ID proteins protect against oxidative stress by regulating the antioxidant-mitochondrial response in beta-cells

Mohammed Bensellam[1], Magdalene Montgomery[2], Jude Luzuriaga[1], Jeng Yie Chan[1] and D. Ross Laybutt[1]

From the [1]Garvan Institute of Medical Research, Sydney, Australia; and [2]University of New South Wales, Sydney, Australia

Corresponding Author:

D. Ross Laybutt

Garvan Institute of Medical Research

384 Victoria St, Darlinghurst, NSW 2010, Australia.

Tel: 61-2-9295-8228

Fax: 61-2-9295-8201

Email: r.laybutt@garvan.org.au

Running title: IDs regulate the antioxidant-mitochondrial response

Number of characters: 38430

Number of figures: 10

Abstract

Oxidative stress is implicated in beta-cell glucotoxicity in type 2 diabetes. ID proteins are transcriptional regulators induced by hyperglycemia in islets, but the mechanisms involved and their role in beta-cells are not clear. Here we show that IDs are oxidative stress-responsive genes that promote beta-cell survival. ID1-4 expression was upregulated *in vivo* in the islets of diabetic mice and stimulated *in vitro* by ribose and H_2O_2. Id1/3 inhibition reduced the expression of multiple antioxidant genes and potentiated oxidative stress-induced apoptosis. This was associated with increased intracellular H_2O_2 levels, mitochondrial fragmentation and reduced expression of TFAM and respiratory chain components. Id1/3 inhibition also reduced the expression of small Maf proteins, interacting partners of NFE2L2, master regulator of the antioxidant response. Inhibition of Maf(F/G/K) mimicked the effects of Id1/3 inhibition. These results suggest that IDs are key regulators of the adaptive antioxidant-mitochondrial response that promotes beta-cell survival under oxidative stress through a novel link to the NFE2L2-Maf pathway.

Introduction

Type 2 diabetes (T2D) is principally defined by chronic hyperglycemia that arises if pancreatic beta-cells fail to provide sufficient insulin to compensate for insulin resistance. Beta-cell failure in T2D is characterized by dysfunctional insulin secretion and a reduction in beta-cell mass, which has been linked to an increased rate of apoptosis (Sakuraba et al., 2002;Yoon et al., 2003;Deng et al., 2004;Rahier et al., 2008;Butler et al., 2003). Several lines of evidence underscore a role of chronic hyperglycemia -termed as glucotoxicity- in the increase in beta-cell apoptosis. Thus, elevated glucose concentrations trigger apoptosis in cultured islets and beta-cell lines (Ling et al., 1994;Maedler et al., 2001;Piro et al., 2002;DelGuerra et al., 2007;Hou et al., 2008;Bensellam et al., 2009;Jonas et al., 2009;McKenzie et al., 2010;Wali et al., 2014) and in animal models of T2D (Donath et al., 1999;Finegood et al., 2001;Song et al., 2008;Kluth et al., 2011).

Oxidative stress has been proposed as a central mechanism of hyperglycemia-induced beta-cell demise (Robertson and Harmon, 2006;Bensellam et al., 2012). Elevated glucose or ribose levels have been shown to increase reactive oxygen species (ROS) production in beta-cells (Tanaka et al., 2002;Bindokas et al., 2003;Sakai et al., 2003;Duprez et al., 2012;Wali et al., 2014) and islets of diabetic mice exhibit higher ROS content, mitochondrial dysfunction and oxidative damage (Ihara et al., 1999;Gorogawa et al., 2002;Bindokas et al., 2003;Nakayama et al., 2005;Lu et al., 2010).

An optimal redox environment is crucial for proper biological function. Many cell types are equipped with a battery of antioxidant defense genes to neutralize ROS and prevent macromolecular damage. The vulnerability of beta-cells to oxidative stress may owe to their low expression of several key antioxidant genes, namely glutathione peroxidase 1 (*Gpx1*), superoxide dismutase 1-2 (*Sod1-2*) and catalase (Lenzen et al., 1996;Tiedge et al., 1997). However, other genes of the antioxidant arsenal display strong expression in beta-cells [e.g. other *Gpx* isoforms, heme oxygenase 1 (*Hmox1*), sulfiredoxine 1 (*Srxn1*), peroxiredoxins (*Prdxs*), glucose-6-phosphate dehydrogenase (*G6pdx*)], or are markedly upregulated under oxidative stress conditions (Laybutt et al., 2002;Bensellam et al., 2009;Jonas et al., 2009;Lacraz et al., 2009). The signaling pathways that regulate redox status and antioxidant gene expression in beta-cells are only partially understood. Nuclear Factor E2-related factor 2 (NFE2L2 also known as NRF2) is a primary regulator of the antioxidant response and its activation has been shown to protect beta-cells against oxidative damage (Yagishita et al., 2014). NFE2L2 activation of antioxidant gene expression requires its dimerization with small Maf proteins (MafF, MafG and MafK) (Katsuoka et al., 2005;Hirotsu et al., 2012;Tanigawa et al., 2013).

The inhibitors of differentiation (ID) proteins are transcriptional regulators that play important roles in both physiology and pathology, including stem cell homeostasis and fate commitment, tumorigenesis, cancer progression and chemotherapeutic drug resistance. They have been proposed to act as repressors of basic helix-loop-helix transcription factors thereby modulating cell differentiation and proliferation (Ling et al., 2006;Lasorella et al., 2014;Nair et al., 2014). Previous reports have demonstrated that the expression of ID1 and ID3 is

induced by glucose stimulation in human islets and beta-cell lines (Webb et al., 2000;Wice et al., 2001). Further studies link the upregulation of *Id1* mRNA levels to hyperglycemia in islets of diabetic mice (Kjorholt et al., 2005) and also suggest an influence of *Id1* expression in insulin secretion (Akerfeldt and Laybutt, 2011). However, the mechanisms underlying the induction of the ID proteins by hyperglycemia and their precise role in beta-cell pathophysiology are not clear.

In the present study, we demonstrate that IDs are novel oxidative stress-responsive genes in beta-cells. We also identify an unexpected role of ID expression in the induction of the antioxidant response under oxidative stress conditions. Inhibition of *Id1* and/or *Id3* reduces the expression of multiple antioxidant genes and leads to increased ROS production and apoptosis and altered mitochondrial function and morphology. Our studies also suggest a mechanism for these effects via a previously unrecognized interaction in any cell system of IDs with the NFE2L2-Maf antioxidant pathway. Our results therefore suggest that IDs are key regulators of the adaptive antioxidant-mitochondrial response that promote beta-cell survival under oxidative stress.

Results

IDs expression is increased in the islets of diabetic mice in parallel with that of NFE2L2 and antioxidant response genes

By immunofluorescence on histological pancreatic sections, we observed a marked increase in the expression of ID1 and ID3 in the islets of diabetic db/db mice in comparison with normoglycemic control db/+ mice (Fig.1 A-D). These effects were paralleled by increased expression of NFE2L2, a master transcriptional regulator of antioxidant gene expression (Fig.1 E-F). In agreement with these observations, mRNA levels of *Id1-4* were upregulated in the islets of db/db mice (Fig.1 G-J). These changes were accompanied by increased mRNA levels of the antioxidant genes *Hmox1*, *Gpx1-2*, thioredoxin reductase 1 (*Txnrd1*), *G6pdx*, *Srxn1* and *Prdx1-4* (Fig.1 K-T). These results demonstrate an *in vivo* association between the upregulation of IDs and the antioxidant response in islets of diabetic mice.

IDs are induced by oxidative stress in beta-cells

We tested whether IDs are oxidative stress-responsive genes in beta-cells. MIN6 beta-cells were treated with the pro-oxidant agents H_2O_2 or ribose. The latter is a sugar that produces ROS more potently and in a shorter time in comparison to glucose and is an established model to study beta-cell glucotoxicity *in vitro* (Kaneto et al., 1996;Matsuoka et al., 1997;Tanaka et al., 2002). We found that H_2O_2 treatment increased the mRNA levels of *Id1-4* in a concentration-dependent manner in parallel with similar changes in the mRNA levels of *Hmox1* (Fig. 2A-E). Ribose treatment also increased the mRNA levels of *Id1-4* and *Hmox1* in a concentration-dependent manner (Fig. 2F-J). Accordingly, ribose treatment increased the (peri)nuclear expression of ID1 (Fig. 3A-B) and ID3 proteins (Fig. 3C-D).

To assess the kinetics of *Ids* induction in comparison with antioxidant genes, we performed a time-course experiment (0-48h) in the absence or presence of ribose. We found that *Id1* and *Id3* mRNA levels were induced by ribose in a time-dependent manner. The effect of ribose was significant at 6h and reached a maximum at 48h. The mRNA levels of antioxidant genes were also induced by ribose in a time-dependent manner and displayed various profiles. Thus, *Hmox1*, *Srxn1* and to a lesser extent *Gpx2* were induced at 6h and reached the maximum at 24h (Fig. 4C-E) while *Prdx1* was significantly induced between 24-48h and reached the maximum at 48h (Fig. 4F). Interestingly, the mRNA levels of the proapoptotic gene Bcl2 associated x protein (*Bax*) were upregulated by ribose treatment between 24-48h. These findings suggest a close association between *Id* and antioxidant gene expression under oxidative stress conditions in beta-cells.

Ids expression is prevented by antioxidant treatment under oxidative stress

To further demonstrate that *Ids* are oxidative stress-responsive genes in beta-cells, we tested the effects of the antioxidant MnTBAP on the expression of antioxidant genes and *Ids* in ribose-treated MIN6 cells. We found that ribose-induced upregulation of the mRNA levels of *Hmox1*, *Gpx1-2*, *Srxn1*, *Prdx1* and *Prdx3* was prevented by antioxidant treatment (Fig. 5A-F). In parallel, antioxidant treatment prevented ribose-induced upregulation of the mRNA levels

of *Id1-3* and to a lesser extent *Id4* (Fig. 5G-J). Together these results demonstrate that *Ids* are novel oxidative stress-responsive genes in beta-cells.

Inhibition of Id1 and/or Id3 leads to global attenuation of the antioxidant response

To explore the role of *Ids* induction under oxidative stress in beta-cells, we used siRNA to inhibit the expression of *Id1* and/or *Id3*. Thus, the siRNA against *Id1* (si-Id1) specifically reduced the mRNA levels of *Id1* but not *Id3* and the siRNA against *Id3* (si-Id3) specifically reduced the mRNA levels of *Id3* but not *Id1* while the combination of both (si-Id1/3) reduced the mRNA levels of both genes (Fig. 6A-B). Interestingly, in ribose-treated cells, inhibition of *Id1* significantly reduced the mRNA levels of *Hmox1*. When both genes were inhibited, a stronger effect was observed (Fig. 6C). We also observed that the inhibition of *Id1* or *Id3* had a slight effect on the mRNA levels of *Gpx1* but a strong additive effect was observed when both isoforms were inhibited (Fig. 6D). This additive inhibitory effect was observed for all the other antioxidant genes studied including *Gpx2*, *Srxn1*, *Prdx1-4*, *Txnrd1* and *G6pdx* (Fig. 6E-L). In agreement with these findings, we found that *Id1* inhibition decreased H_2O_2- and ribose-induced upregulation of the protein levels of HMOX1 and this reduction was stronger after inhibition of both isoforms (Fig. 6M). We also found that inhibition of *Id1/3* reduced the ribose-induced upregulation of the protein levels of SOD2. Moreover, ribose-induced upregulation of GPX activity was partially prevented by ~21% after inhibition of *Id1/3* (Fig. 6N). These results indicate a striking association between *Id1/3* inhibition and a global attenuation of the antioxidant response.

Inhibition of Id1 and/or Id3 increased ROS levels, apoptosis and oxidative damage

We next assessed the influence of *Id* inhibition on ROS generation and cell viability. The attenuated antioxidant response may occur as a consequence of reduced ROS levels or it may lead to an increase in ROS generation. To verify this, we assessed H_2O_2 levels using DFCDA probe. As expected, ribose treatment increased H_2O_2 levels by ~1.7-fold in comparison to the control condition. However, *Id1/3* inhibition strongly increased basal and ribose-stimulated H_2O_2 levels by ~2.4- and ~1.8-fold respectively (Fig. 7A). In line with these results, H_2O_2 and ribose treatment increased apoptosis by ~3.2- and ~5.8-fold respectively while *Id1/3* inhibition potentiated basal, H_2O_2- and ribose-induced apoptosis by ~2.1-, ~2.0- and ~1.9-fold respectively (Fig. 7B). Moreover, in cultured islets, basal and ribose-induced apoptosis were potentiated by ~1.7- and ~1.25-fold in *Id1*-KO islets and by ~3.0- and ~1.6-fold in *Id3*-KO respectively (Fig. 7C). The increase in ROS levels and potentiation of apoptosis observed in the absence of *Id1/3* were accompanied by increased mRNA levels of the pro-apoptotic gene *Bax* and DNA-damage inducible transcript 2 (*Ddit2*) (Fig. 7D-E), and reduced mRNA levels of the anti-apoptotic genes *Bcl2* and *Bcl-xl* (Fig. 7F-G). Accordingly, DNA-damage was also increased after inhibition of *Id1/3*. Thus, 8-hydroxyguanosine (8OHdG) immunostaining was barely detected in untreated cells and markedly increased by ribose treatment (Fig. 7H-I). This staining was further increased upon *Id1/3* inhibition in control and ribose-treated cells (Fig. 7J-K). Interestingly, 8OHdG staining was detected in the nucleus, but was mainly cytosolic reflecting thereby oxidative damage of mitochondrial DNA. The latter is particularly vulnerable to oxidative damage due to the absence of histones and poor DNA-repair capacity

(Yakes and Van, 1997). In addition, the cytosolic 8OHdG staining was not homogenous and was stronger in some elliptical structures of different sizes that may reflect fragmented mitochondria.

Altogether, these results demonstrate that the inhibition of *Id1/3* leads to increased ROS levels, oxidative damage and apoptosis. These alterations may stem from the attenuation of the antioxidant response, but in addition, an alteration of mitochondrial homeostasis may be involved.

Inhibition of Id1 and/or Id3 impaired mitochondrial homeostasis

Mitochondria are vital cellular organelles involved in energy production as well as the regulation of numerous cellular processes, including redox homeostasis and apoptosis. It has been shown in islet cells of diabetic animals that there is an association between increased ROS generation and altered mitochondrial function (Bindokas et al., 2003;Lu et al., 2010). To assess the latter, we measured O_2 consumption in control and ribose-treated cells in the presence or absence of *Id1/3*. We found that ribose treatment tended to reduce O_2 consumption. After inhibition of *Id1/3*, O_2 consumption tended to be lower in untreated cells and was significantly reduced by ~20% after ribose treatment (Fig. 8A).

In parallel to this defect, we observed changes in the expression of components of the electron transport chain (ETC). Thus, western blot using Mitomix antiserum revealed that H_2O_2 and ribose treatment induced upregulation of Complex (C) I-V expression (Fig. 8B). However, after inhibition of *Id1/3*, we found a slight decrease in the expression of CI (subunit NDUFB8), CII (subunit SDHB) and CIII (subunit UQCRC2), and a noticeable strong decrease in the expression of CIV (subunit MTCO1), and to a lesser extent CV (subunit ATP5A) (Fig. 8B). On the other hand, the expression of *Ucp2* was not affected by *Id1/3* inhibition (Fig. S1A). MTCO1 is a catalytic subunit of cytochrome c oxidase encoded by mitochondrial DNA. Its expression is regulated by the transcription factor A mitochondrial (TFAM). The mRNA levels of the latter were upregulated by ribose treatment (Fig. 8C) as well as in the islets of diabetic db/db mice (Fig. S1B). Interestingly, inhibition of *Id1/3* significantly reduced the mRNA levels of TFAM (Fig. 8C).

Evidence suggests a relationship between increased ROS production and mitochondrial fragmentation (Picard et al., 2013). Therefore, we assessed mitochondrial morphology using Mitotracker probe. We found that the classical tubular structure of mitochondria observed under control conditions (Fig. 8D) was markedly altered by ribose treatment as evidenced by increased fragmentation (Fig. 8E). Interestingly, *Id1/3* inhibition induced mitochondrial fragmentation in untreated cells (Fig. 8F) and further enhanced fragmentation after ribose treatment (Fig. 8E). Similarly, in primary beta-cells from wild type mice, the tubular structure of mitochondria (Fig. 8H) was fragmented by ribose treatment (Fig. 8I). In beta-cells from *Id3*-KO mice, fragmentation was observed under control and ribose conditions (Fig. 8J-K).

Together, these results show that the inhibition of *Id1/3* is associated with altered mitochondrial function and morphology. These alterations were associated with reduced

expression of components of the ETC, particularly MCTO1, as well as the upstream transcription factor TFAM.

Thus, the increased ROS generation and subsequent mitochondrial damage and cell death observed in the absence of *Ids* may stem on the one hand from altered mitochondrial homeostasis, at least in part via an effect on TFAM, and on the other hand, from reduced expression of antioxidant genes.

Inhibition of Id1 and Id3 reduced the expression of Mafs, interacting partners of NFE2L2

NFE2L2 is a master regulator of antioxidant gene expression. Here, we assessed whether *Id1/3* inhibition altered the expression or localization of NFE2L2 by immunofluorescence. As expected, under control conditions, NFE2L2 was barely detected in the cytosol while ribose stimulation increased its nuclear localization (Fig. 9A-B). Surprisingly, inhibition of *Id1/3* increased NFE2L2 nuclear expression under control conditions and after ribose treatment (Fig. 9C-D). This observation is in agreement with increased oxidative stress in the absence *of Id1/3* but does not explain the inhibitory effect on antioxidant gene expression. We therefore hypothesized that *Ids* may affect an interacting partner of NFE2L2. We used cytoscape and MiMI plugin to retrieve the known interacting partners of NFE2L2 (Fig. 9E). Among them, a potential candidate was *Atf4* (He et al., 2001). The mRNA levels of the latter were upregulated by ribose treatment but were not significantly affected by *Id1/3* inhibition (Fig. S2A). Another potential candidate was *c-Jun* (Newman and Keating, 2003). Western blot analysis of phospho-c-JUN protein levels revealed that it was increased by ribose treatment but unaffected by *Id1/3* inhibition (Fig. S2B). The other potential candidates were the small *Maf* proteins (Newman and Keating, 2003;Katsuoka et al., 2005;Hirotsu et al., 2012;Tanigawa et al., 2013). We found that ribose treatment markedly increased *MafF* and *MafK* mRNA levels. Interestingly, *Id1/3* inhibition strongly prevented this increase (Fig. 9F-G). *MafG* mRNA levels were slightly increased by ribose treatment and slightly reduced by *Id1/3* inhibition (Fig. 9H). Changes in the expression of *MafK* were confirmed at the protein level by western blot (Fig. 9I). These findings demonstrate that the inhibition of *Id1/3* leads to reduced expression of small Maf proteins. Confirming the potential physiological relevance of these findings, *MafF* and *MafK* mRNA levels were markedly increased in the islets of diabetic db/db mice while the mRNA levels of *MafG* were not significantly affected (Fig. 9J-L). These findings suggest a novel mechanism whereby *Ids* regulate antioxidant gene expression by modifying the expression of small Maf proteins, NFE2L2-interacting partners.

Inhibition of Mafs reduces antioxidant gene expression and potentiates apoptosis, recapitulating the effects of Id1/3 inhibition

To verify whether the inhibition of small Maf proteins recapitulate the effects of *Id1/3* inhibition on antioxidant gene expression, we used siRNA against *MafF*, *MafK* and *MafG* (si-FGK). By this means, the ribose-mediated upregulation of *MafF*, *MafK* and *MafG* was inhibited to a similar extent to that observed after inhibition of *Id1/3* (Fig. 10A-C). Strikingly, the inhibition of *Mafs* resulted in reduced mRNA levels of several antioxidant genes, including *Hmox1*, *Gpx1*, *Txnrd1*, *Prdx2-3*, and to a lesser degree *G6pdx* (Fig. 10D-I). Furthermore, inhibition of *Mafs* potentiated ribose-induced apoptosis by ~1.8-fold (Fig. 10J).

These results demonstrate for the first time the importance of small Maf proteins F, K and G in the regulation of antioxidant gene expression and beta-cell survival under conditions of oxidative stress. Our results strongly suggest that *Id1/3* regulate antioxidant gene expression via the regulation of *Mafs* expression, thereby providing a novel mechanism of the cellular antioxidant response.

Discussion

In the present study, we have unveiled a novel cellular role for IDs in the regulation of redox status, mitochondrial integrity and cell survival under oxidative stress conditions. Mechanistically, our studies suggest that IDs are required for cellular mitochondrial adaptation and the antioxidant response by maintaining expression of the mitochondrial transcription factor *Tfam* and the NFE2L2-MAF signaling pathway. We have shown that IDs are novel oxidative stress-responsive genes in pancreatic beta-cells. The inhibition of *Id1/3* results in 1) reduced expression of *Tfam* and components of the ETC, altered mitochondrial respiration and increased mitochondrial fragmentation; and 2) global attenuation of the antioxidant response as evidenced by reduced expression of small *Mafs* and antioxidant gene expression. These alterations correlate with increased intracellular ROS levels, oxidative damage and apoptosis. Thus, our studies provide strong evidence for IDs to be crucial components of mitochondrial adaptation and the antioxidant response that promotes beta-cell survival under oxidative stress.

IDs and oxidative stress

Besides their important developmental role (Ruzinova and Benezra, 2003), ID proteins have been extensively studied in the field of cancer. Deregulation of their expression has been associated with several malignancies and has been proposed to play key roles in dedifferentiation, proliferation, angiogenesis and metastasis (Lasorella et al., 2014;Nair et al., 2014). Interestingly, few reports have also suggested a link between IDs induction and the cellular redox status. Thus, ID1 overexpression in neonatal cardiac myocytes increased ROS generation (Tanaka et al., 1998). On the other hand, UV treatment increased the mRNA levels of *Id3* in immortalized keratinocytes in a ROS-dependent manner (Trabosh et al., 2009). Moreover, *Id3* induction in proliferating vascular smooth muscle cells has been shown to be ROS-dependent (Nickenig et al., 2002).

Oxidative stress is an important mechanism implicated in numerous pathological situations, such as tumorigenesis (Costa et al., 2014), neurodegeneration (Chaturvedi and Flint, 2013), vascular injury (Stocker and Keaney, Jr., 2004), and beta-cell glucotoxicity (Robertson, 2004;Bensellam et al., 2012). In beta-cells, ID1/3 were induced by elevated glucose concentrations in isolated human islets and beta-cell lines (Webb et al., 2000;Wice et al., 2001). We have previously shown that *Id1* mRNA levels were specifically induced by hyperglycemia in the islets of diabetic db/db mice. Indeed, this induction was prevented when animals were treated with phlorizin, a drug that lowers plasma glucose levels (Kjorholt et al., 2005). Here, we have confirmed and extended this observation by showing that the four members of the ID family were upregulated in the islets of db/db mice. This induction was paralleled by a global upregulation of the antioxidant response. Since oxidative stress is a major downstream mechanism of beta-cell glucotoxicity (Bensellam et al., 2012), we verified whether IDs are induced by pro-oxidant agents in beta-cells. We found that H_2O_2 and ribose treatment induced *Id1-3*, and to a lesser extent *Id4*, in a time- and concentration-dependent manner in parallel with antioxidant genes in MIN6 beta-cells. Furthermore, antioxidant

treatment prevented IDs induction in parallel with antioxidant genes, thereby demonstrating for the first time that IDs are oxidative stress responsive genes in beta-cells.

Oxidative stress-responsive genes can be divided into three main subcategories: antioxidant genes that restore cellular homeostasis by scavenging and detoxifying supraphysiological ROS levels, oxidative damage-induced genes that activate pathways to repair macromolecular damage, and proapoptotic genes that trigger cell death if the two previous strategies fail. The parallelism between *Ids* induction and antioxidant gene expression suggested a potential link with the antioxidant response.

IDs and the antioxidant response

We have shown that *Id1* and *Id3* regulate antioxidant gene expression under oxidative stress via a specific interaction with the NFE2L2-MAF antioxidant pathway. The inhibition of *Id1*/*3* reduced the expression of multiple antioxidant genes in parallel with increased intracellular ROS levels, oxidative damage, and cell death. This observation suggested that *Id1/3* may regulate the antioxidant response by an upstream mechanism.

Mammalian cells are endowed with a set of oxidative stress sensors that activate different signaling pathways to restore cellular homeostasis or otherwise, trigger apoptosis (Marinho et al., 2014). Among them, NFE2L2 is a key regulator of antioxidant gene expression. Under basal conditions, it is continuously targeted for proteasomal degradation by the cytoplasmic adaptor protein Kelch-like ECH associated protein 1 (KEAP1). Under oxidative stress, the interaction between KEAP1 and NFE2L2 is impaired leading to the stabilization of the latter and its nuclear translocation (Itoh et al., 2004). Unexpectedly, we found that the inhibition of *Id1/3* markedly increased NFE2L2 expression and nuclear localization. This interesting observation, which is in line with the increased ROS accumulation under these conditions, suggested that *Id1/3* may affect an interacting partner rather than NFE2L2 itself. Indeed, we found that inhibition of *Id1/3* inhibited the expression of small Maf proteins F, K and G, known interacting partners of NFE2L2 that play an important role in the regulation of antioxidant gene expression (Itoh et al., 1997;Katsuoka et al., 2005;Hirotsu et al., 2012;Yamazaki et al., 2012;Tanigawa et al., 2013). Interestingly, inhibition of *MafF/G/K* reduced the expression of several antioxidant genes and potentiated ribose-induced apoptosis, thereby mimicking the effects of *Id1/3* inhibition.

These striking findings provide a novel regulatory mechanism of the antioxidant response. Our results strongly suggest that *Id1/3* regulate antioxidant gene expression through the regulation of small *Maf* protein expression. Besides, our results highlight the important role of small Maf proteins in beta-cells. While recent reports have focused on the role of NFE2L2 in diabetes and beta-cell pathophysiology (Uruno et al., 2013;Li et al., 2014;Yagishita et al., 2014), less attention has been given to the role of small Maf proteins (Shimohata et al., 2006). Yet, our results show that increased expression and nuclear accumulation of NFE2L2 alone, in the absence of *Mafs*, was insufficient to maintain adequate expression of antioxidant genes under oxidative stress. Interestingly, *MafF* and *MafK* mRNA levels were significantly upregulated in the islets of db/db mice suggesting a possible involvement *in vivo* in the regulation of antioxidant gene expression.

Defective antioxidant response is one plausible explanation for the increase in ROS levels and cellular damage and death observed in the absence of *Id1/3*. Previous reports have proposed that beta-cells are vulnerable to oxidative stress because of their low intrinsic antioxidant defenses. However, these reports did not explore the full antioxidant arsenal of beta-cells. We previously observed that a large set of antioxidant genes, such as several isoforms of *Gpx*, metallothioneins, *Prdxs* and *G6pdx* which produces NADPH, the principal cellular reductant, were expressed in rat islets and some of them, such as *Prdxs*, had strong signals on microarray chips (Bensellam et al., 2009). Moreover, although some antioxidant genes have moderate signal under basal conditions, such as *Hmox1*, they were markedly upregulated by high glucose levels *in vitro* and *in vivo*, and may contribute to beta-cell resistance to hyperglycemia-induced apoptosis in some animal models (Laybutt et al., 2002;Jonas et al., 2003;Elouil et al., 2005;Bensellam et al., 2009;Lacraz et al., 2009). It is however unknown whether *Id* expression is affected in these models. In the present study, we have shown that several antioxidant genes were expressed in the well differentiated MIN6 beta-cells and mouse islets and were markedly stimulated by oxidative stress *in vitro* and *in vivo*. Alteration of their expression under oxidative stress by *Id1/3* inhibition had a profound effect on the cellular redox status and beta-cell survival.

IDs and mitochondria

Mitochondria play a fundamental role in beta-cells by coupling glucose metabolism to insulin secretion (Jitrapakdee et al., 2010). The mitochondrial ETC is the principal cellular source of ROS (Turrens, 2003). At physiological levels, ROS, in particular H_2O_2, have been proposed to play a role in cell signaling (Rhee, 2006), including the potentiation of insulin secretion (Pi et al., 2007). However, excessive production in pathology, that could result from either alteration of electron flow in the ETC or deficiency in antioxidant defenses or both, has been associated with mitochondrial dysfunction and altered expression of ETC components (Bindokas et al., 2003;Lu et al., 2008;Lu et al., 2010). We found that *Id1/3* inhibition uncovered an inhibitory effect of ribose on oxygen consumption. Interestingly, this effect was associated with reduced expression of ETC components and the mitochondrial transcription factor *Tfam*.

Besides 22 tRNAs and 2 rRNAs, mitochondrial DNA (mtDNA) encodes 13 polypeptides that constitute subunits of complex I, III, and IV in the ETC, and complex V. Point mutations and deletions in mtDNA have been associated with impaired respiratory chain function and have been involved in several pathologies including mitochondrial diabetes. Besides, depletion of mtDNA by *Tfam* deletion resulted in deficient oxidative phosphorylation and increased apoptosis (Park and Larsson, 2011). Mitochondria have been shown to be a primary target for oxidative damage. Thus, acute exposure of rat islets and INS1 beta-cell to H_2O_2 has been shown to impair mitochondrial function in parallel with reduced expression of components of the ETC, especially MTCO1, as well as reduced expression of *Tfam*, subsequent production of mitochondrial ROS and increased apoptosis (Li et al., 2009). These observations mirror ours and suggest that the global attenuation of antioxidant gene expression induced by *Id1/3* inhibition may lead to ROS accumulation and mitochondrial alterations. The latter could result in enhanced generation of mitochondrial ROS, thus further exacerbating oxidative

stress and inducing oxidative damage and apoptosis. Alternatively, it is possible that the inhibition of *Id1/3* alters the expression of *Tfam*, thereby reducing the expression of ETC component. The resulting slow down of electron flow may lead to enhanced ROS generation. Indeed, it is well established that beta-cells generate high ROS levels when cultured in the presence of low non-stimulatory glucose concentration in association with increased expression of oxidative stress responsive genes, such as *Hmox1*, and increased apoptosis (Martens et al., 2005;Bensellam et al., 2009;Roma et al., 2012). The resulting oxidative stress is further aggravated by the global attenuation of the antioxidant response and further alters mitochondria. This view is supported by the observation that enhanced ROS formation, increased oxidative damage and potentiation of apoptosis are observed under basal conditions in the absence of any ribose or H_2O_2 stimulation. Moreover, in our model, we observed that chronic stimulation with H_2O_2 or ribose do not inhibit the expression of components of the ETC and *Tfam* but rather increased it. Furthermore, the mRNA levels of *Tfam* were also upregulated in the islets of diabetic mice despite the presence of oxidative stress in these islets. Either way, these alterations were associated with mitochondrial fragmentation. The latter has been observed in beta-cells of T2D animal models and human subjects as well as in *in vitro* models of nutrient oversupply and oxidative stress (Bindokas et al., 2003;Anello et al., 2005;Li et al., 2009;Molina et al., 2009;Dlaskova et al., 2010;Lu et al., 2010).

Potential exploitation of these new findings to protect beta-cells

Although antioxidant supplementation has been shown to be promising in T2D animal models, it was overall ineffective in humans and even risky in some cases. Consequently, the activation of endogenous antioxidant genes could be a more promising strategy to reduce ROS-mediated apoptosis in beta-cells. Understanding the mechanisms of regulation of antioxidant response and the effectors involved is a key step towards this goal. The identification of *Ids* as key upstream regulators of the antioxidant response, besides their parallel role in mitochondria, may serve for the development of a novel therapeutic strategy to protect beta-cells against oxidative stress. *Ids* are known downstream targets of the BMP signaling pathway. Emerging evidence indicates an important role of this pathway in beta-cells (Goulley et al., 2007;Scott et al., 2009;Bonner et al., 2011). However, one should keep in mind that the activation of *Ids* may also interfere with beta-cell differentiation. In a previous report, we have shown that *Id1* was induced by elevated lipids in beta-cells and plays a role in lipid-induced impairment of insulin secretion and differentiation (Akerfeldt and Laybutt, 2011). A moderate physiological stimulation of this pathway may exert beneficial effects under oxidative stress while avoiding the alteration of the beta-cell phenotype. Interestingly, our other studies show that low concentrations of recombinant BMP stimulated the expression of *Id1/3* in parallel with a significant protection against ribose-induced apoptosis in MIN6 beta-cells (MB-RL, unpublished results).

In conclusion, we have identified *Ids* as a novel family of oxidative stress responsive genes in beta-cells that play an important physiological role under oxidative stress. We have demonstrated that *Id1/3* are crucial for the maintenance of an adaptive mitochondrial-antioxidant response that promote beta-cell survival under oxidative stress via a novel link to the NFE2L2-MAF signaling pathway. Besides their importance for basic cellular biology,

these novel findings may help for the development of therapeutic strategies to protect beta-cells against oxidative stress.

Material and methods

Reagents

Ribose was from Sigma (St Louis, MI, USA). Manganese(III)tetrakis (4-benzoicacid) porphyrin (MnTBAP) was from Alexis Biochemicals (Lausen, Germany). 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) and Deep Red Mitotracker were from Invitrogen (Carlsbad, CA, USA).

Antibodies

The following primary antibodies were used: anti-β-ACTIN (A5441), anti-α-TUBULIN (T90267) and anti-INSULIN (I2018; Sigma, St Louis, MI, USA); anti-HMOX1 (5061; Cell Signaling Technology, Danvers, MA, USA); anti-ID1 (BCH-1/37-2) and anti-ID3 (BCH-4/17-3; Biocheck, Foster City, CA, USA); anti-14-3-3 (sc-629), anti-ID1 (sc-488), anti-NFE2L2 (sc-13032) and anti-SOD2 (sc-30080; Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-8-hydroxyguanosine (ab48508) and anti-MAFK (ab50322; Abcam, Cambridge, UK); anti-mitochondrial respiratory chain subunits (CI: NDUFB6, CII: SDHB, CIII: UQCRC2, and CV: ATP5A) (MS601; Mitosciences, Eugene, OR) and anti-CIV: MCTO1 (A6403; Invitrogen).

Mice

C57BL/KsJ db/db and their age-matched lean control db/+ mice were taken from the Garvan Institute breeding colonies at the age of 14-16 weeks. Wild-type (C57BL/6/129/Sv), $Id1^{-/-}$ and $Id3^{-/-}$ mice were bred in-house using animals provided by Professor Robert Benezra (Memorial Sloan-Kettering Cancer Center, New York, NY) and used at the age of 8-10 weeks. Animals were kept under conventional conditions with free access to food and water. All experiments were approved by the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee, following guidelines issued by the National Health and Medical Research Council of Australia.

Islet isolation and culture

db/db and db/+ islets were isolated with liberase RI (Roche Diagnostics, Castle Hill, Australia) digestion of the pancreas and further separated with a Ficoll-Paque PLUS gradient (Amersham Biosciences) and handpicked under a stereomicroscope. Immediately after collection, islets were used for extraction of RNA. Wild-type, $Id1^{-/-}$ and $Id3^{-/-}$ islets were precultured 48h in RPMI medium containing 11.1 mmol/L glucose (Invitrogen) and supplemented with 0.2 mmol/L glutamine, 10% heat-inactivated FBS 100 units/mL penicillin, and 100 mg/mL streptomycin. Then, islets were further cultured 48h under control condition or in the presence of 50 mmol/L ribose. At the end of culture, islets were used for apoptosis measurements. For assessment of mitochondrial morphology, islets were dispersed with trypsin and cells seeded on poly-L-lysine-treated glass coverslips before treatment.

Cell culture

MIN6 beta-cells (P26-39) were grown in Dulbecco's modified Eagle's medium (Invitrogen) containing 25 mmol/L glucose, 10 mmol/L HEPES, 10% FCS, 50 units/mL penicillin, and 50 mg/mL streptomycin. Cells were cultured in the presence of H_2O_2 (100-300 µmol/L) or ribose (5-25 mmol/L) to induce oxidative stress.

RNA intereference

To downregulate gene expression, cells were transfected with either 100 nmol/L control Non-Targeting siRNA and Id1 and/or Id3 ON-TARGETplus SMARTpool siRNA using DharmaFECT3 according to manufacturer instructions (Thermo Fisher Scientific, Lafayette, CO). To mimic the effects of Id1/3 inhibition on MafF/MafG/MafK expression, cells were first transfected for 6h with either 100 nmol/L control Non-Targeting siRNA or 50 nmol/L of MafF and 50 nmol/L of MafK ON-TARGETplus SMARTpool siRNA. Then, cells were transfected a second time with either 150 nmol/L control Non-Targeting siRNA or 50 nmol/L of MafF, 50 nmol/L of MafK and 50 nmol/L of MafG ON-TARGETplus SMARTpool siRNA. After 24h, the transfection medium was changed and cells were cultured for 48h in the absence or presence of H_2O_2 or ribose. At the end of culture, cells were used for RNA and protein extraction, ROS, apoptosis and oxygen consumption measurements and immunocytochemistry.

Real-time RT-PCR

Total RNA extraction, cDNA synthesis and Real-time PCR were performed as previously described (Chan et al., 2013). Primer sequences are listed in Supplementary Table 1. The value obtained for each specific gene product was normalized to the control gene cyclophilin A and expressed as a fold change of the value in control condition.

Western blot

MIN6 cells were lysed in normal lysis buffer (50 mM Hepes, 150 mM NaCl, 10% glycerol, 1% Triton X100, 1.5 mM $MgCl_2$, 1 mM EGTA, 10 mM pyrophosphate, 100 mM NaF) supplemented with 2 mM PMSF, 1 mM sodium orthovanadate and 1% protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Proteins (15-30 µg) were then separated by NuPage SDS-PAGE gels (Invitrogen) and transferred onto PVDF membrane. After blocking with 5% non-fat milk, the membranes were incubated with primary antibodies overnight at 4°C. The next day, membranes were incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and the signal was revealed by enhanced chemiluminescence (Perkin Elmer, Wellesley, MA, USA).

GPx activity

GPx activity was determined indirectly as the decrease in NADPH absorption at 340 nm and 30°C. A reaction cocktail was prepared by adding azide buffer (9.2 ml) [50 mM NaH_2PO_4, 0.4 mM EDTA (pH 7.0), 1 mM Na-azide], glutathione reductase (100 µl, 100 u/ml), and GSH

(50 µl, 200 mM) into a 1 mg β-NADPH vial (N0411; Sigma). Sample and reaction cocktail were mixed and H_2O_2 (5 µl, 0.042% (v/v)) was added to start the reaction. Results were normalized to protein content.

Apoptosis

Cytoplasmic histone-associated DNA fragments were measured in MIN6 cells and islets with the Cell Death Detection ELISA^{PLUS} kit (Roche Diagnostics) according to manufacturer instructions as previously described (Bensellam et al., 2009). Results were normalized to DNA content.

Oxygen consumption

After culture, cells were trypsinized and resuspended in 1ml of warm medium containing the same composition as during culture. Oxygen consumption was measured at 37°C using an Oxytherm electrode unit (Hansatech, Norfolk, UK). This Clark-type oxygen electrode system monitors the dissolved oxygen concentration in a sealed measurement chamber over time. The measurements were exported to a computerized graph recorder (Oxygraph 1.01, Hansatech, Norfolk, UK) to calculate the rate of oxygen consumption. Experiments were done in duplicates and results were normalized to protein content.

ROS measurement

Cellular ROS levels were measured using chloromethyl-2'7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA) probe following the manufacturer's instructions (Invitrogen). Briefly, cells were seeded in black 96 well plates with clear flat bottom. After siRNA tansfection and 48h culture in the absence or presence of ribose, cells were washed and incubated with 2µM CM-H_2DCFDA or DMSO for 30 min at 37°C. Then, cells were washed and incubated in culture medium for 10 min at 37°C. Fluorescence intensity was measured (excitation at 485nm and emission at 520nm) using a plate reader (FluoStar OPTIMA, BMG Lab Technologies) and normalized to that measured in cells incubated with DMSO. Experiments have been done in quintuplicate for each condition.

Immuofluorescence

Pancreata were fixed in 10% buffered formalin (Sigma) and embedded in paraffin. Antigen retrieval was performed by using citrate buffer (S1699; Dako, Glostrup, Denmark). Cells cultured on coverslips were fixed in freshly prepared 4% paraformaldehyde (Electron Microscopy Sciences; Washington, PA) and permeabilized with 1% Triton X-100. For 8OHdG staining, additional steps of RNAse treatment, proteinase K treatment and DNA denaturation were performed. Pancreatic sections and cells were incubated with 5% normal goat serum-1% BSA (Sigma) for 1h, and then overnight at 4°C with primary antibodies. Alexa fluor conjugated secondary antibodies (Molecular probes) were used to visualize immunostaining. Cells and sections were counterstained by DAPI and images were acquired using Leica SP8 confocal microscope.

Statistical analysis

Results are means±SEM for the indicated number of experiments. Statistical significance of differences between groups was assessed by unpaired two-tailed student t-test, one-way ANOVA and a post-test of Newman–Keuls or two-way ANOVA and a post-test of Bonferroni.

Acknowledgements

This work was supported by grants from the National Health and Medical Research Council (NHMRC) of Australia and Australian research Council (ARC). MB was supported by a Post-doctoral Fellowship from the Société Francophone du Diabète (SDF, Paris, France).

MB and DRL conceived, designed and performed experiments and wrote the manuscript. MM, JL and JYC performed experiments and reviewed the manuscript.

The authors declare no competing financial interests

Abbreviations list

8OHdG: 8-hydroxyguanosine

ID: Inhibitor of Differentiation

NFE2L2: Nuclear Factor E2-related factor 2

ROS: Reactive Oxygen Species

Tfam: Transcription factor A mitochondrial

Figure legends

Figure 1. IDs expression is upregulated in the islets of diabetic mice in parallel with oxidative stress response genes.

(A-F) Immunostaining of ID1 (A-B), ID3 (C-D) and NFE2L2 (E-F) in the islets of normoglycemic db/+ and diabetic db/db mice. Pancreatic sections were analyzed by confocal microscopy using anti-insulin antibody (green), anti-ID1, anti-ID3, anti-NFE2L2 antibodies (red) and DAPI (blue). Images are representative of 3 animals for each group. (G-T) Changes in the mRNA levels of *Id1-4*, *Hmox1*, *Gpx1-2*, *Txnrd1*, *G6pdx*, *Srxn1* and *Prdx1-4* in the islets of normoglycemic db/+ and diabetic db/db mice were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the levels in islets of db/+ mice. Data are means±SEM for 6-10 animals in each group. *p<0.05, **p<0.01, ***p<0.001 for the effect of genotype (unpaired two-tailed student t-test).

Figure 2. Oxidative stress increases the mRNA levels of Ids in beta-cells.

MIN6 beta-cells were cultured for 48h in the absence or presence of increasing concentrations of H_2O_2 (100-300 µM) (A-E) or ribose (5-50 mM) (F-J) to induce oxidative stress. Changes in the mRNA levels of *Hmox1* and *Id1-4* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels, and expressed relative to the control condition. Data are means±SEM for 3 experiments. *p<0.05, **p<0.01, ***p<0.001 vs. control condition (one-way ANOVA+test of Newman-Keuls).

Figure 3. Oxidative stress increases the (peri)nuclear localization of Id1 and Id3.

Immunostaining of ID1 (A-B) and ID3 (C-D) in MIN6 beta-cells cultured 48h in the absence or presence of 50 mM ribose. Cells were fixed and analyzed by confocal microscopy using anti-ID1 antibody (green), anti-ID3 antibody (red) and DAPI (blue). Images are representative of four experiments.

Figure 4. Oxidative stress time-dependently increases the mRNA levels of Id1 and Id3 in parallel with oxidative stress response genes.

MIN6 beta-cells were cultured from 0 to 48h in the absence or presence of 50 mM ribose to induce oxidative stress. Changes in the mRNA levels of *Id1*, *Id3*, *Hmox1*, *Gpx2*, *Srxn1*, *Prdx1* and *Bax* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition at time point 0. Open circles: untreated cells; closed circles: ribose treated cells. Data are means±SEM for 3 experiments. *p<0.05, **p<0.01, ***p<0.001 for the effect of time and #p<0.05, ##p<0.01, ###p<0.001 for the effect of ribose (two-way ANOVA+test of Bonferroni).

Figure 5. Antioxidant treatment prevents the ribose-induced upregulation of Id mRNA levels.

MIN6 beta-cells were cultured for 48h in the absence or presence of 50 mM ribose. The antioxidant MnTBAP was added to a final concentration of 10 µM to oppose ribose-induced

oxidative stress. Changes in the mRNA levels of *Hmox1*, *Gpx1-2*, *Srxn1*, *Prdx1*, *Prdx3* (A-F) and *Id1-4* (G-J) were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 3 experiments. *p<0.05, ***p<0.001 for the effect of ribose and #p<0.05, ##p<0.01, ###p<0.001 for the effect of antioxidant treatment (two-way ANOVA+test of Bonferroni).

Figure 6. Inhibition of Id1 and/or Id3 attenuates the global antioxidant response.

MIN6 beta-cells were transfected with either control siRNA (si-C), siRNA against *Id1* (si-Id1), *Id3* (si-Id3) or both (si-Id1/3) for 24h, then further cultured for 48h in the absence or presence of 50 mM ribose to induce oxidative stress. (A-L) Changes in the mRNA levels of *Id1*, *Id3*, *Hmox1*, *Gpx1-2*, *Srxn1*, *Prdx1-4*, *Txnrd1* and *G6pdx* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 3 experiments. *p<0.05, **p<0.01, ***p<0.001 for the effect of ribose and #p<0.05, ##p<0.01, ###p<0.001 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (M) Changes in the protein levels of ID1, ID3, SOD2, HMOX1 and control genes were analysed by western blot. Blots are representative for 3 to 5 experiments. C: control; H: H_2O_2 100 µM; R: ribose 50 mM. (N) Changes in enzymatic activity of GPx. Data are means±SEM for 5 experiments. *p<0.05, ***p<0.001 for the effect of ribose (two-way ANOVA+test of Bonferroni).

Figure 7. Inhibition of Id1 and Id3 increases ROS levels and apoptosis.

MIN6 beta-cells were transfected with either control siRNA (si-C) or siRNA against Id1 and Id3 (si-Id1/3) for 24h, then further cultured for 48h in the absence or presence of 50 mM ribose or 100 µM H_2O_2. (A) ROS levels were measured using the DCFDA probe. Fluoresence was normalized to that measured in cells incubated with DMSO and expressed relative to the control condition. Data are means±SEM for 3 experiments. ##p<0.01 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (B) Apoptosis was assessed by measurement of DNA fragmentation by ELISA and normalized to DNA content. Data are means±SEM for 4 experiments. *p<0.05, ***p<0.001 for the effect of treatment and ###p<0.001 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (C) Isolated WT, Id1-KO and Id3-KO mouse islets were cultured for 48h in the absence or presence of 50 mM ribose. At the end of culture, DNA fragmentation was assessed by ELISA and normalized to DNA content. Data are means±SEM for 4 experiments. ***p<0.001 for the effect of ribose and ##p<0.01 for the effect of genotype (two-way ANOVA+test of Bonferroni). (D-G) Changes in the mRNA levels of *Bax*, *Ddit2*, *Bcl2* and *Bcl-XL* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 3-4 experiments. **p<0.01, ***p<0.001 for the effect of ribose and #p<0.05, ###p<0.001 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (H-K) Immunostaining of 8OHdG. Cells were fixed and analyzed by confocal microscopy using anti-8OHdG antibody (red) and DAPI (blue). Images are representative of three experiments.

Figure 8. Inhibition of Id1 and Id3 impairs mitochondrial homeostasis and induces mitochondrial fragmentation.

MIN6 beta-cells were transfected with either control siRNA (si-C) or siRNA against *Id1* and *Id3* (si-Id1/3) for 24h, then further cultured for 48h in the absence or presence of 50 mM ribose. (A) Changes in oxygen consumption were assessed by Clark electrode, normalized to protein content and expressed relative to the control condition. Data are means±SEM for 5 experiments. Experiments were performed in duplicates. ***p<0.001 for the effect of ribose and ##p<0.01 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (B) Changes in the protein levels of components of the electron transport chain complexes and control genes were analysed by western blot. Blots are representative for 3 experiments. C: control; H: H_2O_2 100 µM; R: ribose 50 mM. (C) Changes in the mRNA levels of the mitochondrial transcription factor *Tfam* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 3 experiments. **p<0.01 for the effect of ribose (two-way ANOVA+test of Bonferroni). (D-G) Mitochondrial morphology was assessed by Red Mitotracker probe used at a final concentration of 300 nM (Red). Cells were fixed and analyzed by confocal microscopy. DAPI was used to stain nuclei (blue). Images are representative of three experiments. (H-K) Isolated WT and Id3-KO mouse islets were dispersed and cultured for 48h in the absence or presence of 50 mM ribose. At the end of culture, mitochondrial morphology was assessed by Red Mitotracker probe used at a final concentration of 300 nM (red). Cells were fixed and analyzed by confocal microscopy using anti-insulin antibody (green) and DAPI (blue). Images are representative of three experiments.

Figure 9. Inhibition of Id1 and Id3 increases the nuclear accumulation of NFE2L2 while reducing the expression of its interacting partners Mafs.

MIN6 beta-cells were transfected with either control siRNA (si-C) or siRNA against *Id1* and *Id3* (si-Id1/3) for 24h, then further cultured for 48h in the absence or presence of 50 mM ribose to induce oxidative stress. (A-D) Immunostaining of NFE2L2. Cells were fixed and analyzed by confocal microscopy using anti-NFE2L2 antibody (green) and DAPI as a nuclear stain (blue). Images are representative of three experiments. (E) Analysis of NFE2L2 interacting partners using Cytoscape (2.8.3) and MiMl plugin. (F-H) Changes in the mRNA levels of *MafF*, *MafG* and *MafK* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 4 experiments. ***p<0.001 for the effect of ribose and ##p<0.01 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (I) Changes in the protein levels of MAFK and control gene were analysed by western blot. Blots are representative for 4 experiments. C: control; R: ribose 50 mM. (J-L) Changes in the mRNA levels of *MafF*, *MafG* and *MafK* in the islets of normoglycemic db/+ and diabetic db/db mice were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the levels in islets of db/+ mice. Data are means±SEM for 6 animals in each group. *p<0.05, ***p<0.001 for the effect of genotype (unpaired two-tailed student t-test).

Figure 10. Inhibition of Mafs reproduces the effects of Id1/3 inhibition on antioxidant gene expression and apoptosis.

MIN6 beta-cells were transfected with either control siRNA (si-C) or siRNA against *MafF*, *MafK* and *MafG* (si-FGK) as detailed in material and methods. Cells were then further cultured for 48h in the absence or presence of 50 mM ribose to induce oxidative stress. (A-I) Changes in the mRNA levels of *MafF*, *MafG*, *MafK*, *Hmox1*, *Prdx2-3*, *Txnrd1*, *Gpx1* and *G6pdx* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 4 experiments. **p<0.01, ***p<0.001 for the effect of ribose and #p<0.05, ##p<0.01, ###p<0.001 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (J) Apoptosis was assessed by measurement of DNA fragmentation by ELISA and normalized to DNA content. Data are means±SEM for 3 experiments. **p<0.01, ***p<0.001 for the effect of ribose and ##p<0.01 for the effect of siRNA (two-way ANOVA+test of Bonferroni). (G) The proposed model: prolonged hyperglycemia increases ROS generation by defective mitochondria. The subsequent induction of IDs promotes beta-cell survival via the maintenance of an adaptive mitochondrial-antioxidant response. The latter consists of 1) the upregulation of the expression of the mitochondrial transcription factor *Tfam* and components of the ETC and 2) upregulation of the expression of NFE2L2-interacting partners small Maf proteins and subsequent induction of downstream antioxidant genes.

Figure S1.

(A) MIN6 beta-cells were transfected with either control siRNA (si-C) or siRNA against Id1 and Id3 (si-Id1/3) for 24h, then further cultured for 48h in the absence or presence of 50 mM ribose. Changes in the mRNA levels of *Ucp2* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 3 experiments. **p<0.01 for the effect of ribose (two-way ANOVA+test of Bonferroni).

(B) Changes in the mRNA levels of *Tfam* in the islets of normoglycemic db/+ and diabetic db/db mice were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the levels in islets of db/+ mice. Data are means±SEM for 6 animals in each group. *p<0.05, **p<0.01, ***p<0.001 for the effect of genotype (unpaired two-tailed student t-test).

Figure S2.

MIN6 beta-cells were transfected with either control siRNA (si-C) or siRNA against Id1 and Id3 (si-Id1/3) for 24h, then further cultured for 48h in the absence or presence of 50 mM ribose. (A) Changes in the mRNA levels of *Atf4* were analyzed by real-time RT-PCR, normalized to cyclophilin A mRNA levels and expressed relative to the control condition. Data are means±SEM for 4 experiments. **p<0.01 for the effect of ribose (two-way ANOVA+test of Bonferroni). (B) Changes in the protein levels of phospho-c-JUN (P-c-JUN) and control gene were analysed by western blot. Blots are representative for 4 experiments. C: control; R: ribose 50 mM.

References

Akerfeldt, M.C., and D.R.Laybutt. 2011. Inhibition of Id1 augments insulin secretion and protects against high-fat diet-induced glucose intolerance. *Diabetes* 60:2506-2514.

Anello, M., R.Lupi, D.Spampinato, S.Piro, M.Masini, U.Boggi, S.D.Prato, A.M.Rabuazzo, F.Purrello, and P.Marchetti. 2005. Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. *Diabetologia*.

Bensellam, M., D.R.Laybutt, and J.C.Jonas. 2012. The molecular mechanisms of pancreatic beta-cell glucotoxicity: recent findings and future research directions. *Mol. Cell Endocrinol.* 364:1-27.

Bensellam, M., L.Van Lommel, L.Overbergh, F.C.Schuit, and J.C.Jonas. 2009. Cluster analysis of rat pancreatic islet gene mRNA levels after culture in low-, intermediate- and high-glucose concentrations. *Diabetologia* 52:463-476.

Bindokas, V.P., A.Kuznetsov, S.Sreenan, K.S.Polonsky, M.W.Roe, and L.H.Philipson. 2003. Visualizing superoxide production in normal and diabetic rat islets of Langerhans. *J. Biol. Chem.* 278:9796-9801.

Bonner, C., A.M.Farrelly, C.G.Concannon, H.Dussmann, M.Baquie, I.Virard, H.Wobser, D.Kogel, C.B.Wollheim, M.Rupnik, M.M.Byrne, H.G.Konig, and J.H.Prehn. 2011. Bone morphogenetic protein 3 controls insulin gene expression and is down-regulated in INS-1 cells inducibly expressing a hepatocyte nuclear factor 1A-maturity-onset diabetes of the young mutation. *J. Biol. Chem.* 286:25719-25728.

Butler, A.E., J.Janson, S.Bonner-Weir, R.Ritzel, R.A.Rizza, and P.C.Butler. 2003. β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102-110.

Chan, J.Y., J.Luzuriaga, M.Bensellam, T.J.Biden, and D.R.Laybutt. 2013. Failure of the adaptive unfolded protein response in islets of obese mice is linked with abnormalities in beta-cell gene expression and progression to diabetes. *Diabetes* 62:1557-1568.

Chaturvedi, R.K., and B.M.Flint. 2013. Mitochondrial diseases of the brain. *Free Radic. Biol. Med.* 63:1-29.

Costa, A., A.Scholer-Dahirel, and F.Mechta-Grigoriou. 2014. The role of reactive oxygen species and metabolism on cancer cells and their microenvironment. *Semin. Cancer Biol.* 25:23-32.

DelGuerra, S., M.Grupillo, M.Masini, R.Lupi, M.Bugliani, S.Torri, U.Boggi, C.M.Del, F.Vistoli, F.Mosca, P.S.Del, and P.Marchetti. 2007. Gliclazide protects human islet beta-cells from apoptosis induced by intermittent high glucose. *Diabetes Metab Res. Rev.* 23:234-238.

Deng, S., M.Vatamaniuk, X.Huang, N.Doliba, M.M.Lian, A.Frank, E.Velidedeoglu, N.M.Desai, B.Koeberlein, B.Wolf, C.F.Barker, A.Naji, F.M.Matschinsky, and J.F.Markmann. 2004. Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. *Diabetes* 53:624-632.

Dlaskova, A., T.Spacek, J.Santorova, L.Plecita-Hlavata, Z.Berkova, F.Saudek, M.Lessard, J.Bewersdorf, and P.Jezek. 2010. 4Pi microscopy reveals an impaired three-dimensional mitochondrial network of pancreatic islet beta-cells, an experimental model of type-2 diabetes. *Biochim. Biophys. Acta* 1797:1327-1341.

Donath, M.Y., D.J.Gross, E.Cerasi, and N.Kaiser. 1999. Hyperglycemia-induced β-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. *Diabetes* 48:738-744.

Duprez, J., L.P.Roma, A.F.Close, and J.C.Jonas. 2012. Protective antioxidant and antiapoptotic effects of $ZnCl_2$ in rat pancreatic islets cultured in low and high glucose concentrations. *PLoS. ONE.* 7:e46831.

Elouil, H., A.K.Cardozo, D.L.Eizirik, J.C.Henquin, and J.C.Jonas. 2005. High glucose and hydrogen peroxide increase c-Myc and haeme-oxygenase 1 mRNA levels in rat pancreatic islets without activating NFκB. *Diabetologia* 48:496-505.

Finegood, D.T., M.D.McArthur, D.Kojwang, M.J.Thomas, B.G.Topp, T.Leonard, and R.E.Buckingham. 2001. β-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. *Diabetes* 50:1021-1029.

Gorogawa, S., Y.Kajimoto, Y.Umayahara, H.Kaneto, H.Watada, A.Kuroda, D.Kawamori, T.Yasuda, M.Matsuhisa, Y.Yamasaki, and M.Hori. 2002. Probucol preserves pancreatic β-cell function through reduction of oxidative stress in type 2 diabetes. *Diabetes Res. Clin. Pract.* 57:1-10.

Goulley, J., U.Dahl, N.Baeza, Y.Mishina, and H.Edlund. 2007. BMP4-BMPR1A signaling in beta cells is required for and augments glucose-stimulated insulin secretion. *Cell Metab* 5:207-219.

He, C.H., P.Gong, B.Hu, D.Stewart, M.E.Choi, A.M.Choi, and J.Alam. 2001. Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. *J. Biol. Chem.* 276:20858-20865.

Hirotsu, Y., F.Katsuoka, R.Funayama, T.Nagashima, Y.Nishida, K.Nakayama, J.D.Engel, and M.Yamamoto. 2012. Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. *Nucleic Acids Res.* 40:10228-10239.

Hou, Z.Q., H.L.Li, L.Gao, L.Pan, J.J.Zhao, and G.W.Li. 2008. Involvement of chronic stresses in rat islet and INS-1 cell glucotoxicity induced by intermittent high glucose. *Mol. Cell Endocrinol.* 291:71-78.

Ihara, Y., S.Toyokuni, K.Uchida, H.Odaka, T.Tanaka, H.Ikeda, H.Hiai, Y.Seino, and Y.Yamada. 1999. Hyperglycemia causes oxidative stress in pancreatic β-cells of GK rats, a model of type 2 diabetes. *Diabetes* 48:927-932.

Itoh, K., T.Chiba, S.Takahashi, T.Ishii, K.Igarashi, Y.Katoh, T.Oyake, N.Hayashi, K.Satoh, I.Hatayama, M.Yamamoto, and Y.Nabeshima. 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* 236:313-322.

Itoh, K., K.I.Tong, and M.Yamamoto. 2004. Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. *Free Radic. Biol. Med.* 36:1208-1213.

Jitrapakdee, S., A.Wutthisathapornchai, J.C.Wallace, and M.J.MacDonald. 2010. Regulation of insulin secretion: role of mitochondrial signalling. *Diabetologia* 53:1019-1032.

Jonas, J.C., M.Bensellam, J.Duprez, H.Elouil, Y.Guiot, and S.M.Pascal. 2009. Glucose regulation of islet stress responses and β-cell failure in type 2 diabetes. *Diabetes Obes. Metab* 11 Suppl 4:65-81.

Jonas, J.C., Y.Guiot, J.Rahier, and J.C.Henquin. 2003. Haeme-oxygenase 1 expression in rat pancreatic β-cells is stimulated by supraphysiological glucose concentrations and by cyclic AMP. *Diabetologia* 46:1234-1244.

Kaneto, H., J.Fujii, T.Myint, N.Miyazawa, K.N.Islam, Y.Kawasaki, K.Suzuki, M.Nakamura, H.Tatsumi, Y.Yamasaki, and N.Taniguchi. 1996. Reducing sugars trigger oxidative modification and apoptosis in pancreatic β-cells by provoking oxidative stress through the glycation reaction. *Biochem. J.* 320 (Pt 3):855-863.

Katsuoka, F., H.Motohashi, T.Ishii, H.Aburatani, J.D.Engel, and M.Yamamoto. 2005. Genetic evidence that small maf proteins are essential for the activation of antioxidant response element-dependent genes. *Mol. Cell Biol.* 25:8044-8051.

Kjorholt, C., M.C.Akerfeldt, T.J.Biden, and D.R.Laybutt. 2005. Chronic hyperglycemia, independent of plasma lipid levels, is sufficient for the loss of β-cell differentiation and secretory function in the *db/db* mouse model of diabetes. *Diabetes* 54:2755-2763.

Kluth, O., F.Mirhashemi, S.Scherneck, D.Kaiser, R.Kluge, S.Neschen, H.G.Joost, and A.Schurmann. 2011. Dissociation of lipotoxicity and glucotoxicity in a mouse model of obesity associated diabetes: role of forkhead box O1 (FOXO1) in glucose-induced beta cell failure. *Diabetologia* 54:605-616.

Lacraz, G., F.Figeac, J.Movassat, N.Kassis, J.Coulaud, A.Galinier, C.Leloup, D.Bailbe, F.Homo-Delarche, and B.Portha. 2009. Diabetic β-cells can achieve self-protection against oxidative stress through an adaptive up-regulation of their antioxidant defenses. *PLoS. ONE.* 4:e6500.

Lasorella, A., R.Benezra, and A.Iavarone. 2014. The ID proteins: master regulators of cancer stem cells and tumour aggressiveness. *Nat. Rev. Cancer* 14:77-91.

Laybutt, D.R., H.Kaneto, W.Hasenkamp, S.Grey, J.C.Jonas, D.C.Sgroi, A.Groff, C.Ferran, S.Bonner-Weir, A.Sharma, and G.C.Weir. 2002. Increased expression of antioxidant and antiapoptotic genes in islets that may contribute to β-cell survival during chronic hyperglycemia. *Diabetes* 51:413-423.

Lenzen, S., J.Drinkgern, and M.Tiedge. 1996. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radic. Biol. Med.* 20:463-466.

Li, N., T.Brun, M.Cnop, D.A.Cunha, D.L.Eizirik, and P.Maechler. 2009. Transient oxidative stress damages mitochondrial machinery inducing persistent beta-cell dysfunction. *J. Biol. Chem.* 284:23602-23612.

Li, W., W.Wu, H.Song, F.Wang, H.Li, L.Chen, Y.Lai, J.S.Janicki, K.W.Ward, C.J.Meyer, X.L.Wang, D.Tang, and T.Cui. 2014. Targeting Nrf2 by dihydro-CDDO-trifluoroethyl amide enhances autophagic clearance and viability of beta-cells in a setting of oxidative stress. *FEBS Lett.* 588:2115-2124.

Ling, M.T., X.Wang, X.Zhang, and Y.C.Wong. 2006. The multiple roles of Id-1 in cancer progression. *Differentiation* 74:481-487.

Ling, Z., J.C.Hannaert, and D.Pipeleers. 1994. Effect of nutrients, hormones and serum on survival of rat islet β cells in culture. *Diabetologia* 37:15-21.

Lu, H., V.Koshkin, E.M.Allister, A.V.Gyulkhandanyan, and M.B.Wheeler. 2010. Molecular and metabolic evidence for mitochondrial defects associated with β-cell dysfunction in a mouse model of type 2 diabetes. *Diabetes* 59:448-459.

Lu, H., Y.Yang, E.M.Allister, N.Wijesekara, and M.B.Wheeler. 2008. The identification of potential factors associated with the development of type 2 diabetes: a quantitative proteomics approach. *Mol. Cell Proteomics.* 7:1434-1451.

Maedler, K., G.A.Spinas, R.Lehmann, P.Sergeev, M.Weber, A.Fontana, N.Kaiser, and M.Y.Donath. 2001. Glucose induces β-cell apoptosis via upregulation of the Fas receptor in human islets. *Diabetes* 50:1683-1690.

Marinho, H.S., C.Real, L.Cyrne, H.Soares, and F.Antunes. 2014. Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox. Biol.* 2:535-562.

Martens, G.A., Y.Cai, S.Hinke, G.Stange, C.M.Van de, and D.Pipeleers. 2005. Glucose suppresses superoxide generation in metabolically responsive pancreatic β cells. *J. Biol. Chem.* in press, published online.

Matsuoka, T., Y.Kajimoto, H.Watada, H.Kaneto, M.Kishimoto, Y.Umayahara, Y.Fujitani, T.Kamada, R.Kawamori, and Y.Yamasaki. 1997. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. *J. Clin. Invest* 99:144-150.

McKenzie, M.D., E.Jamieson, E.S.Jansen, C.L.Scott, D.C.Huang, P.Bouillet, J.Allison, T.W.Kay, A.Strasser, and H.E.Thomas. 2010. Glucose induces pancreatic islet cell apoptosis that requires the BH3-only proteins Bim and Puma and multi-BH domain protein Bax. *Diabetes* 59:644-652.

Molina, A.J., J.D.Wikstrom, L.Stiles, G.Las, H.Mohamed, A.Elorza, G.Walzer, G.Twig, S.Katz, B.E.Corkey, and O.S.Shirihai. 2009. Mitochondrial networking protects beta-cells from nutrient-induced apoptosis. *Diabetes* 58:2303-2315.

Nair, R., W.S.Teo, V.Mittal, and A.Swarbrick. 2014. ID Proteins Regulate Diverse Aspects of Cancer Progression and Provide Novel Therapeutic Opportunities. *Mol. Ther.*

Nakayama, M., T.Inoguchi, T.Sonta, Y.Maeda, S.Sasaki, F.Sawada, H.Tsubouchi, N.Sonoda, K.Kobayashi, H.Sumimoto, and H.Nawata. 2005. Increased expression of NAD(P)H oxidase in islets of animal models of Type 2 diabetes and its improvement by an AT1 receptor antagonist. *Biochem. Biophys. Res. Commun.* 332:927-933.

Newman, J.R., and A.E.Keating. 2003. Comprehensive identification of human bZIP interactions with coiled-coil arrays. *Science* 300:2097-2101.

Nickenig, G., S.Baudler, C.Muller, C.Werner, N.Werner, H.Welzel, K.Strehlow, and M.Bohm. 2002. Redox-sensitive vascular smooth muscle cell proliferation is mediated by GKLF and Id3 in vitro and in vivo. *FASEB J.* 16:1077-1086.

Park, C.B., and N.G.Larsson. 2011. Mitochondrial DNA mutations in disease and aging. *J. Cell Biol.* 193:809-818.

Pi, J., Y.Bai, Q.Zhang, V.Wong, L.M.Floering, K.Daniel, J.M.Reece, J.T.Deeney, M.E.Andersen, B.E.Corkey, and S.Collins. 2007. Reactive oxygen species as a signal in glucose-stimulated insulin secretion. *Diabetes* 56:1783-1791.

Picard, M., O.S.Shirihai, B.J.Gentil, and Y.Burelle. 2013. Mitochondrial morphology transitions and functions: implications for retrograde signaling? *Am. J. Physiol Regul. Integr. Comp Physiol* 304:R393-R406.

Piro, S., M.Anello, C.Di Pietro, M.N.Lizzio, G.Patane, A.M.Rabuazzo, R.Vigneri, M.Purrello, and F.Purrello. 2002. Chronic exposure to free fatty acids or high glucose induces apoptosis in rat pancreatic islets: possible role of oxidative stress. *Metabolism* 51:1340-1347.

Rahier, J., Y.Guiot, R.M.Goebbels, C.Sempoux, and J.C.Henquin. 2008. Pancreatic β-cell mass in European subjects with type 2 diabetes. *Diabetes Obes. Metab* 10 Suppl 4:32-42.

Rhee, S.G. 2006. Cell signaling. H_2O_2, a necessary evil for cell signaling. *Science* 312:1882-1883.

Robertson, R.P. 2004. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet β cells in diabetes. *J. Biol. Chem.* 279:42351-42354.

Robertson, R.P., and J.S.Harmon. 2006. Diabetes, glucose toxicity, and oxidative stress: A case of double jeopardy for the pancreatic islet β cell. *Free Radic. Biol. Med.* 41:177-184.

Roma, L.P., S.M.Pascal, J.Duprez, and J.C.Jonas. 2012. Mitochondrial oxidative stress contributes differently to rat pancreatic islet cell apoptosis and insulin secretory defects after prolonged culture in a low non-stimulating glucose concentration. *Diabetologia* 55:2226-2237.

Ruzinova, M.B., and R.Benezra. 2003. Id proteins in development, cell cycle and cancer. *Trends Cell Biol.* 13:410-418.

Sakai, K., K.Matsumoto, T.Nishikawa, M.Suefuji, K.Nakamaru, Y.Hirashima, J.Kawashima, T.Shirotani, K.Ichinose, M.Brownlee, and E.Araki. 2003. Mitochondrial reactive oxygen species reduce insulin secretion by pancreatic β-cells. *Biochem. Biophys. Res. Commun.* 300:216-222.

Sakuraba, H., H.Mizukami, N.Yagihashi, R.Wada, C.Hanyu, and S.Yagihashi. 2002. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. *Diabetologia* 45:85-96.

Scott, G.J., M.K.Ray, T.Ward, K.McCann, S.Peddada, F.X.Jiang, and Y.Mishina. 2009. Abnormal glucose metabolism in heterozygous mutant mice for a type I receptor required for BMP signaling. *Genesis.* 47:385-391.

Shimohata, H., K.Yoh, N.Morito, H.Shimano, T.Kudo, and S.Takahashi. 2006. MafK overexpression in pancreatic beta-cells caused impairment of glucose-stimulated insulin secretion. *Biochem. Biophys. Res. Commun.* 346:671-680.

Song, B., D.Scheuner, D.Ron, S.Pennathur, and R.J.Kaufman. 2008. Chop deletion reduces oxidative stress, improves β cell function, and promotes cell survival in multiple mouse models of diabetes. *J Clin Invest* 118:3378-3389.

Stocker, R., and J.F.Keaney, Jr. 2004. Role of oxidative modifications in atherosclerosis. *Physiol Rev.* 84:1381-1478.

Tanaka, K., J.B.Pracyk, K.Takeda, Z.X.Yu, V.J.Ferrans, S.S.Deshpande, M.Ozaki, P.M.Hwang, C.J.Lowenstein, K.Irani, and T.Finkel. 1998. Expression of Id1 results in apoptosis of cardiac myocytes through a redox-dependent mechanism. *J. Biol. Chem.* 273:25922-25928.

Tanaka, Y., P.O.Tran, J.Harmon, and R.P.Robertson. 2002. A role for glutathione peroxidase in protecting pancreatic β cells against oxidative stress in a model of glucose toxicity. *Proc. Natl. Acad. Sci. U. S. A* 99:12363-12368.

Tanigawa, S., C.H.Lee, C.S.Lin, C.C.Ku, H.Hasegawa, S.Qin, A.Kawahara, Y.Korenori, K.Miyamori, M.Noguchi, L.H.Lee, Y.C.Lin, C.L.Steve Lin, Y.Nakamura, C.Jin, N.Yamaguchi, R.Eckner, D.X.Hou, and K.K.Yokoyama. 2013. Jun dimerization protein 2 is a critical component of the Nrf2/MafK complex regulating the response to ROS homeostasis. *Cell Death Dis.* 4:e921.

Tiedge, M., S.Lortz, J.Drinkgern, and S.Lenzen. 1997. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* 46:1733-1742.

Trabosh, V.A., A.Daher, K.A.Divito, K.Amin, C.M.Simbulan-Rosenthal, and D.S.Rosenthal. 2009. UVB upregulates the bax promoter in immortalized human keratinocytes via ROS induction of Id3. *Exp. Dermatol.* 18:387-395.

Turrens, J.F. 2003. Mitochondrial formation of reactive oxygen species. *J. Physiol* 552:335-344.

Uruno, A., Y.Furusawa, Y.Yagishita, T.Fukutomi, H.Muramatsu, T.Negishi, A.Sugawara, T.W.Kensler, and M.Yamamoto. 2013. The Keap1-Nrf2 system prevents onset of diabetes mellitus. *Mol. Cell Biol.* 33:2996-3010.

Wali, J.A., D.Rondas, M.D.McKenzie, Y.Zhao, L.Elkerbout, S.Fynch, E.N.Gurzov, S.Akira, C.Mathieu, T.W.Kay, L.Overbergh, A.Strasser, and H.E.Thomas. 2014. The proapoptotic BH3-only proteins Bim and Puma are downstream of endoplasmic reticulum and mitochondrial oxidative stress in pancreatic islets in response to glucotoxicity. *Cell Death Dis.* 5:e1124.

Webb, G.C., M.S.Akbar, C.Zhao, and D.F.Steiner. 2000. Expression profiling of pancreatic β cells: glucose regulation of secretory and metabolic pathway genes. *Proc. Natl. Acad. Sci. U. S. A* 97:5773-5778.

Wice, B.M., E.Bernal-Mizrachi, and M.A.Permutt. 2001. Glucose and other insulin secretagogues induce, rather than inhibit, expression of Id-1 and Id-3 in pancreatic islet beta cells. *Diabetologia* 44:453-463.

Yagishita, Y., T.Fukutomi, A.Sugawara, H.Kawamura, T.Takahashi, J.Pi, A.Uruno, and M.Yamamoto. 2014. Nrf2 protects pancreatic beta-cells from oxidative and nitrosative stress in diabetic model mice. *Diabetes* 63:605-618.

Yakes, F.M., and H.B.Van. 1997. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc. Natl. Acad. Sci. U. S. A* 94:514-519.

Yamazaki, H., F.Katsuoka, H.Motohashi, J.D.Engel, and M.Yamamoto. 2012. Embryonic lethality and fetal liver apoptosis in mice lacking all three small Maf proteins. *Mol. Cell Biol.* 32:808-816.

Yoon, K.H., S.H.Ko, J.H.Cho, J.M.Lee, Y.B.Ahn, K.H.Song, S.J.Yoo, M.I.Kang, B.Y.Cha, K.W.Lee, H.Y.Son, S.K.Kang, H.S.Kim, I.K.Lee, and S.Bonner-Weir. 2003. Selective β-cell loss and α-cell expansion in patients with type 2 diabetes mellitus in Korea. *Journal of Clinical Endocrinology and Metabolism* 88:2300-2308.

Figure 1

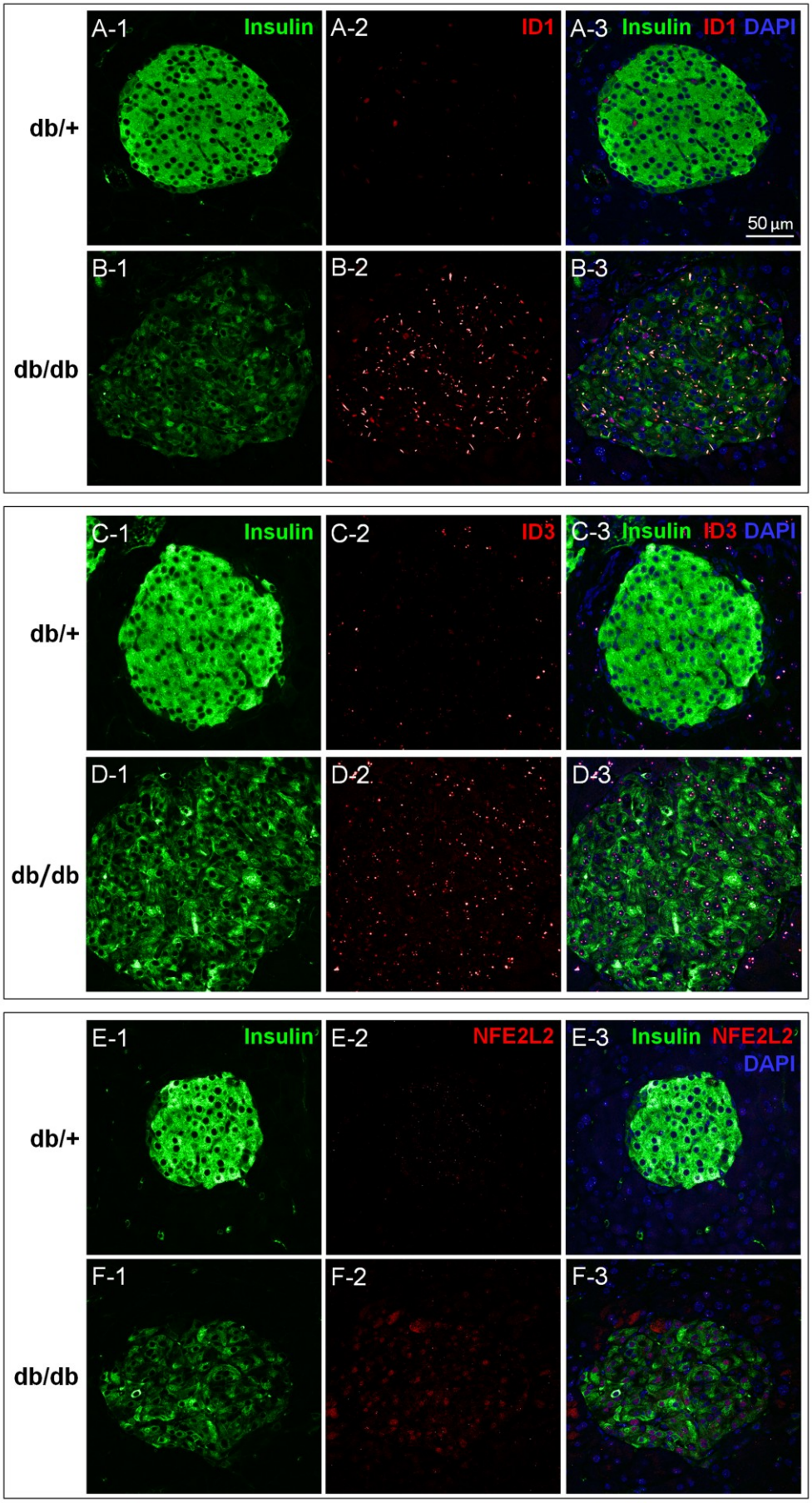

A-1
Insulin
A-2
ID1
A-3
Insulin
ID1
DAPI
50 μm
db/+
B-1
B-2
B-3
db/db
C-1
Insulin
C-2
ID3
C-3
Insulin
ID3
DAPI
db/+
D-1
D-2
D-3
db/db
E-1
Insulin
E-2
NFE2L2
E-3
Insulin
NFE2L2
DAPI
db/+
F-1
F-2
F-3
db/db

Figure 1

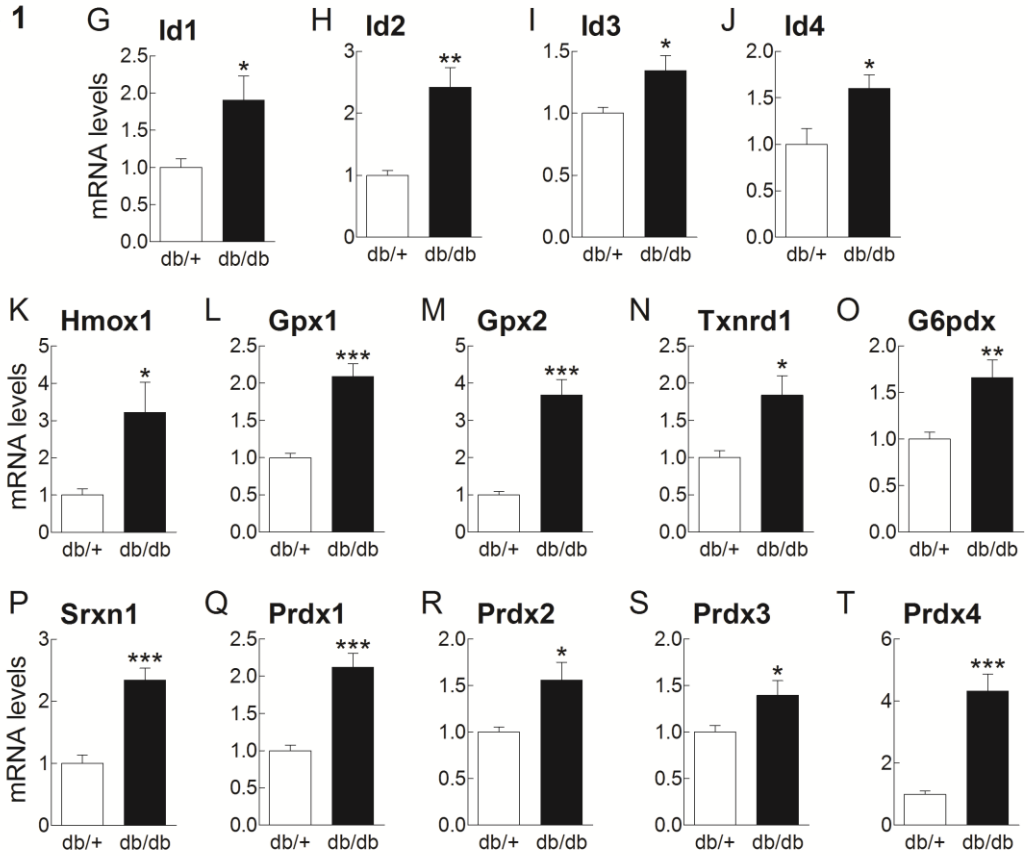

G
Id1
mRNA levels
2.5
2.0
1.5
1.0
0.5
0.0
*
db/+
db/db
H
Id2
3
2
1
0
**
db/+
db/db
I
Id3
1.5
1.0
0.5
0.0
*
db/+
db/db
J
Id4
2.0
1.5
1.0
0.5
0.0
*
db/+
db/db
K
Hmox1
mRNA levels
5
4
3
2
1
0
*
db/+
db/db
L
Gpx1
2.5
2.0
1.5
1.0
0.5
0.0
***
db/+
db/db
M
Gpx2
5
4
3
2
1
0
***
db/+
db/db
N
Txnrd1
2.5
2.0
1.5
1.0
0.5
0.0
*
db/+
db/db
O
G6pdx
2.0
1.5
1.0
0.5
0.0
**
db/+
db/db
P
Srxn1
mRNA levels
3
2
1
0
***
db/+
db/db
Q
Prdx1
2.5
2.0
1.5
1.0
0.5
0.0
***
db/+
db/db
R
Prdx2
2.0
1.5
1.0
0.5
0.0
*
db/+
db/db
S
Prdx3
2.0
1.5
1.0
0.5
0.0
*
db/+
db/db
T
Prdx4
6
4
2
0
***
db/+
db/db

Figure 2

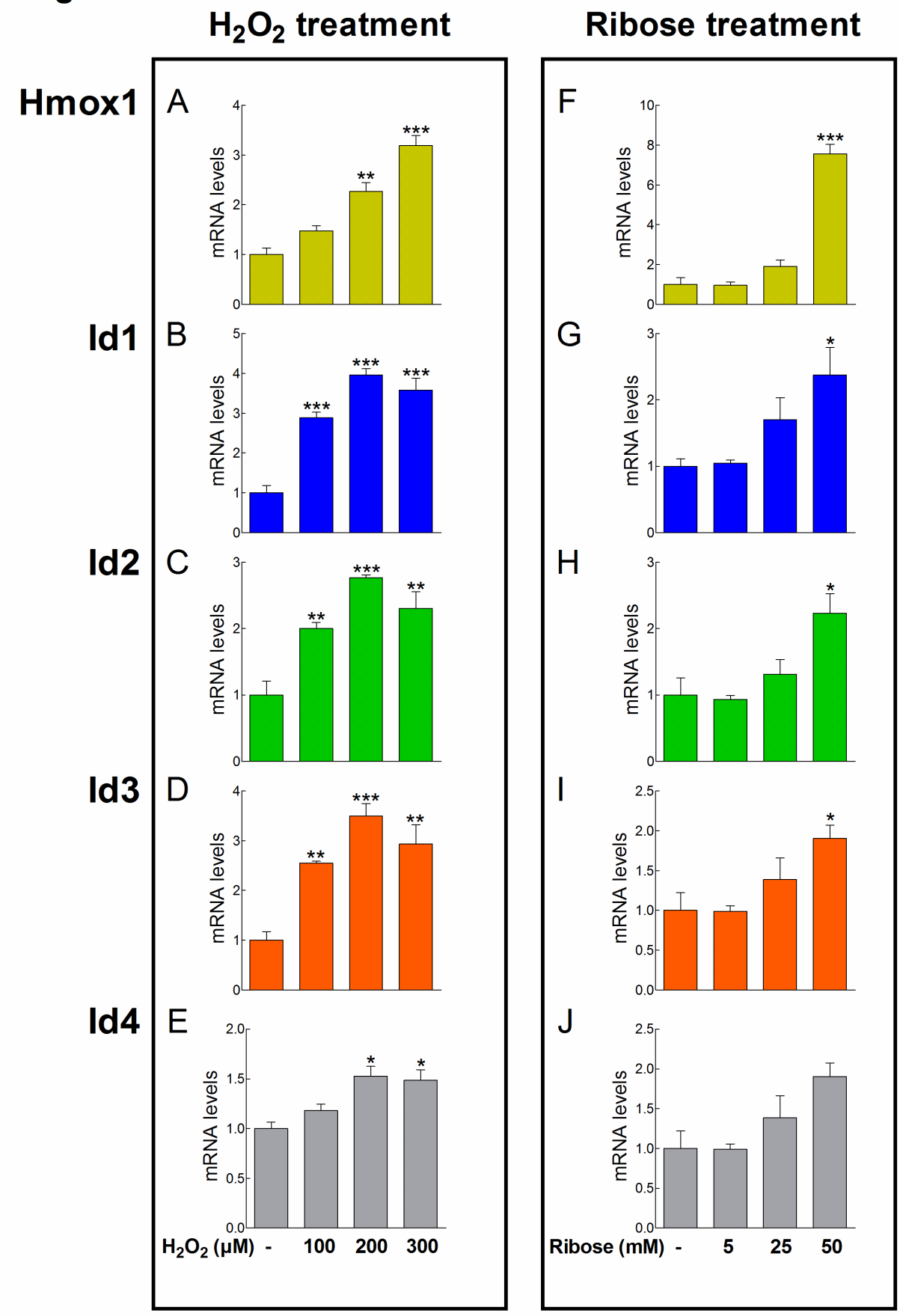

H2O2 treatment
Ribose treatment
Hmox1
Id1
Id2
Id3
Id4
A
B
C
D
E
F
G
H
I
J
mRNA levels
H2O2 (μM) - 100 200 300
Ribose (mM) - 5 25 50

Figure 3

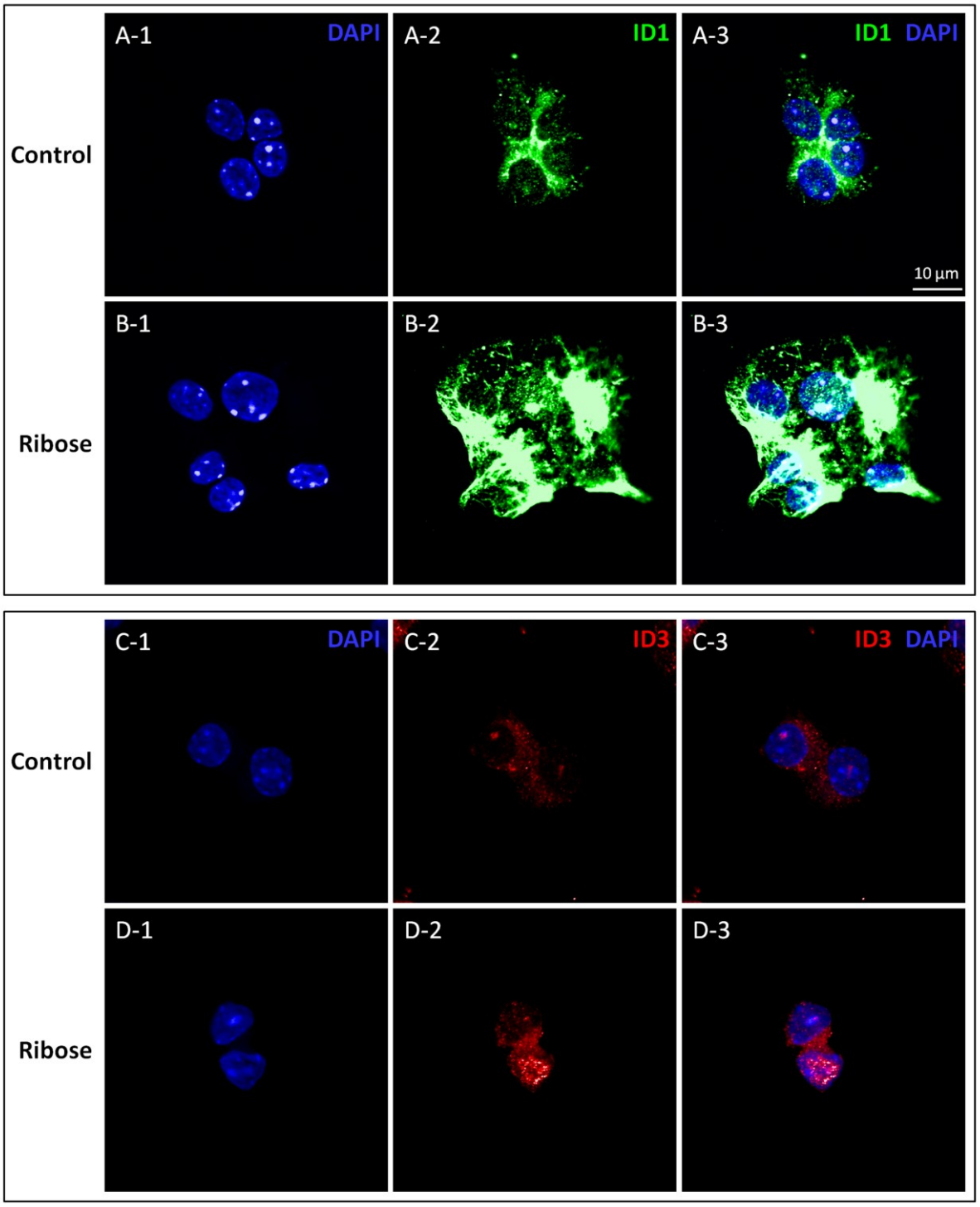

Control
A-1
DAPI
A-2
ID1
A-3
ID1 DAPI
10 µm
Ribose
B-1
B-2
B-3
Control
C-1
DAPI
C-2
ID3
C-3
ID3 DAPI
Ribose
D-1
D-2
D-3

Figure 4

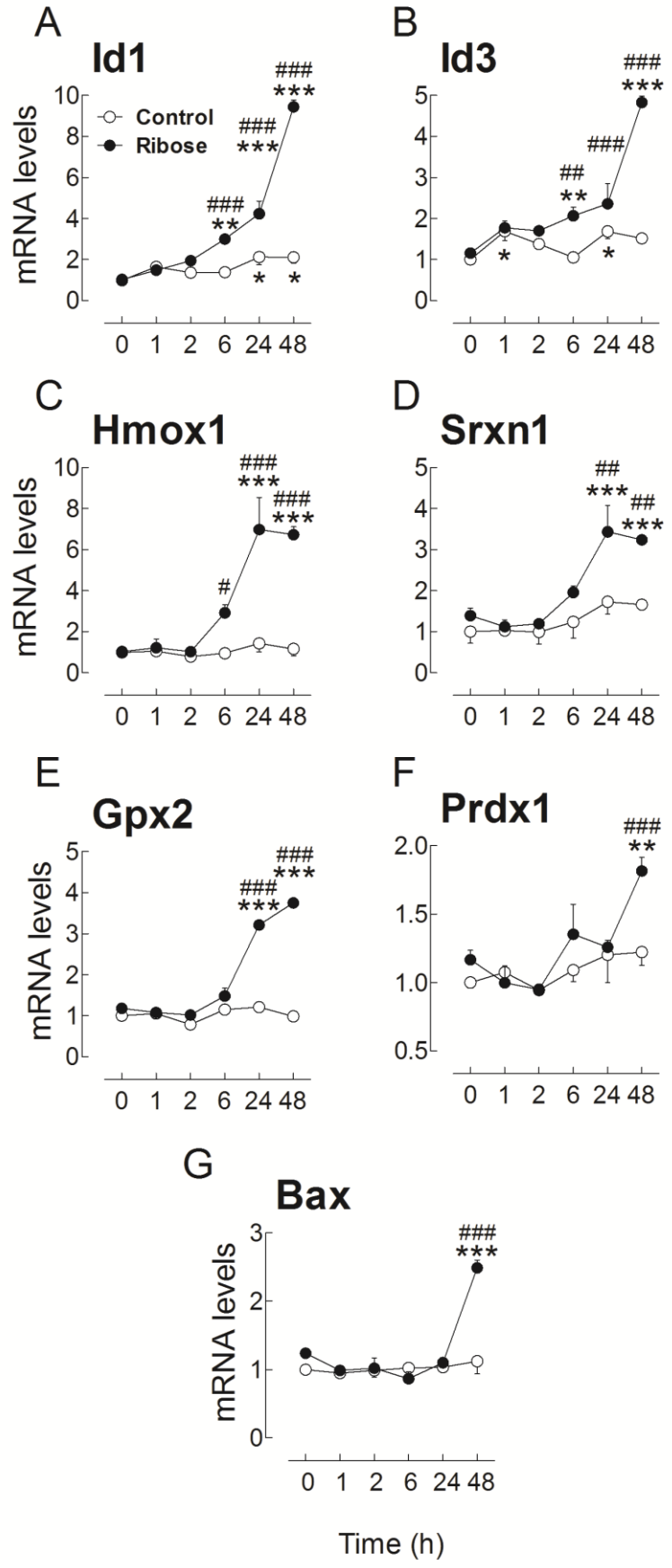

A
Id1
Control
Ribose
mRNA levels
B
Id3
C
Hmox1
D
Srxn1
E
Gpx2
F
Prdx1
G
Bax
Time (h)

Figure 5

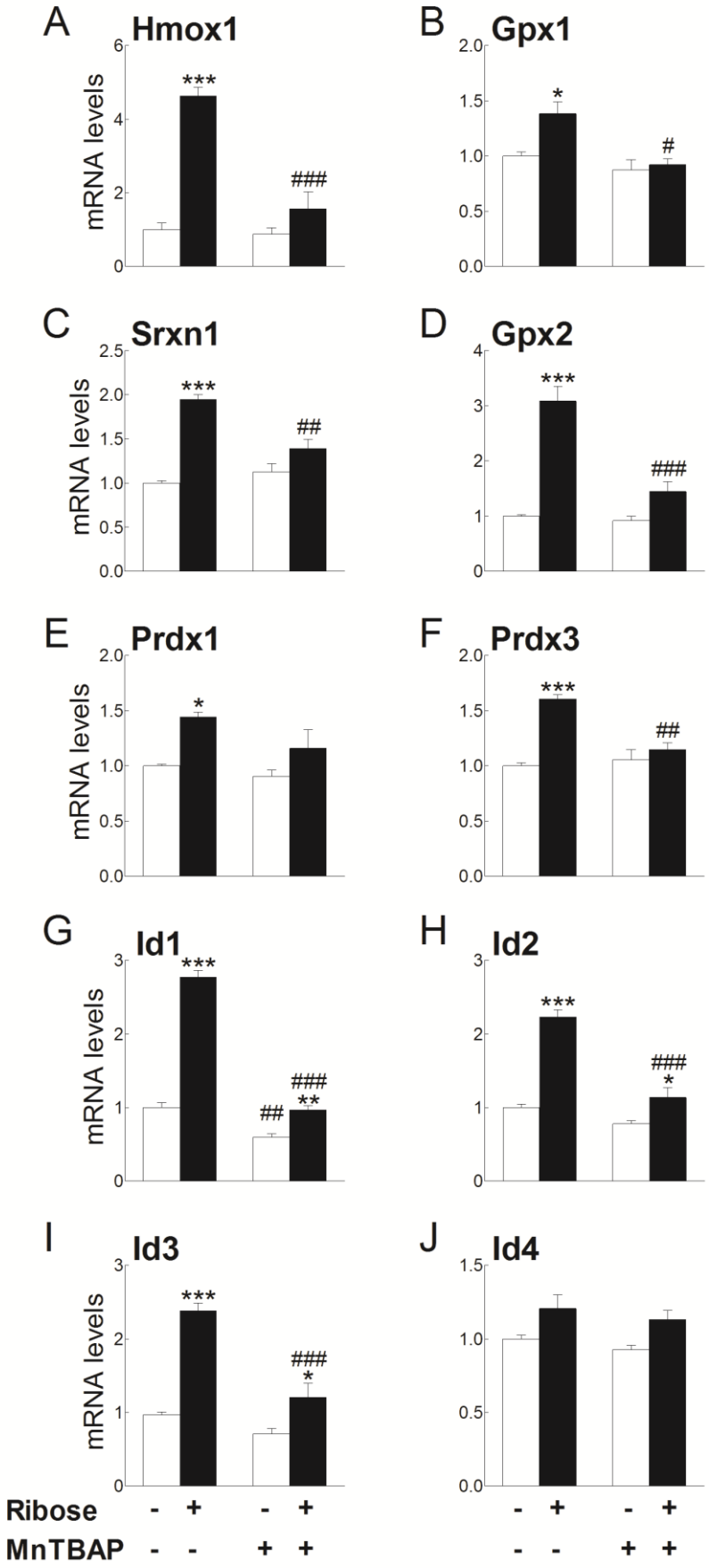

A
Hmox1
mRNA levels
***
###
B
Gpx1
*
#
C
Srxn1
***
##
D
Gpx2
***
###
E
Prdx1
*
F
Prdx3
***
##
G
Id1
***
###
**
##
H
Id2
***
###
*
I
Id3
***
###
*
J
Id4
Ribose - + - +
MnTBAP - - + +

Figure 6

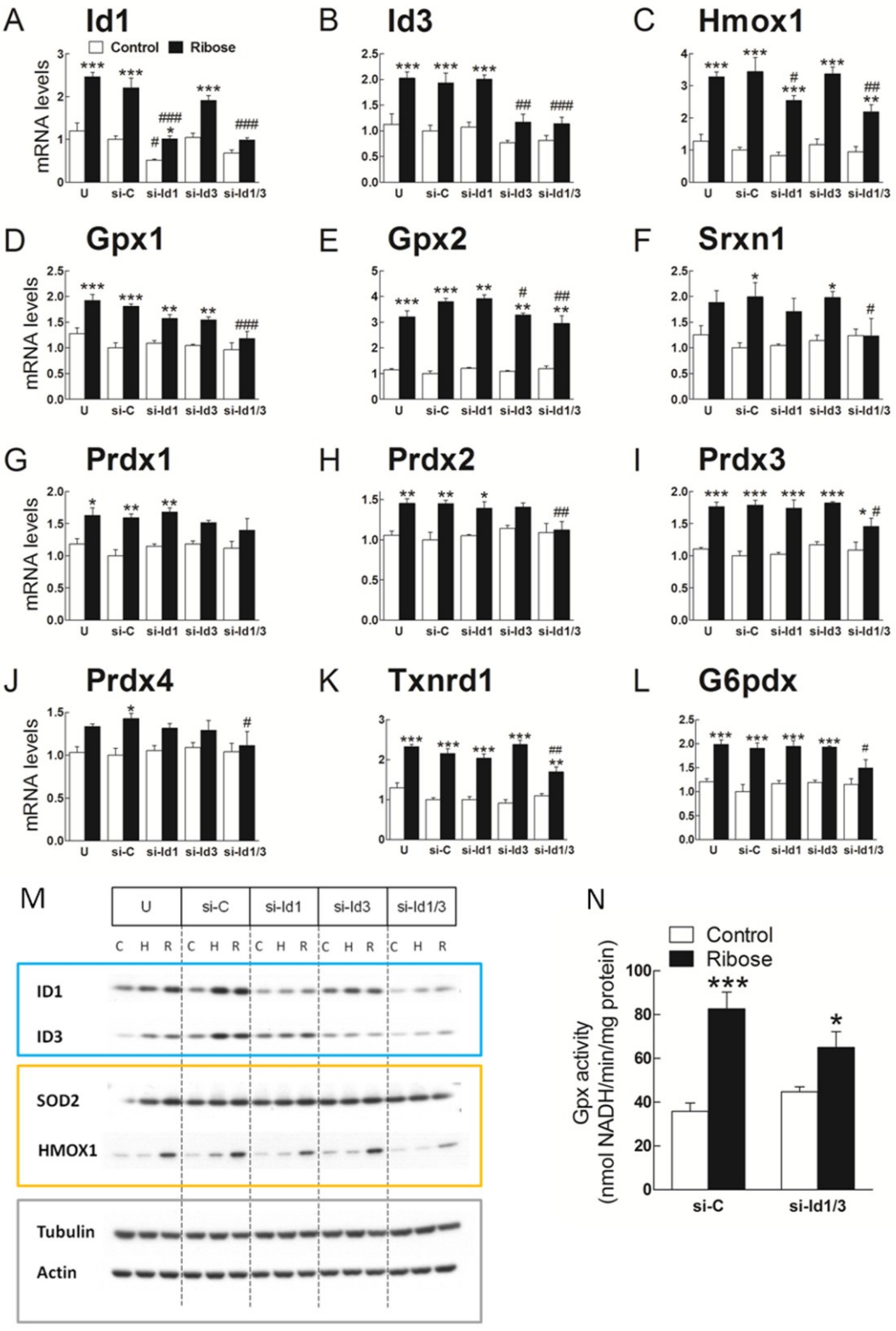

A
Id1
Control
Ribose
mRNA levels
B
Id3
C
Hmox1
D
Gpx1
E
Gpx2
F
Srxn1
G
Prdx1
H
Prdx2
I
Prdx3
J
Prdx4
K
Txnrd1
L
G6pdx
U
si-C
si-Id1
si-Id3
si-Id1/3
M
C H R
ID1
ID3
SOD2
HMOX1
Tubulin
Actin
N
Control
Ribose
Gpx activity
(nmol NADH/min/mg protein)
si-C
si-Id1/3

Figure 7

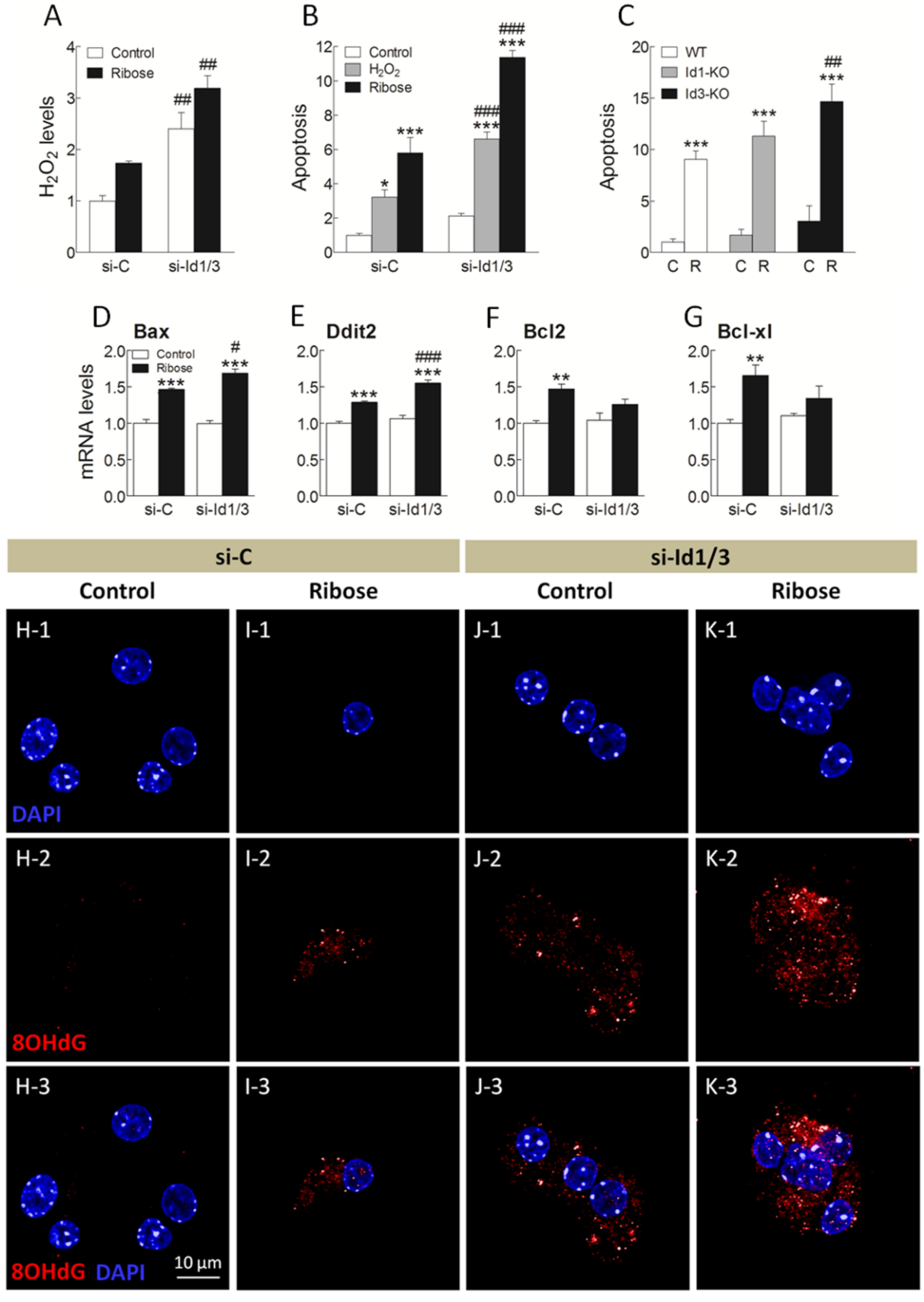

A
Control
Ribose
H_2O_2 levels
si-C
si-Id1/3
B
Control
H_2O_2
Ribose
Apoptosis
si-C
si-Id1/3
C
WT
Id1-KO
Id3-KO
Apoptosis
C R C R C R
D
Bax
mRNA levels
E
Ddit2
F
Bcl2
G
Bcl-xl
si-C
si-Id1/3
Control
Ribose
Control
Ribose
H-1
I-1
J-1
K-1
DAPI
H-2
I-2
J-2
K-2
8OHdG
H-3
I-3
J-3
K-3
8OHdG DAPI
10 μm

Figure 8

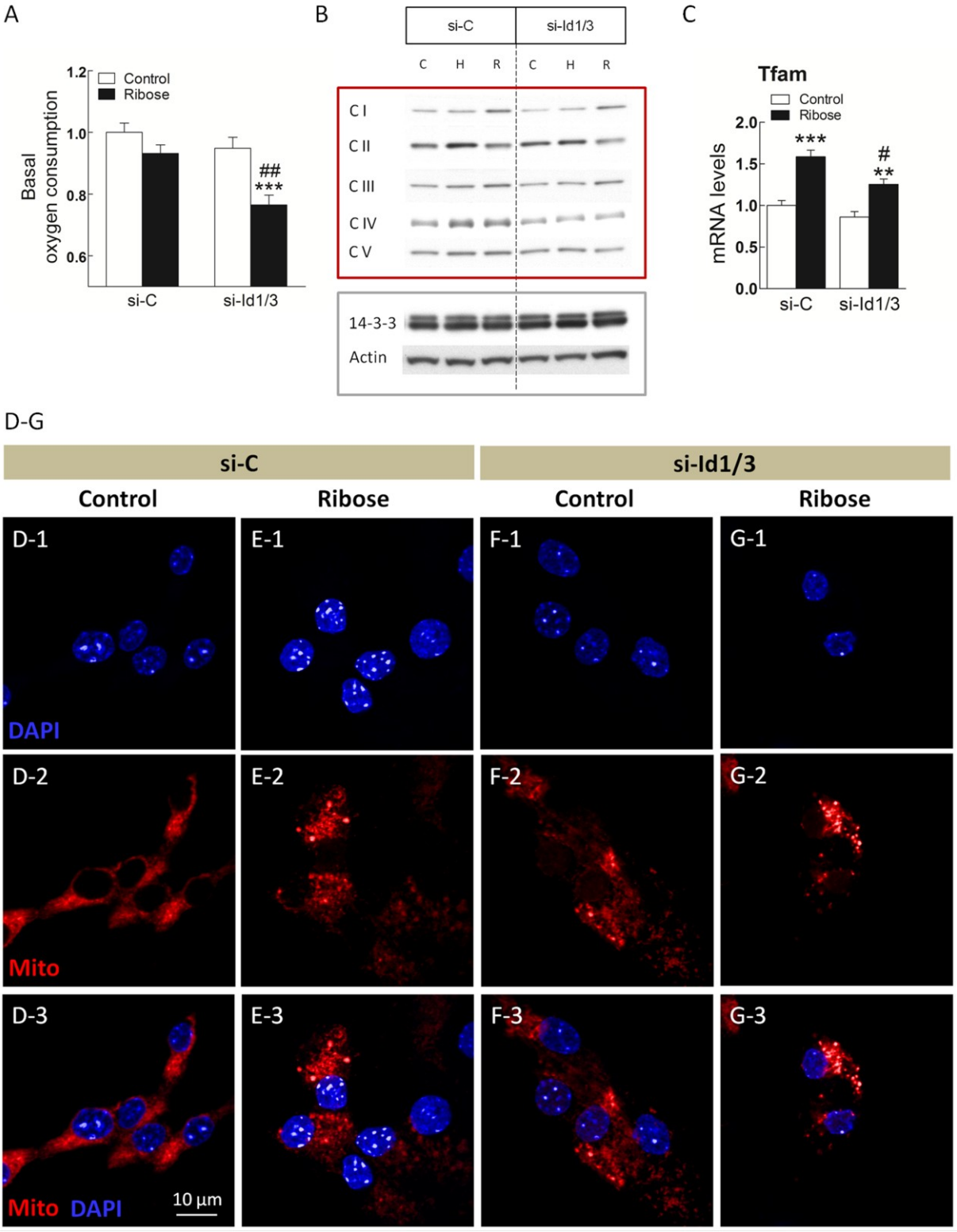

A
Basal oxygen consumption
Control
Ribose
1.2
1.0
0.8
0.6
##
***
si-C
si-Id1/3
B
si-C
si-Id1/3
C H R C H R
C I
C II
C III
C IV
C V
14-3-3
Actin
C
Tfam
Control
Ribose
mRNA levels
2.0
1.5
1.0
0.5
0.0
***
#
**
si-C
si-Id1/3
D-G
si-C
si-Id1/3
Control
Ribose
Control
Ribose
D-1
E-1
F-1
G-1
DAPI
D-2
E-2
F-2
G-2
Mito
D-3
E-3
F-3
G-3
Mito DAPI
10 μm

Figure 8

H-K

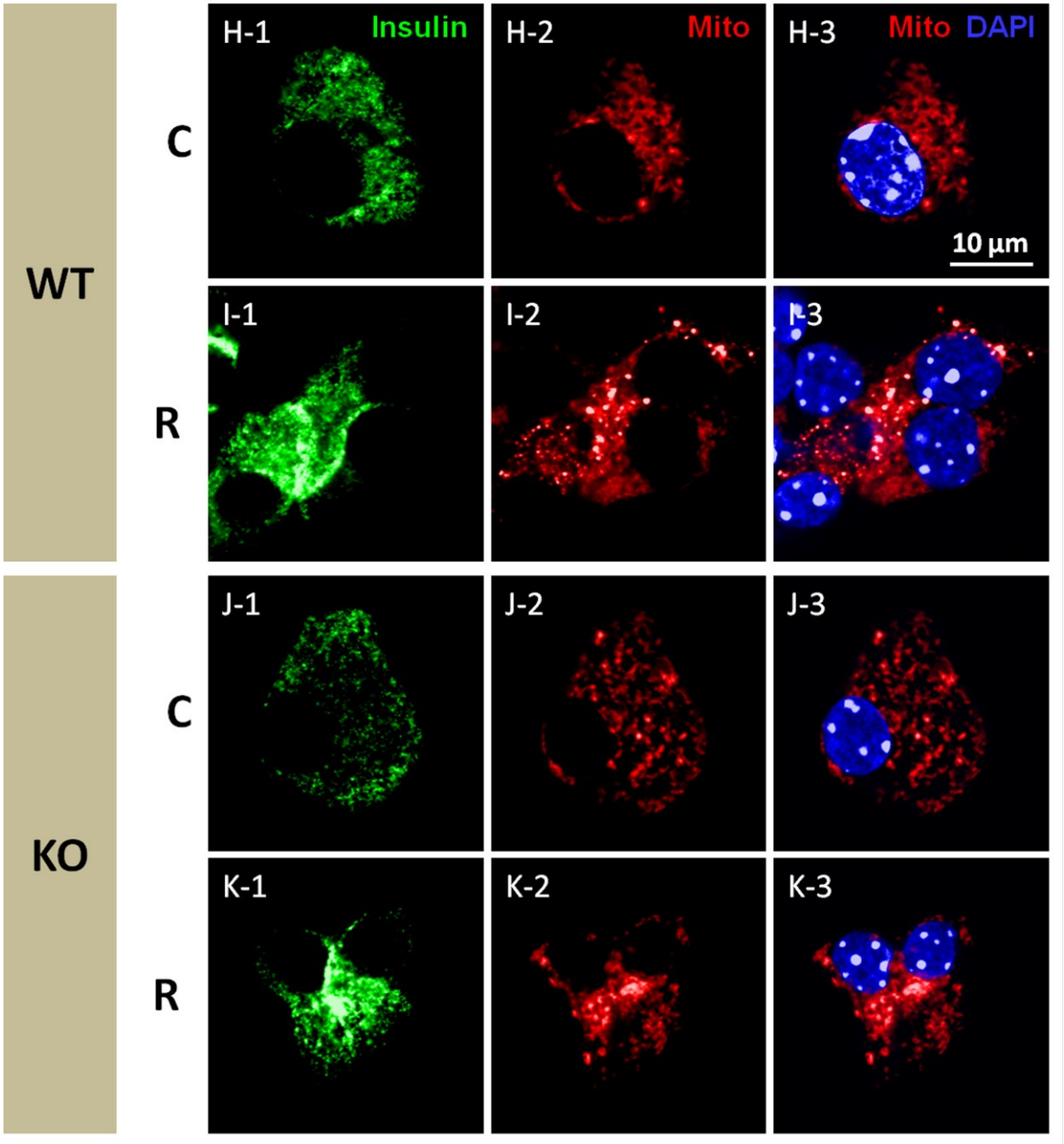

WT
KO
C
R
C
R
H-1
Insulin
H-2
Mito
H-3
Mito DAPI
10 µm
I-1
I-2
I-3
J-1
J-2
J-3
K-1
K-2
K-3

Figure 9

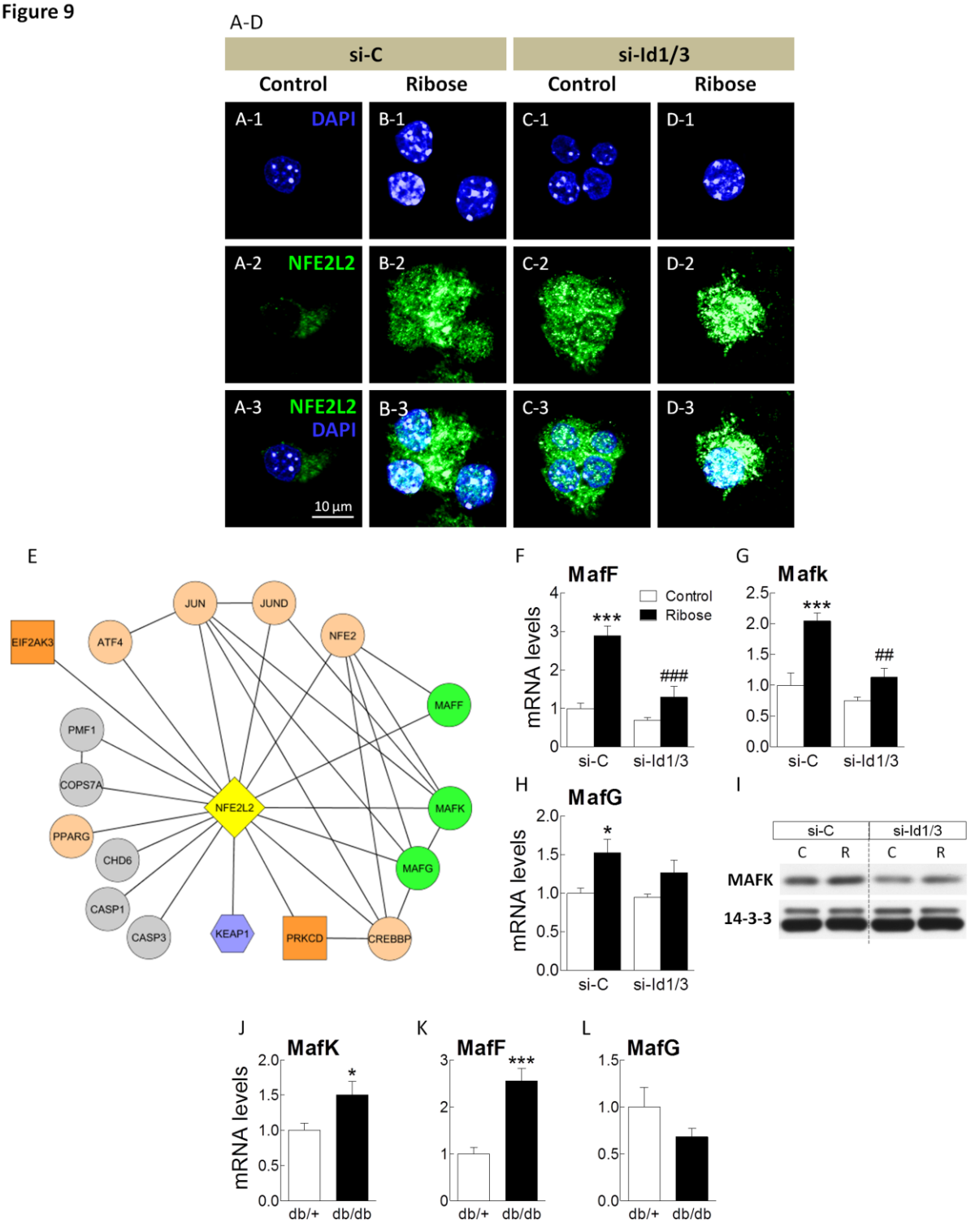

A-D
si-C
si-Id1/3
Control
Ribose
Control
Ribose
A-1 DAPI
B-1
C-1
D-1
A-2 NFE2L2
B-2
C-2
D-2
A-3 NFE2L2 DAPI
B-3
C-3
D-3
10 µm
E
JUN
JUND
EIF2AK3
ATF4
NFE2
MAFF
PMF1
COPS7A
NFE2L2
MAFK
PPARG
CHD6
MAFG
CASP1
CASP3
KEAP1
PRKCD
CREBBP
F
MafF
Control
Ribose
mRNA levels
***
###
si-C
si-Id1/3
G
Mafk
***
##
si-C
si-Id1/3
H
MafG
mRNA levels
*
si-C
si-Id1/3
I
si-C
si-Id1/3
C
R
C
R
MAFK
14-3-3
J
MafK
mRNA levels
*
db/+
db/db
K
MafF
***
db/+
db/db
L
MafG
db/+
db/db

Figure 10

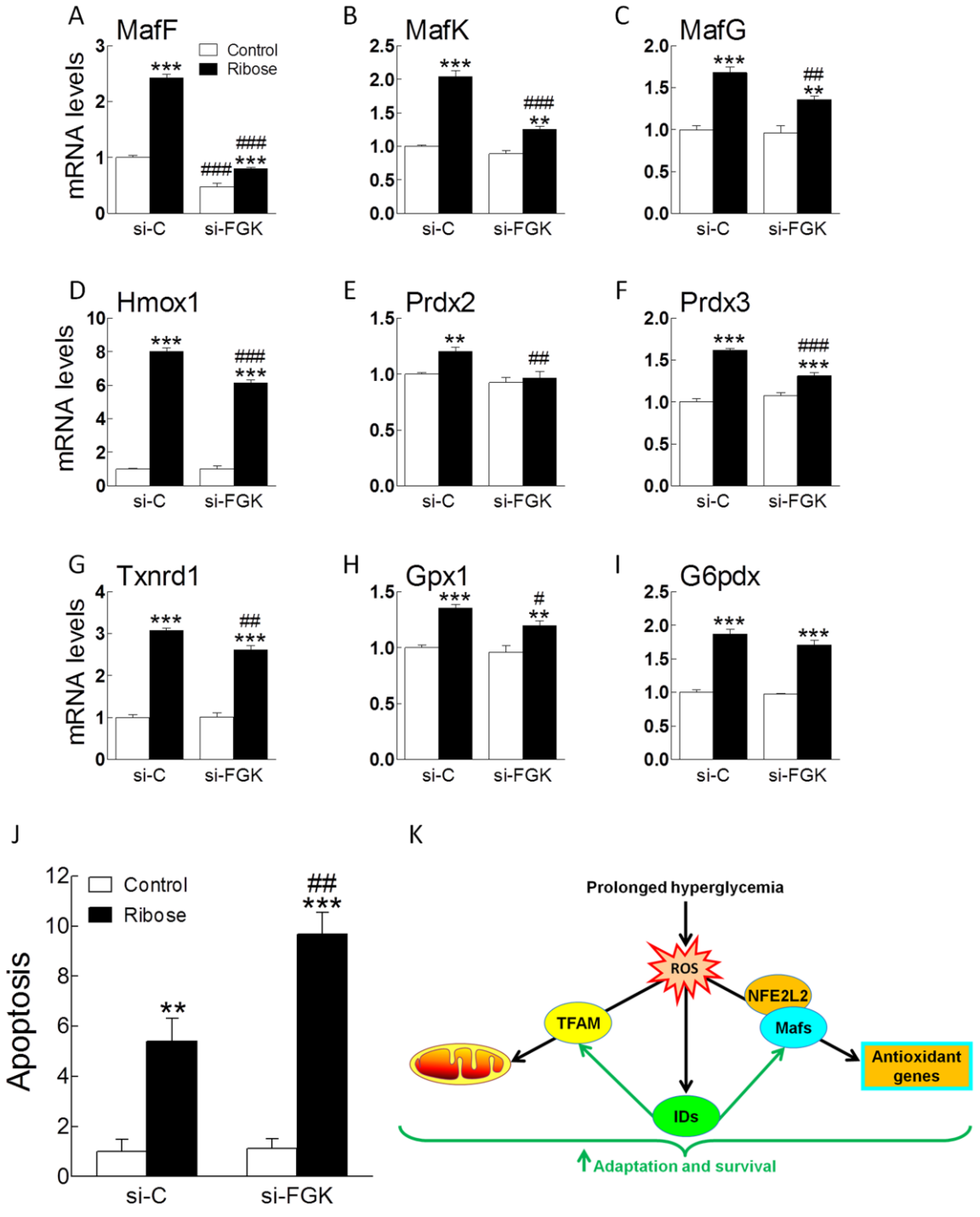

A
MafF
Control
Ribose
mRNA levels
B
MafK
C
MafG
D
Hmox1
E
Prdx2
F
Prdx3
G
Txnrd1
H
Gpx1
I
G6pdx
si-C
si-FGK
J
Apoptosis
K
Prolonged hyperglycemia
ROS
NFE2L2
TFAM
Mafs
Antioxidant genes
IDs
Adaptation and survival

Figure S1

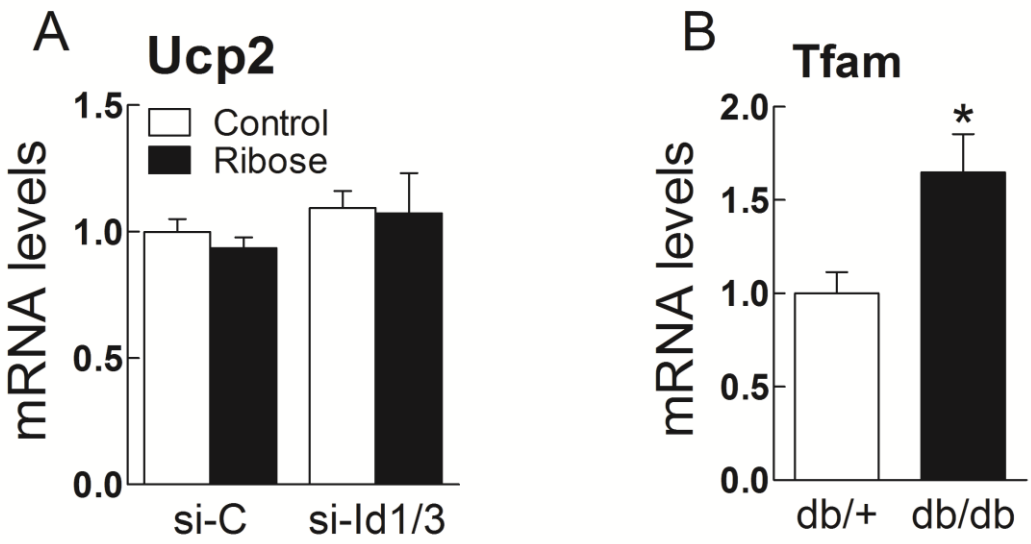
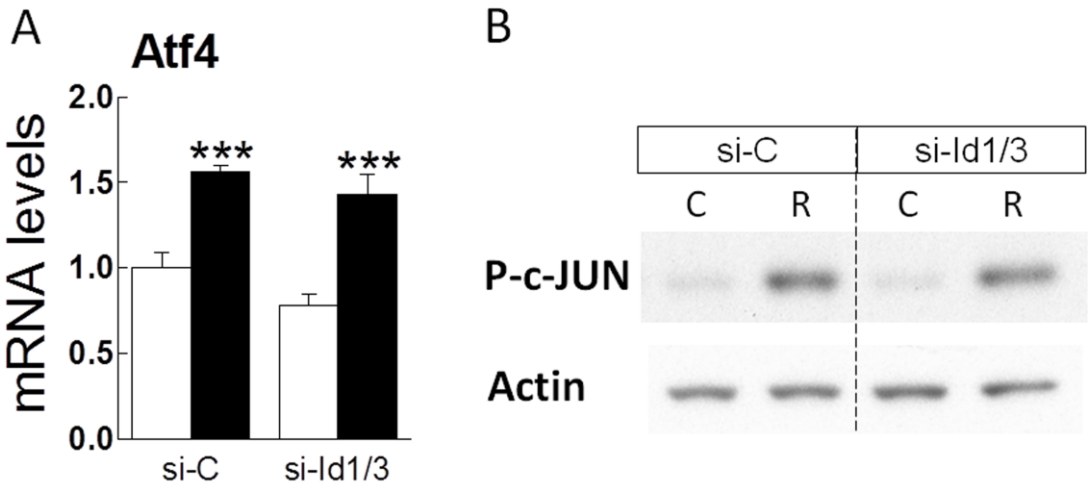

A
Ucp2
Control
Ribose
mRNA levels
1.5
1.0
0.5
0.0
si-C
si-Id1/3
B
Tfam
*
mRNA levels
2.0
1.5
1.0
0.5
0.0
db/+
db/db

Figure S2


A
Atf4
mRNA levels
2.0
1.5
1.0
0.5
0.0
***
***
si-C
si-Id1/3
B
si-C
si-Id1/3
C
R
C
R
P-c-JUN
Actin

Supplementary Table S1. Sequences of oligonucleotide primers (5'-3')

Gene symbol	5' Oligonucleotide	3' Oligonucleotide
Bax	TGCAGAGGATGATTGCTGAC	GATCAGCTCGGGCACTTTAG
Bcl2	TCTGAAGGATTGATGGCAGA	CATCAGCCACGCCTAAAAGT
Bcl-xl (Bcl2l2)	CCATTGCTACCAGGAGAACC	AGGAGCTGGTTTAGGGGAAA
Cyclophilin (Cypa, Ppia)	TGTGCCAGGGTGGTGACTTTAC	TGGGAACCGTTTGTGTTTGG
Ddit2 (Gadd45g)	AATGTGGACCCTGACAATGTGAC	GCTCTCCTCGCAGAACAAACTG
G6pdx (G6pd)	CCCACAGTCTATGAAGCAGTCACC	CCATCTCTTTGCCCAGGTAGTG
Gpx1 (GPx, CGPx)	ACAGTCCACCGTGTATGCCTTC	CTCTTCATTCTTGCCATTCTCCTG
Gpx2	TGATTGAGAATGTGGCGTCACTC	TTTGGGTAAGACTAAAGGTGGGC
Hmox1 (HO-1, Hsp32)	CCACACAGCACTATGTAAAGCGTC	GTTCGGGAAGGTAAAAAAAGCC
Id1	TTGGTCTGTCGGAGCAAAGC	GCAGGTCCCTGATGTAGTCGATTAC
Id2	CCTCCTACGAGCAGCATGAAAG	TGGTGATGCAGGCTGACGATAG
Id3	AACCCAGCCCTCTTCACTTACC	CCCATTCTCGGAAAAGCCAG
Id4	GAAGAGCAATCGTGAATCCAACC	AGAAAAAGTTCCCCGCCCTG
MafF	AGCAAAGCCCTGAAGGTCAAG	TGACGATGGTGATGACGCTG
MafG	TTAAAGGTGAAGCGGGAGCC	GCTGGATGATCTCTTCCTTGGAG
MafK	GCCCCTGTTCTTAGCGATGATG	GCGTAGCCTCTGTTCTTGAGTGTG
Prdx1	CTTTACTTTTGTGTGTCCCACGG	GTCCCAATCCTCCTTGTTTCTTG
Prdx2	AGTGTCTGTGGACTCTCAGTTCACC	CCCTGTAAGCAATGCCCTCATC
Prdx3	TTCCCACTTCAGTCATCTTGCC	TTCCAACAGCACTCCGTAGTCTC
Prdx4	GTGTGTCCAACTGAAATCATCGC	TGGGTAAACTGAGAGTCAACAGAGC
Srxn1	TACCAATCGCCGTGCTCATC	AAAGGAATAGTAGTAGTCGCCACCC
Tfam (Tcf6, Tcf6l2)	GGAATGTGGAGCGTGCTAAAAG	ACAAGACTGATAGACGAGGGGATG
Txnrd1	TGAAGAGCAGACCAATGTGC	AGCCTCCATACAGCCTCTGA
Ucp2	TCTCATCACTTTCCCTCTGGATACC	GAGCCCTTGGTGTAGAACTGTTTG

Aliases of gene symbols given in parentheses