Inactivation of Both foxo and reaper Promotes Long-Term Adult Neurogenesis in Drosophila

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Figure S1. Some Neuroblasts Are Eliminated by Cell Death and Not by Pros-Dependent Terminal Differentiation

(A,B) Some CB neuroblasts other than mb neuroblasts also undergo cell death. Top two rows, single channel images with pseudo-colored overlay in bottom row, showing co-localization of markers in neuroblasts. AC3, activated caspase 3.

(C,D) Wild type (WT) mb neuroblasts after their terminal cell division fail to localize Pros to the nucleus. Mb neuroblasts before (C) and during (D) nuclear envelope breakdown.

(E) A single Dpn-expressing cell persists in H99 mutant mb neuroblast clones. All GFP-expressing cells are H99 homozygous mutant, including the mb neuroblast which only weakly expresses GFP.

(F,G) worniu transcription is attenuated in young RHG miRNA mb neuroblasts. Two RHG miRNA mb neuroblasts from the same brain lobe. Only one of the two mb neuroblasts expresses high levels of the mCD8:GFP reporter. Top three panels are single channel image and bottom row is overlay. (G). Neuroblasts in brackets. worniuGAL4 is used to induce UAS transgene expression (F,G).

Scale bar (A,F) equals 10 μm.
Figure S2. RHG miRNA Targets \textit{rpr}, \textit{hid}, and \textit{grim} for Inactivation.
(A) Generation of UAS-RHG miRNA using the mir6.1 technique.
(B) Validation of RHG miRNA transgene. Overexpression of RHG miRNA can suppress \textit{reaper}, \textit{hid}, and \textit{grim} induced cell death in the eye. GMR, glass multimer reporter.
Figure S3. Mb Neuroblasts Persist in Young rpr Mutant Adults and Generate a Few New Adult mb Neurons
(A-G) Young (3-5 days old) rpr mutant adult mb neuroblasts (brackets) and their progeny (arrows, I-L).
(A-E, I-J) Top two rows, single channel images with colored overlay in bottom row, showing co-localization of markers in mb neuroblasts (A-E) and progeny (I,J).
(F-H) Mb neuroblasts incorporate BrdU and generate new progeny during a 24 (F) or 48 hour (G) BrdU pulse.
(H) Quantitation of the number of BrdU positive progeny generated during a BrdU pulse for indicated times. Number of BrdU positive mb neuroblasts scored, white numbers in black columns. Mean and std. dev.
(I,J) New adult progeny are Elav, Ey positive mb neurons, identified as BrdU positive progeny (arrow) or in clones (arrows, J) following activation of a tau:LacZ transgene in adult mb neuroblasts.
(K) Immature neurites (arrowheads) form from new adult mb neurons two days after tau:LacZ activation.
(L) A 50μm axon bundle extends through the mb peduncle at five days after tau:LacZ activation.
(A-E,I-L) and (F,G) same scale.
Scale bar (A,F) is 10μm.
Figure S4. Reduced Levels of Insulin/PI3 Kinase Signaling Restricts rpr Mutant Adult mb Neuroblast Survival and Proliferation

(A) Z-projection of the dorsal brain surface showing persisting adult mb neuroblasts (arrowheads) near the mb calyx (white outline) following insulin/PI3 kinase pathway activation. Scale bar equals 10 μm.

(B) Number of BrdU progeny generated in 24 hours relative to mb neuroblast cell size. Mb neuroblast cell size and proliferation rate do not correlate in young Dp110o/e,rpr adults. R²=coefficient of determination for a linear correlation. worniuGAL4 induces Dp110 overexpression (o/e) in mb neuroblasts.