Supplement Figure1

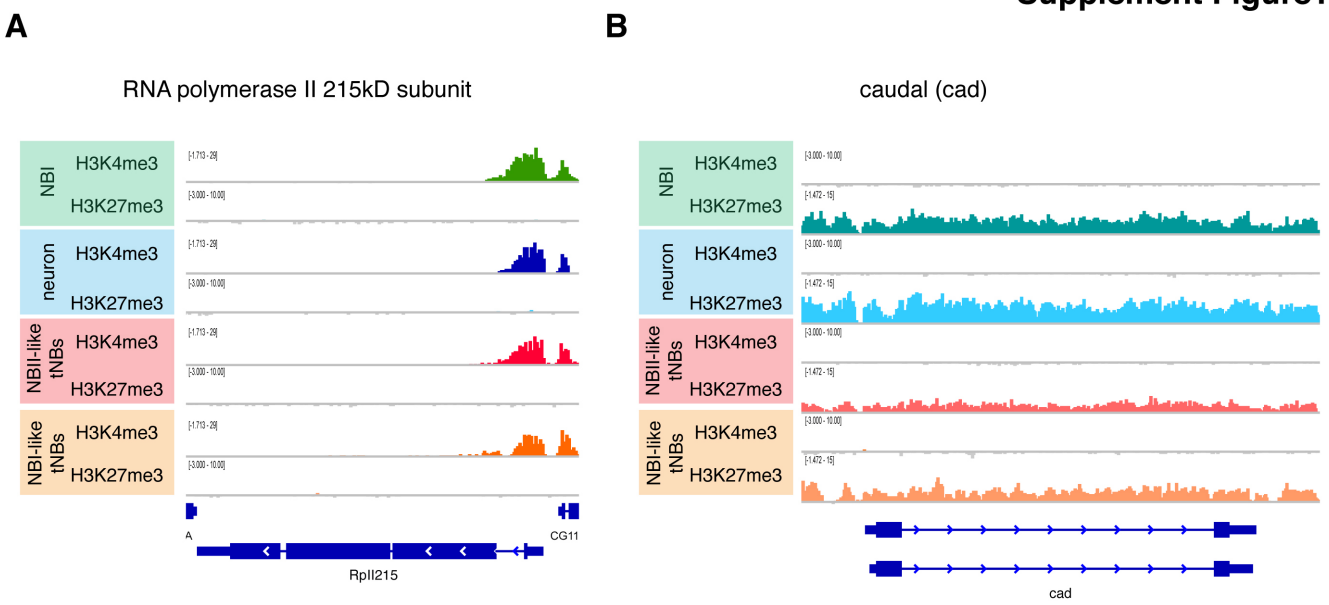

Figure S1. Examples of H3K4me3 and H3K27me3-dependent gene loci.

H3K4me3 and H3K27me3 distribution of RpLL215 (A), caudal (B).

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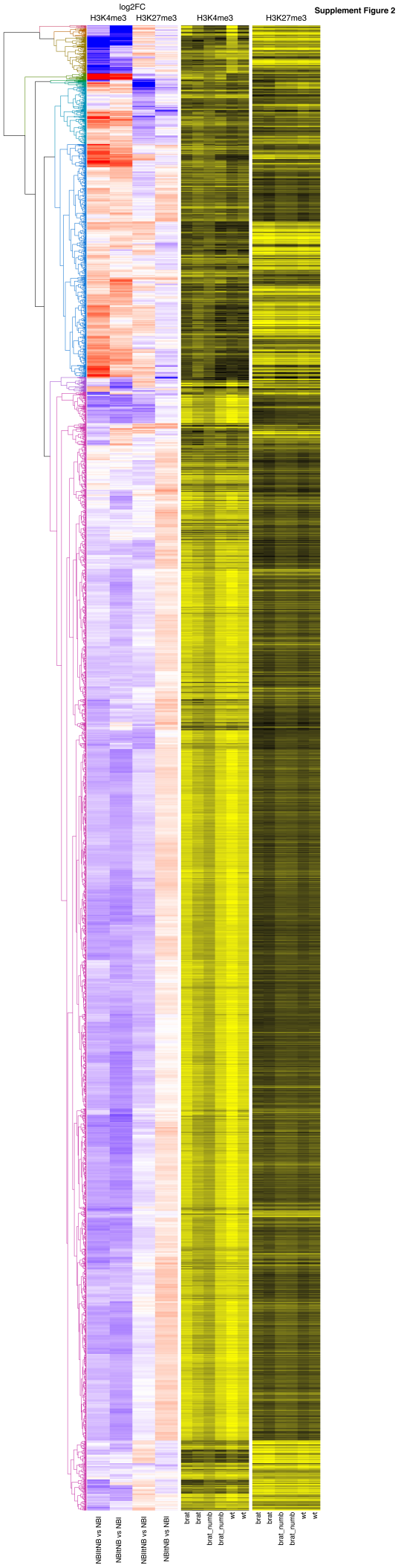

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Figure S2. Full heatmap of H3K4me3 and H3K27me3 changes between NB samples.

Comprehensive hierarchical clustering analysis for NB samples presented in Figure 3.

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Supplement Figure 3

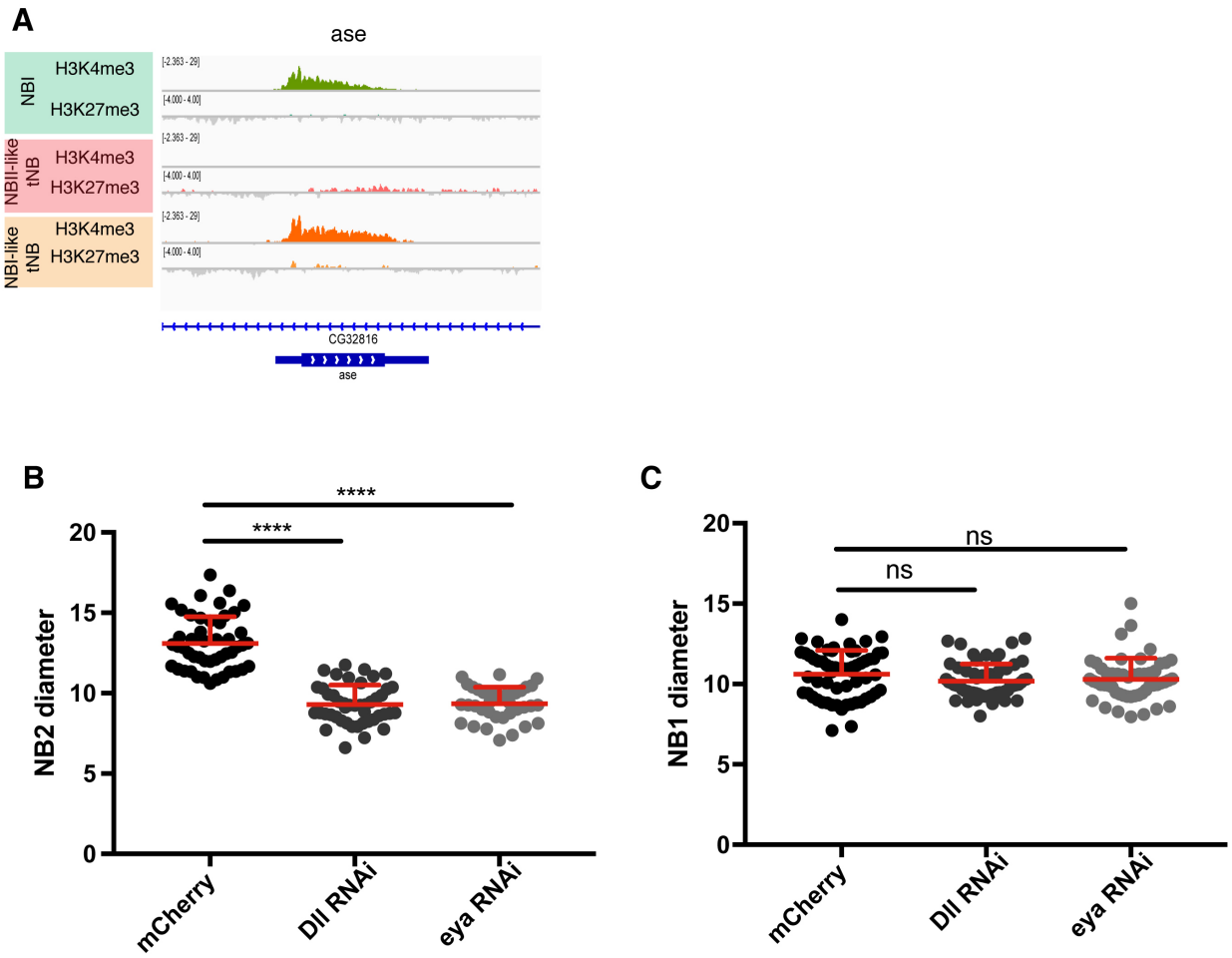

Figure S3. Dll and eya knockdown affect NBII but not NBI size.

(A) H3K4me3 and H3K27me3 distribution at the *Asense* locus. Measurements of NBII (B) and NBI (C) diameter. Driver line used was UAS-*dicer2*; *insc*-Gal4, UAS-CD8::GFP. Mean ± SD is shown. (for NBII diameter (B) control = 13.09±1.66 (n = 48), *Dll* = 9.29±1.21 (n = 45) and *eya* = 9.34±1.02 (n = 37), and for NBI diameter (C) control = 10.61±1.48 (n=60), *Dll* = 10.17± 1.07 (n=60) and *eya* = 10.28±1.33 (n=52)). One-way ANOVA test was used and ****p < 0.0001, ns = not significant. n numbers are lineages quantified.

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Supplement Figure 4

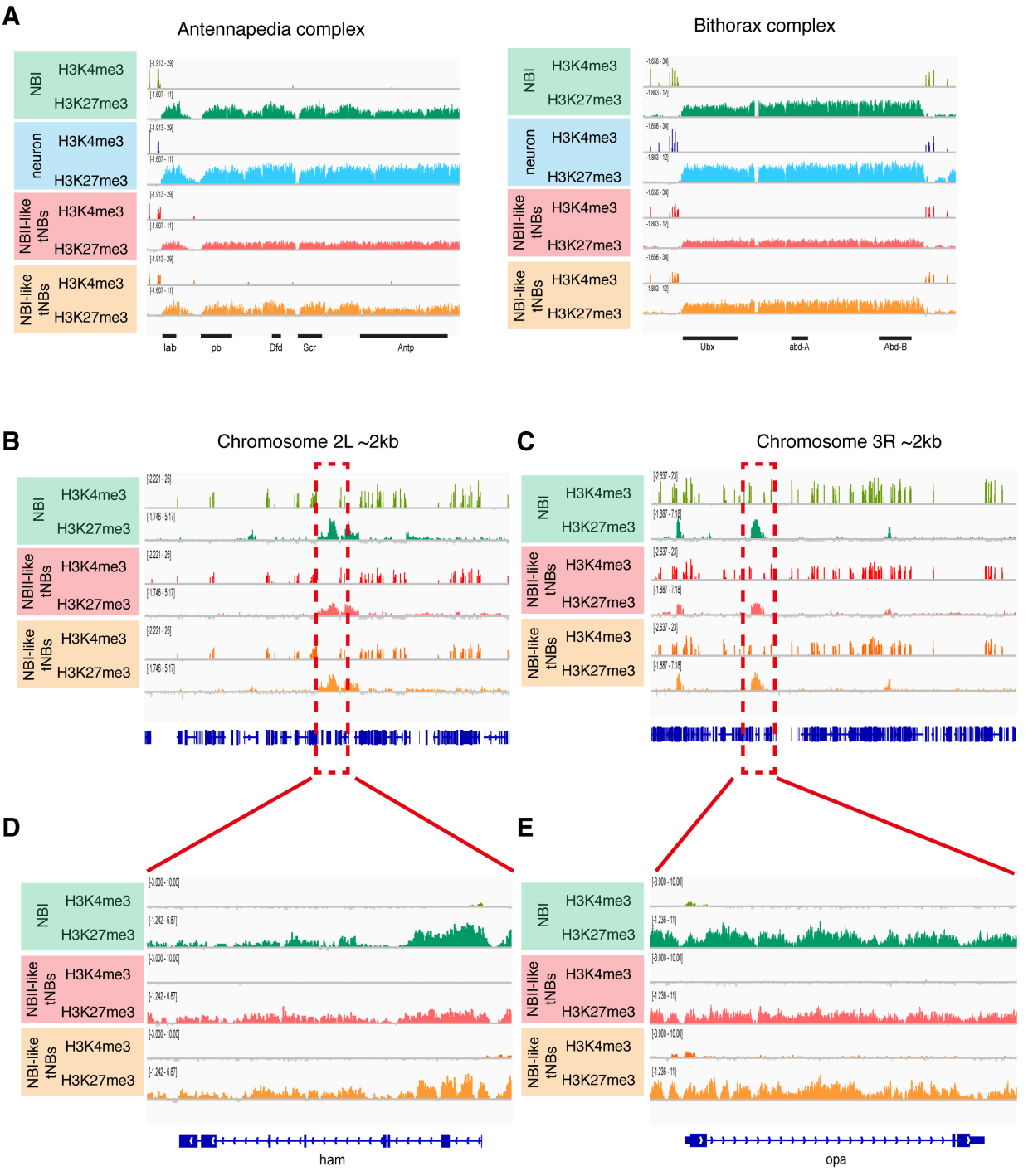

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Figure S4. Examples of regions marked heavily with H3K27me3.

(A) ChIP-seq tracks of the HOX gene clusters; Antennapedia and Bithorax. H3K4me3 and H3K27me3 distribution of 2 kb region of chromosome 2L (B) and 3R (C). H3K4me3 and H3K27me3 distribution at hamlet locus on chromosome 2L (D) and odd-paired locus on chromosome 3R (E).

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Supplement Figure 5

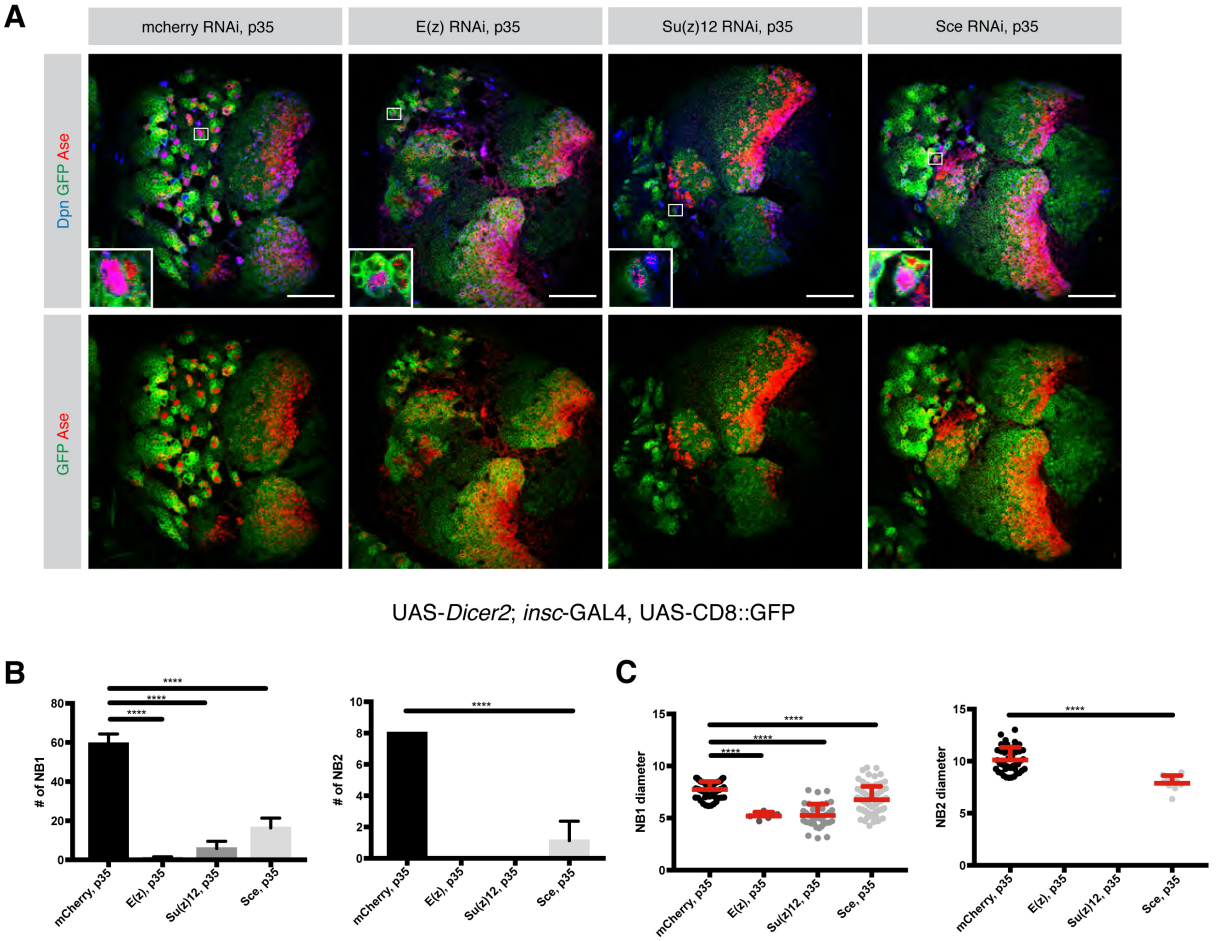

Figure S5. NBI and NBII neurogenesis depend on PcG with different sensitivity

(A) Pupal brains expressing apoptosis inhibitor P35 together with mCherry, *E(z), Su(z)* and *Sce* RNAi constructs. PcG loss causes smaller NBI (blow-ups). Scale bar 50 μm. Driver line used was UAS-*dicer2*; *insc*-Gal4, UAS-CD8::GFP. (B) Quantification of NBI and NBII numbers in (A). Mean ± SD is shown (for NBI, mCherry+P35 = 59.43±4.93 (n=7), *E(z)*+P35 = 0.62±0.91 (n=8), *Su(z)12*+P35 = 5.85±3.62 (n=8) and *Sce*+P35 = 16.38±4.98 (n=8) and for NBII, mCherry+P35 = 8 (n=7), *E(z)*+P35 = NA, *Su(z)12*+P35 = NA and *Sce*+P35 = 1.12±1.24 (n=8)). One-way ANOVA test was used and ****p < 0.0001. (C) Quantification of NBI and NBII diameter in (A). Mean ± SD (for NBI, mCherry+P35 = 7.74±0.76 (n=60), *E(z)*+P35 = 5.19±0.37 (n=5), *Su(z)12*+P35 = 5.15±0.92 (n=41) and *Sce*+P35 = 5.74±0.82 (n=80) and for NBII, mCherry+P35 = 10.12±1.2 (n=43), *E(z)*+P35 = NA, *Su(z)12*+P35 = NA and *Sce*+P35 = 7.88±0.73 (n=9)) One-way ANOVA test was used and ****p < 0.0001.

Supplement Figure 6

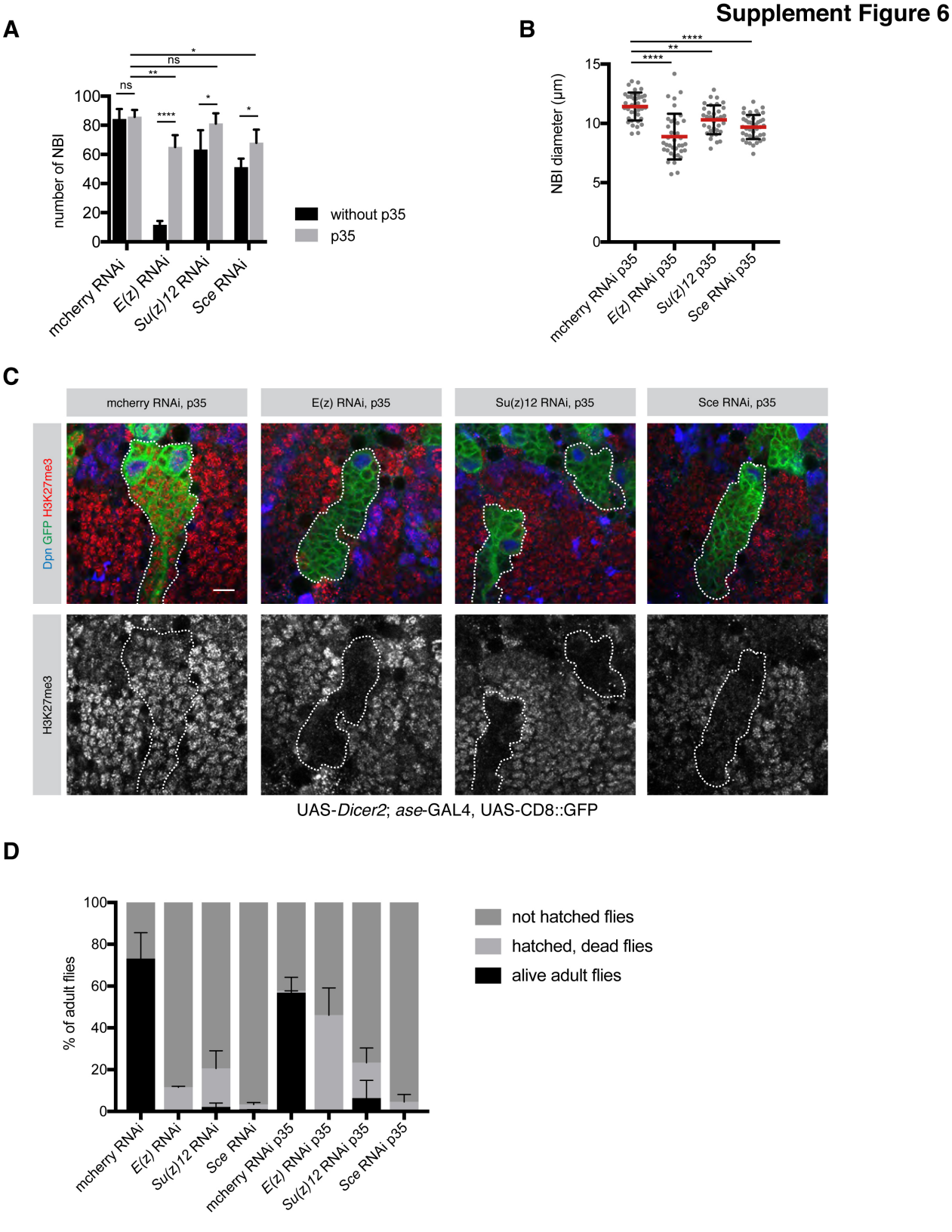

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Figure S6. Blocking apoptosis in PcG depleted NB lineages does not restore neurogenesis.

(A) Number of NBI in L3 larval brains upon RNAi-depletion of PcG genes with and without blocking apoptosis with p35. Mean ± SD. ANOVA test and * $p<0.05$, ** $p<0.005$, *** $p<0.0005$, ns not significant. n= 5 brain lobes, except for *E(z)* RNAi and *Su(z)12* RNAi n=4 brain lobes. Driver line UAS-*dicer2*; *ase*-GAL4, UAS-CD8::GFP was used. (B) Quantification of the NBI cell size of apoptosis-blocked mCherry (n=38), *E(z)* (n=37), *Su(z)12* (n=34), *Sce* (n=41) RNAi-depleted NBIs. n numbers are NBI numbers, each time from 3 different brain lobes. Driver line used was UAS-*dicer2*; *ase*-GAL4, UAS-CD8::GFP. Mean ± SD. ANOVA test and ** $p<0.005$, **** $p<0.00005$. (C) H3K27me3 levels upon the knockdown of PcG genes. Exemplarily NBI lineages are outlined. Driver line used was UAS-*dicer2*; *ase*-GAL4, UAS-CD8::GFP. Scale bar 10μm. (D) Quantification of adult flies that are alive, hatched but dead and did not hatch. For all conditions n=3 independent viability assays. UAS-*dicer2*; *ase*-GAL4. Mean ± SD.

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Table S1: GO-term analysis of genes with decreased H3K4me3 in neurons compared to NBIs.

GO-term enrichment analysis for genes of cluster 4 (related to Fig. 2).

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