Supplementary Materials

Cell-Specific Epithelial Senescence in Human Trisomy 21: Implications for Impaired Lung Development

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Table S1. Antibodies used in this study.

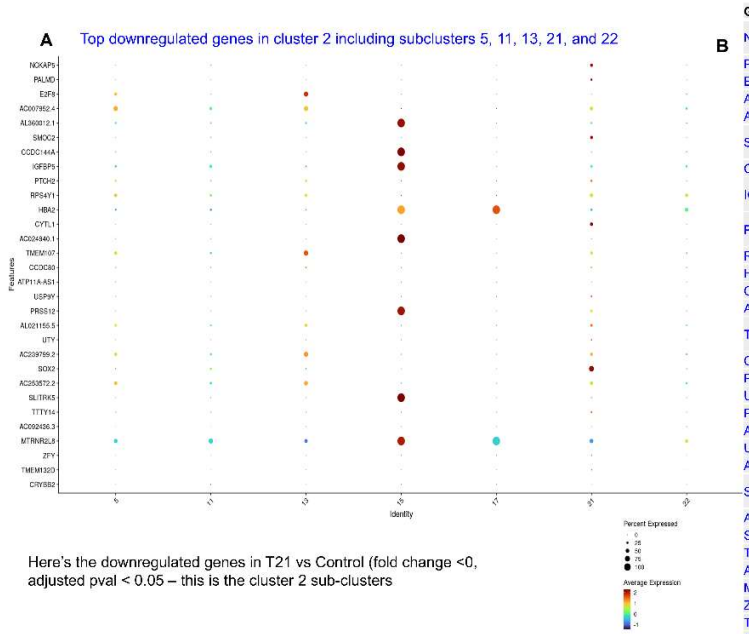
Antibody	Catalog #	Company	Dilution
Lamin B1	MA1-06103	Invitrogen	1:1000
γ-H2AX	ab22551	Abcam	2 µg/mL
RAGE	AF1179	R&D Systems	10 µg/mL
UGRP1	PA5-120195	Invitrogen	1:100
CD31	3528S	Cell Signaling Technology	1:800
VWF	A0082	Dako	1:200
594 Goat Anti-Rabbit	A11012	Invitrogen	2 µg/mL
488 Goat Anti-Mouse	A11001	Invitrogen	1 µg/mL
488 Donkey Anti-Mouse	715-545-150	Jackson ImmunoResearch	1:500
594 Donkey Anti-Goat	705-585-147	Jackson ImmunoResearch	1:500
594 Donkey Anti-Rabbit	711-585-152	Jackson ImmunoResearch	1:500
594 Donkey Anti-Mouse	715-585-150	Jackson ImmunoResearch	1:500
488 Goat Anti-Rabbit	A11008	Invitrogen	10 µg/mL
488 Donkey Anti-Goat	A11055	Invitrogen	10 µg/mL

Table S2. Proteins expressed only in the euploid group.

Proteins	Biological functions	Related with lung
EMILIN2, TIMP3, ADAMTSL4, SCARA5, ENPP1	Extracellular Matrix (ECM), Matrix Remodeling & Structural Support	Branching morphogenesis, alveolar architecture, vessel stabilization
MYH3, KATNA1, TXLNG, MID1, SPATA5	Cytoskeleton, Contractility & Cell Migration	Branch extension, epithelial remodeling, endothelial sprouting
TYMP, EMILIN2, TIMP3, EGLN1, SCARA5	Angiogenesis & Vascular–Epithelial Coupling	Capillary formation, alveolar–vascular alignment
LGALS9, ALDH1A3, CAB39L, PTPN2, BRD7	Progenitor Cell Maintenance & Developmental Plasticity	Distal lung progenitors, developmental competence
RRM2, ANAPC5, TELO2, UTP15, RBM19	Cell Proliferation, Cell Cycle & Growth Control	Tissue expansion, controlled growth
TAF2, BRD7, RBM19, GTPBP6	Transcriptional, Epigenetic & RNA Processing Regulators	Developmental gene expression programs

Table S3. Proteins expressed only in the trisomy 21 group.

Proteins	**Functional Protein Group**	**Mechanistic Role in Senescence**
MORC3, ZMYND11, FOCAD, BCL9L, PTPN13, KRAS	DNA damage response & genome stability	Chromatin remodeling defects, replication stress, defective checkpoint signaling
PGLYRP2, C9, C8A, LRG1, REL, ADAM17, PROCR, IFI35	Inflammation & innate immune signaling	NF-κB activation, complement signaling, cytokine amplification
CYB5B, DHRS11, CRYAA, PDXDC1, CASTOR1, SLC30A7	Oxidative stress & redox homeostasis	ROS accumulation, impaired redox buffering, metabolic stress sensing
NAGLU, TOM1L2, ANXA8	Endolysosomal/proteostasis stress	Reduced autophagic flux, lysosomal expansion
PCDH1, OCLN, DMTN, LDB3, MYZAP, DLG3, VANGL1, RALGAPA2	Structural stress & growth arrest	Junctional instability, polarity loss, mechanotransduction defects
CASTOR1, CYB5B, PDXDC1	Metabolic stress signaling	Aberrant mTORC1 and metabolic checkpoint regulation

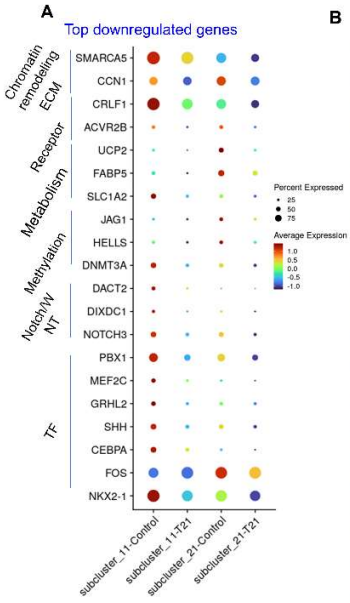


B

Gene	Function / Role
NCKAP5	Cytoskeleton regulation; neuronal development (poorly characterized).
PALMD	Palmdelphin-actin cytoskeleton regulation; cell motility.
E2F8	Transcription factor regulating cell proliferation and DNA replication.
AC007952.4	lncRNA, function not well defined.
AL360012.1	lncRNA, unknown physiological role.
SMOC2	Extracellular matrix glycoprotein; angiogenesis and tissue remodeling.
CCDC144A	Coiled-coil protein, unknown function.
IGFBP5	Insulin-like growth factor binding protein, growth regulation, senescence.
PTCHD2	Putative patched domain protein, possible Hedgehog signaling involvement.
RPS4Y1	Ribosomal protein S4 Y-linked, translation machinery.
HBA2	Hemoglobin alpha subunit, oxygen transport.
CYTL1	Cytokine-like protein, immune modulation; cartilage development.
AC024940.1	lncRNA, uncharacterized.
TMEM107	Membrane protein; primary cilia structure & Hedgehog signaling regulation.
CCDC80	Extracellular matrix protein; adipogenesis, fibrosis signaling.
PTPLA-AS1	Antisense transcript, function unclear.
USP9Y	Y-linked deubiquitinase, spermatogenesis; protein turnover.
PRSS12	Serine protease neurotrypsin, synaptic plasticity and learning.
AL021155.5	lncRNA, uncharacterized.
UTY	Y-linked demethylase, chromatin regulation.
AC239799.2	lncRNA, unknown function.
SOX2	Stem cell transcription factor maintaining pluripotency and epithelial identity.
AC253728.2	lncRNA, unknown function.
SLITRK5	Neuronal synapse development.
TTTY14	Y-linked ncRNA, poorly defined function.
AC092436.3	lncRNA, uncharacterized.
MTRNRL8	Mitochondrial RNA-related transcript.
ZFY	Y-linked zinc finger transcription factor.
TMEM132D	Membrane protein.
CRYBB2	Beta-crystallin—lens structural protein (eye development).

Figure S1. Developmental and epithelial regulatory genes are reduced in trisomy 21 fetal lung epithelial cells. (**A**) Dot plot showing representative genes significantly downregulated in T21 epithelial cells compared with euploid controls across epithelial subclusters. Dot size represents the percentage of cells expressing each gene, and color indicates average expression. Downregulated genes include regulators of epithelial identity and differentiation (SOX2), extracellular matrix organization and tissue remodeling (SMOC2 and CCDC80), growth regulation (IGFBP5), Hedgehog-related signaling (PTCHD2 and TMEM107), and chromatin regulation (UTY). (**B**) Functional annotation of representative downregulated genes highlighting their roles in epithelial differentiation, developmental signaling, extracellular matrix remodeling, growth regulation, and tissue organization.

A

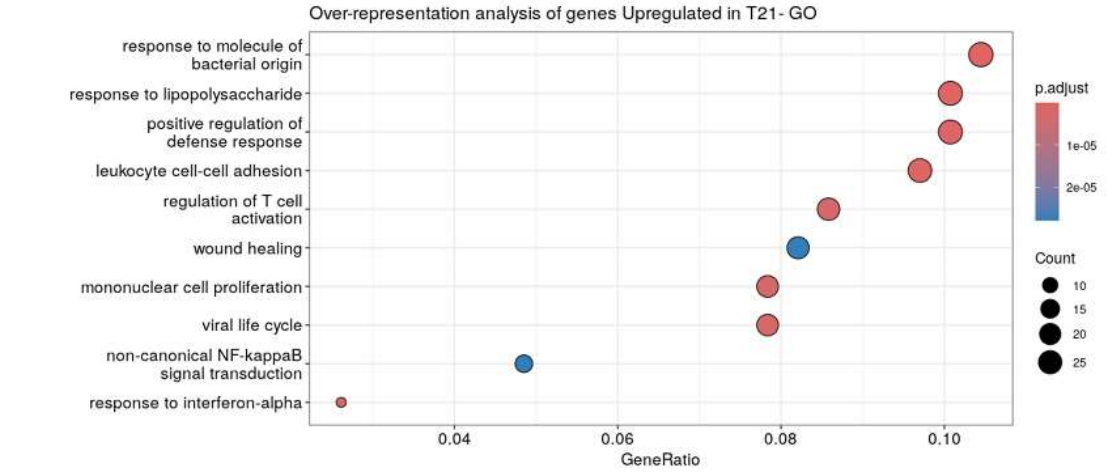


B

Gene	Core Function in Lung Development/Homeostasis
SMARCA5	Regulates gene expression programs necessary for the correct differentiation of lung cells by making DNA accessible (Chromatin Remodeling).
CCN1	Promotes the remodeling of the lung extracellular matrix and supports angiogenesis (new blood vessel formation), which is vital for gas exchange structure.
CRLF1	A cytokine signaling receptor that may be involved in anti-fibrotic signaling and modulating inflammatory responses within the lung tissue.
ACVR2B	Controls cell growth and size in the mesenchymal tissues (like the airway smooth muscle) by sensing growth factors.
UCP2	Regulates cellular energy and protects lung cells, especially type II pneumocytes, from oxidative stress during development and maturation.
FABP5	Transports fatty acids required for generating cellular membranes and surfactant production in developing alveolar cells.
SLC1A2	Likely maintains the chemical environment by transporting signaling molecules, potentially important for cell-to-cell signaling in airway epithelia.
JAG1	A Notch signaling ligand crucial for airway branching morphogenesis and deciding the fate of epithelial and vascular cells during lung formation.
HELLS	Essential for epigenetic fidelity during rapid cell proliferation and differentiation, ensuring lung progenitor cells properly mature.
DNMT3A	Establishes long-term gene silencing patterns (DNA methylation) that are critical for stabilizing the cell identity of specialized lung cells (e.g., alveolar cells).
DACT2	Acts to modulate Wnt signaling, which is key for epithelial-mesenchymal interactions that guide the patterning of the developing lung.
DIXDC1	Aids in cell shape changes and movement necessary for the correct migration and organization of cells into the lung's structured architecture.
NOTCH3	A Notch receptor that controls the proliferation and differentiation of endothelial cells and smooth muscle cells, influencing vascular development in the lung.
PBX1	A master transcription factor that cooperates with other factors to establish the correct pattern and structure during lung organogenesis.
MEF2C	Regulates genes involved in the development and maintenance of the pulmonary vasculature (blood vessels) and smooth muscle surrounding airways.
GRHL2	Maintains the integrity and barrier function of the airway and alveolar epithelial lining, essential for homeostasis and protection.
SHH	A primary morphogen signal that guides airway branching and patterning by communicating between epithelial and mesenchymal cells.
CEBPA	Drives the terminal differentiation of specific lung cell types, potentially including alveolar macrophages or certain epithelial cell lineages.
FOS	A rapid-response transcription factor that quickly translates environmental or stress signals into changes in gene expression for cellular adaptation.
NKX2-1	The master regulator of lung identity; absolutely required for lung tissue formation and regulates genes for surfactant production (e.g., in Type II pneumocytes)

Figure S2. Key developmental and homeostatic programs are suppressed in type I alveolar and Club cells in trisomy 21 fetal lungs. (**A**) Dot plot showing representative genes downregulated in type I alveolar cells (subcluster 11) and Club cells (subcluster 21) from T21 fetal lungs compared with their respective euploid controls. Dot size represents the percentage of cells expressing each gene, and color indicates average expression. Downregulated genes include regulators of chromatin remodeling and extracellular matrix organization (SMARCA5 and CCN1), metabolism (UCP2 and FABP5), DNA methylation (HELLS and DNMT3A), Notch/Wnt signaling (JAG1, NOTCH3, DACT2, and DIXDC1), and transcriptional control of lung development and epithelial identity (PBX1, GRHL2, SHH, CEBPA, and NKX2-1). (**B**) Functional annotation of representative downregulated genes highlighting their roles in lung development, epithelial differentiation, tissue homeostasis, and cellular metabolism.

(**A**)



(**B**)

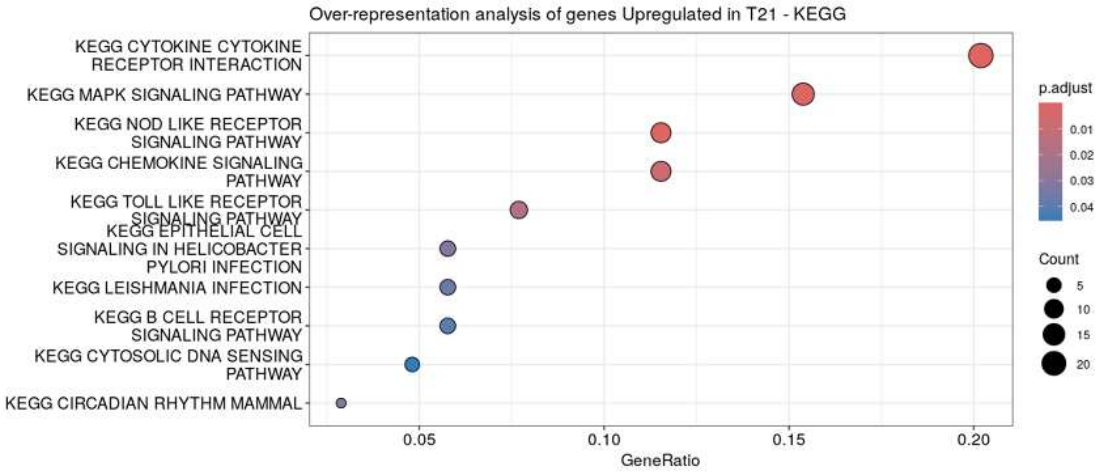
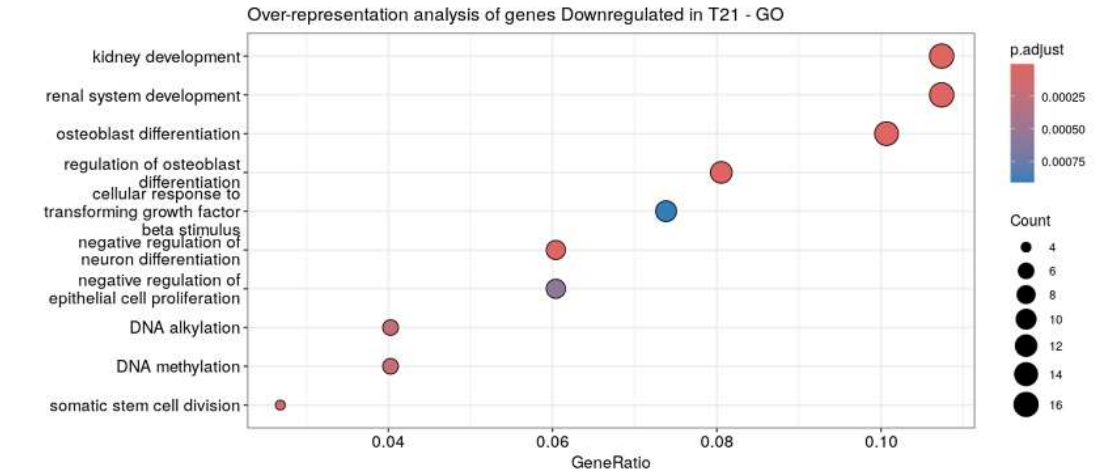


Figure S3. Upregulated genes in trisomy 21 fetal lungs are enriched in inflammatory and immune signaling pathways. Over-representation analysis of genes upregulated in T21 fetal lungs compared with euploid controls. (**A**) Gene Ontology (GO) analysis showing enrichment of biological processes associated with responses to bacterial products, defense responses, leukocyte adhesion, T-cell activation, mononuclear cell proliferation, NF-κB signaling, and interferon responses. (**B**) KEGG pathway analysis showing enrichment of cytokine-cytokine receptor interaction, MAPK, NOD-like receptor, chemokine, Toll-like receptor, B-cell receptor, and cytosolic DNA-sensing pathways. Dot size represents the number of genes contributing to each pathway, and color indicates the adjusted P value.

(**A**)



(**B**)

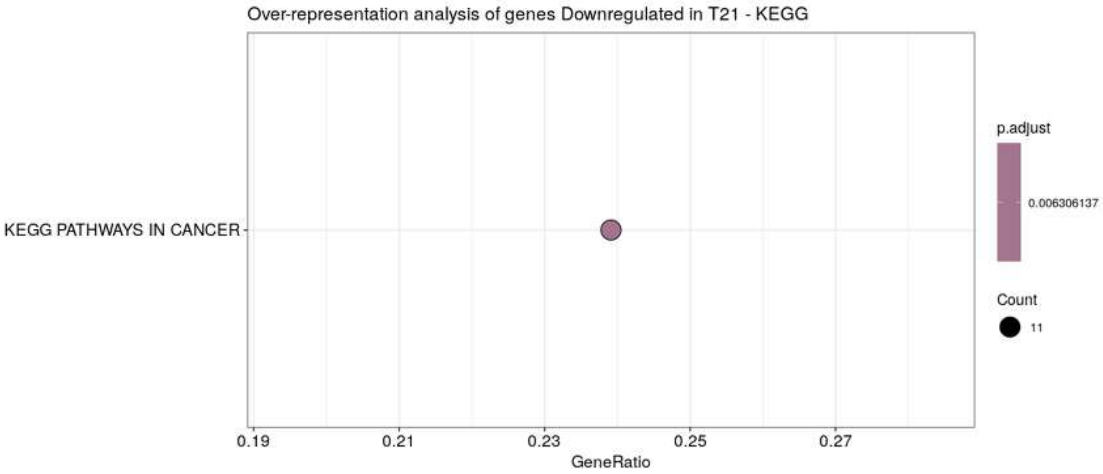


Figure S4. Downregulated genes in trisomy 21 fetal lungs are enriched in developmental, differentiation, and epigenetic regulatory processes. Over-representation analysis of genes downregulated in T21 fetal lungs compared with euploid controls. (**A**) Gene Ontology (GO) analysis showing enrichment of biological processes associated with development, cellular differentiation, epithelial cell proliferation, TGF-β responses, DNA methylation, and somatic stem cell division. (**B**) KEGG pathway analysis showing significant enrichment of the pathways in cancer category. Dot size represents the number of genes contributing to each pathway, and color indicates the adjusted P value.

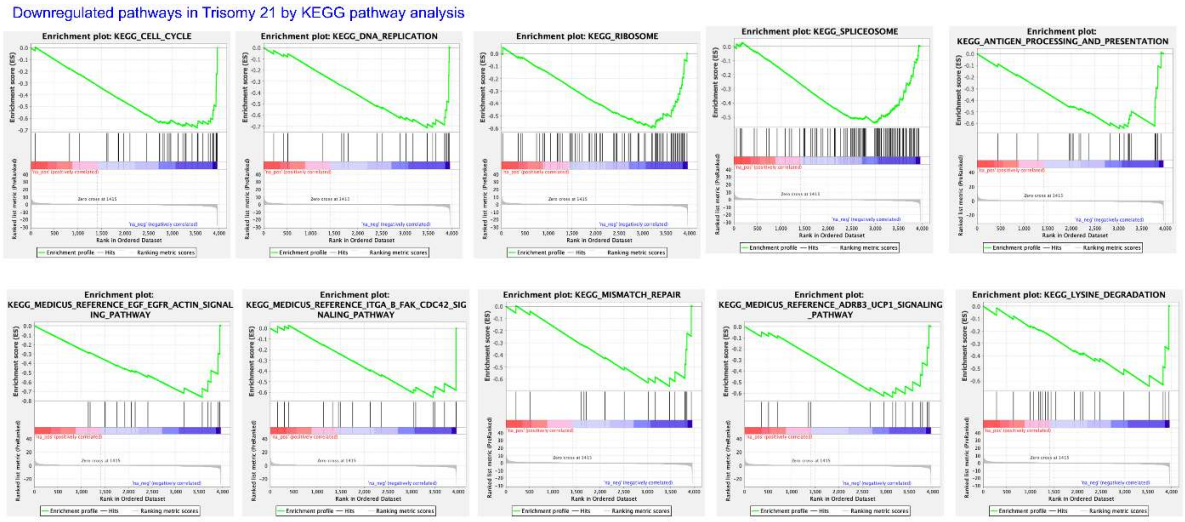


Figure S5. Proteomic KEGG pathway analysis reveals suppression of cell proliferation, DNA replication and repair, and protein processing pathways in trisomy 21 fetal lungs. Gene set enrichment analysis of proteins decreased in T21 fetal lungs compared with euploid controls using KEGG pathways. Downregulated pathways include cell cycle, DNA replication, mismatch repair, ribosome, spliceosome, antigen processing and presentation, EGF/EGFR signaling, integrin/FAK/CDC42 signaling, and lysine degradation. These findings indicate coordinated suppression of proliferative, genome-maintenance, protein synthesis and processing, and cellular signaling programs in T21 fetal lungs.

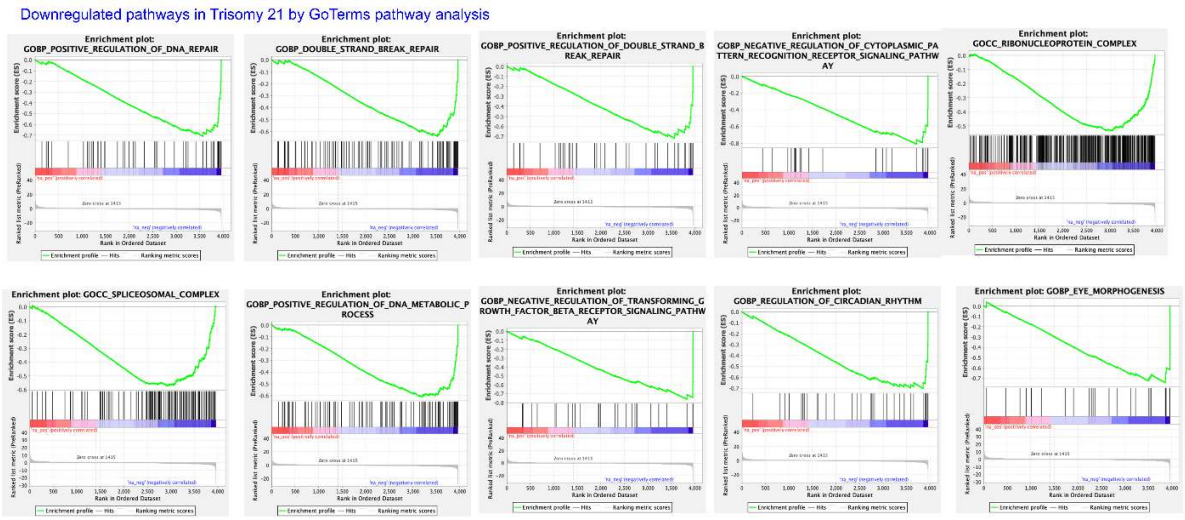


Figure S6. Proteomic Gene Ontology analysis reveals suppression of DNA repair, protein processing, and developmental regulatory pathways in trisomy 21 fetal lungs. Gene set enrichment analysis of proteins decreased in T21 fetal lungs compared with euploid controls using Gene Ontology (GO) terms. Downregulated processes include DNA repair and double-strand break repair, DNA metabolic processes, ribonucleoprotein and spliceosomal complexes, TGF-β receptor signaling, circadian rhythm regulation, and morphogenesis. These findings indicate coordinated impairment of genome-maintenance, RNA processing, and developmental regulatory programs in T21 fetal lungs.