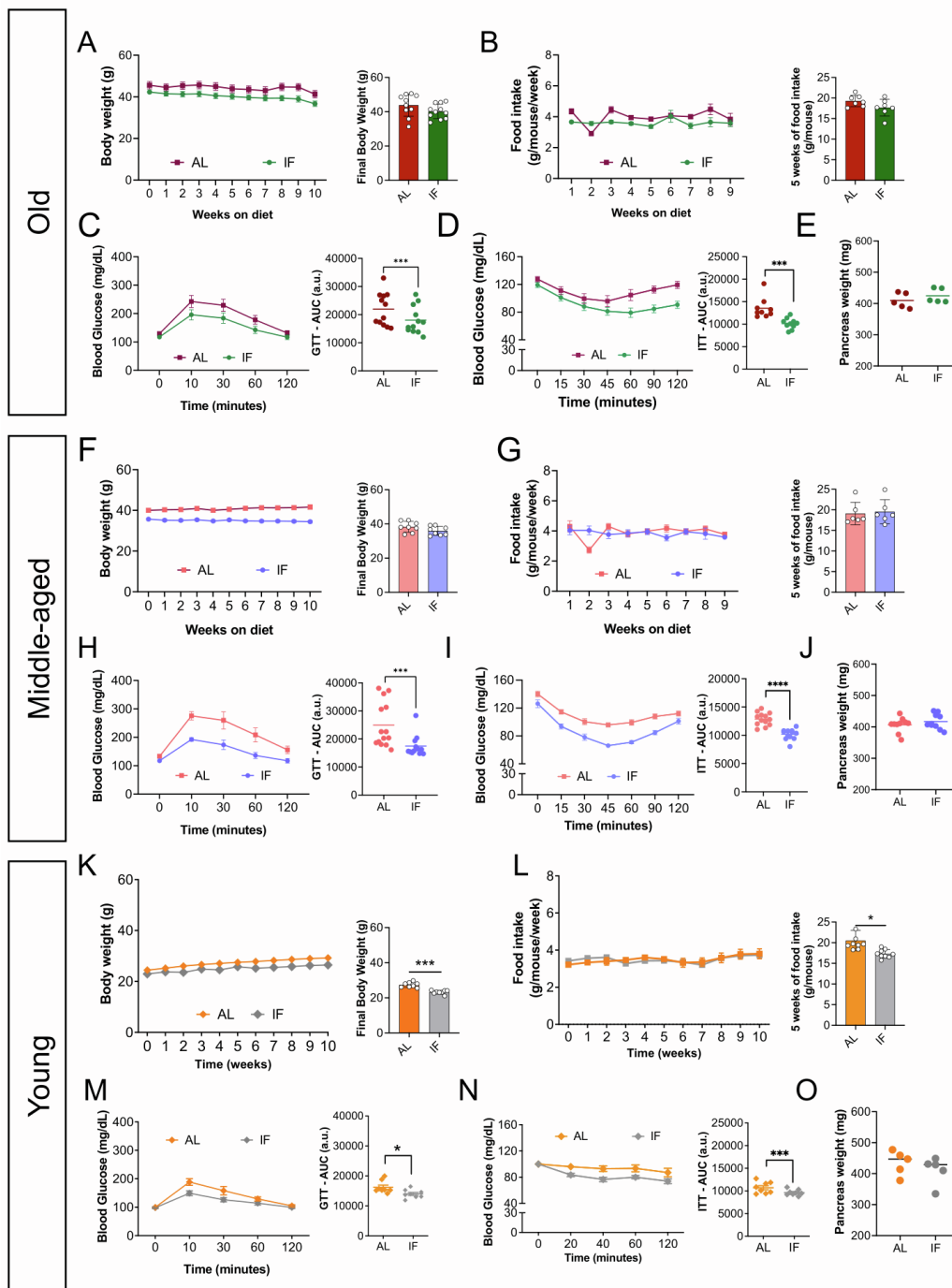


**Supplemental information**

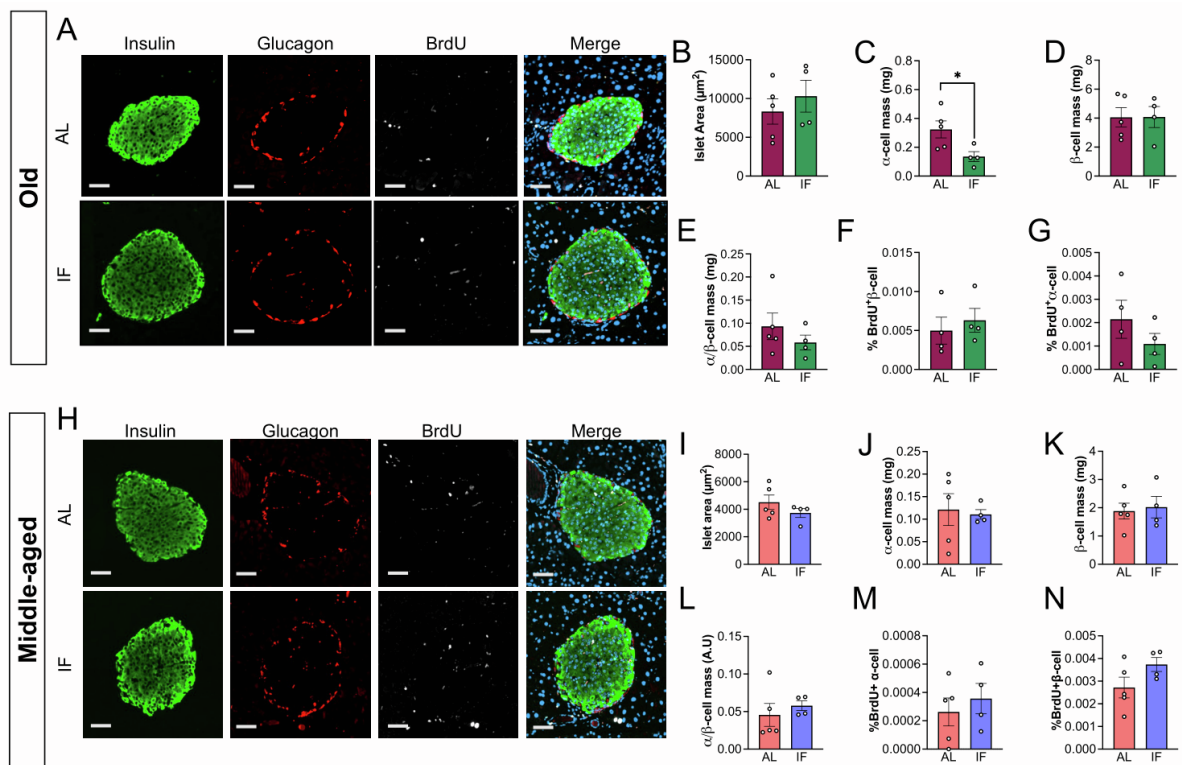
**Chronic intermittent fasting impairs  $\beta$  cell  
maturation and function in adolescent mice**

**Leonardo Matta, Peter Weber, Suheda Erener, Alina Walth-Hummel, Daniela Hass, Lea K. Bühler, Katarina Klepac, Julia Szendroedi, Joel Guerra, Maria Rohm, Michael Sterr, Heiko Lickert, Alexander Bartelt, and Stephan Herzig**

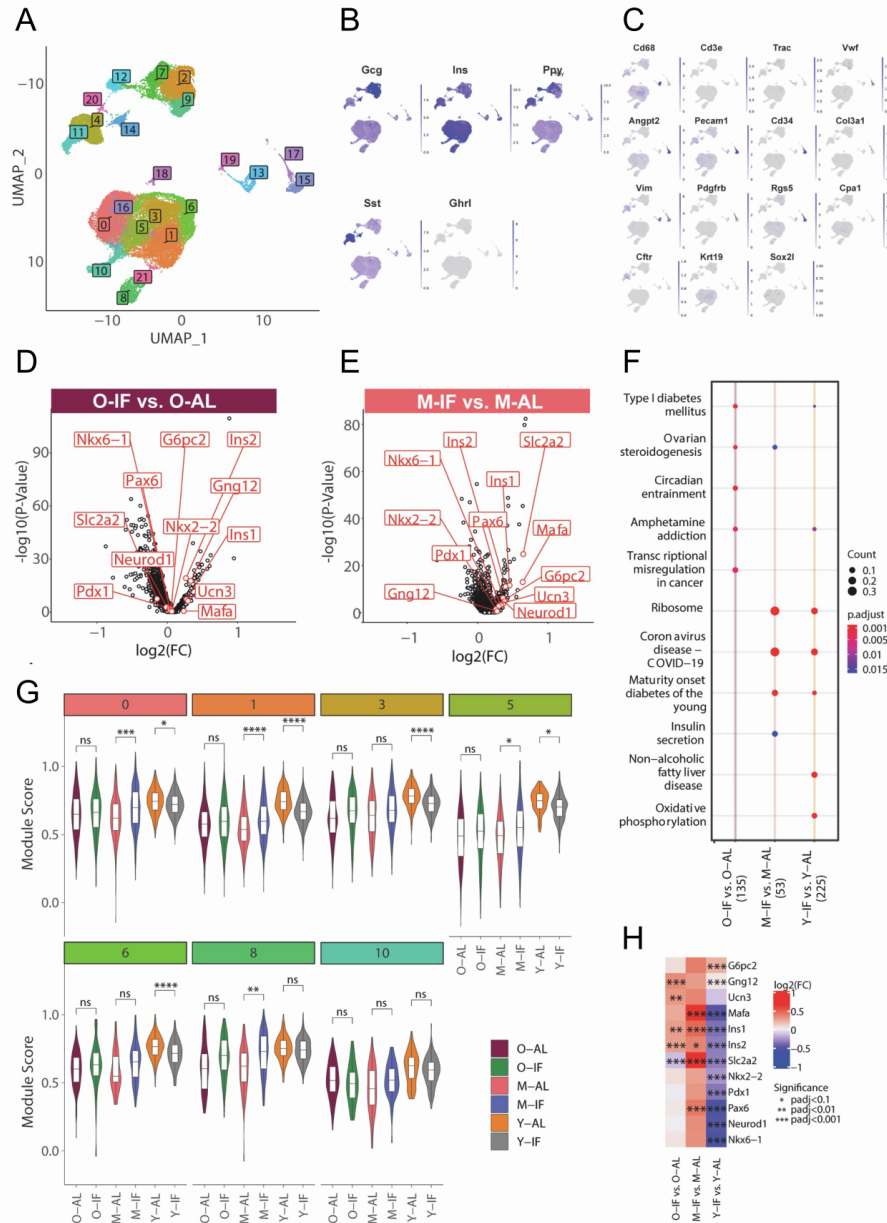
## Supplementary Material



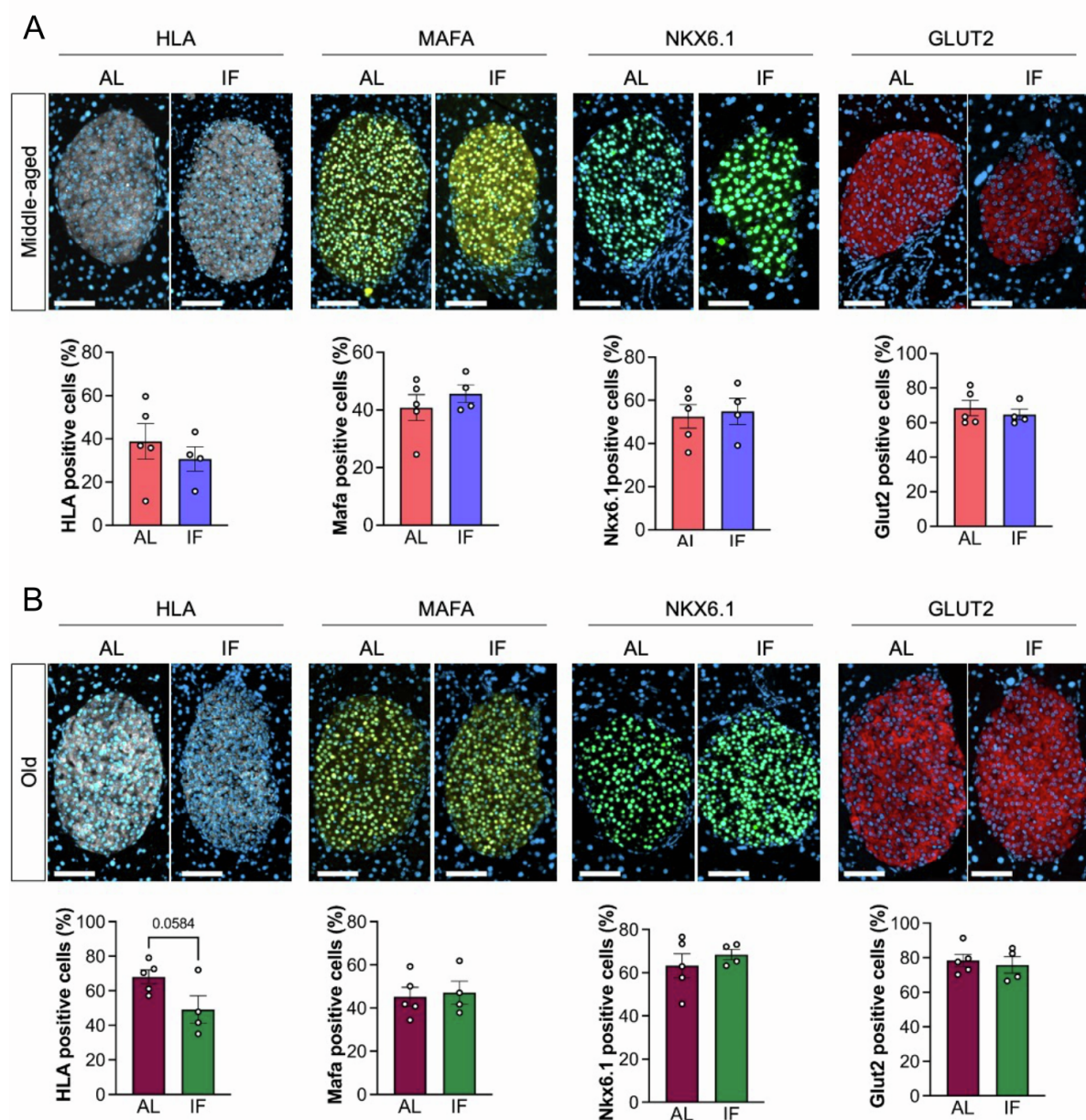
**Supplementary figure 1. A short period of intermittent fasting intervention improves glucose homeostasis in all ages.** Body weight during and at the end of 5 weeks of dietary intervention (Old: a, Middle-aged: f, and young: k); Weekly and cumulative food intake (Old: b, Middle-aged: g, and young: l); Glucose tolerance test (2.5 mg/kg) after 5 weeks of IF intervention (Old: c, Middle-aged: h, and young: m); Insulin tolerance test (0.8 U/kg) after 5 weeks of IF intervention (Old: d, Middle-aged: i, and young: n); Pancreas Weight (Old: e, Middle-aged: j, and young: o). AL: Ad libitum; IF: Intermittent fasting; GTT: Glucose tolerance test; ITT: Insulin tolerance test and AUC: Area under curve. The data were expressed as the mean  $\pm$  standard error of the mean (Old: n = 9/group; middle-aged: n = 8/group; young mice: n = 8/group). Statistical differences, \* $p < 0.05$ , \*\*\* $p < 0.001$ .



**Supplementary Figure 2. Long Intermittent fasting intervention does not affect islet morphology in old and middle-aged mice.** Immunofluorescence from pancreas of old- and middle-aged mice exposed to 10 weeks of intermittent fasting (a, h); Islet area  $\mu$ m<sup>2</sup> (b, i);  $\alpha$ -cell mass (c, j);  $\beta$ -cell mass (d, k);  $\alpha$ - and  $\beta$ - cell ratio (e, l); mass of  $\alpha$ - and  $\beta$ -cells positively stained for BrdU – proliferation (old: f and g; young m and n, respectively). Insulin positive cells: Green; Glucagon positive cells: Red; BrdU positive cells: White; and Nuclear staining (DAPI): Blue – Scale bar 100 $\mu$ m. AL: Ad libitum; IF: Intermittent fasting. The data is expressed as the mean  $\pm$  standard error of the mean (n=5 in the AL group and 3 in the IF group). Statistical differences, \*p<0.05, \*\*p <0.01.



**Supplementary Figure 3. Young mice exposed to long IF intervention present reduced transcriptomic activity scores for maturation and development also in subpopulations of  $\beta$ -cell.** UMAPs of scRNA-seq data indicating 22 statistical clusters (a), and expression of main markers for endocrine (b) as well as non-endocrine (c) cell types. Results of differential gene expression analysis upon IF vs. AL in old (d) and middle old (e) animals. Volcano plots show biological effect size ( $\log_2(\text{FC})$ ) on the x-axis and statistical significance ( $-\log_{10}(\text{P-value})$ ) on the y-axis. Comparison of over-representation analysis results of KEGG pathways in differentially expressed genes between all age groups (f). Comparison of module scores for beta cell maturation markers between IF and AL in all age groups for all beta cell clusters (g). Beta cell clusters with only a few cells have been excluded from this analysis (Clusters 16, 18 21). Heatmap of differential gene expression results ( $\log_2(\text{FC})$ ) of feeding intervention for all age groups (h). Feeding regimen-based experimental groups are intermittent fasting (IF) vs ad libitum (AL) in old (O-IF vs. O-AL), middle-aged (M-IF vs. M-AL), and young animals (Y-IF vs. Y-AL). Statistical significance is indicated with asterisks. \*  $\text{padj} < 0.05$ ; \*\*  $\text{padj} < 0.01$ ; \*\*\*  $\text{padj} < 0.001$ ; \*\*\*\*  $\text{padj} < 0.0001$  in (g) and (h).



**Supplementary Figure 4. Long Intermittent fasting intervention does not affect maturation-related protein expression in old and middle-aged mice.** Immunofluorescence from Pancreas from old- and middle-aged mice exposed to 10 weeks of intermittent fasting. HLA, Mafa, NKX6.1 and Glut 2 representative images and individual dot plot graphs from Middle-aged (a), and (b) old mice. Scale bar 50µm. AL: Ad libitum; IF: Intermittent fasting. The data is expressed as the mean  $\pm$  standard error of the mean.