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	mCES2 samples									
	30	ng	60	ng	120 ng					
Time (sec)	average	S.E.M	average	S.E.M	average	S.E.M				
0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000				
10	0.025467	0.000720	0.051200	0.002265	0.097800	0.003859				
20	0.043867	0.001785	0.093000	0.002868	0.167533	0.004907				
30	0.062200	0.003559	0.120333	0.001785	0.211867	0.006706				
40	0.082533	0.000720	0.145667	0.013411	0.289200	0.003266				
50	0.102000	0.001633	0.171267	0.014350	0.329000	0.005354				
60	0.101467	0.000544	0.201867	0.014631	0.340467	0.001361				
	NTC samples									
	30	ng	60	ng	120 ng					
Time (sec)	average	S.E.M	average	S.E.M	average	S.E.M				
0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000				
10	0.015133	0.002880	0.023467	0.005761	0.069133	0.001515				
20	0.029200	0.001247	0.064200	0.003399	0.131867	0.004481				
30	0.037867	0.003954	0.091533	0.001785	0.176867	0.003839				
40	0.054867	0.004119	0.118867	0.004907	0.231533	0.003067				
50	0.077333	0.003839	0.143667	0.004380	0.284333	0.002373				
60	0.072800	0.000817	0.153467	0.005664	0.306467	0.003345				

Table 1: Esterase activity of mCES2 (mouse Carboxyl ESterase 2) and NTC (Non-Template Control) samples using PNPA method. Data table of Fig 3 c representing time dependent and dose-dependent curve shown above as absorbance at 410 nm, n=3.



Figure1: SERCA activity measured by fluorescence spectroscopy: Fluorescence activity measured with the *m*CES2 microsomes pre-loaded with Ca²⁺ under different experimental conditions. SERCA activity in the presence of ATP (SERCA + ATP), SERCA activity in the presence of TG and ATP (SERCA +TG+ATP) and SERCA activity measured in the presence of 100 nM TG without any ATP (SERCA+TG-ATP), n=2, 10 µg of *m*CES2 protein each well, data represented mean +/- S.E.M. Clear inhibition of SERCA activity is observed with TG in samples with and without ATP.



Figure 2: Inhibitory effects of dantrolene on caffeine induced calcium release via ryanodine receptor (RyR2) activation in *m*CES2 microsomes, n=5 each. ** indicate significant difference of caffeine sample dataset before (150 s) and after adding caffeine (600 sec) with p value = 0.002, paired t test, two tailed. # indicate the significant difference between the two sample groups at 600 sec with p value = 0.0159, Mann Whitney test, two tailed. The fluorescence value is represented as Δ F/F *100 of Fluo 5N-AM +/- S.E.M. All the samples were calcium loaded with ATP for 60 min, 37°C prior to caffeine and dantrolene experiments. For caffeine experiments, 1 mM caffeine was used for activation of ryanodine channel at 5 min. For inhibition experiments, the samples were pre-incubated with dantrolene minimum 20 min prior to start of the experiments and also additionally maintained in the baseline buffer from time 0 to 5 min. No calcium release induced by caffeine added at 5 min was observed in samples in the presence of dantrolene.



Fig3: Gradual leakage caused due to change in the buffer from 200 μ M to 300 nM Ca²⁺, n=5; data represented mean +/- S.E.M.



Fig 4: a) Protein yields of bTRPV3, bovine Transient Receptor Potential Channel Vanilloid Receptor member 3. by scintillation counting via CECF reaction for 24 h, 27 °C in Sf21 system; suspension and pellet correspond to translation mix and vesicular fraction correspondingly. The plasmid construct was similar to hTRPV1 construct as mentioned in this work. Data is represented as mean+/- S.E.M, n = 3, TM- Translation mix, SN- Supernatant, VF- Vesicular fraction. b) Autoradiogram of proteins run on SDS MES gel, no band was observed in Non-template Control microsomes and 91 kDa protein was observed in bTRPV3 samples; c) Calcium imaging of mCES2 and bTRPV3-mCES2 cell-free synthesized microsomes with menthol, 200 µM for activation of bTRPV3 functionality. Ca2+ release was observed only in bTRPV3mCES2 microsomes, data is represented as deltaF/F *100 +/- S.E.M, of dye fluorescence, n=5 each. ### indicate the significant difference between the mCES2 samples and bTRPV3-mCES2 samples at 600 s, p value < 0.0001, unpaired t test, two tailed. *** indicate the significant difference of bTRPV3-mCES2 sample data at 150 s and 600 s, before and after menthol addition, p < 0.0001, Paired t test, two tailed.