

Supplementary Information

Aging Related Transcriptomic Signatures Associated with Recurrence Following Resection of Stage I Lung Adenocarcinoma

Fares Darawshy et al.

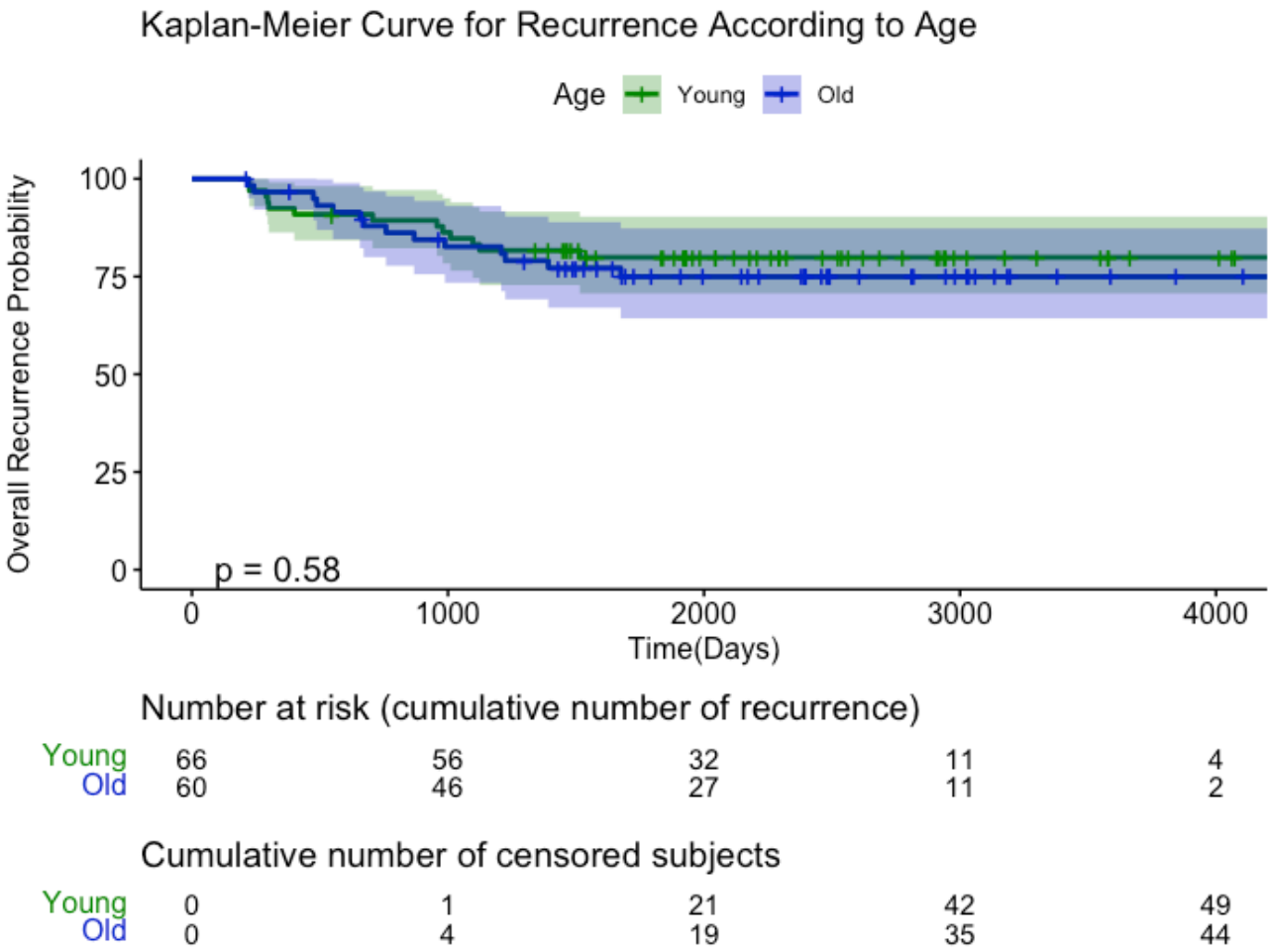
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- Supplementary Data Legends

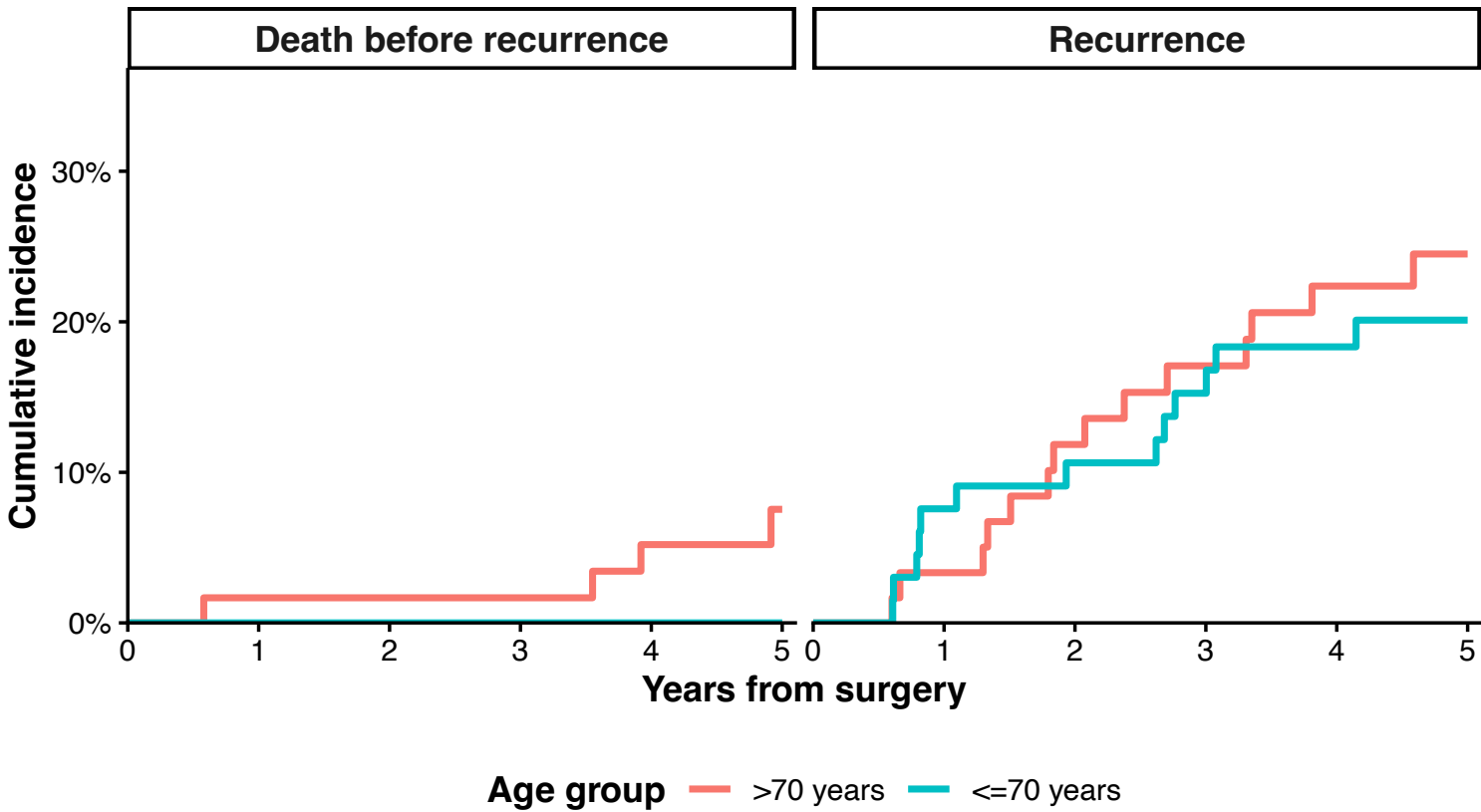
Supplementary Figure 1

NYU Cohort

A)



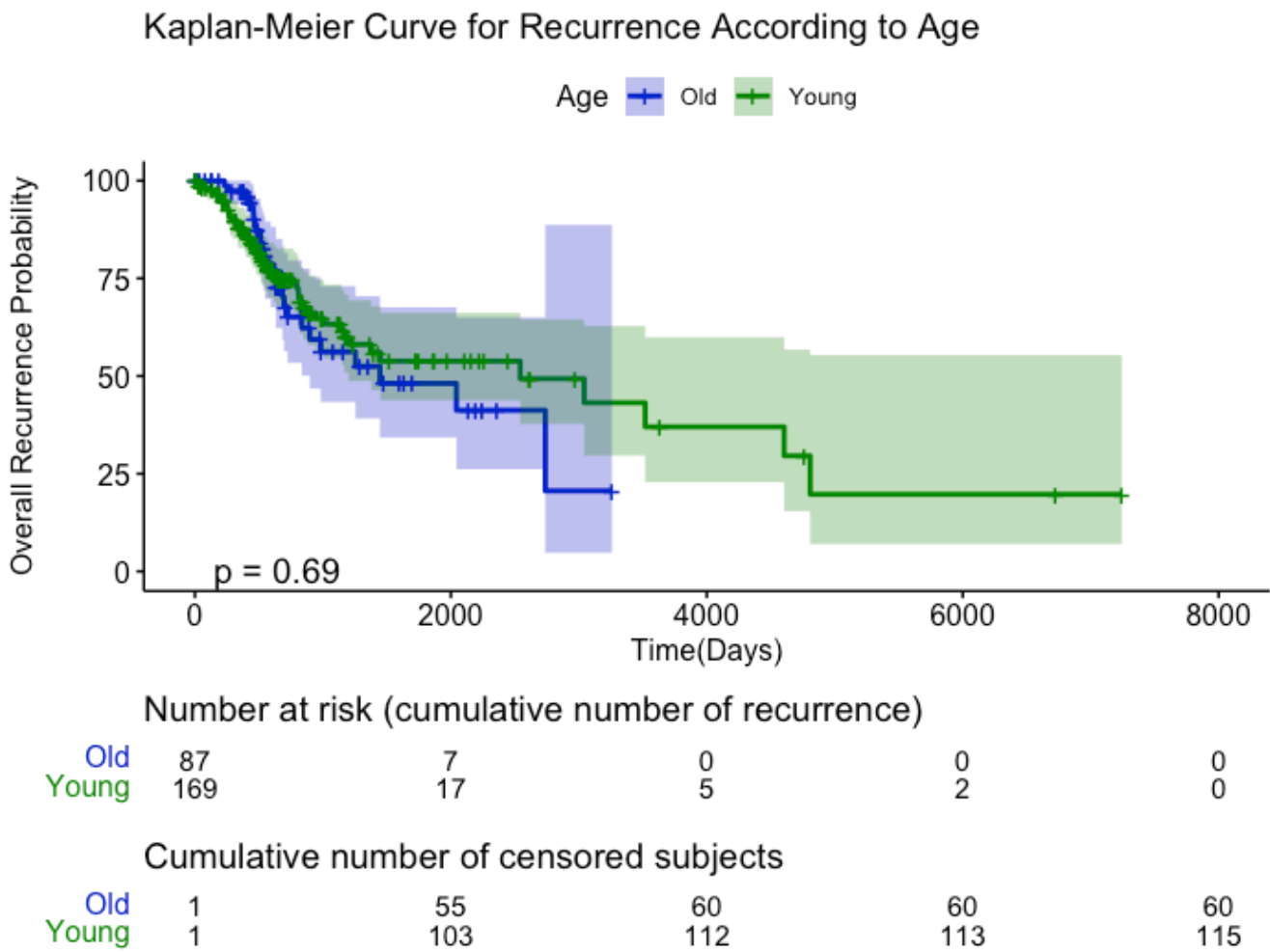
B)



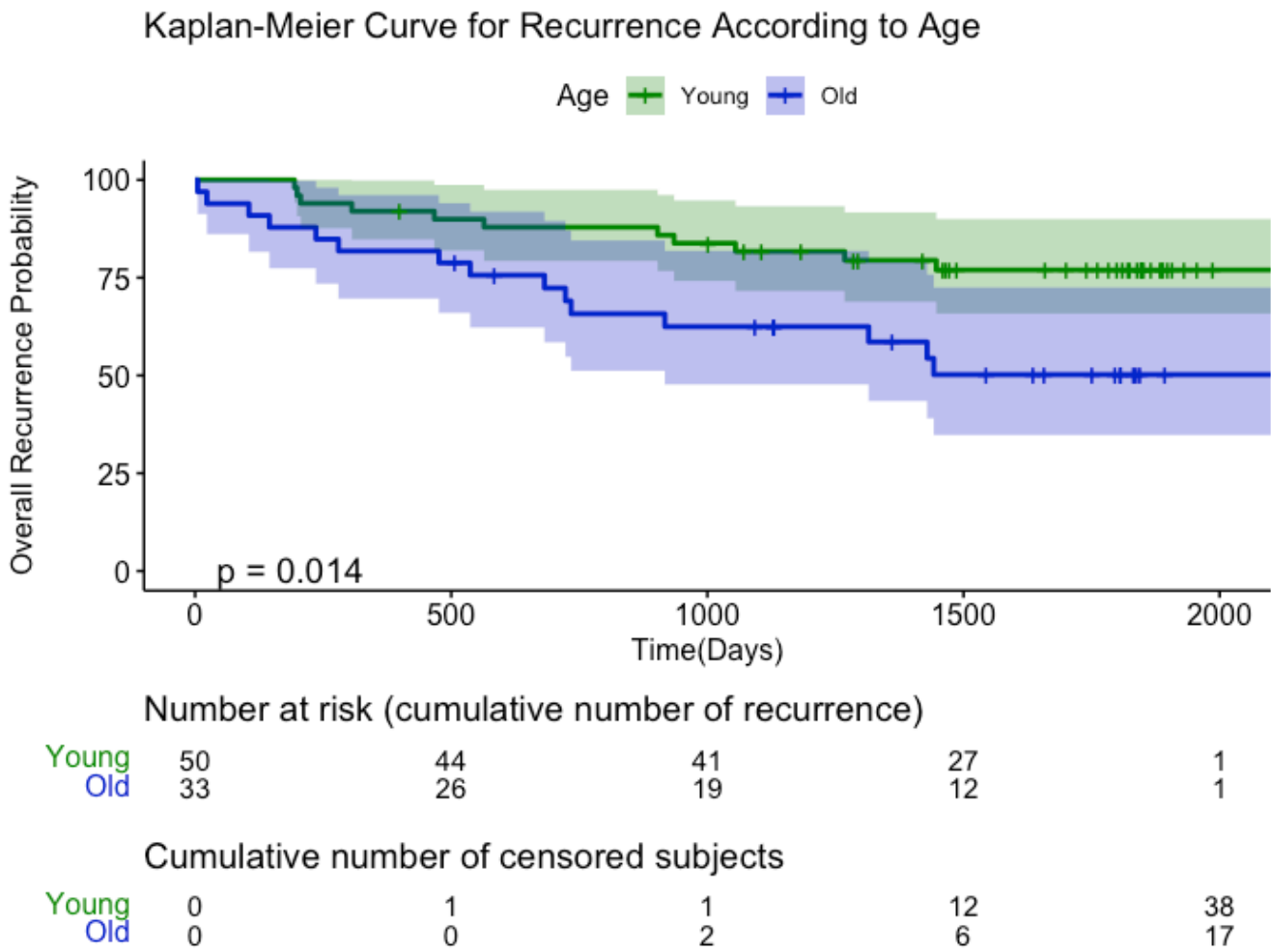
Supplementary Figure 1: A) Kaplan–Meier curve of recurrence according to age in the NYU cohort. Patients were stratified into young (≤ 70 years) and old (> 70 years) groups. No significant differences in recurrence-free survival were observed between groups (log rank test, $p = 0.58$). Numbers at risk and censored subjects are shown below the plot. B) Cumulative incidence of 5-years recurrence and 5-years death before recurrence by age group (5-years).

Supplementary Figure 2

A) TCGA Cohort



B) TRACERx Cohort

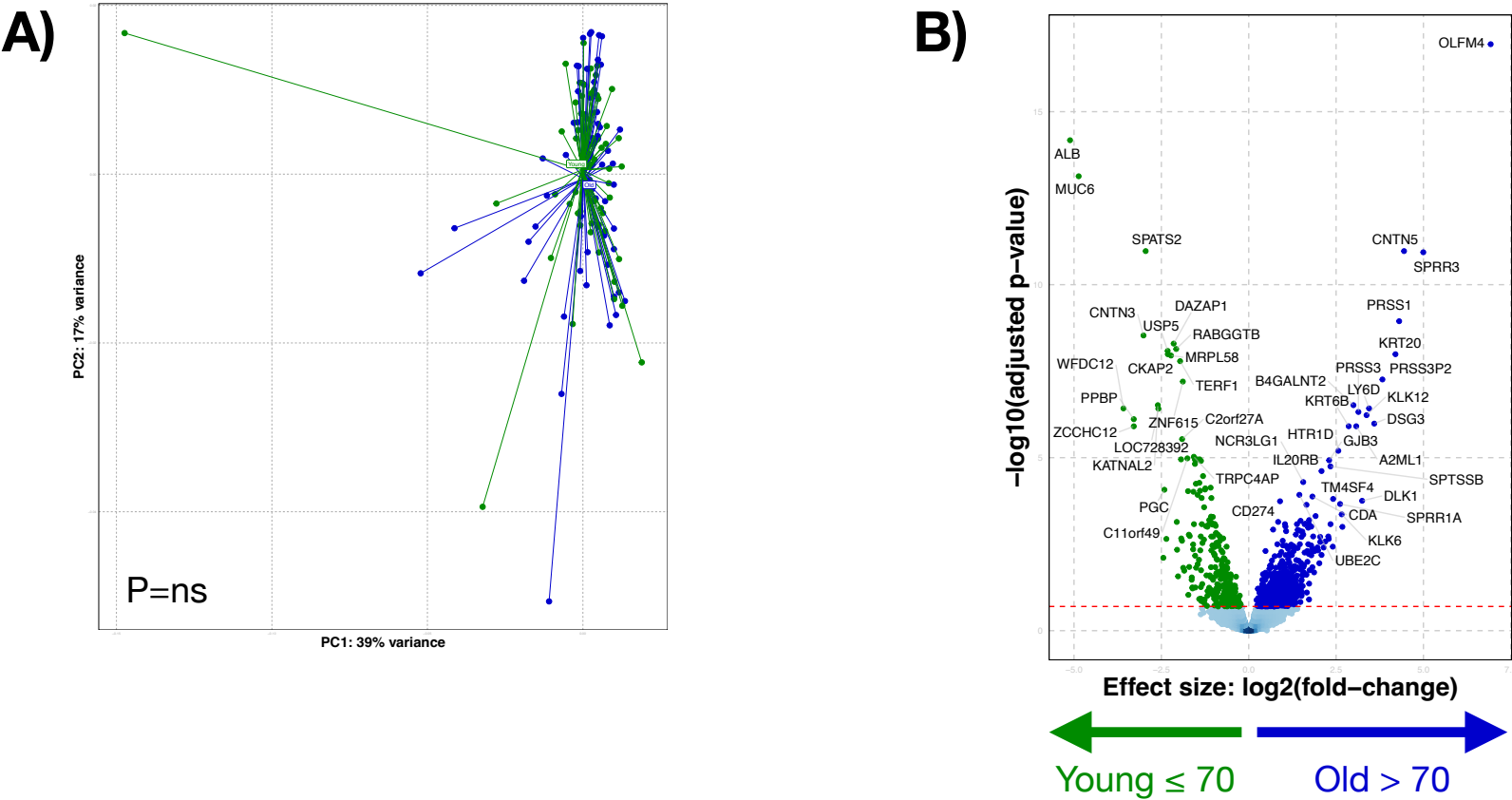


Supplementary Figure 2: Kaplan–Meier curve of recurrence according to age in external cohorts. (A) No significant differences in recurrence-free survival were observed between patients young (≤ 70 years) and old (> 70 years) in TCGA cohort (log rank test, $p = 0.69$). (B) TRACERx cohort results showing significantly higher recurrence rate among older patients ($p = 0.014$). Numbers at risk and censored subjects are shown below the plot.

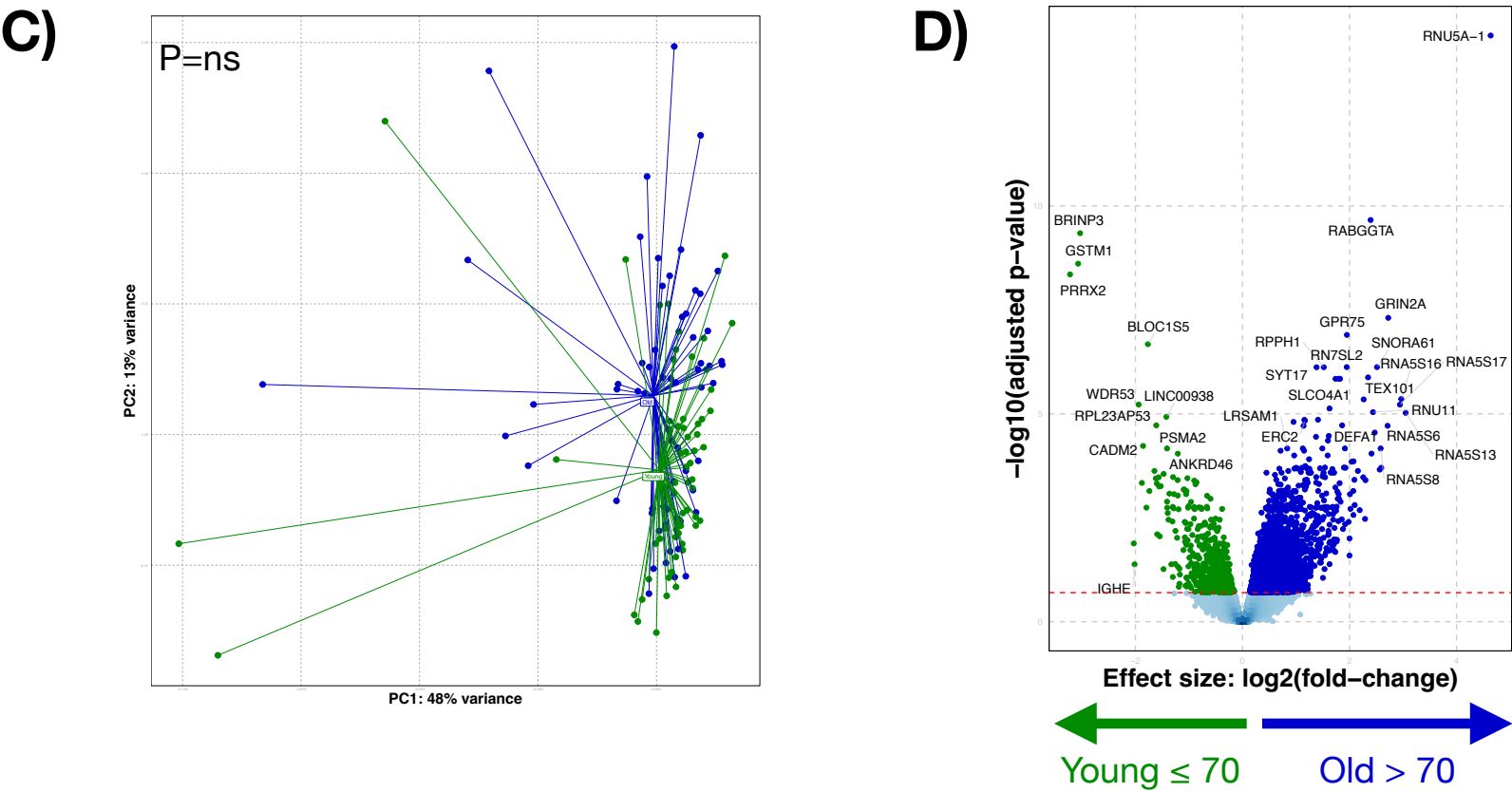
Supplementary Figure 3

NYU Cohort

Tumor



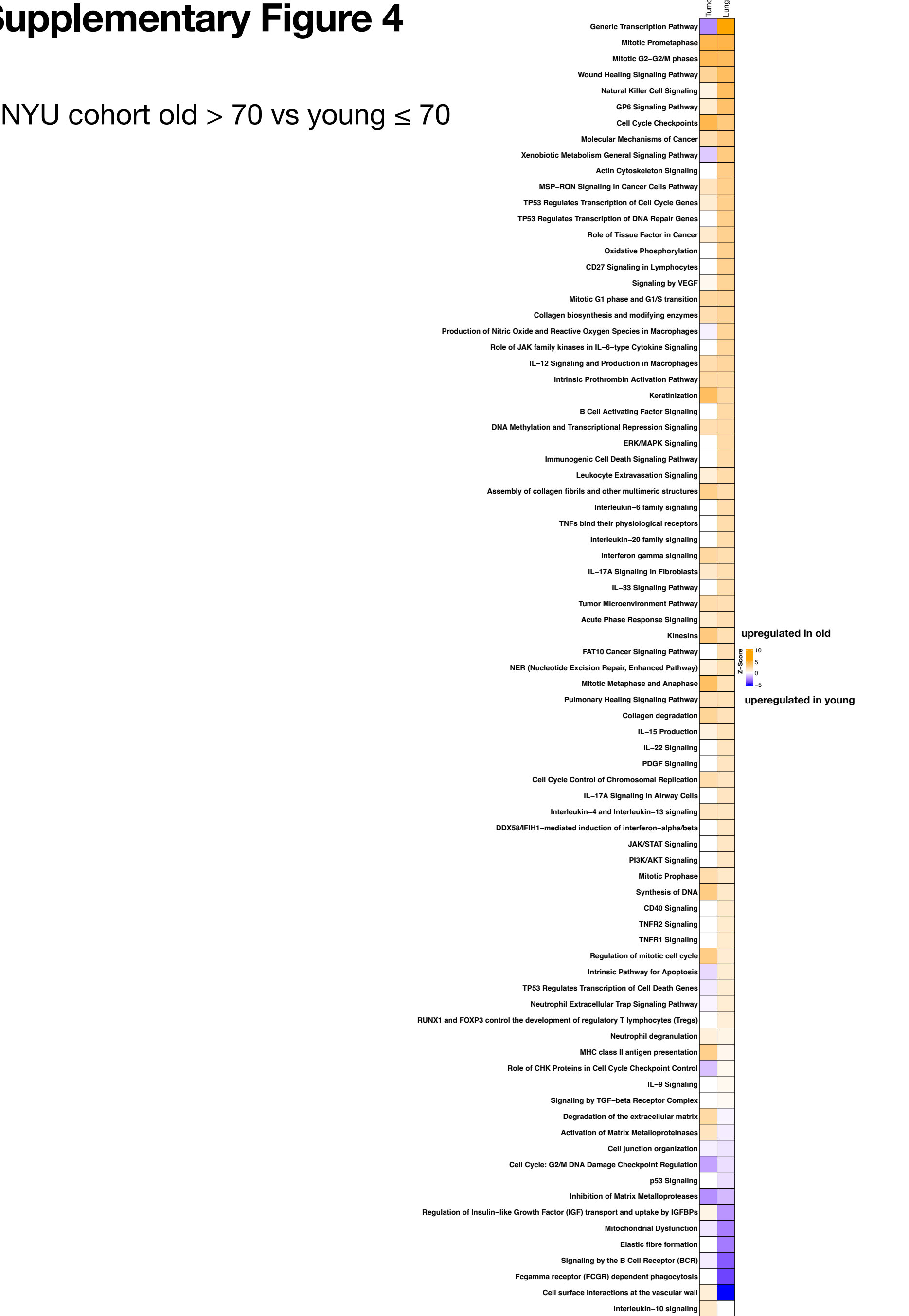
Lung



Supplementary Figure 3: Transcriptomic signatures associated with age in tumors and adjacent non-tumor lung (NAT) samples from the NYU cohort. (A) Principal coordinate analysis (PCoA) showing overall transcriptomic differences between young (≤ 70 years) and old (> 70 years) patients in tumor tissue (PERMANOVA, $p = \text{ns}$). (B) Volcano plots of differentially expressed genes between young and old groups in tumor tissue. Genes upregulated in older patients are shown in blue, and those in younger patients in green. (C) PCoA showing overall transcriptomic differences between young (≤ 70 years) and old (> 70 years) patients in NAT (PERMANOVA, $p = \text{ns}$). (D) Volcano plots of differentially expressed genes between young and old groups in NAT. Genes upregulated in older patients are shown in blue, and those in younger patients in green.

Supplementary Figure 4

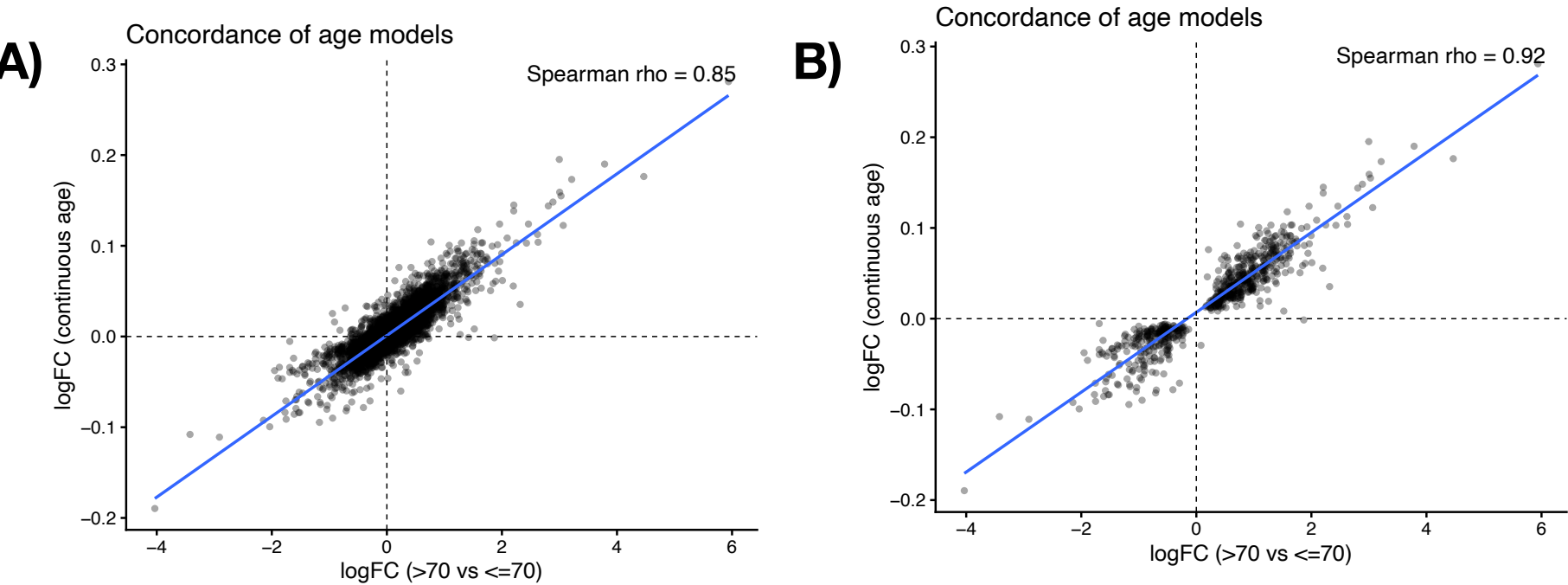
NYU cohort old > 70 vs young ≤ 70



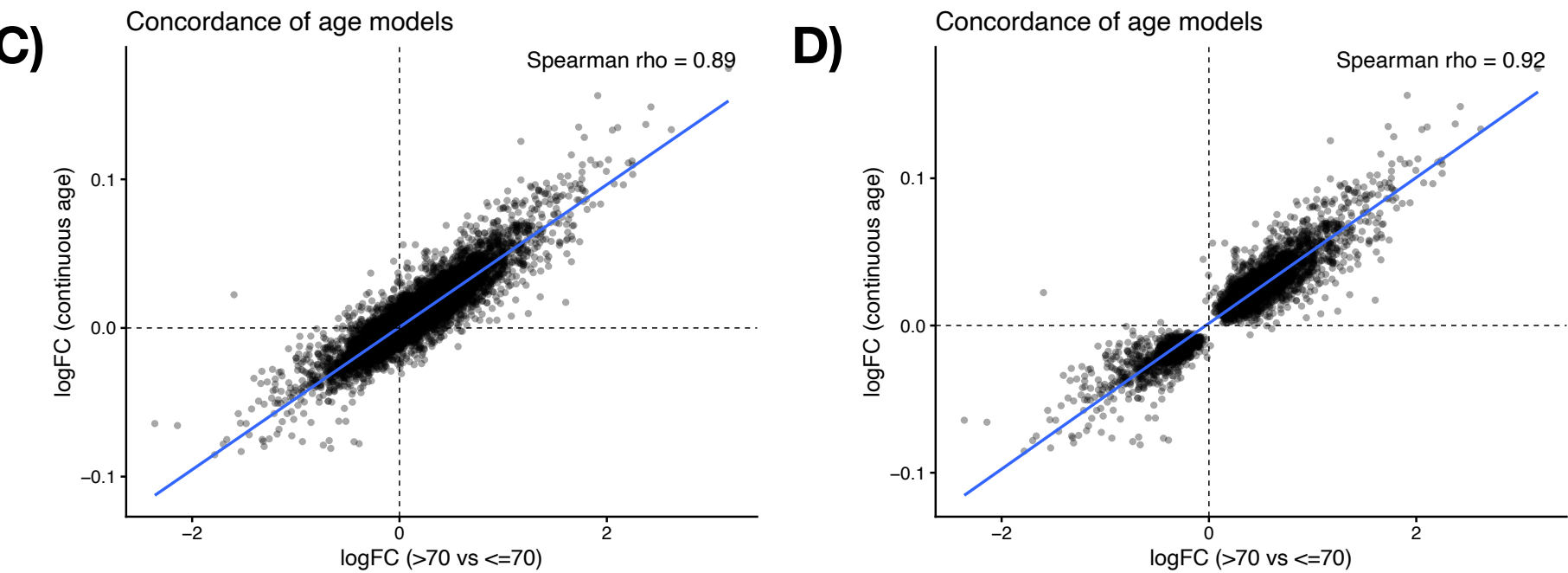
Supplementary Figure 4: Canonical pathway analysis by Ingenuity Pathways Analysis (IPA) of age-associated differential expression in the NYU cohort. A heatmap of canonical pathway analysis based on IPA comparing old (>70 years) versus young (≤ 70 years) patients in both tumor and normal adjacent tissue. Yellow shows upregulation of pathway, and blue shows downregulation.

Supplementary Figure 5

Tumor



Lung



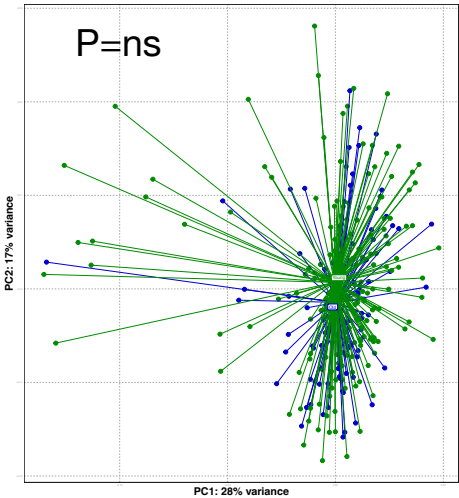
Supplementary Figure 5: Concordance between dichotomized and continuous age models. Scatter plots comparing gene-level log fold changes (logFC) derived from age modeled as a dichotomous variable (>70 vs ≤70 years; x-axis) versus age modeled as a continuous variable (y-axis). Each point represents a gene. Panels A–B show tumor samples and panels C–D show normal adjacent lung (NAT) samples. Panels A and C include all genes, while panels B and D are restricted to genes meeting the predefined significance threshold (FDR < 0.2). Spearman correlation coefficients (ρ) indicate strong concordance between modeling approaches, supporting robustness of age-associated transcriptomic findings.

Supplementary Figure 6

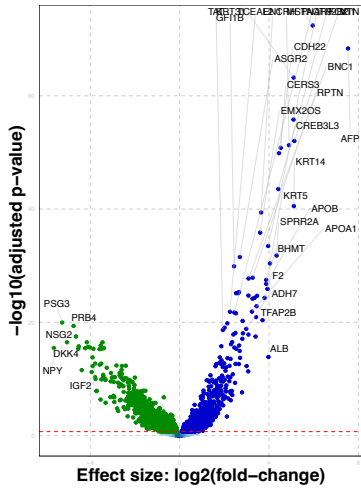
TCGA Cohort

Tumor

A)



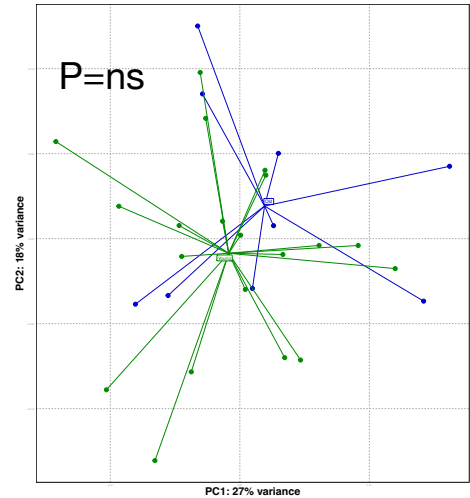
B)



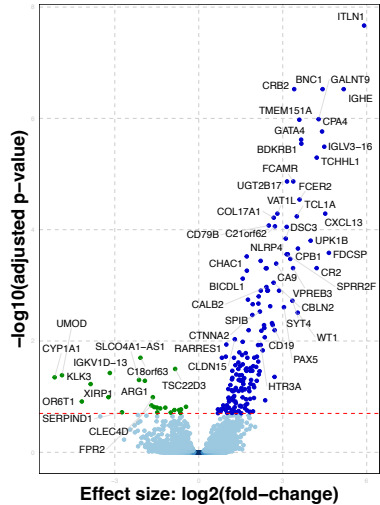
Young ≤ 70 Old > 70

Lung

C)

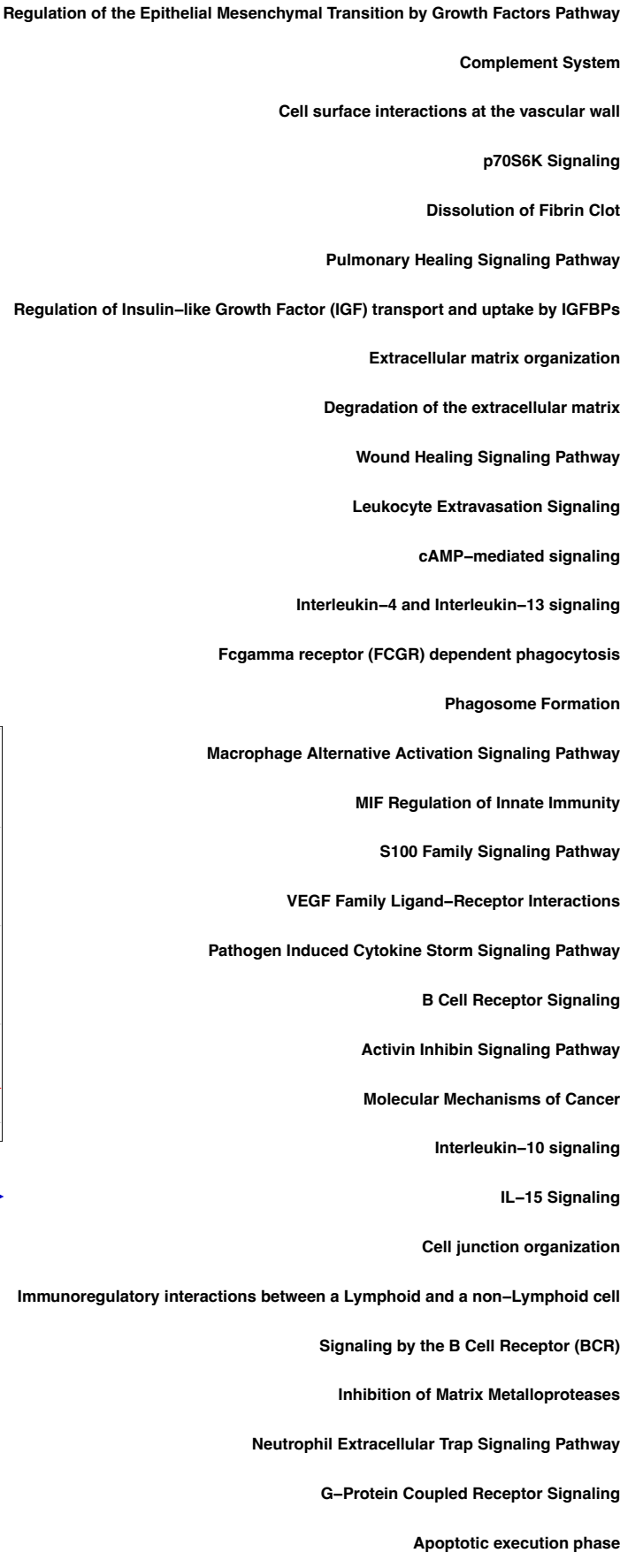


D)



Young ≤ 70 Old > 70

E)

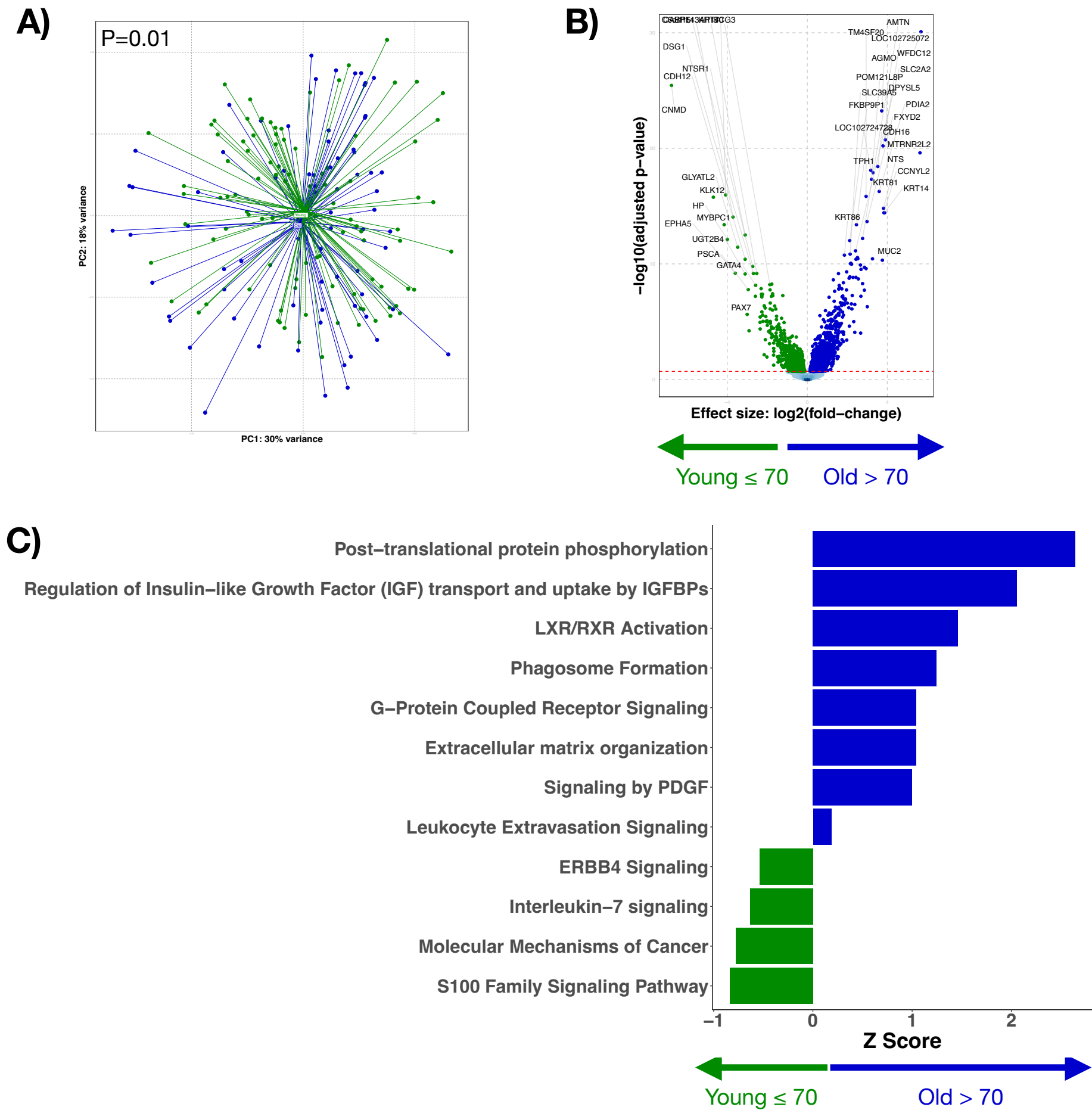


Supplementary Figure 6: Transcriptomic signatures associated with age in tumors and adjacent non-tumor lung (NAT) samples in the TCGA cohort. (A) Principal coordinate analysis (PCoA) showing overall transcriptomic differences between young (≤ 70 years) and old (> 70 years) patients in tumor tissue (PERMANOVA, $p = ns$). (B) Volcano plots of differentially expressed genes between young and old groups in tumor tissue. Genes upregulated in older patients are shown in blue, and those in younger patients in green. (C) PCoA showing overall transcriptomic differences between young (≤ 70 years) and old (> 70 years) patients in NAT (PERMANOVA, $p = ns$). (D) Volcano plots of differentially expressed genes between the young and old groups in NAT. Genes upregulated in older patients are shown in blue, and those in younger patients are shown in green. (E) A heatmap of canonical pathway analysis based on IPA comparing old (> 70 years) versus young (≤ 70 years) patients in both tumor and normal adjacent tissue. Yellow shows upregulation of pathways, and blue shows downregulation.

Supplementary Figure 7

TRACERxCohort

Tumor



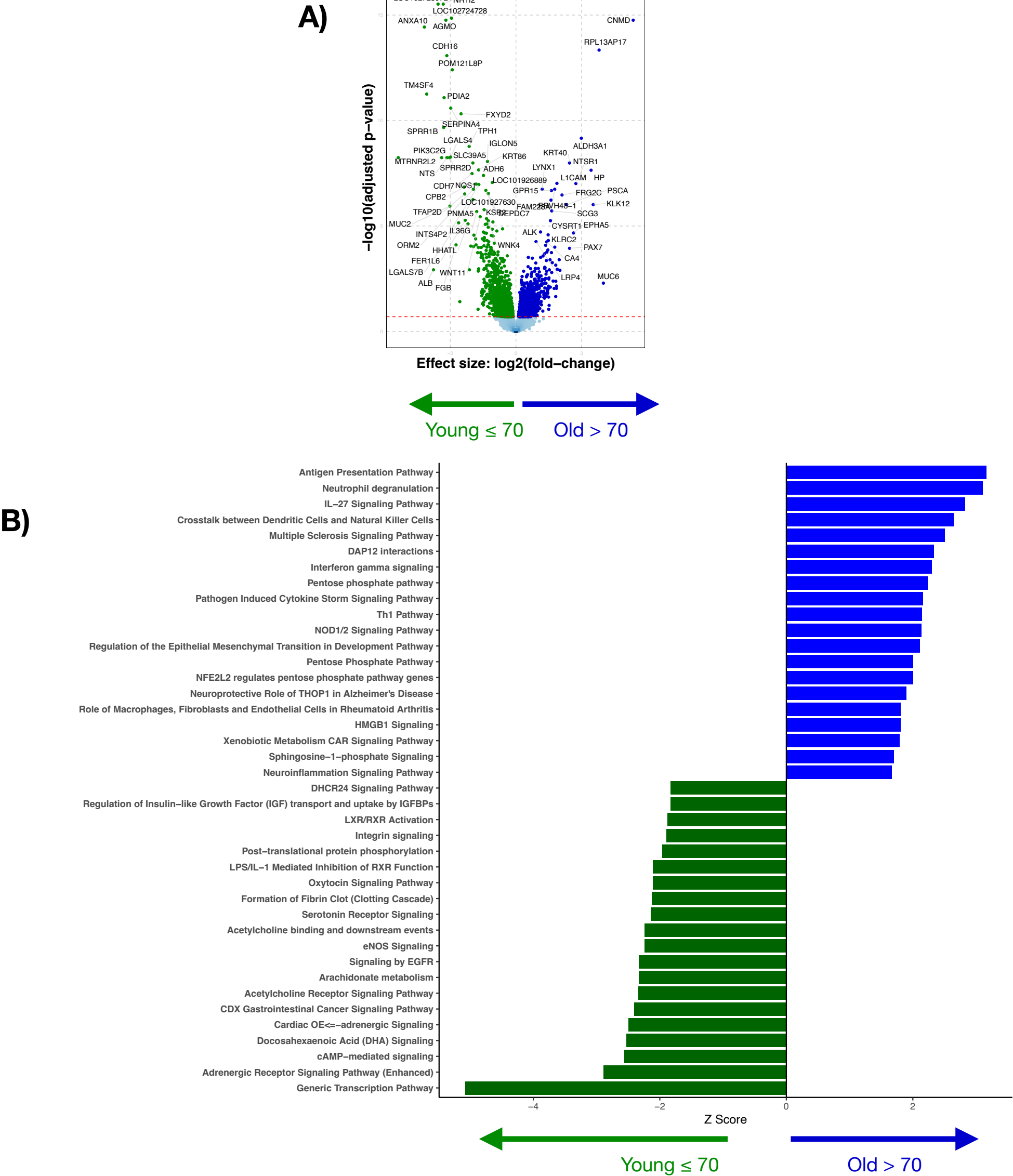
Supplementary Figure 7: Age-associated transcriptomic differences in tumors from the TRACERx cohort. (A) Principal coordinate analysis (PCoA) showing overall transcriptomic differences between young (≤ 70 years) and old (> 70 years) patients (PERMANOVA, $p=0.01$). (B) Volcano plot of differentially expressed genes by age group; Genes upregulated in older patients are shown in blue, and those in younger patients in green. (C) A barplot based on IPA showing z-score values of pathways upregulated in old vs. young.

upregulated in
young

Supplementary Figure 8: Transcriptomic signatures associated with age in tumors and adjacent non-tumor lung (NAT) samples from the NYU and TCGA cohorts following adjusting for clinical covariates. (A) Volcano plots of differentially expressed genes between young and old groups in tumor tissue. Genes upregulated in older patients are shown in blue, and those in younger patients in green. (B) Volcano plots of differentially expressed genes between young and old groups in NAT. Genes upregulated in older patients are shown in blue, and those in younger patients in green. (C) Canonical pathway analysis by Ingenuity Pathways Analysis (IPA) of age-associated differential expression in the NYU cohort. A heatmap of canonical pathway analysis based on IPA comparing old (>70 years) versus young (≤70 years) patients in both tumor and normal adjacent tissue. Yellow shows upregulation of pathway, and blue shows downregulation. (D) Differentially expressed genes between young and old groups in tumor tissue, TCGA cohort. (E) Differentially expressed genes between young and old groups in NAT tissue, TCGA cohort. (F) Canonical pathway analysis of age associated differentially expressed genes in the TCGA cohort. A heatmap of canonical pathway analysis based on IPA comparing old (>70 years) versus young (≤70 years) patients in both tumor and normal adjacent tissue

Supplementary Figure 9

TRACERxCohort



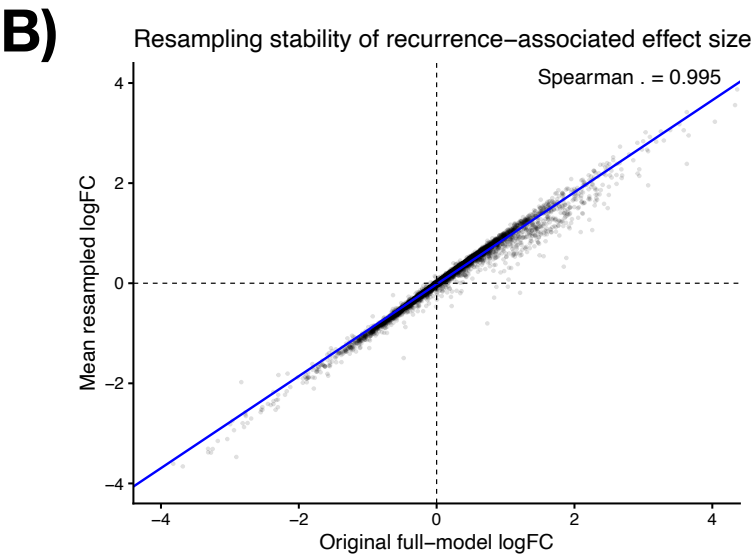
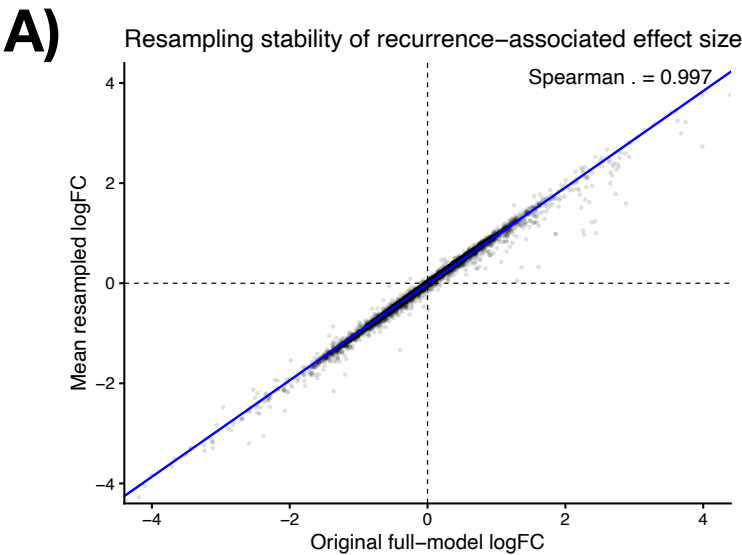
Supplementary Figure 9: Age-associated transcriptomic differences in tumors from the TRACERx cohort after adjusting for clinical covariates (A) Volcano plot of differentially expressed genes by age group; Genes upregulated in older patients are shown in blue, and those in younger patients in green. (B) A barplot based on IPA showing z-score values of pathways upregulated in old vs. young.

Supplementary Figure 10

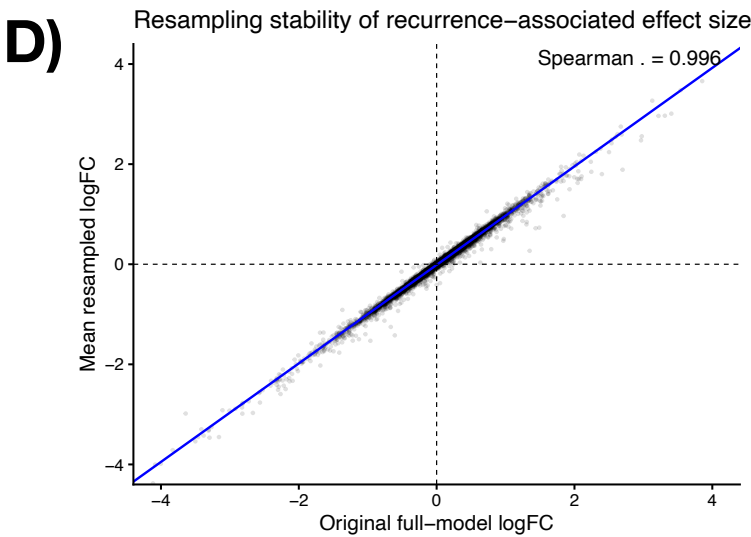
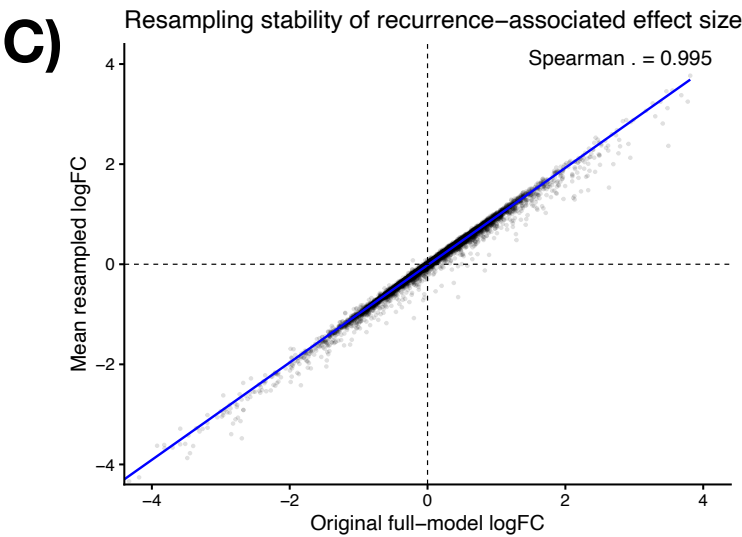
Tumor

Old

Young



Lung



Tumor

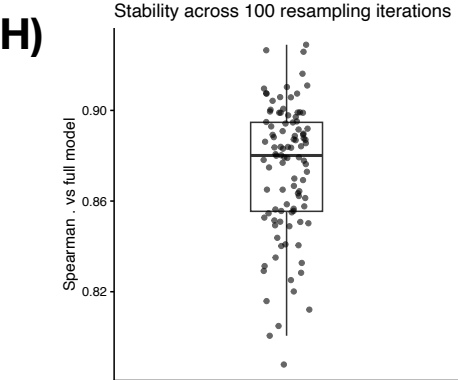
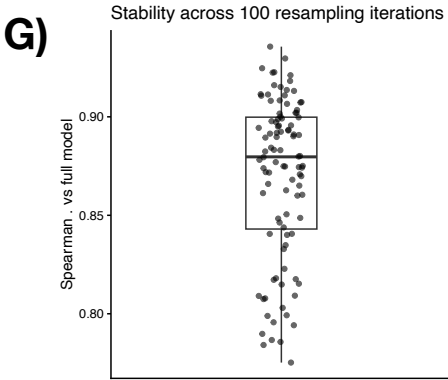
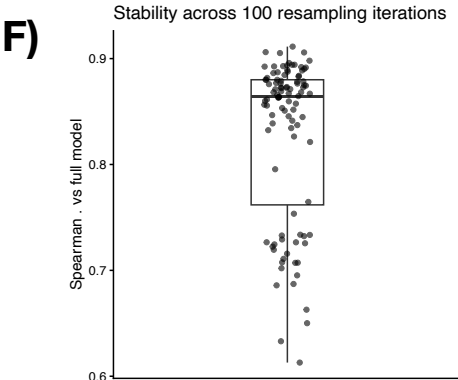
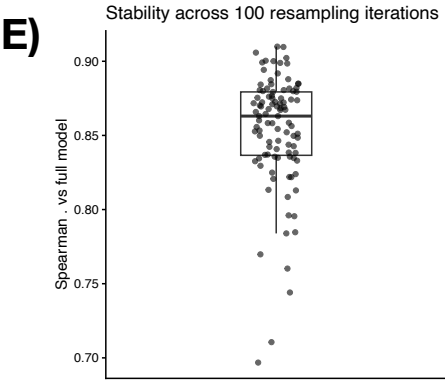
Lung

Old

Young

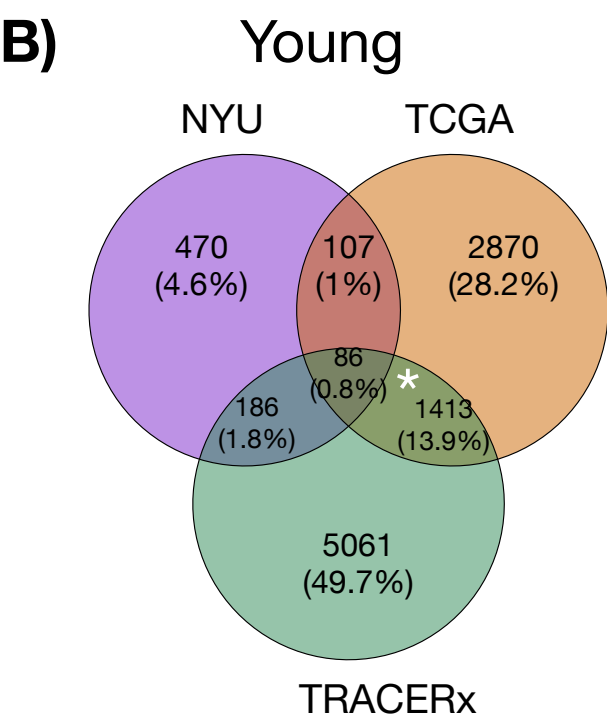
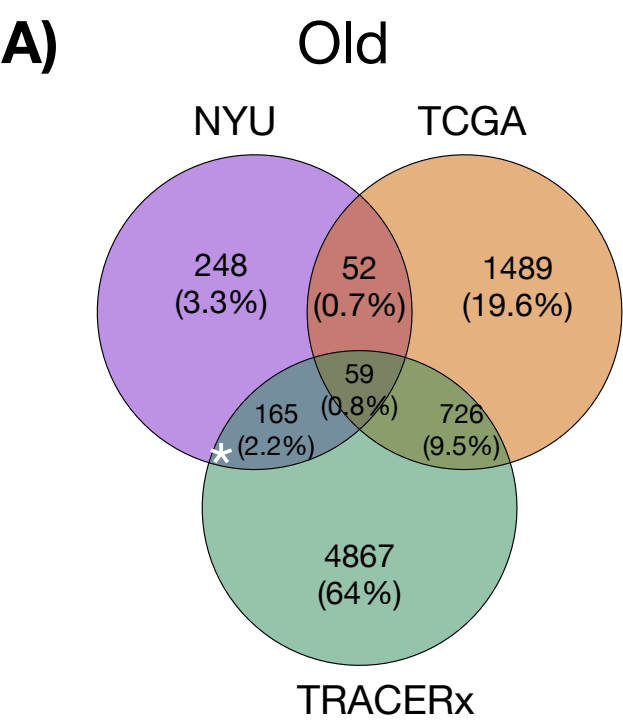
Old

Young



Supplementary Figure 10: Resampling-based stability of recurrence-associated transcriptomic signals. Panels A–D show the concordance between mean resampled log fold changes and the original full-cohort model for tumor and normal adjacent lung (NAT) samples stratified by age group. Panels E–H show the distribution of Spearman correlation coefficients across 100 resampling iterations. High concordance across all analyses ($\rho \approx 0.85$ – 0.90 across iterations; mean-profile $\rho \approx 0.995$ – 0.997) demonstrates robustness of recurrence-associated transcriptomic effects

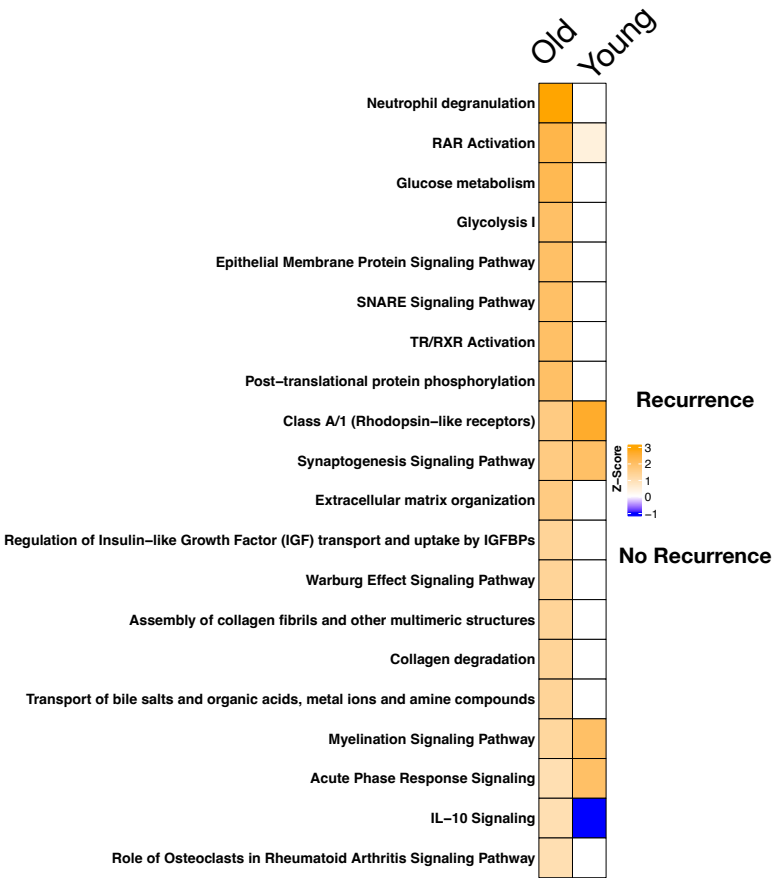
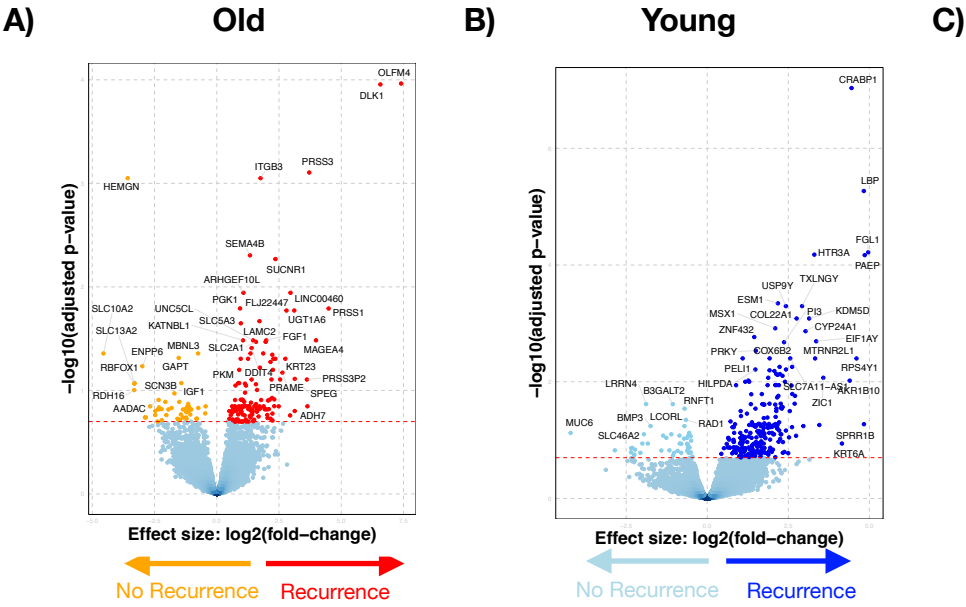
Supplementary Figure 11



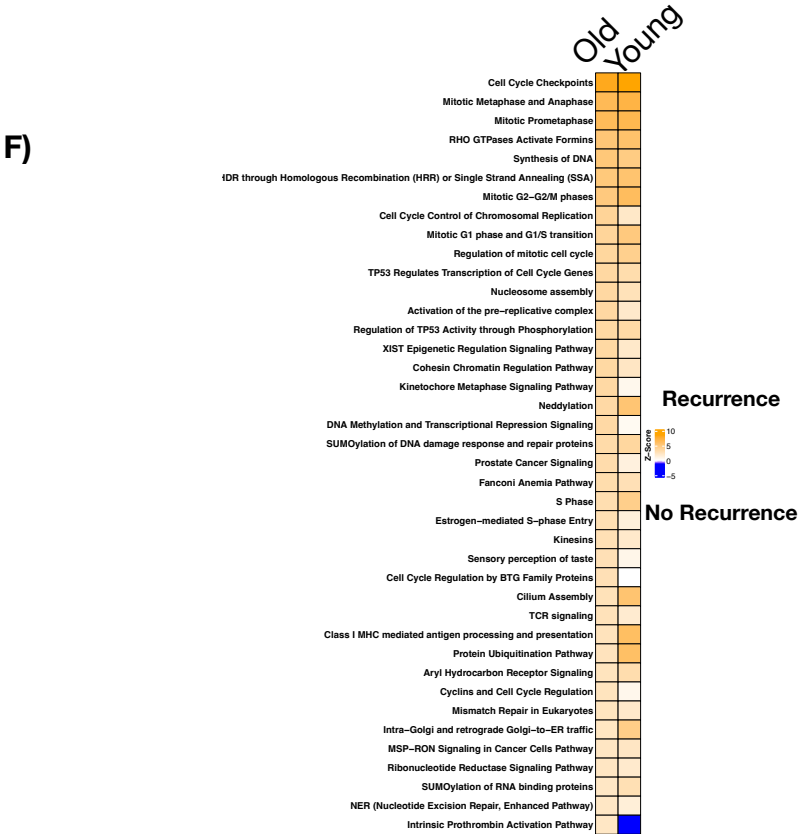
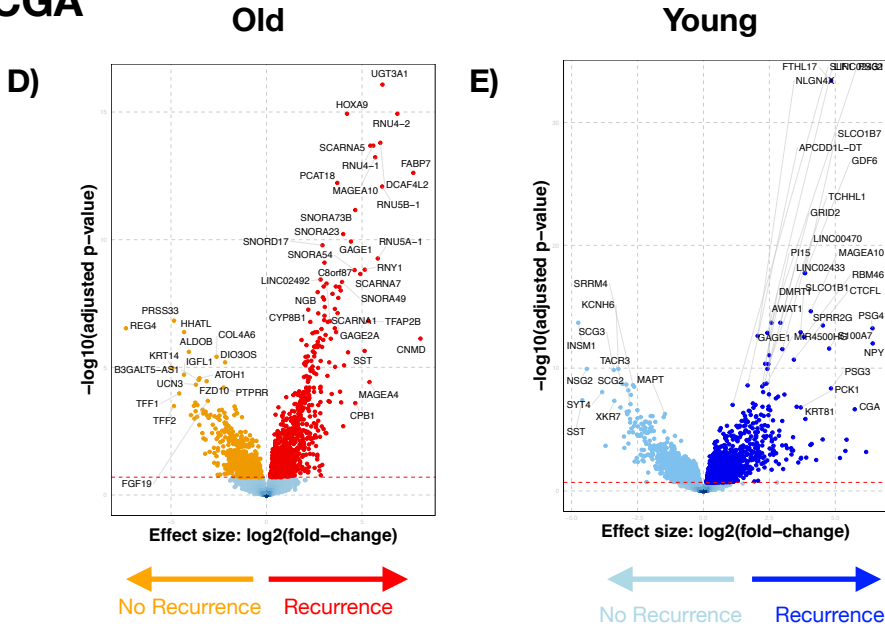
Supplementary Figure 11: Shared genes between the NYU, TCGA and TRACERx cohorts' gene datasets associated with recurrence among A) old and B) young patients. Asterisks resemble statistically significant GSEA analysis (FDR <0.05) showing a significant overlap between two groups.

Supplementary Figure 12

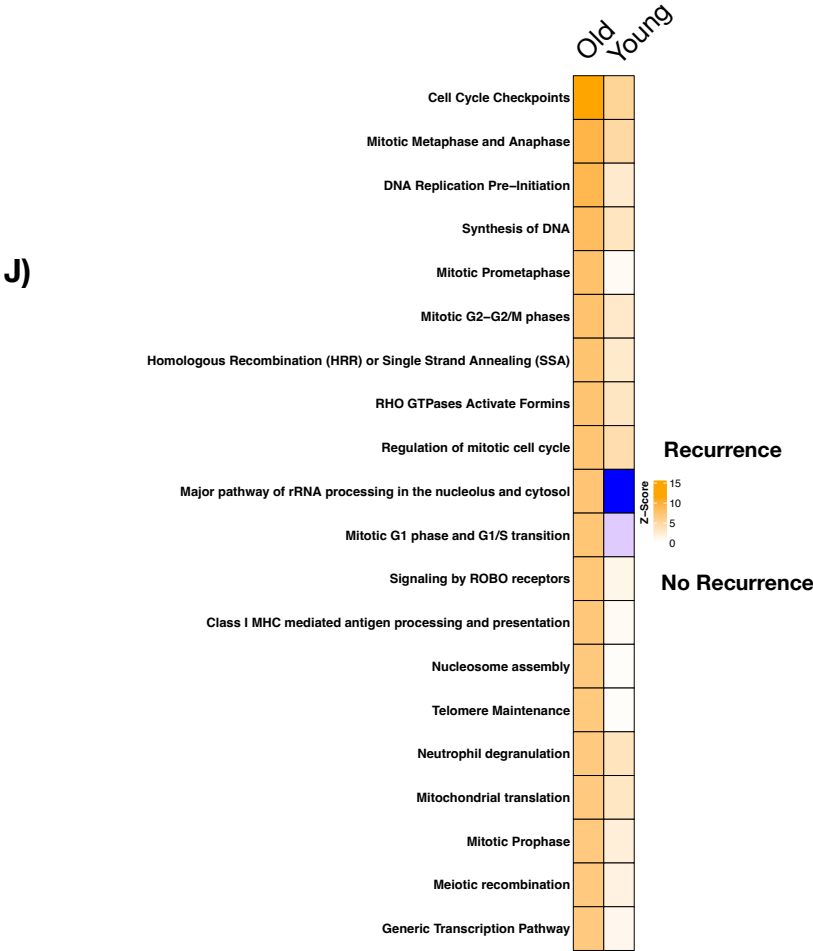
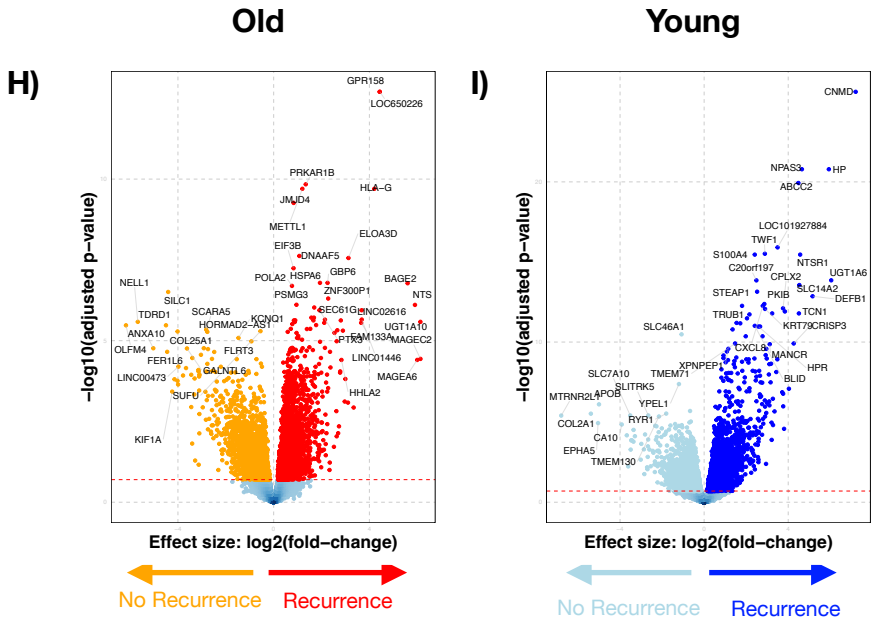
NYU



TCGA

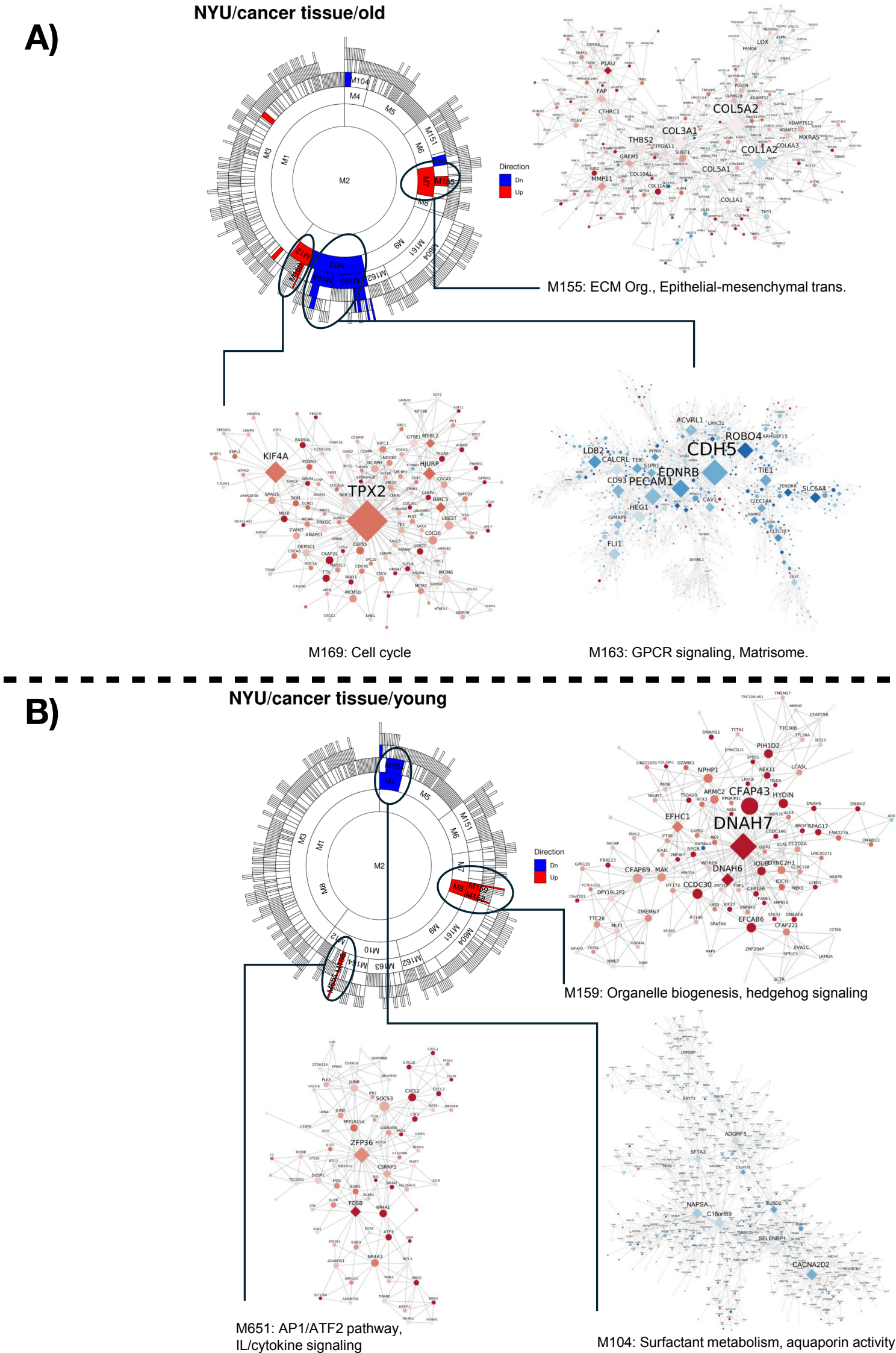


TRACERx



Supplementary Figure 12: Transcriptomic signatures associated with recurrence in tumor tissue following adjusting for clinical covariates. (A, B) volcano plots of differentially expressed genes (FDR <0.2) between subjects with recurrence and without recurrence in old and young patients in the NYU cohort, respectively. (C) heatmap of canonical pathway analysis based on Ingenuity Pathway Analysis (IPA) comparing recurrence vs. no recurrence groups in old and young patients within the NYU cohort. Yellow shows upregulation of pathways, and blue shows downregulation. Similar analysis pattern in (D-F) TCGA cohort, and (H-J) TRACERx cohort.

Supplementary Figure 13

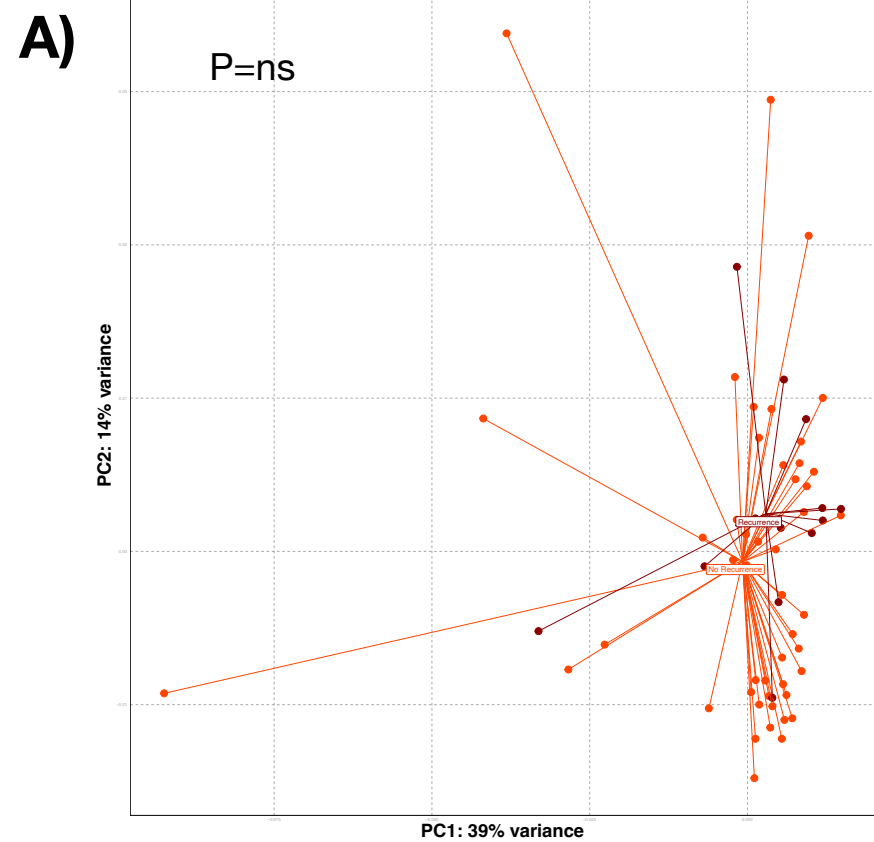


Supplementary Figure 13: MEGENA-based co-expression network analysis of tumor samples in the NYU cohort. Sunburst plots show genes co-expression modules identified by MEGENA analysis that are associated with recurrence in (A) old and (B) young patients. Red shows upregulation of module, and blue downregulation of module. Major modules are highlighted: (A) Modules associated with recurrence in older patients include ECM remodeling (M155), cell cycle (M169), and GPCR/matrisome signaling (M163). (B) Modules associated with recurrence in younger patients include organelle biogenesis (M159), surfactant metabolism (M104), and AP1/ATF2/IL-cytokine signaling (M651).

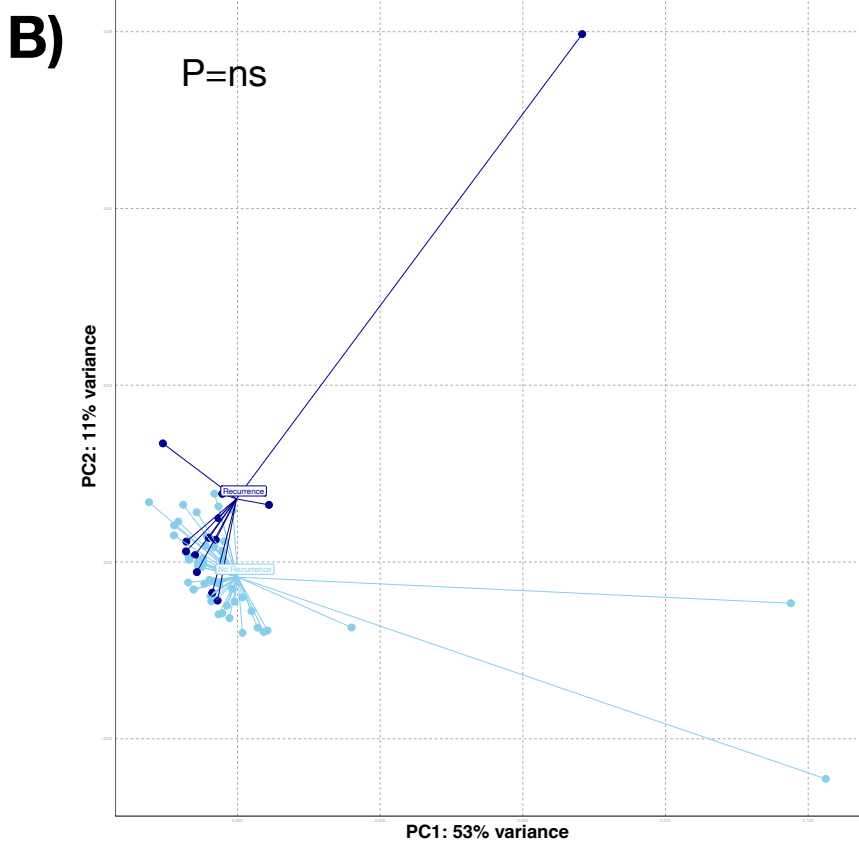
Supplementary Figure 14

NYU Cohort - NAT

Old > 70

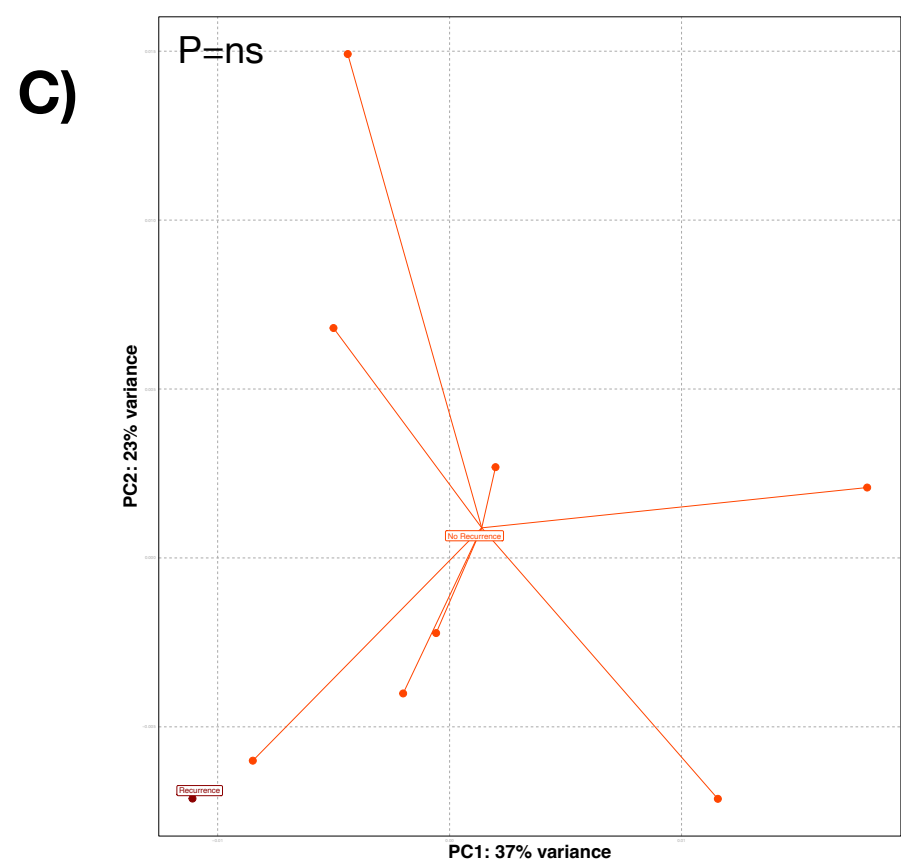


Young ≤ 70

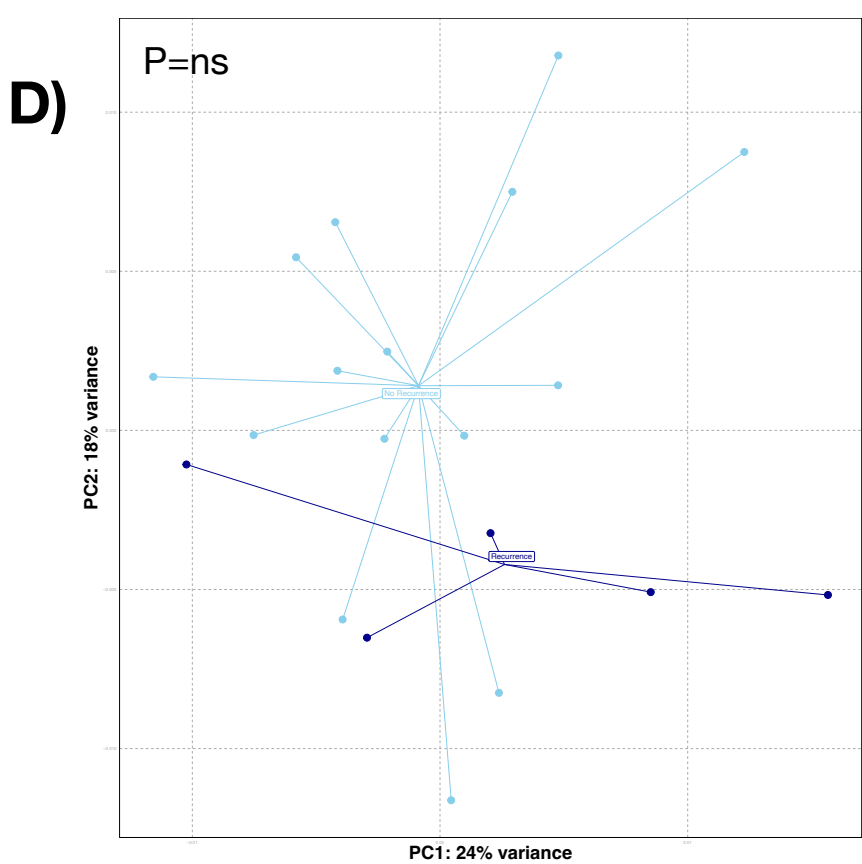


TCGA Cohort - NAT

Old > 70

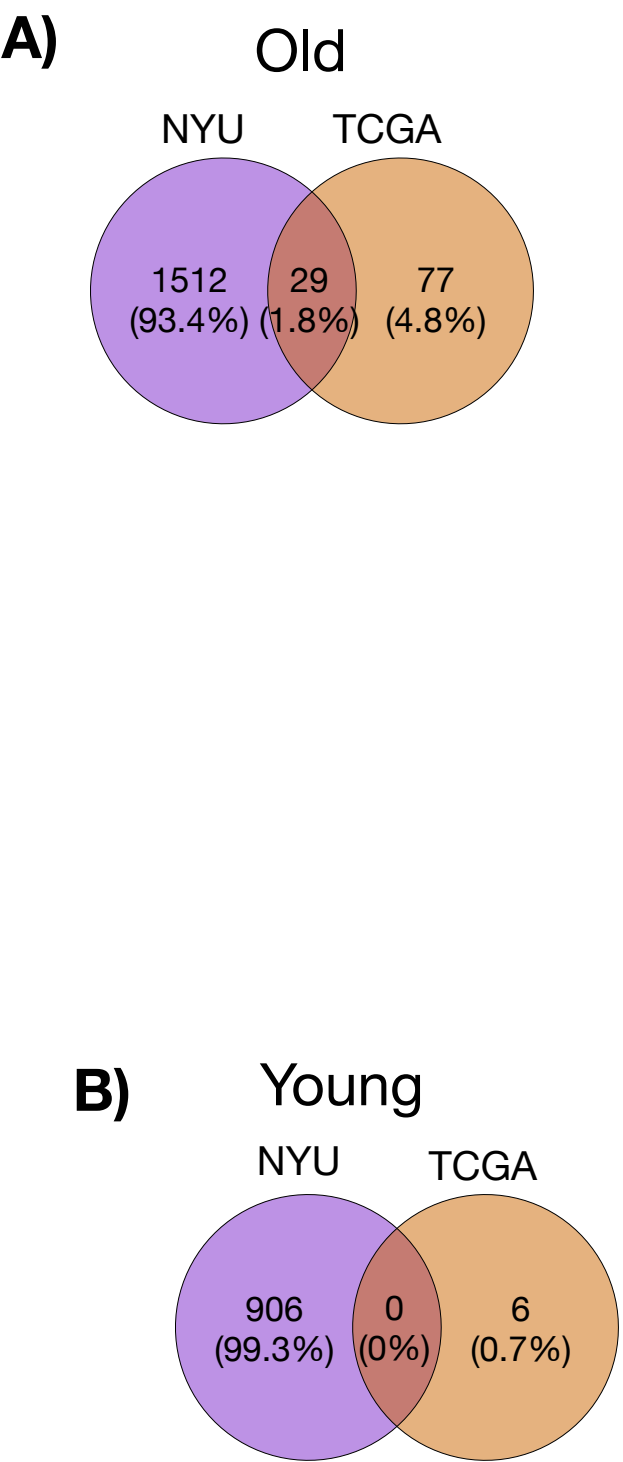


Young ≤ 70



Supplementary Figure 14: Recurrence associated transcriptomic in normal adjacent tissue samples (NAT) stratified by age. (A, B) Principal Coordinate analysis (PCoA) comparing patients with recurrence vs. no recurrence in NYU cohort NAT samples from old (>70 years) and young (≤70 years) patients. (C, D) PCoA comparing patients with recurrence vs. no recurrence in TCGA cohort NAT samples from old (>70 years) and young (≤70 years) patients. No significant global separation by recurrence was observed in either cohort (PERMANOVA, p=ns).

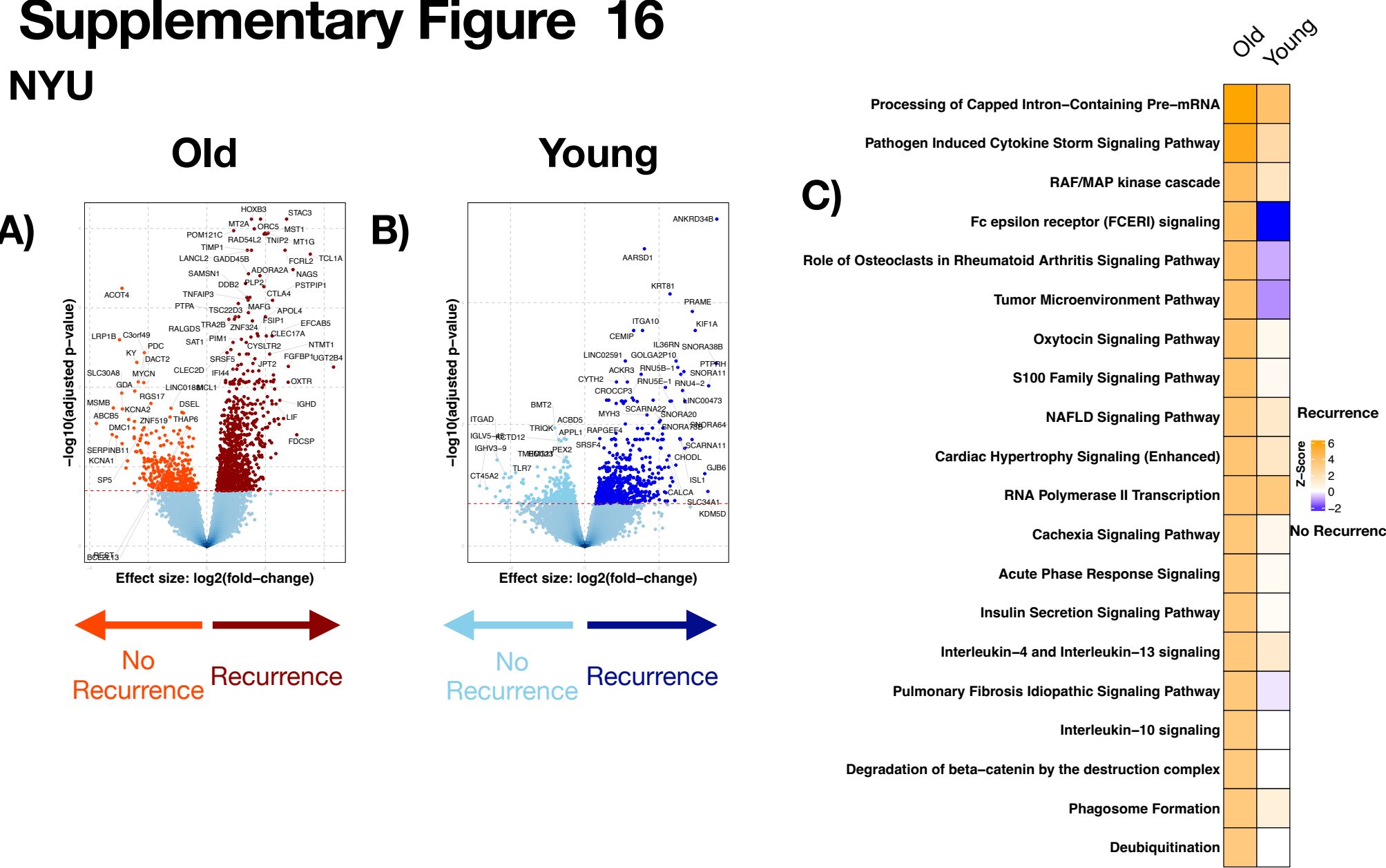
Supplementary Figure 15



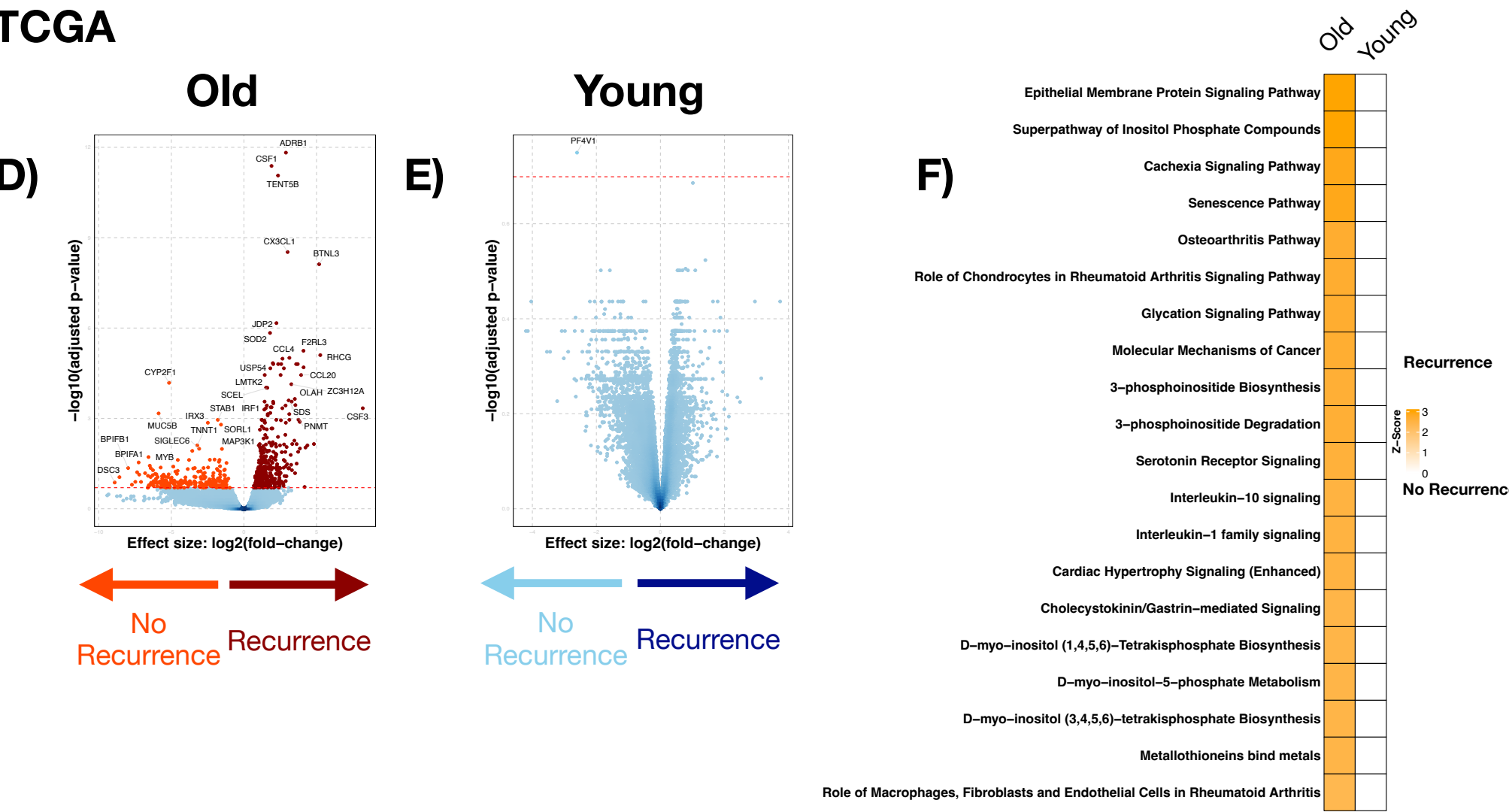
Supplementary Figure 15: Shared genes between the NYU and TCGA cohorts associated with recurrence among A) old and B) young patients. Asterisks resemble statistically significant GSEA analysis ($FDR \leq 0.05$) showing a significant overlap between two groups.

Supplementary Figure 16

NYU



TCGA

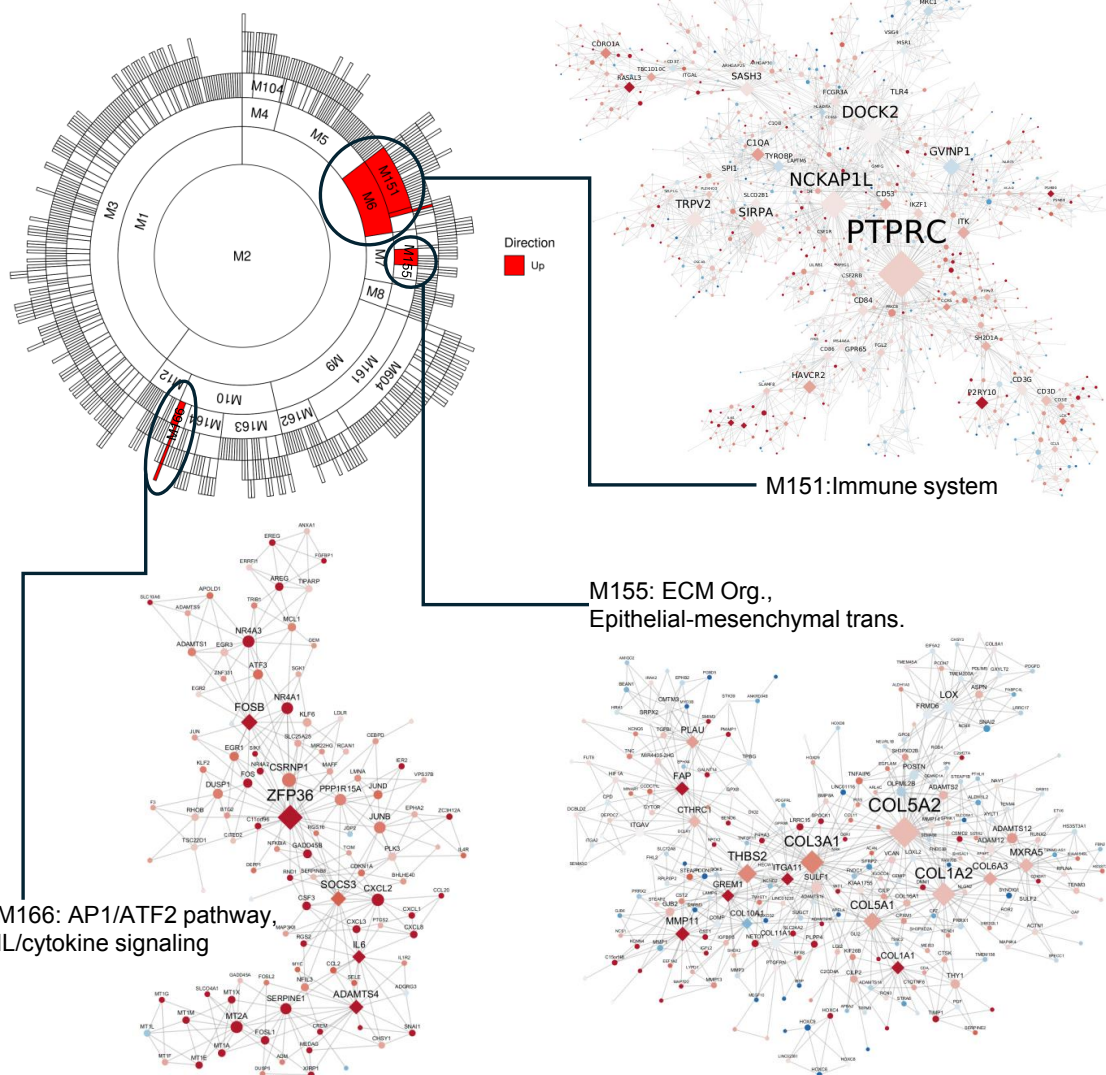


Supplementary Figure 16: Transcriptomic signatures associated with recurrence in NAT tissue following adjusting for clinical covariates. (A, B) volcano plots of differentially expressed genes (FDR <0.2) between subjects with recurrence and without recurrence in old and young patients in the NYU cohort, respectively. (C) heatmap of canonical pathway analysis based on Ingenuity Pathway Analysis (IPA) comparing recurrence vs. no recurrence groups in old and young patients within the NYU cohort. Yellow shows upregulation of pathways, and blue shows downregulation. Similar analysis pattern in (D-F) TCGA cohort.

Supplementary Figure 17

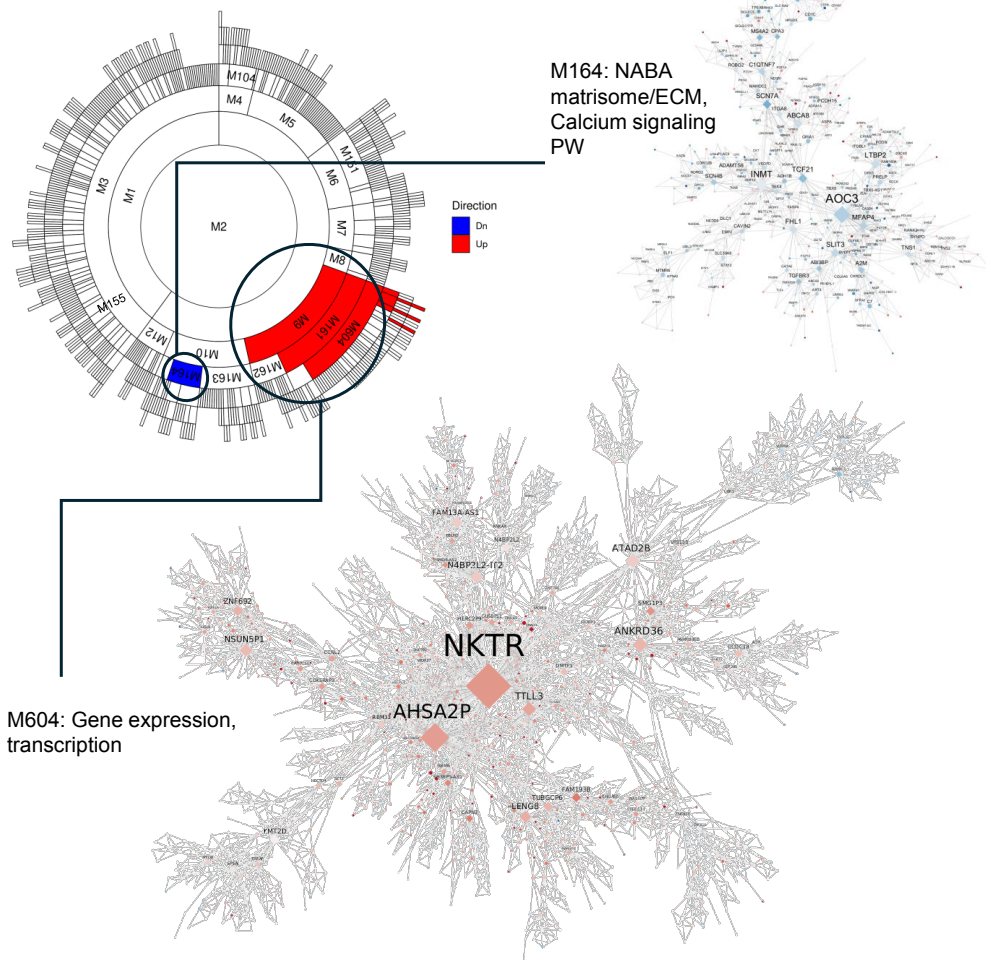
A)

NYU/normal tissue/old



B)

NYU/normal tissue/young



Supplementary Figure 17: MEGENA based co-expression network analysis of normal adjacent tissue samples (NAT) in the NYU cohort. Sunburst plots show genes co-expression modules identified by MEGENA analysis that are associated with recurrence in (A) old and (B) young patients. Red shows upregulation of module, and blue downregulation of module. Major modules are highlighted: (A) Modules associated with recurrence in older patients include immune-related (M151), ECM remodeling (M155), and cytokine/AP1-ATF2 (M168) modules. (B) Modules associated with recurrence in younger patients include enrichment of ECM/matrisome (M164) and transcriptional regulation (M604).

Supplementary data file

Supplementary Data file is an excel file that contains supplementary data as tables organized as follows:

- **Sheet 01:** Cohorts demographics.
- **Sheets 02-08:** Age analysis: comparison of old vs. young, continuous age and adjusted model analyses.
- **Sheets 09–14:** Tumor Recurrence analysis: differential regulated genes comparison of recurrence among old and young patients. NYU, TCGA and TRACERx analyses and adjusted models..
- **Sheets 15-17:** Tumor MEGENA network analysis
- **Sheets 18-19:** Lung Recurrence analysis: differential regulated genes comparison of recurrence among old and young patients. NYU and TCGA analyses and adjusted models
- **Sheets 20-21:** Lung MEGENA network analysis

Tables legends

Supplementary data 1: Demographic and patients characteristics of TCGA and TRACERx cohorts stage I

Supplementary data 2: Genomic differential expression between old and young patients in tumor and lung samples from NYU cohort

Supplementary data 3: Z-score values of Ingenuity Pathway Analysis (IPA) while comparing old vs. young groups in NYU cohort among tumor and lung samples

Supplementary data 4: Genomic differential expression according to continuous age in tumor and lung samples from NYU cohort

Supplementary data 5: Genomic differential expression between old and young patients in tumor and lung samples from TCGA and TRACERx stage I cohorts

Supplementary data 6: Z-score values of Ingenuity Pathway Analysis (IPA) while comparing old vs. young groups in TCGA and TRACERx stage I cohort among tumor and lung samples tumor samples

Supplementary data 7: Genomic differential expression between old and young patients in all cohorts after adjusting for clinical covariates

Supplementary data 8: Z-score values of Ingenuity Pathway Analysis (IPA) while comparing old vs. young groups in all cohorts following adjusting for clinical covariates

Supplementary data 9: Genomic differential expression between recurrence and no recurrence groups among old and young patients in tumor and lung samples from NYU cohort

Supplementary data 10: Z-score values of Ingenuity Pathway Analysis (IPA) while comparing recurrence to no recurrence among old and young groups in in NYU cohort among tumor and lung samples

Supplementary data 11: Genomic differential expression between recurrence and no recurrence groups among old and young patients in tumor and lung samples from TCGA and TRACERx stage I cohort

Supplementary data 12: Z-score values of Ingenuity Pathway Analysis (IPA) while comparing old vs. young groups in TCGA and TRACERx stage I cohort among tumor and lung samples

Supplementary data 13: Genomic differential expression between patients with recurrence and no recurrence among old and young patients in tumor samples in all cohorts after adjusting for clinical covariates

Supplementary data 14: Z-score values of Ingenuity Pathway Analysis (IPA) while comparing recurrence vs. no recurrence groups among old and young patients in tumor samples of all cohorts following adjusting for clinical covariates

Supplementary data 15: MEGENA modules functional enrichments in all cohorts

Supplementary data 16: MEGENA modules enrichment with recurrence associated differentially expressed genes in tumor

Supplementary data 17: Enrichment of co-expression modules with recurrence-associated DEGs in tumor tissue using discrete and continuous age interaction model

Supplementary data 18: Genomic differential expression between patients with recurrence and no recurrence among old and young patients in NAT samples in all cohorts after adjusting for clinical covariates

Supplementary data 19: Z-score values of Ingenuity Pathway Analysis (IPA) while comparing recurrence vs. no recurrence groups among old and young patients in NAT samples in NYU and TCGA cohorts following adjusting for clinical covariates

Supplementary data 20: MEGENA modules enrichment with recurrence associated differentially expressed genes in normal adjacent tissue (NAT)

Supplementary data 21: Enrichment of co-expression modules with recurrence-associated DEGs in NAT tissue using discrete and continuous age interaction model