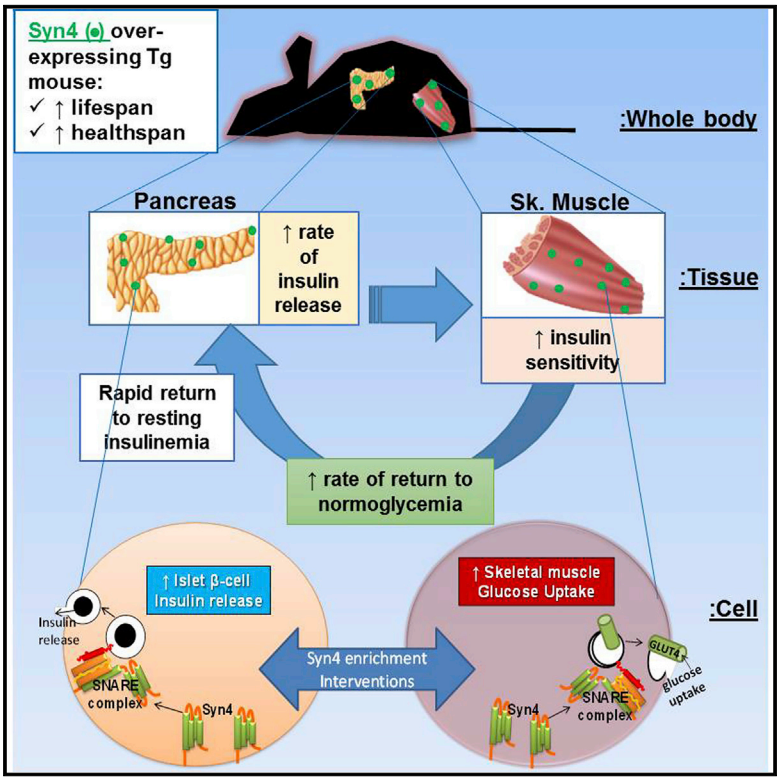
Short Article

Cell Metabolism

# Syntaxin 4 Overexpression Ameliorates Effects of Aging and High-Fat Diet on Glucose Control and Extends Lifespan

## Graphical Abstract




## Highlights

- Syntaxin 4 abundance is inversely related to aging
- Aged Syntaxin-4-enriched mice retain youthful glucose and insulin tolerances
- Syntaxin 4 enrichment protects from high-fat-induced muscle and islet derangements

## Authors

Eunjin Oh, Richard A. Miller, Debbie C. Thurmond

## Correspondence

dthurmond@coh.org

## In Brief

Oh et al. show that mice overexpressing the exocytosis protein Syntaxin 4 have increased insulin sensitivity and exhibit marked lifespan extension (+33%). These long-lived mice are protected from age- or diet-induced insulin resistance and loss of functional β cell.

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# Syntaxin 4 Overexpression Ameliorates Effects of Aging and High-Fat Diet on Glucose Control and Extends Lifespan

Eunjin Oh,[1,3] Richard A. Miller,[2] and Debbie C. Thurmond[1,3,*]
[1]Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, 46202, USA
[2]Department of Pathology and Geriatrics Center, University of Michigan School of Medicine, Ann Arbor, MI 48109-2200, USA
[3]Present address: Department of Molecular and Cellular Endocrinology, Beckman Research Institute of City of Hope, Duarte, CA 91010, USA
*Correspondence: dthurmond@coh.org
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## SUMMARY

**Indirect evidence suggests that improved insulin sensitivity may contribute to improved lifespan of mice in which aging has been slowed by mutations, drugs, or dietary means, even in stocks of mice that do not show signs of late-life diabetes. Peripheral responses to insulin can be augmented by overexpression of Syntaxin 4 (Syn4), a plasma-membrane-localized SNARE protein. We show here that Syn4 transgenic (Tg) mice with high level expression of Syn4 had a significant extension of lifespan (33% increase in median) and showed increased peripheral insulin sensitivity, even at ages where controls exhibited age-related insulin resistance. Moreover, skeletal muscle GLUT4 and islet insulin granule exocytosis processes were fully protected in Syn4 Tg mice challenged with a high-fat diet. Hence, high-level expressing Syn4 Tg mice may exert better glycemic control, which slows multiple aspects of aging and extends lifespan, even in non-diabetic mice.**

## INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2D) increases substantially with age. Both aging and T2D are accompanied by deleterious physiological changes, such as the development of insulin resistance, diminished glucose tolerance, fat accumulation, and loss of muscle mass (Biddinger and Kahn, 2006; Saltiel and Kahn, 2001). Despite a strong correlation between aging and T2D, the molecular events linking aging and T2D are unknown.

There are many mouse models in which slow aging and long life are associated with higher insulin sensitivity. This includes GHRKO, Snell and Ames dwarf, calorie restriction, and the crowded litter mice (Sadagurski et al., 2014). Further, the GHRKO and pituitary dwarf mutations slow many aspects of aging. This has led to a model in which excellent, and preserved, insulin sensitivity is postulated as a key causal factor (Bartke et al., 2003) in extended longevity in many kinds of experimental mice. Fat-specific deletion of insulin receptor (IR) also leads to longevity in mice, as does deletion of IR or IRS in some brain regions (Blüher et al., 2003; Taguchi et al., 2007). This too hints at links between insulin sensitivity and whole animal aging.

One clue from studies of centenarians demonstrates that this population displays increased peripheral insulin sensitivity and reduced circulating insulin concentrations and has reduced adipose tissue mass when compared to younger generations (Barbieri et al., 2003; Biddinger and Kahn, 2006; Klöting and Blüher, 2005; Paolisso et al., 1996; Rozing et al., 2010; Wijsman et al., 2011), suggesting that insulin signaling and/or synthesis genes may link diabetes and aging. Moreover, people with a mutation in GHR were protected against cancer and diabetes (Guevara-Aguirre et al., 2011). Several proteins thought to play a role in regulation of aging, including insulin like growth factor (IGF-1), mammalian target of rapamycin (mTOR), and mammalian homolog of the Silent information regulator 2 (SIRT1), have also been implicated in the control of glucose homeostasis and diabetes pathogenesis. In humans, genetic variation causing reduced insulin/IGF-1 signaling is associated with extended longevity (van Heemst et al., 2005). Similarly, IGF-1-receptor-deficient knockout mice as well as a model of reduced local IGF activity (pregnancy-associated plasma protein-A knockout mice) show increased longevity (Conover et al., 2008; Xu et al., 2014). Depletion of S6K1 (a downstream target protein of mTOR) in mice increased lifespan and maintained insulin sensitivity (Selman et al., 2009; Um et al., 2004). SIRT1 overexpression in brain-specific transgenic mice was recently shown to increase lifespan by ~11% (Satoh et al., 2013). However, while increased SIRT1 selectively in pancreatic β cells of transgenic mice enhanced glucose-stimulated insulin secretion and improved glucose tolerance at 8 months of age, these benefits were lost by 18 months of age (Moynihan et al., 2005; Ramsey et al., 2008). Reduced insulin sensitivity has also, in some circumstances, been associated with lifespan extension in mice, including that mediated by rapamycin (Harper et al., 2005; Miller et al., 2014). Hence, whether improved insulin secretion and/or increased insulin sensitivity are sufficient to promote extended lifespan remains in question.

Syn4 is a SNARE protein that functions at the distal end of the insulin signaling cascade to promote clearance of excess glucose from the blood. Syn4 is similarly required for pancreatic β cell insulin secretion in response to elevated blood glucose.

Therefore, changes in Syn4 protein levels affect two of the most important processes required to regulate blood glucose levels and maintain glucose homeostasis. Importantly, Syn4 protein levels are significantly reduced in human T2D islets (Oh et al., 2014), as are Syn4 mRNA levels in ob/ob (Keller et al., 2008) and STZ-diabetic mice (Yechoor et al., 2002); mice deficient in Syn4 exhibit insulin resistance and dysfunctional insulin secretion (Spurlin et al., 2004; Spurlin and Thurmond, 2006; Yang et al., 2001). Thus, deficiency of Syn4 may be associated with an increased susceptibility of the aged or obese to develop T2D. In the current study, we report the existence of a relationship between Syn4 abundance, healthspan and aging, pertinent to insulin-secreting and insulin-responsive cellular mechanisms affecting glucose homeostatic changes.

## RESULTS

### Relationship between Syn4 Abundance and Aging

Given the current queries into the relationship between longevity and glucose homeostasis, we investigated abundances of exocytosis proteins in pancreas lysates of differentially aged mice and found Syn4 to be selectively attenuated in a manner inversely related to that of the known aging-induced p-mTOR (Figure 1A). No differences in pancreatic abundances of Syn4-based SNARE partners VAMP2, SNAP23, or Munc18c were observed with aging (Figure S1). This observation led to a longitudinal study of Syn4 transgenic (Syn4 Tg) mice. The Syn4 Tg mice express higher levels of Syn4 protein specifically in the pancreas, skeletal muscle, and adipose tissues and exhibit enhanced insulin sensitivity and accelerated glucose clearance (Spurlin et al., 2004) and have the capacity to release ~30% more insulin per islet while retaining biphasic kinetics (Spurlin and Thurmond, 2006). Remarkably, the Syn4 Tg mice lived ~33% longer than wild-type control (Con) littermates ($p < 0.001$), with similar effects in male and female mice (Figures 1B–1D). In this regard, significant upregulation of Syn4 yields lifespan extension similar to that produced by caloric restriction, and more than rapamycin or acarbose treatments (Harrison et al., 2014; Miller et al., 2014). The overall lifespan of the control (Con) mice was somewhat shorter than expected for this strain of mice (Ikeno et al., 2005). Aging-related gene products such as SIRT1 or mTOR were unchanged in pancreata of Syn4 Tg mice relative to littermate controls (Figure 1E), despite the continued expression of the transgene in the aged Tg mice (Figure 1F; 2.1-fold more Syn4 protein compared with Con, $n = 4$). While the amount of phosphorylated FoxO1 (Ser256) was also unchanged in Syn4 Tg pancreata, total FoxO1 levels were 2-fold greater, thus decreasing the relative level of phosphorylated FoxO1 (Figure 1E). Given the trend for reduced Syn4 protein in aged Con mouse muscle (Figure S2) and the persistence of elevated Syn4 levels in the muscle of the aged Tg mice, (Figure 1F; 3.3-fold more Syn4 protein compared with Con, $n = 4$), we used microarray to assess potential relative changes in skeletal muscle genes. In those genes with absolute fold change $\geq 1.2$, Ingenuity pathway analysis revealed 12 pathways that were significantly changed (Table S1). Pathways identified included AMPK signaling, leptin signaling, cAMP, and nNOS signaling in skeletal muscle. Quantitative real-time PCR was used to confirm a set of seven differentially expressed genes from these pathways; of these, four were statistically significant (Table S2). These data collectively suggested the existence of a specific inverse relationship between aging and Syn4 abundance.

### Glucose Homeostatic Mechanisms in Long-Lived Syn4 Tg Mice

Comparing the insulin tolerance tests of 25-month-old mice with the response of this strain at 4–6 months of age (Spurlin et al., 2004), the Syn4 Tg mice showed enhanced insulin sensitivity (Figure 2A). Increased insulin sensitivity was particularly notable in Syn4 Tg males (Figure 2B), since aged C57BL/6 males are characteristically insulin resistant (Lee et al., 2010). Consistent with this, aged Syn4 Tg mice were significantly more glucose tolerant than controls (Figures 2C and 2D). Syn4 upregulation was not associated with dwarfism and occurred independent of caloric restriction, as evidenced by body weight and food intake measures (Figures 2E and 2F). Metabolic caging studies showed no significant differences between 12-month-old Con and Tg mice for respiratory quotient (RQ) and movement/activity level (Figures 2G and 2H). Although body fat content was also similar (Figure 2I), serum triglycerides were slightly but significantly diminished in the Syn4 Tg mice (Figure 2J). These data suggest that increasing the capacity for exocytosis in skeletal muscle is sufficient to protect against age-induced insulin resistance, and by extrapolation, it could be speculated that an age-generated deficiency in exocytosis might underlie the development of insulin resistance with aging.

Glucose tolerance and homeostasis is substantially affected by functional β cell mass. Serum insulin content in fasted Syn4 Tg versus Con mice was reduced at 18 months of age (Figure 3A), rising in both strains by age 24 months ($p = 0.0275$ by two-way ANOVA). Following a glucose injection, 6-month-old Syn4 Tg mice exhibited a left-shifted insulin response in vivo, showing a peak in serum insulin content by 5 min, as opposed to that seen in control mice (Figure 3B); serum glucagon levels were not different between Syn4 Tg and Con mice under fasted or glucose-stimulated conditions (Figure 3C). The rapid serum insulin response of the Syn4 Tg mice is consistent with an amplified glucose-stimulated insulin secretory response from their islets ex vivo (Figure 3D) and was not affiliated with changes in islet insulin content or distribution of β and α cells within the islets (Figures 3E and 3F). The percentage of β cell area remained similar between 18-month-old Syn4 Tg and Con mice (Figure 3G), although both were elevated by ~2-fold relative to that reported for 6-month-old Syn4 Tg and Con littermate mice (Oh et al., 2014). These data suggest that islet function was increased in the Syn4 Tg mice, independent of insulin content and islet morphometry.

### Syn4-Induced Resistance to Obesity-Induced Dysfunction

The onset of pre/type 2 diabetes with aging is associated with increasing adiposity/obesity in humans. Activities of proteins associated with lifespan and longevity, such as FoxO3, FoxO1, mTOR, IRS1, AMPK, and SIRT1, reportedly change with high-fat diet (HFD) (Battiprolu et al., 2012; Chalkiadaki and Guarente, 2012; Rivas et al., 2009). To assess whether Syn4 Tg and Con mice differ in responses to dietary fat, mice were fed a diabetogenic 60% HFD or regular chow diet (RD) for 10 weeks. Both

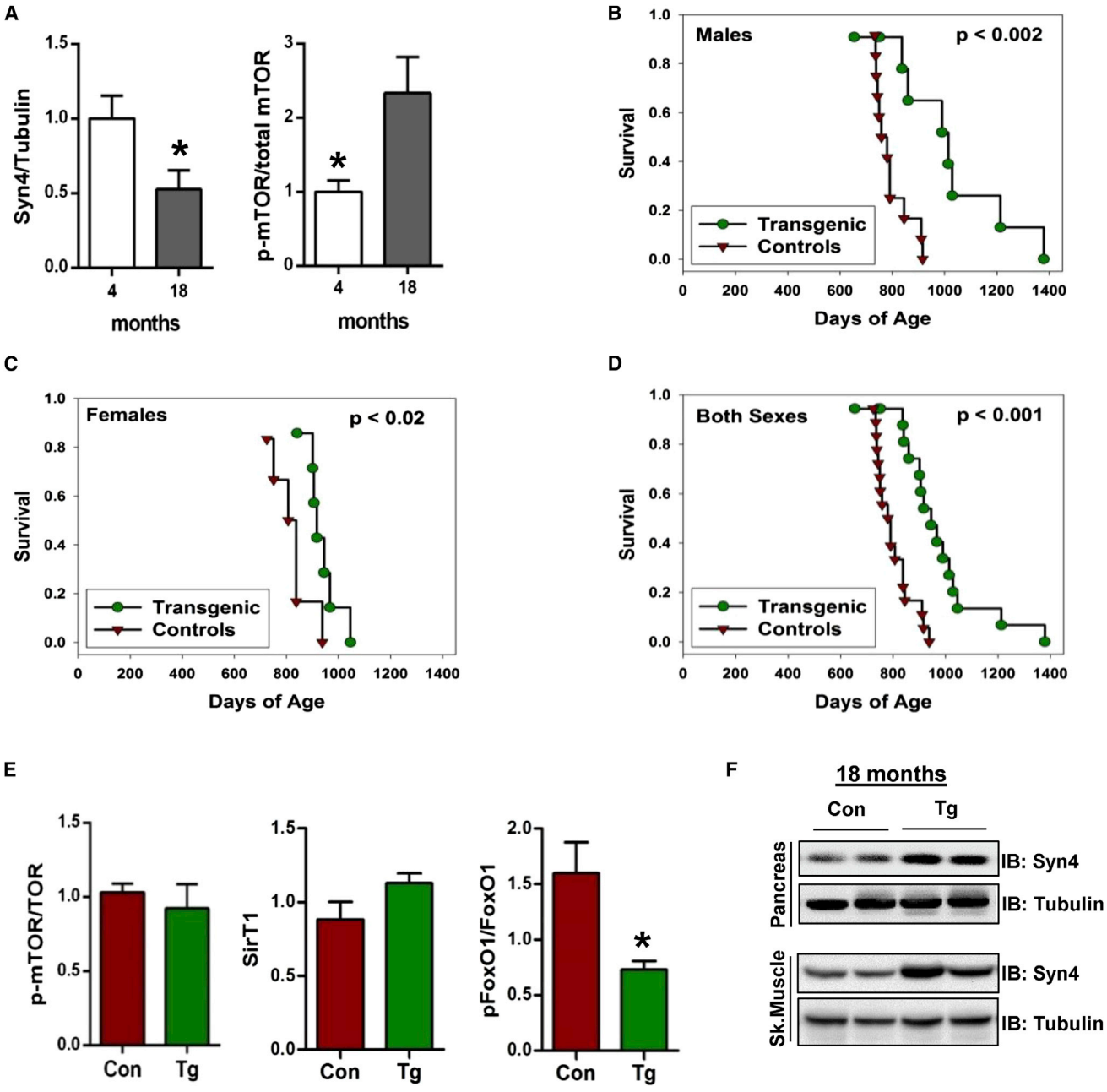


**Figure 1. Syn4 Abundance and Aging**

(A) Protein levels in pancreata of 4- and 18-month-old C57BL/6J mice: Syn4 relative to tubulin (loading control), phosphorylated mTOR relative to total mTOR. Data represent the mean ± SE of five sets of pancreata. *$p < 0.05$.

(B–D) Proportion of all mice surviving, for males (12 Con and 11 Syn4 Tg), females (7 Con and 6 Syn4 Tg), versus both sexes pooled (19 Con and 17 Syn4 Tg).

(E) Abundance/activation of proteins associated with aging in three paired sets of pancreata from male mice: phosphorylated/total mTOR, total SIRT1, and phosphorylated/total FoxO1 (pSer256).

(F) Levels of transgene expression in aged male pancreatic and skeletal muscle (Sk. Muscle) mouse lysates, representative of n = 4/group.

Syn4 Tg and Con mice showed equivalently increased body weight on either RD or HFD (Figure 4A). The relative fat pad weights of HFD-fed Tg and Con mice were similarly elevated above that of RD-fed mice (0.022 g/g BW for RD-Con and 0.024 RD–Tg, n = 4/group) (Figure 4B). Weights of other tissues, including liver, were similar between HFD-fed Tg and Con mice (Figure 4B). This is consistent with similarities in hepatic glucose uptake function and insulin action in Syn4 Tg and Con mice in hyperinsulinemic-euglycemic clamp analyses (Spurlin et al., 2004). Food intake was similar between Con and Tg mice on either RD or HFD (Figure 4C). Despite the obesity, HFD-fed Syn4 Tg mice retained the normal 2-fold increase in insulin-stimulated GLUT4

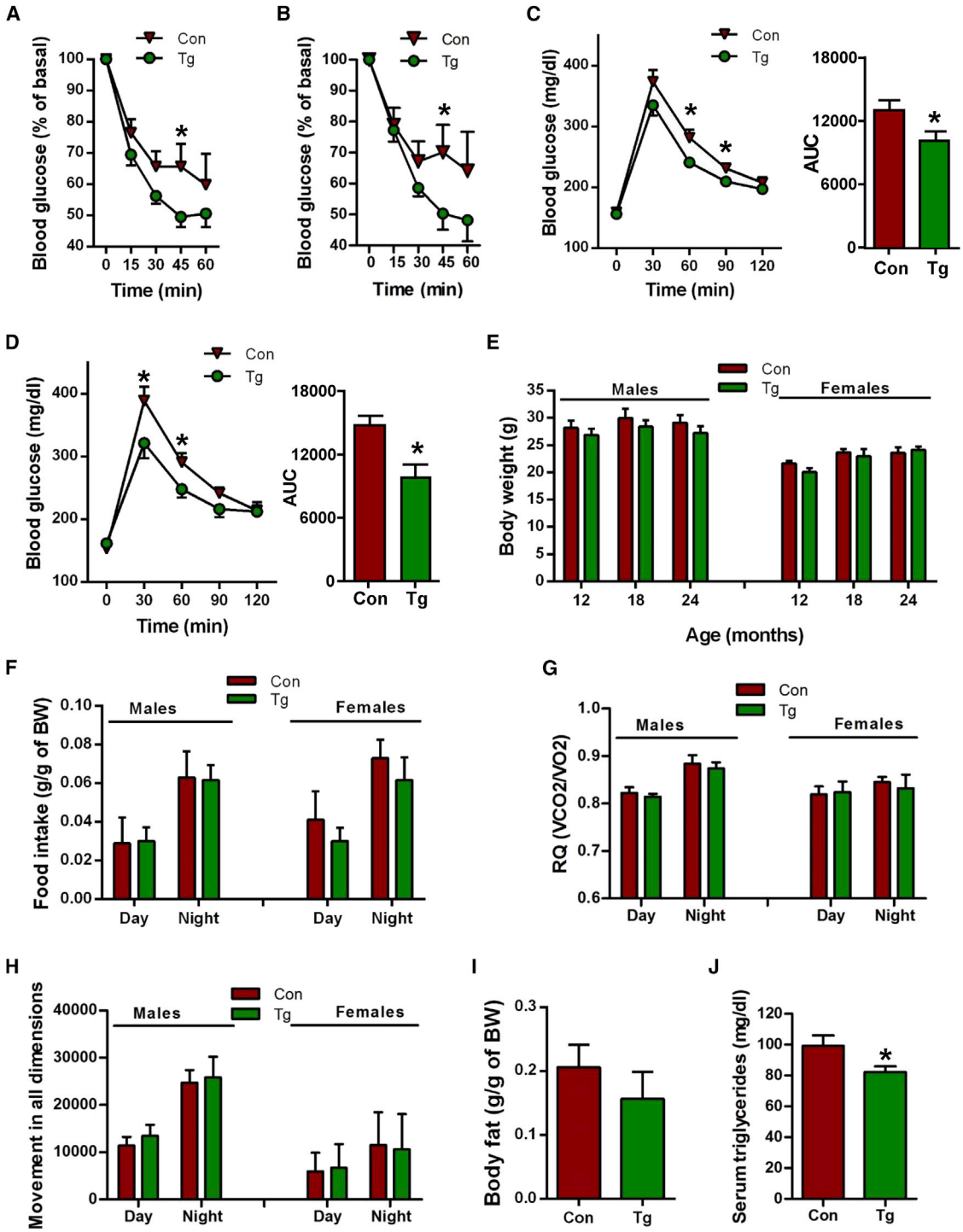


**Figure 2. Improved Insulin and Glucose Tolerance in Aged Syn4 Tg Mice**

(A and B) Insulin tolerance tests were assessed in 25-month-old mice with both sexes combined (A) or males alone (B); *p < 0.05 ([A], 8 Con and 12 Tg mice; [B], 6 Con and 8 Tg mice).

(C and D) Glucose tolerance tests were performed in 24-month-old mice, both with sexes combined (C), and in males alone (D) with bar graphs denoting area under the curve (AUC); *p < 0.05 ([C], 12 Con and 16 Tg; [D], 8 Con and 9 Tg mice).

(E–H) Metabolic caging studies, wherein data represent the average ± SE for four to nine pairs of 12-month-old male and female mice: body weights (E), food intake (F), RQ (G), and physical activity (H).

(I) DEXA scanning quantified body fat in four pairs of 8-month-old male mice. Serum triglycerides (18-month-old 7 Con and 7 Tg, sexes combined) (J); *p < 0.05. BW, body weight.

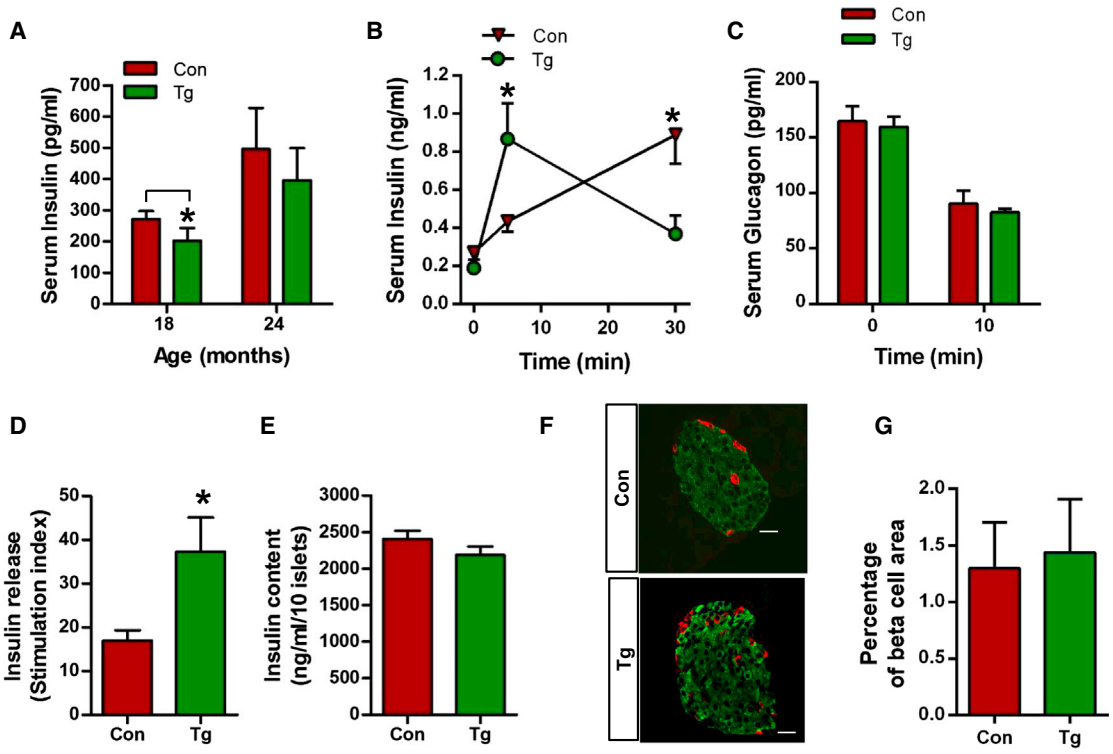


**Figure 3. Syn4 Tg Mice Show Robust Islet Function**

(A) Fasting serum insulin content in aged mice. Data represent the average ± SE (18 months: 7 Con, 6 Tg mice; 24 months: 5 Con and 5 Tg).

(B and C) Serum insulin and glucagon contents during glucose tolerance testing in 6-month-old male mice. Data represent the average ± SE of 6–8 mice per group; *p < 0.05 versus Con at same time point. Glucagon in serum from mice fasted (0) and after glucose stimulation (10 min).

(D and E) Static insulin release from mouse islets ex vivo and insulin content therefrom (n = 3 to 4 islet batches from 6-month-old mice); *p < 0.05.

(F and G) Islet cell distributions (red, glucagon stained α cell; green, insulin stained β cell) and percentage of β cell area in 18-month-old mouse islets (n = 3/group).

accumulation in skeletal muscle t-tubules/sarcolemma reported for similarly aged chow-fed control mice (dashed line) (Spurlin et al., 2004); by contrast, HFD-fed Con mice failed to respond to insulin (Figure 4D).

Within just 2 weeks on the HFD, fasting serum insulin levels of the Syn4 Tg mice were significantly higher than those of HFD-fed Con mice (Figure 4E), elevated relative to those reported for similarly aged chow-fed mice (Spurlin et al., 2004). Islets from HFD-fed Syn4 Tg mice also retained the 16-fold increase in glucose-stimulated insulin secretion seen in the RD-Con mice (dashed line), while the response of the Con-HFD islets ex vivo fell by 50% (Figure 4F). In terms of islet insulin content, HFD-fed Syn4 Tg mice showed an increase relative to RD-fed Tg mice, whereas HFD-Con and RD-Con showed no differences (Figure 4G). β cell area was similar between the HFD-Con and HFD-Syn4 Tg mice (Figure 4H), with both being increased by nearly 2-fold relative to age-matched RD-fed mice (RD-Con and RD-Tg, both 0.7% in Oh et al. [2014]), and rising to levels otherwise seen in the aged mice of Figure 3G. These data suggest that Syn4 enrichment preserves exocytotic activities in skeletal muscle and islet β cells with aging and with obesity.

## DISCUSSION

These data demonstrate a previously unrecognized role for an exocytosis factor in promoting the extension of lifespan. Insight into how Syn4 promotes healthspan stems from data showing that Syn4 Tg mice exhibit accelerated and enhanced capacity for glucose uptake and insulin release, as well as faster "re-equilibration" of glycemia and insulinemia following a glucose challenge. When challenged with HFD-induced obesity, skeletal muscle GLUT4 and islet insulin granule exocytosis processes were fully protected in the Syn4 Tg mice, matching levels equivalent to those of chow-fed Con mice; HFD-Con mice showed significant lapses in both exocytotic processes. Intriguingly, the Migration inhibition factor knockout mouse (MIF KO) showed a 15% extension of lifespan (Harper et al., 2010), and MIF deficiency is linked to protection from age-related insulin resistance (Verschuren et al., 2009) and from inflammation in islets associated with type 1 and type 2 diabetes (Stojanovic et al., 2012; Stosic-Grujicic et al., 2008). Despite the similarities with MIF deficiency in terms of lifespan extension and improvements in insulin secretion and insulin action, the Syn4-enriched Tg mice did not exhibit differences in inflammation, as assessed via serum levels of inflammatory factors TNFα or IL-6, with aging or under HFD-induced inflammatory conditions (Tables S3 and S4). However, finding that the capacity of islets of HFD-fed Syn4 Tg mice to increase insulin content, without increasing β cell mass, suggests that this exocytotic protein may play an otherwise unappreciated role in insulin biosynthesis. The increased capacity to synthesize and secrete insulin may be unrelated to the increased lifespan of

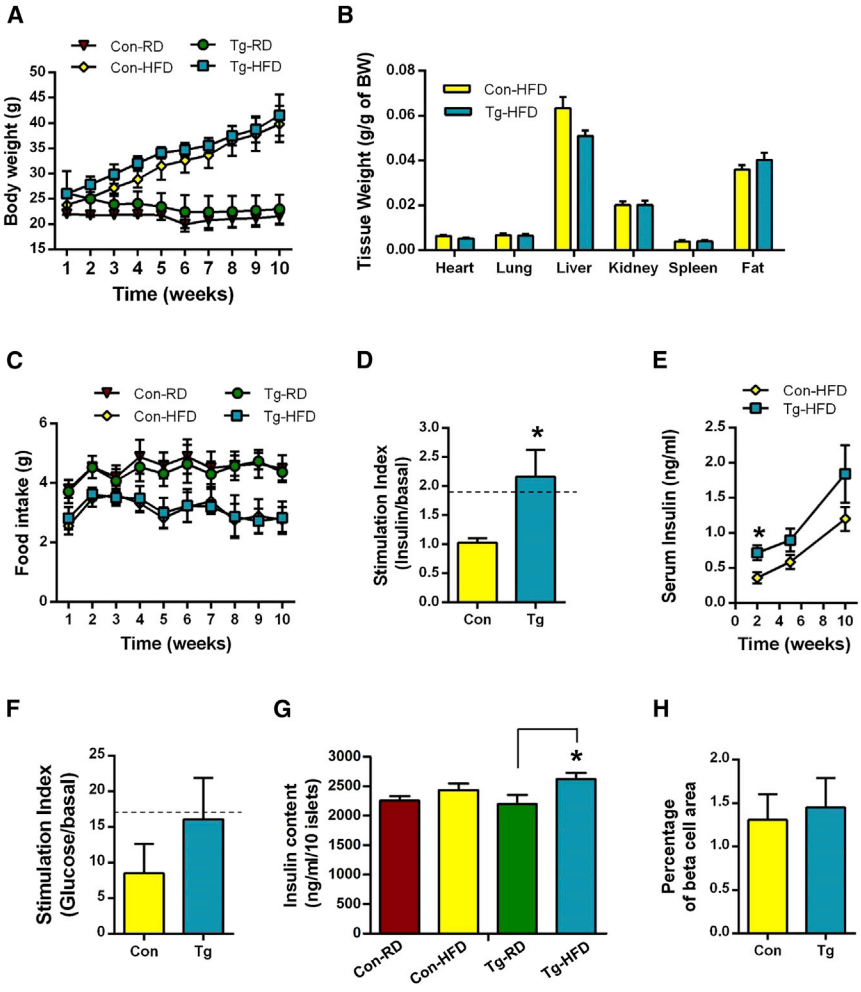


**Figure 4. Protection of Skeletal Muscle and Islet Function in High-Fat-Fed Syn4 Tg Male Mice**

(A–C) Body weight (A), tissue weights (B), and food intake (C) in control (Con) and Syn4 Tg mice ([A] and [C], n = 8–12 per group; [B], n = 4 to 5 per group).

(D) Skeletal muscle GLUT4 accumulated at the cell surface (stimulation index = insulin/basal levels of GLUT4 at cell surface). Data represent the average ± SE of four matched sets of mice/group; *p < 0.05). Dashed line, average RD-Con mice.

(E) Fasting serum insulin content during the HFD study (n = 6 mice/group; *p < 0.05).

(F) Glucose-stimulated insulin release (stimulation index = glucose stimulated/basal) of islets from mice fed HFD for 10 weeks (n = 3 mice/group). Dashed line, average RD-Con mice; p = 0.07, RD-Con versus HFD-Con.

(G) Islet insulin content after 10 weeks on HFD; data represent the average ± SE of six mice/group; *p < 0.05 versus Tg fed the RD.

(H) β cell area for 6-month-old mice fed HFD for 10 wks; n = 3 pancreata/group. Body weight, BW.

the Syn4 Tg mouse; however, since the long-lived GHRKO mouse has marked reductions in these aspects (Bartke and Westbrook, 2012; Coschigano et al., 2003; Junnila et al., 2013; List et al., 2011).

Microarray analysis of muscle from Syn4 Tg and Con mice pointed to changes in pathways of leptin and AMPK signaling, as well as cAMP and nNOS signaling in skeletal muscle, and components of these pathways are affiliated with lifespan extension (Tables S1 and S2). At the protein level, relative phosphorylated FoxO1 in pancreata from the Syn4 Tg mice was reduced, consistent with the association of health and longevity with decreased pFoxO1 in liver (Laurent et al., 2008; Tomobe et al., 2013). Relatedly, pFoxO1 is elevated in cardiac tissue of diabetic and HFD-fed mice (Battiprolu et al., 2012). Our findings of "normal" insulin sensitivity in aged Syn4 Tg mice provides a clue suggestive of increased "healthspan" in addition to longevity, although more evidence will be required to demonstrate broad improvement in late-life health in these mice. Preservation of insulin sensitivity into old age may reflect enhanced responsiveness by the skeletal muscle to the insulin signal, itself a consequence of more rapid GLUT4 vesicle accumulation at the t-tubule/sarcolemmal membranes, leading to faster clearance of circulating glucose. This is consistent with reports citing hyperglycemia as damaging to cells and leading to shorter lifespan (Fitzenberger et al., 2013; Yang et al., 2005). Additionally, this enhanced muscle response to insulin was maintained in obese Syn4 Tg mice. The decreased serum triglyceride content of the aged Syn4 Tg mice was unexpected, particularly given that 3- to 6-month-old Syn4 Tg mice or HFD-fed Syn4 Tg mice failed to show differences from paired Con mice in triglyceride contents (Spurlin et al., 2004). While Syn4 Tg mice at 3 to 4 months of age also show normal hepatic insulin signaling and glucose uptake, and normal adipocyte glucose uptake and glycolytic functions (Spurlin et al., 2004), it remains possible that crosstalk from the tissues expressing the transgene in this model (pancreas, skeletal muscle, and fat) to the liver, or functions directly in the fat, affect serum triglyceride levels with increasing age.

Syn4 may also act through changes in NAD metabolism in one or more of these tissues expressing the transgene. Increased NAD is associated with increased healthspan and longevity (reviewed in Verdin, 2014), dependent upon SIRT1 (Mitchell et al., 2014), and NAD-mediated PARP inhibition in *C. elegans* (Mouchiroud et al., 2013). However, SIRT1 protein and transcript levels of all sirtuins detected were normal, and although PARP could not be detected in the islet by microarray methods, it is their activity that NAD influences acutely, and hence may be impacted somehow by increased Syn4. Another possibility is that Syn4 indirectly impacts IR function in aging via its ability to bind and potentially chaperone translation of the IR substrate protein, Munc18c (Jewell et al., 2011; Spurlin et al., 2004; Yang et al., 2001), and that this alters IR signaling in aging at a post-transcriptional level to extend lifespan. The current report is also accompanied by a second site replicate cohort conducted at the University of Michigan, which revealed potential "site

effects'' and transgene dosage effects upon lifespan (Thurmond et al., 2015). Hence, efforts to sort out the potential roles for Syn4 in aging will require tissue-specific Syn4 transgenic models to clarify the individual tissue-related beneficial contributions to the longevity phenotype. For example, would a β-cell-specific Syn4 transgenic mouse show improved peripheral insulin sensitivity? Indeed, reports from other models suggest that improved β cell function could be sufficient to control peripheral insulin resistance (Buchanan et al., 2002; Li et al., 2004). Alternatively, a skeletal-muscle-specific Syn4 mouse protected from age-induced insulin resistance could obviate the need for compensatory elevation of β cell insulin release. Examination of such models may otherwise reveal that it is the simultaneous enrichment of Syn4 in the β cell and skeletal muscle that confers the extended healthspan and longevity phenotype, which would be important going forward with strategies to target Syn4 expression and activation.

## EXPERIMENTAL PROCEDURES

### Animals

All animal studies were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee (IACUC). The rat Syntaxin 4 cDNA inserted into the pUC-Combi targeting vector (Schultze et al., 1996) was used to generate heterozygous transgenic mice on the C57BL/6J strain background as described (Spurlin et al., 2004); wild-type littermates served as controls. Detailed methods are available in the Supplemental Materials.

### Metabolic Studies

Mice were fasted for IPGTT (2 g glucose/kg BW) and ITT (0.75 U/kg BW) studies, as described (Spurlin et al., 2004). Indirect calorimetric measurement was performed using a TSE systems LabMaster Metabolism Research Platform. Body fat percentage was measured by DEXA. Serum analytes were quantified by the Hormone Assay and Analytical Services Core at Vanderbilt University.

### GLUT4 Translocation

Fasted male mice were injected intraperitoneally with 21 U/kg BW Humulin or vehicle (saline) and sacrificed for removal of the hindquarter muscles for homogenization and subsequent differential centrifugation and detection of GLUT4 as described (Zhou et al., 1998).

### Islet Isolation and Immunofluorescence, and Tissue Immunohistochemistry and Immunoblotting

Mouse islets were isolated as described (Spurlin et al., 2004). Islet morphometry was used to determine β cell area as described (Wang et al., 2011); data shown represent three sections per pancreata, using three pancreata from each group. Pancreatic and skeletal muscle hindlimb lysate proteins were resolved for immunoblot detection as described (Oh et al., 2014).

### Microarray

Hindlimb skeletal muscle was dissected from 5-month-old Con and Syn4 Tg mice for RNA isolation. RNA was labeled using the Ambion WT expression kit and hybridized to Affymetrix Mouse Gene 1.0 ST arrays. Differentially expressed genes were analyzed using the 2014 Fall Release of QIAGEN's Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, http://www.ingenuity.com). Pathway, Functional and Upstream analysis were performed using Genes that had absolute fold changes $\geq$1.2. Data for the microarrays are deposited in NCBI's GEO with series accession number GEO: GSE63780.

### Statistical Analysis

All values are presented as means ± SE. Differences were analyzed by Student's t test or two-way ANOVA.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2015.07.023.

## AUTHOR CONTRIBUTIONS

E.O. and D.C.T. researched data, contributed to discussion, and wrote/reviewed/edited the manuscript. R.A.M. performed survival analyses, contributed to discussion, and reviewed/edited the manuscript. D.C.T. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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