

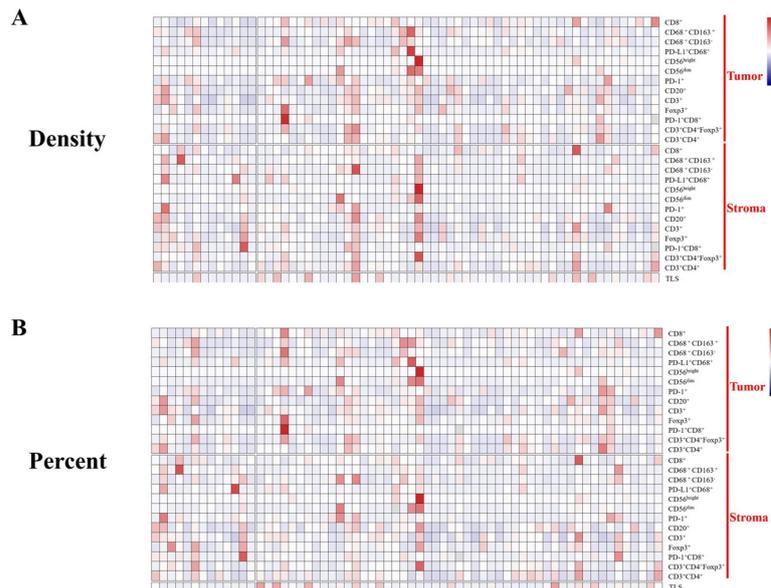
**Table S1** List of genes in the 3DMed 35-gene panel

*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*

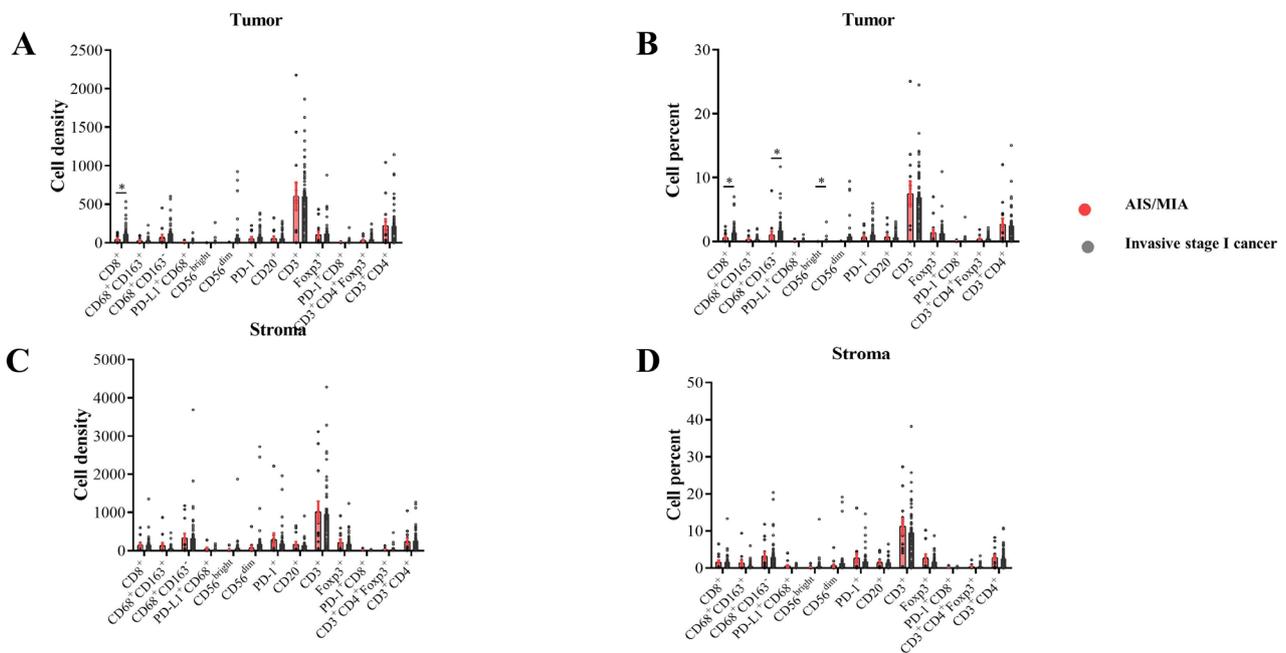
**Table S2** Antibodies used in the study

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

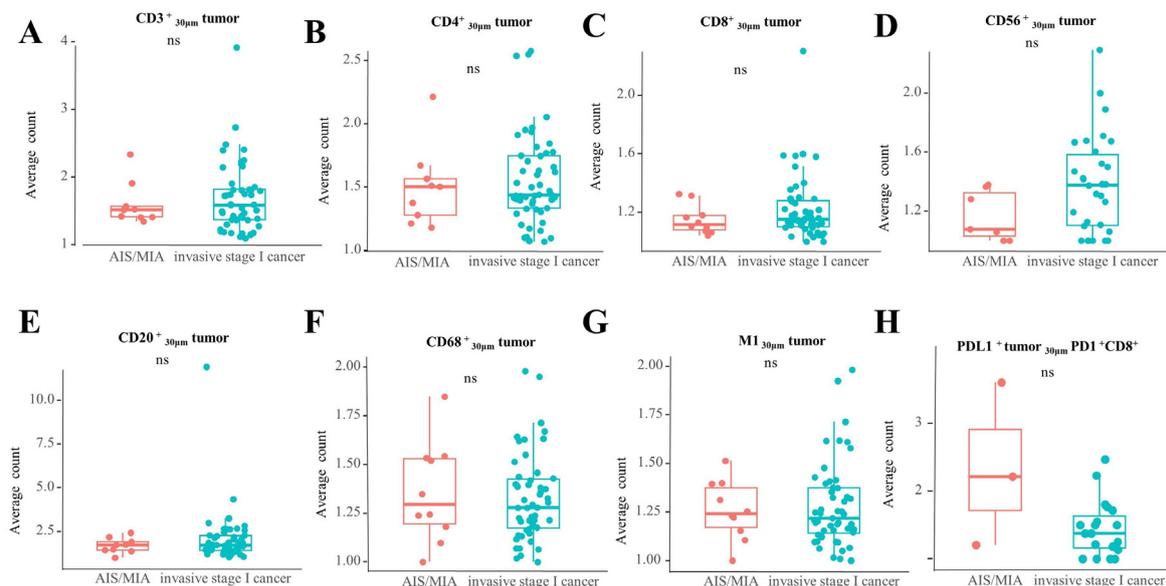
CK, cytokeratin.



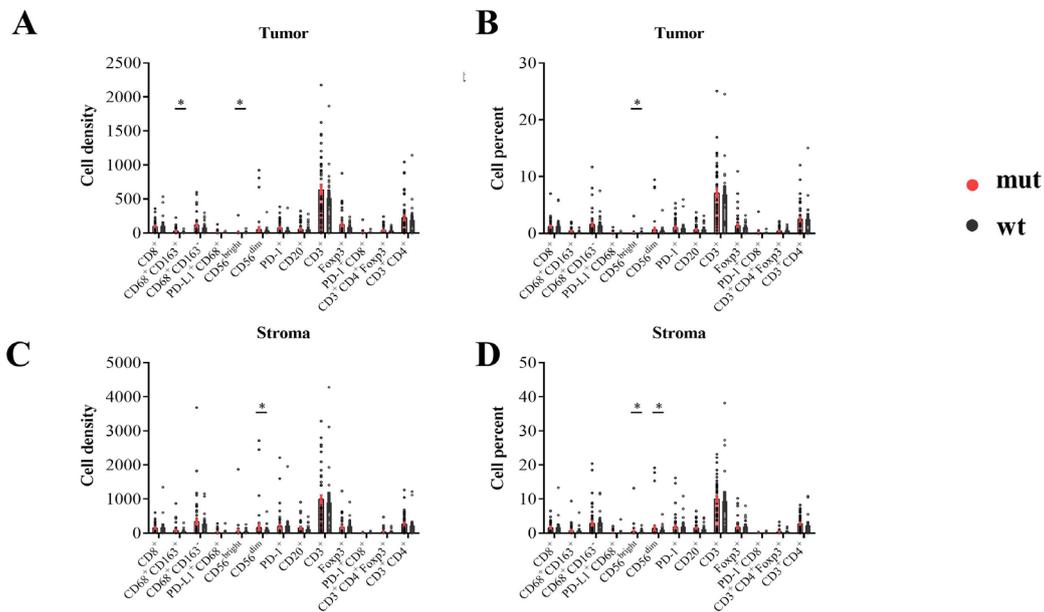
**Figure S1** Heatmap demonstrating the landscape of immune cell infiltration in NSCLC patients (n=64) with AIS (n=3), MIA (n=10), or stage I invasive cancer (n=51). (A) Heatmap showing the density of immune cell subsets in tumor and stromal areas of 64 patients. (B) Heatmap showing the percentage of 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.



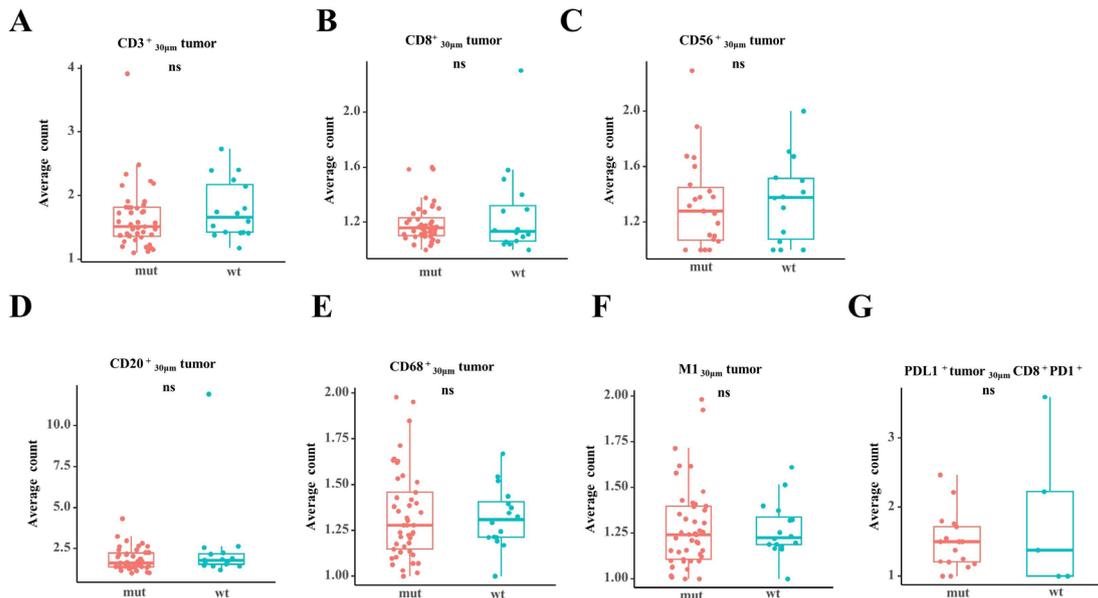
**Figure S2** Comparison of density and percentage of immune cells in the TIME between AIS/MIA (n=13) and stage I invasive cancer patients (n=51). (A,C) Comparison of density of tumoral immune cells and stromal immune cells between AIS/MIA and stage I invasive cancer samples. (B,D) Comparison of percentage 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.



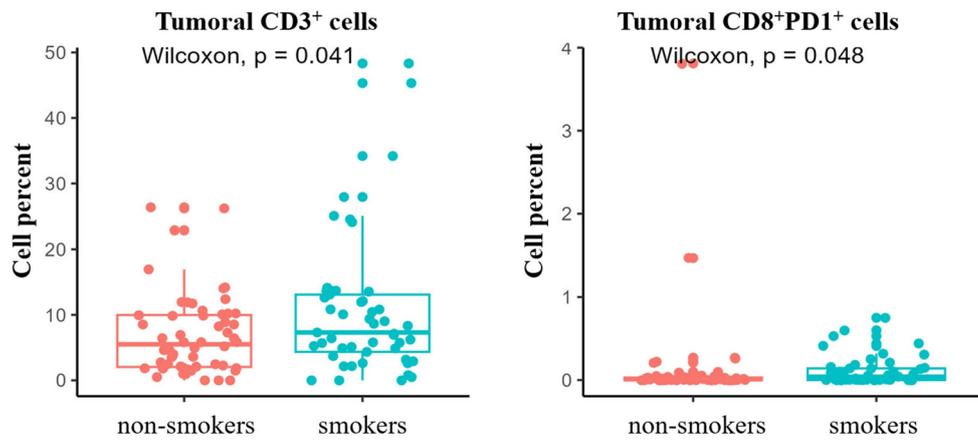
**Figure S3** Comparison of spatial proximity of lung cancer cells and specific immune cells in the TIME between AIS/MIA (n=13) and stage I invasive cancer patients (n=51). (A-H) Comparison of the average number of CD3<sup>+</sup> T cells (A), CD4<sup>+</sup> T cells (B), CD8<sup>+</sup> T cells (C), 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.



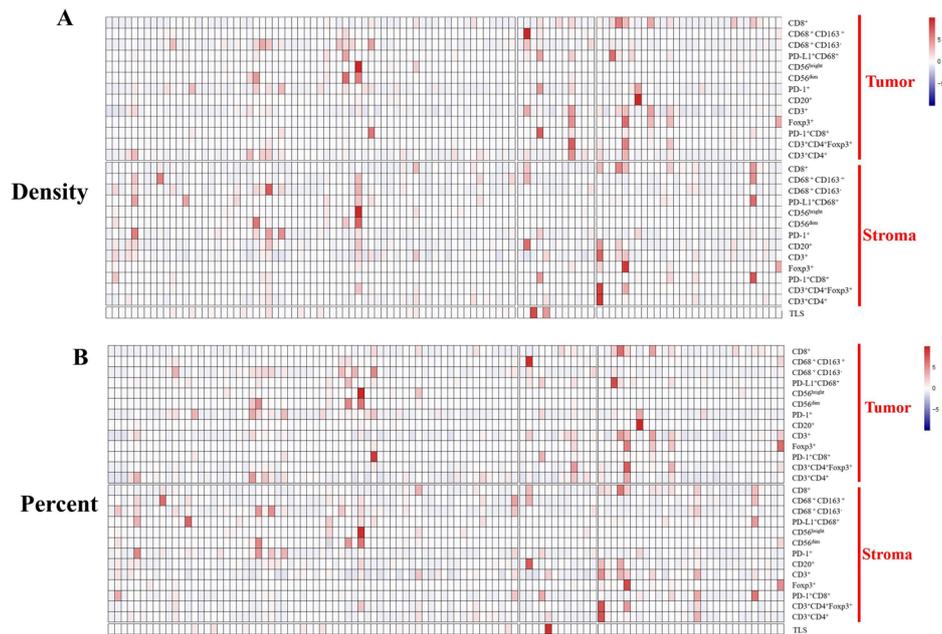
**Figure S4** Comparison of density and percentage of immune cells in the TIME between patients with *EGFR*-mutated (n=46) and wild-type AIS/stage I NSCLC (n=18). (A,C) Comparison of density of tumoral immune cells and stromal immune cells between patients with *EGFR*-mutated and wild-type AIS/stage 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. \*, 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.



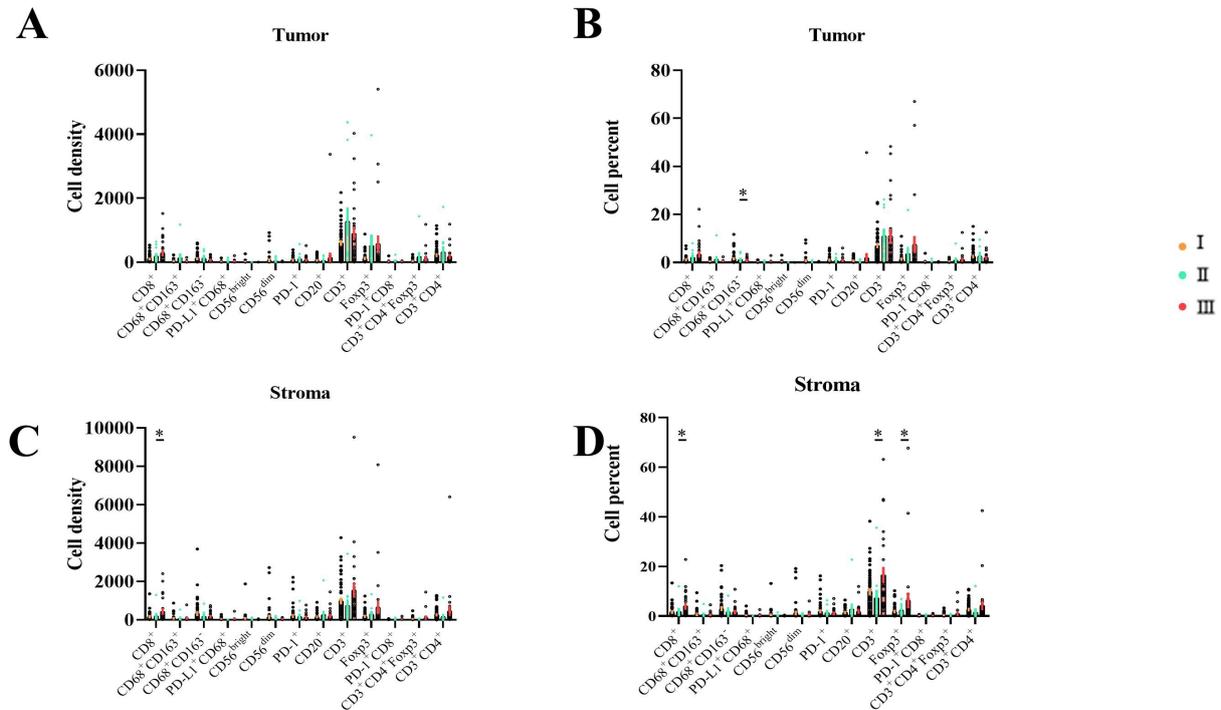
**Figure S5** Comparison of spatial proximity of lung cancer cells and specific immune cells in the TIME between patients with *EGFR*-mutated (n=46) and wild-type AIS/stage I NSCLC (n=18). (A-G) Comparison of the average number of CD3<sup>+</sup> T cells (A), CD8<sup>+</sup> T cells (B), CD56<sup>+</sup> (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.



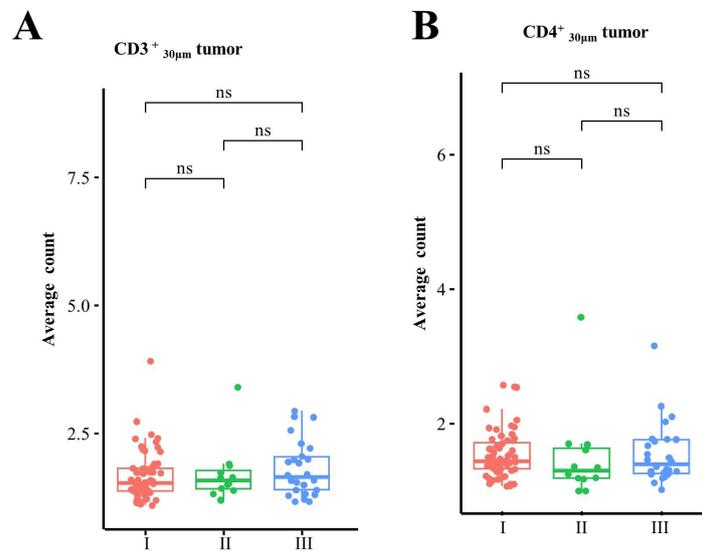
**Figure S6** Comparison of immune cells between smokers and non-smokers.



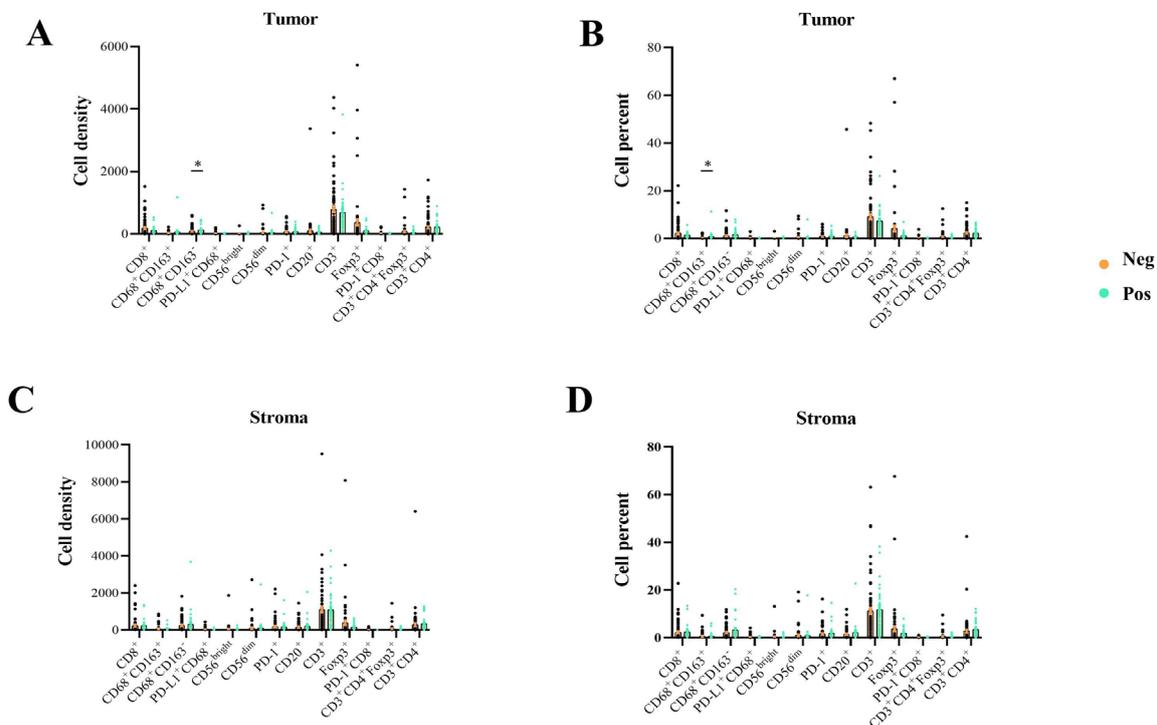
**Figure S7** Heatmap demonstrating the landscape of immune cell infiltration in NSCLC patients (n=102) with MIA or invasive cancer. (A) Heatmap showing the density of immune cells in tumor and stromal areas in all patients. (B) Heatmap showing the percentage of immune cells in tumor and stromal areas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



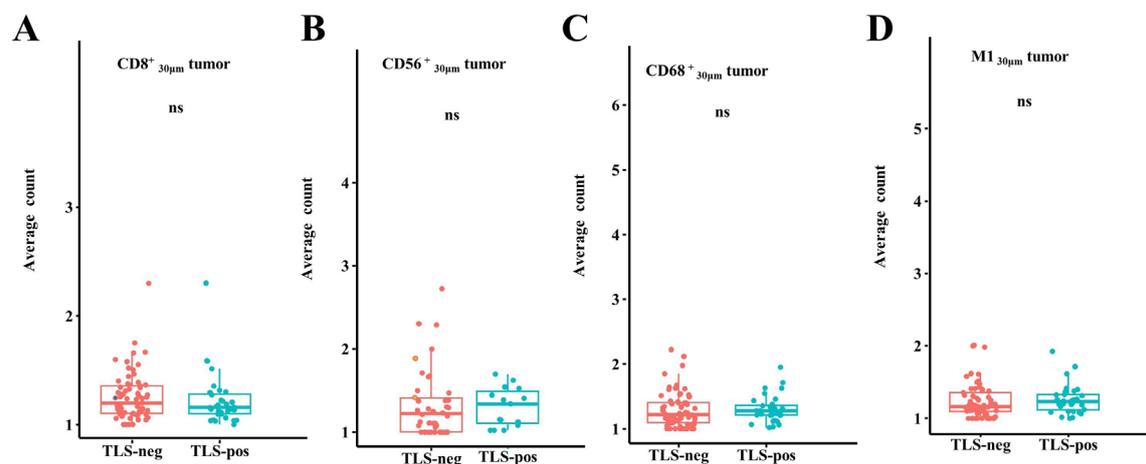
**Figure S8** Comparison of density and percentage of immune cells in the TIME among patients with stage I, II, and III (n=102). (A,C) Comparison of density of tumoral immune cells and stromal immune cells among patients with stage I, II, and III. (B,D) Comparison of percentage of tumoral immune cells 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<sup>+</sup> T cells (A) and CD4<sup>+</sup> 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.

