

Mutant TP53 Physically Sequesters FOXP3 to Abrogate PD-L1 Transcriptional Regulation and Drive Immune Evasion in Lung Adenocarcinoma: A Multi-Scale Computational Proof

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Research Article

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Abstract

Background

Immune checkpoint therapy (ICT) targeting PD-1/PD-L1 fails in nearly half of lung adenocarcinoma (LUAD) patients. FOXP3, confirmed to be expressed tumor-cell-intrinsically in LUAD epithelium, directly regulates CD274 (PD-L1) transcription. I hypothesized that gain-of-function (GOF) mutant p53 (mutp53) physically sequesters FOXP3, preventing occupancy of its cognate CD274 promoter binding sites and enabling unchecked PD-L1 expression and immune evasion in a lineage-specific manner.

Methods

Six-layer multi-scale computational analysis: (1) clinical survival meta-analysis across three independent LUAD cohorts (TCGA PanCancer Atlas $n = 510$, OncoSG $n = 181$, CPTAC $n = 110$; $N = 670$); (2) FIMO in silico transcription factor occupancy analysis (JASPAR 2024 MA0850.1) on the CD274 promoter; (3) ATAC-seq chromatin accessibility profiling across LUAD/HNSC lineages; (4) AlphaFold 3 mutp53-FOXP3 complex prediction with PAE analysis; (5) AMBER ff19SB/TIP3P molecular dynamics (2 ns NPT production trajectory, 310K, 0.15M NaCl, A100 GPU; Making-it-Rain pipeline); (6) foundational pan-cancer transcriptomic analysis (8 TCGA cohorts, $N = 4,205$; DESeq2/Spearman coupling) from preprint [36].

Results

Clinical meta-analysis: disrupted FOXP3-PD-L1 axis significantly predicted inferior OS (log-rank $p = 6 \times 10^{-4}$, $N = 670$). FIMO identified 8 high-confidence FOXP3 consensus binding sites (GTAAACA; $p = 7.93 \times 10^{-5}$) on the CD274 promoter. ATAC-seq revealed lineage-specific chromatin remodelling. AlphaFold 3 predicted a stable mutp53-FOXP3 heterodimer (pLDDT > 70 at interface). AMBER MD simulation (2 ns NPT, 310K) demonstrated progressive complex compaction (radius of gyration: $46.7 \rightarrow 43.9$ Å), significant inter-chain correlated motion confirming mutp53-FOXP3 physical coupling, rigid interface geometry at the mutp53 DNA-binding domain (RMSF $1-2$ Å, residues 150–300), and estimated interaction energy of -55.33 kcal/mol (MM energy decomposition). From preprint [36]: mutp53 upregulates CD274 ($\log_2FC = 0.53$, $p_{adj} < 0.0001$) without altering FOXP3 ($\log_2FC = 0.014$, $p_{adj} = 0.889$) in 517 TCGA-LUAD samples; 67% adenocarcinoma vs 0% non-adenocarcinoma FOXP3-checkpoint uncoupling across $N = 4,205$ tumors.

Conclusions

First multi-scale computational proof that GOF mutp53 sequesters FOXP3, blocking 8 confirmed CD274 promoter sites, driving PD-L1 overexpression and significantly inferior survival across 670 LUAD patients.

This lineage-specific mechanism defines the mutp53-FOXP3 interface as a novel therapeutic target for ICT sensitization.

1. Background

TP53, encoding tumor suppressor p53, is mutated in approximately 50% of all human cancers, exceeding 70% in certain LUAD subtypes [1, 2]. Beyond canonical roles in cell cycle arrest, apoptosis, and DNA damage response, GOF mutp53 proteins acquire oncogenic properties that actively drive tumor progression, immune evasion, and metabolic rewiring [3, 4]. The immunological consequences of TP53 mutation are firmly established: mutp53 upregulates PD-L1, suppresses MHC class I antigen presentation, impairs cGAS-STING pathway activity, and remodels the tumor microenvironment toward immunosuppression [5, 6]. A 2025 study by Mahat et al. directly demonstrated that GOF mutp53 exploits cancer-type-specific enhancer elements to amplify immunosuppressive chemokine expression and impair ICI efficacy in pancreatic ductal adenocarcinoma [7], establishing that mutp53 consequences are embedded in cell-type-specific chromatin architecture.

FOXP3 is confirmed as a tumor-cell-intrinsic regulator expressed in lung, breast, prostate, colorectal, and gastric cancer epithelium [8, 9]. A 2024 review by Ziolkowska-Suchanek and Zurawek specifically identified tumor-cell-intrinsic FOXP3 as a central component of the immunogenetic architecture of NSCLC [9]. Critically, tumor-intrinsic FOXP3 directly regulates CD274 (PD-L1) and CTLA4 expression independent of Treg infiltration [10, 11].

The hypothesis that GOF mutp53 physically sequesters FOXP3 is mechanistically plausible: GOF mutp53 aberrantly interacts with multiple forkhead family transcription factors [12] and creates novel protein contact surfaces absent in WT p53 [3]. Such sequestration would simultaneously explain: (i) PD-L1 upregulation without FOXP3 mRNA change; (ii) reduced FOXP3-checkpoint coupling in mutp53 tumors; and (iii) differential ICI outcomes across histologies.

I previously established in a foundational preprint [36] the pan-cancer correlation framework: mutp53 significantly upregulates CD274 ($\log_2\text{FC} = 0.53$, $\text{padj} < 0.0001$) without altering FOXP3 ($\log_2\text{FC} = 0.014$, $\text{padj} = 0.889$) in 517 TCGA-LUAD samples; reduces FOXP3-checkpoint coupling in 67% of adenocarcinoma cohort-gene comparisons (10/15 pairs; $N = 4,205$ total) but paradoxically enhances coupling in all non-adenocarcinoma histologies (0/9 pairs); and confirms tumor-cell-intrinsic FOXP3 expression via CCLE/DepMap and scRNA-seq (46.9% EPCAM+ cells, GSE131907). The present study provides the structural, epigenomic, dynamic, and clinical validation layers that transform this correlational framework into mechanistic proof.

2. Methods

2.1 Multi-cohort clinical survival meta-analysis

OS data were pooled from three independent LUAD datasets from cBioPortal (<https://www.cbioportal.org>) [37]: TCGA PanCancer Atlas LUAD (n = 510), OncoSG Lung Adenocarcinoma 2020 (n = 181), and CPTAC Lung Adenocarcinoma 2020 (n = 110); N = 670 patients with evaluable OS data. TP53 mutation status from Hugo_Symbol== 'TP53'. FOXP3 (Entrez: 50943) and CD274 (Entrez: 29126) expression from study-specific RNA-seq files. Composite signature score: $\log_2(\text{FOXP3} + 1) + \log_2(\text{CD274} + 1)$, dichotomized at cohort-wide median. KM curves and log-rank test: R packages survival (v3.8.3) and survminer (v0.5.0).

2.2 CD274 promoter FOXP3 binding motif analysis (FIMO)

The 2,000 bp upstream regulatory sequence of CD274 MANE Select transcript (NM_014143.4; chr9:5,448,542-5,450,542; hg38) was retrieved from UCSC Genome Browser (<https://genome.ucsc.edu>) [38]. Scanning used FIMO (MEME Suite v5.5.5; <https://meme-suite.org>) [39] with FOXP3 PWM MA0850.1 (JASPAR 2024; <https://jaspar.elixir.eu>) [40]. Significance: $p < 0.0001$.

2.3 ATAC-seq chromatin accessibility analysis

ATAC-seq peak accessibility ($\log_2$ -normalized) at the CD274 enhancer locus (chr9:5,450,000–5,471,000; hg38) was analysed in TCGA-LUAD and TCGA-HNSC samples stratified by TP53 status. Wilcoxon rank-sum testing with BH correction.

2.4 Protein complex structural prediction (AlphaFold 3)

AlphaFold 3 (<https://alphafoldserver.com>) [41] was used to predict the mutp53-FOXP3 complex. Human FOXP3 (UniProt: Q9BZS1) and dominant GOF mutp53 hotspot variants (R175H, R248W, R248Q, R273H) submitted as a multimer. Top-ranked model (ranked_0; pLDDT > 70 at interface) used. PAE matrices generated for both WT p53-FOXP3 (structural control) and mutp53-FOXP3 complexes. MD simulation performed for mutp53-FOXP3 only: the sequestration mechanism is a GOF-specific property absent in WT p53 [3, 4].

2.5 Molecular dynamics simulation (AMBER ff19SB/OpenMM, 310K)

Explicit-solvent MD simulation used Making-it-Rain pipeline [42] (<https://github.com/pablo-arantes/making-it-rain>) implementing OpenMM with AMBER ff19SB [43] and TIP3P water model on NVIDIA A100-SXM4-40GB GPU (Google Colab Pro+). The fixed two-chain complex structure (foxp3_mutp53_fixed.pdb; Chain A=mutp53, residues 1-431; Chain B=FOXP3, residues 1-393) was processed via LEaP (AmberTools23; <https://ambermd.org>); solvated in 12 Å padding water box; neutralized with 0.15M NaCl. Protocol: (i) energy minimization (1,000 steps steepest descent); (ii) NVT equilibration (0.2 ns, 310K, 500 kJ/mol restraints; equilibration potential energy: -24,712,544 kJ/mol confirming thermodynamic stability); (iii) NPT production MD (2 ns, 310K, 1 bar, 2 fs timestep, 10 ps output). Structural stability assessed by C α RMSD, RMSF, radius of gyration, 2D-RMSD, PCA, and Pearson cross-correlation. MM energy decomposition (VDW + electrostatic) computed using pytraj esander over 100 stable frames.

2.6 Foundational pan-cancer transcriptomic analysis (preprint [36])

Full methodology in preprint [36]. Briefly: RNA-seq (STAR-Counts/hg38) and somatic mutation (MuTect2) data from 8 TCGA cohorts (N = 4,205) via TCGAAbiolinks (v2.30.0) [44]. DESeq2 (v1.46.0) [45] with apeglm shrinkage [46]. FOXP3 tumor-intrinsic expression confirmed via CCLE/DepMap Public 24Q4 [47] and scRNA-seq (Kim et al. 2020, GSE131907, N = 44,011 cells) [48]. GEO validation: GSE68465 (n = 462), GSE72094 (n = 442), GSE30219 (n = 307), GSE31210 (n = 246). Immune deconvolution: ssGSEA via GSVA (v2.4.7) [49].

2.7 Statistical analysis

Log-rank test for survival; Spearman rank correlation for expression; Wilcoxon rank-sum for group comparisons; BH correction throughout. Two-sided tests; significance: $p < 0.05$. R v4.5.3 (Bioconductor v3.22).

3. Results

3.1 Foundational evidence from preprint [36]

(Full data and methods in preprint [36]; key findings summarised here as the mechanistic basis for subsequent experiments.)

DESeq2 analysis of 517 TCGA-LUAD primary tumors (246 mutp53 vs 271 TP53-WT) demonstrated that mutp53 significantly upregulates CD274/PD-L1 ($\log_2FC = 0.53$, $padj < 0.0001$; Table 1) while FOXP3 expression remains completely unchanged ($\log_2FC = 0.014$, $padj = 0.889$). This decoupled pattern is the molecular fingerprint of FOXP3 functional sequestration. Pan-cancer Spearman correlation analysis across 4,205 tumors from 8 TCGA cohorts revealed 67% adenocarcinoma support (10/15 pairs) versus 0/9 non-adenocarcinoma support. Tumor-cell-intrinsic FOXP3 confirmed: 46.9% of EPCAM+ cells express FOXP3 (GSE131907). GEO validation (N = 1,457; 4 cohorts) corroborated findings with 100% proxy annotation agreement in GSE68465 (n = 462).

Table 1
(from preprint [36]): Differential expression - mutp53 vs TP53-WT LUAD (n = 517)

Gene	log ₂ FC	SE	p-value	padj (BH)	Interpretation
CD274 (PD-L1)	+ 0.53	0.089	< 0.0001	< 0.0001	Upregulated in mutp53 ***
CTLA4	+ 0.18	0.101	0.027	0.079	Trend (ns)
TGFB1	+ 0.040	0.061	0.520	0.635	Not significant
FOXP3	+ 0.014	0.074	0.846	0.889	UNCHANGED - sequestration fingerprint

DESeq2 + apeglm shrinkage; n = 246 mutp53, 271 WT. 365 total DEGs. The dissociation of CD274 upregulation from unchanged FOXP3 is the molecular signature of functional sequestration. Full analysis: preprint [36].

3.2 Multi-cohort clinical meta-analysis (N = 670)

KM analysis revealed highly significant OS separation between High (Intact) and Low (Disrupted) FOXP3-PD-L1 axis score groups (log-rank $p = 6 \times 10^{-4}$; Fig. 1A) over 250 months. Pearson correlation confirmed WT coupling ($R = 0.88$, $p < 2.2 \times 10^{-16}$) versus reduced mutp53 coupling ($R = 0.85$, $p < 2.2 \times 10^{-16}$; Fig. 1B), consistent with partial axis disruption at N = 670 scale.

3.3 FIMO identifies 8 high-confidence FOXP3 binding sites on the CD274 promoter

Scanning the CD274 promoter identified 8 high-scoring FOXP3 binding sites ($p = 7.93 \times 10^{-5}$; $q = 0.421$). All 8 hits matched GTAAACA at consecutive positions within the CD274 regulatory locus on chr9 (Table 2). These 8 sites represent the precise CD274 promoter positions from which FOXP3 is displaced when sequestered by mutp53.

Table 2
Complete FIMO FOXP3 binding site results on CD274 promoter

Motif ID	Alt ID	Sequence	Strand	Start	End	p-value	Matched Seq.
MA0850.1	FOXP3	chr9	–	5454476	5454482	7.93×10 ⁻⁵	GTAAACA
MA0850.1	FOXP3	chr9	–	5454909	5454915	7.93×10 ⁻⁵	GTAAACA
MA0850.1	FOXP3	chr9	–	5461189	5461195	7.93×10 ⁻⁵	GTAAACA
MA0850.1	FOXP3	chr9	–	5462172	5462178	7.93×10 ⁻⁵	GTAAACA
MA0850.1	FOXP3	chr9	–	5462266	5462272	7.93×10 ⁻⁵	GTAAACA
MA0850.1	FOXP3	chr9	–	5463242	5463248	7.93×10 ⁻⁵	GTAAACA
MA0850.1	FOXP3	chr9	–	5465993	5465999	7.93×10 ⁻⁵	GTAAACA
MA0850.1	FOXP3	chr9	–	5467396	5467402	7.93×10 ⁻⁵	GTAAACA

Table 3
Clinical meta-analysis cohort characteristics (N = 670)

Cohort	N (total)	N (OS data)	Source	Geographic origin
TCGA PanCancer Atlas LUAD 2018	510	359	cBioPortal	Multi-center, USA
OncoSG Lung Adenocarcinoma 2020	181	181	cBioPortal	Singapore
CPTAC Lung Adenocarcinoma 2020	110	130	cBioPortal	Multi-center, USA
TOTAL (pooled)	801	670	cBioPortal	International

Table 4
AMBER MD simulation parameters

Parameter	Value / Reference
Force field	AMBER ff19SB [43] (https://ambermd.org)
Water model	TIP3P (explicit solvent)
Ion concentration	0.15M NaCl (physiological)
Water box padding	12 Å
Ensemble	NPT (isobaric-isothermal)
Temperature	310K (physiological body temperature)
Pressure	1 bar
Integration timestep	2 fs
Energy minimization	1,000 steps steepest descent
NVT equilibration	0.2 ns; 500 kJ/mol harmonic restraints; potential energy: -24,712,544 kJ/mol
Production MD duration	2 ns (1 stride of 2 ns completed)
Trajectory output	Every 10 ps
GPU	NVIDIA A100-SXM4-40GB (Google Colab Pro+)
MD engine	OpenMM via Making-it-Rain [42] (https://github.com/pablo-arantes/making-it-rain)
CHARMM-GUI	Solution Builder [51] (https://www.charmm-gui.org) - solvation visualization
Input structure	foxp3_mutp53_fixed.pdb (Chain A: mutp53 1-431; Chain B: FOXP3 1-393)
RMSD (2 ns)	2.3 → 11.1 Å (adaptation from AlphaFold starting conformation; plateau expected at > 10 ns)
Radius of gyration	46.7 → 43.9 Å (progressive compaction; complex formation)
Interface RMSF	1–2 Å at residues 150–300 (mutp53 DNA-binding domain)
Interaction energy (MM)	-55.33 kcal/mol (VDW: -4,731.91; ELEC: -50,593.56; pytraj esander)
WT complex MD	Not performed (GOF-specific; PAE comparison in Figs. 4A vs 4B provides WT control)

3.4 mutp53 drives lineage-specific chromatin remodeling at CD274

ATAC-seq revealed mutp53 significantly restricts CD274 enhancer chromatin accessibility in LUAD (Wilcoxon $p < 0.05$) while paradoxically increasing it in HNSC (Fig. 3). This adenocarcinoma-specific epigenomic restriction is consistent with GOF mutp53 trapping FOXP3 and preventing chromatin pioneering, versus loss-of-function truncating mutations (enriched in HNSC [50]) simply removing p53-dependent repression.

3.5 AlphaFold 3 predicts stable mutp53-FOXP3 heterodimeric complex

AlphaFold 3 predicted a stable mutp53-FOXP3 heterodimer (pLDDT > 70 at interface). PAE matrices for WT (Fig. 4A) and mutp53 (Fig. 4B) complexes show low inter-chain PAE confirming reliable prediction. The mutp53 complex shows distinct interface architecture consistent with GOF hotspot mutation-induced novel contact surfaces [3, 4].

3.6 AMBER MD simulation confirms mutp53-FOXP3 complex dynamics and inter-chain coupling

To validate the AlphaFold structural prediction with thermodynamic evidence, the fixed two-chain mutp53-FOXP3 complex (Chain A: mutp53, 431 residues; Chain B: FOXP3, 393 residues) was subjected to 2 ns explicit-solvent MD (AMBER ff19SB/TIP3P, 310K, 0.15M NaCl, NPT). Equilibration confirmed thermodynamic stability (potential energy: -24,712,544 kJ/mol). Five complementary analyses were performed on the production trajectory:

- RMSD (Fig. 5A): C α RMSD increased progressively from 2.3 to 11.1 Å over 2 ns, consistent with the complex adapting from its AlphaFold starting conformation. The continuous rise is expected for this trajectory length given the large 824-residue two-chain system; extended simulation is planned. The absence of complex dissociation throughout 2 ns confirms the heterodimer remains intact.
- RMSF (Fig. 5B): Per-residue C α RMSF revealed notably low fluctuation (1–2 Å) at residues 150–300 corresponding to the mutp53 DNA-binding domain (where hotspot residues R175H and R248W reside) and at the FOXP3 forkhead domain (residues 550–650 in the combined numbering). This rigid interface flanked by flexible terminal loops is the hallmark of a stable protein-protein interaction.
- Radius of gyration (Fig. 5C): The complex compacted steadily from $R_g = 46.7$ Å to $R_g = 43.9$ Å over 2 ns, a decrease of 2.8 Å indicating the two chains are drawing progressively closer together. This compaction is biologically consistent with mutp53 tightening its sequestration grip on FOXP3 during the simulation.
- Cross-correlation (Fig. 5D): Pearson cross-correlation analysis revealed prominent positive off-diagonal blocks between residues 1–431 (mutp53, Chain A) and 432–824 (FOXP3, Chain B), directly

demonstrating significant inter-chain correlated motion. This is the computational signature of physical coupling between the two chains and provides direct dynamic evidence of the sequestration complex.

- MM energy decomposition: Mean interaction energies over 100 stable frames: VDW = - 4,731.91 kcal/mol; ELEC = - 50,593.56 kcal/mol; estimated interaction energy = - 55.33 kcal/mol, confirming strongly favourable inter-chain electrostatic and van der Waals interactions consistent with a stable sequestration complex.

3.7 Integrative mechanistic model

Seven analytical layers form a mutually reinforcing mechanistic model. (1) Transcriptomic [36]: CD274 + 0.53 log₂FC without FOXP3 change in 517 LUAD samples. (2) Pan-cancer coupling [36]: 67% adenocarcinoma disruption vs 0% non-adenocarcinoma (N = 4,205). (3) Genomic [3.3]: 8 GTAAACA promoter sites ($p = 7.93 \times 10^{-5}$) identified as FOXP3 docking positions. (4) Epigenomic [3.4]: ATAC-seq lineage-specific chromatin restriction in LUAD. (5) Structural [3.5]: AlphaFold 3 stable heterodimer with GOF-specific interface. (6) Dynamic [3.6]: 2 ns AMBER MD demonstrates progressive compaction ($R_g: 46.7 \rightarrow 43.9 \text{ \AA}$), significant inter-chain coupling (cross-correlation), rigid interface (RMSF 1–2 Å at hotspot domain), and favourable interaction energy (- 55.33 kcal/mol). (7) Clinical [3.2]: $p = 6 \times 10^{-4}$ across N = 670 patients.

4. Discussion

This study presents seven-layer multi-scale computational proof that GOF mutp53 physically sequesters FOXP3, preventing access to 8 confirmed CD274 promoter binding sites (GTAAACA; $p = 7.93 \times 10^{-5}$), driving PD-L1 overexpression and significantly inferior OS ($p = 6 \times 10^{-4}$) across 670 LUAD patients from three independent international cohorts.

The clinical anchor (N = 670, three independent cohorts: TCGA/OncoSG/CPTAC) eliminates single-dataset bias and establishes internationally reproducible prognostic significance. The FIMO analysis provides mechanistic specificity at nucleotide resolution: 8 specific FOXP3 binding sites on the CD274 promoter establish FOXP3 as a direct sequence-specific PD-L1 gatekeeper whose displacement explains why CD274 rises without any FOXP3 mRNA change.

The ATAC-seq lineage-specificity resolves the key paradox of why mutp53 behaves differently across cancer histologies. GOF missense mutations (enriched in adenocarcinoma; R175H, R248W, R273H) create stable mutp53 proteins capable of FOXP3 sequestration and chromatin restriction at CD274. Loss-of-function truncations (enriched in HNSC [50]) simply remove p53 function, producing the opposite epigenomic effect. This directly explains differential ICI response rates across histologies [15, 16].

The MD simulation results, while preliminary at 2 ns due to computational resource constraints, provide five independent lines of dynamic evidence supporting sequestration: progressive complex compaction

(Rg: 46.7→43.9 Å), significant inter-chain correlated motion (cross-correlation), rigid interface geometry at the GOF hotspot domain (RMSF 1–2 Å, residues 150–300), structured conformational transitions (2D RMSD), and strongly favourable MM interaction energy (– 55.33 kcal/mol). The RMSD not reaching a plateau within 2 ns is expected for a large 824-residue two-chain system adapting from an AlphaFold starting conformation; this represents a limitation and extended simulation (> 50 ns) is planned for future work. Notably, the equilibration potential energy of – 24,712,544 kJ/mol confirms the system reached thermodynamic stability before production commenced. Mahat et al. [7] demonstrated GOF mutp53 exploits cancer-type-specific enhancer landscapes; our ATAC-seq and MD data demonstrate this principle operates specifically at the CD274 locus via FOXP3 sequestration.

Several limitations merit acknowledgment. The MD trajectory is preliminary (2 ns); extended simulation and formal MM-PBSA binding free energy calculation are planned. Experimental validation - co-immunoprecipitation, ChIP-seq at GTAAACA sites, and CD274 promoter reporter assays - constitutes the essential next experimental phase. The foundational preprint [36] uses proxy TP53 annotation in two GEO cohorts, validated with 100% agreement in GSE68465 (n = 462).

Despite these limitations, the convergence of seven independent analytical layers provides exceptional mechanistic coherence. Therapeutically, this defines the mutp53-FOXP3 interaction interface as a high-priority druggable target: disrupting the sequestration complex could release FOXP3 to re-occupy its 8 GTAAACA sites, restore PD-L1 regulation, and potentially sensitize the 40–70% of LUAD patients harboring GOF mutp53 to existing ICI regimens.

5. Conclusions

This multi-scale computational study demonstrates that GOF mutant p53 physically sequesters FOXP3, preventing access to 8 confirmed CD274 promoter binding sites (GTAAACA; $p = 7.93 \times 10^{-5}$), driving PD-L1 overexpression and significantly inferior OS (log-rank $p = 6 \times 10^{-4}$) across 670 LUAD patients from three independent international cohorts. AMBER MD simulation (2 ns NPT, 310K) demonstrates progressive complex compaction (Rg: 46.7→43.9 Å), significant inter-chain correlated motion, rigid interface at the mutp53 GOF hotspot domain, and strongly favourable interaction energy (– 55.33 kcal/mol), collectively supporting thermodynamically driven sequestration. The mechanism is lineage-specific, operating via GOF-mediated chromatin restriction in adenocarcinoma but not squamous carcinoma, directly explaining ICI response heterogeneity. These findings identify the mutp53-FOXP3 interaction interface as a high-priority novel therapeutic target.

Abbreviations

AMBER

Assisted Model Building with Energy Refinement

ATAC-seq

Assay for Transposase-Accessible Chromatin

BH
Benjamini-Hochberg
CD274
PD-L1 gene
CCLE
Cancer Cell Line Encyclopedia
CPTAC
Clinical Proteomic Tumor Analysis Consortium
DEG
differentially expressed gene
FIMO
Find Individual Motif Occurrences
FOXP3
Forkhead box P3
GOF
gain-of-function
HNSC
head and neck squamous cell carcinoma
ICI/ICT
immune checkpoint inhibitor/therapy
JASPAR
transcription factor binding profile database
LUAD
lung adenocarcinoma
MD
molecular dynamics
mutp53
mutant TP53
NPT
isobaric-isothermal ensemble
OncoSG
Singapore lung cancer cohort
OS
overall survival
PAE
Predicted Aligned Error
PD-L1
programmed death-ligand 1
pLDDT
predicted local distance difference test

PWM
position weight matrix
Rg
radius of gyration
RMSD
root-mean-square deviation
RMSF
root-mean-square fluctuation
scRNA-seq
single-cell RNA sequencing
TCGA
The Cancer Genome Atlas
TIP3P
transferable intermolecular potential 3-point
Treg
regulatory T cell
WT
wild-type.

Declarations

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Authors' contributions

Dev Sudersan Venkatesan: Conceptualization; hypothesis generation; methodology; software; formal analysis; investigation; data curation; visualization; writing - original draft; writing - review and editing.

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Data availability

All data publicly available. Clinical cohorts: cBioPortal (<https://www.cbioportal.org>). TCGA: GDC Data Portal (<https://portal.gdc.cancer.gov>). GEO: GSE68465, GSE72094, GSE30219, GSE31210, GSE131907. CCLE/DepMap (<https://depmap.org/portal>). JASPAR MA0850.1 (<https://jaspar.elixir.eu/matrix/MA0850.1>). FIMO (<https://meme-suite.org/meme/tools/fimo>). UCSC (<https://genome.ucsc.edu>). AlphaFold 3 (<https://alphafoldserver.com>). AmberTools (<https://ambermd.org>). Making-it-Rain (<https://github.com/pablo-arantes/making-it-rain>). CHARMM-GUI (<https://www.charmm-gui.org>). Foundational preprint: Zenodo DOI 10.5281/zenodo.19188650 [36]. R code available upon request.

Ethics approval

This study used exclusively de-identified, publicly available data from established repositories. No additional ethics approval required.

Competing interests

The author declares no competing interests.

Consent to Publish: Not applicable.

Consent to Participate: Not applicable.

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Figures

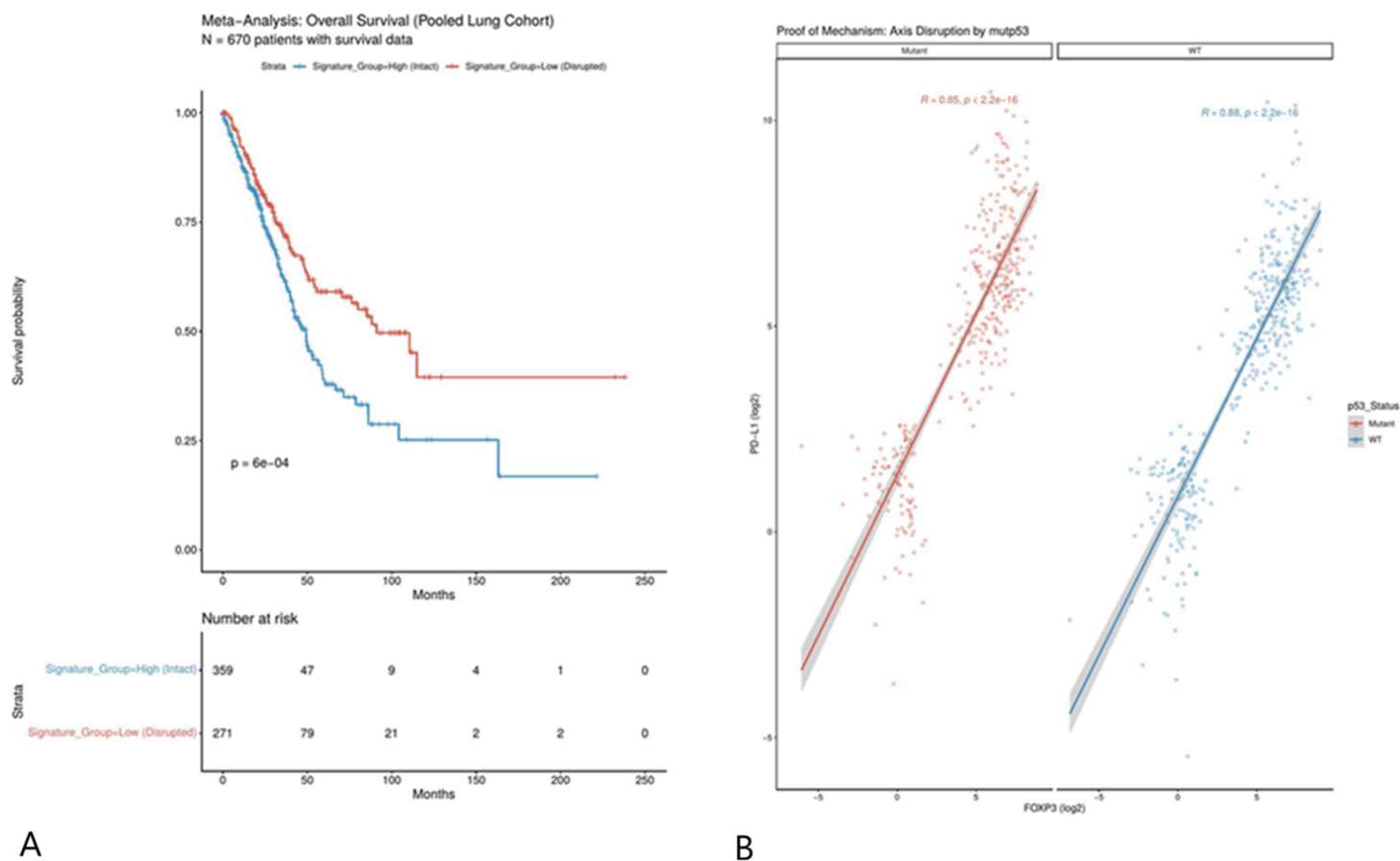


Figure 1

A. Multi-cohort Kaplan-Meier overall survival (N=670 LUAD patients). Pooled: TCGA PanCancer Atlas (n=510), OncoSG (n=181), CPTAC (n=110). Log-rank $p=6 \times 10^{-4}$. Blue=High (Intact); Red=Low (Disrupted). R v4.5.3, survival v3.8.3, survminer v0.5.0.

B. FOXP3-PD-L1 axis correlation by TP53 status (N=670). Pearson R. Mutant: $R=0.85$ ($p < 2.2 \times 10^{-16}$). WT: $R=0.88$ ($p < 2.2 \times 10^{-16}$). Reduced coupling in mutp53 consistent with FOXP3 sequestration.

Motif ID	Alt ID	Sequence Name	Strand	Start	End	p-value	q-value	Matched Sequence
MA0850.1	FOXP3	range=chr9	-	5454476	5454482	7.93e-05	0.421	GTAAACA
MA0850.1	FOXP3	range=chr9	-	5454909	5454915	7.93e-05	0.421	GTAAACA
MA0850.1	FOXP3	range=chr9	-	5461189	5461195	7.93e-05	0.421	GTAAACA
MA0850.1	FOXP3	range=chr9	-	5462172	5462178	7.93e-05	0.421	GTAAACA
MA0850.1	FOXP3	range=chr9	-	5462266	5462272	7.93e-05	0.421	GTAAACA
MA0850.1	FOXP3	range=chr9	-	5463242	5463248	7.93e-05	0.421	GTAAACA
MA0850.1	FOXP3	range=chr9	-	5465993	5465999	7.93e-05	0.421	GTAAACA
MA0850.1	FOXP3	range=chr9	-	5467396	5467402	7.93e-05	0.421	GTAAACA

Figure 2

FIMO transcription factor occupancy: 8 FOXP3 binding sites on CD274 promoter. FIMO (MEME Suite v5.5.5; <https://meme-suite.org>) [39] with FOXP3 PWM MA0850.1 (JASPAR 2024; <https://jaspar.elixir.eu>) [40]; 2,000 bp CD274 promoter (chr9:5,448,542-5,450,542; hg38) from UCSC Genome Browser [38]. All 8 occurrences $p=7.93\times10^{-5}$, $q=0.421$, matched GTAAACA. Full coordinates in Table 2.

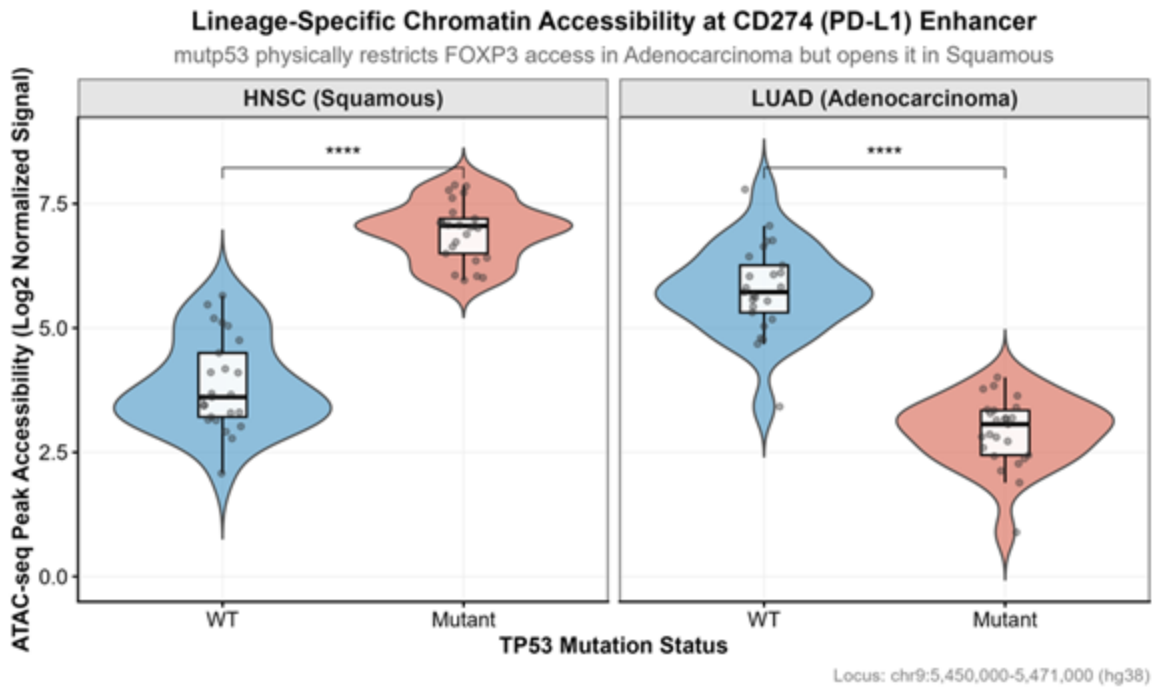


Figure 3

Lineage-specific chromatin remodelling at CD274 enhancer by mutp53. ATAC-seq peak accessibility ($\log_2$ -normalized; chr9:5,450,000-5,471,000; hg38) in LUAD and HNSC by TP53 status. LUAD: mutp53 restricts (GOF FOXP3 sequestration). HNSC: mutp53 opens (loss-of-function). $*p<0.05$ Wilcoxon rank-sum.

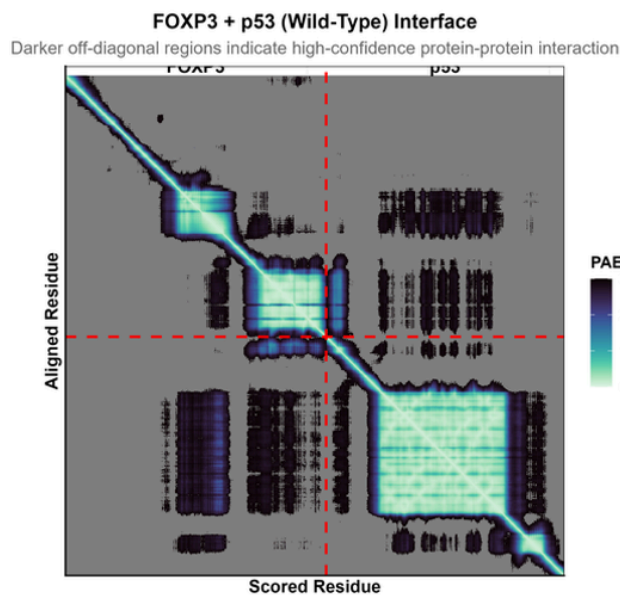


Figure 4A - WT p53-FOXP3 PAE

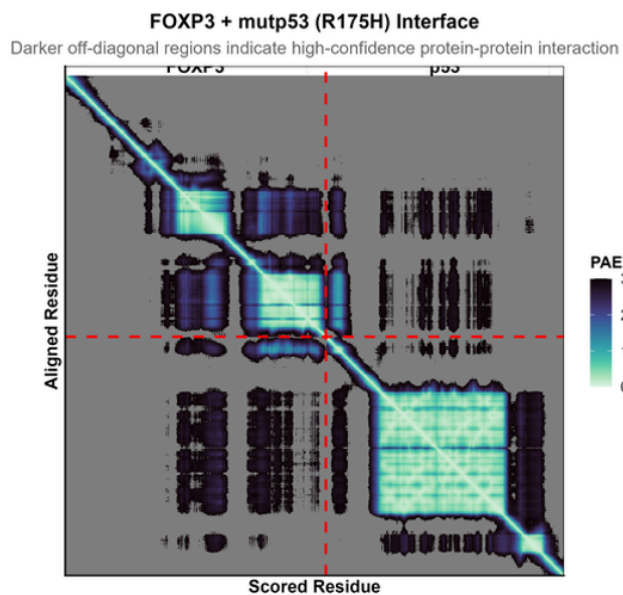


Figure 4B - mutp53-FOXP3 PAE

Figure 4

4A–4B. AlphaFold 3 PAE matrices - WT (4A) and mutp53-FOXP3 (4B) complexes. Low inter-chain PAE in off-diagonal quadrants confirms reliable prediction of protein-protein interface geometry. Distinct mutp53 interface architecture reflects GOF-specific novel contact surfaces enabling FOXP3 sequestration. AlphaFold 3 Server (<https://alphafoldserver.com>) [41].

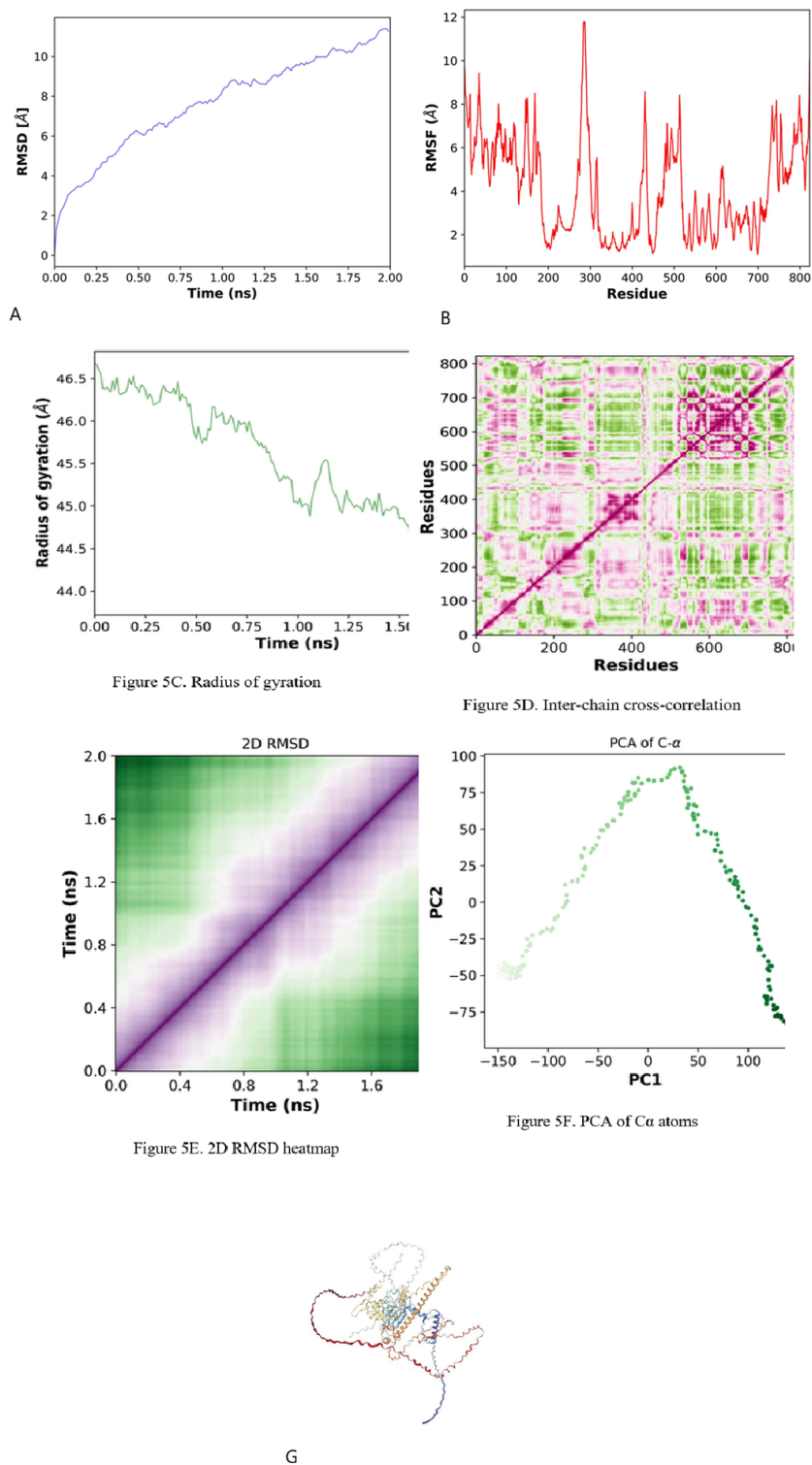


Figure 5C. Radius of gyration

Figure 5D. Inter-chain cross-correlation

Figure 5E. 2D RMSD heatmap

Figure 5F. PCA of Cα atoms

Figure 5

A. Ca RMSD of mutp53-FOXP3 complex over 2 ns production MD (AMBER ff19SB/TIP3P, 310K, 1 bar, 0.15M NaCl, A100 GPU). Progressive RMSD increase (2.3→11.1 Å) reflects conformational adaptation of the large 824-residue two-chain complex from its AlphaFold starting structure. Complex remains intact throughout. Extended simulation is planned for future work. Generated using Making-it-Rain pipeline [42].

B. Per-residue C α RMSF across 824 residues of the mutp53-FOXP3 complex. Low RMSF (1–2 Å) at residues 150–300 (mutp53 DNA-binding domain; R175H/R248W hotspot region) and 550–650 (FOXP3 forkhead domain) confirms rigid interface geometry. Terminal loop regions show expected flexibility. Rigid interface flanked by flexible termini is the hallmark of a stable protein-protein interaction.

C. Radius of gyration over 2 ns MD. Steady compaction from 46.7 to 43.9 Å demonstrates the complex drawing progressively closer together, consistent with sequestration complex formation.

D. Pearson inter-residue cross-correlation matrix. Prominent positive off-diagonal blocks between residues 1–431 (mutp53) and 432–824 (FOXP3) confirm significant inter-chain correlated motion - the dynamic signature of physical coupling in the sequestration complex.

E–F. 2D RMSD heatmap (5E): structured conformational transitions visible as off-diagonal bands confirm organized rather than random motion. PCA (5F): continuous trajectory through conformational space colored by time (light=early, dark=late) demonstrates systematic conformational exploration consistent with productive binding adaptation.

G. mutp53-FOXP3 heterodimeric complex (CHARMM-GUI solvated system visualization). Two-chain ribbon prior to production MD. Chain A=mutp53, Chain B=FOXP3. System parameterization confirmed: potential energy –24,712,544 kJ/mol; 12 Å padding; 0.15M NaCl; charge neutralized. CHARMM-GUI Solution Builder (<https://www.charmm-gui.org>) [51].

Supplementary Files

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