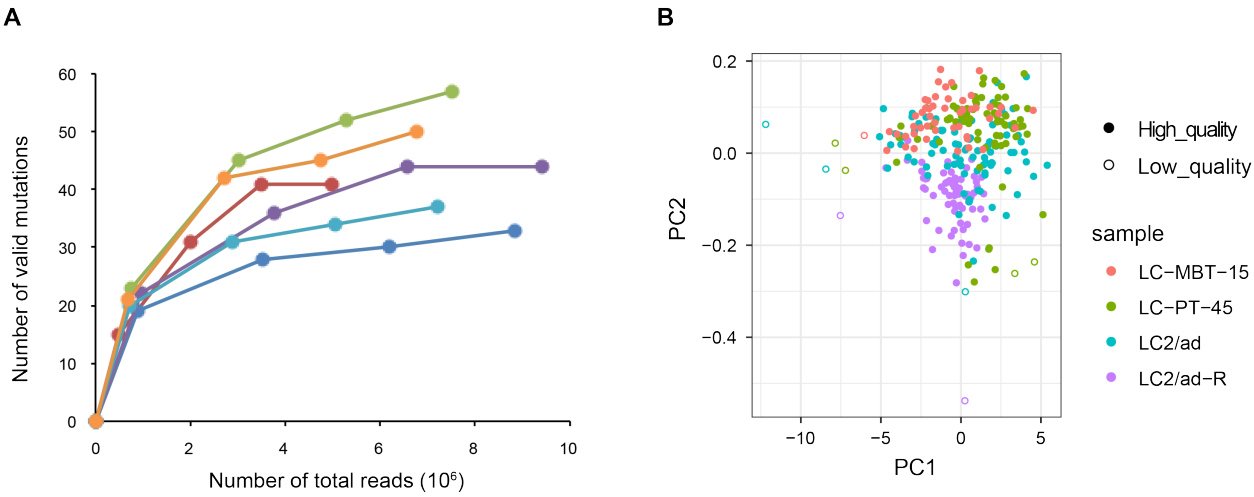
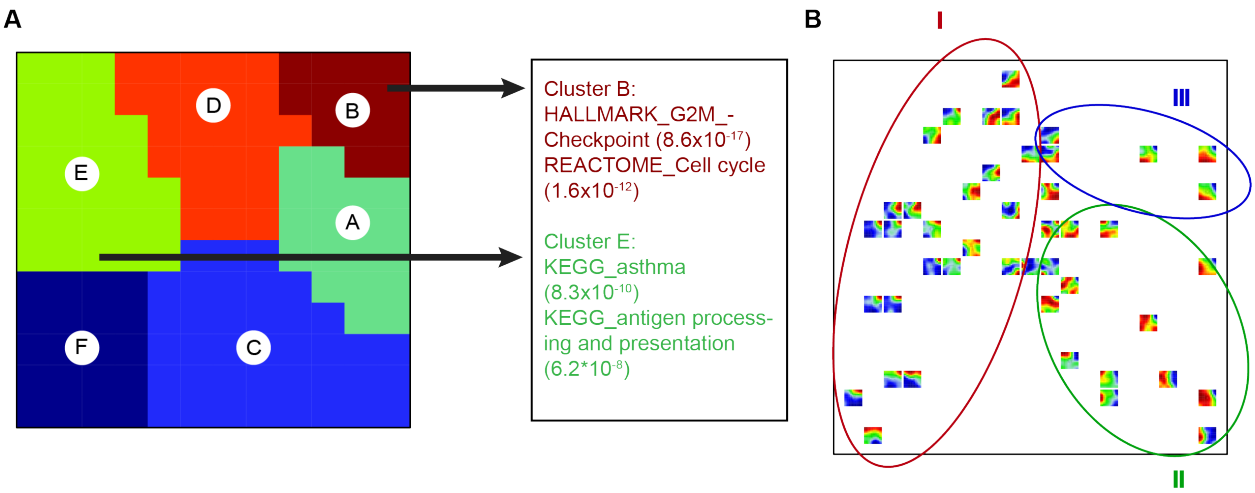
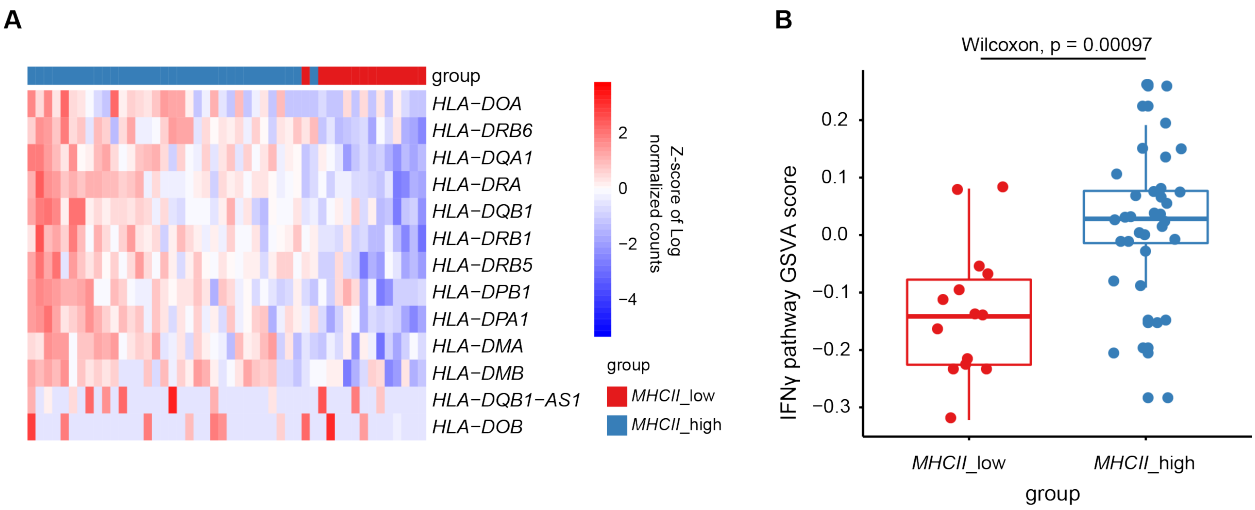


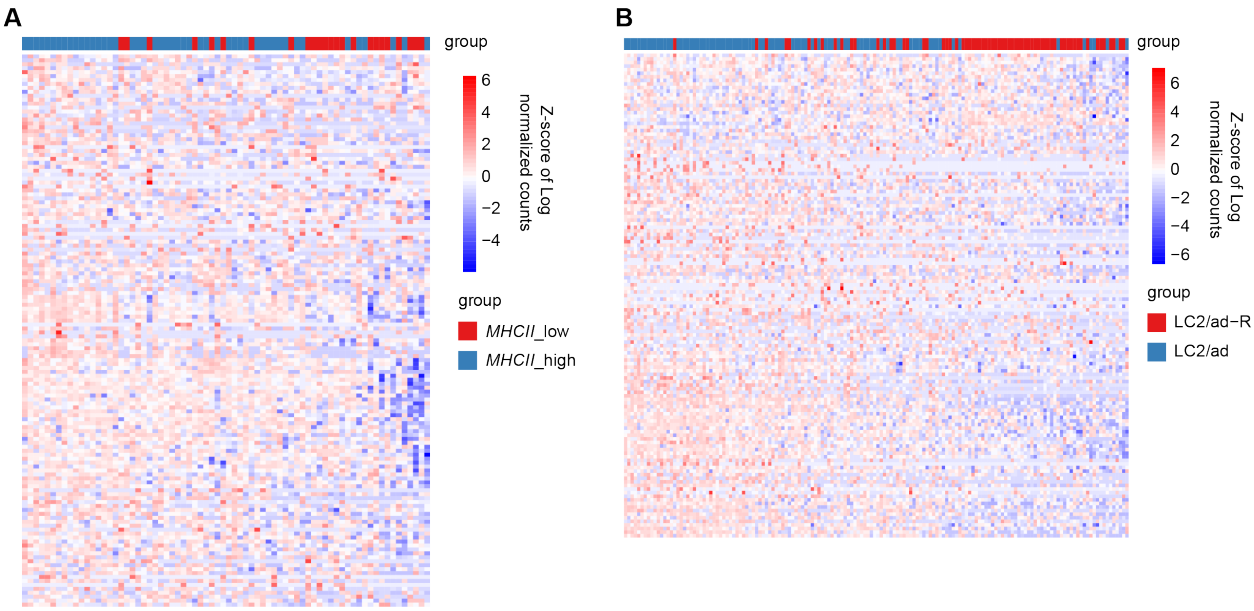
Supplementary Fig. S1. Quality control of single cell RNA-seq data. A. Rarefaction analysis on number of sequencing reads needed to saturate number of valid somatic mutations (6 single cells were shown here). B.Low quality cells identified in four data sets used in our study accounts for a small percentage. Low quality cells are identified as outliers in PCA plot, using R package “mvoutlier”.



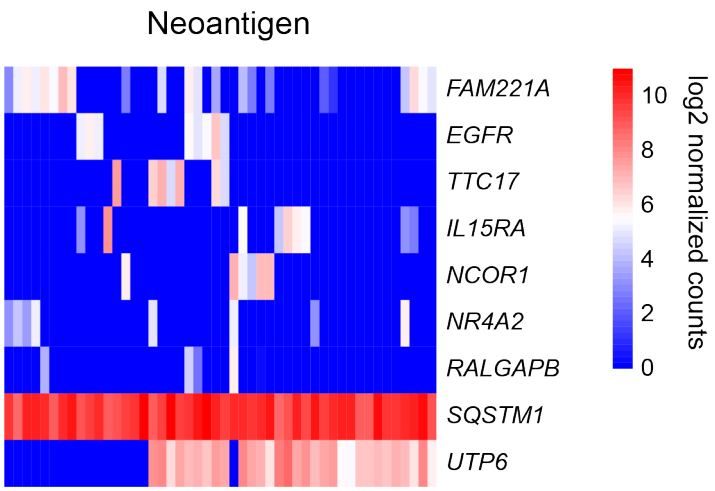
Supplementary Fig. S2. **Expression of cell cycle genes and *MHCII* genes in single cancer cells from LC-MBT-15.** A. Gene pathway clusters from metagene analysis of single cells from PDX LC-MBT-15. K-means clustering of metagenes on SOMs into 6 clusters. Hypergeometric test was then performed on each cluster of metagenes to determine enrichment of canonical pathways. Cluster B is enriched for cell cycle pathways, while cluster E is enriched for antigen presentation pathways. P-values in the bracket are false discovery rate corrected. B. Second level clustering of SOMs for 49 single cells from PDX LC-MBT-15. Same as Figure 2, Co-expression of cell cycle pathway and antigen presentation pathway further separate cells into three major groups: cell cycle pathway high (I), antigen presentation pathway high (II), and both pathways low (III).



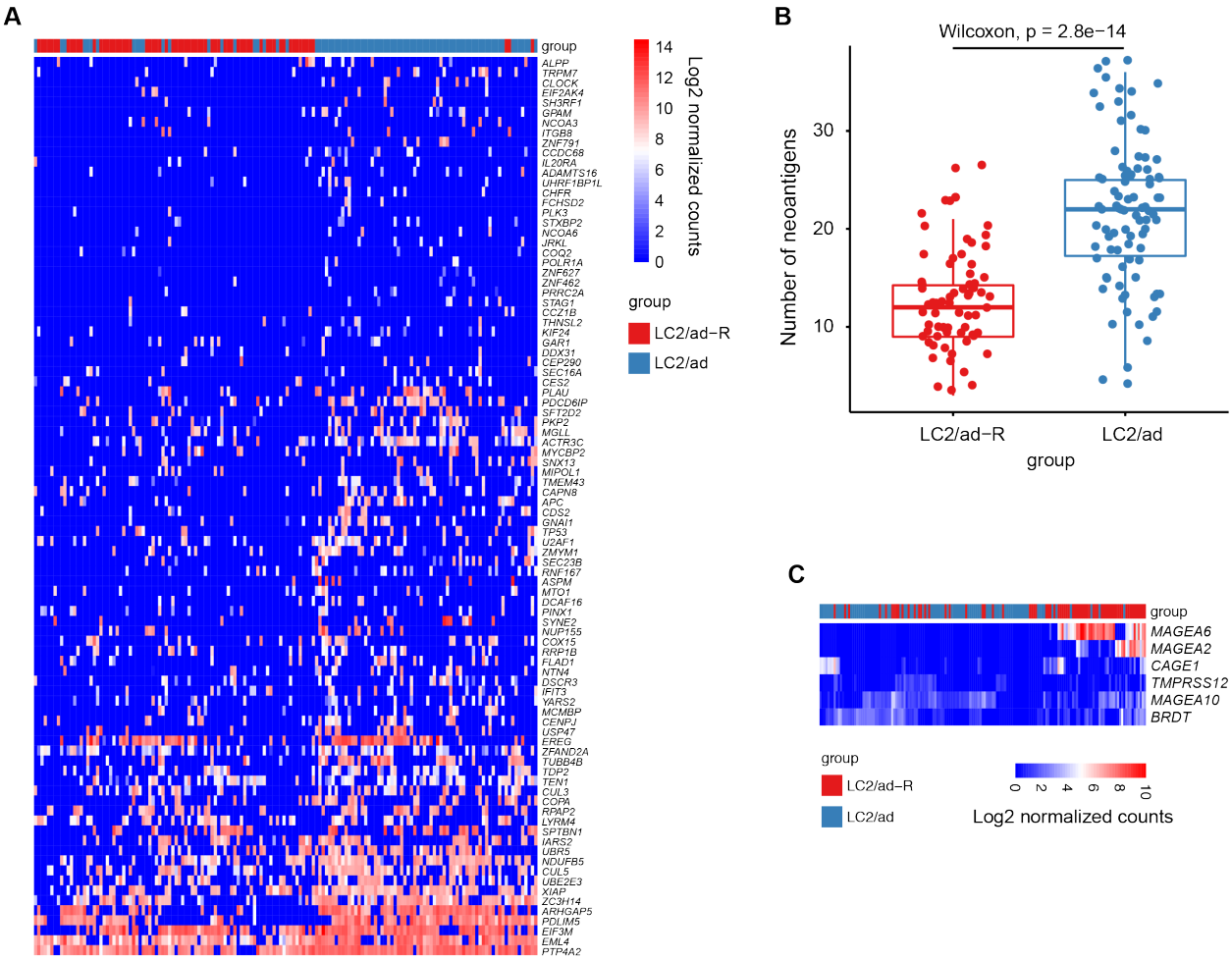
Supplementary Fig. S3. Co-expression of IFN γ signaling pathways in LC-MBT-15. A. Heatmap of *MHCII* genes. K-means clustering was performed to cluster all single cells into two clusters, *MHCII* low and *MHCII* high. B. Gene Set Variation Analysis (GSVA) scores of IFN γ signaling pathway were calculated for each individual cell. Non-parametric Wilcoxon test were performed between *MHCII* low and *MHCII* high groups.



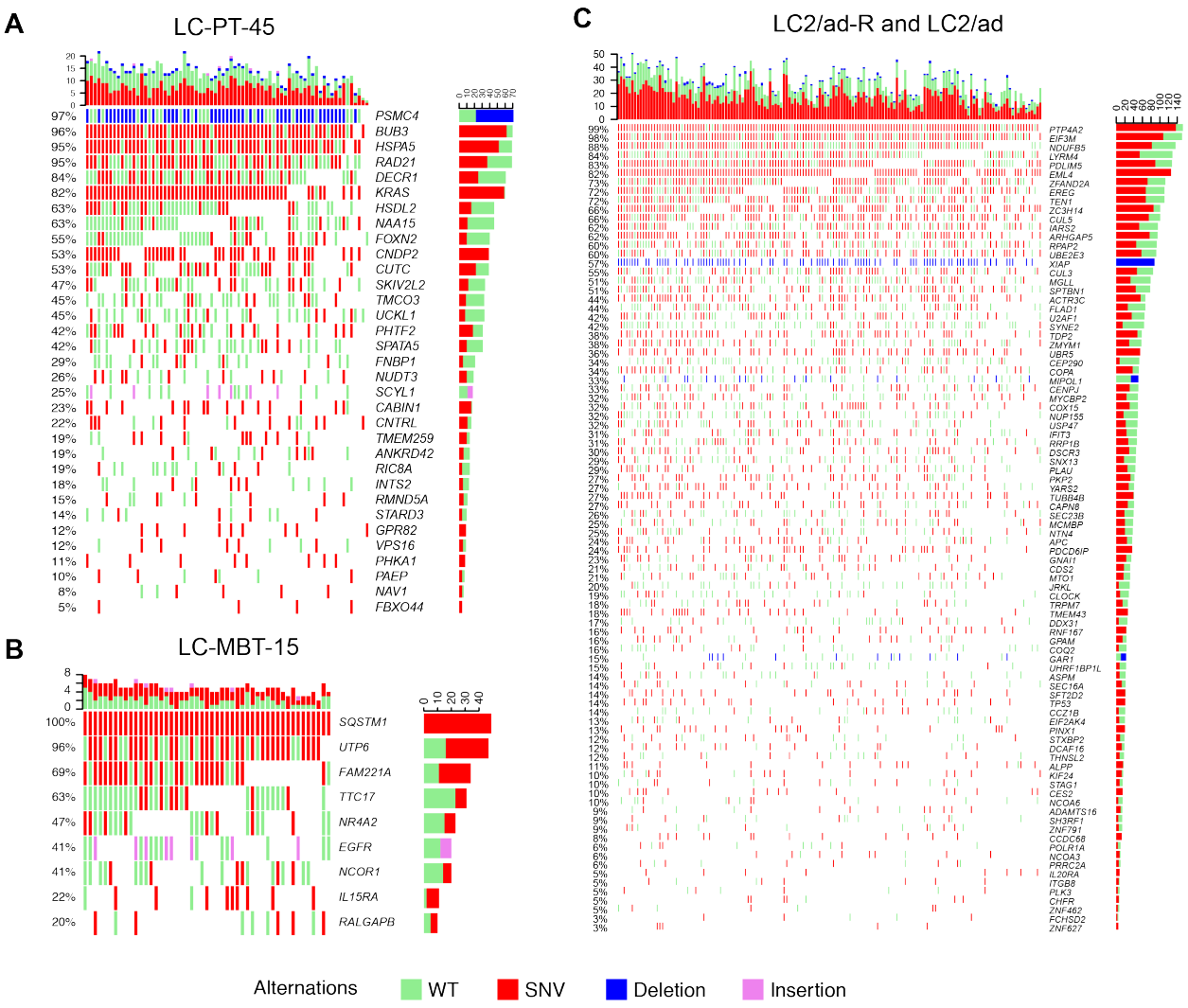
Supplementary Fig. S4. Co-expression of IFN γ signal pathways. A. Heatmap of genes in IFN γ signaling pathway, which are curated from gene ontology (GO) and REACTOME databases, among single cells from LC-PT-45 patient. B. Heatmap of genes in IFN γ signaling pathway among LC2/ad-R and LC2/ad cell lines.



Supplementary Fig. S5. Heterogeneous expression of neoantigens in single cancer cells from LC-MBT-15. Heatmap showing the expression of neoantigens in single cells from LC-MBT-15. Log2 normalized counts being 0 represents either no expression or no somatic mutation detected. Cells were included if a neoantigen is detected regardless of its corresponding wildtype antigen detection status. Only neoantigens detected in more than three cells were selected.



Supplementary Fig. S6. **Heterogeneous expression of neoantigens and CTAs in single cancer cells from LC2/ad-R and LC2/ad cell lines.** A. Heatmap showing the expression of neoantigens in single cells from LC2/ad-R and LC2/ad cell lines. Log2 normalized counts being 0 represents either no expression or no somatic mutation detected. Only neoantigens detected in more than three cells were selected. B. Boxplot of number of neoantigens in each individual cells. Non-parametric Wilcoxon test were performed between LC2/ad-R and LC2/ad cell lines. C. Heatmap showing the expression of CTAs in single cells from LC2/ad-R and LC2/ad cell lines. CTAs whose expressions are transcriptionally silent in normal non-germline tissues based on Genotype-Tissue Expression (GTEx) data and with normalized counts > 0 in more than 2 cells were selected.



Supplementary Fig. S7 OncoPrint profiles of neoantigen-associated SNV and INDEL in high-quality single cells from (A) LC-PT-45; (B) LC-MBT-15; and (C) LC2/ad-R and LC2/ad cell lines. Cells used as in Figure 4 and Supplementary Fig. S5, S6.