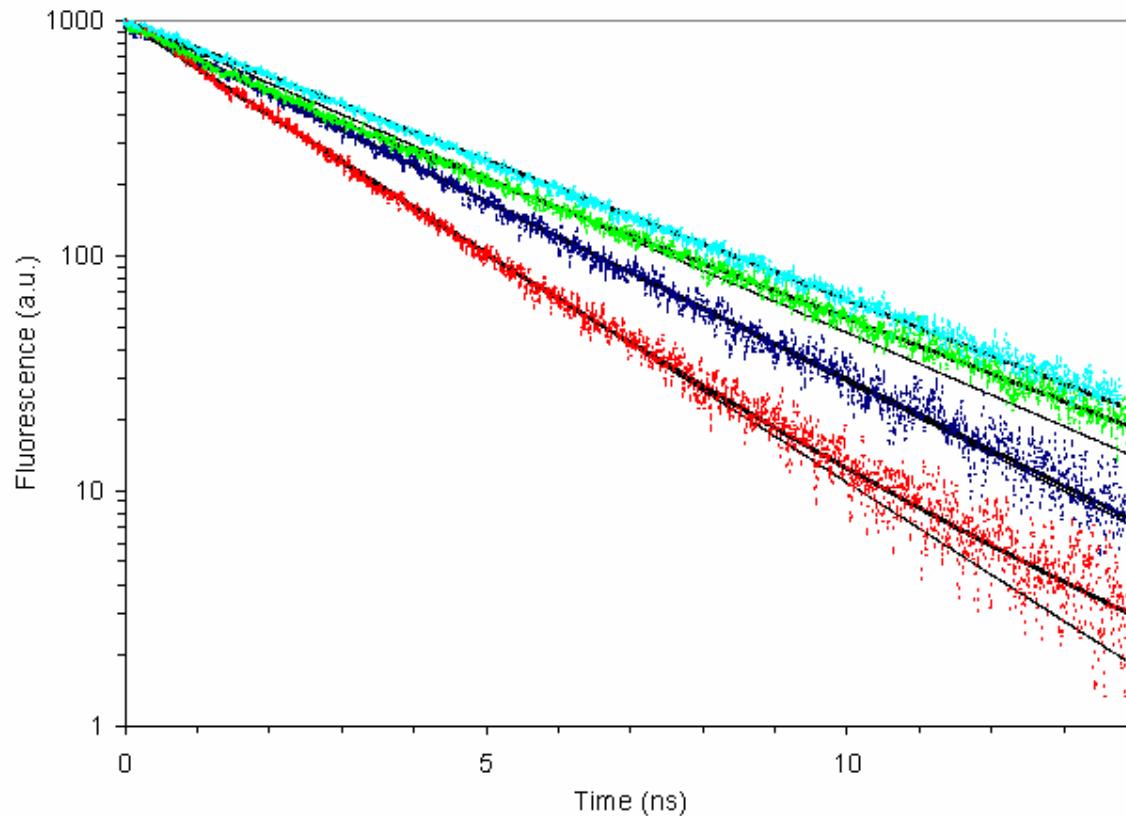


Supplementary Figure 3. Fluorescence lifetime analysis of BFP mutants for BFP (blue), BFP+F64L/V163A (red), A5 (green), Azurite (cyan). Lines indicate fit to monoexponential curve (thin) and double exponential (bold). Monoexponential and double exponential lines are indistinguishable for Azurite.



| Clone | Monoexponential Fit | | Double Exponential Fit | | | | |
|-----------------|---------------------|------------|------------------------|---------------|------------|-----------------------|------------|
| | τ (ns) | χ^2_R | α_1 | τ_1 (ns) | α_2 | τ_2 (ns) | χ^2_R |
| BFP | 2.82 | 0.61 | 0.97 | 2.87 | 0.03 | 1.00×10^{-9} | 0.54 |
| BFP(F64L/V163A) | 2.21 | 0.61 | 0.04 | 4.39 | 0.96 | 2.10 | 0.45 |
| A5 | 3.27 | 1.50 | 0.80 | 3.70 | 0.20 | 1.06 | 0.31 |
| Azurite | 3.65 | 0.29 | 1.00 | 3.65 | 0.00 | 9.17×10^{-4} | 0.29 |