

Supplementary Information

Monitoring TRPC7 Conformational Changes by BRET Following GPCR Activation

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Table S1: PCR primers used for TRPC7 amplification.

Biosensor	Primer	DNA sequence
RLUCII-TRPC7-GFP10	Forward	gagcaggatccgggtaccATGTTGAGGAACAGCACC
	Reverse	tgctcaccatggtggcgaaagcttAATGTCTTGCCCTGTTC
GFP10-TRPC7-RLUCII	Forward	tacaaggatccgggtaccATGTTGAGGAACAGCACC
	Reverse	tgctggcatggtggcgaaagcttAATGTCTTGCCCTGTTC

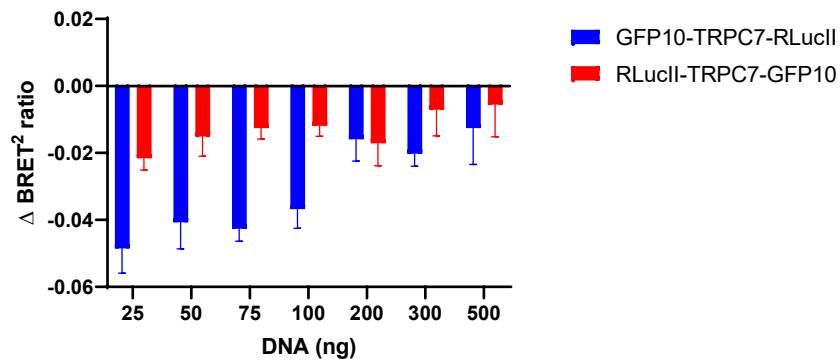


Figure S1: Δ BRET² ratio of double-tagged TRPC7 biosensors after G α_q -DREADD activation by CNO. HEK293 cells were co-transfected with plasmids encoding RLucII-TRPC7-GFP10 or GFP10-TRPC7-RLucII biosensor and G α_q -DREADD or co-transfected with an empty vector containing RLucII tag and G α_q -DREADD. BRET signal was measured after stimulation with 1 μ M 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.

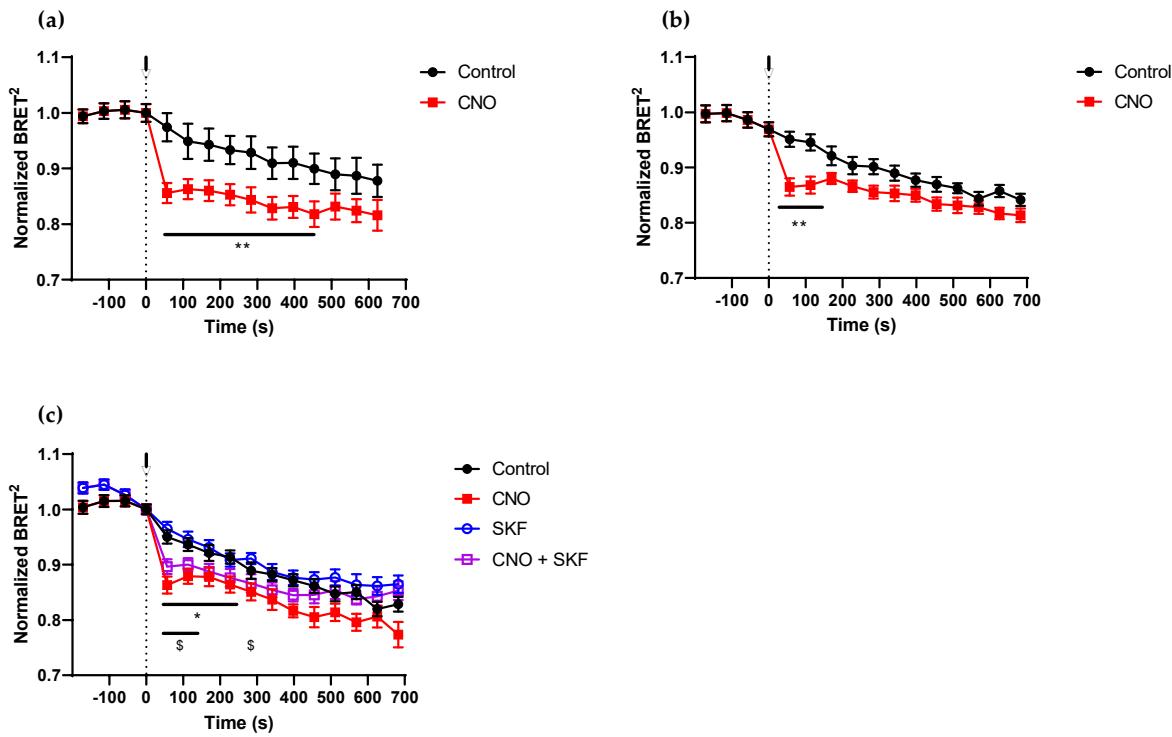


Figure S2: Modulation of BRET ratio in calcium-free conditions and response to pharmacological calcium entry blockade. HEK293 cells were co-transfected with plasmids encoding GFP10-TRPC7-RLucII (75 ng), G α q-DREADD (500 ng) and were stimulated with CNO, 1 μ M or vehicle as a control. The BRET signal 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 μ M), a non-selective TRPC channel calcium entry blocker before BRET measurement and stimulation with CNO (1 μ M) 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 \pm 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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Sequence S2: DNA sequence of RlucII-TRPC7-GFP10 biosensor.

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