# FOXO-transcriptional programs regulate the protein homeostasis to protect the cell against aging

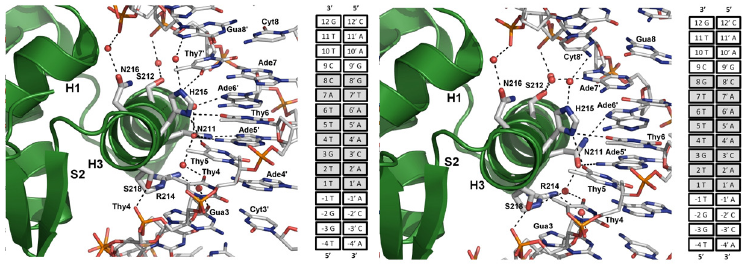
**Forkhead box O (FOXO) transcription factors are involved in the regulation of several homeostatic processes. FOXO activity also affects stem cell maintenance, life span as well as age-related diseases, such as Alzheimer, OPDM, and polyglutamine (Q) diseases. Multiple upstream pathways regulate FOXO transcription through post-translational modifications and nuclear-cytoplasmic shuttling of both FOXO and regulators. As FOXO has multiple upstream pathways, it has multiple downstream effects. FOXO predominantly responds to stress conditions. This response is the upregulation of several protein homeostatic genes, including, heat shock proteins, autophagy associated genes and proteasome and immunoproteasome subunits to maintain the cell homeostasis. This protein homeostatic effect activated by FOXO can be linked to protection of age-related diseases and longevity.**

## Introduction

The forkhead box O (FOXO) family are a family of transcriptional regulators characterized by a highly conserved 110 amino acid DNA-binding domain, also known as "forkhead box" or "winged-helix domain". The family of FOXO proteins are found in species ranging from yeast to humans. There are four main groups of mammalian FOXOs: FOXO1, FOXO3, FOXO4, and FOXO6. FOXO2 was originally identified as a separate paralogue, but is homologous to FOXO3, and FOXO5 is only expressed in *Danio rerio* (FOXO3b). These FOXO transcription factors control a wide array of genes, which are all linked by common mechanisms in that they are involved in metabolism control, cell survival, cellular proliferation, DNA damage repair response, and stress resistance [for a review, see REF 1].

Two evolu­tionarily conserved signalling pathways are the main regulators of FOXO activity: in the presence of growth factors, FOXOs are negatively regulated by the insulin/IGF-1 (Insulin Like Growth Factor 1) signal­ling pathway through phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB) [2,3,4,5]; and FOXOs are acti­vated in the presence of oxidative stress through Jun N-terminal kinase (JNK) signalling [6]. These pathways regulate through a number of post-translational modifications (PTMs), including phosphorylation, acetylation, methylayion, ubiquitination, and O‑linked‑d-*N*‑acetylglucosamine addition [for a review, see REF 7,8,9]. These PTMs change the recognized sequence of FOXOs, leading to a different transcription of genes. The insulin/IGF-1 signal­ling pathway leads to recognition of the sequence: 5′-(C/A)(A/C)AAA(C/T)AA-3′ present in the IGFBP-1 promoter region and known as the insulin-responsive sequence (IRE). Furthermore, with higher affinity the Jnk signalling leads to recognition of the sequence: 5′-GTAAA(T/C)AA-3′, known as the DAF-16 (FOXO homolog in *C. elegans*) family member-binding element. Both sequences are closely related and include the core sequence 5′-(A/C)AA(C/T)A-3′, recognized by all forkhead proteins [10,11] (Figure 1).

Figure 1: Stereoview of the interactions between the recognition helix H3of FoxO1 and the DNA containing consensus sequences. A). The Daf-16 family member-binding element 5′-GTAAA(T/C)AA-3′. B). The insulin-responsive sequence 5′-(C/A)(A/C)AAA(C/T)AA-3. The sequence of the DNA used for co-crystallization is shown on the right. Water molecules are represented as red spheres. Polar contacts important for the recognition and the FOXO–DNA complex stability are represented by dashed black lines. [Original figure is from T.Obsil and V.Obsilova., 2011. REF 10]



**A**

**B**

The insulin/IGF-1 pathway influences life span in worms, flies and mammals [13]. This pathway was first linked to life span in *Caenorhabditis elegans (C. elegans).* Mutations of Daf-2, a receptor ortholog of Insulin/Igf-1, were found to double life span [14]. The life span extension caused by Daf-2 mutations required the activity of Daf-16 (homolog of FOXO). The Daf-2 receptor activates a conserved Pi3k and Pkb signalling pathway that negatively regulates the nuclear localization of Daf-16 [15,16,17,]. In addition to Daf-16, HSF-1, the *C. elegans* heat shock transcription factor, is also required completely for Daf-2 mutations to extend life span [19]. Like Daf-16, HSF-1 delays aging and extends life span. In Daf-2 mutants, HSF-1 promotes longevity by activating specific longevity genes, including genes that encode small heat shock proteins [for a review, see REF 20,21,22]. Daf-16 is also activating HSP16, a gene that encodes a small heat shock protein that can increase life span in *C. Elegans* [23]. The mechanisms how Daf-16 and HSPs can affect life span are not yet known. However, most hypotheses are linked to protein homeostasis. Also in mammals FOXOs act as a regulator of homeostasis, particularly in response to stress.

In this review, we focus on the role of FOXOs in regulating the protein homeostasis We will look at recent developments on FOXO signalling, regulation of FOXOs as transcription factors, and FOXO specific cell functions. Finally, we will discuss the role of FOXO in protein folding, proteasomal degradation and autophagy in stress related protein diseases as Huntington's and Alzheimer's disease.

## Upstream regulation of FOXO activity

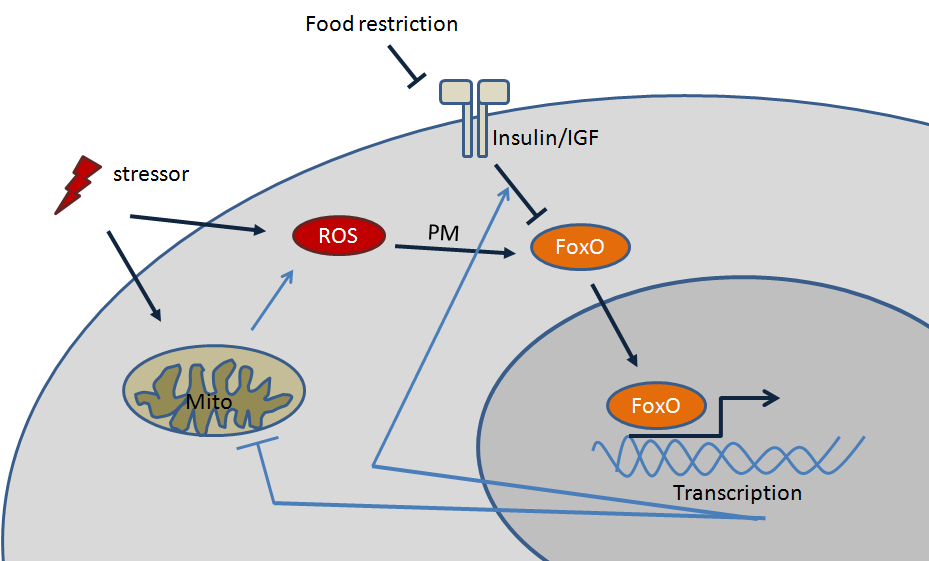
Generally, FOXOs are inactive when cells are growing under nor­mal conditions, and this requires negative regulation by insulin/IGF signalling. insulin/IGF-1 signalling acts through PI3K which activates a second messenger (phosphatidylinositol-3-phosphate) that activates phosphoinositide-dependent kinase 1 (PDK1) and PKB. Active PKB translocates to the nucleus and phosphorylates FOXO at three conserved residues, resulting in increased binding of FOXO to the regulator 14‑3‑3 and cytoplasmic localization of both. Subsequently, this leads to inhibition of transcriptional binding of FOXO [2,24, for a review, see REF 25]. During periods of starvation this inhibition is abolished and FOXO signalling is activated. A conserved mechanism during FOXO signalling is downstream activation of the PI3K–PKB axis, which results in the inhibition of FOXO activity [26,27,28]. This negative feedback loop probably allows a fast and/or increased response to the reappearance of growth factors after periods of starvation (Figure 1).   
Cellular stress activates FOXO transcription in the opposite way, especially when high levels of reactive oxygen species (ROS) are generated. ROS activates JNK, and JNK inhibits insulin signalling at multiple levels by decreasing insulin recep­tor substrate (IRS) activity and by inducing the release of FOXO from 14‑3‑3, thereby overriding growth factor-induced FOXO inhibition [29]. Through JNK-mediated phosphorylation of FOXO, FOXO translocates to the nucleus and thereby induces FOXO transcriptional activity. Both the PI3K-PKB and the JNK signalling are evolutionarily conserved [7]. Besides the negative feedback loop from FOXO to downstream activation of the PI3K–PKB axis, there is another feedback loop from FOXO to downstream detoxification of ROS, through regulate mitochondrial ROS production through inhibition of c-MYC function and alters the hypoxia response [ 30] (Figure 1).

Figure 2: Double negative feedback loop of FoxO activation. Food restriction leads to inactive insulin/IGF signalling and active FoxO transcription. FoxO activates the PI3K–PKB axis downstream of insulin/IGF receptor. This activation leads to inactivation of FoxO. Extracellular and intracellular stress leads to ROS induction which is an activator of FoxO transcription. FoxO transcription leads to a decrease in mitochondrial ROS production through inhibition of c-Myc function and alters the hypoxia response.

In addition to these signalling mechanisms that can control FOXO transcription, there are numerous other signal events and other post-translational modifications (PTMs) which are less established that can regulate FOXO transcription as well. This overflow of signal events and PTMs increases the complexity of the regulation of FOXO and suggests that an array of PTMs and signal events influence FOXO function. In this way, FOXO can be guided in different transcriptional acting to maintain tissue homeostasis

One of these signal events that also can regulate FOXO, is AMP-activated protein kinase (AMPK). AMPK phosphorylates FOXO(3) on at least three serine residues (Ser413, Ser588 and Ser626) [31]. This phosphorylation induces FOXO activity without affecting sub-cellular localization and is independent of growth factors or stress that induce nuclear translocation of FOXO. AMPK is known to control cell growth and energy balance. AMPK steers the transcriptional action of FOXO in a way that activates alternative energy sources and stress resistance [32,33]. (A similar pathway oper­ates in *C. elegans* to confer life span extension under conditions of caloric restriction [34]). Other protein kinases that can regulate FOXO activity by phosphorylation (Ser249) are cyclin-dependent kinases (CDK) [35,36]. The regulation of FOXO activity by Cdk is cell dependent. FOXO1 in neuronal cells is activated by Cdk1, in other cells Cdk1 induces cytoplasmic localization and thus its inhibition [36,37]. CDKs are key regulators of cell cycle progress and it seems that DNA damage response activates FOXOs via CDKs. Besides CDKs also MAPK-activated protein kinase 5 (MK5) phosphorylates and activates FOXO in response to DNA damage [38].   
Besides phosphorylation, FOXO can be regulated by acetylation, ubiquitination and methylation. An example of FOXO regulation by acetylation is by histone acetyl­transferases (HATs) including sirtuins (SIRTs) and histon deacetylases (HDACs) [39]. An example of FOXO regulation by ubiquitination is by ubiquitin-specific-processing protease 7 (USP7) and Mouse double minute 2 homolog (MDM2) (also known as E3 ubiquitin-protein ligase) [40,41 ].   
 FOXOs can also be methylated at arginine residues; this modification of FoxO generally blocks a protein or DNA binding. For example, protein arginine methyltransferase 1 (PRMT1) methylates FOXO at Arg resi­dues within the PKB consensus site, thereby inhibiting PKB-mediated phosphorylation [42]. The *C. elegans* homo­log of PRMT1 also methylates Daf‑16, and its deletion increases life span in a Daf‑16‑dependent manner [43]. Methylation in the DNA-binding domain (by the Lys methyltransferase SET domain-containing protein 7) is shown in FOXO(3) as well and thereby inhibits FOXO function [44].  
Besides acetylation, ubiquitination and methylation, FOXO activity can be regulated by   
O-GlcNAcylation, cysteine (cis) oxidation. O‑linked‑d-*N*‑acetylglucosamine (O-GlcNAc), a monosaccharide derivative of glucose that is added as post-translational modification, can be added on serine and threonine residues of FOXO and thereby oppose phosphorylation. However, the O-GlcNAc-modified residues did not yet correspond to any known FOXO phosphorylation sites. The thiol group of Cys residues can be easily oxidized, which can result in the formation of disulphide bonds with a Cys residue in the same or another protein. This occurs in normal signal transduction. Reversible Cys oxidation of FOXOs induces binding of CREB-binding protein (CPB) and E1A binding protein p300 through Cys disul­phide bonds and thereby regulates FOXO transcription [45]. There are more proteins that seem to have a bond with FOXOs. There are some proteins that shuttle between the nucleus and the cytosol together with FOXO. Most of them are regulators of FOXO such as JNK, PKB, SIRT1, MDM2, and HDAC which were already described earlier in this paper. -catenin also shuttles between the nucleus and the cytosol. -catenin is translocated to the nucleus when it is phosphorylated by JNK. In the nucleus it acts as a co-activator of FOXO transcriptional activity [46].

-catenin is not the only factor that assists with transcriptional activity of FOXOs. DNA binding of FOXO does not directly result in gene activation, but it requires additional factors like -catenin or HATs and HDACs [9]. The transactivation domain of FOXO functions in a classic manner by recruiting HATs and HDACs. Opening of compacted chromatin (pioneer factor) has been suggested as an additional mechanism for FOXO1‑mediated tran­scriptional regulation. The amino-terminal and C‑terminal regions of FOXO1 mediate histone H3 and H4 binding, and the N terminus is required for chromatin opening [47]. By opening compacted chromatin, FOXO might allow other transcription factors to bind. In addition, FOXOs showed to have interactions with several other transcription factors, nuclear receptors, metabolic regulators and other forkhead box members [for a review, see REF 48]. These interactions can be guided by PTMs on FOXOs and/or their binding partner or, alter­natively, FOXOs can cooperate with other transcription factors through simultaneous binding to regulatory sequences in a target gene. One possibility is that this cooperation results from a pioneer function of FOXO, in which FOXO binding, allows binding of other transcription factors. These multiple interactions could explain the multiple out-stream of proteins when FOXO is activated.

Here, we briefly highlight additional interactions with the transcription factor HSF-1. As previously discussed, FOXOs can be activated by ROS through JNK, and FOXOs are negatively regulated by insulin/IGF signalling. Like FOXO, HSF-1 is negatively regulated by insulin/IGF signalling as well (through Daf-16-dependent longevity (Ddl-1 and Ddl-2)) and is indirectly activated by ROS [49 and for a review, see 50]. ROS causes protein unfolding in the cell. These unfolded proteins attract heat shock proteins (HSPs). HSF-1 (in non-stress conditions) is constitutively expressed in the cytoplasm and is maintained in an inactive state by association with HSPs [50]. Due to ROS cell exposure, HSF-1 is released from HSPs, which enables HSF-1 to undergo trimerization, activation and translocation to the nucleus, where it binds to conserved heat shock elements (HSEs) in the promoters of HSP genes and activates their transcription [50]. In *C.elegans*, FOXO acts in concert with HSF-1 to regulate small HSPs (sHSPs) induction in response to stress and upon reduced insulin/Igf signalling. In addition, consensus FOXO-binding sites are present in most *C. elegans* and human sHSP gene promoters (see Table 1 and [51]). In *C.elegans*, HSF-1 and Daf-16/FOXO stimulate the expression of genes required for longevity and stress resistance. These same upstream regulations of FOXO and HSF-1 plus the similar transcription targets suggest a cooperation between these two transcription factors through indirect (from a pioneer function of FOXO) or direct binding.

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| --- | --- | --- | --- | --- | --- | --- | --- |
| Promoter regions of: | Predicted FOXO binding site |  | Promoter regions of: | Predicted FOXO binding site |  | Promoter regions of: | Predicted FOXO binding site |
| HSPB1 | CAAAACAA(\*) |  | HSPB7 | CAACA |  | DNAJA4 | AAACA/AAATA |
| HSPB2 | AAACA |  | HSPB8 | AAATA |  | DNAJB4 | AAAAATAA |
| HSPB3 | AAACA/AAATA |  | HSPB9 | (no Bindingsite) |  | DNAJB9 | AAACA(A) |
| HSPB4 | (no Bindingsite) |  | HSPB10 | AAACA |  | DNAJC11 | CCAAACAA |
| HSPB5 | AAACA(A) |  | HSPBAP1 | CAAAACAA |  | PSMB8 (IP) | AAACA |
| HSPB6  Table 1: An overview of some heat shock proteins tested on a FoxO DNA binding site in the human promoter region. The promoter regions are from the Transcriptional Regulatory Element Database and are experimentally verified and stated explicitly in GenBank records. The yellow colour shows an 8 letter binding sequence code inside the promoter of this protein. HSPB1 (\*) can be found in mice promoter region, in human, the region is smaller. Blue shows the subunits of the immunoproteasome. | AAATA |  | DNAJA3 | AAATA/ CAACA |  | PSMB9 (IP) | (no Bindingsite) |

## FOXO transcriptional output

Above, we discussed the cooperation with FOXO and other transcription factors. Here, we highlight the transcriptional output of FOXO with and without the influence of secondary transcription factors. Generally, the transcriptional output of FOXO can be classified into effects on diverse cellular processes depending on the multiple upstream pathways and/or cell specific functions. Most studies conducted on the output of FOXO transcription are in the field of two major research topics. On the one hand, cancer, because FOXOs play an important role in cell-cycle arrest, DNA repair, and apoptosis (cell functions that go wrong in cancer). On the other hand, longevity, because FOXOs have been shown to prolong life span by promoting resistance to oxidative stress, pathogens, and damage to protein structure. It is thought that FOXOs are homeostasis regulators that coordinate this balance between longevity and tumor suppression. The effects of FOXOs on stem cell maintenance, combined with specialized functions of FOXOs in the immune system and neuropeptides, also indicate that FOXOs might contribute to tissue homeostasis more generally.

In this paper we discuss recent insights into the *in vivo* functions of FOXOs from genetic studies in *C. elegans, D. melanogaster* and mice, which suggest that FOXOs are involved in protein homeostasis as part of the metabolic stress response (starvation). Starvation of a cell activates FOXO through the insulin/ IGF signalling pathway and in the presence of ROS as discussed. ROS or another stressor leads to protein toxicity and disturbed protein homeostasis. FOXOs are emerging as factors that protect against protein toxicity through effects on protein aggregation and degradation.

In a protein aggregation model of Alzheimer's disease, where *C. elegans* expresses the Aβ1–42 peptide, Daf-16 expression induces the formation of high molecular weight aggregates. Consistent with this, these *C.elegans* shows longevity on a Daf-16-dependent manner [52]. A late activation of Daf-16 during adulthood still protects against Aβ1–42 peptide aggregation but has little effect on life span [53]. This indicates that this function can be separated from longevity. In another aggregation formation model of polyglutamine disease, such as Huntington's disease, FOXO is found to substantially decrease polyglutamine aggregates [54]. In addition, HSF-1, a hypothetical cooperator of transcription of FOXO discussed above, shows a strong inhibitory effect on polyglutamine aggregate formation in a mouse model (*in vivo* and *in vitro*) [55]. Another protein which has shown a protective effect in Huntington's disease is X-Box-binding protein 1 (XBP1). XBP1 upregulates genes related to protein folding, quality control, ER translocation, and ER-mediated degradation (ERAD). Recently, XBP1 was shown to control a dynamic crosstalk with FOXO1 and the autophagy pathway to modulate Huntington's disease pathogenesis [56]. In addition, FOXO1 and FOXO3a have been shown to be able to transcriptionally upregulate the expression of several autophagy-related genes such as ATG12, GABARAPL1, BNIP3, and BCEN1 [57,58]. Interestingly, FOXOs also regulate transcription of PTEN-induced putative kinase protein 1 (PINK1), a kinase that regulates mitophagy, suggesting that FOXOs regulate autophagy and mitophagy in parallel [59]. To come back to neurodegenerative disease, accumulated evidence has also demonstrated a neuroprotective role of autophagy and mitophagy in mediating the degradation of aggregated proteins that are causative of various neurodegenerative diseases [for a review, see REF. 60]. In addition to direct regulation of autophagy genes, FOXOs can also inhibit mTORC1 [61] a major regulator of protein homeostasis, and this pro­vides an alternative mechanism for FOXOs to regulate autophagy. Moreover, a new model has been proposed that FOXO1 can activate Atg7, an autophagy regulating protein, on a non-transcriptional manner [62]. However, this model should be taken with some caution because it does not address several important questions [63].

Aside from autophagy and mitophagy, there is another major pathway for clearing damaged proteins and has been demonstrated as well to have a neuroprotective role, namely proteasomal degradation. In *C. elegans* germline deficient mutants, Daf‑16 is required for the upregulation of the gene encod­ing the proteasome subunit RPN‑6 and concomitantly increases proteasome activity [64]. Interestingly, in human embryonic kidney cells (HEK293), FOXO upregulated subunits of the immunoproteasomes PSMB8, PSMB9 and PSMB10 [54]. The immunoproteasome is well established in normal conditions in the cells of the immune system and associated tissues, such as B-cells, Treg cells, macrophages, monocytes, thymus and spleen but is also expressed in normal conditions in the colon, intestine, retina, placenta, embryonic cells, liver, kidney, endothelial cells, astrocytes, and in neurons. In neurons, it is suggested to play a role in the cellular stress response pathway in a non-immune manner. Furthermore, in abnormal conditions such as in neurodegenerative diseases, high level of immunoproteasome subunits in the mouse brain revealed signs of neurodegeneration [65]. In addition, induction of the immunoproteasome was also found in Huntington's and Alzheimer's diseased postmortem brains [65,66,67]. The immunoproteasome also plays a role in oculopharyngeal muscular dystrophy (OPMD). OPMD is a late onset progressive muscle disorder where protein aggregation attenuate muscle symptoms. The mechanisms are largely unknown; however, it is believed that the natural decrease in the proteasome and Immunoproteasome expression and its activity during muscle aging contributes to the onset of the disease. Also in injury (which does not involve an inflammatory response) of the retina and brain, the immunoproteasome is upregulated [68]. In addition, knock out or mutated versions of the subunits; PSMB8 and PSMB9 of the immunoproteasome show increased levels of protein oxidation and accumulation of oxidized and poly-ubiquitylated proteins [69], suggesting that the immunoproteasome is more prone than the (16S) proteasome to eliminate oxidized proteins. A protective role of immunoproteasome against cellular oxidative damage is also reinforced by the observation that these subunits are constitutively expressed in cells which are frequently challenged with reactive oxygen radicals such as in phagocytic cells (i.e., DC and macrophages) during the respiratory burst process [for a review, see 70,71]. Also, mouse embryo fibroblasts cells (MEF) from a PSMB8 knock out mouse showed to be less prone than those of their wildtype counterparts in clearing aggresome-induced like structures (ALIS) (following IFN-γ stimulation) [72].

Up to now, the mechanisms by which the incorporation of the inducible subunits allows immunoproteasome to degrade ubiquitin substrates faster than (16S) proteasome are unclear and require further investigations. One possible explanation may be the increased chymotryptic-like activity observed following incorporation of PSMB8 [73]. This, in turn, might be due to a conformational change that in some way favors the entry of poly-ubiquitylated substrates into the catalytic core. Another point for further investigations is how the immunoproteasome is upregulated. A possible explanation may be the transcriptional upregulation through FOXOs, since the upregulation in HEK293 cells, as well as the regulation in expression in Treg cells [supplementary data of REF.74] and the activation of FOXO by ROS.

Another role of FOXOs in protein homeostasis is the increase in life span due to heat shock proteins (HSPs). In both *Drosophila melanogaster* and *C. elegans*, activation of the JNK pathway can increase life span in a FOXO-dependent manner through FOXO activation of small HSP gene expression. *D. melanogaster* FOXO regulates expression of the small HSP, l(2)efl, and overexpression of l(2)efl during development and adulthood has been reported to increase fly life span [75]. *C. elegans* FOXO regulates expression of the small HSP, HSP16.Taken together, this suggests a possible conserved mechanism of life span regulation. In human cells (HEK293), FOXO1 regulates the expression of small HSPs: HSPB2, HSPB4 and HSPB6. However, FOXO1 as well as FOXO4 and FOXO6 do not appear to play a significant role in the ability to reach old age in Europeans [76]. In contrast, FOXO1 appears to play a role in the ability to reach old age in Han Chinese [77]. The discrepant association findings in Europeans and Chinese may be explained by their different FOXO1 linkage disequilibrium structures and could indicate a Chinese- or Asian-specific effect. Interestingly, FOXO3a has repeatedly been found to influence survival into old age in numerous populations worldwide [78-84]. If FOXO3a (in humans) regulates the expression of small HSPs is unclear. However, a ChIP-seq study of FOXO3a showed transcriptional binding with the small HSPs: HSPB1 and HSPB11 [supplementary data of 85]  
Another HSP that showed to be involved in life span is HSPA9a, also known as mortalin. HSPA9a is a mitochondrial HSP that is related to HSP70 but it is not induced by heat stress. HSPA9a can increase replicate life span when overexpressed in human foreskin fibroblasts [86]. In addition, in *C. Elegans*, HSPA9a-related gene HSP70F was reported to increase life span as well [87]. However, HSP70 itself is found to be suppressed by FOXO3a in human umbilical vein endothelial cells (HUVEC) [88]. This fits the general idea that longevity correlates with lower basal levels of HSP gene expression and a more robust heat shock response. However, both basal and stress-induced HSP70 protein expression is suppressed by FOXO3a. Moreover, FOXO3a induced caspase-9-dependent apoptosis in HUVEC, and cotransduction with HSP70 rescued endothelial cells from FOXO3a-induced apoptosis under basal and stress conditions [88]. Besides HSP70, clusterin (sHSP-related protein) and HSP27 are as well shown to be anti-apoptotic. Aging is associated with a misregulation of apoptosis in several ways. For example, certain replicating cell types from old mammals have a reduced ability to undergo apoptosis [89]. The precise molecular mechanism that causes extension in life span is not yet known. However, there are several possibilities how FOXO together with HSPs might be directly involved in modulating these phenotypes. One of these possibilities is the apoptotic pathway that is balanced by FOXO3a and HSP70 as described above. Another possibility is the role in protection of proteotoxicity, FOXOs upregulate the immunoproteasome and proteasome proteins. These are involved in the degradation of toxic proteins. Besides proteins of the immunoproteasome and proteasome, HSPs are well known to reduce the toxic effect of human disease genes by proteasomal degradation, stress protection and refolding. Besides degradation of toxic proteins by the immunoproteasome and proteasome, toxic proteins are removed by autophagy. HSPs play an important role in autophagy by unfolding proteins to facilitate their translocation into the lysosome (chaperone-mediated autophagy). Activity of proteins involved in the chaperone-mediated autophagy declines during aging [90]. In *C. elegans*, autophagy has been reported to be required for life span extension in response teach of three life span pathways: reduced insulin/IGF signalling [91], dietary restriction [92,93], and reduced mitochondrial gene function [94]. Reduced insulin/IGF signalling and dietary restriction lead to activation of FOXOs. FOXO activation leads to reduced mitochondrial gene function.

Another possibility of extension in life span is by FOXO and HSPs through the maintenance of stem cells (SCs). As the regenerative process of a living organism is determined by the ability and potential of its SCs to replace damaged tissue or worn out cells, a living organism is therefore as old as its SC. FOXOs have a crucial role in several types of adult SCs. In mice, FOXOs maintain self-renewal of the haematopoietic stem cell (HSC). Moreover, acute deletion of *FOXO1, FOXO3* and *FOXO4* in adult murine bone marrow led to the expansion of both the myeloid and lymphoid lineages coupled with increased cell cycling of the long-term HSCs and exhaustion of the HSC pool [ 95, for a review see REF. 96]. This indicates that FOXOs regulate the proliferation and self-renewal of these SCs. In addition, deletion of *FOXO3* in female mice leads to the early depletion of the follicular pool and premature infertility [97]. Also, in the neural stem cells (NSCs) FOXO maintains the stem cell population in time [98,99]. Moreover, FOXO3‑deficient NSCs from middle-aged mice were deficient in the generation of different neural lineages [98,99]. A key mechanism by which FOXOs mediate self-renewal and SC maintenance seems to be through tran­scriptional regulation of cell cycle arrest and oxidative stress resistance. FOXO is also necessary to maintain pluripotency in the embryonic stem cells (ESCs) and directly controls the expression of the transcrip­tion factors OCT4, SOX2, and perhaps other pluripotency genes. HSPs as well as FOXO play a role in maintenance of SC. HSPs have a unique expression profile in ESCs, mesenchymal stem cells (MSCs) and NSCs [for a review see, REF. 100,101]. Furthermore, all types of SCs shared high expression levels of HSPA5, HSPA8 and Stip1 (HOP). Moreover, HSPs are downregulated during differentiation of the ESCs to embryoid bodies. Expression patterns of three members of the HSP25 (HSPB) familie change during the first 24 hours of differentiation until expression is decreased to levels that are barely detectable at 4 days following differentiation [102]. in addition, HSP90, heat shock cognate 70,(HSC) and HSPA9a were eliminated when embryonic stem cells underwent differentiation [103]. In certain mouse embryonic stem cells, HSP70, HSP60 and Hop are downregulated as well [104]. In mesenchymal stem cells isolated from the bone marrow of rhesus monkeys (rBMSCs), HSP70 and HSF-1 were decreased in expression in middle and old rBMSCs, compared to young rBMSCs. This downregulationof HSP70 and HSF-1 was coupled with an increased level of ROS generation. Other heat shock proteins and stress proteins (HSF-2, HSP27, HSP47, HSP60, HSP90A, and HSP90B) were not significantly changed in young, middle and old rBMSCs [105]. This data suggests that high expression levels of HSPs and co-factors in ESC exert a buffering effect against external and internal stressors, thereby maintaining their stemness. Another role in SC maintenance is has been proposed. HSP90 is activated in unstressed cells by the JAK/STAT3 pathway. The JAK/STAT3 pathway is required for the maintenance, pluripotency, and survival of mouse ESC. This suggests a role of HSP90Bin the JAK/STAT3 pathway s to maintain pluripotency, and survival of mouse ESC [101]. In conclusion, both HSPs and FOXOs are important in SC maintenance, pluripotency, protection, proliferation and survival, processes that are important for survival of SC, and so, important for healthy aging of an organism.

Taken together, the role of FOXO together with ( or without) the role of HSPs in aging-related diseases, stem cell maintenance, autophagy, (immune) proteasomal degradation and life span promises to be an active and productive area for future research.

## Conclusion and perspectives

It has become clear that FOXOs mainly function to maintain cellular and protein homeostasis. Their biological role is therefore predominantly to respond to stress conditions rather than being an essential media­tor of normal physiology. To control this protein homeostatic function, FOXO activation occurs through metabolic and oxidative stress and by the absence of growth factors. Regulation of FOXO activity is accomplished through nuclear-cytoplasmic locali­zation of both FOXO and its regulators and through active regulation by various post-translational modifications (PTMs) (phosphorylation, acetylation, methylation, o-GlcNAc modification and cis-oxidation). Activated FOXO ensures tran­scriptional regulation by opening of compacted chromatin and by recruiting HATs and HDACs and other transcriptional factors. This results in diversity in FOXO-regulated gene programmes, depending on the PTMs and the upstream pathway of FOXO. Unfortunately, we do not exactly know which upstream pathways lead to which PTMs, and which PTMs lead to which FOXO transcription. In addition to FOXOs upstream pathways, many regula­tors of FOXO are under spatio-temporal control. One possibility is that this spatio-temporal control might result in different combinations of FOXO regula­tors being present within a cellular compartment and thereby allowing them to integrate upstream signalling pathways into a specific FOXO-mediated functional outcome   
[9]. A point to look into besides these regulators, is the variety of FOXO modifications. We could speculate whether we found all interaction proteins and PTMs of FOXO, however, it is likely we only know the tip of the iceberg. Another point which requires much more knowledge to understand the transcription of FOXO is the cooperation of FOXOs with other transcription factors, including HSF-1.

The different activation pathways, the different PTMs and co-transcription factors make FOXO transcription hard to predict. However, a huge function of FOXO is the transcription of genes involved in degradation of toxic proteins, such as a bunch of autophagy associated genes, proteasome subunits and associated genes and heat shock genes (HSPs). Interestingly, this FOXO-regulated gene program of maintaining the protein homeostasis protects the cell against age-related diseases such as Alzheimer's, OPMD and polyQ diseases. In addition, these FOXO regulated genes, at least HSP16, showed extension in life span in *C. elegans.* In other organisms like *D. melanogaster*, and mouse, FOXO expression leads to longevity as well. However, in humans, only expression of FOXO3a (in some populations also FOXO1) is related with higher age. How FOXO leads to longevity in humans is not known, however, it can be suggested that it is a conserved mechanism. Further research should elaborate if HSPs and other FOXO regulated proteins (that are involved in regulation of homeostasis) are involved in longevity in humans.

Finally, in this hypothetical model I want to show how FOXO activation leads to longevity (figure3). In addition, this figure also shows the missing knowledge, which hopefully can be filled in, in detail after intense research. So we all can extend our lifetimes to do more research.

Insulin/IGF

stressor

Proteins ?

PM

PM

TF?

PM

?

?

?

TF?

PM

PM

Transcription

Protein homeostasis

?

?

?

?

?

Autophagy

Mitophagy

Hsps

(Immuno-) proteasomal degradation

**Longevity**

Food restriction

Mito

Figure 3: a simplified figure of the upstream and downstream pathways of FoxOs with as a result protein homeostasis and in the end longevity. The darker blue arrows are the known pathways to active FoxO transcription including the transcription of protein homeostasis related genes. These related genes can be separated in autophagy, mitophagy and genes of the (immuno) preasomal pathway. Heat shock proteins and maybe other chaperones are supporting or involved in all of these protein homeostatsis pathways. The lighter blue arrows are upstream or downstream signals which are suggestively involved. However the exact mechanism and the exact proteins which are involved, are not yet known. (TF;Transcritpion factor. PM; Post modifictation. Mito; Mitochondria. HSPs; Heat shock proteins. ?; uncertain connection.

## Glossary:

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| **Aggregates:** is a biological phenomenon in which mis-folded proteins aggregate (i.e., accumulate and clump together) either intra- or extracellularly.  **Alzheimer's disease:** A protein misfolding disease (proteopathy), caused by accumulation of abnormally folded amyloid beta and amyloid tau proteins in the brain. Autophagy: is the basic catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the lysosomal machinery.  **Autophagy:** tightly regulated catabolic process (also known as autophagocytosis) that involves the degradation of cellular components through the lysosomal machinery. **Catalytic core:** A barrel shape formed particle of the proteasome composed by four stacked rings, each exist out of seven subunits. The active sites are sequestered in the inner chamber of the catalytic core and thus shielded from the intracellular medium. For degradation of a folded polypeptide to occur, it must be recognized, unfolded, and translocated into the catalytic core. **Chymotryptic-like activity:** The activity of enzymes that catalyzes the hydrolysis of certain proteins into polypeptides and amino acids.  **Dietary restriction:** is based on low calorie intake. "Low" can be defined relative to the subject's previous intake before intentionally restricting calories, or relative to an average person of similar body type.  **Heat shock proteins:** are a group of proteins induced by heat shock, the most prominent members of this group are a class of functionally related proteins involved in the folding and unfolding of other proteins.  **HEK293 cells:** Human Embryonic Kidney 293 cells, are a specific cell line originally derived from human embryonic kidney cells grown in tissue culture. HEK 293 cells are very easy to grow and transfect very readily and have been widely used in cell biology research for many years.  Homeostasis: The ability of an organism, or its constituent cells and organs, to establish and maintain an equilibrium that stabilizes its internal milieu and optimizes its ability to deal with moderate external changes  **Huntington's disease:** a neurodegenerative genetic disorder caused by “Huntingtin” gene with an expansion of a CAG triplet repeat, results in a different (mutant) form of the protein, which gradually damages cells in the brain, through mechanisms that are not fully understood. That affects muscle coordination and leads to cognitive decline and psychiatric problems.  **IFN-γ:** Interferon-gamma favors the breakdown of protein carbonyls by 26S proteasomes because of the concomitant increased activation of the ubiquitin-conjugation system in response to this cytokine. **Immunoproteasome:** alternative catalytic subunits of the 20S proteasome variant known as the Immunoproteasome The Immunoproteasome is normally associated with antigen-presenting cells where it provides peptides of an appropriate length for antibody generation; however, it is as well involved in non immune related cell types.   **Longevity:** The word "longevity" is sometimes used as a synonym for "life expectancy" in demography - however, the term "longevity" is sometimes meant to refer only to especially long lived members of a population, whereas "life expectancy" is always defined statistically as the average number of years remaining at a given age.  **Mitophagy:** An autophagy pathway that selectively degrades mitochondria  **OPMD:** Oculopharyngeal muscular dystrophy (OPMD) is a lateonset progressive muscle disorder for which the underlying molecular mechanisms are largely unknown. OPMD is caused by expansion of a homopolymeric alanine (Ala) stretch at theN-terminus of the Poly(A) Binding Protein Nuclear 1 (PABPN1) by two to seven additional Ala residues protein. In OPMD models disaggregation approaches attenuate muscle symptoms.  **Oxidized proteins:** Oxidative modifications to proteins can lead to cross-linking, peptide fragmentation, modified residues, and the conversion of one amino acid to another.if sufficient protein damage accumulates, cell death will occur. Although several antioxidant defense systems have evolved to prevent ROS damage, oxidized proteins appear to accumulate with age and may represent 30-50% of the total protein in old cells  **Pioneer factor:** Although the precise definition is still debated, pioneer factors are thought to be transcription factors that can initially bind regulatory sequences, allowing binding of other factors by opening compacted chromatin, ultimately enabling transcriptional activation.  **Pluripotency:** from the Latin plurimus, meaning “very many”, and potens, meaning “having power” refers to a stem cell that has the potential to differentiate into any of the three germ layers (endoderm, mesoderm, ectoderm)  **Poly-ubiquitylated proteins:** Ubiquitination is an enzymatic, protein post-translational modification process in which the carboxylic acid of the terminal glycine from the di-glycine motif in the activated ubiquitin forms an amide bond to the epsilon amine of the lysine in the modified protein. This ubiquitination is a cascade, which the ubiquitin forms a chain attached to the protein this chain is a label for destruction of the protein.  **Post-translational modifications:** After translation of a protein, the posttranslational modification of amino acids extends the range of functions of the protein by attaching it to other biochemical functional groups (such as acetate, phosphate, various lipids and carbohydrates), changing the chemical nature of an amino acid (e.g. citrullination), or making structural changes (e.g. formation of disulfide bridges). Also, enzymes may remove amino acids from the amino end of the protein, or cut the peptide chain in the middle. Other modifications, like phosphorylation, are part of common mechanisms for controlling the behavior of a protein, for instance activating or inactivating an enzyme.  **Proteasome:** a cylindrical complex containing a "core" of four stacked rings forming a central pore. The role of the proteasome is to degrade unneeded or damaged proteins. The degradation process yields peptides of about seven to eight amino acids long, which can then be further degraded into shorter amino acid sequences and used in synthesizing new proteins. **Respiratory burst proces**s: is the rapid release of reactive oxygen species superoxide radical and hydrogen peroxide from different types of cells. **ROS:** reactive oxygen species form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress, ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress.  **Self-renewal:** refers to a stem cell that has the potential to go through numerous cycles of cell division while maintaining the undifferentiated state. |

## Reference

1. Carlsson, P. & Mahlapuu, M. Forkhead transcription factors: key players in development and metabolism. Dev. Biol. 250, 1–23 (2002).
2. [Brunet A](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Brunet%20A%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), [Bonni A](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Bonni%20A%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), [Zigmond MJ](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Zigmond%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), Lin MZ, [Juo P](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Juo%20P%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), [Hu LS](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Hu%20LS%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), [Anderson MJ](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Anderson%20MJ%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), [Arden KC](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Arden%20KC%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), [Blenis J](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Blenis%20J%5BAuthor%5D&cauthor=true&cauthor_uid=10102273), [Greenberg ME](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Greenberg%20ME%5BAuthor%5D&cauthor=true&cauthor_uid=10102273). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 96, 857–868 (1999).
3. [Kops GJ](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Kops%20GJ%5BAuthor%5D&cauthor=true&cauthor_uid=10217147), [de Ruiter ND](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=de%20Ruiter%20ND%5BAuthor%5D&cauthor=true&cauthor_uid=10217147), De Vries-Smits AM, [Powell DR](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Powell%20DR%5BAuthor%5D&cauthor=true&cauthor_uid=10217147), [Bos JL](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Bos%20JL%5BAuthor%5D&cauthor=true&cauthor_uid=10217147), [Burgering BM](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Burgering%20BM%5BAuthor%5D&cauthor=true&cauthor_uid=10217147).Direct control of the Forkhead transcription factor AFX by protein kinase B. Nature 398, 630–634 (1999).
4. Biggs, W. H., Meisenhelder, J., Hunter, T., Cavenee, W. K. & Arden, K. C. Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. Proc. Natl Acad. Sci. USA 96, 7421–7426 (1999).
5. Tang, E. D., Nunez, G., Barr, F. G. & Guan, K. L. Negative regulation of the forkhead transcription factor FKHR by Akt. J. Biol. Chem. 274, 16741–16746 (1999).
6. [Essers MA](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Essers%20MA%5BAuthor%5D&cauthor=true&cauthor_uid=15538382), [Weijzen S](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Weijzen%20S%5BAuthor%5D&cauthor=true&cauthor_uid=15538382), [de Vries-Smits AM](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=de%20Vries-Smits%20AM%5BAuthor%5D&cauthor=true&cauthor_uid=15538382), [Saarloos I](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Saarloos%20I%5BAuthor%5D&cauthor=true&cauthor_uid=15538382), [de Ruiter ND](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=de%20Ruiter%20ND%5BAuthor%5D&cauthor=true&cauthor_uid=15538382), [Bos JL](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Bos%20JL%5BAuthor%5D&cauthor=true&cauthor_uid=15538382), [Burgering BM](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed?term=Burgering%20BM%5BAuthor%5D&cauthor=true&cauthor_uid=15538382).FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. EMBO J. 23, 4802–4812 (2004).
7. van der Horst, A. & Burgering, B. M. Stressing the role of FoxO proteins in lifespan and disease. Nature Rev. Mol. Cell Biol. 8, 440–450 (2007).
8. Calnan, D. R. & Brunet, A. The FoxO code. Oncogene 27, 2276–2288 (2008).
9. Eijkelenboom A, Burgering BM. FOXOs: signalling integrators for homeostasis maintenance. Nat Rev Mol Cell Biol. Feb;14(2):83-97(2013). ,
10. Obsil T, Obsilova V. [Structural basis for DNA recognition by FOXO proteins.](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed/21146564) Biochim Biophys Acta. Nov;1813(11):1946-53. (2011)
11. Brent MM, Anand R, Marmorstein R. [Structural basis for DNA recognition by FoxO1 and its regulation by posttranslational modification.](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed/18786403) Structure. Sep 10;16(9):1407-16. (2008)
12. Takahashi, Y. et al. Asymmetric arginine dimethylation determines life span in C. elegans by regulating forkhead transcription factor DAF‑16. Cell Metab. 13, 505–516 (2011).
13. Tatar, M., Bartke, A., and Antebi, A. The endocrine regulationof aging by insulin-like signals. Science 299, 1346–1351. (2003).
14. Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. A C. elegans mutant that lives twice as long as wild type. Nature 366, 461–464. (1993)
15. Henderson, S.T., and Johnson, T.E. daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. Curr. Biol. 11, 1975–1980 (2001).
16. Lee, S.S., Kennedy, S., Tolonen, A.C., and Ruvkun, G. DAF- 16 target genes that control C. elegans life-span and metabolism. Science 300, 644–647. (2003)
17. Lin, K., Hsin, H., Libina, N., and Kenyon, C. Regulation of the C. elegans Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat. Genet. 28, 139–145. (2001)
18. Hsu, A.L., Murphy, C.T., and Kenyon, C. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science 300, 1142–1145. (2003).
19. Morley, J.F., and Morimoto, R.I. Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones. Mol. Biol. Cell 15, 657–664. (2004).
20. Kenyon, C. The plasticity of aging: insights from long-lived mutants. Cell 120, 449–460 (2005)
21. Tower J. [Hsps and aging.](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed/19394247) Trends Endocrinol Metab. Jul;20(5):216-22. (2009)
22. Tower J. [Heat shock proteins and Drosophila aging.](http://www.ncbi.nlm.nih.gov.proxy-ub.rug.nl/pubmed/20840862) Exp Gerontol. May;46(5):355-62 (2011)
23. Rea, S.L. et al. A stress-sensitive reporter predicts longevity in isogenic populations of Caenorhabditis elegans. Nat. Genet. 37, 894–(2005)
24. V. Obsilova, J. Vecer, P. Herman, A. Pabianova, M. Sulc, J. Teisinger, E. Boura, T. Obsil 14-3-3 Protein interacts with nuclear localization sequence of forkhead transcription factor FoxO4 Biochemistry, 44 pp. 11608–11617(2005),
25. Tzivion G, Dobson M, Ramakrishnan G. [FoxO transcription factors; Regulation by AKT and 14-3-3 proteins.](http://www.ncbi.nlm.nih.gov/pubmed/21708191) Biochim Biophys Acta. Nov;1813(11):1938-45. (2011)
26. Hui RC, Gomes AR, Constantinidou D, Costa JR, Karadedou CT, Fernandez de Mattos S, Wymann MP, Brosens JJ, Schulze A, Lam EW. The forkhead transcription factor FOXO3a increases phosphoinositide‑3 kinase/Akt activity in drug-resistant leukemic cells through induction of PIK3CA expression. Mol. Cell. Biol. 28, 5886–5898 (2008).
27. Ide T, Shimano H, Yahagi N, Matsuzaka T, Nakakuki M, Yamamoto T, Nakagawa Y, Takahashi A, Suzuki H, Sone H, Toyoshima H, Fukamizu A, Yamada N.SREBPs suppress IRS‑2‑mediated insulin signalling in the liver. Nature Cell Biol. 6, 351–357 (2004). \
28. Puig, O. & Tjian, R. Transcriptional feedback control of insulin receptor by dFOXO/FOXO1. Genes Dev. 19, 2435–2446 (2005).
29. van den Berg, M. C. & Burgering, B. M. Integrating opposing signals toward forkhead box O. Antioxid. Redox Signal. 14, 607–621 (2011).
30. Peck B, Ferber EC, Schulze A. [Antagonism between FOXO and MYC Regulates Cellular Powerhouse.](http://www.ncbi.nlm.nih.gov/pubmed/23630664) Front Oncol. Apr 25;3:96 (2013)
31. [Greer EL](http://www.ncbi.nlm.nih.gov/pubmed?term=Greer%20EL%5BAuthor%5D&cauthor=true&cauthor_uid=17711846), [Oskoui PR](http://www.ncbi.nlm.nih.gov/pubmed?term=Oskoui%20PR%5BAuthor%5D&cauthor=true&cauthor_uid=17711846), [Banko MR](http://www.ncbi.nlm.nih.gov/pubmed?term=Banko%20MR%5BAuthor%5D&cauthor=true&cauthor_uid=17711846), [Maniar JM](http://www.ncbi.nlm.nih.gov/pubmed?term=Maniar%20JM%5BAuthor%5D&cauthor=true&cauthor_uid=17711846), [Gygi MP](http://www.ncbi.nlm.nih.gov/pubmed?term=Gygi%20MP%5BAuthor%5D&cauthor=true&cauthor_uid=17711846), [Gygi SP](http://www.ncbi.nlm.nih.gov/pubmed?term=Gygi%20SP%5BAuthor%5D&cauthor=true&cauthor_uid=17711846), [Brunet A](http://www.ncbi.nlm.nih.gov/pubmed?term=Brunet%20A%5BAuthor%5D&cauthor=true&cauthor_uid=17711846).. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. J. Biol. Chem. 282, 30107–30119 (2007).
32. Hardie DG, Ross FA, Hawley SA. [AMPK: a nutrient and energy sensor that maintains energy homeostasis.](http://www.ncbi.nlm.nih.gov/pubmed/22436748) Nat Rev Mol Cell Biol. Mar 22;13(4):251-62. (2012)
33. Hardie DG, Salt IP, Hawley SA, Davies SP. [AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge.](http://www.ncbi.nlm.nih.gov/pubmed/10051444) Biochem J. Mar 15;338 ( Pt 3):717-2(1999)
34. Greer, E. L. et al. An AMPK–FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. Curr. Biol. 17, 1646–1656 (2007).
35. Huang, H., Regan, K. M., Lou, Z., Chen, J. & Tindall, D. J. CDK2‑dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. Science 314, 294–297 (2006).
36. Yuan, Z. et al. Activation of FOXO1 by Cdk1 in cycling cells and postmitotic neurons. Science 319, 1665–1668 (2008).
37. Liu, P., Kao, T. P. & Huang, H. CDK1 promotes cell proliferation and survival via phosphorylation and inhibition of FOXO1 transcription factor. Oncogene 27, 4733–4744 (2008).
38. Yata, K. & Esashi, F. Dual role of CDKs in DNA repair: to be, or not to be. DNA Repair (Amst.) 8, 6–18 (2009).
39. [Mihaylova MM](http://www.ncbi.nlm.nih.gov/pubmed?term=Mihaylova%20MM%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Vasquez DS](http://www.ncbi.nlm.nih.gov/pubmed?term=Vasquez%20DS%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Ravnskjaer K](http://www.ncbi.nlm.nih.gov/pubmed?term=Ravnskjaer%20K%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Denechaud PD](http://www.ncbi.nlm.nih.gov/pubmed?term=Denechaud%20PD%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Yu RT](http://www.ncbi.nlm.nih.gov/pubmed?term=Yu%20RT%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Alvarez JG](http://www.ncbi.nlm.nih.gov/pubmed?term=Alvarez%20JG%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Downes M](http://www.ncbi.nlm.nih.gov/pubmed?term=Downes%20M%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Evans RM](http://www.ncbi.nlm.nih.gov/pubmed?term=Evans%20RM%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Montminy M](http://www.ncbi.nlm.nih.gov/pubmed?term=Montminy%20M%5BAuthor%5D&cauthor=true&cauthor_uid=21565617), [Shaw RJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Shaw%20RJ%5BAuthor%5D&cauthor=true&cauthor_uid=21565617).Class IIa Histone Deacetylases Are Hormone-Activated Regulators of FOXO and Mammalian Glucose Homeostasis. Cell 145, 607-621(2011)
40. Fu W, Ma Q, Chen L, Li P, Zhang M, Ramamoorthy S, Nawaz Z, Shimojima T, Wang H, Yang Y, Shen Z, Zhang Y, Zhang X, Nicosia SV, Zhang Y, Pledger JW, Chen J, Bai W. MDM2 acts downstream of p53 as an E3 ligase to promote FOXO ubiquitination and degradation. J. Biol. Chem. 284, 13987–14000 (2009).
41. Brenkman, A. B., de Keizer, P. L., van den Broek, N. J., Jochemsen, A. G. & Burgering, B. M. Mdm2 induces mono-ubiquitination of FOXO4. PLoS ONE 3, e2819 (2008).
42. Yamagata K, Daitoku H, Takahashi Y, Namiki K, Hisatake K, Kako K, Mukai H, Kasuya Y, Fukamizu A. [Arginine methylation of FOXO transcription factors inhibits their phosphorylation by Akt.](http://www.ncbi.nlm.nih.gov/pubmed/18951090) Mol Cell. Oct 24;32(2):221-31. (2008)
43. [Takahashi Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Takahashi%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Daitoku H](http://www.ncbi.nlm.nih.gov/pubmed?term=Daitoku%20H%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Hirota K](http://www.ncbi.nlm.nih.gov/pubmed?term=Hirota%20K%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Tamiya H](http://www.ncbi.nlm.nih.gov/pubmed?term=Tamiya%20H%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Yokoyama A](http://www.ncbi.nlm.nih.gov/pubmed?term=Yokoyama%20A%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Kako K](http://www.ncbi.nlm.nih.gov/pubmed?term=Kako%20K%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Nagashima Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Nagashima%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Nakamura A](http://www.ncbi.nlm.nih.gov/pubmed?term=Nakamura%20A%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Shimada T](http://www.ncbi.nlm.nih.gov/pubmed?term=Shimada%20T%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Watanabe S](http://www.ncbi.nlm.nih.gov/pubmed?term=Watanabe%20S%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Yamagata K](http://www.ncbi.nlm.nih.gov/pubmed?term=Yamagata%20K%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Yasuda K](http://www.ncbi.nlm.nih.gov/pubmed?term=Yasuda%20K%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Ishii N](http://www.ncbi.nlm.nih.gov/pubmed?term=Ishii%20N%5BAuthor%5D&cauthor=true&cauthor_uid=21531333), [Fukamizu A](http://www.ncbi.nlm.nih.gov/pubmed?term=Fukamizu%20A%5BAuthor%5D&cauthor=true&cauthor_uid=21531333).Asymmetric arginine dimethylation determines life span in C. elegans by regulating forkhead transcription factor DAF‑16. Cell Metab. 13, 505–516 (2011).
44. [Xie Q](http://www.ncbi.nlm.nih.gov/pubmed?term=Xie%20Q%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [Hao Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Hao%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [Tao L](http://www.ncbi.nlm.nih.gov/pubmed?term=Tao%20L%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [Peng S](http://www.ncbi.nlm.nih.gov/pubmed?term=Peng%20S%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [Rao C](http://www.ncbi.nlm.nih.gov/pubmed?term=Rao%20C%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [Chen H](http://www.ncbi.nlm.nih.gov/pubmed?term=Chen%20H%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [You H](http://www.ncbi.nlm.nih.gov/pubmed?term=You%20H%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [Dong MQ](http://www.ncbi.nlm.nih.gov/pubmed?term=Dong%20MQ%5BAuthor%5D&cauthor=true&cauthor_uid=22402663), [Yuan Z](http://www.ncbi.nlm.nih.gov/pubmed?term=Yuan%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=22402663).Lysine methylation of FOXO3 regulates oxidative stress-induced neuronal cell death. EMBO Rep. 13, 371–377 (2012).
45. De Keizer PL, Burgering BM, Dansen TB.[Forkhead box o as a sensor, mediator, and regulator of redox signaling.](http://www.ncbi.nlm.nih.gov/pubmed/20626320) Antioxid Redox Signal. Mar 15;14(6):1093-106 (2011).
46. Essers MA, de Vries-Smits LM, Barker N, Polderman PE, Burgering BM, Korswagen HC. Functional interaction between β-catenin and FOXO in oxidative stress signaling. Science 308, 1181–1184 (2005).
47. Hatta, M. & Cirillo, L. A. Chromatin opening and stable perturbation of core histone:DNA contacts by FoxO1. J. Biol. Chem. 282, 35583–35593 (2007).
48. van der Vos, K. E. & Coffer, P. J. FOXO-binding partners: it takes two to tango. Oncogene 27, 2289–2299 (2008).
49. Chiang WC, Ching TT, Lee HC, Mousigian C, Hsu AL. [HSF-1 regulators DDL-1/2 link insulin-like signaling to heat-shock responses and modulation of longevity.](http://www.ncbi.nlm.nih.gov/pubmed/22265419) Cell. Jan 20;148(1-2):322-34. (2012)
50. Tower J. [Hsps and aging.](http://www.ncbi.nlm.nih.gov/pubmed/19394247) Trends Endocrinol MetabJul;20(5):216-22. (2009)
51. Walker GA, White TM, McColl G, Jenkins NL, Babich S, Candido EP, Johnson TE, Lithgow GJ. [Heat shock protein accumulation is upregulated in a long-lived mutant of Caenorhabditis elegans.](http://www.ncbi.nlm.nih.gov/pubmed/11445592) J Gerontol A Biol Sci Med Sci. Jul;56(7):B281-7. (2001)
52. Cohen, E., Bieschke, J., Perciavalle, R. M., Kelly, J. W. & Dillin, A. Opposing activities protect against age-onset proteotoxicity. Science 313, 1604–1610 (2006).
53. Cohen, E. [Du D](http://www.ncbi.nlm.nih.gov/pubmed?term=Du%20D%5BAuthor%5D&cauthor=true&cauthor_uid=20003171), [Joyce D](http://www.ncbi.nlm.nih.gov/pubmed?term=Joyce%20D%5BAuthor%5D&cauthor=true&cauthor_uid=20003171), [Kapernick EA](http://www.ncbi.nlm.nih.gov/pubmed?term=Kapernick%20EA%5BAuthor%5D&cauthor=true&cauthor_uid=20003171), [Volovik Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Volovik%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=20003171), [Kelly JW](http://www.ncbi.nlm.nih.gov/pubmed?term=Kelly%20JW%5BAuthor%5D&cauthor=true&cauthor_uid=20003171), [Dillin A](http://www.ncbi.nlm.nih.gov/pubmed?term=Dillin%20A%5BAuthor%5D&cauthor=true&cauthor_uid=20003171). Temporal requirements of insulin/IGF‑1 signaling for proteotoxicity protection. Aging Cell 9, 126–134 (2010).
54. J. Yang, S.Carra, W.Zhu, H.H.Kampinga. FOXO1 inhibits polyglutamine aggregation. (Manuscript in preparation)
55. Fujimoto M, Takaki E, Hayashi T, Kitaura Y, Tanaka Y, Inouye S, Nakai A. [Active HSF1 significantly suppresses polyglutamine aggregate formation in cellular and mouse models.](http://www.ncbi.nlm.nih.gov/pubmed/16051598) J Biol Chem. Oct 14;280(41):34908-16. (2005)
56. Vidal R.L, Figueroa A, Court FA, Thielen P, Molina C, Wirth C, Caballero B, Kiffin R, Segura-Aguilar J, Cuervo AM, Glimcher LH, Hetz C. Targeting the UPR transcription factor XBP1 protects against Huntington's disease through the regulation of FoxO1 and autophagy. Hum Mol Genet. May 15;21(10):2245-62. (2012)
57. Zhao, J. [Brault JJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Brault%20JJ%5BAuthor%5D&cauthor=true&cauthor_uid=18054316), [Schild A](http://www.ncbi.nlm.nih.gov/pubmed?term=Schild%20A%5BAuthor%5D&cauthor=true&cauthor_uid=18054316), [Cao P](http://www.ncbi.nlm.nih.gov/pubmed?term=Cao%20P%5BAuthor%5D&cauthor=true&cauthor_uid=18054316), [Sandri M](http://www.ncbi.nlm.nih.gov/pubmed?term=Sandri%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18054316), [Schiaffino S](http://www.ncbi.nlm.nih.gov/pubmed?term=Schiaffino%20S%5BAuthor%5D&cauthor=true&cauthor_uid=18054316), [Lecker SH](http://www.ncbi.nlm.nih.gov/pubmed?term=Lecker%20SH%5BAuthor%5D&cauthor=true&cauthor_uid=18054316), [Goldberg AL](http://www.ncbi.nlm.nih.gov/pubmed?term=Goldberg%20AL%5BAuthor%5D&cauthor=true&cauthor_uid=18054316). FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell Metab. 6, 472–483 (2007).
58. Mammucari, C. [Milan G](http://www.ncbi.nlm.nih.gov/pubmed?term=Milan%20G%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Romanello V](http://www.ncbi.nlm.nih.gov/pubmed?term=Romanello%20V%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Masiero E](http://www.ncbi.nlm.nih.gov/pubmed?term=Masiero%20E%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Rudolf R](http://www.ncbi.nlm.nih.gov/pubmed?term=Rudolf%20R%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Del Piccolo P](http://www.ncbi.nlm.nih.gov/pubmed?term=Del%20Piccolo%20P%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Burden SJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Burden%20SJ%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Di Lisi R](http://www.ncbi.nlm.nih.gov/pubmed?term=Di%20Lisi%20R%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Sandri C](http://www.ncbi.nlm.nih.gov/pubmed?term=Sandri%20C%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Zhao J](http://www.ncbi.nlm.nih.gov/pubmed?term=Zhao%20J%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Goldberg AL](http://www.ncbi.nlm.nih.gov/pubmed?term=Goldberg%20AL%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Schiaffino S](http://www.ncbi.nlm.nih.gov/pubmed?term=Schiaffino%20S%5BAuthor%5D&cauthor=true&cauthor_uid=18054315), [Sandri M](http://www.ncbi.nlm.nih.gov/pubmed?term=Sandri%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18054315). FoxO3 controls autophagy in skeletal muscle in vivo. Cell Metab. 6, 458–471 (2007)
59. Mei, Y. [Zhang Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Zhang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=19276113), [Yamamoto K](http://www.ncbi.nlm.nih.gov/pubmed?term=Yamamoto%20K%5BAuthor%5D&cauthor=true&cauthor_uid=19276113), [Xie W](http://www.ncbi.nlm.nih.gov/pubmed?term=Xie%20W%5BAuthor%5D&cauthor=true&cauthor_uid=19276113), [Mak TW](http://www.ncbi.nlm.nih.gov/pubmed?term=Mak%20TW%5BAuthor%5D&cauthor=true&cauthor_uid=19276113), [You H](http://www.ncbi.nlm.nih.gov/pubmed?term=You%20H%5BAuthor%5D&cauthor=true&cauthor_uid=19276113). FOXO3a‑dependent regulation of Pink1 (Park6) mediates survival signaling in response to cytokine deprivation. Proc. Natl Acad. Sci. USA 106, 5153–5158 (2009).
60. Nedelsky NB, Todd PK, Taylor JP. [Autophagy and the ubiquitin-proteasome system: collaborators in neuroprotection.](http://www.ncbi.nlm.nih.gov/pubmed/18930136) Biochim Biophys Acta. Dec;1782(12):691-9. (2008)
61. Chen, C. C. [Jeon SM](http://www.ncbi.nlm.nih.gov/pubmed?term=Jeon%20SM%5BAuthor%5D&cauthor=true&cauthor_uid=20412774), [Bhaskar PT](http://www.ncbi.nlm.nih.gov/pubmed?term=Bhaskar%20PT%5BAuthor%5D&cauthor=true&cauthor_uid=20412774), [Nogueira V](http://www.ncbi.nlm.nih.gov/pubmed?term=Nogueira%20V%5BAuthor%5D&cauthor=true&cauthor_uid=20412774), [Sundararajan D](http://www.ncbi.nlm.nih.gov/pubmed?term=Sundararajan%20D%5BAuthor%5D&cauthor=true&cauthor_uid=20412774), [Tonic I](http://www.ncbi.nlm.nih.gov/pubmed?term=Tonic%20I%5BAuthor%5D&cauthor=true&cauthor_uid=20412774), [Park Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Park%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=20412774), [Hay N](http://www.ncbi.nlm.nih.gov/pubmed?term=Hay%20N%5BAuthor%5D&cauthor=true&cauthor_uid=20412774). FoxOs inhibit mTORC1 and activate Akt by inducing the expression of Sestrin3 and Rictor. Dev. Cell 18, 592–604 (2010).
62. Zhao Y, Yang J, Liao W, Liu X, Zhang H, Wang S, Wang D, Feng J, Yu L, Zhu WG. [Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity.](http://www.ncbi.nlm.nih.gov/pubmed/20543840) Nat Cell Biol. Jul;12(7):665-75 (2010).
63. Medema RH. Jäättelä M. [Cytosolic FoxO1: alive and killing.](http://www.ncbi.nlm.nih.gov/pubmed/20596046) Nat Cell Biol. Jul;12(7):642-3. (2010)
64. Vilchez D, Morantte I, Liu Z, Douglas PM, Merkwirth C, Rodrigues AP, Manning G, Dillin A. [RPN-6 determines C. elegans longevity under proteotoxic stress conditions.](http://www.ncbi.nlm.nih.gov/pubmed/22922647) Nature. Sep 13;489 (7415) (2012).
65. Díaz-Hernández M, Hernández F, Martín-Aparicio E, Gómez-Ramos P, Morán MA, Castaño JG, Ferrer I, Avila J, Lucas JJ. [Neuronal induction of the immunoproteasome in Huntington's disease.](http://www.ncbi.nlm.nih.gov/pubmed/14684867) J Neurosci. Dec 17;23,37(2003)
66. Orre M, Kamphuis W, Dooves S, Kooijman L, Chan ET, Kirk CJ, Dimayuga Smith V, Koot S, Mamber C, Jansen AH, Ovaa H, Hol EM. [Reactive glia show increased immunoproteasome activity in Alzheimer's disease.](http://www.ncbi.nlm.nih.gov/pubmed/23604491) Brain. May;136(Pt 5):1415-31(2013)
67. Mishto M, Bellavista E, Santoro A, Stolzing A, Ligorio C, Nacmias B, Spazzafumo L, Chiappelli M, Licastro F, Sorbi S, Pession A, Ohm T, Grune T, Franceschi C. [Immunoproteasome and LMP2 polymorphism in aged and Alzheimer's disease brains.](http://www.ncbi.nlm.nih.gov/pubmed/16298241) Neurobiol Aging. Jan;27(2006)
68. Anvar SY, 't Hoen PA, Venema A, van der Sluijs B, van Engelen B, Snoeck M, Vissing J, Trollet C, Dickson G, Chartier A, Simonelig M, van Ommen GJ, van der Maarel SM, Raz V. [Deregulation of the ubiquitin-proteasome system is the predominant molecular pathology in OPMD animal models and patients.](http://www.ncbi.nlm.nih.gov/pubmed/21798095) Skelet Muscle. Apr 4;1(1):15. (2011)
69. Hussong SA, Kapphahn RJ, Phillips SL, Maldonado M, Ferrington DA. [Immunoproteasome deficiency alters retinal proteasome's response to stress.](http://www.ncbi.nlm.nih.gov/pubmed/20345760) J Neurochem. Jun;113(6): (2010)
70. Ebstein F, Kloetzel PM, Krüger E, Seifert U. [Emerging roles of immunoproteasomes beyond MHC class I antigen processing.](http://www.ncbi.nlm.nih.gov/pubmed/22382925) Cell Mol Life Sci. Aug;69 (2012)
71. Groettrup M, Kirk CJ, Basler M. [Proteasomes in immune cells: more than peptide producers?](http://www.ncbi.nlm.nih.gov/pubmed/20010787) Nat Rev Immunol. Jan;10 (2010)
72. Seifert U, Bialy LP, Ebstein F, Bech-Otschir D, Voigt A, Schroter F, Prozorovski T, Lange N, Steffen J, Rieger M, Kuckelkorn U, Aktas O, Kloetzel PM, Kruger E Immunoproteasomes preserve protein homeostasis upon interferon-induced oxidative stress. Cell 142(4):613–624 (2010)
73. Sijts AJ, Ruppert T, Rehermann B, Schmidt M, Koszinowski U, Kloetzel PM. Efficient generation of a hepatitis B virus cytotoxic T lymphocyte epitope requires the structural features of immunoproteasomes. J Exp Med 191(3):503–514(2000).
74. Ouyang W, Liao W, Luo CT, Yin N, Huse M, Kim MV, Peng M, Chan P, Ma Q, Mo Y, Meijer D, Zhao K, Rudensky AY, Atwal G, Zhang MQ, Li MO [Novel Foxo1-dependent transcriptional programs control T(reg) cell function.](http://www.ncbi.nlm.nih.gov/pubmed/23135404) Nature. Nov 22;491(7425):554-9. (2012).
75. Wang MC, Bohmann D, Jasper H. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. Cell.;121:115–25. (2005).
76. Kleindorp R, Flachsbart F, Puca AA, Malovini A, Schreiber S, Nebel A. [Candidate gene study of FOXO1, FOXO4, and FOXO6 reveals no association with human longevity in Germans.](http://www.ncbi.nlm.nih.gov/pubmed/21388494) Aging Cell. Aug;10(4):622-8. (2011)
77. Li Y, Wang WJ, Cao H, Lu J, Wu C, Hu FY, Guo J, Zhao L, Yang F, Zhang YX, Li W, Zheng GY, Cui H, Chen X, Zhu Z, He H, Dong B, Mo X, Zeng Y, Tian XL. Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. Hum. Mol. Genet. 18, 4897–4904. (2009).
78. Christensen K, Johnson TE, Vaupel JW The quest for genetic determinants of human longevity: challenges and insights. Nat. Rev.Genet. 7, 436–448. (2006)
79. Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD FOXO3A genotype is strongly associated with human longevity. Proc. Natl Acad. Sci. USA 105, 13987–13992. (2008)
80. Anselmi CV, Malovini A, Roncarati R, Novelli V, Villa F, Condorelli G, Bellazzi R, Puca AA Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. Rejuvenation Res. 12, 95–104. (2009)
81. Flachsbart F, Caliebe A, Kleindorp R, Blanche H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A Association of FOXO3A variation with human longevity confirmed in German centenarians. Proc. Natl Acad. Sci. USA 106, 2700–2705. (2009)
82. Li Y, Wang WJ, Cao H, Lu J, Wu C, Hu FY, Guo J, Zhao L, Yang F, Zhang YX, Li W, Zheng GY, Cui H, Chen X, Zhu Z, He H, Dong B, Mo X, Zeng Y, Tian XL Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. Hum. Mol. Genet. 18, 4897–4904. (2009)
83. Pawlikowska L, Hu D, Huntsman S, Sung A, Chu C, Chen J, Joyner AH, Schork NJ, Hsueh WC, Reiner AP, Psaty BM, Atzmon G, Barzilai N, Cummings SR, Browner WS, Kwok PY, Ziv E Association of common genetic variation in the insulin ⁄ IGF1 signaling pathway with human longevity. Aging Cell 8, 460–472(2009)
84. Soerensen M, Dato S, Christensen K, McGue M, Stevnsner T, Bohr VA, Christiansen L Replication of an association of variation in the FOXO3A gene with human longevity using both case–control and longitudinal data. Aging Cell 9, 1010–1017. (2010)
85. Eijkelenboom A, Mokry M, de Wit E, Smits LM, Polderman PE, van Triest MH, van Boxtel R, Schulze A, de Laat W, Cuppen E, Burgering BM. [Genome-wide analysis of FOXO3 mediated transcription regulation through RNA polymerase II profiling.](http://www.ncbi.nlm.nih.gov/pubmed/23340844) Mol Syst Biol. Jan 22;9:638 (2013).
86. Kaul, S.C. [Deocaris CC](http://www.ncbi.nlm.nih.gov/pubmed?term=Deocaris%20CC%5BAuthor%5D&cauthor=true&cauthor_uid=17188442), [Wadhwa R](http://www.ncbi.nlm.nih.gov/pubmed?term=Wadhwa%20R%5BAuthor%5D&cauthor=true&cauthor_uid=17188442). Three faces of mortalin: a housekeeper, guardian and killer. Exp. Gerontol. 42, 263–274(2007)
87. Yokoyama, K. [Fukumoto K](http://www.ncbi.nlm.nih.gov/pubmed?term=Fukumoto%20K%5BAuthor%5D&cauthor=true&cauthor_uid=11959102), [Murakami T](http://www.ncbi.nlm.nih.gov/pubmed?term=Murakami%20T%5BAuthor%5D&cauthor=true&cauthor_uid=11959102), [Harada S](http://www.ncbi.nlm.nih.gov/pubmed?term=Harada%20S%5BAuthor%5D&cauthor=true&cauthor_uid=11959102), [Hosono R](http://www.ncbi.nlm.nih.gov/pubmed?term=Hosono%20R%5BAuthor%5D&cauthor=true&cauthor_uid=11959102), [Wadhwa R](http://www.ncbi.nlm.nih.gov/pubmed?term=Wadhwa%20R%5BAuthor%5D&cauthor=true&cauthor_uid=11959102), [Mitsui Y](http://www.ncbi.nlm.nih.gov/pubmed?term=Mitsui%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=11959102), [Ohkuma S](http://www.ncbi.nlm.nih.gov/pubmed?term=Ohkuma%20S%5BAuthor%5D&cauthor=true&cauthor_uid=11959102). Extended longevity of Caenorhabditis elegans by knocking in extra copies of hsp70F, a homolog of mot-2 (mortalin)/mthsp70/Grp75. FEBS Lett. 516, 53–57(2002)
88. Kim HS, Skurk C, Maatz H, Shiojima I, Ivashchenko Y, Yoon SW, Park YB, Walsh K. [Akt/FOXO3a signaling modulates the endothelial stress response through regulation of heat shock protein 70 expression.](http://www.ncbi.nlm.nih.gov/pubmed/15784720) FASEB J. Jun;19(8):1042-4. (2005)
89. Leroi AM, Bartke A, De Benedictis G, Franceschi C, Gartner A, Gonos ES, Fedei ME, Kivisild T, Lee S, Kartaf-Ozer N, Schumacher M, Sikora E, Slagboom E, Tatar M, Yashin AI, Vijg J, Zwaan B. What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mech. Ageing Dev. 126, 421–429 (2005)
90. Mizushima, N. [Levine B](http://www.ncbi.nlm.nih.gov/pubmed?term=Levine%20B%5BAuthor%5D&cauthor=true&cauthor_uid=18305538), [Cuervo AM](http://www.ncbi.nlm.nih.gov/pubmed?term=Cuervo%20AM%5BAuthor%5D&cauthor=true&cauthor_uid=18305538), [Klionsky DJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Klionsky%20DJ%5BAuthor%5D&cauthor=true&cauthor_uid=18305538). Autophagy fights disease through cellular self-digestion. Nature 451, 1069–1075(2008)
91. Melendez, A. [Tallóczy Z](http://www.ncbi.nlm.nih.gov/pubmed?term=Tall%C3%B3czy%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=12958363), [Seaman M](http://www.ncbi.nlm.nih.gov/pubmed?term=Seaman%20M%5BAuthor%5D&cauthor=true&cauthor_uid=12958363), [Eskelinen EL](http://www.ncbi.nlm.nih.gov/pubmed?term=Eskelinen%20EL%5BAuthor%5D&cauthor=true&cauthor_uid=12958363), [Hall DH](http://www.ncbi.nlm.nih.gov/pubmed?term=Hall%20DH%5BAuthor%5D&cauthor=true&cauthor_uid=12958363), [Levine B](http://www.ncbi.nlm.nih.gov/pubmed?term=Levine%20B%5BAuthor%5D&cauthor=true&cauthor_uid=12958363). Autophagy genes are essential for dauer development and life-span extension in C. elegans. Science 301, 1387–1391(2003)
92. Jia, K. and Levine, B. Autophagy is required for dietary restriction-mediated life span extension in C. elegans. Autophagy 3,597–599(2007)
93. Hansen, M [Chandra A](http://www.ncbi.nlm.nih.gov/pubmed?term=Chandra%20A%5BAuthor%5D&cauthor=true&cauthor_uid=18282106), [Mitic LL](http://www.ncbi.nlm.nih.gov/pubmed?term=Mitic%20LL%5BAuthor%5D&cauthor=true&cauthor_uid=18282106), [Onken B](http://www.ncbi.nlm.nih.gov/pubmed?term=Onken%20B%5BAuthor%5D&cauthor=true&cauthor_uid=18282106), [Driscoll M](http://www.ncbi.nlm.nih.gov/pubmed?term=Driscoll%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18282106), [Kenyon C](http://www.ncbi.nlm.nih.gov/pubmed?term=Kenyon%20C%5BAuthor%5D&cauthor=true&cauthor_uid=18282106).A role for autophagy in the extension of lifespan by dietary restriction in C. elegans. PLoS Genet. 4, e24(2008)
94. Toth, M.L. [Sigmond T](http://www.ncbi.nlm.nih.gov/pubmed?term=Sigmond%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Borsos E](http://www.ncbi.nlm.nih.gov/pubmed?term=Borsos%20E%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Barna J](http://www.ncbi.nlm.nih.gov/pubmed?term=Barna%20J%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Erdélyi P](http://www.ncbi.nlm.nih.gov/pubmed?term=Erd%C3%A9lyi%20P%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Takács-Vellai K](http://www.ncbi.nlm.nih.gov/pubmed?term=Tak%C3%A1cs-Vellai%20K%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Orosz L](http://www.ncbi.nlm.nih.gov/pubmed?term=Orosz%20L%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Kovács AL](http://www.ncbi.nlm.nih.gov/pubmed?term=Kov%C3%A1cs%20AL%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Csikós G](http://www.ncbi.nlm.nih.gov/pubmed?term=Csik%C3%B3s%20G%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Sass M](http://www.ncbi.nlm.nih.gov/pubmed?term=Sass%20M%5BAuthor%5D&cauthor=true&cauthor_uid=18219227), [Vellai T](http://www.ncbi.nlm.nih.gov/pubmed?term=Vellai%20T%5BAuthor%5D&cauthor=true&cauthor_uid=18219227). Longevity pathways converge on autophagy genes to regulate life span in Caenorhabditis elegans. Autophagy 4, 330–338(2008).
95. [Tothova Z](http://www.ncbi.nlm.nih.gov/pubmed?term=Tothova%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Kollipara R](http://www.ncbi.nlm.nih.gov/pubmed?term=Kollipara%20R%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Huntly BJ](http://www.ncbi.nlm.nih.gov/pubmed?term=Huntly%20BJ%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Lee BH](http://www.ncbi.nlm.nih.gov/pubmed?term=Lee%20BH%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Castrillon DH](http://www.ncbi.nlm.nih.gov/pubmed?term=Castrillon%20DH%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Cullen DE](http://www.ncbi.nlm.nih.gov/pubmed?term=Cullen%20DE%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [McDowell EP](http://www.ncbi.nlm.nih.gov/pubmed?term=McDowell%20EP%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Lazo-Kallanian S](http://www.ncbi.nlm.nih.gov/pubmed?term=Lazo-Kallanian%20S%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Williams IR](http://www.ncbi.nlm.nih.gov/pubmed?term=Williams%20IR%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Sears C](http://www.ncbi.nlm.nih.gov/pubmed?term=Sears%20C%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Armstrong SA](http://www.ncbi.nlm.nih.gov/pubmed?term=Armstrong%20SA%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Passegué E](http://www.ncbi.nlm.nih.gov/pubmed?term=Passegu%C3%A9%20E%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [DePinho RA](http://www.ncbi.nlm.nih.gov/pubmed?term=DePinho%20RA%5BAuthor%5D&cauthor=true&cauthor_uid=17254970), [Gilliland DG](http://www.ncbi.nlm.nih.gov/pubmed?term=Gilliland%20DG%5BAuthor%5D&cauthor=true&cauthor_uid=17254970). FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell;128(2):325-339. (2007).
96. [Tothova Z](http://www.ncbi.nlm.nih.gov/pubmed?term=Tothova%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=18371346), [Gilliland DG](http://www.ncbi.nlm.nih.gov/pubmed?term=Gilliland%20DG%5BAuthor%5D&cauthor=true&cauthor_uid=18371346). FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. [Cell Stem Cell.](http://www.ncbi.nlm.nih.gov/pubmed/18371346) Aug 16;1(2):140-52. (2007)
97. Castrillon DH, Miao L, Kollipara R, Horner JW, DePinho RA. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. Science 301:215–8. (2003)
98. Paik, J. H. [Ding Z](http://www.ncbi.nlm.nih.gov/pubmed?term=Ding%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Narurkar R](http://www.ncbi.nlm.nih.gov/pubmed?term=Narurkar%20R%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Ramkissoon S](http://www.ncbi.nlm.nih.gov/pubmed?term=Ramkissoon%20S%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Muller F](http://www.ncbi.nlm.nih.gov/pubmed?term=Muller%20F%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Kamoun WS](http://www.ncbi.nlm.nih.gov/pubmed?term=Kamoun%20WS%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Chae SS](http://www.ncbi.nlm.nih.gov/pubmed?term=Chae%20SS%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Zheng H](http://www.ncbi.nlm.nih.gov/pubmed?term=Zheng%20H%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Ying H](http://www.ncbi.nlm.nih.gov/pubmed?term=Ying%20H%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Mahoney J](http://www.ncbi.nlm.nih.gov/pubmed?term=Mahoney%20J%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Hiller D](http://www.ncbi.nlm.nih.gov/pubmed?term=Hiller%20D%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Jiang S](http://www.ncbi.nlm.nih.gov/pubmed?term=Jiang%20S%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Protopopov A](http://www.ncbi.nlm.nih.gov/pubmed?term=Protopopov%20A%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Wong WH](http://www.ncbi.nlm.nih.gov/pubmed?term=Wong%20WH%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Chin L](http://www.ncbi.nlm.nih.gov/pubmed?term=Chin%20L%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [Ligon KL](http://www.ncbi.nlm.nih.gov/pubmed?term=Ligon%20KL%5BAuthor%5D&cauthor=true&cauthor_uid=19896444), [DePinho RA](http://www.ncbi.nlm.nih.gov/pubmed?term=DePinho%20RA%5BAuthor%5D&cauthor=true&cauthor_uid=19896444). FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. Cell Stem Cell 5, 540–553 (2009).
99. Renault, V. M. Rafalski VA, Morgan AA, Salih DA, Brett JO, Webb AE, Villeda SA, Thekkat PU, Guillerey C, Denko NC, Palmer TD, Butte AJ, Brunet A. FoxO3 regulates neural stem cell homeostasis. Cell Stem Cell 5, 527–539 (2009).
100. Baharvand H, Fathi A, van Hoof D, Salekdeh GH. [Concise review: trends in stem cell proteomics.](http://www.ncbi.nlm.nih.gov/pubmed/17495109)Stem Cells. Aug;25(8):1888-903. (2007).
101. Prinsloo E, Setati MM, Longshaw VM, Blatch GL. [Chaperoning stem cells: a role for heat shock proteins in the modulation of stem cell self-renewal and differentiation?](http://www.ncbi.nlm.nih.gov/pubmed/19274656) Bioessays. Apr;31.4 (2009).
102. Battersby A, Jones RD, Lilley KS, McFarlane RJ, Braig HR, Allen ND, Wakeman JA. [Comparative proteomic analysis reveals differential expression of Hsp25 following the directed differentiation of mouse embryonic stem cells.](http://www.ncbi.nlm.nih.gov/pubmed/17030443) Biochim Biophys Acta. Feb;1773 (2007).
103. Hernebring M, Brolén G, Aguilaniu H, Semb H, Nyström T. [Elimination of damaged proteins during differentiation of embryonic stem cells.](http://www.ncbi.nlm.nih.gov/pubmed/16672370) Proc Natl Acad Sci U S A. May 16;103.20 (2006).
104. Baharvand H, Fathi A, Gourabi H, Mollamohammadi S, Salekdeh GH. [Identification of mouse embryonic stem cell-associated proteins.](http://www.ncbi.nlm.nih.gov/pubmed/18047272) J Proteome Res. Jan;7(1):412-23. (2008).
105. Yu JM, Wu X, Gimble JM, Guan X, Freitas MA, Bunnell BA. [Age-related changes in mesenchymal stem cells derived from rhesus macaque bone marrow.](http://www.ncbi.nlm.nih.gov/pubmed/20969724) Aging Cell. Feb;10(1):66-79. (2011)