

Supplementary Figure 1: Prolonged IFN- β signaling causes retinal degeneration

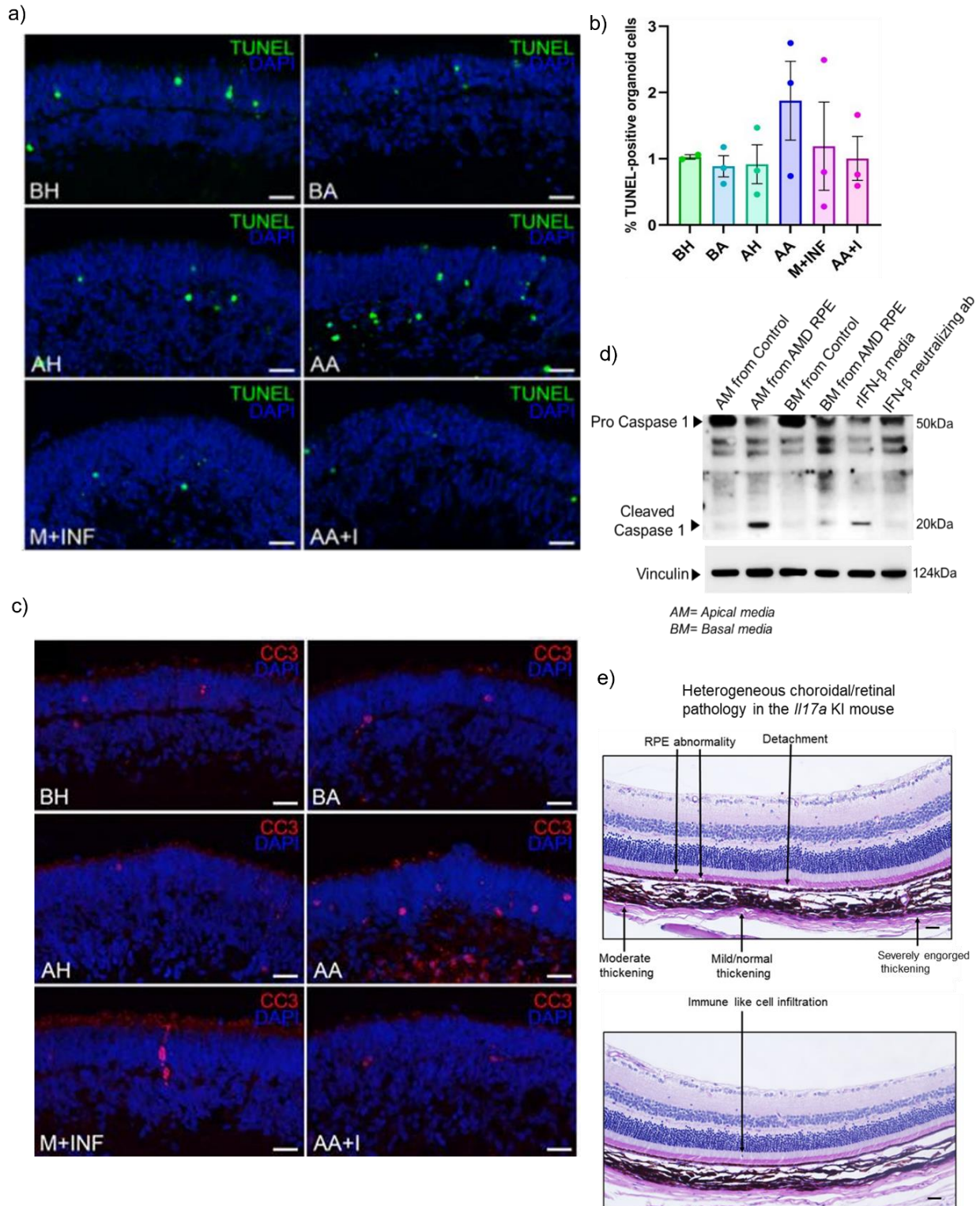


Figure S1: Prolonged IFN- β signaling causes photoreceptor death and retinal degeneration. a) TUNEL staining of retinal organoids after 72 h of treatment with conditioned media: BH (basal media from healthy individuals), BA (basal media from AMD patients), AH (apical media from healthy individuals), AA (apical media from AMD patients), M+INF (regular media with recombinant IFN- β), and AA+I (apical media from

AMD patients pretreated with IFN- β antibodies). b) Quantification of TUNEL-positive cells in retinal organoids after 72 h of treatment (n = 3). c) Immunofluorescence showing cleaved caspase-3 (CC3) expression in retinal organoids treated with conditioned media. Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; CC3, cleaved caspase-3; d) Western blot showing increased pyroptotic cleaved caspase-1 expression in D180 hiPSC-derived retinal organoids upon exposure to apical media from human AMD iPSC-RPE or media supplemented with recombinant IFN- β . Neutralization with anti-IFN- β antibody prevented the increased cleaved caspase-1 expression upon exposure to apical media from human AMD iPSC-RPE media. e) H&E staining of retinal tissue showing the heterogenous choroidal and retinal pathology observed in the *Il17a*-KI mice

Supplementary Figure 2: **Gene set enrichment analysis (GSEA) interferon in all retinal cells** identified an upregulation of type I

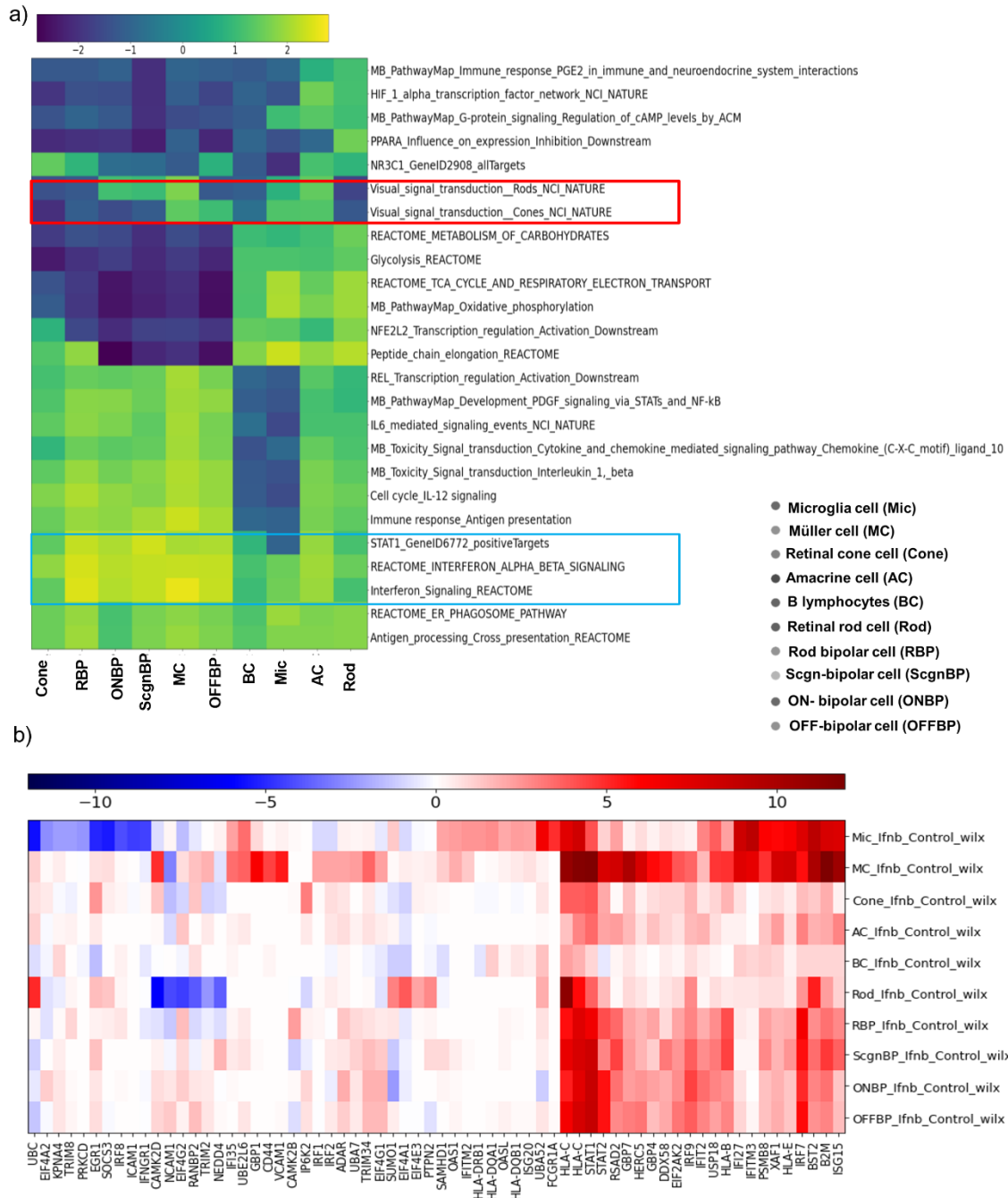


Figure S2: GSEA indicated a global upregulation of type I interferon responses in all retinal cells. a) Among REACTOM enriched pathways, interferon signaling was upregulated across annotated retinal cells (blue box). Down-regulated visual signaling (red box) was observed in photoreceptors, bipolar and B cells (n=4). b) The heat map of wilcoxon test statistical values genes in the interferon signaling network were upregulated across retinal cells, especially in Müller and bipolar cells (n=4).

Supplementary Figure 3. **scRNAseq analysis showed visual transduction genes reduced in rods and Müller cells of IFN- β -AAV2 induced (10 weeks) mouse eyes.**

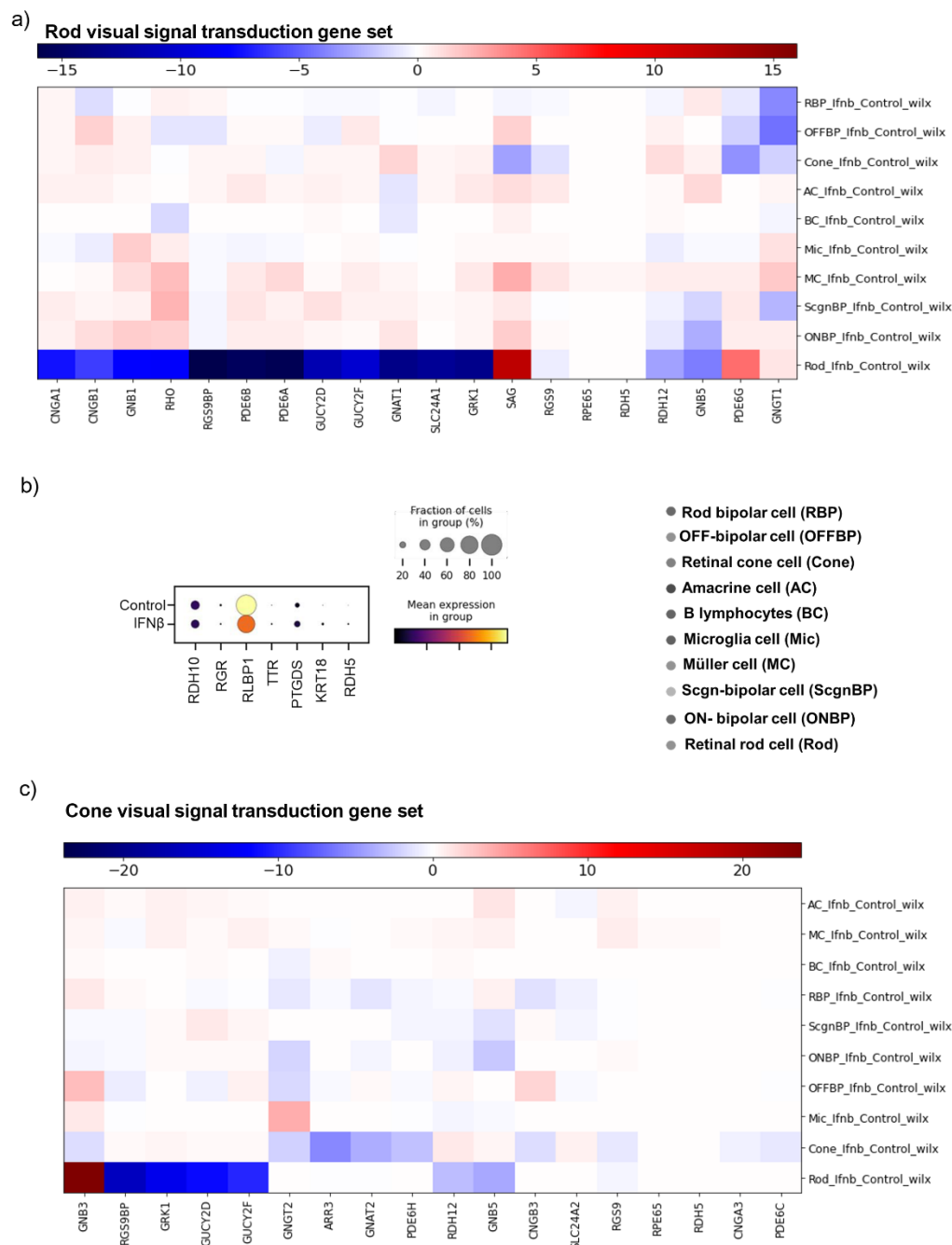


Figure S3: scRNA-seq analysis revealed a reduction in visual transduction gene expression in rod photoreceptors and Müller glia of IFN- β -AAV2-treated mouse eyes at 10 weeks. a) Rod visual signal transduction genes, including *RPE65* and *RDH5*, displayed in a heat map of Wilcoxon test statistical values, showed lower expression in rods of IFN- β -AAV2 induced eyes. b) Müller cell group from the scRNA analysis (Fig. 4b) shows a reduction of *CRALBP1/RLBP1* gene in IFN- β -AAV induced eyes compared with Null-AAV

eyes; however, no changes were observed in other retinal metabolism genes. c) Cone visual signal transduction gene set showed minimal changes with IFN- β stimulation, indicating a rod-specific response upon IFN- β induction in RPE (n=4).

Supplementary Figure 4. **Chronic IFN- β expression in mouse eyes leads to significant RPE degeneration by 20 weeks**

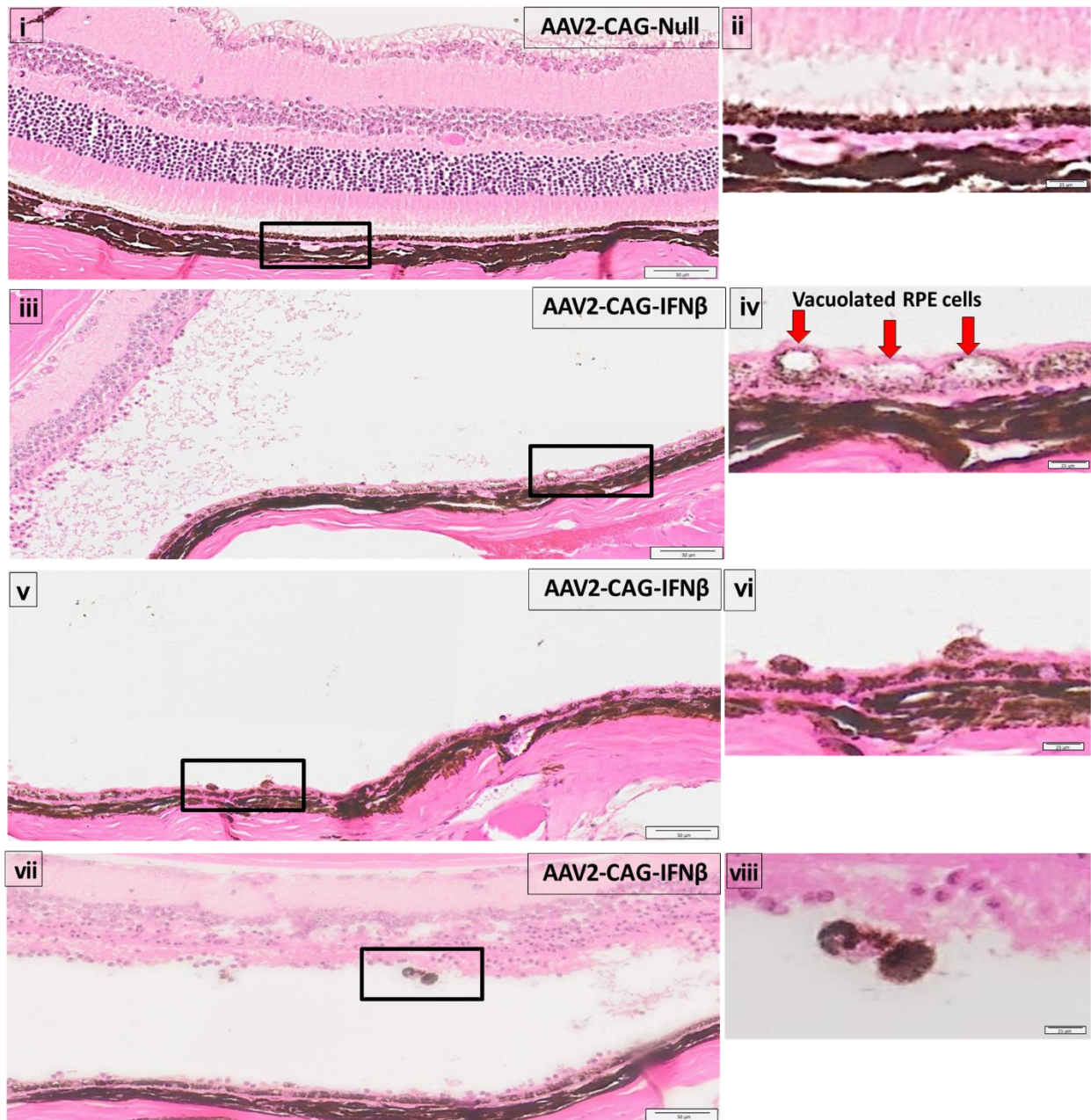


Figure S4: H&E staining displayed profound retinal and RPE degeneration in IFN- β induced mouse eyes at 20 weeks post AAV injection. a) Comparing with AAV2-CAG-Null mouse eyes, AAV2-CAG-IFN-induced eyes demonstrated degeneration of the photoreceptor layer (i, ii). AAV2-CAG-IFN induced eyes showed swollen RPE cells with vacuolated cytoplasm (iii, iv), pigment-laden cells attached to the surface of RPE cells (v, vi) and (vii, viii) and in the sub-retinal space (vii, viii) and intra-retinal microcysts (n=5 per group). Scale bar= 50 μ m (Zoomed insets= 25 μ m).

Supplementary Figure 5. Chronic IFN- β stimulation of primary human RPE (7 days) reduces RPE cellularity, inflammation, increases senescence and its pharmacological inhibition lowers key NF- κ B-driven proinflammatory cytokines.

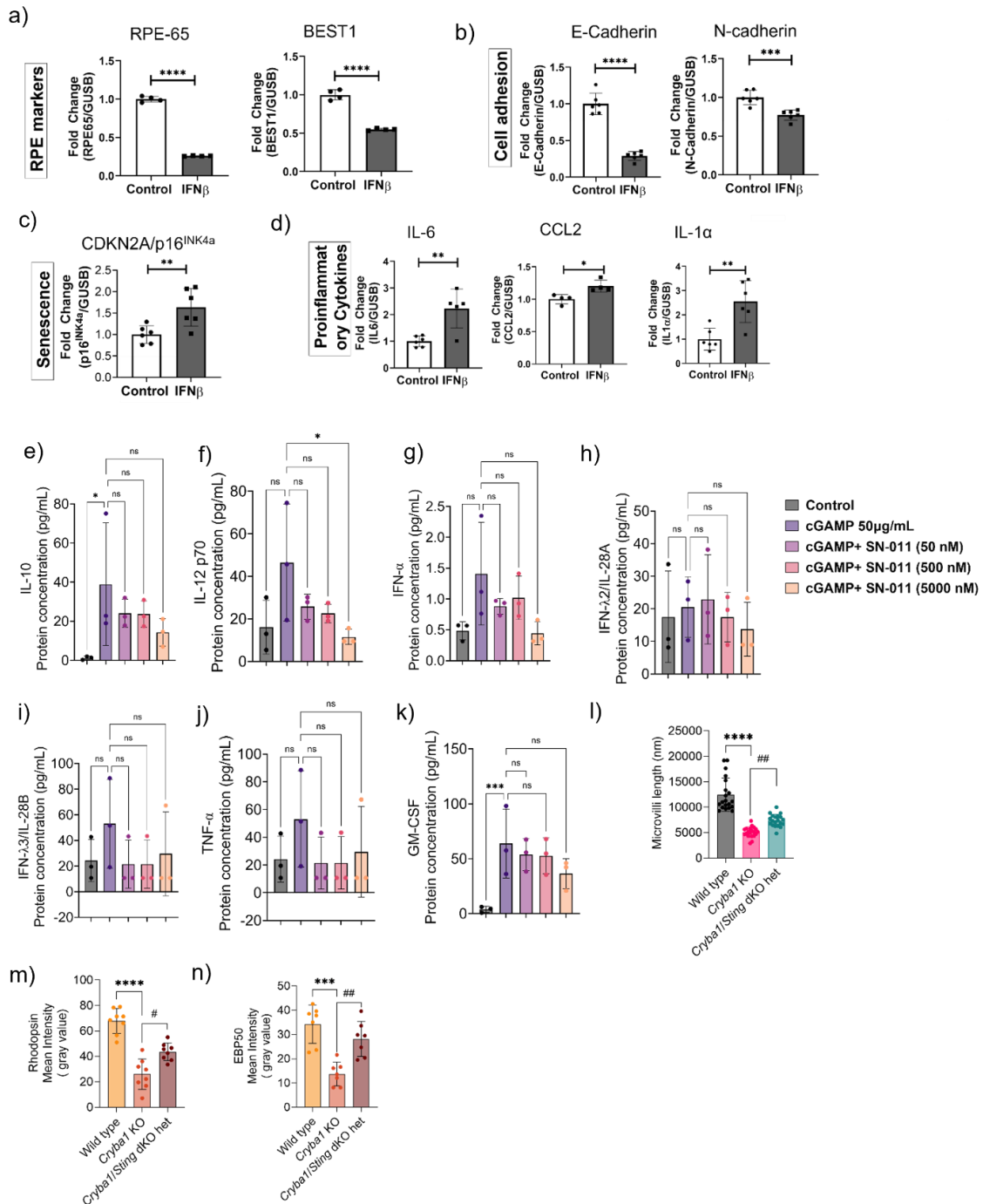


Figure S5: Chronic IFN- β stimulation of primary human RPE cells for 7 days leads to reduced RPE cellularity, increased senescence, and enhanced secretion of senescence-associated secretory phenotypes (SASP). a) Gene expression analysis of human RPE cells exposed to IFN- β (5000 IU/mL) for 7 days revealed a

reduction in RPE-specific markers *RPE65* and *BEST1* (n=4). b) Alterations in cell adhesion molecule expression were observed, with decreased E-cadherin and N-cadherin levels upon IFN- β induction (n=6). c) Expression of the senescence marker CDKN2A/p16^{INK4a} was elevated (n=6). d) Proinflammatory cytokines IL-6, CCL2, and IL-1 α were upregulated. Mature human RPE (hRPE) cells were treated with 50 μ g/mL cGAMP and varying concentrations of the STING inhibitor SN-011 for 24 h (n=6). Supernatants were collected and cytokine levels (pg/mL) were quantified using a Luminex assay: e) IL-10, f) IL-12p70, g) IFN- α , h) IFN- λ 2/IL-28A, i) IFN- λ 3/IL-28B, j) TNF- α and k) GM-CSF. cGAMP increased the levels of all cytokines, and the effect of cGAMP was blunted by the STING inhibitor SN-011. Values represent mean $\pm$ s.d from n=3 independent experiments. Statistical analyses were performed using an unpaired *t*-test (for comparisons between two groups) or two-way ANOVA with Dunnett's multiple comparisons test (for more than two groups). ns= not significant. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. l) Bar graphs represent phalloidin microvilli length in Wild type, *Crybal* KO and *Crybal/Sting* dhet retinal sections, which was significantly lower in *Crybal* KO mice, compared to wild type. But was rescued in age-matched dhet mice, relative to *Crybal* KO, indicating rescue of structural changes in the dhet retina. n=3. mean microvilli length measured at six regions per section $\pm$ s.d. (n = 3). Statistical analysis was performed using the Kruskal–Wallis test. *****p* < 0.0001, ## *p* < 0.01. Mean immunofluorescence intensity of m) rhodopsin n) EBP50 measured in Wild type, *Crybal* KO and *Crybal/Sting* dhet retinal sections shows significant rescue in the levels of both proteins in dhet retina compared to *Crybal* KO, indicating towards rescue of both photoreceptor loss and RPE apical changes upon partial *Sting* deletion and *Crybal* upregulation. Values represent the mean intensity $\pm$ s.d. (n = 3). Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparisons test. *****p* < 0.0001, ****p* < 0.001, # *p* < 0.05.