# The MIDAS motif and glycosylation of CACHD1 are important for expression and function as a $Ca_V3.1$ voltage-gated calcium channel modulator

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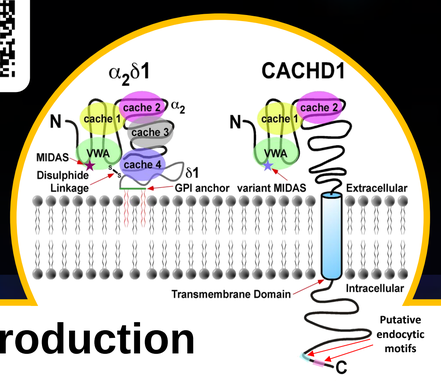


## 1. Introduction

### α2δ auxiliary subunits

- Represent important therapeutic targets for gabapentinoids[1].
- Modulate high-voltage-activated voltage-gated calcium channels (VGCCs)
  - ➡ Enhance channel trafficking and function[1,2].
- Within the α2δ family, **CACHD1** recently emerged as a modulator of low-voltage-activated (LVA) VGCCs[3].
  - ➡ Increases channel open probability and surface expression.

**How does CACHD1 modulate LVA VGCCs?**

### MIDAS motif

- Metal ion-dependent adhesion site motif.
- Essential for the trafficking and synaptic function of α2δ and VGCCs[1,2].
- α2δ subunits contain fully conserved MIDAS motif (DxSxS).
- CACHD1 has a variant MIDAS motif ($D^{234}$x**G**xS).

### Glycosylation

- Plays crucial role in α2δ function, stability, and surface expression.
- N136 and N184 necessary for α2δ function
  - ➡ Affect interaction with other subunits and VGCCs.
  - ➡ Role in channel modulation.

## 2. Methods

**PCR mutagenesis**

MIDAS mutants (G236S, AxAxA); Glycosylation mutants (N145/329/373/587/940/985Q, NΔ6Q)

➡

**Immunocytochemistry**

Assessed CACHD1 subcellular localisation

➡

**Western blotting & Densitometry**

Confirmed protein expression and molecular mass; Quantified expression levels

➡

**Whole-cell patch clamp**

Voltage-clamp recordings using 10 mM $Ba^{2+}$ as charge carrier to assess CACHD1 effects on rat $Ca_V3.1$ ($rCa_V3.1$) channel function.

## 3. Results - MIDAS motif

### Disruption of the CACHD1 MIDAS motif reduces expression and trafficking to the cell surface

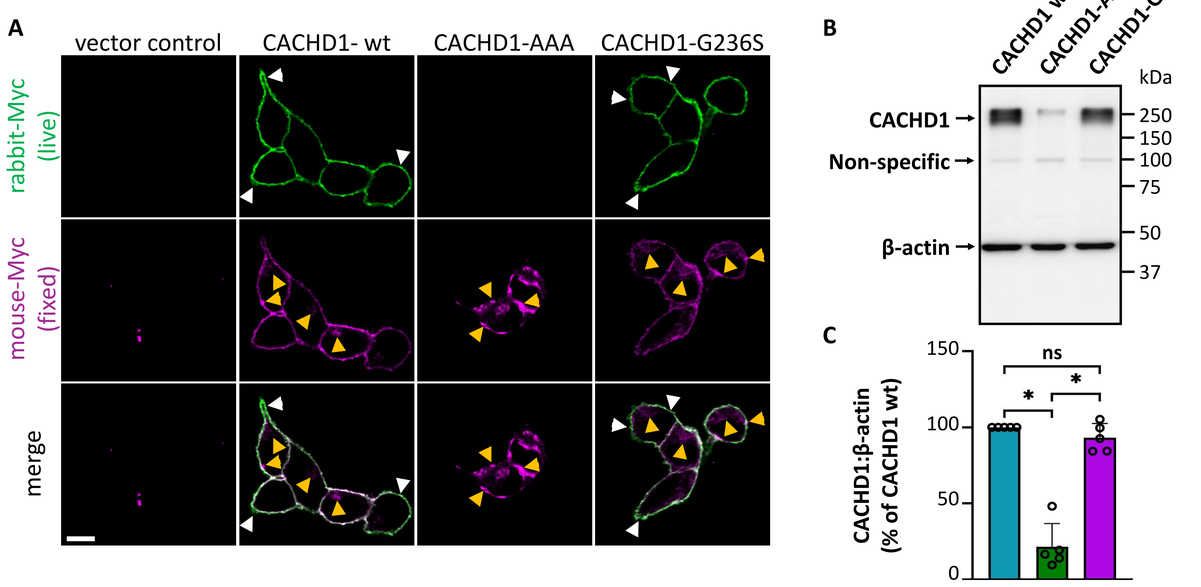


**Fig. 1: (A)** 'AAA' MIDAS motif mutation impaired CACHD1 expression at cell surface. Myc-tagged CACHD1-wt and CACHD1-G236S detected in intracellular compartments (yellow arrows) and at cell surface (white arrows), while CACHD1-AAA detected in intracellular compartments only; (scale bar 10 µm; n=5). **(B)** Immunoreactive Myc-tagged CACHD1 proteins detected at 150-170 kDa. **(C)** Significant reduction in expression levels of CACHD1-AAA (21.3±6.8%), but not CACHD1-G236S (93.3±4.2%); (mean±SEM; One-way ANOVA, Tukey's *post hoc* test, *p<0.05, ns – non-significant; n=5).

### CACHD1 modulation of $Ca_V3.1$ is dependent on an intact MIDAS motif

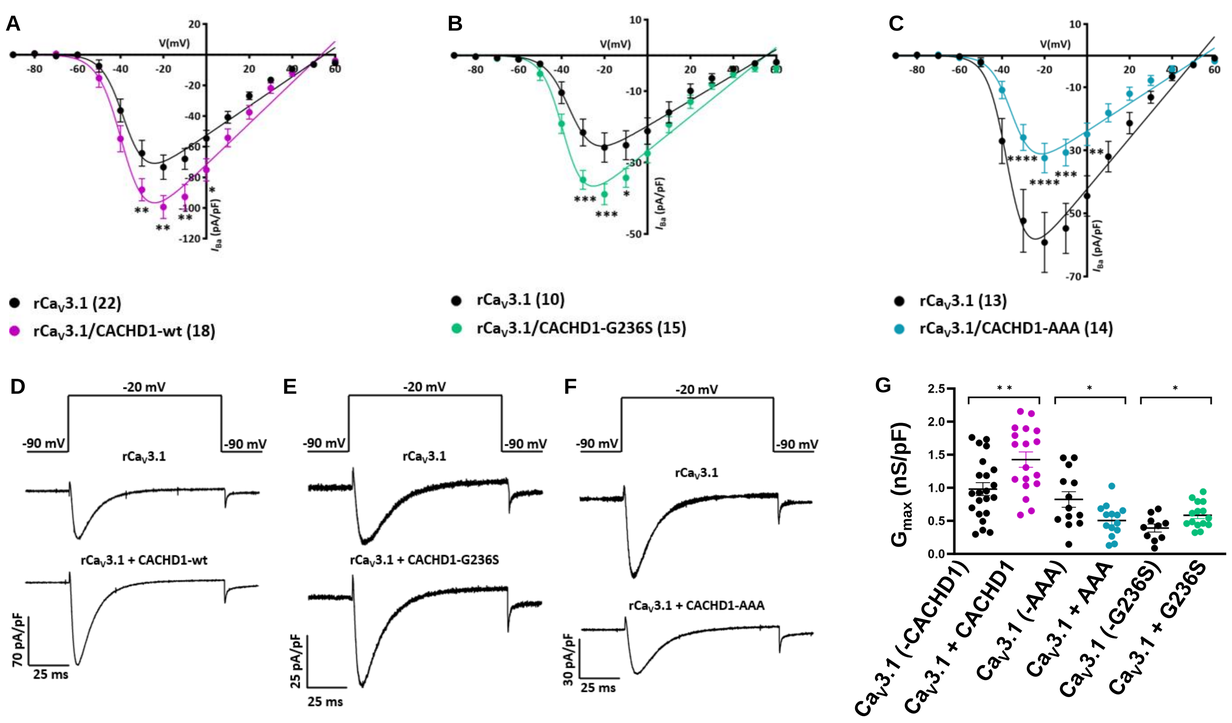


**Fig. 2:** 'AAA' mutation in CACHD1 MIDAS motif prevents CACHD1-mediated $rCa_V3.1$ current increase and further decreases $rCa_V3.1$ T-type currents by 0.55-fold. **(A, B, C)** I-V relationship (holding potential -90 mV; two-way ANOVA, Bonferroni *post hoc* test, curve fitted with modified Boltzmann). **(D, E, F)** Representative current density traces at -20 mV. **(G)** Maximal conductance (two-tailed unpaired Student's *t* test); (*$p$<0.05, **$p$<0.01, ***$p$<0.001, ****$p$<0.0001).

## 4. Results - Glycosylation

### Disruption of CACHD1 glycosylation shows no effect on CACHD1 trafficking

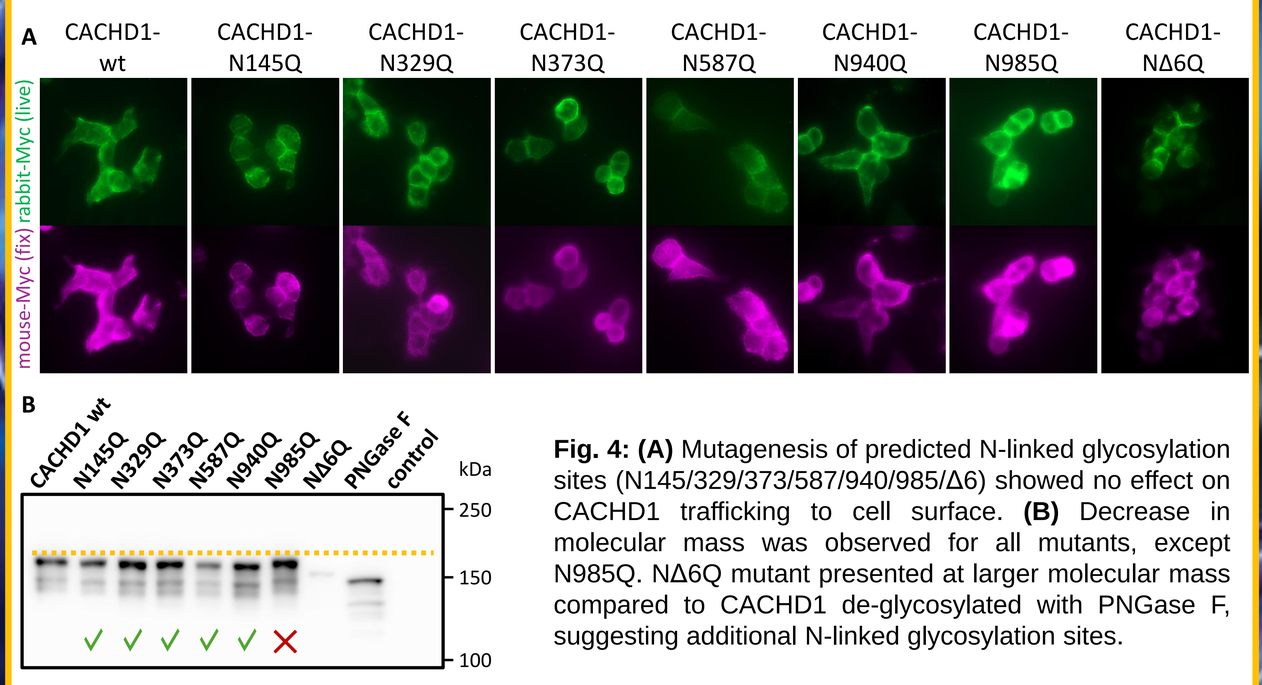


**Fig. 4: (A)** Mutagenesis of predicted N-linked glycosylation sites (N145/329/373/587/940/985/Δ6) showed no effect on CACHD1 trafficking to cell surface. **(B)** Decrease in molecular mass was observed for all mutants, except N985Q. NΔ6Q mutant presented at larger molecular mass compared to CACHD1 de-glycosylated with PNGase F, suggesting additional N-linked glycosylation sites.

## 5. Proposed mechanism of CACHD1-$Ca_V3.1$ interaction

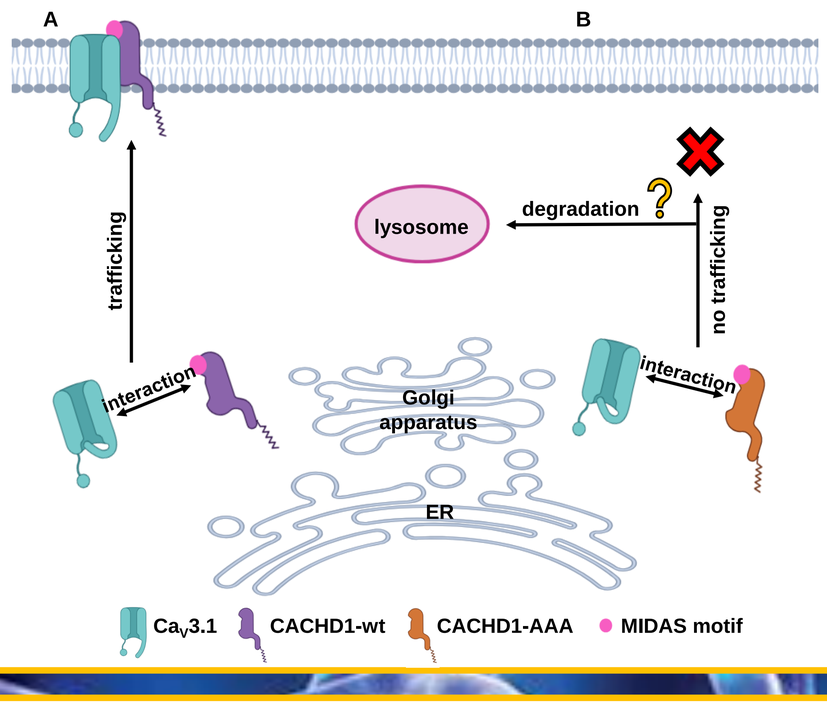


**Fig. 5: Proposed mechanism of CACHD1-$Ca_V3.1$ interaction.**

**(A)** CACHD1 and $Ca_V3.1$ interact intracellularly, forming a complex that is trafficked to the membrane. CACHD1 promotes $Ca_V3.1$ cell surface localisation[3] and modulates $Ca_V3.1$ electrophysiological properties.

**(B)** Mutagenesis of MIDAS motif (DxGxS to AxAxA), disrupts CACHD1 membrane trafficking, leading to intracellular retention of CACHD1/$Ca_V3.1$ complex, resulting in reduction of $Ca_V3.1$ T-type currents.

## 6. Conclusions

- The MIDAS motif plays an important role in CACHD1-mediated increase of T-type currents in $Ca_V3.1$ VGCCs.
- Mutagenesis of the MIDAS motif significantly reduces CACHD1 expression and cell surface trafficking, similar to effects seen in α2δ subunits.
- Glycosylation does not appear to have a key role in CACHD1 trafficking, and further investigation is needed to assess its effect on CACHD1 function
- CACHD1 represents a promising therapeutic target for modulating $Ca_V3$ VGCCs in hyperexcitability conditions (e.g., pain, epilepsy), similar to gabapentinoid targeting of α2δ subunits.

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**References:**

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**2** Hoppa, M. B., *et al.* (2012). *Nature*, *486*(7401), 122–125.
**3** Cottrell, G. S., *et al.* (2018). *Journal of Neuroscience*, *38*(43), 9186–9201.