SUPPLEMENTAL MATERIALS

Wang et al.

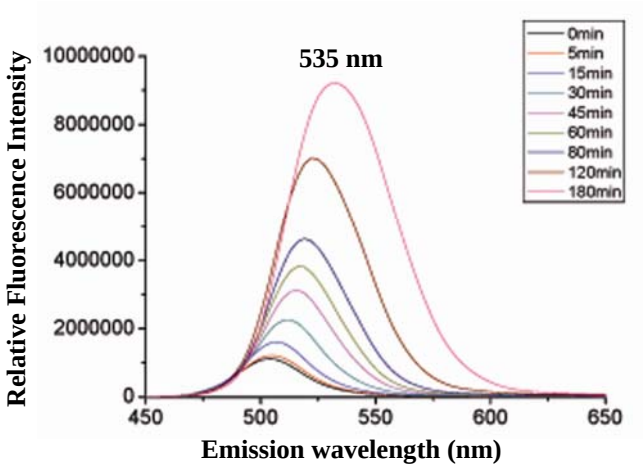

FIGURE S1. **CdTe quantum dot (QD) preparation displays photostability and symmetric emission spectra.**

A preparation of water-soluble 3-mercaptopropionic acid (MPA)-capped CdTe quantum dots (5 nm diameter) was generated using the hydrothermal method (Zhang *et al*. [2003] Preparation of stable fluorescence CdTe material by hydrothermal method. *Chem J Chinese Univ.* 24:662-665.) Briefly, sodium hydrogen telluride (NaHTe) was prepared by mixing sodium borohydride and tellurium (molar ratio of 2:1), in water, and N_2-saturated deionized water was then added to yield a final concentration of 47 mM NaHTe. Next, a precursor solution was prepared by dissolving $CdCl_2$ (1 mM) and MPA (1.2 mM) in 50 mL of deionized water; the pH value was adjusted to 9.0 by the stepwise addition of NaOH solution. Next, an aliquot (100 μL) of oxygen-free solution containing fresh NaHTe, cooled to 0◦C, was added to 10 mL of precursor solution and vigorously stirred. The resultant solution was put into a Teflon-lined stainless steel container and autoclaved at the reaction temperature for periods ranging from 5 to 180 min. Solutions containing the MPA-capped CdTe quantum dots (QDs) were then cooled, to room temperature, and the fluorescence emission spectrum measured on a Fluorolog-3 spectrofluorometer (Edison, France).

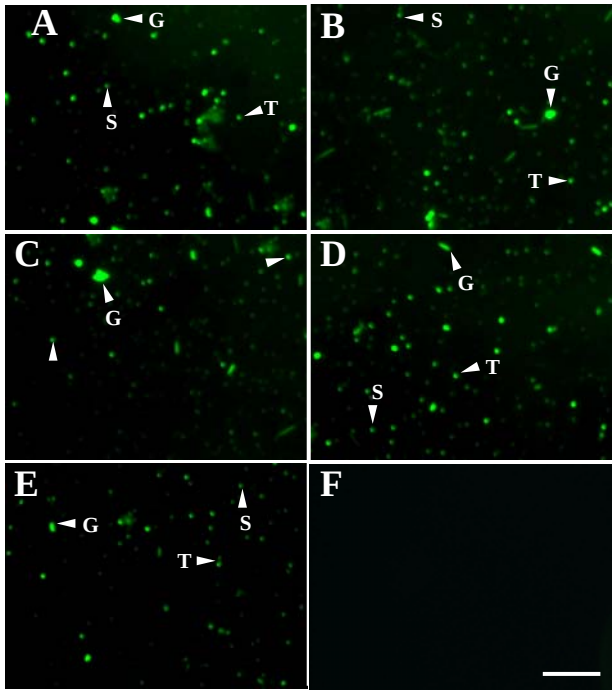

FIGURE S2. **Conjugation of test proteins to MPA-capped QDs carried out by EDC-NHS mediated coupling reactions performed on silanized slides.**

The MPA-capped QDs were treated with mercaptopropionic acid (MPA), a hydrophilic ligand, to exchange the surfactant, making CdTe water-soluble. The MPA thiol group was used as a means to conjugate the QDs to proteins (Chan and Nie [1998]: Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 281: 2016–2018; Dahan *et al*. [2003]: Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. *Science* 302:442-445; Vu *et al*. [2005]: Peptide-conjugated quantum dots activate neuronal receptors and initiate downstream signaling of neurite growth. *Nano Lett.* 5:603–607.). To this end, the cross-linking reagent, ethyl-3-(dimethylaminopropyl) carbodiimide (EDC), was used to synthesize QD-CaM, QD-BSA, QD-CmPP16, QD-CaMTR$_1$C and QD-CaMTR$_2$C luminescent probes. Cross-linking was catalyzed at room temperature by N-hydroxysuccinimide (NHS) and a suitable yield of luminescent probes was generally acquired after 4 h, except for QD-BSA which required 8 h. These bio-conjugates were then dispersed by adding β-mercaptoethanol, which neutralized any unreacted EDC and increased the stability of the QDs in aqueous solution.

To confirm that the five test proteins were conjugated to the QD nanoparticles, EDC-mediated coupling reactions were also performed on silanized slides that were coated with NHS. Slides were washed extensively, to terminate the reaction, and then observed with a Bio-Rad MRC 600 laser-scanning confocal microscope. Detection of strong fluorescence signals with QD-CaM (A), QD-CmPP16 (B), QD-BSA (C), QD-CaMTR$_1$C (*D*) and QD-CaMTR$_2$C (E) indicated that these test proteins were successfully bio-conjugated to the surface of the QDs. Note that no fluorescent signal was detected when the coupling reaction was performed in the presence of only the QDs (F), suggesting that QDs not conjugated to the peptide could be removed by washing with deionized water, thereby ensuring the absence of non-specific signals. All images are at the same magnification; scale bar = 0.5 μm. Abbreviations are as follows: S, single QD; T, twin QDs; G, group of QDs.

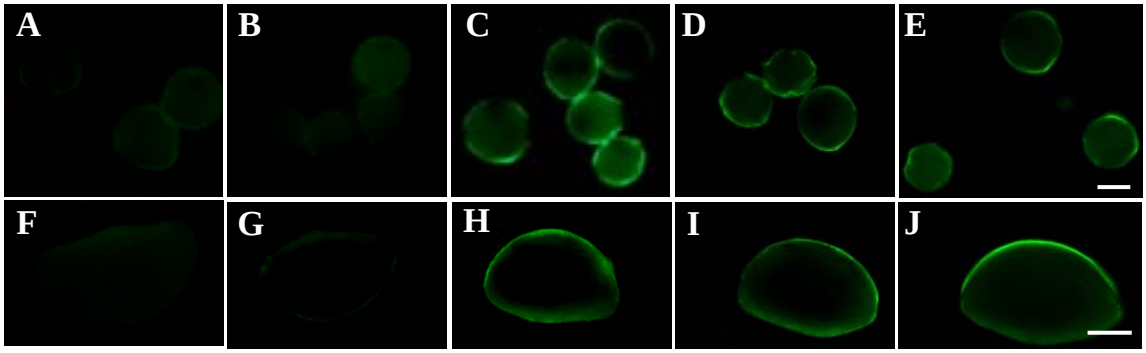

FIGURE S3. **General non-specific binding of QD−protein probes to pollen cell walls**.

Tobacco and lily pollen grains were incubated in control medium or medium supplemented with QD, or protein-conjugated QD probes. After extensive washing with control medium, pollen grains were examined under a fluorescence microscope. All images shown were obtained with the same microscopy parameters.

(A) and (B) Tobacco pollen grains incubated in medium without or with 1 μM QDs, respectively. Note the absence of fluorescent signal in (B) indicating that QD, alone, do not bind to the matrix of the cell wall.
(C) – (E) Tobacco pollen grains incubated in medium containing 1 μM QD-CaM, QD-CmPP16 or QD-BSA, respectively. Note that in all three treatments, strong fluorescent signals were detected in the pollen cell walls.
(F) and (G) Lily pollen grains incubated in medium without or with 1 μM QDs, respectively. Note the absence of fluorescent signal in (G) indicating that QD, alone, do not bind to the matrix of the cell wall.
(H) – (J) Lily pollen grains incubated in medium containing 1 μM QD-CaM, QD-CmPP16 or QD-BSA, respectively. Note that in all three treatments, strong fluorescent signals were detected in the pollen cell walls.
The magnification of (A) – (D) are the same as (E), and (F) – (I) are the same as (J); scale bars = 5 μm.

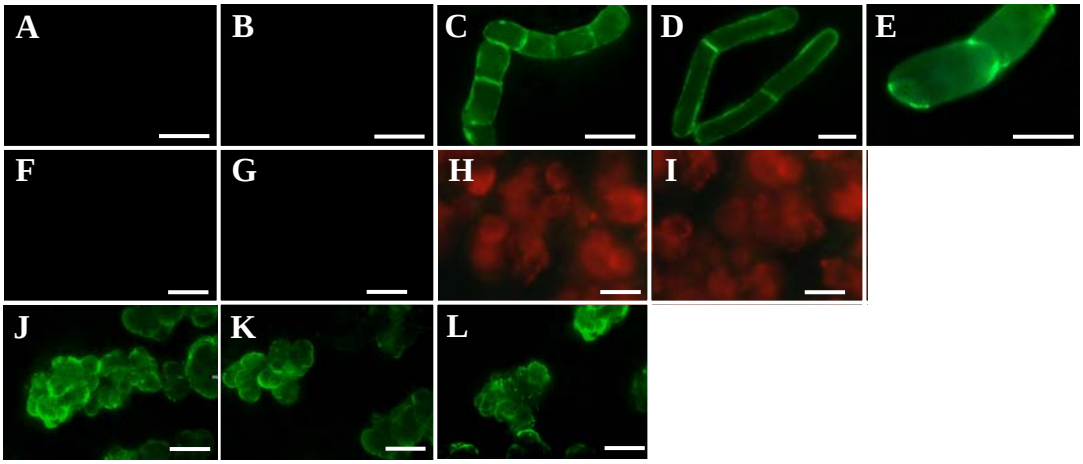

FIGURE S4. **General non-specific binding of QD-protein probes to tobacco BY-2 and *Arabidopsis thaliana* suspension-cultured cell walls.**

Tobacco BY-2 and *Arabidopsis thaliana* suspension-cultured cells were incubated in control medium or medium supplemented with QD, or protein-conjugated QD probes. After extensive washing with control medium, pollen grains were examined under a fluorescence microscope. All images shown were obtained with the same microscopy parameters.

(A) and (B) Tobacco BY-2 cells incubated in medium without or with 1 μM QDs, respectively. Note the absence of fluorescent signal in (B) indicating that QD, alone, do not bind to the matrix of the cell wall.
(C) – (E) Tobacco BY-2 cells incubated in medium containing 1 μM QD-CaM, QD-CmPP16 or QD-BSA, respectively. Note that in all three treatments, strong fluorescent signals were detected in the cell walls.
(F) and (G) *Arabidopsis* cells incubated in medium without or with 1 μM QDs, respectively. Note the absence of fluorescent signal in (G) indicating that QD, alone, do not bind to the matrix of the *Arabidopsis* cell wall.
(H) and (I) Chlorophyll autofluorescence images of the same cells as in (F) and (G).
(J) – (L) *Arabidopsis* cells incubated in medium containing 1 μM QD-CaM, QD-CmPP16 or QD-BSA, respectively. Note that in all three treatments, strong fluorescent signals were detected in the *Arabidopsis* cell walls.
Scale bars = 2.5 μm.

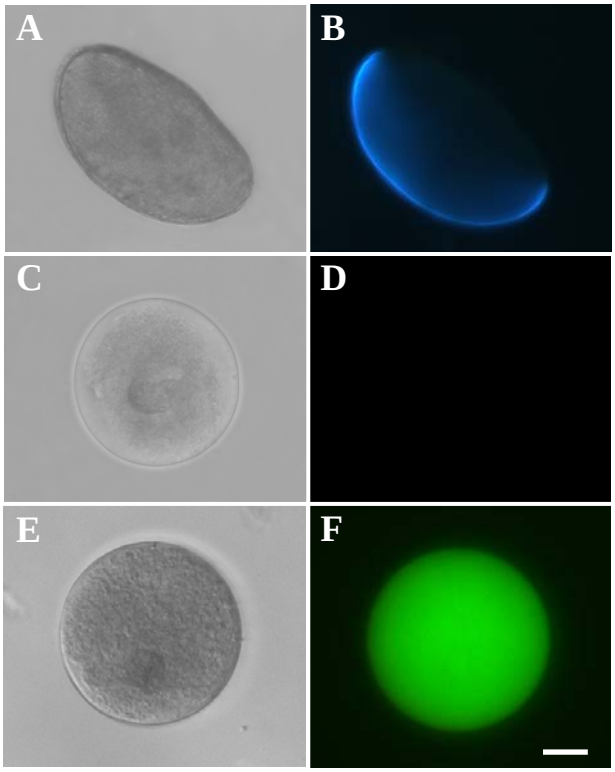

FIGURE S5. **Protoplasts as a model experimental system to test for the binding of exogenous CaM.**

Mature pollen protoplasts from lily and tobacco were isolated using the procedure of Yu *et al*. (2006). In hydrated mature pollen grains (A), the wall is heavily stained with Calcofluor White (B). Enzymatic digestion of the pollen grains for 3h completely removed the cell wall, as indicated by the absence of Calcofluor White associated fluorescence around the pollen protoplasts (C and D). Hence, pollen protoplasts isolated by this method were suitable for fluorescence studies as they are devoid of cell wall materials that could otherwise yield a spurious fluorescent signal. The viability of pollen protoplast preparations was tested using the vital stain, fluorescein diacetate (FDA). Following a 20 min incubation of protoplasts in FDA, most lily (F) and tobacco pollen protoplasts were found to emit a bright green fluorescent signal, indicative of physiologically competent cells and were thus deemed suitable for CaM binding studies.

(A), (C) and (E) Lily pollen grains and protoplasts imaged under bright field microscopy. (B), (D) and (F) Lily pollen grains and protoplasts imaged under fluorescence microscopy. All images are at the same magnification; scale bar = 5 μm.

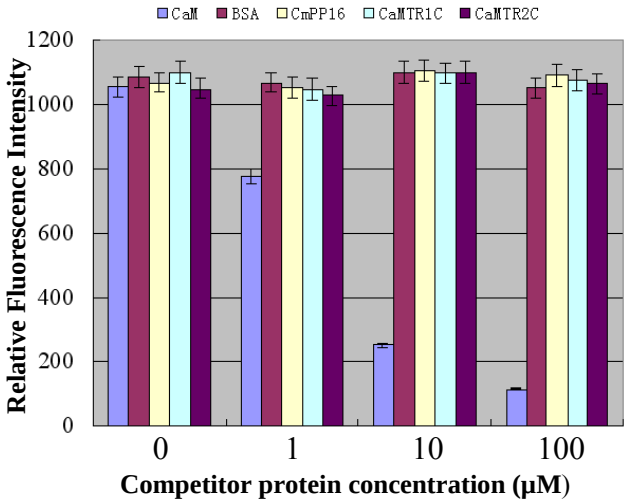

FIGURE S6. **Competition assays establish the specificity of QD-CaM binding to the surface of lily pollen protoplasts.**

Equal quantities of lily pollen protoplasts were either incubated in medium containing 1 μM QD-CaM probe, or were preincubated with 0, 1, 10 or 100 μM CaM, BSA, CmPP16, CaM TR_1C or CaM TR_2C separately, then transferred to medium containing 1 μM QD-CaM probe. Protoplast-associated fluorescence was quantified by fluorimetry, using an F-4500 spectrofluorometer (Hitachi, Japan).

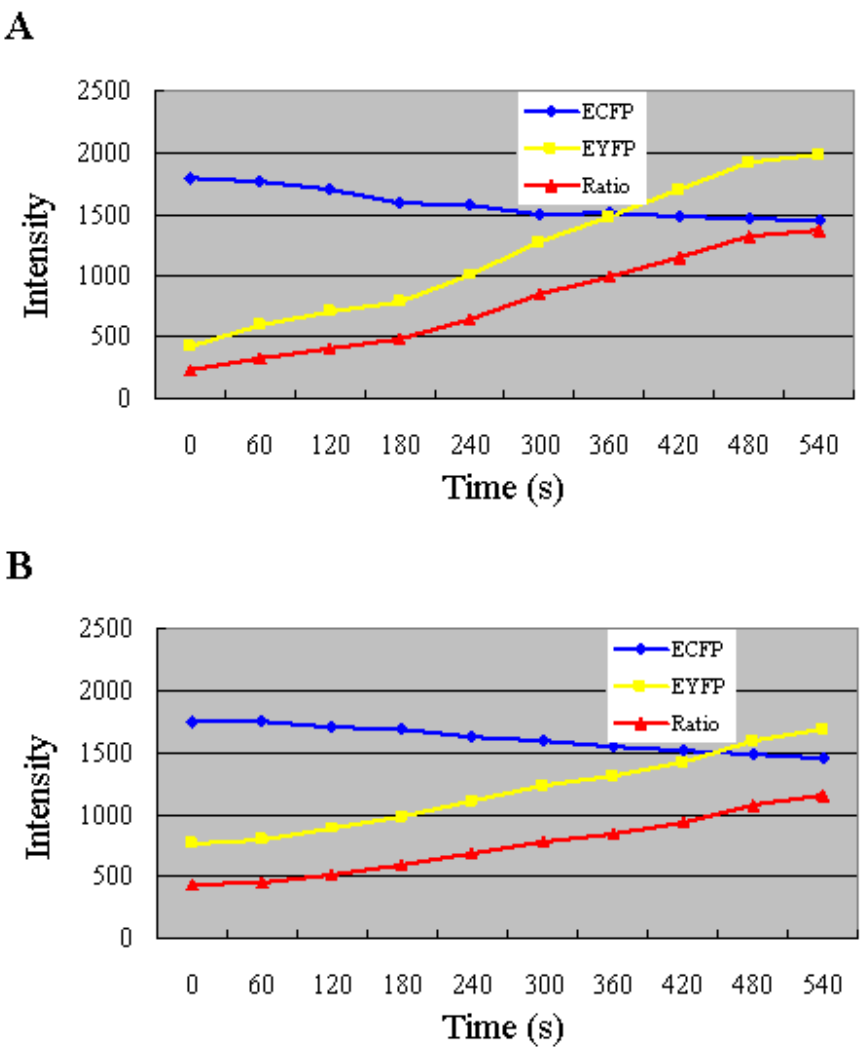

FIGURE S7. **Changes in ECFP and EYFP fluorescence following treatment of lily pollen protoplasts with 1 µM CaM (A) or 1 µM QD-CaM (B).**

FRET ratios were calculated from the data presented in Fig. 6 using imageJ software (http://rsbweb.nih.gov), as the ratio of EYFP to ECFP intensity, according to the following formula: ratio = 1000 x·EYFP/ECFP.