Missense mutations in the WD40 domain of *AHI1* cause non-syndromic retinitis pigmentosa

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Supplementary Figures S1-S5.

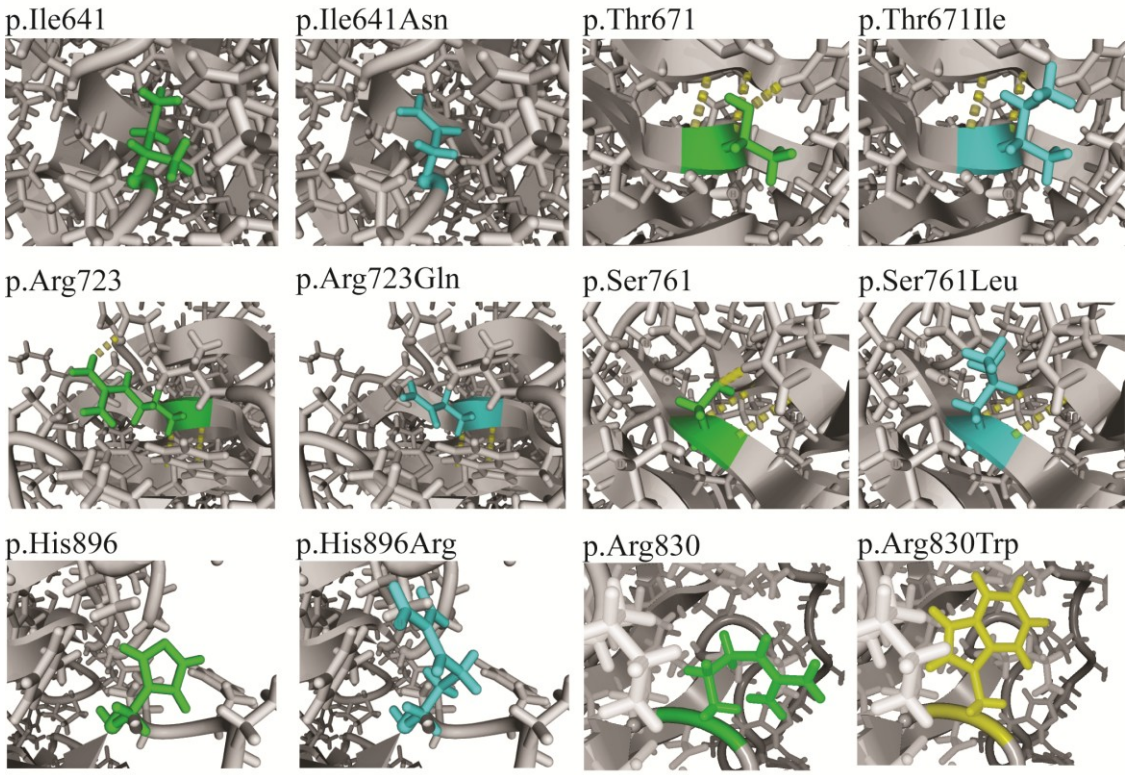

Figure S1. Predicted effect of the amino acid change of JBTS missense variants (p.Ile641Asn, p.Thr671Ile, p.Arg723Gln, p.Ser761Leu and p.His896Arg) and the only polymorphism in the WD40 domain (p.Arg830Trp) in a protein model of the WD40 domain of Jouberin. In green the wild-type amino acids, in cyan the amino acid changes of a JBTS associated missense variants and in yellow the amino acid change of a polymorphism in the WD40 domain of jouberin are depicted.

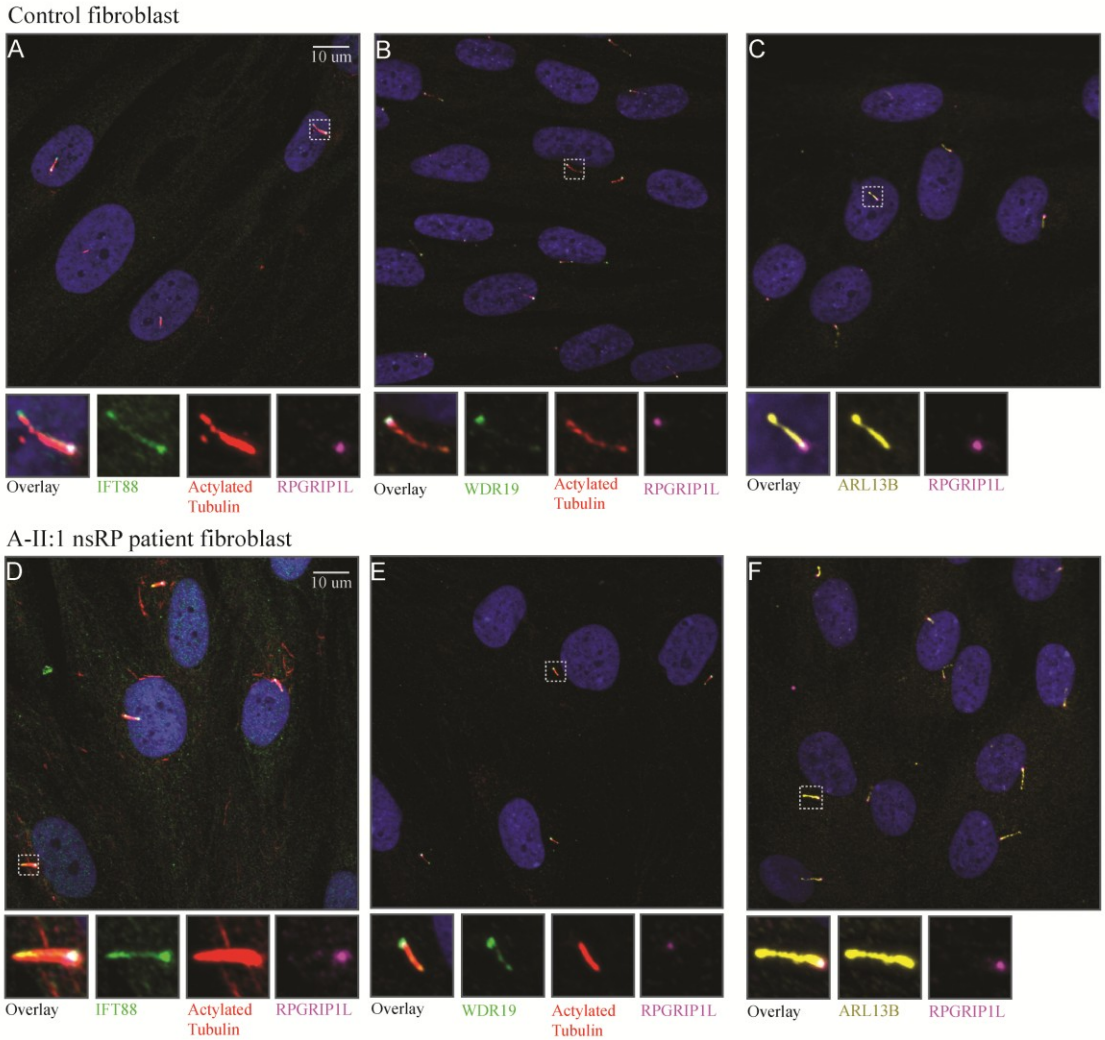

Figure S2. *AHI1* variants did not impair core ciliary parameters in patient fibroblasts. Representative images used in the analysis depicted in Figure 4 are shown. Ciliary parameters were analyzed by immunofluorescence staining of either acetylated tubulin (red) or ARL13B (yellow), marking the ciliary axoneme, RPGRIP1L (purple), marking the ciliary transition zone, the IFT-B marker IFT88 (green), and the IFT-A marker WDR19/IFT144 (green). **(A, C, E)** Control fibroblasts (CL10-00010); **(B, D, F)** Fibroblasts from RP patient A-II:1 (CL14-00043).

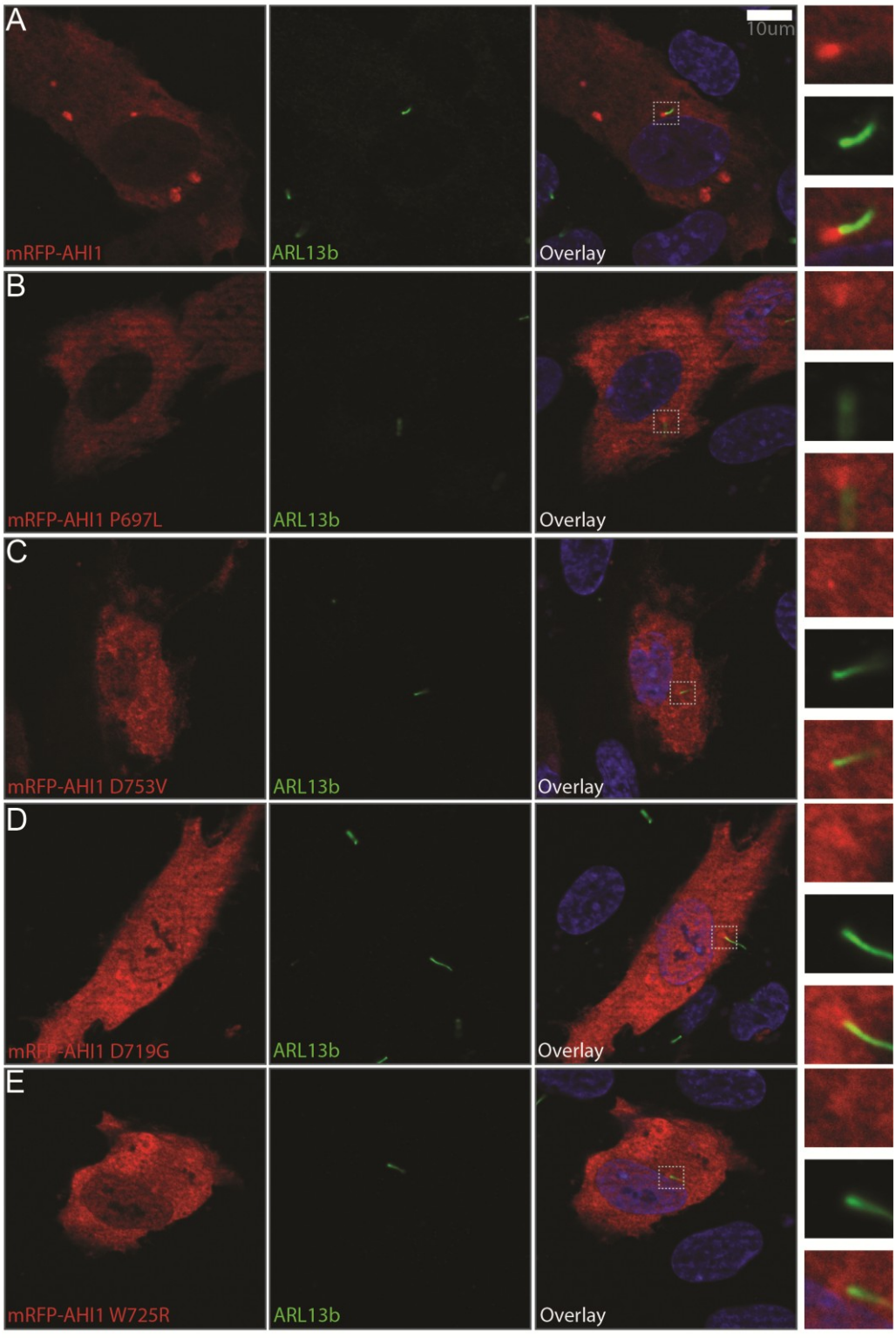

Figure S3. Localization of AHI1 wild type and mutants in hTERT-RPE1 cells. mRFP-AHI1 localized to the cytoplasm and enriched at the base of primary cilia in hTERT-RPE1 cells **(A)**. RP-associated AHI1 mutants, N-terminally fused to mRFP, also localized to the cell cytoplasm but significantly decreased their enrichment to the ciliary base **(B, C)**, similar to JBTS associated AHI1 mutants **(D, E)**. ARL13B staining was used as a marker for primary cilia, and DAPI for nuclei.

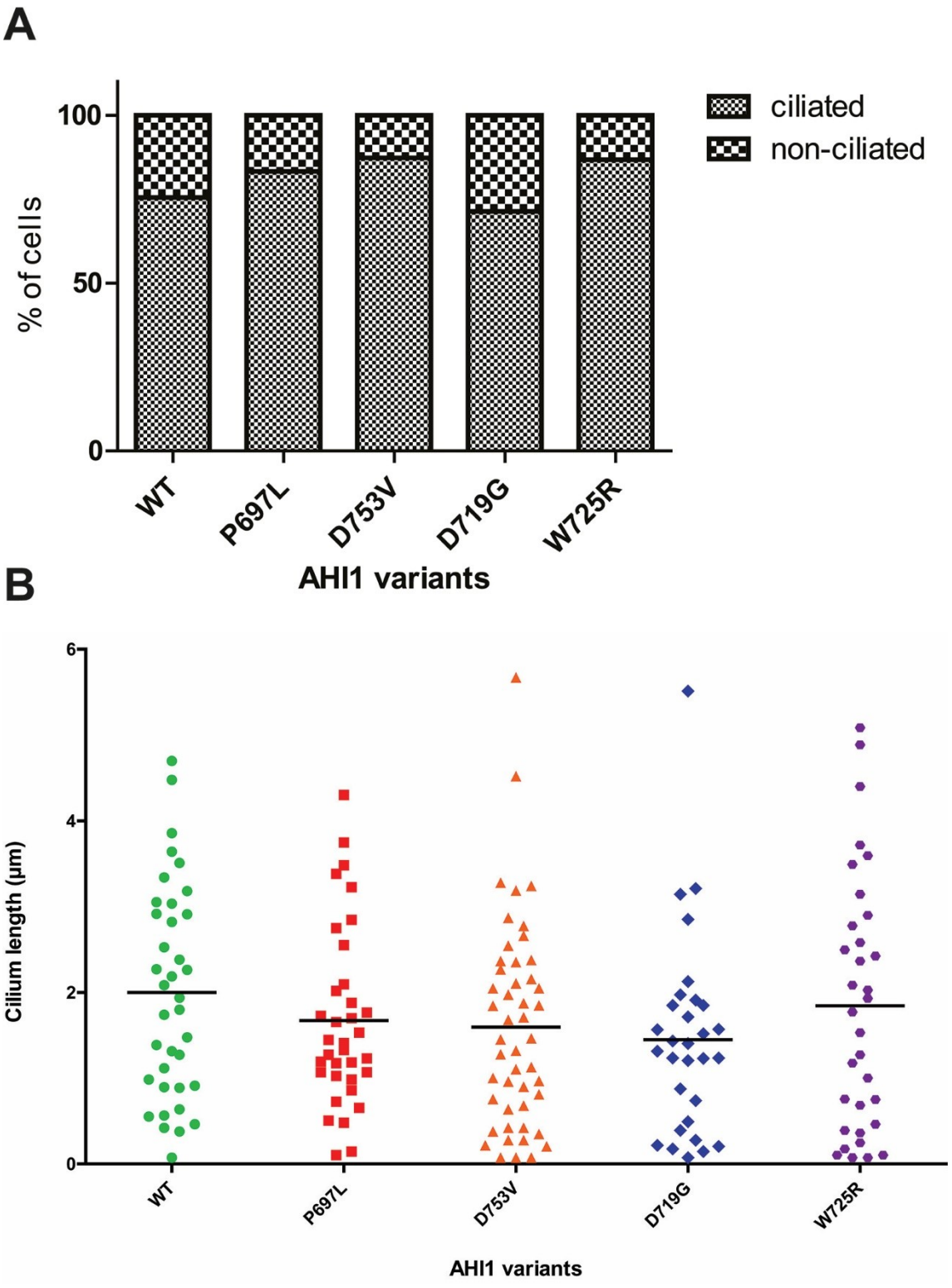

Figure S4. Ciliation in hTERT-RPE1 cells is not disturbed upon overexpression of Jouberin. **(A)** Cells in which recombinant Jouberin was expressed (WT) did not show a significantly different percentage of ciliated cells compared to the mutated recombinant proteins (variants indicated); **(B)** The length of the primary cilia in these cells also did not vary significantly, and shows an equally wide distribution with all variants as with wild-type Jouberin.

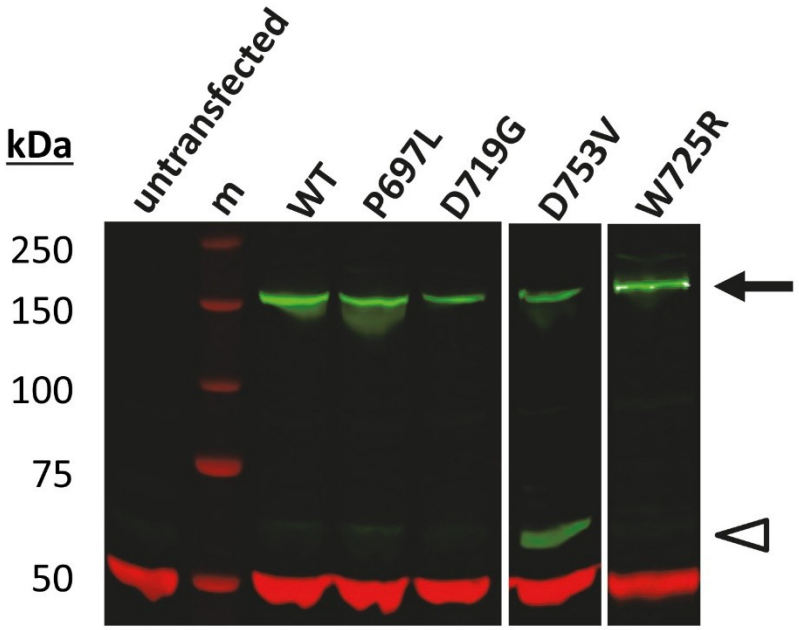

Figure S5. Validation of the correct size (162 kDa, arrow) of the mRFP-AHI1/Jouberin fusion proteins (wild-type (WT) and different indicated variants) by immunoblotting, using anti-RFP antibodies (green). Staining against anti-α-tubulin (red) was used as a loading control, m is a size marker. A protein of 60 kDa (open arrow), here more prominently visible with the p.D753V variant, likely represents a degradation product.