Supplementary materials

New synthetic red- and orange-emitting luciferases to upgrade in vitro and 3D cell biosensing

Maria Maddalena Calabretta[a,b], Denise Gregucci[a,b] and Elisa Michelini[a,b,c]*

[a]Department of Chemistry "Giacomo Ciamician", University of Bologna, Via Selmi 2, 40126, Bologna, Italy

[b]Center for Applied Biomedical Research (CRBA), Azienda Ospedaliero-Universitaria Policlinico S. Orsola-Malpighi, 40138 Bologna, Italy

[c]Health Sciences and Technologies Interdepartmental Center for Industrial Research (HSTICIR), University of Bologna, 40126, Bologna, Italy

*Correspondence:

Prof. Dr. Elisa Michelini
Departement of Chemistry "Giacomo Ciamician"
University of Bologna
Via Selmi 2, 40126 Bologna, Italy
Tel.: +39 051 20 9 9533
e-mail: elisa.michelini8@unibo.it

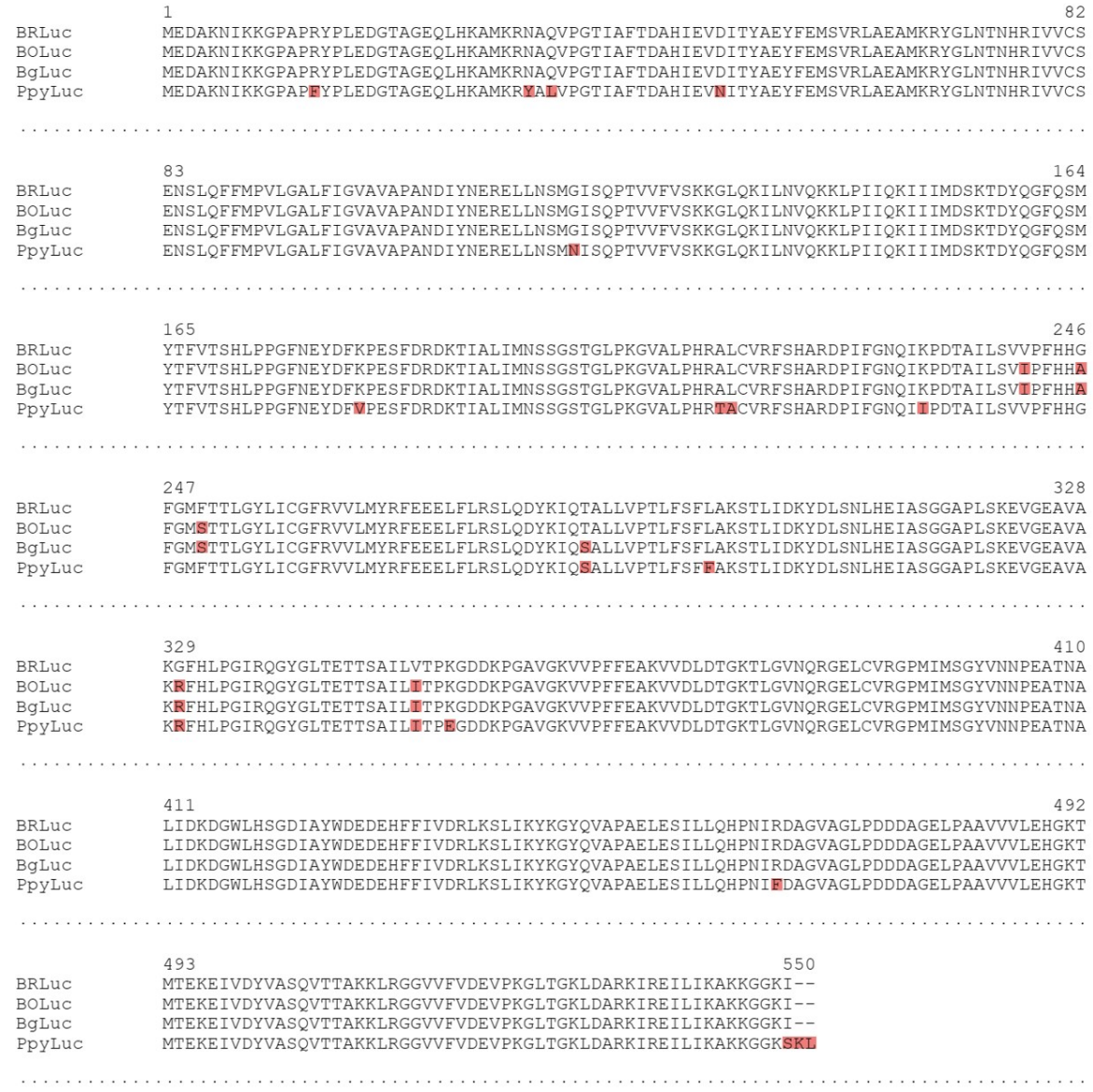

Figure S1: Amino acid alignment of the BrLuc, BoLuc, BgLuc and PpyLuc luciferases. The red highlighted residues indicate the mutated amino acids.

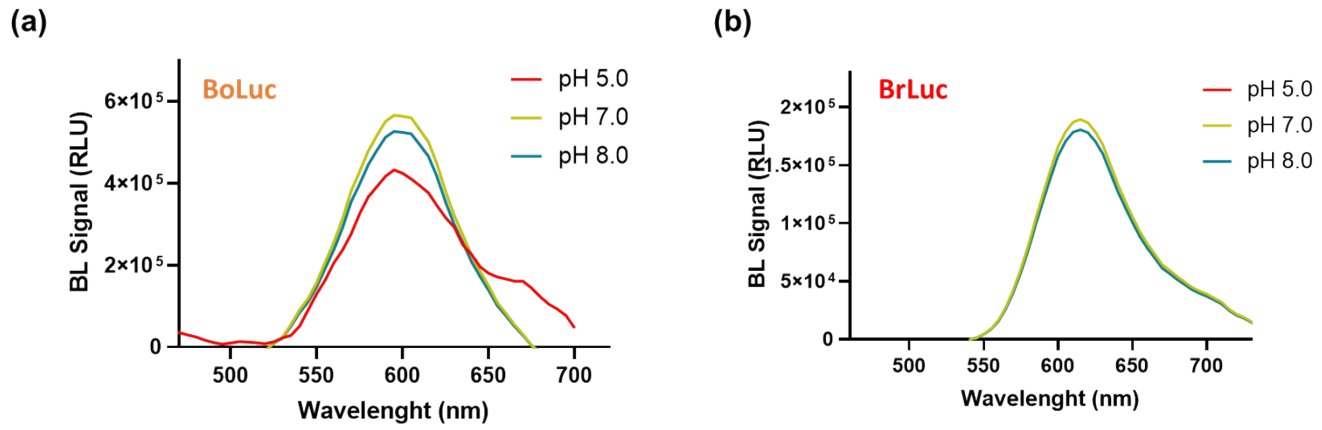

Figure S2: (a) BoLuc mutant and (b) BrLuc mutant emission spectra obtained at different pH (5.0, 7.0 and 8.0) with D-Luciferin substrate. BrLuc emission at pH 5.0 was not detectable.

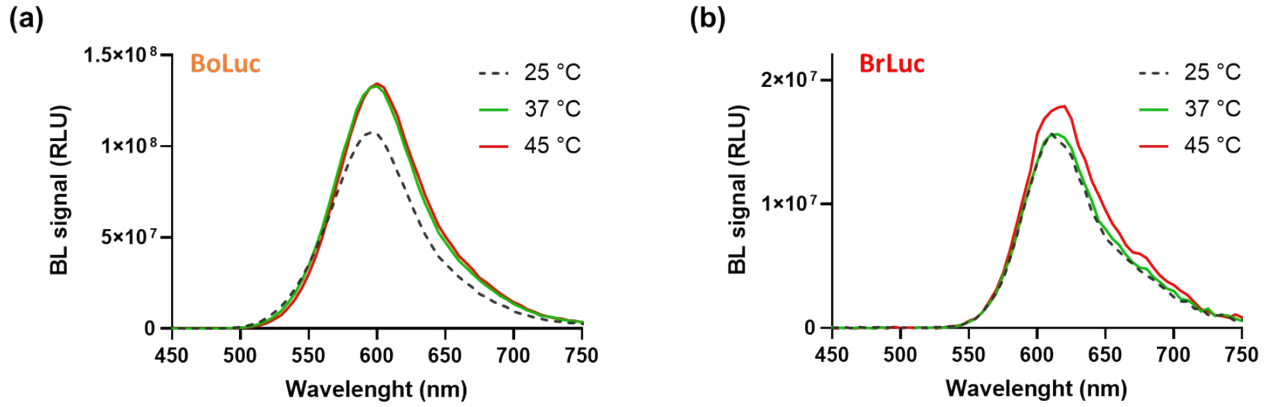

Figure S3: (a) BoLuc mutant and (b) BrLuc mutant emission spectra obtained at different temperature (25°, 37° and 45°C) with the D-Luciferin substrate.

Table S1. Kinetic Parameters of BgLuc, BoLuc and BrLuc mutants

Luc enzyme	Km (µM)	Kcat (cps/M)*	Kcat/ Km
BgLuc	20.9 ± 0.5	4.91 x 10^8	2.93 x 10^{10}
BoLuc	8.2 ± 0.2	4.97 x 10^8	6.06 x 10^7
BrLuc	196 ± 11	1.50 x 10^8	7.92 x 10^5

*Apparent kcat values were calculated by dividing the apparent Vmax (cps) by the luciferase concentration (6.6 x 10^{-6} M)