

**Supplementary Table 1.** Photostability of selected fluorescent proteins.

Fluorescent protein	Excitation/emission maxima (nm)	Fluorescence quantum yield	Molar extinction coefficient, ( $M^{-1}cm^{-1}$ )	Laser line used (nm)	$t_{0.5}$ for bleach, s <sup>a</sup>
Cerulean	433/475	0.62 (0.56 <sup>b</sup> )	43,000 (35,000 <sup>b</sup> )	458 nm	287
EGFP	484/510	0.60	55,000	488 nm	1167
EYFP	513/527	0.61	83,400 (72,000 <sup>b</sup> )	514 nm	417
TagRFP	555/584	0.48	100,000	543 nm	364
DsRed-Express	557/579	0.40	30,000	543 nm	1304
mCherry	587/610	0.22	72,000 (78,000 <sup>b</sup> )	543 nm	352
mPlum	590/649	0.10	41,000 (22,000 <sup>b</sup> )	543 nm	300

<sup>a</sup>See legend for the **Supplementary Fig. 3**.

<sup>b</sup>Our data. Fluorescence quantum yield and molar extinction coefficient of Cerulean were measured in direct comparison with ECFP (EC = 32,500  $M^{-1}cm^{-1}$ , Fluorescence quantum yield = 0.4, Clontech data).

**Legend to Supplementary Fig. 3:**

Photobleaching was performed in living HeLa cells using Leica SP2 confocal microscope, excitation by laser lines of equalized output power (tuned to 40 $\mu$ W in a parked laser mode). Following parameters were used: beam expander 1 (providing high intensity of excitation light), zoom 4x, scan frequency 400 Hz, format 512x512, 1 image per 1.63 s. 458 nm laser line was used for Cerulean, 488 nm laser line for EGFP, 514 nm laser line for EYFP, 543 nm laser line for TagRFP, DsRed-Express, mCherry, and mPlum. Each curve is an averaged result from at least three cells, from at least two independent experiments. TagRFP displayed a biphasic rate of photobleaching, with a half-time similar to that of mCherry, mPlum, EYFP or Cerulean. Dashed gray lines indicate time to bleach 50% of TagRFP fluorescence.