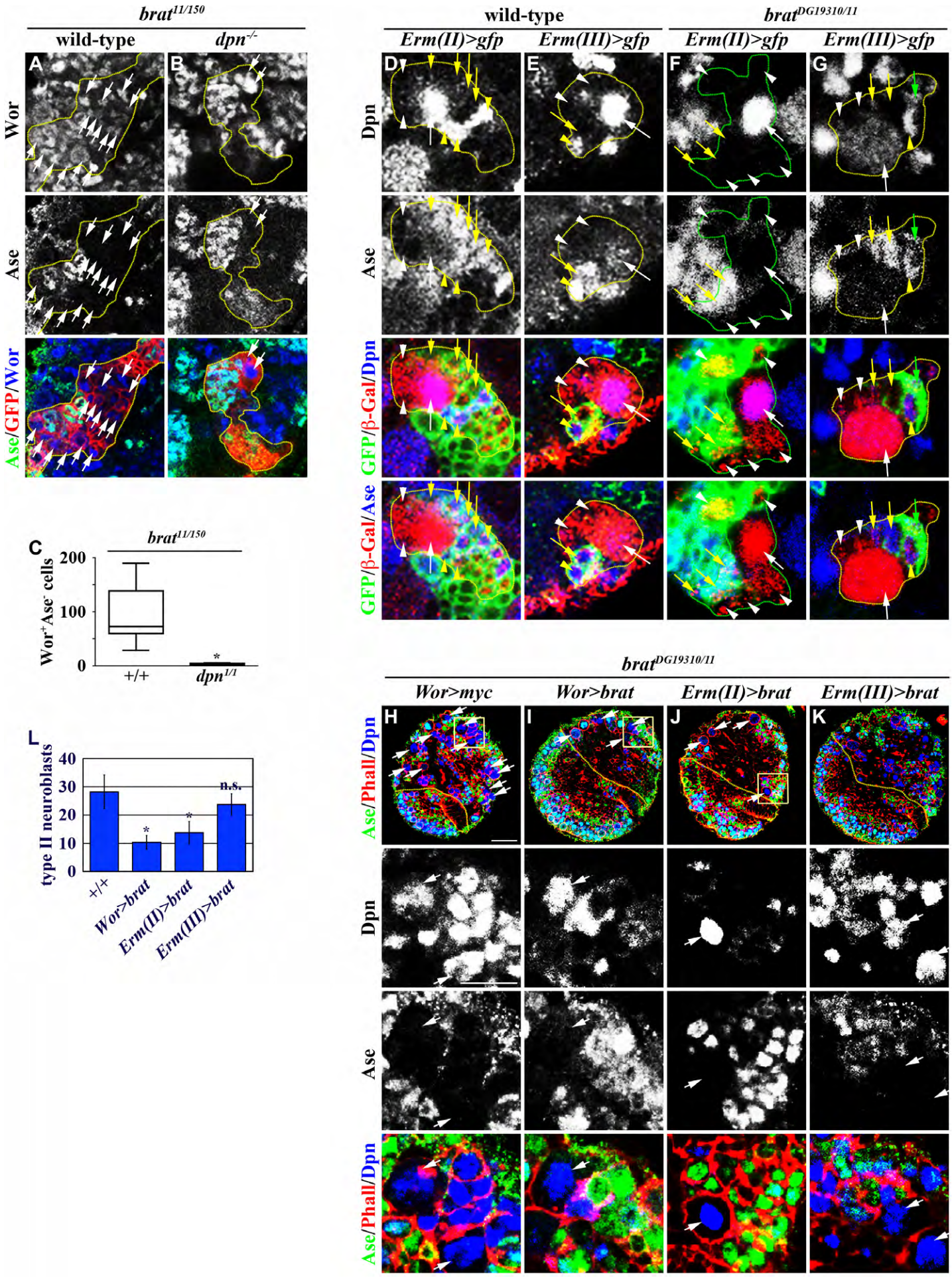
brat11/150
wild-type
dpn-/-
A
B
Wor
Ase
Ase/GFP/Wor
C
brat11/150
Wor+Ase- cells
200
100
0
+/+
dpn1/1
*
L
type II neuroblasts
40
30
20
10
0
n.s.
*
*
+/+
Wor>brat
Erm(II)>brat
Erm(III)>brat
wild-type
bratDG19310/11
Erm(II)>gfp
Erm(III)>gfp
Erm(II)>gfp
Erm(III)>gfp
D
E
F
G
Dpn
Ase
GFP/β-Gal/Dpn
GFP/β-Gal/Ase
bratDG19310/11
Wor>myc
Wor>brat
Erm(II)>brat
Erm(III)>brat
H
I
J
K
Ase/Phall/Dpn
Dpn
Ase
Ase/Phall/Dpn

Fig. S1 Brat functions in the newly born immature INP or Ase^{-} immature INP to suppress the formation of supernumerary neuroblasts.
(A-C) Removal of *dpn* function suppressed the supernumerary neuroblast phenotype in *brat* null brains. (C) The quantification of supernumerary neuroblasts in wild-type or *dpn* mutant type II neuroblast clones in *brat* null brains.
(D-G) The expression pattern of *Erm-Gal4(II)* and *Erm-Gal4(III)* appeared indistinguishable between wild-type and *brat* hypomorphic brains. wild-type or *brat*$^{DG19310/11}$ larvae carrying the *UAS-mCD8-GFP*, *hs-flp*, Act-FRT-FRT-lacZ, *Erm-Gal4 (II)* or *Erm-Gal4(III)* transgenes were genotyped at hatching, and heat-shocked at 37^{o}C for 90 minutes at 24 hours after hatching to induce the lineage clones. Larvae were dissected and processed for immunofluorescent staining at 96 hours after hatching. The specificity of *Erm-Gal4(II)* or *Erm-Gal4(III)* expression was examined in the β-Gal-marked lineage clones (outlined in yellow) derived from single type II neuroblasts in wild-type or *brat*$^{DG19310/11}$ brains.
(H-L) Over-expression of *brat* in neuroblasts or in Ase^{-} immature INPs suppressed the supernumerary neuroblast phenotype in *brat* hypomorphic brains, but over-expression of *brat* in Ase^{+} immature INPs had no effect. The high magnification image of the boxed area in the low magnification image is shown below. Scale bars, 40 μm in the low magnification image and 10 μm in the high magnification image. (L) The quantification of the average number of type II neuroblasts per brain lobe of the indicated genotypes.
Key: White arrow: type II neuroblast. White arrowhead: newly born immature INP and Ase^{-} immature INP. Yellow arrow: Ase^{+} immature INP. Yellow arrowhead: INP. The dotted yellow line separates the brain from the optic lobe (OL). 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 significant.

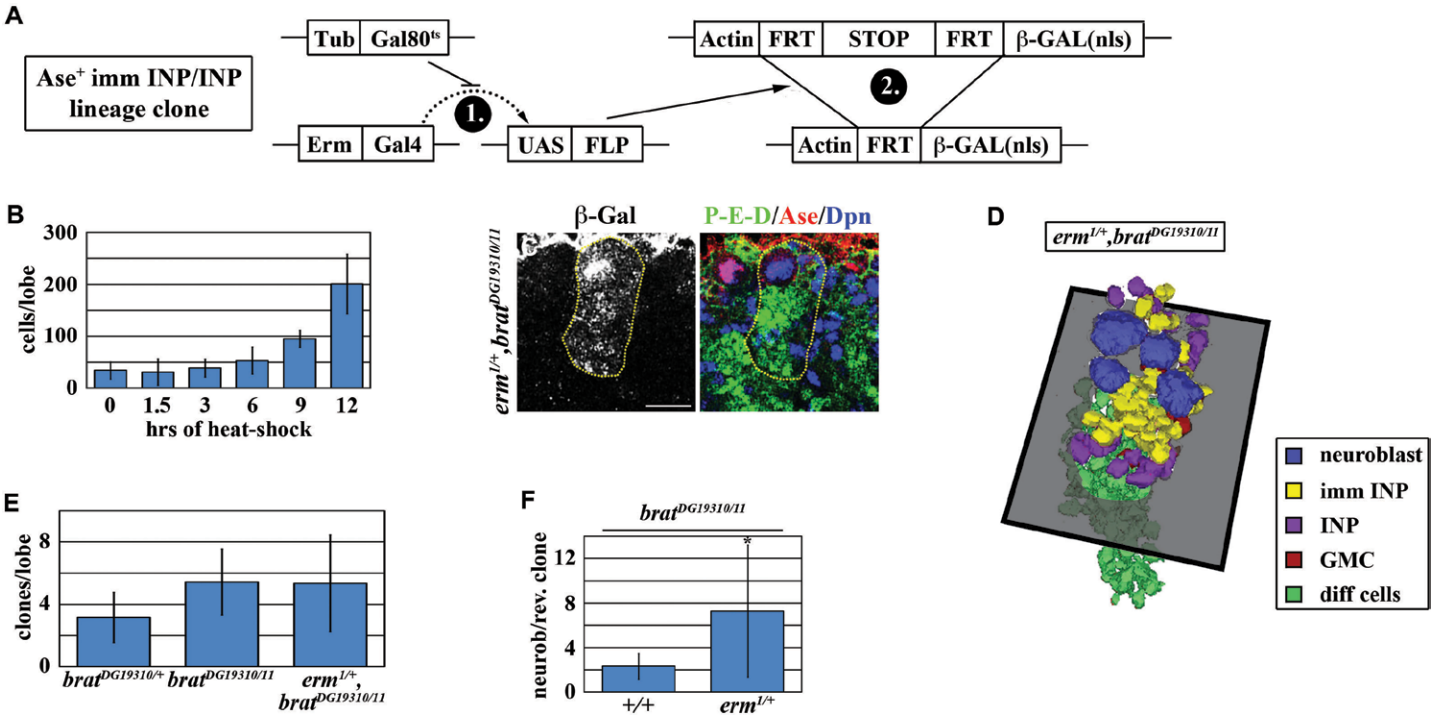

Fig. S2 Empirical determination of the condition required for inducing a low number of clones derived from single Ase$^+$ immature INPs or INPs per brain lobe.
(A) The scheme used to induce clones derived from single Ase$^+$ immature INPs or INPs.
(B) The total number of β-Gal-marked cells per brain lobe when larvae of the genotype in A. are heat-shocked at 30^{O} C for the indicated number of hrs.
(C) The image of a reverted clone derived from a single Ase$^+$ immature INP or an INP in *brat* hypomorphic brains heterozygous for *erm*.
(D) A three-dimensional reconstruction of a reverted clone shown in C.
(E) The total number of clones per brain lobe induced by the basal leaky expression of *Erm-Gal4(III)* at 25^{o}C in the genotype indicated.
(F) The average number of supernumerary neuroblasts in the reverted clones in $brat^{DG19310/11}$ brains or $brat^{DG19310/11}$ brains heterozygous for *erm*.

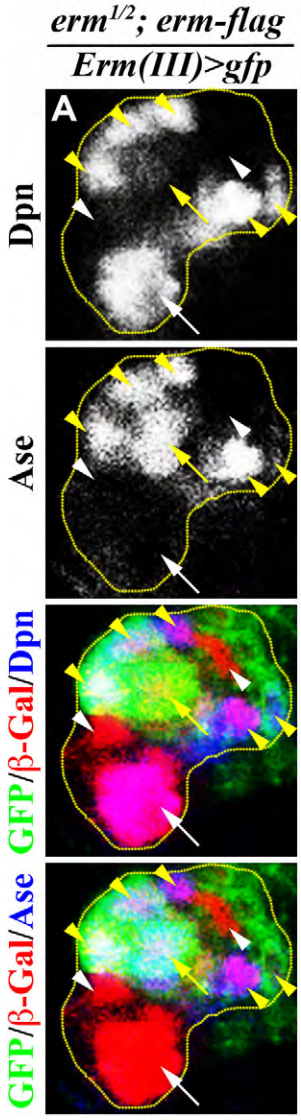

Fig. S3 The expression pattern of *Erm-Gal4(III)* was not affected in *erm* hypomorphic brains. *erm$^{1/2}$; erm-flag4C/+* larvae carrying the *UAS-mCD8-GFP*, *hs-flp*, Act-FRT-FRT-lacZ, *Erm-Gal4(III)* transgenes were genotyped at hatching, and heat-shocked at 37°C for 90 minutes at 24 hours after hatching to induce the lineage clones. Larvae were dissected and processed for immunofluorescent staining at 96 hours after hatching. The specificity of *Erm-Gal4(III)* expression was examined in the β-Gal-marked lineage clones (outlined in yellow) derived from single type II neuroblasts in *erm$^{1/2}$* brains.