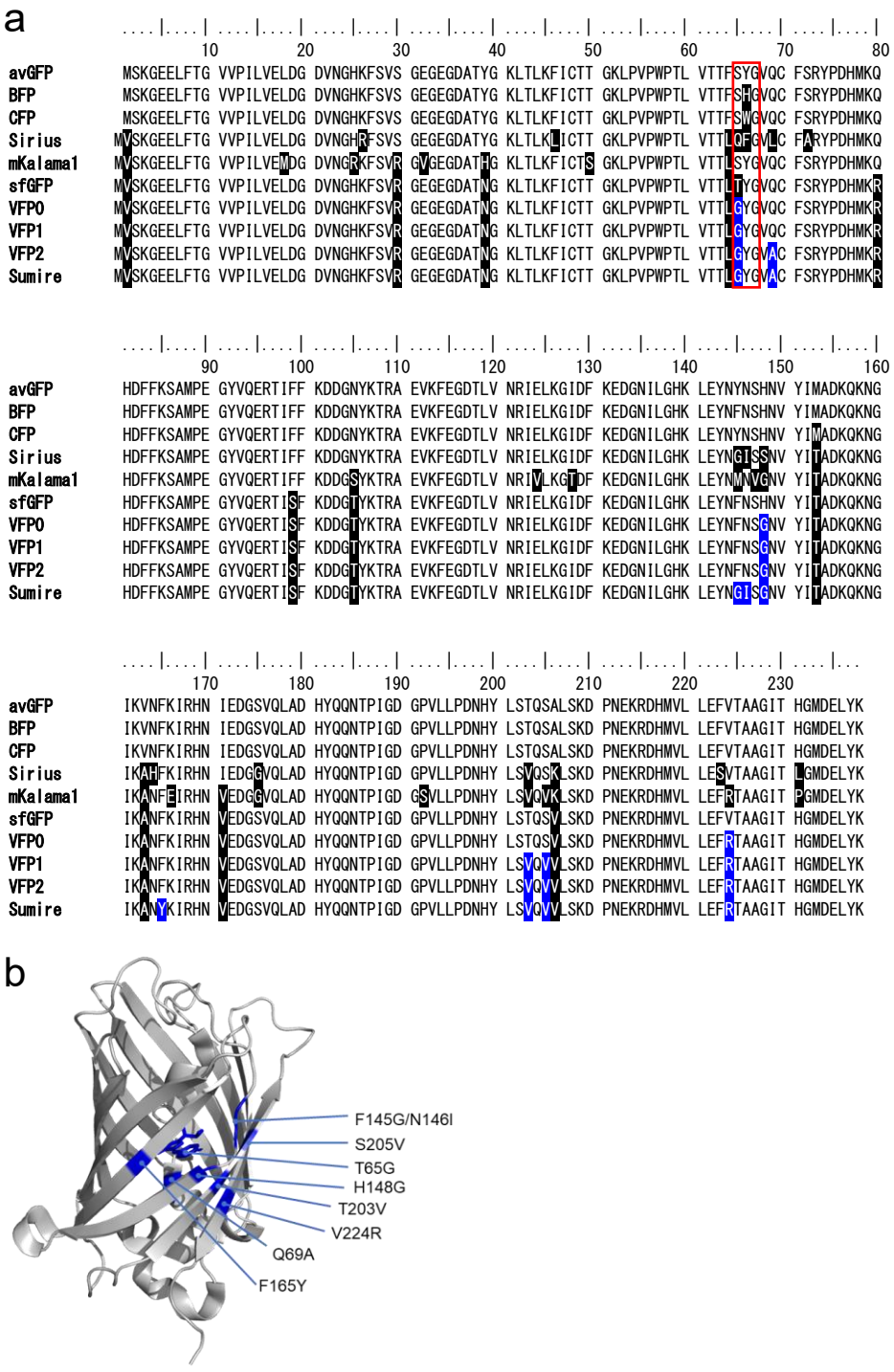
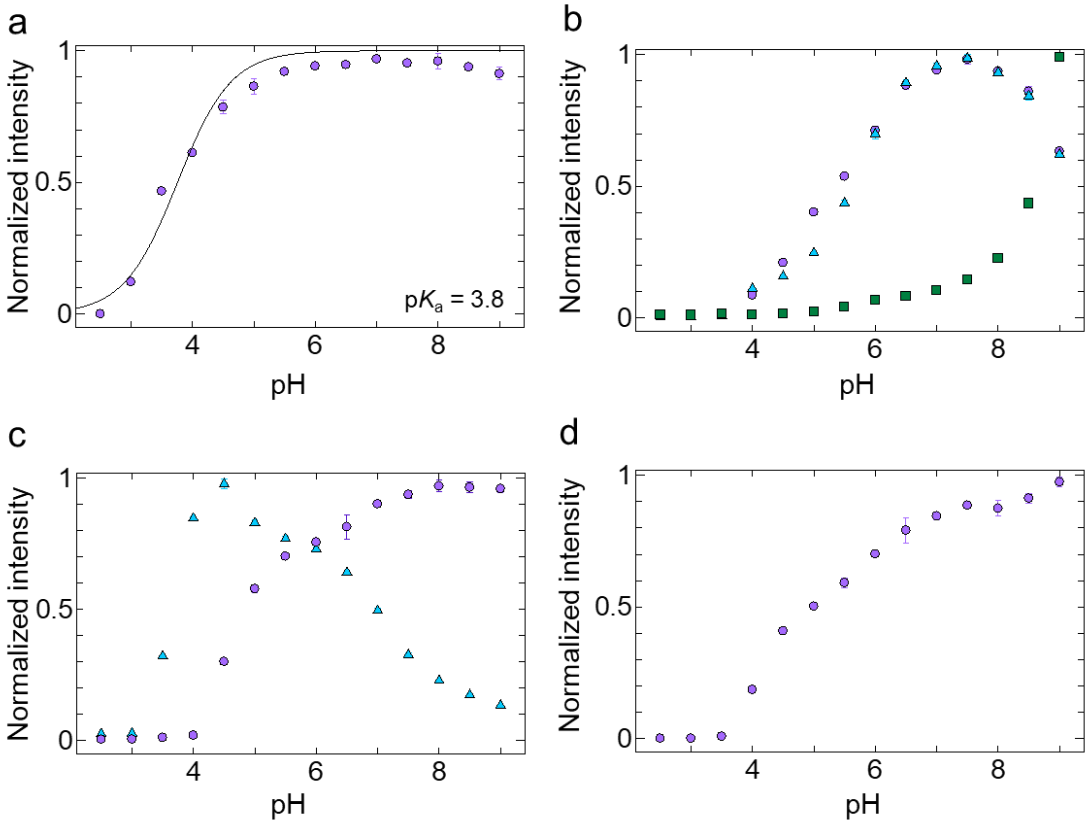
Extension of the short wavelength side of fluorescent proteins using hydrated chromophores, and its application

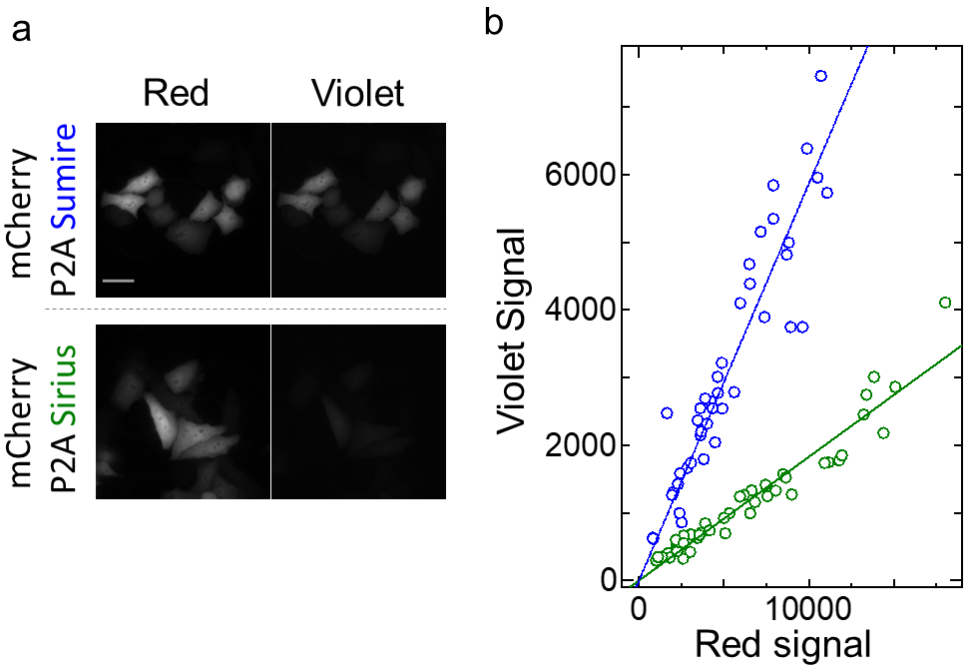
Supplemental Information



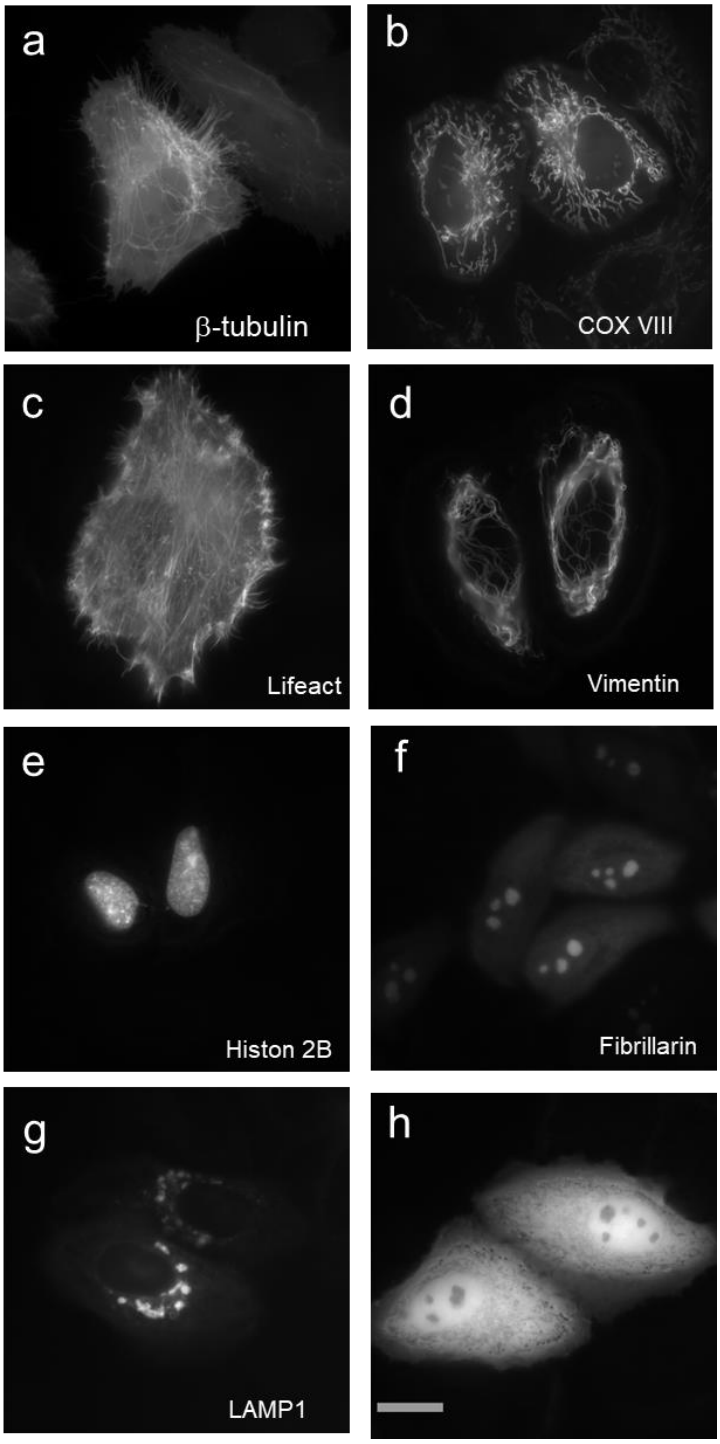
Supplementary Figure 1. The mutation points of Sumire. (a) Amid acid sequences of short wavelength mutants of avGFP. Mutations to avGFP are shown in black, and mutations introduced in this study are shown in blue. Chromophores are framed in red. (b) Mutational introduction site of Sumire on the crystal structure of sfGFP (PDB ID: 2b3p) (11)



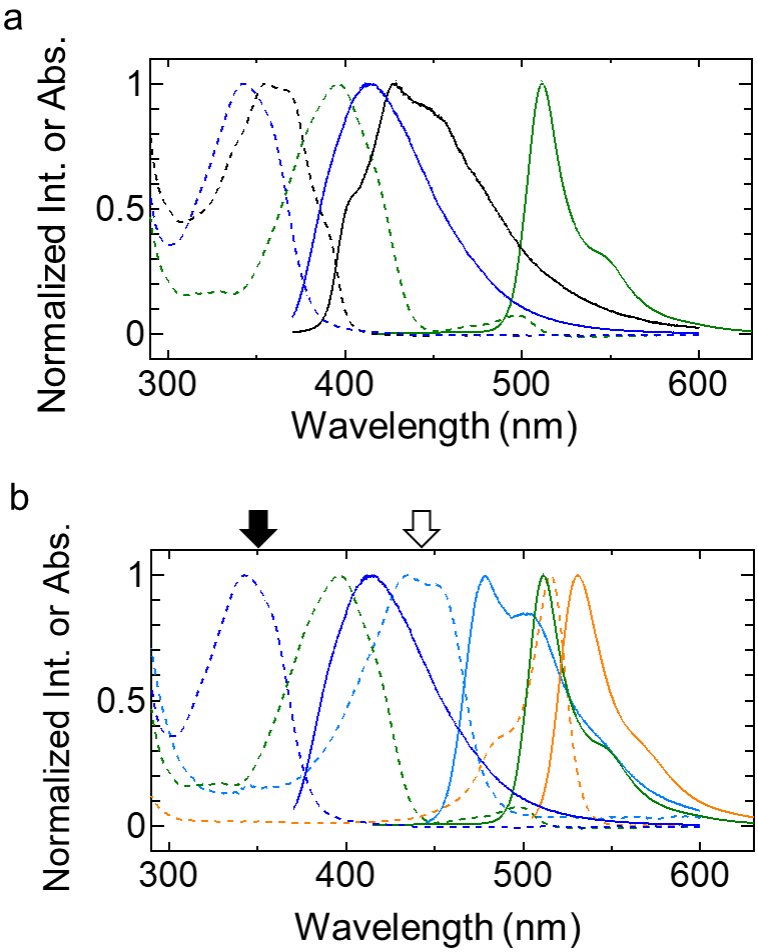
Supplementary Figure 2. The pH sensitivity of Sumire emission. The relative fluorescence intensities of Sumire (a), VFP0 (b), VFP1 (c) and VFP2 (d) were measured in 0.5 increments over a pH range of 3-9 in the buffer containing 30 mM trisodium citrate and sodium tetraborate, pH adjusted with HCl. Violet cycle, cyan triangle and green square indicate fluorescence intensities expected to be derived from hChr, nChr and iChr respectively.



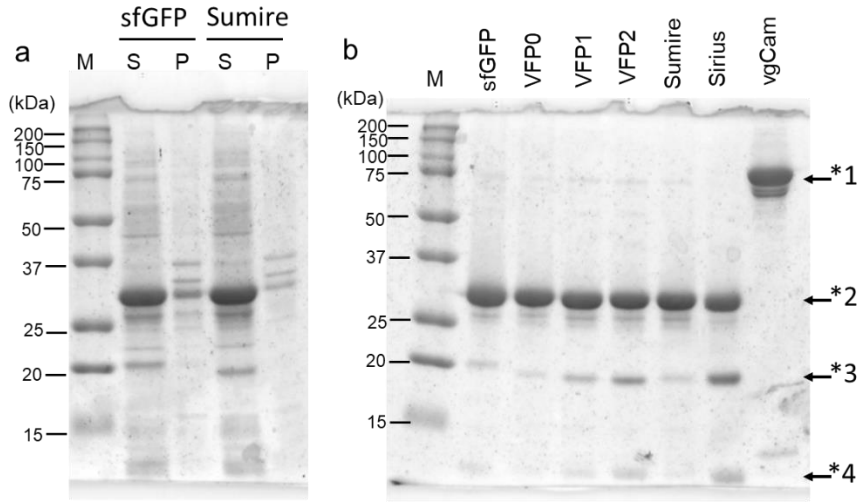
Supplementary Figure 3. Comparison of fluorescence intensity of Sumire and Sirius in HeLa cells using P2A assay. (a) Images of HeLa cells expressing mCherry P2A Sumire or Sirius under a microscope. Scale bar: 40 μm. (b) Signal intensity plots of violet (Sumire or Sirius) channel versus red (mCherry) channel. Solid line was an approximation using linear function of Sumire (blue) and Sirius (green). The slopes were 0.59 (Sumire) and 0.18 (Sirius). Three dishes each transfected with mCherry-P2A-Sumire and mCherry-P2A-Sirius were prepared and 42 and 45 cells were measured, respectively.



Supplementary Figure 4. Fluorescence localization. Fluorescence localization images of Sumire fused to (a) β-tubulin (mycrotubule) (b) CoxVIII signal peptide (mitochondria), (c) LifeAct (cytoskeleton), (d) vimentin (cytoskeleton), (e) H2B (nucleus), (f) fibrillarin (nucleoli), (g) LAMP1 (external part of lysosomes), and (h) non-fused sumire. Scale bars, 20 mm.



Supplementary Figure 5. Normalized spectra. (a, b) Normalized emission (solid line) and absorption spectra (dash line) of Sumire (blue), Sirius (black), T-Sapphire (green), ECFP (cyan), and Venus (yellow).



Supplementary Figure 6. Solubility and purity check. (a) Solubility check of Sumire. M: marker, S: supernatant, P: pellet. (b) 5 μg purified proteins were applied to the SDS page. *1: vgCam *2: FPs, *3 and *4: FP fragments expected to break the main chain in front of the chromophore.