# Supplemental Data

# Conversion of Red Fluorescent Protein into a Bright Blue Probe

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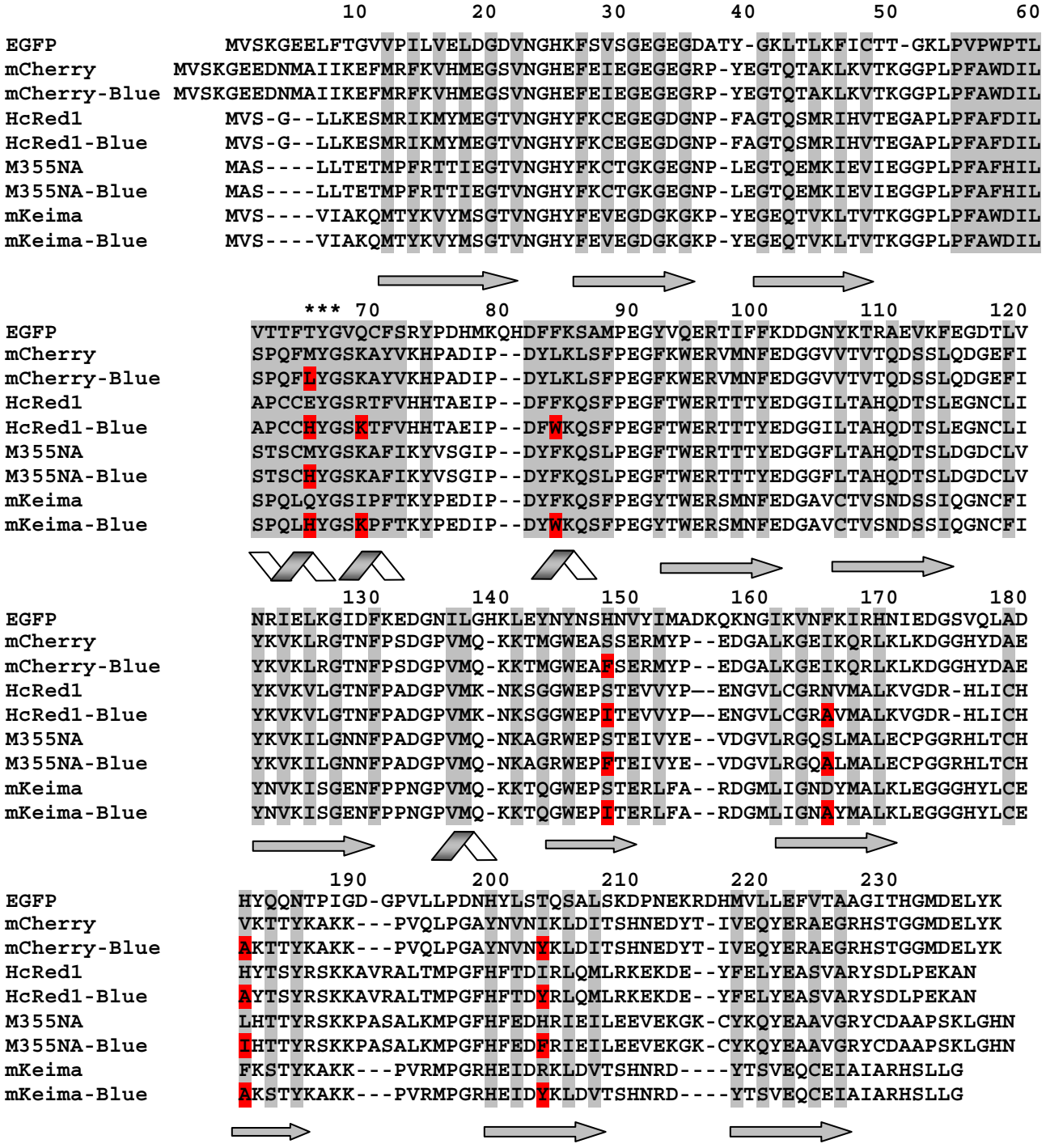


**Figure S1. Alignment of the Amino Acid Sequences of the Best Blue Mutants of mCherry, HcRed1, M355NA, and mKeima with Their Red Analogs and EGFP**

Structurally important regions are highlighted in grey, β-strands are shown with arrows, α-helixes are shown with ribbons. The chromophore forming residues are marked with asterisks. Site-specific mutations resulted in conversion of RFP into the blue mutants are shown in red. The alignment numbering follows that for EGFP.

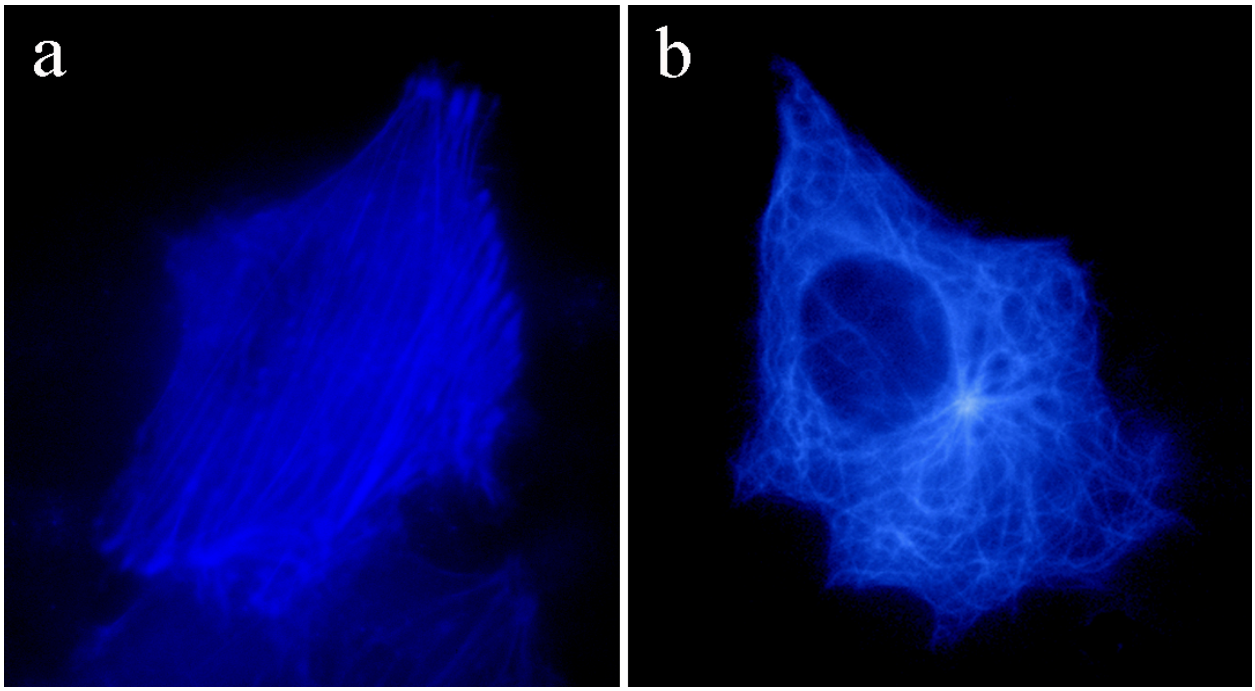


**Figure S2. mTagBFP-β-Actin and mTagBFP-α-Tubulin Fusion Proteins Expressed in Mammalian Cells**

(a and b) Live HeLa cells expressing (a) mTagBFP-β-actin and (b) mTagBFP-α-tubulin fusion constructs were imaged 48 hours after transfection using Leica AFLX 6000 inverted fluorescent microscope equipped with 63x glycerol objective lens. The fusions exhibit the monomeric behavior and are well incorporated into the endogenous cytoskeleton structures. Statistical quality of mTagBFP in fusions with β-actin and α-tubulin was compared to that of the respective EGFP fusions for approximately 200 transfected cells. Both EGFP and mTagBFP fusions gave about the same 70% of positive cells determined as clear visibility of prolonged filaments in the case β-actin or microtubules in the case of α-tubulin EGFP and mTagBFP fusions.

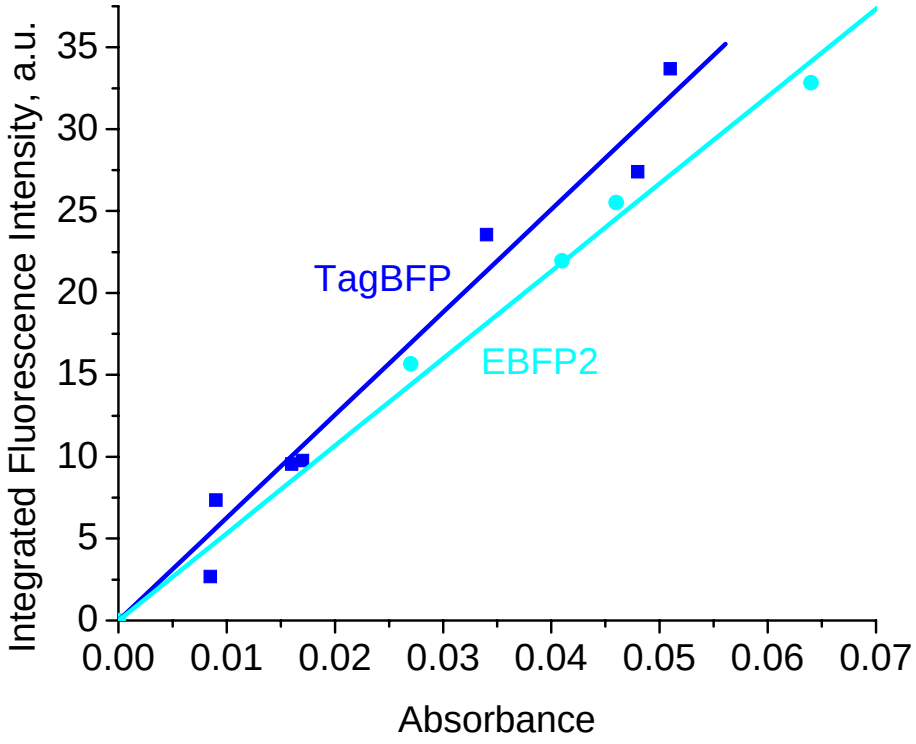


**Figure S3. Determination of Quantum Yields for mTagBFP and EBFP2**
The linear plots of integral fluorescence versus absorbance for mTagBFP and EBFP2. The gradient for each fluorescent protein is proportional to the quantum yield. The experiments were repeated three times.

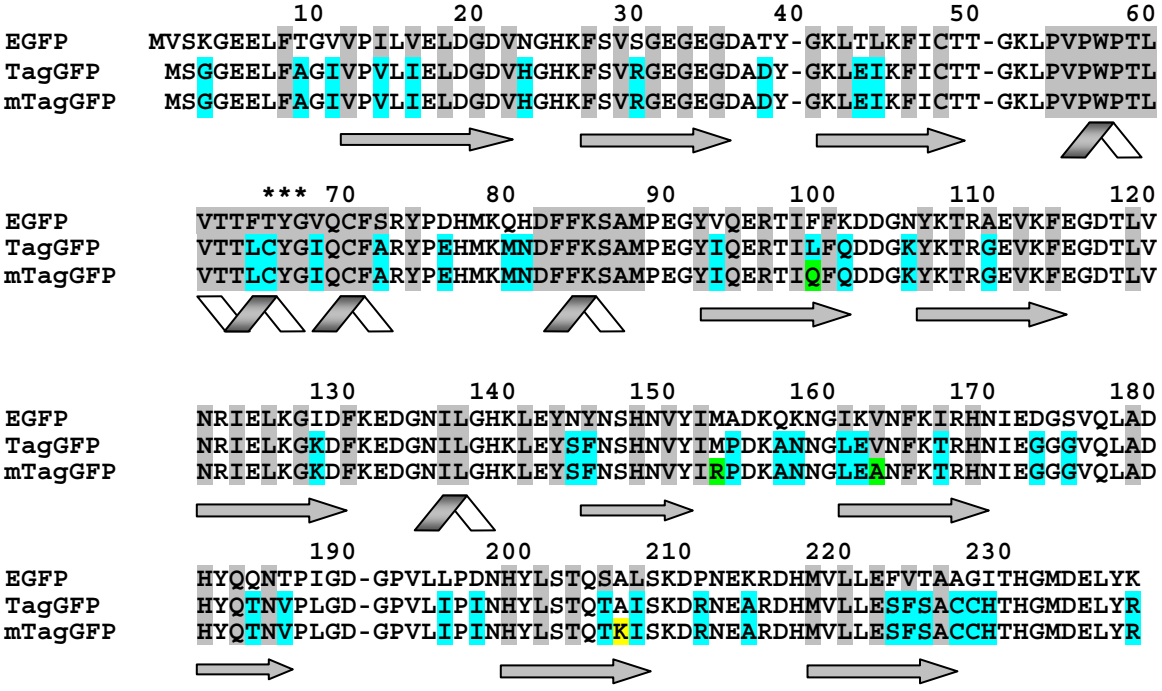


**Figure S4. Alignment of the Amino Acid Sequences of mTagGFP with EGFP and TagGFP**
Structurally important regions are highlighted in grey, beta-strands are shown with arrows, and alpha-helixes are shown with ribbons. The chromophore forming residues are marked with asterisks.

Monomerizing A203K mutation in mTagGFP is shown yellow. Mutations induced by site-directed saturated mutagenesis that enhance mTagGFP maturation rate and extinction coefficient are shown green. The sequence differences of TagGFP and mTagGFP from EGFP are highlighted by cyan. The alignment numbering follows that for EGFP.

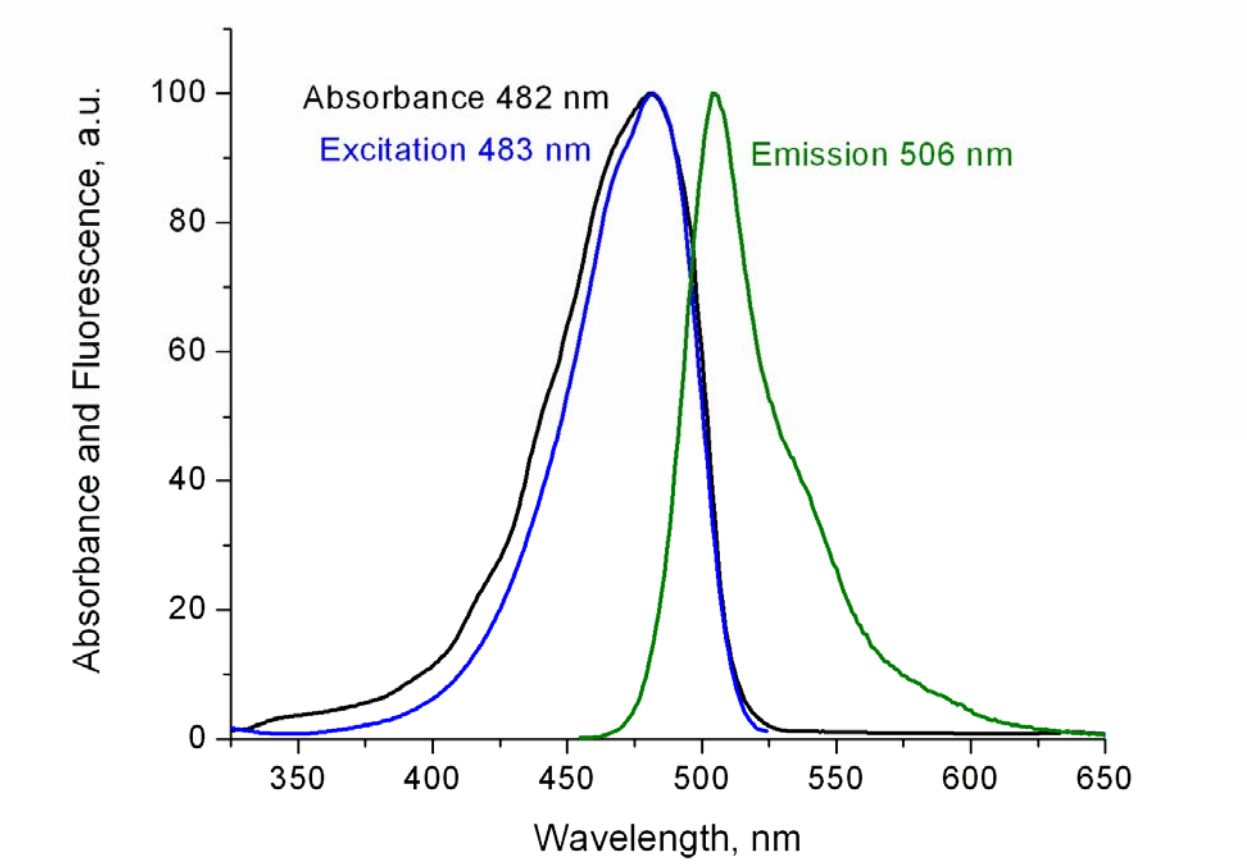


**Figure S5. Absorbance and Fluorescence Spectra of mTagGFP**
Normalized absorbance (black curve), fluorescence excitation (blue curve) and fluorescence emission (dark green curve) spectra for freshly purified mTagGFP are shown. Maxima of the absorbance, excitation and emission spectra are indicated.

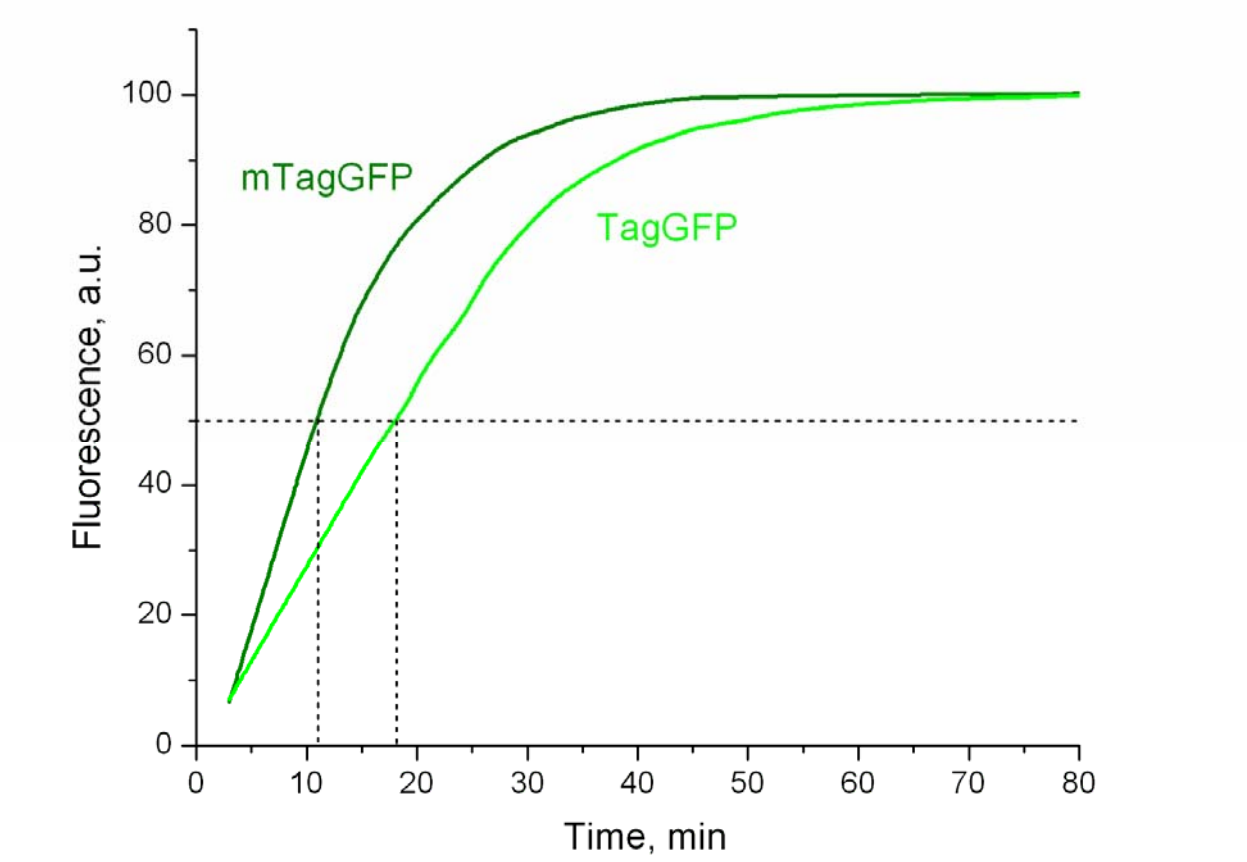


**Figure S6. Maturation Curves for mTagGFP and Parental TagGFP**
Dashed lines indicate maturation half-times of 11 min and 18 min for mTagGFP (dark green curve) and TagGFP (light green curve), respectively. Recording of protein maturation was started when about 7% from their maximal fluorescence has been detected. Time point “0” was defined using an approximation of the beginning of the maturation curves with straight lines.

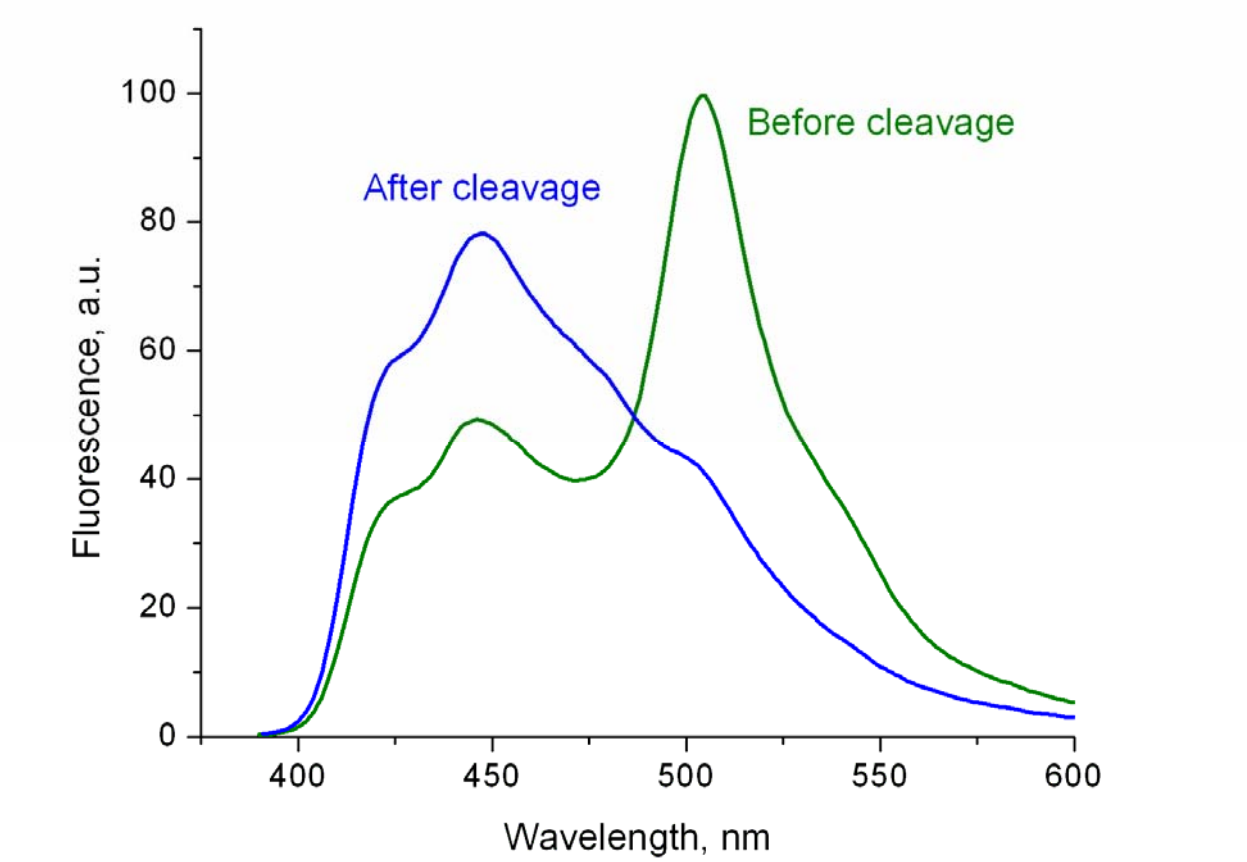


**Figure S7. Fluorescence Spectra of the Purified EBFP2-mTagGFP Fusion Construct**
Emission spectra of the purified EBFP2-mTagGFP construct before and after its cleavage with trypsin were measured when excited at 383 nm (maximum of the absorbance for EBFP2 donor).

**Table S1. Properties of the Purified mTagGFP in Comparison with Parental TagGFP and EGFP**

| Protein | Excitation maximum, nm | Emission maximum, nm | Extinction coefficient, $M^{-1}cm^{-1}$ | Quantum yield | Brightness relative to EGFP | Effective p$K_a$ | Reference |
|---|---|---|---|---|---|---|---|
| **EGFP** | 489 | 508 | 55,000 | 0.60 | 1.00 | 5.9 | (Patterson et al., 2001) |
| **TagGFP** | 481 | 505 | 55,000 ± 500 | 0.60 ± 0.02 | 1.00 | 5.0 ± 0.1 | this paper |
| **mTagGFP** | 483 | 506 | 56,500 ± 300 | 0.61 ± 0.02 | 1.05 | 5.0 ± 0.1 | this paper |

## Supplemental Reference

Patterson, G.H., Day, R.N., and Piston, D.W. (2001). Fluorescent protein spectra. J. Cell Sci. *114*, 837-838.