

Supporting information

Bioluminescent Ratiometric Indicator for Analysis of Water Hardness in Household Water

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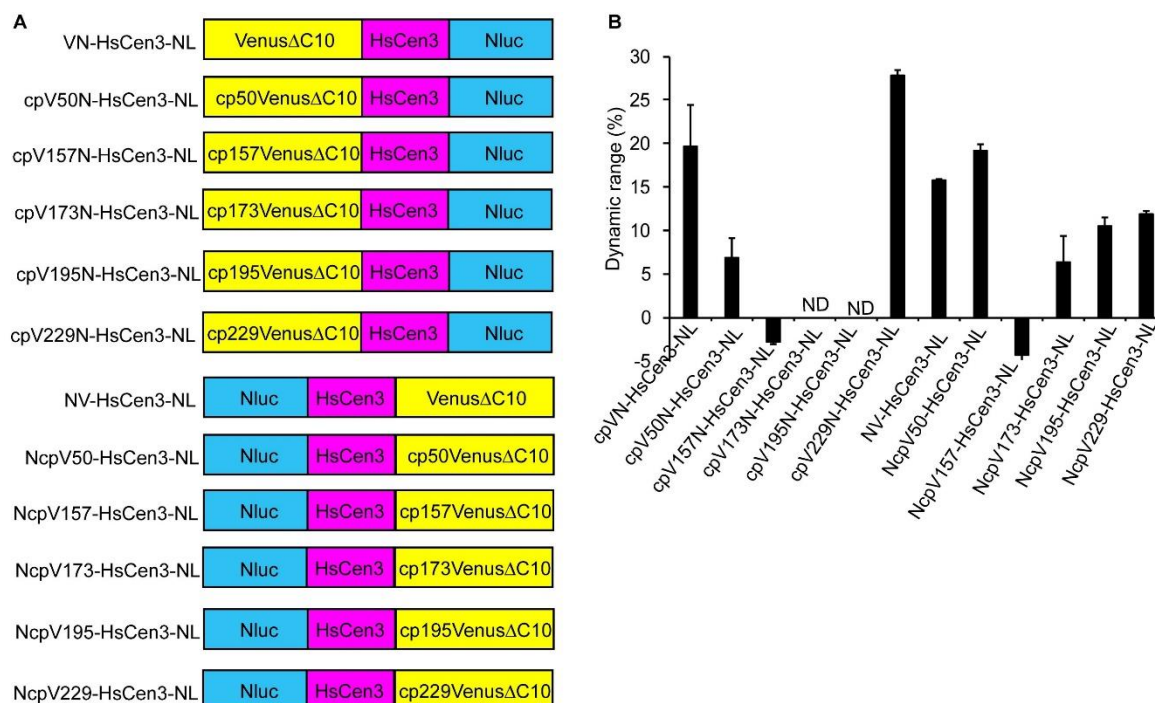
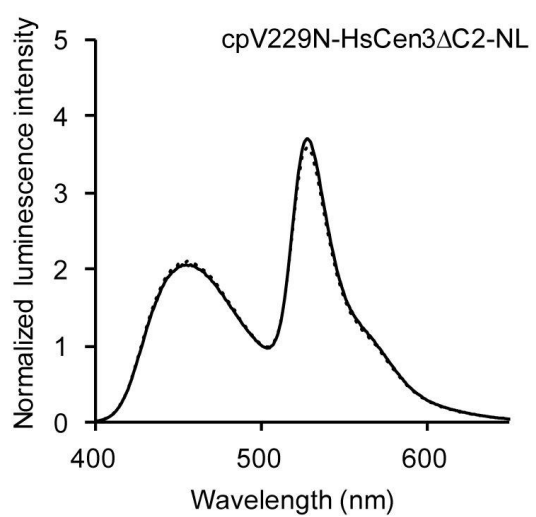
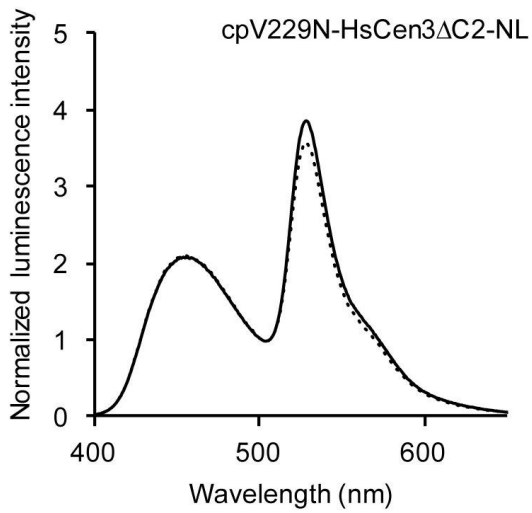
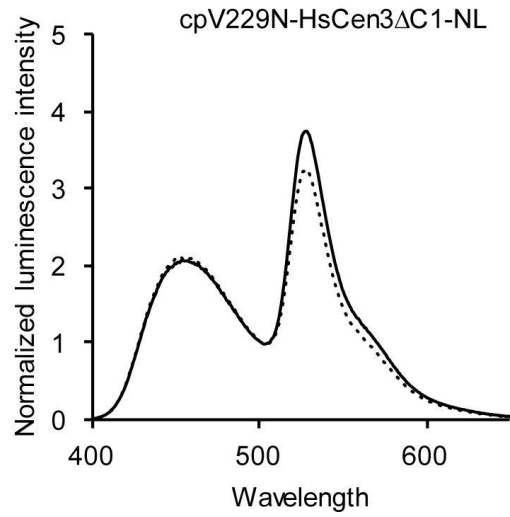
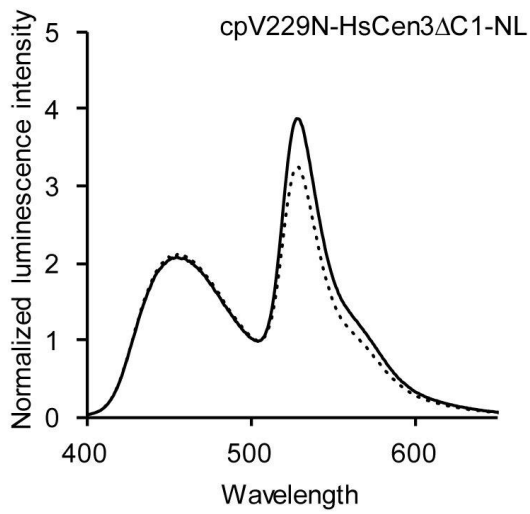
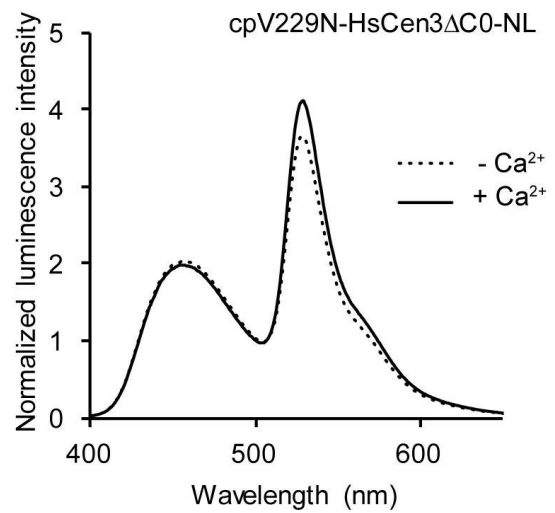
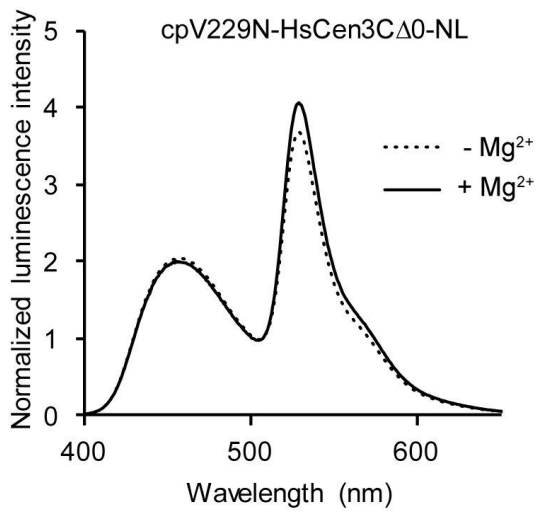
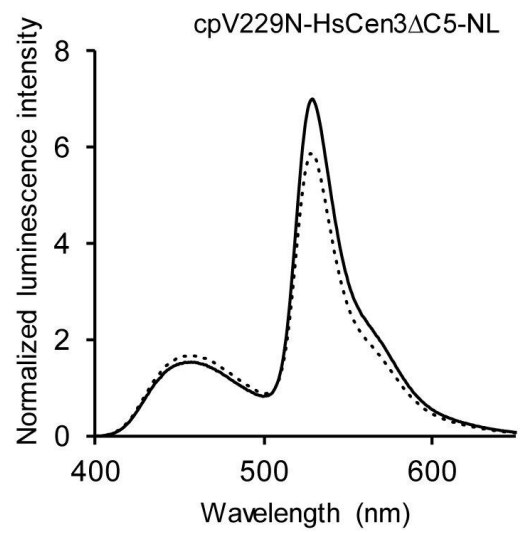
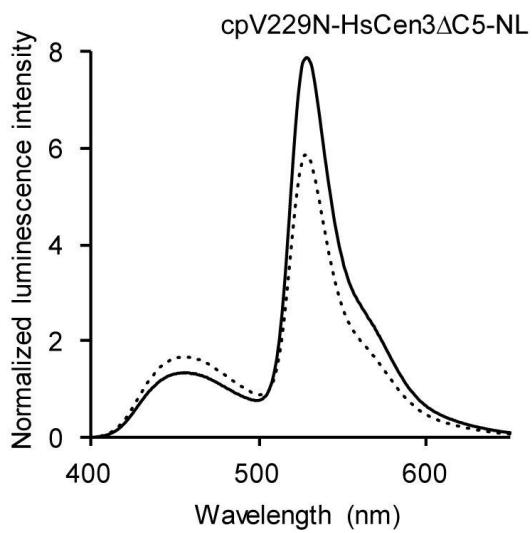
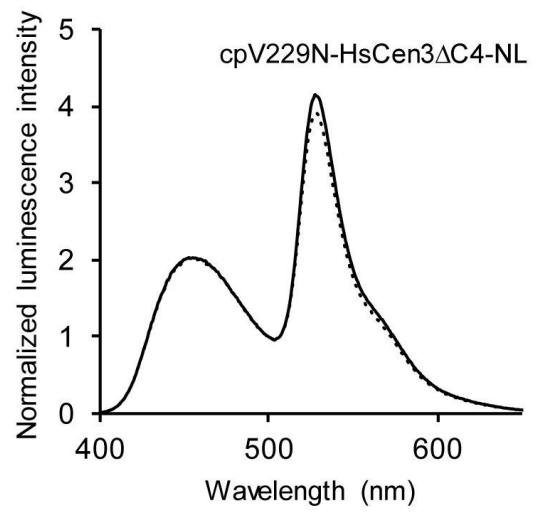
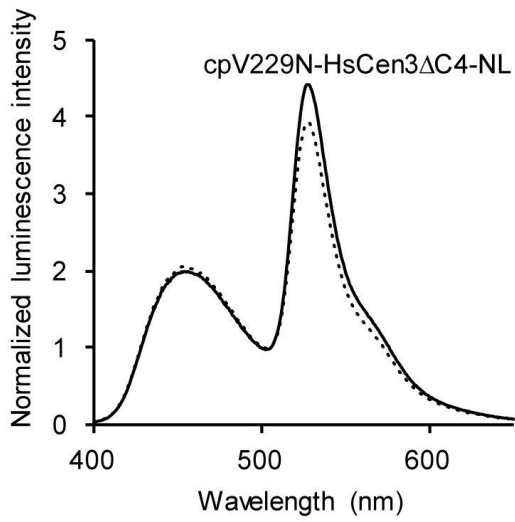
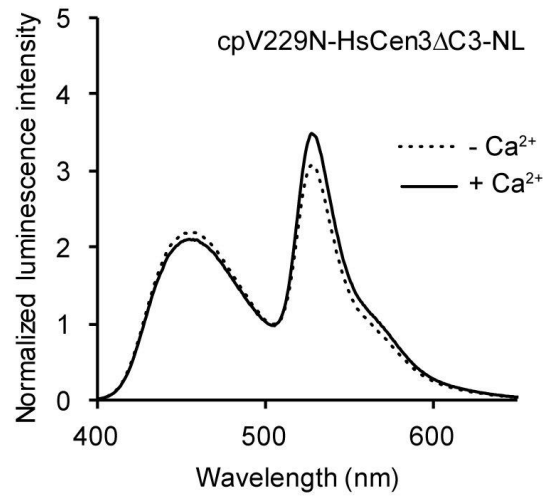
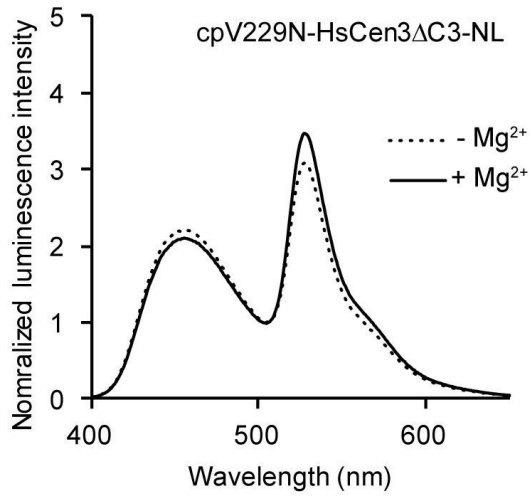
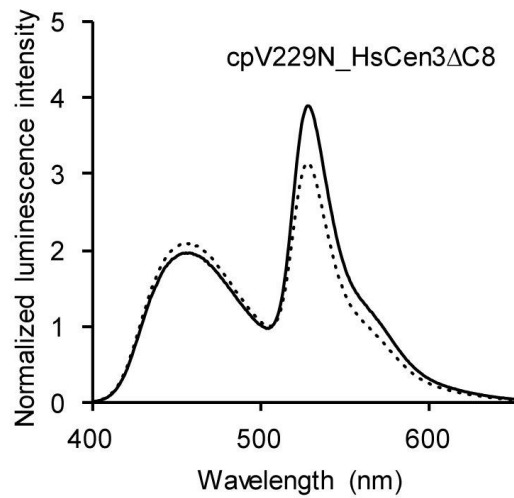
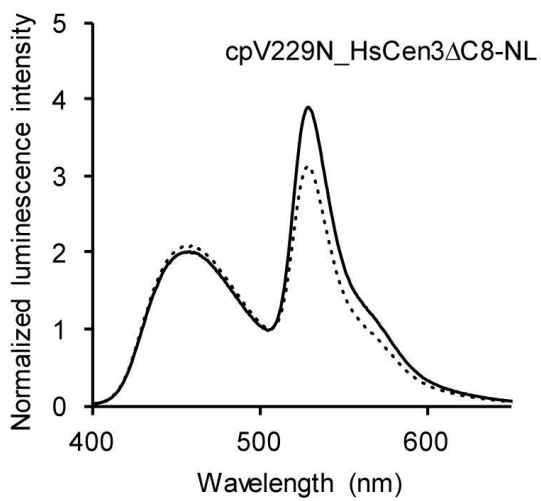
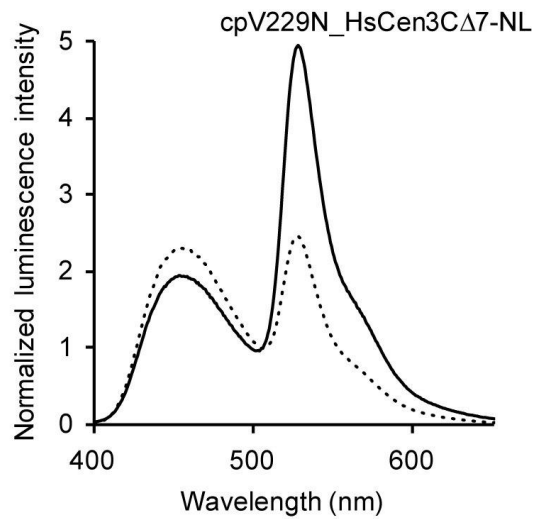
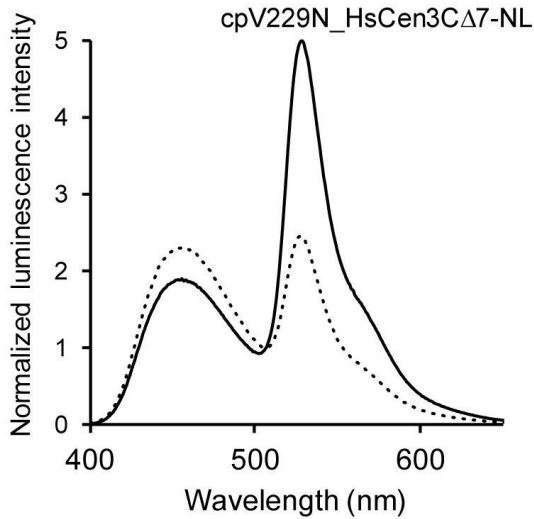
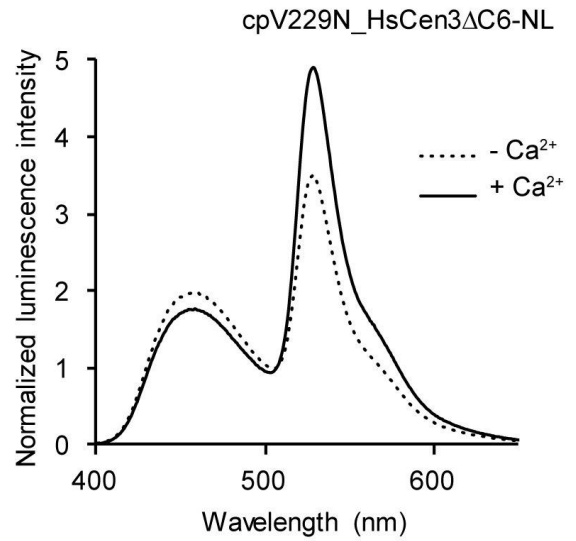
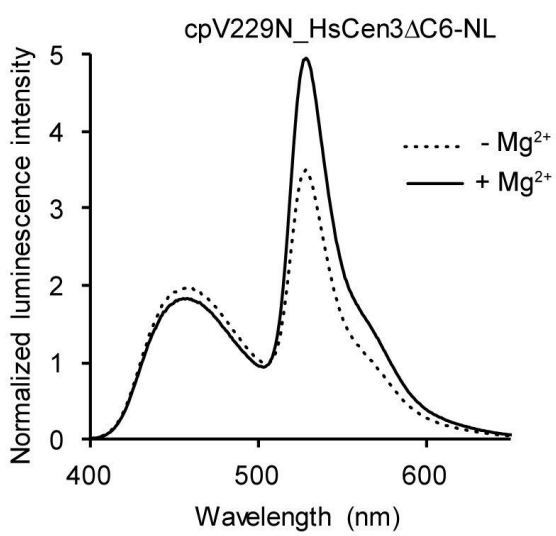


Figure S1. Construction and screening of LOTUS-W candidates (A) Domain structures of the LOTUS-W candidates. Venus or cpVenus (cp50, 157, 173, 195, or 229) was connected to the N-terminal or C-terminal of HsCen3. Nluc was connected to the other side. (B) Dynamic range for Mg²⁺ in candidates calculated in the presence and absence of 10 mM Mg²⁺ emission ratio (525 nm/455 nm). Each dynamic range is shown as a percentage (%).







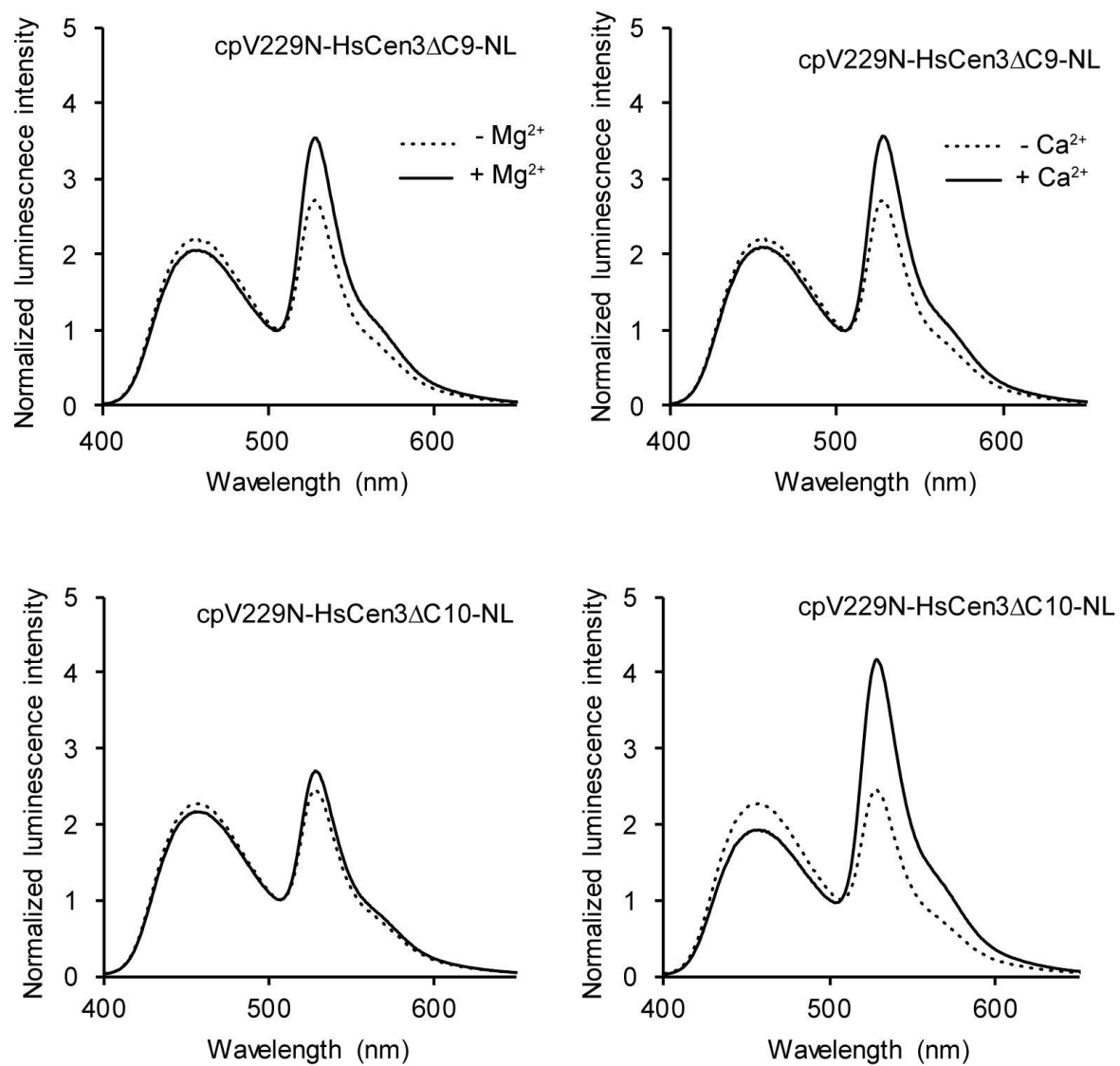


Figure S2. *In vitro* properties of cpV229N-HsCen3-NL variants. Bioluminescence spectra of cpV229N-HsCen3-N3 variants are shown in the presence (solid line) or absence (dotted line) of 10 mM Mg²⁺ or Ca²⁺.

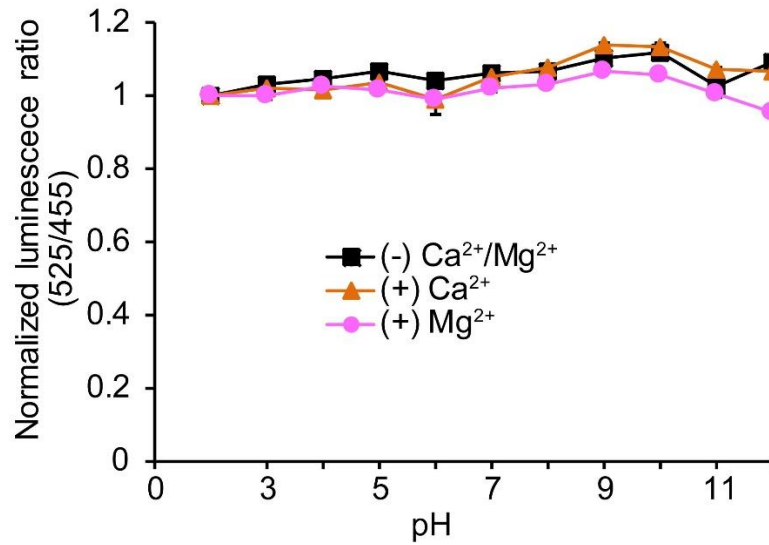


Figure S3. pH dependence of cpV229N_HsCen3ΔC7-NL. Bioluminescence intensity ratios of cpV229N_HsCen3ΔC7-NL were confirmed at different pH values. These counts were measured in the presence and absence of 10 mM Ca²⁺ or 10 mM Mg²⁺. All measurements were performed three times (n = 3).

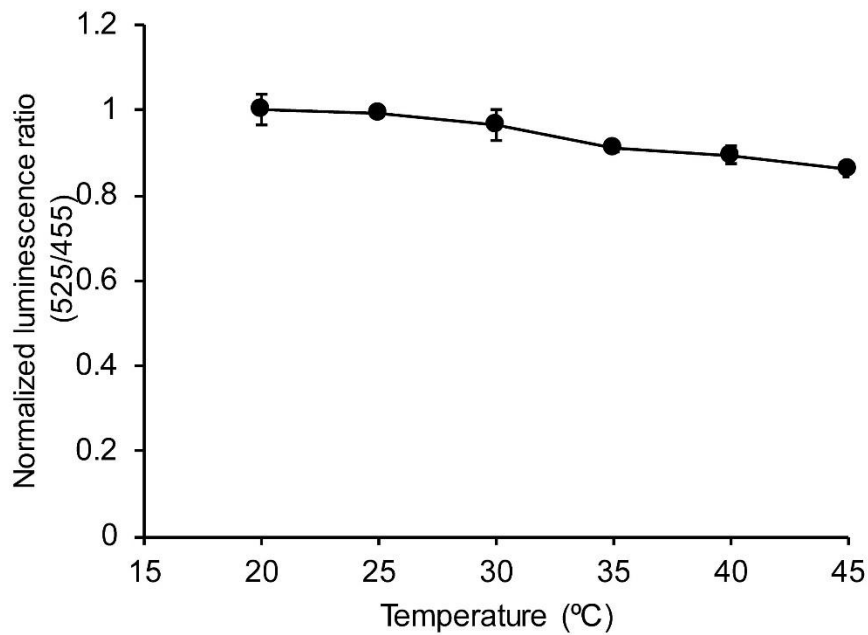


Figure S4. Temperature dependence of cpV229N_HsCen3ΔC7-NL. Bioluminescence intensity ratio at different temperatures.

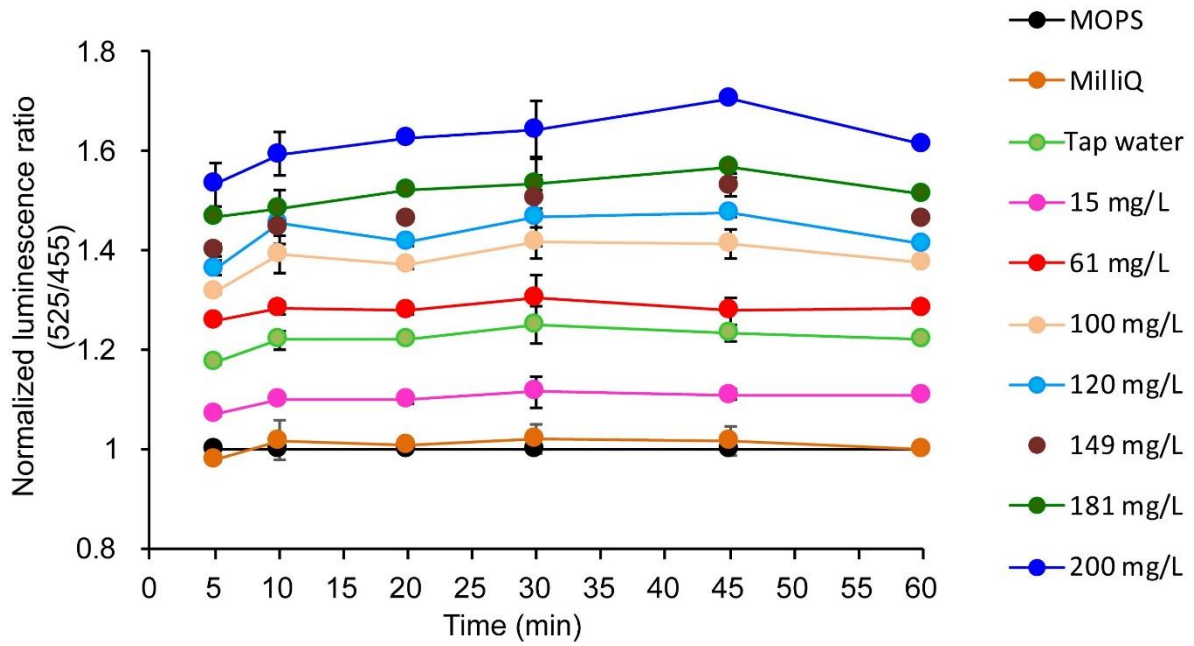


Figure S5. Time course variation of the LOTUS-W ratio. Bioluminescence intensities of LOTUS-W were measured at each time point with different water hardness (CaCO₃) conditions and the ratio was calculated (525 nm/455 nm). All ratios were normalized to 10 mM MOPS without added ions.

Table S1. Oligonucleotide sequence.

Name	Sequence (5'--3')
F-BH1-Venus_1	GCGGATCCGATG GTGAGCAAGGGCGAG
F-BH1-M-ven_50	AAGGATCCGATGACCGGCAAGCTGCCC
F-BH1-M-ven_157	AAGGATCCGAGCAGAAGAACGGCATC
F-BH1-M-ven_173	AAGGATCCGATGGACGGCGGCGTGC
F-BH1-M-ven_195	TTGGATCCGATGCTGCCCGACAACCAC
F-BH1-M-ven_229	AAGGATCCGATGATCACTCTCGGCATG
R-ven_49-SphI	ATTGCATGCGGGTGCAGATCAGCTTCAGGG
R-ven_156-SphI	GACGCATGCGCTTGTTCGGCGGTGATATAGA
R-ven_172-SphI	ATTGCATGCGCTCGATGTTGTGGCGGATCT
R-ven_194-SphI	ATTGCATGCGCAGCACGGGGCCGTCGCCGA
R-ven_228-SphI	TATGCATGCGCCCCGGCGGCGGTCACGAACT
F-SacI-gfp_1	TTTGAGCTCATGGTGTGAGCAAGGGCGAGGA
F-SacI-M-ven_50	AATGAGCTCATGACCGGCAAGCTGCCCGTGCCC
F-SacI-M-ven_157	AATGAGCTCATGCAGAAGAACGGCATCAAGGCCAAC
F-SacI-M-ven_173	AATGAGCTCATGGACGGCGGCGTGCAGCTCGCC
F-SacI-M-ven_195	AATGAGCTCATGCTGCCCGACAACCCTACCTGAGC
F-SacI-M-ven_229	TTTGAGCTCATGATCACTCTCGGCATGGA
R-ven_49-x-EcoRI	ATTGAATTCTTAGGTGCAGATCAGCTTCAG
R-ven_156-x-EcoRI	ATTGAATTCTTACTTGTTCGGCGGTGATATA
R_ven_172-x-EcoRI	ATTGAATTCTTACGATGTTGTGGCGGATCT
R-ven_195-x-EcoRI	ATTGAATTCTTACAGCACGGGGCCGTCGCC
R-ven_229-x-EcoRI	ATTGAATTCTTACCCGGCGGCGGTCACGAA
R-cp229Venus-Sph1	ATAGCATGCCCCGGCGGCGGTCACGAA
R-Nluc-171-X-ER1	TATGAATTCTTACGCCAGAATGCGTTCGC
R-Nluc-171-g-SphI	ATTGCATGCGCGCCAGAATGCGTTCGCACA
R-BH1-g-Nluc	AATGGATCCGATGGTCTTCACACTCGAAG
F-SacI_Nanoluc	ATCGAGCTCGATGGTCTTCACACTCGA
R_SacI_hsCen3-ΔC1	TGGGAGCTCATCTCTTTCCAATATCCAGTCTG
R_SacI_hsCen3-ΔC2	TGGGAGCTCTCTTTCCAATATCCAGTCTGTC
R_SacI_hsCen3-ΔC3	TGGGAGCTCTTCCAATATCCAGTCTGTAC
R_SacI_hsCen3-ΔC4	TGGGAGCTCCAATATCCAGTCTGTCACAAC
R_SacI_hsCen3-ΔC5	TGGGAGCTCTATCCAGTCTGTCACAACCTC
R_SacI_hsCen3-ΔC6	TGGGAGCTCCCAGTCTGTCACAACCTTCATT
R_SacI_hsCen3-ΔC7	TGGGAGCTCCCAGTCTGTCACAACCTTCATT
R_SacI_hsCen3-ΔC8	TGGGAGCTCTGTCACAACCTTCATTAAAATCTTCA
R_SacI_hsCen3-ΔC9	TGGGAGCTCCACAACCTTCATTAAAATCTTCAAAGG
R_SacI_hsCen3-ΔC10	TGGGAGCTCAACTTCATTAAAATCTTCAAAGGTGA

Table S2. WHO/USGS-proposed water hardness scale based on CaCO₃.

Scale of WH (mg/L)	Classification
0–60	Soft
61–120	Moderately hard
121–180	Hard
181<	Very hard

Table S3. Water hardness of commercially available mineral water.

Product name	Scale of WH (mg/L)	Classification	Mg ²⁺ (mg/L)	Ca ²⁺ (mg/L)	Other minerals	pH	Company name, Country
Natural Mineral Water	10	Soft	0.5	3	—	6.4	AEON CO., LTD., Japan
Okudaisan no Tennensui	20	Soft	1~3	2~7	Na ⁺ , K ⁺	7	Suntory Holdings Limited, Japan
Minami-Alps no Tennensui	30	Soft	1~3	6~15	Na ⁺ , K ⁺	7	Suntory Holdings Limited, Japan
CRYSTAL Geyser	38	Soft	3.4	6.4	Na ⁺ , K ⁺	—	Crystal Geyser Water Company, USA
Irohasu	40	Soft	5.5	7.1	Na ⁺ , K ⁺	7	The Coca-Cola Company, Japan
Volvic	60	Soft	8	10.15	Na ⁺ , K ⁺	7	Danone S.A., France
Ogeu	172	Hard	10.2	40.6	K ⁺	—	Coron Vert CO., LTD., France
Beaupre	240	Very hard	20.4	50.8	—	—	CMA CGM, France
Evian	304	Very hard	20.6	80	Na ⁺	7.2	Danone S.A., France
Vittle	315	Very hard	20	90.4	—	—	Nestlé S.A., France
Perlage	389	Very hard	20.4	110.7	K ⁺	5.3	Nałęczów Zdrój Sp. z o.o, Poland
S.Pellegrino	610	Very hard	40.9	160.4	So ₄ ²⁻ , HCO ₃ ⁻ , Cl ⁻	—	Sanpellegrino S.p.A, Italy
Gerolsteiner	1310	Very hard	100	360	Na ⁺ , K ⁺	6.4	GmbH & Co. KG, Germany
Contrex	1468	Very hard	70.45	460.8	K ⁺	—	Nestlé S.A., France

Table S4. *in vitro* properties of cpV229N_HsCen3-NL variants.

Variants name	R_{\min} for Mg^{2+}/Ca^{2+}	R_{\max} for Mg^{2+}	R_{\max} for Ca^{2+}	Dynamic range for Mg^{2+} (%)	Dynamic range for Ca^{2+} (%)
cpV229N_Cen3-NL	1.8	2	2	13	15
cpV229N_Cen3ΔC1-NL	1.5	1.9	1.8	23	18
cpV229N_Cen3ΔC2-NL	1.7	1.9	1.8	9	6
cpV229N_Cen3ΔC3-NL	1.3	1.6	1.7	18	18
cpV229N_Cen3ΔC4-NL	1.9	2.2	2	15	5
cpV229N_Cen3ΔC5-NL	3.4	5.8	4.5	66	30
cpV229N_Cen3ΔC6-NL	1.7	2.7	2.8	52	56
cpV229N_Cen3ΔC7-NL	1	2.6	2.6	145	140
cpV229N_Cen3ΔC8-NL	1.5	1.9	2	29	31
cpV229N_Cen3ΔC9-NL	1.2	1.7	1.7	40	38
cpV229N_Cen3ΔC10-NL	1	1.2	2.2	16	101