

## ONLINE SUPPLEMENTARY DATA

# Prognostic phenotypes of early-stage lung adenocarcinoma

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## SUPPLEMENTARY TABLES

**Supplementary Table E1.** Comparison of the patients selected for the present study with the originating cohort.

|                       | <b>All patients (n = 366)</b> |            | <b>Selected patients (n = 200)</b> |            | <b>Probability</b><br>$\chi^2$ test |
|-----------------------|-------------------------------|------------|------------------------------------|------------|-------------------------------------|
|                       | Patients                      | Percentage | Patients                           | Percentage |                                     |
| <b>Smoking status</b> |                               |            |                                    |            |                                     |
| Never                 | 75                            | 20         | 43                                 | 21         | 0.8850                              |
| Former                | 130                           | 36         | 67                                 | 34         |                                     |
| Current               | 161                           | 44         | 90                                 | 45         |                                     |
| <b>Age</b>            |                               |            |                                    |            |                                     |
| < 45 years            | 11                            | 3          | 7                                  | 3          | 0.9434                              |
| 45 – 65 years         | 160                           | 44         | 88                                 | 44         |                                     |
| > 65 years            | 195                           | 53         | 105                                | 53         |                                     |
| <b>Sex</b>            |                               |            |                                    |            |                                     |
| Female                | 181                           | 49         | 98                                 | 49         | 0.9301                              |
| Male                  | 185                           | 51         | 102                                | 51         |                                     |
| <b>Tumor location</b> |                               |            |                                    |            |                                     |
| Right lung            | 163                           | 45         | 98                                 | 49         | 0.1723                              |
| Left lung             | 133                           | 36         | 76                                 | 38         |                                     |
| Other                 | 70                            | 19         | 26                                 | 13         |                                     |
| <b>pTNM7 stage</b>    |                               |            |                                    |            |                                     |
| Ia                    | 78                            | 21         | 42                                 | 21         | 0.4335                              |
| Ib                    | 60                            | 16         | 43                                 | 21         |                                     |
| IIa                   | 54                            | 15         | 25                                 | 13         |                                     |
| IIb                   | 36                            | 10         | 17                                 | 9          |                                     |
| IIIa                  | 97                            | 26         | 59                                 | 29         |                                     |
| IIIb                  | 10                            | 3          | 5                                  | 3          |                                     |
| IV                    | 31                            | 9          | 9                                  | 4          |                                     |
| <b>Histology</b>      |                               |            |                                    |            |                                     |
| Lepidic               | 16                            | 4          | 7                                  | 4          | 0.9841                              |
| Acinar                | 14                            | 39         | 75                                 | 37         |                                     |
| Papillary             | 70                            | 19         | 39                                 | 19         |                                     |
| Solid                 | 126                           | 34         | 72                                 | 36         |                                     |
| Other                 | 13                            | 4          | 7                                  | 4          |                                     |

**Supplementary Table E2.** Antibodies used in the present study.

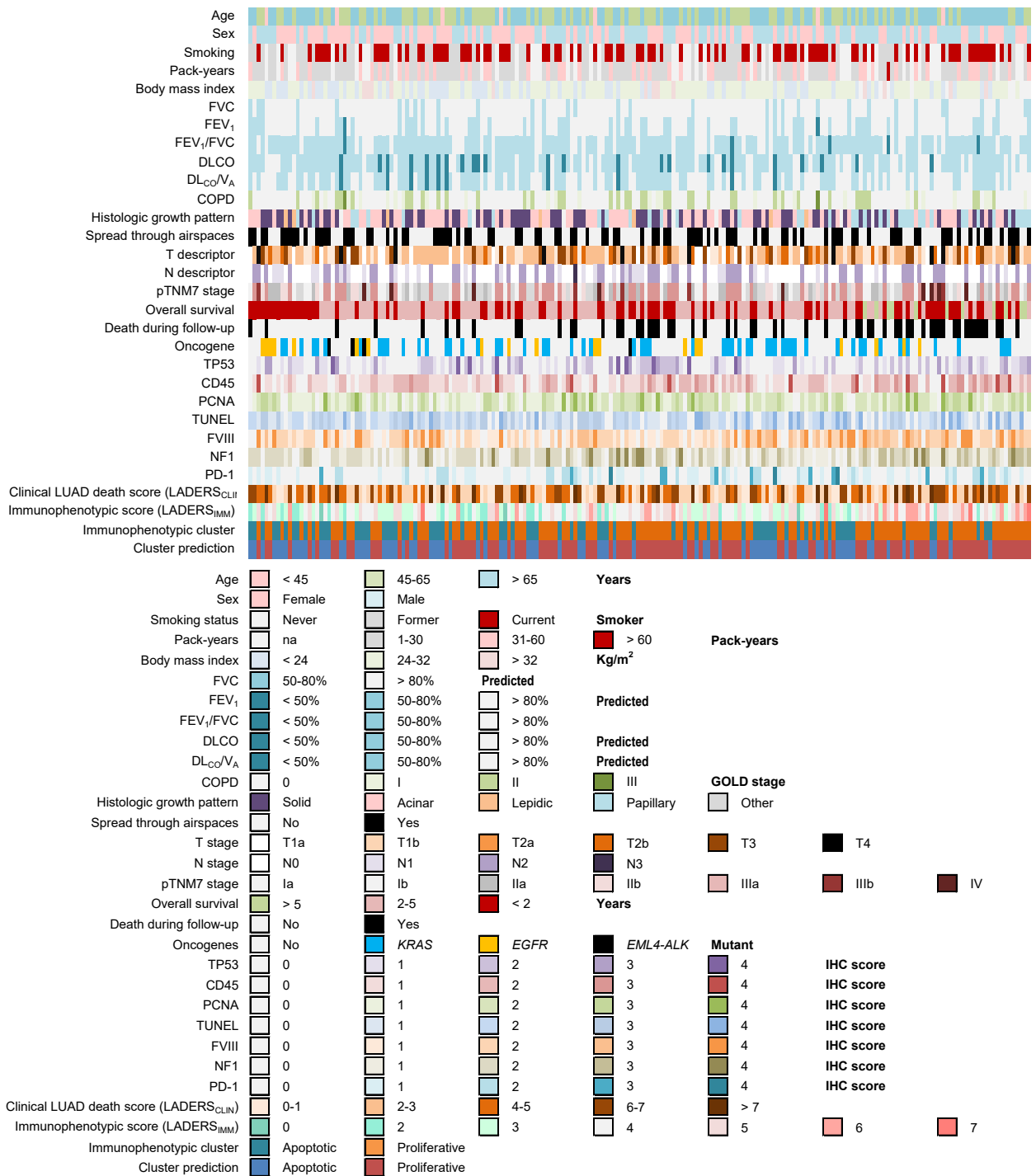
| Target                                   | Host   | Provider                             | RRID        | Dilution | Conjugate              | Incubation       |
|--|--------|--------------------------------------|-------------|----------|------------------------|------------------|
| <b>Primary</b>                           |        |                                      |             |          |                        |                  |
| Proliferating cell nuclear antigen, PCNA | Rabbit | Sigma-Aldrich, St. Louis, MO         | AB_1855078  | 1:100    | -                      | 2h00 – 37°C      |
| Tumor protein 53, TP53                   | Mouse  | Thermo Fisher, Waltham, MA           | AB_10989883 | 1:100    | -                      | overnight– 4°C   |
| Cluster of differentiation 45, CD45      | Rabbit | Thermo Fisher, Waltham, MA           | AB_2174009  | 1:250    | -                      | overnight – 4°C  |
| Anti-hemophillic factor VIII, FVIII      | Sheep  | Thermo Fisher, Waltham, MA           | AB_2262541  | 1:500    | -                      | 2h00 – 20°C      |
| Neurofibromin 1, NF1                     | Rabbit | Thermo Fisher, Waltham, MA           | AB_2149657  | 1:500    | -                      | overnight – 20°C |
| Programmed cell death-1, PD-1            | Mouse  | Elabscience, Houston, TX             | AB_2891227  | 1:100    | -                      | overnight– 4°C   |
| <b>Secondary</b>                         |        |                                      |             |          |                        |                  |
| Mouse IgG/IgM                            | Goat   | Jackson, Cambridge, UK               | AB_2338505  | 1:1000   | horseradish peroxidase | 1h30 – 20°C      |
| Rabbit IgG                               | Mouse  | Abcam, London, UK                    | AB_2650595  | 1:5000   | horseradish peroxidase | 1h30 – 20°C      |
| Goat IgG with Sheep reactivity           | Mouse  | Santa Cruz Biotechnology, Dallas, TX | AB_628490   | 1:50     | horseradish peroxidase | 1h00 – 20°C      |

RRID, research reagent identification.

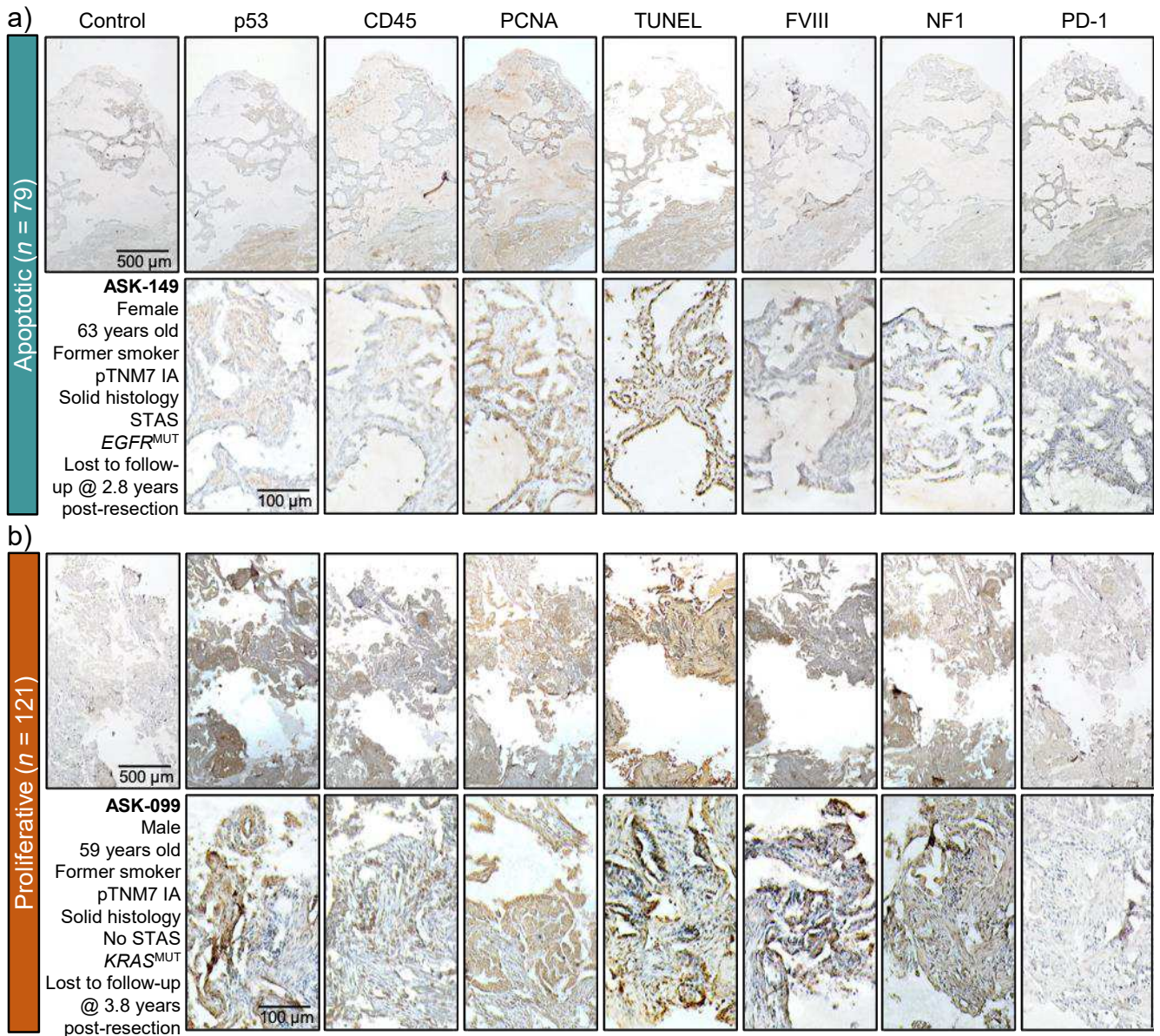
Ig, Immunoglobulin.

**Supplementary Table E3.** Raw data obtained from 200 resected lung adenocarcinoma donors.

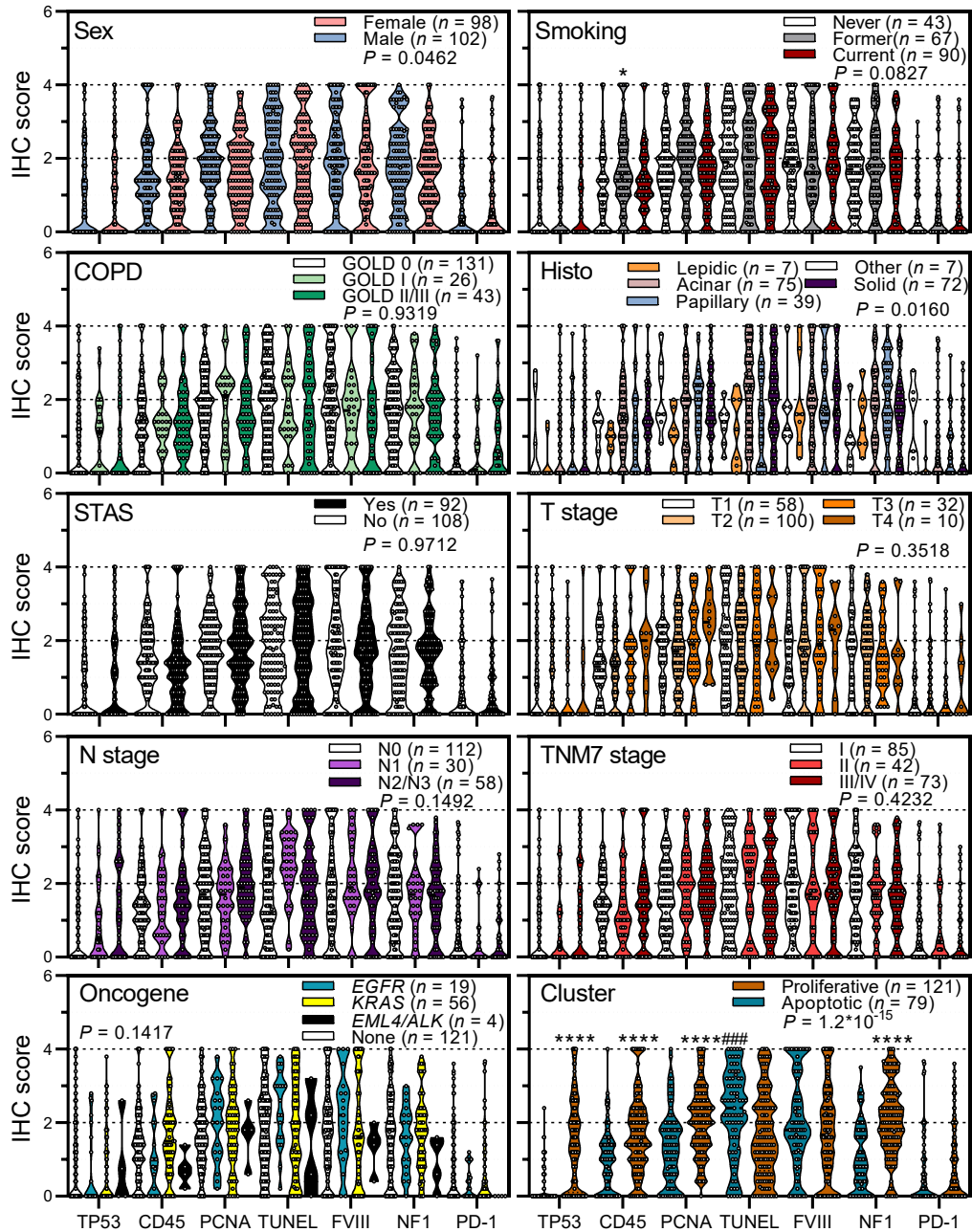
Provided as a separate \*.xlsx file. Clinical and molecular variables (color-coded columns) of 200 lung adenocarcinoma donors where each row represents a patient. pTNM7, pathological tumor-node-metastasis staging system seventh edition; STAS, spread through the airspaces; TP53, tumor protein 53; CD45, cluster of differentiation 45; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; FVIII, coagulation factor VIII; NF1, neurofibromatosis 1; PD-1, Programmed cell death protein 1; KRAS, KRAS proto-oncogene, GTPase; EGFR, epidermal growth factor receptor; numbers 0–4, semi-quantitative immunohistochemistry scores; LADERS<sub>CLIN</sub>, clinical LUAD death score; LADERS<sub>IMM</sub>, immunophenotypic score.



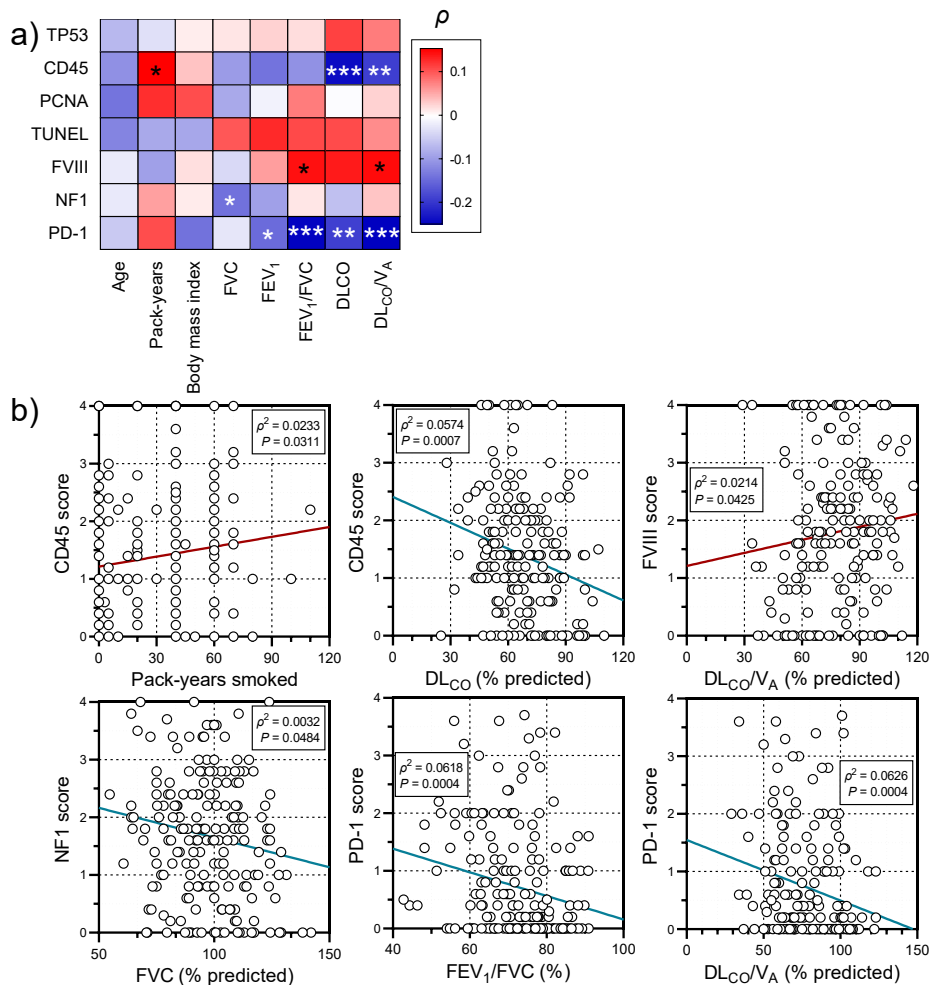
**Supplementary Figure E1. Main findings of the study.** Color-coded clinical and molecular variables (columns) of 200 lung adenocarcinoma donors where each column represents a patient. pTNM7, pathological tumour-node-metastasis staging system seventh edition; TP53, tumour protein 53; CD45, cluster of differentiation 45; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyltransferase dUTP nick-end labelling; FVIII, coagulation factor VIII; NF1, neurofibromatosis 1; PD-1, Programmed cell death protein 1; *KRAS*, *KRAS* proto-oncogene, GTPase; *EGFR*, epidermal growth factor receptor; numbers 0–4, semi-quantitative immunohistochemistry scores; LADERS<sub>CLIN</sub>, clinical LUAD death score; LADERS<sub>IMM</sub>, immunophenotypic score.



**Supplementary Figure E2. Representative patients' immunoreactivity.** Shown are representative immunohistochemistry microphotographs of one patient from each immunophenotype.

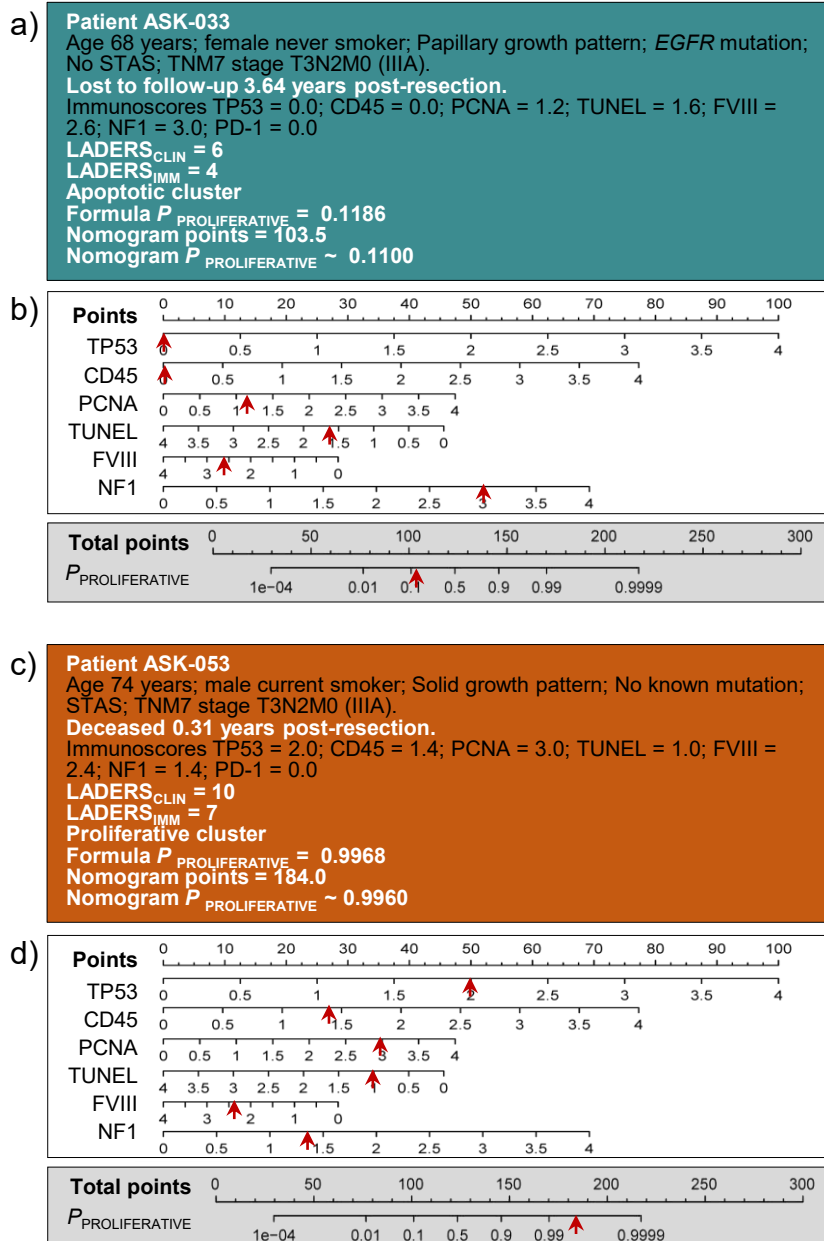


**Supplementary Figure E3. Immunoreactivity of early-stage LUAD for seven cancer hallmarks.** Immunoreactivity scores of tumour tissues of  $n = 200$  patients with LUAD for seven cancer hallmarks stratified by clinicopathologic features shows phenotypic cluster to be the main defining factor of hallmark expression. Data are shown as patient numbers ( $n$ ), raw data points (circles), rotated kernel density distributions (violins), medians (dashed lines), quartiles (dotted lines), and  $P$ , 2-way ANOVA probability values ( $P$ ). \*,  $P < 0.05$  for comparison between former and never-smokers; \*\*\*,  $P < 0.0001$  for comparison between proliferative over never-smokers; ###,  $P < 0.001$  for comparison between apoptotic over proliferative patients; ####,  $P < 0.0001$  for comparison between apoptotic over proliferative patients; Sidak's post-test. TP53, tumour protein 53; CD45, cluster of differentiation 45; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl nick-end labelling; FVIII, anti-hemophilic factor; NF1, neurofibromatosis 1; PD-1, programmed cell death-1.



**Supplementary Figure E4. Correlations between clinical variables and immunoreactivity of early-stage LUAD for seven cancer hallmarks.** Immunoreactivity scores of tumor tissues of  $n = 200$  patients with LUAD for seven cancer hallmarks were determined and were correlated with clinical and laboratory features. Data in **a)** are shown as heatmap of Spearman's correlation coefficients ( $\rho$ ). \*, \*\*, and \*\*\*,  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively, Spearman's correlation. Data in **b)** are shown as raw data points (circles), linear regression lines (coloured lines), and squared Spearman's correlation coefficients ( $\rho^2$ ) and probabilities ( $P$ ) of some representative correlations. TP53, tumour protein 53; CD45, cluster of differentiation 45; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl nick-end labelling; FVIII, anti-hemophilic factor; NF1, neurofibromatosis 1; PD-1, programmed cell death-1.





**Supplementary Figure E5. Exemplary patient cluster prediction using the formula and nomogram provided. a, c)** Clinical and immunoreactivity features, clinical (LADERS<sub>CLIN</sub>) and immunoreactivity (LADERS<sub>IMM</sub>) risk scores and probability of belonging to the proliferative phenotype ( $P_{\text{PROLIFERATIVE}}$ ) of two representative patients, as derived from the formula and the nomogram using a cut-off of  $P_{\text{PROLIFERATIVE}} > 0.538$ . **b, d)** Exemplary uses of the nomogram with red arrows in white boxes indicating individual hallmark scores corresponding to points and red arrows in grey boxes indicating total hallmark scores corresponding to  $P_{\text{PROLIFERATIVE}}$ : TP53, tumour protein 53; CD45, cluster of differentiation 45; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl nick-end labelling; FVIII, anti-hemophilic factor; NF1, neurofibromatosis 1; PD-1, programmed cell death-1; LADERS<sub>CLIN</sub>, clinical LUAD death score; LADERS<sub>IMM</sub>, immunophenotypic score..