## Table S1 List of genes in the 3DMed 35-gene panel

AKT1, ALK, ARAF, BRAF, EGFR, ERBB2, ESR1, FGFR1, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MET, MTOR, NRAS, NRG1, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, RET, ROS1, CYP2C19, DPYD, TPMT, UGT1A1, BCL2L11, PDGFB

## Table S2 Antibodies used in the study

Panel	Target	Source	Dilution Opal fluorophores		Color
Panel 1	CD163	Abcam, ab182422	1:500	Opal 620	Red
	CD8	Abcam, ab178089	1:200	Opal 690	Magenta
	CD68	Abcam, ab213363	1:1,000	Opal 480	Cyan
	PD-1	CST, D4W2J, 86163S	1:200	Opal 520	Green
	PD-L1	CST, E1L3N, 13684S	1:400	Opal 570	Yellow
Panel 2	CD20	DAKO, L26, IR604	1:1	Opal 620	Green
	CD3	DAKO, A0452	1:1	Opal 690	Magenta
	CD56	Abcam, ab75813	1:1,000	Opal 480	Cyan
	CD4	Abcam, ab133616	1:100	Opal 520	Red
	FOXP3	Abcam, ab20034	1:100	Opal 570	Yellow
Detection in common	Pan-CK	Abcam, ab7753	1:100	Opal 780	White

CK, cytokeratin.



**Figure S1** Heatmap demonstrating the landscape of immune cell infiltration in NSCLC patients (n=64) with AIS (n=3), MIA (n=10), or stage I invasive cancer (n=51). (A) Heatmap showing the density of immune cell subsets in tumor and stromal areas of 64 patients. (B) Heatmap showing the percentage of immune cell subsets in tumor and stromal areas of 64 patients. TLS, tertiary lymphoid structure; NSCLC, non-small cell lung cancer; AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma.



**Figure S2** Comparison of density and percentage of immune cells in the TIME between AIS/MIA (n=13) and stage I invasive cancer patients (n=51). (A,C) Comparison of density of tumoral immune cells and stromal immune cells between AIS/MIA and stage I invasive cancer samples. (B,D) Comparison of percentage of tumoral immune cells and stromal immune cells between AIS/MIA and stage I invasive cancer samples. \*, P<0.05. AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; TIME, tumor immune microenvironment.



**Figure S3** Comparison of spatial proximity of lung cancer cells and specific immune cells in the TIME between AIS/MIA (n=13) and stage I invasive cancer patients (n=51). (A-H) Comparison of the average number of CD3<sup>+</sup> T cells (A), CD4<sup>+</sup> T cells (B), CD8<sup>+</sup> T cells (C), CD56<sup>+</sup> (NK) cells (D), CD20<sup>+</sup> B cells (E), CD68<sup>+</sup> cells (F), and M1 macrophages (G) within 30 µm of a tumor cell and tumor cells expressing PD-L1 (H) within 30 µm of a PD-1<sup>+</sup>CD8<sup>+</sup> T cell between AIS/MIA and stage I invasive cancer samples. AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; TIME, tumor immune microenvironment; NK, natural killer; ns, not significant.



**Figure S4** Comparison of density and percentage of immune cells in the TIME between patients with *EGFR*-mutated (n=46) and wild-type AIS/stage I NSCLC (n=18). (A,C) Comparison of density of tumoral immune cells and stromal immune cells between patients with *EGFR*-mutated and wild-type AIS/stage I NSCLC. (B,D) Comparison of percentage of tumoral immune cells and stromal immune cells between patients with *EGFR*-mutated and wild-type AIS/stage I NSCLC. (B,D) Comparison of percentage of tumoral immune cells and stromal immune cells between patients with *EGFR*-mutated and wild-type AIS/stage I NSCLC. (B,D) Comparison of percentage of tumoral immune cells and stromal immune cells between patients with *EGFR*-mutated and wild-type AIS/stage I NSCLC. \*, P<0.05. Mut, mutated; Wt, wild-type; TIME, tumor immune microenvironment; *EGFR*, epidermal growth factor receptor; AIS, adenocarcinoma in situ; NSCLC, non-small cell lung cancer.



**Figure S5** Comparison of spatial proximity of lung cancer cells and specific immune cells in the TIME between patients with *EGFR*mutated (n=46) and wild-type AIS/stage I NSCLC (n=18). (A-G) Comparison of the average number of CD3<sup>+</sup> T cells (A), CD8<sup>+</sup> T cells (B), CD56<sup>+</sup> (NK) cells (C), CD20<sup>+</sup> B cells (D), CD68<sup>+</sup> cells (E), and M1 macrophages (F) within 30 µm of a tumor cell and tumor cells expressing PD-L1 (G) within 30 µm of a PD-1<sup>+</sup>CD8<sup>+</sup> T cell between patients with *EGFR*-mutated and wild-type AIS/stage I NSCLC. Mut, mutated; Wt, wild-type; TIME, tumor immune microenvironment; *EGFR*, epidermal growth factor receptor; AIS, adenocarcinoma in situ; NSCLC, non-small cell lung cancer; NK, natural killer; ns, not significant.



Figure S6 Comparison of immune cells between smokers and non-smokers.



**Figure S7** Heatmap demonstrating the landscape of immune cell infiltration in NSCLC patients (n=102) with MIA or invasive cancer. (A) Heatmap showing the density of immune cells in tumor and stromal areas in all patients. (B) Heatmap showing the percentage of immune cells in tumor and stromal areas in all patients. TLS, tertiary lymphoid structure; NSCLC, non-small cell lung cancer; MIA, minimally invasive adenocarcinoma.



**Figure S8** Comparison of density and percentage of immune cells in the TIME among patients with stage I, II, and III (n=102). (A,C) Comparison of density of tumoral immune cells and stromal immune cells among patients with stage I, II, and III. (B,D) Comparison of percentage of tumoral immune cells and stromal immune cells among patients with stage I, II, and III. \*, P<0.05. TIME, tumor immune microenvironment.



**Figure S9** Comparison of spatial proximity of lung cancer cells and specific immune cells in the TIME among patients with stage I, II, and III (n=102). (A,B) Comparison of the average number of  $CD3^+T$  cells (A) and  $CD4^+T$  cells (B) within 30 µm of a tumor cell among patients with stage I, II, and III. TIME, tumor immune microenvironment; ns, not significant.



**Figure S10** Comparison of density and percentage of immune cells in the TIME between patients with or without TLS (n=102). (A,C) Comparison of density of tumoral immune cells and stromal immune cells between patients with or without TLS. (B,D) Comparison of percentage of tumoral immune cells and stromal immune cells between patients with or without TLS. \*, P<0.05. Neg, no TLSs; TLS, tertiary lymphoid structure; pos, exhibiting TLSs; TIME, tumor immune microenvironment.



**Figure S11** Comparison of spatial proximity of lung cancer cells and specific immune cells in the TIME between patients with or without TLS (n=102). Comparison of the average number of CD8<sup>+</sup> T cells (A), CD56<sup>+</sup> (NK) cells (B), CD68<sup>+</sup> cells (C), and M1 macrophages (D) within 30 µm of a tumor cell between patients with or without TLS. TLS, tertiary lymphoid structure; neg, negative; pos, positive; TIME, tumor immune microenvironment; NK, natural killer; ns, not significant.

AIS		MIA		MIA		MIA	
123420501 HE	127285501_HE	123580501_HE	125953S01_HE	135230501_HE	142733501_HE	148417501_HE	148484S01_HE
		120049501_HE	134516501_HE	145743S01_HE	147980501_HE		
Invasive stage I		Invasive stage I		Invasive stage I		Invasive stage I	
123387501_HE	123511S01_HE	124866S01_HE	125556S01_HE	127284S01_HE	128208501_HE	130202S01_HE	130303S01_HE
124633501_HE	124640501_HE	125859501_HE	126033501_HE	129589501_HE	130088501_HE	132021501_HE	132349501_HE
Invasive stage I		Invasive stage I	The Car States	Invasive stage I		Invasive stage I	
132364501_HE	132729501_НЕ	133237501_HE	134356501_HE	135997501_HE	136296501_HE	138576501_HE	140344501_HE
Invasive stage I	100114001_NL	Invasive stage I	194010001_NL	Invasive stage I	is southing	Invasive stage I	1007201_112
141469501_HE	142058S01_HE	142744501_HE	143678501_HE	145992501_HE	146357501_HE	146992501_HE	147002502_HE
142452501_HE	LAZYASSO2_HE	145243501_HE	145724501_HE	146381501_HE	146742501_HE	147760501_HE	148292501_HE
Invasive stage I		Stage II-III		Stage II-III		Stage II-III	and the second sec
148421501_HE	149728S01_HE	10782501_HE	120704S01_HE	120707501_HE	120708501_HE	120711S01_HE	120712501_HE
150327501_HE		120705501_HE	120706501_HE	120709501_HE	120710501_HE	120713501_HE	120714501_HE
Stage II-III		Stage II-III		Stage II-III		Stage II-III	TAYS' MARINE
120715S01_HE	120716501_HE	120719501_HE	120720501_HE	120723501_HE	120724501_HE	120727501_HE	120728501_HE
	See a less				W. Ferry		100 A



Figure S12 Images of HE (100×). AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; HE, hematoxylin and eosin.