Supplementary Information

Monitoring TRPC7 Conformational Changes by BRET Following GPCR Activation

Cécile Pétigny [1,2,3], Audrey-Ann Dumont [2,3,4], Hugo Giguère [2,3,4], Audrey Collette [1,2,3], Brian J. Holleran [1,2,3], Mircea Iftinca [5], Christophe Altier [5], Élie Besserer-Offroy [6,7,8], Mannix Auger-Messier [2,3,4] and Richard Leduc [1,2,3,*]

1 Department of Pharmacology-Physiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC J1H 5N4, Canada; cecile.petigny@usherbrooke.ca (C.P.); audrey.collette@usherbrooke.ca (A.C.); brian.holleran@usherbrooke.ca (B.J.H.)
2 Institut de Pharmacologie de Sherbrooke, Université de Sherbrooke, Sherbrooke, QC J1H 5N4, Canada
3 Centre de recherche du Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, QC J1H 5N4, Canada
4 Department of Medicine, Division of Cardiology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC J1H 5N4, Canada; audrey-ann.dumont@usherbrooke.ca (A.-A.D.); hugo.giguere@usherbrooke.ca (H.G.); mannix.auger-messier@usherbrooke.ca (M.A.-M.)
5 Department of Physiology and Pharmacology, Inflammation Research Network-Snyder Institute for Chronic Diseases and Alberta Children's Hospital Research Institute, University of Calgary, Calgary, AB T2N 1N4, Canada; miftinca@ucalgary.ca (M.I.); altier@ucalgary.ca (C.A.)
6 Department of Molecular and Medical Pharmacology, Ahmanson Translational Theranostics Division, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA 90095, USA; ebesserer@ucla.edu (É. B.-O.)
7 Jonsson Comprehensive Cancer Center, University of California, Los Angeles, CA 90095, USA
8 California NanoSystems Institute, Los Angeles, CA 90095, USA
* Correspondence: richard.leduc@usherbrooke.ca (R.L.)

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Table S1: PCR primers used for TRPC7 amplification.

Biosensor	Primer	DNA sequence
RLUCII-TRPC7-GFP10	Forward	gagcagggatccgccggtaccATGTTGAGGAACAGCACC
	Reverse	tgctcaccatggtggcgggaagcttAATGTCTTTGCCCTTGTTC
GFP10-TRPC7-RLUCII	Forward	tacaagggatccgccggtaccATGTTGAGGAACAGCACC
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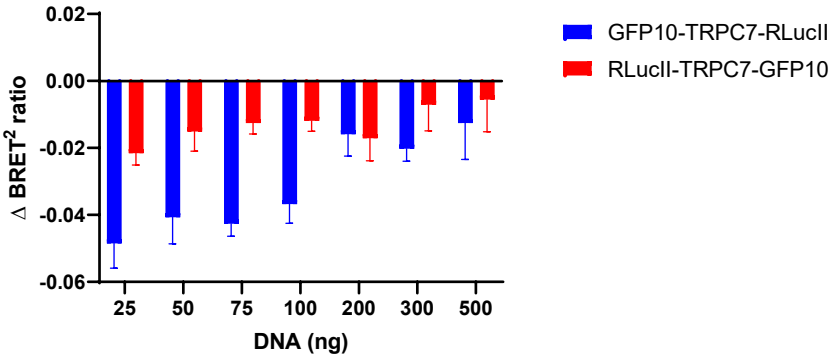

Figure S1: ΔBRET² ratio of double-tagged TRPC7 biosensors after Gα_q-DREADD activation by CNO. HEK293 cells were co-transfected with plasmids encoding RLucII-TRPC7-GFP10 or GFP10-TRPC7-RLucII biosensor and Gα_q-DREADD or co-transfected with an empty vector containing RLucII tag and Gα_q-DREADD. BRET signal was measured after stimulation with 1 μM of Clozapine-N-Oxide (CNO) or vehicle as a control. BRET was calculated by subtracting background luminescence of cells only expressing RLucII, approximately 60s after stimulation.

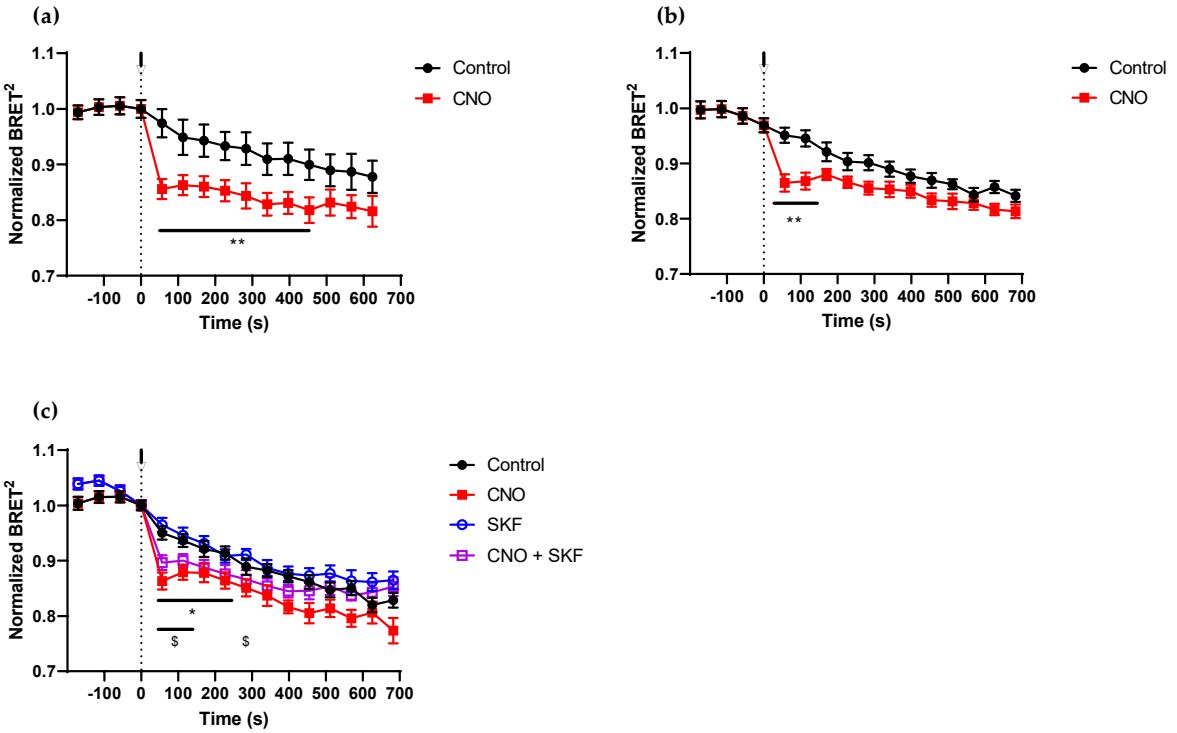

Figure S2: Modulation of BRET ratio in calcium-free conditions and response to pharmacological calcium entry blockade. HEK293 cells were co-transfected with plasmids encoding GFP10-TRPC7-RLucII (75 ng), Gαq-DREADD (500 ng) and were stimulated with CNO, 1 µM or vehicle as a control. The BRET signal was measured for 10 minutes in HBSS buffer containing calcium (a) or in calcium-free HBSS buffer replaced 5 min before acquisition (b). (c) HEK293 cells were transfected in the same conditions as in (a) and were pre-incubated 10 minutes with SKF96365 (SKF; 10 µM), a non-selective TRPC channel calcium entry blocker before BRET measurement and stimulation with CNO (1 µM) or vehicle as a control. BRET signal was measured for 10 minutes. Each data set represents the mean of three independent experiments, which were each done in triplicate, and expressed as the mean ± S.E.M. Statistical analyses were performed using a Two-Way ANOVA with multiple comparisons followed by a Sidak's post-hoc test. * $p < 0.05$, ** $p < 0.01$ for control *vs.* CNO; $ $p < 0.05$ for SKF *vs.* CNO + SKF.

Sequence S1: DNA sequence of GFP10-TRPC7-RlucII biosensor.

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