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Supplemental information

IGF1 deficiency integrates stunted growth and neurodegeneration in Down syndrome

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES AND LEGENDS

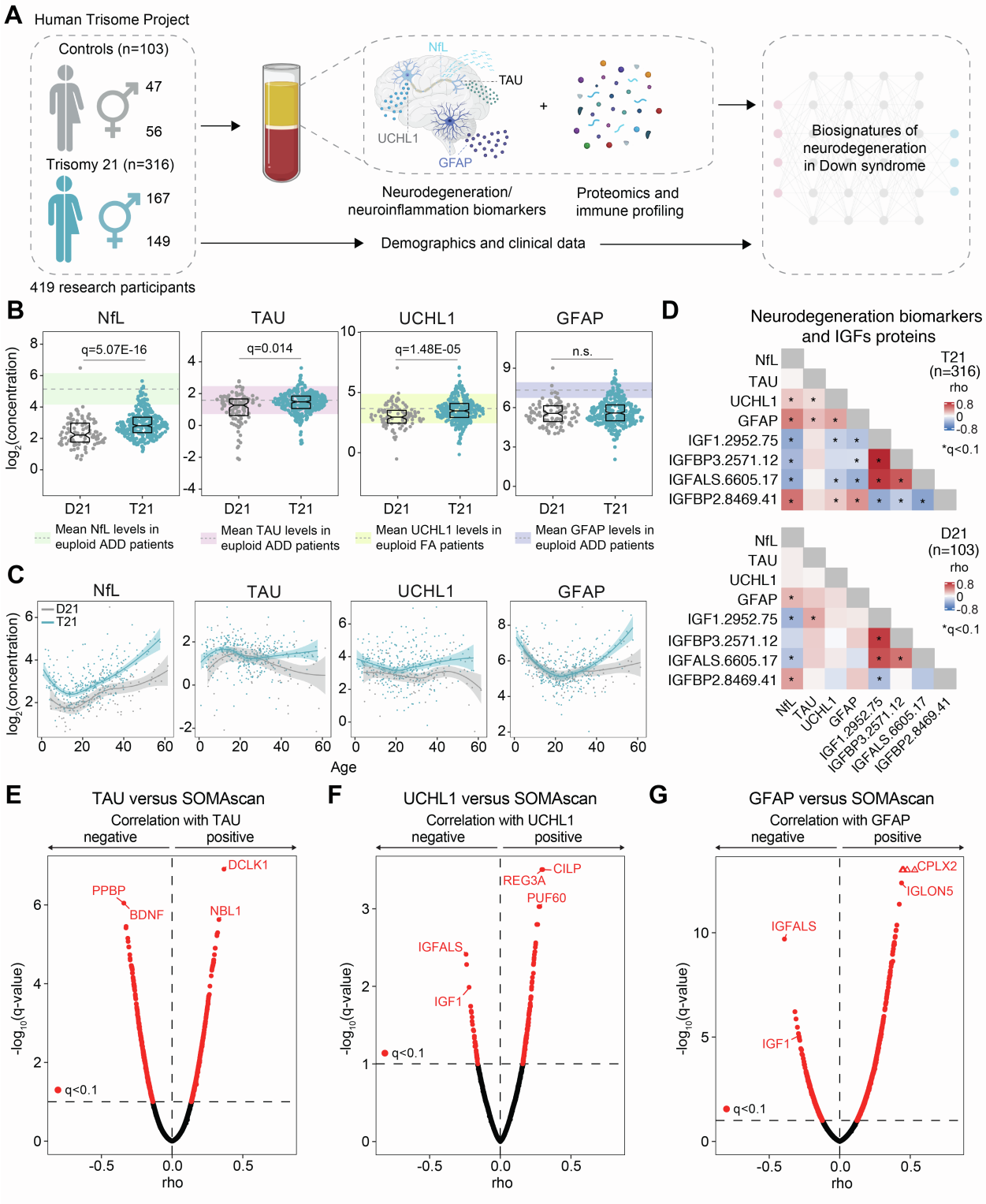

Figure S1. Multi-omics analysis of biosignatures associated with neurodegeneration in Down syndrome, related to Figure 1. (**A**) Overview of experimental approach. Levels of

neurodegeneration/neuroinflammation biomarkers were measured in plasma samples from 419 research participants, 316 of them with trisomy 21 (T21) versus 103 age- and sex-matched euploid controls (D21) using SIMOA® technology. Matched plasma proteomics data was generated via SOMAscan® proteomics and Meso Scale Discovery (MSD) technology. Data was then analyzed to define biosignatures of neurodegeneration. (**B**) Sina plots displaying levels of neurodegeneration biomarkers in individuals with and without T21. Data are presented as sina plots with boxes indicating median and interquartile range. Differences between groups were determined with a multivariable linear regression with age, sex, and source as covariables, with Benjamini-Hochberg (BH) correction of p-values and an estimated FDR 10%, q<0.1. Dashed lines indicate the mean levels of each neurodegeneration/neuroinflammation biomarker in diverse studies of Alzheimer's disease and other neurodegenerative conditions in the general population[S1-S4], with shaded areas representing the standard deviation. ADD: Alzheimer's disease dementia. FA: Friedreich Ataxia. (**C**) LOESS plots displaying the age trajectories of neurodegeneration/neuroinflammation biomarkers in individuals with and without Down syndrome. (**D**) Heatmap showing the correlation between neurodegeneration/neuroinflammation biomarkers and IGF1, IGFBP3, IGFALS, and IGFBP2 in individuals with T21 (top) and euploid controls (D21, bottom). Values displayed represent rho values from Spearman correlations and asterisks indicate a significant correlation after multiple hypothesis correction (q<0.1, BH method). (**E-G**) Volcano plots for Spearman correlations between circulating TAU (**E**), UCHL1 (**F**), and GFAP (**G**) levels versus proteins measured by SOMAscan®. The horizontal dashed lines indicated the statistical cutoff of q<0.1. Features passing the statistical cutoff in the left and right quadrants are highlighted in red.

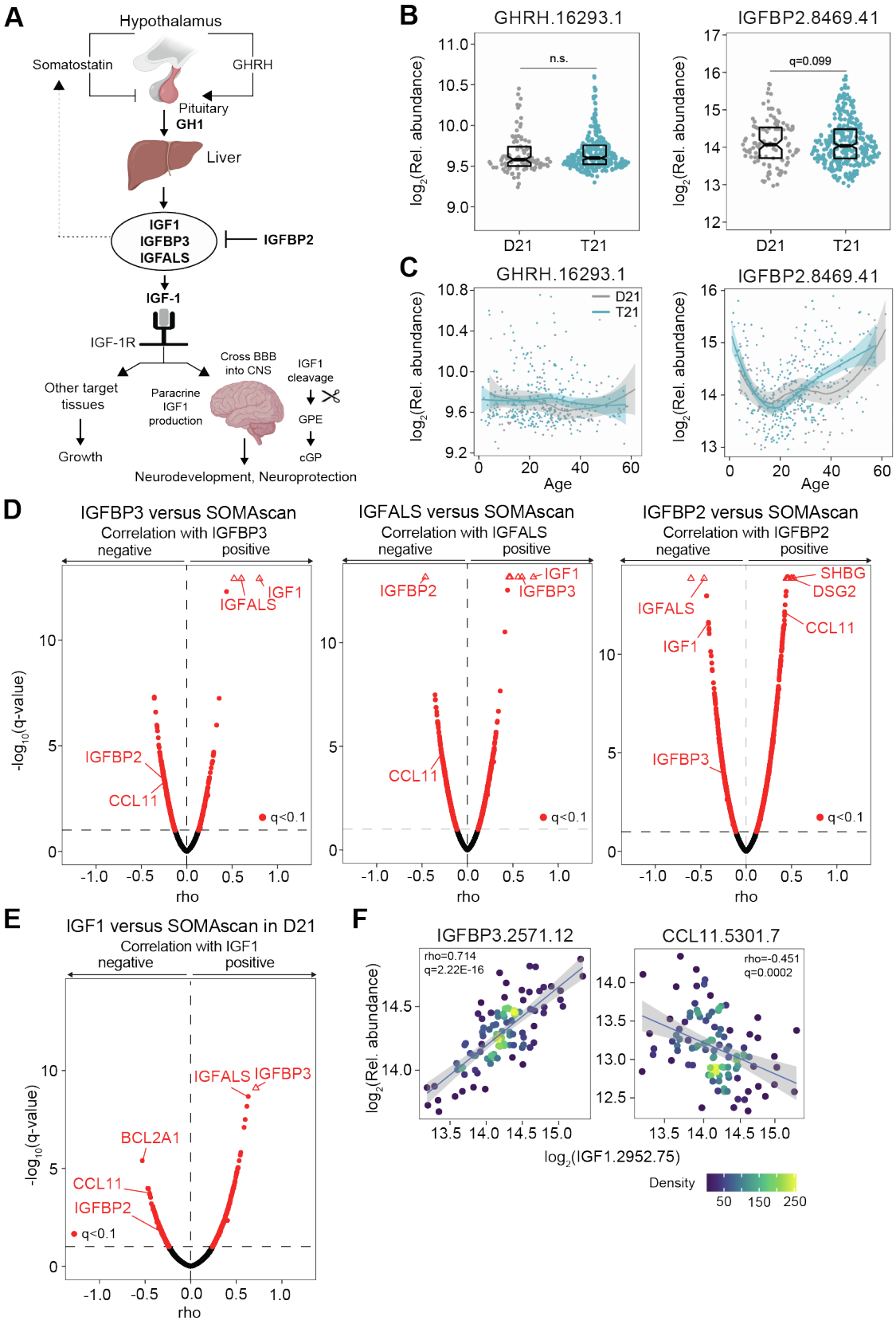

Figure S2. Trisomy 21 disrupts the GH1/IGF1 signaling pathway across the lifespan, related to Figure 2. Levels of factors in the GH1/IGF1 signaling pathway were measured in plasma samples from

419 research participants, 316 of them with trisomy 21 (T21) versus 103 age- and sex-matched euploid controls (D21) using SOMAscan® proteomics technology. (**A**) Schematic representation of the GH1/IGF1 signaling pathway. Hepatic production of IGF1 occurs in response to GH1 secretion from somatotrophs in the anterior pituitary. GHRH released from the hypothalamus stimulates pituitary GH1 release, while IGF1 and somatostatin can inhibit GH release. IGFBPs have high affinity for IGF1 and modulate its stability and bioavailability. IGFBP2 is a major antagonist of IGF1. IGFBP3 and IGFALS bind to IGF1 to form the IGF1 ternary complex found in circulation, enhancing the effects of IGF1. IGF1 signals mostly through the IGF1R present in different tissues to promote growth but can also cross the blood-brain barrier (BBB) to promote brain development and function. In the CNS, IGF1 is proteolytically cleaved at its N-terminus, leading to production of the tripeptide Glycine-Proline-Glutamate (GPE), which is further metabolized into cyclic Glycine-Proline (cGP), both of which exhibit neuroprotective effects independent of the IGF1R. (**B**) Sina plots showing levels of GHRH and IGFBP2 in individuals with and without T21. Data are presented as sina plots with boxes indicating median and interquartile range. Differences between groups were determined with a multivariable linear regression with age, sex, and source as covariables, with Benjamini-Hochberg (BH) correction of p-values and an estimated FDR of 10%, $q<0.1$. (**C**) Age trajectory plots with line from simple LOESS regression showing levels of GHRH and IGFBP2 in individuals with and without T21. (**D**) Volcano plots for Spearman correlations between circulating IGFBP3, IGFALS, and IGFBP2 levels versus proteins measured by SOMAscan® in individuals with DS. The horizontal dashed lines indicated the statistical cutoff of $q<0.1$. Features passing the statistical cutoff in the left and right quadrants are highlighted in red. (**E**) Volcano plots for Spearman correlations between circulating IGF1 levels versus SOMAscan® proteins in D21. The horizontal dashed line indicates the statistical cutoff of $q<0.1$. Features passing the statistical cutoff in the left and right quadrants are highlighted in red. (**F**) Scatter plots showing levels of IGFBP3 and CCL11 (Eotaxin) proteins correlated with levels of IGF1 among euploid controls (D21). Values shown represent rho values from Spearman correlations and q-values calculated with BH method. Points are colored by density. Lines represent a simple linear regression with 95% confidence interval. Abbreviations: GH1 - Growth Hormone 1; GHRH – Growth Hormone releasing hormone; IGF1 –

Insulin-Like Growth Factor 1; IGFBPs – Insulin-Like Growth Factor Binding Proteins; ALS: Acid Labile Subunit; IGF1R - Insulin-Like Growth Factor 1 receptor; BBB – Blood Brain Barrier; CNS: central nervous system; GPE - Glycine-Proline-Glutamate; cGP cyclic Glycine-Proline.

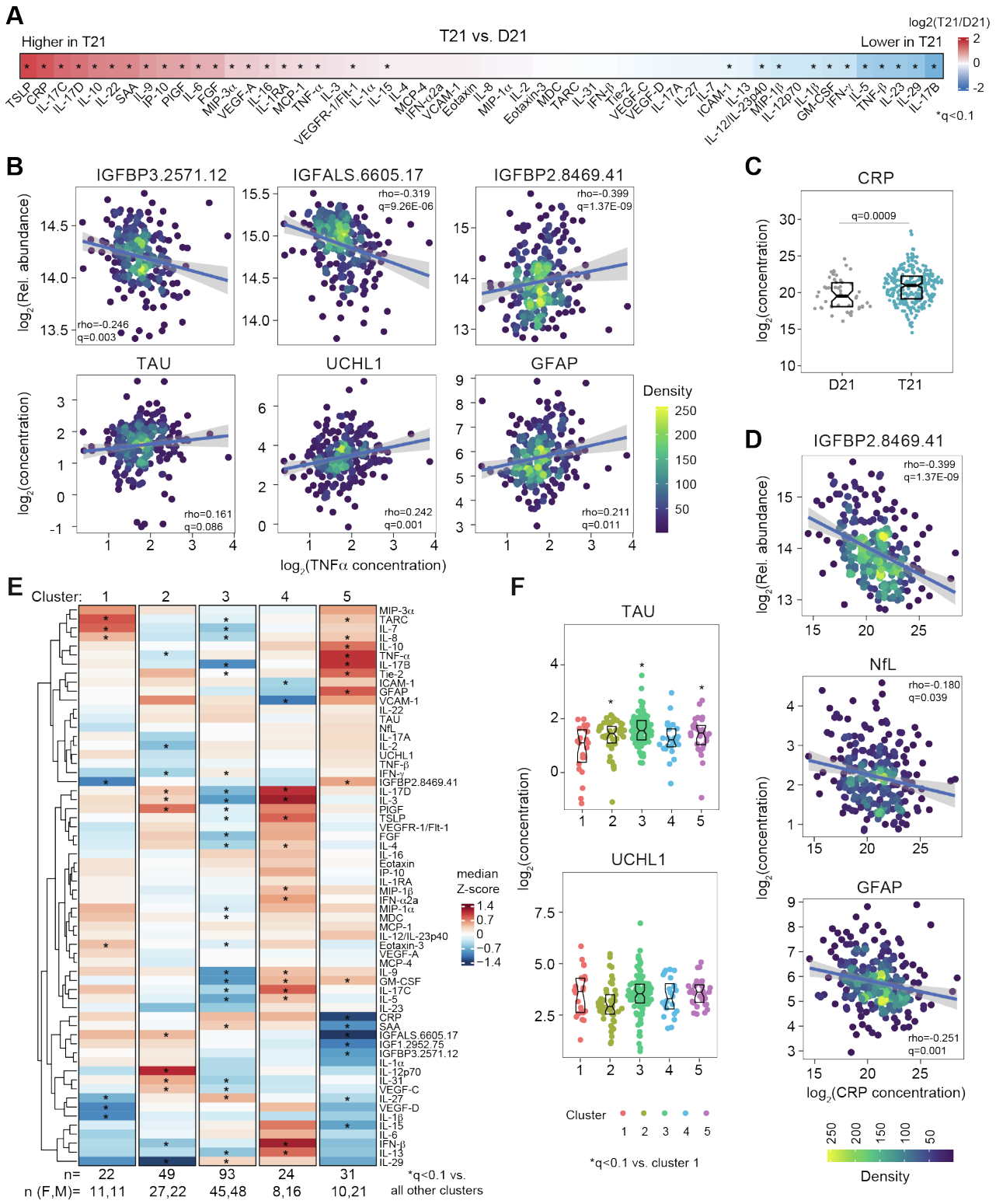

Figure S3. Interplay between inflammation, IGF1 deficiency, and neurodegeneration markers in Down syndrome, related to Figure 3. 54 immune factors were measured with Meso Scale Discovery (MSD) technology in plasma samples from 309 research participants, 259 of them with trisomy 21 (T21) versus 50 age- and sex-matched euploid controls (D21) for whom matched measurements of IGF1

signaling factors and neurodegeneration markers were available. (**A**) Heatmap displaying the differences between the circulating levels of immune factors in individuals with T21 compared with typical controls. Differences between T21 and D21 were determined with a multivariable linear regression with age, sex, and source as covariables with Benjamini-Hochberg (BH) correction of p-values and an estimated FDR 10%, q<0.1. (**B**) Scatter plots showing the adjusted values for levels of indicated proteins correlated with levels of TNFα among individuals with T21. Values shown represent rho values from Spearman correlations and q-values (BH method). Points are colored by density. Lines represent a simple linear regression with 95% confidence interval. (**C**) Sina plots displaying levels of CRP in individuals with and without T21. Data are presented as sina plots with boxes indicating median and interquartile range. Differences between groups were determined with a multivariable linear regression with age, sex, and source as covariables with BH correction of p-values and an estimated FDR 10%, q<0.1. (**D**) Scatter plots showing the adjusted values for levels of indicated proteins correlated with levels of CRP among individuals with T21. Values shown represent rho values from Spearman correlations and q-values (BH method). Points are colored by density. Lines represent a simple linear regression with 95% confidence interval. (**E**) Heatmap displaying median Z-scores for cytokines/chemokines, four neurodegeneration biomarkers, and plasma proteins in each of 5 consensus clusters identified among 259 participants with T21. Total number of participants and numbers of males and females are listed at the bottom of the heatmap. Asterisks indicate q<0.1 (10% FDR) for Mann-Whitney tests for differences in the levels of various factors in each individual cluster versus all other clusters combined. (**F**) Sina plots displaying distributions of indicated proteins across the five clusters identified in **Figure S3E**. Asterisks indicate q<0.1 (10% FDR) for Mann-Whitney tests of each cluster against Cluster 1. Boxes in sina plots represent interquartile ranges and medians, with notches approximating 95% confidence intervals.

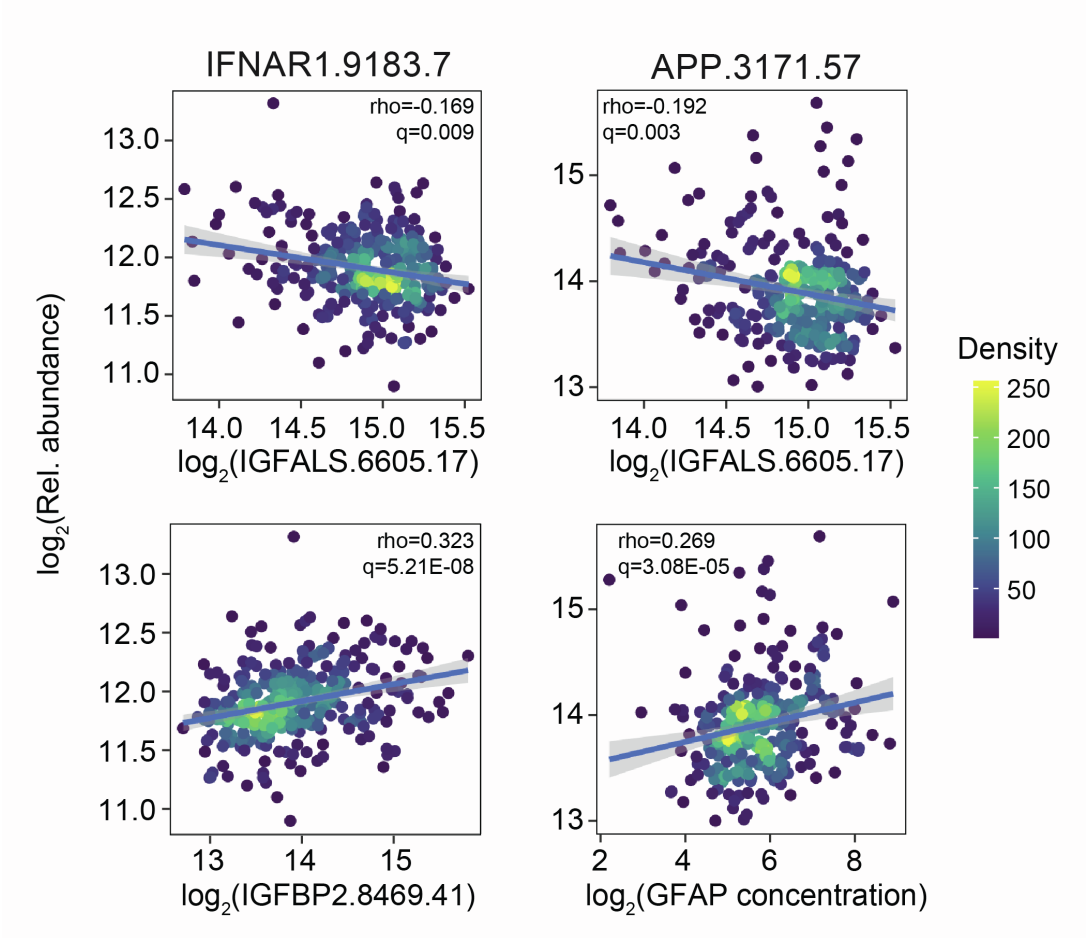

Figure S4. Interplay between overexpression of chr21 proteins, IGF1 deficiency and markers of neurodegeneration in Down syndrome, related to Figure 4. Levels of proteins encoded on chromosome 21 (chr21) were measured in plasma samples from 419 research participants, 316 of them with trisomy 21 (T21) versus 103 age- and sex-matched euploid controls (D21) using SOMAscan® proteomics technology. Scatter plots showing the adjusted values for levels of indicated proteins encoded on chr21 (IFNAR1, APP) correlated with levels of IGFALS, IGFBP2, and/or GFAP among individuals with Down syndrome. Values shown represent rho values from Spearman correlations, q-values (q<0.1, BH method), and points are colored by density. Lines represent a simple linear regression with 95% confidence interval.

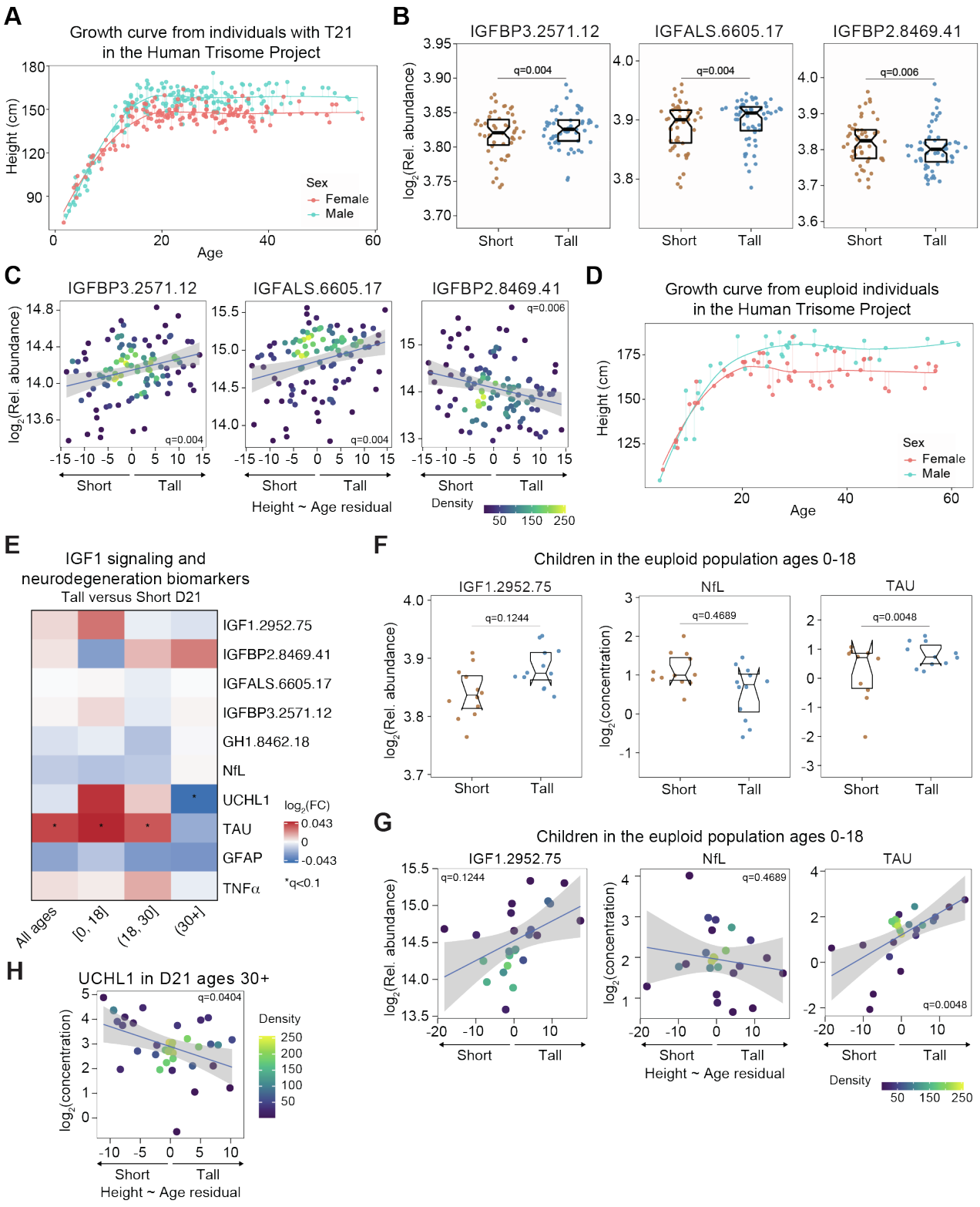

Figure S5. Analysis of stunted growth, IGF1 deficiency, and markers of neurodegeneration in Down syndrome, related to Figure 5. Relationship between stature, IGF1 signaling, and neurodegeneration was explored in 314 individuals with Down syndrome (DS). (**A**) Growth curve

showing the height versus age LOESS curve from females (n=148, red) and males (n=166, turquoise) with DS in the Human Trisome Project. (**B**) Sina plots displaying unadjusted levels of IGFBP3, IGFALS and IGFBP2 in short and tall individuals with trisomy (T21) (n=50-51 and n=55-56, respectively). Data are presented as sina plots with boxes indicating median and interquartile range. Differences between groups were determined with a multivariable linear regression with age, sex, and source as covariables with correction of p-values using the Benjamini-Hochberg method (BH). Significance is defined as FDR 10%, q<0.1. (**C**) Scatter plots showing levels of IGFBP3, IGFALS and IGFBP2 associated with the Tall and Short groups in individuals with T21. Values shown represent rho values from Spearman correlations. q-values were calculated with the BH method. Points are colored by density. Lines represent a simple linear regression with 95% confidence interval. (**D**) Growth curve showing the height versus age LOESS curve from euploid (D21) females (n=53, red) and males (n=40, turquoise) in the Human Trisome Project. (**E**) Heatmap representing differences in levels of GH1/IGF1 signaling proteins, the indicated neurodegeneration/neuroinflammation biomarkers, and TNFα per unit of residual value from the Height ~ Age LOESS regression in euploid controls (D21). Values displayed are log2 fold change of concentration levels per unit of residual and asterisks indicate a significant correlation after multiple hypothesis correction with the BH method (FDR10%, q<0.1). (**F**) Sina plots showing the levels of IGF1, NfL and TAU in short and tall children (0-18) in the euploid cohort (D21) (Short, n= 12 versus Tall, n= 12). Data are presented as sina plots with boxes indicating median and interquartile range. Differences between groups were determined as in (B). (**G**) Scatter plots for levels of IGF1, NfL and TAU versus the residuals from the Height ~ Age LOESS regression in children with DS. q-values were calculated as in (E). Points are colored by density. Lines represent a simple linear regression with 95% confidence interval. (**H**) Scatter plots showing the levels of UCHL1 in short and tall older adults (30+) in the euploid cohort (D21) (Short, n= 14 versus Tall, n= 19). q-value calculated as in (C). Points are colored by density. Lines represent a simple linear regression with 95% confidence interval.

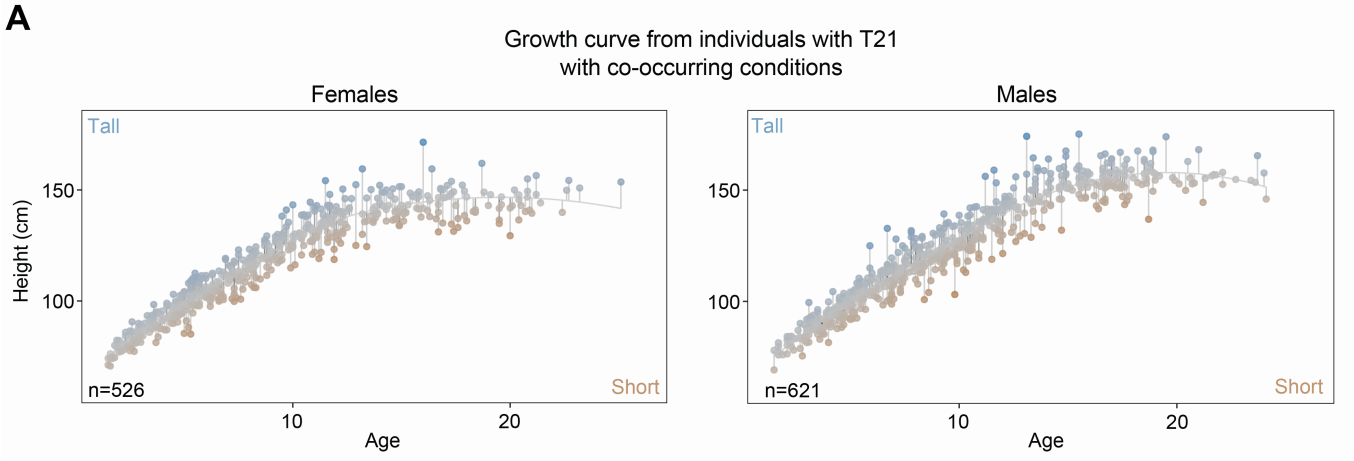

Figure S6. Analysis of stunted growth versus prevalence of co-occurring conditions in Down syndrome, related to Figure 6. Associations between stature and co-occurring conditions was evaluated in individuals with trisomy 21. (**A**) Growth curve showing the height versus age LOESS curve from females (n=526, left) and males (n=621, right) with Down syndrome from the Anna and John J. Sie Center for Down Syndrome (SCDS) at Children's Hospital Colorado (CHCO).

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