Adult neural stem cells and neurogenesis are resilient to intermittent fasting

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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

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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). 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 and S1A, 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 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 production, associated with thermogenesis in these cells (Ricquier, 2011). After 3 months of

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

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

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

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

before 3 months or after 5 months of age in these mice. Nevertheless, the number of NSCs in IF mice were indistinguishable from those of ad libitum fed mice (Figure 3C, D).

These data indicate that NSCs are protected from proliferation bursts that could lead to exhaustion, making IF a safe option for potentially increasing neurogenesis through diet-based interventions.

Adult neurogenesis is mildly and transiently impaired by IF

Since neither the pool nor the activity of NSCs change upon IF, we hypothesized that the previously reported increase in neurogenesis laid in subsequent stages of neurogenesis. We set out to determine at which step/s of the neurogenic lineage IF influences adult neurogenesis. NSCs and neuroblasts are predominantly out of the cell cycle, meaning that the vast majority of proliferating cells in the SGZ are IPCs (Hodge et al., 2008; Kronenberg et al., 2003). We therefore used Ki67 as a proxy for IPC identification. As previously reported, we observed a decrease in IPC abundance over time (Figure 4A, B). IF neither changed the number of IPCs after 1 month nor prevented their decline after 3 months (Figure 4A, B).

Next, we quantified the number of neuroblasts, identified by doublecortin (DCX) immunoreactivity. Our results show that neuroblasts were more abundant in younger mice and that they are generated at the same rate in IF mice and ad libitum fed mice at both time points (Figure 4C, D).

Finally, we measured the number of new neurons generated during the intervention. We used colocalization of the YFP reporter and the marker for mature neurons NeuN to identify newly born neurons. In accordance with the cumulative labelling of neurons upon lineage tracing, mice had many more newly born neurons labelled with the YFP reporter after 3 months than after 1 month (Figure 4E, F). Unexpectedly, we found a reduction in the number of newly born neurons in IF mice compared to ad libitum fed mice after 1 month of IF (Figure 4E, F). Cell death, measured by the number of picnotic nuclei in the SGZ, was comparable in control and IF mice (Figure S6A). Since neuroblasts were produced at the same rate (Figure 4D), we reasoned that this decrease in neurogenesis could be due to a transient drop in neurogenesis at an earlier time point. We made use of the label retention experiment in Figure 2A to ask if neuronal production was impaired two weeks after starting IF. Indeed, we found that fewer neurons had been produced at that time in mice subjected to IF compared to control mice (Figure S6B). One month of IF is therefore not only insufficient to increase adult neurogenesis but in fact decreases the neuronal output of adult NSCs. The decrease of neurogenesis was transient, as the number of newly born neurons went back to control levels in the 3-month intervention (Figure 4E, F), suggesting that the process of adult neurogenesis is robust enough to buffer initial changes in neuronal production.

Our findings show that 1 or 3 months of IF do not increase adult neurogenesis. This seemingly contradicts previous reports and puts a question mark on the suitability of IF as a tool to increase neurogenesis in the adult hippocampus.

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

We next asked which intrinsic variables of our study could explain the contradicting results with previously published studies.

The metabolism of male and female mice has been shown to react in a sex-specific manner to fasting and refeeding (Freire et al., 2020). While most studies on IF and neurogenesis were conducted with only male or only female mice, differences in the response to caloric restriction of adult hippocampal neurogenesis have been reported between sexes

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

Discussion

Fasting is a promising non-pharmacological intervention to increase life span and counteract ageing that has been associated with increased adult neurogenesis. However, few studies have investigated the specific effects of fasting on adult NSCs and other adult stem cell populations. This is important not only because impaired adult stem cell function is regarded as one of the hallmarks of ageing, but also because alterations in the adult stem cell population will have long-lasting consequences for tissue homeostasis. Here, we used genetic lineage tracing to determine the effects of every-other-day IF on adult NSCs.

We found that IF does not affect the proliferation or maintenance of adult NSCs. This suggested that NSCs are preserved upon IF and prompted us to investigate which further steps of neurogenesis might be increased by fasting. We carefully monitored the progression of the neurogenic lineage upon 1 or 3 months of IF and found no differences between control and fasted mice. This is in contrast with previous work reporting a sharp increase in proliferative cells (BrdU labelled) in the DG after 3 months of every-other-day IF (Dias). One possible explanation is that Dias et al. analysed the mice after 5 days of a recovery period with ad libitum feeding, while our mice were still fasting at the time of analysis. Alternating fasting periods with normal feeding has been proposed to be a crucial mechanism by which IF and CR exert their beneficial effects (Brandhorst et al., 2015). It would be very interesting to determine whether returning to ad libitum feeding from IF rather than fasting on its own can promote cell proliferation along the neurogenic lineage. However, a recent report using a similar intervention consisting of two fasting days per week (known as 5:2 diet) shows no effect on proliferation in the DG, similar to our results, even after a refeeding period (Roberts et al., 2022).

Our results were therefore in line with numerous reports which did not find differences in cell proliferation upon fasting (Kitamura et al., 2006; Lee et al., 2002b; Lee et al., 2002a). Those studies found instead an increased survival of newly born cells 4 weeks after fasting. We used lineage tracing to quantify the neuronal output of hippocampal NSCs and found no difference between control mice and mice fasted for 3 months. Classically, the effects of IF on adult neurogenesis were evaluated 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 method evaluates only narrow waves of neurogenesis and, when several time points are used, can be useful to identify changes in the effects of the intervention over time. However, it also increases the variability of the results and, unlike lineage tracing, does not provide information on the total number of new neurons produced during the whole intervention. We nevertheless performed a classical label retention experiment to our mice and again found no increase in neuronal production upon IF for 3 or 4 months. Of note, 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, either using label retention or short (1-month) lineage tracing. They could therefore be a consequence of the high degree of intrinsic variability in adult neurogenesis, questioning their functional importance. Since other cells proliferate in the dentate gyrus and can uptake the label (e.g. astrocytes, oligodendrocyte precursors or microglia), the interpretation of label-retention data is challenging unless combined with neuronal markers. In addition, the thymidine analogue positive population by the end of the labelling period depends not only on uptake of the label by proliferating cells but also on their differentiation and survival

(Kronenberg 2003), hampering the interpretation of the results. By using lineage tracing combined with immunofluorescence and thymidine analogues in label-retention experiments, we could separately evaluate different steps along the neurogenic lineage and faithfully quantify changes – or the lack of them - in neurogenic output.

To perform lineage tracing of adult NSCs, we used GlastCre mice that have a mixed genetic background. However, C57BL6/J is the most commonly used strain in fasting studies (REFS). In accordance with different strains presenting different levels of adult neurogenesis (Goodrick et al., 1990; Kempermann et al., 2006; Kim et al., 2017; Koehl, 2015; Wiget et al., 2017), we did observe differences in the number of neuroblasts between control GlastCre mice and control C57BL/6J at 5 months of age (Figure S8). Therefore, we repeated our experiments using C57BL/6J mice. Our results again showed that 3 or 4 months of fasting did not increase neurogenesis in the hippocampal niche. However, since variations can occur even within the C57BL/6J strain and the response to dietary interventions is strain and even substrain-specific (Bachmann et al., 2022; Hwang et al., 2008; Mitchell et al., 2016; Siersbæk et al., 2020), we cannot rule out that small differences in the animals' background could have contributed to the discrepancies between previous studies and ours.

Differences in the metabolic response to dietary interventions have also been observed between male and female mice (Bachmann et al., 2022; Mitchell et al., 2016), making it crucial to include both sexes when analysing the effects of any potential clinically relevant interventions. Although increased neurogenesis upon fasting has been reported for both sexes, we wondered whether differences in basal levels and response to the diet could be masking the effects of fasting in our data. Splitting our data by sex showed no increase in adult neurogenesis upon fasting in either male of female mice. We observed one parameter that was affected by sex: the number of new neurons after 1 month of IF (Figure 5A, 5B and S7). Since sex differences are particularly important for stress-related factors (Yagi and Galea, 2019), we hypothesize this discrepancy could be linked to metabolic or stress-related differences in handling the change from ad libitum feeding to fasting between male and female mice. Of note, while female mice are often excluded arguing higher variability in the results, variability was comparable in both sexes in our data (Figure S7).

Although some reports indicate that weight loss is not necessarily linked to the benefits of IF and CR, it remains plausible that the degree of caloric restriction and weight loss induced by IF could influence its subsequent effects on adult neurogenesis. Both Lee et al. and Kim et al. reported weight loss upon every-other-day IF. Our protocol, in contrast, did not affect the final weight of the mice or their growth curve. This is despite a reduction in calorie consumption of more than 20% and the presence of clear weight oscillations between fasting and feeding days. Our mice also showed signs of broad systemic changes induced by fasting (inguinal adipose tissue browning and liver remodelling), but we cannot rule out that these were not strong enough to induce changes in adult neurogenesis.

While establishing our every-other-day IF protocol, we found that daytime IF disrupts the circadian activity pattern of mice, introducing a confounding variable to IF that could affect adult neurogenesis (Bouchard-Cannon et al., 2013; Draijer et al., 2019; Schouten et al., 2020). A very recent report supports this notion, as circadian entraining can boost the beneficial effects of caloric restriction on longevity (Acosta-Rodríguez et al., 2022). This calls for a close monitoring of potential circadian disruptions during fasting interventions and for careful reporting of fasting protocols. However, circadian disruption alone does not explain the differences between our results and previous studies, as both day and night-time IF have been shown to increase neurogenesis in young, healthy mice (M. Mattson and S. Thuret, personal communication).

There are multiple other variables that we did not test for in our study which can modulate the impact of fasting on neurogenesis. These include mice stress and overall fitness (Snyder et al., 2011; Toda et al., 2019), pathological states (Cao et al., 2022; Li et al., 2020; Wu et al., 2008), age (Brandhorst et al., 2015; Park et al., 2013) and even cage bedding (Gregor et al., 2020) and diet composition (Cryan and Dinan, 2012; Ribeiro et al., 2020; Wei et al., 2021). We also did not assess differences in behavioural performance in our mice, which could be very interesting to determine the involvement of adult neurogenesis in the cognitive benefits of fasting. Of note, a recent report showing no effect on adult neurogenesis upon a 5:2 fasting intervention in mice also showed a lack of cognitive improvement in those mice (Roberts et al., 2022).

In summary, the specific combination of sex, strain, health status, housing conditions and fasting protocol can influence the outcome of dietary interventions on adult neurogenesis, putting a question mark on IF as a strategy to boost neurogenesis. Despite this, 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). The effects of fasting on adult neurogenesis are, however, very diverse, ranging from variable positive impact on the number of newly generated neurons (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) to no effect or even a mild impairment (Bondolfi et al., 2004; Roberts et al., 2022; Staples et al., 2017). We propose the variability is due to a combination of different factors (and most likely different combinations of factors in each case). This fits with a model in which IF can render NSCs and other cells along the neurogenic lineage more susceptible to further interventions such as circadian disruption or sudden re-feeding (Urbán, 2022). In addition, publication bias might be playing a role in skewing the literature on fasting and neurogenesis towards reporting positive results. 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.

Materials and Methods

Animal husbandry

Mice were housed in groups in ventilated cages (TECNIPLAST 1285L NEXT) provided with HEPA filtered air covered with Aspen-wood-derived bedding (Kliba Nafag, ABEDD 4063 OM G10). They were kept under a 12:12 h light/dark cycle (light period 0600 – 1800 h CET), unless stated otherwise. Mice had ad libitum access to food (Ssniff, V1184-300) and water. To generate a mouse line that allowed lineage tracing of adult NSCs with a fluorescent reporter (Andersen et al., 2014), we crossed Glast-CreER^{T2} (Slc1a3$^{tm1(cre/ERT2)Mgoe}$, (Mori et al., 2006)) mice and RYFP (Gt(ROSA)26Sor$^{tm1(EYFP)Cos}$, (Srinivas et al., 2001)) mice. All Glast-CreER^{T2};RYFP mice were of mixed genetic background. 4–6-week-old C57BL/6J (Charles

River) mice were obtained from the Comparative Medicine facility at IMBA/IMP. Experimental groups were formed by randomly assigning mice from different litters within each mouse strain and all experiments were conducted in male and female mice, except stated otherwise.

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

Intermittent fasting

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

Automated home cage mouse phenotyping

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

Before each run, CO_2 and O_2 sensors were calibrated against a defined mix of CO_2 and O_2. The respiratory exchange ratio (RER) was calculated as the ratio of VCO_2 produced to the VO_2 consumed by the PhenoMaster software. Mouse locomotor activity was monitored using the ActiMot2 Activity module equipped with 3-dimensional infrared sensors. An estimate of movement (DistD, cm) was calculated using the PhenoMaster software. Data acquired in 15-min intervals are expressed as means of 48h cycles. For grouped housed mice (daytime IF), DistD was divided by the number of mice in each cage. Total mouse locomotor activity was calculated as the sum of cm throughout the whole intervention and segregated by time periods to depict differences between diurnal and nocturnal behaviour during fasting and feeding days. For daytime IF, where the mice were kept in a 14:10 LD cycle, the data was divided in ZT 0-14-24-38-48 h. For night-time IF, where the mice were kept in a 12:12 LD cycle, the data was divided in ZT 0-12-24-36-48 h.

The automated home cage mouse phenotyping was performed by the Preclinical Phenotyping Facility at Vienna BioCenter Core Facilities (VBCF), member of the Vienna BioCenter (VBC), Austria.

Tamoxifen and EdU administration

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

471 *Tissue preparation and immunohistochemistry*

472 Mice were transcardially perfused with phosphate buffered saline (PBS) for 3 min,
473 followed by 4% paraformaldehyde (PFA, Sigma, P6148) in PBS for 15 min.
474 Brains were post-fixed in 4% PFA for 2-6 h at 4°C with rocking, washed with PBS and
475 stored in PBS with 0.02% Sodium Azide (ITW Reagents, A1430). Brains were coronally
476 sectioned at a thickness of 40 µm using a vibratome (Leica, VT1000S). Immunofluorescence
477 of brain tissue was performed in free-floating sections. Sections were blocked in 1%Triton-
478 PBS with 10% normal donkey serum (DS, Jackson Immuno Research, JAC0170001210) for
479 2 h at room temperature. Primary and secondary antibody solutions were prepared in
480 0.1%Triton-PBS with 10% DS. Sections were incubated in primary antibody solution at 4°C
481 overnight, washed 3 x 15 min with 0.1%Triton-PBS and incubated with secondary antibody
482 solution at room temperature for 2 h. Following 1 x 0.1%Triton-PBS and 2 x PBS 15 min
483 washes, sections were incubated with 1 µg/mL DAPI (Sigma, D9542) in 1:1 PBS:H_2O at room
484 temperature for 30 min. EdU was detected before DAPI incubation using Click-iT™ Plus Alexa
485 Fluor™ Picolyl Azide tool kits with 488 and 647 dyes (Invitrogen, C10641 and C10643
486 respectively). All incubations and washes were performed under gentle rocking.
487 Inguinal adipose tissue and livers were dissected and post-fixed in 4%PFA at room
488 temperature with rocking for 24h prior to paraffin embedding. Eight random mice per condition
489 were chosen from the original cohort of mice for immunohistochemical analysis. Fixed tissue
490 was process in a Donatello Automatic Sample Processor (Diapath) and embedded in paraffin.
491 2µm thick sections were collected on glass slides. For staining of the inguinal adipose tissue
492 antigen retrieval with tri-sodium citrate (pH=6) was carried out for 30 min prior to a 1-h-
493 incubation with the primary antibody at room temperature. The 2 steps HRP polymer Kit with
494 DAB (DCX, PD000POL-K) was used for detection followed by hematoxylin counterstaining.
495 Liver sections were stained with hematoxylin and eosin in an Erpedia Gemini AS strainer
496 (Fisher Scientific).
497 Primary and secondary antibodies and concentrations are listed in Table S1. Processing
498 and staining of adipose tissue and liver was performed by the Histology facility at the VBCF.

499 *Imaging*

500 Images of brain immunofluorescence were acquired using an inverted Axio Observer
501 microscope (ZEISS) equipped with a CSU X1 confocal scanning unit (ZEISS) and an EM-CCD
502 camera (Hamamatsu, C9100-13). 40x and 63x oil Plan-Apochromat objective lenses (ZEISS)
503 were used to image through whole 40 µm sections with a z-step of 1 µm. Images were stitched
504 using ZEN Blue software (ZEISS).
505 Images of adipose tissue and liver were acquired using a Pannoramic FLASH 250 II
506 (3DHISTECH) equipped with a CIS VVC FC60FR19CL camera (Vital Vision Technology) and

a 20x Plan-Apochromat objective. Representative images were cropped using the CaseViewer software.

Image quantifications

Quantifications of cells in the neurogenic lineage were performed using the Fiji software (Schindelin 2012) with the Cell Counter plugin. NSCs were identified by the localisation of a DAPI$^+$ nucleus in the SGZ, the extension of a single GFAP$^+$ vertical projection towards the molecular layer and the help of the YFP lineage tracing reporter (except in C57BL/6J mice), that is brighter in NSCs than other cells. IPCs were identified as Ki67$^+$ cells in the SGZ. Neuroblasts were identified as DCX$^+$ cells in the SGZ. Newly born neurons were identified as NeuN$^+$ cells that colocalised with YFP (in GlastCreER^{T2};RYFP mice) or EdU (in C57BL/6J mice) in the SGZ and granule cell layer of the DG. In GlastCreER^{T2};RYFP mice, NeuN counts were normalised to the recombination rate. New cells in the DG were identified by EdU labelling. For cell numbers, 1 DG per sample was quantified (two for NSCs). The freehand line tool was used to measure the length of the SGZ, to which the total cell counts were normalised. For percentage of proliferating or EdU-labelled NSCs, at least 120 cells from two DG were counted in young mice, and 70 in older mice. All data were counted with group blinding within each experiment.

The appropriate sample size was based on previous publications studying NSC behaviour (Andersen 2014, Urban 2016, Blomfield 2019, Austin 2021), accounting for the increased variability of dietary interventions compared to genetic ones and considering female and male mice as separate groups. For the quantifications based on the YFP reporter, samples with a recombination rate lower than 80% were excluded. Samples with poor staining quality were also excluded from the quantifications.

Statistical analysis

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

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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.

566 **References**

567 **Acosta-Rodríguez, V., Rijo-Ferreira, F., Izumo, M., Xu, P., Wight-Carter, M., Green, C. B.**
568 **and Takahashi, J. S.** (2022). Circadian alignment of early onset caloric restriction
569 promotes longevity in male C57BL/6J mice. *Science* **376**, 1192-1202.

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

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

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

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

583 **Austin, S. H. L., Gabarró-Solanas, R., Rigo, P., Paun, O., Harris, L., Guillemot, F. and**
584 **Urbán, N.** (2021). Wnt/β-catenin signalling is dispensable for adult neural stem cell
585 homeostasis and activation. *Development* **148**, dev199629.

586 **Bachmann, A. M., Morel, J.-D., El Alam, G., Rodríguez-López, S., Imamura de lima, T.,**
587 **Goeminne, L. J. E., Benegiamo, G., Loric, S., Conti, M., Sleiman, M. B., et al.**
588 (2022). Genetic background and sex control the outcome of high-fat diet feeding in
589 mice. *iScience* **25**, 104468.

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

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

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

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

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

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

606 **Bouchard-Cannon, P., Mendoza-Viveros, L., Yuen, A., Kærn, M. and Cheng, H.-Y. M.**
607 (2013). The Circadian Molecular Clock Regulates Adult Hippocampal Neurogenesis
608 by Controlling the Timing of Cell-Cycle Entry and Exit. *Cell Reports* **5**, 961–973.

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

613 **Cao, S., Li, M., Sun, Y., Wu, P., Yang, W., Dai, H., Guo, Y., Ye, Y., Wang, Z., Xie, X., et al.**
614 (2022). Intermittent fasting enhances hippocampal NPY expression to promote
615 neurogenesis after traumatic brain injury. *Nutrition* **97**, 111621.

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

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

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

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

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

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

632 **Freire, T., Senior, A. M., Perks, R., Pulpitel, T., Clark, X., Brandon, A. E., Wahl, D.,**
633 **Hatchwell, L., Le Couteur, D. G., Cooney, G. J., et al.** (2020). Sex-specific metabolic
634 responses to 6 hours of fasting during the active phase in young mice. *J Physiol* **598**,
635 2081–2092.

636 **Froy, O., Chapnik, N. and Miskin, R.** (2009). Effect of intermittent fasting on circadian
637 rhythms in mice depends on feeding time. *Mechanisms of Ageing and Development*
638 **130**, 154–160.

639 **Fusco, S. and Pani, G.** (2013). Brain response to calorie restriction. *Cell. Mol. Life Sci.* **70**,
640 3157–3170.

641 **Goodell, M. A. and Rando, T. A.** (2015). Stem cells and healthy aging. *Science* **350**, 1199–
642 1204.

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

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

649 **Harris, L., Rigo, P., Stiehl, T., Gaber, Z. B., Austin, S. H. L., Masdeu, M. del M., Edwards,**
650 **A., Urbán, N., Marciniak-Czochra, A. and Guillemot, F.** (2021). Coordinated
651 changes in cellular behavior ensure the lifelong maintenance of the hippocampal stem
652 cell population. *Cell Stem Cell* **28**, 863-876.e6.

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

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

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

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

667 **Kaptan, Z., Akgün-Dar, K., Kapucu, A., Dedeakayoğulları, H., Batu, Ş. and Üzüm, G.**
668 (2015). Long term consequences on spatial learning-memory of low-calorie diet during
669 adolescence in female rats; hippocampal and prefrontal cortex BDNF level, expression
670 of NeuN and cell proliferation in dentate gyrus. *Brain Research* **1618**, 194–204.

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

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

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

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

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

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

688 **Kronenberg, G., Reuter, K., Steiner, B., Brandt, M. D., Jessberger, S., Yamaguchi, M.**
689 **and Kempermann, G.** (2003). Subpopulations of proliferating cells of the adult
690 hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol*
691 **467**, 455–463.

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

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

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

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

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

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

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

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

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

716 **Li, W., Wu, M., Zhang, Y., Wei, X., Zang, J., Liu, Y., Wang, Y., Gong, C.-X. and Wei, W.**
717 (2020). Intermittent fasting promotes adult hippocampal neuronal differentiation by
718 activating GSK-3β in 3xTg-AD mice. *Journal of Neurochemistry* **155**, 697–713.

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

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

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

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

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

731 **Mitchell, S. J., Matute, J. M., Scheibye-Knudsen, M., Fang, E., Aon, M., González-Reyes,**
732 **J. A., Cortassa, S., Kaushik, S., Gonzalez-Freire, M., Patel, B., et al.** (2016). Effects
733 of Sex, Strain, and Energy Intake on Hallmarks of Aging in Mice. *Cell Metabolism* **23**,
734 1093–1112.

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

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

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

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

744 **Park, H. R. and Lee, J.** (2011). Neurogenic contributions made by dietary regulation to
745 hippocampal neurogenesis. *Annals of the New York Academy of Sciences* **1229**, 23–
746 28.

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

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

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

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

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

761 **Roberts, L. D., Hornsby, A. K. E., Thomas, A., Sassi, M., Kinzett, A., Hsiao, N., David, B.**
762 **R., Good, M., Wells, T. and Davies, J. S.** (2022). The 5:2 diet does not increase adult
763 hippocampal neurogenesis or enhance spatial memory in mice. bioRxiv
764 2022.10.03.510613.

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

768 **Schouten, M., Bielefeld, P., Garcia-Corzo, L., Passchier, E. M. J., Gradari, S., Jungenitz,**
769 **T., Pons-Espinal, M., Gebara, E., Martín-Suárez, S., Lucassen, P. J., et al.** (2020).

Circadian glucocorticoid oscillations preserve a population of adult hippocampal neural stem cells in the aging brain. *Mol Psychiatry* **25**, 1382–1405.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

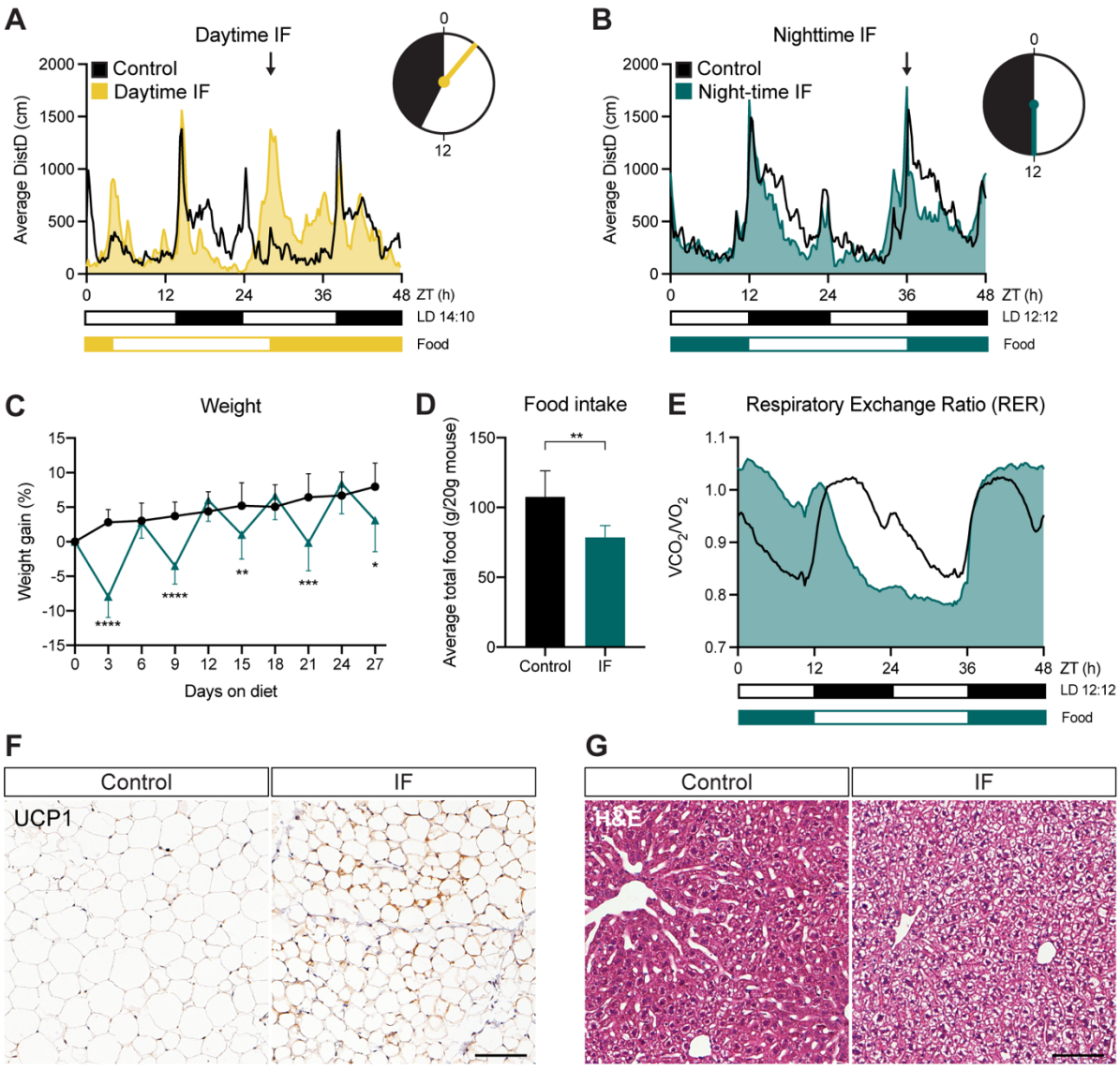
Wu, P., Shen, Q., Dong, S., Xu, Z., Tsien, J. Z. and Hu, Y. (2008). Calorie restriction ameliorates neurodegenerative phenotypes in forebrain-specific presenilin-1 and presenilin-2 double knockout mice. *Neurobiology of Aging* **29**, 1502–1511.

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

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

825 **Figures and Figure Legends**

826 **Figure 1**

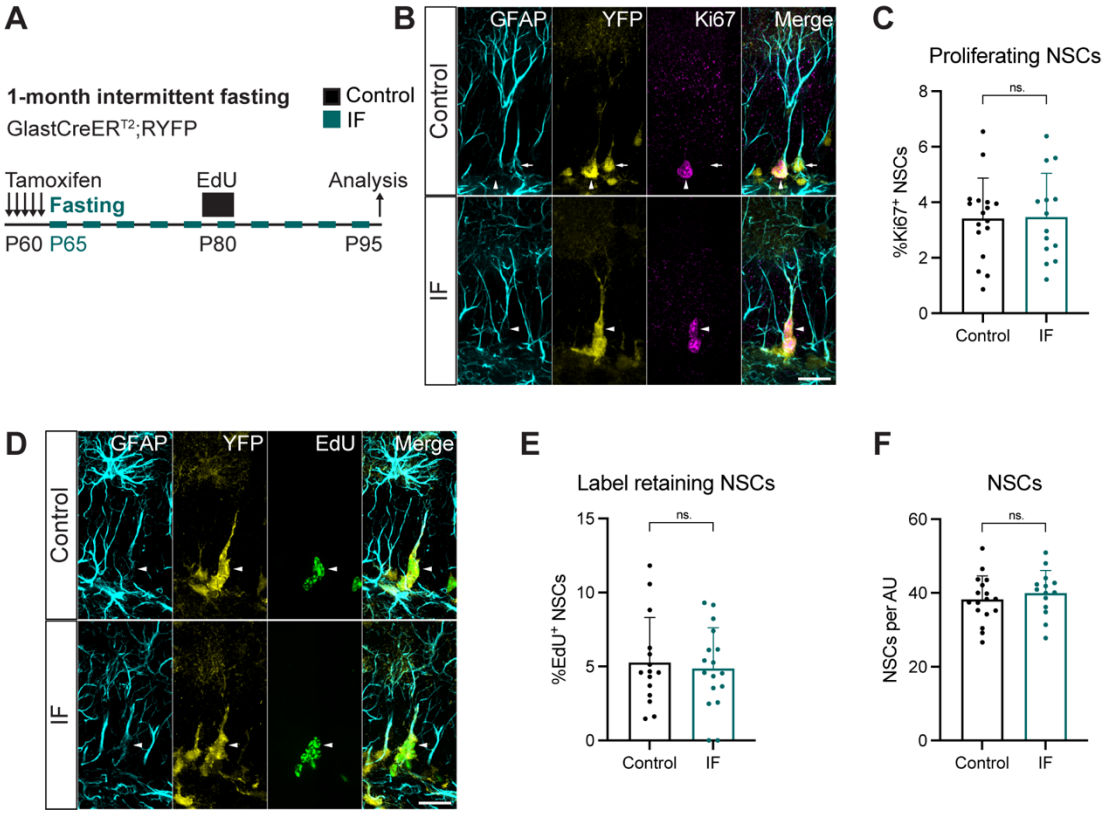


827

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

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

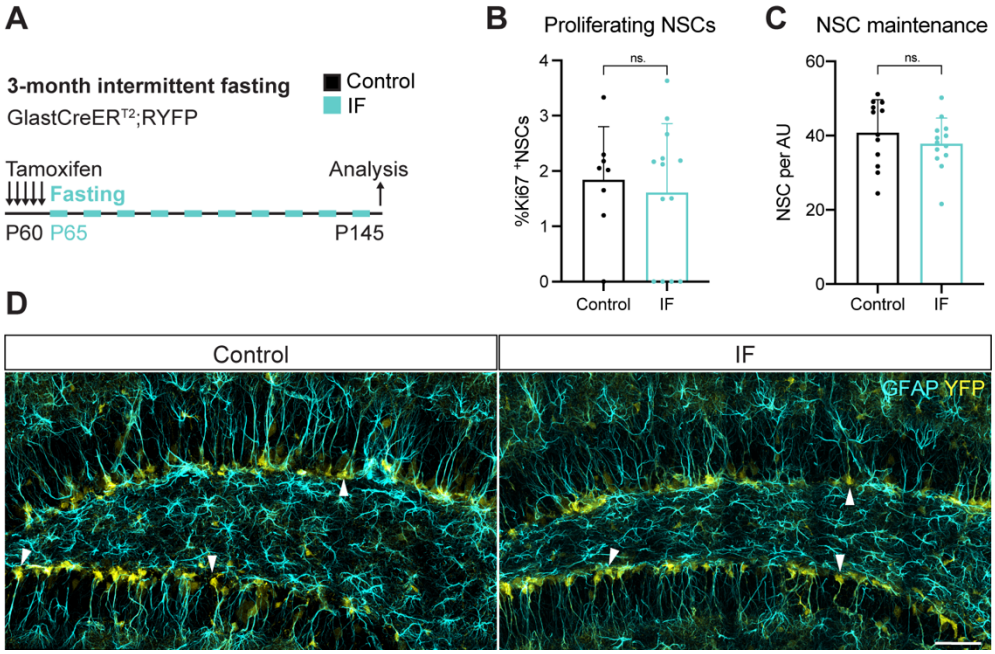
854 **Figure 2**



855

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

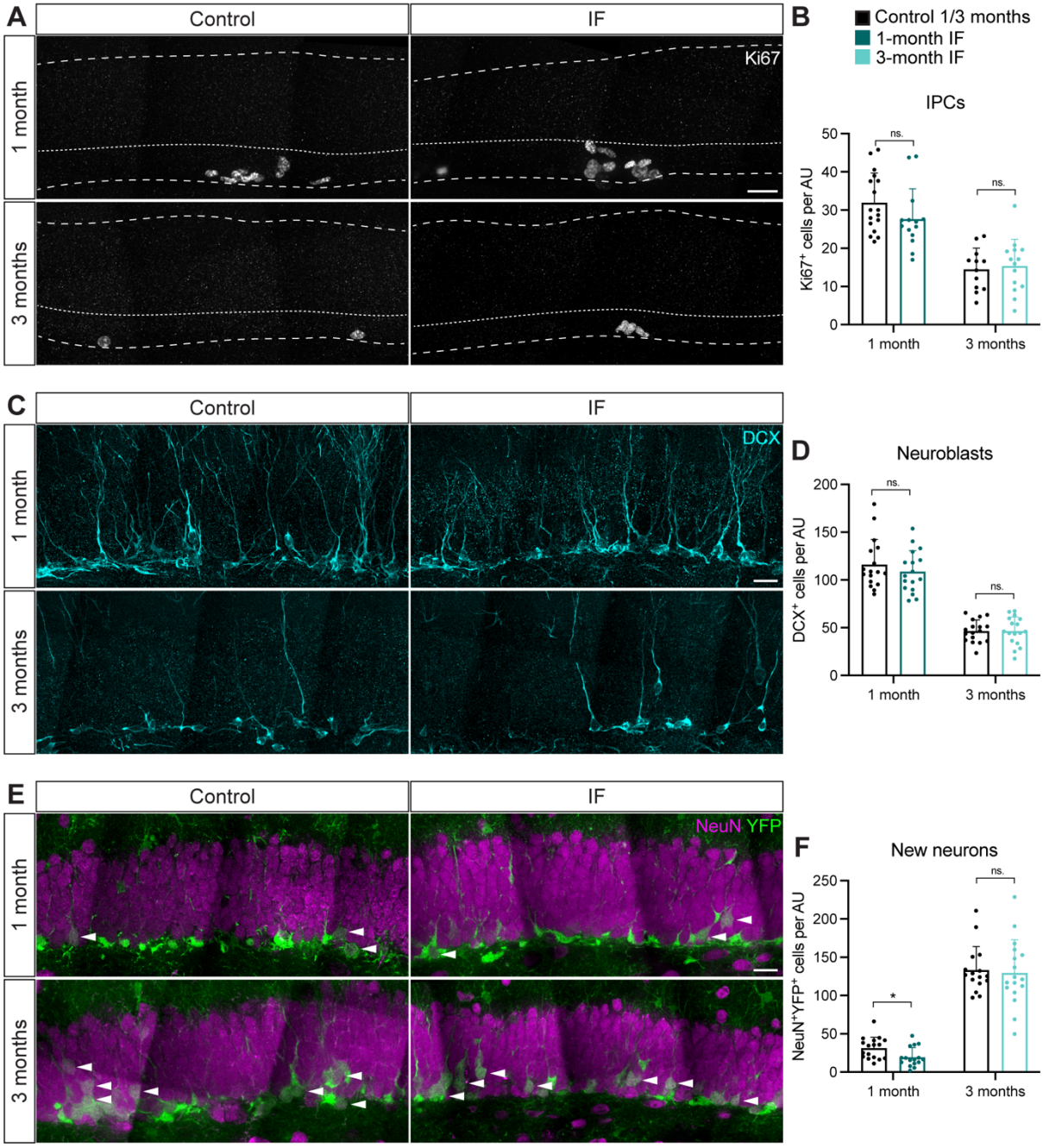
870 **Figure 3**



871

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

881 **Figure 4**



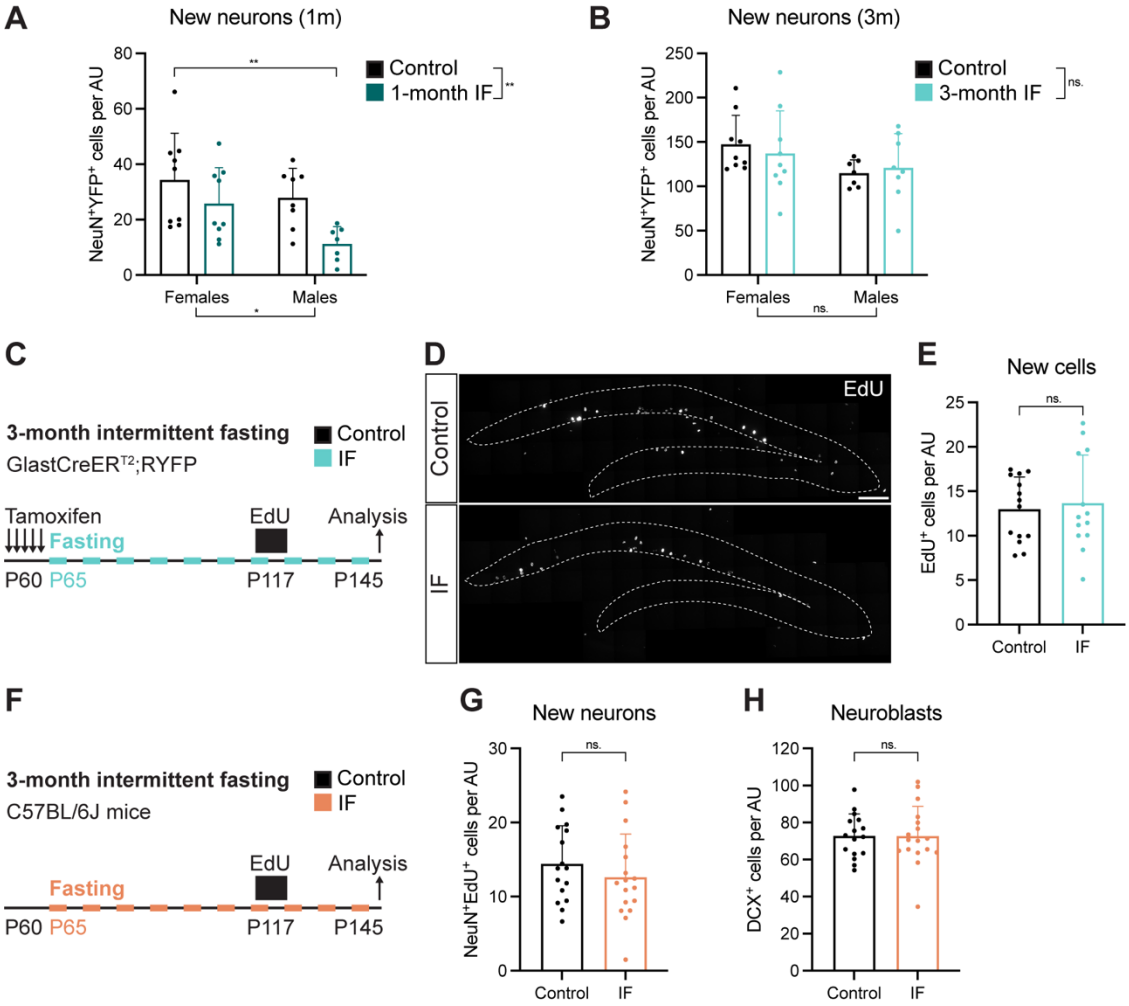
882

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

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

896

897 **Figure 5**



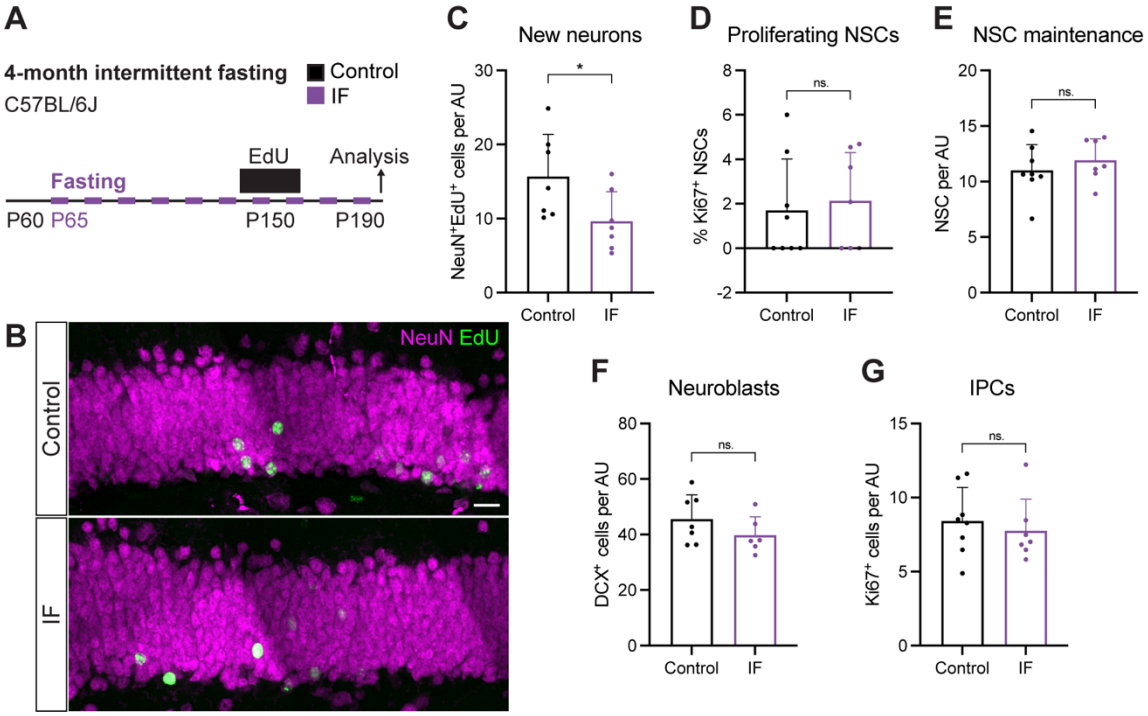
898

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

917 arbitrary unit. Black: control (ad libitum) for each experiment, light blue: 3-month IF, orange:
918 3-month IF C57BL/6J.

919

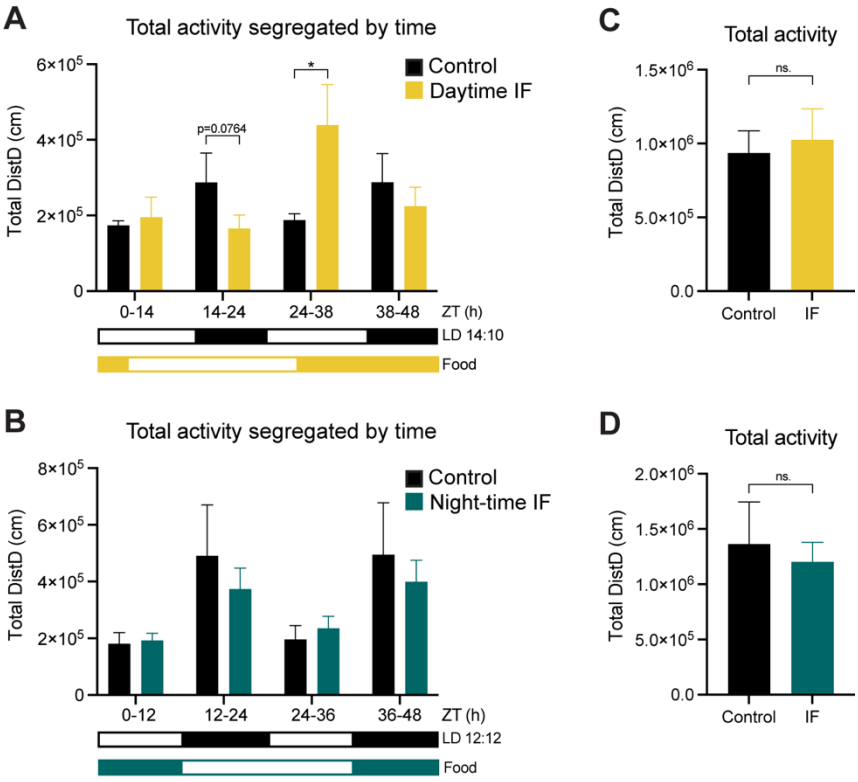
920 **Figure 6**



921

922 **Figure 6.** 4 months of every-other-day IF do not increase adult neurogenesis. (**A**) 2-month-
923 old male C57BL/6J mice were subjected to 4 months of IF, as in previous studies that reported
924 increases in adult neurogenesis. A survival assay with a thymidine analogue (12-day labelling
925 – 1 month chase) was used to evaluate neurogenesis. (**B**, **C**) Image and quantification of
926 newly born neurons identified by colocalization of EdU and the marker for mature neurons
927 NeuN. 4 months of IF induce a decrease in EdU-labelled neurons. (**D**) Quantification of
928 proliferating NSCs. (**E**, **F**, **G**) Quantification the total number of NSCs, neuroblasts and IPCs
929 normalised to DG length. The neurogenic lineage is not affected by 4 months of IF. Bars and
930 error bars represent mean + SD; dots represent individual mice. Significance values: ns,
931 p>0.05; *, p<0.05. Scale bar: 20µm. AU: arbitrary unit. Black: control (ad libitum), purple: IF 4-
932 months C57BL/6J.

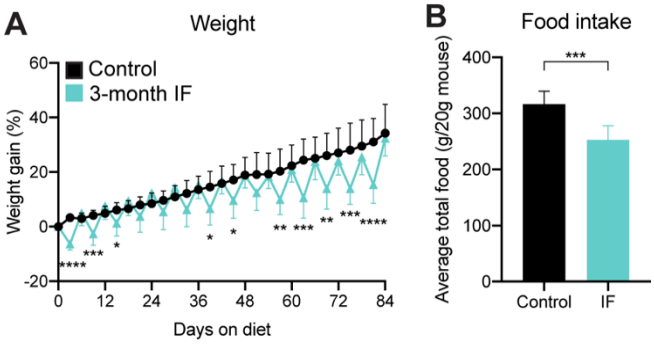
933 **Supplementary figure 1**



934

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

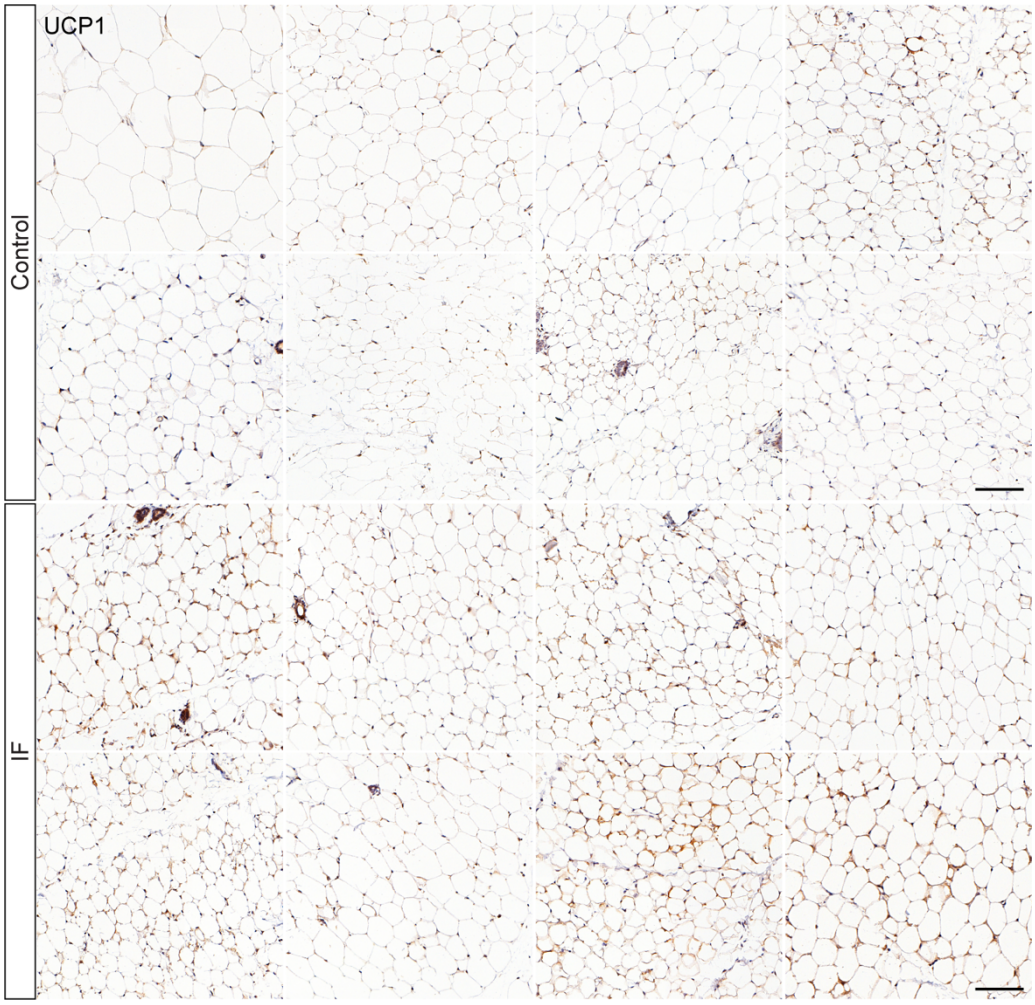
947 **Supplementary figure 2**



948

949 **Figure S2.** Mouse weight and food intake of mice during 3 months of IF. (**A**) Mouse weight
950 shown as the percentage of weight difference to the first day of the diet. Weight oscillations
951 persist throughout the 3 months of IF and there is no weight loss on refeeding days compared
952 to control mice. n = 17. (**B**) Average total food consumed for 3 months shown as g of food per
953 20g of mouse weight (weight reference of mice at P65). n = 17. Significance values: no symbol,
954 p>0.05; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001. Black: control (ad libitum), light
955 blue: 3-month IF.

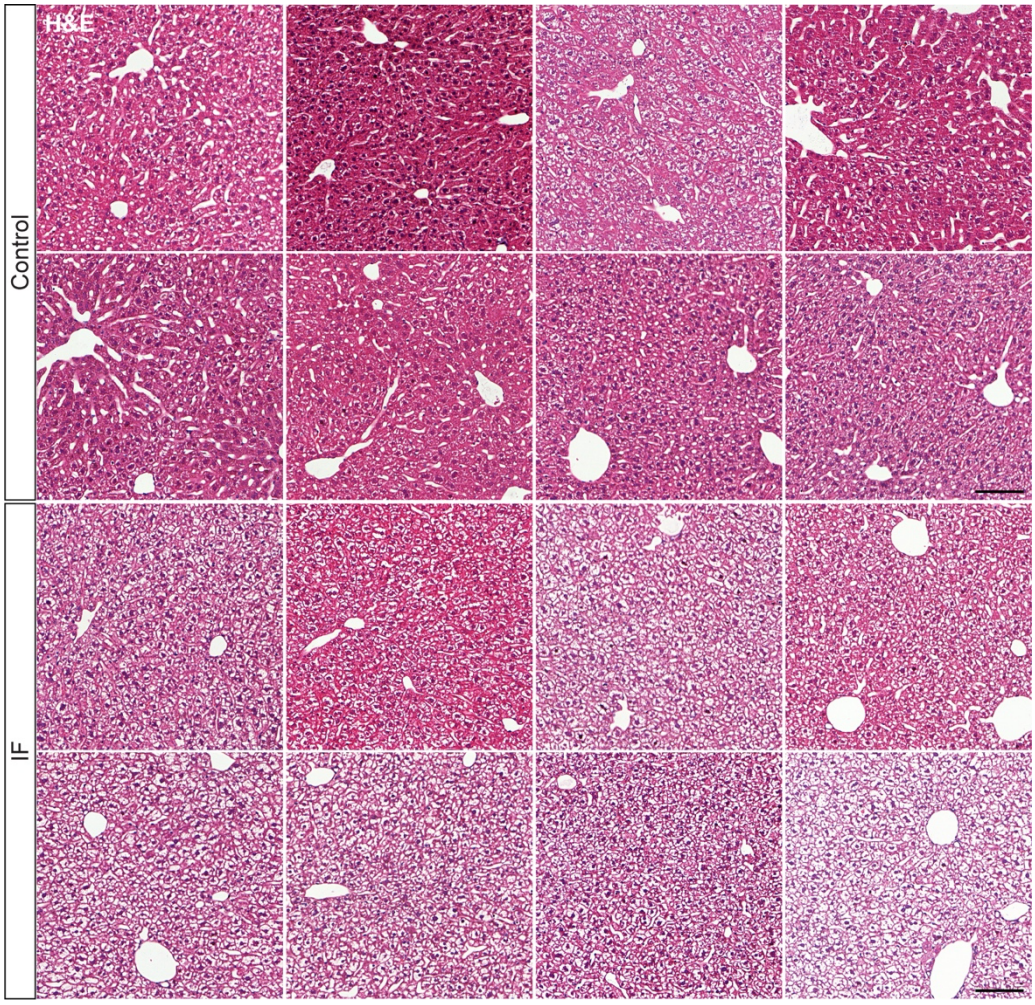
956 **Supplementary figure 3**



957

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

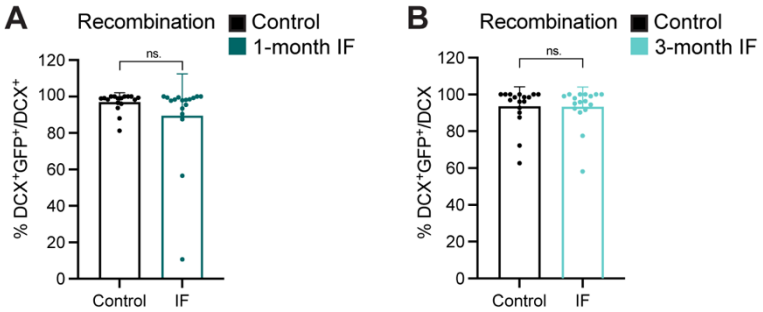
963 **Supplementary figure 4**



964

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

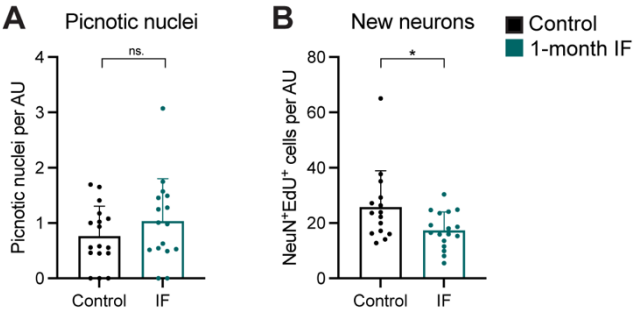
969 **Supplementary figure 5**

970 

971 **Figure S5.** High levels of recombination in Glast-CreER^{T2};RYFP throughout experiments and
972 treatments. (**A**, **B**) Recombination of the YFP reporter in Glast-CreER^{T2};RYFP upon tamoxifen
973 induction shown as YFP^{+} neuroblasts over the total number of neuroblasts. (**A**) Recombination
974 levels evaluated at the end of the 1-month treatment. (**B**) Recombination levels evaluated at
975 the end of the 3-month treatment. Black: control (ad libitum) for each experiment, dark blue:
976 1-month IF, light blue: 3-month IF. Bars and error bars represent mean + SD; dots represent
977 individual mice. Significance values: ns, $p>0.05$.

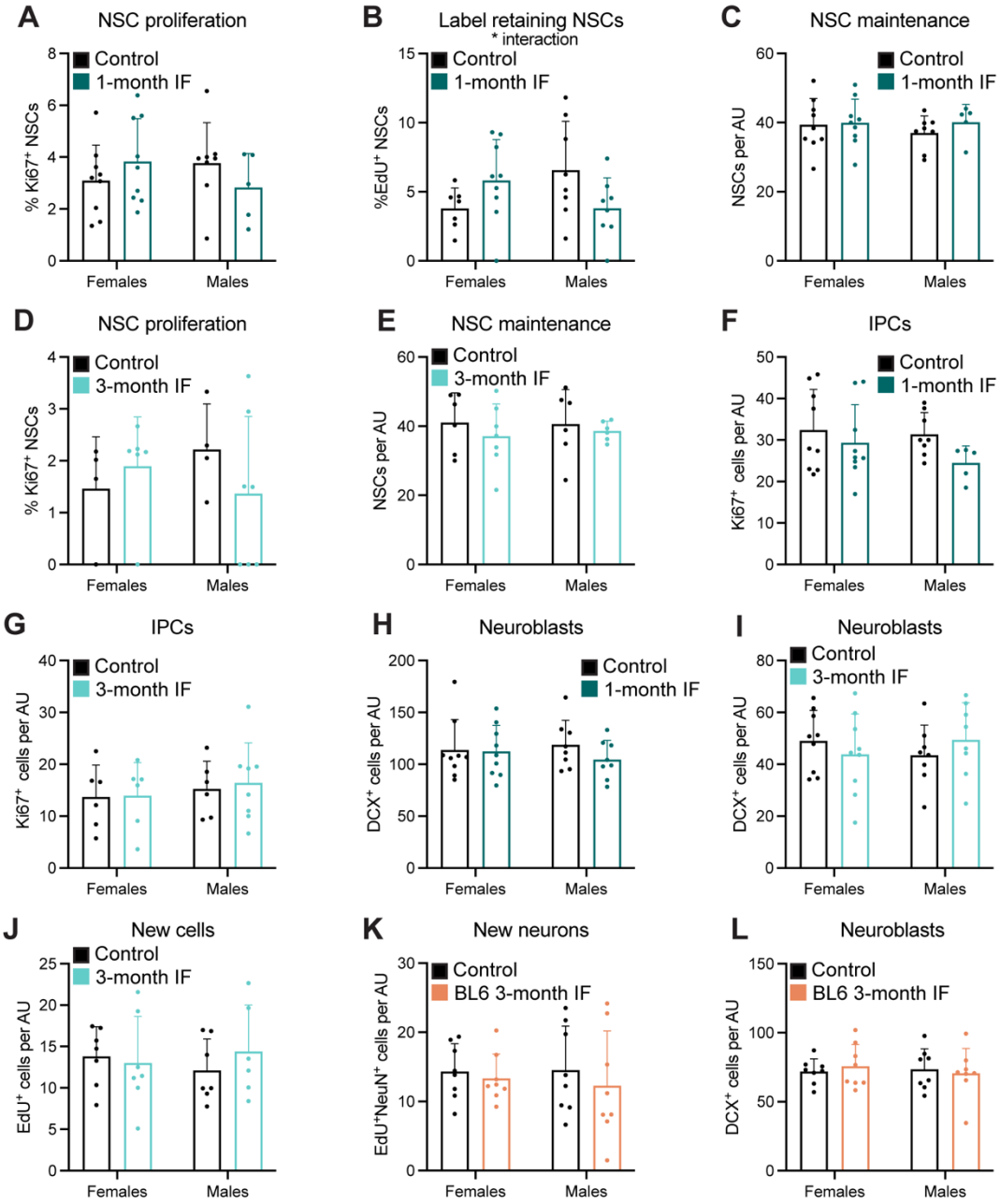
978

979 **Supplementary figure 6**

980 

981 **Figure S6.** Transient decrease in the generation of new neurons upon 1 month of IF. See
982 experimental design in **Figure 2A**. (**A**) Picnotic nuclei normalised to DG length as a proxy for
983 cell death, which was not affected by IF. (**B**) Number of EdU-labelled neurons (as $NeuN^+$ cells)
984 after a 10-day chase, showing a decrease in the number of new neurons generated. Bars and
985 error bars represent mean + SD; dots represent individual mice. Significance values: ns,
986 $p>0.05$; *, $p<0.05$. Black: control (ad libitum), dark blue: IF.

987 **Supplementary figure 7**

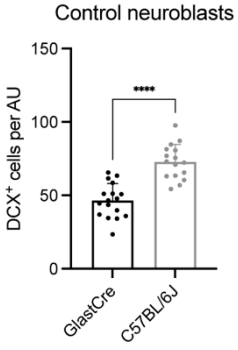


988

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

997

998 **Supplementary figure 8**



999

1000 **Figure S8.** Differences in basal levels of neurogenesis between mouse strains. Neuroblasts
1001 (DCX$^+$ cells) in Glast-CreER^{T2};RYFP and C57BL/6J mice. The neurogenic rate (shown as
1002 number of neuroblasts) is a 40% lower in 5-month-old Glast-CreER^{T2};RYFP mice with a mixed
1003 genetic background than in C57BL/6J mice of matched age. Bars and error bars represent
1004 mean + SD, dots represent individual mice. Significance values: *, $p<0.0001$. AU: arbitrary
1005 unit. Black: 5-month-old Glast-CreER^{T2};RYFP control, gray: 5-month-old C57BL/6J control.

1006 **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

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