(Supplementary data)

Histone deacetylase (Rpd3) regulates *Drosophila* early brain development via regulation of Tailless

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Running title: Rpd3 and Tailless in brain development

Figure S1. Higher-resolution frontal Z-projections showing the changes in the expression of Tailless and Fas2 in the third instar larval brain.

A. The Z-projections of frontal sections of the late third instar larval brains of the wild-type as well as *Rpd3* heteroallelic mutants were captured using a confocal microscope at 40x magnification. They were immunostained with the Tailless antibody (shown in red) and visualized under higher magnifications. Tailless in the wild-type (*CS*) larval brains localize in the central brain, the Outer Proliferation Centers (OPCs) and the IPCs. In the *Rpd3* heteroallelic mutant larval brain, the expression of Tailless is significantly reduced in the neuropils, particularly in the OPCs and the IPCs. Scale bar, 20µm.

B. When *Rpd3* heteroallelic larval mutant brains were dissected and immunostained using the Fas2 antibody (shown in red), Z-projections stacked together, there was a reduction in the expression of Fas2 in the mutant brain compared to the wild-type (*CS*). The structures of the various lobes of the mushroom body were also altered in the heteroallelic *Rpd3* larval mutant brains, with reduced expression in the calyx, β, β', α, α' and γ lobes. VCX, Ventral calyx; CX, Calyx. Scale bar, 20µm.

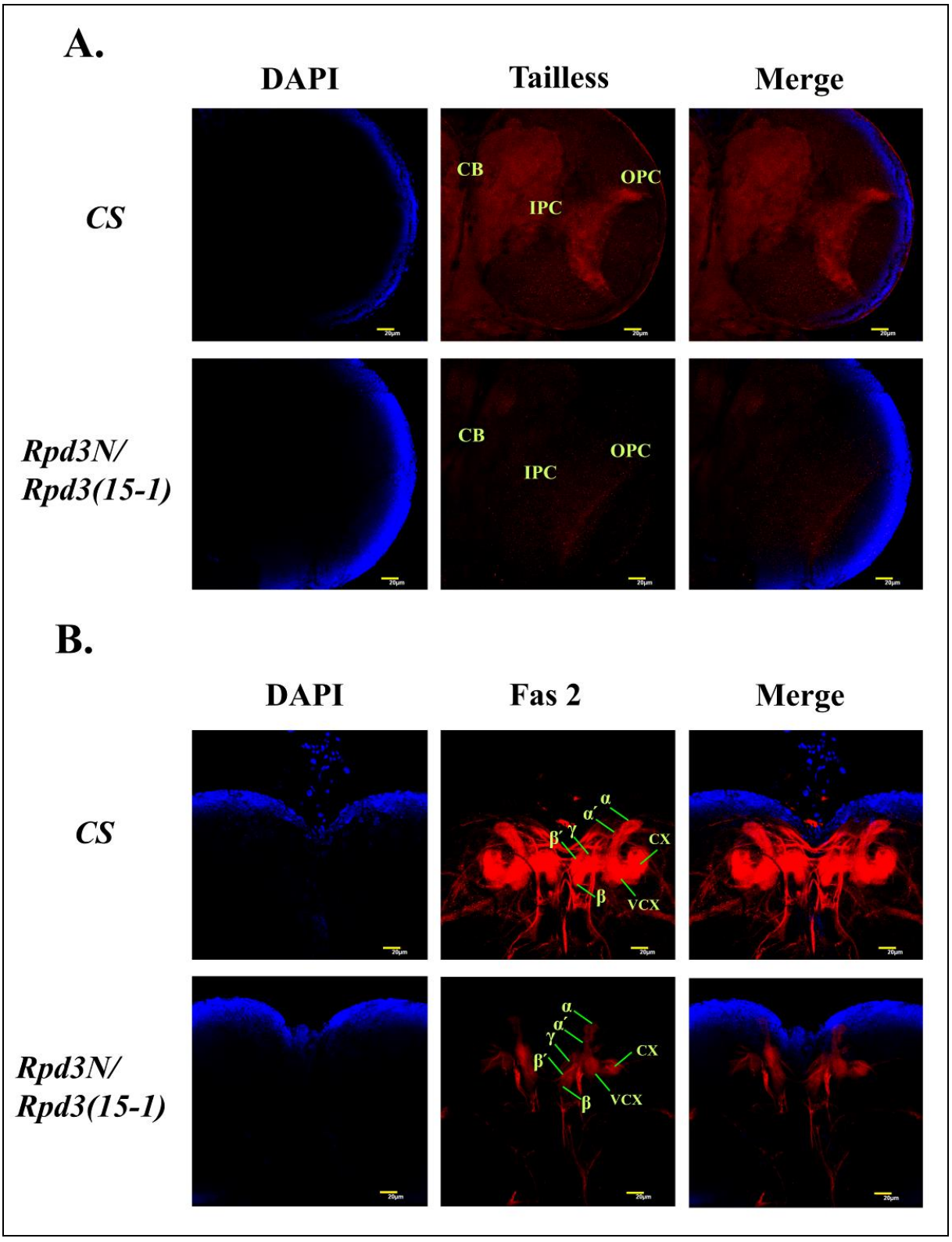

A.
DAPI
Tailless
Merge
CS
CB
OPC
IPC
Rpd3N/
Rpd3(15-1)
CB
OPC
IPC
B.
DAPI
Fas 2
Merge
CS
α
α´
β´
γ
CX
β
VCX
Rpd3N/
Rpd3(15-1)
α
α´
γ
β´
CX
β
VCX

Figure S2. The variation in the expression of Tailless and Fas2 in the interacting genotypes larval brains of the polycomb protein interacting partners of Rpd3: The age-synchronized third instar larval brains of the wild-type (*CS*) and the interacting genotype combinations of *Rpd3* and the polycomb protein mutants, *Pc* and *ph* were immunostained with Tailless and Fas2.

A and B. The late third instar larval brains of the individual mutants for *Pc* and *ph* as well as the interacting genotypes with *Rpd3,* were dissected and immunostained with the Tailless antibody (Tll in red). The images are all Z-projections stacked together. Compared to the wild type (*CS*), there was a decrease in the expression of Tailless in the interacting genotypes for *RpdN/Rpd3(15-1), Pc/+* relative to the individual mutant, *Pc/+*.

C and D. The Z-projections of the late third instar larval brains of the individual mutants for *Pc* and *ph* as well as the interacting genotypes with *Rpd3* that were dissected and immunostained with Fas2 antibody (in red). An increase in the expression of Fas2 was seen in the interacting genotypes *RpdN/Rpd3(15-1), Pc/+* compared to the individual mutant, *Pc/+*. Scale bar, 100µm.

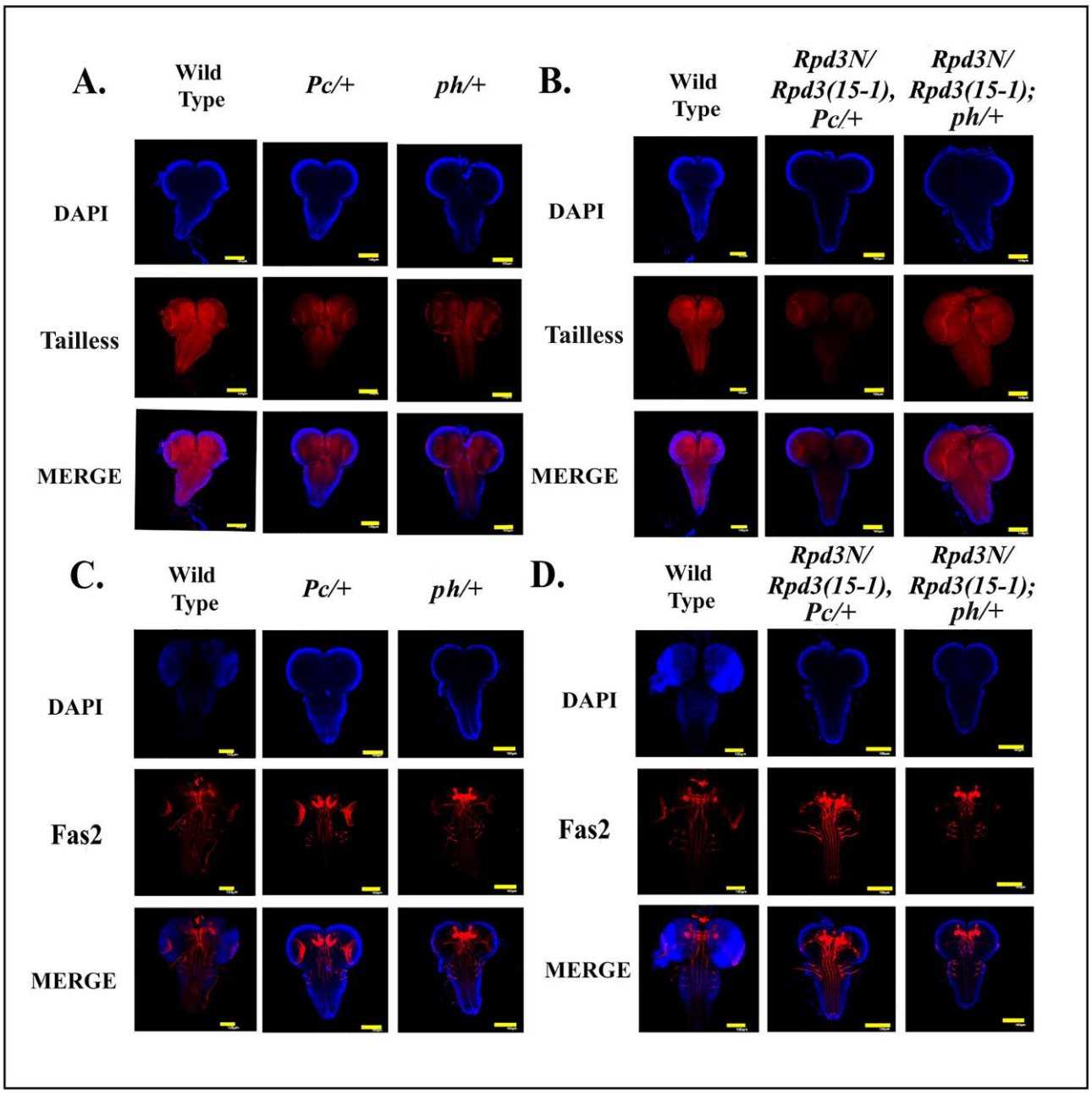

A.
Wild Type
Pc/+
ph/+
DAPI
Tailless
MERGE
B.
Wild Type
Rpd3N/ Rpd3(15-1), Pc/+
Rpd3N/ Rpd3(15-1); ph/+
DAPI
Tailless
MERGE
C.
Wild Type
Pc/+
ph/+
DAPI
Fas2
MERGE
D.
Wild Type
Rpd3N/ Rpd3(15-1), Pc/+
Rpd3N/ Rpd3(15-1); ph/+
DAPI
Fas2
MERGE

Figure S3. Morphology, size, and shape of same-aged synchronized embryos of *Rpd3* heteroallelic mutants.

The age-synchronized embryos of *Rpd3* heteroallelic mutant combination were compared to wild-type (*CS*). The images of each set of embryos (n = 3) were captured at 10x magnification using a confocal microscope and are Z-projections. The genotypes of each combination were marked at the top of the panel.

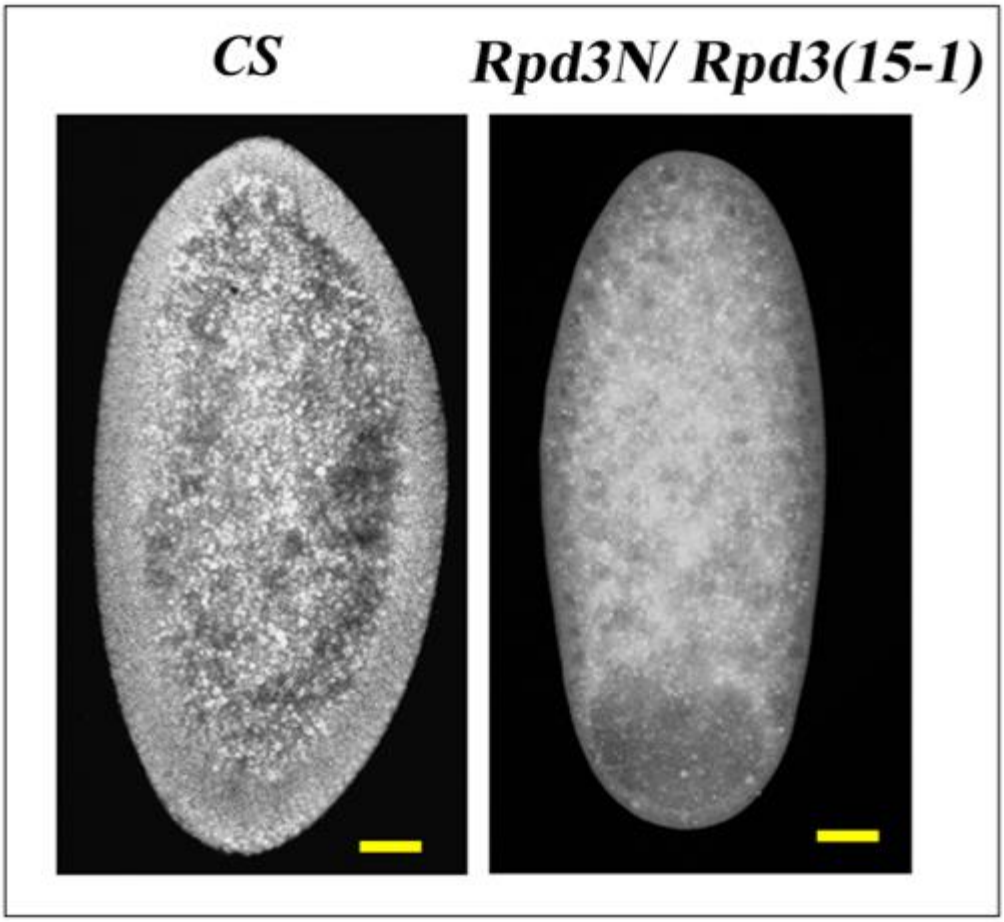
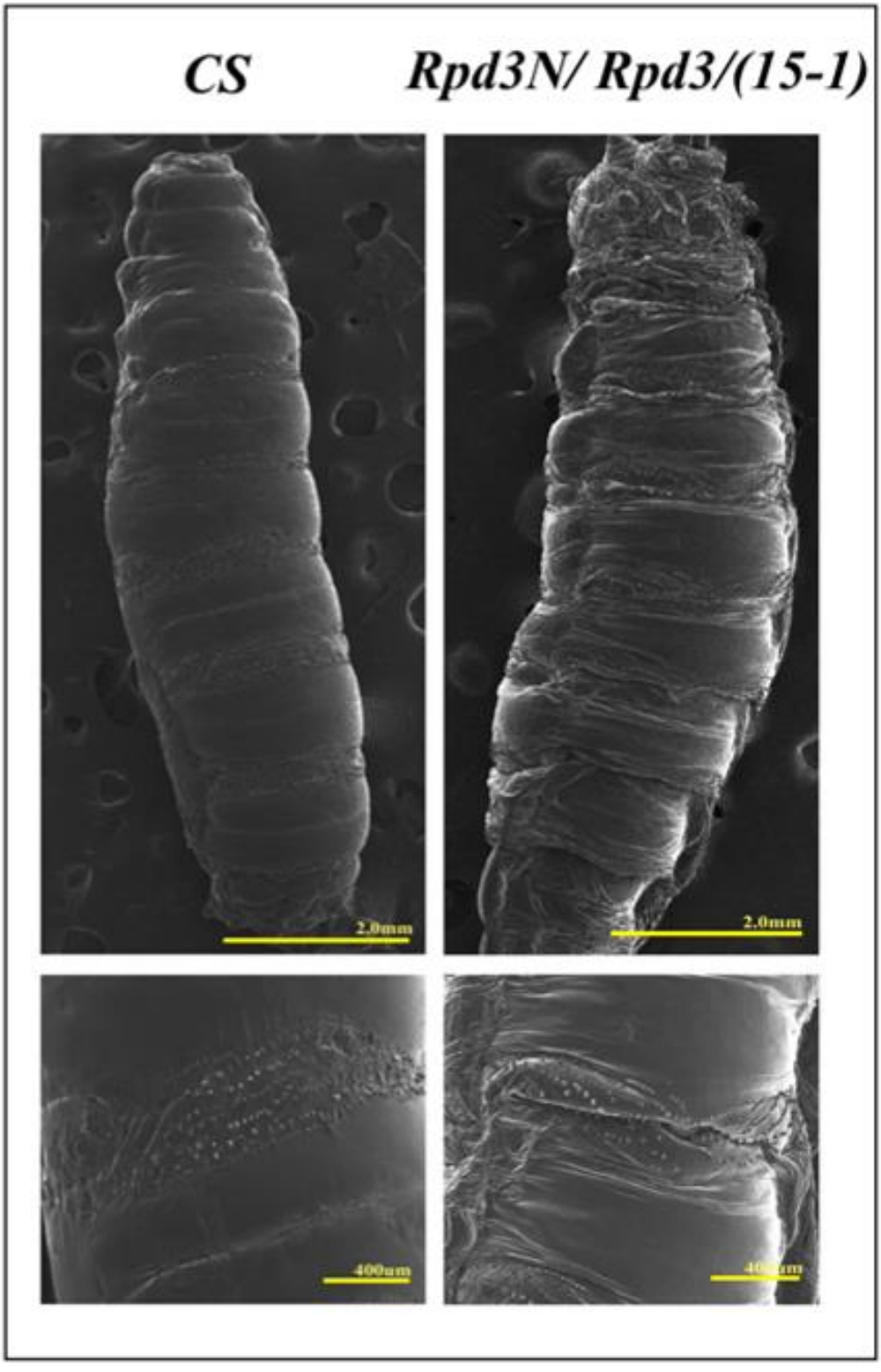

CS
Rpd3N/ Rpd3(15-1)

Table 1.The widths, lengths, and volume of the *Rpd3* mutant embryos of *Drosophila melanogaster*.

Genotype	**Embryo width (mm)**	**Embryo length (mm)**	**Embryos volume (mm^3)x (10^{-3})**
***CS* (wild-type)**	**0.21±0.001**	**0.57±0.0029**	**8.65±0.137**
Rpd3N/Rpd3(15-1)	**0.15±0.001**	**0.54±0.0026**	**5.45±0.111***

Figure S4. Alteration in the cuticular structure of the larva and changes in pattern, distribution, and shape of the denticles.

The ventral epidermal surface of the *Rpd3* heteroallelic combination of larvae was imaged under a scanning electron microscope (SEM) at 27x magnification and compared to that of wild-type (*CS*). The genotypes have been mentioned on top of the panel and underneath each of them, an image was taken at 150x magnification indicating a band of denticles that have been provided for comparison. (Scale bar, 2.0mm for 27x and 400μm for 150x)


CS
Rpd3N/ Rpd3/(15-1)
2.0mm
2.0mm
400um