A TORC1-histone axis regulates chromatin organisation and non-canonical induction of autophagy to ameliorate ageing

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Abstract Age-related changes to histone levels are seen in many species. However, it is unclear whether changes to histone expression could be exploited to ameliorate the effects of ageing in multicellular organisms. Here we show that inhibition of mTORC1 by the lifespan-extending drug rapamycin increases expression of histones H3 and H4 post-transcriptionally through eIF3-mediated translation. Elevated expression of H3/H4 in intestinal enterocytes in Drosophila alters chromatin organisation, induces intestinal autophagy through transcriptional regulation, and prevents age-related decline in the intestine. Importantly, it also mediates rapamycin-induced longevity and intestinal health. Histones H3/H4 regulate expression of an autophagy cargo adaptor Bchs (WDFY3 in mammals), increased expression of which in enterocytes mediates increased H3/H4-dependent healthy longevity. In mice, rapamycin treatment increases expression of histone proteins and Wdfy3 transcription, and alters chromatin organisation in the small intestine, suggesting that the mTORC1-histone axis is at least partially conserved in mammals and may offer new targets for anti-ageing interventions.

Introduction

Ageing leads to the functional decline of cells, tissues, and organs, and is the primary risk factor for the most common, fatal human diseases, including cancer, cardiovascular disease, and neurodegeneration (Harman, 1991; Niccoli and Partridge, 2012). The mechanisms driving ageing are becoming increasingly well-understood, and conserved hallmarks of ageing, present in the aetiology of age-related diseases, have been described (López-Otín et al., 2013). Understanding how these physiological changes interact with each other, including which features are causative in age-related decline, represents a major challenge to the field (López-Otín et al., 2013; Partridge et al., 2018).

Two prominent cellular processes identified as key players in organismal ageing are alteration of the epigenetic machinery and dysregulation of the insulin/Igf (IIS)/mechanistic target of rapamycin (mTOR) nutrient-sensing network (Alic and Partridge, 2011; Benayoun et al., 2015; Johnson et al., 2013; López-Otín et al., 2013; López-Otín et al., 2013; Pal and Tyler, 2016).

Alteration of the epigenetic machinery, including DNA methylation, post-translational modification of histones, and chromatin remodelling, can be driven by diverse stimuli during ageing (Benayoun et al., 2015). Multiple lines of evidence suggest that epigenetic alterations and perturbations can trigger progeroid syndromes or affect longevity in model organisms (Pal and Tyler, 2016; Sen et al., 2016). Enzymatic systems regulating epigenetic patterns, including DNA methylation and histone modifications, have been intensively studied. Beyond enzymatic regulation, there is growing
evidence that expression levels of histone proteins play a key role during the ageing process (Benayoun et al., 2015). Histone proteins pack and order genomic DNA into structural units called nucleosomes, and they constitute the major protein components of chromatin. Histones include the core histones H2A, H2B, H3, and H4, which form the nucleosome core, and the linked histone H1. Histone H3 protein levels decrease in aged yeast (Feser et al., 2010), the nematode worm Caenorhabditis elegans (Ni et al., 2012), and human senescent cells (Ivanov et al., 2013). Concordantly, over-expression of core histones H3 and H4 extended replicative lifespan in yeast, potentially attenuating the age-related loss of nucleosomes, transcriptional dysfunction, and genomic instability in aged yeast cells (Feser et al., 2010; Hu et al., 2014). Studies in yeast suggest that histone-driven loss of nucleosomes could contribute to ageing in other organisms, particularly given that histones have a high degree of structural and functional conservation in eukaryotes. However, almost nothing is known about the role of histone expression in longevity in multicellular organisms.

Dysregulation of the IIS/mTOR network at late ages also has substantial effects on organismal ageing (López-Otín et al., 2013). This network integrates multiple environmental inputs, including nutrient availability, to regulate metabolism, growth, stress resistance, immune responses, reproduction, and lifespan (Alic and Partridge, 2011; Regan et al., 2020; Saxton and Sabatini, 2017). Lowered activity of the IIS/mTOR network by nutritional, genetic, or pharmacological interventions can extend lifespan and reduce age-related pathologies in multiple organisms (Fontana et al., 2010; Kenyon, 2010; Niccoli and Partridge, 2012). Linkage studies of human longevity families and genome-wide association studies (GWAS) of populations suggest that the IIS/mTOR network is associated with longevity in humans (Broer et al., 2015; Deelen et al., 2019; Johnson et al., 2015; Passtoors et al., 2013; Suh et al., 2008).

mTOR is a serine/threonine protein kinase in the PI3K-related kinase family that forms two distinct protein complexes, mTOR Complex 1 (mTORC1) and 2 (mTORC2). Reduction of mTORC1 activity by genetic manipulation of key components of mTORC1, TOR or Raptor, extends lifespan in yeast, nematode worms C. elegans, the fruit fly Drosophila melanogaster, and mice (Johnson et al., 2013). The FDA-approved drug rapamycin directly targets mTORC1 and lowers its activity. Rapamycin treatment extends lifespan in diverse organisms, including mice, and attenuates a broad spectrum of age-related functional decline and diseases (Johnson et al., 2013; Li et al., 2014). In humans, rapamycin has been used clinically at high doses as an immunosuppressant to suppress tissue graft rejection, although these clinical doses are associated with negative metabolic side effects such as hyperglycaemia and insulin resistance. Recent studies, including those showing the beneficial effects of low-dose, short-term treatments with rapamycin analogs (‘rapalogs’) on response to vaccination in the elderly, without significant adverse side effects, suggest its therapeutic potential as a geroprotective compound (Mannick et al., 2014; Mannick et al., 2018; Partridge et al., 2020). Lifespan extension by rapamycin in Drosophila requires reduced S6K activity and increased autophagy downstream of mTORC1 (Bjedov et al., 2010). Consistently, genetic manipulations that reduce S6K activity (Kapahi et al., 2004; Selman et al., 2009) or activate autophagy (Pyo et al., 2013; Ulgherait et al., 2014) extend lifespan in both Drosophila and mice. More generally, activating expression of autophagy-related genes can prevent age-related dysfunction in a variety of tissues; for instance, limiting intestinal barrier dysfunction, memory impairment, and muscular dystrophy in animal models (Hansen et al., 2018). Given the promise of rapamycin and rapalogs to treat age-related decline in humans, understanding how the drug regulates autophagy, in which tissues, and how this leads to increased longevity, is crucial. This will allow for the development of more precise pharmacological treatments that circumvent unwanted side effects (Arriola Apelo and Lamming, 2016; Li et al., 2014).

Here we uncover an unexpected link between histone levels and mTORC1 signalling in Drosophila and mice. Rapamycin treatment increased expression of histone proteins through non-canonical eukaryotic initiation factor 3 (eIF3)-mediated translation in the intestine of Drosophila. Rapamycin treatment, or over-expression of histones H3 and H4, specifically in the enterocytes (ECs) of the fly intestine, caused chromatin rearrangement and heterochromatin relocation in EC nuclei. Increased expression of histones in ECs was a key step for rapamycin-dependent longevity and gut homeostasis. Importantly, direct expression of H3/H4 in ECs was sufficient to extend lifespan and improve intestinal health during ageing. Increased expression of H3/H4 in ECs activated autophagy by epigenetic, transcriptional regulation of expression of autophagy-related genes, including Blue Cheese (Bchs), a selective autophagy cargo adaptor, which we demonstrated to be required and sufficient
for the effects of increased histone levels on intestinal autophagy, gut health, and lifespan. In mice, rapamycin treatment increased expression of histone proteins and the mammalian Bchs homolog WdHy3 transcript in the small intestines of aged individuals and altered the chromatin architecture in intestinal ECs, suggesting that the mTORC1-histone axis is at least partially conserved in mammals. Our findings unveil an mTORC1-histone axis as a crucial pro-longevity mechanism that can offer new directions for therapeutic anti-ageing interventions.

Results

Expression of core histones in the fly intestine is increased during ageing and by rapamycin treatment

To address a possible role for histones in the extension of lifespan induced by lowered mTORC1 activity in response to rapamycin treatment (Bjedov et al., 2010; Figure 1A; Supplementary file 1), we measured expression of histone proteins H3 and H4 during ageing in rapamycin-treated and control flies, in brain, muscle, fat body, and intestine (Figure 1B). In brain, muscle, and fat, neither rapamycin treatment nor age affected the expression of H3 or H4 protein (Figure 1—figure supplement 1A–C). In contrast, in the intestine rapamycin induced a marked increase in expression of both H3 and H4 proteins at all ages assessed, and there was also a slight increase in expression of these
proteins with age in control, untreated flies (Figure 1C). The intestine had much lower basal expression of H3 and H4 than did the other three tissues (Figure 1—figure supplement 1D). In the intestine, rapamycin increased expression of histone proteins by 2 days after the start of treatment (Figure 1—figure supplement 2A). The expression of core histones thus increased slightly during ageing in control flies and was strongly increased at all ages by rapamycin treatment, specifically in the intestine.

Previous studies have shown that dietary restriction (DR) has some similar effects on organismal physiology to rapamycin treatment (Unnikrishnan et al., 2020). We therefore tested if DR affected expression of histone proteins in the fly intestine. There was no difference in expression of H3 and H4 between intestines of flies fed control food and those fed food with a doubled yeast content (Figure 1—figure supplement 3). Increased histone protein expression was thus specific to treatment with rapamycin.

**Rapamycin treatment did not affect cell composition or EC polyploidy in the intestine**

The fly intestine contains four major cell types: intestinal stem cells (ISCs), which are mitotically active throughout the life course, multipotent enteroblasts, secretory enteroendocrine cells, and polyploid ECs that are the major differentiated cell type (Lemaitre and Miguel-Aliaga, 2013). The increase in expression of histone proteins in the intestine in response to rapamycin treatment could have been attributable to a change in cell composition or to the extent of polyploidisation of ECs. We therefore assessed the ratio of all cell types and of EC ploidy and found that neither was affected by rapamycin treatment (Figure 2—figure supplement 1A–C), suggesting that increased histone protein expression in response to rapamycin was not caused by changes to intestinal epithelial architecture or EC polyploidy.

Expression of core histones is increased in response to rapamycin treatment through eIF3 activity. mTORC1 is a central signalling hub that maintains cellular homeostasis through downstream effectors by transcriptional and post-transcriptional regulation (Saxton and Sabatini, 2017). To determine whether increased histone protein expression in response to rapamycin treatment was mediated transcriptionally, we measured expression of histones H3 and H4 transcripts in the intestines of flies treated with rapamycin. In young flies, up to day 10, transcript levels did not change in controls, and rapamycin treatment had no effect (Figure 1—figure supplement 2B, C). However, there was a marked age-related increase in controls at days 30 and 50, which was strongly attenuated by rapamycin treatment (Figure 1—figure supplement 2C). These results suggest that the age-related increase in histone protein levels may have been a consequence of increased transcript abundance, but that the rapamycin-dependent increase in histone H3 and H4 protein levels (Figure 1C, Figure 1—figure supplement 1A) was not and was instead mediated in a post-transcriptional manner through regulation of translation or protein stability.

We next tested whether rapamycin regulated histone protein levels through effects on their translation. Cycloheximide, which inhibits protein synthesis, abolished the increase in histone protein levels in response to rapamycin treatment (Figure 2A). This indicated that increased histone translation occurred in response to rapamycin treatment, which is not intuitive given that mTORC1 attenuation is known to suppress translation (Saxton and Sabatini, 2017). However, previous studies have demonstrated notable exceptions to this translational suppression, including histones, which can undergo increased translation via a non-canonical, eIF3-mediated mechanism (Lee et al., 2015; Lee et al., 2016; Thoreen et al., 2012). To test for the role of this mechanism, we knocked down expression of elf3d or elf3g in adult ECs by RNAi, which abolished the rapamycin-induced increased expression of histone proteins (Figure 2B, C), suggesting that the elf3 protein complex was required. In addition, inhibiting the canonical mTORC1- eIF4 translation cascade, by knock-down of elf4e in adult ECs by RNAi, recapitulated the rapamycin-induced increased expression of histone proteins (Figure 2D). This result was in line with the previous study showing that inhibition of eIF4 components can enforce mRNA translation through an eIF3-specialised pathway (Lee et al., 2016).

We examined whether rapamycin also regulated histone proteins through protein turnover. Neither perturbation of autophagy by ubiquitously reducing expression of Atg5 by RNAi (Bjedov et al., 2010) nor inhibition of proteasome activity by treatment with bortezomib, a proteasome inhibitor (Tain et al., 2017), interfered with increased expression of histones in response to rapamycin.
Figure 2. Expression of core histones in the fly intestine in response to rapamycin treatment and inhibition of translation or translation factors eukaryotic initiation factor (eIF)3 and eIF4. (A) Adult-onset cycloheximide treatment (1 mM) alone had no effect on histone expression but blocked increased expression of histones H3 and H4 in response to rapamycin treatment in intestines of flies at 2 days of age ($n = 4$ biological replicates of 10 intestines per replicate, two-way ANOVA, interaction, H3 $p<0.05$, H4 $p<0.01$; post-hoc test, $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$). (B, C) Adult-onset, enterocyte (EC)-specific knock-down of eIF3d or eIF3g by RNAi alone had no effect on histone expression but blocked increased expression of H3 and H4 in response to rapamycin treatment in intestine of flies at 20 days of age ($n = 4$ biological replicates of 10 intestines per replicate, two-way ANOVA, interaction, eIF3d RNAi H3 $p<0.01$, H4 $p<0.001$; eIF3g RNAi H3 $p<0.05$, H4 $p<0.05$; post-hoc test, $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$). (D) Adult-onset, EC-specific knock-down of eIF4e by RNAi alone increased expression of H3 and H4 to the same extent as did rapamycin treatment, with no additional effect of their combination in intestine of flies at 20 days of age ($n = 4$ biological replicates of 10 intestines per replicate, two-way ANOVA, interaction, H3 and H4 $p<0.01$; post-hoc test, $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$). The amount of protein was normalised to DNA, shown by stain-free blot.

The online version of this article includes the following source data and figure supplement(s) for figure 2:

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Figure 2 continued on next page
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Source data 1. Source data pertaining to Figure 2.

Figure supplement 1. Rapamycin did not affect cell composition or enterocyte (EC) polyploidisation in the fly intestine.

Figure supplement 1—source data 1. Source data pertaining to Figure 2—figure supplement 1.

Figure supplement 2. Perturbation of autophagy or proteosome activity has no effect on expression of core histones in the fly intestine in response to rapamycin treatment.

Figure supplement 2—source data 1. Source data pertaining to Figure 2—figure supplement 2.

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Figure 3. Increased histone expression in response to rapamycin treatment causes chromatin rearrangement and heterochromatin expansion across the nucleus in intestinal enterocytes (ECs). (A) Rapamycin induced a substantial accumulation of chromatin at the nuclear envelope in ECs (linear mixed model, interaction, p>0.05; post-hoc test, ***p<0.001). (B) Adult-onset, EC-specific expression of H3/H4 by the 5966GS driver recapitulated the effect of rapamycin on the accumulation of chromatin at the nuclear envelope in intestine of flies at 20 days of age (linear mixed model, interaction, p<0.001; post-hoc test, ***p<0.001). (C) Adult-onset, EC-specific expression of H3/H4 by the 5966GS driver had no effect on the total amount of HP1 in the presence or absence of rapamycin (linear mixed model, interaction, p>0.05; post-hoc test, NS p>0.05), but it altered the distribution of HP1 in the nucleus in the intestine of flies at 20 days of age (linear mixed model, interaction, p<0.001; post-hoc test, ***p<0.001). The yellow arrow indicates the expansion of HP1 to the whole nucleus. Each data point (n = 4 intestines) represents an average value from 3 to 5 ECs per intestine.

The online version of this article includes the following source data and figure supplement(s) for figure 3:

Source data 1. Source data pertaining to Figure 3.

Figure supplement 1. Increased histones are required for rapamycin-induced chromatin rearrangement and heterochromatin expansion in the fly intestine.

Figure supplement 1—source data 1. Source data pertaining to Figure 3—figure supplement 1.
Increased expression of histones in ECs in response to rapamycin treatment alters chromatin architecture

Histones are basic proteins that help package genomic DNA to form chromatin. In yeast, loss of histones with age causes a decline in global nucleosome occupancy (Hu et al., 2014). Conversely, increased expression of histones can trigger a cytotoxic response to cytoplasmic-free histones (Singh et al., 2010) or result in an increase in the number of nucleosomes and altered chromatin structure (Hu et al., 2014). We observed that histone H3 remained in chromatin in intestines of both control and rapamycin-treated flies (Figure 3—figure supplement 1A), suggesting that rapamycin did not disturb histone incorporation into chromatin. We further examined whether the increase in expression of histones from rapamycin treatment resulted in altered chromatin structure. Micrococcal nuclease (MNase) cleaves and digests linker regions between nucleosomes, allowing the nucleosome number (occupancy) to be estimated. The number of mono-, di-, and tri-nucleosomes in the intestine of rapamycin-treated flies was substantially higher than in controls after a short (1 min) MNase digest. An extended digestion time led to the generation of more mononucleosomes from di- and tri-nucleosomes, revealing an even greater difference in mononucleosome number between rapamycin-treated and control intestines (Figure 3—figure supplement 1B). Over-expression of histones H3 and H4 in ECs elevated the number of nucleosomes in intestines as much as did rapamycin treatment, with no further increase in the combined treatment (Figure 3—figure supplement 1B). Thus, increased expression of histones resulted in increased nucleosome occupancy.

One consequence of increased nucleosome occupancy is a change in higher-order chromatin architecture (Hauer and Gasser, 2017; Luger et al., 2012). Interestingly, rapamycin treatment induced a substantial chromatin rearrangement in ECs, with marked accumulation of chromatin at the nuclear envelope in both young (10-day-old) and middle-aged (40-day-old) flies (Figure 3A). To determine if rapamycin induced this chromatin rearrangement by increasing histone expression, we either abolished increased histone expression by RNAi or directly overexpressed histones, and assessed the interaction with rapamycin treatment. Knock-down of either histone H3 or H4 in adult ECs by RNAi blocked the rapamycin-induced chromatin rearrangement (Figure 3—figure supplement 1C, D). Conversely, EC-specific over-expression of H3 and H4 recapitulated the effect of rapamycin treatment, with no further effect in the presence of rapamycin (Figure 3B). These results indicate that the increase in histone expression mediated the effect of rapamycin on chromatin arrangement.

Nucleosome occupancy and higher-order chromatin architecture eventually affect chromatin state. Heterochromatin is a tightly packed form of chromatin and is marked by heterochromatin protein 1 (HP1) (Ebert et al., 2004; Grewal and Jia, 2007). To investigate whether altered chromatin architecture led to heterochromatinisation in ECs, we examined the amount and the distribution of HP1 in EC nuclei. Rapamycin did not affect the amount of HP1, but it altered its distribution, by expanding it across the nucleus in ECs. Blocking-increased expression of H3 or H4 in response to rapamycin treatment did not affect the amount of HP1 but abolished HP1 expansion to the whole nucleus in response to rapamycin treatment (Figure 3—figure supplement 1E, F). Furthermore, over-expression of H3 and H4 in ECs recapitulated the effect of rapamycin treatment on this phenotype, with no additional effect in the presence of rapamycin (Figure 3C). Together, these results suggest that increased histone expression mediated the effects of rapamycin on higher-order chromatin architecture.

Increased expression of histones in ECs mediates increased longevity and intestinal homeostasis in response to rapamycin

ECs play a key role in modulating ageing and age-related pathologies (Bolukbasi et al., 2017; Guo et al., 2014; Lemaitre and Miguel-Aliaga, 2013; Resnik-Docampo et al., 2017; Salazar et al., 2018). We therefore examined whether increased histone expression in ECs in the intestine mediated the effects of rapamycin on lifespan. Adult-onset knock-down of H3 or H4 by the 5966GS driver alone had no effect on lifespan of control flies, but completely blocked the lifespan extension by rapamycin (Figure 4A, B; Supplementary file 2). Age-related intestinal pathologies are driven by
both unregulated ISC division (Biteau et al., 2008; Choi et al., 2008) and loss of homeostasis in ECs (Bolukbasi et al., 2017; Resnik-Docampo et al., 2017; Salazar et al., 2018), both of which reduce lifespan. Rapamycin reduces age-associated ISC proliferation, attenuating intestinal dysplasia (Fan et al., 2015). To determine whether increased histone expression in ECs mediated the effects of rapamycin on intestinal homeostasis, we measured ISC proliferation. In line with the previous study (Fan et al., 2015), rapamycin treatment reduced pH3-positive cell number, a proxy for ISC proliferation (Biteau et al., 2010), in the intestine (Figure 4D–F). Knock-down of H3 or H4 in adult ECs substantially attenuated the effect of rapamycin on ISC proliferation (Figure 4D, E) and on intestinal dysplasia in old flies (50-day-old) (Figure 4G). Increased longevity and intestinal homeostasis from rapamycin treatment thus both required the increased expression of histone proteins.

Reciprocally, over-expression of H3/H4 by the 5966GS driver resulted in a marked extension of lifespan and did not further extend lifespan in rapamycin-treated flies (Figure 4C;
Histones in ECs activate autophagy by mediating a transcriptional change upon rapamycin treatment

Changes in nucleosomes and chromatin mediate transcriptional responses, which can in turn affect ageing and health (Hu et al., 2014; Larson et al., 2012; Sen et al., 2016). To investigate whether these changes in ECs in response to rapamycin treatment were associated with changes in RNA expression, we compared RNA expression profiles of intestines of rapamycin-treated flies with controls. Rapamycin had a substantial impact on the entire transcriptome in the intestine, which increased with age (Figure 5—figure supplement 1A), with modest changes in gene expression at day 10, and substantial changes at days 30 and 50 (Figure 5—figure supplement 1B). Although we did not detect any significant enrichment of specific biological processes by Gene Ontology (GO) analysis, we noticed that expression of autophagy-related genes (e.g., Bchs, Diabetes and obesity regulated (DOR), Stat92E, Atg4a, and Atg8a) were affected by rapamycin treatment (Figure 5—figure supplement 1C–E). This is in line with previous studies showing that mTORC1 can influence expression of autophagy-related transcripts (Di Malta et al., 2019; Martina et al., 2012).

Autophagy plays an important role in gut health and longevity (Hansen et al., 2018). We therefore tested whether increased expression of histones in ECs could mediate transcriptional regulation of autophagy-related genes. Quantitative RT-PCR on RNA isolated from fly intestines showed that EC-specific knock-down of H3 by RNAi abolished the effect of rapamycin on expression of the Bchs and DOR transcripts but not the Stat92E transcript (Figure 5—figure supplement 2A). Conversely, over-expression of H3 and H4 altered the expression of Bchs and DOR transcripts similarly to rapamycin treatment, with no additional effect of their combination (Figure 5A), suggesting that increased histone expression mediated increased expression of transcripts of autophagy-related genes Bchs and DOR in response to rapamycin.

Altered histone modifications (e.g., H3K9me3 and H3K27me3) regulate autophagy-related gene expression (An et al., 2017; Wei et al., 2015). We investigated whether increased histone levels affected the enrichments of histone modifications and HP1 on the Bchs, DOR, and Stat92E gene loci. ChIP-qPCR showed that expression of H3 and H4 altered the enrichment of H3K4me3, H3K9me3, H3K27me3, and HP1 on the Bchs and DOR gene loci, but not the Stat92E gene locus, similarly to rapamycin treatment, with no additional effect of their combination (Figure 5B). Taken together, these results show that increased histone expression regulated expression of transcripts of autophagy-related genes Bchs and DOR through altering the enrichment of histone modifications (H3K4me3, H3K9me3, and H3K27me3) and HP1 on these gene loci in response to rapamycin.

Lowered mTORC1 activity can activate autophagy, either through transcriptional changes (Martina et al., 2012) or through mediating the phosphorylation status of Atg1, to regulate the activity of the Atg1/ULK1 autophagic complex and subsequent autophagic processes (Jung et al., 2010). To determine if increased histone levels induced autophagy by altering mTORC1 activity, we examined phospho-S6K levels, a direct output. As expected, rapamycin greatly decreased phospho-S6K levels, but EC-specific expression of H3/H4 did not affect phospho-S6K levels, in either the presence or the absence of rapamycin (Figure 5—figure supplement 3A). Furthermore, rapamycin treatment resulted in hyperphosphorylation of Atg1, shown by a slower-migrating band on western blot (Figure 5—figure supplement 3B), in line with previous studies (Memisoglu et al., 2019; Yeh et al., 2010). However, EC-specific expression of H3/H4 alone did not cause this effect (Figure 5—figure supplement 3B). Taken together, these results suggest that increased histone expression mediated autophagy through transcriptional change, rather than by affecting mTORC1 activity or phosphorylation status of Atg1.

The (macro)autophagy process is mediated by a number of autophagy-related proteins, which form double-membrane vesicles called autophagosomes that engulf cytoplasmic material and subsequently fuse with lysosomes to form autolysosomes, where engulfed material is degraded (Mizushima et al., 2010). To determine the effect of increased histone expression on autophagy, we
Figure 5. Increased histone expression in enterocytes from rapamycin treatment activates autophagy by altered histone marks and maintains gut barrier function. (A) Expression of H3/H4 in adult enterocytes (ECs) regulated expression of Bchs and DOR in the intestine of flies at 20 days of age (n = 4 biological replicates of 15 intestines per replicate, two-way ANOVA, post-hoc test, compared to controls, *p<0.05, **p<0.01). (B) Expression of H3/H4 in adult ECs mediated enrichment of H3K4me3, H3K9me3, H3K27me3, and HP1 on Bchs and DOR transcriptional start sites in the intestine of flies at 20 days of age (n = 3 biological replicates of 25 intestines per replicate, two-way ANOVA; post-hoc test, compared to controls, *p<0.05, **p<0.01, ***p<0.001). (C) Expression of H3/H4 in adult ECs decreased the amount of Atg8a-II and Ref(2)P (n = 4 biological replicates of 10 intestines per replicate, two-way ANOVA, interaction, p<0.05; post-hoc test, *p<0.05, **p<0.01, ***p<0.001). (D) Expression of H3/H4 in adult ECs substantially increased the number of LysoTracker-stained puncta and had no effect on the number of Cyto-ID-stained puncta in the intestine (n ≥ 6 intestines per condition; n = 2–3 pictures per intestine, data points represent the average value per intestine; linear mixed model, interaction, LysoTracker-stained puncta p<0.01; post-hoc test, *p<0.05, **p<0.01). (E) Expression of H3/H4 in adult ECs improved maintenance of coracle at septate junctions between ECs in the intestine of flies at 50 days of age. The ratio of septate junction (SJ)/cytoplasm fluorescence for coracle was high in the intestine of flies fed RU486 and/or rapamycin (n ≥ 9 intestines per condition; n = 3–5 ECs were observed per intestine, linear mixed model, interaction, p<0.01; post-hoc test, **p<0.01, ***p<0.001). (F) The number of Smurfs was significantly reduced in response to increased expression of H3/H4 in ECs and/or rapamycin at 60 days of age. Bar charts with n = 10 biological replicates of 15–20 flies per replicate (two-way ANOVA, interaction, p<0.05; post-hoc test, **p<0.01, ***p<0.001).

The online version of this article includes the following source data and figure supplement(s) for figure 5:

Source data 1. Source data pertaining to Figure 5.

Figure supplement 1. Rapamycin mediates a transcriptional response of autophagy-related genes in the fly intestine.

Figure supplement 2—source data 1. Source data pertaining to Figure 5—figure supplement 1.

Figure supplement 2. Increased histone expression in enterocytes is required for activation of autophagy and maintenance of gut barrier function from rapamycin treatment.

Figure supplement 2—source data 1. Source data pertaining to Figure 5—figure supplement 2.

Figure supplement 3. Increased histone expression does not affect mTORC1 activity.

Figure supplement 3—source data 1. Source data pertaining to Figure 5—figure supplement 3.
measured the levels of Atg8 and the Drosophila p62 homolog Ref(2)P. Atg8a-II, the active form of Atg8a, is a marker of autophagy, reflecting the number of autophagosomes (Nagy et al., 2015), while Ref(2)P is a cargo receptor for ubiquitinated proteins destined for degradation. Both are reduced upon persistently excessive autophagy (Mizushima et al., 2010). EC-specific expression of H3/H4 decreased the amount of Atg8a-II and Ref(2)P to the same degree as did rapamycin treatment, with no additional effect of their combination (Figure 5C), suggesting that increased histone expression mimicked the effect of rapamycin treatment on autophagy activation. To further assess the effect of increased histones on autophagy, we performed co-staining with LysoTracker, a fluorescent dye labelling acidic organelles, including autolysosomes, and Cyto-ID, a fluorescent dye labelling autophagosomes (Oeste et al., 2013). In line with a previous study (Bjedov et al., 2010), rapamycin treatment increased the number of LysoTracker-stained puncta in intestines (Figure 5—figure supplement 2B) while EC-specific knock-down of H3 by RNAi abolished the increase (Figure 5—figure supplement 2B). Reciprocally, expression of H3/H4 increased the number of LysoTracker-stained puncta to the same extent as did rapamycin treatment, and neither treatment affected the number of Cyto-ID-stained puncta (Figure 5D), suggesting that expression of H3/H4 in ECs did not disturb autophagic flux. Together, these data suggest that increased expression of histones in ECs activated autophagy.

Increased expression of histones in ECs improves gut barrier function

Activation of autophagy promotes increased intestinal junction and barrier integrity in worms and flies, and these play an important role in healthy longevity (Hansen et al., 2018). Rapamycin treatment attenuated the age-related loss of the bicellular junctional protein coracle (Figure 5—figure supplement 2C; Resnik-Docampo et al., 2017; Salazar et al., 2018). EC-specific knock-down of H3 by RNAi abolished the effect of rapamycin on maintenance of coracle levels at EC junctions, while expression of H3/H4 resulted in maintenance of coracle similarly to rapamycin treatment, without further effect of their combination (Figure 5E). These results suggest that increased expression of histones in response to rapamycin treatment led to better junction maintenance in the intestine of old flies. To further investigate whether activation of autophagy improved intestinal barrier integrity in old flies, we fed aged flies with a blue dye that normally does not leak out of the intestine into the body and scored the number of flies with extra-intestinal accumulation of the blue dye (the ‘Smurf’ phenotype; Clark et al., 2015; Rera et al., 2012). Rapamycin treatment resulted in a reduction of barrier function loss, and this effect was abolished by knock-down of H3 in adult ECs (Figure 5—figure supplement 2D). Expression of H3/H4 in ECs resulted in a modest, but significant, reduction in the number of Smurf flies and had no further effect in the presence of rapamycin (Figure 5F). Taken together, these results suggest that increased histone expression in ECs in response to rapamycin treatment improved the maintenance of EC junctions and overall intestinal integrity in old flies, which may in turn have promoted systemic health and increased lifespan.

Autophagy is required downstream of the mTORC1-histone axis for increased health and survival

Autophagy activation is necessary for lifespan extension in response to rapamycin in flies (Bjedov et al., 2010). To elucidate whether autophagy activation mediates the effects of increased histone expression in ECs on lifespan and intestinal homeostasis, we inhibited autophagy in ECs by knock-down of Atg5 expression by RNAi (Bjedov et al., 2010). Reduction of Atg5 substantially reduced the number of LysoTracker-stained puncta following increased expression of H3/H4 in ECs (Figure 6A), suggesting that Atg5 was required for the effect of increased histone expression on autophagy activation. We next examined whether autophagy activation was required for the beneficial effects of increased histone expression in ECs on survival and intestinal health. EC-specific knock-down of Atg5 alone did not affect lifespan, but it abolished the increase in response to increased expression of H3/H4 (Figure 6B; Supplementary file 4). Furthermore, EC-specific knock-down of Atg5 completely blocked the effects of increased expression of H3/H4 on intestinal dysplasia and maintenance of gut integrity (Figure 6C, D). Interestingly, we obtained similar results by EC-specific knock-down of expression of Atg1, a key gene with multiple roles in autophagy, including in autophagy initiation, through its phosphorylation, and in autophagosome formation and/or fusion with lysosomes (Kraft et al., 2012; Nakatogawa et al., 2012; Noda and Fujioka, 2015). Knock-
down of Atg1 inhibited the increase in autophagy in response to over-expression of H3/H4 (Figure 6—figure supplement 1A) and blocked the beneficial effects of increased expression of H3/H4 on gut health (Figure 6—figure supplement 1B). Together, these results suggest that increased autophagy is required for the beneficial effects of increased histone expression in response to rapamycin treatment for the increases in gut health and longevity.

The selective autophagy cargo adaptor Bchs mediates the effects of rapamycin and histones on the intestine and lifespan

Autophagy not only functions as a bulk degradation pathway, but also contributes to selective clearance of unwanted cellular material, including aggregated proteins, damaged mitochondria, and
invading pathogens (Zaffagnini and Martens, 2016). WDFY3 is a cargo adaptor for selective degradation of ubiquitinated protein aggregates and physically interacts with Atg5 and p62 (Clausen et al., 2010; Filimonenko et al., 2010). Mutants in the Drosophila Wdfy3 homolog Bchs show shortened lifespan and neurodegeneration (Finley et al., 2003; Sim et al., 2019). Given that the expression of Bchs was increased in response to rapamycin treatment or over-expression of H3/H4 in ECs, we examined if Bchs was required for the effects of these treatments on the intestine and lifespan. Reduction of Bchs expression by RNAi in combination with either rapamycin treatment or over-expression of H3/H4 in ECs blocked the increase of LysoTracker-stained puncta (Figure 7A, Figure 7—figure supplement 1A) in response to H3/H4, suggesting that Bchs was crucial for the effects of increased histone expression on autophagy activation. Knock-down of Bchs alone had no effect on lifespan, but it abolished the effects of both rapamycin treatment and histone over-expression on lifespan (Figure 7B, Figure 7—figure supplement 1B; Supplementary files 5 and 6). It also abolished the effects of these treatments on intestinal dysplasia and gut integrity (Figure 7C, D, Figure 7—figure supplement 1C, D). Conversely, EC-specific over-expression of Bchs was sufficient to recapitulate the effects of these treatments on autophagy, lifespan, and intestinal homeostasis (Figure 7E–H; Supplementary file 7). Moreover, we found that neither knocking down nor over-expressing Bchs in ECs influenced mTORC1-mediated phosphorylation of Atg1 (Figure 7—figure supplement 2A, B). Taken together, these data suggest that Bchs is a required target for the effects of increased expression of histones on autophagy and longevity, and acts independently of mTORC1-mediated phosphorylation of Atg1.

**Rapamycin treatment increases expression of histones and alters the chromatin structure in the small intestine of mice**

There are many physiological and functional similarities between the fly and mammalian intestine, especially the signalling pathways that regulate intestinal regeneration and disease (Apidianakis and Rahme, 2011; Jiang and Edgar, 2012). To investigate whether the mTORC1-histone axis is conserved between fly and mammals, we examined whether rapamycin increased the expression of histones in small intestines in mice. Expression of all of the core histones (H2A, H2B, H3, and H4) in the small intestine of female mice was significantly increased by rapamycin treatment at 12 months and 22 months of age (Figure 8A, B), consistent with our results from flies. In mammals, intestinal villi are small projections that extend into the lumen of the small intestine, and they are predominantly composed of ECs (Sancho et al., 2015). Rapamycin treatment induced a modest, but significant, chromatin rearrangement in epithelial cells in villi, with marked accumulation of chromatin at the nuclear envelope in cells of rapamycin-fed mice at 12 months and 22 months of age (Figure 8C). Rapamycin treatment also increased nucleosome occupancy in 22-month-old rapamycin-fed mouse intestines (Figure 8D). Furthermore, expression of Wdfy3 transcript in the small intestine in 22-month-old mice increased in response to rapamycin treatment (Figure 8E). Taken together, these results suggest that the mTORC1-histone axis may respond to mTORC1 inhibition in similar ways in flies and mammals.

**Discussion**

Changes in histone expression levels during ageing is a common phenomenon in diverse organisms (Benayoun et al., 2015). In yeast, over-expression of histones H3 and H4 prevents age-related nucleosome loss and transcriptional dysfunction, and extends replicative lifespan (Feser et al., 2010; Hu et al., 2014). Therefore, it is important to understand how histones contribute to longevity and in which tissues of multicellular organisms they play such a role. In this study, we have shown that histones H3 and H4 act downstream of mTORC1 to play a critical role in gut ECs in mediating autophagy to promote intestinal health and lifespan extension.

The expression of histones dynamically responds to cellular and environmental stresses in order to alter nuclear architecture, both to protect genomic DNA from damage and to orchestrate transcriptional programmes (Feser et al., 2010; Matilainen et al., 2017; Maze et al., 2015). Both nutrient-sensing pathways and chromatin regulation, including that mediated by histones, affect longevity, and perturbations to either of them can cause age-associated pathologies (López-Otín et al., 2013). However, it is unknown whether these processes act together to affect the ageing process. Here, we focused on females because their lifespan is increased much more than is that of...
Figure 7. Bchs is a required target for autophagy activation, lifespan extension, and intestinal homeostasis from the mTORC1-histone axis. (A) Knock-down of Bchs abolished the effect of expression of H3/H4 in enterocytes (ECs) on induction of LysoTracker-stained puncta in the intestine of flies at 20 days of age (n = 7 intestines per condition; n = 3 images per intestine, data points represent the average value per intestine; linear mixed model, interaction, p<0.001; post-hoc test, ***p<0.001). (B) Knock-down of Bchs abolished the effects of expression of H3/H4 in adult ECs on lifespan. 5966GS>H3/H4 females showed increased lifespan in the presence of RU486 (log-rank test, p=7.55E-08), but 5966GS>H3/H4 Bchs\textsuperscript{(RNAi)} females did not (p=0.90). See also Figure 7 continued on next page
males upon rapamycin treatment (Bjedov et al., 2010) and they show age-related intestinal decline that is attenuated by rapamycin treatment (Fan et al., 2015; Regan et al., 2016). Our findings reveal an interaction between mTORC1 signalling and histones, which determines longevity. Lowered mTORC1 activity by rapamycin treatment caused increased expression of histones in the intestine in Drosophila and mice, and changes in nuclear architecture of ECs and transcription of autophagy-related genes. Interestingly, the basal protein expression level of histones was substantially higher in brain, muscle, and fat than in intestine in Drosophila, and rapamycin did not further increase histone levels in these three tissues, possibly because their chromatin is already fully occupied by histones. Our findings therefore elucidate a novel intestine-specific mechanism connecting nutrient-sensing pathways and histone-driven chromatin alterations in ageing, which can be regulated by mTORC1 attenuation through rapamycin treatment.

The Drosophila intestine consists of four main cell types that have distinct physiological functions and genomic DNA content. Our findings show that increased expression of core histones in response to rapamycin treatment was not caused by either cell composition change or EC polyploidisation, and the drop in ISC proliferation may instead reflect increased health and persistence of ECs. Given the crucial role of histone proteins in packaging genomic DNA into nucleosomes to form chromatin, it is essential to finely regulate histone levels in the cell. In line with a previous study demonstrating that the expression of histone transcripts and proteins is uncoupled in aged yeast (Feser et al., 2010), we found that in Drosophila ECs lowered activity of mTORC1 by rapamycin treatment-elevated histone protein expression, independent of the abundance of histone transcripts. In mice also, the increase in lifespan from rapamycin treatment can be dissociated from the reduction in global translational activity (Garelick et al., 2013). Previous studies suggest that histones are exceptions to translation suppression upon mTOR attenuation, with their translational efficiencies increased through translation factor eIF3 (Lee et al., 2015; Lee et al., 2016; Thoreen et al., 2012). Here, we reveal that increased histones in the fly intestine in response to rapamycin treatment is regulated through translation, specifically via the activity of eIF3 in ECs. Together, these findings suggest that regulation of expression of specific protein subsets, including histones, is a key effector for rapamycin-induced longevity.

Global histone loss accompanied with nucleosome reduction occurs in aged budding yeast, and over-expression of H3/H4 ameliorates age-related nucleosome loss and extends replicative lifespan (Hu et al., 2014). Although we did not observe age-related histone loss in the Drosophila or mouse tissues that we examined, increased histone expression from rapamycin treatment, or EC-specific expression of H3/H4, caused the number of nucleosomes to increase. Furthermore, this resulted in a higher-order chromatin structural rearrangement in intestinal ECs. Importantly, our findings show this chromatin rearrangement did not happen over ageing, possibly because the ageing-induced
Figure 8. Rapamycin treatment upregulates expression of histones and Wdfy3 transcript, alters chromatin structure, and increases the number of nucleosomes in the small intestine of mice. (A) Female mice were sacrificed at 12 months and 22 months of age, and the jejunum of the small intestine was dissected. (B) Rapamycin substantially increased expression of H2A, H2B, H3, and H4 compared to controls in the small intestine of mice (n = 3 jejunums, two-way ANOVA; treatment *p<0.05; ***p<0.001). The amount of protein was normalised to DNA, shown by stain-free blot. (C) Rapamycin induced a substantial accumulation of chromatin at the nuclear envelope in cells in villi of the small intestine of mice at 12 months and 22 months of age (n = 3 jejunums per condition; n = 40–45 cells were observed per intestine, linear mixed model; post-hoc test, NS p>0.05, *p<0.05). (D) The number of nucleosomes in the intestine increased markedly in response to rapamycin treatment in mice at 22 months of age. Gel electrophoresis of 5 min micrococcal nuclease (MNase) digestions showed that the majority of nucleosomes after digestion were trinucleosomes (tri), dinucleosomes (di), and mononucleosomes (mo). The number of nucleosomes was normalised to input (0 min) (n = 3 jejunums, two-way ANOVA, post-hoc test,

Figure 8 continued on next page
increase of histone proteins was subtle and much lower than that induced by rapamycin treatment. Chromatin organisation plays an essential role in cellular senescence and organismal ageing. For instance, profound chromatin change has been reported in senescent fibroblasts, including the formation of senescence-associated heterochromatin foci (SAHF) (Chandra et al., 2015; Chandra et al., 2012), and these changes to chromatin structure can directly affect transcriptional programmes (Finlan et al., 2008; Zuin et al., 2014). Regulation of histone expression levels in ECs hence may be important for mediating their transcriptional programme.

Interestingly, we found that increased histone expression in ECs led to activation of autophagy in the fly intestine, accompanied by attenuation of age-related intestinal pathologies and extension of lifespan. Autophagy plays a crucial role in a number of conserved longevity paradigms, including reduced IIS/mTOR network and DR in multiple organisms (Hansen et al., 2018). Furthermore, genetically inducing autophagy globally, or activating selective autophagy mechanisms, extends lifespan in worms (Kumsta et al., 2019), flies (Aparicio et al., 2019; Ulgherait et al., 2014), and mice (Pyo et al., 2013). Generally, autophagy is considered to be regulated by mTORC1 by altering phosphorylation status of the Atg1/ULK1 complex (Jung et al., 2010). However, autophagy can be also controlled by epigenetic and transcriptional mechanisms, and several lines of evidence suggest that epigenetic regulation of autophagy-related genes activates autophagy, and is key for somatic homeostasis (Fullgrabe et al., 2016; Lapierre et al., 2015). In line with these previous studies, we found that the increased histones activated autophagy by altering enrichment of H3K4me3, H3K9me3, H3K27me3, and HP1 at the loci of autophagy-related genes, including Bchs, a selective autophagy cargo adaptor, to mediate their transcriptional expression and activate autophagy without affecting mTORC1 activity or phosphorylation status of Atg1.

The age-related decline of structure and function in the intestine has been shown to lead to intestinal pathologies and mortality (Regan et al., 2016; Rera et al., 2012; Resnik-Docampo et al., 2017; Salazar et al., 2018). Given the importance of the intestine for health and longevity, it is crucial to preserve its structure and function during ageing. We demonstrate that the histone protein levels in intestinal ECs mediate intestinal health and longevity in response to rapamycin treatment. Importantly, over-expressing histones H3/H4 in adult ECs recapitulated the effects of rapamycin treatment, which attenuated age-related structural and functional decline in the intestine and extended lifespan. Consistent with several previous studies showing that activation of autophagy promotes maintenance of cell-cell junctions and barrier function in the intestine (Hansen et al., 2018), we found that activation of autophagy was required for, and is sufficient to recapitulate, the effects on barrier integrity by increased levels of histones in ECs.

Atg1 has multiple functions in autophagy process. While its phosphorylation is essential for autophagy initiation (Jung et al., 2010), its protein (i.e., AIM/LIR sequence) can interact with Atg8a and therefore contributes to autophagosome formation and/or fusion with lysosomes (Kraft et al., 2012; Nakatogawa et al., 2012; Noda and Fujioka, 2015). In line with these findings, we found that hyperphosphorylation of Atg1, which is induced by rapamycin, was unaffected by increased histones H3/H4. Instead Atg1 protein functioned downstream of increased histones in ECs. Increased transcription of Bchs, which was sufficient to mediate autophagy (Sim et al., 2019), was a key downstream effector of histone-induced intestinal health and longevity.

In sum, the simplest model to integrate the role of rapamycin, histones, and autophagy in extension of lifespan and preservation intestinal health is presented in Figure 8F. We propose that lowered mTORC1 activity by rapamycin increases expression of histone proteins in intestinal ECs in a post-transcriptional manner through the activity of elf3. This increased expression of histones in ECs alters chromatin architecture and transcriptional output in ECs, including of autophagy-related genes that activate intestinal autophagy, resulting in preserved gut health and extended lifespan. This mTORC1-histone axis can activate autophagy via epigenetic and transcriptional regulation of Bchs...
which subsequently works together with other autophagy-related proteins, for example, Atg1, Atg5, and Atg8a, bypassing the canonical mTORC1-mediated phosphorylation of Atg1 autophagy initiation.

Importantly, we found that the effects of rapamycin treatment on histone protein levels, Wdfy3 transcript, and chromatin architecture were conserved in mice. Rapamycin treatment increased expression of all core histones, nucleosome occupancy, and expression of Wdfy3 transcript in the small intestines of mice and altered higher-order chromatin structure in intestinal villi cells. Several lines of evidence from previous studies have suggested that rapamycin affects histone methylation and chromatin states in aged mice ([Gong et al., 2015; Wang et al., 2017]). Furthermore, in humans, rapamycin affects chromatin organisation in fibroblasts from normal individuals in a way that mimics that seen in fibroblasts from centenarians ([Lattanzi et al., 2014]), further supporting the idea that the mTORC1-histone axis is a pro-longevity mechanism in mammals. Our study highlights the mTORC1-histone axis as a novel, pharmacological target that requires further investigation for its potential role in geroprotection.

Materials and methods

Fly husbandry
The wildtype D. melanogaster stock, Dahomey, was collected in 1970 in Dahomey (now Benin), and since then it has been maintained in large population cages with overlapping generations on a 12L:12D cycle at 25°C. The white Dahomey (wDah) stock was derived by incorporation of the white gene deletion from w1118 into the outbred Dahomey background by successive backcrossing. All mutants were backcrossed for at least six generations into the wild type, wDah, maintained in population cages. Stocks were maintained and experiments conducted at 25°C on a 12 hr:12 hr light/dark cycle at 60% humidity, on food (1/C2 SYA) containing 10% (w/v) brewer’s yeast, 5% (w/v) sucrose, and 1.5% (w/v) agar unless otherwise noted. The following stocks were used in this study and are listed in the Key resources table. UAS-H3/H4 strain was generated by combining the UAS-H3 and UAS-H4 strains. UAS-H3 strain was generated by cloning the H3 cDNA into the pUAST attb vector. pUAST attb H3 was inserted into the fly genome by the jC31 and attP/attB integration system using the attP40 landing site.

Mouse husbandry
Female mice of the genetically heterogeneous UM-HET3 stock (CByB6F1 × C3D2F1) were used in this study. They were bred, housed, and given ad libitum access to normal or rapamycin-containing chow under specific pathogen-free conditions. Rapamycin was added to the food at concentration of 14 ppm (mg of drug per kg of food). Mice were fasted for 18 hr before euthanasia at the age of 12 months and 22 months, and small intestines were dissected into different parts, including duodenum, jejunum, and ileum, then snap-frozen in liquid nitrogen and embedded in paraffin. The jejunum part was used in this study. The mouse work was approved by the University of Michigan’s Institutional Committee on the Use and Care of Animals.

Lifespan assay
For lifespan assays and all other experiments, flies were reared at standard density before being used for experiments. Crosses were set up in cages with grape juice agar plates. Embryos were collected in PBS and dosed into bottles at 20 µl per bottle to achieve standard density. The flies were collected over a 24 hr period and allowed 48 hr to mate after eclosing as adults. Flies were subsequently lightly anaesthetised with CO2, and females were sorted into vials. RU486 (Sigma) and/or rapamycin (LC Laboratories) dissolved in ethanol was added to food at appropriate concentrations (RU486 100 µM, rapamycin 200 µM). For control food, ethanol alone was added. Flies were maintained continuously on the appropriate food.

Cycloheximide/bortezomib treatment
Cycloheximide (Sigma) or bortezomib (Sigma) dissolved in ethanol was added to food at appropriate concentrations (cycloheximide 1 mM, bortezomib 2 µM) with or without rapamycin. For control food,
ethanol alone was added. Flies were kept continuously on the appropriate food until being dissected.

**Gut barrier assay (‘Smurf’ assay)**

Flies were aged on standard 1 × SYA food and then switched to SYA food containing 2.5% (w/v) Brilliant Blue FCF (Sigma). Flies were examined after 48 hr, as previously described (Martins et al., 2018; Regan et al., 2016; Rera et al., 2012).

**RNA isolation and quantitative RT-PCR**

Tissue of female flies was dissected, frozen on dry ice, and stored at −80°C. Total RNA from guts of 10 flies was extracted using TRIzol (Invitrogen) according to the manufacturer’s instructions. mRNA was reverse transcribed using random hexamers and the SuperScript III First Strand system (Invitrogen). Quantitative PCR was performed using Power SYBR Green PCR (Applied Biosystems) on a QuantStudio 6 instrument (Applied Biosystems) by following the manufacturer’s instructions. Primers used are listed in the Key resources table.

**Immunoblotting**

Female fly tissues were homogenised in 100 μl 1× RIPA Lysis and Extraction Buffer (Thermo Fisher) containing PhosSTOP (Roche) and cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail (Roche). Extracts were cleared by centrifugation, protein content determined by using Pierce BCA Protein Assay (Thermo Fisher), and DNA content determined by using Qubit dsDNA HS Assay (Invitrogen). Approximately 10 μg of protein extract or 100 ng of DNA extract was loaded per lane on polyacrylamide gel (4–20% Criterion, Bio-Rad). Proteins were separated and transferred to PVDF membrane. HRP-conjugated secondary antibodies (Invitrogen) were used. Blots were developed using the ECL detection system (Amersham). Immunoblots were analysed using Image Lab program (Bio-Rad laboratories).

**Subcellular isolation**

Fly guts were homogenised in 100 μl 1% Triton X-100 lysis buffer containing PhosSTOP (Roche) and cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail (Roche), then centrifuged for 15 min at 4°C. The supernatant contains cytoplasmic and necleoplasmic faction, and the pellet contains chromatin faction.

**MNase assay**

Fly guts were homogenised in 200 μl Nuclei Prep buffer (Zymo Research). Extracts were pelleted by centrifugation, resuspended in 120 μl MN Digestion buffer (Zymo Research), and DNA content determined by using Qubit dsDNA HS Assay (Invitrogen). Approximately 50 ng of DNA extract was used for enzymatic treatment. DNA was digested using 0.0025 U MNase. Treatment was stopped at different time points (1, 2, 5, 10 min). Nucleosomal DNA purification was done by following the manufacturer’s instructions. DNA fragments were analysed using High Sensitivity D5000 ScreenTape (Agilent Technologies) in a 4200 TapeStation instrument (Agilent Technologies).

**Chromatin immunoprecipitation (ChIP)**

Guts were dissected in PBS and immediately cross-linked in 1% formaldehyde for 10 min, fixation was subsequently stopped with 0.125 M glycine and washed in PBS, centrifuged at 4°C. Pellets were homogenised in Lysis buffer, centrifuged at 4°C, suspended in Shearing buffer, and sonicated by Covaris M220 sonicator. The following antibodies for immunoprecipitation were used: anti-histone H3 (Abcam #ab1791), anti-H3K4me3 (Abcam #ab8580), anti-H3K9me3 (Abcam #ab8898), and anti-HP1 (DSHB #C1A9). The pre-immune serum was used as mock control. Enrichment after IP was measured relative to input with qPCR. Primers used are listed in the Key resources table.

**Cyto-ID and LysoTracker staining, imaging, and image analysis**

Cyto-ID staining selectively labels autophagic vacuoles, and LysoTracker dye accumulates in low pH vacuoles, including lysosomes and autolysomes. Combination of both gives a better assessment of the entire autophagic process (Oeste et al., 2013). For the dual staining, complete guts were
dissected in PBS and stained with Cyto-ID (Enzo Life Sciences, 1:1000) for 30 min, then stained with LysoTracker Red DND-99 (Thermo Fisher, 1:2000) with Hoechst 33342 (1 mg/ml, 1:1000) for 3 min. For the experiment only with LysoTracker staining, guts were stained with LysoTracker Red and Hoechst 33342 directly after dissection. Guts were mounted in Vectashield (Vector Laboratories, H-1000) immediately. Imaging was performed immediately using a Leica TCS SP8 confocal microscope with a 20× objective plus 5× digital zoom in. Three separate images were obtained from each gut. Settings were kept constant between images. Images were analysed by Imaris 9 (Bitplane).

**Immunohistochemistry and imaging of the Drosophila intestine**

The following antibodies were used for immunohistochemistry of fly guts. Primary antibodies: anti-PH3 (Cell Signaling #9701, 1:200), anti-Lamin C (DSHB #LC28.26, 1:250), anti-HP1 (DSHB #C1A9, 1:500), anti-Coracle (DSHB #C615.16, 1:100), and anti-Prospero (DSHB #MR1A, 1:250). Secondary antibodies: Alexa Flour 488 goat anti-mouse (A11001, 1:1000) and Alexa Flour 594 goat anti-rabbit (A11012, 1:1000). Guts were dissected in PBS and immediately fixed in 4% formaldehyde for 30 min, and subsequently washed in 0.1% Triton-X/PBS (PBST), blocked in 5% BSA/PBST, incubated in primary antibody overnight at 4˚C, and in secondary antibody for 1 hr at room temperature (RT). Guts were mounted in Vectashield, scored, and imaged as described above. For dysplasia measurement, the percentage of intestinal length was blind-scored from luminal sections of the R2 region of intestines.

**Immunohistochemistry and imaging of the mouse intestine**

Staining was performed on 5-μm-thick sections of formalin-fixed paraffin-embedded (FFPE) jejunum samples of 12- and 22-month-old rapamycin-treated and control animals. Deparaffinised, heat-mediated antigen retrieval with 10 mM sodium citrate buffer (pH 6) and blocking with IHC blocking buffer (5% FBS, 2.5% BSA in 1/2 PBS) were carried out according to standard protocols. Primary antibody incubations were performed overnight at 4˚C in reaction buffer (0.25% BSA, 5% FBS, 2 g NaCl, and 0.1 g Triton X-100 in 1/2 PBS) using the primary antibody Lamin A/C (CST #2032, 1:50). Secondary antibody incubations were performed 1 hr at RT using Alexa Flour 594 goat anti-rabbit (A11012, 1:400), followed by washing and DAPI staining (1 μg/ml). Samples were washed in PBS 0.5% Triton or PBS and mounted in Vectashield (Vector Laboratories H-1000).

**Library preparation and RNA sequencing**

For transcriptomic analysis, guts were dissected from control and rapamycin-treated females at the age of 10 days, 30 days, and 50 days. Total RNA was extracted from 25 guts (three replicates) using Trizol (Thermo Fisher) following standard protocols. DNA concentrations were evaluated using a Qubit 2.0 fluorometer (Life Technologies) before DNase I treatment (Thermo Fisher). After adjusting final RNA concentration to 100 ng/μl, 2–3 μl ERCC ExFold RNA Spike-In Mixes (Life Technologies) was added for normalisation to the DNA content of the sample. Ribosomal RNA depletion libraries were generated at the Max Planck Genome Centre Cologne (MPGCC). RNA sequencing was performed with an Illumina HighSeq2500 with 150 bp read length read at MPGCC. At least 37.5 million single-end reads were obtained for each sample.

**RNA sequencing data analysis**

Raw sequence reads were quality-trimmed using Flexbar (v2.5.0) and aligned using HiSat (v2.0.14) against the Dm6 reference genome (Dodd et al., 2012; Kim et al., 2015). Mapped reads were filtered using SAMtools (v1.2) (Li et al., 2009), and guided transcriptome assembly was done using StringTie (v1.04) (Pertea et al., 2015). Merging of assembled transcriptomes and differential gene expression was performed using deseq2 analysis after ERCC normalised. The data are accessible through (GEO: GSE148002).

**Quantification and statistical analysis**

Statistical analyses were performed in Prism (GraphPad) or R (version 3.5.5) except for log-rank test using Excel (Microsoft). For the quantification of the chromatin arrangement, Leica LAS X-3D (Leica) was used to measure the fluorescence intensity of the DAPI and Lamin C staining. For the quantification of the total amount of HP1, Fiji was used to measure the sum of fluorescence intensity from the
nucleus, and the amount of HP1 per cell in all treatments was compared to controls. The amount of HP1 in peripheral location in nucleus was divided by the total amount of HP1 to obtain the amount of HP1 expansion. Sample sizes and statistical tests used are indicated in the figure legends, and Tukey post-hoc test was applied to multiple comparisons correction. Error bars are shown as standard error of the mean (SEM). The criteria for significance are NS (not significant) p>0.05; *p<0.05; **p<0.01, and ***p<0.001.

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Additional files
Supplementary files
• Supplementary file 1. Inhibition of mTORC1 activity by rapamycin treatment extends lifespan in females. Related to Figure 1.
• Supplementary file 2. Knock-down of histone H3 or H4 in adult enterocytes blocks rapamycin-induced lifespan extension. Related to Figure 4.
• Supplementary file 3. Over-expression of H3/H4 in enterocytes recapitulates rapamycin-induced lifespan extension. Related to Figure 4.
• Supplementary file 4. Knock-down of Atg5 associated with the expression of H3/H4 in enterocytes abolished the benefits of increased histones on lifespan extension. Related to Figure 6.
• Supplementary file 5. Knock-down of Bchs associated with the expression of H3/H4 in enterocytes abolished the benefits of increased histones on lifespan extension. Related to Figure 7.
• Supplementary file 6. Knock-down of Bchs in enterocytes abolished rapamycin-induced lifespan extension. Related to Figure 7—figure supplement 1.
• Supplementary file 7. Over-expression of Bchs in enterocytes extends lifespan in females. Related to Figure 7.
• Transparent reporting form

Data availability
Sequencing data have been deposited in GEO under accession code GSE148002.

The following dataset was generated:

| Author(s) | Year | Dataset title | Dataset URL | Database and Identifier |
|-----------|------|---------------|-------------|-------------------------|
| Lu Y, Regan JC, Eßer J, Drews LF, Weiriseis T, Stimm J, Hahn Q, Miller RA, Grönke S, Partridge L | 2021 | A TORC1/histone axis regulates chromatin organization and non-canonical induction of autophagy to ameliorate ageing | https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148002 | NCBI Gene Expression Omnibus, GSE148002 |

References
Alic N, Partridge L. 2011. Death and dessert: nutrient signalling pathways and ageing. *Current Opinion in Cell Biology* 23:738–743. DOI: https://doi.org/10.1016/j.ceb.2011.07.006, PMID: 21835601
An PNT, Shimaji K, Tanaka R, Yoshida H, Kimura H, Fukusaki E, Yamaguchi M. 2017. Epigenetic regulation of starvation-induced autophagy in *Drosophila* by histone methyltransferase G9a. *Scientific Reports* 7:7343. DOI: https://doi.org/10.1038/s41598-017-07566-1, PMID: 28779125
Aparicio R, Rana A, Walker DW. 2019. Upregulation of the autophagy adaptor p62/SQSTM1 prolongs health and lifespan in Middle-Aged *Drosophila*. *Cell Reports* 28:1029–1040. DOI: https://doi.org/10.1016/j.celrep.2019.06.070, PMID: 31340141
Apidianakis Y, Rahme LG. 2011. *Drosophila melanogaster* as a model for human intestinal infection and pathology. *Disease Models & Mechanisms* 4:21–30. DOI: https://doi.org/10.1242/dmm.003970, PMID: 21183483
Arriola Apelo SI, Lamming DW. 2016. Rapamycin: an InhibiTOR of aging emerges from the soil of easter island. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 71:841–849. DOI: https://doi.org/10.1093/gerona/glw090, PMID: 27208895

Benayoun BA, Pollina EA, Brunet A. 2015. Epigenetic regulation of aging: linking environmental inputs to genomic stability. *Nature Reviews Molecular Cell Biology* 16:593–610. DOI: https://doi.org/10.1038/nrm4048, PMID: 26373265

Biteau B, Hochmuth CE, Jasper H. 2008. JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. *Cell Stem Cell* 3:442–455. DOI: https://doi.org/10.1016/j.stem.2008.07.024, PMID: 18940735

Biteau B, Karpac J, Supoyo S, Degennaro M, Lehmann R, Jasper H. 2010. Lifespan extension by preserving proliferative homeostasis in Drosophila. *PLOS Genetics* 6:e1001159. DOI: https://doi.org/10.1371/journal.pgen.1001159, PMID: 20976250

Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, Foley A, Partridge L. 2010. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metabolism* 11:35–46. DOI: https://doi.org/10.1016/j.cmet.2009.11.010, PMID: 20074526

Bolukbasi E, Khericha M, Regan JC, Ivanov DK, ADCott J, Dyson MC, Nespital T, Thornton JM, Alic N, Partridge L. 2017. Intestinal fork head regulates nutrient absorption and promotes longevity. *Cell Reports* 21:641–653. DOI: https://doi.org/10.1016/j.celrep.2017.09.042, PMID: 29045833

Broer L, Buchman AS, Deelen J, Evans DS, Faul JD, Lunetta KL, Sebastiani P, Smith JA, Smith AV, Tanaka T, Yu L, Arnold AM, Aspelund T, Benjamin EJ, De Jager PL, Eiriksdottir G, Evans DA, Garcia ME, Hofman A, Kaplan RC, et al. 2015. GWAS of longevity in CHARGE consortium confirms APOE and FOXO3 candidacy. *The Journals of Gerontology: Series A* 70:110–118. DOI: https://doi.org/10.1093/gerona/glq166, PMID: 25199915

Chandra T, Kirschner K, Thuret JY, Pope BD, Ryba T, Newman S, Ahmed K, Samarajiwa SA, Salama R, Carroll T, Stark R, Janky R, Narita M, Xue L, Chicas A, Núñez S, Janknecht R, Hayashi-Takanaka Y, Wilson MD, Marshall A, et al. 2012. Independence of repressive histone marks and chromatin compaction during senescent heterochromatin layer formation. *Molecular Cell* 47:203–214. DOI: https://doi.org/10.1016/j.molcel.2012.06.010, PMID: 22795131

Chandra T, Ewels PA, Schoenfelder S, Furlan-Magaril M, Wingett SW, Kirschner K, Thuret JY, Andrews S, Fraser P, Reik W. 2015. Global reorganization of the nuclear landscape in senescent cells. *Cell Reports* 10:471–483. DOI: https://doi.org/10.1016/j.celrep.2014.12.055, PMID: 25640177

Choi N-H, Kim J-G, Yang D-J, Kim Y-S, Yoo M-A. 2008. Age-related changes in Drosophila midgut are associated with PVF2, a PDGF/VEGF-like growth factor. *Aging Cell* 7:318–334. DOI: https://doi.org/10.1111/j.1474-9726.2008.00380.x

Clark RI, Salazar A, Yamada R, Fitz-Gibbon S, Morrelli M, Alcaraz J, Rana A, Rera M, Pellegrini M, Ja WW, Walker DW. 2015. Distinct shifts in Microbiota composition during Drosophila aging impair intestinal function and drive mortality. *Cell Reports* 12:1656–1667. DOI: https://doi.org/10.1016/j.celrep.2015.08.004, PMID: 26321641

Clausen TH, Lamark T, Isakson P, Finley K, Larsen KB, Brech A, Øvervatn A, Stenmark H, Bjørkøy G, Simonsen A, Lamark T, Isakson P, Finley KD, Anderson M, Jeong H, Melia TJ, Bartlett B, Myers KM, Birkeland HCG, Lamark T, Krainc D, Brech A, Stenmark H, Simonsen A, Yamamoto A. 2010. Selective macroautophagic degradation of aggregated proteins requires the PI3P-Binding protein alfy. *Molecular Cell* 38:265–279. DOI: https://doi.org/10.1016/j.molcel.2010.04.007

Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, Ylstra B, Chubb JR, Bickmore WA. 2008. Recruitment to the nuclear periphery can alter expression of genes in human cells. *PLOS Genetics* 4:e1000039. DOI: https://doi.org/10.1371/journal.pgen.1000039, PMID: 18369458
Finley KD, Edeen PT, Cumming RC, Mardahl-Dumesnil MD, Taylor BJ, Rodriguez MH, Hwang CE, Benedetti M, McKeown M. 2003. Blue cheese mutations define a novel, conserved gene involved in progressive neural degeneration. The Journal of Neuroscience 23:1254–1264. DOI: https://doi.org/10.1523/JNEUROSCI.23-04-01254.2003, PMID: 12598614
Fontana L, Partridge L, Longo VD. 2010. Extending healthy life span—from yeast to humans. Science 328:321–326. DOI: https://doi.org/10.1126/science.1172539, PMID: 20399504
Füllgrabe J, Ghislat G, Cho DH, Rubinsztein DC. 2016. Transcriptional regulation of mammalian autophagy at a glance. Journal of Cell Science 129:3059–3066. DOI: https://doi.org/10.1242/jcs.189820, PMID: 27528204
Garell MG, Mackay VL, Yanagida A, Academia EC, Schreiber KH, Ladiges WC, Kennedy BK. 2013. Chronic rapamycin treatment or lack of S6K1 does not reduce ribosome activity in vivo. Cell Cycle 12:2493–2504. DOI: https://doi.org/10.10001/cc.25512, PMID: 23839034
Gong H, Qian H, Erdl R, Astle CM, Wang GG, Harrison DE, Xu X. 2015. Histone modifications change with age, dietary restriction and rapamycin treatment in mouse brain. Oncotarget 6:15882–15890. DOI: https://doi.org/10.18632/oncotarget.4137, PMID: 26021816
Grewal SI, Jia S. 2007. Heterochromatin revisited. Nature Reviews Genetics 8:35–46. DOI: https://doi.org/10.1038/nrg2008, PMID: 17173056
Guo L, Karpac J, Tran SL, Jasper H. 2014. PGRPC-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell 156:109–122. DOI: https://doi.org/10.1016/j.cell.2013.12.018
Hansen M, Rubinsztein DC, Walker DW. 2018. Autophagy as a promoter of longevity: insights from model organisms. Nature Reviews Molecular Cell Biology 19:579–593. DOI: https://doi.org/10.1038/s41580-018-0033-y, PMID: 30006559
Harman D. 1991. The aging process: major risk factor for disease and death. PNAS 88:5360–5363. DOI: https://doi.org/10.1073/pnas.88.12.5360, PMID: 2052612
Hauer MH, Gasser SM. 2017. Chromatin and nucleosome dynamics in DNA damage and repair. Genes & Development 31:2204–2221. DOI: https://doi.org/10.1101/gad.307702.117, PMID: 29284710
Hu Z, Chen K, Xia Z, Chavez M, Pal S, Seol JH, Chen CC, Li W, Tyler JK. 2014. Nucleosome loss leads to global transcriptional up-regulation and genomic instability during yeast aging. Genes & Development 28:396–408. DOI: https://doi.org/10.1101/gad.233221.113, PMID: 24532716
Ivanov A, Pawlikowski J, Manoharan I, van Tuyn J, Nelson DM, Rai TS, Shah PP, Hewitt G, Korolchuk VI, Passos JF, Wu H, Berger SL, Adams PD. 2013. Lysosome-mediated processing of chromatin in senescence. Journal of Cell Biology 202:129–143. DOI: https://doi.org/10.1083/jcb.20121211
Jiang H, Edgar BA. 2012. Intestinal stem cell function in Drosophila and mice. Current Opinion in Genetics & Development 22:354–360. DOI: https://doi.org/10.1016/j.gde.2012.04.002, PMID: 22608824
Johnson SC, Rabinovitch PS, Kaeberlein M. 2013. mTOR is a key modulator of ageing and age-related disease. Nature 493:338–345. DOI: https://doi.org/10.1038/nature11861, PMID: 23325216
Johnson SC, Dong X, Vig J, Suh Y. 2015. Genetic evidence for common pathways in human age-related diseases. Aging Cell 14:809–817. DOI: https://doi.org/10.1111/acel.12362, PMID: 26077337
Jung CH, Ro S-H, Cao J, Otto NM, Kim D-H. 2010. mTOR regulation of autophagy. FEMS Letters 584:1287–1295. DOI: https://doi.org/10.1016/j.femsle.2010.01.017
Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. 2004. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Current Biology 14:885–890. DOI: https://doi.org/10.1016/j.cub.2004.03.059, PMID: 15186745
Kenyon CJ. 2010. The genetics of ageing. Nature 464:504–512. DOI: https://doi.org/10.1038/nature08980, PMID: 20336132
Kim D, Langmead B, Salzberg SL. 2015. HISAT: a fast spliced aligner with low memory requirements. Nature Methods 12:357–360. DOI: https://doi.org/10.1038/nmeth.3317
Kraft C, Kijanska M, Kalie E, Siergiejuk E, Lee SS, Semplicio G, Stoffel I, Truong SL, Jasper H. 2014. PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell 156:109–122. DOI: https://doi.org/10.1016/j.cell.2013.12.018
Langmead B, Salzberg SL. 2015. HISAT: a fast spliced aligner with low memory requirements. Nature Methods 12:357–360. DOI: https://doi.org/10.1038/nmeth.3317
Kraft C, Kijanska M, Kalie E, Siergiejuk E, Lee SS, Semplicio G, Stoffel I, Truong SL, Jasper H. 2014. PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell 156:109–122. DOI: https://doi.org/10.1016/j.cell.2013.12.018
Lapiere LR, Kumsta C, Sandri M, Ballabio A, Hansen M. 2015. Transcriptional and epigenetic regulation of autophagy in aging. Autophagy 11:867–880. DOI: https://doi.org/10.1080/15548627.2015.1034410, PMID: 25836756
Lee AS, Kranzusch PJ, Cate JH. 2015. eIF3 targets cell-proliferation messenger RNAs for translational activation or repression. Nature 522:111–114. DOI: https://doi.org/10.1038/nature14267, PMID: 25849773

Lu et al. eLife 2021;10:e62233. DOI: https://doi.org/10.7554/eLife.62233
Lee AS, Kranzusch PJ, Doudna JA, Cate JH. 2016. eLF3d is an mRNA cap-binding protein that is required for specialized translation initiation. *Nature* **536**:96–99. DOI: https://doi.org/10.1038/nature18954, PMID: 27462815

Lemaitre B, Miguel-Aliaga I. 2013. The digestive tract of Drosophila Melanogaster. *Annual Review of Genetics* **47**:377–404. DOI: https://doi.org/10.1146/annurev-genet-111212-133343, PMID: 24016187

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* **25**:2078–2079. DOI: https://doi.org/10.1093/bioinformatics/btp352, PMID: 19505943

Li J, Kim SG, Blenis J. 2014. Rapamycin: one drug, many effects. *Cell Metabolism* **19**:373–379. DOI: https://doi.org/10.1016/j.cmet.2014.01.001, PMID: 24508508

López-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. *Cell* **153**:1194–1217. DOI: https://doi.org/10.1016/j.cell.2013.05.039, PMID: 23746838

Luger K, Dechassa ML, Tremethick DJ. 2012. New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? *Nature Reviews Molecular Cell Biology* **13**:436–447. DOI: https://doi.org/10.1038/nrm3382

Mannick JB, Del Giudice G, Lattanzi M, Valiante NM, Praestgaard J, Huang B, Lonetto MA, Maeker HT, Kovarik J, Carson S, Glass DJ, Klickstein LB. 2014. mTOR inhibition improves immune function in the elderly. *Science Translational Medicine* **6**:288ra179. DOI: https://doi.org/10.1126/scitranslmed.3009892

Mannick JB, Morris M, Hockey HP, Roma G, Beibel M, Kulmatycki K, Watkins M, Shavlakadze T, Zhou W, Quinn D, Glass DJ, Klickstein LB. 2018. TORC1 inhibition enhances immune function and reduces infections in the elderly. *Science Translational Medicine* **10**:eaau1564. DOI: https://doi.org/10.1126/scitranslmed.aau1564, PMID: 29997249

Martina JA, Chen Y, Gucel M, Puertollano R. 2012. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy* **8**:903–914. DOI: https://doi.org/10.4161/auto.19653, PMID: 22576015

Martins RR, McCracken AW, Simons MJ, Henriques CM, Rera M. 2018. How to catch a smurf? - Ageing and beyond. In *In vivo Assessment of Intestinal Permeability in Multiple Model Organisms*. *Bio-Protocol* **8**:e2722. DOI: https://doi.org/10.21769/BioProtoc.2722, PMID: 29457041

Matilainen O, Sleiman MSB, Quiros PM, Garcia S, Auwerx J. 2017. The chromatin remodeling factor ISW-1 integrates organismal responses against nuclear and mitochondrial stress. *Nature Communications* **8**:1818. DOI: https://doi.org/10.1038/s41467-017-01903-8, PMID: 29180639

Maze I, Wenderski W, Noh KM, Bagot RC, Tzavaras N, Purushothaman I, Elsässer SJ, Guo Y, Ionesc e, Hurd YL, Tamminga CA, Ha lene T, Farrelly L, Soshnev AA, Wen D, Rafii S, Birtwistle MR, Akbarian S, Buchholz BA, Blitzer RD, et al. 2015. Critical role of histone turnover in neuronal transcription and plasticity. *Neuron* **87**:77–94. DOI: https://doi.org/10.1016/j.neuron.2015.06.014, PMID: 26139371

Memisoglu G, Eapen VV, Yang Y, Kli omsky DJ, Haber JE. 2019. PP2C phosphatases promote autophagy by dephosphorylation of the Atg1 complex. *PNAS* **116**:1613–1620. DOI: https://doi.org/10.1073/pnas.1817078116, PMID: 30655342

Mizushima N, Yoshimori T, Levine B. 2010. Methods in mammalian autophagy research. *Cell* **140**:313–326. DOI: https://doi.org/10.1016/j.cell.2010.01.028, PMID: 20144757

Nagy P, Varga Á, Kovács AL, Takáts S, Juhasz G. 2015. How and why to study autophagy in Drosophila: it’s more than just a garbage chute. *Methods* **75**:151–161. DOI: https://doi.org/10.1016/j.ymeth.2014.11.016, PMID: 25481477

Nakatogawa H, Ohbayashi S, Sakoh-Nakatogawa M, Kakuta S, Suzuki SW, Kirisako H, Kondo-Kakuta C, Noda NN, Yamamoto H, Ohsumi Y. 2012. The autophagy-related protein kinase Atg1 interacts with the ubiquitin-like protein Atg8 via the Atg8 family interacting motif to facilitate autophagosome formation. *Journal of Biological Chemistry* **287**:28503–28507. DOI: https://doi.org/10.1074/jbc.C112.387514, PMID: 22778255

Ni Z, Ebata A, Alipanahiramandi E, Lee SS. 2012. Two SET domain containing genes link epigenetic changes and aging in Caenorhabditis elegans. *Aging Cell* **11**:315–325. DOI: https://doi.org/10.1111/j.1474-9726.2011.00785.x, PMID: 22212395

Niccoli T, Partridge L. 2012. Ageing as a risk factor for disease. *Current Biology* **22**:R741–R752. DOI: https://doi.org/10.1016/j.cub.2012.07.024, PMID: 22975005

Noda NN, Fujioka Y. 2015. Atg1 family kinases in autophagy initiation. *Cellular and Molecular Life Sciences* **72**:3083–3096. DOI: https://doi.org/10.1007/s00018-015-1917-z, PMID: 25948417

Oeste CL, Secco E, Patton WF, Boya P, Pérez-Sala D. 2013. Interactions between autophagic and endo-lysosomal markers in endothelial cells. *Histochemistry and Cell Biology* **139**:659–670. DOI: https://doi.org/10.1007/s00418-012-1057-6, PMID: 23203316

Pal S, Tyler JK. 2016. Epigenetics and aging. *Science Advances* **2**:e1600584. DOI: https://doi.org/10.1126/sciadv.1600584, PMID: 27482540

Partridge L, Deelen J, Slagboom PE. 2018. Facing up to the global challenges of ageing. *Nature* **561**:45–56. DOI: https://doi.org/10.1038/s41586-018-0457-8, PMID: 30185958

Partridge L, Fuentealba M, Kennedy BK. 2020. The quest to slow ageing through drug discovery. *Nature Reviews Drug Discovery* **19**:513–532. DOI: https://doi.org/10.1038/s41573-020-0067-7, PMID: 32467649

Passtoors WM, Beekman M, Abelen J, van der Breggen R, Matus AB, Guigas B, Derhavenessian E, van Heemst D, de Craen AJ, Gunn DA, Pawelec G, Slagboom PE. 2013. Gene expression analysis of mTOR pathway association with human longevity. *Aging Cell* **12**:24–31. DOI: https://doi.org/10.1111/acel.12015, PMID: 23061800
Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. 2015. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nature Biotechnology* 33:290–295. DOI: https://doi.org/10.1038/nbt.3122, PMID: 25690850

Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, Jung S, Jung YK. 2013. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nature Communications* 4:2300. DOI: https://doi.org/10.1038/ncomms3300, PMID: 23939249

Regan JC, Khericha M, Dobson AJ, Bolukbasi E, Rattanavirokul N, Partridge L. 2016. Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. *eLife* 5:e10956. DOI: https://doi.org/10.7554/eLife.10956, PMID: 26878574

Regan JC, Froy H, Walling CA, Moatt JP, Nussey DH. 2020. Dietary restriction and insulin-like signalling pathways as adaptive plasticity: a synthesis and re-evaluation. *Functional Ecology* 34:107–128. DOI: https://doi.org/10.1111/1365-2435.13418

Ren C, Finkel SE, Tower J. 2009. Conditional inhibition of autophagy genes in adult Drosophila impairs immunity without compromising longevity. *Experimental Gerontology* 44:228–235. DOI: https://doi.org/10.1016/j.exger.2008.10.002, PMID: 18955126

Rera M, Clark RJ, Walker DW. 2012. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. *PNAS* 109:21528–21533. DOI: https://doi.org/10.1073/pnas.1215849110, PMID: 23236133

Resnik-Docampo M, Koehler CL, Clark RI, Schinaman JM, Sauer V, Wong DM, Lewis S, D’Alterio C, Walker DW, Jones DL. 2017. Tricellular junctions regulate intestinal stem cell behaviour to maintain homeostasis. *Nature Cell Biology* 19:52–59. DOI: http://dx.doi.org/10.1038/ncb3454, PMID: 27992405

Salazar AM, Resnik-Docampo M, Ulgherait M, Clark RI, Shirasu-Hiza M, Jones DL, Walker DW. 2018. Intestinal snailksen limits microbial dysbiosis during aging and promotes longevity. *iScience* 9:229–243. DOI: https://doi.org/10.1016/j.isci.2018.10.022, PMID: 30419503

Sancho R, Cremona CA, Behrens A. 2015. Stem cell and progenitor fate in the mammalian intestine: notch and lateral inhibition in homeostasis and disease. *EMBO Reports* 16:571–581. DOI: https://doi.org/10.15252/embr.201540188, PMID: 25855643

Saxton RA, Sabatini DM. 2017. mTOR signaling in growth, metabolism, and disease. *Cell* 168:960–976. DOI: https://doi.org/10.1016/j.cell.2017.02.004, PMID: 28283069

Scott RC, Schuldiner O, Neufeld TP. 2004. Role and regulation of starvation-induced autophagy in the Drosophila fat body. *Developmental Cell* 7:167–178. DOI: https://doi.org/10.1016/j.devcel.2004.07.009, PMID: 15296714

Selman C, Tullet JM, Wieser D, Irvine E, Lingard SJ, Choudhury AL, Claret M, Al-Qassab H, Carmignac D, Ramadani F, Woods A, Robinson JC, Schuster E, Battachary RL, Kozma SC, Thomas G, Carling D, Okkenhuag K, Thornton JM, Partridge L, et al. 2009. Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science* 326:140–144. DOI: https://doi.org/10.1126/science.1177221, PMID: 19797661

Sen P, Shah PP, Nativio R, Berger SL. 2016. Epigenetic mechanisms of longevity and aging. *Cell* 166:822–839. DOI: https://doi.org/10.1016/j.cell.2016.07.050, PMID: 27518561

Sim J, Osborne KA, Arquelo Garcia I, Matysik AS, Kraut R. 2019. The BEACH domain is critical for blue cheese functionality in a spatial and epistatic autophagy hierarchy. *Frontiers in Cell and Developmental Biology* 7:129. DOI: https://doi.org/10.3389/fcell.2019.00129, PMID: 31428609

Singh RK, Liang D, Gajalalalavari UR, Kabbaj MH, Paik J, Gunjan A. 2010. Excess histone levels mediate cytotoxicity via multiple mechanisms. *Cell Cycle* 9:4236–4244. DOI: https://doi.org/10.4161/cc.9.20.13636, PMID: 20948314

Suh Y, Atzmon G, Cho MO, Hwang D, Liu B, Leahy DJ, Barzilai N, Cohen P. 2008. Functionally significant insulin-like growth factor I receptor mutations in centenarians. *PNAS* 105:3438–3442. DOI: https://doi.org/10.1073/pnas.0705467105, PMID: 18316275

Tain LS, Sehlikre R, Jain C, Chokkalingam M, Nagaraj N, Essers P, Rasner M, Grönke S, Froelich J, Dieterich C, Mann M, Alic N, Beyer A, Partridge L. 2017. A proteomic atlas of insulin signalling reveals tissue-specific mechanisms of longevity assurance. *Molecular Systems Biology* 13:939. DOI: https://doi.org/10.15252/msb.20177663, PMID: 28916641

Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM. 2012. A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature* 485:109–113. DOI: https://doi.org/10.1038/nature11083, PMID: 22552098

Ulgherait M, Rana A, Rera M, Graniel J, Walker DW. 2014. AMPK modulates tissue and organismal aging in a non-cell-autonomous manner. *Cell Reports* 8:1767–1780. DOI: https://doi.org/10.1016/j.celrep.2014.08.006, PMID: 25199830

Unnikrishnan A, Kurup K, Salmon AB, Richardson A. 2020. Is rapamycin a dietary restriction mimetic? *The Journals of Gerontology: Series A* 75:4–13. DOI: https://doi.org/10.1093/gerona/glz060, PMID: 30854544

Wang T, Tsui B, Kreisberg JF, Robertson NA, Gross AM, Yu MK, Carter H, Brown-Borg HM, Adams PD, Ideker T, et al. 2017. Epigenetic aging signatures in mice livers are slowed by dwarfism, calorie restriction and rapamycin treatment. *Genome Biology* 18:57. DOI: https://doi.org/10.1186/s13059-017-1186-2, PMID: 28351423

Wei FZ, Cao Z, Wang X, Wang H, Cai MY, Li T, Hattori N, Wang D, Yu Y, Song B, Cao LL, Shen C, Wang L, Wang H, Yang Y, Xie D, Wang F, Ushijima T, Zhao Y, Zhu WG. 2015. Epigenetic regulation of autophagy by the methyltransferase EZH2 through an MTOR-dependent pathway. *Autophagy* 11:2309–2322. DOI: https://doi.org/10.1080/15548627.2015.1177734, PMID: 26735435
Yeh YY, Wrasman K, Herman PK. 2010. Autophosphorylation within the Atg1 activation loop is required for both kinase activity and the induction of autophagy in Saccharomyces cerevisiae. Genetics 185:871–882. DOI: https://doi.org/10.1534/genetics.110.116566, PMID: 20439775

Zaffagnini G, Martens S. 2016. Mechanisms of selective autophagy. Journal of Molecular Biology 428:1714–1724. DOI: https://doi.org/10.1016/j.jmb.2016.02.004, PMID: 26876603

Zuin J, Dixon JR, van der Reijden MI, Ye Z, Kolovos P, Brouwer RW, van de Corput MP, van de Werken HJ, Knoch TA, van Laken WF, Grosveld FG, Ren B, Wendt KS. 2014. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. PNAS 111:996–1001. DOI: https://doi.org/10.1073/pnas.1317788111, PMID: 24335803
## Appendix 1—key resources table

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|------------------------------------|-------------|---------------------|-------------|------------------------|
| Antibody                           | Histone H2A antibody (rabbit polyclonal) | Active Motif | Cat# 39209 RRID:AB_2793184 | WB (1:1000) |
| Antibody                           | Anti-histone H2B antibody – ChIP Grade (mouse monoclonal) | Abcam | Cat# ab52484 RRID:AB_1139809 | WB (1:10,000) |
| Antibody                           | Anti-histone H3 antibody – ChIP Grade (rabbit polyclonal) | Abcam | Cat# ab1791 RRID:AB_302613 | WB (1:10,000) ChIP (2 µg) |
| Antibody                           | Histone H4 antibody (pAb) (rabbit polyclonal) | Active Motif | Cat# 39269 RRID:AB_2636967 | WB (1:3000) |
| Antibody                           | Lamin C (mouse monoclonal) | Developmental Studies Hybridoma Bank | Cat# LC28.26 RRID:AB_528339 | WB (1:3000) |
| Antibody                           | Atg1 (rabbit polyclonal) | From Jun Hee Lee’s lab, USA | WB (1:2000) |
| Antibody                           | Atg8a (rabbit polyclonal) | From Peter Nagy’s lab, Hungary | WB (1:3000) |
| Antibody                           | Phospho-Drosophila p70 S6 Kinase (Thr398) (rabbit polyclonal) | Cell Signaling | Cat#9209 RRID:AB_2269804 | WB (1:1000) |
| Antibody                           | Total S6K (rabbit polyclonal) | Self-made | WB (1:1000) |
| Antibody                           | Anti-histone H3 (trimethyl K4) antibody – ChIP Grade (rabbit polyclonal) | Abcam | Cat# ab8580 RRID:AB_306649 | ChIP (2 µg) |
| Antibody                           | Anti-histone H3 (trimethyl K9) antibody – ChIP Grade (rabbit polyclonal) | Abcam | Cat# ab8898 RRID:AB_306848 | ChIP (2 µg) |
| Antibody                           | Anti-trimethyl-histone H3 (Lys27) Antibody (rabbit polyclonal) | Merck Millipore | Cat# 07-449 RRID:AB_310624 | ChIP (2 µg) |
| Antibody                           | HP1 (mouse monoclonal) | Developmental Studies Hybridoma Bank | Cat# C1A9 RRID:AB_52276 | IF (1:500) |
| Antibody                           | Mouse (G3A1) mAb IgG1 Isotype Control (mouse monoclonal) | Cell Signaling | Cat# 5415 RRID:AB_10829607 | ChIP (2 µg) |
| Antibody                           | Rabbit IgG, polyclonal - Isotype Control (rabbit polyclonal) | Abcam | Cat# ab171870 RRID:AB_2687657 | ChIP (2 µg) |
| Antibody                           | Coracle (mouse monoclonal) | Developmental Studies Hybridoma Bank | Cat# C615.16 RRID:AB_1161644 | IF (1:100) |
| Antibody                           | Phospho-Histone H3 (Ser10) (rabbit polyclonal) | Cell Signaling | Cat# 9701 RRID:AB_331535 | IF (1:500) |

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### Appendix 1—key resources table continued

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Antibody                         | Lamin A/C (rabbit polyclonal) | Cell Signaling | Cat# 2032 RRID:AB_2136278 | IF (1:500) |
| Antibody                         | Prospero (mouse monoclonal) | DSHB | Cat# MR1A RRID:AB_S28440 | IF (1:200) |
| Antibody                         | Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | Thermo Fisher Scientific | Cat# A11001 RRID:AB_2534069 | IF (1:1000) |
| Antibody                         | Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 | Thermo Fisher Scientific | Cat# A11012 RRID:AB_2534079 | IF (1:1000) |
| Chemical compound, drug          | Rapamycin | LC Laboratories | Cat# R-5000 | |
| Chemical compound, drug          | RU486 (Mifepristone) | Sigma | Cat# M8046 | |
| Chemical compound, drug          | Cycloheximide | Sigma | Cat# 239763 | |
| Chemical compound, drug          | Bortezomib | Sigma | Cat# 5043140001 | |
| Chemical compound, drug          | Brilliant Blue FCF | Sigma | Cat# 80717 | |
| Commercial assay or kit          | TRizol Reagent | Invitrogen | Cat#15596018 | |
| Commercial assay or kit          | SuperScript III First Strand Master Mix | Invitrogen | Cat#15596018 | |
| Commercial assay or kit          | High Sensitivity D5000 ScreenTape | Agilent Technologies | Cat# 5067-5593 | |
| Commercial assay or kit          | VECTASHIELD Antifade Mounting Medium | Vector Laboratories | Cat# H-1000 RRID:AB_2336789 | |
| Commercial assay or kit          | RIPA Lysis and Extraction Buffer | Thermo Scientific | Cat# 89901 | |
| Commercial assay or kit          | 4–20% Criterion TGX Stain-Free Protein Gel, 26 well, 15 μl | Bio-Rad | Cat# 5678095 | |
| Commercial assay or kit          | Amersham Hybond P Western blotting membranes, PVDF | GE Healthcare Amersham | Cat# GE10600023 | |
| Commercial assay or kit          | PhosSTOP | Roche | Cat# 04906837001 | |
| Commercial assay or kit          | cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail | Roche | Cat# 11836170001 | |
| Commercial assay or kit          | Qubit dsDNA HS Assay Kit | Invitrogen | Cat# Q32854 | |
| Commercial assay or kit          | Qubit dsRNA HS Assay Kit | Invitrogen | Cat# Q32852 | |
| Commercial assay or kit          | Pierce BCA Protein Assay Kit | Thermo Scientific | Cat# 23227 | |
| Commercial assay or kit          | Power SYBR Green PCR Master Mix | Applied Biosystems | Cat# 4367659 | |
| Commercial assay or kit          | TaqMan Gene Expression Master Mix | Applied Biosystems | Cat# 4369016 | |
| Commercial assay or kit          | EZ Nucleosomal DNA Prep Kit | Zymo Research | Cat# DS220 | |

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### Appendix 1—key resources table continued

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Commercial assay or kit           | LysoTracker Red DND-99 | Invitrogen | Cat# L7528 |                        |
| Commercial assay or kit           | Cyto-ID Autophagy detection kit 2.0 | Enzo Life Sciences | Cat# ENZ-KIT175 |                        |
| Strain, strain background | w¹¹¹     | This lab |             |                        |
| Genetic reagent (D. melanogaster) | DaGS       | Bloomington Drosophila Stock Center | 8641 |                        |
| Genetic reagent (D. melanogaster) | 5966GS     | Guo et al., 2014 |             |                        |
| Genetic reagent (D. melanogaster) | esgGal4    | Kyoto Stock Center | 104863 |                        |
| Genetic reagent (D. melanogaster) | UAS-H3[RNAi] | Vienna Drosophila Resource Center | KK109374 |                        |
| Genetic reagent (D. melanogaster) | UAS-H4[RNAi] | Vienna Drosophila Resource Center | KK109059 |                        |
| Genetic reagent (D. melanogaster) | UAS-Bchs[RNAi] | Vienna Drosophila Resource Center | KK110785 |                        |
| Genetic reagent (D. melanogaster) | UAS-Bchs.HA | Bloomington Drosophila Stock Center | 51636 |                        |
| Genetic reagent (D. melanogaster) | UAS-Atg5[RNAi] | Scott et al., 2004; Ren et al., 2009 |             |                        |
| Genetic reagent (D. melanogaster) | UAS-Atg1[RNAi] | Bloomington Drosophila Stock Center | 26731 |                        |
| Genetic reagent (D. melanogaster) | UAS-eIF3d[RNAi] | Vienna Drosophila Resource Center | 330545 |                        |
| Genetic reagent (D. melanogaster) | UAS-eIF3g[RNAi] | Vienna Drosophila Resource Center | GD28937 |                        |
| Genetic reagent (D. melanogaster) | UAS-eIF4e[RNAi] | Vienna Drosophila Resource Center | GD7800 |                        |
| Genetic reagent (D. melanogaster) | UAS-H3/H4 | This lab |             |                        |
| Genetic reagent (D. melanogaster) | UAS-mCD8::GFP.L | Bloomington Drosophila Stock Center | 5137 |                        |
| Strain, strain background (mouse) | UM-HET3 stock (CByB6F1 x C3D2F1) | This lab |             |                        |
| Sequence-based reagent           | Primer for Q-RT-PCR Act5C_F1: AGGC CAACCGTGAGAAGATG | This paper |             |                        |
| Sequence-based reagent           | Primer for Q-RT-PCR Act5C_R1: GGGG AAGGGCATAAAAACCCTC | This paper |             |                        |
| Sequence-based reagent           | Primer for Q-RT-PCR His3_F1: CCACGCA AACAACTGGCTAC | This paper |             |                        |
| Sequence-based reagent           | Primer for Q-RT-PCR His3_R1: TGCGGAT TAGAAGCTCGGTG | This paper |             |                        |

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### Appendix 1—key resources table continued

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|----------------------------------|-------------|---------------------|-------------|------------------------|
| Sequence-based reagent Primer for Q-RT-PCR | His4_F1: CGGATAGC AGGCTTCTGTGAT | This paper |             |                        |
|                                  | His4_R1: GGTGCTGT GAAAAGGAGGC | This paper |             |                        |
| Sequence-based reagent Primer for Q-RT-PCR | Bchs_F1: AGCCTCAC CACGCTAAAGA | This paper |             |                        |
|                                  | Bchs_R1: CTCATGTC GTTGACGGGAC | This paper |             |                        |
| Sequence-based reagent Primer for Q-RT-PCR | DOR_F1: CTTGATCT GTGGGTTGTGAC | This paper |             |                        |
|                                  | DOR_R1: CTTCAAC TGTACGGCGCAT | This paper |             |                        |
| Sequence-based reagent Primer for Q-RT-PCR | Stat92_F1: AAGCTG CTTGCCCAAAACTAC | This paper |             |                        |
|                                  | Stat92_R1: GACGC ATTGTGAGTACGATGG | This paper |             |                        |
| Sequence-based reagent Primer for Q-RT-PCR | B2m_Mouse_F1: TTCT GGTCCTGTCCTCACTGA | This paper |             |                        |
|                                  | B2m_Mouse_R1: CAG TATGTTCGGCTTCCCATTC | This paper |             |                        |
| Sequence-based reagent Primer for Q-RT-PCR | Wdfy3_Mouse_F1: GAGGCTCTGGA GTGTGATTACG | This paper |             |                        |
|                                  | Wdfy3_Mouse_R1: GTGGCCGTCT CCTTCAGTG | This paper |             |                        |
| Sequence-based reagent Primer for ChIP-Q-PCR | Bchs_ChIP_F1: AGAACAGCTG TCTCGACAA | This paper |             |                        |
|                                  | Bchs_ChIP_R1: CTTACATAAGCG AGCAAGCG | This paper |             |                        |
| Sequence-based reagent Primer for ChIP-Q-PCR | DOR_ChIP_F1: ATTCGCTCGTC AGTCTTGTT | This paper |             |                        |
|                                  | DOR_ChIP_R1: TAACTGACGGG GGTGAGAT | This paper |             |                        |

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| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|--------------------|-------------|------------------------|
| Sequence-based reagent            | Primer for ChIP-Q-PCR Stat92E_ChIP_F1: AAGCGATCCA CATGGGATACT | This paper |             |                        |
| Sequence-based reagent            | Primer for ChIP-Q-PCR Stat92E_ChIP_R1: TCCTATCTTCC CGGTTTGGC | This paper |             |                        |
| Recombinant DNA reagent           | Plasmid: pUAST attb H3 | This paper |             |                        |
| Software, algorithm               | Microsoft Excel | Microsoft |             | [https://www.microsoft.com/en-gb/](https://www.microsoft.com/en-gb/) |
| Software, algorithm               | GraphPad Prism | GraphPad |             | [https://www.graphpad.com/scientific-software/prism/](https://www.graphpad.com/scientific-software/prism/) |
| Software, algorithm               | Adobe Illustrator | Adobe |             | [https://www.adobe.com/uk/products/illustrator.html](https://www.adobe.com/uk/products/illustrator.html) |
| Software, algorithm               | ImageJ | ImageJ |             | [https://imagej.nih.gov/ij/](https://imagej.nih.gov/ij/) |
| Software, algorithm               | Leica LAS X-3D | Leica |             | [https://www.leica-microsystems.com/de/produkte/mikroskop-software/details/product/leica-las-x-3d/](https://www.leica-microsystems.com/de/produkte/mikroskop-software/details/product/leica-las-x-3d/) |
| Software, algorithm               | Imaris | Bitplane |             | [http://www.bitplane.com/imaris](http://www.bitplane.com/imaris) |
| Software, algorithm               | Flexbar | [Dodt et al., 2012](https://doi.org/10.7554/eLife.62233) |
| Software, algorithm               | HiSat | [Kim et al., 2015](https://www.sciencedirect.com/science/article/pii/S0167483815001736) |
| Software, algorithm               | SAMtools | [Li et al., 2009](http://samtools.sourceforge.net/) |
| Software, algorithm               | StringTie | [Pertea et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4427524/) |
| Software, algorithm               | R statistics package | R Core Team |             | [https://www.r-project.org/](https://www.r-project.org/) |
| Other                             | Rapamycin gut RNA-seq analysed data | This paper | GEO: GSE148002 |