Table S1. Gene list of the 1021-gene panel

1.1 All 4847 exon regions of 312 genes

ABL1	ACVR1B	AKT1	AKT2	AKT3	ALK	APC	AR	ARAF	ARID1A
ARID1B	ARID2	ASXL1	ATM	ATR	ATRX	AURKA	AURKB	AXIN1	AXIN2
AXL	B2M	BAP1	BARD1	BCL2	BCL2L1	BCOR	BLM	BMPR1A	BRAF
BRCA1	BRCA2	BRD4	BRIP1	BTK	CARD11	CASP8	CBFB	CBL	CCND1
CCND2	CCND3	CCNE1	CD274	CDC73	CDH1	CDK12	CDK4	CDK6	CDK8
CDKN1A	CDKN1B	CDKN2A	CDKN2B	CDKN2C	CEBPA	CHEK1	CHEK2	CIC	CREBBP
CRKL	CSF1R	CTCF	CTNNA1	CTNNB1	CUL3	CYLD	DAXX	DDR1	DDR2
DICER1	DNMT3A	DOT1L	EGFR	EIF1AX	C11orf30	EP300	EPAS1	EPCAM	EPHA2
EPHA3	EPHA5	EPHB1	EPHB6	ERBB2	ERBB3	ERBB4	ERCC1	ERCC3	ERCC4
ERCC5	ERG	ERRFI1	ESR1	EXT1	EXT2	EZH2	FAM123B	FAM175A	FANCA
FANCC	FANCD2	FANCE	FANCF	FANCG	FANCL	FANCM	FAS	FAT1	FAT2
FBXW7	FGF19	FGF3	FGF4	FGFR1	FGFR2	FGFR3	FGFR4	FH	FLCN
FLT1	FLT3	FLT4	FOXA1	FOXL2	FOXP1	FUBP1	GALNT12	GATA3	GNA11
GNAQ	GNAS	GRIN2A	GRM3	HDAC1	HGF	HNF1A	HOXB13	HRAS	IDH1
IDH2	IFNG	IFNGR1	IGF1R	IKBKE	IKZF1	IL7R	INPP4B	IRF2	IRS2
JAK1	JAK2	JAK3	JUN	KDM5A	KDM5C	KDM6A	KDR	KEAP1	KIT
KRAS	LRP1B	MAF	MAP2K1	MAP2K2	MAP2K4	MAP3K1	MAPK1	MAX	MCL1
MDM2	MDM4	MED12	MEF2B	MEN1	MET	MITF	MLH1	MLH3	MLL
MLL2	MLL3	MPL	MRE11A	MS4A1	MSH2	MSH3	MSH6	MST1R	MTOR
MUTYH	MYC	MYCL1	MYCN	MYD88	NBN	NCOR1	NF1	NF2	NFE2L2
NFKBIA	NKX2-1	NOTCH1	NOTCH2	NOTCH3	NPM1	NRAS	NSD1	NTHL1	NTRK1
NTRK2	NTRK3	PALB2	PARK2	PARP1	PAX5	PBRM1	PCK1	PDCD1	PDCD1LG2
PDGFRA	PDGFRB	PDK1	PIK3CA	PIK3CB	PIK3CG	PIK3R1	PIK3R2	PMS1	PMS2
POLD1	POLE	POT1	PPP2R1A	PRDM1	PRKAR1A	PTCH1	PTCH2	PTEN	PTPN11
PTPRD	RAC1	RAD50	RAD51	RAD51B	RAD51C	RAD51D	RAD52	RAD54L	RAF1
RARA	RB1	RBM10	RECQL	RECQL4	RET	RHOA	RICTOR	RINT1	RNF43
ROS1	RPTOR	RUNX1	SDHA	SDHAF2	SDHB	SDHC	SDHD	SERPINB3	SERPINB4
SETD2	SF3B1	SLX4	SMAD2	SMAD3	SMAD4	SMARCA4	SMARCB1	SMO	SOCS1
SOX2	SOX9	SPOP	SRC	STAG2	STAT3	STK11	SUFU	SYK	TBX3
TCF7L2	TERC	TET2	TGFBR2	TMEM127	TMPRSS2	TNFAIP3	TNFRSF14	TOP1	TOP2A
TP53	TSC1	TSC2	TSHR	U2AF1	VEGFA	VHL	WRN	WT1	XPO1
XRCC2	ZMAT3								

1.2 Introns, promoter, or fusion breakpoint area of 38 genes

ALK	BCL2L11	BRAF	BRCA1	BRD4	CD74	EGFR	EML4	ERG	ETV6
EZR	FGFR1	FGFR2	FGFR3	KIF5B	KIT	MAML2	MET	MSH2	MYC
MYCL1	NCOA4	NOTCH2	NTRK1	NTRK2	NTRK3	PDGFRA	RAF1	RET	ROS1
RSPO2	SDC4	SLC34A2	TERT	TFE3	TMPRSS2	TPM3	PMS2		

1.3 A total of 1778 selected coding regions of 709 genes

ABCA13	ABCB1	ABCC1	ABCC11	ABCC2	ABCG2	ABL2	ACACA	ACIN1	ACTB
ACTG1	ACTG2	ACVR2A	ACVRL1	ADAM29	ADAMTS5	ADCY1	AFF1	AFF2	AFF3
AHNAK	AKAP9	ALB	AMOT	ANGPT1	ANK3	ANKRD11	ANKRD30A	ANKRD30B	APEX1
APOBEC3B	ARAP3	ARFGEF1	ARFGEF2	ARHGAP29	ARHGAP35	ARID4B	ARID5B	ARNT	ASCL4
ASH1L	ASMTL	ASPM	ASTN1	ASXL2	ATIC	ATP11B	ATP12A	ATP1A1	ATP2B3
BAZ2B	BBC3	BBS9	BCAS1	BCL10	BCL11A	BCL11B	BCL2A1	BCL2L11	BCL3
BCL6	BCL9	BCORL1	BCR	BIRC3	BMPR2	BNC2	BPTF	BRD2	BRD3
BRSK1	BRWD1	BTLA	BUB1	C15orf23	C15orf55	C1QA	C1S	C3orf70	C7orf53
C8orf34	CACNA1E	CADM2	CALR	CAMTA1	CASP1	CASQ2	CBLB	CBR1	CBR3
CCDC168	CCNA1	CCNB3	CCT3	CCT5	CCT6B	CD22	CD33	CD5L	CD74
CDA	CDH11	CDH18	CDH23	CDK13	CHD1	CHD1L	CHD4	CHD6	CHD8
CHD9	CHFR	CHI3L1	CHN1	CIITA	CLDN18	CLP1	CLSPN	CLTC	CNOT3
CNOT4	CNTN1	CNTN5	CNTNAP1	CNTNAP5	COL1A1	COL2A1	COL5A1	COL5A2	COL5A3
COPS2	CPS1	CRIPAK	CRLF2	CRNKL1	CRTC1	CSF1	CSF3R	CSMD1	CSMD3
CSNK1A1	CSNK1G3	CTLA4	CTNNA2	CTNND1	CUX1	CXCR4	CYBA	CYP19A1	CYP1A1
CYP1B1	CYP2A13	CYP2C8	CYP2D6	CYP3A4	CYP3A5	DCC	DDX3X	DDX5	DEK
DHX35	DHX9	DIAPH1	DIS3L2	DLC1	DMD	DNAH6	DNAJB1	DNM2	DNMT1
DNMT3B	DOCK2	DOCK7	DPYD	DRGX	DTX1	DUSP22	DYSF	E2F3	EBF1
ECT2L	EED	EEF1A1	EGFL7	EGR3	EIF2AK3	EIF2C3	EIF3A	EIF4A2	EIF4G3
ELAC2	ELF1	ELF3	ELMO1	ELN	EME2	EMID2	EML4	EPC1	EPHA1
EPHA4	EPHA7	EPHB2	EPHB4	EPOR	EPPK1	EPS15	ERBB2IP	ERCC2	ESR2
ETS1	ETV1	ETV5	ETV6	EWSR1	EZR	F8	FAM131B	FAM135B	FAM157B
FAM46C	FAM5C	FAP	FASLG	FAT3	FAT4	FCGR1A	FCGR2A	FCGR2B	FCGR3A
FCRL4	FGF10	FGF12	FGF14	FGF23	FGF6	FLG	FLI1	FLNC	FMN2
FN1	FNDC4	FOXA2	FOXO1	FOXO3	FOXQ1	FRMPD4	FUS	FXR1	FYN
FZD1	G3BP1	G3BP2	GAB2	GABRA6	GATA1	GATA2	GFRAL	GIGYF1	GKN2
GLB1L3	GLI1	GLI2	GLI3	GMPS	GNA13	GNG2	GPC3	GPR124	GPS2
GPX1	GRB7	GSK3B	GSTM5	GSTP1	GUSB	H3F3A	H3F3B	H3F3C	HCLS1
HCN1	HDAC4	HDAC9	HECW1	HEY1	HIST1H1C	HIST1H1D	HIST1H1E	HIST1H2AC	HIST1H2G
HIST1H2AL	HIST1H2AM	HIST1H2BC	HIST1H2BD	HIST1H2BJ	HIST1H2BK	HIST1H2O	HIST1H3B	HIST1H3C	HIST1H3D
HIST1H3F	HIST1H3G	HIST1H3H	HIST1H3I	HIST1H4I	HIST3H3	HLA-A	HLA-B	HLA-C	HLF
HMCN1	HNF1B	HNRPDL	HOXA11	HOXA13	HOXA3	HOXA9	HOXC13	HOXD11	HOXD13
HSD3B1	HSP90AA1	HSP90AB1	HSPA8	HSPD1	HSPH1	ICK	ICOSLG	ID3	IFITM3
IGF1	IGF2	IGF2R	IGLL5	IKZF2	IKZF3	IL10	IL1RAPL1	IL21R	IL6
IL6ST	IMPG1	ING1	INHBA	INPP4A	INPPL1	INSR	IRF4	IRF6	IRS1
ITGB3	ITK	ITSN1	JARID2	KALRN	KAT6A	KAT6B	KCNJ5	KCNQ2	KDM2B
KEL	KIF5B	KLF4	KLHL6	KLK1	KRTAP5-5	L3MBTL1	LAMA2	LATS1	LATS2
LCP1	LEF1	LGALS8	LIFR	LPHN2	LPP	LRP2	LRP4	LRP5	LRP6
LRRC7	LRRK2	LYN	LZTS1	MACF1	MAD1L1	MAGI2	MAML2	MAML3	MAP3K13
MAPK3	MCC	MCM3	MDC1	MECOM	MEF2C	MGA	MIB1	MIOS	MKL1
MLL4	MLLT3	MMP11	MMP2	MN1	MNDA	MNX1	MSH4	MSN	MSR1
MTHFR	MTRR	MUC5B	MYH11	MYH14	MYH9	MYO3A	MYOD1	NAP1L1	NAV3

NCAM2	NCF2	NCF4	NCK1	NCOA3	NCOA4	NCOR2	NCSTN	NDUFA13	NFATC4
NFE2L3	NKX3-1	NLRC3	NOD1	NOS3	NOTCH4	NQO1	NR1I2	NR2F2	NR4A2
NRG1	NRP2	NRXN1	NTM	NUMA1	NUP107	NUP210	NUP93	NUP98	OBSCN
OGDH	OMD	OPCML	OR11G2	OR2T4	OR4A15	OR4C6	OR5L2	OR6F1	P2RY8
P4HB	PABPC1	PABPC3	PAG1	PAK1	PAK3	PASK	PAX3	PAX7	PC
PCDH18	PCSK6	PCSK7	PDCD11	PDE4DIP	PDGFB	PDILT	PER1	PGR	PHF1
PHF6	PIK3C2A	PIK3C2B	PIK3C2G	PIK3C3	PIM1	PKD1L2	PKHD1	PLAG1	PLCB1
PLCG1	PLCG2	PLK1	PLXNA1	PLXNB2	PNRC1	POLQ	POM121	POM121L12	POU2AF1
PPM1D	PPP1R17	PPP6C	PRDM16	PREX2	PRF1	PRKAA1	PRKCB	PRKCI	PRKDC
PRRX1	PRX	PSG2	PSIP1	PSMB1	PSMB5	PTGS1	PTGS2	PTPN13	PTPN2
PTPRB	PTPRK	PTPRO	PTPRS	PTPRT	PTPRU	RAB35	RAC2	RAD21	RAD54B
RANBP2	RASA1	RASGRP1	RBL1	REL	RELN	RFC1	RGS3	RHEB	RHOH
RHOT1	RIT1	RNASEL	ROBO1	ROBO2	ROBO3	ROCK1	RPGR	RPS6KB1	RPS6KB2
RSPO2	RSPO3	RUNX1T1	RUNX2	RXRA	RYR1	RYR2	SBDS	SCUBE2	SDC4
SEC31A	SEMA3A	SEMA3E	SEMA6A	SERPINA7	SETBP1	SETDB1	SF1	SF3A1	SFPQ
SGCZ	SGK1	SH2B3	SH2D1A	SH3PXD2A	SHH	SI	SIN3A	SLC16A1	SLC1A2
SLC22A16	SLC22A18	SLC22A2	SLC22A3	SLC34A2	SLCO1B3	SLIT1	SLIT2	SMARCD1	SMARCE1
SMC1A	SMC1B	SNCAIP	SNTG1	SNX29	SOD2	SOS1	SOX10	SOX17	SPEN
SPRR3	SPSB4	SPTA1	SRD5A2	SRGAP1	SRGAP3	SRSF2	SRSF7	STAG1	STAT1
SUCLG1	SUCLG2	SULT1A1	SUZ12	SVEP1	SYNCRIP	SYNE1	TAF1	TAF15	TAF1L
TAL1	TBL1XR1	TBX15	TBX22	TCEB1	TCF12	TCF3	TCF4	TCL1A	TEC
TENM3	TERT	TET1	TFDP1	TFDP2	TFE3	TGFBR1	THBS2	TJP1	TLE1
TLL2	TLR4	TLX3	TMEM13D	TNFSF11	TNN	TP53BP1	TP63	TP73	TPM3
TPR	TRAF2	TRAF7	TRIM24	TRIM58	TRIO	TRPC5	TRRAP	TSHZ2	TSHZ3
TTF1	TUBA3C	TUBB3	TUSC3	TXNIP	TYMS	TYR	UBE2D2	UBR5	UGT1A1
UMPS	UPF3B	USH2A	USP6	USP8	VEZF1	VIM	VTCN1	WASF3	WDR90
WDTC1	WHSC1	WHSC1L1	WIPF1	WNK1	WNT5A	WSCD2	WWOX	WWP1	WWP2
XIAP	XPC	XRCC1	XRCC3	YAP1	YY1AP1	ZBTB16	ZC3H11A	ZFHX3	ZFP36L1
ZFP36L2	ZFPM2	ZIC3	ZNF217	ZNF384	ZNF521	ZNF638	ZNF750	ZNF804B	

Table S2 Putative driver genes shared among multiple lesions in 61 MPLCs patients

Genes	Shared mutation patterns	Numbers of patients
EGFR	L858R, 19del, L861Q, S768I, G719A, T790M, 19indel, 20ins, 20indel, 18indel	45
ERBB2	20ins, exon17 V659E, exon19 L755P	6
KRAS	G12C, Q61H, G12R, G12F, G12V	5
BRAF	K601E, G469A, L597R, N581S, D594H, E586K, T599dup, S37L	6
MAP2K1	Q58_E62del, E41_F53del, E102_I103del	1

Note: Two patients shared two kinds of mutated driver genes among multiple lesions, this is, EGFR and ERBB2 mutations; EGFR and KRAS mutations.

Table S3 Clinicopathological characteristics according to shared mutated genes

Characteristics	Different mutant gene, n (%)	Same mutant gene, n (%)	P value	Characteristics	Different mutant gene, n (%)	Same mutant gene, n (%)	P value
Sex			0.477	Lobes of tumor			0.659
female	12 (40.0)	18 (60.0)		Upper lobe	66 (44.3)	83 (55.7)	
male	39 (47.6)	43 (52.4)		Middle lobe	7 (36.8)	12 (63.2)	
Age			0.370	Lower lobe	34 (39.1)	53 (60.9)	
<60	39 (48.1)	42 (51.9)		Radiology			0.817
≥60	12 (38.7)	19 (61.3)		GGO	95 (42.2)	130 (57.8)	
Smoking			0.522	Solid	12 (40.0)	18 (60.0)	
no	44 (44.4)	55 (55.6)		Histopathology			0.426
yes	7 (53.9)	61 (54.5)		AIS	15 (53.6)	13 (46.4)	
Malignancy			0.624	MIA	16 (39.0)	25 (61.0)	
no	50 (46.3)	58 (53.7)		ADC	75 (41.0)	108 (59.0)	
yes	1 (25.0)	3 (75.0)		Other	1 (33.3)	2 (66.7)	
Family malignancy			0.308	Diameter			0.326
no	46 (47.4)	51 (52.6)		≤1cm	71 (43.8)	91 (56.2)	
yes	5 (33.3)	10 (66.7)		1<X≤2cm	30 (41.7)	42 (58.3)	
Highest T stage			**0.027***	>2cm	6 (28.6)	15 (71.4)	
T1a	18 (56.3)	14 (43.8)		T stage			**0.038***
T1b	21 (51.2)	20 (48.8)		T1a	69 (46.3)	60 (53.7)	
≥T1c	12 (30.8)	27 (69.2)		T1b	25 (43.9)	32 (56.1)	
Highest N stage			0.591	≥T1c	13 (26.5)	36 (73.5)	
N0	49 (45.0)	60 (55.0)		N stage			0.574
N1	2 (66.7)	1 (33.3)		N0	105 (41.7)	147 (58.3)	
Highest TNM stage			**0.039***	N1	2 (66.7)	1 (33.3)	
IA1	18 (56.3)	14 (43.7)		TNM stage			**0.044***
IA2	20 (50.0)	20 (50.0)		IA1	69 (46.3)	80 (53.7)	
≥IA3	13 (32.5)	27 (67.5)		IA2	24 (42.9)	32 (57.1)	
				≥IA3	14 (28.0)	36 (72.0)	

Table S4 Clinicopathological, radiological, and molecular characteristics of 25 MPLCs patients sharing the same driver mutation among at least 2 lesions

ID	Sex	Age	Lesion, n	Tumor location	Tumors sharing mutation	Histopathology, radiology,	Shared mutation	Specific mutations, n
2	male	58	4	bilateral	2A, 2D	2A: ADC, GGO 2D: AIS, GGO	EGFR p.L858R	2A: 8 2D: 0
4	female	57	2	ipsilateral	4A, 4B	ADC, GGO	EGFR p.L858R	4A: 4 4B: 1
5	male	79	2	lobe	5A, 5B	ADC, GGO	KRAS p.G12C	5A: 8 5B: 10
21	female	43	4	ipsilateral	21A, 21D	ADC, GGO	ERBB2 p.Y772_A775dup	21A: 3 21D: 1
23	male	65	2	bilateral	23A,	23A: ADC, GGO	EGFR p.L858R	23A: 3

					23B	23B: MIA, GGO		23B: 4
27	female	65	4	bilateral	27B, 27C	27B: AIS, GGO 27C: AIS, GGO	EGFR p.L858R	27B: 1 27C: 4
35	female	65	3	ipsilateral	35A, 35B	35A: ADC, GGO 35B: ADC, solid	EGFR p.L858R	35A: 9 35B: 0
39	female	36	4	bilateral	39A, 39C	ADC, GGO	EGFR p.A767_V769dup	39A: 3 39C: 0
41	female	73	4	bilateral	41C, 41D	41C: ADC, GGO 41D: ADC, GGO	EGFR p.L861Q TNFRSF14 p.S186_W187insR	41C: 3 41D: 5
50	male	55	2	lobe	50A, 50B	50A: ADC, GGO 50B: ADC, solid	EGFR 19del	50A: 2 50B: 2
61	female	57	2	bilateral	61A, 61B	61A: MIA, GGO 61B: ADC, GGO	EGFR 19del	61A: 2 61B: 2
66	male	44	2	ipsilateral	66A, 66B	ADC, GGO	ERBB2 p.Y772_A775dup	0
68	female	59	3	bilateral	68B, 68C	68B: ADC, solid 68C: ADC, GGO	EGFR p.L858R	68B: 2 68C: 0
69	female	52	2	bilateral	69A, 69B	69A: ADC, GGO 69B: MIA, GGO	EGFR p.L858R	69A: 3 69B: 2
76	female	56	3	ipsilateral	76A, 76B, 76C	76A: ADC, GGO 76B: ADC, GGO 76C: MIA, GGO	BRAF p.K601E	76A: 1 76B: 6 76C: 3
77	male	37	2	ipsilateral	77A, 77B	MIA, GGO	ERBB2 p.Y772_A775dup	0
80	female	57	3	lobe	80A, 80C	ADC, GGO	EGFR p.L858R	80A: 5 80C: 3
81	male	47	4	ipsilateral	81B, 81C, 81D	ADC, GGO	EGFR 19del	81B: 3 81C: 4 81D: 4
91	female	59	2	bilateral	91A, 91B	91A: ADC, solid 91B: ADC, GGO	EGFR p.L858R	91A: 10 91B: 5
92	female	57	3	bilateral	92A, 92C	ADC, GGO	EGFR p.L858R	92A: 5 92C: 3
98	female	58	2	ipsilateral	98A, 98B	98A: ADC, solid 98B: ADC, GGO	EGFR p.L858R	98A: 10 98B: 4
108	female	76	2	lobe	108A, 108B	ADC, GGO	EGFR p.L858R KRAS p.G12R	108A: 1 108B: 3
111	male	52	2	bilateral	111A, 111B	ADC, GGO	EGFR 19delins	111A: 5 111B: 1
112	male	51	3	lobe	112B, 112C	112B: ADC, GGO 112C: NEC, GGO	KRAS p.G12V	112B: 21 112C: 32
114	male	64	2	ipsilateral	114A, 114B	ADC, GGO	EGFR 19del	114A: 2 114B: 2

MPLCs: multiple primary lung cancers; GGO: ground-glass opacity; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; ADC: adenocarcinoma; NEC: neuroendocrine tumors.

Table S5 Association of PD-L1 and clinicopathological features and high-frequency mutated genes

Characteristics	PD-L1 TPS, n (%)		P value	PD-L1 CPS, n (%)		P value
	<1%	≥1%		<1	≥1	
Tumor location			0.507			0.561
Upper lobe	91 (94.8)	5 (5.2)		42 (43.8)	54 (56.3)	
Middle lobe	8 (100.0)	0 (0.0)		4 (50.0)	4 (50.0)	
Lower lobe	52 (98.1)	1 (1.9)		28 (52.8)	25 (47.2)	
Radiology			**0.004***			0.771
GGO	133 (98.5)	2 (1.5)		63 (46.7)	72 (53.3)	
Solid	18 (81.8)	4 (18.2)		11 (50.0)	11 (50.0)	
Histopathology			**0.004***			0.882
AIS	11 (100.0)	0 (0.0)		6 (54.6)	5 (45.5)	
MIA	29 (100.0)	0 (0.0)		15 (51.7)	14 (48.3)	
ADC	110 (95.7)	5 (4.3)		52 (45.2)	63 (54.8)	
Other	1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)	
Diameter			**<0.001***			0.538
≤1cm	84 (97.7)	2 (2.3)		44 (51.2)	42 (48.8)	
≤2cm	52 (100.0)	0 (0.0)		22 (42.3)	30 (57.7)	
>2 cm	15 (79.0)	4 (21.1)		8 (42.1)	11 (57.9)	
pTNM stage			**0.010***			0.312
IA1	72 (98.6)	1 (1.4)		38 (52.1)	35 (48.0)	
IA2	39 (100.0)	0 (0.0)		19 (48.7)	20 (51.3)	
≥IA3	40 (88.9)	5 (11.1)		17 (37.8)	28 (62.2)	
EGFR mutations			0.631			0.287
no	48 (96.0)	2 (4.0)		27 (54.0)	23 (46.0)	
non-sensitive	25 (92.6)	2 (7.4)		13 (48.1)	14 (51.9)	
sensitive	64 (97.0)	2 (3.0)		26 (39.4)	40 (60.6)	
ERBB2 mutations			0.563			**0.046***
no	124 (96.1)	5 (3.9)		56 (43.3)	73 (56.6)	
yes	13 (92.9)	1 (7.1)		10 (71.4)	4 (28.6)	
TP53 mutations			0.079			0.461
no	123 (96.9)	4 (3.1)		60 (47.2)	67 (52.8)	
yes	14 (87.5)	2 (12.5)		6 (37.5)	10 (62.5)	
BRAF mutations			0.563			0.794
no	124 (96.1)	5 (3.9)		60 (46.5)	69 (53.5)	

yes	13 (92.9)	1 (7.1)		6 (42.9)	8 (57.1)	
RBM10 mutations			0.392			0.613
no	122 (95.3)	6 (4.7)		60 (46.9)	68 (53.1)	
yes	15 (100.0)	0 (0.0)		6 (40.0)	9 (60.0)	
KRAS mutations			0.510			0.560
no	125 (96.2)	5 (3.8)		59 (45.4)	71 (54.6)	
yes	12 (92.3)	1 (7.7)		7 (53.8)	6 (46.2)	
MAP2K1 mutations			0.600			1.000
no	131 (95.6)	6 (4.4)		63 (46.0)	74 (54.0)	
yes	6 (100.0)	0 (0.0)		3 (50.0)	3 (50.0)	
LRP1B mutations			**0.001***			0.451
no	132 (97.1)	4 (2.9)		64 (47.1)	72 (52.9)	
yes	5 (71.4)	2 (28.6)		2 (28.6)	5 (71.4)	
MED12 mutations			0.634			0.662
no	132 (95.7)	6 (4.3)		63 (45.7)	75 (54.3)	
yes	5 (100.0)	0 (0.0)		3 (60.0)	2 (40.0)	
PIK3CA mutations			0.172			0.704
no	131 (96.3)	5 (3.7)		62 (45.6)	74 (54.4)	
yes	6 (85.7)	1 (14.3)		4 (57.1)	3 (42.9)	

PD-L1: programmed death ligand 1; TPS: tumor proportion score; CPS: combined positive score; GGO: ground-glass opacity; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; ADC: adenocarcinoma.

Table S6 Associations of TILs with clinicopathological characteristics

Characteristics	CD3-CT, median (Q1-Q3)	P value	CD3-IM, median (Q1-Q3)	P value	CD8-CT, median (Q1-Q3)	P value	CD8-IM, median (Q1-Q3)	P value
Tumor location		0.405		**0.041***		0.759		0.062
Upper lobe	1508 (847-2283)		550 (389-667)		513 (319-745)		185 (131-297)	
Middle lobe	2103 (1398-3165)		846 (759-959)		551 (406-1010)		214 (169-672)	
Lower lobe	1601 (779-2329)		552 (344-678)		530 (300-880)		154 (113-269)	
Histopathology		0.202		0.583		0.105		0.811
AIS	842 (654-2134)		465 (385-664)		426 (255-476)		186 (151-249)	
MIA	1522 (485-2038)		552 (345-680)		384 (256-694)		157 (131-264)	
ADC	1589 (973-2340)		560 (368-725)		555 (325-782)		170 (125-292)	
Diameter		**<0.001***		**0.004***		**<0.001***		**0.008***
≤1cm	1229 (619-1960)		462 (334-648)		416 (255-694)		151 (104-225)	
≤2cm	1663 (1091-2335)		568 (412-755)		557 (325-757)		201 (156-303)	
>2cm	2385 (1747-2846)		698 (553-1042)		813 (602-1427)		192 (156-409)	
pTNM stage		**0.003***		**0.035***		**0.006***		**0.010***
IA1	1043 (585-2037)		512 (319-657)		413 (248-676)		152 (104-234)	
IA2	1741 (1179-2334)		571 (422-760)		540 (357-731)		213 (157-317)	
≥IA3	1705 (1187-2409)		565 (368-889)		689 (384-850)		172 (142-293)	

TILs: tumor-infiltrating lymphocytes; CT: central tumor; IM: invasive margin; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; ADC: adenocarcinoma.

Table S7 Associations of TAMs with clinicopathological characteristics

Characteristics	CD68-CT, median (Q1-Q3)	P value	CD68-IM, median (Q1-Q3)	P value	CD163-CT, median (Q1-Q3)	P value	CD163-IM, median (Q1-Q3)	P value
Tumor location		0.466		0.730				0.142
Upper lobe	1110 (810-1424)		694 (564-900)		692 (536-878)	0.974	374 (266-508)	
Middle lobe	1346 (1192-1614)		722 (589-829)		773 (541-900)		474 (334-651)	
Lower lobe	1124 (887-1373)		776 (570-938)		702 (545-829)		417 (316-528)	
Histopathology		0.093		0.121				0.587
AIS	881 (604-1299)		564 (392-890)		656 (483-874)	0.365	414 (237-547)	
MIA	1045 (733-1287)		756 (656-890)		682 (528-766)		337 (288-486)	
ADC	1152 (852-1448)		740 (568-913)		717 (543-879)		409 (298-521)	
Diameter		**0.003***		0.064		**0.009***		**0.022***
≤1cm	1042 (732-1286)		687 (546-892)		677 (512-832)		374 (281-519)	
≤2cm	1214 (892-1494)		740 (594-878)		719 (565-838)		392 (308-496)	
>2cm	1361 (1227-1691)		887 (675-1151)		879 (698-1200)		527 (411-633)	
pTNM stage		**0.002***		0.434		**0.033***		0.217
IA1	1035 (710-1278)		687 (545-893)		671 (490-829)		395 (288-525)	
IA2	1198 (872-1425)		740 (591-910)		742 (540-838)		361 (260-497)	
≥IA3	1329 (995-1622)		776 (605-944)		734 (607-961)		417 (332-557)	

TAMs: tumor-associated macrophages; CT: central tumor; IM: invasive margin; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; ADC: adenocarcinoma.

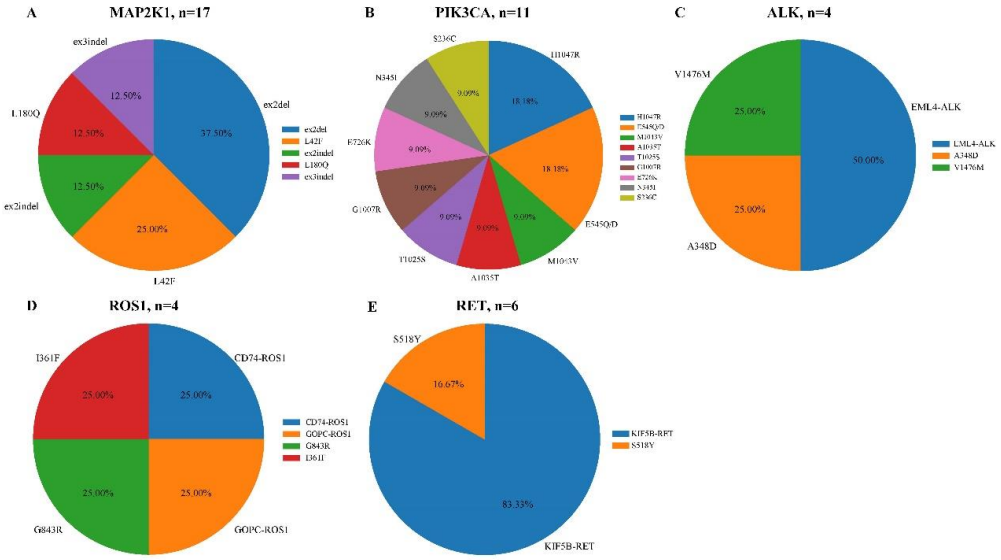
Table S8 P values of non-parametric tests between immune indexes and frequent mutations

	CD3-CT	CD3-IM	CD8-CT	CD8-IM	CD68-CT	CD68-IM	CD163-CT	CD163-IM
EGFR mutations	**0.005***	**0.001***	0.240	0.171	0.478	0.759	0.714	0.626
ERBB2 mutations	**0.040***	**0.027***	**0.061**	**0.058**	**0.016***	0.561	0.280	0.679
TP53 mutations	**0.043***	0.64	**0.002***	**0.015***	0.083	0.084	0.065	0.241
BRAF mutations	0.123	0.071	0.356	0.603	0.288	0.582	0.211	0.302
RBM10 mutations	**0.030***	**0.036***	0.271	0.774	0.056	0.937	0.273	0.483
KRAS mutations	0.715	0.305	0.430	0.284	0.833	0.388	0.594	0.450
MAP2K1 mutations	**0.007***	0.100	**0.033***	0.233	0.229	0.368	**0.017***	0.944
LRP1B mutations	0.822	0.914	0.235	0.650	0.335	0.877	0.660	0.772
MED12 mutations	0.705	0.921	0.135	0.058	0.448	0.144	0.524	0.195
PIK3CA mutations	0.948	0.197	0.881	0.885	0.372	0.892	0.996	0.647
TMB	0.118	**0.012***	0.075	0.249	0.131	0.564	0.650	0.513

CT: central tumor; IM: invasive margin; TMB: tumor mutation burden.

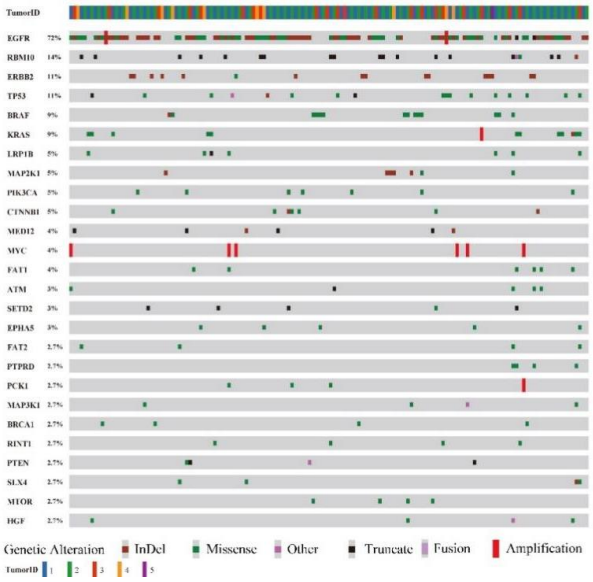
Figure S1 Mutation subtypes of other genes.

The frequency distribution of MAP2K1 (A), PIK3CA (B), ALK (C), ROS1 (D), and RET (E) mutation subtypes.



MPLCs: multiple primary lung cancers.

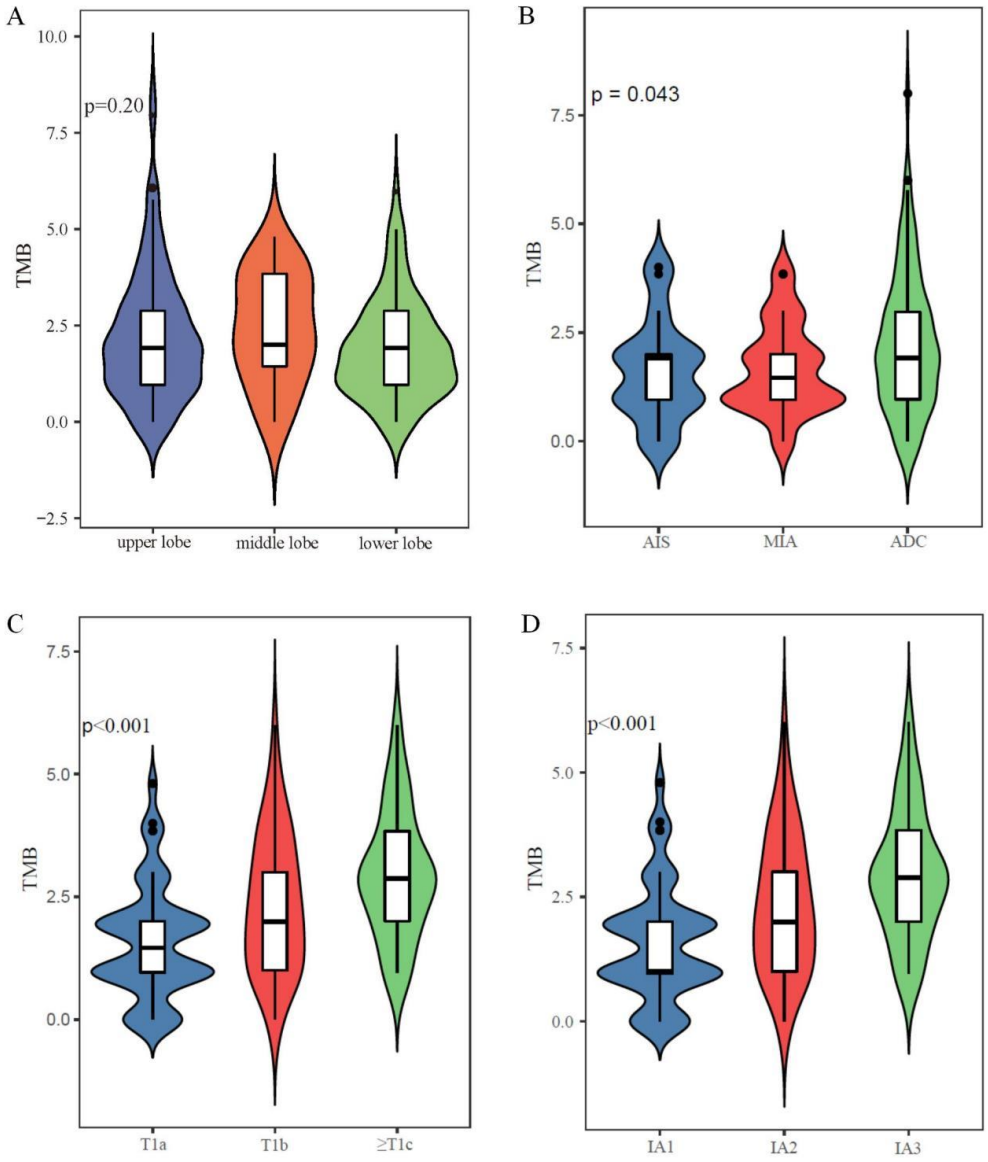
Figure S2 Genomic profile and clinicopathological features of 61 MPLCs patients sharing at least one putative driver gene.



MPLCs: multiple primary lung cancers.

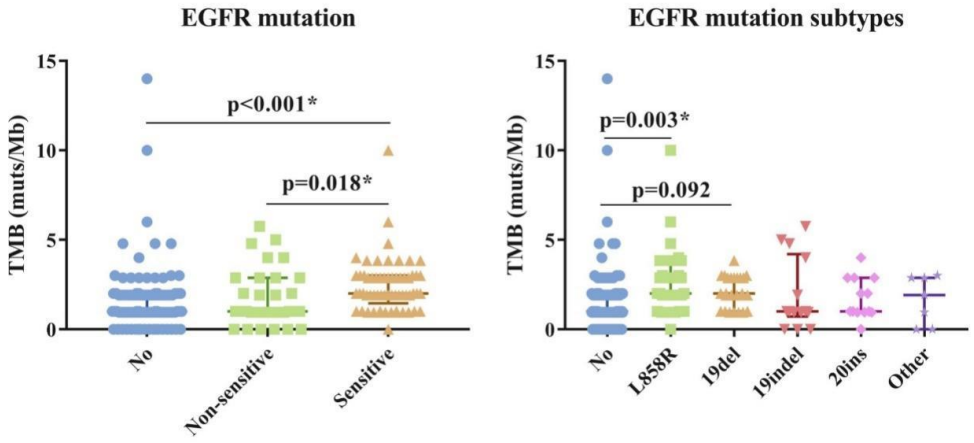
Figure S3 Association of TMB with clinicopathological features.

Association of TMB with tumor location (A), histology subtypes (B), T stages (C), and pTNM stages (D).



TMB: tumor mutation burden; AIS: adenocarcinoma in situ; MIA: minimally invasive adenocarcinoma; ADC: adenocarcinoma.

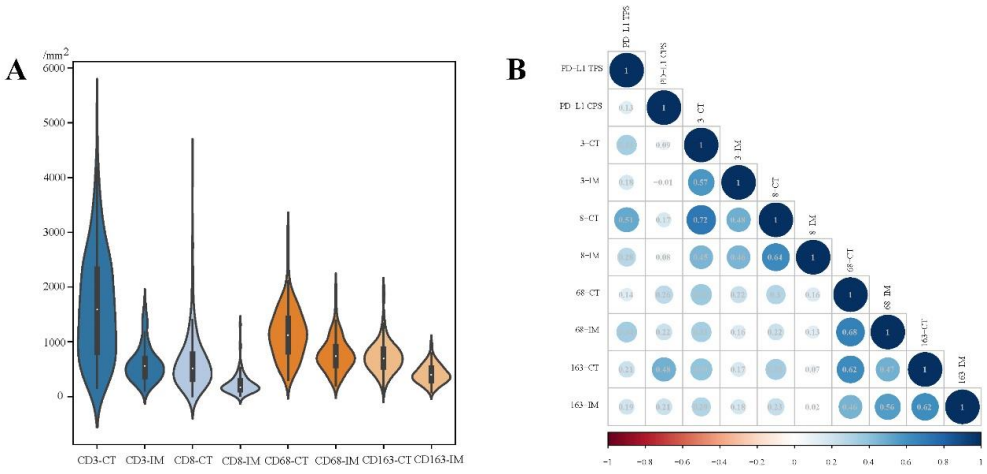
Figure S4 Association of TMB with EGFR mutations in tumors without TP53/RBM10/LRP1B mutation.



TMB: tumor mutation burden.

Figure S5 The distribution and correlation analysis of tumor-infiltrating immune cells.

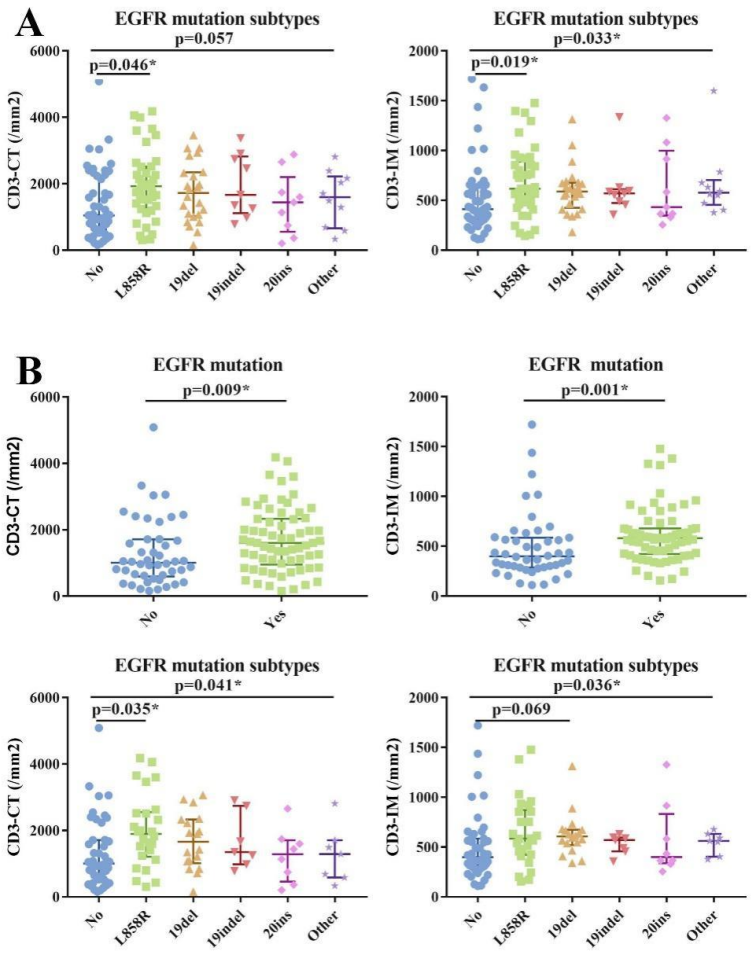
(A) The distribution of CD3+/CD8+TILs and CD68+/CD163+TAMs at CT and IM. (B) Correlation analysis of immune indicators.



PD-L1: programmed death ligand 1; TPS: tumor proportion score; CPS: combined positive score; CT: central tumor; IM: invasive margin.

Figure S6 Association of EGFR mutations with CD3+TILs in tumors with or without TP53/RBM10 mutation.

(A) Association of EGFR mutation subtypes with CD3+TILs in 143 tumors. (B) Association of EGFR mutation and its subtypes with CD3+TILs in 112 tumors without TP53/RBM10 mutation.



TILs: tumor-infiltrating lymphocytes; CT: central tumor; IM: invasive margin.

Methods S1. Sample Processing and DNA Extraction

The genomic DNA (gDNA) in formalin-fixed, paraffin-embedded (FFPE) tissue samples and peripheral blood lymphocytes (PBL) was extracted by using the QIAamp DNA FFPE Tissue & Blood Mini Kit (Qiagen, Hilden, Germany). DNA concentrations in tissue samples was measured with a Qubit fluorometer and the Qubit dsDNA HS (High Sensitivity) Assay Kit (Invitrogen, Carlsbad, CA USA), while DNA concentrations in PBL was with the Qubit 3.0 fluorometer and the Qubit dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific Inc., Carlsbad, CA, USA).

Methods S2. Target Capture and Next-Generation Sequencing (NGS)

The custom-designed biotinylated oligonucleotide probes (Roche NimbleGen, Madison, WI, USA) covering ~1.4 Mbp coding region of genomic sequence of 1021 cancer-related genes (supplementary table S1) was designed. For library construction, 1.0 μg of PBL and tissue DNA were sheared to 300-bp fragments with a Covaris S2 ultrasonicator (Covaris, Woburn, MA, USA). Libraries were constructed using the KAPA DNA Library Preparation Kit (Kapa Biosystems, Wilmington, MA, USA). Captured libraries were measured using an Agilent 2100 Bioanalyzer and an Applied Biosystems 7500 real-time PCR system (Thermo Fisher Scientific Inc., Carlsbad, CA, USA). DNA sequencing was carried out on the HiSeq3000 Sequencing System (Illumina, San Diego, CA, USA) with 2×100 bp paired-end reads.

Methods S3. Next-Generation Sequencing Analysis

From raw sequencing data, terminal adaptor sequences and low-quality reads were removed. The reads were aligned to the human genome build GRCh37 using BWA (a Burrows-Wheeler aligner)[1]. To mark PCR duplicates, Picard tools (http://broadinstitute.github.io/picard/) were used. SNVs and Indels were called using MuTect (version 1.1.4)[2] and GATK (version 3.4-46-gbc02625)[3], respectively. PBL sequencing results were used to filter germline variations. All candidate somatic mutations identified by the bioinformatics pipeline were manually reviewed in the

Integrative Genomics Viewer (IGV)[4] through assessing the quality of base calls, the mapping quality of the reads, and the overall read depth at each mutation site. Mutations were annotated to genes by ANNOVAR software[5] to identify the mutated protein-coding position and filtered intronic and silent changes. Variant allele fraction (VAF) = sequencing read count of altered alleles/(sequencing read count of reference alleles + sequencing read count of altered alleles) ×100%. For tissue, a mutation was identified according to these standards: VAF ≥ 1.0%, and at least 5 high-quality reads (Phred score ≥ 30, mapping quality ≥ 30, and without paired-end reads bias).

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