Tsutsui et al., Supplementary Information

The residues for site-directed random mutagenesis on KikG/D62H were Met^{10} and Leu^{12} (1^{st} strand); Gly^{27} and Gly^{29} (2^{nd} strand); Gln^{38} and Met^{40} (3^{rd} strand); Tyr^{87} and Trp^{89} (4^{th} strand); Asn^{105} and Ile^{107} (5^{th} strand); Tyr^{119} and Ile^{121} (6^{th} strand); Ser^{145} and Glu^{147} (7^{th} strand); Val^{160} and Met^{162} (8^{th} strand); Phe^{176} and Thr^{178} (9^{th} strand); His^{196} and Ile^{198} (10^{th} strand); and Leu^{212} and Glu^{214} (11^{th} strand).

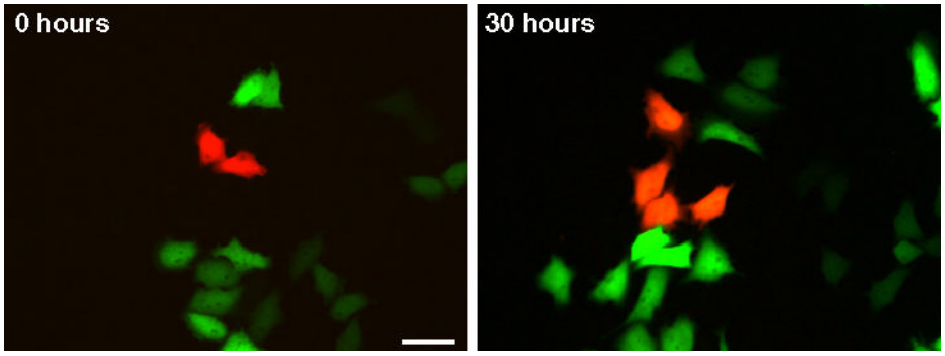
Data collection and refinement statistics table

Data collection statistics	
Space group	C2
Wavelength (Å)	0.7
Unit cell dimensions (Å)	a=96.7; b=119.1; c=49.3; β=120.72°
Resolution range (Å)[a]	100-1.6 (1.69-1.60)
Number of observations	203807
Number of unique reflections	53570
Completeness (%)[a]	95.0 (94.0)
R sym (%)[ab]	7.2 (12.5)
Mean I / σ (%)[a]	5.9 (4.3)
Refinement statistics	
Resolution range for refinement (Å)	100-1.6
R work (%)[b]	19.7
R free (%)[b]	22.9
R.m.s. deviations from ideal	
Bond length (Å)	0.005
Bond angle (degree)	1.4
Average B-factor (Å^2)	15.8
Number of water molecules	573

[a] Values in parentheses are for the highest-resolution shell.

[b] R sym = $\Sigma|(I\ hkl - I\ <hkl>)| / (I\ hkl)$, where $I\ <hkl>$ is the mean intensity of all reflections equivalent to reflection hkl by symmetry; R work (R free) = $\Sigma\ ||F\ o| - |F\ c|| / \Sigma\ |F\ o|$; 5% of data were used for R free.

Figure S1. Division of highlighted cells



Among HeLa cells expressing KikGR, two were photoconverted to provide maximum green/red contrast. Both the cells underwent normal cell division, ensuring insignificant toxicity of cell labeling by KikGR photoconversion (Scale, 50 μm).

Table S1. Protein expression and folding efficiencies of KikGR and Kaede in mammalian cells.

	Kaede (*16,120 pixels)	KikGR(*21,439 pixels)
Myc-tag intensity [a.u.]	310 ± 6.24	715 ± 6.75
Green intensity [a.u.]	236 ± 10.2	660 ± 9.65
Green/myc [a.u.]	1.75 ± 0.063	2.46 ± 0.046

*Number of pixels comprising the cells and quantified.

HeLa cells were transfected with the cDNA for C-terminal myc-tagged KikGR or Kaede. After 24 hours, cells were fixed and subject to immunocytochemistry using anti-myc antibody conjugated with Alexa583. Total and fluorescent proteins were quantified by measuring fluorescence intensities at 583 and 517 nm, respectively. Folding efficiency was estimated as the ratio of 517 / 583 nm. Data are presented as mean ± standard error.