



Short Communication

Comprehensive mapping of primary cilia dynamics throughout mouse development and aging

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Primary cilia are microtubule-based organelles involved in a variety of fundamental biological processes, including cell cycle regulation, cell polarity, and signal transduction, which are crucial for proper tissue formation and function [1,2]. The characteristics of primary cilia, such as the percentage of ciliated cells, cilia type transformation, and cilia length, are dynamic and tightly regulated during development and in response to signals [3,4]. Abnormalities in these features can contribute to disease mechanisms, such as defective signaling pathways in ciliopathies or altered cellular responses to stimuli in cancer [5]. Despite previous studies have characterized cilia distribution in specific tissues or organs, a comprehensive understanding of how cilia presence varies across different tissues and developmental stages remains incomplete [6,7]. To address this, we systematically map the dynamic distribution of primary cilia in mice from early embryogenesis through adulthood and aging.

A mouse can live up to two years, from embryonic stages (including implantation, gastrulation, and organogenesis) through postnatal stages (weaning, juvenile, adulthood, middle age, and old age). We selected several timepoints covering these critical developmental stages across major organs for investigation. Gastrulation, which begins at embryonic day 6.5 (E6.5), marks a pivotal phase of early embryonic development. During this stage, the three germinal layers—endoderm, mesoderm, and ectoderm—are established, forming the foundation for all organ systems [8]. To elucidate the dynamics of cilia distribution during this period, we selected four key time points (E6.5–E8.0) and conducted immunostaining on embryo sections. All animal experimental pro-

cedures conducted in this research were approved by the Institutional Animal Care and Use Committee (IACUC-20240918-01). Ciliary structures were visualized using markers *Arl13b* and γ -tubulin, which label the ciliary membrane and basal body, respectively. Additionally, *FoxA2*, *Brachyury*, and *Otx2* or *Sox2* were used to identify the endoderm, mesoderm, and ectoderm, respectively. Quantitative analysis of cilia frequency and length revealed that primary cilia were abundant in the mesoderm and ectoderm across the examined time points (E6.5–E8.0), with only slight variations in distribution (Fig. 1a, b, Fig. S1a online). In contrast, cilia were initially sparse in the endoderm at E6.5 and E7.0 but showed a marked increase to approximately 18%–25% between E7.5 and E8.0 (Fig. 1a, b, Fig. S1a online). This increase suggests that endodermal cilia play a critical role in cell proliferation and differentiation during later organogenesis. Across all layers, cilia were relatively short ($\sim 2 \mu\text{m}$) but uniform in length (Fig. S1b online).

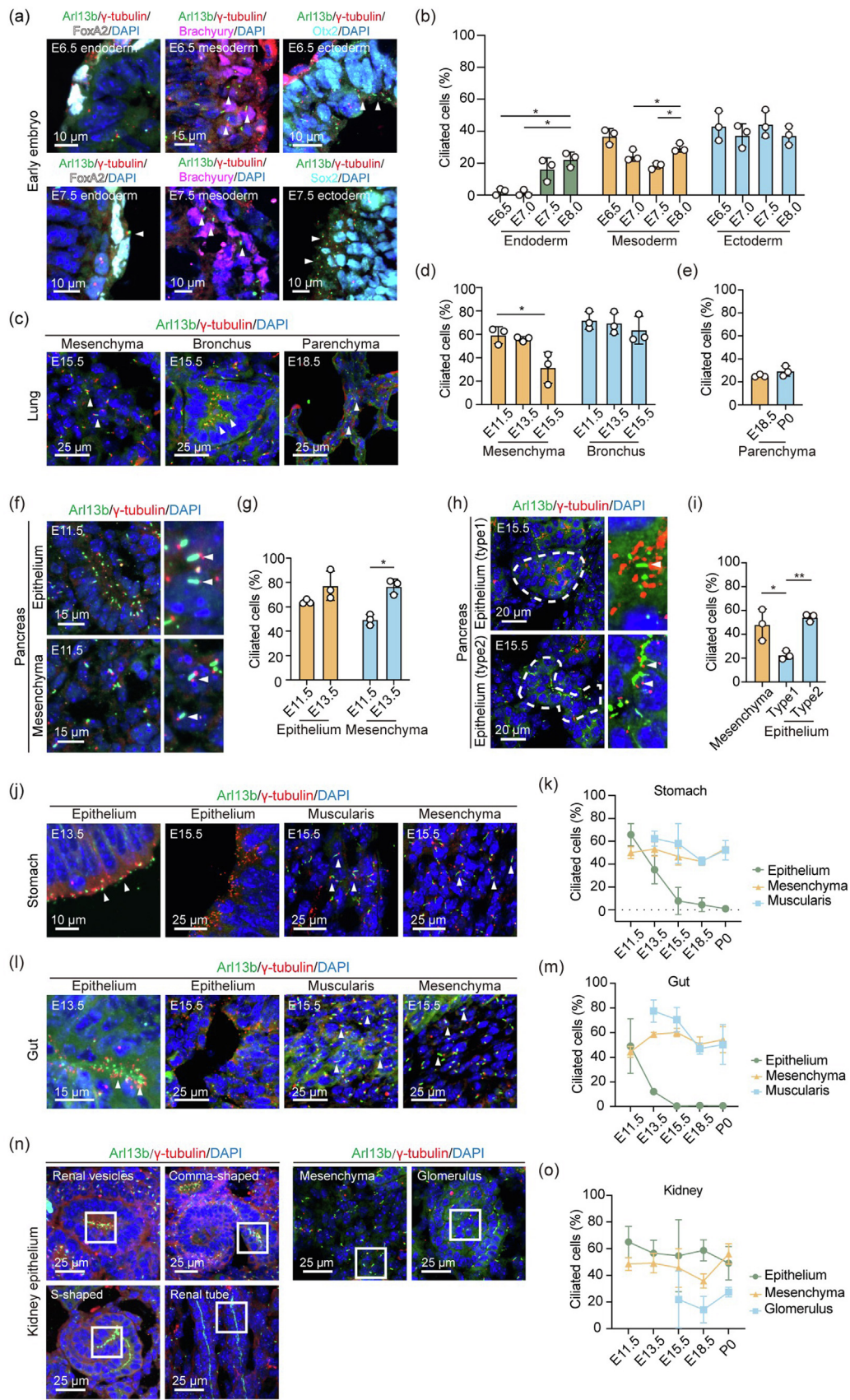
As development progresses to E8.5, organogenesis begins, marking a transition from the establishment of germinal layers to the differentiation and formation of specialized tissues and organs. At this stage, primary cilia were observed in tissues derived from all three germinal layers. For instance, cilia were detected in endoderm-derived structures (e.g., the developing gut), mesoderm-derived tissues (e.g., somites and the heart), and ectoderm-derived regions (e.g., the prospective brain) (Fig. S1c online). Additionally, extraembryonic structures like the allantois and amnion exhibited widespread ciliation, reflecting the ubiquitous role of primary cilia during early organogenesis (Fig. S1d online).

Tissue-specific ciliary patterns became more distinct as organogenesis advanced. By E10.5, lung airway structures emerge, surrounded by mesenchymal cells. From E11.5 to E15.5, immunofluorescence staining showed primary cilia in bronchial epithelial and mesenchymal cells (Fig. 1c, Fig. S2a online). While

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cilia in bronchial epithelial cells were polarized and extended into the airway lumen, mesenchymal cilia lacked a specific orientation. Over time, the proportion of ciliated mesenchymal cells decreased, and epithelial cilia became predominant (Fig. 1d). Cilia length in the lung during these stages is generally 1–2 μm , with mesenchymal cilia being longer than those in bronchial cells (Fig. S2b online). By E18.5, the lung had transitioned to a more mature state. The frequency of cilia in the parenchyma stabilized at approximately 30%, accompanied by an increase in cilia length (Fig. 1e, Fig. S2c, d online), which may reflect a shift in the sensory function of cilia as the lung matures from a developmental to a functional state.

Liver development followed a different trajectory. Beginning at E9.5 with the formation of the liver bud [9], the liver exhibited relatively low ciliation rates (2%–6%) throughout embryogenesis without significant changes in length (Fig. S3a–c online).

The pancreas bud originates at E9.5, undergoing differentiation into tip and trunk multipotent progenitor cells (MPCs) by E12.5 [10,11]. By E15.5, epithelial cells were classified into type 1 (low ciliation, rounded lumen) and type 2 (high ciliation, elongated lumen), the latter corresponding to duct progenitor cells. Both mesenchyma and epithelium possessed a high proportion of cilia during E11.5 and E15.5 (Fig. 1f–i, Fig. S4a–d online). At E18.5, mature pancreatic structures exhibited high ciliation in islet and duct cells, with cilia lengths of 3–5 μm , occasionally exceeding 13 μm , whereas acinar cells displayed consistently low ciliation (<5%) with shorter cilia (Fig. S4e–g online). The higher ciliation in duct cells may support epithelial morphogenesis and progenitor cell maintenance, enhancing signaling essential for pancreatic development.

The gastrointestinal tract develops through multiple layers, including the mucosa (epithelial cells), lamina propria (mesenchymal cells), muscularis, and serosa. From E11.5 to P0, primary cilia were broadly distributed, particularly in mesenchymal and muscle cells, while epithelial ciliation decreased after E13.5 (Fig. 1j–m, Fig. S5a, b online). Epithelial cells in the gastrointestinal tract exhibited a marked reduction in cilia after E13.5, which may be associated with the specialization of these cells for absorption and secretion as the tissue matures. Cilia length ranges from 1–3 μm , declining moderately during embryonic development (Fig. S5c, d online).

Kidney development progresses through renal vesicle, comma-shaped body, S-shaped body, and mature glomerulus stages [12]. From E11.5 to P0, our data captured different stages of renal body development, with mature glomeruli appearing around E15.5 (Fig. 1n). We identified tubular epithelial and mesenchymal cells with high ciliation, whereas renal bodies exhibited lower ciliation (Fig. 1n, o, Fig. S6a online). The length of primary cilia in tubular epithelium increased with kidney maturation, reaching up to 8 μm at birth (Fig. S6b online).

Postnatally, changes in ciliary dynamics varied across tissues. Recent studies show that mesenchymal cells are uniformly ciliated during early lung development, but this is restricted to PDGFR α + fibroblasts in the adult alveoli [13]. We observed that type 1 alveolar cells (AT1), type 2 alveolar cells (AT2) and mesenchymal cells possessed primary cilia (Fig. 2a). While the proportion of ciliated cells in AT1 and AT2 cells was low, the proportion of primary cilia in mesenchymal cells reached over 40% at P7 and declined notably to ~10% by 3 weeks (Fig. 2b–d, Fig. S7a–c online), with no significant change in cilia length throughout development and aging (Fig. S7d–f online). It has been reported that the lung mesenchymal niche is crucial for epithelial self-renewal and growth [14]. The observed decline suggests that primary cilia may play important roles in regulating cell self-renewal, particularly during early development.

In the postnatal liver, the tissue comprises cholangiocytes (bile duct cells) and non-cholangiocytes, including hepatocytes, Kupffer cells, hepatic stellate cells, and endothelial cells. Cholangiocytes display a high density of primary cilia (>50%), which are oriented toward the duct lumen. In contrast, non-cholangiocytes exhibit a much lower ciliation rate (~5%) (Fig. 2e–g). Cholangiocyte ciliation remains consistently high across the lifespan, with cilia lengths averaging 4–5 μm , significantly longer than the 2–3 μm observed on non-cholangiocytes (Fig. S8 online). This high and sustained ciliation in cholangiocytes may be essential for bile secretion and maintaining ductal architecture.

In the pancreas, primary cilia are retained in approximately 60% of islet and duct cells postnatally, with minimal variation across the lifespan. Acinar cells exhibit consistently low ciliation rates (<5%) (Fig. 2h–k). The cilia on islet and duct cells average 3–5 μm in length, with some exceeding 13 μm , while cilia on acinar cells remain significantly shorter at 2–3 μm (Fig. S9 online). Cilia in islet and duct cells likely play roles in regulating insulin secretion and ductal fluid transport, with longer cilia possibly involved in mechanosensation and signaling.

In the gastrointestinal tract, the lamina propria remains one of the thickest layers postnatally. Vimentin staining identified lamina propria mesenchymal cells, which exhibited consistently high ciliation, while epithelial cells displayed low ciliation throughout life. The ciliary frequency in stomach mesenchymal cells decreased from ~40% at P7 to ~15% by 3 weeks of age, stabilizing thereafter (Fig. 2l, m). Epithelial ciliation remained extremely low (<5%) throughout life (Fig. 2n). Primary cilia length remained consistent across the lifespan, measuring 1–2 μm in both stomach epithelial and mesenchymal cells (Fig. S10a, b online). Similar patterns were observed in the small intestine and colon, where ciliary frequency declined from P7 to 3 weeks and stabilized thereafter, while cilia length remained steady (Fig. S10c–l online). Infant mice are weaned at 3 weeks when the gastrointestinal tract begins to acquire metabolic functions. The decline in mesenchymal cilia frequency and stable cilia length may reflect their shifting toward

Fig. 1. Characteristics of cilia in embryonic stages. (a) Immunofluorescence staining of primary cilia in embryos from E6.5 and E7.5. Arl13b labels cilia and γ -tubulin labels the basal body. FoxA2 marks endoderm, Brachyury marks mesoderm, and Otx2 or Sox2 marks ectoderm, respectively. White arrows indicate primary cilia. (b) Quantification of the proportion of primary cilia across germinal layers during E6.5 to E8.0. (c) Immunofluorescence staining of primary cilia in mesenchymal and bronchial cells of the lung at E15.5, and parenchymal cells of the lung at E18.5. (d, e) The proportion of primary cilia in mesenchymal and bronchial cells from E11.5 to E15.5 (d), and in parenchymal cells from E18.5 to P0 (e). (f) Immunofluorescence staining of primary cilia in the pancreatic primordium at E11.5. (g) The proportion of primary cilia in mesenchymal and epithelial cells from E11.5 to E13.5. (h) Immunofluorescence staining of primary cilia in the pancreas at E15.5, showing two distinct epithelial cell types (indicated by the white dotted line). (i) The proportion of primary cilia in mesenchymal and two types of epithelial cells at E15.5. (j) Immunofluorescence staining of primary cilia in different layers of the stomach, including epithelial, mesenchymal, and muscle cells. (k) The proportion of primary cilia in the stomach from E11.5 to P0. (l) Immunofluorescence staining of primary cilia in different layers of the gut, including epithelial, mesenchymal, and muscle cells. (m) The proportion of primary cilia in the gut from E11.5 to P0. (n) Immunofluorescence staining of primary cilia in the kidney across different developmental stages. Renal epithelial and mesenchymal cells are analyzed, with mature glomeruli appearing post-E15.5. (o) The proportion of primary cilia in the kidney from E11.5 to P0. Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using two-way ANOVA with Tukey's test (b, d, g), one-way ANOVA with Tukey's multiple comparisons test (i) or unpaired two-tailed Student's *t*-test (e), using three mice per group.

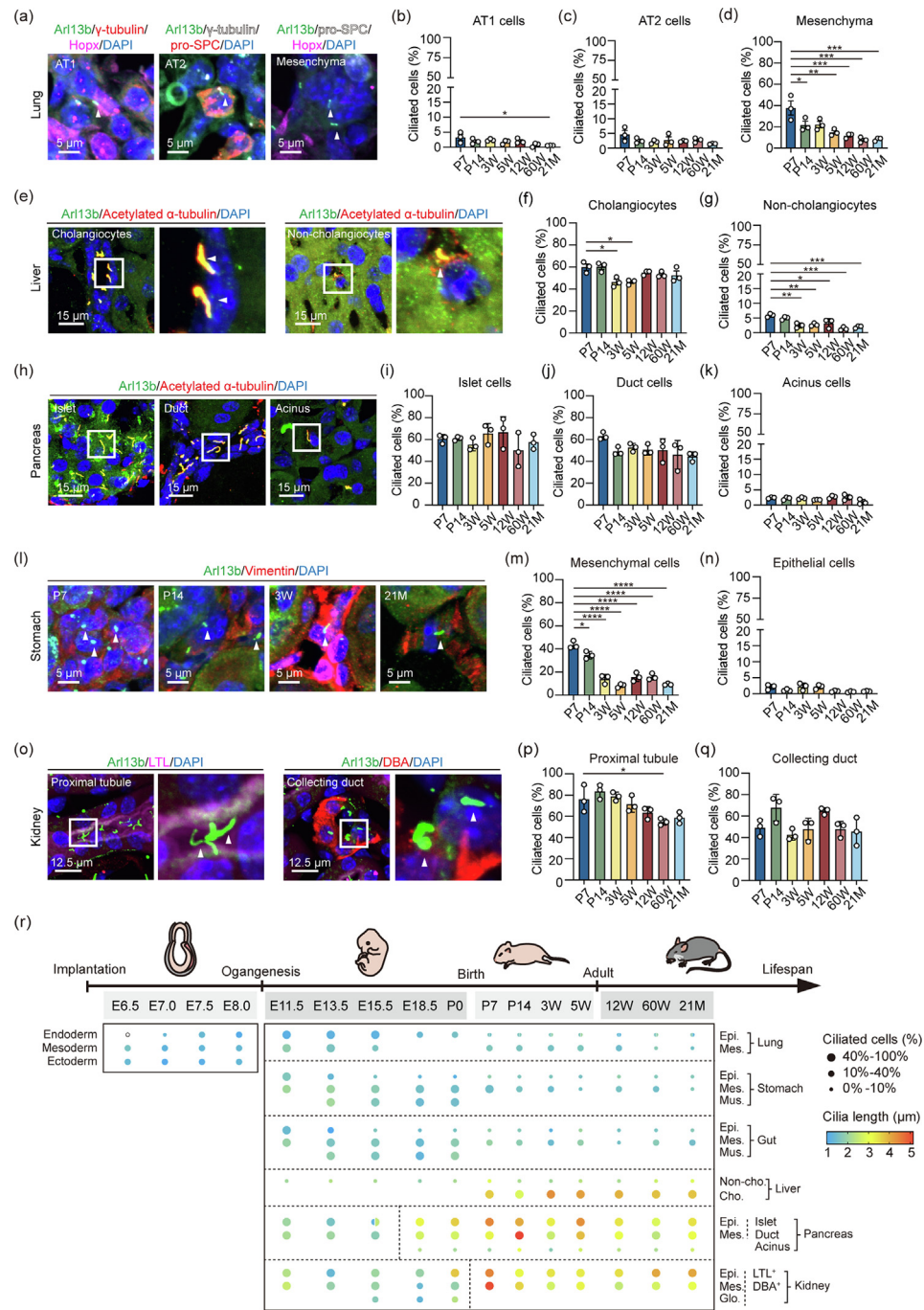


Fig. 2. Characteristics of cilia after birth. (a) Immunofluorescence staining of primary cilia in lung AT1 cells, AT2 cells, and mesenchymal cells (Hopx⁺pro-SPC⁺) at P7. Arl13b labels cilia and γ -tubulin labels the basal body, Hopx labels AT1 cells, pro-SPC labels AT2 cells. White arrows indicate cilia. (b–d) Temporal changes in ciliary proportion from P7 to 21 months in the lung AT1 cells (b), AT2 cells (c), and mesenchymal cells (Hopx⁺pro-SPC⁺) (d). (e) Immunofluorescence staining of primary cilia in cholangiocytes and non-cholangiocytes in P14 liver sections. Arl13b labels cilia and acetylated- α -tubulin labels the ciliary axoneme. White arrows indicate primary cilia. (f, g) The proportion of primary cilia in cholangiocytes (f) and non-cholangiocytes (g) throughout development, from P7 to 21 months. (h) Immunofluorescence staining of cilia in islet, duct, and acinar cells in a 12-week-old mouse. (i–k) Temporal changes in ciliary proportion from P7 to 21 months in islet cells (i), duct cells (j), and acinus cells (k). (l) Immunofluorescence staining of primary cilia in stomach lamina propria mesenchymal cells. (m, n) The proportion of primary cilia in the stomach mesenchymal (m) and epithelial cells (n) over the lifespan, from P7 to 21 months. (o) Immunofluorescence staining of primary cilia in a 5-week-old mouse kidney, showing proximal tubules (LTL⁺) and collecting ducts (DBA⁺). (p, q) Changes of cilia proportion in renal proximal tubules (p) and collecting ducts (q) from P7 to 21 months. (r) Summary diagram of cilia dynamics throughout the mouse lifespan from embryos to aged. Dot color gradient shows the scaled mean cilia length from short (blue) to long (red). Dot sizes show the percentage of ciliated cells in different tissues. The two semi-circular dots on the left and right, which represent lung epithelial cells after birth, correspond to AT1 and AT2. As for the two semi-circular dots of pancreatic epithelial cells at E15.5, they represent type 1 and type 2 epithelial cells, respectively. Data are presented as mean $\pm$ SD. Statistical analyses were performed using one-way ANOVA with Tukey's multiple comparisons test, using three mice per group unless otherwise stated.

regulating intestinal peristalsis and digestion as the gastrointestinal tract matures.

After birth, the kidney's structure differentiates into the outer cortex (containing glomeruli and renal tubules) and the inner

medulla (composed of collecting ducts). Using Lotus tetragonolobus lectin (LTL) and Dolichos biflorus agglutinin (DBA) markers, we found that primary cilia were abundant in proximal tubules and collecting ducts but rare in glomerular cells (Fig. 2o,

Fig. S11a online). The frequency of primary cilia in proximal tubules decreased slightly from ~80% in young mice to ~60% in older mice, while it remained stable in collecting ducts (Fig. 2p, q). With aging, primary cilia length decreased in both proximal tubules and collecting ducts (Fig. S11b, c online).

In summary, our comprehensive mapping of primary cilia across various murine tissues at ten embryonic time points (E6.5–P0) and seven postnatal time points (P7–21 months) reveals several key patterns (Fig. 2p, Fig. S12 online). First, tissue-specific ciliary dynamics were observed, with mesenchymal and epithelial cells exhibiting distinct ciliation patterns throughout development. Second, ciliary frequency and length varied in response to developmental cues: certain tissues displayed increased ciliation during organogenesis, while others showed a decline as cells specialized for particular functions. Notably, ciliary length often correlated with the functional maturation of organs, suggesting a shift in ciliary roles over time. For example, in the kidney, longer cilia in proximal tubules and collecting ducts are essential for mechanosensation and fluid homeostasis, while in pancreatic islets, extended cilia may facilitate insulin secretion [15]. Postnatally, ciliary dynamics stabilized in many tissues, while mesenchymal cells exhibited age-dependent reductions in ciliation. This decline may reflect changes in tissue remodeling and homeostasis, particularly in organs such as the lung and gastrointestinal tract, where mesenchymal-epithelial interactions regulate regeneration and function. In contrast, tissues that retain high ciliation throughout life, such as cholangiocytes and pancreatic ductal cells, likely depend on primary cilia for sustained fluid regulation and mechanosensation. Recent studies have shown that primary cilia are dynamically regulated in response to developmental and environmental cues, with their resorption or elongation affecting key signaling pathways such as Hedgehog, Wnt, and mTOR [16]. Given the strong correlation between ciliary characteristics and tissue function, further exploration of cilia-mediated signaling pathways may provide critical insights into disease mechanisms and therapeutic strategies. Our findings lay the groundwork for future studies on how ciliary alterations contribute to tissue dysfunction and age-related diseases, offering potential translational applications in regenerative medicine and targeted therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

Zhouyuanjing Shi carried out the experiments, analyzed the data, and wrote the manuscript. Zhangqi Xu and Xiyang Chen helped with the experiments. Hao Jin and Feng Yang provided guidance on the experiments. Chunxiao Huo and Zhigang Liu helped edit the manuscript. Shanshan Xie, Tianhua Zhou, and Liangjing Wang supervised the project and designed experiments. All authors reviewed the paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scib.2025.04.031>.

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