

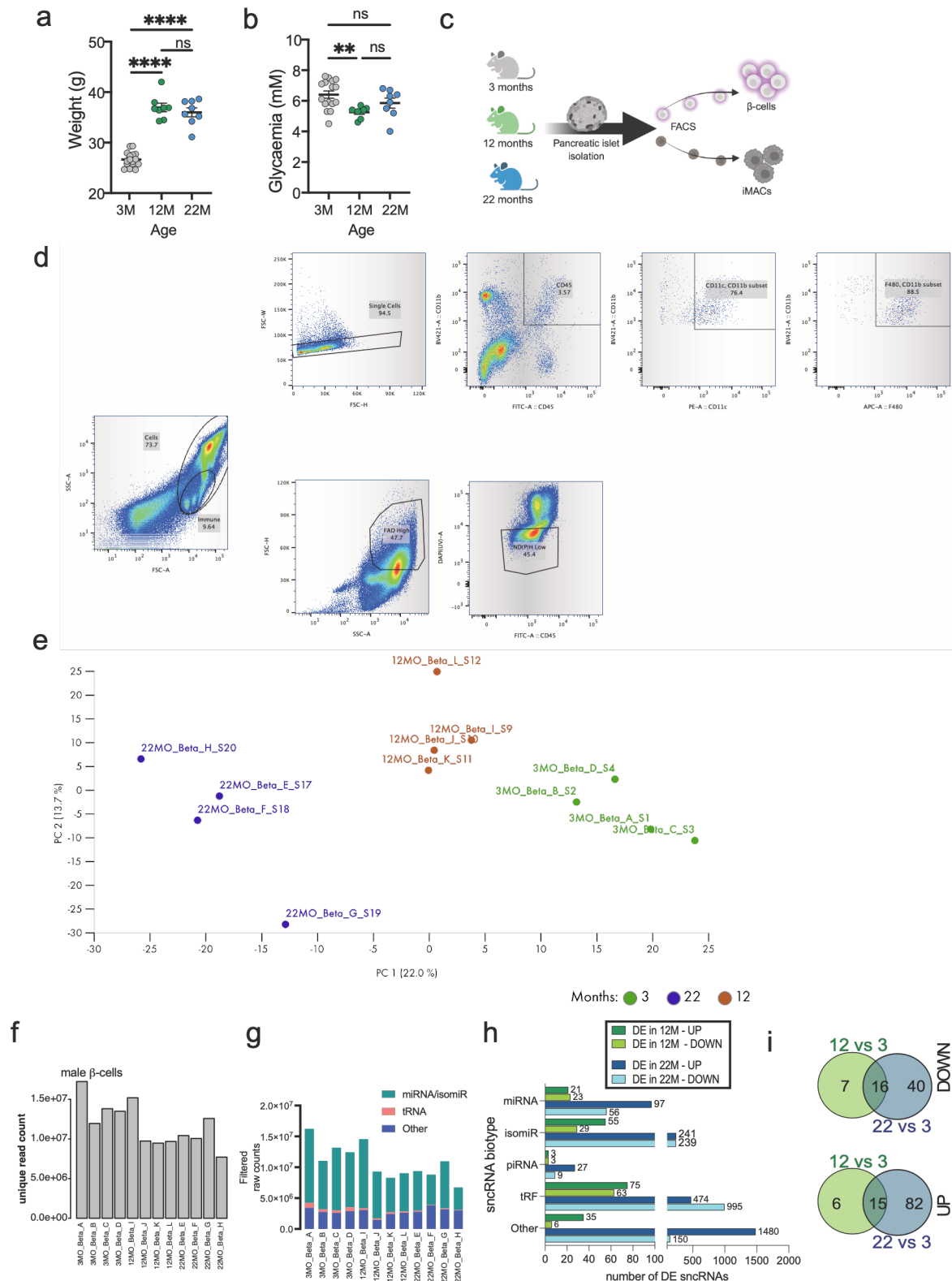
Supplementary materials
for manuscript COMMSBIO-25-12700

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

Supplementary figures

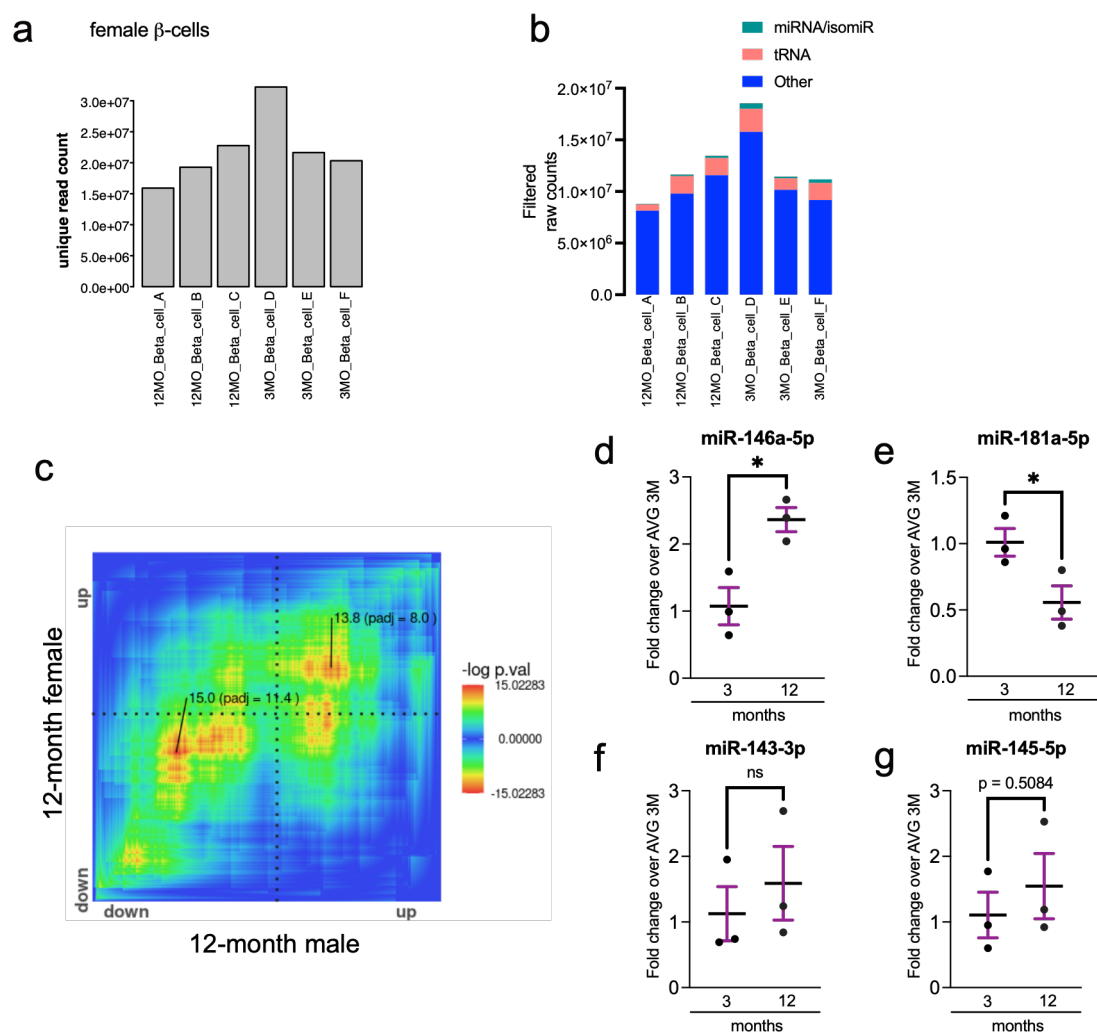
Supplementary tables

Supplementary Figures



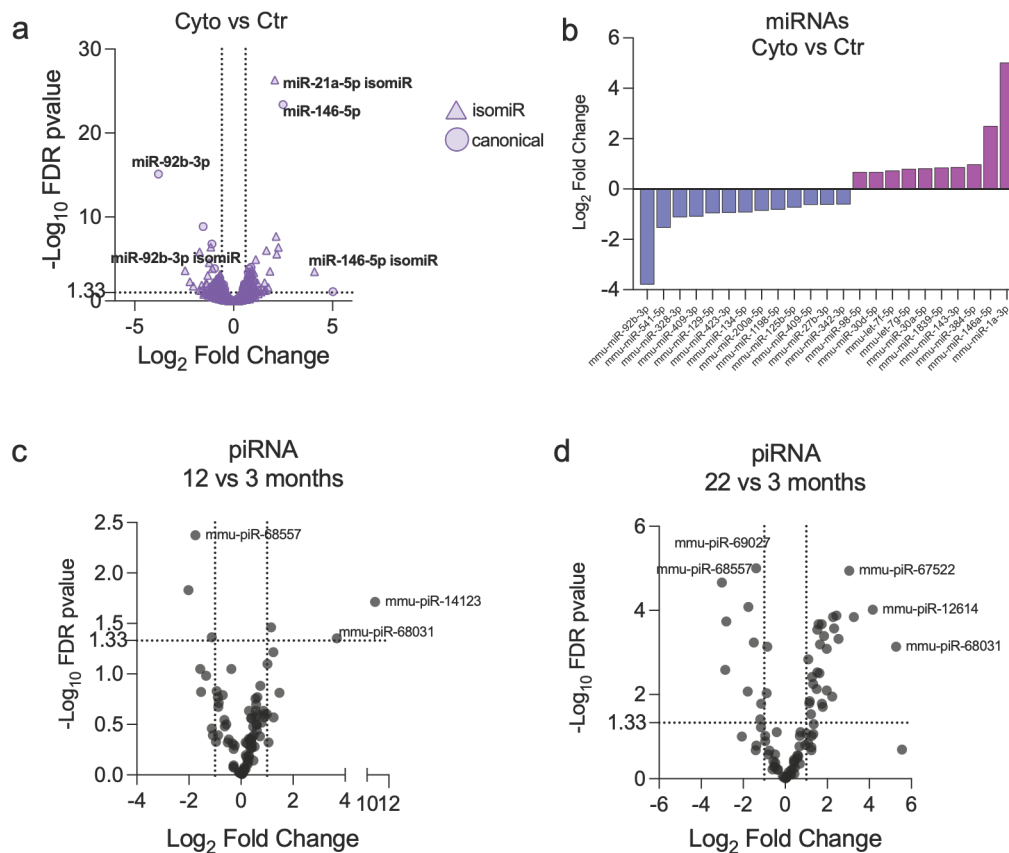
Suppl. FIGURE 1 a) Body weight and b) Glycemia of 3-, 12- and 22-month-old male mice in fed state. c) Experimental design: Pancreatic islets were isolated from 3-, 12- and 22-month-old mice, dispersed and FACS sorted to collect enriched β-cell (99%) and islet-resident macrophage (iMACs) fractions. d) Gating strategy:

Immune cells were sorted based on the expression of CD45, Cd11b, F4/80 and Cd11c markers (top panel) while β -cells were sorted based on autofluorescence (lower panel). e) Principal component analysis of small RNA seq in aging β -cell samples showing clustering based on age. f-g) Read count in β -cell samples before filtering for expression (f) and retained for statistical analysis (g) with indication of reads mapping to miRNA/isomiR (green), tRNA (orange) and other genomic locations (blue). h) Differentially expressed (DE) sncRNA biotypes (miRNAs, siomiRs, piRNAs, tRFs and others) in middle-aged (12 months – green bars) and elderly (22 months-blue bars) β -cells compared to young adult (3 months). Dark green/blue bars correspond to increased levels while light green/blue bars correspond to decreased levels of sncRNAs. Differential expression was computed using a negative binomial generalized linear model with two-sided t-test and Benjamini–Hochberg FDR correction. Fold change ≥ 2 and $FDR \leq 0.05$ were considered significant (number of significant changes for each biotype and aged condition is indicated next to the bar). i) Venn diagram showing overlap between miRNAs significantly modulated (Fold change ≥ 2 and $FDR \leq 0.05$) in β -cells from 12 months and 22 months of age.

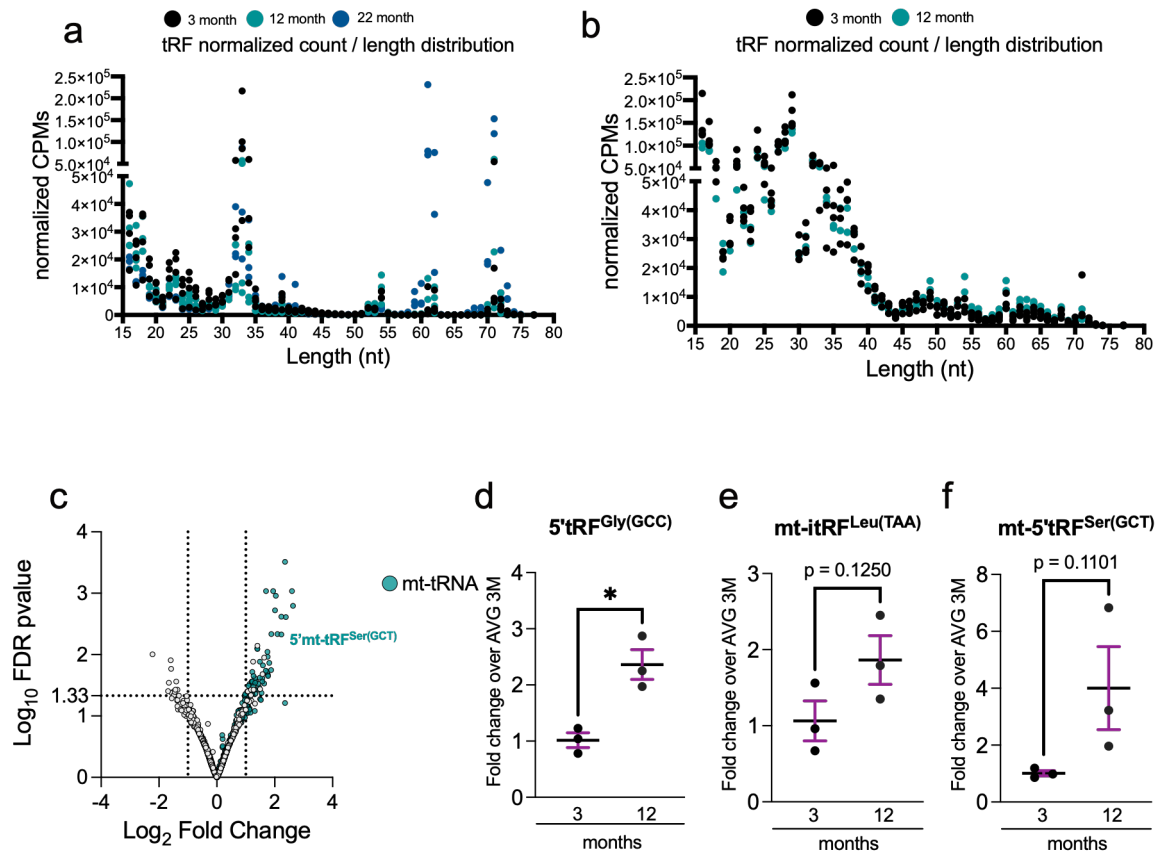


Suppl. FIGURE 2) Small RNA sequencing of β -cells from female mice: a-b) Read count in β -cell samples pretreated for RNA modification removal before expression filtering (a) and retained for statistical analysis (b) with indication of reads mapping to miRNA/isomiR (green), tRNA (orange) and other genomic locations (blue). c) heatmap of the rank-rank hypergeometric overlap between differentially expressed miRNA datasets from β -cells derived from 12-month-old male and female mice. d-g) qPCR analysis of selected miRNAs in β -cells from

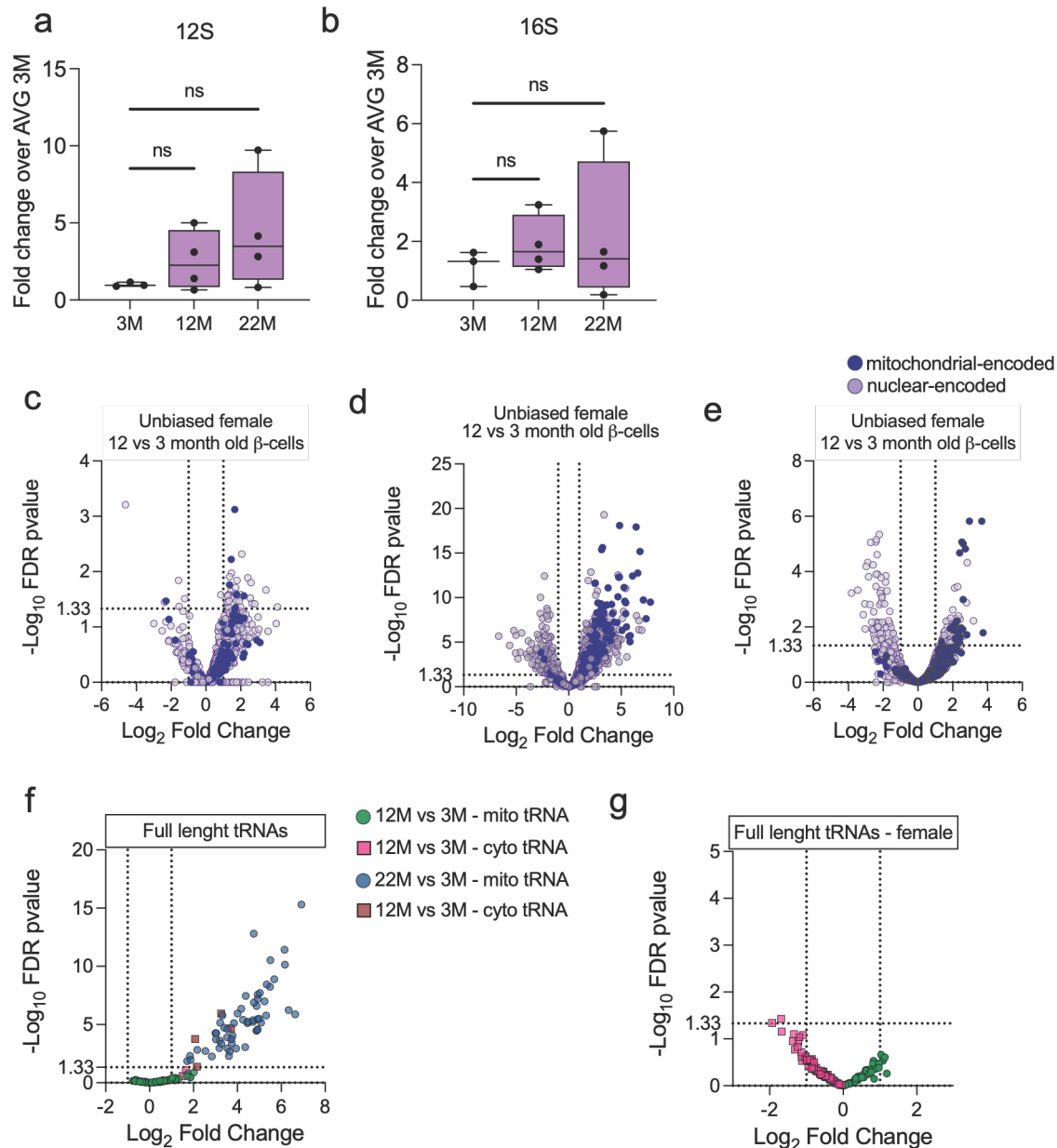
3-month and 12-month-old female mice (n=3 independent experiments). Each dot represents one independent experiment. Data are presented as mean $\pm$ SEM. * p-value <0.05; p-values <0.1 are indicated in figure.



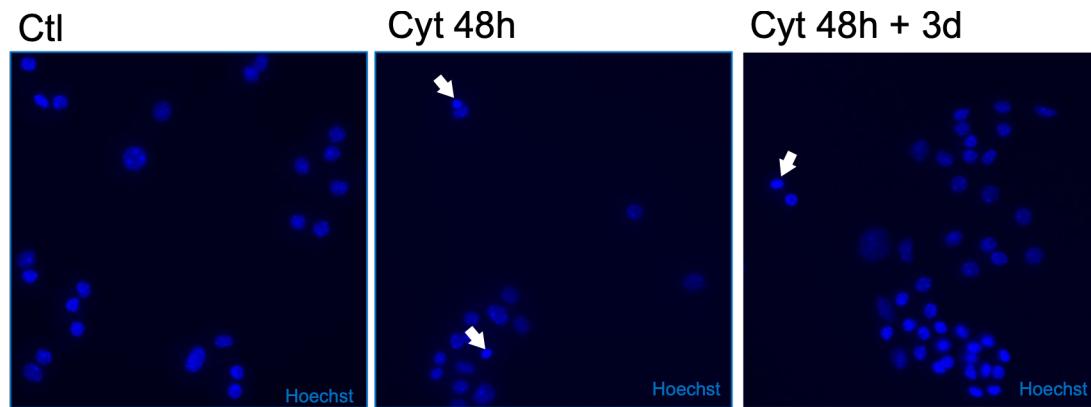
Suppl. FIGURE 3) IsomiR signature of mouse islets treated with cytokines for 24 hours (a-b). Volcano plot shows changes in isomiR and miRNA expression. Differential expression was computed using a negative binomial generalized linear model with two-sided t-test and Benjamini–Hochberg FDR correction. Dotted lines indicate thresholds of FDR pvalue<0.1 ($-\text{Log}_{10}$ FDR>1) and Fold change>1.5 (Log_2 FC>0.6). Bar plot shows changes in significantly modulated miRNAs (b). Age-associated piRNA signature was analyzed in 12-month (c) and 22-month-old (d) mice versus 3-month. Differential expression was computed using a negative binomial generalized linear model with two-sided t-test and Benjamini–Hochberg FDR correction. Volcano plots show differential expression and dotted lines are thresholds of FDR pvalue<0.05 ($-\text{Log}_{10}$ FDR>1.33) and Fold change>2 (Log_2 FC>1).



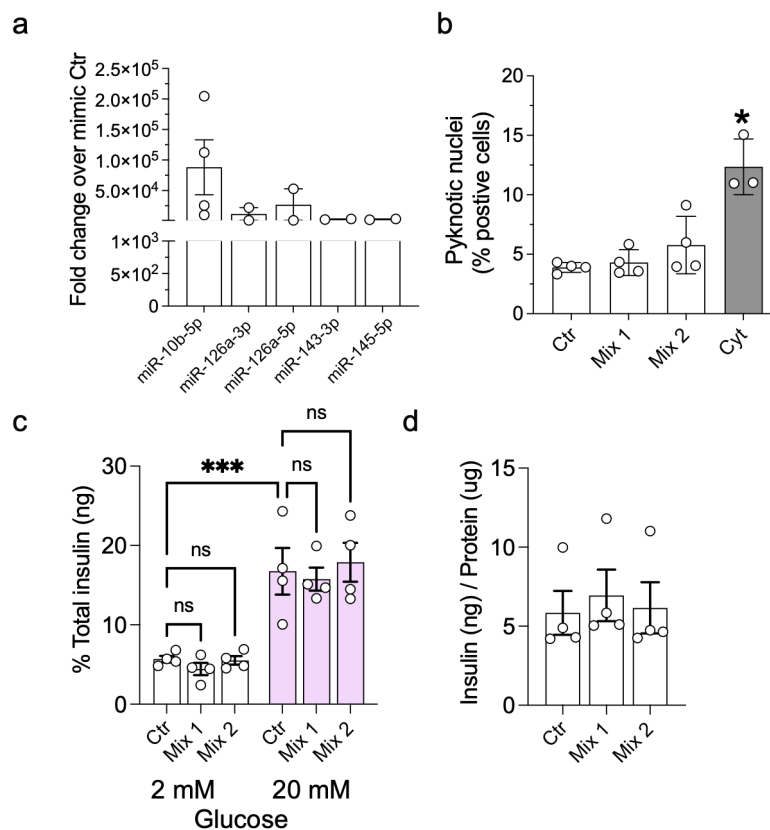
Suppl. FIGURE 4) a-b) tRF normalized read count distribution over tRF nucleotide length in datasets from FACS-sorted β -cells from male (a) and female (b) mice. c-f) tRF modulation in β -cells from female 12- compared to 3-month-old mice. a) Volcano plots show differential expression. Differential expression was computed using a negative binomial generalized linear model with two-sided t-test and Benjamini–Hochberg FDR correction. Dotted lines are thresholds of FDR pvalue<0.05 ($-\log_{10}$ FDR>1.33) and Fold change>2 ($\log_2$ FC>1). d-f) qPCR analysis of selected tRFs (n=3 independent experiments). Each dot represents one independent experiment. Data are presented as mean $\pm$ SEM. *p value<0.05 by unpaired Student t-test. Only p-values <0.1 are indicated in figure.



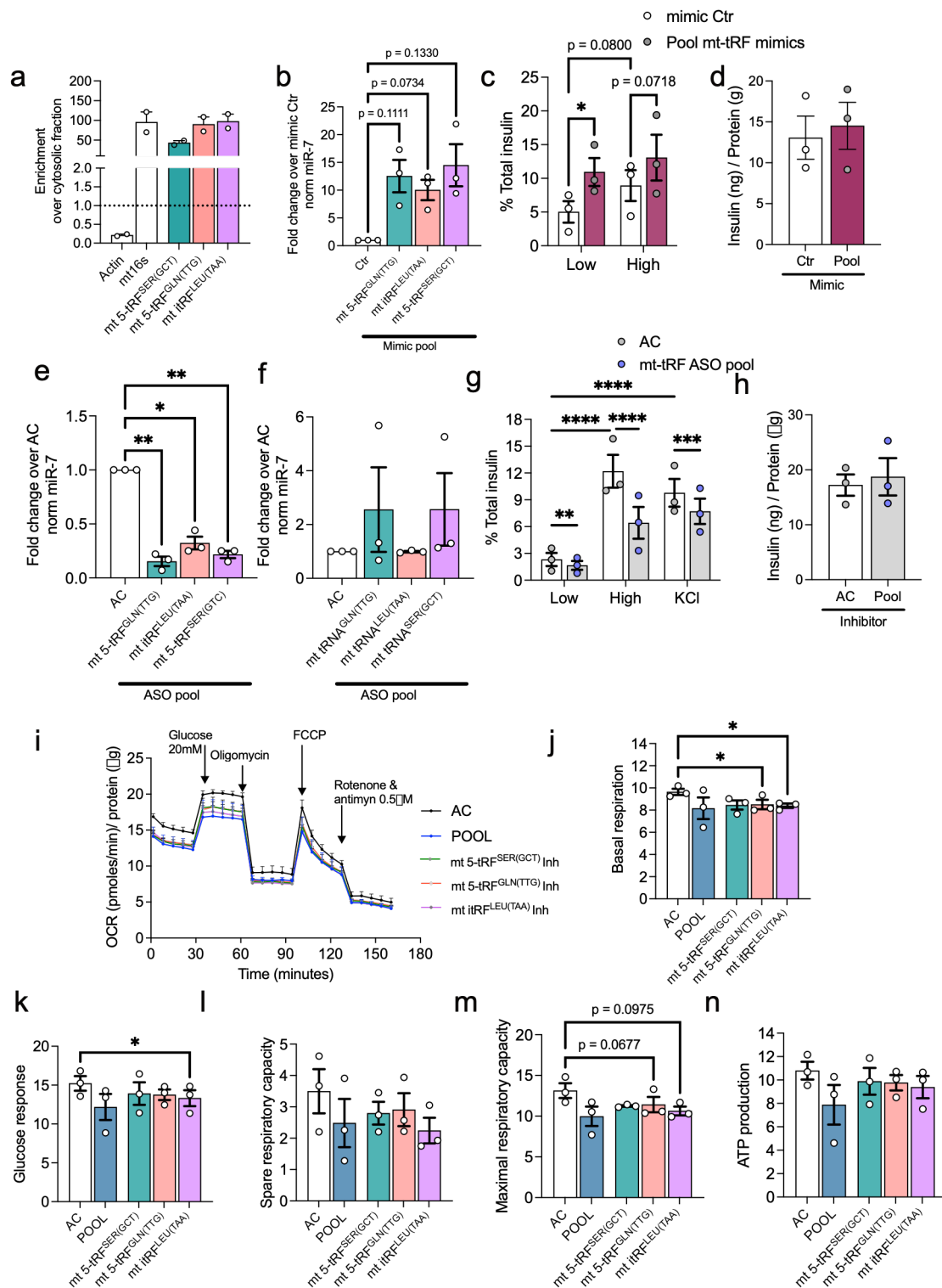
Suppl. FIGURE 5) a) 12S and b) 16S expressions in FACS-sorted β -cells of 3-, 12- and 22-month-old mice. Ribosomal RNA levels were measured by qPCR, normalized to HPRT and expressed as fold change over the average value of 3-month-old β -cells. Box plots show the median (center line), the 25th and 75th percentiles (box limits), and the minimum and maximum values (whiskers). Individual data points are shown. Statistical analysis was performed by one-way ANOVA, Dunnett's post hoc test ($n=4$ independent experiments). c-e) Differential expression of sequences mapping to mitochondrial- (dark purple) or nuclear-encoded (light purple) RNA transcripts in β -cells from c) 12-month-old or d) 22-month-old male mice and e) 12-month-old female mice. f-g) Differential expressions of full length tRNA transcripts detected in small RNAseq from β -cells from male mice (f) and from female mice (g). Circles: mitochondrial-encoded tRNA changes in aged β -cells of 12- (green) or 22- (blue) month-old mice versus young 3-month-old β -cells. Squares: Nuclear-encoded tRNA changes in aged β -cells of 12- (pink) or 22- (red) month-old mice versus young 3-month-old β -cells. Volcano plots show differential expression and dotted lines are thresholds of FDR p value <0.05 ($-\log_{10}$ FDR >1.33) and Fold change >2 ($\log_2$ FC >1). Differential expression was computed using a negative binomial generalized linear model with two-sided t-test and Benjamini–Hochberg FDR correction.



Suppl. FIGURE 6) Representative images of Hoechst staining of dispersed mouse islet cells cultured in control conditions (Ctl) or exposed to cytokines for 48 hours without (Cyt 48h) or with 3 days wash out period (Cyt 48h + 3d). Pyknotic nuclei are indicated by white arrows.

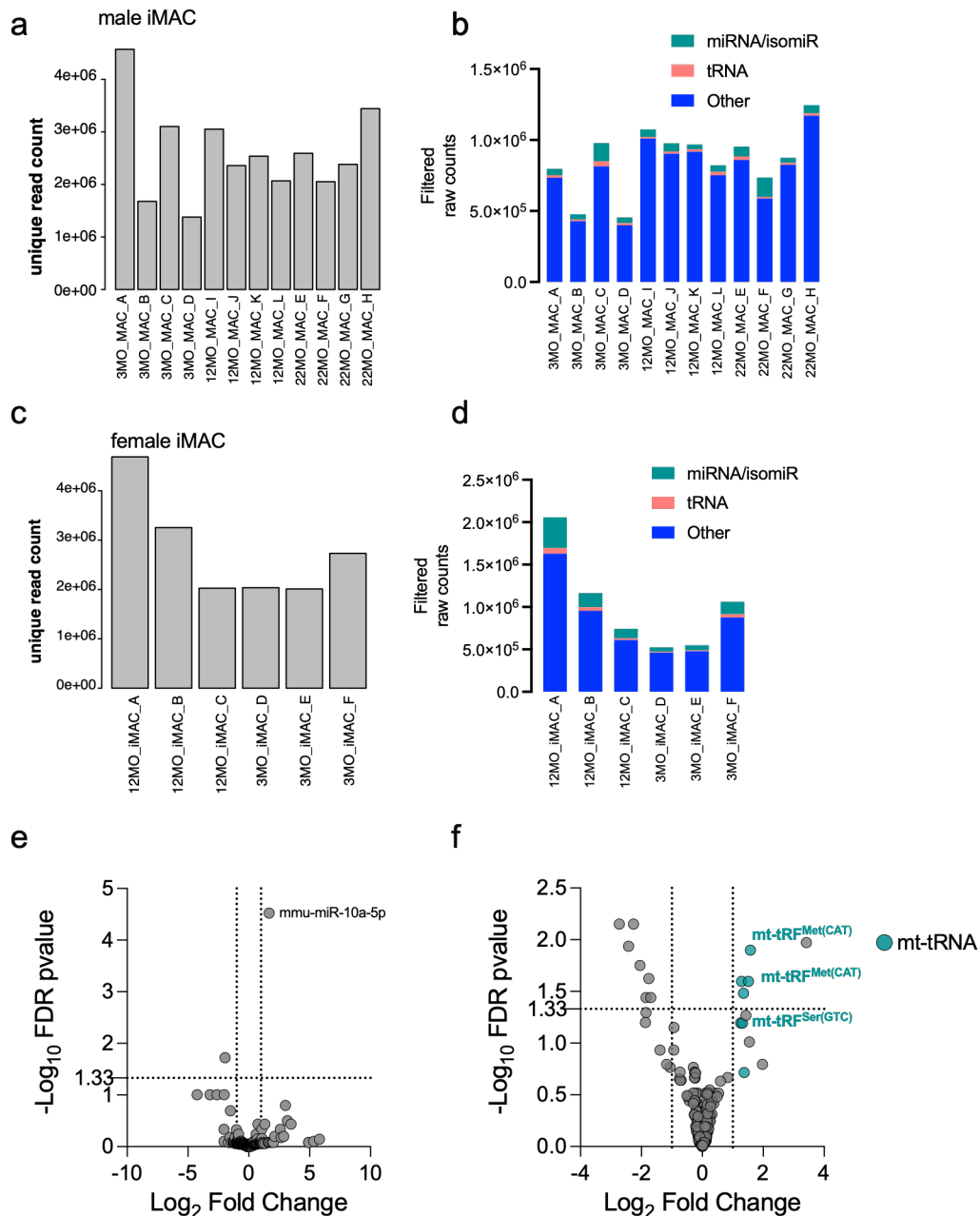


Suppl. FIGURE 7) Overexpression of miRNAs was achieved by transfection of mouse islet cells with mimics and assessed by qPCR (a; n=2-4 independent experiments). b): Apoptotic cells were identified by nuclear staining with Hoechst and identification of pyknotic nuclei in mouse islet cells transfected with mimic of control (Ctl) and different mix of miRNA mimics (Mix 1: miR-10b-5p, miR-126a-3p, miR-126a-5p and Mix2: miR-143-3p and miR-145-5p) (n=4 independent experiments). Glucose-stimulated insulin secretion (c) and insulin content (d) were assessed in mouse islet cells upon transfection (n=4 independent experiments). Each dot represents one independent experiment. Data are presented as mean ± SEM. *p-value <0.05. ***p-value <0.001 by one-way ANOVA, Dunnett's post hoc test. Only p-values <0.1 are indicated in figure.



Suppl. FIGURE 8) The enrichment of mt-tRFs was assessed by qPCR in MIN6B1 by comparing Ct values in mitochondrial fraction to the one in cytosolic fractions (a; n=2 independent experiments). Cytosolic marker *Actin* and mitochondrial marker *mt16s* were quantified in the same fractions as controls (a). Mt-tRF overexpression was assessed by qPCR (b) in mouse islet cells transfected with a pool of synthetic oligos mimicking the mt-tRF sequence. In parallel, cells were transfected with a mimic of control (Ctr) (n=3 independent experiments). b-c) Glucose-stimulated insulin secretion (c) and insulin content (d) were assessed in mouse islet cells transfected with

Ctr or mt-tRF mimic pool (n=3 independent experiments). Mouse islet cells were transfected with ASOs targeting specific mt-tRFs or ASO of control (AC) and downregulation of mt-tRFs (e) and the levels of parental full-length tRNAs (f) was assessed by qPCR (n=3 independent experiments). G-h) Glucose and KCl -stimulated insulin secretion and insulin content were assessed in transfected cells (n=3 independent experiments). i-n) Mitochondrial function was analyzed by Seahorse mito-stress assay in MIN6B1 cells transfected with AC, the ASO pool or individual ASOs. The oxygen consumption rate was assessed in the medium over 120 minutes with sequential injections (i) and parameters of mitochondrial function were derived from OCR measures (j-n) (n=3 independent experiments). Each dot represents one independent experiment. Data are presented as mean $\pm$ SEM. *p-value <0.05. *p<0.05 by one-way ANOVA, Dunnett's post hoc test (n=3 independent experiments). Only p-values <0.1 are indicated in figure.



Suppl. FIGURE 9 a-d) Read count in male (a-b) and female (c-d) iMAC samples before expression filtering (a and c) and after filtering (b and d) with indication of reads mapping to miRNA/isomiR (green), tRNA (orange) and other genomic locations (blue). e-f) miRNA and tRF differential expression analysis in FACS-sorted iMACs derived from 12-month female mice compared to 3-month. 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 $p\text{value} < 0.05$ ($-\text{Log}_{10} \text{FDR} > 1.33$) and Fold change > 2 ($\text{Log}_2 \text{FC} > 1$).

Supplementary Tables

Suppl. TABLE 1: Characteristics of C57BL/6J male mice and number of FACS-sorted β -cells and iMACs.

Islet Pool ID	Mouse ID	Age months	Weight g	Glycemia mM	Number of FACS sorted	
					Beta cells	iMACs
A	7176	3	27.72	5.9	96976	259
	7177	3	29.25	4.5		
	7178	3	27.59	6.1		
	7179	3	27.93	5.8		
B	7180	3	25.75	5.8	49892	249
	7181	3	25.78	7.5		
	7182	3	27.35	6.9		
	7183	3	25.80	5.9		
C	7184	3	26.24	7.2	129870	420
	7185	3	24.76	5.3		
	7186	3	24.70	7.1		
	7187	3	26.30	7.3		
D	7188	3	29.31	7.6	118026	211
	7189	3	24.64	6.9		
	7190	3	26.72	7.4		
	7191	3	26.66	5.3		
I	7200	12	37.96	6.3	60228	449
J	7202	12	36.92	6.4	198221	531
	7203	12	36.42	6.8		
K	7204	12	34.26	4.0	69581	369
	7205	12	36.90	5.5		
L	7206	12	42.01	5.9	71480	669
	7207	12	36.72	5.2		
E	7192	22	35.65	5.7	106419	627
	7193	22	38.14	5.5		
F	7194	22	31.12	5.3	111694	475
	7195	22	38.65	4.6		
G	7196	22	34.20	5.4	88891	460
	7197	22	36.81	4.8		
H	7198	22	35.20	5.4	129244	510
	7199	22	38.20	5.3		

Suppl. TABLE 2: Characteristics of C57BL/6NRj female mice and number of FACS-sorted β -cells and iMACs.

Islet Pool ID	Mouse ID	Age months	Weight g	Number of FACS sorted	
				Beta cells	iMACs
D	6963	3	21.6	86896	408
	6964	3	24.4		
	7004	3	21.9		
	7005	3	20.9		
E	6965	3	20.6	31168	216
	6975	3	25.8		
	7006	3	19.8		
	7007	3	23.5		
F	6976	3	25.2	76079	732
	6977	3	21.6		
	6978	3	21.7		
	7003	3	22.0		
A	6652	12	37.8	75987	2072
	6653	12	40.8		
	6654	12	38.9		
B	6647	12	26.2	96374	1042
	6648	12	46.4		
C	6655	12	41.8	88349	869
	6656	12	39.7		

Suppl. TABLE 3: Number of FACS-sorted SA- β Gal positive and negative β -cells from 7–8-month male mice.

Pool ID	FACS-sorted SA- β gal	
	Positive	Negative
1	190000	270000
2	49838	43702
3	185977	131016
4	214514	241493