Supplemental Information

Genome-Wide Analysis of Self-Renewal in Drosophila Neural Stem Cells by Transgenic RNAi

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Table showing 38 genes, identified in the course of the re-screen, for which non-overlapping GD and KK RNAi lines exist and for which insc-Gal4 mediated knock down results in similar phenotypes.

**Figure S1, related to Figure 2.**

Table showing 38 genes, identified in the course of the re-screen, for which non-overlapping GD and KK RNAi lines exist and for which insc-Gal4 mediated knock down results in similar phenotypes.
Neumuller et al., Figure S2

**Figure S2, related to Figure 3.**

(A) Knock down with *lola-all-RNAi* in type II lineages results in the formation of ectopic Nbs. All brains are stained for Mira and Pros. (See also Fig. S5 for explanation of type II specific driver line.)
Figure S3, related to Figure 4.

Heat map displaying over- and underrepresentation of GO terms in the groups Nb_huge, Overproliferation, Nb_loss_small, Nb_loss_large and Nb_loss_normal (Colour code represents Z-score with colours from red (underrepresented) to blue (overrepresented) (see materials for details)).
Figure S4 related to Figure 5.

(A) Network of all genes in the Nb_huge group. The node shape refers to the comparison of our data set with the gene set of the MSigDB v2.5 (see Fig. 2E for details). Edges denote interactions/associations between genes and the edge width reflects the evidence count: thick edges represent multiple evidences of interactions.

(B,C) Knock down of genes previously implicated in cytokinesis (B) and of alphaTub67C and betaTub60D (C) result in similar phenotypes. All brains are stained for Actin and DNA. Cells are massively enlarged (Actin) and the DNA (DAPI) content is increased.
**Figure S5, related to Figure 6.**

(A) *Insc-Gal4* mediated knock down of *alpha-Adaptin, CG6066, Ada1-2, hermaphrodite (her)* and *ccn* result in the formation of ectopic Nbs. All brains are stained with Mira and Pros whereas CD8::GFP marks the *insc*-Gal4 expression area.

(B,C) Knock down of *alpha-Adaptin* and *AP2-sigma* in type I (B) or type II (C) lineages results in the formation of ectopic Nbs. All brains are stained for Mira and Pros.

(D) Self-renewal defects are observed in *α-Adaptin* mutant MARCM clones. Clones are marked by GFP expression and all brains are stained for Mira and Pros.

(E) Establishment of a type II Nb specific Gal4 line. The expression pattern of the Gal4-line is marked by CD8-GFP. All brains are stained for Mira and Pros. Knock down of *brat* and *numb* in type II lineages using *wor*-Gal4, *ase*-Gal80 results in the formation of ectopic Nbs.
Naumuller et al., Figure S6
Figure S6, related to Figure 7.

(A) *Insc*-Gal4 mediated knock down of *barc* using an independent non-overlapping RNAi line results in the formation of ectopic cells positive for Mira.

(B) *Insc*-Gal4 mediated knock down of *barc* results in the formation of ectopic cells positive for Dpn.

(C) The Barc antibody recognizes a band of approximately 75kD in a wt embryo lysate on a Western Blot that can be blocked by the antigenic peptide.

(D and E) Endogenous Barc localizes to the nucleus in interphase and is expressed in INPs, GMCs, neurons and predominantly in Nbs. Barc staining is reduced upon *insc*-Gal4 mediated knock down of *barc* (D).
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Genome-Wide RNAi Screen

The RNAi driver line was established by recombining a UASt-CD8::GFP onto the inscuteable-Gal4 (1407-Gal4) chromosome. Thereafter a heat shock inducible hid gene (hs-hid) on the Y chromosome and a homozygous viable UAS-Dicer-2 on the X chromosome were crossed into the insc-Gal4 background. Heterozygous driver line virgins (balanced over CyO) were isolated following 2 consecutive 1 hour heat shocks at embryonic stages and crossed to males carrying a UAS-RNAi transgene derived from the VDRC. Crosses were set up on 25°C and after one day transferred to 29°C. In case of homozygous RNAi lines, crosses were flipped after four days. Lethality was determined in the first cross by the presence of balancer chromosome flies only. In case of lethality larvae of the right genotype were identified by insc-Gal4 driven CD8::GFP (brain and salivary gland) using a fluorescence microscope. 6 larvae per genotype were dissected in PBS and fixed in 5%PFA in PBS for 20 minutes. After mounting the specimen in Vectashield, a confocal stack of the brain was recorded using a ZEISS LSM confocal microscope.

For RNAi lines on the X chromosome or heterozygous RNAi lines, lethality was determined by the presence of males only for the X chromosome and otherwise as before. In case of lethality for the X chromosome CD8::GFP positive female larvae were selected for dissection. In case of lethality for the heterozygous RNAi lines, UAS-RNAi flies were crossed against a balancer chromosome visible at larval stages (Tm6b for the 3rd chromosome and CyO, carrying a ubiquitously expressed His2A::mRFP1, for the 2nd chromosome). The rebalanced heterozygous RNAi lines were crossed again with the RNAi driver line and larvae of the right genotype were identified by CD8::GFP and the absence of the balancer chromosome.
All phenotypic abnormalities were recorded and stored in a database (http://neuroblasts.imba.oeaw.ac.at)

**Phenotypic Annotations**

For cell size measurements, the maximal diameter of 10 to 15 Nbs or GMCs/INPs was determined: In the Nb_large phenotypes 0 corresponds to ~15μm reflecting a wt Nb diameter. The biggest cell diameter was found upon *cdc45* (TID 41084) (~27μm) knock down which corresponds to the score 9. Lines with Nbs bigger than 30μm diameter were set to 10. Lines where Nb size exceeds two cell diameters were additionally scored as abnormally large cells (see online database). In the Nb_small phenotypes 0 corresponds to ~15μm reflecting a wt Nb diameter. The smallest cell diameter was found upon *omd* (TID 44969) (~7μm) knock down which corresponds to the score 10.

In the cell count phenotypes (Nb_more/less and GMC_more/less) cell number was quantified on the posterior brain hemisphere. In the Nb_less/ GMC_less phenotypes, 0 corresponds to ~30 Nbs (and ~4 CD8::GFP expressing daughter cells per Type I lineage) per posterior brain hemisphere and lines that result in a complete absence of CD8::GFP expressing cells score with 10. Lines with intermediate phenotypes are scored between 2 and 10 depending on their Nb/GMC number. The Nb/GMC_more phenotypes were quantified in a similar manner. As Nb/GMC number cannot be reliably quantified in strong phenotypes (e.g. *pros*-RNAi), phenotypic scores in these cases reflect an approximation.

Intracellular CD8::GFP aggregates were quantified by determining their number and size within cells. A score of 10 in the GFP_aggregate_number reflects lines in which the entire cell is filled with aggregates. Maximum size of CD8::GFP aggregates
was observed upon knock down of \textit{Trxr-1} (TID 47306), which hence marks the maximum score. Deformations of Nbs and GMC from the wt spherical appearance were similarly expressed in numeric values ranging from 0 – 10 (2-5 = mildly deformed, 6-10 = extremely deformed).

\textbf{Constructs}

\textit{Barricade} (CG6049) was cloned from total mixed stage cDNA with the primers:

\begin{align*}
\text{5'}- & \ GGGGACAAAGTTTGTACAAAAAAGCAGGCTTAATGAGCGACGAAGGTGGC- \\
& \text{3'} \ 	ext{and} \\
\text{5'}- & \ GGGGACCACCTTTGTACAAGAAAGCTGGGTACTAAGGGTGCGTCTCC-3'
\end{align*}

and recombined into the Gateway pDONOR221 vector. The resulting entry clone was used to generate a pUAS\textit{-barricade} destination vector, which was used to generate transgenic flies by using standard P-element transformation.

The \textit{barricade} independent RNAi line was created by amplifying a fragment using the primers 5'- CGCGAATTCTTACCATCCGACCACACTTGGCC-3’ and 5'- GCCTCTAGAGCGGAAGCTGCTATGTCTC-3’.

We combined \textit{wor}\textsuperscript{-Gal4} that expresses in type I and type II Nbs with the Gal4 inhibitor Gal80 expressed under the \textit{ase} promoter to obtain a line that drives expression of UAS constructs specifically in type II Nb lineages. The \textit{ase} promoter fragment was amplified using the primers: 5’- GATCGAATTCCAGTATGTTTCCACGCTAGC-3’ (containing an EcoRI site) and 5’-CTGAGGATCCTAAGGCGGCCATAATTAAGT-3’ (containing a BamHI site). The \textit{gal80} was amplified from flies carrying a functional \textit{gal80} as a transgene using the primers 5’-CTGAGGATCCATGGACTACAACAAGAGATC-3’ (containing a
BamHI site) and 5'-GCATCTCTAGACTATAAATACTATAATGCGAGA-3'
(containing a XbaI site). SV40 polyA was amplified using the primers 5'-
GGATCTCTAGAGATCATAATCAGCCATAACCA-3' (containing a XbaI site) and
5'-CGATCTGCAGGATCCAGACATGATAAGATA-3' (containing a PstI site).
Thereafter, the promoter fragment, gal80 and SV40 were subsequently cloned into the
pCaSpeR vector and transgenic flies were established by conventional P-element
mediated fly transformation.

Histone::RFP was cloned into a fly transformation vector containing a
ubiquitous promoter. This construct was injected into Pin/CyO flies and a line in
which the transgene maps to the CyO chromosome was selected for all further
experiments.

**RNAi-Resistant Barc Construct**

The barricade RNAi resistant sequence was generated using a custom made
Perl script (available upon request) and the resulting fragment (sequence available
upon request) was de novo synthesized (Mr. Gene). This fragment, covering the RNAi
resistant part, was cloned into the pDONOR221-barricade backbone using the
enzymes Apai and PstI. This vector was used to generate a pUAS\textit{st-barricade}^{RNAi-
resistant} construct.

**Type II-Specific Driver Line**

We generated a line that drives expression of UAS constructs specifically in
type II Nb lineages by combining \textit{wor\textit{nui}}-Gal4 (\textit{wor}-Gal4, active in all Nbs) with a
transgene expressing the Gal4 inhibitor Gal80 from the \textit{ase} promoter, which is only
expressed in type I Nbs (Fig. S5E).
Gene Expression Analysis

Total RNA was isolated from third instar larval brains of either wild type or brat-RNAi (GD31333 and KK105054) crossed to UAS-Dicer-2; insc-Gal4, UAS-CD8::GFP/CyO. The experiments were done in triplicates. For each sample brains from 50-70 animals were dissected in PBS on ice and RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturers instructions. The following experimental steps were done by the Microarray DNA Facility of the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden. The two principle steps were the amplification and biotin-labeling of the RNA using MessageAmp™III RNA Amplification Kit from Ambion and the hybridization of the RNA to the GeneChip® Drosophila Genome 2.0 from Affymetrix. The analysis of Affymetrix microarray data was performed using the R environment for statistical computing. Raw intensity values from three biological replicates of a control and two different brat-RNAi fly lines were log-transformed and normalised with the variance stabilising normalisation algorithm (vsnrma function, vsn package). Differentially expressed genes were identified using the LIMMA package. P-values were adjusted for multiple testing to control the false discovery rate. Only transcripts with a log2 fold-change (log2FC) > 0.4 and an adjusted p-value < 0.05 were considered for further analysis (Isoform B log2FC 0.58, adj. p-value 0.0012; Isoform C log2FC 0.66, adj. p-value 5.30x10^-5; Isoform D log2FC -0.49, adj. p-value 0.0033; Isoform H log2FC -0.43, adj. p-value 0.0057; Isoform S log2FC 0.47, adj. p-value 0.00021).

Bioinformatic Analysis

Functional annotation of phenotypically defined gene sets was obtained using 'Over Representation Analyses' (ORA). The probability for a particular functional category to be enriched or depleted from a gene set—compared to the reference set of
all screened genes—was assessed using the hypergeometric test with Benjamini-Hochberg correction for multiple testing. Non-specific filtering removed genes lacking any mapping for a particular category from further consideration. Standardized difference scores (Z-scores) are shown as heat maps, where values above 2 and below -2 signify statistical over-abundance and under-representation. Annotations such as the association of genes to Gene Ontology (GO) terms were obtained from FlyBase release 2008_04, the Gene Ontology Consortium (Ashburner et al., 2000) and the Gene ontology website curated by the GO consortium (http://www.geneontology.org/). Gene-based expression information, such as the tissue-specific transcriptional upregulation in comparison to whole fly, was obtained from flyatlas microarray data analysis. We used the Drosophila Transcription Factor Database (FlyTF.org) and their datasets „equivalent to release v1 - putative TFs (754 Genes)“ and „proteins involved in chromatin-related processes (160 Genes)“ to identify all putative and known chromatin associated genes and transcription factors among the genes associated with loss of Nbs (Adryan and Teichmann, 2006). To identify potential orthologs between Drosophila and mouse and between Drosophila and human, we used pre-computed orthology predictions obtained from compara49, inparanoid6.1, homologene08, and orthomclv2 (Kuzniar et al., 2008) databases. If multiple potential orthologs were predicted for an individual Drosophila gene a single ortholog with best support was chosen for MSigDB v2.5 overlap analysis.

Interaction maps among proteins of phenotypically defined gene sets were based on the Drosophila interactome as in (Mummery-Widmer et al., 2009). The interactome included direct and indirect (functional) associations from DroID (www.droidb.org) (v5), STRING (v7.0 and v8.2) and BioGRID (v2.0.40). Resulting networks were drawn using Cytoscape (http://www.cytoscape.org). We used the
clustering algorithms „Molecular Complex Detection“ MCODE (Bader and Hogue, 2003) and „Markov Cluster“ MCL (Enright et al., 2002) to identify complexes within the networks. Genes are shown as nodes, and the node color reflects the phenotype where blue nodes denote underproliferation and grey nodes GFP aggregates. Overproliferation phenotypes were confirmed in a rescreen with Mira and Pros immunofluorescence stainings and are shown as red nodes. In case of lola and Pp4-19C the overproliferation phenotype is displayed and nodes are in red. The intensity of the colour denotes the phenotypic strength. Edges denote the interaction/association of the nodes based on two-hybrid, biochemical, interolog and genetic interactions between Drosophila genes text-mining tools (edge width reflects evidence count: thick edges represent multiple evidences of interactions).
SUPPLEMENTAL REFERENCES

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