Supporting Material

Figure Legends

Figure S-1 **Experimental setup**

Schematic showing the experimental setup for in vitro EGFP-CALI. An unfocused beam from a continuous wave (cw) Argon ion laser is reflected off a mirror into a single well of a Teflon chamber containing FP-GST sample. Each individual well of the sample chamber has 5 mm diameter, close to the laser beam diameter (3 mm) to insure irradiation of the entire sample volume (100 µl).

Figure S-2 **Reciprocity in flux and time of irradiation for EYFP-GST.**
EYFP fluorescence (A) and chromophore (B) photobleaching depends on the illumination dose only, with laser beam power and time of illumination being reciprocal (1200 mW and 600mW at 514 nm). The results are presented as a ratio of sample fluorescence signal after illumination ($F_{illuminated}$) and before illumination ($F_{control}$). Correspondingly, chromophore photobleaching is measured as ratio of sample absorbance at 514 nm after illumination ($A_{illuminated}$) and before illumination ($A_{control}$).

Figure S-3 **CALI depends on the power that is used to deliver a given dose.**
Both the CALI and fluorophore and chromophore photobleaching is more pronounced at similar doses when GST-EGFP is illuminated with 1200 mW (A) than with 600 mW (B) at 488 nm laser power. Figure S-3A is reproduced from text figure 1B for reader convenience. All measurements (activity, blue; fluorescence, red and absorbance, green) were normalized to the initial values of non-illuminated sample to give relative values on the ordinate.

Comment [OU1]: Zenon: change ordinate as in Fig 1 in paper.

Figure S-4 **CALI of EGFP-GST in bacterial cell lysates.**

GST-EGFP activity (open bars) and EGFP fluorescence (full bars) decrease with increasing illumination dose, showing the same trend as purified protein. The presence of NaN_3 diminishes CALI effect while the decrease in fluorescence remains comparable to that without NaN_3. Concentration of GST-EGFP in lysate is estimated to be ~ 12μM based on enzyme activity. All measurements (activity, open bars) and fluorescence (filled bars) were normalized to the initial values of non-illuminated sample (control) to give relative values on the ordinate.

Comment [JJ2]: Zao and Zenon: you need to define what the ordinate is in the legend and on the figure.

Comment [OU3]: Zenon: change ordinate as in Fig 1 in paper.

Comment [JJ4]:

Figure S-5 **Effect of singlet oxygen quencher sodium azide on ECFP & EYFP-mediated CALI.**

A and B: Enzymatic activity of GST–EYFP decreases after 144J dose illumination with 514 nm laser light. Enzymatic activity of GST-EYFP is protected from CALI inactivation in the presence of 100mM NaN3 (panel A). A similar effect is observed for GST-ECFP (216J of 457.9nm laser light) (panel B). C and D: Fluorophore bleaching in the presence and absence of sodium azide for GST-ECFP and GST-EYFP, respectively. For A-D, all measurements were normalized to the initial values of non-illuminated sample (control) to give relative values on the ordinate. E and F: Absorbance spectra of GST-ECFP (panel E) and GST-EYFP (panel F) show the chromophore photobleaching in the presence and absence of sodium azide. These results are similar to those of GST-EGFP (Figure 3B) where CALI of GST activity is inhibited in the presence of azide yet the bleaching of the chromophore /fluorophore is relatively unaffected.

Comment [JJ5]: Again, for A, B, C, D you need to define ordinate in legend and on Figure.

Comment [OU6]: Zenon: change ordinate as in Fig 1 in paper.

Comment [JJ7]: In E, you need to state why you do control in presence of azide!

Figure S-6 **CALI is not due to laser heating-induced thermal denaturation of GST.**

(A) Laser illumination of 15.2 μM of EGFP-GST increases the sample temperature (blue diamonds) but not of buffer alone (red triangles). (B) EGFP-GST activity (triangles), as well as the chromophore (empty squares) and the

fluorophore (full squares) are thermally stable up to 50 C. (C) SDS PAGE analysis of EGFP-GST shows no crosslinking or degradation products after incubating EGFP-GST at different temperatures, 49 C, 52 C and 55 C for 5 min demonstrating that molecular integrity of EGFP-GST is retained after laser illumination.