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Supplementary Material

corresponding to:

Repression of differentiation genes by Hes transcription factors fuels neural tumour growth in Drosophila

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Table S1. Transcriptome analysis of T0 vs primary (FACS) DM tumours. RNA-seq differential analysis complete output by SARTools/edgeR.

Table S2. Transcriptome analysis of T3 vs primary (FACS) DM tumours. RNA-seq differential analysis complete output by SARTools/edgeR.

Table S3. Transcriptome analysis of T0 vs T3 DM tumours. RNA-seq differential analysis complete output by SARTools/edgeR.

Table S4. Metascape analysis of GO terms enriched in DEG lists from allografted tumours compared to the primary (FACS) tumour. Up- and down-regulated genes from T0 and T3 (4 lists) were used and are indicated in the output.

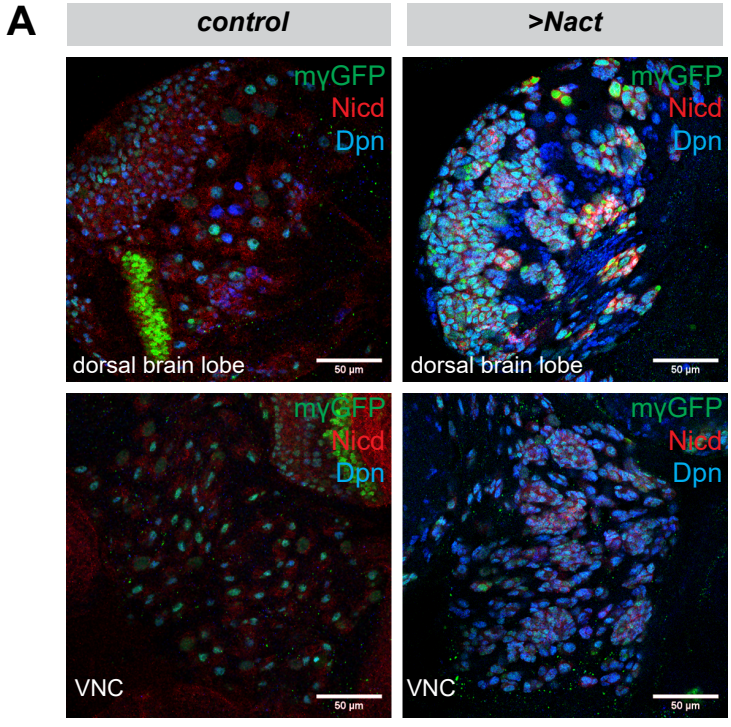


Fig. S1. The expression pattern of *Hes* genes *E(spl)mγ* and *dpn* in larval neural stem cell hyperplasias. Confocal images of brain lobes (top) or VNCs (bottom) form control (left) or NΔecd hyperplastic (right) larval central nervous system. Notch hyperplasias were generated by driving a constitutively active form of Notch (NΔecd) via a neuroblast specific Gal4 driver (*grhNB-Gal4*) for 48h. E(spl)mγ-GFP (green) and Dpn (blue) are expressed in neural stem cells and ectopic NSC-like cells. High levels of NΔecd (using anti-Nicd, an antibody against the intracellular domain of Notch, red) are present in hyperplastic lineages. Scale bar 50µm.

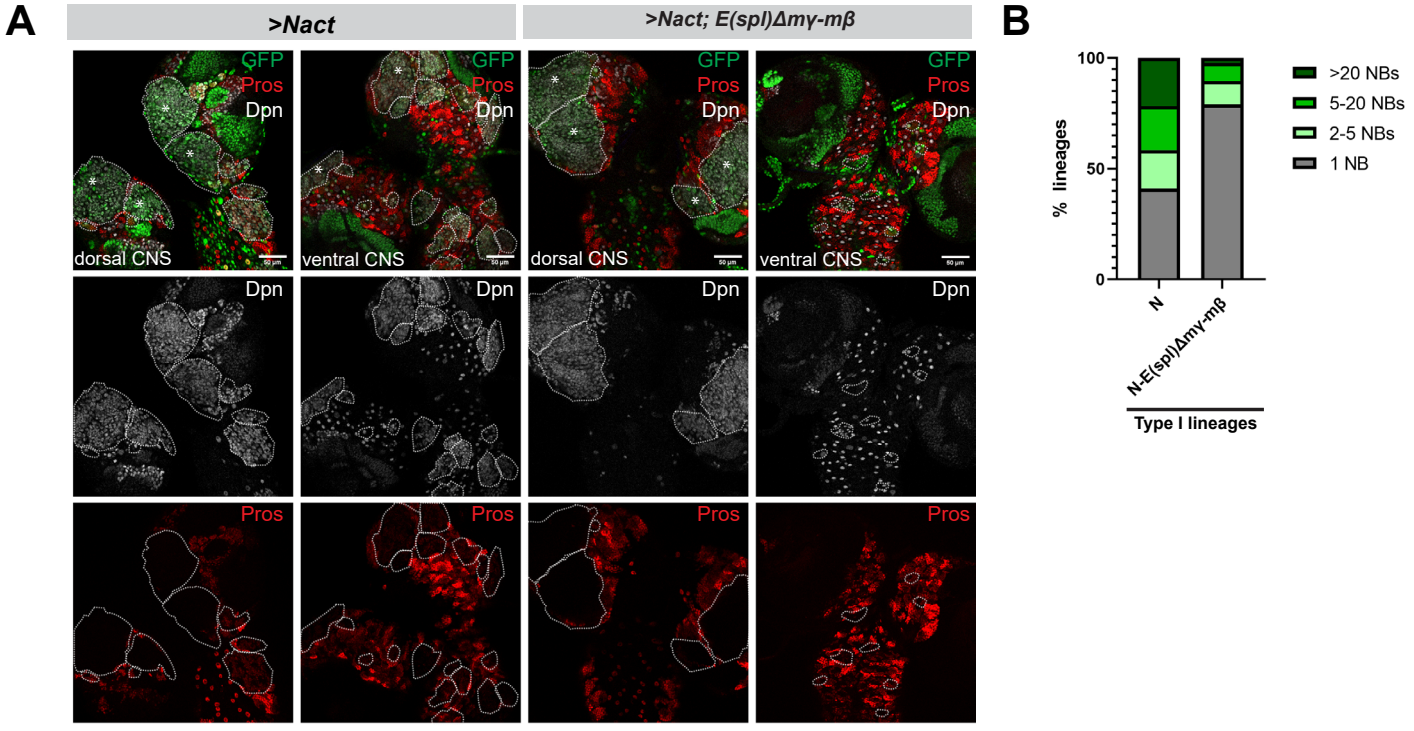


Fig. S2. Loss of *E(spl)mγ* and *E(spl)mβ* mildly affects the Notch-induced hyperplasia in the larval central nervous system. (A) Confocal images of larval brains carrying *act-F/O* clones (marked by GFP and white dotted outlines) of the indicated genotypes [*NΔecd* in *wt* vs *N* in *E(spl)Δmγ-mβ*) background] stained for Dpn (grey) and the early neuronal marker Pros (red). Scale bar 50µm. Type II clones are marked with asterisks. **(B)** Diagram depicting the frequency of Type I neuroblast clones with one or multiple Dpn-positive cells per clone in the two genotypes described above: *NΔecd* in *wt* (202 clones from 7 animals were scored) vs *NΔecd* in *E(spl)Δmγ-mβ*) (381 clones from 8 animals were scored). Clones were scored at 4 days after clone induction.

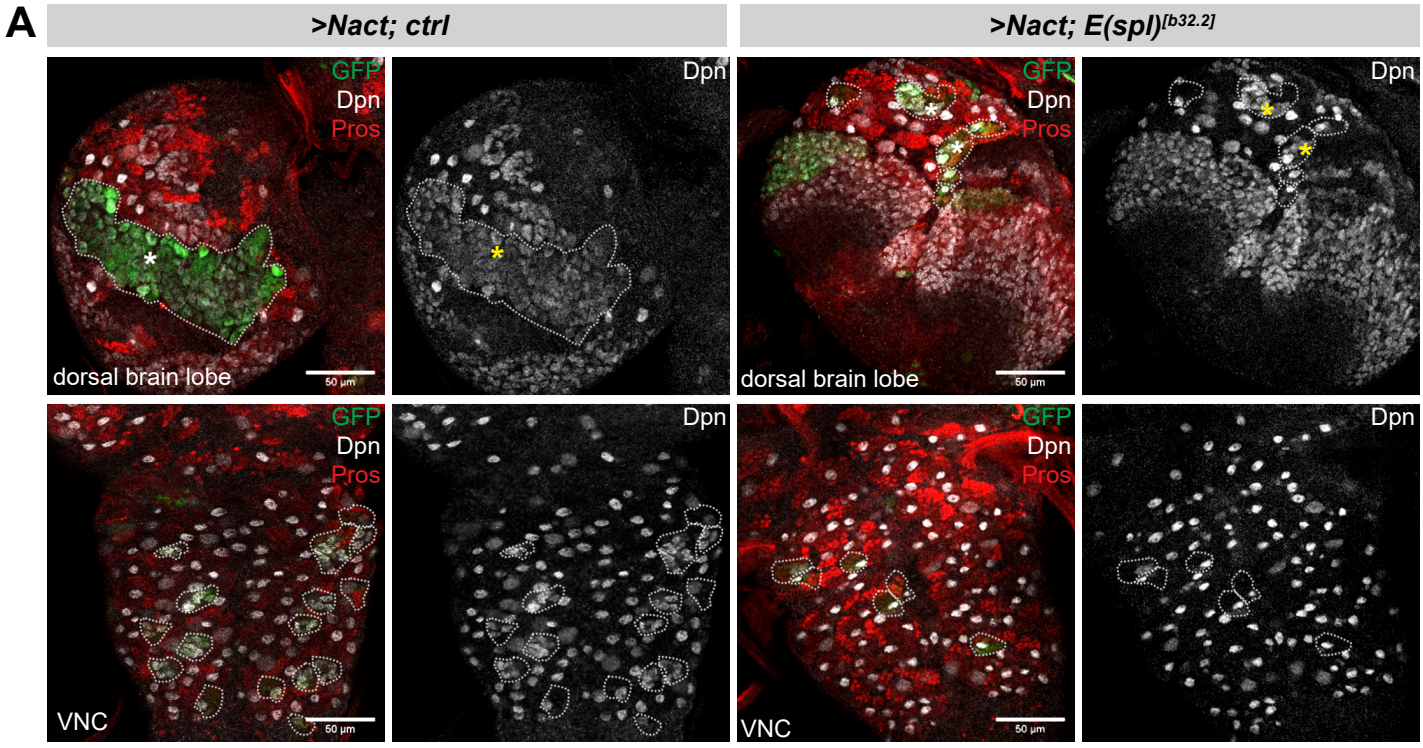


Fig. S3. Loss of the entire *E(spl)* locus severely affects both Type I and Type II *NΔecd* hyperplastic lineages in the larval central nervous system. (A) Confocal images of brain lobes (top) and VNCs (bottom) from larval brains with MARCM clones (marked by GFP and white dotted outlines) of the indicated genotypes [*NΔecd* in *ctrl* vs *NΔecd in E(spl)*$^{b32.2}$ background]. Dpn (grey) stains neural stem cells and neural stem cell-like cells and Pros (red) is an early neuronal marker. Note that Type II *NΔecd-E(spl)*$^{b32.2}$ lineages (marked by asterisks) have fewer neuroblast-like cells (Dpn-positive) than control, whereas almost all Type I lineages revert to having a single Dpn-positive cell. Scale bar 50µm.

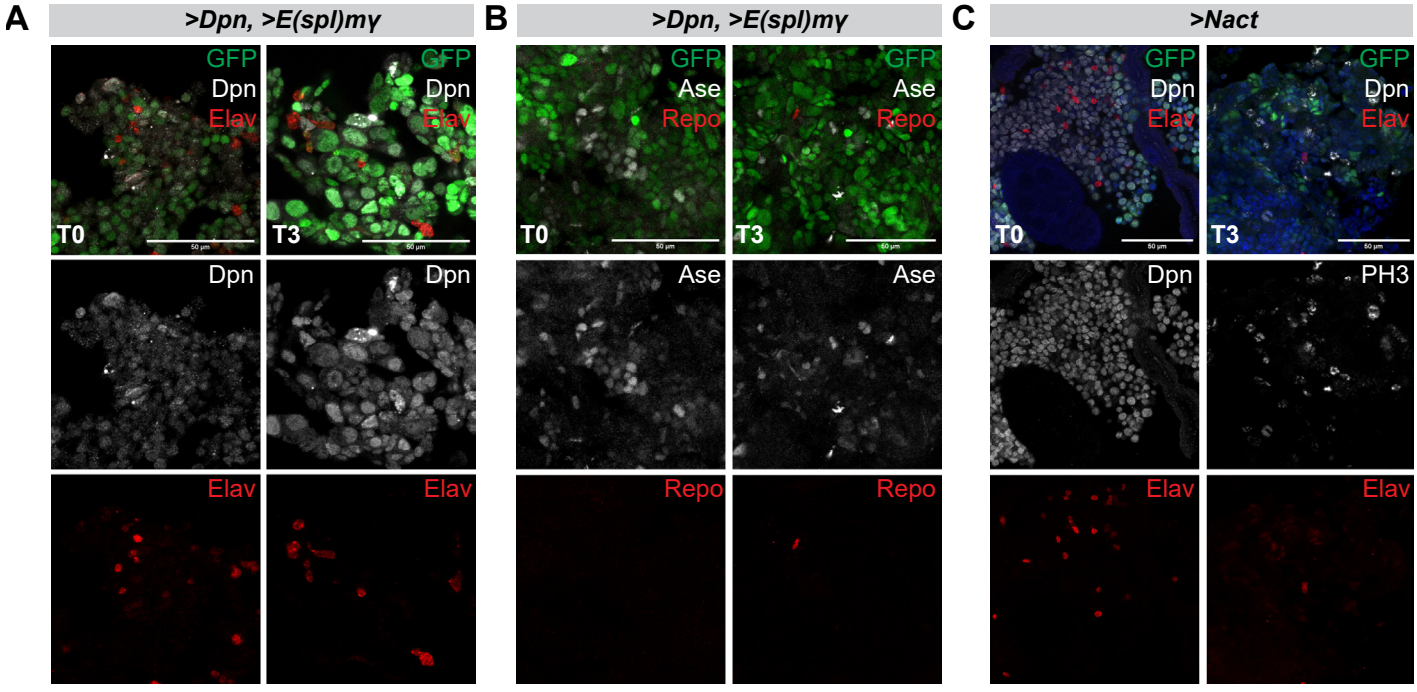
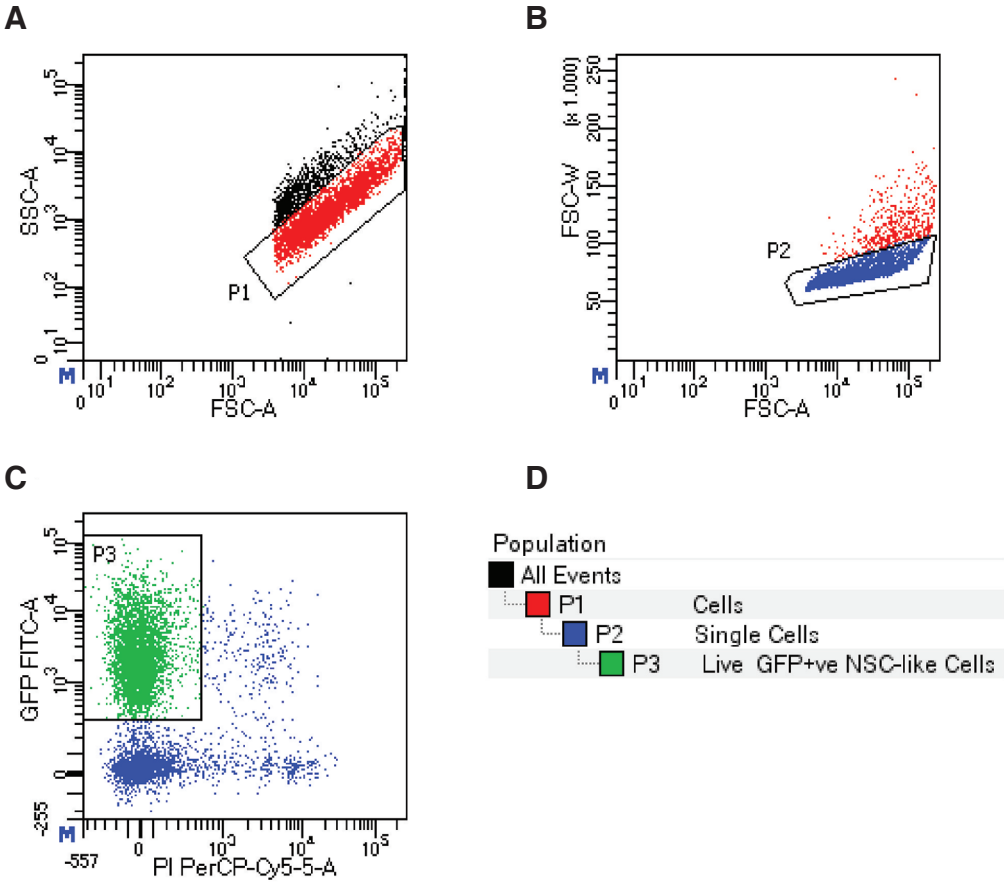


Fig. S4. Hes allograft neural stem cell tumours exhibit limited neurogenesis and no gliogenesis. (A-C) Confocal images of DM (A-B) or NΔecd (C) allograft tumour fragments recovered from the abdomen of host flies after the first (T0) or fourth (T3) serial round of transplantation. These fragments are stained for Dpn (grey) or Ase (grey) to mark neural stem cell-like cells and for the neuronal marker Elav (red) or the glia marker Repo (red). Note that T3 allograft tumours have many cells that are larger in size compared to T0 allograft tumours. Scale bar 50µm.



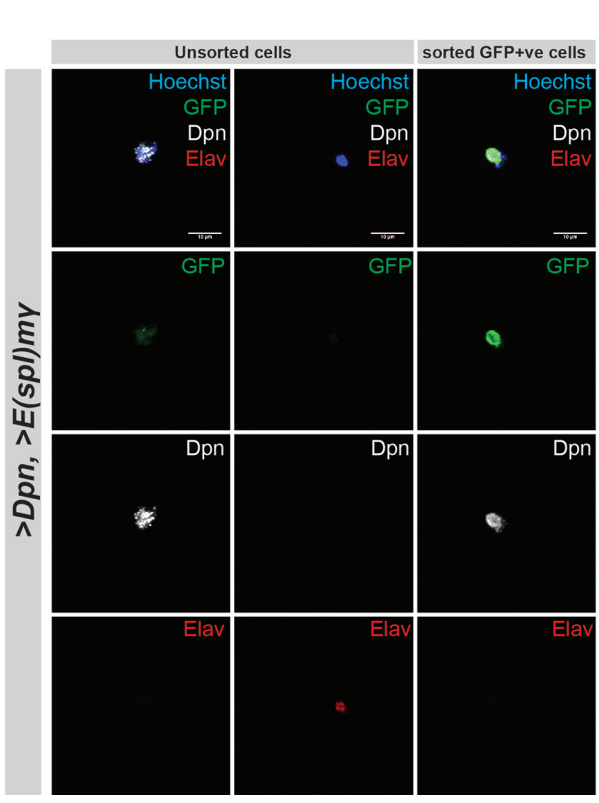


Fig. S5. FACS purification of larval GFP positive NSC-like cells. (A-D) Plots from representative flow cytometry data illustrating the gating strategy and hierarchy for FACS purification of larval NSC-like cells. **(A)** SSC-A vs FSC-A, log plot. The lower SSC-A signal population is chosen to define the Cell sorting gate P1 and exclude debris (higher SSC-A signal). **(B)** FSC-W vs FSC-A, log plot. The population with a low FSC width (W) versus area (A) signal is chosen to define the Single Cell sorting gate P2 and exclude cell aggregates. **(C)** GFP-FITC-A vs propidium iodide (PI PerCP-Cy5.5-A), log plot. The population with a higher GFP signal and a low PI signal is chosen to define the final Live, GFP-positive, NSC-like Cell sorting gate P3. **(D)** Gating hierarchy. **(E)** Cells from the dissociated larval CNS before and after FACS sorting. Dpn (grey) stains for *DM* overexpressing neural stem cells and NSC-like cells and Elav (red) for neurons. Hoechst (blue) marks nuclei. Scale bar 10µm.