**Supplementary Information for**

**"Neuromedin U promotes lung adenocarcinoma progression by enhancing nucleotide metabolism and potentiating fibrosis in the tumor microenvironment"**

**Neuromedin U promotes lung adenocarcinoma progression by enhancing nucleotide metabolism and potentiating fibrosis in the tumor microenvironment**

Shih-Miao Li, Chi-Shuan Fan, Tzu-Yu Chen, Wen-Tsen Fang, Fang-Yu Tsai, Hui-Yu Jao, Po-Tsun Shen, Zih-Syuan Cao, Yueh-Feng Wen, Yi-Ju Wu, Po-Jung Huang, Tze-Sing Huang, Ya-Wen Chen, Ching-Chuan Kuo, B. Linju Yen, Ko-Jiunn Liu, Chao A. Hsiung, I-Shou Chang, Shih Sheng Jiang*

This file contains supplementary figures, tables, and additional methodological details referenced in the main manuscript.
The materials are organized in the following order:

- Supplementary Tables S1
- Supplementary Figures S1 - S5

All supplementary materials are cited in the main text, and figure/table legends are included with each item.
Please refer to these materials as needed for additional data supporting the findings in the main manuscript.

**Supplementary Table S1. Nucleic acid sequences of the probes and primers for the RT-qPCR experiment.**

| Oligonucleotide information | | | |
|---|---|---|---|
| **Oligonucleotide name** | **UPL probe ROCHE** | **Forward primer (5'-3')** | **Reverse primer (5'-3')** |
| NMU | #25 (cat. no. 04686993001) | **CGTCTTTTCTGTCCATTGATTCT** | **GCATTCCCATAATCATAAAGCAA** |
| IMPDH1 | #22 (cat. no. 04686969001) | **CCATGGCCTGCACTCTTA** | **GTGGACACTGGGGTGCAT** |
| GMPS | #78 (cat. no. 04689011001) | **GCTCTGTGCAACGGAGACT** | **TGGTGGTGGCCATCCTTA** |
| RRM1 | #49 (cat. no. 04688104001) | **CCACATAATGGCAAACACTCTC** | **GAGAAATCTCGGTCATAGATAATAGCA** |
| NTSR1 | #50 (cat. no. 04688112001) | **GGAGACAGCCCGAGGAAC** | **CTCCGGACTCCCAGCTTC** |
| ATIC | #89 (cat. no. 04689143001) | **GAGGGACTGCAAAAGCTCTC** | **GGAAATCCCGTCAACTCAGA** |

## Supplementary Figure S1

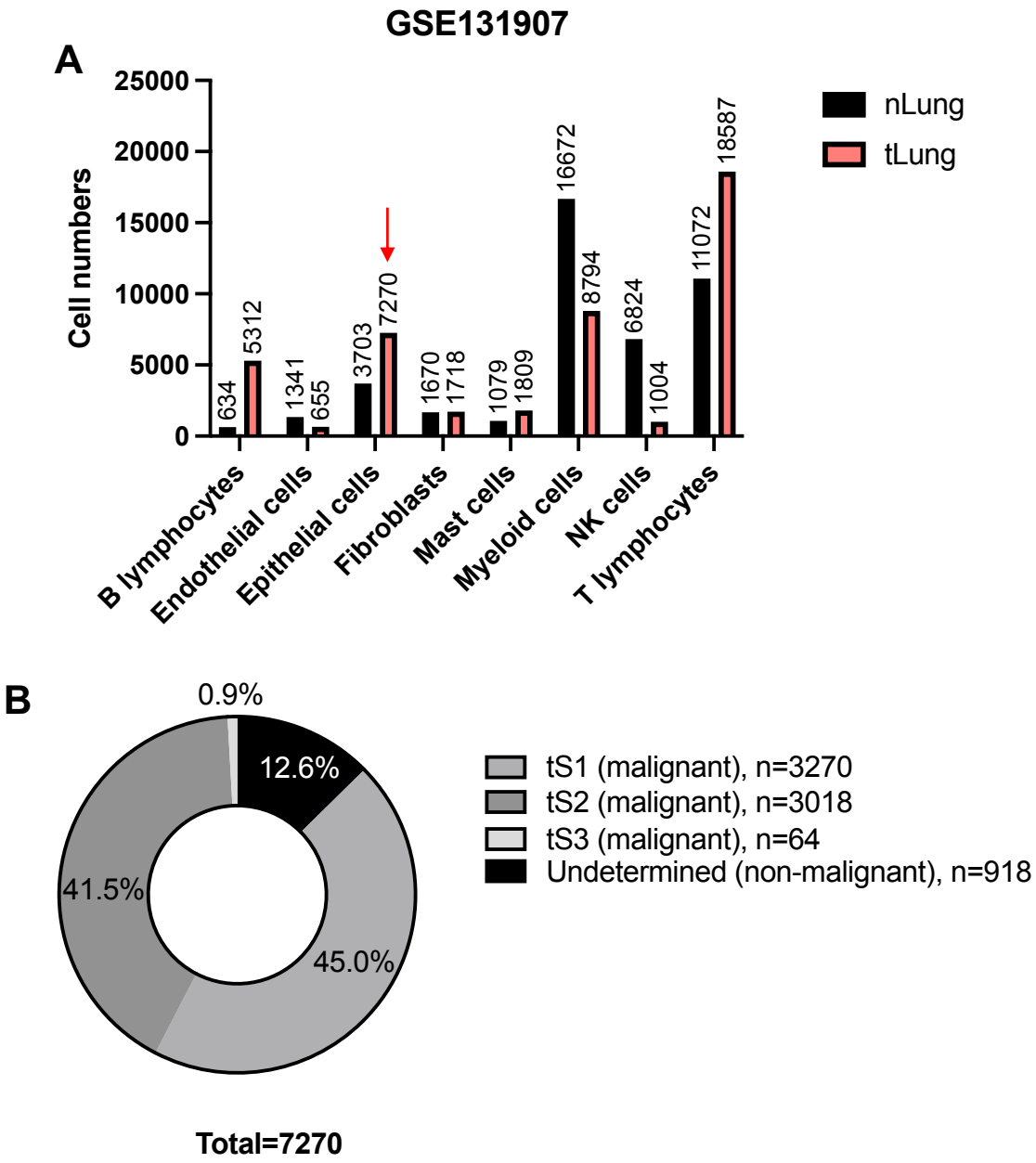



**Figure S1. Landscape of cell types analyzed by scRNA-seq dataset GSE131907.**

(A) Distribution of major cell types in the GSE131907 dataset, with 7270 epithelial cells indicated by red arrow identified in the tumor lung (tLung) group.

(B) The total number and proportion of tumor epithelial cells, further categorized into tS1, tS2, tS3, and undetermined cell types.

# Supplementary Figure S2

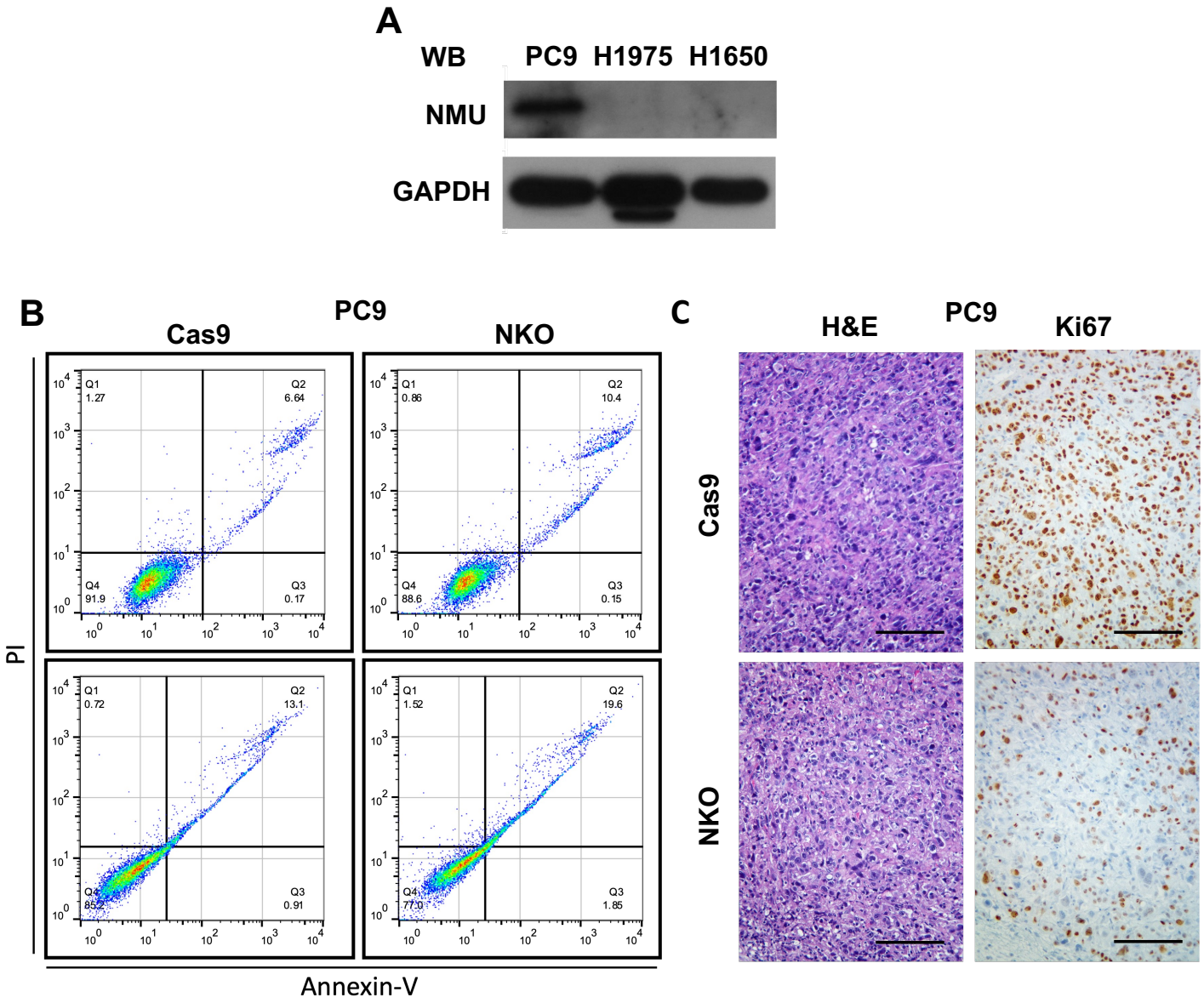



**Figure S2. Effects of *NMU* knockout on cell apoptosis and xenograft tumor proliferation.**

(A) Western blot analysis of NMU protein expression profile in NSCLC cell lines.

(B) FACS analysis of apoptosis in PC9-Cas9 and PC9-NKO clones. Upper panel: 24 hours post seeding, lower panel: 48 hours post seeding.

(C) Hematoxylin staining and Ki67 expression in PC9-Cas9 and in PC9-NKO tumors. Scale bar, 100 μm.

## Supplementary Figure S3

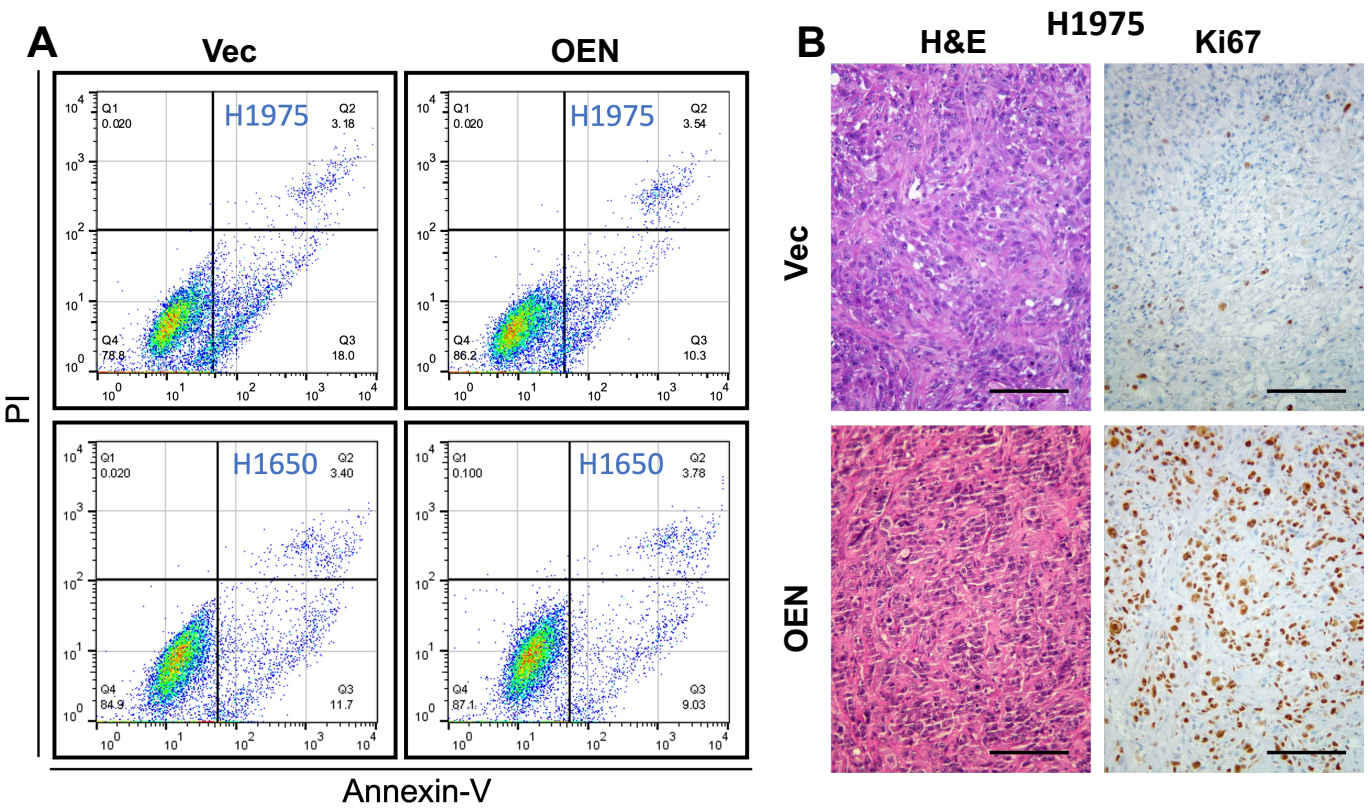



**Figure S3. Effects of *NMU* overexpression on cell apoptosis and xenograft tumor proliferation.**

(A) FACS analysis of apoptosis. Upper panels: H1975 cells; lower panels: H1650 cells.

(B) Hematoxylin staining and Ki67 expression in tumors from H1975 cells overexpressing *NMU*. Scale bar, 100 μm.

# Supplementary Figure S4

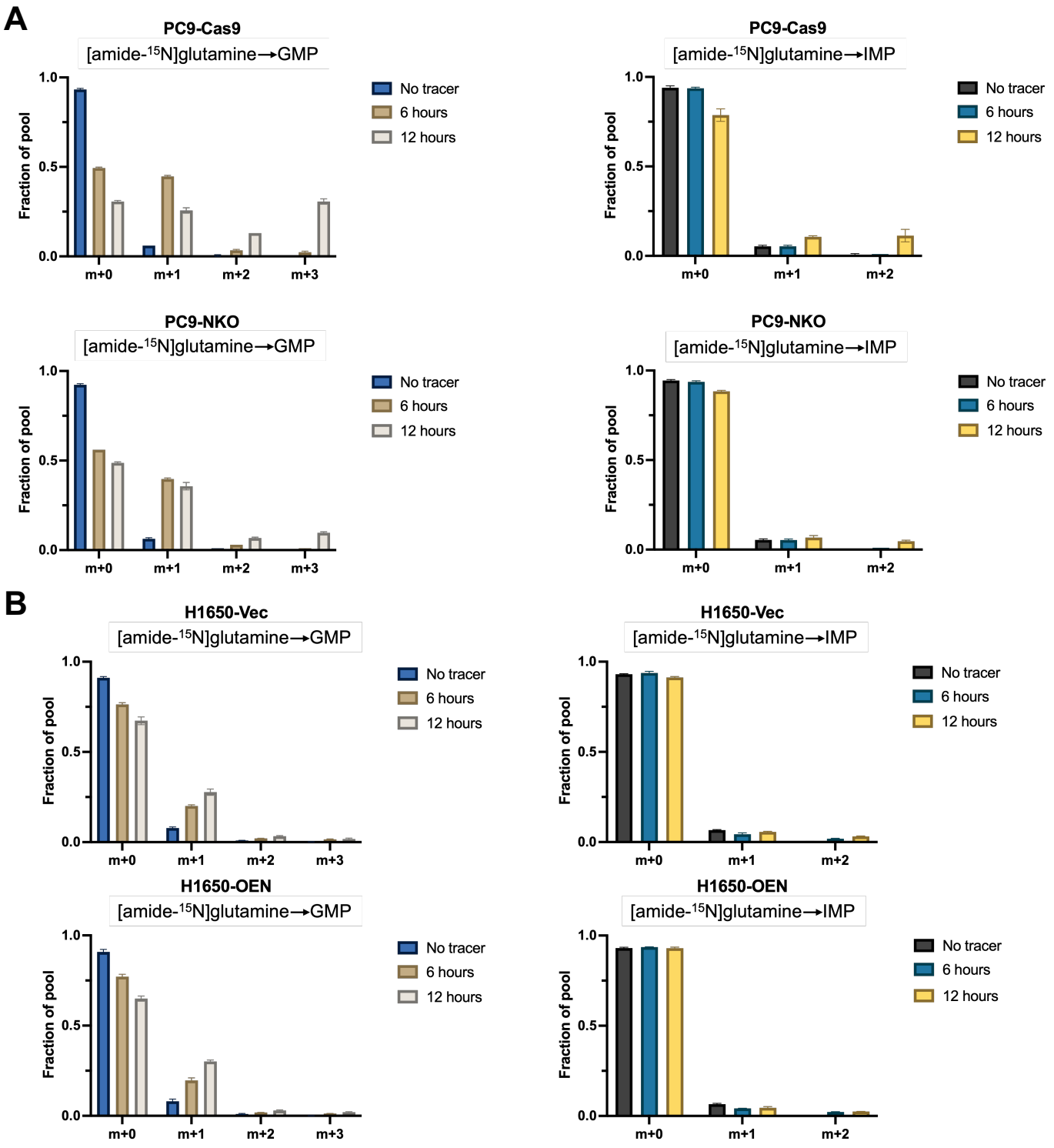
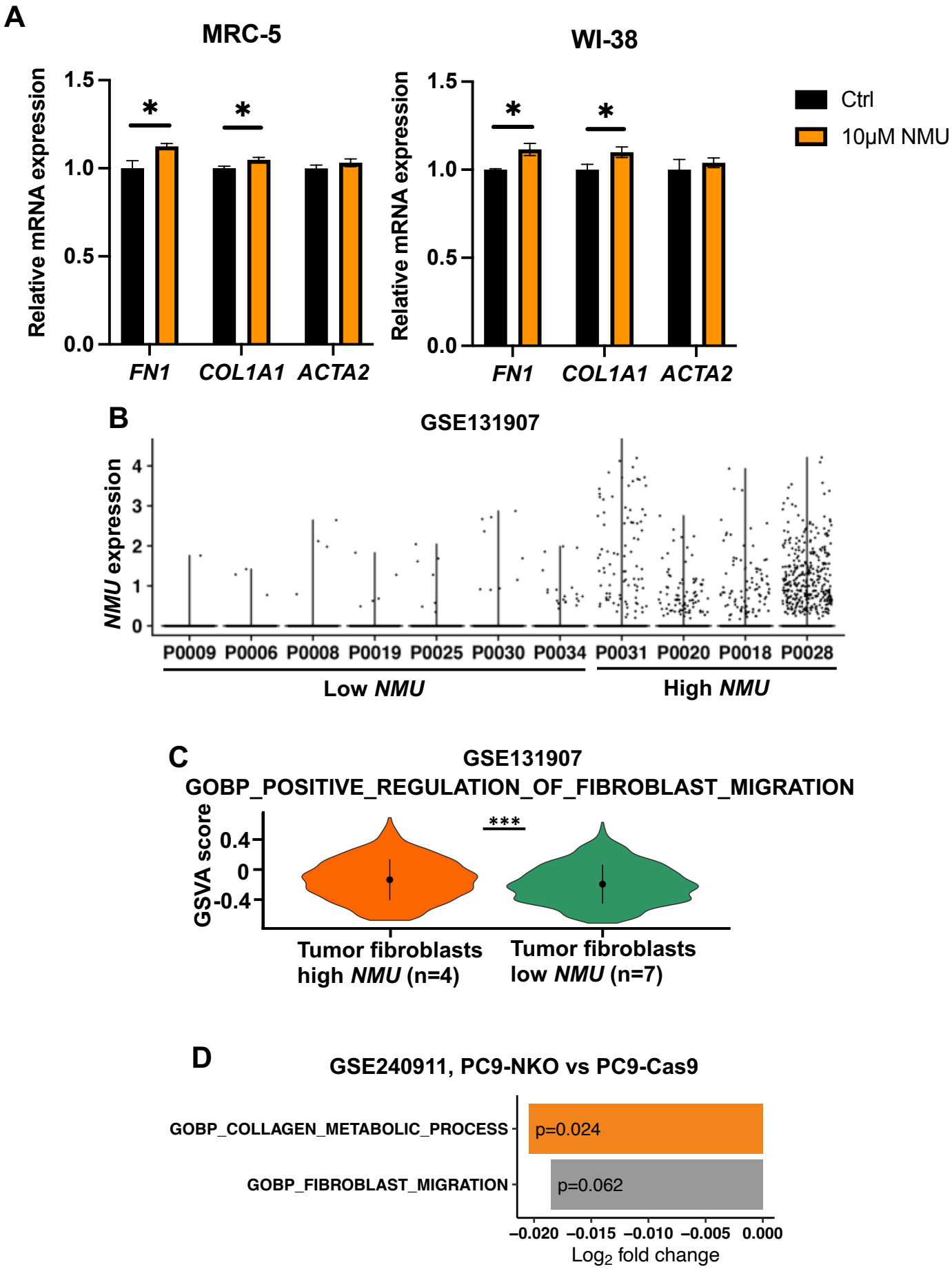



**Figure S4. Fractional $^{15}$N-labeling of purine nucleotides in PC9 and H1650 cells**

(A) Fractional enrichment of $^{15}$N-labeled IMP and GMP isotopologues (m+1, m+2, and m+3) in PC9 control and *NMU* knockout clones following 6 h and 12 h incubation with [amide-$^{15}$N]glutamine, as determined by LC-MS.

(B) Fractional enrichment of $^{15}$N-labeled IMP and GMP isotopologues in H1650 control and *NMU*-overexpressing clones under the same labeling conditions.

## Supplementary Figure S5




(To be continued)

(Continued)

**Figure S5. Integrated analysis of NMU-driven fibroblast activation using qPCR, scRNA-seq, and GSVA.**

(A) Relative mRNA expression of *FN1*, *COL1A1*, and *ACTA2* in human lung fibroblast cell lines (MRC-5 and WI-38) treated with NMU peptide (10 μM, 24 h), as measured by quantitative PCR

(B) Violin plots showing *NMU* expression levels across 11 LUAD tumor samples, ranked by the percentage of *NMU* expression per sample in scRNA-seq dataset GSE131907.

(C) GSVA analysis of fibroblast showing significant increase in the GOBP_POSITIVE_REGULATION_OF_FIBROBLAST_MIGRATION gene set score in the high *NMU* expression group.

(D) GSVA analysis of fibroblasts co-cultured with NSCLC cells, revealing significant downregulation of the collagen metabolic process pathway and a decreasing trend in fibroblast migration pathway activity in PC9-NKO compared to PC9-Cas9 clones.