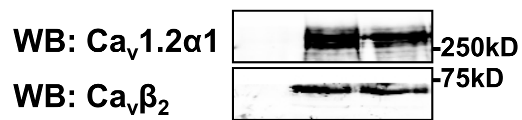
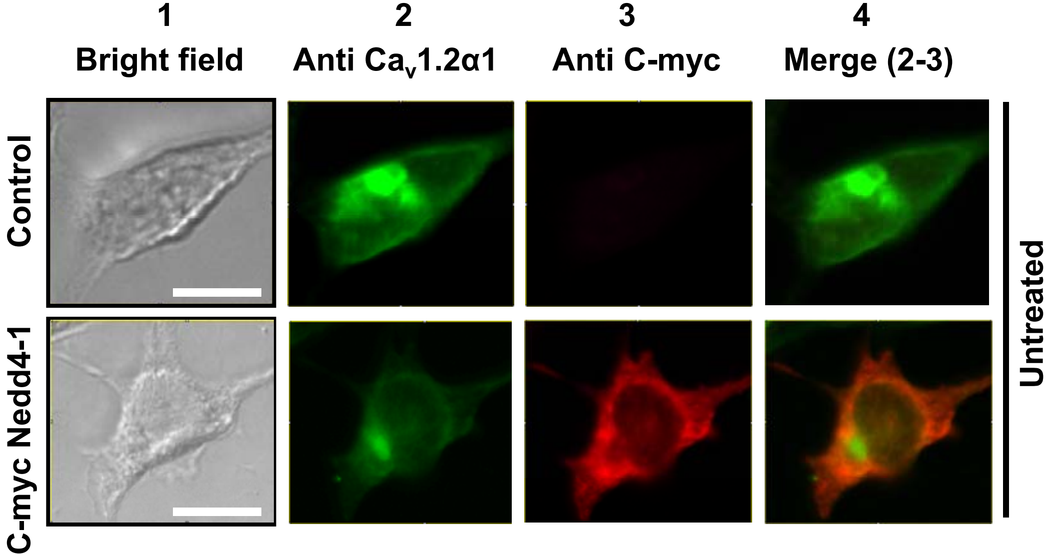
Supplemental figure 1.

IP: $Ca_v1.2\alpha1$

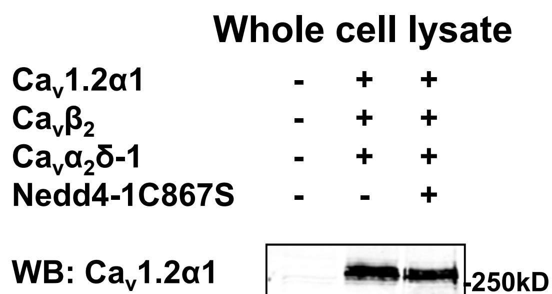
$Ca_v1.2\alpha1$	-	+	+
$Ca_v\beta_2$	-	+	+
$Ca_v\alpha_2\delta$-1	-	+	+
Nedd4-1	-	-	+



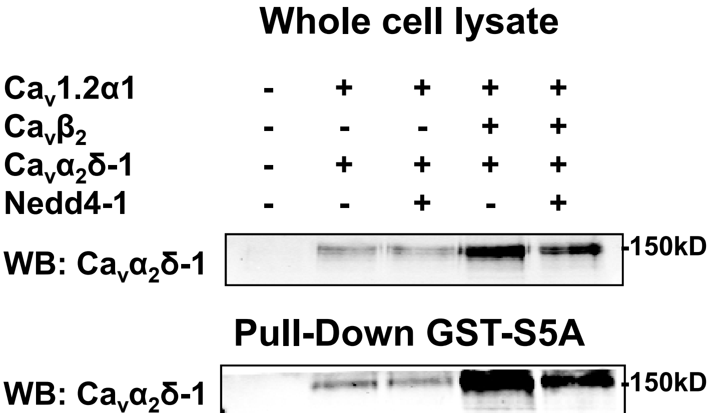
Supplemental Figure 2



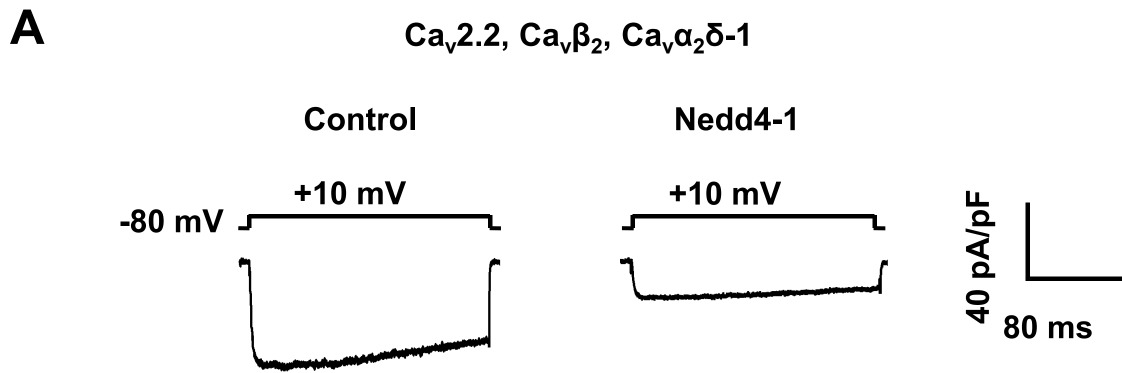
Supplemental figure 3.

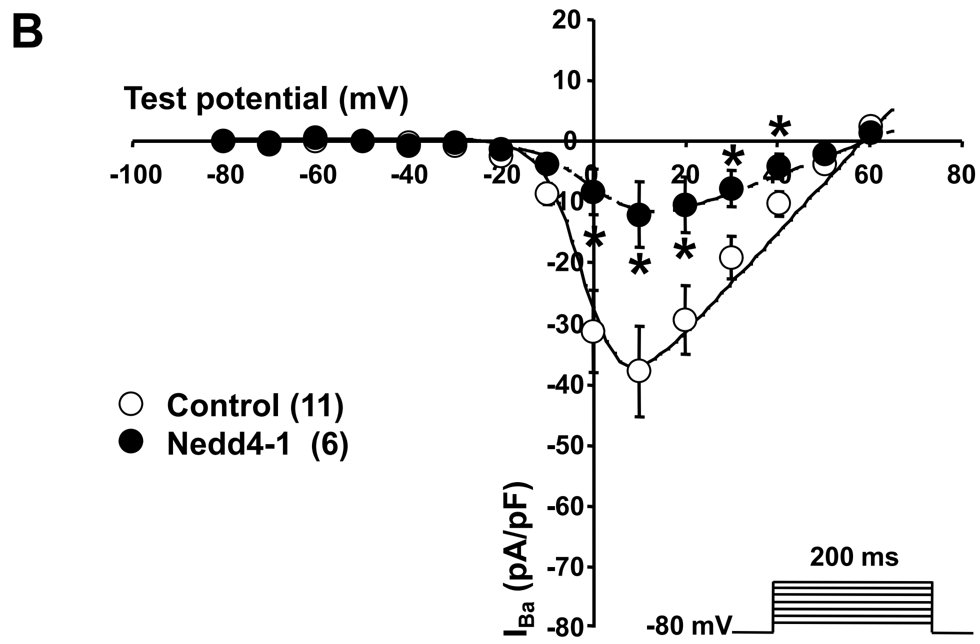


Supplemental figure 4.



Supplemental figure 5.





Supplemental Table 1. Effect of Nedd4-1 and Nedd4-1C867S on Ca_v biophysical properties. $Ca_v1.2\alpha1/\alpha_2\delta$-1 channels were expressed alone or together with $Ca_v\beta_1$, $Ca_v\beta_2$, $Ca_v\beta_2$Y221A, $Ca_v\beta_3$, $Ca_v\beta_4$. $Ca_v1.2\alpha1/\alpha_2\delta$-1 channels were expressed with $Ca_v\beta_2$. All experiments were performed with 5 mM $BaCl_2$ with the exception of $Ca_v1.2\alpha1/\alpha_2\delta$-1 currents recorded in 20 mM$BaCl_2$ extracellular solution. The upper and middle horizontal panels indicate the biophysical parameters corresponding to steady-state activation (SSA) and steady-state inactivation (SSI). V_{50} values indicate the respective voltage at which 50% of the channels are activated (V_{50} act), or inactivated (V_{50} inact), and k the slopes of the corresponding Botzmann-fitted curves described in Material and Methods. The lower panel shows the corresponding current densities and percentage of variation (%) from the controls (100%). Values are means ± SEM. The number of cells is indicated in parentheses. Values were compared versus control cells transfected with the channels only. *P<0.05, **P<0.01 and ***P<0.001. ND: Not Determined.

Supplemental Fig. 1. Co-immunoprecipitation of $Ca_v\beta_2$ with $Ca_v1.2\alpha1$ subunits. $Ca_v1.2\alpha1$ was immunoprecipitated with anti-$Ca_v1.2\alpha1$antibodies. Western-blots show that the amount of $Ca_v\beta_2$ co-immunoprecipitated with $Ca_v1.2\alpha1$ was similar in control and Nedd4-1-transfected cells. Experiments were performed in duplicate.

Supplemental Fig.2. Immunocytochemistry and confocal imaging experiments showing the distribution of $Ca_v1.2$ channels in control and Nedd4-1-transfected cells. $Ca_v1.2$ was detected using an anti-$Ca_v1.2$-ATTO-488 fluorescent primary antibody. Successful co-expression of c-myc-Nedd4-1 was assessed using mouse anti-c-myc primary and AlexaFluor594 anti-mouse secondary antibodies. Scale bars are 10 μm.

Supplemental Fig. 3. Nedd4-1C867S fails to regulate the ubiquitylation of $Ca_v1.2\alpha1$. Ubiquitylated proteins were pulled-down using ubiquitin-binding GST-S5A and the presence of $Ca_v1.2\alpha1$ in the isolated fraction was assessed by Western-blotting using anti-$Ca_v1.2\alpha1$ antibodies. Western-blots show that Nedd4-1C867S did not modify the amount of $Ca_v1.2\alpha1$ proteins recovered from whole-cell lysates or GST-S5A pulled-down fractions. Experiments were performed in duplicate.

Supplemental Fig.4. $Ca_v\alpha_2\delta$-1 is not ubiquitylated by Nedd4-1. Ubiquitylated proteins were pulled-down using ubiquitin-binding GST-S5A and the presence of $Ca_v\alpha_2\delta$-1 in the isolated fraction was assessed by Western-blotting using anti-$Ca_v\alpha_2\delta$-1 antibodies. Representative Western-blot showing that $Ca_v\alpha_2\delta$-1 is endogenously ubiquitylated and co-expressing $Ca_v\beta$ promotes its ubiquitylation, however there was no effect of Nedd4-1 on the basal ubiquitylation of $Ca_v\alpha_2\delta$-1(five independent experiments). Note that as for $Ca_v1.2\alpha1$, Nedd4-1 similarly reduced the amount of $Ca_v\alpha_2\delta$-1 recovered in whole-cell lysates and GST-S5A pulled-down fractions, when expressed together with $Ca_v\beta$.

Supplemental Fig.5. Nedd4-1 down-regulates $Ca_v2.2$ channels. A, Representative whole-cell current traces and B, I-V relationships recorded from tsA-201 cells transfected with $Ca_v2.2\alpha1/Ca_v\beta_2/Ca_v\alpha_2\delta$-1 channels alone (Control, ○) or together with Nedd4-1 (●). The number of cells recorded from is indicated in parentheses. *P<0.05 when compared with control cells transfected with $Ca_v2.2\alpha1/Ca_v\beta_2/Ca_v\alpha_2\delta$-1 channels alone.

Supplemental Material and Methods:

Immunocytochemistry: HEK-293 cells were plated on fibronectin-coated glass-bottom dishes (MatTek) 24 hours before experiment. Forty-eight hours post-transfection, cells were washed once with PBS then fixed using 4% paraformaldehyde PBS pH7.4 for 10 minutes at room temperature. Cells were permeabilized with PBS containing 0.02% Triton X100 (twice for 7 minutes), followed by a 15-minute block with TBS containing 10% goat serum. (Alomone, Jerusalem, Israel) and mouse anti-C-myc (M4439; Sigma-Aldrich Chemie, Postfach, Switzerland) antibodies were used at a dilution of 1/20 and 1/100, respectively and incubated overnight at 4°C. AlexaFluor594 goat anti-rabbit secondary antibodies were incubated 2 hours at room temperature. Confocal images were acquired with a Zeiss LSM-510 confocal laser scanning microscope, using a 60X (1.4 NA) oil-immersion objective. Optical slices were 0.8µm thick. Images were acquired using an argon laser (excitation: 488 nm; emission: BP505−530 nm) and a He-Ne laser (excitation: 543 nm; emission: LP650 nm). Image J was used for analysis.