Cell Research (2014) 24:1137-1140.

www.nature.com/cr

LETTER TO THE EDITOR

The IRE1α-XBP1 pathway regulates metabolic stress-induced compensatory proliferation of pancreatic β-cells

Cell Research (2014) **24**:1137-1140. doi:10.1038/cr.2014.55; published online 6 May 2014

Dear Editor,

In eukaryotes, increased protein folding demand at the endoplasmic reticulum (ER) activates the unfolded protein response (UPR) [1], which plays a pivotal role in control of cellular functions and survival under ER stress [2]. Chronic ER stress is thought to contribute to the pathogenic progression of diabetes [3, 4]. Inositol-requiring enzyme 1 (IRE1), an ER-resident transmembrane Ser/Thr protein kinase and endoribonuclease, is the most conserved ER stress sensor that mediates a key branch of the UPR [1]. In mammals, activation of IRE1α results in non-conventional splicing of the mRNA encoding the transcription factor X-box binding protein 1 (XBP1), generating a spliced active form of XBP1 (XBP1s) to initiate a major UPR program [1]. The IRE1-XBP1 pathway has been implicated in the homeostatic regulation of pancreatic islet β-cells. Whereas glucose-stimulated IRE1α activation is coupled to insulin production [5-7], IRE1α also degrades insulin mRNAs under severe ER stress conditions [8]. Interestingly, genetic deletion of XBP1 in β-cells of mice was reported to result in a feedback hyperactivation of IRE1α, causing defective proinsulin processing and insulin secretion [9]. However, the precise role *in vivo* of IRE1α in integrating metabolic ER stress signals to regulate β-cell functions remains largely elusive.

To investigate the metabolic actions of IRE1α in β-cells, we generated mice (denoted *Ire1α$^{f/f}$:Cre*) with specific Ire1α deletion in β-cells by intercrossing *Ire1α$^{f/f}$* mice [10] with RIP-*Cre* transgenic mice (denoted *Cre*) that express Cre recombinase under the control of the rat insulin II promoter. Because no significant differences were observed in body weight and fed blood glucose levels between wild-type and *Cre* littermates (Supplementary information, Figure S1A), we used *Cre* and *Ire1α$^{f/f}$* mice as the control. In *Ire1α$^{f/f}$:Cre* mice, IRE1α protein levels were substantially decreased in primary islets (by ~75%) and hypothalamus (by ~50%) as compared to their *Ire1α$^{f/f}$:Cre* or *Cre* counterparts, while no distinguishable changes were observed in their livers (Supplementary information, Figure S1B). This indicates the presence of IRE1α protein in other islet cells than β-cells, as well as deletion of *Ire1α* in RIP-Cre neurons within the hypothalamus. When maintained on a normal chow (NC) diet, fasted male *Ire1α$^{f/f}$:Cre* animals exhibited significantly elevated glucose levels and decreased serum insulin levels relative to control mice (Figure 1A), despite similar body weight and daily food intake (Supplementary information, Figure S1C). Glucose and insulin tolerance tests revealed impaired glucose clearance (Figure 1B) but unaltered effectiveness of insulin in lowering blood glucose (Supplementary information, Figure S1D) in *Ire1α$^{f/f}$:Cre* mice. Similarly, female *Ire1α$^{f/f}$:Cre* mice also showed hyperglycemia and glucose intolerance without changes in body weight (Supplementary information, Figure S1E and S1F). These data implied a more predominant metabolic role for IRE1α in β-cells rather than in hypothalamus under these experimental conditions. We then examined the islet morphology from pancreatic sections of *Ire1α$^{f/f}$:Cre* and *Cre* mice. Unlike the reported β-cell-specific XBP1-deficient mice showing disorganization of islet structure with loss of β-cells [9], *Ire1α$^{f/f}$:Cre* mice did not exhibit alterations in pancreatic architecture or islet morphology (Supplementary information, Figure S1G). Moreover, they displayed similar abundance of β-cells (Supplementary information, Figure S1G), normal intracellular localization pattern of Glut2 glucose transporter protein (Supplementary information, Figure S1G), comparable islet mass and density (Supplementary information, Figure S1H), and unaltered cellular proliferation as determined by Ki67 immunostaining (Supplementary information, Figure S1I). However, primary *Ire1α$^{f/f}$:Cre* islets had significantly decreased insulin content (by ~35%; Supplementary information, Figure S1J) and reduced capacity for glucose-stimulated insulin secretion (by ~36%; Supplementary information, Figure S1K). As the islets of NC-fed *Ire1α$^{f/f}$:Cre* mice showed significantly reduced Xbp1 mRNA splicing, we wondered whether this is coupled to the expression of cyclins D1, D2 and A1, the critical cell cycle regulators either implicated in β-cell proliferation/expansion [11, 12] or regulated by

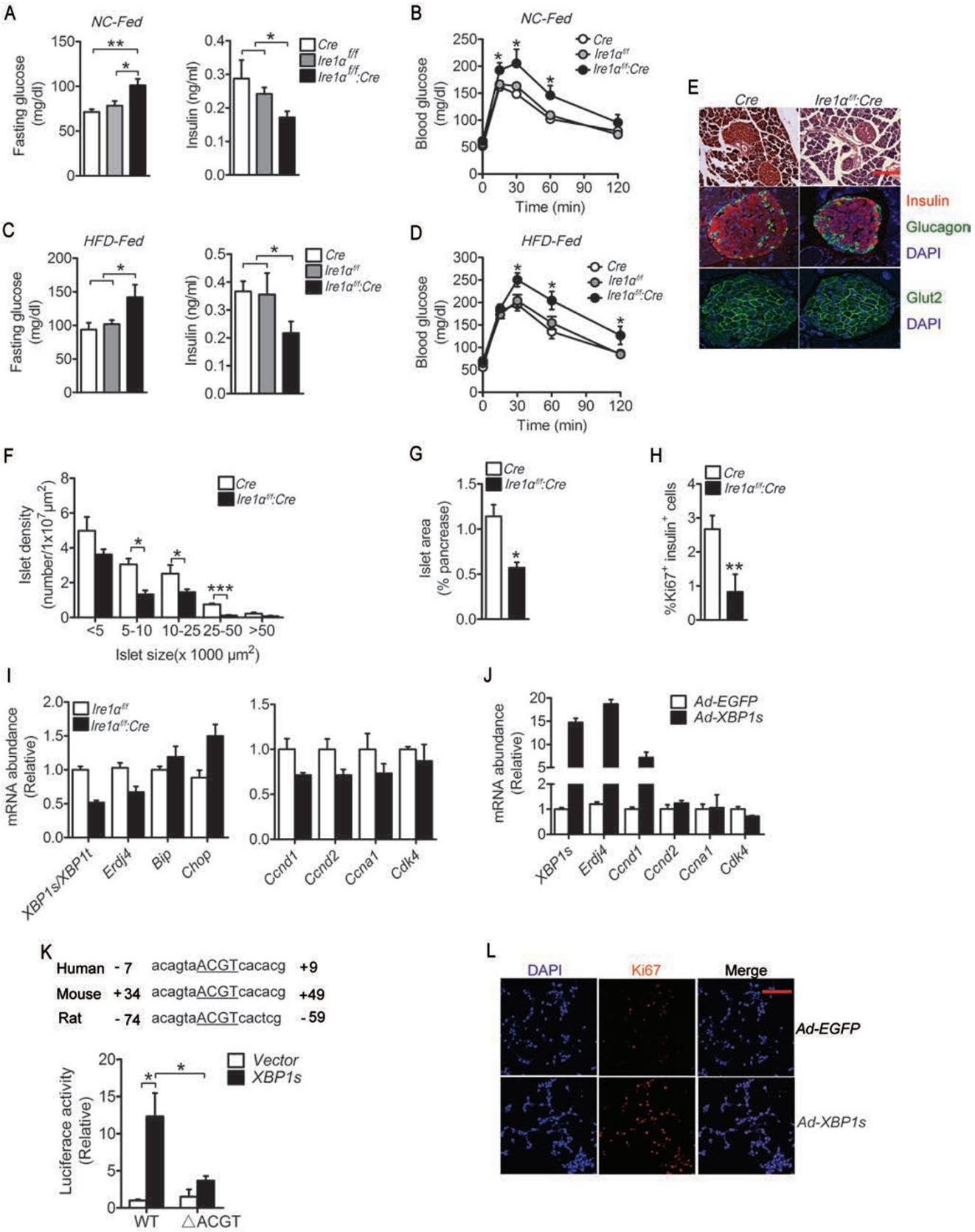
A
NC-Fed
Fasting glucose (mg/dl)
Insulin (ng/ml)
Cre
Ire1a f/f
Ire1a f/f:Cre
B
NC-Fed
Blood glucose (mg/dl)
Time (min)
C
HFD-Fed
D
HFD-Fed
E
Cre
Ire1α f/f:Cre
Insulin
Glucagon
DAPI
Glut2
DAPI
F
Islet density (number/1x10^7 μm^2)
Islet size(x 1000 μm^2)
<5
5-10
10-25
25-50
>50
G
Islet area (% pancrease)
H
%Ki67+ insulin+ cells
I
mRNA abundance (Relative)
Ire1a f/f
Ire1a f/f:Cre
XBP1s/XBP1t
Erdj4
Bip
Chop
Ccnd1
Ccnd2
Ccna1
Cdk4
J
Ad-EGFP
Ad-XBP1s
XBP1s
K
Human -7 acagtaACGTcacacg +9
Mouse +34 acagtaACGTcacacg +49
Rat -74 acagtaACGTcactcg -59
Luciferace activity (Relative)
Vector
XBP1s
WT
△ACGT
L
DAPI
Ki67
Merge
Ad-EGFP
Ad-XBP1s

Figure 1 Ablation of *Ire1α* in β-cells disrupts glucose homeostasis and impairs HFD-induced compensatory proliferation of β-cells. **(A**, **B)** Male *Ire1α*$^{f/f}$, *Cre* and *Ire1α*$^{f/f}$*:Cre* mice were maintained on a normal chow (NC) diet (*n* = 5-6 per genotype). Blood glucose and serum insulin levels were measured after a 6-hours fast in mice at 6 and 9 weeks of age, respectively **(A)**. Glucose tolerance tests. Blood glucose was determined for mice at 10 weeks of age at the indicated time points after i.p. injection of 1 g/kg glucose **(B)**. Results are presented as means ± SEM. **P* < 0.05, ***P* < 0.01 by one-way ANOVA. **(C**, **D)** Male mice of the indicated genotype at 12 weeks of age (n = 5-6 per genotype) were challenged with a high-fat diet (HFD) for 8 or 10 weeks. Blood glucose and serum insulin levels after a 6-hours fast **(C)**. Glucose tolerance tests **(D)**. Results are shown as means ± SEM. **P* < 0.05 by one-way ANOVA. **(E-H)** Male *Cre* and *Ire1α*$^{f/f}$*:Cre* mice were fed HFD for 10 weeks (*n* = 4 per genotype). Representative images of hematoxylin and eosin (HE) staining (the scale bar, 200 μm) and immunohistochemical staining of pancreatic sections using antibodies against insulin (red), glucagon (green) and Glut2 (green). Nuclei were visualized by DAPI staining **(E)**. Distribution of islets of various sizes, shown as the average number of islets per 1 × 10^7 μm^2 of pancreas area **(F)**. Islet areas shown as the percentage of total pancreas areas examined **(G)**. β-cell proliferation was estimated by quantification of Ki67-positive β-cells in pancreas. Shown are percentage ratios of insulin and Ki67 double-positive cells to insulin-positive cells **(H)**. Data are presented as means ± SEM (10 sections per mouse, *n* = 3 mice). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by unpaired two-tailed Student's *t*-test. **(I)** Primary islets were pooled from male mice fed HFD for 10 weeks. Quantitative RT-PCR analyses of Xbp1 mRNA splicing, shown as spliced (s) relative to total (t), and the mRNA abundance of genes encoding ERdj4, BiP and CHOP, as well as cyclin D1/D2/A1 and CDK4. 18S ribosomal RNA was used as the internal control. Results are shown as means ± SEM (*n* = 2 independent experiments, each using islets pooled from three mice). **(J)** Primary islets from four *Ire1α*$^{f/f}$*:Cre* mice were infected in triplicates with Ad-EGFP and Ad-XBP1s for 2 days. The mRNA abundance of the indicated genes was determined by quantitative RT-PCR using 18S ribosomal RNA as the internal control. **(K)** Sequence alignment of the putative UPR element of the *Ccnd1* promoter from human, rat and mouse. The ACGT core is indicated. Luciferase reporter assays were performed by co-transfection of 293T cells with the empty control vector or pCMV-XBP1s plasmid together with Luc constructs under the control of the human *Ccnd1* promoter (WT) or that with the ACGT core deleted (ΔACGT). Results are from three independent experiments. Data are shown as means ± SEM. **P* < 0.05 by one-way ANOVA. **(L)** Representative images for DAPI staining or Ki67 immunostaining of INS-1 cells infected for 48 h with Ad-XBP1s or Ad-EGFP (the scale bar, 100 μm).

XBP1 in prostate cancer cell lines [13]. Notably, we only detected a marginal decrease in the mRNA abundance of *Ccnd1* (Supplementary information, Figure S1L). These data suggest that ablation of *Ire1α* in β-cells caused impairment of insulin production, resulting in hyperglycemia, hypoinsulinemia and glucose intolerance. This supports a *in vivo* role for IRE1α in modulating insulin biosynthesis, consistent with findings in cultured β-cell lines [6, 7]. In the absence of overt ER stress in NC-fed mice, IRE1α deficiency does not affect islet growth or survival, in accordance with a reported conditional Ire1α knockout mouse model [5]. Notably, NC-fed *Ire1α*$^{f/f}$*:Cre* mice did not exhibit disruption of pancreatic structure or dramatically decreased islet areas as XBP1-deficient mice [9]. These differences may be attributable to the unknown actions of unspliced XBP1 protein; alternatively, XBP1 deficiency-induced hyperactivation of IRE1α may exert deleterious effects on islet architecture maintenance without XBP1 to restore ER homeostasis.

Obesity and insulin resistance can elicit compensatory responses in β-cells, including augmented insulin biosynthesis and β-cell proliferation. When challenged with a high-fat diet (HFD), mice developed obesity and glucose intolerance (Supplementary information, Figure S1M). Their pancreatic islets exhibited significantly increased *Xbp1* mRNA splicing, upregulated expression of ER stress-related genes and cell cycle regulators (Supplementary information, Figure S1N), and elevated levels of BiP protein and eIF2α phosphorylation (Supplementary information, Figure S1O), indicating a state of metabolic ER stress along with compensatory proliferation. Despite comparable body weight and food intake relative to their *Ire1α*$^{f/f}$ or *Cre* counterparts (Supplementary information, Figure S1P), HFD-fed *Ire1α*$^{f/f}$*:Cre* mice had significantly elevated fasting glucose levels and reduced serum insulin levels (Figure 1C), and showed impaired glucose tolerance (Figure 1D) with unaltered insulin sensitivity (Supplementary information, Figure S1Q). Whereas analyses of pancreatic sections showed no apparent changes in islet morphology, insulin-producing β-cells or Glut2 protein localization (Figure 1E), quantitative morphometric assessments revealed greater reductions in the number of larger islets (Figure 1F), marked decreases in islet mass (Figure 1G) and total islet density (Supplementary information, Figure S1R), but insignificant changes in β-cell sizes (Supplementary information, Figure S1R) in HFD-fed *Ire1α*$^{f/f}$*:Cre* mice. This indicates that IRE1α deficiency led to defective islet expansion, most likely resulting from decreased proliferation of β-cells. Indeed, examination of islet cell proliferation in *Ire1α*$^{f/f}$*:Cre* mice showed a marked reduction in Ki67-positive cells out of insulin-producing β-cells (Figure 1H). Thus, IRE1α is likely to sense HFD-induced metabolic stress to promote the compensatory proliferation of β-cells.

We then investigated whether IRE1α exerted its proliferative effect through an XBP1-dependent mechanism under HFD-induced ER stress. Interestingly, HFD-fed *Ire1α*$^{f/f}$*:Cre* islets showed a ~50% reduction in Xbp1 mRNA splicing with considerably increased expression of Bip and Chop, which paralleled decreased expression

of *Ccnd1*, *Ccnd2* and *Ccna1* (Figure 1I). Curiously, adenoviral overexpression of XBP1s in *Ire1α$^{f/f}$:Cre* islets and INS-1 β-cells increased the expression of *Ccnd1* but not *Ccnd2*, *Ccna1* or *Cdk4* (Figure 1J and Supplementary information, Figure S1S), suggesting that *Ccnd1* is an XBP1s-regulated gene as documented in hepatoma cells [14]. Similarly, adenovirus-directed shRNA knockdown of IRE1α in INS-1 cells significantly decreased the expression of *Ccnd1*, and simultaneously restored expression of XBP1s rescued this suppressive effect of IRE1α knockdown (Supplementary information, Figure S1T). Additionally, analyses of the *Ccnd1* promoter identified a conserved putative UPR element containing the "ACGT" core sequence (Figure 1K), a potential XBP1s-binding site [15]. Co-transfection and reporter assays showed that XBP1s transactivation of the *Ccnd1* promoter required this "ACGT" core sequence (Figure 1K). Consistently, XBP1s-expressing INS-1 cells showed specifically increased protein expression of cyclin D1 (Supplementary information, Figure S1U), and significantly augmented numbers of proliferative Ki67-positive cells (by ~80%; Figure 1L and Supplementary information, S1V) or BrdU-incorporating cells (Supplementary information, Figure S1V). These results indicate that IRE1α can promote β-cell proliferation through, at least in part, XBP1s upregulation of cyclin D1, which is known to drive cells from the G1 into the S-phase of the cell cycle [11]. This links IRE1α to the cell cycle checkpoint control, which may represent a general mechanism for the UPR regulation of cellular proliferation in other cell types. It is currently unclear how the proliferative action of XBP1s is dynamically regulated, as prolonged overproduction of XBP1s was shown to be detrimental to β-cell functions [16].

Together, our findings revealed multiple actions by IRE1α in regulation of β-cell functions: not only is IRE1α involved in insulin biosynthesis, but also linked to the cell cycle machinery when coping with metabolic ER stress, thereby promoting the compensatory proliferation of β-cells in the face of obesity and insulin resistance. Under insulin resistance states, loss of the ability of β-cells to undergo compensatory proliferation/expansion exacerbates the derangement of glucose homeostasis. It remains to be further dissected *in vivo* whether IRE1α deficiency can also cause impairment of the secretory machinery of β-cells, or whether chronic, excessive ER stress can disrupt the IRE1α-XBP1s-cyclin D1 axis to hinder islet expansion, ultimately leading to β-cell loss and failure. A better understanding of the molecular components that connect the UPR signaling to the proliferative control of β-cells can shed light upon new avenues for the therapeutic treatment of type 2 diabetes.

Acknowledgments

This work was supported by grants from the Ministry of Science and Technology of China (973 Program; 2012CB524900 and 2011CB910900), the National Natural Science Foundation of China (31230036, 81321062 and 91213306) and the Chinese Academy of Sciences (The Knowledge Innovation Program No. KSCX2-EW-R-09) to YL; the National Natural Science Foundation of China (81170814) and the Ministry of Education of China for PhD program (20100131110037) to XW; and the National Natural Science Foundation of China (81200562) to LY.

Tongfu Xu[1, *], Liu Yang[2, *], Cheng Yan[2], Xiaoxia Wang[2], Ping Huang[2], Feng Zhao[2], Liyun Zhao[2, 3], Mingliang Zhang[4], Weiping Jia[4], Xiangdong Wang[1], Yong Liu[2, 5, 6]

[1]*Department of Cell Biology, Shandong University School of Medicine, 44 Wenhua Road West, Jinan, Shandong 250012, China;* [2]*Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China;* [3]*China Novartis Institutes for BioMedical Research, Shanghai 201203, China;* [4]*Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China;* [5]*School of Life Science and Technology, ShanghaiTech University, Shanghai 200031, China;* [6]*University of the Chinese Academy of Sciences, Shanghai 201203, China*

*These two authors contributed equally to this work.

Correspondence: Yong Liu[a], Xiangdong Wang[b]

[a]E-mail: liuy@sibs.ac.cn

[b]E-mail: xdongw@sdu.edu.cn

References

1 Ron D, Walter P. *Nat Rev Mol Cell Biol* 2007; **8**:519-529.
2 Hetz C. *Nat Rev Mol Cell Biol* 2012; **13**:89-102.
3 Eizirik DL, Cardozo AK, Cnop M. *Endocr Rev* 2008; **29**:42-61.
4 Back SH, Kaufman RJ. *Annu Rev Biochem* 2012; **81**:767-793.
5 Iwawaki T, Akai R, Kohno K. *PLoS One* 2010; **5**:e13052.
6 Lipson KL, Fonseca SG, Ishigaki S, *et al*. *Cell Metab* 2006; **4**:245-254.
7 Qiu YF, Mao T, Zhang YL, *et al*. *Sci Signal* 2010; **3**:ra7.
8 Pirot P, Naamane N, Libert F, *et al*. *Diabetologia* 2007; **50**:1006-1014.
9 Lee AH, Heidtman K, Hotamisligil GS, *et al*. *Proc Natl Acad Sci USA* 2011; **108**:8885-8890.
10 Shao M, Shan B, Liu Y, *et al*. *Nat Commun* 2014; **5**:3528.
11 Zhang XB, Gaspard JP, Mizukami Y, *et al*. *Diabetes* 2005; **54**:712-719.
12 Kushner JA, Ciemerych MA, Sicinska E, *et al*. *Mol Cell Biol* 2005; **25**:3752-3762.
13 Thorpe JA, Schwarze SR. *Cell Stress Chaperones* 2010; **15**:497-508.
14 Na B, Huang ZM, Wang Q, *et al*. *PLoS One* 2011; **6**:e26240.
15 Acosta-Alvear D, Zhou Y, Blais A, *et al*. *Mol Cell* 2007; **27**:53-66.
16 Allagnat F, Christulia F, Ortis F, *et al*. *Diabetologia* 2010; **53**:1120-1130.

(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)