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RAPID COMMUNICATION

Targeted down-regulation of *Hipp1* ameliorates tau-induced deficits in *Drosophila melanogaster*

Tauopathies, such as Alzheimer's disease (AD), are neurodegenerative diseases characterized by the deposition of neurofibrillary tangles comprising hyperphosphorylated tau protein in the human brain.[1] Given that abnormal epigenetic alterations in heterochromatin configuration have been documented in AD patients and transgenic animal models of AD,[2] we investigated the roles of novel heterochromatin-associated interactors[3] in tauopathies. Using transgenic flies via UAS-Gal4 binary system, we found that knockdown of *Hipp1*(HP1a and insulator partner protein-1)[3] ameliorates tau^{R406W} (referred to as "tau" hereafter for simplicity)[2]-induced locomotion defects, reduced lifespan, and degeneration of brain tissues. Intriguingly, *Hipp1* knockdown restored tau-driven aberrant expression of putative insulator targets and aberrant insulator-mediated epigenetic alterations. HIPP1 may have a role as an insulator-binding partner regarding being implicated in tau-induced neurodegeneration. Moreover, *Hipp1* knockdown in flies overexpressing tau restored the aberrant expression of AD susceptibility genes. These observations provide new insights into the roles of insulator proteins in tauopathies.

Relaxation of heterochromatin plays a crucial role as a mechanistic driver of neurodegeneration in tauopathies[2]; thus, to evaluate the neuronal effects of loss-of-function alleles of novel heterochromatin-associated interactors[3] in flies overexpressing tau via pan-neuronal *elav*-Gal4, we performed the climbing assay. While control flies retained 90% of their climbing ability on day 15, only 40%–50% of tau-expressing flies climbed up the vials. However, deletion mutation of *Hipp1* ($Hipp1^{\Delta DsR}$) in flies overexpressing tau significantly ameliorated the locomotion defect, leading to 60%–70% climbing ability, while loss-of-function mutation of either *HP1a* or *dADD1* in flies overexpressing tau did not significantly affect climbing ability (Fig. S1A). Accordingly, RNAi-mediated reduction of *Hipp1* in flies overexpressing tau significantly ameliorated the locomotion defect, leading to 70%–80% climbing ability (Fig. S1B). We validated the knockdown of *Hipp1* by the *Hipp1* RNAi line via qRT-PCR and the knockdown of its protein level was confirmed, using a GFP-tagged derivative of the endogenous gene ($Hipp1^{GFP}$) generated by CRISPR mutagenesis (Fig. S1B). To validate that the effect on climbing ability was not due to genetic background effects, multiple control RNAi constructs were expressed in flies overexpressing tau, resulting in no significant effect on climbing ability, compared with flies overexpressing tau alone (Fig. S1C). *Hipp1* knockdown in flies overexpressing tau in the neurons did not significantly affect the levels of total tau and phosphorylated tau (S262 and T231) (Fig. S2). Furthermore, significant amelioration of the locomotion defect by knockdown of *Hipp1* in flies overexpressing tau was sustained until day 20; these flies exhibited 60%–70% climbing ability, compared with 20% of flies overexpressing tau alone (Fig. 1A). The median lifespan of tau-expressing flies was significantly reduced to 28 days, compared with 42 days in control flies. However, the median lifespan of flies overexpressing tau with *Hipp1* knockdown was extended to 35 days. Knockdown of *Hipp1* alone slightly increased lifespan (Fig. 1B). Compared with control brain tissue, pan-neuronal expression of tau resulted in extensive perforation and large vacuoles in brain tissue, while *Hipp1* knockdown in flies overexpressing tau ameliorated vacuolization (Fig. 1C). HIPP1, which was recently discovered as a novel HP1a-interacting protein,[3] is well-colocalized with HP1a in heterochromatin on the chromosome. Given that tau promotes neuronal death through heterochromatin loss,[2] we examined whether *Hipp1* knockdown ameliorates tau-induced defects by repairing heterochromatin loss in flies overexpressing tau in their central nervous system. Unexpectedly, *Hipp1* knockdown failed to restore tau-induced heterochromatin loss (data not shown). This indicates that another mechanistic

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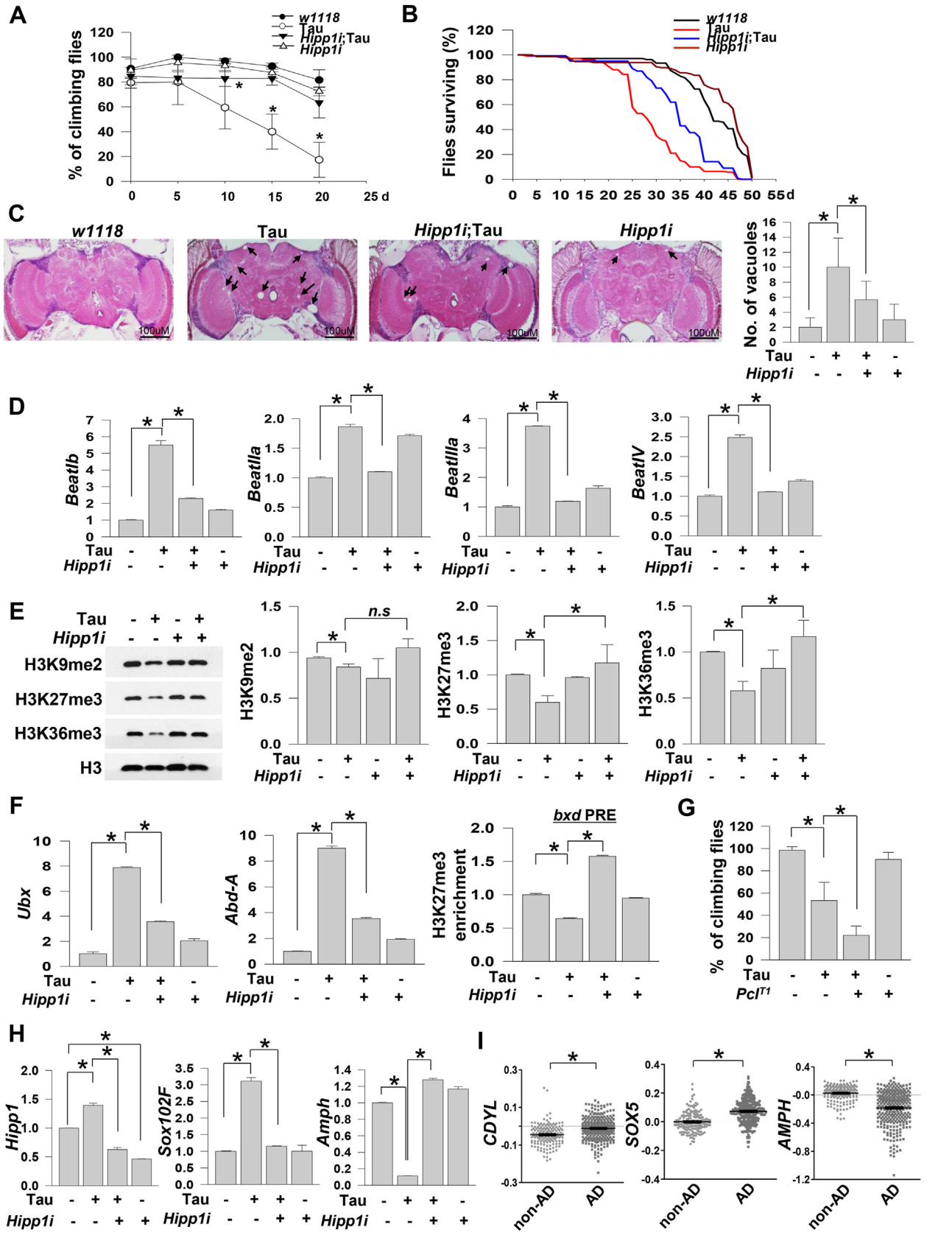

Figure 1 Targeted down-regulation of *Hipp1* ameliorates tau-induced deficits in *Drosophila melanogaster*. **(A)** Locomotor activity from each of the indicated genotypes [*elav*-Gal4/+ (*w1118*), *elav*-Gal4/+; *UAS*-tau^{R406W}/+ (Tau), *elav*-Gal4/*UAS-Hipp1RNAi*; *UAS*-tau^{R406W}/+ (*Hipp1i*;Tau), and *elav*-Gal4/*UAS-Hipp1RNAi* (*Hipp1i*)] was monitored in every consecutive interval of 5 days until 20 days after eclosion (10 flies and 10 repeats per group, respectively; 15 cm/15 s; $n > 5$). **(B)** The survival rate from each of the

driver may be implicated in such amelioration by knocking down *Hipp1*.

HIPP1 has been shown to physically stabilize the interaction between CP190 and SU(HW) as an insulator binding partner in euchromatin[3]; thus, we examined whether the amelioration of tau-induced defects by *Hipp1* knockdown is involved with the insulator mechanism. Using publicly available ChIP-*chip* and ChIP-seq data,[3,5] we confirmed that HIPP1 is well-colocalized with insulator proteins, such as SU(HW), CTCF, CP190, and MOD2.2 on the chromosome (Fig. S3A). We also identified putative target genes with the enrichment of insulator proteins nearby. In particular, the *beat* family genes, which have crucial roles in controlling motor axon guidance in neuromuscular junctions, exhibited multiple binding peaks involving HIPP1, SU(HW), CTCF, CP190, and MOD2.2 (Fig. S3B). Notably, *beat Ib*, *beat IIa*, *beat IIIa*, and *beat IV* expression levels were abnormally increased in flies overexpressing tau. However, *Hipp1* knockdown ameliorated tau-induced abnormal overexpression of these genes (Fig. 1D). Therefore, these observations suggested that overexpression of tau in neurons may interfere with the configuration or mechanism of insulator proteins, resulting in abnormal overexpression of *beat* family genes, whereas *Hipp1* knockdown in a tauopathy background restores their expression.

Because insulators have been characterized as boundary elements to prevent spreading or influencing the chromatin state of the flanking domain which is mediated by distinct sets of epigenetic factors,[4,5] the above results suggested that histone code alteration may occur in tau-expressing flies. As expected, the levels of H3K9me2, H3K27me3, and H3K36me3 were significantly decreased in tau-expressing flies (Fig. 1E). However, *Hipp1* knockdown restored the level of H3K27me3 and H3K36me3 in the tauopathy background (Fig. 1E). The *Ubx-AbdA* locus is normally silenced by H3K27me3 enrichment mediated through the PRC (Polycomb repressive complex)[4,5] and also a well-known target of the insulator complex including HIPP1 which clearly demarcates the boundary of the H3K27me3 binding trail (Fig. S4)[3–5]; this specific region is the most useful readout to monitor the alteration of H3K27me3 binding, likely due to the aberrant configuration of insulator proteins by overexpressing tau. Therefore, we examined whether the reduction of H3K27me3 level in flies overexpressing tau affects the expression of the *Ubx-AbdA* locus. As expected, the expression levels of *Ubx-AbdA* genes were abnormally increased in flies overexpressing tau, while *Hipp1* knockdown in flies overexpressing tau suppressed this derepression, due to restoring the binding level of H3K27me3 (Fig. 1F). Using ChIP assay, we confirmed that the FM6 region flanking the *bxd*-PRE (Polycomb response element) in the *Ubx-AbdA*[4] locus showed reduced binding with anti-H3K27me3 antibody in flies overexpressing tau, while *Hipp1* knockdown in flies overexpressing tau restored the binding level of H3K27me3 (Fig. 1F). Consistently, a loss-of-function allele of *Pcl* (*Polycomb like*), a component of PRC[4,5] exacerbated tau-induced locomotion defects (Fig. 1G). Therefore, these observations suggested that tauopathies may disrupt the binding of H3K27me3, likely due to perturbation of the insulator-mediated boundary, subsequently altering the expression of targets. However, *Hipp1* knockdown in a tauopathy background may restore the insulator-mediated boundary of the H3K27me3 binding trail.

Intriguingly, the expression of *Hipp1* and AD susceptibility gene, *sox102F* was increased in tau-expressing flies; this change was suppressed by *Hipp1* knockdown in flies overexpressing tau (Fig. 1H). Similarly, we found that the expression of human *CDYL*[3] which is homologous to *Hipp1*, and human *SOX5* which is homologous to *sox102F* was significantly higher in postmortem brain tissue from AD patients, using bioinformatics analyses (Fig. 1I). The expression of another AD susceptibility gene, *Amph* was strongly decreased in tau-expressing flies; this change was ameliorated by *Hipp1* knockdown in flies overexpressing tau (Fig. 1H). Interestingly, the level of *AMPH* expression was significantly lower in postmortem brain tissue from AD patients (Fig. 1I). Therefore, *Hipp1* knockdown in flies overexpressing tau may ameliorate tau-induced defects probably by restoring the altered expression of AD susceptibility genes.

indicated genotypes (abovementioned) was monitored for 50 days after eclosion at 28 °C. The numbers of flies analyzed per group were as follows: *w1118* ($n = 135$); tau ($n = 140$); *Hipp1i*;Tau ($n = 120$); *Hipp1i* ($n = 146$). **(C)** Neurodegeneration from each of the indicated genotypes (abovementioned) was monitored 15 days after eclosion. Histological analysis in paraffin sections of adult brain stained with hematoxylin and eosin. Quantification of brain vacuoles ($n = 10–15$ hemispheres). Black arrowheads indicate the brain vacuoles. Scale bar = 100 μM. **(D)** Analysis of *beat* family gene expression in adult heads from each of the indicated genotypes (abovementioned). Each expression level, normalized relative to *RpL32*, is shown relative to the control *w1118*. **(E)** Western blotting analysis of the levels of histone codes in adult heads from each of the indicated genotypes (abovementioned). Quantification of the levels of histone codes ($n = 5$). Each level is shown relative to the control *w1118*. **(F)** Analysis of *Ubx-AbdA* gene expression in adult heads from each of the indicated genotypes (abovementioned). Each expression level, normalized relative to *RpL32*, is shown relative to the control *w1118*. Levels of H3K27me3 on the FM6 amplicon from the *bxd*-PRE were determined by ChIP with chromatin in adult heads from each of the indicated genotypes (abovementioned). **(G)** Locomotor activity from each of the indicated genotypes [*elav*-Gal4/+ (*w1118*), *elav*-Gal4/+; *UAS*-tau^{R406W}/+ (Tau), *elav*-Gal4/*Pcl*^{T1}; *UAS*-tau^{R406W}/+ (*Pcl*^{T1};Tau), and *elav*-Gal4/*Pcl*^{T1} (*Pcl*^{T1})] was monitored 15 days after eclosion (10 flies and 10 repeats per group, respectively; 15 cm/15 s; $n = 5$). **(H)** Analysis of *Hipp1*, *Sox102F*, and *Amph* expression in adult heads from each of the indicated genotypes [*elav*-Gal4/+ (*w1118*), *elav*-Gal4/+; *UAS*-tau^{R406W}/+ (Tau), *elav*-Gal4/*UAS-Hipp1RNAi*; *UAS*-tau^{R406W}/+ (*Hipp1i*;Tau), and *elav*-Gal4/*UAS-Hipp1RNAi* (*Hipp1i*)]. Each expression level, normalized relative to *RpL32*, is shown relative to the control *w1118*. **(I)** Scatter diagrams presenting the mRNA expression levels of human *CDYL* (homologous to *Drosophila Hipp1*), human *SOX5* (homologous to *Drosophila Sox102F*), and human *AMPH* in brain tissues from controls without dementia and Alzheimer's disease patients (Table S1). *$P < 0.05$; ns, $P > 0.05$ between indicated groups.

Author contributions

Conception and experimental design: Sung Yeon Park, Seungbok Lee, and Yang-Sook Chun; Data acquisition: Sung Yeon Park, Jieun Seo, Seulbee Lee, and Joohyung Kim; Data analysis: Sung Yeon Park, Jieun Seo, Seungbok Lee, Sang Jeong Kim, and Yang-Sook Chun; Manuscript drafting: Sung Yeon Park, Seungbok Lee, Sang Jeong Kim, and Yang-Sook Chun. All authors read and concurred with the content of the manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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Data availability

All data sets reported in this study have been submitted to the NCBI Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) under accession numbers GSE20768, GSE20781, GSE20808, GSE20815, GSE23489, GSM1363101, GSM1363107 and modENCODE Chromatin Consortium site (http://modencode.org).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.009.

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