

RESEARCH ARTICLE

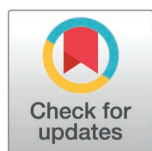
# Comprehensive profiling of circRNAs reveals stimulus-specific networks and core regulators of cellular senescence

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## Abstract

Cellular senescence, a hallmark of aging and age-related disorders, is characterized by irreversible cell cycle arrest and profound molecular alterations. Although previous transcriptomic studies have largely focused on protein-coding genes, the expression landscape of circular RNAs (circRNAs) during senescence remains poorly defined. Here, we systematically profiled circRNA expression across multiple senescence models using two human fibroblast lines (WI38 and IMR90) subjected to four distinct senescence-inducing stimuli: replicative senescence (RS), oncogene-induced senescence (OIS), doxorubicin-induced senescence (DOX), and ionizing radiation (IR). Through rigorous analysis, we identified 39,187 high-confidence circRNAs, classifying them into stimulus-specific (SS-circRNAs) and general senescence-associated circRNAs (GS-circRNAs). Among them, 24 GS-circRNAs exhibited conserved expression trends across different senescence models, and eight core circRNAs displayed consistent expression changes in both fibroblast lines, suggesting their potential as universal senescence biomarkers. Functional enrichment and co-expression network analyses revealed that SS-circRNAs participated in pathway-specific processes such as ribosome biogenesis, mitochondrial regulation, ubiquitin-mediated signaling, and RNA metabolism. Collectively, our findings provide a comprehensive atlas of circRNA dynamics across diverse senescence programs and identify candidate circRNAs that may serve as novel diagnostic or therapeutic targets for age-related diseases.

## Introduction

Cellular senescence, characterized by an irreversible arrest of cell growth, plays a crucial role in a wide range of physiological and pathological processes [1–3]. This phenomenon, initially described by Hayflick and Moorhead in 1961 [4], is marked by several key features, including constitutive DNA damage response (DDR) signaling,

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**Abbreviations:** RS: Replicative senescence; OIS: Oncogene-induced senescence; DOX: Doxorubicin-induced senescence; IR: Ionizing radiation-induced senescence; SS-circRNAs: Stimulus-specific senescence-associated circRNAs; GS-circRNAs: General senescence-associated circRNAs across stimuli

senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) activity, increased expression of cyclin-dependent kinase (CDK) inhibitors such as p16INK4A (CDKN2A) and p21CIP1 (CDKN1A), and the senescence-associated secretory phenotype (SASP), which involves the secretion of various bioactive factors [2,5–7]. In addition, the expression of the nuclear lamina protein LaminB1 (LMNB1) is typically reduced in senescent cells [8]. These markers have been widely recognized as universal indicators of cellular senescence.

However, the senescent phenotype is highly variable, and methods for clearly identifying senescent cells are still limited. Recent studies addressing this gap have sought to identify robust markers of senescence by analyzing diverse cell types and stimuli [9]. These analyses revealed a core set of 55 genes consistently linked to senescence, alongside 50 upregulated and 18 down-regulated RNAs common to all senescence models, highlighting both the complexity and heterogeneity of the senescent transcriptome [9,10]. Circular RNA (circRNA) is a class of non-coding RNAs formed by back-splicing of pre-mRNAs, resulting in a covalently closed structure [11]. In recent years, circRNAs have emerged as important regulators in various biological and physiological processes [12–19]. Their unique characteristics, such as high stability, conservation, and tissue specificity, make them promising biomarkers and therapeutic targets [20–24]. Notably, a variety of circRNAs have been found to play functional roles in organismal aging and aging-related diseases [25–28]. While changes in circRNA expression profile during cellular senescence are rarely reported, and the potential of circRNA molecules as features for the identification of senescent cells remains unexplored.

In the present study, we collected and analyzed the whole-transcriptome data from two fibroblast cell lines, WI38 and IMR90, subjected to four different senescence-inducing stimuli: replicative senescence (RS), oncogene-induced senescence (OIS), doxorubicin-induced senescence (Dox), and ionizing radiation-induced senescence (IR). We identified the general expression patterns of circRNAs across different types of cellular senescence and the specific expression profiles associated with certain senescence-inducing stimulus. Our findings provide valuable insights into the dynamic circRNA expression profiles of various senescence programs and contribute to the identification of circRNA signatures for senescent cells.

## Materials and methods

### Data collection

Raw RNA-seq data were obtained from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE130727 and GSE130306 [10,29]. The first dataset (GSE130727) comprises two human fibroblast cell lines, WI38 (lung fibroblasts) and IMR90 (foreskin fibroblasts), exposed to multiple senescence-inducing stimuli. Specifically, WI38 cells were analyzed under four senescence conditions: replicative senescence (RS), oncogene-induced senescence (OIS), doxorubicin-induced senescence (DOX), and ionizing radiation-induced senescence (IR). The DOX condition consisted of two independent batches (study 1 and study 2), both treated with 2  $\mu$ g/mL doxorubicin (Sigma) for 24h. IMR90 cells

were examined under two senescence conditions (RS and IR). For each condition, both young (proliferating) and senescent cells were included, with two biological replicates per group. The second dataset (GSE130306) consists of WI38 cells subjected to RS- and OIS-induced senescence. In the RS condition, three biological replicates were available for both young and senescent cells, whereas in the OIS condition, three biological replicates for young cells and two biological replicates for senescent cells were included.

The corresponding raw sequencing data were downloaded using the SRA Toolkit v2.10.8 (<https://github.com/ncbi/sra-tools/wiki/01.-Downloading-SRA-Toolkit>). All samples were processed using the same standardized bioinformatics pipeline to ensure data comparability across cell types and senescence models.

### Quality Control and Alignment of Sequencing Datasets

To ensure the reliability and reproducibility of downstream analyses, all raw sequencing reads were subjected to stringent quality control procedures. Adapter trimming and low-quality base removal were performed using fastp (v0.20.1) [30], and data quality was further assessed using FastQC v0.11.9 (<https://github.com/s-andrews/FastQC>) on all raw data. Clean reads were subsequently aligned to the human reference genome (hg19) using the HISAT2 (v2.1.0) aligner [31].

To quantify gene-level expression, aligned reads were processed using FeatureCounts from the Subread (v1.6.0) package [32]. Raw read counts were normalized to Transcripts Per Million (TPM) to account for sequencing depth and gene length. Only genes with TPM  $\geq 1$  in more than 50% of samples were retained for further analyses to ensure robust signal detection.

### Identification and quantification of circRNAs

High-confidence circRNA candidates were identified using the CIRCexplorer pipeline [33], which detects back-spliced junction reads derived from rRNA-depleted RNA-seq data. To minimize false positives, only circRNAs supported by at least two unique back-spliced reads and present in  $\geq 50\%$  of samples within each experimental condition were retained. The expression levels of circRNAs were quantified based on back-spliced junction reads and normalized using the “DESeqDataSetFromMatrix” function implemented in the DESeq2 R package [34]. To ensure cross-sample comparability, normalization was performed jointly across all datasets (WI38 and IMR90). The resulting circRNA expression matrix was used for subsequent analysis.

### Differential Expression analysis and combined analysis of Fibroblasts

To identify circRNAs consistently associated with cellular senescence, we first conducted differential expression analysis on each type of senescent WI38 cells independently using the DESeq2 package. Statistical significance was determined *a priori* at  $p$ -value of  $< 0.05$ . CircRNAs that were significantly up- or down-regulated in a specific senescence condition were designated as Stimulus-specific senescence-associated circRNAs (SS-circRNAs).

Given the inherent heterogeneity of cellular senescence across stimuli, we further conducted a combined analysis to identify circRNAs consistently regulated across all senescence programs of WI38 cells. Three independent methods were applied following the approach of Hernandez-Segura et al. [9]: (1) Negative binomial generalized linear model (GLM): implemented in DESeq2 with the design formula  $\sim$  senescence\_type + condition, where senescence\_type represented the four induction modes (RS, OIS, DOX, IR) and condition indicated senescent versus proliferating states. (2) Fisher  $p$  value combination and (3) Inverse Normal  $p$  value combination, both conducted using the MetaRNASeq R package (<https://cran.r-project.org/web/packages/metaRNASeq/index.html>).

Only circRNAs meeting the criteria of  $p < 0.05$  in the GLM and combined  $p < 0.05$  in both combination methods were retained as General Senescence-associated circRNAs (GS-circRNAs). These circRNAs were considered robust senescence-associated transcripts independent of stimulus type or cell context.

## Construction of Stimulus-Specific circRNA-Gene Co-expression Networks in Induced Senescence and functional enrichment analysis

To illustrate the function of stimulus-specific senescence-associated circRNAs (SS-circRNAs), we constructed specific circRNA-gene co-expression networks for each senescence model. P Pearson correlation coefficients ( $r$ ) between SS-circRNAs and differentially expressed genes (DEGs) expression levels were calculated using the R statistical environment. Thresholds Benjamini-Hochberg (BH)-corrected  $p$ -value of  $< 0.05$  were employed to obtain genes strongly co-expressed with SS-circRNAs.

Genes significantly co-expressed with SS-circRNAs were subjected to Gene Ontology (GO) function enrichment analysis using the clusterProfiler v4.15.1 package [35] and visualized using the 'ggplot2' package in R. Enriched GO terms and KEGG pathways were filtered at  $p < 0.01$ . For comprehensive pathway-level comparison across multiple senescence types, overlapping gene sets from the co-expression networks were further analyzed using the Metascape [36] (<https://metascape.org>). The co-expression networks plots were visualized using Cytoscape (v3.10.0) [37].

### Visualization

All plots and visual representations were generated using R (version 4.4.2) and related packages. Volcano plots were created using the "EnhancedVolcano" package, while intersection analyses of circRNAs and genes were visualized using "venn" and "UpSetR". Hierarchical clustering and heatmaps were generated with the "pheatmap" package based on z-score normalized expression values. The circRNA structural annotation was retrieved from the Circular RNA Interactome Database (CSCD) (<http://gb.whu.edu.cn/CSCD/>).

## Results

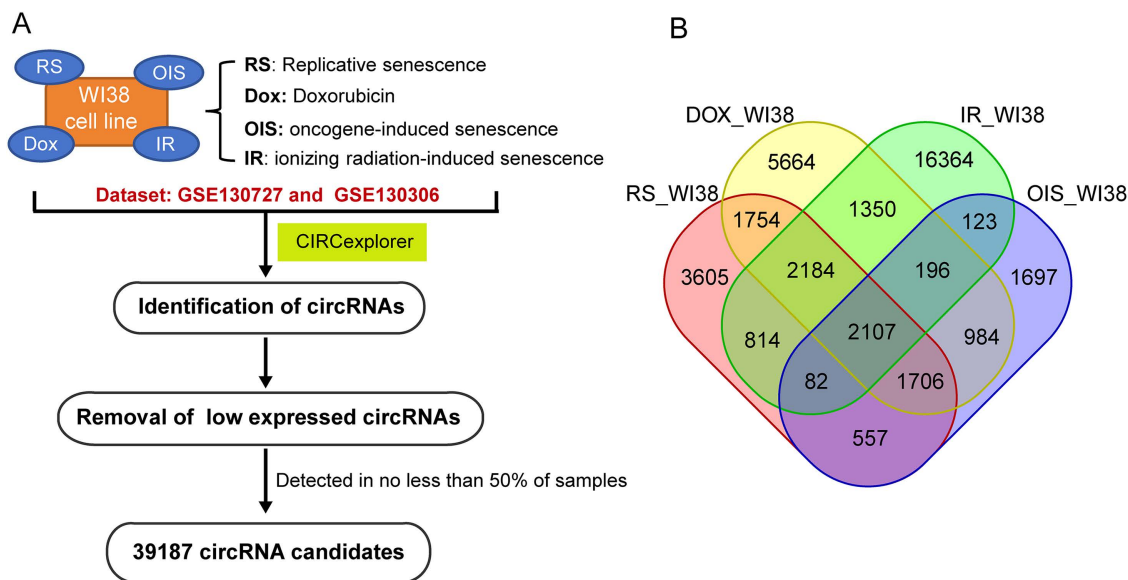
### Identification of circRNAs in senescent cells subjected to different senescence-inducing stimuli

Transcriptome signatures of cellular senescence, mainly protein-coding genes, have been characterized in prior studies. For a better understanding on regulation of cellular senescence from the perspective of circRNAs, we collected two rRNA-depleted RNAseq datasets of human fibroblast (WI38 cell line) subjected to different senescence-inducing stimuli, including replicative senescence (RS), doxorubicin-induced senescence (DOX), ionizing radiation-induced senescence (IR), and oncogene-induced senescence (OIS). We identified 39,187 circRNAs with high confidence in total (Fig 1A and S1 Table).

To explore the diversity of circRNAs expression across various senescence types, we compared the circRNAs expressed in different types of senescent WI38 cells. The results showed that some circRNAs specifically expressed in certain senescence program. To be specific, 3,605 circRNAs only expressed in replicative senescent cells, 1,697 circRNAs only expressed in oncogene-induced senescent cells, while 16,364 circRNAs in ionizing radiation-induced senescent cells and 5,664 circRNAs in doxorubicin-induced senescent process (Fig 1B). In addition to the distinctness, we found that 2,107 circRNAs expressed in all senescence types, which indicates these circRNAs might serve as fundamental role in cellular biological process.

### Heterogeneity and consistency of circRNA expression in diverse senescence types

To investigate the dynamic changes in circRNA expression profiles during fibroblast proliferation and senescence, we performed comprehensive differential expression analysis of circRNA transcripts. Given the molecular heterogeneity of cellular senescence across different stimuli, we first grouped the samples according to their specific senescence-inducing stimuli. The results revealed that circRNAs exhibited senescence-type-specific expression patterns during the transition from young to senescent cells, which we termed as Stimulus-specific senescence-associated circRNAs (SS-circRNAs). In replicative senescence, we identified 304 differentially expressed circRNAs,



**Fig 1. Identification of circRNAs in senescent fibroblast subjected to different stimuli.** (A) Schematic representation of the workflow in this study. (B) Comparison between circRNAs detected in distinct types of senescent WI38 cells. RS: Replicative senescence; DOX: Doxorubicin-induced senescence; OIS: oncogene-induced senescence; IR: ionizing radiation-induced senescence.

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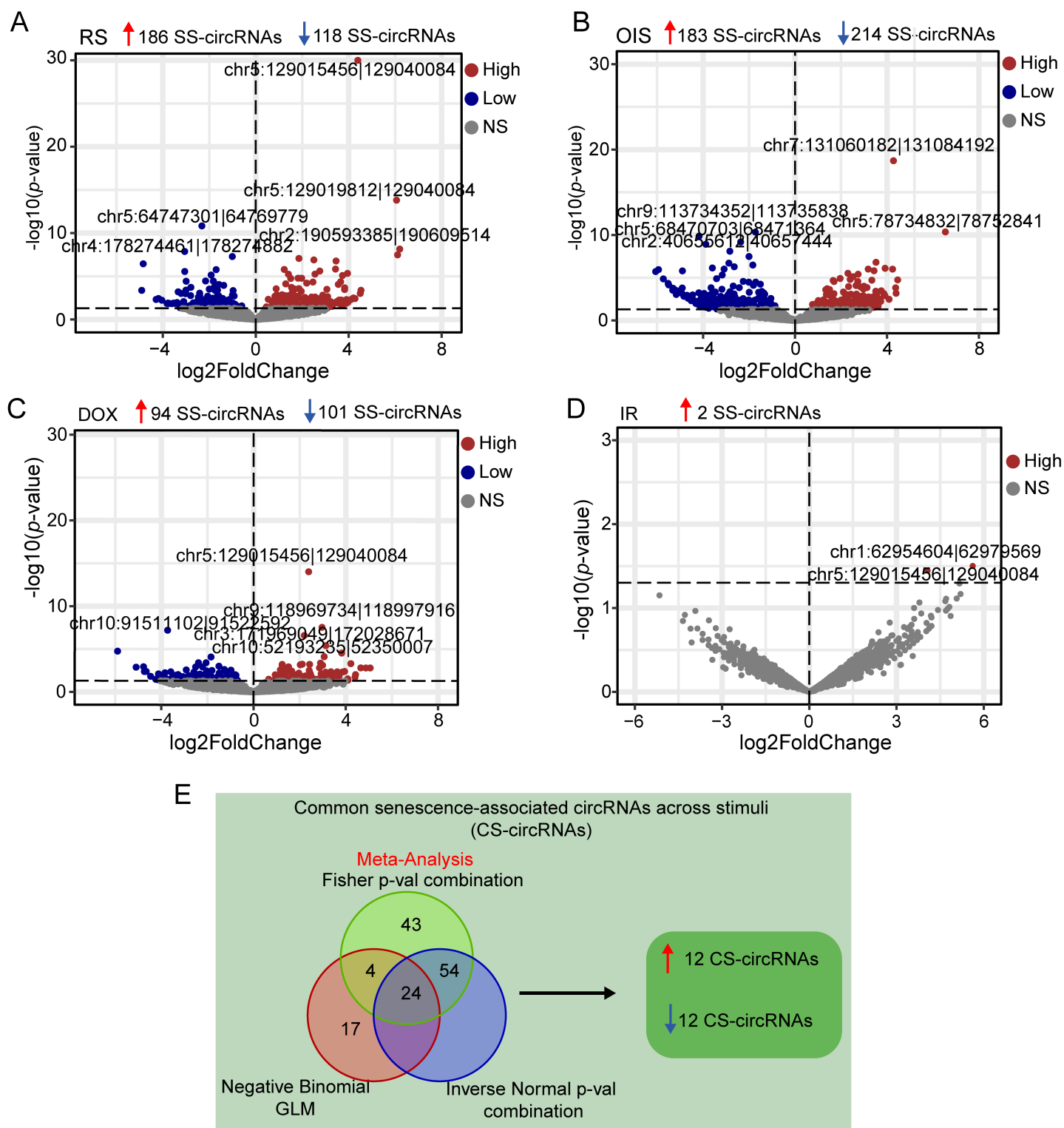
comprising 186 upregulated and 118 downregulated circRNAs during the aging process (Fig 2A). Regarding oncogene-induced senescence (OIS), 183 circRNAs demonstrated significant upregulation, while another 214 showed marked downregulation (Fig 2B). Analysis of doxorubicin-induced senescent cells revealed 94 significantly upregulated circRNAs, along with 101 significantly down-regulated circRNAs in senescent cells, respectively (Fig 2C). Notably, in the ionizing radiation-induced senescence (IR) dataset, only 2 circRNAs displayed differential expression between senescent and proliferating cells (Fig 2D), a phenomenon potentially attributable to inherent limitations within the dataset itself.

To delineate the core senescence signatures, we performed a combined analysis on sample groups subjected to diverse senescence stimuli. In the integrated analysis of three methods, senescence type was included as a covariate (see Methods). To minimize false positive discoveries, we implemented a statistical threshold of nominal  $p < 0.05$ , thereby retaining only those circRNAs demonstrating consistent differential expression across all three analytical approaches. Consequently, we identified 24 circRNAs exhibiting consistently and significantly differential expression patterns across various senescence types (adjusted  $p$ -value  $< 0.05$ ), which we designated as General Senescence-associated circRNAs across stimuli (GS-circRNAs) (Fig 2E and S2 Table). Among them, 12 GS-circRNAs were progressively upregulated while the other 12 were down-regulated throughout the senescence process. These findings suggest that these 24 circRNAs may serve as potential discriminative features for cellular senescence.

### SS-circRNAs are involved in regulating distinct functional pathways across various types of cellular senescence

Given the molecular heterogeneity among different types of cellular senescence, we sought to further elucidate the underlying functions of stimulus-specific circRNAs (SS-circRNAs) across distinct senescence programs. Based on the well-documented regulatory functions of circRNAs in previous studies, we constructed circRNA-gene (mRNA) co-expression network using Pearson correlation analysis. Our analysis revealed 61 SS-circRNAs exhibiting strong co-expression relationships (BH-adjusted  $p$ -value  $< 0.05$ ) with 315 genes in replicative senescence. Similarly, we identified





**Fig 2. Expression changes of circRNAs in WI38 cells undergoing different types of senescence.** Volcano plots depict circRNAs that are upregulated and downregulated during the aging process in replicative senescence (A), oncogene-induced senescence (B), doxycycline (dox)-induced senescence (C), and ionizing radiation-induced senescence (D). Red dots represent up-regulated circRNAs, and blue dots represent down-regulated circRNAs. A  $p$ -value threshold of  $< 0.05$  was set a priori to determine statistical significance. (E) general signature of senescent WI38 cells regardless of the stimulus. Combined analysis with three methods was performed for detecting general senescence-associated circRNAs across stimuli (see Methods for details).

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78 and 81 SS-circRNAs significantly co-expressed with 664 and 1750 genes in oncogene-induced senescence (OIS) and doxorubicin-induced senescence (DOX), respectively (Fig 3A and S3-S4 Table).

To further investigate the functional implications of these co-expression networks, we performed comprehensive functional enrichment analysis across the three senescence programs. Comparative analysis revealed that genes within the distinct circRNA-gene co-expression networks exhibited significant and program-specific enrichment in diverse biological pathways ( $p$ -value < 0.01) (S5 Table). Specifically, genes co-expressed with SS-circRNAs in replicative senescence were predominantly enriched in biological processes related to cytosolic ribosome function, cytoplasmic translation and DNA-binding transcription repressor activity (Fig 3B). While SS-circRNAs associated with oncogene-induced senescence (OIS) showed strong co-expression relationships with genes involved in mitochondrial function regulation, cytoskeleton organization, and protein localization regulation (Fig 3C). Notably, SS-circRNAs identified in doxorubicin-induced senescence (DOX) were primarily linked to genes regulating ribonucleoprotein complex biogenesis and RNA metabolic processes, including RNA splicing (Fig 3D).

Intriguingly, our analysis revealed that genes co-expressed with SS-circRNAs across all three senescence programs were consistently associated with fundamental regulatory functions, including developmental processes, regulation of biological processes, and cellular localization. Notably, SS-circRNAs specific to doxorubicin-induced senescence (DOX) demonstrated unique co-expression patterns with genes involved in homeostatic process and circadian rhythm regulation (Fig 3E). These findings suggest that senescence type-specific circRNAs may exert their functional roles in cellular aging through distinct physiological mechanisms and regulatory networks.

### Core senescence-associated circRNAs across diverse cell types and inducing stimuli

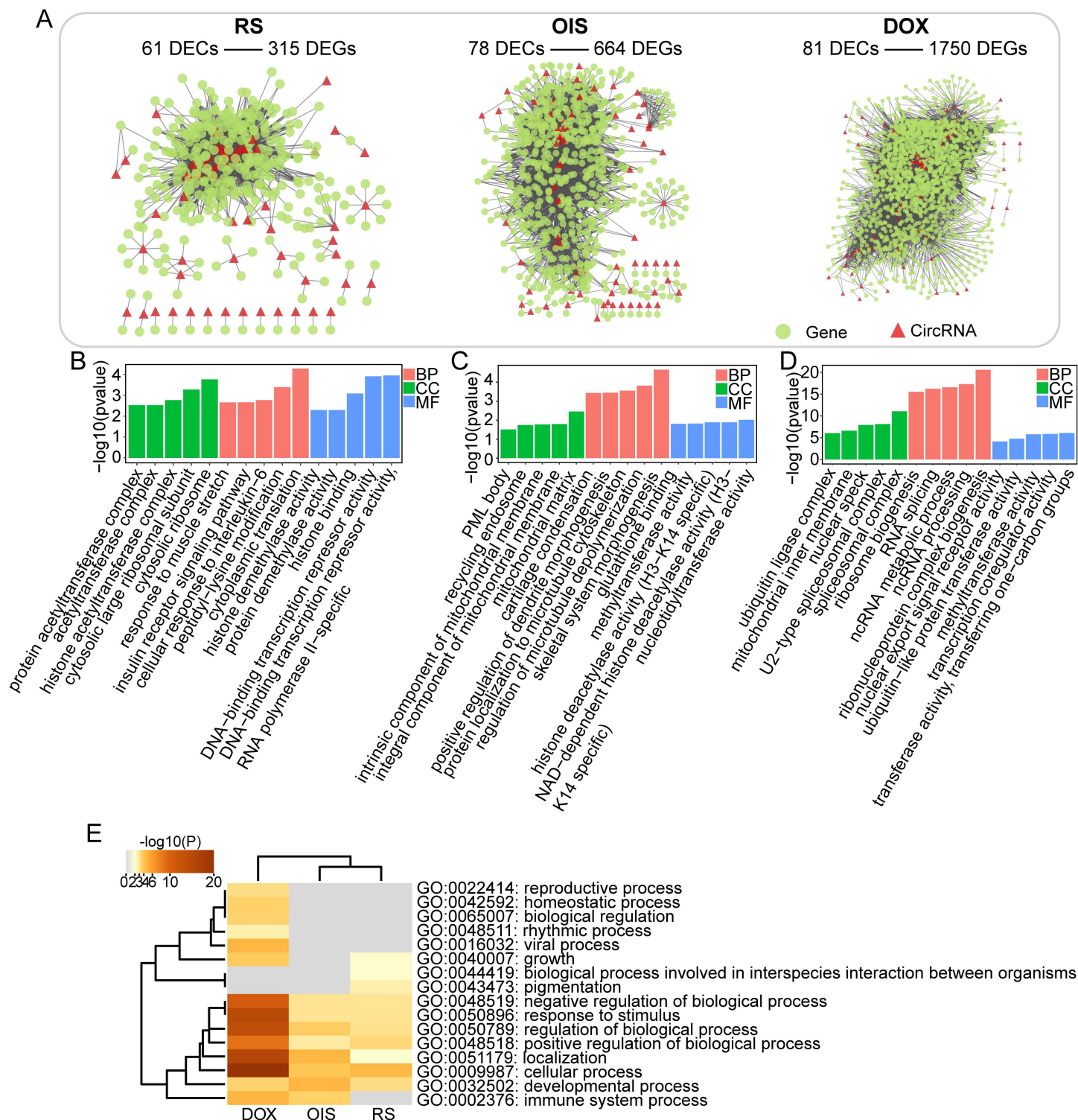
To determine whether the circRNA expression patterns were confined to the WI38 cell line or also existed in other cells, we analyzed circRNA expression of human fibroblast (IMR90) subjected to RS and IR. Subsequently, we refined and screened the specificity of SS-circRNAs in diverse cellular contexts and under different senescence-inducing conditions. Our analysis revealed that 12 circRNAs exhibited consistent and significant expression changes during replicative senescence across both cell lines, indicating their potential utility as universal markers for replicative senescence regardless of cell type. Additionally, our study uncovered stimulus-specific circRNA signatures, with 251, 382, and 125 unique circRNAs being identified in DOX-treated, OIS-induced, and IR-exposed senescent cells, respectively (Fig 4A and S6 Table).

While the identification of stimulus-specific circRNA signatures was an important aspect of our study, we placed particular emphasis on discovering core circRNA markers that maintain consistent expression patterns across different senescence contexts. We therefore conducted a comprehensive evaluation of the combined analysis-derived candidate GS-circRNAs, examining their expression patterns across multiple cell lines and senescence models. Through rigorous filtering criteria requiring consistent statistical significance in at least five independent analyses, we pinpointed six critical circRNAs (Fig 4B). The expression analysis revealed that four consistently downregulated circRNAs (hsa\_circ\_0000994, hsa\_circ\_0000591 and hsa\_circ\_0072688) in senescent WI38 and IMR90 cells. A particularly interesting finding was the cell line-dependent regulation of hsa\_circ\_0002111, which displayed opposing expression trends in WI38 (upregulation) and IMR90 (downregulation) cell lines during senescence (Fig 4B and 4C).

### Discussion

In this study, we systematically investigated the dynamic expression profiles of circRNAs during cellular senescence, revealing both heterogeneous and universal patterns across different senescence types and cell lines. Our findings underscore the potential of circRNAs as core signatures for cellular senescence, while also highlighting the complexity and heterogeneity of circRNA regulation in different senescence programs.

Our analysis identified a substantial number of Stimulus-specific senescence-associated circRNAs (SS-circRNAs) with distinct expression profiles in different senescence models. SS-circRNAs identified in our study further emphasize



**Fig 3. Functional analysis of stimulus-specific circRNAs (SS-circRNAs).** (A) Construction of circRNA-gene (mRNA) co-expression networks in three senescence types (RS, OIS and DOX). Genes exhibiting strong co-expression with circRNAs were identified using a Benjamini-Hochberg (BH)-adjusted  $p$ -value threshold of  $< 0.05$ . Red triangles and green dots represent circRNAs and genes, respectively. (B-D) Functional enrichment of SS-circRNAs-correlated genes in three co-expression networks. The top five significantly enriched Gene Ontology (GO) terms from each category



(Biological Process [BP], Cellular Component [CC], and Molecular Function [MF]) are presented. **(B)** RS. **(C)** OIS. **(D)** DOX. **(E)** Comparison of enriched GO terms in different senescence types. Metascape was employed for multi-gene list enrichment analysis, with significant pathways defined by  $p < 0.01$ , minimum 3 genes, and enrichment factor  $> 1.5$ .

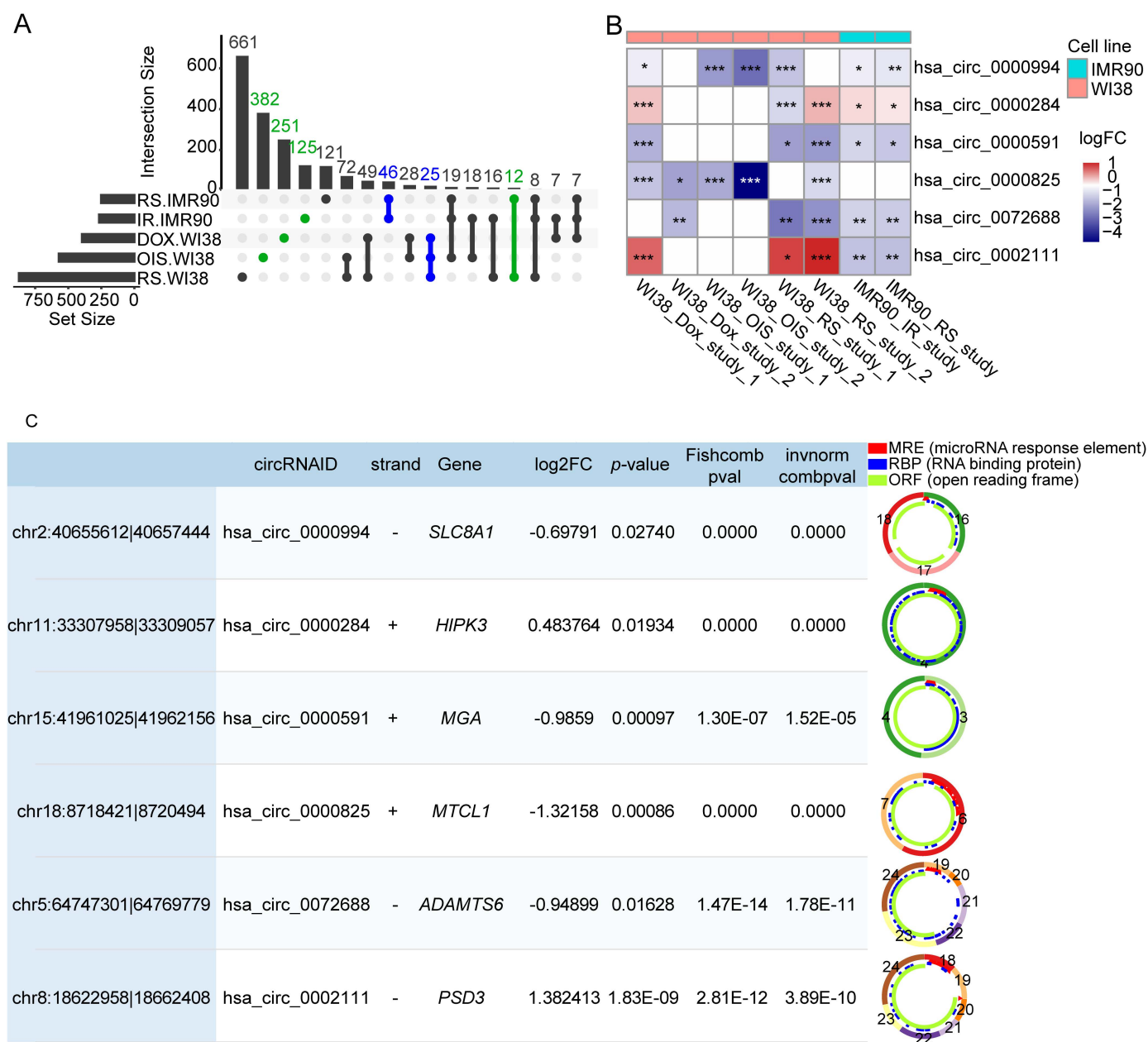
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the molecular heterogeneity of cellular senescence. The construction of circRNA-gene co-expression networks provided insights into the functional roles of SS-circRNAs in different senescence programs. In RS-associated co-expression network, SS-circRNAs were strongly co-expressed with genes involved in cytosolic ribosome function and DNA-binding transcription repressor activity, suggesting they may regulate protein synthesis and gene expression during this senescence type. Indeed, prior studies have proved that diminished ribosome biogenesis could lead to cell cycle arrest [38,39]. Whereas, the enrichment of genes involved in ribonucleoprotein complex biogenesis and RNA splicing in DOX-associated circRNA networks. These pathways have been previously implicated in the regulation of protein homeostasis and RNA processing, both of which are critical for maintaining cellular function during stress-induced senescence [40–42]. The unique co-expression patterns of DOX-specific circRNAs with homeostatic process and circadian rhythm regulation further suggest that these circRNAs may play specialized roles in coordinating cellular responses to genotoxic stress. Similarly, the association of OIS-specific circRNAs with mitochondrial function and cytoskeleton organization reflects the known impact of oncogenic signaling on cellular metabolism and structural integrity [43–45]. These findings suggest that circRNAs may act as molecular hubs that integrate diverse senescence-inducing signals into specific cellular responses.

A particularly intriguing observation was the cell line-dependent regulation of certain circRNAs, such as hsa\_circ\_0002111, which exhibited opposing expression trends in WI38 and IMR90 cell lines (Fig 4B). This phenomenon underscores the importance of considering cellular context when interpreting circRNA functions, as cell type-specific factors may significantly influence circRNA expression and activity. This finding is consistent with previous studies that have highlighted the tissue- and cell type-specific nature of circRNA regulation [46].

Beyond the heterogeneity, we identified 24 General senescence-associated circRNAs (GS-circRNAs) that exhibited consistent differential expression across all senescence types and cell lines examined. Previous studies have reported that circPVT1 and circCCNB1 are down-regulated in senescent cells [47,48]. We further validated the expression of these two circRNAs in our datasets and found that they were significantly down-regulated only in senescent WI38 cells, whereas no significant changes in their expression were observed during the senescence process in IMR90 cells (S1 Fig). These results suggest that the roles of circPVT1 and circCCNB1 in the regulation of cellular senescence may exhibit cell-type-specific heterogeneity.

Among the 24 GS-circRNAs, several have been reported to participate in the initiation and progression of diverse malignancies, including hsa\_circ\_0006916 [49], hsa\_circ\_0001098 [50], hsa\_circ\_0058495 [51], hsa\_circ\_0000228 [52], hsa\_circ\_0000260 [53], hsa\_circ\_0000825 [54], and hsa\_circ\_0000591 [55]. Given the well-recognized interplay between cellular senescence, organismal aging, and tumorigenesis, these findings suggest that the GS-circRNA signature identified in this study may capture molecular features relevant to aging-associated pathological processes rather than representing cell-type-specific artifacts. Moreover, a subset of GS-circRNAs originates from host genes with established roles in senescence-related pathways, although direct functional characterization of the circRNAs themselves remains limited. Notably, *SATB2*-derived circRNAs (hsa\_circ\_0003915 and hsa\_circ\_0008265) warrant attention, as increased *SATB2* expression has been reported to suppress endothelial cell senescence and to be involved in the regulation of bone marrow mesenchymal stem cell aging and age-related bone loss [56,57]. Similarly, GS-circRNAs derived from *DNA2*, *TRPC1*, *NSD1* and *NSD2* originate from genes that regulate cell proliferation and cell-cycle progression and have been implicated in cellular senescence and senescence-associated osteoarthritis [58–61]. Collectively, these observations suggest that the GS-circRNA signature is broadly consistent with current findings in aging-related disorders at the level of host gene function and disease association, while also highlighting the need for future mechanistic studies to directly elucidate the functional roles of these circRNAs in aging and senescence.



**Fig 4. Essential circRNAs linked to senescence across multiple cell types and stimuli.** (A) Comparative analysis of senescence-associated circRNAs across diverse stimuli and cell types. (B) Heatmap plot present the expression changes of key eight senescence-associated circRNAs in different cell types and stimuli. Heatmap visualization of the core senescence-associated circRNAs expression changes during cellular senescence. Each cell in the heatmap is color-coded to represent the log2 fold change (log2FC) value. Red indicates upregulation in senescent conditions, while blue indicates downregulation. The intensity of the color corresponds to the magnitude of the fold change, with darker shades reflecting greater differences. WI38\_DOX\_study\_1 and \_2 are two batches from GSE130727 treated with 2  $\mu$ g/mL doxorubicin for 24 h. WI38\_OIS/RS\_study\_1 are from GSE130727 (OIS: HRASG12V lentivirus, MOI=10, with puromycin), whereas WI38\_OIS/RS\_study\_2 are from GSE130306 (OIS: 20 nM 4-hydroxy-tamoxifen for 10 days). \*:  $p$ -value < 0.05; \*\*:  $p$ -value < 0.01; \*\*\*:  $p$ -value < 0.001. (C) The genomic information of the core senescence-associated circRNAs. Graphical representation of circRNA structure (right) sourced from CSCD database (<http://gb.whu.edu.cn/CSCD/>) with exon-specific color mapping.

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This study is primarily based on transcriptomic analyses, and the identified senescence-associated circRNAs have not yet been experimentally validated. Therefore, the functional roles and regulatory mechanisms of these circRNAs in cellular senescence remain to be determined. Future studies incorporating direct experimental approaches will be necessary to clarify whether these circRNAs actively contribute to senescence or represent downstream transcriptional changes.

In conclusion, our study provides a comprehensive landscape of circRNA expression in cellular senescence, revealing both universal and stimulus-specific circRNA signatures. These findings not only advance our understanding of the regulatory roles of circRNAs in senescence but also highlight their potential as biomarkers and therapeutic targets for age-related diseases. Future studies should focus on elucidating the mechanistic roles of these circRNAs in senescence regulation and exploring their translational potential in aging and disease contexts.

## Supporting information

**S1 Fig. Expression profiles of circPVT1 and circCCNB1 in WI-38 and IMR-90 cells under different types of cellular senescence.** \*:  $p$ -value < 0.05; \*\*:  $p$ -value < 0.01; \*\*\*:  $p$ -value < 0.001.

(TIF)

**S1 Table. Genomic information of identified circRNAs in this study.**

(XLSX)

**S2 Table. Information of 24 Senescence-associated circRNAs across stimuli (GS-circRNAs).**

(XLSX)

**S3 Table. Correlation analysis results of SS-circRNAs and genes across distinct cellular senescence types.**

(XLSX)

**S4 Table. DEGs identified in senescent WI38 cells compared to young cells.**

(XLSX)

**S5 Table. Enrichment analysis results of genes co-expressed with SS-circRNAs in distinct senescent types.**

(XLSX)

**S6 Table. Stimulus-specific circRNA signatures in diverse cellular senescence inductions.**

(XLSX)

## Author contributions

**Conceptualization:** Mingxia Ge.

**Data curation:** Mingxia Ge.

**Formal analysis:** Mingxia Ge, Zimo Liu, Xiaoxiao Zhao.

**Funding acquisition:** Mingxia Ge, Shan Jiang.

**Investigation:** Xiaoxiao Zhao.

**Methodology:** Mingxia Ge.

**Project administration:** Shan Jiang.

**Resources:** Shan Jiang.

**Software:** Mingxia Ge.

**Supervision:** Shan Jiang.

**Validation:** Mingxia Ge.

**Visualization:** Mingxia Ge.

**Writing – original draft:** Mingxia Ge, Zimo Liu.

**Writing – review & editing:** Mingxia Ge, Xiaodong Zhan, Shan Jiang.

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