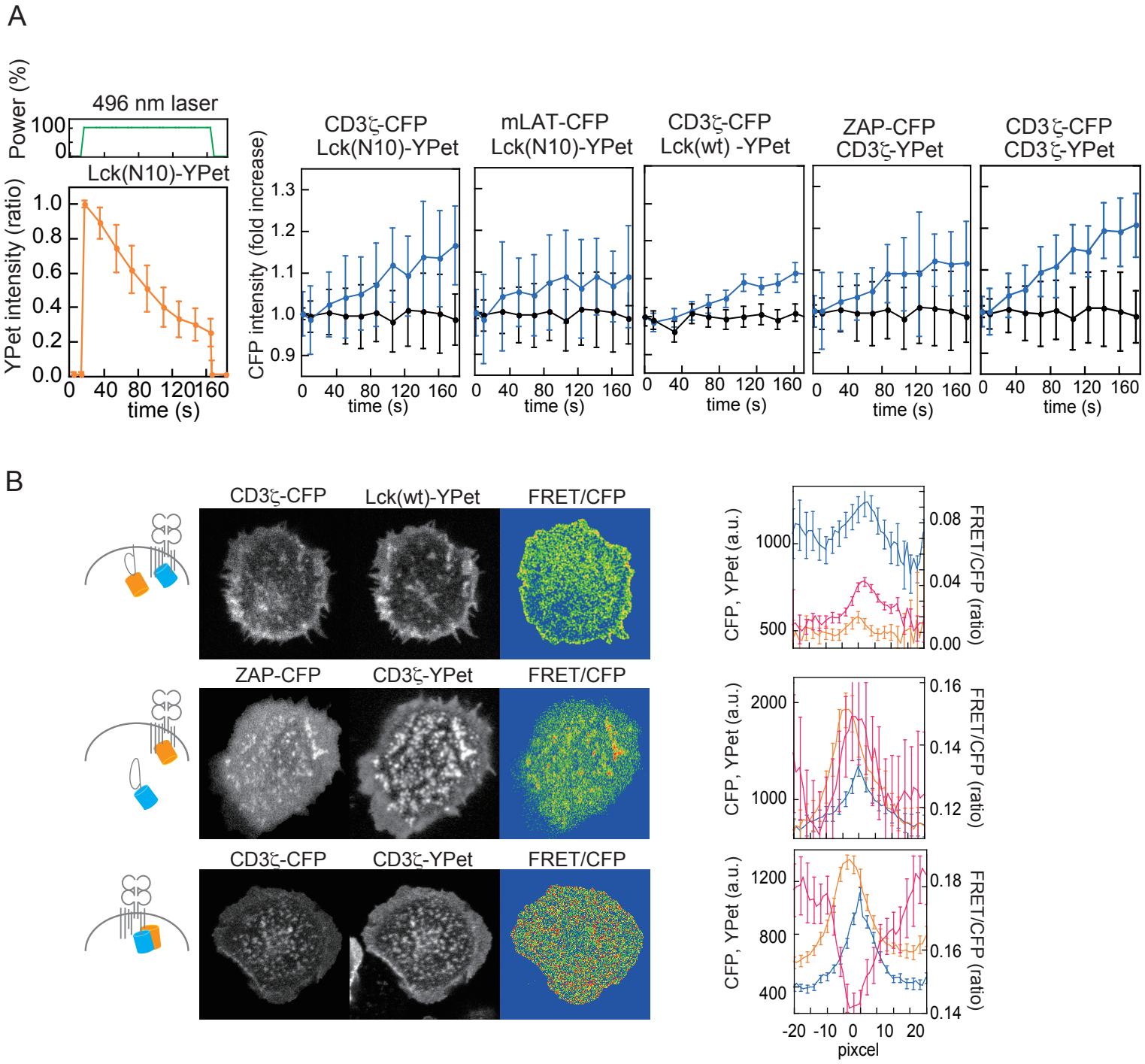

Fig. S2. Supplemental data for FRET analysis. (A) Acceptor bleaching method for FRET evaluation. After the cells had expanded on the bilayer, they were fixed with 4% paraformaldehyde and illuminated with a 496 nm laser to detect and bleach only YPet. CFP was excited by a 405 nm laser and detected every 22 seconds. The time course of the 496 laser power and fold decrease of YPet intensities are shown in the left panel. The time course of fold increase of CFP intensities (blue) is plotted. AND-TCR hybridoma cells expressing only CD3ζCFP (black) are included as a negative control in each plot. All the pairs showed significant increases of CFP intensities after YPet bleaching. * $p < 0.05$
(B) CD3ζ-CFP and Lck(wt)-YPet, ZAP-CFP and CD3ζ-YPet or CD3ζ-CFP and CD3ζ-YPet were analyzed as control for the FRET analysis. Representative images and intensity profiles of 10 x 41 pixels around the peak of Lck(wt)-YPet, CD3ζ-CFP, ZAP-CFP or CD3ζ-YPet were arranged and plotted. Data are mean ± standard error of the mean (S.E.M.) of 40-50 peaks from 3 cells. The blue line indicates CFP intensities, orange is YPet and pink is FRET/CFP. The diagrams in the left column depict the labeled molecules in each experimental condition.