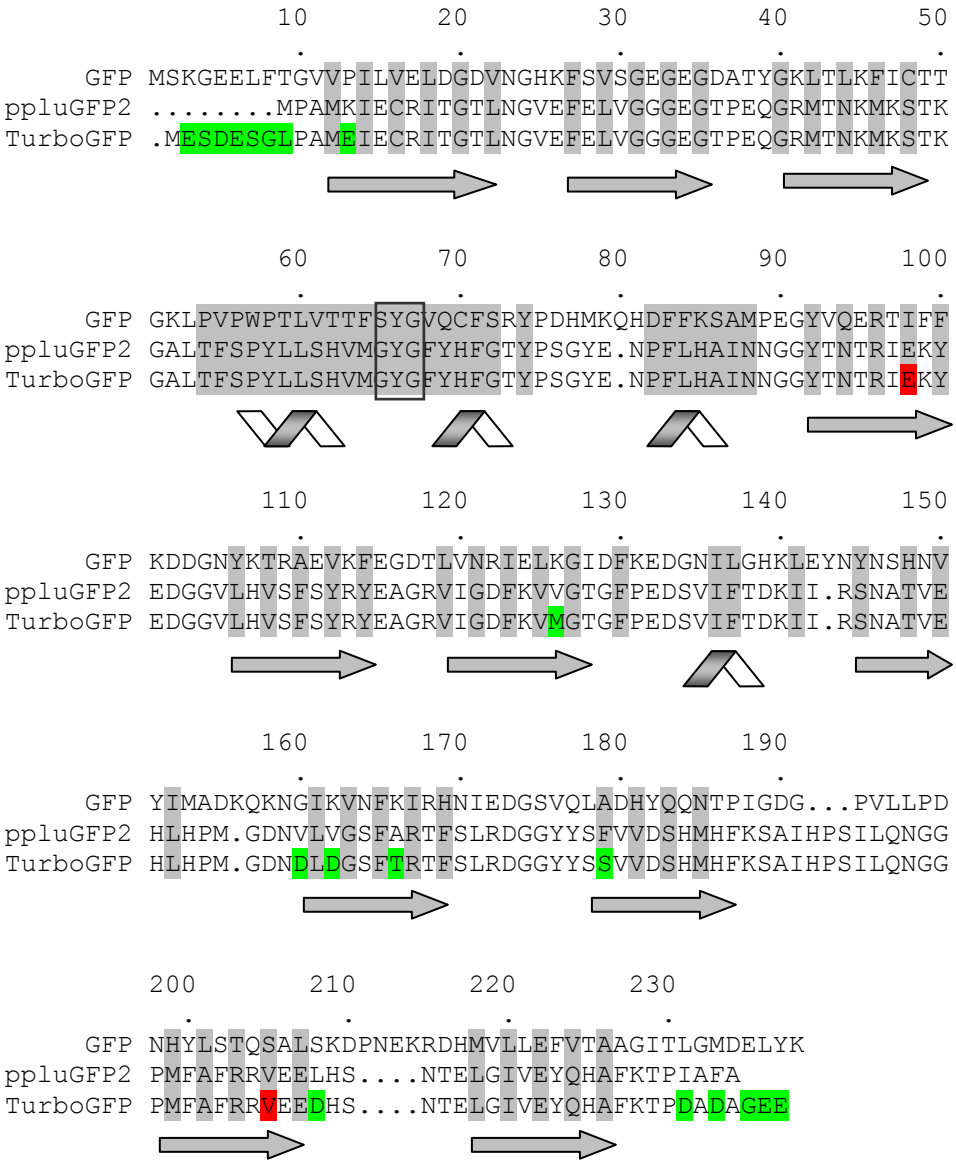
Supplementary Table 1

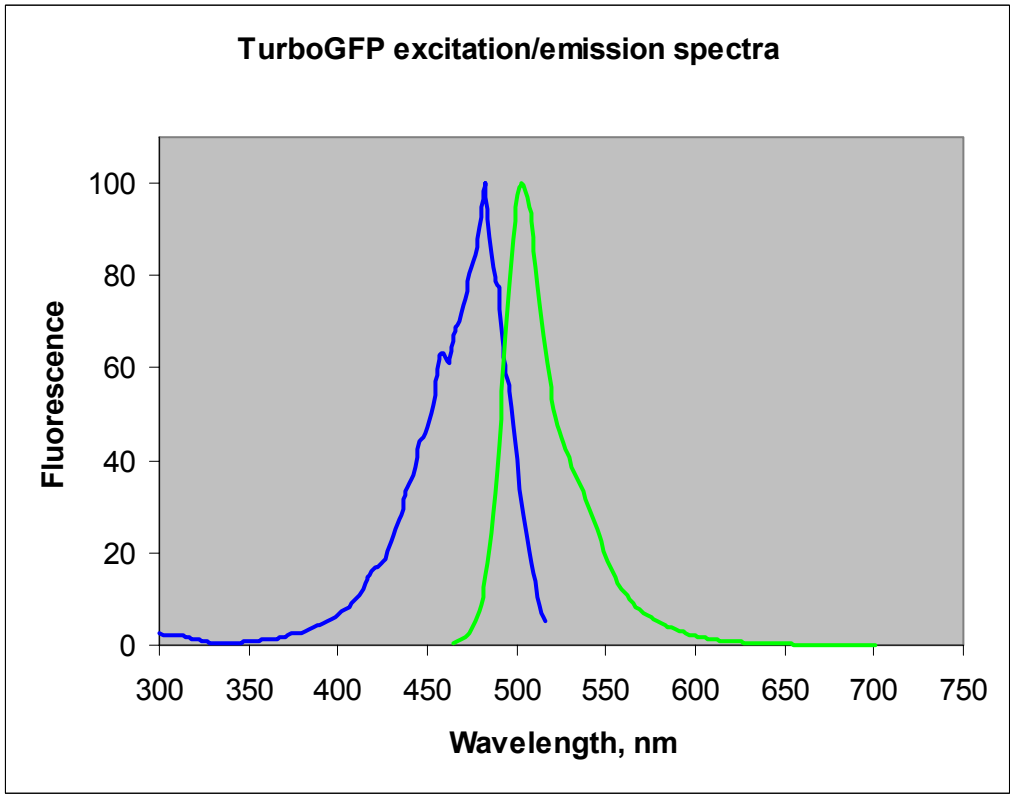
Data collection and refinement statistics (Molecular Replacement)

	Crystal form 1	Crystal form 2
Data collection		
Space group	$P2_1$	$C222_1$
Cell dimensions		
a, *b*, *c* (Å)	68.0, 97.16, 75.15	97.75, 97.91, 172.32
α, β, γ (°)	90, 96.47, 90	90, 90, 90
Resolution (Å)	100-1.6 (1.7-1.6) *	2.1 (2.2-2.1)
R_{sym} or R_{merge}	0.045 (0.40)	0.052 (0.31)
$I / \sigma I$	14.9 (2.2)	15.4 (3.3)
Completeness (%)	96.5 (86.0)	98.9 (98.7)
Redundancy	2.2 (1.7)	4.2 (3.0)
Refinement		
Resolution (Å)	100-1.6 (1.7-1.6)	2.1 (2.2-2.1)
No. reflections	116015 (8078)	45982 (3476)
R_{work} / R_{free}	0.17/0.23	0.19/0/24
No. atoms		
Protein	6897	6897
Ligand/ion	76	76
Water	1138	210
B-factors		
Protein	38	34
Ligand/ion	19	19
Water	45	39
R.m.s deviations		
Bond lengths (Å)	0.009	0.024
Bond angles (°)	1.6	3.0

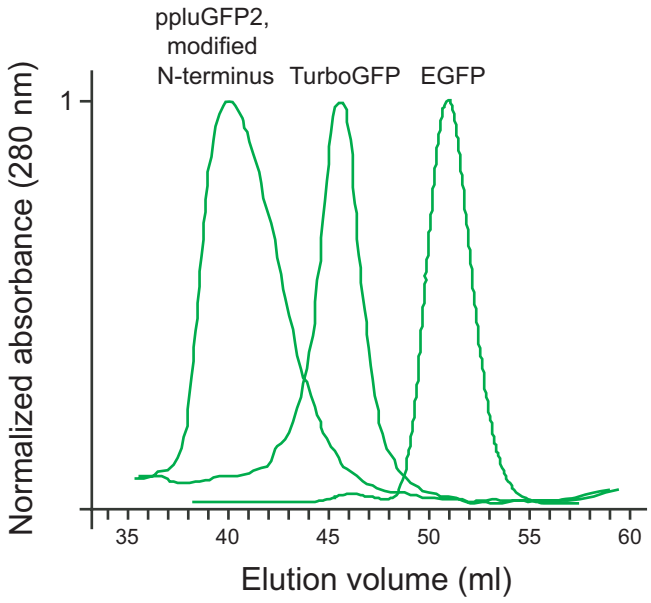
* Single crystal of each form was used for data collection, highest resolution shell is shown in parenthesis.



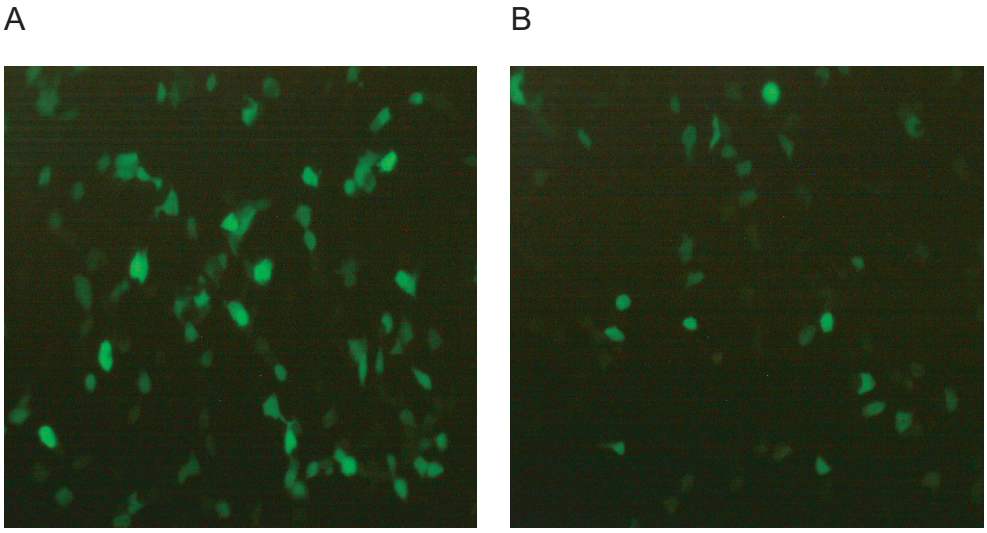
Supplementary Figure 1. *Aequorea victoria* GFP, ppluGFP2 and TurboGFP protein sequences alignment. Numbering is given according to *Aequorea victoria* GFP. Structurally important regions are highlighted in grey, beta-strands are shown with arrows, alpha-helixes are shown with ribbons. Chromophore forming residues are boxed. Mutations that resulted in TurboGFP are highlighted in green. Positions E89 and V197, studied in the work, are highlighted in red.



Supplementary Figure 2. TurboGFP fluorescence excitation/emission spectra. Excitation spectrum was measured for 530 nm emission. Emission spectrum was measured upon excitation at 450 nm.



Supplementary Figure 3. Gel filtration of EGFP, TurboGFP and low-aggregating variant of ppluGFP2.



Supplementary Figure 4. Degradation of destabilized TurboGFP. Mammalian cells expressing destabilized TurboGFP were treated with DMSO (A) or 100 mkg/ml cycloheximide (B) for 1.5 hr.