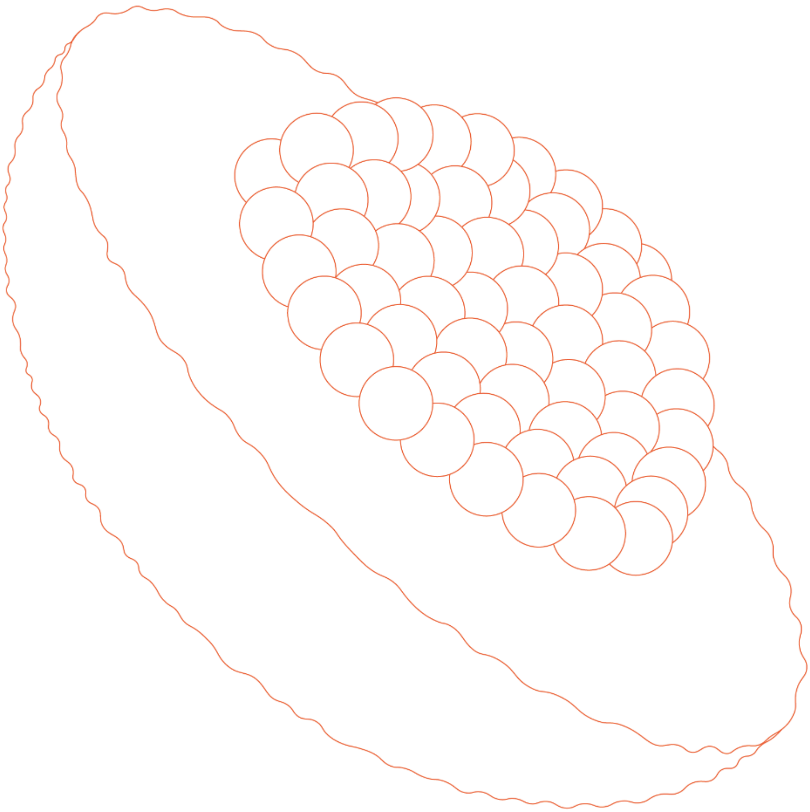
Molecular regulation of neural stem cell quiescence by FoxO transcription factors

Draijer, S.

Publication date
2022

Chapter 7
General discussion



General discussion

Summary of key findings

Molecular regulation of neural stem cell (NSC) quiescence is key to maintain NSC populations throughout life. Aberrant regulation of quiescence can result in their increased exhaustion, expansion or dysfunction. In this thesis, I set out to investigate the function of FoxO3 and FoxO6 in NSC maintenance in the adult and aging brain. FoxO3 is a known regulator of NSC maintenance but its various modes of action, through the dynamic regulation of target genes in the NSC lineage are not clear. FoxO6 is the most recently discovered FoxO isoform and the least studied. It is interesting that its shuttling abilities are drastically impaired compared to the other FoxOs but the functional consequences have not yet been determined. An increased understanding of their roles in NSC fates could offer important insights into the age-related changes in adult neurogenesis and the oncogenic transformation of NSCs towards brain cancer stem cells that give rise to gliomas.

We found that FoxO6 is an important regulator of NSC fates. In **Chapter 2**, we demonstrate that FoxO6 suppresses NSC quiescence during aging. This is a novel role of FoxO6 and opposite from FoxO3-dependent regulation. We suggest that FoxO6 negatively regulates FoxO3-dependent gene programs related to quiescence. In **Chapter 3**, we built on these data to show that FoxO6 is likely involved in the oncogenic transformation of neural stem cells towards brain cancer stem cells that give rise to gliomas. NSCs lacking FoxO6 exhibit a glioma-like molecular signature whereas FoxO6 expression is a prognostic factor for glioma patients. In **Chapter 4**, we focussed on *Hmgn2*, a nucleosomal binding protein that epigenetically regulates progression through the NSC lineage. Whereas expression of *Hmgn2* is down-regulated in FoxO6-deficient NSCs, we found that FoxO3 directly binds within an intron. In **Chapter 5**, we reviewed the literature on circadian regulation of NSCs and suggested that disruption of the circadian clock or its weaking during aging, results in loss of NSC quiescence. In **Chapter 6**, we demonstrated that FoxO3 regulates expression of circadian clock component *Clock* in NSCs and is necessary for the correct timing of circadian proliferation of NSCs in the hippocampus.

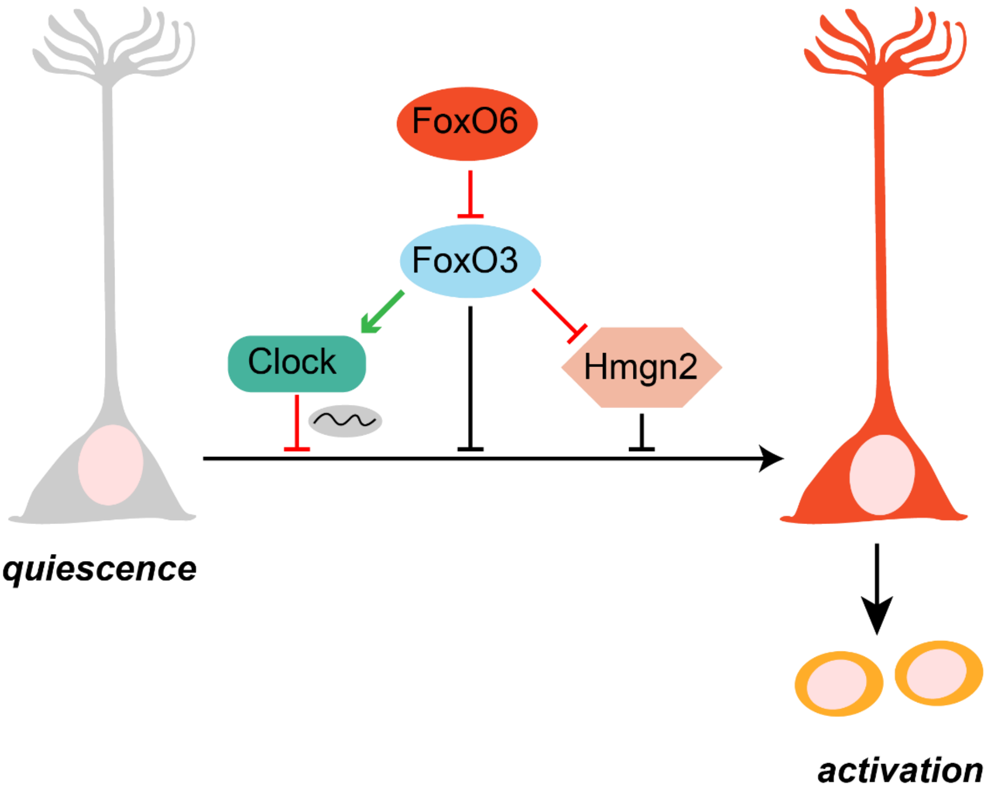


Figure 1: Proposed model of how FoxO6 and FoxO3 are in control of a gene regulatory network that regulates NSC fates

FoxO6: black sheep among FoxOs and neural stem cell regulators

The work presented here suggests that FoxO3 and FoxO6 have different roles to play in the regulation of the adult NSC pool. Whereas FoxO3 promotes NSC quiescence, we found that FoxO6, surprisingly, *suppressed* NSC quiescence. As such, FoxO3 and FoxO6 are together responsible for a healthy balance between NSC activation and quiescence. NSC activation is necessary for adult neurogenesis but results in a declining NSC population (Encinas et al., 2011). NSC quiescence limits this decline during aging but excessive NSC quiescence impairs neurogenesis (Urbán et al., 2019; Tümpel & Rudolph, 2019). Interestingly, among the many described regulators of NSC quiescence, only few are described in such a suppressive way (**Table 1**). As such, FoxO6 is not only a *black sheep* within the FoxO transcription factor family but also among intrinsic neural stem cell regulators. Interestingly, we found that FoxO6 represses FoxO3 activity. Whereas FoxOs do show functional diversification (Hosaka et al., 2004), they have predominantly been reported as working redundantly or cooperatively (Paik et al., 2007, 2009; Zhou et al., 2012; Yadav et al., 2018) and are often lumped together as a result. That our findings indicate an opposing function of FoxO isoforms is therefore a novel perspective on FoxO function. As FoxOs are essential regulators of cellular homeostasis, aging and cancer biology, it would be interesting to determine if such relationships exist in other tissues and cell types.

Table 1: FoxO6 is among the few intrinsic regulators of neural stem cell quiescence that promotes activation

Pro-activation	*Pro-quiescence*
Ascl1[1]	$p21^{WAF1/Cip1}$ [3]
Tlx[2] **FoxO6**	$p27^{Kip1}$ [4] p53[5]
	p57[6]
	PTEN[7]
	Sirt1[8]
	CSP-α[9]
	Id4[10]
	Lrig1[11] Bmal1[12] Huwe1[13]
	FoxO1[14]
	FoxO3[14,15]
	FoxO4[14]

1, Blomfield et al., 2019; 2, Qu et al., 2010; 3, Kippin et al., 2005; 4, Andreu et al., 2015; 5, Meletis et al., 2005; 6, Furutachi et al., 2013; 7, Groszer et al., 2006; 8, Ma et al., 2014; 9, Nieto-González et al., 2019; 10, Zhang et al., 2019; 11, Marqués-Torrejón et al., 2021; 12, Bouchard-Cannon et al., 2013; 13, Urbán et al., 2016; 14, Paik et al., 2009; 15, Renault et al., 2009.

FoxO3 activity: could there be too much of a good thing?

Our findings revealed a potential role for FoxO6 in the oncogenic transformation of neural stem cells. Interestingly, we found that NSCs lacking FoxO6 expressed transcriptional signatures of brain cancer stem cells. This was surprising as our data indicate that NSCs lacking FoxO6 have increased activity of FoxO3, which is typically considered as a tumor suppressor. However, the view of FoxOs as a double-edged sword is now also gaining recognition in cancer biology (Hornsveld et al., 2018; Yadav et al., 2018). Although long regarded as *bona fide* tumor suppressor, FoxOs could promote tumor development by maintaining cancer stem cells and by reactivating PI3K-Akt signaling and, clinically, by promoting drug resistance. As such, the association of low FoxO6 expression and poor clinical outcome for glioma patients fits within our model of a hyperactive FoxO3 promoting quiescence and brain cancer stem cell identity.

Importantly, there is a close association between the circadian clock and the development and progression of various cancers, including gliomas, through the regulation of metabolism and cell cycle (Sahar and Sassone-Corsi, 2009, 2012; Arafa and Amara, 2020). Whereas single nucleotide polymorphisms of clock genes are associated with an increased risk to develop gliomas, gliomas also altered expression of many core clock genes (Madden et al., 2014; De La Cruz Minyeti et al., 2021). Interestingly, rather than a disrupted circadian clock, many of these clock genes exhibit increased expression, which promotes stemness and cancer-favouring metabolism in glioma (Arafa and Amara, 2020). In a recent study, Dong et al. (2019) demonstrated that targeting *BMAL1* or *CLOCK* resulted in down-regulated expression of tricarboxylic acid cycle genes, decreased expression of stem cell factors and reduced glioblastoma growth. Although core clock genes are typically considered to function as tumour suppressors (Sahar and Sassone-Corsi, 2009), this indicates a tumour promoting role for the circadian clock by promoting stem cell homeostasis. As such, FoxO3-dependent regulation of the circadian clock may also promote brain cancer stem cell identity.

Since the discovery of neural stem cells in the adult brain, research has focused on the consequences of a loss of quiescence as this results in a depletion of the stem cell population (Urbán et al., 2019) but the consequences of excessive NSC quiescence on NSC populations are less clear. Stem cell dysfunction is present in many aging tissues (Tümpel and Rudolph, 2019) and old SVZ NSCs have lysosomal defects that impair their activation (Leeman et al., 2018). The work presented here, therefore, could serve as an example illustrating NSC quiescence gone awry. It is also an example of why increased FoxO3 activity could be *too much of a good thing* when it comes to NSC maintenance. While FoxO3 is necessary to limit quiescence, its presumed hyperactivity would result in an impaired NSC activation. An analogy I have used in the various presentations of my work throughout the course of my PhD is to consider FoxO3 as a smoke alarm. Whereas a smoke alarm is essential to detect a house fire and stop it in its tracks (e.g. cell cycle arrest, apoptosis, senescence), a smoke alarm that sets off the sprinkler system when a candle is burning would be detrimental. In this model, FoxO6 limits the sensitivity of FoxO3 so that it only acts when it is crucial to protect cellular homeostasis without harming tissue homeostasis. This is reminiscent of FoxO4 action. In a seminal paper, Baar et al. (2017) showed that FoxO4 can promote senescence by interacting with p53 following DNA-damage. While inducing senescence is a safe alternative compared to risking accumulation of mutations in DNA, senescent cells are thought to impair tissue function and to accelerate aging as they develop a pro-inflammatory phenotype or senescence-associated secretory phenotype (Coppé et al., 2008; de Keizer, 2017). Targeted apoptosis of senescent cells by inferring with the FoxO4-p53 axis, therefore, restored fitness, hair density and renal function in aged mice (Baar et al., 2017).

Relevance of adult hippocampal neural stem cells in the old brain

Whereas we found that FoxO6 modulates NSC quiescence during aging and that the Hmgn2+ population of neural stem/progenitor cells is diminished in old brains, circadian function in NSCs declines during aging. As such, the work presented here is closely connected to the presence of adult neural stem cells in the aging brain, with the implication that alterations in NSC fates likely affect adult neurogenesis.

Conflicting reports about whether adult hippocampal neurogenesis occurs in humans has raised controversy over its significance for human health (Sorrells et al., 2018; Boldrini et al., 2018). With subsequent studies showing the presence of new neurons in old humans, the consensus seems to have reverted to the *pre-Sorrells* school of thought; that adult hippocampal neurogenesis contributes to learning and memory processes, and promotes stress regulation (Snyder et al., 2019; Lucassen et al., 2020). Interestingly, brains of Alzheimer's patients show low levels of hippocampal neurogenesis, which correlated with memory deficits (Tobin et al., 2019; Moreno-Jimenez et al., 2020). A recent study has now shown that levels of microRNA miR-132 are reduced in the brains of Alzheimer's disease patients but that its replacement restored adult hippocampal neurogenesis and improved memory deficits in a mouse model of the disease (Walgrave et al., 2021). In other words, the existence of neural stem cells in old brains has tangible benefits.

Therefore, the preservation of this population through modulation of quiescence seems beneficial (Kalamakis et al., 2019; Harris et al., 2021; Ibrayeva et al., 2021). However, from an evolutionary perspective, there would be no selection pressures on stem cell activity after the adult reproductive years (Goodell and Rando, 2015). In others words, it is hard to imagine that the preservation of neural stem cells for neurogenesis in old brains is subjected to evolutionary pressure. In fact, a sizeable population of quiescent NSC might never become activated (Harris et al., 2021). As such, the preservation of adult neural stem cells during aging might be the logical consequence of increased quiescence due to stem cell dysfunction or aging-related changes of the surrounding niche. For instance, both the accumulation of protein aggregates and a pro-inflammatory niche impair the activation of old NSCs (Leeman et al., 2018; Kalamakis et al., 2019; Dulken et al., 2019).

Conversely, the existence of adult neural stem cells also poses a risk for their oncogenic transformation. Since the discovery of NSCs in brain tumours (Singh et al., 2004), an increasing body of knowledge demonstrates that SVZ NSCs are the main cell-of-origin for human brain tumours (Matarredonna and Pastor, 2019; Kim et al., 2021). Mutations in oncogenes and tumour suppressors drive the transformation of, mainly, neural stem cells towards a brain cancer stem cell, characterized by sustained self-renewal and proliferation, which gives rise to the various cell types that constitute a brain tumour (Lathia et al., 2015; Lee et al., 2018; Kim et al., 2021). These tumours, of which glioblastoma multiforme is the most common and most lethal, are almost impossible to treat and most patients die within the first years following diagnosis (Louis et al., 2007; McLendon & Halperin, 2003; Jaeckle et al., 2011). This is partially due to recurrence of the tumours driven by a slow-cycling population of NSCs that survives chemotherapy (Chen et al., 2012.). As such, adult NSCs are responsible for one of the most lethal human cancers while many of them likely never generate new neurons.

Among the key regulators of NSC quiescence (Urbán et al., 2019) are many tumour suppressors with key functions in cancer biology, which are likely to be subjected to strong evolutionary pressure. The prevalence of NSC quiescence might stem from the activity of these tumour suppressors rather than to functionally preserve NSCs for the aging brain. Alternatively, quiescence of young NSCs is likely important to limit levels of adult neurogenesis. Newborn hippocampal neurons integrate into the existing neuronal circuitry, which promotes brain plasticity (Gonçalves et al., 2016). Whereas neural circuits in young brains show high levels of plasticity, this is later stabilized (Takesian and Hensch, 2013). A mechanism favouring NSC quiescence might therefore start at a young age, not to preserve NSCs but to promote a balance between brain plasticity and stability.

Therefore, I suggest that neurogenesis is beneficial for old brains but that this is not likely the reason for its existence. Rather, neurogenesis is a logical consequence of the persistence of neural stem cells in old brains and the mechanisms in place regulating their quiescence. In this view, adult neural stem cells persist as a leftover of the neurodevelopmental period. Their increased quiescence during aging allowed them to exist longer than is expected in a purely “division-coupled depletion” model (Harris et al., 2021). However, from an evolutionary perspective, it is more likely that mechanisms favouring tumour suppression and stability of neuronal circuitry in combination with age-related stem cell dysfunction and pro-inflammatory neurogenic niches drive this quiescent state rather than these mechanisms have developed to functionally preserve neurogenesis in old brains. In other words, the process is the evolutionary driver rather than the outcome.

For example, almost all mammals contain seven cervical vertebrae, not because this is necessarily the perfect number of cervical vertebrae for a mammal but because of developmental constraints (Galis, 1999). Patterning of the skeleton is regulated by Hox genes, which are key regulators during development and cancer biology in adult tissues. As such, variation in the number of vertebrae in the neck may not seem that harmful, abnormalities in Hox genes are.

Regulation by FoxO6 and FoxO3: like clockwork?

Additionally, we discovered novel modes of FoxO3-dependent regulation in NSCs: the control of circadian rhythms and the control of *Hmgn2* expression. FoxO3 promotes NSC quiescence through the induction of transcriptional programmes that directly limit cell cycle progression (e.g. *p21*, *p27*) and its activity is dependent on the cellular metabolic state (Renault et al., 2009; Ro et al., 2013). The circadian clock is closely connected to both the cell cycle and metabolism (Asher & Schibler, 2011; Gaucher et al., 2018). Our finding that FoxO3 regulates *Clock,* an essential component of the cellular clock, adds another layer to the regulation of NSC fates. Interestingly, we found that, in the liver, circadian clock components rhythmically bound to the *FoxO3* gene in a publically available ChIP-seq dataset (Koike et al., 2012), indicating that FoxO3 is an integral part of the circadian gene regulatory network. This raises the question whether FoxO6 is also part of this network. Interestingly, the *FoxO6* gene was not bound by any circadian clock components in the liver and FoxO6 knockdown did also not significantly change circadian clock dynamics on a single cell level. Nevertheless, FoxO6 overexpression clearly reduced the amplitude of circadian oscillations in both NIH3T3 cells (Chaves et al., 2014) and in our experiments in neural stem cells. Conversely, FoxO6-/- mice exhibit fragmented circadian behaviour with an increased period, similar to the circadian behaviour of old mice (Chaves et al., unpublished). Moreover, we found altered expression of clock genes - *Per1, Per2, Rev-Erbα/Nr1d1, Cry1, Cry2* (Draijer et al., unpublished) - in FoxO6-deficient NSCs. As such, FoxO6 seems to be involved in circadian regulation but likely through its regulation of FoxO3 or target genes and not, as in the case of FoxO3, as an integral part of the circadian regulatory network.

Hmgn2: a transcriptomic smoking gun

Importantly, we found that FoxO3 regulated expression of the nucleosomal binding protein Hmgn2. This line of inquiry started with the observation that *Hmgn2* expression was drastically reduced in neurospheres derived from FoxO6-deficient mice, both adult and embryos compared to wild types. When I started my PhD, Hmgn2 had just been reported as highly up-regulated in neural progenitor cells and belonging to a group of *"transcription factors whose function has not been described in the context of adult hippocampal neural development"* (Artegiani et al., 2017). As such, Hmgn2 seemed to be an important *smoking gun* in figuring out why loss of FoxO6 resulted in a quiescent-like signature.

Therefore, we set out to examine its spatial expression *in vivo*, as that had not been done at the time, and assessed *Hmgn2* expression in many available datasets as well as in our own model of NSC quiescence. Together, these results all indicated that *Hmgn2* expression sharply increases following NSC activation, peaks in neural progenitor cells and is then down-regulated during differentiation towards neuroblasts.

A question that remains is whether the drastic reduction of *Hmgn2* expression in FoxO6-deficient neurospheres is due to either the loss of neural progenitor cells, which highly express *Hmgn2*, or due to FoxO3 hyperactivity repressing *Hmgn2* or is caused by the absence of direct FoxO6 regulation. Whereas we found a clear reduction in the number of Hmgn2+ cells in FoxO6-deficient mice, adult hippocampal neural stem cells *in vitro* - arguably a more homogeneous culture than neurospheres – also exhibit diminished *Hmgn2* expression. Conversely, direct FoxO6-dependent regulation seems unlikely as we did not find a FoxO6 binding site at the *Hmgn2* gene in our ChIP-seq data. Moreover, this would mean that FoxO6 and FoxO3 would both bind to a DBE within *Hmgn2* but differentially regulate its expression. This is possible depending on their transcriptional co-regulators but it seems more likely that *Hmgn2* expression is so drastically reduced in FoxO6-deficient neurospheres because 1) increased quiescence at the expense of neural progenitor cells results in a proportionally reduced number of cells with high *Hmgn2* expression and 2) FoxO3 hyperactivity results in increased repression of *Hmgn2*. In addition, a dynamic balance between FoxO3- and Ascl1-dependent transcription likely regulates *Hmgn2* expression, among the many shared target genes of FoxO3 and Ascl1 (Webb et al., 2013). Since we found that FoxO6 regulates FoxO3, FoxO6 would indirectly affect Ascl1-dependent transcription of not only *Hmgn2* but also of many other target genes.

Limitations of this work

An obvious ethical and experimental concern is the use of mouse models throughout the chapters in this thesis. Although we have aimed to minimize the use of mice, their sacrifice was inherent to many experiments involving post-mortem analysis. The use of a primary neural stem cell line provides an ideal model to study cell cycle dynamics outside of a mouse brain and only necessitates a single isolation. Nevertheless, a crucial aspect of the research outlined here involves aging. We observed age-related changes in FoxO6-deficient mice as well as age-related changes in the number of Hmgn2+ neural stem/progenitor cells. Such studies are impossible *in vitro*. Similarly, loss of FoxO6 affected the quiescence of hippocampal NSCs but not of NSCs in the SVZ of the lateral ventricles. In contrast, both hippocampal NSCs and whole forebrain neurospheres, which likely predominantly originate from SVZ NSCs, that lacked FoxO6, were more quiescent in culture. This indicates niche-specific features that are hard to recapitulate *in vitro*. Finally, we found a phase-shift in the peak of circadian proliferation in mice lacking FoxO3. As the circadian clock within cells is synchronized by cues from the SCN and by metabolic signals, such an effect would have likely been missed *in vitro*. Because of these reasons and others, the use of human brain tissue would not have been adequate to carry out the research as performed in this thesis. Whereas the use of post-mortem brain tissue would not have been able to answer causative questions about FoxO3 and FoxO6, the use of human neural stem cell cultures would have been challenging for the reasons mentioned above.

As such, it remains challenging to translate the findings in this thesis to the human brain. In recent years, the debate around adult neurogenesis in humans has been reinvigorated after a study reporting negligent neurogenesis in adult human brains sparked controversy (Sorrells et al., 2018). This was quickly met with rebuttals questioning the experimental methodology (Lucassen et al., 2019) and new studies that did find adult neurogenesis to persist in old human brains (Boldrini et al., 2019; Tobin et al., 2019). Nevertheless, we also observed that the number of proliferating NSCs in old mouse brains was a fraction of the number of proliferating NSCs in young mice. As such, adult neurogenesis in mice and humans seem to be comparable when the organism's development and lifespan are accounted for (Snyder et al., 2019).

This does not withstand the fact that there are limitations of the mouse models used in this work. We used transgenic mouse mutants lacking FoxO3 or FoxO6. These mutant mice were full knockouts, meaning that many cells throughout the body had been lacking either of these transcription factors from conception onwards. This poses two problems: first, the lack of FoxO3 or FoxO6 in other tissues than the brain may affect NSCs maintenance and, secondly, the absence of these transcription factors during brain development may affect NSCs in the adult brain.

For instance, FoxO3 regulates circadian rhythms in the liver (Chaves et al., 2014) whereas FoxO6, now that it is increasingly studied, is emerging as a regulator of gluconeogenesis in the liver (Kim et al., 2013). NSCs are positioned close to blood vessels and - in the SVZ - cerebrospinal fluid and therefore are dependent on systemic signals deriving from the niche environments for their activation and cell fates (Doetsch, 2003; Obernier and Alvarez-Buylla, 2019). Additionally, FoxO3 is a known regulator of embryonic NSCs (Paik et al., 2009; Schmidt-Strassburger et al., 2012) whereas FoxO6 expression is abundant in the developing brain (Hoekman et al., 2006) and affects neuronal migration during cortical development (Paap et al., 2016). As such, loss of FoxO3 or FoxO6 in other tissues may indirectly alter NSC homeostasis while their absence during development could affect the generation of adult NSCs. A solution would be to use a brain-specific and/or temporally specific conditional transgenic knockout mouse. For instance, by crossing a transgenic Cre recombinase mouse driven by the brain-specific *Nestin* promoter with a floxed mouse: a transgenic mouse that has a gene - in this case *FoxO6* or *FoxO3* - sandwiched in between lox P sites. This then allows for the deletion of these genes using Cre-Lox recombination. To study the acute effects of a FoxO6 deletion in the adult brain, a Nestin-Cre^{ERT2} mouse model can be used, in which a deletion with tamoxifen activates Cre recombinase. However, a floxed *FoxO6* mouse is currently lacking and challenging to generate in-house.

Concluding remarks

The work presented in this thesis reveals novel roles for FoxO6 and FoxO3 in the regulation of NSC fates but, above all, it demonstrates that a proper balance between NSC activation and quiescence is key. While FoxO3 is indisputably an essential regulator of NSC quiescence, we show here that FoxO6 is necessary to keep its activity in check to allow NSC activation. Quiescence is necessary to maintain adult NSC populations but without their activation, their function as stem cells is arguably abrogated. Similarly, FoxO3 is an important regulator of cancer biology but as it promotes stemness of neural stem cells, it can likewise promote stemness of brain cancer stem cells. FoxO6 may therefore act as a tumour suppressor in an unusual way: by keeping another tumour suppressor, FoxO3, in check. Hmgn2 seems to fit within this framework. Its expression is up-regulated following NSC activation but its function is not pro-neurogenic but rather promotes stem cell identity.

Circadian regulation of NSC fates is emerging as an important factor in balancing NSC fates. Not only does the circadian clock modulate the decision *whether* to enter or exit the cell cycle, it also modulates *when*. That FoxO3 - and by association FoxO6 - are interconnected within the cellular circadian clock machinery adds to the complexity of NSC regulation.

Together, all these findings illustrate that quiescence is not inherently *good* or *bad* but that its active regulation is necessary to strike a balance between exhaustion, expansion and stem cell dysfunction. Aging heavily affects this balance, both by intrinsic and extrinsic changes, which leaves neural stem cells -and other somatic stem cells - continuously poised between these three evils. To understand healthy aging, it is, therefore, important to understand quiescence.

REFERENCES

Arafa, Kholoud, and Marwan Emara. 2020. “Insights About Circadian Clock and Molecular Pathogenesis in Gliomas.” *Frontiers in Oncology* 10 (February): 1–16. https://doi.org/10.3389/fonc.2020.00199

Artegiani, Benedetta, Anna Lyubimova, Mauro Muraro, Johan H. van Es, Alexander van Oudenaarden, and Hans Clevers. 2017. “A Single-Cell RNA Sequencing Study Reveals Cellular and Molecular Dynamics of the Hippocampal Neurogenic Niche.” *Cell Reports* 21 (11): 3271–84. https://doi.org/10.1016/j.celrep.2017.11.050.

Asher, Gad, and Ueli Schibler. 2011. “Crosstalk between Components of Circadian and Metabolic Cycles in Mammals.” *Cell Metabolism* 13 (2): 125–37. https://doi.org/10.1016/J.CMET.2011.01.006.

Baar, Marjolein P., Renata M.C. Brandt, Diana A. Putavet, Julian D.D. Klein, Kasper W.J. Derks, Benjamin R.M. Bourgeois, Sarah Stryeck, et al. 2017. “Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging.” *Cell* 169 (1): 132-147.e16. https://doi.org/10.1016/j.cell.2017.02.031.

Berg, Daniel A., Yijing Su, Dennisse Jimenez-Cyrus, Aneek Patel, Nancy Huang, David Morizet, Stephanie Lee, et al. 2019. “A Common Embryonic Origin of Stem Cells Drives Developmental and Adult Neurogenesis.” *Cell* 177 (3): 654-668.e15. https://doi.org/10.1016/j.cell.2019.02.010.

Blomfield, Isabelle M., Brenda Rocamonde, Maria del Mar Masdeu, Eskeatnaf Mulugeta, Stefania Vaga, Debbie L.C. van den Berg, Emmanuelle Huillard, François Guillemot, and Noelia Urbán. 2019. “Id4 Promotes the Elimination of the Pro-Activation Factor Ascl1 to Maintain Quiescence of Adult Hippocampal Stem Cells.” *ELife* 8: 1–26. https://doi.org/10.7554/elife.48561.

Boldrini, Maura, Camille A. Fulmore, Alexandria N. Tartt, Laika R. Simeon, Ina Pavlova, Verica Poposka, Gorazd B. Rosoklija, et al. 2018. “Human Hippocampal Neurogenesis Persists throughout Aging.” *Cell Stem Cell* 22 (4): 589-599.e5. https://doi.org/10.1016/j.stem.2018.03.015.

Bouchard-Cannon, Pascale, Lucia Mendoza-Viveros, Andrew Yuen, Mads Kærn, and Hai-Ying M. Cheng. 2013. “The Circadian Molecular Clock Regulates Adult Hippocampal Neurogenesis by Controlling the Timing of Cell-Cycle Entry and Exit.” *Cell Reports* 5 (4): 961–73. https://doi.org/10.1016/j.celrep.2013.10.037.

Chaves, Inês, Gijsbertus T.J. van der Horst, Raymond Schellevis, Romana M. Nijman, Marian Groot Koerkamp, Frank C.P. Holstege, Marten P. Smidt, and Marco F.M. Hoekman. 2014. “Insulin-FOXO3 Signaling Modulates Circadian Rhythms via Regulation of Clock Transcription.” *Current Biology*. Vol. 24. https://doi.org/10.1016/j.cub.2014.04.018.

Chen, Jian, Yanjiao Li, Tzong Shiue Yu, Renée M. McKay, Dennis K. Burns, Steven G. Kernie, and Luis F. Parada. 2012. “A Restricted Cell Population Propagates Glioblastoma Growth after Chemotherapy.” *Nature* 488 (7412): 522–26. https://doi.org/10.1038/nature11287.

Coppé, Jean-Philippe, Christopher K Patil, Francis Rodier, Yu Sun, Denise P Muñoz, Joshua Goldstein, Peter S Nelson, Pierre-Yves Desprez, and Judith Campisi. 2008. “Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the P53 Tumor Suppressor.” Edited by Julian Downward. *PLoS Biology* 6 (12): e301. https://doi.org/10.1371/journal.pbio.0060301.

Doetsch, Fiona. 2003. “A Niche for Adult Neural Stem Cells.” *Current Opinion in Genetics & Development* 13 (5): 543–50. https://doi.org/10.1016/j.gde.2003.08.012.

Dong, Zhen, Guoxin Zhang, Meng Qu, Ryan C. Gimple, Qiulian Wu, Zhixin Qiu, Briana C. Prager, et al. 2019. “Targeting Glioblastoma Stem Cells through Disruption of the Circadian Clock.” *Cancer Discovery* 9 (11): 1556–73. https://doi.org/10.1158/2159-8290.CD-19-0215.

Dulken, Ben W., Matthew T. Buckley, Paloma Navarro Negredo, Naresha Saligrama, Romain Cayrol, Dena S. Leeman, Benson M. George, et al. 2019. “Single-Cell Analysis Reveals T Cell Infiltration in Old Neurogenic Niches.” *Nature*. https://doi.org/10.1038/s41586-019-1362-5.

Encinas, Juan M, Tatyana V Michurina, Natalia Peunova, June-Hee Park, Julie Tordo, Daniel A Peterson, Gord Fishell, Alex Koulakov, and Grigori Enikolopov. 2011. “Division-Coupled Astrocytic Differentiation and Age-Related Depletion of Neural Stem Cells in the Adult Hippocampus.” *Cell Stem Cell* 8 (5): 566–79. http

Fuentealba, Luis C., Kirsten Obernier, and Arturo Alvarez-Buylla. 2012. “Adult Neural Stem Cells Bridge Their Niche.” *Cell Stem Cell*. Cell Press. https://doi.org/10.1016/j.stem.2012.05.012.

Furutachi, Shohei, Akinobu Matsumoto, Keiichi I Nakayama, and Yukiko Gotoh. 2013. “P57 Controls Adult Neural Stem Cell Quiescence and Modulates the Pace of Lifelong Neurogenesis.” *The EMBO Journal* 32 (7): 970–81. https://doi.org/10.1038/emboj.2013.50.

Galis, Frietson. 1999. “Why Do Almost All Mammals Have Seven Cervical Vertebrae? Developmental Constraints, Hox Genes, and Cancer.” *Journal of Experimental Zoology* 285 (1): 19–26. https://doi.org/10.1002/(SICI)1097-010X(19990415)285:1<19::AID-JEZ3>3.0.CO;2-Z.

Gaucher, Jonathan, Emilie Montellier, and Paolo Sassone-Corsi. 2018. “Molecular Cogs: Interplay between Circadian Clock and Cell Cycle.” *Trends in Cell Biology* 28 (5): 368–79. https://doi.org/10.1016/j.tcb.2018.01.006.

Gonçalves, J. Tiago, Simon T. Schafer, and Fred H. Gage. 2016. “Adult Neurogenesis in the Hippocampus: From Stem Cells to Behavior.” *Cell*. Cell Press. https://doi.org/10.1016/j.cell.2016.10.021.

Goodell, Margaret A., and Thomas A. Rando. 2015. “Stem Cells and Healthy Aging.” *Science* 350 (6265): 1199–1204. https://doi.org/10.1126/science.aab3388.

Groszer, M., R. Erickson, D. D. Scripture-Adams, J. D. Dougherty, J. Le Belle, J. A. Zack, D. H. Geschwind, X. Liu, H. I. Kornblum, and H. Wu. 2006. “PTEN Negatively Regulates Neural Stem Cell Self-Renewal by Modulating G0-G1 Cell Cycle Entry.” *Proceedings of the National Academy of Sciences* 103 (1): 111–16. https://doi.org/10.1073/pnas.0509939103.

Harris, Lachlan, Piero Rigo, Thomas Stiehl, Zachary B. Gaber, Sophie H.L. Austin, Maria del Mar Masdeu, Amelia Edwards, Noelia Urbán, Anna Marciniak-Czochra, and François Guillemot. 2021. “Coordinated Changes in Cellular Behavior Ensure the Lifelong Maintenance of the Hippocampal Stem Cell Population.” *Cell Stem Cell*, February. https://doi.org/10.1016/j.stem.2021.01.003.

Hoekman, Marco F.M., Frank M.J. Jacobs, Marten P. Smidt, and J. Peter H. Burbach. 2006. “Spatial and Temporal Expression of FoxO Transcription Factors in the Developing and Adult Murine Brain.” *Gene Expression Patterns* 6 (2): 134–40. https://doi.org/10.1016/j.modgep.2005.07.003.

Hosaka, Taisuke, William H. Biggs, David Tieu, Antonia D. Boyer, Nissi M. Varki, Webster K. Cavenee, and Karen C. Arden. 2004. “Disruption of Forkhead Transcription Factor (FOXO) Family Members in Mice Reveals Their Functional Diversification.” *Proceedings of the National Academy of Sciences of the United States of America* 101 (9): 2975–80. https://doi.org/10.1073/pnas.0400093101.

Ibrayeva, Albina, Maxwell Bay, Elbert Pu, David J. Jörg, Lei Peng, Heechul Jun, Naibo Zhang, et al. 2021. “Early Stem Cell Aging in the Mature Brain.” *Cell Stem Cell* 28 (5): 955-966.e7. https://doi.org/10.1016/j.stem.2021.03.018.

Jaeckle, K. A., P. A. Decker, K. V. Ballman, P. J. Flynn, C. Giannini, B. W. Scheithauer, R. B. Jenkins, and J. C. Buckner. 2011. “Transformation of Low Grade Glioma and Correlation with Outcome: An NCCTG Database Analysis.” *Journal of Neuro-Oncology* 104 (1): 253–59. https://doi.org/10.1007/s11060-010-

0476-2.

Kalamakis, Georgios, Daniel Brüne, Srikanth Ravichandran, Jan Bolz, Wenqiang Fan, Frederik Ziebell, Thomas Stiehl, et al. 2019. "Quiescence Modulates Stem Cell Maintenance and Regenerative Capacity in the Aging Brain." *Cell*, February. https://doi.org/10.1016/J.CELL.2019.01.040.

Keizer, Peter L.J. de. 2017. "The Fountain of Youth by Targeting Senescent Cells?" *Trends in Molecular Medicine*. Elsevier Ltd. https://doi.org/10.1016/j.molmed.2016.11.006.

Kim, Hyun Jung, Jung Won Park, and Jeong Ho Lee. 2021. "Genetic Architectures and Cell-of-Origin in Glioblastoma." *Frontiers in Oncology* 10 (January): 1–6. https://doi.org/10.3389/fonc.2020.615400.

Kippin, Tod E, David J Martens, and Derek Van Der Kooy. 2005. "P21 Loss Compromises the Relative Quiescence of Forebrain Stem Cell Proliferation Leading to Exhaustion of Their Proliferation Capacity," 756–67. htt

Koike, Nobuya, Seung Hee Yoo, Hung Chung Huang, Vivek Kumar, Choogon Lee, Tae Kyung Kim, and Joseph S. Takahashi. 2012. "Transcriptional Architecture and Chromatin Landscape of the Core Circadian Clock in Mammals." *Science* 338 (6105): 349–54. https://doi.org/10.1126/science.1226339.

Lathia, Justin D., Mack, Stephen C., Mulkearns-Hubert, Erin E., Valentim, Claudia L.L., Rich, and Jeremy N. Rich2. 2015. "Cancer Stem Cells in Glioblastoma." *Genes and Development*, 113–20. https://doi.org/10.1007/978-94-007-1709-1_14.

Lee, Joo Ho, Jeong Eun Lee, Jee Ye Kahng, Se Hoon Kim, Jun Sung Park, Seon Jin Yoon, Ji Yong Um, et al. 2018. "Human Glioblastoma Arises from Subventricular Zone Cells with Low-Level Driver Mutations." *Nature* 560 (7717): 243–47. https://doi.org/10.1038/s41586-018-0389-3.

Leeman, Dena S., Katja Hebestreit, Tyson Ruetz, Ashley E. Webb, Andrew McKay, Elizabeth A. Pollina, Ben W. Dulken, et al. 2018. "Lysosome Activation Clears Aggregates and Enhances Quiescent Neural Stem Cell Activation during Aging." *Science* 359 (6381): 1277–83. https://doi.org/10.1126/science.aag3048.

Louis, David N., Hiroko Ohgaki, Otmar D. Wiestler, Webster K. Cavenee, Peter C. Burger, Anne Jouvet, Bernd W. Scheithauer, and Paul Kleihues. 2007. "The 2007 WHO Classification of Tumours of the Central Nervous System." *Acta Neuropathologica* 114 (2): 97–109. https://doi.org/10.1007/s00401-007-0243-4.

Lucassen, Paul J., Nicolas Toni, Gerd Kempermann, Jonas Frisen, Fred H. Gage, and Dick F. Swaab. 2020. "Limits to Human Neurogenesis—Really?" *Molecular Psychiatry* 25 (10): 2207–9. https://doi.org/10.1038/s41380-018-0337-5.

Ma, C.-y., M.-j. Yao, Q.-w. Zhai, J.-w. Jiao, X.-b. Yuan, and M.-m. Poo. 2014. "SIRT1 Suppresses Self-Renewal of Adult Hippocampal Neural Stem Cells." *Development* 141 (24): 4697–4709. https://doi.org/10.1242/dev.117937.

Madden, Melissa H., Gabriella M. Anic, Reid C. Thompson, L. Burton Nabors, Jeffrey J. Olson, James E. Browning, Alvaro N. Monteiro, and Kathleen M. Egan. 2014. "Circadian Pathway Genes in Relation to Glioma Risk and Outcome." *Cancer Causes and Control* 25 (1): 25–32. https://doi.org/10.1007/s10552-013-0305-y.

Marqués-Torrejón, María Ángeles, Charles A.C. Williams, Benjamin Southgate, Neza Alfazema, Melanie P. Clements, Claudia Garcia-Diaz, Carla Blin, et al. 2021. "LRIG1 Is a Gatekeeper to Exit from Quiescence in Adult Neural Stem Cells." *Nature Communications* 12 (1): 1–15. https://doi.org/10.1038/s41467-021-22813-w.

Matarredona, Esperanza R., and Angel M. Pastor. 2019. "Neural Stem Cells of the Subventricular Zone as the Origin of Human Glioblastoma Stem Cells. Therapeutic Implications." *Frontiers in Oncology* 9 (AUG). https://doi.org/10.3389/fonc.2019.00779.

McLendon, Roger E., and Edward C. Halperin. 2003. "Is the Long-Term Survival of Patients with Intracranial

Glioblastoma Multiforme Overstated?” *Cancer* 98 (8): 1745–48. https://doi.org/10.1002/cncr.11666.

Meletis, K. 2005. “P53 Suppresses the Self-Renewal of Adult Neural Stem Cells.” *Development* 133 (2): 363–69. https://doi.org/10.1242/dev.02208.

Microenvironment, Tumor, Patient Survival, Julianie De La, Cruz Minyety, Dorela D Shuboni-mulligan, Nicole Briceno, Demarrius Young, Mark R Gilbert, Orieta Celiku, and Terri S Armstrong. 2021. “Association of Circadian Clock Gene Expression with Glioma,” no. grades 4.

Moreno-Jiménez, Elena P., Miguel Flor-García, Julia Terreros-Roncal, Alberto Rábano, Fabio Cafini, Noemí Pallas-Bazarra, Jesús Ávila, and María Llorens-Martín. 2019. “Adult Hippocampal Neurogenesis Is Abundant in Neurologically Healthy Subjects and Drops Sharply in Patients with Alzheimer’s Disease.” *Nature Medicine* 25 (4): 554–60. https://doi.org/10.1038/s41591-019-0375-9.

Nieto-González, Jose L., Leonardo Gómez-Sánchez, Fabiola Mavillard, Pedro Linares-Clemente, María C. Rivero, Marina Valenzuela-Villatoro, José L. Muñoz-Bravo, Ricardo Pardal, and Rafael Fernández-Chacón. 2019. “Loss of Postnatal Quiescence of Neural Stem Cells through MTOR Activation upon Genetic Removal of Cysteine String Protein-α.” *Proceedings of the National Academy of Sciences of the United States of America* 116 (16): 8000–8009. https://doi.org/10.1073/pnas.1817183116.

Obernier, Kirsten, and Arturo Alvarez-Buylla. 2019. “Neural Stem Cells: Origin, Heterogeneity and Regulation in the Adult Mammalian Brain.” *Development (Cambridge)* 146 (4). https://doi.org/10.1242/dev.156059.

Paap, Ricardo H., Saskia Oosterbroek, Cindy M. R. J. Wagemans, Lars von Oerthel, Raymond D. Schellevis, Annemarie J. A. Vastenhouw-van der Linden, Marian J. A. Groot Koerkamp, Marco F. M. Hoekman, and Marten P. Smidt. 2016. “FoxO6 Affects Plxna4-Mediated Neuronal Migration during Mouse Cortical Development.” *Proceedings of the National Academy of Sciences* 113 (45): E7087–96. https://doi.org/10.1073/pnas.1609111113.

Paik, Ji-hye, Zhihu Ding, Rujuta Narurkar, Shakti Ramkissoon, Florian Muller, Walid S Kamoun, Sung-Suk Chae, et al. 2009. “FoxOs Cooperatively Regulate Diverse Pathways Governing Neural Stem Cell Homeostasis.” *Cell Stem Cell* 5 (5): 540–53. https://doi.org/10.1016/j.stem.2009.09.013.

Paik, Ji-Hye, Ramya Kollipara, Gerald Chu, Hongkai Ji, Yonghong Xiao, Zhihu Ding, Lili Miao, et al. 2007. “FoxOs Are Lineage-Restricted Redundant Tumor Suppressors and Regulate Endothelial Cell Homeostasis.” *Cell* 128 (2): 309–23. https://doi.org/10.1016/j.cell.2006.12.029.

Qu, Qiuhao, Guoqiang Sun, Wenwu Li, Su Yang, Peng Ye, Chunnian Zhao, Ruth T. Yu, Fred H. Gage, Ronald M. Evans, and Yanhong Shi. 2010. “Orphan Nuclear Receptor TLX Activates Wnt/Β-Catenin Signalling to Stimulate Neural Stem Cell Proliferation and Self-Renewal.” *Nature Cell Biology* 12 (1): 31–40. https://doi.org/10.1038/ncb2001.

Renault, Valérie M, Victoria A Rafalski, Alex A Morgan, Dervis A M Salih, Jamie O Brett, Ashley E Webb, Saul A Villeda, et al. 2009. “FoxO3 Regulates Neural Stem Cell Homeostasis.” *Cell Stem Cell* 5 (5): 527–39. https://doi.org/10.1016/j.stem.2009.09.014.

Ro, Seung-Hyun, Debra Liu, Hyeonju Yeo, and Ji-hye Paik. 2013. “FoxOs in Neural Stem Cell Fate Decision.” *Archives of Biochemistry and Biophysics* 534 (1–2): 55–63. https://doi.org/10.1016/j.abb.2012.07.017.

Sahar, S., and P. Sassone-Corsi. 2009. “Metabolism and Cancer: The Circadian Clock Connection.” *Nature Reviews Cancer* 9 (12). https://doi.org/10.1038/nrc2747.

Sahar, Saurabh, and Paolo Sassone-Corsi. 2012. “Regulation of Metabolism: The Circadian Clock Dictates the Time.” *Trends in Endocrinology and Metabolism: TEM* 23 (1): 1–8. https://doi.org/10.1016/j.tem.2011.10.005.

Schmidt-Strassburger, Uta, Tobias G. Schips, Harald J. Maier, Katharina Kloiber, Francesca Mannella, Kerstin E. Braunstein, Karlheinz Holzmann, et al. 2012. “Expression of Constitutively Active FoxO3 in Murine Forebrain Leads to a Loss of Neural Progenitors.” *FASEB Journal* 26 (12): 4990–5001. https://doi.org/10.1096/fj.12-208587.

Singh, Sheila K., Cynthia Hawkins, Ian D. Clarke, Jeremy A. Squire, Jane Bayani, Takuichiro Hide, R. Mark Henkelman, Michael D. Cusimano, and Peter B. Dirks. 2004. “Identification of Human Brain Tumour Initiating Cells.” *Nature* 432 (7015): 396–401. https://doi.org/10.1038/nature03128.

Snyder, Jason S. 2019. “Recalibrating the Relevance of Adult Neurogenesis.” *Trends in Neurosciences*. Elsevier Ltd. https://doi.org/10.1016/j.tins.2018.12.001.

Sorrells, Shawn F., Mercedes F. Paredes, Arantxa Cebrian-Silla, Kadellyn Sandoval, Dashi Qi, Kevin W. Kelley, David James, et al. 2018. “Human Hippocampal Neurogenesis Drops Sharply in Children to Undetectable Levels in Adults.” *Nature* 555 (7696): 377–81. https://doi.org/10.1038/nature25975.

Takesian, Anne E., and Takao K. Hensch. 2013. “Balancing Plasticity/Stability across Brain Development.” In *Progress in Brain Research*, 207:3–34. Elsevier B.V. https://doi.org/10.1016/B978-0-444-63327-9.00001-1.

Tobin, Matthew K., Kianna Musaraca, Ahmed Disouky, Aashutosh Shetti, Abdullah Bheri, William G. Honer, Namhee Kim, et al. 2019. “Human Hippocampal Neurogenesis Persists in Aged Adults and Alzheimer’s Disease Patients.” *Cell Stem Cell* 24 (6): 974-982.e3. https://doi.org/10.1016/j.stem.2019.05.003.

Tümpel, Stefan, and K. Lenhard Rudolph. 2019. “Quiescence: Good and Bad of Stem Cell Aging.” *Trends in Cell Biology*. https://doi.org/10.1016/j.tcb.2019.05.002.

Urbán, Noelia, Debbie L C van den Berg, Antoine Forget, Jimena Andersen, Jeroen A A Demmers, Charles Hunt, Olivier Ayrault, and François Guillemot. 2016. “Return to Quiescence of Mouse Neural Stem Cells by Degradation of a Proactivation Protein.” *Science* 353 (6296): 292–95. https://doi.org/10.1126/science.aaf4802.

Urbán, Noelia, Isabelle Maria Blomfield, and François Guillemot. 2019. “Quiescence of Adult Mammalian Neural Stem Cells: A Highly Regulated Rest.” *Neuron*. https://doi.org/10.1016/j.neuron.2019.09.026.

Walgrave, Hannah, Sriram Balusu, Sarah Snoeck, Elke Vanden Eynden, Katleen Craessaerts, Nicky Thrupp, Leen Wolfs, et al. 2021. “Restoring MiR-132 Expression Rescues Adult Hippocampal Neurogenesis and Memory Deficits in Alzheimer’s Disease.” *Cell Stem Cell*, May. https://doi.org/10.1016/j.stem.2021.05.001.

Webb, Ashley E, Elizabeth A Pollina, Thomas Vierbuchen, Noelia Urbán, Duygu Ucar, Dena S Leeman, Ben Martynoga, et al. 2013. “FOXO3 Shares Common Targets with ASCL1 Genome-Wide and Inhibits ASCL1-Dependent Neurogenesis.” *Cell Reports* 4 (3): 477–91. https://doi.org/10.1016/j.celrep.2013.06.035.

Zhang, Runrui, Marcelo Boareto, Anna Engler, Angeliki Louvi, Claudio Giachino, Dagmar Iber, and Verdon Taylor. 2019. “Id4 Downstream of Notch2 Maintains Neural Stem Cell Quiescence in the Adult Hippocampus.” *Cell Reports* 28 (6): 1485-1498.e6. https://doi.org/10.1016/j.celrep.2019.07.014.

Zhou, Jingyi, Wenjuan Liao, Jing Yang, Ke Ma, Xue Li, Yachen Wang, Donglai Wang, et al. 2012. “FOXO3 Induces FOXO1-Dependent Autophagy by Activating the AKT1 Signaling Pathway.” *Autophagy* 8 (12): 1712–23. https://doi.org/10.4161/auto.21830.