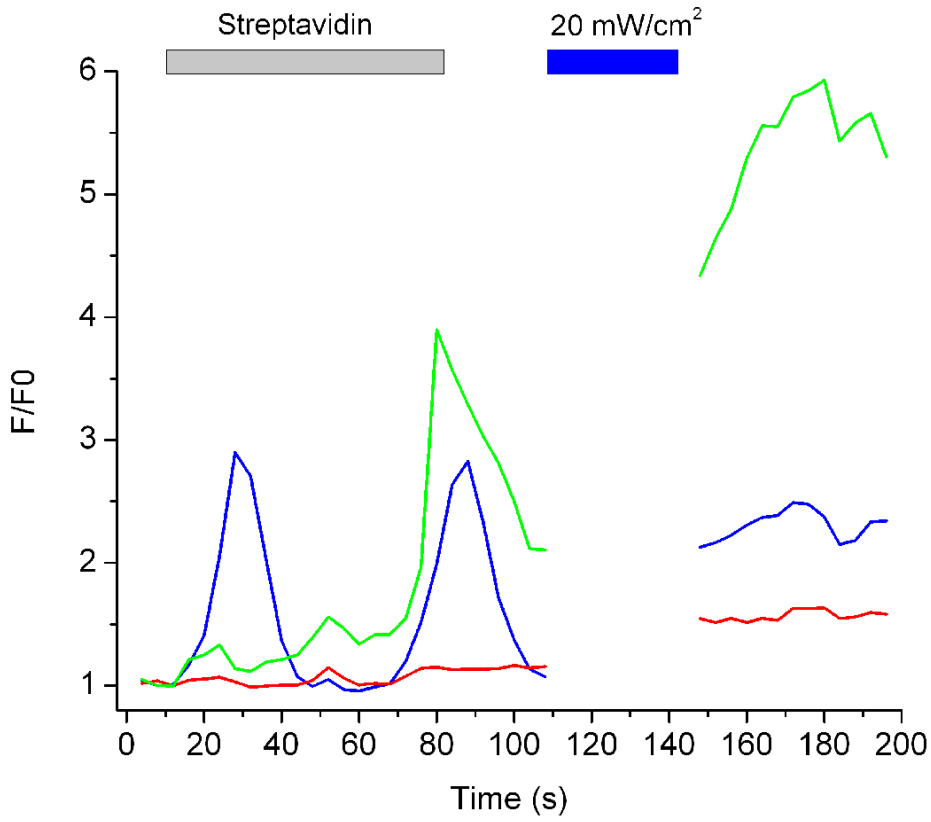
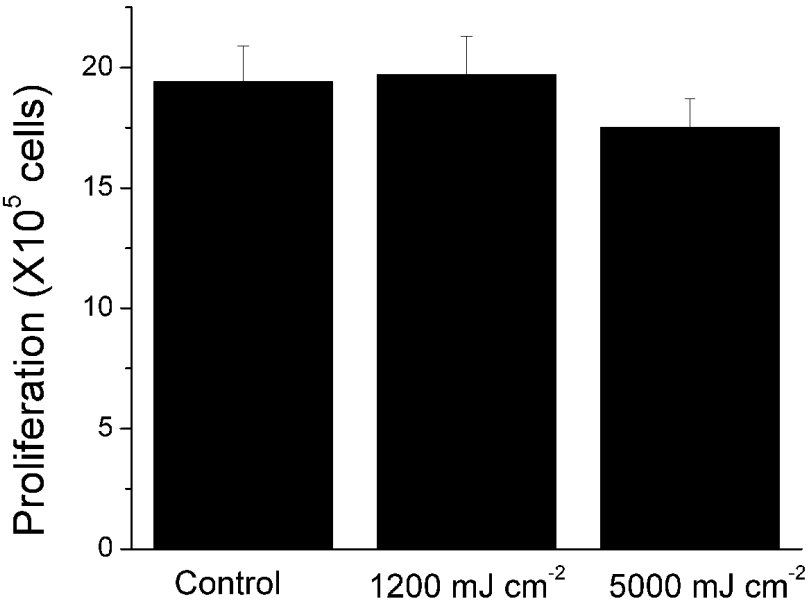
## Supplementary Information

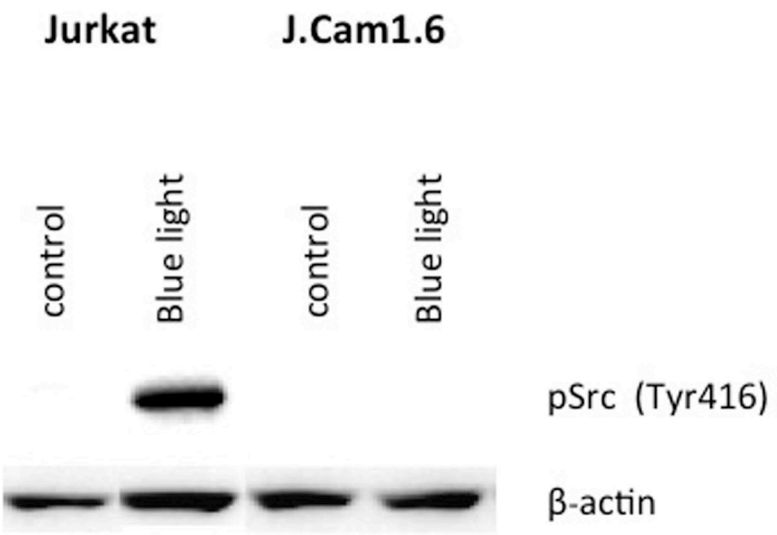
"Intrinsic Photosensitivity Enhances Motility of T Lymphocytes" by Phan X. Thieu, Barbara Jaruga, Sandeep C. Pingle, Bidhan C. Bandyopadhyay, & Gerard P. Ahern



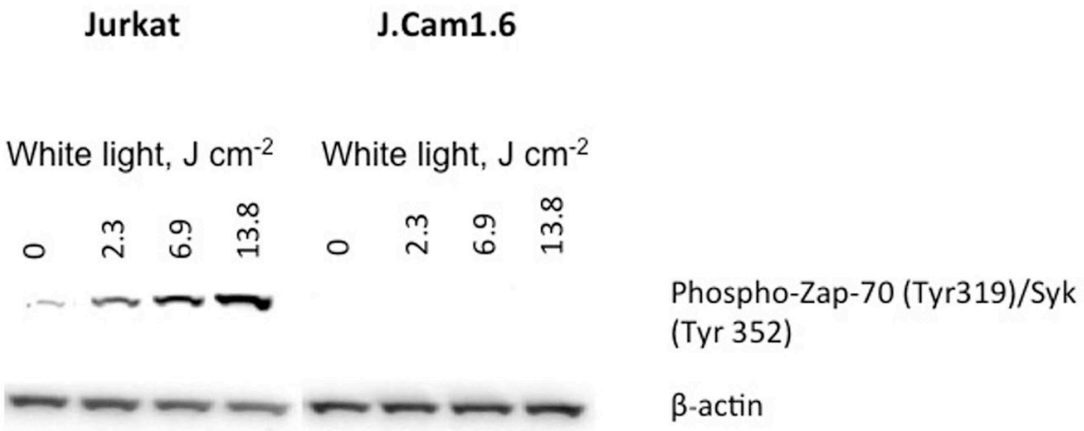

**Supplementary Figure 1. Comparison of $Ca^{2+}$ responses evoked by T cell receptor mediated signaling and blue light irradiation.** Mouse CD4+ T cells were incubated with 5 μg/ml biotinylated anti-CD3 antibody (BD Biosciences) for 10 min at RT followed by cross-linking with 5 μg/ml streptavidin (Pierce Chemical Co.) as indicated. A 30 s pulse of blue light (20 mW $cm^{-2}$) was used to trigger photosignaling. $Ca^{2+}$ imaging was performed in Fluo4 loaded cells. The dye was excited with low intensity blue light (0.8 mW $cm^{-2}$)




**Supplementary Figure 2. Light treatment does not affect T cell proliferation.** Mouse splenocytes were stimulated with plate bound anti-CD3 antibody (10 μg/ml) for 3 days with or without daily irradiation with full spectrum light. Cell were counted using MTT assay.

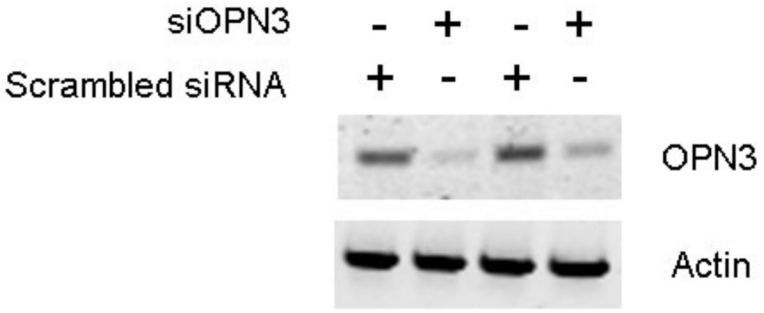



**Supplementary Figure 3. Lck activation by light in Jurkat and Lck-null Jurkat cells**. Blue light induces phosphorylation of Src kinase as detected with a generic pSrc antibody (mouse Y416). The prominent immunoreactive band is absent in J.Cam1.6 (Lck-null) cells confirming predominant activation of Lck.

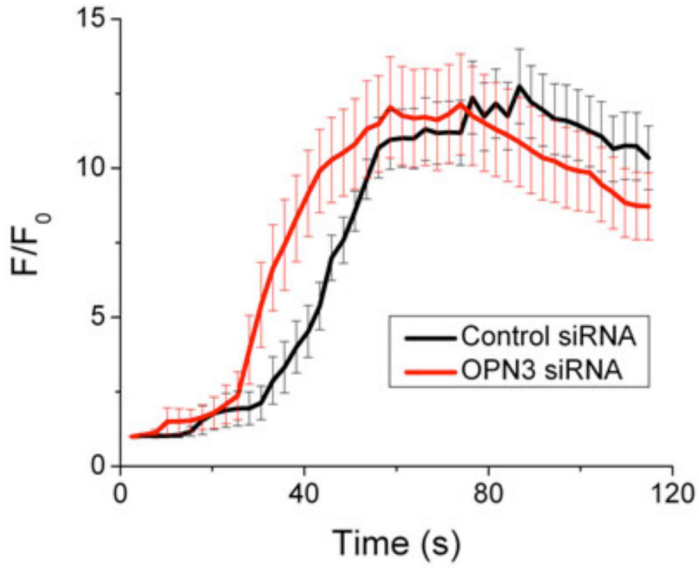



**Supplementary Figure 4. White light activates Zap-70 in a Lck-dependent manner**. Induction of phospho-Zap-70 by white light in Jurkat and J.Cam1.6 (Lck-deficient) cells.

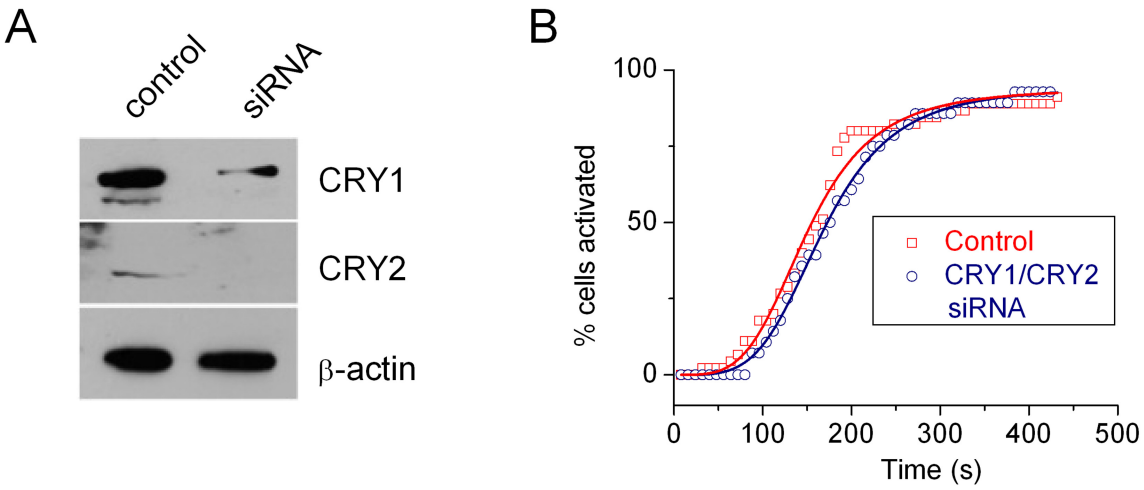
A



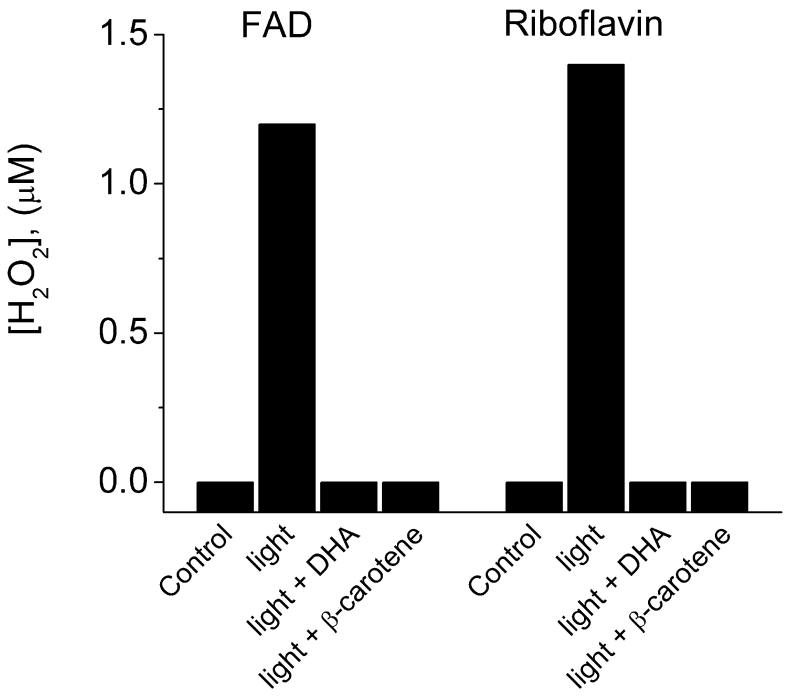

B



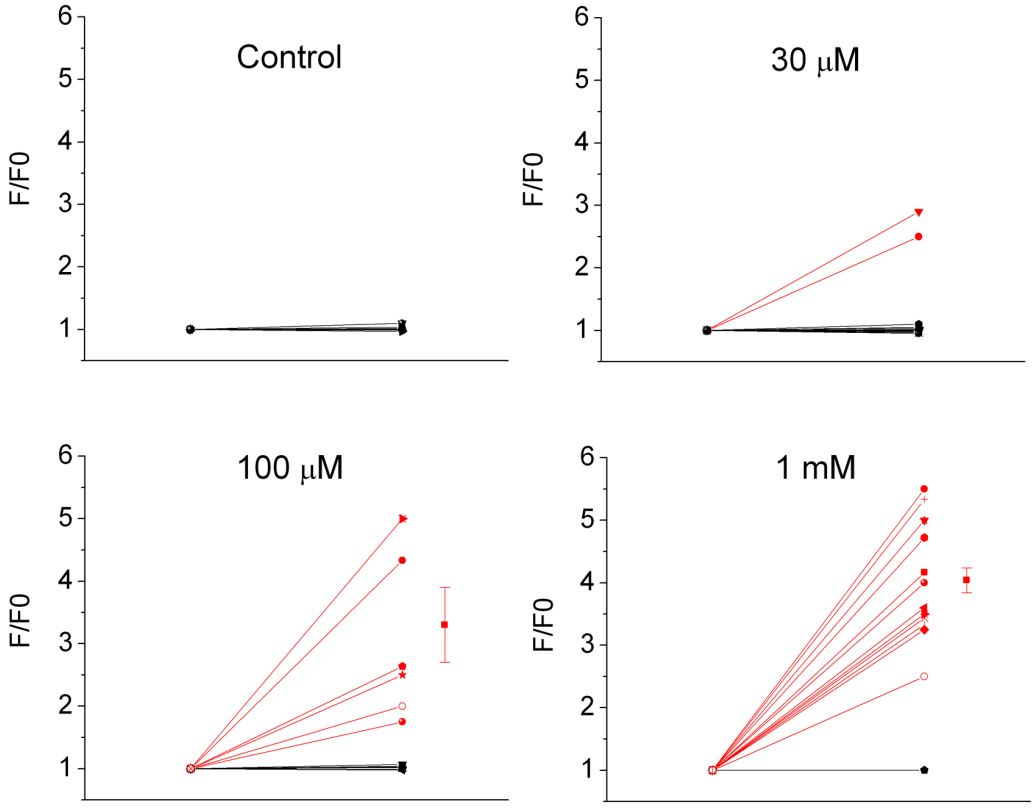

**Supplementary Figure 5. Opsin 3 (OPN3) does not contribute to light signaling in Jurkat cells**. **A**, inhibition of Opsin 3 mRNA expression in Jurkat cells by siRNA. **B**, inhibition of Opsin 3 mRNA expression does not disrupt blue light-evoked $Ca^{2+}$ signaling.



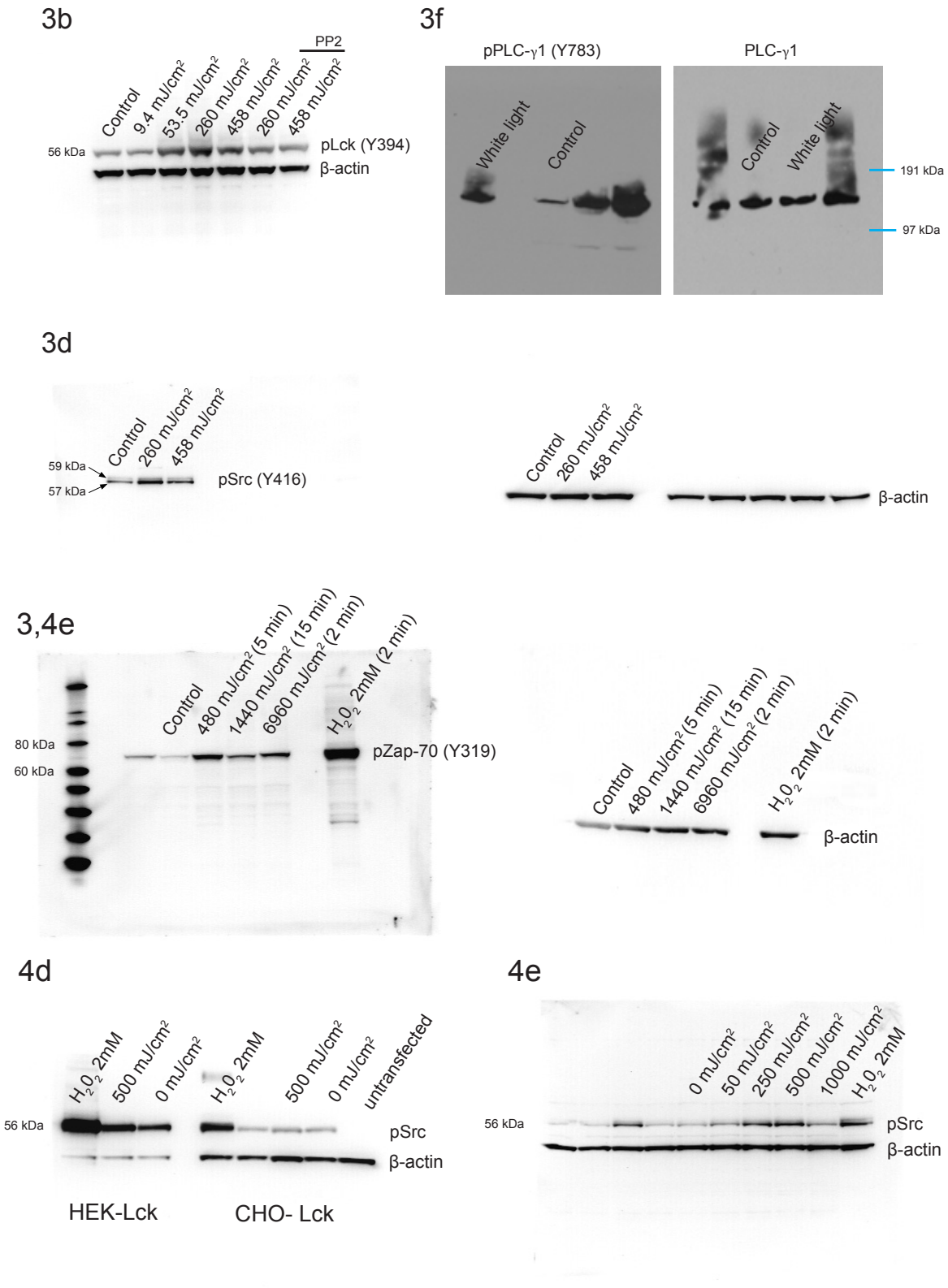

**Supplementary Figure 6. Light activates $Ca^{2+}$ independently of Cryptochrome proteins.** Inhibiting protein expression of cryptochrome 1&2 (CRY1&2) (A) in Jurkat cells does not affect light-evoked $Ca^{2+}$ signaling (B).




**Supplementary Figure 7. Blue light irradiation of riboflavin and flavin adenine dinucleotide generates $H_20_2$.** Solutions containing 5 μM of riboflavin or flavin adenine dinucleotide were irradiated with 600 mJ $cm^{-2}$ blue light with or without docosahexoenoic acid or β-carotene (10 μM). [$H_20_2$] was measured using a colorimetric Oxired assay (Abcam).




**Supplementary Figure 8. $H_20_2$ increases intracellular [$Ca^{2+}$] in Jurkat cells.** Fluo4 fluorescence in individual cells before and after a two-minute exposure to 0, 30, 100 μM and 1 mM $H_20_2$ (n=12-15 cells). Non-responding and responding cells are denoted as black and red respectively. Note that $H_20_2$ in a concentration dependent manner increases the number of responding cells. Mean $F/F_o$ (3.3 ± 0.6 and 4.1 ±0.2) was not significantly different (P=0.15) for 100 μM and 1 mM treatments. $F_0$ was measured using <10 mJ $cm^{-2}$ blue light that did not affect subsequent fluorescence measured 2 minutes later.




**Supplementary Figure 9. Full-sized immunoblots for Figures 3b-f, 4d&4e.**

**Supplementary Movie 1.** Blue light irradiation (5 mW $cm^{-2}$) stimulates an increase in [$Ca^{2+}$] in Jurkat T cells; 7.5x speed.

**Supplementary Movie 2.** Single cell $Ca^{2+}$ and motility response to blue light (488 nm laser 2.7 mW $cm^{-2}$; 20x speed).

**Supplementary Movie 3.** Blue light triggers extension of lamellipodia in Jurkat cells (488 nm laser 2.7 mW $cm^{-2}$, 37ºC, 20x speed).