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„Suppressing tumor growth in *Drosophila melanogaster* brains, using an RNA interference screen“

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Content

Abstract

Stem cells simultaneously self-renew and generate differentiated progeny. Asymmetric cell division provides a basic mechanism to achieve this remarkable task. The segregation of cell fate determinants into only one daughter cell allows its commitment to lineage-specific cell fate. Maintaining the balance between stem cells and differentiated cells requires a precise regulation of asymmetric cell division and failure to do so can cause severe stem cell overgrowth ultimately leading to tumorigenesis. *Drosophila* neural stem cells, called neuroblasts (NBs), provide a fruitful model to investigate molecular details underlying this regulatory mechanism. Larval type II NBs self-renew and give rise to an intermediate neural precursor (INP). The well-known tumor suppressor *brain tumor* (brat) segregates into the differentiating daughter cell where it specifies INP cell fate. Lacking *brat* prevents INPs from adapting to its fate but instead; INPs revert back into a NB-like stage, turning them into tumor-initiating cells. These cells reenter mitosis and overproliferate, which leads to the formation of tumors. Subsequently, the tumor overgrows the brain and causes premature death of the fly.

In this study, we are using *Drosophila brat* tumors to study tumor requirements. We performed a tumor-suppressor screen to identify genes, on which ectopic, but not wild-type NBs depend on. RNA interference (RNAi) was used to knockdown *brat* function in type II NBs of *Drosophila* 3rd instar larvae. Using an RNAi library, 1182 genes that have been identified to be upregulated in *brat* tumors were systematically knocked down simultaneously to *brat*. We used the adult survival rate as a read out of the screen and identified 266 genes, which led to a significant increase in the survival rate. Systematic confocal fluorescence microscopy was performed to examine type II NB lineage behavior on a single knockdown level of these candidate genes. 75 genes did not alter the type II NB lineage formation upon knockdown and thus, were chosen for further analysis. Intriguingly, all candidates of interest showed tumor initiation in larval brains upon double knockdown with *brat*. However, some of them showed a clear decrease in tumor size in adult stages. The mitochondrial ribosomal protein L46 (*mRpL46*) was identified as a gene, which did not alter the type II NB lineage upon knockdown, but dramatically decreased the *brat* tumor in adult flies. Thus, mRpL46 may be a potential target for therapy development in cancer research.

Introduction

Stem cells and tumorigenesis

Stem cells (SCs) are pluri- or multipotent cells, which give rise to more differentiated progeny while maintaining their self-renewing capacity. This task can be achieved by a process, which is called asymmetric cell division. During development, SCs generate all different tissues that make up an entire organism, whereas in adult organisms, they mainly act as a repair system and are required to maintain tissue homeostasis.

Misregulation of the key factors that control asymmetric cell division can lead to the formation of abnormal cells, which proliferate in an uncontrolled manner and give rise to stem cell-derived tumors (Al-Hajj and Clarke, 2004). Misregulation in asymmetric cell division can be caused by intrinsic defects like altered segregating proteins, leading to a wrong distribution of cell fate determinants.

SCs, which generate the variety of cell types that can be found in a tumor, are called cancer stem cells (CSCs). They are located within the tumor and drive tumor growth (Kreso and Dick, 2014). Although the concept of CSCs is increasingly gaining attention, not many ways to specifically target CSCs are known. The fact that CSCs are often expressing similar surface markers and rely on similar signaling pathways as regular SCs makes a specific targeting challenging (Reya et al., 2001)(Lobo et al., 2007).

Thus, so far, in human therapy CSCs are only inefficiently targeted by conventional cancer therapies and often remain in a so-called stem cell niche. This can lead to a tumor relapse after a seemingly successful therapy (Michor et al., 2005).

Asymmetric cell division

The generation of multicellular organisms requires the asymmetric division of cells. This mechanism allows self-renewing stem cells to generate differentiating progeny. To determine different cell fates between mother- and daughter cells, cell fate determinants are asymmetrically partitioned between the mother cell and its progeny. RNA as well as proteins can act as cell fate determinants. Asymmetric segregation of proteins requires a polarity axis, which is established by partitioning defective (Par) proteins during interphase (Knoblich, 2001). In mitosis, this polarity axis determines the localization of cell fate determinants and spindle orientation. In telophase, the cell fate determinants are segregated into the differentiating cell, whereas the Par proteins remain in the self-renewing cell (Knoblich, 2010).

Drosophila neuroblasts as a stem cell model

In order to investigate characteristics of tumor cells, it is necessary to use a convenient model system. It is estimated that around 75% of all known disease related genes have functional *Drosophila* homologues (Reiter and Bier, 2002). *Drosophila* larval brains have proven themselves to offer a way of studying stem cell derived tumors (Betschinger et al., 2006).

The larval central nervous system consists of two brain lobes and the ventral nerve cord. Neural stem cells in the *Drosophila* brain are called neuroblasts (NBs). NBs arise by delamination from the neuroectoderm during embryonic development. Proneural clusters in the neuroectoderm specify NBs by Notch-dependent lateral inhibition from neighboring non-neuroblast cells. This process restricts proneural gene expression to the NB cells (Campos-Ortega, 1993; Hartenstein and Wodarz, 2013).

NBs produce neurons and glia cells during the larval stage and disappear during pupariation. They repeatedly divide along the apical-basal axis, enabling extrinsic factors to interfere with the angle of division and hereby the segregation of cell fate determinants (Figure 1B) (Knoblich, 2010).

The 3rd instar larval central brain contains two different types of NBs. Type I NBs are mainly located at the anterior side of the brain, whereas type II NBs are found at the posterior side. The ventral nerve cord (VNC), which is a part of the 3rd instar larval brain as well, is harboring different NBs, namely thoracic and abdominal NBs. Besides

about 100 type I NBs, there are only eight type II NBs in one brain lobe of a 3rd instar *Drosophila* larvae (Figure 1A).

Type I NBs are characterized by their presence of the transcription factors *asense* (*ase*) and *deadpan* (*dpn*) in the nucleus as well as *prospero* (*pros*) in the cytoplasm (Figure 1 C) (Brand et al., 1993; Bier et al., 1992; Choksi et al., 2006). These factors are utilized as markers to determine the different stages of the lineage. Type I NBs divide asymmetrically so that the offspring consists of another NB and a ganglion mother cell (GMC). GMCs are characterized by their presence of *pros* and *ase* in the nucleus (Boone and Doe, 2008). They divide into two *pros*$^+$ glia cells or neurons. Type II NBs are easily to distinguish from type I NBs by their absence of *ase* and *pros* expression (Figure 1 D). Type II NBs resemble the mammalian NB lineages in a closer way than type I NBs do, due to the presence of transit amplifying cells (Bello et al., 2008; Boone et al., 2008; Bowman et al., 2008; Izergina et al., 2009). They divide asymmetrically to self-renew and to generate intermediate neural progenitors (INPs). INPs undergo a division-free maturation process. First they turn off *dpn* expression, followed by expression of *pros* in the cytoplasm and *ase* in the nucleus. Finally they reexpress *dpn* and reenter mitosis. This final stage is then called mature INP (mINP). mINPs themselves are able to transiently self-renew, which allows eight NBs to give rise to a huge amount of neurons and glia cells (Homem et al., 2013). Asymmetric division of mINPs leads to the formation of an INP and a GMC, which expresses nuclear *ase* and *pros* and gives rise to *pros*$^+$ glia cells and/or neurons. Each INP cell can generate 6-12 neurons. This strict hierarchy and the fact that NB progenies do not migrate makes NB lineages an attractive model to trace both the origin and the behavior of the various cell types.

A crucial step in type II NB lineage formation is the transition from NBs to imINP cells. During mitosis, the NB establishes an apical-basal polarity. So called cell fate determinants localize to the basal side of the cell membrane (Figure 2 A). Known cell fate determinants of type II NBs are the Notch-repressor Numb (Berdnik et al., 2002) and the translational repressor Brat (Arama et al. 2000). Within the differentiating daughter cell, these determinants act together to prevent self-renewal and induce differentiation in the INP cells, leading to the correct development of the type II NB lineage. Their ability to limit self-renewal makes these cell fate determinants act as tumor suppressors. Mutations in these suppressors lead to the formation of tumors. Upon transplantation these tumors become immortal and evade other tissues

(Caussinus and Gonzalez, 2005). Limitless replicative potential and tissue invasion are hallmarks of cancer, which are also observed in human tumors (Hanahan and Weinberg, 2000), supporting the relevance of *brat* tumors as tumor model.

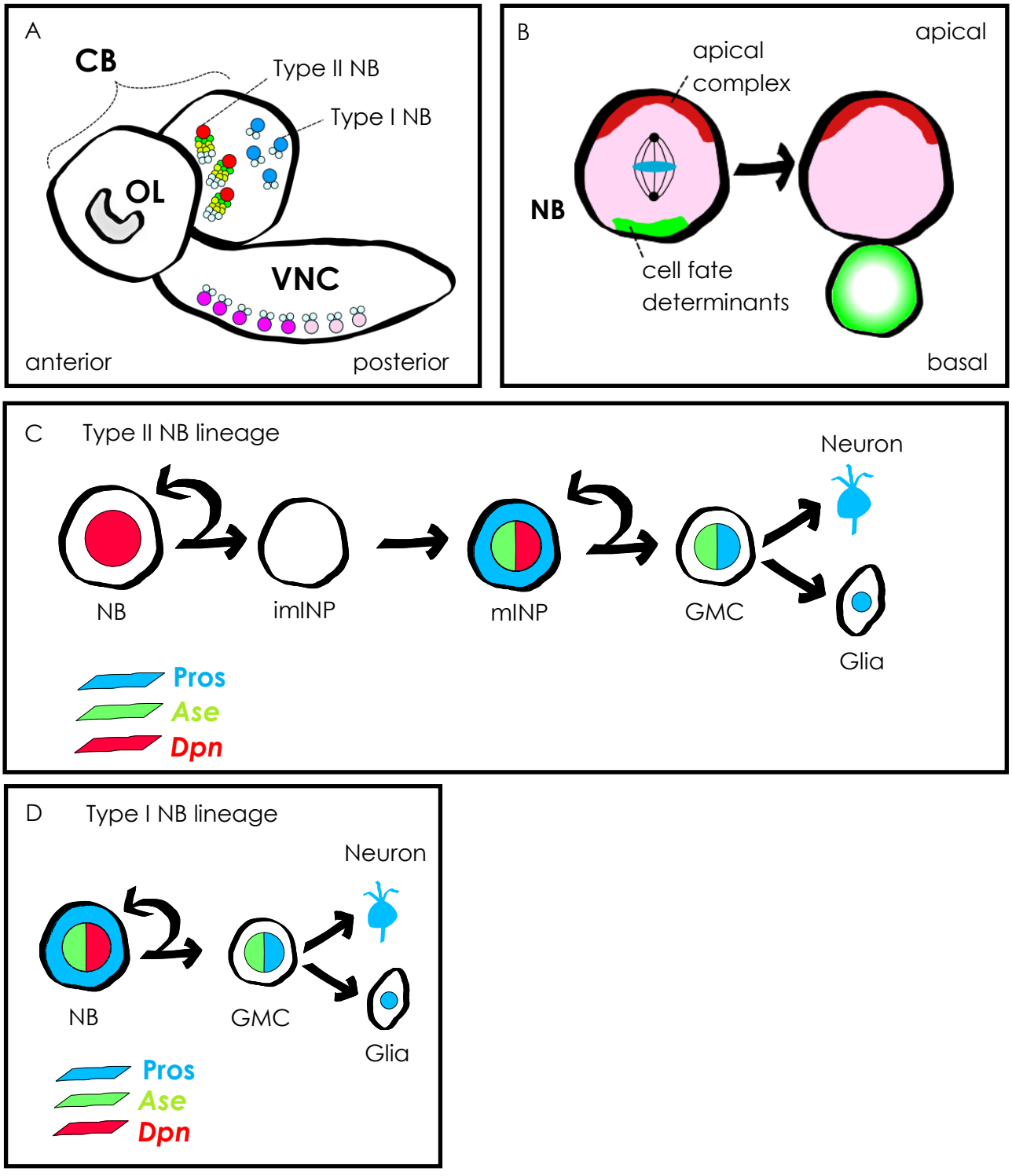


Figure 1 - Neuroblasts in the Drosophila larval brain

(A) The central nervous system of *D. melanogaster* larvae consists of two brain lobes and a ventral nerve cord (VNC). The brain lobes contain a definite optic lobe (OL). A brain hemisphere harbors about 100 type I NBs at the anterior side and eight type II NBs at the posterior side of the CB.
(B) Located at the apical side of *Drosophila* NBs are the partitioning defective proteins PAR3, PAR6 and aPKC. The adaptor protein Inscuteable links the PAR proteins to the partner of Inscuteable (PINS), the G protein a_i (Ga_i) and MUD. Together they form the apical complex. In mitosis, the apical complex organizes the mitotic spindle orientation and directs the basal components, which include cell fate determinants like Brat, Numb and in type I NBs Pros, to the basal side of the cell. After mitosis, the cell fate determinants in the daughter cell act together to prevent self-renewal and establish cell fate.
Panel (C) shows the expression pattern of the type II NB lineage. Panel (D) shows the expression pattern of the type I NB lineage.

Tumor formation in brat mutant larval brains

Misregulation of asymmetric cell division is a known cause for tumorigenesis in *Drosophila*. Previous research had shown that defects in cell fate determinants like Prospero and Numb can lead to tumor formation, as well as defects in proteins of the segregation machinery like l(2)gl (Caussinus and Gonzalez, 2005). Numb, Miranda, Pros and Pins deficient *Drosophila* tumors displayed genomic instability upon tumor transplantation to the adult abdomen (Caussinus and Gonzalez, 2005). Genomic instability is also observed in human tumors.

Brat is a cell fate determinant which asymmetrically segregates from the NB to its progeny. It encodes a member of the Trim-NHL family, which plays a major role in development and differentiation (Loedige et al., 2014). During embryo patterning, Brat interacts with Nanos and Pumilio to repress the translation of hunchback mRNA (Sonoda and Wharton, 2001). In mitotic NBs, Brat localizes to the basal site of the cell and segregates into the differentiating daughter cell during telophase (Betschinger et al., 2006). During larval brain development, Brat acts as a tumor suppressor and regulates self-renewal by post-transcriptionally inhibiting the growth factor dMyc (Betschinger et al., 2006). Recently it has been shown that *brat* tumors arise from type II NB lineages (Bowman et al., 2008). *brat* mutant type II NBs divide asymmetrically, however, the smaller daughter cell does not mature into an INP. Instead, it blocks its cell cycle in G2 for 48 hours. It then regrows to NB size, reenters mitosis (Bowman et al., 2008) and reverts back to a NB-like cell, undergoing an indefinite number of

divisions (Figure 2 B). Ectopic NBs share many similarities with normal type II NBs, e.g. expressing the same self-renewing proteins Dpn, Klu and Notch. However, in contrast to regular NBs, *brat* mutant NBs give rise to tumor initiating cells (TICs), which are unable to establish their INP fate. TICs regrow after an initial G2 cell cycle bock. They fail to turn off the expression of the Notch-target Dpn, which mimics a Notch pathway overexpression phenotype. In type II NBs this leads to uncontrolled cell division and tumor overgrowth (San-Juán and Baonza, 2011). Brat induced tumor growth leads to significantly enlarged *Drosophila* brains. In fact, Brat deficient NBs generate transplantable tumors and cause premature death of the fly (Caussinus and Gonzalez, 2005). The molecular mechanism of how Brat prevents INPs from turning into TICs is not completely understood. Recently it has been shown that Brat specifies INP cell identity by attenuating beta-catenin/Armadillo activity to extinguish the function of the self-renewal factor Klumpfuss (Komori et al., 2014).

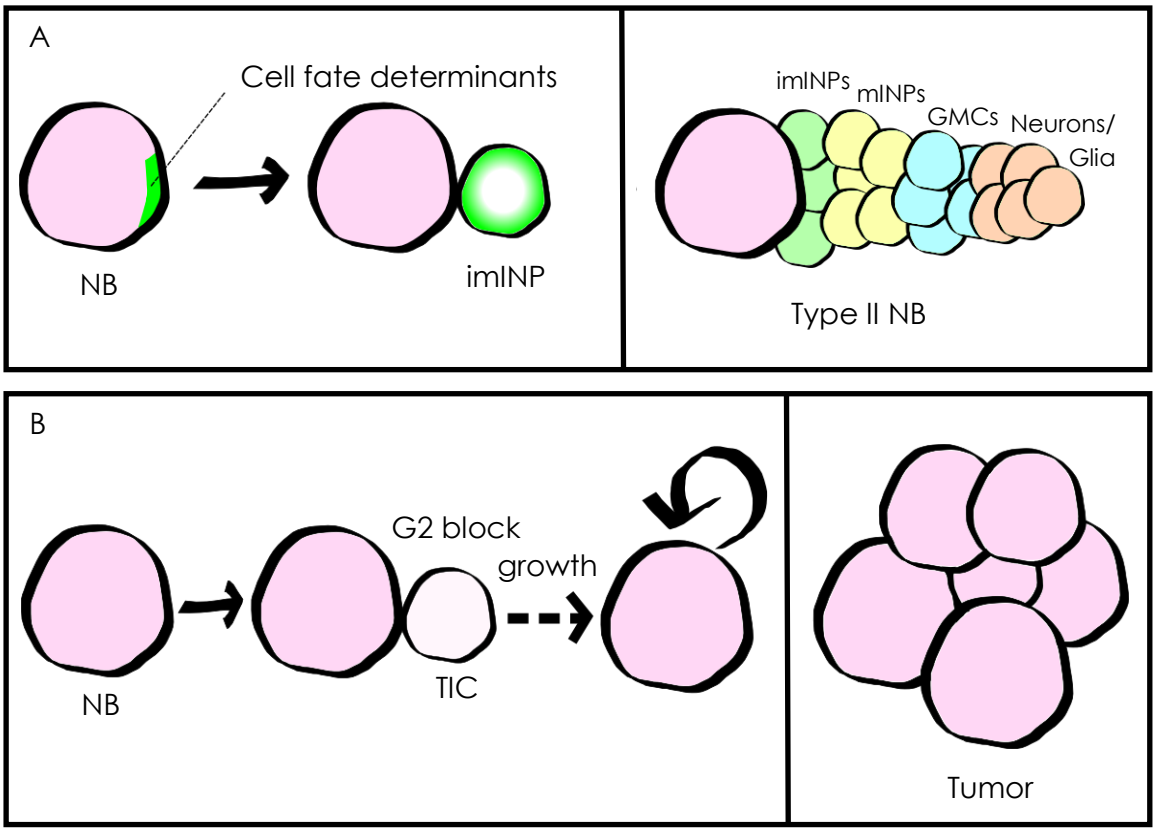


Figure 2 - Tumor formation

(A) During mitosis of type II NBs, the cell fate determinants Brat and Numb are segregated to the basal side of the cell. They are transmitted to the daughter cell to establish INP cell fate, which results in the regular formation of the type II NB lineage.
(B) Upon damage to the tumor suppressor Brat, the daughter cell of the NB is not able to establish its fate as an INP cell, but instead reverts back into a NB-like state, described as tumor initiating cell (TIC). TICs block their cell cycle in G2, regrow to NB size and subsequently start to proliferate, leading to the formation of a brain overgrowing tumor.

Aim of this study

CSCs share many features of normal stem cells but have lost the ability to control their mode of cell division, resulting in tumor formation. There is growing evidence that CSCs exist in a wide array of tumors and contribute to tumor heterogeneity (Dontu et al., 2003). However, whether tumors arise from differentiating cells SCs is still an ongoing debate. Brat tumors arise from deregulated stem cells, providing a fruitful tool to investigate CSCs. To understand mechanisms regulating the self-renewing divisions of regular SCs could provide insights into how those mechanisms are disrupted in CSCs.

In 2011, RNAi experiments showed that ectopic *Drosophila* NBs are stronger depending on the eukaryotic translation initiation factor 4E (*eIF4E*) than regular type II NBs (Song and Lu, 2011). This finding clarified the potential of an RNAi screen to discover genes that the tumor is especially depending on.

The first aim of this study is to identify genes, which are required for *brat* tumorigenesis. These genes will be further referred to as candidate genes. The second aim of this study is to analyze the overall requirement of these candidate genes in type II NBs. A type II NB specific RNAi system allowed for a targeted gene knockdown in type II NBs. Candidate gene knockdowns will be referred to as single knockdowns (SKDs), double knockdowns (DKDs) describe candidate gene knockdowns in addition to *brat*. An ideal target for cancer therapy would be a gene that tumor stem cells are dependent on whereas normal stem cells are not. We are screening for genes which suppress the brat phenotype without altering the regular type II NB lineage.

Results

Candidate gene selection - 1182 genes are upregulated in brat tumors

The *Drosophila* genome contains about 14000 genes (Adams, 2000). Tumors tend to upregulate genes, which are especially important to them. To identify genes, which are potentially important for a *brat* tumor, transcriptome analysis was performed. 1182 genes (Supplementary Table 1) were identified to be upregulated under the following three conditions of *brat* tumor formation:

I) Wild type type I and *brat* RNAi NBs of 3rd instar LBs were FACS sorted and their RNA was analyzed using deep-sequencing technology.

II) *brat* RNAi was induced in 3rd instar type II NBs. 24h after induction, the brains got FACS sorted to isolate the tumor cells. Subsequently the RNA was isolated and compared to the transcriptome of FACS sorted wt 3rd instar type II NBs using deep-sequencing technology. A *brat* RNAi induction of 24h was chosen to ensure that the lineage stages of the compared cells were analogical.

III) The transcriptome of whole wt adult brains was compared to the adult brain transcriptome of hypomorphic $brat^{K06028}$ mutants.

Candidate genes were annotated in a FileMaker database, which was specifically created for this screen (Figure 3).

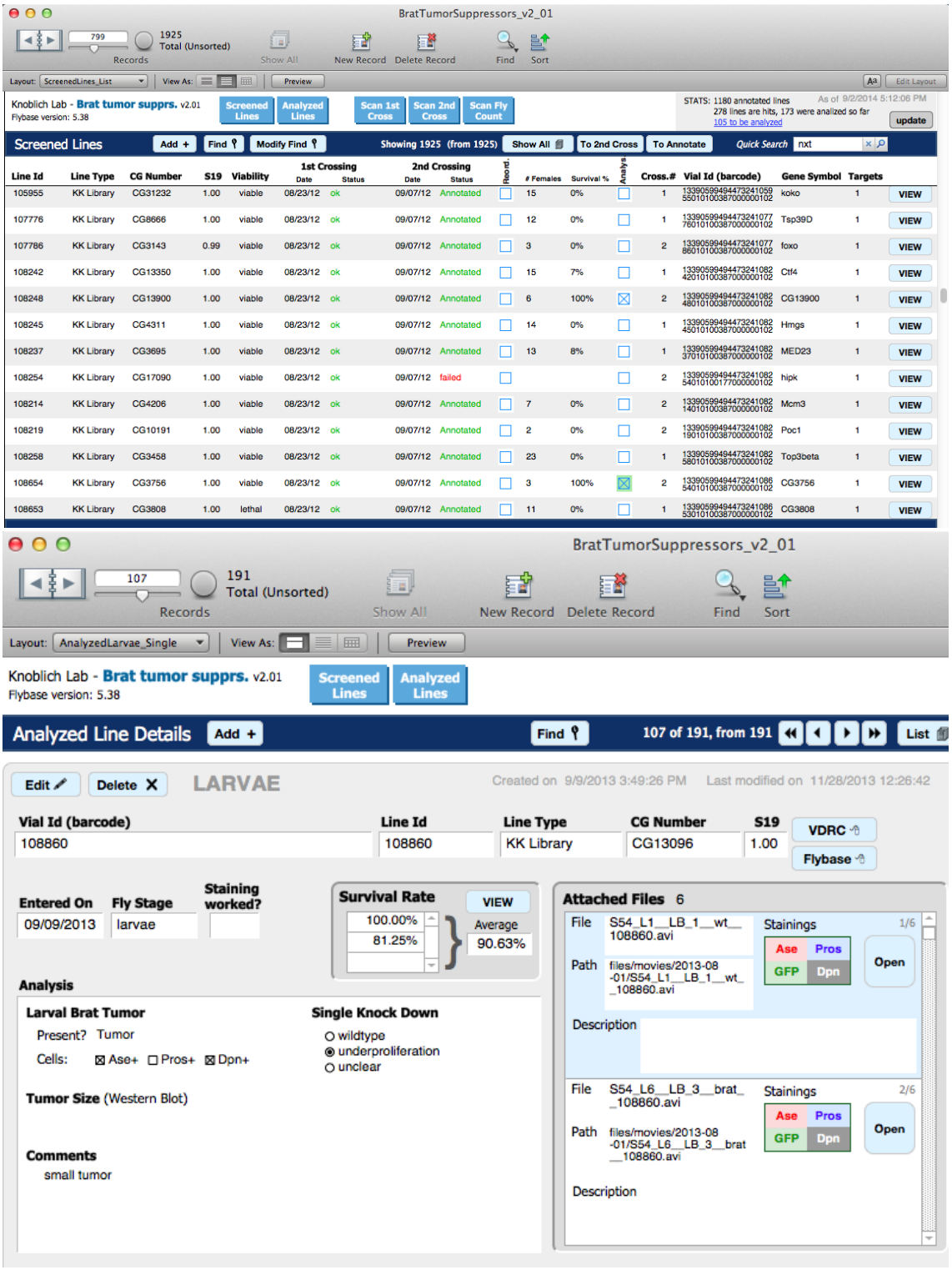


Figure 3 - Candidate gene database

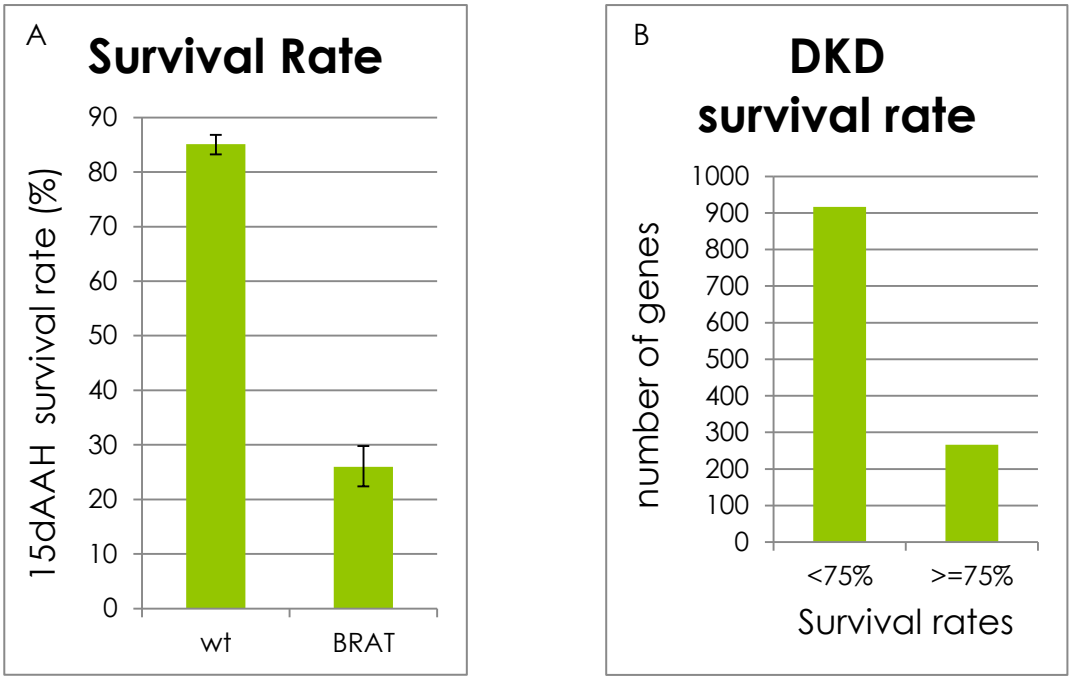
A FileMaker database was created to document the screening results. The candidate genes from the primary screen were annotated including the transformant identification number (TID) and the survival rate. Genes which were later chosen for the secondary screen were annotated in further detail, including tumor size and Z-stack movies of three SKD and DKD brains per gene.

Primary screen - 266 candidate genes prolong the survival rate

DKD flies were created using an F2 generation screen (Figure 4C). An upstream activating sequence (UAS) limits the expression of a gene to cells in which the yeast-derived transcription factor Gal4 is expressed. To allow cell-specific expression of genes, driver-lines activate Gal4 expression in desired cell types using specific promotors. Crossing a driver line to a UAS-GeneX containing line leads to the cell-specific expression of GeneX.

In the parental cross of our screen, a fly stocks containing homozygous UAS-*brat* RNAi on the first chromosome was crossed to fly stocks containing UAS-candidate gene RNAi on the second chromosome. The resulting F1 generation contained UAS-*brat* RNAi on the first, and UAS-candidate gene RNAi on the second chromosome. In the F2 cross, these flies were crossed to a PAN specific Gal4 driver line. PAN stands for "posterior *asense* negative" and describes the type II NB specific driver line, which was used for all mentioned experiments. It contains a *worniu*-Gal4, *ase*-Gal80 construct which allows for type II NB specific candidate gene expression. *worniu* is expressed in type I and type II NBs to prevent Elav-induced differentiation. A *worniu* promotor targets both kinds of NBs. *ase* is expressed in type I, but not in type II NBs. In the driver line, an *ase* promotor was linked to Gal80, which is an inhibitor of Gal4. Crossing our F1 generation to this driver line led to a type II NB specific expression of candidate genes. In addition, the PAN driver line contains an UAS-DCR2 construct to enhance the knockdown efficiency and UAS-CD8::GFP to label the cell surface of type II NBs and brain tumors.

One of the most important aspects of a tumor treatment is a prolonged survival of the cancer patient. We chose to use a prolonged survival rate of *brat* tumor flies upon candidate gene knockdown as the first readout to test the effectiveness of our candidates. An examination of wt flies showed that 15 days after adult hatching (15dAAH), about 88% of all wt flies are still alive (Figure 4 A). In contrast, *brat* RNAi flies showed only a survival rate of 25% 15dAAH. In order to examine the benefit of a candidate gene knockdown in a *brat* background, a threshold was set. All candidate genes, which upon knockdown raised the fly's survival rate in DKD to ≥ 75% 15dAAH, were further considered as promising candidates. 266 genes passed this viability screen (Figure 4 B). The rest of the 1182 genes was discarded, since they did not show a crucial survival benefit upon knockdown.



C

$$\boldsymbol{P}\ brat-RNAi;\frac{Pin}{CyO-RFP};\ X\ ;GeneX-RNAi;$$

$$\mathbf{F1}\ \frac{bratRNAi}{y};\frac{GeneX-RNAi}{CyO-RFP};X\ \frac{DCR2}{DCR2};\frac{PAN-Gal4}{PAN-Gal4};\frac{CD8::GFP}{CD8::GFP}$$

$$\mathbf{F2}\ \frac{brat-RNAi}{DCR2};\frac{GeneX-RNAi}{PAN-Gal4};\frac{CD8::GFP}{+}$$

Figure 4 - Primary screen

(A) The survival rates of wt adults and *brat* flies were determined 15dAAH. 88% of all wt *Drosophila* flies were still alive. In contrast, only 25% of *brat* flies survived the first 15dAAH. A total of 555 *brat* control flies and 1148 wt control flies were examined. Error bars indicate the confidence intervals.
(B) 916 double knockdown mutants showed a survival rate <75%. 266 candidate gene knockdowns rescued the survival to the threshold of 75% or more and were chosen for further analysis.
(C) The crossing scheme leading to the SKD and DKD flies. Males are shown in blue, females in red. Female larvae of the F2 generation are DKDs, male are SKDs.

Database confirmation – exclusion of 81 candidate genes

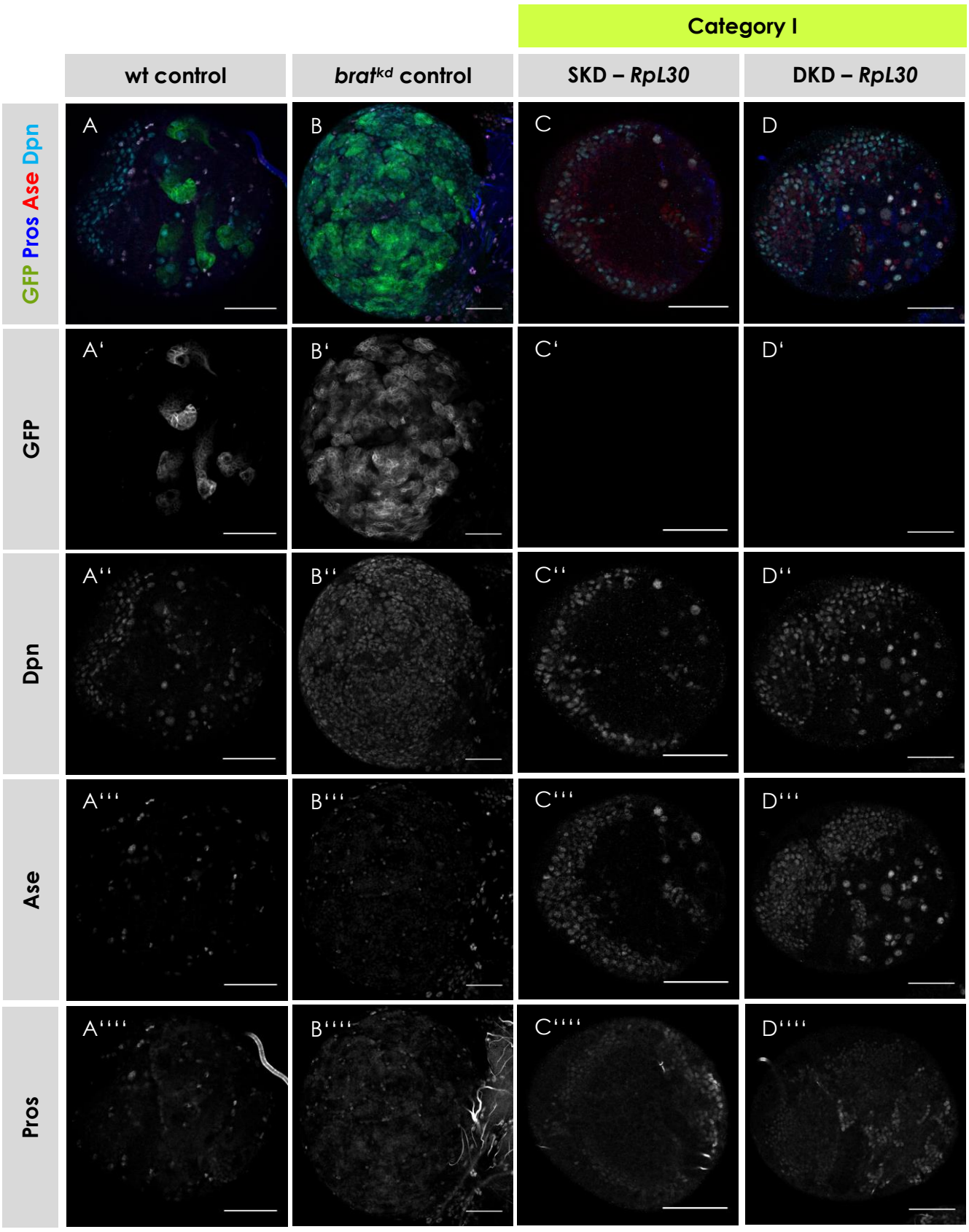
Previous research conducted in the Knoblich laboratory examined different gene knockdowns in NBs and annotated the phenotypes (Neumüller et al., 2011). The annotations including movies of knockdowns are available at the neuroblast screen database (Neuroblast Screen Database). 81 of the 266 remaining candidate genes from the primary screen were discarded, since they have been shown to cause an underproliferation phenotype in *Drosophila* NBs, indicating that these genes are required for NB development. The 185 remaining candidate genes (Supplementary Table 2), which either did not show a phenotype upon knockdown in type II NBs or have not been analyzed in the previous NB screen, were chosen for further analysis in the following secondary screen.

Secondary screen – 75 candidate genes did not alter the type II NB lineage

To determine the effects of candidate gene knockdowns on tumor development in *brat* tumor flies, a secondary screen including the 185 remaining candidate genes was performed. An important requirement for candidate gene knockdowns is that they do not alter the type II NB lineage in 3rd instar LBs, since this would indicate a role in basic cellular functions. Remaining candidates who fit those criteria are interesting, since we have already shown that they prolong the fly's life span. To examine these properties, confocal microscopy of type II NB lineages was performed with all 185 remaining candidate genes. They were observed in two different backgrounds, using long hairpin *RNAi* on the second chromosome. SKDs were used to analyze the effects of candidate gene knockdowns on the type II NB lineage. DKDs were examined to observe the effects of the candidate gene knockdowns on the tumor development. Three brains of every SKD and DKD were imaged using four different markers.

Expression of the markers *ase, dpn, pros* and GFP distinguishes cell types in larval neuroblast lineages. GFP was linked to CD8 on the cell surface of *worniu*$^{+}$, *ase*$^{-}$ cells (type II NBs, tumor cells), using a *worniu*-Gal4, *ase*-Gal80 – CD::GFP driver line. *Ase* is expressed in the nucleus of type I NBs and GMCs as well as the nucleus of type II mINPs and GMCs. Dpn is expressed in the nucleus of type I and type II NBs and mINPs. Using a dpn antibody, *dpn*$^{+}$, *ase*$^{-}$ labeled cells allow the identification of type II NBs within the lineage. Through dedifferentiation into a NB-like state, *dpn* expression is re-activated in the tumor cells. The cell fate determinant p*ros* is expressed in the nucleus of GMCs and neurons.

The results were divided into three categories, for each of which an example is shown in Figure 5. Category I, which involves 61 genes, showed no type II NB lineage in the SKD and no tumor in the DKD (Figure 5 C-C'''', D-D''''). Category II, which involves 124 genes, showed an altered type II NB lineage upon SKD and a small tumor in the DKD (Figure 5 E-E'''', F-F''''''). Category III includes 75 genes and showed a wt type II NB lineage upon SKD and a regular tumor in the DKD (Figure 5 G-G'''', H-H''''''). There were no candidate genes which showed a wt lineage in the SKD and no tumor in the DKD. The category III genes are the most interesting subgroup, since they do not alter the regular type II NB lineage, but prolong the survival of the flies.


Category I
wt control
bratkd control
SKD – RpL30
DKD – RpL30
GFP Pros Ase Dpn
GFP
Dpn
Ase
Pros
A
B
C
D
A‘
B‘
C‘
D‘
A‘‘
B‘‘
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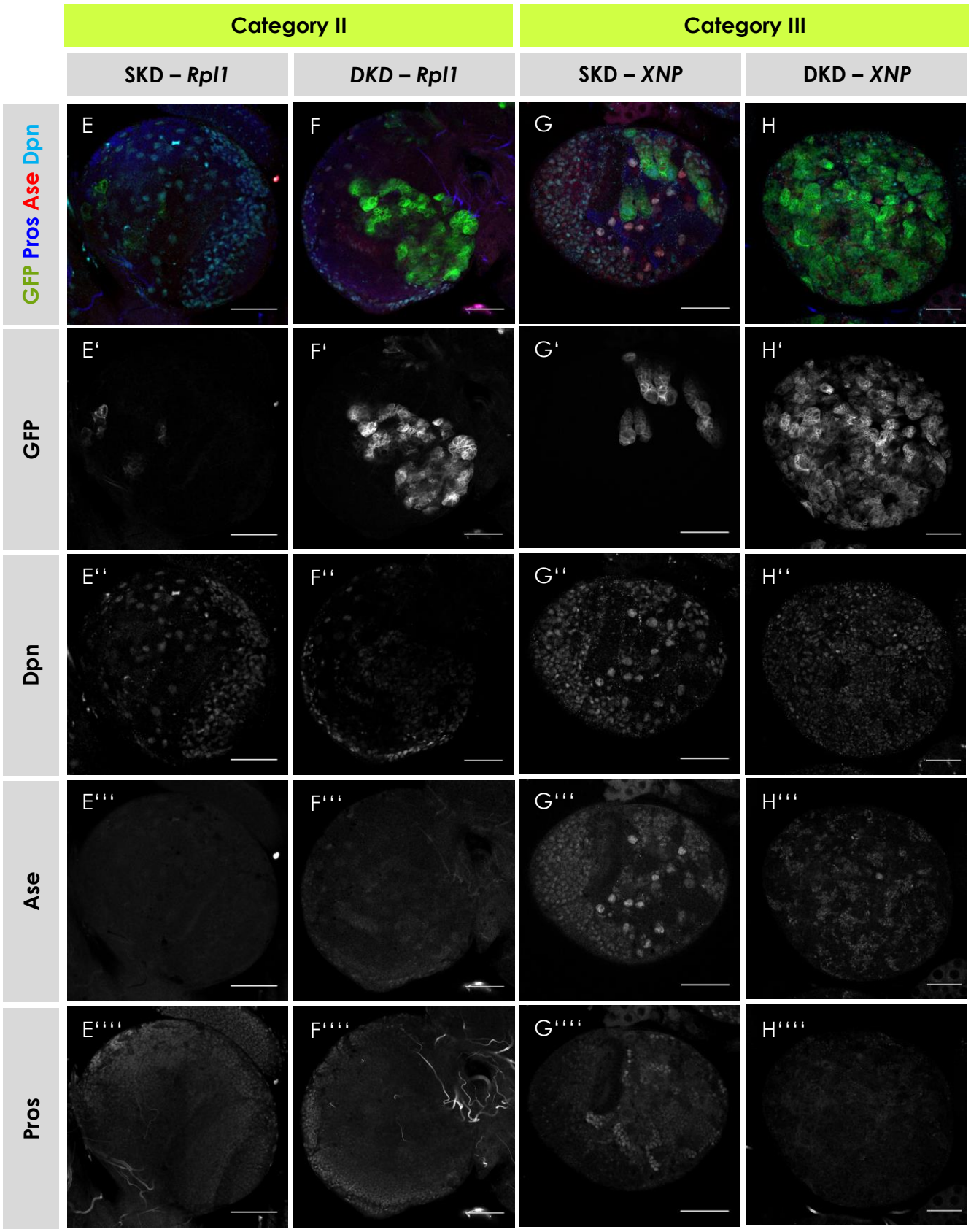
Category II
Category III
SKD – Rpl1
DKD – Rpl1
SKD – XNP
DKD – XNP
GFP Pros Ase Dpn
GFP
Dpn
Ase
Pros
E
F
G
H
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Figure 5 - Categories of secondary screen DKDs

The 185 remaining candidate genes were tested by knocking them down as SKDs and DKDs. Representative confocal microscopy images are shown. (A-A'''') wt type II NBs showed a regular lineage, whereas brat control brains were overgrown with tumor cells (B-B''''). (C-C'''') Category I genes showed no type II NB lineage upon SKD and no tumor upon DKD. The gene RpL30 is shown as a representative example. (D-D'''') Category II genes showed an altered type II NB lineage upon SKD and an oftentimes smaller tumor in the DKD. Rpl1 is shown as representative gene. (E-E'''') Category III genes did not alter the type II NB lineage upon SKD, but showed a regular sized tumor in the DKD. XNP is shown as an example for this category. Scale bars: 50 µm

Confirmation screen – 85% confirmation rate

A limitation of RNAi is the possibility of hitting off-target genes. Long hairpin miRNAs, which were used in the primary screen, are more susceptible to off-target effects than short hairpin miRNAs (shmiRs). A confirmation screen was performed for the category III candidate genes (Table 1) using fly lines with candidate gene shmiRs on the third chromosome.

The confirmation lines of the candidate genes were tested using confocal microscopy, whereas three different brains per gene were imaged. A confirmation of the secondary screen is defined by an unaltered type II NB lineage in a SKD 3rd instar larval brain. 40 out of the 75 candidates were tested (53.3%), whereas all but six tested genes confirmed the phenotype of the secondary screen, which is a confirmation rate of 85%.

75 category III hits							
APC4	*CG13624*	*CG31648*	*chico*	*kermit*	*MYPT-75D*	*Rbf2*	*U2A*
ase	*CG14274*	*CG32280*	*crc*	*klu*	*ncd*	*RpL24-like*	*wor*
BL26089	*CG14471*	*CG42321*	*dgt6*	*l(1)G0007*	*Nup133*	*Sip1*	*xmas-2*
bsf	*CG14667*	*CG4553*	*dpn*	*l(2)37Cc*	*Nxt1*	*Smg1*	*XNP*
cas	*CG15651*	*CG5414*	*Fak56D*	*ldbr*	*Pdk1*	*spn-E*	*yps*
CG10512	*CG16723*	*CG6569*	*FOXO(oe)*	*mRpL23*	*PGRP-LE*	*Ssl1*	
CG11337	*CG16758*	*CG8038*	*fu12*	*mRpL37*	*40 out of 75*	*Tfb4*	
CG11406	*CG17724*	*CG8142*	*galectin*	*mRpL46*	*Pi3K92E*	*Top3alpha*	
CG12018	*CG2691*	*CG9336*	*GLaz*	*mRpL53*	*Pi3K92E(DN)*	*ttm50*	
CG13126	*CG30398*	*CG9630*	*Jhl-21*	*Myb*	*Rae1*	*tyf*	

Confirmed phenotype

Altered NB lineage

Table 1 - Category III hits confirmation

40 out of 75 candidates were tested and 34 of them confirmed the genotype of the secondary screen (85%). Confirmed genes are highlighted green; genes showing a type II NB alteration in the confirmation screen are highlighted red. Orange genes were not jet tested in the confirmation screen at the end of the thesis.

Tumor mass quantification - l(1)G0007, mRpL46 and bsf reduce tumor size

According to our observations of double knockdown experiments, the candidates did show a *brat* tumor during larval development, however, rescued the viability. It is possible that some of the knockdowns interfere with the tumor maintenance at a later stage rather than with tumor initiation. In order to investigate whether adult flies still show tumors or whether the tumor disappears during development, the tumor size was quantitated. 15 3rd instar larval brains and 10 adult heads 2dAAH of every candidate gene DKD were collected and lysates were used for Western Blot. To quantify the tumor size we used an anti-GFP antibody, since tumor cells are GFP labeled. A representative Blot is shown in Figure 6 A. Additionally an anti-Miranda antibody was used, which labels proliferating cells. Miranda is an anchoring protein in NBs that keeps Prospero at the basal cortex to assure its asymmetric segregation into the GMC. After the generation of GMCs, Miranda releases Prospero and becomes degraded (Ikeshima-Kataoka et al., 1997). In a wild-type adult brain, Miranda is not present, whereas tumor brains show high amounts of Miranda. A Lamin antibody was used as a loading control.

The pixel intensities of the bands were measured using Fiji. The different bands were normalized to Lamin and put in relation to the *brat* control (Figure 6 B). The intensity of the *brat* control is defined as one on the y-axis. Intensity values higher than one indicate an up regulation compared to the *brat* control. Lower values indicate less abundant protein.

When analyzing type II NBs of SKDs using confocal microscopy, it is hardly possible to recognize a slower cell cycle progression, which could slow down tumor development in a *brat* background. Interesting candidates are those who do not alter the regular type II NB development in any way. Given the high number of brains in one Western Blot sample, a slower cell cycle progression in the DKDs should be visible as a smaller amount of GFP in the larval brains, compared to the *brat* control. To exclude this possibility, a criterion for an interesting gene was that the amount of GFP in the larval brain DKD should be around the same as in the larval *brat* control. To assess a relative shrinkage of the tumor in the DKDs, the levels of Brat and Miranda in the adult brain should be reduced, compared to the *brat* control.

Three of the examined genes - *l(1)G0007*, *mRpL46* and *bsf* - fit those criteria.

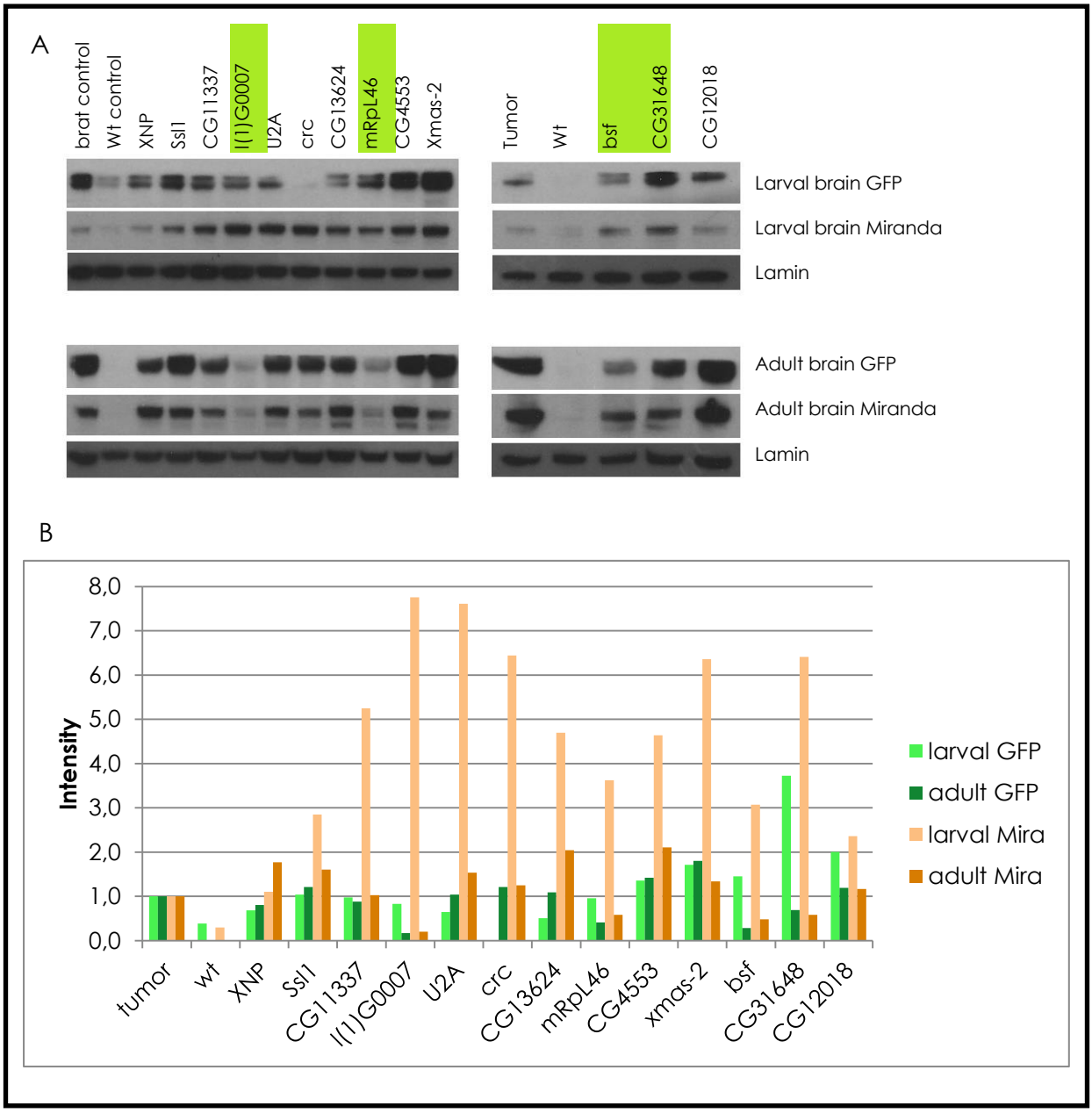


Figure 6 - Tumor mass quantification

The tumor development of the category III candidate genes was examined using Western Blotting (A). 15 3rd instar larval brains and 10 adult brains 2dAAH of each candidate gene were processed. Tumor mass was assessed using a GFP and a Miranda antibody. Signal intensities of the candidate gene DKDs were normalized to Lamin and put in relation to the brat control (B).
Three genes showed a strong GFP and Miranda reduction in the DKD of adult brains compared to the adult brain brat control, indicating a reduced tumor size in the adult stage. These genes - *l(1)G0007*, *mRpL46* and *bsf* - are highlighted green in panel A.

Adult brain microscopy - mRpL46^{kd} potently reduces tumor mass

To get a visual conformation of the Western Blot data, the three genes which showed a reduced tumor in the adult stage 2dAAH were further analyzed using confocal microscopy. Tumor size of the DKDs was measured by the amount of GFP and Deadpan in the brains. Several brains of the candidate gene DKDs were examined. Representative images are shown. Adult wt control brains did not show GFP staining and only background in the antibody labeled Dpn staining (Figure 7 A,A',A''). *brat* control brains were overgrown with GFP^{+} tumor cells which also feature a Dpn^{+} staining. The candidate genes *l(1)G0007* (Figure 7 C,C',C'') and *bsf* (Figure 7 D,D',D'') showed at most a small reduction in tumor size, concerning GFP and Dpn staining. The *mRpL46* adult DKD was comparable to the wt control, verifying a massive tumor reduction (Figure 7 E,E',E'').

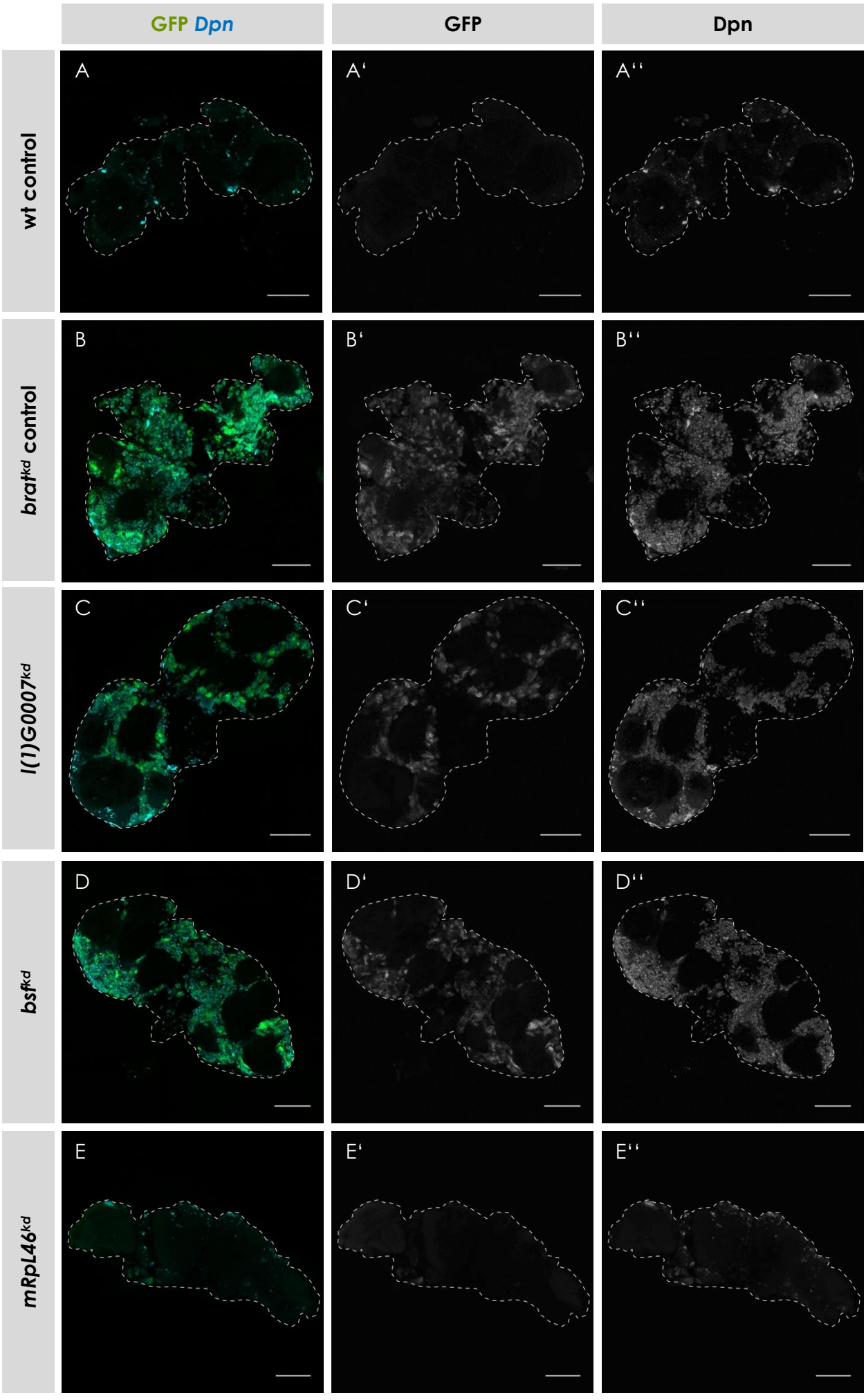

GFP Dpn
GFP
Dpn
wt control
brat^kd control
l(1)G0007^kd
bsf^kd
mRpL46^kd
A
A'
A''
B
B'
B''
C
C'
C''
D
D'
D''
E
E'
E''

Figure 7 - Adult tumor staining

Adult head stainings of the 3 most interesting candidate genes 2dAAH. Tumor mass was examined using a Dpn antibody and GFP labeling of tumor cells. (A-B) Images of control brains. wt control brains showed no GFP or Dpn staining (A, A', A''), whereas *brat* control brains showed many GFP^+, Dpn^+ cells (B, B', B''). *l(1)0007* (C, C', C'') and *bsf* (D, D', D'') knockdown brains showed no significant reduction in tumor mass. However in the *mRpL46* DKD (E, E', E''), Dpn and GFP stainings were comparable to the wt control, indicating a massive tumor shrinkage during development.

Discussion

Screening background

The targeting of tumor initiating cells without harming regular stem cells is an important task, considering future sustainable cancer therapy. Since tumors initiating cancer stem cells and regular stem cells mostly share the same surface markers and signaling pathways, this specific targeting is a challenging task. Systematic RNAi knockdowns are providing a potent tool to screen for different dependencies of regular type II NBs in contrast to ectopic, tumor initiating NBs.

In 2011, Song and Lu discovered the elevated dependency of tumor initiating NBS on the eukaryotic translation initiation factor 4E – eIF4E, using RNAi (Song and Lu, 2011). They showed that overexpression of Notch and loss of Brat both result in the up-regulation of eIF4E, which forms a feedback regulatory loop with the growth regulator dMyc. Ectopic NBs were shown to have a higher dependence on eIF4E than regular type II NBs. In this background, brain tumor phenotypes were successfully suppresses by feeding the animals fly food containing the antiviral drug Ribavirin, which is an inhibitor of eIF4E (Kentsis et al., 2004).

However, eIF4E was in none of the 3 datasets of upregulated genes in our screen. This could be due to a different inclusion-threshold. In our screen we paid much attention to the premise, that candidate genes should not alter the regular NB lineage. According to previous experiments from the Knoblich laboratory, eIF4E is annotated to cause neuroblast lineage alterations, as shown in the neuroblast database (Neuroblast Screen database - eIF4E). A possibility why this did not appear in the Song and Lu paper could be the usage of a weaker RNAi lineage. Also a less

efficient knockdown due to other temperatures, which are determining the efficiency of the Gal4 system, is possible.

A thinkable limitation of our screen is that genes, which were not upregulated in our dataset, could also able to specifically inhibit ectopic NB formation. However, limiting the screen to tumor-upregulated genes was a rational way of receiving a manageable amount of promising candidate genes.

75 genes prolong the life span without reducing the larval tumor size

The initial aim of this study was to search for genes, which the *brat* tumor is depending on, but the wt type II NBs are not. The most straight forward way to do that was to search for gene knockdowns, which show a regular type II NB lineage in the SKD, but no tumor in the DKD larval brain. None of the tumor up-regulated genes, which upon knockdown prolonged the lifespan of the *brat* tumor flies, was able to fit these criteria. All genes which showed no type II NB alterations upon SKD also showed a tumor phenotype of regular size in the DKD larval brain. Nevertheless 75 of these candidate genes led to a prolonged lifespan. There are different possible explanations for this phenomenon.

Candidate gene knockdowns could alter the genetic program in a way that does not reduce tumor mass, but helps the fly to better tolerate the tumor. It is not known what actually leads to the death of the tumor flies. Possibilities are e.g. mechanical pressure in the brain or tumor secreted toxic substances. One cannot rule out the possibility that candidate gene knockdowns reduce the production of fly harming substances.

Knockdowns leading to a slightly slower cell cycle could look ordinary in the SKD. Since confocal microscopy only shows a single time point of lineage development, one cannot distinguish between a younger lineage and an older lineage with slower lineage progression. A decelerated tumor progression could elevate the survival rate to the threshold of 75% 15dAAH.

Candidate genes could be required for the tumor maintenance at a later stage of the fly's development, e.g. the pupa or adult stage. This could lead to the subsequent reduction of tumor mass upon knockdown.

The last possibility would allow a specific interference with the tumor progression without altering regular NBs. Also concerning human therapy the aim is to eliminate a

grown tumor. To test this possibility, Western Blotting was performed on the 75 candidates to track the development of tumor mass beyond the larval stage. Western Blotting was preferred over immune fluorescence microscopy, since it is a more quantitative method. The GFP staining of tumor cells becomes instable over time, for which reason the adult brains were dissected 2dAAH, although the primary screen looked at the prolonged survival rate 15dAAH. A possible problem with this approach is that since the RNAi is only activated in type II NBs, 2dAAH candidate gene protein from precursor cells could still be present, due to high protein stability. 15dAAH the RNAi is inhibiting the candidate gene expression for a longer period of time, enabling the cell to further reduce protein remains. This could lead to a prolonged survival rate 15dAAH without altering the tumor phenotype 2dAAH.

As a further conformation, a Miranda antibody was used. The most interesting Western Blot findings were later verified by immune fluorescence microscopy, showing that knocking down *mRpL46* makes the tumor disappear in a later stage as measured by the absence of GFP and Dpn.

In the primary screen, *mRpL46* DKDs had a survival rate of 94% 15dAAH, in contrast to the 25% survival rate of *brat* tumor flies, indicating a huge survival benefit.

Limitations of the candidate gene selection

The limitation of the comparison between wt type I and *brat* RNAi NBs of 3rd instar LBs (Dataset I of the candidate gene selection) is that brat tumors arise from type II NBs and are here compared to type I NBs. This method was chosen because of the higher quantity of type I NBs over type II NBs in a wild type larval brain. It is to be expected that this analysis will include genes, which naturally differ in their expression between these cell types. Nevertheless it is likely that genes, which are upregulated in brat tumors, are included in these results as well, whereas genes of lower interest will be excluded from the screen at a later stage.

A downside of the comparison between the transcriptome of whole wt adult and the adult brain transcriptome of hypomorphic $brat^{K06028}$ mutants (Dataset II) is that it also contrasts the expression in neurons (adult brain) to the expression in neuroblasts (larval brain). Since inappropriate candidate genes will later be excluded, this

transcriptome analysis further elevates the chance of finding specific genes, which the tumor is depending on.

mRpl46 is required for tumor maintenance

Mitochondrial ribosomal proteins are encoded by nuclear genes and required for the mitochondrial protein synthesis. Our screen shows that the mitochondrial ribosomal protein L46 - *mRpL46* - plays an important role in the maintenance of *brat* induced tumors. *mRpL46* encodes a 258 AA protein and is located at the left arm of the 3rd chromosome (3L) of *Drosophila melanogaster*. During the pupa stage, regular NBs disappear, whereas the ectopic, *brat* induced tumor cells remain. We have shown that regular type II NBs are not depending on *mRpL46* and that a knockdown of *mRpL46* does not alter the tumor size up to the 3rd instar larval brain, but effectively reduces it during, or shortly after pupariation. This finding indicates that *mRpL46* plays a role in maintaining the tumor at a later stage. Few is known about the molecular function of *mRpL46*. Electronic annotations suggest a hydrolase activity and structural similarities suggest it to be a structural constituent of ribosome (Koc et al., 2001) which is involved in mitochondrial translation.

Besides mRpL46, three more mitochondrial ribosomal proteins – mRpL23, mRpL37 and mRpL53 - were included in the 75 candidate genes for the secondary screen, indicating an important role of the mitochondria in tumor function. Mitochondrial ribosomes translate among others genes which regulate the energy generation of the cell. Cancer cells are known to sometimes switch from oxidative phosphorylation to aerobic glycolysis (Warburg, 1956). Latter accumulated lactate even in the presence of oxygen. But also other metabolic changes in tumor mitochondria are documented, including decreased oxidation of substrates and altered control of apoptosis. It is possible that the knockdown of the four mitochondrial ribosomal proteins in our screen interferes with the metabolic switch from oxidative phosphorylation to aerobic glycolysis and thereby reduces the fitness of the tumor.

In human, specific inhibition of mitochondrial ribosomes was shown to selectively kill leukemia cells (Järås and Ebert, 2011).

mRpl46 and brat have human orthologs

Since the discovery of human brain tumor stem cells, the importance of appropriate tumor stem cell models is increasing (Singh et al., 2004). The Brat protein is conserved among metazoa. TRIM3 shares many similarities with the *Drosophila* Brat. TRIM3 acts as a tumor suppressor that regulates asymmetric cell division and stem cell properties in glioblastoma (Chen et al., 2014). In human glioblastoma samples, neurospheres and cell lines, TRIM3 expression was reduced. Like Brat, TRIM3 is a suppressor of c-Myc. These similarities lend relevance to Brat related research in *Drosophila*. There is a human ortholog of the *Drosophila mRpl46* gene with the identical name. The fact that there are *mRpL46* orthologs in many model organisms indicates that it serves a very basic mitochondrial function. In mice, the Brat homologue TRIM32 suppresses self-renewal, induces neuronal differentiation and thereby acts as tumor suppressor (Schwamborn et al., 2009). This presents a possibility for further *mRpL46* research in a mammalian model.

Further research

When this thesis was written, the project was not completed jet. Some experiments still have to be conducted in order to confirm and extend the knowledge about the role of *mRpL46* in *Drosophila* tumor maintenance. Some of them are already ongoing.

A survival assay, similar to the one which was performed with the initial long hairpin *miRNA* lines will be conducted with the short hairpin mRNA confirmation lines to determine the similarity of effectiveness. A confirmation line for *mRpL46* is already ordered.

Different approaches allow controlling for RNAi off-target effects. In or confirmation screen we used short hairpin miRNA to confirm the results of the long hairpin miRNA lines in the secondary screen. As an additional control, promising candidate gene knockdowns will be examined in a hypomorphic $brat^{KO6028}$ background. A further confirmation that the tumor disappears due to a specific RNAi targeting of the candidate genes would be RNAi resistant constructs. A prolonged survival rate of candidate gene RNAi resistant DKDs would indicate that the observed effects are due to off-targets.

Knockdown efficiency of the utilized candidate gene knockdown lines will be examined using qPCR and a housekeeping-gene driver line.

Tumor-rescue experiments are conducted to examine the possibility of a tumor mass reduction when knocking down candidate genes after tumor initiation. A stock containing a hypomorphic *brat* allele will be crossed to a candidate gene RNAi line and kept on 18°C. After tumor initiation, the larvae will be shifted to 29°C to activate the Gal4 system and knock down the candidate gene. Tumor rescue ability would further enhance the relevance of this model for therapy purposes. In patients, one cannot interfere with the initiation of a tumor, but has to eliminate it afterwards.

Material and Methods

Fly strains

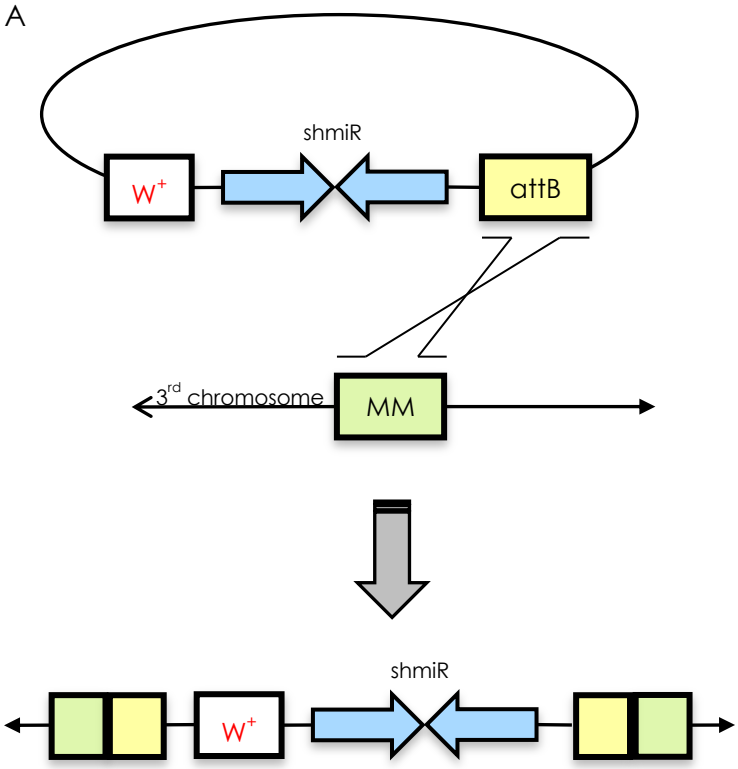
Flies were raised on a standard agar medium. RNAi lines were purchased either from the Bloomington Stock Center or the VDRC Stock Center. The following fly strains were used: wt control - VDRC *TID 60100;* brat^{kd} –VDRC TID 105054; mRpL46^{kd} – VDRC TID 110327; type II NB driver line – UAS-Dicer2; worGal4, *ase*Gal80; UAS-CD8::GFP (Neumüller et al., 2011). Stocks were maintained either at 25°C or at 18°C. RNAi lines crossed to a driver line were kept on 25°C for 24h, then shifted to 29°C for optimal Gal4 conditions.

ShmiR line generation

The 75 category III candidate genes were confirmed either using ordered small hairpin RNAi lines or self-created ones. For genes, which were not available as shmiR line, 24 transgenic fly lines were created. Gene specific primers were designed using the online tool "Primer3Plus". A list of the ordered primers is shown in Supplementary Table 3. The VBC fly house facility cloned the primers into vectors, introduced them into *E.coli* bacteria for expansion and extracted them using midiprep. The vectors were injected into *Drosophila* embryos by the VBC fly facility (Supplementary Figure 1 A).

A backcrossing scheme led to the creation of stable shmiR transgenic fly stocks including larval and adult marker genes. Further crosses allowed the selection of SKDs and DKDs (Supplementary Figure 1 B).

A



B

$$\boldsymbol{P}\ \frac{Integrase}{y};;\frac{W+,shmiR}{+}\ X\ ;\frac{IF}{CyO};\frac{Sb}{TM3,Ser}$$

$$\boldsymbol{F1}\ \frac{+}{y};\frac{IF}{+};\frac{W+,shmiR}{TM3,Ser}\ X\ ;\frac{IF}{CyORFP};\frac{Sb}{TM3,Ser}$$

$$\boldsymbol{F3}\ \frac{+}{y};\frac{IF}{CyORFP};\frac{W+,shmiR}{TM3,Ser}\ X\ ;brat-RNAi;\frac{Dr}{TM6,tb}$$

$$\boldsymbol{F4}\ ;\frac{brat-RNAi}{CyORFP};\frac{W+,shmiR}{TM6,tb}\ X\ \frac{DCR2}{DCR2};\frac{PAN-Gal4}{PAN-Gal4};\frac{CD8-GFP}{CD8-GFP}$$

$$\boldsymbol{F5\ DKD}\ \frac{DCR2}{+};\frac{brat-RNAi}{PAN-Gal4};\frac{W+,shmiR}{CD8-GFP}$$

$$\boldsymbol{F5\ SKD}\ \frac{DCR2}{+};\frac{CyORFP}{PAN-Gal4};\frac{W+,shmiR}{CD8-GFP}$$

Supplementary Figure 1

To generate shmiR lines for the confirmation screen of the category III candidate genes, primer were designed in the "Primer 3Plus" online tool and cloned into vectors carrying a W+ marker and an attB landing site (A). The vectors were injected into *Drosophila* embryos where they integrated into an MM landing site on the third chromosome of germline cells. To generate stable, transgenic stocks and to add larval and adult marker genes, the injected flies were crossed to balancer-chromosome containing stocks. This allowed the selection of SKDs and DKD larvae, when crossed to a type II NB driver line (B).

Primary viability screen

To determine the effects of candidate gene knockdowns on the survival rate of *brat* tumor flies, DKDs were kept in vial-boxes on 29°C with each box containing about 100 different DKD stocks as well as wt - and $brat^{kd}$ controls.

The survival rate was determined by counting and flipping the DKD and control flies of each genotype into new vials 4dAAH. To prevent them from breeding they were kept on yeast negative media and flipped in regular intervals. 15dAAH the number of living flies in each vial was counted and the survival rate was expresses in percent.

Larval brain microscopy preparation

3rd instar larval brains were dissected in PBS and fixed with 5% PFA in PBS (Sigma-Aldrich) for 20min at room temperature. Afterwards the brains were washed 2x with PBST (0.1% Triton-X100 in PBS). Brains were blocked for 1h using a blocking solution containing 0.1% PBST and 1% NDS (normal donkey serum). Primary antibodies were put in blocking solution to stain the brains over night at 4°C. Three washing steps with 0.1% PBST for 15min removed the primary antibodies from the solution. Brains were put into secondary antibodies in blocking solution for 2h at room temperature. Three washing steps with 0.1% PBST for 15min removed the secondary antibodies from the solution. Two washing steps with PBS removed the Triton from the solution. After removing the PBS, 3 drops of Equilibrium buffer (Life technologies – Antifade Kit Component C) were added to the brains for 5min. After removing the equilibrium buffer, 1 drop of Antifade buffer (Life technologies – Antifade Kit Component A) was added to the brains for 5min. Brains were then mounted on glass slides in Vectashield (VECTOR laboratories). The stainings resulted from the following markers. Neuroblasts and tumor cells were labeled using a CD8::GFP producing driver line (Neumüller et al., 2011). For additional stainings different primary antibodies were utilized.

Guinea pig anti-*dpn* 1:1000 (Antibody from Jim Skeath - unpublished)
Rat anti-*ase*: 1:100 (made by Knoblich lab)
Rabbit anti-*pros*: 1:1000 (Vaessin et al., 1991)
Secondary antibodies were ordered from life technologies and diluted 1:600
Alexa Flour 647 goat anti-guinea pig IgG
Alexa Flour 568 goat anti-rat IgG
Alexa Fluor 405 goat anti-rabbit IgG

Adult brain microscopy preparation

Adult brains 2dAAH were dissected in Grace's insect media and incubated in 300µl formaldehyde solution (4% paraformaldehyde in PBS + 1% Triton-X) for 20min at room temperature. Three washing steps in 0.1%, 0.3% and 0.5% Triton-X in PBS were conducted for 15min each. Brains were put on 4°C over night in 300µl blocking solution (5% normal goat serum + 0.3% PBST). The brains were put in 100µl primary antibody solution (Guinea pig anti-*dpn* 1:1000 in 100µl blocking solution) over night at 4°C. Three washing steps in 500µl PBST (0.3% Triton-X in PBS) were performed for 15min at room temperature. Brains were spun down for 5min and incubated in secondary antibody solution (Alexa Flour 647 goat anti-guinea pig IgG 1:600 in blocking solution) for 24h at 4°C. 3 washing steps to 15min each were performed using PBST. An additional PBST washing step was performed over night at 4°C. Brains were washed in PBS for 15min. Three drops of Equilibrium buffer (Life technologies – Antifade Kit Component C) were added to the brains for 5 minutes. After removing the Equilibrium buffer, 1 drop of Antifade buffer (Life technologies – Antifade Kit Component A) was added to the brains for 5 minutes. Brains were then mounted on glass slides in Vectashield (VECTOR laboratories).

Western blotting

15 larval brains and 10 adult brains of each sample were collected in 50µl Laemmli Sample Buffer (Bio Rad). 1µl Benzonase Nuclease (Novagen) was added to the samples which then were thoroughly grinded and put onto 95°C for 2min. Samples were snap frozen and stored at -80°C. The samples were unstitched using Novex NuPAGE 4-12% Bis-Tris Protein gels and transferred to Amersham Hybond ECL

Nitrocellulose membranes. Thermo Scientific PageRuler Prestained Protein Ladder was used as a marker. Different primary antibodies were used for detection.
Mouse anti-Lamin 1:2000
Mouse anti JL-8 GFP 1:1000 (Clonetech)
Rabbit anti-Miranda 1:1000
HRP bearing secondary antibodies were used.
ECL™ Anti-Rabbit IgG HRP linked whole antibody from donkey (GE Healthcare)
ECL™ Anti-Mouse IgG HRP linked whole antibody from sheep (GE Healthcare)
Pierce ECL Plus Western Blotting Substrate (Thermo scientific) was used as HRP substrate for detection.

Contributions

The 1182 tumor upregulated genes were determined by three datasets of comparative transcriptome analysis. Dataset I was created by Heike Harzer. Dataset II was created by Ilka Reichardt and dataset III was created by Christoph Jüschke. The primary viability screen, the database confirmation and the majority of the secondary screen were performed by Ilka Reihchardt and Lisa Landskron. The large part of my contribution was the creation of confirmation shmiR lines, the execution of the confirmation screen, the tumor mass quantification and the adult brain examination.

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Supplemental Material

1182 brat upregulated genes											
18w	*CG10932*	*CG15029*	*CG30338*	*CG42574*	*CG7339*	*CycB3*	*GluRIIB*	*Mat1*	*Nup58*	*RpI12*	*stau*
Aats-ala	*CG10933*	*CG15047*	*CG30349*	*CG42588*	*CG7352*	*CycE*	*Glycogenin*	*mbm*	*Nup62*	*RpII18*	*stc*
Aats-asn	*CG10979*	*CG15083*	*CG30354*	*CG42593*	*CG7357*	*CycH*	*Gnf1*	*mbt*	*Nup98*	*RpL12*	*stck*
Aats-gly	*CG11019*	*CG15098*	*CG30383*	*CG42613*	*CG7386*	*Cyp6t3*	*Got2*	*Mcm10*	*nxf2*	*RpL15*	*Su(var)2-HP2*
Aats-his	*CG11030*	*CG15107*	*CG30398*	*CG42709*	*CG7457*	*Cyp9c1*	*Gp150*	*Mcm2*	*Nxt1*	*RpL19*	*sug*
Aats-lys	*CG11071*	*CG1516*	*CG30409*	*CG42797*	*CG7504*	*D12*	*Gpo-1*	*Mcm3*	*okr*	*RpL21*	*Sug*
achi	*CG11095*	*CG15173*	*CG30497*	*CG4281*	*CG7627*	*dan*	*Gr43a*	*Mcm5*	*olf413*	*RpL24-like*	*Surf6*
Acp1	*CG11120*	*CG15220*	*CG3061*	*CG42863*	*CG7637*	*daw*	*grh*	*Mcm7*	*Optix*	*RpL27*	*Taf8*
Acph-1	*CG11125*	*CG15317*	*CG31028*	*CG43066*	*CG7728*	*Dbp73D*	*grim*	*Mdh2*	*Or42b*	*RpL28*	*Takl1*
Actr13E	*CG11133*	*CG15343*	*CG31055*	*CG43313*	*CG7730*	*Dbp80*	*Grip128*	*Mdr49*	*orb*	*RpL30*	*tam*
Adam	*CG11180*	*CG15368*	*CG31125*	*CG43340*	*CG7744*	*Dbx*	*Grip71*	*MED23*	*Orc1*	*RpL35*	*Tango13*
Aef1	*CG11257*	*CG1542*	*CG31370*	*CG43367*	*CG7841*	*Dcp-1*	*grn*	*mei-218*	*Orc2*	*RpL36A*	*Tango4*
agt	*CG11337*	*CG15535*	*CG31373*	*CG4364*	*CG7922*	*del*	*grp*	*mei-38*	*Orc4*	*RpL39*	*Tango7*
Ahcy13	*CG11403*	*CG15561*	*CG31462*	*CG4476*	*CG7993*	*desat1*	*GstD10*	*mei-41*	*Orc6*	*RpL40*	*Tao-1*
ald	*CG11406*	*CG15602*	*CG31473*	*CG4500*	*CG8010*	*Det*	*GstD2*	*mei-P26*	*osp*	*RpL5*	*tap*
ana3	*CG11417*	*CG15639*	*CG31525*	*CG4553*	*CG8038*	*Dgp-1*	*GstD3*	*mei-S332*	*Ote*	*RpL7*	*Tcp-1eta*
AP-2	*CG11505*	*CG15646*	*CG3160*	*CG4554*	*CG8097*	*dgt3*	*GstD4*	*melt*	*Pak3*	*RpL7A*	*Tcp-1zeta*
APC4	*CG11583*	*CG15651*	*CG31635*	*CG4570*	*CG8142*	*dgt4*	*GstD9*	*Mes2*	*Parg*	*RpLP0-like*	*tefu*
aPKC	*CG11596*	*CG15738*	*CG31648*	*CG4658*	*CG8145*	*dgt6*	*GstE1*	*Mes4*	*park*	*RpLP2*	*TepI*
apt	*CG11660*	*CG1575*	*CG31673*	*CG4709*	*CG8173*	*Dhod*	*GstE6*	*Mes-4*	*path*	*Rpp30*	*TepIV*
Arc1	*CG11825*	*CG15771*	*CG31688*	*CG4726*	*CG8177*	*Dic1*	*GstE7*	*mfrn*	*pav*	*RpS12*	*Tfb1*
Arc-p34	*CG11837*	*CG15784*	*CG31694*	*CG4901*	*CG8184*	*dikar*	*GstE8*	*Mical*	*pch2*	*RpS16*	*Tfb4*
armi	*CG11873*	*CG15881*	*CG31710*	*CG4936*	*CG8306*	*Dll*	*gwl*	*Mio*	*Pcl*	*RpS20*	*TfIIFalpha*
Arp8	*CG11882*	*CG1607*	*CG31873*	*CG4951*	*CG8399*	*dm*	*Hcs*	*Mira*	*pcx*	*RpS27*	*Tgt*
Art3	*CG11920*	*CG1622*	*CG31918*	*CG4973*	*CG8478*	*dmrt99B*	*hdc*	*Mis12*	*Pde8*	*RpS27A*	*TH1*
Art7	*CG11943*	*CG1667*	*CG32104*	*CG5017*	*CG8503*	*dmt*	*Hex-A*	*mit(1)15*	*Pdk1*	*RpS28b*	*Thor*
ase	*CG11986*	*CG1671*	*CG3216*	*CG5033*	*CG8531*	*DNA-ligI*	*hig*	*Mkrn1*	*Pen*	*RpS30*	*thr*
ash1	*CG12010*	*CG16713*	*CG32185*	*CG5079*	*CG8538*	*DNApol-alpha180*	*HipHop*	*Mlh1*	*pen*	*RpS3A*	*timeout*
ash2	*CG12016*	*CG1673*	*CG32198*	*CG5104*	*CG8545*	*DNApol-alpha73*	*hipk*	*Mmp2*	*Pepck*	*Rpt3R*	*tio*
asp	*CG12018*	*CG16753*	*CG3226*	*CG5114*	*CG8549*	*DNApol-delta*	*Hmgs*	*mms4*	*pex12*	*Rrp1*	*tld*
asrij	*CG12112*	*CG16756*	*CG32280*	*CG5149*	*CG8557*	*DNApol-eta*	*Hmx*	*mnd*	*Pfk*	*Rrp4*	*tll*
atms	*CG12128*	*CG16799*	*CG32344*	*CG5205*	*CG8611*	*dnk*	*HP5*	*Mnn1*	*Pgd*	*Rrp40*	*Tmhs*
ato	*CG12170*	*CG16879*	*CG32392*	*CG5254*	*CG8613*	*Dok*	*Hr78*	*MP1*	*Pgm*	*Rrp45*	*Top2*
AttB	*CG12179*	*CG16888*	*CG32479*	*CG5270*	*CG8771*	*Dp1*	*Hsc70-5*	*Mpk2*	*PGRP-LE*	*Rrp46*	*Top3alpha*
Atu	*CG12204*	*CG16892*	*CG3251*	*CG5335*	*CG8778*	*dpa*	*Hsp68*	*mrn*	*phr6-4*	*Rrp6*	*Top3beta*
Atx-1	*CG12206*	*CG16908*	*CG32532*	*CG5380*	*CG8801*	*Dph5*	*Hsp70Ba*	*mRpL20*	*Pi3K21B*	*Rs1*	*Traf4*
aur	*CG12269*	*CG16935*	*CG32533*	*CG5414*	*CG8891*	*Dpit47*	*ial*	*mRpL23*	*pie*	*S6k*	*trbl*
az2	*CG12299*	*CG16989*	*CG32576*	*CG5525*	*CG8920*	*dpn*	*IM14*	*mRpL28*	*Pink1*	*Sas10*	*Treh*
baf	*CG12301*	*CG17018*	*CG32625*	*CG5535*	*CG8939*	*dpr12*	*Imp*	*mRpL37*	*pita*	*Sas-4*	*trem*
ball	*CG12316*	*CG1703*	*CG32712*	*CG5589*	*CG9065*	*dre4*	*ImpL2*	*mRpL38*	*pix*	*sas-6*	*Tret1-1*
barr	*CG12325*	*CG17032*	*CG32767*	*CG5664*	*CG9086*	*drongo*	*ImpL3*	*mRpL46*	*Pkn*	*sba*	*Trf*
beat-IIIc	*CG1234*	*CG17162*	*CG32792*	*CG5665*	*CG9125*	*Dscam2*	*Incenp*	*mRpL53*	*plexB*	*SC35*	*Trf2*
beta4GalNAcTA	*CG12370*	*CG17219*	*CG32982*	*CG5690*	*CG9215*	*dUTPase*	*Ino80*	*mRpS31*	*Pmm45A*	*scat*	*Trn*
beta4GalNAcTB	*CG12432*	*CG17265*	*CG33051*	*CG5705*	*CG9238*	*Dys*	*Inos*	*Mrtf*	*Pms2*	*scny*	*trpm*
BHD	*CG12496*	*CG17266*	*CG33056*	*CG5728*	*CG9246*	*E(Pc)*	*insc*	*msb1l*	*pnt*	*scra*	*Trx-2*
bip1	*CG12531*	*CG17273*	*CG33062*	*CG5757*	*CG9272*	*Eaf*	*Ip259*	*msd1*	*Poc1*	*sdk*	*Ts*
Bj1	*CG1265*	*CG17294*	*CG33082*	*CG5800*	*CG9305*	*Eap*	*Ir100a*	*Msh6*	*pog*	*sea*	*Tsp39D*
bor	*CG12702*	*CG17327*	*CG33092*	*CG5808*	*CG9336*	*eap*	*Ir40a*	*msk*	*polo*	*Sec16*	*Tsp42Ef*
Br140	*CG12708*	*CG1753*	*CG33123*	*CG5835*	*CG9363*	*ebd2*	*Ir76a*	*Msp-300*	*pon*	*Sep.05*	*Tsp68C*
bsf	*CG12822*	*CG17556*	*CG33158*	*CG5853*	*CG9384*	*eco*	*Irbp*	*mst*	*por*	*sff*	*ttm50*
btd	*CG12909*	*CG17658*	*CG33172*	*CG5857*	*CG9422*	*eIF2B-epsilon*	*Jhl-1*	*MTF-1*	*ppa*	*shg*	*tum*
Bub3	*CG12911*	*CG17724*	*CG33230*	*CG5886*	*CG9576*	*eIF-2beta*	*Jhl-21*	*mthl10*	*ppan*	*shn*	*tyf*
bys	*CG12975*	*CG17726*	*CG33260*	*CG5924*	*CG9619*	*eIF5B*	*Jhl-26*	*MtnA*	*Ppat-Dpck*	*shtd*	*U2A*
c11.1	*CG13096*	*CG17764*	*CG33293*	*CG5932*	*CG9630*	*eIF6*	*jigr1*	*mtSSB*	*Ppcs*	*shu*	*U2af50*

Ca-beta	*CG13126*	*CG17765*	*CG33331*	*CG5953*	*CG9634*	*Eip71CD*	*kek1*	*mu2*	*pr-set7*	*sif*	*U3-55K*
cac	*CG13185*	*CG17803*	*CG3358*	*CG6023*	*CG9650*	*Eip74EF*	*kis*	*mus101*	*ps*	*Sip1*	*U4-U6-60K*
Cad96Ca	*CG13287*	*CG17838*	*CG34126*	*CG6066*	*CG9674*	*Eip75B*	*Klp3A*	*mus205*	*Psf1*	*Sirt4*	*Uba2*
Cad96Cb	*CG13305*	*CG1785*	*CG34135*	*CG6073*	*CG9705*	*Eip93F*	*Klp61F*	*mus209*	*PsGEF*	*Six4*	*Ubpy*
Caf1	*CG13330*	*CG1789*	*CG34243*	*CG6154*	*CG9727*	*Elf*	*Klp67A*	*mus210*	*Ptth*	*skap*	*unk*
Caf1-105	*CG13367*	*CG17896*	*CG34250*	*CG6171*	*CG9752*	*Elp1*	*klu*	*mus301*	*Pvf2*	*Ski6*	*Upf3*
CalpC	*CG1344*	*CG17999*	*CG34263*	*CG6188*	*CG9772*	*Elp2*	*koko*	*mus308*	*pxb*	*sky*	*uri*
CanA-14F	*CG13623*	*CG18171*	*CG3430*	*CG6191*	*CG9784*	*enc*	*kug*	*mxc*	*pyd*	*Slbp*	*vfl*
CanB2	*CG13624*	*CG18273*	*CG34356*	*CG6234*	*CG9839*	*Ercc1*	*kz*	*Myb*	*qkr58E-2*	*sle*	*Vha100-2*
Cas	*CG13690*	*CG18302*	*CG34371*	*CG6241*	*CG9890*	*Ets97D*	*l(1)dd4*	*Myd88*	*r*	*slp1*	*VhaAC45*
cas	*CG1371*	*CG18324*	*CG34430*	*CG6272*	*CG9940*	*eya*	*l(1)G0007*	*Myo61F*	*rad50*	*SmB*	*VhaPPA1-1*
cav	*CG13741*	*CG18343*	*CG34449*	*CG6310*	*CG9967*	*Fancd2*	*l(1)G0045*	*MYPT-75D*	*Rad9*	*Smc5*	*VhaSFD*
Cct5	*CG13766*	*CG18347*	*CG34450*	*CG6353*	*CG9973*	*Fancl*	*l(1)G0222*	*N*	*Rae1*	*SmD1*	*vig*
Cctgamma	*CG13887*	*CG1850*	*CG34454*	*CG6424*	*CG9975*	*Fatp*	*l(2)03659*	*nab*	*RagA*	*SmD3*	*vig2*
CD98hc	*CG13895*	*CG18508*	*CG3476*	*CG6425*	*CG9987*	*f-cup*	*l(2)03709*	*NaCP60E*	*Rapgap1*	*SmE*	*vls*
cdc23	*CG13900*	*CG18528*	*CG3573*	*CG6426*	*CG9993*	*fd102C*	*l(2)09851*	*nAcRalpha-34E*	*Rbcn-3A*	*SmG*	*vret*
CdGAPr	*CG13901*	*CG18536*	*CG3605*	*CG6459*	*Chd64*	*Fdxh*	*l(2)35Df*	*Nap1*	*Rbcn-3B*	*Smg1*	*vri*
Cdk7	*CG13907*	*CG18547*	*CG3645*	*CG6470*	*cher*	*Fen1*	*l(2)37Cc*	*Nat1*	*Rbf2*	*smid*	*vvl*
CecA2	*CG13926*	*CG18600*	*CG3679*	*CG6486*	*chico*	*feo*	*l(2)k09022*	*Ncc69*	*Rbm13*	*Smox*	*wb*
Cenp-C	*CG14023*	*CG18605*	*CG3704*	*CG6490*	*cid*	*Fer1HCH*	*l(2)k10201*	*ncd*	*Rcd5*	*Smr*	*wdb*
CG10133	*CG14042*	*CG1868*	*CG3756*	*CG6512*	*CkIIalpha-i3*	*ferrochelatase*	*l(2)not*	*ncm*	*rdgBbeta*	*sni*	*WDR79*
CG10195	*CG14059*	*CG1972*	*CG3788*	*CG6550*	*Cks30A*	*Fib*	*l(3)07882*	*Ndc80*	*RecQ4*	*Snm1*	*wds*
CG10260	*CG14073*	*CG2006*	*CG3808*	*CG6569*	*Clp*	*FK506-bp1*	*La*	*neb*	*ref(2)P*	*sno*	*wibg*
CG10286	*CG14117*	*CG2051*	*CG3817*	*CG6614*	*CLS*	*fkh*	*lack*	*nej*	*REG*	*Snoo*	*Wnt2*
CG10336	*CG14210*	*CG2064*	*CG3829*	*CG6693*	*clu*	*Fmr1*	*lama*	*NFAT*	*rempA*	*so*	*wor*
CG10341	*CG14230*	*CG2083*	*CG3918*	*CG6695*	*cn*	*fok*	*larp*	*NK7.1*	*rept*	*SoxN*	*wus*
CG10365	*CG14231*	*CG2260*	*CG3919*	*CG6712*	*cno*	*foxo*	*ldbr*	*Nle*	*RfC3*	*Sp1*	*xmas-1*
CG10414	*CG14234*	*CG2321*	*CG3975*	*CG6762*	*Coop*	*frc*	*lds*	*Nmd3*	*RfC38*	*Spargel*	*xmas-2*
CG10418	*CG14274*	*CG2617*	*CG3984*	*CG6770*	*Corin*	*Frq2*	*Lig4*	*Nmdmc*	*RfC4*	*Spc105R*	*XNP*
CG10425	*CG14434*	*CG2662*	*CG40002*	*CG6776*	*corn*	*fru*	*ligatin*	*nmdyn-D7*	*RhoBTB*	*spd-2*	*Xpac*
CG10445	*CG14438*	*CG2691*	*CG4036*	*CG6790*	*Corp*	*Fs(2)Ket*	*lilli*	*noc*	*RhoGAP102A*	*spdo*	*Xpd*
CG10462	*CG14441*	*CG2794*	*CG4038*	*CG6845*	*cp309*	*fu12*	*Liprin-alpha*	*Nop60B*	*RhoGAP54D*	*SpdS*	*XRCC1*
CG10463	*CG14471*	*CG2811*	*CG4041*	*CG6905*	*Cpr66D*	*fw*	*lok*	*norpA*	*RhoGAP92B*	*spen*	*Xrp1*
CG10494	*CG14544*	*CG2982*	*CG4210*	*CG6937*	*Cpsf73*	*fzy*	*LpR1*	*Npc2a*	*RhoGEF4*	*Spn42Db*	*y*
CG10496	*CG14545*	*CG2990*	*CG4213*	*CG6961*	*crc*	*G9a*	*LpR2*	*Nrx-1*	*Ric*	*Spn42Dc*	*yps*
CG10508	*CG14561*	*CG30000*	*CG42303*	*CG6985*	*CrebA*	*GABA-B-R1*	*Lrr47*	*ns2*	*r-l*	*spn-B*	*yu*
CG10512	*CG14614*	*CG30001*	*CG42321*	*CG7006*	*crm*	*Gadd45*	*Lrt*	*ns3*	*rnh1*	*spn-E*	*zfh2*
CG10565	*CG14632*	*CG30005*	*CG42329*	*CG7033*	*crn*	*galectin*	*Lsp1gamma*	*Nuf2*	*RnrL*	*Spx*	*zip*
CG10631	*CG14654*	*CG3004*	*CG42342*	*CG7082*	*csul*	*Galpha49B*	*mad2*	*Nup107*	*robls54B*	*sqd*	*zuc*
CG10777	*CG14667*	*CG3008*	*CG42358*	*CG7110*	*Ctf4*	*Gdh*	*mahj*	*Nup133*	*rok*	*Ssadh*	
CG10801	*CG14684*	*CG3011*	*CG42360*	*CG7130*	*Cul-2*	*gem*	*mamo*	*Nup153*	*roq*	*Sse*	
CG10802	*CG14696*	*CG30127*	*CG4238*	*CG7182*	*cv-c*	*Gem2*	*Map205*	*Nup154*	*RPA2*	*Ssl1*	
CG10803	*CG14709*	*CG30183*	*CG4239*	*CG7246*	*cwo*	*Gem3*	*Mapmodulin*	*Nup358*	*RpA-70*	*ssp*	
CG10916	*CG14814*	*CG30184*	*CG4250*	*CG7262*	*CycA*	*GLaz*	*mars*	*Nup43*	*Rpb5*	*Ssrp*	
CG10927	*CG15019*	*CG30269*	*CG42553*	*CG7294*	*CycB*	*glu*	*mask*	*Nup54*	*Rpl1*	*stai*	

Supplementary Table 1 – Tumor upregulated candidate genes

List of 1182 genes which were found to be upregulated in tumor cells compared to wt neuroblasts

185 candidate genes for secondary screen					
Aats-ala	*CG1542*	*chico*	*Mes2*	*Rpb5*	*Rs1*
Aats-asn	*CG15651*	*crc*	*mRpL23*	*RpI1*	*scny*
Aats-gly	*CG1575*	*CycB*	*mRpL28*	*RpI12*	*Sec16*
Aats-his	*CG16723*	*dgt6*	*mRpL37*	*RpII18*	*Sip1*
Aats-lys	*CG16758*	*DNA-ligI*	*mRpL46*	*RpL12*	*Ski6*
APC4	*CG1703*	*DNApol-alpha73*	*mRpL53*	*RpL15*	*SmB*
ase	*CG1750*	*dpn*	*msk*	*RpL19*	*SmD3*
ash1	*CG17724*	*eIF-2beta*	*Myb*	*RpL21*	*SmE*
baf	*CG2691*	*eIF5B*	*MYPT-75D*	*RpL24-like*	*Smg1*
BL26089	*CG30349*	*eIF6*	*N*	*RpL27*	*spn-E*
BL8288	*CG30398*	*Elf*	*ncd*	*RpL30*	*Ssl1*
bor	*CG31648*	*Fak56D*	*ncm*	*RpL35*	*Taf8*
bsf	*CG32280*	*Fer1HCH*	*Nle*	*RpL39*	*Tango4*
Caf1	*CG32579*	*Fib*	*ns2*	*RpL40*	*Tango7*
cas	*CG3605*	*FOXOoe*	*Nup107*	*RpL5*	*Tcp-1zeta*
Cctgamma	*CG3756*	*Fs(2)Ket*	*Nup133*	*RpL7*	*Tfb4*
CG10512	*CG42321*	*fu12*	*Nup154*	*RpL7A*	*thr*
CG11030	*CG4553*	*galectin*	*Nup62*	*RpLP0-like*	*Top2*
CG11337	*CG5414*	*GLaz*	*Nup98*	*RpLP2*	*Top3alpha*
CG11406	*CG5728*	*glu*	*Nxt1*	*RpS12*	*ttm50*
CG11920	*CG6569*	*Hsc70-5*	*Orc4*	*RpS16*	*tyf*
CG12018	*CG6712*	*JhI-21*	*Pdk1*	*RpS20*	*U2A*
CG1234	*CG7006*	*kermit*	*PGRP-LE*	*RpS27*	*U4-U6-60K*
CG12975	*CG8038*	*klu*	*Pi3K21B*	*RpS27A*	*Uba2*
CG13096	*CG8142*	*l(1)G0007*	*Pi3K92E*	*RpS28b*	*wibg*
CG13126	*CG8545*	*l(1)G0222*	*pix*	*RpS30*	*wor*
CG13185	*CG8801*	*l(2)03709*	*Rae1*	*RpS3A*	*xmas-2*
CG13624	*CG8939*	*l(2)37Cc*	*Rbf2*	*Rrp4*	*XNP*
CG14274	*CG9336*	*l(3)07882*	*Rcd5*	*Rrp45*	*Xpd*
CG14471	*CG9630*	*ldbr*	*RecQ4*	*Rrp46*	*yps*
CG14667	*CG9947*	*mahj*	*RpA-70*	*Rrp6*	

Supplementary Table 2 – candidate genes in the secondary screen

List of the 185 candidate genes which were examined as DKDs and SKDs in the secondary screen.

Primer for shmiR generation		
tyf	F	CTAGCAGTTGGAGGCAGTGTCAACTATAATAGTTATATTCAAGCATATTATAGTTGACACTGCCTCCAGCG
	R	AATTCGCTGGAGGCAGTGTCAACTATAATATGCTTGAATATAACTATTATAGTTGACACTGCCTCCAACTG
l(1)G0007	F	CTAGCAGTCGAGTCCGATCACCTGACCTATAGTTATATTCAAGCATATAGGTCAGGTGATCGGACTCGGCG
	R	AATTCGCCGAGTCCGATCACCTGACCTATATGCTTGAATATAACTATAGGTCAGGTGATCGGACTCGACTG
Aats-gly	F	CTAGCAGTAGCGTTAAGCACTCTACGAAATAGTTATATTCAAGCATATTTCGTAGAGTGCTTAACGCTGCG
	R	AATTCGCAGCGTTAAGCACTCTACGAAATATGCTTGAATATAACTATTTCGTAGAGTGCTTAACGCTACTG
Nup107	F	CTAGCAGTTCGGCAGTTGTACAAAGTCTATAGTTATATTCAAGCATATAGACTTTGTACAACTGCCGAGCG
	R	AATTCGCTCGGCAGTTGTACAAAGTCTATATGCTTGAATATAACTATAGACTTTGTACAACTGCCGAACTG
CG2691	F	CTAGCAGTAGCAGCTAATCAAATCTATTATAGTTATATTCAAGCATATAATAGATTTGATTAGCTGCTGCG
	R	AATTCGCAGCAGCTAATCAAATCTATTATATGCTTGAATATAACTATAATAGATTTGATTAGCTGCTACTG
CG8939	F	CTAGCAGTAGAAGGATGTACTACAGAATATAGTTATATTCAAGCATATATTCTGTAGTACATCCTTCTGCG
	R	AATTCGCAGAAGGATGTACTACAGAATATATGCTTGAATATAACTATATTCTGTAGTACATCCTTCTACTG
Rrp4	F	CTAGCAGTCGAGGGAGGATTCGCTCAGAATAGTTATATTCAAGCATATTCTGAGCGAATCCTCCCTCGGCG
	R	AATTCGCCGAGGGAGGATTCGCTCAGAATATGCTTGAATATAACTATTCTGAGCGAATCCTCCCTCGACTG
CG16758	F	CTAGCAGTCAACAAGGACCTAATCAACAATAGTTATATTCAAGCATATTGTTGATTAGGTCCTTGTTGGCG
	R	AATTCGCCAACAAGGACCTAATCAACAATATGCTTGAATATAACTATTGTTGATTAGGTCCTTGTTGACTG
Nup133	F	CTAGCAGTACGCGAAGAGTTTATAACGAATAGTTATATTCAAGCATATTCGTTATAAACTCTTCGCGTGCG
	R	AATTCGCACGCGAAGAGTTTATAACGAATATGCTTGAATATAACTATTCGTTATAAACTCTTCGCGTACTG
CG16723	F	CTAGCAGTTGGTAAGAGAATACGAGGAAATAGTTATATTCAAGCATATTTCCTCGTATTCTCTTACCAGCG
	R	AATTCGCTGGTAAGAGAATACGAGGAAATATGCTTGAATATAACTATTTCCTCGTATTCTCTTACCAACTG
CG13624	F	CTAGCAGTGCGATAACTACGACGATTGTATAGTTATATTCAAGCATATACAATCGTCGTAGTTATCGCGCG
	R	AATTCGCGCGATAACTACGACGATTGTATATGCTTGAATATAACTATACAATCGTCGTAGTTATCGCACTG
dgt6	F	CTAGCAGTTGGATGCTAATGACCACTACATAGTTATATTCAAGCATATGTAGTGGTCATTAGCATCCAGCG
	R	AATTCGCTGGATGCTAATGACCACTACATATGCTTGAATATAACTATGTAGTGGTCATTAGCATCCAACTG
CG1750	F	CTAGCAGTTGGATACTATTAACAACCTATTAGTTATATTCAAGCATAATAGGTTGTTAATAGTATCCAGCG
	R	AATTCGCTGGATACTATTAACAACCTATTATGCTTGAATATAACTAATAGGTTGTTAATAGTATCCAACTG
CG11337	F	CTAGCAGTCGACAAGATGTCTCGTGACAATAGTTATATTCAAGCATATTGTCACGAGACATCTTGTCGGCG
	R	AATTCGCCGACAAGATGTCTCGTGACAATATGCTTGAATATAACTATTGTCACGAGACATCTTGTCGACTG
bor	F	CTAGCAGTACGGAAAGACGTGTTCGAAGATAGTTATATTCAAGCATATCTTCGAACACGTCTTTCCGTGCG
	R	AATTCGCACGGAAAGACGTGTTCGAAGATATGCTTGAATATAACTATCTTCGAACACGTCTTTCCGTACTG
ncm	F	CTAGCAGTCGAAGATGACCTACCGACTAATAGTTATATTCAAGCATATTAGTCGGTAGGTCATCTTCGGCG
	R	AATTCGCCGAAGATGACCTACCGACTAATATGCTTGAATATAACTATTAGTCGGTAGGTCATCTTCGACTG
ttm50	F	CTAGCAGTAGCAGCAGTACCTCCGACTATTAGTTATATTCAAGCATAATAGTCGGAGGTACTGCTGCTGCG
	R	AATTCGCAGCAGCAGTACCTCCGACTATTATGCTTGAATATAACTAATAGTCGGAGGTACTGCTGCTACTG
CG42321	F	CTAGCAGTCGAAGCTGATGAAGAACTCTATAGTTATATTCAAGCATATAGAGTTCTTCATCAGCTTCGGCG
	R	AATTCGCCGAAGCTGATGAAGAACTCTATATGCTTGAATATAACTATAGAGTTCTTCATCAGCTTCGACTG
Xpd	F	CTAGCAGTTCGCTACAAGCTTCTATTCATTAGTTATATTCAAGCATAATGAATAGAAGCTTGTAGCGAGCG
	R	AATTCGCTCGCTACAAGCTTCTATTCATTATGCTTGAATATAACTAATGAATAGAAGCTTGTAGCGAACTG
crc	F	CTAGCAGTCGCTCACTGACTCAAACGATATAGTTATATTCAAGCATATATCGTTTGAGTCAGTGAGCGGCG
	R	AATTCGCCGCTCACTGACTCAAACGATATATGCTTGAATATAACTATATCGTTTGAGTCAGTGAGCGACTG
ncd	F	CTAGCAGTAAGCAATACATGAGAAAGTAATAGTTATATTCAAGCATATTACTTTCTCATGTATTGCTTGCG
	R	AATTCGCAAGCAATACATGAGAAAGTAATATGCTTGAATATAACTATTACTTTCTCATGTATTGCTTACTG
thr	F	CTAGCAGTAAGAAGAATCAGACTACAAAATAGTTATATTCAAGCATATTTTGTAGTCTGATTCTTCTTGCG
	R	AATTCGCAAGAAGAATCAGACTACAAAATATGCTTGAATATAACTATTTTGTAGTCTGATTCTTCTTACTG
kermit	F	CTAGCAGTTCCACGACCAGTCCCAAGTAATAGTTATATTCAAGCATATTACTTGGGACTGGTCGTGGAGCG
	R	AATTCGCTCCACGACCAGTCCCAAGTAATATGCTTGAATATAACTATTACTTGGGACTGGTCGTGGAACTG
Sip1	F	CTAGCAGTAGGTGAAACCAGGACAGTTTATAGTTATATTCAAGCATATAAACTGTCCTGGTTTCACCTGCG
	R	AATTCGCAGGTGAAACCAGGACAGTTTATATGCTTGAATATAACTATAAACTGTCCTGGTTTCACCTACTG

Supplementary Table 3 – shmiR line primers

List of the primers used to generate 24 shmiR lines for the secondary screen. Designed in the online tool "Primer3Plus".
F – forward primer
R – reverse primer

Attachments

Summary

In this study, we are using *Drosophila* to study tumor requirements. Tumors were induced by knocking down the tumor suppressor *brat* in type II neuroblasts (NBs) of *Drosophila* larval brains. We performed a tumor-suppressor screen to identify genes, on which ectopic, but not wild-type neuroblasts depend on. RNA interference (RNAi) was used to knockdown *brat* function in type II NBs of *Drosophila* 3rd instar larvae. Using an RNAi library, 1182 genes that have been identified to be upregulated in *brat* tumors were systematically knocked down simultaneously to *brat*. We used the adult survival rate as a read out of the screen and identified 266 genes, which led to a significant increase in the survival rate. Systematic confocal fluorescence microscopy was performed to examine type II NB lineage behavior on a single knockdown level of these candidate genes. 75 genes did not alter the type II NB lineage formation upon knockdown and thus, were chosen for further analysis. Intriguingly, all candidates of interest showed tumor initiation in larval brains upon double knockdown with *brat*. However, some of them showed a clear decrease in tumor size in adult stages. The mitochondrial ribosomal protein L46 (*mRpL46*) was identified as a gene, which did not alter the type II NB lineage upon knockdown, but dramatically decreased the *brat* tumor in adult flies. Thus, mRpL46 may be a potential target for therapy development in cancer research.

Zusammenfassung

Diese Arbeit handelt von einem Tumor-suppressor screen in *Drosophila* Larven Hirnen. Knockdown des Tumor-suppressors *brat* in type II Neuroblasten führt zu einem Defekt in der asymmetrischen Zellteilung und einer Überwucherung des *Drosophila* Hirns. Ziel dieser Arbeit ist das Aufspüren von Genen, auf welche die Tumorzellen angewiesen sind, nicht aber die regulären Neuroblasten. Zu diesem Zweck wurden mittels Transkriptomanalyse 1182 Gene ermittelt, welche in den Tumorzellen - verglichen mit regulären type II Neuroblasten - hochreguliert sind. Systematisch wurden diese Gene zusätzlich zu *brat* mittels RNAi ausgeschalten. Knockdowns, welche zu einer verlängerten Überlebensrate der Tumor-Fliegen führten, wurden als Kandidaten beibehalten. Gene, welche laut einer Neuroblasten-Datenbank zu einem Phänotypen in type II Neuroblasten führen wurden aus der Liste entfernt. Die übrigen Kandidaten wurden als Doppel-Knockdown – *brat* RNAi + Kandidaten Gen RNAi – mikroskopisch untersucht. 75 Gene, welche als Einzel-Knockdown keinen Phänotypen in type II Neuroblasten aufwiesen, wurden als Kandidaten beibehalten. Als Kontrolle der Resultate wurden weitere RNAi Linien bestellt, bzw. hergestellt, mit welchen ebenso verfahren wurde. Die Entwicklung des Tumors wurde mittels Western Blot bis ins adulte Stadium verfolgt. Hierbei zeigten drei Gene - *l(1)G0007*, *mRpL46* und *bsf*, eine Tumorreduktion zu einem späteren Entwicklungszeitpunkt. Mikroskopie der adulten Hirne zeigte eine deutliche Tumorreduktion bei Knockdown des Gens *mRpL46*. Demnach könnte *mRpL46* ein interessantes Ziel für weitere Tumorforschung sein.

Curriculum Vitae – Martin Moder

Martin Moder, BSc

Education

Bachelor: Microbiology & Genetics
Master: Molecular Biology – Specialization: Molecular Medicine

2013/2014	Master thesis at the Institute of Molecular Biotechnology (IMBA) at the laboratory of Dr. Jürgen Knoblich – Topic: "Suppressing tumor growth in Drosophila melanogaster brains, using an RNA interference screen"
2012	Reception of the title BSc; Start of the masters program Molecular Biology; specialization: Molecular Medicine
2012	Internship at the Institute of Molecular Pathology (IMP) - Examining the molecular mechanisms of asymmetric mitochondria segregation in *C. elegans* development
2011	Bachelor thesis at the General Hospital Vienna (AKH) - Examining the effects of the immunosuppressive agent rapamycin on dendritic cells in vitro
2009	Internship at the department of population ecology - Investigating the phylogenetics of tropical butterflies
2007	Start of the bachelor studies - Microbiology & Genetics
2006-2007	Basic military service
1998-2006	High School - BRG Stockerau – Main focus on Informatics