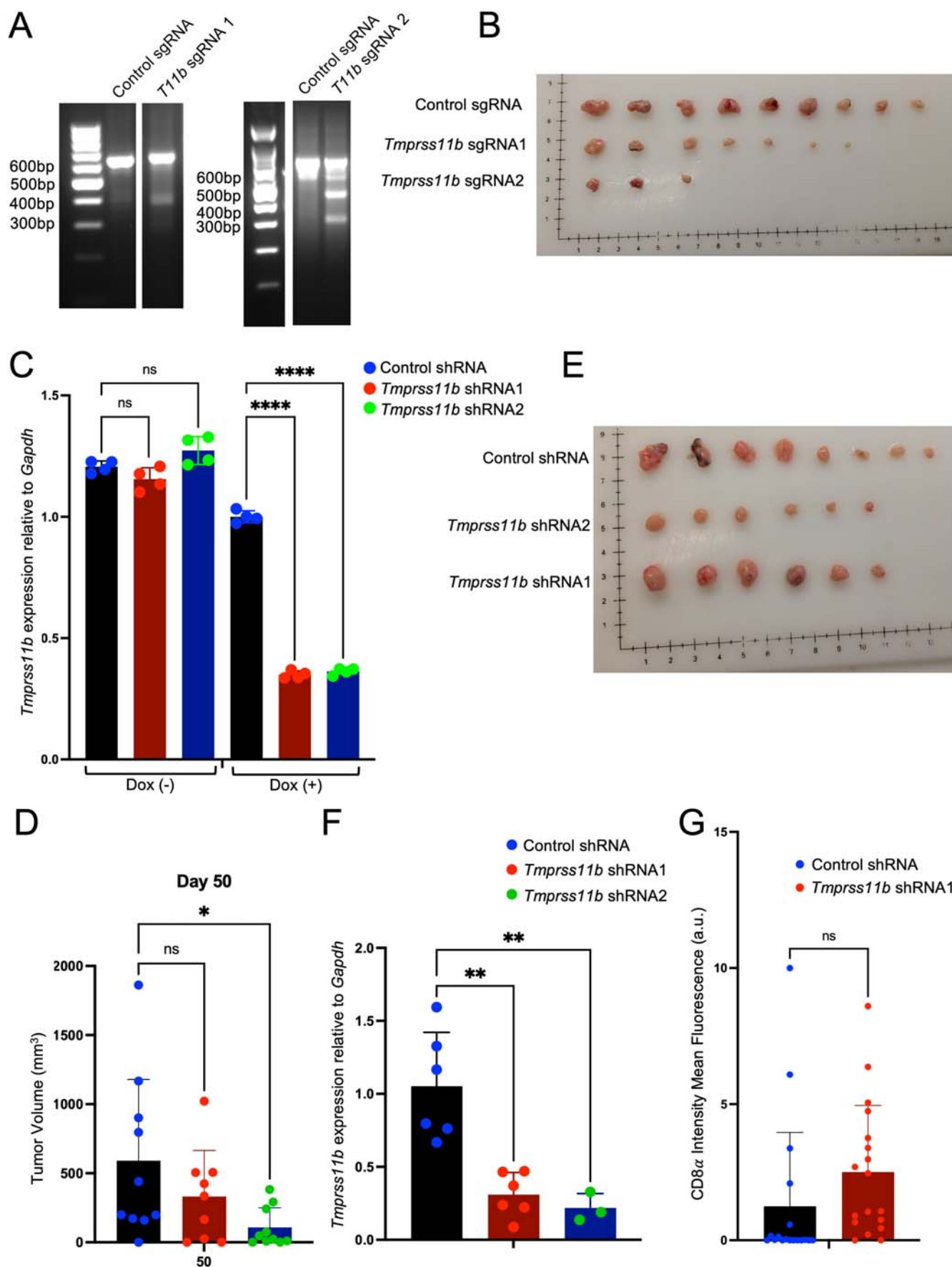
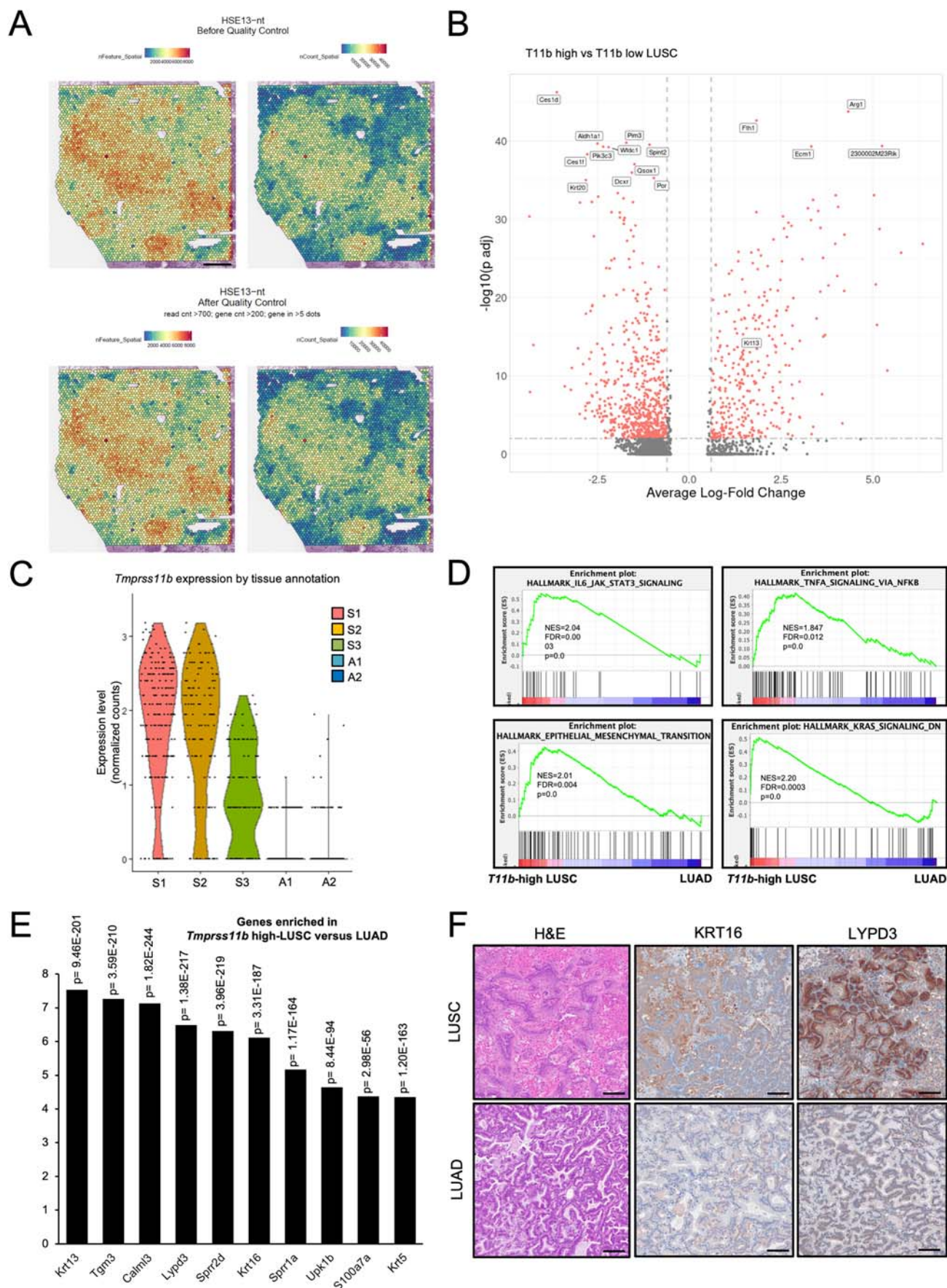


## Expanded View Figures

### Figure EV1. *Tmprss11b* depletion inhibits tumor burden in a syngeneic mouse model of LUSC.

(A) Agarose gel electrophoresis images of PCR amplified products from the Surveyor assay performed on the genomic DNA isolated from KLN205 cells expressing control or *Tmprss11b* sg1 or *Tmprss11b* sg2. The assay was repeated two times with different surveyor primers to confirm the observations (biological replicates). (B) Image showing the resected tumors at endpoint from the syngeneic experiment in Fig. 1C. (C) qRT-PCR analysis of *Tmprss11b* mRNA in KLN205 cells expressing doxycycline-inducible control shRNA or two independent shRNA sequences targeting *Tmprss11b*. Brown-Forsythe and Welch ANOVA test with Dunnett's T3 multiple comparisons test was used for the statistical analysis, \*\*\*\* $P < 0.0001$ . Plot represents mean  $\pm$  SD;  $n = 4$  per group (technical replicates). Experiment was repeated two times for confirmation (biological replicates). (D) Quantification of tumor volumes of KLN205 cells expressing doxycycline-inducible control or *Tmprss11b* shRNA on day 50 (terminal measurement) post injection in syngeneic DBA/2 wild-type mice ( $n = 10$  control shRNA mice;  $n = 8$  *Tmprss11b* sh1 mice;  $n = 10$  *Tmprss11b* sh2 mice, biological replicates). Ordinary one-way ANOVA with Dunnett's multiple comparisons test was used for the statistical analysis,  $P = 0.0235$ . Plot represents mean  $\pm$  SD. (E) Image showing the resected tumors from the syngeneic experiment in Fig. 1D. (F) qRT-PCR analysis of *Tmprss11b* mRNA in the resected KLN205 tumors from D). Brown-Forsythe and Welch ANOVA test with Dunnett's T3 multiple comparisons test was used for the statistical analysis,  $P = 0.0055$  (*T11b* shRNA1),  $P = 0.0041$  (*T11b* shRNA2). Plot represents mean  $\pm$  SD;  $n = 3$  technical replicates,  $n = 3$ –6 tumors per group, biological replicates. (G) Quantification of CD8a staining from fluorescent immunohistochemistry (IHC-F) performed on KLN205 tumor sections. An unpaired t test with Welch's correction was used for the statistical analysis ( $n = 10$ –14 fields per tumor section, 3 tumors per group, biological replicates). Plot represents mean  $\pm$  SD. Source data are available online for this figure.

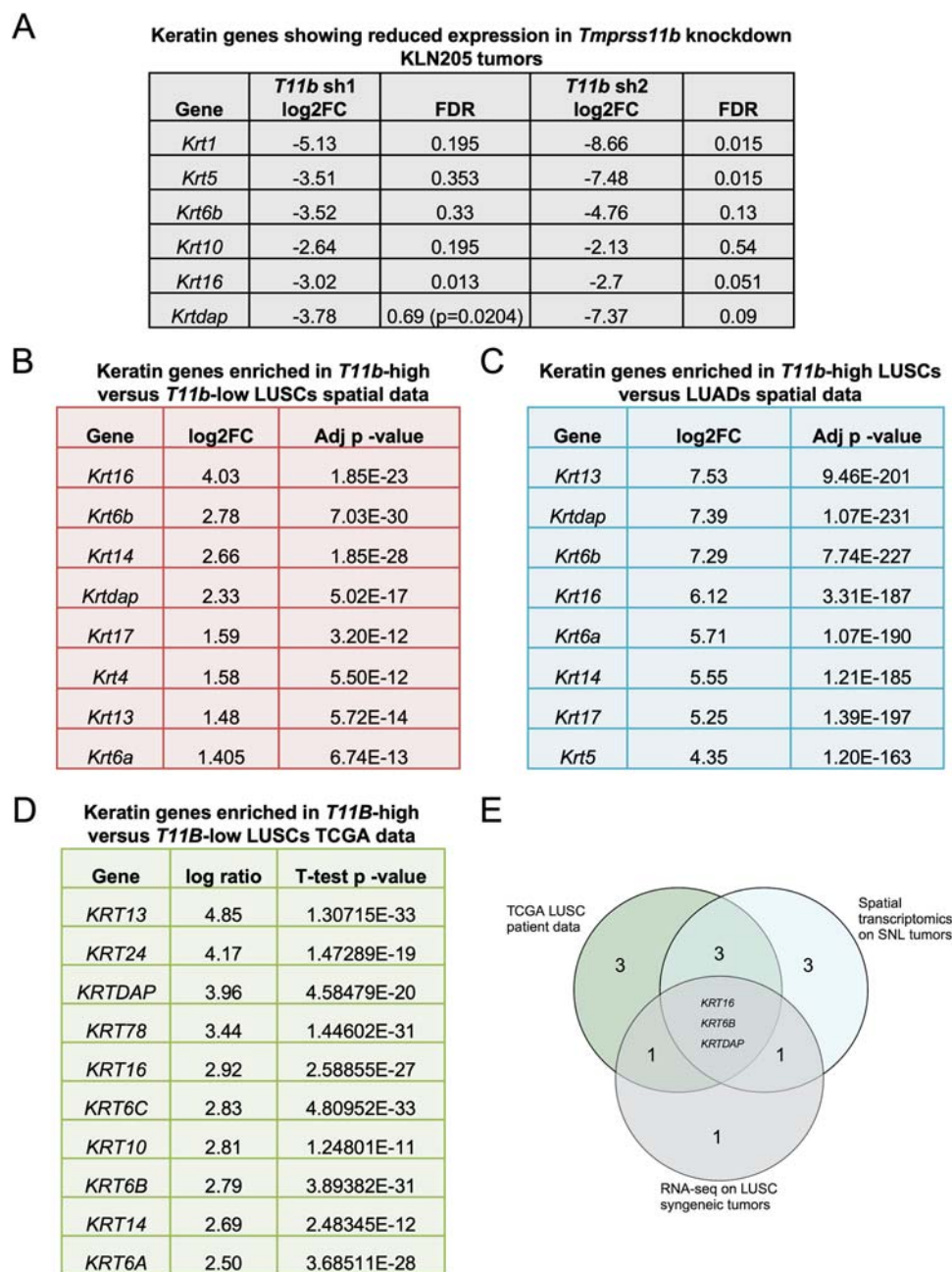




**Figure EV2. *Tmprss11b*-high squamous tumors have increased expression of oncogenes and squamous markers.**

(A) Spatial plots depicting the read counts before and after the quality control process. Scale bar, 1 mm. (B) Volcano plot showing the top differentially expressed genes in the *Tmprss11b*-high versus low LUSCs spatial data. A total of 4090 genes are plotted, of which 970 pass filter (colored). A two-tailed Wilcoxon rank-sum test with Bonferroni correction was used for the statistical analysis ( $n = 4280$  Visium spots post-QC, biological replicates). (C) Violin plot depicting normalized counts for *Tmprss11b* transcript in the annotated regions (from Fig. 2B) ( $n = 1597$ , biological replicates). (D) Gene set enrichment analysis (GSEA) of the *Tmprss11b*-high LUSC versus LUAD spatial data with normalized enrichment scores (NES), false discovery rate (FDR) and  $P$  values for the indicated gene signatures. The nominal  $P$  and FDR values were obtained from the “GSEA Preranked” tool (from Broad Institute) using a weighted scoring scheme. Gene sets were evaluated based on the default normalized enrichment score method, and statistical significance was determined by bootstrapping with 1000 permutations. (E) Top squamous markers and known oncogenes from the differential gene expression (DEG) analysis of the *Tmprss11b*-high LUSC versus LUAD spatial data. Differential gene expression was calculated using Seurat’s FindAllMarkers function, and direct comparisons between two classes and corresponding  $P$  values were obtained using Seurat’s FindMarkers function with the Wilcoxon rank-sum test with Bonferroni  $P$  value correction. (F) Representative H&E and immunohistochemistry (IHC) validation for KRT16 and LYPD3 in LUSC (top) and mucinous LUAD (bottom). The staining was repeated with lung sections from different mice ( $n = 2-3$ , biological replicates). Scale bar, 100  $\mu\text{m}$ . Source data are available online for this figure

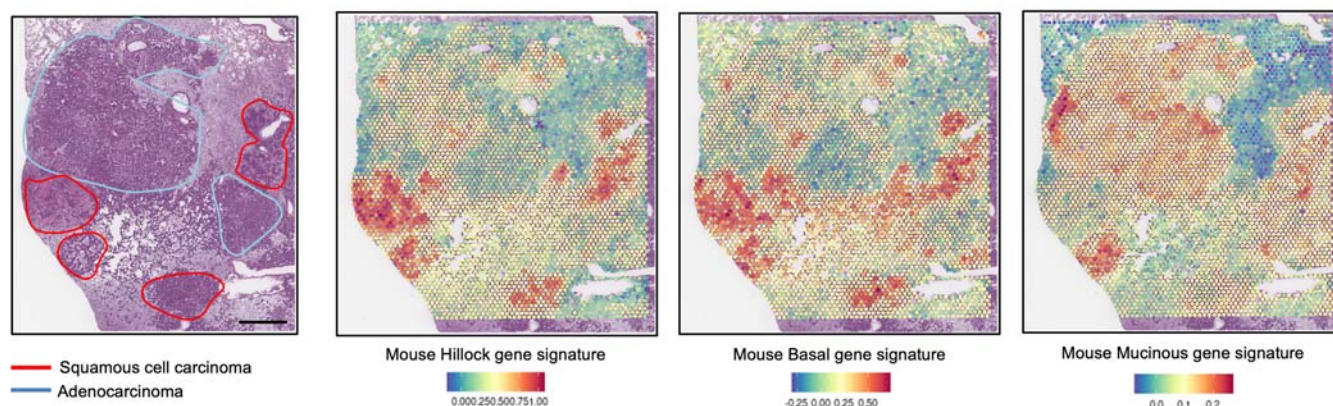




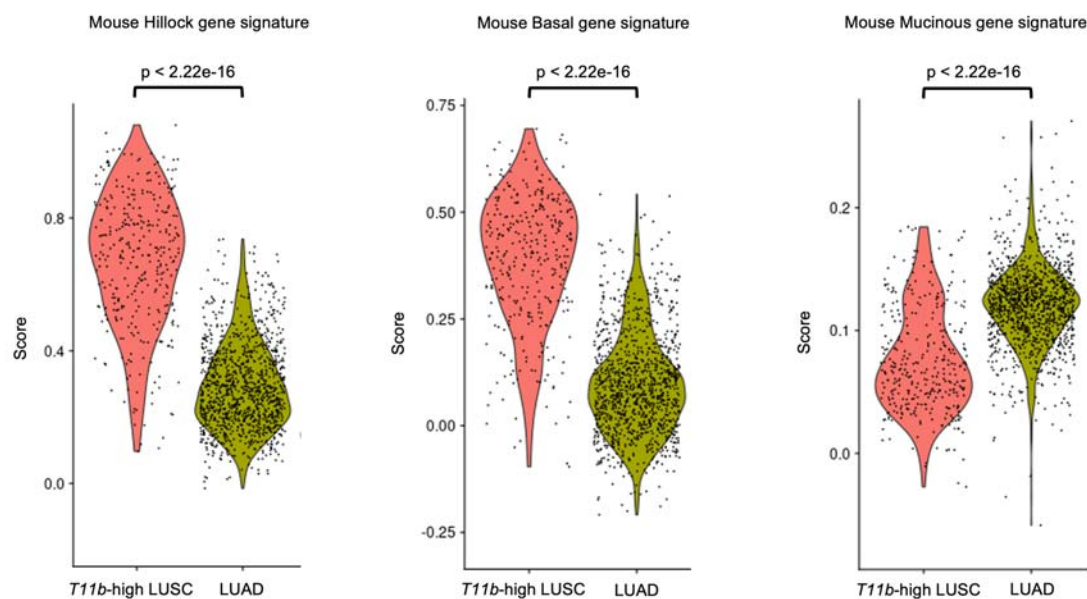
**Figure EV3. Modulation of TMPRSS11B expression is accompanied by coordinated changes in expression of Keratin genes.**

(A) Top downregulated Keratin genes from differential gene expression analysis of control shRNA versus *Tmprss11b* shRNA bulk RNA sequencing from the KLN205 syngenic experiment in Fig. 1D. The log2FC change depicts the reduction in expression of the indicated genes in the *Tmprss11b* knockdown tumors compared to the control. (B) Top Keratin genes from the differential gene expression (DEG) analysis of the *Tmprss11b*-high versus low in LUSC spatial data from SNL lung tumors. (C) Top keratin genes from the differential gene expression (DEG) analysis of the *Tmprss11b*-high LUSC versus LUAD spatial data from SNL lung tumors. (D) Top Keratin genes from the differential gene expression (DEG) analysis of *TMPPRSS11B*-high versus low LUSC human tumors from TCGA. (E) Venn diagram depicting overlapping Keratin genes from the gene lists in (A–D). Source data are available online for this figure

A



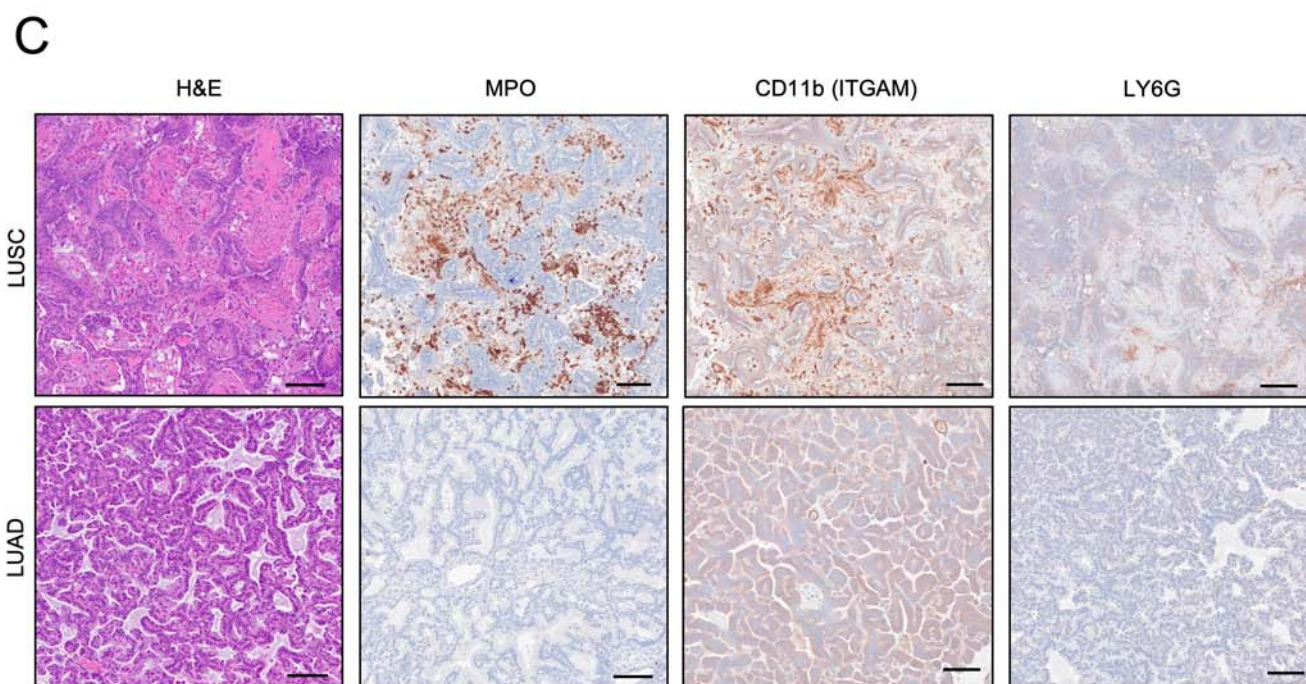
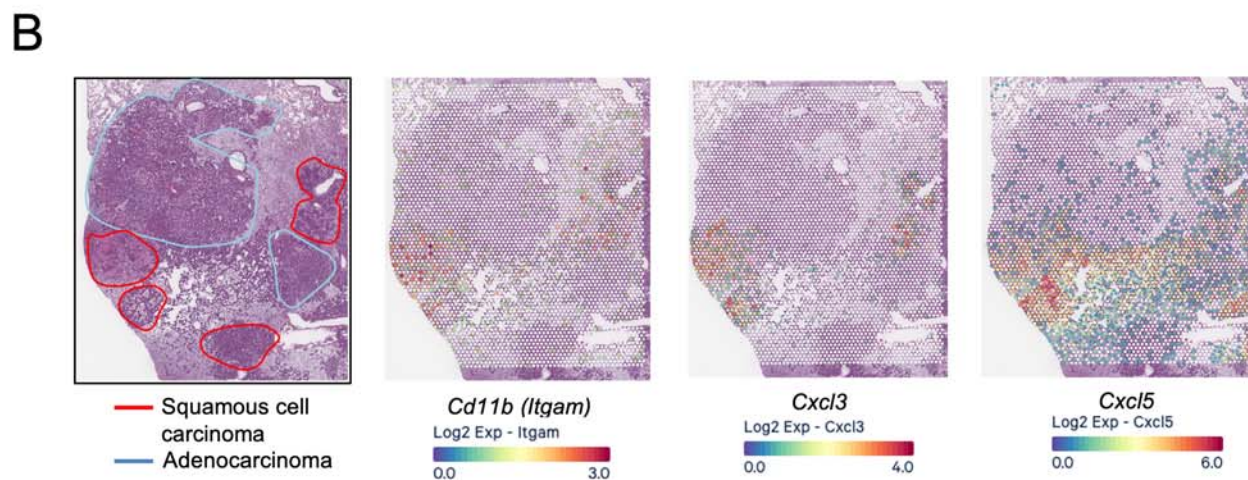
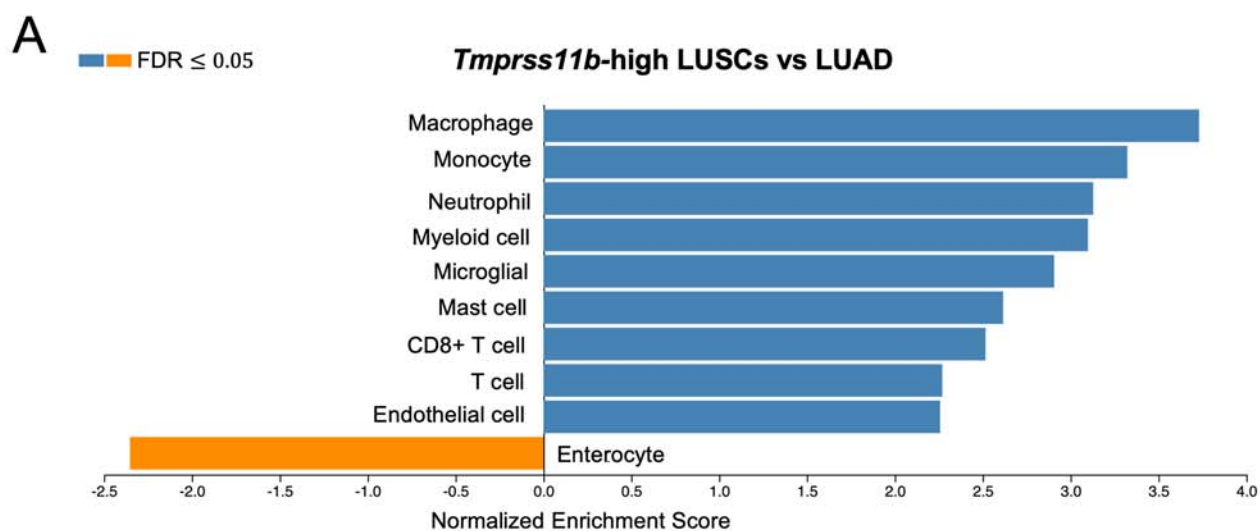
B



**Figure EV4. *Tmprss11b*-high squamous tumors show enrichment for hillock and basal gene signatures.**

(A) H&E image of the lung section from 3 A), annotated with regions of LUSC and LUAD, (left) and spatial plots from the transcriptomic data depicting the distribution of the indicated gene signatures (right). Scale bar, 1 mm. (B) Violin plots representing the enrichment for the indicated gene signatures in *Tmprss11b*-high LUSC and LUAD. A two-tailed Wilcoxon rank-sum test was used for the statistical analysis ( $n = 1492$ , biological replicates).  $P < 2.22 \times 10^{-16}$  for all comparisons. Source data are available online for this figure

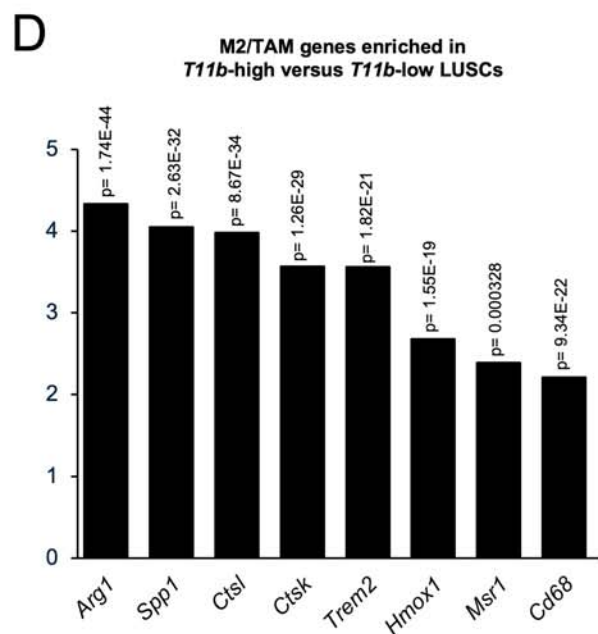
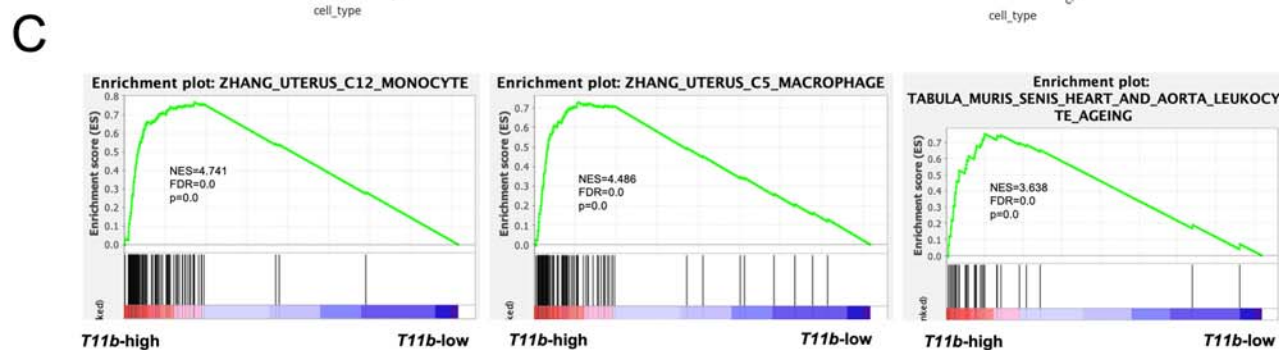
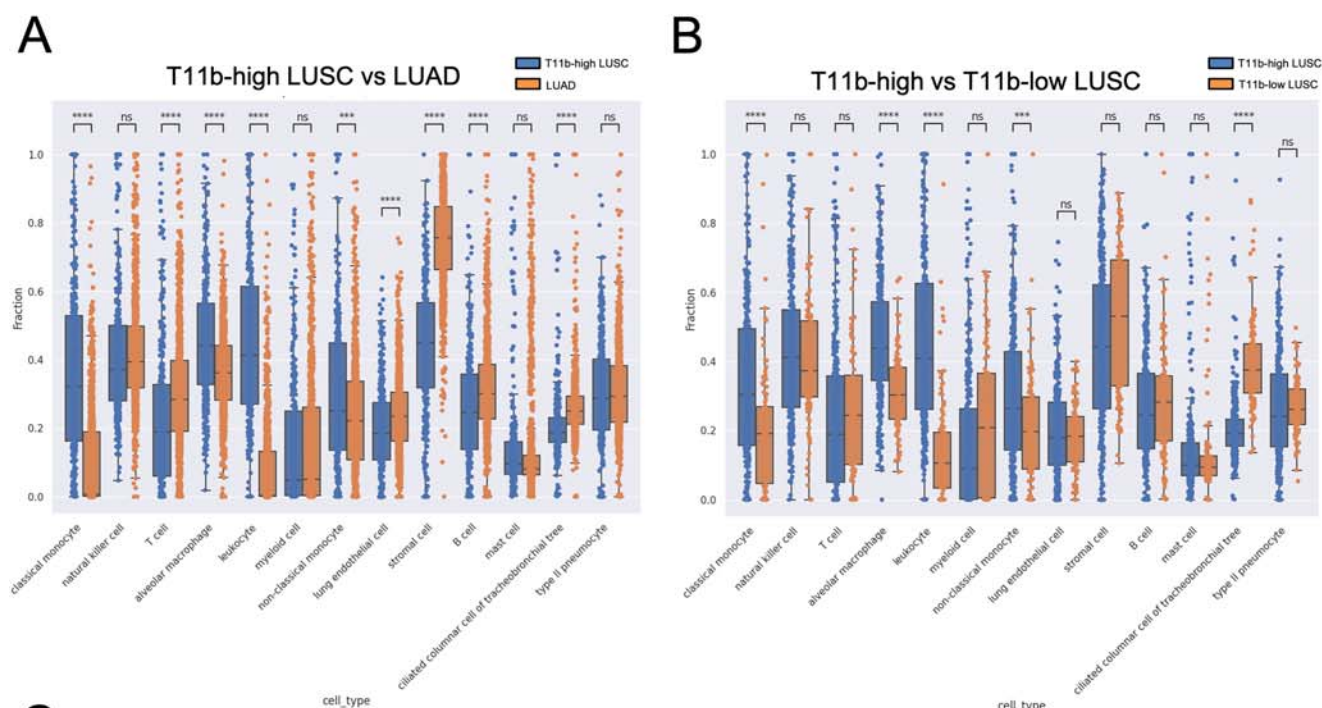




**Figure EV5. *Tmprss11b*-high squamous tumors have higher infiltration of neutrophils.**

(A) Gene set enrichment analysis (GSEA) of *Tmprss11b*-high LUSC versus LUAD spatial transcriptomics data with normalized enrichment scores (NES) and false discovery rate (FDR) for the indicated gene signatures; analysis performed using WEB-based Gene Set Analysis Toolkit. (B) H&E image of the lung section from Fig. 3A), annotated with regions of LUSC and LUAD, and corresponding spatial plots depicting the distribution of the indicated mRNAs (neutrophil markers). (C) Representative H&E and immunohistochemistry (IHC) validation for MPO, CD11b (ITGAM) and LY6G in LUSC (top) and mucinous LUAD (bottom). The staining was repeated with lung sections from different mice ( $n = 2$ , biological replicates). Scale bar, 100  $\mu\text{m}$ . Source data are available online for this figure





**Figure EV6. *Tmprss11b*-high squamous tumors show enrichment for M2-like macrophage markers.**

(A) Quantification of immune cell populations in *Tmprss11b*-high LUSC vs. LUAD using cell deconvolution analysis of the spatial data. A two-sided Mann-Whitney-Wilcoxon test was used for the statistical analysis (*T11b*-high LUSC  $n = 344$ , LUAD  $n = 1148$ , biological replicates), \*\*\*\* $P = 7.96E-78$  (Classical monocyte), \*\*\*\* $P = 7.63E-16$  (T cell), \*\*\*\* $P = 4.74E-17$  (Alveolar macrophage), \*\*\*\* $P = 6.04E-133$  (Leukocyte), \*\*\* $P = 2.31E-05$  (Non-classical monocyte), \*\*\*\* $P = 2.84E-10$  (Lung endothelial cell), \*\*\*\* $P = 1.28E-111$  (Stromal cell), \*\*\*\* $P = 3.92E-09$  (B cell), \*\*\*\* $P = 4.41E-44$  (Ciliated columnar cell of tracheobronchial tree). The box plots represent the distribution of values for each group, extending from the 25th percentile to the 75th percentile; *T11b*-high LUSC: Classical monocyte (minima=0.00035, median=0.32265, maxima=1, Q1=0.16193, Q3=0.52806), Natural killer cell (minima=0.04763, median=0.37114, maxima=0.82722, Q1=0.28091, Q3=0.49943), T cell (minima=0, median=0.18974, maxima=0.72977, Q1=0.06047, Q3=0.32819), Alveolar macrophages (minima=0.01914, median=0.44117, maxima=0.91769, Q1=0.32846, Q3=0.56415), Leukocyte (minima=0.987E-05, median=0.41385, maxima=1, Q1=0.27138, Q3=0.61336), Myeloid cell (minima=0.00012, median=0.04951, maxima=0.61895, Q1=0.00328, Q3=0.24955), Non-classical monocyte (minima=0.00036, median=0.25014, maxima=0.92007, Q1=0.13621, Q3=0.44976), Lung endothelial cell (minima=0, median=0.18464, maxima=0.52498, Q1=0.10807, Q3=0.27484), Stromal cell (minima=0, median=0.44905, maxima=0.94097, Q1=0.31863, Q3=0.56757), B cell (minima=0, median=0.24525, maxima=0.69056, Q1=0.13767, Q3=0.35883), Mast cell (minima=0.00046, median=0.09658, maxima=0.30038, Q1=0.06603, Q3=0.15977), Ciliated columnar cell of tracheobronchial tree (minima=0.05124, median=0.18792, maxima=0.34003, Q1=0.15953, Q3=0.23173), Type II pneumocyte (minima=0, median=0.28766, maxima=0.71305, Q1=0.19541, Q3=0.40246); LUAD: Classical monocyte (minima=0, median=0.00788, maxima=0.47288, Q1=0.00108, Q3=0.18980), Natural killer cell (minima=0.04933, median=0.39471, maxima=0.76680, Q1=0.31838, Q3=0.49774), T cell (minima=0.00029, median=0.28494, maxima=0.70883, Q1=0.19111, Q3=0.39820), Alveolar macrophages (minima=0.04437, median=0.36127, maxima=0.67967, Q1=0.28261, Q3=0.44143), Leukocyte (minima=0, median=0.00264, maxima=0.33064, Q1=0.00058, Q3=0.13260), Myeloid cell (minima=0, median=0.05014, maxima=0.64748, Q1=0.00252, Q3=0.26050), Non-classical monocyte (minima=0, median=0.22165, maxima=0.68314, Q1=0.10763, Q3=0.33783), Lung endothelial cell (minima=0, median=0.23560, maxima=0.51640, Q1=0.16346, Q3=0.30464), Stromal cell (minima=0.39036, median=0.75595, maxima=1, Q1=0.66414, Q3=0.84667), B cell (minima=0, median=0.30153, maxima=0.62486, Q1=0.22758, Q3=0.38650), Mast cell (minima=0, median=0.08179, maxima=0.20366, Q1=0.06479, Q3=0.12034), Ciliated columnar cell of tracheobronchial tree (minima=0.08851, median=0.25076, maxima=0.41768, Q1=0.21195, Q3=0.29424), Type II pneumocyte (minima=0, median=0.29401, maxima=0.63087, Q1=0.21685, Q3=0.38246). (B) Quantification of immune cell populations in *Tmprss11b*-high vs. *Tmprss11b*-low LUSC using cell deconvolution analysis of the spatial data. A two-sided Mann-Whitney-Wilcoxon test was used for the statistical analysis (*T11b*-high LUSC  $n = 344$ , *Tmprss11b*-low LUSC  $n = 104$ , biological replicates), \*\*\*\* $P = 8.89E-08$  (Classical monocyte), \*\*\*\* $P = 3.22E-15$  (Alveolar macrophage), \*\*\*\* $P = 1.25E-32$  (Leukocyte), \*\*\* $P = 6.54E-05$  (Non-classical monocyte), \*\*\*\* $P = 2.42E-36$  (Ciliated columnar cell of tracheobronchial tree). The box plots represent the distribution of values for each group, extending from the 25th percentile to the 75th percentile; *T11b*-high LUSC: Classical monocyte (minima=0.00029, median=0.30549, maxima=1, Q1=0.15744, Q3=0.49527), Natural killer cell (minima=0, median=0.41140, maxima=0.97370, Q1=0.26653, Q3=0.5490), T cell (minima=0, median=0.18983, maxima=0.81820, Q1=0.05084, Q3=0.35778), Alveolar macrophages (minima=0.00293, median=0.43913, maxima=0.91408, Q1=0.34461, Q3=0.57240), Leukocyte (minima=0.00068, median=0.40974, maxima=1, Q1=0.26157, Q3=0.62635), Myeloid cell (minima=4.55E-05, median=0.09014, maxima=0.65331, Q1=0.00217, Q3=0.26263), Non-classical monocyte (minima=1.67E-05, median=0.26527, maxima=0.85779, Q1=0.14324, Q3=0.42906), Lung endothelial cell (minima=0, median=0.17896, maxima=0.55746, Q1=0.09927, Q3=0.28255), Stromal cell (minima=0, median=0.44430, maxima=1, Q1=0.26373, Q3=0.62252), B cell (minima=0, median=0.24471, maxima=0.69114, Q1=0.14794, Q3=0.36522), Mast cell (minima=0, median=0.10069, maxima=0.30702, Q1=0.06993, Q3=0.16477), Ciliated columnar cell of tracheobronchial tree (minima=0.03638, median=0.19021, maxima=0.35185, Q1=0.15468, Q3=0.23355), Type II pneumocyte (minima=0, median=0.24117, maxima=0.67966, Q1=0.1531, Q3=0.36374); *Tmprss11b*-low LUSC: Classical monocyte (minima=0.00020, median=0.19138, maxima=0.60530, Q1=0.04595, Q3=0.26969), Natural killer cell (minima=0, median=0.37341, maxima=0.84796, Q1=0.29730, Q3=0.51756), T cell (minima=7.77E-05, median=0.24393, maxima=0.74889, Q1=0.10171, Q3=0.36058), Alveolar macrophages (minima=0.08051, median=0.30265, maxima=0.60637, Q1=0.23290, Q3=0.38229), Leukocyte (minima=0, median=0.10504, maxima=0.43837, Q1=0.03357, Q3=0.19549), Myeloid cell (minima=0.00048, median=0.20803, maxima=0.90453, Q1=0.00571, Q3=0.36524), Non-classical monocyte (minima=0.00013, median=0.19676, maxima=0.60907, Q1=0.08942, Q3=0.29728), Lung endothelial cell (minima=0, median=0.18352, maxima=0.39994, Q1=0.10900, Q3=0.23955), Stromal cell (minima=0.10516, median=0.53043, maxima=0.88675, Q1=0.32928, Q3=0.69341), B cell (minima=0.00077, median=0.28140, maxima=0.64062, Q1=0.17045, Q3=0.35852), Mast cell (minima=0, median=0.09511, maxima=0.21496, Q1=0.06911, Q3=0.12745), Ciliated columnar cell of tracheobronchial tree (minima=0.13684, median=0.37548, maxima=0.66299, Q1=0.30862, Q3=0.45037), Type II pneumocyte (minima=0.06470, median=0.26157, maxima=0.47461, Q1=0.21841, Q3=0.32089). (C) Gene set enrichment analysis (GSEA) of the *Tmprss11b*-high versus low LUSC spatial data with normalized enrichment scores (NES), false discovery rate (FDR) and  $p$  values for the indicated immune cell gene signatures. The nominal  $P$  and FDR values were obtained from the "GSEA Preranked" tool (from Broad Institute) using a weighted scoring scheme. Gene sets were evaluated based on the default normalized enrichment score method, and statistical significance was determined by bootstrapping with 1000 permutations. (D) Bar graph representing top M2-like/TAM genes from the differential gene expression (DEG) analysis of *Tmprss11b*-high versus low LUSC spatial transcriptomics data. Differential gene expression was calculated using Seurat's FindAllMarkers function, and direct comparisons between two classes and corresponding  $P$  values were obtained using Seurat's FindMarkers function with the Wilcoxon rank-sum test with Bonferroni  $P$  value correction. Source data are available online for this figure