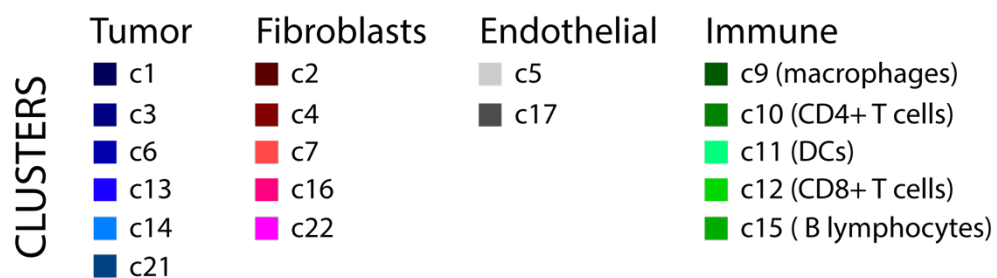
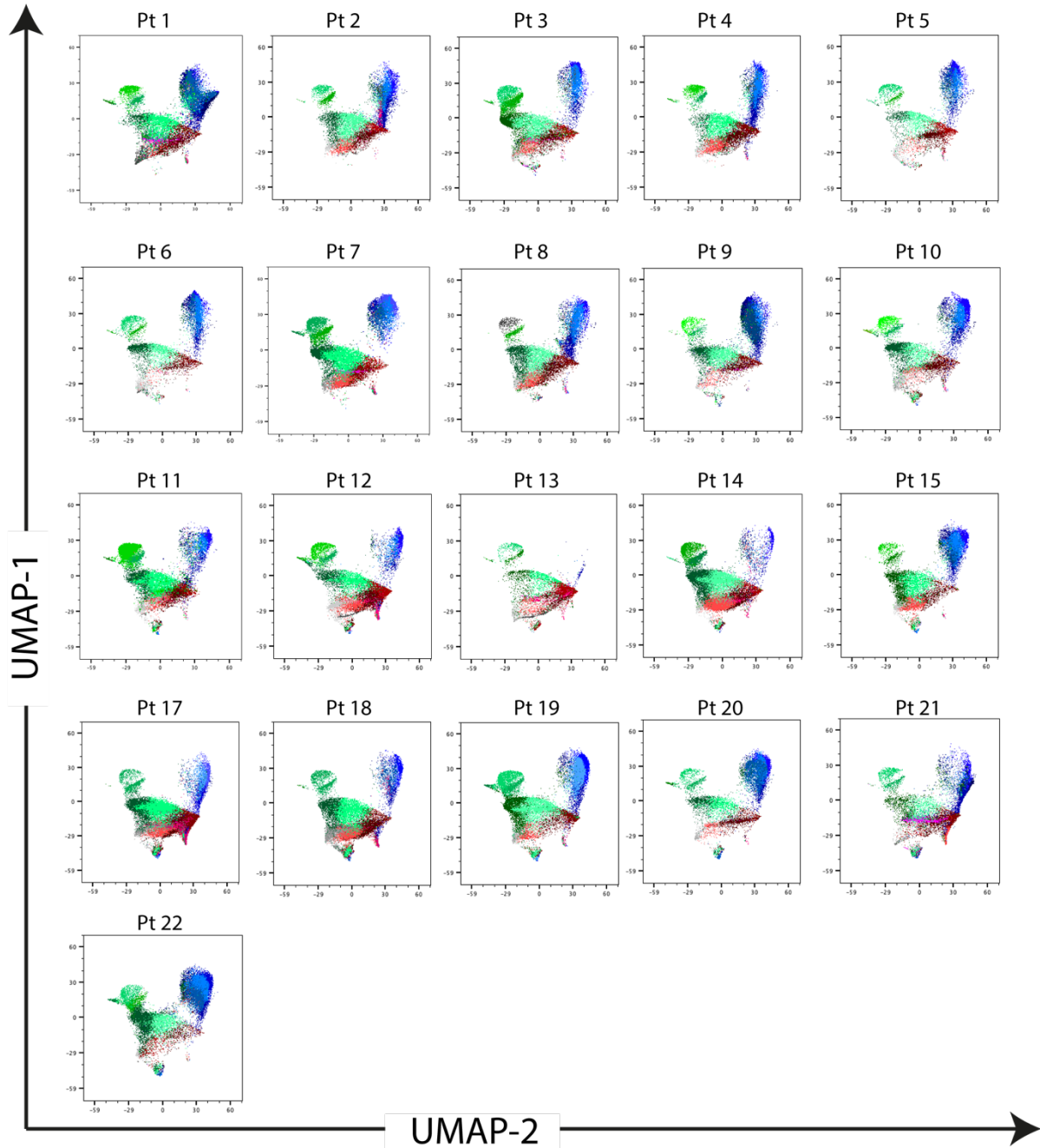


Supplementary Material

The phenogenomic landscapes of pleural mesothelioma tumor microenvironment predict clinical outcomes

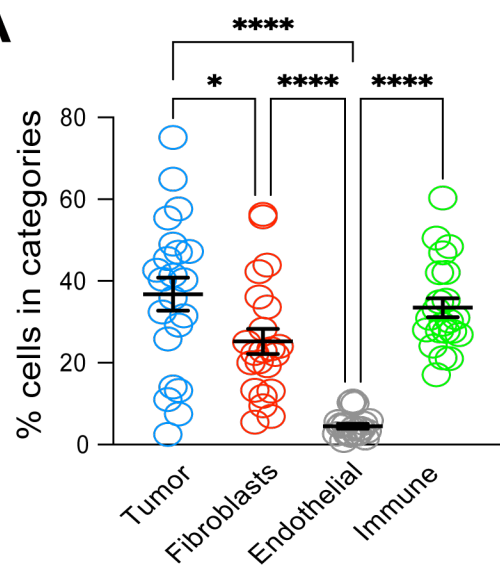
Supplementary Figure 1

A

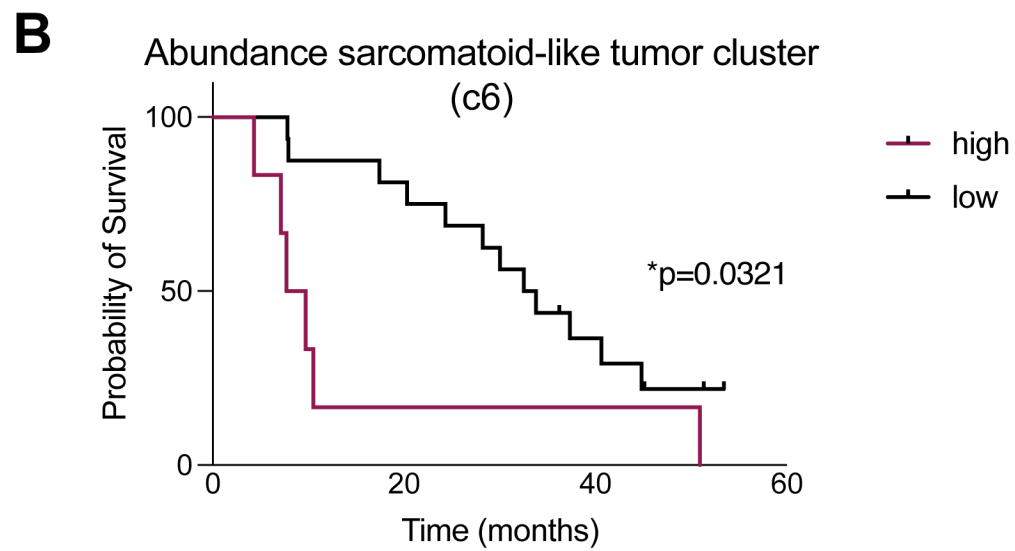
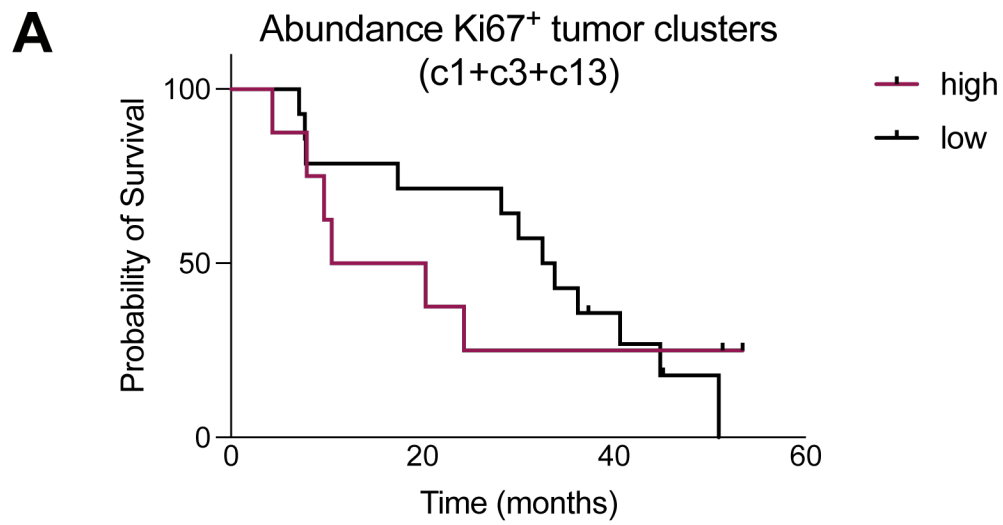


Supplementary Figure 2

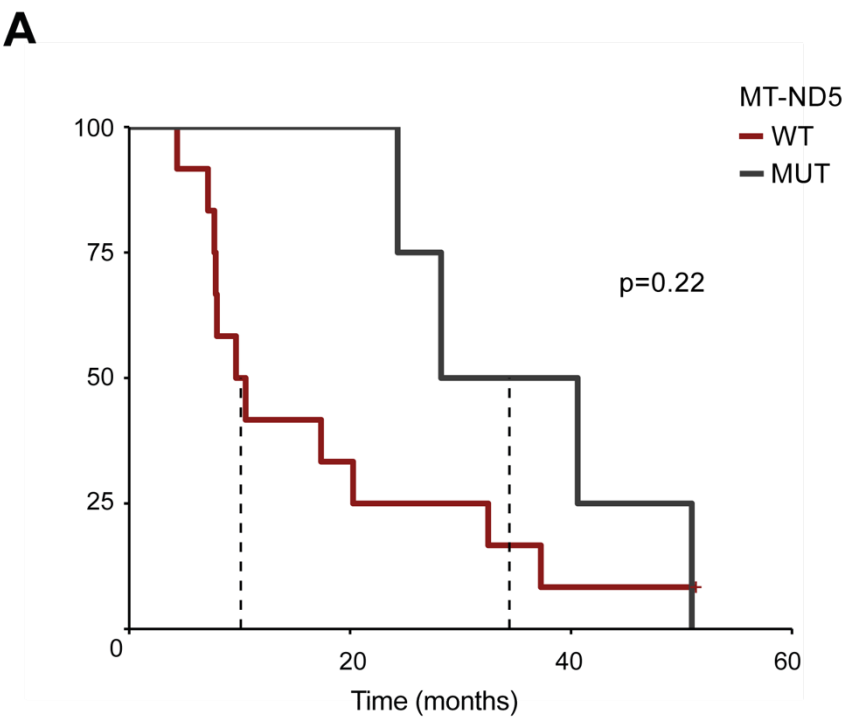
A



Supplementary Figure 3

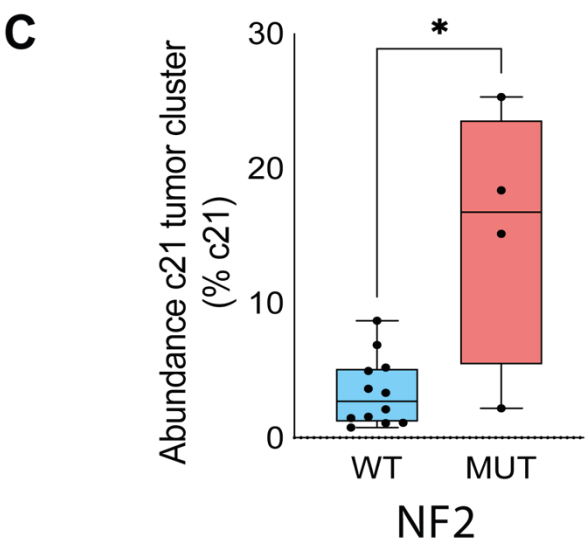


Supplementary Figure 4



B

Gene	HR	CI 95%	P value
BAP1	1.67	0.56-5	0.362
NF2	1.44	0.44-4.72	0.543
MT-ND5	0.48	0.15-1.57	0.226
TTN	1.68	0.43-6.56	0.457



Supplementary Figure Legends

Figure S1. UMAP plot of all analyzed MPM patient samples.

Figure S2. Frequency distribution of the identified cellular categories (tumor, fibroblast, endothelial and immune cells) across the analyzed mesothelioma samples.

Figure S3. Kaplan-Meyer survival curves for patients stratified for Ki67⁺ tumor cell clusters (c1+c3+c13) abundance (**A**) or sarcomatoid cluster (c6) frequency (**B**).

Figure S4. (**A**) Kaplan-Meyer survival curves for patients stratified according to MT-ND5 mutation (WT vs mutated -MUT-). (**B**) Table showing the most frequent mutations detected in the MPM cohort. (**C**) Box and whisker plots showing the frequency of the tumor cluster c6 in NF2^{WT} vs NF2^{MUT} patients.

Table S1. Clinical characteristics of the analyzed mesothelioma patients

Characteristics	Numbers	Percentage
Age median range	72.5	n.a.
Sex		
Male	17	77.3
Female	5	22.7
Performance status		
0	3	13.6
1	13	59.1
2	4	18.2
3	1	4.5
Asbestos exposure		
No	2	9.1
Yes	18	81.8
Unknown	2	9.1
Smoking history		
Smoker	0	0.0
Ex smoker	17	77.3
Non smoker	5	22.7
Symptoms		
SOB	18	81.8
Chest Pain	4	18.2
Others	0	0.0
Histology		
Epithelioid	19	86.4
Biphasic	3	13.6
Side		
Right	12	54.5
Left	10	45.5
Clinical T		
T1	3	13.6
T2	5	22.7
T3	13	59.1
n.d.	1	4.5
Clinical N		
N0	10	45.5
N1	10	45.5
n.d.	2	9.1
MulTimodality treatment		
NO	1	4.5
YES	21	95.5

Resection status			
	R1	16	72.7
	R2	6	27.3

Table S2. List of antibodies and reagents used for IMC.

IMC Antibody list					
Metal	Target	Clone	Cat No	Manufacturer	Dilution
89Y	CD45	HI30	3089003B	Standard Biotoools	1:600
141Pr	αSMA	1A4	3141017D	Standard Biotoools	1:6000
143Nd	Vimentin	RV202	3143029D	Standard Biotoools	1:3000
145Nd	CD4	RPA-T4	300502	Biologend	1:200
146Nd	CD8	RPA-T8	3146001B	Standard Biotoools	1:800
147Sm	CD68	KP1	ab233172	Abcam	1:1000
148Nd	Pan-keratin	C11	3148020D	Standard Biotoools	1:6000
149Sm	CD3	UCHT1	300402	Biologend	1:800
150Nd	PD-L1	E1L3N	3150031D	Standard Biotoools	1:120
151Eu	CD31	EPR3094	ab207090	Abcam	1:300
153Eu	Wilm's tumour 1	CAN-R9(IHC)-56-2	ab216646	Abcam	1:40
154Sm	CD163	GHI/61	3154007B	DVS	1:400
156Gd	CD20	H1	555677	BD Bioscience	1:1000
158Gd	e-Cadherin	24E10	3158029D	Standard Biotoools	1:1000
159Tb	FAP	EPR20021	ab240989	Abcam	1:100
160Gd	Podoplanin	Sheep polyclonal	AF3670	RnD Systems	1:400
161Dy	CD90	5E10	3161009B	Standard Biotoools	1:800
164Dy	Thymidylate synthetase	EPR4545	ab108995	Abcam	1:40
166Er	Mesothelin	EPR19025-42	ab227810	Abcam	1:1500
168Er	Ki67	B56	3168022D	Standard Biotoools	1:1500
169Tm	Collagen I	Goat polyclonal	3169023D	Standard Biotoools	1:6000
171Yb	Granzyme B	GB11	3171002B	Standard Biotoools	1:200
174Yb	VEGF	23410	MAB2931	R&D Systems	1:40
175Lu	CD11c	EP1347Y	ab216655	Abcam	1:400
176Yb	CD56	HCD56	3176001B	Standard Biotoools	1:75
Reagents					
Reagent or Resource	Manufacturer	Catalogue number	Application		
Maxpar Multimetal Labeling Kit	Standard BioTools	201300	IMC		
Antibody Stabilization solution	Candor Bioscience	131 050	IMC		
Human TruStain FcX	Biologend	422301	IMC		
Human IgG	Baxter	5mg/ml KIOVIG	IMC		
Cell-ID™ Intercalator-Ir	Standard BioTools	201192B	IMC		
Software and algorithms					
Reagent or Resource	Manufacturer	Reference	Application		
FlowJo, v10.8.1	FlowJo	https://www.flowjo.com/solutions/flowjo , RRID:SCR_015246	IMC		
MCD Viewer	Fluidigm	Software Fluidigm https://www.standardbio.com/products/mcd-viewer	IMC		
Prism	GraphPad	https://www.graphpad.com , RRID:SCR_002798	Statistical analysis		
Ilastik		DOI:10.1038/s41592-019-0582-9, http://ilastik.org/ , RRID:SCR_015246	IMC		
CellProfiler	Broad Institute	http://cellprofiler.org , RRID:SCR_007358	IMC		
R package IMC	-	https://www.r-project.org/	IMC		

Table S3. Number of ablated 1mm² ROIs for each sample

Patient	Number of ablated ROIs
Pt 1	6
Pt 2	6
Pt 3	7
Pt 4	5
Pt 5	4
Pt 6	5
Pt 7	9
Pt 8	5
Pt 9	5
Pt 10	12
Pt 11	9
Pt 12	10
Pt 13	16
Pt 14	12
Pt 15	5
Pt 16	5
Pt 17	24
Pt 18	13
Pt 19	7
Pt 20	4
Pt 21	5
Pt 22	5

Table S4. Specific phenotype of each identified cell cluster

Cluster n	Cell type	Phenotype
c1	Tumor	Mesothelin ⁺ eCadherin ^{high} Ki67 ⁺ PD-L1 ⁺
c2	Stroma	Collagen I ⁺ Vimentin ^{dim}
c3	Tumor	Mesothelin ⁺ eCadherin ^{high} Pankeratin ⁺ PDPN ⁺ Wilms tumor ⁺ Thymidylate Synthase ⁺ Ki67 ⁺ PD-L1 ⁺
c4	Stroma	Collagen I ^{dim}
c5	Endothelial cells	CD31 ⁺ CD90 ^{high} VEGF ⁺ Vimentin ⁺ Thymidylate Synthase ⁺ Collagen I ^{dim} PD-L1 ^{dim}
c6	Tumor	Mesothelin ^{low} eCadherin ^{low} Pankeratin ^{low} PDPN ^{low} Wilms tumor ^{low} Thymidylate Synthase ^{low} Ki67 ^{low}
c7	Stroma	Collagen I ⁺ Vimentin ^{dim} VEGF ⁺
C9	Macrophages	CD68 ⁺ CD163 ⁺ CD11c ⁺ CD45 ⁺ CD4 ⁺ VEGF ⁺ PD-L1 ^{dim}
c10	T lymphocytes CD4 ⁺	CD4 ⁺ CD3 ⁺ CD45 ⁺ PD-L1 ^{dim}
c11	DCs	CD11c ⁺ CD68 ^{dim} CD163 ^{dim} CD45 ^{dim}
c12	T lymphocytes CD8 ⁺	CD8 ⁺ CD3 ⁺ CD45 ⁺ GrB ⁺ PD-L1 ^{dim}
c13	Tumor	Mesothelin ⁺ eCadherin ^{high} Pankeratin ⁺ PDPN ⁺ Wilms tumor ⁺ Thymidylate Synthase ⁺ Ki67 ^{high} PD-L1 ⁺
c14	Tumor	eCadherin ^{dim} Pankeratin ⁺ PDPN ^{dim} Wilms tumor ⁺ Thymidylate Synthase ^{dim} Ki67 ^{dim} PD-L1 ⁺
c15	B lymphocytes	CD20 ⁺ CD45 ⁺ GrB ^{dim}
c16	Stroma	PDPN ⁺
c17	Endothelial	CD31 ⁺ SMA ⁺ PD-L1 ⁺
c21	Tumor	Mesothelin ^{low} eCadherin ^{dim} Pankeratin ^{dim} PDPN ⁺ Wilms tumor ^{dim} Thymidylate Synthase ^{dim} Ki67 ^{dim} PD-L1 ^{dim}
c22	Stroma	FAP ⁺ Collagen I ⁺