Supplemental Figure Legends

Supplemental Figure S1. Number of predicted neoantigens. A) Number of total (IC50<500nM) **B)** and high affinity (IC50<50nM) predicted MHC I neoantigens in each patient. Red patient IDs = patients who have relapsed; Blue patient IDs = patients who have not relapsed. **C)** Number of total (IC50<500nM) and **D)** high affinity (IC50<50nM) predicted MHC II neoantigens in each patient. Red patient IDs = patients who have relapsed; Blue patient IDs = patients who have not relapsed.

Supplemental Figure S2. Association between expressed branch predicted neoantigens and relapse. A) Proportion of trunk (green) and branch (purple) expressed MHC I neoantigens in each patient. **B)** Proportion of branch expressed MHC I neoantigens in patients who have recurred (red) and those who have not (blue). **C)** Disease-free survival of patients with a high proportion of branch expressed MHC I neoantigens (above median, green) versus those with a low proportion of branch expressed MHC I neoantigens (below median, purple). **D)** Proportion of trunk (green) and branch (purple) expressed MHC II neoantigens in each patient. **E)** Proportion of branch expressed MHC II neoantigens in patients who have recurred (red) and those who have not (blue). **F)** Disease-free survival of patients with a high proportion of branch expressed MHC II neoantigens (above median, green) versus those with a low proportion of branch expressed MHC II neoantigens (below median, purple).

Supplemental Figure S3. Substantial ITH in the number of unique T cell rearrangements. A) Average number of unique T cell rearrangements in each tumor as defined by ImmunoSeq. **B)** Number of unique T cell rearrangements in each tumor region. **C)** Maximal difference in the number of unique T cell rearrangements defined as the difference between highest and lowest number of unique T cell rearrangements across regions within the same tumor expressed as a percent difference. Red patient IDs = patients who have relapsed; Blue patient IDs = patients who have not relapsed.

Supplemental Figure S4. Substantial ITH in tumor-enriched T cell repertoire in 8 patients whose blood samples were available. A) Average T cell density in each tumor defined by ImmunoSeq based on T cell clones significantly enriched in tumor compared to paired blood samples from the same patient (tumor-enriched T cell clones). **B)** T cell density in each tumor region based on tumor-enriched T cell clones. **C)** Maximal difference in T cell density based on tumor-enriched T cell clones, defined as the difference between highest and lowest T cell density across regions within the same tumor expressed as a percent difference. **D)** Average T cell clonality in each patient defined by ImmunoSeq based on tumor-enriched T cell clones. **E)** T cell clonality in each tumor region based on tumor-enriched T cell clones. **F)** Maximal difference in T cell clonality based on tumor-enriched T cell clones defined as the difference between highest and lowest T cell clonality across regions within the same tumor expressed as a percent difference. Red patient IDs = patients who relapsed; Blue

patient IDs = patients who have not relapsed.

Supplemental Figure S5. A minority of T cell clones are shared between different regions within a tumor. Average percentage of shared T cell clones by pairwise comparison between different regions of a tumor. Red patient IDs = patients who have relapsed; Blue patient IDs = patients who have not relapsed.

Supplemental Figure S6. The top 5 clones within each tumor are conserved across all regions of that given tumor. The top 5 most frequent T cell clones in each patient are colored in red (#1), blue (#2), green (#3), orange (#4), and purple (#5). Grey represents the added frequency of remaining clones. Red patient IDs = patients who have relapsed; Blue patient IDs = patients who have not relapsed.

Supplemental Figure S7. Tumor-enriched MOI in patients with available paired blood. A) MOI showing the overlap in the T cell repertoire between different regions of a tumor (T-T) or tumor regions and paired peripheral blood (T-B). **B)** Correlation between MOI and tumor-enriched MOI. **C)** Quantification of tumor-enriched MOI between distinct tumor regions. The color scale indicates the MOI between two tumor regions. Red patient IDs = patients who relapsed; Blue patient IDs = patients who have not relapsed.

Supplemental Figure S8. CD4-dominant T cell infiltrate in localized lung adenocarcinomas by immunohistochemical (IHC) staining. A) CD4 T cell density in each localized lung adenocarcinoma sample. Dots represent measurements in individual regions within the sample. **B)** CD8 T cell density in each localized lung adenocarcinoma sample. Dots represent measurements in individual regions within the sample. **C)** Ratio of CD4 to CD8 T cells based on average density across regions within each localized lung adenocarcinoma sample. Red patient IDs = patients who have relapsed; Blue patient IDs = patients who have not relapsed. **D)** Correlation between ITH in CD3+ T cell density by IHC and TCR density by ImmunoSeq.

Supplemental Figure S9. Correlation between predicted neoantigen burden and TCR metrics. Correlation between predicted MHC I neoantigen burden and **A)** T cell density, **B)** T cell entropy, and **C)** T cell clonality. Correlation between predicted MHC II neoantigen burden and **D)** T cell density, **E)** T cell entropy, and **F)** T cell clonality.

Supplemental Figure S10. Association between intratumor TCR heterogeneity and patient relapse. A) Percent difference in T cell clonality in patients who recurred (red) and those who have not (blue) and disease-free survival based on high (above median, green) versus low (below median, purple) difference in clonality. **B)** Percent difference in CD3 density by IHC in patients who recurred (red) and those who have not (blue) and disease-free survival based on high (above median, green) versus low (below median, purple) difference in CD3 density. **C)** Percent difference in TCR density by ImmunoSeq

in patients who recurred (red) and those who have not (blue) and disease-free survival based on high (above median, green) versus low (below median, purple) difference in TCR density. **D)** Percent difference in tumor-enriched T cell density in patients who recurred (red) and those who have not (blue) and disease-free survival based on high (above median, green) versus low (below median, purple) difference in tumor-enriched T cell density in the 8 patients whose blood samples were available. **E)** Percent difference in tumor-enriched T cell clonality in patients who recurred (red) and those who have not (blue) and disease-free survival based on high (above median, green) versus low (below median, purple) difference in tumor-enriched T cell clonality in the 8 patients whose blood samples were available. **F)** Tumor-enriched MOI in patients who recurred (red) and those who have not (blue) and disease-free survival based on high (above median, green) versus low (below median, purple) tumor-enriched MOI in the 8 patients whose blood samples were available.

Supplemental Figure S11. Follow up time (months) in patients who recurred and those who have not. n.s., not significant.