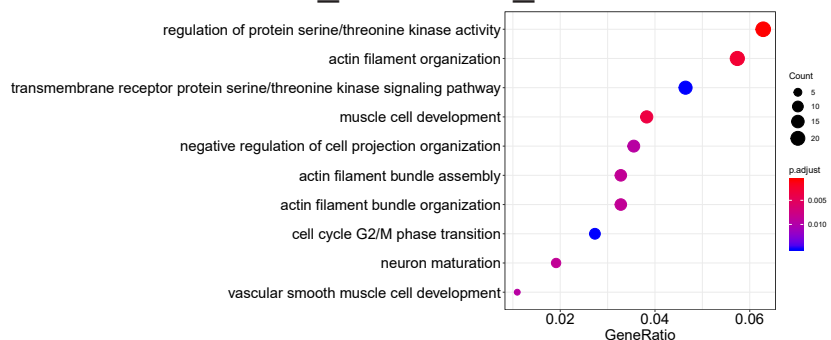


# SUPPLEMENTAL 1

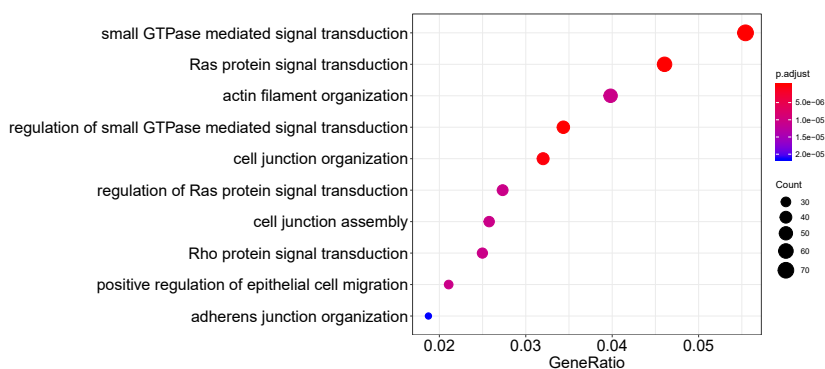
**A**

## FGF\_decreased\_H3K27ac



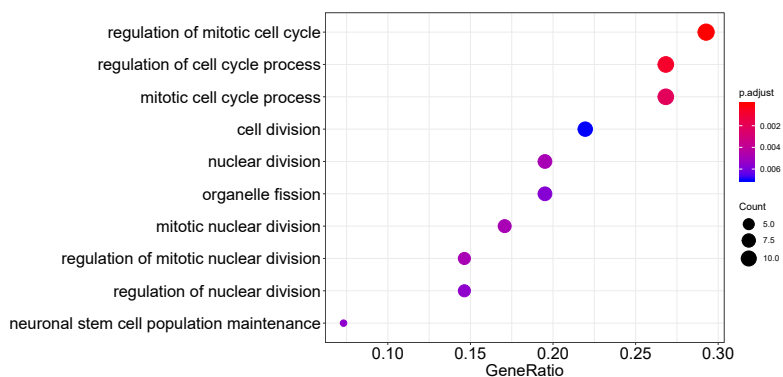
**B**

## FGF\_increased\_H3K27ac



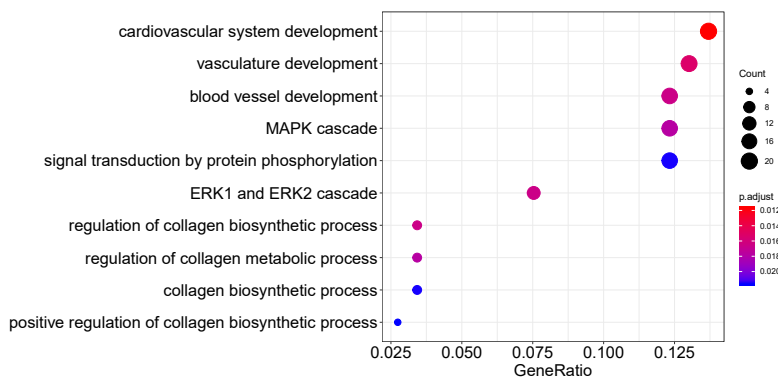
**C**

## FGF\_decreased\_concordant\_H3K27ac\_RNA



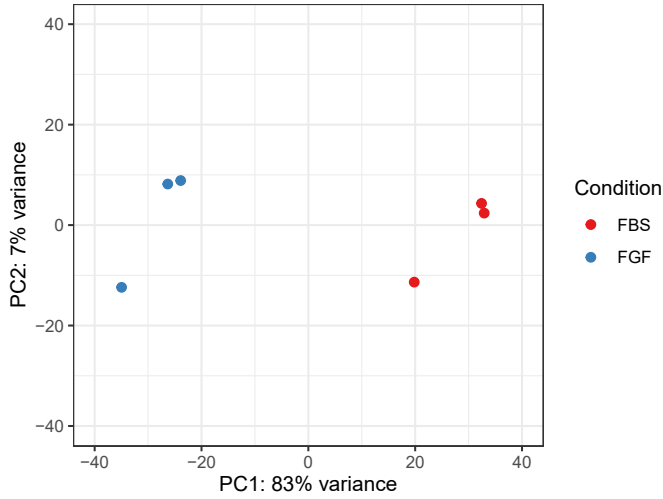
**D**

## FGF\_increased\_concordant\_H3K27ac\_RNA

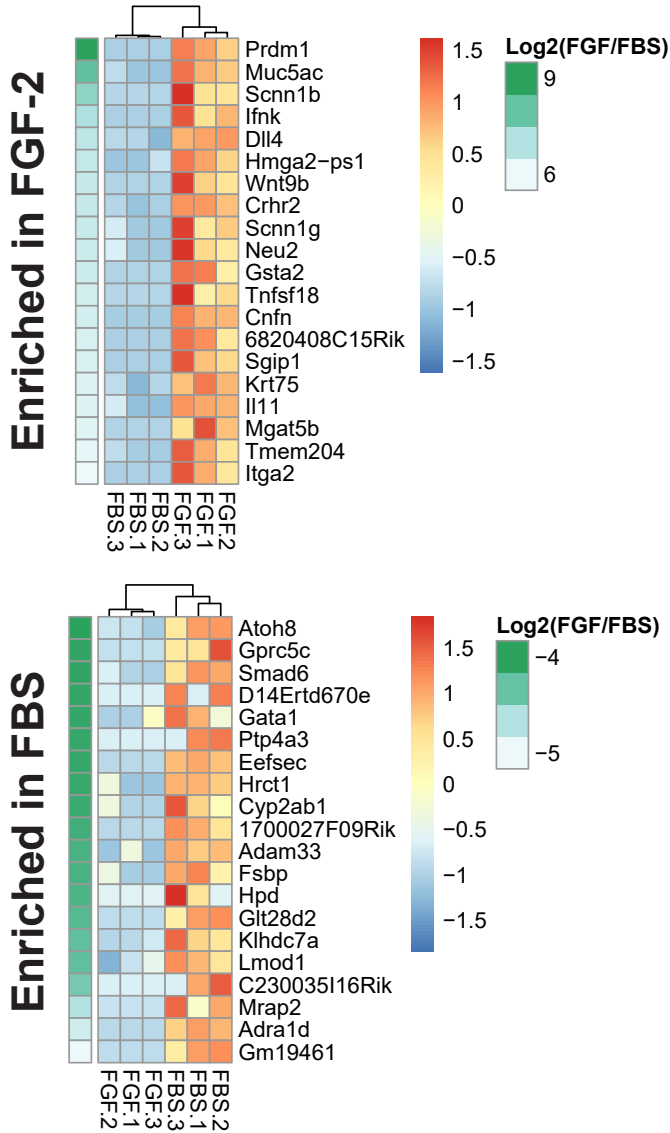


# SUPPLEMENTAL 2

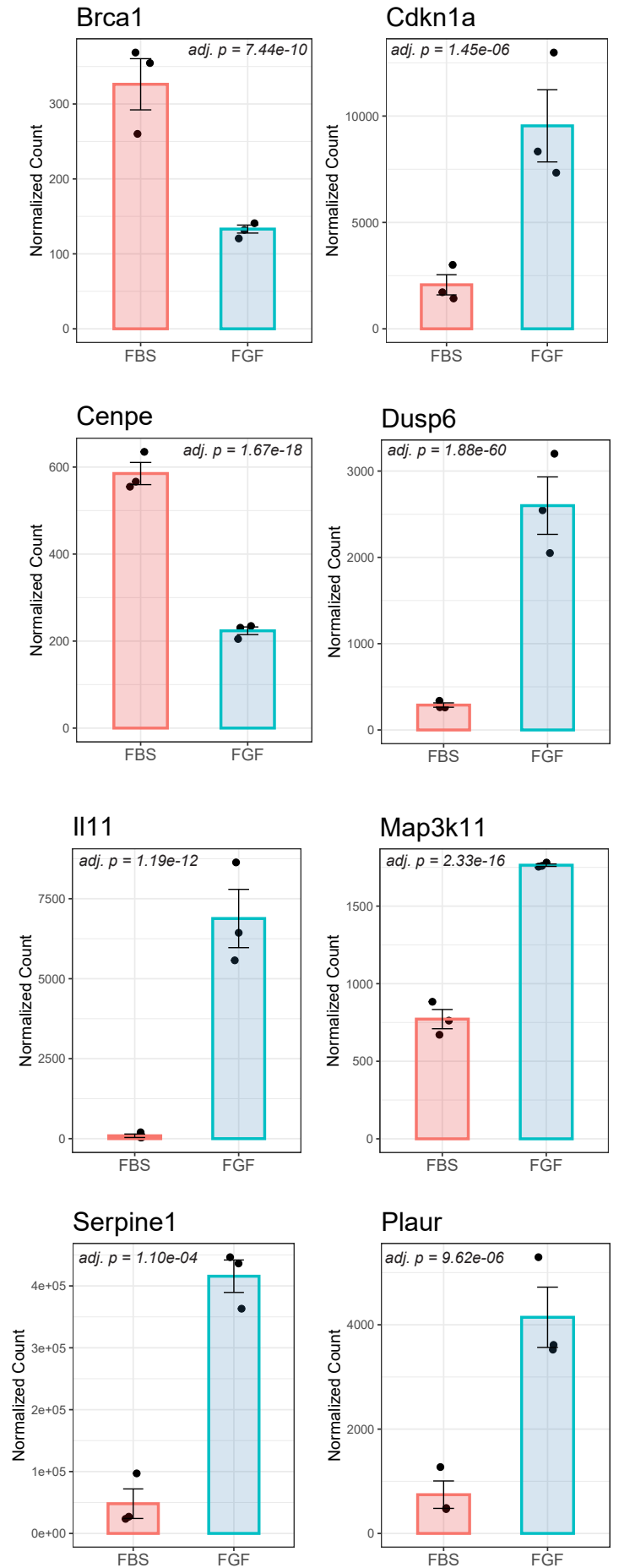
**A**



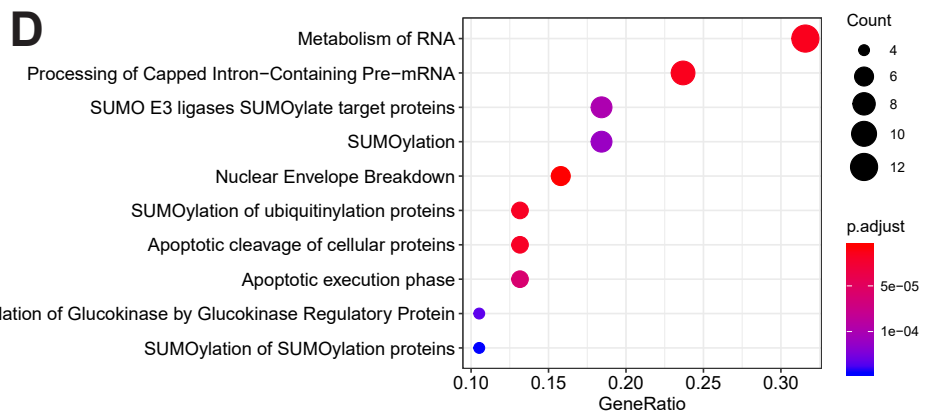
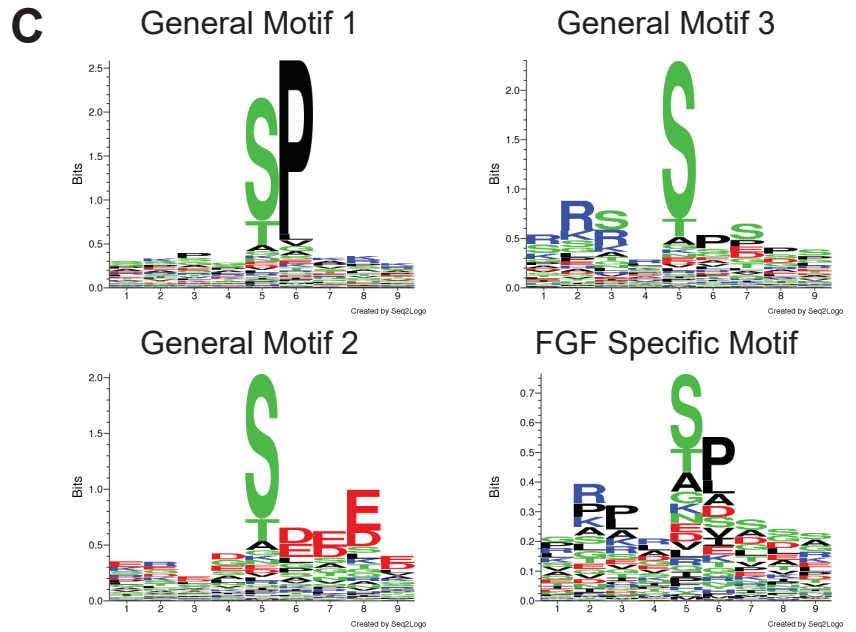
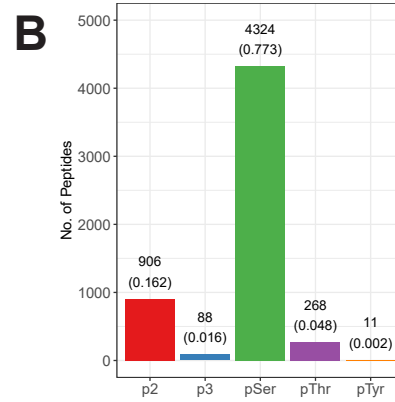
**B**



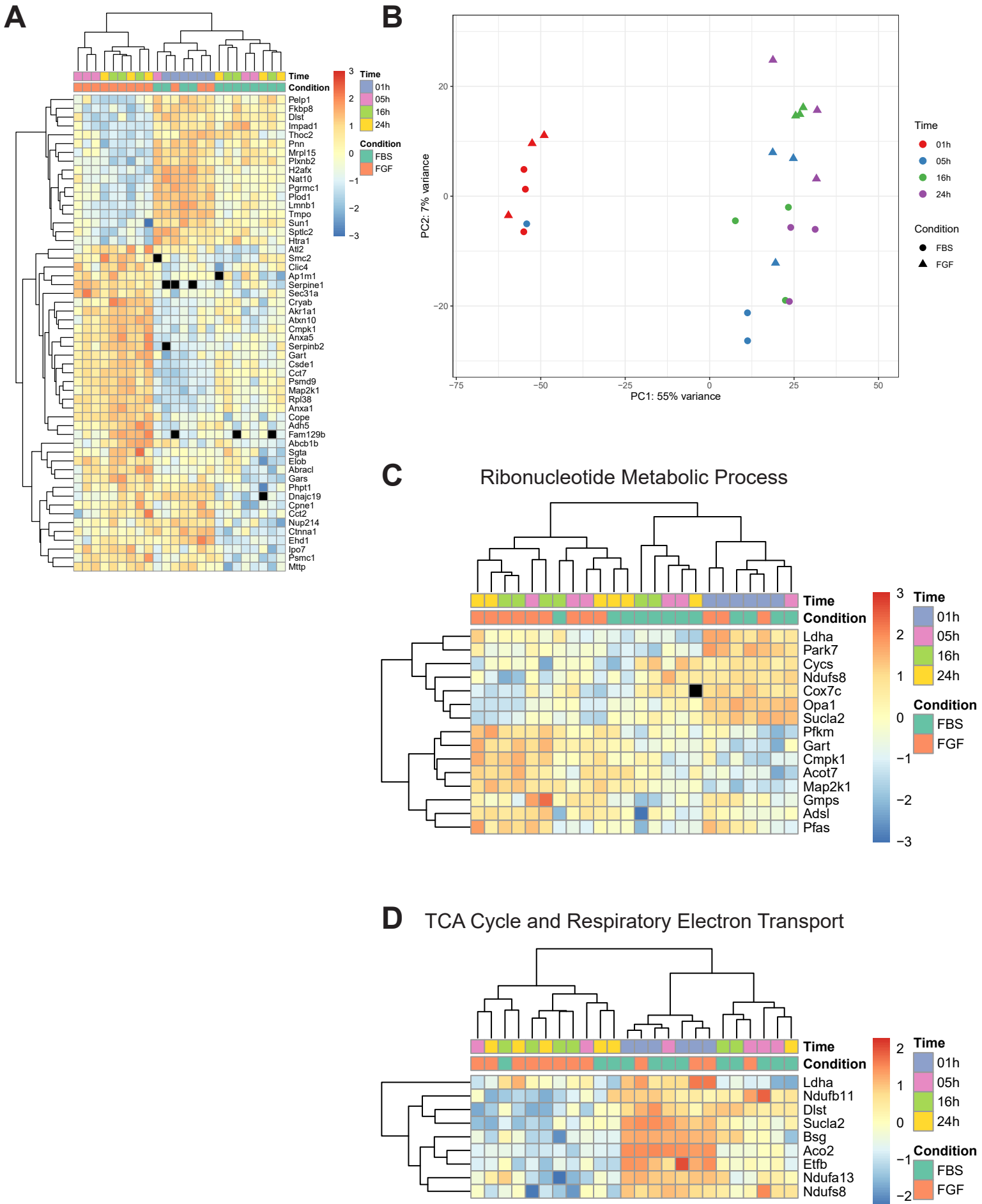
**C**



# SUPPLEMENTAL 3

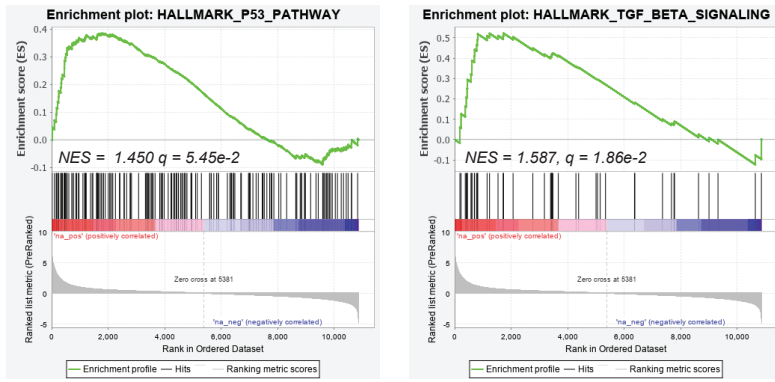


# SUPPLEMENTAL 4

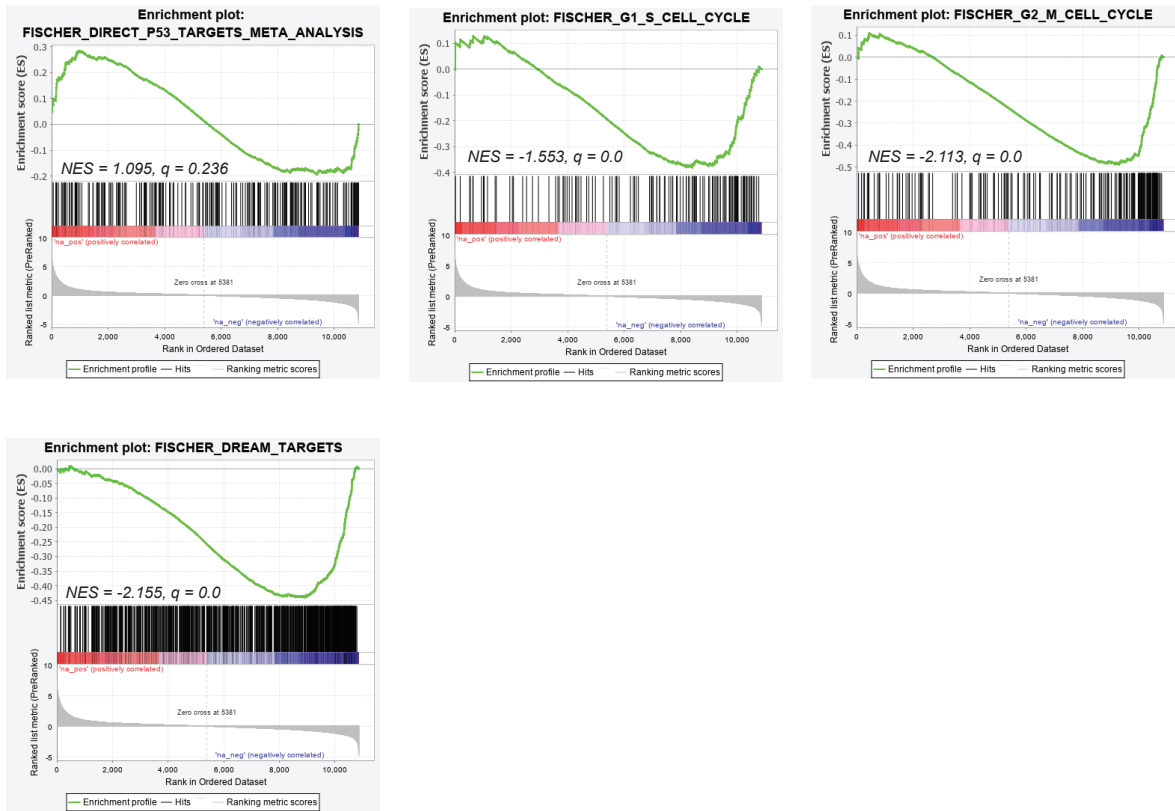


# SUPPLEMENTAL 5

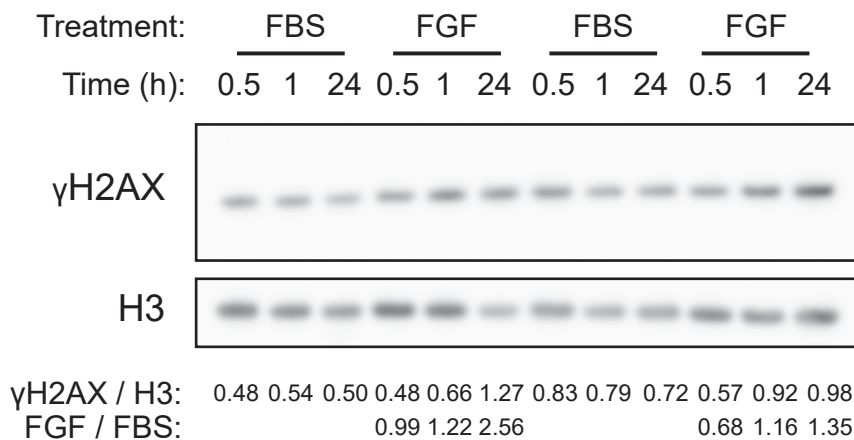
**A**



**B**



**C**



## **SUPPLEMENTAL FIGURE LEGENDS**

### **Supplemental Figure 1. Gene ontology analysis of H3K27ac peaks with differential abundance after FGF-2 treatment and gene ontology analysis of genes with matching trends in levels of RNA and H3K27ac.**

A,B) H3K27ac peaks with quantitative differences between the FGF treatment and the FBS control were annotated with the nearest gene. GO enrichment analysis was performed for peaks with lower intensity in the FGF condition (A, 502 peaks representing 415 genes,  $\log_2(\text{FGF}/\text{FBS}) < -1$  at  $\text{FDR} < 0.05$ ) and separately for peaks with higher intensity in the FGF condition (B, 2924 peaks representing 1421 genes,  $\log_2(\text{FGF}/\text{FBS}) > 1$  at  $\text{FDR} < 0.05$ ). The top 10 GO terms are displayed. The entire genome served as background.

C,D) Gene ontology analysis was performed on genes showing concordant trends in RNA and H3K27ac levels. Enriched terms are presented for (C) genes with decreased RNA expression and H3K27ac intensity (42 genes with H3K27ac  $\log_2(\text{FGF}/\text{FBS}) < 0$  at  $\text{FDR} < 0.05$  and RNA  $\log_2(\text{FGF}/\text{FBS}) < 0$  at  $p\text{-adj} < 0.05$ ) and for (D) genes with increased RNA expression and H3K27ac intensity (157 genes with H3K27ac  $\log_2(\text{FGF}/\text{FBS}) > 0$  at  $\text{FDR} < 0.05$  and RNA  $\log_2(\text{FGF}/\text{FBS}) > 0$  at  $p\text{-adj} < 0.05$ ).

### **Supplemental Figure 2. Summary of RNA-seq results and plots of selected genes demonstrating differential expression.**

A) Replicates from the RNA-seq analysis were clustered by PCA (11,952 genes with valid adjusted p-values). Each point represents an individual replicate ( $n = 3$ ). The FBS condition is shown in red while the FGF condition is shown in blue.

B) Heatmaps of the 20 genes showing the largest changes in  $\log_2(\text{FGF}/\text{FBS})$  ( $p\text{-adj} < 0.05$ ) are presented.

C) Normalized RNA-seq counts are presented for several genes related to MAPK signaling, cell cycle regulation, and the senescence-associated secretory phenotype. Bar charts display the

group mean  $\pm$  s.e.m. with individual replicates as points ( $n = 3$ ). Adjusted  $p$ -values are shown from the DESeq2 analysis.

**Supplemental Figure 3. Summary of phospho-proteome results and additional analyses.**

A) A heatmap of phospho-peptides with significant changes ( $p < 0.05$ ,  $\text{abs}(Z\text{-score of } \log_2(\text{FGF}/\text{FBS})) > 2$ , quantified in all replicates in both conditions for at least 1 timepoint) in intensities is presented. Unlike Fig. 4A, phospho-peptide intensity is not normalized to the intensity of the respective protein. Missing values are shown in black. Row names indicate the gene symbol, starting and ending positions of the peptide, and the phosphorylation sites. The asterisk for Ybx3 indicates an ambiguous protein assignment due to an identical sequence in Ybx1. The na for Bnip3l indicates an ambiguous phospho-serine assignment.

B) The numbers of phospho-peptides (total = 5,597) bearing single phosphorylation events at serine, threonine, or tyrosine are plotted as well as those bearing two or three simultaneous phosphorylation events. The frequency is shown in parentheses below the absolute number of unique phospho-peptides. The phospho-peptide HEPsSEEGDSHRR is not included in the counts.

C) Motif analysis was performed on 1,547 general phospho-sites that were detected in all 3 replicates of both FBS and FGF conditions in at least 1 timepoint. This analysis was repeated for 47 phospho-sites that were significantly elevated in the FGF condition at 30 or 60 mins to search for motifs specific to the FGF treatment.

D) Pathway analysis (Reactome) was performed on proteins ( $n = 67$ ) with protein-normalized phospho-peptides showing significant changes across conditions ( $p < 0.05$  and absolute value of  $Z\text{-score of } \log_2[\text{FGF}/\text{FBS}] > 2$  at 5, 10, 30, or 60 mins).

**Supplemental Figure 4. Summary of proteome results.**

A) A heatmap is presented showing proteins with significant changes in intensity across conditions ( $p < 0.05$  for both 16 and 24 hrs with consistent sign in Z-scores of  $\log_2[\text{FGF}/\text{FBS}]$  at 5, 16, and 24 hrs). Missing values are shown in black.

B) Proteins with complete sets of measurements across all conditions and replicates ( $n = 1742$ ) were used for PCA. Individual replicates ( $n = 3$ ) are plotted with a different color representing each timepoint and a different shape representing each condition.

C, D) Proteins related to ribonucleotide metabolism (C) or the TCA cycle (D) by GO analysis and showing significant changes ( $p < 0.05$  at 16 or 24 hrs for  $\log_2[\text{FGF}/\text{FBS}]$  and consistent sign in Z-score of  $\log_2[\text{FGF}/\text{FBS}]$  at 5, 16, and 24 hrs) across conditions are presented in a heatmap.

### **Supplemental Figure 5. GSEA of p53, TGFb, DREAM, and cell cycle pathways and staining for $\gamma\text{H2AX}$ .**

A) Enrichment plots for the hallmark p53 and TGFb pathways from the initial GSEA.

B) Enrichment plots from a targeted GSEA focusing on direct p53 transcriptional targets, DREAM targets, and genes with peak expression at the G1/S and G2/M transitions based on a published meta-analysis.<sup>1</sup>

C) Levels of  $\gamma\text{H2AX}$  were analyzed in acid-extracted histones from cells treated with FBS or FGF for the indicated timepoints. H3 was used as a loading control. Two replicates are represented.

### **Table 1. Analysis of histone PTMs in Y1 cells by bottom-up mass spectrometry**

Histones from Y1 cells treated with FBS or FGF-2 for various amounts of time were analyzed by bottom-up mass spectrometry. Detailed data regarding the peak area, peak retention time (RT), relative abundances (area of given peptide species divided by sum of all modified and unmodified forms of that peptide sequence), t-test p-values (two-tailed, unpaired), and  $\log_2$  fold-changes (FGF/FBS) are presented for uniquely modified peptides down the rows with samples across the columns. The column labeled RT flags indicates the number of samples with inconsistent RTs



(defined as  $\text{abs}[\text{sampleRT} - \text{medianRT}] > 3 * [\text{median absolute deviation}]$ ). A high number of RT flags suggests potential issues with peak assignments or sensitivity for that peptide species.

### **Table 2. Analysis of H3K27ac ChIP-seq data**

This table reports qualitative and quantitative information regarding the consensus H3K27ac peaks that were called by MACS2 and processed by DiffBind in R. Genomic annotation was provided by the ChIPseeker package in R. Peaks were assigned to FGF\_up, FGF\_down, and Unchanged subsets based on  $\text{FDR} < 0.05$  and Fold (calculated as  $\log_2[\text{FGF}/\text{FBS}] > 1$  or  $< -1$ ). The Unchanged subset contains 37,346 peaks instead of 37,357 due to 11 peaks with ChrUn designation not receiving a genomic annotation. FBS1 and FBS2 represent the two replicates from the FBS condition while FGF1 and FGF2 represent the two replicates from the FGF-2 condition.

### **Table 3. Integration of RNA-seq with H3K27ac ChIP-seq.**

This table reports information regarding the integrated analysis of the H3K27ac ChIP-seq and RNA-seq datasets after separate processing by DiffBind and DESeq2. H3K27ac peaks were assigned to FGF\_up, FGF\_down, and Unchanged subsets based on  $\text{FDR} < 0.05$  and Fold (calculated as  $\log_2[\text{FGF}/\text{FBS}] > 1$  or  $< -1$ ). The Unchanged subset contains 37,346 peaks instead of 37,357 due to 11 peaks with ChrUn designation not receiving a genomic annotation. FBS1 and FBS2 represent the two replicates from the FBS condition while FGF1 and FGF2 represent the two replicates from the FGF-2 condition. H3K27ac peaks were required to be within 10 kb of the TSS. For genes with multiple H3K27ac peaks, the peak closest to the TSS was selected and in the case of ties, the most intense peak was selected. Genes were also required to have defined adjusted p-values and fold-changes from the DESeq2 analysis. Genes with concordant trends ( $\text{FDR} < 0.05$  and  $\log_2[\text{FGF}/\text{FBS}] > 0$  or  $< 0$  for both RNA-seq and ChIP-seq) were used for GO analyses.

**Table 4. Intensities of phospho-peptides before and after normalization to protein intensities**

This table reports the run-normalized intensity measurements from MsStats for the identified phospho-peptides, which are uniquely identified based on concatenation of the protein accession, peptide sequence, and modification site assignments (AccessionModSeq). The phospho-peptide values are matched to run-normalized protein intensity measurements across the rows with the final set of columns representing protein-normalized phospho-peptide intensities. The peptides have not been filtered for consistent detection across replicates. Additional peptide information (ambiguity, accessions, modifications, missed cleavages, sequence) originated from ProteomeDiscoverer. The peptide HEPsSSEEGDSHRR has no intensity measurements after processing by MsStats, most likely due to low intensities reported by ProteomeDiscoverer.

**Table 5. Statistical analysis of phospho-peptides with and without normalization to protein levels**

This table reports statistics, based on MsStats and other tools in R, for the identified phospho-peptides, which are uniquely identified based on concatenation of the protein accession, peptide sequence, and modification site assignments (AccessionModSeq column). Phospho-peptides either include (columns with Incl.Norm) or exclude (columns with Excl.Norm) normalization to protein levels as indicated. Significance values are derived from unpaired Student's t-tests on intensity values from MsStats. N indicates the number of replicates in which the phospho-peptide was detected. Additional peptide information (ambiguity, accessions, modifications, missed cleavages, sequence) originated from ProteomeDiscoverer.

**Table 6. Statistical analysis of phospho-peptides based on number of detections across timepoints and conditions**

This table reports information regarding the chi square analysis of phospho-peptides. For each peptide, a contingency table was constructed to test for skewed partitioning of the number of detections in replicates of a condition independent of time (FBS or FGF; 12 detections possible for each) or of a timepoint independent of condition (5 min, 10 min, 30 min, or 60 min; 6 detections possible for each).

### **Table 7. Statistical analysis of proteome data**

This table reports information regarding the statistical analysis of the proteome data performed with MsStats and additional tools in R. NA values originate from proteins with undefined fold-changes at a given timepoint. Normalized protein intensity values as calculated by MsStats are also included.

## **REFERENCES**

- 1 M. Fischer, P. Grossmann, M. Padi and J. A. DeCaprio, *Nucleic Acids Res.*, 2016, **44**, 6070–6086.