

Tau Protein Modulates an Epigenetic Mechanism of Cellular Senescence

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19 Abstract

20 Progressive Tau deposition in neurofibrillary tangles and neuropil threads is the hallmark of
21 tauopathies, a disorder group that includes Alzheimer's disease. Since Tau is a microtubule-
22 associated protein, a prevalent concept to explain the pathogenesis of tauopathies is that abnormal
23 Tau modification contributes to dissociation from microtubules, assembly into multimeric β -sheets,
24 proteotoxicity, neuronal dysfunction and cell loss. Tau also localizes in the cell nucleus and evidence
25 supports an emerging function of Tau in DNA stability and epigenetic modulation. To better
26 characterize the possible role of Tau in regulation of chromatin compaction and subsequent gene
27 expression, we performed a bioinformatics analysis of transcriptome data obtained from Tau-
28 depleted human neuroblastoma cells. Among the transcripts deregulated in a Tau-dependent manner,
29 we found an enrichment of target genes for the polycomb repressive complex 2. We further describe
30 decreased cellular amounts of the core components of the polycomb repressive complex 2 complex
31 and a lower histone 3 trimethylation activity in Tau deficient cells. Among the de-repressed
32 polycomb repressive complex 2 target gene products, IGFBP3 protein was found to be linked to
33 increased senescence induction in Tau-deficient cells. Our findings propose a mechanism for Tau-
34 dependent epigenetic modulation of cell senescence, a key event in pathologic aging.

35 1 Introduction

36 Tau pathology is the hallmark of tauopathies, a neurodegenerative disorder group that includes
37 Alzheimer's disease (AD), where progressive Tau deposition in neurofibrillary tangles and neuropil
38 threads correlates with a deteriorating clinical course (Long and Holtzman, 2019). Autosomal dominant
39 mutations in the *MAPT* gene encoding for Tau lead to a relatively small group of frontotemporal lobar
40 degenerations (FTLD-Tau), which are classified among frontotemporal dementia (Josephs, 2018)
41 diagnosed mostly at 45-65 years of age (Hutton et al., 1998, Spillantini et al., 1998). With Tau being a
42 microtubule-binding protein, a prevalent concept to explain the pathogenesis of tauopathies is that
43 abnormal Tau modification e.g., phosphorylation and folding, contributes to Tau dissociation from
44 microtubules, assembly into multimeric β -sheets, proteotoxicity, neuronal dysfunction and cell loss
45 (Jeganathan et al., 2006, Ludolph et al., 2009). In addition to its well-characterized role in
46 neurodegeneration, studies reporting a correlation between *MAPT* gene products and survival in
47 various types of tumors endorse an implication of Tau in cancer (Papin and Paganetti, 2020, Gargini
48 et al., 2020, Gargini et al., 2019, Cimini et al., 2022, Rossi et al., 2018). The mechanisms underlying
49 these findings may involve microtubule-unrelated Tau functions.

50 Tau exerts non canonical functions e.g., it localizes in the cell nucleus and binds DNA (Cross et al.,
51 1996, Greenwood and Johnson, 1995, Loomis et al., 1990, Thurston et al., 1996). Heat or oxidative
52 stress cause nuclear translocation of Tau, which may favor its role in DNA protection (Sultan et al.,
53 2011). Neurons knocked-out for *MAPT* display enhanced DNA damage (Violet et al., 2014), and
54 induced DNA damage correlates with nuclear translocation and dephosphorylation of Tau (Ulrich et
55 al., 2018). Chromosomal abnormalities in AD fibroblasts (Rossi et al., 2008) and frequent DNA
56 damage in AD brains (Lovell and Markesbery, 2007, Mullaart et al., 1990), both reinforce the emerging
57 function of Tau in DNA stability. Tau depletion also modulates the induction of apoptosis and cell
58 senescence in response to DNA damage by a mechanism involving P53, the guardian of the genome
59 (Sola et al., 2020). Additional functions of Tau in epigenetic modulation were also reported (Rico et
60 al., 2021). Upon binding to histones, Tau stabilizes chromatin compaction (Montalbano et al., 2021,
61 Frost et al., 2014, Rico et al., 2021, Montalbano et al., 2020) and affects global gene expression during
62 the neurodegenerative process (Frost et al., 2014, Klein et al., 2019). A meta-analysis of dysregulated
63 DNA methylation in AD identified over hundreds genomic sites in cortical regions (Shireby et al.,
64 2022); growing to thousands when looking at the dentate gyrus of oldest old patients (Lang et al.,
65 2022).

66 DNA and histone modification is an effective mechanism to regulate gene activity. Hence, with the
67 aim to investigate a possible participation of Tau in gene expression, we performed a bioinformatics
68 analysis of transcriptome data obtained from Tau-depleted human neuroblastoma cells. Among the
69 transcripts deregulated in a Tau-dependent manner, we found an enrichment of target genes for the
70 Polycomb Repressive Complex 2 (PRC2), a result confirmed by decreased PRC2 protein and histone
71 3 (H3) methylation in Tau deficient cells. Notably, among the de-repressed gene products, Insulin
72 Growth Factor Binding Protein 3 (IGFBP3) was linked to senescence induction. Our findings propose
73 a Tau-driven mechanism for epigenetic modulation of cell senescence, a key event in pathologic aging.

74 2 Materials and methods

75 2.1 Cell culture

76 Human SH-SY5Y cells (94030304, Sigma-Aldrich) were cultured in complete Dulbecco's
77 Modified Eagle Medium (61965-059, Gibco) supplemented with 1% non-essential amino acids
78 (11140035, Gibco), 1% penicillin-streptomycin (15140122, Gibco) and 10% fetal bovine serum (FBS;

79 10270106, Gibco). Cells were grown at 37°C with saturated humidity and 5% CO₂ and maintained in
80 culture for less than one month. *MAPT* knock-out cells were as described (Sola et al., 2020).

81 **2.2 RNAseq**

82 Total RNA extraction with the TRIzol™ Reagent (15596026, Invitrogen) was done according to
83 the instructions of the manufacturer. Extracted RNA was processed with the NEBNext Ultra
84 Directional II RNA library preparation kit for Illumina and sequenced on the Illumina NextSeq500
85 with single-end, 75 base pair long reads. The overall quality of sequencing reads was evaluated using
86 a variety of tools, namely FastQC (Wingett and Andrews, 2018), RSeQC (Wang et al., 2012), AfterQC
87 (Chen et al., 2017) and Qualimap (García-Alcalde et al., 2012). Sequence alignments to the reference
88 human genome (GRCh38) was performed using STAR (v.2.5.2a) (Dobin et al., 2013). Transcript
89 expression was quantified at gene level with the comprehensive annotations v27 release of the Gene
90 Transfer File (GTF) made available by Gencode (Harrow et al., 2012). Raw-counts were further
91 processed in the R Statistical environment and downstream differential expression analysis was
92 performed using the DESeq2 pipeline (Love et al., 2014). Transcripts characterized by low mean
93 normalized counts were filtered out by the Independent Filtering feature embedded in DESeq2 (alpha
94 = 0.05). The RNA-Seq data have the accession no. E-MTAB-8166 and were uploaded on
95 [https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-8166?key=64a67428-adb9-4681-](https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-8166?key=64a67428-adb9-4681-99c9-98910b78ed4c)
96 [99c9-98910b78ed4c](https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-8166?key=64a67428-adb9-4681-99c9-98910b78ed4c). The genes differentially expressed between WT and Tau-KO cells (734) were
97 used to interrogate a possible gene-set enrichment utilizing the transcription tool of the EnrichR portal
98 (Chen et al., 2013, Kuleshov et al., 2016, Xie et al., 2021).

99 **2.3 Pseudoviral particle production and transduction**

100 Pseudolentiviral particles were produced by transient transfection of HEK293FT cells with 2 µg of
101 the pSIF-H1-puro-IGFBP3 shRNA-2 or of the control plasmid pSIF-H1-puro-luciferase shRNA and 8
102 µg of the feline immunodeficiency virus (FIV) packaging plasmid mix (pFIV-34N & pVSV-G); all
103 plasmids were kindly provided by Prof. Yuzuru Shiio (Greehey Children's Cancer Research Institute,
104 University of Texas). Cell conditioned medium was collected 2 days after transfection and cleared by
105 centrifugation at 300 g for 5 min, 4°C. Pseudo-lentiviruses were 20-fold concentrated with centrifugal
106 filters (MWCO 30 kDa, UFC903024, Amicon) at 3'000 g for 30-45 min, 4°C, aliquoted and stored at
107 -80°C.

108 Human neuroblastoma SH-SY5Y cells (1 x10⁵) were seeded into a 24-well plate coated with poly-D-
109 lysine (p6407, Sigma-Aldrich) one day before pseudo-lentiviral particle transduction. One day after
110 transduction, cells were supplemented with fresh complete medium and selected in the presence of 2.5
111 µg/mL puromycin (P8833, Sigma-Aldrich) for two weeks.

112 **2.4 Drugs and cell treatments**

113 Treatment of SH-SY5Y cells with Tazemetostat (CAS No. 1403254-99-8, S7128, Selleckchem)
114 was performed at 10 µM for four days starting from a 10 mM stock solution in DMSO; vehicle 0.1%
115 DMSO was added to the controls.

116 **2.5 Western blot and immune precipitation**

117 For direct analysis by western blot, total lysates from cells cultured in 6-well plates were prepared
118 in 50 µL of SDS-PAGE sample buffer (1.5% SDS, 8.3% glycerol, 0.005% bromophenol blue, 1.6% β-
119 mercaptoethanol and 62.5 mM Tris pH 6.8) and incubated for 10 min at 100°C. 15 µL per lane of the
120 sample was loaded on SDS-polyacrylamide gels (SDS-PAGE).

121 For immune isolation, the cells were rinsed with PBS and lysed on ice in 100 μ L of AlphaLisa Lysis
122 Buffer (AL003, PerkinElmer) supplemented with protease and phosphatase inhibitor cocktails (S8820
123 & 04906845001, Sigma-Aldrich). Cell lysates were treated with benzonase (707463, Novagen) for 15
124 min at 37°C, centrifuged at 20'000 g for 10 min at 4°C and supernatants were collected as cell extracts.
125 These latter were diluted in HiBlock buffer (10205589, PerkinElmer) and incubated overnight at 4°C
126 with 0.5 μ g of primary antibodies against SUZ12 (3737, Cell Signaling Technology), or EZH2 (5246,
127 Cell Signaling Technology). Protein G-Sepharose beads (101241, Invitrogen) were added for 1 h at
128 room temperature (RT) and the beads were washed three times in PBS with 0.1% Tween-20. Bead-
129 bound proteins were eluted in SDS-PAGE sample buffer by boiling for 10 min at 100°C.

130 After SDS-PAGE, PVDF membranes with transferred proteins were incubated with primary
131 antibodies: 0.084 μ g/mL SUZ12, 0.421 μ g/mL EZH2, 0.18 μ g/mL GAPDH (ab181602, Abcam), 0.1
132 μ g/mL H3K27me3 (C15410069, Diagenode), 0.02 μ g/mL H3 (ab176842, Abcam), or 0.4 μ g/mL
133 IGFBP3 (sc365936, Santa Cruz Biotechnology). Primary antibodies were revealed with anti-mouse
134 IgG coupled to IRDye RD 680 or anti-rabbit IgG coupled to IRDye 800CW (Licor Biosciences, 926–
135 68070 & 926–32211) on a dual infrared imaging scanner (Licor Biosciences, Odyssey CLx 9140) and
136 quantified with the software provided (Licor Biosciences, Image Studio V5.0.21, 9140–500).

137 **2.6 Immune staining**

138 For immune staining, cells were grown on poly-D-lysine coated 8-well microscope slides (80826-
139 IBI, Ibbidi). Cells were fixed in 4% paraformaldehyde and stained (Ulrich et al., 2018) with primary
140 antibodies: 0.168 μ g/mL SUZ12, 0.842 μ g/mL EZH2, 1.6 μ g/mL H3K27me3 or 1.5 μ g/mL p16
141 (ab108349, Abcam). Detection by fluorescent laser confocal microscopy (Nikon C2 microscope) was
142 done with 2 μ g/mL secondary antibodies anti-mouse IgG Alexa594, anti-rabbit IgG -Alexa 488 or anti-
143 rabbit IgG-Alexa 647 (A-11032, A-11034, A21245, Thermo Fisher Scientific). Nuclei were
144 counterstained with 0.5 μ g/mL DAPI (D9542, Sigma-Aldrich). Images were acquired by sequential
145 excitations (line-by-line scan) with the 405 nm laser (464/40 emission filter), the 488 nm laser
146 (525/50 nm filter), the 561 nm laser (561/LP nm filter) and the 650 nm laser (594/633 emission filter).
147 ImageJ was used for all image quantifications.

148 **2.7 RNA extraction and RT-qPCR**

149 Total RNA extraction using the TRIzol™ Reagent (15596026, Invitrogen) and cDNA synthesis
150 using the GoScript Reverse Transcription Mix Random Primers (A2800, Promega) were done
151 according to the instructions of the manufacturer. Amplification was performed with SsoAdvanced
152 Universal SYBR Green Supermix (1725271, BioRad) with 43 cycles at 95°C for 5 sec, 60°C for 30
153 sec and 60°C for 1 min using specific primers for *EZH2*, *SUZ12*, *IGFBP3*, *GPR37*, *ITGA3*, *MRC2* and
154 *IRF6* gene transcripts (**Supplementary Table IV**). Relative RNA expression was calculated using the
155 comparative Ct method and normalized to the geometric mean of the GAPDH and HPRT1 mRNAs.

156 **2.8 SA- β Gal assay**

157 Senescence-associated β -galactosidase (SA- β Gal) staining was determined on cells grown in 6-well
158 plates, fixed with 2% paraformaldehyde for 10 min at RT and washed twice with gentle shaking for 5
159 min at RT. Cells were then incubated with 1 mg/mL X-gal (20 mg/mL stock in DMF; B4252, Sigma-
160 Aldrich,) diluted in pre-warmed 5 mM $K_3[Fe(CN)_6]$ (P-8131, Sigma-Aldrich), 5 mM
161 $K_4[Fe(CN)_6] \cdot 3H_2O$ (P-3289, Sigma-Aldrich), and 2 mM $MgCl_2$ (M-8266, Sigma-Aldrich) in PBS at
162 pH 6.0. Acquisition and quantification of the images for SA- β Gal activity and cell area were done with
163 an automated live cell imager (Lionheart FX, BioTek).

164 2.9 LysoTracker

165 For LysoTracker staining, cells were seeded in poly-D-lysine coated 8-well microscope slides,
166 incubated for 10 min at 37°C with 0.25 µM LysoTracker Red (L7528, Thermo Fischer Scientific).
167 Nuclei were counterstained with 2.5 µg/mL Hoechst (H3570, Invitrogen) for 10 min at 37°C,
168 afterwards cells were washed with complete medium. Images of living cells were acquired on a
169 fluorescent laser confocal microscope (C2, Nikon) by sequential excitations (line-by-line scan) with
170 the 405 nm laser (464/40 nm emission filter), and the 561 nm laser (561/LP nm filter). ImageJ was used
171 for all image quantifications. Both the lysosomes area and the mean number of lysosomes per cells and
172 per images were determined.

173 3 Results

174 3.1 Transcriptomics analysis of Tau-KO cells

175 We performed a next-generation transcription (RNAseq) analysis of human SH-SY5Y
176 neuroblastoma cells knocked-out for Tau when compared to normal Tau-expressing cells (**Fig 1A**).
177 The sequences obtained from the six samples analyzed (three Tau expressing cell lines, three Tau-KO
178 cell lines) mapped reliably to ~16'000 genes. Additional 14'000 transcripts were expressed at low
179 levels and were not included in the differential expression analysis. The primary data were stored at
180 <https://www.ebi.ac.uk/biostudies/> with open access (E-MTAB-8166). When filtering for differentially
181 expressed transcripts in Tau-KO cells, 1388 RNAs displayed a significant change (Adj P <.05), of
182 which 723 RNAs were up-regulated in the log₂(FC) range between 0.31 and 11.05 (between 1.24 and
183 ~2000 fold higher than in control Tau expressing cells) (**Supplementary Table I**).

184 We selected these 723 differentially expressed genes to interrogate a possible gene-set enrichment
185 utilizing the transcription tool of the EnrichR portal (Chen et al., 2013, Kuleshov et al., 2016, Xie et
186 al., 2021). The CHIP-sequencing datasets (ChEA 2016) identified an overrepresentation of Polycomb
187 Repressive Complex-associated proteins among the 68 datasets showing a significant (Adj P <.01)
188 difference. Indeed, almost half of the enriched CHIP-sequencing datasets were obtained from core
189 components or known regulators of PRC2 (32%) or of PRC1 (16%) (**Fig 1B; Supplementary Table**
190 **II**). PRC2 actively catalyzes the trimethylation of histone 3 (H3) at lysin 27 (H3K27me3) (Laugesen
191 et al., 2016, Moritz and Trievel, 2018). In agreement with the identification of PRC2 in the CHIP-
192 sequencing datasets, mining of the epigenomics roadmap (HM CHIP-seq) resulted in a 73% enrichment
193 of the H3K27me3 signature (Adj P <.01) in 45 datasets (**Fig 1C; Supplementary Table III**).
194 Altogether, analysis of the RNAseq data suggested that up-regulation of transcription of a specific set
195 of genes in Tau-depleted neuroblastoma SH-SY5Y cells might ensue from a relief of gene activity
196 decline controlled by PRC2.

197 3.2 Reduced expression and activity of PRC2 in Tau-depleted cell

198 We subsequently analyzed the amount of two PRC2 core components in Tau-KO cells by western
199 blot. In agreement with what suggested by the transcriptomics data, we observed reduced amounts of
200 the catalytic subunit EZH2 and the scaffold subunit SUZ12 of PRC2 in Tau-KO cells when compared
201 to Tau-expressing cells (**Fig 2A**). Reduced proteins were found also by quantitative immune staining
202 of the cells utilizing specific antibodies (**Fig 2B**). RT-qPCR analysis excluded that the effect on PRC2
203 protein resulted from reduced transcription since no difference was found for the EZH2 and SUZ12
204 mRNAs (**Fig 2C**), data that suggested a Tau-dependent effect on PRC2 protein stability. Nonetheless,
205 co-immune isolation revealed the presence of the EZH2-SUZ2 core complex of PRC2 in Tau-KO cells,
206 albeit at reduced levels when compared to controls (**Fig 2D**).

207 PRC2 enzymatic activity was determined by western blot and quantitative immune staining of
208 H3K27me3, an epigenetic mark produced by the histone methyl transferase activity of PRC2 (Guo et
209 al., 2021). Confirming the lower amounts of the PRC2 complex, we found that Tau-KO cells display
210 reduced H3K27me3 (**Fig 3A-B**). Among the upregulated transcripts found by RNA-seq in Tau-KO
211 cells, we selected five known PRC2 targets displaying close to average signals (**Supplementary Table**
212 **I**): *IGFBP3* (19.0x of WT, adjP .004), *GPR37* (9.3x, .015), *ITGA3* (6.3x, .017.3x), *MRC2* (5.1x, .016),
213 and *IRF6* (3.2x, .0498). Determination of mRNA expression by RT-qPCR validated their up-regulation
214 (**Fig 3C**); indicating again that Tau-depletion relieved repression of transcription caused by reduced
215 PRC2 activity in Tau-depleted cells.

216

217 **3.3 PRC2-dependent overproduction of IGFBP3 in Tau-KO cells**

218 IGFBP3 protein is a component of the senescence-associated secreted phenotype (SASP) (Basisty
219 et al., 2020). Having previously reported that Tau-depletion favored cellular senescence (Sola et al.,
220 2020), we interrogated the role of IGFBP3 in this process. As anticipated from the mRNA data, a strong
221 overproduction of endogenous IGFBP3 was present in Tau-KO cells (**Fig 4A**). Reinforcing the link
222 between PRC2 and IGFBP3, treatment of SH-SY5Y cells with Tazemetostat, a specific blocker of the
223 histone methyl transferase activity of EZH2 (Straining and Eighmy, 2022), reduced H3K27me3 and
224 increased IGFBP3 (**Fig 4B**). Lower EZH2 and SUZ12 and higher IGFBP3 was confirmed in an
225 independent Tau-KO cell line (**Supplementary Fig 1**).

226 **3.4 Tau/PRC2/IGFBP3 triad in senescence**

227 Increased cellular expression of IGFBP3 is associated with autocrine and paracrine senescence
228 induction (Elzi et al., 2012), and reduced PRC2 is also linked to increased cellular senescence (Ito et
229 al., 2018). Furthermore, increased senescence is observed in Tau-KO cells (Sola et al., 2020). Thus,
230 we postulated that PRC2-dependent de-repression of IGFBP3 in Tau-depleted cells may explain the
231 induction of cellular senescence. We observed first that Tau depletion as well as PRC2 inhibition both
232 increased the percentage of SH-SY5Y cells entering in senescence, as assessed by three independent
233 markers: P16, senescence-associated β -galactosidase (SA- β gal), and the number and size of lysosomes
234 labelled with the acidotrophic LysoTracker dye (**Fig 5A**). Next, we reduced IGFBP3 expression in
235 Tau-KO cells by a shRNA-based approach (**Fig 5B**) and found that this reduced senescence induction
236 in Tau-KO cells (**Fig 5C**). Thus, we validated our hypothesis that increased senescence in Tau-KO SH-
237 SY5Y cells was likely the consequence of decreased PRC2-dependent repression of IGFBP3
238 expression.

239 **4 Discussion**

240 We report data demonstrating a non-canonical role of Tau as a modulator of the epigenetic activity
241 of PRC2 inducing cellular senescence in neuroblastoma SH-SY5Y cells. In our study we identified a
242 prevalent PRC2 signature for shared modulation of upregulated transcripts in Tau-depleted cells. PRC1
243 and PRC2 are multi-subunit transcriptional repressors that crucially modulate chromatin structure and
244 gene expression by distinct enzymatic activities (Vijayanathan et al., 2022). PRC1 is an E3 ubiquitin
245 ligase that catalyzes H2A ubiquitination at lysine119, whereas PRC2 acts as a methyltransferase that
246 generates H3K27me3 with some cross-talk between the two complexes (Guerard-Millet et al., 2021).
247 Confirming the bioinformatics results, Tau depletion caused reduced cellular amounts of PRC2 and its
248 product H3K27me3. Increased senescence status upon Tau-depletion was reproduced through

249 pharmacological inhibition of PRC2 in Tau-expressing cells. Finally, we report that reversing the up-
250 regulation of the PRC2-target IGFBP3, impaired senescence induction in Tau-depleted cells.

251 Evidence exists for the implication of Tau in chromatin remodeling. A pioneering study investigated
252 chromatin conformation in mouse and drosophila models of AD as well as in human diseased brain,
253 whereby a general loss of heterochromatin was associated with aberrant gene expression in all three
254 paradigms (Frost et al., 2014). More recently, binding of Tau to histones was linked to the maintenance
255 of condensed chromatin (Rico et al., 2021). Thus, Tau may favor chromatin compaction for preventing
256 aberrant gene transcription. Misfolding, hyperphosphorylation or sequestration of Tau in oligomers
257 and fibrils, typical hallmarks of tauopathies, could all result in a negative regulation of this non-
258 canonical function of Tau. In our study, we show that in addition to the direct interaction between Tau
259 and histones (Rico et al., 2021), an indirect mechanism involving PRC2 is an additional instrument for
260 modulating chromatin compaction.

261 The role of PRC2 in senescence was shown by findings indicating that impairment of its catalytic
262 activity induces a delayed decrease in H3K27me3 at the CDKN2A locus, which upregulates p16, the
263 SASP phenotype, and senescence (Ito et al., 2018). This function of PRC2 represents a target for
264 anticancer therapies e.g., through EED inhibition associated to de-repression of SASP-encoding genes
265 and entry of proliferative cancer cells in a senescent state (Chu et al., 2022). Beside the paradoxical
266 implication in cancer (Yang et al., 2021), senescence contributes to neurodegenerative diseases.
267 Senescent neurons, microglia, astrocytes and neuronal stem cells were found during the pathogenic
268 process (Si et al., 2021). A recent study in a tauopathy mouse model supported a causal link between
269 cell senescence and cognitive decline linked to neuronal loss. Indeed, p16INK4A-positive senescent
270 glial cells were found associated to Tau lesions, and, strikingly, the clearance of these cells prevented
271 Tau hyperphosphorylation, Tau fibril deposition, whilst preserving neuronal survival and cognitive
272 functions (Bussian et al., 2018). We describe now a conceivable mechanism linking depletion of
273 functional Tau in tauopathies and senescence induction.

274 Among the PRC2 targets, we identified IGFBP3 as a main driver of senescence resulting from Tau-
275 depletion. Ectopic expression of the SASP component IGFBP3 or its administration to MCF7 or IMR-
276 90 cells is sufficient to induce senescence, whereas IGFBP3 knock-down impairs doxorubicin-induced
277 senescence (Elzi et al., 2012). Although the role of PRC2 and IGFBP3 were established independently,
278 to our knowledge our study is the first one showing IGFBP3 as a main executor of PRC2-dependent
279 senescence induction and its modulation by Tau protein levels.

280 PRC2 has numerous functions in the developing central nervous system, with many neurogenesis-
281 linked genes regulated by the PRC2/H3K27 axis (Liu et al., 2017). PRC2 is essential in preserving
282 neural progenitor cell identity and neuroepithelial integrity (Akizu et al., 2016). PRC2 deficiency in
283 mice leads to aberrant gastrulation and lack of neural tissue (Schumacher et al., 1996). Later in
284 development, a transcription pattern with a PRC2 signature drives neuronal migration and is essential
285 for the organization of neural circuits (Zhao et al., 2015). The rare Weaver syndrome linked to
286 developmental cognitive deficits is caused by autosomal dominant mutations in any one of the three
287 PRC2 core components EZH2, EED and SUZ12 (Deevy and Bracken, 2019). However, PRC2 is also
288 involved in neurodegeneration. PRC2 deficiency in striatal neurons of mice reactivates the deleterious
289 expression of transcripts that are normally suppressed in these cells, ultimately causing premature
290 lethality (Von Schimmelmänn et al., 2016). Additional studies implicated PRC2 in ataxia-
291 telangiectasia (Li et al., 2013), Parkinson's disease, Huntington's disease and AD (Kuehner and Yao,
292 2019). A meta-analysis of differentially methylated regions in prefrontal neocortex at different disease
293 stages has identified in AD several hypermethylated regions, which were significantly enriched in
294 polycomb repressed regions (Zhang et al., 2020). These data also link PCR2-dependent methylation of

295 H3 with that of CpG islands of the genome, another epigenetic mechanism of gene repression (Phillips,
296 2008).

297 PRC2 lacks sequence-specific DNA-binding ability and therefore relies on accessory proteins for
298 targeting specific loci. Factors contributing to selective PRC2 recruitment to chromatin are the
299 interaction with sequence-specific transcription factors or RNAs, and or discerning chromatin features
300 (Blackledge and Klose, 2021). In the fly, PRC2 activity is regulated through the interaction with
301 transcription factors binding to polycomb response elements often located in proximal promoter
302 regions of developmental genes (Kassis and Brown, 2013). However, the orthologue system was not
303 found in mammals (Bauer et al., 2016). Rather, it is maybe replaced by the evolution of a mechanism
304 based on non-methylated CpG islands (Ku et al., 2008) and the action of DNA-binding proteins binding
305 to them. Proteins with such features are the PRC1.1 complex member KDM2B, or the PRC2-members
306 PHF1, MTF2 and PHF19 (Owen and Davidovich, 2022).

307 PRC histone modifications are heritable over mitotic cell division providing an epigenetic memory
308 for stable cell identity and adequate response to stress (Reinig et al., 2020). Thus, PRC2 dysfunction is
309 frequently associated with neoplastic progression and is a target for anticancer therapy (Comet et al.,
310 2016). Expression of its catalytic subunit EZH2 correlates with cell proliferation, and its aberrant
311 overexpression is frequent in many types of cancer cells (Liu and Liu, 2022). However, in line with a
312 role in tumor suppression, loss-of-function of PRC2 is also involved in cancer (Liu et al., 2017). PRC2
313 modulation by Tau implicates this latter in the pathogenesis of cancer, supporting the observation that
314 the Tau mRNA correlates with survival in several tumors (Gargini et al., 2019, Papin and Paganetti,
315 2020). The mechanism explaining this correlation is unknown but may involve the non-canonical role
316 of Tau in modulating chromatin compaction and senescence induction. This may open new therapeutic
317 opportunities for neurodegenerative diseases and cancer.

318 **5 Conflict of Interest**

319 The authors declare that the research was conducted in the absence of any commercial or financial
320 relationships that could be construed as a potential conflict of interest.

321 **6 Author Contributions**

322 Conceptualization: SP, PP
323 Methods and investigations: CM, MS, EP, MB, AR
324 Supervision: SP, PP
325 Writing (original draft): SP
326 Writing (review & editing): all co-authors

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332 **9 Data Availability Statement**

333 The RNA-Seq data have the accession no. E-MTAB-8166 and were uploaded on
334 [https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-8166?key=64a67428-adb9-4681-](https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-8166?key=64a67428-adb9-4681-99c9-98910b78ed4c)
335 [99c9-98910b78ed4c](https://www.ebi.ac.uk/biostudies/arrayexpress/studies/E-MTAB-8166?key=64a67428-adb9-4681-99c9-98910b78ed4c).
336

337 **References**

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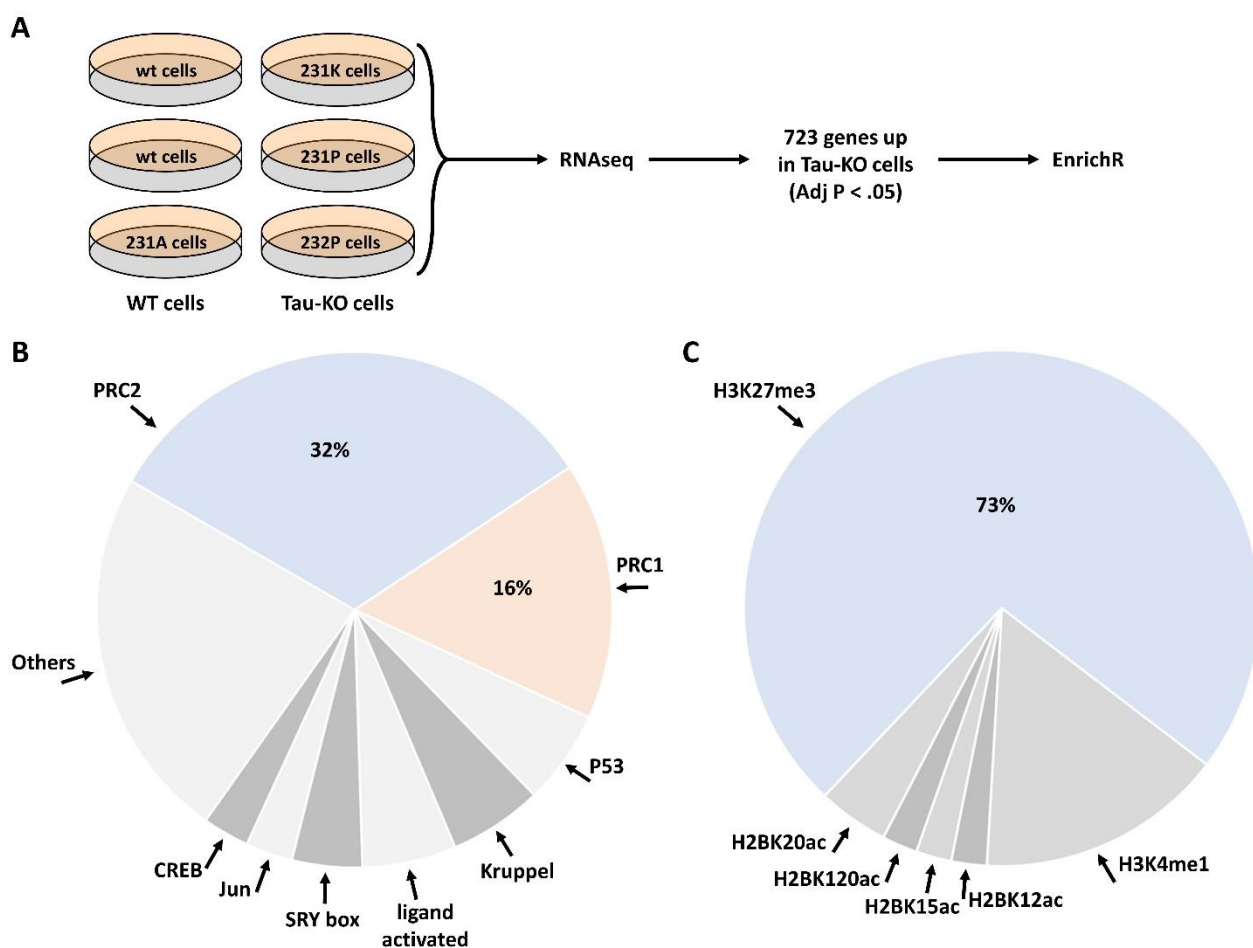
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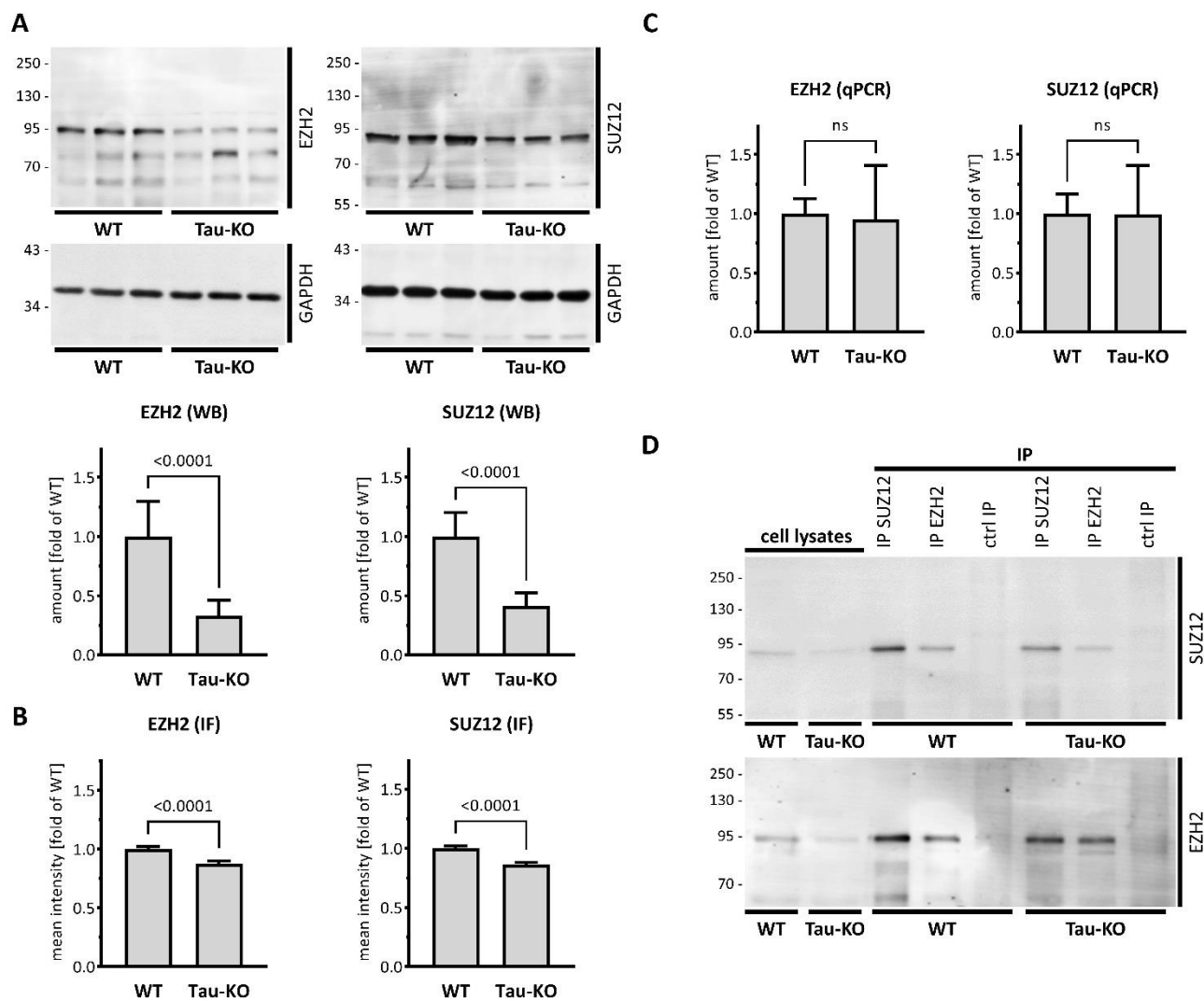
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539

540 **Figure 1. Deregulation of the PRC2 pathway in human Tau-KO SH-SY5Y neuroblastoma cells.**
 541 (A). Scheme of the procedure for the RNAseq and EnrichR analyses in Tau-expressing (WT) and
 542 Tau-knock-out (Tau-KO) cells. (B-C). The EnrichR analysis based on 723 upregulated genes in Tau-
 543 KO cells resulted in the enrichment of the PRC2 pathway with the ChIP datasets (B) and of
 544 H3K27me3 with the epigenomics datasets (C).

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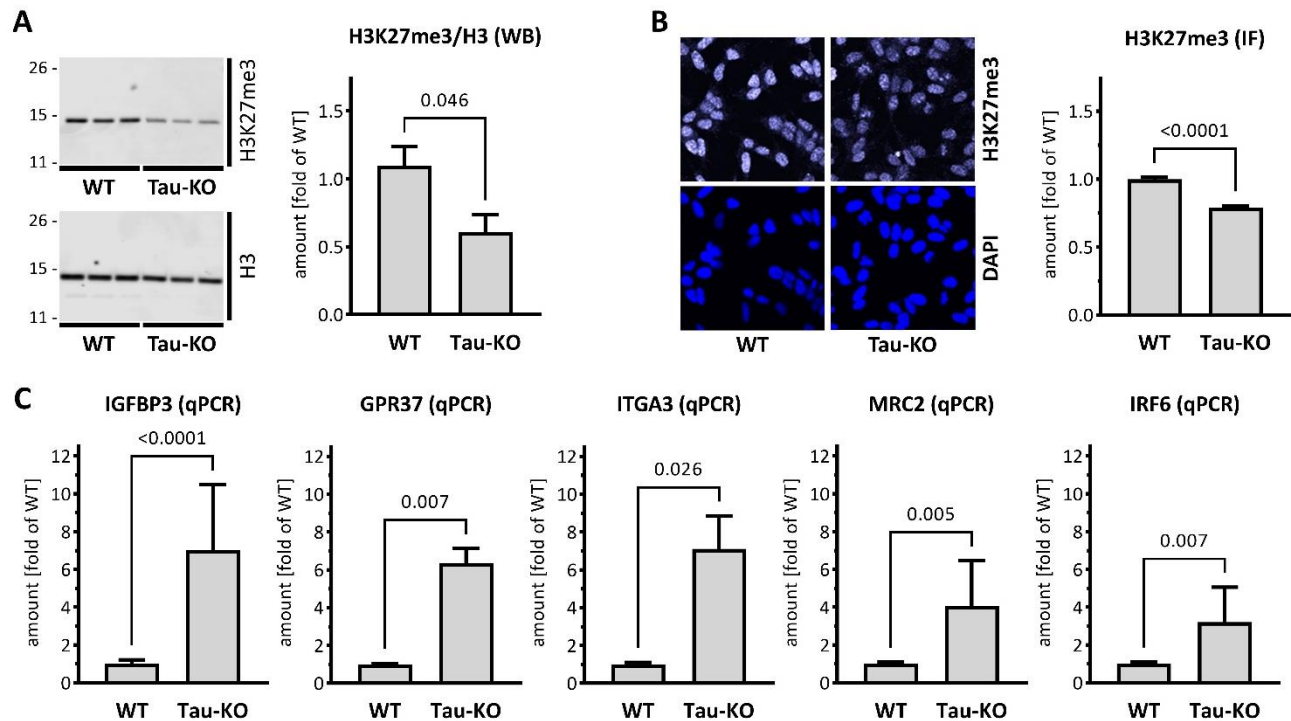


546

547 **Figure 2. Reduced PRC2 complex in Tau-KO SH-SY5Y cells.**

548 (A). Shown are matched protein amounts of parental (WT) or Tau-KO cell lysates analyzed by
 549 western blot (biological triplicates on a single gel) with EZH2, SUZ12 or GAPDH primary
 550 antibodies and anti-rabbit IgG IRDye 800CW secondary antibody. The EZH2 and SUZ12 signals
 551 were normalized on the respective GAPDH signals and reported as fold of WT; mean \pm SD of 9
 552 biological replicates, unpaired Mann-Whitney test. (B). Determination of nuclear EZH2 or SUZ12
 553 mean fluorescent intensity analyzed by immune fluorescence staining and laser confocal microscopy
 554 with EZH2 or SUZ12 antibodies revealed with an anti-rabbit AlexaFluor 488 antibody. Data obtained
 555 with a DAPI nuclear mask (ImageJ) are reported as fold of WT; mean \pm SEM of 292-475 nuclei from
 556 two (EZH2) or three (SUZ12) independent experiments, unpaired Mann-Whitney test. (C). Shown
 557 are RT-PCR determination of mRNA with specific primers for EZH2 or SUZ12. Normalization was
 558 performed on the geometric mean of the GAPDH and HPRT1 mRNA values and reported as fold of
 559 WT; mean \pm SD of 12 biological replicates, unpaired Mann-Whitney test. (D). Shown are matched
 560 protein amounts of cell lysates subjected to immune isolation (IP) with EZH2 or SUZ12 antibodies or
 561 matched amounts of control antibodies (ctrl IP). Samples were resolved on a single same gel and
 562 analyzed by western blot with EZH2 or SUZ12 antibodies and secondary anti-rabbit IgG IRDye
 563 800CW antibody.

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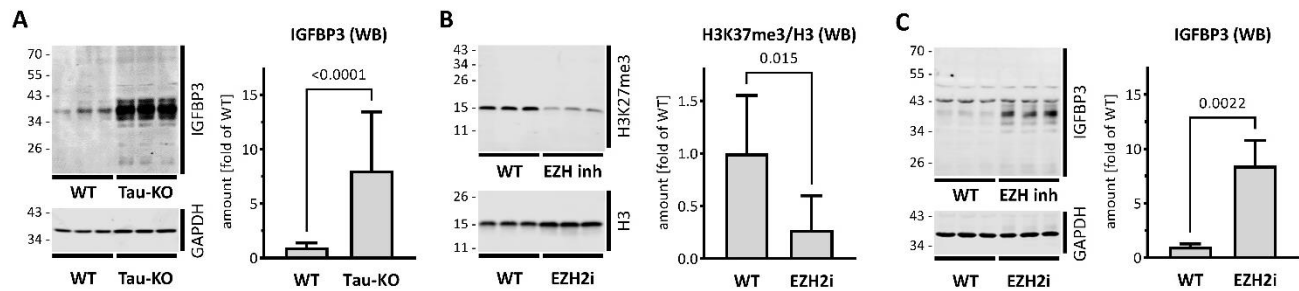


565

566 **Figure 3. Reduced PRC2 activity in Tau-KO SH-SY5Y cells.**

567 (A). Shown are matched protein amounts of parental (WT) or Tau-KO cell lysates analyzed by
568 western blot (biological triplicates on a single gel) with H3K27me3, H3 or GAPDH primary
569 antibodies and anti-rabbit IgG IRDye 800CW secondary antibody. The H3K27me3 and H3 signals
570 were normalized for GAPDH and reported as fold of WT for the H3K27me3/H3 ratios; mean \pm SD
571 of 8-9 biological replicates, unpaired Mann-Whitney test. (B). Nuclear H3K27me3 mean fluorescent
572 intensity was determined by immune fluorescence staining and laser confocal microscopy. Data
573 obtained with a DAPI nuclear mask (ImageJ) are reported as fold of WT; mean \pm SEM of 695-716
574 nuclei from five independent experiments, unpaired Mann-Whitney test. (C). Shown are RT-PCR
575 determination of mRNA with specific primers as indicated. Normalization was performed on the
576 geometric mean of the GAPDH and HPRT1 mRNA values and reported as fold of WT; mean \pm SD
577 of 3-12 biological replicates, unpaired Mann-Whitney test.

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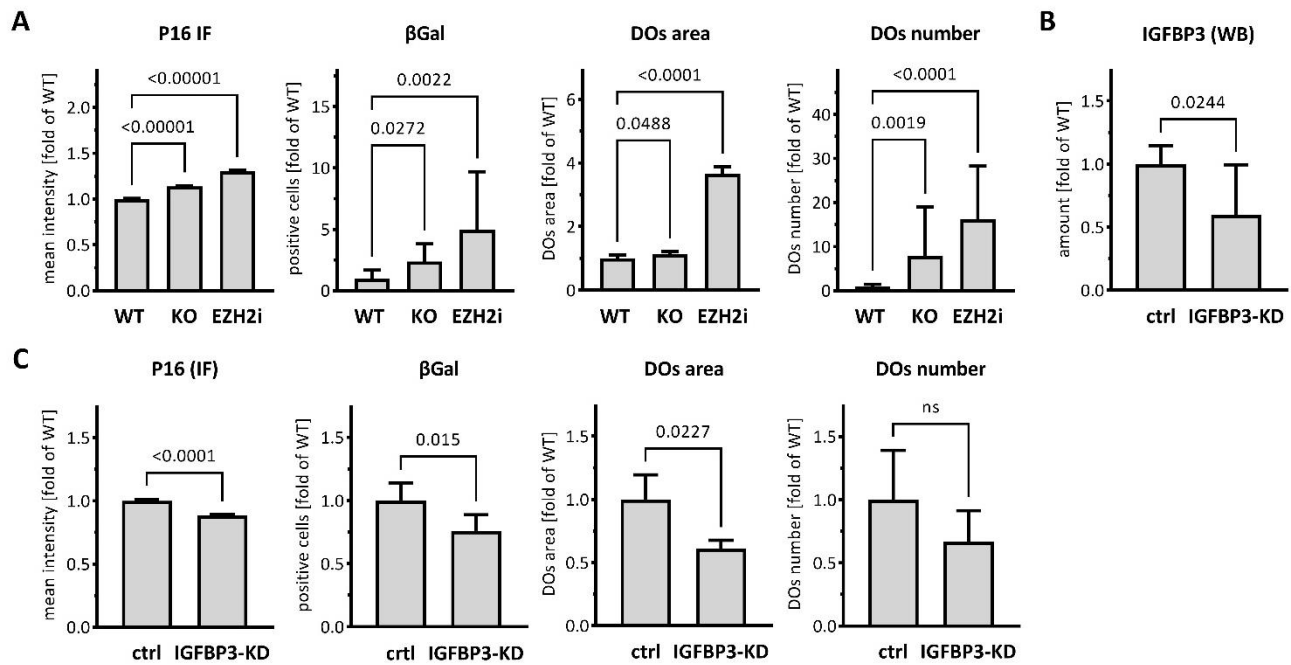


579

580 **Figure 4. Increased IGFBP3 in SH-SY5Y cells after Tau-KO or PRC2 inhibition.**

581 (A). Shown are matched protein amounts of parental (WT) or Tau-KO cell lysates analyzed by
582 western blot (biological triplicates on a single gel) with IGFBP3 or GAPDH primary antibodies and
583 anti-mouse IgG IRDye 680RD or anti-rabbit IgG IRDye 800CW secondary antibodies. The IGFBP3
584 signals were normalized for GAPDH and reported as fold of WT; mean \pm SD of 8-9 biological
585 replicates, unpaired Mann-Whitney test. (B-C). Shown is a western blot of matched protein amounts
586 of SH-SY5Y cells treated for 4 days in the absence (WT) or presence of 10 μ M Tazemetostat
587 (EZH2i). Biological triplicates on a single gel were probed (B) with H3K27me3, H3 or GAPDH
588 primary antibodies and anti-rabbit IgG IRDye 800CW secondary antibody or (C) with IGFBP3 or
589 GAPDH antibodies. Protein signals were normalized for GAPDH and reported as fold of WT; mean
590 \pm SD of 6 biological replicates, unpaired Mann-Whitney test.

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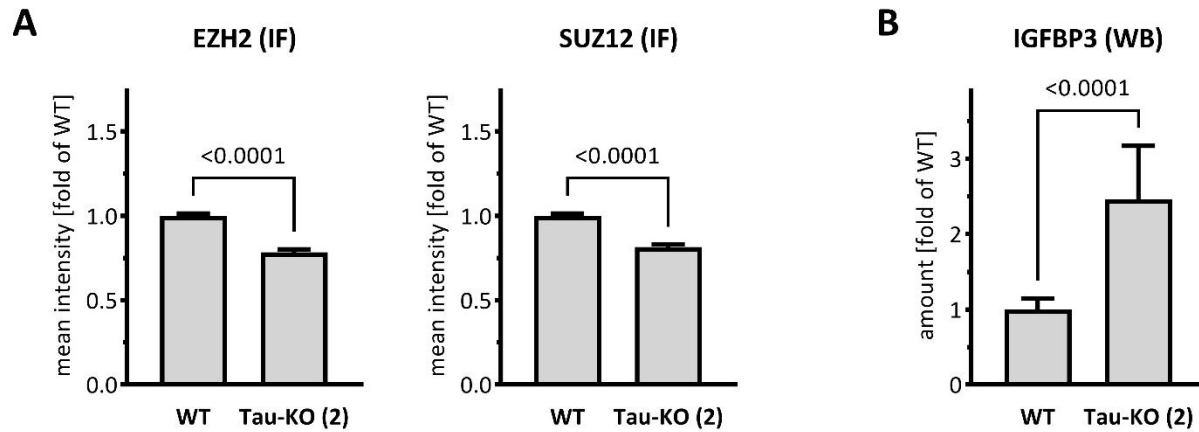
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593 **Figure 5. Increased IGFBP3-induced senescence in cells.**

594 (A). Senescence markers were analyzed in parental (WT), Tau-KO or 10 μ M Tazemetostat-treated
595 parental (EZH2i) cells. The nuclear P16 fluorescent intensity (P16 IF) was determined with a DAPI
596 nuclear mask (ImageJ) by immune fluorescence staining and laser confocal microscopy and reported
597 as fold of WT; mean \pm sem of 1991-3153 nuclei. The percent of senescence-associated β Gal positive
598 cells (β Gal) was determined by automated cell imaging; mean \pm SD of 7-14 fields. Living cells
599 labeled with the acidotrophic dye LysoTracker (DOs) were imaged on a laser confocal microscope
600 and analyzed for the size (area of 643-3429 DOs) and mean number per cell (13-18 fields) of
601 LysoTracker-positive organelles (ImageJ). Values are reported as fold of WT; mean \pm sem (area) or
602 \pm SD (number), non-parametric Krustal-Wallis and Dunn's multiple comparison test. (B). Matched
603 protein amounts of SH-SY5Y cells transduced with mock (ctrl) or IGFBP3 shRNA (IGFBP3-KD)
604 pseudo lentiviral particles were analyzed by western blot (biological triplicates on a single gel) with
605 IGFBP3 or GAPDH primary antibodies and anti-mouse IgG IRDye 680RD or anti-rabbit IgG IRDye
606 800CW secondary antibodies. The IGFBP3 signals were normalized for GAPDH and reported as fold
607 of WT; mean \pm SD of 9 biological replicates, unpaired Mann-Whitney test. (C). As in (A) for SH-
608 SY5Y cells transduced with mock (ctrl) or IGFBP3 shRNA (IGFBP3-KD) pseudo lentiviral particles.
609 P16 IF: mean \pm sem of 669-881 nuclei; β Gal: mean \pm SD of 6 fields. DOs; area of 456-551 DOs,
610 mean number per cell (5 fields) of LysoTracker-positive organelles (ImageJ). Values are reported as
611 fold of WT; mean \pm sem (area) or \pm SD (number); unpaired Mann-Whitney test.

612

613 Supplementary Figure 1



614

615 **Supplementary Figure 1. Reduced EZH2/SUZ12 and increased IGFBP3 in an independent**
616 **Tau-KO SH-SY5Y cell line.**

617 (A). Nuclear EZH2 or SUZ12 mean fluorescent intensity was analyzed by immune fluorescence
618 staining and laser confocal microscopy with EZH2 or SUZ12 antibodies revealed with an anti-rabbit
619 AlexaFluor 488 antibody. Data obtained with a DAPI nuclear mask (ImageJ) are reported as fold of
620 WT; mean \pm sem of 202-208 nuclei (EZH2) or 182-184 nuclei (SUZ12), unpaired Mann-Whitney
621 test. (B). IGFBP3 was determined by western blot for matched protein amounts of parental (WT) or
622 Tau-KO (2) cell lysates (biological triplicates on a single gel). Data are reported as fold of WT, mean
623 \pm SD of 9 biological replicates, unpaired Mann-Whitney test.

624 **Supplementary Table I. Transcripts upregulated in human Tau-KO SH-SY5Y cells (Adj P**
 625 **<.05) listed in order of decreasing significance**

Symbol	log2FC	Symbol	log2FC	Symbol	log2FC	Symbol	log2FC
CDKN1A	2.24	TMEM63A	2.19	WWC3	1.39	PACSIN2	0.39
C22orf34	8.85	PDGFA	4.48	RELB	1.75	AGA	1.82
SLC12A7	2.93	ASB9	3.14	LGR5	2.09	PRCP	0.58
EVC2	7.23	ZFP36L2	0.76	GPX7	0.86	NTRK2	3.13
DCN	10.43	CILP	2.70	SLC10A3	1.17	SSPO	2.79
TNFRSF1A	11.05	LOC100507156	2.69	ARHGEF40	0.99	XYLT1	2.74
HMCN2	2.05	CAVIN2	4.65	CXCL16	4.90	APLP2	0.77
APOBEC3C	3.27	HAS2	6.02	SPATA20	1.04	CBR3	1.69
COL3A1	8.26	TGFB1I1	1.04	TBX2	0.91	ABCA7	0.85
MGP	6.99	CDC42EP1	2.48	IGFBP2	1.21	PDLIM7	1.00
RGS11	1.70	PSMB10	2.44	IL11RA	1.40	MEST	4.65
CFLAR	1.38	OBP2A	5.69	NFKBIE	1.27	EDN3	5.11
RHBDF1	4.25	TRIL	1.74	LRP1B	5.68	ZBTB46	1.51
KCNG1	1.90	DDR1	1.48	PDE4A	1.27	BTG2	0.57
SEL1L3	6.30	SSH3	1.33	RBPM52	0.72	TCTN3	0.50
S100A11	6.39	CRACR2B	1.93	AVIL	2.59	ABLIM3	1.62
CSF1	1.95	CLU	4.18	BHLHE40	5.44	TESK1	0.76
CCDC80	7.90	VCAN	5.01	HSD3B7	2.55	TNFRSF9	3.24
CD9	3.13	TNS2	1.97	TPBG	2.67	PXDC1	2.20
TIMP1	7.39	S100A10	7.90	ZNF425	0.73	LRP10	1.27
PTGS1	6.25	NT5E	4.88	MCAM	1.92	SERTAD1	1.10
RBPM5	6.08	RDH10	2.62	KIAA0040	1.38	SLC26A11	0.82
LTBP2	1.56	GNG11	1.04	FOXI3	3.60	FMN1	2.87
EVA1A	7.27	MTUS2	5.11	C1QTNF1-AS1	1.16	FSTL1	2.17
S100A16	7.04	LAT2	3.32	PGGHG	1.12	FAM46C	3.27
GNG3	2.71	ST6GAL1	2.61	ORMDL2	0.85	POP5	0.64
TRIM17	1.53	HLX	2.95	KRT17	4.14	NUAK1	3.11
GLIS2	1.32	LUM	4.89	CERCAM	1.17	ZDHHC1	0.84
MAB21L2	9.87	PEAR1	0.78	POLD4	1.71	IRF2BPL	0.84
MR1	5.41	S1PR3	1.65	PLK2	2.05	SEMA3B	1.63
ARHGAP36	3.04	GPX3	1.59	ANTXR1	2.22	RAB3IL1	0.96
TUBB6	1.31	RBM47	6.48	VIM	1.83	BMP1	1.13
A2M	9.55	NQO1	1.89	ZC3H12A	1.50	NOTCH4	1.41
PHLDA3	1.23	ARSA	1.29	RBM24	4.66	LOC101927752	1.11
SYT9	4.48	SPSB2	1.29	PANX2	1.75	STAC	1.11
INSRR	0.79	PTN	3.99	SERPINE1	3.14	COCH	1.22
HGF	10.53	TMEM254	0.86	HECW1	2.42	CRLF1	3.75
ABCC3	6.27	CCL2	4.38	CDH2	2.33	METTL7A	0.78
MOCOS	3.48	ITPKB	2.24	AAED1	1.72	TMEM150C	2.10
C7	2.90	TP53I13	1.31	ANXA7	0.73	TMEM253	1.84
LCAT	1.19	GPNMB	4.78	EPS8L2	1.48	SIRPA	2.87
ITGA5	5.60	ID4	3.92	SERPINB8	2.17	CYTH3	0.49
IDUA	1.64	KIRREL	8.84	SAMD14	1.06	ATXN1	2.37
PRR16	1.84	TRIM5	5.31	CYSTM1	2.26	GJC2	1.61
HS3ST3B1	6.17	KLHL36	1.20	PPCS	0.49	TMED1	0.81
OSGIN1	1.67	SDC4	2.74	SLC7A4	2.57	SLC39A11	0.51

NACC2	1.42	ABI3BP	5.64	RAB26	1.28	RTL5	0.99
CELSR1	5.30	ITM2A	4.28	PLEKHG5	0.76	FOXD1	2.76
GPRC5C	3.49	KCNH2	0.84	FAM110B	0.74	LRRC29	1.27
KCNMB4	1.25	CORIN	7.09	CHRNA9	4.51	WIPI1	1.22
CAVIN1	5.39	GALNT6	2.81	RUSC1	0.72	ADAMTS2	2.13
CHRD	5.54	THSD4	2.39	ROM1	0.78	FBLIM1	1.58
LOXL2	3.68	IER5L	1.47	FEZ1	0.92	SIK2	1.73
ERVMER34-1	3.40	LDLRAP1	1.47	KIF26A	1.00	ZNF467	2.73
TRIOBP	1.19	PLEKHA4	1.34	NPC2	1.71	PTCH2	1.38
MSX2	1.10	ACADS	1.12	METRNL	1.77	NRBP2	0.59
NOTCH1	1.59	DHRS1	0.91	AGRN	1.20	MYO15B	1.06
SPHK1	4.05	APIG2	0.67	HYI	1.14	CXCL12	3.16
GBP2	6.29	SP5	6.48	SMIM3	3.00	CMTM3	1.20
LAMB3	4.06	HEG1	3.46	FN1	2.99	SLC27A3	1.07
APLNR	2.94	CPVL	1.43	C1orf204	2.44	ARSB	0.82
FAM129A	3.95	COL1A2	2.84	BOK	1.29	LINC00890	7.03
MMP15	2.44	GAD1	4.94	SIX1	6.68	HES1	1.39
TFEB	2.32	ARSD	1.46	SPOCK2	2.08	PPM1M	0.73
IL13RA1	5.07	ORAI3	1.69	FAM124A	1.32	HBEGF	2.73
SLC12A4	1.35	ARID3A	0.63	IGF2BP2	8.80	COL4A1	1.16
FBXL7	0.60	ITGA6	4.65	NRIP2	3.93	PPL	1.31
TGFBI	6.17	NRP1	4.96	CARNS1	3.78	PCOLCE	1.01
VTN	4.11	FOXC1	2.15	EYA4	3.23	C12orf75	1.42
PGF	2.43	SHROOM1	1.40	IL10RB	1.10	CHGB	1.34
VWA5B2	3.59	ACPP	6.17	DNALI1	0.60	POPDC2	1.29
SP110	4.59	ARSJ	4.43	HEXIM1	0.72	GPRC5A	3.64
FGFRL1	5.65	COL27A1	2.11	TGFBR2	2.89	ANXA4	0.74
ACHE	1.92	CA12	5.72	BOC	0.74	NFKB2	1.13
JDP2	1.88	ACADVL	1.17	SNTB1	7.16	MAP3K12	0.51
IGSF11	4.11	NTNG2	1.37	SIX2	4.04	SLC27A1	0.92
LGALS3	1.36	IFITM2	5.59	FGFR2	2.79	HS3ST5	3.81
SPRN	4.16	SERPINF1	2.90	C1QTNF1	2.14	WHRN	1.47
LHFP	2.96	SIX5	2.27	FAM114A1	1.54	ZNF582-AS1	1.37
TGM2	2.51	NTNG1	3.74	ENDOD1	1.20	GNAI2	1.12
ASL	1.33	TGFB3	4.20	CTDSPL	1.10	NPY	1.04
LAYN	3.12	CRYM	4.02	TRADD	1.02	SLIT2	2.86
HSPB7	3.51	RRAS	2.37	PDGFRL	2.76	DUSP3	0.86
NEDD9	4.45	MB21D2	3.10	GALNT2	0.67	LMF2	0.88
MST1	1.09	TMBIM1	3.99	BDKRB2	1.96	NCK2	0.45
HTRA1	5.98	HTR4	7.33	MYO3B	4.59	MCHR1	3.23
SH2D2A	5.79	TAPBP	1.30	CDH11	1.80	FNDC5	2.27
PTGER2	5.63	CTSS	4.36	IFT43	0.89	CST3	1.06
MAP3K6	1.26	RAMP1	1.22	LRPAP1	0.73	TSPAN9	0.78
TNFRSF12A	4.22	COL6A2	1.79	IAH1	0.69	GNB5	0.41
PTPN14	3.56	PGAP3	0.96	S100A2	2.09	UBA7	2.28
PAQR6	0.97	PPIC	2.63	EFEMP2	1.79	ZFP36	1.81
SLC6A9	2.24	TPST1	1.15	HLA-C	2.16	RBM3	0.44
PLA2G4C	3.06	LRRC4C	5.91	PLXDC2	5.96	GBP3	4.67
NOL3	1.08	GLIPR2	1.19	SRPX	2.62	SNX21	0.57
SPON2	3.88	FECH	0.45	SLC9A1	1.38	HPCA	1.66
LTBP1	3.18	CASP4	4.53	ACCS	0.59	LRP1	1.00

ATP8A2	6.49	SDK2	2.94	SVIL	2.37	CHPF2	0.72
SLC16A4	5.40	PLAU	3.08	KIAA1217	2.06	RNH1	0.93
KCNAB1	5.26	MIR100HG	5.42	MTCL1	0.95	CD59	1.69
CORO2B	3.38	OSBPL5	2.68	DHRS12	0.67	SHISA4	1.16
PIEZO1	3.24	SLC22A18	1.61	AFAP1L1	1.70	KCNJ2	3.81
KIF1C	2.03	CYBA	1.16	SHISA5	1.81	TLR2	2.98
INPP1	0.80	GADD45A	0.59	ELMO1	1.06	CD274	3.08
RGS3	2.54	LMNA	0.93	RAB7A	0.31	EVC	1.05
RNASET2	1.04	SCUBE2	2.86	COL6A3	2.51	CEP170B	0.90
PLAT	3.14	RELN	4.74	MARCH2	0.84	CHST14	0.69
COL7A1	2.37	PTPN3	3.75	RABEP2	0.84	HGSNAT	0.80
C10orf10	6.27	ARPIN	0.99	TIRAP	0.59	FZD7	2.12
PLPP4	5.73	COL13A1	6.17	SELENOM	1.15	MYO1D	1.41
AGT	5.19	C1QTNF2	2.27	CYBRD1	2.96	CNTNAP1	1.07
IGFBP3	4.25	IL4R	5.70	IGSF1	1.13	TPM2	1.25
MELTF	1.12	SLC31A2	2.09	RAB38	2.34	HOMER3	1.07
EHBP1L1	1.80	TAP1	3.16	VPS9D1	1.01	LOC339803	0.59
SYT12	7.06	PRSS23	3.16	SFXN5	0.73	SFRP1	1.65
CYTOR	7.48	SIPA1	0.93	TRABD2B	5.52	GDF15	1.39
TFPI2	2.36	IL32	3.61	ADORA2A	2.14	BOLA3	0.64
TNC	5.01	ZNF185	2.03	BAALC	2.28	GAP43	1.98
NLRC5	3.23	PAPPA	6.17	B4GALT1	1.57	ARHGAP6	2.71
ANXA2	2.03	FAM19A5	3.34	DKK1	1.25	DYNC1I1	3.00
ADAMTS15	4.93	SYTL4	2.99	MARVELD1	0.81	AHNAK	2.52
SYK	1.39	EDN1	4.66	GBX2	6.09	FAM131B	4.24
SOX9	4.19	SYNGR3	1.10	C6orf1	0.81	IFI6	1.03
COL5A1	4.66	DGKQ	1.15	IL33	5.88	TGFB1	2.76
GPC3	5.16	DEPTOR	4.82	TMEFF2	1.82	IL11	1.75
FAM89A	5.33	GLI1	4.44	PDLIM1	1.75	RARB	2.70
LOC101927809	3.62	EPDR1	0.73	FBXL8	1.16	CMKLR1	3.78
GJA1	8.42	TRAF1	3.60	TMEM8A	0.89	ZYX	1.22
OLFML3	4.44	ASTN1	0.66	OSMR	6.51	SIAE	1.13
DRAM1	2.79	EPS8L1	1.20	FBN1	2.85	RPN2	0.53
MMP11	1.97	FSTL3	2.82	SLC39A13	0.76	LOC645166	1.28
METRN	1.60	FKRP	0.59	STARD8	1.71	KLF2	2.54
SCN4B	1.30	DGKA	1.22	LOC101927204	3.44	SPARCL1	6.88
EWSAT1	4.66	KIAA1211L	2.37	SPOCD1	4.23	NTAN1	0.64
SGPP2	3.40	TBX18	6.79	DEGS1	0.58	ANO10	0.51
SH3TC1	1.70	PID1	3.47	PNPLA3	4.00	HHAT	0.75
ZFYVE21	0.80	B3GNT9	1.06	ISYNA1	0.96	CREB3L2	1.28
MIR4435-2HG	5.83	TNFRSF19	1.60	IFITM3	4.94	C19orf66	1.17
ANGPTL4	5.80	GPR37	3.22	PLTP	0.86	PLXNB1	0.48
SH3RF3	2.56	PTHLH	4.93	KDEL3	2.27	SCN9A	3.17
CD82	1.91	DNAJC22	2.70	CASP8	4.29	SPATA2L	1.12
DKK2	4.40	CD151	1.45	CEL	1.40	ATG16L2	0.73
NTRK1	1.27	GAL3ST1	1.48	NOTCH3	1.28	NUAK2	2.39
ECM1	5.15	LOXL4	1.15	SPRY4	1.16	SPEG	0.66
B3GNT4	2.91	PMP22	0.93	COLEC11	0.80	CEMIP	5.15
SERPINE2	7.09	SVEP1	4.68	PHYKPL	0.50	GFRA1	3.04
TXNDC11	0.65	LSR	1.98	MUM1	0.48	UXS1	1.07
TCF7L1	2.16	DOCK2	4.75	RAB20	1.93	DNASE1L2	1.75

PPP1R13B	1.32	PXDN	0.95	AJUBA	1.25	B3GAT3	0.81
CLCF1	5.16	DUSP10	2.50	CPXM1	1.04	POLR2L	0.90
TRIM21	2.73	MRC2	2.35	NPAS3	4.29	MAN2B2	0.66
YAP1	5.61	DUSP6	1.02	KREMEN1	1.21	CTTN	0.31
KRT18	5.21	ABTB1	1.03	BDKRB1	2.90	SORBS3	0.74
GABARAPL1	1.91	ABHD15	1.19	LGALS1	1.15	BATF3	2.42
GRIN2C	4.75	TRPM4	1.26	LTBP3	0.93	SEMA3D	4.22
RHOC	1.79	EPN3	4.24	LOC100506258	3.75	B4GALNT3	0.94
LRP4	1.91	MAN1A1	3.33	TRIB2	1.95	ADAM19	1.85
EVA1B	2.66	TMEM108-AS1	3.36	SGSH	0.80	EFHD1	1.77
ICAM1	4.82	SCARF2	1.30	IL17RA	2.84	AK5	1.40
TAGLN	1.82	PTPRR	2.22	FAM120AOS	0.48	FMNL1	1.19
PLPPR3	1.26	MIPEP	0.69	PTGR1	2.65	ALKAL2	3.24
SSC5D	4.72	MVP	2.02	ITGA7	2.23	MSRB3	2.38
ADAMTSL4	3.43	CORO2A	2.43	VIPR2	2.17	SH2B3	0.86
PTPRB	2.84	OLFML2A	1.95	TMSB4X	1.66	GFRA2	2.31
RET	0.49	ITGA3	2.65	SUMF1	0.81	CYB5R2	4.47
PTPRE	4.26	CPNE2	1.00	XYLT2	0.76	TMEM59L	0.81
THBS2	9.60	PQLC3	1.46	TMEM100	3.47	DSTNP2	0.94
AQP3	3.09	RPS6KA4	0.88	COL11A2	1.94	BRINP1	2.33
FAM20C	6.85	C1QTNF6	1.51	ITPR3	1.45	SPON1	4.72
BCL3	2.89	SHISA2	5.42	CDC42BPG	2.45	LOC101926941	0.91
RENBP	1.88	MPP4	5.47	SRD5A3	0.47	ID1	0.82
ALS2CL	1.85	GPC4	4.09	HSPB8	3.13	SH3BGRL3	0.92
ECEL1	3.02	LPAR1	3.99	LTK	1.73	CRELD1	0.78
PHLDA1	3.70	NME3	1.43	FLI1	5.30	PSMB8	3.05
FAM111A	4.50	PBXIP1	2.07	LHX9	4.59	ADAMTS7	0.84
ACTA2	1.68	WTIP	3.04	TSPAN8	2.52	RBCK1	0.64
TPM1	1.89	SSBP2	2.20	SMAGP	2.37	IRF6	1.68
IL13RA2	4.48	CHST1	2.05	FAM196B	5.05	DRAXIN	1.28
MOV10L1	3.23	EMILIN1	1.28	SPRY1	1.64	NOD2	1.93
TMEM150A	1.15	CPT1A	0.76	ASIC3	0.87	CAPS	0.73
SPARC	2.82	C1R	2.74	TTLL3	0.81		

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627

628 **Supplementary Table II. ChIP datasets identified with 723 upregulated transcripts in human**
 629 **Tau-KO cells (Adj P<.01). Analysis performed August 4th 2022.**

ChEA_2016 Term	Adj P	Comment
SUZ12 20075857 ChIP-Seq MESC's Mouse	1.22E-31	PRC2 core
MTF2 20144788 ChIP-Seq MESC's Mouse	3.73E-21	PRC2.1 facultative subunit
ELK3 25401928 ChIP-Seq HUVEC Human	1.83E-17	TX factor
RELA 24523406 ChIP-Seq FIBROSARCOMA Human	1.27E-12	TX factor - NFKB subunit - EZH2 interactor
SUZ12 18974828 ChIP-Seq MESC's Mouse	1.89E-12	PRC2 core
TCF21 26020271 ChIP-Seq SMOOTH MUSCLE Human	1.90E-12	TX factor - basic helix-loop-helix
RACK7 27058665 Chip-Seq MCF-7 Human	1.78E-11	TX regulator - RACK receptor - PRC2 regulator
SUZ12 27294783 Chip-Seq ESCs Mouse	3.39E-11	PRC2 core
EGR1 20690147 ChIP-Seq ERYTHROLEUKEMIA Human	4.40E-11	TX regulator - C2H2 zink finger
SUZ12 18692474 ChIP-Seq MEFs Mouse	9.20E-11	PRC2 core
KDM2B 26808549 Chip-Seq K562 Human	2.20E-10	PRC1 core
EZH2 27294783 Chip-Seq ESCs Mouse	2.20E-10	PRC2 core
JARID2 20075857 ChIP-Seq MESC's Mouse	6.20E-10	PRC2.2 facultative subunit
RNF2 18974828 ChIP-Seq MESC's Mouse	6.31E-10	PRC1 core
EZH2 18974828 ChIP-Seq MESC's Mouse	6.31E-10	PRC2 core
WT1 20215353 ChIP-ChIP NEPHRON PROGENITOR Mouse	6.31E-10	TX factor
RING1B 27294783 Chip-Seq ESCs Mouse	6.75E-10	PRC1 core
SUZ12 18692474 ChIP-Seq MESC's Mouse	1.05E-09	PRC2 core
SUZ12 18555785 ChIP-Seq MESC's Mouse	4.07E-09	PRC2 core
KDM2B 26808549 Chip-Seq SUP-B15 Human	4.83E-09	PRC1 core
SRY 25088423 ChIP-ChIP EMBRYONIC GONADS Mouse	7.06E-09	TX factor - SRY-Box
KLF4 26769127 Chip-Seq PDAC-Cell line Human	3.39E-08	TX - Kruppel
RNF2 27304074 Chip-Seq ESCs Mouse	5.77E-08	PRC1 core
WT1 25993318 ChIP-Seq PODOCYTE Human	7.32E-08	TX factor
JARID2 20064375 ChIP-Seq MESC's Mouse	9.08E-08	PRC2.2 facultative subunit
SOX2 20726797 ChIP-Seq SW620 Human	1.31E-07	TX factor - SRY-Box
RARG 19884340 ChIP-ChIP MEFs Mouse	2.46E-07	TX factor - ligand activated
UBF1/2 26484160 Chip-Seq HMEC-DERIVED Human	3.52E-07	TX factor - RNA transcription
RUNX2 24764292 ChIP-Seq MC3T3 Mouse	6.32E-07	TX factor
TP53 20018659 ChIP-ChIP R1E Mouse	9.40E-07	TX factor - P53 family
SMC1 22415368 ChIP-Seq MEFs Mouse	1.09E-06	cohesin subunit - PRC1 regulator
ZNF217 24962896 ChIP-Seq MCF-7 Human	2.05E-06	TX factor - repressor - PRC2 regulator
SA1 22415368 ChIP-Seq MEFs Mouse	3.08E-06	cohesin subunit
KDM2B 26808549 Chip-Seq JURKAT Human	3.08E-06	PRC1 core
RING1B 27294783 Chip-Seq NPCs Mouse	3.08E-06	PRC1 core
KLF5 25053715 ChIP-Seq YYC3 Human	3.08E-06	TX - Kruppel
ESR2 21235772 ChIP-Seq MCF-7 Human	4.87E-06	TX factor - hormone activated
KDM2B 26808549 Chip-Seq DND41 Human	9.18E-06	PRC1 core
EOMES 21245162 ChIP-Seq HESCs Human	1.05E-05	TX factor - T-box
P300 27058665 Chip-Seq ZR-75-30cells Human	1.49E-05	HAT
JUN 26020271 ChIP-Seq SMOOTH MUSCLE Human	1.49E-05	TX factor - proto-oncogene
KLF6 26769127 Chip-Seq PDAC-Cell line Human	2.39E-05	TX - Kruppel
CJUN 26792858 Chip-Seq BT549 Human	2.39E-05	TX factor - proto-oncogene
CTCF 27219007 Chip-Seq ERYTHROID Human	2.39E-05	TX regulator - zink finger - PRC2 regulator
ATF3 23680149 ChIP-Seq GBM1-GSC Human	2.70E-05	TX factor - CREB

BACH1 22875853 ChIP-PCR HELA AND SCP4 Human	3.63E-05	TX factor - CNC-bZip - PRC2 regulator
KDM2B 26808549 Chip-Seq SIL-ALL Human	6.35E-05	PRC1 core
ZFP281 27345836 Chip-Seq ESCs Mouse	6.35E-05	TX factor - repressor - PRC2 regulator
CEBPD 21427703 ChIP-Seq 3T3-L1 Mouse	7.41E-05	TX factor - bZIP
P63 26484246 Chip-Seq KERATINOCYTES Human	0.00010	TX factor - P53 family
TP63 17297297 ChIP-ChIP HaCaT Human	0.00013	TX factor - P53 family
EED 16625203 ChIP-ChIP MESC's Mouse	0.00014	PRC2 core
NFI 21473784 ChIP-Seq ESCs Mouse	0.00016	TX factor - CTF/NF-I family
EZH2 27304074 Chip-Seq ESCs Mouse	0.00021	PRC2 core
ELF3 26769127 Chip-Seq PDAC-Cell line Human	0.00025	TX factor
LXR 22292898 ChIP-Seq THP-1 Human	0.00025	TX factor - ligand activated
ESR1 21235772 ChIP-Seq MCF-7 Human	0.00033	TX factor - hormone activated
BRD4 25478319 ChIP-Seq HGPS Human	0.00037	TX factor - bromodomain
SMC3 22415368 ChIP-Seq MEFs Mouse	0.00037	cohesin subunit - PRC1 regulator
CLOCK 20551151 ChIP-Seq 293T Human	0.00037	TX factor - regulation of circadian rhythms
CTCF 27219007 Chip-Seq Bcells Human	0.00037	TX regulator - zink finger - PRC2 regulator
SUZ12 16625203 ChIP-ChIP MESC's Mouse	0.00057	PRC2 core
CREB1 26743006 Chip-Seq LNCaP-abl Human	0.00057	TX factor - CREB
CTCF 21964334 Chip-Seq Bcells Human	0.00057	TX regulator - zink finger - PRC2 regulator
TP53 23651856 ChIP-Seq MEFs Mouse	0.00071	TX factor - P53 family
KLF4 18358816 ChIP-ChIP MESC's Mouse	0.00091	TX - Kruppel
SOX9 24532713 ChIP-Seq HFSC Mouse	0.00097	TX factor - SRY-Box
NUCKS1 24931609 ChIP-Seq HEPATOCYTES Mouse	0.00097	TX regulator - dna repair

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631 **Supplementary Table III. Epigenomic datasets identified with 723 upregulated transcripts in**
 632 **human Tau-KO cells (Adj P<.01). Analysis performed August 4th 2022.**

Epigenomics Roadmap HM ChIP Term	Adj P	Comment
H3K27me3 H1	6.4E-23	H3K27me3
H3K27me3 Colonic Mucosa	1.2E-18	H3K27me3
H3K27me3 Mobilized CD34 Primary Cells	2.9E-16	H3K27me3
H3K27me3 Fetal Brain	1.8E-15	H3K27me3
H3K27me3 iPS-20b	1.2E-11	H3K27me3
H3K27me3 iPS DF 19.11	7.3E-11	H3K27me3
H3K27me3 CD34 Primary Cells	3.1E-10	H3K27me3
H3K27me3 H9	4.2E-10	H3K27me3
H3K27me3 CD8 Memory Primary Cells	3.1E-09	H3K27me3
H3K27me3 iPS DF 6.9	1.4E-08	H3K27me3
H3K27me3 CD4+ CD25- CD45RA+ Naive Primary Cells	2.2E-08	H3K27me3
H3K27me3 CD8 Naive Primary Cells	3.2E-08	H3K27me3
H3K27me3 Rectal Smooth Muscle	4.0E-08	H3K27me3
H3K27me3 H1 BMP4 Derived Mesendoderm Cultured Cells	9.7E-08	H3K27me3
H2BK20ac IMR90	1.7E-07	H2BK20ac
H3K27me3 CD4+ CD25int CD127+ Tmem Primary Cells	2.6E-07	H3K27me3
H3K27me3 Duodenum Mucosa	3.1E-07	H3K27me3
H3K27me3 iPS-15b	1.3E-06	H3K27me3
H3K27me3 H1 BMP4 Derived Trophoblast Cultured Cells	1.9E-06	H3K27me3
H3K4me1 CD4+ CD25- CD45RA+ Naive Primary Cells	9.5E-06	H3K4me1
H3K27me3 Rectal Mucosa	1.6E-05	H3K27me3
H3K4me1 H1	3.2E-05	H3K4me1
H3K4me1 Brain Germinal Matrix	3.4E-05	H3K4me1
H2BK20ac H1	3.5E-05	H2BK20ac
H3K27me3 Brain Germinal Matrix	4.6E-05	H3K27me3
H3K4me1 IMR90	5.8E-05	H3K4me1
H3K27me3 CD4+ CD25- CD45RO+ Memory Primary Cells	1.4E-04	H3K27me3
H3K27me3 Pancreatic Islets	1.9E-04	H3K27me3
H3K27me3 CD3 Primary Cells	2.4E-04	H3K27me3
H3K27me3 Neurosphere Cultured Cells Cortex Derived	2.5E-04	H3K27me3
H2BK12ac IMR90	2.7E-04	H2BK12ac
H3K4me1 CD4+ CD25- Th Primary Cells	2.7E-04	H3K4me1
H3K4me1 Fetal Brain	2.8E-04	H3K4me1
H3K27me3 CD4 Naive Primary Cells	3.2E-04	H3K27me3
H3K27me3 Stomach Mucosa	4.3E-04	H3K27me3
H3K27me3 CD4+ CD25+ CD127- Treg Primary Cells	5.2E-04	H3K27me3
H3K27me3 Fetal Lung	7.6E-04	H3K27me3
H3K27me3 CD4+ CD25- Th Primary Cells	8.1E-04	H3K27me3
H3K27me3 Duodenum Smooth Muscle	0.0011	H3K27me3
H2BK15ac IMR90	0.0018	H2BK15ac
H3K27me3 Brain Hippocampus Middle	0.0021	H3K27me3
H3K27me3 CD4 Memory Primary Cells	0.0028	H3K27me3
H2BK120ac IMR90	0.0031	H2BK120ac
H3K27me3 Brain Substantia Nigra	0.0031	H3K27me3
H3K4me1 iPS DF 6.9	6.6E-03	H3K4me1

633 **Supplementary Table IV: qPCR primers**

mRNA	Forward primer (5'-3')	Reverse primer (5'-3')
EZH2	GACCTCTGTCTTACTTGTGGAGC	CGTCAGATGGTGCCAGCAATAG
SUZ12	CCATGCAGGAAATGGAAGAATGTC	CTGTCCAACGAAGAGTGAAGTGC
IGFBP3	CGCTACAAAGTTGACTACGAGTC	GTCTTCCATTTCTCTACGGCAGG
GPR37	TTCTGCCTTCCGCTGGTCATCT	TGAAGGTGGTGACTCCCAGAGA
ITGA3	GCCTGACAACAAGTGTGAGAGC	GGTGTTTCGTCACGTTGATGCTC
MRC2	GGCAAGGACAAGAAGTGCGTGT	CTTTGGTGACGTTGCTGCGCTT
IRF6	AGAGAAGCAGCCACCGTTTGAG	GATCATCCGAGCCACTACTGGA

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