Supplementary Figure 1. The gating strategy of cancer cells and CSCs in LNs aspirates. A) The population of CD45- cells (non-lymphoid origin). B) CD45-/CD184+/EpCAM+ gate – cancer cells (14.1% of all cells). C) Among cancer cells 29.5% cells express markers of stemness: CD44 and CD133. D) Among CD45-/CD184+/EpCAM+/CD44+/CD133+ cells 75% of cells express additional marker of stemness CD90. CSCs represent 3.5% of all cells in LN aspirate. Finally, 36.5% of CSCs are PDL-1+. E) The highest frequencies of PD-L1+ CSCs were observed in patients with confirmed mutations (p=0.465; not significant) F) The frequencies of PD-L1+ CSCs in patients with positive metastatic disease versus patients with no metastatic disease.
Supplementary Figure 2. Lymphocyte gating strategy. A-D) Distinguishing main lymphocyte subsets: T cells, CD4 T cells, CD8 T cells and Tregs. E-K) Gating CD4+ T cells positive for immunomodulatory molecules. L-R) Gating CD8+ T cells positive for immunomodulatory molecules.
Supplementary figure 3. Correlations between cancer cells, CSCs and T cell subsets in metastatic LNs. Red dots are representative for patients with confirmed mutations. A) Cancer cells are positively correlated with CD8 T cells. $r=0.6025$, $p=0.0174$. B) Cancer cells are positively correlated with Tregs $r=0.5317$, $p=0.0436$. C) Cancer cells are negatively correlated with CD4 T cells. $r=-0.5989$, $p=0.0303$. D) CSCs are negatively correlated with CD4 T cells $r=-0.6320$, $p=0.0253$. 
Supplementary figure 4. A heatmap of Pearson correlation coefficients in R values over all investigated lymphocyte subpopulations and cancer cells subpopulations (described as a percentage of all cancer cells). The percentage of cancer cells positively correlated with the percentage of Tregs ($r=0.5862$, $p=0.0362$). PD-L1+ cancer cells positively correlated with Tregs ($r=0.5885$, $p=0.0291$) and CD8+ T cells ($r=0.5267$). PD-L1+ CSCs correlated positively with the percentage of CD8+ T cells ($r=0.6225$, $p=0.0298$), Tregs ($r=0.6257$, $p=0.0286$), PD-1+ CD4+ T cells ($r=0.6747$, $p=0.0233$), and Tim3+ CD4+ T cells ($r=0.6161$, $p=0.0198$). PD-L1+ CSCs negatively correlated with CD4+ T cells and CD28+ CD4+ T cells ($r=-0.7243$, $p=0.0095$ and $r=-0.6204$, $p=0.0236$ respectively).
| metastases | Histological subtype | TNM     | Stage | Sex | Age | NGS result                                                                 | Pack years | treatment | follow-up |
|------------|-----------------------|---------|-------|-----|-----|----------------------------------------------------------------------------|------------|-----------|-----------|
| 1.0        | SQCLC                 | T4N0M0  | IIIC  | F   | 59  | 0                                                                            | 80 (ex)    | C-R       | NE        |
| 2.0        | NOS                   | T1bN1M0 | IIIB  | F   | 64  | 0                                                                            | 0          | C         | PD        |
| 3.0        | ASC                   | T2N1M0  | IIIB  | M   | 65  | 0                                                                            | 30         | C         | PR        |
| 4.0        | ADC                   | T2bN0M0 | IIA   | M   | 63  | KRAS exon2: c.38G>A, HER2 amplification                                      | 70         | none      | PR        |
| 5.0        | ADC                   | T1aN0M0 | IA    | M   | 56  | PIK3CA c.3140A>G, p.H1047R                                                  | 50         | none      | PR        |
| 6.1        | SQCLC                 | T2N2M1  | IV    | M   | 72  | 0                                                                            | 0          | C         | PD        |
| 7.1        | SQCLC                 | T4N3M0  | IIIC  | M   | 69  | 0                                                                            | 64 (ex)    | C         | PD        |
| 8.1        | ADC                   | T4N1M1  | IV    | M   | 78  | TP53 exon 5: c.473G>T; p.R158L                                             | 70         | C-R       | PD        |
| 9.1        | ADC                   | T4N1M0  | IIIA  | F   | 65  | KRAS exon 2: c.34G>T; p.G12C, TP53 exon 6: c.594del; p.G199Es*48            | 90         | C-I       | PD        |
| 10.1       | ADC                   | T3N3M0  | IIIA  | F   | 40  | HER2 exon 20: c.2313_2324dup; p.Y772_A775dup                               | 0          | C-I       | PD        |
| 11.1       | ADC                   | T4N1M0  | IIIA  | F   | 76  | KRAS exon 2 + EGFR exon 21 c.2573T>G                                       | 60         | TKI       | PD        |
| 12.1       | ADC                   | T4N2M1  | IV    | F   | 71  | KRAS exon 2: c.35G>C;                                                      | 60         | C-R       | PR        |
| 13.1       | ADC                   | T4N3M1  | IV    | M   | 71  | MET exon 14: c.3082+1_3082+3delinsTT; p.?, MET exon 19: c.3736G>A; p.D1246N.| 50 (ex)    | I         | PR        |
| 14.1       | ADC                   | T2aN2M1 | IV    | M   | 63  | EGFR ex19; c.22352249del; p.Glu746_Ala750del                                | 50         | TKI       | PR        |
| 15.1       | LCNEC                 | T1N2M0  | IIIA  | M   | 86  | TP53 exon 8: c.845G>C; p.R282P, TERT promoter: n.*1095475G>A, GNAQ exon 5: c.674C>G; p.S225 | 0          | C         | PR        |
| 16.1       | SQCLC                 | T2aN2M1a| IVA   | M   | 62  | 0                                                                            | 30         | C         | SD        |
| 17.1       | SQCLC                 | T2aN1M0 | IIIB  | M   | 77  | 0                                                                            | 80         | C         | SD        |
| 18.1       | ADC                   | T2aN3M0 | IIIB  | F   | 58  | 0                                                                            | 0          | C         | SD        |
| 19.1       | ADC                   | T3N3M0  | IIIC  | F   | 70  | HER2 exon 20: A775_G776insYVMA                                             | 50         | C         | SD        |
| 20.1       | ASC                   | T3N2M1  | IV    | M   | 73  | 0                                                                            | 48         | none      | NE        |

Supplementary Table 1. Patient characteristics. PR = partial response, SD = stable disease, PD = progressive disease, NE = not evaluable; C = Chemotherapy, TKI = tyrosine kinase inhibitor, I = immunotherapy, C-I = combined chemo- and immunotherapy, C-R = combined chemo- and radiotherapy; ADC- adenocarcinoma, SQCLC- squamous cell carcinoma, ASC- adenosquamous carcinoma, LCNEC- large cell neuroendocrine carcinoma, NOS- not otherwise specified.
| Coding sequence | CDKN2A |  |
|-----------------|--------|---|
| PTEN            |        |  |
| TP53            |        |  |
| **Mutation hotspots** |        |  |
| AKT1 (exon 3)   | IDH2 (exon: 4) |  |
| ALK (exon: 20, 22-25) | KIT (exon: 8, 9, 11, 13, 14, 17) |  |
| APC (exon 14)   | KRAS (exon: 2,3,4) |  |
| ARAF (exon 7)   | MAP2K1 (exon: 2 and 3) |  |
| BRAF (exon: 11, 15) | MET (exon: 2, 14, 19) |  |
| CTNNB1 (exon: 3, 7, 8) | MYD88 (exon 5) |  |
| EGFR (exon: 18-21) | NOTCH1 (exon: 26, 27) |  |
| HER2 (exon: 19-21) | NRAS (exon: 2,3,4) |  |
| EZH2 (exon: 16) | PDGFRA (exon: 12, 14, 18) |  |
| FBWX7 (exon: 9, 10) | PIK3CA (exon: 10 and 21) |  |
| FGFR1 (exon: 4, 7, 12) | POLD1 (exon: 12) |  |
| FGFR2 (exon: 7, 9, 12) | POLE (exon: 9, 13) |  |
| FGFR3 (exon: 7, 9) | RAF1 (exon: 7) |  |
| FOXL2 (exon 1) | RET (exon: 11, 16) |  |
| GNA11 (exon: 4, 5) | RNF43 (exon: 3, 4, 9) |  |
| GNAQ (exon: 4, 5) | ROS1 (exon: 38, 41) |  |
| GNAS (exon: 8, 9) | SMAD4 (exon: 3, 9, 12) |  |
| HRAS (exon: 2,3,4) | STK11 (exon: 4, 5, 8) |  |
| IDH1 (exon: 4) |        |  |

| In situ hybridization | NTRK1 |  |
|-----------------------|-------|---|
|                       | ROS1  |  |
|                       | RET   |  |
|                       | MET   |  |

| Immunohistochemistry  | ALK gene rearrangement |  |

Supplementary Table 2. Targeted NGS Custom made diagnostics V4 panel
### Supplementary Table 3. Antibodies used for flow cytometry identification of PD-L1+ CSCs

| Target       | Conjugate | Company      | Clone |
|--------------|-----------|--------------|-------|
| CD44         | FITC      | BD Biosciences | L178  |
| CD133        | PE        | BD Biosciences | W6B3  |
| CD184 (CXCR4)| PE-Cy7    | BD Biosciences | 1G7G5 |
| CD90         | APC       | BD Biosciences | 5E10  |
| CD326 (EpCAM)| BV785     | Biolegend     | 9G4   |
| CD45         | APCeF700  | eBioscience   | HI30  |
| PD-L1        | PeCf594   | BD Biosciences | M1H1  |

### Supplementary Table 4. Antibodies used for flow cytometry analysis of lymphocyte repertoire.

| Target       | Conjugate   | Company      | Clone |
|--------------|-------------|--------------|-------|
| CD45         | PeCf594     | BD Biosciences | HI30  |
| CD3          | APCeF700    | eBioscience   | UCHT1 |
| CD8          | AF700       | Biolegend     | SK1   |
| CD4          | BV785       | BD Biosciences | M-A251|
| CD134 (OX40) | FITC       | BD Biosciences | ACT35 |
| CD366 (TIM3) | PE         | Biolegend     | F38-2E2|
| CD223 (LAG3) | PerCp-Cy5.5| Biolegend     | C9B7W |
| CD95 (FAS)   | APC         | eBioscience   | DX2   |
| CD27         | BV421       | BD Biosciences | M-T272|
| CD28         | BV605       | BD Biosciences | CD28.2|
| CD127        | BV650       | BD Biosciences | hIL7R-M21|
| CD279 (PD-1) | BV711       | BD Biosciences | EH12.1|