# Supporting Information for

# Analysis of Pancreatic Extracellular Matrix Protein Post-Translational Modifications via Electrostatic Repulsion-Hydrophilic Interaction Chromatography Coupled with Mass Spectrometry

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**KEYWORDS**

electrostatic repulsion-hydrophilic interaction chromatography (ERLIC), post-translational modifications (PTM), phosphopeptide, glycopeptide, mass spectrometry, pancreas, extracellular matrix

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**Table S1.** UPLC-MS/MS method details.

| Chromatography and ESI-MS Instrument Acquisition | | | |
|---|---|---|---|
| Resuspension volume | 13 µL (E1 and E2, 3% ACN, 0.1% FA); 20 µL (W, 3% ACN, 0.1% FA); 400 µL (FT, 0.1% FA) | MS1 Maximum IT | 100 ms |
| Injection volume | 1.5 µL (E1, E2), 2 µL (FT, W) | RF Lens (%) | 30 |
| Stationary phase | Bomb-packed BEH C18 column (75 µm i.d. x 360 um o.d., ~15 cm of 1.7 µm beads, capped with 3 µm beads) | Isolation | Quadrupole |
| LC solvent A | 0.1% FA in H2O | Isolation window | 1.6 *m/z* |
| LC solvent B | 0.1% FA in 100% ACN (E1 and E2) or 95% ACN (FT and W) | Charge states | 2-8, undetermined |
| Gradient ramp and duration | 3-30% B in 90 min | Dynamic exclusion duration (after 1 time) | 30 s |
| Flow rate | 0.3 µL/min | MS2 resolution | 30000 |
| Mass spectrometer | Thermo Orbitrap Fusion Lumos Tribrid | MS2 AGC target | 5E4 |
| Spray voltage | 2 kV | Minimum intensity requirement | 2.5E4 |
| MS1 detection | Orbitrap | MS2 acquisition | Data dependent, centroid, top 20 |
| MS1 scan range | 400-2000 *m/z* | MS2 fragmentation | Stepped HCD (22, 30, 38%) |
| MS1 resolution | 120000 | MS2 detection | Orbitrap |
| MS1 AGC target | 2E5 | MS2 fixed first mass | 120 *m/z* |

**Table S2.** Proteome Discoverer 2.1 data analysis method details.

| Data analysis settings | | | |
|---|---|---|---|
| Precursor mass tolerance | 10 ppm | Static modifications | Carbamidomethylation (+57.02146 Da) @ C |
| Fragment mass tolerance | 0.01 Da | Dynamic modifications | Oxidation (+15.99492 Da, rare1) @ M; deamidation (+0.984016 Da, rare1) @ N, Q; glycosylation (common1) @ N; phosphorylation (+79.96633, common2) @ S, T, Y |
| Target FDR | 1% | Total common mods max. | 1 |
| Min. peptide length | 4 residues | Total rare mods max. | 2 |
| Enzyme | Trypsin | Missed cleavages | <3 |

**Table S3.** Donor information.

| Pancreas | Gender | Donor age (years) | DCD/DBD | BMI (kg/m$^2$) | CIT (hours) |
|---|---|---|---|---|---|
| 20 | Female | Range: 7-61, Average: 47 | DBD | Range: 14.7 - 27.2, Average: 22.4 | Average: 10.5 |
| 21 | Female | | DBD | | |
| 22 | Male | | DCD | | |
| 24 | Female | | DBD | | |

**Fig. S1.** ERLIC enrichment comparison of PSMs between fractions and enrichment specificities. The ERLIC enrichment proceeded with two separate elutions (E1 and E2), with the flow-through (FT) and wash (W) fractions also analyzed. Error bars reflect standard deviations of four biological replicates per tissue condition. PSMs were compared in each fraction between native and decellularized samples in terms of A) N-glycopeptides, B) phosphopeptides, and C) "other" peptides without glyco- or phospho- modifications. D) compares the enrichment specificity (PSM count for a specific PTM/total PSMs) between tissue condition and PTM among fractions.

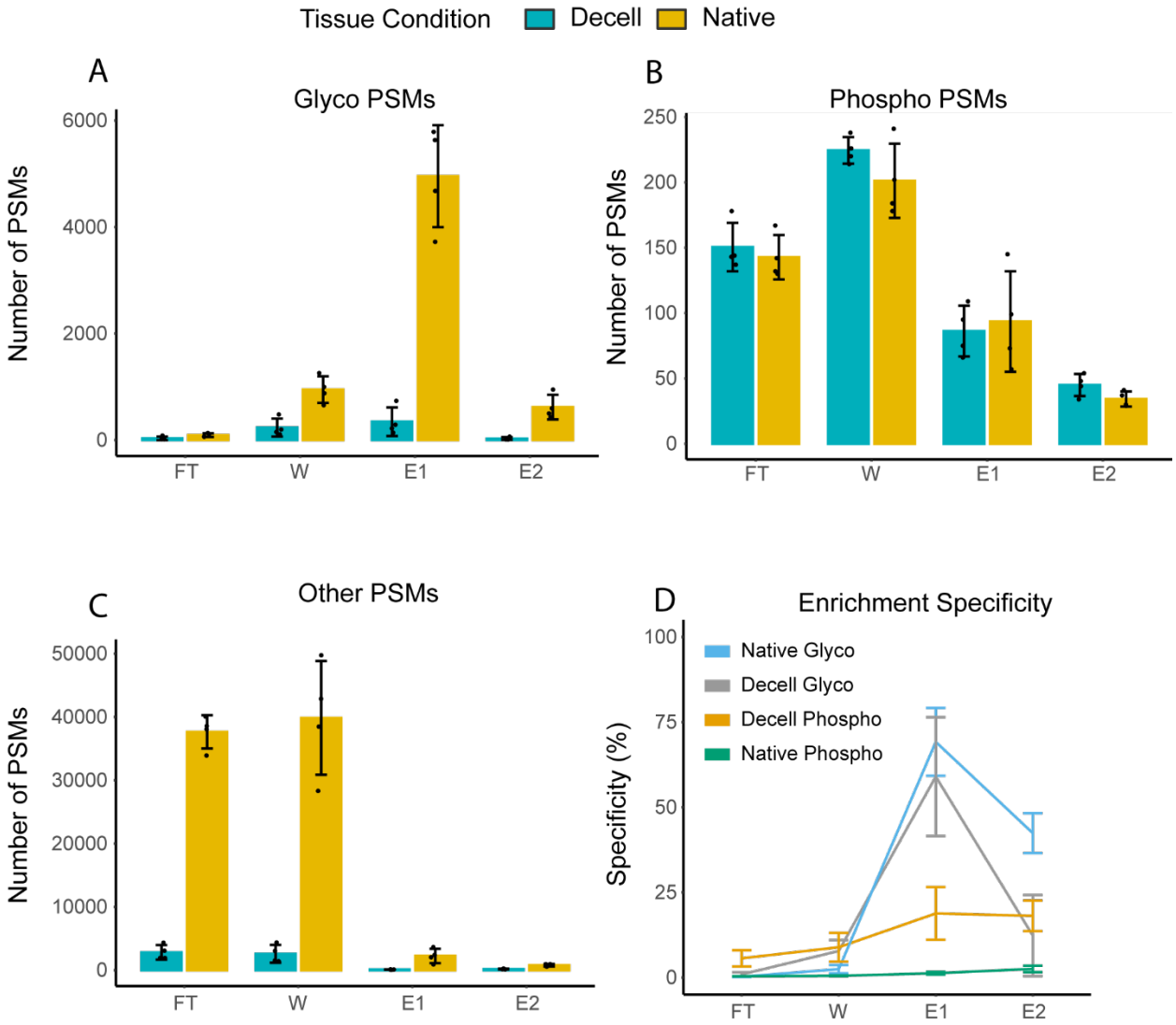


**Fig. S2.** Network of statistically enriched terms from identified glycoproteins generated using Metascape ([http://metascape.org]).[1]

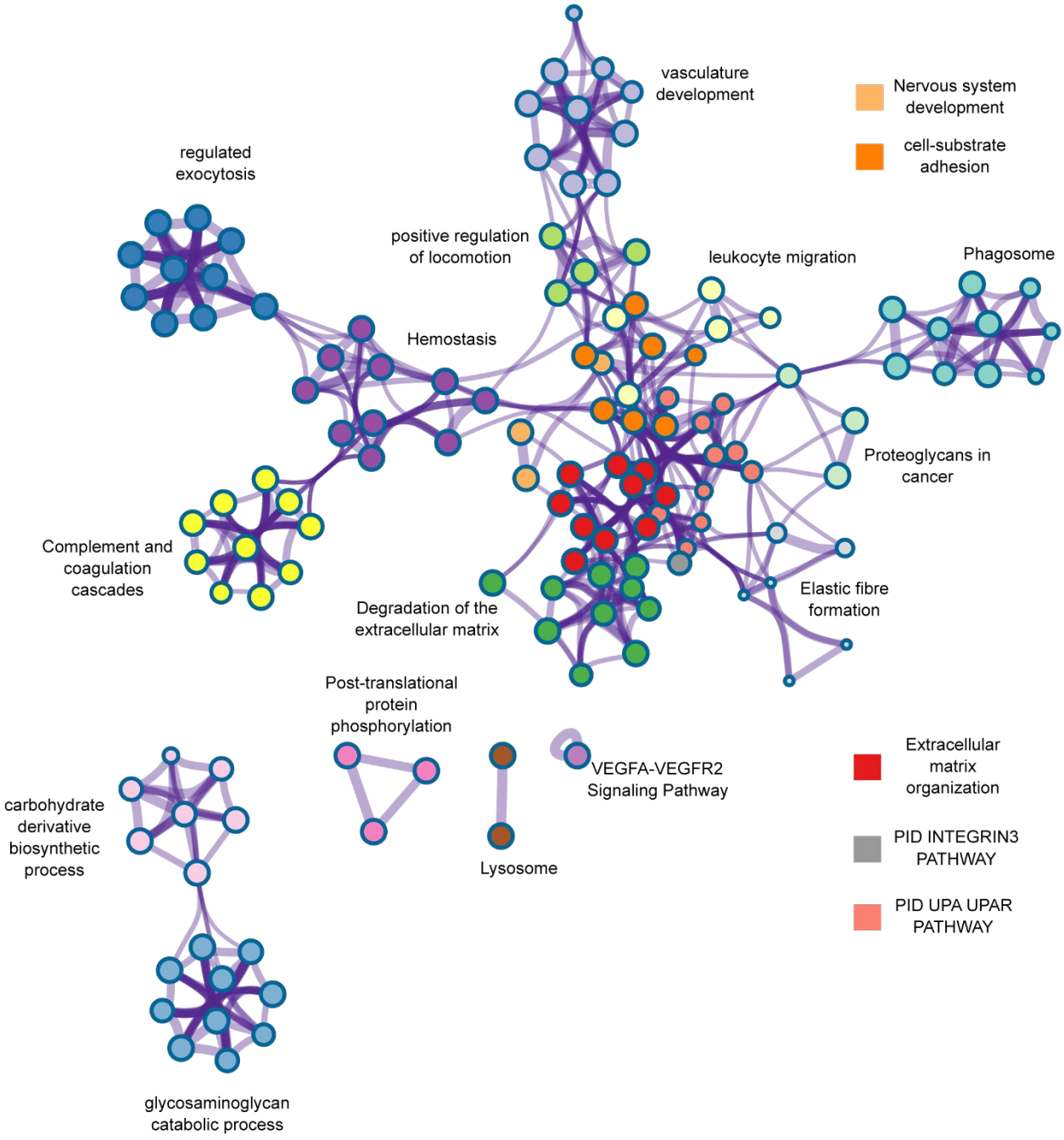


**Fig. S3.** Network of statistically enriched terms from identified phosphoproteins.

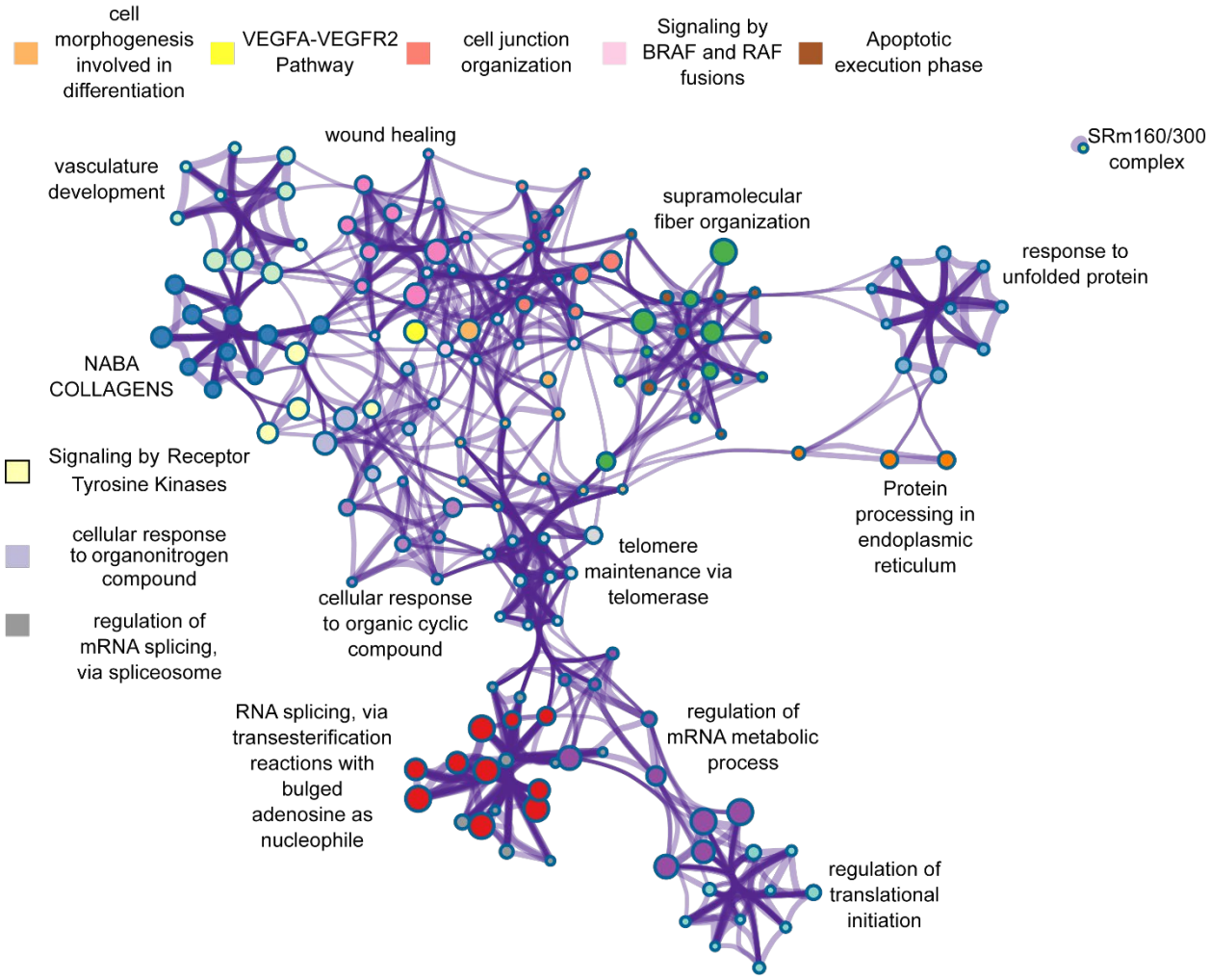


**Fig. S4.** Network of statistically enriched terms from identified proteins bearing both glycosylation and phosphorylation.

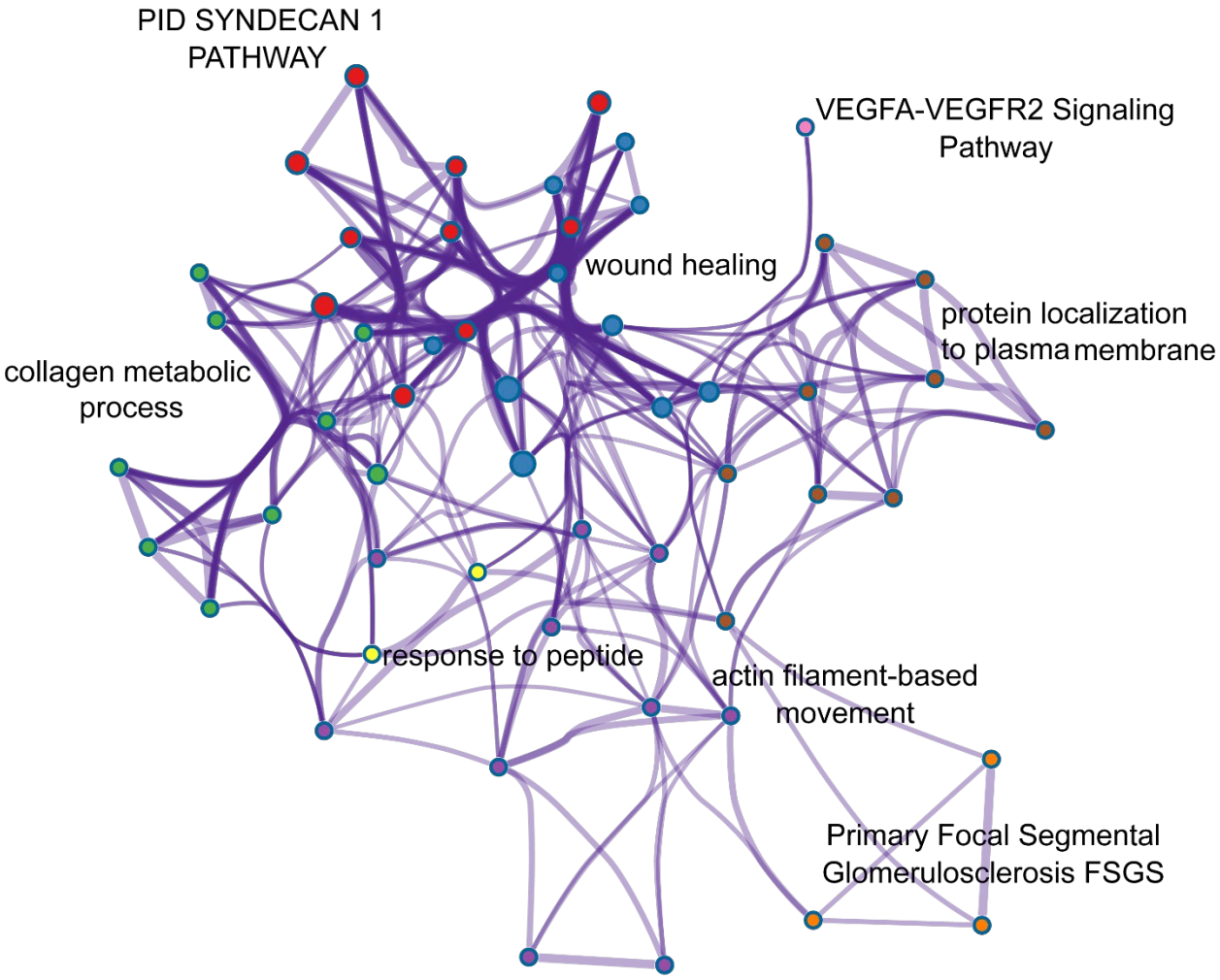


**Fig. S5.** Network of statistically enriched terms from identified M6P-containing glycoproteins.

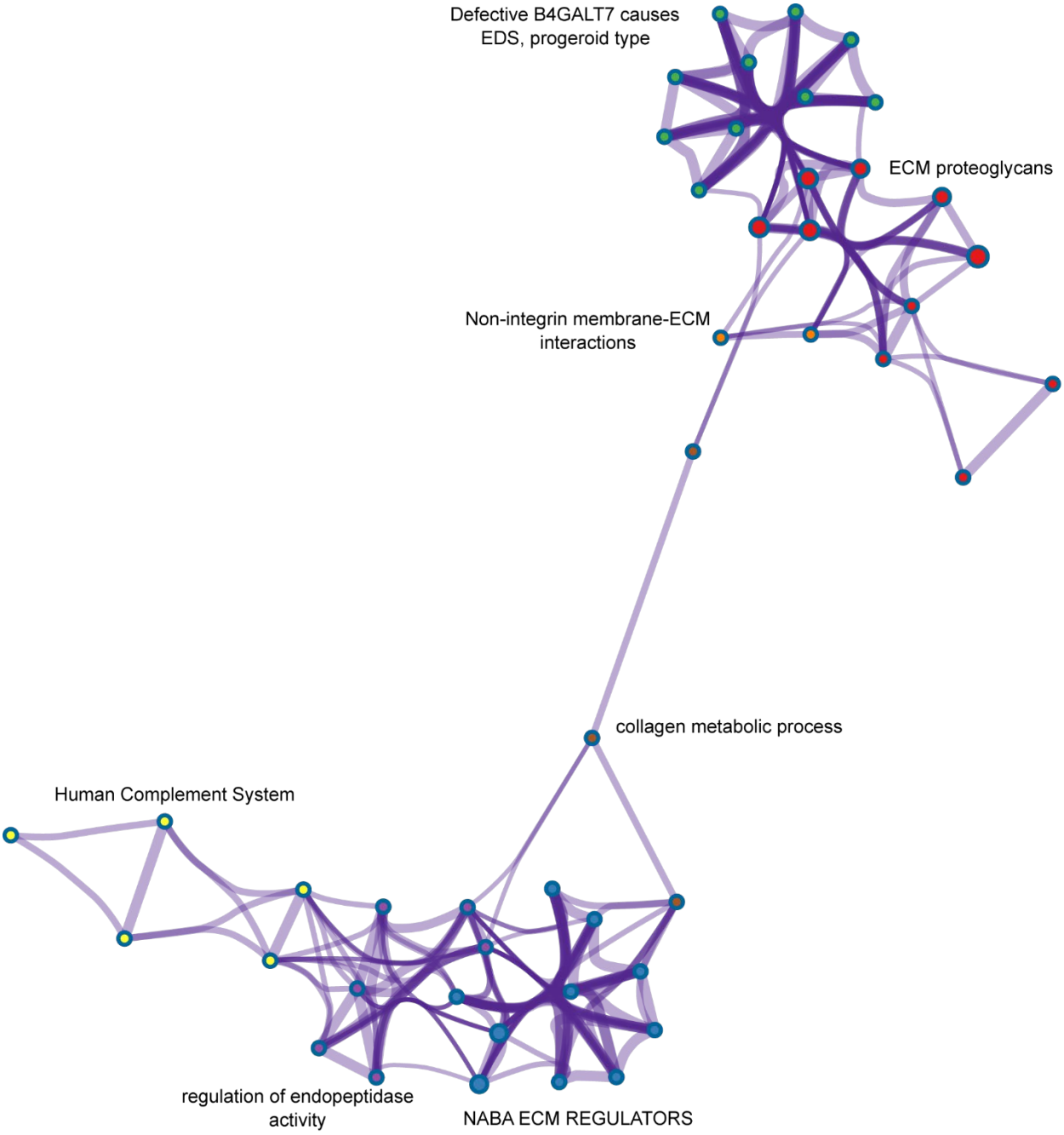


**Fig S6.** Comparison of hydrophobicity of peptide sequences via grand average of hydropathicity (GRAVY) scores of peptide sequences identified in native samples versus decellularized samples using the Kidera[2] and Kyte-Doolittle[3] hydrophobicity scales.

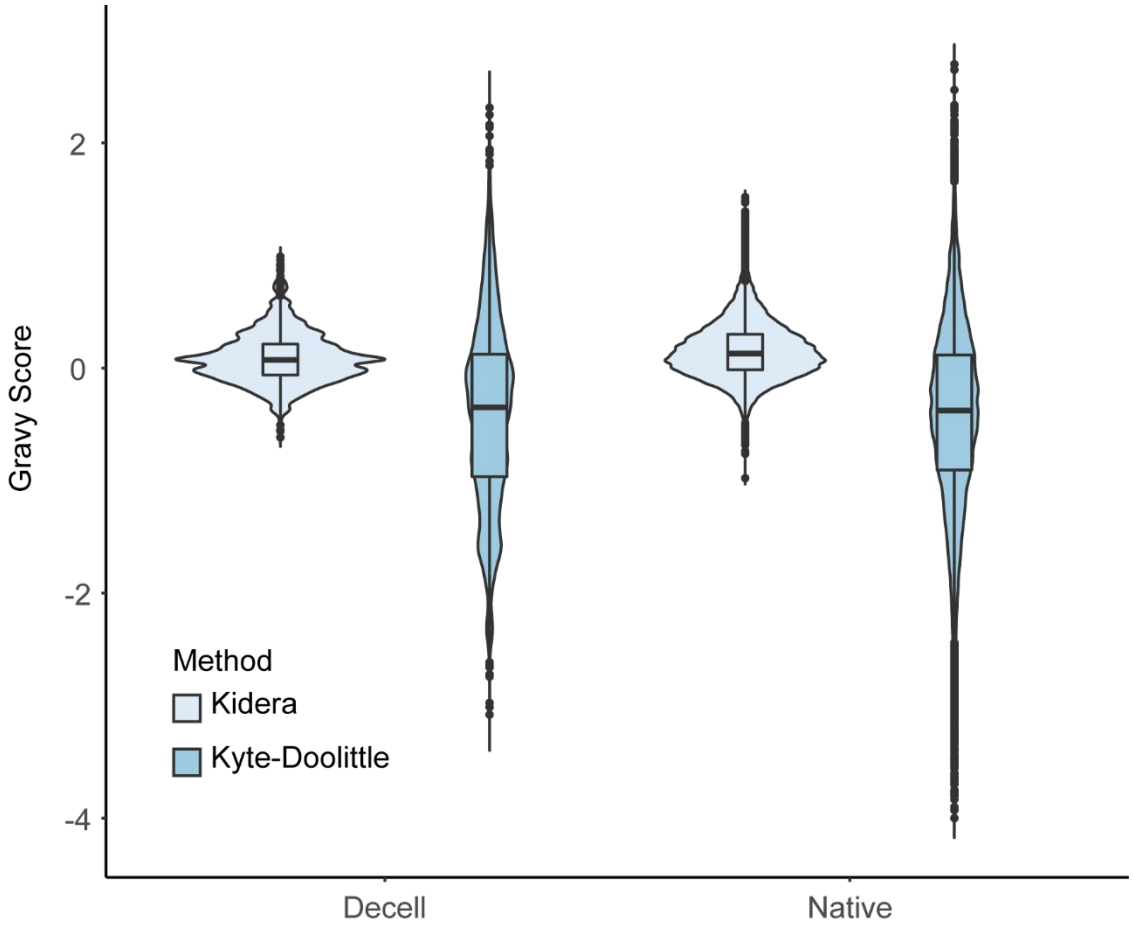
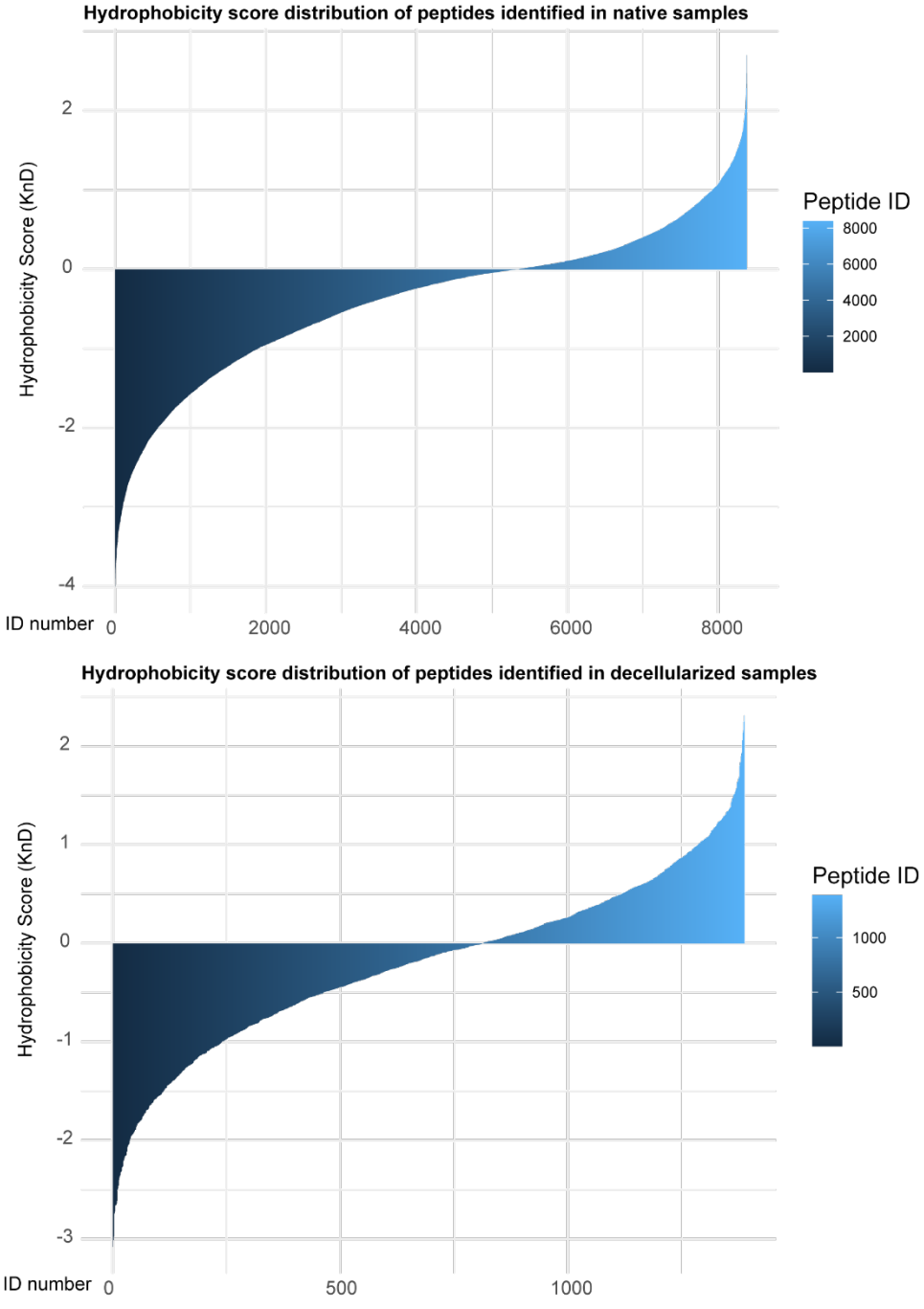


**Fig S7.** Comparison of hydrophobicity scores (Kyte-Doolittle; KnD) of peptide sequences identified in native samples versus decellularized samples plotted against peptide ID number (ranked from least to most hydrophobic).



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