Cell Reports, Volume *41*

Supplemental information

Glycemic control releases regenerative potential of pancreatic beta cells blocked by severe hyperglycemia

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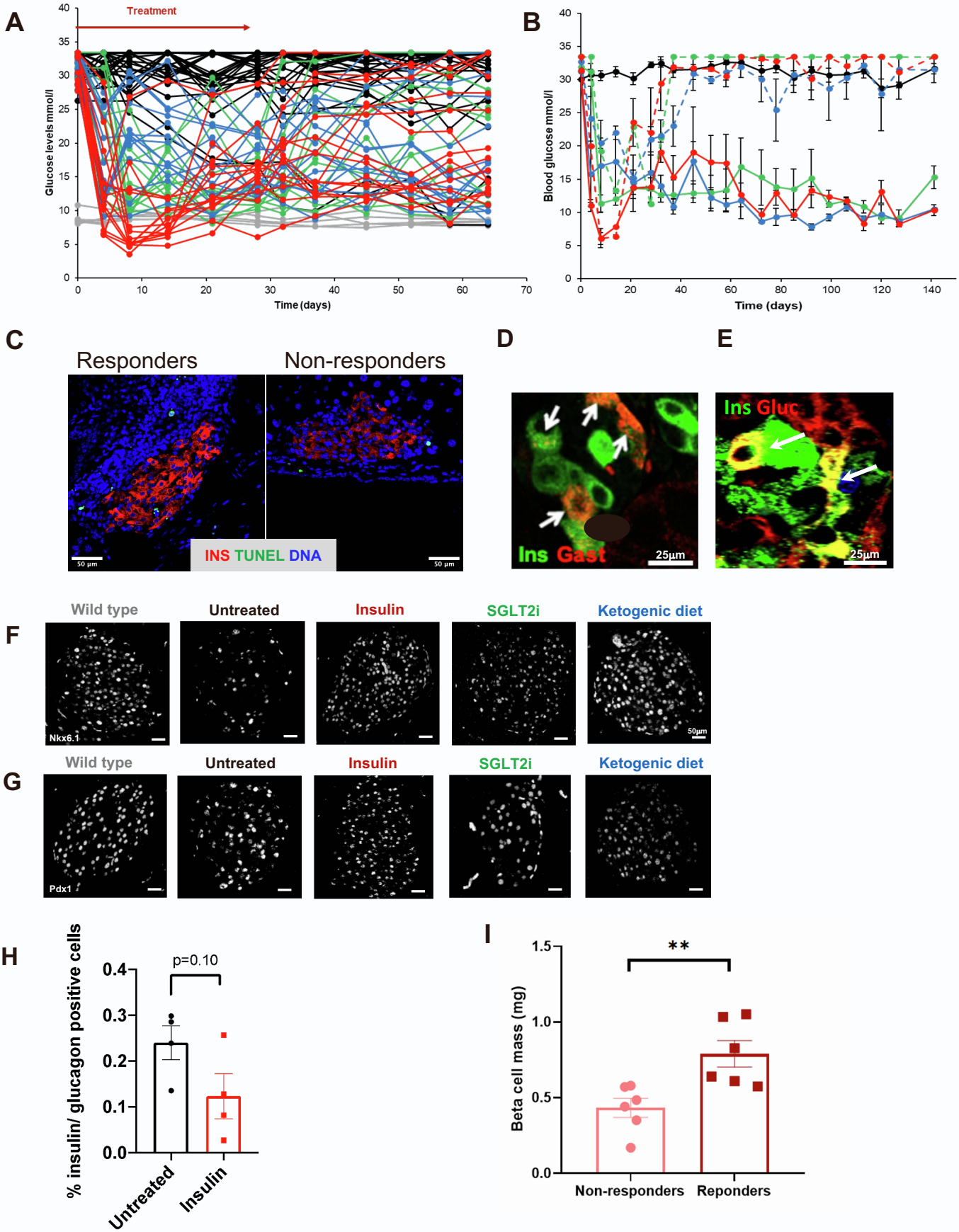
Supplementary Figure S1: Extreme hyperglycemia results in reversible failure to recover, associated with decreased expression of Nkx6.1 and appearance of bihormonal islet cells.



Supplemental Figure 1:

A. Individual mouse data during treatment and follow-up. Glucose levels are shown for WT (gray n=5) and untreated beta cell ablated (black n=32) mice as well as mice treated with insulin pellets (red), SGLT2 inhibitor (green) and ketogenic diet (blue).

B. A different cohort of mice treated as in panel A, but followed for an additional 5 months demonstrating that responders maintained near-normal glucose levels whereas non-responders returned to and remained at pre-treatment glycemic levels. Untreated beta cell ablated mice (n=9), mice treated with insulin pellets (responders=2 non-responders=2), SGLT2 inhibitor (responders=3 non-responders=1) and ketogenic diet (responders=3 non-responders=2). Color coding as in panel A.

C. TUNEL immunostaining for responders and non-responder groups of mice was detected in less than 0.1% of the cells (TUNEL-green, Insulin-red, DNA-blue).

D. Representative immunofluorescence image of islets co-stained for insulin (green) and gastrin (red) in untreated mice. Bi-hormonal cells expressing both insulin and gastrin are marked by arrows. In wild type mice, bi-hormonal cells were not detected.

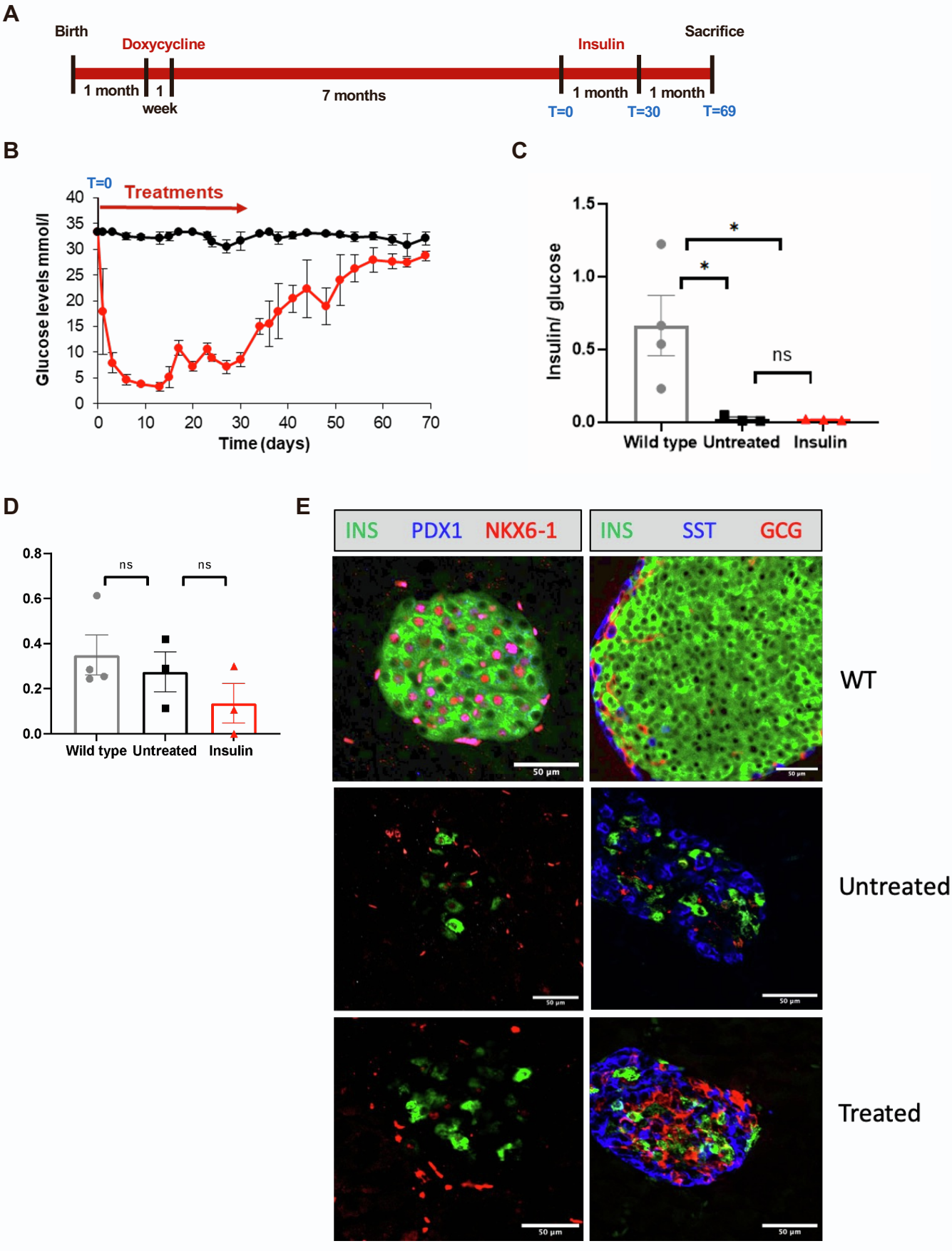
E. Representative immunofluorescence image of islets co-stained for insulin (green) and glucagon (red) in untreated mice. Bi-hormonal cells expressing both insulin and glucagon are marked by arrows.

F.G Representative immunofluorescence image of islets stained for NKX6.1 (**F**) and PDX1 (**G**) in WT, untreated and treated mice, showing a decrease in NKX6.1 but not PDX1 levels in untreated mice, which returned to normal after treatments to restore normoglycemia.

H. Percentage of bi-hormonal cells expressing both insulin and glucagon, 2 month after beginning of treatment (n=4 for each group).

I. Morphometric assessment of beta cell mass immediately upon completion of treatment in animals with blood glucose levels below 16 mmol/l at 3 weeks (responder group) compared to those with glucose levels >16 mmol/l at 3 weeks (non-responders group). Graph represents the fraction of pancreas tissue area stained for insulin multiplied by pancreas weight. Each bar represents the mean± SE of 6 responder and 6 non-responder mice.

Supplementary Figure S2: The ability to recover from severe hyperglycemia decreases with duration of hyperglycemia.



S2- The ability to recover from severe hyperglycemia decreases with duration of hyperglycemia.

A. Study design: One month old insulin-rtTA;TET-DTA mice were treated with doxycycline in the drinking water for 1 week and followed for 7 months. After 7 months, mice with glucose levels >30 mmol/l were divided into two groups: one treated with insulin pellets for 1 month and the other an untreated control group. One month after completion of treatment the mice were sacrificed. Data represent the mean of 3 mice per each group ± SE

B. Glucose levels in insulin treated (red) and untreated (black). After termination of treatment, glucose levels increased to pre-treatment levels.

C. Insulin/glucose ratio levels (ratio x 100) indicate that there no improvement of beta cell function. Each bar represents the mean of 3 mice per each group ± SE.

D. Quantification of beta cell proliferation (number Ki67-positive, insulin positive cells/ insulin positive cells x100). Data represent the mean of 3 mice per each group ± SE.

E. Left column: immunostaining for insulin (green), pdx1 (blue) and nkx6.1 (red) in wild type, treated and untreated mice following 7 months of hyperglycemia and 1 month if insulin treatment. Right column: immunostaining for insulin (green) somatostatin (blue) and glucagon (red) in the same mice as in the left column.