A FRET-facilitated photoswitching using an orange fluorescent protein with the fast photoconversion kinetics

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SUPPORTING INFORMATION

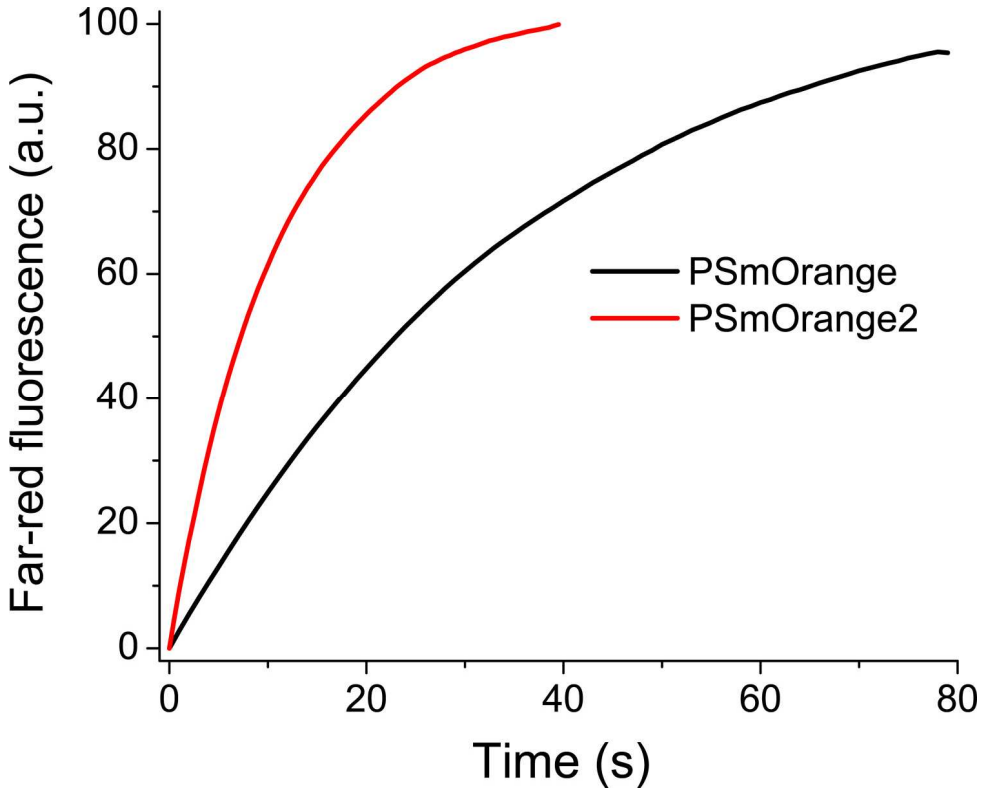

Figure S1. Photoswitching kinetics of the PSmOrange and PSmOrange2 purified proteins without adding oxidant. The PSmOrange and PSmOrange2 proteins (1 mg/ml) in phosphate buffered saline (PBS) were photoconverted using 480/40 nm light at 1,050 W cm^{-2} at the sample.

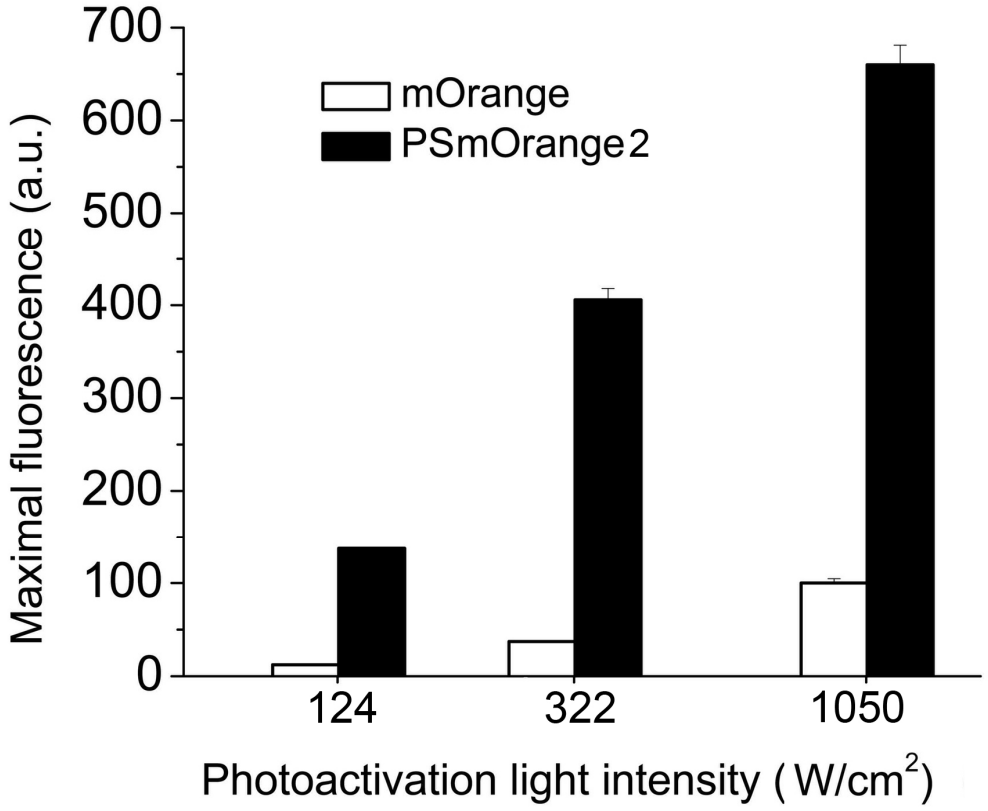

Figure S2. Dependence of the maximal far-red fluorescence on the power of photoswitching light. The 9-fold decrease in the power density of photoswitching light resulted in almost 6-fold decrease in the maximal far-red fluorescence.

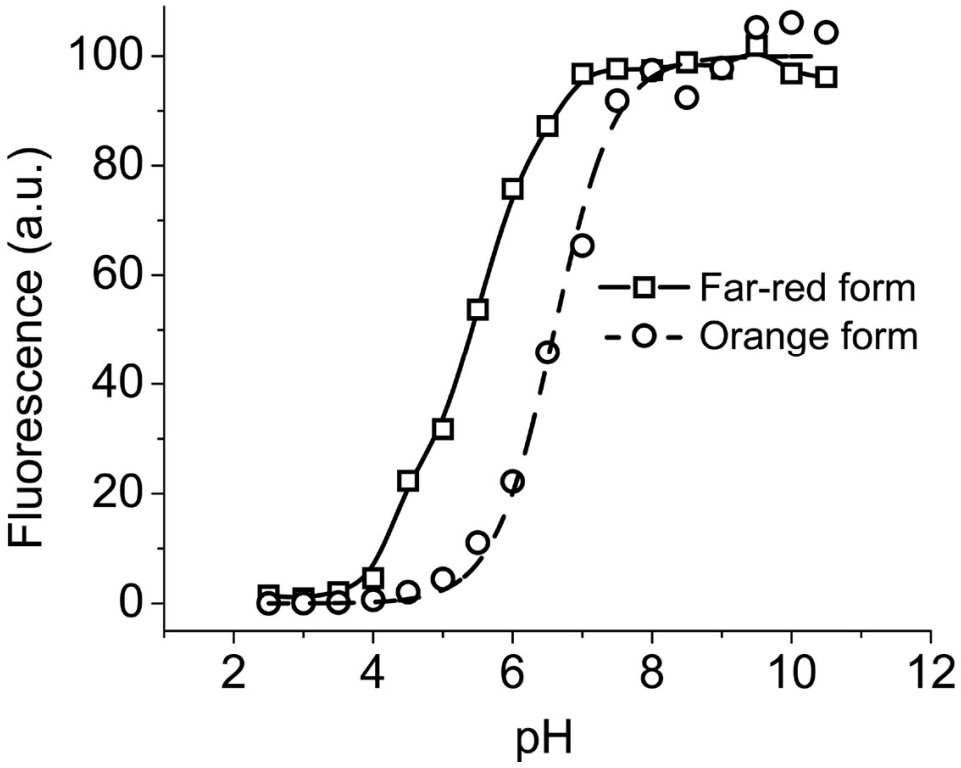

Figure S3. The pH dependence of the purified PSmOrange2 protein. The equilibrium pH dependences for fluorescence of the PSmOrange orange form (circles) and its far-red form (squares) are shown.

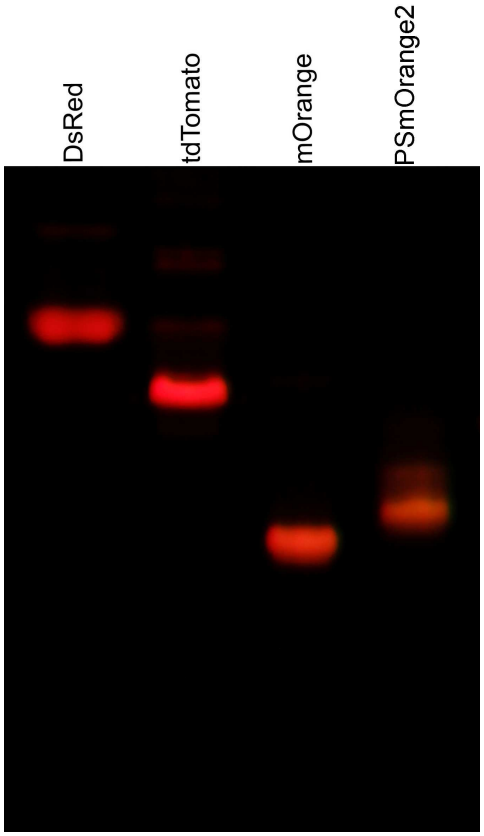

Figure S4. Semi-native polyacrylamide gel with the purified PSmOrange2 protein. 10 μg of the freshly purified fluorescent proteins were applied without heating in 10 μl aliquots onto the 15% polyacrylamide gel containing 0.1% SDS. The DsRed [1], tdTomato [2] and mOrange [2] proteins were applied as the teterameric, dimeric and monomeric native protein standards, respectively.

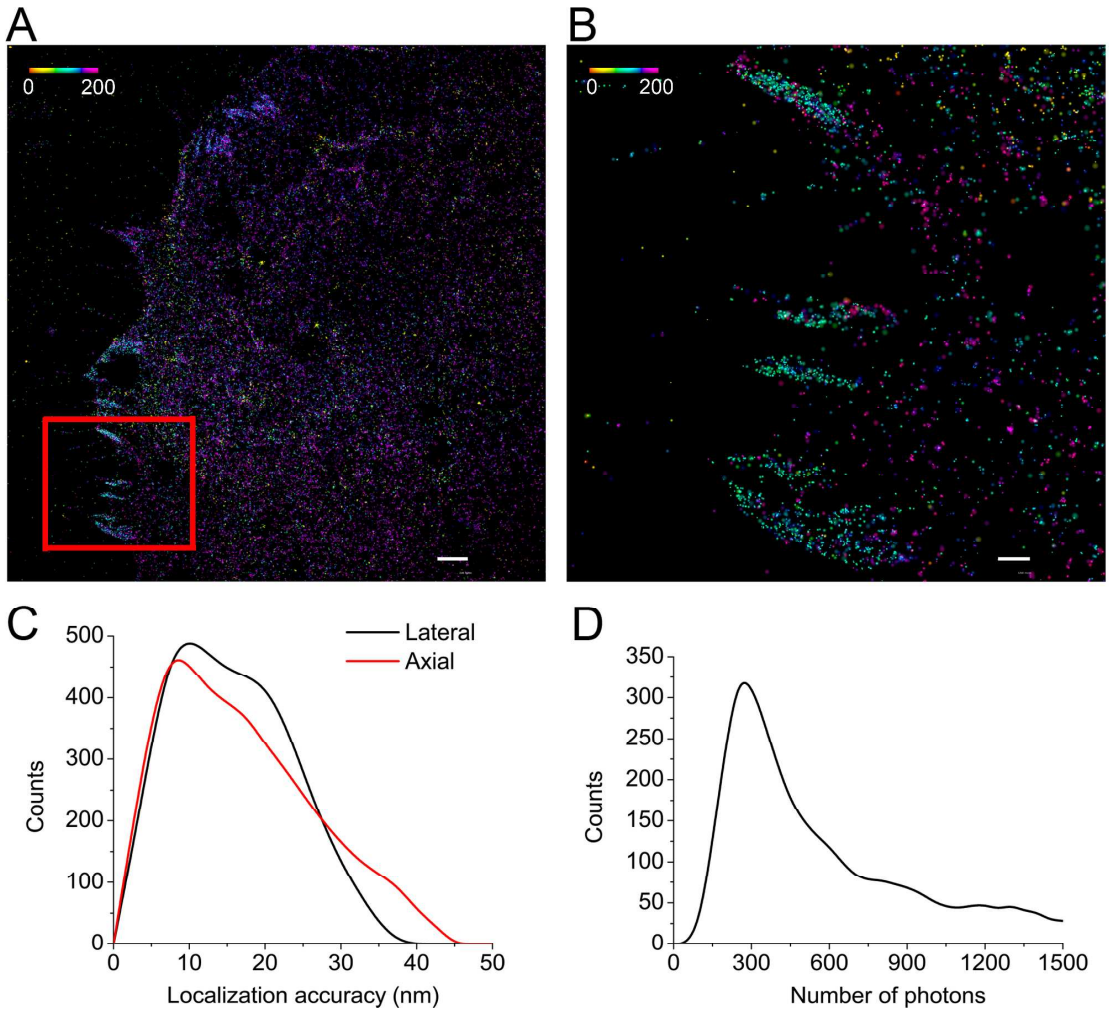

Figure S5. iPALM imaging of HeLa cells expressing a paxillin-PSmOrange2 fusion protein. (A) iPALM image of a representative cell. White scale bar, 5 µm. Vertical coordinates relative to the coverglass surface are indicated by a color scale from red to purple (z = 0–200 nm). (B) The zoomed area is marked as a red box in A. White scale bar, 1 µm. Vertical coordinates relative to the coverglass surface are indicated by a color scale from red to purple (z = 0–200 nm). (C) Distribution of the lateral (black) and axial (red) localization accuracies for the fluorescent particles in focal adhesion areas inside the red box in A. With iPALM, this brightness allows for localization accuracy of typically 10-20 nm in both lateral (xy) and axial (z) directions. (D) Distribution of detected number of photons for the fluorescent particles in focal adhesion areas inside the red box in A.

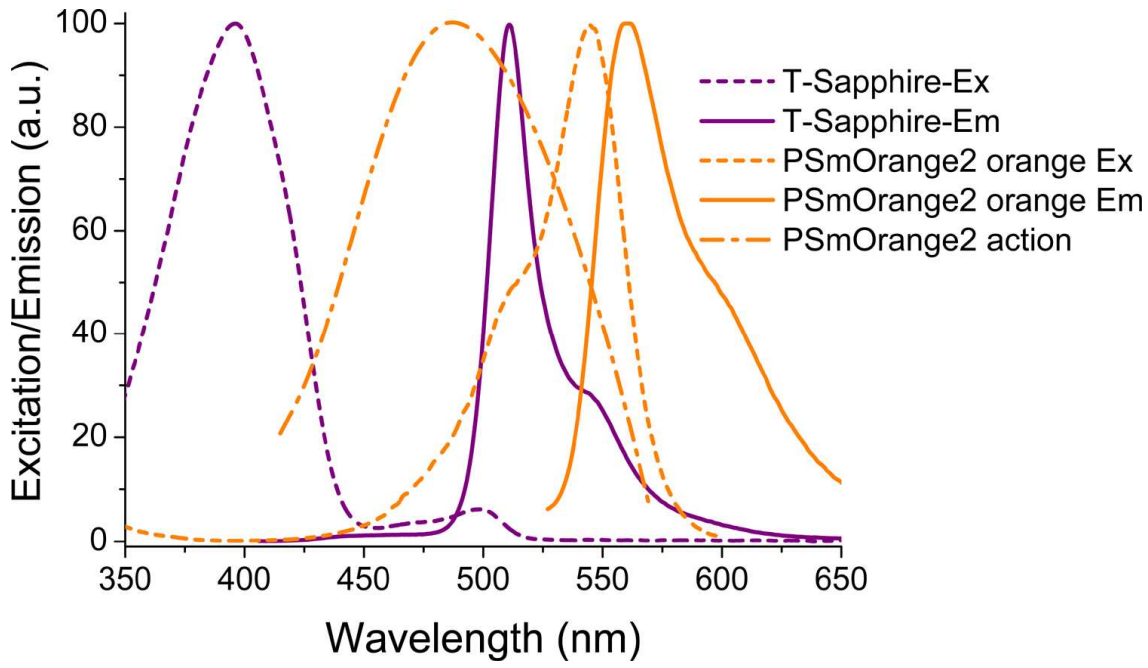

Figure S6. Excitation and emission spectra of T-Sapphire overlaid with those of the orange form of PSmOrange2 and action spectrum of PSmOrange2. Emission spectrum of T-Sapphire has a good overlap with photoswitching action spectrum of PSmOrange2. That means that T-Sapphire is a good FRET donor for PSmOrange2 photoswitching. The photoswitching action spectrum of PSmOrange2 was determined by measurement of initial photoswitching rates of PSmOrange2 using different excitation filters (415/30, 436/20, 480/40, 540/20 and 570/30 nm).

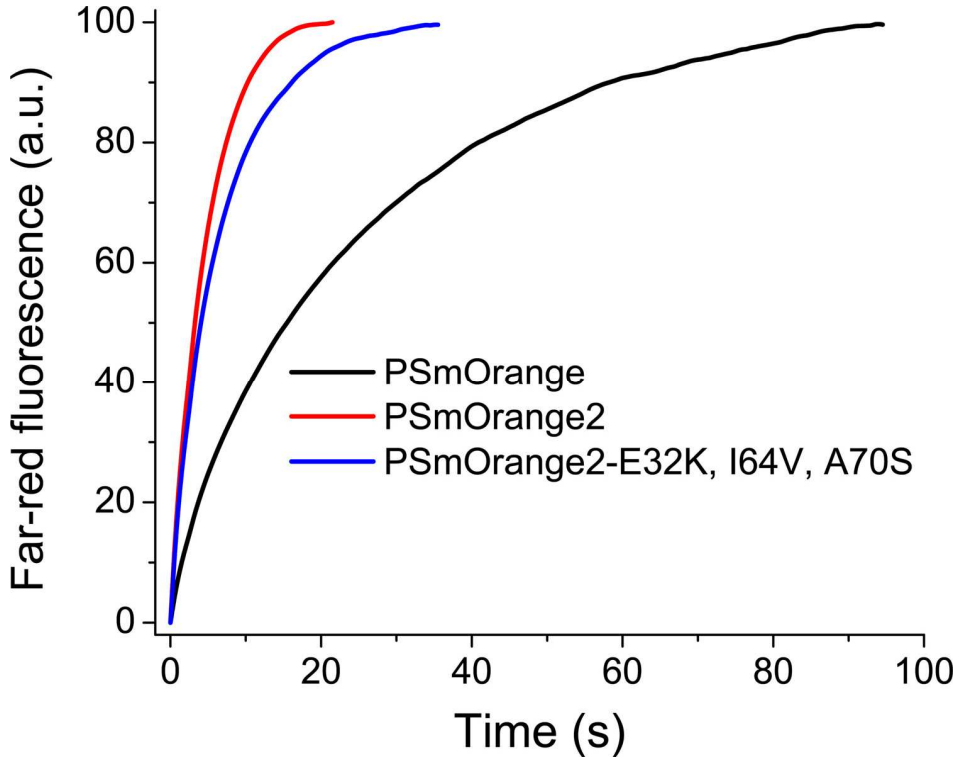

Figure S7. Photoswitching kinetics of PSmOrange, PSmOrange2, and PSmOrange2/E32K/I64V/A70S proteins. Similarly to PSmOrange2 its mutant containing I64V substitution has the fast photoswitching kinetics. Other substitutions in this mutant are external to the β-can and far from the chromophore (E32K), or remote from the bond that is cleaved upon photoconversion (A70S). Thus, mainly Val64 causes the fast photoswitching of PSmOrange2/E32K/I64V/A70S.

Table S1. Initial rates of PSmOrange2 photoswitching in different sets of fusion constructs in presence and absence of T-Sapphire FRET donor.

Fusion constructs		**Photoswitching wavelength, nm**	**Initial rate of photoswitching, s^{-1}**
In vitro			
T-Sapphire-L2-PSmOrange2		415/30	9.7 ± 0.7
T-Sapphire-L7-PSmOrange2			10.8 ± 0.6
T-Sapphire-L14-PSmOrange2			8.4 ± 0.4
T-Sapphire-L20-PSmOrange2			7.7 ± 0.3
T-Sapphire-L7-PSmOrange			1.02 ± 0.01
T-Sapphire-L7-mOrange			0.87 ± 0.02
T-Sapphire-L7-PSmOrange2		390/40	11.2 ± 1.1
T-Sapphire-L7-PSmOrange2		436/20	2.3 ± 0.1
PSmOrange2 alone		415/30	0.72 ± 0.02
Mammalian cells			
T-Sapphire-L7-PSmOrange2	fusion	415/30	16.6 ± 2.8
	PSmOrange2 alone		2.7 ± 0.3
T-Sapphire-FKBP and PsmOrange2-FRB	interacting		10.5 ± 1.6
	apart		3.2 ± 0.4
EGFR –T-Sapphire and Grb2-PSmOrange2	interacting		12.9 ±2.2
	apart		5.6 ± 0.7

Table S2. Comparison of different FRET methods to detect protein-protein interactions.

FRET method	Advantages	Drawbacks
FRET-facilitated photoswitching	1. Easy to detect FRET by fast increase of far-red fluorescence. 2. Possibility to observe a part of molecules, which previously interacted with other molecules. 3. Allows for accumulation of weak signals over time.	1. Necessary to compare with negative controls. 2. Not suitable for dynamic interactions of the same population.
Sensitized emission	1. Suitable for dynamic interactions.	1. Necessary to compare with negative controls. 2. Complex filter set for detection of FRET. 3. Necessary to subtract the cross-talk components.
Acceptor photobleaching	1. Requires only one sample without controls.	1. Not suitable for dynamic interactions. 2. Incorrect when acceptor photobleaching is not complete. 3. High light intensities may damage the donor.
Fluorescence lifetime imaging	1. Not sensitive to donor-acceptor cross-talk artifacts. 2. Can be applied for non-fluorescent acceptors.	1. Complicated instrumentation for measurement. 2. Slow, requires several minutes for each image. 3. Not suitable for fluorescent molecules with multi-exponential lifetimes. 4. Sensitivity to environmental factors (such as change in pH).
Polarization anisotropy imaging	1. FRET measurement with high signal-to-noise ratio and high rate.	1. Sensitive to cross-excitation of the acceptor. 2. Limited to imaging with objective lenses with numerical aperture less than 1.0.

Supporting References

(1) Matz, M. V.; Fradkov, A. F.; Labas, Y. A.; Savitsky, A. P.; Zaraisky, A. G.; Markelov, M. L.; Lukyanov, S. A. *Nat Biotechnol* **1999**, *17*, 969.

(2) Shaner, N. C.; Campbell, R. E.; Steinbach, P. A.; Giepmans, B. N.; Palmer, A. E.; Tsien, R. Y. *Nat. Biotechnol.* **2004**, *22*, 1567.