

Supplementary Materials

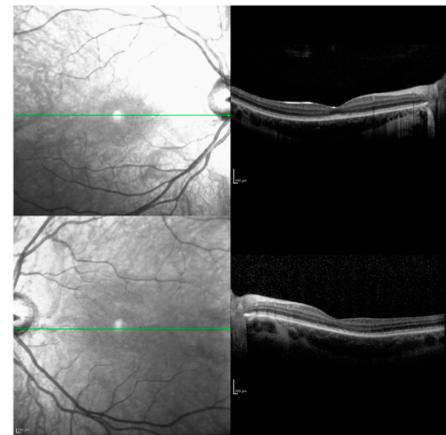
# A Novel *GUCA1A* Variant Associated with Cone Dystrophy Alters cGMP Signaling in Photoreceptors by Strongly Interacting with and Hyperactivating Retinal Guanylate Cyclase

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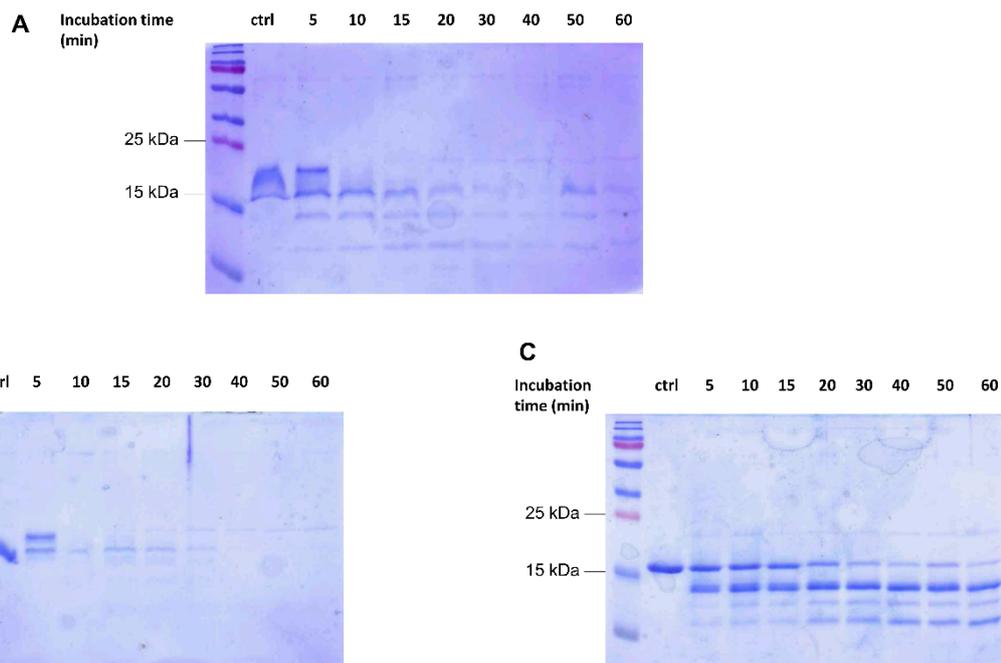
**A**



**B**

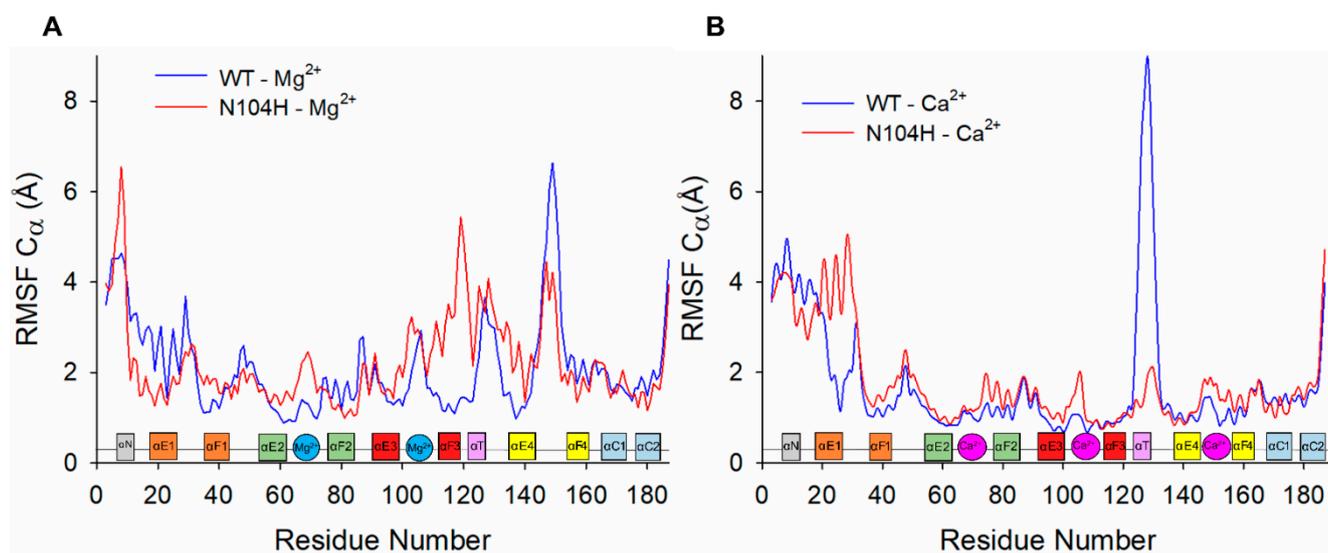


**Figure S1.** (A) Fundus image of the right eye and (B) SD-OCT scans of right and left eye of patient II:2 at the age of 10. Line corresponding to interdigitation zone is shown in green.

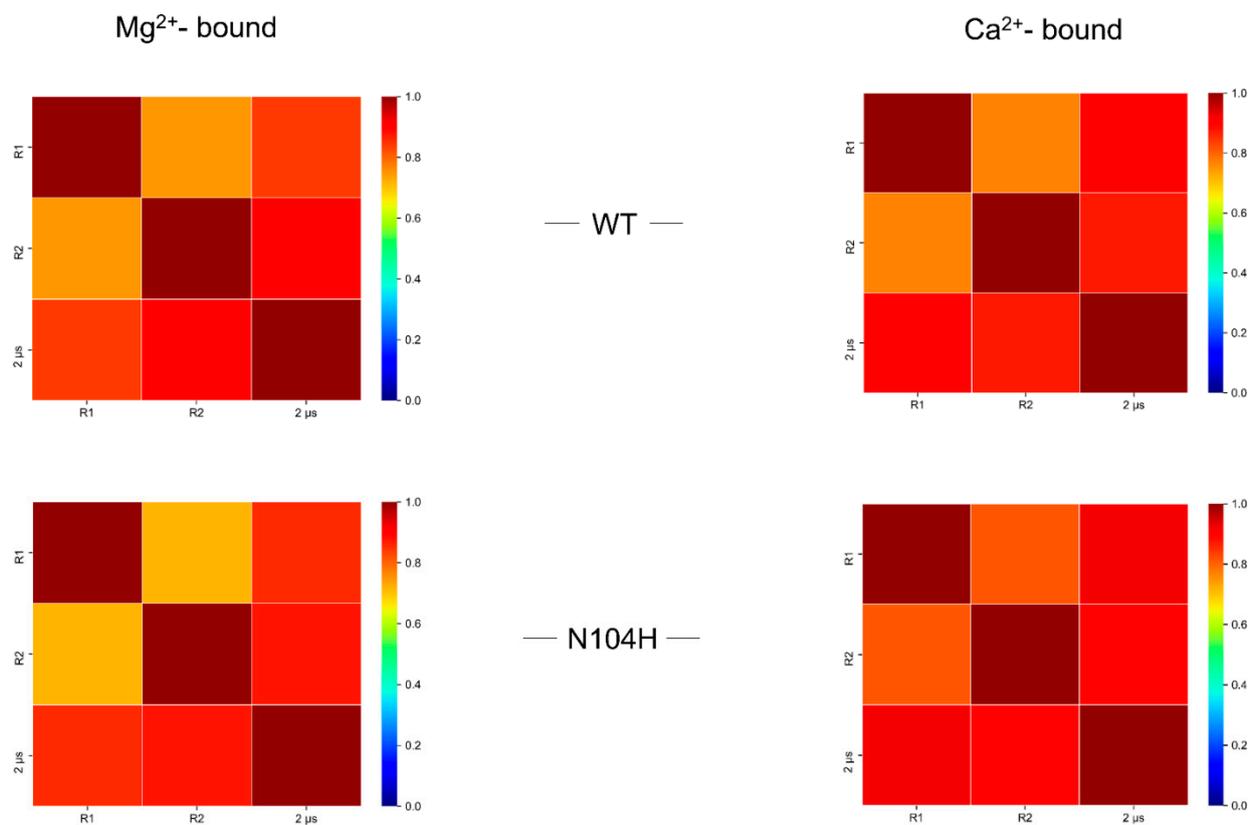


**Figure S2.** Time-dependent limited proteolysis of 20  $\mu$ M GCAP1 WT in the presence of (A) 2 mM EDTA (ctrl) or 2 mM EDTA + 0.3  $\mu$ M trypsin, (B) 1 mM EGTA + 1.1 mM  $Mg^{2+}$  (ctrl) or 1 mM EGTA + 1.1 mM  $Mg^{2+}$  + 0.3  $\mu$ M trypsin, (C) 1 mM

Mg<sup>2+</sup> and 1 mM Ca<sup>2+</sup> (ctrl) or 1 mM Mg<sup>2+</sup> and 1 mM Ca<sup>2+</sup> + 0.3 μM trypsin. Incubation times were (from left to right): 5-10-15-20-30-40 and 60 minutes for each condition.



**Figure S3.** C $\alpha$ -RMSF profiles (calculated over 2  $\mu$ s MD simulations) of (A) Mg<sup>2+</sup>-bound and (B) Ca<sup>2+</sup>-loaded WT (blue line) and N104H (red line) GCAP1. Insets show secondary structure elements represented with the same coloring scheme as Fig. 5. Ca<sup>2+</sup> and Mg<sup>2+</sup> ions are represented respectively as pink and blue circles.



**Figure S4.** RMSIP of the first 20 principal components of the two 1  $\mu$ s MD simulation replicas (R1 and R2) and of the concatenated trajectories of WT- and N104H-GCAP1 in their Mg<sup>2+</sup>-bound and Ca<sup>2+</sup>-loaded forms.