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Supplemental Information

Full-Spectral Multiplexing of Bioluminescence Resonance Energy Transfer in Three TRPV Channels

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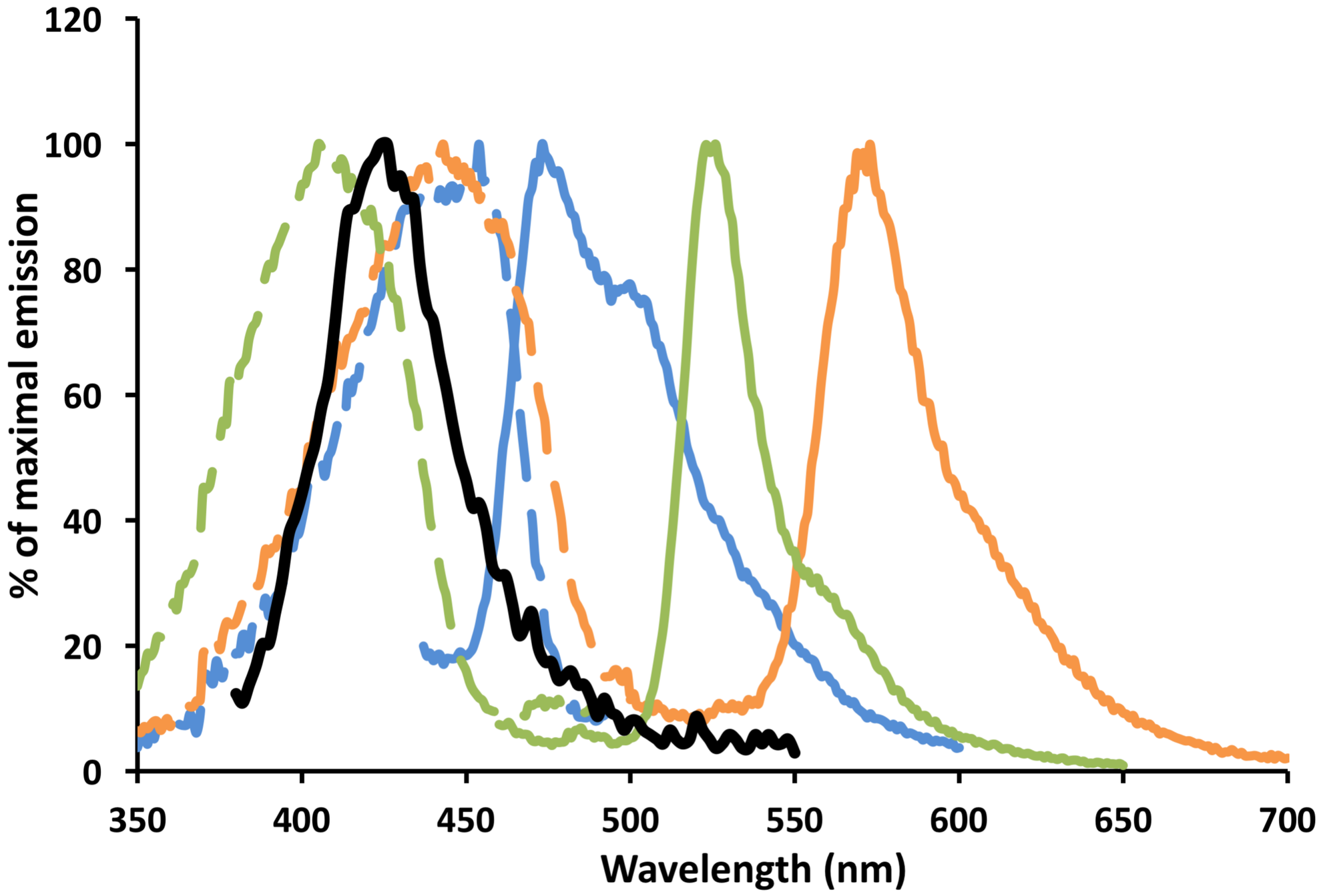


FIGURE S1 Compatibility of the emission spectrum of Luc (in the presence of purple coelenterazine substrate, black line) and the absorption (dotted lines) and emission spectra (full lines) of aquamarine (blue lines), mAmetrine (green lines), and Lss-mOrange (orange lines).

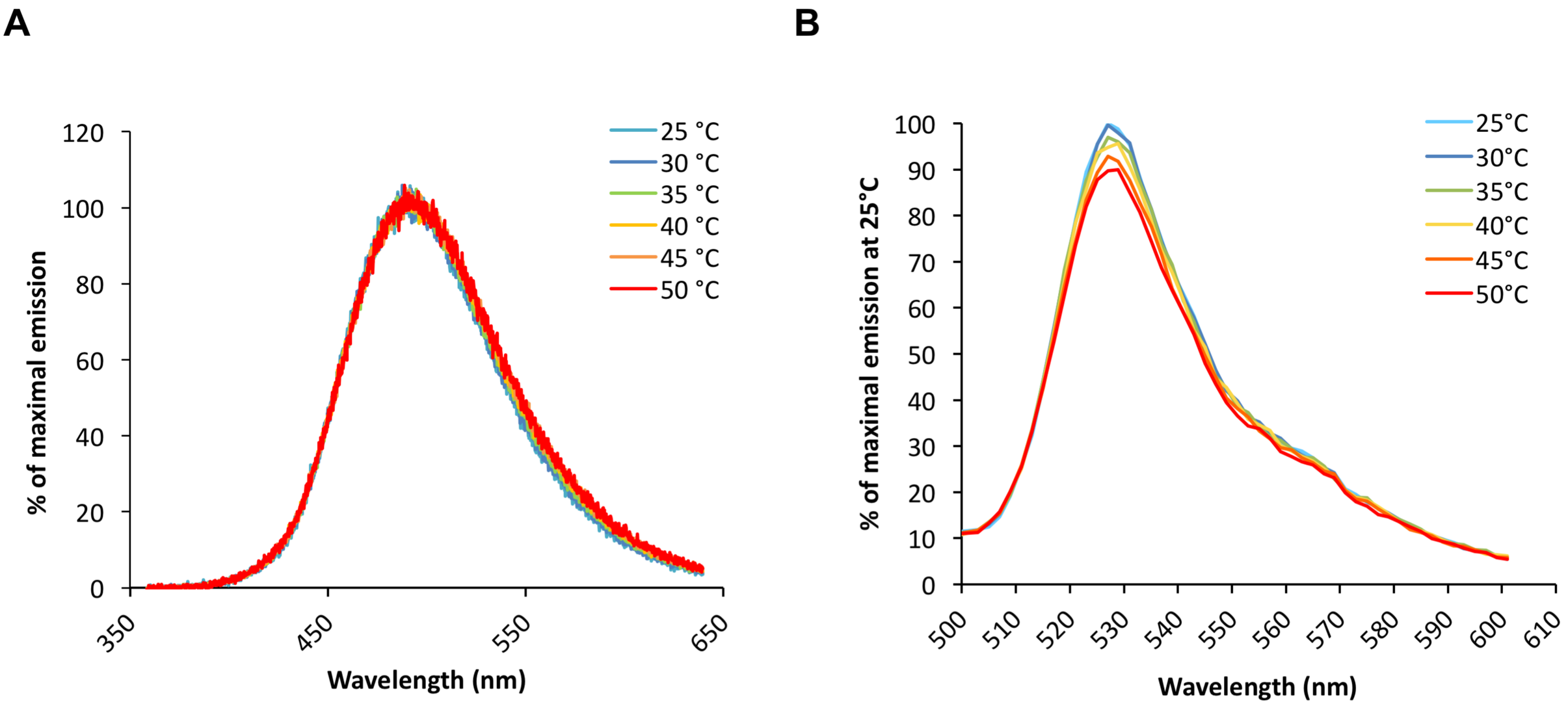


FIGURE S2 Emission spectra of Luc (in presence of Coelenterazine H) (A) and YFP (B) at temperatures ranging from 25 to 50 °C.

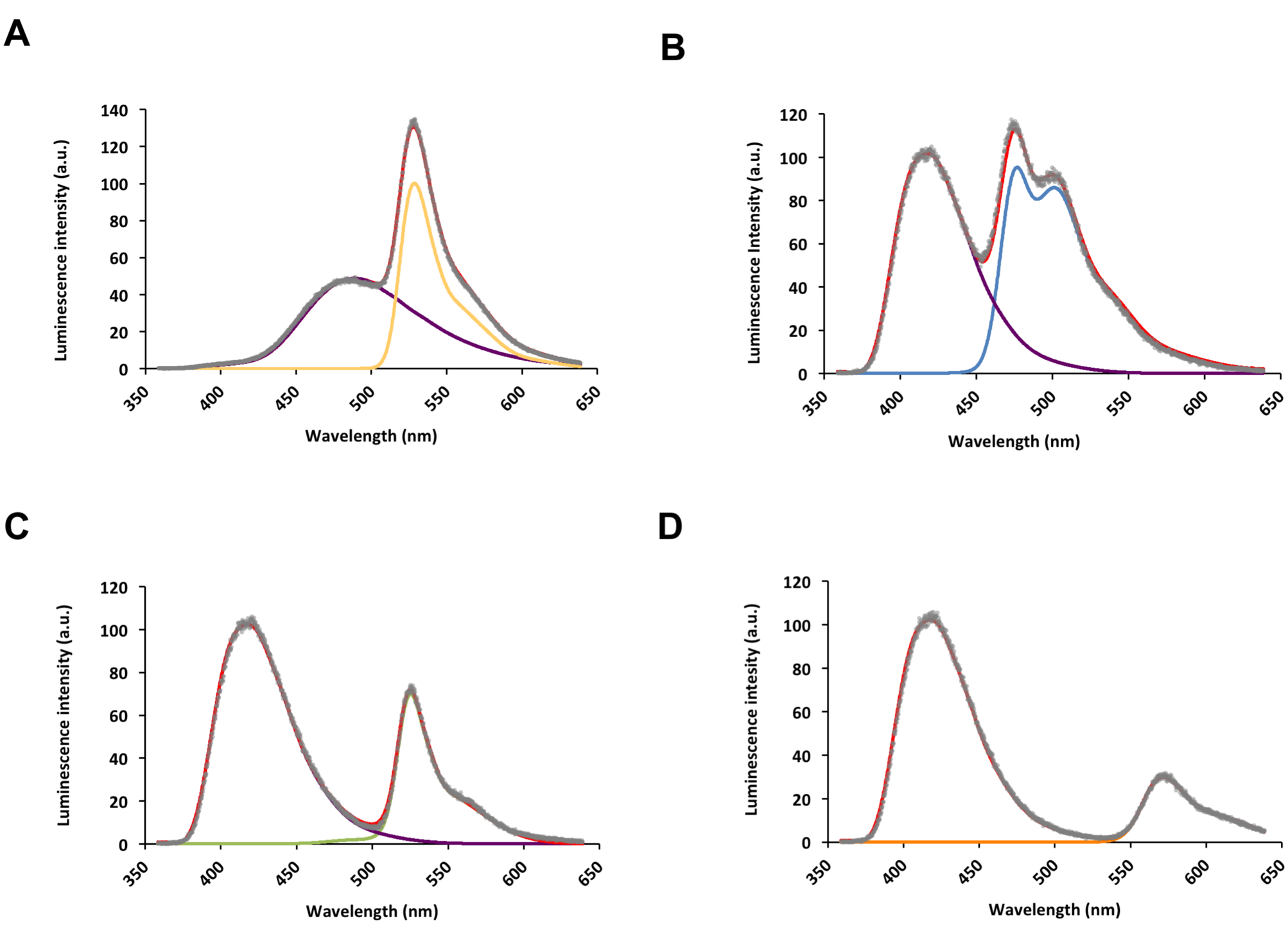


FIGURE S3 Signal decomposition of the bioluminescent spectra measured from HEK293T cells expressing YFP-Luc (A), aquamarine-Luc (B), mAmetrine-Luc (C), or LSSmOrange-Luc (D). Based on the experimental data (red line), the LabVIEW interface was used to calculate the shape of the BRET signal and separate the Luc emission spectrum (purple line) from those of YFP (yellow line, A), aquamarine (blue line, B), mAmetrine (green line, C), and LSSmOrange (orange line, D). The BRET ratio was then calculated by dividing the area under the acceptor spectrum by that under the donor spectrum, thus assuring its independence from any contamination by that of the donor or other acceptors. Net BRET for each FP-Luc is as follow: 0.82 for YFP-Luc, 1.09 for CFP-Luc, 0.43 for mAmetrine-Luc and 0.24 for LssmOrange-Luc. Coelenterazine H was used as a substrate in A, while purple coelenterazine was used as a substrate in B-D.

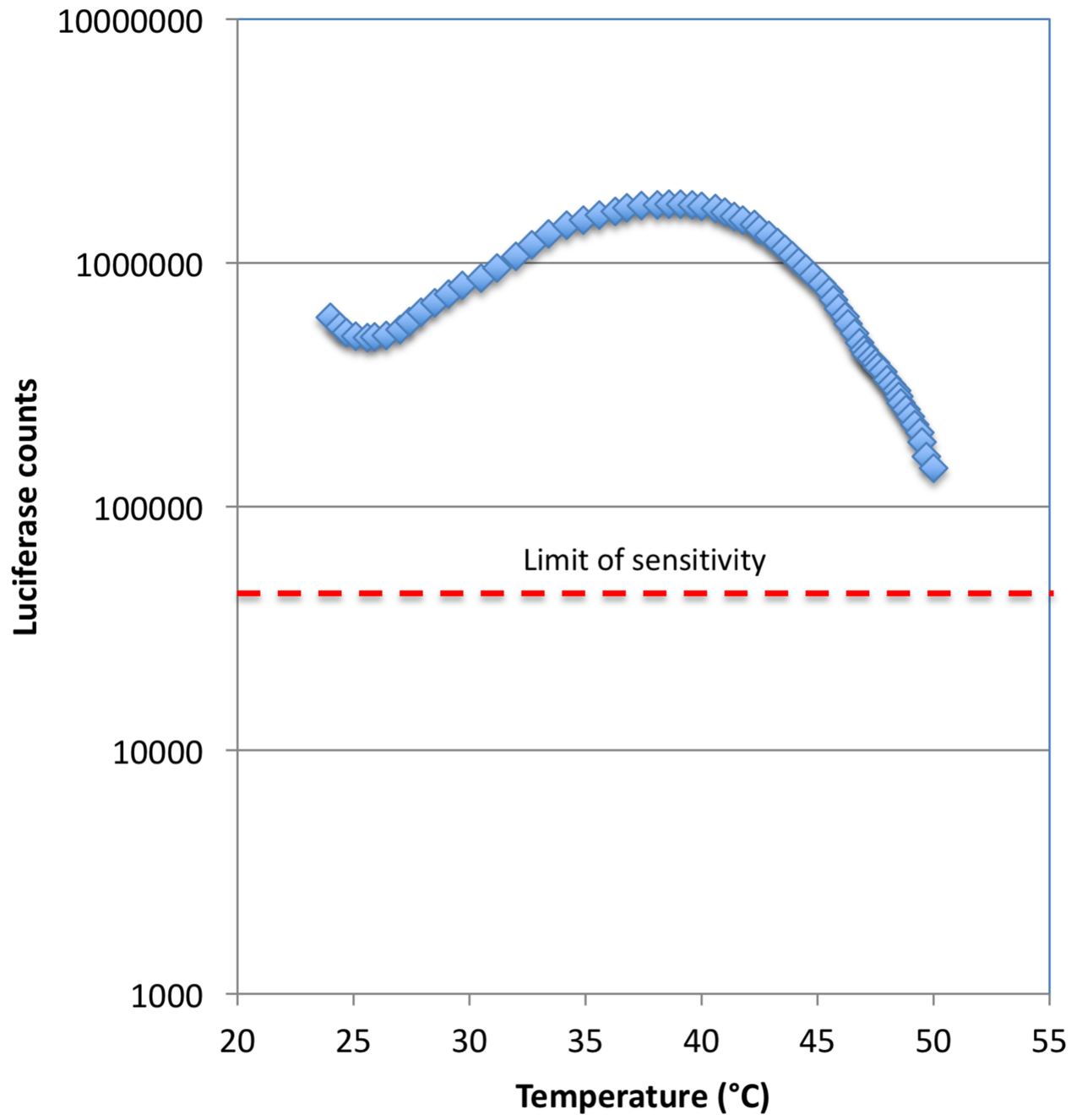


Figure S4 Effect of temperature on bioluminescence measured from live and adherent HEK293T cells expressing TRPV1-Luc. The total luciferase counts from the Luc spectra were measured after addition of 5 µM of Coelenterazine H. The measurement was performed using the Acton Spectrapro2300i. The limit of sensitivity of the spectrometer, e.g. lower number of counts below which the Luc spectra is not efficiently decomposed, is indicated as a red dashed line.

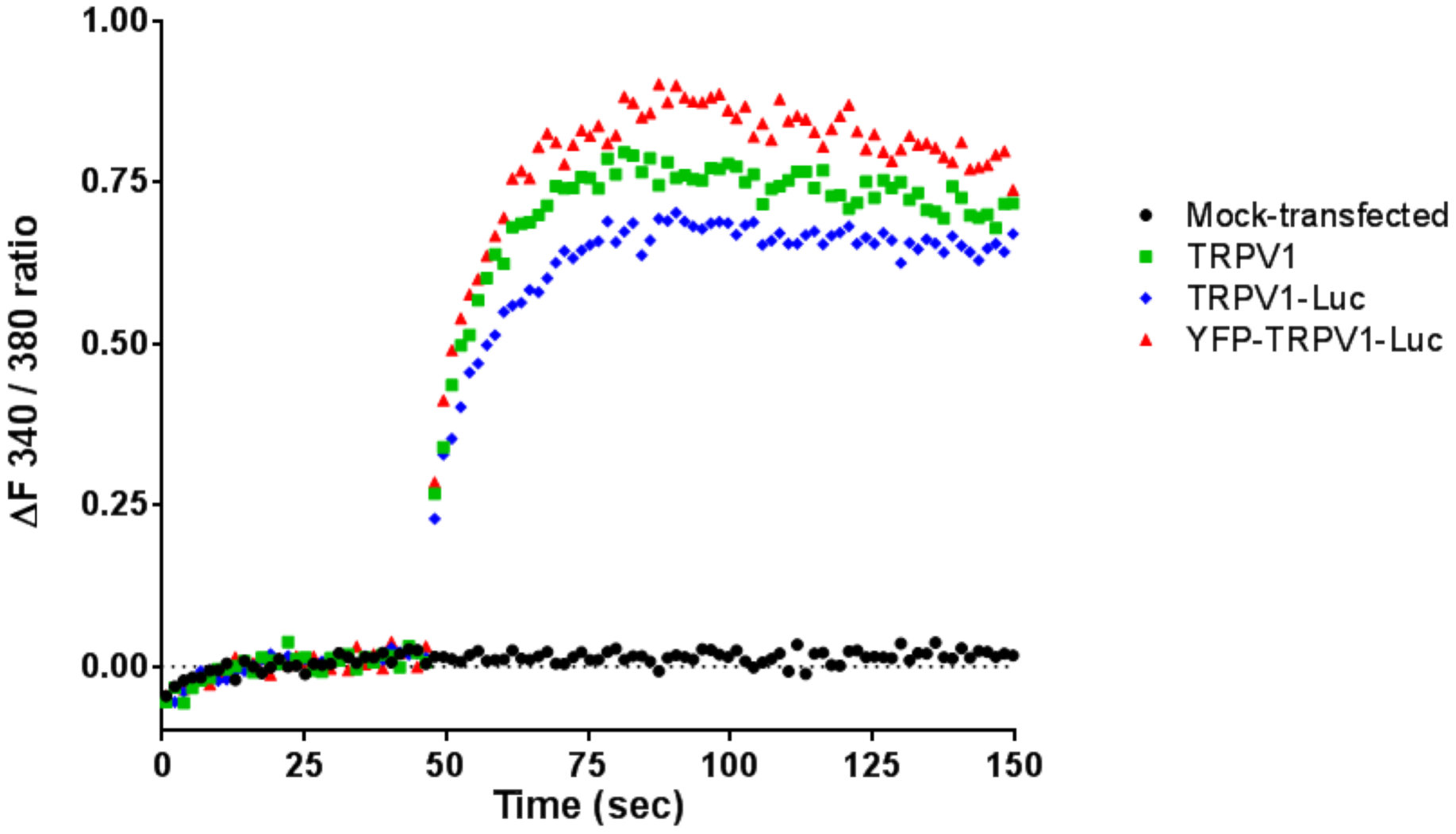


FIGURE S5 Ca^{2+} influx was induced by 20 μM capsaicin stimulation of human embryonic kidney (HEK) cells, mock-transfected or transiently expressing TRPV1, TRPV1-Luc, or YFP-TRPV1-Luc. Ca^{2+} entry was measured as a change in fluorescence intensity, before and after addition of the agonist (applied at 45 sec). The plotted signal corresponds to the difference between the 340/380 nm ratio for each dot, and the basal ratio measured in the absence of stimulation. Data represent the average of three independent experiments. Analysis yielded similar time constants under all TRPV1 transfected conditions with $\tau = 4.71 \pm 0.44$ sec for TRPV1, $\tau = 6.38 \pm 0.43$ sec for TRPV1-Luc, and $\tau = 5.02 \pm 0.51$ sec for YFP-TRPV1-Luc.

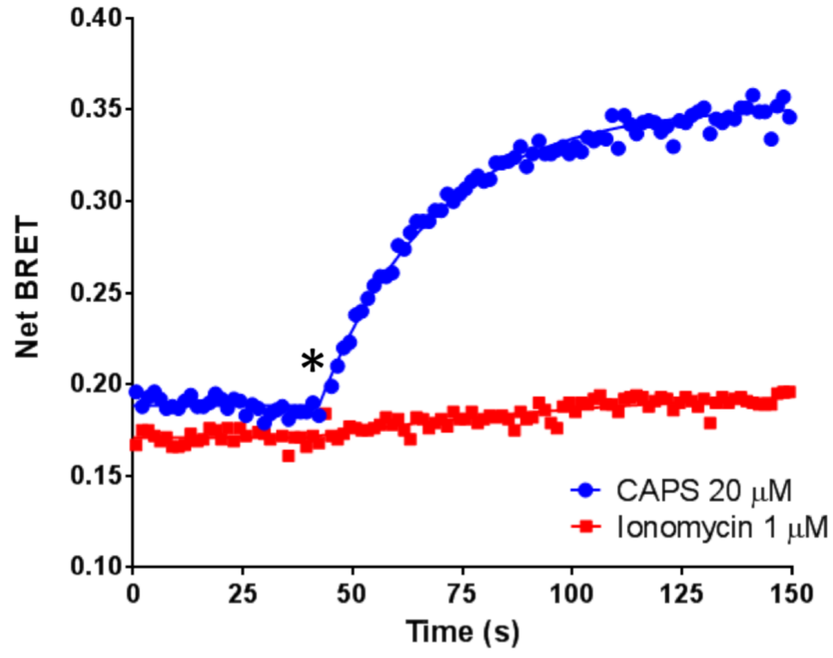
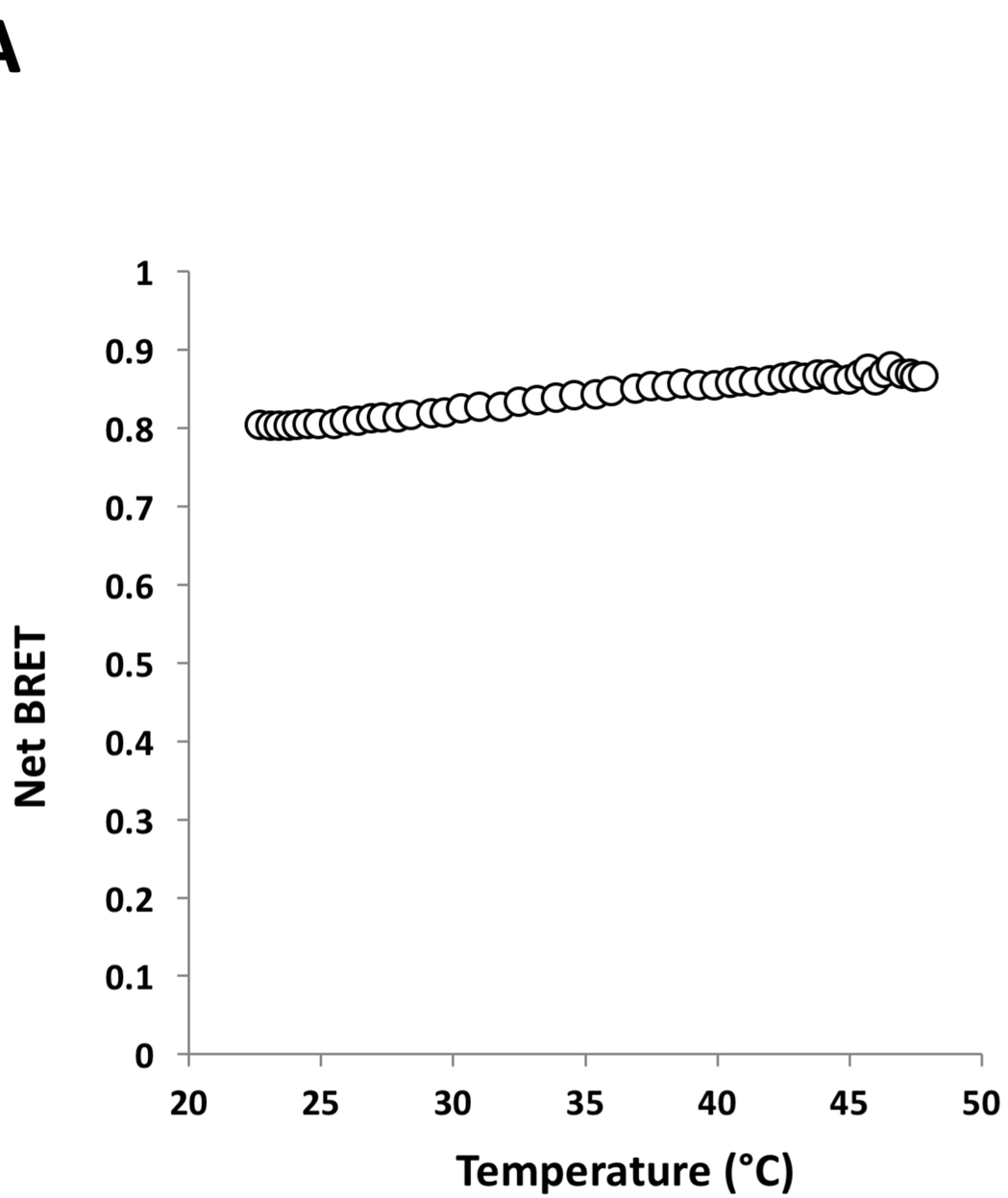


FIGURE S6 Kinetic measurement of the effect of 20 µM CAPS or 1 µM Ionomycin on cells expressing the TRPV1-Luc/YFP-CaM constructs. Compounds were injected at the time indicated by a star. Data represent one out of five independent experiments. The time-constant (τ) of the BRET increase induced by CAPS is 26.28 ± 0.95 s, n=5.



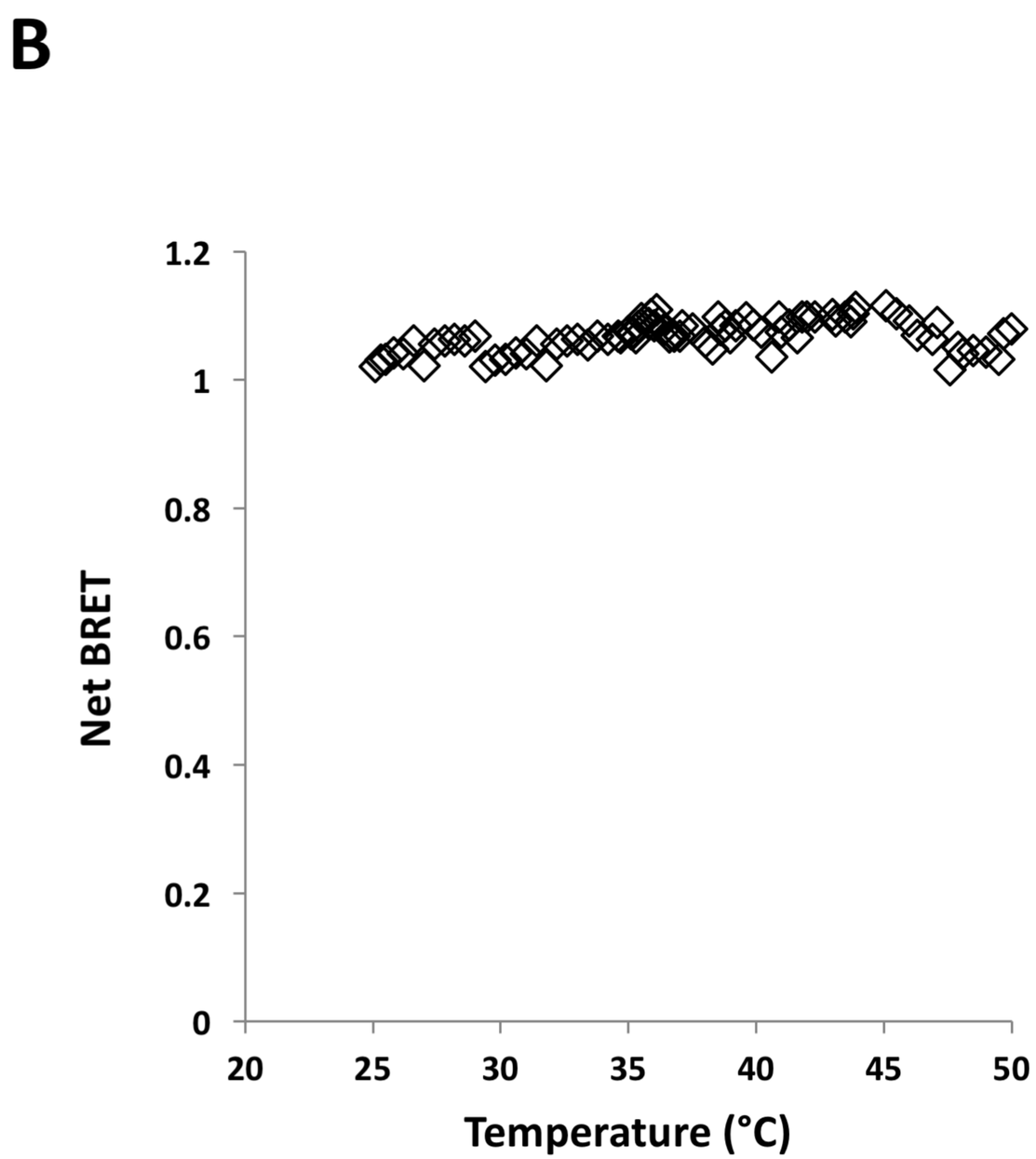


FIGURE S7 Effect of temperature on Net BRET measured on HEK293T cells transfected with YFP-Luc (A) or CD95-Luc/CD95-YFP (B).

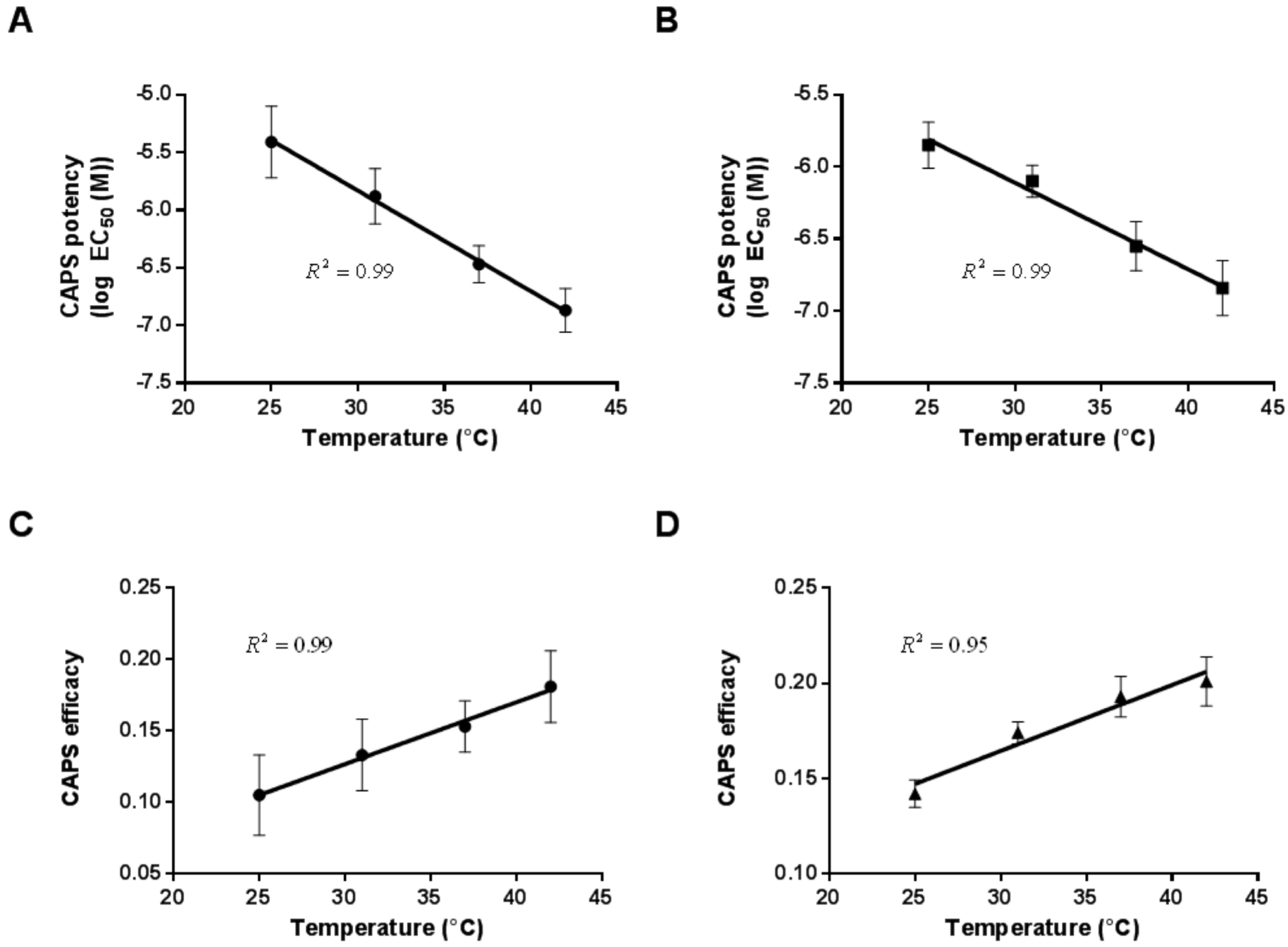


FIGURE S8 Pharmacological parameters derived from CAPS-dose response curves carried out at different temperatures in HEK293T cells expressing either YFP-TRPV1-Luc (A and C) or TRPV1-Luc/YFP-CaM BRET probes (B and D). CAPS Potency (A and B) is expressed as Log EC_{50} (M) and CAPS efficacy (B and D) is expressed as the net BRET variation between the minimum and maximum BRET values derived from sigmoidal dose-response curve fitting. Values represent the mean ± standard error of five independent experiments performed in duplicate. For each set of data, a linear regression has been performed between either CAPS potency or efficacy and the temperature. The goodness of the fit, R^2, is indicated.

Table S1 CAPS potency derived from BRET assays carried out in HEK293T expressing either YFP-TRPV1-Luc or TRPV1-Luc/YFP-CaM, activated with increasing doses of CAPS, with or without inhibitors (vehicle, CPZ 1μM, or AMG517 1 μM). BRET assays were analyzed by nonlinear regression using the GraphPad-Prism software. Potency, expressed as Log EC_{50} (M), was derived from sigmoidal dose-response curve fitting. Values represent the mean ± standard error of four independent experiments performed in duplicate. Data were analyzed against the control condition (no inhibitors) for significance using unpaired Student's t test analysis with Prism software. Asterisks indicate statistical significance of the difference between the inhibitors conditions and control condition with ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$. "ns" indicates no significant differences with the no inhibitor group ($p > 0.05$).

	Inhibitors			
	None (CAPS alone)	Vehicle	AMG517	CPZ
YFP-TRPV1-Luc	−6.50 ± 0.11	-6.44 ± 0.11^{ns}	$-5.07 \pm 0.10^{***}$	$-4.57 \pm 0.16^{**}$
TRPV1-Luc/YFP-CaM	−6.53 ± 0.16	-6.64 ± 0.14^{ns}	$-4.78 \pm 0.09^{****}$	$-4.29 \pm 0.19^{**}$