Two types of BRET constructs were used:

(1) mNeonGreen (mNeonG) and nanoluciferase (nLuc) were incorporated into mammalian expression vectors encoding BRET-based calcium sensors fused to the N-terminus of TRPV1, TRPV4 and TRPM8 (mNeonG-Calflux-nLuc-TRPV1, mNeonG-Calflux-nLuc-TRPV4 and mNeonG-Calflux-nLuc-TRPM8). These expression vectors were generated using cDNA "bricks" obtained by gene synthesis (Genescript, Leiden, The Netherlands), containing sequences for mNeonG (brick 1.1), the calflux calcium ion sensor (brick 1.2), nLuc (brick 1.3), and the TRP ion channel (TRPV1, TRPV4, or TRPM8) (bricks 2 and 3).

(2) Expression vectors encoding rGFP-TRP-rLuc fusion proteins were similarly constructed using cDNA bricks containing sequences for rGFP (brick 1), TRPV1, TRPV4, or TRPM8 (bricks 2 and 3), and rLuc (brick 4).

The cDNA bricks were assembled in-frame and in the correct order into the pcDNA3.1(+)-Lac Z vector using the BsmBI type IIS enzyme and T4 DNA ligase, enabling direct visualization of assembly efficiency through an alpha-complementation colorimetric test. The ligation reaction contained 2 µL of 10× T4 DNA ligase buffer, 1 U of BsmBI enzyme, 1 U of T4 DNA ligase, 0.4 mM ATP, 4 mM dithiothreitol, 200 ng of the pcDNA3.1(+)-Lac Z vector, and 100 ng of cDNA bricks (bricks 1–4). The reaction was subjected to 35 cycles of alternating incubations at 37°C for 1 min and 16°C for 1 min, followed by enzyme inactivation at 55°C for 5 min. The ligation mix was then used to transform Escherichia coli DH5α by thermal shock.

1) Cell culture

Materials:

- **Cells:** XP6BE fibroblast line was supplied by the Coriell Institute (Camden, NJ).
- **Culture medium:** DMEM/F12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F12, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin and 100 µg/mL streptomycin and 3,6 mL of glucose solution (Gibco, Ref A2490-01)
- PBS (Phosphate-Buffered Saline)
- Trypsin-EDTA solution (0.5 g/L EDTA)

Cells are maintained at 37 °C in a humidified incubator with 5% CO_2.

Procedure:

1. **Remove the complete medium** by gently tilting the flask and aspirating the medium (avoid direct suction on cells)
2. **Add 10 mL sterile PBS** along the wall opposite to the cell layer. Perform a gentle wash to remove residual medium and floating cells. Discard the PBS.
3. **Add 1 mL of Trypsin-EDTA**, gently spreading it across the entire cell surface.
4. Incubate the flask for **5 minutes at 37°C** to allow cell detachment.
5. **Tap the flask firmly by hand** to help release the cells from the surface.
6. Add **x mL of complete medium** (volume depends on the desired final cell density).
7. **Resuspend the cells thoroughly** by pipetting up and down 4–5 times to ensure a homogeneous suspension.
8. Distribute the cell suspension into the desired number of plates or flasks. Always **mix the suspension well before seeding** to maintain consistency.
9. Adjust the final volume in each plate to **15 mL with complete medium**.
10. Place the plates **flat in the incubator at 37°C**, ensuring even distribution.

2) Transfection

24 hours before transfection, cells were seeded at a density of 500,000 cells per well in T25 culture flask. Transient transfections were performed using X-tremeGENE HP DNA Transfection Reagent (Ref 06366546001, Sigma-Aldrich) with a X-treme:DNA ratio of 2:1.

After overnight incubation, transfected cells were then detached, resuspended in DMEM w/o red phenol (Ref 21063-029, ThermoFisher scientific, Waltham, MA, USA) and replated at a density of 250 000 cells per mL onto 12 mm diameter glass coverslips (Knittel Glass, Braunschweig, Germany). Cells were left in culture for 24 h before being processed for the BRET assay.

The final concentrations of reagents vary depending on the experimental design. Unless otherwise specified, all compounds are freshly diluted in HBSS solution. All reagents are applied directly to cells in pre-warmed medium and incubated under standard culture conditions (37°C, 5% CO_2) unless specified otherwise.

TRP Agonist:

-Capsaicin (CAPS, was from Tocris (Bristol, UK): final concentration ranges from 3 nM to 10 µM, depending on the experimental conditions.
-GSK1016790A (GSK101) was from Sigma (Lyon, France): used at final concentrations ranging from 0.1 nM to 300 nM.
-WS-12 (was from Tocris (Bristol, UK): applied at final concentrations from 100 nM to 10 µM.

TRP Antagonist:

-Capsazepine (CPZ), RN1734, HC067047 and M8B hydrochloride were all from Tocris (Bristol, UK) and used at a final concentration of 20 µM.

-AMG517 was from Medchemexpress (Princeton, NJ) and used at a final concentration of 20 µM.

Substrat:

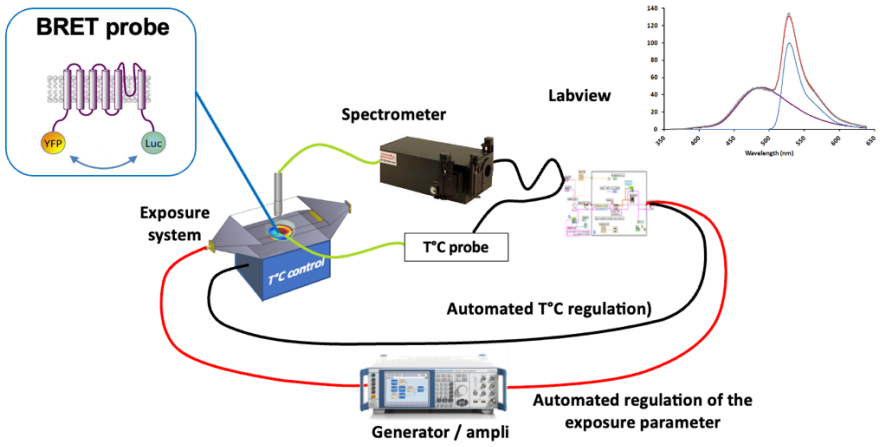
Coelenterazine H (Nanolight Technology, Pinetop, AZ) and Hikarazine-108 (provided by Dr Yves L. Janin) were added to a final concentration of 10 µM.

RF-EMF Exposure System:

The Teflon chamber hosting the fibroblast cells was placed on the ground plane inside a transverse electromagnetic (TEM) cell in which the RF-EMF signal was propagating. Two 10 mm diameter holes were drilled in the septum and top ground plate of the TEM cell to insert the optic fiber for BRET signal measurements. All tested RF-EMF signals were delivered to the TEM cell using a vector generator (SMBV100A, Rohde & Schwarz, Munich, Germany) connected to a 10-W power preamplifier in series with a 200-W power amplifier (RF14002600-10 and RFS1800-200, respectively; RFPA, Artigues-Pres-Bordeaux, France).

Spectral BRET setup:

Full BRET spectra were acquired using an optical fiber linked to a Spectra Pro 2300i spectrometer (Princeton Instruments, Acton, MA), equipped with a liquid-nitrogen-cooled charge-coupled device camera for recording the full visible spectrum (Acton Optics). The bioluminescent signal was recorded from transfected cells seeded onto a glass coverslip and placed into an HBSS solution. The temperature of the cell buffer was regulated using a home-made system and measured in real time using a fiber-optic temperature measurement Luxtron 812 system (Lumasense Technologies, Santa Clara, CA).



Spectral BRET recording:

Using the LabView programming language (National Instruments, Austin, TX), an interface was developed to acquire the bioluminescent spectra and perform real-time spectral decomposition of the BRET signal into its various components.

The BRET signal was determined by calculating the ratio of the emission intensity measured in the acceptor window (ImNeonG or rGFP) over the emission intensity measured in the donor window (InLuc or rLuc).