



TROP2 and PTEN are biomarkers of primary resistance to TUSC2 gene therapy in non-small cell lung cancer (NSCLC)

Ismail M Meraz¹, Renduo Song¹, Shuhong Wu¹, Yi Xu¹, Meng Feng¹, Lihui Gao¹, Chenghui Ren¹, Qi Wang², Jun Li³, Mourad Majidi¹, Jing Wang², Mark Berger⁴, and Jack A Roth¹
 Thoracic and Cardiovascular Surgery¹, ²Bioinformatics and Computational Biology, MD Anderson Cancer Center, Houston, TX; ³School of Data Science and Society, University of North Carolina, Chapel Hill; ⁴Genprex inc., Austin, TX

Abstract

Primary resistance to targeted therapies, immunotherapies, and gene therapies in NSCLC continues to be a significant challenge. *TUSC2* tumor suppressor gene therapy has shown promising anti-tumor efficacy by overcoming resistance to targeted therapy and enhancing checkpoint blockade immunotherapy, including in a mutant *KRAS/LKB1*-driven immunotherapy-resistant NSCLC model. *TUSC2* protein expression is downregulated or absent in over 80% of NSCLC and 100% of SCLC cases. *TUSC2* mediates cancer cell death through several mechanisms: inhibiting MAPK and mTOR signaling pathways, arresting cell growth, inducing programmed cell death, and activating immune responses. We established models primarily resistant to *TUSC2* gene therapy to find biomarkers indicative of *TUSC2* gene therapy resistance in NSCLC patient-derived xenografts (PDXs), PDX-derived organoids (PDXOs), and cell lines. A panel of 10 NSCLC cell lines screened for *TUSC2* sensitivity showed resistance in 50% of the cell lines, as assessed by annexin V staining and colony formation assays. We evaluated *TUSC2* sensitivity in 12 NSCLC PDXOs using ATP-based viability assays in 3D culture following *TUSC2* or empty vector transfection. While some PDXOs were highly responsive to *TUSC2* within 72 hours post-transfection, 50% of PDXOs exhibited primary resistance. We developed TC314AR (Acquired Resistance) PDX tumors and xenograft models (A549, H1299, H23AR) in NSG mice and treated them with *TUSC2* gene therapy. 20-30% of tumors in every model showed resistance, with no significant reduction in size compared to the control tumors after treatment. Protein expression profiling using reverse-phase protein array (RPPA) analysis of 500 proteins showed distinct expression signatures, with several candidate biomarkers significantly altered in resistant cell lines and PDXOs. RPPA analysis of residual tumors from both the xenograft and PDX models revealed significant but model-specific alterations in protein expression between responders and non-responders. Comparative analyses across the three models showed low expression of TROP2 and high expression of PTEN as potential biomarkers of primary resistance. Overexpression of TROP2 in H1299 and H460 cells increased *TUSC2*-induced apoptosis. These findings suggest that TROP2 and PTEN may serve as biomarkers to predict *TUSC2* response and guide therapeutic strategies in NSCLC.

Primary resistance to TUSC2 gene therapy in NSCLC cell lines

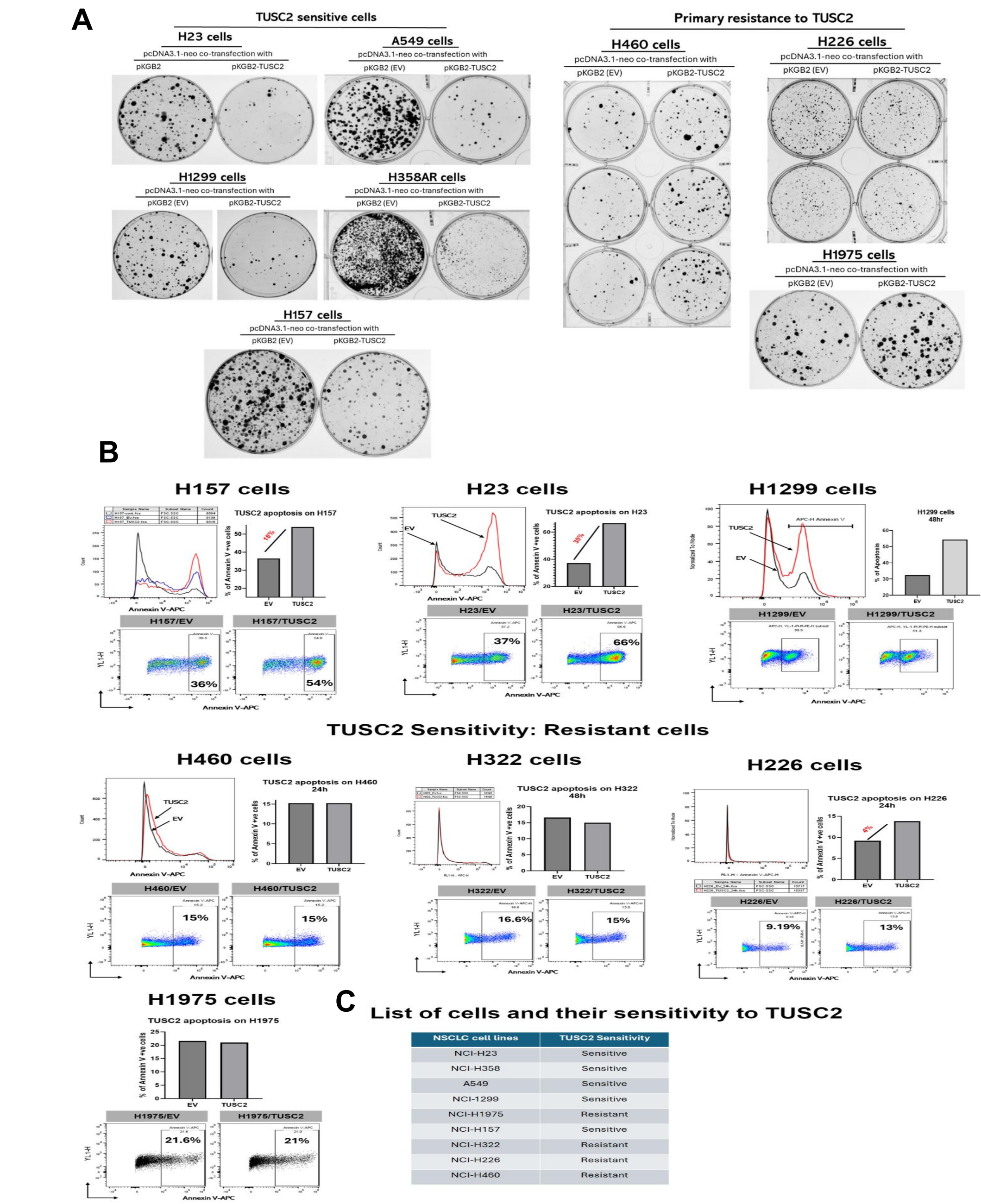


Fig 2. Primary resistance to TUSC2 gene therapy in NSCLC cell lines. A panel of 10 NSCLC cell lines was screened for response to *TUSC2* treatment via inhibition of colony formation (A) and induction of apoptosis (B). C) Table listing *TUSC2*-sensitive and resistant cell lines identified through colony formation and apoptosis assays.

PTEN, a potential biomarker, for TUSC2 primary resistance in NSCLC cell lines

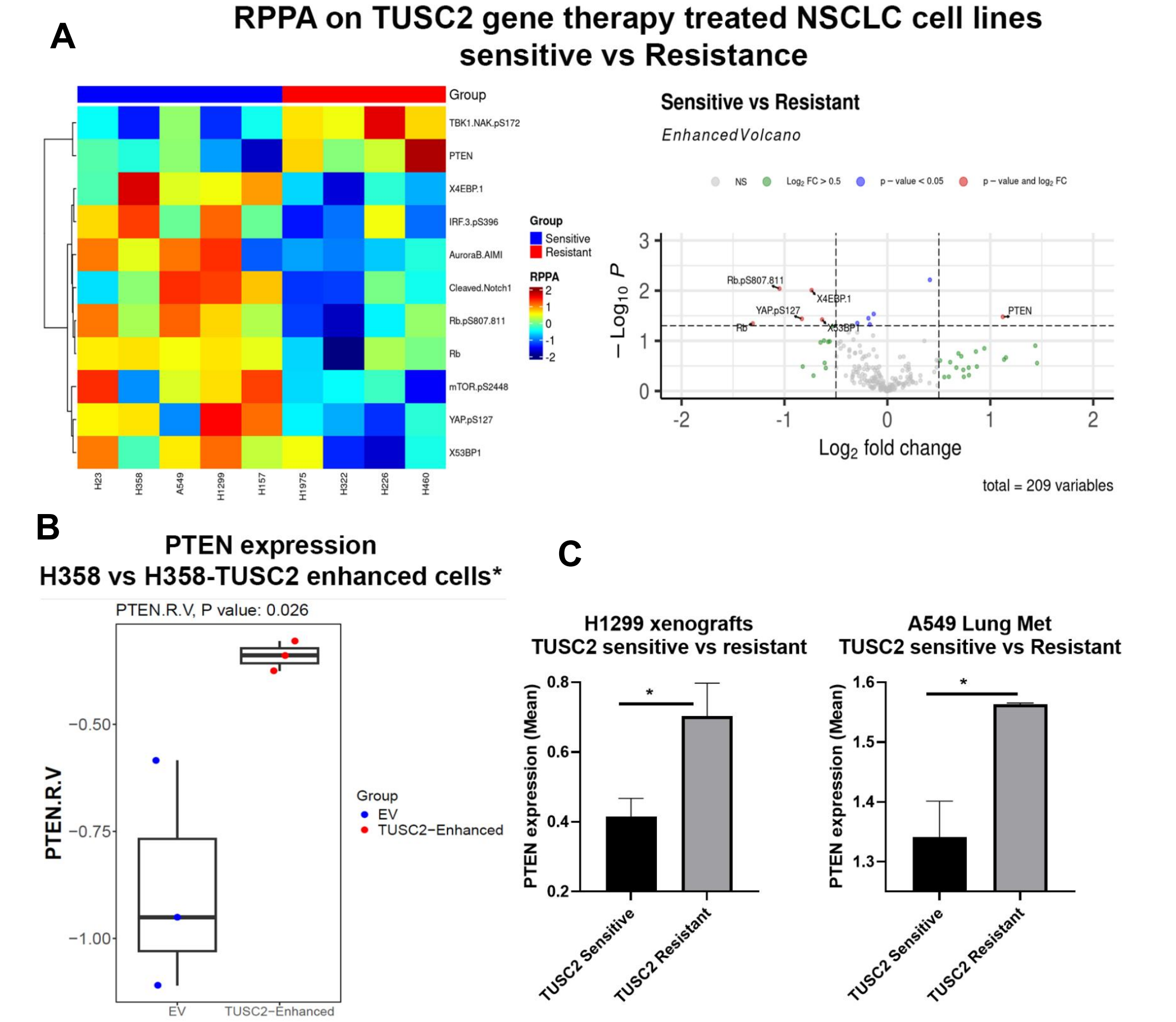


Fig 3. High-throughput analysis on TUSC2-resistant cells. A) RPPA data from TUSC2-resistant and TUSC2-sensitive cell lines were analyzed. The heatmap highlights proteins that were significantly upregulated or downregulated. A volcano plot identifies PTEN as a key protein associated with primary resistance to *TUSC2*. B) PTEN expression in TUSC2-resistant H358-*TUSC2* enhanced cells, which was generated through repeated delivery of *TUSC2* by lentivirus; C) A significant increase in PTEN was observed in non-responsive tumors and lung metastases.

Primary resistance to TUSC2 gene therapy in NSCLC PDXOs

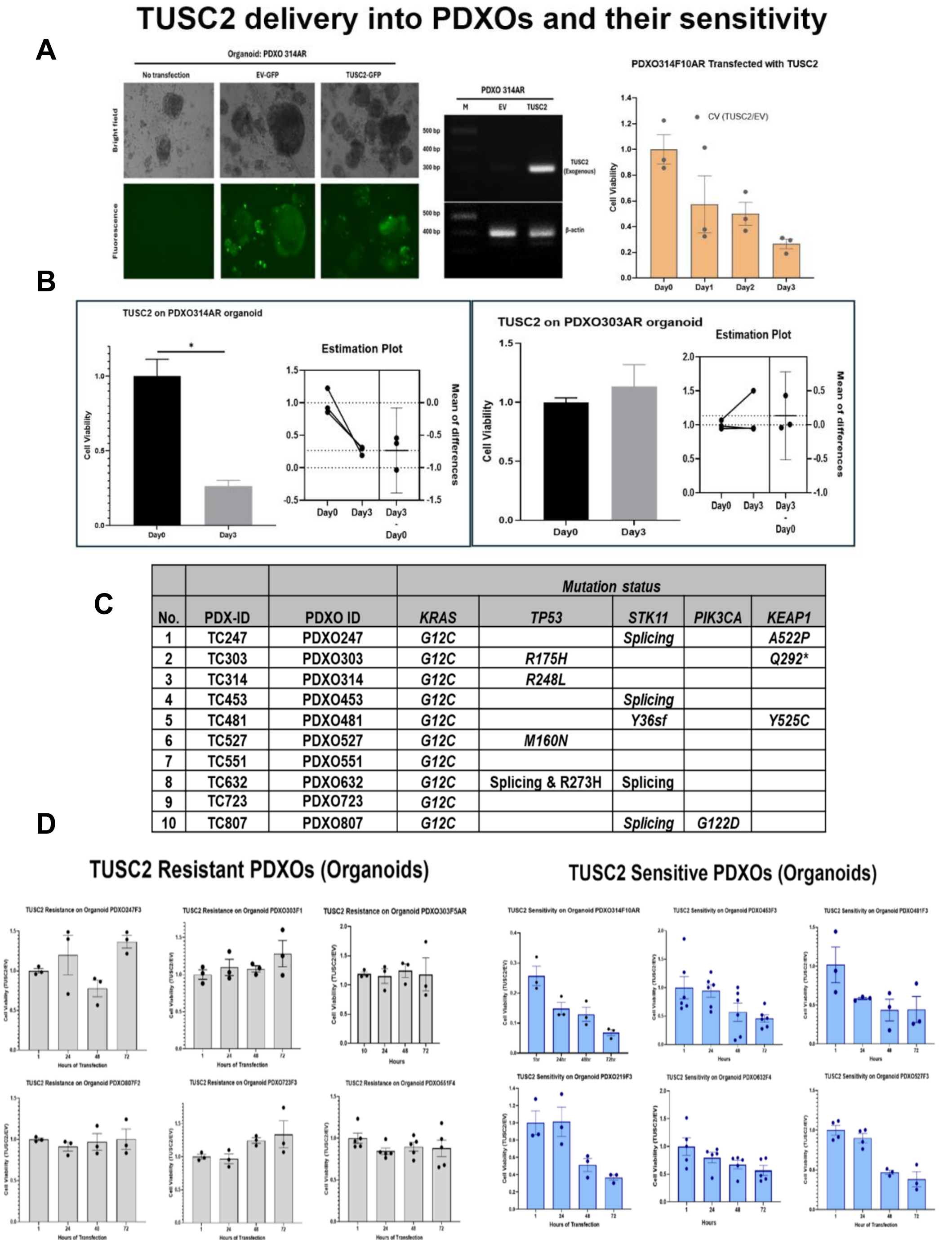


Fig 4. Primary resistance to TUSC2 gene therapy in NSCLC PDXOs. A panel of 12 NSCLC patient-derived xenograft organoids (PDXOs) was established from their respective PDXs. A) Optimization of *TUSC2* gene delivery into the 3D organoid model, verified by imaging and RT-PCR. *TUSC2* cytotoxicity was measured at multiple time points using an ATP-based CellTiter-Glo assay. B) Statistical significance of *TUSC2* efficacy in PDXOs. C) Molecular profiling of PDXOs and their corresponding parental PDXs. D) *TUSC2* screening categorized PDXOs into *TUSC2*-sensitive and resistant groups. (* means $p < 0.05$).

Low expression of TROP2 as a potential biomarker of TUSC2 primary resistance in NSCLC organoids

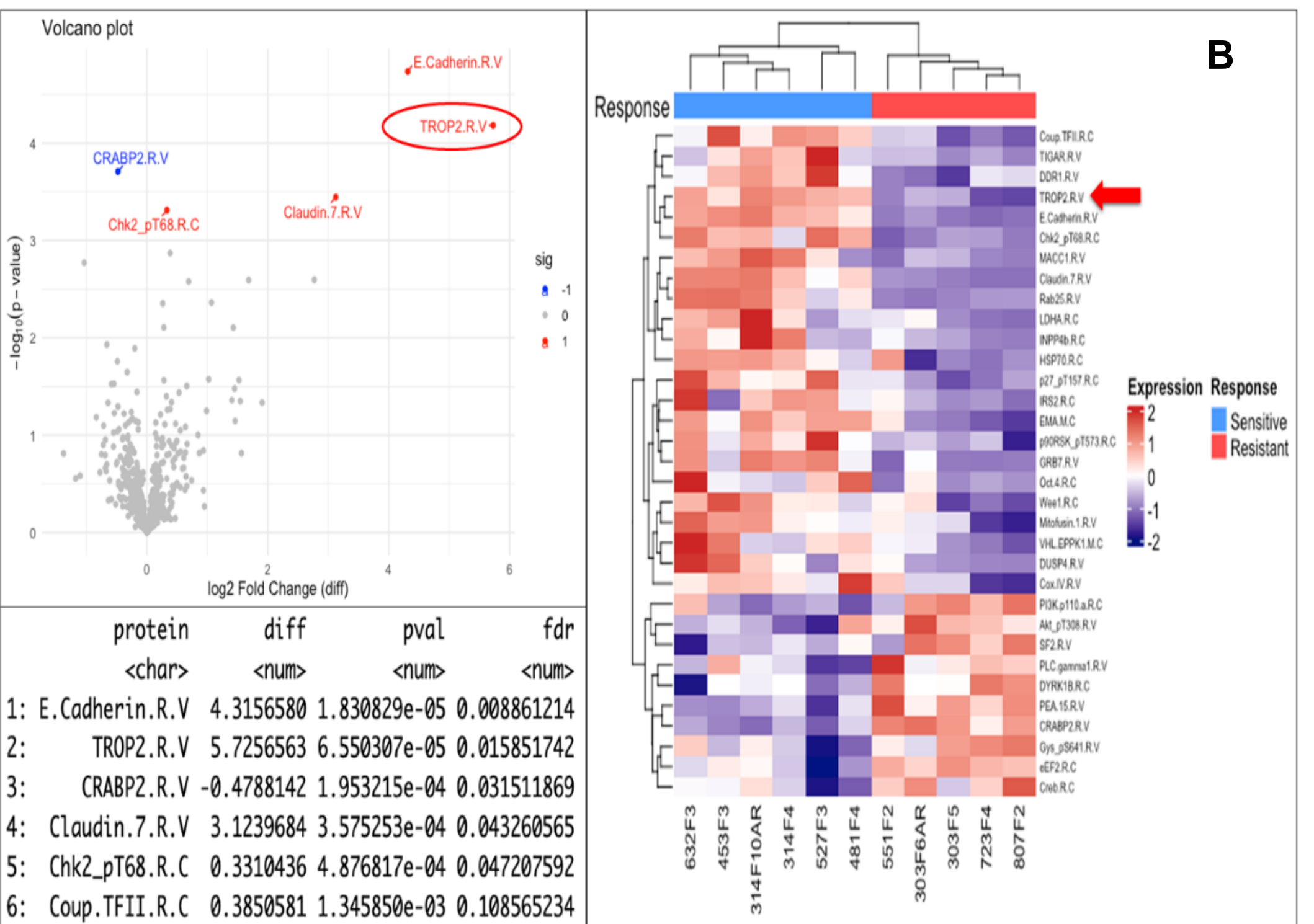


Fig 5. High-throughput analysis on protein expression in TUSC2-sensitive and resistant organoids. RPPA was performed on TUSC2-sensitive and resistant PDXOs, and the resulting data were statistically analyzed to identify associated biomarkers. A) Volcano plot identifying TROP2 as a significantly altered protein. B) Heatmap showing sets of proteins significantly upregulated or downregulated in sensitive versus resistant PDXOs.

Delivery of TROP2 with TUSC2 synergistically increases the cytotoxicity in NSCLC

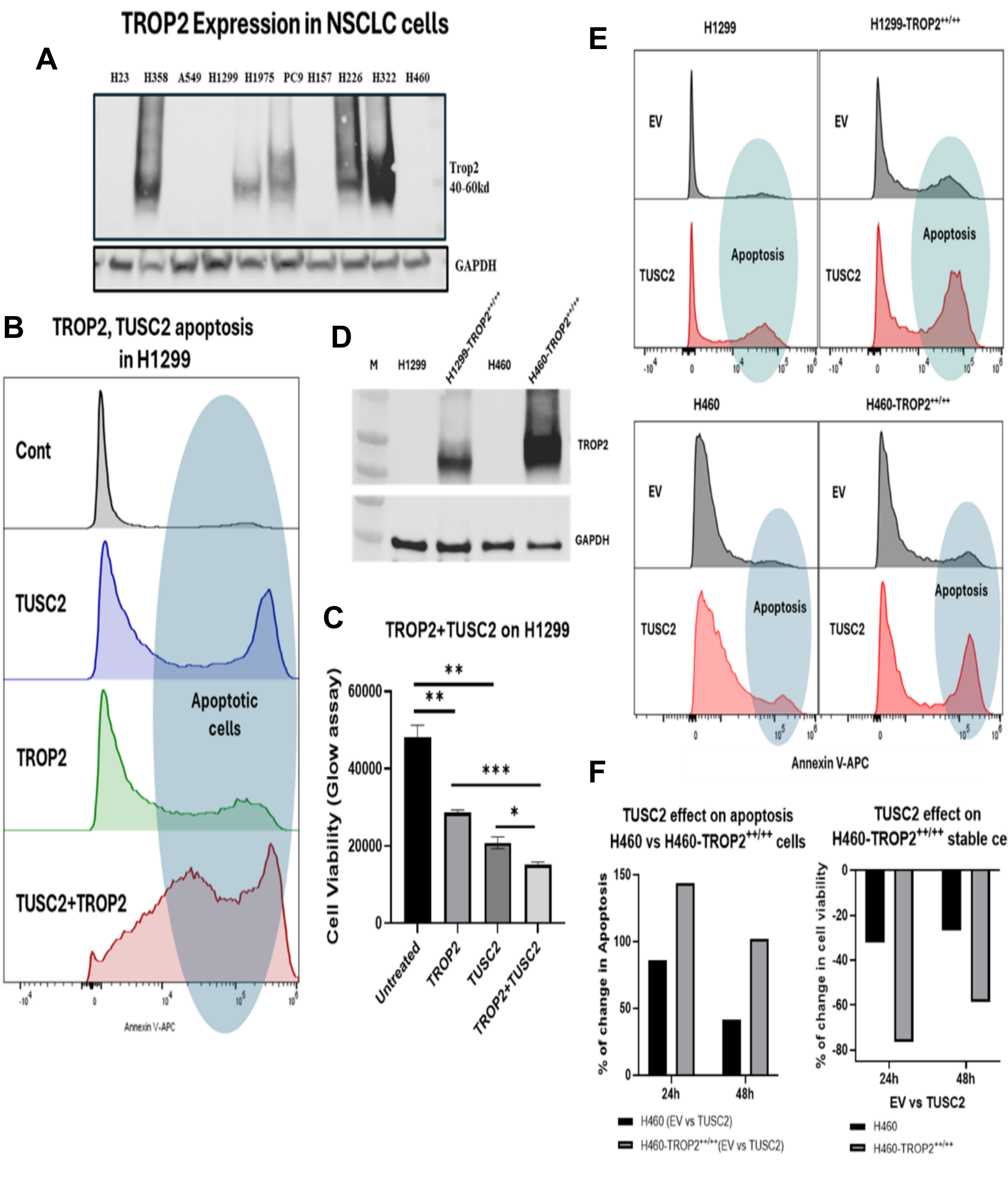


Fig 6. Re-expression of TROP2 increases the sensitivity of NSCLC to TUSC2 gene therapy. A) Evaluation of TROP2 expression in 10 NSCLC cell lines. B) TROP2 and *TUSC2* were delivered as single agents or in combination into the TROP2-low H1299 cell line. Flow cytometry was performed to detect Annexin V-positive apoptotic cells. C) Cell viability assays evaluating the synergistic cytotoxicity of TROP2 and *TUSC2*. D) Establishment of TROP2-overexpressing stable cell lines in low-expressing H1299 and H460 cells. E) Apoptosis assays following *TUSC2* gene therapy in TROP2-overexpressing stable cells (H1299-TROP2^{+/+} and H460-TROP2^{+/+}). F) Evaluation of the percentage change in cell viability and apoptosis comparing parental vs. TROP2-overexpressing stable cells after *TUSC2* delivery. (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$).

Conclusions

- Although *TUSC2* gene therapy was effective in reducing tumors in various NSCLC in vivo models, 20-30% of tumors in every model showed resistance, with no significant reduction in size compared to the control tumors after treatment.
- Protein expression profile on residual tumors showed that a set of proteins is significantly altered in *TUSC2*-treated sensitive vs resistant tumors
- A panel of 10 NSCLC cell lines screened for *TUSC2* sensitivity showed resistance in 50% of the cell lines, as assessed by annexin V staining and colony formation assays.
- *TUSC2* sensitivity was screened in 12 NSCLC PDXOs using ATP-based viability assays in 3D culture following *TUSC2* or empty vector transfection. While some PDXOs were highly responsive to *TUSC2* within 72 hours post-transfection, 50% of PDXOs exhibited primary resistance.
- High throughput analysis on RPPA data of 500 proteins showed distinct expression signatures, with several candidate biomarkers significantly altered in resistant cell lines and PDXOs.
- Comparative analyses across the three models showed low expression of TROP2 and high expression of PTEN as potential biomarkers of primary resistance.
- Delivery of TROP2 in TROP2-low expressing H1299 and H460 cells increased *TUSC2*-induced apoptosis.
- *TUSC2* sensitivity is significantly increased in TROP2-overexpressing H1299 and H460 stable cells

Disclosures

Jack A. Roth is a consultant and stock owner (including pending patents) in Genprex, Inc. All other authors have declared that no competing interests exist.

In-vivo resistance to TUSC2 gene therapy in NSCLC PDX and xenograft models

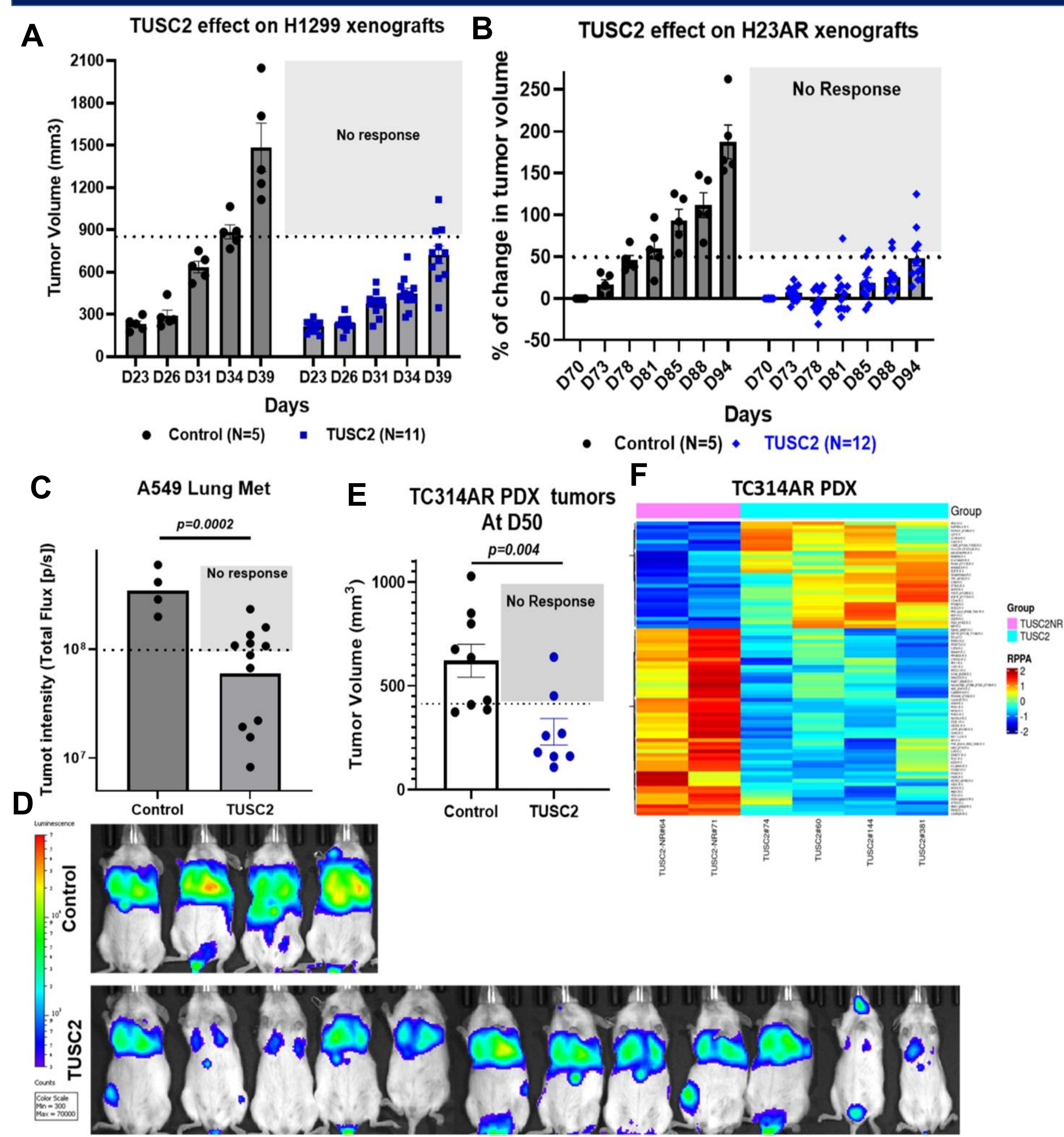


Fig 1. In vivo resistance to TUSC2 gene therapy in NSCLC. H1299 and H23AR xenograft tumors, H314AR PDX tumors, and A549 lung metastases were developed in NSG mice. Once established, tumors were treated with *TUSC2* gene therapy for three weeks to evaluate treatment efficacy. A subset of tumors that did not respond significantly compared to others was categorized as resistant (indicated by gray boxes). A) H1299 xenograft tumor model; B) H23AR xenografts; C) A549 lung metastasis model; D) IVIS images showing tumor burden of A549 lung metastasis; E) TC314AR PDX tumor model; F) RPPA analysis of TUSC2-responsive vs. non-responsive H314AR PDX tumors.