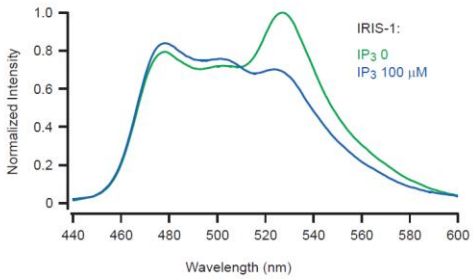
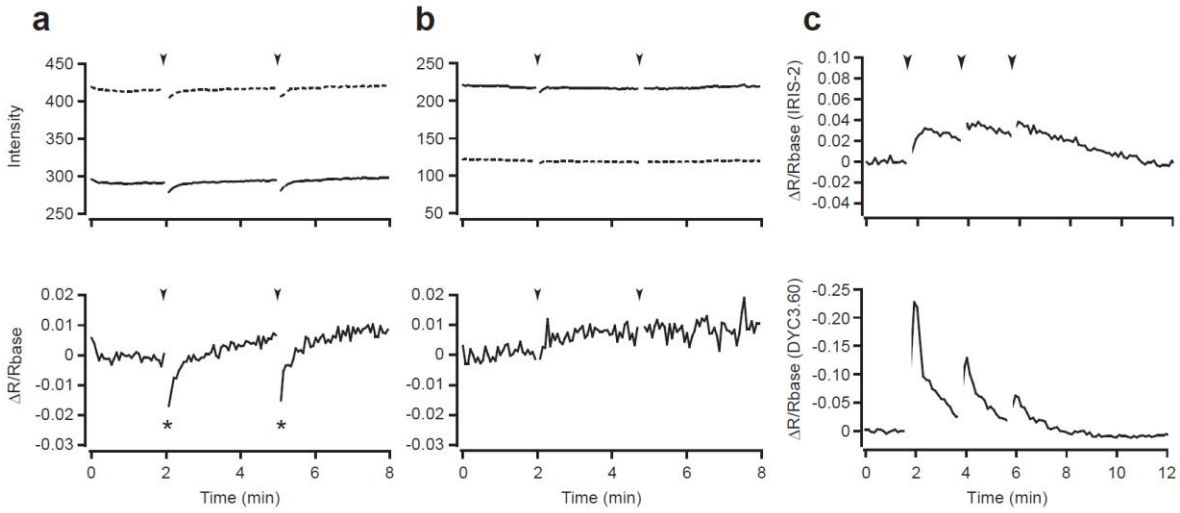
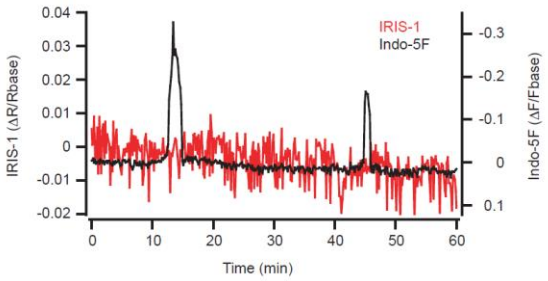


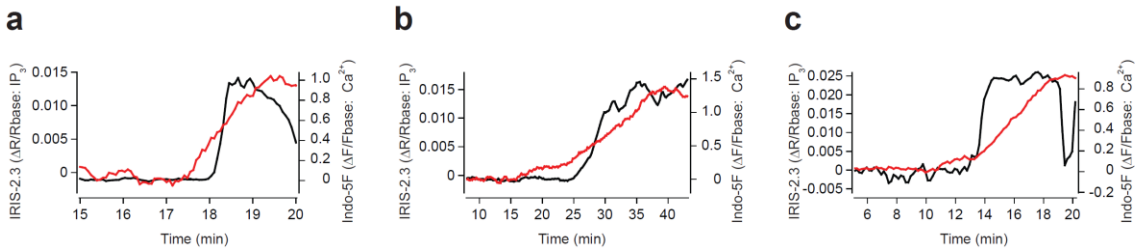
Supplementary Figure 1. Emission spectra of IRIS-1. Spectrum measurements of purified IRIS-1 were obtained with (blue line) and without (green line) addition of IP_3.



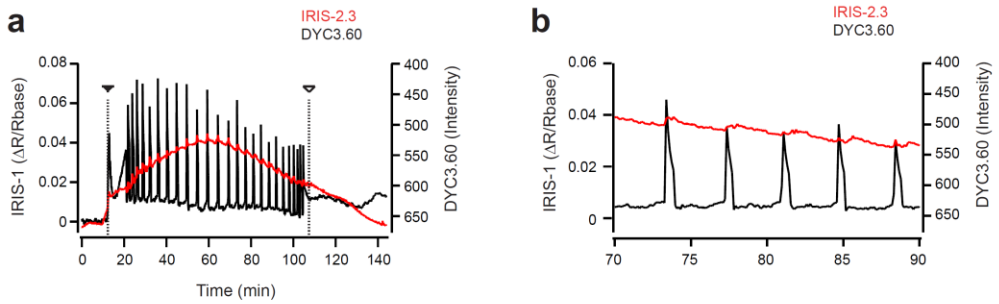
Supplementary Figure 2. Uncaged-IP_3 was detected by IRIS-2_{TMR} expressed in HeLa cells. (a and b) The effect of UV on the fluorescence of IRIS-1 (a) and IRIS-2_{TMR} (b). Arrow heads indicate the timing of UV irradiations. Upper panels show fluorescent signals of FRET donors (continuous lines; ECFP and EGFP in a and b, respectively) and acceptors (broken lines; Venus and TMR in a and b, respectively) in HeLa cells. Lower panels show FRET ratio of IRIS-1 (a) and IRIS-2 (b). (c) IRIS-2_{TMR} detected UV-uncaged IP_3 which induce Ca^{2+} release from IP_3R. Fluorescent ratio of IRIS-2_{TMR} (upper panel) and DYC3.60 (lower panel) are plotted.



Supplementary Figure 3. Fluorescent ratio changes of IRIS-1 during fertilization in mouse eggs. Signals of IRIS-1 are plotted with red lines. Fertilization induced Ca^{2+} spikes were detected by Indo-5F (black lines). Sperm was added at the time of zero. Fluorescent images were acquired each 4 sec.



Supplementary Figure 4. [IP_3] and [Ca^{2+}] traces obtained with Indo-5F and IRIS-2.3$_{TMR}$ at the onset of first Ca^{2+} spike. (a-c) Three independent imaging experimental results are shown. [IP_3] (red lines) and [Ca^{2+}] traces (black lines) were obtained with IRIS-2.3$_{TMR}$ and DYC3.60, respectively.



Supplementary Figure 5. Dual FRET imaging of whole Ca^{2+} oscillations at fertilization. (**a**) Emission changes of DYC3.60 (black line) and ratio changes of IRIS-2.3$_{TMR}$ (red line) are shown. Initiation of the first Ca^{2+} spike and the termination of Ca^{2+} oscillations were marked with dashed line with closed and open triangles, respectively. Sperm was added at time zero. (**b**) The enlarged graph of **a** shows delays in [IP_3] rises.