SHORT COMMUNICATION

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Polr3b heterozygosity in mice induces both beneficial and deleterious effects on health during ageing with no effect on lifespan

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Abstract

The genetic pathways that modulate ageing in multicellular organisms are typically highly conserved across wide evolutionary distances. Recently RNA polymerase III (Pol III) was shown to promote ageing in yeast, *C. elegans* and *D. melanogaster*. In this study we investigated the role of Pol III in mammalian ageing using C57BL/6N mice heterozygous for Pol III (*Polr3b*$^{+/-}$). We identified sexually dimorphic, organ-specific beneficial as well as detrimental effects of the *Polr3b*$^{+/-}$ mutation on health. Female *Polr3b*$^{+/-}$ mice displayed improved bone health during ageing, but their ability to maintain an effective gut barrier function was compromised and they were susceptible to idiopathic dermatitis (ID). In contrast, male *Polr3b*$^{+/-}$ mice were lighter than wild-type (WT) males and had a significantly improved gut barrier function in old age. Several metabolic parameters were affected by both age and sex, but no genotype differences were detected. Neither male nor female *Polr3b*$^{+/-}$ mice were long-lived compared to WT controls. Overall, we find no evidence that a reduced Pol III activity extends mouse lifespan but we do find some potential organ- and sex-specific benefits for old-age health.

KEYWORDS
Aging, C57BL/6N, healthspan, longevity, RNA polymerase III

The ageing process is associated with a profound decline in physiological function and increased prevalence in multiple pathologies (Figueira et al., 2016). It is well established that lifespan can be extended through dietary, pharmacological, and genetic means (Fontana & Partridge, 2015; Gems & Partridge, 2013; Mannick & Lamming, 2023), with several of these interventions also delaying and/or reducing age-related pathology (Selman & Withers, 2011). RNA polymerase III (Pol III) is one of three nuclear RNA polymerases found in eukaryotes. It transcribes a number of short non-coding RNAs (e.g., tRNAs, snRNAs, 5S rRNA (Kulaberoglu et al., 2021)), and is estimated to account for ~15% of total cellular transcription (Moir & Willis, 2013). Pol III inhibition extends lifespan in yeast, *C. elegans* and *D. melanogaster*, acting through the intestine/intestinal stem cells to achieve this in worms and flies respectively (Filer et al., 2017). Inhibition also preserves age-related health in flies and acts downstream of mTORC1 (Filer et al., 2017).

In mammals, Pol III consists of 17 subunits, of which Polr3a and Polr3b are the largest and form the catalytic subunit of the polymerase (Choquet et al., 2019; Kulaberoglu et al., 2021). Given that Pol III can modulate lifespan in invertebrate models and that this phenotype appears not to be subunit-specific in flies (Filer et al., 2017), we examined longevity and aspects of age-related health in mice heterozygous for *Polr3b* (*Polr3b*$^{+/-}$) which encodes the second largest catalytic subunit of Pol III (Figure S1A,B); global homozygous loss of *Polr3b* causes embryonic lethality (see Methods Data S1). Mice bred with expected Mendelian frequencies (Figure 1a) and hepatic expression of *Polr3b* was reduced in both female and male

Polr3b$^{+/-}$ mice (Figure 1b; Hepatic POLR3b protein levels were also reduced in female, but not male, mice) (Figure S2A,B). Performing extensive phenotyping at different ages, we observed both beneficial and detrimental, sexually dimorphic, organ-specific effects of the heterozygous *Polr3b* mutation on health. Akin to humans, mice exhibit age-related bone loss (Jilka, 2013) and this loss can be assessed by microCT (Selman et al., 2009). Female *Polr3b*$^{+/-}$ mice showed increased trabecular bone relative to WT females particularly evident at mid-life (Figure 1c–e; Figure S3), similar to our previously findings in long-lived IIS and mTOR mutants (Selman et al., 2008, 2009), indicating that reduced Pol III activity may help maintain bone health
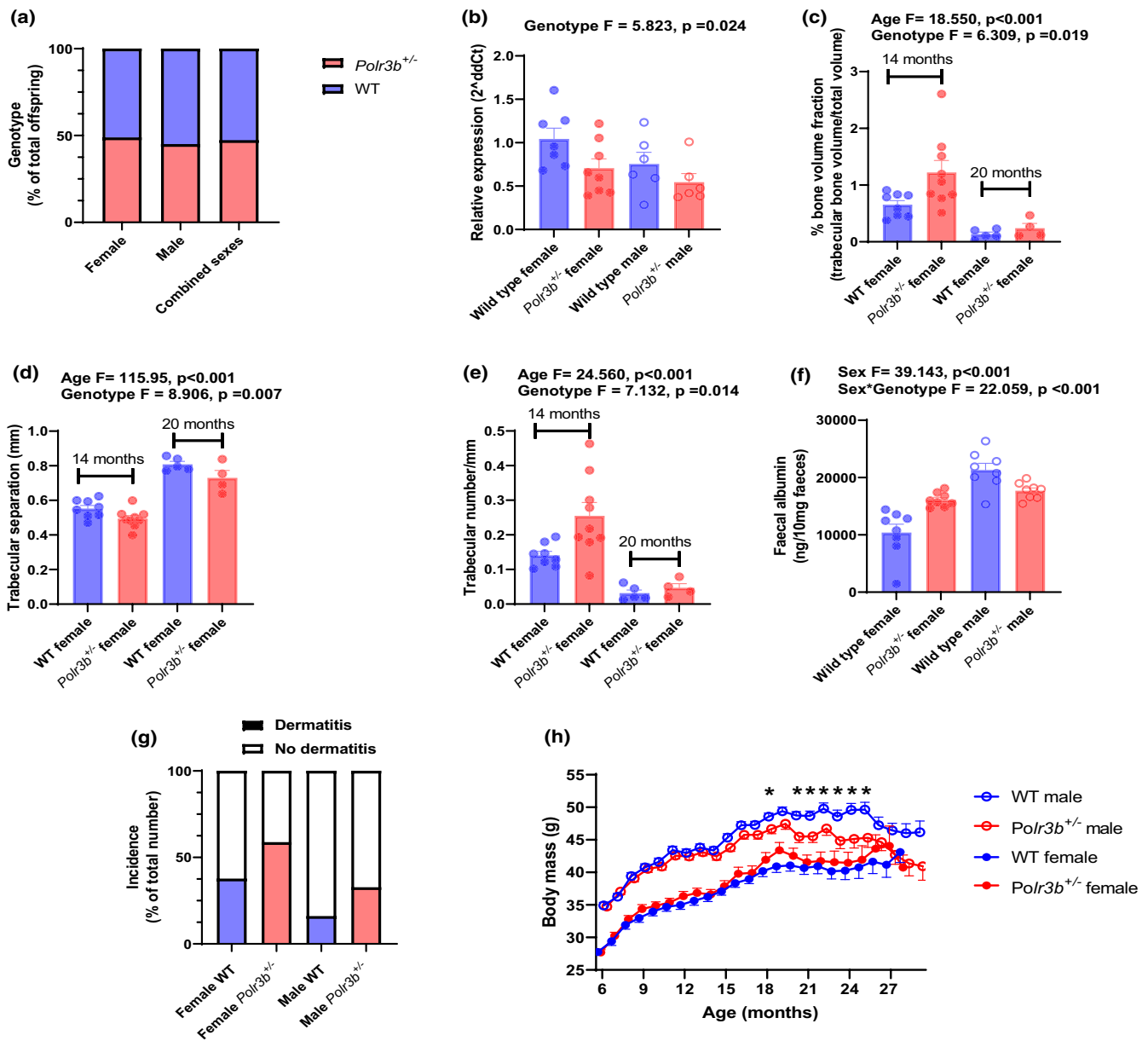

FIGURE 1 (a) Mendelian frequencies of mice born from heterozygous *Polr3b*$^{+/-}$ x wild-type (WT) parents. (b) Hepatic *Polr3b* expression in 14-month-old mice. (c) Percentage bone volume, (d) Trabecular separation, (e) Trabecular number in female mouse femurs at 14 and 20 months. (f) Faecal albumin levels in female and male mice at 22 months. (g) Percentage of mice (of total number) presenting with idiopathic dermatitis. (h) Body mass (mean ± SEM) across the life-course in female and male WT and *Polr3b*$^{+/-}$ mice. Histograms denote mean ± SEM, with sample sizes indicated by individual points within a group. For (b–f), a two-way ANOVA was used to test for age/sex and genotype effects (both main and interaction effects). Only significant main and/or interaction effects are reported within the figures.

during ageing in female mice. No phenotypic differences in bone characteristics were observed in males (Figure S4A–C).

With age, the barrier function of the mouse gut becomes compromised, and this leakiness can be observed through an increase in faecal albumin (Wang et al., 2021). In contrast to the preserved gut barrier function observed in older flies with attenuated Pol III activity (Filer et al., 2017), female $Polr3b^{+/-}$ mice showed increased gut permeability compared to age-matched WT controls (Figure 1f). These females were also susceptible to ID (Figure 1g). On the other hand, $Polr3b^{+/-}$ males were lighter (Figure 1h) and their gut barrier function was preserved in old age (Figure 1f), relative to WT controls. For several other metabolic phenotypes (Figure S4d–i) and for grip strength (Figure S4j), no genotypic differences were detected at any age in either sex, although several significant age and sex effects were seen.

We evaluated lifespan in female and male C57BL/6N WT mice compared to $Polr3b^{+/-}$ mice. Combined data from both sexes (Figure 2a) showed no significant effect of genotype on median or maximum lifespan (oldest 10% of cohort, Figure S5A; Table S1). Similarly, when each sex was analysed separately neither female nor male $Polr3b^{+/-}$ mice were long-lived (Figure 2b,c; Figure S5B,C; Table S1). Male WT mice lived significantly longer than female WT mice ($X^2 = 10.420$, $p = 0.001$), with a similar sex-specific trend seen in $Polr3b^{+/-}$ mice ($X^2 = 3.810$, $p = 0.051$). Median lifespan of our C57BL/6N WT mice was shorter than our previously published data for C57BL/6J mice (Selman et al., 2008, 2009). C57BL/6J and 6N mice differ in a range of metabolic parameters (Selman & Swindell, 2018), but there is a current dearth of published lifespan

data for C57BL/6N mice. However, our lifespans compare favourably with published lifespans for this strain (Reid et al., 2023; Tang et al., 2021). No genotype difference in cancer incidence upon postmortem was identified in our ageing cohorts (Figure 2d). Note that censoring female mice euthanised for ID did not alter the lifespan outcome (Figure S5D; $X^2 = 0.001$, $p = 0.970$).

Overall, we observed an indication of organ- and sex-specific benefits for old-age health achieved by a partial loss-of-function in Pol III with no effect on lifespan. In support, pathogenic conditions associated with Pol III appear to show pronounced tissue-specific responses following perturbation in Pol III transcription (Watt et al., 2023). The difference to the net beneficial effect on invertebrate lifespan may result from a more complex physiological role of Pol III in mammals. For example, Pol III plays a critical noncanonical role in viral and bacterial DNA sensing within the innate immune response (Chiu et al., 2009). It is possible that the appropriate response to bacterial and viral challenge at the skin surface is impaired in female $Polr3b^{+/-}$ mice causing ID. Our mice were heterozygous for $Polr3b$, and at the level of both protein and gene expression the reduction in $Polr3b$ observed was variable both within and between sexes. It is possible that a more substantial reduction in Polr3b expression in specific cell types is required to obtain a longevity phenotype. Still, the observation of some beneficial effects, for example, on bone health in females, suggests that cell-type specific attenuation of Pol III function may have positive effect on aspects of mammalian ageing, as indicated by a recent analysis of human genetic data (Javidnia et al., 2022).
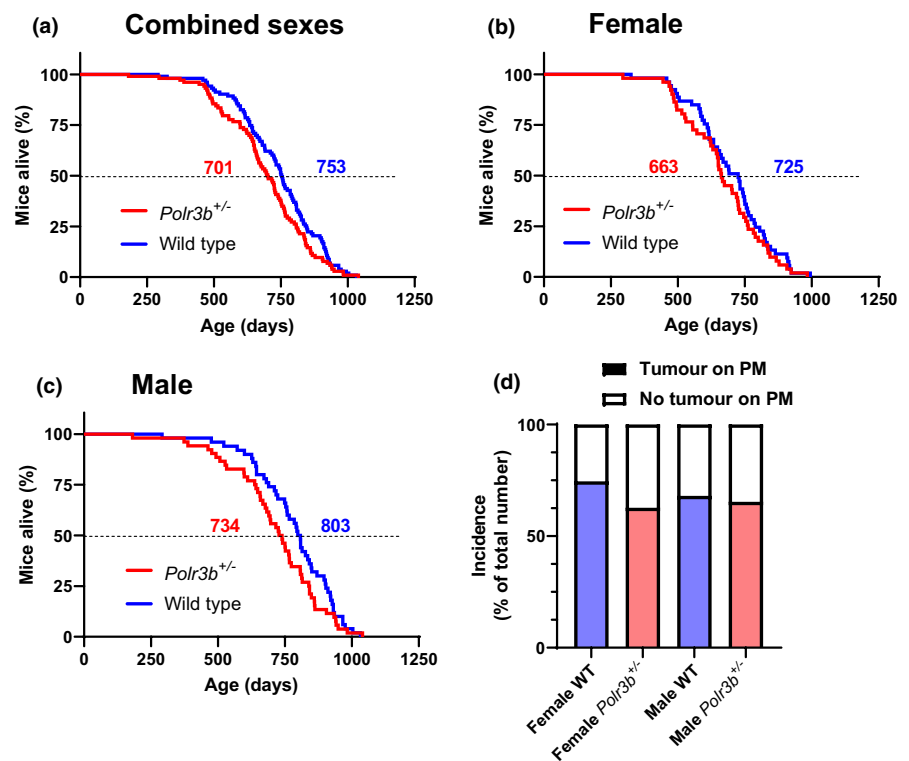

FIGURE 2 (a) Kaplan–Meier survival curves for combined female and male wild-type (WT) and $Polr3b^{+/-}$ mice (log-rank $X^2 = 3.084$, $p = 0.079$, $n = 103$ for WT, $n = 103$ for $Polr3b^{+/-}$). Survival curves for female (b; log-rank $X^2 = 1.040$, $p = 0.308$, $n = 53$ for WT, $n = 51$ for $Polr3b^{+/-}$) and male (c; log-rank $X^2 = 2.355$, $p = 0.125$, $n = 50$ for WT, $n = 52$ for $Polr3b^{+/-}$) mice. Numbers denote median lifespan (days). (d) Percentage of mice presenting postmortem with macroscopic tumours.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All individual lifespan data are included within the supplementary material Table S3).

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REFERENCES

Chiu, Y. H., Macmillan, J. B., & Chen, Z. J. (2009). RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell*, *138*(3), 576–591. https://doi.org/10.1016/j.cell.2009.06.015

Choquet, K., Pinard, M., Yang, S., Moir, R. D., Poitras, C., Dicaire, M. J., Sgarioto, N., Larivière, R., Kleinman, C. L., Willis, I. M., Gauthier, M. S., Coulombe, B., & Brais, B. (2019). The leukodystrophy mutation Polr3b R103H causes homozygote mouse embryonic lethality and impairs RNA polymerase III biogenesis. *Molecular Brain*, *12*(1), 59. https://doi.org/10.1186/s13041-019-0479-7

Figueira, I., Fernandes, A., Mladenovic Djordjevic, A., Lopez-Contreras, A., Henriques, C. M., Selman, C., Ferreiro, E., Gonos, E. S., Trejo, J. L., Misra, J., Rasmussen, L. J., Xapelli, S., Ellam, T., & Bellantuono, I. (2016). Interventions for age-related diseases: Shifting the paradigm. *Mechanisms of Ageing and Development*, *160*, 69–92. https://doi.org/10.1016/j.mad.2016.09.009

Filer, D., Thompson, M. A., Takhaveev, V., Dobson, A. J., Kotronaki, I., Green, J. W. M., Heinemann, M., Tullet, J. M. A., & Alic, N. (2017). RNA polymerase III limits longevity downstream of TORC1. *Nature*, *552*(7684), 263–267. https://doi.org/10.1038/nature25007

Fontana, L., & Partridge, L. (2015). Promoting health and longevity through diet: From model organisms to humans. *Cell*, *161*(1), 106–118. https://doi.org/10.1016/j.cell.2015.02.020

Gems, D., & Partridge, L. (2013). Genetics of longevity in model organisms: Debates and paradigm shifts. *Annual Review of Physiology*, *75*, 621–644. https://doi.org/10.1146/annurev-physiol-030212-183712

Javidnia, S., Cranwell, S., Mueller, S. H., Selman, C., Tullet, J. M. A., Kuchenbaecker, K., & Alic, N. (2022). Mendelian randomization analyses implicate biogenesis of translation machinery in human aging. *Genome Research*, *32*(2), 258–265. https://doi.org/10.1101/gr.275636.121

Jilka, R. L. (2013). The relevance of mouse models for investigating age-related bone loss in humans. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *68*(10), 1209–1217. https://doi.org/10.1093/gerona/glt046

Kulaberoglu, Y., Malik, Y., Borland, G., Selman, C., Alic, N., & Tullet, J. M. A. (2021). RNA polymerase III, ageing and longevity. *Frontiers in Genetics*, *12*, 705122. https://doi.org/10.3389/fgene.2021.705122

Mannick, J. B., & Lamming, D. W. (2023). Targeting the biology of aging with mTOR inhibitors. *Nature Aging*, *3*(6), 642–660. https://doi.org/10.1038/s43587-023-00416-y

Moir, R. D., & Willis, I. M. (2013). Regulation of pol III transcription by nutrient and stress signaling pathways. *Biochimica et Biophysica Acta*, *1829*(3–4), 361–375. https://doi.org/10.1016/j.bbagrm.2012.11.001

Reid, K., Daniels, E. G., Vasam, G., Kamble, R., Janssens, G. E., Hu, I. M., Green, A. E., Houtkooper, R. H., & Menzies, K. J. (2023). Reducing mitochondrial ribosomal gene expression does not alter metabolic health or lifespan in mice. *Scientific Reports*, *13*(1), 8391. https://doi.org/10.1038/s41598-023-35196-3

Selman, C., Lingard, S., Choudhury, A. I., Batterham, R. L., Claret, M., Clements, M., Ramadani, F., Okkenhaug, K., Schuster, E., Blanc, E., Piper, M. D., Al-Qassab, H., Speakman, J. R., Carmignac, D., Robinson, I. C., Thornton, J. M., Gems, D., Partridge, L., & Withers, D. J. (2008). Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *The FASEB Journal*, *22*(3), 807–818. https://doi.org/10.1096/fj.07-9261com

Selman, C., & Swindell, W. R. (2018). Putting a strain on diversity. *The EMBO Journal*, *37*(22). https://doi.org/10.15252/embj.2018100862

Selman, C., Tullet, J. M., Wieser, D., Irvine, E., Lingard, S. J., Choudhury, A. I., Claret, M., Al-Qassab, H., Carmignac, D., Ramadani, F., Woods, A., Robinson, I. C., Schuster, E., Batterham, R. L., Kozma, S. C., Thomas, G., Carling, D., Okkenhaug, K., Thornton, J. M., … Withers, D. J. (2009). Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. *Science*, *326*(5949), 140–144. https://doi.org/10.1126/science.1177221

Selman, C., & Withers, D. J. (2011). Mammalian models of extended healthy lifespan. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *366*(1561), 99–107. https://doi.org/10.1098/rstb.2010.0243

Tang, J., Ju, A., Li, B., Zhang, S., Gong, Y., Ma, B., Jiang, Y., Liu, H., Fu, Y., & Luo, Y. (2021). Young and undamaged rMSA improves the healthspan and lifespan of mice. *Biomolecules*, *11*(8). https://doi.org/10.3390/biom11081191

Wang, H., Wang, G., Banerjee, N., Liang, Y., Du, X., Boor, P. J., Hoffman, K. L., & Khan, M. F. (2021). Aberrant gut microbiome contributes to intestinal oxidative stress, barrier dysfunction, inflammation and systemic

autoimmune responses in MRL/lpr mice. *Frontiers in Immunology, 12,* 651191. https://doi.org/10.3389/fimmu.2021.651191

Watt, K. E., Macintosh, J., Bernard, G., & Trainor, P. A. (2023). RNA polymerases I and III in development and disease. *Seminars in Cell & Developmental Biology, 136,* 49–63. https://doi.org/10.1016/j.semcdb.2022.03.027

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