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Supplementary information

Universal transcriptomic hallmarks of mammalian ageing and mortality

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Supplementary Information for

Universal Transcriptomic Hallmarks of Mammalian Aging and Mortality

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Supplementary Discussion

A central motivation of this study was to bridge three properties that are often only partially met by existing aging biomarkers: broad generalizability across organs and species, mechanistic interpretability of observed effects, and more direct reflection of health status and burden of damage. By constructing transcriptomic clocks of expected mortality across >11,000 samples from mouse, rat, macaque and human, we provide a unified framework that integrates age-associated transcriptional change with the direction and magnitude of lifespan modulation by interventions, enabling a quantitative and biologically interpretable readout of health status. While chronological clocks captured many detrimental conditions (e.g., chronic diseases or certain short-lived genetic models), they were comparatively less responsive to lifespan-extending interventions, consistent with reports that longevity treatments often only modestly reverse age-associated transcriptomic signatures[1,2]. In contrast, mortality clocks robustly distinguished both short- and long-lived models and showed strong associations with human time-to-death, approaching the predictive performance of second-generation DNAm mortality clocks while remaining biologically interpretable.

These results help position transcriptomic clocks within the broader biomarker landscape. Epigenetic clocks, from early pan-tissue models[3] to pan-mammalian clocks[4], provide high accuracy and broad applicability across tissues and species, establishing a foundational framework for quantifying biological age. Proteomic clocks, particularly plasma-based models, capture systemic and organ-level dysfunction with features that are often biologically interpretable[5,6]. Transcriptomic clocks combine advantages of both: they generalize across tissues, species, and cell types, while remaining based on functionally annotated genes and enabling module-specific readouts that reflect the state of individual cellular subsystems. This combination makes

transcriptomic clocks useful both as standalone biomarkers and as a complement to epigenetic and proteomic measures, especially when interpretability and pathway resolution are priorities.

Our results also support and extend DNAm-based observations that heterochronic parabiosis in old animals and early embryogenesis can induce molecular age deceleration[7–9]. During embryogenesis, transcriptomic age showed a pronounced U-shaped trajectory with a minimum around ~E10, consistent with a developmental "ground zero" hypothesis[10]. Lineage reconstruction in single-cell data suggested that this rejuvenation is largely restricted to intraembryonic lineages, whereas extraembryonic endoderm did not show comparable transcriptomic rejuvenation, consistent with elevated eAge in extraembryonic tissues at similar stages[7]. In old heterochronic parabionts, mortality clocks detected a sustained tAge reduction that persisted for 2 months after detachment from young partners. Together, these findings support the view that early development contains a conserved rejuvenation-like phase and that systemic environment can partially reset molecular age later in life.

A major conceptual outcome of this work is that transcriptomic signatures of aging and mortality have a modular organization. Modules capturing innate immunity/inflammation, interferon signaling, mitochondrial translation/oxidative phosphorylation, chromatin organization, ECM/EMT, and other processes jointly explain much of the mortality-associated variation and enable accurate module-specific clocks. These module clocks responded to interventions in ways that align with known biology: CR primarily affected mitochondrial and metabolic modules; LPS acutely accelerated immune modules; *Klotho* deficiency preferentially perturbed mitochondrial and Nrf2-related programs; and chronic diseases showed broad pro-mortality shifts dominated by inflammation-related modules. This suggests that organismal aging is not a single trajectory but a composite of partially dissociable pathway-level processes, each of which can be selectively accelerated or decelerated by distinct perturbations. It also helps explain why some interventions elicit strong effects in particular subsystems while leaving others relatively unchanged, and why composite biomarkers can mask mechanistically informative pathway-level heterogeneity.

At single-cell resolution, most mammalian cell types, including stem cell populations, showed pro-aging and pro-mortality shifts with age. These shifts converged on upregulation of genes associated with senescence, inflammation, and apoptosis and downregulation of regenerative and ECM-related factors, suggesting broad conservation of damage-associated transcriptional hallmarks across cell types. These trends likely reflect a combination of cell-

intrinsic changes and responses to shared environmental cues in aged organisms; heterochronic parabiosis provides a direct demonstration that systemic environment can modulate molecular age. The observation that clocks trained on bulk tissue detect aging-associated changes at the cell-type level further supports the presence of conserved, cross-scale transcriptional programs that are not solely driven by shifts in cell composition.

Transcriptomic mortality signatures were also consistent across *in vivo* and *in vitro* damage models. Replicative senescence in primary fibroblasts and WI-38 cells, γ-irradiation in rodent fibroblasts, and metabolic stress all accelerated tAge, indicating that transcriptomic clocks respond to damage-associated states at both cellular and tissue levels. This is consistent with the deleteriome concept[11], in which diverse molecular lesions converge on shared deterioration-associated phenotypes. Similarly, age-deceleration contexts, including heterochronic parabiosis, CR, and early embryogenesis, showed coordinated anti-mortality shifts, suggesting partially shared damage-suppressive transcriptional signatures. Together, these patterns argue that transcriptomic clocks capture a component of biological aging that is tightly linked to physiological state and can respond to both acute stress and sustained systemic interventions.

Across most examined models, a compact set of genes repeatedly emerged as robust pro-mortality biomarkers, including *Gpnmb* and *Lgals3* (inflammation and fibrosis modulators elevated in multiple pathologies[12–17]) as well as *Cdkn1a*/*p21* (a canonical marker of DNA damage and senescence[18,19]). These genes were consistently upregulated in aging, chronic diseases, and cellular stress models and downregulated in age-deceleration contexts. Conversely, *Nrep* implicated in wound healing and scar formation[20] was downregulated with age, negatively associated with mortality, and induced by certain age-deceleration models (e.g., heterochronic parabiosis). Plasma levels of GPNMB, CDKN1A, and LGALS3 were positively associated with human mortality and multiple age-related diseases, extending these markers across molecular layers and supporting their relevance to organismal health status.

Despite broad convergence on inflammation, stress, and metabolic decline, individual interventions can elicit divergent responses across subsystems. Thus, *Klotho* knockout accelerates molecular age in most kidney and brain cell types but not microglia, and HCC exhibits a youth-associated tAge reduction in the ECM/EMT module while showing pro-aging features in inflammatory and metabolic modules, consistent with tumor-associated dedifferentiation and ECM remodeling along with inflammation and metabolic dysregulation[21,22]. These examples

emphasize that organismal aging and mortality reflect a heterogeneous yet interconnected landscape of cell-type- and pathway-specific trajectories, and that a given intervention may yield both pro- and anti-aging changes across subsystems.

Our results also clarify how transcriptomic mortality biomarkers relate to epigenetic aging. In human blood, transcriptomic mortality clocks and multiple DNAm clocks showed concordant deviations after adjustment for age and sex, with the strongest correlations observed for the transcriptomic module linked to chromatin modification, consistent with mechanistic interplay between chromatin state and DNA methylation[23]. At the same time, γ-irradiation and hTERT overexpression had minimal effects on DNAm age[24] but strongly altered tAge, supporting a model in which DNAm integrates slower, more cumulative (and partly stochastic) changes, whereas gene expression captures more dynamic functional states and acute damage or repair responses that may influence the epigenome via chromatin-modifying pathways.

Our study has several limitations. First, clocks and signatures are correlative and likely reflect a mixture of causal drivers, downstream consequences, and compensatory responses. Integrating perturbation data and causal inference approaches[25] will be essential to disentangle these components. Second, associations between plasma protein concentrations and health outcomes in UK Biobank are observational and may be influenced by co-morbidities, lifestyle, and socioeconomic confounders. Third, functional interpretation of co-regulated transcriptional modules relies on existing gene ontologies and will benefit from refinement through targeted perturbations and improved pathway annotation in specific tissues and cell types.

Despite these caveats, this work establishes an interpretable framework for quantifying molecular age and mortality risk across species, tissues, and cell types. By enabling both composite and module-level readouts, these universal transcriptomic biomarkers can help identify candidate aging-modulating interventions, dissect mechanisms of aging, disease, and rejuvenation, and inform development of therapies aimed at extending healthspan and lifespan. The accompanying TACO web application and *tAge* R package should facilitate broad adoption and further refinement of these tools by the community.

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Supplementary Table Legends

Supplementary Table 1. Datasets used in the study.

(A) RNA-seq of mouse livers derived from the ITP project. Each row corresponds to an individual animal.

(B) Aggregated mouse and rat gene expression meta-dataset. Each row corresponds to a single experimental group.

(C) Aggregated human and crab-eating macaque gene expression meta-dataset. Each row corresponds to a single experimental group.

Supplementary Table 2. Gene expression signatures of aging, expected mortality, and expected maximum lifespan.

(A-G) Liver-specific signatures estimated from the ITP cohort. Signatures were calculated using linear models with *edgeR* package.

(H-N) Multi-tissue signatures estimated from the aggregated rodent meta-dataset. Signatures were calculated using linear mixed-effects models with *metafor* package.

(O-R) Multi-tissue aging signatures estimated separately for each species. Signatures were calculated using linear mixed-effects models with *metafor* package.

Supplementary Table 3. Functional enrichment (GSEA) of gene expression signatures of aging, expected mortality, and maximum lifespan.

(A) Enrichment of liver-specific signatures estimated from the ITP cohort. KEGG, Reactome, and Hallmark ontologies were utilized.

(B) Enrichment of multi-tissue signatures estimated from the aggregated rodent meta-dataset. KEGG, Reactome, and Hallmark ontologies were utilized.

(C) Enrichment of species-specific multi-tissue aging signatures estimated separately for each species. KEGG, Reactome, and Hallmark ontologies were utilized.

Supplementary Table 4. Performance of mammalian transcriptomic clocks of aging and mortality.

(A) Characteristics, performance metrics, and number of non-zero features (for Elastic Net models) for composite tissue-specific and multi-tissue clocks of chronological age, normalized age, and expected mortality. Clock accuracy was evaluated on test set using a nested cross-validation framework (with 10 iterations). Median MAE, Pearson's r, and R^2 values across iterations are reported.

(B) Characteristics, performance metrics, and number of non-zero features for module-specific rodent multi-tissue clocks of chronological age and expected mortality. Accuracy was evaluated on test sets using leave-one-fold-out framework (with 10 folds).

(C) Characteristics, performance metrics, and number of non-zero features for module-specific multi-species multi-tissue clocks of chronological age and expected mortality. Accuracy was evaluated on test sets using leave-one-fold-out framework (with 10 folds).

Supplementary Table 5. Coefficients of Elastic Net transcriptomic clocks.

(A) Coefficients of composite tissue-specific and multi-tissue clocks of chronological age, normalized age, and expected mortality.

(B) Coefficients of module-specific rodent multi-tissue clocks of chronological age and expected mortality.

(C) Coefficients of module-specific multi-species multi-tissue clocks of chronological age and expected mortality.

Supplementary Table 6. Functional and upstream regulator annotation of co-regulated transcriptomic modules of aging and longevity.

(A) Functional and upstream regulator enrichment (Fisher's exact test) of rodent multi-tissue co-regulated gene modules.

(B) Gene set size and annotation dictionary of rodent multi-tissue modules, summarized by top representative functions.

(C) Functional and upstream regulator enrichment of multi-species multi-tissue co-regulated gene modules.

(D) Gene set size and annotation dictionary of multi-species multi-tissue modules, summarized by top representative functions.

Supplementary Table 7. GSEA-based pathway and module enrichment of gene expression signatures from biological models of transcriptomic age acceleration or deceleration. Analyses were performed using KEGG, Hallmark, Reactome, and Module gene set ontologies.

(A) Signatures induced by caloric restriction (CR) in mouse liver and by lipopolysaccharide (LPS) injection in mouse brain.

(B) Signatures induced by *Klotho* knockout (KO) in mouse kidney and skeletal muscle.

(C) Signatures induced by γ-irradiation in mouse and NMR fibroblasts (embryonic and primary skin fibroblasts).

(D) Signatures induced by cellular reprogramming, oligomycin treatment, and 2-deoxyglucose (2-DG) in primary human fibroblasts.

(E) Signatures associated with rodent models of aging-related diseases.

(F) Signatures induced by heterochronic parabiosis in livers from young and old animals.

(G) Signatures associated with early (up to E10) and late (after E10) stages of mouse embryogenesis.

Supplementary Table 8. Associations between plasma concentrations of GPNMB, CDKN1A, and LGALS3, and health outcomes in the UK Biobank proteomics database. Only outcomes with at least 150 recorded incidences among patients with available proteomic measurements were included in the analysis.

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