

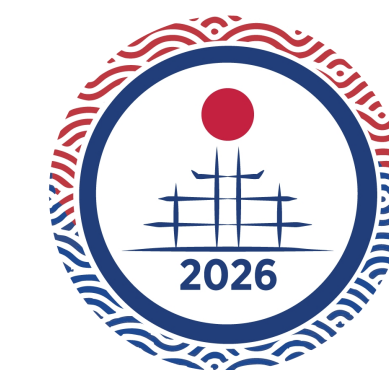


P3.155: An intact tumor microenvironment in patient-derived NSCLC organoids facilitates subtype-specific drug profiling

Zihao Chen*, Hongdou Ding, Haifeng Wang, Xinyu Ding

Department of Thoracic Surgery, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai 200433, China;

*Correspondence: giochan@163.com



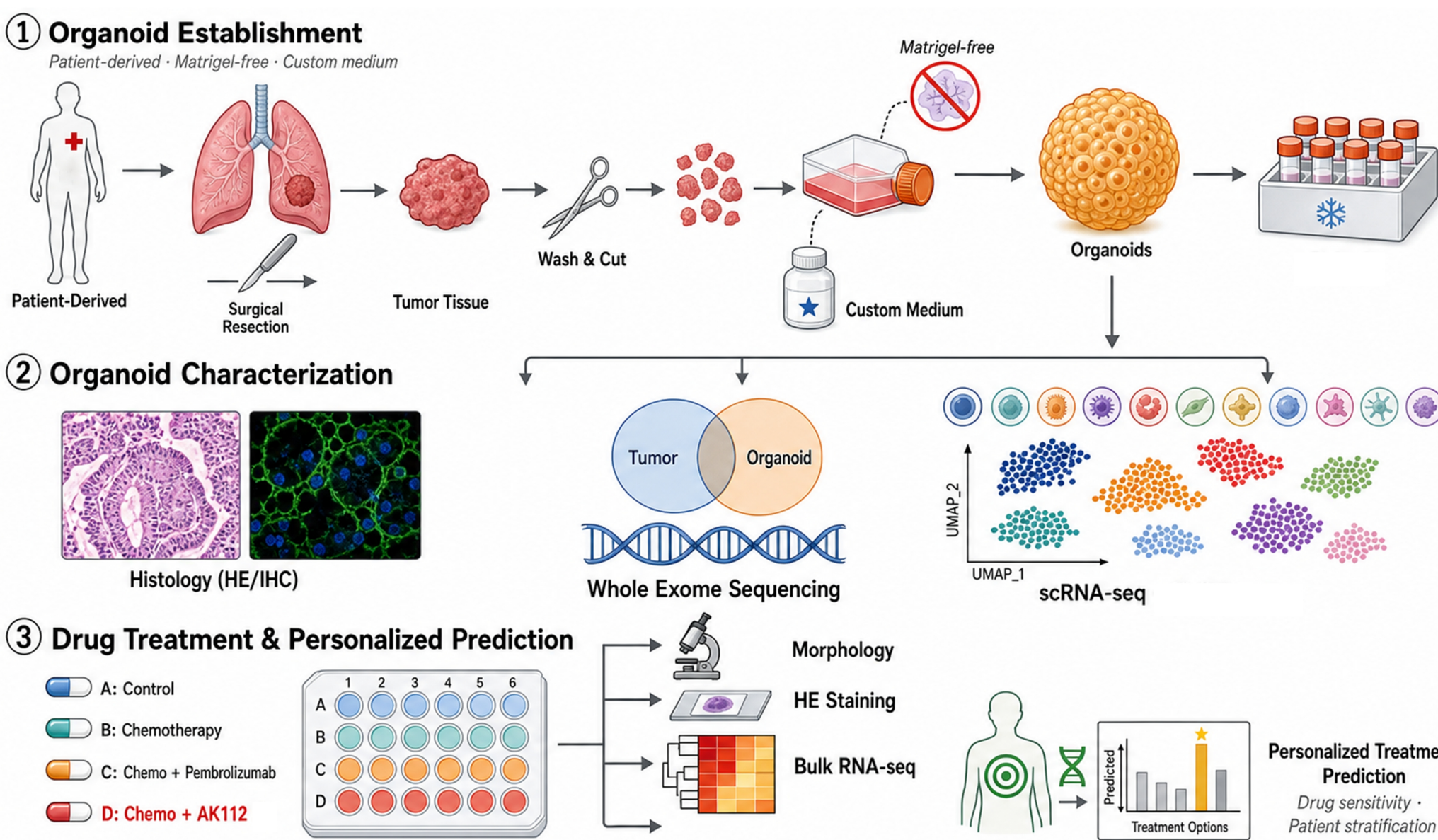
IASLC 2026 World
Conference on Lung Cancer

SEPTEMBER 12 - 15, 2026
SEOUL, REPUBLIC OF KOREA

INTRODUCTION

- NSCLC is heterogeneous**
Different histological subtypes exhibit distinct biological features and therapeutic vulnerabilities.
- Current models are imperfect**
2D cultures lack tumor complexity, while PDX models are costly and time-consuming.
- PDOs have translational potential**
However, conventional organoid systems often depend on Matrigel and enzymatic dissociation, which can compromise the native TME.
- Our approach**
A **Matrigel-free, tissue-fragment-based platform** was developed to preserve tumor architecture, genomic features, and native stromal/immune components for **subtype-specific drug profiling**.

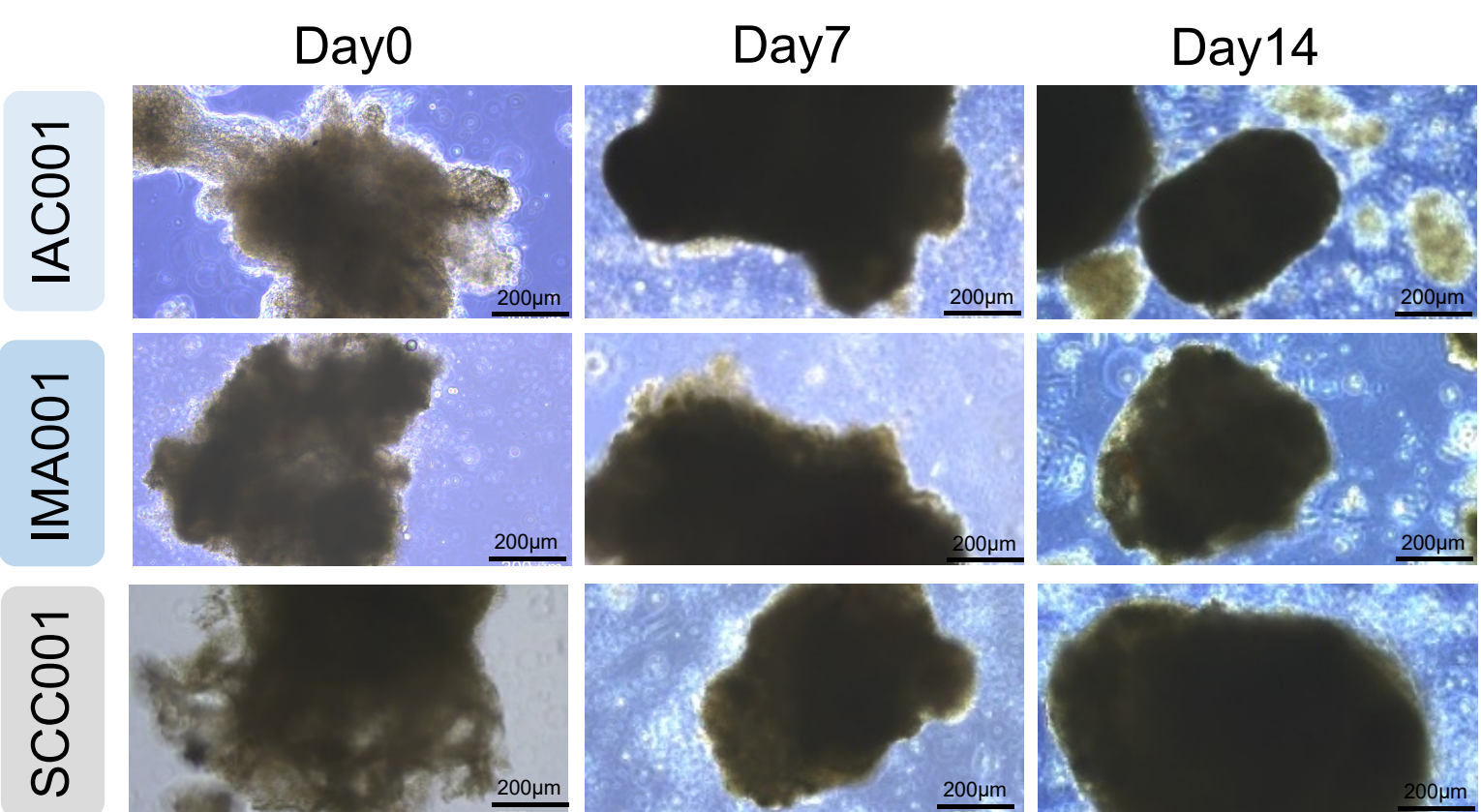
METHODS



- Organoids Establishment**
 - Treatment-naïve, freshly resected NSCLC tissues were mechanically fragmented into **500–1,000 µm** pieces.
 - Direct culture in **custom medium**, without **Matrigel** or **enzymatic digestion**.
 - Organoid morphology and growth were monitored longitudinally.
- Organoids Characterization**
 - H&E/IHC**: Histological fidelity
 - scRNA-seq**: Tumor, stromal and immune composition
 - WES**: Genomic concordance, CNV and mutational signatures
 - Bulk RNA-seq**: Treatment-associated transcriptional remodeling
- Drug Treatment & Personalized Drug Profiling**
 - 7-day organoids** were treated with chemotherapy alone or in combination with **pembrolizumab** or **AK112**.
 - Chemotherapy regimen: **ADC**: Cisplatin + pemetrexed; **SCC**: Cisplatin + nab-paclitaxel.
 - Responses were assessed by **morphology, H&E and transcriptomics**.
 - DESeq2 + Hallmark GSEA** identified patient- and histology-specific response patterns.

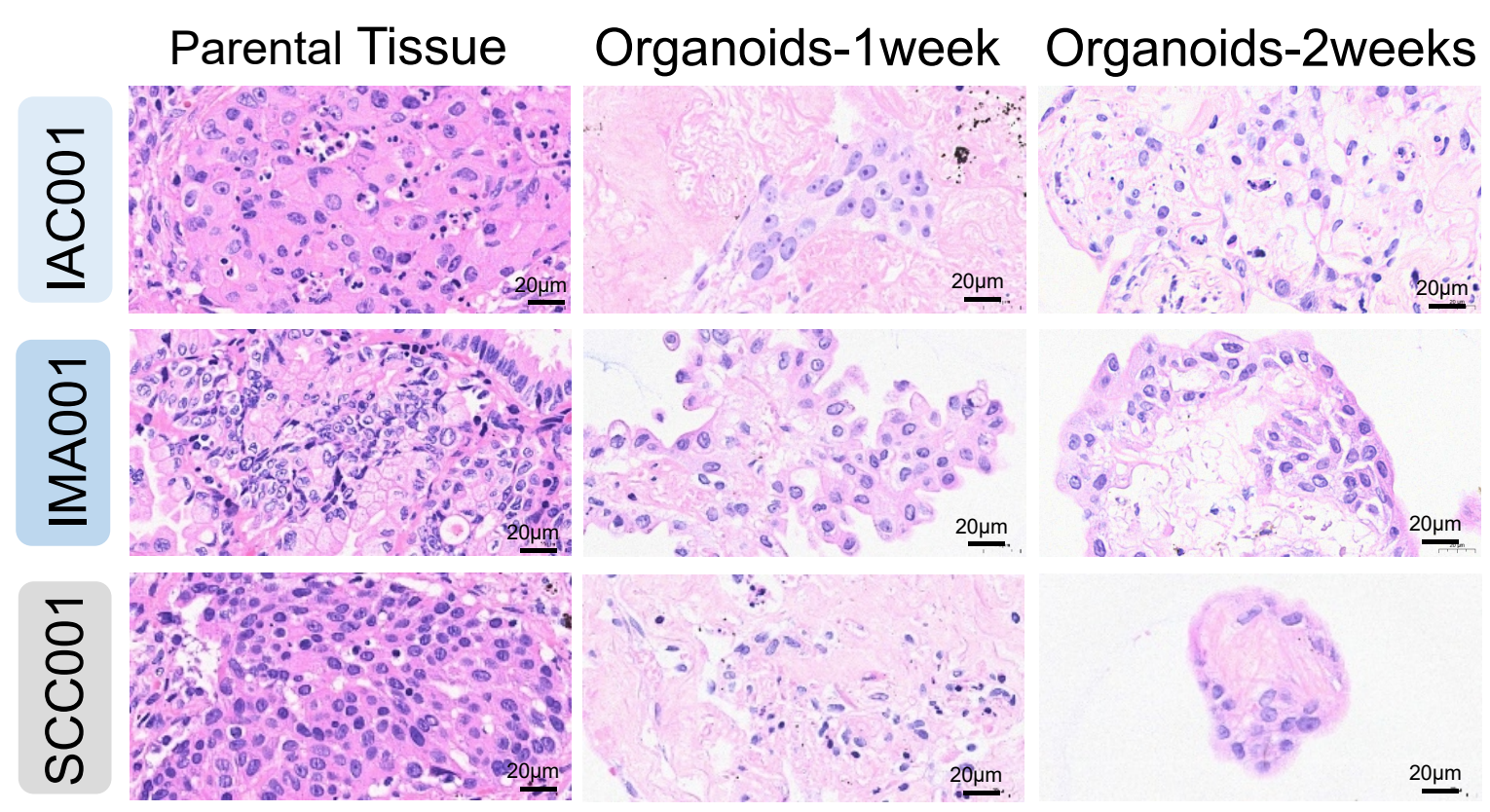
RESULTS

① Organoids Establishment

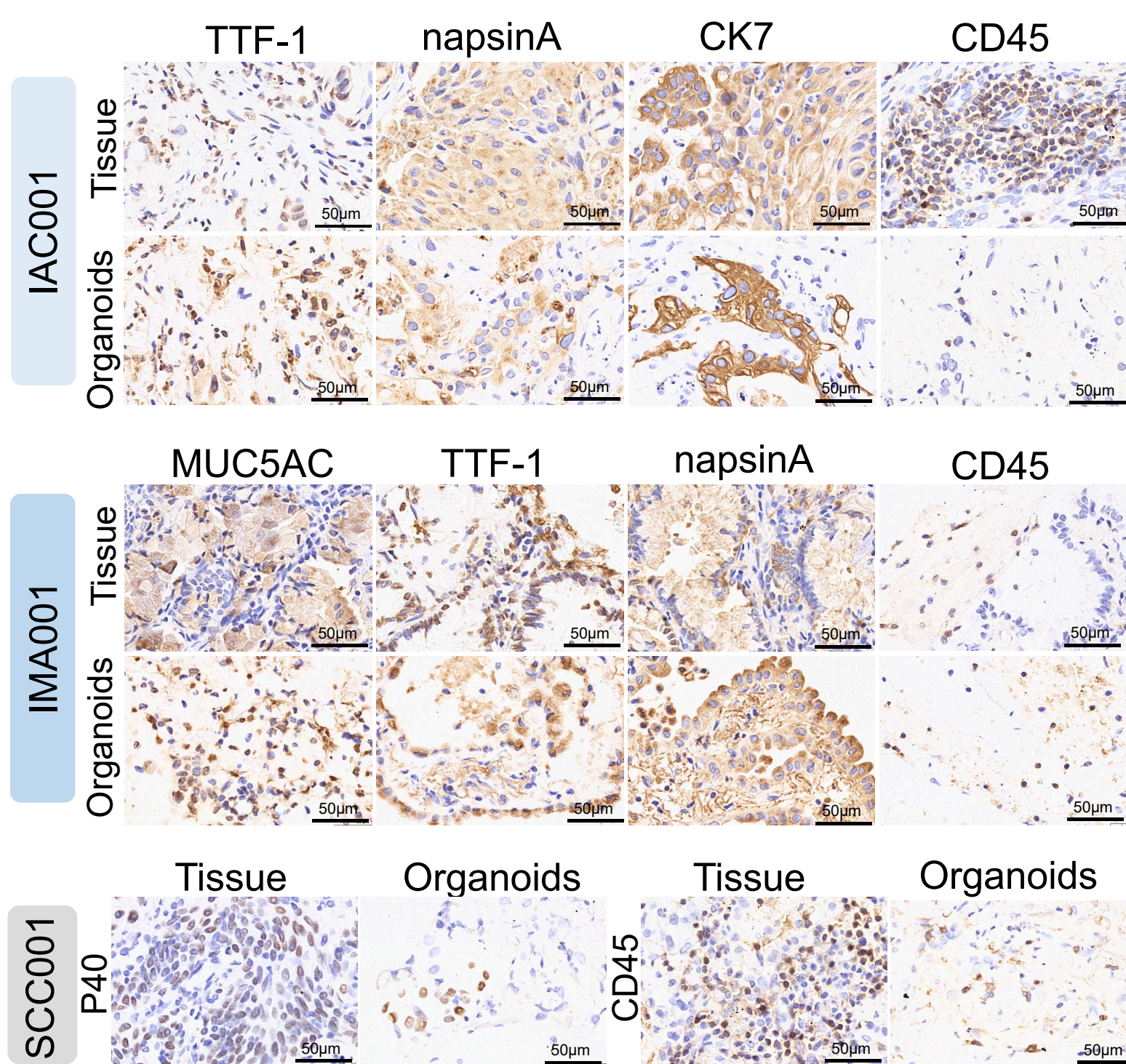


- Representative brightfield micrographs of organoids derived from lung adenocarcinoma (IAC), invasive mucinous adenocarcinoma (IMA), and squamous cell carcinoma (SCC).
- During 14 days of culture, the tissue fragments progressively rounded, with increased circularity and sustained surface cell survival and outgrowth.

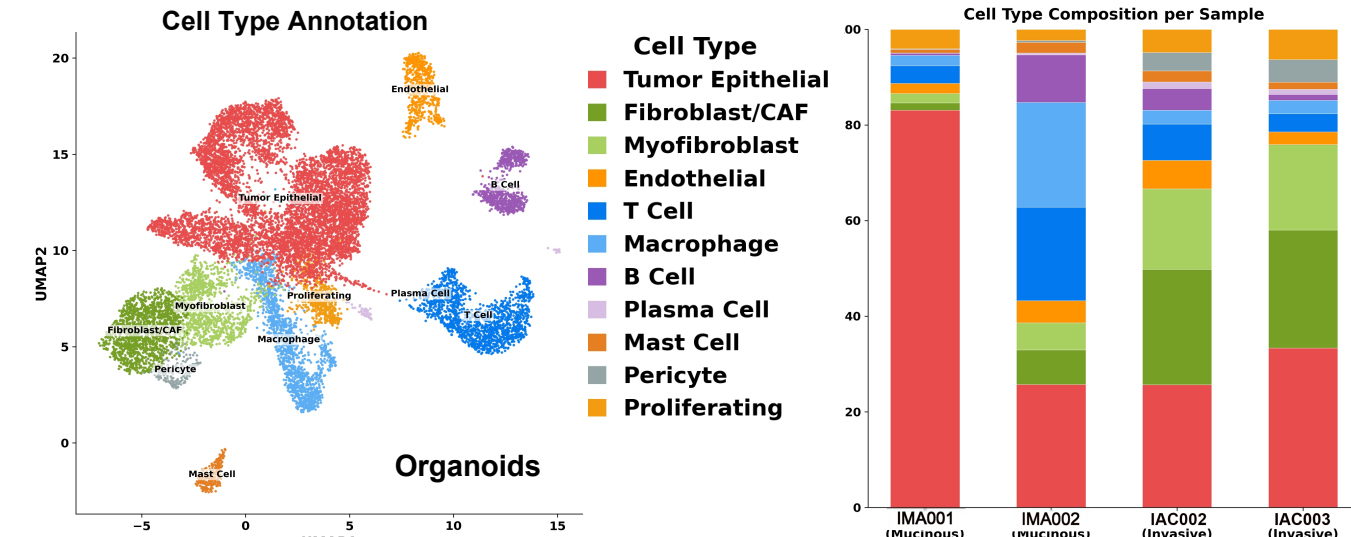
② Organoids Characterization



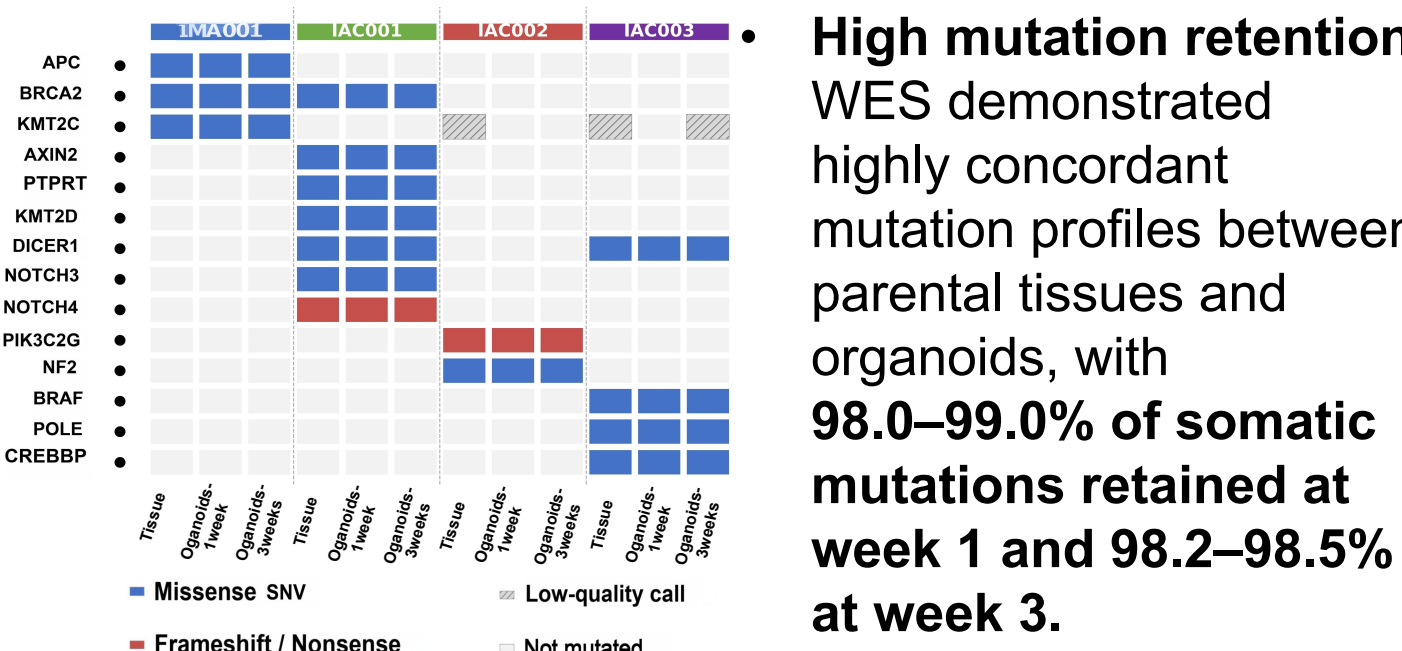
- H&E staining demonstrated that organoids derived from IAC, IMA, and SCC recapitulated the characteristic histoarchitectural features of their parental tumors at both 1 and 2 weeks of culture.



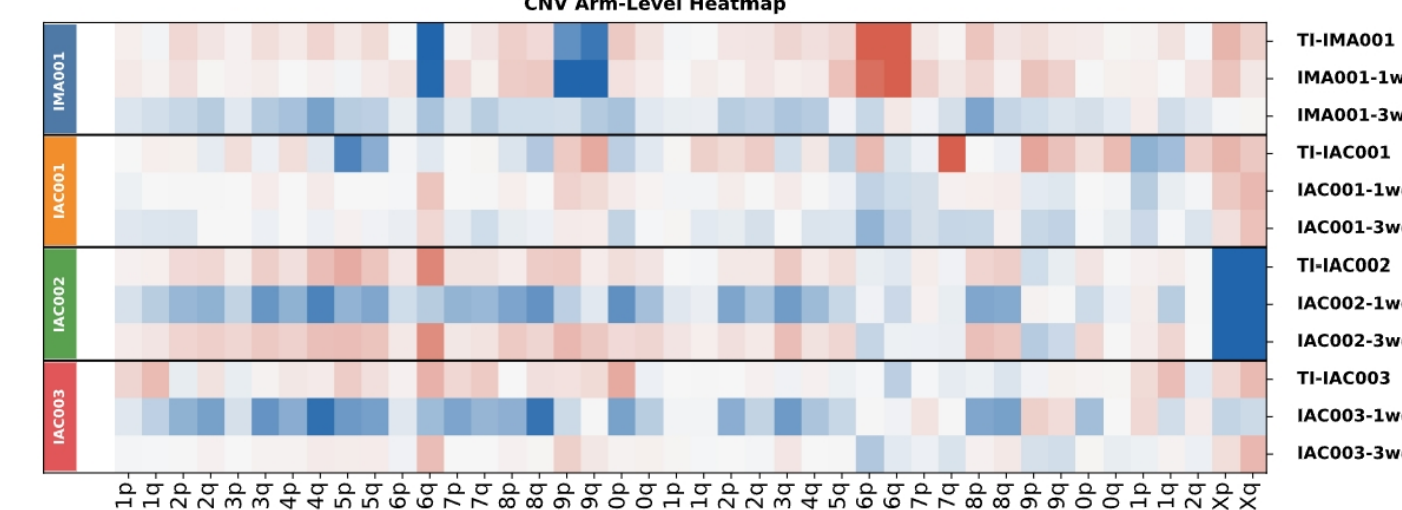
- IHC profiling confirmed the retention of subtype-specific molecular features in organoids, with concordant expression of canonical markers including TTF-1, Napsin A, CK7, P40 and MUC5AC.
- CD45+ immune cells were also preserved within organoids after 1 week of culture.



- scRNA-seq profiling** identified 11 major cell populations in four adenocarcinoma organoids, spanning tumor epithelial, stromal, immune, and proliferating compartments.
- Tumor epithelial cells predominated, whereas stromal and immune compartments exhibited **substantial interpatient heterogeneity**.



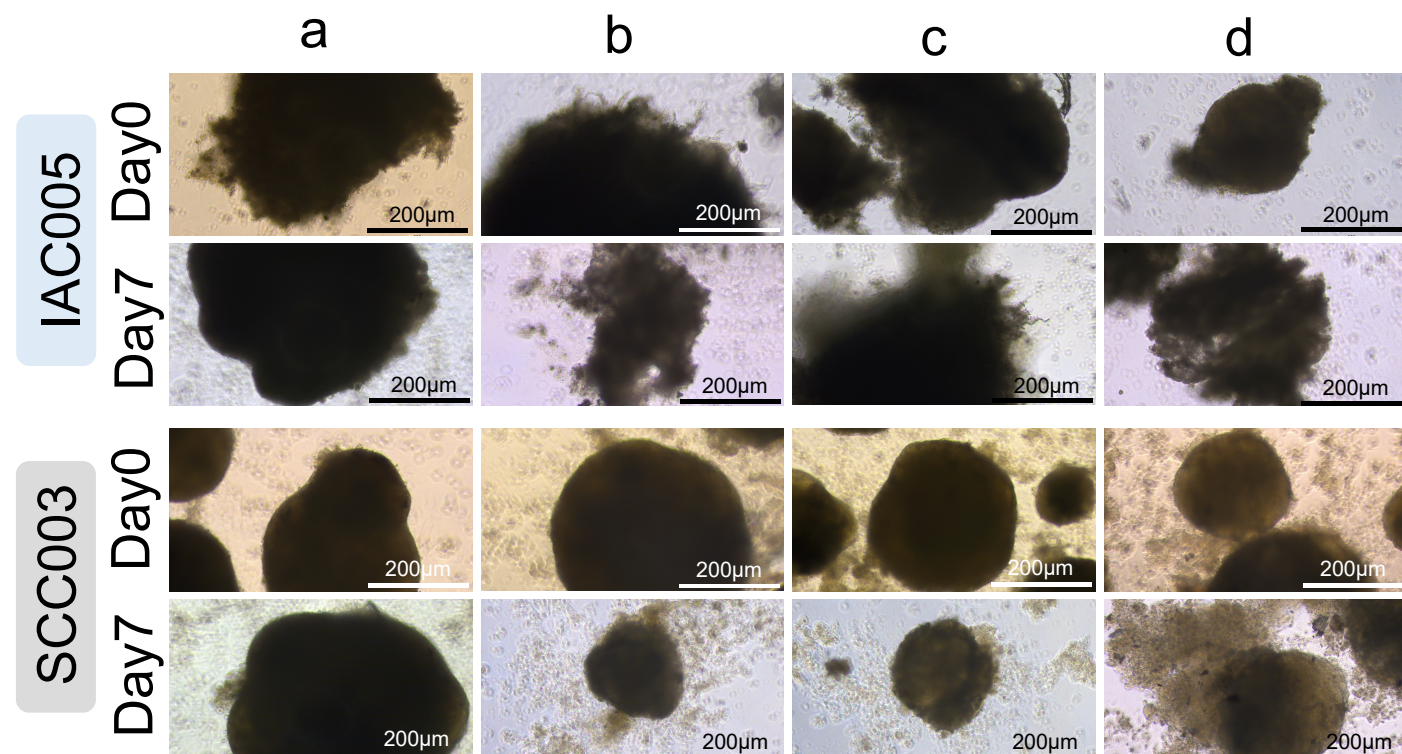
High mutation retention: WES demonstrated highly concordant mutation profiles between parental tissues and organoids, with **98.0–99.0% of somatic mutations retained at week 1 and 98.2–98.5% at week 3**.



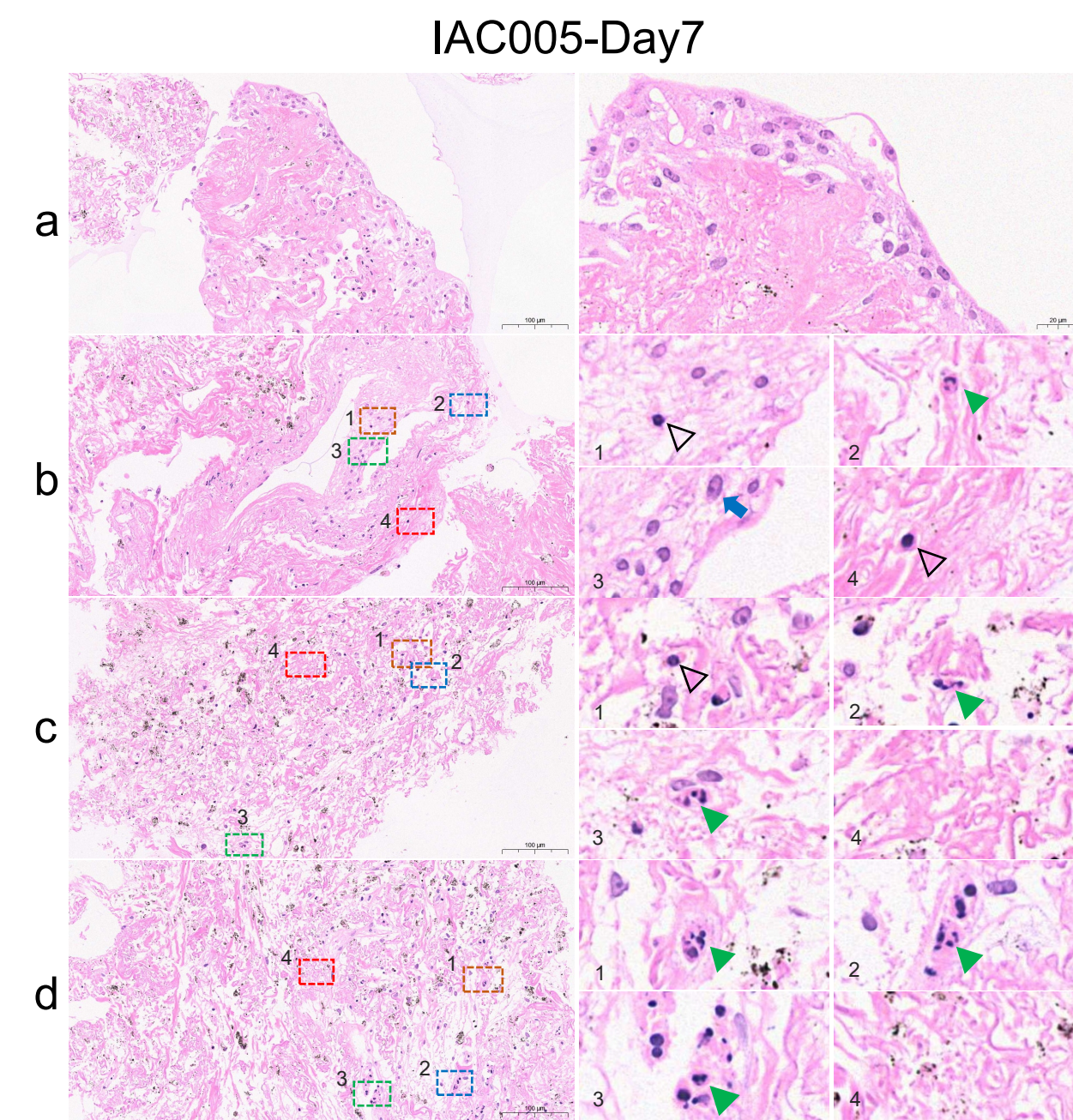
- Stable CNV profiles:** Arm-level copy number alterations were consistently preserved between parental tissues and organoids throughout 3 weeks of culture, supporting sustained genomic fidelity.

③ Drug Treatment & Personalized Drug Profiling

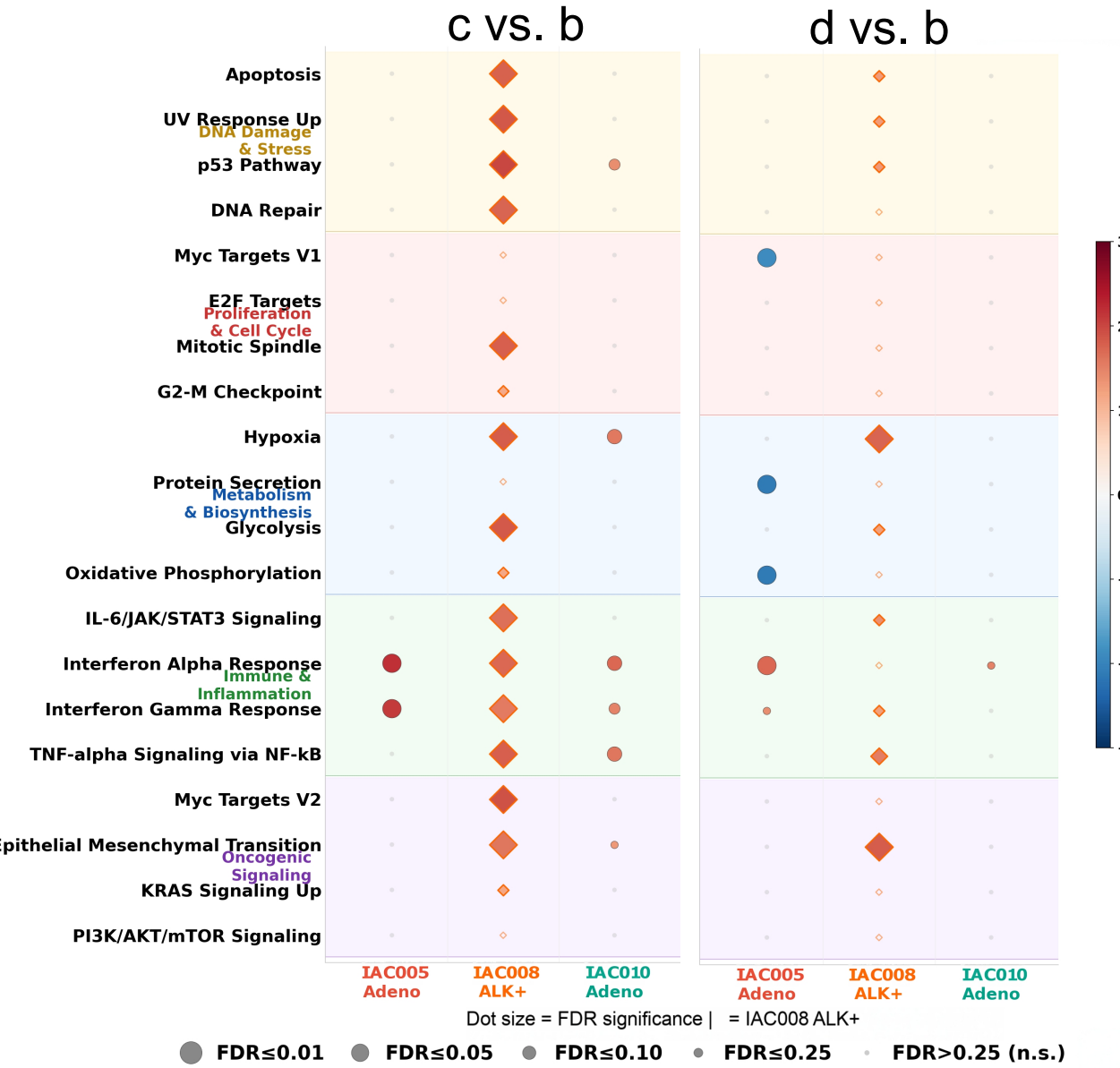
Group	Drug Regimen	
	Adenocarcinoma	Squamous Cell Carcinoma
a	Control	Control
b	Cisplatin + Pemetrexed	Cisplatin + nab-Paclitaxel
c	b + Pembrolizumab	b + Pembrolizumab
d	b + AK112	b + AK112



- Drug sensitivity was assessed in patient-derived organoids from EGFR/ALK/ROS1 negative ADC and SCC.
- Bright-field microscopy revealed distinct morphological responses to different treatment regimens after 7 days.
- Compared with the compact spheroidal architecture maintained in the control group (a), treated organoids (b–d) showed marked structural disintegration and fragmentation.

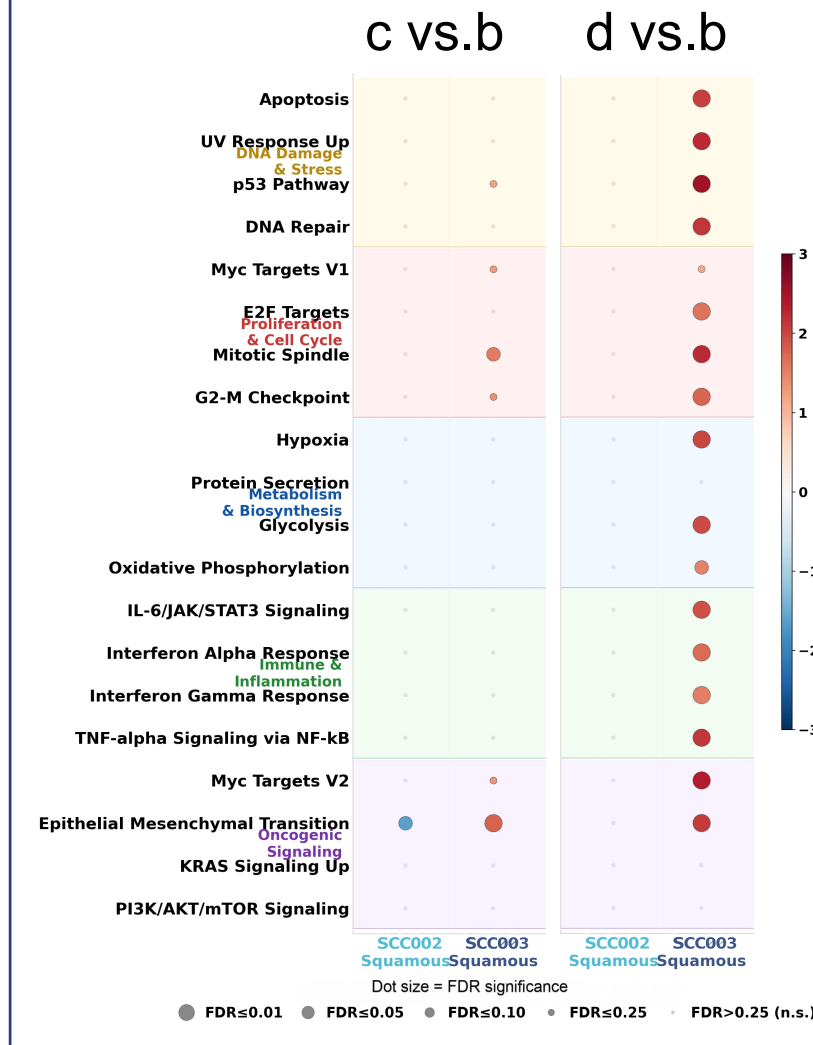


- Control(a):** Preserved organoid architecture and viable tumor cell density.
- Treatment groups(b–d):** Reduced cellularity, disrupted tissue organization, cytoplasmic vacuolization, and extensive apoptotic changes.
- Apoptosis:** Nuclear pyknosis (black triangles) and karyorrhexis (green arrowheads) were prominent.
- Severe injury:** Region “4” showed extensive architectural collapse and apoptotic degeneration.
- Overall:** Chemotherapy-based regimens induced substantial tumor cell injury and death.



GSEA revealed distinct transcriptional responses to pembrolizumab- and AK112-based regimens.

- Pembrolizumab + chemotherapy (c vs. b):** IAC008 (ALK+) showed a unique **near-pan-Hallmark transcriptional activation**, involving DNA damage/apoptosis, cell cycle, metabolism, immune signaling, and oncogenic pathways.
- IAC005:** Response was mainly restricted to **IFN-α/γ signaling**, while IAC010 showed only modest activation of p53, hypoxia, and TNF-α pathways.
- AK112 + chemotherapy (d vs. b):** Induced a **distinct and attenuated transcriptional response**. IAC005 showed suppression of oxidative phosphorylation, protein secretion, and Myc targets, with IFN-α activation.
- IAC008:** AK112 induced only limited **Hypoxia** and **EMT** enrichment, without the broad immune and DNA damage response observed with pembrolizumab.



GSEA revealed a distinct treatment-response pattern in squamous cell carcinoma organoids.

- Pembrolizumab + chemotherapy (c vs. b)** induced **minimal transcriptional changes** in both SCC002 and SCC003, with **no significant immune pathway activation**.
- AK112 + chemotherapy (d vs. b)** triggered **broad transcriptional remodeling** in SCC003, involving DNA repair, cell cycle, metabolism, immune/inflammatory, and oncogenic pathways, whereas SCC002 showed no significant enrichment.
- These findings highlight a histotype-specific contrast**, with AK112 eliciting broader transcriptional responses than pembrolizumab in squamous organoids.

DISCUSSION AND CONCLUSION

- Faithful tumor modeling**
Multi-histotype NSCLC PDOs preserved parental tumor architecture, genomic alterations, and subtype-specific biology.
- Native TME preservation**
scRNA-seq identified **11 major epithelial, stromal, and immune populations**, enabling patient-specific modeling of tumor–microenvironment interactions.
- Patient- and histotype-specific drug responses**
Pembrolizumab and AK112 induced distinct transcriptional programs across adenocarcinoma and squamous PDOs, revealing marked inter-patient and histotype heterogeneity.
- Functional precision oncology**
TME-preserving PDOs enable high-resolution profiling of chemo-immunotherapy responses and provide a potential **ex vivo platform for personalized treatment selection**.

Conclusion

TME-preserving, multi-histotype NSCLC PDOs provide a physiologically relevant platform for patient-specific drug profiling and may support personalized treatment selection.

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