SUPPLEMENTARY MATERIAL

Thermostabilization of firefly luciferase by *in vivo* directed evolution

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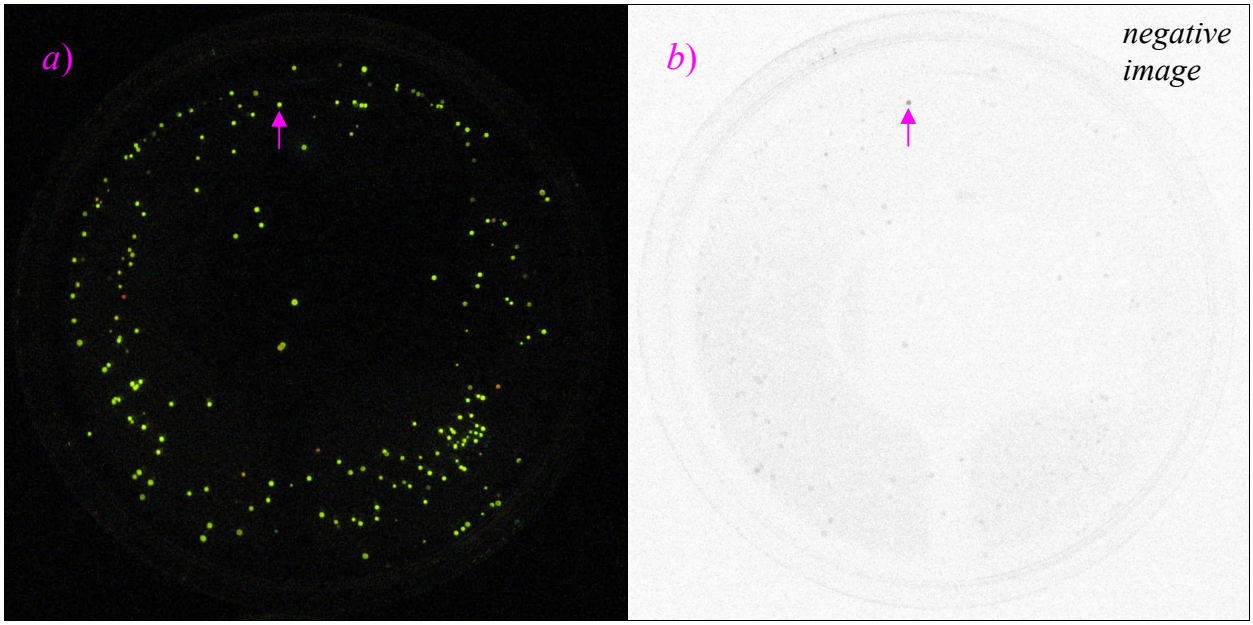

Fig. S1. Typical screening of the 90 mm plate with mutant *E. coli* colonies for thermostability (second cycle of random mutagenesis). *In vivo* bioluminescence before (*a*) and after (*b*) incubation of the plate at 50°C. The thermostable mutant 2T1 is marked by the arrow. Green bioluminescence of most of the colonies is due to the presence of the Y35N mutation in the cloning vector used at 2nd-4th cycles.

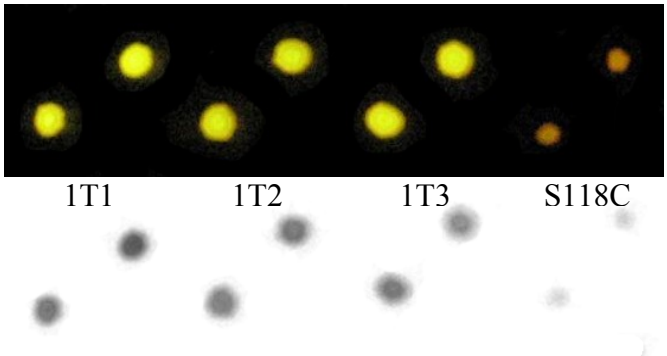

Fig. S2. *In vivo* bioluminescence of *E. coli* XL1blue colonies expressing the luciferase mutants 1T1-1T3 and their parent enzyme S118C. Bioluminescence was induced after the growth of cells at 37°C.

Table S1. Constants of irreversible thermal inactivation of WT Lml and the mutant 4TS at 37-55°C

Enzyme	Buffer	k_{in}, h^{-1}					
		37°C	42°C	45°C	47°C	50°C	55°C
WT	TsB1	0.95 (to 50%) 0.58 (further)	4.57	15.1	n.d.[1)]	68	n.d.
	TsB2	0.02	0.73	3.27	n.d.	18.6	n.d.
4TS	TsB1	0.04 (to 80%) 0.0037 (further)	0.07	0.95	2.83	11.1	99
	TsB2	n.d.	n.d.	0.021	0.047	0.94	31
$\frac{k_{in}(WT)}{k_{in}(4TS)}$	TsB1	168	65	16	-	6	-
	TsB2	-	-	155	-	20	-

[1)] n.d. – not determined

Note: The error associated with k_{in} falls within 10% of the value.

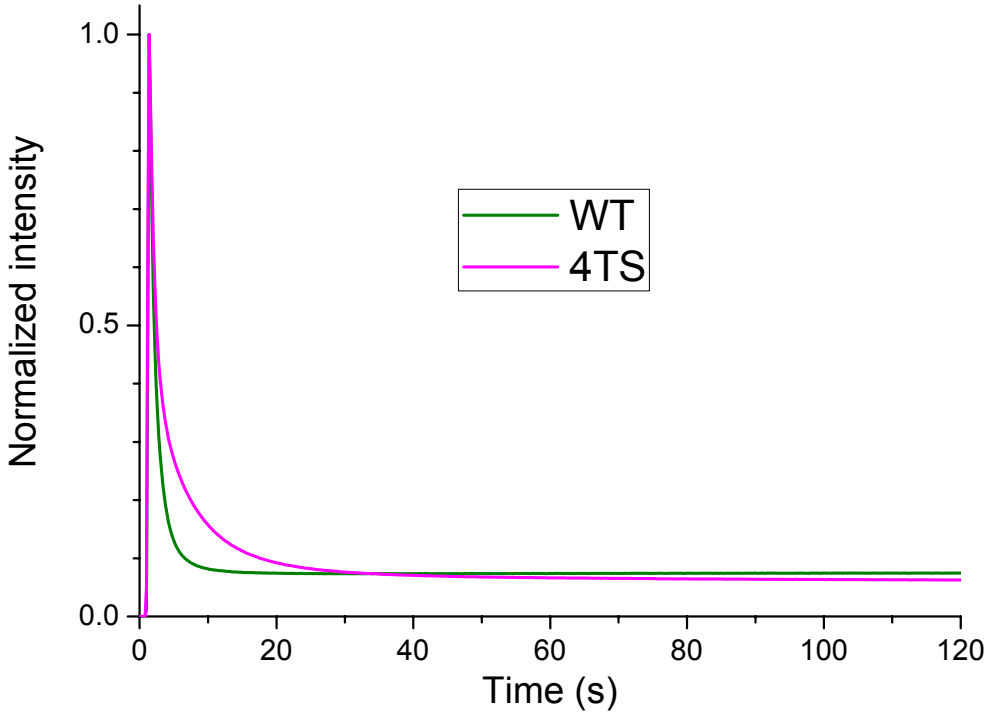

Fig. S3. Normalized light emission kinetic profiles of WT Lml and the mutant 4TS. Each curve is a mean of 6 (WT) or 8 (4TS) measurements. Flash heights and integrated intensities were within 10% of the mean value. Reactions were conducted at the saturating concentrations of the substrates as described in Materials and Methods. The final reaction mixture contained $2 \cdot 10^{-6}$ mg/ml luciferase, 0.15 mM LH_2 and 1.2 mM ATP in 50 mM Tris-acetate (pH 7.8) buffer containing 10 mM $MgSO_4$ and 2 mM EDTA.

Color versions of the figures presented in the main text

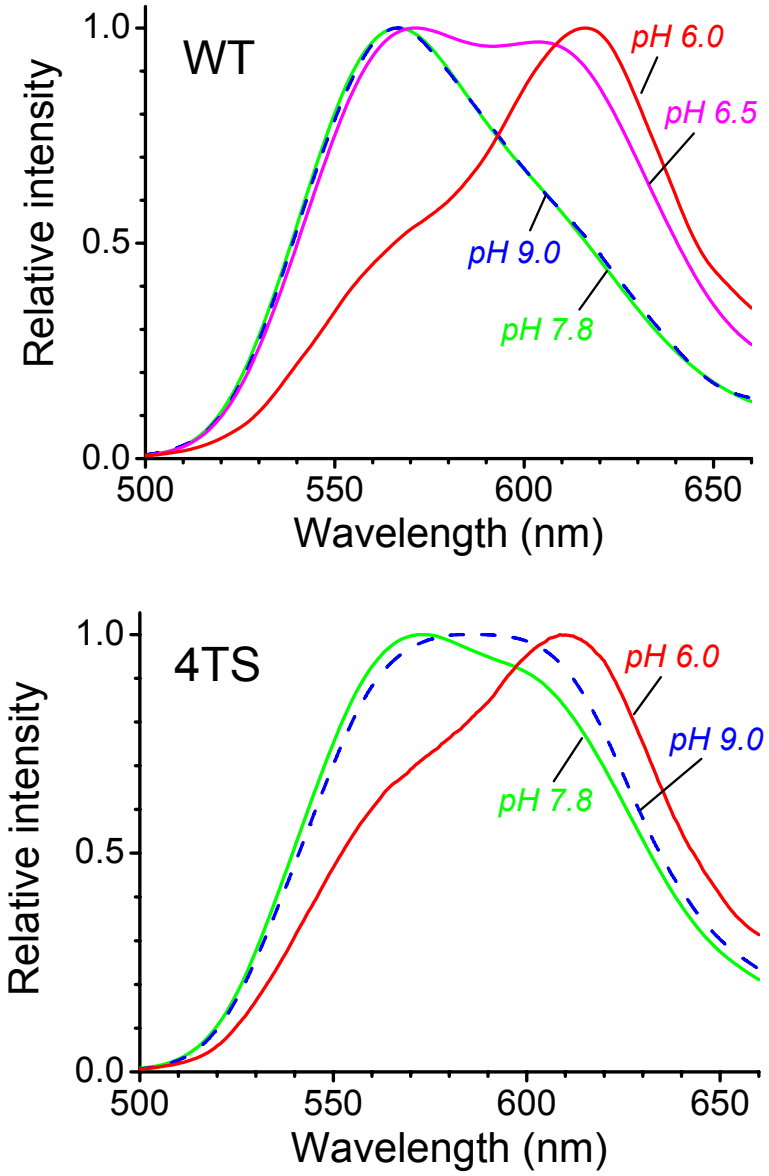

Fig. 2. Bioluminescence spectra of WT luciferase and the mutant 4TS at different pH (t = 25°C).

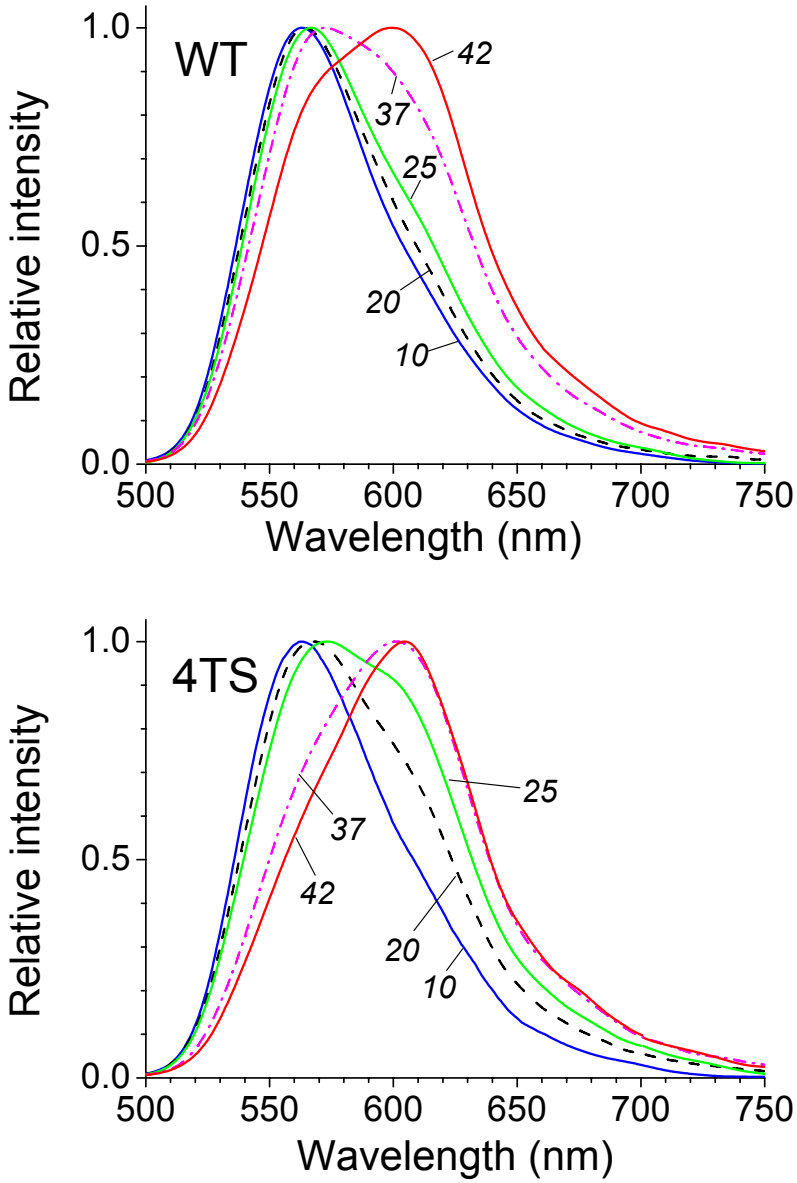

Fig. 3. Bioluminescence spectra of WT luciferase and the mutant 4TS at temperatures 10, 20, 25, 37, 42°C (pH 7.8).

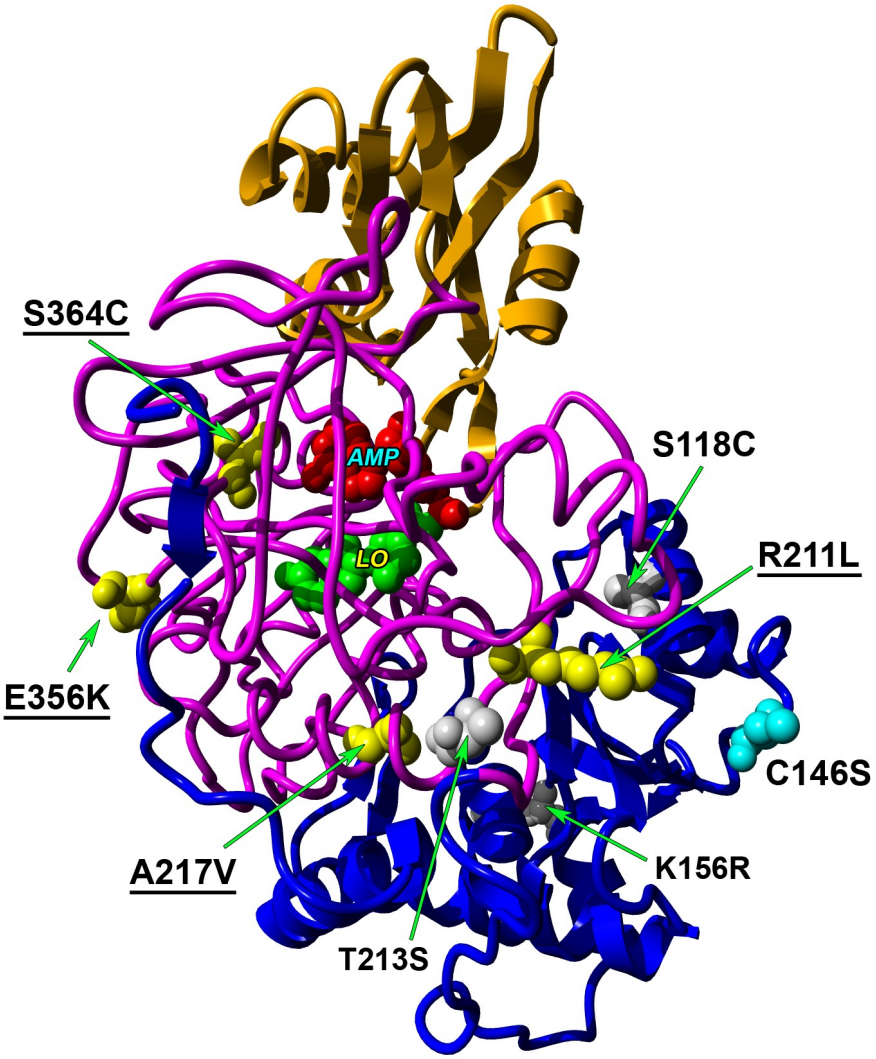

Fig. 7. Homology model of Lml showing the location of substitutions in the mutant 4TS. Four key thermostabilizing mutations are underlined. LO and AMP – luciferyl and adenylate groups of DLSA (5'-*O*-[N-(dehydroluciferyl)-sulfamoyl] adenosine). Subdomains A, B and C are depicted in blue, magenta and orange respectively.