

Supplementary Materials

Lineage Plasticity and Spatial Immune Redistribution Patterns in TTF-1/p40 Dual-Positive NSCLC: A Multi-Omics Study

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Supplementary Figure S1. Mutation frequencies of the top 20 most frequently altered genes by lineage group.

Supplementary Figure S2. HRR-pathway mutation burden score by lineage group. NOTE: This score is a mutation-count descriptor, not based on the established LOH+LST+TAI methodology, and has not been benchmarked against whole-genome HRD assays. It is

presented for exploratory reference only and should not be interpreted as a validated clinical HRD score.

Supplementary Figure S3. Patient-level spatial comparisons (per-patient regional means) for key immune metrics.

Supplementary Figure S4. Sensitivity analysis excluding mixed-phenotype cases: pure TTF-1 only versus pure diffuse regions.

Supplementary Methods: Detailed Analytical Protocols for mIHC and Spatial Topology

1. Multispectral Image Unmixing and Cell Segmentation Algorithms (inForm)

Multispectral images acquired with the KR-HT5 scanning system were processed using inForm Advanced Image Analysis Software (version 2.4.0, Akoya Biosciences). To eliminate autofluorescence and distinguish discrete fluorophores, a synthetic spectral library was generated from single-stained control slides.

Tissue Segmentation: An active learning-based tissue segmentation algorithm was trained by the pathologist. The tumor parenchyma was defined as regions with continuous PanCK-positive staining, whereas the tumor stroma was defined as DAPI-dense, PanCK-negative regions with characteristic stromal morphology.

Cell Segmentation: Adaptive cell segmentation was performed using a watershed algorithm. Nuclear boundaries were initially identified using the DAPI signal with a standardized nuclear counterstain threshold and a typical nuclear radius of 3-6 μm (adjusted for tissue morphology). Cellular boundaries (membrane/cytoplasm) were subsequently delineated by expanding outward from the nuclear mask to a maximum radius of 2 μm , guided by membrane markers (e.g., CD8, PanCK, and CD4). Positive cell phenotyping was determined based on the mean fluorescence intensity (MFI) from the segmented single-cell data.

2. Mathematical Modeling of CSR and Biological Affinity

To objectively evaluate spatial interactions (Attraction vs. Avoidance) among immune cells, we transformed the single-cell Cartesian coordinates (x, y) into spatial point pattern objects (ppp class) using the spatstat package (version 4.6.0) in R.

To correct for the inherent geometric bias that highly dense cell populations tend to exhibit shorter NNDs, we normalized the empirical distance ($robs$) against a theoretical baseline derived from a homogeneous Poisson process (representing CSR).

1. Step 2.1: Expected Distance Calculation. For each target cell phenotype with an absolute density of λ (cells/ mm^2), the expected NND $E(r)$ under CSR was calculated using

Equation 1:

$$E(r) = \frac{500}{\sqrt{\lambda}}$$

2. **Step 2.2: Interaction Weight (W) Computation.** The true biological affinity was quantified using a log2-transformed ratio as shown in Equation 2:

$$W = \log_2 \left(\frac{E(r)}{\bar{r}_{obs}} \right)$$

3. **Step 2.3: Z-score Normalization and Edge Correction.** To determine statistical significance, a Z-score was computed based on the variance of the nearest-neighbor distribution. An interaction was deemed biologically meaningful (non-random) only if $|Z| > 1.96$ ($P < 0.05$). Isotropic edge correction (Ripley's circumference method) was applied across all X-Y boundary calculations to prevent underestimation of cell densities and artificial inflation of NNDs near the ROI periphery.

The resulting adjacency matrices (W scores) were input into the *circlize* R package to generate the red-blue spatial interaction chord diagrams (Figure 2G), clearly distinguishing $W > 0$ (biological attraction) from $W < 0$ (structural exclusion).

3. Genomic Variant Annotation and Extended Feature Mapping

Raw NGS reads were aligned to the human reference genome (*GRCh37/hg19*). Variant annotation was performed without strictly masking off-target reads. This bioinformatics strategy deliberately retains biologically relevant variants located in adjacent intergenic regions or lncRNAs (e.g., *AC018692.2*) captured via probe flanking effects. Variants derived from genome-wide backbone SNP probes, which are required for establishing the baseline somatic ploidy and calculating the LOH and HRR scores, were retained in the MAF files for global mutational landscape mapping and subsequent spatial interaction analysis.

Supplementary Tables

Supplementary Table S1

Supplementary Table S1. Primary antibodies and mIHC panel specifications.

Target Marker	Clone	Catalog Number	Manufacturer	Dilution	Antigen Retrieval (pH)	TSA Fluorophore
PanCK	AE1/AE3	ZM-0069	ZSGB-BIO	Ready-to-use	AR6 15MIN	Opal 570
CD8	SP16	ZA-0508	ZSGB-BIO	Ready-to-use	AR9 15MIN	Opal 480
CD4	EP204	ZA-0519	ZSGB-BIO	Ready-to-use	AR9 15MIN	Opal 690
FOXP3	B21	ZA-0682	ZSGB-BIO	Ready-to-use	AR9 15MIN	Opal 520
CD25	EPR6452	AB128955	Abcam	1: 500	AR9 15MIN	Opal 620

Note: TSA, Tyramide Signal Amplification; PanCK, Pan-Cytokeratin. Microwave-assisted heat-induced epitope retrieval was used for antigen retrieval. The multiplex fluorescent staining kit (Cat# RF05525; KR Pharmtech, Shanghai, China) provided the universal polymer HRP-conjugated secondary antibody (ready-to-use), TSA fluorophores (1:100), and DAPI (1:500).

Supplementary Table S2

Supplementary Table S2. Clinicopathological features of the 110 NSCLC patients by TTF-1/p40 expression status.

Characteristic	Total Cohort (n = 110)	TTF-1 Only Positive (n = 35)	TTF-1/p40 Focal Positive (n = 36)	TTF-1/p40 Diffuse Positive (n = 39)	P value
Age, mean±SD	60.65±12.28	62.03±12.52	62.69±10.59	57.51±13.16	0.136
Sex, n (%)					0.929
- Male	50(45.45)	15(42.86)	17(47.22)	18(46.15)	
- Female	60(54.55)	20(57.14)	19(52.78)	21(53.85)	
Smoking history, n (%)					0.577
- Present	38(34.55)	13(37.14)	14(38.89)	11(28.21)	
- Absent	72(65.45)	22(62.86)	22(61.11)	28(71.79)	
AJCC 9th Stage, n (%)					0.661
- Stage I	81(73.64)	27(77.14)	27(75.00)	27(69.23)	
- Stage II	18(16.36)	5(14.29)	7(19.44)	6(15.38)	
- Stage III	11(10.00)	3(8.57)	2(5.56)	6(15.38)	
Predominant Histology, n (%)					0.034
- Lepidic / Acinar / Papillary/ Micropapillary	97(88.18)	35(100.00)	29(80.56)	33(84.62)	
- Solid	13(11.82)	0(0.00)	7(19.44)	6(15.38)	
IASLC grading system, n (%)					0.758
- MIA	2(1.82)	0(0.00)	0(0.00)	2(5.13)	
- Grade 1	4(3.64)	1(2.86)	1(2.78)	2(5.13)	

Characteristic	Total Cohort (n = 110)	TTF-1 Only Positive (n = 35)	TTF-1/p40 Focal Positive (n = 36)	TTF-1/p40 Diffuse Positive (n = 39)	P value
- Grade 2	52(47.27)	17(48.57)	15(41.67)	20(51.28)	
- Grade 3	52(47.27)	17(48.57)	20(55.56)	15(38.46)	
Key Genomic Drivers, n (%)					
- <i>EGFR</i> Mutated	74(74.75)	25(75.76)	22(66.67)	27(81.82)	0.362
- <i>TP53</i> Mutated	24(24.24)	6(18.18)	13(39.39)	5(15.15)	0.043

Note: Categorical variables were compared using Pearson's Chi-square test or Fisher's exact test, and continuous variables using ANOVA or Kruskal-Wallis test. $P < 0.05$ are in bold.

Supplementary Table S3

Supplementary Table S3. List of 68 genes in the targeted NGS panel.

Gene Symbol	transcript_used_FULLNAME	Gene Symbol	transcript_used_FULLNAME
AKT1	NM_001014432.1	IGF1R	NM_000875.4
ALK	NM_004304.4	JAK1	NM_001320923.1
APC	NM_000038.5	JAK2	NM_004972.3
AR	NM_000044.3	KDR	NM_002253.2
ARAF	NM_001256196.1	KIT	NM_000222.2
ATM	NM_000051.3	<i>KRAS</i>	NM_033360.3
AXL	NM_021913.4	MAP2K1	NM_002755.3
BCL2L11	NM_001204107.1	MET	NM_000245.3
BRAF	NM_004333.4	MTOR	NM_004958.3
BRCA1	NM_007294.3	MYC	NM_002467.4
BRCA2	NM_000059.3	NF1	NM_000267.3
CCND1	NM_053056.2	NOTCH1	NM_017617.4
CDK4	NM_000075.3	NRAS	NM_002524.4
CDK6	NM_001145306.1	NRG1	NM_001322205.1
CDKN2A	NM_000077.4	NTRK1	NM_001007792.1
CTNNB1	NM_001904.3	NTRK2	NM_006180.4
CYP2D6	NM_000106.5	NTRK3	NM_001012338.2
DDR2	NM_001014796.1	PDGFRA	NM_006206.4
DPYD	NM_000110.3	PIK3CA	NM_006218.3
<i>EGFR</i>	NM_005228.3	PTCH1	NM_000264.3
ERBB2	NM_004448.3	PTEN	NM_000314.6
ERBB3	NM_001982.3	RAF1	NM_002880.3
ERBB4	NM_005235.2	RB1	NM_000321.2
ESR1	NM_000125.3	RET	NM_020975.4
FGF19	NM_005117.2	ROS1	NM_002944.2
FGF3	NM_005247.2	SMAD4	NM_005359.5
FGF4	NM_002007.2	SMO	NM_005631.4
FGFR1	NM_023110.2	STK11	NM_000455.4
FGFR2	NM_000141.4	TOP2A	NM_001067.3

Gene Symbol	transcript_used_FULLNAME	Gene Symbol	transcript_used_FULLNAME
FGFR3	NM_000142.4	<i>TP53</i>	NM_000546.5
FLT3	NM_004119.2	TSC1	NM_000368.4
HRAS	NM_005343.3	TSC2	NM_000548.4
IDH1	NM_005896.3	UGT1A1	NM_000463.2
IDH2	NM_001289910.1		

Note: This table lists the 68 core actionable genes targeted by the primary assay design. The downstream bioinformatics annotation pipeline additionally identified and retained variants from genomic loci extending beyond these primary targets. These extended mappings include flanking non-coding regions captured via off-target hybridization (e.g., the lncRNA *AC018692.2*), non-target fusion partners, and genome-wide backbone SNP probes used to assess baseline ploidy for LOH and HRR evaluation.

Supplementary Table S4

Supplementary Table S4. Per-case morphology, immunophenotype, grading, and molecular annotations of the 110 NSCLC patients.

One row per case. Histologic components are reported as fractions of the total tumor area; predominant subtype and grade follow the WHO 2021 classification and the IASLC grading system. *EGFR/TP53* mutation status derives from clinical reports (1 = mutated, 0 = wild-type). Specimen type: 1 = wedge resection, 2 = segmentectomy, 3 = lobectomy.

Case ID	P40 group	Subtype	IASLC grade	Lepidic %	Acinar %	Cribriform %	Papillary %	Micropap. %	Solid %	Ki-67 %	PD-L1 TPS	<i>EGFR</i>	<i>TP53</i>	Stage	Specimen
Case 1	Diffuse	Acinar	Grade 2	0.2	0.8	0	0	0	0	0.1	0.25	1	0	I	3
Case 2	Diffuse	Acinar	Grade 2	0	0.5	0	0.3	0.2	0	0.1	0.02	1	0	I	3
Case 3	Diffuse	Acinar	Grade 2	0.1	0.5	0	0.4	0	0	0.02	0.01	1	0	I	3
Case 4	Diffuse	Papillary	Grade 2	0.3	0.2	0	0.5	0	0	0.05	0.01	1	0	I	3
Case 5	Diffuse	Solid	Grade 3	0	0.1	0	0	0	0.9	0.9	0.4	0	1	II	1
Case 6	Diffuse	Acinar	Grade 2	0.1	0.6	0	0.3	0	0	0.05	0.01	1	0	I	3
Case 7	Diffuse	Acinar	Grade 2	0.2	0.4	0	0.4	0	0	0.05	0.01			I	3
Case 8	Diffuse	Papillary	Grade 2	0.1	0.4	0	0.5	0	0	0.05	0.01	1	0	I	2
Case 9	Diffuse	Acinar	Grade 2	0.2	0.8	0	0	0	0	0.05	0.01	0	0	I	2
Case 10	Diffuse	Acinar	Grade 2	0.3	0.7	0	0	0	0	0.05	0.01	0	0	I	1
Case 11	Diffuse	Complex glandular	Grade 3	0	0.75	0.5	0	0.05	0.2	0.75	0.01	1	1	III	2
Case 12	Diffuse	Minimally invasive		1.0	0	0	0	0	0	0.03	0.01	1	0	I	1
Case 13	Diffuse	Lepidic	Grade 1	0.55	0.2	0	0.25	0	0	0.18	0.01	1	0	I	1
Case 14	Diffuse	Lepidic	Grade 1	0.5	0.4	0	0.1	0	0	0.03	0.01	1	0	I	1
Case 15	Diffuse	Micropapillary	Grade 3	0	0.4	0.1	0.5	0.1	0	0.25	0.01	1	0	II	3
Case 16	Diffuse	Minimally invasive		1.0	0	0	0	0	0					I	1
Case 17	Diffuse	Solid	Grade 3	0	0	0	0	0	1	0.3	0.2	1	0	III	3
Case 18	Diffuse	Acinar	Grade 2	0.3	0.55	0	0.1	0.05	0	0.15	0.01			I	1
Case 19	Diffuse	Solid	Grade 3	0	0.6	0	0	0	0.4	0.65	0.01	1	0	II	1

Case ID	P40 group	Subtype	IASLC grade	Lepidic %	Acinar %	Cribriform %	Papillary %	Micropap. %	Solid %	Ki-67 %	PD-L1 TPS	EGFR	TP53	Stage	Specimen
Case 20	Diffuse	Acinar	Grade 2	0.35	0.45	0	0.2	0	0	0.05	0.01	1	0	I	2
Case 21	Diffuse	Acinar	Grade 2	0.2	0.8	0	0	0	0	0.08	0.01			I	2
Case 22	Diffuse	Complex glandular	Grade 3	0	0.8	0.2	0.2	0	0	0.2	0.01	1	0	I	3
Case 23	Diffuse	Solid	Grade 3	0	0.2	0.1	0	0.1	0.7	0.7	0.98	1	0	III	1
Case 24	Diffuse	Acinar	Grade 2	0.4	0.6	0	0	0	0	0.03	0.01	0	0	I	3
Case 25	Diffuse	Solid	Grade 3	0	0	0	0	0	1	0.6	0.95	0	1	I	3
Case 26	Diffuse	Micropapillary	Grade 3	0	0.6	0.2	0	0.4	0	0.3	0.01	0	0	III	3
Case 27	Diffuse	Acinar	Grade 2	0	0.5	0	0.4	0.1	0	0.1	0.01	1	0	I	3
Case 28	Diffuse	Complex glandular	Grade 3	0	0.8	0.15	0.15	0.05	0	0.3	0.01	1	0	I	3
Case 29	Diffuse	Micropapillary	Grade 3	0	0.7	0.3	0	0.3	0	0.75	0.01	1	1	III	3
Case 30	Diffuse	Solid	Grade 3	0	0	0	0	0	1	0.4	1	1	0	II	1
Case 31	Diffuse	Acinar	Grade 2	0.3	0.5	0	0.2	0	0	0.1	0.01	1	0	I	2
Case 32	Diffuse	Papillary	Grade 2	0.1	0.4	0	0.5	0	0	0.05				I	2
Case 33	Diffuse	Complex glandular	Grade 3	0	0.6	0.15	0.35	0.05	0	0.2		1	0	I	3
Case 34	Diffuse	Acinar	Grade 2	0.1	0.7	0.15	0.2	0	0	0.08		1	0	II	2
Case 35	Diffuse	Complex glandular	Grade 3	0	0.6	0.4	0	0.3	0.1	0.75		1	1	II	3
Case 36	Diffuse	Micropapillary	Grade 3	0	0.3	0.3	0.2	0.5	0	0.5	0.05	1	0	III	3
Case 37	Diffuse	Acinar	Grade 2	0	0.8	0	0.15	0.05	0	0.1	0.01	1	0	I	3
Case 38	Diffuse	Acinar	Grade 2	0	0.9	0	0.1	0	0	0.05	0.01	1	0	I	3
Case 39	Diffuse	Papillary	Grade 2	0	0.1	0	0.9	0	0	0.08	0.01			I	2
Case 40	Focal	Acinar	Grade 2	0.4	0.6	0	0	0	0			0	1	II	1
Case 41	Focal	Solid	Grade 3	0	0.1	0.05	0	0.35	0.55	0.4		0	1	II	1

Case ID	P40 group	Subtype	IASLC grade	Lepidic %	Acinar %	Cribriform %	Papillary %	Micropap. %	Solid %	Ki-67 %	PD-L1 TPS	EGFR	TP53	Stage	Specimen
Case 42	Focal	Solid	Grade 3	0	0.2	0	0	0	0.8			1	1	II	1
Case 43	Focal	Solid	Grade 3	0	0.1	0	0	0	0.9	0.7	0.01	0	1	I	1
Case 44	Focal	Complex glandular	Grade 3	0	0.5	0.4	0.3	0.2	0	0.55	0.01	0	1	II	1
Case 45	Focal	Acinar	Grade 2	0.2	0.8	0.15	0	0	0	0.15	0.01	1	0	I	2
Case 46	Focal	Acinar	Grade 2	0	0.7	0.1	0.3	0	0	0.15	0.01	1	0	I	1
Case 47	Focal	Complex glandular	Grade 3	0	0.7	0.15	0.15	0.15	0	0.08	0.01	0	0	I	2
Case 48	Focal	Acinar	Grade 2	0.2	0.6	0	0.2	0	0	0.15	0.01	1	0	I	3
Case 49	Focal	Micropapillary	Grade 3	0.1	0.35	0.2	0	0.35	0.2	0.3	0.01	1	1	III	3
Case 50	Focal	Solid	Grade 3	0	0.3	0	0	0	0.7	0.8	0.98	0	0	I	3
Case 51	Focal	Solid	Grade 3	0	0.3	0.15	0.3	0.05	0.35	0.4	0.03	1	1	I	3
Case 52	Focal	Acinar	Grade 2	0	1	0	0	0	0	0.05	0.01	1	0	I	2
Case 53	Focal	Acinar	Grade 2	0.3	0.7	0	0	0	0	0.15	0.01			I	1
Case 54	Focal	Micropapillary	Grade 3	0.05	0.3	0.1	0.4	0.25	0	0.03	0.01	1	0	II	1
Case 55	Focal	Solid	Grade 3	0	0.1	0	0	0	0.9	0.4	0.7	0	0	I	3
Case 56	Focal	Complex glandular	Grade 3	0	0.8	0.5	0.1	0.1	0	0.1	0.01	1	1	I	1
Case 57	Focal	Lepidic	Grade 1	0.55	0.45	0	0	0	0	0.05	0.01			I	1
Case 58	Focal	Acinar	Grade 2	0	0.6	0	0.4	0	0	0.05	0.01	1	0	I	3
Case 59	Focal	Solid	Grade 3	0	0.6	0	0	0	0.4	0.6	0.01			II	3
Case 60	Focal	Complex glandular	Grade 3	0.2	0.8	0.2	0	0	0	0.03	0.01	1	0	I	2
Case 61	Focal	Papillary	Grade 2	0	0.3	0.05	0.65	0.05	0	0.15	0.01	0	0	I	3
Case 62	Focal	Complex glandular	Grade 3	0	0.7	0.4	0.1	0.2	0	0.15	0.03	1	0	II	3
Case 63	Focal	Complex glandular	Grade 3	0	0.8	0.3	0	0.1	0.1	0.15	0.01	1	0	I	1

Case ID	P40 group	Subtype	IASLC grade	Lepidic %	Acinar %	Cribriform %	Papillary %	Micropap. %	Solid %	Ki-67 %	PD-L1 TPS	EGFR	TP53	Stage	Specimen
Case 64	Focal	Acinar	Grade 2	0.3	0.7	0	0	0	0			1	0	I	2
Case 65	Focal	Acinar	Grade 2	0.4	0.6	0	0	0	0	0.05	0.01	0	0	I	1
Case 66	Focal	Papillary	Grade 2	0.3	0.3	0	0.4	0	0	0.1	0.01	1	0	I	3
Case 67	Focal	Complex glandular	Grade 3	0	0.6	0.4	0.3	0.1	0	0.45	0.01	1	1	I	3
Case 68	Focal	Micropapillary	Grade 3	0	0.3	0	0.5	0.2	0	0.08	0.01	0	0	I	1
Case 69	Focal	Acinar	Grade 2	0	0.8	0.1	0.2	0	0	0.05		1	0	I	2
Case 70	Focal	Acinar	Grade 2	0.1	0.9	0.1	0	0	0	0.1		0	1	I	1
Case 71	Focal	Acinar	Grade 2	0	1	0	0	0	0	0.08		1	0	I	3
Case 72	Focal	Micropapillary	Grade 3	0	0.3	0.05	0.4	0.3	0	0.35	0.01	1	1	I	2
Case 73	Focal	Acinar	Grade 2	0	0.6	0.05	0.35	0.05	0	0.05	0.01	1	1	I	2
Case 74	Focal	Complex glandular	Grade 3	0	0.8	0.6	0	0	0.2	0.85	0.55	1	1	I	3
Case 75	Focal	Complex glandular	Grade 3	0	0.7	0.1	0.1	0.1	0.1	0.25	0.01	1	0	III	3
Case 76	TTF-1 only	Acinar	Grade 2	0	0.9	0	0.1	0	0	0.05	0.01	1	1	II	2
Case 77	TTF-1 only	Acinar	Grade 2	0	0.6	0	0.4	0	0	0.1	0.01	1	0	I	1
Case 78	TTF-1 only	Micropapillary	Grade 3	0	0	0	0.7	0.3	0	0.05	0.01	1	0	I	1
Case 79	TTF-1 only	Micropapillary	Grade 3	0	0.5	0	0.3	0.2	0	0.7	0.01	1	0	I	1
Case 80	TTF-1 only	Complex glandular	Grade 3	0	0.95	0.3	0	0.05	0	0.15				I	1
Case 81	TTF-1 only	Complex glandular	Grade 3	0	0.5	0.2	0.3	0.1	0.1	0.15		0	0	I	1
Case 82	TTF-1 only	Papillary	Grade 2	0	0.2	0	0.7	0.1	0	0.05		0	0	I	2
Case 83	TTF-1 only	Micropapillary	Grade 3	0	0.4	0.2	0.1	0.3	0.2	0.3		1	0	I	3

Case ID	P40 group	Subtype	IASLC grade	Lepidic %	Acinar %	Cribriform %	Papillary %	Micropap. %	Solid %	Ki-67 %	PD-L1 TPS	EGFR	TP53	Stage	Specimen
Case 84	TTF-1 only	Micropapillary	Grade 3	0.1	0.35	0	0.15	0.4	0	0.05		1	0	I	1
Case 85	TTF-1 only	Acinar	Grade 2	0.1	0.35	0.05	0.5	0.05	0	0.1	0.01	1	0	I	3
Case 86	TTF-1 only	Micropapillary	Grade 3	0	0.25	0.2	0.35	0.35	0.05	0.4		1	1	III	3
Case 87	TTF-1 only	Papillary	Grade 2	0.1	0.35	0.05	0.5	0.05	0	0.1		0	0	I	1
Case 88	TTF-1 only	Complex glandular	Grade 3	0	0.7	0.35	0.2	0.1	0	0.35	0.01	1	0	III	3
Case 89	TTF-1 only	Micropapillary	Grade 3	0	0.4	0.1	0.5	0.1	0	0.3		1	0	I	1
Case 90	TTF-1 only	Micropapillary	Grade 3	0	0.3	0.3	0.1	0.5	0.1	0.08	0.01	0	0	I	3
Case 91	TTF-1 only	Acinar	Grade 2	0	0.8	0.1	0.15	0.05	0	0.3	0.01	1	1	I	3
Case 92	TTF-1 only	Micropapillary	Grade 3	0	0.5	0.2	0.2	0.3	0	0.08	0.05	0	1	II	3
Case 93	TTF-1 only	Acinar	Grade 2	0.3	0.7	0	0	0	0	0.2	0.01	0	0	I	1
Case 94	TTF-1 only	Micropapillary	Grade 3	0	0	0	0.6	0.4	0	0.6	0.03	0	0	III	3
Case 95	TTF-1 only	Micropapillary	Grade 3	0	0	0	0	1	0	0.4	0.01	1	0	I	2
Case 96	TTF-1 only	Acinar	Grade 2	0	0.6	0	0.3	0	0.1	0.08	0.01	1	0	I	3
Case 97	TTF-1 only	Acinar	Grade 2	0.1	0.85	0.1	0	0.05	0	0.1		1	0	I	3
Case 98	TTF-1 only	Acinar	Grade 2	0.2	0.8	0	0	0	0	0.05	0.01	1	0	I	1
Case 99	TTF-1 only	Complex glandular	Grade 3	0	0.7	0.2	0.2	0.1	0	0.3	0.05	1	1	II	2
Case 100	TTF-1 only	Acinar	Grade 2	0.3	0.7	0	0	0	0	0.15	0.01	1	0	I	2
Case 101	TTF-1	Papillary	Grade 2	0	0.1	0	0.9	0	0	0.1	0.01	1	0	I	3

Case ID	P40 group	Subtype	IASLC grade	Lepidic %	Acinar %	Cribriform %	Papillary %	Micropap. %	Solid %	Ki-67 %	PD-L1 TPS	EGFR	TP53	Stage	Specimen
Case 102	only TTF-1 only	Complex glandular	Grade 3	0	0.7	0.3	0.2	0.1	0	0.45	0.03	1	0	I	1
Case 103	TTF-1 only	Acinar	Grade 2	0	0.75	0	0.2	0.05	0	0.15	0.01	1	0	I	2
Case 104	TTF-1 only	Acinar	Grade 2	0.1	0.6	0	0.3	0	0	0.08	0.01			I	1
Case 105	TTF-1 only	Lepidic	Grade 1	0.5	0.4	0	0.1	0	0	0.15	0.01	1	0	I	1
Case 106	TTF-1 only	Complex glandular	Grade 3	0	0.7	0.1	0.2	0.1	0	0.1	0.01	0	0	I	2
Case 107	TTF-1 only	Papillary	Grade 2	0	0.1	0	0.9	0	0	0.3	0.01	1	1	II	1
Case 108	TTF-1 only	Micropapillary	Grade 3	0	0.1	0	0.05	0.85	0	0.3	0.01	1	0	II	3
Case 109	TTF-1 only	Acinar	Grade 2	0	0.6	0	0.3	0.1	0	0.15	0.01	1	0	I	2
Case 110	TTF-1 only	Acinar	Grade 2	0	0.8	0.05	0.2	0	0	0.1	0.01	1	0	I	2

Machine-readable Excel file: [Supplementary_Table_S4.xlsx](#)

Supplementary Table S5

Supplementary Table S5. Patient-level re-analysis of spatial density, ratio, and nearest-neighbor-distance (NND) metrics (750 ROI nested in 30 patients).

Panel A: patient-level comparison of per-patient mean densities using independent-sample t tests (nominal, exploratory; no adjustment for multiple comparisons). The full patient-level aggregated dataset is provided in the Excel file (sheet 'Patient-level aggregated data'). Four additional sheets report sensitivity analyses (pure-group comparison, original binary assignment, and three-group comparison) and heterogeneity metrics (CV and stromal proportions).

Panel A. Patient-level comparison.

Metric	n_TTF1_Only	n_Diffuse	Median_TTF1_Only	Median_Diffuse	Mean_diff_Diffuse_minus_TTF1
CD8+ total density	21	20	65.97	102.2	22.6
CD8+ parenchymal density	21	20	27.81	54.35	23.84
CD8+ stromal density	21	20	118.6	200.5	52.57
FOXP3+ total density	21	20	42.11	26.88	-12.14
FOXP3+ parenchymal density	21	20	11.84	11.17	-2.166
FOXP3+ stromal density	21	20	70.03	50.79	-17.98
CD4+FOXP3+ total density	21	20	6.792	3.571	-2.822
CD4+FOXP3+ parenchymal density	21	20	1.398	1.587	0.5592
CD4+FOXP3+ stromal density	21	20	14.3	9.931	-5.024
CD25+CD4+FOXP3+ total density	21	20	2.284	0.9131	-1.868
CD25+CD4+FOXP3+ parenchymal density	21	20	1.029	0.3825	-0.04189
CD25+CD4+FOXP3+ stromal density	21	20	4.633	1.879	-3.707
Tumor area fraction (%)	21	20	0.5689	0.6405	0.05851
FOXP3+ stroma/parenchyma density ratio	20	18	6.454	4.705	0.7266
CD4+FOXP3+ stroma/parenchyma density ratio	19	16	5.848	4.358	-6.548
CD25+CD4+FOXP3+ stroma/parenchyma density ratio	16	13	4.42	3.609	0.5518

Machine-readable Excel file: *Supplementary_Table_S5.xlsx*

Supplementary Table S6

Supplementary Table S6. NGS mutation re-analysis.

Panel A: mutation frequencies of the three key genes (*EPHA5*, *HSPBP1*, *AC018692.2*) by group with Wilson 95% confidence intervals and pairwise Fisher tests (transposed layout; original table in the Excel file). Panel B: per-variant allele fraction (VAF) and read-depth inspection of all key-gene calls. Panel C: genome-wide Fisher tests across 1,568 genes for the diffuse-positive group versus the remaining samples, with Benjamini–Hochberg FDR (top 30 genes by p value shown; full table in the Excel file). Panel D: driver-versus-passenger annotation of nominally significant genes.

Panel A. Key genes (transposed: rows = statistics, columns = genes).

Statistic	<i>EPHA5</i>	<i>HSPBP1</i>	<i>AC018692.2</i>
Driver_Annotation	Driver(candidate)	Passenger/Unknown	Passenger/Unknown
TTF1 Only Positive n/N	0/13	2/13	1/13
TTF1 Only Positive freq%	0	15.4	7.7
TTF1 Only Positive Wilson95CI	[0.000,0.228]	[0.043,0.422]	[0.014,0.333]
TTF1/P40 Focal Positive n/N	1/21	3/21	0/21
TTF1/P40 Focal Positive freq%	4.8	14.3	0
TTF1/P40 Focal Positive Wilson95CI	[0.008,0.227]	[0.050,0.346]	[0.000,0.155]
TTF1/P40 Diffuse Positive n/N	5/11	7/11	5/11
TTF1/P40 Diffuse Positive freq%	45.5	63.6	45.5
TTF1/P40 Diffuse Positive Wilson95CI	[0.213,0.720]	[0.354,0.848]	[0.213,0.720]
p TTF1 Only Positive vs TTF1/P40 Focal Positive	1	1	0.3824
OR TTF1 Only Positive vs TTF1/P40 Focal Positive	0.506	1.149	5.16
ORCI TTF1 Only Positive vs TTF1/P40 Focal Positive	[0.02,13.37]	[0.19,6.83]	[0.19,136.54]
p TTF1 Only Positive vs TTF1/P40 Diffuse Positive	0.01087	0.03274	0.0608
OR TTF1 Only Positive vs TTF1/P40 Diffuse Positive	0.044	0.13	0.142

Statistic	<i>EPHA5</i>	<i>HSPBP1</i>	<i>AC018692.2</i>
ORCI TTF1 Only Positive vs TTF1/P40 Diffuse Positive	[0.00,0.92]	[0.02,0.79]	[0.02,1.09]
p TTF1/P40 Focal Positive vs TTF1/P40 Diffuse Positive	0.01122	0.01283	0.002294
OR TTF1/P40 Focal Positive vs TTF1/P40 Diffuse Positive	0.086	0.114	0.027
ORCI TTF1/P40 Focal Positive vs TTF1/P40 Diffuse Positive	[0.01,0.65]	[0.02,0.58]	[0.00,0.57]
TTF1 Only Positive vs Rest p	0.1601	0.4596	0.6556
TTF1 Only Positive vs Rest OR	0.151	0.466	0.6
TTF1 Only Positive vs Rest ORCI	[0.01,2.89]	[0.10,2.20]	[0.09,4.11]
TTF1/P40 Focal Positive vs Rest p	0.1927	0.1011	0.02319
TTF1/P40 Focal Positive vs Rest OR	0.259	0.309	0.066
TTF1/P40 Focal Positive vs Rest ORCI	[0.04,1.75]	[0.08,1.25]	[0.00,1.26]
TTF1/P40 Diffuse Positive vs Rest p	0.001985	0.003471	0.001985
TTF1/P40 Diffuse Positive vs Rest OR	18.9	8.939	18.9
TTF1/P40 Diffuse Positive vs Rest ORCI	[2.58,138.42]	[2.03,39.33]	[2.58,138.42]

Panel B. VAF details.

Gene	Group	Tumor_Sample_Barcode	HGVSp	Variant_Classification	VAF	Depth_ADsum	DP
<i>EPHA5</i>	TTF1/P40 Focal Positive	AE24002605FFP	ENSP00000273854.3:p.His959Arg	Missense_Mutation	0.584	125	125
<i>EPHA5</i>	TTF1/P40 Diffuse Positive	AE23001636FFP	ENSP00000273854.3:p.His959Arg	Missense_Mutation	0.5445	191	192
<i>EPHA5</i>	TTF1/P40 Diffuse Positive	AE24001604FFP	ENSP00000273854.3:p.His959Arg	Missense_Mutation	0.4724	127	128
<i>EPHA5</i>	TTF1/P40 Diffuse Positive	AE24002512FFP	ENSP00000273854.3:p.His959Arg	Missense_Mutation	0.5294	102	102
<i>EPHA5</i>	TTF1/P40 Diffuse Positive	AE24002804FFP	ENSP00000273854.3:p.His959Arg	Missense_Mutation	0.6667	123	123

Gene	Group	Tumor_Sample_Barcode	HGVSp	Variant_Classification	VAF	Depth_ADsum	DP
<i>EPHA5</i>	TTF1/P40 Diffuse Positive	AE23004129FFP	ENSP00000273854.3:p.Asp430Asn	Missense_Mutation	0.3952	248	248
<i>HSPBP1</i>	TTF1 Only Positive	AE24002823FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	1	1
<i>HSPBP1</i>	TTF1 Only Positive	AE24003301FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	2	2
<i>HSPBP1</i>	TTF1/P40 Focal Positive	AE23002057FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	0.3333	3	3
<i>HSPBP1</i>	TTF1/P40 Focal Positive	AE23003459FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	2	2
<i>HSPBP1</i>	TTF1/P40 Focal Positive	AE24000524FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	1	1
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	AE23000546FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	0.5	2	2
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	AE23001636FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	1	1
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	AE23004129FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	0.25	8	8
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	AE23005026FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	1	1
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	AE24001604FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	1	1
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	AE24001850FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	2	2
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	AE24002512FFP	ENSP00000255631.4:p.Gly28_Gly30dup	In_Frame_Ins	1	1	1
<i>AC018692.2</i>	TTF1 Only Positive	AE24002595FFP	ENSP00000471653.1:p.Phe23Leu	Missense_Mutation	1	1	1
<i>AC018692.2</i>	TTF1/P40 Diffuse Positive	AE23000540FFP		Splice_Site	1	1	1
<i>AC018692.2</i>	TTF1/P40 Diffuse Positive	AE23000546FFP	ENSP00000471653.1:p.Phe23Leu	Missense_Mutation	1	2	2
<i>AC018692.2</i>	TTF1/P40 Diffuse Positive	AE23004129FFP	ENSP00000471653.1:p.Phe23Leu	Missense_Mutation	1	1	1
<i>AC018692.2</i>	TTF1/P40 Diffuse Positive	AE23005026FFP	ENSP00000471653.1:p.Phe23Leu	Missense_Mutation	1	1	1

Gene	Group	Tumor_Sample_Barcode	HGVSp	Variant_Classification	VAF	Depth_ADsum	DP
<i>AC018692.2</i>	TTF1/P40 Diffuse Positive	AE24002804FFP	ENSP00000471653.1:p.Phe23Leu	Missense_Mutation	1	1	1
<i>AC018692.2</i>	TTF1/P40 Diffuse Positive	AE23000540FFP	ENSP00000471653.1:p.Val22Leu	Missense_Mutation	1	1	1

Panel C. Genome-wide Fisher tests with Benjamini–Hochberg FDR (top 30 of 1,568 genes by p value).

Hugo_Symbol	Group1	Group2	n_mutated_group1	n_mutated_group2	p_value	OR	OR_low	OR_high	k1	k2	fdr	Driver_Annotation
<i>AC018692.2</i>	TTF1/P40 Diffuse Positive	Rest	5 of 11	1 of 34	0.001985	27.5	2.58	138.4	5	1	0.9679	Passenger/Unknown
<i>EPHA5</i>	TTF1/P40 Diffuse Positive	Rest	5 of 11	1 of 34	0.001985	27.5	2.58	138.4	5	1	0.9679	Driver(candidate)
<i>HSPBP1</i>	TTF1/P40 Diffuse Positive	Rest	7 of 11	5 of 34	0.003471	10.15	2.032	39.33	7	5	0.9679	Passenger/Unknown
GATA2	TTF1/P40 Diffuse Positive	Rest	5 of 11	30 of 34	0.007365	0.1111	0.02768	0.5631	5	30	0.9679	Passenger/Unknown
PM20D1	TTF1/P40 Diffuse Positive	Rest	4 of 11	1 of 34	0.009562	18.86	1.795	100	4	1	0.9679	Passenger/Unknown
ARID3A	TTF1/P40 Diffuse Positive	Rest	3 of 11	0 of 34	0.01163	inf	1.336	604	3	0	0.9679	Passenger/Unknown
ATXN3	TTF1/P40 Diffuse Positive	Rest	3 of 11	0 of 34	0.01163	inf	1.336	604	3	0	0.9679	Passenger/Unknown
APOBEC3F	TTF1/P40 Diffuse Positive	Rest	3 of 11	0 of 34	0.01163	inf	1.336	604	3	0	0.9679	Passenger/Unknown
GON4L	TTF1/P40 Diffuse Positive	Rest	3 of 11	0 of 34	0.01163	inf	1.336	604	3	0	0.9679	Passenger/Unknown
C11orf42	TTF1/P40 Diffuse Positive	Rest	3 of 11	0 of 34	0.01163	inf	1.336	604	3	0	0.9679	Passenger/Unknown
MUC22	TTF1/P40	Rest	3 of 11	0 of 34	0.01163	inf	1.336	604	3	0	0.9679	Passenger/Unknown

Hugo_Symbol	Group1	Group2	n_mutated_group1	n_mutated_group2	p_value	OR	OR_low	OR_high	k1	k2	fdr	Driver_Annotation
ZFHX2	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	3 of 11	0 of 34	0.01163	inf	1.336	604	3	0	0.9679	Passenger/Unknown
YBX2	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	5 of 11	3 of 34	0.01408	8.611	1.562	37.12	5	3	0.9679	Passenger/Unknown
KLHDC7B	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	4 of 11	2 of 34	0.02471	9.143	1.372	44.35	4	2	0.9679	Passenger/Unknown
OR8B4	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	4 of 11	2 of 34	0.02471	9.143	1.372	44.35	4	2	0.9679	Passenger/Unknown
DDHD1	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	4 of 11	2 of 34	0.02471	9.143	1.372	44.35	4	2	0.9679	Passenger/Unknown
AP001888.1	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	4 of 11	26 of 34	0.02555	0.1758	0.04741	0.7812	4	26	0.9679	Passenger/Unknown
OR10AC1P	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	5 of 11	4 of 34	0.02751	6.25	1.272	25.87	5	4	0.9679	Passenger/Unknown
MYLK	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	5 of 11	4 of 34	0.02751	6.25	1.272	25.87	5	4	0.9679	Passenger/Unknown
FOXD4	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	3 of 11	1 of 34	0.03987	12.38	1.177	71.83	3	1	0.9679	Passenger/Unknown
PTPRN2	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	3 of 11	1 of 34	0.03987	12.38	1.177	71.83	3	1	0.9679	Passenger/Unknown
KIAA1210	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	3 of 11	1 of 34	0.03987	12.38	1.177	71.83	3	1	0.9679	Passenger/Unknown
C6orf223	Diffuse Positive TTF1/P40 Diffuse Positive	Rest	3 of 11	1 of 34	0.03987	12.38	1.177	71.83	3	1	0.9679	Passenger/Unknown
BPIFB4	TTF1/P40	Rest	3 of 11	1 of 34	0.03987	12.38	1.177	71.83	3	1	0.9679	Passenger/Unknown

Hugo_Symbol	Group1	Group2	n_mutated_group1	n_mutated_group2	p_value	OR	OR_low	OR_high	k1	k2	fdr	Driver_Annotation
	Diffuse Positive											
ANKS1A	TTF1/P40 Diffuse Positive	Rest	3 of 11	1 of 34	0.03987	12.38	1.177	71.83	3	1	0.9679	Passenger/Unknown
NOC2L	TTF1/P40 Diffuse Positive	Rest	3 of 11	1 of 34	0.03987	12.38	1.177	71.83	3	1	0.9679	Passenger/Unknown
CLASRP	TTF1/P40 Diffuse Positive	Rest	5 of 11	5 of 34	0.04766	4.833	1.059	19.45	5	5	0.9679	Passenger/Unknown
TMEM44	TTF1/P40 Diffuse Positive	Rest	5 of 11	5 of 34	0.04766	4.833	1.059	19.45	5	5	0.9679	Passenger/Unknown
SORBS3	TTF1/P40 Diffuse Positive	Rest	7 of 11	31 of 34	0.04958	0.1694	0.03709	0.9245	7	31	0.9679	Passenger/Unknown
KRTAP10-11	TTF1/P40 Diffuse Positive	Rest	4 of 11	3 of 34	0.04958	5.905	1.082	26.96	4	3	0.9679	Passenger/Unknown

Panel D. Driver annotation.

Hugo_Symbol	Group1	Group2	n_mutated_group1	n_mutated_group2	p_value	OR	OR_low	OR_high	k1	k2	fdr	Driver_Annotation
RET	TTF1 Only Positive	Rest	8 of 13	9 of 32	0.04808	4.089	1.031	14.18	8	9	1	Driver(candidate)
KEAP1	TTF1/P40 Focal Positive	Rest	5 of 21	0 of 24	0.01666	inf	0.845	315.7	5	0	1	Driver(candidate)
RET	TTF1/P40 Focal Positive	Rest	4 of 21	13 of 24	0.02968	0.1991	0.05966	0.8043	4	13	1	Driver(candidate)
EPHA5	TTF1/P40 Diffuse Positive	Rest	5 of 11	1 of 34	0.001985	27.5	2.58	138.4	5	1	0.9679	Driver(candidate)

Machine-readable Excel file: [Supplementary_Table_S6.xlsx](#)

Supplementary Table S7

Supplementary Table S7. Additional re-analyses: smoking and driver-gene distributions by group, median PFS and 12-/24-month PFS rates, CONSORT counts, IASLC grade and predominant-subtype distributions, patient-level NND comparisons, and per-patient ROI breakdown.

Smoking

Group	Non-smoker	Smoker
Diffuse	28	11
Focal	22	14
TTF-1 only	22	13

EGFR mutation

Group	Wild-type	Mutated
Diffuse	6	27
Focal	11	22
TTF-1 only	8	25

TP53 mutation

Group	Wild-type	Mutated
Diffuse	28	5
Focal	20	13
TTF-1 only	27	6

KRAS mutation

Group	Wild-type	Mutated
Diffuse	31	2
Focal	29	4
TTF-1 only	31	2

Median PFS

Group	n	Events	Median PFS	Median PFS CI lower	Median PFS CI upper	PFS rate 12- month	PFS rate 24- month
TTF-1 only	35	5	NR	13.3	NR	0.84	0.747
Focal	36	5	NR	14.7	NR	0.835	0.759
Diffuse	39	1	NR	inf	NR	0.967	0.967

CONSORT

Full cohort	NGS	mIHC	Both platforms	Clinical only
110	45	30	9	44

IASLC grade

Group	IASLC grade 1	IASLC grade 2	IASLC grade 3
Diffuse	2	20	15
Focal	1	15	20
TTF-1 only	1	17	17

Predominant subtype

Group	Papillary	Complex glandular	Solid	Micropapillary	Minimally invasive	Acinar	Lepidic
Diffuse	4	5	6	4	2	16	2
Focal	2	9	7	4	0	13	1
TTF-1 only	4	6	0	11	0	13	1

NND patient-level

Metric	Median_TTF1_Only	Median_Diffuse	Mean difference
STROMA CD8+ to PANCK+	108.6	136.5	1.222
STROMA CD4+ to PANCK+	119.8	133.5	-2.783
STROMA FOXP3+ to PANCK+	112	140.8	-0.668
STROMA CD25+ to PANCK+	110.2	120.1	1.302
STROMA CD25+/CD4+ to PANCK+	133.4	142.2	24.32
STROMA CD25+/FOXP3+ to PANCK+	109.5	107.7	-7.358
STROMA CD4+/FOXP3+ to PANCK+	103.3	128.9	11.2
STROMA CD25+/CD4+/FOXP3+ to PANCK+	102.6	133.5	29.93
TUMOR CD8+ to PANCK+	10.43	9.884	-0.5862
TUMOR CD4+ to PANCK+	10.27	9.644	-0.804
TUMOR FOXP3+ to PANCK+	10.12	9.707	-0.267
TUMOR CD25+ to PANCK+	10.42	10.51	0.1196
TUMOR CD25+/CD4+ to PANCK+	10.58	10.23	-0.5075
TUMOR CD25+/FOXP3+ to PANCK+	10.97	10.89	-0.193
TUMOR CD4+/FOXP3+ to PANCK+	10.84	10.09	-0.9344

Metric	Median_TTF1_Only	Median_Diffuse	Mean difference
TUMOR CD25+/CD4+/FOXP3+ to PANCK+	10.4	11.94	-0.6933

ROI per patient

Case ID	ROI count	TTF-1 only ROIs	Diffuse ROIs
Case 1	25	0	25
Case 3	30	6	24
Case 4	25	0	25
Case 26	25	0	25
Case 15	25	19	6
Case 16	25	0	25
Case 20	25	0	25
Case 21	25	0	25
Case 22	25	1	24
Case 28	25	4	21
Case 29	25	9	16
Case 30	25	0	25
Case 31	25	0	25
Case 78	25	25	0
Case 79	25	25	0
Case 33	25	21	4
Case 71	25	22	3
Case 84	25	25	0
Case 85	25	25	0
Case 86	25	25	0
Case 35	25	19	6
Case 36	25	21	4
Case 72	25	25	0
Case 88	25	25	0
Case 89	25	25	0
Case 91	25	25	0
Case 74	25	24	1
Case 39	25	0	25
Case 95	25	25	0
Case 75	20	18	2

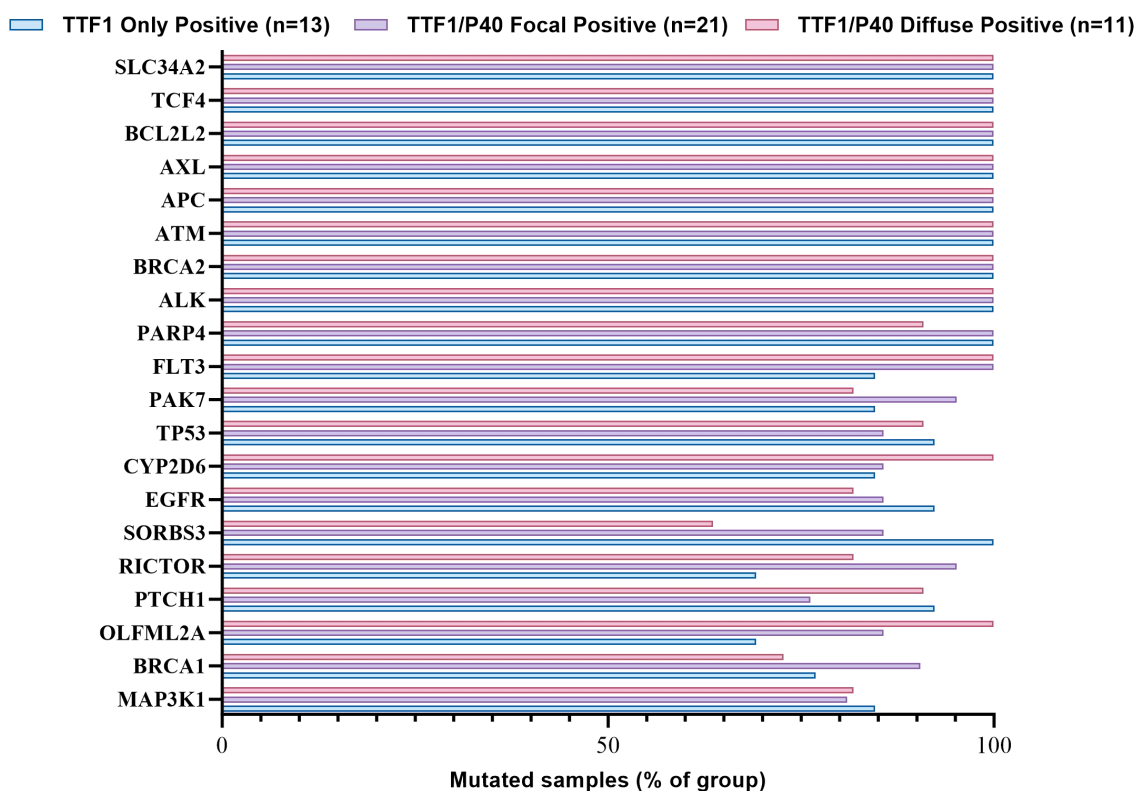
Machine-readable Excel file: *Supplementary_Table_S7.xlsx*

Supplementary Figures

Supplementary Figure S1

Supplementary Figure S1. Mutation frequencies of the top 20 most frequently altered genes across the three lineage groups (TTF-1 only, n = 13; focal, n = 21; diffuse, n = 11).

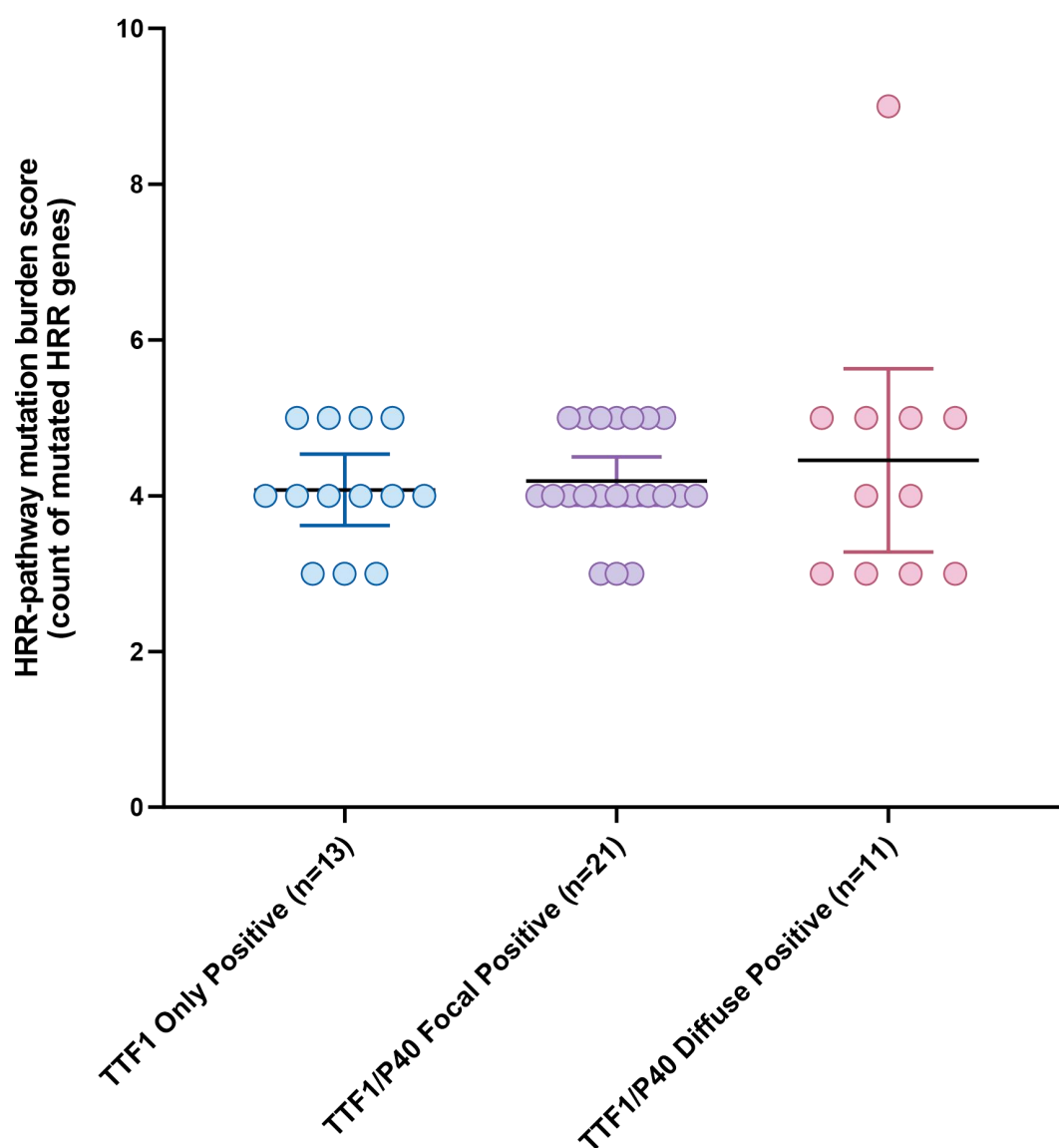
Frequencies are computed at the sample level from non-synonymous variant calls. The call set is unfiltered and retains germline polymorphisms and low-depth calls; the displayed frequencies therefore overestimate clinically confirmed mutation rates and should be interpreted as exploratory.



Supplementary Figure S2

Supplementary Figure S2. HRR-pathway mutation burden score by lineage group (TTF-1 only, n = 13; focal, n = 21; diffuse, n = 11).

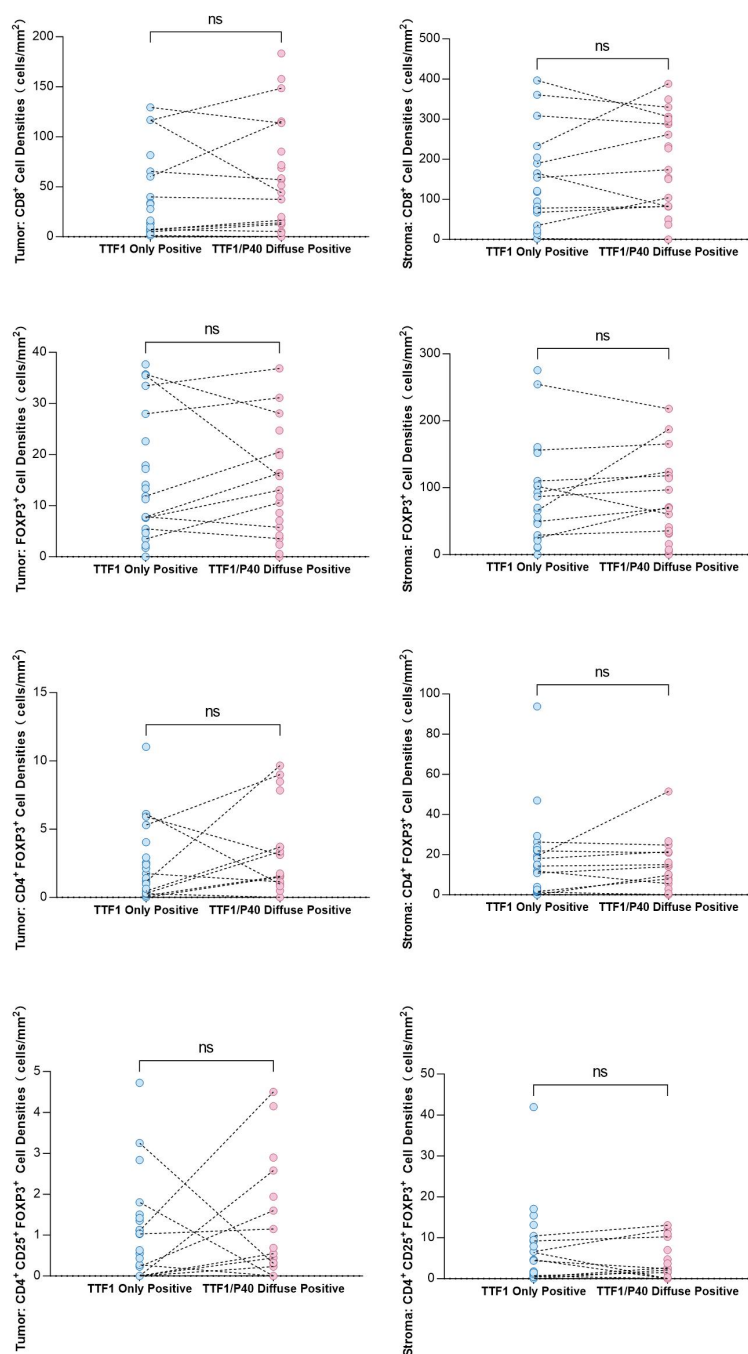
The score is defined as the per-sample count of non-synonymous mutations in prespecified homologous recombination repair-related genes. NOTE: This is a mutation-count descriptor, not based on the established LOH+LST+TAI methodology, and has not been benchmarked against whole-genome HRD assays. It is presented for exploratory reference only and should not be interpreted as a validated clinical HRD score.



Supplementary Figure S3

Supplementary Figure S3. Patient-level re-analysis of spatial immune metrics.

Per-patient regional means were computed from 750 regions of interest (ROI) in 30 patients (TTF-1 only regions from 21 patients; diffuse-positive regions from 20 patients). Gray connecting lines indicate the 11 mixed-phenotype patients who contributed both region types. Eight compartment-specific metrics are shown as dot plots with per-patient points: CD8⁺, FOXP3⁺, CD4⁺FOXP3⁺, and CD25⁺CD4⁺FOXP3⁺ densities in tumor and stromal compartments, and stromal area fraction. Groups were compared with independent-sample t tests on per-patient means; comparisons are nominal, exploratory, and were not adjusted for multiple comparisons. No metric reached nominal significance (all $P > 0.05$); these data are descriptive.



Supplementary Figure S4

Supplementary Figure S4. Sensitivity analysis excluding the 11 mixed-phenotype cases.

Patient-level means of immune cell densities in pure TTF-1 only ($n = 10$) versus pure diffuse ($n = 9$) regions. The same eight metrics are shown as dot plots with overlaid points. Nominal independent-sample t-test P values are shown, without adjustment for multiple comparisons. Pure diffuse regions were descriptively CD8-high and Treg-low.

