SUPPLEMENTAL MATERIAL

Expanded Methods

Cell preparation and transient expression of wt and mutant ZASP1

Wt and mutant ZASP1 were generated as previous described.[1] Cell preparation and transient transfection were performed as previously described. Sarcomere disturbances were carried out by the incubation of HEK-293 cells for 30 minutes in the medium containing 30 μM ML-7 (Sigma-Aldrich, MO) or Cytochalasin D (Cyto-D, Sigma-Aldrich, MO). The effects of wt and mutant ZASP1 on I_K were studied in CHO cells which stably expressed KCNQ1 and KCNE1, or KCNH2 and KCNE2 with transient expression of ZASP1-wt or ZASP1-D117N.

Neonatal rat cardiomyocyte (NRCM) isolation and transient expression of wt and mutant ZASP1

All procedures were approved by the Institutional Animal Care and Use Committee at the Texas Heart Institute. The neonatal rat cardiomyocytes were isolated according to the procedure as previously described.[10] Briefly, neonates (<3 days after birth) were deeply anesthetized with pentobarbiturate (i.p.). The hearts were excised quickly and rinsed in ice-cold PBS (Dulbecco's phosphate-buffered saline, Invitrogen, Carlsbad, CA). The ventricles were transferred to a dry Petri dish and minced with a scalpel blade. The minced hearts were digested in a warm collagenase II and procaine. The digested cells were resuspended in adhesion medium: DMEM (ATCC, Manassas, VA) supplemented with 10% FBS and 2 mM glutamate. The cells were

placed on a 100 mm Petri dish and incubated for 1-1.5 hours at 37˚C with 5% CO_2. After fibroblasts were settled and stick to the bottom of the dish, the supernatants were collected. Cardiomyocytes (1-2 millions) were aliquot into a 1.5 ml Eppendorf tube and centrifuged. The cells were resuspended in a transfection buffer (VPE-1002, Amaxa, Gaithersburg, MD) with 10 µg plasmid pcDNA3.1-CT-GFP-TOPO containing either *ZASP1*-wt or *ZASP1*-D117N cDNA and transferred into cuvettes. The transfection (nucleofection) was performed with an electroporator according to the company's protocol (Nucleofector-I Amaxa, Gaithersburg, MD). The cells were incubated for 24 hours at 37˚C with 5% CO_2. The cells showing green fluorescence were selected to recode sodium currents (I_{Na}).

Patch-clamp

Experiments were carried out at ambient temperature unless otherwise stated. For I_{Na} recording, the pipette solution contained (in mM): 5 NaF, 115 CsF, 20 CsCl, 10 EGTA and 10 HEPES (pH 7.35 with CsOH), and the bath solution contained (in mM): 145 NaCl, 1.0 $MgCl_2$, 10 tetraethylammonium (TEA) chloride, 5 CsCl, 10 HEPES, and 10 glucose (pH 7.35 with NaOH). Whole-cell currents amplitude and gating kinetics were analyzed with Clampfit (Axon Instruments, Sunnyvale, CA) and Igor software (WaveMetrics, Lake Oswego, OR).

Immunohistochemistry

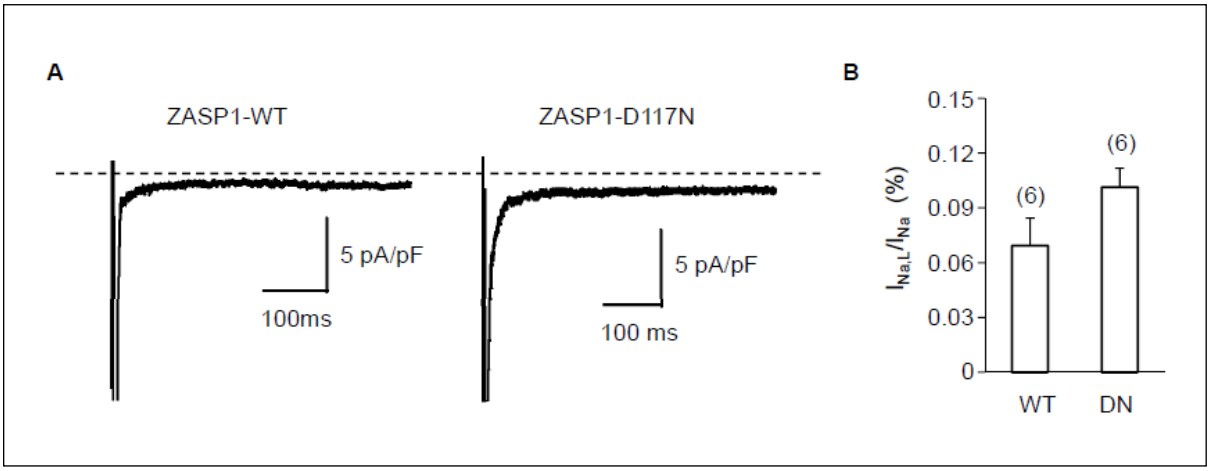
Following antibodies were used: (1) mouse monoclonal anti-telethonin/T-Cap (1:100,

Sigma-Aldrich); (2) mouse monoclonal anti-ACTN2 (1:200); (3) rabbit polyclonal anti-dystrophin (DMD, 1:200); and (4) rabbit polyclonal anti- α_1-syntrophin (SNTA1, 1:200). F-actin was labeled with fluorescent phalloidin (1:500, Invitrogen). The nucleus was labeled with To-pro 633 nm (Invitrogen).

***In vitro* interaction pull-down assay**

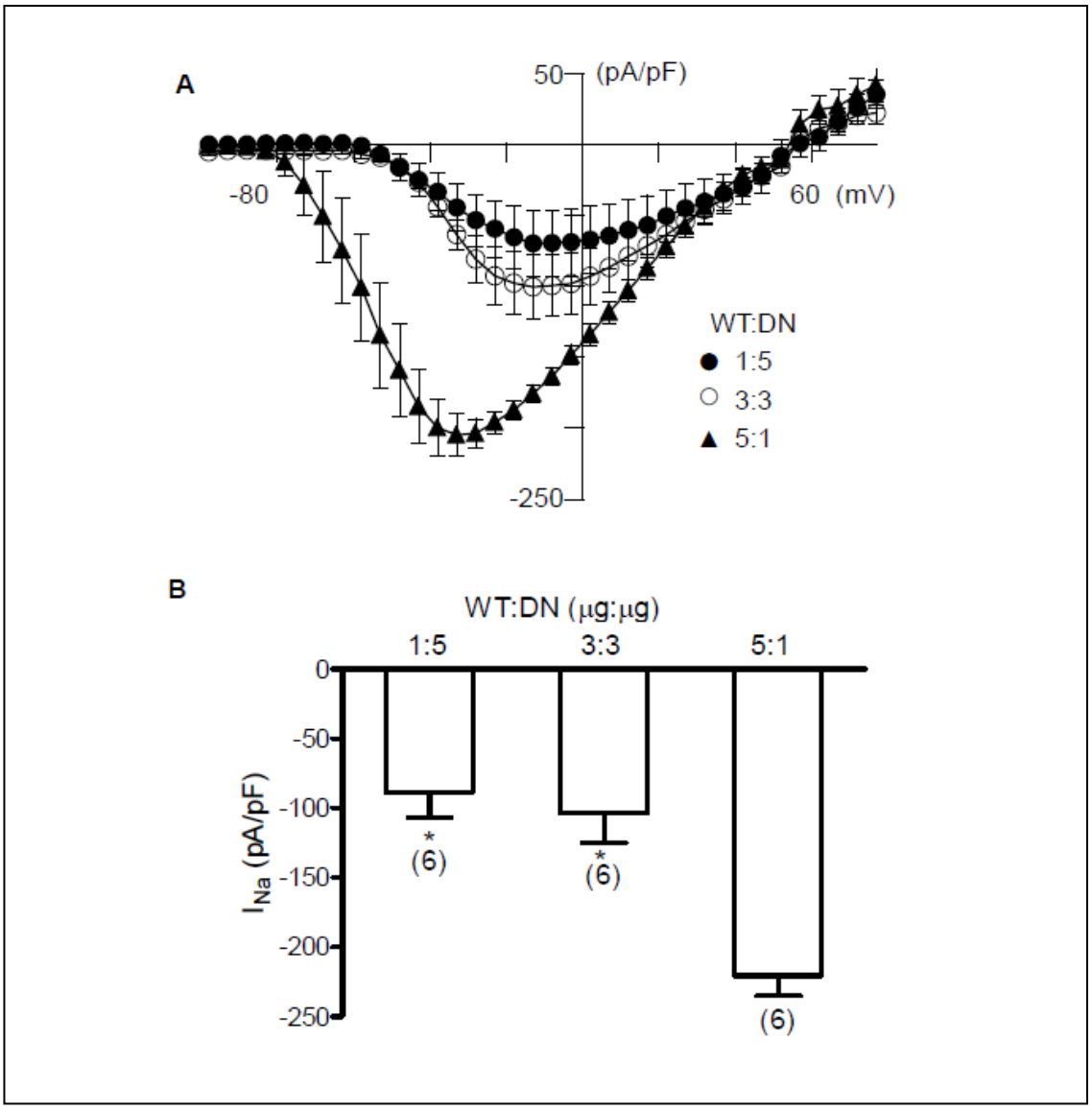
To produce ZASP and $Na_v1.5$, E. coli were transformed with pcDNA 3.1/V5-His containing *ZASP1*-wt or *ZASP1*-D117N, and *SCN5A*. The purified ZASP1-wt (His) or ZASP1-D117N (His) was incubated with preys: the purified $Na_v1.5$ from HEK-293 cells, and the lysate from NRCMs. The eluted proteins were separated by SDS–PAGE, and stained with anti-pan voltage-gated sodium channel (1:100), anti-telethonin (1:100), and anti-α-actinin antibodies (1:100). The pcDNA3.1/V5-His-TOPO/lacZ (Invitrogen, Carlsbad, CA) was used for negative controls.

Supplemental Experiments



Supplemental Figure S-I. Effects of ZASP1-wt and ZAP1-D117N on the late I_{Na}.

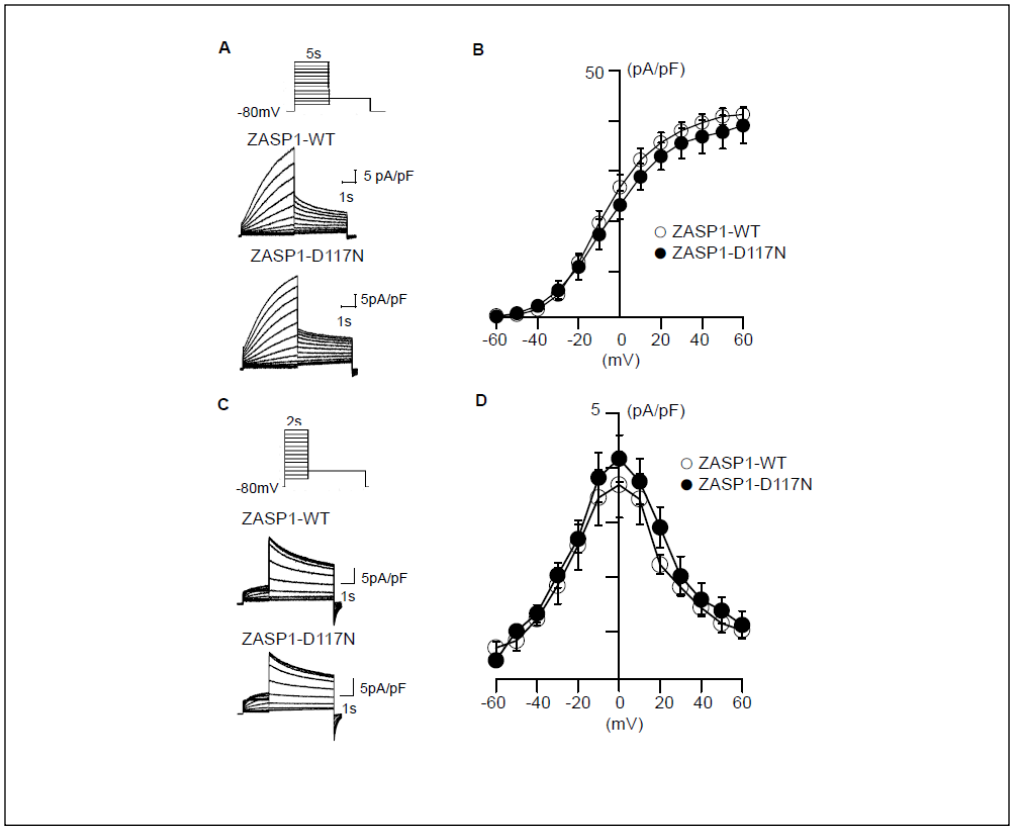
(A) Representative traces of late I_{Na} ($I_{Na,L}$) obtained from the cells stably expressing $Na_v1.5$ transfected with ZASP-wt or ZASP-D117N. $I_{Na,L}$ was induced with a long depolarization pulse (-30 mV for 1000 ms from a holding potential of -80 mV). (B) Bar graph demonstrates the normalized amplitudes of $I_{Na,L}$ obtained from the cells transfected with ZASP1-wt or ZASP1-D117N. $I_{Na,L}$ were measured at 200 to 220 ms of the test pulse, and normalized by the peak I_{Na}. The numbers in the parenthesis depict the number of cells studied. The error bars represent SD.



Supplemental Figure S-II. Effects of different ratios of ZASP1-wt and ZASP1-D117N plasmids on $Na_v1.5$. (A) The I-V relationships of peak I_{Na} obtained from the cells transfected with various combinations of ZASP1-wt and ZASP1-D117N plasmids: 1 μg/5 μg; 3 μg/3 μg; 5 μg/1 μg. (B) The bar graphs of peak I_{Na} for each condition. The numbers in the parenthesis depict the number of cells studied. The error bars represent SD. *p<0.05 vs. 5 μg/1 μg.

Effects of ZASP1-D117N on delayed rectifier potassium channels

Since abnormal repolarization may also cause cardiac conduction disturbances, we sought to investigate whether ZASP1-D117N can affect the function of two major components of delayed rectifier potassium channels (the slow component, I_{Ks} and the rapid component, I_{Kr}). The experiments were conducted in the CHO cells stably-transfected with *KCNQ1* with *KCNE1* or *KCNH2* with *KCNE2*, which mimics I_{Ks} and I_{Ks}, respectively. **Supplemental Figure S-III** showed that the steady-state peak I_{Ks} (Panel A) and I_{Kr} (Panel C) were not significantly affected by ZASP1-D117N. The I-V relationships of the steady-state I_{Ks} (Panel B) and I_{Kr} (Panel D) were almost identical between the cells expressing ZASP1-D117N and ZASP1-wt. These results indicate that ZASP1-D117N specifically altered the $Na_v1.5$ functions. **Supplemental Table S-I summarizes the parameters.**



Supplemental Figure S-III. The effect of ZASP1-wt and ZASP1-D117N on slow delayed rectify potassium currents (I_{Ks}) and rapid delayed rectifier current (I_{Kr}). (A) Representative current traces of I_{Ks}. (B) Current-voltage (I-V) relationship of I_{Ks} activation. The current amplitude was measured at the end of five second pulse between -60 mV and 60 mV from a holding potential of -80 mV. (C) Representative current traces of I_{Kr}. (D) I-V relationship of I_{Kr} activation. The current amplitude was measured at the end of second pulse between -60mV and 60 mV from a holding potential of -80 mV.

Table S-I. Parameters of K^+ channels

	I_{Ks}		I_{Kr}	
	WT (n=15)	D117N (n=11)	WT (n=10)	D117N (n=9)
Peak current density (pA/pF)	40.6 [36.5; 47.2] (at 60mV)	35.2 [33.8; 41.3] (at 60mV)	2.4 [1.0; 3.3] (at 0mV)	3.2 [0.5; 5.7] (at 0mV)
Tail current density at (pA/pF)	-15.0 [-24.0; -11.7] (at -40mV)	-12.1 [-35.9; -6.7] (at -40mV)	2.7 [0.4; 4.2] (at -40mV)	3.6 [1.4; 5.5] (at -40mV)

Data are presented as median [25th percentile; 75th percentile].