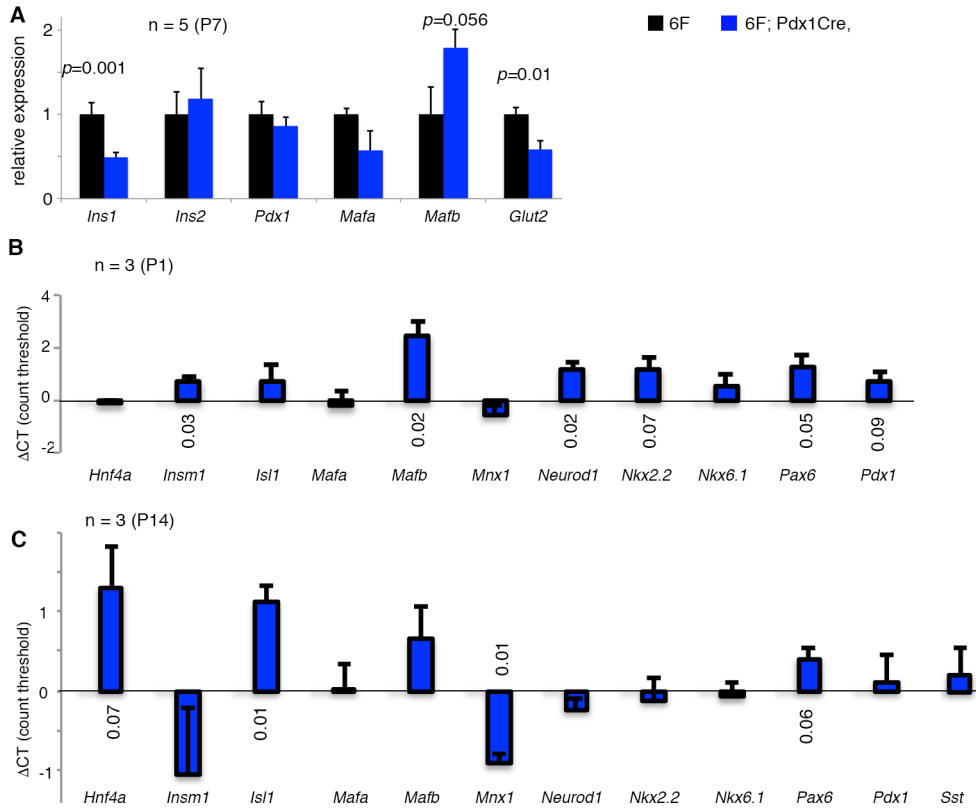


**Figure S1: Related to Figure 1. Quality control tests in *6F; Pdx1<sup>Cre</sup>* mice.** Mice were derived from inter-cross of *Myt1<sup>F/+</sup>; Myt2<sup>F/+</sup>; Myt3<sup>F/+</sup>; Pdx1<sup>Cre</sup>* mice or between *6F* and *Myt1<sup>F/+</sup>; Myt2<sup>F/F</sup>; Myt3<sup>F/F</sup>; Pdx1<sup>Cre</sup>* or *Myt1<sup>F/F</sup>; Myt2<sup>F/+</sup>; Myt3<sup>F/F</sup>; Pdx1<sup>Cre</sup>* mice.

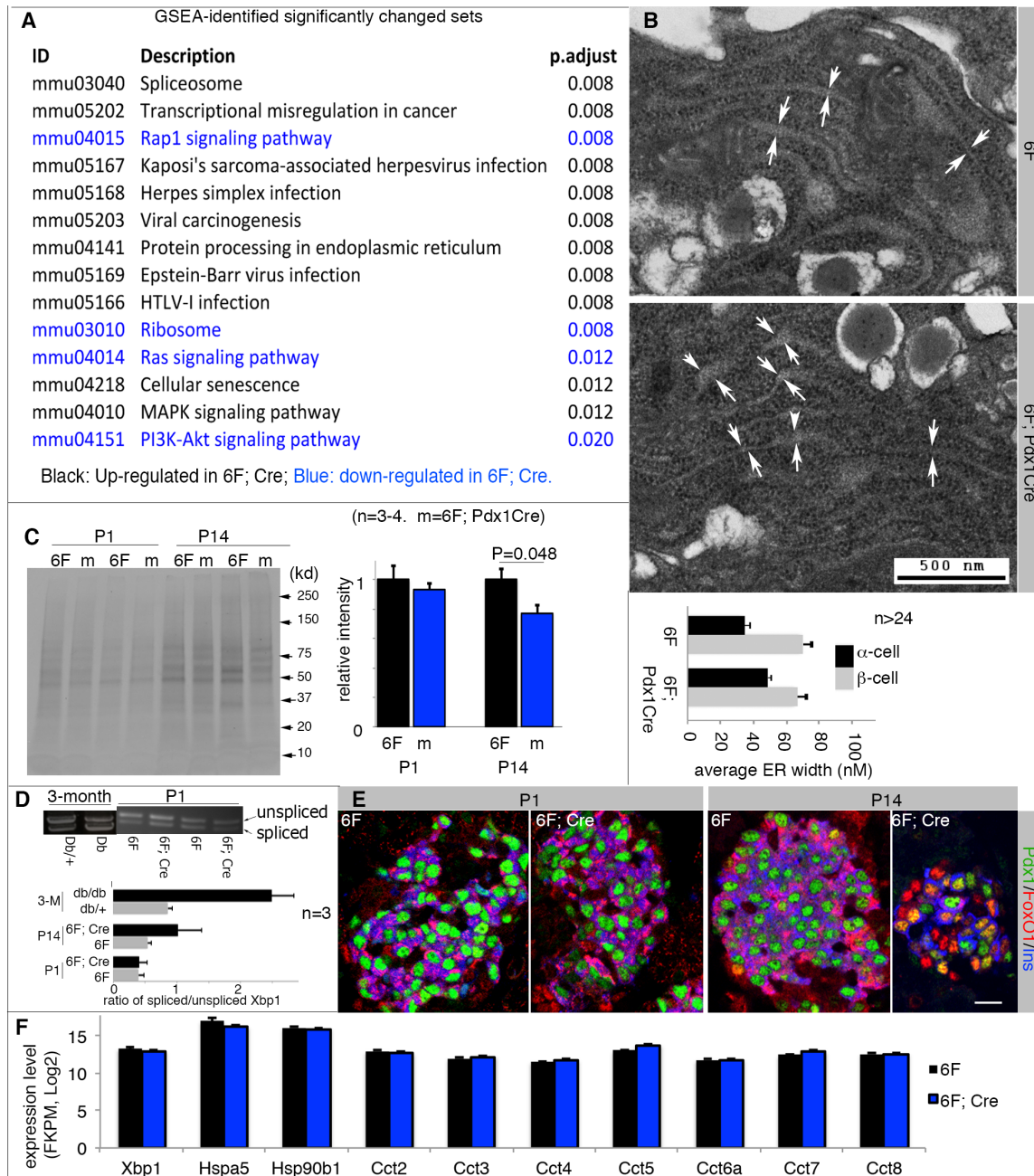
(A) Myt TF deletion efficacy in *6F; Pdx1<sup>Cre</sup>* islets, with a *6F* control presented (P14). Pdx1 signals (green) were used to identify the location of islets. Scale bars = 20  $\mu$ m.

(B) Myt TF detection in the hypothalamus regions of P14 *6F* and *6F; Pdx1<sup>Cre</sup>* mice, labeled for each Myt TF and DAPI. Scale bars = 50  $\mu$ m.

(C) The morphology of pancreatic exocrine tissues in P14 *6F* and *6F; Pdx1<sup>Cre</sup>* mice. Islets were labeled with HRP signals (brown), visualized by Eosin counter-staining (pink). Scale bars = 100  $\mu$ m.



**Figure S2: Related to Figure 2. Neonatal Myt TF mutant islets have several mis-regulated  $\beta$ -cell factors assayed via qPCR.** Mice were derived from crosses between *6F* and *Myt1<sup>F/+</sup>*; *Myt2<sup>F/F</sup>*; *Myt3<sup>F/F</sup>*; *Pdx1<sup>Cre</sup>* or *Myt1<sup>F/F</sup>*; *Myt2<sup>F/+</sup>*; *Myt3<sup>F/F</sup>*; *Pdx1<sup>Cre</sup>* mice. The day of birth was counted as P1. The *p*-values (type 2, 2-tailed t-test) smaller than 0.10 were marked on top of corresponding assays. Error bars, SEM. “n”, the number of independent islet preparations (from 1-2 mice each) used. (A-C) QPCR assays of P7, P1 and P14 islets, using *Gapdh* as controls. Data are presented as relative expression (A) or differences in threshold count (CT in panels B, C). Note at P1, the islet-based assay detected no *Mnx1* expression change, although RNA-seq in purified  $\beta$ -cells showed its upregulation in *6F*; *Pdx1<sup>Cre</sup>*  $\beta$ -cells. Similarly, we detected increased *Mafb* transcription in *6F*; *Pdx1<sup>Cre</sup>* islets here but not *6F*; *Pdx1<sup>Cre</sup>*  $\beta$ -cells via RNA-seq. These are likely attributable to *Mafb/Mnx1* expression differences amongst different islet cell types.



**Figure S3: Related to Figure 3.  $6F; Pdx1^{Cre}$  islet cells lacked global cellular features of UPR before defective glucose homeostasis.** Mice were derived from crosses between  $6F$  and  $Myt1^{F/+}; Myt2^{F/F}; Myt3^{F/F}; Pdx1^{Cre}$  or  $Myt1^{F/F}; Myt2^{F/+}; Myt3^{F/F}; Pdx1^{Cre}$  mice. The  $p$ -values were calculated via type 2, 2-tailed t-test. Error bars, SEM. "n", the number of independent islet preparations or culture assays.  $6F; Cre=6F; Pdx1^{Cre}$ .

(A) Pathways differentially expressed in P1  $6F$  and  $6F; Pdx1^{Cre}$   $\beta$ -cells, identified via Gene Set Enrichment Assays. Pathways with adjusted  $p$  value  $\leq 0.02$  were listed.

(B) ER-width in P1  $\beta$ -cells assayed via transmission electron microscopy. The presented quantification data were from double-blind tests, counting the distance

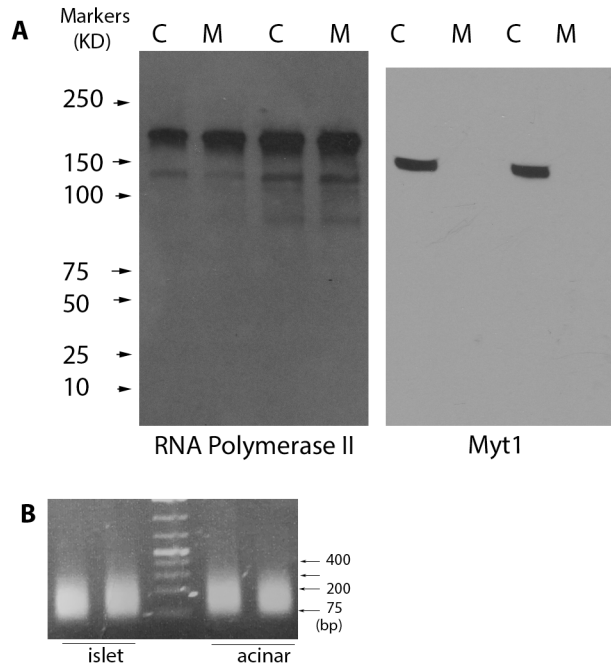
between two membranes of ER (distance between paired arrows).  $\alpha$ -cells were included as controls.

(C) Radio-labeling ( $^{35}\text{S}$ ) and quantification of newly made proteins in *6F* and *6F; Pdx1<sup>Cre</sup>* islets within a four-hour window. Quantification was from Image J-aided assays from exposed protein gels.

(D) *Xbp1* mRNA splicing assays. Islets from 3-month old *db/+* and *db/db* mice were included as positive controls. The ratios between spliced (lower bands) and unspliced (upper bands) bands were presented.

(E) Foxo1 activation in *6F; Pdx1<sup>Cre</sup>*  $\beta$ -cells. Pdx1 signals were used to identify  $\beta$ -cells. Note the presence of yellow nuclei (Foxo1 activation) only in the P14 sample. Scale bars = 20  $\mu\text{m}$ .

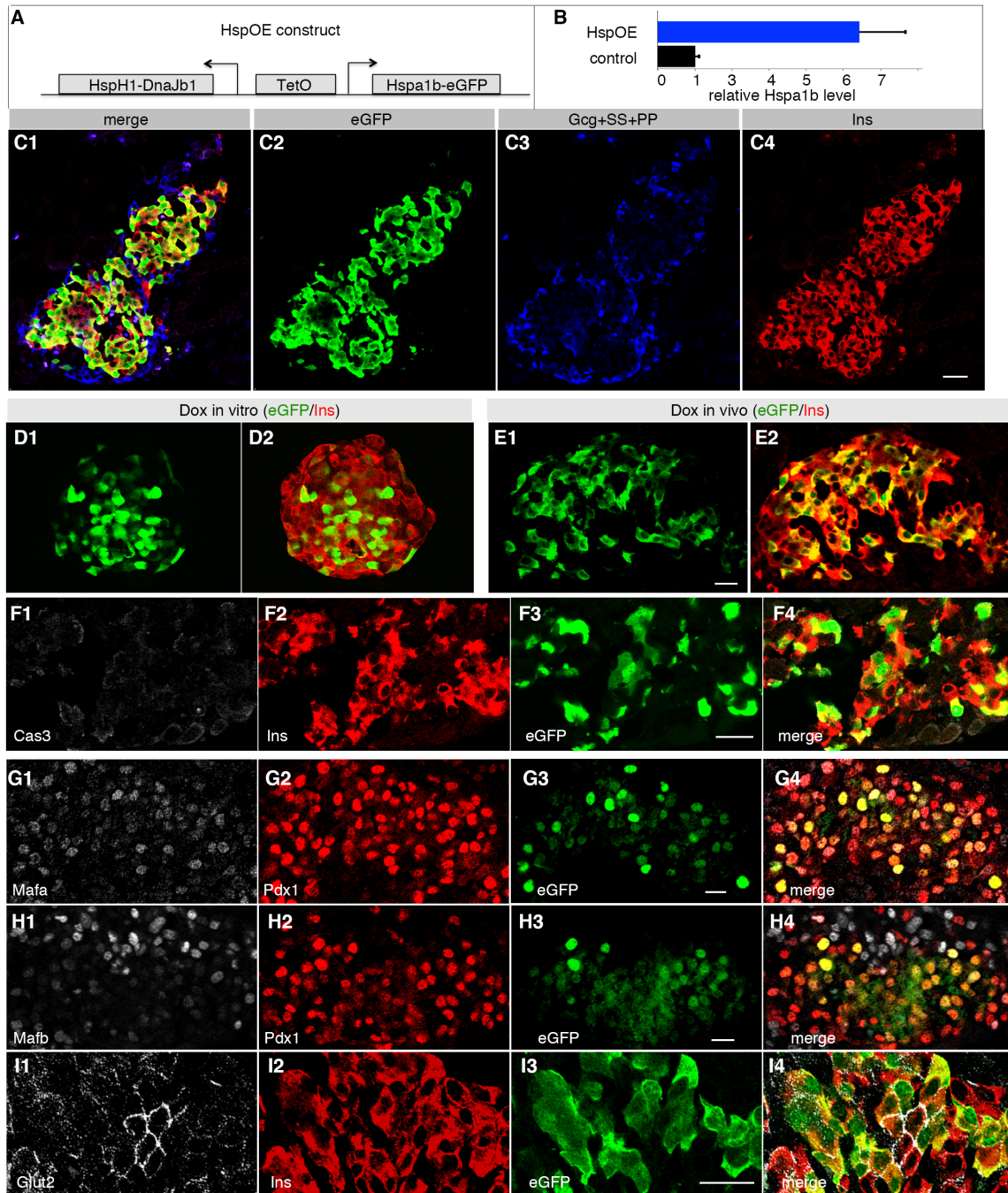
(F) Expression of several stress markers in P1 control and *6F; Pdx1<sup>Cre</sup>*  $\beta$ -cells assayed via RNA-seq, presented as Log2 (fragments per kb per million reads).



**Figure S4: Related to Figure 3. Myt1 CHIP assay controls.**

(A) Western blot to test the specificity of the Myt1 antibodies used in ChIP-PCR (“C”, 6F control islets. M, 6F; *Pdx1<sup>Cre</sup>* mutant islets). An RNA polymerase II antibody was used as a positive loading/transfer control.

(B) Examples of fragmented chromatin from adult islet and acinar cells.



**Figure S5. Related to Figure 4. The derivation and some characterization of *Hsp*-OE  $\beta$ -cells.** For inducing OE, *TetO<sup>3H</sup>* and *Rip<sup>rTTA</sup>* mice were crossed. Dox was administered in the drinking water of pregnant females starting from E16.5 (16<sup>th</sup> day after the observation of vaginal plug). Dox application continued to the date of tissue collection.

(A) The structure of *Hsp*-OE (*TetO<sup>3H</sup>*) transgene. Note that two mRNA will be transcribed under the control of a bi-directional TetO promoter. Each mRNA will

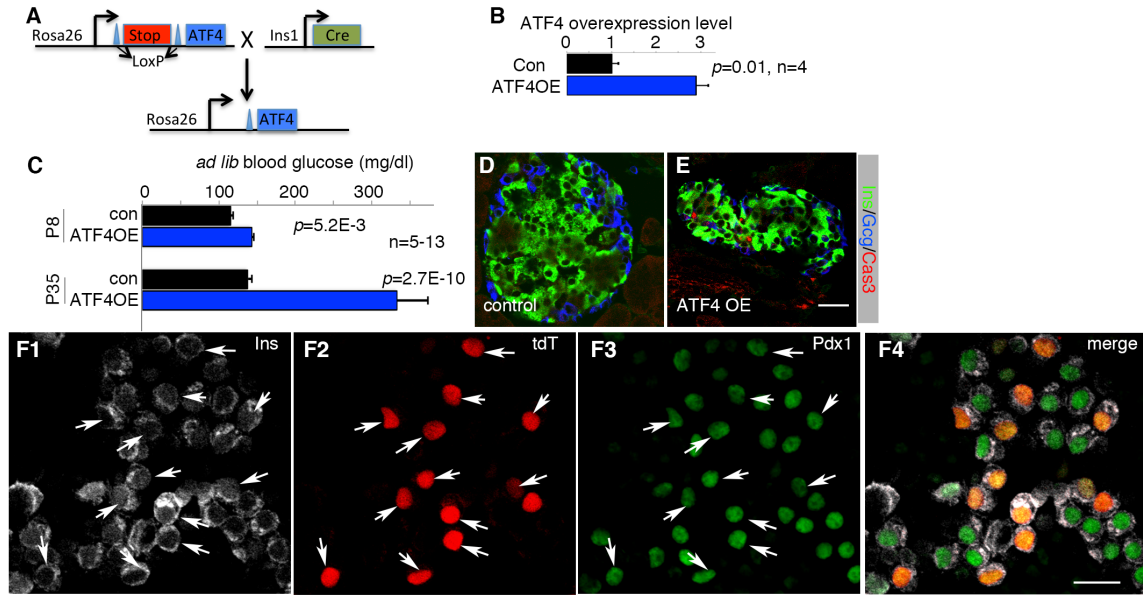
produce two proteins, with T2A peptide included between Hsph1 and Dnajb1 or Hspa1b and eGFP.

(B) Level of *Hspa1b* OE in P2 *Hsp-OE* islets ( $p=0.01$ ). Results were from four ( $n=4$ ) islet preps. Error bars, SEM.

(C) Restricted transgene expression, by virtue of monitoring eGFP expression, in  $\beta$ -cells of *Hsp-OE* islets (P4). Scale bars = 20  $\mu\text{m}$ .

(D, E) *Hsp-OE* in 8-week old islets (E) or mice (F) in animals without prior exposure to Dox but treated with Dox for two days. Islets were isolated from mice and treated with 5 ng/ml Dox for two days. For *in vivo* activation, two-month-old mice were injected with 10  $\mu\text{g}$  Dox via intra-peritoneal route.

(F-I) Expression of Cleaved Caspase 3 (Cas3) and several  $\beta$ -cell markers in P10 *Hsp-OE* islets. Scale bars = 20  $\mu\text{m}$ .



**Figure S6. Related to Figure 5. *ATF4* overexpression (OE) in mouse  $\beta$ -cells.**

(A) The cross scheme used to activate *ATF4*-OE in  $\beta$ -cells.

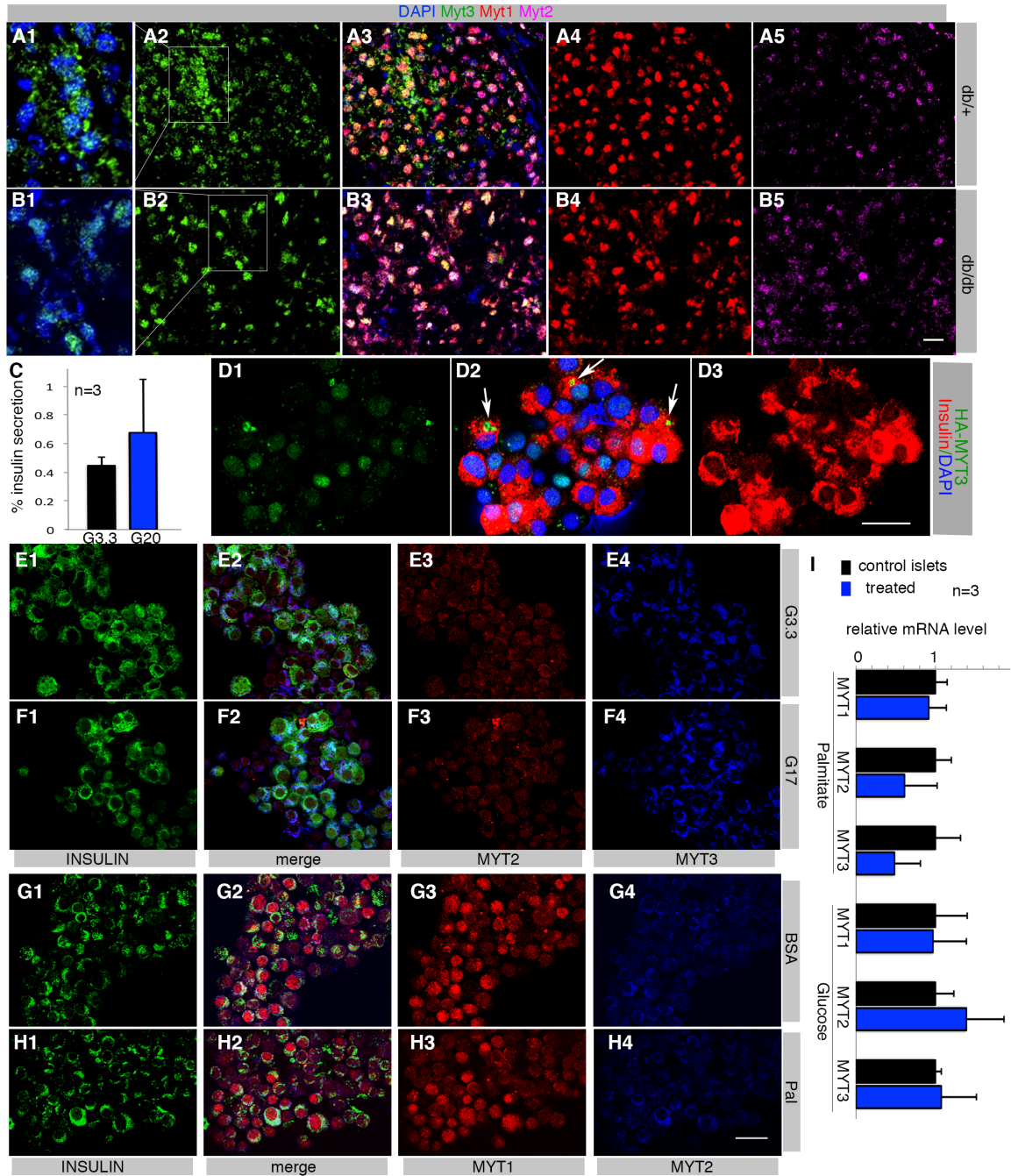
(B) Level of *ATF4* OE in P2 *ATF4*-OE islets. Results were from four islet preps (n=4). The *p*-values (type 2, 2-tailed t-test) were included in the panel. Error bars, SEM.

(C) *Ad lib* blood glucose in control and *ATF4*-OE mice before and after weaning. The controls include wild-type, *Ins1*<sup>Cre</sup>, and single *Rosa26-ATF4*<sup>LoxTG</sup> mice, both sexes. The *p*-values (type 2, 2-tailed t-test) were included in the panel. Error bars, SEM.

(D, E) Cas3 activation in one-month old *ATF4*-OE islet. The few red dots in the *ATF4*-OE panels are likely blood cells, which localize in the interstitial space between hormone-expressing cells. Scale bar = 20  $\mu$ m.

(F) Insulin and Pdx1 production in P35 control and tdTomato (tdT)-expressing  $\beta$ -cells. tdT activation was initiated from ~E16.5, using a *Pdx1*<sup>CreER</sup>-based activation in an *Ai9* Cre-reporter mouse line. Arrows point to the tdT+  $\beta$ -cells. Scale bar = 20  $\mu$ m.





**Figure S7. Related to Figures 6 and 7. *Myt*(*MYT*) regulation in very old mice and human islet/ $\beta$ -cells.**

(A, B) *Myt* TF detection in 8-month-old *db/+* (A) or *db/db* mouse islet cells (B). The cytoplasmic/nuclear localization of *Myt3* was highlighted in A1, A2, B1, and B2. Scale bar = 20  $\mu$ m.

(C) GSIS results of T2D islets ( $p=0.26$ ), % of total insulin secretion within a 30-minute window.

(D) Localization of HA epitope-tagged MYT3 in human  $\beta$ -cells, five days after lentivirus-mediated transduction. Arrows point to several cells with cytoplasmic HA-MYT3 localization, detected using anti-HA antibodies. Scale bar = 20  $\mu$ m.

(E, F) MYT2 and MYT3 in islet  $\beta$ -cells treated with 17 mM glucose for  $\sim$ 40 hours. Starting materials were functional human islets.

(G, H) MYT1 and MYT2 in islet  $\beta$ -cells treated with 0.6 mM palmitate for  $\sim$ 40 hours. Starting materials were functional human islets. Scale bar = 20  $\mu$ m.

(I) QPCR assays in human islets treated with (17 mM glucose) G17 or 0.6 mM palmitate for  $\sim$ 40 hours. Whole islets were used for all assays.  $P > 0.05$  in all comparisons, according to student t-test (type 2, 2-tails).

**Table S3. Related to Figure 2, S2.** The expression levels of several TFs in P1 control and *Myt TF* deficient  $\beta$  cells (mutant), assayed via RNAseq. The logFC {log(2) [(average of mutant) - (average of control)]/(average of control)}, *p*-values, adjusted *p*-values, and the log(2) transformed levels (FPKM, fragment per kilobase million) of three biological repeats of control and mutants are shown.

| Symbol  | logFC  | P.Value | adj. P.Val | LogFPKM mutant |          |          | LogFPKM control |          |          |
|---------|--------|---------|------------|----------------|----------|----------|-----------------|----------|----------|
|         |        |         |            | mutant         | repeat 1 | repeat 2 | control         | repeat 1 | repeat 2 |
| Hnf4a   | -0.143 | 0.722   | 0.820      | 5.682          | 7.210    | 5.814    | 6.480           | 6.221    | 6.429    |
| Insm1   | 0.958  | 0.001   | 0.016      | 8.564          | 8.854    | 8.297    | 7.716           | 7.354    | 7.765    |
| Isl1    | 0.726  | 0.102   | 0.223      | 7.262          | 6.590    | 8.254    | 6.713           | 6.461    | 6.736    |
| Mafa    | 1.098  | 0.022   | 0.083      | 7.767          | 7.590    | 7.074    | 6.852           | 5.454    | 6.764    |
| Mafb    | -0.653 | 0.012   | 0.057      | 8.577          | 7.866    | 8.526    | 8.986           | 8.963    | 8.980    |
| Mnx1    | 0.784  | 0.006   | 0.039      | 5.911          | 6.396    | 5.923    | 5.440           | 4.935    | 5.452    |
| Neurod1 | 0.874  | 0.000   | 0.008      | 9.208          | 9.078    | 8.872    | 8.099           | 8.288    | 8.151    |
| Nkx2.2  | 1.224  | 0.000   | 0.002      | 8.111          | 8.150    | 8.355    | 7.035           | 6.901    | 7.004    |
| Nkx6.1  | 0.124  | 0.245   | 0.400      | 8.988          | 9.127    | 8.980    | 8.920           | 8.880    | 8.924    |
| Pax6    | 0.505  | 0.006   | 0.039      | 9.468          | 9.899    | 9.648    | 9.119           | 9.205    | 9.176    |
| Pdx1    | 0.092  | 0.687   | 0.793      | 8.732          | 9.530    | 8.734    | 8.935           | 8.859    | 8.925    |

**Table S4. Related to Figure 6.** The expression changes of several stress-response genes in islets of 3-months-old db/db and db/+ mice. The Log(2)-fold change (Fc), *p*- or adjusted *p*-values are shown.

| <b>genes</b> | <b>Logfc (db/dbt vs db/+)</b> | <b>p.val (db/dbt vs db/+)</b> | <b>p.adj (db/dbt vs db/+)</b> |
|--------------|-------------------------------|-------------------------------|-------------------------------|
| Atf1         | -0.118                        | 0.467                         | 0.623                         |
| Atf1-ps      | -0.009                        | 0.914                         | 0.953                         |
| Atf2         | -1.025                        | 0.005                         | 0.038                         |
| Atf3         | 0.833                         | 0.225                         | 0.379                         |
| Atf4         | -0.163                        | 0.291                         | 0.456                         |
| Atf5         | -0.240                        | 0.054                         | 0.151                         |
| Atf6         | -0.801                        | 0.160                         | 0.308                         |
| Atf6b        | -0.545                        | 0.017                         | 0.074                         |
| Atf7         | -0.436                        | 0.078                         | 0.188                         |
| Atf7ip       | -0.604                        | 0.184                         | 0.334                         |
| Atf7ip2      | -0.342                        | 0.417                         | 0.583                         |
| Cat          | 1.235                         | 0.072                         | 0.180                         |
| Cct2         | -0.056                        | 0.951                         | 0.972                         |
| Cct3         | -0.595                        | 0.009                         | 0.052                         |
| Cct3-ps1     | -0.400                        | 0.477                         | 0.631                         |
| Cct4         | -0.344                        | 0.068                         | 0.174                         |
| Cct5         | -0.802                        | 0.009                         | 0.053                         |
| Cct6a        | -0.634                        | 0.004                         | 0.035                         |
| Cct6b        | 0.118                         | 0.402                         | 0.569                         |
| Cct7         | -0.434                        | 0.053                         | 0.150                         |
| Cct8         | -0.339                        | 0.058                         | 0.157                         |
| Ddit3        | -0.407                        | 0.620                         | 0.750                         |
| Ddit4        | -0.107                        | 0.183                         | 0.334                         |
| Ddit4l       | 0.886                         | 0.173                         | 0.325                         |
| Derl1        | 0.093                         | 0.746                         | 0.842                         |
| Derl2        | -1.358                        | 0.127                         | 0.262                         |
| Derl3        | 2.090                         | 0.001                         | 0.018                         |
| Dnaja1       | -0.634                        | 0.002                         | 0.027                         |
| Dnaja1-ps    | -0.063                        | 0.932                         | 0.961                         |
| Dnaja2       | -0.212                        | 0.100                         | 0.221                         |
| Dnaja3       | -0.412                        | 0.051                         | 0.145                         |
| Dnaja4       | 0.090                         | 0.517                         | 0.665                         |
| Dnajb1       | -1.312                        | 0.001                         | 0.018                         |
| Dnajb11      | 1.283                         | 0.213                         | 0.364                         |
| Dnajb12      | -0.023                        | 0.841                         | 0.910                         |
| Dnajb13      | -0.262                        | 0.395                         | 0.562                         |

|            |        |       |       |
|------------|--------|-------|-------|
| Dnajb14    | -0.477 | 0.006 | 0.043 |
| Dnajb2     | -0.758 | 0.001 | 0.019 |
| Dnajb3     | 0.010  | 0.987 | 0.991 |
| Dnajb4     | -0.183 | 0.158 | 0.306 |
| Dnajb5     | -0.822 | 0.001 | 0.020 |
| Dnajb6     | -1.621 | 0.067 | 0.172 |
| Dnajb9     | -0.074 | 0.787 | 0.872 |
| Dnajc1     | 1.017  | 0.020 | 0.082 |
| Dnajc10    | 0.538  | 0.080 | 0.191 |
| Dnajc11    | -0.863 | 0.002 | 0.024 |
| Dnajc12    | 0.935  | 0.050 | 0.143 |
| Dnajc13    | -6.116 | 0.001 | 0.017 |
| Dnajc14    | -0.513 | 0.007 | 0.048 |
| Dnajc15    | 0.081  | 0.551 | 0.695 |
| Dnajc16    | -0.536 | 0.007 | 0.047 |
| Dnajc17    | -1.006 | 0.010 | 0.056 |
| Dnajc18    | -0.228 | 0.721 | 0.824 |
| Dnajc19    | -0.363 | 0.017 | 0.074 |
| Dnajc19-ps | 0.186  | 0.066 | 0.171 |
| Dnajc2     | -0.211 | 0.082 | 0.194 |
| Dnajc21    | -0.591 | 0.004 | 0.034 |
| Dnajc22    | 1.095  | 0.007 | 0.047 |
| Dnajc24    | -1.417 | 0.000 | 0.011 |
| Dnajc25    | 0.151  | 0.402 | 0.569 |
| Dnajc27    | -0.838 | 0.001 | 0.021 |
| Dnajc28    | -0.264 | 0.635 | 0.762 |
| Dnajc3     | 0.394  | 0.033 | 0.110 |
| Dnajc30    | -0.392 | 0.576 | 0.717 |
| Dnajc4     | 0.293  | 0.175 | 0.328 |
| Dnajc5     | -0.219 | 0.089 | 0.205 |
| Dnajc6     | -0.161 | 0.129 | 0.264 |
| Dnajc7     | -0.818 | 0.064 | 0.168 |
| Dnajc8     | -0.592 | 0.005 | 0.041 |
| Dnajc9     | -0.531 | 0.021 | 0.084 |
| Edem1      | 0.967  | 0.037 | 0.119 |
| Edem2      | 1.222  | 0.012 | 0.061 |
| Edem3      | 0.851  | 0.028 | 0.100 |
| Eif2ak3    | 0.760  | 0.013 | 0.066 |
| Ern1       | 0.791  | 0.173 | 0.325 |
| Ern2       | 1.128  | 0.100 | 0.222 |
| Hsp90aa1   | -0.631 | 0.006 | 0.042 |
| Hsp90ab1   | -0.640 | 0.002 | 0.028 |

|           |        |       |       |
|-----------|--------|-------|-------|
| Hsp90b1   | 0.396  | 0.067 | 0.172 |
| Hspa12a   | -4.368 | 0.001 | 0.018 |
| Hspa12b   | -0.161 | 0.379 | 0.550 |
| Hspa13    | 0.672  | 0.222 | 0.375 |
| Hspa14    | -0.191 | 0.542 | 0.688 |
| Hspa1a    | -0.585 | 0.001 | 0.022 |
| Hspa1b    | -1.093 | 0.003 | 0.031 |
| Hspa1l    | -1.167 | 0.008 | 0.051 |
| Hspa2     | -0.585 | 0.010 | 0.055 |
| Hspa4     | -0.520 | 0.009 | 0.052 |
| Hspa4l    | -0.541 | 0.056 | 0.155 |
| Hspa5     | 1.324  | 0.098 | 0.218 |
| Hspa8     | -1.056 | 0.001 | 0.021 |
| Hspa9     | -1.059 | 0.050 | 0.144 |
| Hspa9-ps1 | -0.493 | 0.011 | 0.059 |
| Hspb1     | -0.653 | 0.010 | 0.055 |
| Hspb11    | -0.252 | 0.201 | 0.353 |
| Hspb2     | 1.610  | 0.075 | 0.183 |
| Hspb6     | -1.581 | 0.082 | 0.194 |
| Hspb7     | -0.421 | 0.039 | 0.123 |
| Hspb8     | -0.413 | 0.012 | 0.060 |
| Hspbap1   | -0.484 | 0.030 | 0.103 |
| Hspbp1    | -0.629 | 0.025 | 0.094 |
| Hspd1     | -0.596 | 0.003 | 0.031 |
| Hspd1-ps3 | -0.694 | 0.067 | 0.172 |
| Hspd1-ps4 | -0.157 | 0.855 | 0.919 |
| Hspe1     | -1.561 | 0.001 | 0.021 |
| Hspg2     | -0.785 | 0.200 | 0.351 |
| Hsph1     | -1.289 | 0.004 | 0.036 |
| Nfe2l2    | -0.885 | 0.103 | 0.226 |
| Pdia2     | 1.944  | 0.047 | 0.138 |
| Pdia3     | 0.308  | 0.158 | 0.306 |
| Pdia4     | 1.968  | 0.024 | 0.092 |
| Pdia5     | -0.267 | 0.109 | 0.236 |
| Pdia6     | 0.269  | 0.007 | 0.048 |
| Sod1      | 1.229  | 0.074 | 0.182 |
| Sod2      | -0.888 | 0.010 | 0.057 |
| Sod3      | 3.945  | 0.023 | 0.088 |
| Txnip     | 2.761  | 0.000 | 0.010 |
| Xbp1      | -1.187 | 0.146 | 0.289 |

**Table S5. Related to Figure 6.** Trends of gene-expression change in a pseudotime order from human functional  $\beta$  to non-functional  $\beta$  cells.

| Beta cell T2D trajectory |         |              |                |                |              |
|--------------------------|---------|--------------|----------------|----------------|--------------|
|                          | Gene    | Slope        | adjusted.pvalu | Direction      |              |
| MYT TFs                  | ST18    | -0.009552881 | 0.000689667    | Down-regulated |              |
|                          | HSP90B1 | 0.099623923  | 0.000701885    | Up-regulated   |              |
| HSPs                     | HSPA5   | 0.078881827  | 7.89653E-07    | Up-regulated   |              |
|                          | HSPA8   | 0.046666698  | 0.000372293    | Up-regulated   |              |
|                          | HSPB1   | 0.025065974  | 0.006448528    | Up-regulated   |              |
|                          | HSPA9   | 0.016106986  | 0.00600379     | Up-regulated   |              |
|                          | HSPE1   | -0.020456345 | 0.000104408    | Down-regulated |              |
|                          | DNAJC12 | 0.02720265   | 0.002680748    | Up-regulated   |              |
|                          | DNAJC1  | 0.024054916  | 0.000252719    | Up-regulated   |              |
|                          | DNAJC10 | 0.016418118  | 0.005643945    | Up-regulated   |              |
|                          | DNAJC2  | 0.0120836    | 0.005010186    | Up-regulated   |              |
|                          | DNAJB9  | 0.01018867   | 0.00092521     | Up-regulated   |              |
| DNAJs                    | DNAJB6  | 0.009346537  | 0.000522079    | Up-regulated   |              |
|                          | DNAJB2  | 0.008060908  | 0.001535664    | Up-regulated   |              |
|                          | DNAJC9  | 0.007839499  | 0.00116952     | Up-regulated   |              |
|                          | DNAJC22 | 0.007742143  | 0.008308862    | Up-regulated   |              |
|                          | DNAJA1  | -0.032440054 | 0.000825183    | Down-regulated |              |
|                          | DNAJC8  | -0.031413516 | 1.10178E-07    | Down-regulated |              |
|                          | ATFs    | ATF4         | 0.02233869     | 0.000429695    | Up-regulated |
|                          |         | ATF6         | 0.009440338    | 0.007479231    | Up-regulated |
|                          |         | ATF3         | 0.0082767      | 0.004181079    | Up-regulated |