

Supplementary Material

1 Supplementary Figures

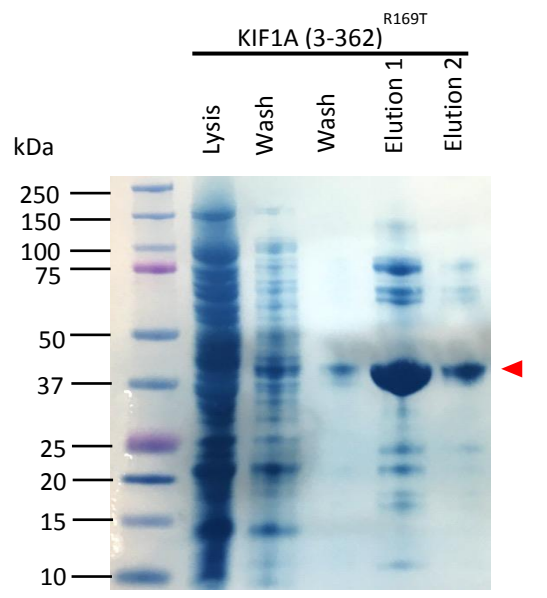
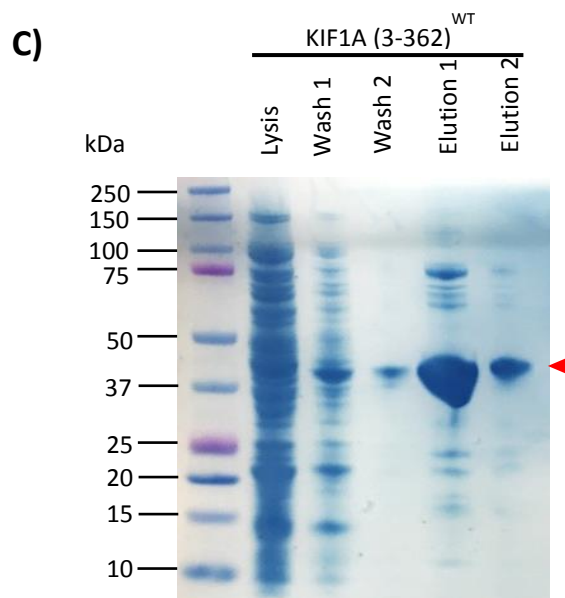
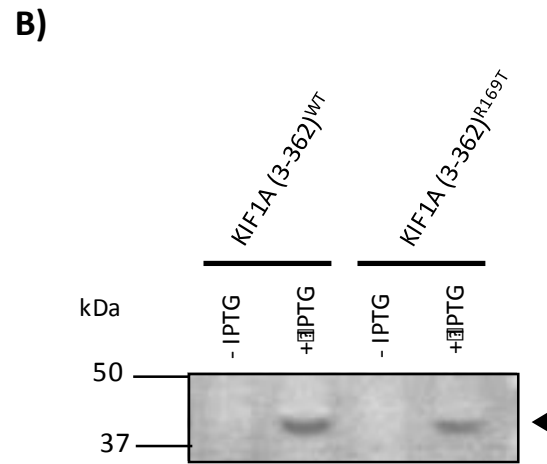
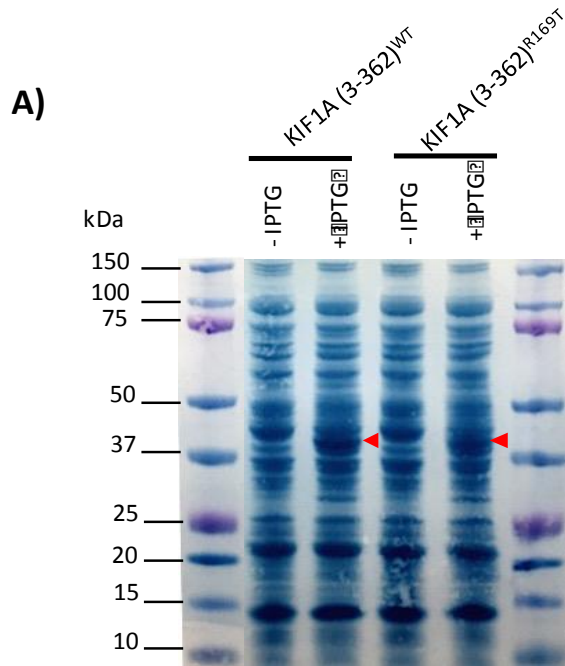


Figure 1: Detection and purification of His tagged KIF1A motor domain. **A)** Expression of KIF1A motor domain WT and R169T after 1mM IPTG induction at 18 °C overnight of BL21 cell culture. 10 µl of the cell pellet resuspended with Laemmli Buffer 2x was loaded. Red arrowheads indicate the recombinant protein corresponding to KIF1A motor domain. **B)** Western blot that shows the specific expression of the recombinant motor domain of KIF1A that carry a six-histidine tail. A penta-his antibody (1/1000) was used to detect the recombinant proteins. **C)** Steps on the purification of KIF1A WT and R169T motor domains. Red arrowheads indicate the purified recombinant proteins.

2 Supplementary Tables

Table 1. Site directed mutagenesis primers.

Primer name	Primer sequence (5' → 3')	Length	Tm (° C)
KIF1A_R169T_F	GGGTGCTCCGTCACGCGAAGGTTGCC	27	84,8
KIF1A_R169T_R	GGGCAACCTTCGCGTGACGGAGCACCC	27	84,8

Gene	Genomic coordinates (hg19)	Nucleotide change	Amino acid change	Variant type	ACMG classification	Gene function
<i>KIF1A</i>	Chr2:241725854C>G	NM_001244008.2:c.506G>C	NP_001230937.1:p.(Arg169Thr)	Missense	Likely pathogenic	KIF1A belongs to the kinesin-3 family of kinesins and is involved in fast anterograde transport of synaptic vesicle precursors along microtubules.
<i>RCC2</i>	Chr1:17748698C>A	NM_001136204.3:c.744+1G>T	-	Splicing	Likely pathogenic	<i>RCC2</i> encodes for a multifunctional protein that regulates the activity of small GTPases and is required for normal progress of the cell cycle during interphase and mitosis.
<i>ZNF2</i>	Chr2:95847733G>A	NM_021088.4:c.1160G>A	NP_066574.2 :p.(Arg387Gln)	Missense	Variant of uncertain significance	ZNF2 may be involved in DNA binding and transcriptional regulation.

Table 2. Variants identified after filtering WES trio data for a *de novo* pattern of inheritance.