

Lineage-Specific Rewiring of the JAK-STAT Signalling Axis by Mutant TP53 Drives Pan-Cancer Immune Evasion: A Comprehensive Analysis of 5,775 TCGA Samples

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Research Article

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Abstract

Background

Mutant TP53 (mutp53) is the most frequent oncogenic alteration in human cancer, yet its capacity to reshape tumour immunology across tissue lineages remains incompletely understood. I hypothesized that mutp53-driven rewiring of the JAK-STAT axis would exhibit lineage-specific patterns with distinct immune and clinical consequences.

Methods

Pan-cancer analysis integrating RNA-sequencing, somatic mutation, mutational signature, and clinical data from 5,775 tumour samples across 10 TCGA cohorts. JAK-STAT activity was scored by ssGSEA; immune infiltration estimated across ten populations; mutational signatures fitted to COSMIC v3.2; survival analysed by stratified Cox regression.

Results

mutp53 rewired JAK-STAT signalling lineage-dependently across all ten cohorts. In adenocarcinoma, mutp53 paradoxically activated STAT1 ($\text{FDR} = 4.81 \times 10^{-23}$) and IRF1 ($\text{FDR} = 3.89 \times 10^{-19}$) while suppressing JAK2 ($\text{FDR} = 1.08 \times 10^{-10}$), revealing JAK2-independent STAT1 activation. CXCL10 was massively upregulated ($\text{delta} = +0.93$, $\text{FDR} = 9.08 \times 10^{-47}$) and PD-L1 elevated ($r = 0.433$, $p < 2.2 \times 10^{-16}$). In squamous lineages, IRF1 was paradoxically suppressed ($\text{FDR} = 0.013$) despite STAT1 activation, revealing transcription factor-level divergence. GOF mutp53 drove opposing survival outcomes: increased mortality in gastric adenocarcinoma ($\text{HR} = 2.13$, 95% CI 1.21–3.74, $p = 0.008$) versus survival benefit in lung squamous carcinoma ($\text{HR} = 0.61$, 95% CI 0.39–0.95, $p = 0.027$). mutp53 tumours showed elevated Tregs, T cell exhaustion, doubled TMB (median 109 vs 53, $p = 2.35 \times 10^{-73}$), and 3–4-fold SBS4 enrichment.

Conclusions

mutp53 is a lineage-context-dependent master regulator of JAK-STAT-mediated immune evasion. Tissue-of-origin determines immune exclusion strategy with direct clinical consequences, providing a framework for lineage-stratified immunotherapy in TP53-mutant cancers.

Background

TP53 is mutated in approximately 50% of all human cancers, making it the most frequently altered tumour suppressor gene^[1]. Beyond simple loss of function, specific missense mutations confer gain-of-function (GOF) properties that actively promote oncogenesis, immune evasion, and therapeutic

resistance^[2, 3]. The six most common GOF hotspot mutations — R175H, R248Q, R273H, R248W, R282W, and Y220C — collectively account for the majority of missense mutations observed clinically and exhibit properties qualitatively distinct from simple p53 loss^[4, 5]. Despite extensive characterization of mutp53 transcriptional programs, the degree to which tissue-of-origin determines the functional consequences of specific mutations remains incompletely understood.

The JAK-STAT signalling axis is a central mediator of innate and adaptive immune responses in the tumour microenvironment (TME). Canonical JAK-STAT activation, initiated by interferon-gamma (IFN- γ) binding to its receptor, proceeds through JAK1/JAK2 phosphorylation, STAT1 dimerization, and transcriptional activation of interferon-stimulated genes (ISGs) including IRF1, CXCL10, and CD274 (PD-L1)^[6]. This pathway represents a fundamental mechanism by which tumours both respond to immune pressure and paradoxically upregulate immune checkpoint molecules to evade destruction^[7, 8]. The JAK-STAT pathway is also a critical determinant of immunotherapy response and resistance^[9].

Previous work established that wild-type p53 modulates JAK-STAT signalling through transcriptional regulation of ISGs and direct interaction with STAT proteins^[10]. A 2025 study in hepatocellular carcinoma demonstrated that mutp53 disrupts the IFN- γ /JAK/STAT/IRF1/PD-L1 axis^[11]. However, whether this rewiring is lineage-specific has not been examined systematically. Our prior work demonstrated striking lineage specificity in mutp53-mediated transcriptional reprogramming, with a 67% versus 0% differential effect between adenocarcinoma and non-adenocarcinoma histologies on FOXP3 regulation^[12]. I therefore hypothesized that mutp53 rewiring of JAK-STAT signalling would exhibit lineage-dependent patterns with distinct molecular mechanisms and clinical consequences across adenocarcinoma versus squamous cell carcinoma contexts.

Here I report a pan-cancer analysis of 5,775 TCGA samples across 10 cancer types, integrating transcriptomic, genomic, mutational signature, and clinical data to systematically characterize mutp53-driven JAK-STAT rewiring and its immunological and prognostic consequences across lineages.

Methods

Data acquisition and cohort selection

RNA-sequencing data (STAR-aligned counts and TPM-normalized expression) and somatic mutation data (masked somatic mutation MAF files) were obtained from The Cancer Genome Atlas (TCGA) through the NCI GDC Data Portal (<https://portal.gdc.cancer.gov>) using TCGAbiolinks R package (v2.30.0)^[13]. Ten cohorts were analyzed: TCGA-LUAD (n = 601), TCGA-LUSC (n = 562), TCGA-BRCA (n = 1,231), TCGA-COAD (n = 524), TCGA-STAD (n = 448), TCGA-BLCA (n = 431), TCGA-HNSC (n = 566), TCGA-OV (n = 434), TCGA-PRAD (n = 554), and TCGA-LIHC (n = 424), totalling 5,775 samples. Cohorts were classified as adenocarcinoma lineage (LUAD, BRCA, COAD, STAD, OV, PRAD, LIHC; n = 4,216) or squamous lineage (LUSC, HNSC, BLCA; n = 1,559).

TP53 mutation classification

TP53 somatic mutations were extracted from MAF files filtered to missense, nonsense, frameshift, and splice site variants. Samples were classified as mutp53 (n = 2,348, 40.7%) or WTP53 (n = 3,427, 59.3%). GOF mutations were defined as established hotspot missense mutations: R175H (n = 90), R248Q (n = 57), R273H (n = 49), R248W (n = 42), R282W (n = 42), Y220C (n = 36), R273C (n = 35), V157F (n = 28), H193R (n = 20), R158L (n = 20), R273L (n = 20), and Y163C (n = 21), totalling 453 confirmed GOF samples [2, 4].

JAK-STAT pathway scoring

A 20-gene JAK-STAT pathway signature was constructed comprising: JAK1, JAK2, STAT1, STAT3, IRF1, IFNG, CD274, SOCS1, SOCS3, MX1, OAS1, IFIT1, CXCL10, IFITM1, IRF9, PIAS1, PTPN2, IL6, IL10, and STAT2. Per-sample activity was scored using single-sample gene set enrichment analysis (ssGSEA) implemented in the GSVA Bioconductor package (v1.52.0) with Gaussian kernel [14]. Expression matrices were $\log_2(\text{TPM} + 1)$ normalized prior to scoring. All 20 genes were detected in all 10 cohorts.

Individual gene expression analysis

Key JAK-STAT component genes (STAT1, JAK1, JAK2, IRF1, CXCL10, CD274) were analyzed from $\log_2(\text{TPM} + 1)$ normalized data. Differential expression between mutp53 and WTP53 groups was assessed per lineage using Wilcoxon rank-sum tests with Benjamini-Hochberg false discovery rate (FDR) correction.

Immune cell deconvolution

Immune cell infiltration was estimated using gene signature-based ssGSEA scoring for ten populations: CD8 + T cells (CD8A, CD8B, GZMA, GZMB, PRF1, IFNG, TBX21, EOMES), CD4 + T cells, regulatory T cells (FOXP3, IL2RA, CTLA4, IKZF2, TNFRSF18), NK cells (NCAM1, NKG7, GNLY, KLRB1, KLRD1), M1 macrophages (CD68, CD80, CD86, IL6, TNF, NOS2, CXCL9), M2 macrophages (MRC1, CD163, IL10, TGFB1, ARG1), B cells, dendritic cells, neutrophils, and exhausted T cells (PDCD1, LAG3, TIGIT, HAVCR2, CTLA4, TOX, ENTPD1). Signatures derived from established reference sets [15, 16].

Tumour mutational burden

Tumour mutational burden (TMB) was calculated as total somatic mutation count per sample from MAF files and $\log_2(n + 1)$ transformed for correlation analyses. Differential TMB between mutp53 and WTP53 groups was assessed by Wilcoxon rank-sum test per lineage.

Mutational signature analysis

Somatic single nucleotide variants (SNVs) were processed using the MutationalPatterns Bioconductor package (v3.14.0) ^[17] against the hg38 reference genome. Mutation matrices (96 trinucleotide contexts) were fitted to COSMIC Mutational Signatures v3.2 reference signatures ^[18] by non-negative least squares. Key signatures analyzed: SBS1 (aging), SBS2 and SBS13 (APOBEC), SBS3 (homologous recombination deficiency), SBS4 (tobacco), SBS17a/b (gastric-associated), and SBS40. A total of 4,830 samples with adequate SNV counts were included.

Survival analysis

Overall survival data were obtained from TCGA clinical databases. Cox proportional hazards regression included GOF mutp53 status (binary), scaled JAK-STAT score, and their interaction term as covariates, stratified by cohort to control for baseline hazard. GOF analyses compared confirmed hotspot samples (n = 453) versus WTp53 (n = 3,427). A total of 5,668 samples with available survival data were analyzed (1,832 events).

Statistical analysis

All analyses were performed in R v4.4.3. Differential pathway activity was assessed by Student t-test and Wilcoxon rank-sum test with Benjamini-Hochberg FDR correction. Pearson correlation was used for continuous associations. Statistical significance was defined as FDR < 0.05. Plots were generated with ggplot2 (v3.5.0), survminer, and ggpubr R packages.

Data availability

The datasets analyzed during the current study are publicly available from The Cancer Genome Atlas (TCGA) through the NCI GDC Data Portal at <https://portal.gdc.cancer.gov>. No new patient data were generated. All processed data and analysis code are available from the corresponding author upon reasonable request and will be deposited to a public repository upon acceptance.

Results

Pan-cancer landscape of mutp53 JAK-STAT rewiring

To characterize mutp53-driven JAK-STAT rewiring, I analyzed 5,775 tumour samples spanning 10 TCGA cohorts (Table 1). TP53 mutations were identified in 2,348 samples (40.7%), with mutation frequency ranging from 9.9% (PRAD) to 69.6% (LUSC). All 20 JAK-STAT signature genes were detected in each cohort enabling consistent cross-cohort analysis.

Table 1
Cohort characteristics and TP53 mutation frequencies

Cohort	Lineage	Total n	mutp53 n	WTP53 n	mutp53%	Mean JAK (mutp53)	Mean JAK (WTP53)
TCGA-LUAD	Adeno	601	257	344	42.8%	2.47	2.38
TCGA-LUSC	Squamous	562	391	171	69.6%	2.55	2.43
TCGA-BRCA	Adeno	1,231	334	897	27.1%	2.52	2.44
TCGA-COAD	Adeno	524	240	284	45.8%	2.29	2.35
TCGA-STAD	Adeno	448	177	271	39.5%	2.34	2.42
TCGA-BLCA	Squamous	431	196	235	45.5%	2.53	2.44
TCGA-HNSC	Squamous	566	340	226	60.1%	2.51	2.45
TCGA-OV	Adeno	434	259	175	59.7%	2.44	2.39
TCGA-PRAD	Adeno	554	55	499	9.9%	2.32	2.38
TCGA-LIHC	Adeno	424	99	325	23.3%	2.38	2.41
Total	Mixed	5,775	2,348	3,427	40.7%	—	—

mutp53 was significantly associated with altered JAK-STAT pathway activity in a lineage-dependent manner (Fig. 1). Adenocarcinoma cohorts with hormone-driven or proliferative biology (BRCA, OV, LUAD) demonstrated positive JAK-STAT delta scores indicating pathway activation, while GI adenocarcinomas (COAD, STAD, LIHC) showed suppression. Within the squamous lineage, LUSC showed significant activation (FDR = 0.009) while HNSC remained neutral. GOF mutp53 was associated with significantly higher JAK-STAT scores versus WTP53 in both adenocarcinoma (delta = 0.060, $p = 0.0011$) and squamous (delta = 0.098, $p = 0.025$) contexts (Fig. 2). Mutation-specific analysis revealed V157F had the strongest JAK-STAT elevation in adenocarcinoma (mean = 2.58 vs 2.28, $p = 0.041$), while other hotspot mutations showed heterogeneous effects (Fig. S3), confirming lineage context dominates over mutation identity.

Paradoxical JAK2 suppression with downstream STAT1 activation

Individual component analysis revealed a striking mechanistic paradox (Table 2). JAK2 was significantly suppressed in mutp53 tumours in both adenocarcinoma (delta = - 0.186, FDR = 1.08×10^{-10}) and squamous lineages (delta = - 0.185, FDR = 2.93×10^{-5}), while downstream STAT1 was simultaneously and significantly upregulated in adeno (delta = + 0.379, FDR = 4.81×10^{-23}) and squamous (delta = + 0.406, FDR = 5.47×10^{-9}) contexts. JAK1 was also modestly suppressed in adenocarcinoma (delta = - 0.079, FDR = 0.003). This paradoxical dissociation indicates that mutp53 activates STAT1 through a JAK2-independent mechanism not previously described.

Table 2

JAK-STAT component gene expression by mutp53 status and lineage Values in log2(TPM + 1). Delta = mean(mutp53) - mean(WTp53). Wilcoxon rank-sum test with Benjamini-Hochberg FDR. Adeno: n = 1,388 mutp53, 2,760 WTp53; squamous: n = 919 mutp53, 624 WTp53.

Gene	Lineage	Mean mutp53	Mean WTp53	Delta	FDR	Sig.
STAT1	Adeno	6.52	6.15	+ 0.379	4.81×10^{-23}	***
STAT1	Squamous	6.79	6.38	+ 0.406	5.47×10^{-9}	***
JAK2	Adeno	2.91	3.10	-0.186	1.08×10^{-10}	***
JAK2	Squamous	2.92	3.11	-0.185	2.93×10^{-5}	***
JAK1	Adeno	5.26	5.34	-0.079	2.55×10^{-3}	**
IRF1	Adeno	4.39	4.11	+ 0.279	3.89×10^{-19}	*** ↑
IRF1	Squamous	4.38	4.52	-0.137	0.013	* ↓
CXCL10	Adeno	6.09	5.16	+ 0.931	9.08×10^{-47}	***
CXCL10	Squamous	6.16	5.70	+ 0.453	6.43×10^{-4}	***
CD274 (PD-L1)	Adeno	2.14	1.93	+ 0.205	1.24×10^{-5}	***
CD274 (PD-L1)	Squamous	3.21	3.00	+ 0.217	0.013	*
↑ activated in adeno; ↓ suppressed in squamous — key mechanistic divergence. ** FDR < 0.01; *** FDR < 0.001.						

The mechanistic divergence between lineages was most apparent at the IRF1 level. In adenocarcinoma, mutp53 significantly activated IRF1 (delta = + 0.279, FDR = 3.89×10^{-19}), consistent with STAT1-mediated transcription. In squamous lineages, IRF1 was paradoxically suppressed (delta = - 0.137, FDR = 0.013) despite equivalent STAT1 upregulation (Fig. 3). This lineage-specific IRF1 uncoupling from STAT1 suggests that lineage-defining transcription factors — most likely TP63 in squamous epithelium — create chromatin environments that prevent STAT1-driven IRF1 transcription in squamous contexts, while TTF-1/NKX2-1 in adenocarcinoma permissively allows this coupling.

JAK-STAT rewiring drives effector dysregulation and PD-L1 upregulation

The downstream functional consequences were most pronounced at the effector gene level (Table 2). CXCL10 showed the strongest and most statistically significant upregulation in mutp53 adenocarcinomas (mean 6.09 vs 5.16 log2 TPM, delta = + 0.931, FDR = 9.08×10^{-47}) and squamous tumours (delta = + 0.453, FDR = 6.43×10^{-4}). PD-L1 (CD274) was significantly elevated in mutp53 tumours across both lineages (adeno: delta = + 0.205, FDR = 1.24×10^{-5} ; squamous: delta = + 0.217, FDR = 0.013). Per-cohort analysis revealed significance in LUAD (***), LUSC (**), BLCA (****), BRCA (*), and LIHC (*) (Fig. S4).

JAK-STAT pathway scores were strongly correlated with PD-L1 expression across all groups (Fig. 5). The correlation was substantially stronger in mutp53 versus Wtp53 adenocarcinoma tumours ($r = 0.433$ vs $r = 0.307$, both $p < 2.2 \times 10^{-16}$), indicating that mutp53 amplifies the coupling between JAK-STAT activity and PD-L1 output. In squamous Wtp53 tumours, this correlation was particularly strong ($r = 0.633$), suggesting that the squamous lineage context intrinsically links JAK-STAT to PD-L1 regulation more tightly.

GOF mutation-specific survival effects are lineage-dependent

Cox proportional hazards regression examining the interaction between GOF mutp53 and JAK-STAT activity on overall survival across 5,668 samples revealed three cohorts with statistically significant interaction terms (Table 3, Fig. 6). In TCGA-STAD ($n = 296$, events = 126), the interaction conferred significantly increased mortality (HR = 2.13, 95% CI 1.21–3.74, $p = 0.008$). Conversely, in TCGA-LUSC ($n = 236$, events = 118), the same interaction was associated with paradoxical survival benefit (HR = 0.61, 95% CI 0.39–0.95, $p = 0.027$). TCGA-BRCA showed a protective interaction (HR = 0.53, 95% CI 0.29–0.98, $p = 0.043$). These opposing HR directions are consistent with the divergent IRF1 regulation and confirm that survival consequences are determined by tissue context rather than mutation identity.

Table 3

GOF mutp53 × JAK-STAT interaction hazard ratios for overall survival per cohort Cox proportional hazards regression with GOF mutp53 status, JAK-STAT score, and interaction term, stratified by cohort. n total = 5,668; n events = 1,832.

Cohort	Lineage	n total	n events	HR	95% CI	p-value
TCGA-STAD	Adeno	296	126	2.13	1.21–3.74	0.008 **
TCGA-LUSC	Squamous	236	118	0.61	0.39–0.95	0.027 *
TCGA-BRCA	Adeno	946	156	0.53	0.29–0.98	0.043 *
TCGA-BLCA	Squamous	267	127	0.69	0.47–1.00	0.052 ns
TCGA-LUAD	Adeno	360	131	0.82	0.36–1.88	0.635 ns
TCGA-LIHC	Adeno	334	131	0.58	0.17–2.01	0.387 ns
TCGA-HNSC	Squamous	283	124	0.89	0.59–1.34	0.565 ns
TCGA-OV	Adeno	230	145	0.99	0.68–1.45	0.963 ns
TCGA-COAD	Adeno	350	68	1.04	0.64–1.70	0.871 ns
HR, hazard ratio; CI, confidence interval. ** $p < 0.01$; * $p < 0.05$; ns, not significant. GOF mutations: R175H, R248Q, R273H, R248W, R282W, Y220C, R273C, V157F, H193R, R158L, R273L, Y163C.						

mutp53 remodels the tumour immune microenvironment

Immune cell deconvolution revealed that mutp53 significantly remodelled the TME without directly depleting CD8 + T cells, which showed no statistically significant change in either lineage (Fig. 7). Instead, mutp53 created immunosuppression through expansion of regulatory populations: Treg infiltration was significantly elevated in adenocarcinoma (delta = + 0.022, FDR = 7.64×10^{-5}) and squamous (delta = + 0.044, FDR = 3.28×10^{-8}) mutp53 tumours. T cell exhaustion scores were similarly elevated (adeno: FDR = 9.89×10^{-5} ; squamous: FDR = 2.82×10^{-4}). M1 macrophage infiltration was elevated in mutp53 adeno (FDR = 1.72×10^{-11}) while dendritic cells were reduced (FDR = 2.08×10^{-10}). The complete immune landscape is shown in Fig. S7.

JAK-STAT pathway scores were exceptionally strongly correlated with CD8 + T cell infiltration across all groups. In WTP53 adenocarcinoma, this correlation reached $r = 0.772$ ($p < 2.2 \times 10^{-16}$), and in squamous WTP53 tumours $r = 0.864$ ($p < 2.2 \times 10^{-16}$). In mutp53 tumours, the correlation was attenuated (adeno: $r = 0.711$; squamous: $r = 0.735$), quantifying for the first time how mutp53 partially uncouples JAK-STAT-driven chemokine production from effective cytotoxic T cell recruitment, instead favouring a Treg-dominated exhausted microenvironment consistent with the immune-excluded phenotype associated with poor immunotherapy response ^[20].

Mutational signatures link tobacco and APOBEC mutagenesis to JAK-STAT rewiring

Mutational signature analysis across 4,830 samples revealed striking enrichment in mutp53 tumours (Figs. S8, S9). SBS4 (tobacco) showed the most dramatic enrichment: mean 41.1 versus 12.1 in adeno ($p = 5.81 \times 10^{-10}$) and 44.6 versus 15.2 in squamous ($p = 3.02 \times 10^{-18}$), a 3–4-fold enrichment. SBS13 (APOBEC) was significantly elevated in mutp53 adenocarcinomas ($\Delta = +1.5$, $\text{FDR} = 1.22 \times 10^{-43}$) but not squamous tumours, revealing a lineage-specific APOBEC-mutp53 interaction. JAK-STAT scores correlated positively with SBS4 in mutp53 adenocarcinomas ($r = 0.396$, $p = 2.86 \times 10^{-53}$), suggesting tobacco-associated mutagenesis amplifies JAK-STAT rewiring.

mutp53 tumours showed significantly elevated TMB in both lineages: median 109 versus 53 mutations per sample in adeno ($p = 2.35 \times 10^{-73}$) and median 215 versus 148 in squamous ($p = 1.33 \times 10^{-13}$) (Fig. S5). The positive correlation between TMB and JAK-STAT in adeno mutp53 ($r = 0.476$, $p = 4.59 \times 10^{-81}$) but negative correlation in squamous mutp53 ($r = -0.25$, $p = 1.02 \times 10^{-14}$) (Fig. S6) further illustrates the lineage-dependent relationship between genomic instability and immune pathway activation.

Discussion

This study provides the most comprehensive characterization to date of mutp53-driven JAK-STAT rewiring across human cancer types, analyzing 5,775 TCGA samples across 10 cohorts. The principal finding — that mutp53 remodels JAK-STAT signalling in a lineage-context-dependent manner with distinct molecular mechanisms, immune microenvironment consequences, and clinical outcomes — has important implications for understanding cancer immunology and for developing rational immunotherapy strategies in TP53-mutant cancers.

The most mechanistically novel observation is the paradoxical dissociation between JAK2 suppression and STAT1 activation in mutp53 tumours. Wild-type p53 regulates the IFN- γ signalling cascade^[10], and these data suggest that mutp53 GOF activity specifically uncouples canonical JAK2-mediated STAT1 phosphorylation while maintaining or enhancing alternative STAT1 activation pathways. Non-canonical STAT1 activation has been described through receptor tyrosine kinases, EGFR, and direct protein-protein interactions^[21], and mutp53 is known to interact with transcriptional coactivators including p300/CBP with broad chromatin regulatory activities^[22]. Future studies using ChIP-sequencing and co-immunoprecipitation in isogenic cell systems should establish whether mutp53 recruits STAT1 to JAK-STAT target gene promoters independently of upstream kinase signalling.

The lineage-specific divergence at the IRF1 level is the most clinically significant finding of this study. In squamous tumours, despite STAT1 upregulation, IRF1 is paradoxically suppressed. TP63, the master lineage transcription factor of squamous epithelium, antagonizes p53 family member functions and remodels chromatin at shared target loci^[23]. I propose that TP63-mediated chromatin compaction at IRF1 regulatory elements creates an epigenetic barrier that prevents STAT1-driven IRF1 transcription in

squamous contexts. ATAC-sequencing and ChIP-sequencing in isogenic cell lines with controlled mutp53 expression across lineage backgrounds can directly test this hypothesis.

The CXCL10 paradox is immunologically important. CXCL10 is a critical CD8 + T cell recruitment chemokine associated with immunotherapy response ^[24]. The massive upregulation ($\Delta = +0.93$, $p = 7.56 \times 10^{-48}$) would be expected to enhance CD8 + T cell infiltration, yet deconvolution reveals no increase in CD8 + T cell scores but significant Treg and exhausted T cell elevation. This represents the immune-excluded phenotype ^[20] in which CXCL10 production is decoupled from effective cytotoxic recruitment, likely through concurrent Treg expansion and PD-L1-mediated T cell exhaustion ^[27]. The quantification of the attenuated JAK-STAT/CD8 correlation in mutp53 versus Wtp53 tumours ($r = 0.71$ vs 0.77 in adeno; $r = 0.74$ vs 0.86 in squamous) provides the first quantitative measure of this uncoupling.

The opposing survival effects of GOF mutp53 $\times$ JAK-STAT interaction in STAD versus LUSC confirm that clinical intervention strategies in mutp53 cancers must be lineage-stratified. The HR = 2.13 in STAD suggests that gastric mutp53 JAK-STAT activation amplifies an already immunosuppressive microenvironment ^[25]. The paradoxical protection in LUSC may reflect context-specific inflammatory consequences of JAK-STAT activation in a tobacco-dominated mutational landscape ^[26] where APOBEC and smoking signatures create distinct neoantigen profiles that partially override mutp53-driven immune exclusion, consistent with our prior demonstration that mutp53 hotspot variants differentially rewire downstream regulatory networks across cancer lineages ^[28].

The SBS4 enrichment (3–4-fold in mutp53 vs Wtp53) and its positive correlation with JAK-STAT scores ($r = 0.396$ in mutp53 adeno) suggest that tobacco-induced mutagenesis not only generates TP53 mutations through C > A transversion at hotspot codons but also creates a broader genomic and epigenomic context that amplifies JAK-STAT rewiring. Patients with tobacco-associated mutp53 cancers may require distinct immunotherapy approaches accounting for both mutp53-driven immune exclusion and the high TMB that would theoretically favour immunotherapy response.

Several limitations merit consideration. Bulk RNA-sequencing cannot resolve cell-type-specific JAK-STAT activation; single-cell studies are needed. Functional validation in mutp53-expressing cell lines is required to establish causal relationships. Our BLCA classification as squamous lineage represents a histological simplification. Unmeasured confounders including treatment heterogeneity may influence survival analyses. As a computational study, all findings represent associative evidence requiring experimental validation.

Conclusions

mutp53 rewires JAK-STAT signalling in a lineage-context-dependent manner with IRF1 serving as the key mechanistic divergence point. The combination of JAK2-independent STAT1 activation, CXCL10 upregulation without CD8 + T cell recruitment, Treg expansion, and lineage-dependent IRF1 regulation creates a coherent model of mutp53-driven immune exclusion with tissue-specific nuances. These

findings support lineage-stratified approaches to immunotherapy in TP53-mutant cancers and provide a mechanistic rationale for prospective clinical studies examining TP53 mutation status and histological lineage as co-predictors of immunotherapy response.

Declarations

Ethics approval and consent to participate

Not applicable. This study used exclusively de-identified, publicly available data from The Cancer Genome Atlas (TCGA), which are governed by the TCGA Data Use Certification Agreement. No new patient data were collected and no additional ethics approval was required.

Consent for publication

Not applicable.

Competing interests

The author declares no competing interests.

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Authors' contributions

DSV conceived and designed the study, performed all data acquisition, statistical analyses, figure generation, and manuscript writing. The author has read and approved the final manuscript.

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Availability of data and materials

The datasets analyzed during the current study are publicly available from The Cancer Genome Atlas (TCGA) through the NCI Genomic Data Commons Data Portal at <https://portal.gdc.cancer.gov>. All

custom analysis code is available from the corresponding author upon reasonable request and will be deposited to GitHub upon publication.

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Figures

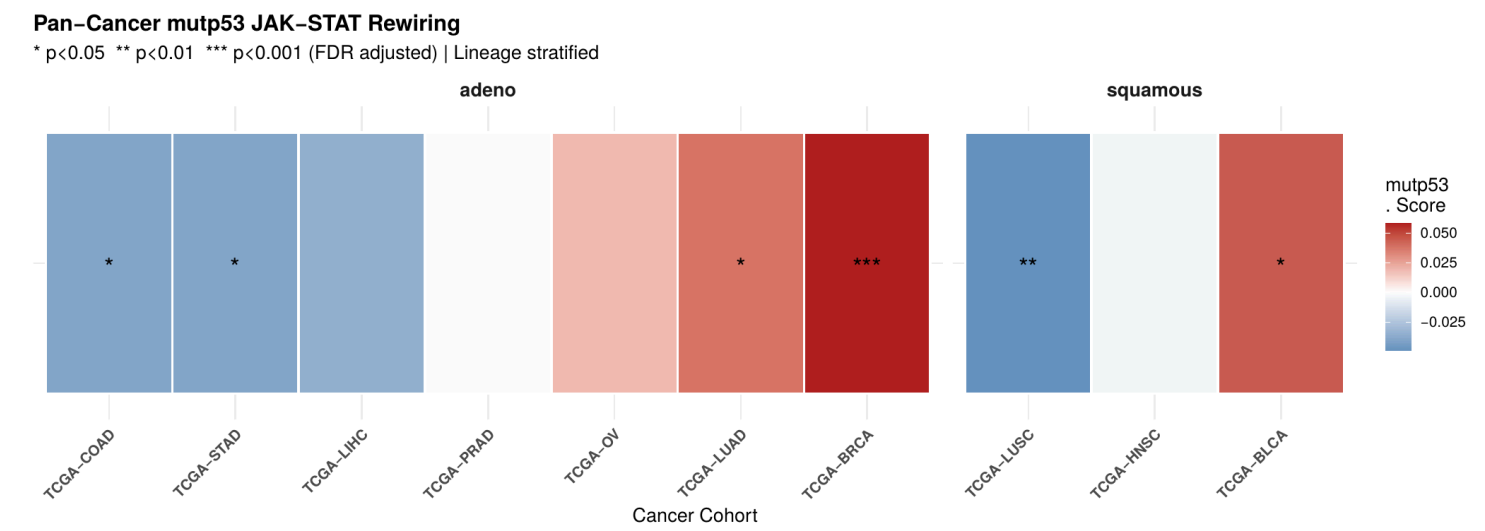


Figure 1

Pan-cancer mutp53 JAK-STAT rewiring heatmap. Delta scores represent the difference in mean ssGSEA JAK-STAT pathway scores between mutp53 and Wtp53 samples per cohort. Red indicates mutp53-associated pathway activation; blue indicates suppression. Cohorts are grouped by lineage (adeno vs squamous). Asterisks indicate FDR-adjusted significance (* p<0.05, ** p<0.01, *** p<0.001; Benjamini-Hochberg). n=5,775 samples, 10 TCGA cohorts.

Lineage-Specific JAK-STAT Rewiring by mutp53

Key finding: Adeno vs Squamous dichotomy

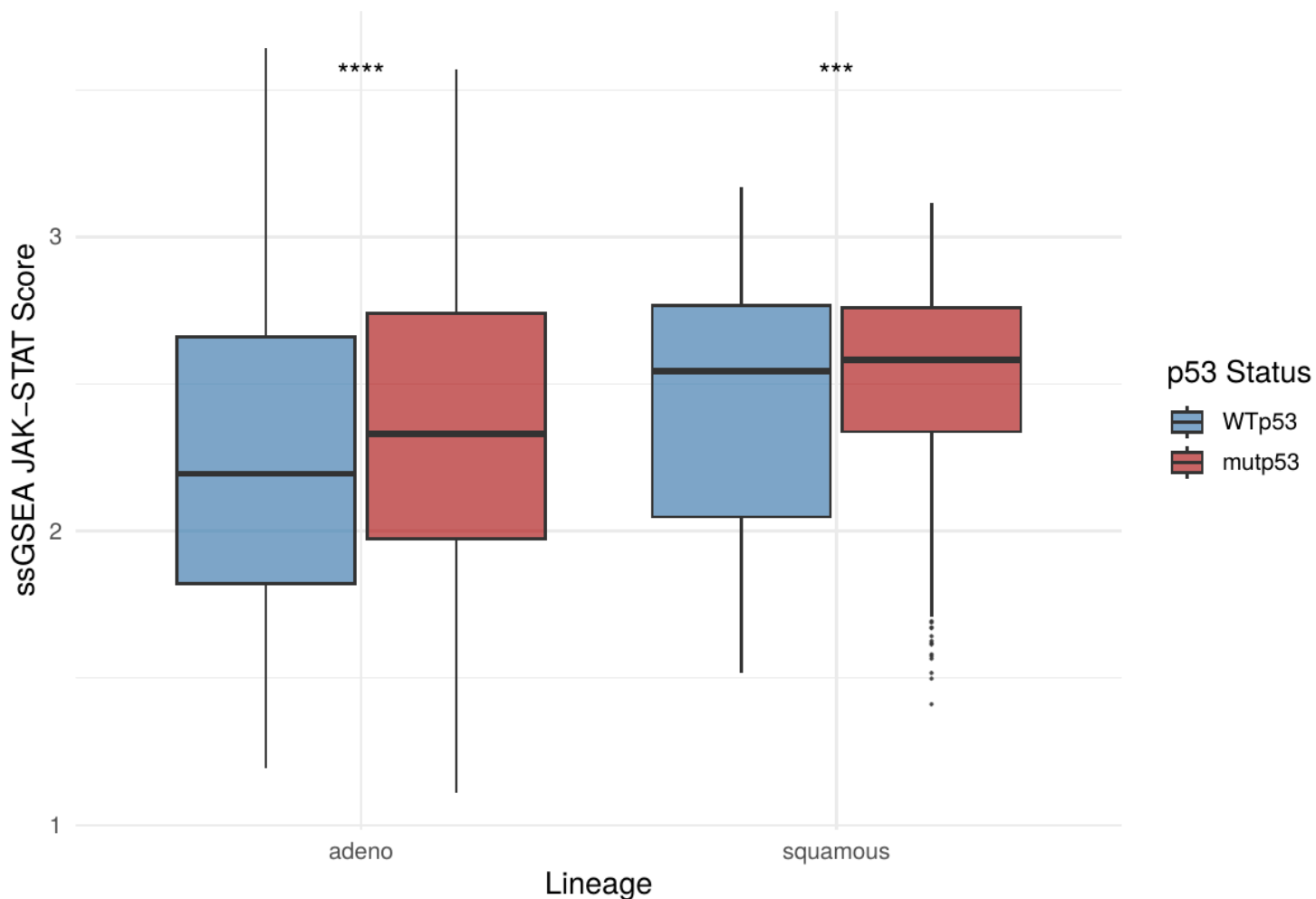


Figure 2

Lineage-specific JAK-STAT score distribution. Boxplots showing ssGSEA JAK-STAT pathway scores stratified by mutp53 status (red: mutp53, blue: WTp53) and lineage. Both lineages show significant differences (**** $p < 0.0001$, *** $p < 0.001$) by Wilcoxon rank-sum test, confirming lineage-dependent rewiring.

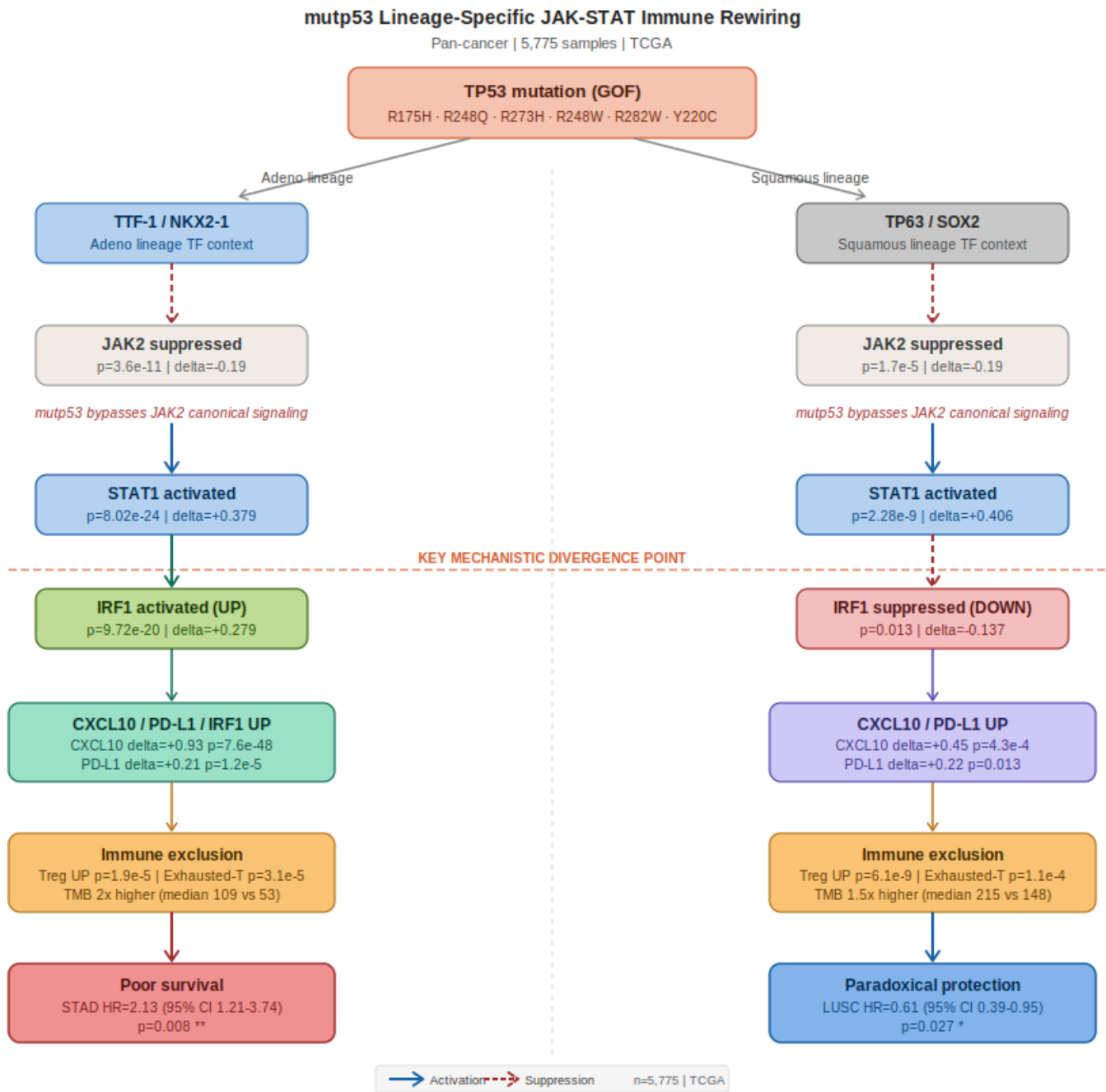


Figure 3

Schematic of mutp53 lineage-specific JAK-STAT immune rewiring. mutp53 GOF mutations suppress JAK2 in both adenocarcinoma (TTF-1/NKX2-1 lineage TF context, left) and squamous (TP63/SOX2 TF context, right) lineages while paradoxically activating STAT1 through a JAK2-independent mechanism. The critical mechanistic divergence occurs at IRF1: activated (green) in adenocarcinoma but suppressed (red dashed) in squamous. Differential IRF1 output drives distinct immune microenvironment remodelling and opposing survival outcomes (STAD HR=2.13 vs LUSC HR=0.61). Solid arrows: activation; dashed red arrows: suppression. All statistical values derived from 5,775 TCGA samples.

JAK-STAT Effectors: IRF1, CXCL10, PD-L1

mutp53 vs WTp53 | Adeno vs Squamous

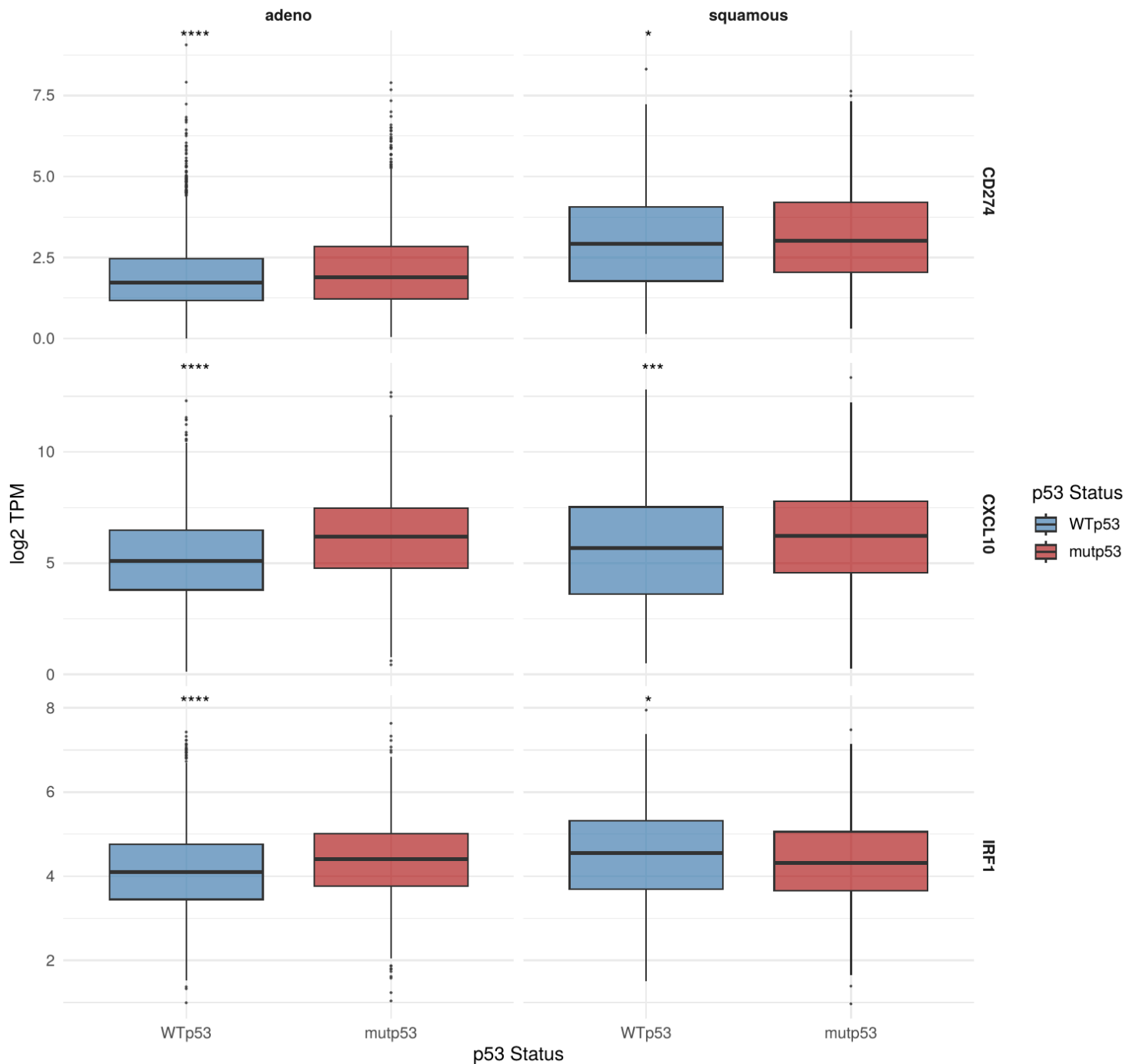


Figure 4

JAK-STAT effector gene dysregulation by mutp53. Violin and boxplots showing log2 TPM expression of IRF1, CXCL10, and CD274 (PD-L1) in mutp53 (red) versus WTp53 (blue) tumours stratified by lineage (adeno vs squamous). Significance by Wilcoxon rank-sum test with FDR correction (**** $p < 0.0001$, *** $p < 0.001$, * $p < 0.05$). The opposite IRF1 direction in adeno (UP, $\Delta = +0.279$) versus squamous (DOWN, $\Delta = -0.137$) represents the key mechanistic divergence. CXCL10 shows the largest absolute effect size ($\Delta = +0.931$ in adeno, $\text{FDR} = 9.08 \times 10^{-47}$).

JAK-STAT Score vs PD-L1 (CD274)

Pearson | mutp53 vs WTp53 | By lineage

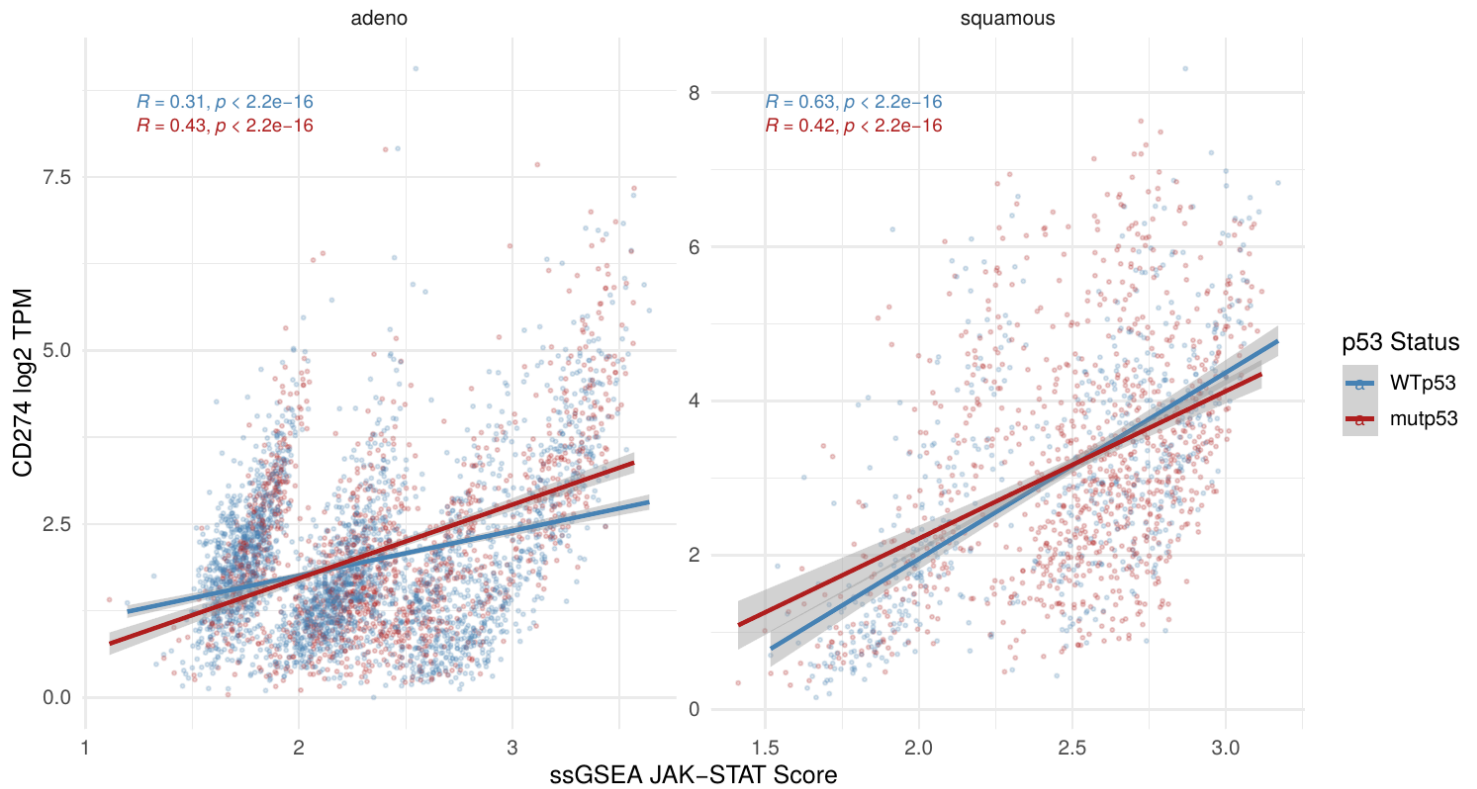


Figure 5

JAK-STAT score correlates with PD-L1 (CD274) expression. Scatter plots showing Pearson correlation between ssGSEA JAK-STAT score and $\log_2(\text{CD274 TPM}+1)$ stratified by mutp53 status (red: mutp53, blue: WTp53) and lineage (adeno vs squamous). All correlations highly significant ($p < 2.2 \times 10^{-16}$). mutp53 amplifies the JAK-STAT to PD-L1 coupling in adenocarcinoma ($r=0.433$ vs $r=0.307$ in WTp53). Squamous WTp53 shows the tightest coupling ($r=0.633$).

GOF-mutp53 x JAK-STAT: Survival HR Per Cohort

Hotspot GOF mutations only (R175H, R248Q, R273H etc.)

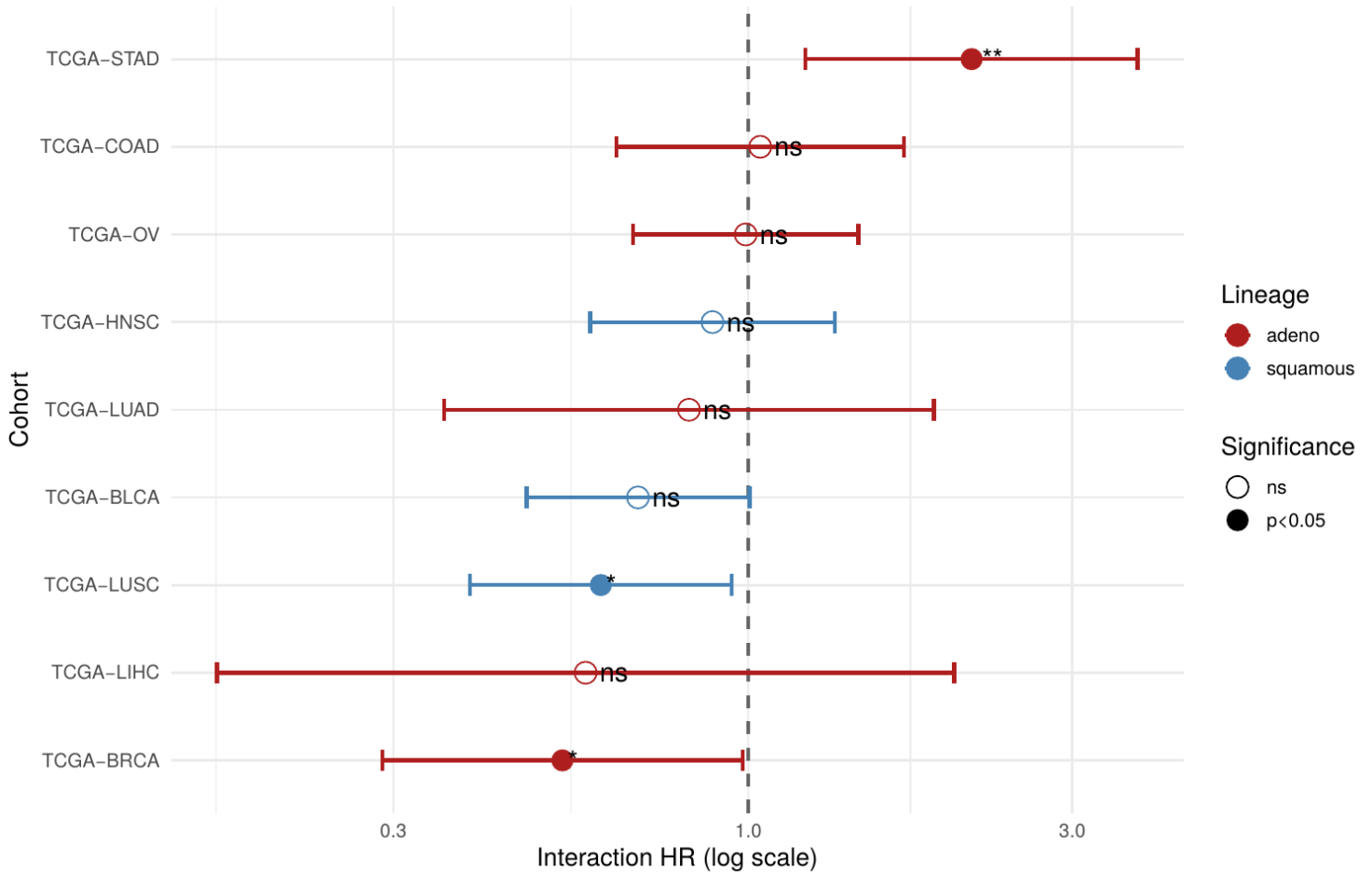


Figure 6

GOF-mutp53 × JAK-STAT interaction hazard ratios for overall survival per cohort. Forest plot showing Cox regression interaction term hazard ratios for confirmed GOF hotspot mutp53 (R175H, R248Q, R273H, R248W, R282W, Y220C) × JAK-STAT score interaction per cohort. Error bars: 95% confidence intervals. Filled circles: p<0.05. Colors denote lineage (red: adeno, blue: squamous). TCGA-STAD shows significantly increased mortality risk (HR=2.13, 95% CI 1.21–3.74, p=0.008, **); TCGA-LUSC shows paradoxical protection (HR=0.61, 95% CI 0.39–0.95, p=0.027, *); TCGA-BRCA shows protection (HR=0.53, p=0.043, *).

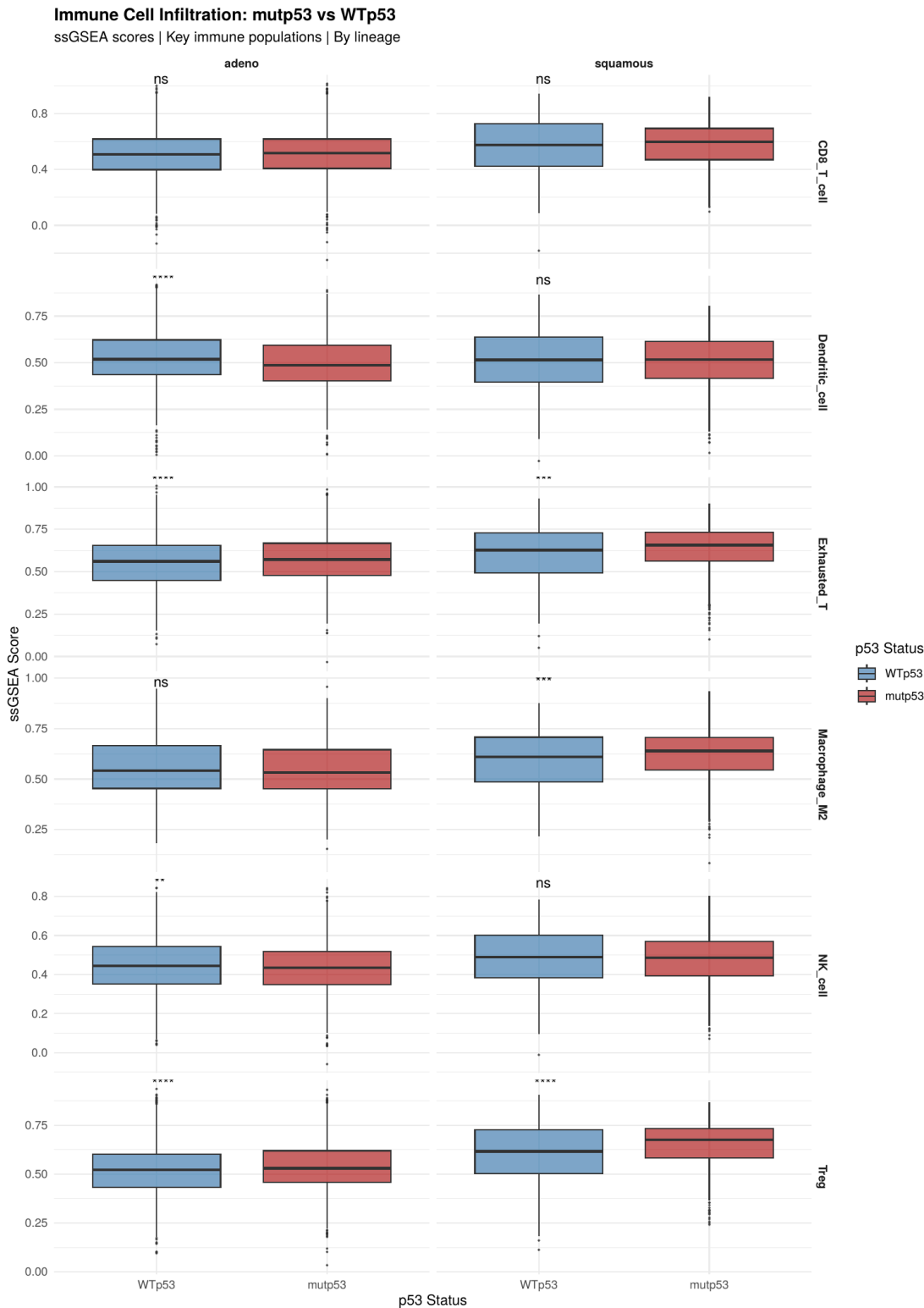


Figure 7

mutp53 remodels the tumour immune microenvironment. Boxplots showing ssGSEA immune cell infiltration scores for key populations (CD8+ T cells, exhausted T cells, Tregs, NK cells, M2 macrophages, dendritic cells) stratified by mutp53 status (red: mutp53, blue: WTp53) and lineage. Significance by Wilcoxon rank-sum test with FDR correction (**** FDR<0.0001, *** FDR<0.001, ** FDR<0.01, * FDR<0.05, ns: not significant). Note: CD8+ T cells show no significant direct change despite Treg and exhausted T

cell elevation, indicating immunosuppression through regulatory expansion rather than cytotoxic depletion.