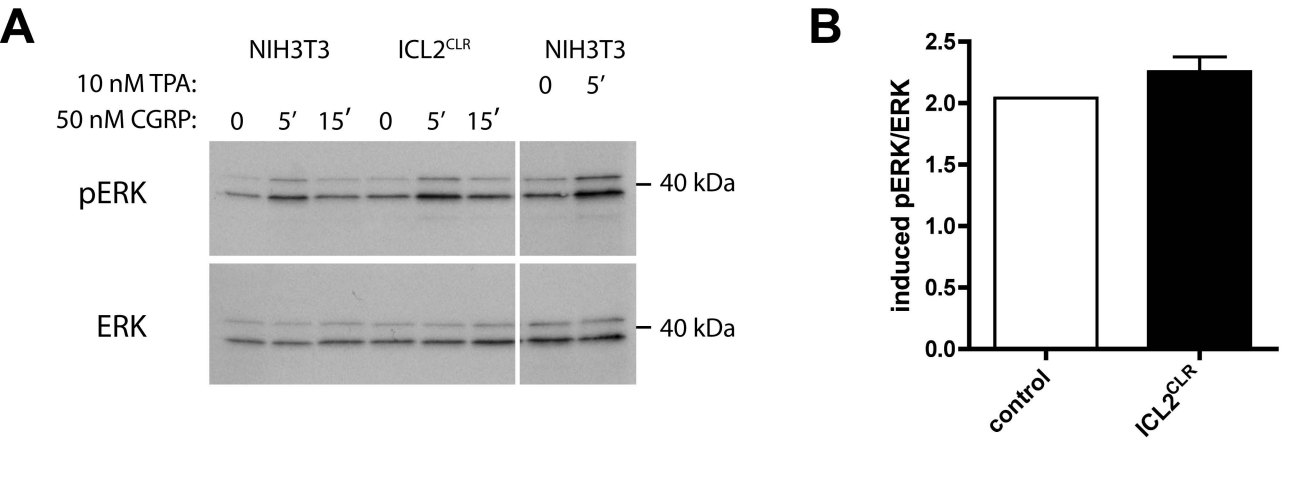
Supplemental Figure 1. Expression of ICL2^{CLR} does not alter CGRP-mediated ERK phosphorylation in NIH3T3 cells. NIH3T3 cells were plated in 6-well plates at a density of 62,000 cells per well. Forty-eight hours after plating cells were transfected with ICL2^{CLR}-GFP (n=7) or GFP alone. Twenty-four hours after transfection cells were serum-starved for 16 hrs by changing media to Optimem (Invitrogen) supplemented with 1% BSA. Cells were approximately 90% confluent when incubated with Optimem supplemented with either 50 nM CGRP or 10 nM 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 5' or 15'. After incubation cells were immediately placed on ice, rinsed once with cold PBS, and extracted into 200 ul Laemmlli loading buffer. One tenth of the cell lysate from each well was analyzed in duplicate by 15% SDS-PAGE, transferred to PVDF membranes (Bio-Rad Laboratories, Inc.), and immunoblotted with antibodies against ERK1/2 (Cell Signaling #4695S diluted 1:2000) or phospho-ERK1/2 (Cell Signaling #4370S diluted 1:4000) using methods described. Autoradiograms were scanned and bands quantified using Kodak 1D image analysis software. Maximal pERK response was observed at 5' incubation with CGRP. Phospo-ERK was normalized to ERK, and increase of normalized pERK at t=5' compared to t=0 was calculated. A) Representative blot for one of the replicate ICL2^{CLR} transfections B) quantified data for all replicates, expressed as fold increase of pERK at t=5' compared to t=0. No statistical difference was observed between the two conditions using a Student's t-test.


A
NIH3T3
ICL2CLR
NIH3T3
10 nM TPA:
0 5′
50 nM CGRP:
0 5′ 15′ 0 5′ 15′
pERK
40 kDa
ERK
40 kDa
B
induced pERK/ERK
2.5
2.0
1.5
1.0
0.5
0.0
control
ICL2CLR