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Supplemental Data

Defects in the Cell Signaling Mediator β-Catenin Cause the Retinal Vascular Condition FEVR

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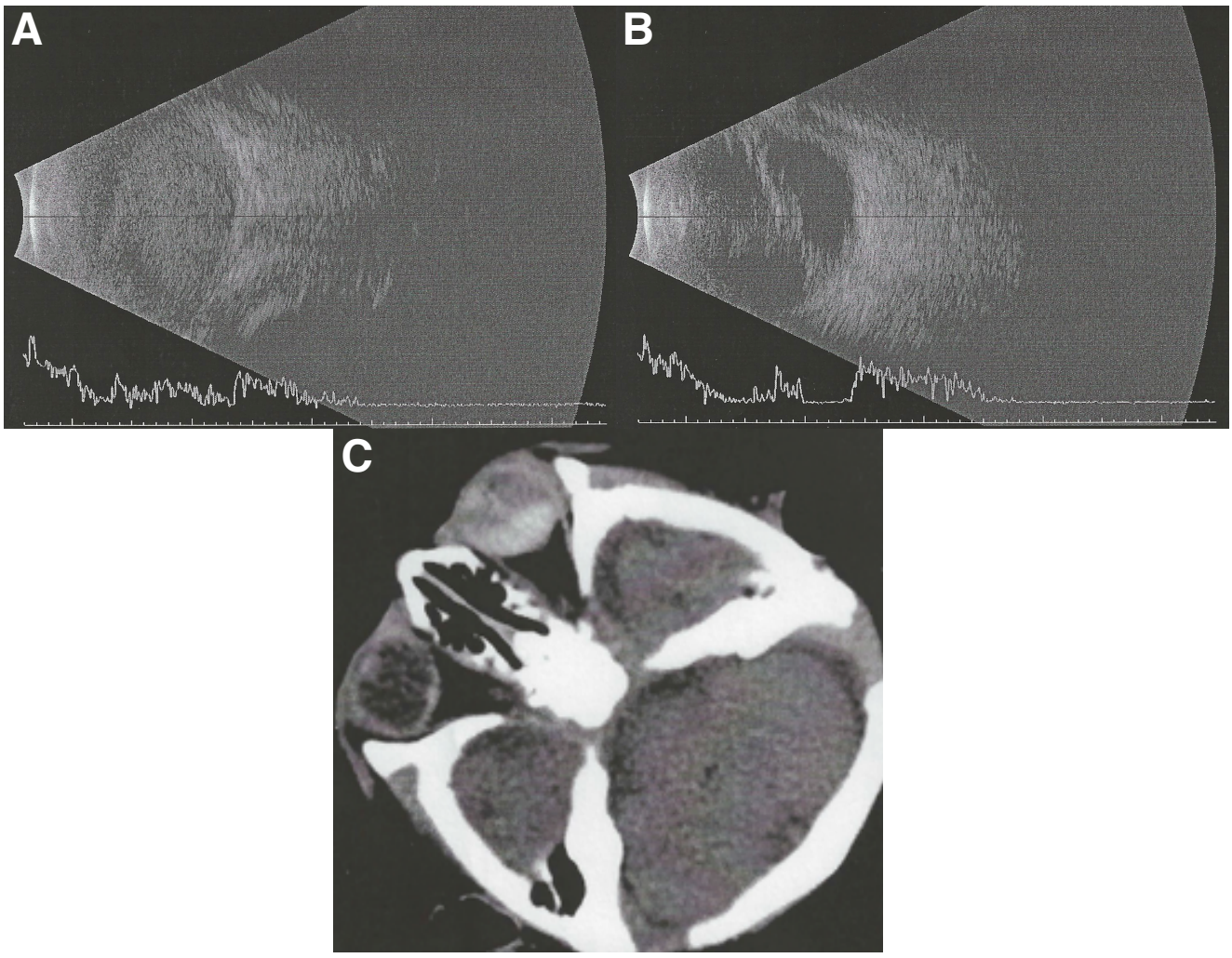

Figure S1. Clinical appearance of the proband from family F1321 aged one month. (A) Ultrasound biomicroscopy photograph of the left eye showing vitreous hemorrhage. (B) Ultrasound biomicroscopy photograph of the right eye showing a funnel-shaped retinal detachment and vitreous hemorrhage. (C) Computerized tomography scan of the brain showing hyper-dense left globe content and no acute intracranial hemorrhage.

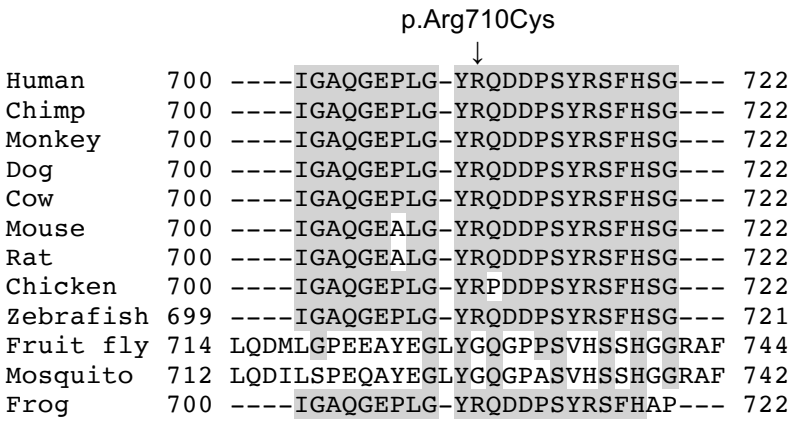

Figure S2. Protein sequence alignment of human β-catenin with homologues. Multiple protein alignment was calculated using HomoloGene (http://www.ncbi.nlm.nih.gov/homologene) [Edgar, R.C. (2004) Nucleic Acids Res. 32, 1792-7]. Thirty amino acid residues surrounding the p.Arg710Cys variant are shown. Conserved amino acid residues are shaded. Accession numbers: Human NP_001091679.1; Chimpanzee XP_001138023.1; Monkey NP_001244847.1; Dog NP_001131124.1; Cow NP_001069609.1; Mouse NP_001159374.1; Rat NP_445809.2; Chicken NP_990412.1; Zebrafish NP_571134.2; Fruit fly (armadillo) NP_996328.1; Mosquito (Arm_Anoga) XP_309245.5; Frog NP_001016958.1.

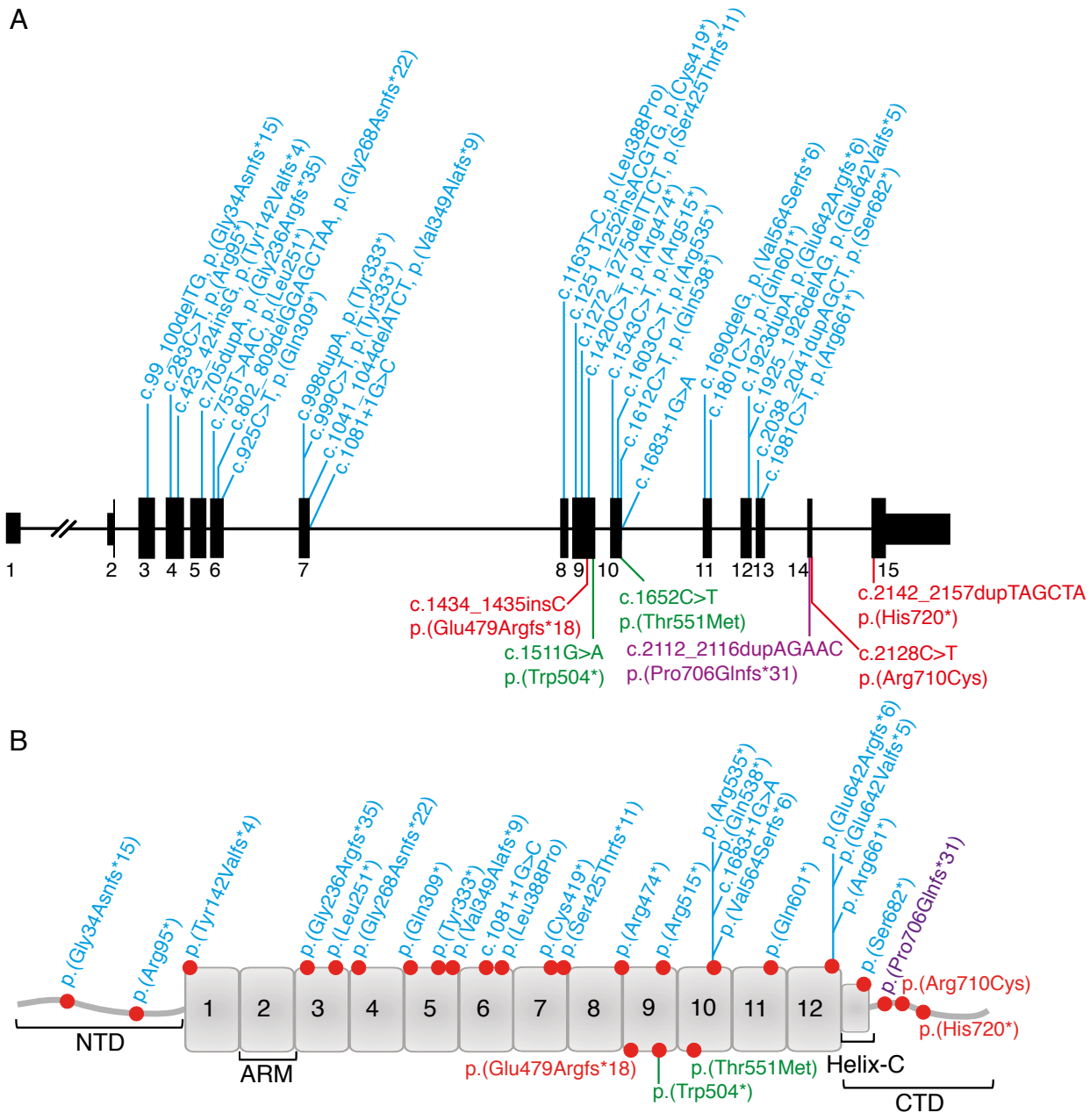

Figure S3. Published intragenic heterozygous *CTNNB1* mutations causing syndromic intellectual disability (ID) and autism spectrum disorder (ASD). Schematic representation of the *CTNNB1* gene and protein showing the location of *CTNNB1* mutations identified in individuals with ID syndrome (blue) and ASD (green) (de Ligt et al. 2012; O'Roak et al. 2012; Tucci et al. 2014; Kuechler et al. 2015; Kharbanda et al. 2017) alongside the mutations identified in the present study (red). The mutation in purple was identified in a child with ID syndrome but with an eye phenotype consistent with a diagnosis of FEVR (Dixon et al 2016). The amino-terminal domain (NTD) spans amino acids 1-137, 12 armadillo repeats (ARM) span amino acids 138-664, the carboxy-terminal domain (CTD) spans 667-781 and the Helix-C domain spans amino acids 667-683. All mutations are written using the standard recommended guidelines (http://varnomen.hgvs.org). The frameshift mutations reported in Kharbanda et al. 2017 (c.1038_1044delGCTATCTinsGCT; c.1038_1044delGCTATCTinsGCT; c.799_809delGAAGGAGCTAAinsGAA) have therefore been changed to fit these standards.

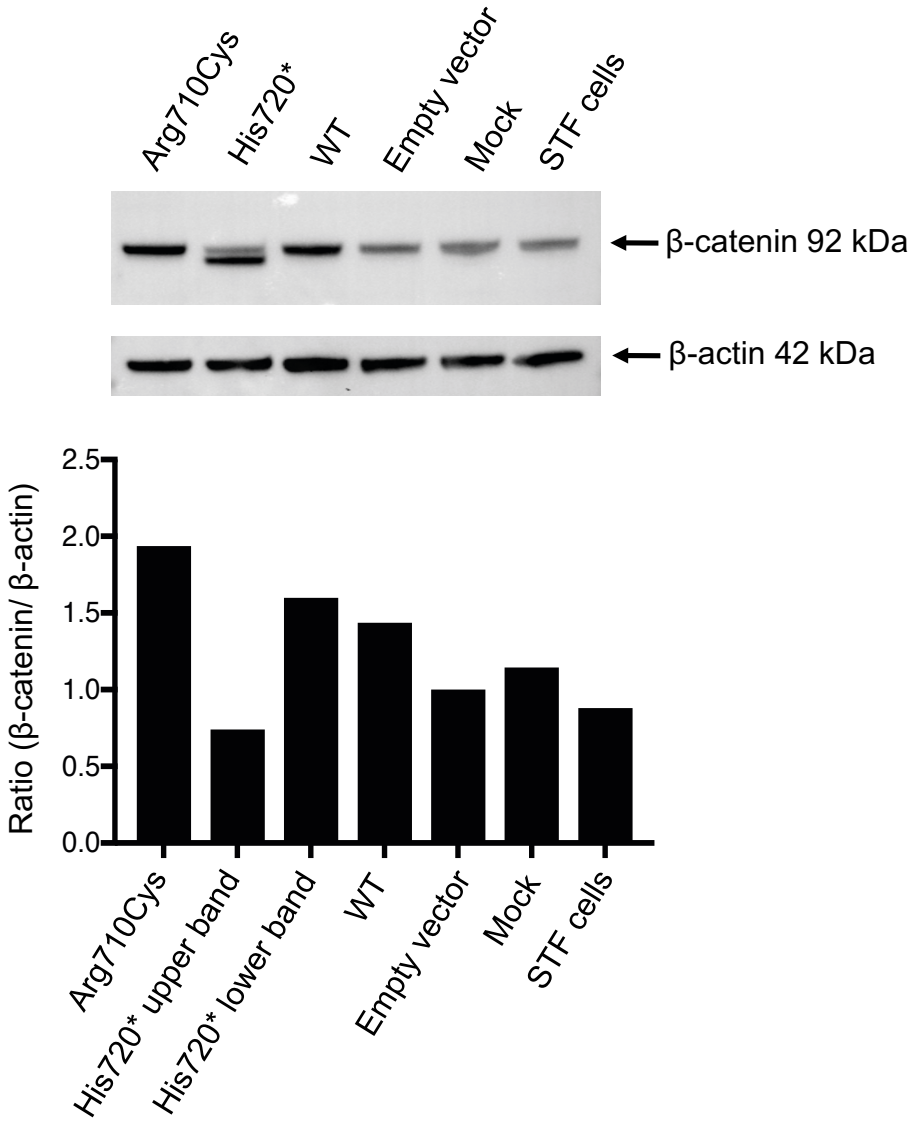

Figure S4. Western blot analysis and quantitative densitometry to confirm the expression of the wildtype and mutant β-catenin constructs. (A) Western blot of *CTNNB1* expression constructs. STF cells were transiently transfected with expression vectors for wild-type (WT) or mutant β-catenin (Arg710Cys or His720*) or empty vector (pDEST40). Mock transfection (Mock) and untreated STF cells were additional controls. Forty-eight hours after transfection, whole cell lysates were prepared and subjected to western blot analysis with anti-β-catenin polyclonal antibody (#9562 Cell Signalling Technology). Anti-β-actin monoclonal antibody (A5441 Sigma Aldrich) was used as the loading control. (B) Western band intensities were measured using Image Lab 5.2.1 (Bio-Rad) and quantified relative to the cells transfected with empty vector. Results are shown as ratios to the loading control. Note that this experiment was only performed once and the samples are not normalized for transfection efficiency but to total number of cells.

Table S1. *CTNNB1* primers for PCR/sequencing.

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)
2	CAGGTATCCCAGTGACTTAGGAG	GCAGAAAATGGAGCAAAAGG	268
3&4	TGCTTTTCTTGGCTGTCTTTC	AGTTTTCAAGTACTGGTATTGGGT	913
5&6	TGAAGTAAATGCTCAAGGGGA	CCACAACCCATTCATGGAAA	758
7	GCAAGCTGGCTGAAATTCTT	TCAGTAGTTAAAGTTCTACCACCTTTT	351
8&9	CAGAAGGACACCTCCTAAGGC	CCCTATCGCAGCCATACTTC	761
10	GCGATAGGGGTAAGATTCTGAAAT	CTCTTCAGGAAGACGGATGG	411
11	TTACGGGGAACTTCGGGTAT	TCATAAAATTAAATGTTGGTAACCC	345
12&13	TGTGAATGCCTCTTGCACTC	CAATGCAAATGAATGTGTACTAAGTG	569
14	TTGTTCCTTTTGTAATCTGAAAGTATG	CTGCCAACACTGGTTTCCC	267
15	TTTGGATGCCCTAACCTCAG	TAGCCTAAACCACTCCCACC	419

Mutation	PolyPhen2	MutationTaster	CADD*
c.1434_1435insC p.Glu479Argfs*18	N/A	Disease causing (prediction probability 1)	Score 35
c.2128C>T p.Arg710Cys	Possibly damaging (score 0.54)	Disease causing (prediction probability 0.999)	Score 25.4
c.2142_2157 dup p.His720*	N/A	Disease causing (prediction probability 1)	Score 35

Table S2. Summary of bioinformatics analyses undertaken to predict the pathogenic nature of the *CTNNB1* mutations. URLs: PolyPhen2, http://genetics.bwh.harvard.edu/pph2/ [Adzhubei, I.A. et al. (2010). Nat. Methods 7, 248-9]; Mutationtaster, http://www.mutationtaster.org/ [Schwarz, J.M. et al. (2010). Nat. Methods 7, 575-6]; Scaled CADD (Combined Annotation Dependent Depletion) scores generated using version 1.3 http://cadd.gs.washington.edu [Kircher, M. et al. (2014). Nat. Genet. 46, 310-5]. *Scaled CADD scores of 20 means that the variant is amongst the top 1% of deleterious variants in the human genome and a score of 30 means that the variant is in the top 0.1%.