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MOG1 Rescues Defective Trafficking of $Na_v1.5$ Mutations in Brugada Syndrome and Sick Sinus Syndrome

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Abstract

Background—Loss-of-function mutations in $Na_v1.5$ cause sodium channelopathies, including Brugada syndrome (BrS), dilated cardiomyopathy (DCM), and sick sinus syndrome (SSS), however, no effective therapy exists. MOG1 increases plasma membrane (PM) expression of $Na_v1.5$ and sodium current (I_{Na}) density, thus we hypothesize that MOG1 can serve as a therapeutic target for sodium channelopathies.

Methods and Results—Knockdown of *MOG1* expression using siRNAs reduced $Na_v1.5$ PM expression, decreased I_{Na} densities by 2-fold in HEK/$Na_v1.5$ cells and nearly abolished I_{Na} in mouse cardiomyocytes. MOG1 did not affect $Na_v1.5$ PM turnover. *MOG*1 siRNAs caused retention of $Na_v1.5$ in endoplasmic reticulum, disrupted the distribution of $Na_v1.5$ into caveolin3-enriched microdomains, and led to redistribution of $Na_v1.5$ to non-caveolin-rich domains. MOG1 fully rescued the reduced PM expression and I_{Na} densities by $Na_v1.5$ trafficking defective mutation D1275N associated with SSS/DCM/atrial arrhythmias. For BrS mutation G1743R, MOG1 restored the impaired PM expression of the mutant protein, and restored I_{Na} in a heterozygous state (mixture of wild-type and mutant $Na_v1.5$) to a full level of a homozygous wild-type state.

Conclusions—Use of MOG1 to enhance $Na_v1.5$ trafficking to PM may be a potential personalized therapeutic approach for some patients with BrS, DCM and SSS in the future.

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Disclosures
None.

Keywords

cardiac sodium channel $Na_v1.5$; *SCN5A*; *MOG1*; cell surface expression; ion channel trafficking

Introduction

The cardiac sodium channel $Na_v1.5$ (encoded by the *SCN5A* gene) is required for the initiation and conduction of the cardiac action potential. Loss-of-function mutations cause Brugada syndrome (BrS), cardiac conduction disease (CCD), sick sinus syndrome (SSS), dilated cardiomyopathy (DCM), and atrial fibrillation (AF).[12] However, no effective treatments are available for these disorders except for invasive implantation of defibrillators or pacemakers in some cases.

Recent studies have started to unravel the molecular mechanism for regulation of $Na_v1.5$ function, which may lead to the development of new therapeutic strategies for prevention or management of diseases associated with $Na_v1.5$ mutations. In 2008, we reported the [3] identification of MOG1 as a new factor that interacts with and regulates the function of $Na_v1.5$. MOG1 is a small, 187 amino acid protein which interacts with Ran, the Ras family GTPase involved in nuclear import and export.[4] MOG1 is expressed in both lateral sarcolemma and intercalated disks in atrial and ventricular cardiomyocytes, and overexpression of *MOG1* enhances cell surface expression of $Na_v1.5$ and sodium current (I_{Na}) densities.[3] Interestingly, a dominant negative missense mutation E83D in MOG1 was found to be associated with BrS.[5]

Defects in cell surface trafficking of ion channels have been demonstrated to be a novel molecular mechanism underlying the pathogenesis of a variety of arrhythmic disorders. Many loss-of-function $Na_v1.5$ mutations, including D1275N associated with SSS, AF and DCM and G1743R associated with BrS, are due to defective trafficking of[267] Identification of new $Na_v1.5$.mechanisms that can be targeted to increase trafficking of $Na_v1.5$ to plasma membranes (PM) may have a potential therapeutic implication. In this study, we assessed whether MOG1 canenhance PM trafficking of mutant sodium channels and rescue the reduced I_{Na} associated with $Na_v1.5$ mutations.

Methods

Details for each method are presented in the Online Supplements.

Membrane Fractionations and Western Blot (WB) Analysis

Isolation of subcellular fractions including PM, a rough endoplasmic reticulum (RER)-enriched fraction, and caveolin-enriched fractions, and WB analyses were performed as described earlier[3] and details are in the Online Supplement.

RNA Interference

MOG1-specific small interfering RNAs (siRNAs) (designed using Dharmacon software) and control scrambler siRNAs (designed by GenScript) were synthesized by Dharmacon RNAi Technologies. Sequences of the siRNAs are shown in Supplemental Table 1.

Electrophysiological Analysis

HEK293 cells that stably overexpress $Na_v1.5$ (HEK/$Na_v1.5$) or neonatal cardiomyocytes transfected with fluorescent conjugated siRNAs were identified by the presence of green fluorescence and used for whole-cell voltage clamp recordings of I_{Na} as described earlier by

us.[3] Details for recordings of the late sodium current (I_{NaL}), the transient outward potassium current (I_{To}) and the L-type calcium current ($I_{Ca\text{-}L}$) are in the Online Supplements.

tsA201 cells (a kind gift from Charles Antzelevitch) were transfected with expression plasmids and pmax*EGFP* (Lonza Inc). Cells with green fluorescence were used for whole-cell voltage clamp recordings of I_{Na} as described previously by us.[3]

Assays for Stability of the PM Fraction of $Na_v1.5$

Stability analysis of $Na_v1.5$ on PM was performed by a cell surface biotinylation assay adapted from the endocytosis assay by Morimoto *et al*,[8] and details are in the Online Supplements.

Statistical Analysis

Data are presented as mean±SEM. Statistical analysis was performed using a two-tailed Student's t-test to compare means between two groups and significance was set at $P < 0.05$ unless otherwise indicated.

Results

Effects of Knockdown of *MOG1* Expression on Cardiac I_{Na}

We used RNA interference (siRNA) to knock *MOG1* expression down in HEK/$Na_v1.5$ cells and determined its effect on I_{Na} density. The genomic region for *MOG1* on chromosome 17p13.1 overlaps with that for a much larger gene *SLC25A35,* which is transcribed in the opposite direction from the reverse strand (Figure 1A). To prevent the non-specific knockdown of the *SLC25A35* gene, we have selected and tested the siRNAs that specifically targeted *MOG1*, but not *SLC25A35*. Transfection of two independent siRNAs against *MOG1* into HEK/$Na_v1.5$ cells significantly decreased the expression level of *MOG1* mRNA (Figure 1B) or protein (Figure 1C), but not the level of *SLC25A35* mRNA (Figure 1D). *MOG1*-specific siRNA1 decreased I_{Na} densities across the range of test potentials compared to scrambler siRNA1 (scrm1) (Figure 2A–B). Identical results were obtained for *MOG*1-specific siRNA2 (Figure S1). The peak current density was reduced by >2-fold by both siRNA1 and siRNA2 (Figure S2, Supplemental Table 3). No significant alteration in the steady state activation/inactivation kinetics and recovery from inactivation was observed for *MOG1* siRNAs (Figure 2C–D, Supplemental Table 3).

We then tested whether MOG1 also affects the late I_{Na} generated by WT or ΔKPQ mutation, which was known to generate a larger late I_{Na}.[9] Overexpression of MOG1 did not have any significant effect on the late I_{Na} (Figure S3). Thus, the effect of MOG1 is specific to the peak I_{Na}.

More dramatic effects were found for *MOG1* siRNA1 on I_{Na} in cardiomyocytes. When *MOG1* expression was knocked down in mouse neonatal cardiomyocytes by siRNA1, I_{Na} was almost diminished (Figure 3). Identical results were obtained with *MOG1*-specific siRNA2 (Figure S4).

We performed similar electrophysiological analyses on the L-type voltage-dependent calcium current ($I_{Ca\text{-}L}$) and the transient outward potassium current (I_{To}) in mouse neonatal cardiomyocytes. *MOG1* siRNA1 and siRNA2 did not have any significant effect on nifedipine- sensitive $I_{Ca\text{-}L}$ (Figure S5) or I_{To} in neonatal cardiomyocytes (Figure S6).

MOG1 Is Required for the Cell Surface Expression of $Na_v1.5$

To explore the potential mechanism by which reduced *MOG1* expression decreased I_{Na}, we determined the effects of *MOG1* siRNA1 and siRNA2 on the expression level of $Na_v1.5$ on PM. Figure 4A showed that knockdown of *MOG1* expression in HEK/$Na_v1.5$ cells significantly decreased the level of $Na_v1.5$ on PM. The decreased PM expression of $Na_v1.5$ was not caused by decreased *SCN5A* gene expression since the total amounts of *SCN5A* mRNA and $Na_v1.5$ protein did not decrease significantly in the cells (Figure 4B–C). Therefore, down-regulation of *MOG1* expression led to a decrease of the cell surface expression of $Na_v1.5$.

Molecular Mechanism by which MOG1 Regulates PM Expression of $Na_v1.5$

The steady state level of $Na_v1.5$ in the PM is determined by the rate of trafficking to the PM and the rate of internalization back to the intracellular compartments. To identify the molecular mechanism by which MOG1 increases PM expression of $Na_v1.5$, we tested whether MOG1 affects the stability of $Na_v1.5$ on PM. PM proteins of tsA201 cells co-transfected with an *SCN5A* expression plasmid and *MOG1* expression plasmid *pcMOG1* (empty vector *pcDNA* as control) were biotinylated and then allowed to internalize for 3, 6, and 9 hrs. At each time point, one set of cells were stripped off biotin and lysed (pool 1 of internalized biotinylated $Na_v1.5$), and another set of cells were lysed directly (pool 2 of both PM and the internalized $Na_v1.5$). Subtraction of pool 1 $Na_v1.5$ from pool 2 results in the PM pool of biotinylated $Na_v1.5$. The PM pool of $Na_v1.5$ reduced by more than 50% by 3 hrs (Figure 5), suggesting that the turnover of $Na_v1.5$ is considerably fast. No significant difference on the amount of the PM pool of $Na_v1.5$ was found with and without overexpression of *MOG1* at all three time points (P=0.84, 0.33, 0.57, Figure 5). These results suggest that MOG1 does not modulate the stability and/or internalization of $Na_v1.5$ on PM.

Exclusion of an effect of MOG1 on the turnover of PM $Na_v1.5$ suggests that increased PM expression of $Na_v1.5$ by MOG1 may be due to enhanced trafficking of $Na_v1.5$ to PM. Trafficking of $Na_v1.5$ to PM consists of transport from the ER to the Golgi apparatus and from the Golgi apparatus to PM. MOG1 was previously shown to interact with RAN[4] in the nucleocytoplasmic transport system which is close to the ER. Therefore, we tested whether MOG1 is critical to the trafficking of $Na_v1.5$ from the ER to Golgi apparatus. HEK/$Na_v1.5$ cells were transfected with *MOG1* specific siRNAs and scrambler siRNAs as controls for 48 hrs and lysed. The lysates enriched with RER were used to determine the amount of $Na_v1.5$. Knockdown of *MOG1* significantly increased the amount of $Na_v1.5$ in RER (Figure 6A). Thus, knockdown of *MOG1* expression resulted in retention of $Na_v1.5$ in RER.

Because MOG1 interacts with $Na_v1.5$, we examined whether MOG1 was also present in RER. WB analysis detected the presence of MOG1 in the RER-enriched fraction of HEK/$Na_v1.5$ cell lysates (Figure S7). Overexpression of *MOG1* increased the MOG1 level in RER, while *MOG1* siRNA dramatically decreased the MOG1 level in that fraction (Figure S7).

Knockdown of *MOG1* Expression Leads to Redistribution of $Na_v1.5$ in a Caveolin-3-Enriched Pool to Other Areas

Different ion channels are trafficked to their respective membrane subdomains to efficiently exert their functional effects. Caveolae, the specialized caveolin-enriched compartments, have been implicated in the cellular trafficking of PM proteins. It was shown that $Na_v1.5$ was associated with caveolin-3 in the caveolin-rich membranes.[10] Since MOG1 interacts with $Na_v1.5$,[3] MOG1 might also be present in the caveolin-rich membranes. Protein extracts from HEK293 cells with co-expression of $Na_v1.5$ and caveolin-3 were fractionated through

a sucrose density gradient into 12 different fractions. WB analysis showed that MOG1 indeed co-localized with $Na_v1.5$ in the cavolin-3-rich membrane fractions (Figure 6B). We then determined whether MOG1 affects localization of $Na_v1.5$ in caveolar membrane compartments. $Na_v1.5$ was normally associated with caveolar fractions (Figure 6B). However, MOG1 knockdown caused redistribution of $Na_v1.5$ to non-caveolar fractions and caveolar $Na_v1.5$ content from caveolin-3-rich fractions 4–7 was decreased significantly (84.19±8.90 % vs. 52.03±9.63%, P=0.0035, n=3) (Figure 6B). These data suggest that knockdown of *MOG1* expression disrupts localization of $Na_v1.5$ onto caveolin-3-rich fractions and led to redistribution of $Na_v1.5$ to non-caveolin-3-rich fractions, defining a significant role of MOG1 in regulating and/or maintaining $Na_v1.5$ localization in caveolin-3-rich membrane microdomains.

MOG1 Can Rescue the Disrupted PM Expression of $Na_v1.5$ and Marked I_{Na} Reduction by Trafficking-Defective Mutation D1275N Associated with SSS, Atrial Arrhythmias and DCM

Because overexpression of *MOG1* can significantly increase the PM expression of $Na_v1.5$,[3] we hypothesized that overexpression of *MOG1* might rescue the trafficking defect of $Na_v1.5$ caused by mutation D1275N. WB analyses showed that mutation D1275N significantly reduced PM expression of $Na_v1.5$ (Figure 7A, compare Lane 1 and 2), and MOG1 significantly increased PM expression of mutant D1275N $Na_v1.5$ (Figure 7A, compare Lane 2 and 3). The expression level of $Na_v1.5$/D1275N on PM in the presence of MOG1 was at the comparable level as that of the WT $Na_v1.5$ alone (Figure 7A Lane 3 vs. 1, P=0.98). Mutation D1275N significantly reduced the I_{Na} densities over a range of test potentials, and *MOG1* fully rescued this defect (Figure 7B–C). The peak I_{Na} density was significantly lower for D1275N mutant channels than for wild type channels (P=0.016; Figure S8). In the presence of MOG1, no significant difference of the peak I_{Na} density was detected between D1275N mutant channels and wild type channels (P=0.81, Figure S8, note that peak currents were at potentials of −30 mV and −15 mV for WT and D1275N channels, respectively), indicating that MOG1 restored the function of D1275N mutant channels. The mean maximal conductance values were 55.11±4.43 nS and 85.68±3.58 (P=0.005) at the membrane potential of −30 mV for WT channels in the absence and presence of MOG1, respectively, and 41.67±4.82 ns and 73.53±5.39 (P=0.001) at the membrane potential of −15 mV for D1275N channels in the absence and presence of MOG1, respectively.

Mutation D1275N affected channel kinetics and shifted the steady state activation and inactivation to more positive potentials (Figure 7D–F). However, overexpression of *MOG1* did not alter channel kinetics of either wild-type (WT) or mutant D1275N $Na_v1.5$ (Figure 7D–F, Supplemental Table 4).

Effect of *MOG1* Overexpression on BrS Mutation G1743R

Although previous attempt in using high concentrations of sodium channel blockers quinidine and especially mexiletine has been shown to restore the I_{Na} density of sodium channels with BrS mutation G1743R to some extent, but <16% of the wild-type I_{Na} density,[7] the potential side-effects of the high doses present a safety concern. Here we examined whether *MOG1* overexpression could rescue defects associated with mutation G1743R. WB analyses showed that mutation G1743R significantly reduced PM expression of $Na_v1.5$ (Figure 8A, compare Lane 1 and 2), and MOG1 significantly increased PM expression of mutant G1743R $Na_v1.5$ (Figure 8A, compare Lane 2 and 3). The expression level of $Na_v1.5$/G1743R on PM in the presence of MOG1 was at the comparable level as that of the WT $Na_v1.5$ alone (Figure 8A Lane 3 vs. 1, P=0.67). Functionally, mutant G1743R completely eliminated I_{Na} (compare WT to G1743R in the absence of *MOG1* overexpression, Figure 8B). Overexpression of *MOG1* in cells co-transfected with an equal amount of wild-type and mutant G1743R expression constructs generated a comparable

level of I_{Na} as the cells transfected with wild-type *SCN5A* plasmid alone (Figure 8B–C). Similarly, the peak I_{Na} density for mixed G1743R+WT sodium channels in the presence of MOG1 was similar to WT sodium channels (P=0.77, Figure S9). The mean maximal conductance values at the membrane potential of −25 mV were 36.31±3.67 nS and 51.92±3.73 (P=0.0013) for WT channels in the absence and presence of MOG1, respectively, and 20.56±3.26 ns and 35.16±3.56 ns (P=0.0058) for WT/G1743R channels in the absence and presence of MOG1, respectively. Thus, overexpression of *MOG1* can restore the level of I_{Na} densities in the heterozygous state (G1743R+WT) to that of homozygous wild-type state.

The steady state activation/inactivation as well as recovery from inactivation were not significantly affected by *MOG1* overexpression for either WT or mixture of WT+G1743R channels (Figure 8D–F, Supplemental Table 5).

Discussion

We have previously reported the identification of MOG1 as a $Na_v1.5$-modulating protein that interacts with $Na_v1.5$ and enhances I_{Na} density.[3] Here, we show that knockdown of *MOG1* in HEK/$Na_v1.5$ cells and mouse cardiomyocytes leads to a significant decrease in the amount of $Na_v1.5$ on PM and a significant decrease in I_{Na} density. These results were previously published as an Abstract.[11] Recently, Kattygnarath *et al* also found that *MOG1* siRNA decreased I_{Na} density in HEK293 cells,[5] but its effect on $Na_v1.5$ trafficking to PM was not studied. Furthermore, their study did not examine the effect of *si-MOG1* either in cardiomyocytes or on expression of *SLC25A35* gene, which overlaps with *MOG1*. In this study we carefully demonstrate that *MOG1*-specific siRNAs inhibit $Na_v1.5$ expression on PM and result in reduced I_{Na} both in HEK293 cells overexpressing $Na_v1.5$ and in mouse cardiomyocytes, indicating the essential regulatory roles of MOG1 in $Na_v1.5$ expression on PM and physiological function.

The data in this study suggest that the molecular mechanism by which MOG1 increases $Na_v1.5$ expression on PM is through increased trafficking of $Na_v1.5$ onto PM, but not by affecting $Na_v1.5$ turnover on PM. Because knockdown of *MOG1* expression by siRNA led to retention of $Na_v1.5$ in the RER (Figure 6A), MOG1 may be involved in ER-to-Golgi transport. Most interestingly, *MOG1*-specific siRNA disrupts localization of $Na_v1.5$ to caveolin-3 rich sarcolemma subdomains (caveolae). The results suggest that MOG1 not only regulates $Na_v1.5$ trafficking to the plasma membrane, but also directs $Na_v1.5$ into caveolae microdomains. However, the underlying mechanism by which $Na_v1.5$ is targeted to and retained in caveolae remains unknown and warrants future studies.

Bioinformatic analysis showed that the intracellular loop II between transmembrane domains II and III of $Na_v1.5$ did not have any homology with the L-type calcium channel $Ca_v1.2$ (*CACNA1C*) or potassium channel proteins $K_v4.1$, $K_v4.2$, or $K_v4.3$ that encode I_{To}. This may explain why knockdown of *MOG1* expression did not affect the I_{CaL} and I_{To} from neonatal cardiomyocytes. No homology was identified between $Na_v1.5$ loop II and KCNQ1 (encoding the I_{Ks} potassium current) or KCNH2 (I_{Kr} potassium current). Thus, MOG1 may not affect I_{Ks} or I_{Kr}. Recent studies showed that $Na_v1.5$ and Kir2.1 shared a common trafficking mechanism involving their respective protein complex formation with SAP97.[12] However, we found that in tsA201 cells, co-expression of Kir2.1 with MOG1 did not increase PM expression of Kir2.1 compared to expression of Kir2.1 alone (P=0.64, Figure S10), suggesting that MOG1 may not be involved in trafficking of Kir2.1 to PM. Interestingly, homology was identified between $Na_v1.5$ loop II and $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$, $Na_v1.7$, $Na_v1.8$, $Na_v1.9$, and $Na_v1.10$. It will be interesting to systematically

examine whether MOG1 has any effect on the functions of other sodium channels that share homology with $Na_v1.5$ in the future.

One of the major findings of the present study is that MOG1 can rescue $Na_v1.5$ trafficking defects and I_{Na} decreases identified in patients with BrS, DCM, and SSS. Implantation of an implantable cardioverter defibrillator (ICD) and a pacemaker is the only therapeutic option for BrS and SSS patients, respectively, at the present time. The invasive ICD therapy may cause painful shocks or initiate rhythm disturbances due to inappropriate activations, may not prevent syncope and death, and may have devastating impact on quality of life. Thus, alternative therapeutic strategies are needed for BrS and SSS. Data in this study suggest that MOG1- enabled trafficking of $Na_v1.5$ to PM may serve as a novel mechanism that can be used to explore potential therapies for BrS and SSS patients with loss-of-function mutations in $Na_v1.5$. Moreover, reduced sodium current densities were frequently associated with acquired cardiac disorders, such as myocardial infarctions, myocardial ischemia, and heart failure. The causes are heterogeneous, but identification of MOG1 as the molecule that can enhance I_{Na} without affecting the kinetics of sodium currents may help the development of therapeutic strategies for such patients with reduced expression of $Na_v1.5$.

Similar to the report by Gui *et al*[2], we found that mutation D1275N reduced I_{Na} densities at more negative membrane potentials from −10 mV to −50 mV (Figure 7C). The results are consistent with those from Western blot studies (Figure 7A) utilizing cultured tsA201 cells that were at physiological, resting membrane potentials reported to be from −20 mV to −40 mV for this type of cells.[13] Similarly, mutation D1275N markedly reduced I_{Na} densities in ventricular cardiomyocytes isolated from mice with the mutation.[14] Mutation D1275N altered the gating properties by shifting the voltage dependence of both activation and inactivation to more positive potentials (Figure 7D and 7E). On the other hand, in ventricular myocytes, D1275N did not affect the voltage dependence of activation and positively, but slightly, shifted the inactivation curve.[14] Therefore, we predict that MOG1 is expected to rescue D1275N-associated phenotypes in mice. Ongoing studies in transgenic mice harboring the D1275N mutation will test this hypothesis. For mutation G1743R, the mutant remained non-functional even after its PM expression level was restored to the wild type level (Figure 8A and 8B). Interestingly, when co-expressed with WT $Na_v1.5$, a situation mimicking the heterozygous state in human patients, we found that MOG1 restored I_{Na} densities to cells with WT $Na_v1.5$ (a situation mimicking normal people). We postulate that MOG1 may drive the cytoplasmic pool of $Na_v1.5$ to PM, resulting in increased I_{Na} densities. The results may have a general implication that MOG1 may be a potential strategy to increase I_{Na} densities in patients with haploinsufficiency of $Na_v1.5$ (e.g. nonsense mutations, frame-shift mutations) or with pore mutations that inactivate $Na_v1.5$ and mutations in other genes that lead to a reduction of $Na_v1.5$ cell surface expression.

One of the limitations of this study is the difficulties in achieving high transfection efficiency in cardiomyocytes. Our optimized condition achieved only 10–30% of transfection efficiency for siRNAs in mouse neonatal cardiomyocytes using oligofectamine. This was not a major problem for electrophysiological analysis since the transfected cells could be identified by the green fluorochrome dye that was conjugated to the siRNAs. However, the low transfection efficiency did compromise our biochemical analysis. This explains why *MOG1* siRNAs almost eliminated the sodium current in neonatal cardiomyocytes, while *MOG1* expression levels were knocked down by 30–50%. There is another important limitation with the present study. In addition to caveolae, $Na_v1.5$ is also targeted to other membrane subdomains such as intercalated disks and T-tubules. Our study was not able to address whether MOG1 also affects $Na_v1.5$ targeting to intercalated disks or T-tubules. MOG1 is expressed in both sarcolemma and intercalated disks,[3] thus it will be interesting to explore whether MOG1 is involved in targeting of $Na_v1.5$ to intercalated

disks. Future studies with adult cardiomyocytes isolated from *MOG1* knockout mice are required to address this issue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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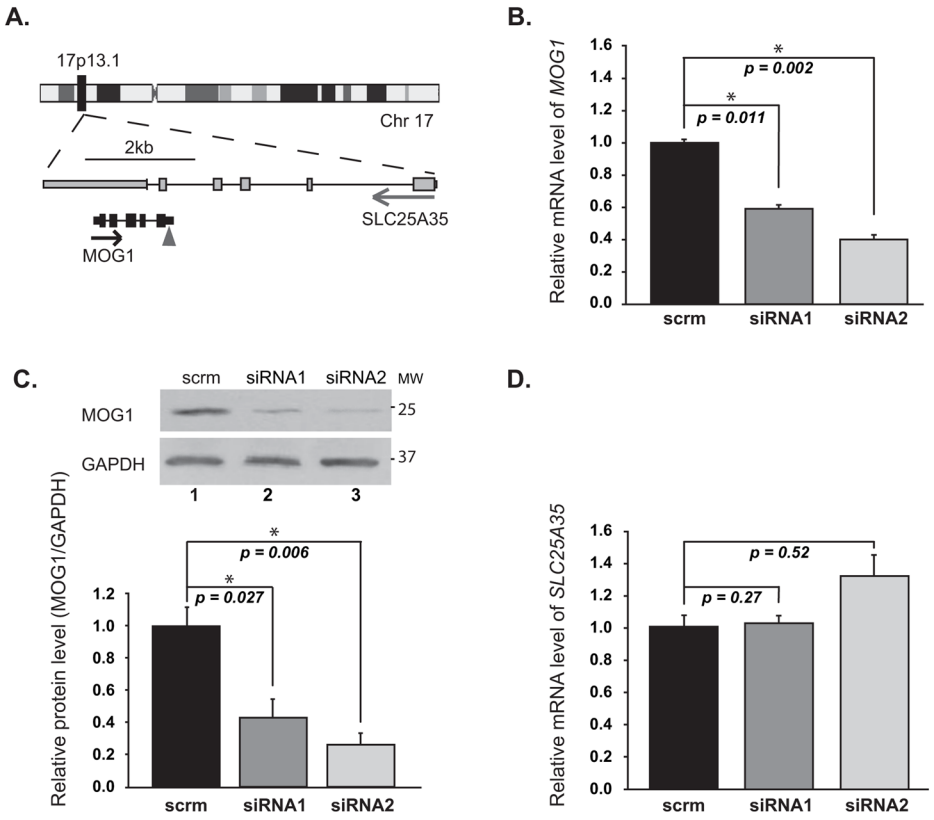

Figure 1. ***MOG1*** **siRNA specifically knocks the expression of** ***MOG1*** **down but not of** ***SLC25A35*** **in HEK/Na_V1.5 cells**

A. *MOG1* and *SLC25A35* reside in the same genomic location, but are transcribed from the opposite strand. The triangle points to the siRNA target site. **B**. Relative mRNA levels of *MOG1* analyzed by qRT-PCR. scrm, scrambler siRNA. **C.** WB analysis for MOG1. GAPDH, loading control. **D.** Relative mRNA levels of *SLC25A35* analyzed by qRT-PCR.

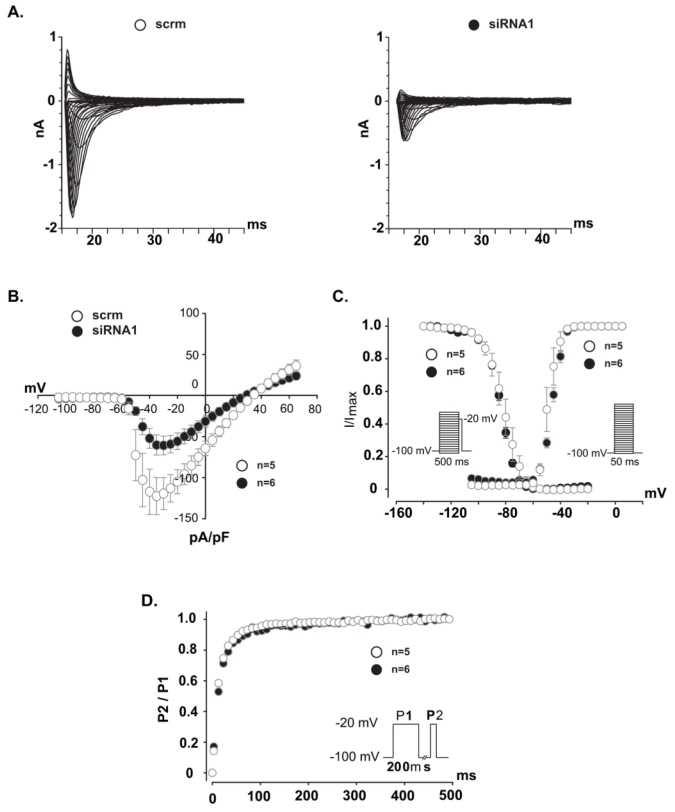

Figure 2. Knockdown of *MOG1* expression by siRNAs significantly decreased I_{Na} density in HEK/$Na_V1.5$ cells

A. Raw traces of sodium currents. **B.** Effects of siRNA1 on the current- voltage relationship of $Na_V1.5$. The current amplitudes were normalized to cell capacitance (pA/pF, abscissa). **C.** Effects of siRNA1 on steady-state activation (Right) and inactivation (Left) of $Na_V1.5$. **D.** Effects of siRNA1 on recovery from inactivation of $Na_V1.5$.

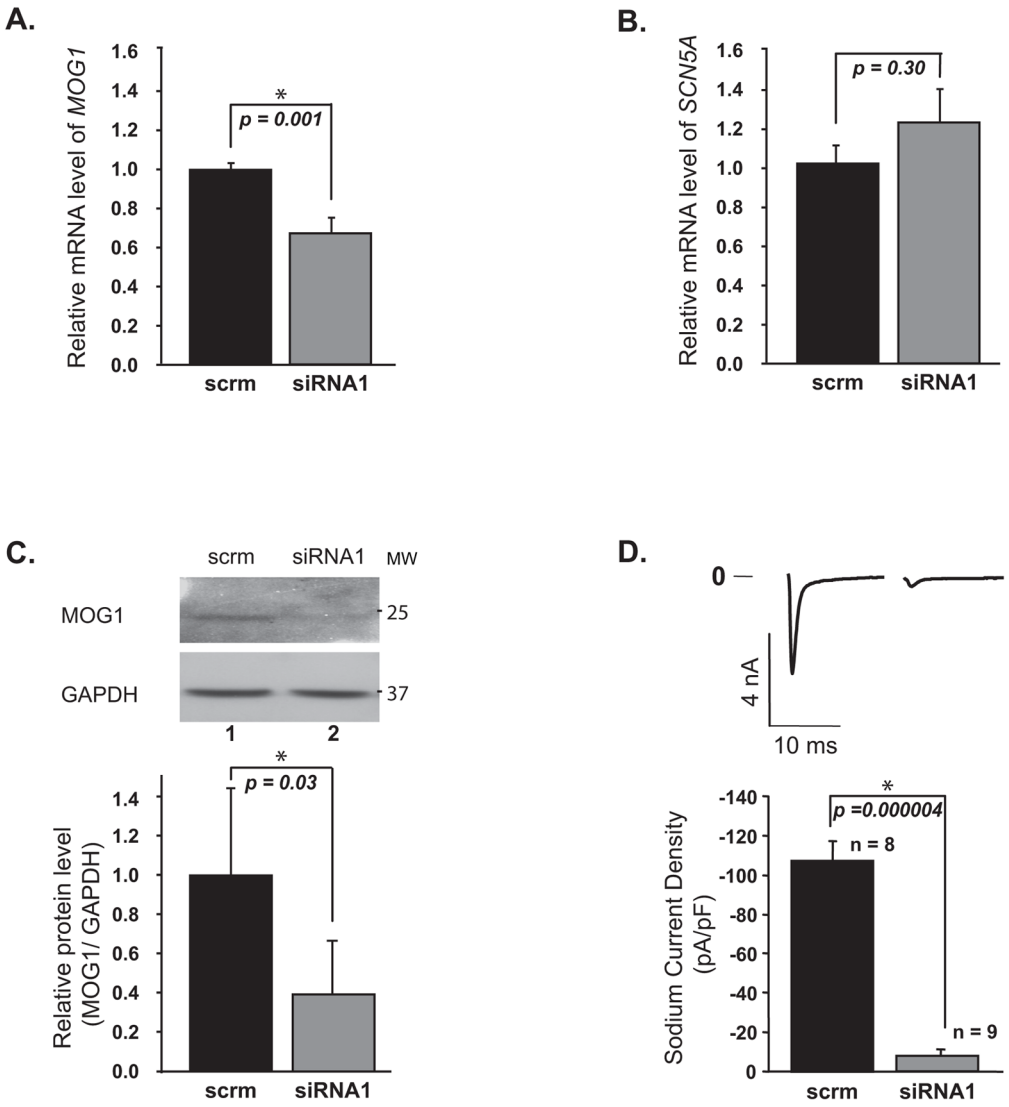

Figure 3. Knockdown of *MOG1* expression markedly reduced I_{Na} densities in neonatal cardiomyocytes

Relative mRNA levels of *MOG1* (**A**) and *SCN5A* (**B**) were analyzed by qRT-PCR. **C.** Relative protein levels of MOG1 analyzed by WB analysis (12% gel, Top) and quantified by densitometry (Bottom). **D.** Raw traces of peak I_{Na} and current densities (pA/pF). Current densities (pA/pF) were 8.37±3.15 (siRNA1) and 107.24±10.13 pF (scrm) (P=4.31×10^{-6}).

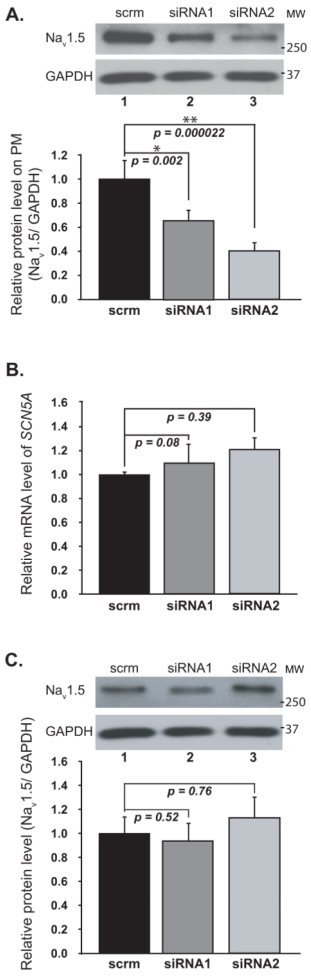

Figure 4. Knockdown of *MOG1* expression significantly decreased the PM expression of $Na_V1.5$ without affecting the expression of total $Na_V1.5$

A. Relative amounts of $Na_V1.5$ in the PM of HEK/$Na_V1.5$ cells analyzed by WB analysis. Inputs, 1/50 of total cell extracts used for PM protein isolation; GAPDH, loading control. **B.** Relative amounts of *SCN5A* mRNA analyzed by qRT-PCR. **C.** Relative amounts of $Na_V1.5$ protein in the total cell extracts analyzed by WB analysis.

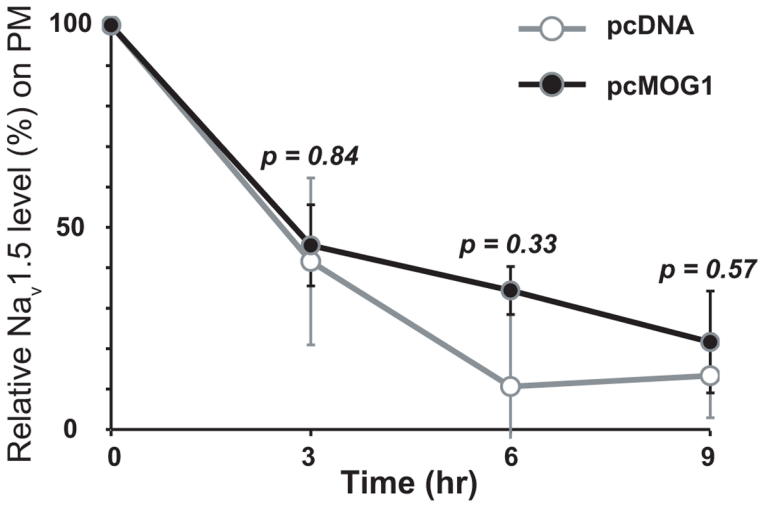

Figure 5. MOG1 does not affect the stability of PM $Na_v1.5$

A relative amount of biotinylated plasma membrane $Na_v1.5$ from tsA201 cells overexpressing *SCN5A* and *MOG1 (pcMOG1)* or vector as a control (*pcDNA3.1*) was analyzed by cell surface biotinylation assays. The experiment was repeated 3 times.

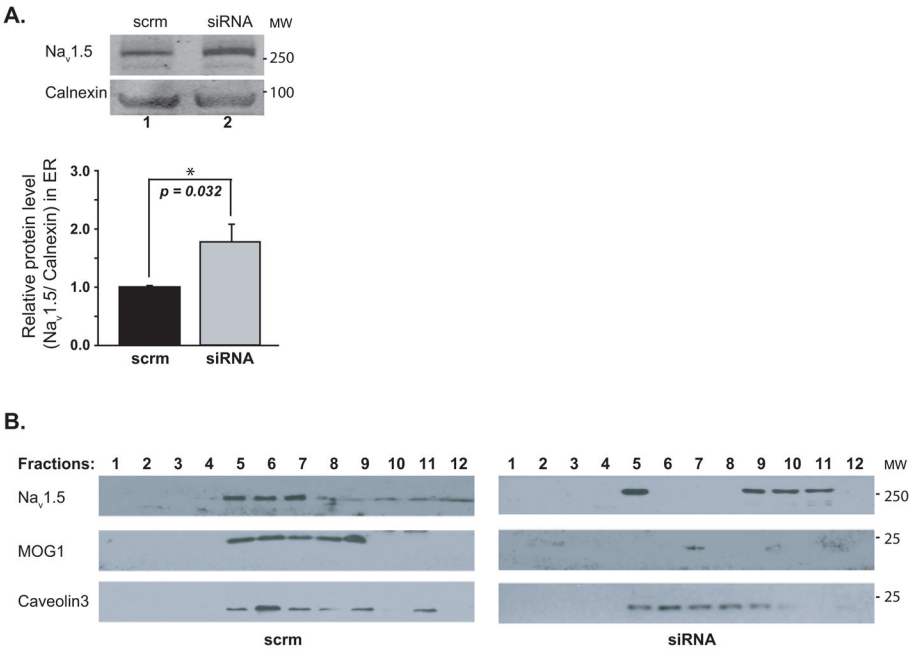

Figure 6. Knockdown of *MOG1* expression causes retention of $Na_V1.5$ in the ER and the redistribution of $Na_V1.5$ from caveolin-3 enriched fractions and other fractions

A. Relative amounts of $Na_V1.5$ in the RER-enriched fractions of HEK/$Na_V1.5$ cells analyzed by WB analysis (10% gel). Calnexin (an ER marker), loading control. **B.** HEK/$Na_V1.5$ cells were transiently transfected with an expression plasmid for caveolin-3. Cell lysates were fractionated, one ml of fractions (a total of 12 fractions) were collected from the top of the gradient and analyzed by WB (10% gel) with antibodies against $Na_V1.5$, MOG1 or caveolin-3 (Cav-3). The experiment was repeated three times.

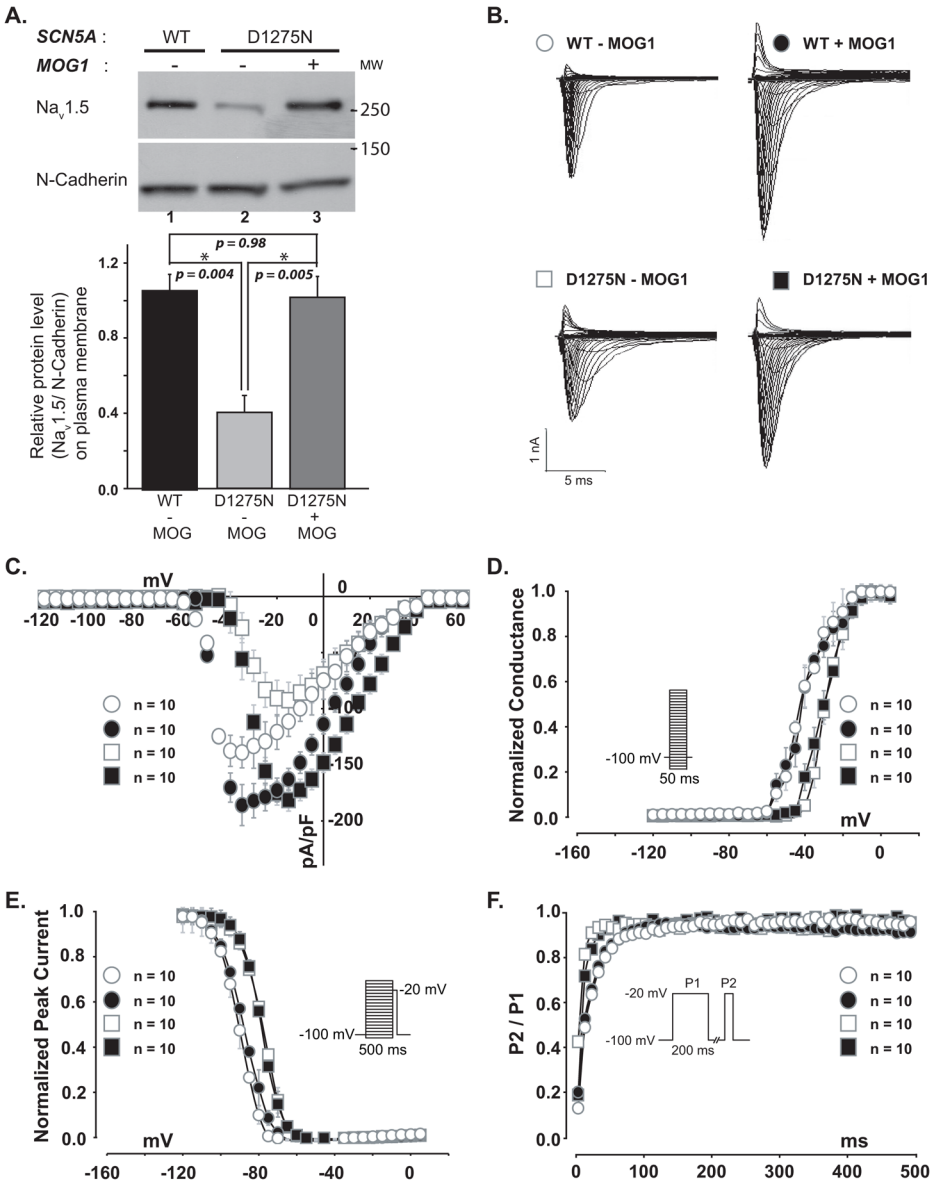

Figure 7. MOG1 rescued PM expression and function of $Na_V1.5$/D1275N mutant

A. WB analysis of $Na_V1.5$ on the PM from tsA201 cells co-transfected with the wild-type (WT) or mutant (D1275N) *SCN5A* gene with (+) or without (−) *MOG1*. N-cadherin, loading control. **B.** Raw traces of a family of I_{Na} in cells transfected with WT (Top) or mutant (Bottom) *SCN5A* expression constructs without (−) or with (+) *MOG1*. **C.** Effects of MOG1 on the current-voltage relationship of WT and mutant $Na_V1.5$. The current amplitudes were normalized to cell capacitance (pA/pF, abscissa). **D.** Effects of MOG1 on steady-state activation of WT and mutant $Na_V1.5$. **E.** Effects of MOG1 on steady-state inactivation of WT and mutant $Na_V1.5$. **F.** Effects of MOG1 on recovery from inactivation of WT and mutant $Na_V1.5$.

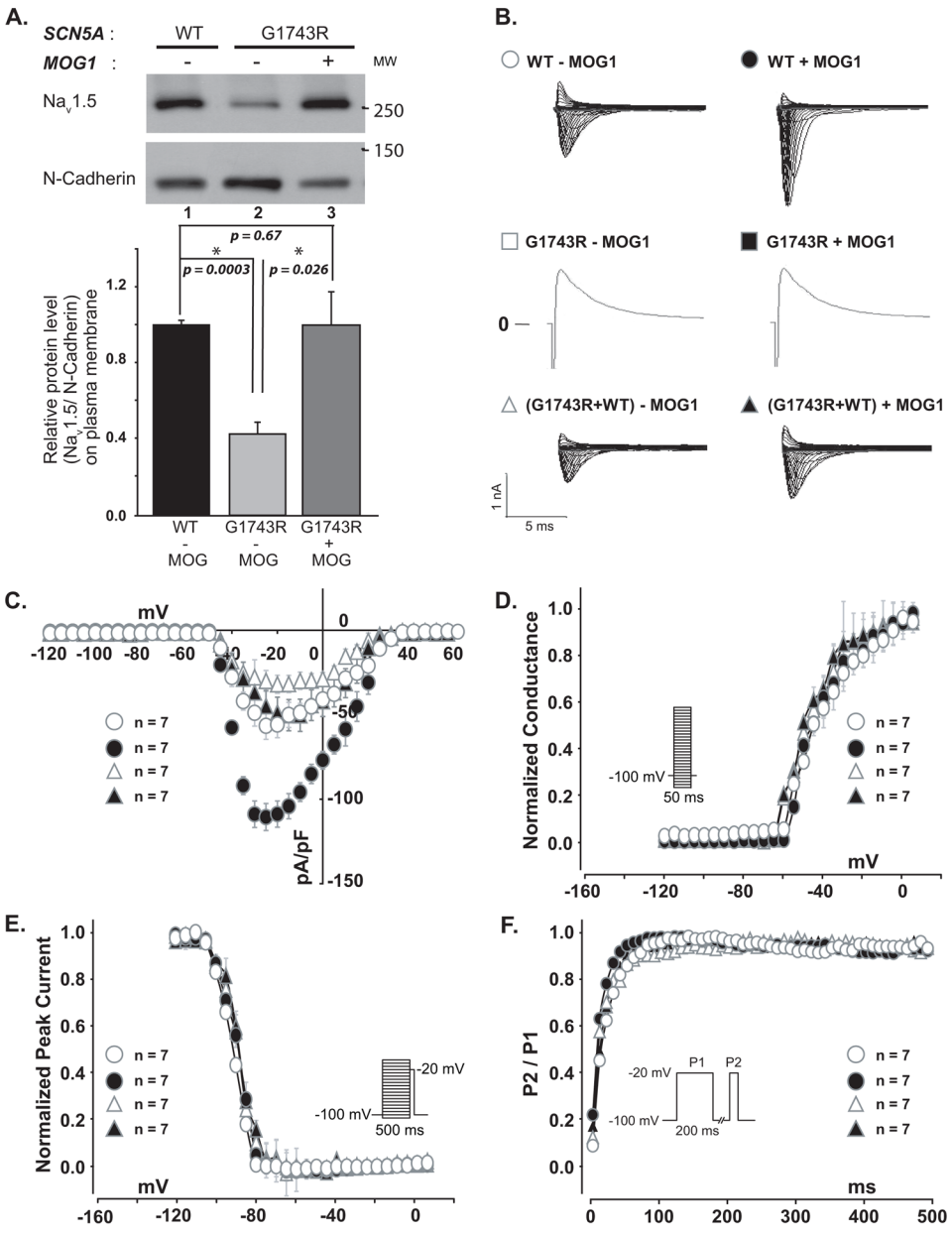

Figure 8. Effects of MOG1 on PM expression and function of mutant $Na_v1.5$/G1743R in the presence/absence of wild-type $Na_v1.5$

A. WB analysis of PM $Na_v1.5$ level for WT or mutant (G1743R) channels with (+) or without (−) *MOG1*. N-cadherin, loading control. **B.** Raw traces of I_{Na} in cells transfected with WT *SCN5A* (Top), mutant *SCN5A* (G1743R, Middle) or an equal amount of mutant +WT (Bottom) without (−) or with (+) *MOG1*. **C.** Effects of MOG1 on the current-voltage relationship. **D.** Effects of MOG1 on steady-state activation. **E.** Effects of MOG1 on steady-state inactivation. **F.** Effects of MOG1 on recovery from inactivation.