

Supplementary Results

Different islet cell composition in diabetes-resistant OB and diabetes-susceptible NZO mice

Consistent with earlier findings [1], body weights remained comparable between strains throughout the experiment (Figure S2B-D). Blood glucose levels were initially similar, but increased on +CH diet as expected [1], particularly in NZO mice (Figure S2E). Feeding +CH diet also raised plasma insulin levels in both mouse strains (Figure S2F). In line with previous reports [1, 2], NZO islets were smaller than their diet-matched OB counterparts, and contained significantly less β -cells and more α -cells (Figure S3A-D). Although extended +CH feeding induces β -cell proliferation and islet hyperplasia in OB mice [2], endocrine cell numbers did not increase significantly in the two-day feeding regimen (Figure S3A-D).

Despite stringent quality control, the possibility of cell-free RNA contamination partially contributing to the percentage of polyhormonal cells (up to 10 %) could not be ruled out completely.

Predisposition of NZO *Beta1* cells to metabolic stress

Network analysis by ingenuity pathway analysis (IPA) demonstrated that targets of the transcription factor *Pdx1* were more abundant in OB cells (Figure 2D), including transcripts of secreted proteins (*Ucn3*, *Iapp*, *Scg2*), plasma membrane proteins (*Slc2a2*, *Camk2n1*), and the cytoplasmic proteins *Mt1* and *Txnip* (Figure 2D). In NZO *Beta1* cells, gene expression of chaperone proteins (*Hspa5*, *Hsp90aa1*, *Hspa13*) and the ATP synthase subunit *Atp5e* was upregulated. Similar patterns emerged for *Ckb*, a molecular marker for cellular energy homeostasis [3]; *Atf5*, stress-induced pro-survival transcription factor [4]; and *Sec61a1*, involved in ER protein transport [5] (Figure 2D).

β-cell clusters of diabetes-prone mice are less capable of mitigating effects of diabetogenic diet

Expression of the anti-apoptotic factor *Tmbim4* [6] and genes promoting N-glycosylation and counteracting ER stress, such as the oligosaccharyl transferase subunit *Ddost* and chaperones *Calr* and *Hspa5*, were highly upregulated on the *Beta1* to *Beta4* trajectory in OB as opposed to NZO islets (Figure S6A-E). At the same time, OB-enriched *Beta4* cells undergo a reduction in *Slc30a8* and *Mafa* expression not seen to the same extent in NZO β-cells (Figure 3B-C, Figure S6E).

Since lower GLUT2 expression in OB islets might also indicate a lower degree of β-cell maturity, we evaluated a reference dataset of the top 500 immaturity marker genes [7] to calculate a score for the β-cell clusters *Beta1-4*. The OB-enriched *Beta4* cluster exhibited the highest immaturity score (Figure S7A; Supplementary Table 6). This was apparent from increased expression of MYC target genes related to ribosomal biogenesis and function (*Eef1b2*, *Rpl18*, *Rpl6*, *Rplp0*, *Rps2*, *Rps3*, *Rps5*; Supplementary Table 6), indicative of a dedifferentiated state poised for growth and proliferation [8].

Supplementary References

- [1] Kluth O, Matzke D, Schulze G, Schwenk RW, Joost HG, Schürmann A (2014) Differential Transcriptome Analysis of Diabetes-Resistant and -Sensitive Mouse Islets Reveals Significant Overlap With Human Diabetes Susceptibility Genes. *Diabetes* 63(12): 4230-4238. 10.2337/db14-0425
- [2] Kluth O, Matzke D, Kamitz A, et al. (2015) Identification of Four Mouse Diabetes Candidate Genes Altering β -Cell Proliferation. *PLoS Genet* 11(9): e1005506. 10.1371/journal.pgen.1005506
- [3] Ju TC, Lin YS, Chern Y (2012) Energy dysfunction in Huntington's disease: insights from PGC-1alpha, AMPK, and CKB. *Cell Mol Life Sci* 69(24): 4107-4120. 10.1007/s00018-012-1025-2
- [4] Juliana CA, Yang J, Cannon CE, Good AL, Haemmerle MW, Stoffers DA (2018) A PDX1-ATF transcriptional complex governs β cell survival during stress. *Mol Metab* 17: 39-48. 10.1016/j.molmet.2018.07.007
- [5] Lloyd DJ, Wheeler MC, Gekakis N (2010) A point mutation in Sec61alpha1 leads to diabetes and hepatosteatosis in mice. *Diabetes* 59(2): 460-470. 10.2337/db08-1362
- [6] Carrara G, Parsons M, Saraiva N, Smith GL (2017) Golgi anti-apoptotic protein: a tale of camels, calcium, channels and cancer. *Open Biol* 7(5). 10.1098/rsob.170045
- [7] Qiu WL, Zhang YW, Feng Y, Li LC, Yang L, Xu CR (2017) Deciphering Pancreatic Islet β Cell and α Cell Maturation Pathways and Characteristic Features at the Single-Cell Level. *Cell Metab* 25(5): 1194-1205 e1194. 10.1016/j.cmet.2017.04.003
- [8] Puri S, Roy N, Russ HA, et al. (2018) Replication confers beta cell immaturity. *Nat Commun* 9(1): 485. 10.1038/s41467-018-02939-0