Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary material to

Viable protoplast formation of the coral endosymbiont alga Symbiodinium spp. in a microfluidics platform

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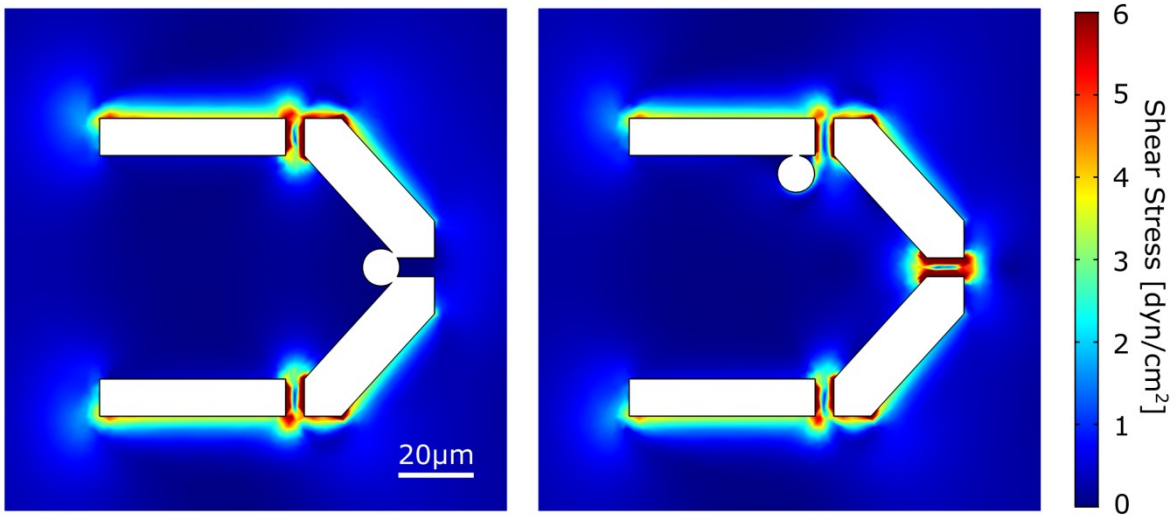


Fig. S1 Calculated shear stress within the largest traps containing cells. Calculations were done for 60 µL/h overall flow rate in the device.

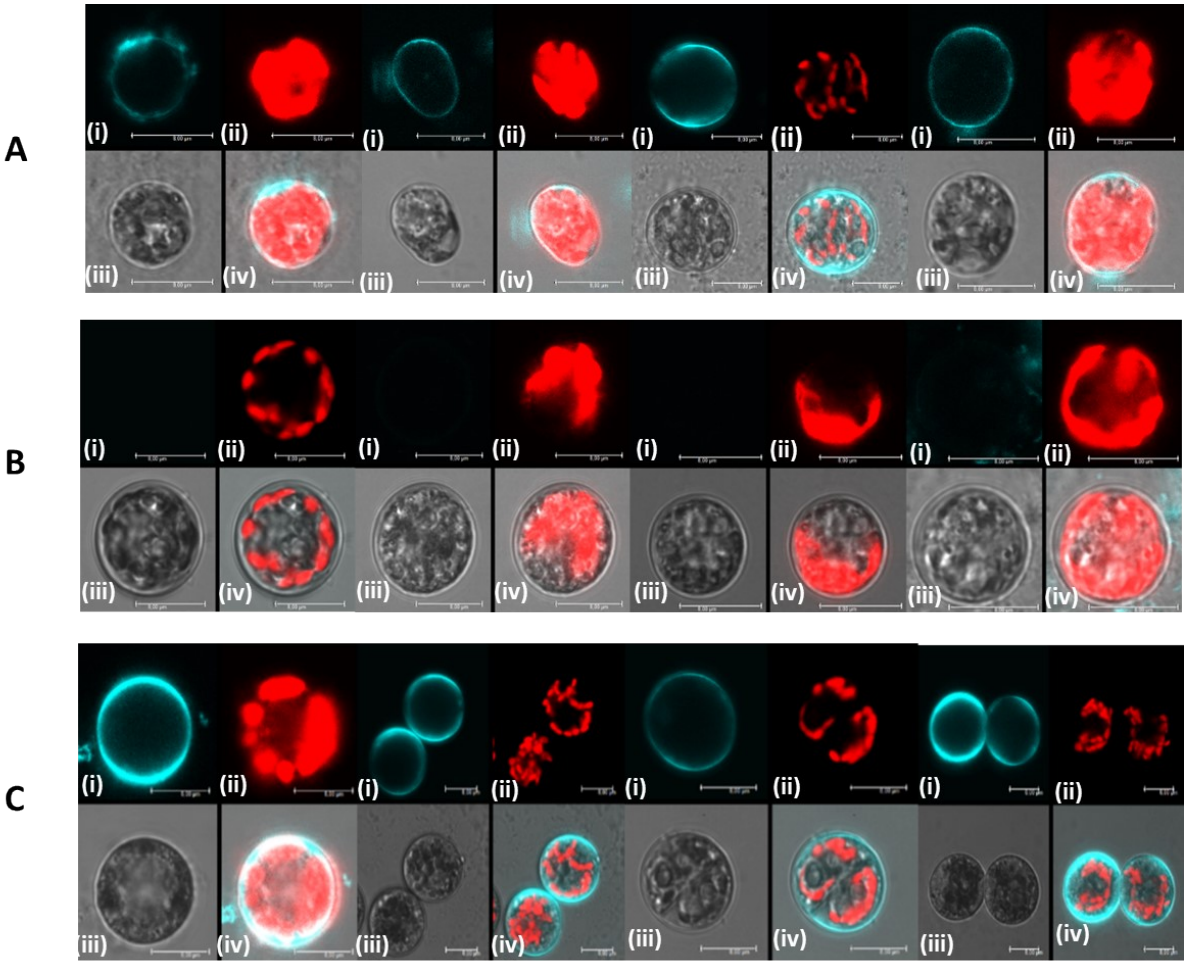
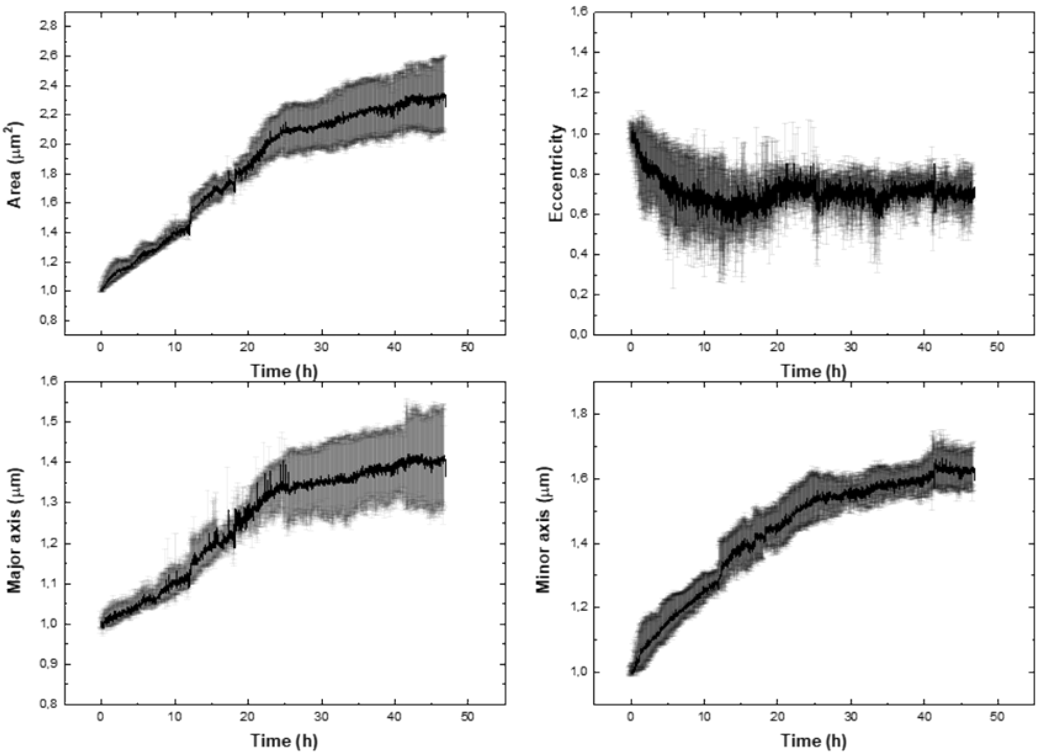
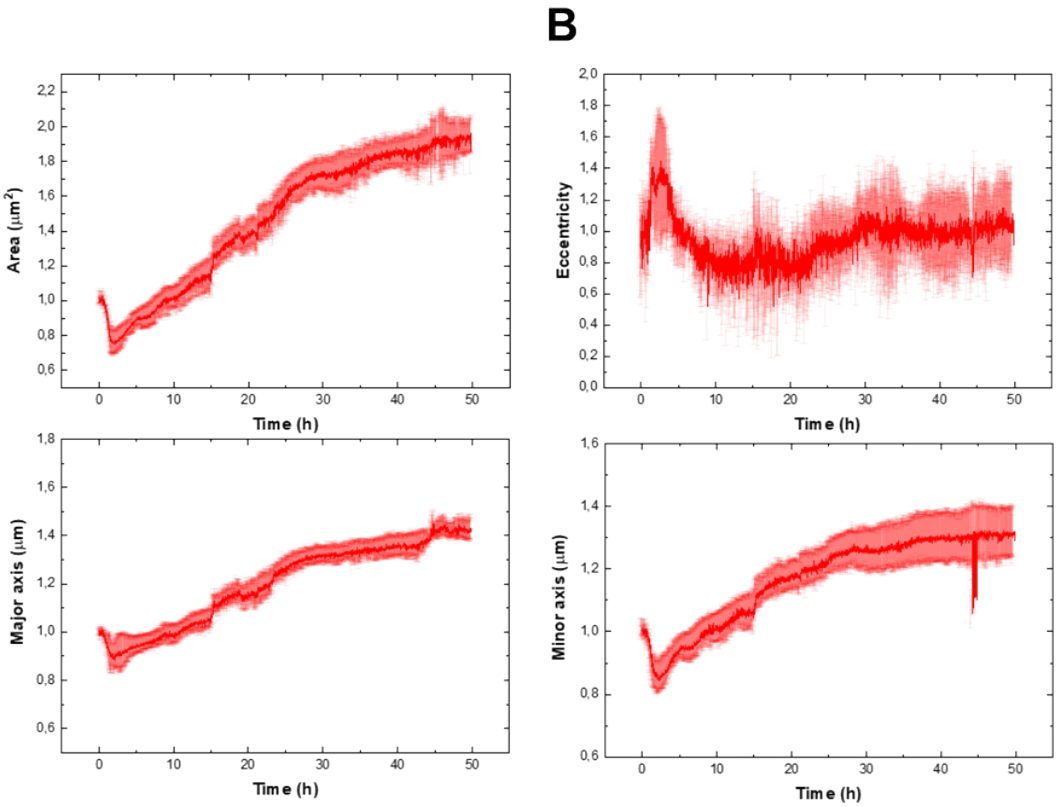
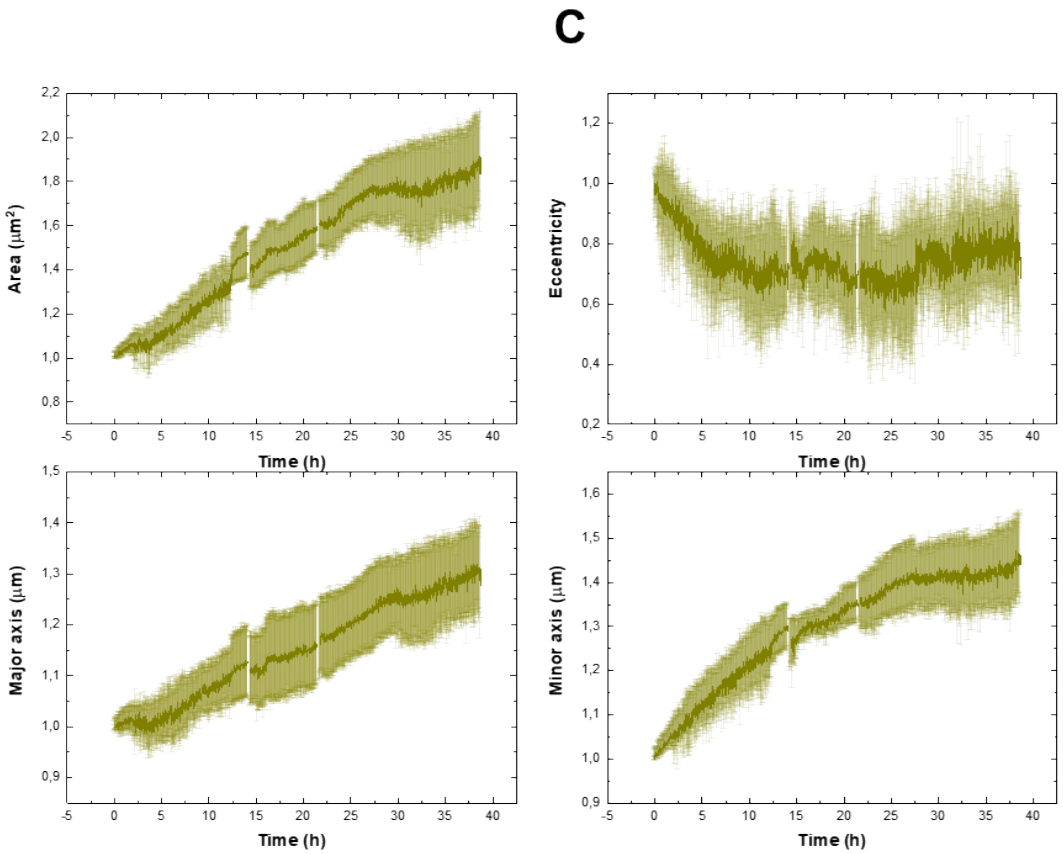


Fig. S2 (A) Control cells stained with CFW (B) Protoplast stained with CFW (C) CFW staining after regeneration of cell wall (In each panel, 1st channel (i) shows CFW fluorescence, 2nd (ii) is chlorophyll fluorescence, 3rd (iii) is transmission and 4th (iv) is merged image). Scale bar represents 8 µm.

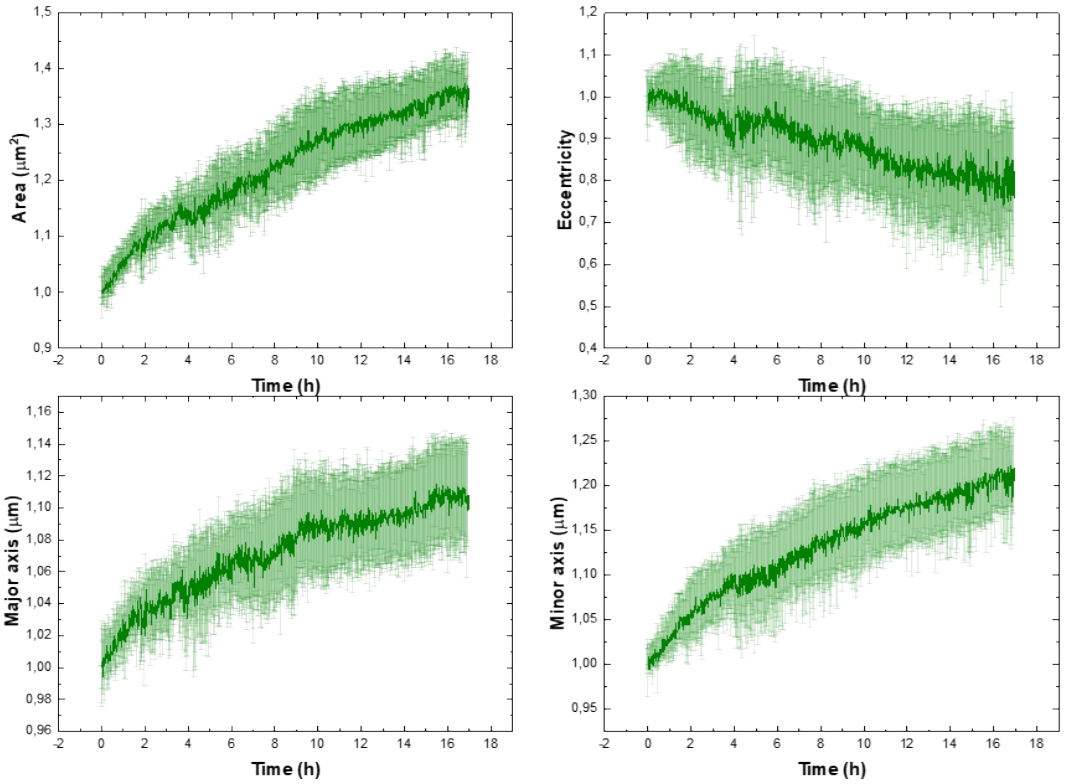
A


Area (μm²)
Time (h)
Eccentricity
Time (h)
Major axis (μm)
Time (h)
Minor axis (μm)
Time (h)


B
Area (μm²)
Time (h)
Eccentricity
Time (h)
Major axis (μm)
Time (h)
Minor axis (μm)
Time (h)


C
Area (μm²)
Time (h)
Eccentricity
Time (h)
Major axis (μm)
Time (h)
Minor axis (μm)
Time (h)

D


Area (μm²)
Time (h)
Eccentricity
Time (h)
Major axis (μm)
Time (h)
Minor axis (μm)
Time (h)

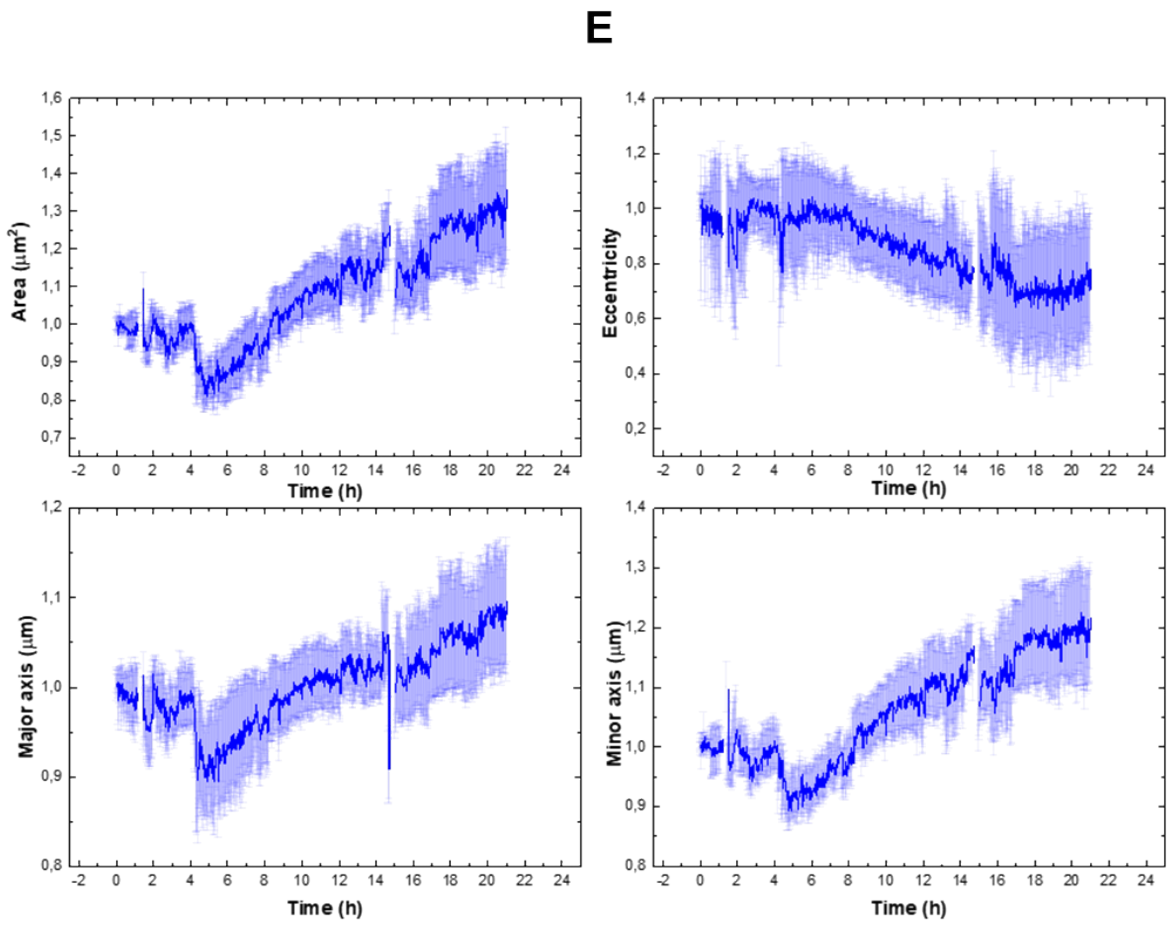


Fig. S3 The time course of the relative changes in area, eccentricity, major and minor axis of protoplast at different flow rates determined with morphometric analysis of protoplast by using Matlab software. (A) morphology changes under 20 µL/h flow rate, (B) morphology changes under 40 µL/h flow rate, (C) morphology changes under 60 µL/h flow rate, (D) morphology changes under 80 µL/h flow rate, (E) morphology changes under 100 µL/h flow rate (mean ± S.D., n = 10). The plateau phase in each flow rates shows the stage when protoplast formation is completed and cells attained maximum increase in diameter and eccentricity. With 20 µL/h flow rate the process of protoplast formation completed in approx. 40 h, with 40 µL/h flow rate protoplast formation occurred in 35 h, while with 60 µL/h, 80 µL/h and 100 µL/h, the protoplast formation occurred in 30 h, 18 h and 15 h respectively.

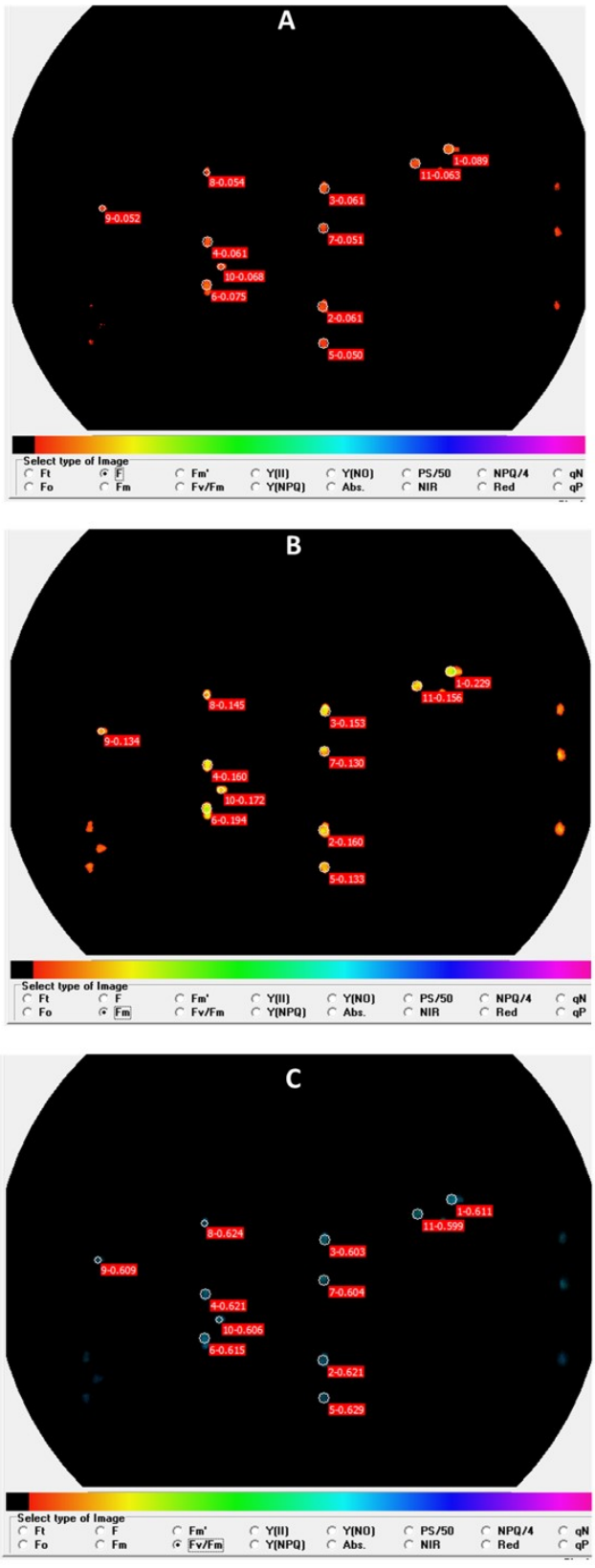


Fig. S4 Original microscope PAM images of F_0 (A), F_m (B) and F_v/F_m (C) of intact *Symbiodinium* cells.

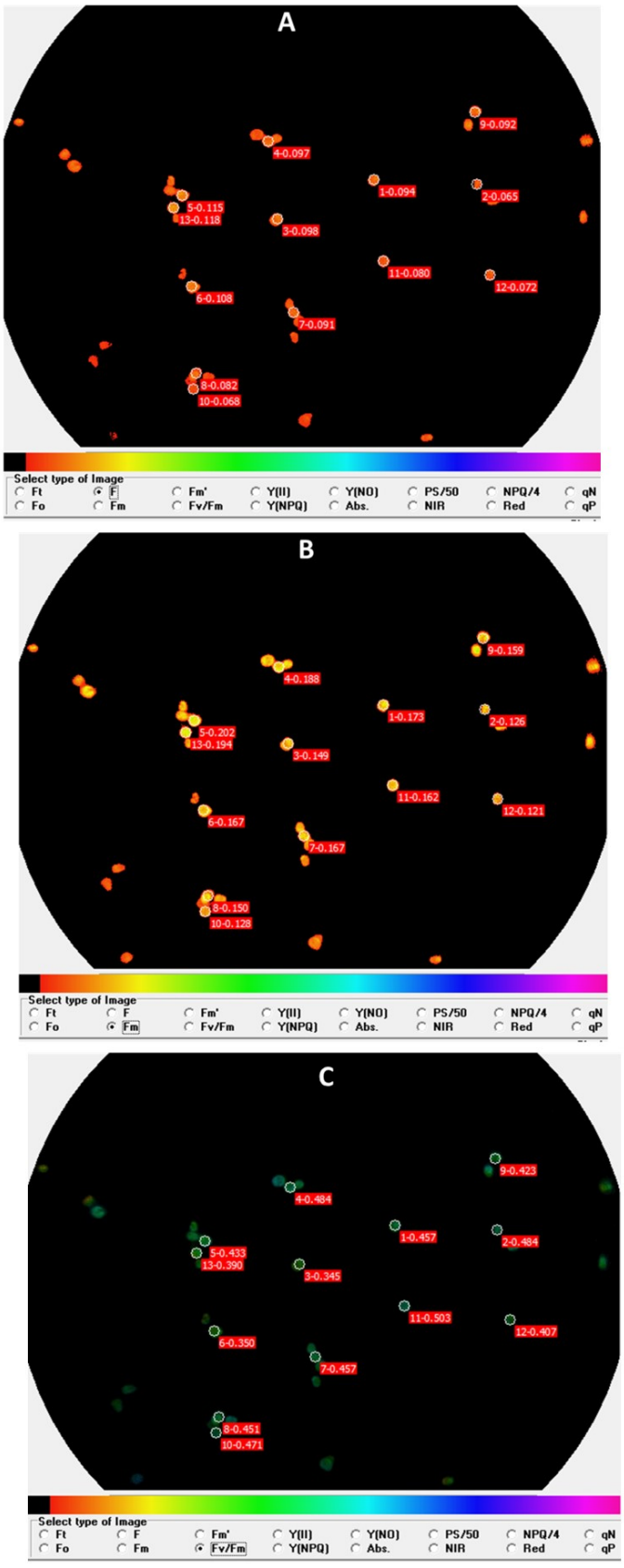


Fig. S5 Original microscope PAM images of F_0 (A), F_m (B) and F_v/F_m (C) of *Symbiodinium* protoplasts

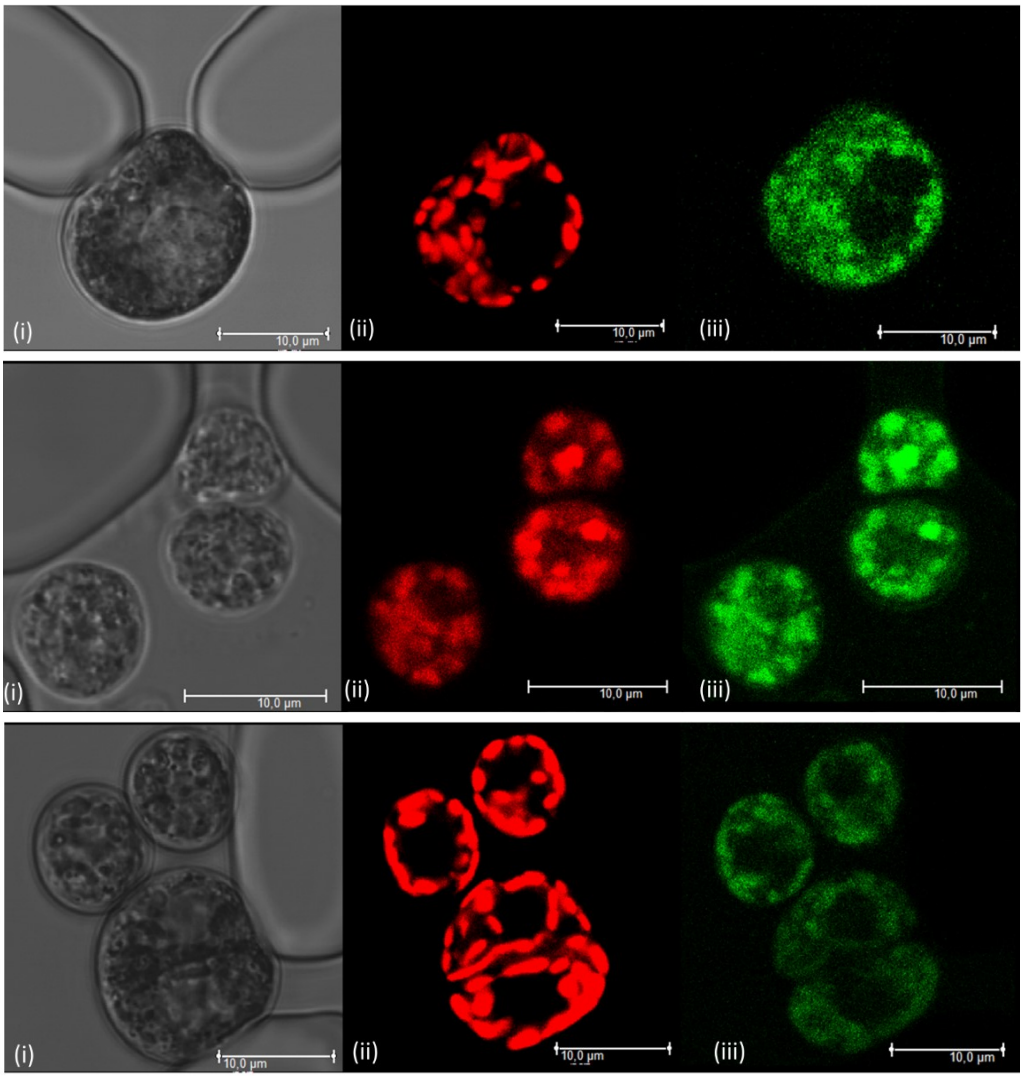


Fig. S6 More examples of co-localization of Chl and SOSG fluorescence using LSM imaging of SOSG stained protoplasts. (i) transmission image; (ii) chlorophyll fluorescence obtained in non-illuminated cells; (iii) SOSG fluorescence image obtained after illumination with 2300 µmol photons m^{-2} s^{-1} light for 5 min. Scale bar represents 10 µm.

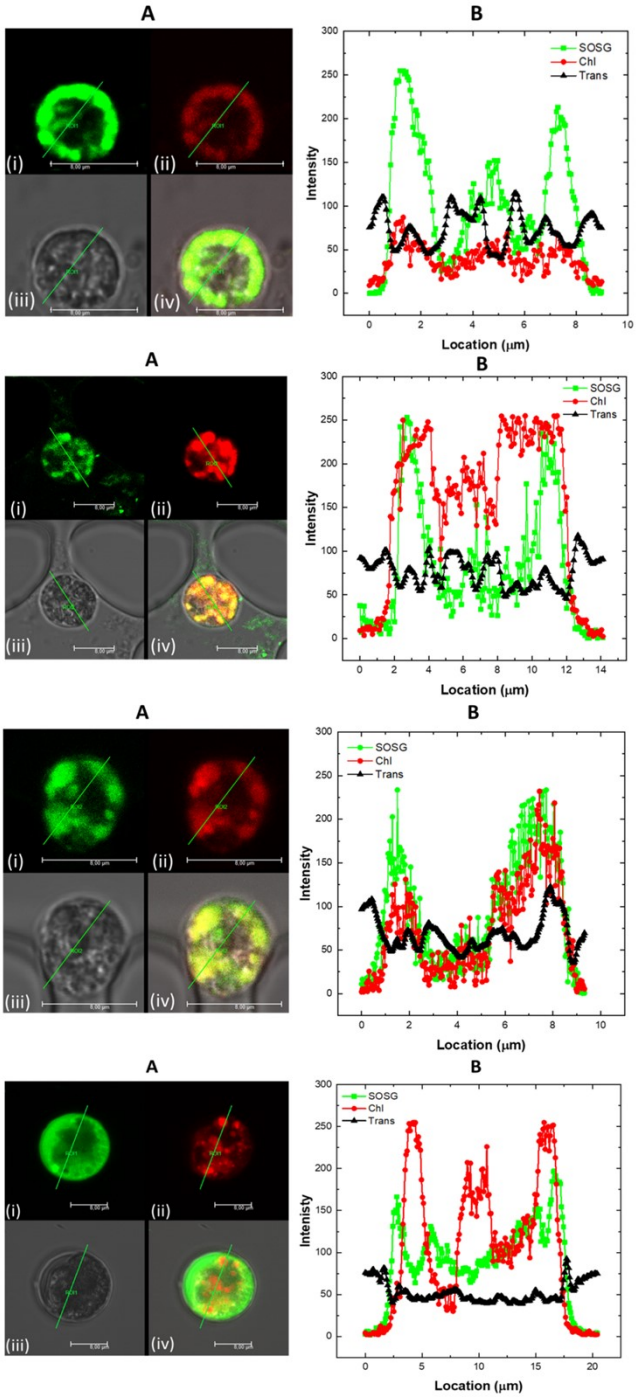


Fig. S7 Confocal microscopy imaging of *Symbiodinium* protoplast stained with singlet oxygen sensor green (SOSG) and illuminated with light showing SOSG fluorescence co-localized with chlorophyll fluorescence. Four detection channels are represented: (i) SOSG fluorescence; (ii) chlorophyll (Chl) fluorescence; (iii) transmission; (iv) merged images of the three signals (scale bar 8 μm). (A) Laser scanning microscopy (LSM) imaging of protoplast which were exposed to light (2300 μmol photons m^{-2} s^{-1}) for 5 min. (B) showing the intensity distribution of the three detection channels at the indicated transect lines for (i), (ii) and (iii) respectively. Scale bar represents 8 μm.

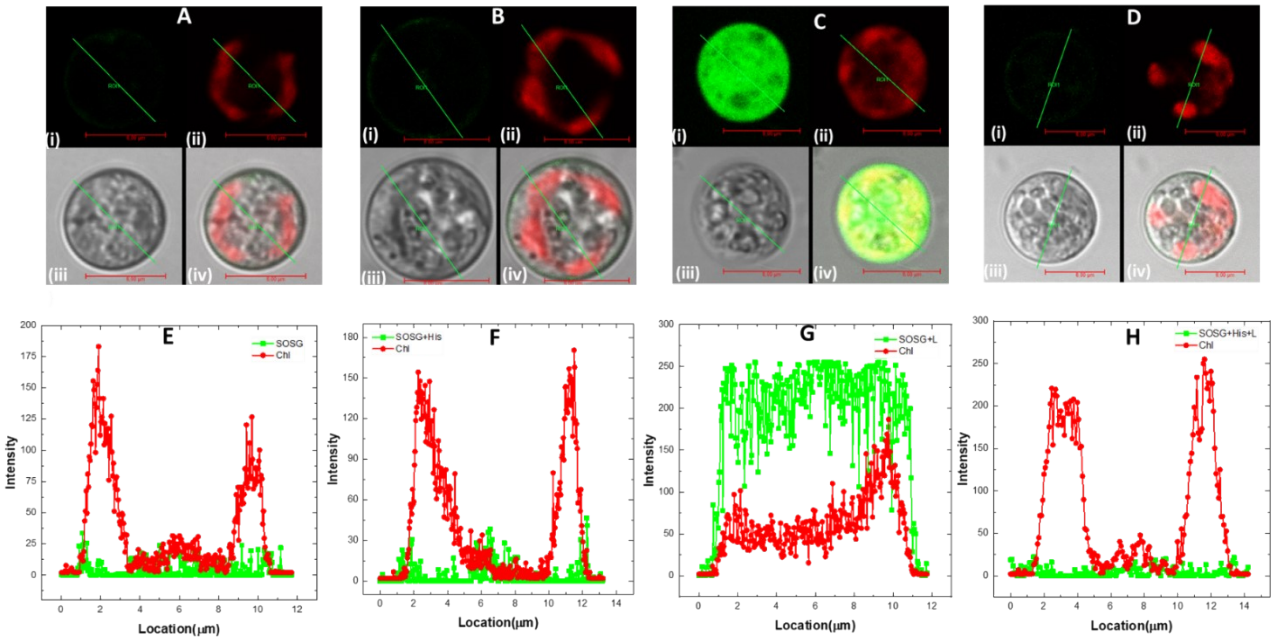


Fig. S8 Confocal microscopy imaging of *Symbiodinium* protoplast stained with singlet oxygen sensor green (SOSG) with and without histidine (His). Four detection channels are represented: (i) SOSG fluorescence; (ii) chlorophyll (Chl) fluorescence; (iii) transmission; (iv) merged images of the three signals. Laser scanning microscopy (LSM) imaging of (A) SOSG stained protoplast without light treatment, (B) SOSG+His stained protoplast without light treatment, (C) SOSG stained protoplast which were exposed to light (2300 µmol photons m^{-2} s^{-1}) for 5 min, (D) SOSG+His stained protoplast which were exposed to light (2300 µmol photons m^{-2} s^{-1}) for 5min. (E), (F) (G) and (H) show the intensity distribution of the four detection channels at the indicated transect lines for (A), (B), (C), and (D), respectively. Scale bar represents 8 µm.

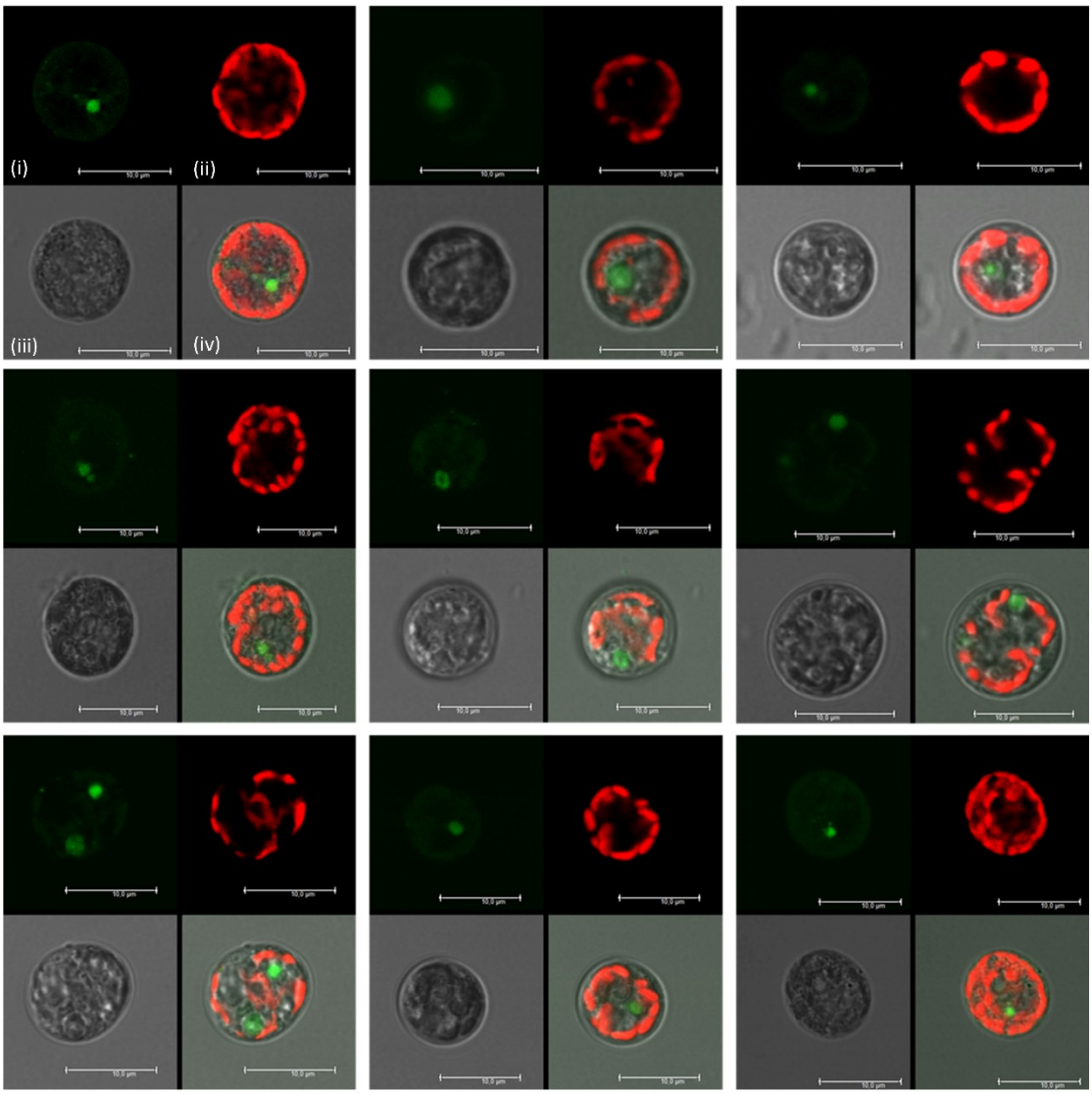


Fig. S9 LSM imaging of FAM-ODN labeling of *Symbiodinium* protoplast. (i) FAM (green) fluorescence; (ii) chlorophyll (Chl) fluorescence; (iii) transmission; (iv) merged images of the three signals. Scale bar represents 10 μm.

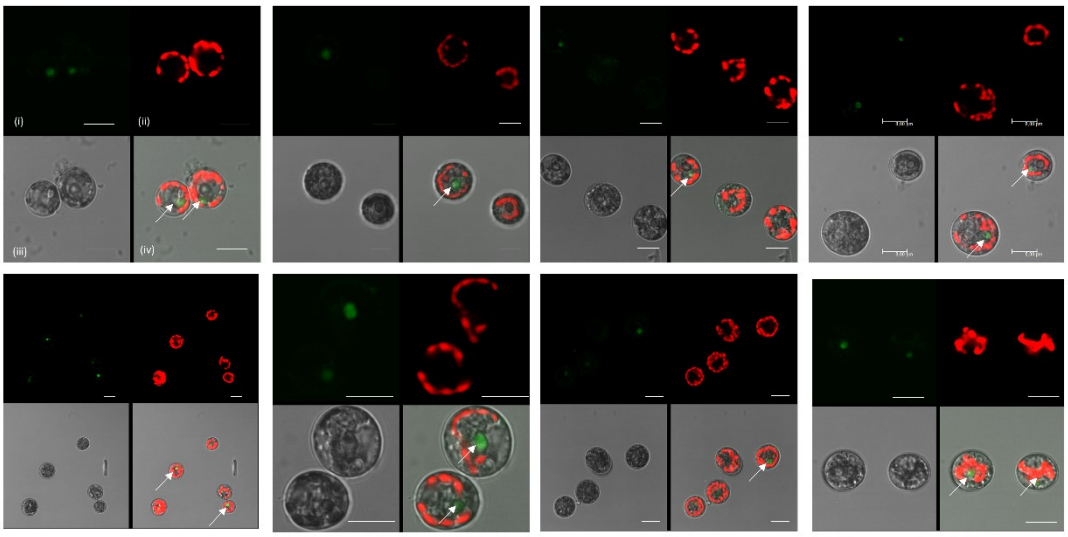


Fig. S10 LSM imaging of FAM-ODN labeling of several *Symbiodinium* protoplasts. (i) FAM (green) fluorescence; (ii) chlorophyll (Chl) fluorescence; (iii) transmission; (iv) merged images of the three signals. White arrows in the merged images indicate the green spot of FAM fluorescence. Scale bar represents 8 μm.

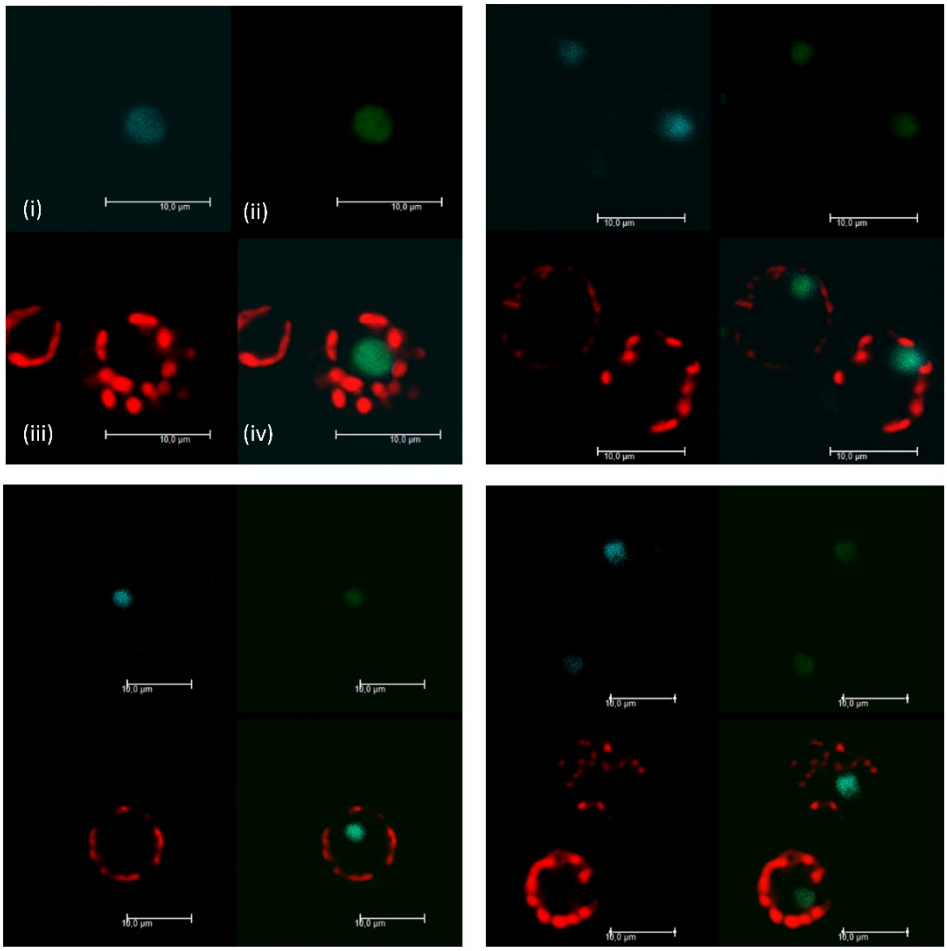


Fig. S11 Co-localization of FAM-ODN fluorescence and DAPI fluorescence in *Symbiodinium* protoplasts. (i) DAPI (blue) fluorescence, (ii) FAM (green) fluorescence; (iii) chlorophyll (Chl) fluorescence; (iv) merged images of the three signals. The applied concentration of the DAPI dye was 10 µg/ml. Scale bar represents 10 µm.