

Interferon regulates neural stem cell function at all ages by orchestrating mTOR and cell cycle

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Abstract

Stem cells show intrinsic interferon signalling, which protects them from viral infections at all ages. In the ageing brain, interferon signalling also reduces the ability of stem cells to activate. Whether these functions are linked and at what time interferons start taking on a role in stem cell functioning is unknown. Additionally, the molecular link between interferons and activation in neural stem cells and how this relates to progenitor production is not well understood. Here we combine single-cell transcriptomics, RiboSeq and mathematical models of interferon to show that this pathway is important for proper stem cell function at all ages in mice. Interferon orchestrates cell cycle and mTOR activity to post-transcriptionally repress Sox2 and induces quiescence. The interferon response then decreases in the subsequent maturation states. Mathematical simulations indicate that this regulation is beneficial for the young and harmful for the old brain. Our study establishes molecular mechanisms of interferon in stem cells and interferons as genuine regulators of stem cell homeostasis and a potential therapeutic target to repair the ageing brain.

Keywords ageing; dormancy; interferon; mTOR; stem cells

Subject Categories Neuroscience; Stem Cells & Regenerative Medicine

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Introduction

In the adult brain, stem cells residing in the ventricular-subventricular zone (vSVZ) generate olfactory bulb interneurons that are crucial for fine-tuning odour discrimination. For neuronal production, neural stem cells (NSCs) transit from a dormant to an activation state to produce transient amplifying progenitors (TAPs) and finally neuroblasts (Urbán *et al*, 2019). These neuroblasts migrate along the rostral migratory stream towards the olfactory bulb (OB), where they mature into olfactory bulb interneurons. As the animal ages, activation of NSCs decreases, while interferon signalling increases (Baruch *et al*, 2014; Kalamakis *et al*, 2019). This age-related interferon response is highest in the neighbouring cells but also visible in NSCs (Kalamakis *et al*, 2019). Apart from their function in homeostasis, NSCs become activated upon injury to produce neurons and other glial cells (Delgado *et al*, 2021). This injury response is in part mediated by interferons (Kyritsis *et al*, 2012; Llorens-Bobadilla *et al*, 2015). Interferons (IFNs) are cytokines known to modulate the innate and adaptive immune response upon infections and injury (Mazewski *et al*, 2020). The interferon family is composed of type I, II and III IFNs. While type I and II IFNs are sensed ubiquitously in the body, type III IFN response is restricted to immune and epithelial cells. Type I and II IFNs activate the canonical JAK/STAT signalling pathway through IFN receptor α (IFNAR) and γ (IFNGR), respectively, leading to the transcription of a subset of interferon stimulated genes (ISGs; Alspach *et al*, 2019; Stanifer *et al*, 2020). Despite the ubiquitous expression of IFNAR and IFNGR in stem cells, they show an attenuated response to IFN compared to differentiated counterparts (Wu *et al*, 2018). Instead, stem cells, including neural stem cells (NSCs), rely on intrinsic expression of ISGs to prevent viral infection (Wu *et al*, 2018).

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Whether this intrinsic interferon signalling that is observed in NSCs already in young animals regulates stem cell function has not been addressed. In addition, whether the age-related increase in interferon response is independent of the intrinsic interferon response is similarly unexplored. Interestingly, recent ageing studies hinted at a faint basal interferon response already in the young homeostatic brain, albeit they were technically unable to characterise it (Kalamakis *et al*, 2019). Moreover, previous studies focused only on transcriptional control of ISGs while the post-transcriptional regulation of stemness factors (Baser *et al*, 2019) upon IFN exposure in NSCs remains elusive. Understanding how the positive and negative functions of interferon are molecularly wired in stem cells in the young and old brain is mandatory to provide regenerative therapy for a better ageing.

Here, single-cell RNA sequencing (scRNAseq) of IFNAGR^{KO} and wt NSCs reveals that already at young ages NSCs exhibit an IFN-response, as opposed to previous reports suggesting that this response is only found in ageing NSCs (Baruch *et al*, 2014; Dulken *et al*, 2019; Kalamakis *et al*, 2019). Interestingly, already committed immature TAPs and neuroblasts are resilient to IFNs, revealing a hierarchy of interferon responsiveness along the stem cell lineage. To address the molecular underpinnings of NSCs IFN response, we performed Ribo-Seq in NSCs exposed to IFNs. Our data indicate that type-I IFN induces a transient up- and a late down-regulation of mTORC1 activity and a concomitant gradual inhibition of cell cycle. This biphasic control of mTORC1 is mediated by the crosstalk of the JAK-STAT and PI3K-Akt signalling pathways. In addition, late phosphorylation of eIF2 α and Cdk4/6-mediated TSC2 inhibition contribute to the shutdown of mTORC1 and protein translation. Together, this IFN-response leads to the post-transcriptional inhibition of Sox2 expression. Mathematical modelling of NSC dynamics *in vivo* uncovers interferons as regulators of neural stem cell activation and self-renewal at all ages. Consequently, modelling predicts that inhibition of interferon is detrimental in the young and beneficial in the old brain.

Results

Interferon regulates neural stem cells in the young and ageing brain

Interferons are known regulators of NSCs reaction to injury and infection. To characterise a potential role of IFNs in NSCs homeostasis, we first examined the individual transcriptomes of NSC and

their progeny in mice lacking the type-I (IFNA) and -II (IFNG) interferon receptors (IFNAGR^{KO}) and in wild-type mice (IFNAGR^{WT}). To capture changes in NSCs dynamics across ages we examined young (2–3 months old) and old mice (17–24 months old). To this end, we profiled the transcriptomes of 15,548 individual NSCs, their progeny (Tlx-mediated eYFP⁺ cells) and neighbouring microglia and endothelial cells isolated from vSVZ, the RMS and the olfactory bulb (Figs 1A and EV1A). Using previously defined scRNAseq markers (Llorens-Bobadilla *et al*, 2015; Kalamakis *et al*, 2019) we identified dormant NSC (qNSC1), primed-quiescent NSCs (qNSC2), active NSC (aNSC), TAPs and neuroblasts (NBs; Figs 1B and EV1B). Next, we aimed at assessing the strength of the interferon signalling in cells along the different transitions into neuronal differentiation. Previous single-cell analysis on NSCs proved scRNAseq to be underpowered to categorise basal inflammatory signatures in the brain (Kalamakis *et al*, 2019). To maximise the power of our interferon response analysis, we explored the specific NSC type-I IFN response by treating NSCs with IFN- β *ex-vivo*. First, applying Cycleflow (Jolly *et al*, 2022) to evaluate cell cycle progression, we show that IFN- β arrested NSCs in the G₀ quiescent state *ex-vivo* (Figs 1C and EV2A–C), mimicking the effect of IFN-increased quiescence as already suggested for the old brain (Kalamakis *et al*, 2019). We thereafter addressed the molecular response at transcriptional and post-transcriptional level of NSCs to IFN- β *ex-vivo* via Ribo-Seq (Fig 1D). Analysis of the transcriptional response identified a strong upregulation of ISGs in NSCs (Fig 1E), opposed to the suggested attenuated capacity of stem cells to build interferon responses (Wu *et al*, 2018). The 300 highest-expressed genes (Fig 1E and Dataset EV1) were used to generate a NSC-specific type-I IFN response signature further used for detection of this response in our single cell NSC-lineage from IFNAGR^{WT} and IFNAGR^{KO} young and old vSVZ.

Scoring of the NSC type-I interferon response signature indicated that the interferon response is already present in young WT individuals and it fluctuates dynamically along the lineage (Figs 1F, and EV1D and E). While intermediate progenitors (TAPs and NBs) score the lowest, stem cells and mature neurons score the highest type-I interferon response. Interestingly, stem cells and neurons are also particularly responsive in the old brain, while TAPs and NBs remain unaffected by the age-related increase of IFNs (Fig 1F, lower panel). Of note, we found expression of type-I IFN receptors in all cell types along the lineage (Fig EV1C), in addition to the recently reported expression of type-II IFN (Dulken *et al*, 2019). This underscores the relevance of interferons both in the young and the old brain and reveals neural stem cells as their preferential target to modulate neurogenesis. In agreement, stem cells lacking interferon receptors

Figure 1. Interferon signalling regulates stem cells in the young and old brain and decreases in neural progenitors.

- A Experimental layout for scRNA-Seq in young and old mice lacking interferon receptors. All mice represent T1CY (Tlx reporter, see Materials and Methods) that are either IFNAGR WT or KO. TAM (Tamoxifen). See also Fig EV1.
- B UMAP embedding showing the 15,548 single cells in this analysis with their cell types. See also Fig EV1. $n = 2$ biological replicates per age and genotype.
- C Cell cycle fractions inferred from Cycleflow in IFN- β -treated NSCs. See also Fig EV2. $n = 3$ biological replicates.
- D Schematic representation of Ribo-Seq pipeline in NSCs.
- E MA plot showing differential expression between IFN- β 16 h treated bulk cells and Control cells. Light red dots are significantly upregulated (one-sided Wald test), dark red dots are the top 300 genes selected as the “NSC Type-I Interferon Response”. $n = 4$ biological replicates.
- F Scores computed for the NSC Type-I Interferon Response signature displayed in the UMAP embedding for young cells (with colours clipped to the range seen in the lineage cells) and averaged for the cell types in our analysis at varying ages in IFNAGR^{WT} and IFNAGR^{KO} cells. $n = 2$ biological replicates per age and genotype.
- G Scores computed for the Wu *et al* (2018) intrinsic interferon response gene set displayed in the UMAP embedding for young cells (with colours clipped to the range seen in the lineage cells) and averaged for the cell types in our analysis at varying ages in IFNAGR^{WT} and IFNAGR^{KO} cells. $n = 2$ biological replicates per age and genotype.

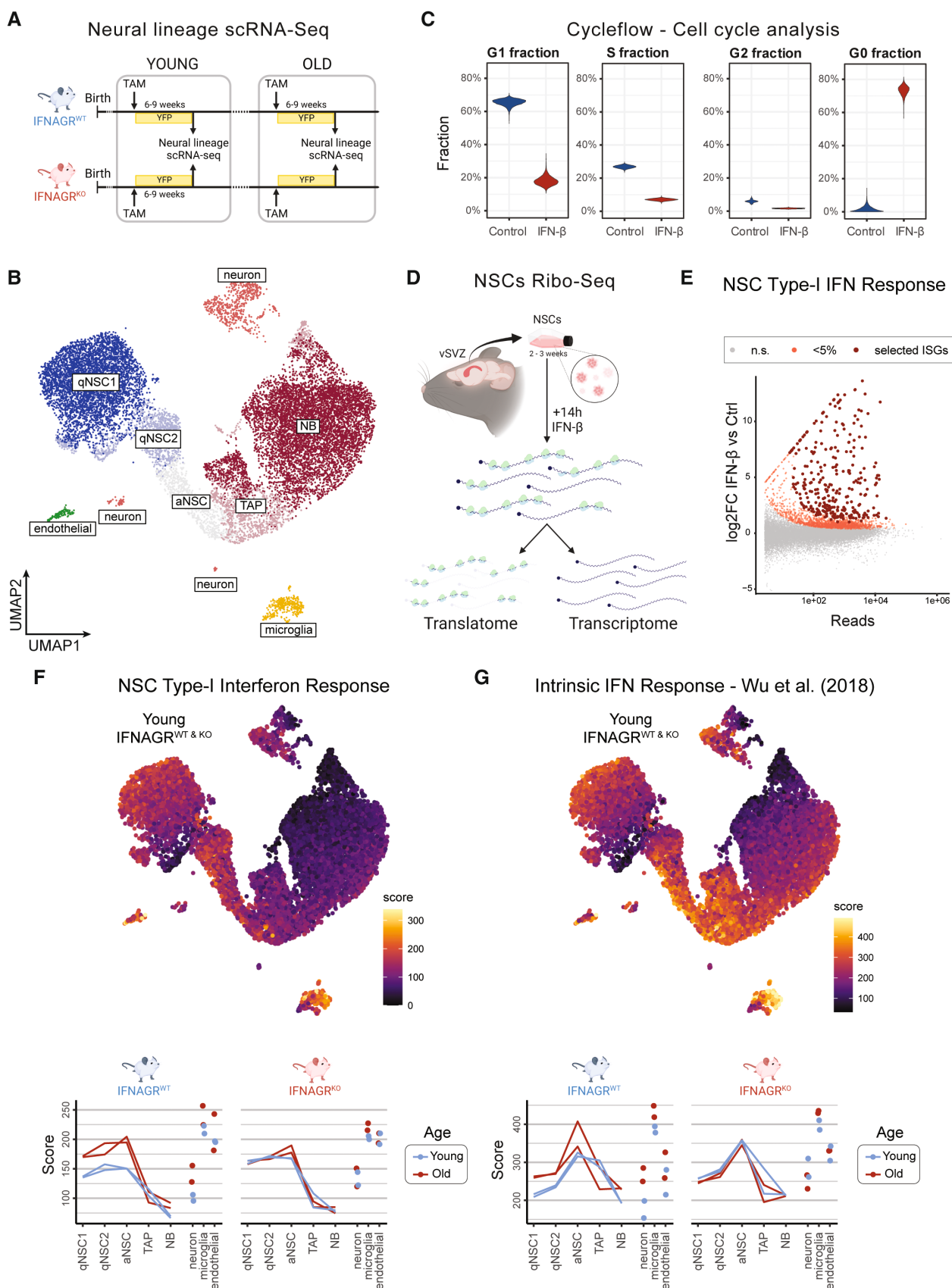


Figure 1.

(IFNAGR^{KO}) display a dysregulated type-I interferon response which remains oblivious to ageing (Fig 1F, lower panel). Strikingly, even in IFNAGR^{KO} mice, stem cells retain a higher type-I interferon response than TAPs and NBs. This supports the notion from Wu *et al* (2018) that stemness confers an intrinsic interferon response (Wu *et al*, 2018) as compared to their progeny. However, Wu *et al* (2018) proposed stem cells to be refractory to IFNs, while we show that NSCs *in-vivo* display an interferon response that relies on IFN receptors both in the young and old brain (Fig 1G). Moreover, loss of IFN receptors increased the intrinsic levels of IFN signalling. This confirms our hypothesis that interferon modulates neural stem cells already in young adults, albeit the molecular underpinnings of such regulation remains elusive.

IFN- β exerts a biphasic control of mRNA translation in NSCs

To describe the molecular mechanisms downstream of IFN in NSCs, we focused on the post-transcriptional changes exerted by these cytokines. IFNs can modulate mRNA translation in differentiated cells (Mazewski *et al*, 2020), a process that is of key importance in the regulation of quiescence in stem cells (Blanco *et al*, 2016; Baser *et al*, 2017, 2019; Tahmasebi *et al*, 2019). We thus hypothesised that IFN- β could also affect mRNA translation in NSCs, leading to their quiescence induction through G₀ arrest (Fig 1C). To address this, we examined polysome profiles and Ribo-Seq of NSCs following short (2 h) or long (14 h) exposure to IFN- β (Appendix Fig S1). Analysis of the polysome profiles of IFN- β -treated NSCs revealed a mild and transient increase followed by a strong decrease of the heavy polysomal fractions, as compared to untreated controls (Fig 2A and B). Accordingly, OPP (O-propargyl-puromycin) incorporation revealed a transient early increased followed by a profound decrease of global protein synthesis following exposure to IFN- β (Fig EV2D and E). Despite this IFN- β 's biphasic control of global translation, effects varied per gene (Fig 2C and D). Gene set enrichment analysis of translational efficiency (TE) ranked genes identified specific subsets of transcripts exhibiting either a steady enhanced or repressed translation (Figs 2C and D, and EV3A and B, Dataset EV2). mRNAs encoding DNA replication and cell cycle-related proteins were steadily repressed, in agreement with the detected arrest in G₀. Conversely, interferon stimulated genes (ISGs), crucial components of the antiviral response (Mazewski *et al*, 2020), exhibited a gradual increase of TE. This enhanced translation was confirmed by RT-qPCR of the polysomal fractions for a subset of ISGs including *Ifit3* and *Irf9* (Fig EV3C). Notably, IFN-mediated translation of ISGs had always been linked to upregulation of cap-dependent translation (Kroczyńska *et al*, 2014;

Mazewski *et al*, 2020). How translation of these ISGs is maintained despite a global downregulation of translation remains unclear. Overall, this shows that interferon induces a biphasic control of mRNA translation in NSCs beyond transcriptional activation of ISGs.

mTOR contributes to the IFN-induced biphasic control of mRNA translation

To dissect the molecular drivers of the IFN-induced biphasic control of mRNA translation, we examined the implication of mTOR. mTOR is a key checkpoint in the control of metabolic pathways, including regulation of cap-dependent translation (Liu & Sabatini, 2020). Type-I, -II and -III IFNs have been reported to activate the PI3K/mTOR pathway in differentiated cell types (Lekmine *et al*, 2003, 2004; Ivashkiv & Donlin, 2014; Syedbasha *et al*, 2020). There are also recent reports on mTOR inhibition by IFN- β and IFN- γ (Su *et al*, 2015; Vigo *et al*, 2019). To assess the impact of mTOR in the observed IFN- β 's biphasic control of global translation in NSCs, we inspected the TE of mRNAs containing the 5' terminal oligopyrimidine (TOP) motif, referred to as TOP-mRNAs (Avni *et al*, 1996). Translation of TOP-mRNAs is susceptible to mTOR activity (Thoreen *et al*, 2012), which makes it a faithful readout of mTOR activity. We observed that IFN- β controlled TOP-mRNAs in a biphasic manner, exhibiting a tendency to early upregulation and a significant downregulation of translation index (Figs 2E and F, and EV3D and Dataset EV3). Further inspection of the Ribo-Seq profiles of expressed TOP-mRNAs confirmed this biphasic regulation with a differential abundance of footprints in their coding sequence (CDS) upon IFN- β treatment (Fig 2G and H). To fully confirm the implication of mTOR, we next examined the phosphorylation state of mTOR pathway components. The same biphasic regulation of phosphorylation was observed in the downstream substrates of mTOR, ribosomal protein S6 kinase (S6K), ribosomal protein S6 (S6) and eIF4E-binding protein (4E-BP1; Fig 2I and Appendix Fig S2). We checked p-4E-BP1^{Ser65} as other 4E-BP1 sites such as Thr37 and Thr46 are priming sites and might not fully reflect the activity of 4E-BP1 (Qin *et al*, 2016). In addition, p-4 E-BP1^{Ser65} exhibits a higher degree of sensitivity to Rapamycin compared to other priming sites, serving as a better read-out for mTORC1 activity (Gingras *et al*, 2001). In particular, Thr389 phosphorylation of S6K, a direct target of mTORC1 (Ma & Blenis, 2009), confirmed the specific modulation of mTORC1 by IFN- β in NSCs.

We additionally assessed whether LARP1, a key repressor of TOP-mRNA translation, would be regulated by IFN- β in NSCs. We find that p-LARP1^{Ser498} (human^{Ser521}) and p-LARP1^{Thr492}

Figure 2. IFN- β controls mTORC1 shaping a biphasic regulation of mRNA translation in NSCs.

- A, B Representative polysome profiles of NSCs treated with IFN- β for 2 and 14 h treatment. Arrows indicate the 40S, 60S and 80S subunits of the ribosome.
- C, D Ribo-Seq results depicting translation efficiency as the interaction of log₂ fold changes (LFC) between footprints mapped to the CDS, referred to as "CDS counts", and total RNA after 2 or 14 h IFN- β treatment. FDR < 10%, LR-Test in DESeq2. Genes with a *P* value < 0.1 after FDR correction are highlighted. For associated GO terms see also Fig EV3. *n* = 4 biological replicates.
- E, F Ribo-Seq results of 2 and 14 h IFN- β treatments. 5' terminal oligopyrimidine motif-containing mRNAs (TOP-mRNAs) highlighted in blue. *n* = 4 biological replicates.
- G, H Coverage profile of ribosome-protected reads (footprints) of TOP-mRNAs in 2 and 14 h IFN- β treatment. Nucleotide 0 depicts the start codon (AUG) marking the interface between 5' untranslated region (5' UTR) and coding sequence (CDS).
- I Representative WB and phosphorylation levels quantification (log₂FC) of the mTOR-related proteins ribosomal protein S6 kinase (S6K), ribosomal protein S6 (S6) and eIF4E-binding protein 1 (4 E-BP1) from NSCs treated with IFN- β and normalised to control (*t* = 0 h). Bars represent the mean value. One-way ANOVA for the biphasic response test (See Materials and Methods for details). p-4 E-BP1^{S65} * (*P* = 0.021), p-S6^{S235/236} & p-S6^{S240/244} *** (*P* < 0.001), p-S6K^{T389} ** (*P* = 0.004). *n* = biological replicates specified. *P* $\equiv$ *P*-value.

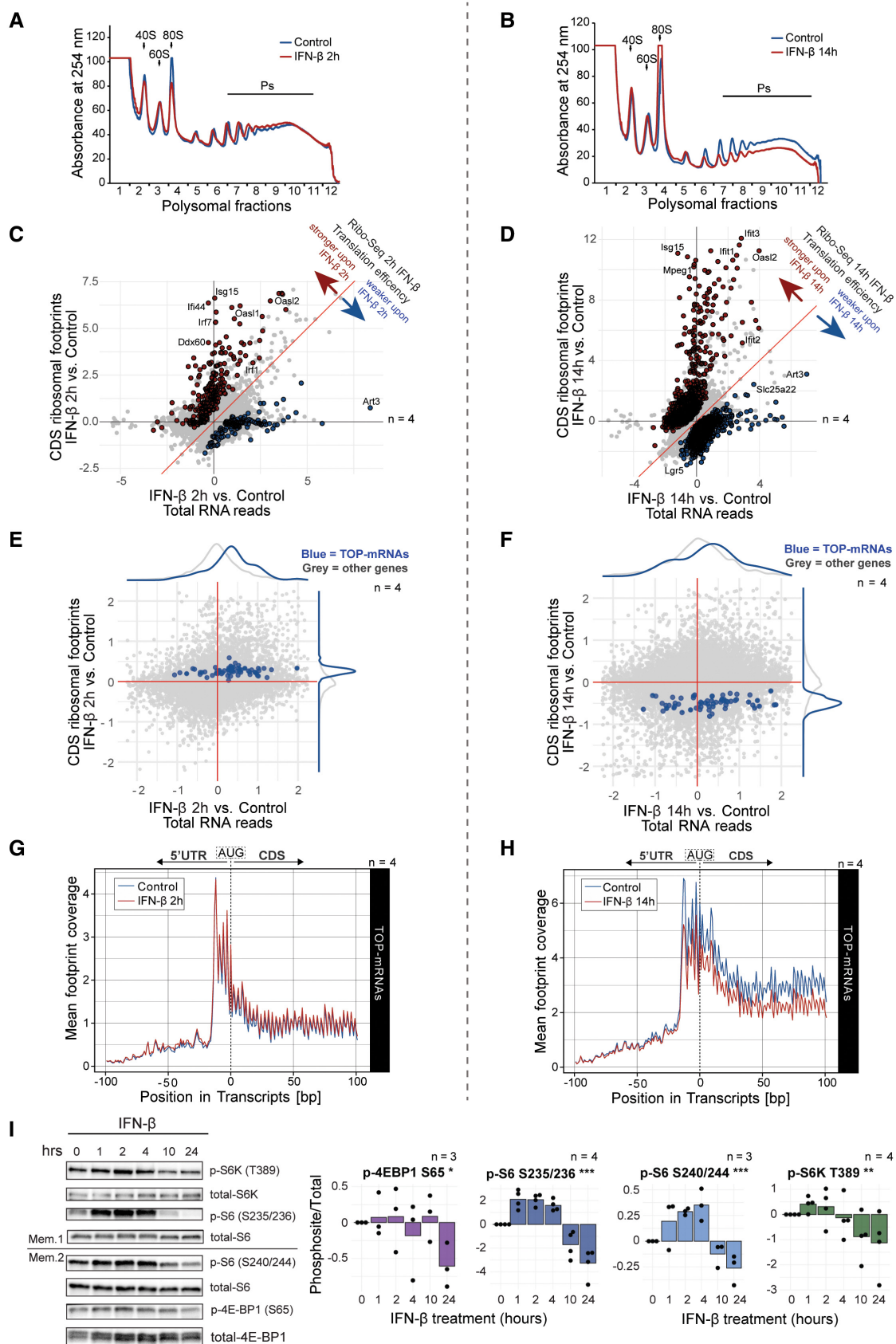


Figure 2.

(human^{Thr515}) are differentially phosphorylated at 16 h of IFN- β treatment, and no change in total LARP1 protein (Fig EV3E and Table EV1). However, as the biological relevance of these phosphosites is unclear (Jia *et al*, 2021), the role of LARP1 in regulation of TOP mRNAs translation in NSCs remains elusive.

The biphasic control of mRNA translation by IFN- β involves modulation of mTORC1 and p-eIF2 α

We next set out to address the molecular underpinnings of mTOR biphasic modulation. The control of mTOR by type-I IFN in different cell systems mainly relies on the crosstalk of the IFN-activated JAK/STAT pathway with the PI3K pathway (Platanias, 2005; Mazewski *et al*, 2020). In this scenario, IFN activates JAK1/TYK2 kinases that interact with the insulin receptor substrate 1 (Irs1), which modulates the activity of the PI3K-pathway components Akt and TSC1/2 (Platanias, 2005; Kim & Guan, 2019). We found that upon IFN- β treatment, phosphorylation of Akt^{Thr308} and TSC2^{Thr1462} not only increased, but followed a bimodal mode of regulation (Fig 3A), as for mTORC1 (Fig 2). Notably, the mTORC2-dependent p-Akt^{Ser473} site remained unaffected by IFN- β treatment (Fig EV3F). This indicates that the early crosstalk between IFN- β and the PI3K pathway upregulates mTORC1 selectively, with no effect on mTORC2 at any time of interferon exposure in NSCs. The biphasic regulation of Akt^{Thr308} suggests that prolonged IFN- β exposure in NSCs induces negative feedback loops acting upstream of Akt that ultimately inhibit mTORC1 (Hsu *et al*, 2011; Fig 3B). According to our results, these two temporal components of the biphasic regulation of mTOR rely on TSC1/2's modulation by Akt. To validate this, we generated four heterozygous TSC2 mutant NSC clones by CRISPR/Cas9 which halved their levels of TSC2 (labelled TSC2^{mut}). In addition, two NSC clones where CRISPR/Cas9 did not reduce TSC2 protein levels were used as control (labelled TSC2^{ctrl}) to exclude off-target or manipulation-derived artefacts (Fig EV4A). TSC2^{mut} NSC clones had a basally increased mTOR activity and displayed a milder inhibition of global translation in response to IFN- β (Fig EV4B

and Appendix Fig S3). As hypothesised, the biphasic regulation of mTORC1 activity, measured by p-S6K^{Thr389}, was strongly blunted in TSC2^{mut} NSCs (Fig EV4B). Interestingly, the biphasic regulation of global protein synthesis was not detected in TSC2^{mut} NSCs. These mutant cells only exhibited a downregulation of global protein synthesis upon longer exposure to IFN (Fig EV4C). This uncovers the PI3K-crosstalk and inhibitory feedback loops converging on TSC1/2 as the main drivers of the early regulation, while unveiling additional mechanisms contributing to the late shut-down.

Along with the inhibitory feedback loops, transcriptionally upregulated ISGs, such as PKR, contribute to the late inhibition of global mRNA translation induced by mTORC1 (Ivashkiv & Donlin, 2014). PKR (*Eif2ak2*) is activated by dsRNA and phosphorylates the translation factor eIF2 α at Ser51, resulting in a global inhibition of mRNA translation initiation (Gal-Ben-Ari *et al*, 2019). Certainly, NSCs exposed to IFN- β induced an increase in p-eIF2 α ^{Ser51} (Fig 3C), which intensifies the inhibition of mRNA translation already exerted by late inhibition of mTORC1. Of note, extended exposure to IFN- β also increased PKR protein levels and phosphorylation in poorly-described residues (Piazzi *et al*, 2020; Fig EV4D and Table EV1), suggesting a late PKR activation. In addition, activated PKR can inhibit Irs1, contributing to the late mTORC1 feedback inhibitory loops (Nakamura *et al*, 2010). Indeed, selective inactivation of the p-eIF2 α ^{Ser51} response by the Intrinsic Stress Response Inhibitor (ISRIB; Sidrauski *et al*, 2015) reduced the late downregulation of global protein synthesis in IFN- β -treated NSCs (Fig EV4E). This shows how the late phosphorylation of eIF2 α upon sustained IFN- β exposure contributes to the biphasic control of mRNA translation and suggests PKR as its upstream regulator.

In addition to the regulation of global mRNA translation, p-eIF2 α ^{Ser51} is associated with the alternative use of upstream open reading frames (uORFs) located in the 5' untranslated region (5'UTR; Young & Wek, 2016). IFN- β increased local 5'UTR footprint density in a subset of genes while maintaining unaltered levels in the CDS, indicating alternative uORF usage (Fig 3D). Notably, ISGs were not among these transcripts with increased uORF usage.

Figure 3. IFN- β 's biphasic control of mTORC1 uncouples growth and cell cycle and represses Sox2 translation via its 5' PRM motif.

- Representative WB image and quantification (log2FC) of p-TSC2^{Thr1462} & p-Akt^{Thr308} from IFN- β -treated NSCs normalised to control ($t = 0$ h). Bars represent the mean value. One-way ANOVA for the biphasic response test (See [Materials and Methods](#) for details). p-TSC2^{Thr1462} ***($P < 0.001$), p-Akt^{Thr308} **($P = 0.003$). $n = 4$ biological replicates.
- Schematic representation of the type-I interferon signalling pathway and its crosstalk with the receptor tyrosine kinase (RTK) pathway. Activatory (blue) and inhibitory (red) phosphosites are depicted. Red inhibitory lines depict late mTORC1-derived inhibitory feedback loops.
- Representative WB images and quantifications (log2FC) of p-eIF2 α ^{Ser51} in IFN- β -treated NSCs normalised to control ($t = 0$ h). Bars represent the mean value. Spearman's rank correlation test. p-eIF2 α ^{Ser51} **($P = 0.003724$). $n = 4$ biological replicates.
- Coverage profile of ribosome-protected reads (footprints) in genes with upregulated canonical (upper) or alternative upstream (lower) ORFs upon extended IFN- β treatment. Nucleotide 0 depicts the start codon (AUG) marking the interface between 5' untranslated region (5' UTR) and coding sequence (CDS).
- Representative WB image and quantifications (log2FC) of p-Rb1^{Ser780} from IFN- β -treated NSCs normalised to control ($t = 0$ h). Bars represent the mean value. Spearman's rank correlation test. p-Rb1^{Ser780} ***($P = 5.37 \times 10^{-6}$). $n = 3$ biological replicates.
- Schematic representation of the prevailing signalling pathways driving the early inhibition and late activation of mTORC1.
- Polysome profiling (RT-qPCR) of Sox2 mRNA upon 2 and 14 h IFN- β treatment. Hyphens represent mean of biological replicates. Arrows represent the 40S, 60S and 80S subunits of the ribosome. Two-way ANOVA with Šídák's multiple comparison test was computed. Two hours fraction 11 * ($P = 0.0206$), 14 h fraction 3 ** ($P = 0.0042$), fraction 9 ** ($P = 0.0028$), fraction 10 *** ($P = 0.0003$). $n = 4$ (2 h) or 3 (14 h) biological replicates.
- Experimental layout of IFN and Torin1 treatments (upper panel). Torin1 was administered 12 and 9 h before collection. Schematic representation (lower panel) of the 5'UTR constructs priming the *renilla* luciferase. TOP = 5'Terminal Oligopyrimidine motif; PRM = 5' Pyrimidine Rich Motif.
- Luciferase activity controlled by the upstream 5'UTR fragment from Sox2 (WT and mutant), *Rps21* (WT and mutant) and *Actb*. Treatments were applied for 12 h. Data are normalised to vehicle and are represented as mean $\pm$ SD. $n = 6$ biological replicates. Two-way ANOVA with Tukey's multiple comparisons test (P -value specified).

Data information: $P \equiv P$ -value.

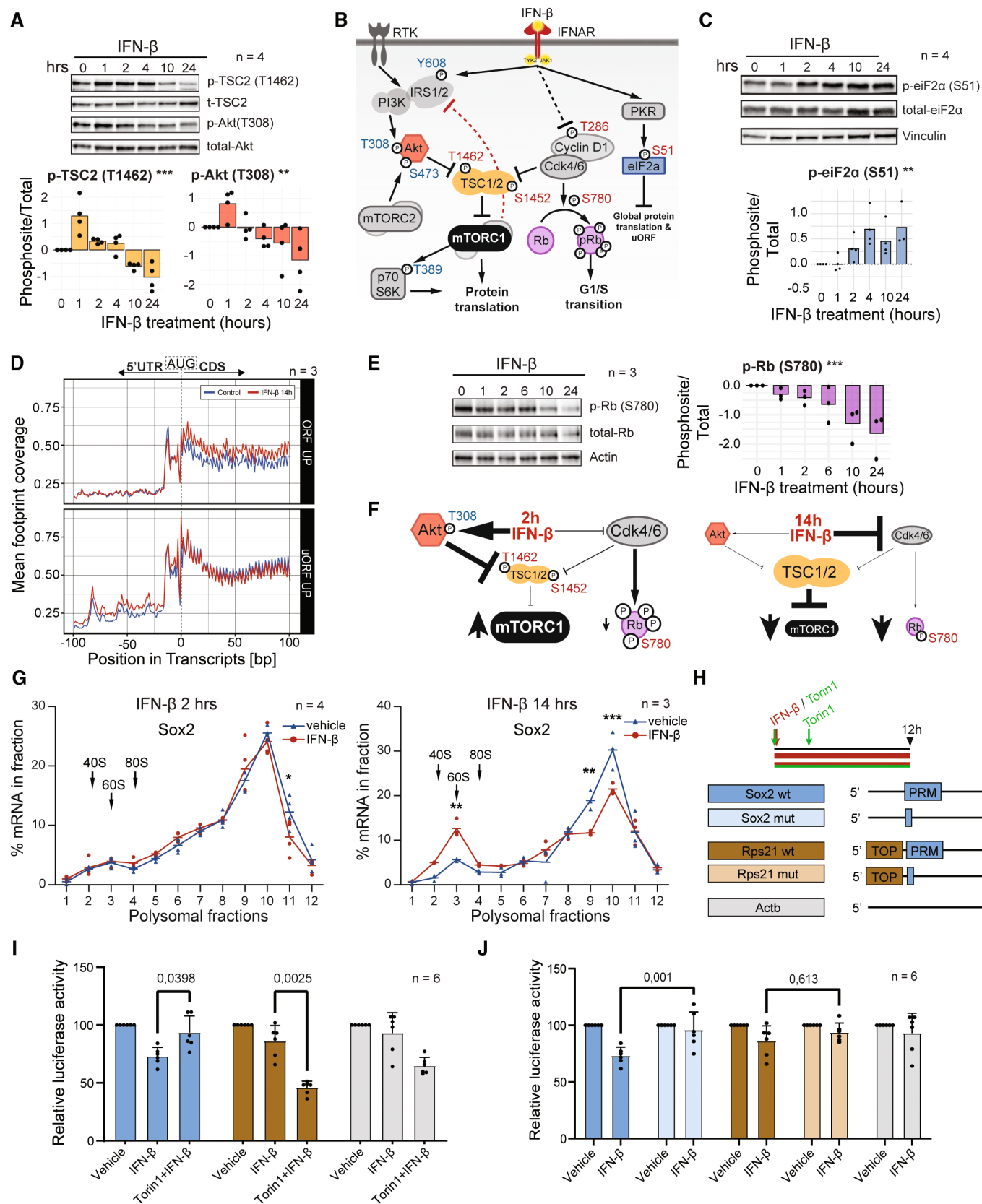


Figure 3.

Consistently, while translation upregulation in the CDS shifted mRNAs to heavy polysomes, a higher ribosomal density in uORFs shifted the associated mRNAs into light polysomes (Fig EV4F).

IFN- β transiently uncouples mTOR and cell cycle in NSCs

It did not remain unnoticed that the transient increase of mRNA translation induced by IFN coincides with the inhibition of cell cycle in NSCs (Fig 1C). We therefore assessed how the type-I IFN response uncouples translation and cell cycle in NSCs. Given that NSCs remained significantly longer in G₁ upon IFN- β treatment (Fig EV2A), we hypothesised that IFN could trigger the inhibition of the G₁/S checkpoint regulators Cdk4/6. Cdk4/6 have recently emerged as a dual regulator of cell growth and proliferation via TSC2, and together with CyclinD1 they modulate proliferation of NSCs and neurogenic progenitors in the adult brain (Lange *et al*, 2009; Artegiani *et al*, 2011; Romero-Pozuelo *et al*, 2020). Cdk4/6 control the G₁/S transition by phosphorylation of Rb1 and consequent expression of S-phase genes (Topacio *et al*, 2019). We found that IFN- β reduced p-Rb^{Ser780}, a Cdk4/6-dependent phosphosite, in a progressive manner in NSCs *ex-vivo* (Fig 3E). Similarly, IFN- β also reduced the Cdk4/6-dependent phosphorylation at TSC2^{Ser1452} (Romero-Pozuelo *et al*, 2020), consistent with the IFN-mediated inhibition of Cdk4/6 (Fig EV4G). This confirms that NSCs transiently uncouple mRNA translation and cell proliferation upon exposure to IFN- β . Interestingly, IFN- β treatment also led to the phosphorylation of CyclinD1 at Thr286 (Table EV1). This phosphosite is involved in cytoplasmic translocation and degradation of CyclinD1 (Guo *et al*, 2005), presenting cyclinD1 as a direct target of the IFN response in NSCs.

Overall, our data provide molecular insights of the biphasic control of mTOR by IFNs in stem cells. We show that an early activation of PI3K-Akt masks a gradual inhibition of Cdk4/6 that only gains relevance upon extended IFN- β incubation and complements the late inhibitory feedback loops shutting down mTOR (Fig 3F).

Dual control of mTOR and cell cycle by IFN- β represses Sox2 translation via its 5' pyrimidine-rich motif

A dual regulation of mTOR and cell cycle is needed to repress translation of Sox2 and allow exit of an activated stem cell state (Baser *et al*, 2019). However, homeostatic cues orchestrating this dual regulation in NSCs are yet missing. We therefore wondered whether type-I IFN, via its dual effect on TOR and cell cycle, would be one upstream post-transcriptional regulator of Sox2 expression. To test this, we first examine expression of Sox2 in polysomal profiles obtained from untreated and IFN- β -treated NSCs. As opposed to TOP-mRNAs (Fig EV3D), Sox2 remained oblivious to the initial increase of mTORC1 activity and even exhibited a subtle repression at 2 h after IFN- β (Fig 3G, left panel). Notably, Sox2 translation was strongly repressed in NSCs following long exposure to IFN- β (Fig 3G, right panel). This repression was also reflected in a mild reduction in Sox2 protein (Fig EV5A). Since IFN- β -mediated repression was much stronger than the one we previously measured following TOR inhibition and cell cycle arrest (Baser *et al*, 2019), we wondered whether the early decoupling of mTORC1 and cell cycle exerted by IFN- β would be required to more effectively repress Sox2 translation. In order to evaluate this hypothesis, we blocked the

early activation of mTOR with Torin1 (Fig 3H). To simultaneously examine the involvement of the 5'UTR of Sox2 in this repression, we established a luciferase assay in which we cloned the 5'UTR region of Sox2 as well as of the TOP-containing mRNA *Rps21* upstream of the *Renilla* luciferase CDS (Fig 3H). Sole mTOR inhibition by Torin1 treatment effectively repressed translation of the TOP-mRNA *Rps21* but failed to repress Sox2 (Fig EV5B and C), in agreement with previous findings (Baser *et al*, 2019). Conversely, dual control of mTOR and cell cycle by IFN- β treatment effectively repressed Sox2 (Fig 3I). However, IFN- β -mediated repression of Sox2 was prevented by Torin1 (Fig 3I). We decided to use Torin1 as, differently to rapamycin, it fully inhibits mTORC1 activity (Thoreen *et al*, 2012). However, we still observe the same results using rapamycin (Fig EV5D and E) confirming the selective relevance of mTORC1 in the IFN- β response in NSCs. In addition, given the effect of increased p-eIF2 α ^{Ser51} in protein synthesis in the IFN- β response in NSCs (Fig 3C), we wondered if p-eIF2 α would also influence Sox2 translation. To test this, we blocked the effect of p-eIF2 α ^{Ser51} using ISRIB (Sidrauski *et al*, 2015). Blocking the ISR in the presence of IFN- β restored global translation levels (Fig EV4E) and rescued the repression of translation of *Rps21*, but failed to significantly affect Sox2 translation (Fig EV5F). This underscores the importance of IFN- β as a *bona-fide* regulator of Sox2 translation by selective uncoupling of cell cycle and mTORC1 activity in NSCs.

Furthermore, we recently proposed that a pyrimidine-rich motif (PRM) present in the 5'UTR of relevant stem genes could be responsible for post-transcriptional repression of factors involved in stem cell exit (Baser *et al*, 2019). PRM is present in different mRNAs downregulated at the onset of differentiation such as Sox2 and Pax6 and can co-localise with the TOP motif as in the case of *Rps21* (Baser *et al*, 2019). To evaluate the contribution of the PRM to the repression of Sox2, we mutated the PRM using *Rps21* as control (Fig 3H). The deletion of the PRM attenuated the repression of Sox2 exerted by IFN- β in NSCs (Fig 3J) and did not affect expression of the PRM-mutated *Rps21*, driven by the prevalent TOP motif. This highlights the role of the 5'UTR PRM motif in regulating translation of pioneer factors such as Sox2 in stem cells.

Interferon regulates stem cell function and fine-tunes progenitor production across all ages

Altogether we have shown that IFN targets NSCs in the young and old brain and that IFN blocks stem cell activation through a biphasic control of mRNA translation and repression of Sox2. In addition, we show that the interferon response of NSCs in the natural environment of the brain relies on external interferons both in young and aged individuals (Fig 1F). In order to examine how IFNs fine-tune the adult vSVZ's neurogenesis across the animal's life-span, we examined NSC dynamics in young and old IFNAGR KO mice. To this end, we quantified the pool of NSCs (Prom1⁺GLAST⁺) within the vSVZ via flow cytometry and compared them to wild-type (WT) controls. In addition, we used the fraction of active NSCs, that is, the number of cycling cells among the BrdU-retaining cells, previously acquired in WT and IFNAGR KO animals³. These data were analysed using a mathematical model previously established and validated in wild-type animals (Kalamakis *et al*, 2019). This model describes the dynamics of quiescent, active and neurogenic progenitor populations and allows linking those to age-dependent changes

in the cell parameters: (i) activation rate and (ii) self-renewal probability (Fig 4A and Appendix Supplementary Methods). For wild-type data, as already reported (Kalamakis *et al*, 2019), it pointed to a profound reduction of the NSC activation rate and a slight increase in self-renewal probability as the parameters responsible for a deceleration in NSC decline in old mice. Notably, this deceleration is needed to maintain a minimal amount of NSCs that otherwise would be fully depleted at older ages. In IFNAGR KO, NSC depletion exhibited faster dynamics and yet stabilised at counts of around 150 cells per mouse, as in WT mice (Fig 4B). The mathematical model applied now to the IFNAGR KO data predicted a constant activation rate of NSCs and an earlier time-dependent regulation of the

probability of NSC self-renewal (Fig 1C). The latter explained the observed saturation in the NSC decline. This unveils self-renewal as a key second layer of regulation, following modulation of the activation rate, which had been previously neglected (Kalamakis *et al*, 2019).

Last, given the relevance of extrinsic IFNs for NSCs in youth and ageing (Fig 1), we further explored its potential role as a therapeutic target. In our models, we know that the activation rate of NSCs directly influences the production of progenitors (Appendix Supplementary Methods). Young IFNAGR KO mice have a lower activation rate of NSCs than WT, suggesting a decreased progenitor production. Older IFNAGR KO mice however have a higher activation rate

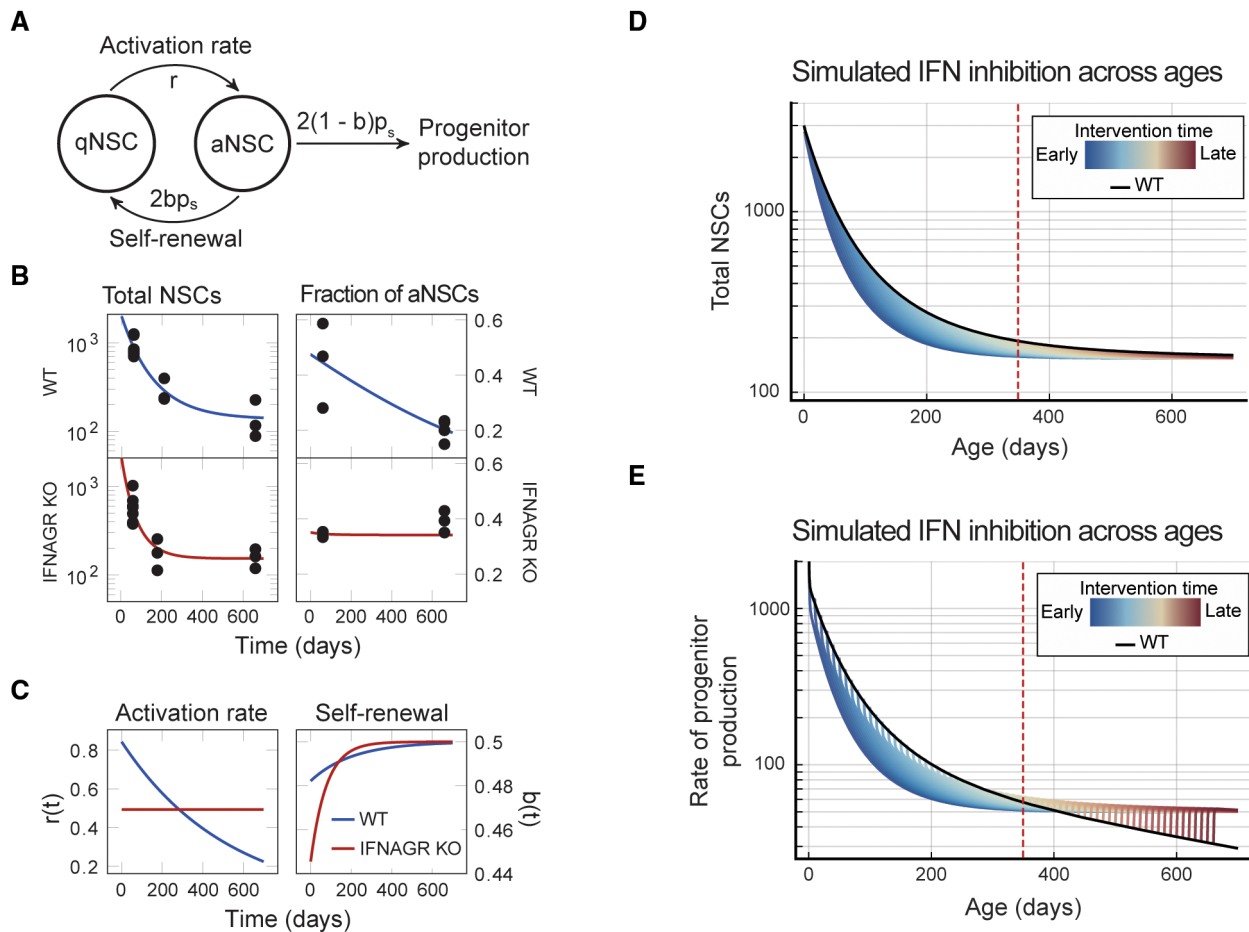


Figure 4. Interferon regulates stemness and fine-tunes progenitor production at all ages.

- A** Graphical depiction of the mathematical model describing the activation (at rate r) of quiescent neural stem cells (qNSC) into active NSC (aNSC) followed by their progression through cell cycle (at rate p_s) into either two downstream progenitors (at probability $1 - b$) or two NSCs (at probability b) that return to quiescence. In models with time-dependent parameters, $b \equiv b(t)$ and $r \equiv r(t)$. See Appendix Supplementary Methods for details.
- B** vSVZ niche FACS quantifications of total NSCs (Total NSCs) & immunofluorescence quantification of aNSCs (Fraction of aNSCs) in WT or IFNAGR KO mice at 2, 7 and 22 months old (MO). Data from individual mice are plotted. Inferred dynamics are depicted as a blue (WT) or red (IFNAGR KO) line over time. Sample sizes: WT total at 60 days: 8 mice, WT total at 210 days: 3 mice, WT total at 660 days: 3 mice, WT fraction aNSCs at 60 days: 3 mice, WT fraction aNSCs at 660 days: 4 mice, IFNAGRKO total at 60 days: 8 mice, IFNAGRKO total at 180 days: 3 mice, IFNAGRKO total at 660 days: 3 mice, IFNAGRKO fraction aNSCs at 60 days: 3 mice, IFNAGRKO fraction aNSCs at 660 days: 3 mice.
- C** Inferred parameters are depicted as a blue (WT) or red (IFNAGR KO) line over time.
- D** Induced interferon knockout simulations (coloured, each colour represents a different intervention timepoint) against wild-type simulations (black) showing the total number of stem cells across age. Dashed red line denotes age 350 days.
- E** The rate at which progenitors are produced ($2.25emp_s \cdot 25em(1-b) \cdot 25emaNSC$) from NSCs for induced interferon knockout simulations with interferon dependent self-renewal (coloured, each colour represents a different intervention timepoint) and WT simulations (black). Dashed red line denotes age 350 days.

than WT, suggesting increased progenitor production (Fig 4C). This response suggests a possibility for improving progenitor production in WT animals by selecting an optimal time point to block IFN function. To investigate this, we simulated a modified model with IFN-dependent switches in model parameters at arbitrary time points (Appendix Supplementary Methods). The intervention was modelled by switching the activation rate and self-renewal parameters estimated from WT to the parameters estimated for IFNAGR KO mice at a given intervention time point. This best mimic an acute clinical intervention for systemic neutralisation of interferons. Interventions simulated at young ages resulted in a significant loss of NSCs, dropping from thousands to hundreds in the short term (Fig 4D). However, this effect diminished with time resulting in similar cell counts in old age (Figs 4D and EV5A). Since total NSCs are not necessarily indicative of proper functioning, we also computed the cell flux from aNSC to progenitors (progenitor production). Simulations of young age interventions showed drastically reduced progenitor production on a short time scale, which ultimately changed to an increased progenitor production in old ages (Fig 4E). Strikingly, interventions in older mice (after ~300 days) showed to always be beneficial. We next compared the summary effect of an intervention in terms of the life-long progenitor production. The interventions at young ages had diminished progenitor production when compared to WT, while the maximal benefit of the life-long production was achieved by intervention at about 350 days of age (Figs 4E and EV5G). Curiously, this was also the age at which the activation rate for WT dropped below the constant IFNAGR KO activation rate (Fig 4C).

Overall, this shows that there may be benefits to progenitor production from blocking interferon response at increasing ages, while it may have strong adverse effects on short-term progenitor production and stem cell counts when intervening too early. In agreement with our predictions, local blockade of CXCL10 signalling in the aged brain as well as neutralisation of IFN (Baruch *et al*, 2014) increased progenitor production from vSVZ-NSCs (Blank *et al*, 2016; Kalamakis *et al*, 2019). CXCL10 is induced in endothelial cells upon systemic IFN- β , which itself is not able to cross the blood–brain barrier (Blank & Prinz, 2017). Altogether, our predictions show that only a late intervention blocking IFN signalling is beneficial and unveils the relevance of homeostatic interferons for a life-long healthy adult neurogenesis.

Discussion

Interferons are bona fide regulators of stem cell homeostasis at all ages and potential candidates to repair the ageing brain

Stem cells exhibit an intrinsic IFN signature, that is, constitutive expression of ISGs independent of interferon receptor stimulation, which is unique to stem cells and protects them against viral infections (Wu *et al*, 2018). In addition, Wu *et al* also reported that stem cells are refractory to extrinsic IFNs. Our study shows that in the natural environment of the brain, type-I interferon ISGs are highest expressed in NSCs but also present, albeit to a lower extent, in fully differentiated new-born neurons. This IFN signature in neural stem cells relies on expression of IFN receptors both in the old and the young brain and modulates neural progenitor production along the

lifespan of the individual. Our data also reveal that neural stem cells are the preferred target and indeed responsive to IFNs both *in-* and *ex-vivo*, as it is the case for their fully differentiated counterparts. It is however the intermediate maturation stages that shutdown expression of these ISGs. As opposed to previous reports claiming that the ability to respond to IFN ligands is acquired upon exit of stemness (Wu *et al*, 2018), our single cell transcriptome data show that intermediate progenitors are not responding to interferons neither in the young nor in the ageing brain. Thus, intermediate progenitor stages are refractory to extrinsic interferon and probably represent the most sensitive cells to viral infections along the neural lineage. We believe that during brain development the high number of intermediate progenitors could contribute to viral infections being a life-threatening risk at this age. In addition, our models reveal the blockade of external IFN signalling to be harmful for the young vSVZ albeit beneficial in ageing. This is supported by previous reports blocking IFN signalling in ageing (Baruch *et al*, 2014; Kalamakis *et al*, 2019) despite the recently reported IFN memory in stem cells (Haas *et al*, 2017). Altogether, our combined single-cell transcriptomics and mathematical modelling underscore the relevance of defining a critical time of intervention and unveils a previously neglected role of interferon signalling in the homeostasis of the young adult brain.

Type-I IFN induces stem cell quiescence by a biphasic control of mRNA translation and cell cycle exit

IFNs modulate stem cells across different tissues but the molecular underpinnings of this regulation are yet poorly described (Kalamakis *et al*, 2019; Zhang *et al*, 2020; Demerdash *et al*, 2021; Hou *et al*, 2021). Performing polysome profiles and ribosome footprinting on IFN- β treated NSCs we find that IFN- β induces a biphasic control of mRNA translation. This regulation involves a transient activation of mTORC1 followed by its inhibition, as opposed to the previously reported unidirectional modulation of mTOR by type-I IFN (Mazewski *et al*, 2020). Of note, recent studies highlighted the importance of a precise pharmacological regulation of mTOR activity in NSCs, since hyperactive mTOR is associated with Alzheimer's Disease both in patients and mouse models (Nicaise *et al*, 2020). Future studies should address the potential role of this biphasic regulation of mTOR in brain repair. We further show that the transient activation of mTORC1 is the result of the crosstalk between IFN- β signalling and PI3K. This transient activation of mTORC1 triggers inhibitory feedback loops acting upstream of Akt that, together with a delayed activation of PKR and phosphorylation of eIF2 α , ultimately lead to the late and profound inhibition of mRNA translation and global protein synthesis. Whether this biphasic response is a hallmark of stem cells beyond the brain will be subject for future investigations.

Additionally, our data suggest that IFN- β treatment in NSCs not only induces the expression but also activates PKR in a dsRNA-independent manner. This is an undescribed feature of IFNs in stem cells as a similar response was only proposed in an interferon-selected carcinoma line harbouring oncogenic mutations (Su *et al*, 2007). Interestingly, high levels of p-eIF2 α are also associated to self-renewal and quiescence induction in embryonic and adult stem cells (Friend *et al*, 2015; Zismanov *et al*, 2016). As a whole, these pathways converge in a profound shut down of translation that

keeps NSCs dormant. Similarly, chronic type-I IFN promotes quiescence after a transient activation of haematopoietic stem cells (HSCs; Pietras *et al*, 2014). Future studies should address whether this transient activation of HSCs by IFN is also driven by a biphasic modulation of mTOR.

Supporting the induction of dormancy, we find that IFN- β shifts NSCs to a quiescent G₀ state, as opposed to the previously reported increase in cell-cycle length in quiescent NSCs (Daynac *et al*, 2016). Remarkably, inhibition of Cdk4/6 upon IFN- β treatment transiently uncouples mTOR and cell cycle in NSCs. A similar uncoupling of proliferation and protein synthesis was observed upon differentiation of skin stem cells, but the drivers of this response remained elusive (Blanco *et al*, 2016). Of note, senescent cells, a hallmark of ageing (Fulop *et al*, 2018; Zhu *et al*, 2021), steadily uncouple mRNA translation and cell cycle (Payea *et al*, 2021). Differently to NSCs, the late coupling of translation and cell cycle has not been described in senescence and might reveal a stem cell-related feature to avoid senescence induction. Intriguingly, IFN- β is one of the main drivers of senescence. Cytosolic chromatin fragments present in senescent cells activate cyclic GMP-AMP synthase (cGAS) that, in turn, triggers the production of inflammatory factors including IFN- β , thereby inducing paracrine senescence (Glück & Ablasser, 2019). Although the prevalence of senescent cells increases with age in the neurogenic niches in the brain (Molofsky *et al*, 2006; Fernández-Fernández *et al*, 2012; Ogrodnik *et al*, 2019; Jin *et al*, 2021), how NSCs are protected from senescence remains unknown. Overall, our study describes the molecular underpinnings of interferon signalling in NSCs and reveals previously undescribed features of the IFN- β response in stem cells.

Type-I IFN induces a biphasic control of mTOR that represses Sox2 translation in NSCs

We recently reported that a dual regulation of mTOR and cell cycle is needed to repress translation of Sox2 in NSCs (Baser *et al*, 2019). We now identify type-I IFN as a regulator of this response. Furthermore, we narrow down the nature of this repression to the transient activation of mTORC1 and the presence of the recently described pyrimidine-rich motif (PRM; Baser *et al*, 2019) present on the 5'UTR of Sox2. In this recent study, we addressed the role of repression of Sox2 translation in the transition of an activated stem cell into a differentiated neuroblast (Baser *et al*, 2019). Sox2 levels are however likewise reduced in quiescent NSCs to avoid replicative stress (Marqués-Torrejón *et al*, 2013). The determinants of directionality into a differentiated progenitor or a quiescent stem cell state following repression of Sox2 remain subject of future studies. Notably, Sox2 expression is reduced in the ageing brain, correlating with increased quiescence in NSCs (Carrasco-Garcia *et al*, 2019).

Collectively, our findings unveil interferons as *bona fide* regulators of stem cell function during homeostasis as well as in ageing. Mechanistically, IFN- β arrests NSCs at G₀ of the cell cycle and drives a transient TOR activation followed by a profound decrease of TOR activity and global protein synthesis. This biphasic regulation is orchestrated by mTORC1, PKR and p-eIF2 α . In addition, we show that the transient increase of TOR activity is required for post-transcriptional repression of PRM-containing transcripts, such as Sox2, that contribute to the induction of quiescence in stem cells. This exit from activation after a life-long exposure to interferons

results in a more quiescent old brain, when interferon signalling is found at highest. Future studies should unveil the potential uniqueness of this biphasic response in stem cells across different tissues and its relevance for the prevention of senescence in stem cells. Our study indicates that IFN's control of the activation rate and self-renewal of NSCs adapts the output of differentiated progenitors to demand. In young brains, with high demand, interferon would increase the output, and vice versa, decrease the number of generated progenitors in the less active ageing brain. Therefore, while the loss of IFN signalling is beneficial in the old brain, it is detrimental in young individuals. The fact that the intrinsic expression of ISGs required as antiviral defence even slightly increases after blocking interferon receptors, discloses IFNs as potential therapeutic target to improve stem cell homeostasis and repair in the brain without compromising antiviral response. However, intervention should be timed only after reaching advanced age.

Materials and Methods

Mice

C57BL/6 male mice were purchased from Janvier or bred in-house at the DKFZ Center for Preclinical Research. Male IFNAGRKO (Huang *et al*, 1993; Müller *et al*, 1994; IFNAR^{-/-}IFNGR^{-/-}) were backcrossed to a C57BL6 background. Male TiCY mice (Tlx-CreERT2-YFP; Liu *et al*, 2008; TiCY [B6-Tg(Nr2e1-Cre/ERT2)1GscGt (ROSA)26Sortm1(EYFP)CosFastm1Cgn/Amv]) were crossed with IFNAGRKO mice to generate TiCY-IFNAGRKO mice ([B6-Tg(Nr2e1-Cre/ERT2)1Gsc Gt(ROSA)26Sortm1(EYFP)CosFastm1CgnIfnar1tm1AgtIfngr1tmAgt/Amv]). Animals were housed under standard conditions and fed *ad libitum*. All procedures were approved by the Regierungspräsidium Karlsruhe.

Cell culture and treatments

For NSC isolation, the subventricular zones from 8 to 12 weeks old male C57BL/6 mice were isolated as described (Mirzadeh *et al*, 2010). The tissue was dissociated using the Neural Tissue Dissociation Kit with Papain (Miltenyi Biotec) following the manufacturer's instructions. Cells were cultured in Neurobasal A Medium supplemented with 2% B27 Supplement serum-free, 1% L-Glutamine (all from ThermoFisher), 2 μ g/ml of heparin, 20 ng/ml of human basic FGF (ReliaTech) and 20 ng/ml of human EGF (Promokine). Cells were maintained in a 37°C, 5% CO₂ incubator.

For the treatment with interferon, mouse recombinant IFN- β (Millipore) diluted in DPBS with 0.1% bovine serum albumin BSA (Roche) were added directly to the culture media at final concentration of 40 μ g/ml for the indicated time. Control cells were treated with the same volume of 0.1% BSA in PBS.

For the treatment with Torin1 (Cay10997), Torin1 was dissolved in 100% DMSO and added to cells at 250 nM for the indicated times. Control cells were treated with the same volume of DMSO. For the treatment with Rapamycin (Cay13346), Rapamycin was dissolved in 100% DMSO and added to cells at 100 nM for the indicated times. Control cells were treated with the same volume of DMSO. For the treatment with ISRIB (Sigma), ISRIB (Sidrauski *et al*, 2015) was dissolved in 100% DMSO and added to cells at 500 nM for the

indicated times. Control cells were treated with the same volume of DMSO.

CRISPR-mediated generation of TSC2^{+/-} NSCs

CRISPR-Cas9 gRNAs targeting exon 2 of *Tsc2* (ENSMUSG000 00002496) were designed using the CCTop target online predictor (Stemmer *et al*, 2015) and cutting efficiency was evaluated by T7EI Assay using T7 Endonuclease I (NEB). We selected the TSC2Ex2-gRNA (TGTTGGGATTGGGAACATCGAGG) and cloned it into the Cas9-containing plasmid pSpCas9(BB)-2A-GFP (Addgene) as described (Ran *et al*, 2013; see Table EV2).

For nucleofection, 1×10^6 NSCs were mixed with 2.5 µg of corresponding DNA using Amaxa P4 Primary Cell 4D-Nucleofector X Kit S (Lonza) with CA137 programme in a 4D-Nucleofector X Unit (Lonza). Cells were incubated for 24 h at 37°C and 5% CO₂. GFP-positive single NSCs were FACS-sorted into 96-well plates. Single NSC colonies were expanded and TSC2 abundance was checked by western blot (see section below).

Flow cytometry analysis

For the flow cytometry analysis, vSVZ single cell suspensions from mice at the specified ages were generated and stained for flow cytometry analysis as described (Llorens-Bobadilla *et al*, 2015). Briefly, vSVZ was microdissected using the Neural Tissue Dissociation kit with Trypsin and Gentle MACS dissociator (Miltenyi). Cells were stained with the following antibodies: O4 APC-Cy7 (1:100), GLAST (ACSA-1)-PE (1:50) (all from Miltenyi), Ter119 APC-Cy7 (Biolegend; 1:100), CD45 APC-Cy7 (BD; 1:200), Prominin1-APC (eBioscience; 1:75), Alexa488::EGF (Invitrogen; 1:100), and Sytox Blue (Invitrogen, 1:500). For analysis, we size selected the vSVZ cells and excluded for doublets, dead cells and CD45⁺/Ter119⁺/O4⁺ cells as recently described (Kalamakis *et al*, 2019). Signal acquisition was performed on a BD FACSCanto II at the DKFZ Flow Cytometry Facility and results were analysed using FlowJo v.10.

Cell cycle analysis – CycleFlow

Cell cycle was assessed using CycleFlow as described (Jolly *et al*, 2022) with some modifications. CycleFlow was applied to NSCs with or without a pre-treatment of IFN-β. Twenty-four hours after cell seeding, either vehicle or IFN-β was added to NSCs for 17 h (this step was skipped in the non-pre-treated NSCs). Then, EdU from the Click-iT Plus EdU Flow Cytometry Assay Kit (ThermoFisher) was added at 10 µM. Cells were incubated 1 h at 37°C 25% CO₂. Cells were collected, washed with DPBS and resuspended in pre-warmed medium containing either vehicle or IFN-β. Cells were then divided in different wells to be incubated and collected at the indicated incubation times (Fig EV2B). At the collection time, cells were collected, dissociated (Accutase[®], Sigma) and stained for viability with Zombie Red (Biolegend) following the manufacturer's recommendations. After that, cells were washed and PFA-fixed using the Click-iT Plus EdU Flow Cytometry Assay Kit (ThermoFisher). Fixed cells were kept in 90% methanol DPBS at -20°C until completion of the time-course. Upon all collections, the Click-iT reaction was performed following the manufacturer's recommendations in combination with a final staining with the DNA dye FxCycle Violet

reagent (ThermoFisher; 1:1,000). Signal acquisition was performed on a BD LSRFortessa Flow Cytometer and results were analysed using FlowJo v.10. Doublets and non-viable cells were excluded from the analysis. Doublets were discriminated using the DNA stain area versus width. Cell-cycle progression mathematical inference was performed using uniform priors as already described (Jolly *et al*, 2022).

Global protein synthesis O-propargyl-puromycin (OPP) assay

Eight-well glass chambers (lab-tek) were pre-coated with poly-D Lysine (Gibco) overnight followed by additional coating with laminin (Sigma). Cells were plated and treated 2 days after with IFN-β or ISRIB for the indicated times. OPP (Thermo) was dissolved in DMSO and added 1 h before collection to all conditions at a concentration of 50 µM. Cells were processed using the Click-iT Plus OPP Alexa Fluor 488 Protein Synthesis Assay Kit (Thermo) following the manufacturer's instructions excluding the DNA staining. Cells were finally mounted using fluoromount G with DAPI (ebioscience) for DNA staining. Five representative images were acquired per sample using a Leica TCS SP5 II confocal microscope with 40× magnification. OPP intensity was quantified using Fiji and normalised to the vehicle-treated samples. Control treatments consisting of no-OPP (negative control), N2 supplement (Gibco; positive control) and cycloheximide (CHX; Sigma; negative control) were always run and processed in parallel.

Polysome profiling

NSCs were incubated with either vehicle or IFN-β as above explained. Right before cell collection, 100 µg/ml cycloheximide (CHX, EDM Millipore) in H₂O was added to cells and they were incubated 5 min at 37°C 25% CO₂. Cells were collected by centrifugation, washed with ice-cold DPBS containing 100 µg/ml cycloheximide (CHX), and lysed in the Polysome lysis buffer (20 mM Tris-HCl, pH 7.4, 120 mM KCl, 5 mM MgCl₂, 14 mM β-mercaptoethanol, 1% NP-40, 100 µg/ml CHX, 1× complete protease inhibitor cocktail (Roche), 100 µ/ml RNase Inhibitor (Thermo Fisher)). Lysates of differently treated NSC were loaded at equal amounts for total RNA onto preformed 17.5–50% gradient of sucrose prepared in 20 mM Tris-HCl pH 7.4, 120 mM KCl, 5 mM MgCl₂, 2 mM DTT, 100 µg/ml CHX. Free mRNPs, ribosome subunits, and polysome complexes were resolved by centrifugation for 2.5 h at 210,000 g (at r_{max}) in a SW41 rotor. The content of the tubes was fractionated with the Density Gradient Fractionation System (Teledyne ISCO) allowing for the recording of absorbance at 254 nm. One milliliter fractions were collected for RT-qPCR processing.

RT-qPCR

Total RNA was extracted from cell cultures with the Arcturus PicoPure RNA Isolation Kit (Applied Biosystems). For the fractions of polysome profiling, RNA was purified via phenol/chloroform extraction as described (Faye *et al*, 2014). cDNA was synthesised with the SuperScript VILO cDNA synthesis kit (Thermo Fisher). DNA amplification and detection was performed with the PowerSYBR Green PCR MasterMix (Applied Biosystems) in C1000 Touch Thermal Cycler (Bio-Rad) using QuantiTect (Qiagen) primers (see Table EV2:

RT-qPCR primers). For the calculation of relative gene expression, the ΔCt method was used. The distribution of analysed mRNAs across polysome fractions was presented as a percentage of the total amount of the mRNAs for all fractions.

Ribosomal footprinting

Cells were treated with vehicle or IFN- β 48 h after seeding and incubated at 37°C 25% CO₂ for the corresponding incubation time. Then, NSCs were incubated for 5 min with 0.1 mg/ml CHX at 37°C 25% CO₂. Cells were collected, washed with DPBS supplemented with CHX, and lysed in 150 μ l of the Polysome lysis buffer (see Polysome profiling section). Lysates containing 40 μ g of total RNA were treated with 100 U of RNaseI (Ambion) for 45 min at room temperature, and the reaction was stopped by addition of 50 U of SUPERaseIn (Ambion). Generated 80S monosomes were collected via centrifugation through 25% sucrose cushion at 434,513 g (at r_{max}) for 1 h in a TLA100.2 rotor, and the pelleted RNA was extracted with the Direct-Zol RNA Miniprep kit (Zymo). Ribosomal RNA was depleted with the Gold Ribo-Zero kit (Illumina). Resulted RNA samples were resolved by electrophoresis in a 15% NOVEX TBE-Urea gel (Thermo Fisher). Gel slices including ribosome footprints corresponding to a fuzzy band of the 26–34 nt size range were cut out from the gel, crushed by centrifugation in 0.5 ml gel-breaker tubes (Segmatic) and extracted in 0.5 ml 10 mM Tris-HCl pH 7.0 by incubation for 15 min at 70°C. RNA was recovered by precipitation with isopropanol in presence of 0.3 M sodium acetate pH 5.5 and 20 μ g Glycoblue (Thermo Scientific). Recovered RNA was dephosphorylated with T4 PNK (NEB) in the absence of ATP. Indexed libraries were generated using the SMARTer smRNA-Seq kit for Illumina (Takara).

For the parallel total mRNA sequencing, we used the same NSC lysates prepared as described above at the amount of 20 μ g of total RNA. RNA was extracted with phenol/chloroform as described (Faye *et al*, 2014). RNA samples were treated with 3 U of TURBO DNase (Ambion) for 15 min at 37°C. RNA was again extracted with phenol/chloroform and recovered with ethanol precipitation in the presence of 0.3 M sodium acetate pH 5.2. Ribosomal RNA was depleted with the Gold Ribo-Zero kit (Illumina). After depletion 80 ng of each RNA sample was used to synthesised cDNA and make a library using the NEBNext Ultra Directional RNA library kit for Illumina (NEB).

Libraries quality was assessed using the Bioanalyzer 2100 (Agilent) and were sequenced in HiSeq 2000 v4.

For the analysis, Reads were trimmed applying the tool TrimGalore version 0.4.4_dev. The adaptor sequence “AGATCGGAAGAGC” (Illumina TruSeq, Sanger iPCR; auto-detected) as well as 3 bp from the 5' end and 15 bp from the 3' end were removed. In addition, sequences that became shorter than 18 bp (after quality trimming) were removed using TrimGalore's default settings. After trimming reads had a length of 33 bp on average. Subsequently, reads were mapped to the mm10 transcriptome build GRC38.93 from ENSEMBL using boWTie version 0.12.7 with its standard options. Reads falling into genes were counted from BAM files applying a suited R/Bioconductor workflow (function SummarizeOverlaps with mode “Union”). Duplicated reads were removed. For ribosomal footprinting samples, reads in the whole gene body or in the coding part of genes (CDS) were counted separately, for total RNA samples reads

in the whole gene body were counted. Next, to get an estimation of the translation efficiency (TE) per gene, log-fold-changes between ribosome protected reads from the CDS and total RNA samples were computed applying DESeq2. DESeq2 was chosen as it is considered a standard tool for modelling negative-binomial distributions arising in sequencing experiments. The likelihood ratio test (LRT) was applied on the TE analysis upon IFN- β treatment. Genes with positive fold-changes are considered to be enhanced, those with negative ones to be repressed. The gene set enrichment analysis was performed using the clusterProfiler tool from Bioconductor using fold-changes for all expressed genes. Gene ontology terms related to “biological process” with an FDR < 0.05 and highest significance were selected for the plots. TOP-mRNAs (Dataset EV3) were defined as mRNAs with a cytidine immediately after the 5' cap, followed by an uninterrupted stretch of four to 14 pyrimidines (Thoreen *et al*, 2012).

Proteome and phosphoproteome

Cells were treated with vehicle or IFN- β and incubated at 37°C 25% CO₂ for the time specified in the figures. Cell lysates for the proteome and phosphoproteome analysis were prepared as described (Potel *et al*, 2018) with some modifications. Briefly, cells were collected, washed and weighted to determine the mass of cells pellets. One volume of the cell pellet was resuspended in six volumes of proteome lysis buffer composed of 100 mM Tris-HCl pH 8.5, 7 M Urea, 1 mM MgCl₂, 1 mM sodium ortovanadate, 1% Triton X-100, 1 $\times$ PhosphoSTOP inhibitor (Roche), 1 $\times$ Complete EDTA free protease inhibitor (Roche). To improve lysis, cells suspension was sonicated three times for 10 s (1 s on, 1 s off) with 30 s pause at 40% output using the Fisherbrand Model 120 Sonic Dismembrator (Fisher Scientific). The lysate was clarified by centrifugation at 21,000 g, for 1 h at 4°C. Lysates were incubated for 2 h at room temperature. Protein concentration was determined with the Pierce BCA Protein Assay Kit (Thermo Fisher) and 400 μ g of total protein for each sample were subjected to metal-affinity enrichment and mass spectrometry at the DKFZ Genomics and Proteomics Facility. Proteins and phosphopeptides were quantified and analysed for differential abundance using Perseus45.

Western blot analysis

Cells were collected, washed with DPBS and resuspended in 100 μ l of 0.2% SDS, 1 mM vanadate, 1 $\times$ cComplete Protease inhibitor cocktail (Roche), 1 $\times$ PhosphoSTOP inhibitor (Roche) and pass several times through a 23G syringe needle. Fifteen microgram of total protein from cellular lysates were resolved in NuPAGE 10% gels (Thermo Fisher) and transferred to nitrocellulose membranes. The membranes were blocked in 5% BSA in TBS-T buffer and probed overnight with primary antibody. Primary antibodies used: Akt (#9272), phospho-Akt S473 (#9271), 4 E-BP1 (#9644; clone 53H11), phospho-4 E-BP1 S65 (#9451), eIF2alpha (#5324S; clone D7D3), phospho-eIF2 α S51 (#3597; clone 119A11), Rb (#9313; clone D20), phospho-Rb S780 (#8180; clone D59B7), p70S6K (#2708; clone 49D7), phospho-p70S6K T389 (#9234; clone 108D2), phospho-Rps6 S235/236 (#4857; clone 91B2), phospho-Rps6 S240/244 (#2215), TSC2 (#4308; clone D93F12), phospho-TSC2 T1462 (#3617; clone 5B12) and LARP1 (#14763) are all rabbit antibodies from Cell

Signalling. Rps6 (#ab40820) and Vinculin (#ab129002; clone EPR8185) are rabbit antibodies from Abcam. Rabbit anti-actin (#sc-1616-R; clone I-19) from Santa Cruz. Mouse anti-phospho-Akt T308 (#A1124-07R) from USBiological. Rabbit Ifitm3 (11714-1-AP) from ptglab. Rabbit phospho-TSC2 S1452 was kindly provided by Aurelio Telemán. Membranes were washed and reprobed with secondary anti-rabbit or anti-mouse HRP-antibody (Dianova). The signals were developed with the Western Lightning Plus ECL substrate (Perkin Elmer) and scanned using the ChemiDoc Touch Imaging System (Bio-Rad). Loading controls were always run on the same blot than the quantified phospho- or total protein. The Image Lab version 5.2 (Bio-Rad) was used for band intensity quantification.

Luciferase assay

For nucleofection, 3×10^6 NSCs were mixed with 5 µg of corresponding DNA with the Amaxa P4 Primary Cell 4D-Nucleofector X Kit S (Lonza) and pulse was delivered using the CA137 programme in a 4D-Nucleofector X Unit (Lonza). Afterwards, the cells were washed, resuspended in medium and separated in three technical replicates. After recovery, cells were treated with IFN-β, Torin1, a combination of both or their corresponding vehicles as stated in the figures. Then, cells were washed and lysed in 1× Passive Lysis buffer from the Dual-Luciferase Reporter Assay System (Promega). Lysis was proceeded on an orbital shaker for 15 min at room temperature. Luciferase Assay reagent was added to each sample, mixed and all volume was transferred into a 96 well White Cliniplate (Thermo Fisher). For *Firefly* luciferase activity the plate was scanned in a Synergy LX multi-mode reader (BioTek). Then 100 µl of Stop&Glo reagent was added and the plate was scanned again for *Renilla* luciferase. *Renilla* luminescence was normalised to *Firefly* luminescence (with technical triplicates) and the final results were presented as fold change to the control samples.

Plasmid construction

DNA constructs with different WT and mutated 5'UTRs of mouse *Sox2* mRNA were assembled in the psiCheck-2 vector (Promega) including synthetic *Renilla* luciferase gene (hRluc) driven by SV40 early enhancer/promoter and synthetic *Firefly* luciferase gene (hluc+) under HSV-TK promoter. The UTRs flank hRluc open reading frame. The full-length 5'UTRs of mouse *Sox2* (NM_011443.4) and *Rps21* (variant2, NM_025587.2) as well as their mutated variants carrying the deletions of PRM in *Sox2* (5UTRmut *Sox2*; CTCTT deleted), PRM in *Rps21* (5UTRmut *Rps21*; TCCTTTC deleted) were synthesised and inserted into pEX-K4 (*Sox2*) or pEX-A2 (*Rps21*) by Eurofins. The plasmids were used to amplify the UTRs with Phusion High-Fidelity DNA polymerase (NEB) using corresponding primers carrying SfiI and NheI restriction sites to allow the inserts between SV40 promoter and the reading frame of hRluc. The used primers are listed in Table EV2. The full-length 5'UTR of mouse beta actin (*Actb*) mRNA (NM_007393.5) was generated by amplification of cDNA library prepared from NSCs isolated from C57BL/6 mice. For generation of the 5'UTR *Actb*, the used PCR primers were flanked with SfiI and NheI sites to allow the insertion in front of hRluc reading frame in psiCheck-2 vector (Promega). The accuracy of cloning was verified by sequencing of the inserts in all generated plasmids

from both directions with corresponding primers for sequencing (see Table EV2).

Single-cell transcriptomics

To characterise the single-cell transcriptomics of NSCs and their progeny, we made use of TiCY (referred to as IFNAGR^{WT}) and TiCY-IFNAGR KO mice (referred to as IFNAGR^{KO}). In these mice, tamoxifen-induced Cre recombination takes place in neural stem cells in the vSVZ, which express *Tlx* (*Nr2e1*; Liu *et al*, 2008), and will stably activate the production of eYFP labelling NSCs and their progeny.

Tamoxifen (TAM) injection was done in 3 days as follows: two doses daily of 1 mg of tamoxifen in 100 µl of a solution of 10 mg/ml of TAM in EtOH 10% diluted on sunflower seed oil. TiCY-IFNAGR^{WT} young mice were injected with TAM at 10 weeks old and were sacrificed 6 weeks afterwards. TiCY-IFNAGR^{KO} old mice (71 weeks old), TiCY-IFNAGR^{KO} young (7 weeks old) and old mice (85 and 98 weeks old) were injected with TAM and were sacrificed 9 weeks afterwards. After animal sacrifice, the vSVZ, striatum, rostral migratory stream and olfactory bulb was isolated. These latter three tissues were pooled together and referred to as Rest of the Brain (RoB). Tissues were processed as described previously (Kremer *et al*, 2021) and sorted in a BD FACSaria II at the DKFZ Flow Cytometry Facility. For sorting, we size-selected the vSVZ or RoB cells and excluded for doublets, dead cells and CD45⁺/Ter119⁺/O4⁺ cells as recently described (Kalamakis *et al*, 2019). We sorted vSVZ eYFP⁺ cells and GLAST⁺ cells. From the RoB, cells that were eYFP⁺ and eYFP[−]/PSANCAM^{low} were sorted. For every condition, two mice were used whose cells were labelled with Hashtags oligos (HTO) using the Cell Hashing protocol from Biolegend (TotalSeq-A). All the sorted cells were pooled on the same tube and processed in the Chromium Next GEM Chip G using Chromium Next GEM Single Cell 3' v3.1.

Gene expression libraries were prepared following the manufacturer's protocol (Chromium Next GEM Single Cell 3' v3.1) at the Single-cell open lab at DKFZ and sequenced on a NextSeq2000 V3 PE 100 bp at the Sequencing open lab provided by the DKFZ Genomics and Proteomics Core Facility. Each condition (genotype and age combination) was processed on a separate 10× reaction. Hashtags libraries were processed according to the manufacturer's instruction (Biolegend) and were sequenced on a NextSeq 500 Mid output PE 75 bp at the DKFZ Genomics and Proteomics Core Facility.

Single-cell transcriptomic data analysis

To derive a "NSC type I Interferon Response" signature we used the 16 h IFN-β treated Ribo-Seq transcriptomic libraries. Reads were trimmed using trimalore 0.6.6 using default settings and mapped and quantified using STAR 2.7.7a with --quantMode GeneCounts. Star outputs were concatenated and read into R 4.0.5 where DESeq2 1.30.1 was used to compute differential expression between treated and untreated samples. The genes with the 300 most significantly changed genes after multiple-testing correction from a one-sided Wald test (increased expression) were chosen as our "NSC Type I Interferon Response" gene set.

For the single-cell libraries analysis, we filtered them using the 10× index-hopping-filter 1.0.1 and thereafter quantified using kallisto/bustools's lamanno workflow (kb_python 0.26.3). Hashtag

libraries were quantified using CITE-seq-Count 1.4.5. Count matrices were read into scanpy 1.6.0 and preprocessed by filtering out genes present in less than three cells and cells with less than 100 genes. Doublets were called and removed with scrublet 0.2.1. Cells with less than 10% mitochondrial UMIs, 70–6,000 genes and 1,000–20,000 UMIs were retained as quality cells. Counts were normalised (to a target sum of 10,000) and log1p transformed using scanpy methods. A UMAP was computed from 50PCs computed on scaled counts. Main clusters were identified in UMAP using DBSCAN (scikit-learn 0.22.2.post1). An auxiliary Leiden clustering was computed. The largest DBSCAN cluster was identified as the lineage cluster based on marker expression. Non-subventricular astrocytes were identified and removed from this cluster by inspecting the fraction of SVZ hashtags in each cell. Other clusters were named by summed expression of celltype specific-markers (Pecam1, Prom1 and Cldn5 for endothelials, Itgam and Ptpcr for microglia, Rbfox3, Calb2, Nefl and Th for neurons). Diffusion pseudotime (from scanpy) was run on the lineage cluster using the cell with the highest Aqp4 expression as the root cell. Pseudotime was then binned into qNSC1, qNSC2, aNSC, TAP and NB based on visual inspection of the expression of well-known markers Aqp4, Egfr, Dcx, Mki67, Sl100b. Hashtag samples were called for each cell as the maximum posterior from a multinomial mixture model (R 4.0.5 package mixtools 1.2.0) with five components (one for each hashtag and an extra component for no hashtags).

Gene set scores were then computed for each cell by summing up the normalised (per cell to 1,000,000 UMIs) and log1p-transformed expression of genes from a given set. Group-wise gene set scores were then computed as the average of all of the cell's scores in a given group of interest. Scores were computed for each replicate, celltype, age and genotype combination for MSigDB's "HALLMARK_INFLAMMATORY_RESPONSE", "HALLMARK_INTERFERON_ALPHA_RESPONSE" genesets, as well as the gene set identified by Wu *et al*, 2018 (Wu *et al*, 2018) and our "NSC Type I Interferon Response".

IFNAGR KO modelling

We built on the previously established mathematical model of cell population dynamics (Kalamakis *et al*, 2019), describing counts of quiescent (non-cycling) and active (cycling) stem cells. The model is given by a system of ordinary differential equations:

$$\begin{aligned}\frac{d}{dt} \text{qNSC} &= -r \cdot \text{qNSC} + 2 \cdot b \cdot p_s \cdot \text{aNSC}, \\ \frac{d}{dt} \text{aNSC} &= r \cdot \text{qNSC} - p_s \cdot \text{aNSC}.\end{aligned}$$

The parameter r describes the activation rate that may exponentially decrease in time

$$r(t) = r_0 \cdot e^{-\beta_r \cdot t}.$$

Self-renewal is modelled by a function b

$$b(t) = \frac{1}{2} (1 + e^{-\beta_b \cdot t} \cdot (2 \cdot b_0 - 1)).$$

The choice of this function follows the assumptions that self-renewal probability is not larger than $\frac{1}{2}$ and may be a constant or increasing in time function.

The remaining parameter $p_s = \log(2)/(17.5/24) \approx 0.9506$ corresponds to the cell cycle rate. It was chosen from literature, following our previous publication (Kalamakis *et al*, 2019). In summary, the model is characterised by free parameters r_0 , b_0 , β_b and β_r . The initial value additionally provides the parameter NSC_0 which was used to compute initial values for the specific compartments from the steady state ratio (Appendix Supplementary Methods).

Parameters were estimated using a multi-start box-constrained weighted-least-squares approach. The objective functions were built using new FACS quantifications for the total numbers of stem cells and the fraction of actively cycling neural stem cells from our previous publication. We applied solvers provided and chosen by the DifferentialEquations.jl package. Weights were computed as the inverse standard deviation for each age and genotype combination. Starting values were sampled from within reasonable bounds using Latin Hypercubes computed by the LatinHypercubeSampling.jl library, each was then optimised using a box constrained optimizer from the Optim.jl library. The best parameter estimates were kept as the optimum. To aid in model selection, AIC_c was computed for each model and dataset combination.

Finally, we estimated parameters for models with population-dependent self-renewal probabilities, described by a Hill function:

$$b(\text{NSC}) = 1 - \frac{1}{1 + \left(\frac{k_b}{\text{NSC}}\right)^{n_b}}.$$

We estimated these with a single k_b for both genotypes. The parameter n_b was estimated individually per-genotype (IFN-dependent self-renewal) or shared (IFN-independent self-renewal) to provide the two scenarios of self-renewal dependence on interferons.

IFNAGR KO simulations at arbitrary time points

To simulate the age-specific dynamics of the IFNAGR KO, we built a model with a time-dependent switch in the parameter functions. At the intervention age, the parameter functions are switched from the previously estimated WT parameter functions to their IFNAGR KO counterparts. We simulated this intervention model for varying intervention ages. For each simulation we computed the number of stem cell loss at 660 days' age compared to the wildtype. We also computed the progenitor production as the cell flux from active neural stem cells to progenitors. We then integrated progenitor production from 0 to 700 as the total life-long progenitors produced.

Statistical analysis

Biological replicates "n" in the figures refer to biological replicates as of either different mice or different NSCs cultures extracted from different mice. Plotted bars represent mean and plotted error bars represent standard deviation from different biological replicates. Only biological replicates were considered for statistical analyses.

The paper explained

Problem

Interferons represent not only a first line of defence against viral infections but also a major component of ageing-related functional decline of brain stem cells. Whether these two functions are linked, and whether regulation of stem cell function by interferon occurs only in the ageing brain remains elusive. We apply mathematical modelling, single-cell RNA-Seq and Ribo-Seq to describe the population dynamics and molecular underpinnings of the interferon response in NSCs across the adult lifespan of the animal.

Results

We observe an interferon response in stem cells already at young ages that is shut down in committed neurogenic progenitors and comes back in end differentiated neurons. Interferon control stem cell homeostasis through orchestration of mTORC1 activity and cell cycle, which represses translation of the stemness factor Sox2 and retain cells in a G₀ quiescent state. The component of interferon response triggered by interferon ligands controls stem cell activation and self-renewal and is beneficial in the young but detrimental in the old brain.

Impact

The selective response to interferon of NSCs but not in neural progenitors indicates that the latter are intrinsically less protected against viral infections. This could explain the higher susceptibility of embryos and infants to brain viral infections (e.g. Zika virus) and should be further explored. In addition, the results establish the molecular underpinnings of the interferon response in NSCs revealing potential blocking targets of this response that might be relevant to other adult stem cell system. Last, the influence of stemness by the novel biphasic regulation of mTOR might be key to control activation and escape senescence. Our data reveal that the optimal time for interventions targeting interferons to revert detrimental ageing-related stem cell dysfunction is at middle-aged adults.

Statistical tests were performed as indicated in each figure legend with a significance level of $\alpha = 0.05$. To test for biphasic response in western blot data, linear models explaining log₂ fold changes were fitted with a single explanatory variable of time being greater than a change time. To find this change time, models were fitted for varying change times and the model with the lowest AIC was chosen. To determine the significance of this biphasic response, one-way ANOVA was performed on this model.

Data availability

The datasets and compute code produced in this study are available in the following databases:

- Single-cell RNA-seq and Ribo-Seq data have been deposited at the Gene Expression Omnibus (GEO) and are publicly available with GEO Accession Number GSE197217 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE197217>).
- All original code is deposited at <https://github.com/Martin-Villalba-lab/Data/> and publicly available as of the date of publication.

Any additional material, data or information required to reanalyse the data reported in this paper are available from the lead contact upon request. Illustrations were created with Adobe Illustrator or with [BioRender.com](https://www.biorender.com).

Expanded View for this article is available [online](#).

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Author contributions

Damian Carvajal Ibañez: Conceptualization; resources; formal analysis; validation; investigation; visualization; methodology; writing – original draft; project administration; writing – review and editing. **Maxim Skabkin:** Conceptualization; resources; formal analysis; investigation; visualization; methodology; project administration; writing – review and editing. **Jooa Hooli:** Conceptualization; resources; data curation; software; formal analysis; validation; visualization; writing – original draft; writing – review and editing. **Santiago Cerrizuela:** Resources; investigation; methodology; writing – review and editing. **Manuel Göpferich:** Resources; data curation; software; formal analysis; visualization. **Adrien Jolly:** Resources. **Katrin Volk:** Validation. **Marc Zumwinkel:** Investigation. **Matilde Bertolini:** Resources. **Gianluca Figlia:** Resources. **Thomas Höfer:** Resources; supervision. **Gunter Kramer:** Resources; supervision. **Simon Anders:** Resources; supervision. **Aurelio A Teleman:** Resources; supervision. **Anna Marciniak-Czochra:** Conceptualization; supervision; writing – original draft; project administration; writing – review and editing. **Ana Martin-Villalba:** Conceptualization; resources; supervision; funding acquisition; writing – original draft; project administration; writing – review and editing.

Disclosure and competing interests statement

The authors declare that they have no conflict of interest. Ana Martin-Villalba is an EMBO member. This has no bearing on the editorial consideration of this article for publication.

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Expanded View Figures

Figure EV1. Inference of IFN signatures in the vSVZ niche.

- A Experimental layout for scRNA-Seq in young and old mice lacking interferon receptors. All mice represent TiCY (Tlx reporter, see [Materials and Methods](#) and Fig EV1) that are either IFNAGR WT or KO. vSVZ (Ventricular Subventricular Zone), RMS (Rostral Migratory Stream), OB (Olfactory Bulb), TAM (Tamoxifen).
- B Relative gene expression of relevant markers for cell types along pseudotime. Black lines denote cuts between cell types.
- C Relative expression of type-I IFN receptors in young and old wildtype cells over pseudotime coloured by lineage cell types.
- D Scores computed for the Hallmark Interferon Alpha Response signature displayed in the UMAP embedding for young cells (with colours clipped to the range seen in the lineage cells) and averaged for the cell types in our analysis at varying ages in IFNAGR^{WT} and IFNAGR^{KO} cells. $n = 2$ biological replicates per age and genotype.
- E Scores computed for the Hallmark Inflammatory Response signature displayed in the UMAP embedding for young cells (with colours clipped to the range seen in the lineage cells) and averaged for the cell types in our analysis at varying ages in IFNAGR^{WT} and IFNAGR^{KO} cells. $n = 2$ biological replicates per age and genotype.

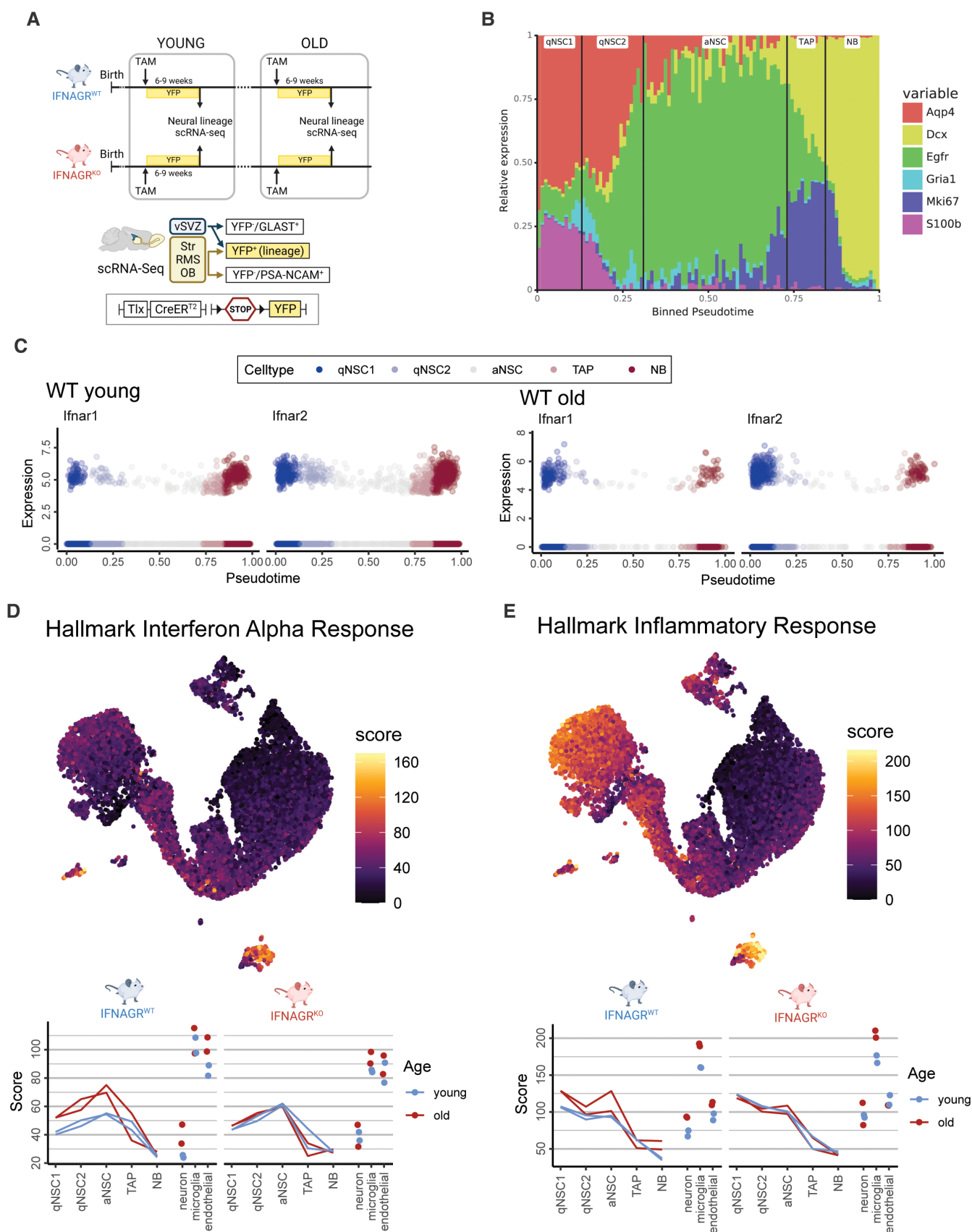


Figure EV1.

Figure EV2. Cycleflow and OPP analysis: IFN- β induce cell cycle exit and a biphasic control of protein translation in NSCs.

- A Gating strategy for quantification of cell cycle states for Cycleflow.
- B EdU and IFN- β exposure experimental scheme for Cycleflow.
- C Cell cycle properties inferred from Cycleflow in IFN- β -treated NSCs. $n = 3$ biological replicates.
- D Representative images of the OPP (O-propargyl-puromycin) incorporation assay in WT NSCs treated with IFN- β at 2 and 16 h. N2 supplement or Cycloheximide (CHX) were added as controls (see [Materials and Methods](#)). Bar scale represents 50 μm .
- E OPP (O-propargyl-puromycin) incorporation assay quantification in WT NSCs treated with IFN- β at 2 and 16 h. $n = 5$ biological replicates. One-way ANOVA with Dunnett's multiple comparison test was computed (P -values specified) using vehicle-treated NSCs as control group.

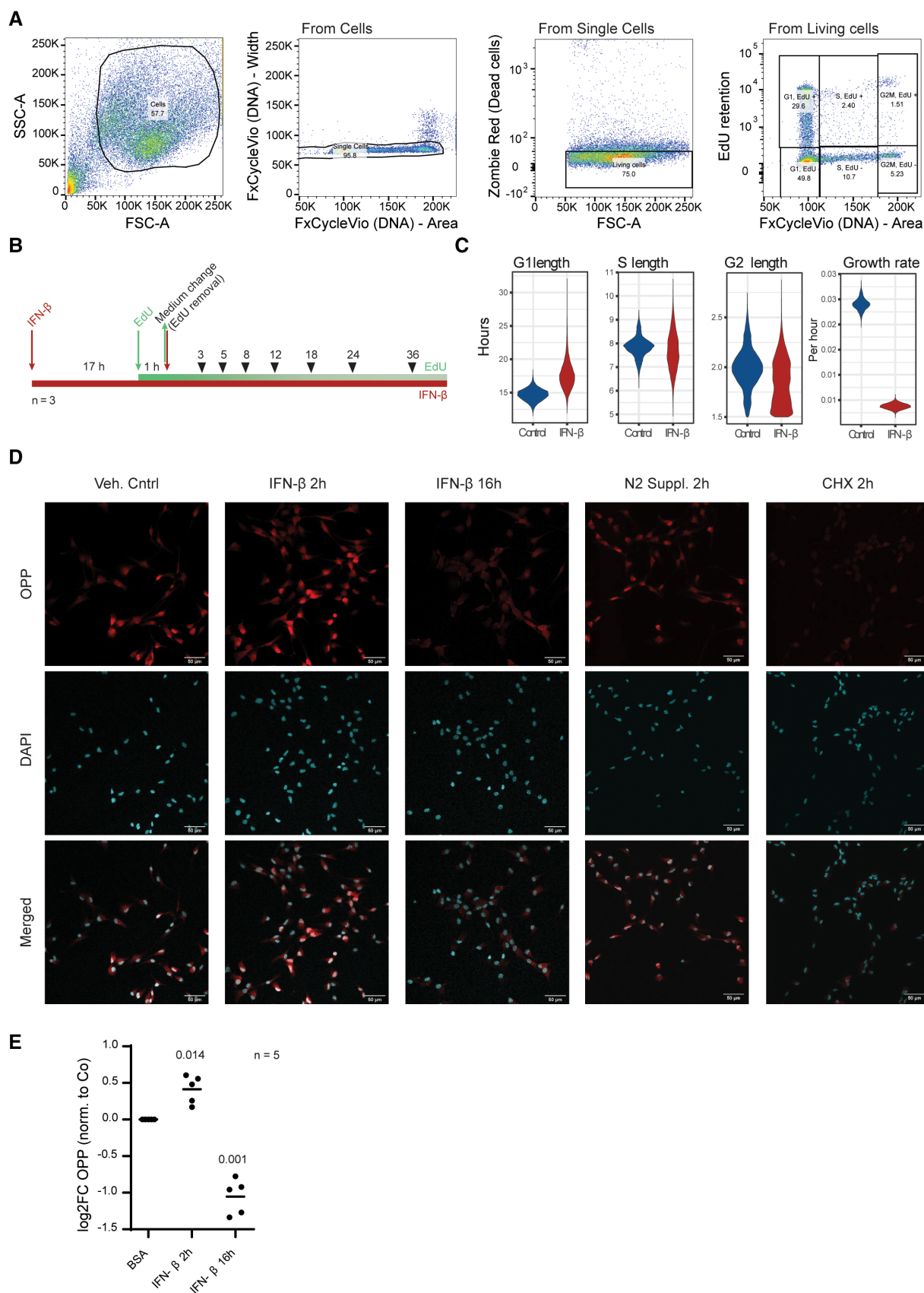


Figure EV2.

Figure EV3. IFN- β induce a biphasic control of mRNA translation in NSCs.

- A, B Results of Ribo-Seq depicting translation efficiency as the interaction of log2 fold changes (LFC) between footprints (ribosome protected reads) and total RNA at 2 and 14 h IFN- β treatment. FDR < 10%, LR-Test. Genes with a *P*-value < 0.1 after FDR correction are highlighted. The associated GO terms are depicted. *n* = 3 biological replicates.
- C Polysome profiling (RT-qPCR) of actin beta and of the interferon stimulated genes (ISGs) *Ifit3* and *Irf9* upon 2 and 14 h IFN- β treatment. Hyphens represent mean of biological replicates. Arrows indicate the 40S, 60S and 80S subunits of the ribosome. Two-way ANOVA with Šídák's multiple comparison test was computed. Two hours *Ifit3* fraction 4 ***(*P* < 0.0001), fraction 10 **(*P* = 0.0024); 2 h *Irf9* fractions 5, 6, 10 ***(*P* < 0.0001), fraction 9 **(*P* = 0.0091). Fourteen hours *Actb* fractions 9, 10 ***(*P* < 0.0001), fraction 11 ***(*P* = 0.0002); 14 h *Ifit3* fraction 10 ***(*P* < 0.0001), fraction 11 ***(*P* = 0.0003); 14 h *Irf9* fraction 1 *(*P* = 0.0093), fractions 2, 3, 5 ***(*P* < 0.0001), fraction 4 *(*P* = 0.0104), fraction 6 *(*P* = 0.0263), fraction 10 **(*P* = 0.0092). *n* = 3–4 biological replicates. Outliers of fraction 1 from *Ifit3* were excluded from the statistical analysis.
- D Polysome profiling (RT-qPCR) of *Rps17* and *Rpl34* upon 2 and 14 h IFN- β treatment. Hyphens represent mean of biological replicates. Arrows indicate the 40S, 60S and 80S subunits of the ribosome. Two-way ANOVA with Šídák's multiple comparison test was computed. Two hours *Rpl34* fraction 7 *(*P* = 0.037). Fourteen hours *Rps17* fraction 4 ***(*P* = 0.0002), fraction 5 **(*P* = 0.003), fraction 7 ***(*P* < 0.0001), fraction 8 **(*P* = 0.0018); 14 h *Rpl34* fractions 4, 7 ***(*P* < 0.0001), fraction 5 (*P* = 0.0047), fraction 8 **(*P* = 0.0042). *n* = 3–4 biological replicates.
- E Representative WB image and quantification (log2FC) of LARP1 from IFN- β -treated NSCs normalised to control (*t* = 0 h). Bars represent the mean value. *n* = 4 biological replicates.
- F Representative WB image and quantification (log2FC) of p-Akt^{Ser473} from IFN- β -treated NSCs normalised to control (*t* = 0 h). Bars represent the mean value. One sample *t*-test was computed, no significant results. *n* = 3 biological replicates.

Data information: *P* $\equiv$ *P*-value.

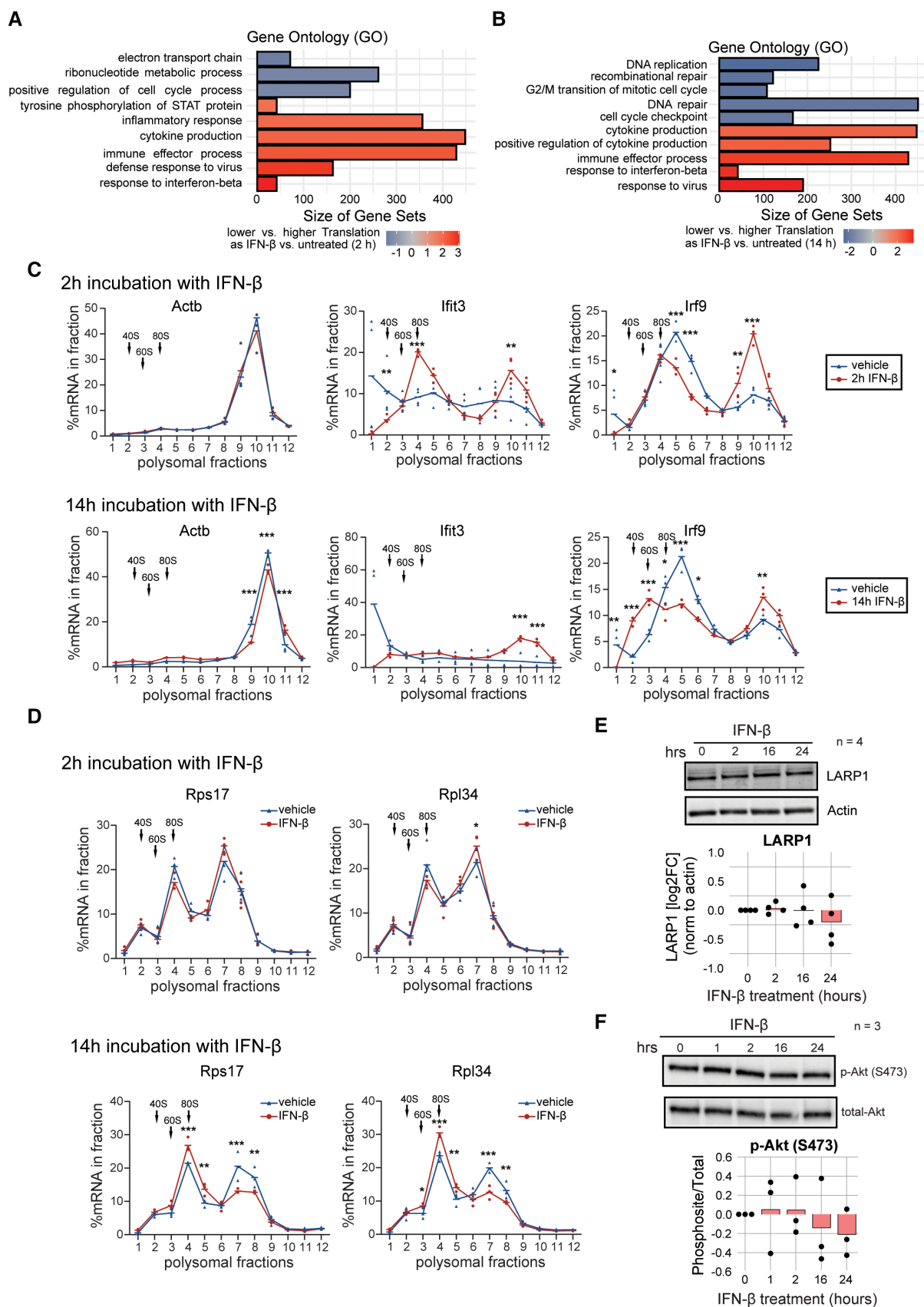


Figure EV3.

Figure EV4. The biphasic control of mTORC1 by IFN- β relies on TSC2, PKR and eIF2 α .

- A WB images and quantifications of TSC2 relative to actin beta and normalised to TSC2^{WT} NSCs in different CRISPR-mutated (TSC2^{mut}) or CRISPR-non-mutated (TSC2^{ctrl}) NSC clones.
- B Representative WB images and quantifications (log2FC) of p-p70S6K^{Thr389} in TSC2^{WT}, TSC2^{ctrl} & TSC2^{mut} NSCs treated with IFN- β and normalised to vehicle ($t = 0$ h) TSC2^{WT}. Bars represent the mean value. $n = 2$ –4 clonal replicates.
- C OPP (O-propargyl-puromycin) incorporation assay quantification in WT, TSC2^{ctrl} & TSC2^{mut} NSCs treated with IFN- β at 2 and 16 h. Two-way ANOVA with Dunnett's multiple comparison test was computed (P -values specified). $n = 5$ biological replicates for WT NSCs and 2–3 clonal replicates for TSC2^{ctrl} & TSC2^{mut} NSCs, respectively.
- D Relate label-free quantification (LFQ) of proteomics from WT NSCs untreated or treated with IFN- β for 2 or 16 h. $n = 5$ biological replicates.
- E OPP (O-propargyl-puromycin) incorporation assay quantification in WT NSCs treated with IFN- β at 16 h in the presence or absence of the integrated stress response inhibitor (ISRIB). One-way ANOVA with Tukey's multiple comparison test was computed (P -values specified). $n = 3$ biological replicates.
- F Polysome profiling (RT-qPCR) of genes displaying upregulation of footprints in the ORF (Trex1 and Ifit2) compared to genes with an upregulation in uORFs (Slfn9 and Rbms2) upon 14 h IFN- β treatment. Hyphens represent mean of biological replicates. Arrows indicate the 40S, 60S and 80S subunits of the ribosome. Two-way ANOVA with Šídák's multiple comparison test was computed. Trex1 fraction 1 **($P = 0.0019$), fractions 2, 6, 7, 9, 10 ***($P < 0.0001$), fraction 5 **($P = 0.0016$). Ifit2 fractions 3, 8, 11 ***($P < 0.0001$), fraction 7 **($P = 0.0044$), fraction 9 **($P = 0.0031$), fraction 10 **($P = 0.0063$). Slfn9 fraction 6 *($P = 0.0357$). Outliers of fraction 1 from Ifit2, Slfn9 and Rbms2 were excluded from the statistical analysis. $n = 3$ biological replicates.
- G Representative WB image and quantification (log2FC) of p-TSC2^{Ser1452} from IFN- β -treated NSCs normalised to control ($t = 0$ h). Bars represent the mean. One sample t -test was computed. One hour *($P = 0.017$), 2 h **($P = 0.00565$), 16 h *($P = 0.0268$). $n = 3$ biological replicates.

Data information: $P \equiv P$ -value.

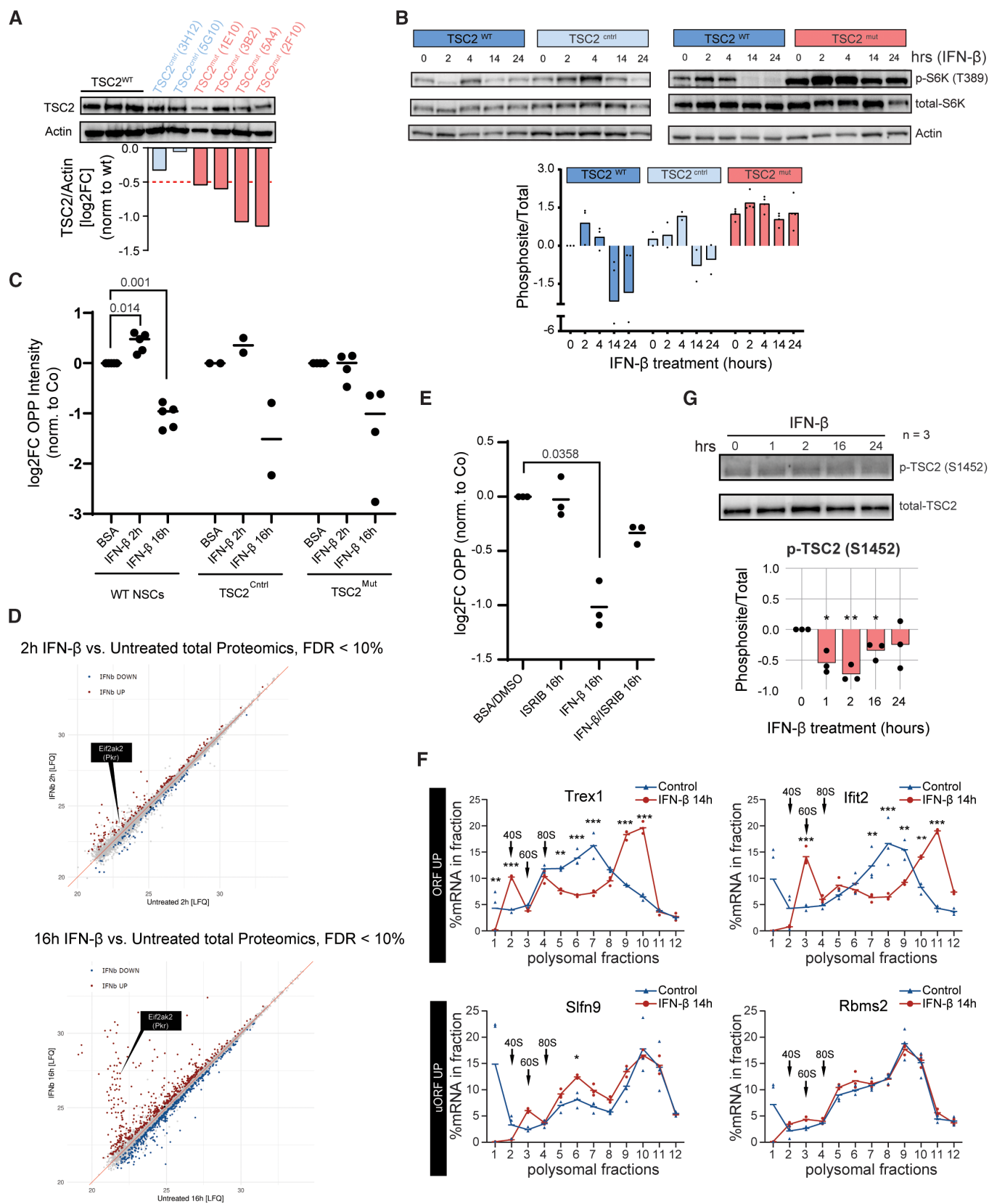


Figure EV4.

Figure EV5. Effect of interferon on Sox2 translation and modelling of interferon receptors removal along lifespan of animals.

- A Representative WB image and quantification (log2FC) of Sox2 from IFN- β -treated NSCs normalised to control ($t = 0$ h). Bars represent the mean value. One sample t -test was computed. Sixteen hours $^*(P = 0.0219)$, 24 h $^{**}(P = 0.03)$, 48 h ($P = 0.056$). $n = 4$ biological replicates.
- B 5'UTR constructs priming *renilla* luciferase controlled by the upstream 5'UTR fragment from Sox2, Rps21 and Actb. TOP = 5'Terminal Oligopyrimidine motif; PRM = 5'Pyrimidine Rich Motif.
- C Luciferase activity assay in NSCs treated with Torin1. Data are normalised to vehicle and are represented as mean $\pm$ SD. $n = 3$ biological replicates. Two-way ANOVA test with Šidák's multiple comparison test (P -values specified).
- D Luciferase activity assay in NSCs treated with Rapamycin (Rapa) and IFN- β . Data are normalised to vehicle and are represented as mean $\pm$ SD. $n = 7$ biological replicates. Two-way ANOVA test with Šidák's multiple comparison test (P -value specified).
- E Luciferase activity assay in NSCs treated with Rapamycin. Data are normalised to vehicle and are represented as mean $\pm$ SD. $n = 7$ biological replicates. Two-way ANOVA test with Šidák's multiple comparison test (P -values specified).
- F Luciferase activity assay in NSCs treated with the integrated stress response inhibitor (ISRIB) and IFN- β . Data are normalised to vehicle and are represented as mean $\pm$ SD. $n = 5$ biological replicates. Two-way ANOVA test with Šidák's multiple comparison test (P -values specified).
- G Induced interferon knockout simulations (coloured, each colour represents a different intervention timepoint) of relative loss of stem cells (upper panel) across age compared to wildtype simulations (black; 100%). The relative rate (lower panel) at which progenitors are produced ($2.25\text{emp}_s, 25\text{em}(1-b), 25\text{emaNSC}$) from NSCs for induced interferon knockout simulations with interferon dependent self-renewal compared to WT simulations. Dashed red line denotes age 350 days.

Data information: $P \equiv P$ -value.

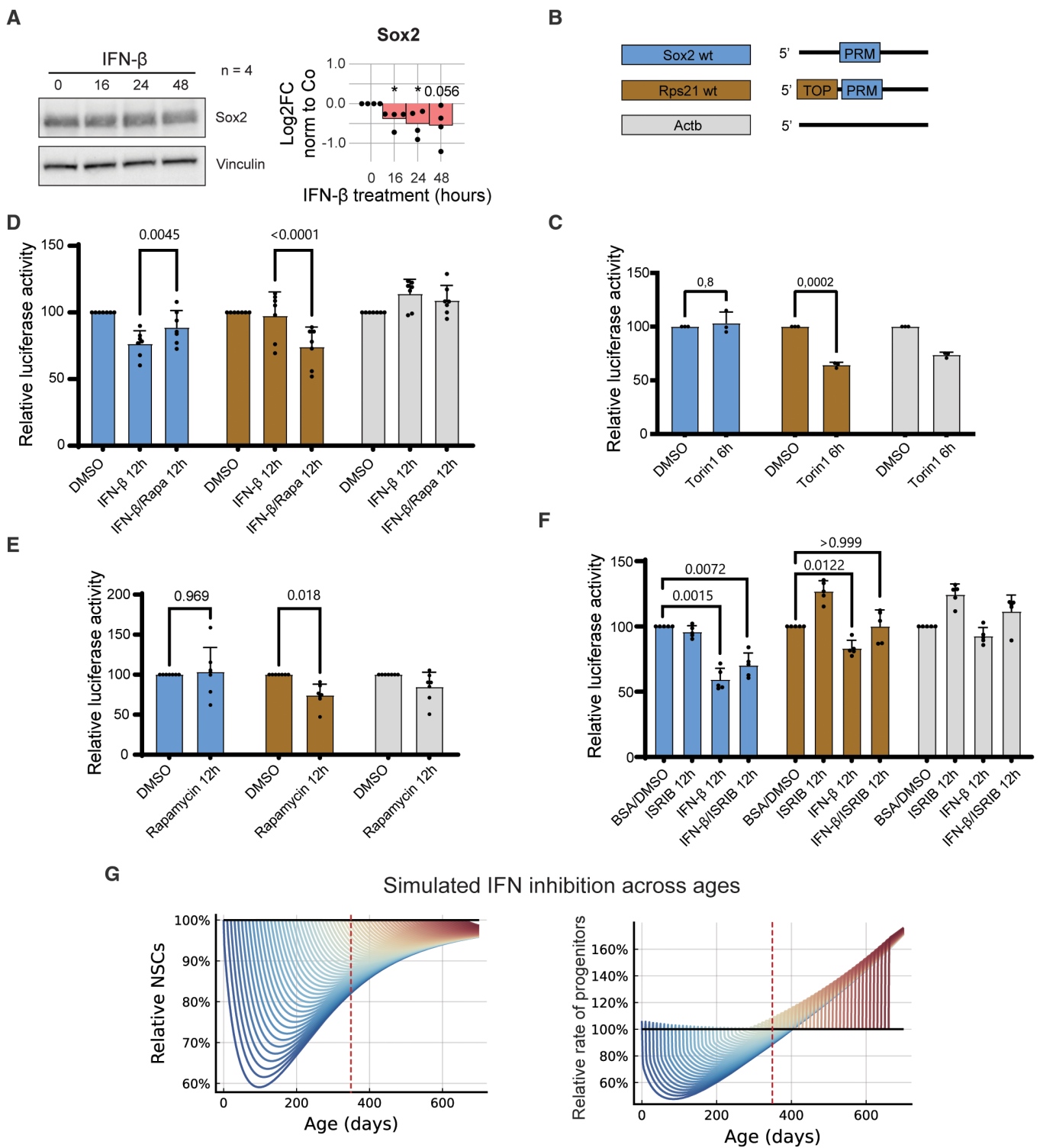


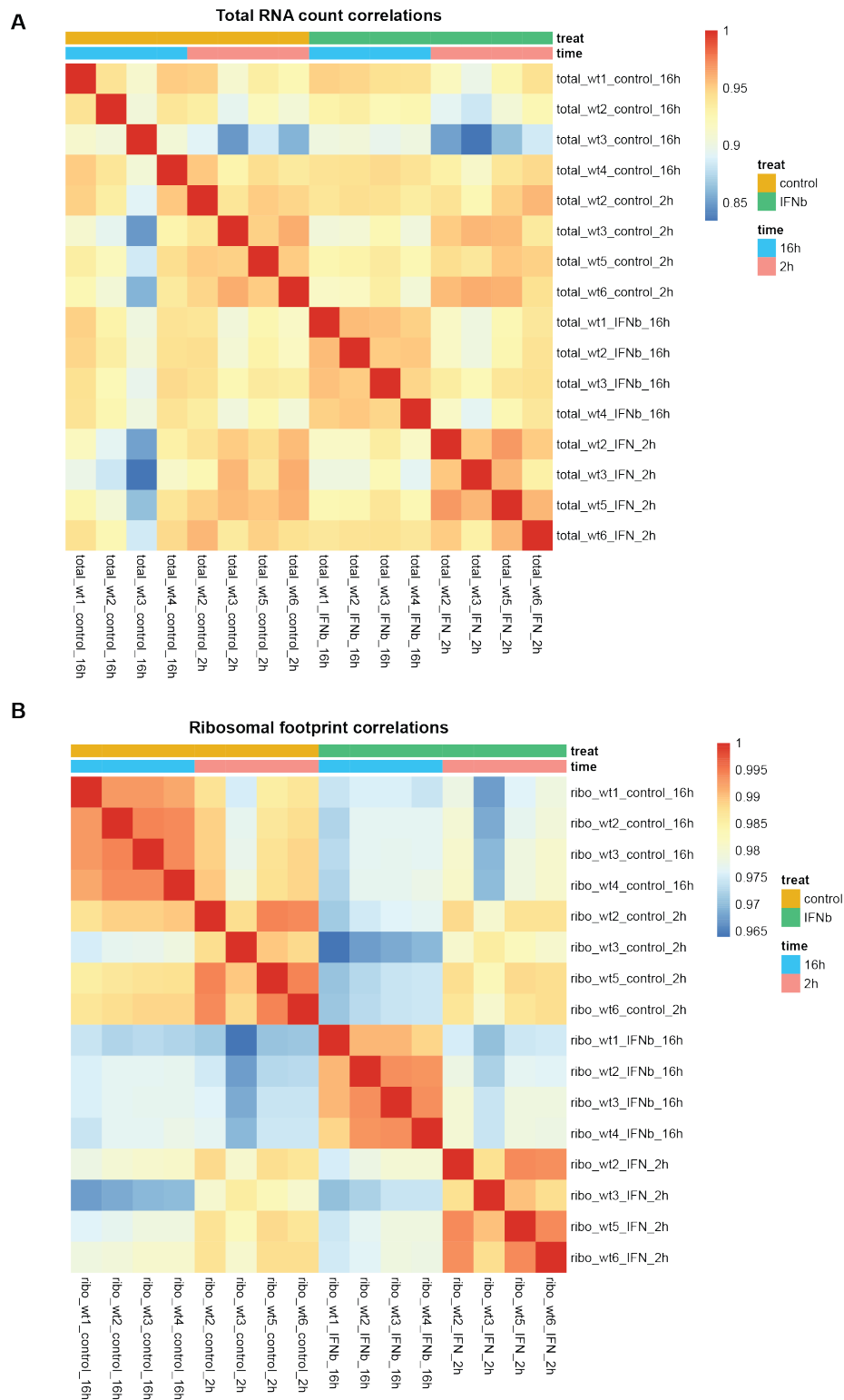
Figure EV5.

Appendix Data

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Appendix Figure S1: Ribo-Seq sample correlation quality control

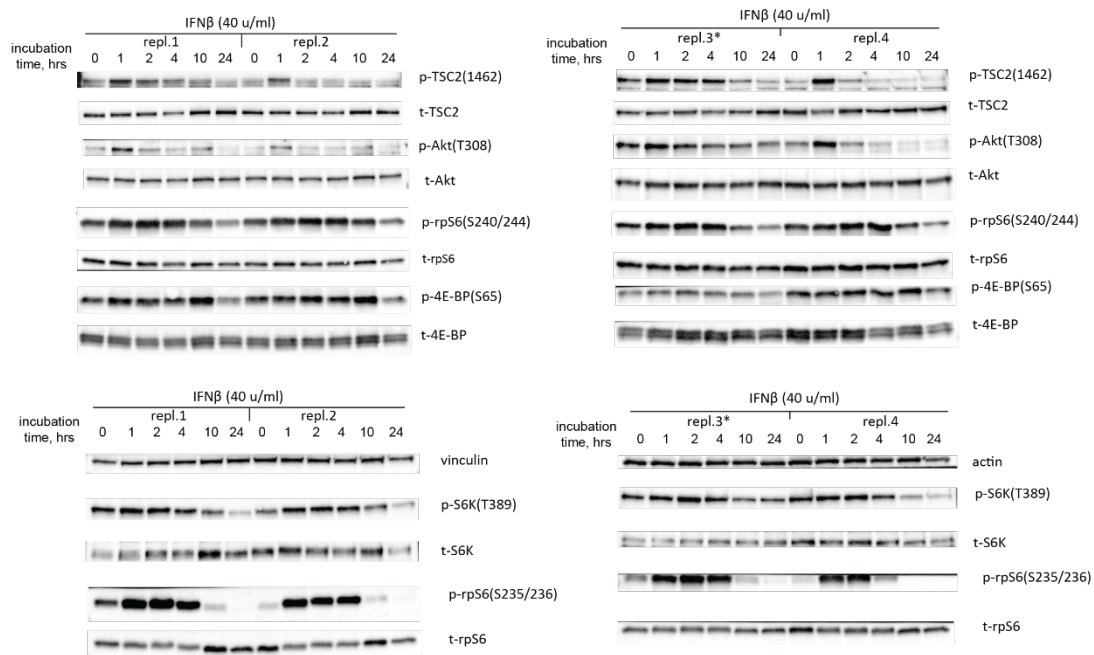


Appendix Figure S1. Ribo-Seq sample correlation quality control

A. Correlation matrix for ribosomal footprint libraries depicting pearson correlation coefficients for all samples. $n = 4$ biological replicates.

B. Correlation matrix for total RNA libraries depicting pearson correlation coefficients for all samples. $n = 4$ biological replicates.

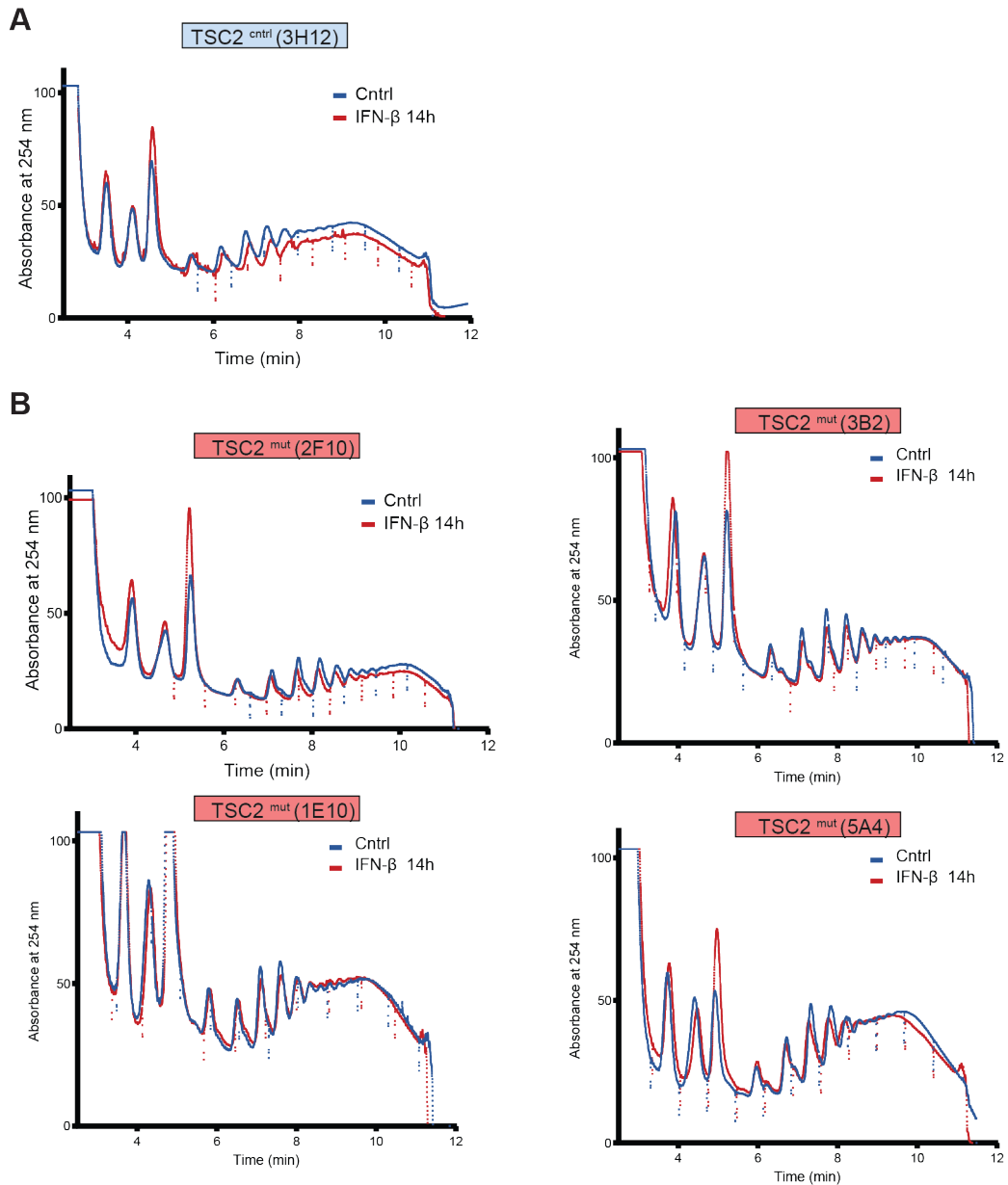
Appendix Figure S2: Collection of western blot membranes for the biphasic response of mTOR



Appendix Figure S2. Collection of western blot membranes for assessing the biphasic response of mTOR caused by IFN-β treatment – Related to Fig 2I, 3A.

Collection of western blot membranes from 4 biological replicates of NSCs treated with IFN-β for the specified times. *Replicate 3 was used as representative blots for Figures 2I, 3A.

Appendix Figure S3: Polysome profiles of CRISPR-mediated TSC2 targeting of NSCs upon IFN- β treatment



Appendix Figure S3. Polysome profiles of CRISPR-mediated TSC2 targeting of NSCs upon IFN- β treatment – Related to Fig EV4

A. Polysome profiles of CRISPR-mediated TSC2^{ctrl} NSC clone treated with IFN- β (red) as compared to control (blue).

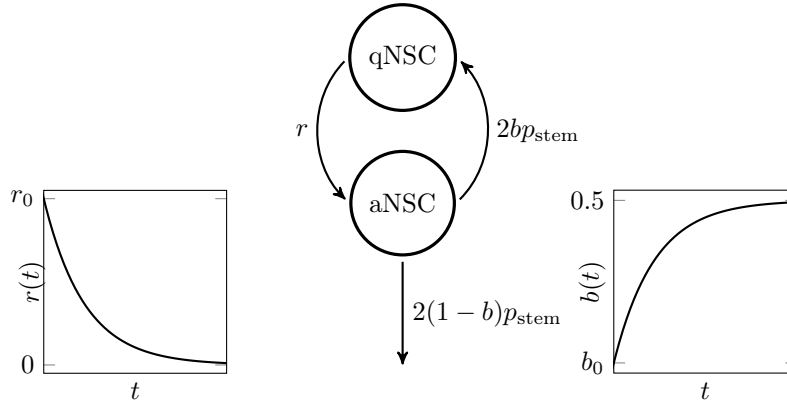
B. Polysome profiles of CRISPR-mediated TSC2^{mut} NSC clones treated with IFN- β (red) as compared to control (blue).

Appendix supplementary methods: Modelling of neural stem cell dynamics in interferon knockout mice

Modelling of neural stem cell dynamics in interferon knockout mice

1 Model

The work is based on the models which were previously established for neurogenesis of the hippocampus [1–3] and recently extended and quantified for the ventricular sub-ventricular zone [4, 5]. We adapt the model to describe the NSC dynamics in the ventricular-subventricular zone (V-SVZ) of Interferon α and γ receptor knockout (IFNAGR KO) mice.



Appendix Figure S4: Graphical representation of the model and plots for parameter functions. qNSC activate at rate r , at which point they progress through cell cycle at rate p_{stem} , to either produce two qNSC at probability b , or at probability $1 - b$ undergo differentiation.

The model consists of a system of ordinary differential equations

$$\frac{d}{dt} \text{qNSC}(t) = -r \cdot \text{qNSC}(t) + 2 \cdot b \cdot p_{\text{stem}} \cdot \text{aNSC}(t) \quad (1)$$

$$\frac{d}{dt} \text{aNSC}(t) = r \cdot \text{qNSC}(t) - p_{\text{stem}} \cdot \text{aNSC}(t) \quad (2)$$

where qNSC and aNSC describe the number of quiescent (non-cycling) and active (cycling) stem cells. Quiescent stem cells (qNSC) enter cell cycle at rate r , becoming active stem cells (aNSC). Parameter p_{stem} describes the division rate and b the fraction of self-renewal, reflecting the probability that a progeny cell stays a stem cell (qNSC) (Appendix Figure S4). Consequently, progenitor cells arise at the probability $1 - b$.

In line with previous models, we assume a constant cell division rate, based on observed cell cycle lengths from Ponti et al. [6]:

$$p_{\text{stem}} = \log(2) \cdot 24 \cdot 17.5^{-1} \approx 0.95 \text{ days}^{-1}.$$

and introduce time-dependent parameters (plots in Appendix Figure S4),

$$r(t) = r_0 \cdot e^{-t \cdot \beta_r} \quad (3)$$

$$b(t) = \frac{1}{2} (1 + e^{-t \cdot \beta_b} (2 \cdot b_0 - 1)). \quad (4)$$

In the remainder of this paper, models with the time-dependent r are called the quiescence models, while the models with time-dependent b are referred to as the self-renewal models. The models with constant r and b are called no ageing models, as they assume that cell properties do not change during aging (Appendix Table S1).

Appendix Table S1: Model variants with fully time-dependent parameters fitted. Free parameters list which parameters were estimated in model fitting. NSC(0) is the sum of both NSC compartments, at time point zero.

Name	Description	Free parameters
No aging	No time-dependence	NSC(0), r , b
Quiescence	Decreasing activation rate	NSC(0), β_r , r_0 , b
Self-renewal	Increasing self-renewal probability	NSC(0), r , β_b , b_0
Self-renewal and Quiescence	Increasing self-renewal probability and decreasing activation rate	NSC(0), β_r , r_0 , β_b , b_0

ODE initial values were picked from the steady-state ratio of the system with constant parameters. The steady state ratio can be found by solving

$$\frac{d}{dt} \frac{\text{qNSC}}{\text{aNSC}} = 0$$

using (1) and (2), which reveals that

$$\frac{\text{qNSC}}{\text{aNSC}} = \frac{p_{\text{stem}} - r}{2r} + \sqrt{\left(\frac{p_{\text{stem}} - r}{2r}\right)^2 + 2\frac{bp_{\text{stem}}}{r}}. \quad (5)$$

This gives the full model the free parameters NSC₀, r_0 , b_0 , β_r , β_b (See Appendix Table S1 for exact parameters in each model).

2 IFNAGR KO parameter estimates

2.1 Data

We obtained data from two separate experiments. In the first experiment, fluorescence activated cell sorting (FACS) was used to quantify the total number of

neural stem cells (NSC) in the ventricular sub-ventricular zone. FACS quantifications for wildtype mice were taken from Kalamakis et al. Some FACS quantifications (Appendix Table S2) were acquired in pools of mice instead of individual mice, here referred to as ‘quantifications’. IFNAGR KO NSC population estimates were obtained in eight quantifications at two months old, three quantifications at six month old and three quantifications at 22 months old (Appendix Table S2). In the second experiment, immunohistochemistry (IHC) was used to quantify the fraction of cycling (i.e. active) stem cells. This data is from Kalamakis et al. [4, Fig.5G]. By quantifying $\text{Sox2}^+\text{Dcx}^-\text{BrdU}^+\text{Mki67}^+$ as a fraction of $\text{Sox2}^+\text{Dcx}^-\text{BrdU}^+$ cells in a pulse-chase labelling regime this dataset avoids labelling cycling pro-genitors. Due to this, the fraction of cycling stem cells can now be computed as $a\text{NSC}(t)/\text{NSC}(t)$ from model outputs.

Appendix Table S2: Data used. Age is given in months. Numbers marked with † indicate that four of these quantifications were actually pools of two mice and four were individual quantifications. ‡ Indicates that all of these quantifications were pools of three mice.

Genotype	Age	Quantifications	
		FACS	IHC
IFNAGR KO	2	8†	3
	6	3	—
	22	3	4
Wildtype	2	8†	3
	7	3	—
	22	3‡	3

2.2 Parameter estimation

Parameter estimates were obtained using multi-start weighted least-squares optimisation. Optimisation starting values were sampled from optimised Latin Hypercubes using `LatinHypercubeSampling.jl` and scaled to optimisation bounds (Appendix Table S3). Initial value problems were created on the previously described steady-state ratios and solved using `DifferentialEquations.jl`’s solvers. Objective functions were built based on weighted least-squares. Weights used were the inverse of the standard deviation for each age and genotype combination. Optimisation of objective functions was then performed using box-constrained optimisers from the `Optim.jl` library. The minimum optimum for each model and genotype combination was chosen as the best optimum. For model comparison, the low-sample size adjusted Akaike Information Criterion (AIC_c) was calculated for each model and genotype combination. The Akaike Information Criterion is an information-theoretic approach for model comparison that penalises extra parameters and rewards lower residuals. Lower AIC_c values correspond to “better” models.

2.3 Time-dependent models

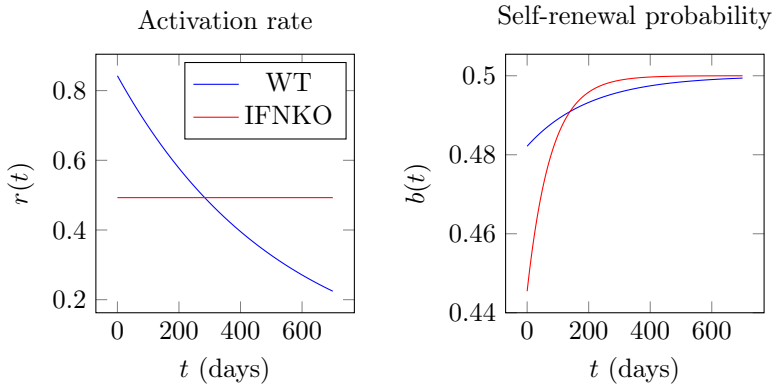
Wildtype data was best explained by models with increasing quiescence or increasing quiescence and self-renewal (Appendix Table S4). Models with only increasing self-

Appendix Table S3: Allowed parameter ranges for box-bounded optimisation and start-ing values.

Parameter	Lower	Upper
NSC(0)	0.0	2500.0
r_0	0.0	1.0
b_0	0.0	0.5
β_b	0.0	0.1
β_r	0.0	0.1

renewal failed to account for the variation in active stem cell fractions. While including self-renewal in a quiescence model provides better residuals, the change in AIC_c is not large. Previously, this, along with the minute change in self-renewal probability, was considered insufficient evidence to assume that both are taking place. These parameter estimates (Appendix Table S5) nicely recapitulate the results from our previous publication [4].

Data for IFNAGR KO mice was not explainable by time-dependent decreases in activation (“Quiescence” model). Since wildtype mice show this decrease and have IFNAGR, this indicates that IFNAGR do indeed play a role in age-related activation changes. Should these changes in activation be the only mechanism at play, we would expect IFNAGR KO to have no more time-dependent effects. However, as there is a population of stem cells remaining in old IFNAGR KO mice, models where no parameters are changing in time (“No ageing” model) are not able to explain this data. To explain this data where no age-dependent activation changes can take place, yet a population of stem cells remains in age, a time-dependent self-renewal probability is necessary. Indeed, models with such a time-dependent increase in self-renewal are the best-performing models for this dataset when ranked using residuals or AIC_c (Appendix Table S4).



Appendix Figure S5: Comparison of parameter estimates for combined quiescence and self-renewal models. The left panel shows the rate of activation, r , the right panel the probability of self-renewal, b .

While it was previously argued that this small change in self-renewal probability is not meaningful due to the much larger changes in activation rate, changes on this scale are in fact meaningful for populations and sufficient to maintain

them (Appendix Figures S5-6). With time-dependent self-renewal improving fits in both wildtype and interferon deficient mice as well as it's ability to maintain stem cell populations, it seems likely that time-dependent (or population-dependent) self-renewal is a second layer of stem cell maintenance that exists in wildtype mice as well and becomes obvious once ageing-induced quiescence is removed, as is the case in IFNAGR KO mice.

2.4 Population-dependent self-renewal

Stem cell populations approach the plateau of approximately 150 cells in wild-type mice faster than they do in knockout mice (Appendix Figure S6), which coincides with a faster increase in self-renewal probability in models that allow for changing self-renewal. This hints at a possible population-dependence of the self-renewal term. To address this issue, we additionally fit models where self-renewal was expressed in terms of total stem cells. To do this, we employed a Hill function:

$$b(\text{NSC}) = 1 - \frac{1}{1 + \left(\frac{k_b}{\text{NSC}}\right)^{n_b}} \quad (6)$$

This parameterisation of the Hill function exposes the population size at $b = 0.5$ as k_b . As this system has a steady state at $b = 0.5$, k_b is where the population will plateau.

To address if IFNAGR have a possible effect on b , we fit both data sets at the same time. To do this, we created two different models: In the first all parameters of b are shared. This models self-renewal not being affected by interferon (IFN-independent self-renewal). In the second we allowed n_b to vary with genotype. As the plateau of both populations was the same, we did not allow k_b to change with genotype. This simulates the case that interferon has an effect on self-renewal (IFN-dependent self-renewal).

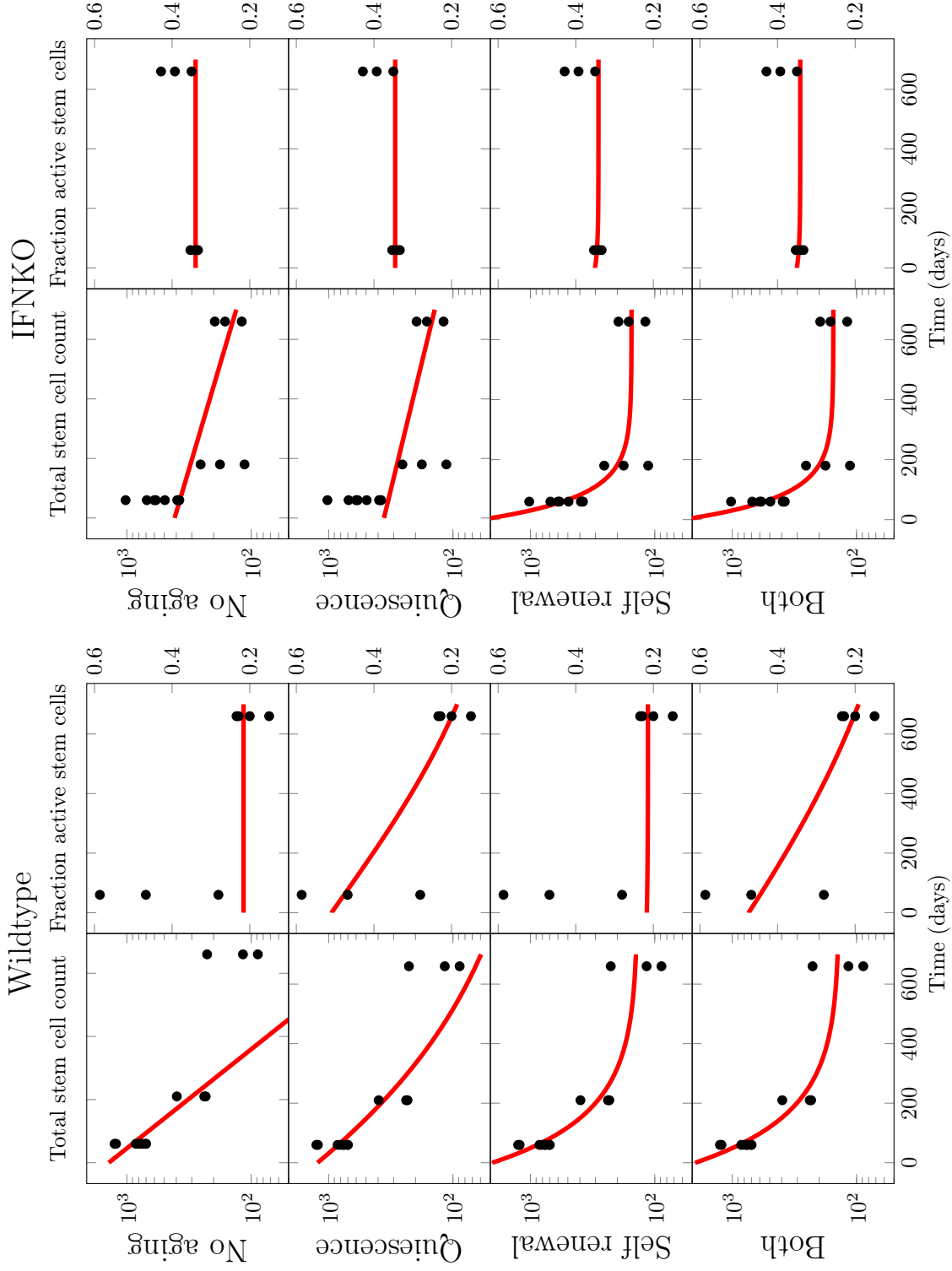
Both models were capable of explaining the observed data. The IFN-dependent model provides a small benefit to the fit at the cost of one extra parameter. Because of this, at this time it is not clear if IFNAGR additionally affects self-renewal and further experiments are required to determine this.

3 Conditional knockout simulation

To investigate the age-dependent effects of interferon knockouts, we built models that simulate an Interferon receptor knockout induced at a specific age. These models work the same as our wildtype models with population-dependent self-renewal up to some timepoint of intervention t_1 . At this timepoint, the parameter functions are switched to the functions quantified on the IFNAGR KO data and the model continues running with these. Due to the uncertain nature of the effects of IFNAGR KO on self-renewal we simulated this model with both the shared $b(\text{NSC})$ and IFN-dependent $b(\text{NSC})$ individually.

We considered two metrics of proper niche functioning: The size of the population as well as the production of progenitors from stem cells. This rate of progenitor production follows from (1) as

$$a\text{NSC}(t) \cdot (1 - b) \cdot 2 \cdot p_s. \quad (7)$$



Appendix Figure S6: Models fitted to wildtype (left column) and interferon knockout (right column) data. Panels on the left show total amounts of stem cells, qNSC + aNSC. Panels on the right show the fraction of cycling cells among these, aNSC/NSC. Red lines represent model solutions and black dots are measurements. Each row is a separate model, “Both” is the model with both time-dependent self-renewal and quiescence.

Appendix Table S4: Model fit comparison. ΔAIC_c is the change in AIC_c provided by this model compared to the best model (lowest AIC_c) for the given genotype. r_w are the weighted residuals that were optimised for. Models are sorted by ΔAIC_c , putting the top-performing models at the top.

	Model	r_w	ΔAIC_c
IFNKO	Self renewal	20.28	0.0
	Self renewal and Quiescence	20.28	1.8
	No ageing	36.66	10.25
	Quiescence	34.95	10.89
Wildtype	Self renewal and Quiescence	16.0	0.0
	Quiescence	21.61	4.55
	Self renewal	22.94	5.81
	No ageing	33.1	11.93

Appendix Table S5: Obtained parameter estimates for wildtype and interferon knock-out data. Values at ‘—’ are not present and can be read as 0.

	parameter	no ageing	quiescence	self-renewal	both
IFNAGR KO	NSC ₀	412.9	359.0	2500.0	2500.0
	r_0	0.4878	0.5006	0.4928	0.4928
	β_r	—	$2.56 \cdot 10^{-10}$	—	$1.94 \cdot 10^{-12}$
	b_0	0.4975	0.4987	0.4455	0.4455
	β_b	—	—	0.01288	0.01288
Wildtype	NSC ₀	1397.0	1228.0	2031.0	1986.0
	r_0	0.2598	0.9786	0.2587	0.8426
	β_r	—	0.00216	—	0.001891
	b_0	0.4821	0.4932	0.4596	0.4822
	β_b	—	—	0.006139	0.004862

Appendix Table S6: Residuals for models with population dependent self-renewal. The number of free parameters is denoted k , the residuals r_w and the difference in low-sample size corrected Akaike Information Criterion ΔAIC_c .

Self-renewal	k	r_w	ΔAIC_c
IFN-independent	8	39.0	
IFN-dependent	9	36.6	

To assess the life-long effects of this conditional knockout we also computed the integral of this progenitor production from 0 to 700 days, here called “life-long progenitor production”.

3.1 IFN-dependent self-renewal

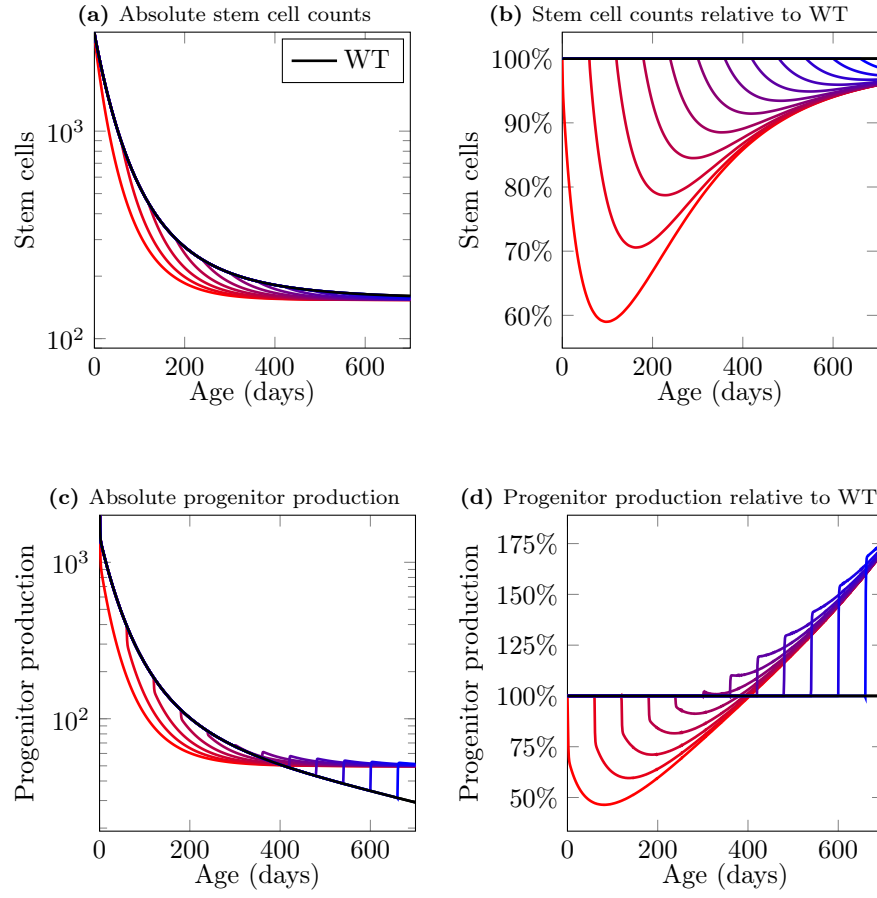
Conditional knockout simulations with IFN-dependent self-renewal showed a decrease of stem cell populations upon intervention for all intervention ages. This difference was most pronounced in young ages, where the population was down to 60% of wildtype in the worst case. In old ages, this effect was less pronounced, with mice retaining most of their stem cells (Appendix Figure S7a-b). In young ages, progenitor production was also seriously affected. This was down to 50% of the wildtype in the worst cases, but eventually recovered to yield a higher progenitor production than in wildtype for old ages, up to 175% of the wildtype production (Appendix Figure S7c-d). At ages beyond approximately 100 days, the total number of cells produced was higher than in wildtype, peaking out around 300 days intervention age. Overall, this shows that IFNAGR KO could increase the productivity of the niche at the cost of a few cells, when done in sufficiently old ages.

3.2 IFN-independent self-renewal

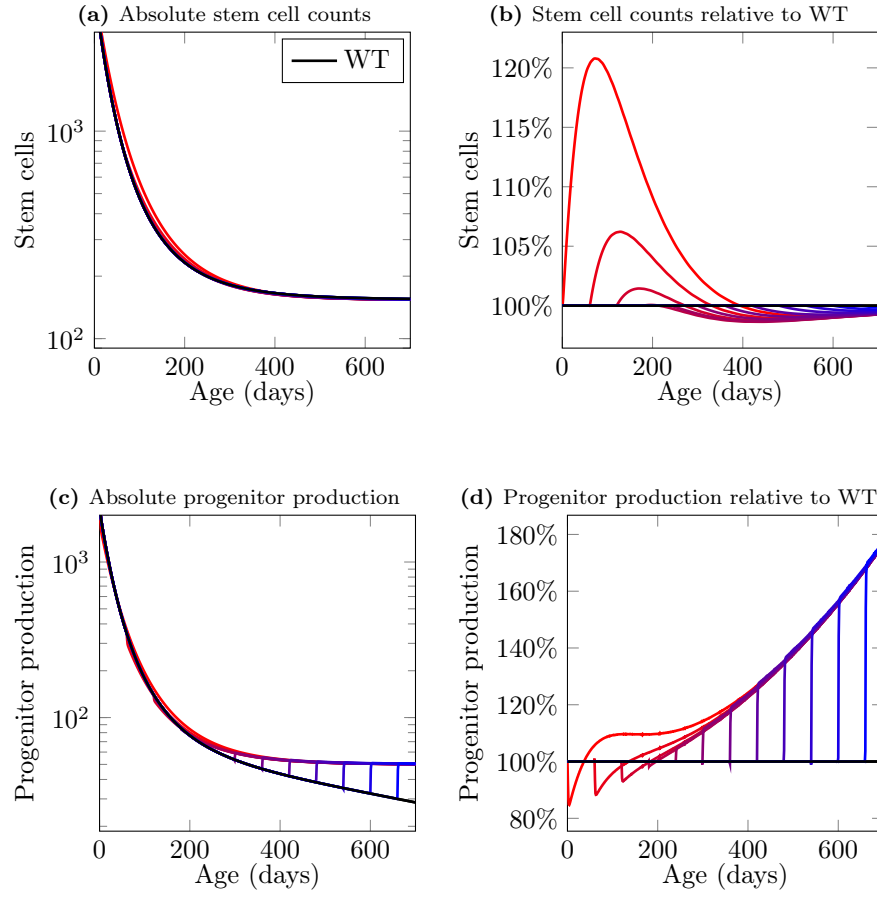
Simulations with IFN-independent self-renewal show the same trend of increased progenitor production in older ages, with some decrease in stem cell numbers in old ages as the IFN-dependent self-renewal models (Appendix Figure S8a). Contrary to those however, there is no loss of stem cells in young interventions. Instead, stem cell populations increase in size for interventions in young ages (Appendix Figure S8b). Further, there is no strong loss of progenitor production in early ages either (Appendix Figure S8c-d). Thus, should IFNAGR also have an effect on self-renewal, progenitor production would still be increased in age, with no obvious adverse effects in young ages (Appendix Figure S9).

References

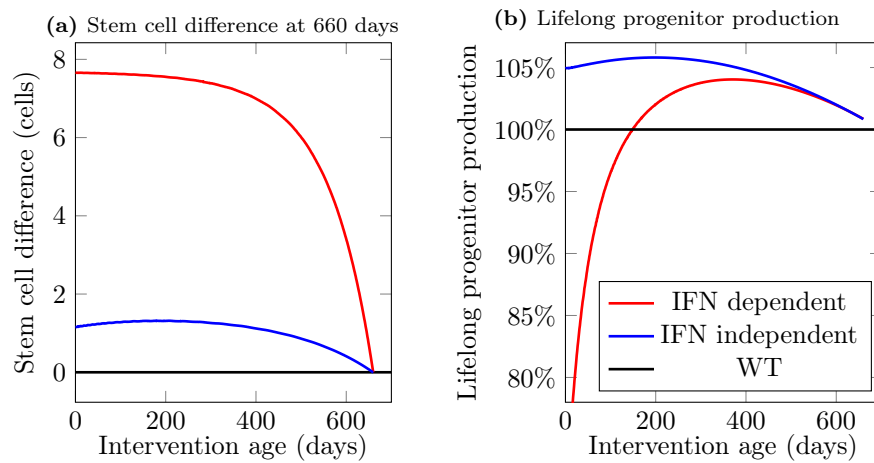
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Appendix Figure S7: IFN-dependent conditional knockout simulations. Stem cell counts are the total stem cells for the wildtype (black) and simulations at varying timepoints (coloured from red to blue). Progenitor production is the rate of progenitors produced from stem cells. Relative values are expressed relative to the WT simulation.



Appendix Figure S8: IFN-independent conditional knockout simulations. Stem cell counts are the total stem cells for the wildtype (black) and simulations at varying timepoints (coloured from red to blue). Progenitor production is the rate of progenitors produced from stem cells. Relative values are expressed relative to the WT simulation.



Appendix Figure S9: Stem cell differences at old age (660 days) for varying intervention ages and life-long progenitor production as percentage of wildtype. Both a scenario with interferon dependent (red) and interferon independent (blue) self-renewal are shown compared to the wildtype (black).

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