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Intratumoral influenza A virus infection drives platinum resistance and invasive reprogramming in NSCLC

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Key words: lung cancer, influenza virus, drug metabolism, chemoresistance, EMT, invasion

Abstract

Respiratory viral infections are frequent in patients with non-small cell lung cancer (NSCLC), yet whether intratumoral viral presence directly influences tumor behavior and therapeutic response remains unknown. Here, we identify a respiratory virus-associated drug metabolism program (REVIS-DM), predominantly expressed by tumor cells and linked to poor clinical outcome. Across multiple NSCLC cohorts, a subgroup of tumors with concurrent high REVIS-DM, interferon-stimulated gene and antiviral signatures was associated with reduced survival and increased relapse after chemotherapy. Consistent with this antiviral profile, Influenza A virus (IAV) transcripts were detected in NSCLC and associated with poor outcome and relapse. Mechanistically, IAV infection induced xenobiotic detoxification, epithelial-to-mesenchymal transition, invasion, and platinum resistance *in vitro* and *in vivo*. These effects required viral sensing and NLRP3 inflammasome signaling but were independent of type I/III interferon pathways. Together, our findings establish intratumoral IAV infection as a previously unrecognized driver of tumor-cell plasticity and chemoresistance in NSCLC, revealing virus-induced metabolic vulnerabilities with potential therapeutic relevance.

Introduction

Non-small-cell lung cancer (NSCLC) remains the leading cause of cancer-related mortality worldwide, with a 5-year survival rate below 15% despite major therapeutic advances, including perioperative chemotherapy, immunotherapy, and targeted therapies ¹.

Resistance to treatment and metastases occurrence remain the principal drivers of therapeutic failure ². Multiple mechanisms contribute to chemoresistance, including enhanced DNA repair, epigenetic reprogramming, resistance to apoptosis, drug efflux, and epithelial-mesenchymal transition (EMT) ³. Yet whether exogenous environmental factors, particularly microbial ones, can trigger or amplify such programs in the tumor cell has received limited attention.

The tumor microenvironment (TME) is a critical determinant of NSCLC progression and therapeutic response ^{4,5}. Beyond its immune composition, accumulating evidence indicates that microorganisms shape the TME and influence cancer evolution. The gut microbiota modulates responses to immunotherapy, while intratumoral lung microbiota contributes to tumor progression ^{6,7}. Epidemiological studies further suggest that recurrent respiratory viral infections, particularly with influenza virus, increase lung cancer risk through chronic inflammation and tissue remodeling ⁸. In other hand, in NSCLC patients, these infections are frequent comorbidities associated with more severe disease course and viral persistence ^{9–11}.

Influenza A virus (IAV) can directly infect tumor cells and alter their phenotype ¹², yet the consequences of such infections on TME features, tumor progression, and response to treatments remain poorly understood. IAV single-stranded RNA (ssRNA) is sensed by pattern recognition receptors, including TLR7, triggering NF- κ B and type I/III interferon (IFN) signaling ^{13–15}. We previously demonstrated that TLR7 is highly expressed by tumor cells within the lung TME and that its activation promotes tumor growth, metastasis, and chemoresistance ^{16–18}. More recently, we showed that IAV infection of lung tumor-bearing mice induces sustained intratumoral viral persistence, extensive TME remodeling, and accelerated

tumor progression ¹⁹. Transcriptomic analysis of IAV-infected tumors identified a respiratory virus-associated signature (REVIS) enriched in genes involved in xenobiotic metabolism, detoxification, oxidative stress response, and chemoresistance ¹⁹. Here, we investigate whether IAV infection promotes chemoresistance in NSCLC through induction of such program.

Integrating transcriptomic analyses of human NSCLC cohorts, intratumoral viral transcript detection, single-cell RNA sequencing, functional chemoresistance assays, siRNA and CRISPR/Cas9 gene editing, and 3D invasion models, we identify a previously unrevealed link between intratumoral IAV infection, xenobiotic metabolism, EMT-associated reprogramming, and platinum resistance. Mechanistically, we demonstrate that viral sensing and inflammasome signaling, but not canonical IFN signaling, drive this chemoresistant state, defining a novel vulnerability at the intersection of virology and oncology.

Methods

Cell Lines

Human NSCLC cell lines A549, H1355, and SK-MES, and the nonmalignant bronchial epithelial cell line Beas-2B, were obtained from ATCC. H1975, H1650, and HCC827 cells were kindly provided by S. Mouillet-Richard (Centre de Recherche des Cordeliers, Paris, France). Cells were cultured at 37°C under 5% CO₂ in DMEM/F12 (Gibco, 11320033) supplemented with 10% heat-inactivated FBS (Eurobio, CVFSVF00), 1% Penicillin/Streptomycin (Gibco, 15140122), 1% L-glutamine (Gibco, 25030081), 1% Non-Essential Amino-Acid (Gibco, 11140050), 1% sodium pyruvate (Gibco, 11360070), and 1% HEPES (Gibco, 15630080). Cisplatin-resistant A549 cells, generated by long-term culture in the presence of increasing concentrations of cisplatin, were kindly provided by G. Kroemer's laboratory (Centre de Recherche des Cordeliers, Paris, France). Murine KP lung adenocarcinoma cells derived from *Kras*^{LSL-G12D/+}*Trp53*^{flox/flox} tumors were kindly provided by L Menger (Institut Gustave Roussy, Paris, France). KP cells were cultured in RPMI 1640 Glutamax (Gibco, 61870036) supplemented with 10% heat-inactivated FBS, 1% penicillin/streptomycin, and 1% HEPES. Cells were used for fewer than 15 passages. All cell lines were routinely tested for mycoplasma contamination (Ozyme, MORV0011). Cell lines were not authenticated.

Influenza A Virus Strains

Influenza A virus (IAV) strains A/Scotland/20/74 (H3N2) and A/PR/8/34 (H1N1) were provided by J.M. Sallenave (INSERM UMR 1152, Université Paris Cité, Paris, France). Viruses were propagated in MDCK cells and purified from culture supernatants by sucrose gradient centrifugation.

Reagents

Cisplatin, carboplatin, oxaliplatin, vinorelbine, paclitaxel, fluorouracil, and gemcitabine were obtained from the pharmacy of Cochin hospital (Paris, France).

In vitro IAV Infection

For 2D cultures, A549 cells (1.5×10^5 cells/well) were infected with IAV (MOI = 1) for 2 h, washed, and cultured for the indicated times. For 3D cultures, cells were infected in suspension (1×10^5 cells/mL, MOI = 0.1) for 2 h, washed, and seeded in ultra-low attachment 96-well plates (200 cells/well) for 2-3 days.

RNA Extraction and RT-qPCR

RNA was extracted from cell lysates using NucleoSpin RNA plus or NucleoSpin RNA plus XS kits (Macherey-Nagel, 740984, 740990). RNA from FFPE human tumor samples was isolated using the Maxwell 16 FFPE tissue LEV Purification kit (Promega, AS1260). RNA quality and concentration were assessed using an Agilent 2100 Bioanalyzer.

cDNA synthesis was performed using the high-capacity cDNA reverse transcription kit (Applied Biosystem, 4374966). cDNA concentration was measured using the Qubit ssDNA Assay kit (Invitrogen, Q10212). Quantitative PCR was performed using TaqMan universal master mix II (Applied Biosystems, 4440040) and TaqMan probes listed in **Supplementary Table 1**. Gene expression was normalized to HPRT1, and relative expression was calculated using the $\Delta\Delta C_q$ method. Fold-change was determined by the formula: $FC = 2^{-\Delta\Delta C_q}$.

Multiplex Gene Expression Profiling

Multiplex gene expression analyses were performed using the QuantiGene Plex Gene Expression Assay (Invitrogen). Two custom panels were designed containing genes related to antiviral responses, drug detoxification, EMT, and invasion (**Supplementary Tables 2,3**).

Plates were analyzed on a Luminex FlexMap3D system. Data were normalized using housekeeping genes with Quantigene Plex software.

Flow Cytometry

Cells were harvested 24 h after IAV infection, washed with PBS containing 10% FBS and incubated with anti-mouse CD16/CD32 antibody (1:50; BD Pharmingen, 553142) to block Fc receptors. Cells were then stained with anti-IAV nucleoprotein (NP-FITC) antibody (Invitrogen, clone MA1-7322) following fixation and permeabilization (eBioscience, 88-8824-00). Dead cells were excluded using Live/Dead Aqua staining (Invitrogen, L34965). Samples were acquired on a BD LSRFortessa X-20 flow cytometer (BD Biosciences) and analyzed using FlowJo software v10 (BD Biosciences).

***In vitro* Chemoresistance Assays**

Cells (5×10^3 /well) were seeded in 96-well plates and infected with IAV (MOI = 1) for 2 h. Cells were washed and treated with chemotherapeutic agents at various concentrations either immediately or 24 h postinfection. Cell viability and cytotoxicity were assessed after 48-72 h using XTT (CyQuant XTT, Invitrogen, X12223) and LDH assays (CyQuant LDH, Invitrogen, C20300), respectively. Absorbance was measured using an Infinite 200 pro microplate reader (Tecan). For fluorescence-based cell counting, cells were fixed with 4% paraformaldehyde during 15 min, stained with Hoechst 33342 (NucBlue, Invitrogen, R37605), and imaged using an Operetta CLS high-content imaging system. Cell viability was normalized to untreated controls.

siRNA-Mediated Gene Silencing

Cells were transfected with siRNAs (FlexiTube, Qiagen) targeting *MYD88*, *MAVS*, *STAT1*, or *IL28RA* using Lipofectamine RNAiMAX (Invitrogen, 13778150) at a final concentration of 10 μ M. After 24 h, cells were cultured in fresh medium for an additional 24 h before IAV infection

(H3N2, MOI = 1). After 2 h of incubation, cells were washed to remove unbound viral particles. RNA was collected 24 h post-infection for RT-qPCR analysis. For chemoresistance assays, cells were treated with cisplatin (20 μ M) and viability was assessed after 72 h using XTT assays.

CRISPR/Cas9-Mediated IFNAR1 Knockout

IFNAR1 knockout A549 cells were generated by CRISPR/Cas9 with guide sequence ACAGGAGCGAUGAGUCUGUC (Transcript ID: ENST00000270139.8; Synthego). Single-cell clones were obtained by limiting dilution. Functional IFNAR1 knockout was validated by IFN- β stimulation (100 ng/mL, 18 h) followed by ISG15 quantification.

***In vivo* Chemoresistance Assay**

B6(C)/Rj-Tyr^{c/c} mice (Janvier Labs) were subcutaneously injected with 1×10^5 KP cells. Once tumors became palpable (day 16), mice were randomized into four groups and intratumorally injected with IAV (H3N2, 150 PFU in 40 μ L PBS) or PBS. Mice subsequently received intratumoral cisplatin injections (100 μ M, 40 μ L) on days 18, 21, and 24. Tumor growth was monitored every 2–3 days using caliper measurements. Tumor volume was calculated as (length $\times$ width $\times$ height) $\times \pi/6$.

A549 Spheroid Invasion Assays

Non-infected or infected (H3N2, MOI = 1) spheroids of 200 cells (A549) were formed in U-bottom ultra-low attachment (Thermo scientific, Nunclon Sphera, 174925) plates were and embedded in 2 mg/mL collagen I hydrogels (prepared from rat-tail collagen provided by C Helary, Laboratoire de Chimie de la Matière Condensée de Paris, Sorbonne Université, CNRS, Paris, France), and supplemented with 10x M199 medium (Sigma-Aldrich, Saint-Louis MO, USA). The collagen solution was buffered at pH 6.5 at 20°C using NaHCO₃. Embedded spheroids were cultured for 2 days in FB96 phenoplates (Revvity, Waltham, MA, USA) in supplemented DMEM/F12 as previously described. Then, spheroids were stained with

phalloidin as described previously ²⁰ and imaged using a spinning-disk confocal microscopy (Orion platform, College de France, Paris). Invasion was quantified using a custom ImageJ plugin developed by the Orion imaging platform. The plugin was based on a Sholl analysis, in which concentric circles are constructed around the spheroids and the number of nuclei and phalloidin-positive areas are quantified within each radial distance.

Detection of IAV in A549 Spheroids

A polyclonal anti-HA antibody (Invitrogen, PA5-34930) was conjugated to Alexa Fluor (AF) 488 (Invitrogen, A10235) and validated by flow cytometry. Spheroids were fixed in 4% paraformaldehyde for 30 min, permeabilized with PBS-Triton 0.5% for 30 min at room temperature and stained with phalloidin conjugated to AF-555 (Invitrogen, A34055; 1:200) and anti-HA-AF488 antibody (1:50) overnight at 4°C. Spheroids were then embedded in 1% low-melting agarose, cleared overnight in RapiClear 1.52 (Sunjin Lab, RC152001), and imaged using spinning-disk confocal microscopy (Orion platform, College de France, Paris). Images were analyzed using ImageJ.

Transcriptomic Analyses of NSCLC Cohorts

Gene expression (log-transformed FPKM) and clinical data from the TCGA LUAD cohort (n = 473) were downloaded from the Genomic Data Commons portal (<https://portal.gdc.cancer.gov/>). Differentially expressed genes were identified using Wilcoxon rank-sum tests. Fold-changes were obtained from log-transformed expression values as differences in median expression between groups. Bulk RNA-seq datasets from two independent NSCLC cohorts, Onco-Cochin ²¹ and Onco-HEGP ²², were analyzed as validation cohorts. These cohorts included patients treated with perioperative chemotherapy (neo-adjuvant or adjuvant chemotherapy in the Onco-HEGP cohort and neoadjuvant chemotherapy in the Onco-Cochin cohort). Differentially expressed genes were identified using the package *DESeq2* and normalized gene expression matrices were obtained using the VST method. Associated

clinical annotations were obtained from the corresponding studies. Gene set enrichment analysis (GSEA) was performed using the *clusterProfiler* package with KEGG and GO databases or custom signatures. Immune cell deconvolution was performed using MCP-Counter. The IAV-imprint signature was derived from an initial gene set comprising the ISG and Influenza A signatures using penalized logistic regression (glmnet package; elastic net, $\alpha = 0.4$). The model contrasted IAV-positive and relapsing patients against all remaining patients, with cross-validation to optimize performance. The final signature retained the top 75% of positively weighted coefficients. Gene signature scores were then computed either as the mean of normalized expression values across genes within each signature or using single-sample gene set enrichment analysis (ssGSEA).

Single-cell RNA sequencing Analysis

Single-cell RNA-seq data from GSE123904 were obtained from the Gene Expression Omnibus and analyzed using the R package *Seurat* v4. The dataset is composed of 6,180 cells including 530 epithelial cells (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE123904>).

Viral Sequence Detection

RNA-seq data from the Onco-HEGP cohort were analyzed to identify respiratory viral sequences, with a particular focus on IAV. Viral detection was performed using VirusBreakend version griss_2.13.2 (<https://github.com/PapenfussLab/VIRUSBreakend>) within a Singularity containerized environment. VirusBreakend implements a host-subtraction approach to detect non-human sequences in high-throughput sequencing data. Briefly, RNA-seq reads were aligned to the human reference genome (GRCh38) using STAR, and unmapped reads were retained for downstream viral analysis. Unmapped reads were subsequently classified against the VirusBreakend reference database (virusbreakenddb_20210401), using a k-mer-based taxonomic classification strategy relying on Kraken-like algorithms. Taxonomic assignments were aggregated at multiple levels (genus, species, and assigned taxa), and summarized in the

summary.tsv output file. This file includes taxonomic identifiers, lineage information, and quantitative metrics such as the number of supporting reads (e.g., reads_assigned, reads_species_tree) and coverage estimates. These metrics were used to assess the confidence of viral detection. Only samples with consistent viral assignments and detectable coverage were considered positive.

To validate these findings, an independent analysis was performed using the nf-core/viralmetagenome pipeline version 1.0.1 (<https://github.com/nf-core/viralmetagenome>) an untargeted metagenomic workflow designed for viral detection, genome reconstruction, and intra-host variant analysis. In this workflow, reads were first filtered against a human Kraken2 database (kraken2_human), and remaining reads were subsequently classified against the Virosaurus vertebrate viral database (Virosaurus; virosaurus90 Vertebrate-20200330). Viral assignments were extracted from the Kraken2 reports (*kraken2.report.txt), which provides hierarchical taxonomic assignments along with read abundance estimates.

Using both approaches, IAV transcripts were consistently detected in 33 of 167 samples, with complete concordance between pipelines. The use of two independent host-depletion and viral-classification strategies reduced the likelihood of false-positive viral identification.

All analyses were performed in a Linux high-performance computing environment using Singularity containers and the SLURM workload manager.

Statistical analyses

Statistical analyses were performed using R v4.5.2. Two-group comparisons were performed using Wilcoxon rank-sum tests unless otherwise indicated. Heatmap and hierarchical clustering (dendrograms) were generated using the *pheatmap* R package. Correlations were performed using *Hmisc* and *Corrplot*. Survival analyses were performed using *survival*, *survminer* and *ggsurvfit* packages. Survival analyses were conducted using Kaplan–Meier estimates and log-

rank tests. Figures were plotted using *ggplot2*, *ggallin* and *ggpubr* and statistics performed with *ggsignif* or *rstatix*. *P*-values $P < 0.05$ were considered statistically significant.

Results

An Interferon Stimulated Gene-associated drug metabolism signature identifies NSCLC patients with poor clinical outcome

Within the REVIS gene expression signature identified in lung tumors from IAV-infected mice¹⁹ and enriched in genes involved in xenobiotic metabolism, detoxification, oxidative stress response, and chemoresistance, we defined a 13-gene drug metabolism sub-signature, termed REVIS-DM, comprising transcription factors (*NR1I2*, *HNF4A*, *HNFAG*), detoxification enzymes (*CYP3A4*, *CYP3A5*, *CYP3A7*, *SULT1B1*, *GSTA1*, *CES2*, *AKR1B10*, *UGT2A3*), and ROS-associated genes (*DUOX2*, *DUOX2A2*), several of which have previously been implicated in platinum resistance and tumor progression.

We first investigated the expression of this signature in 473 NSCLC patients from the LUAD TCGA cohort. The 13 genes displayed strong co-expression across samples, as revealed by correlation analysis (**Extended data Fig. 1a**). A REVIS-DM score, calculated as the mean expression of the signature genes, identified a subset of patients with high REVIS-DM expression (**Extended data Fig. 1b**).

To explore the potential link between REVIS-DM expression and prior viral exposure, we next analyzed the expression of type-I IFN-related genes and ISGs, which are known to be induced by the influenza infection¹⁵. We defined an ISG signature composed of 49 genes curated from the literature (**Supplementary Table 4**), including canonical antiviral effectors such as 2'-5'-oligoadenylate synthetase (OAS), protein kinase R (PKR) and Mx1. These genes were highly co-expressed (**Extended data Fig. 1c**), and gene set enrichment analysis using the KEGG database confirmed a strong association with IAV infection pathways (**Extended data Fig. 1d**), supporting the use of this signature as a surrogate marker of antiviral signaling in the TME.

An ISG score was computed for each patient, and its relationship with REVIS-DM was assessed. Although no linear correlation was observed between the two signatures, a subset of patients exhibited concurrent high expression of REVIS-DM and ISG scores (**Fig. 1a**). Unsupervised K-means clustering identified three patient groups: group 1 (green, 39 patients, 8.2%) characterized by high REVIS-DM and ISG expression, group 2 (orange, 245 patients, 53.5%) with high ISG expression alone, and group 3 (blue, 189 patients, 38.3%) with low expression of both signatures (**Fig. 1b**). Importantly, patients with concurrent high REVIS-DM and ISG expression (group 1) exhibited significantly worse overall survival compared to the other groups, while patients with elevated ISG expression alone (group 2) also showed reduced survival relative to group 3 (log-rank test, $p = 0.018$) (**Fig. 1c**). These results indicate that the combination of antiviral signaling and drug metabolism programs identifies a subset of NSCLC patients with particularly poor prognosis.

Consistent with this observation, groups 1 and 2 patients displayed increased expression of the interferon-related DNA damage resistance signature (IRDS), previously associated with resistance to chemo- and radiotherapy²³, as well as elevated Influenza A (KEGG signature) score (**Extended Data Fig. 1e**).

A xenobiotic metabolism program defines the TME of high-risk LUAD patients

We next investigated the TME of patients with concurrent high REVIS-DM and ISG expression (group 1). Differential gene expression analysis identified 407 genes significantly upregulated in this group compared to other patients. In addition to the REVIS-DM genes, these included multiple enzymes involved in xenobiotic metabolism and drug detoxification, such as *CYP2C9*, *CYP2C18*, *CYP2C19*, UGT1A family members including *UGT1A1*, *UGT1A3*, *UGT1A6*, *UGT1A8*, and *UGT1A10*, and *SULT1C3* (**Fig. 1d**). Gene set enrichment analysis using Gene Ontology Biological Process databases identified 157 significantly enriched pathways (adjusted $P < 0.05$, NES > 1) (**Supplementary Table 5**), including the top 12 shown in **Fig. 1e**. A

substantial proportion of these pathways was related to xenobiotic metabolism (14%), lipid metabolism (13.4%), and immune responses (10.8%) (**Fig. 1f**). Together, these results indicate that high-risk NSCLC tumors are characterized by a coordinated activation of xenobiotic metabolism and detoxification programs within the TME, consistent with a potential role in therapeutic resistance.

A neutrophil-enriched immune TME characterizes high-risk NSCLC patients

Given that 10.8% of the enriched pathways were related to immune response, we next characterized the immune landscape of group 1 tumors. Enriched immune signatures were mainly associated with immune cytotoxicity, antibacterial defense, and acute inflammation (**Extended Data Fig. 2a**). Immune deconvolution using MCP-counter revealed distinct immune landscapes across patient groups. Tumors with high ISG and low REVIS-DM expression (group 2) exhibited increased immune infiltration, including T cells, CD8⁺ T cells, cytotoxic lymphocytes, NK cells, B cells, myeloid dendritic cells and monocytic lineage, compared to the two other groups (**Extended Data Fig. 2b**). In contrast, group 1 tumors were specifically enriched in neutrophils, whose abundance positively correlated with REVIS-DM expression (**Extended Data Fig. 2b,c**). Finally, unsupervised clustering based on immune infiltration identified two patient groups, immune-high (54%) and immune-low (46%) (**Extended Data Fig. 2d**). As expected, immune-high tumors were associated with improved overall survival (**Extended Data Fig. 2e**). Although REVIS-DM/ISG groups significantly associated with immune stratification (χ^2 test, $p = 1.87 \times 10^{-9}$), only group 2 was enriched in immune-high tumors (standardized residual = 5.80), whereas group 3 was enriched in immune-low tumors (standardized residual = 6.27) (**Extended Data Fig. 2f**). In contrast, group 1 showed no preferential distribution between immune classes, indicating that its poor prognosis cannot be explained by differences in overall immune infiltration alone.

Clinical features of REVIS-DM/ISG-defined patient groups

Clinical characteristics were compared across the three patient groups defined by REVIS-DM and ISG expression (**Supplementary Table 6**). Patients with concurrent high REVIS-DM and ISG expression (group 1) were enriched for males and older individuals. In contrast, no significant differences were observed in tumor stage, tumor size, or major oncogenic alterations, including *EGFR* and *KRAS* mutation and *EML4/ALK* translocations. No differences in smoking history were observed between groups, indicating that concurrent REVIS-DM and ISG expression is independent of tobacco exposure.

Response to chemotherapy and relapse are associated with response to viral infection, xenobiotic detoxification, and EMT signatures.

Given the concurrent expression of xenobiotic metabolism and ISG programs, we hypothesized that viral infection-associated transcriptional responses may contribute to chemotherapy resistance and tumor relapse. We therefore investigated whether NSCLC patients with poor response to chemotherapy or post-treatment relapse displayed enrichment of antiviral and drug detoxification signatures. Transcriptomic analysis of the Onco-Cochin cohort (n = 13 early-stage NSCLC patients treated with neo-adjuvant chemotherapy; **Supplementary Table 7**) revealed that non-responders to chemotherapy, as well as patients who subsequently relapsed, were significantly enriched for signatures related to viral infection, chemoresistance (xenobiotic detoxification), and metastasis (epithelio-mesenchymal transition) (**Fig. 2a; Extended Data Fig. 3a,b; Supplementary Tables 8-9**). These findings were independently validated in the Onco-HEGP cohort (n = 43 patients treated with neoadjuvant or adjuvant chemotherapy; **Supplementary Table 10**), which confirmed the association between poor clinical outcome and enrichment of antiviral, detoxification, and EMT programs (**Fig. 2b; Extended Data Fig. 3c,d; Supplementary Tables 11-12**). Additional pathways associated with relapse (Onco-HEGP cohort) and non-response to chemotherapy (Onco-Cochin cohort) included DNA

replication and cell cycle progression, mRNA processing and protein synthesis, cell metabolism and respiration, and immune-related pathways (**Extended Data Fig. 4**). Together, these results indicate that chemotherapy resistance and relapse in NSCLC are associated with a transcriptional program combining antiviral signaling, xenobiotic metabolism, and mesenchymal reprogramming.

Intratumoral IAV detection is associated with relapse after chemotherapy

Since viral infection-related pathways were enriched in non-responders and relapsing patients, we investigated the presence of viral transcripts in tumor samples. Analysis of bulk RNA-seq data from the Onco-HEPG cohort using the VirusBreakend and nf-core/viralmetagenome pipelines detected respiratory viral sequences in 45% of tumors, with IAV representing the most frequently identified virus (46% of respiratory virus-positive cases) (**Fig. 3a**). To assess the biological impact of intratumoral IAV, we compared the transcriptomic profiles of IAV-positive and respiratory virus-negative tumors from patients treated with chemotherapy alone. Differential gene expression analysis identified widespread transcriptional changes (**Fig. 3b**) and enrichment of 340 biological pathways (**Extended Data Fig. 5a; Supplementary Table 13**). IAV-positive tumors displayed strong enrichment of pathways related to viral replication and host antiviral responses, including ISG, Influenza A (KEGG) and IRDS signatures (**Fig. 3c; Extended Data Fig. 5a,b**), together with pathways related to EMT and oxidative stress (**Fig. 3d; Extended Data Fig. 5a,b**), supporting the biological relevance of viral detection within the tumor. Clinically, IAV-positive patients exhibited poorer outcomes following chemotherapy. Although the overall proportion of relapsing patients did not differ according to viral status (**Extended Data Fig. 6a**), IAV-positive patients exhibited significantly shorter disease-free survival (DFS) and a trend toward reduced five-year progression-free survival (PFS) (**Fig. 3e,f**).

We then compared IAV⁺ patients who relapsed to those without relapse, and identified differentially expressed genes between the two groups (**Extended Data Fig. 6b**). Relapsing patients showed enrichment of antiviral signaling (including the ISG and the Influenza A signatures), EMT (tissue remodeling and TGF- β signaling), oxidative stress and detoxification (**Extended Data Fig. 6c-f; Supplementary Table 14**). To further independently identify the best predictive signature of IAV infection and post-chemotherapy relapse, we generated an IAV-imprint signature using penalized logistic regression (LASSO) to discriminate IAV-positive relapsing tumors from all other patients (**Supplementary Table 15; Extended Data Fig. 7a**). This signature was specifically enriched in IAV-positive patients who relapsed compared with both non-infected and IAV-positive patients without relapse (**Extended Data Fig. 7b**) and independently associated with an increased risk of relapse in treated patients in multivariable Cox analysis (HR > 1, P = 0.0236) (**Extended Data Fig. 7c**).

Together, these findings demonstrate that intratumoral IAV detection is associated with relapse after chemotherapy and support a link between viral persistence, antiviral signaling, and treatment resistance, and identifies patients at increased risk of relapse following perioperative chemotherapy.

REVIS-DM is predominantly expressed by tumoral cells within the TME

Next, to identify the cells expressing REVIS-DM within the TME, we analyzed publicly available single-cell RNA sequencing data from NSCLC tumors (GSE123904). UMAP visualization enabled the identification of major cell populations including: myeloid cells, T and B lymphocytes, NK cells, mast cells, fibroblasts, endothelial cells, and tumor cells (**Fig. 4a**). Cell-type annotation was performed based on canonical marker gene expression (**Supplementary Fig. 1**). The majority of REVIS-DM genes were selectively expressed in tumor cells, including *GSTA1*, *CES2*, *CYP3A5*, *AKR1B10*, *DUOX2*, *DUOX2*, *NR1H2*, *HNF4A*,

HNF4G, *SULT1B1*, and *CYP3A4*, whereas viral sensor genes and ISGs were broadly expressed across multiple cell types within the TME (**Fig. 4b**).

Together, these results indicate that antiviral signaling is broadly distributed across the tumor microenvironment, whereas the REVIS-DM program is predominantly tumor cell-intrinsic. The coexistence of these two programs in a subset of patients with poor clinical outcome suggests a potential mechanistic link between antiviral responses and the induction of tumor cell drug metabolism. We therefore hypothesized that Influenza A virus directly induces a drug metabolism program in tumor cells, promoting chemoresistance. To test this hypothesis, we infected NSCLC tumor cells with Influenza A virus and assessed the induction of drug metabolism programs and response to chemotherapy.

IAV infection of lung tumor cells induced the expression of REVIS-DM

To determine whether IAV directly induces REVIS-DM expression in tumor cells, A549 lung adenocarcinoma cells were infected with the H3N2 (A/Scotland/20/74) strain. Efficient infection was confirmed by flow cytometry (70-77% infected cells at 24 h, MOI 0.5-1) and RT qPCR, along with robust induction of antiviral response genes, including *IFNB1* and *ISG15* (**Supplementary Fig. 2a,b**).

Time-course analysis (8-48 h post-infection) using a multiplex gene expression assay revealed coordinated activation of viral sensing pathways, with upregulation of *TLR3*, *TLR7*, *TLR8*, *DDX58* (RIG-I), and *IFIH1* (MDA5), and downstream *MYD88* and *TICAM1* signaling components, as well as type I IFNs (**Fig. 4c**), with a peak activity 24 h post-infection.

Strikingly, IAV infection induced the expression of multiple REVIS-DM genes, including *NR1I2*, *CYP3A4*, *CYP3PA7*, *CES2*, *SULT1B1*, *GSTA1*, *UGT2A3*, *DUOX2* and *DUOXA2*, *HNF4G* and *CES2* (**Fig. 4d**). This was accompanied by broader activation of drug metabolism and detoxification pathways, with induction of the transcription factor *NR1I3* (CAR), multiple

P450 enzymes, including various *CYP450* isoforms, phase II detoxification enzymes such as *GST* and *UGT*, and the efflux transporter *ABCB1* (MDR1) (**Fig. 4e**). In contrast, other transcription factors involved in xenobiotic sensing (*AHR*, *NR1H3*, *NR1H4*, *RXRA*), phase III enzymes, and those involved in glutathione metabolism were not significantly induced (with the exception of *ABCB1*) (**Fig. 4e**).

The overexpression of selected genes, including *CYP2C8*, *DUOX2*, *GSTA1*, and *NR1I2* was validated by RT-qPCR, and was reproduced using a distinct IAV strain (H1N1, PR8) (**Supplementary Fig. 2c**).

IAV infection promotes platinum chemoresistance

To assess the functional consequences of IAV-induced drug metabolism programs, we investigated the response of lung tumor cells to various drugs commonly employed in patient treatment. A549 cells were infected with IAV (MOI = 1) and subsequently treated with platinum-based drugs, including cisplatin, carboplatin, and oxaliplatin. As expected, platinum treatment induced a dose-dependent decrease of cell viability at 48 and 72 h. However, IAV infection significantly reduced platinum-induced cytotoxicity at both time points, indicating the acquisition of chemoresistance (**Fig. 5a**).

These findings were corroborated using complementary approaches, including nuclear quantification using high-content imaging (Operetta CLS™) and LDH release assay, both showing increased cell survival and reduced cytotoxicity in infected cells following cisplatin treatment (**Fig. 5b-d**). Similar results were obtained with another strain of IAV (H1N1 PR8), and in an additional adenocarcinoma cell line (H1355) (**Extended Data Fig. 8**). This effect was not observed, or minimal, in non-tumoral bronchial epithelial cells (Beas-2B), in EGFR-mutant and KRAS-WT adenocarcinoma cells (H1650, HCC827, H1975), and in squamous carcinoma cells (SK-MES), indicating tumor subtype specificity (**Extended Data Fig. 8b,c**).

To evaluate whether this phenotype extended to combination therapies, cells were treated with second-generation chemotherapeutic agents, commonly used with platinum salts. While IAV infection did not confer resistance to these agents when used alone (vinorelbine, paclitaxel, fluorouracil, gemcitabine), resistance was maintained when combined with cisplatin (**Fig. 5e**), indicating a platinum-specific effect. Delayed treatment experiments further showed that chemoresistance persisted when platinum administration was initiated 24 h after infection, although to a lesser extent (**Extended Data Fig. 8d,e**). These data suggest that early viral-induced reprogramming contributes to this phenotype.

Finally, we assessed the expression of IAV-induced genes in response to cisplatin, alone or in combination with IAV infection. This analysis revealed a partial overlap in the transcriptional response to cisplatin and IAV infection, with *DUOX2*, *NR1I2*, and *ISG15* being upregulated by both conditions, whereas *IFNB1* was induced exclusively by IAV infection. Concomitant infection and cisplatin treatment in A549 cells did not alter the expression of *DUOX2* or *NR1I2* and only slightly attenuated the overexpression of *IFNB1* and *ISG15* (**Supplementary Fig. 2d**).

To assess the *in vivo* relevance of these findings, KP tumor-bearing mice, a tumor model in which we previously demonstrated the pro-tumoral effects of IAV ¹⁹, were intratumorally infected with IAV (H3N2) and treated with cisplatin. As expected, cisplatin induced tumor regression in non-infected mice. In contrast, IAV-infected tumors failed to respond to cisplatin, with sustained tumor growth despite treatment (**Fig. 5f**). These results demonstrate that IAV infection promotes resistance to cisplatin *in vivo*. Collectively, these findings establish that IAV infection induces a tumor cell-intrinsic program that selectively confers resistance to platinum-based chemotherapy.

Viral sensing pathways drive IAV-induced platinum resistance independently of IFN signaling

To dissect the mechanism underlying IAV-induced chemoresistance, we silenced key components of antiviral signaling. Given that MyD88 and MAVS activate NF- κ B signaling and promote inflammasome activation, we also investigated the contribution of the NLRP3 inflammasome. *MYD88*, *MAVS*, *STAT1*, *IL28RA*, and *NLRP3* were silenced using siRNA, whereas *IFNAR1* was disrupted by CRISPR/Cas9. Efficient inhibition was confirmed by RT-qPCR following IAV infection for *MYD88*, *MAVS*, and *STAT1* (**Supplementary Fig. 3a**), or following IFN- β or IFN- λ stimulation for *IFNAR1* and *IL28RA*, respectively, using *ISG15* expression as a readout (**Supplementary Fig. 3b,c**). To thoroughly inhibit IFN signaling, *IFNAR1*-deficient cells were further transfected with *IL28RA*-targeting siRNA. Combined inhibition of type I and type III IFN pathways significantly reduced *MX1* and *OAS1* induction following IAV infection, whereas *ISG15* expression remained partially preserved, consistent with IFN-independent regulation (**Supplementary Fig. 3d**).

Multiplex gene expression analysis further confirmed the biological impact of pathway inhibition, as silencing *MYD88*, *MAVS*, or *NLRP3* markedly reduced the induction of viral sensors, inflammatory cytokines, ISGs, and REVIS-DM genes following infection (**Fig. 6a**).

Functional assays revealed distinct contributions of these pathways to platinum resistance. Silencing *MYD88*, *STAT1*, or *NLRP3* abolished IAV-induced chemoresistance, as evidenced by the absence of increased viability in infected and cisplatin-treated cells, and by the lack of reduction in LDH release. Inhibition of *MAVS* significantly attenuated, but did not fully suppress the IAV-induced chemoresistance. In contrast, *IFNAR1* knockout alone had no detectable effect, and combined inhibition of type I and type III IFN signaling produced only a modest reduction in cisplatin resistance without abolishing the increased viability of infected cells (**Fig. 6b-d**). Collectively, these findings demonstrate that IAV-induced platinum

resistance depends on additive mechanisms involving viral sensing and inflammasome pathway, whereas type I and type III IFN signaling is largely dispensable.

IAV induces a mesenchymal-like phenotype and invasiveness of lung tumor cells

Because EMT has been linked to chemoresistance^{24,25}, we assessed whether IAV infection promotes mesenchymal features in tumor cells. Cisplatin-resistant A549 cells (**Fig. 7A**) displayed a mesenchymal-like morphology, characterized by spindle-shaped cells and dispersed growth, whereas sensitive cells retained an epithelial cobblestone appearance (**Fig. 7b**). Notably, microscopic examination suggested that IAV-infected cells more often exhibited a mesenchymal-like morphology (**Fig. 7b**).

Consistently, IAV infection induced transcriptional reprogramming toward an EMT phenotype. Expression of epithelial markers *CDH1* and *KRT19* was reduced, while mesenchymal markers *VIM* and *ZEB2*, and EMT-associated transcription factor *SNAIL3* were upregulated (**Fig. 7c**). This was accompanied by differential expression of extracellular matrix remodeling genes (*MMP2*, *MMP7*, and *MMP9*), collagens (*COL18A1*, *COL1A2* and *COL4A3*), pro-invasive markers (*LYZ*, *ACTB* and *GREM1*), integrins (*ITGA5*, *ITGAV*, *ITGB1*), cytokines and growth factors associated with EMT (*IL6*, *IL8*, *IL33*, *CXCL9*, *IFNG*, *TNF*, *TFGA*, *TGFB2*, and *TGFB3*), and multiple fibroblast growth factors (**Fig. 7c**). Together, these findings indicate that IAV infection drives EMT-like reprogramming of tumor cells, consistent with the acquisition of a chemoresistant phenotype.

To investigate whether IAV-induced EMT also enhances the invasiveness of A549 cells, we established a 3D culture model using A549 spheroids embedded in collagen I hydrogels²⁰. A549 cells were infected with IAV (MOI = 0.1) just before spheroid formation or 24 h after. Infection was confirmed by qPCR (**Supplementary Fig. 4a**) and 3D microscopy using a spinning-disk confocal microscope (**Supplementary Fig. 4b**). When cultured in cell-repellent plates, A549 cells spontaneously form spheroids and exhibit limited invasive capacity when

embedded in collagen I hydrogels. Five days after embedding, spheroids derived from IAV-infected cells displayed prominent invasive protrusions, indicating that IAV enhances A549 cell migration within the hydrogel (**Fig. 7d**). Spheroid area was quantified on 36 spheroids from six independent experiments, and spheroid perimeter was measured on 23 spheroids from three independent experiments using total stack projections. These analyses confirmed significant increases in both area and perimeter in IAV-infected spheroids compared to controls (**Fig. 7e**). Invasion was further quantified on 19 spheroids from three independent experiments using Sholl analysis, which confirmed that IAV infection significantly increased spheroid invasiveness relative to non-infected controls (**Fig. 7f**).

Discussion

Our study identifies intratumoral IAV infection as a previously unrecognized driver of platinum resistance and invasive reprogramming in NSCLC. By integrating transcriptomic analyses of human cohorts with functional *in vitro* and *in vivo* models, we demonstrate that IAV infection induces a drug metabolism program, termed REVIS-DM, associated with poor clinical outcome, relapse after chemotherapy, and increased tumor cell invasiveness.

Analysis of the TCGA LUAD cohort revealed a subgroup of patients exhibiting concurrent high REVIS-DM and antiviral ISG signatures associated with reduced survival. Importantly, detection of viral transcripts in an independent cohort further linked intratumoral IAV sequences with relapse after perioperative chemotherapy, supporting the clinical relevance of respiratory viral persistence in NSCLC patients. Single-cell RNA-seq analyses demonstrated that REVIS-DM is predominantly expressed by tumor cells, indicating that virus-induced metabolic reprogramming occurs directly within the malignant compartment.

Together, these findings suggest that intratumoral IAV persistence reshapes the NSCLC tumor microenvironment to promote chemotherapy resistance and invasive progression. These observations complement recent studies showing that IAV infection reactivates dormant metastatic breast cancer cells in lungs thereby promoting lung metastasis ²⁶. Moreover, IAV exerts pro-tumoral effects in NSCLC through neutrophil-dependent remodeling of the TME when infection occurs prior to tumor initiation ¹¹ in line with the observed correlation between REVIS-DM expression and neutrophil infiltration, further highlighting the major clinical consequences of IAV infection in lung cancer.

Mechanistically, IAV infection induced broad activation of xenobiotic detoxification pathways, including upregulation of the transcriptional regulators *PXR/NR1I2*, *CAR/NR1I3*, *HNF4A*, and *HNF4G*, together with phase I cytochrome P450 enzymes (*CYP3A4*, *CYP3A5*, *CYP3A7*, and *CYP2C8*) and phase II detoxification enzymes, including *GSTA1* and *UGT2A3*. These pathways

coordinate xenobiotic metabolism and drug detoxification and have been broadly implicated in multidrug resistance, including resistance to platinum-based chemotherapy^{27–30}. In contrast, phase III transporters were minimally modulated, with the notable exception of *ABCB1*, a major mediator of multidrug chemoresistance. These findings suggest that viral infection promotes a selective metabolic adaptation favoring platinum tolerance rather than a generalized multidrug resistance program.

Oxidative stress likely contributes to this reprogramming. Reactive oxygen species (ROS) are known to activate NRF2-dependent antioxidant responses, leading to induction of detoxification pathways including GSTs, GPXs, AKRs, NQO1, HO-1, CYPs, UGTs, and drug efflux transporters^{31–34}. Consistent with this mechanism, we observed marked upregulation of DUOX2 and DUOXA2 following IAV infection.

These ROS producing enzymes are involved in antiviral responses^{35,36} and have previously been associated with chemoresistance and EMT in several cancer models^{37–39}. In parallel, IAV infection induced multiple antioxidant and xenobiotic metabolism genes, suggesting activation of adaptive oxidative stress responses in infected tumor cells. Although ROS contribute to the cytotoxic effects of many chemotherapeutic agents, persistent oxidative stress can also promote survival programs associated with platinum resistance³⁴. Together, these findings support a model in which virus-induced oxidative stress contributes to both chemoresistance and mesenchymal reprogramming in NSCLC cells.

Our findings further identify viral sensing pathways as central mediators of IAV-induced chemoresistance. Because chronic IFN exposure and persistent ISG induction have previously been associated with therapeutic resistance⁴⁰, and resistance to DNA-damaging agents through the IRDS²³, we initially hypothesized that type I or type III IFN signaling could mediate the resistant phenotype observed following infection. Unexpectedly, however, inhibition of *IFNAR1* and *IL28RA* signaling failed to fully reverse IAV-induced chemoresistance, despite

efficient suppression of ISG induction. In contrast, silencing of *MYD88*, *MAVS*, *STAT1* or *NLRP3* abolished or reduced platinum resistance, demonstrating that chemoresistance depends primarily on innate viral sensing rather than canonical type I or type III IFN signaling. These findings suggest that persistent activation of inflammatory and stress-response pathways downstream of viral recognition may be sufficient to drive tumor cell adaptation independently of IFN-mediated antiviral programs.

Inflammatory signaling likely contributes to the mesenchymal and invasive reprogramming induced by IAV infection. Inflammation within the TME is known to contribute to tumor progression through production of cytokines and growth factors that support tumor cell survival, EMT, and invasion ^{41,42}. Consistent with this model, infected A549 cells exhibited increased expression of multiple inflammatory mediators, growth factors, and TGF- β signaling components known to initiate EMT programs ⁴³. The association between EMT and resistance to therapy has been consistently observed across human studies and experimental ^{24,25,44,45}. Here, IAV infection induced a mesenchymal-like phenotype characterized by decreased epithelial marker expression and increase of mesenchymal markers, consistent with EMT induction. These findings align with previous studies emphasizing that IAV disrupts epithelial integrity and promotes EMT through neuraminidase-dependent activation of Snail and TGF- β signaling and in lung epithelial cells ⁴⁶. Our findings reveal that A549 cells resistant to cisplatin exhibit a mesenchymal phenotype, consistent with observations that mesenchymal clones of lung tumor cells generally exhibit reduced sensitivity to cisplatin ²⁵.

At functional level, IAV infection enhanced tumor cell invasiveness in 3D spheroids embedded in collagen I hydrogel. Increased protrusions were associated with upregulation of extracellular matrix remodeling molecules, including MMP2, MMP3, MMP9, COL1A2, and COL3A1. These molecules have previously been linked to NSCLC aggressiveness, metastasis, poor clinical outcomes, and resistance to cisplatin ⁴⁷⁻⁴⁹. We additionally observed the increased

expression of several pro-invasive markers, including *ACTB*, *ITGA5*, *ITGB1*, *LYZ*, and *GREM1*, further supporting the acquisition of an invasive phenotype following infection.

Collectively, our study uncovers respiratory viral persistence as a clinically relevant determinant of therapeutic resistance and tumor progression in NSCLC. These findings reveal that intratumoral IAV infection directly reprograms tumor cells toward a chemoresistant and invasive state and suggest that targeting virus-induced inflammatory and metabolic pathways may represent a therapeutic opportunity in lung cancer.

Conclusion

Collectively, these findings underscore the potent role of IAV infection in shaping the TME towards a more invasive one, and conferring resistance to chemotherapy. This highlights the importance of considering these factors in the clinical management of NSCLC patients, and confirms the importance of vaccination for both short-term and long-term benefits in healthcare of NSCLC patients. Furthermore, vaccination has been demonstrated to be safe and efficient in lung cancer patients, even those undergoing immune checkpoint blockade therapy⁵⁰. In the long-term, identifying viral infection biomarkers in the TME could facilitate more personalized therapeutic choices.

Authors contribution

IC and PEF designed the study. PEF, NJ, PC, FH, EF, KK, CC, VR, LB, IG and AR performed the *in vitro* 2D and 3D experiments and analyses. SM performed the *in vivo* experiments and analyses. AS, AML, DD and MA. provided the Onco-Cochin cohort. SMR provided lung tumor cells. SG and HB provided the Onco-HEGP cohort. IGV prepared and provided IAV. PEF, TB, HG, CG, ADR performed bioinformatic analyses. PEF generated the figures and tables. PEF and IC wrote the manuscript. PEF, DS, SMR, ADR, CM, HB and IC reviewed the manuscript. All the authors discussed the results and commented on the manuscript.

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Figure 1

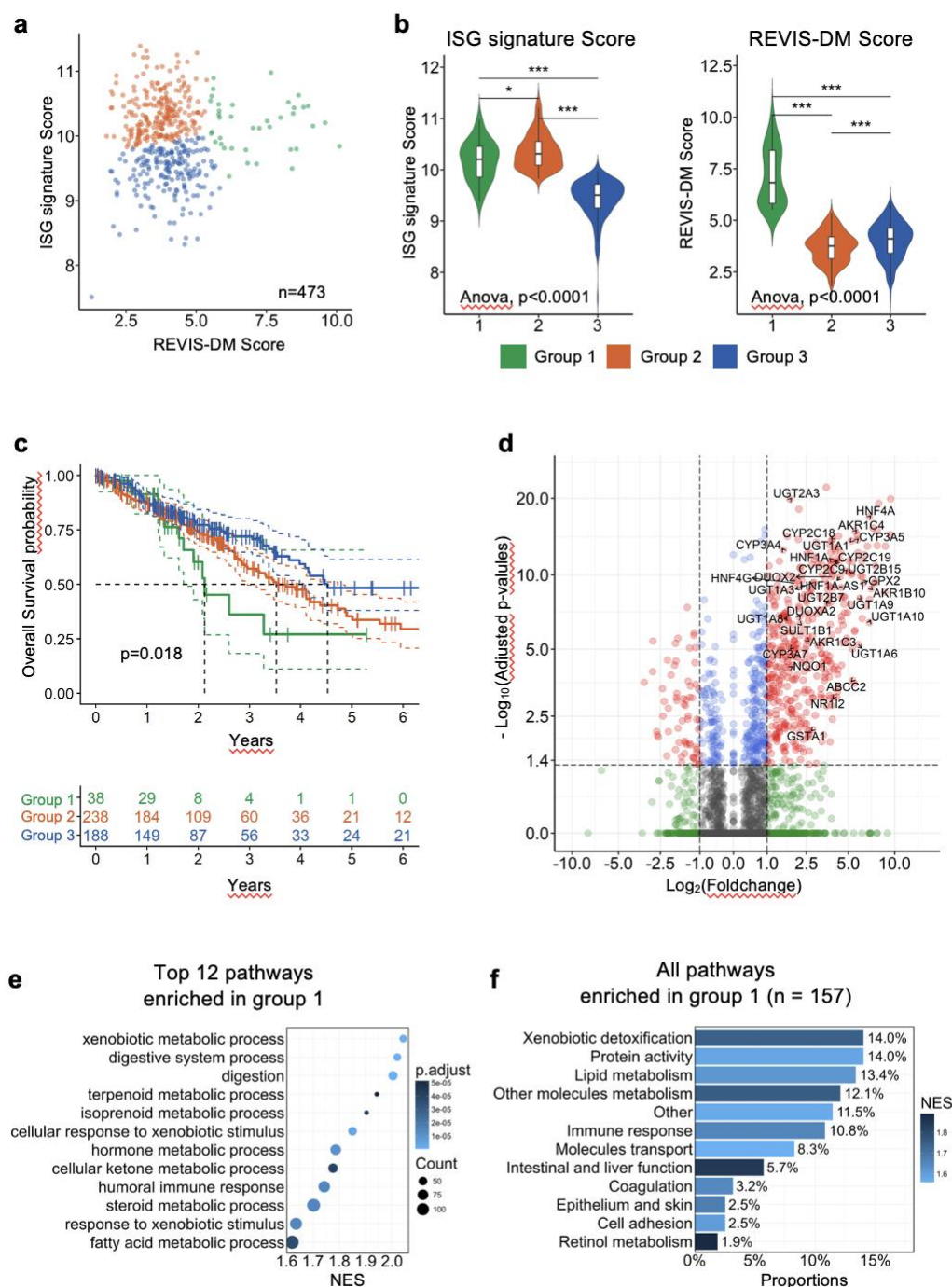
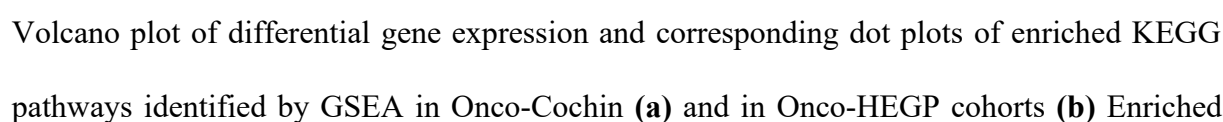


Figure 1: An Interferon Stimulated Gene-associated drug metabolism signature identifies NSCLC patients with poor clinical outcome

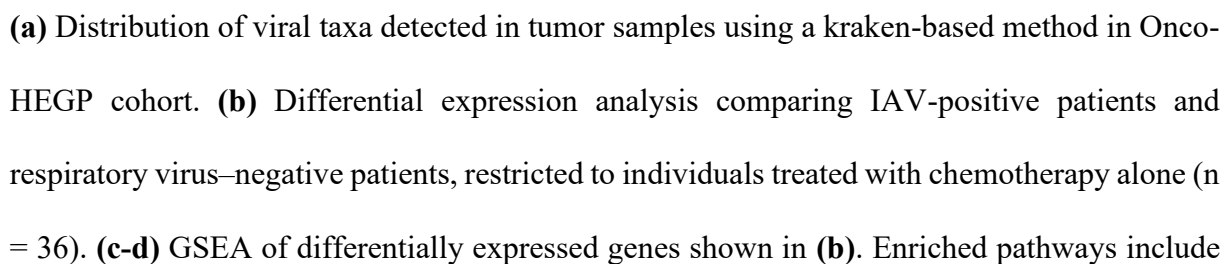
(a) Scatterplot of REVIS-DM and ISG scores across 473 LUAD patients from the TCGA cohort. Each dot corresponds to one patient. Scores were calculated as the mean expression of genes within each signature. Colors indicate k-means clustering based on both scores. **(b)** Violin plots showing REVIS-DM and ISG signature scores among patient groups. *P*-values were calculated using the Wilcoxon test. **(c)** Kaplan-Meier overall survival curves for the three patient groups. *P*-value was calculated using the log-rank test. **(d)** Volcano plot of differentially expressed genes (DEGs) between group 1 and the two other groups. Red dots indicate significant DEGs (adjusted *P*-values < 0.05; Fold-change >2 or < -2). *P*-values were calculated using the Wilcoxon test and adjusted with the Bonferroni method. **(e)** Top 12 enriched pathways identified by GSEA using Gene Ontology Biological Process databases (adjusted *P*-values < 0.05; NES > 0). **(f)** Distribution of enriched GO terms classified by main biological functions.

Figure 2: Response to chemotherapy and relapse are associated with response to viral infection, xenobiotic detoxification, and EMT signatures.



pathways include antiviral response, chemoresistance-associated signatures (detoxification, xenobiotic metabolism, and oxidative stress), and metastasis (epithelial cell migration, TGF- β signaling, and matrix remodeling). Comparisons were conducted between non-responders and responders (defined as $\leq 20\%$ viable tumor cells after neoadjuvant chemotherapy) and between patients with or without relapse.

Figure 3: Intratumoral IAV is associated with relapse after chemotherapy



antiviral responses **(c)** and programs associated with mesenchymal transition, matrix remodeling, xenobiotic export and oxidative stress detoxification **(d)**. **(e)** Disease-free survival stratified by viral status (IAV-positive vs respiratory virus–negative patients). **(f)** Progression-free survival stratified by viral status.

Figure 4

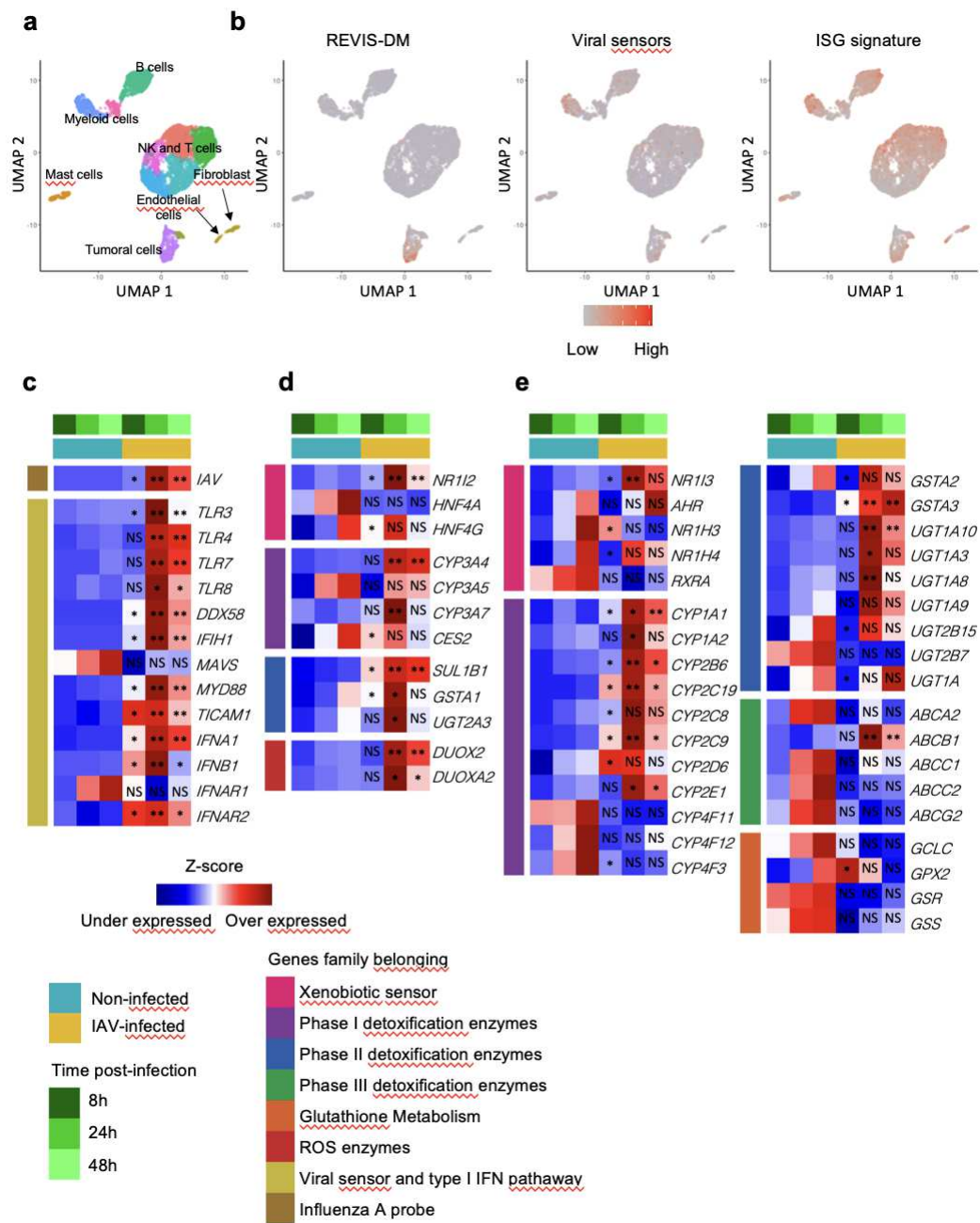


Figure 4: REVIS-DM in predominantly expressed by tumoral cells within the TME and induced by IAV infection.

(a) UMAP projection of sc-RNA-seq data (GSE123904) showing major cell populations and cluster annotations. (b) UMAP feature plots showing mean expression of REVIS-DM, viral sensing genes, and ISG signatures across cell populations. (c-e) Heatmaps showing relative

gene expression (z-scores) in non-infected and IAV-infected A549 cells at 8, 24, and 48 h post-infection. Panels display viral sensing and type I IFN pathway genes **(c)**, REVIS-DM genes **(d)**, and drug detoxification genes **(e)**. Corresponding *P*-values (one-sided Wilcoxon test) are indicated. Data represent *n* = 3 replicates (8 h) and *n* = 6 replicates (24 and 48 h). **P* < 0.05; ***P* < 0.01.

Figure 5

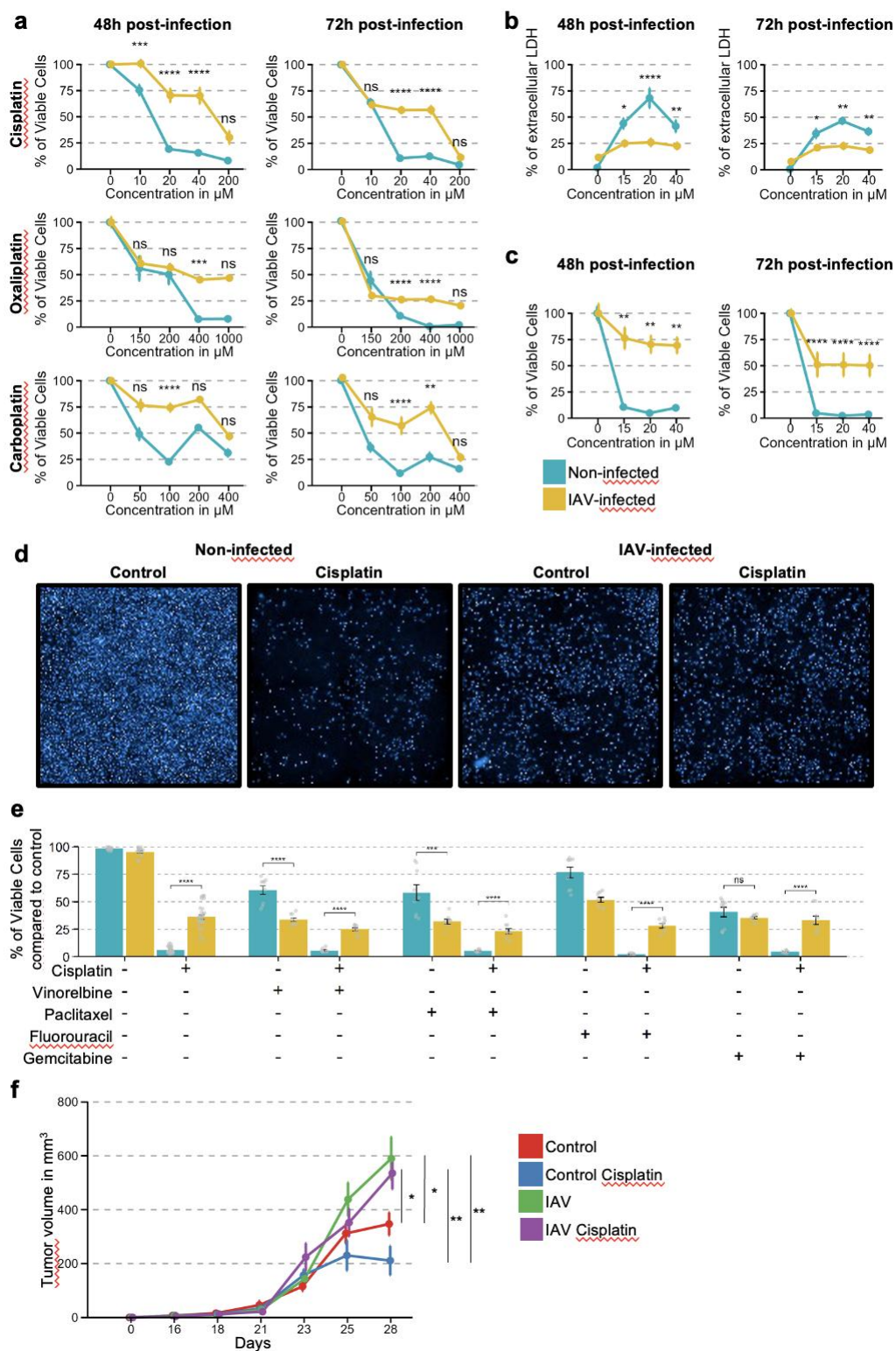


Figure 5: IAV infection promotes platinum chemoresistance

(a-d) A549 cells were infected with IAV (H3N2, MOI = 1) for 2 h, washed, and treated with Cisplatin, Carboplatin, or Oxaliplatin at the indicated concentrations for 48 or 72 h. **(e)** Cells were treated with Cisplatin in combination with second-generation chemotherapeutic agents as indicated. **(a, e)** Cell viability relative to untreated controls, assessed by XTT assay. **(b)** Cell cytotoxicity measured by extracellular LDH release. **(c)** Cell viability relative to untreated controls, assessed by nuclei quantification by the Operetta CLS™. **(d)** Representative Hoechst-stained images acquired by the Operetta CLS™. **(f)** *In vivo* chemoresistance assay. B6(C)/Rj-Tyrc/c mice were subcutaneously injected with 1×10^5 KP cells. At day 16, tumors were intratumorally injected with IAV (H3N2; 150 pfu) or PBS, followed by intratumoral Cisplatin (100 μ M) on days 18, 21, and 24. Tumor growth was monitored every 2–3 days. Tumor volume was calculated as $V \text{ (mm}^3\text{)} = (\text{length} \times \text{width} \times \text{height})/6 \times \pi$.

Statistical analyses were performed using the Wilcoxon test comparing infected versus non-infected conditions at each drug concentration. Data represent > 3 independent experiments.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

Figure 6

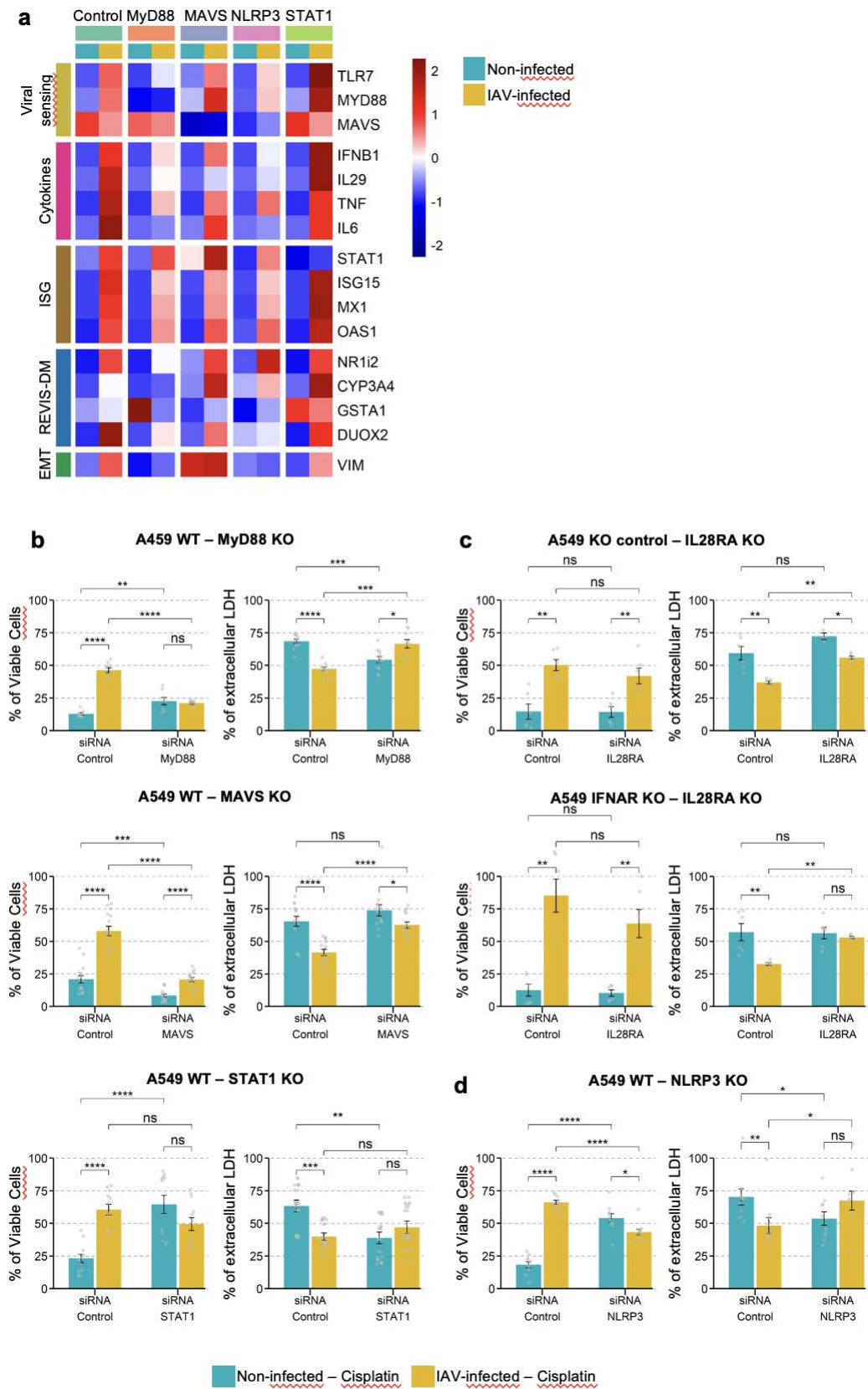


Figure 6: Viral sensing pathways drive IAV-induced platinum resistance independent of type I/III IFN signaling.

(a) Heatmap of relative gene expression (z-scores) in control or siRNA-transfected A549 cells (MYD88, MAVS, NLRP3, STAT1), with or without IAV infection. **(b-d)** Cell viability (XTT assay) and cell cytotoxicity (LDH release) in control (A549 WT) and IFNAR1 knockout A549 cells transfected with siRNA targeting MYD88, MAVS, STAT1, IL28RA **(c)**, or NLRP3 **(d)**, as indicated. Cells were infected with IAV (H3N2, MOI = 1) for 2 h, and treated with Cisplatin (20 μ M) for 72 h. Statistical analyses were performed using the Wilcoxon test comparing infected versus non-infected conditions. Data represent three independent experiments performed in triplicate. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

Figure 7

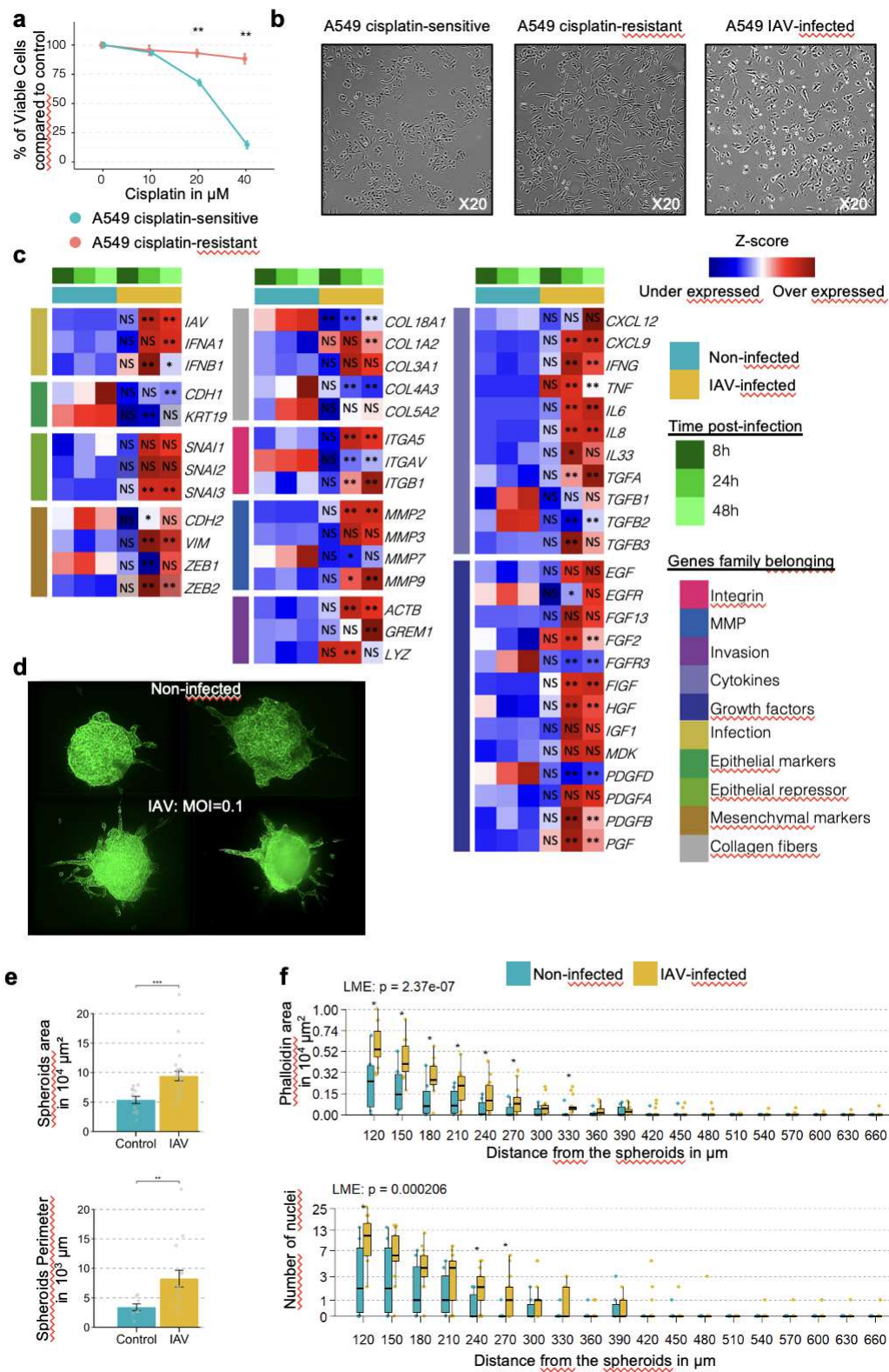
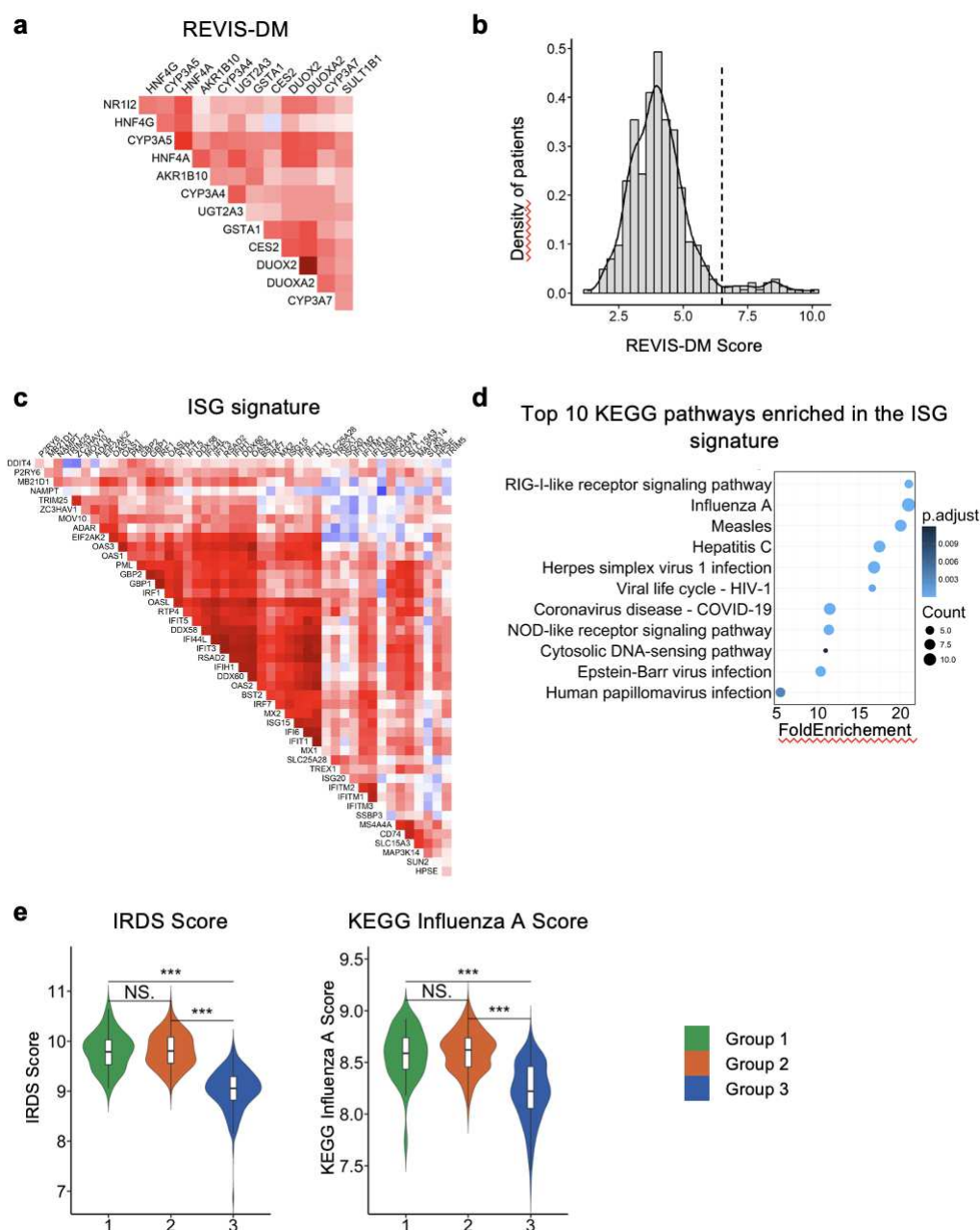


Figure 7: IAV induces a mesenchymal-like phenotype and invasiveness of lung tumor of A549 cells

(a) Dose-response curves of Cisplatin-treated A549 Cisplatin-sensitive and Cisplatin-resistant cells. Cell viability was assessed by XTT assay after 72 h.

(b) Bright-field images of cisplatin-sensitive, cisplatin-resistant, and IAV-infected A549 cells, showing morphological features consistent with epithelial and mesenchymal phenotypes. **(c)** Heatmaps of the relative gene expression (z-scores) of EMT markers, invasion-associated genes, cytokines, and growth factors in non-infected and IAV-infected A549 cells at 8, 24, and 48 h post-infection. **(d)** Representative images of A549 spheroids (infected or not with IAV) embedded in collagen I and stained with phalloidin, acquired by spinning-disk confocal microscopy. **(e)** Quantification of spheroid area and perimeter is shown **(f)** Sholl analysis of spheroid invasion. Quantification of nuclei number and phalloidin-positive area as a function of distance from the spheroid center. Statistical comparisons between infected and non-infected conditions were performed using the Wilcoxon test; global *P*-values were estimated using a linear mixed-effects model.

Extended Data Figure 1

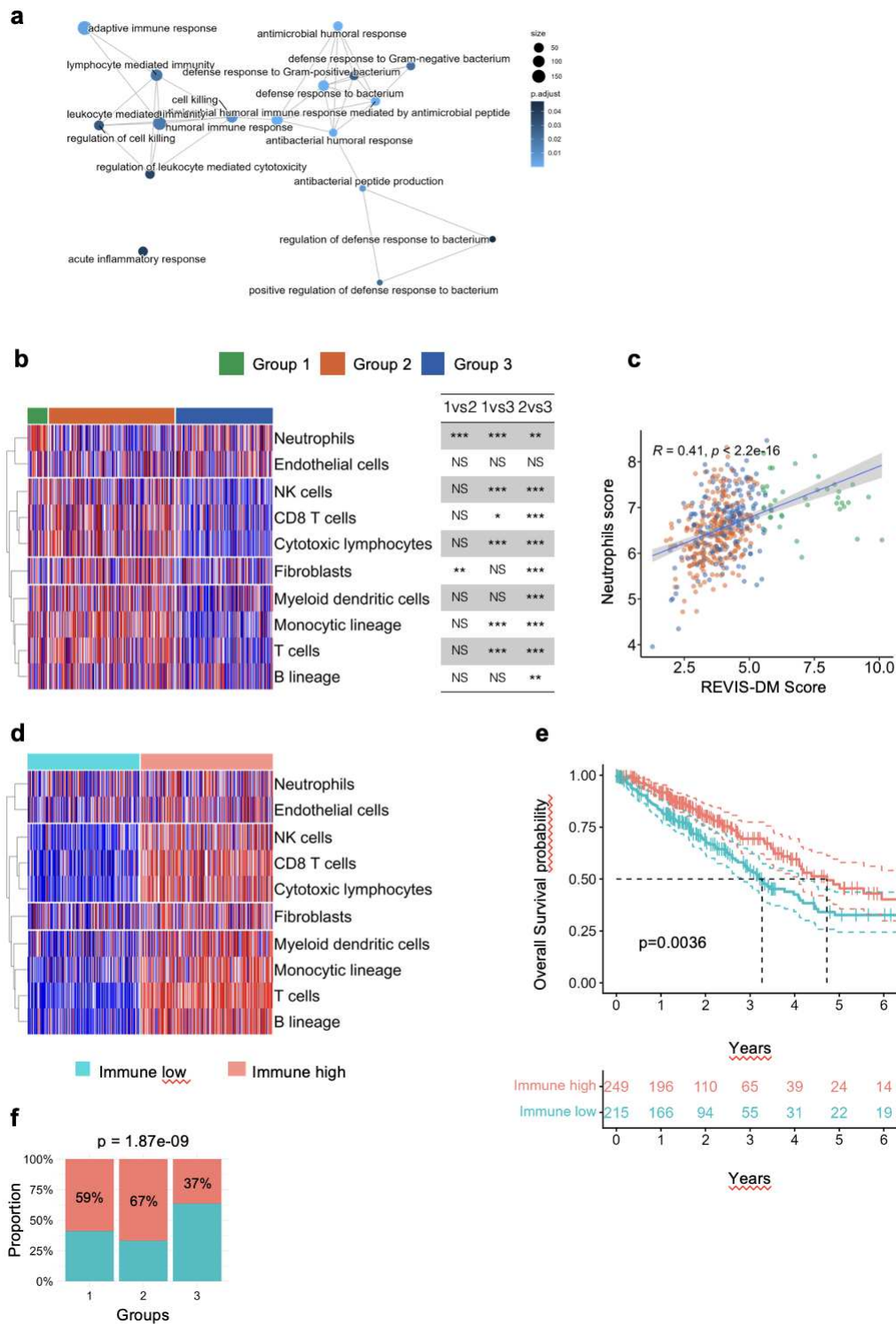


Extended Data Figure 1: REVIS-DM and ISG signature expression in NSCLC patients (TCGA LUAD cohort).

(a) Correlation matrix of REVIS-DM genes across samples. Pearson correlation coefficients are shown. **(b)** Distribution of REVIS-DM scores in patients. **(c)** Correlation matrix of ISG signature genes. Pearson correlation coefficients are shown. **(d)** KEGG pathway enrichment

analysis of ISG signature genes performed using ClusterProfiler. **(e)** Violin plots showing IRDS and KEGG Influenza A signature scores across the three patient groups. Scores were calculated as the mean expression of the genes within each signature.

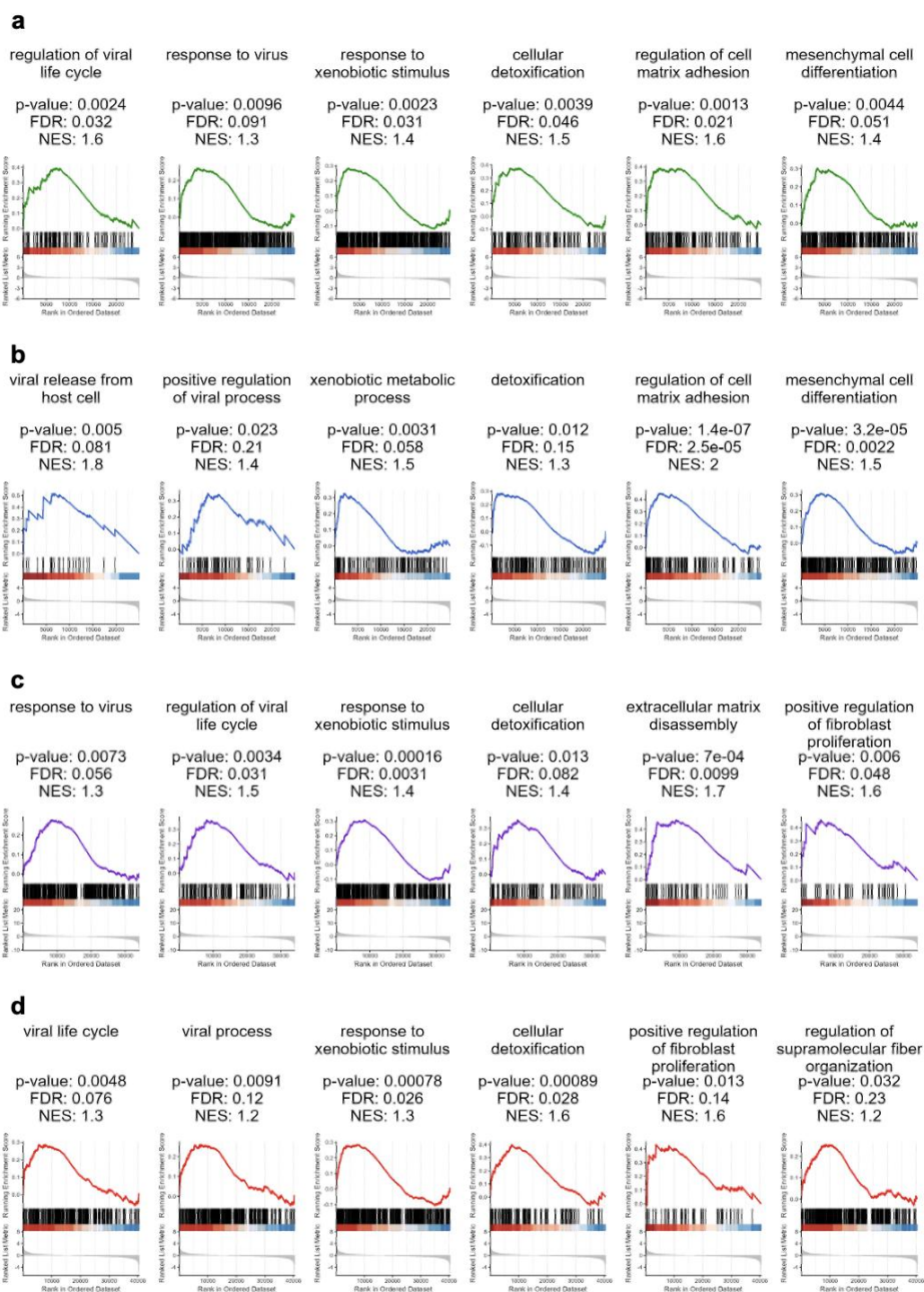
Extended Data Figure 2



Extended Data Figure 2: Distinct immune landscape characterizes REVIS-DM/ISG patient group (TCGA LUAD cohort).

(a) Enrichment map of immune-related GO terms identified by GSEA. Connections between GO terms are proportional to the number of overlapping genes, with a similarity threshold of 20% for connecting two nodes. **(b)** Heatmap of MCP-counter immune cell infiltration scores (z-scores) across patient groups. Corresponding *P*-values were calculated using the Wilcoxon test. **(c)** Scatterplot showing the Spearman correlation between REVIS-DM and neutrophils scores. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, NS: non-significant. **(d)** Heatmap of MCP-counter immune cell infiltration scores (z-scores). Unsupervised clustering identified two patient groups, termed immune-high and immune-low. **(e)** Kaplan-Meier overall survival curves for immune-high and immune-low patients. *P*-value was calculated using the log-rank test. **(f)** Stacked bar plot showing the proportion of immune high and low within each group. Group differences were assessed using Pearson's chi-square test of independence.

Extended Data Figure 3



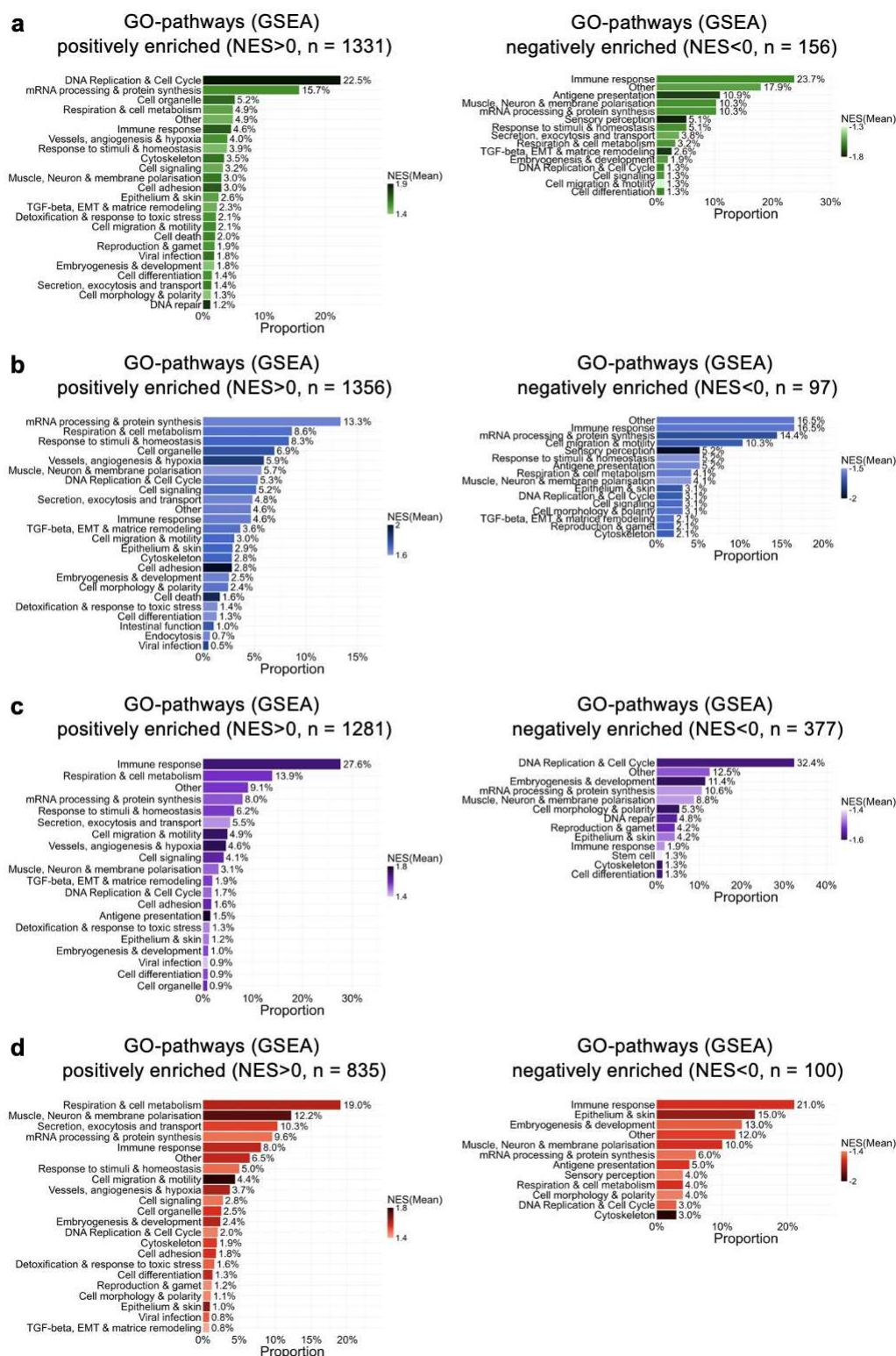
Extended Data Figure 3: GSEA reveals enrichment of response to viral infection, chemoresistance, and metastasis-associated pathways.

Selected GO terms identified by GSEA from differential expression analyses performed in NSCLC cohorts treated with chemotherapy.

(a) Responders versus non-responders in the Onco-Cochin cohort treated with neoadjuvant chemotherapy. **(b)** Non-relapsing versus relapsing patients in the Onco-Cochin cohort treated with neoadjuvant chemotherapy. **(c)** Non-relapsing versus relapsing patients in the Onco-HEGP cohort treated with neoadjuvant chemotherapy. **(d)** Non-relapsing versus relapsing patients in the Onco-HEGP cohort treated with adjuvant chemotherapy.

Pathways with positive NES correspond to pathways enriched in Responder or Non-relapsing patients whereas pathways with negative NES correspond to pathways enriched in Non-Responder or relapsing patients.

Extended Data Figure 4

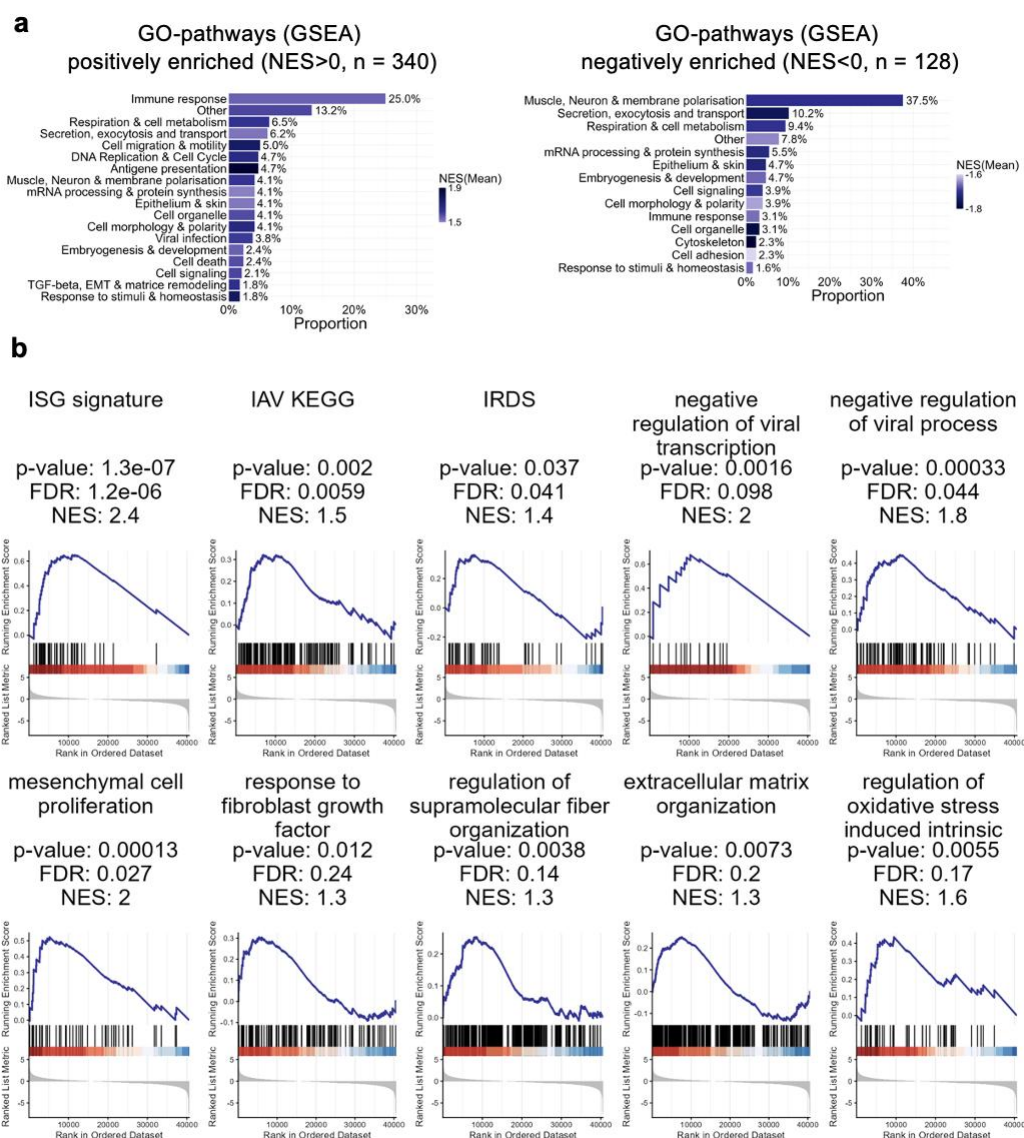


Extended Data Figure 4: Enriched biological programs associated with chemotherapy resistance and relapse.

(a) Responders versus non-responders in the Onco-Cochin cohort treated with neoadjuvant chemotherapy. **(b)** Non-relapsing versus relapsing patients in the Onco-Cochin cohort treated with neoadjuvant chemotherapy. **(c)** Non-relapsing versus relapsing patients in the Onco-HEGP cohort treated with neoadjuvant chemotherapy. **(d)** Non-relapsing versus relapsing patients in the Onco-HEGP cohort treated with adjuvant chemotherapy.

Pathways with positive NES correspond to pathways enriched in Responder or Non-relapsing patients whereas pathways with negative NES correspond to pathways enriched in Non-Responder or relapsing patients.

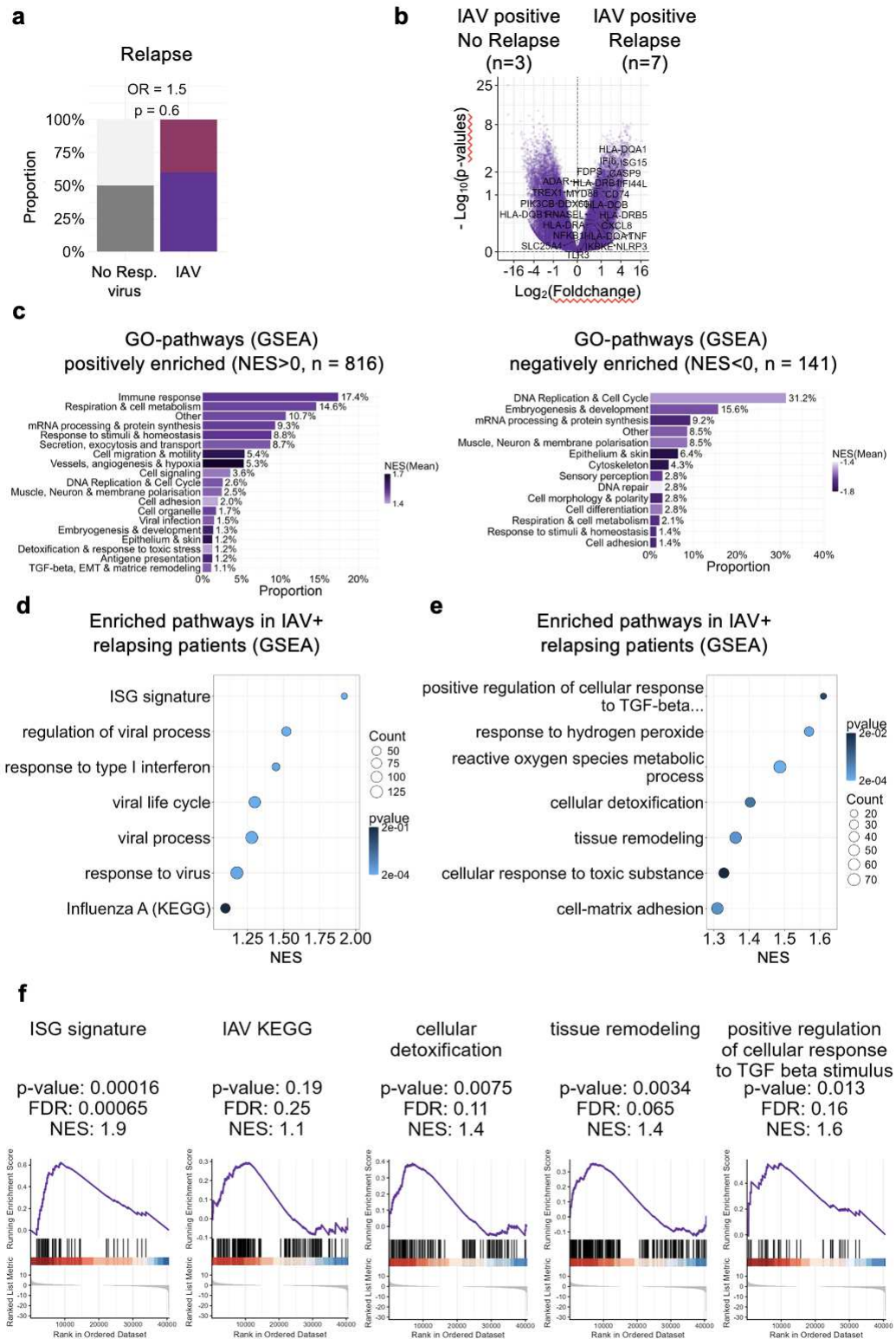
Extended Data Figure 5



Extended Data Figure 5: Viral infection and EMT pathways are enriched in IAV-positive patients compared to respiratory virus negative patients.

(a) Enriched biological pathways in IAV+ tumors. **(b)** Selected signatures identified by GSEA in IAV+ tumors.

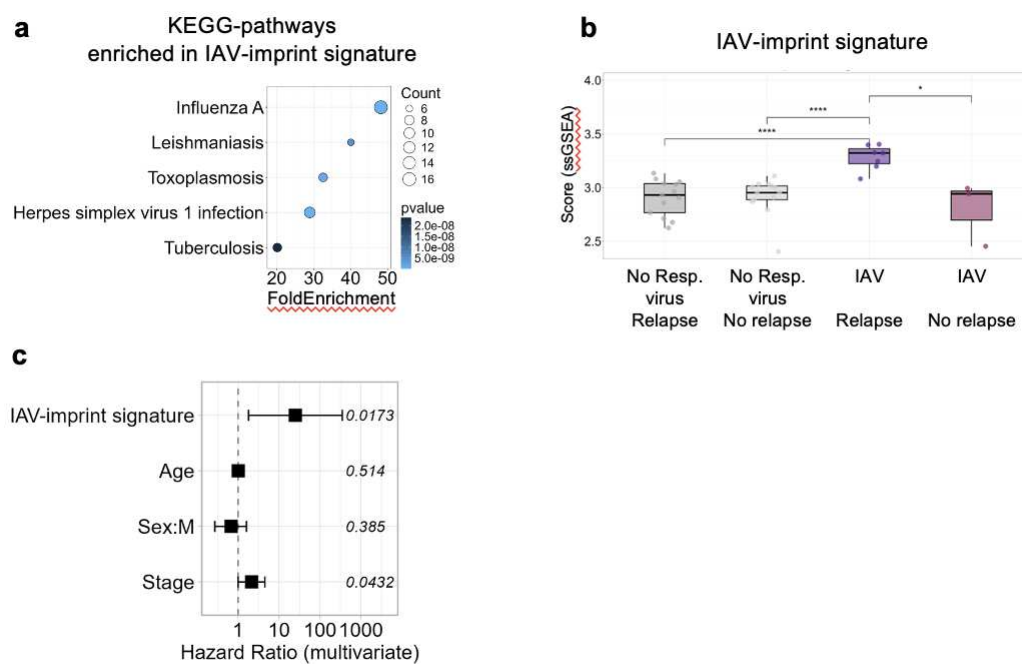
Extended Data Figure 6



Extended Data Figure 6: Viral response, EMT, and oxidative stress pathways are enriched in IAV-positive and relapsing patients.

(a) Proportion of relapse and non-relapse cases among IAV-positive and respiratory virus-negative patients. **(b)** Differential gene expression analysis between IAV-positive relapsing patients and IAV-positive non-relapsing patients. **(c)** Gene set enrichment analysis of differentially expressed genes showing enrichment of biological programs associated with IAV infection and relapse. **(d)** Dotplot of selected enriched virus-related pathways. **(e)** Dotplot of selected enriched EMT-related and oxidative-stress-related pathways enriched in IAV-positive relapsing patients. **(f)** Representative GSEA plots illustrating pathways associated with IAV detection and relapse.

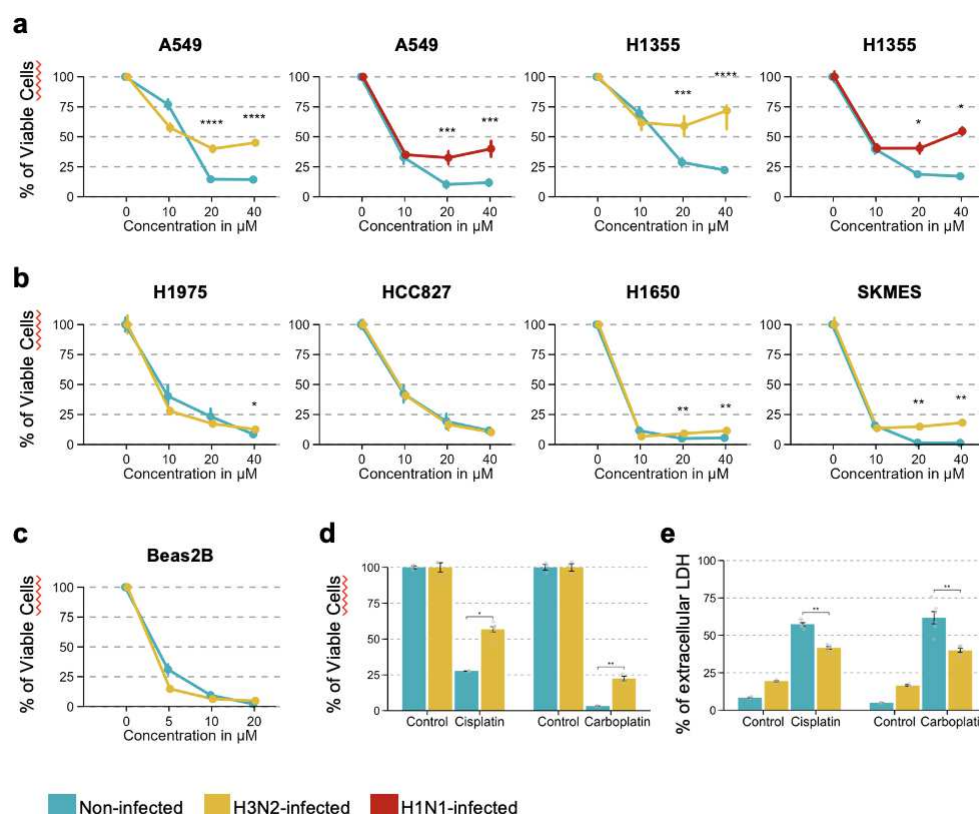
Extended Data Figure 7



Extended Data Figure 7: An IAV-imprint signature identifies relapsing IAV-positive patients.

(a) KEGG-pathways enriched in the IAV-imprint signature genes identified by LASSO. **(b)** ssGSEA scores of the IAV-imprint signature according to IAV detection status and relapse. **(c)** Multivariable Cox regression analysis for prognostic value of the IAV-imprint signature.

Extended Data Figure 8



Extended Data Figure 8: IAV-induced chemoresistance depends on tumor cells, viral strain and treatment kinetics.

(a) A549 and H1355 cells were infected for 2 h with IAV H3N2 (MOI = 1) or H1N1 (MOI = 0.05), followed by cisplatin treatment for 72 h. (b-c) H1975, HCC827, H1650, SK-MES-1, and BEAS-2B cells were infected with IAV H3N2 (MOI = 1) and treated with cisplatin for 72 h. (d-e) A549 cells were infected with IAV H3N2 (MOI = 1), cultured for 24 h, and subsequently treated with cisplatin or carboplatin for 48 h. (a-d) Cell viability relative to untreated controls assessed using XTT assay. (e) LDH release assay showing cytotoxicity levels. All data represent at least three independent replicates. Statistical significance was determined using the Wilcoxon test.

Supplementary Files

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