Supplementary Fig. S1. TIME scoring on another gene sets and associations with clinical features and experimental data in lung cancer patients. A, Another gene sets regarding T- and S-scores and TIME scores tested. B, Correlogram showing correlations between each gene signature of the TIME factors. The size of the circles indicates the degree of correlation. C, Correlogram showing correlations between each TIME score (T-score, I-score and S-score). Association of clinicopathological findings or experimental data with T-score (D) and S-score (E) are shown. TIME, tumor immune microenvironment; PET, positron emission tomography; SUVmax, maximum standardized uptake value; FAP, fibroblast activation protein. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
**Supplementary Fig. S2. Flow cytometry gating strategy.**

The gating strategy is shown including initial gates (SSC singlet, FSC singlet, and live cells) followed by CD3⁺ and CD8⁺CD39⁺CD103⁺ cells in Fig. 1e and Fig. 3j and k (A) and fibroblast activation protein (FAP)⁺, CD31⁺ and CD19⁺ cells in Fig. 1e and f (B).
Supplementary Fig. S3. Correlation of I-score and immune cell composition.

Correlation between I-score and immune cell (lymphocyte) compositions from quanTIseq (A), xCell (B) and CIBERSORT (C).
Supplementary Fig. S4. Association of S-score with the expression of genes related to immunosuppression.

The S-score was correlated with macrophage M2, stroma cell and endothelial cell abundance inferred from Xcell (A), expression of anti-inflammatory cytokine genes (TGFB1 and IL10) (B) and immune checkpoint molecule genes (CTLA4, PDCD1, HAVCR2 and LAG3) (C). *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Supplementary Fig. S5. Immune cell composition and immune-related molecule gene expression

Immune cell composition evaluated by quanTI-seq, xCell and CIBERSORT (upper) and immune-related molecule gene expression (lower) in 8 groups was shown.
Supplementary Fig. S6. The correlation of mutations with TIME score.
The T-score, I-score, and S-score are compared between EGFR mutant and wild-type tumors (A) and TP53 mutant and wild-type tumors (B). *, P < 0.05; **, P < 0.01; ***, P < 0.001; not statistically significant.
Supplementary Fig. S7

Supplementary Fig. S7. SNVs and neoantigens compared between T\textsubscript{lo} (G1-4) and T\textsubscript{hi} (G5-8).

Comparison of the numbers of SNV (A), p-neoAg (B) and e-neoAg (C) between T\textsubscript{lo} (G1-4) and T\textsubscript{hi} (G5-8) groups. *, $P < 0.05$; ns, not statistically significant.
Supplementary Fig. S8. Subgroup analyses of Fig. 3.

Comparison of the ratio of observed to expected neoantigen between T\textsuperscript{lo} (G1-4) and T\textsuperscript{hi} (G5-8) (A), and among 4 groups stratified according to low or high of I- and T-scores (T\textsuperscript{lo}/I\textsuperscript{lo}, T\textsuperscript{lo}/I\textsuperscript{hi}, T\textsuperscript{hi}/I\textsuperscript{lo}, and T\textsuperscript{hi}/I\textsuperscript{hi}) (B) in TCGA cohort.

Following analyses were performed using ACC cohort. Comparison of the ratio of p-neoantigen to missense mutation between T\textsuperscript{lo} and T\textsuperscript{hi} (C), and among 4 groups stratified according to low or high of I- and T-scores (D). Comparison of neoantigen expression ratio (e-neoAg/p-neoAg) between T\textsuperscript{lo} and T\textsuperscript{hi} (E), and among 4 groups stratified according to low or high of I- and T-scores (T\textsuperscript{lo}/I\textsuperscript{lo}, T\textsuperscript{lo}/I\textsuperscript{hi}, T\textsuperscript{hi}/I\textsuperscript{lo}, and T\textsuperscript{hi}/I\textsuperscript{hi}) (F).

The score of CYT (G), IFNG mRNA level (H), proportion of CD3\textsuperscript{+} (I) and CD8\textsuperscript{+}CD39\textsuperscript{+}CD103\textsuperscript{+} (J) cells in FTD, and specific IFN-\gamma production (K) among the 4 groups are shown. ns, not statistically significant.
Supplementary Fig. S9

Supplementary Fig. S9. IFN-γ production from cultured tumor-infiltrating lymphocytes (TILs) co-cultured autologous tumor cells.

The fold change (FC) of IFN-γ concentration in the TILs plus FTD with respect to TILs only was calculated in each well and the maximum value was plotted in Fig. 3l and Supplementary Fig. 5g. Only wells showing the production of IFN-γ more than 20 pg/ml from FTD plus TILs were evaluated. Representative data from two cases are shown. FCs are 1.62 and 14.9 in LK059 (G5) and Mchu002 (G6), respectively.
Supplementary Fig. S10.

A. Flow chart of case selection in TCGA data set. B. Correlogram showing the correlation between each gene signature in TCGA cohort. The size of the circles indicates the degree of correlation. C. Correlogram showing the correlation between each TIME score (T-score, I-score and S-score) in TCGA cohort. D. The heatmap of each gene set normalized by z-score in 990 lung cancers and 8 groups (G1-8) stratified according to high or low of T-, I- and S-scores are shown. Top bars show sex, histology, and classification of TIME. TCGA, The Cancer Genome Atlas; TIME, tumor immune microenvironment.
Supplementary Fig. S11. Subgroup analyses of Fig. 4D.
Kaplan-Meier curves for OS in T<sub>lo</sub> and T<sub>hi</sub> group between I<sub>hi</sub> and I<sub>lo</sub> (A) or between S<sub>hi</sub> and S<sub>lo</sub> (B).
Supplementary Fig. S12. Immune cell composition in the presence or absence of alterations in oncogenic signaling pathways. Immune cell compositions evaluated by QuanTSeq, xCell, and CIBERSORT between the cases with and without alterations in oncogenic signaling pathways (p53, RTK-RAS, PI3K, MYC and WNT).
Supplementary Fig. S13

**A.** Principal component analysis (PCA) of each TIME score using RNA seq data from lung cancer and other 9 different types of cancer in TCGA. **B.** 3D plot of T-, I- and S-scores is shown.

**Supplementary Fig. S13. TIME score across different cancer types from TCGA.**

A. Principal component analysis (PCA) of each TIME score using RNA seq data from lung cancer and other 9 different types of cancer in TCGA. B. 3D plot of T-, I- and S-scores is shown.
Supplementary Fig. S14

**Melanoma**

**A** Overall survival rate (Group1-8)

**B** T-score

**C** Age, Sex, T-score, I-score, S-score

**Head and neck cancer**

**A** Overall survival rate (Group1-8)

**B** T-score

**C** Age, Sex, Stage, T-score, I-score, S-score

**Pancreatic cancer**

**A** Overall survival rate (Group1-8)

**B** T-score

**C** Age, Sex, Stage, T-score, I-score, S-score

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Supplementary Fig. S14 (continued)
Supplementary Fig. S14. Prognostic impact of TIME score in 9 different cancer types from TCGA.

Kaplan-Meier curves for OS in 8 groups (A), Kaplan-Meier curves for OS in each group divided by T-score, I-score, and S-score (B), and Forrest plot to evaluate OS using multivariate analysis of the Cox proportional hazards model (C) are shown in each cancer type.
Supplementary Fig. S15. TIME scores and prediction of therapeutic efficacy of immune checkpoint inhibition. 

A, Heatmap of the 8 groups in melanoma patients treated with immune checkpoint inhibitors (ICI) classified as in Fig. 2A. B, Kaplan-Meier curves for OS of melanoma patients. C, Disease control rate in ICI therapy. D, ROC curves for TIME score, Tumor Inflammatory Signature (TIS) and CD274 expression. E, Kaplan–Meier survival curves for the ICI treatment dataset, with high versus low TIME score, TIS, or CD274 expression (using the optimal cut-off as a threshold differentiating between the high and low groups). The P values were computed via a two-sided log-rank test.