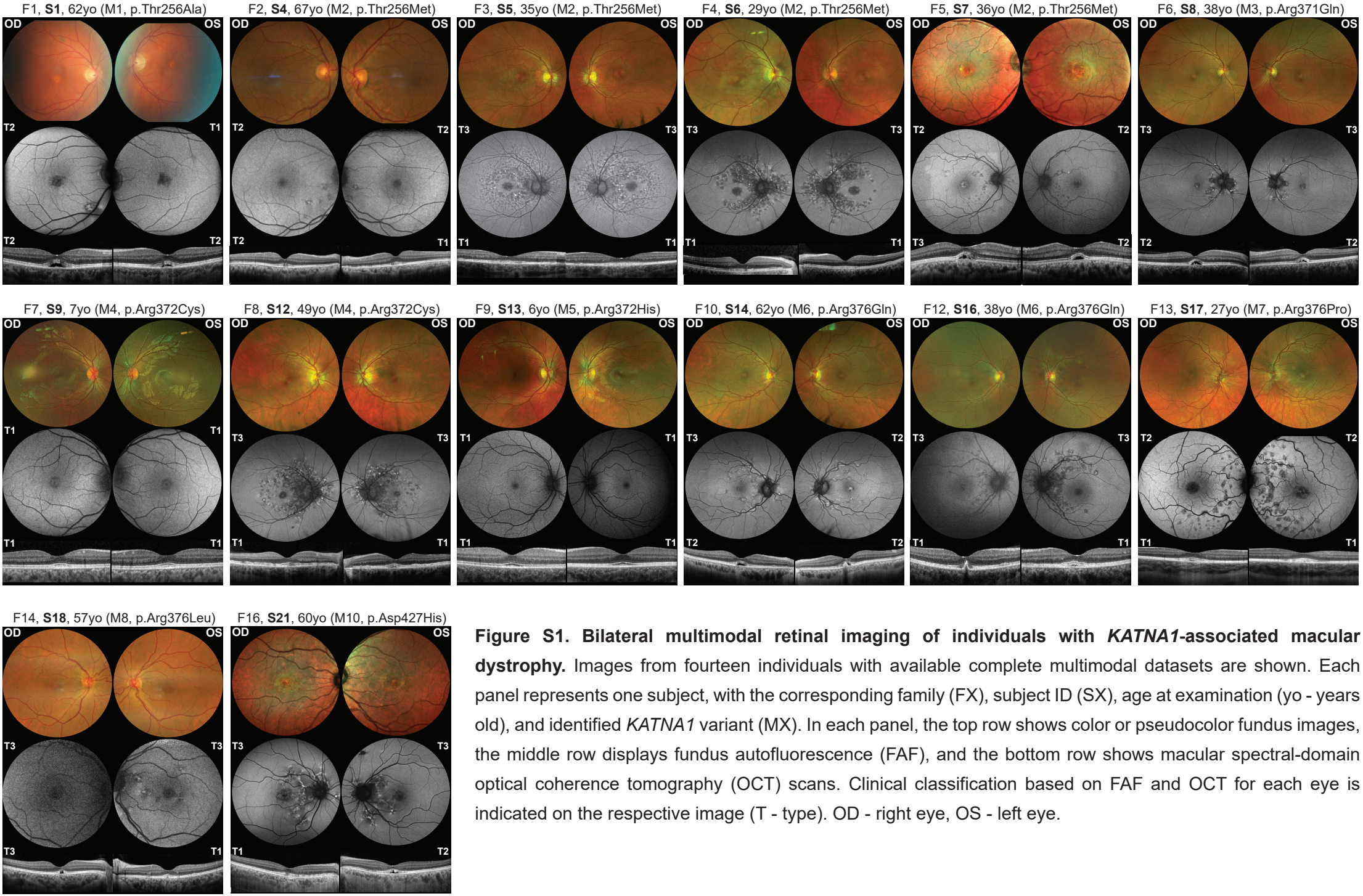

Figure S1. Bilateral multimodal retinal imaging of individuals with *KATNA1*-associated macular dystrophy. Images from fourteen individuals with available complete multimodal datasets are shown. Each panel represents one subject, with the corresponding family (FX), subject ID (SX), age at examination (yo - years old), and identified *KATNA1* variant (MX). In each panel, the top row shows color or pseudocolor fundus images, the middle row displays fundus autofluorescence (FAF), and the bottom row shows macular spectral-domain optical coherence tomography (OCT) scans. Clinical classification based on FAF and OCT for each eye is indicated on the respective image (T - type). OD - right eye, OS - left eye.

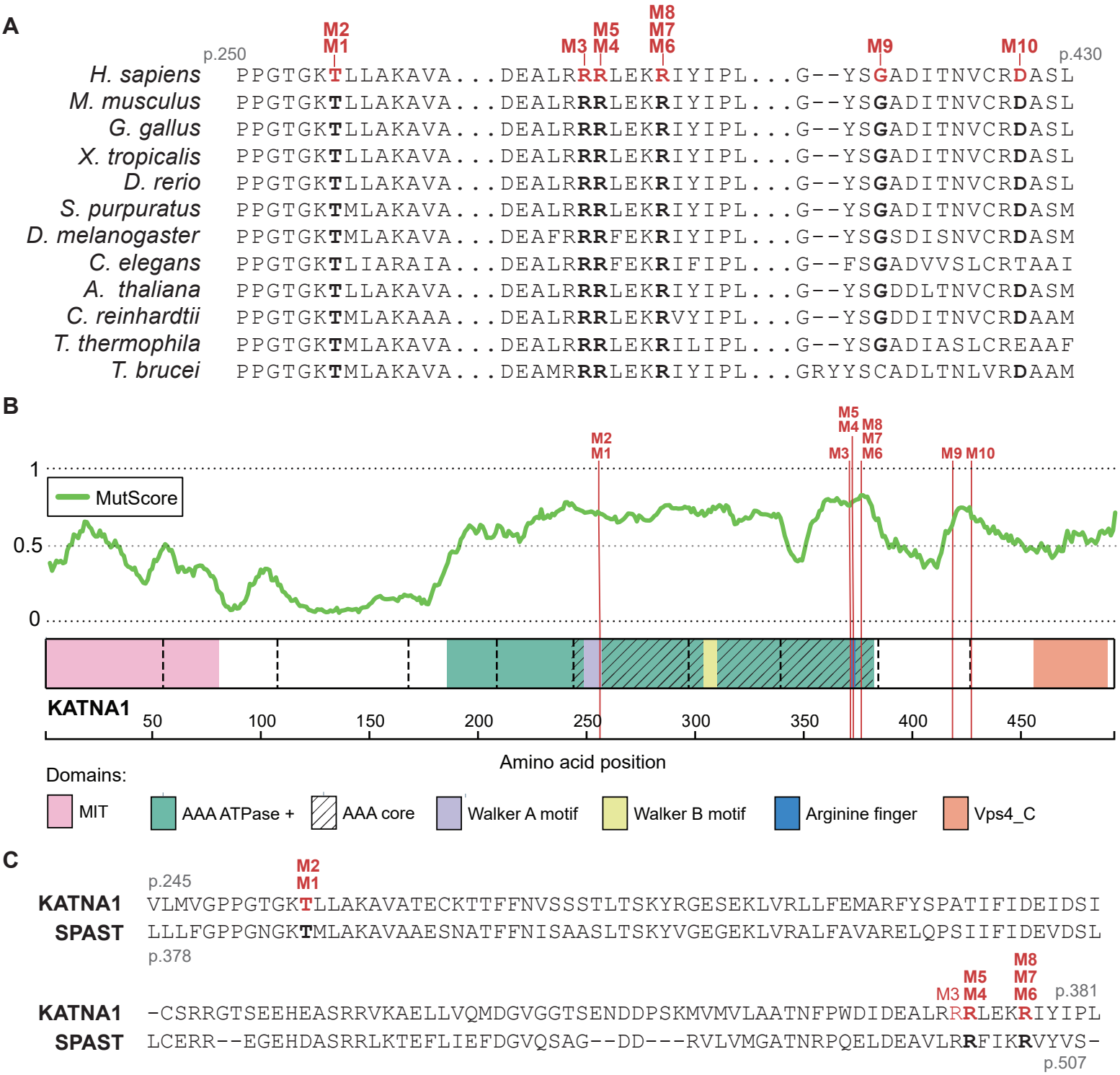

Figure S2: Conservation and homology of the identified KATNA1 variants. A. Sequence alignment of parts of KATNA1 with orthologues from vertebrates, invertebrates, plants, and unicellular organisms. Variants detected in this study (M1-M10) are shown in red, and the corresponding conserved residues across species are highlighted in bold. All missense changes affect residues that are either fully or highly conserved across all organisms. **B.** Mutational constraint across the full KATNA1 protein, shown as a plot of running MutScore average per ten amino acids (green line; range 0-1, where 1 indicates the highest predicted deleteriousness). Domain organization of KATNA1 is depicted below with color-coded features. Protein positions of the variants identified in this study are marked by red lines. **C.** Alignment of the AAA core domain (Pfam ID: PF00004) sequences of KATNA1 and SPAST. KATNA1 variants identified in this study within this domain (M1-M8) are shown in red, and homologous residues in SPAST with reported pathogenic mutations are marked in bold.

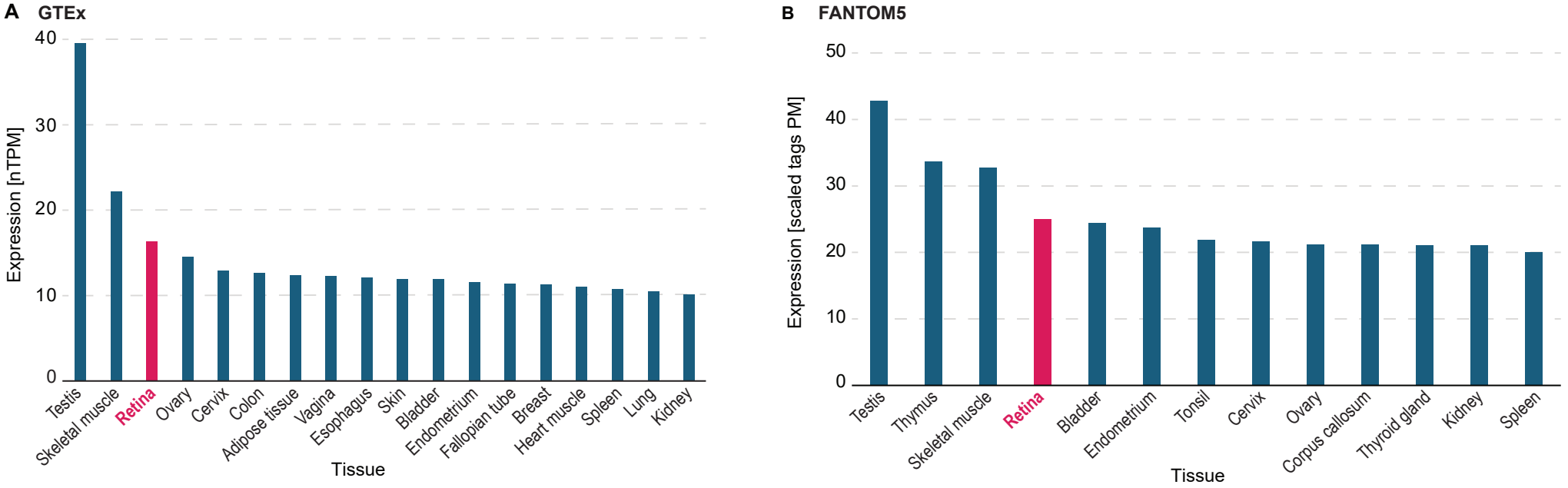

Figure S3: Expression of *KATNA1* in human tissues. Expression values across selected human tissues and organs from the Human Protein Atlas (bulk RNAseq), based on the GTEx (**A**) and FANTOM5 (**B**) datasets. Only tissues with *KATNA1* expression ≥10 nTPM (normalized transcripts per million, A) or ≥20 scaled tags PM (B) are shown.

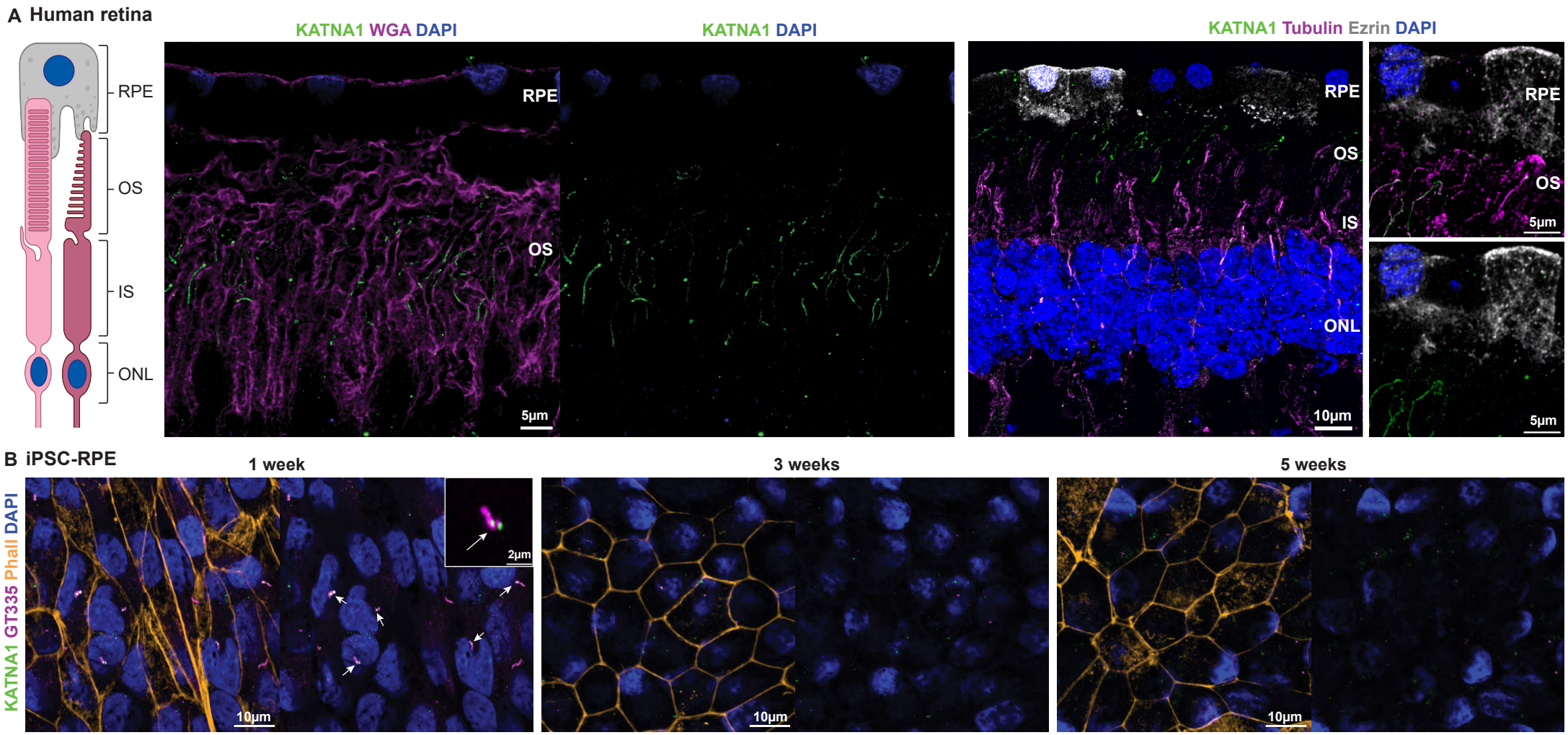

Figure S4: Immunofluorescence detection of KATNA1 in human retina and iPSC-RPE cells. A. Schematic representation of the photoreceptor and RPE layers of the retina. Human retina sections with preserved RPE were stained with KATNA1 (green) together with WGA (magenta, left panels), or tubulin (magenta), and Ezrin (grey) (right panels). KATNA1 immunoreactivity was detected in the outer segments (OS) of photoreceptors, with no specific signal observed in the RPE layer. WGA highlights rod OS, while Ezrin labels the microvilli of the RPE. Nuclei were counterstained with DAPI (blue). Scale bars are indicated in the respective panels. Abbreviations: RPE – retinal pigment epithelium; OS – photoreceptor outer segments, IS – inner segments; ONL – outer nuclear layer. **B.** Monolayers of iPSC-derived RPE cells, analyzed at 1, 3, and 5 weeks of maturation. Staining included KATNA1 (green), GT335 (magenta, labeling glutamylated tubulin), phalloidin (orange, marking the actin cytoskeleton), and DAPI (blue). At week one, arrows highlight retained primary cilia co-stained with GT335 and KATNA1, consistent with early RPE maturation. At later stages (3 and 5 weeks), cells acquired mature epithelial organization, characterized by prominent actin outlining hexagonal cell borders, and no specific KATNA1 signal was observed. Scale bars are indicated in the respective panels.