Stac adaptor proteins regulate trafficking and function of muscle and neuronal L-type Ca\(^{2+}\) channels

Alexander Polster, Stefano Perni, Hicham Bichraoui, and Kurt G. Beam

Department of Physiology and Biophysics, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

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Excitation–contraction (EC) coupling in skeletal muscle depends upon trafficking of CaV_{1.1}, the principal subunit of the dihydropyridine receptor (DHPR) (L-type Ca\(^{2+}\) channel), to plasma membrane regions at which the DHPRs interact with type 1 ryanodine receptors (RyR1) in the sarcoplasmic reticulum. A distinctive feature of this trafficking is that CaV_{1.1} expresses poorly or not at all in mammalian cells that are not of muscle origin (e.g., tsA201 cells), in which all of the other nine Ca\(_v\) isoforms have been successfully expressed. Here, we tested whether plasma membrane trafficking of CaV_{1.1} in tsA201 cells is promoted by the adapter protein Stac3, because recent work has shown that genetic deletion of Stac3 in skeletal muscle causes the loss of EC coupling. Using fluorescently tagged constructs, we found that Stac3 and CaV_{1.1} traffic together to the tsA201 plasma membrane, whereas CaV_{1.2} is retained intracellularly when Stac3 is absent. Moreover, L-type Ca\(^{2+}\) channel function in tsA201 cells coexpressing Stac3 and CaV_{1.1} is quantitatively similar to that in myotubes, despite the absence of RyR1. Although Stac3 is not required for surface expression of CaV_{1.2}, the principle subunit of the cardiac/brain L-type Ca\(^{2+}\) channel, Stac3 does bind to CaV_{1.2} and, as a result, greatly slows the rate of current inactivation, with Stac2 acting similarly. Overall, these results indicate that Stac3 is an essential chaperone of CaV_{1.1} in skeletal muscle and that in the brain, Stac2 and Stac3 may significantly modulate CaV_{1.2} function.

Stac adaptor protein \(\mid\)-type Ca\(^{2+}\) channel \| excitation–contraction coupling

Voltage-gated calcium channels serve to couple membrane electrical activity to various downstream signaling cascades, whose properties are strongly influenced by the spatial interrelationships between the calcium channels, effector proteins, and cellular organelles that comprise these signaling pathways. For example, in skeletal muscle the link between muscle excitation and contraction [excitation–contraction (EC) coupling] depends upon triadic or dyadic junctions between the plasma membrane and the sarcoplasmic reticulum (SR), with L-type Ca\(^{2+}\) channels (dihydropyridine receptors, DHPRs) and type 1 ryanodine receptors (RyR1) localized in junctional domains of the plasma membrane and the SR, respectively. The DHPRs, which contain CaV_{1.1} as their principal subunit, are arranged into groups of four (“tetrads”), evidently as a consequence of yet-to-be identified, physical links between the DHPRs and the four homomeric subunits of RyR1 (1, 2). These links are thought to be necessary for the bidirectional functional interactions between the DHPR and RyR1. In particular, the DHPR is thought to be conformationally coupled to RyR1 with the result that movements of the CaV_{1.1} S4 helices in response to depolarization of the plasma membrane cause RyR1 to release Ca\(^{2+}\) from the SR. In addition to this orthograde signal, which is necessary for EC coupling, there is also a retrograde signal whereby the association with RyR1 causes the L-type Ca\(^{2+}\) current via CaV_{1.1} to be larger and to activate more rapidly. Thus, Ca\(^{2+}\) currents are substantially reduced in size in dyspedic myotubes genetically null for RyR1 (3, 4).

Given the structural and functional interactions between CaV_{1.1}-containing DHPRs and RyR1, it would seem necessary that the trafficking of CaV_{1.1} to the plasma membrane be precisely regulated. As for other high-voltage activated Ca\(^{2+}\) channels, the auxiliary β-subunit is important for membrane trafficking (5, 6). However, it is clear that an additional factor(s) is also crucial. Specifically, CaV_{1.1} expresses poorly (7) or not at all in mammalian cells that are not of muscle origin (e.g., tsA201 cells), whereas the closely related cardiac/neuronal L-type Ca\(^{2+}\) channel, CaV_{1.2}, is readily expressed in such cells (8–10).

The absence of RyR1 seems insufficient to account for the difficulty of expressing CaV_{1.1} in tsA201 cells because CaV_{1.1} expresses well in dyspedic myotubes, albeit producing less current per channel (3). Here we explored the hypothesis that the adapter protein Stac3 is important for membrane trafficking of CaV_{1.1}. Stac3 is one of three isoforms of the Stac family of adapter proteins, so named because they contain src homology 3 (SH3) and cysteine-rich domains. Based on quantitative PCR of adult mice, transcripts for Stac3 are found at high levels in skeletal muscle and at low levels in the cerebellum, forebrain, and eye, whereas transcripts for Stac2 are found in these same, three, neurally rich regions at levels comparable to those of Stac3 in skeletal muscle (11). Transcripts for Stac1 (often designated simply as Stac) are present in cerebellum, fore-/midbrain, and eye (at levels 1/10th or less those of Stac2) and also present in the bladder and adrenal gland (11). In skeletal muscle, the absence of Stac3 causes the failure of EC coupling in both mice (11) and fish (12). Moreover, Stac3 appears to interact with CaV_{1.1} based on both localization at triad junctions (11, 12) and coimmunoprecipitation (12). Our results now indicate that Stac3 is a chaperone protein that binds directly to CaV_{1.1} and is necessary for its plasma membrane expression. Stac3 and Stac2 also bind to CaV_{1.2}, which is not required for membrane trafficking.

Significance

Voltage-gated calcium channels are essential for diverse cellular functions. For example, CaV_{1.1} channels trigger skeletal muscle contraction and CaV_{1.2} channels regulate neural gene expression in response to neuronal activity. Thus, it is important to understand the cellular mechanisms that regulate delivery of these channels to the plasma membrane and that govern calcium movements via the membrane-inserted channels. Here we show that the cellular adapter protein “Stac3” participates in both processes. Specifically, Stac3 binds to both CaV_{1.1} and CaV_{1.2}. This binding is essential for efficient delivery of CaV_{1.1} to the plasma membrane, but not for CaV_{1.2}. However, binding of Stac3, or the related protein Stac2, to CaV_{1.2} causes a dramatic slowing of inactivation, thereby increasing calcium entry via CaV_{1.2}.


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1To whom correspondence should be addressed. Email: Kurt.Beam@ucdenver.edu.

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Results

Fig. 1 compares the subcellular distribution of fluorescently tagged CaV1.2 and CaV1.1, after coexpression in tsA201 cells with the auxiliary β_{1a}- and αδ-subunits. CaV1.2 appeared to be associated with the surface membrane (Fig. 1A, Left), but CaV1.1 had a reticular distribution (Fig. 1A, Right) consistent with retention in the endoplasmic reticulum (ER) (13). Recently, Stac3 was reported to localize to plasma membrane/SR junctions (triads) in skeletal muscle, to be necessary for skeletal-type EC coupling (not requiring entry of extracellular Ca^{2+}), and to be highly expressed in skeletal muscle but not in cardiac muscle. Thus, we decided to test whether Stac3 would affect the trafficking of CaV1.1. Strikingly, the presence of Stac3 caused CaV1.1 to associate with the cell surface (Fig. 1B). To determine whether this surface-associated CaV1.1 was actually inserted into the plasma membrane, we used a monoclonal antibody to CaV1.1 (14). This antibody stained intact tsA201 cells coexpressing CaV1.1 and Stac3, but not cells expressing only CaV1.1 (Fig. S1). An obvious feature of cells like those illustrated in Fig. 1B was that Stac3, like CaV1.1, was associated with the surface. On the basis of photobleaching analysis, such surface binding was not an intrinsic property of Stac3, but depended instead on CaV1.1 also being present (Fig. S2). Moreover, even with neither β_{1a} nor αδ, Stac3 appeared able to colocalize at the surface with CaV1.1 and to promote the insertion of CaV1.1 into the plasma membrane (Fig. S3). Taken together, these results strongly suggest that Stac3 binds directly to CaV1.1 and that this binding is necessary for efficient delivery of CaV1.1-containing DHPRs to the plasma membrane.

Whole-cell patch clamping was used to test whether CaV1.1 was functional after its cotransfection with Stac3 in tsA201 cells. As shown in Fig. 2, CaV1.1 in the absence of Stac3 produced neither Ca^{2+} currents nor membrane-bound charge movements (gating currents), whereas, in the presence of Stac3, CaV1.1 produced robust Ca^{2+} currents and charge movements. Fig. 2 also compares the peak current−voltage and charge−voltage relationships for CaV1.1 in tsA201 cells with those of CaV1.1 expressed in dysgenic myotubes (“mdg”; null for endogenous CaV1.1). We had expected that CaV1.1 function would differ between the two cell types because RyR1 is present in dysgenic myotubes but not in tsA201 cells and because the absence of RyR1 in dyspedic myotubes (null for endogenous CaV1.1) has been shown to cause Ca^{2+} currents via CaV1.1 to activate more rapidly (4) and to be severalfold smaller in relationship to charge movement (\(G_{\text{max}}/Q_{\text{max}}\)) (3, 4). However, double-exponential fits (Eqs. 3) revealed no significant differences between tsA201 cells and dysgenic myotubes in the rate of Ca^{2+} current activation (Fig. 3). Additionally, based on fits of Boltzmann expressions (Eqs. 1 and 2) there were no significant differences between the two cell types in the magnitude of the ratio \(G_{\text{max}}/Q_{\text{max}}\) (Fig. 3). Thus, the function of CaV1.1 coexpressed with Stac3β_{1a}/αδ in tsA201 cells is remarkably similar to that of CaV1.1 as part of the DHPR complex in myotubes.

In addition to binding to the DHPR, it has been suggested that Stac3 may also bind to RyR1 and thus serve as a functional link between the two proteins (11, 12). As a test of this possibility, we determined whether Stac3-YFP had the distribution expected for targeting to plasma membrane/SR junctions, which are present with a punctate distribution in both dyspedic (15, 16) and dysgenic myotubes (17). Stac3-YFP had a punctate distribution in dysgenic myotubes (Fig. 4A, Left), which contain DHPRs but lack RyR1. This result is consistent with the apparent interaction between Stac3 and DHPRs in tsA201 cells (Fig. 1B, extended data in Fig. 2). By contrast, Stac3-YFP had a diffuse distribution in dysgenic myotubes (Fig. 4A, Right), which contain RyR1 but not CaV1.1. This latter result could mean either that Stac3-YFP
The YFP-CaV1.1 expressing dysgenic myotubes (solid bars, respectively, to yield values of $A_{max}$ for dysgenic myotubes (open bars, $n = 10$). YFP-CaV1.1 expressing dysgenic myotubes (solid bars, $n = 10$), and in YFP-CaV1.1/ 
Stac3 expressing tsA201 cells (shaded bars, $n = 12$). Based on one-way ANOVA, there was no statistically significant difference between the tsA201 cells and dysgenic myotubes in either the time constants ($\tau_{slow}, P = 0.461; \tau_{fast}, P = 0.421$) or amplitudes ($A_{slow}, P = 0.398; A_{fast}, P = 0.400$) of the slow and fast components of current activation. (D) Peak current-voltage (peak $I-V$) and charge-voltage ($Q-V$) relationships were fitted with Eqs. 1 and 2, respectively, to yield values of $G_{max}, Q_{max},$ and $G_{max}/Q_{max}$ for individual cells. Mean values ($\pm$SEM) are shown for dysgenic myotubes (open bars, $n = 6$), YFP-CaV1.1 expressing dysgenic myotubes (solid bars, $n = 6$), and YFP-CaV1.1/ 
Stac3 expressing tsA201 cells (shaded bars, $n = 8$). Data shown were obtained 
only from cells in which the two proteins were expressed at high levels and shows images obtained before and after bleaching YFP within the indicated region of interest. This bleaching would not have been 
expected to affect any Stac3-YFP that remained bound to RyR1. Alternatively, it may be 
that the previously described coimmunoprecipitation was a consequence of a ternary complex of Stac3, the DHPR, and RyR1. 

As described above, the membrane trafficking of CaV1.2 in 
tsA201 cells does not depend on the presence of Stac proteins (Fig. 1A). However, low levels of Stac3 transcripts and high levels of Stac2 are present in the cerebellum and fore- and midbrain (11), where CaV1.2 is also present (18). Thus, we tested whether Stac3 and/or Stac2 might interact with CaV1.2. As shown in Fig. 5A, both Stac3-YFP and Stac2-YFP colocalized with CFP-CaV1.2 at the surface of tsa201 cells. Furthermore, this interaction alters CaV1.2 function. Specifically, in addition to causing modest changes in Ca$^{2+}$ current magnitude, both Stac2 and Stac3 caused a profound slowing of the rate of inactivation (Fig. 5B).

**Discussion**

If, as our results suggest, Stac3 is required for efficient membrane trafficking of CaV1.1, then it stands to reason that EC coupling would fail in animals that lack it. However, it seems likely that its continued presence is required for maintaining the EC coupling apparatus in a functional state. In particular, EC coupling is greatly impaired in muscle fibers of Stac3-null zebrafish embryos at 48 h postfertilization (hpf) even though there is a near-normal level of triadic CaV1.1 (12). Moreover, these Stac3-null embryos displayed some motile activity at 2 hpf, which could be attributed to the presence of Stac3 translated from maternally deposited mRNA. Thus, one could speculate that the maternally derived Stac3 supported the triadic insertion of CaV1.1, but that the subsequent loss of Stac3 protein caused CaV1.1 to become inoperative for EC coupling. An important goal for future research will be to identify the domains of CaV1.1 and Stac3 that are important for their interactions with one another. It will also be important to determine whether the modulation of Ca$^{2+}$ channel function that we have shown to be produced by Stac2 and Stac3 also occurs for Stac1 and to determine whether such modulation actually occurs in specific neuronal populations.

The ability to achieve high-level expression of CaV1.1 in 
tsA201 cells provides a new experimental tool that will facilitate the study of skeletal muscle DHPRs without the time and expense involved in maintaining colonies of animals heterozygous for mutations of DHPR subunits. The relative absence of contaminating currents is also an advantage. For example, the ability to study how mutations alter the voltage dependence of CaV1.1 charge movements is hampered in dysgenic myotubes by the variable presence of gating currents arising from other ion channels, whereas background gating currents are essentially nonexistent in tsA201 cells (Fig. 2B, Right). Moreover, experiments on tsA201 cells may provide unexpected insights. For example, it was previously hypothesized that the loss of a “current-enhancing” signal from RyR1 to CaV1.1 accounted for the reduced size of Ca$^{2+}$ currents in RyR1-null myotubes (3). However, this hypothesis appears to be incompatible with the near equivalence of CaV1.1 Ca$^{2+}$ currents in tsA201 cells and dysgenic myotubes (Figs. 2 and 3). Thus, it may be that Ca$^{2+}$ currents via CaV1.1 are inhibited by other junctional proteins unless RyR1 is present to relieve this inhibition. In principle, this idea and other aspects of DHPR signaling in skeletal muscle

**Fig. 3.** L-type Ca$^{2+}$ currents are similar for YFP-CaV1.1 expressed in dysgenic myotubes and tsA201 cells. (A) Comparison of peak Ca$^{2+}$ currents ($I_{Ca}$) in a naive dysgenic (RyR1-null) myotube; a dysgenic myotube transfected with YFP-CaV1.1; and a tsA201 cell cotransfected with YFP-CaV1.1, $\delta$, $\beta$, and Stac3. Calibrations: 5 pA/pF, vertical; 50 ms, horizontal. (B and C) Activation was fitted with a double-exponential function (Eq. 3) for Ca$^{2+}$ currents at +40 mV in naive dysgenic myotubes (open bars, $n = 10$). YFP-CaV1.1 expressing dysgenic myotubes (solid bars, $n = 10$), and in YFP-CaV1.1/ 
Stac3 expressing tsA201 cells (shaded bars, $n = 12$). Based on one-way ANOVA, there was no statistically significant difference between the tsA201 cells and dysgenic myotubes in either the time constants ($\tau_{slow}, P = 0.461; \tau_{fast}, P = 0.421$) or amplitudes ($A_{slow}, P = 0.398; A_{fast}, P = 0.400$) of the slow and fast components of current activation. (D) Peak current-voltage (peak $I-V$) and charge-voltage ($Q-V$) relationships were fitted with Eqs. 1 and 2, respectively, to yield values of $G_{max}, Q_{max},$ and $G_{max}/Q_{max}$ for individual cells. Mean values ($\pm$SEM) are shown for dysgenic myotubes (open bars, $n = 6$), YFP-CaV1.1 expressing dysgenic myotubes (solid bars, $n = 6$), and YFP-CaV1.1/ 
Stac3 expressing tsA201 cells (shaded bars, $n = 8$). Data shown were obtained 
only from cells in which both peak $I-V$ and $Q-V$ relationships were measured. The $G_{max}/Q_{max}$ ratios for dysgenic and dysgenic myotubes were not 
corrected for any background charge (unrelated to CaV1.1) because this was small in the current experiments: 0.87 ± 0.09 nC/pF, $n = 7$, measured at +40 mV in naive dysgenic myotubes.

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**Fig. 4.** Stac3 does not appear to bind to RyR1 in either myotubes or tsA201 cells. (A) Stac3-YFP (red) had a punctate distribution in dysgenic (RyR1 null) myotubes (Left) and a diffuse distribution in dysgenic (CaV1.1 null) myotubes (Right), indicative of a lack of binding to RyR1 because punctate plasma membrane/SR junctions are present both before (Left) and after (Right) bleaching of YFP within the indicated polygon. Note that CFP-RyR1 is localized in stacks of elongated ER. (Scale bars, 5 μm.)
could be addressed by using tsA201 cells to manipulate the set of junctional proteins present in addition to CaV1.1 and Stac3. Of course, there are many questions about signaling interactions between the DHPR and RyR1 that will continue to require the use of skeletal muscle. However, even those experiments are likely to benefit from insights obtained in experiments on tsA201 cells.

### Materials and Methods

#### Molecular Biology

The construction of the expression plasmids for GFP-tagged, CFP-tagged, YFP-tagged, or unlabeled CaV1.1 was described previously (19, 20). The coding sequence of CaV1.2 (21) was inserted into the compatible ends, was used to produce the expression vector for unlabeled CaV1.1, CFP-RyR1, and unlabeled Stac2 (white symbols, □□□). Images of tsA201 cells coexpressing CFP-CaV1.2 (green) and unlabeled CaV1.1, CFP-RyR1, and unlabeled Stac2 without Stac2 or Stac3 (gray symbols, □□□ + ■□□ + □□□) are indicated for YFP-CaV1.2 without Stac2 or Stac3 at 150 ms (Right) and YFP-CaV1.2 with either Stac3 (black symbols, □□□ + □□□ + □□□) or Stac2-YFP (red, □□□ + □□□ + □□□). (Scale bars, 5 μm.)

#### Immunostaining

Transfected tsA201 cells were washed twice with PBS containing 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4, pH 7.4 with HCl, and fixed in paraformaldehyde [4% (vol/vol) in PBS] for 20 min at room temperature. After fixation, the cells were washed three times with PBS and incubated in 10% (vol/vol) goat serum/1% BSA/PBS for 60 min at room temperature. Incubation in mouse CaV1.1 subunit primary antibody IIF7 (1:50 diluted in 1% BSA/PBS, kindly provided by Kevin P. Campbell, University of Iowa, Iowa City, IA) was overnight at 4 °C, after which the cells were washed three times for 10 min with 1% BSA/PBS. Cells were then incubated in Alexa Fluor 568-labeled secondary antibody (diluted 1:300 in 1% BSA/PBS) for 1 h at room temperature, washed with 1% BSA/PBS, and mounted using Fluorescent Mounting Medium (Molecular Probes) between two glass slides. The images were captured using a Zeiss LSM 710 confocal microscope. Images were obtained as a single optical slice with a 40× [1.3 numerical aperture (NA)] or 63× (1.4 NA) oil immersion objective. Excitation and emission, respectively, were 440 nm (diode laser) and 465–495 nm for CFP, 488 nm (argon laser) and 500–545 nm for GFP, 514 nm (argon laser) and 530–565 nm for YFP, 543 nm (HeNe laser), and either 565–721 nm for DsRed or 596–664 nm for Alexa Fluor 568. Relative to full power output, the excitation was attenuated to ∼4% (440 nm), ∼2% (488 nm and 514 nm), ∼15% (543 nm, DsRed), and ∼3% (543 nm, Alexa Fluor 568). For photobleaching of Stac-YFP in tsA201 cells, a prebleach image was obtained as described above. Within this image, a smaller region of interest was designated to avoid the cell surface and repeatedly scanned for 15–45 s with nonattenuated 514-nm excitation. About 5–10 s after completion of the bleaching, a postbleach image was then obtained with the same settings as those of the prebleach image.

#### Measurement of L-Type Ca2+ Currents and Intracellular Charge Movements

All experiments were performed at room temperature (−25 °C). Pipettes (2.0 MΩ) were fabricated from borosilicate glass and were filled with internal solution, which consisted of 140 mM Cs-aspartate, 10 mM CsCl, EGTA, 5 mM MgCl2, and 10 mM Hepes, pH 7.4, with CsOH. The bath solution contained 145 mM tetraethyammonium-Cl, 10 mM CaCl2, and 10 mM Hepes, pH 7.4, with NaOH. For electrophysiological experiments on
myotubes, 0.003 mM tetrodotoxin (TTX) and 0.1 mM N-benzyl-p-toluene sulphonamide (BTS) were added to the bath solution. To isolate L-type currents in myotubes, voltage was stepped from the holding potential (~80 mV) to ~20 mV for 1 s to inactivate endogenous T-type current. For measurement of intramembrane charge movements attributable to Cav1.1, 0.1 M LaCl₃ and 0.5 M CdCl₂ were added to the bath solution and a prepulse protocol (28) was used to inactivate T-type Ca²⁺ channels and sodium channels. Electronic compensation was used to reduce the effective series resistance to <5 MΩ (time constant <400 μs). Linear components of leak and capacitive current were corrected with −P raw online subtraction protocols. Filtering was at 2–5 kHz and digitization was either at 10 kHz (L-type currents) or at 25 kHz (charge movements). Cell capacitance was determined by integration of a transient elicited by stepping from the holding potential (−80 mV) to −70 mV, using Clampex B.2 (Molecular Devices). It was used to normalize charge movements (nC/F) and ionic currents (pA/pF). Peak current–voltage (I–V) curves were fitted according to

\[ I = \frac{\max (V - V_{rev})}{\tau_{fast} + \tau_{slow}} \left( 1 + \exp \left( \frac{V - V_{1/2}}{k_c} \right) \right) \]  

where \( I \) is the peak current for the test potential, \( V \), \( V_{rev} \) is the reversal potential, \( \max \) is the maximum Ca²⁺ channel conductance, \( V_{1/2} \) is the half-maximal activation potential, and \( k_c \) is the slope factor. Plots of the integral of the ON transient (\( Q_{ON} \)) of intramembrane charge movement as a function of test potential (\( V \)) were fitted according to

\[ Q_{on} - \frac{Q_{max}}{1 + \exp \left( \frac{V - V_{0}}{k_c} \right)} \cdot \tau \]  

where \( Q_{max} \) is the maximal \( Q_{on} \), \( V_0 \) is the potential causing movement of half the maximal charge, and \( k_c \) is a slope parameter. The activation phase of macroscopic ionic currents was fitted as described in ref. 4, using the exponential function

\[ I(t) = A_{fast} \exp \left( \frac{-t}{\tau_{fast}} \right) + A_{slow} \exp \left( \frac{-t}{\tau_{slow}} \right) + C, \]  

where \( I(t) \) is the current at time \( t \) after the depolarization, \( A_{fast} \) and \( A_{slow} \) are the steady-state current amplitudes of each component with their respective time constants of activation \( (\tau_{fast} \) and \( \tau_{slow} \)) and \( C \) represents the peak current. Fractional inactivation of current in 150 ms \( (R_{150}) \) was determined by dividing the peak current into the current 150 ms after the peak.

Analysis. Figures were made using the software program SigmaPlot (version 11.0; SSPS). All data are presented as mean ± SEM. Statistical comparisons were made by one-way ANOVA.

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Fig. S1. Plasma-membrane insertion of YFP-Ca\textsubscript{V}1.1 after coexpression with Stac3 in tsA201 cells. Intact cells expressing YFP-Ca\textsubscript{V}1.1 (green) plus Stac3 immunostained (red) with Ca\textsubscript{V}1.1-specific monoclonal antibody IIF7 and Alexa 568 secondary antibody (Top), whereas those expressing only YFP-Ca\textsubscript{V}1.1 did not (Bottom). (Scale bars, 5 \textmu m.)

Fig. S2. Stac3 stably associates with Ca\textsubscript{V}1.1 in tsA201 cells. Images from tsA201 cells expressing only Stac3-YFP (Top) or Stac3-YFP plus unlabeled Ca\textsubscript{V}1.1 (Bottom) were obtained immediately before (Left) and after (Right) bleaching of YFP within the indicated regions of interest. Note that there was no surface-associated Stac3-YFP after photobleaching unless Ca\textsubscript{V}1.1 was present. (Scale bars, 2 \textmu m.)
The α2δ- and β1α-subunits are not required for Stac3 to bind to CaV1.1 and promote its surface expression. (A) Fluorescence images of a tsA201 cell expressing Stac3-DsRed (red) and GFP-CaV1.1 (green) without α2δ or β1α. The association of CaV1.1 with the surface appeared to be less complete in cells containing Stac3 but lacking α2δ and β1α compared with cells where α2δ and β1α were also present (compare with Fig. 1B). (B) Immunostaining (red) of an intact tsA201 cell with monoclonal antibody IIF7 indicates membrane insertion of YFP-CaV1.1 (green) cotransfected with Stac3 but without α2δ or β1α (Scale bar, 5 μm.)