

**Resolving the spatial and cellular architecture of lung adenocarcinoma by multi-region single-cell sequencing**

**SUPPLEMENTARY DATA FILE 6**

**Supplementary Figures S21-S24**

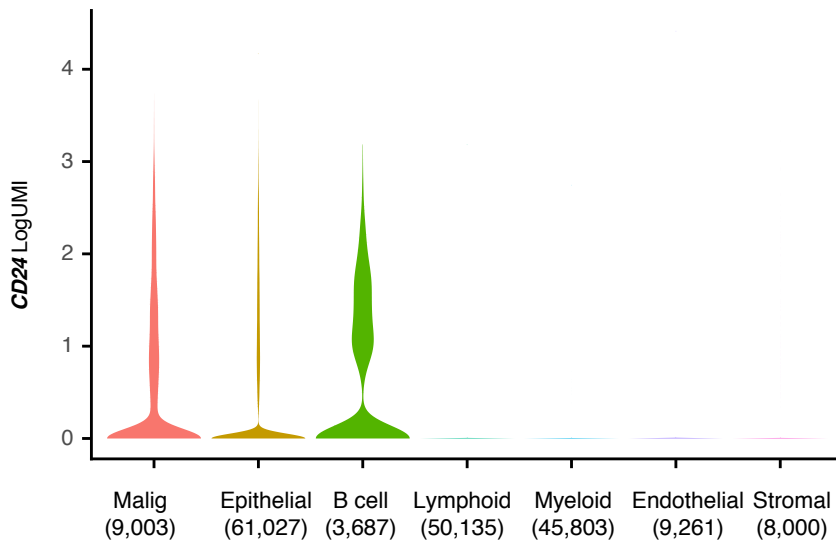


**Supplementary Fig. S21. Spatially enriched chemokine-receptor cell-cell communication networks in the immune microenvironment of early-stage LUAD.**

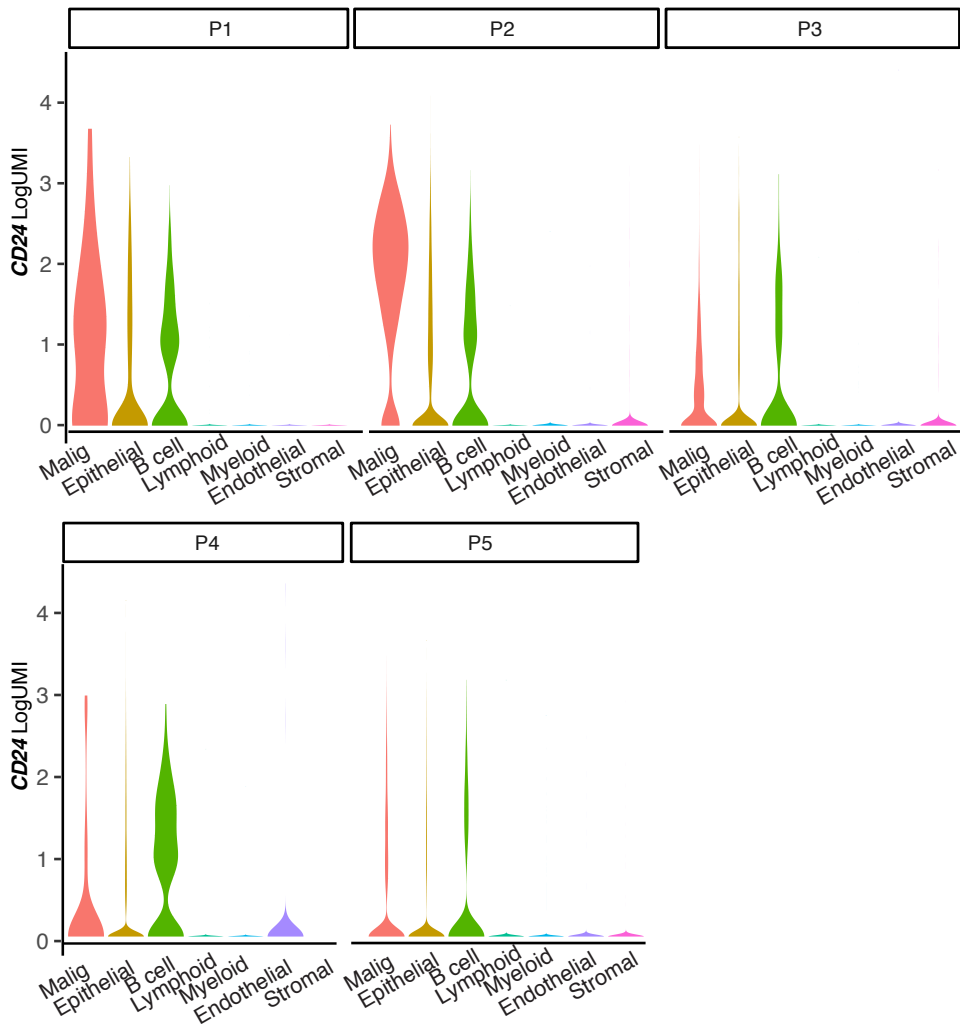
**A**, Circos plots showing modulation of chemokine-mediated ligand-receptor (L-R) pairs between the LUADs and tumor-intermediate normal tissue of P2, P3, and P5. **B-C**, Violin plots showing spatial expression levels of the major chemokine and receptor genes in P3 (B) and P5 (C).

# Supplementary Fig. S22

**A**



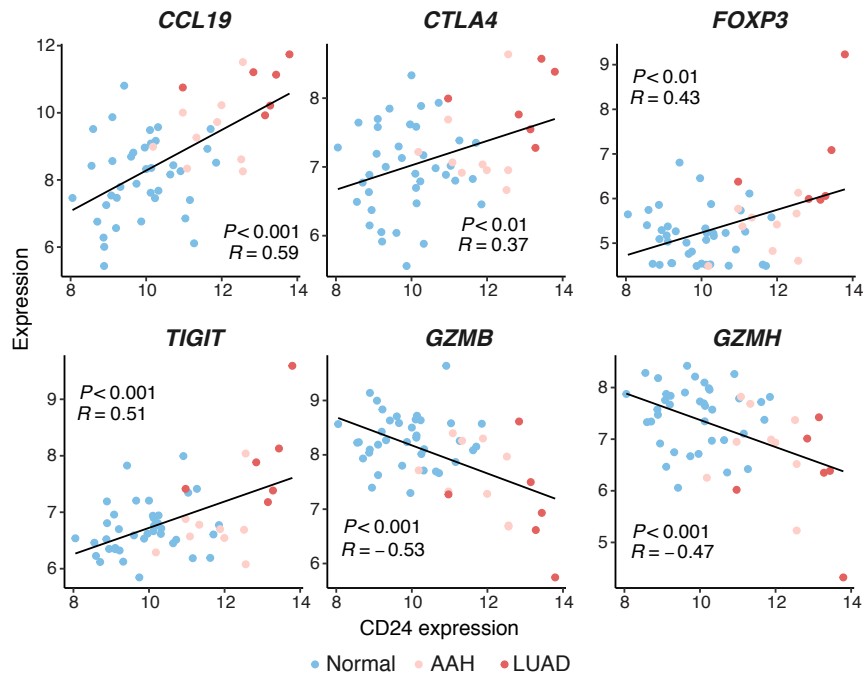
**B**



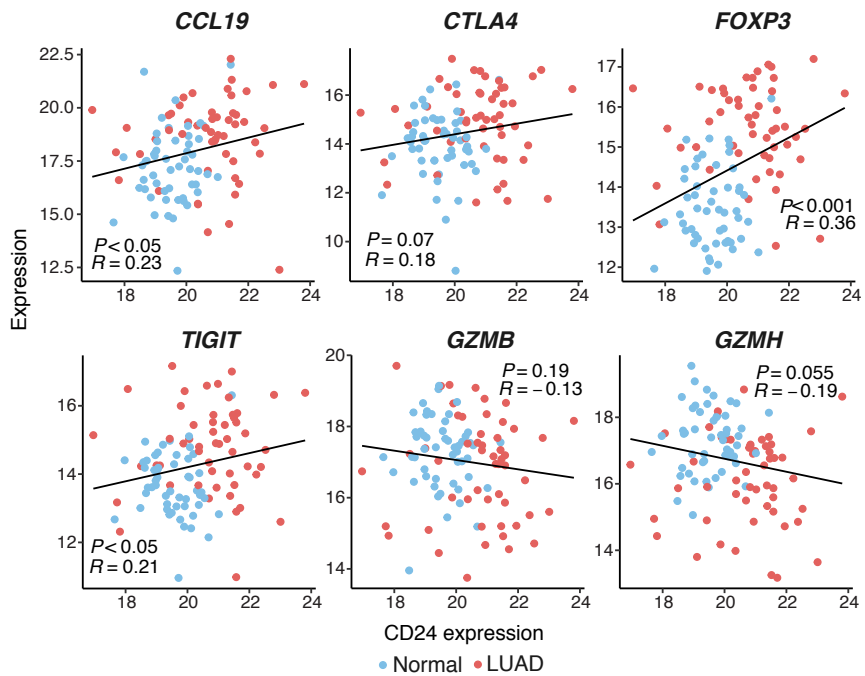
**Supplementary Fig. S22. Single-cell expression patterns of *CD24* in the ecosystem of early-stage LUAD.** **A**, Violin plot showing *CD24* expression levels across major lineages from all patient samples. **B**, Violin plots showing *CD24* expression levels across major lineages for each patient.

# Supplementary Fig. S23

## A



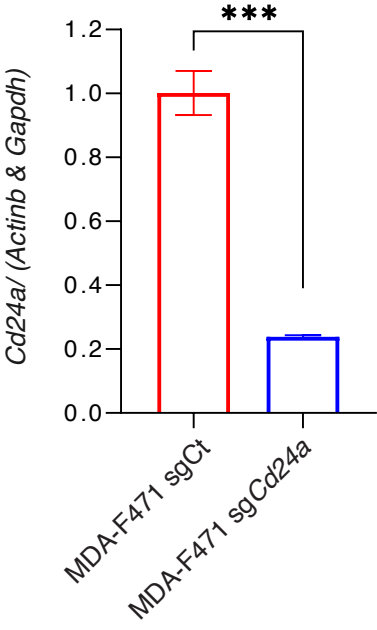
## B



**Supplementary Fig. S23. Correlation of *CD24* expression with immune genes.**

Scatterplots showing gene expression correlation analysis between *CD24* and immune genes in a cohort of normal lung (NL) tissues, premalignant atypical adenomatous hyperplasias (AAH), and invasive LUADs (GSE10251, **A**) and in LUADs and NL from TCGA cohort (**B**). *P* – values were calculated using Pearson’s correlation test.

# Supplementary Fig. S24





**Supplementary Fig. S24. CRISPR-mediated knockdown of *Cd24a* expression in mouse LUAD cells.** Expression of *Cd24a* in MDA-F471 mouse LUAD cells transduced with sgCt or sg*Cd24a* CRISPR constructs and following FACS-sorting for high or low expression of CD24 surface protein, respectively. Gene expression was normalized to both *Gapdh* and *Actinb* using the  $2^{-\Delta\Delta C_t}$  method (\*\*\*,  $P < 0.001$ ; unpaired Student's *t* test).