Inhibition of mTORC1 by ER stress impairs neonatal β-cell expansion and predisposes to diabetes in the Akita mouse

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Figure 1. β-Cell mass, turnover and differentiation in adult Akita mice. Analyses were performed on 2- to 3-month-old Akita mice and age-matched controls. (a) β-cell mass (n = 6 in each group); (b) β-cell proliferation and apoptosis assessed by staining for insulin and Ki67 (n = 6–7 mice in each group; a total of 4909 wild type (WT) and 2523 Akita β-cells were quantified) or TUNEL (n = 4–5 mice in each group; 2592 WT and 1754 Akita β-cells). The percentage of Ki67+ and TUNEL+ β-cells is shown in the table above; (c–d) β-cell differentiation was assessed by lineage tracing. Wild-type and Akita mice were crossed with RIP-Cre:Rosa26-Yfp reporter mice; (c) pancreatic sections of Akita mice were immunostained for insulin and somatostatin or glucagon. Lineage-traced β-cells (YFP+) expressing somatostatin or glucagon is shown in squares and zoomed in; (d) quantification of insulin-expressing β-cells (percentage of insulin+/YFP+ cells), insulin-degranulated β-cells (percentage of insulin-/YFP+ cells) and of cells with misexpression of somatostatin or glucagon (percentage of somatostatin+/YFP+ or glucagon+/YFP+ cells) in WT and Akita mice is shown; *p<0.05.

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**Figure 1—figure supplement 1.** Glycemia and β-cell function in adult Akita and control mice. (a) Fed blood glucose (n = 11–15 in each group); (b) IPGTT- glucose (1.5 g/kg) was injected intraperitoneally after an overnight or 4 hr fast (n = 4–5 in each group); (c) pancreatic insulin content analyzed by ELISA on whole pancreas extracts (n = 4–5 in each group). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

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Figure 1—figure supplement 2. NKX6.1 and PDX-1 expression in adult Akitaβ-cells. Pancreatic sections of 2- to 3-month-old Akita and control mice were stained for NKX6.1 (n = 5 mice in each group; 3348 WT and 2370 Akitaβ-cells) or PDX-1 and insulin (n = 5–7 mice in each group; 4580 WT and 2320 Akitaβ-cells). Values are mean ± SE. **p<0.01, ****p<0.0001.

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Figure 2. Dynamic changes of body and pancreas growth and glycemia, β-cell mass, proliferation and differentiation in Akita and control mice at P1-2. (a) body weight, (b) pancreas weight of wild-type and Akita mice at P1-2, P19-21 and at the age of 2–3 months. (a) P1-2: WT (n = 8); Akita mice (n = 4), P19-21: WT (n = 21), Akita mice (n = 23), 2–3 months: WT (n = 33); Akita mice (n = 39). (b) P1-2: WT (n = 8), Akita mice (n = 4), P19-21: n = 14 in each group, 2–3 months: n = 17 mice in each group. (c) Fed blood glucose (n = 7–8 mice in each group); (d) β-cell mass (n = 5 mice in each group); (e) β-cell proliferation assessed by immunostaining for insulin and Ki67 (n = 4 mice in each group; 1886 WT and 1483 Akita β-cells). The percentage of Ki67⁺ β-cells is shown in the table above; (f–g) quantification of β-cells (insulin⁺) expressing NKX6.1 (n = 3–4 mice in each group; 1148 WT and 1808 Akita β-cells) and PDX-1 (n = 3–5 mice in each group; 1364 WT and 1507 Akita β-cells). *p<0.05, ****p<0.0001.

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Figure 3. Metabolic state, β-cell function and mass in pre-weaning (P19-21) Akita mice and age-matched controls. (a) fed blood glucose (n = 7 in each group); (b) IPGTT- glucose (1.5 g/kg) was injected intraperitoneally after an overnight fast (n = 5 in each group); (c) glucose-stimulated insulin secretion in vivo. Insulin was measured before and 15 min following IP glucose injection (1.5 g/kg); (d) pancreatic insulin content (n = 4–5 in each group); (e) basal (3.3 mmol/L glucose) and stimulated (16.7 mmol/L glucose) insulin secretion and insulin content of Akita and control islets analyzed by static incubations. Islets were divided into 4 batches of 25 islets per group (n = 3); (f) β-cell mass (n = 6 mice in each group). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. DOI: https://doi.org/10.7554/eLife.38472.007
Figure 4. Dynamic changes in β-cell expansion in Akita and control mice. (a) β-cell proliferation assessed by immunostaining for insulin and Ki67 (n = 6 mice in each group; 2541 WT and 3391 Akita β-cells); (b) β-cell size at P1-2 (newborn, n = 4–5 mice in each group; 334 WT and 435 Akita β-cells), P19-21 (pre-weaning, n = 3 mice in each group; 330 WT and 364 Akita β-cells) and in adult mice (2–3 month-old, n = 3 mice in each group; 266 WT and 417 Akita β-cells) assessed by immunostaining for E-cadherin and insulin. Quantifications of β-cell size (c), proliferation (d), and mass (e) are shown. *p<0.05, **p<0.01, ****p<0.0001. DOI: https://doi.org/10.7554/eLife.38472.008
Figure 4—figure supplement 1. Proliferation of β-cells and exocrine cells in pre-weaning Akita and control mice. Analyses were performed on pancreatic sections of Akita mice and age-matched controls at 19–21 days stained for proliferation markers. (a) β-Cell proliferation was assessed by staining for insulin and PCNA (n = 3 mice in each group; 1241 WT and 1944 Akita β cells), or phospho-Histone H3 (H3P n = 3 mice in each group; 1176 WT and 1982 Akita β–cells). (b) Proliferation of pancreatic exocrine cells in pre-weaning Akita and control mice. Pancreatic sections of pre-weaning Akita and control mice were stained for insulin and Ki67 and exocrine cells surrounding the islets were used for quantification. The percentage of proliferating exocrine cells (Ki67+/INS−) is shown (n = 6 mice in each group; 2486 WT and 2670 Akita cells). *p<0.05, **p<0.01.
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**Figure 5.** Transcriptomic analysis of ER stress markers and β-cell gene signature in neonate Akita islets. (a) RNA-seq comparing the transcriptome of islets from P19-21 Akita and age-matched control mice (n = 3 samples in each group, each sample is a pool of islets from three mice). Columns represent pathways that are differentially regulated in Akita mice; (b) expression of UPR and apoptosis genes and of Nkx6.1 and Pdx1 in islets of Akita compared to control mice at P19-21. Spliced and total Xbp1 were also quantified by qPCR. The spliced/total Xbp1 ratio is shown beside (n = 3); (c–d) heat map of genes regulated by NKX6.1 (c) and PDX-1 (d) in Akita islets and controls. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

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Figure 5—figure supplement 1. Islet composition of wild-type and Akita mice. β/α cell ratio and number of β and α cells per islet area in P19-21 wild-type and Akita mice (n = 44 and 35 islets isolated from three to four mice in each group).

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Figure 6. Effects of ER stress on the expression of β-cell transcription factors in neonate Akita islets (P19-21) and islets treated with thapsigargin: (a) PDX-1 and BiP protein level analyzed by Western blotting (n = 3, each sample is a pool of islets from four to six mice); (b) quantification of NKX6.1 (n = 3 mice in each group; 1646 WT and 728 Akita β-cells), and PDX-1 (n = 3 mice in each group; 1534 WT and 844 Akita β-cells) expressing β-cells. Pancreatic sections were immunostained for NKX6.1 or PDX-1 and insulin. The percentage of NKX6.1- and PDX-1-positive β-cells is shown. (c) Islets from young (P19-21) and adult wild-type mice were treated with low-dose thapsigargin (50 nmol/l) and TUDCA (250 µmol/l) or PBA (2.5 mmol/l) for 48 hr with daily media changes and further analyzed by western blotting for PDX-1 and BiP (n = 3, each sample is a pool of islets from six to nine mice); (d) INS-1E cells were treated with 20 nmol/l thapsigargin for 24 and 48 hr followed by western blotting for PDX-1. **p<0.01, ***p<0.001, ****p<0.0001. DOI: https://doi.org/10.7554/eLife.38472.012
Figure 7. Effects of ER stress on IRS2/Akt signaling in Akita islets and in INS-1E treated with low-dose thapsigargin. (a) IRS2/Akt signaling in islets from neonate (P19-21) and adult wild-type and Akita mice. Each sample is a pool of islets from 4 to 15 mice (n = 4 for neonate islets and n = 2 for adult islets). (b–c) INS-1E cells were treated with 20 nmol/l thapsigargin for 24 and 48 hr followed by western blotting for IRS2, total and phosphorylated Akt (Ser473 and Thr308) and S6 (Ser240/244). A representative experiment (b) and quantification (c) are shown (n = 4–6). *p<0.05.

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**Figure 8.** mTORC1 signaling in neonate and adult Akita islets. (a) Western blot analysis of S6 and 4EBP1 phosphorylation in islets of neonate (P19-21) and adult wild-type and Akita mice. Quantification of phosphorylated S6 in neonate Akita compared to control islets is shown (n = 3, each sample is a pool of islets from 4 to 7 mice); (b) immunostaining for phospho-S6 on pancreatic sections of P1-2, P19-21 and adult Akita mice and age-matched controls and quantifications of the percentage of S6⁺ β-cells (P1-2: n = 4 mice in each group; 1159 WT and 1655 Akita β—cells; P19-21: n = 6 mice in each group; 2259 WT and 1567 Akita β—cells; adult: n = 4–5 mice in each group; 2391 WT and 1383 Akita β—cells). Islet boundaries are marked by dotted line; (c) adult Akita mice were treated with 25 mg/kg dapagliflozin in drinking water for 72 hr. Blood glucose in dapagliflozin-treated Akita mice was ~200 mg/dl compared to ~500 mg/dl in control Akita mice. Pancreatic sections were immunostained for insulin and phospho-S6 (n = 3 mice in each group). *p<0.05, **p<0.01, ****p<0.0001.

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Figure 8—figure supplement 1. Effects of chemical chaperones on mTORC1 activity in neonate Akita islets and controls. (a) Islets of P16-19 Akita and wild-type (WT) mice were treated with 250 μmol/l TUDCA or 2.5 mmol/l PBA for 48 hr followed by western blotting for BiP and phosphorylated S6 (n = 3, each sample is a pool of islets from 4 to 9 mice); (b) effects of TUDCA on β-cell proliferation in neonate Akita mice (P18-20). TUDCA (1 mg/kg) was injected IP twice daily for 48 hr followed by immunostaining of pancreatic sections for Ki67 and insulin (n = 4-6 mice in each group; 1403 control Akita and 2183 TUDCA-treated Akitaβ-cells). **p<0.01.
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Figure 9. Effects of mTORC1 activation in neonate Akita β-cells on β-cell size and proliferation. Studies were performed on heterozygous and homozygous βTsc1 knockout Akita mice (RIP-Cre:Tsc1floxflox:Akita (Akita, βTsc1+/−) and RIP-Cre:Tsc1floxflox:Akita (Akita, βTsc1−/−)). Tsc1floxflox:Akita and Tsc1floxflox:Akita were used as Akita controls. RIP-Cre:Tsc1floxflox:Akita, βTsc1+/− and RIP-Cre:Tsc1floxflox:Akita, βTsc1−/− were used as WT controls (a, b).

(a) Western blotting for phospho-S6 on islets from homozygous and heterozygous knockout mice and matched controls (n = 4, each sample is a pool of islets from two to four mice); (b) Western blotting and quantification of BiP expression in wild-type, Akita and Akita, βTsc1+/− mice (n = 4, each sample is a pool of islets from two to four mice); (c) β-cell size was assessed by immunostaining for insulin and E-cadherin (n = 400–500 β-cells per group); (d) β-cell proliferation was assessed by immunostaining for insulin and Ki67 (n = 1200–1400 β-cells per group). Quantifications and representative images are shown. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

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Figure 9—figure supplement 1. Metabolic characterization of RIP-Cre mouse. (a) IPGTT: glucose (1.5 gr/kg) was injected after an overnight fast to adult RIP-Cre and non-transgenic control mice (n = 3); (b) fed blood glucose of adult RIP-Cre mice and RIP-Cre:Akita mice compared to non-transgenic controls (n = 6–8); (c) insulin tolerance test on Akita and RIP-Cre:Akita mice (n = 3). *p<0.05.

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**Figure 10.** Effects of mTORC1 activation in neonate Akita β-cells on diabetes. (a–b) IPGTT at P30-35: glucose (1 g/kg) was injected IP after an overnight fast; (a) heterozygous Tsc1 knockout Akita mice (RIP-Cre:Tsc1^{flox/+};Akita (Akita, βTsc1^{+/+}) and matched controls: Tsc1^{flox/+} mice (Tsc1^{+/+}), RIP-Cre: Tsc1^{flox/+} mice (βTsc1^{+/+}), and Tsc1^{flox/+}:Akita (Akita, βTsc1^{+/+}) (n = 3–5 mice in each group); (b) homozygous Tsc1 knockout Akita mice (RIP-Cre:Tsc1^{flox/flox}:Akita (Akita, βTsc1^{+/+}) and matched controls: Tsc1^{flox/flox} mice (Tsc1^{+/+}), RIP-Cre:Tsc1^{flox/flox}:Akita (Akita, βTsc1^{+/+}), and Tsc1^{flox/flox}:Akita (Akita) (n = 3–5 in each group); (c–d) pancreatic insulin content of heterozygous and homozygous Tsc1 knockout Akita mice and matched controls at P30-35 (WT (n = 7), Akita (n = 11), Akita, βTsc1^{+/+} (n = 3) and Akita, βTsc1^{+/+} (n = 4); (e) islet insulin content. (f–g) Effects of mTORC1 activation in neonate Akita β-cells on insulin secretion in vivo and ex vivo. (f) insulin secretion in response to IP glucose injection (n = 6 mice in each group); (g) islets were isolated from Tsc1^{flox/+} WT mice (WT), Tsc1^{flox/+}:Akita (Akita) and RIP-Cre:Tsc1^{flox/+}:Akita (Akita, βTsc1^{+/+}) mice and insulin secretion assessed following static incubations at basal (3.3 mmol/l) and stimulated (16.7 mmol/l) glucose. (h) a model of the pathophysiology of permanent neonatal diabetes. *p<0.05, ***, p<0.001, ****, p<0.0001.

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Figure 10—figure supplement 1. Effects of mTORC1 activation in Akitaβ-cells on PDX-1 (a, b) and NKX6.1 expression (c, d). Heterozygous (a, c) and homozygous (b, d) βTsc1 knockout Akita and age-matched controls were sacrificed at P18. Pancreatic sections were stained for insulin and PDX-1 (Akita (n = 1760 β-cells); Akita, bTsc1+/- (n = 814 β-cells); Akita, bTsc1-/- (n = 1458 β-cells)) or NKX6.1 (Akita (n = 1500 β-cells); Akita, bTsc1+/- (n = 1438 β-cells); Akita, bTsc1-/- (n = 1584 β-cells)).

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Figure 10—figure supplement 2. Fed blood glucose of Tsc1^{flox/+} mice (WT), Tsc1^{flox/+}:Akita (Akita) and heterozygous Tsc1 knockout RIP-Cre:Tsc1^{flox/+}:Akita (Akita, b Tsc1^{+/−}) mice at the age of 2–3 months. Blood glucose levels are the mean of the last three consecutive glucose measurements.

**p<0.01, ***p<0.001.

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