Supporting Information

Bioluminescent Indicator for Highly Sensitive Analysis of Estrogenic Activity in a Cell-Based Format

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1. Conformational difference between agonist-bound and antagonist-bound ERα.

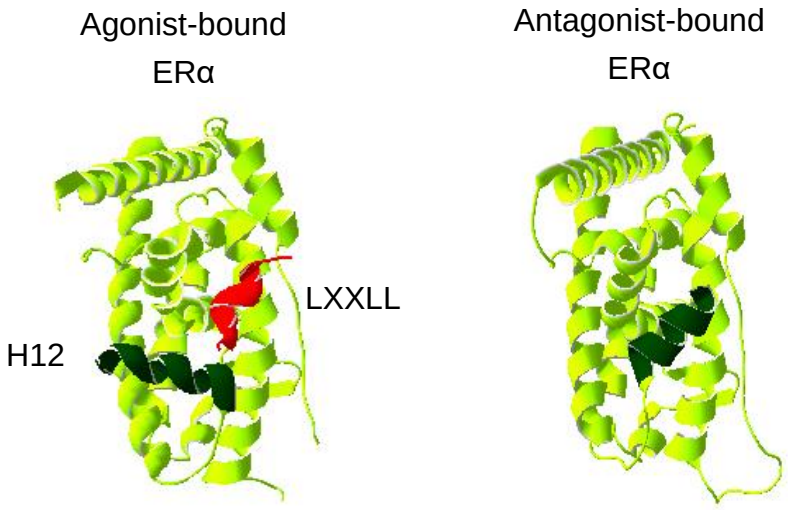

Figure S1. Conformational difference between agonist-bound and antagonist-bound ERα.

A ligand binding domain (LBD, light green) binds to an agonist. Helix 12 (H12, deep green) covers the ligand binding pocket. Coactivators possessing LXXLL sequence (red) interact with the LBD. In contrast, when an antagonist is bound to the LBD, the H12 covers a different position on the LBD, which inhibits the interaction of coactivators. These figures were created using the Swiss-PDB Viewer (PDB ID; 1GWR (agonist-bound ER) and 3ERT (antagonist-bound ER)).

2. Concentration-dependence of E2 on luminescence intensities.

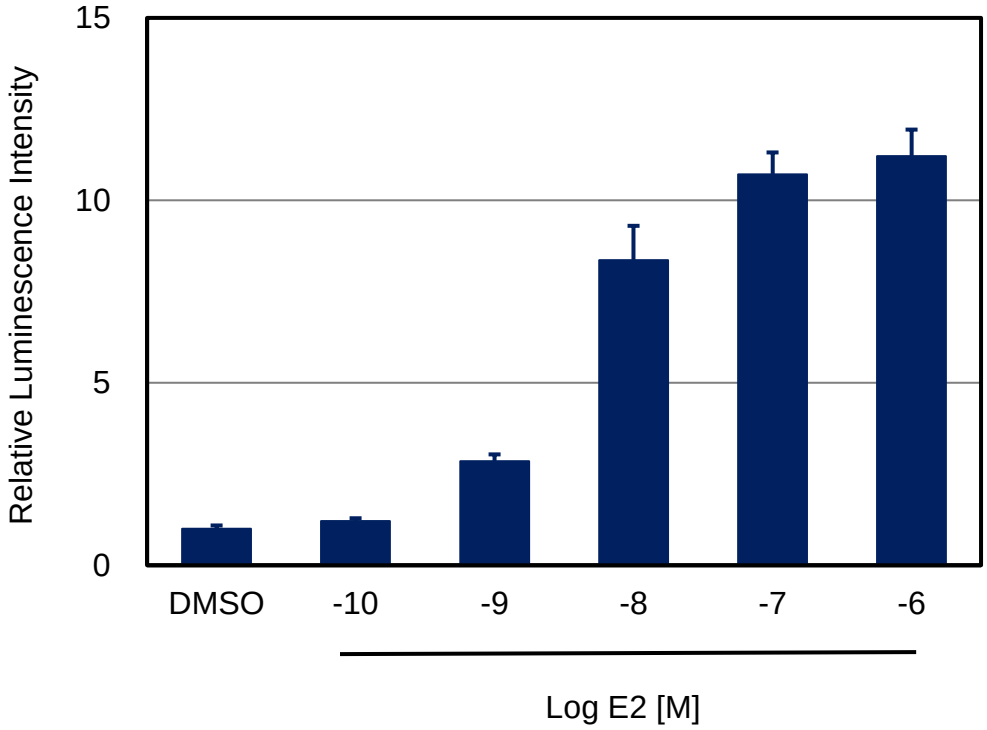

Figure S2. Concentration-dependence of E2 on luminescence intensities.
The COS-7 cells harboring the HR-RLuc were exposed to various concentrations of E2 for 1 h. Luminescence activities were measured for 15 s. Luminescence intensities were normalized against the luminescence intensity upon stimulation of DMSO.

3. Comparison of the luminescence recoveries among different LXXLL sequences using *E.coli*.

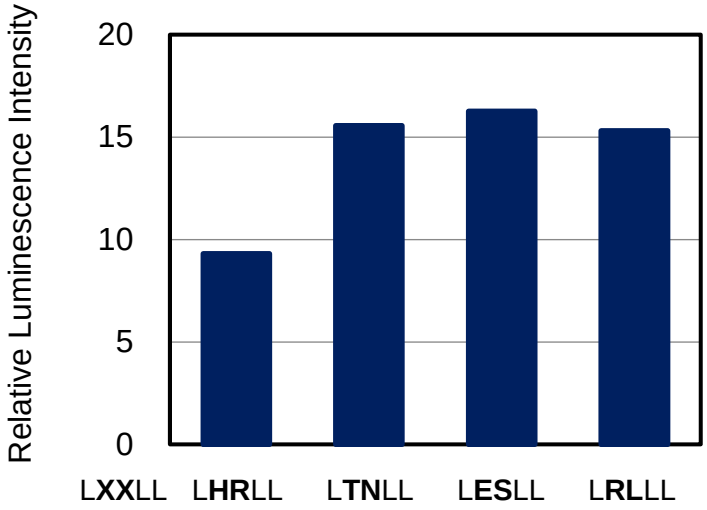

Figure S3. Comparison of the luminescence recoveries among different LXXLL sequences using *E.coli*. Luminescence increases for the mutated indicators in E. coli. The *E. coli* expressing the mutated indicators including the LXXLL sequences were sonicated. The suspension was mixed with 1.0×10^{-6} M E2 or DMSO for 1 h. The luminescence intensities were measured for 5 s.

4. Optimization of the dissection sites in ELuc fragments.

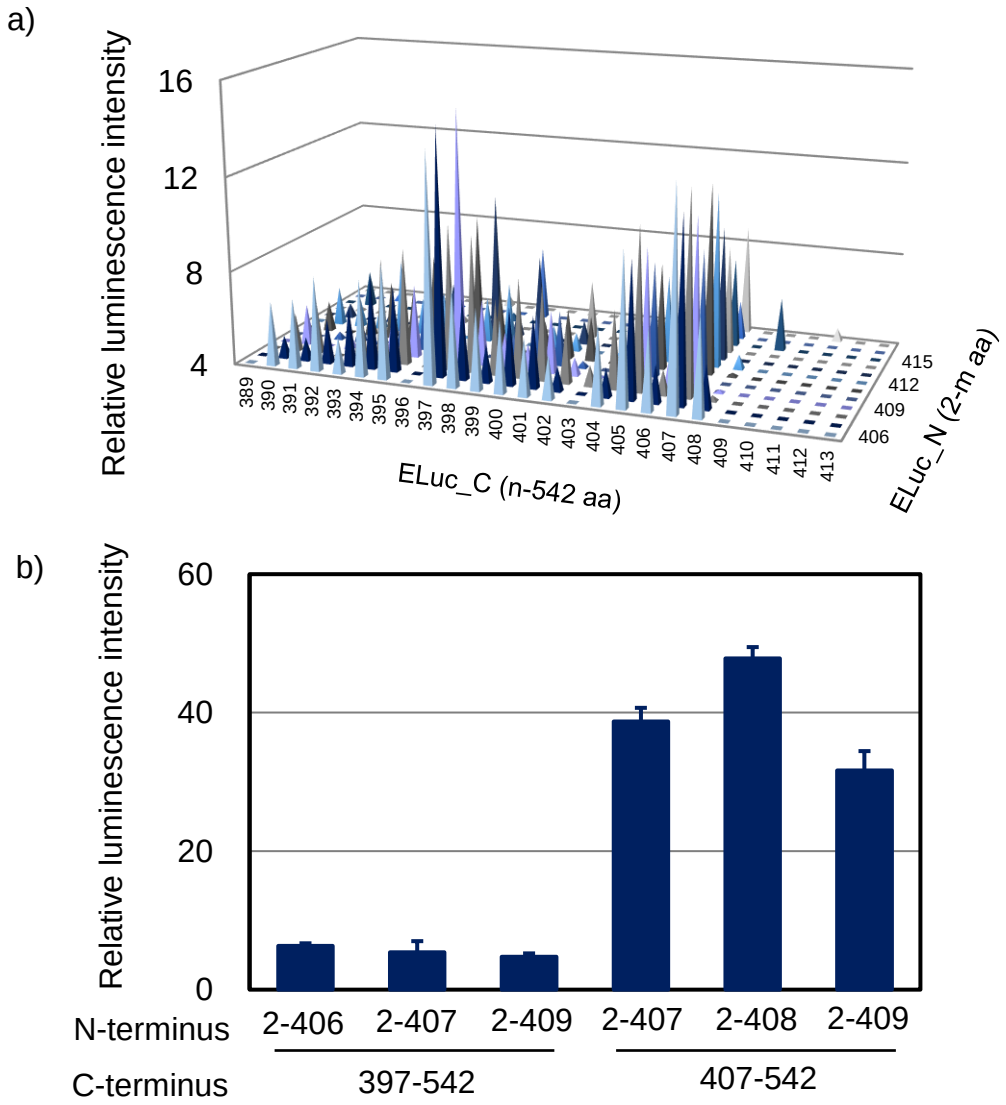

Figure S4. Optimization of the dissection sites in ELuc fragments.

a) Verification of the reactivity of the different dissection sites in the presence of the E2 using *E. coli*. The *E. coli* expressing each indicator were sonicated. The suspension was mixed with 1.0×10^{-6} M E2 or DMSO. The luminescence intensities were measured using a plate reader. The measuring time was 2 s/well. b) Luminescence increases during stimulation with E2 using COS-7 cells. The COS-7 cells were transfected with several responsive indicators and were exposed to 1.0×10^{-6} M E2 for 1 h. The luminescence was measured in a single tube using a luminometer. The measuring time was 15 s/tube. Luminescence intensities were normalized against the luminescence intensity upon stimulation of DMSO.

5. Comparison of luminescence intensities of carboxy-terminal fragments of ELuc and McLuc.

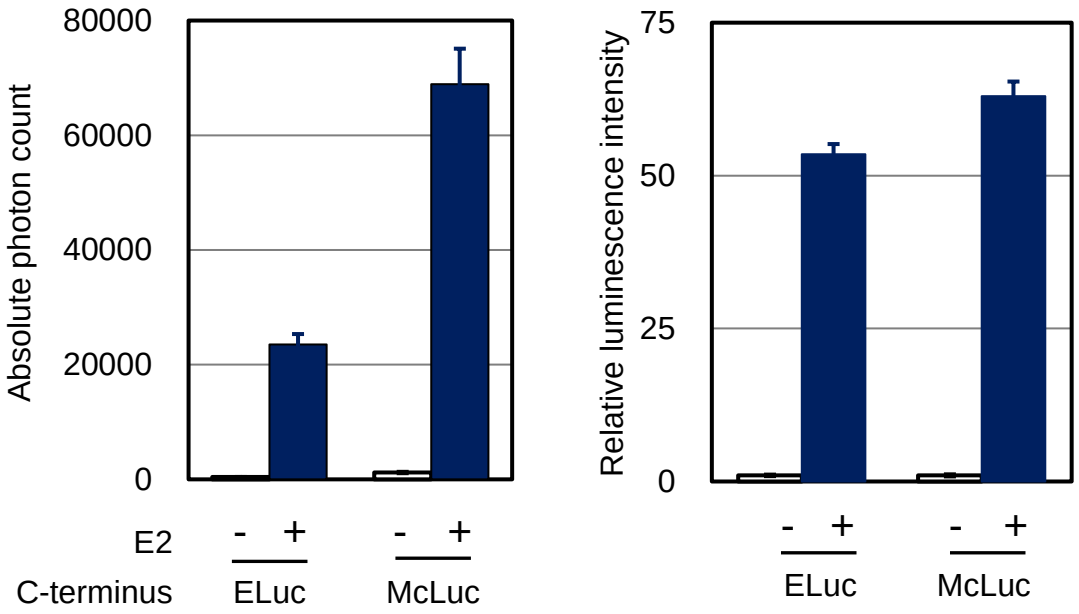

Figure S5. Comparison of the luminescence intensities of carboxy-terminal fragments of ELuc and McLuc.

The COS-7 cells transfected with the indicator in a 24-well microtiter plate were stimulated to 1.0×10^{-6} M E2 or DMSO for 1 h. The luminescence intensities were measured for 15 s. Absolute photon counts of luminescence (left) and their relative luminescence intensities (right) are shown.

6. Evaluation of reactivity of the different dissection sites using *E. coli*

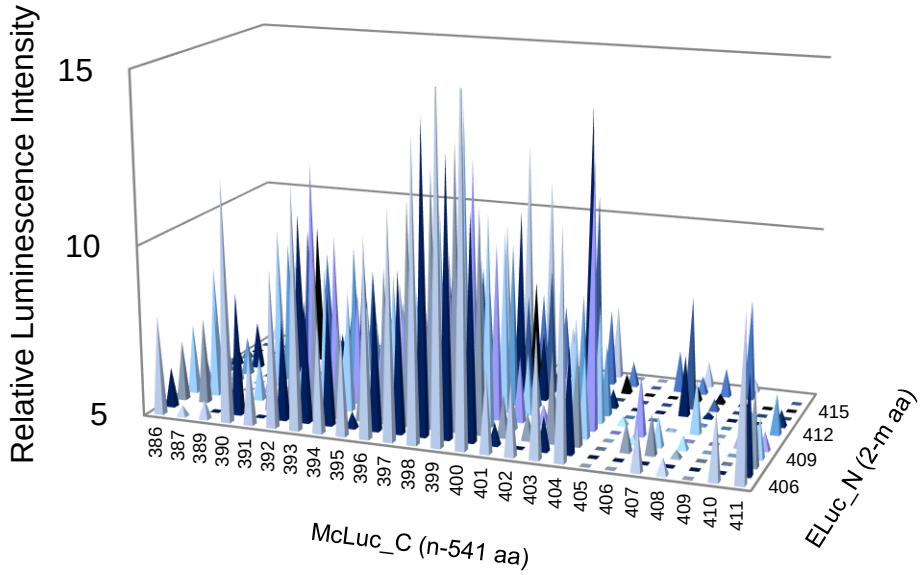

Figure S6. Systematic analysis of the reactivity of the different dissection sites in the presence of 1.0×10^{-6} M E2 using *E. coli*. The E. coli expressing each indicator were sonicated. The suspension was mixed with 1.0×10^{-6} M E2 or DMSO. The luminescence intensities for each suspension were measured using a plate reader. The measuring time was 2 s/well.

7. Inhibitory effects of an ER antagonist on the bioluminescence by E2

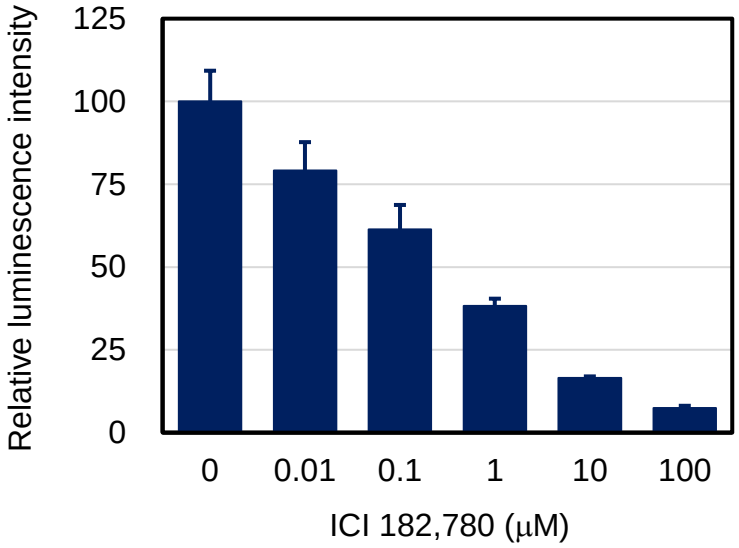

Figure S7. Inhibitory effects of an ER antagonist on the bioluminescence by E2
The COS-7 cells expressing the RL-EMcLuc were exposed to 1.0×10^{-6} M E2 in the presence of various concentrations of ICI 182,780 for 1 h. After lysis, the bioluminescence was measured for 5 s/well (n = 4). The luminescence intensities were normalized against upon the stimulation of E2.

8. Comparison of expression levels of three indicators

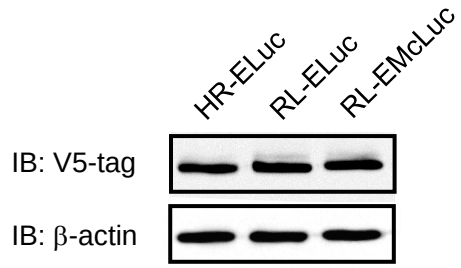

Figure S8. Comparison of expression levels of three indicators. The COS-7 cells were transfected with cDNA coding V5 tag-fused indicators (the HR-ELuc, RL-ELuc and RL-EMcLuc). The cells were incubated for 24 h and lysed in 150 μL of a lysis buffer (125 mM Tris pH 6.8, 10% glycerol, 4% SDS, 0.006% bromophenol blue and 10% mercaptoethanol). The proteins were separated using 10 % acrylamide gel and transferred onto nitrocellulose membrane. The indicators and β-actin were blotted using anti-V5-tag antibody (Life Technologies) and anti-β-actin antibody (Sigma-Aldrich), respectively.

9. Temporal changes in the luminescence upon stimulation of 1 μM E2

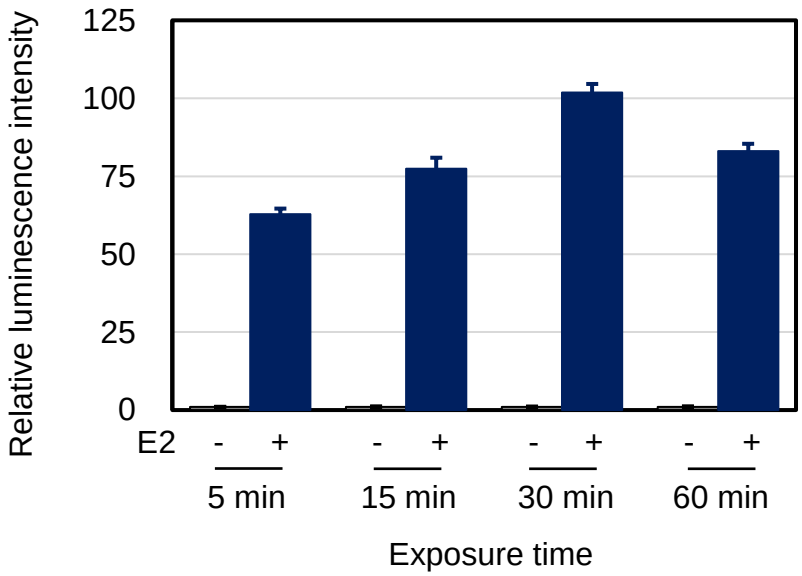

Figure S9. Temporal changes in the luminescence upon stimulation of 1 μM E2. The COS-7 cells expressing the RL-EMcLuc were exposed to 1.0×10^{-6} M E2 for 5, 15, 30 and 60 min. After lysis, the bioluminescence was measured for 5 s (n = 3). The luminescence intensities upon stimulation of 1 μM E2 were normalized against that upon the stimulation of DMSO in each exposure time.

10. Summary of luminescence recovery by estrogenic compounds.

Table S1. Summary of luminescence recovery by tested compounds

compound	Agonist						
	DES	E2	E1	E3	Gen	Dai	BPA
EC5 ($\times 10^{-10}$ M)	2	17	43	81	1538	11249	25971
Relative binding affinity[a]	400	100	7	10	0.5	0.02	0.01
Relative binding affinity[b]	468	100	60	14	5	0.1	0.05

Relative binding affinity values were based on a) Fang et al., Chem. Res. Toxicol. 2001 and b) Kuiper et al., Endocrinology, 1997 and 1998.