Extracellular Ca^{2+}-sensing fluorescent protein biosensor based on a collagen-binding domain

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Supplementary figures S1-S7

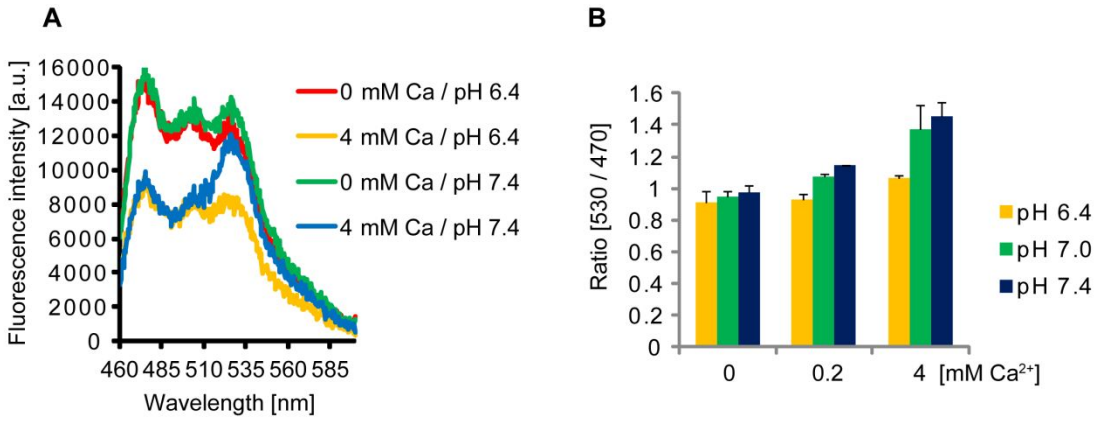

Figure S1. pH sensitivity of ColBD-Twitch protein. The protein was diluted to 0.07 μM in buffers with different pH and concentrations of $CaCl_2$ as indicated and measured on a microplate reader at 470 nm and 530 nm emission wavelengths, and in spectral emission scan modes. A: pH-dependence of emission spectra at 0 and 4 mM $CaCl_2$. B: pH-dependent ratio responses. N=3.

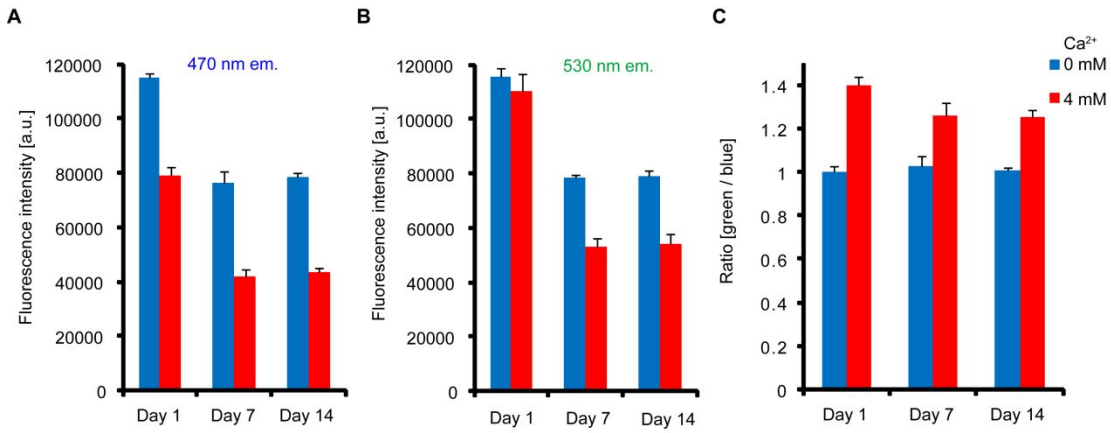

Figure S2. Storage stability of ColBD-Twitch protein. Sample was diluted to 0.1 μM, measured on a microplate reader immediately or after storing at 4 °C for 7 and 14 days. A, B: Fluorescence intensity at different wavelengths. C: Ratio (530/ 470 nm). N=3.

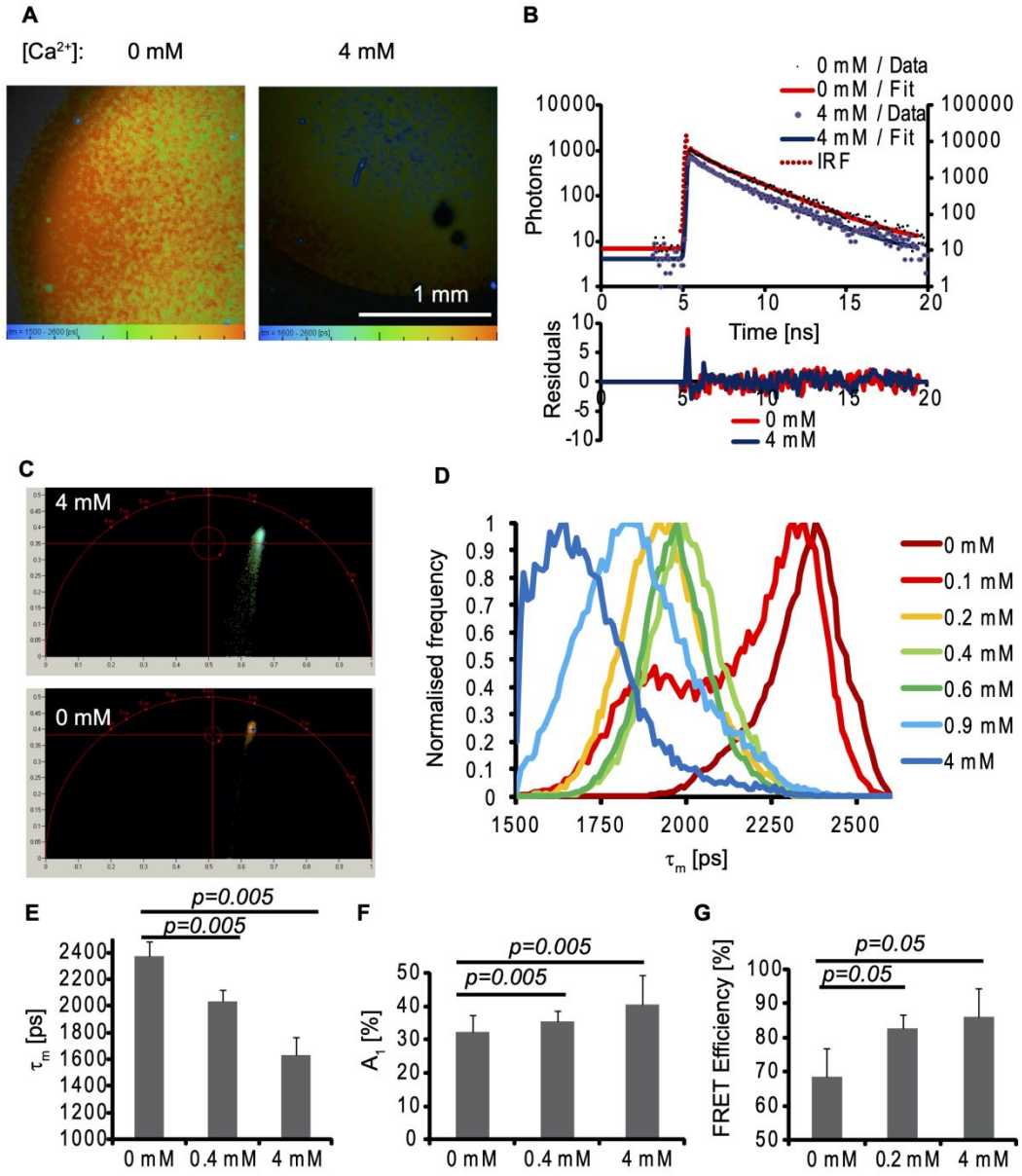

Figure S3. Biosensing properties of ColBD-Twitch protein measured on a confocal FLIM microscope. ColBD-Twitch-stained (1 μM, 3 h) Matrigel matrix was used for measurements at various concentrations of $CaCl_2$ (37 °C). A: Examples of FLIM images for donor channel (405 nm exc., 468 nm em., double-exponential fit, τ_m) of stained Matrigel. B: Example of double-exponential decay fitting. C: Examples of Phasor diagrams. D: Normalized fluorescence lifetime frequency distribution histograms for FLIM images produced at 0-4 mM $CaCl_2$. E, F, G: comparisons of τ_m (double-exponential fit), A_1 fraction (%) and FRET efficiency [%] in response to Ca^{2+} binding.

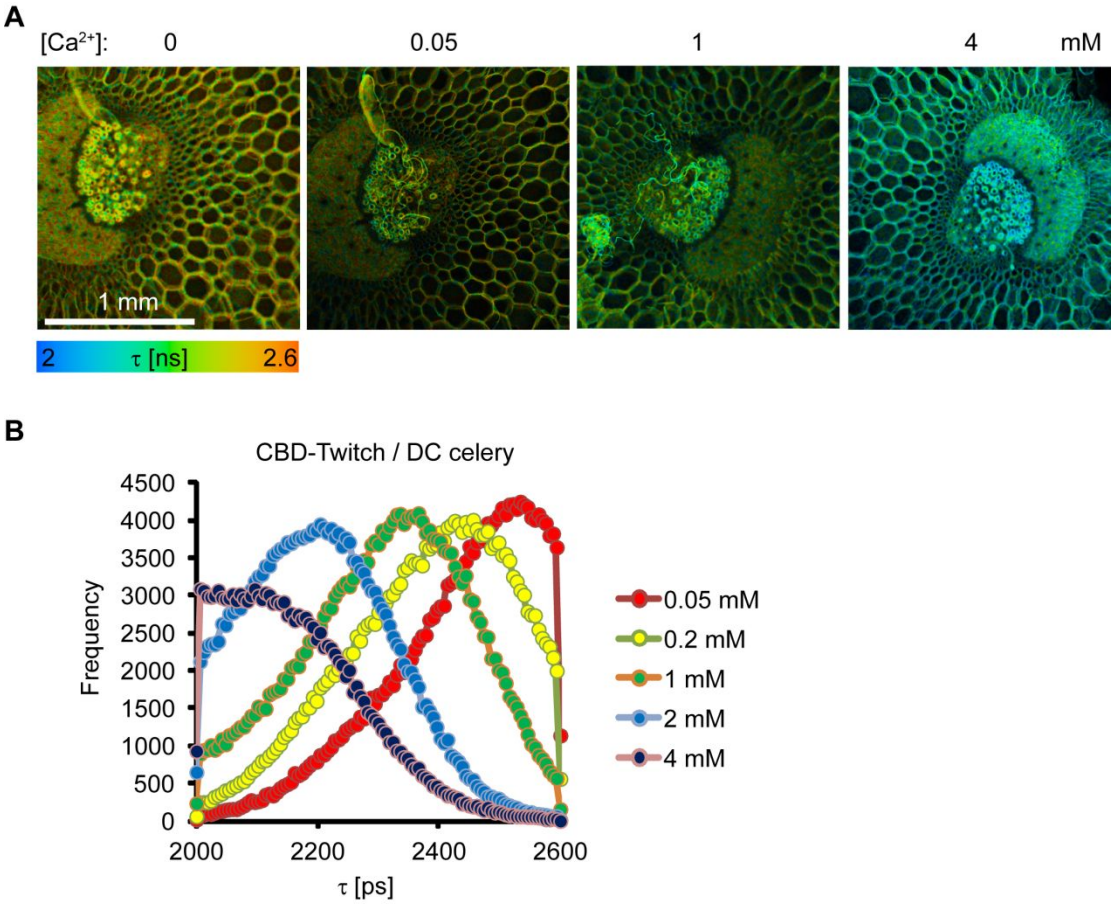

Figure S4. **Ca^{2+}-dependent FLIM response** of decellularized celery matrix stained with CBD-Twitch protein (1 μM in HBSS, overnight incubation) and measured in the presence of different concentrations of $CaCl_2$. Examples of FLIM images (A) and normalized lifetime frequency distribution histograms (B).

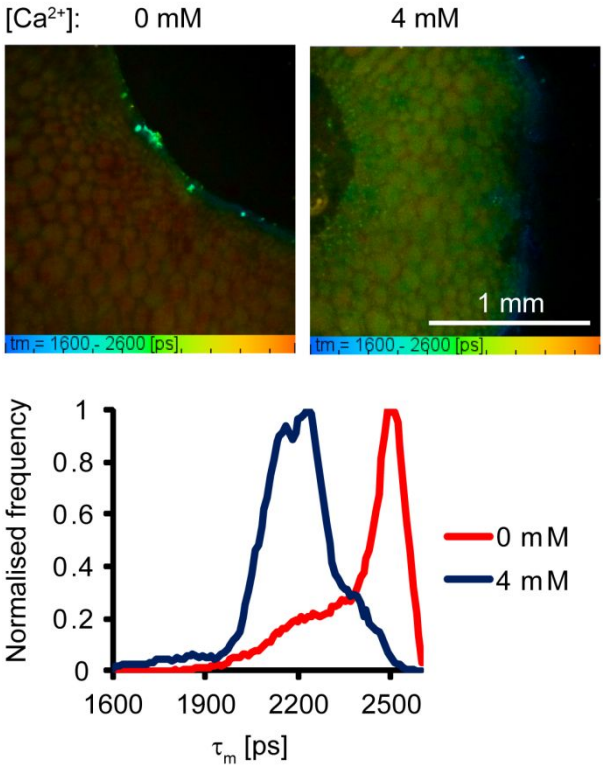

Figure S5. Ca^{2+}-dependent FLIM response with decellularized celery matrix pre-coated with Collagen IV (0.1%, overnight incubation) and stained with ColBD-Twitch protein (1 μM, overnight). Produced matrix was subsequently imaged on FLIM (405 nm exc., 468 nm em.) at 0 and 4 mM $CaCl_2$ in PBS. Below: normalized fluorescence lifetime distribution histograms.

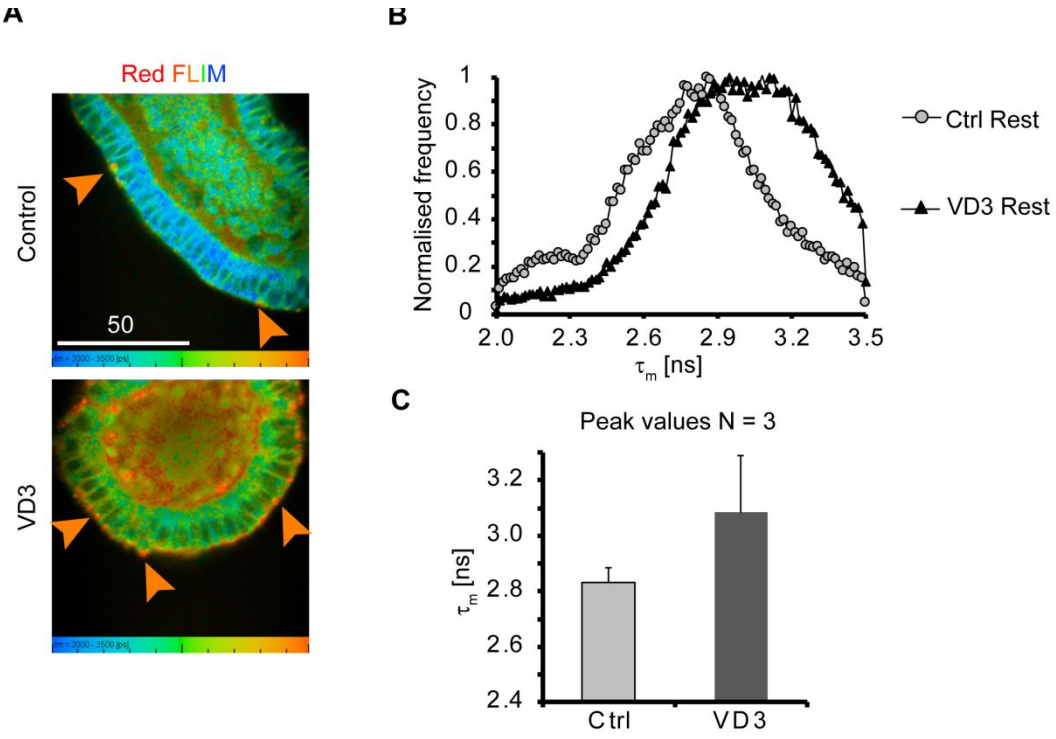

Figure S6: Live confocal FLIM of Nile Red-stained intestinal organoids shows Ca^{2+} and VD3-dependent responses. Organoids grown in presence of VD3 (100 nM, 3 d) were treated with 2.5 mM EGTA. A: FLIM images in red fluorescence (565-605 nm emission of Nile Red) channel. B, C: Lifetime distribution histograms and comparison of peak values for different experimental conditions. Scale bar is in μm.

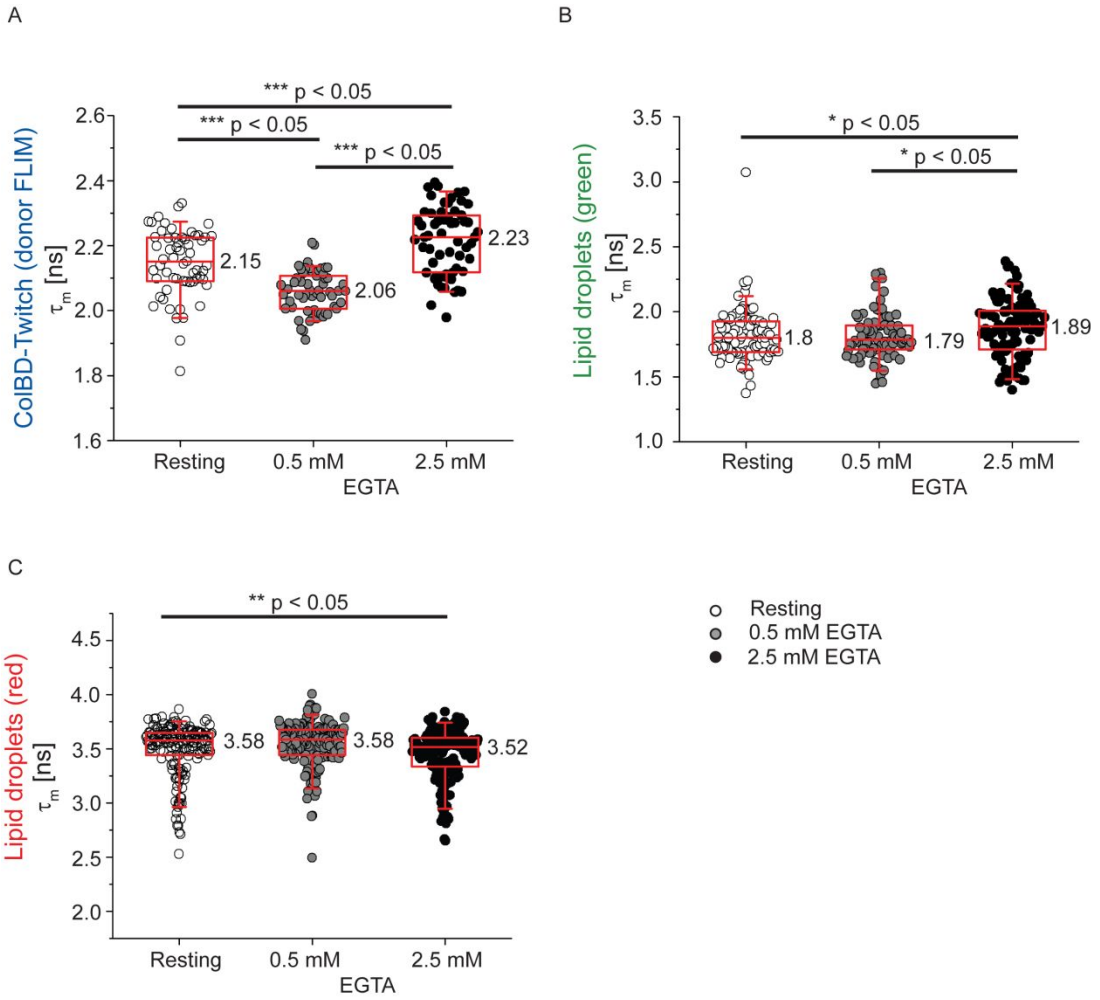

Figure S7. 'General statistical analysis' of fluorescence lifetimes for donor FLIM channel (Matrix) measured with ColBD-Twitch (a), green (b) and red (c) spectral fractions of Nile Red measured and calculated at rest and different concentrations of EGTA added to the culture of live intestinal organoids (**second experiment**). Data distributions correspond to the average lifetime values calculated from ROIs taken from 6 organoid images per each condition. P values indicate statistical significance (Mann-Whitney test): * - p<0.05, ** - p<0.005 and ***- p<0.0005. Box charts correspond to median (values shown in numbers), 25 and 75 percentiles. Whiskers show 5 and 95 percentiles.