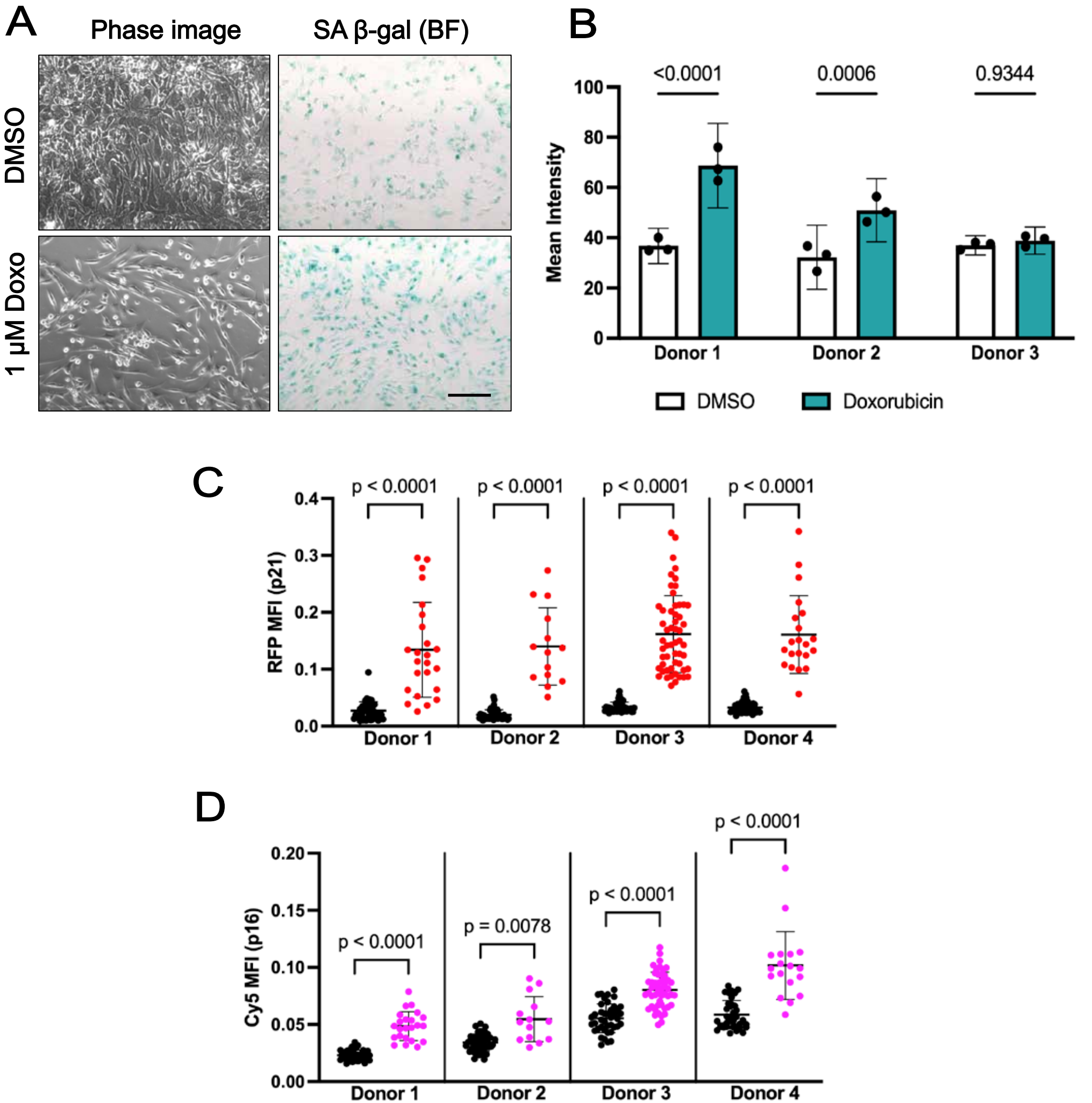
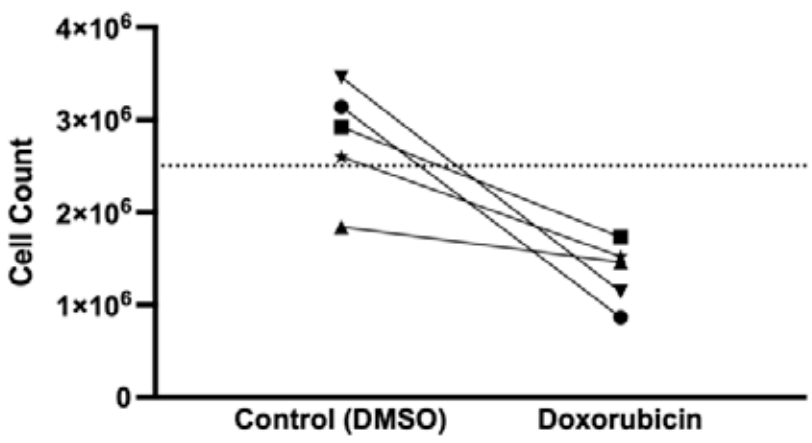
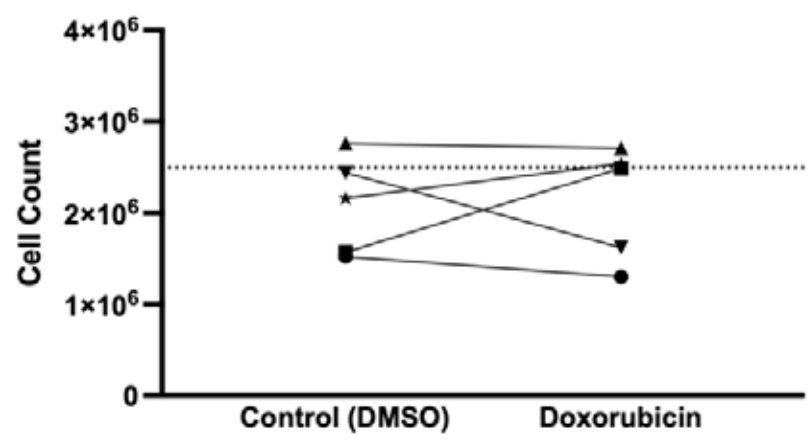
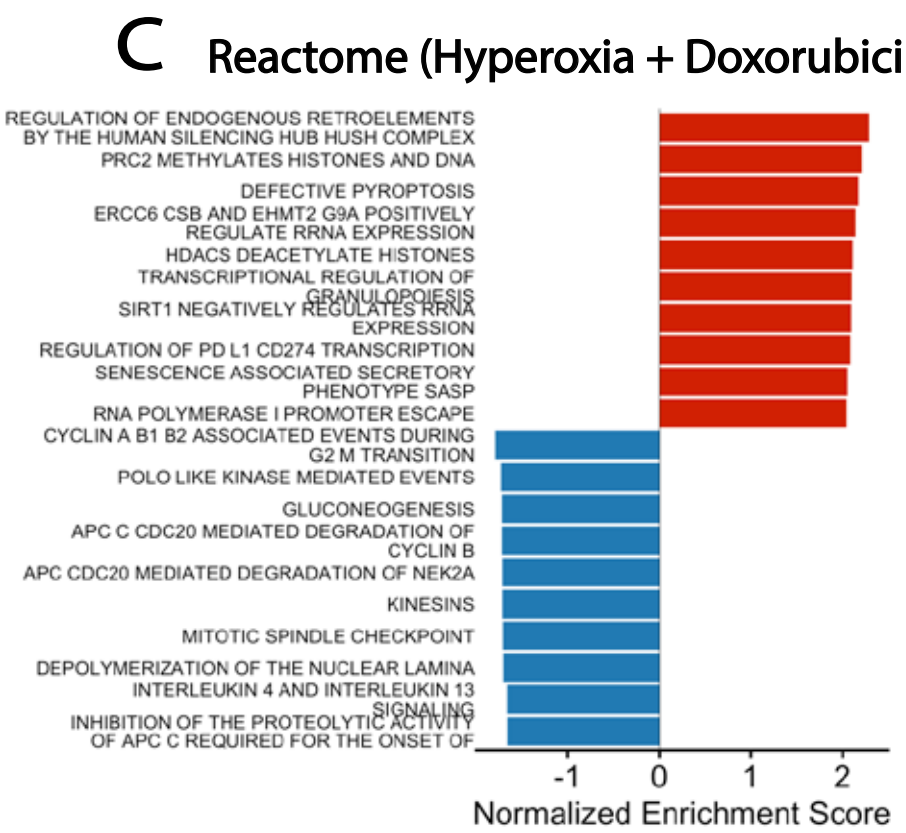
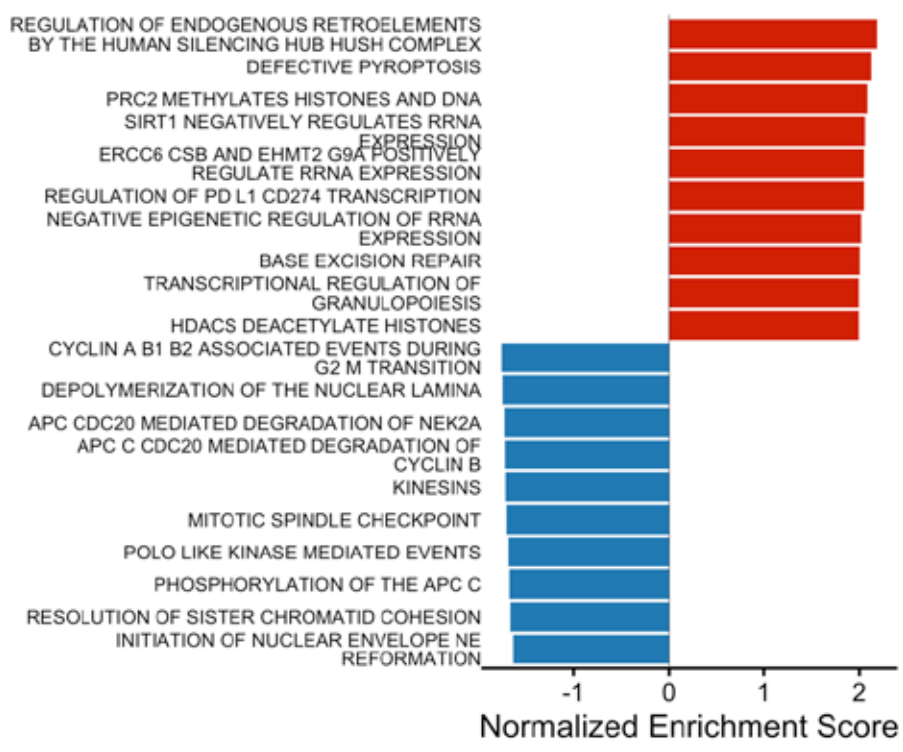


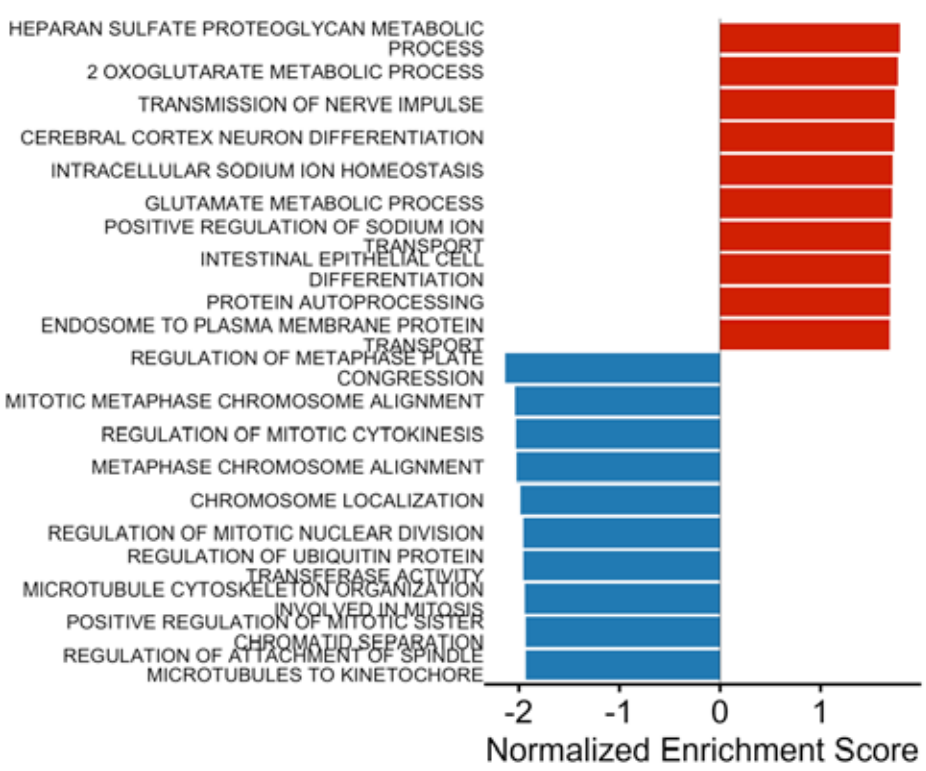
Supplementary Figure 1. SA-β-gal staining and single-cell MFI analysis of p16 and p21.
(A) Representative 20x phase-contrast and SA-β-gal images of human normal chondrocytes treated with DMSO or 1 µM doxorubicin; scale bar: 100 µm. (B) Mean intensity of SA-β-gal staining in 3 independent donors treated with DMSO or doxorubicin. (C) RFP and (D) Cy5 mean fluorescence intensity (MFI) in 4 independent donors. We used one representative frame from each donor for analysis. Each dot represents an individual cell; the number of cells analyzed varied between frames. Statistical test was performed using paired t-test. Error bars indicate standard deviation.

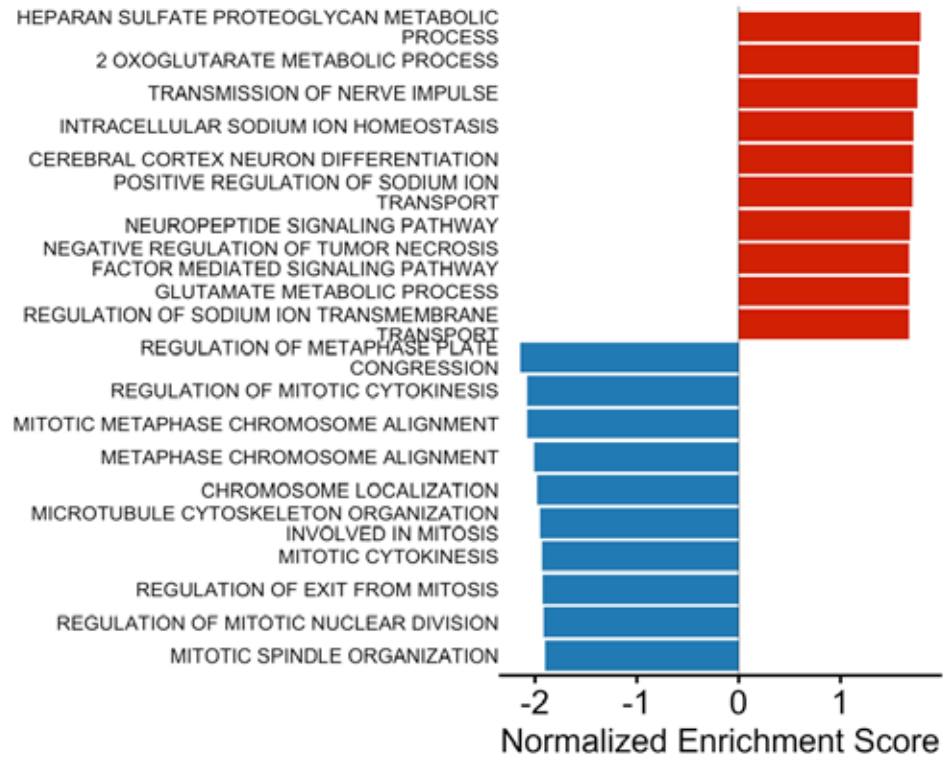


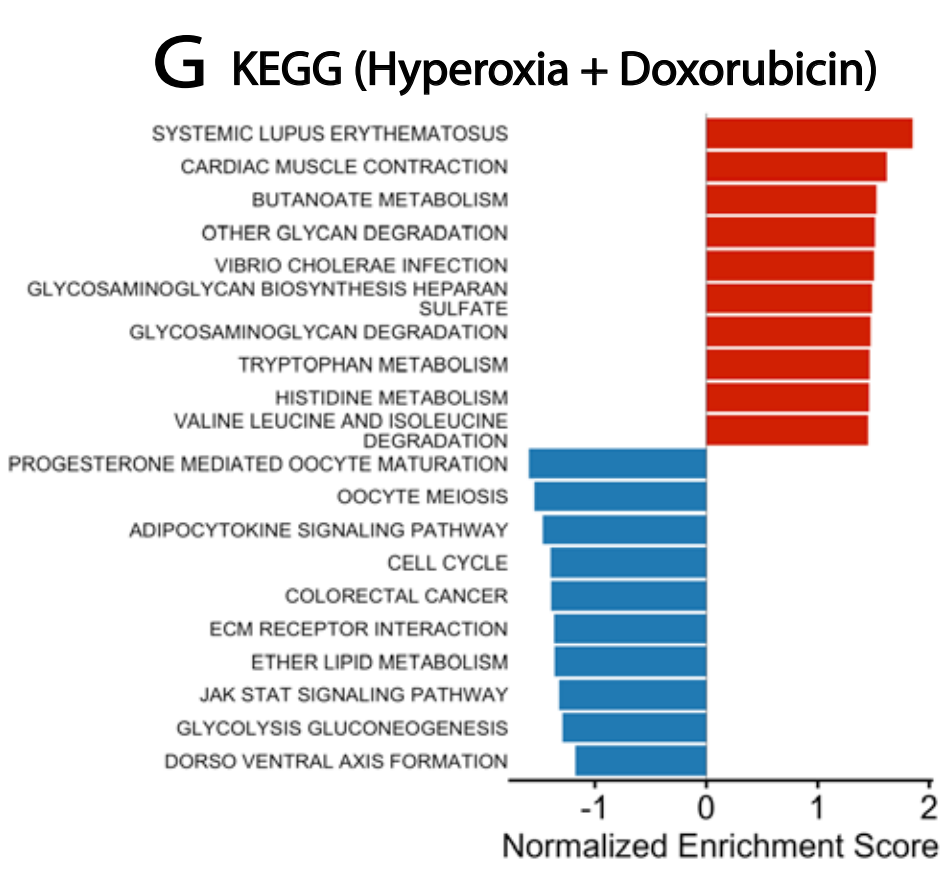


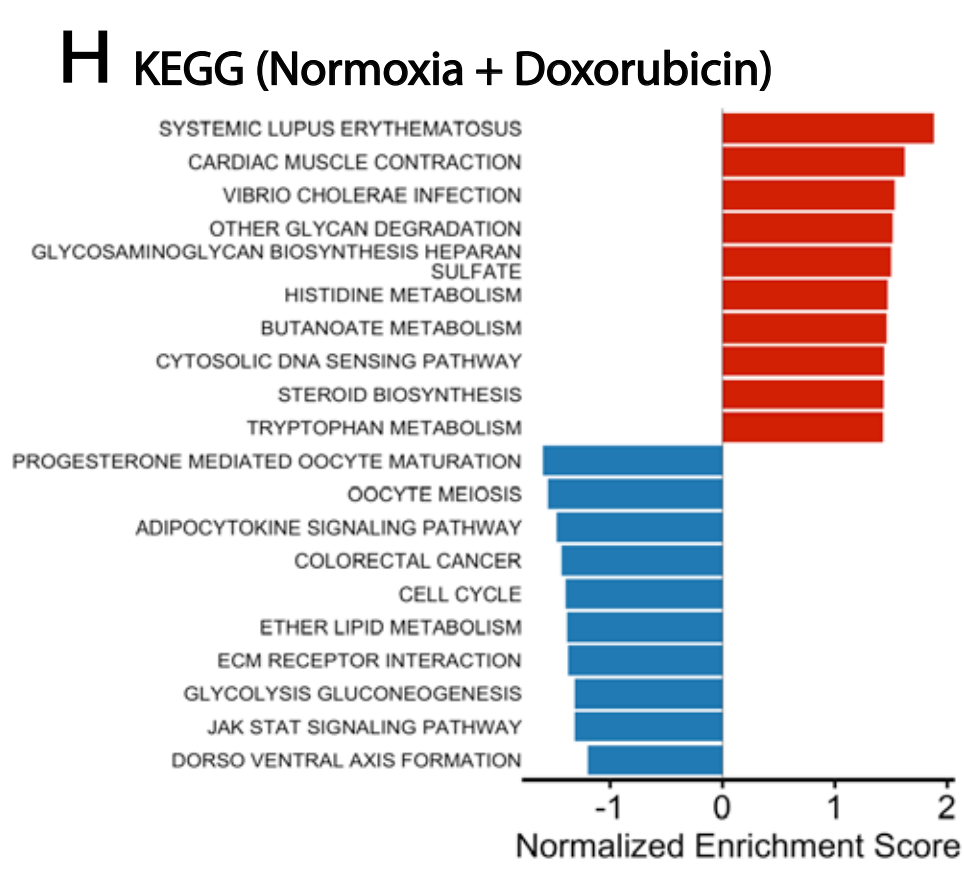




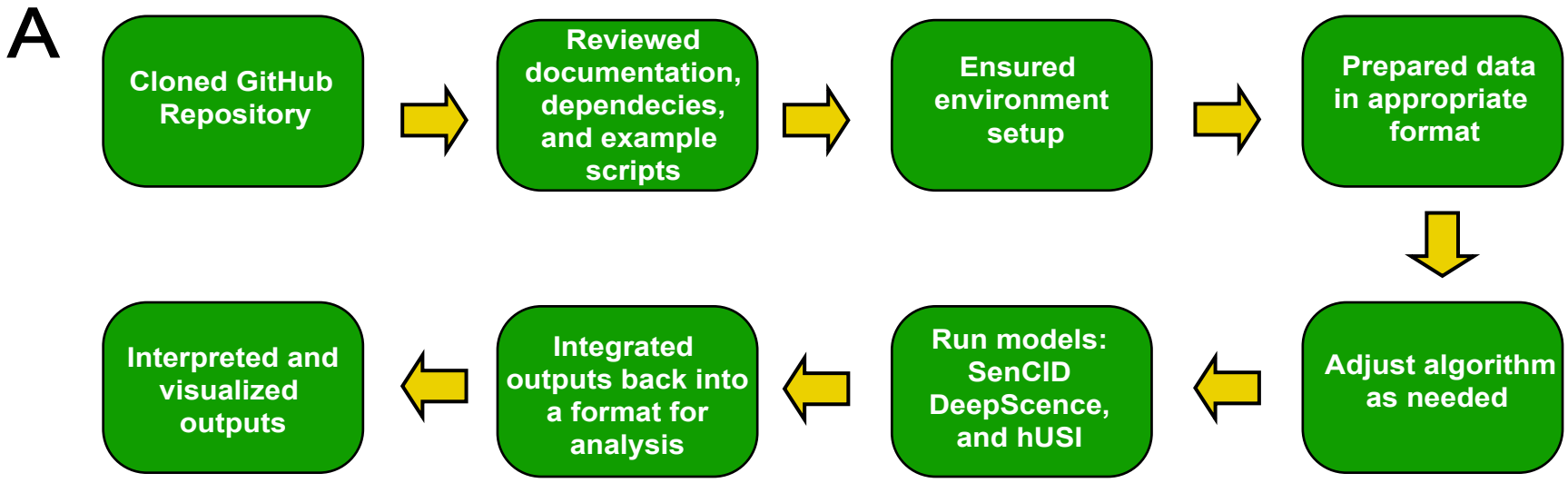


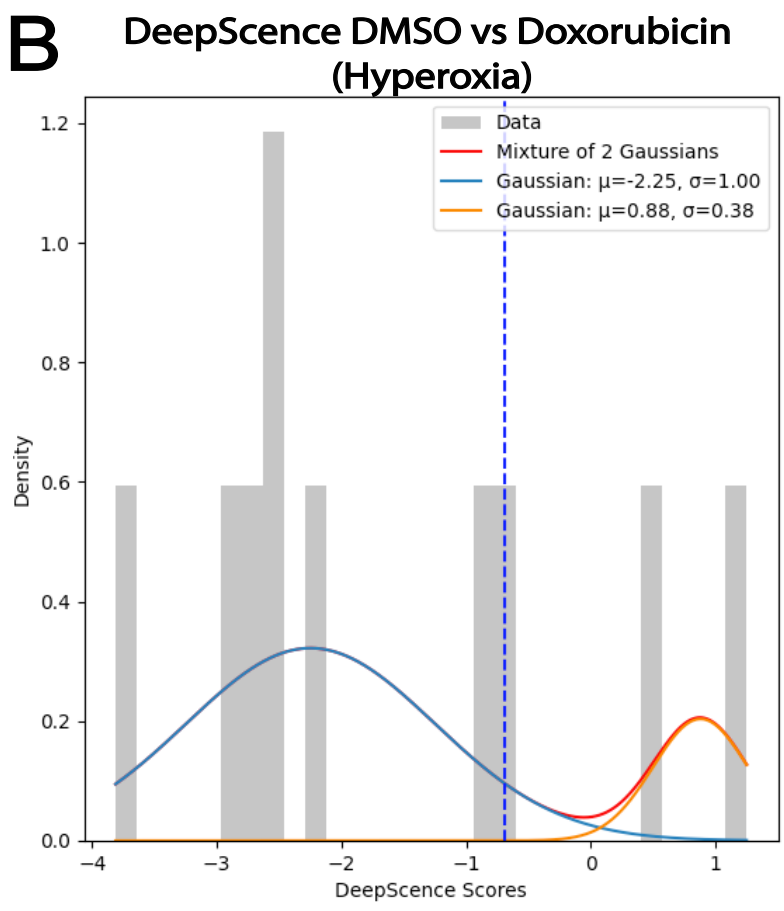


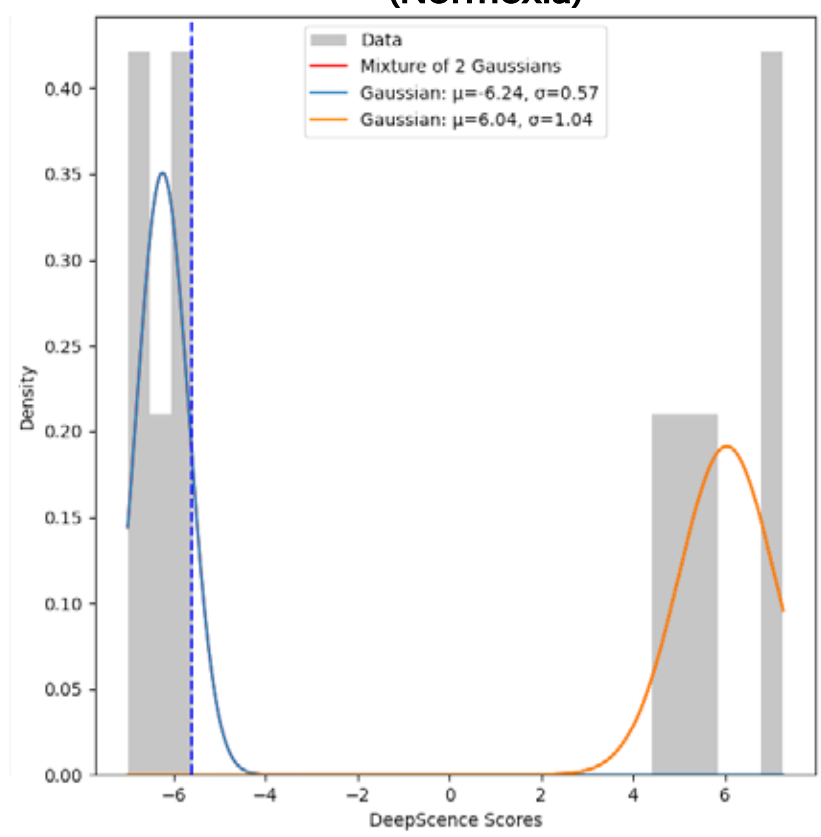


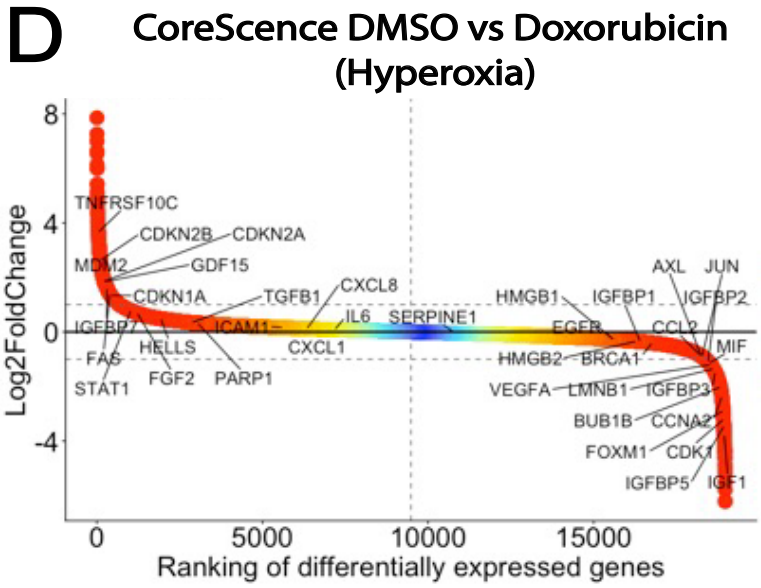


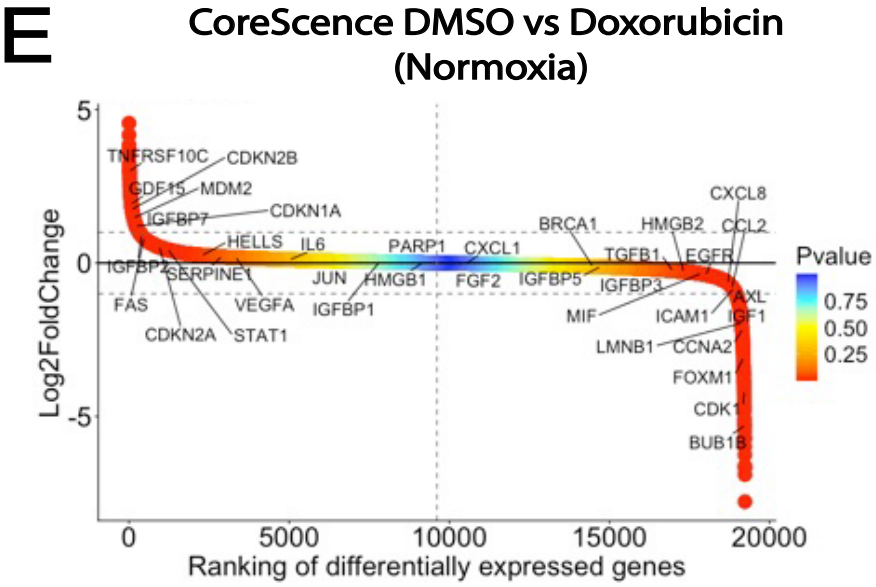
Supplementary Figure 2. Cell counts and pathway enrichment analysis of doxorubicin-treated chondrocytes. (A) Cell counts following treatment under hyperoxia and (B) normoxia conditions. The horizontal dotted line indicates the starting cell density: 2.5×10^6. Top 10 upregulated and bottom 10 downregulated Reactome terms for (C) hyperoxia with 1 µM doxorubicin and (D) normoxia with 0.5 µM doxorubicin. Top 10 upregulated Gene Ontology biological processes (GO B) and bottom 10 downregulated KEGG terms for (E) hyperoxia with 1 µM doxorubicin and (F) normoxia with 0.5 µM doxorubicin. Top 10 upregulated and bottom 10 downregulated KEGG terms for (G) hyperoxia with 1 µM doxorubicin and (H) normoxia with 0.5 µM doxorubicin.











Supplementary Figure 3. Senescence scoring pipeline and DeepScence and CoreScence analyses. (A) Pipeline for senescence scoring using multiple models applied to the hyperoxia and normoxia datasets. DeepScence senescence score distributions and Gaussian mixture model-derived thresholds for (B) hyperoxia and (C) normoxia. CoreScence genes (39 genes) identified in the gene-ranking dot plots for (D) hyperoxia with 1 μM doxorubicin and (E) normoxia with 0.5 μM doxorubicin.

A

Hyperoxia

Total of 2152 proteins detected via LC-MS after removal of KRT containminants and blank proteins

338 proteins were filtered out
1814 proteins
Hyperoxia Control + treatments proteins

Missing protein abundance values imputed with 1/5 of the lowest detected sample

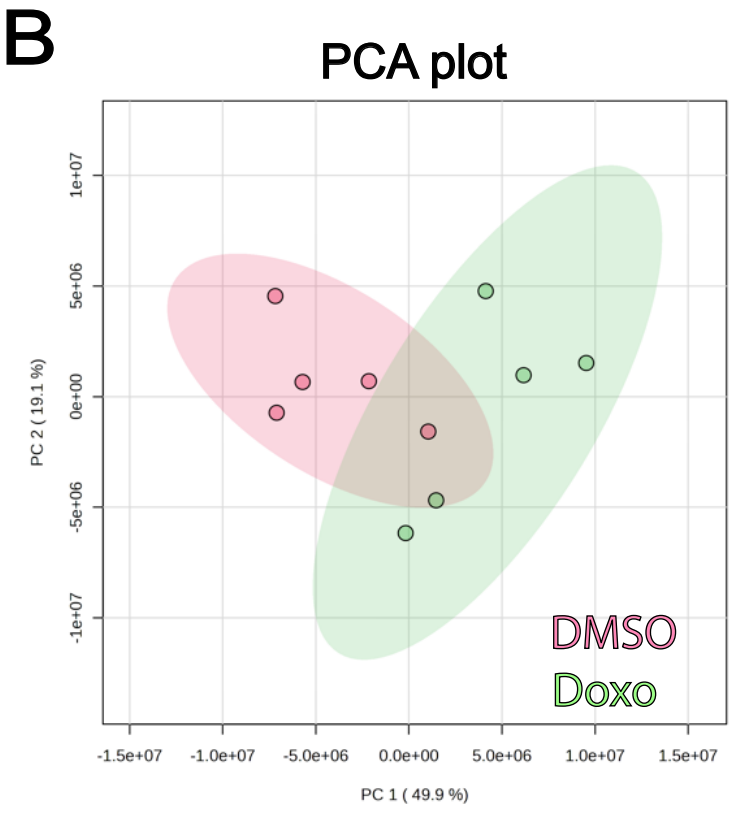
Normoxia

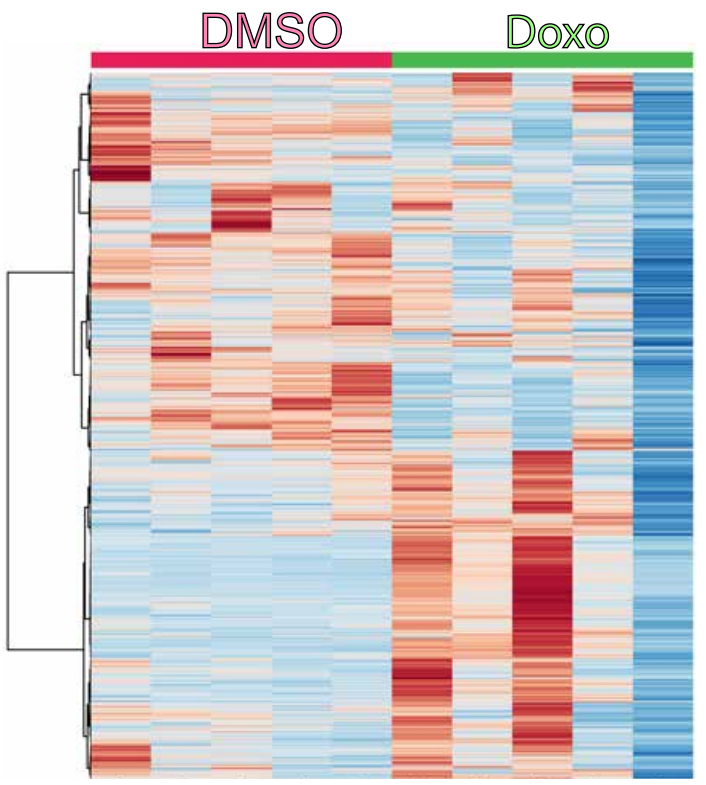
Total of 1953 proteins detected via LC-MS after removal of KRT containminants and blank proteins

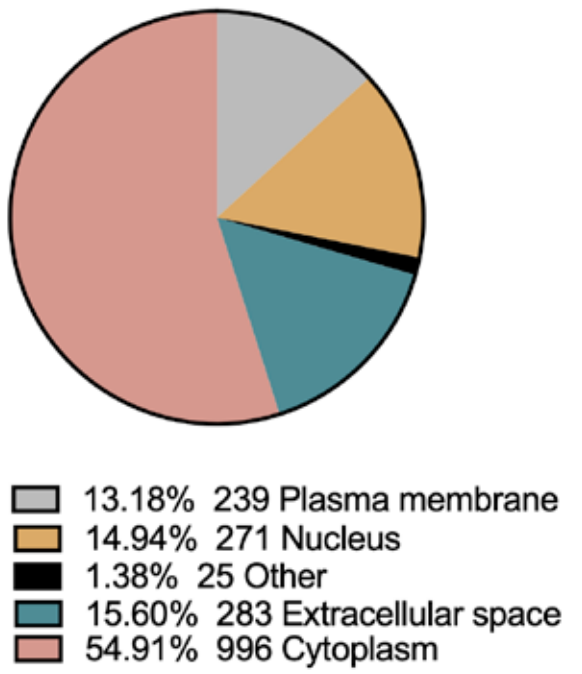
345 proteins were filtered out
1608 proteins
Normoxia control + treatments proteins

Missing protein abundance values imputed with 1/5 of the lowest detected sample

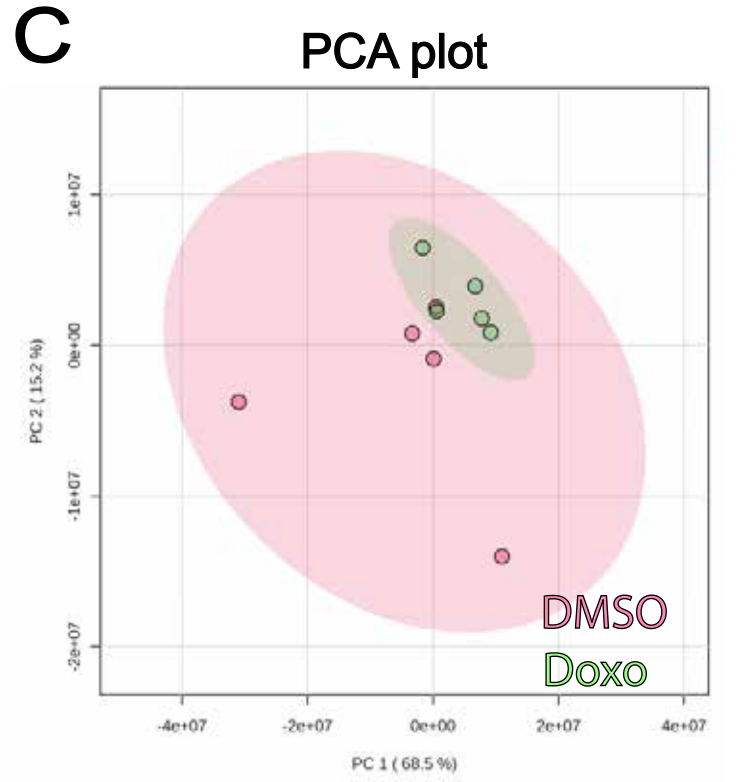
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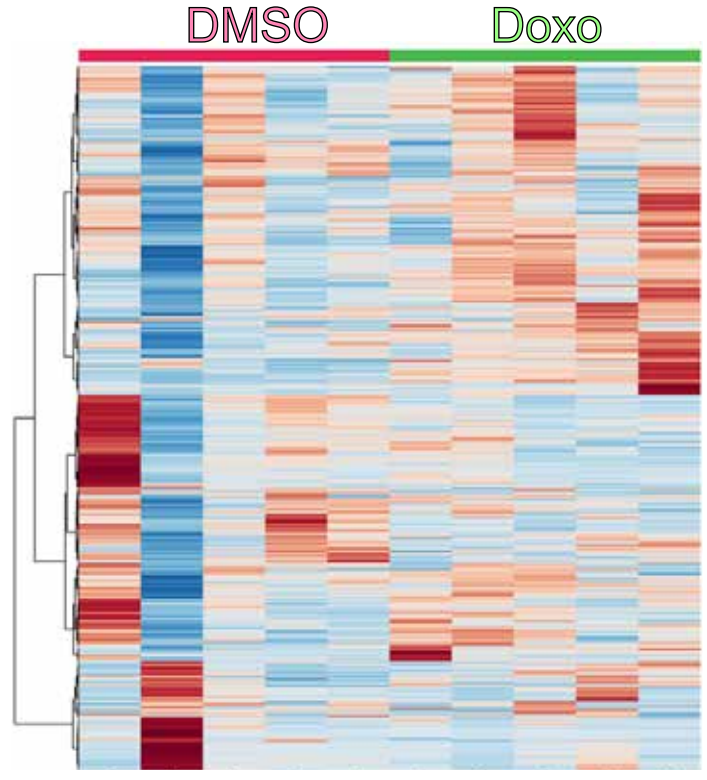


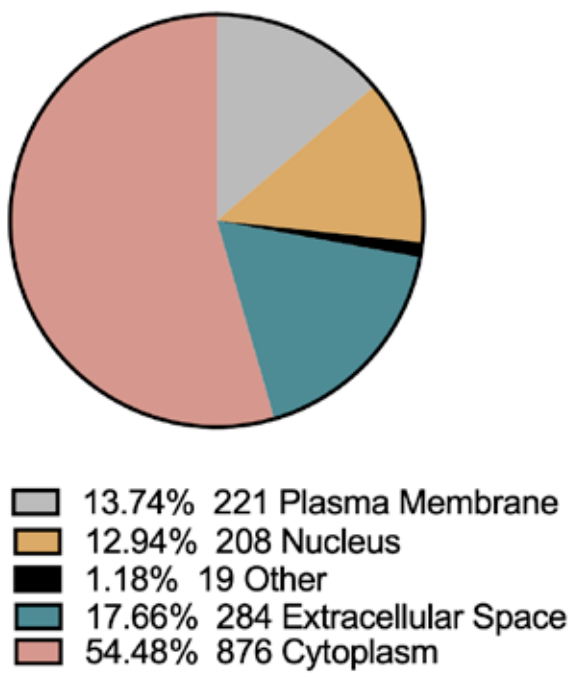




C

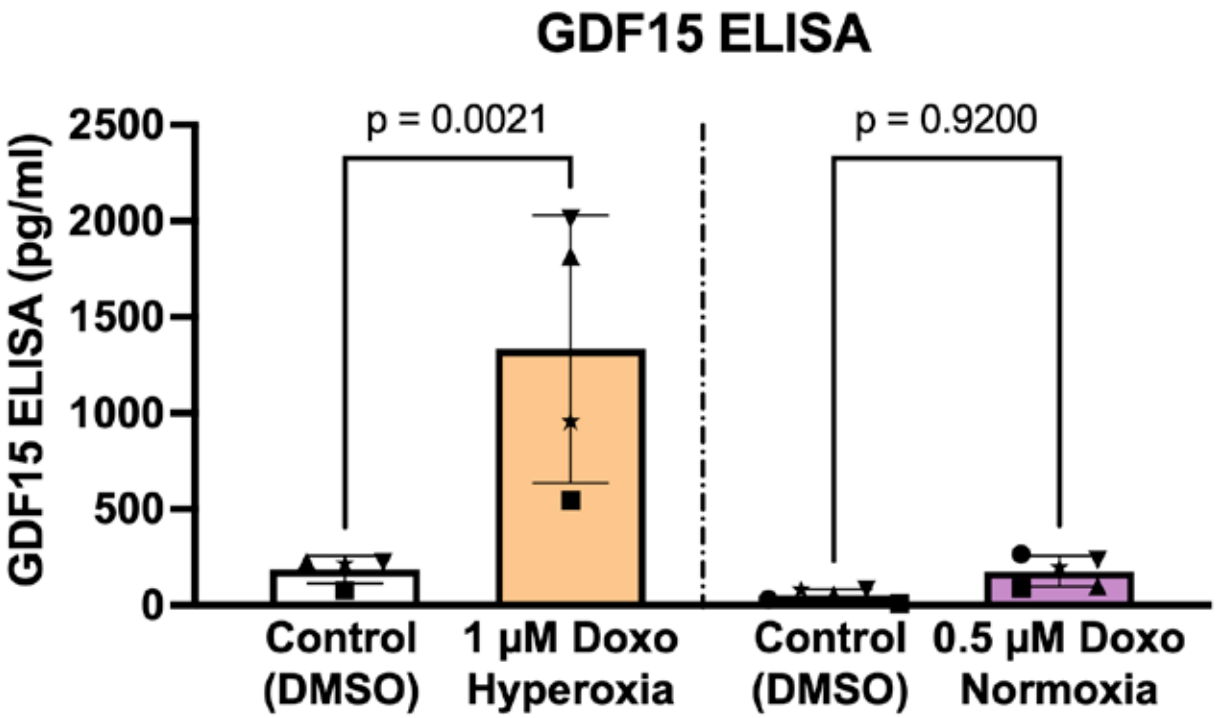




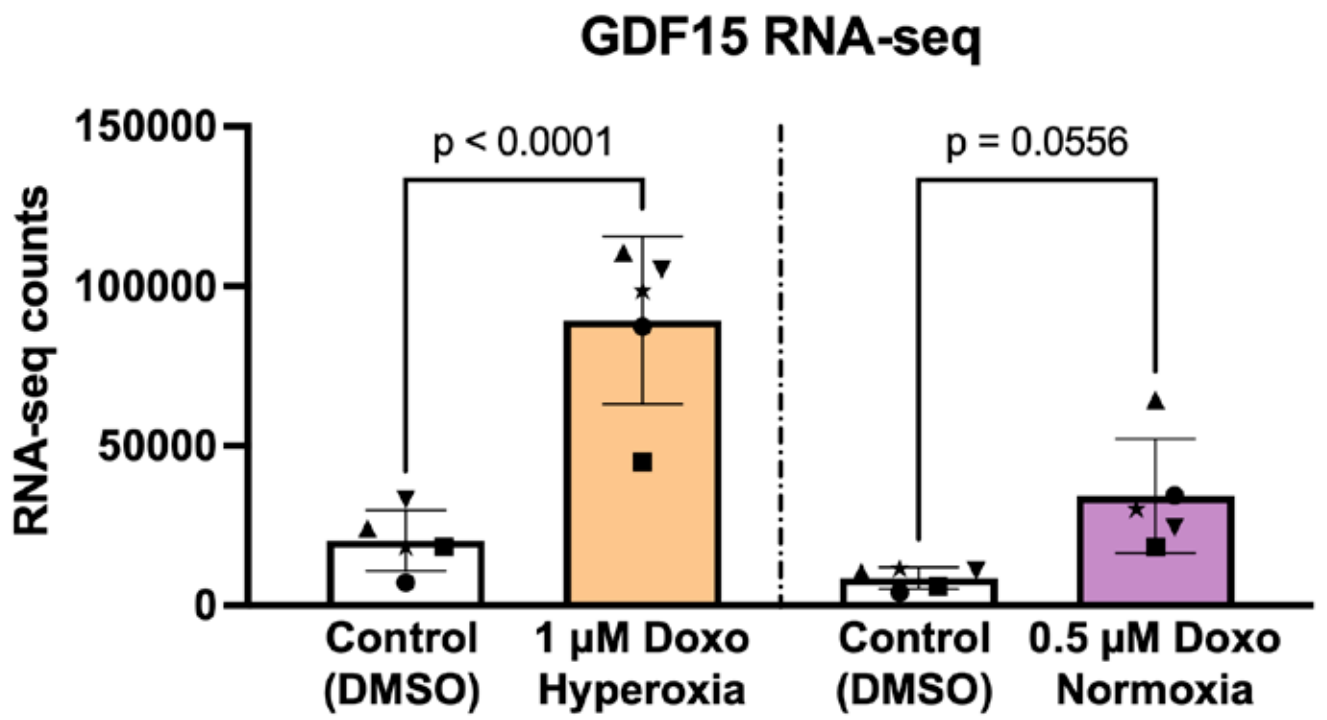


Supplementary Figure 4. Proteomic analysis of doxorubicin-treated chondrocytes. (A) Protein identification and filtering workflow for hyperoxia (top) and normoxia (bottom). (B) Hyperoxia proteomic analysis showing PCA plot (left) and heatmap (middle) retrieved from MetaboAnalyst, and protein categorization (right) retrieved from Ingenuity Pathway Analysis (IPA). (C) Normoxia proteomic analysis showing PCA plot (left) and heatmap (middle) retrieved from MetaboAnalyst, and protein categorization (right) retrieved from Ingenuity Pathway Analysis (IPA). DMSO (contol): dimethyl sulfoxide, Doxo: doxorubicin.

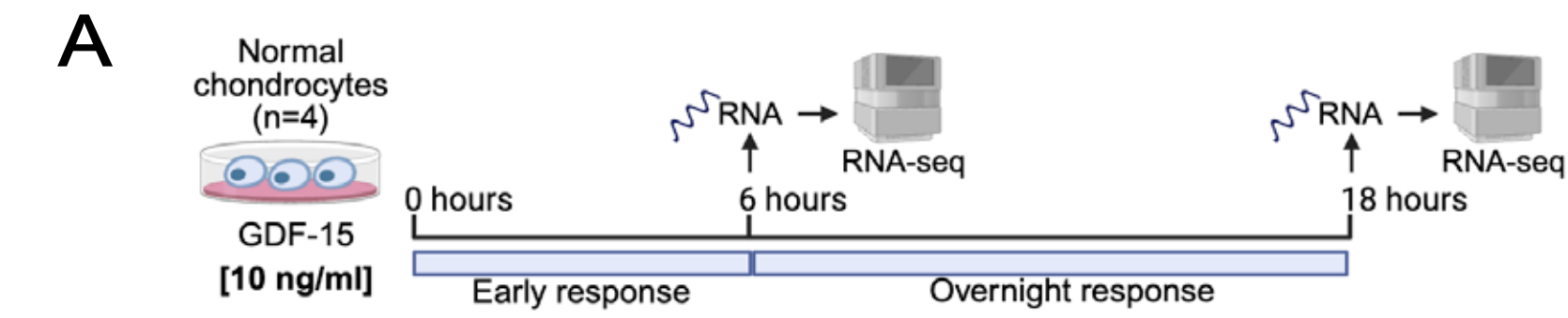
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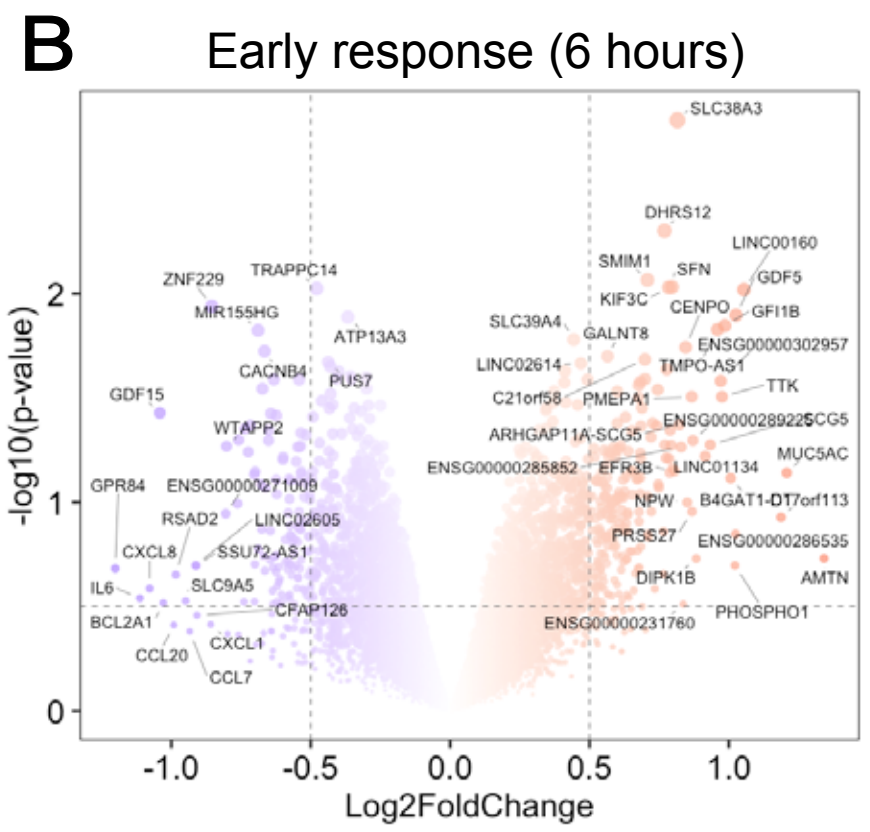


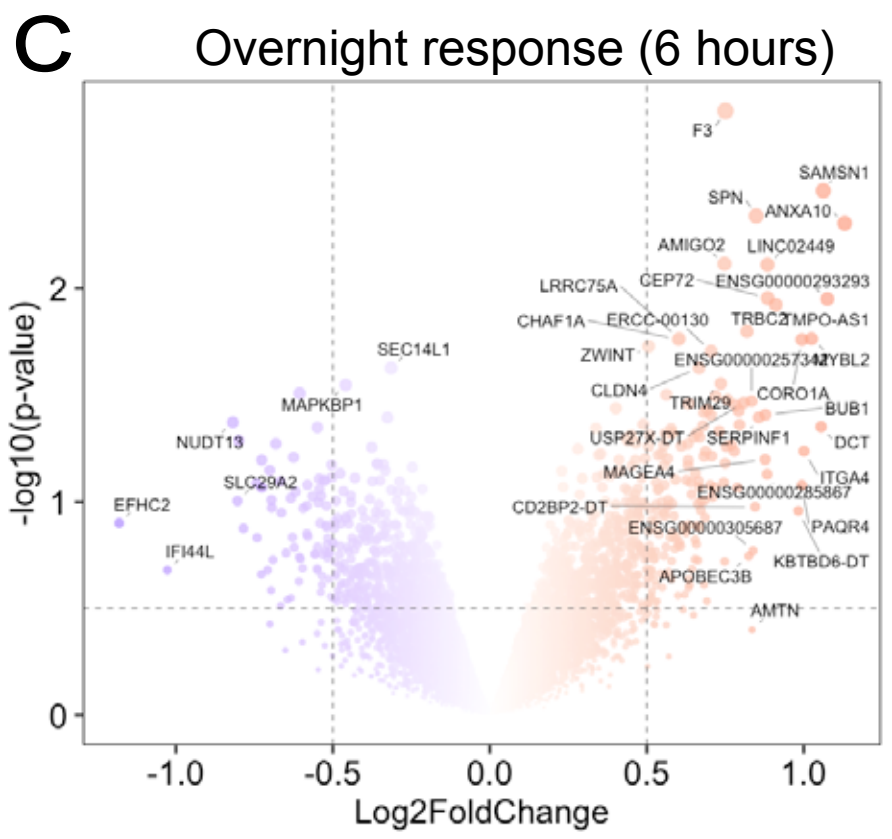
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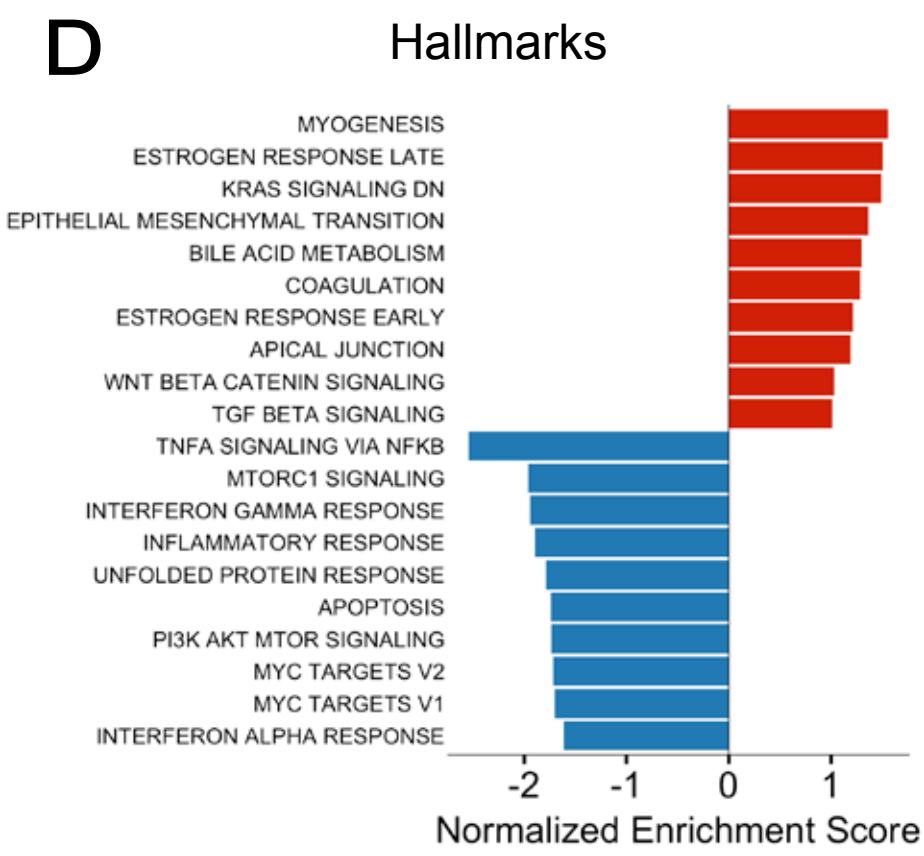


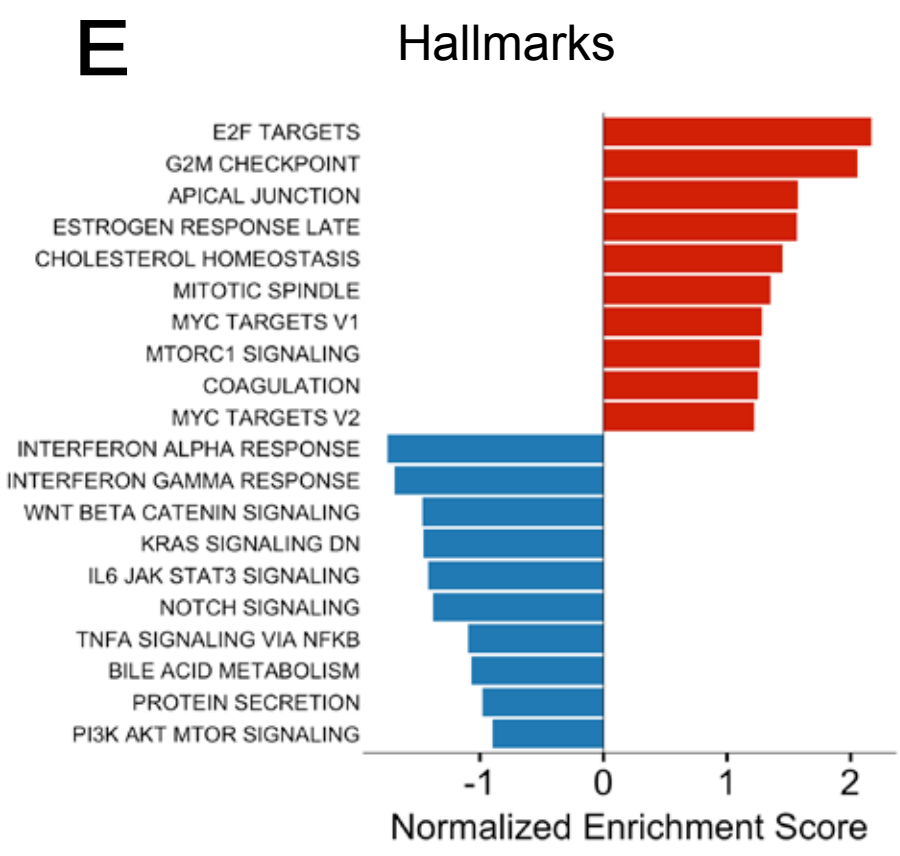
Supplementary Figure 5. GDF15 ELISA and RNA-seq expression analysis. (A) GDF15 protein levels measured by ELISA in conditioned medium from doxorubicin-treated normal chondrocytes. (B) GDF15 RNA-seq expression counts in doxorubicin-treated normal chondrocytes. Statistical analysis was performed using paired t-tests. Error bars indicate standard deviation.

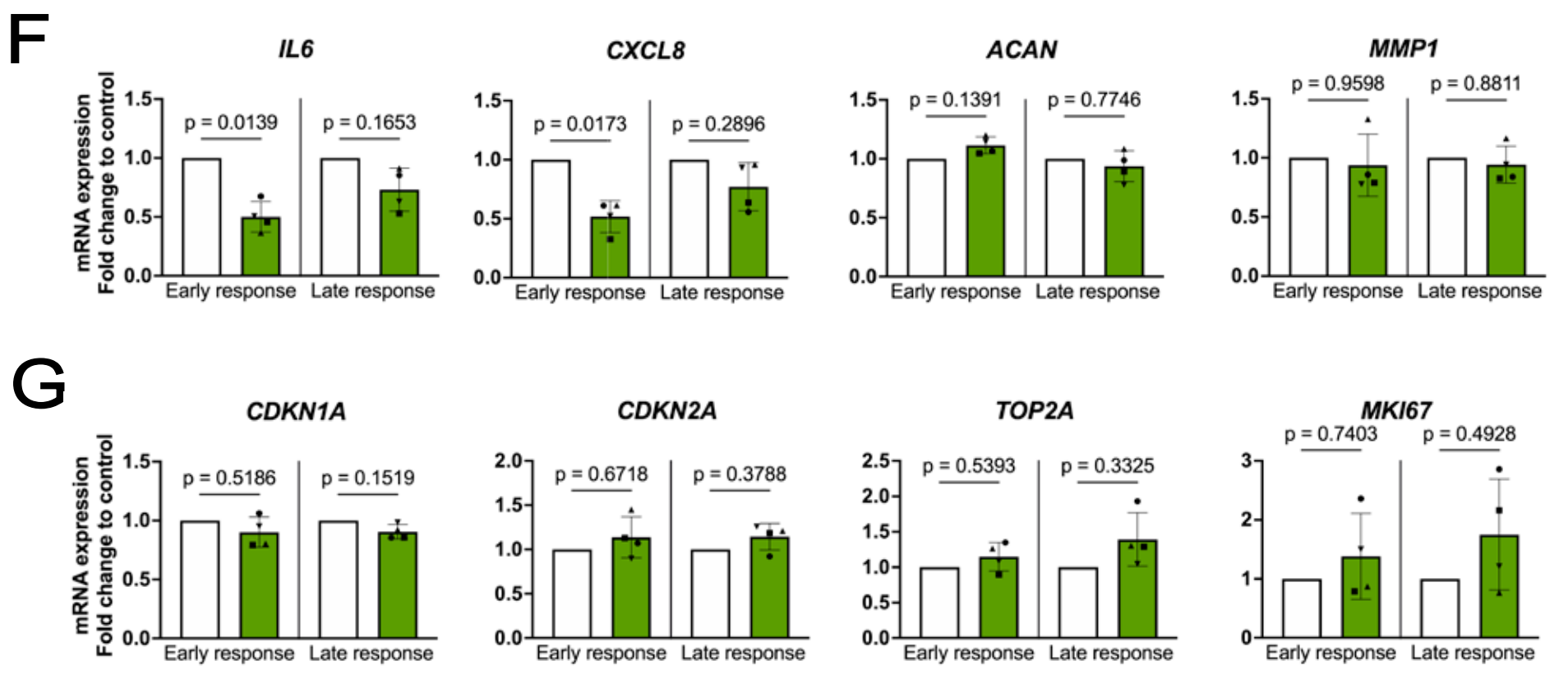


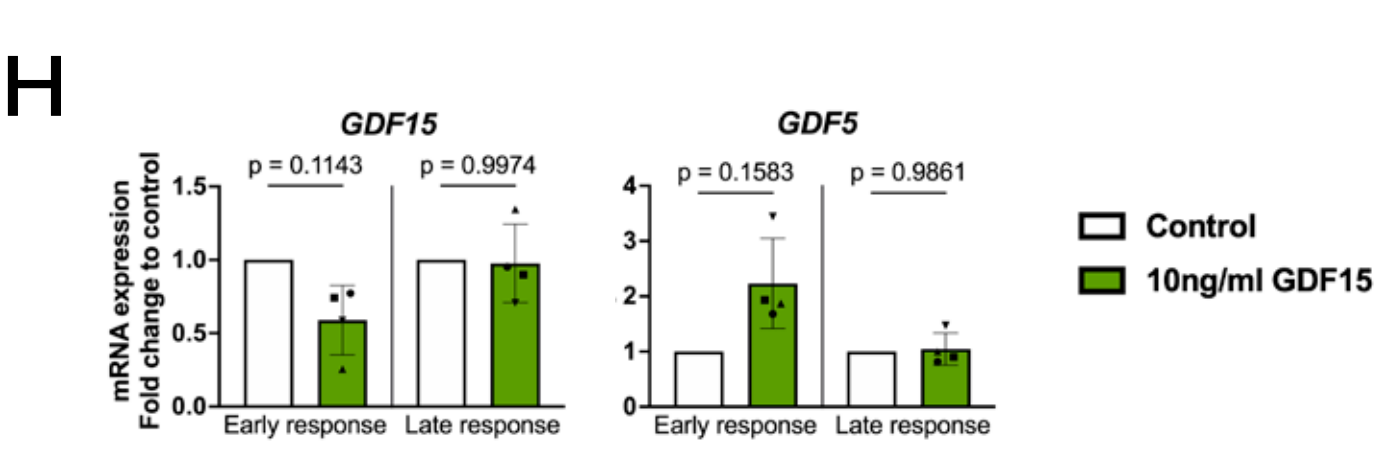












Supplementary Figure 6. Effects of 10ng/ml GDF15 treatment on early and late chondrocyte responses. (A) Experimental schematic of GDF15 treatments with GDF15 for 6 hours (early response) or 18 hours (overnight response) with 10ng/ml. Volcano plots of differentially expressed genes following treatment with 10 ng/ml GDF15 for (B) 6 hours (early response) and (C) 18 hours (late response) in normal human chondrocytes (n = 4). Top 10 Reactome-enriched upregulated and downregulated terms following treatment with 10 ng/ml GDF15 for (D) 6 hours and (E) 18 hours. Fold-change expression of (F) inflammatory and matrix-related genes *IL6*, *CXCL8*, *ACAN*, and *MMP1*; (G) cell-cycle and proliferation markers *CDKN1A, CDKN2A, MKI67*, and *TOP2A*; and (H) *GDF15* and *GDF5*. Statistical analysis was performed using paired t-tests. Error bars indicate standard deviation.