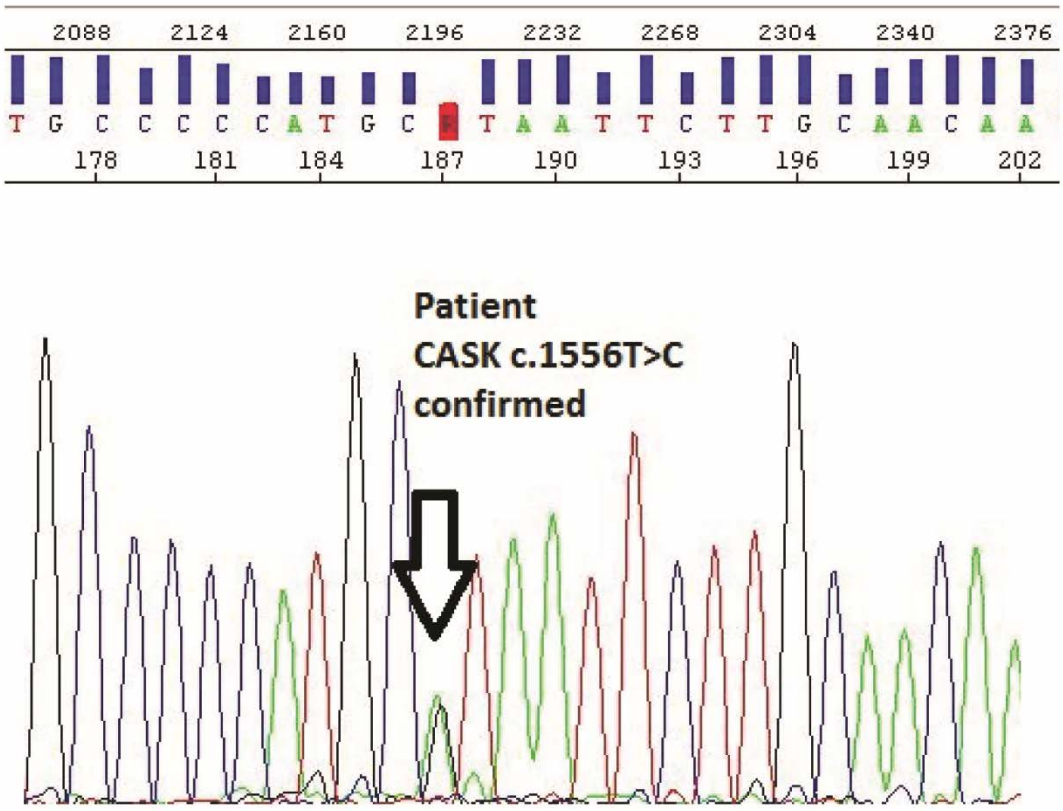
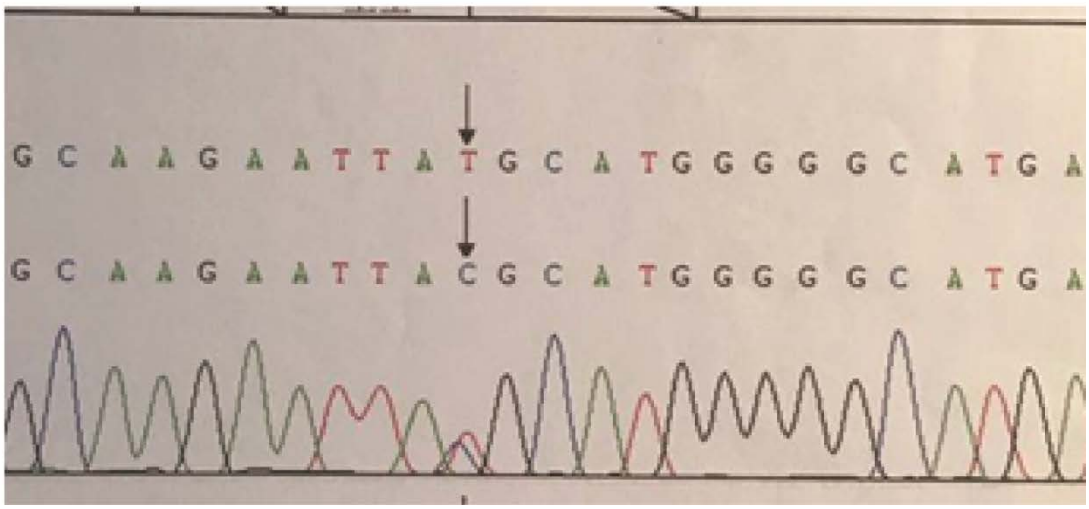
Supplemental Figure 1. Sanger sequencing results confirm a non-maternally inherited heterozygous variant in exon 16 of the X-linked CASK gene, c.1556T>C (p.M519T) in both Subject 1 and Subject 2.

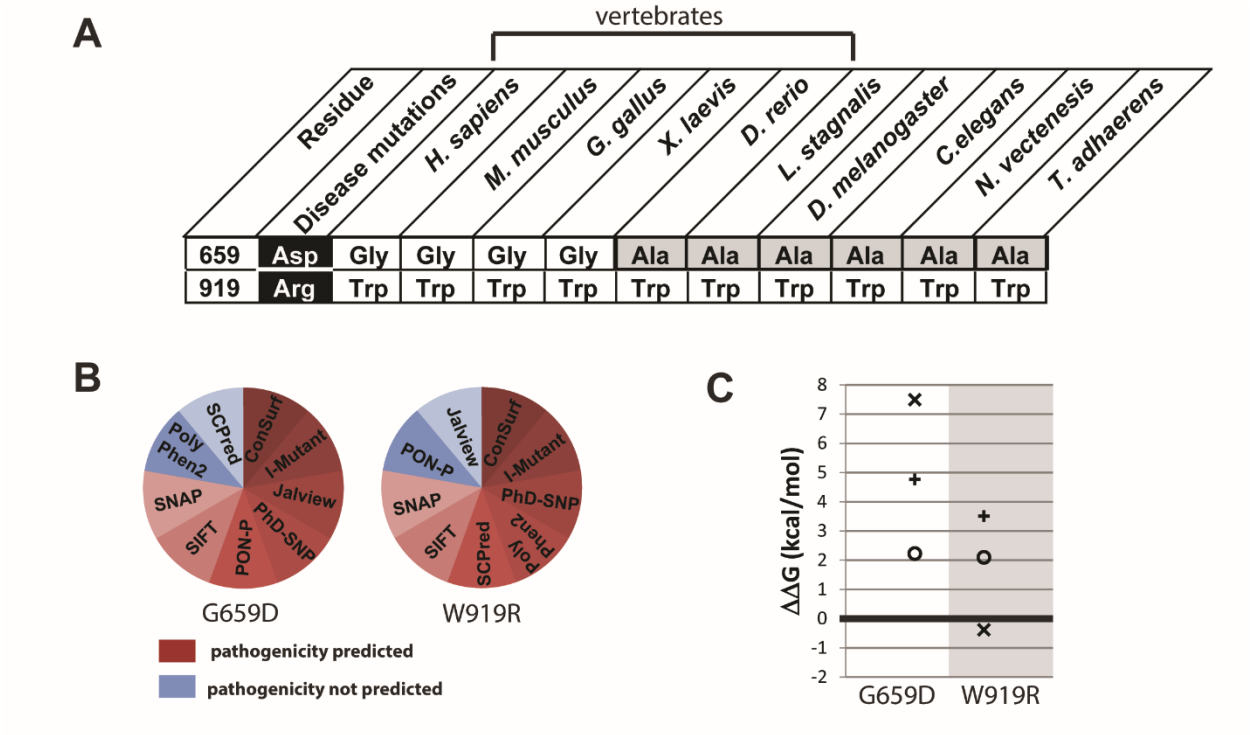
M519T Case 1



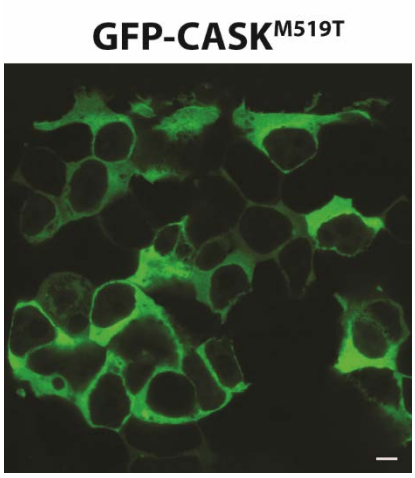
M519T Case 2



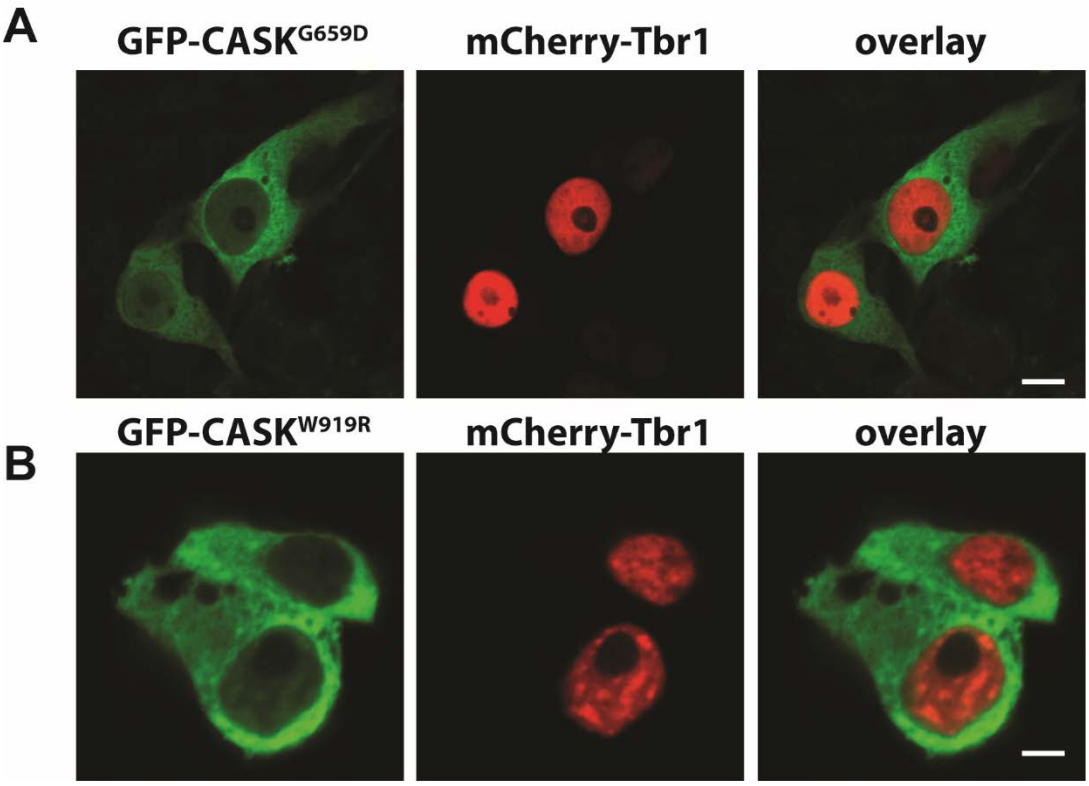
Supplemental Figure 2. A) Sequence conservation in CASK orthologs from nine species at the amino acid position associated with two CASK mutations of interest, G659D and W919R. Conserved residues are in white boxes; gray boxes indicate residue differences from the wild-type human sequence. **B**) Sequence-based predictions of pathogenicity for CASK mutations G659D and W919R from nine web-based bioinformatics algorithms. Algorithms that predict a particular mutation is damaging are shown in shades of red; algorithms that do not predict pathogenicity are in shades of blue. C) Change in protein stability induced by either the G659D mutation or W919R mutation as indicated by ΔΔG (kcal/mol). ΔΔG values were calculated using PoPMuSiC (o), FoldX (+), and Eris (x).



Supplemental Figure 3. Representative images of HEK293FT cells expressing GFP-CASK^{M519T}, scale bar = 10μm. No aggregates were noted.



Supplemental Figure 4. Images of HEK293FT cells co-transfected with A) GFP-CASK^{G659D} with mCherry Tbr1 and B) GFP-CASK^{W919R} with mCherry Tbr1. Scale bars = 5μm.



Supplemental Figure 5. Images of HEK293FT cells transfected with GFP-CASK^{R28L}, GFP-CASK^{Y278H}, GFP-CASK^{P396S}, or GFP-CASK^{Y728C} plasmid DNA co-expressed with neurexin-1β-FLAG (A,B). After 48 hours, cells were fixed, permeabilized and immunostained for neurexin. Scale bar = 5μm.