SHANGHAI MODEL ORGANISMS

# Phenotypic characterization of *Zmpste24*-null mouse model and its potential in ageing research

## Introduction

Ageing is a process that gradually increases the organism's vulnerability to death. While ageing affects different biological pathways, its cellular mechanisms are complex and challenging to investigate. As a result of the growing disease burden of ageing populations, increasing efforts are being invested to understand the underlying mechanisms of ageing. It is realized that instead of a passive decline of physiological functions, ageing has become the result of a complex interconnection of genetic and biochemical mechanisms. Although it is still far from a complete understanding of the ageing-related, intrinsic network of pathways, *in vivo* studies in a variety of animal models have proven the feasibility of modulating the ageing process via genetic manipulations[1].

Mouse represents an attractive model for studying mammalian biology due to the genetic manageability of its genome, ease of breeding, and the wide availability of baseline phenotypic data. Besides, mouse models have relative short lifespan, which allows the monitoring of the ageing process in a reasonable time window.

Disruption of the nuclear lamina, one of the cellular changes involved in natural ageing, underlies several different syndromes known as progeroid laminopathies. Exemplified by the Hutchinson-Gilford progeria syndrome, most if not all those diseases are caused by lamin A maturation defects due to the mutations in the *Lmna* and *Zmpste24* loci, the latter encoding the metalloproteinase required for prelamin A processing. In 2002, the pathological consequences of *Zmpste24* deficiency in mice was described for the first time. *Zmpste24*$^{-/-}$ mice gain weight slowly, appear to be malnourished and suffer from progressive hair loss. In addition, those mice manifested muscle weakness and spontaneous bone fractures[2]. Lastly but most importantly, mice deficient in *Zmpste24* recapitulate multiple features of ageing, making it an attractive rodent model to study the molecular mechanisms of ageing as well as the pathogenesis of ageing-related diseases.

## Results

CRISPR-Cas9 revolutionizes genomics by enabling efficient site-directed genome editing in a wide variety of biological systems. Upon the creation of double-stranded breaks via CRISPR-Cas9, mammalian cells use endogenous cellular machinery to repair the broken sites, via the canonical non-homologous end joining (NHEJ) pathway or homology-directed repair (HDR) pathway[3]. Here, scientists at Shanghai Model Organisms Center independently developed a mouse model deficient in the *Zmpste24* gene (referred to as *Zmpste24*$^{-/-}$ mice in this article) via the CRISPR/Cas9 technology. Specifically, two guide RNAs were simultaneously introduced to generate a 2547-base deletion *in situ*, resulting in a frameshift and premature termination of the target gene.

## Animal growth

The growth phenotypes of the *Zmpste24*$^{-/-}$ mice were characterized. At birth, *Zmpste24*$^{-/-}$ mice were indistinguishable from their wild-type (*Zmpste24*$^{+/+}$ mice) littermates and appeared healthy until 4 weeks of age. Subsequently, null mice could be distinguished from wild-type by size and weight (Figure 1A and B). Within 4-6 weeks, their growth rate was significantly reduced. They stopped growing despite normal feeding habits by week 6-7 (Figure 1B). In addition, *Zmpste24*$^{-/-}$ mice died prematurely, with an average lifespan of 20 weeks (Figure 1C).

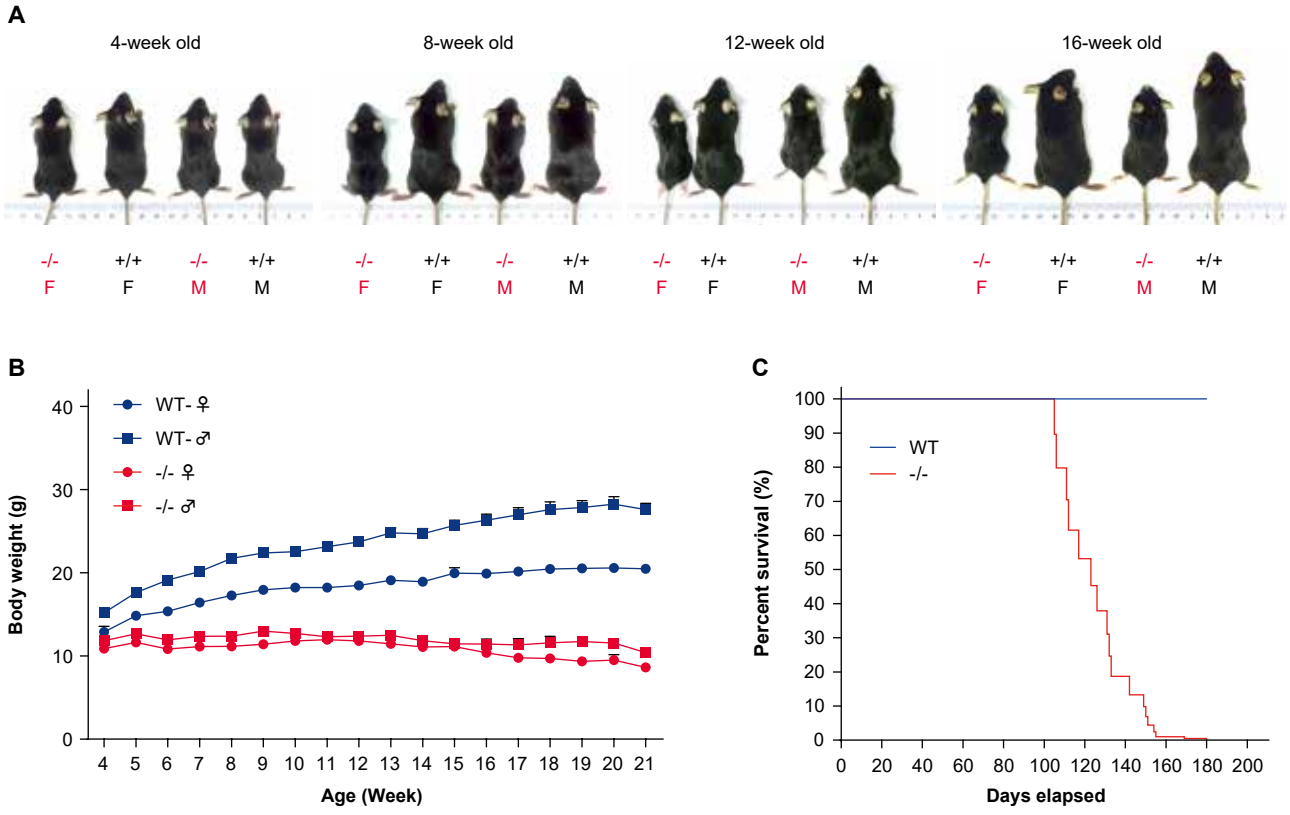


**Figure 1. Growth phenotype of *Zmpste24*$^{-/-}$ mice. A.** Photograph of *Zmpste24*$^{-/-}$ mice and their wild-type siblings at 4, 8, 12 and 16 weeks of age. **B.** Cumulative plot of body weight versus age. Dots represent mean values (n=28). **C.** Survival curve of *Zmpste24*$^{-/-}$ mice versus their WT littermates (n=22).

## WBC count

White blood cells or leukocytes produce, transport and distribute antibodies as a part of the immune system response[4]. While in WT mice the white blood cell (WBC) count falls into the normal range of 2000-10,000 per microliter, the WBC appears to be significantly less in *Zmpste24*$^{-/-}$ mice by the age of 12 weeks (Figure 2).

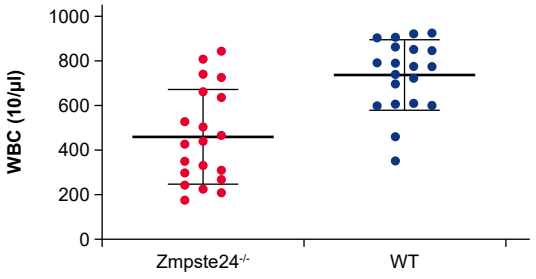


**Figure 2. WBC count was measured and compared between *Zmpste24*$^{-/-}$ mice and their wild-type counterparts.**

## Characterization of the behavior of *Zmpste24*$^{-/-}$ mice

### New object recognition test

New object recognition test, developed in the late 1980s, is now among the most commonly used behavioral tests for mice. New object recognition test is based on the natural tendency of mice to investigate a novel object instead of a familiar one, as well as their innate tendency to restart exploring when they are presented with a novel environment. The choice to explore the novel object, as well as the reactivation of exploration after object displacement, reflects the learning and recognition memory process[5]. Specifically, in this study a mouse is presented with two similar objects during the first session, and then one of the two objects is replaced by a new object during the second session. The amount of time taken to explore the new object provides an index of recognition memory. This approach has been widely adapted for research on memory, for instance, brain lesions or ageing studies[6]. At all the ages tested in this study (4 8 and 12 weeks), we did not observe a significant difference between the *Zmpste24*$^{-/-}$ and wild-type group in terms of the discrimination index, suggesting that the constitutive loss of the *Zmpste24* gene does not seem to have a negative impact upon the mouse memory (Figure 3).

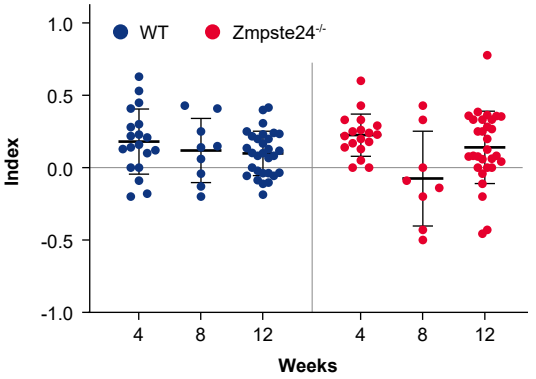


**Figure 3. *Zmpste24* deficient mice show normal object recognition.**

## Open field test

The open field test (OFT) is a common measure of exploratory behavior and general activity in rodent animals, where both the quality and quantity of the activity can be measured. It is used primarily to examine motor functions by means of measuring spontaneous activities in an open field by automated computer program[7]. General movements and preference for particular sections can be calculated to examine behaviors and activities. In this study, we specifically measured the time the mice spent in the central and peripheral areas (Center Time and Peripheral Time) as well as the accumulative distance the mice moved during the test (Center Distance and Peripheral Distance). Mouse locomotor activities reflected by each measurement were statistically compared between the WT and *Zmpste24*$^{-/-}$ group using analysis of variance (Figure 4A-D). Strikingly, at old ages *Zmpste24*$^{-/-}$ mice exhibited decreased locomotor activities relative to their age-matched controls, implicating that *Zmpste24* may be involved in the age-related behavior changes.

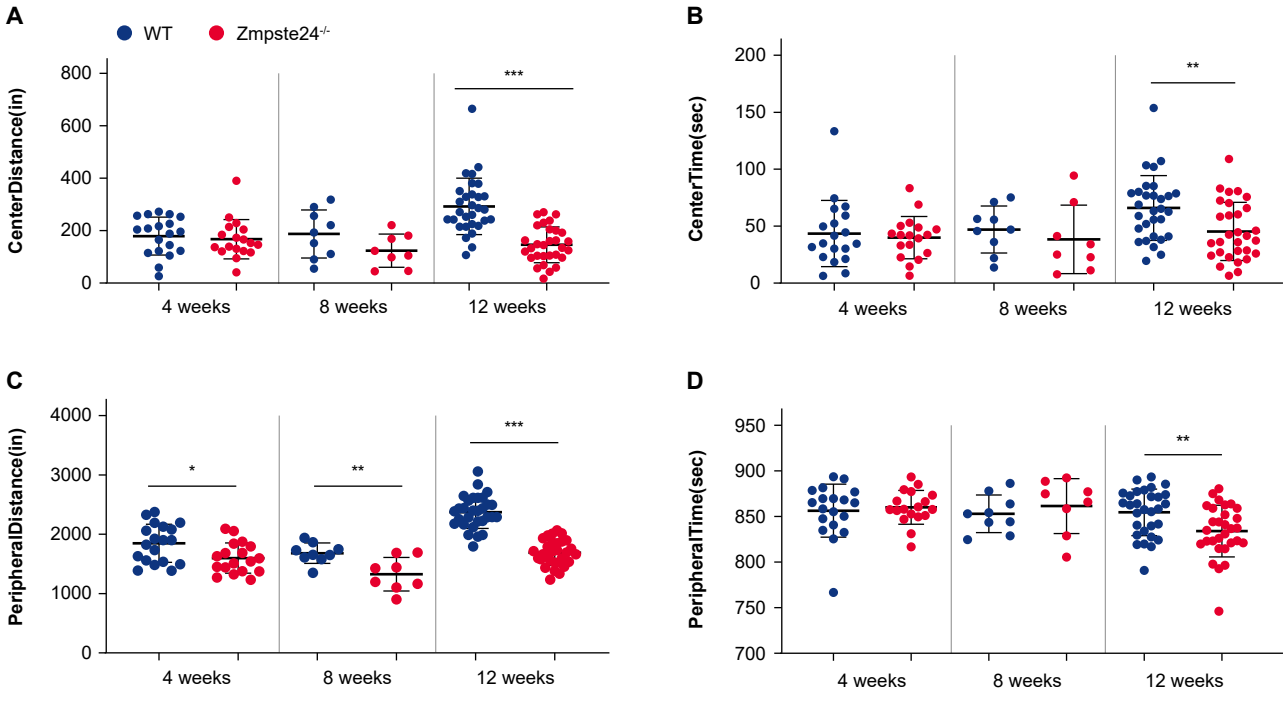


**Figure 4. Decreased locomotor activity in *Zmpste24*$^{-/-}$ mice, measured by OFT. A.** distance travelled in the central area; **B.** time spent in the central area; **C.** distance travelled in the peripheral area; **D.** time spent in the peripheral area. *p<0.05, **p<0.01, ***p<0.001.

## Pole test

The pole test assesses the agility of animals. A vertical wooden pole with a rough surface was placed in the home cage. Mice placed head-up on top of the pole, orient themselves downward and descend the pole back into their home cage[8]. Time spent to orient downward (Turn Latency) and the time to descend (Descend Latency) were measured. While *Zmpste24*$^{-/-}$ mice displayed normal performance on the pole test at 4 weeks of age, the null mice spent more time to descend at 8 weeks of age (Figure 5A and B). At 12 weeks, *Zmpste24*$^{-/-}$ mice could not grip the pole to finish the test, indicating an impaired muscle strength (data not shown).

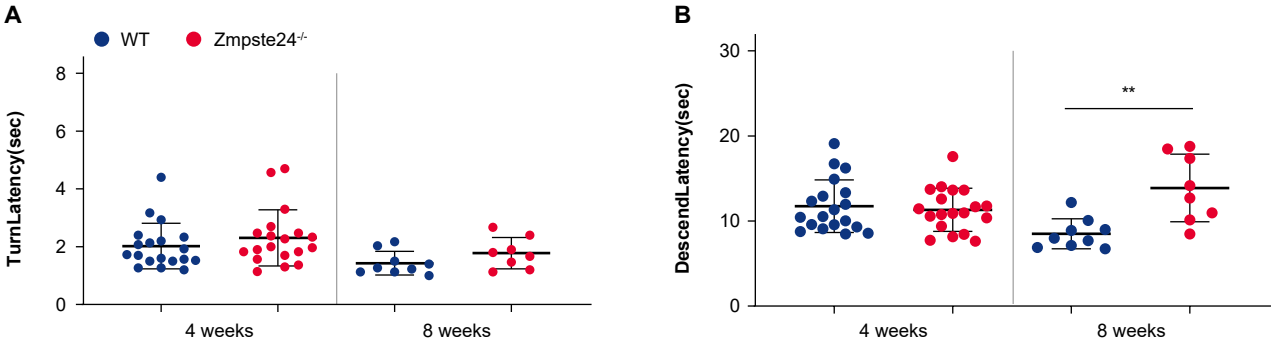


**Figure 5.** Time to orient down (Turn latency) and total time to descend the pole (Descent latency) were measured for *Zmpste24*$^{-/-}$ mice and wild-type controls at 4 and 12 weeks of age (data not shown). ** p<0.01.

## Beam walking assay

Lastly, we performed the beam walking assay to assess fine motor coordination and balance in *Zmpste24*$^{-/-}$ mice. The goal of this test is for the mouse to stay upright and walk across an elevated narrow beam to a safe platform[9]. Performance on the beam is quantified by measuring the time it takes for the mouse to traverse the beam. Here, *Zmpste24*$^{-/-}$ and littermate wild-type control mice were tested on the beam when they reached the age of 12 weeks. Not surprisingly, the null animals took much longer to traverse the beam, revealing a significant effect of genotype on mouse motor coordination (Figure 6).

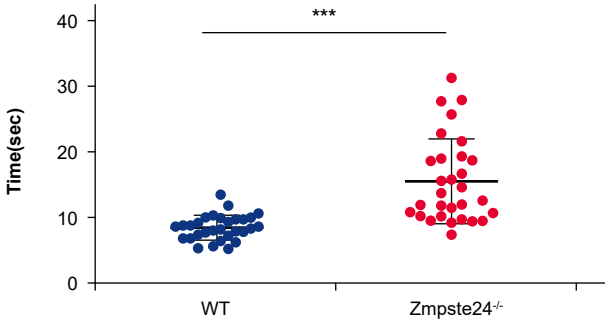


**Figure 6.** Motor performance and coordination was measured in *Zmpste24*$^{-/-}$ and wild-type mice using the challenging beam. At 12 weeks of age, *Zmpste24*$^{-/-}$ mice took longer to traverse the beam compared to wild-type animals. *** p<0.001.

## Discussion

Nuclear lamins constitute the scaffold required for the maintenance of a myriad of nuclear protein complexes responsible for chromatin organization and gene regulation. Since nuclear architecture defects result in genome instability, it is not surprising that mutations in lamins or lamin-binding proteins cause a variety of human diseases, including the Hutchinson-Gilford progeria syndrome and other genetic disorders where severe progeroid features are found[10]. The generation of mouse models deficient in the enzyme responsible for its accurate proteolytic processing and the conversion of prelamin A into the mature protein, such as the *Zmpste24*$^{-/-}$ mouse model characterized in this article, mimics the genetic defect found in some of the progeria patients and provide further evidence that alterations in nuclear structural components cause age-related phenotypes.

## Summary

As shown in this study, *Zmpste24*$^{-/-}$ mice display robust locomotor impairments and other abnormalities in a variety of behavior measures, including:

- Retarded growth and shorter lifespan
- Low white blood cell count
- Normal recognition memory
- Decreased locomotor activities and impaired muscle strength
- Impaired motor coordination and balance



**References**

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