

Highlighted Ca²⁺ imaging with a genetically-encoded ‘caged’ indicator

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Supplementary Information

Supplementary Figure 1

Emission spectrum of the TN-XL based indicator with red fluorescent protein as an FRET acceptor.

Supplementary Figure 2

Absorption and emission spectrum of dim variants of Venus.

Supplementary Figure 3

Photoactivation property of TN-XL-based constructs.

Supplementary Table 1

Spectral characteristics of dim variants of Venus.

Supplementary Video 1

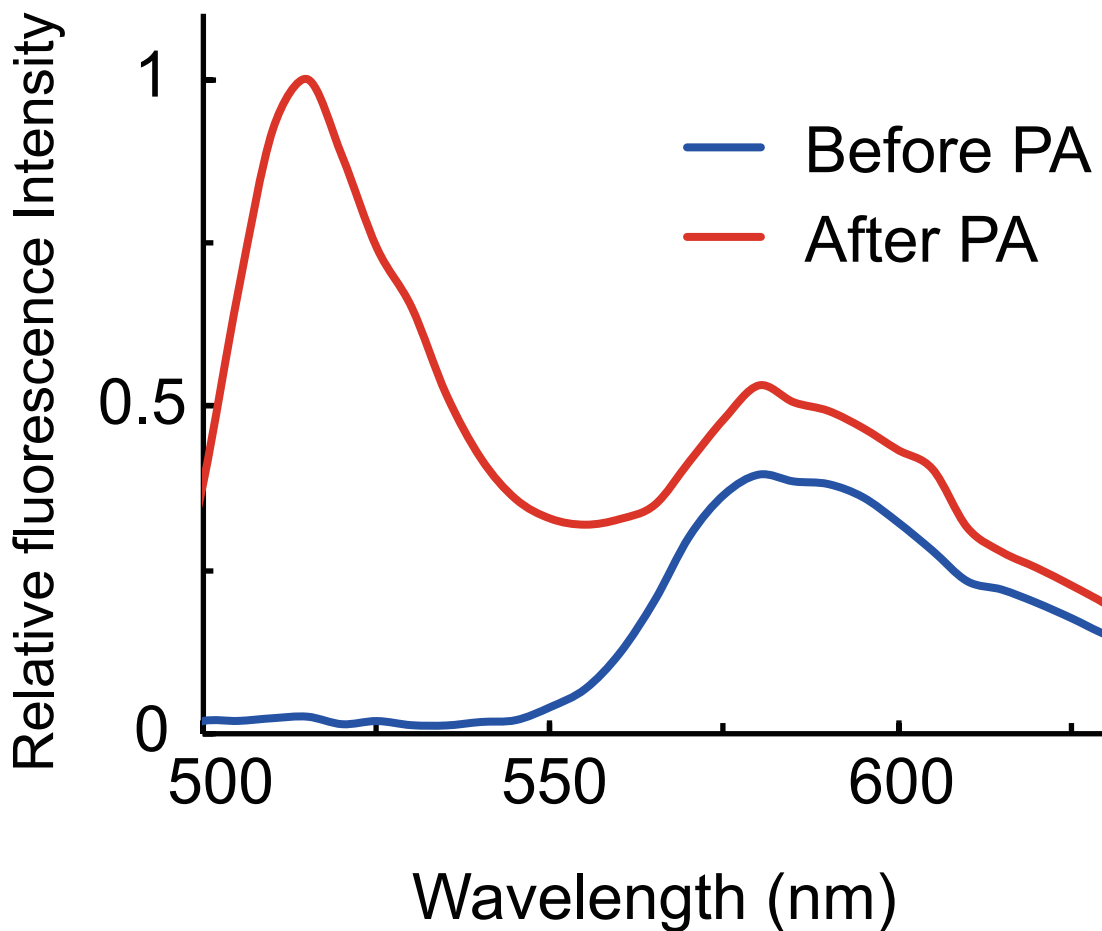
Photoactivation and Ca²⁺ imaging by PA-TNXL in HeLa cells.

Figure S1. Matsuda et al

a

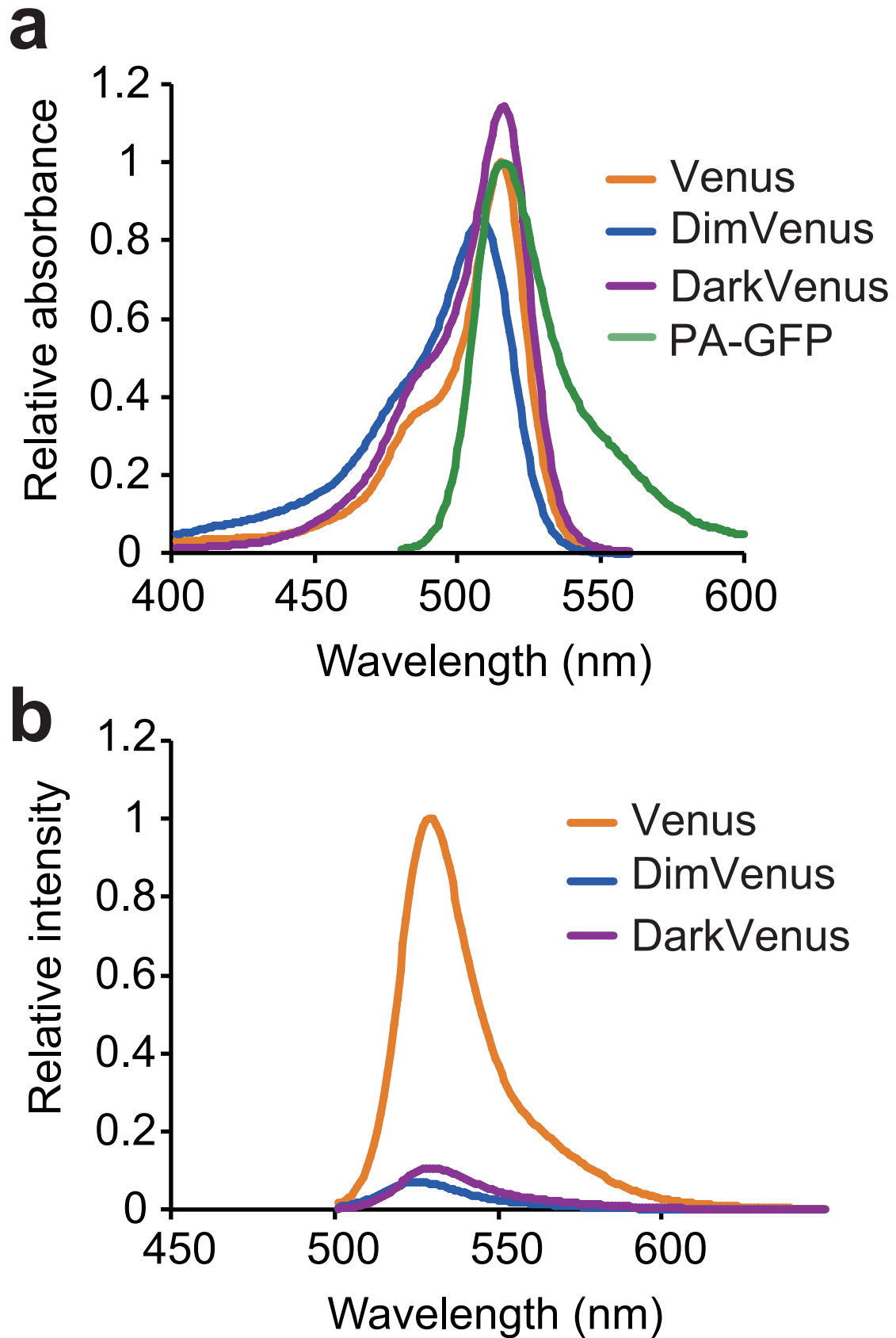


b



Supplementary Figure 1. Emission spectrum of the TN-XL based indicator with red fluorescent protein as an FRET acceptor. a, Domain structure of the construct. PA-GFP with C-terminal 11 amino acids deleted, Ca²⁺ binding moiety of TN-XL and full-length DSRed.T3 were tandemly linked (Bevis, BJ. & Glick, BS. Rapidly maturing variants of the *Discosoma* red fluorescent protein (DsRed). *Nat. Biotechnol.* **20**, 83-87 (2002).) . **b**, Emission spectrum of the construct before and after photoactivation.

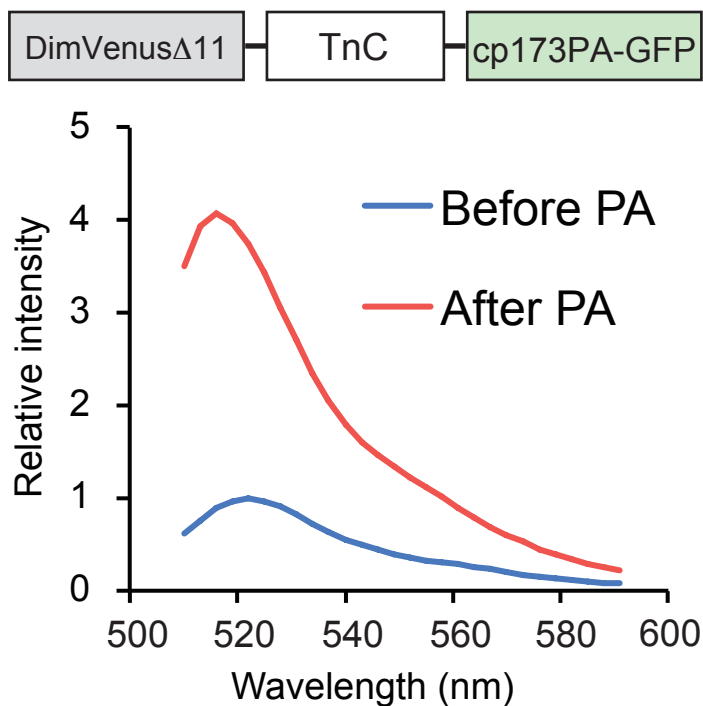
Figure S2. Matsuda et al



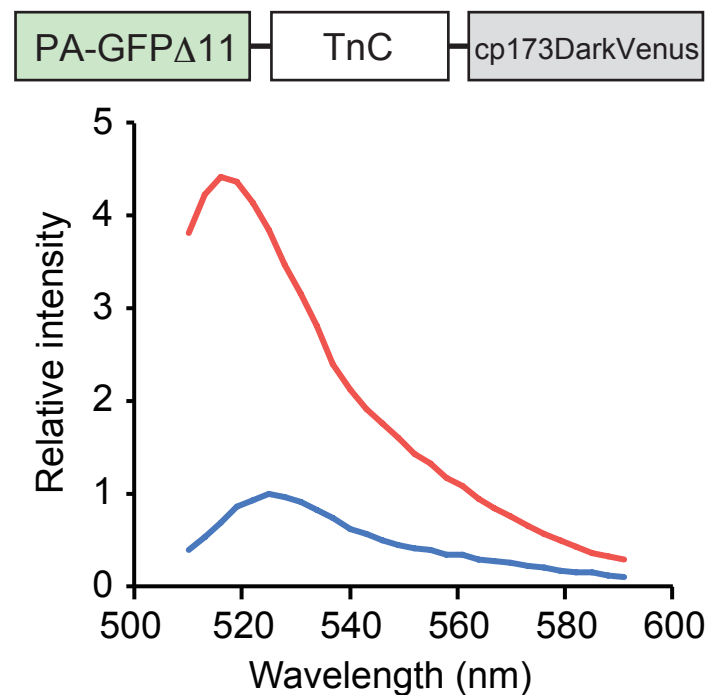
Supplementary Figure 2. Absorption and emission spectrum of dim variants of Venus. a, Absorption spectra of Venus, DimVenus and DarkVenus. Emission spectrum of PA-GFP is also shown. **b,** Emission spectrum of Venus, DimVenus and DarkVenus.

Figure S3. Matsuda et al

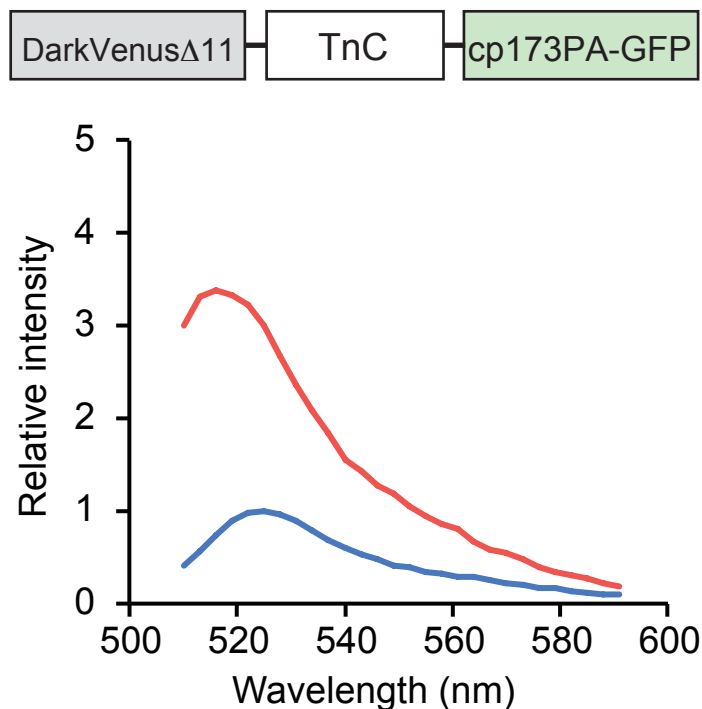
a



b



c



Supplementary Figure 3. Photoactivation property of TN-XL-based constructs. Domain structures and emission spectrum before and after photoactivation of TN-XL-based photoactivatable Ca²⁺ indicators.

Supplementary Table 1. Spectral characteristics of dim variants of Venus

Protein	Substitutions	Absorption max (nm)	ϵ^a ($10^3\text{M}^{-1}\text{cm}^{-1}$)	Emission max (nm)	Φ^a	Brightness^b ($10^3\text{mM}^{-1}\cdot\text{cm}^{-1}$)
Venus	—	515	92.2	528	0.57	52.6
DimVenus	Y145W	508	55.4	525	0.03	1.71
DarkVenus	H148V/Y145W	515	74.4	528	0.05	3.73

^aThe extinction coefficients (ϵ) and quantum yields (Φ) were determined as described^{S1}.

^bBrightness is defined as the product of ϵ and Φ .