



Small RNA profiling reveals inflammatory and mitochondrial changes in aging β cells and islet macrophages



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Aging is associated with functional decline and senescence of pancreatic β -cells. Modulation of post-transcriptional mechanisms is a hallmark of aging. Here we analyze the small non-coding RNA (sncRNA) repertoire of FACS-sorted β -cells and islet-resident macrophages (iMACs) from 3-, 12-, and 22-month-old mice, and of senescence-associated β -galactosidase-positive β -cells from 8-month-old mice. We show that senescent β -cells display distinct sncRNA signatures partially overlapping with aging-associated ones. MiRNAs linked to diabetic conditions and islet inflammation are commonly modulated in both contexts. Cytokine exposure followed by washout partially recapitulates the aging sncRNA profile in vitro. Upregulation of inflammatory miR-146a-5p and downregulation of proliferative miR-181a-5p are shared across aged metabolic tissues, whereas upregulation of the miR-143-3p/miR-145-5p cluster, which is associated with insulin responses, is β -cell specific. Aging also remodels the tRNA-derived fragment (tRF) repertoire, with mitochondrial tRFs markedly increased. iMACs from aged mice show elevated anti-inflammatory associated miRNAs and mitochondrial tRFs, suggesting adaptive reprogramming. Overall, our findings reveal extensive sncRNA remodeling during islet aging.

Biological aging results from the accumulation of cellular damage that leads to a progressive decline in physiological functions, favoring disease development and mortality. While being a fundamental process, aging is influenced by genetic predisposition and environmental factors. With the global increase in life expectancy, understanding the complex cellular mechanisms linked to aging is crucial to favor healthy aging and reduce disease outcomes in elderly people. Aging represents a risk factor for metabolic diseases like diabetes because of the establishment of insulin resistance in insulin target tissues and the progressive decline of pancreatic β -cell ability to compensate^{1–3}. Several hallmarks have been associated with aging⁴ including cellular senescence, altered intercellular communication and epigenetic alterations.

Accumulation of senescent β -cells has been observed in both elderly humans and mice, and their proportion increase in response

to glucose intolerance and to cellular stress^{5–7}. Importantly, clearance of senescent β -cells using senolytic drugs and/or by genetic ablation prevented both type 1 (T1D) and type 2 (T2D) diabetes development in different mouse models^{6,8}.

An important alteration in islet intercellular communication is the establishment of low-grade chronic inflammation⁴, an aging hallmark shared with obesity, another common risk factor for T2D. Among the mechanisms possibly involved, islet-resident macrophages (iMACs) emerge as new players^{9,10}. Under physiological conditions, iMACs exhibit a unique pro-inflammatory profile that favors β -cell function, while insulin resistant conditions induce iMAC expansion and transcriptional reprogramming that exacerbates β -cell dysfunction. Increased macrophage numbers have been observed in aged mouse islets¹¹, however their activation phenotype remains to be investigated.

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As part of age-related epigenetic alterations, recent evidence shows that the correlation between mRNA and protein expression is progressively lost during aging¹², pointing to a deregulation of the post-transcriptional regulatory network. Non-coding RNAs orchestrate a variety of post-transcriptional gene regulatory mechanisms. ncRNAs are classified based on nucleotide length: long non-coding RNAs (>200 nt) and small ncRNAs (sncRNAs, <200 nt). Beside well described sncRNA classes like microRNAs (miRNAs) and PIWI-interacting RNA (piRNAs), other categories of sncRNAs with specific mode of actions in gene regulation are continuously discovered. Fragments derived from the cleavage of transfer RNAs (tRFs) have been identified as functional sncRNAs binding to different protein and RNA interactors¹³. Next-generation sequencing studies revealed the existence of isoforms of miRNAs (isomiRs) that differ by one or more nucleotides from the canonical miRNA sequence, resulting in differences in target recognition or stability^{14,15}. Both tRFs and isomiRs have been identified in pancreatic β -cells and have been proposed to be involved in diabetes development^{16–19}. miRNA deregulation in aging has been scarcely studied. We previously performed a microarray on aged rat islets and observed that an increase of miR-34a-5p and a decrease of miR-181a-5p were associated with impaired β -cell proliferation in elderly rat islets²⁰. The contribution of other classes of sncRNAs to age-related alterations in islet function remains to be established.

Here, we took advantage of small RNA sequencing technologies and refined bioinformatics pipelines to obtain a comprehensive sncRNA profiling of aging β -cells and iMACs. Aged β -cells showed changes in the level of several miRNAs that are involved in the control of β -cell functions and are deregulated in T1D and/or T2D. Globally, aged β -cells exhibited an inflammatory miRNA signature while iMACs displayed a miRNA profile reflecting the acquisition of a tissue homeostatic reparative phenotype. Both β -cells and iMACs showed increased mitochondrial tRF levels in aging, suggesting metabolic adaptation. Senescent β -cells in adult mice displayed a sncRNA expression profile partially overlapping with that of aged β -cells, with activation of inflammatory miRNA-dependent mechanisms and anti-proliferative signals. In addition to the analysis of the miRNA and tRF patterns associated with aging, we also provide data on piRNAs, isomiRs and other less characterized sncRNAs modulated during aging.

Results

Comprehensive analysis of the changes in the small non-coding RNA profile in aging β -cells

Pancreatic islets were isolated from young adult (3-month-old), middle-aged (12-month-old) and elderly (22-month-old) wild-type C57BL/6J male mice. Body weight was higher in 12- and 22-month-old mice, while fed blood glucose levels were similar between age groups (Suppl. Fig. 1a, b). To obtain a comprehensive dataset of the sncRNAs expressed in insulin-secreting cells, we performed a small RNA sequencing on 4 independent preparations of FACS-sorted β -cells from these 3 age groups (Suppl. Fig. 1c, d). Principal component analysis highlighted the clustering of samples based on mouse age (Suppl. Fig. 1e). Sequencing reads were then annotated to the mouse genome and mapped to reference databases for miRNA (Suppl. Data 1), isomiR (Suppl. Data 2), piRNA (Suppl. Data 3), and tRF (Suppl. Data 4) identification. In addition, reads not matching reference sncRNAs but unambiguously mapping to one genomic location were used for differential expression analysis (Suppl. Data 5). More than 98% of the total unique read counts were retained for statistical analysis (Suppl. Fig. 1f), of which ~70% mapped to miRNAs and 1–5% to tRNAs (Suppl. Fig. 1g). This systematic and unbiased analysis revealed major modulation in the sncRNA profile of β -cells occurring during aging. Using a cut-off of FDR p -value < 0.05, Fold Change > 2, we could detect age-dependent changes in all different sncRNA biotype categories in middle-aged and elderly β -cells compared to young adults (Suppl. Fig. 1h), suggesting that sncRNA rewiring might represent a hallmark of β -cell aging.

Differential miRNA and isomiR expression in aged β -cells

We further characterized the modulated miRNA signature because of their crucial role in islet homeostasis and diabetes development^{21–23}. In FACS-sorted β -cells, 1151 sequences were annotated as miRNAs. Upon expression filtering, 588 miRNAs in 12- versus 3- and 821 miRNAs in 22- versus 3-month-old were retained for statistical multiple-testing correction, respectively (Suppl. Data 1). We identified 44 and 153 miRNAs that were differentially expressed in 12- and in 22-month-old mice, respectively, compared to young 3-month-old mice (FDR p -value < 0.05, Fold Change > 2; Fig. 1a, b). Among the age-modulated miRNAs, 16 were downregulated and 15 were upregulated in β -cells of both 12- and 22-month groups (Suppl. Fig. 1i and Suppl. Data 1). Notably, miR-146a-5p, a miRNA associated with human inflammation and aging²⁴ was downregulated at 12 months but upregulated in older mice (22 months) (Suppl. Data 1). To gain a broader view of the pathways potentially affected by the modulated miRNAs, we performed a functional enrichment analysis using a more inclusive list of differentially expressed miRNAs selected with less stringent thresholds (FDR p -value < 0.1, Fold Change > 1.5). Downregulated miRNAs in 22-month-old β -cells were associated with FoxO regulation and mTOR signaling (Fig. 1c). Notably, these pathways control many essential cellular processes, such as cell cycle, survival, metabolism and proliferation, and contribute to cellular aging by modulating autophagy and oxidative stress. Other overrepresented KEGG pathways in downregulated miRNAs were insulin resistance and autophagy, while miRtar Base Gene Ontology enrichment highlighted the downregulation of miRNAs involved in cellular responses to hypoxia and to insulin resistance (Fig. 1d). These results suggest that a modulation of the miRNome may contribute to the metabolic adaptation of β -cells to aging. The annotation of upregulated miRNAs did not return any significant pathway. We then analyzed whether the miRNAs modified during aging present a validated functional link with β -cell processes and diabetes development. Based on a meta-analysis of the existing literature (Suppl. Data 6), we identified groups of miRNAs associated with islet inflammation (Fig. 1e), β -cell proliferation (Fig. 1f), β -cell maturation (Fig. 1g), insulin secretion (Fig. 1h), and T1D and T2D (Fig. 1i–l). As shown in the heatmap plots, miRNAs that have been reported to promote islet inflammation generally increase with age, while miRNAs associated with β -cell proliferation and maturation tend to change to favor a highly mature β -cell phenotype with limited proliferative capacity. At least part of the changes detected in β -cells of elderly animals overlapped with those observed during T1D and T2D pathogenesis. Reference annotations of the literature meta-analysis are included in Suppl. Data 6. Altogether, the age-associated miRNA signature in β -cells points to a progressive loss of regenerative capacity accompanied by the establishment of a pro-inflammatory microenvironment.

To determine whether these age-related miRNA changes were sex-independent, β -cells were FACS-sorted from 3 independent preparations of islets from female mice aged 3 or 12 months and analyzed by small RNA-seq. Enzymatic removal of RNA modifications in the female dataset markedly shifted library composition, reducing the proportion of miRNA reads from ~70 to ~2% while increasing the reads mapping to tRNA and other RNA species (Suppl. Fig. 2a, b). However, sufficient miRNA coverage was retained for robust differential expression analysis. Similarly to the male dataset, 688 of the 1960 miRNAs detected passed expression filtering. RRHO analysis revealed a significant overlap between miRNA changes in middle-aged male and female mice, defining a conserved age-associated remodeling of the β -cell miRNA landscape (Suppl. Fig. 2c and Suppl. Data 7). To confirm these findings, we validated selected miRNAs previously implicated in inflammation (miR-146-5p), aging and proliferation (miR-181a-5p), and insulin sensitivity and glucose homeostasis (miR-143-3p and miR-145-5p, which are transcribed as a cluster) (Suppl. Fig. 2d–g). qPCR validation of selected miRNAs confirmed that miR-146-5p was induced in middle-aged female β -cells, suggesting earlier activation of inflammatory pathways in females. miR-181a-5p levels were significantly reduced at 12 months, while miR-143-3p and miR-145-5p showed a trend

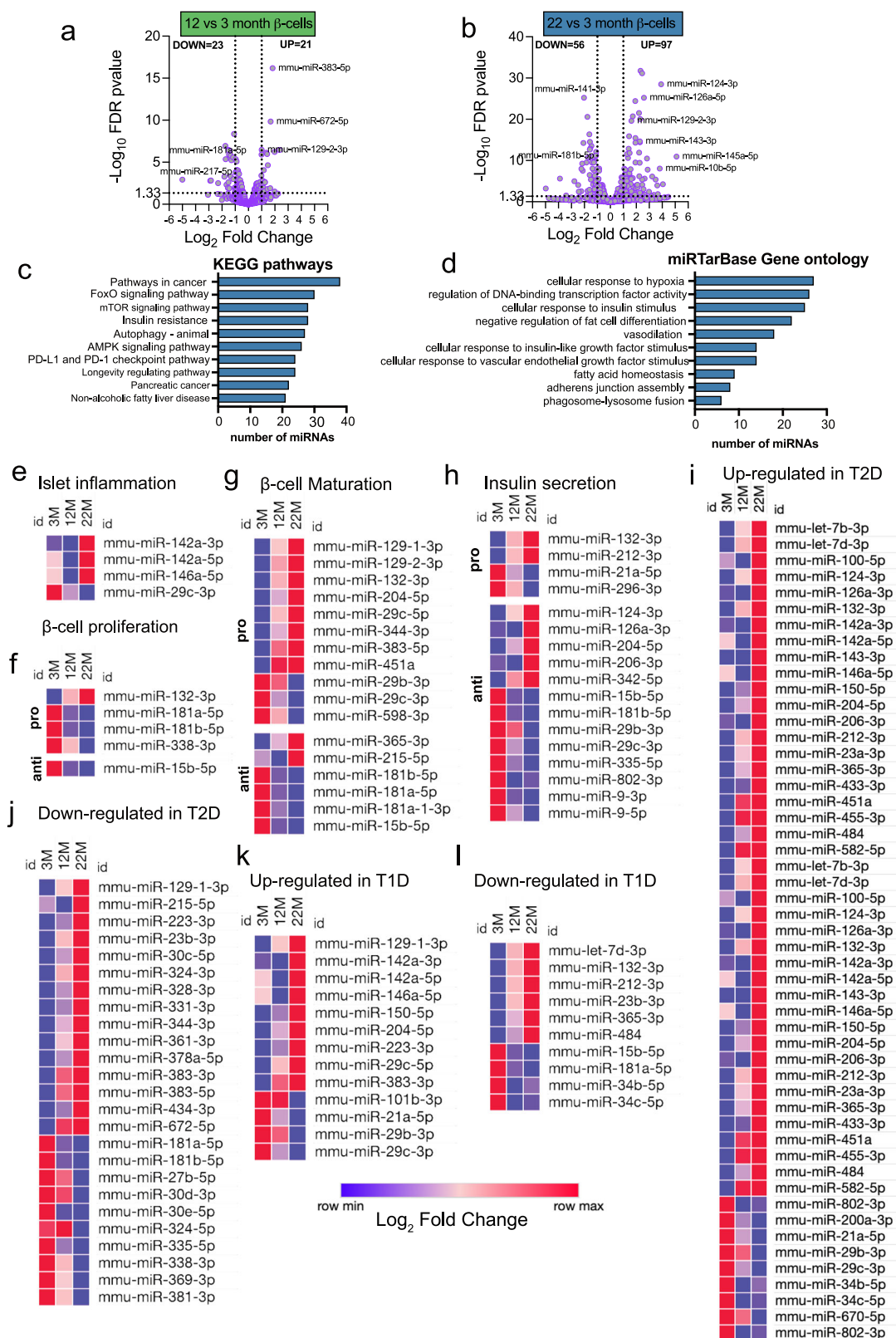
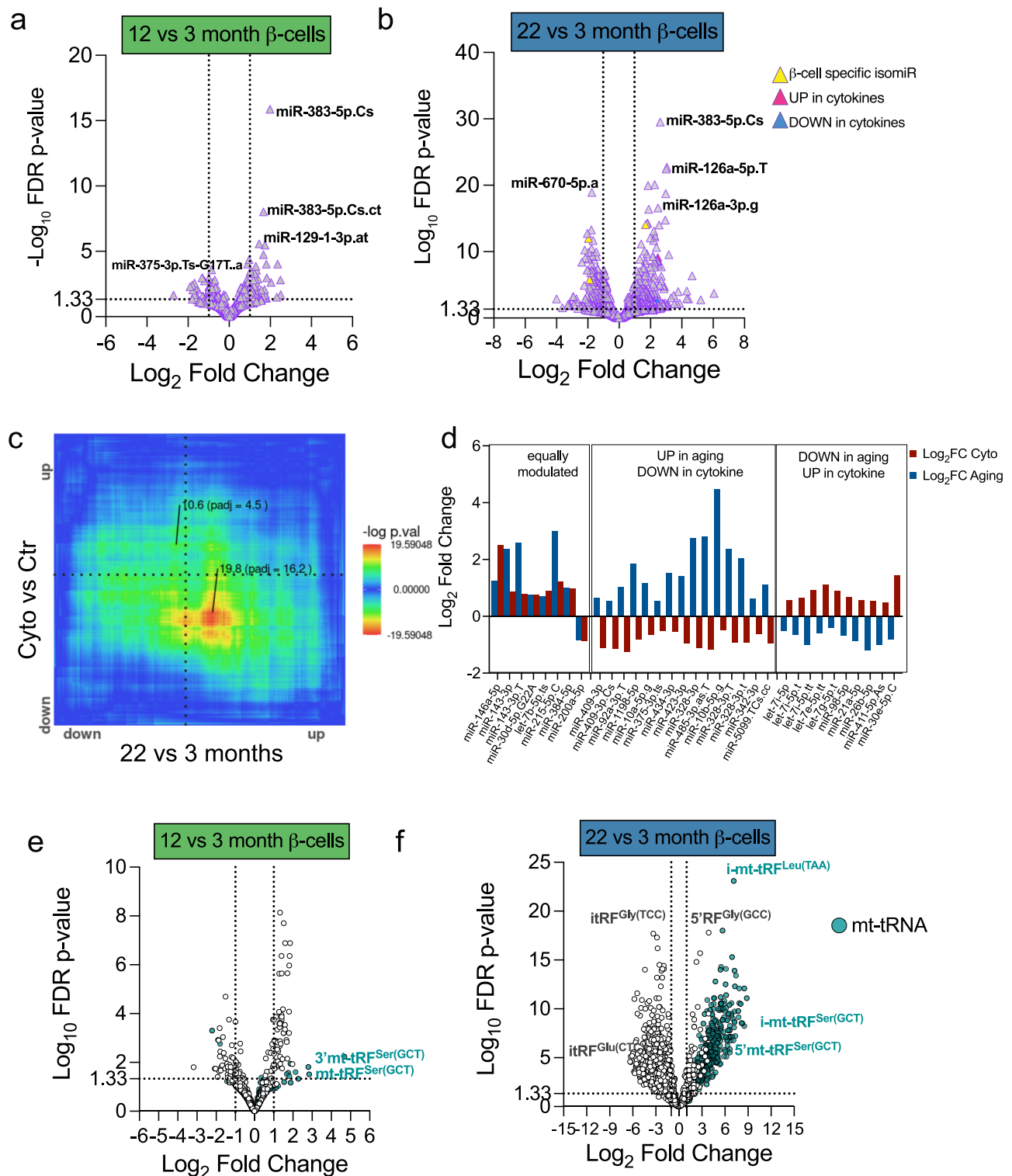


Fig. 1 | Differential miRNA expression in aged β -cells. Small RNA-seq on FACS-sorted β -cells from young adult (3-month), middle-aged (12-month) and elderly (22-month) C57BL/6J male mice ($n = 4$ independent experiments). **a, b** Volcano Plots show changes in miRNA expression in 12-month (a) or 22-month (b) versus 3-month-old β -cells. Differential expression was computed using a negative binomial generalized linear model with two-sided t -test and Benjamini-Hochberg FDR correction. Dotted lines define thresholds of FDR p -value < 0.05 ($-\text{Log}_{10}$ FDR > 1.33) and Fold Change > 2 (Log_2 FC > 1). Functional enrichment was

performed on miRNAs with FDR p -value < 0.1 and Fold change > 1.5 (c, d). **c** Overrepresented KEGG pathways and **d** miRTar Base Gene Ontology enrichments associated with miRNA downregulation of 22-month-old β -cells. **e–l** Heatmap plots showing miRNAs significantly deregulated in 22-month-old β -cells that were previously reported in the literature to be associated with islet inflammation (e), β -cell proliferation (f), β -cell maturation (g), or insulin secretion (h), or deregulated in islet from T2D (i, j) or T1D (k, l) models. References provided in Suppl. Data 6.



toward increased expression that did not reach statistical significance, mirroring observations in male mice.

Beyond canonical miRNAs, we identified 97328 isomiRs, of which 3722 were retained for statistical testing (Suppl. Data 2). Eighty-four were differentially expressed in β -cells from 12-month-old mice and 480 in 22-month-old mice (FDR p -value < 0.05, Fold Change > 2; Suppl. Data 2, Suppl. Fig. 1h, and Fig. 2a, b). Deregulated isomiRs predominantly displayed 3' alterations and base switching, while 5' alterations were less represented (Suppl. Data 2). Notably, isomiRs of miR-383-5p, miR-126a-5p, and miR-

126a-3p were more abundant in aged β -cells, and among β -cell-enriched isomiRs¹⁶, mmu-miR-221-3p.c and miR-204-5p.G were upregulated while miR-141-3p.g and miR-335-5p.t were downregulated with aging (Fig. 2b).

Since several aging-deregulated miRNAs are linked to inflammation, we assessed the impact of acute 24 h pro-inflammatory cytokine treatment of whole mouse islets on miRNA and isomiR levels using small RNA-seq. (Suppl. Fig. 3a). Cytokine treatment downregulated 13 miRNAs and 46 isomiRs and upregulated 10 miRNAs and 62 isomiRs (FDR < 0.1, FC > 1.5), confirming the upregulation of inflammation-associated miR-146a-5p^{25,26}

Fig. 2 | Differential isomiR and tRF levels in aged β -cells. Small RNA-seq on FACS-sorted β -cells from young adult (3 months), middle-aged (12 months) and elderly (22 months) C57BL/6J male mice ($n = 4$ independent experiments). **a, b** Volcano Plots show differential levels of isomiRs in 12-month (a) or 22-month (b) versus 3-month-old β -cells; Differential expression was computed using a negative binomial generalized linear model with two-sided t -test and Benjamini–Hochberg FDR correction. Dotted lines define thresholds of FDR p -value < 0.05 ($-\log_{10}$ FDR > 1.33) and Fold change > 2 ($\log_2$ FC > 1). Yellow triangles highlight isomiRs previously found to be β -cell specific, and pink and blue triangles isomiRs upregulated or downregulated, respectively, in islets treated with pro-inflammatory cytokines. **c** RRHO analysis comparing miRNA and isomiR modulation in islets exposed for 24 h to pro-inflammatory cytokines ($n = 3$ independent experiments) versus age-associated changes in FACS-sorted β -cells of 22-month mice ($n = 4$ independent

experiments). A predominant overlap can be observed in inversely correlated changes. **d** Graphical representation of miRNAs and isomiRs that are significantly modulated (FDR p -value threshold of < 0.1 , FC > 2) in response to pro-inflammatory cytokines and during aging, either in the same direction (left part of the graph) or in opposite direction (middle and right part of the graph). **e, f** Volcano Plots show differential levels of tRFs in 12-month (e) or 22-month (f) versus 3-month-old β -cells; Differential expression was computed using a negative binomial generalized linear model with two-sided t -test and Benjamini–Hochberg FDR correction. Dotted lines define thresholds of FDR p -value < 0.05 ($-\log_{10}$ FDR > 1.33) and Fold change > 2 ($\log_2$ FC > 1). White circles correspond to tRFs derived from nuclear-encoded tRNAs and turquoise circles refer to tRFs derived from mitochondrially-encoded tRNAs.

and T1D-associated miR-1a-3p²⁷ (Suppl. Fig. 3b). RRHO analysis comparing acute cytokine-induced ($n = 3$ independent experiments) and age-associated ($n = 4$ independent experiments) changes revealed that, although significant correlations existed in concordant directions, the predominant overlap occurred in inversely correlated changes (Fig. 2c), suggesting that acute cytokine exposure and physiological aging induce partially distinct and sometimes opposing small RNA regulatory programs. Applying an FDR p -value threshold of < 0.1 , we identified a set of miRNAs and isomiRs that were significantly modulated in both datasets (Fig. 2d). Among small RNAs modulated in both conditions, eight were altered in the same direction, including upregulated miR-146-5p, miR-143-3p, and the isomiR miR-143-3p.T. Fifteen miR/isomiRs were downregulated by cytokines but upregulated during aging, including miR-409-3p, previously reported to be decreased in T1D and increased in T2D patients' plasma^{28,29}. Conversely, ten miR/isomiRs upregulated by cytokines were downregulated in aged β -cells, including miR-21a-5p, implicated in cytokine-induced β -cell apoptosis²⁶, and let-7i-5p, a negative regulator of insulin secretion³⁰, along with its truncated isomiRs.

We also profiled piRNAs, which guide PIWI proteins to transposable elements to maintain genomic stability. Although most abundant in germ cells, PIWI proteins and piRNAs are expressed in pancreatic islets, where they regulate insulin secretion. Altered piRNA profiles have been reported in rodent T2D models³¹. In β -cells from male mice, we detected 0.5–1% piRNA-mapping reads, corresponding to 262 piRNAs, (Suppl. Data 3), and 89 were retained for statistical analysis. Six were significantly modulated at 12 months and 36 at 22 months (FDR p -value < 0.05 , Fold Change > 2 ; Suppl. Fig. 3c, d). None of the age-related piRNA changes overlapped with those previously reported in T2D rat islets³¹.

Impact of aging on the β -cell tRF profile

tRNA cleavage generates functional sncRNA fragments. Given the emerging role of tRFs in islet gene regulation^{9,17,18,32}, we further characterized tRFs modulated during aging. Small RNA-seq analysis retained 2222 sequences mapping to genomic tRNA loci passing expression filtering, including 1723 from nuclear-encoded and 499 from mitochondrially-encoded tRNAs (Suppl. Data 4). Reads derived from tRFs accounted for 1–5% of the total filtered counts. Considering tRF ranging up to 55 nucleotides in length, the highest read abundance was detected for fragments of 16–25 nt and 32–34 nt (Suppl. Fig. 4a). However, our sequencing settings also enabled the detection of longer fragments up to 77 nt, including some corresponding to full-length mature tRNAs (Suppl. Fig. 4a). Differential expression analysis revealed a marked age-dependent remodeling of the tRF landscape: 138 tRFs were modulated at 12 months and 1469 at 22 months compared to 3-month-old mice (FDR p -value < 0.05 , Fold Change > 2 ; Fig. 2e, f). Among upregulated tRFs, we identified a 5'tRF generated by cleavage of tRNA^{Gly(GCC)} at the anticodon loop, a tRF class typically associated with stress responses. We previously demonstrated that 5'tRF^{Gly(GCC)} is upregulated in islets of T2D patients and in β -cells exposed to fatty acids⁹ and its extracellular vesicle-mediated transfer from T cells induces apoptosis in recipient β -cells¹⁷. Mitochondrial tRFs accounted for a substantial proportion of age-associated

increases in tRF abundance: of the 75 and 474 tRFs upregulated at 12 and 22 months, 10 and 396 originated from mitochondrially-encoded tRNAs (mt-tRNAs) (Suppl. Data 4). Upregulated mt-tRFs included an internal tRF from mt-tRNA^{Leu(TAA)}, previously shown to regulate mitochondrial respiration and glucose-induced insulin secretion and 5'tRF^{Ser(GCT)}, which is repressed in islets from diabetic db/db mice³².

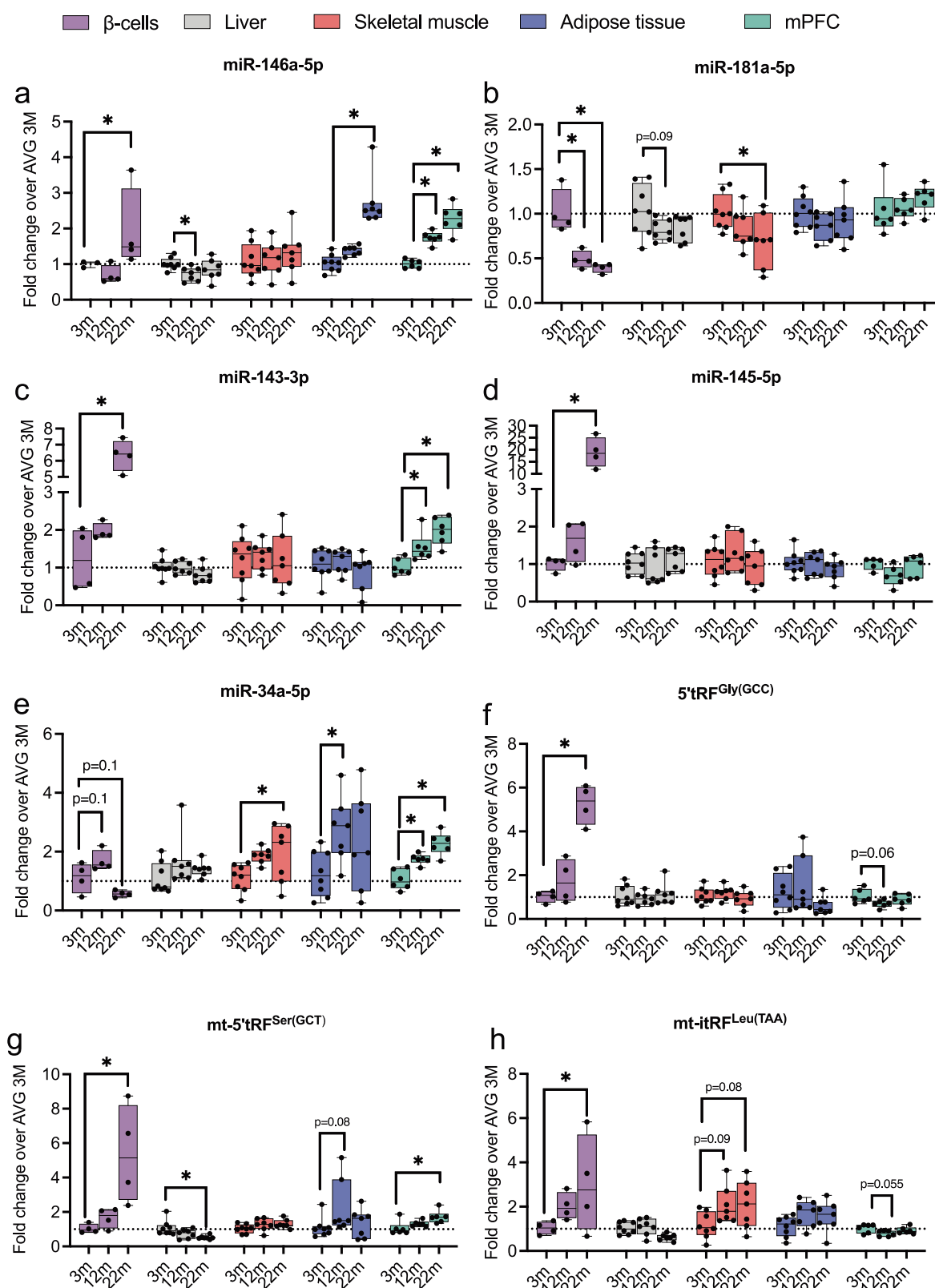
To assess whether similar changes occurred in female β -cells, small RNA-seq was performed on β -cells from 3- and 12-month-old female mice using modification-depleted RNA to improve tRF detection^{33,34}. This yielded 7–14% tRNA-mapping counts (Suppl. Fig. 2a, b), corresponding to 4269 tRFs, including 1057 of mitochondrial origin (Suppl. Data 4). As observed in male mice, fragments up to 77 nt of length were detected, with the highest normalized read counts corresponding to fragments of 15 to 40 nt (Suppl. Fig. 4b). Differential expression analysis (FDR p -value < 0.05 , Fold Change > 2) identified 86 significantly modulated tRFs at 12 months, of which 59 originated from mt-tRNAs (Suppl. Fig. 4c), further supporting an age-dependent remodeling of the mitochondrial tRF repertoire. qPCR validation confirmed the upregulation of tRNA^{Gly(GCC)}, and mt-tRF^{Leu(TAA)} and 5'tRF^{Ser(GCT)} showed a trend toward increased expression in middle-aged female β -cells (Suppl. Fig. 4d–f).

To assess whether the increase in mt-tRFs reflects greater mitochondrial mass, we measured mitochondrially encoded 16S and 12S ribosomal RNAs, which showed a non-significant tendency to increase with age (Suppl. Fig. 5a, b). Unbiased analysis further revealed age-dependent overexpression of mitochondrial RNA transcripts, including ribosomal RNAs and protein-coding genes in both male and female mice-derived datasets (Suppl. Fig. 5c–e and Suppl. Data 5).

In the male dataset, full-length transcripts from 18 of the 22 mitochondrial tRNAs were detected across all age groups, whereas only four cytosolic full-length tRNAs were identified (Suppl. Data 4). This likely reflects more extensive nucleotide modifications on cytosolic tRNAs, which impair reverse transcription and reduce sequencing efficiency^{33,34}. Consistent with this, modification removal in the female dataset markedly increased cytosolic full-length tRNA detection from 4 to 15 species, while mitochondrial tRNA detection remained comparable to the male dataset (17 of 22 species; Suppl. Data 4). Differential expression analysis (FDR p -value < 0.05 , Fold Change > 2) revealed no significant age-dependent changes in full-length mt-tRNA relative abundance in 12-month-old male or female mice (Suppl. Fig. 5f and g). However, the relative abundance of all detected full-length tRNAs was significantly higher in β -cells of 22-month-old male mice (Suppl. Fig. 5f), indicating a possible global accumulation of mt-tRNAs in advanced aging.

miRNA and tRF modulation in other aging tissues

We next assessed whether the observed deregulation of miRNAs and tRFs is specific for β -cells or is shared with other metabolic tissues. For this purpose, selected miRNAs and tRFs identified by RNA-seq were measured by qPCR in FACS-sorted β -cells, liver, skeletal muscle (SM), adipose tissue (AT) and in medial pre-frontal cortex (mPFC), a brain region involved in age-related cognitive decline (Fig. 3). As described above, we selected miRNAs



differentially expressed in β -cell RNA-seq, involved in key pathways of metabolic aging, such as inflammation (miR-146-5p), aging and cell proliferation (miR-181a-5p) and insulin sensitivity and glucose homeostasis (cluster miR-143-3p/miR-145-5p). Although unchanged in the aging β -cell datasets, we also included miR-34a-5p that has been extensively associated to p53 signaling in cancer and aging tissues³⁵. In addition, we tested the

stress-associated 5'tRF^{Gly(GCC)} and the mitochondrial mt-tRFs Ser^(GCT) and Leu^(TAA). None of the tested snRNAs showed a universal pattern in response to aging. We found that the inflammatory marker miR-146a-5p was mainly upregulated in β -cells (confirming our sequencing data), in AT and mPFC (Fig. 3a). As previously reported in elderly rat islets²⁰, miR-181a-5p was decreased in FACS-sorted mouse β -cells but also in SM, and a similar

Fig. 3 | miRNA and tRF levels in aged metabolic tissues. FACS-sorted β -cells were isolated from mouse islet pools (purple, $n = 4$ independent experiments). Tissues were isolated from individual from 3-, 12-, and 22-month-old C57BL/6J male mice: liver (grey; $n = 6$ independent experiments), skeletal muscle (red; $n = 7$ independent experiments), adipose tissue (blue; $n = 7$ independent mice) and medial pre-frontal cortex (mPFC, green; $n = 6$ independent experiments). RNA was extracted and **a–e** miRNA and **f–h** tRF levels were measured by qPCR, normalized on let-7a-5p and let-7b-5p and expressed as fold change over the average value of the specific tissue from 3-month-old mice. Box plots show the median (center line), the 25th and 75th percentiles (box limits), and the minimum and maximum values (whiskers). Individual data points are shown. * p -value < 0.05 by one-way ANOVA, Dunnett's post hoc test for each tissue. **a** miR-146a-5p * $p = 0.0499$ 22 vs 3 months in β -cells,

* $p = 0.0419$ 12 vs 3 months in liver, * $p < 0.0058$ 22 vs 3 months in adipose tissue, * $p = 0.0008$ 12 vs 3 months in mPFC, * $p < 0.0001$ 22 vs 3 months in mPFC. **b** miR-181a-5p * $p = 0.0019$ 12 vs 3 months in β -cells, * $p = 0.0006$ 22 vs 3 months in β -cells, * $p = 0.0354$ 22 vs 3 months in skeletal muscle. **c** miR-143-3p * $p < 0.0001$ 22 vs 3 months in β -cells, * $p = 0.0295$ 12 vs 3 months in mPFC, * $p = 0.0003$ 22 vs 3 months in mPFC. **d** miR-145-5p * $p < 0.0001$ 22 vs 3 months in β -cells. **e** miR-34a-5p * $p = 0.0437$ 22 vs 3 months in skeletal muscle, * $p = 0.0032$ 12 vs 3 months in mPFC, * $p < 0.0001$ 22 vs 3 months in mPFC. **f** 5'tRF^{Gly(GCC)} * $p < 0.0001$ 22 vs 3 months in β -cells. **g** mt-5'tRF^{Ser(GCT)} * $p = 0.0101$ 22 vs 3 months in β -cells, * $p = 0.0060$ 22 vs 3 months in liver, * $p = 0.0067$ 22 vs 3 months in mPFC. **h** mt-itRF^{Leu(TAA)} * $p = 0.0436$ 22 vs 3 months in β -cells. p -values between >0.05 and <0.1 not reaching statistical significance are also indicated on the figure.

tendency was observed in liver (Fig. 3b). The levels of miR-143-3p and miR-145-5p were strongly upregulated in aging β -cells, an effect that was not observed in any other tissue except for mPFC in which a smaller increase of miR-143-3p was detected (Fig. 3c, d). A progressive induction of miR-34a-5p was observed in most analyzed tissues, including SM, AT, and mPFC. In FACS-sorted β -cells, miR-34a-5p tended to increase at 12 months and to decrease at 22 months of age (Fig. 3e). Concerning the tRFs, we found that the induction of 5'tRF^{Gly(GCC)} was specific to β -cells (Fig. 3f). In contrast, albeit not statistically significant, a trend to overexpression of the mitochondrial itRF^{Leu(TAA)} was also detected in SM, while a significant decrease of tRFs 5'tRF^{Ser(GCT)} was found in the liver of 22-month-old mice (Fig. 3g, h).

miRNA and tRF profiling of senescent β -cells

Since senescence is an important component of the aging phenotype, we next investigated if miRNA and tRF profiles of senescent β -cells overlap with those observed in β -cells of aged mice. To distinguish sncRNA signatures associated with aging, we performed small RNA sequencing of senescent β -Gal⁺ and non-senescent β -Gal⁻ β -cells isolated from the same islet preparations of adult 7/8-month-old male mice⁸ (Suppl. Data 8). Among the 469 miRNAs retained for statistical testing, we identified 41 differentially expressed miRNAs in senescent β -cells, 36 of which upregulated (Fig. 4a). Comparing Fold changes (>2) in β -Gal⁺ vs β -Gal⁻ β -cells and aged vs young β -cells revealed that 19 senescence-associated miRNAs were also modulated during aging (Fig. 4b). Inflammation-associated miRNAs, including miR-146a-5p and miR-142-3p/-5p, were induced to a comparable or greater extent in senescent β -cells than in 22-month-old β -cells. Notably, miR-155-5p was exclusively upregulated in senescent β -cells (Fig. 4b and d), suggesting that early induction of inflammatory signals may promote senescence acquisition in a β -cell subset. The miR-143/miR-145 cluster, associated with insulin resistance³⁶, was similarly upregulated in both β -Gal⁺ and 22-month-old β -cells. In contrast, miR-181a-5p and miR-181b-5p, which decrease with aging, were increased in senescent β -cells (Fig. 4b and d). In rat β -cells, miR-181b-5p targets key metabolic genes, including glycerol-3-phosphate dehydrogenase 2 (*Gpd2*) and malate dehydrogenase 1 (*Mdh1*), and also sirtuin 1 (*Sirt1*) and transforming growth factor β receptor 1 (*Tgfbri*)³⁷. The elevated miR-181b-5p levels in senescent β -cells correlate with reduced expression of *Sirt1*, previously linked to senescence acquisition and premature aging^{5,38}. Twenty-two miRNAs were specifically deregulated in senescent β -cells without overlap with aging-associated changes (Fig. 4c). Among these, miR-184-3p was induced in senescent β -cells, where it may limit their proliferative capacity. (Fig. 4d). Indeed, miR-184-3p was previously found to be strongly downregulated in obese rodent models and in T2D patients, where it favors an adaptive response to insulin resistance by stimulating β -cell proliferation and survival^{39,40}. While aging was associated with profound tRF remodeling, senescence had only a modest impact on tRF levels (Fig. 4e and Suppl. Data 9). The stress-associated 5'tRF^{Gly(GCC)}, upregulated in aged β -cells, was slightly downregulated in β -Gal⁺ β -cells (Fig. 4f) as was 5'tRF from tRNA^{Glu(CTC)} recently implicated in T2D pathogenesis and β -cell gene expression regulation. Levels of mt-tRFs were unaffected in senescent β -cells (Fig. 4f).

Transient inflammatory stimuli drive age-associated miRNA and tRF modulation

We next sought to establish an in vitro model recapitulating, at least in part, age- and/or senescence-associated changes. Inflammation appears to contribute to both aging and cellular senescence; however, we observed a divergence between the aging sncRNA signature and the one induced by acute inflammatory stimuli. We hypothesized that transient cytokine exposure followed by a recovery period could induce persistent cellular responses mimicking the chronic low-grade inflammation associated with aging and senescence. Dispersed mouse islet cells were exposed for 48 h to pro-inflammatory cytokines (30 ng/ml IFN γ , 10 ng/ml TNF α and 1 ng/ml IL-1 β), followed or not, by a 3-day washout (Fig. 5a). While both conditions tended to increase cell death, only acute cytokine exposure was close to significance (Fig. 5b and Suppl. Fig. 6). We next assessed the acquisition of aging/senescent traits by measuring the mRNA expression of *Cdkn1a* (p21^{Cis}), a known marker of cell cycle arrest via the activation of the p53-p21 axis, *IL6* and *Serpine1* as part of the acquisition of the senescence-associated secretory phenotype (SASP), and *Bambi* as a β -cell aging marker interfering with the TGF- β and BMP signaling. The level of the senescent markers *Cdkn1a* (p21^{Cis}) and *IL6* was upregulated already after 48 h, while *Bambi* and *Serpine1* were upregulated only after washout (Fig. 5c–f). Regarding sncRNAs, miR-142-3p, miR-146-5p, and miR-181a-5p were altered upon acute cytokine treatment, whereas miR-10b-5p, miR-143-3p, miR-145-5p, and miR-184-3p were significantly modulated only after washout (Fig. 5g–m). Among tRFs, 5'tRF^{Gly(GCC)} levels raised after the acute treatment, while mt-5'tRF^{Ser(GCT)} and mt-itRF^{Leu(TAA)} were upregulated only following washout (Fig. 5n–p). While this in vitro cytokine model doesn't fully recapitulate the complex aging or senescence profile, it indicates that prolonged cytokine exposure can partially reproduce the sncRNA changes observed in senescent and aged β -cells.

Functional roles of age-associated miRNAs and tRFs in β -cell homeostasis

Several miRNAs displaying changes in aging and/or senescent cells have already been shown to regulate the activity of β -cells (Fig. 1e–l and Suppl. Data 6). To investigate the functional contribution of age- and senescence-associated miRNAs not yet linked to β -cell physiology, we overexpressed miR-10b-5p, miR-126a-3p, and miR-126a-5p (Mix1), or miR-143-3p and miR-145-5p (Mix2) in dispersed mouse islet cells (Suppl. Fig. 7a). In fact, different members of the miR-143/145 cluster, and of the miR-10 and the miR-126 families were consistently upregulated in senescence and/or aging conditions. Neither of the two miRNA mimic mixes significantly affected cell survival or glucose-stimulated insulin secretion (Suppl. Fig. 7b–d).

We next assessed the functional roles of age-regulated mt-tRFs, focusing on mt-itRF^{Leu(TAA)}, mt-5'tRF^{Ser(GTC)}, and mt-5'tRF^{Gln(TGG)}, fragments previously found to be altered in diabetic mouse models and cytokine-treated islets^{17,32}. Their mitochondrial localization was confirmed by qPCR enrichment in mitochondrial versus cytosolic fractions of MIN6B1 cells (Suppl. Fig. 8a). Overexpression of a mt-tRF mimic pool in islet cells from young 3-month-old mice resulted in a trend toward enhanced β -cell

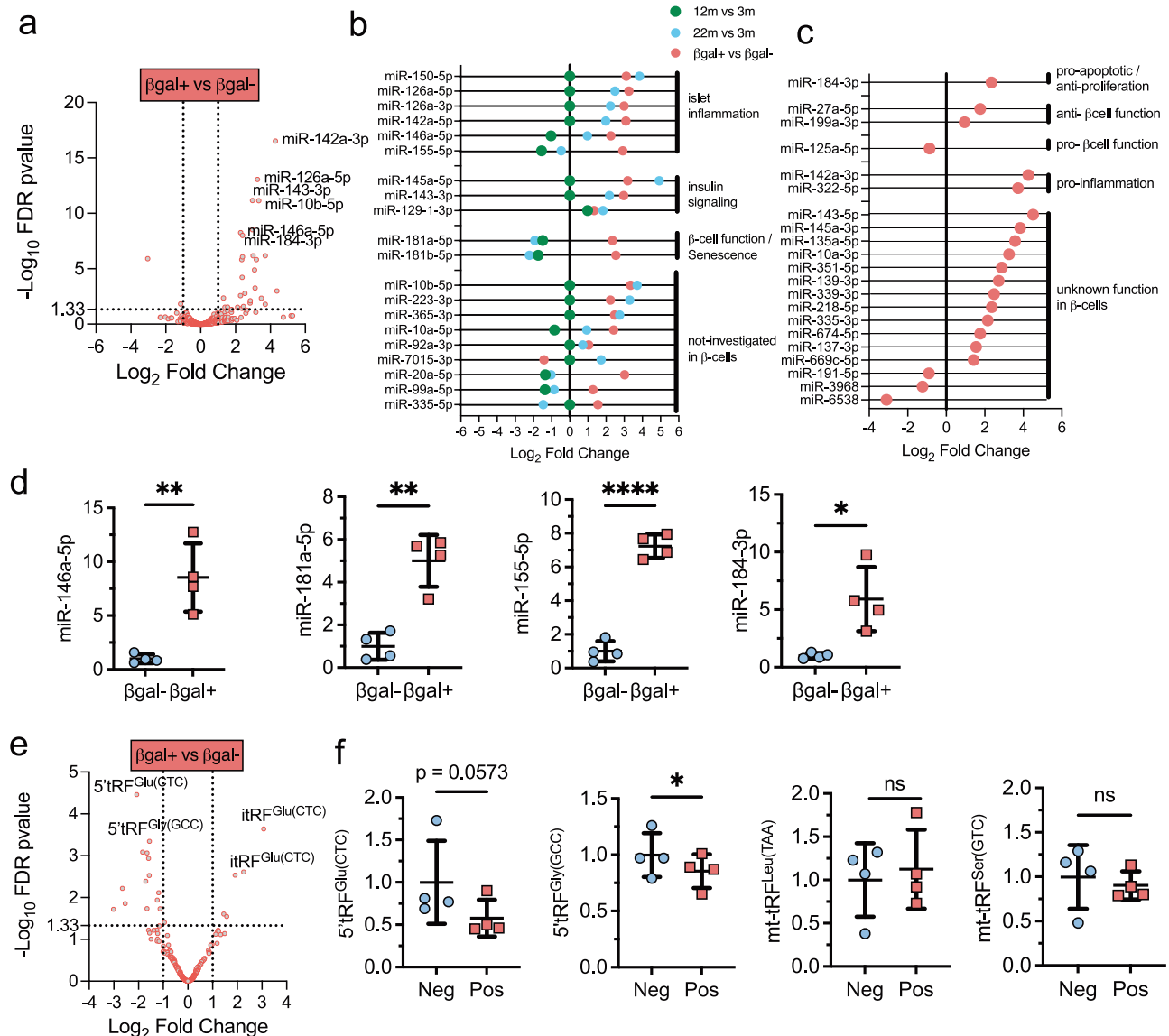


Fig. 4 | Senescence induced miRNA and tRFs changes in β -cells. Small RNAseq on FACS-sorted SA- βGal positive versus SA- βGal negative β -cells isolated from 7/8-month-old male mice ($n = 4$ independent experiments). **a** Volcano Plot shows differentially expressed miRNAs in senescent (βGal^+) versus control (βGal^-) cells; Differential expression was computed using a negative binomial generalized linear model with two-sided t -test and Benjamini–Hochberg FDR correction. Dotted lines define thresholds of FDR p -value < 0.05 ($-\log_{10}$ FDR > 1.33) and Fold change > 2 ($\log_2$ FC > 1). **b** Schematic representation of specific miRNA expression changes in aged or senescent β -cells and grouped by cellular functions, if known in the literature. **c** Schematic representation of miRNA changes in senescent β -cells and clusters by cellular functions. **d** miRNA changes confirmed by qPCR. miRNA levels were normalized on miR-7a-5p and let-7b-5p and expressed as fold change over the average value of βGal^- cells. ($n = 4$ independent experiments). Each dot represents

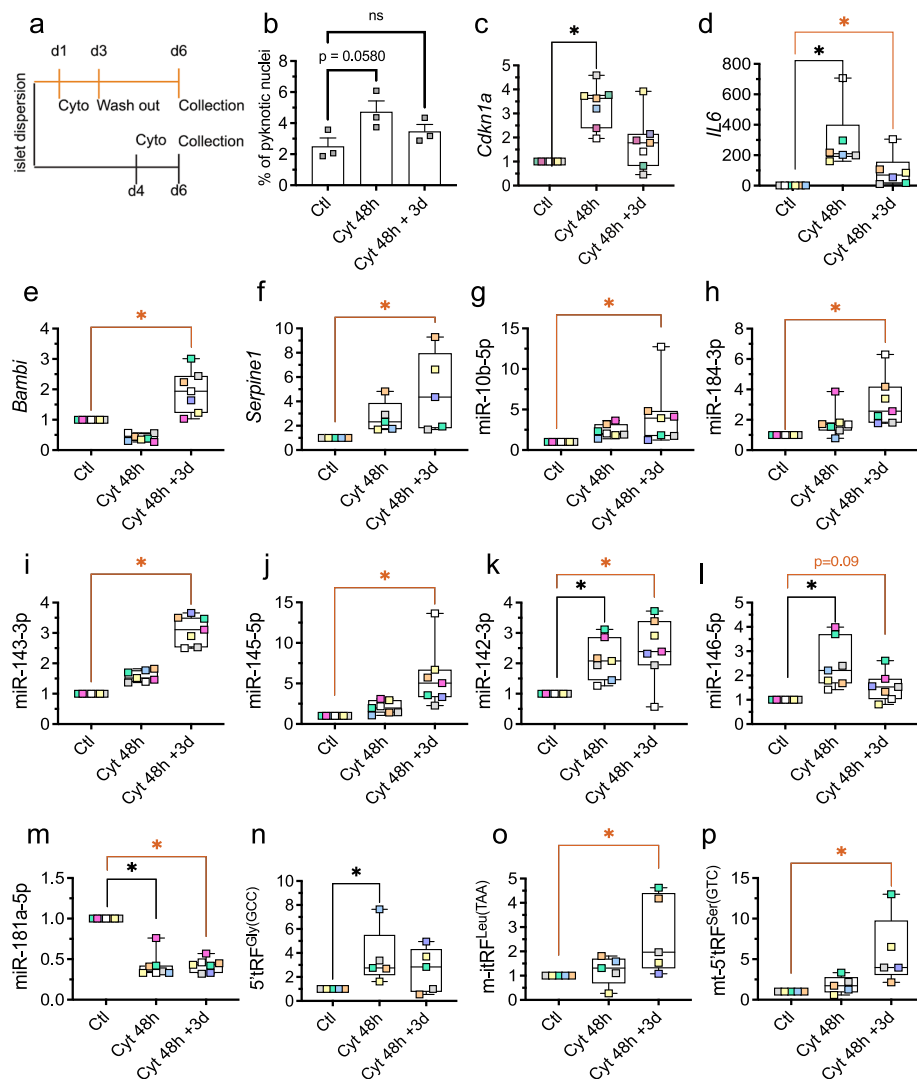
one independent experiment. Data are presented as mean $\pm$ SEM. * p -value < 0.05 , ** $p < 0.01$, **** $p < 0.0001$ by Student's t test. For miR-146a-5p $p = 0.0033$, for miR-181a-5p $p = 0.0011$, for miR-155-5p $p < 0.0001$, for miR-184-3p $p = 0.0126$. **e** Volcano Plot shows differential tRF levels in senescent (βGal^+) versus control (βGal^-) cells; Differential expression was computed using a negative binomial generalized linear model with two-sided t -test and Benjamini–Hochberg FDR correction. Dotted lines define thresholds of FDR p -value < 0.05 ($-\log_{10}$ FDR > 1.33) and Fold change > 2 ($\log_2$ FC > 1). **f** tRF changes confirmed by qPCR. tRF levels were normalized on miR-7a-5p and let-7b-5p levels and expressed as fold change over the average value of βGal^- cells ($n = 4$ independent experiments). Each dot represents one independent experiment. Data are presented as mean $\pm$ SEM. * p -value < 0.05 by Student's t -test. For 5'tRF^{Gly}(GCC) $p = 0.0330$. p -values between > 0.05 and < 0.1 not reaching statistical significance are also indicated on the figure.

function and improved glucose responsiveness, consistent with our previous findings³², while insulin content remained unchanged (Suppl. Fig. 8b–d). In complementary loss-of-function experiments, antisense oligonucleotides (ASOs) targeting the three mt-tRFs selectively reduced tRF abundance without affecting the levels of their parental full-length mt-tRNAs (Suppl. Fig. 8e, f). Functional analysis revealed that mt-tRF depletion reduced both basal and glucose-stimulated insulin secretion, with a modest effect on KCl-stimulated release, while insulin content remained unchanged (Suppl. Fig. 8g, h). These findings indicate that combined mt-tRF inhibition impairs both glucose responsiveness and basal secretory function. Seahorse mito-

stress assays in MIN6B1 cells further showed that mt-tRF ASO transfection reduced oxygen consumption rate (OCR), with a more pronounced effect observed with the ASO pool than with individual ASOs (blue line; Suppl. Fig. 8i). Modest but significant decreases in basal and glucose-induced respiratory responses were detected, and although other mitochondrial parameters did not reach statistical significance, they consistently trended toward impairment (Suppl. Fig. 8i–n). Collectively, these results demonstrate that in vitro perturbation of mt-tRFs can influence mitochondrial function and insulin secretion in adult mouse islets.

Fig. 5 | In vitro exposure of islet cells to pro-inflammatory cytokines induced miRNA and tRF changes related to aging and senescence.

a Experimental design. Mouse islet cells were isolated and dispersed. Islets from 3 mice were pooled to obtain one independent experiment. The next day (d1), a group of dispersed cells (Cyt 48 h + 3d) were exposed to pro-inflammatory cytokines (30 ng/ml IFN γ , 10 ng/ml TNF α and 1 ng/ml IL-1 β) for 48 h and media was changed on d3 to normal media for 72 h (washout period). In parallel, another group of cells were first incubated in normal media for 4 days and exposed to pro-inflammatory cytokines for the last 48 h (Cyt 48 h). Control (Ctl) cells were cultured with normal media for 6 days. On d6, RNA was extracted at the same time for all cell conditions. Apoptotic cells were detected by counting pyknotic nuclei stained with Hoechst (b). **c–f** mRNA, **g–m** miRNA and **n–p** tRF levels were measured by qPCR, normalized on HPRT (mRNA) or let-7a-5p and let-7b-5p levels (miRNAs and tRFs) and expressed as fold change over the control group. Data from $n = 3$ to 7 independent experiments. Box plots show the median (center line), the 25th and 75th percentiles (box limits), and the minimum and maximum values (whiskers). Individual data points are shown. * $p < 0.05$ by one-way ANOVA, Dunnett's post hoc test. In (c) $p21$ * $p = 0.0040$ cyt 48 h vs Ctl, in (d) IL6 * $p < 0.0001$ cyt 48 h vs Ctl, * $p = 0.0293$ cyt 48 h + 3d vs Ctl, in (e) Bambl * $p = 0.0291$ cyt 48 h + 3d vs Ctl, in (f) Serpine1 * $p = 0.0210$ cyt 48 h + 3d vs Ctl, in (g) miR-10b-5p * $p = 0.0020$ cyt 48 h + 3d vs Ctl, in (h) miR-184-3p * $p = 0.0008$ cyt 48 h + 3d vs Ctl, in (i) miR-143-3p * $p < 0.0001$ cyt 48 h + 3d vs Ctl, in (j) miR145-5p * $p < 0.0001$ cyt 48 h + 3d vs Ctl, in (k) miR142-3p * $p = 0.0153$ cyt 48 h vs Ctl, * $p = 0.0053$ cyt 48 h + 3d vs Ctl, in (l) miR-146-5p * $p = 0.0008$ cyt 48 h vs Ctl, in (m) miR-181a-5p * $p = 0.0015$ cyt 48 h vs Ctl, * $p = 0.0095$ cyt 48 h + 3d vs Ctl, in (n) 5'tRF^{Gly(GCC)} * $p = 0.0428$ cyt 48 h vs Ctl, in (o) mt-tRF^{Leu(TAA)} * $p = 0.0212$ cyt 48 h + 3d vs Ctl, in (p) mt-5'tRF^{Ser(GTC)} * $p = 0.0037$ cyt 48 h + 3d vs Ctl. p -values between >0.05 and <0.1 not reaching statistical significance are also indicated on the figure.

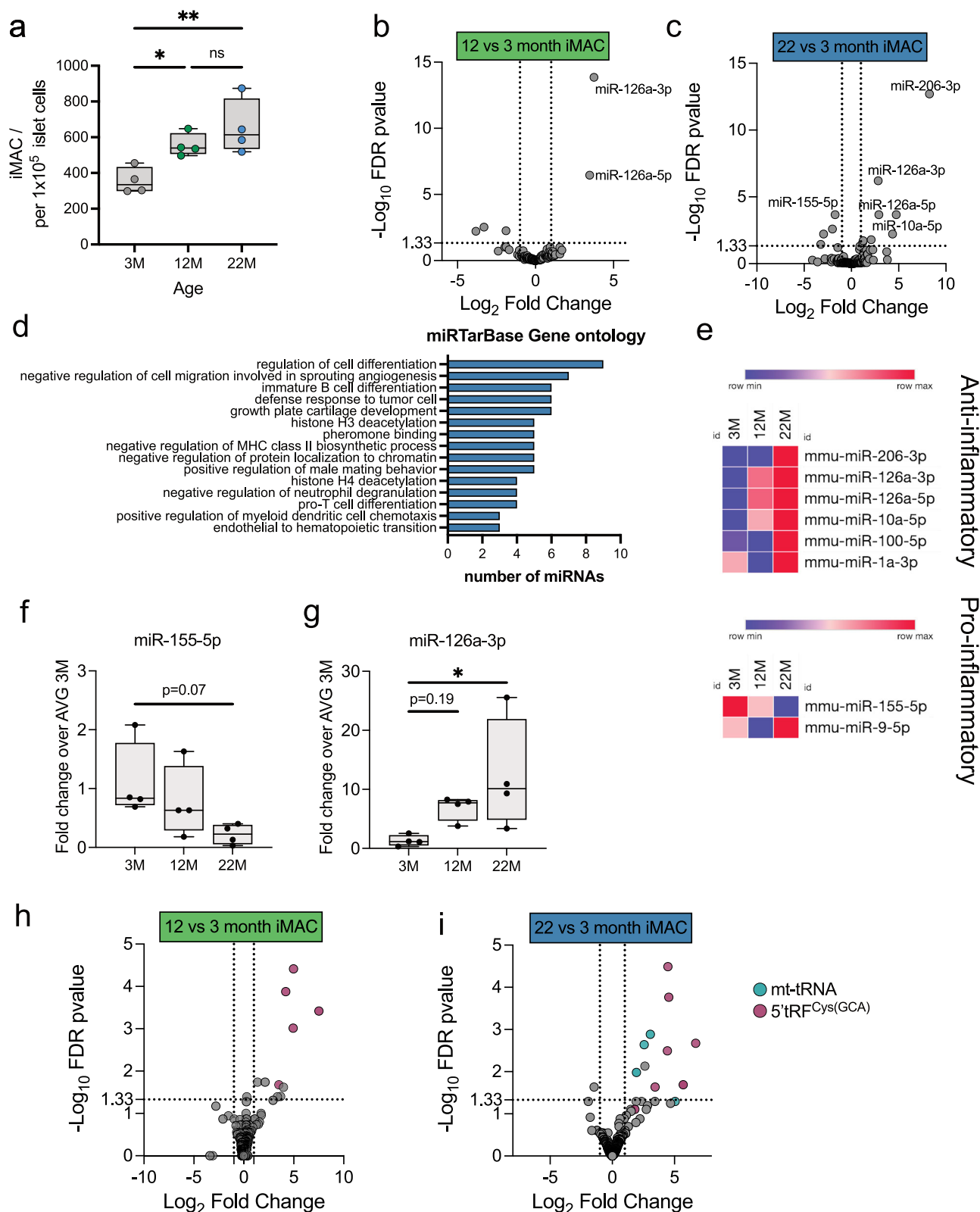


Effect of aging on the small RNA profile of islet-resident macrophages

Islet resident macrophages display a peculiar inflammatory-like phenotype and interact closely with β -cells, sensing and influencing their functional and metabolic state. These plastic cells respond to changes in islet micro-environment by transcriptional and metabolic remodeling to support tissue adaptation^{9,10}. To assess whether the sncRNA repertoire of iMACs is modified during aging, we optimized the FACS-sorting protocol to collect in parallel iMACs and β -cells from our mouse aging model (Suppl. Fig. 1c, d). As reported for other macrophage populations¹¹, we found that the proportion of iMACs per islet cells increases during aging (Fig. 6a). We then investigated age-dependent miRNA changes in iMACs potentially influencing their activation state (Suppl. Data 10). The miRNA-mapping counts accounted for the 5–20% of the total filtered reads, yielding 131 and 225 miRNA transcripts in 12- and 22-month-old mice, respectively. Differential expression analysis identified five miRNAs in 12-month-old mice and 12 miRNAs in 22-month-old mice that were significantly dysregulated relative to young mice (FDR p -value < 0.05 , Fold Change > 2 ; Fig. 6b, c). Functional enrichment analysis showed an overrepresentation of miRNAs involved in cell division, migration and immune cell differentiation (Fig. 6d). Overall, we observed an increase in the level of anti-inflammatory associated

miRNAs and a decrease of those linked to pro-inflammatory actions (Fig. 6e). The most pronounced age-dependent effect was observed for miR-206-3p that was upregulated >300 folds at 22 months. This miRNA has been shown to inhibit inflammation, targeting the TLR4/NF- κ B/NLRP3 pathway⁴¹. Upregulation of miR-126a-5p and miR-126a-3p was already detected at the age of 12 months and was maintained at 22 months. MiR-126a has been reported to induce the switch from pro-inflammatory to anti-inflammatory macrophage phenotype, and to promote efferocytosis, a phagocytic mechanism by which tissue macrophages eliminate apoptotic cells⁴². MiR-10a-5p, which represses pro-inflammatory gene expression⁴³, was upregulated in 22-month-old mice, while inflammation-associated miR-155-5p was downregulated. Key miRNA changes were confirmed by qPCR (Fig. 6f, g). Sequencing of iMACs from female mice confirmed $\sim 15\%$ of miRNA-mapping reads (Suppl. Fig. 9c, d). In iMACs from middle-aged female mice, only two miRNAs were significantly modulated, with miR-10a-5p being the sole upregulated hit (Suppl. Fig. 9e and Suppl. Data 10).

The profiling of tRFs yielded $\sim 3\%$ tRNA-mapping reads in both male and female iMAC datasets (Suppl. Fig. 9c, d). Statistical testing revealed an upregulation of 5'tRFs derived from cytosolic tRNA^{Cys(GCA)} at 12 months and from mitochondrial tRNAs at 22-months, with mt-tRF upregulation also detected in the female datasets (Fig. 6h–l, Suppl. Fig. 9f, and Suppl. Data 11).



Modulation of 5'tRFs from tRNA^{Glu(CTC)} in iMACs has been recently linked to their activation state⁹, but the function of other tRFs, in particular mt-tRFs, remains unexplored.

Discussion

This study provides a comprehensive characterization of sncRNA changes in aging β -cells, complementing previous work on age-associated alterations in gene expression and β -cell function. It directly compares miRNA signatures of physiological aging and cellular senescence, revealing shared and

Fig. 6 | Effect of aging on islet-resident macrophage miRNA and tRF profiles.

Islet-resident macrophages (iMACs) were FACS-sorted from young adult (3-month), middle-aged (12-month) and elderly (22-month) C57BL/6J male mice ($n = 4$ independent experiments). **a** Number of FACS-sorted iMACs increased with age ($n = 4$ independent experiments). Box plots show the median (center line), the 25th and 75th percentiles (box limits), and the minimum and maximum values (whiskers). Individual data points are shown. $*p < 0.05$ by one-way ANOVA, Dunnett's post hoc test. $*p = 0.0491$ 12M vs 3M, $*p = 0.0091$ 22M vs 3M. **b, c** Volcano Plots show differentially expressed miRNAs in 12-month (**b**) or 22-month (**c**) versus 3-month-old iMACs. Differential expression was computed using a negative binomial generalized linear model with two-sided t -test and Benjamini–Hochberg FDR correction. Dotted lines define thresholds of FDR p -value < 0.05 ($-\log_{10}$ FDR > 1.33) and Fold change > 2 ($\log_2$ FC > 1). **d** miRTar Base Gene Ontology enrichments associated with miRNA downregulation of 22-month-old iMACs. **e** Heatmap plots showing miRNAs significantly deregulated in 22-

month-old iMACs that were previously reported in the literature to be associated with macrophage anti-inflammatory or pro-inflammatory states. **f, g** miRNA changes confirmed by qPCR. miRNA levels were normalized on miR-16-5p and expressed as fold change over the average value of 3-month-old iMACs ($n = 4$ independent experiments). Box plots show the median (center line), the 25th and 75th percentiles (box limits), and the minimum and maximum values (whiskers). Individual data points are shown. $*p < 0.05$ by one-way ANOVA, Dunnett's post hoc test. For miR-126a-3p $*p = 0.0422$ 22M vs 3M. p -values between >0.05 and <0.2 , not reaching statistical significance, are indicated on the figure. **h, i** Volcano Plots show differentially expressed tRFs in 12-month (**h**) or 22-month (**i**) versus 3-month-old iMACs (FDR p -value < 0.1 , FC > 1.5 ; $n = 4$ independent experiments). Differential expression was computed using a negative binomial generalized linear model with two-sided t -test and Benjamini–Hochberg FDR correction. Purple circles correspond to tRFs derived from nuclear encoded tRNAs, while turquoise circles refer to tRFs derived from mitochondrial encoded tRNAs.

distinct molecular features. Moreover, parallel analysis of iMACs offers insights into their adaptation to the aging islet microenvironment.

miRNA profiling of FACS-sorted β -cells from middle-aged and aged male mice revealed progressive remodeling of this gene-regulatory class. Most miRNAs dysregulated at 22 months already showed changes in the same direction at 12 months, albeit with smaller effect sizes. A substantial overlap in age-associated miRNA changes was also observed between male and female mice, suggesting that a core miRNA aging signature is established in β -cells independently of sex, despite well-known sex-dependent differences in metabolic regulation^{44,45}. Similarly, plasma profiling in a large human cohort observed a more pronounced influence of age as compared to sex on miRNA deregulation⁴⁶. Several changes previously observed in aged rat islets²⁰ were confirmed, including downregulation of miR-181a-5p and upregulation of miR-124-3p, miR-129-1-3p, miR-383-3p, and miR-488-3p. Notably, the level of miR-124-3p and miR-29b/c that are deregulated in aged β -cells, are also altered in aged brain, kidney, and liver⁴⁷. These miRNAs have been shown to be driven by p53 and to promote cellular senescence by targeting the p21 antagonist Ccna2⁴⁷, suggesting a conserved role in age-related senescence across tissues.

Meta-analysis of miRNAs with validated roles in β -cell homeostasis revealed elevation of several inflammation-associated miRNAs (inflammamiRs), including miR-142-3p/-5p, miR-146a-5p, and miR-150-5p, mirroring changes seen in T1D islet models and supporting chronic low-grade inflammation as a prominent feature of β -cell aging. Age-associated changes were also identified in miRNAs linked to obesity and T2D, including the miR-132-3p/miR-212 cluster, the miR-29 family, miR-184-3p and miR-802-3p, previously implicated in insulin secretion in rodent and human islets^{39,48–53}, and miR-204-5p that was linked to insulin secretion in mouse islets. Notably, the role of miR-132-3p, miR-184-3p, miR-29 family and miR-802-3p in insulin secretion was confirmed in functional studies in primary human islets. In humans, aging impairs glucose-stimulated insulin secretion, while rodent studies globally show a trend towards elevated basal secretion (reviewed by Aguayo-Mazzucato³), increasingly recognized as an early indicator of β -cell dysfunction predisposing to insulin resistance^{54,55}. Beyond its role in insulin secretion, the miR-132/212 cluster has been implicated in insulin resistance in peripheral tissues⁵⁶, though the consequences of its deregulation in β -cells may differ given the distinct insulin signaling mechanisms operating in these cells.

Most miRNAs promoting proliferation, including miR-181a/b-5p and miR-338-3p^{20,37}, were reduced in aged β -cells, while miRNAs inhibiting β -cell maturation (miR-15b-5p, miR-181a/b) were downregulated and those promoting maturation (miR-129-3p, miR-132-3p, miR-204-5p, miR-344-3p, miR-383-5p, miR-415a) were upregulated. Together, these findings suggest that 22-month-old β -cells exhibit a fully mature phenotype with limited proliferative capacity.

Beyond canonical miRNAs, substantial remodeling of isomiRs was observed. These sequence variants may arise through nucleotide additions, deletions, or substitutions, with 5' variations potentially affecting target

recognition and 3' insertions influencing stability^{15,19}. While specific isomiRs have been shown to regulate β -cell function¹⁶, their biogenesis and roles are only beginning to be understood, and the functional implications of isomiR changes in aged β -cells warrant further investigation.

tRFs represent a functionally important sncRNA class in islet homeostasis⁵⁷, yet their roles in aging and senescence remain poorly understood⁵⁸. The biogenesis of cytosolic 5'tRF has been identified as a stress-response mechanism in T2D⁹ and as a pathogenic feature underlying TRMT10A monogenic diabetes¹⁸. We observed upregulation of the 5'tRF, derived from tRNA^{Gly(GCC)} in aged β -cells, a fragment also increased in NOD mouse islets during early T1D and previously shown to promote β -cell apoptosis by reducing anti-apoptotic Bcl-XL¹⁷. We also identified a global upregulation of mt-tRFs in 22-month-old β -cells, detectable as early as 12 months in female mice. Samples from female mice were pre-treated to remove nucleotide modifications that interfere with reverse transcription, suggesting that the overrepresentation of upregulated mt-tRFs is not attributable to differential modification patterns relative to cytosolic tRF. Several mt-tRFs were previously shown to increase in NOD islets¹⁷ but decrease in db/db islets³². The decrease of the internal fragment mt-itRF^{Leu(TAA)} was associated with impaired mitochondrial respiration and glucose-induced insulin secretion in rat islets, whereas elevated levels of mt-itRF^{Leu(TAA)} favored glucose-induced insulin secretion³². Interestingly, we observed a trend toward decreased mt-itRF^{Leu(TAA)} levels in SM of aged mice, a change previously associated to an increase in insulin sensitivity⁵⁹. Consequently, the reduction of mt-itRF^{Leu(TAA)} in aged mice may contribute to insulin resistance. Here, we validate in vitro murine models the function of mt-itRF^{Leu(TAA)} alongside mt-5'tRF^{Ser(GTC)} and mt-5'tRF^{Gln(TGG)}, collectively supporting a key role for mt-tRFs in modulating mitochondrial respiration and glucose-stimulated insulin secretion. Further work will be required to dissect the molecular mechanisms of individual mt-tRFs and to elucidate the basis of their global upregulation in aging β -cells.

Elevated tRF levels may result from enhanced tRNA synthesis, greater cleavage activity or both⁶⁰. Our sequencing approach captured molecules up to 80 nucleotides, including some full-length tRNAs, most of which were mitochondrially encoded, likely because mt-tRNAs carry fewer modifications than cytosolic tRNAs and are therefore more amenable to detection⁶¹. In female β -cell samples, pre-treatment to remove reverse transcription-impairing modifications enabled detection of additional cytosolic full-length tRNAs. Importantly, all detected mitochondrial tRNAs were upregulated in 22-month-old β -cells. However, dedicated tRNA profiling experiments will be necessary to fully characterize cytosolic and mitochondrial tRNA pool dynamics during β -cell aging.

Unbiased dataset annotation also revealed increased abundance of fragments from mitochondrial ribosomal RNAs and protein-coding genes in aged β -cells. As rRNA-derived fragments have recently been reported to possess regulatory properties⁶² these poorly characterized sncRNAs may represent a new avenue for understanding dysfunction in aging and senescent β -cells.

Senescence represents a distinct cellular state induced by stress, characterized by cell-cycle arrest and a SASP. By profiling β -Gal⁺ β -cells from adult mice, we derived a senescence-specific sncRNA signature only partially overlapping with the aging-associated one. Both states were associated with upregulation of inflammation-associated miRNAs, including miR-146a-5p, a known regulator of inflammation in β -cells^{25,26} and other tissues²⁴, as well as miR-142a-3p/-5p, which are associated to autoimmune inflammation⁶³. However, pro-inflammatory miR-155-5p was elevated in senescent but slightly downregulated in aging β -cells. Proliferation-associated miRNA modulation also differed: miR-184-3p, an inhibitor of β -cell mass expansion⁴⁰, was increased in senescence, yet miR-181a/b-5p, which promote proliferation, were also elevated, suggesting that impaired proliferative capacity in aged and senescent β -cells arises through partially distinct mechanisms.

Comparison of aging β -cell sncRNA profiles with those of cytokine-treated islets revealed broadly divergent signatures, leading us to hypothesize that acute inflammatory bursts may trigger long-term aging- or senescence-like phenotypes. Supporting this, a washout period following acute cytokine exposure was sufficient to recapitulate key aging/senescence features in mouse islets, including induction of senescence markers *Cdkn1a* (p21Cis), *Il6*, *Bambi* and *Serpine1*, accompanied by changes across all sncRNA classes identified in our analysis. Nevertheless, this model captures only the short-term persistence of inflammatory effects and cannot fully recapitulate the cumulative nature of aging-associated inflammation over years. Our findings should therefore be interpreted as reflecting the capacity of inflammatory stress to induce aging-like or persistent stress-associated features in β -cells, rather than as a comprehensive model of physiological aging per se.

Finally, this study provides the characterization of the iMAC sncRNA profile during aging. iMACs from aged mice exhibited reduced expression of pro-inflammatory associated miRNAs alongside increased expression of miRNAs linked to anti-inflammatory and pro-phagocytosis functions. Although transcriptional and functional analyses will be necessary to directly establish iMAC phenotypic shifts in aging islets, our findings may reflect a homeostasis-preserving remodeling of iMAC function in the aging pancreatic environment.

Conclusions

This study provides a comprehensive overview of sncRNA changes during aging in pancreatic islet cells, encompassing miRNAs, isomiRs, piRNAs, and tRFs in β -cells from 12- and 22-month-old mice. We identified aging-associated changes linked to β -cell inflammation and connected alterations in mitochondrial tRF levels to modulation of mitochondrial function. These datasets complement previously published transcriptomic and proteomic data^{1,5,64} and may help distinguish individuals likely to age healthily from those predisposed to metabolic diseases such as diabetes.

Limitations and perspectives

Although the functional interpretation of miRNA changes is consistent with evidence of age-dependent β -cell inflammation and impaired insulin secretion, miRNA functions remain context-dependent. In vivo and ex vivo characterization in larger cohorts and profiling the β -cell sncRNome in elderly individuals will be essential to fully establish translational relevance. Interestingly, a recent study points out increasing disparity between mRNA and protein levels in different aged organs in human¹², suggesting that players in post-transcriptional modifications, like miRNAs and tRFs, could play an important role in the development of the aging phenotype. tRF modulation is emerging as a key regulatory mechanism in islet metabolic adaptation, with mitochondrial tRFs representing promising indicators of β -cell functional homeostasis and potential therapeutic targets. Extensive investigation using orthogonal techniques, such as probe hybridization-based approaches, will be required to define the role and the mode of action of these sncRNAs in aging. Finally, the limited sequence conservation of certain sncRNA classes, such as piRNAs, across species necessitates future

investigations in both mouse and human islets to elucidate their roles in aging-related metabolic adaptation.

Methods

Ethical compliance

All animal procedures were performed in accordance with the NIH guidelines, and protocols were approved by the Swiss Research Councils and Veterinary Offices under the animal authorization number VD2495x4, or by the Animal Care and Use Committee from the Joslin Diabetes Center (Protocol number 2022-01). Minimal number of animals per condition were determined by power analyses and justified in our animal authorization.

Experimental models mice

Wild-type C57BL/6J mice were acquired from Jackson Labs and wild-type C57BL/6NRj mice were obtained from Janvier Laboratories or from our colony, when organ collection or in vitro experiments were ready to be performed. Mice were housed on a 12-h light/dark cycle under standard conditions with *ad-libitum* chow diet. Glycemia and body weight were all taken under fed state between 8 and 10AM. Following euthanasia, aged mice underwent a post-mortem visual examination to ensure the absence of detectable tumors. No mice were excluded from our experiments.

Organ collection and mouse islet dispersion

Animals were euthanized with sodium pentobarbital intraperitoneal injections (100 mg/kg) followed by cervical dislocation. Samples from visceral AT, liver, SM and medial prefrontal cortex were collected. Pancreas was isolated by collagenase injection into the common bile duct⁶⁵. Following pancreas digestion at 37 °C, mouse islets were isolated by Histopaque density gradient to separate them from digested exocrine tissue. At the end of the isolation, islets were hand-picked and incubated for a minimum of 2 h at 37 °C in RPMI 1640-GlutaMAX medium (ThermoFisher) containing 11.1 mM glucose and 2.06 mM L-glutamine and supplemented with 10% fetal calf serum (FCS, Sigma), 10 mM HEPES, pH 7.4, 1 mM sodium pyruvate, 100 mg/mL streptomycin and 100 IU/mL penicillin, and referred as “complete RPMI”. Dissociated islet cells were obtained by incubating the islets in Ca²⁺/Mg²⁺ free phosphate buffered saline, 3 mM EGTA and 0.002% trypsin for 2–3 min at 37 °C. After dispersion, islet cells were FACS-sorted or, for in vitro experiments, seeded and cultured in complete RPMI medium.

Fluorescence-activated cell sorting (FACS) of islet cells

Mouse islet cells were FACS-sorted based on two different strategies as described previously to obtain (1) β -cells and iMACs of aged mice⁹ or (2) senescent versus non-senescent β -cells⁸.

To FACS sort β -cells and iMACs from different aged C57BL/6J and C57BL/6NRj mice, islets from four mice aged of 3 months or two mice aged of 12 or 22 months were pooled to obtain one preparation equivalent to one biological replicate (around 600–800 islets per preparation). Pooling strategy is indicated in Suppl. Table 1. Fractions enriched for β -cells were obtained based on β -cell autofluorescence⁶⁶ while fractions enriched in iMACs were sorted based on the expression of CD45, Cd11b, F4/80, and Cd11c markers. As previously described in ref. 9, dissociated islet cells were washed once with FACS buffer (0.1% BSA, 2 mM EDTA, 11 mM glucose in PBS) and incubated for 5 min with TruStain FcX™ PLUS (anti-mouse CD16/32) Antibody (Cat. 156603, BioLegend) at 4 °C. Cells were incubated for 30 min in the dark at 4 °C with the following antibodies: 1:200 of FITC anti-CD45 (Cat. 103108, BioLegend), 1:100 brilliant violet CD11b (Cat. 101235, BioLegend), 1:100 APC F4/80 (Cat. 123115, BioLegend) and 1:100 PE CD11c (Cat. 117307, BioLegend). Cells were then washed twice with FACS buffer and sorted by FCF-Aria-II (SORP). β -cell purity was assessed as previously described in ref. 63. On average, β -cell fractions contained 99.1 ± 0.9% insulin-positive cells and 0.6 ± 0.6% glucagon-positive cells. FlowJo™ 10 software was used for quantification of iMACs from FACS-

derived data. The number of cells collected after FACS is indicated in Suppl. Table 2.

To obtain senescent β -cells, dispersed islet cells from 7 to 8-month-old C57BL/6J male mice were incubated at 37 °C for 1 h with a fluorescent substrate of the senescence-associated β -galactosidase (SA- β -Gal) (Enzo Life Sciences enz-kit 130-0010). Islet cells were then resuspended in FACS buffer containing 2% FBS (Cellgro, Manassas, VA). Using a Dako Cytomation MoFlo Cytometer (Dako, Ft. Collins, CO) or Aria (BD FACS Aria IIu and BD FACS Aria Special Order Research Product), cells were gated according to forward scatter to obtain an enriched fraction of β -cells, and FACS-sorted based on SA- β -Gal activity. The percentage of β -cells by insulin staining ranged from 80 to 90%. Propidium iodide was used to exclude dead cells. SA- β -Gal positive (pos, +) and SA- β -Gal negative (neg, -) subpopulations were collected for RNA extraction and small non-coding RNA sequencing. Complete FACS gating strategy can be seen in Fig. S1 of reference #8 and the number of cells recovered after FACS is indicated in Suppl. Table 3.

Cell lines

MIN6B1 cells, a subclone of the murine insulin-secreting cell line derived from insulinoma, were kindly provided by Dr. Philippe Halban⁶⁷. MIN6B1 cells were cultured in DMEM-GlutaMAX medium (Cat. 10566016, ThermoFisher Scientific) containing 25 mM glucose and 4 mM L-glutamine, and supplemented with 15% FCS, 70 mM β -mercaptoethanol, 50 mg/mL streptomycin and 50 IU/mL penicillin. MIN6B1 cells were tested negative for mycoplasma contamination.

In vitro cytokine treatment

Whole islets or dispersed islet cells were treated with a pro-inflammatory cytokine mix (30 ng/ml IFN γ , 10 ng/ml TNF α and 1 ng/ml IL-1 β) (Peprotech) for 2 different sets of experiments. For RNAseq profiling (Fig. 2c, d), whole islets were incubated for 24 h with pro-inflammatory cytokines prior to RNA extraction ($n = 3$ independent experiments). To mimic long term aging and senescence, dispersed islet cells were seeded and treated with pro-inflammatory cytokines for 48 h followed by media change and a wash out period of 72 h, as illustrated in Fig. 5a. In parallel, acute treatment with pro-inflammatory cytokines was performed in the last 48 h of the culture. RNA was extracted in all conditions after a total of 6 days of cell culture.

RNA assays

RNA was extracted from islet cells using the miRNeasy micro kit (Qiagen). Mitochondrial and cytosolic fractions were isolated from MIN6B1 cells by differential centrifugation as previously described⁶⁸ and RNA was then extracted using the miRNeasy micro kit (Qiagen). For small RNA-sequencing, cDNA libraries were prepared using QIAseq miRNA Library Kit starting from 5 to 10 ng of RNA. Library size was assessed with Agilent fragment analyzer and ranged between 190 and 230 bp. Single-end sequencing was performed with an AVITI sequencer (Element Bioscience), with a minimum of 25M reads/library and 150 cycles. qPCR analysis of mature miRNA and tRFs was performed using the miRCURY LNA Universal microRNA PCR assays (Qiagen), starting from 50 to 200 ng of RNA. Small non-coding RNA levels were normalized to miRNAs highly expressed in β -cells (miR-7a-5p and let-7b-5p) or macrophages (miR-16-5p) that are not modified under the studied conditions based on NormFind strategy⁶⁹. Tissue small non-coding RNA levels were normalized to let-7a-5p and let-7b-5p, whose expression was stable across the different conditions. Cell fraction enrichment was assessed by normalizing the Ct values of genes and sncRNAs measured in the mitochondrial fraction to the corresponding Ct values measured in the cytosolic fraction. Custom primer sequences are provided in Suppl. Data 12. For mRNA measurement, RNA samples were treated with DNase prior to analysis. Gene expression levels were measured by qPCR (BioRad) and the results were normalized to the housekeeping gene *Hprt*. Custom primer sequences are provided in Suppl. Data 12. All qPCR measurements were performed in duplicates, and expression levels

were analyzed using the 2^{-DDCt} method. Of note, since the amount of RNA extracted from FACS-sorted islet cells was very low, tRNA modifications that may interfere with the quantification of tRFs could not be removed from RNA samples and may therefore reduce the detection of some tRFs.

RNA-seq analysis

For miRNA, piRNA, and isomiR profiling, FASTQ files were uploaded on the Qiagen RNA-seq analysis portal. The average expression threshold was determined automatically by the software to maximize the number of significant differentially expressed features, while excluding lowly expressed miRNAs with limited statistical power from multiple testing correction. The isomiR nomenclature adopted from the Qiagen RNA-seq analysis portal is inspired by http://github.com/miRTop/incubator/blob/master/isomirs/isomir_naming.md. Deletions are in lowercase and insertions are in uppercase. A suffix “s” indicates deletions and insertions at the 5' deletions. Base switch mutations (SNVs) are indicated with reference symbol, position and new symbol. For tRF profiling, as previously described in ref. 17, following removal of adapter sequences, identical reads longer than 16 nts were collapsed based on Unique Molecular Identifiers and aligned to the mouse genome (GRCm38.p6). Aligned reads were mapped to the mature tRNA sequences from the GtRNAdb database (Release 1.0; <http://gtRNAdb.ucsc.edu/>, accessed March 2025) and to the 22 mouse mitochondrial tRNA sequences from <https://www.ncbi.nlm.nih.gov/nuccore/J01420.1> (accessed March 2025) using bowtie (version 1; <http://bowtie.cbcb.umd.edu>). Bowtie parameters were set to output only perfect matches to tRNA sequences. Reads not aligning to the chosen reference sncRNA databases and unambiguously mapping to one genomic location were considered for an unbiased identification of potential novel sncRNAs. Upon sequence annotation, differentially expressed sncRNAs were identified using edgeR (version 4.2.2) in R packages (<http://bioconductor.org>). Only fragments with cpm > 5 in at least 4 samples were retained for statistical analysis. For each sncRNAs, *p*-values and false discovery rates (FDRs) were obtained based on the model of negative binomial distribution. Fold changes of gene expression were also estimated within the edgeR package.

Rank-rank hypergeometric overlap to compare two datasets of differentially expressed sncRNAs was performed using the R-package RedRibbon v.1.3.1⁷⁰.

RNA-seq from mouse islet treated with cytokines

Mouse islets were isolated and cultured for 24 h in control conditions or treated with a mix of pro-inflammatory cytokines (30 ng/ml IFN γ , 10 ng/ml TNF α and 1 ng/ml IL-1 β) (Peprotech). RNA was extracted using the miRNeasy micro kit (Qiagen) and RNA samples were sent to Arraystar Inc (Maryland, US) for sequencing. FASTQ files obtained from the small RNA-seq experiment were analyzed with the sRNAbench online pipeline. Raw reads underwent adapter removal and PCR duplicate filtering, followed by stringent quality assessment to retain only high-fidelity sequences ($Q \geq 30$). Reads were mapped to the mouse genome (GRCm39) using Bowtie with specific parameters and annotated using miRBase release 22.1. Low-quality and ambiguous sequences were filtered, followed by a relative abundance filter based on reads per million (RPM), with sequences contributing less than 1% removed. IsomiRs were classified into different classes according to the post-transcriptional modification compared with the canonical miRNA sequence¹⁶. An overlap analysis was performed across the different datasets to identify shared isomiR sequences in an inflammatory context.

Cell transfection with synthetic oligonucleotides

For gain-of-function experiments, dispersed islet cells derived from 12 to 16-week-old male and female mice and MIN6B1 cells plated in 24 well-plates were transfected with 100 μ l of transfection mix containing: 30 pmol miRNA (Qiagen) or tRF mimics (IDT—custom designed) in combination with 2 μ l lipofectamine 2000 (ThermoFisher Scientific). For loss-of-function experiments, transfection mixes were prepared with 20 pmol of ASO (Qiagen) against control sequences (AC) or specific mt-tRFs in combination with 2 μ l of lipofectamine 2000. For 12-well Seahorse plates, 25 μ l of

transfection mix was used. Upon 8–16 h of incubation, the transfection mix was replaced by a complete cell culture medium. Validation of over-expression or downregulation of miRNA/tRF levels by qPCR and functional assays were performed 48 h post-transfection. The sequences of custom designed mimics and ASOs are in Suppl. Data 12.

Insulin secretion assay

Dispersed mouse islets were pre-incubated for 50 min at 37 °C in low glucose (2 mM) Krebs-Ringer bicarbonate buffer (KRBH) containing 25 mM HEPES, pH7.4, 0.5% BSA (Sigma-Aldrich). Cells were then incubated for 50 min in KRBH containing 2 mM glucose and 0.5% BSA, and the supernatants were collected to assess basal insulin secretion. Subsequently, the cells were incubated for 50 min in KRBH containing 20 mM glucose and 0.5% BSA, and the media were collected to assess stimulated insulin secretion. Total cellular insulin content was extracted using acid-ethanol (0.2 mM HCl in 75% ethanol). Insulin concentrations were measured using an insulin enzyme immunoassay kit (Mercodia). For protein quantification, cells were lysed in RIPA buffer, and total protein content was determined using the Bradford assay (ThermoFisher Scientific). All independent experiments were performed in technical quadruplicate.

Mitochondrial function

Mitochondrial function was assessed using the Agilent Seahorse XF Cell Mito Stress Test kit. MIN6B1 cells were seeded in 12-well Seahorse plates and transfected at 70% confluency with either AC or mt-tRF ASOs overnight. The culture medium was then replaced and the assay performed 24 h later. The OCR was measured in live cells following sequential injections of 20 mM glucose, 5 mM Oligomycin (ATP synthase inhibitor), 2 mM FCCP (uncoupler of oxidative phosphorylation) and 0.5 mM rotenone and antimycin A (inhibitors of mitochondrial transport chain complexes). The residual OCR measured after rotenone/antimycin injection was considered non-mitochondrial respiration. Mitochondrial respiration parameters were calculated as follows: basal respiration was determined as the OCR before glucose injection minus non-mitochondrial respiration; glucose response was calculated as the OCR after glucose injection minus basal respiration; ATP-linked respiration was calculated as the OCR before Oligomycin injection minus the minimum OCR following Oligomycin; maximal respiration was calculated as the OCR after FCCP injection minus non-mitochondrial respiration; spare respiratory capacity was determined as the maximal respiration minus basal respiration.

Cell viability assay

Cell death was assessed by counting the pyknotic nuclei following Hoechst staining. Dispersed mouse islet cells were cultured in 24-well plates and treated as indicated. Hoechst 33342 (ThermoFisher Scientific) was added to the culture medium at a final concentration of 1 ng/ml and incubated for 5 min. Cells displaying pyknotic nuclei were scored under fluorescence microscopy (AxioCam MRc5, Zeiss). At least thousand cells were analyzed for each experimental condition.

Statistics and reproducibility

Data are expressed as mean ± SEM. Statistical significance was determined using unpaired two-tailed Student's *t*-test or, for multiple comparisons, with one-way ANOVA, followed by post-hoc Dunnett test (Graph Pad Prism 10). *P*-values less than 0.05 (*p* < 0.05) were considered statistically significant. Independent experimental replicates were conducted to ensure reproducibility, and statistical analysis was performed on data from at least 3 independent replicates. Independent replicates are defined as tissue samples or pools of samples derived from separate animals or as different cell line passages.

Ethical approval

This study was approved by the Swiss Research Councils and Veterinary Offices (authorization number VD2495x4) and by Animal Care and Use Committee from the Joslin Diabetes Center (Protocol number 2022-01).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

RNA-sequencing data reported in this paper are publicly available as raw FASTQ files and processed raw data on GEO under the accession numbers: GSE313422, GSE313423, GSE313424, and GSE313758, GSE335870, GSE335871. Source data for figures are provided in Suppl. data 13.

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Author contributions

R.R., C.C., and C.G. designed the study, conducted data analysis, and wrote the manuscript. C.C., C.G., J.P., V.M., and A.G. performed experiments and analyzed the data. E.M. and F.A. conducted sncRNA-seq data bioinformatical analyses. S.B.W. and C.A. provided senescence β -cells and scientific expertise. E.A., D.F., F.D., and G.S. provided isomiRs bioinformatical analyses and scientific expertise. R.R., C.C., G.S., and F.D. provided financial support. All authors contributed to the manuscript preparation and approved the final version.

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Competing interests

The authors declare no competing interests.

Additional information

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