Figures and figure supplements

Trithorax maintains the functional heterogeneity of neural stem cells through the transcription factor Buttonhead

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Figure 1. *trx* mutant type II neuroblasts display characteristics of a type I neuroblast. Key for all figures: all clones are outlined in yellow. Wild-type type II neuroblasts or mutant type I neuroblasts (Dpn\(^+\)Ase\(^-\)Pros\(^-\); white arrow); Ase\(^-\) immature INPs (Dpn\(^-\)Ase\(^+\)Pros\(^-\); white arrowhead); Ase\(^+\) immature INPs (Dpn\(^-\)Ase\(^-\)Pros\(^-\); yellow arrow); INPs (Dpn\(^-\)Ase\(^+\)erm-lacZ\(^+\)Pros\(^{cytoplasmic}\); yellow arrowhead); GMC generated by INPs (Ase\(^+\)erm-lacZ\(^+\)Pros\(^{nuclear}\); orange arrow); wild-type type I neuroblasts or mutant type II neuroblasts (Dpn\(^-\)Ase\(^+\)erm-lacZ\(^+\); magenta arrow); GMC generated by wild-type type I neuroblasts or mutant type II neuroblasts (Ase\(^+\)Pros\(^+\)erm-lacZ\(^-\); magenta arrowhead). Single asterisks indicate a statistically significant (p-value <0.05) difference between the marked genotype and the control genotype in the same bar graph, as determined by the Student’s t-test. n.s. indicates that the difference is statistically insignificant. NB: neuroblast. (A–D) *trx* mutant type II neuroblasts progressively acquire a type I neuroblast identity. (A–B) In the 72-hr GFP-marked clone, a wild-type type II neuroblast displays a Dpn\(^+\)Ase\(^-\) marker expression profile whereas a *trx* mutant type II neuroblast displays a Dpn\(^+\)Ase\(^+\) expression profile. Scale bar, 10 \(\mu\)m. (C) Three-dimensionally reconstructed images of type II neuroblasts clones of the indicated genotypes. Scale bar, 10 \(\mu\)m. (D) The frequency of *trx* mutant type II neuroblasts displaying a type I neuroblast maker expression profile (PntP1\(^+\)Ase\(^-\)). N = 10 per time point. (E–H) *trx* mutant type II neuroblasts lose the ability to generate INPs. (E) The average number of INPs per staged type II neuroblast clone of the indicated genotype. N = 10 per time point. (F–G) In the 72-hr GFP-marked clones, a wild-type type II neuroblast is surrounded by INPs and their GMC progeny identified by erm-lacZ expression. In contrast, a *trx* mutant type II neuroblast is surrounded by GMCs that are directly derived from neuroblasts and lack erm-lacZ expression. Scale bar, 10 \(\mu\)m. (H) The average number of GMCs with or without erm-lacZ expression per type II neuroblast clone of the indicated genotypes. DOI: 10.7554/eLife.03502.004
Figure 1—figure supplement 1. A diagram of two distinct neuroblast lineages. A summary of the cell fate marker expression profile in type I and type II neuroblast lineage in the larval brain. NB: neuroblast; GMC: ganglion mother cell; INP: intermediate neural progenitor; imm INP: immature INP.
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Figure 2. *trx* mutant type II neuroblast directly generates GMCs. (A–E) *trx* is required for the expansion of supernumerary type II neuroblasts in the *brat* or *erm* mutant. (A–D) Removing *trx* function suppresses the expansion of supernumerary type II neuroblasts and restores differentiation in the 96-hr *brat* or *erm* mutant type II neuroblast clones. Three-dimensionally reconstructed images of the clones are shown to the right. Scale bar, 10 μm. (E) The average number of type II neuroblasts per clone of the indicated genotypes. (F–G) *trx* mutant type II neuroblasts exclusively distribute Pros to their progenies to specify GMC identity. (H) The frequency of wild-type or *trx* mutant mitotic type II neuroblasts displaying the basal cortical localization of Pros. Scale bar, 10 μm. (I) The average number of type I neuroblasts per type II neuroblast clone of the indicated genotypes at 72 hr after clone induction.

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Figure 3  Trx and the core components of the SET/MLL complex maintain a type II neuroblast functional identity dependently on their catalytic activity for H3K4 methylation. (A–B) The function of trx for the H3K4 methylation is required for the maintenance of a type II neuroblast functional identity. (A–B) In the 72-hr clones, a trx^{21} mutant type II neuroblast displays a type I neuroblast marker expression profile and directly generates GMCs. Scale bar, 10 μm. Three-dimensionally reconstructed images of the clones are shown to the right. Scale bar, 10 μm. (C–K) The function of rbbp5 for the H3K4 methylation is required for the maintenance of a type II neuroblast functional identity. (C–E, H, J) In the 96-hr clones, rbbp5^{null} type II neuroblasts display a type I neuroblast marker expression profile and directly generate GMCs. Over-expression of rbbp5^{FL} but not rbbp5^{SG} restores a type II neuroblast functional identity in rbbp5^{null} type II neuroblasts. Three-dimensionally reconstructed images of the clones are shown to the right. Scale bar, 10 μm. (F) The frequency of type II neuroblasts of the indicated genotypes displaying the type I or type II marker expression profiles. (G, I, K) rbbp5 function is essential for the H3K4 methylation in fly larval brains. Scale bar, 10 μm.

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**Figure 3—figure supplement 1.** Decreasing the function of the core components of the SET1/MLL complex leads to a reduction in type II neuroblasts. (A–E) Knocking down the function of trx, rbbp5, wds or ash2 specifically reduces the number of type II neuroblasts per brain lobe. Scale bar, 20 μm. (F–G) The average number of type II neuroblasts or INPs per brain lobe of the indicated genotypes after knocking down the function of trx, rbbp5, wds, or ash2 for 72 hr.

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**Figure 3—figure supplement 2.** Generation of the rbbp5<sup>5ui</sup> allele and the UAS-rbbp5<sup>SG</sup> transgene. (A) The genomic organization of the rbbp5 locus. The rbbp5<sup>5ui</sup> allele was generated via imprecise excision of the P(EP) G4226 element, which removes the entire rbbp5 coding region. Yellow squares indicate the coding exons of rbbp5 while blue squares indicate the untranslated regions. The red line indicates the molecular lesion induced by the rbbp5<sup>5ui</sup> allele. (B) The average number of INPs per clone of the indicated genotypes at 96 hr after clone induction. (C) An alignment of the hinge region of the yeast, fly, and human Rbbp5 protein. The amino acid substitutions in the Rbbp5<sup>SG</sup> transgenic protein are indicated in red.

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Figure 4: Btd likely acts downstream of Trx to maintain a type II neuroblast functional identity. (A–D) The btd gene is an excellent candidate target of Trx in the type II neuroblast. (A) The btd mRNA is highly enriched in the lysate extracted from larval brain enriched with type II neuroblasts. The elav transcript is highly enriched in differentiated neurons. The quantification represents the average of three biological replicates. (B) Trx directly binds to the type II neuroblast-specific enhancer element as well as the transcription start site (TSS) of the btd gene. The ChIP experiments were performed using the extract isolated from dissected brat mutant brains that are enriched with type II neuroblasts. Quantification of chromatin immunoprecipitated by the indicated antibodies relative to 5% of input. The quantification represents the average of three biological replicates. (C–D) An enhancer element from the btd gene is sufficient to induce type II neuroblast-specific expression of a UAS-mCD8::gfp reporter transgene in wild-type brain, while the enhancer activity of btd-Gal4 was reduced in rbbp5 mutant brain. Scale bar, 20 μm. (E–F) btd is required for maintaining the functional identity but not the molecular signature of a type II neuroblast. (E–F) In the 72-hr clones, btd mutant type II neuroblasts maintain a type II neuroblast marker expression profile and are surrounded by 1–2 immature INP-like cells. Three-dimensionally reconstructed images of the clones are shown below. Scale bar, 10 μm. (G) The average number of INPs per clone of the indicated genotypes. (H) The average number of GMCs with or without erm-lacZ expression per type II neuroblast clones of the indicated genotypes at 72 hr after clone induction. (I–J) The immature INP-like cells generated by btd mutant type II neuroblasts are insensitive to loss of brat function. Removing brat function does not lead to supernumerary neuroblast formation in the 72-hr btd mutant type II neuroblast clones. Scale bar, 10 μm.

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Figure 4—figure supplement 1  Global H3K4 mono- or tri-methylation is not required for maintenance of a type II neuroblast functional identity. (A–H) The core component of the SET1/MLL complex is required for the global methylation of H3K4. (A, C, E, G) Knocking down the function of ash2 or trr leads to global loss of the H3K4 mono-methylation while knocking down the function of dSet1 does not. Scale bar, 10 μm. (B, D, F, H) Knocking down the function of ash2 or dSet1 leads to global loss of the H3K4 mono-methylation while knocking down the function of trr does not. (I–J) trr and dSet1 are dispensable for the maintenance of type II neuroblasts. (I–J) The average number of type II neuroblasts or INPs per brain lobe of the indicated genotypes after knocking down the function of trr or dSet1 for 72 hr. (K–N) trx mutant type II neuroblasts do not display appreciable reduction in the global methylation pattern. Scale bar, 10 μm.
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Figure 4—figure supplement 2  Pnt likely functions to specify an INP identity.  (A) Trx directly binds to transcription start site (TSS) of the pntP1 transcript. Quantification of chromatin immunoprecipitated by the indicated antibodies relative to 5% of input. The quantification represents the average of three biological replicates. The black lines indicate three different pnt transcripts. The magenta lines indicate three UAS-RNAi used to target the common exon of pnt transcripts.  (1) UAS-pntRNAi(7171), (2) UAS-pntRNAi(TRiP.JF02227), and (3) UAS-pntRNAi(TRiP:HMSO1452). (B–C) Expression of the UAS-pntRNAi transgene efficiently reduces PntP1 protein expression throughout the type II neuroblast lineage. (D–E) Knocking down the function of pnt induces supernumerary neuroblast formation. Scale bar, 10 μm.  (F–G) The average number of type II neuroblasts per clone of the indicated genotypes.  DOI: 10.7554/eLife.03502.012
Figure 5. Over-expression of btd is sufficient to instruct a type II neuroblast functional identity in the type I neuroblast. (A–E) Over-expression of btd is sufficient to elicit a type II neuroblast functional identity. (A–D) In the 72-hr clones, 18% of type I neuroblasts over-expressing btd lose Ase expression and are surrounded by INP-like cells. An additional 10% of these neuroblasts maintain Ase expression despite being surrounded by INP-like cells. Three-dimensionally reconstructed images of the clones are shown to the right. Scale bar, 10 μm. (E–H) Progeny of type I neuroblasts over-expressing btd revert back to supernumerary neuroblast in the brat mutant or erm mutant. In the 72-hr clones, removing brat or erm function induces the formation of supernumerary type II neuroblasts derived from the progeny of type I neuroblasts over-expressing btd. Three-dimensionally reconstructed images of clones are shown to the right. Scale bar, 10 μm.

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Figure 6 Over-expression of btd restores a type II neuroblast functional identity in trx mutant type II neuroblasts. (A–D) Overexpression of btd reinstates the ability to generate INPs in trx mutant type II neuroblasts. (A–B) In the 72-hr clones, while the control trx mutant type II neuroblasts are surrounded by GMCs, trx mutant type II neuroblasts over-expressing btd are surrounded by INP progeny. Three-dimensionally reconstructed images of the clones are shown to the right. Scale bar, 10 μm. (C) The neuroblast marker expression profile displayed by type II neuroblasts of the indicated genotypes. (D) The average number of INPs per clone of the indicated genotypes. DOI: 10.7554/eLife.03502.014
**Figure 7** A summary model. The Trx histone methyltransferase complex maintains the type II neuroblast functional identity through the btd gene whereas it promotes INP identity specification through the pnt gene.

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