A Calmodulin/Inositol 1,4,5-Trisphosphate (IP$_3$) Receptor-binding Region Targets TRPC3 to the Plasma Membrane in a Calmodulin/IP$_3$ Receptor-independent Process*

Conformational coupling with the inositol 1,4,5-trisphosphate (IP$_3$) receptor has been suggested as a possible mechanism of activation of TRPC3 channels and a region in the C terminus of TRPC3 has been shown to interact with the IP$_3$ receptor as well as calmodulin (calmodulin/IP$_3$ receptor-binding (CIRB) region). Here we show that internal deletion of 20 amino acids corresponding to the highly conserved CIRB region results in the loss of diacylglycerol and agonist-mediated channel activation in HEK293 cells. By using confocal microscopy to examine the cellular localization of Topaz fluorescent protein fusion constructs, we demonstrate that this loss in activity is caused by faulty targeting of CIRB-deleted mutants to intracellular compartments. Wild type TRPC3 and mutants lacking a C-terminal predicted coiled coil region downstream of CIRB were targeted to the plasma membrane correctly in HEK293 cells and exhibited TRPC3-mediated calcium entry in response to agonist activation. Mutation of conserved YQ and MKR motifs to alanine within the CIRB region in TRPC3-Topaz, which would be expected to interfere with IP$_3$ receptor and/or calmodulin binding, had no effect on channel function or targeting. Additionally, TRPC3 targets to the plasma membrane of DT40 cells lacking all three IP$_3$ receptors and forms functional ion channels. These findings indicate that the previously identified CIRB region of TRPC3 is involved in its targeting to the plasma membrane by a mechanism that does not involve interaction with IP$_3$ receptors.

Calcium entry in nonexcitable cells is most commonly triggered by the activation of a receptor stimulating phospholipase C to cleave phosphatidylinositol 4,5-bisphosphate into diacylglycerol (DAG), which stimulates protein kinase C, and inositol 1,4,5-trisphosphate (IP$_3$), which binds to the IP$_3$ receptor to transient receptor potential family of ion channels, has been shown to be involved by agonist activation of plasma membrane G-protein-coupled receptors, by synthetic diacylglycerols, and by store depletion in some cell types (2–4). A conformational coupling model physically linking TRPC3 with the IP$_3$ receptor has been suggested, and a region in the cytosolic C terminus of TRPC3 has been shown to co-immunoprecipitate with regions of the IP$_3$ receptor (5, 6). An overlapping region of TRPC3 binds to calmodulin (CaM), which is hypothesized to be tethered to the channel and to be displaced competitively by the activated IP$_3$ receptor; the region that commonly binds both IP$_3$ receptor and calmodulin has been termed the calmodulin/IP$_3$ receptor-binding (CIRB) region (7). However, binding of CaM to the isolated CaM-binding region of TRPC3 is dependent on the calcium concentration, which is inconsistent with a model of CaM being tethered to the channel independently of the calcium concentration. Calmodulin binding to a CIRB homologous region in all members of the TRPC family (TRPC1–7) has been demonstrated (8). A second CaM-binding region has been identified in other TRPs. For TRPC4, CaM binds in a region unique to the splice variant TRPC4α, which is not present in the splice variant TRPC4β (8, 9). This region has also been shown to interact with the C terminus of IP$_3$ receptors in a yeast two-hybrid screen and glutathione S-transferase pull-down assays (10). Binding to both CaM-binding domains of TRPC4 occurs only above 10 μM Ca$^{2+}$ with an apparent K$_D$ of 100–200 μM CaM (9). For TRPC1 a second CaM-binding domain has also been reported that overlaps with a predicted coiled coil region common to the C terminus of all TRPCs (11). This second CaM-binding region has been shown to be involved in calcium-dependent inactivation of TRPC1 (11). Our goal was to identify functional roles of the CIRB and other domains of TRPC3 using N- and C-terminal truncation mutants of TRPC3.

**MATERIALS AND METHODS**

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1 The abbreviations used are: DAG, diacylglycerol; TRPC, canonical transient receptor potential; ER, endoplasmic reticulum; IP$_3$, inositol 1,4,5-trisphosphate; OAG, 1-eleotyl-2-acetylsn-glycerol; CIRB, calmodulin/IP$_3$ receptor-binding; CaM, calmodulin; EYFP, enhanced yellow fluorescent protein.

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Calcium containing buffer (20 mM HEPES, 120 mM NaCl, 5.4 mM KCl, 0.8 mM MgCl₂, 11.1 mM glucose, pH 7.4). GdCl₃, methyl- 

were loaded with Fluo4-AM as described above and washed twice with nominally calcium free buffer (20 mM HEPES, 120 mM NaCl, 0.8 mM MgCl₂, 11.1 mM glucose, pH 7.4). GdCl₃, methyl-

fluorescence was organized site 3 of the stop codon was mutated keeping the Ascl site 

fusing TrpC3 to Topaz intact. An Ascl site was introduced into the sequence at the desired site of truncation of TrpC3. The mutants were 

digested with Ascl and religated to yield truncated TrpC3-Topaz fusions. For the internal deletion mutant T3T-CΔ78 oligonucleotides 5′- 
cagattctacaagctgagatgatatcagcctgaaag and 5′-ccttttcattatctggtgtacagcagataatgaaaag and 5′-ccttttcattatcttggtgtacagcagataatgaaaag were used in the mutagenesis reaction with TrpC3-Topaz as template.

Cell Culture and Transfection—For the production of pools of cells stably expressing wild type and mutant TRPC3, HEK293 cells were 

transfected with LipofectAMINE2000® at ~80% confluency according to the manufacturer's instructions. The day after transfection the cells were 

split into selection medium containing 0.5 mg/ml G418 and grown for 4 weeks in the continued presence of selection medium. In parallel, 

cell control transfected only with the fluorescent marker pdsRedmito were selected for G418 resistance as above, and pdsRedmito expression 

was confirmed by their red fluorescence (excitation, 585 nm; emission, 610 nm).

The chicken B lymphocyte cell line DT40 and the mutant variant in which the genes for all three IP3 receptor types were disrupted were 

obtained from the Institute of Physical and Chemical Research (RIKEN; Cell Bank code RB1464 and RB1467). The cells were cul-

tured essentially as described by Sugawara et al. (13). DT40 were 

transiently transfected by electroporation to (14) with the indicated 

amounts of the human isoform of TRPC3 or deletion variants or its 

vector (pcDNA3), along with EYFP-C1 vector (Clontech) as a marker for 

transfection.

The cells were co-transfected with the human Mus 

muscarnic receptor (50 µg/ml, in pcDNA3). The cells were assayed 17–25 h post-transfection. The fluorescence measurements were performed un-

der the conditions indicated with single enhanced yellow fluorescent protein (EYFP)-positive cells, selected by their yellow/green fluores-

cence (excitation, 485 nm; emission, 520 nm). Under the conditions of 

measurement, EYFP expression did not contribute significant 

fluorescence.

Flow Cytometry—Pools of G418-resistant cells expressing wild type or mutant T3T were subjected to flow cytometric analysis to enrich for 

cells with high expression levels of fluorescent protein fusions. The cells were 

were treated with trypsin and analyzed on a FACSVantage SE flow 

microscope equipped with Cell Quest software (Becton-Dickinson, San 

Jose, CA). Fluorescence was assayed using an excitation wavelength of 

488 nm and an emission wavelength of 530 nm.

Calcium Measurement—Intracellular calcium concentration was measured with a real time fluorescence plate reader system (FLIPR-

384; Molecular Devices, Sunnyvale, CA); the cells were plated in poly-

L-lysine-coated, black-walled 96-well plates at ~30–40% confluency and 

incubated overnight at 37 °C to allow attachment of the cells. The cells were loaded for 90 min at 37 °C with 4 µM Fluo-4-AM and washed 

twice with nominally calcium free buffer (20 mM HEPES, 120 mM NaCl, 

5.4 mM KCl, 0.8 mM MgCl₂, 11.1 mM glucose, pH 7.4). GdCl₃ meth-

acholine, and CaCl₂ at final concentrations of 1 µM, 100 µM, and 1.8 mM, 

respectively, were added at the time points indicated in the figure. For 

measurement of 1-oleoyl-2-acetyl-sn-glycerol (OAG) activation, the cells 

were loaded with Fluo4-AM as described above and washed twice with 

calcium containing buffer (20 mM HEPES, 120 mM NaCl, 5.4 mM KCl,
entry, whereas the CIRB deletion, T3T-CΔ78, was inactive. The latter deletion is similar in length to the active T3T-CΔ8, indicating that it is the specific sequence of amino acids in the CΔ78 (CIRB) region that is important for activity. All of the mutants that showed Gd³⁺-insensitive Ca²⁺ entry in response to methacholine could also be activated by OAG (Fig. 6).

To determine whether mutants that showed no Ca²⁺ entry were properly targeted to the plasma membrane, we examined the cellular localization of the respective Topaz fusion proteins by confocal microscopy (Fig. 7). As previously reported for a GFP fusion of TRPC3 (18), wild type T3T was mostly localized in punctate regions in the plasma membrane. There was additional staining in intracellular compartments, possibly in the region of the endoplasmic reticulum (ER) and Golgi apparatus, which may represent newly synthesized protein (Fig. 7). A similar pattern was observed for the conservative and physiologically active deletion mutants, T3T-NΔ0 and T3T-CΔ8, although the labeling appears more uniform and less punctate.

The physiologically inactive N-terminal truncation mutant T3T-NΔ1 and C-terminal truncation mutants T3T-CΔ7 and T3T-CΔ78 appeared to be predominantly located in intracellular compartments with no detectable labeling of the plasma membrane (Fig. 7).

Because the CIRB region appeared to be crucial for plasma membrane targeting of T3T, we also mutated conserved amino acids within the overlapping IP₃ receptor/CaM-binding domain to see whether mutations within this region (T3T-YQ/AA and T3T-MKR/AAA; Fig. 4, bottom panel) could also cause loss of plasma membrane targeting as well as agonist and OAG-induced Ca²⁺ entry seen with deletion of the entire region (Fig. 4, bottom panel). Mutation to alanine of the corresponding residues in L-type Ca²⁺ channels was shown to reduce both CaM binding and Ca²⁺-dependent inactivation (19). All of the T3T mutants were expressed in HEK293 cells, and G418-resistant cells with high expression levels were selected using flow cytometry. However, these mutations within the CIRB region (T3T-YQ/AA and T3T-MKR/AAA) did not reduce Gd³⁺-insensitive Ca²⁺ entry in response to methacholine or OAG as compared with wild type T3T (Fig. 8, A and B). Consistent with this phenotype T3T-YQ/AA was detectable throughout the plasma membrane with some protein in cytosolic compartments, possibly ER/Golgi (Fig. 8C).

The findings to this point demonstrate that deletions of the CIRB region block function of TRPC3 by preventing proper trafficking of the protein to the plasma membrane. Because this region has been shown to interact with IP₃ receptors (20), we considered the possibility that this interaction with IP₃ receptors is involved in proper targeting of TRPC3. To examine the possible role of IP₃ receptors in targeting of TRPC3, we made use of a DT40 chicken B-lymphocyte cell line lacking all three types of IP₃ receptors (DT40-KO). To be able to compare phenotypes of T3T and deletion mutants between HEK cells and DT40 KO cells, we first set out to optimize transfection levels of T3T in wild type DT40 cells, which are by experience harder to transfect than HEK cells, aiming to reach similar expression levels as in HEK cells. We accomplished this by two different strategies: first by increasing the amount of plasmid used for transfection from 10 to 100 µg/ml cells as described recently by Vazquez et al. (21) and second by lowering the incubation temperature after transfection to 30 °C. For DT40 cells grown at 37 °C, no TRPC3-Topaz fluorescence was detectable when applying the settings used for HEK cells in Figs. 7 and 8C (optical slice thickness, 0.5 µm); rather, fluorescent images could be obtained only when the optical slice thickness was maximized, such that the resulting images were not confocal (data not shown). Confocal images of T3T expressing
DT40 cells grown at 30 °C, however, could be obtained using the same settings used for HEK cells (Fig. 9A) and show a punctate pattern in the plasma membrane as seen before in HEK cells. Previous functional studies on TRPC3 expressing cells have suggested that IP3 receptors are not required for agonist induced Ca\(^{2+}\) entry in this cell line (21, 22). Consistent
with the functional data, IP$_3$ receptors also do not appear to be required for plasma membrane targeting of T3T because we observe plasma membrane expression of the Topaz-labeled protein in both wild type and DT40-KO cells (Fig. 9A). As for HEK293 cells, deletion of the CIRB region (C/H90047) resulted in the loss of the distinct punctate pattern of plasma membrane localization (Fig. 9A). For functional studies DT40-KO cells were transfected with cDNAs encoding the M5 muscarinic receptor under the control of the chicken β-actin promoter, EYFP as a transfection marker, and the respective T3T construct (Fig. 9B). DT40-KO cells are known to respond to phospholipase C-coupled receptors with generation of IP$_3$, but they do not generate phospholipase C-linked cytosolic Ca$^{2+}$ signals (13). The addition of carbachol to DT40-KO cells expressing T3T and M5 receptors presumably causes formation of IP$_3$ and DAG but no release of Ca$^{2+}$ from stores because of the lack all three types of IP$_3$ receptors. Despite this lack of IP$_3$ receptors, T3T is able to function in a receptor-operated mode as revealed by significant entry of Ba$^{2+}$ (Fig. 9B). When T3T-CΔ7 and T3T-CΔ8 were expressed in DT40-KO cells, we obtained the same phenotypes as in HEK293 cells; whereas the predicted C-terminal coiled coil region could be deleted in T3T without loss of agonist

**FIG. 5.** Agonist-induced Ca$^{2+}$ entry in Topaz-tagged TRPC3 truncation mutants. HEK cells stably expressing pcDNA3, T3T, or T3T truncation mutants were assayed in a FLIPR as described under “Materials and Methods” to determine Ca$^{2+}$ release and entry in response to treatment with 100 μM methacholine. The protocol is as described in the legend to Fig. 3. The measurements were performed in the presence of 1 μM Gd$^{3+}$ to block endogenous channels. Shown is a representative experiment.

**FIG. 6.** OAG-activated Ca$^{2+}$ entry in Topaz-tagged TRPC3 truncation mutants. HEK cells stably expressing pcDNA3, T3T, or T3T truncation mutants were assayed in a FLIPR. 100 μM OAG was added to cells in the continued presence of 1.8 mM Ca$^{2+}$. A pcDNA3 control trace obtained in the same FLIPR experiment as for the truncation mutants is repeated in each panel. Shown is a representative experiment.
induced entry, additional deletion of the CIRB region results in loss of function, a function that is independent of the presence of IP$_3$ receptors.

Because the CIRB region of TRPC3 has been shown to bind to both the IP$_3$ receptor and CaM, we next set out to test the role of CaM in targeting of TRPC3. We therefore overexpressed T3T in DT40-KO cells together with wild type CaM or a dominant negative CaM mutant (CaMEF1–4mut), in which all four EF hand motifs are mutated (7). We observed no difference in plasma membrane targeting of T3T in CaM versus CaMEF1–4mut expressing cells (not shown). Thus, in aggregate, our data demonstrate that CIRB (or elements contained therein) is necessary for proper targeting of TRPC3 to the plasma membrane, but neither CaM nor the IP$_3$ receptor, the binding partners so far shown to interact with this region, appear to play a role in this regard.

**Discussion**

In recent years several genetic diseases have been linked to mutations within ion channel genes (23). Some of these mutations interfere with proper trafficking of the channel to the plasma membrane, for example, the most common mutation of the cystic fibrosis transport regulator, ΔF508 (24, 25). There seems to be an important trafficking checkpoint in the ER; membrane proteins such as channels/channel subunits may contain an ER retention signal, which is masked by another protein or a part of the same protein. Truncation of a channel or lack of a corresponding subunit can unmask the ER retention signal and thus prevents nonfunctional or improperly assembled channels from reaching the plasma membrane (26). This has recently been demonstrated for a variety of channels including the L-type calcium channel, HERG channel, K$_{ATP}$ channel, N-methyl-D-aspartate receptor, and cyclic-nucleotide-gated channel (27–31). In this study we show that deletion of the CIRB region in TRPC3 (mutants T3T-CΔ78 and T3T-CΔ79) results in trapping of the channel in intracellular compartments possibly ER/Golgi. A predicted C-terminal coiled coil region, which is also conserved in all TRPC channels, is not required for targeting or for OAG and agonist-dependent Ca$^{2+}$ entry. It is interesting to note that C-terminal truncation of TRPC1 up to the start of the transmembrane domains results in loss of function, a function that is independent of the presence of IP$_3$ receptors.

Because the CIRB region of TRPC3 has been shown to bind to both the IP$_3$ receptor and CaM, we next set out to test the role of CaM in targeting of TRPC3. We therefore overexpressed T3T in DT40-KO cells together with wild type CaM or a dominant negative CaM mutant (CaMEF1–4mut), in which all four EF hand motifs are mutated (7). We observed no difference in plasma membrane targeting of T3T in CaM versus CaMEF1–4mut expressing cells (not shown). Thus, in aggregate, our data demonstrate that CIRB (or elements contained therein) is necessary for proper targeting of TRPC3 to the plasma membrane, but neither CaM nor the IP$_3$ receptor, the binding partners so far shown to interact with this region, appear to play a role in this regard.
appear to vary among different isoforms. In this study we show that deletion of the CIRB region in TRPC3 (mutants T3T-C∆78 and T3T-C∆7) results in trapping of the channel in intracellular compartments possibly ER/Golgi. Interestingly, a TRPC1 mutant lacking the CIRB region did not alter Ca\(^{2+}\)-dependent feedback inhibition of SOC in a human submandibular cell line, and from the data presented it is not clear whether there is a phenotype associated with this mutant (11). It is therefore tempting to speculate that this mutant does not target properly to the plasma membrane as is the case for the corresponding Topaz-tagged TRPC3 mutant T3T-C∆78 (Fig. 7). We considered the possibility that CaM binding to CIRB was a prerequisite for targeting TRPC3 to the plasma membrane because it has been shown for Ca\(^{2+}\)-activated K\(^{+}\) channels that CaM regulates the trafficking and surface expression of these ion channels (36). To test this hypothesis, we applied two strategies; we mutated conserved residues within the CIRB region that might be expected to interfere with CaM and/or IP\(_{3}\) receptor binding (T3T-YQ/AA and T3T-MKR/AAA; Fig. 8), and we co-expressed T3T with a dominant negative CaM mutant to see whether we could detect an effect on T3T targeting (see “Results”). However, neither of these strategies disrupted plasma membrane targeting of T3T.

A recent study showed that a coiled coil region in the N terminus of TRPC1, but not a region containing the ankyrin repeats, was able to homodimerize based on a yeast two-hybrid screen (37). Thus, the function of ankyrin repeats in TRPC channels is unclear. As we show here ankyrin repeats appear to be required for targeting of TRPC3 to the plasma membrane (T3T-N\(_{1}\)), whereas N-terminal deletion of TRPC3 up to the ankyrin repeats yields functional and plasma membrane-targeted ion channels (T3T-N\(_{0}\)). A similar trafficking defect has been described for a TRPC6 mutant lacking 131 N-terminal amino acid residues that include the first ankyrin repeat (38). Ankyrin repeats are found in a wide spectrum of proteins, including plant potassium channels, TRPC, and vanilloid TRP subfamilies, and are thought to mediate protein-protein interactions (39). However, ankyrin repeats are able to accommodate a variety of target molecules, making it hard to predict a possible binding partner.

It has been shown that members of the TRPC 3/6/7 subfamily as well as the Drosophila isoforms TRP and TRP-like receptor can be activated by the diacylglycerol analog OAG (40, 41). The exact mechanism of this activation is not known, but OAG may mimic the effects of phospholipase C-induced generation of DAG. Recently Zhang and Saffen (17) discovered a splice variant of TRPC6, TRPC6B, which appeared to be activated in response to agonist but not OAG activation. The TRPC6A splice variant that contains 54 additional amino acids at the N terminus was activated by both OAG and receptor stimulation. These authors (17) concluded that these 54 amino acids are crucial for OAG activation. However, none of the other TRP isoforms that are OAG-activated possess this extended N terminus. In agreement with that, Jung et al. (42) have recently reported OAG activation for the respective TRPC6 splice variant. Similarly, we show here that the N terminus of TRPC3 can be truncated up to the start of the ankyrin repeats without loss of proper targeting, OAG activation, or receptor activation (Figs. 5–7, see mutant T3T-N\(_{1}\)). It remains unknown whether DAG and OAG exert their effect by acting on the TRPC3 channel directly. The well described DAG target protein kinase C does not seem to be involved in activation, because protein kinase C inhibitors do not block OAG activation of TRPC3 or TRPC6 (40, 43). However, protein kinase C is not the only effector of DAG, and several newly described targets of DAG should be investigated as possible intermediates between DAG

![Fig. 9. T3T and T3T-C∆7 expression in wild type or IP\(_{3}\) receptor knockout DT40 cells, and carbachol-induced Ba\(^{2+}\) entry.](image)

A confocal images of wild-type (WT) or IP\(_{3}\) receptor knockout (IP\(_{3}\)-KO) DT40 cells transfected with 100 μg of T3T or T3T-C∆7. The cells were cultured at 30 °C to enhance expression, as described under “Results.” B, Ba\(^{2+}\) entry responses of cell transfected with vector (Control), T3T, T3T-C∆7, or T3T-C∆8, together with the M5 muscarinic receptor. At the times indicated 300 μM carbachol followed by 2 mM Ba\(^{2+}\) was added to cells incubated in the nominal absence of Ca\(^{2+}\). Shown are the averages of traces of 15–20 EYFP-positive or -negative (control) cells.

However, mutation of R\(_{4}\) to A\(_{4}\) in T3T-C∆7 T3T did not rescue plasma membrane targeting (data not shown). More extensive studies will be necessary to identify a putative ER retention signal. Mutation of the ER retention signal would allow the functional study of mutants that would otherwise be trapped in the ER/Golgi and would provide a means to exclude defective trafficking as a reason for lack of function. It is intriguing that the ER/Golgi and would provide a means to exclude defective functional study of mutants that would otherwise be trapped in signal. Mutation of the ER retention signal would allow the studies will be necessary to identify a putative ER retention signal. It has been shown that members of the TRPC 3/6/7 subfamily as well as the Drosophila isoforms TRP and TRP-like receptor can be activated by the diacylglycerol analog OAG (40, 41). The exact mechanism of this activation is not known, but OAG may mimic the effects of phospholipase C-induced generation of DAG. Recently Zhang and Saffen (17) discovered a splice variant of TRPC6, TRPC6B, which appeared to be activated in response to agonist but not OAG activation. The TRPC6A splice variant that contains 54 additional amino acids at the N terminus was activated by both OAG and receptor stimulation. These authors (17) concluded that these 54 amino acids are crucial for OAG activation. However, none of the other TRP isoforms that are OAG-activated possess this extended N terminus. In agreement with that, Jung et al. (42) have recently reported OAG activation for the respective TRPC6 splice variant. Similarly, we show here that the N terminus of TRPC3 can be truncated up to the start of the ankyrin repeats without loss of proper targeting, OAG activation, or receptor activation (Figs. 5–7, see mutant T3T-N\(_{1}\)). It remains unknown whether DAG and OAG exert their effect by acting on the TRPC3 channel directly. The well described DAG target protein kinase C does not seem to be involved in activation, because protein kinase C inhibitors do not block OAG activation of TRPC3 or TRPC6 (40, 43). However, protein kinase C is not the only effector of DAG, and several newly described targets of DAG should be investigated as possible intermediates between DAG

Calmodulin plays a crucial role in the activation, inactivation, or modulation of a variety of ion channels (35). CaM has been shown to bind to the CIRB region of TRPC1–7 with different affinities (8), although its effects on channel activity
and TRPC3 channel activation (44, 45).

Conformational coupling has been suggested as a mechanism of activation of TRPC3, and a N-terminal, IP3-binding fragment of the IP3 receptor has been shown to activate TRPC3 channels (5). The CIRB region of TRPC3 was subsequently identified as a region that not only interacts with CaM but also with two sequences within the cytoplasmic N terminus of the IP3 receptor (7, 8). However, TRPC3-mediated Ba2+ entry into DT40 lacking all three types of IP3 receptors has been reported (21, 22), excluding an absolute requirement for the IP3 receptor in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation. We show here that T3T targets to the plasma membrane of DT40-KO cells, which suggests that the possibility that ryanodine receptors can partially assume the role of IP3 receptors not only exists but also is functional in TRPC3 activation.
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