Adult neural stem cells and neurogenesis are resilient to intermittent fasting

Rut Gabarró-Solanas[1,2], Amarbayasgalan Davaatseren[1], Tatjana Kepčija[1], Iván Crespo-Enríquez[1] and Noelia Urbán[1,*]

[1]Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), Dr. Bohr-Gasse 3, 1030 Vienna, Austria.
[2]Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, A-1030, Vienna, Austria.
*Correspondence: noelia.urban@imba.oeaw.ac.at

Abstract: Intermittent fasting (IF) is a promising non-pharmacological strategy to counteract ageing which has been shown to increase the number of adult-born neurons in the dentate gyrus of mice. However, it is still unclear which steps of the adult neurogenesis process are regulated by IF. The number of adult neural stem cells (NSCs) decreases with age in an activation-dependent manner. To counteract the loss of the stem cell pool, adult NSCs are mostly found in an inactive, quiescent state which ensures their long-term maintenance. We aimed to determine if and how IF impacts the activity and maintenance of adult NSCs in the hippocampus. We chose an every-other-day fasting protocol with food re-administration at night, which we found effectively induces fasting features and preserves the circadian activity pattern of mice. To determine the effects of IF on NSCs and all following steps in the neurogenic lineage, we combined fasting with lineage tracing and label retention assays. We found that IF does not affect NSC activation or maintenance. Contrary to previous reports, we also found that IF does not increase hippocampal neurogenesis. We obtained the same results regardless of strain, sex, diet length, tamoxifen administration or new-born neuron identification method. Our data suggest that NSCs maintain homeostasis upon IF and that this intervention is not a reliable strategy to increase adult neurogenesis.

Keywords: adult neurogenesis; dietary restriction; quiescence

---

Introduction

Adult stem cells are rare cell populations in adult tissues that generate new somatic cells to support tissue turnover and repair upon injury (Li and Clevers, 2010). Proper regulation of stem cell function is crucial to ensure tissue homeostasis. With age, stem cell pools decline and the function of the remaining stem cells is impaired (Goodell and Rando, 2015; López-Otín et al., 2013; Schultz and Sinclair, 2016; Tümpel and Rudolph, 2019). Fasting, either in the form of intermittent fasting or time-restricted feeding, is a dietary intervention that prolongs life and health span (Longo and Panda, 2016; Madeo et al., 2019). The health benefits of fasting are explained, in part, by improved stem cell function throughout the body, including in the intestine, muscle or hematopoietic system (Novak et al., 2021).

The adult mammalian brain harbours neural stem cells (NSCs) in specific locations such as the dentate gyrus (DG) of the hippocampus (Kuhn et al., 2018). There, NSCs are the source of adult-born granule neurons, that integrate into the pre-existing hippocampal circuit offering

an additional layer of plasticity that is crucial for the modulation of memory, learning, mood or emotions (Bond et al., 2015). NSCs in the DG have a limited ability to self-renew and are mostly found in an inactive, quiescent state that prevents their activity-coupled exhaustion (Bonaguidi et al., 2011; Bottes et al., 2021; Encinas et al., 2011; Pilz et al., 2018; Urbán et al., 2016). Interestingly, NSCs can return to quiescence after proliferation, a mechanism that helps preserve the active NSC pool and sustain neurogenesis throughout life (Bottes et al., 2021; Urbán et al., 2016). Yet, the aged brain contains fewer NSCs which, in addition, shift to a deeper quiescent state resulting in a decrease in adult neurogenesis (Harris et al., 2021). Understanding NSC quiescence is therefore crucial to elucidate the mechanisms that control and support neurogenesis during adulthood and ageing.

Fasting is widely regarded as an efficient strategy to increase adult neurogenesis, therefore holding great potential to improve cognitive ability and prevent the age-related neurogenic decline (Fusco and Pani, 2013; Landry and Huang, 2021; Levenson and Rich, 2007; Mattson, 2003; Pani, 2015; Poulose et al., 2017; Van Cauwenberghe et al., 2016; Wahl et al., 2016; Zainuddin and Thuret, 2012). However, we still do not understand how NSCs and other cells along the neurogenic lineage respond to fasting. To generate new neurons, quiescent NSCs first activate and give rise to intermediate progenitor cells (IPCs). These are highly proliferative cells that amplify the progenitor pool and further differentiate into neuroblasts, eventually maturing into functional granule neurons. Fasting has been reported to promote the survival of newly born neurons (Brandhorst et al., 2015; Kim et al., 2015; Kitamura et al., 2006; Lee et al., 2002a; Lee et al., 2002b), with some works also reporting an increase in proliferation in the DG (Brandhorst et al., 2015; Cao et al., 2022; Dias et al., 2021; Park and Lee, 2011). It is therefore not clear whether fasting increases the neuronal output of adult NSCs by promoting maturation and survival of newly born neurons, by increasing proliferation of IPCs and/or by activating quiescent NSCs. A fasting-induced burst in NSC proliferation could lead to the exhaustion of the NSC pool eventually impairing adult neurogenesis. Determining the stages of the neurogenic lineage at which fasting acts to increase adult neurogenesis and whether it affects NSC activation and maintenance is necessary to predict its long-term consequences.

Here we have established an intermittent fasting (IF) protocol that effectively causes systemic changes associated with the benefits of fasting without altering the activity pattern of mice. To study the effects of IF on NSCs, we used genetic lineage tracing and thymidine analogues in combination with immunofluorescence. IF did not affect NSC proliferation or maintenance even after a 4-months long intervention. This could make IF an ideal strategy to increase adult neurogenesis without compromising the stem cell pool. However, we also found that IF did not increase adult neurogenesis at any given time. We investigated experimental variables that could explain why our results did not match previous reports on adult neurogenesis, such as mouse strain, sex, diet length, tamoxifen administration or newly born neuron quantification method. Our results consistently show that adult neurogenesis does not increase upon IF.

Results

Intermittent fasting with daytime refeeding disrupts the circadian activity pattern of mice

We first set out to establish a fasting protocol to study adult neurogenesis. IF is a popular form of fasting that encompasses a group of dietary interventions that rely on alternating fasting and feeding periods (Hofer et al., 2022). One of the most widely used IF interventions -and the one we chose for this study- is every-other-day-fasting (also known as alternate-day-

fasting), which consists of cycles of 24h of food deprivation followed by 24h of free access to food, where food is removed or added always at the same time of the day. The specific time at which food is removed/added respect to the time at which lights are switched on in the animal house (usually referred to as Zeitgeber Time 0 or ZT0) varies among studies, although it is often not specified. Morning (ZT0-ZT4, daytime IF) and evening (ZT12-14, night-time IF) times for removing/adding food are commonly used in IF studies, including those showing effects on adult neurogenesis in young, healthy mice (M. Mattson and S. Thuret, personal communication December 2020 and June 2021 respectively). We monitored mouse behaviour in ad libitum fed (Control, free access to food), day- and night-time intermittently fasted mice for 1 month using automated home cage phenotyping PhenoMaster cages (TSE Systems). Ad libitum fed mice displayed higher activity levels during the night than during the day, with a prominent peak of activity at the beginning of the dark phase, as expected for nocturnal animals (Figure 1A, B). It has been previously shown that IF with daytime refeeding decouples feeding patterns from light cues and daily behaviour, flattening the rhythmic expression of circadian clock genes (Acosta-Rodríguez et al., 2022; Froy et al., 2009). Accordingly, daytime refeeding introduced an additional peak of activity during the light phase and decreased the general activity of mice during the night (Figure 1A and S1A). Night-time IF, on the other hand, synchronised food cues to the light/dark cycle preserving the normal circadian activity pattern of the mice (Figure 1B and S1B). Interestingly, the total activity after 1 month of diet was not affected by day- nor night-time IF compared to control mice (Figure S1C, D). Since adult neurogenesis is influenced by circadian rhythms (Bouchard-Cannon et al., 2013; Draijer et al., 2019; Holmes et al., 2004), we chose the night-time IF protocol to study the effects of IF without introducing circadian rhythm disruption as an additional variable.

Night-time IF induces characteristic features of IF in mice

We then characterised the systemic response of mice to night-time IF (from here on IF unless stated otherwise) during 1 and 3 months. Every 24h of fasting resulted in a weight loss of up to 7% of the original weight, which was recovered during the following 24h of free access to food (Figure 1C, S2A). These oscillations persisted throughout the whole 3-month intervention. We also observed that mice subjected to IF ate nearly twice as much as control mice on feeding days. Quantification of overall food consumption by the end of the treatment showed a caloric restriction of 73% or 79.8% upon 1 or 3 months of IF respectively (Figure 1D, S2B). Of note, fasted mice exhibited a similar growth curve to ad libitum fed mice (Figure 1C) and a comparable weight at the end of the IF protocol even after 3 months of diet (Figure S2A). To characterise the impact of IF on systemic metabolism we performed an indirect calorimetry during a whole month of IF to estimate the fuel source used by the body to produce energy. We used the automated home cage phenotyping system to monitor CO_2 production and O_2 consumption, with which we calculated the respiratory exchange ratio (RER). A RER value close to 0.7 indicates predominant usage of lipid oxidation, characteristic of resting periods, while a value around 1 indicates that carbohydrates are the main source of energy. As expected, the RER of control mice was higher during the night than during the day (Figure 1E). In IF mice, fasting extended the periods in which the body shifted its metabolism towards lipid oxidation, a feature of prolonged fasting, while feeding induced a sharp metabolic shift towards the use of carbohydrates as a fuel source (Figure 1E). Therefore, alternating fasting and feeding days resulted in the periodic metabolic shifts that are proposed to mediate the benefits of IF (Anton et al., 2018). Browning of adipose tissue is another indicator of fasting (Li et al., 2017; Liu et al., 2019). Fat browning is characterised by a reduction in adipocyte size and an upregulation of uncoupling protein 1 (UCP1), which regulates mitochondrial energy

135 production, associated with thermogenesis in these cells (Ricquier, 2011). After 3 months of
136 IF, inguinal adipocytes of IF mice looked smaller and had higher levels of UCP1 than those of
137 mice fed ad libitum, although the effects were mild and variable (Figure 1F and S3). We also
138 observed robust structural changes in the livers of IF mice (Figure 1G and S4), where
139 hepatocytes showed an edematous morphology. This hepatocyte edema resembled the one
140 observed upon a 1-week IF as a compensatory mechanism to fluctuating liver size between
141 the fasting and fed states (Sarkar et al., 2021). Our data shows that liver remodelling persists
142 after 3 months of IF. Together, these results demonstrate that cycles of 24h fasting and 24h
143 feeding induce typical features of IF.

144 *IF does not affect the proliferation or maintenance of adult NSCs in the hippocampus*

145 To specifically characterise how adult NSCs respond to IF, we used Glast-CreER^{T2};RYFP
146 mice, a well-established mouse transgenic model for lineage tracing of NSCs in the adult DG
147 in which Tamoxifen-induced Cre recombination indelibly labels Glast-expressing cells with
148 YFP (Andersen et al., 2014; Mori et al., 2006; Srinivas et al., 2001). Tamoxifen was given to
149 two-month-old mice for the 5 days prior to starting the diet and we examined the mice on a
150 feeding day 1 month after the diet began (Figure 2A). The rate of recombination was very high
151 throughout our experiments and identical in ad libitum-fed and intermittently fasted mice
152 (Figure S5). We identified NSCs by their localisation in the sub-granular zone (SGZ) and the
153 extension of a single glial fibrillary acidic protein (GFAP)$^{+}$ radial projection to the molecular
154 layer with help of the YFP fluorescent reporter. A small fraction of NSCs (~3.4%) was positive
155 for the proliferation marker Ki67 in control mice, as expected for a predominantly quiescent
156 NSC population. This number was unchanged by IF, suggesting that NSC proliferation is not
157 affected by this intervention (Figure 2B, C). NSCs can return to quiescence after proliferating,
158 slowing down the activation-triggered loss of the stem cell pool. We used the thymidine
159 analogue 5-ethynyl-2′-deoxyuridine (EdU) in a label retention experiment to explore potential
160 changes in the ability of adult NSCs to return to quiescence (Urbán et al., 2016). We
161 administered EdU in the drinking water for 5 days to label all cells going through S-phase of
162 the cell cycle during that period. After a 10-day chase period, we quantified NSCs that had
163 incorporated EdU during the 5-day labelling period and had retained both the label and their
164 NSC features (i.e. SGZ localisation and a single GFAP^{+} radial process) (Figure 2A). The
165 percentage of EdU-retaining NSCs was similar in control and IF mice (Figure 2D, E). This,
166 together with the similar NSC proliferation rates in control and IF mice, suggests that NSC
167 transitions between quiescence and activation are not altered by IF. The total number of NSCs
168 was also not changed between IF and ad libitum fed mice (Figure 2F). Together, our results
169 show that adult NSCs are not affected by 1 month of IF.

170 To understand the consequences of longer exposures to IF, we subjected Glast-
171 CreER^{T2};RYFP mice to 3 months of IF (Figure 3A), which has been shown to be enough to
172 increase neuronal production in the DG (Baik et al., 2020; Dias et al., 2021; Kim et al., 2015;
173 Kitamura et al., 2006; Lee et al., 2002b; Lee et al., 2002a; Li et al., 2020). The percentage of
174 proliferating NSCs in the 3-month intervention was halved compared to the same value in the
175 1-month intervention, in accordance with the reported increase in quiescence during ageing
176 (Harris et al., 2021) (Figures 2B, C and 3B). NSC proliferation did not change upon IF (Figure
177 3B). We then examined NSC maintenance by quantifying the total number of NSCs present
178 at the end of the treatment (Figure 3C, D). Despite the well-established decrease of
179 neurogenesis with age, we did not observe a decrease in total NSCs in 5-month-old mice
180 respect to 3-month-old mice (Figures 2F and 3C). This could be explained by batch variability
181 in the stainings or by a shift in the exponential phase of the decline in NSC numbers to either

182 before 3 months or after 5 months of age in these mice. Nevertheless, the number of NSCs in
183 IF mice were indistinguishable from those of ad libitum fed mice (Figure 3C, D).
184 These data indicate that NSCs are protected from proliferation bursts that could lead to
185 exhaustion, making IF a safe option for potentially increasing neurogenesis through diet-based
186 interventions.

187 *Adult neurogenesis is mildly and transiently impaired by IF*

188 Since neither the pool nor the activity of NSCs change upon IF, we hypothesized that the
189 previously reported increase in neurogenesis laid in subsequent stages of neurogenesis. We
190 set out to determine at which step/s of the neurogenic lineage IF influences adult
191 neurogenesis. NSCs and neuroblasts are predominantly out of the cell cycle, meaning that
192 the vast majority of proliferating cells in the SGZ are IPCs (Hodge et al., 2008; Kronenberg et
193 al., 2003). We therefore used Ki67 as a proxy for IPC identification. As previously reported,
194 we observed a decrease in IPC abundance over time (Figure 4A, B). IF neither changed the
195 number of IPCs after 1 month nor prevented their decline after 3 months (Figure 4A, B).
196 Next, we quantified the number of neuroblasts, identified by doublecortin (DCX)
197 immunoreactivity. Our results show that neuroblasts were more abundant in younger mice and
198 that they are generated at the same rate in IF mice and ad libitum fed mice at both time points
199 (Figure 4C, D).
200 Finally, we measured the number of new neurons generated during the intervention. We
201 used colocalization of the YFP reporter and the marker for mature neurons NeuN to identify
202 newly born neurons. In accordance with the cumulative labelling of neurons upon lineage
203 tracing, mice had many more newly born neurons labelled with the YFP reporter after 3 months
204 than after 1 month (Figure 4E, F). Unexpectedly, we found a reduction in the number of newly
205 born neurons in IF mice compared to ad libitum fed mice after 1 month of IF (Figure 4E, F).
206 Cell death, measured by the number of picnotic nuclei in the SGZ, was comparable in control
207 and IF mice (Figure S6A). Since neuroblasts were produced at the same rate (Figure 4D), we
208 reasoned that this decrease in neurogenesis could be due to a transient drop in neurogenesis
209 at an earlier time point. We made use of the label retention experiment in Figure 2A to ask if
210 neuronal production was impaired two weeks after starting IF. Indeed, we found that fewer
211 neurons had been produced at that time in mice subjected to IF compared to control mice
212 (Figure S6B). One month of IF is therefore not only insufficient to increase adult neurogenesis
213 but in fact decreases the neuronal output of adult NSCs. The decrease of neurogenesis was
214 transient, as the number of newly born neurons went back to control levels in the 3-month
215 intervention (Figure 4E, F), suggesting that the process of adult neurogenesis is robust enough
216 to buffer initial changes in neuronal production.
217 Our findings show that 1 or 3 months of IF do not increase adult neurogenesis. This
218 seemingly contradicts previous reports and puts a question mark on the suitability of IF as a
219 tool to increase neurogenesis in the adult hippocampus.

220 *IF does not increase adult neurogenesis regardeless of sex, labelling method, strain,*
221 *tamoxifen usage or diet length.*

222 We next asked which intrinsic variables of our study could explain the contradicting results
223 with previously published studies.
224 The metabolism of male and female mice has been shown to react in a sex-specific
225 manner to fasting and refeeding (Freire et al., 2020). While most studies on IF and
226 neurogenesis were conducted with only male or only female mice, differences in the response
227 to caloric restriction of adult hippocampal neurogenesis have been reported between sexes

228 (Park et al., 2013). In our experimental set up, we used enough males and females to identify
229 sex differences in the response of adult neurogenesis to IF. We found that the transient
230 decrease in newly born neurons after 1 month of IF was affected by sex (Figure 5A). However,
231 we did not observe sex differences at any other point of the study, with neurogenesis not being
232 affected by IF in either male or female mice (Figure 5B and S7). Of note, data variability was
233 also similar in male and female mice (Figure S7).
234 We used genetic lineage tracing to quantify the total number of new neurons generated
235 from NSCs throughout the whole dietary intervention. Instead, previous studies relied on the
236 use of thymidine analogues (BrdU) to label a cohort of proliferating cells and quantify the
237 amount of total $BrdU^+$ cells or $BrdU^+$ neurons 1 month after labelling, obtaining information
238 exclusively on one neurogenic wave at the end of the treatment (Brandhorst et al., 2015; Dias
239 et al., 2021; Kim et al., 2015; Kitamura et al., 2006; Lee et al., 2002b; Lee et al., 2002a). To
240 assess the effects of IF in a comparable way, we used EdU to pulse-label proliferating cells 1
241 month before the end of the 3-month IF protocol (Figure 5C). Again, the number of EdU^+ cells
242 (Figure 5D, E) was unchanged by the diet, showing that IF does not affect neurogenesis after
243 three months.
244 Another particularity of our study is that the GlastCreER^{T2};RYFP mice we used had a
245 mixed genetic background (Austin et al., 2020), while previous studies had used mice from
246 the C57BL/6J strain. Strain-specific differences in the response of adult neurogenesis to
247 dietary interventions such as high-fat diet have been reported (Hwang et al., 2008). In addition,
248 recurrent doses of Tamoxifen alter neurogenesis and induce broad detrimental effects, from
249 damaging the gut epithelia to impairing microglial reactivity (Smith et al., 2022). To test
250 whether strain and tamoxifen could alter how IF modulates adult neurogenesis, we subjected
251 C57BL/6J mice to 3 months of IF, excluding tamoxifen administration (Figure 5F). We did a 1-
252 month EdU-chase, as in our previous experiment with Glast-CreER^{T2};RYFP mice, and
253 analysed the number of EdU^+ cells at the end of the treatment. We identified newly born
254 neurons as EdU^+NeuN^+ cells (Figure 5G) and, again, we did not find any differences caused
255 by the diet. In addition to that, neuroblasts (Figure 5H) were not altered by IF either suggesting
256 that tamoxifen and strain do not explain the discrepancy in results.
257 Lee et al., Kim et al., and Dias et al. reported an increase in neurogenesis in a cohort of
258 cells that was labelled at 3 months of IF and analysed 1 month later, 4 months after the start
259 of the diet (Dias et al., 2021; Kim et al., 2015; Lee et al., 2002a). Since we were interested in
260 determining whether NSCs were at the origin of this increase, we performed 3-month-long
261 experiments, meaning that neuronal production was also analysed after 3 months of IF.
262 Therefore, we next asked whether extending the diet for an additional month would increase
263 neurogenesis. We used male C57BL/6J mice and labelled a cohort of proliferating cells with
264 EdU for 12 days, 3 months after the start of the diet (Figure 6A). We analysed newly born
265 neurons 1 month after, and, to our surprise, observed a decrease in EdU^+NeuN^+ cells (Figure
266 6B, C). We wondered if this was a result of decreased NSC activation, as a mechanism to
267 prevent NSC exhaustion and prolong neurogenesis during aging, but IF did not change NSC
268 proliferation nor maintenance (Figure 6D, E). Other stages of neurogenesis were also
269 unaffected since there were the same number of IPCs and neuroblasts in control an IF mice
270 (Figure 6F, G).
271 Together, these results show that differences in sex, labelling method, strain, tamoxifen
272 usage and diet length do not explain the discrepancies in the response of adult neurogenesis
273 upon IF between our study and others. We conclude that every-other day IF is not a robust
274 strategy to increase adult neurogenesis in the hippocampus.

Discussion

While rejuvenating diets partially exert their effects through modulation of adult stem cells, few studies have investigated their specific effects on them. Here, we used genetic lineage tracing to determine the consequences of every-other-day IF on adult NSCs. We found that up to 4 months of IF do not affect the adult neural stem cell pool, making it a safe intervention to extend life and healthspan. However, we could not replicate previous reports associating IF to an increase in adult neurogenesis, even when controlling for sex, labelling method, strain, tamoxifen administration and IF length. This puts a question mark on IF as a strategy to boost neurogenesis during ageing and highlights the context-dependency of dietary interventions. While we could not pinpoint the source of discrepancy between our study and previous reports, there are several points that we would like to mention.

Methodology for neurogenesis quantification

The effects of IF and other dietary interventions on adult neurogenesis are often evaluated exclusively through neuronal survival assays with thymidine analogues (Kim et al., 2015; Lee et al., 2002a). Thymidine analogues (such as BrdU, CldU, IdU or EdU) are administered to the mice to label proliferating cells, with neurogenesis being evaluated after a chase period by direct quantification of the amount of newly born cells and/or comparison to the initially labelled population. This approach focuses on the analysis of the latest steps of neurogenesis, providing no information on possible effects on adult neural stem cells. In addition, it has several limitations:

-First, it is not fully standardised, with the delivery of thymidine analogs being either i.p. or in the drinking water, and the length of the labelling period being variable between studies. The use of each particular analogue could also have consequences in the number of cells identified (for instance detection of EdU is more sensitive than that of BrdU) as well as differentially affect cell viability.

-Second, it evaluates only narrow waves of neurogenesis. While this can be useful to identify changes in the effects of the intervention over time, when only one time point is chosen, it also increases the variability of the results and does not provide information on the total number of new neurons produced during the whole intervention. We observed high variability in the effects of IF on adult neurogenesis, with an initial decrease followed by no effect and a final further decrease in newly born cells. The effects we observed were small and only emerged when analysing small cohorts of new neurons. They could therefore be produced by the high degree of intrinsic variability in adult neurogenesis, questioning their functional importance.

-Third, interpretation of the data is challenging unless accompanied by multiple other measures. For instance, when not used in combination with neuronal markers, it does not allow cell type identification, as other cells (astrocytes, oligodendrocyte precursors or microglia) also proliferate in the dentate gyrus. Even a difference in the number of final $EdU^{+}NeuN^{+}$ cells cannot be solely explained by an increase in neuronal survival as often interpreted. This is because upon thymidine analogues administration, the initially labelled cell population – which is normally used to directly compare with the population of cells that retained the label after the chase, and also as a measure of proliferation in the DG - includes all proliferating cells at different stages of the neurogenic lineage: NSCs, IPCs and early neuroblasts. This, together with typically long labelling protocols, allows many of these cells to further progress through the lineage during the label administration. Therefore, the label positive population by the end of the labelling period depends not only on uptake of the label

321 by proliferation but also on differentiation and survival, hampering the interpretation of the
322 results.
323 Standardisation of these methods and the combined use of label retention and lineage
324 tracing strategies could help provide a more reliable picture of the effects of dietary (or any)
325 interventions on adult neurogenesis.
326 One alternative way in which the effects of IF on neurogenesis are tested is the use of
327 behavioural tests that measure hippocampal function. We did not evaluate the effects of IF on
328 mouse behaviour, and therefore cannot link the lack of effects on neurogenesis to possible
329 changes in hippocampal function. It would be very interesting to determine whether adult
330 neurogenesis is required for the cognitive benefits reported upon IF.
331

332 *Mice sex, strain and fitness*

333 Differences in adult neurogenesis levels have been observed in different strains (Goodrick
334 et al., 1990; Kempermann et al., 2006; Kim et al., 2017; Koehl, 2015; Wiget et al., 2017). In
335 addition, the response to dietary interventions is also strain and even substrain-specific
336 (Bachmann et al., 2022; Hwang et al., 2008; Mitchell et al., 2016; Siersbæk et al., 2020). In
337 our own hands, we observed differences in the number of neuroblasts between control
338 GlastCre mice and control C57BL/6J at 5 months of age (Figure S8). Therefore, we cannot
339 rule out that small differences in the animals' background could have contributed to the
340 discrepancies between previous studies and ours.
341 Mice stress and overall fitness are often overlooked, although they can heavily influence
342 adult neurogenesis (Snyder et al., 2011; Toda et al., 2019). Stress and fitness levels can vary
343 wildly between facilities and even in the same facility over time, placing them as top candidates
344 to contribute to the variability between studies. The effects of IF may well depend on the pre-
345 condition of the mice, as often beneficial effects of IF on neurogenesis are reported in
346 combination with pathological states such as stroke or neurodegenerative disorders (Cao et
347 al., 2022; Li et al., 2020; Wu et al., 2008), or in aged mice (Brandhorst et al., 2015; Park et al.,
348 2013). The effects of IF might be much more subtle on healthy, relatively young mice.
349 Male and female mice also differ in their metabolic response to dietary interventions
350 (Bachmann et al., 2022; Mitchell et al., 2016), making it crucial to include both sexes when
351 analysing the effects of any potential clinically relevant interventions. Currently many medical
352 advances are based on results obtained exclusively in males, assuming females will react the
353 same way, often resulting in detrimental effects for women's health (Criado Perez, 2020).
354 Traditionally, female mice were excluded arguing higher variability in the results. We do not
355 see this, as variability was equally high in both sexes (Figure S7). From our own data, only
356 the number of new neurons after 1 month of IF was affected by sex (Figure 5A, 5B and S7).
357 Sex differences are particularly important for stress-related factors (Yagi and Galea, 2019).
358 Therefore, the sex differences we observe could be linked to metabolic or stress-related
359 differences in handling the change from ad libitum feeding to fasting between male and female
360 mice.
361 In summary, the specific combination of sex, strain and health status of the mice can
362 influence the outcome of dietary interventions on adult neurogenesis and could partially
363 explain the differences we observe respect to previously publishes studies.
364

365 *Fasting protocol and mouse husbandry*

366 IF strategies are extremely diverse, with the length of fasting and the lag between fasting
367 periods varying wildly between a few hours of fasting every day to a few days of continuous

368 fasting followed by long periods of normal feeding. We chose every-other-day fasting because
369 of its simplicity and its apparent ability to induce changes in adult neurogenesis.
370 Our results show that the time at which mice are re-fed influences circadian activity,
371 therefore adding a confounding variable to IF. We found that daytime IF disrupts the circadian
372 activity pattern of mice, while night-time IF preserves it. A very recent report has corroborated
373 our findings and demonstrated that circadian entraining can boost the beneficial effects of
374 caloric restriction on longevity (Acosta-Rodríguez et al., 2022). Out of previous studies that
375 reported an increase in adult neurogenesis upon every-other-day IF in young, healthy mice,
376 at least one performed their re-feeding in the morning and another one in the evening (M.
377 Mattson and S. Thuret, personal communication). Adult neurogenesis can be disrupted by
378 alterations in circadian rhythms (Bouchard-Cannon et al., 2013; Draijer et al., 2019; Schouten
379 et al., 2020). Therefore, this could partially explain the discrepancy and calls for a close
380 monitoring of potential circadian disruptions during IF interventions.
381 Two aspects that do not usually receive any attention are cage bedding and diet
382 composition. Mice that undergo dietary interventions such as CR eat a considerable amount
383 of bedding, which can be derived from wood, cellulose or corncob. Interestingly, only mice
384 housed in corncob bedding upregulated acyl-ghrelin (Gregor et al., 2020), a hormone that
385 might mediate the increase in $BrdU^{+}NeuN^{+}$ cells in the DG upon CR (Hornsby et al., 2016).
386 Diet composition has a strong influence on gut microbiota (Kolodziejczyk et al., 2019). Both
387 diet specification and microbiome are specific to each animal housing facility and can influence
388 the effects of dietary interventions and directly adult neurogenesis (Cryan and Dinan, 2012;
389 Ribeiro et al., 2020; Wei et al., 2021).
390 Another important consideration is the high similarity between caloric restriction (CR)
391 and IF. CR consists of administering a constant number of calories per day to the mice, which
392 is less than what they would normally eat when fed ad libitum. This normally ranges between
393 50 and 80% of their normal daily calory intake. In most cases, CR protocols add the food to
394 the mice all at once, which prompts the mice to quickly eat it and subsequently fast until the
395 next day. IF protocols, particularly those in which food is restricted for more than 16 hours per
396 day, also induce CR, as mice are not able to fully compensate for the fasting periods.
397 Therefore, CR usually comes associated with IF and the other way round. Our study produced
398 an overall CR of 73-78%, which is very similar to that used in several CR-focused experiments
399 (for example, 70% in the recent (Acosta-Rodríguez et al., 2022) study). Dias et al performed
400 an IF protocol which led to only 10% CR, leaving us wondering whether a 30% CR could have
401 cancelled the potential beneficial effects of IF. Despite reducing calorie consumption more
402 than 20%, our protocol did not affect the weight of the mice or their growth curve. In contrast,
403 both Lee et al. and Kim et al. reported weight loss upon every-other-day IF. Although some
404 reports indicate that weight loss is not necessarily linked to the benefits of IF and CR, it
405 remains plausible that the degree of caloric restriction and weight loss induced by IF could
406 influence its subsequent effects on adult neurogenesis.
407 We found the difference between our results and those of Dias et al. particularly striking
408 as they not only observed an increase in newly generated neurons but also a sharp increase
409 in proliferation in the DG after 3 months of IF (Dias et al., 2021). One potential explanation is
410 that they analysed the mice after a period of 5 days of feeding ad libitum, while our mice were
411 still fasting at the time of analysis. Alternating fasting periods with normal feeding has been
412 proposed to be a crucial mechanism by which IF and CR exert their beneficial effects
413 (Brandhorst et al., 2015). It would be very interesting to determine whether returning to ad
414 libitum feeding from IF rather than fasting on its own can promote cell proliferation along the
415 neurogenic lineage.

In summary, there is no consensus IF protocol. The time of day of food change is not even mentioned in most studies, let alone other important variables such as weight loss, diet composition or type of bedding. This variability and lack of accessibility hinders the progress of the field, not only in relation to adult neurogenesis but to all aspects of fasting. For instance, the divergent results obtained through two independent studies on the effects of CR on monkeys were attributed to differences in study design (Colman et al., 2009; Colman et al., 2014; Mattison et al., 2012; Mattison et al., 2017). Our findings call for a much more rigorous and detailed disclosure of fasting protocols and, in the near future, for a standardization of this type of interventions.

Conclusions

Diet is regarded as an important regulator of adult neurogenesis (Aimone et al., 2014; Denoth-Lippuner and Jessberger, 2021; Urbán et al., 2019), with fasting often highlighted as a positive modulator of neuronal production (Bouchard and Villeda, 2015; de Lucia et al., 2017; Fusco and Pani, 2013; Katsimpardi and Lledo, 2018; Landry and Huang, 2021; Levenson and Rich, 2007; Mattson, 2003; Murphy et al., 2014; Pani, 2015; Park and Lee, 2011; Poulose et al., 2017; Valero et al., 2016; Van Cauwenberghe et al., 2016; Wahl et al., 2016; Zainuddin and Thuret, 2012). Our results are a clear example of the high context-dependency of the effects of dietary interventions on adult neurogenesis and call for an urgent revision of our current knowledge. While the health and lifespan benefits of fasting are widely acknowledged, we still need better tools and further protocol standardisation in order to conclude any direct effects on adult neurogenesis. Lineage tracing or at the very least a detailed identification of the cell types affected by dietary interventions, could help clarify many of the current contradictions.

We could not identify any single variable that could account for the disparity between our findings and those of earlier studies. We believe that our efforts to eliminate any confounding factors, such as circadian disruptions, might have led to our negative results. This means that IF alone is not sufficient to reliably influence adult neurogenesis. Instead, we propose that the discrepancy between our results and previous reports is due to a combination of different factors (and most likely different combinations in each case). This would be consistent with a model in which IF can shift the cellular state of adult NSCs and/or other cells in the neurogenic lineage, rendering them more susceptible to further interventions such as circadian disruption or sudden re-feeding (Urbán, 2022).

Upon close examination of the available literature on IF in rodents, we wonder whether publication bias might be playing a role in skewing it towards reporting positive results. We noticed high diversity in its effects on adult neurogenesis, with an overwhelming majority of reports showing variable positive impact (Apple et al., 2019; Baik et al., 2020; Brandhorst et al., 2015; Cao et al., 2022; Dias et al., 2021; Kaptan et al., 2015; Kim et al., 2015; Kitamura et al., 2006; Kumar et al., 2009; Lee et al., 2000; Lee et al., 2002b; Lee et al., 2002a; Li et al., 2020; Park et al., 2013; Wu et al., 2008) and very few showing no effect or a mild impairment (Bondolfi et al., 2004; Staples et al., 2017). In some reviews, even studies reporting no effect are cited as evidence for improved neurogenesis upon IF. Reporting of negative results, especially those challenging accepted dogmas, and a careful and rigorous evaluation of the publications cited in reviews are crucial to avoid unnecessary waste of resources and to promote the advancement of science.

462 **Materials and Methods**

463 *Animal husbandry*

464 Mice were housed in groups in ventilated cages (TECNIPLAST 1285L NEXT) provided
465 with HEPA filtered air covered with Aspen-wood-derived bedding (Kliba Nafag, ABEDD 4063
466 OM G10). They were kept under a 12:12 h light/dark cycle (light period 0600 – 1800 h CET),
467 unless stated otherwise. Mice had ad libitum access to food (Ssniff, V1184-300) and water.
468 To generate a mouse line that allowed lineage tracing of adult NSCs with a fluorescent reporter
469 (Andersen et al., 2014), we crossed Glast-CreER^{T2} (Slc1a3$^{tm1(cre/ERT2)Mgoe}$, (Mori et al., 2006))
470 mice and RYFP (Gt(ROSA)26Sor$^{tm1(EYFP)Cos}$, (Srinivas et al., 2001)) mice. All Glast-
471 CreER^{T2};RYFP mice were of mixed genetic background. 4–6-week-old C57BL/6J (Charles
472 River) mice were obtained from the Comparative Medicine facility at IMBA/IMP. Experimental
473 groups were formed by randomly assigning mice from different litters within each mouse strain
474 and all experiments were conducted in male and female mice, except stated otherwise.

475 All animals were bred and maintained in accordance with ethical animal license protocols
476 complying with Austrian and European legislation.

477 *Intermittent fasting*

478 Mice were randomly divided into Control or IF groups at postnatal day 65 (P65). Control
479 mice had ad libitum access to food. Mice in the IF group were subjected to an every-other-day
480 fasting regime, comprising 1 day of fasting followed by 1 day of feeding. For night-time IF, food
481 was removed at 1800 h CET coinciding with the time at which the lights went off (ZT12) and
482 reintroduced at 1800 h CET on the following day (after 24 h). For daytime IF, food change
483 happened every day at 0900 h CET (ZT3). Mouse weight was recorded every 3 days in both
484 Control and IF groups and leftover food weighted once per week. Weight gain was calculated
485 as the percentage of the weight difference to the weight at the beginning of the diet (P65).
486 Total food intake was calculated by the difference between supplied and leftover food over the
487 treatment in each cage and divided by the number of animals per cage. The length of each
488 experiment is specified in the text and figure legends. For the 4-months-long experiment
489 depicted in Figure 6, mice were fed automatically on a defined schedule through an internally
490 developed automated system.

491 *Automated home cage mouse phenotyping*

492 Locomotor and metabolic activity were measured using the PhenoMaster system TSE
493 Systems every 15 min during a month. Mice were acclimated to PhenoMaster water bottles
494 for at least 6 days and to the complete PhenoMaster housing (TECNIPLAST GreenLine GM
495 500) for 2 days. Mice in the daytime IF experiment were housed in groups (2-3 mice per cage)
496 in a 14:10 h light/dark cycle (light period 0600-2000 h CET). Mice in the night-time IF
497 experiment were housed individually in a 12:12 h light/dark cycle (light period 0600-1800 h
498 CET). Cages were kept in a climate chamber in the same environmental conditions as the
499 general animal rooms (24°C and 50% humidity). The PhenoMaster cages were programmed
500 to automatically grant or restrict access to the food basket at the designated time.

501 Before each run, CO_2 and O_2 sensors were calibrated against a defined mix of CO_2 and
502 O_2. The respiratory exchange ratio (RER) was calculated as the ratio of VCO_2 produced to the
503 VO_2 consumed by the PhenoMaster software. Mouse locomotor activity was monitored using
504 the ActiMot2 Activity module equipped with 3-dimensional infrared sensors. An estimate of
505 movement (DistD, cm) was calculated using the PhenoMaster software. Data acquired in 15-
506 min intervals are expressed as means of 48h cycles. For grouped housed mice (daytime IF),

507 DistD was divided by the number of mice in each cage. Total mouse locomotor activity was
508 calculated as the sum of cm throughout the whole intervention and segregated by time periods
509 to depict differences between diurnal and nocturnal behaviour during fasting and feeding days.
510 For daytime IF, where the mice were kept in a 14:10 LD cycle, the data was divided in ZT 0-
511 14-24-38-48 h. For night-time IF, where the mice were kept in a 12:12 LD cycle, the data was
512 divided in ZT 0-12-24-36-48 h.
513 The automated home cage mouse phenotyping was performed by the Preclinical
514 Phenotyping Facility at Vienna BioCenter Core Facilities (VBCF), member of the Vienna
515 BioCenter (VBC), Austria.

516 *Tamoxifen and EdU administration*

517 To induce activation of CreER^{T2} recombinase, Glast-CreER^{T2};RYFP mice were
518 intraperitoneally injected with 2 mg (75-100 mg/kg) of Tamoxifen (Sigma, T5648) diluted in
519 corn oil (Sigma, C8267) at postnatal day 60 (P60) for 5 consecutive days. Recombination
520 levels were evaluated by comparing YFP^{+} neuroblasts (DCX^{+} cells) to the total number of
521 neuroblasts (Figure S5). Recombination was very high in both experiments (1 month and 3
522 months – above 90%) and levels were comparable in Control and IF groups.
523 To label cells in S-phase, 5-ethynyl-2'-deoxyuridine (EdU, Carl Roth, 7845) was
524 administered in drinking water (0,1 mg/mL) ad libitum. The length of EdU administration and
525 chase period is specified in the text and figures.

526 *Tissue preparation and immunohistochemistry*

527 Mice were transcardially perfused with phosphate buffered saline (PBS) for 3 min,
528 followed by 4% paraformaldehyde (PFA, Sigma, P6148) in PBS for 15 min.
529 Brains were post-fixed in 4% PFA for 2-6 h at 4°C with rocking, washed with PBS and
530 stored in PBS with 0.02% Sodium Azide (ITW Reagents, A1430). Brains were coronally
531 sectioned at a thickness of 40 µm using a vibratome (Leica, VT1000S). Immunofluorescence
532 of brain tissue was performed in free-floating sections. Sections were blocked in 1%Triton-
533 PBS with 10% normal donkey serum (DS, Jackson Immuno Research, JAC0170001210) for
534 2 h at room temperature. Primary and secondary antibody solutions were prepared in
535 0.1%Triton-PBS with 10% DS. Sections were incubated in primary antibody solution at 4°C
536 overnight, washed 3 x 15 min with 0.1%Triton-PBS and incubated with secondary antibody
537 solution at room temperature for 2 h. Following 1 x 0.1%Triton-PBS and 2 x PBS 15 min
538 washes, sections were incubated with 1 µg/mL DAPI (Sigma, D9542) in 1:1 PBS:H_2O at room
539 temperature for 30 min. EdU was detected before DAPI incubation using Click-iT™ Plus Alexa
540 Fluor™ Picolyl Azide tool kits with 488 and 647 dyes (Invitrogen, C10641 and C10643
541 respectively). All incubations and washes were performed under gentle rocking.
542 Inguinal adipose tissue and livers were dissected and post-fixed in 4%PFA at room
543 temperature with rocking for 24h prior to paraffin embedding. Eight random mice per condition
544 were chosen from the original cohort of mice for immunohistochemical analysis. Fixed tissue
545 was process in a Donatello Automatic Sample Processor (Diapath) and embedded in paraffin.
546 2µm thick sections were collected on glass slides. For staining of the inguinal adipose tissue
547 antigen retrieval with tri-sodium citrate (pH=6) was carried out for 30 min prior to a 1-h-
548 incubation with the primary antibody at room temperature. The 2 steps HRP polymer Kit with
549 DAB (DCX, PD000POL-K) was used for detection followed by hematoxylin counterstaining.
550 Liver sections were stained with hematoxylin and eosin in an Erpedia Gemini AS strainer
551 (Fisher Scientific).

552 Primary and secondary antibodies and concentrations are listed in Table S1. Processing
553 and staining of adipose tissue and liver was performed by the Histology facility at the VBCF.

554 *Imaging*

555 Images of brain immunofluorescence were acquired using an inverted Axio Observer
556 microscope (ZEISS) equipped with a CSU X1 confocal scanning unit (ZEISS) and an EM-CCD
557 camera (Hamamatsu, C9100-13). 40x and 63x oil Plan-Apochromat objective lenses (ZEISS)
558 were used to image through whole 40 µm sections with a z-step of 1 µm. Images were stitched
559 using ZEN Blue software (ZEISS).
560 Images of adipose tissue and liver were acquired using a Pannoramic FLASH 250 II
561 (3DHISTECH) equipped with a CIS VVC FC60FR19CL camera (Vital Vision Technology) and
562 a 20x Plan-Apochromat objective. Representative images were cropped using the
563 CaseViewer software.

564 *Image quantifications*

565 Quantifications of cells in the neurogenic lineage were performed using the Fiji software
566 (Schindelin 2012) with the Cell Counter plugin. NSCs were identified by the localisation of a
567 DAPI$^+$ nucleus in the SGZ, the extension of a single GFAP$^+$ vertical projection towards the
568 molecular layer and the help of the YFP lineage tracing reporter (except in C57BL/6J mice),
569 that is brighter in NSCs than other cells. IPCs were identified as Ki67$^+$ cells in the SGZ.
570 Neuroblasts were identified as DCX$^+$ cells in the SGZ. Newly born neurons were identified as
571 NeuN$^+$ cells that colocalised with YFP (in GlastCreER^{T2};RYFP mice) or EdU (in C57BL/6J
572 mice) in the SGZ and granule cell layer of the DG. In GlastCreER^{T2};RYFP mice, NeuN counts
573 were normalised to the recombination rate. New cells in the DG were identified by EdU
574 labelling. For cell numbers, 1 DG per sample was quantified (two for NSCs). The freehand line
575 tool was used to measure the length of the SGZ, to which the total cell counts were normalised.
576 For percentage of proliferating or EdU-labelled NSCs, at least 120 cells from two DG were
577 counted in young mice, and 70 in older mice. All data were counted with group blinding within
578 each experiment.
579 The appropriate sample size was based on previous publications studying NSC
580 behaviour (Andersen 2014, Urban 2016, Blomfield 2019, Austin 2021), accounting for the
581 increased variability of dietary interventions compared to genetic ones and considering female
582 and male mice as separate groups. For the quantifications based on the YFP reporter,
583 samples with a recombination rate lower than 80% were excluded. Samples with poor staining
584 quality were also excluded from the quantifications.

585 *Statistical analysis*

586 All statistical analyses were conducted using GraphPad Prism version 9.3.1 for macOS
587 (GraphPad Software, San Diego, California, USA). Two-tailed unpaired student t tests were
588 used for the comparison of two conditions. Whenever the samples did not pass a Shapiro-
589 Wilk normality test ($p<0.05$), the non-parametric Mann-Whitney test was conducted (Figures
590 S5A, B and S6A). A two-way ANOVA for repeated measures and multiple comparisons with
591 a Šídák correction was performed to compare the weight of control and IF mice over time in
592 Figure 1C. In Figure S2B, the weight gain data had to be fitted into a mixed effects model
593 because of a missing value. For sex comparisons, a two-way ANOVA followed by Tuckey's
594 multiple comparisons test was performed. Error bars represent mean + standard deviation
595 (SD). Significance is stated as follows: ns, $p>0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****,
596 $p<0.0001$. In graphs displaying weight (Figures 1C and S2B) and in Figures S1A, B and S7

$p>0.05$ is indicated by absence of significance symbol. Independent biological replicates are represented as dots in the bar plots.

Acknowledgments

We thank all facilities at IMBA without which this work would have not been possible, in particular the animal house, bio-optics, histology and the workshop. We are particularly grateful to Mark Mattson, Seungjoon Park and Sandrine Thuret for sharing their experimental details with us. We want to specially thank Sandrine Thuret also for her helpful comments on our manuscript. We also thank all members of the Urbán Lab at IMBA for their critical reading of the manuscript.

Funding

N.U. is supported by Institute of Molecular Biotechnology of the Austrian Academy of Sciences (Österreichischen Akademie der Wissenschaften) and by grants from the Austrian Science Fund (FWF: Fonds zur Förderung der wissenschaftlichen Forschung; DOC72, SFB-F78, SFB-F79 and SMICH). R.G.S. is supported by a DOC fellowship from FWF.

Author Contributions

R.G.S. co-developed the concept, performed experiments, analysed data, and co-wrote the manuscript. A.D., T.K. and I.C.E. performed experiments and edited the manuscript. N.U. conceived the original study, co-developed the concept, and co-wrote the manuscript.

Declaration of interests

The authors declare no competing interests.

621 **References**

622 **Acosta-Rodríguez, V., Rijo-Ferreira, F., Izumo, M., Xu, P., Wight-Carter, M., Green, C. B.**
623 **and Takahashi, J. S.** (2022). Circadian alignment of early onset caloric restriction
624 promotes longevity in male C57BL/6J mice. *Science* e.

625 **Aimone, J. B., Li, Y., Lee, S. W., Clemenson, G. D., Deng, W. and Gage, F. H.** (2014).
626 Regulation and Function of Adult Neurogenesis: From Genes to Cognition.
627 *Physiological Reviews* **94**, 991–1026.

628 **Andersen, J., Urbán, N., Achimastou, A., Ito, A., Simic, M., Ullom, K., Martynoga, B.,**
629 **Lebel, M., Göritz, C., Frisén, J., et al.** (2014). A Transcriptional Mechanism
630 Integrating Inputs from Extracellular Signals to Activate Hippocampal Stem Cells.
631 *Neuron* **83**, 1085–1097.

632 **Anton, S. D., Moehl, K., Donahoo, W. T., Marosi, K., Lee, S. A., Mainous III, A. G.,**
633 **Leeuwenburgh, C. and Mattson, M. P.** (2018). Flipping the Metabolic Switch:
634 Understanding and Applying the Health Benefits of Fasting. *Obesity* **26**, 254–268.

635 **Apple, D. M., Mahesula, S., Fonseca, R. S., Zhu, C. and Kokovay, E.** (2019). Calorie
636 restriction protects neural stem cells from age-related deficits in the subventricular
637 zone. *Aging* **11**, 115–126.

638 **Austin, S. H. L., Harris, L., Paun, O., Rigo, P., Guillemot, F. and Urbán, N.** (2020).
639 Wnt/beta-catenin signalling is dispensable for adult neural stem cell homeostasis and
640 activation. *bioRxiv* 2020.12.29.424694.

641 **Bachmann, A. M., Morel, J.-D., El Alam, G., Rodríguez-López, S., Imamura de lima, T.,**
642 **Goeminne, L. J. E., Benegiamo, G., Loric, S., Conti, M., Sleiman, M. B., et al.**
643 (2022). Genetic background and sex control the outcome of high-fat diet feeding in
644 mice. *iScience* **25**, 104468.

645 **Baik, S.-H., Rajeev, V., Fann, D. Y.-W., Jo, D.-G. and Arumugam, T. V.** (2020). Intermittent
646 fasting increases adult hippocampal neurogenesis. *Brain and Behavior* **10**, e01444.

647 **Bonaguidi, M. A., Wheeler, M. A., Shapiro, J. S., Stadel, R. P., Sun, G. J., Ming, G. and**
648 **Song, H.** (2011). In vivo clonal analysis reveals self-renewing and multipotent adult
649 neural stem cell characteristics. *Cell* **145**, 1142–1155.

650 **Bond, A. M., Ming, G.-L. and Song, H.** (2015). Adult Mammalian Neural Stem Cells and
651 Neurogenesis: Five Decades Later. *Cell Stem Cell* **17**, 385–395.

652 **Bondolfi, L., Ermini, F., Long, J. M., Ingram, D. K. and Jucker, M.** (2004). Impact of age
653 and caloric restriction on neurogenesis in the dentate gyrus of C57BL/6 mice.
654 *Neurobiology of Aging* **25**, 333–340.

655 **Bottes, S., Jaeger, B. N., Pilz, G.-A., Jörg, D. J., Cole, J. D., Kruse, M., Harris, L.,**
656 **Korobeynyk, V. I., Mallona, I., Helmchen, F., et al.** (2021). Long-term self-renewing
657 stem cells in the adult mouse hippocampus identified by intravital imaging. *Nat*
658 *Neurosci* **24**, 225–233.

659 **Bouchard, J. and Villeda, S. A.** (2015). Aging and brain rejuvenation as systemic events.
660 *Journal of Neurochemistry* **132**, 5–19.

Bouchard-Cannon, P., Mendoza-Viveros, L., Yuen, A., Kærn, M. and Cheng, H.-Y. M. (2013). The Circadian Molecular Clock Regulates Adult Hippocampal Neurogenesis by Controlling the Timing of Cell-Cycle Entry and Exit. *Cell Reports* **5**, 961–973.

Brandhorst, S., Choi, I. Y., Wei, M., Cheng, C. W., Sedrakyan, S., Navarrete, G., Dubeau, L., Yap, L. P., Park, R., Vinciguerra, M., et al. (2015). A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. *Cell Metabolism* **22**, 86–99.

Cao, S., Li, M., Sun, Y., Wu, P., Yang, W., Dai, H., Guo, Y., Ye, Y., Wang, Z., Xie, X., et al. (2022). Intermittent fasting enhances hippocampal NPY expression to promote neurogenesis after traumatic brain injury. *Nutrition* **97**, 111621.

Colman, R. J., Anderson, R. M., Johnson, S. C., Kastman, E. K., Kosmatka, K. J., Beasley, T. M., Allison, D. B., Cruzen, C., Simmons, H. A., Kemnitz, J. W., et al. (2009). Caloric Restriction Delays Disease Onset and Mortality in Rhesus Monkeys. *Science* **325**, 201–204.

Colman, R. J., Beasley, T. M., Kemnitz, J. W., Johnson, S. C., Weindruch, R. and Anderson, R. M. (2014). Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun* **5**, 3557.

Criado Perez, C. (2020). *Invisible Women: Exposing data bias in a world designed for men*. Vintage books.

Cryan, J. F. and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* **13**, 701–712.

de Lucia, C., Murphy, T. and Thuret, S. (2017). Emerging Molecular Pathways Governing Dietary Regulation of Neural Stem Cells during Aging. *Frontiers in Physiology* **8**,.

Denoth-Lippuner, A. and Jessberger, S. (2021). Formation and integration of new neurons in the adult hippocampus. *Nat Rev Neurosci* **22**, 223–236.

Dias, G. P., Murphy, T., Stangl, D., Ahmet, S., Morisse, B., Nix, A., Aimone, L. J., Aimone, J. B., Kuro-O, M., Gage, F. H., et al. (2021). Intermittent fasting enhances long-term memory consolidation, adult hippocampal neurogenesis, and expression of longevity gene Klotho. *Mol Psychiatry* 1–15.

Draijer, S., Chaves, I. and Hoekman, M. F. M. (2019). The circadian clock in adult neural stem cell maintenance. *Progress in Neurobiology* **173**, 41–53.

Encinas, J. M., Michurina, T. V., Peunova, N., Park, J.-H., Tordo, J., Peterson, D. A., Fishell, G., Koulakov, A. and Enikolopov, G. (2011). Division-Coupled Astrocytic Differentiation and Age-Related Depletion of Neural Stem Cells in the Adult Hippocampus. *Cell Stem Cell* **8**, 566–579.

Freire, T., Senior, A. M., Perks, R., Pulpitel, T., Clark, X., Brandon, A. E., Wahl, D., Hatchwell, L., Le Couteur, D. G., Cooney, G. J., et al. (2020). Sex-specific metabolic responses to 6 hours of fasting during the active phase in young mice. *J Physiol* **598**, 2081–2092.

Froy, O., Chapnik, N. and Miskin, R. (2009). Effect of intermittent fasting on circadian rhythms in mice depends on feeding time. *Mechanisms of Ageing and Development* **130**, 154–160.

Fusco, S. and Pani, G. (2013). Brain response to calorie restriction. *Cell. Mol. Life Sci.* **70**, 3157–3170.

Goodell, M. A. and Rando, T. A. (2015). Stem cells and healthy aging. *Science* **350**, 1199–1204.

Goodrick, C. L., Ingram, D. K., Reynolds, M. A., Freeman, J. R. and Cider, N. (1990). Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. *Mechanisms of Ageing and Development* **55**, 69–87.

Gregor, A., Fragner, L., Trajanoski, S., Li, W., Sun, X., Weckwerth, W., König, J. and Duszka, K. (2020). Cage bedding modifies metabolic and gut microbiota profiles in mouse studies applying dietary restriction. *Sci Rep* **10**, 20835.

Harris, L., Rigo, P., Stiehl, T., Gaber, Z. B., Austin, S. H. L., Masdeu, M. del M., Edwards, A., Urbán, N., Marciniak-Czochra, A. and Guillemot, F. (2021). Coordinated changes in cellular behavior ensure the lifelong maintenance of the hippocampal stem cell population. *Cell Stem Cell*.

Hodge, R. D., Kowalczyk, T. D., Wolf, S. A., Encinas, J. M., Rippey, C., Enikolopov, G., Kempermann, G. and Hevner, R. F. (2008). Intermediate Progenitors in Adult Hippocampal Neurogenesis: Tbr2 Expression and Coordinate Regulation of Neuronal Output. *J. Neurosci.* **28**, 3707–3717.

Hofer, S. J., Carmona-Gutierrez, D., Mueller, M. I. and Madeo, F. (2022). The ups and downs of caloric restriction and fasting: from molecular effects to clinical application. *EMBO Mol Med* **14**, e14418.

Holmes, M. M., Galea, L. A. M., Mistlberger, R. E. and Kempermann, G. (2004). Adult hippocampal neurogenesis and voluntary running activity: Circadian and dose-dependent effects. *Journal of Neuroscience Research* **76**, 216–222.

Hornsby, A. K. E., Redhead, Y. T., Rees, D. J., Ratcliff, M. S. G., Reichenbach, A., Wells, T., Francis, L., Amstalden, K., Andrews, Z. B. and Davies, J. S. (2016). Short-term calorie restriction enhances adult hippocampal neurogenesis and remote fear memory in a Ghsr-dependent manner. *Psychoneuroendocrinology* **63**, 198–207.

Hwang, I. K., Kim, I. Y., Kim, D. W., Yoo, K.-Y., Kim, Y. N., Yi, S. S., Won, M.-H., Lee, I. S., Yoon, Y. S. and Seong, J. K. (2008). Strain-specific differences in cell proliferation and differentiation in the dentate gyrus of C57BL/6N and C3H/HeN mice fed a high fat diet. *Brain Res* **1241**, 1–6.

Kaptan, Z., Akgün-Dar, K., Kapucu, A., Dedeakayoğulları, H., Batu, Ş. and Üzüm, G. (2015). Long term consequences on spatial learning-memory of low-calorie diet during adolescence in female rats; hippocampal and prefrontal cortex BDNF level, expression of NeuN and cell proliferation in dentate gyrus. *Brain Research* **1618**, 194–204.

Katsimpardi, L. and Lledo, P.-M. (2018). Regulation of neurogenesis in the adult and aging brain. *Current Opinion in Neurobiology* **53**, 131–138.

Kempermann, G., Chesler, E. J., Lu, L., Williams, R. W. and Gage, F. H. (2006). Natural variation and genetic covariance in adult hippocampal neurogenesis. *Proc Natl Acad Sci U S A* **103**, 780–785.

744 **Kim, Y., Kim, S., Kim, C., Sato, T., Kojima, M. and Park, S.** (2015). Ghrelin is required for
745 dietary restriction-induced enhancement of hippocampal neurogenesis: lessons from
746 ghrelin knockout mice. *Endocrine Journal* **62**, 269–275.

747 **Kim, J. W., Nam, S. M., Yoo, D. Y., Jung, H. Y., Kim, I. Y., Hwang, I. K., Seong, J. K. and**
748 **Yoon, Y. S.** (2017). Comparison of Adult Hippocampal Neurogenesis and
749 Susceptibility to Treadmill Exercise in Nine Mouse Strains. *Neural Plasticity* **2017**,
750 e5863258.

751 **Kitamura, T., Mishina, M. and Sugiyama, H.** (2006). Dietary restriction increases
752 hippocampal neurogenesis by molecular mechanisms independent of NMDA
753 receptors. *Neuroscience Letters* **393**, 94–96.

754 **Koehl, M.** (2015). Gene-environment interaction in programming hippocampal plasticity: focus
755 on adult neurogenesis. *Frontiers in Molecular Neuroscience* **8**,.

756 **Kolodziejczyk, A. A., Zheng, D. and Elinav, E.** (2019). Diet–microbiota interactions and
757 personalized nutrition. *Nat Rev Microbiol* **17**, 742–753.

758 **Kronenberg, G., Reuter, K., Steiner, B., Brandt, M. D., Jessberger, S., Yamaguchi, M.**
759 **and Kempermann, G.** (2003). Subpopulations of proliferating cells of the adult
760 hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol*
761 **467**, 455–463.

762 **Kuhn, H. G., Toda, T. and Gage, F. H.** (2018). Adult Hippocampal Neurogenesis: A Coming-
763 of-Age Story. *J. Neurosci.* **38**, 10401–10410.

764 **Kumar, S., Parkash, J., Kataria, H. and Kaur, G.** (2009). Interactive effect of excitotoxic
765 injury and dietary restriction on neurogenesis and neurotrophic factors in adult male
766 rat brain. *Neuroscience Research* **65**, 367–374.

767 **Landry, T. and Huang, H.** (2021). Mini review: The relationship between energy status and
768 adult hippocampal neurogenesis. *Neuroscience Letters* **765**, 136261.

769 **Lee, J., Duan, W., Long, J. M., Ingram, D. K. and Mattson, M. P.** (2000). Dietary restriction
770 increases the number of newly generated neural cells, and induces BDNF expression,
771 in the dentate gyrus of rats. *J Mol Neurosci* **15**, 99–108.

772 **Lee, J., Seroogy, K. B. and Mattson, M. P.** (2002a). Dietary restriction enhances
773 neurotrophin expression and neurogenesis in the hippocampus of adult mice. *Journal*
774 *of Neurochemistry* **80**, 539–547.

775 **Lee, J., Duan, W. and Mattson, M. P.** (2002b). Evidence that brain-derived neurotrophic
776 factor is required for basal neurogenesis and mediates, in part, the enhancement of
777 neurogenesis by dietary restriction in the hippocampus of adult mice. *Journal of*
778 *Neurochemistry* **82**, 1367–1375.

779 **Levenson, C. W. and Rich, N. J.** (2007). Eat Less, Live Longer? New Insights into the Role
780 of Caloric Restriction in the Brain. *Nutrition Reviews* **65**, 412–415.

781 **Li, L. and Clevers, H.** (2010). Coexistence of quiescent and active adult stem cells in
782 mammals. *Science* **327**, 542–545.

783 **Li, G., Xie, C., Lu, S., Nichols, R. G., Tian, Y., Li, L., Patel, D., Ma, Y., Brocker, C. N., Yan,**
784 **T., et al.** (2017). Intermittent Fasting Promotes White Adipose Browning and
785 Decreases Obesity by Shaping the Gut Microbiota. *Cell Metabolism* **26**, 672-685.e4.

786 **Li, W., Wu, M., Zhang, Y., Wei, X., Zang, J., Liu, Y., Wang, Y., Gong, C.-X. and Wei, W.**
787 (2020). Intermittent fasting promotes adult hippocampal neuronal differentiation by
788 activating GSK-3β in 3xTg-AD mice. *Journal of Neurochemistry* **155**, 697–713.

789 **Liu, B., Page, A. J., Hutchison, A. T., Wittert, G. A. and Heilbronn, L. K.** (2019). Intermittent
790 fasting increases energy expenditure and promotes adipose tissue browning in mice.
791 *Nutrition* **66**, 38–43.

792 **Longo, V. D. and Panda, S.** (2016). Fasting, Circadian Rhythms, and Time-Restricted
793 Feeding in Healthy Lifespan. *Cell Metabolism* **23**, 1048–1059.

794 **López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. and Kroemer, G.** (2013). The
795 Hallmarks of Aging. *Cell* **153**, 1194–1217.

796 **Madeo, F., Carmona-Gutierrez, D., Hofer, S. J. and Kroemer, G.** (2019). Caloric Restriction
797 Mimetics against Age-Associated Disease: Targets, Mechanisms, and Therapeutic
798 Potential. *Cell Metabolism* **29**, 592–610.

799 **Mattison, J. A., Roth, G. S., Beasley, T. M., Tilmont, E. M., Handy, A. M., Herbert, R. L.,**
800 **Longo, D. L., Allison, D. B., Young, J. E., Bryant, M., et al.** (2012). Impact of caloric
801 restriction on health and survival in rhesus monkeys from the NIA study. *Nature* **489**,
802 318–321.

803 **Mattison, J. A., Colman, R. J., Beasley, T. M., Allison, D. B., Kemnitz, J. W., Roth, G. S.,**
804 **Ingram, D. K., Weindruch, R., de Cabo, R. and Anderson, R. M.** (2017). Caloric
805 restriction improves health and survival of rhesus monkeys. *Nat Commun* **8**, 14063.

806 **Mattson, M. P.** (2003). Gene–Diet Interactions in Brain Aging and Neurodegenerative
807 Disorders. *Ann Intern Med* **139**, 441–444.

808 **Mitchell, S. J., Matute, J. M., Scheibye-Knudsen, M., Fang, E., Aon, M., González-Reyes,**
809 **J. A., Cortassa, S., Kaushik, S., Gonzalez-Freire, M., Patel, B., et al.** (2016). Effects
810 of Sex, Strain, and Energy Intake on Hallmarks of Aging in Mice. *Cell Metab* **23**, 1093–
811 1112.

812 **Mori, T., Tanaka, K., Buffo, A., Wurst, W., Kühn, R. and Götz, M.** (2006). Inducible gene
813 deletion in astroglia and radial glia—A valuable tool for functional and lineage analysis.
814 *Glia* **54**, 21–34.

815 **Murphy, T., Dias, G. P. and Thuret, S.** (2014). Effects of Diet on Brain Plasticity in Animal
816 and Human Studies: Mind the Gap. *Neural Plasticity* **2014**, e563160.

817 **Novak, J. S. S., Baksh, S. C. and Fuchs, E.** (2021). Dietary interventions as regulators of
818 stem cell behavior in homeostasis and disease. *Genes Dev.* **35**, 199–211.

819 **Pani, G.** (2015). Neuroprotective effects of dietary restriction: Evidence and mechanisms.
820 *Seminars in Cell & Developmental Biology* **40**, 106–114.

821 **Park, H. R. and Lee, J.** (2011). Neurogenic contributions made by dietary regulation to
822 hippocampal neurogenesis. *Annals of the New York Academy of Sciences* **1229**, 23–
823 28.

824 **Park, J.-H., Glass, Z., Sayed, K., Michurina, T. V., Lazutkin, A., Mineyeva, O., Velmeshev,**
825 **D., Ward, W. F., Richardson, A. and Enikolopov, G.** (2013). Calorie restriction
826 alleviates the age-related decrease in neural progenitor cell division in the aging brain.
827 *European Journal of Neuroscience* **37**, 1987–1993.

828 **Pilz, G.-A., Bottes, S., Betizeau, M., Jörg, D. J., Carta, S., Simons, B. D., Helmchen, F.**
829 **and Jessberger, S.** (2018). Live imaging of neurogenesis in the adult mouse
830 hippocampus. *Science* **359**, 658–662.

831 **Poulose, S. M., Miller, M. G., Scott, T. and Shukitt-Hale, B.** (2017). Nutritional Factors
832 Affecting Adult Neurogenesis and Cognitive Function. *Adv Nutr* **8**, 804–811.

833 **Ribeiro, M. F., Santos, A. A., Afonso, M. B., Rodrigues, P. M., Sá Santos, S., Castro, R.**
834 **E., Rodrigues, C. M. P. and Solá, S.** (2020). Diet-dependent gut microbiota impacts
835 on adult neurogenesis through mitochondrial stress modulation. *Brain Commun* **2**,.

836 **Ricquier, D.** (2011). Uncoupling Protein 1 of Brown Adipocytes, the Only Uncoupler: A
837 Historical Perspective. *Frontiers in Endocrinology* **2**,.

838 **Sarkar, A., Jin, Y., Anbarchian, T., Yang, Y., Nguyen, H., Rulifson, E., Fish, M., Logan, C.**
839 **Y., Kaye, A., Wu, P., et al.** (2021). Intermittent fasting induces rapid hepatocyte
840 proliferation. 2021.10.16.464650.

841 **Schouten, M., Bielefeld, P., Garcia-Corzo, L., Passchier, E. M. J., Gradari, S., Jungenitz,**
842 **T., Pons-Espinal, M., Gebara, E., Martín-Suárez, S., Lucassen, P. J., et al.** (2020).
843 Circadian glucocorticoid oscillations preserve a population of adult hippocampal neural
844 stem cells in the aging brain. *Mol Psychiatry* **25**, 1382–1405.

845 **Schultz, M. B. and Sinclair, D. A.** (2016). When stem cells grow old: phenotypes and
846 mechanisms of stem cell aging. *Development* **143**, 3–14.

847 **Siersbæk, M. S., Ditzel, N., Hejbøl, E. K., Præstholm, S. M., Markussen, L. K., Avolio, F.,**
848 **Li, L., Lehtonen, L., Hansen, A. K., Schrøder, H. D., et al.** (2020). C57BL/6J
849 substrain differences in response to high-fat diet intervention. *Sci Rep* **10**, 14052.

850 **Smith, B. M., Saulsbery, A. I., Sarchet, P., Devasthali, N., Einstein, D. and Kirby, E. D.**
851 (2022). Oral and Injected Tamoxifen Alter Adult Hippocampal Neurogenesis in Female
852 and Male Mice. *eNeuro* **9**,.

853 **Snyder, J. S., Soumier, A., Brewer, M., Pickel, J. and Cameron, H. A.** (2011). Adult
854 hippocampal neurogenesis buffers stress responses and depressive behaviour.
855 *Nature* **476**, 458–461.

856 **Srinivas, S., Watanabe, T., Lin, C.-S., William, C. M., Tanabe, Y., Jessell, T. M. and**
857 **Costantini, F.** (2001). Cre reporter strains produced by targeted insertion of EYFP and
858 ECFP into the ROSA26 locus. *BMC Developmental Biology* **1**, 4.

859 **Staples, M. C., Fannon, M. J., Mysore, K. K., Dutta, R. R., Ongjoco, A. T., Quach, L. W.,**
860 **Kharidia, K. M., Somkuwar, S. S. and Mandyam, C. D.** (2017). Dietary restriction
861 reduces hippocampal neurogenesis and granule cell neuron density without affecting
862 the density of mossy fibers. *Brain Research* **1663**, 59–65.

863 **Toda, T., Parylak, S. L., Linker, S. B. and Gage, F. H.** (2019). The role of adult hippocampal
864 neurogenesis in brain health and disease. *Mol. Psychiatry* **24**, 67–87.

865 **Tümpel, S. and Rudolph, K. L.** (2019). Quiescence: Good and Bad of Stem Cell Aging.
866 *Trends in Cell Biology* **29**, 672–685.

867 **Urbán, N.** (2022). Could a Different View of Quiescence Help Us Understand How
868 Neurogenesis Is Regulated? *Frontiers in Neuroscience* **16**,.

869 **Urbán, N., van den Berg, D. L. C., Forget, A., Andersen, J., Demmers, J. A. A., Hunt, C.,**
870 **Ayrault, O. and Guillemot, F.** (2016). Return to quiescence of mouse neural stem
871 cells by degradation of a proactivation protein. *Science* **353**, 292–295.

872 **Urbán, N., Blomfield, I. M. and Guillemot, F.** (2019). Quiescence of Adult Mammalian Neural
873 Stem Cells: A Highly Regulated Rest. *Neuron* **104**, 834–848.

874 **Valero, J., Paris, I. and Sierra, A.** (2016). Lifestyle Shapes the Dialogue between
875 Environment, Microglia, and Adult Neurogenesis. *ACS Chem. Neurosci.* **7**, 442–453.

876 **Van Cauwenberghe, C., Vandendriessche, C., Libert, C. and Vandenbroucke, R. E.**
877 (2016). Caloric restriction: beneficial effects on brain aging and Alzheimer's disease.
878 *Mamm Genome* **27**, 300–319.

879 **Wahl, D., Cogger, V. C., Solon-Biet, S. M., Waern, R. V. R., Gokarn, R., Pulpitel, T., Cabo,**
880 **R. de, Mattson, M. P., Raubenheimer, D., Simpson, S. J., et al.** (2016). Nutritional
881 strategies to optimise cognitive function in the aging brain. *Ageing Research Reviews*
882 **31**, 80–92.

883 **Wei, G. Z., Martin, K. A., Xing, P. Y., Agrawal, R., Whiley, L., Wood, T. K., Hejndorf, S.,**
884 **Ng, Y. Z., Low, J. Z. Y., Rossant, J., et al.** (2021). Tryptophan-metabolizing gut
885 microbes regulate adult neurogenesis via the aryl hydrocarbon receptor. *Proceedings*
886 *of the National Academy of Sciences* **118**, e2021091118.

887 **Wiget, F., van Dijk, R. M., Louet, E. R., Slomianka, L. and Amrein, I.** (2017). Effects of
888 Strain and Species on the Septo-Temporal Distribution of Adult Neurogenesis in
889 Rodents. *Frontiers in Neuroscience* **11**,.

890 **Wu, P., Shen, Q., Dong, S., Xu, Z., Tsien, J. Z. and Hu, Y.** (2008). Calorie restriction
891 ameliorates neurodegenerative phenotypes in forebrain-specific presenilin-1 and
892 presenilin-2 double knockout mice. *Neurobiology of Aging* **29**, 1502–1511.

893 **Yagi, S. and Galea, L. A. M.** (2019). Sex differences in hippocampal cognition and
894 neurogenesis. *Neuropsychopharmacology* **44**, 200–213.

895 **Zainuddin, M. S. A. and Thuret, S.** (2012). Nutrition, adult hippocampal neurogenesis and
896 mental health. *Br Med Bull* **103**, 89–114.

897

Figures and Figure Legends

Figure 1

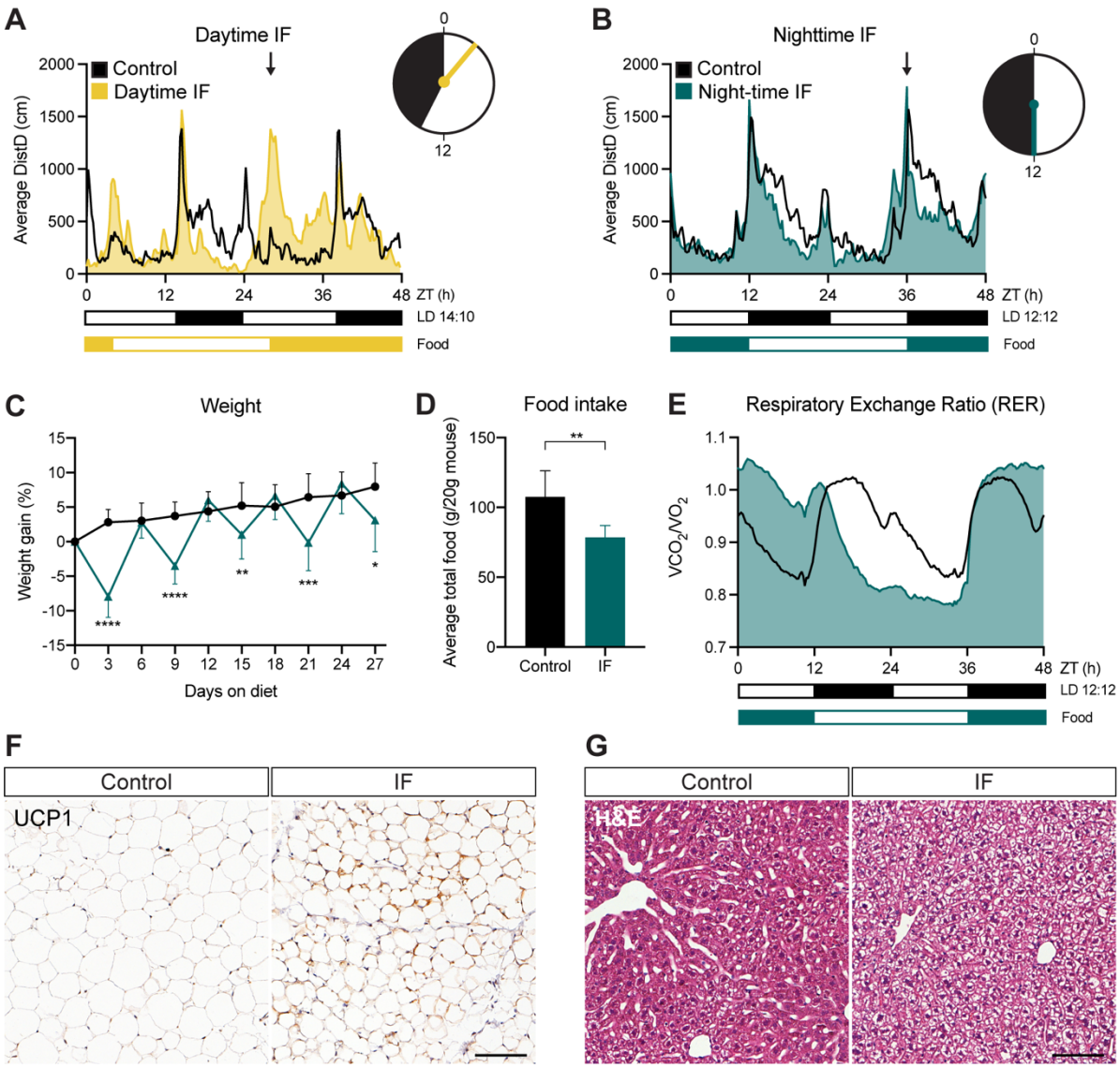
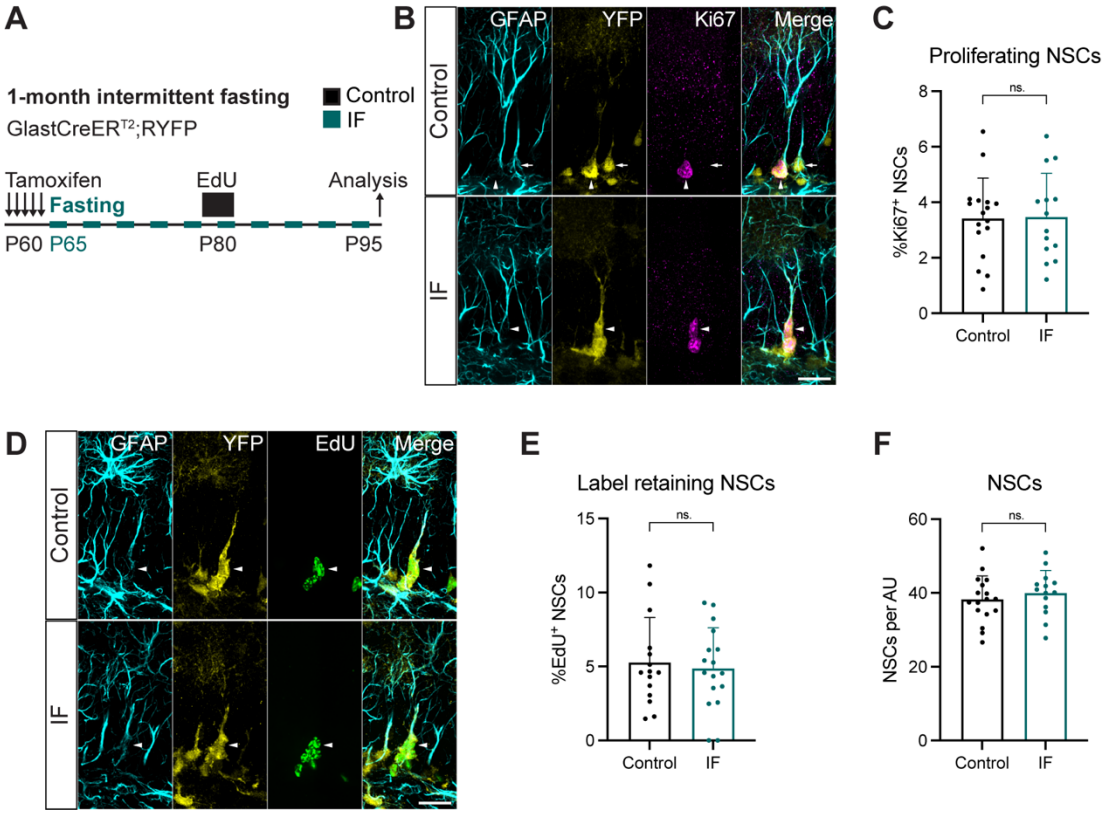


Figure 1. Night-time IF induces systemic features of IF without disrupting the circadian activity pattern of mice. (**A**, **B**) Mouse locomotor activity in control (ad libitum, black, n = 3 in daytime IF and n = 6 in night-time IF), daytime IF (**A**, yellow, n = 4), and night-time IF (**B**, dark blue, n = 6) mice. Activity is displayed as average distance covered by mice in 48 h cycles throughout a 1-month intervention. Black and white boxes under the graphs indicate respectively the dark and light phases of the light:dark (LD) cycle with zeitgeber time (ZT) 0 being the time of lights ON. Yellow/blue and white boxes indicate the presence and absence of food respectively. Arrows indicate the time of food availability onset. The clocks represent 24-h cycles in ZT with light and dark phases filled in white and black respectively. Clock hands indicate time of food change: ZT3 for daytime IF and ZT12 for night-time IF. Daytime IF introduces abnormal peaks of activity during the day disrupting the circadian rhythmicity of mouse activity while night-time IF preserves their normal activity pattern. (**C**) Mouse weight every 3 days shown as the

913 percentage of weight difference to the first day of the diet. Days 3, 9, 15, 21 and 27 show
914 weight on fasting days, and days 6, 12, 18 and 24 on feeding days. Weight oscillates in feeding
915 and fasting days. $n_{Control}$ = 17. n_{IF} = 18. (**D**) Average total food consumed for a month shown
916 as g of food per 20 g of mouse weight (weight reference of mice at the beginning of the diet).
917 IF induces a mild (70%) caloric restriction. $n_{Control}$ = 17. n_{IF} = 18. (**E**) Respiratory Exchange
918 Ratio (RER) as a comparison of CO_2 and O_2 volumes. RER values close to 1 indicate
919 predominant carbohydrate metabolism as energy fuel, while RER values close to 0,7 indicate
920 a shift towards lipid oxidation. RER is displayed as average of 48 h cycles throughout a 1-
921 month intervention. IF induces a longer shift towards lipid oxidation. n = 6. (**F**) Representative
922 images of UCP1-stained inguinal adipose tissue after 3 months of IF. Adipocytes of IF mice
923 look smaller and show a mild increase in UCP1. (**G**) Representative images of liver tissue
924 stained with H&E after 3 months of IF. IF induces hepatocyte swelling. See (**F**) and (**G**) images
925 of all animals in **Figure S2**. Bars and error bars represent mean + SD. Significance values: *,
926 p>0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001. Scale bar: 100µm.

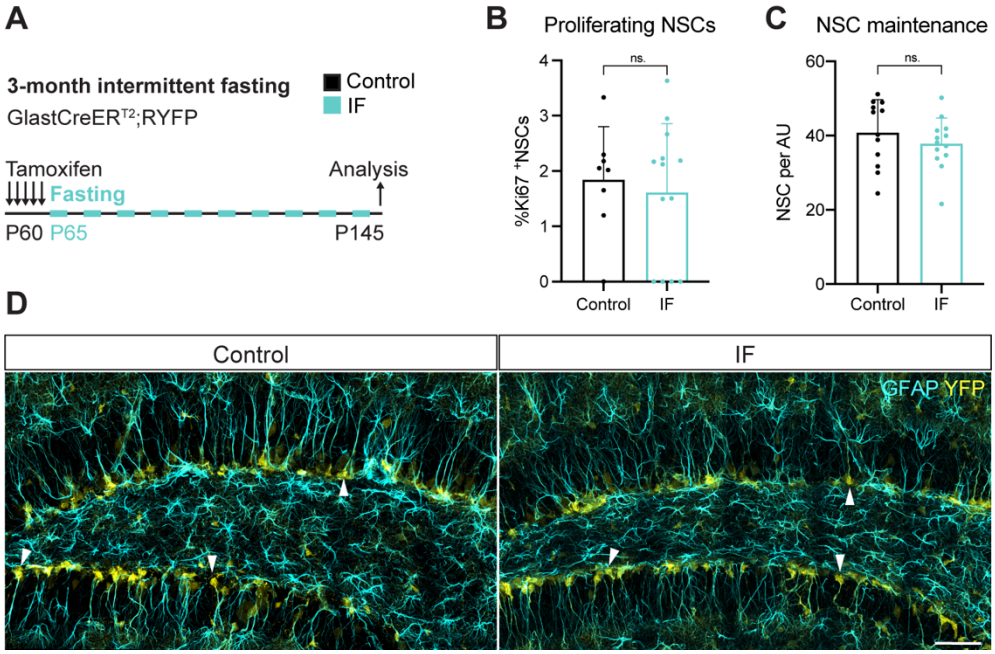
927 **Figure 2**



928

929 **Figure 2.** 1 month of IF does not affect NSC proliferation, quiescence/activation transitions or
930 maintenance. (**A**) 2-month-old GlastCreER^{T2};RYFP mice were administered tamoxifen on 5
931 consecutive days to fluorescently (YFP) label NSCs, after which, they were subjected to every-
932 other-day IF for 1 month. EdU was administered in the drinking water for 5 days, 15 days
933 before the analysis. (**B**, **C**) Proliferating NSCs in control and IF mice, and quantification of the
934 percentage of proliferating NSCs. NSCs were identified by their localisation in the SGZ, the
935 presence of a single GFAP$^+$ vertical projection and the help of YFP. Nuclear colocalisation
936 with Ki67 was used to distinguish proliferating (Ki67$^+$, arrowheads) from quiescent (Ki67$^-$,
937 arrow) NSCs. The percentage of proliferating NSCs was unchanged by IF. (**D**, **E**) EdU
938 retaining NSCs in control and IF mice and quantification of their percentage. Arrowheads
939 indicate EdU$^+$ NSCs. IF did not affect the percentage of label retaining NSCs. (**F**) Total number
940 of NSCs normalised to DG length. The number of NSCs is unaffected by IF. Bars and error
941 bars represent mean + SD; dots represent individual mice. Significance values: ns, p>0.05.
942 Scale bar: 20µm. AU: arbitrary unit. Black: control (ad libitum), dark blue: IF.

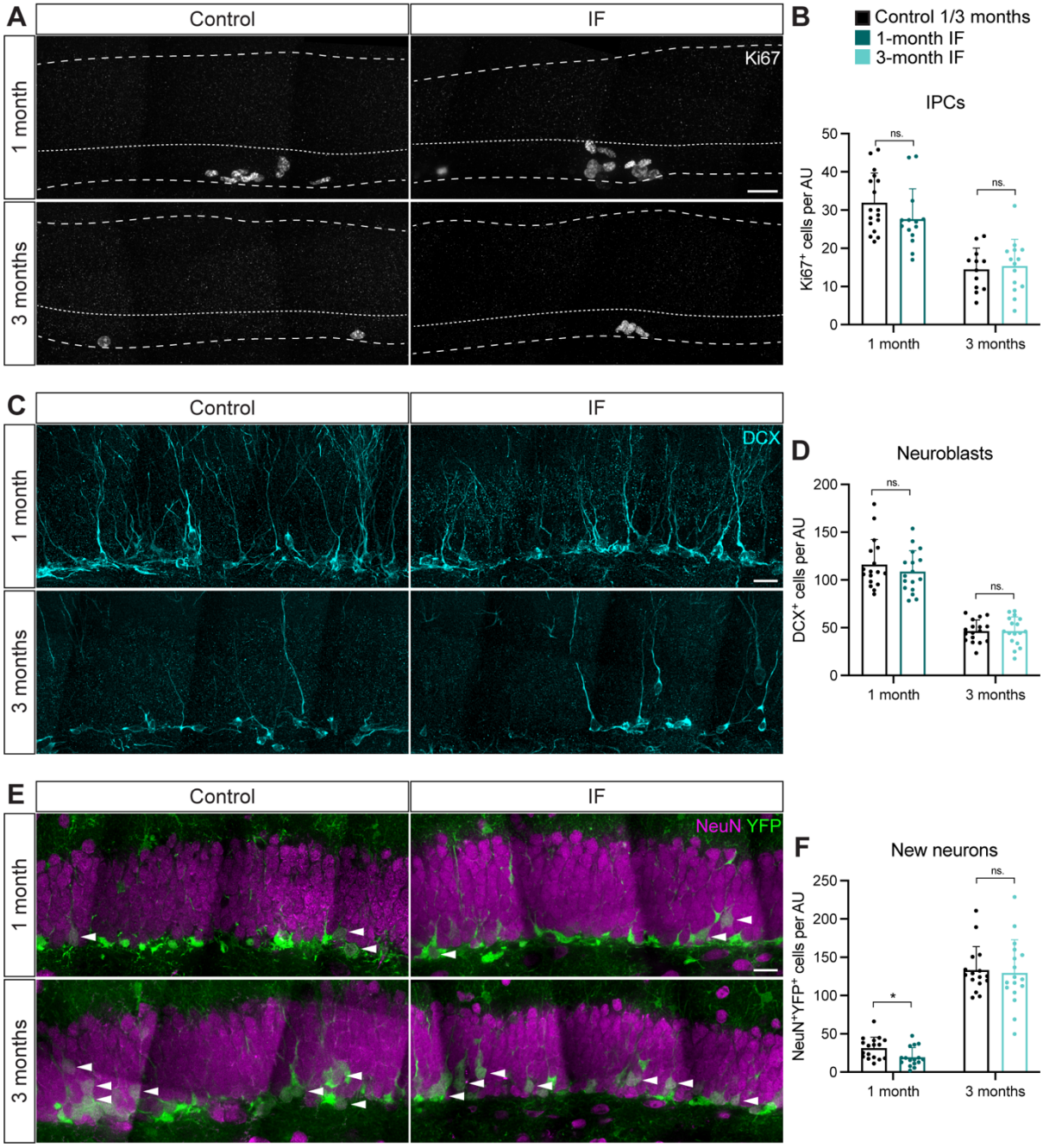
943 **Figure 3**



944

945 **Figure 3.** 3 months of IF do not affect NSC proliferation nor maintenance. (**A**) 2-month-old
946 GlastCreER^{T2};RYFP mice were administered tamoxifen on 5 consecutive days to fluorescently
947 (YFP) label NSCs, after which, they were subjected to every-other-day IF for 3 months. (**B**, **C**)
948 Quantification of the percentage of proliferating NSCs and the total number of NSCs in control
949 and IF mice normalised to DG length. (**D**) NSCs identified by their localisation in the SGZ, the
950 presence of a single GFAP^{+} vertical projection, and the help of YFP. Arrowheads show several
951 NSCs. IF mice show similar levels of proliferating and total NSCs to control mice. Bars and
952 error bars represent mean + SD; dots represent individual mice. Significance values: ns,
953 p>0.05. Scale bar: 50μm. AU: arbitrary unit. Black: control (ad libitum), light blue: IF.

954 **Figure 4**



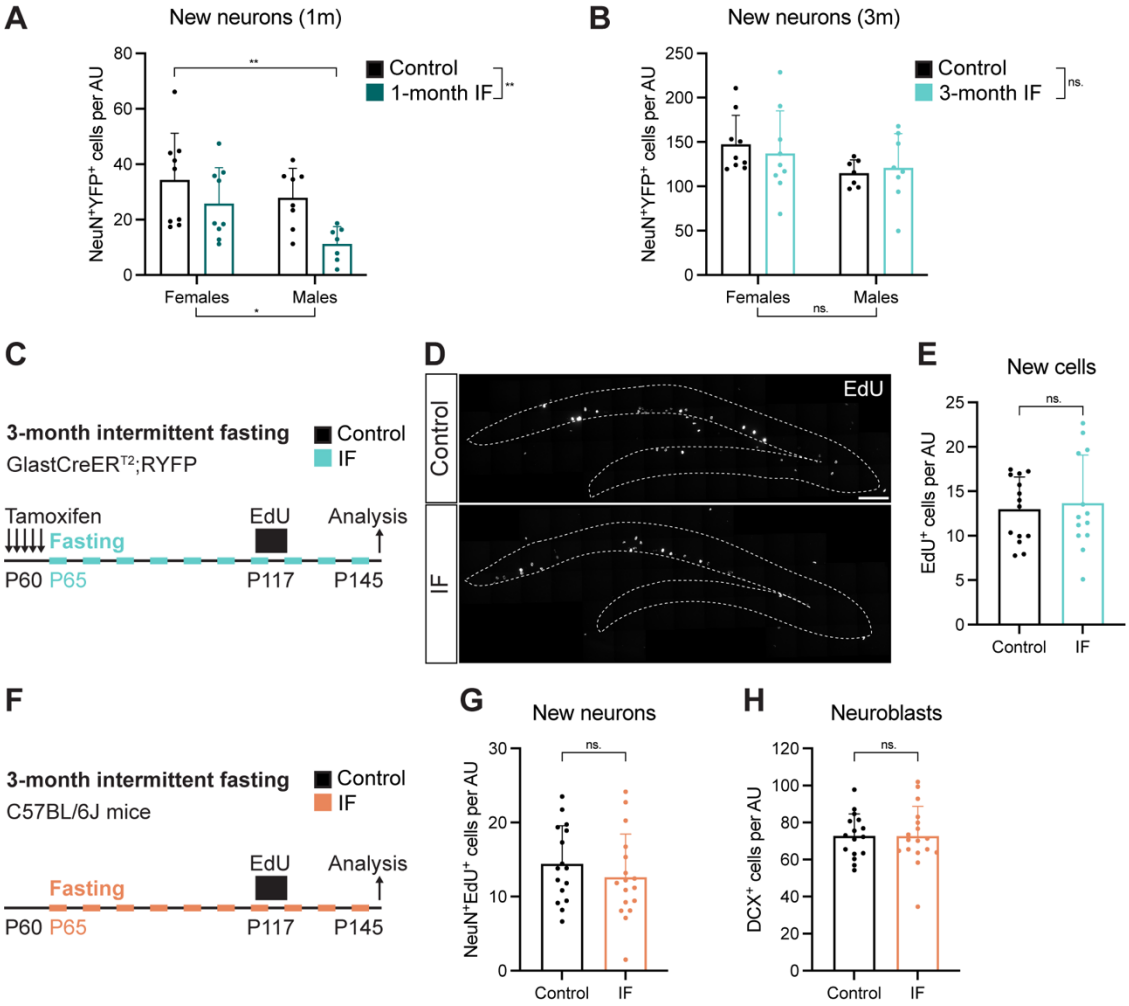
955

956 **Figure 4.** IF does not affect IPCs nor neuroblasts and only mildly and temporarily decreases
957 the neuronal output. (**A**, **B**) Ki67^{+} cells in control and IF mice after a 1- or 3-month intervention
958 as a proxy for IPCs and quantification of IPCs normalised to DG length. Dashed and dotted
959 lines indicate DG area and border between the SGZ and the molecular layer respectively.
960 IPCs were unchanged by IF. (**C**, **D**) Neuroblasts identified by the marker for immature neurons
961 DCX and quantification. IF did not affect the number of neuroblasts. (**E**, **F**) Newly born neurons
962 (arrowheads) identified by the colocalization of the mature neuronal marker NeuN and the
963 YFP reporter, indicating that these neurons were generated from the population of NSCs
964 labelled with YFP at the beginning of the diet. Quantification of newly born neurons normalised

965 to DG length. The number of new neurons transiently decreased upon 1 month of IF, but was
966 unchanged after 3 months. Bars and error bars represent mean + SD; dots represent individual
967 mice. Significance values: ns, $p>0.05$; *, $p<0.05$. Scale bar: 20μm. AU: arbitrary unit. Black:
968 control (ad libitum) for each experiment, dark blue: 1-month IF, light blyue: 3-month IF.

969

970 **Figure 5**



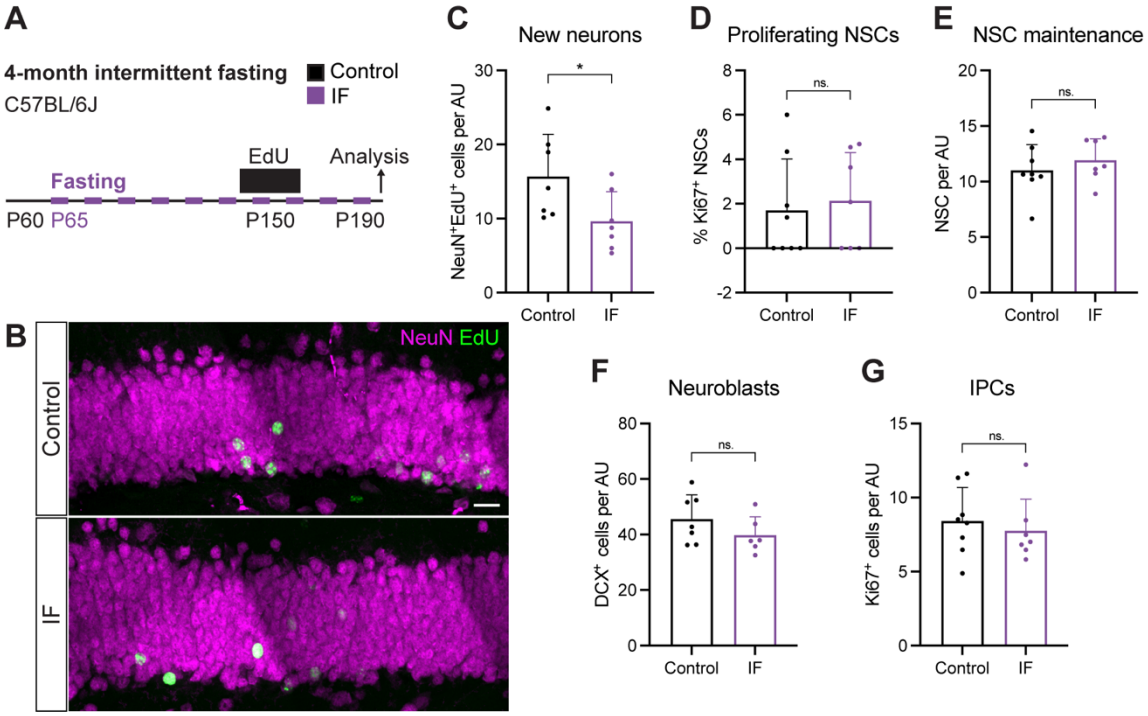
971

972 **Figure 5.** Sex, labelling method, tamoxifen or strain do not alter the neurogenic response to
973 IF. (**A**, **B**) Quantification of newly born neurons after 1 (**A**) or 3 (**B**) months of IF segregated
974 by sex. Significance signs outside the graph indicate result of a two-way ANOVA and sign in
975 the graph indicates result of a Tuckey's multiple comparison test. See all sex-segregated data
976 in **Figure S5**. Sex differences were found only in the number of new neurons at 1 month. (**C**)
977 Survival assay with a thymidine analogue to replicate the labelling method of previous
978 publications. EdU was administered for 4 days to GlastCreER^{T2};RYFP mice (see experiment
979 in **Figure 3**) that had undergone 2 months of IF to label a cohort of proliferating cells that was
980 analysed after a 1 month chase, giving the cells time to progress in the neurogenic lineage.
981 (**D**, **E**) New cells in the DG (enclosed in dashed lines) shown by EdU-labelled cells 1 month
982 after EdU administration. The number of new cells in the DG was unchanged by IF. (**F**)
983 Evaluation of tamoxifen and strain impact in the effects of IF on adult neurogenesis. 2-month-
984 old C57BL/6J mice were subjected to 3 months of IF. A survival assay with a thymidine
985 analogue 1 month before the end of the diet was included. (**G**) Quantification of newly born
986 neurons identified by colocalization of EdU and the marker for mature neurons NeuN. (**H**)
987 Quantification of new neuroblasts. IF did not increase the number of new neurons or
988 neuroblasts in C57BL/6J mice. Bars and error bars represent mean + SD; dots represent
989 individual mice. Significance values: ns, $p>0.05$; *, $p<0.05$; **, $p<0.01$. Scale bar: 100µm. AU:

990 arbitrary unit. Black: control (ad libitum) for each experiment, light blue: 3-month IF, orange:
991 3-month IF C57BL/6J.

992

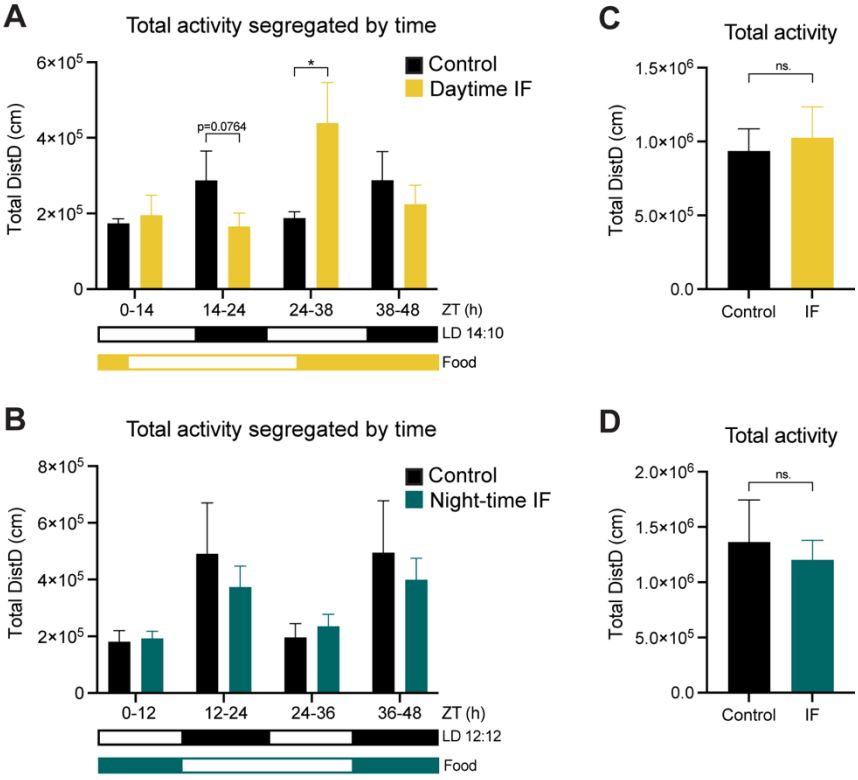
993 **Figure 6**



994

995 **Figure 6.** 4 months of every-other-day IF do not increase adult neurogenesis. (**A**) 2-month-
996 old male C57BL/6J mice were subjected to 4 months of IF, as in previous studies that reported
997 increases in adult neurogenesis. A survival assay with a thymidine analogue (12-day labelling
998 – 1 month chase) was used to evaluate neurogenesis. (**B**, **C**) Image and quantification of
999 newly born neurons identified by colocalization of EdU and the marker for mature neurons
1000 NeuN. 4 months of IF induce a decrease in EdU-labelled neurons. (**D**) Quantification of
1001 proliferating NSCs. (**E**, **F**, **G**) Quantification the total number of NSCs, neuroblasts and IPCs
1002 normalised to DG length. The neurogenic lineage is not affected by 4 months of IF. Bars and
1003 error bars represent mean + SD; dots represent individual mice. Significance values: ns,
1004 p>0.05; *, p<0.05. Scale bar: 20µm. AU: arbitrary unit. Black: control (ad libitum), purple: IF 4-
1005 months C57BL/6J.

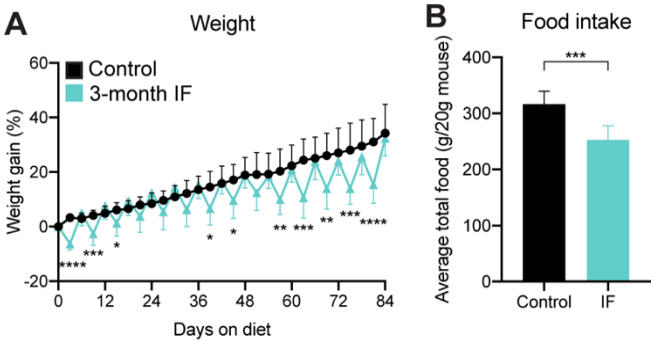
1006 **Supplementary figure 1**



1007

1008 **Figure S1.** Total activity is not affected by day- nor night-time IF. (**A**, **B**) Total mouse locomotor
1009 activity displayed as sum of activity during 1 month of IF segregated by time of the day
1010 (grouped according to the light phase). Black and white boxes under the graphs indicate
1011 respectively the dark and light phases of the light:dark (LD) cycle with zeitgeber time (ZT) 0
1012 being the time of lights ON. Yellow/blue and white boxes indicate the presence and absence
1013 of food respectively. Expected differences between light and dark phases were observed (not
1014 displayed in graph). Daytime IF increased locomotor activity during the feeding day while
1015 night-time IF preserved the activity pattern. (**C**, **D**) Total mouse locomotor activity during 1
1016 month of diet shows no difference between IF an control mice regardless of the time of food
1017 administration. Black: control (ad libitum, n = 3 in daytime IF and n = 6 in night-time IF), yellow:
1018 daytime IF (n = 4), dark blue: night-time IF (n = 6). Bars and error bars represent mean + SD.
1019 Significance values: ns, $p>0.05$; ***, $p<0.001$.

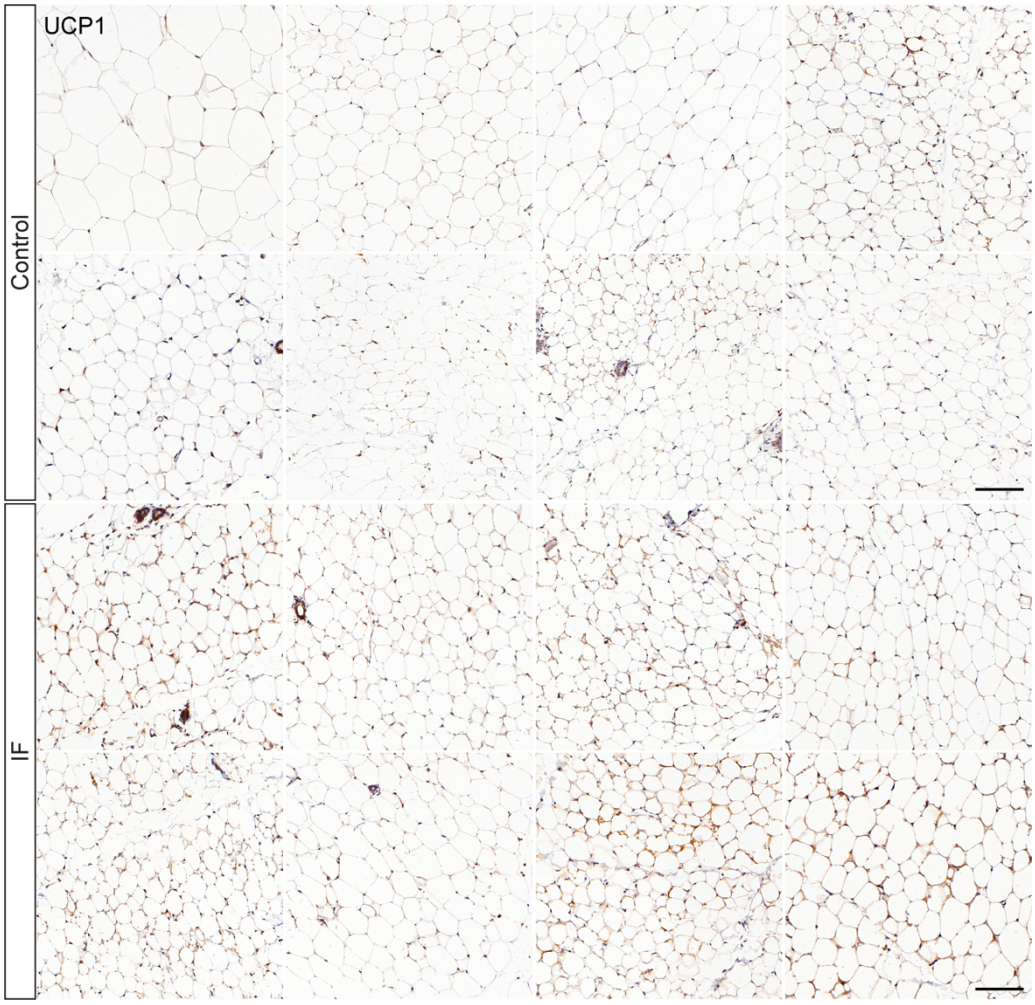
1020 **Supplementary figure 2**



1021

1022 **Figure S2.** Mouse weight and food intake of mice during 3 months of IF. (**A**) Mouse weight
1023 shown as the percentage of weight difference to the first day of the diet. Weight oscillations
1024 persist throughout the 3 months of IF and there is no weight loss on refeeding days compared
1025 to control mice. n = 17. (**B**) Average total food consumed for 3 months shown as g of food per
1026 20g of mouse weight (weight reference of mice at P65). n = 17. Significance values: no symbol,
1027 p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001. Black: control (ad libitum), light
1028 blue: 3-month IF.

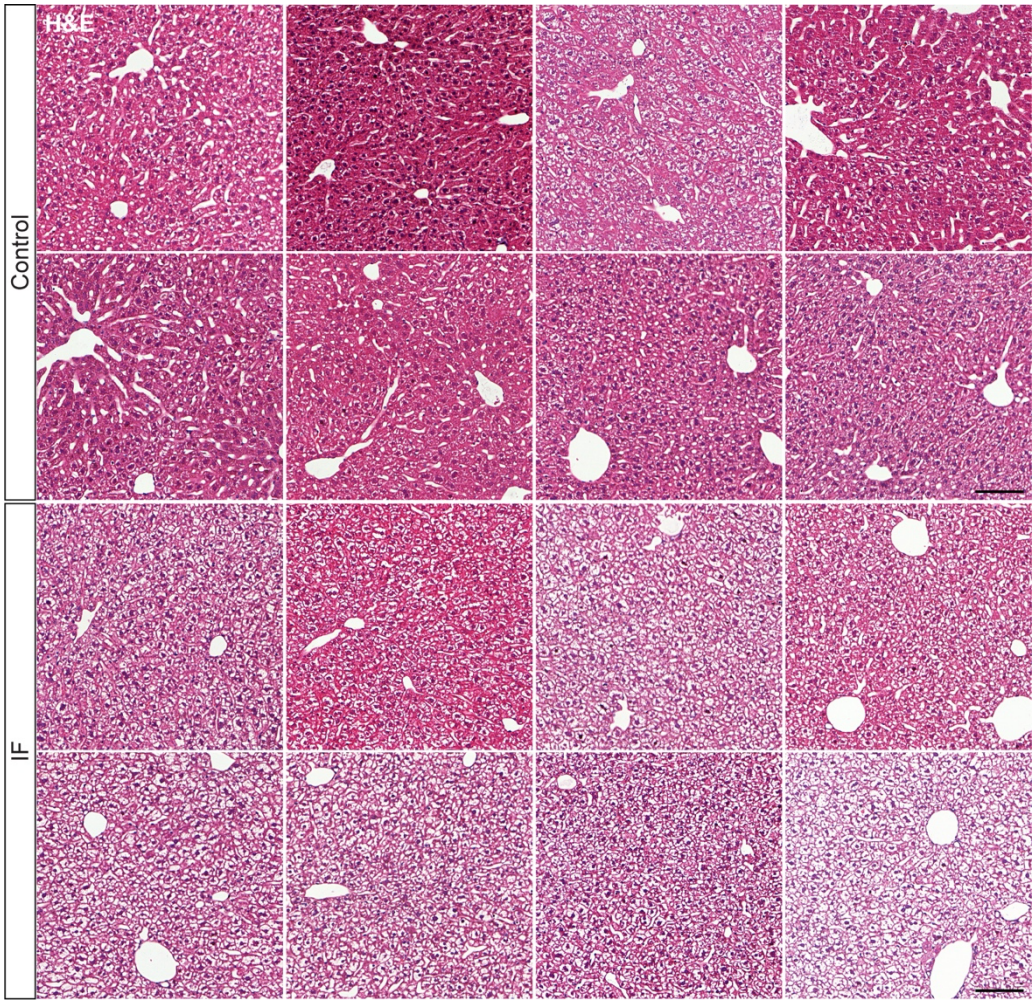
1029 **Supplementary figure 3**



1030

1031 **Figure S3.** Night-time IF promotes mild and variable browning of inguinal adipose tissue.
1032 Images of inguinal adipose tissue stained for uncoupling protein 1 (UCP1) of control and mice
1033 that underwent 3 months of IF. 8 mice per condition were randomly chosen from the
1034 experiment shown in **Figure 3** for immunohistochemical evaluation. Each image corresponds
1035 to an individual mouse. Scale bar: 100µm.

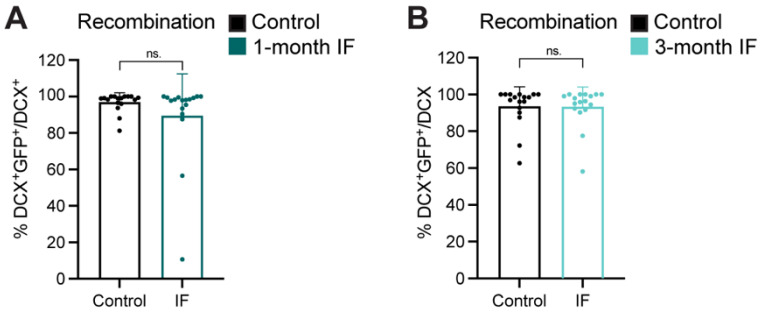
1036 **Supplementary figure 4**



1037

1038 **Figure S4.** Hepatocytes display edematous morphology upon night-time IF. Images of H&E-
1039 stained liver of control and mice that underwent 3 months of IF. 8 mice per condition were
1040 randomly chosen from the experiment shown in **Figure 3** for immunohistochemical evaluation.
1041 Each image corresponds to an individual mouse. Scale bar: 100µm.

1042 **Supplementary figure 5**

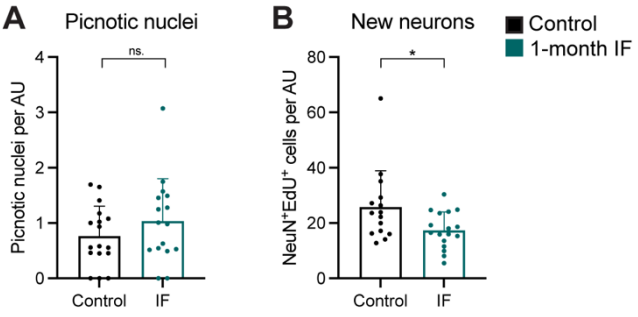


1043

1044 **Figure S5.** High levels of recombination in Glast-CreER^{T2};RYFP throughout experiments and
1045 treatments. (**A**, **B**) Recombination of the YFP reporter in Glast-CreER^{T2};RYFP upon tamoxifen
1046 induction shown as YFP^{+} neuroblasts over the total number of neuroblasts. (**A**) Recombination
1047 levels evaluated at the end of the 1-month treatment. (**B**) Recombination levels evaluated at
1048 the end of the 3-month treatment. Black: control (ad libitum) for each experiment, dark blue:
1049 1-month IF, light blue: 3-month IF. Bars and error bars represent mean + SD; dots represent
1050 individual mice. Significance values: ns, $p>0.05$.

1051

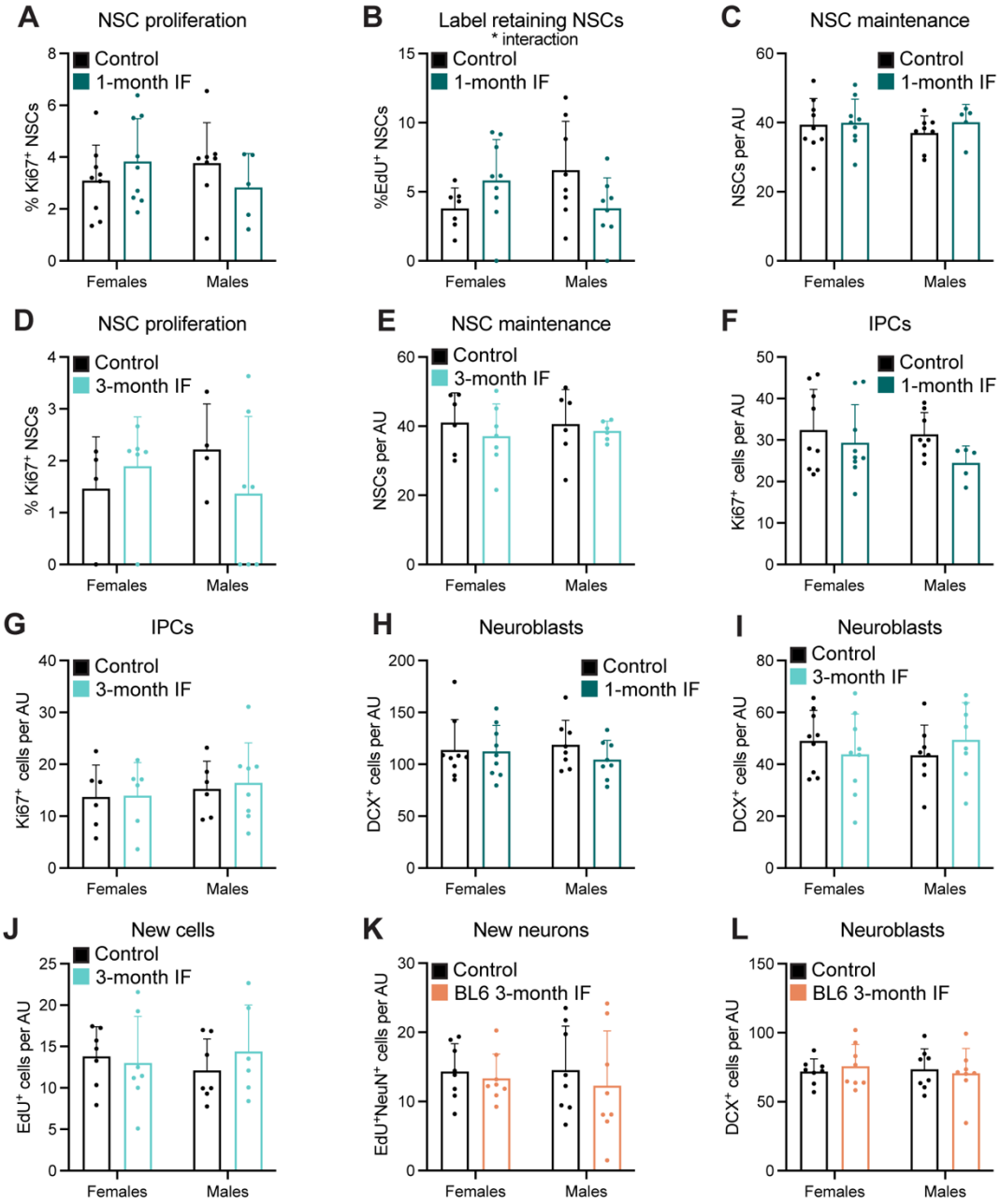
1052 **Supplementary figure 6**



1053

1054 **Figure S6.** Transient decrease in the generation of new neurons upon 1 month of IF. See
1055 experimental design in **Figure 2A**. (**A**) Picnotic nuclei normalised to DG length as a proxy for
1056 cell death, which was not affected by IF. (**B**) Number of EdU-labelled neurons (as $NeuN^+$ cells)
1057 after a 10-day chase, showing a decrease in the number of new neurons generated. Bars and
1058 error bars represent mean + SD; dots represent individual mice. Significance values: ns,
1059 $p>0.05$; *, $p<0.05$. Black: control (ad libitum), dark blue: IF.

1060 **Supplementary figure 7**

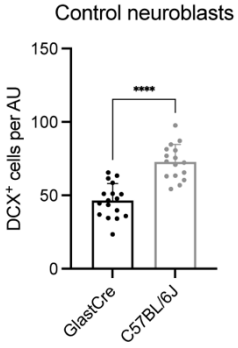


1061

1062 **Figure S7.** Male and female mice show similar responses to IF. (**A-L**) Data of all graphs in the
1063 main figures segregated by sex showing that the response of adult neurogenesis to IF is sex-
1064 independent. The percentage of label retaining NSCs after 1 month of IF (**B**) shows an
1065 interaction between diet and sex that is not translated in the following steps of the neurogenic
1066 lineage. Graphs are displayed in order of appearance on the text. Bars and error bars
1067 represent mean + SD; dots represent individual mice. Significance values: absence of sign,
1068 p>0.05; *, p<0.05. AU: arbitrary unit. Black: controls (ad libitum), dark blue: 1-month IF, light
1069 blue: 3-month IF, orange: C57BL/6J 3-month IF.

1070

1071 **Supplementary figure 8**



1072

1073 **Figure S8.** Differences in basal levels of neurogenesis between mouse strains. Neuroblasts
1074 (DCX$^+$ cells) in Glast-CreER^{T2};RYFP and C57BL/6J mice. The neurogenic rate (shown as
1075 number of neuroblasts) is a 40% lower in 5-month-old Glast-CreER^{T2};RYFP mice with a mixed
1076 genetic background than in C57BL/6J mice of matched age. Bars and error bars represent
1077 mean + SD, dots represent individual mice. Significance values: *, $p<0.0001$. AU: arbitrary
1078 unit. Black: 5-month-old Glast-CreER^{T2};RYFP control, gray: 5-month-old C57BL/6J control.

1079 **Table S1.** List of primary and secondary antibodies used for immunohistochemistry.

Target molecule	Species	Dilution	Company	Catalog number
GFP	chicken	1:5000	Abcam	ab13970
GFAP	rabbit	1:500	DAKO	Z033401-2
GFAP	rat	1:500	Invitrogen	130300
Ki67	rat	1:200	eBioscience	14-5698-82
DCX	rabbit	1:1000	Abcam	ab18723
NeuN	mouse	1:1000	Chemicon	MAB377
UCP1	rabbit	1:1000	Abcam	ab10983
Chicken IgG	donkey – 488	1:500	Jackson Laboratories	703-545-155
Rabbit IgG	donkey – Cy3	1:500	Jackson Laboratories	711-166-152
Rat IgG	donkey – Cy3	1:500	Jackson Laboratories	712-166-153
Rat IgG	donkey – 647	1:500	Jackson Laboratories	712-605-150
Mouse IgG	donkey – 647	1:500	Jackson Laboratories	715-606-151

1080