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Spatial expression of the nonsense-mediated mRNA decay factors UPF3A and UPF3B among mouse tissues

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Nonsense-mediated mRNA decay (NMD) is a highly conserved post-transcriptional gene regulatory mechanism in eukaryotes (Lykke-Andersen and Jensen, 2015; Kurosaki et al., 2019; Yi et al., 2021; Karousis and Mühlemann, 2022; Mailliot et al., 2022). NMD is considered as an RNA surveillance machinery to regulate mRNA abundance and eventual protein expression. NMD can recognize and degrade aberrant mRNAs, which contain premature termination codons (PTCs) located at least 50–55 nucleotides upstream of the last exon–exon junction. This process prevents possible toxic effects from translated truncated proteins (Lykke-Andersen and Jensen, 2015; Kurosaki et al., 2019; Karousis and Mühlemann, 2022; Mailliot et al., 2022). Previous research has shown that NMD can recognize special structures of mRNAs with physiological functions, such as the long 3' untranslated region (3' UTR) and upstream open reading frames of mRNAs, to regulate cell fate (Li et al., 2015). Thus, NMD is essential for embryonic development and tissue homeostasis in mammals (Lykke-Andersen and Jensen, 2015; Hug et al., 2016; Han et al., 2018; Jaffrey and Wilkinson, 2018; Nasif et al., 2018; Popp and Maquat, 2018; Fang et al., 2022).

The phenomenon of NMD was first discovered in *Saccharomyces cerevisiae* (Leeds et al., 1991). The core components of the NMD pathway include the up-frameshift proteins UPF1, UPF2, and UPF3, first identified in the unicellular eukaryotic organism *S. cerevisiae* (Leeds et al., 1991, 1992), as well as the SMG proteins (suppressors with morphological effects on genitalia identified in *Caenorhabditis elegans*) including SMG1, SMG5, SMG6, SMG7, SMG8, and SMG9. SMG proteins were identified and characterized in multicellular organisms (worms, frogs, and mammals) and provide additional layers of regulation on NMD execution (Pulak and Anderson, 1993; Wittkopp et al., 2009; Mailliot et al., 2022; Yi et al., 2022). Interestingly, there is only a single UPF3 in invertebrate organisms, including yeasts, worms, and flies, while UPF3A and UPF3B, two paralogues of UPF3, are thought to have been generated by a gene duplication event during the emergence of vertebrate animals (Shum et al., 2016; Deka et al., 2021). In mice and humans, *UPF3B* is located on the X chromosome. The UPF3B protein is considered the dominant UPF3 paralogue expressed in almost all tissues; *UPF3A* resides in the autosomes of mice and humans, and the UPF3A protein is highly expressed in male germlines (Serin et al., 2001; Tarpey et al., 2007; Shum et al., 2016). In earlier work, Serin et al. (2001) and Tarpey et al. (2007) used the Northern blotting method and showed that *UPF3B* mRNA transcripts are abundantly produced in all major human organs, including bone marrow, brain, lung, testis, and intestine, while *UPF3A* transcripts are highly enriched in the human testis. Only little amounts of *UPF3A* mRNAs are found in human bone marrow, brain, and intestine samples. In mice, *Upf3a* mRNA levels in the testis are approximately 40-fold higher than those in other organs. Consistent with RNA data from human and mouse tissues, Shum et al. (2016) used an antibody-based approach and revealed that UPF3A protein is almost undetectable in the brain, heart, kidney,

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liver, and spleen of adult mice, while UPF3B is abundantly expressed in these tissues. The "underrepresented" UPF3A protein in most mammalian tissues can be partially explained by the hypothesis that UPF3B competes with UPF3A for binding to the NMD factor UPF2, which destabilizes the UPF3A protein (Chan et al., 2009; Shum et al., 2016). In accordance with this hypothesis, the depletion of UPF3B protein results in a dramatic increase of the UPF3A protein (Chan et al., 2009; Nguyen et al., 2012; Shum et al., 2016; Chen et al., 2023). This means that protein expression of UPF3A and UPF3B could be mutually exclusive in most mammalian organs, which supports the functional antagonism between UPF3A and UPF3B (Shum et al., 2016).

We recently constructed a *Upf3a* conditional knockout mouse line, generated a batch of *Upf3a*-inducible knockout embryonic stem cells (ESCs) and rib muscle-derived fibroblasts, and characterized a rabbit monoclonal antibody that can detect mouse UPF3A and UPF3B proteins simultaneously in one immunoblotting reaction (Chen et al., 2023). We found that UPF3A, similar to UPF3B, was adequately expressed in the ESC and somatic cell lines we established. This finding suggests that, under physiological conditions, UPF3A and UPF3B proteins are not mutually exclusive. Thus, in current study, we re-investigated the expression patterns of UPF3A and UPF3B in different tissues from wild-type mice.

Firstly, we examined the mRNA expression of *Upf3a* and *Upf3b* in nine major organs (liver, heart, spleen, lung, kidney, thymus, small intestine, cerebral cortex, and olfactory bulb) and reproductive tissues (testis and ovary) of adult C57BL/6 mice. Real-time quantitative polymerase chain reaction (qPCR) analysis showed that the small intestine had the lowest expression of *Upf3a* and *Upf3b* mRNAs in both sexes (Figs. 1a and 1b). In adult males, the mRNA level of *Upf3a* is the highest in the testis, 20 times more than that in the liver (Fig. 1a), confirming previous findings that mRNA levels of *Upf3a* are the highest in mouse and human testes (Serin et al., 2001; Tarpey et al., 2007; Shum et al., 2016). In hematopoietic organs, including spleen and thymus, mRNAs of *Upf3a* and *Upf3b* are generally lower than those in the liver, heart, lung, kidney, cerebral cortex, and olfactory bulb from males. In females, no significant difference in the mRNA expression of *Upf3a* or *Upf3b* is found between tissues (Fig. 1b), although we noticed that hematopoietic organs have lower expression of *Upf3a* and *Upf3b*. These data are in good agreement with previous findings with the qPCR assay (Shum et al., 2016). Next, we compared the differences in the mRNA expression levels of *Upf3a* and *Upf3b* between female and male mice. In general, *Upf3a* and *Upf3b* mRNA expression levels are comparable between both sexes. However, *Upf3a* mRNA expression in the olfactory bulbs from females and *Upf3b* mRNA expression in the small intestines and thymuses of females are significantly higher than those of male mice ($P<0.05$), while the mRNA expression of *Upf3b* in the lungs of males is higher than that in females ($P<0.01$; Fig. 1c).

In mammalian cells and tissues, the mRNA levels of genes are not always correlated with their protein outputs (Maier et al., 2009). qPCR analysis with gene-specific primers does not allow for the direct comparison of the expression of two genes. Furthermore, antibody-based approaches normally distinguish the relative expression of protein isoforms of a single gene and cannot determine the relative expression of two gene products in a single blot. We previously characterized a commercial antibody that has cross-reactivity with mouse UPF3A and UPF3B proteins (Chen et al., 2023). With this antibody, the protein expression levels of UPF3A and UPF3B between different tissues and the relative amounts of UPF3A vs. UPF3B in a given tissue could be visualized with one western blotting reaction (here, we designated this approach as the "one-tube reaction"). We reasoned here that western blotting analysis with our "one-tube reaction" avoids several western blotting drawbacks: (1) performance variances of individual antibodies against UPF3A and UPF3B, and (2) techniques including signal detection and membrane processing. Western blotting analysis showed that, as expected, UPF3B is abundantly expressed in most mouse tissues, including the liver, spleen, lung, kidney, thymus, cerebral cortex, and olfactory bulb from both sexes and ovaries in females (Fig. 2). However, hearts and small intestines from both sexes have almost negligible expression of UPF3B. UPF3A has the highest expression in male testes. To our surprise, we found that UPF3A is evidently expressed in almost all mouse tissues, including the liver, spleen, lung, thymus, cerebral cortex, and olfactory bulb from both sexes and ovaries in females. Our result is in stark contrast to the finding by Shum et al. (2016), which was done with a different commercial UPF3A antibody. We further

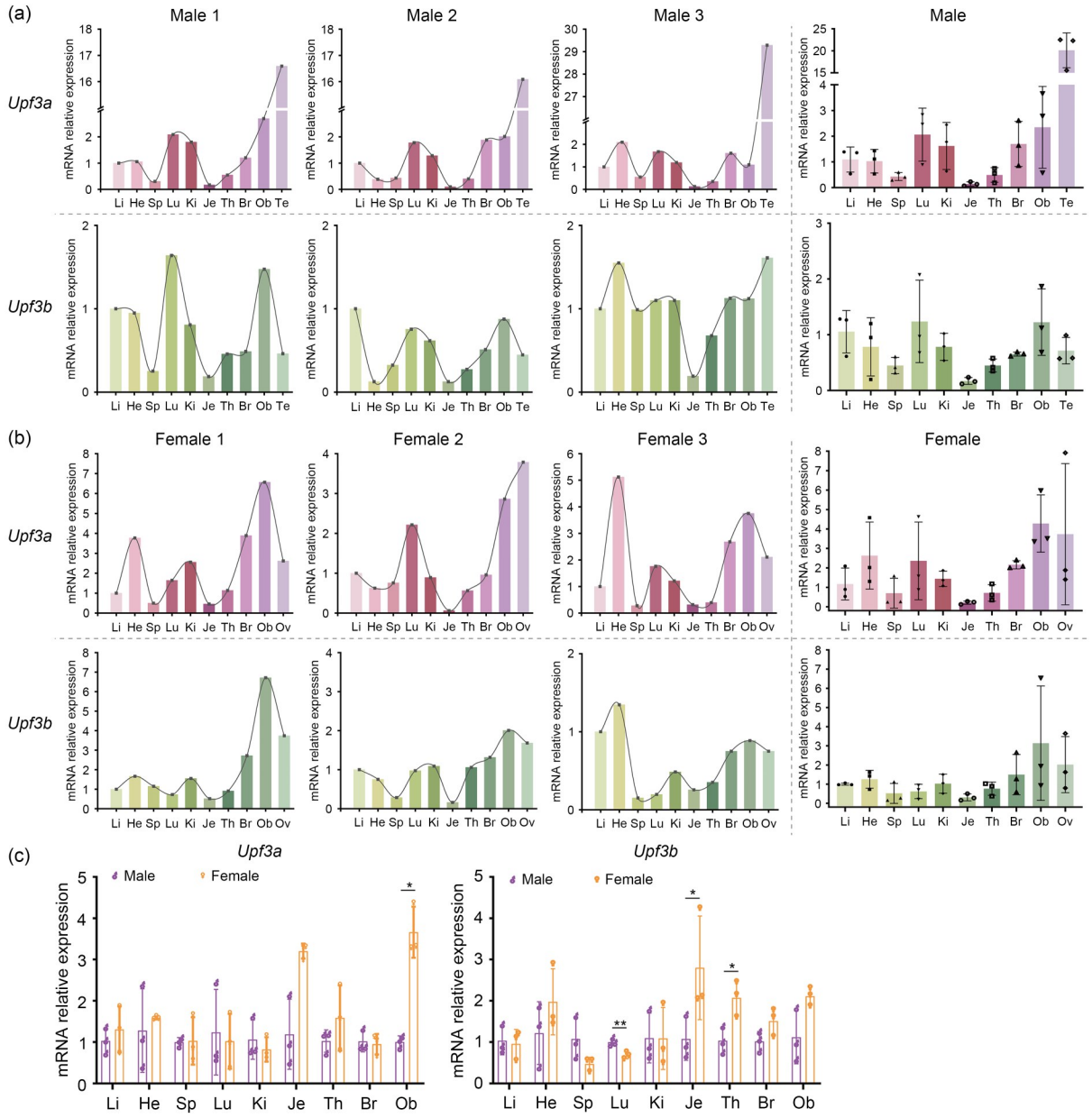

Fig. 1 Relative mRNA expression of *Upf3a* and *Upf3b* in tissues of C57BL/6 mice measured by qPCR. (a) Male mice; (b) Female mice. The right panels of (a) and (b) summarize the mRNA expression of *Upf3a* and *Upf3b* in three male and female mice, respectively. (c) Comparison of male and female mice. The results are represented as mean±standard deviation (SD), *n*=3. * *P*<0.05; ** *P*<0.01. mRNA: messenger RNA; qPCR: real-time quantitative polymerase chain reaction; Li: liver; He: heart; Sp: spleen; Lu: lung; Ki: kidney; Je: jejunum; Th: thymus; Br: brain; Ob: olfactory bulb; Te: testis; Ov: ovary.

investigated the relative expression of UPF3A and UPF3B in all tissues we analyzed. We found that, in spleens and lungs from both sexes, UPF3A protein levels are higher than those of UPF3B. In livers, kidneys, cerebral cortices, and olfactory bulbs from both sexes, UPF3B is the dominant UPF3 paralogue. Intriguingly, a higher protein expression of UPF3A over UPF3B is found in ovaries (Fig. 2). Furthermore, there is no significant difference in the protein expression of UPF3A or UPF3B in all tissues between male and female mice (Fig. 3). Thus, at least four types of tissue-specific expression patterns related to protein levels of UPF3A and UPF3B exist in mice: (1) in spleens and lungs from both sexes and ovaries from adult females, UPF3A is the major UPF3 paralogue (*UPF3A*^{high}); (2) in livers, kidneys, cerebral cortices, and olfactory bulbs

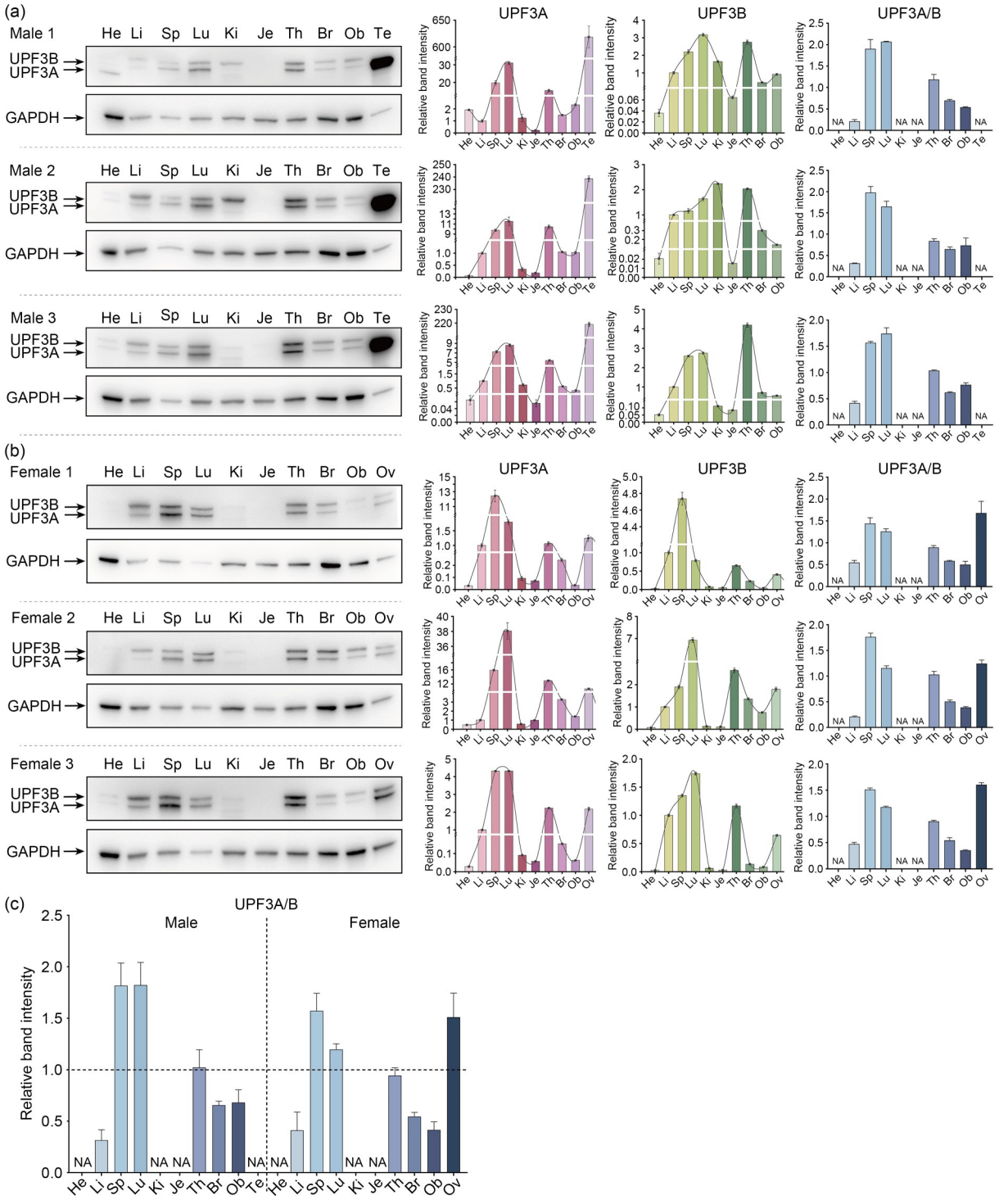

Fig. 2 Expression of UPF3A and UPF3B proteins in tissues from C57BL/6 mice determined by western blotting. GAPDH was used as the loading control. (a) Male mice; (b) Female mice; (c) Relative protein expression of UPF3A/B. The results are represented as mean±standard deviation (SD), *n*=3. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; NA: not applicable; He: heart; Li: liver; Sp: spleen; Lu: lung; Ki: kidney; Je: jejunum; Th: thymus; Br: brain; Ob: olfactory bulb; Te: testis; Ov: ovary.

from both sexes, UPF3B is the dominant form of UPF3 paralogue (*UPF3B*^{high}); (3) in the thymus of both sexes, UPF3A and UPF3B are comparable in protein expression (*UPF3A/B*^{equal}); and (4) hearts and small intestines

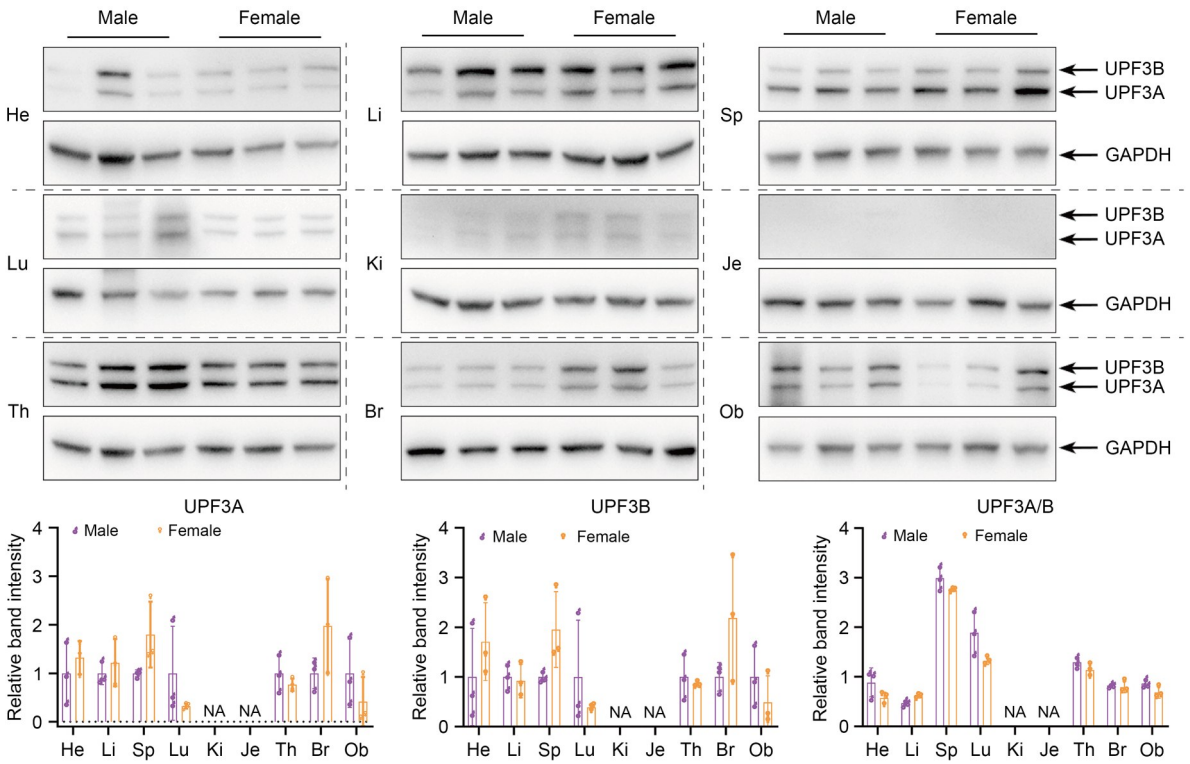

Fig. 3 Comparison of UPF3A and UPF3B proteins in tissues from male and female mice. GAPDH was used as the loading control. The results are represented as mean±standard deviation (SD), *n*=3. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Li: liver; He: heart; Sp: spleen; Lu: lung; Ki: kidney; Je: jejunum; Th: thymus; Br: brain; Ob: olfactory bulb; NA: not applicable.

from both sexes express the least amount of UPF3A and UPF3B (*UPF3A*/*B*^{null}). The biological mechanism of tissue-specific expression of UPF3A and UPF3B is worthy of further detailed characterization. Previous findings suggest that UPF3B competes over UPF3A when binding to UPF2, and thus UPF3A is destabilized and degraded when UPF3B is expressed (Chan et al., 2009; Shum et al., 2016). Our findings suggested that under physiological conditions, UPF3A and UPF3B do not compete or destabilize each other.

In order to further substantiate our unexpected finding that UPF3A, similar to UPF3B, is abundantly expressed in mouse tissues, we retrieved the *Upf3a* and *Upf3b* expression data from the mouse Encyclopedia of DNA Elements (ENCODE) transcriptome and used reads per kilobase per million mapped reads (RPKM) data to reveal the relative mRNA expression of *Upf3a* and *Upf3b* in different tissues. The mouse ENCODE transcriptome dataset further confirmed that *Upf3a* mRNA is highly expressed in the testis. Furthermore, the RPKM values of *Upf3a* are higher than those of *Upf3b* in all tissues investigated (Fig. 4a). The Human Protein Atlas (HPA) RNA sequencing (RNA-seq) data from normal human tissues reveal that *UPF3A* mRNA is the highest in the human testis (Fig. 4b), implying that the expression of UPF3A is conservative. Furthermore, *UPF3A* expression is not lower than *UPF3B* expression in most human or mouse tissues, suggesting that UPF3A is abundantly expressed in humans (Fig. 4). However, the precise protein expression of UPF3A and UPF3B needs to be investigated with human cell and tissue samples.

In summary, in the current study, we characterized the mRNA and protein expression of UPF3A and UPF3B in ten tissues from adult male and female mice. Our data confirm that UPF3B proteins are well represented in most tissues from adult male and female mice. However, the current study reveals that UPF3A proteins are evidently expressed in most murine organs. Furthermore, our data showed that UPF3A and UPF3B proteins are abundantly expressed in murine tissues with distinct patterns. Since UPF3B is a well-recognized weak NMD activator, UPF3A could positively and weakly regulate NMD in mammals (Chen et al., 2023). In addition, the broad expression patterns of UPF3A among mouse tissues indicate that, in

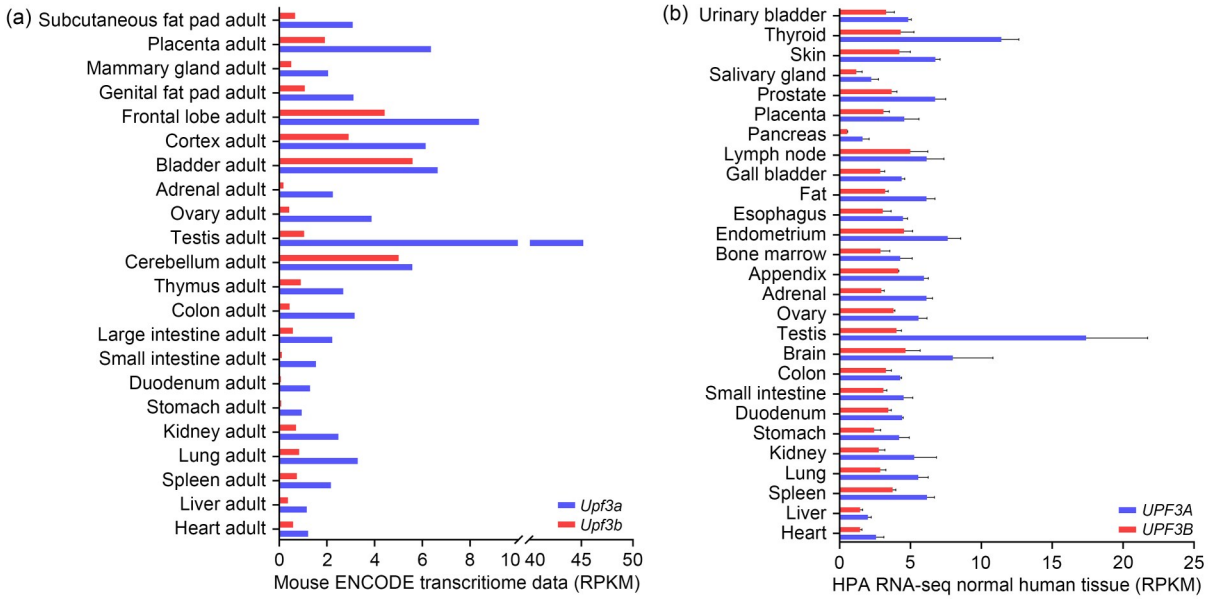

Fig. 4 *Upf3a* and *Upf3b* mRNA levels in different mouse and human organs. ENCODE transcriptome data of adult mouse tissues (a) and HPA RNA-seq data of normal human tissues (b) were retrieved from the NCBI website. The results of (b) are represented as mean±standard deviation (SD) (for each tissue type, 2–7 biological samples were used for the mRNA expression quantification with RNA-seq). mRNA: messenger RNA; ENCODE: Encyclopedia of DNA Elements; HPA: Human Protein Atlas; RNA-seq: RNA sequencing; NCBI: National Center for Biotechnology Information; RPKM: reads per kilobase per million mapped reads.

addition to NMD, UPF3A may actively participate in other NMD-independent mechanisms, such as epigenetic regulation in genetic compensation, to maintain tissue homeostasis (Ma et al., 2019). Since UPF3A and UPF3B are implicated in human development and diseases, such as neurodevelopmental disorders (Tarpey et al., 2007; Nguyen et al., 2012, 2013; Marques et al., 2022), cancers (Michalak et al., 2020), and male infertility (Shum et al., 2016), our findings provide a gene expression framework for future mechanistic characterizations of the biology of UPF3 paralogues with mouse models.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

All datasets analyzed in this study are available from the corresponding author upon request.

Acknowledgments

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Author contributions

Tangliang LI designed the experiments and supervised the project. Xin MA performed the experiments and analyzed the data. Xin MA and Tangliang LI wrote the manuscript, which was edited by Yan LI, Chengyan CHEN, Yanmin SHEN, and Hua WANG. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Xin MA, Yan LI, Chengyan CHEN, Yanmin SHEN, Hua WANG, and Tangliang LI declare that they have no conflicts of interest.

All mice had free access to water and food. All animal care and experiments were performed according to the guidelines of the animal ethics committee in Shandong University, Qingdao, China (license number: SYDWLL-2022-083).

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Supplementary information

Materials and methods