

## 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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**Supplemental Data S1.** Excel file with sheets detailing the identified proteins and whether they are ECM, glycosylated, or phosphorylated; glycan compositions; glycosites and glycoforms; and phosphosites.

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

Chromatography and ESI-MS Instrument Acquisition			
Resuspension volume	13 $\mu$ L (E1 and E2, 3% ACN, 0.1% FA); 20 $\mu$ L (W, 3% ACN, 0.1% FA); 400 $\mu$ L (FT, 0.1% FA)	MS1 Maximum IT	100 ms
Injection volume	1.5 $\mu$ L (E1, E2), 2 $\mu$ L (FT, W)	RF Lens (%)	30
Stationary phase	Bomb-packed BEH C18 column (75 $\mu$ m i.d. x 360 $\mu$ m o.d., ~15 cm of 1.7 $\mu$ m beads, capped with 3 $\mu$ m beads)	Isolation	Quadrupole
LC solvent A	0.1% FA in H <sub>2</sub> O	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 $\mu$ L/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 $m/z$

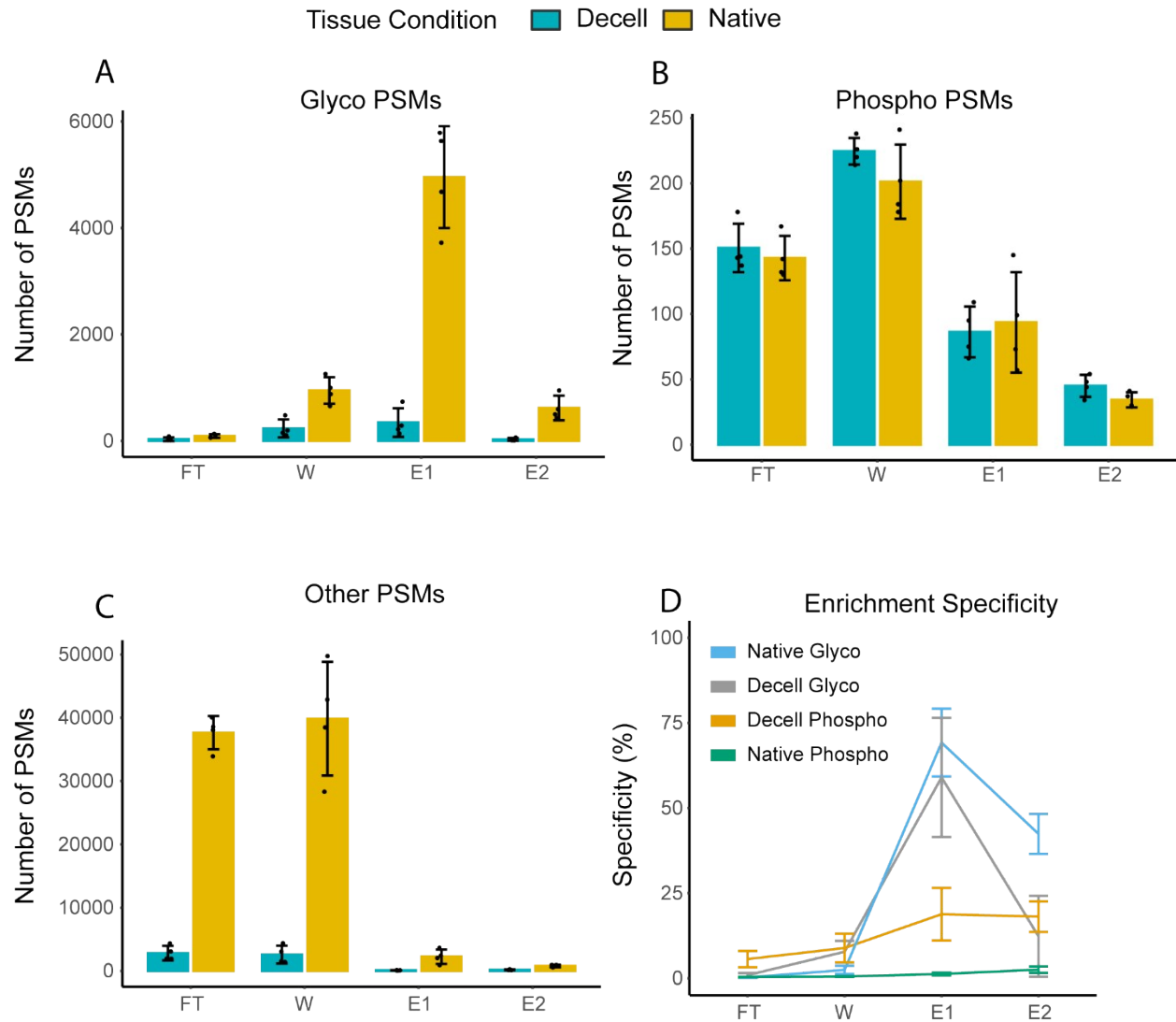
**Table S2.** Proteome Discoverer 2.1 data analysis method details.

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

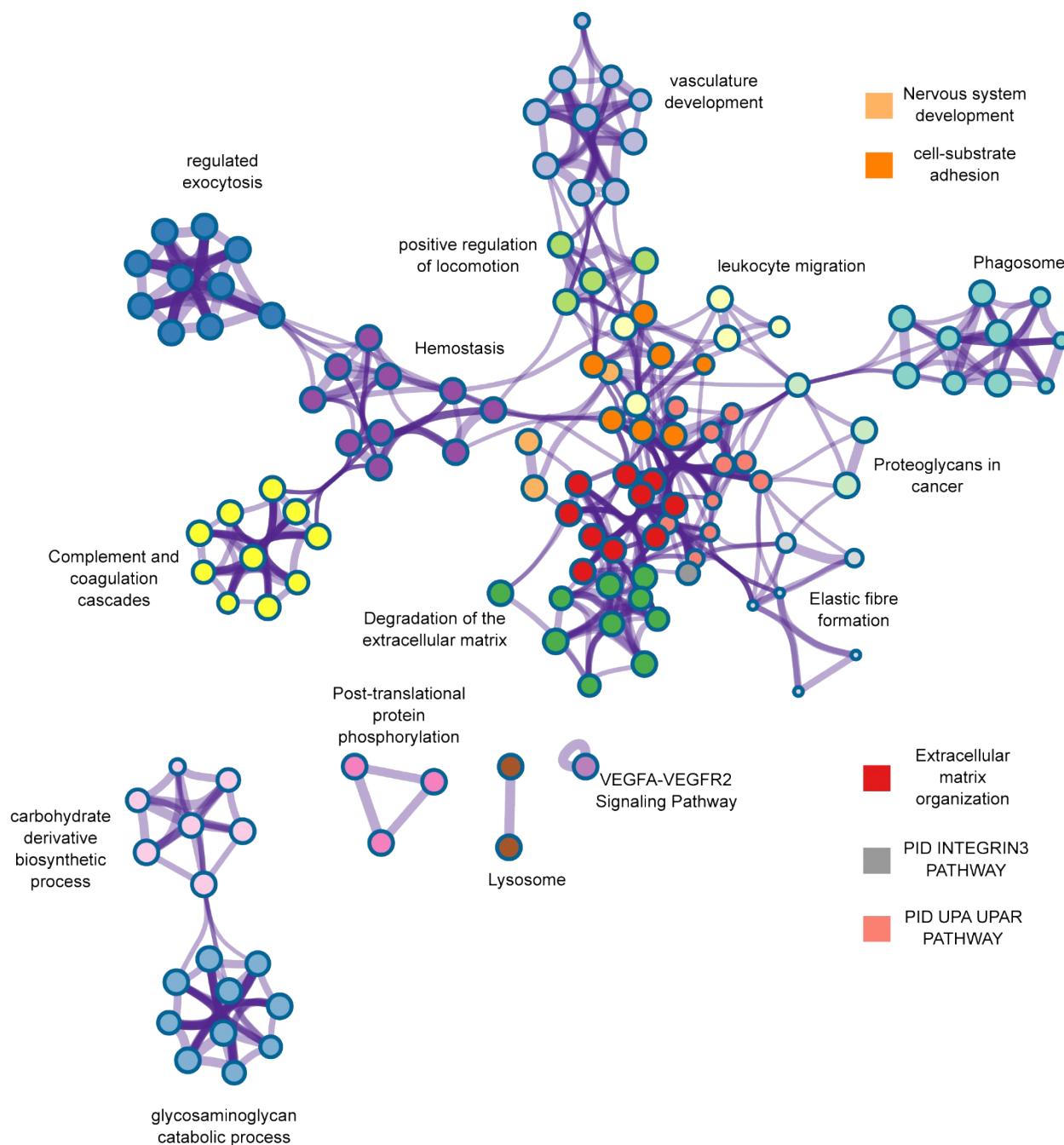
**Table S3.** Donor information.

<b>Pancreas</b>	<b>Gender</b>	<b>Donor age (years)</b>	<b>DCD/DBD</b>	<b>BMI (kg/m<sup>2</sup>)</b>	<b>CIT (hours)</b>
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		

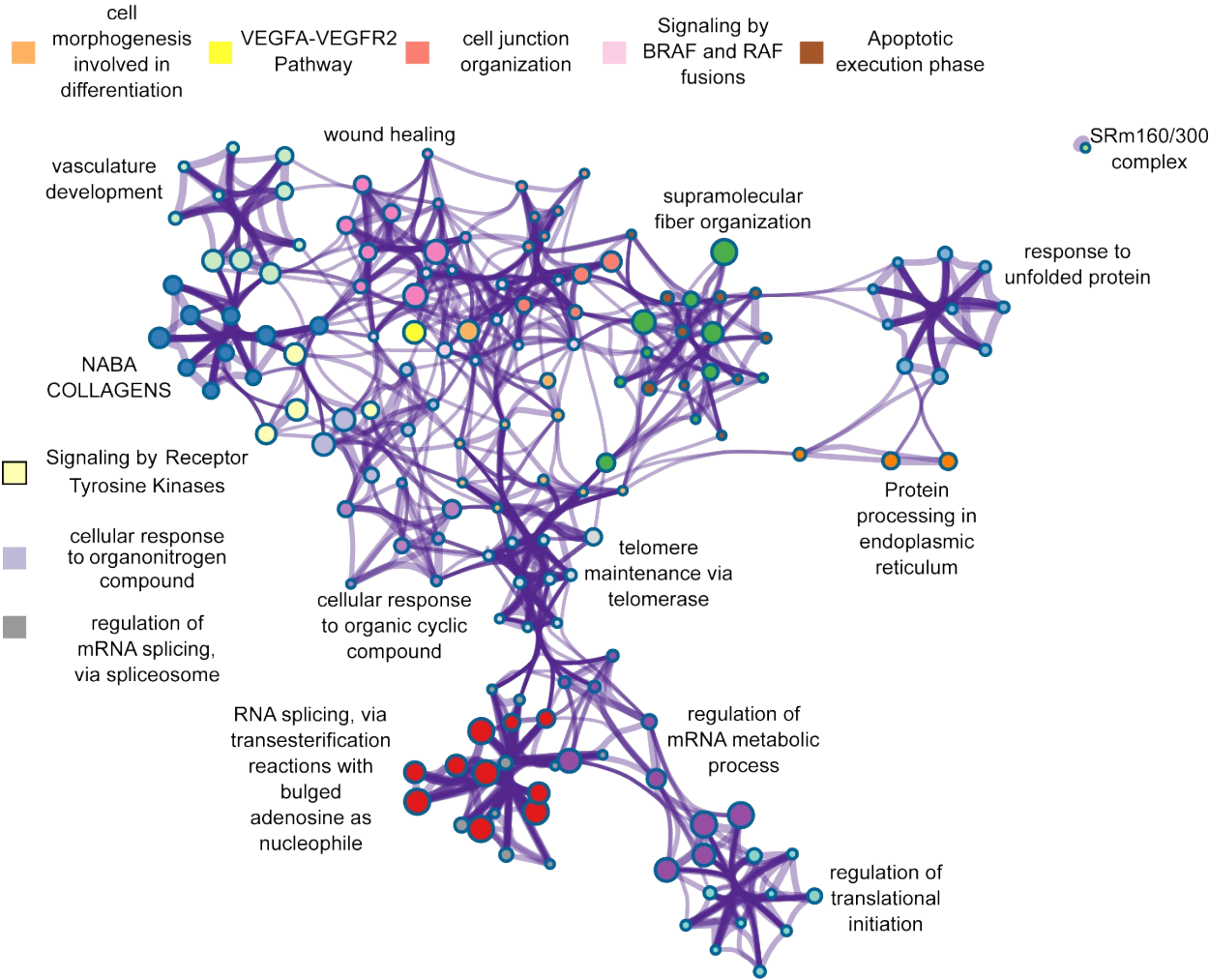
**Fig. S1.** ERLIC enrichment comparison of PSMs between fractions and enrichment specificities. The ERLIC enrichment proceeded with two separate elutions (E1 and E2), with the flow-through (FT) and wash (W) fractions also analyzed. Error bars reflect standard deviations of four biological replicates 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>]).<sup>1</sup>

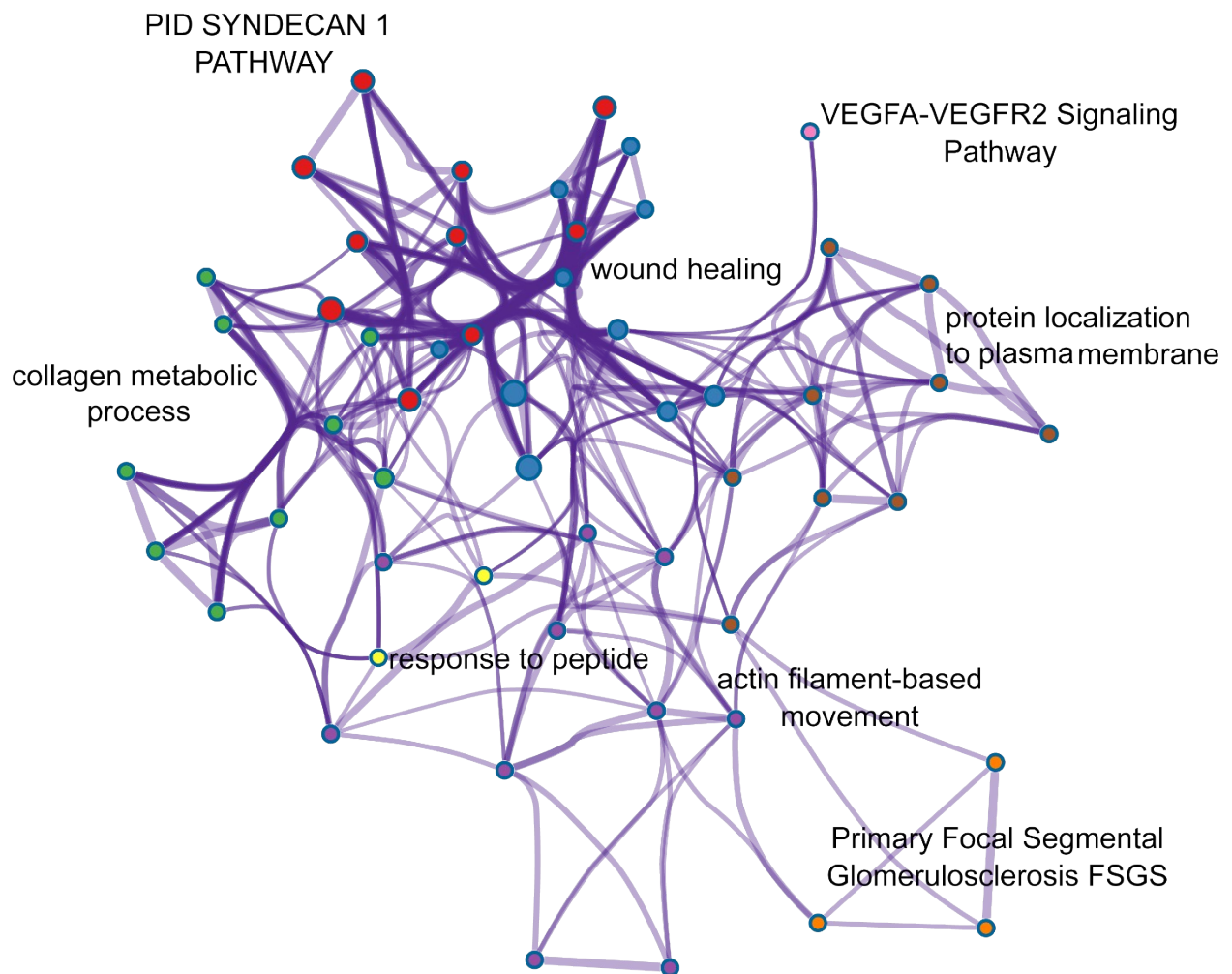


**Fig. S3.** Network of statistically enriched terms from identified phosphoproteins.

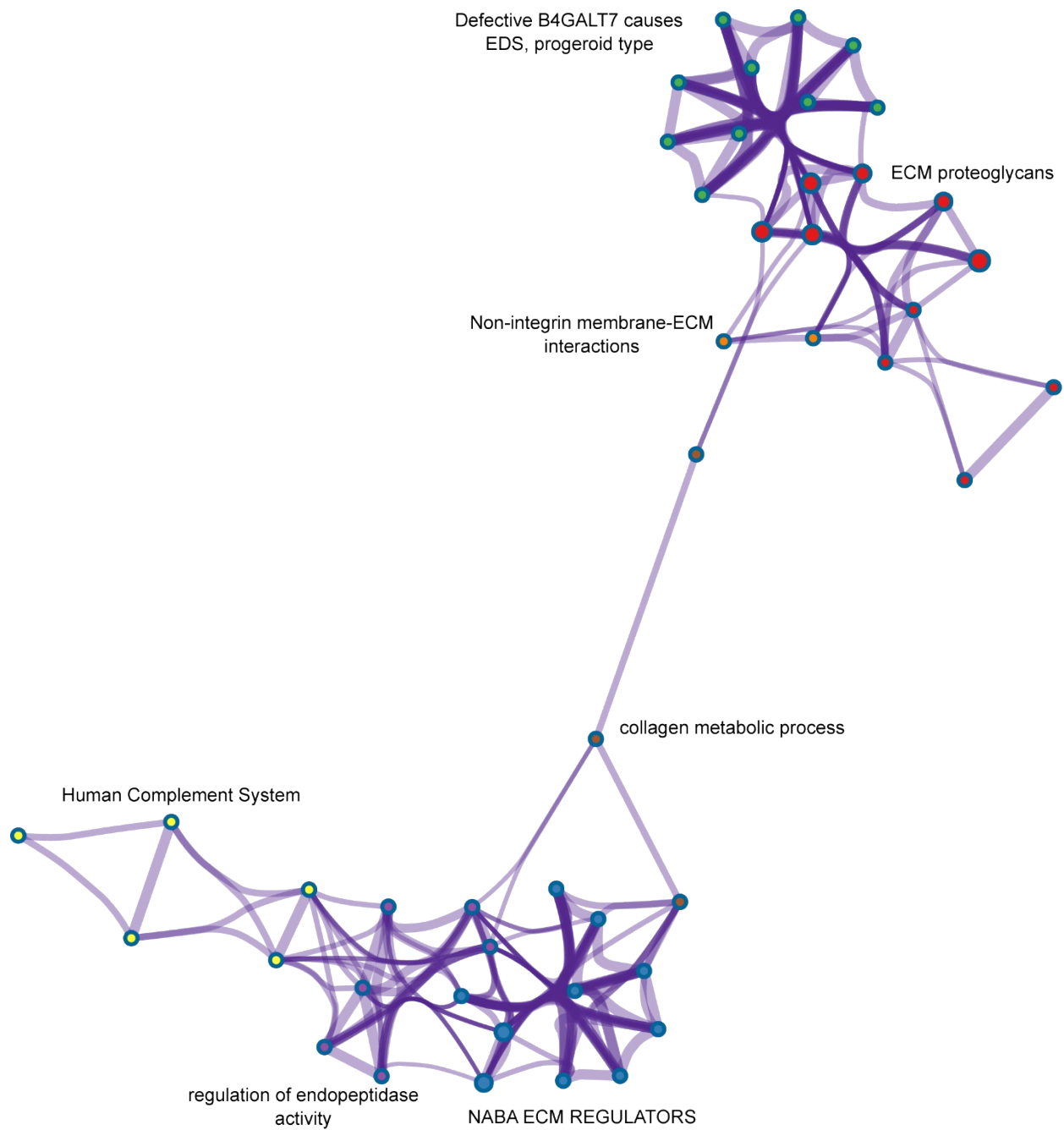




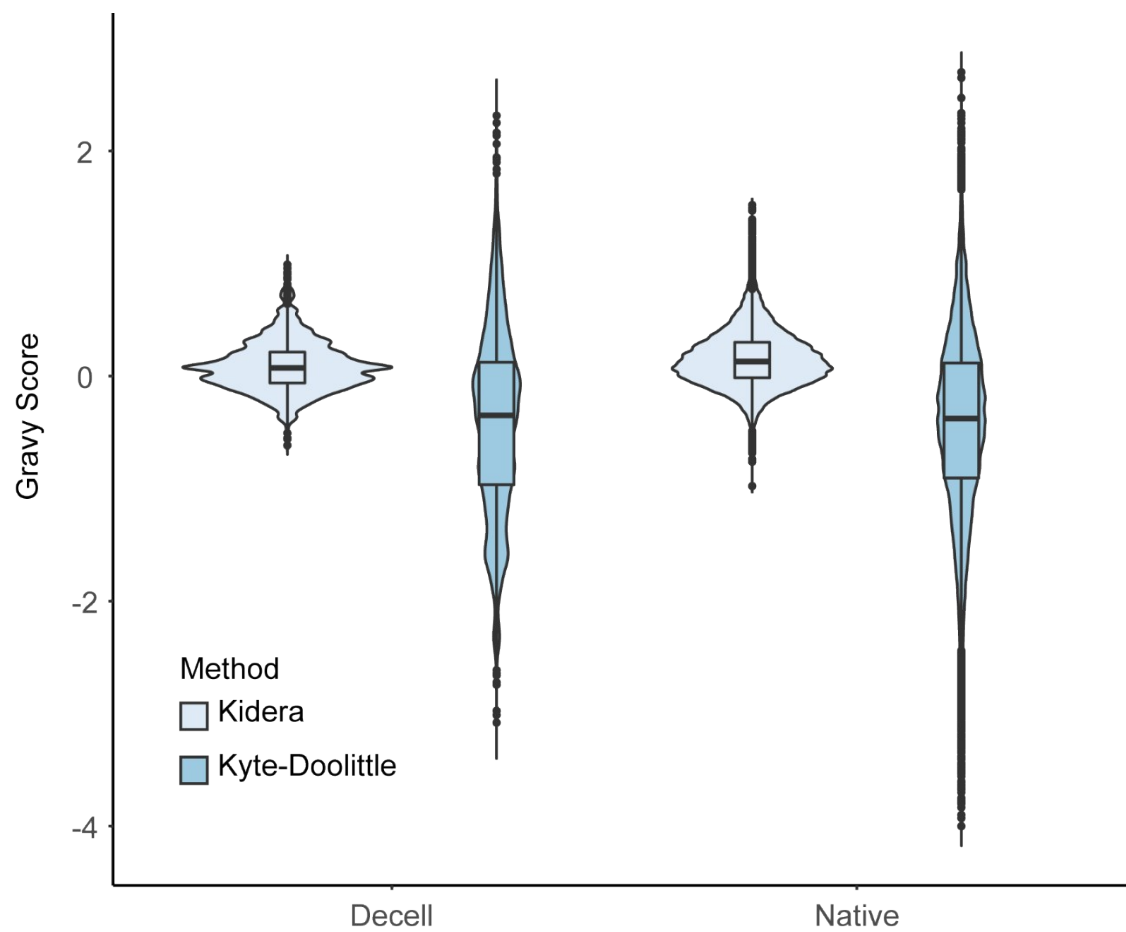
**Fig. S4.** Network of statistically enriched terms from identified proteins bearing both glycosylation and phosphorylation.



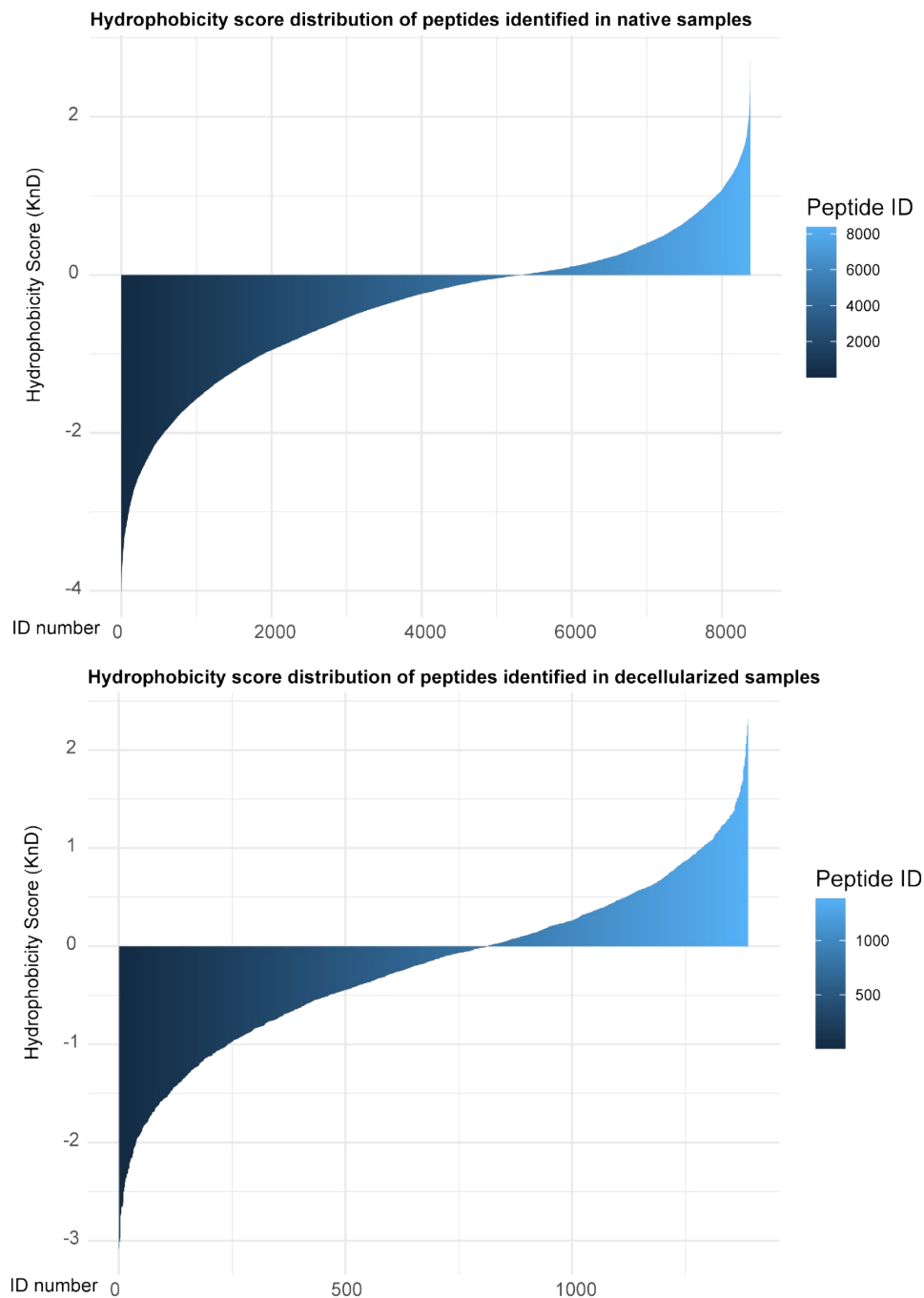
**Fig. S5.** Network of statistically enriched terms from identified M6P-containing glycoproteins.



**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<sup>2</sup> and Kyte-Doolittle<sup>3</sup> 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).



## REFERENCES

1. Y. Zhou, B. Zhou, L. Pache, M. Chang, A. H. Khodabakhshi, O. Tanaseichuk, C. Benner and S. K. Chanda, *Nat Commun*, 2019, **10**, 1523.
2. A. Kidera, Y. Konishi, M. Oka, T. Ooi and H. A. Scheraga, *J. Protein Chem.*, 1985, **4**, 23-55.
3. J. Kyte and R. F. Doolittle, *J. Mol. Biol.*, 1982, **157**, 105-132.