Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

SUPPLEMENTARY MATERIAL

Strategy of mutual compensation of green and red mutants of firefly luciferase identifies a mutation of the highly conservative residue E457 with a strong red shift of bioluminescence

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Table S1. PCR primer sequences

Name	Primer sequence
f_XhoI	5'- GTATTCAGCTCGAGAAAAGGCTTACC -3'
r_T7term	5'- GCTAGTTATTGCTCAGCGG -3'
Forward E457V	5'- GCTGAATTGGTATCCGTTCTTTTGC -3'
Reverse E457V	5'- AGAACGGATACCAATTCAGCAGG -3'
Forward A534R	5'- GGTAAAATTGATCGTAAAGTAATTAGAGAAATTCTTAAG -3'
Reverse A534R	5'- CTAATTACTTTACGATCAATTTTACCAGTTAGACC -3'
Forward E457X	5'- TCCGTTCTTTTGCAACATC -3' (universal primer for mutants of E457)
Reverse E457Q	5'- GTTGCAAAAGAACGGATTGCAATTC -3'
Reverse E457D	5'- GTTGCAAAAGAACGGAATCCAATTC -3'

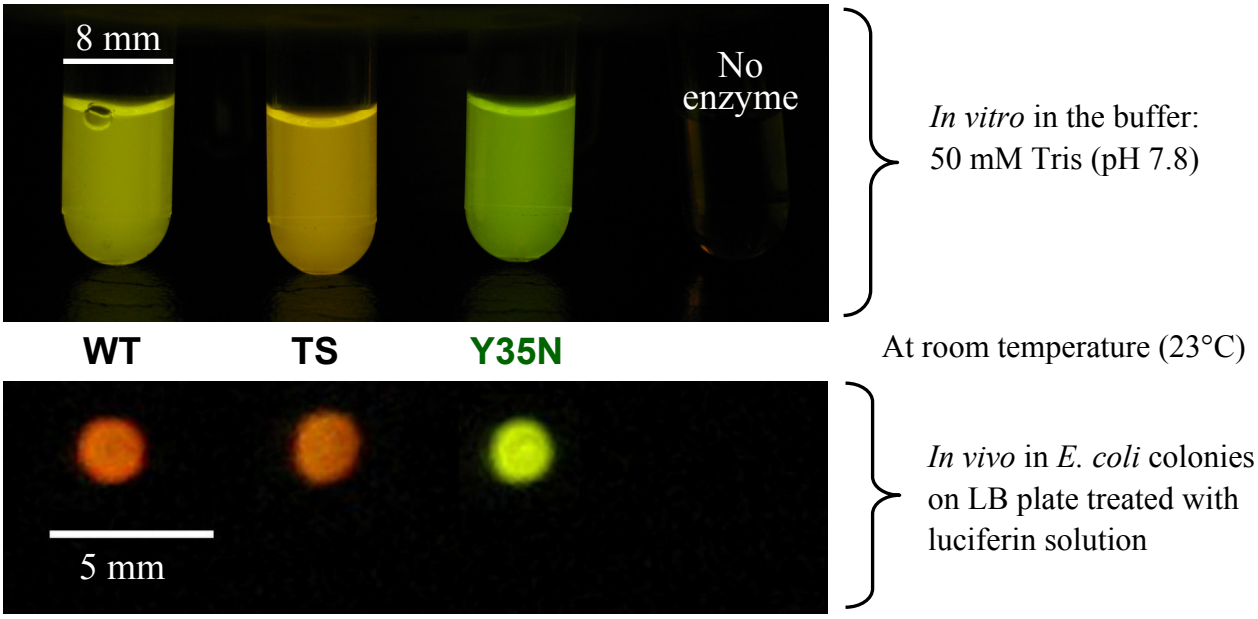

Figure S1. Comparison of *in vitro* bioluminescence in the reaction buffer (7.8) and *in vivo* bioluminescence in *E. coli* cells. For *in vivo* bioluminescence see also Fig. S4.

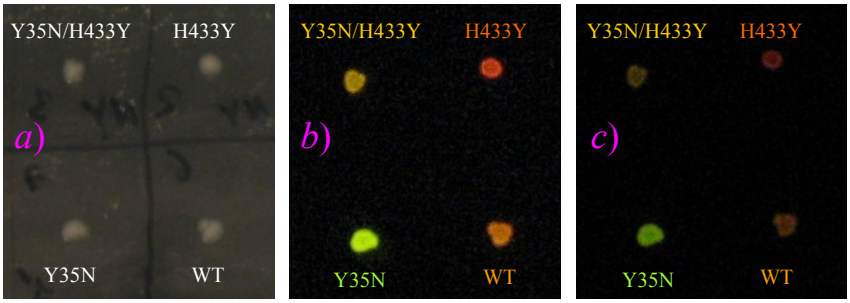

Figure S2. *In vivo* bioluminescence of *E. coli* colonies producing WT luciferase and the mutants Y35N, H433Y and Y35N/H433Y. Mutants were expressed using the pLR4 plasmid in *E. coli* XL1-blue. The plate was photographed: a) under the ambient light; b) in the dark with a higher effective exposure time; c) in the dark with a lower effective exposure time.

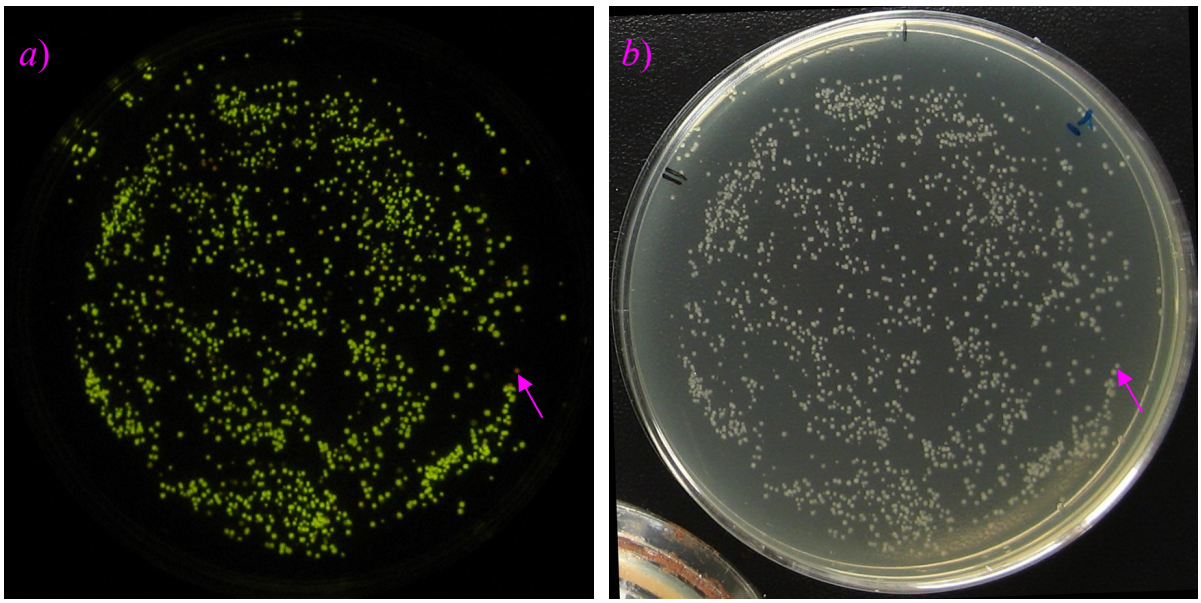

Figure S3. Typical screening of the 90 mm plate with mutant *E. coli* colonies for changed bioluminescence color. *In vivo* bioluminescence (*a*) and the original plate in ambient light (*b*). The parent luciferase for this screening was the mutant Y35N+TS, which produces green bioluminescence. The red-shifted mutant E457K is marked by the arrow.

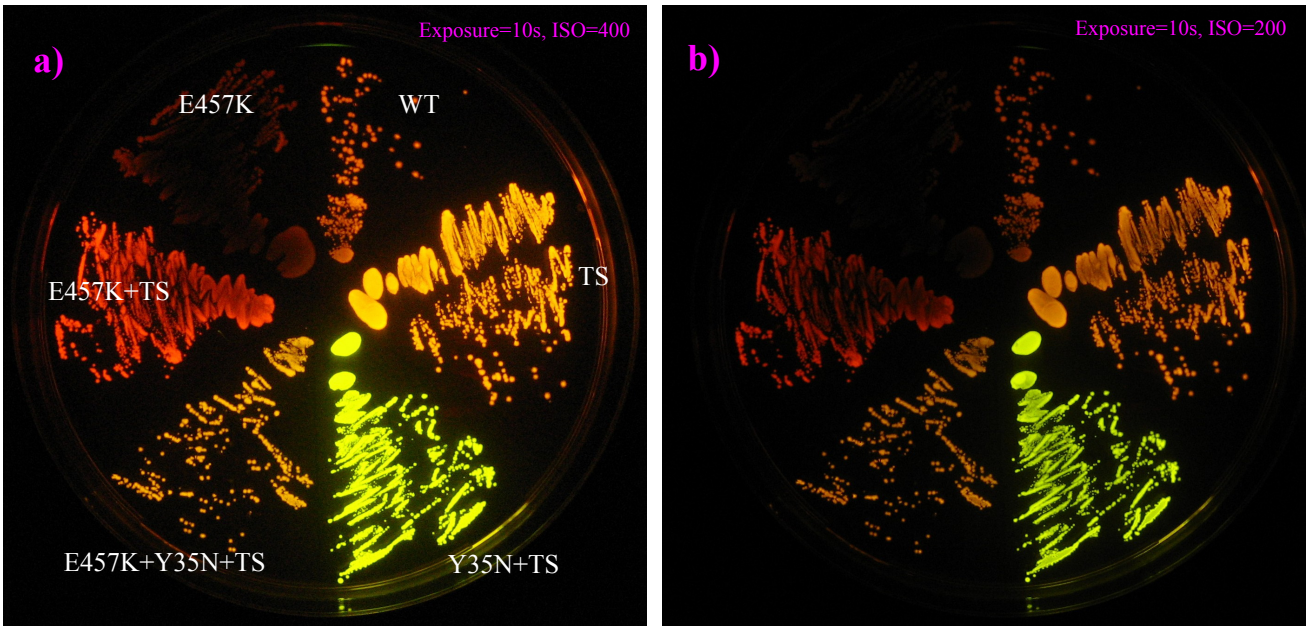

Figure S4. *In vivo* bioluminescence of *E. coli* streaks containing the WT luciferase and the mutants 4TS, E457K, E457K/TS, Y35N/TS and Y35N/E457K/TS. Luciferases were expressed using the pLR4 plasmid in *E. coli* XL1-blue. The 90 mm plate was photographed in the dark with a: a) higher effective exposure time; b) lower effective exposure time.

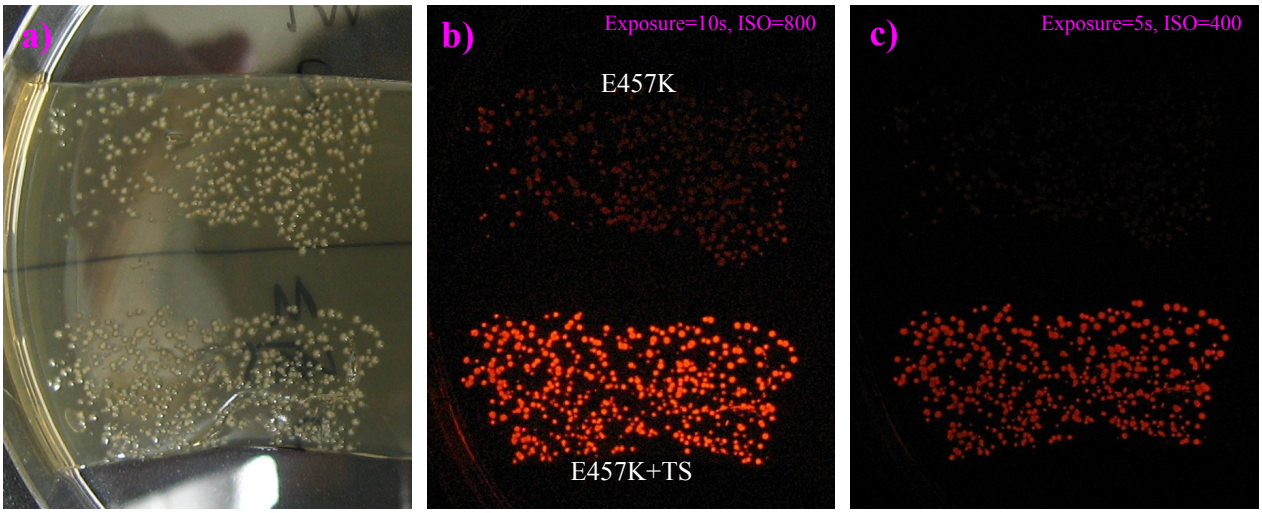

Figure S5. *In vivo* bioluminescence of *E. coli* colonies containing the mutant E457K and E457K/Y35N/TS. The expression vector pETL7 (derived from pET23b) carrying the respective mutant was transformed into *E. coli* strain BL21(DE3). This vector contains the "plain" T7 promoter which results in a considerable level of uninduced expression of luciferase. The 90 mm plate was photographed: a) under the ambient light; b) in the dark with a higher effective exposure time; c) in the dark with a lower effective exposure time.

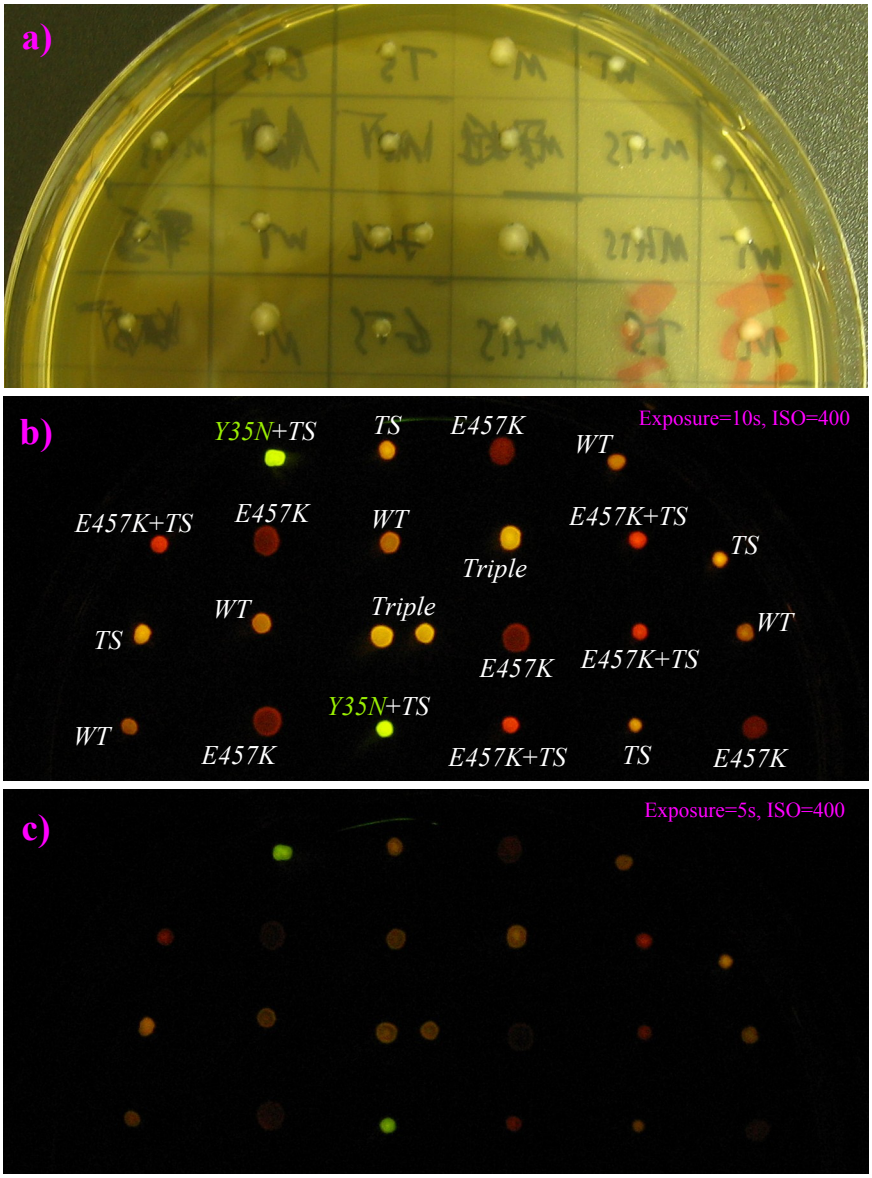

Fig. S6. *In vivo* bioluminescence of *E. coli* colonies containing the WT luciferase and the mutants TS, E457K, E457K/TS, Y35N/TS and Y35N/E457K/TS (*Triple*). Luciferases were expressed using the pLR4 plasmid in *E. coli* XL1-blue. The 90 mm plate was photographed: a) under the ambient light; b) in the dark with a higher effective exposure time; c) in the dark with a lower exposure time.

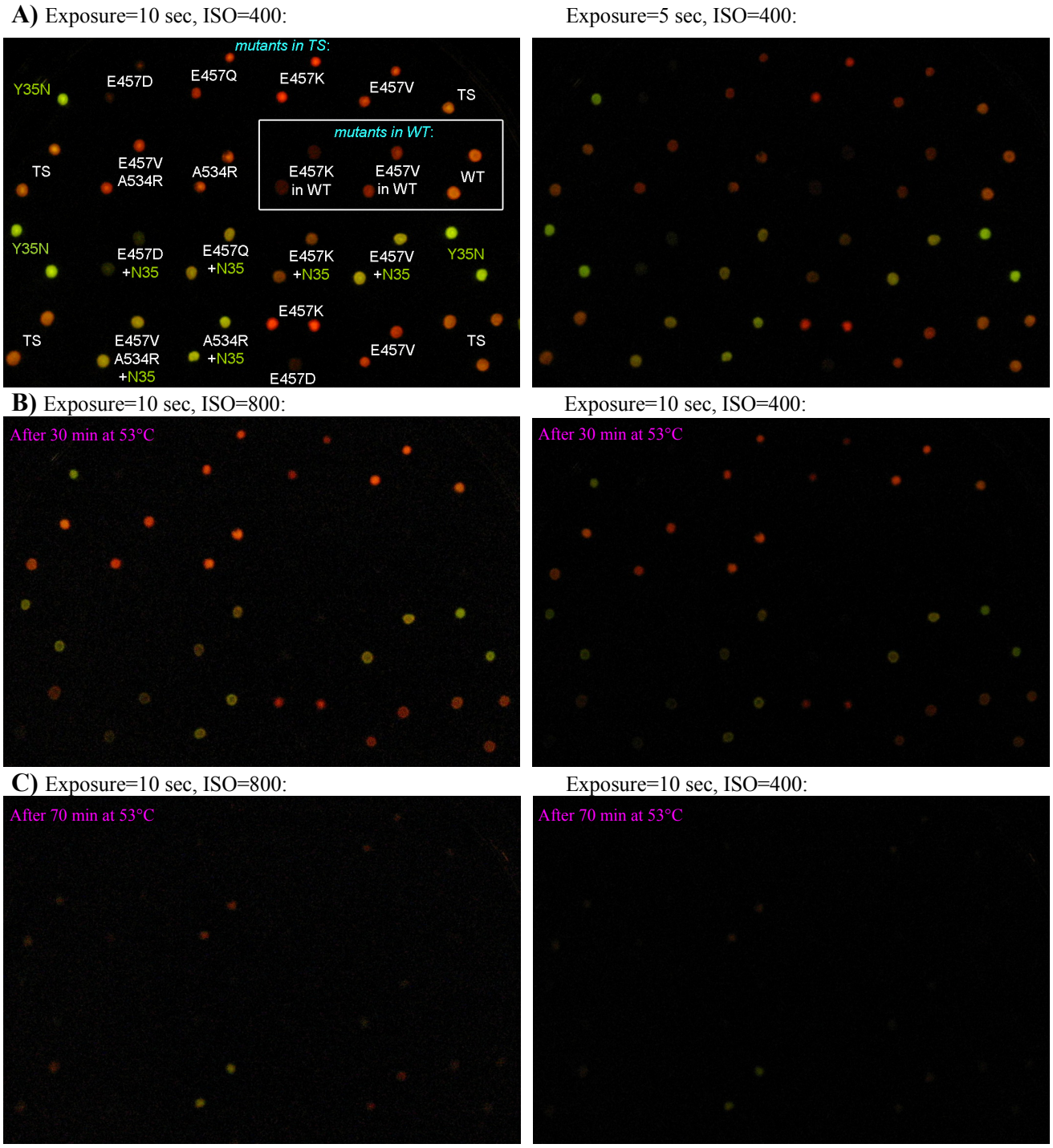

Fig. S7. *In vivo* bioluminescence and thermostability of mutant luciferases in *E. coli* XL1-blue colonies. Luciferases were expressed using pLR4-based plasmids. Bioluminescence was photographed after treating the cells with luciferin solution (**A**). Then the cells were incubated at 53°C and residual bioluminescence was photographed after 30 min (**B**) and after additional 40 min (**C**). The parent form for the mutants is TS except for two mutants in WT that are highlighted by the rectangle. Each time point is represented with photograph at higher and lower effective exposure time for better discrimination of relative differences in intensity and color of bioluminescence of mutant colonies.

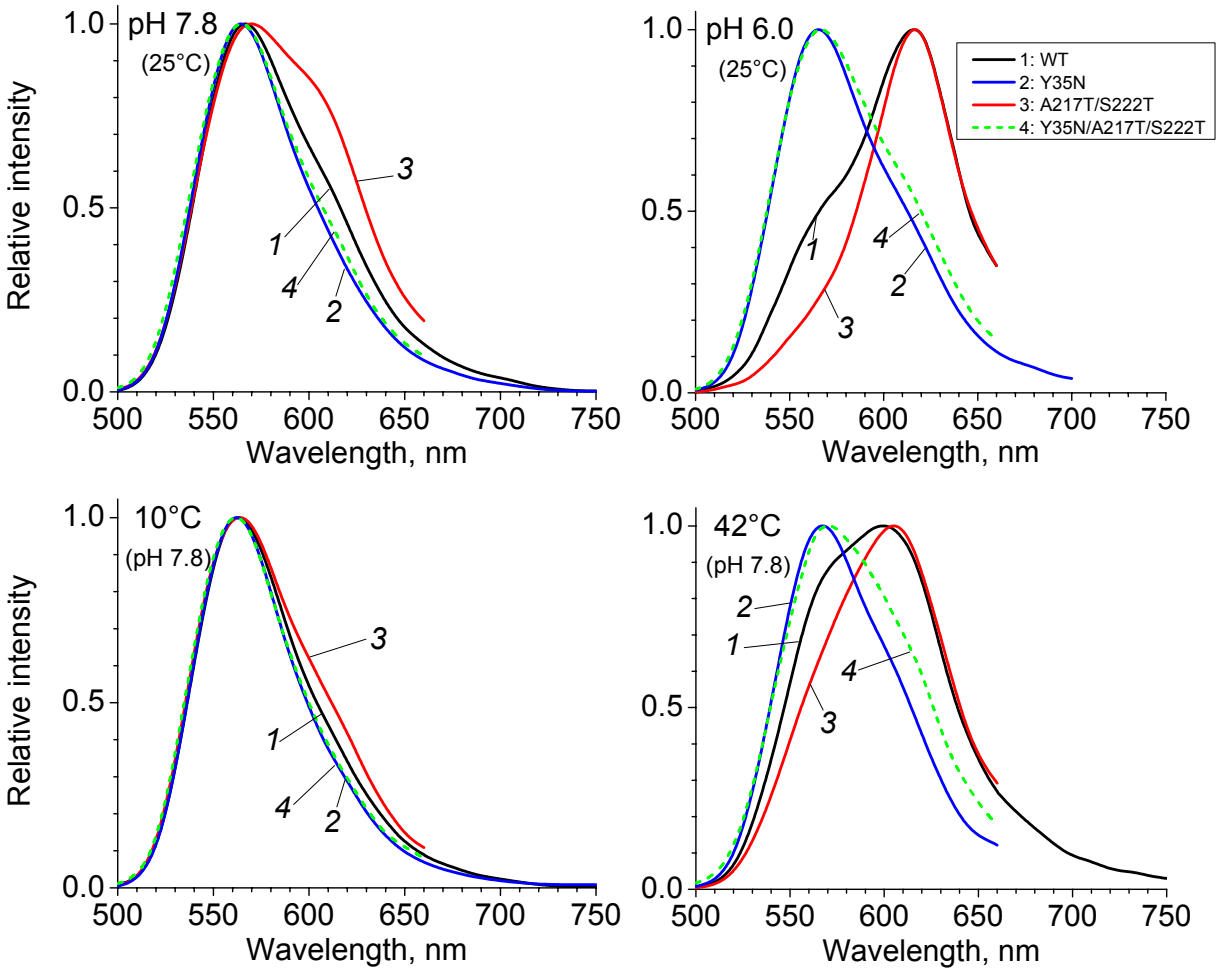

Fig. S8. Combined effect of mutations Y35N and A217T/S222T. Bioluminescence spectra of WT (*1*) luciferase and mutants Y35N (*2*), A217T/S222T (*3*), Y35N/A217T/S222T (*4*) at pH 7.8 (25°C), pH 6.0 (25°C), 10°C (pH 7.8) and at 42°C (pH 7.8).

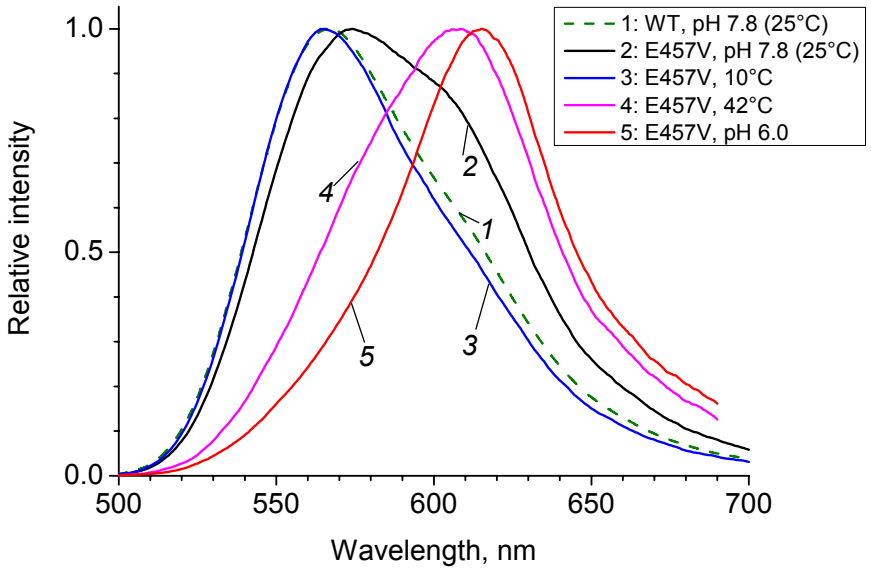

Fig. S9. Effect of the mutant E457V on bioluminescence spectra. Bioluminescence spectra of WT luciferase at pH 7.8 (*1*) and the mutant E457V at pH 7.8 (*2*), 10°C (*3*), 42°C (*4*) and pH 6.0 (*5*). If not specified, the temperature is 25°C and the pH value is 7.8.

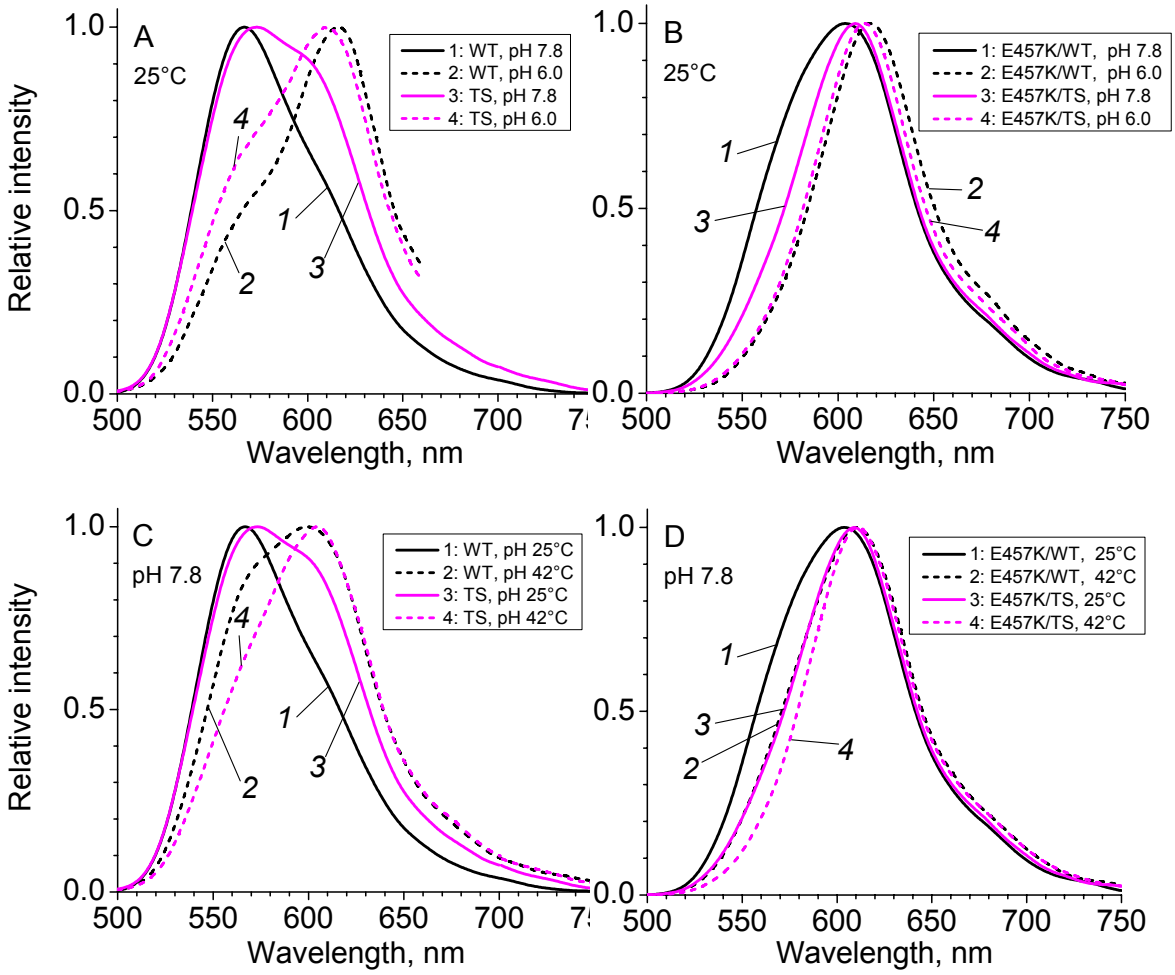

Fig. S10. Effect of the mutant TS on bioluminescence spectra of WT luciferase (A, C) and of the mutant E457K (B, D) at pH 7.8 and pH 6.0 at 25°C (A, B) or at 25 and 42°C at pH 7.8 (C, D).