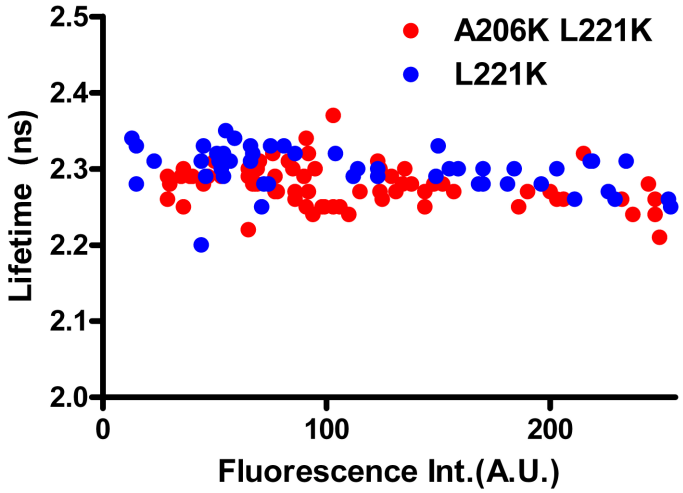
Supplementary Fig. 1 Fluorescence lifetime variation of CFP A206K L221K and CFP L221K



FLIM images of HEK cells expressing CFP A206K L221K and CFP L221K were analyzed using deconvoluting method. There is no significant difference in lifetime between CFP L221K and CFP A206K L221K.

Supplementary Table 1. Photophysical properties of CFP variants

	$\varepsilon \times 10^3$ / $M^{-1}cm^{-1}$ ($\lambda_{max,ab}$ / nm)	Q.Y. ($\lambda_{max,em}$ / nm)	Lifetime (in the cell) / ns	Lifetime (in buffer solution)					
				A_1	τ_1 / ns	A_2	τ_2 / ns	$\langle\tau\rangle$ / ns	χ^2
CFP-L221K	28 (438)	0.28 (477)	2.30±0.04	0.61	0.93	0.39	2.03	1.36	1.71
CFP	29 (436)	0.37 (478)	2.63±0.03	0.48	1.14	0.52	2.95	2.09	1.42
CFP-H148D	28 (435)	0.56 (477)	3.11±0.04			1	3.28	3.28	1.52

$\langle\tau\rangle = A_1 \tau_1 + A_2 \tau_2$ ($\langle\tau\rangle$:average lifetime, A: relative amplitude of each decay component)
ε : molar extinction coefficient, Q.Y.: quantum yield
$\lambda_{max,ab\ or\ em}$: wavelength at absorption or emission maximum

All the decay curves for the FLIM image were fitted using single exponential to simplify the data analysis. All the fluorescence decay curves in buffer solutions were fitted to minimize the χ^2 value. (Lakowicz, 2006). Therefore, fluorescence decay curves of CFP L221K and CFP were fitted by using bi-exponential decay equation.

Ref. > Lakowicz, J.R. Principles of Fluorescence Spectroscopy, 3rd Edition.
(Kluwer Academic/Plenum Publishers, New York, 2006).