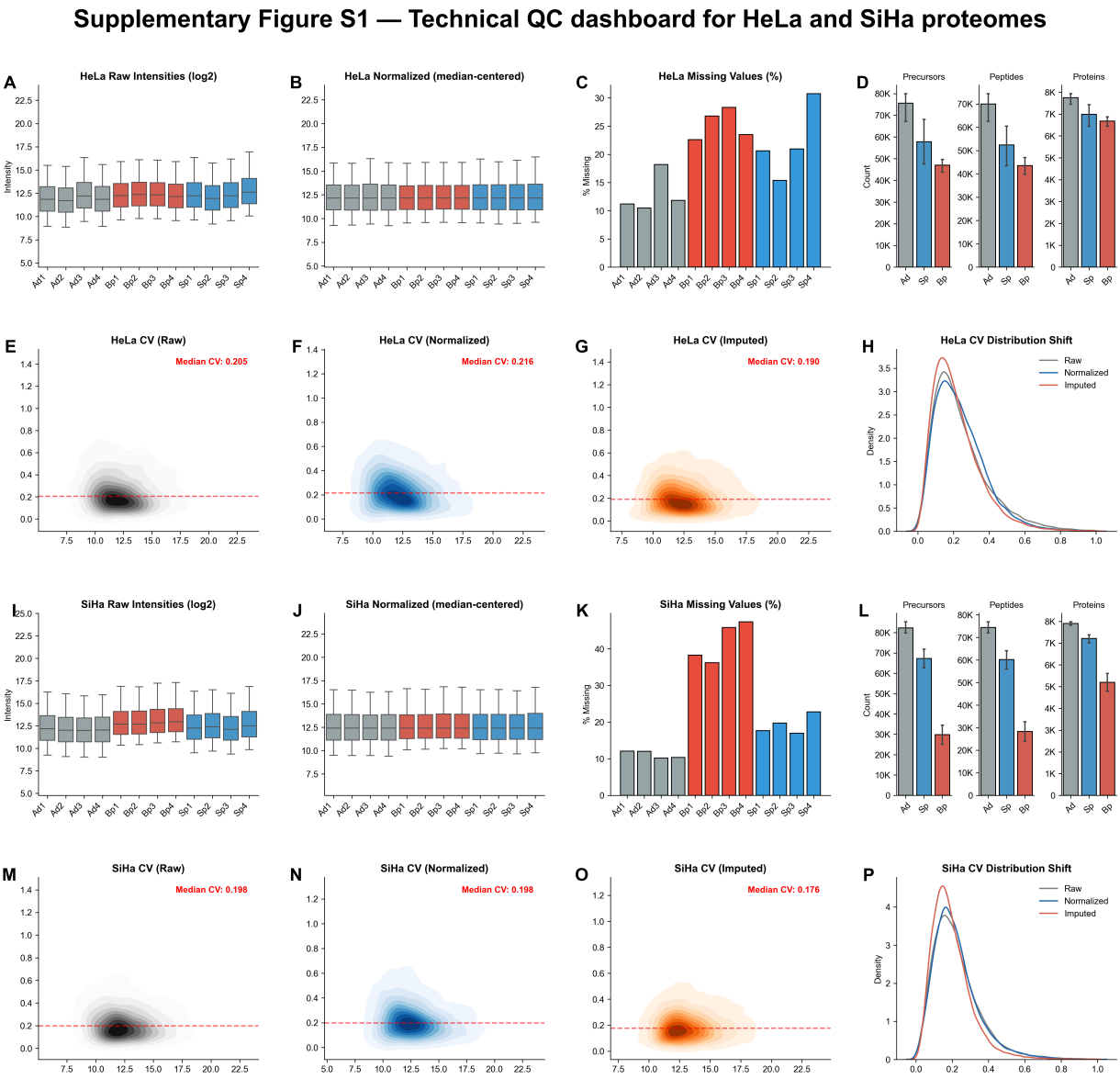
SUPPLEMENTARY INFORMATION

Comparative proteomics reveals distinct architecture-associated states in spheroid and bioprinted cervical cancer models

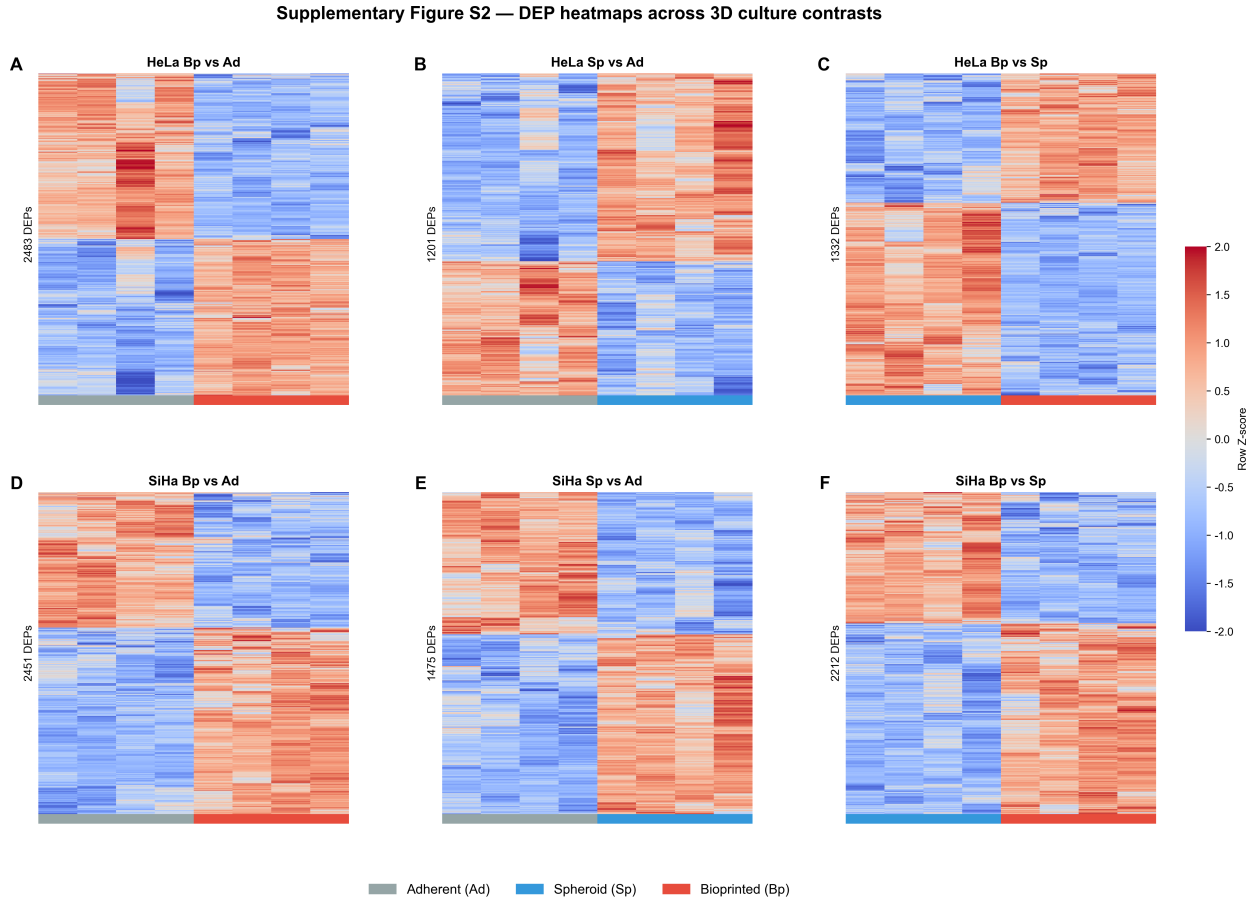
Pablo Saldivia, Carla Villavicencio, María J. Leiva-Carrasco, Francisco Verdugo-Avello, Sergio Hernández, Rodrigo Luengo, Esteban Gutiérrez, Rodrigo San Martín, Mauricio Hernández, Jorge R. Toledo

Supplementary Figure S1



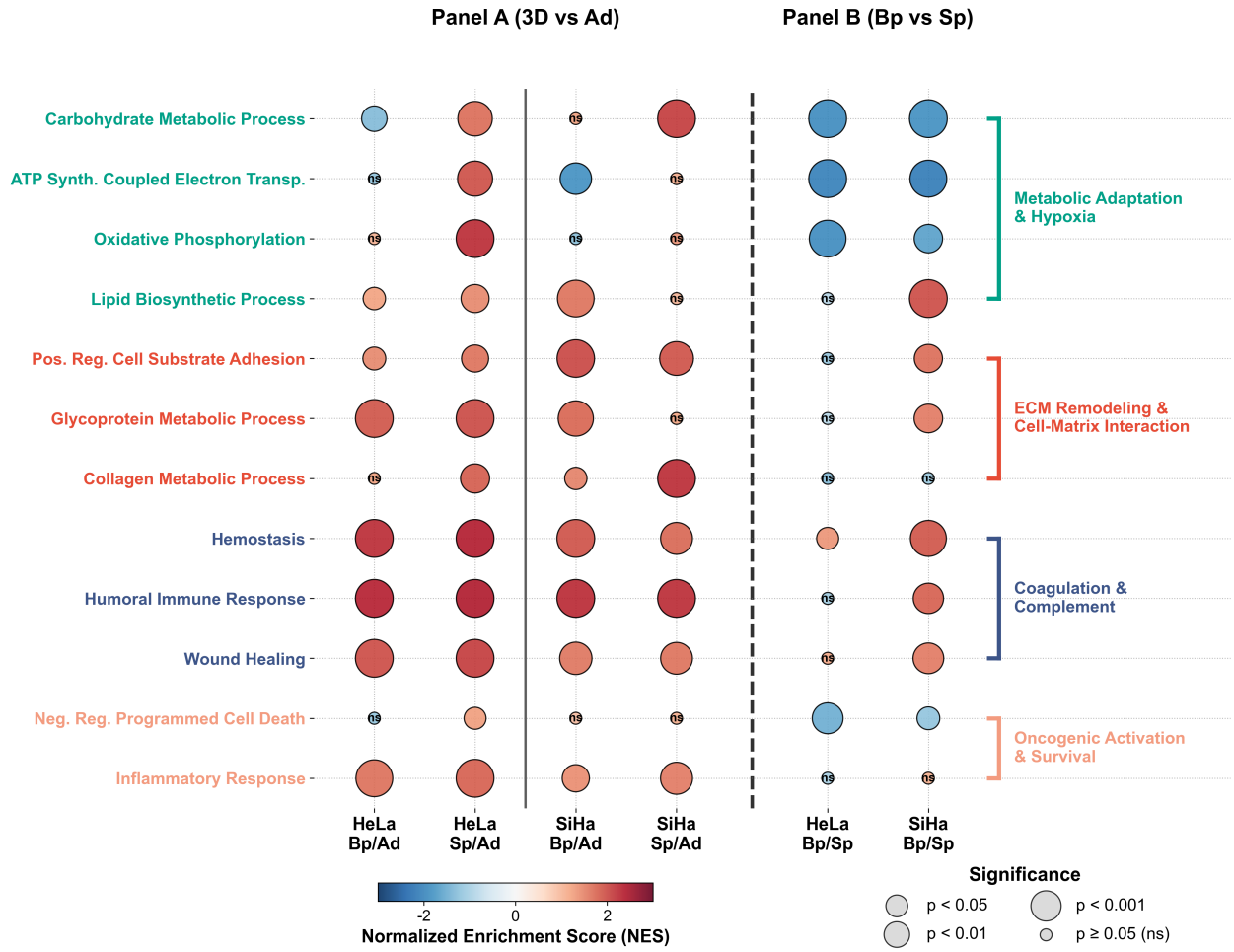
Supplementary Figure S1. Technical quality control dashboard and proteomic identification metrics across culture architectures in HeLa and SiHa cells. (A-H) HeLa cell line: (A) Raw $\log_2$ LFQ intensity distributions across biological replicates in adherent (Ad), spheroid (Sp), and 3D-bioprinted (Bp) models. **(B)** Normalized $\log_2$ LFQ intensity distributions after median centering. **(C)** Proportion of missing values (%) across samples. **(D)** Identification tallies for peptide precursors, stripped sequences, and protein groups across culture architectures. **(E-G)** Two-dimensional kernel density estimation of coefficient of variation (CV) versus $\log_2$ mean intensity for raw (E), normalized (F), and imputed (G) matrices; dashed red line indicates median CV. **(H)** Density distributions illustrating CV distribution shifts across data processing stages. **(I-P) SiHa cell line: (I)** Raw $\log_2$ LFQ intensity distributions. **(J)** Normalized $\log_2$ LFQ intensity distributions. **(K)** Proportion of missing values (%) across samples. **(L)** Identification tallies for precursors, peptides, and protein groups across architectures. **(M-O)** Two-dimensional CV density distributions for raw (M), normalized (N), and imputed (O) matrices. **(P)** CV distribution shifts across processing stages. Detailed sample-level metrics, identification counts, and PRIDE archive identifiers are provided in Supplementary Table S1.

Supplementary Figure S2



Supplementary Figure S2. Unsupervised hierarchical clustering and heatmap profiling of differentially abundant proteins. Heatmaps displaying unsupervised hierarchical clustering of differentially abundant proteins (DEPs; FDR < 0.05, limma empirical Bayes moderated statistics) across adherent (Ad), spheroid (Sp), and 3D-bioprinted (Bp) culture architectures in HeLa and SiHa cervical cancer cells. Protein abundance values are shown as row-scaled Z-scores. Biological replicates predominantly cluster according to culture architecture, reflecting distinct proteomic states between models. Significant differential abundance results (FDR < 0.05), $\log_2$ fold-changes, moderated t-statistics, and adjusted P-values are provided in Supplementary Table S3.

Supplementary Figure S3



Supplementary Figure S3. Gene Ontology Biological Process enrichment across 3D versus adherent and direct bioprinted versus spheroid contrasts. (A) Gene Ontology (GO) Biological Process fast Gene Set Enrichment Analysis (fGSEA) bubble plot for Sp versus Ad and Bp versus Ad comparisons in HeLa and SiHa cells. **(B)** GO Biological Process fGSEA for direct Bp versus Sp comparisons. Bubble color corresponds to Normalized Enrichment Score (NES), where positive NES indicates enrichment in the first condition of the stated contrast and negative NES indicates enrichment in the second (reference) condition. Bubble size reflects statistical significance ($-\log_{10}$ nominal P-value). Full candidate and figure-selected GO Biological Process fGSEA results, alongside over-representation analysis (ORA) and extended GO terms, are provided in Supplementary Table S4.

Index of Supplementary Tables (Supplied as Separate XLSX Files)

Supplementary Table S1: Proteomic identification depth and quality control metrics

File name: **Supplementary_Table_S1.xlsx**

Summary of sample metadata (PRIDE mapping), precursor, peptide, and protein-group counts, missing value distributions, coefficient of variation (CV) distributions across raw, median-normalized, and missForest-imputed matrices, and filtering statistics for HeLa and SiHa samples across Ad, Sp, and Bp architectures (alginate-gelatin bioink; 5 sheets: Description, Sample_metadata, Proteomics_QC_by_sample, Missingness_CV_summary, Protein_group_filtering_summary). Raw MS files processed via DIA-NN v1.9. Supports Figure 2 and Supplementary Figure S1.

Supplementary Table S2: Architecture-associated detected protein subsets and Hallmark over-representation analysis

File name: **Supplementary_Table_S2.xlsx**

Detected protein subsets based on non-imputed presence/absence (LFQ > 0 in >= 1 replicate), condition-restricted protein lists, shared and unique Venn counts across architectures, one-sided hypergeometric Hallmark over-representation analysis (ORA), and STRING interaction network input protein lists (6 sheets: Description, Shared_unique_counts, Protein_subsets, Unique_proteins_by_condition, ORA_architecture_subsets, Figure3_network_inputs). Supports Figure 3.

Supplementary Table S3: Differentially abundant proteins (DEPs) across 3D versus adherent and direct Bp versus Sp comparisons

File name: **Supplementary_Table_S3.xlsx**

Statistically significant differentially abundant proteins (DEPs; Benjamini-Hochberg FDR < 0.05) identified using empirical Bayes moderated t-tests via limma across all six main contrasts: HeLa Bp vs Ad (2,483 DEPs), HeLa Sp vs Ad (1,201 DEPs), HeLa Bp vs Sp (1,332 DEPs), SiHa Bp vs Ad (2,451 DEPs), SiHa Sp vs Ad (1,475 DEPs), and SiHa Bp vs Sp (2,212 DEPs). Contrast sheets contain exclusively significant DEPs (FDR < 0.05). Includes DEP summary tallies, $\log_2$ fold-changes, moderated t-statistics, P-values, and FDR (8 sheets: Description, DEP_summary, HeLa_Bp_vs_Ad, HeLa_Sp_vs_Ad, HeLa_Bp_vs_Sp, SiHa_Bp_vs_Ad, SiHa_Sp_vs_Ad, SiHa_Bp_vs_Sp). Supports Figures 4 and 5.

Supplementary Table S4: Functional gene set enrichment analysis (Hallmark, GO BP fGSEA, ORA, and GO CC/MF) across all contrasts

File name: **Supplementary_Table_S4.xlsx**

Functional enrichment analyses across all six contrasts: preranked fGSEA results for 16 prioritized MSigDB Hallmark gene sets (96 contrast tests; 7 displayed in Figure 4C and 5C heatmaps); Gene Ontology Biological Process (GO BP) fGSEA candidate screening and plotted outputs supporting Supplementary Figure S3 (1,176 candidate rows; 72 plotted entries); over-representation analyses (ORA) supporting Figures 4 and 5; extended GO Cellular Component (CC) and Molecular Function (MF) annotations (4,856 terms; 578 nominal p < 0.05, 224 FDR < 0.05); and biological module definitions for M1-M3 (8 sheets: Description, Enrichment_summary, Hallmark_fGSEA, Bp_vs_Ad_Sp_vs_Ad_enrichment, Direct_Bp_vs_Sp_enrichment, GOBP_fGSEA, GO_CC_MF_extended, Module_Definitions). Supports Figures 4 and 5, and Supplementary Figure S3.

Supplementary Table S5: Selected protein-level assessment values and statistics for representative module markers

File name: **Supplementary_Table_S5.xlsx**

Individual replicate-level normalized $\log_2$ LFQ intensities (288 values), condition-level summary statistics (72 summaries), and unequal-variance Welch's t-test outputs (48 contrast comparisons) for the 12 definitive functional module markers matching Figure 6 across M1 (ENO2, SLC2A1, PKM, ENO1, TPI1), M2 (TIMP2, ILK, TLN1), and M3 (C3, CD47, ITIH2, CLU) in HeLa and SiHa cultures (4 sheets: Description, Marker_values, Marker_summary, Welch_tests). Supports Figure 6 and Table 1.

Note: *Supplementary Tables S1-S5 are supplied as separate multi-sheet Microsoft Excel (.xlsx) workbooks accompanying this submission.*