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Systematic Review, Data Acquisition & FragPipe Analysis

Table S1 Dataset Information LFQ

	DOI	PAPER TITLE	ORG	TISSUE	INJURY	PaperStat	Diff Protein List	Num Raws	mzML Size	Ran IDPicker
PXD020 420	https://doi.org/10.1038/s41598-020-69865-4	Proteomic analysis identifies plasma correlates of remote ischemic conditioning in the context of experimental traumatic brain injury	Mus musculus (mouse)	Blood Plasma	Midline Fluid Percussion Injury (mFPI)	Statistics: obj-For mass	•	39	45.0 GB	•
PXD017 478	https://doi.org/10.3389/fncel.2020.00032	Filling the gaps – a call for comprehensive analysis of extracellular matrix of the glial scar in region- and injury-specific contexts	Mus musculus (mouse)	Brain Tissue	Parasagittal stab wound injury	Statistics: obj-Graphpad	•	70	65.0 GB	9897 clusters; 12525 protein groups; 17656 proteins; 30.17% protein FDR; 477851 spectra
PXD024 317	https://doi.org/10.3390/ijms22052246	Potential Neuroprotective Mechanisms of Methamphetamine Treatment in Traumatic Brain Injury Defined by Large-Scale IonStar-Based Quantitative Proteomics	Rattus norvegicus (rat)	Brain Tissue; Cortex & Hippocampus brain tissue	Lateral Fluid Percussion Injury	Statistics: obj-Generated quantitative	•	78	53.0 GB	•
PXD011 262	https://doi.org/10.1074/mcp.RA119.001604	Mapping Spatiotemporal Microproteomics Landscape in Experimental Model of Traumatic Brain Injury Unveils a link to Parkinson's Disease	Rattus norvegicus (rat)	Brain Tissue; Macrophages; Astrocytes cell lines?	Controlled Cortical Impact (CCI)	Statistics: obj-The	Supp avail, but not clearly marked, unable to know which file a title relates to	97	58.0 GB	
PXD021 869	https://doi.org/10.1534/genetics.120.303776	Survival Following Traumatic Brain Injury in Drosophila Is Increased by Heterozygosity for a Mutation of the NF-κB Innate Immune Response Transcription Factor Relish	Drosophila Melanogaster (Fruit Fly)	Brain Tissue	Subjected to four strikes from the HIT device, with 5 min between strikes.	Statistics: NaN	Suppl => GO DOUBLE CHECK THIS	60	210 GB	•
Project 1707	-	-	Mus musculus (mouse) Cell line	N2A cells	2DG & SDT	•	•	30	9.56 GB	3949 clusters; 4476 protein groups; 7682 proteins; 3.49% protein FDR; 477851 spectra - had to check unmapped for it to work
PXD008 906	https://doi.org/10.15252/ejmbr.201745294	Cross-talk between monocyte invasion and astrocyte proliferation regulates scarring in brain injury	Mus musculus (mouse)	Brain Tissue	parasagittal stab wound			24	16.2 GB	

Table S2 Dataset Information Labelled

	DOI	PAPER TITLE	ORG	TISSUE	INJURY	mzML Size
PXD035289	https://doi.org/10.1186/s12974-022-02654-0	Temporal proteomics of human cerebrospinal fluid after severe traumatic brain injury.	Homo Sapiens (human)	Cerebrospinal Fluid	Medical TBI	54.6 GB
PXD002783	https://doi.org/10.1016/j.brainres.2016.04.008	Protein profile changes in the frontotemporal lobes in human severe traumatic brain injury.	Homo Sapiens (human)	Brain Tissue	Medical TBI	9.43 GB
PXD010602	https://doi.org/10.1177/1759091420914768	Unbiased Proteomic Approach Identifies Pathobiological Profiles in the Brains of Preclinical Models of Repetitive Mild Traumatic Brain Injury, Tauopathy, and Amyloidosis.	Mus musculus (mouse)	Brain, Blood Cell, Astrocyte Of The Cerebral Cortex, Blood Plasma, Neuron Of Cerebral Cortex, Microglial Cell	Controlled Cortical Impact (CCI) ?	
PXD010664	https://doi.org/10.3389/fnagi.2018.00405	Unbiased Proteomic Approach Identifies Unique and Coincidental Plasma Biomarkers in Repetitive mTBI and AD Pathogenesis.	Mus musculus (mouse)	Brain Tissue, Plasma?	Controlled Cortical Impact (CCI) ?	
PXD029312	https://doi.org/10.3389/fnmol.2021.785938	Quantitative Proteomics Reveals the Dynamic Pathophysiology Across Different Stages in a Rat Model of Severe Traumatic Brain Injury	Rattus norvegicus (Rat)	Brain Tissue	Controlled Cortical Impact (CCI)	21.6 GB
PXD014923	https://doi.org/10.1016/j.jep.2020.112826	TMT-based proteomics analysis reveals the protective effects of Xuefu Zhuyu decoction in a rat model of traumatic brain injury	Rattus norvegicus (Rat)	Brain Tissue; Cortex brain tissue	Controlled Cortical Impact (CCI)	
PXD025204	https://doi.org/10.3389/fnmol.2021.688050	Tandem Mass Tag-Based Quantitative Proteomic Analysis Reveals Pathways Involved in Brain Injury Induced by Chest Exposure to Shock Waves	Mus musculus (mouse)	Brain Tissue	Shock Waves	7.34 GB
PXD038607	https://doi.org/10.3390/molecules28196768	Massive Solubility Changes in Neuronal Proteins upon Simulated Traumatic Brain Injury Reveal the Role of Shockwaves in Irreversible Damage	Homo sapiens (human)	human neuroblastoma SH-SY5Y cells	employing a 5.1 kg dummy impact generating a ≤3,300 g-force acceleration and performed system-wide profiling of neuronal cells using Proteome Integral Solubility Alteration (PISA) assay = shockwave	19.6 GB
PXD038675	https://doi.org/10.3390/molecules28196768	Massive Solubility Changes in Neuronal Proteins upon Simulated Traumatic Brain Injury Reveal the Role of Shockwaves in Irreversible Damage	Homo sapiens (human)	human neuroblastoma SH-SY5Y cells	employing a 5.1 kg dummy impact generating a ≤3,300 g-force acceleration and performed system-wide profiling of neuronal cells using Proteome Integral Solubility Alteration (PISA) assay = shockwave	9.08 GB

Table S3 FragPipe Settings Used

PXD	DOI	PAPER TITLE	SPECIE	TISSUE	INJURY	Data Aquisition	Experimental Design	Database Used in Study	MSFRAGGER: PEAK MATCHING	MSFRAGGER: PROTEIN DIGESTION	MSFRAGGER: MODIFICATIONS
PXD020420	https://doi.org/10.1038/s41598-020-69865-4	Proteomic analysis identifies plasma correlates of remote ischemic conditioning in the context of experimental traumatic brain injury	Mus musculus (mouse)	Blood Plasma	Midline Fluid Percussion Injury (mFPI)	- LFQ	Conditions 1. Sham (n = 8) 2. Sham RIC (n= 8) 3. TBI (n = 10) 4. TBI RIC (n= 13) Total = 39	- Mouse UniprotKB/SwissProt 2015 database.		- digested overnight at 37°C using Trypsin Gold	- none mentioned
PXD017478	https://doi.org/10.3389/fncel.2020.00032	Filling the gaps – a call for comprehensive analysis of extracellular matrix of the glial scar in region- and injury-specific contexts	Mus musculus (mouse)	Brain Tissue	Parasagittal stab wound injury	- LFQ - MBR	Conditions: 1. WT_Injured 2. WT_Uninjured 3. CCR2_Injured 4. CCR2_Uninjured	- mouse Uniprot sequence database (uniprot.org)		- at 37 °C for 2h with LysC (1/50) and then with LysC (1/50) and Trypsin (1/25) overnight	- Fixed modifications: Cysteine carbamidomethylation - Variable modifications: hydroxylation of proline and methionine oxidation
PXD024317	https://doi.org/10.3390/ijms22052246	Potential Neuroprotective Mechanisms of Methamphetamine Treatment in Traumatic Brain Injury Defined by Large-Scale IonStar-Based Quantitative Proteomics	Rattus norvegicus (rat)	Brain Tissue; Cortex & Hippocampus brain tissue	Lateral Fluid Percussion Injury	- LFQ	For cortical and hippocampal each: 1) SH, sham 2) TM, mild TBI 3) TS, severe TBI 4) TSm, severe TBI treated	- rat SwissProt+TrEMBL database (ver.201608, 35,953 entries)		- Trypsin	- Fixed modification: carbamidomethylation of cysteine; - Dynamic modification: oxidation of methionine and acetylation of peptide N-terminal
PXD011262	https://doi.org/10.1074/mcp.RA119.001604	Mapping Spatiotemporal Microproteomics Landscape in Experimental Model of Traumatic Brain Injury Unveils a link to Parkinson's Disease	Rattus norvegicus (rat)	Brain Tissue; Macrophages; Astrocytes cell lines?	Controlled Cortical Impact (CCI)	-	-	-	-	-	-
PXD021869	https://doi.org/10.1534/genetics.120.303776	Survival Following Traumatic Brain Injury in Drosophila Is Increased by Heterozygosity for a Mutation of the NF-κB Innate Immune Response Transcription Factor Relish	Drosophila Melanogaster (Fruit Fly)	Brain Tissue	Subjected to four strikes from the HIT device, with 5 min between strikes.	- LFQ - MBR	Conditions: 1. Control @ 10 timepoints (0-24hr) 2. TBI @ 10 timepoints (0-24hr) Replicates: n=3, mostly	- uploaded to PRIDE repo - Uniprot, decoys and isoforms; accessed 8 Aug 2019: 1. Control @ 10 timepoints (0-24hr) 2. TBI @ 10 timepoints (0-24hr)	- high resolution? - max 2 missed cleavages (Orbitrap, resolution of 240 000 in the range of m/z 300 to 1350) => add both enzymes to settings? - No, both have the same cutting places; LysC just more efficient => 10/20 ppm? => only influence fragment mass tolerance or precursor as well?	- LysC & Trypsin - max 2 missed cleavages => These are similar to what I already have as default	- fixed modification for carbamidomethylation of cysteine residues - variable modification for oxidation of methionine, N-terminal acetylation
Project 1707	-	-	Mus musculus (mouse)	Cell culture	neuronal injury through 2-deoxyglucose exposure	- LFQ	Conditions 1. Control 2. Treatment	- mouse reference proteome (MusMusculus_RefProt_UP00000589_55319_280622.fasta) downloaded from UniprotKB on 28/06/2022.		- LysC & Trypsin - max 2 missed cleavages	-

					& neuronal injury through sodium dithionite exposure		3. Injury2DG 4. Injury2DG_AF16 5. InjurySDT 6. InjurySDT_AF16 = 36 samples (?)				
PXD008906	https://doi.org/10.15252/ejmbr.201745294	Cross-talk between monocyte invasion and astrocyte proliferation regulates scarring in brain injury	Mus musculus (mouse)	Brain Tissue	parasagittal stab wound	- LFQ	-	-	-	-	-
PXD002783	https://doi.org/10.1016/j.brainres.2016.04.008	Protein profile changes in the frontotemporal lobes in human severe traumatic brain injury	Homo Sapiens (human)	Brain Tissue	Medical TBI	- 2-plex TMT labeled (TMT6 according to PRIDE) (used TMT10 in FP)	TMT tags: 130 = control 131 = TBI	- human uniprot database	Q Exactive mass spec => HIGH Res	Extracts were then digested with 1:100 (w/w) endopeptidase Lys-C overnight at 37 °C. The extracts were diluted with PBS (pH 8.0) to give a final urea concentration of 1.0 M, and were then digested with trypsin 1:50 (w/w) overnight. => strict trypsin => up to 2 missed cleavages	Var modifications: 1. Methionine oxidation (+15) 2. Acetylation (+42) 3. Hydroxylation of proline (+16) 4. n-terminus Tag (+229) Fixed modifications: 1. Cysteine carbamidomethylation (+57) 2. K (lysine) tag (+229)

Comparison Across Different Species

Table S4 Accession Number Across Species for TBI Candidate Proteins Look Up

Protein Name	Human UniProt Accession (UP000005640.fas)	Mouse UniProt Accession (UP00000589.fas)	Rat UniProt Accession (UP000002494.fas)	Drosophila UniProt Accession (UP00000803.fas)
p62 (SQSTM1)	Q13501	Q64337	O08623	P14199
NBR1	Q14596	Q05BC8; A2A4N5; A2A4N8	A0A0G2K0K7; A0A8I6A311; A0A8I6G713	x
mTOR	P42345	Q9JLN9	P42346	Q9VK45
AMPKa1	Q13131; Q13131-2	Q5EG47	P54645	Q5U118
AMPKa2	P54646	Q8BRK8	Q09137	O18645
AMPK γ 1	P54619	O54950	P80385; A0A8L2R2B3; A0A0G2K913	Q8IGX0
AMPK γ 2	Q9UGJ0; A0A494C068	Q91WG5; Q91WG5-2	A0A140UHX4	Q0KI34; A1A703
AMPK β 3	Q9UGI9	Q8BGM7; Q8BGM7-2	D4A7E4	x
AMPK β 1	Q9Y478	Q9R078	P80386	A1Z7Q8
AMPK β 2	P54646	Q8BRK8	Q09137	O18645
Beclin1	Q14457	O88597; E9PYD6	Q91XJ1	Q9VCE1
LC3A	Q9H492	Q91VR7	Q6XVN8	x Atg8 (Q9W2S2)
LC3B	Q9GZQ8; A6NCE7	Q9CQV6; A0A8L2UNA3; A0A0G2K9Q7	Q62625; Q62625-2	x
Atg5	Q9H1Y0	Q99J83	Q3MQ06	Q9W3R7
Atg7	O95352; O95352-2	Q9D906; A0A0A0MQN4	Q641Y5; A0A8I6AGV7; A0A8I6G565; A0A8I6GJJ9; A0A8L2QWN0; A0A8I6GGN5	Q7JY94
Atg12	O94817	Q9CQY1	Q2TBJ5	Q9VTU1
Caspase3	P42574; C9JXR7	P70677; A0A1B0GRX1	P55213	x
Caspase6	P55212	O08738	O35397; F6Q5I5	x
Caspase7	P55210-3	P97864	Q6IRF9; A0A0G2K9Z4; A0A8I5ZQU7	O01382; O02002
Caspase8	Q14790; Q14790-2; Q14790-4; A0A8Q3SID9	O89110; A0A087WQT6	Q9JHX4	Q8IRY7
Caspase9	P55211	Q8C3Q9	Q9JHK1; Q920G4; A6ITW6	x
Caspase10	Q92851-5	x	x	Q9XYF4
PARP	P09874	P11103; Q921K2	P27008	P35875
Cytochrome C	P99999	P62897	P62898; D4A5L9	P04657; P84029
AIF	O95831; O95831-3; A0A6Q8PFE1; A0A6Q8PFM5	Q9Z0X1; B1AU25	Q924M6; Q9JM53	Q9VQ79; Q9VQ79-1
Bcl-2	P10415; P10415-2	P10417; P10417-2	P49950; A0A8I6AJ02	Q9V9C8
Bax	Q07812	Q07813	Q63690	x
Bak	Q16611	O08734	F7EQF3	x
Calpain1	P07384	O35350	P97571	Q11002
Calpain2	P17655	O08529; Q3U3A8; Q3U8S5	Q07009	Q9VT65
RIPK1	Q13546	Q60855	D3ZYL0	x
RIPK3	Q9Y572; Q9Y572-2	Q9QZL0; A0A2I3BQW9	Q9Z2P5	x
CPLA2	P47712	P47713; Q9DBX5	P50393; A0A0G2KAA9; A0A8I5ZNU3; A0A8I6AFX4; F7EZZ6	x
HMGB1	P09429	P63158; A0A0J9YUZ4	P63159; A0A0G2K6P4; A0A8I6A2H1; D3ZC69; A0A8I5Y7D7; A0A8I6GBU6; A0A8I6GKR4; D3ZXR5	x
MLKL	Q8NB16; Q8NB16-2; I3L2T9	Q9D2Y4; Q9D2Y4-2; D3YV42	A0A8I5ZV82; D3ZKP6	x
Interleukin-6	P05231	P08505	P20607	x

Interleukin-6 α	P08887	P22272	P22273	x
Interleukin-6 β	P40189	Q00560	P40190	x
TNF α	P01375	P06804	P16599	x
CCL2	P13500	P10148	P14844	x
Mitofusin1	Q8IWA4	Q811U4	Q8R4Z9	x
Mitofusin2	O95140	Q80U63	Q8R500	x

Explorative Statistics

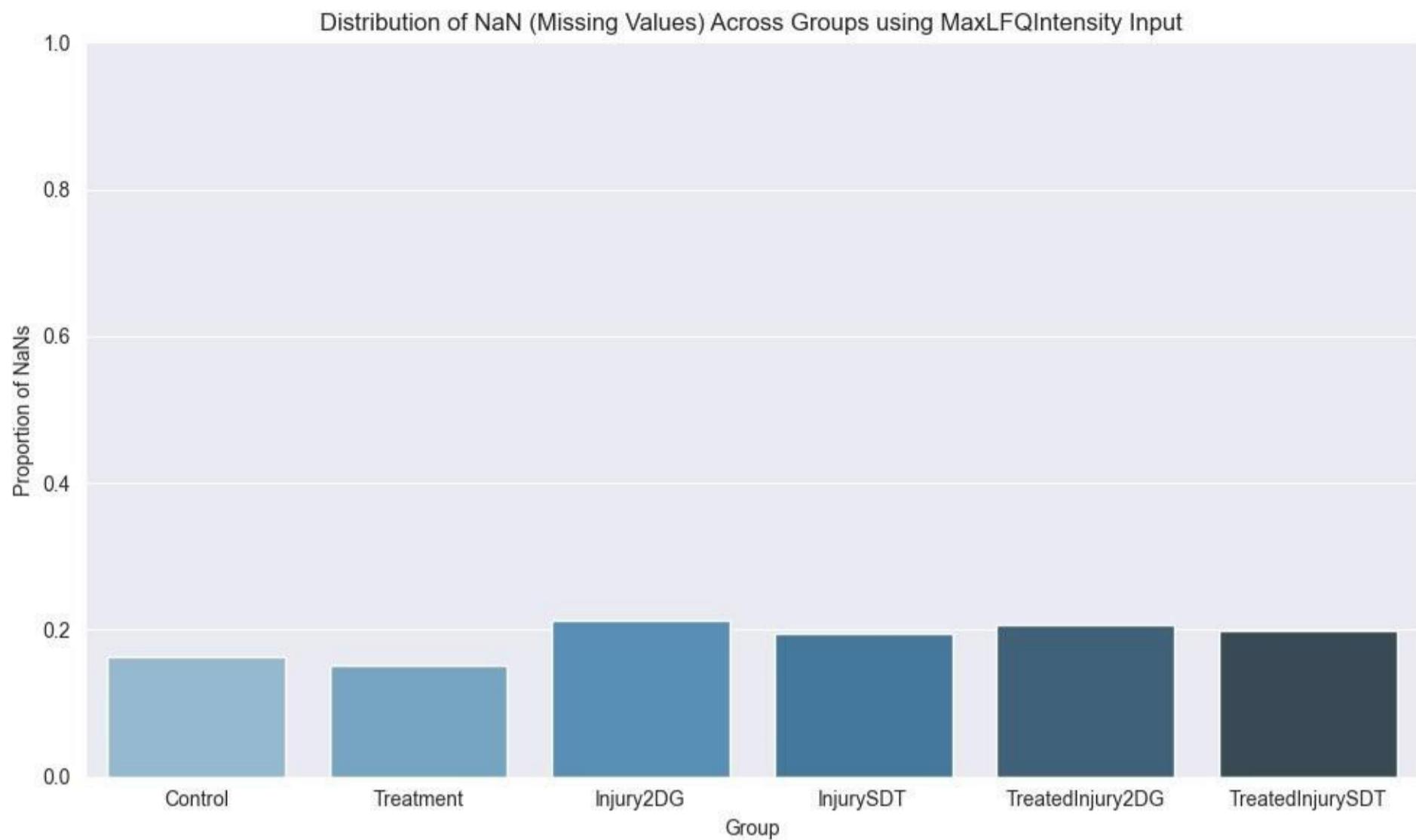


Figure S1 Proportion of missing values (NaNs) across experimental groups based on MaxLFQ intensity data. The groups include Control, Treatment, Injury2DG, InjurySDT, TreatedInjury2DG, and TreatedInjurySDT. The y-axis represents the proportion of NaNs, highlighting the variability in missing data across the groups.

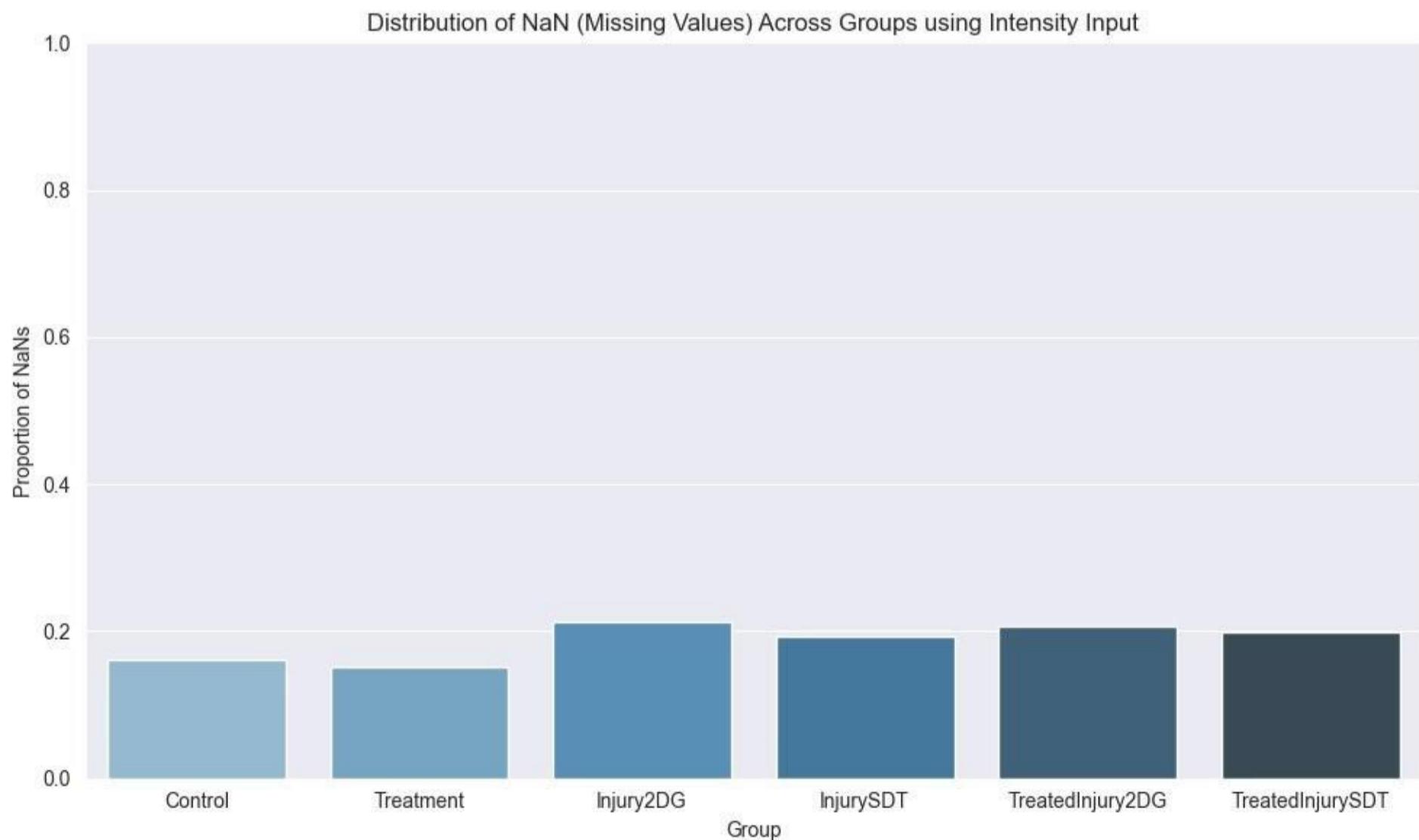


Figure S2 Proportion of missing values (NaNs) across experimental groups based on intensity data. Groups include Control, Treatment, Injury2DG, InjurySDT, TreatedInjury2DG, and TreatedInjurySDT. The y-axis shows the proportion of NaNs, indicating variability in missing data across the groups.

Statistical Assumptions Testing

Statistical assumptions testing revealed that the data did not meet normality or homogeneity of variances. The Shapiro-Wilk test yielded $p < 0.001$ for normality, and Levene's test indicated a violation of equal variances ($p < 0.001$). Therefore, given these violations, a Type III ANOVA was conducted to evaluate the effects of injury type and treatment on protein expression, accommodating the unbalanced nature of the dataset.

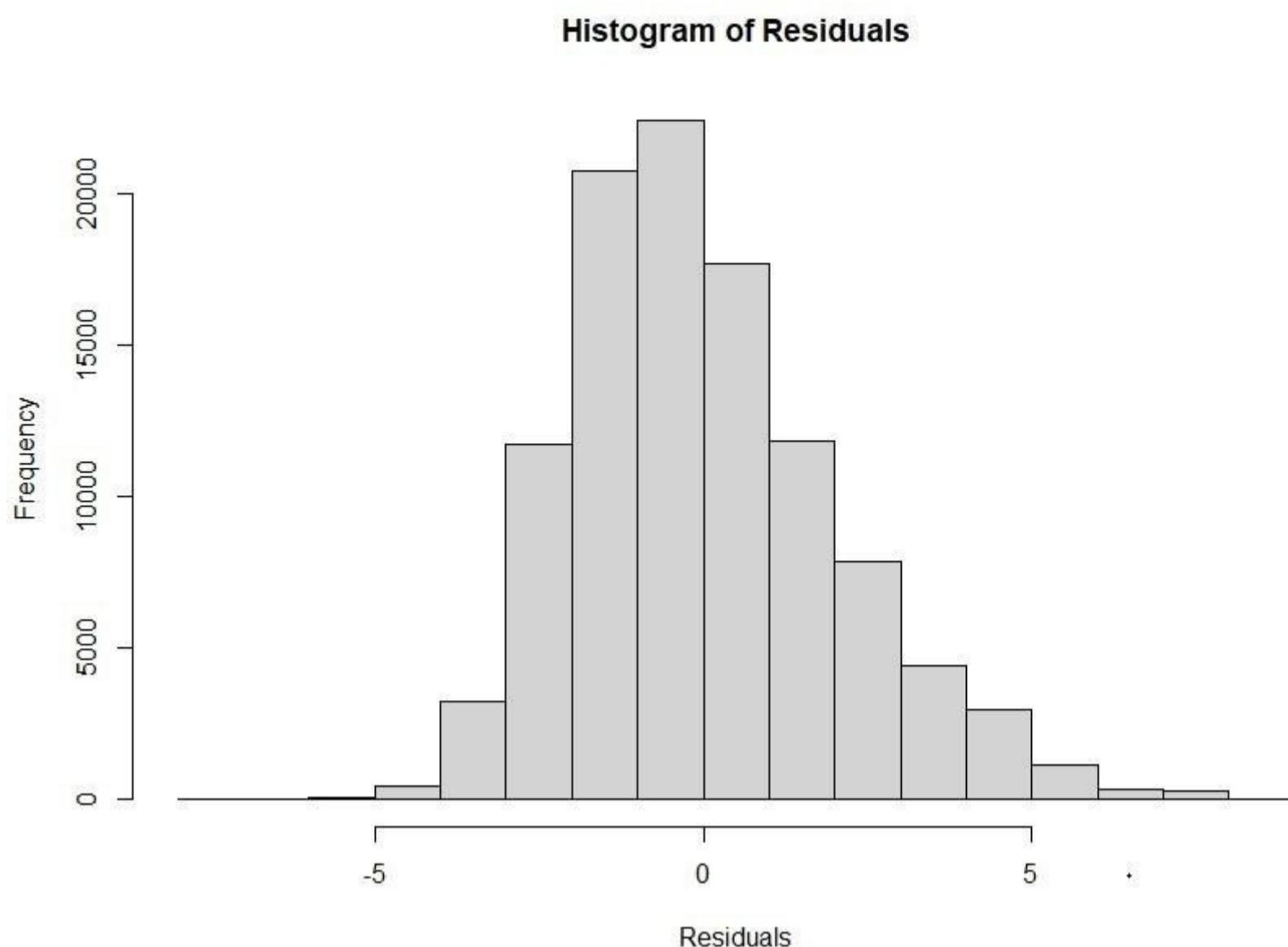


Figure S3 Histogram of residuals from the statistical model. The x-axis represents the residual values, while the y-axis shows their frequency. The distribution appears approximately symmetric and centered around zero, suggesting approximate normality. However, slight deviations and potential outliers indicate that further tests (e.g., Shapiro-Wilk test, Q-Q plot, or skewness and kurtosis analysis) are needed to confirm the assumption of normality.

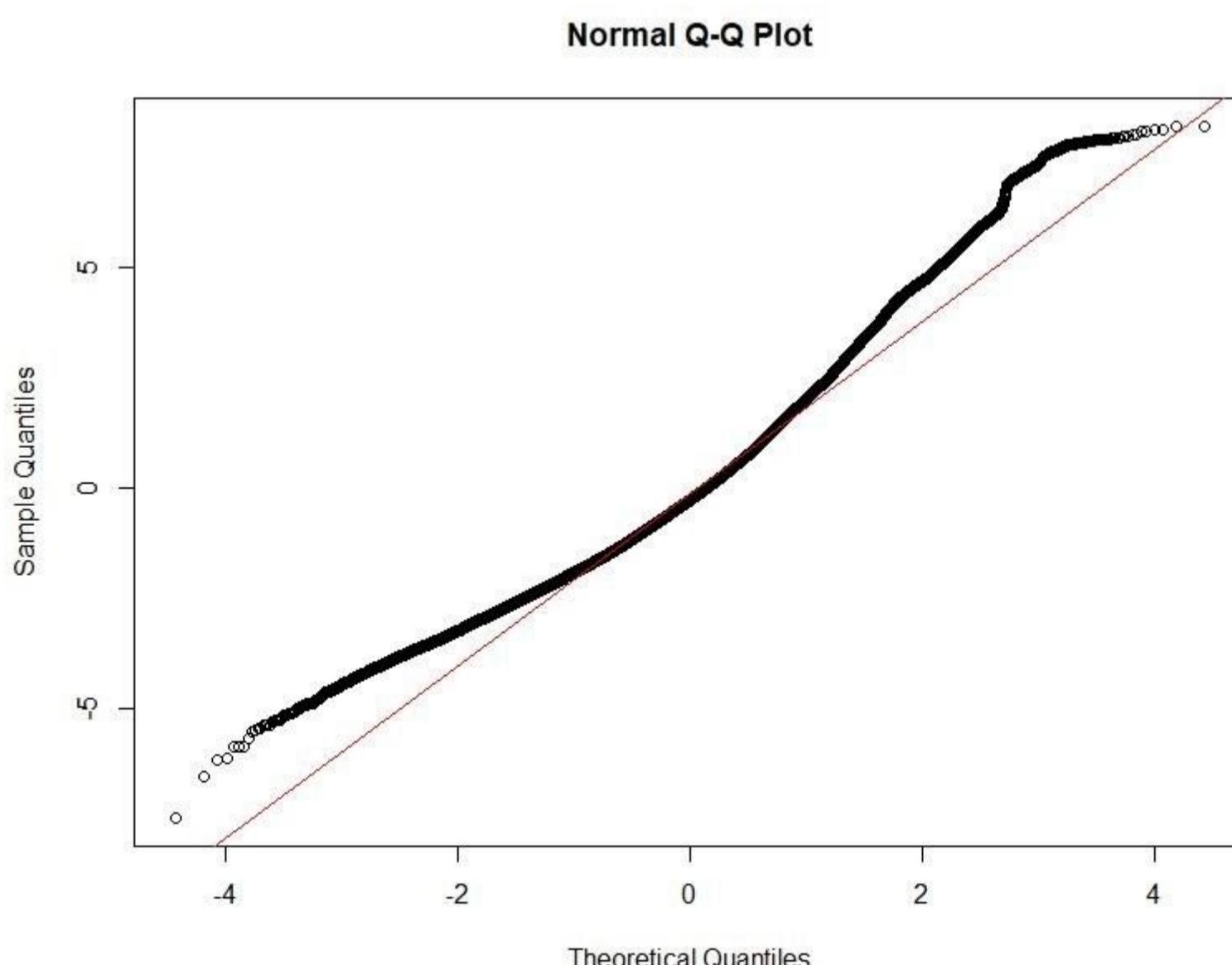
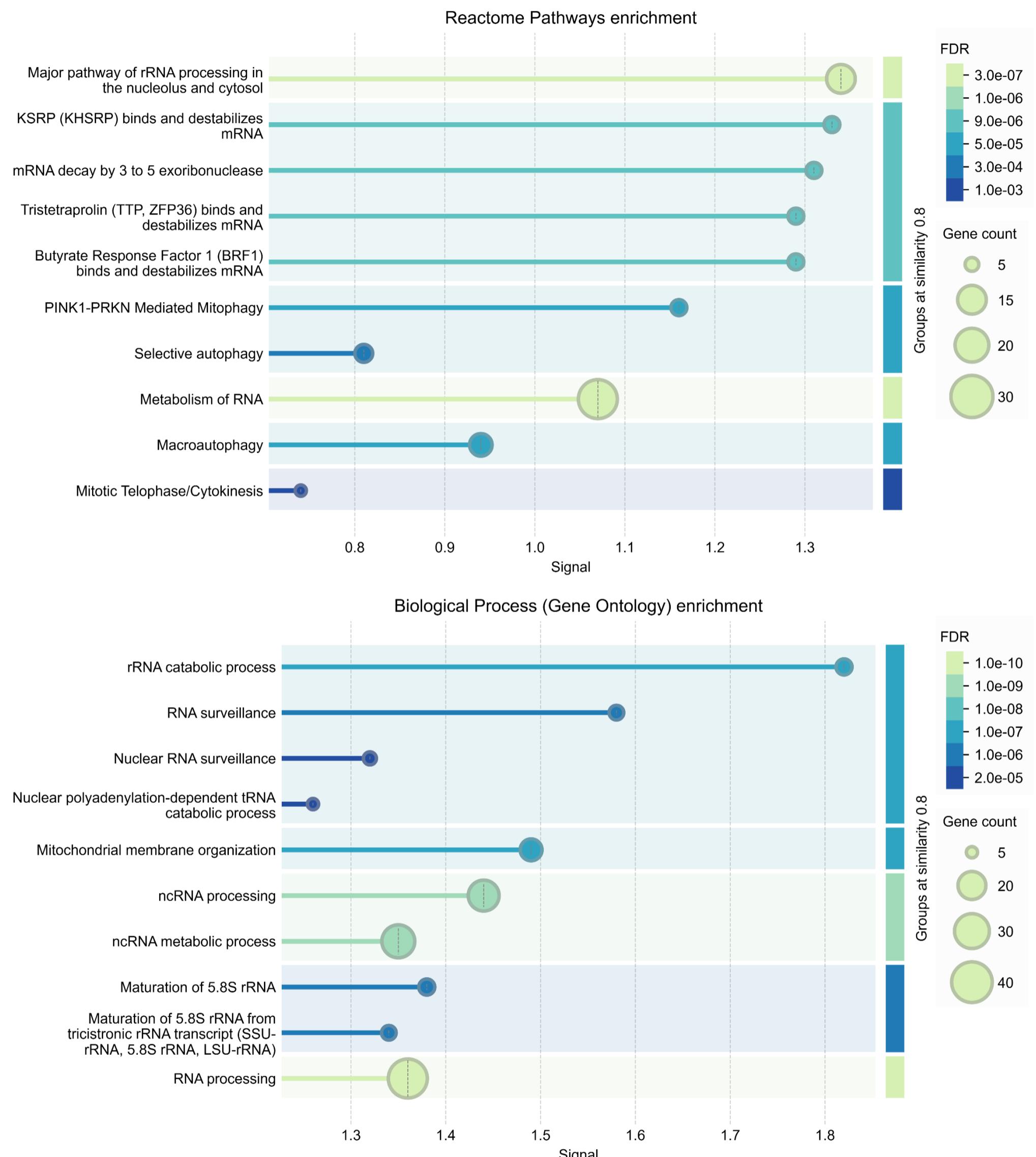


Figure S4 Normal Q-Q plot of residuals from the statistical model. The plot compares the sample quantiles (y-axis) with the theoretical quantiles of a normal distribution (x-axis). While the points generally follow the diagonal line, indicating approximate normality, deviations at the tails suggest potential non-normality. This supports the need for further statistical testing to confirm the assumption of normality.

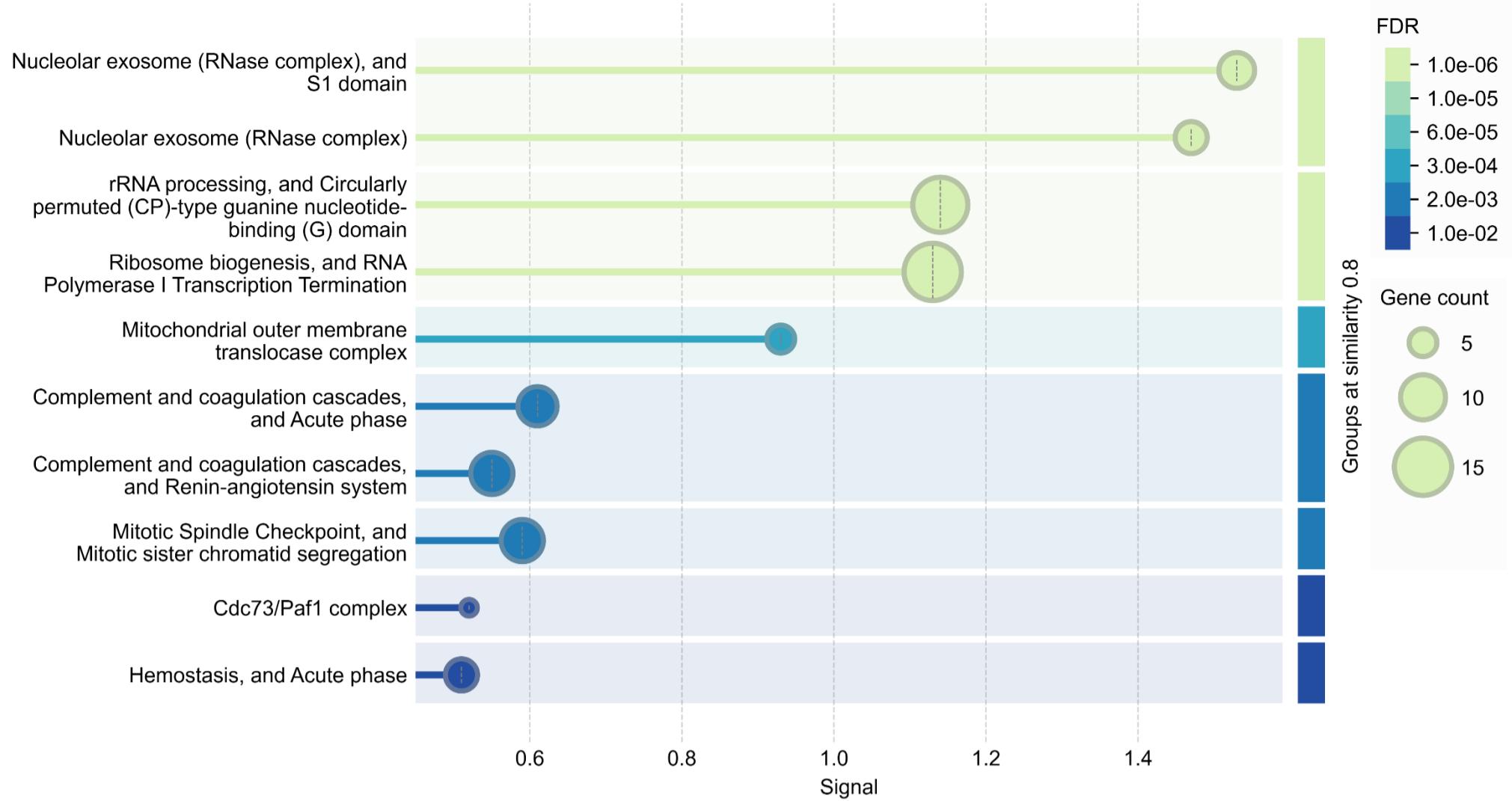
STRING Enrichment Bar Plots

Comparison Injury1 (SDT) vs Injury0 (Control)

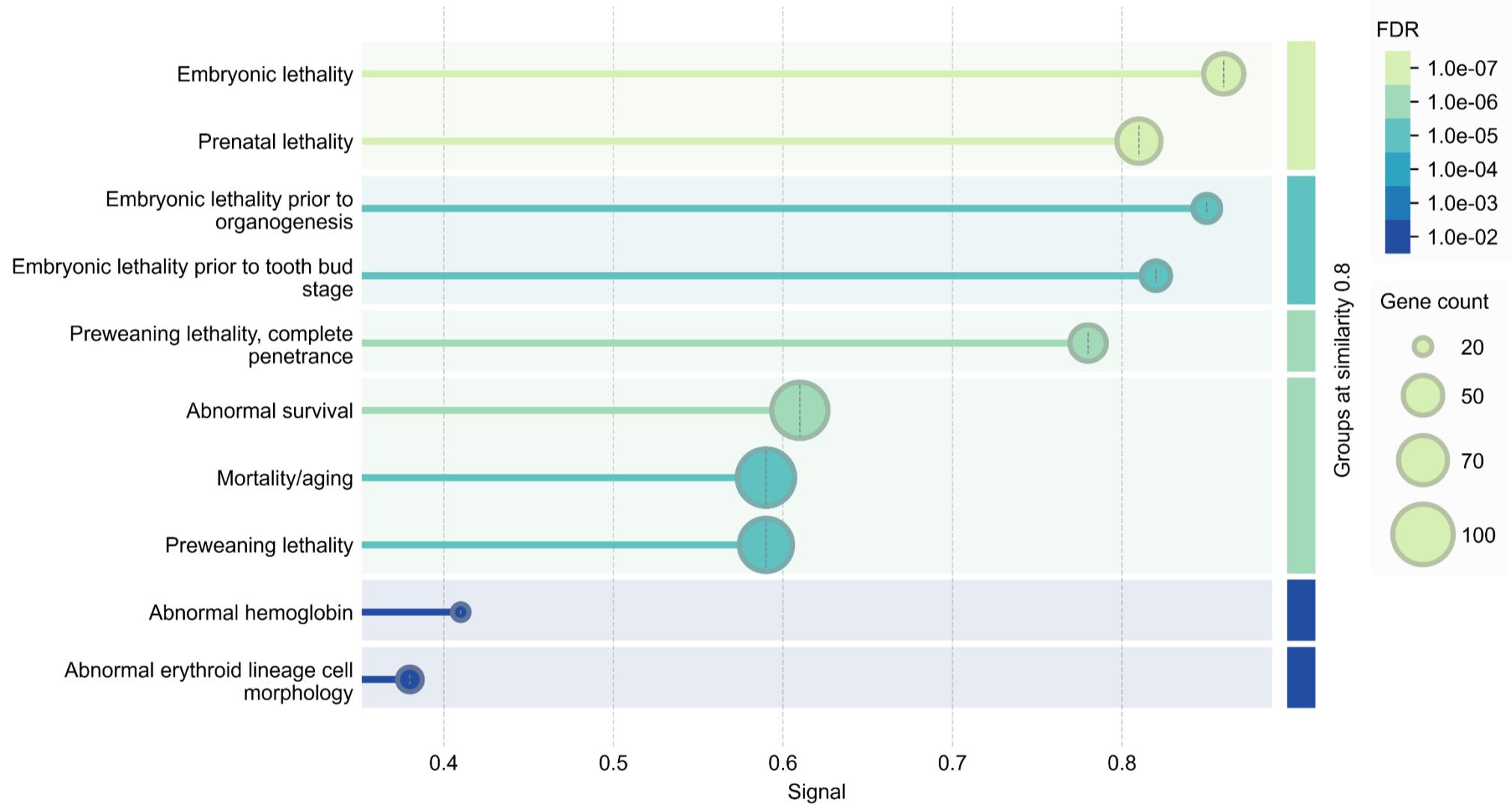
Upregulated



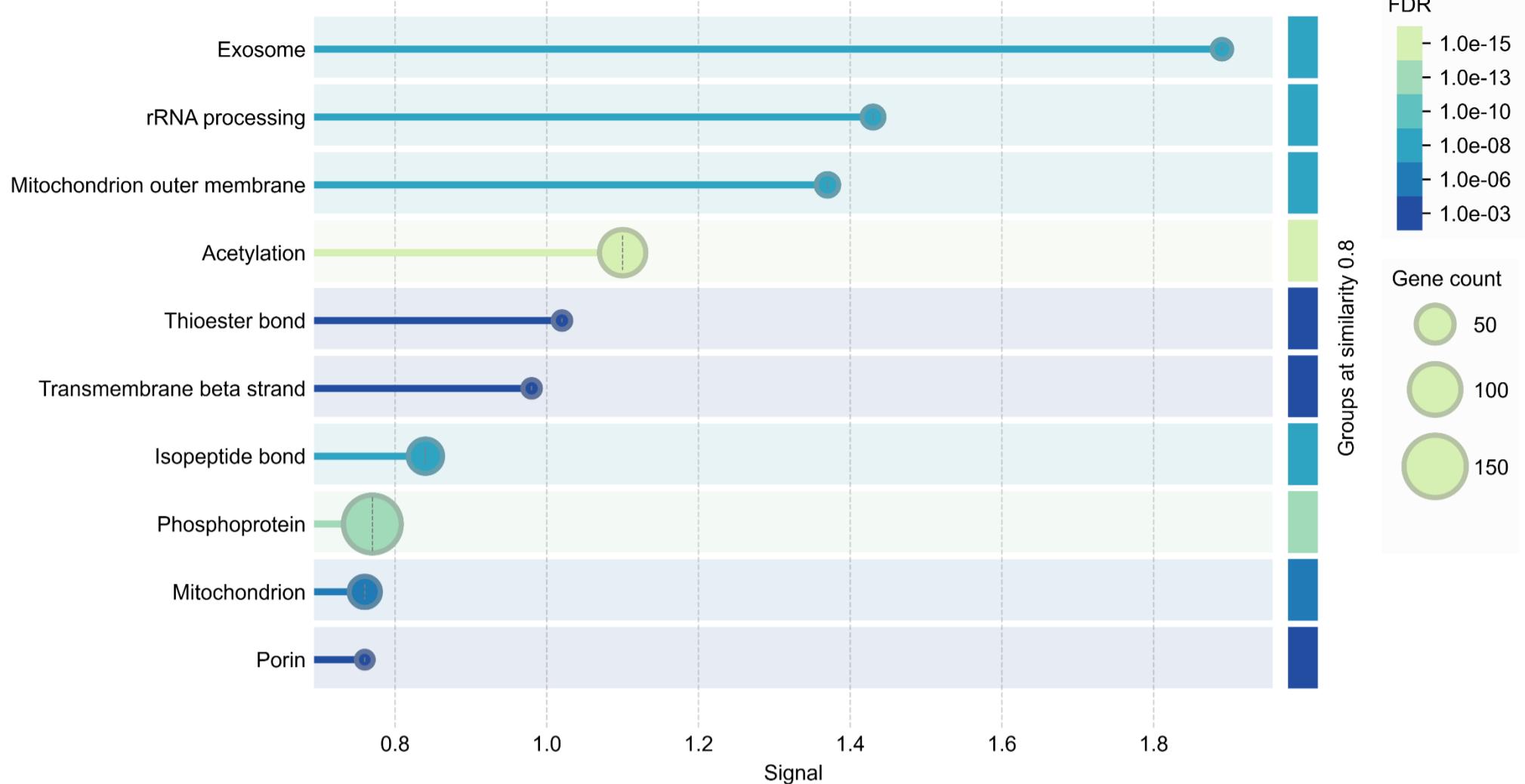
Local Network Cluster (STRING) enrichment



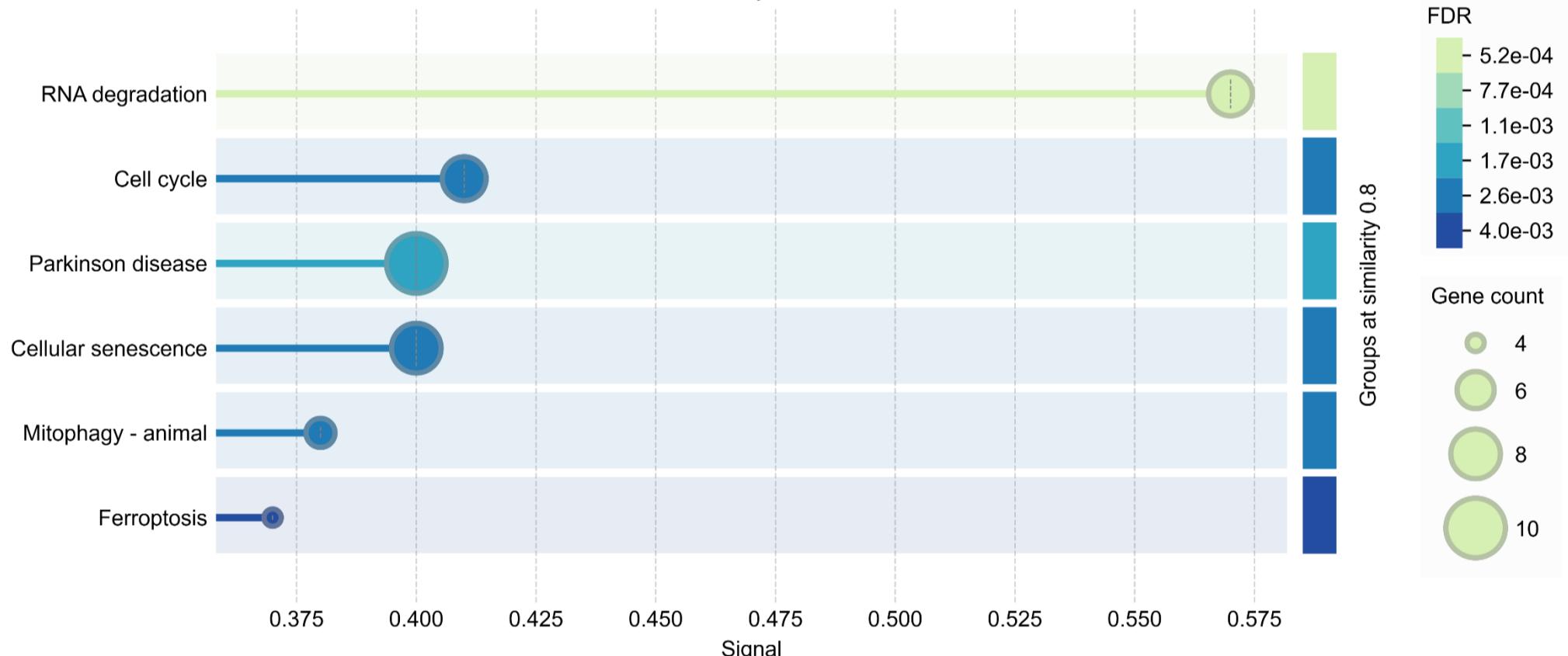
The Mammalian Phenotype Ontology (Monarch) enrichment



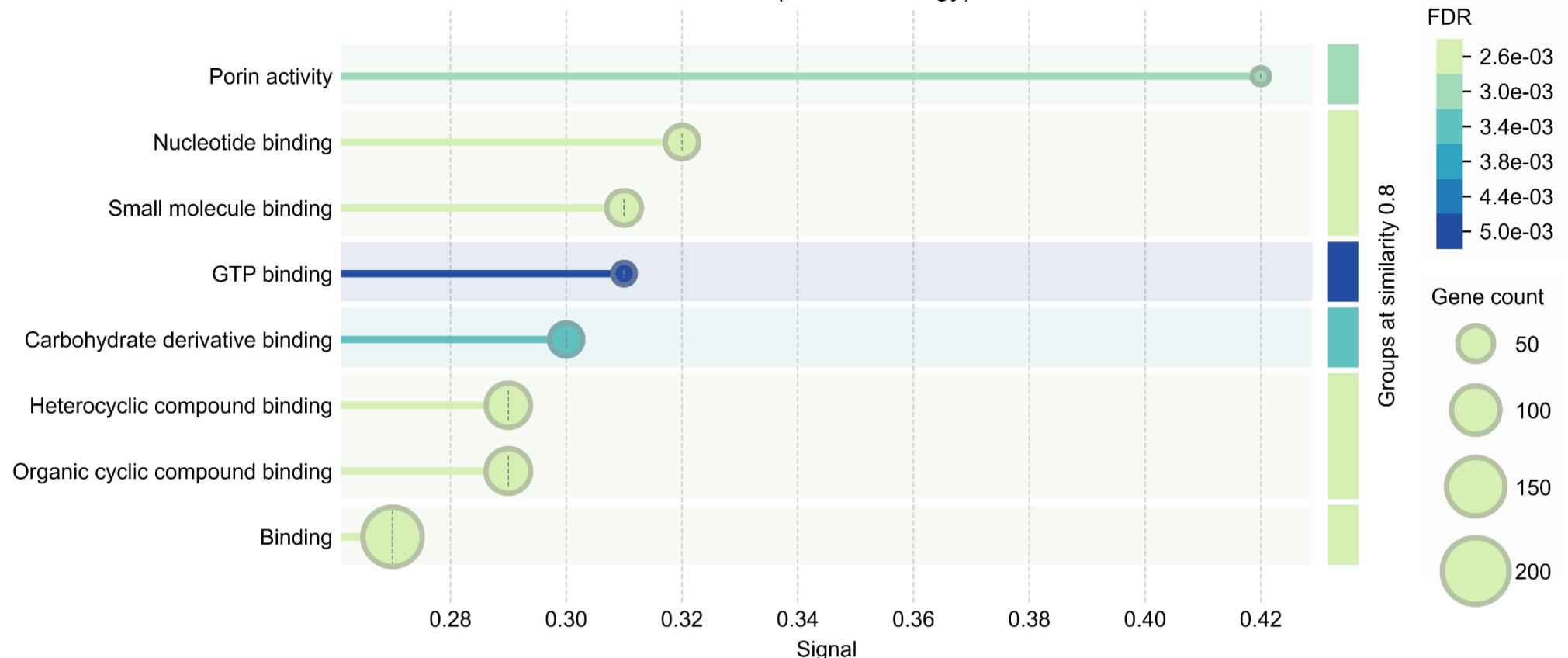
Annotated Keywords (UniProt) enrichment



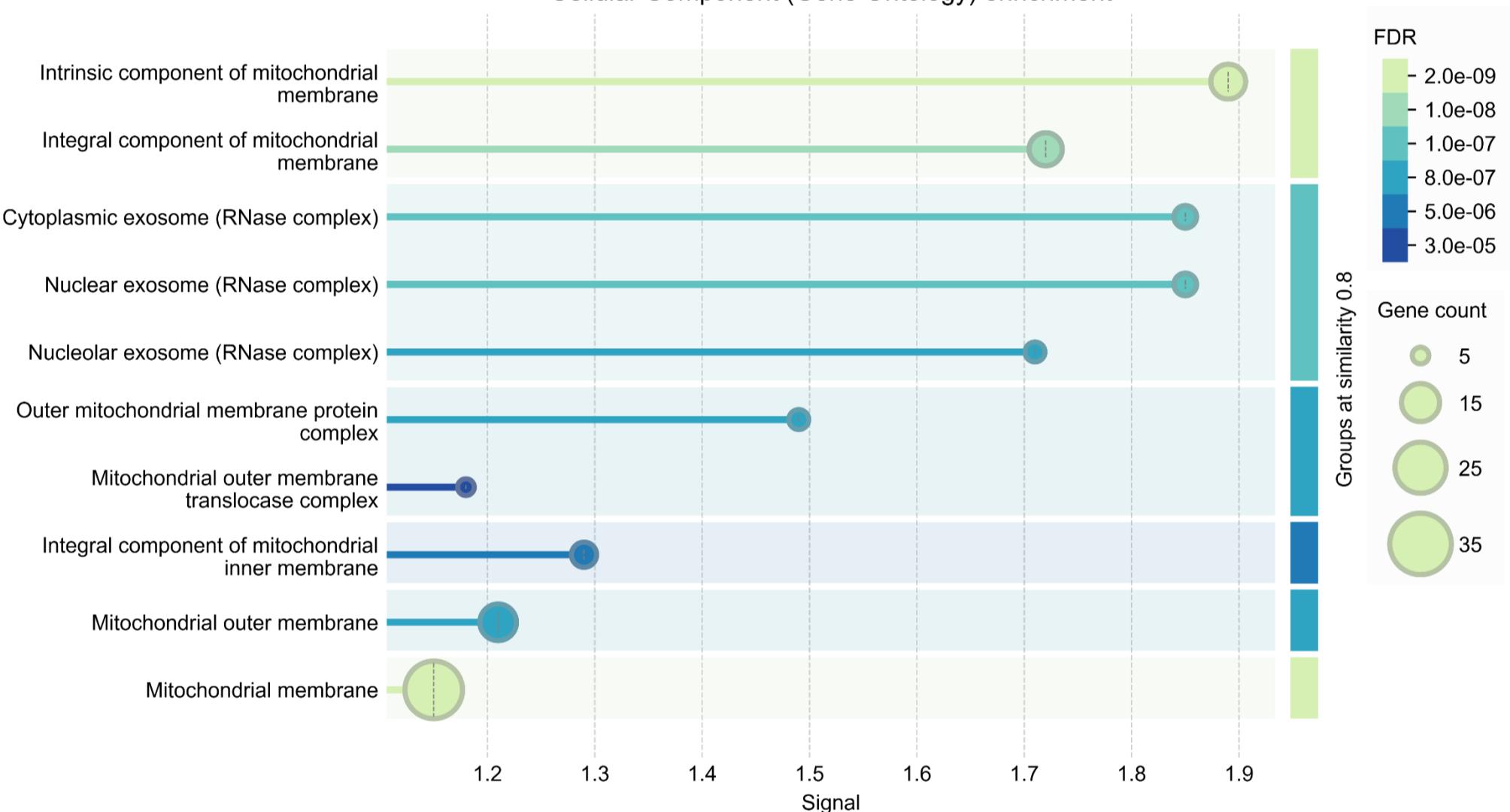
KEGG Pathways enrichment



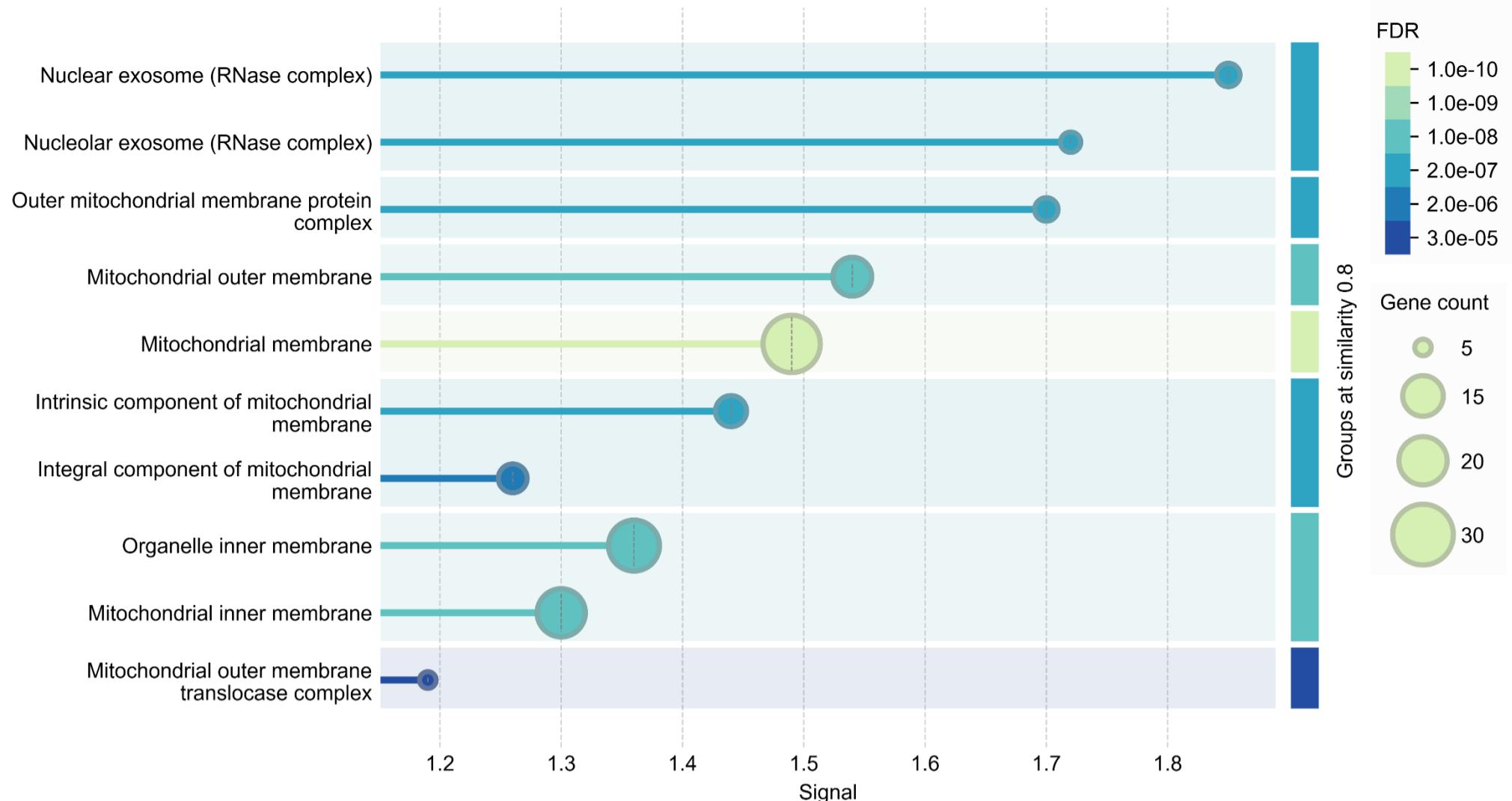
Molecular Function (Gene Ontology) enrichment



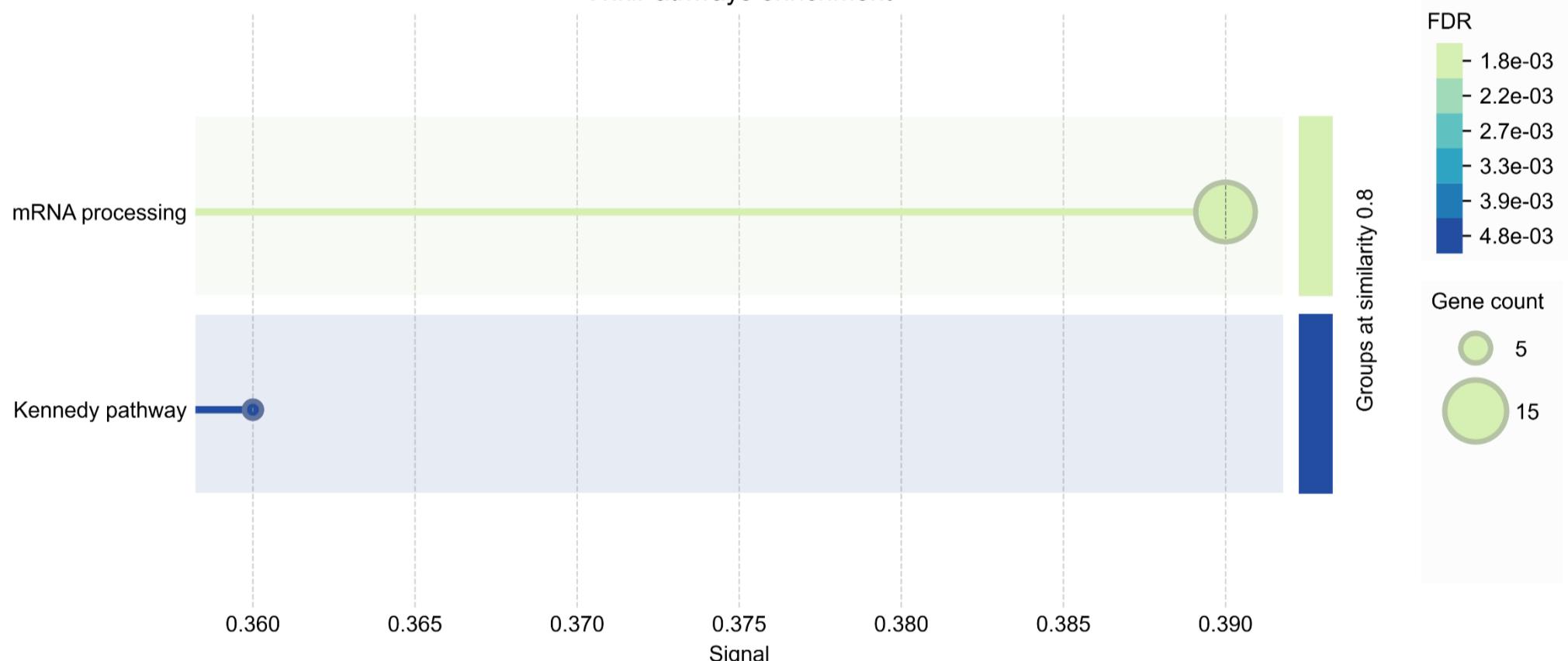
Cellular Component (Gene Ontology) enrichment



Subcellular Localization (COMPARTMENTS) enrichment

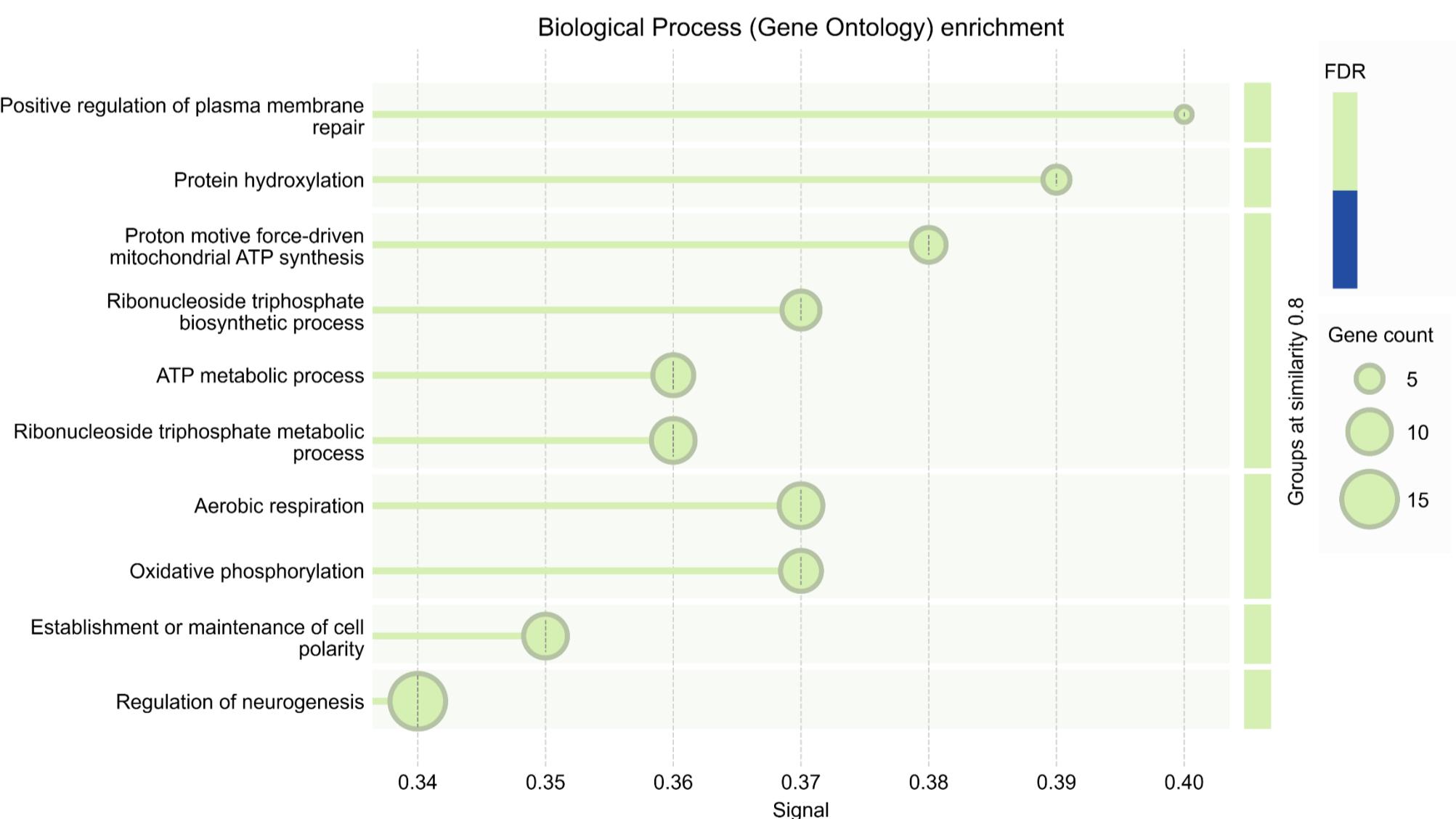
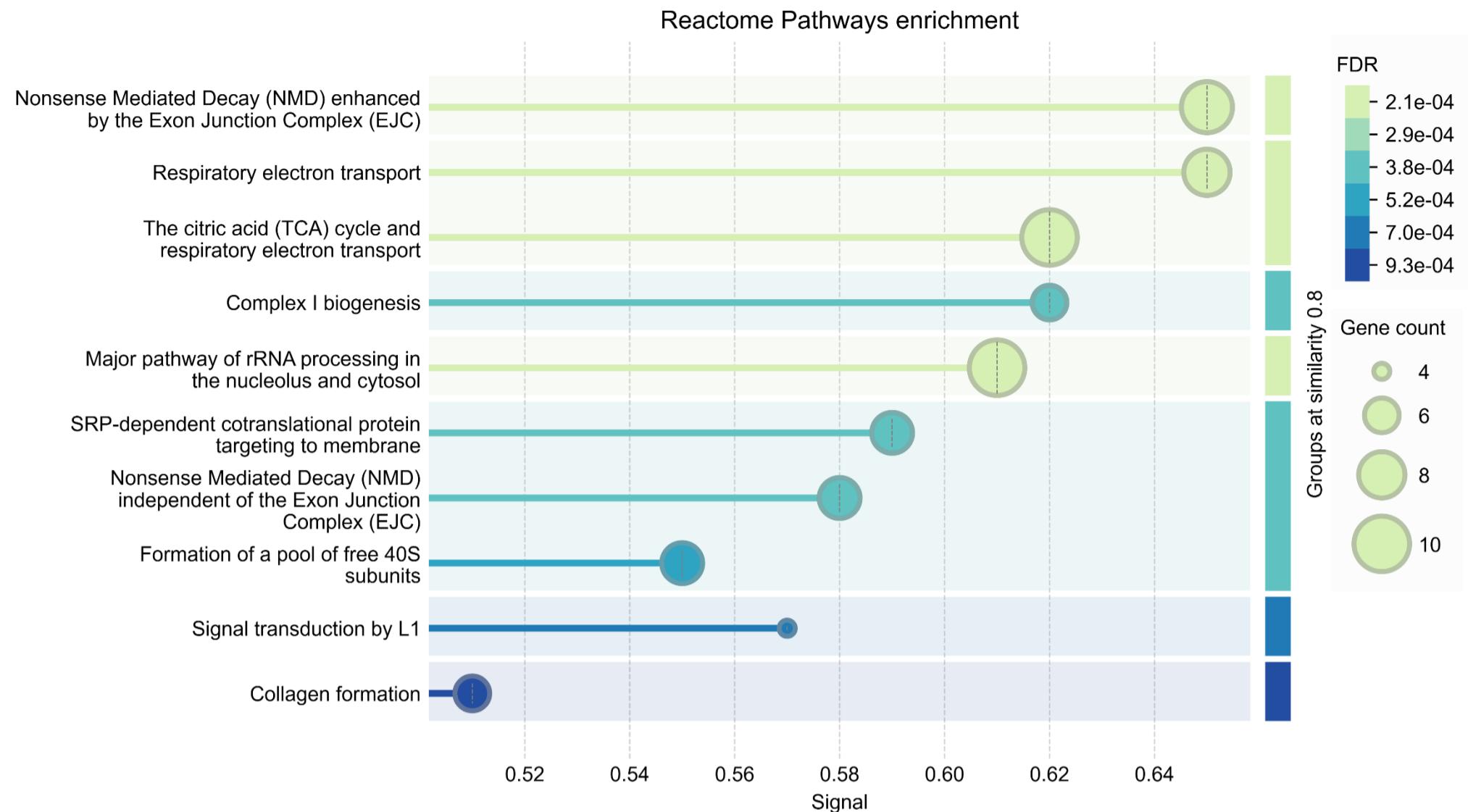


WikiPathways enrichment

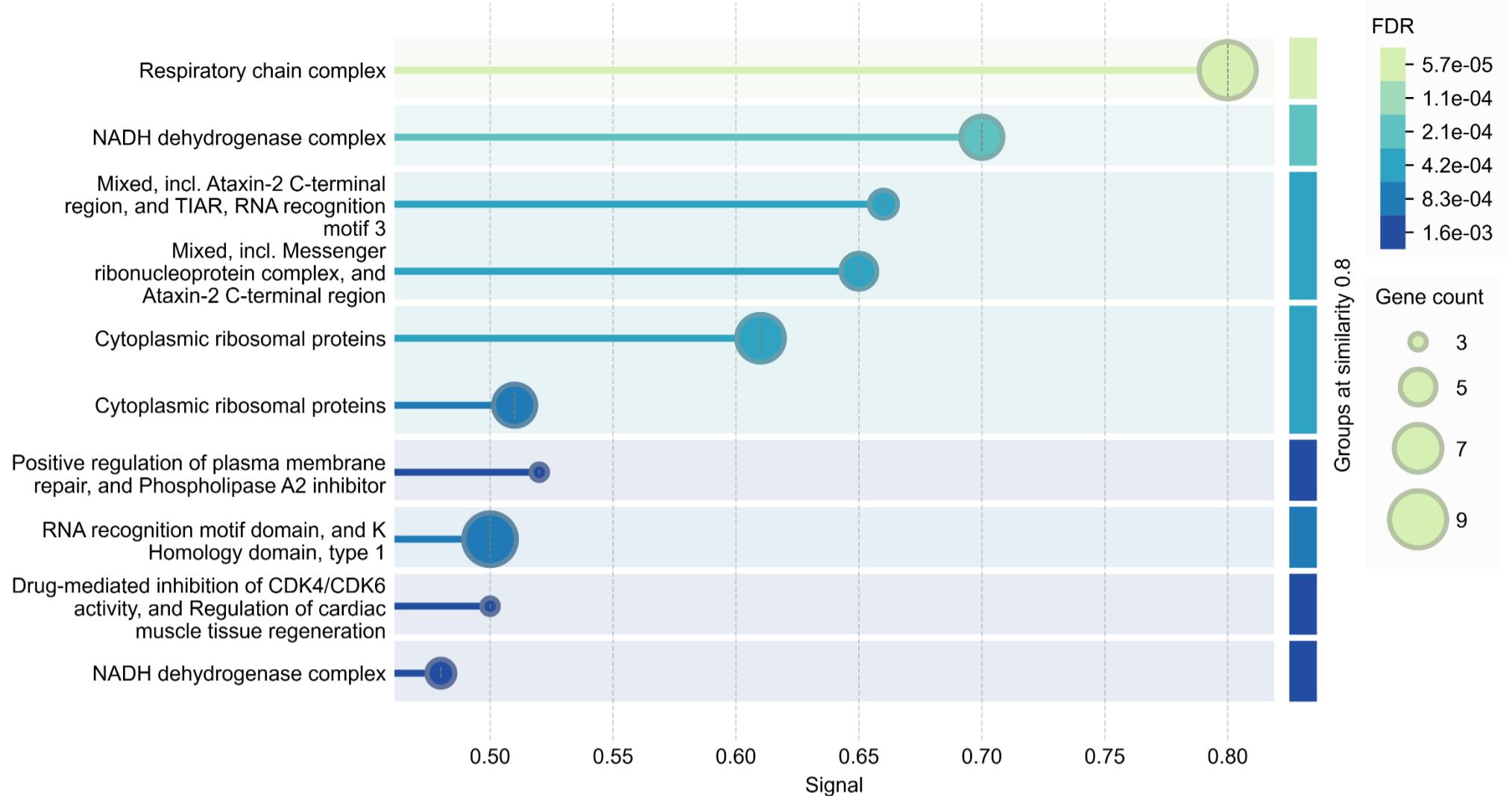


Comparison Injury1 (SDT) vs Injury0 (Control)

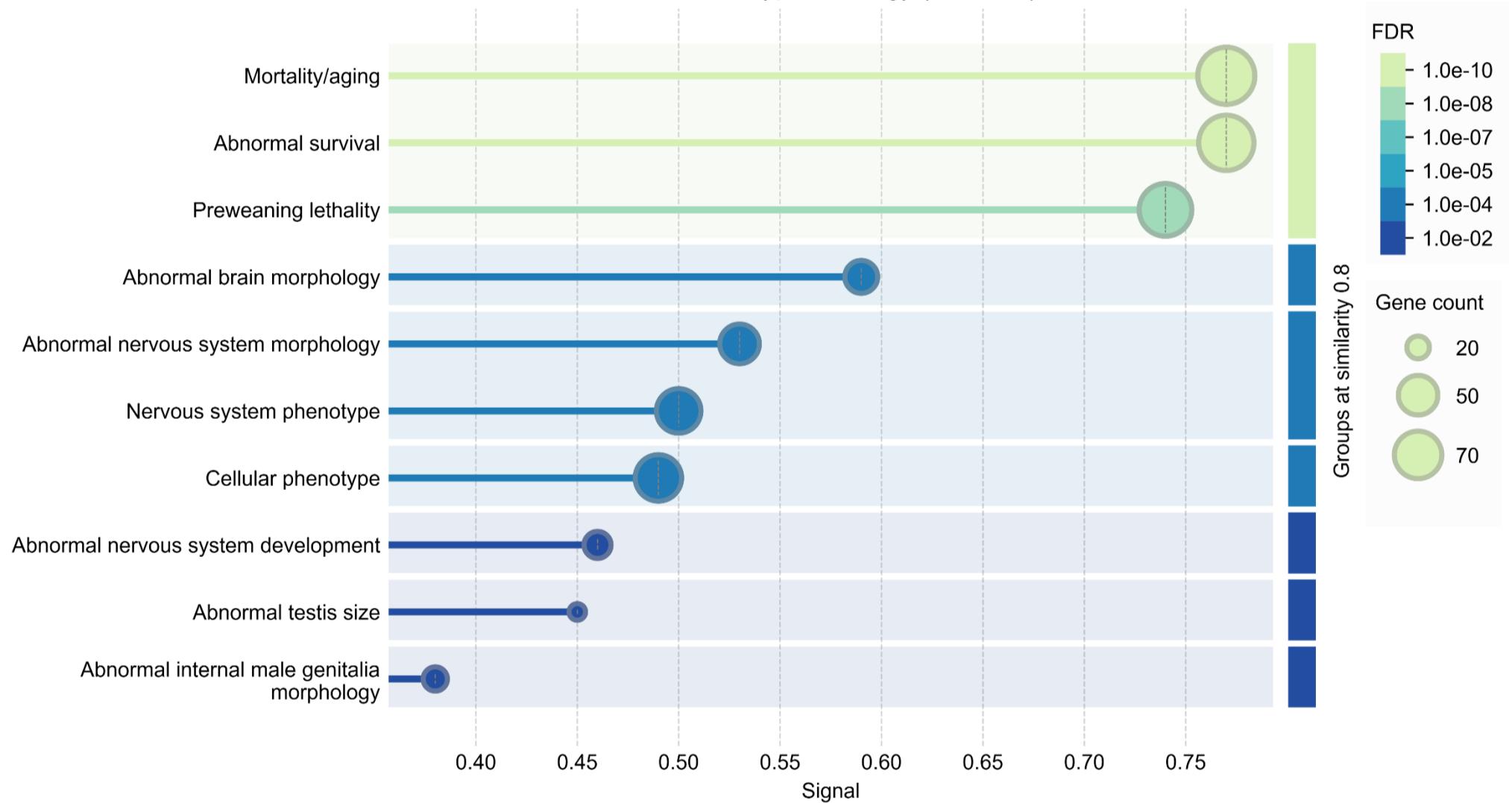
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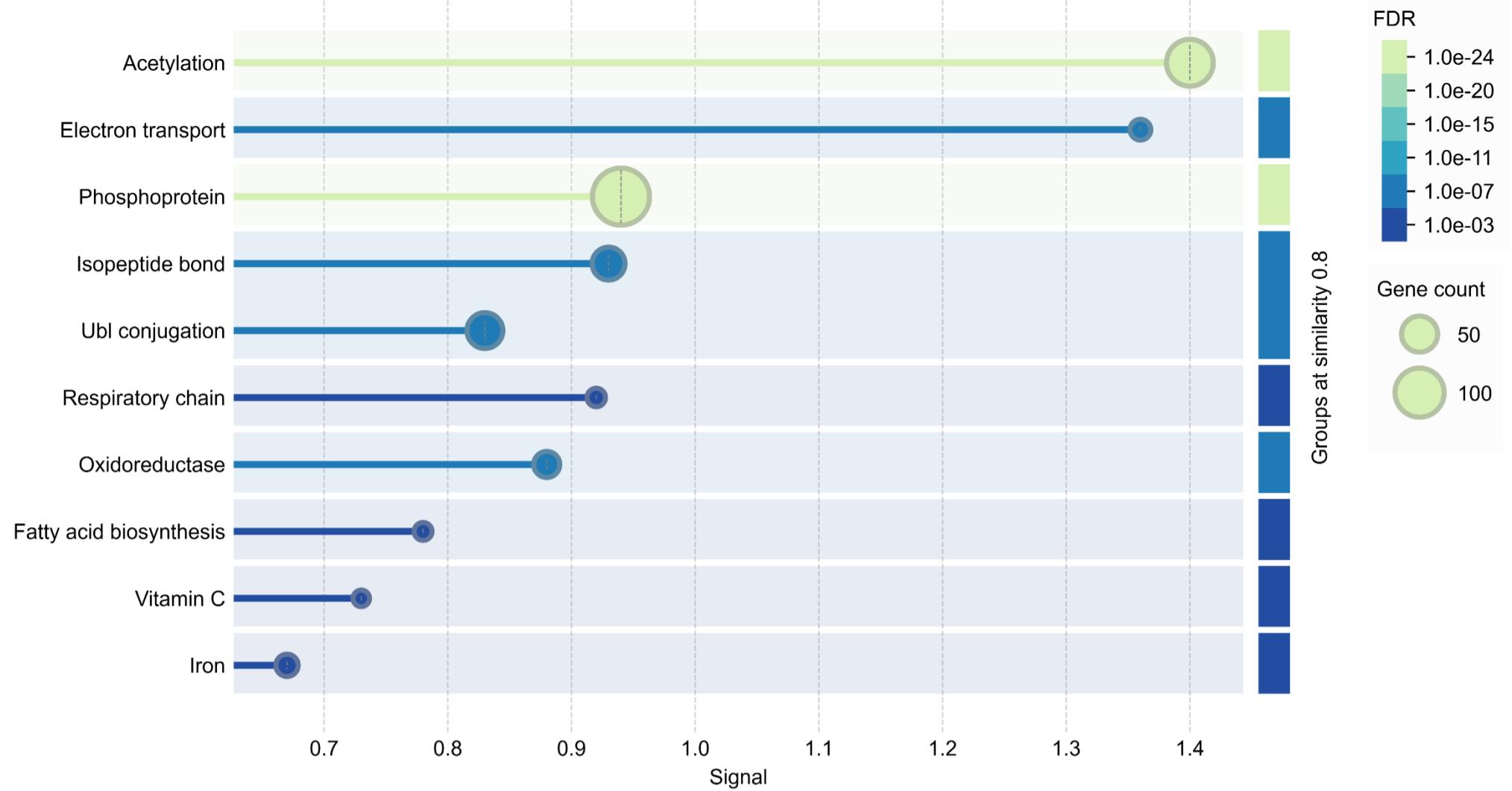
Local Network Cluster (STRING) enrichment



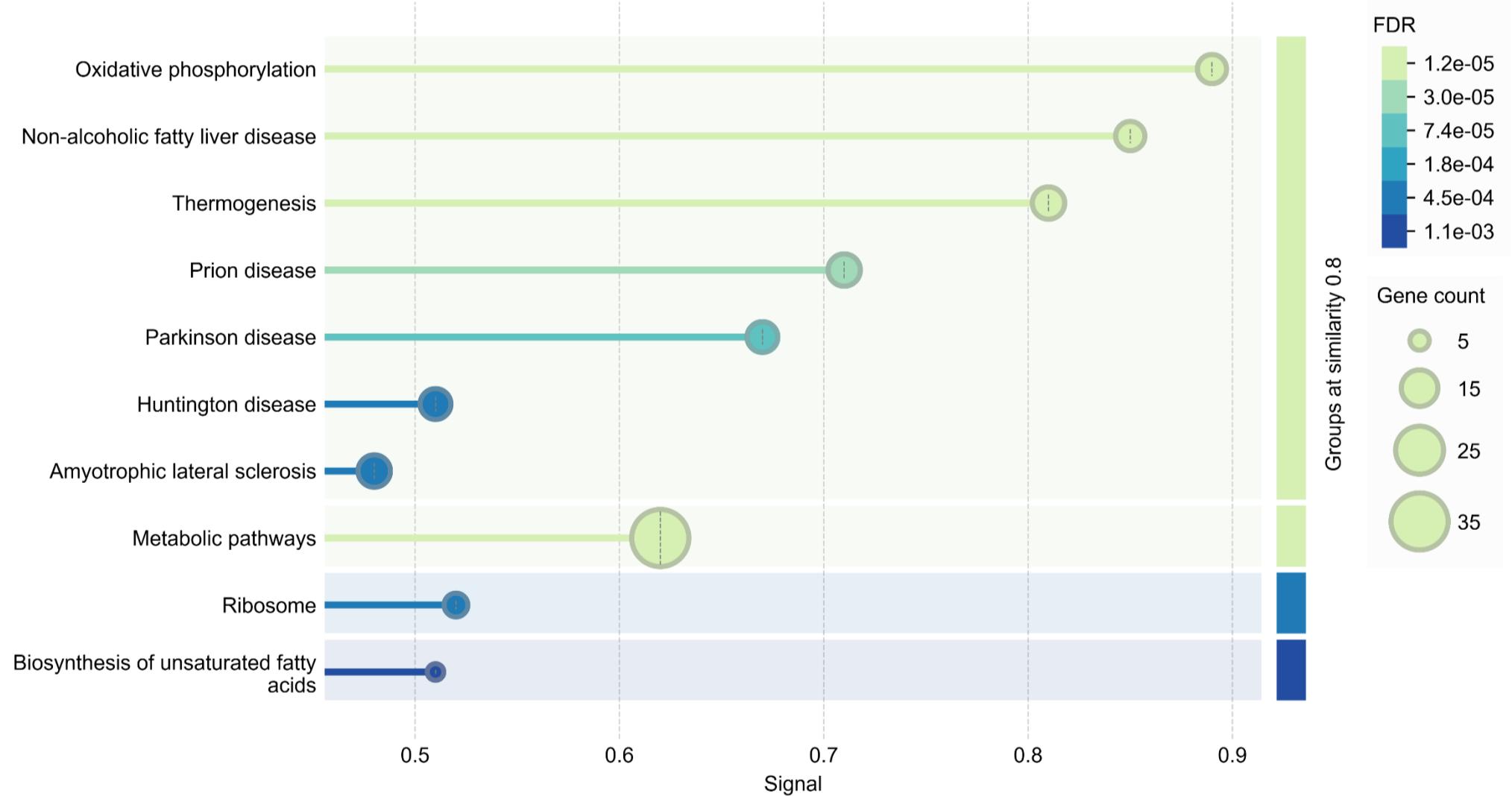
The Mammalian Phenotype Ontology (Monarch) enrichment



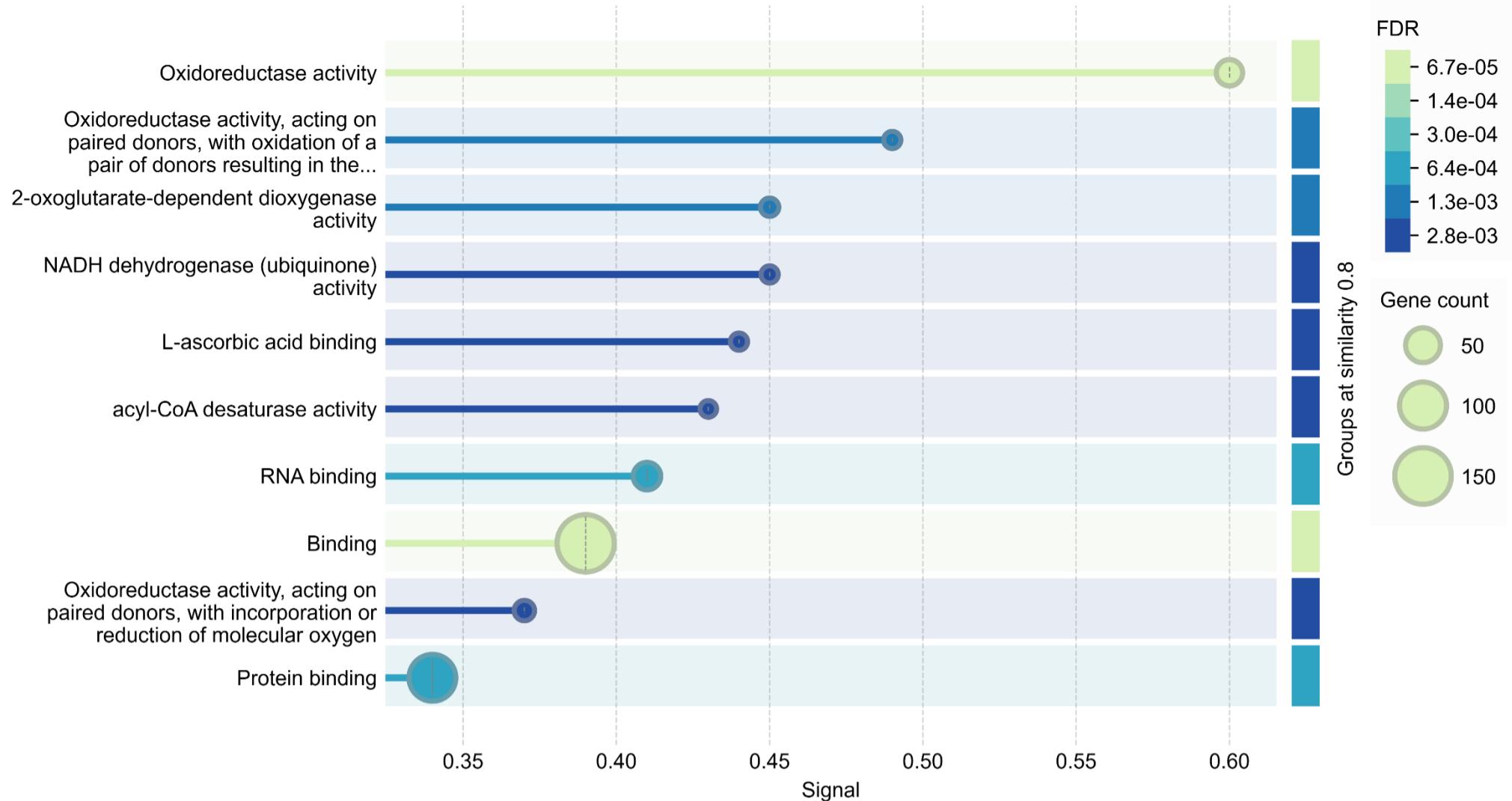
Annotated Keywords (UniProt) enrichment



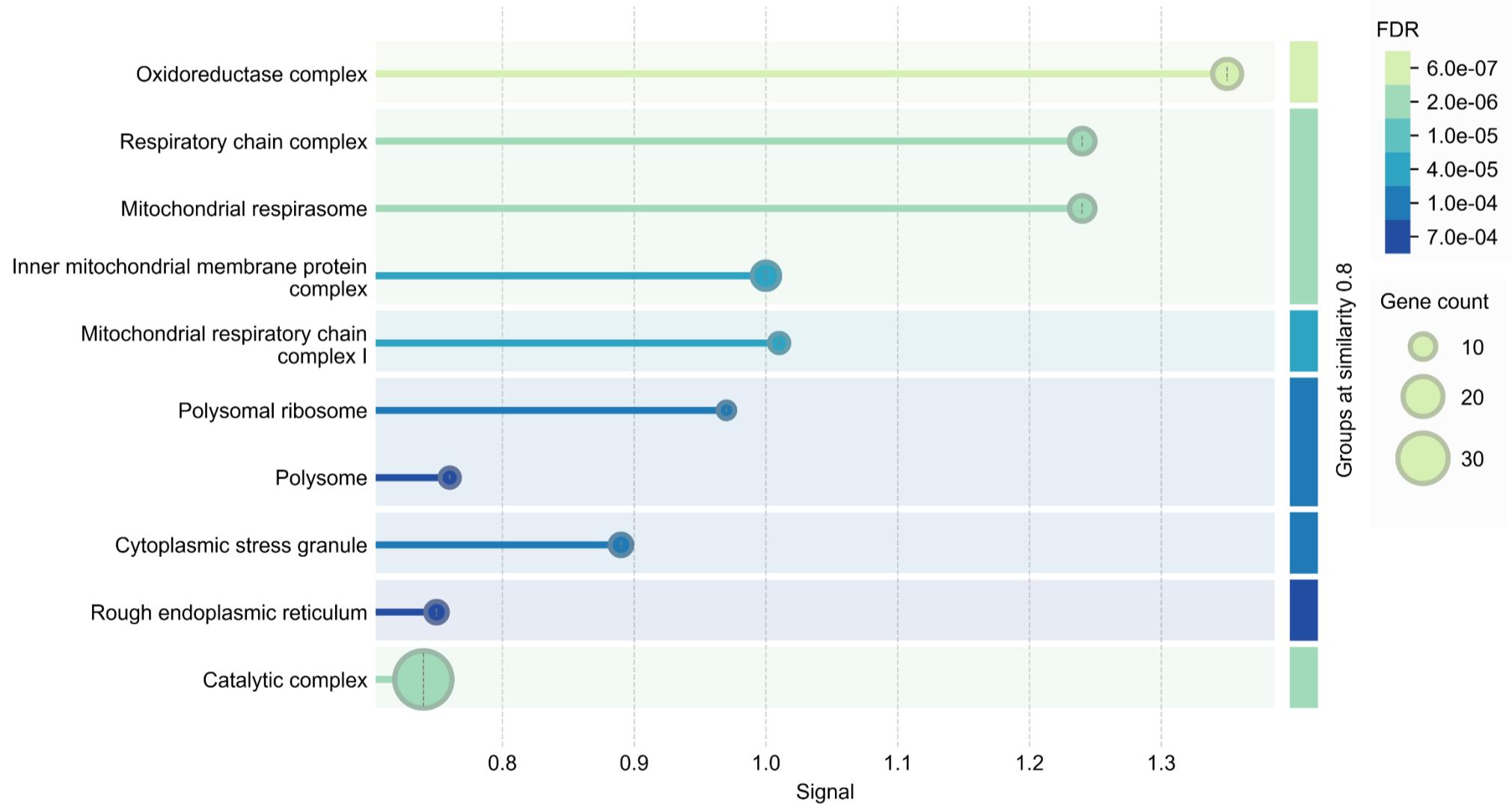
KEGG Pathways enrichment



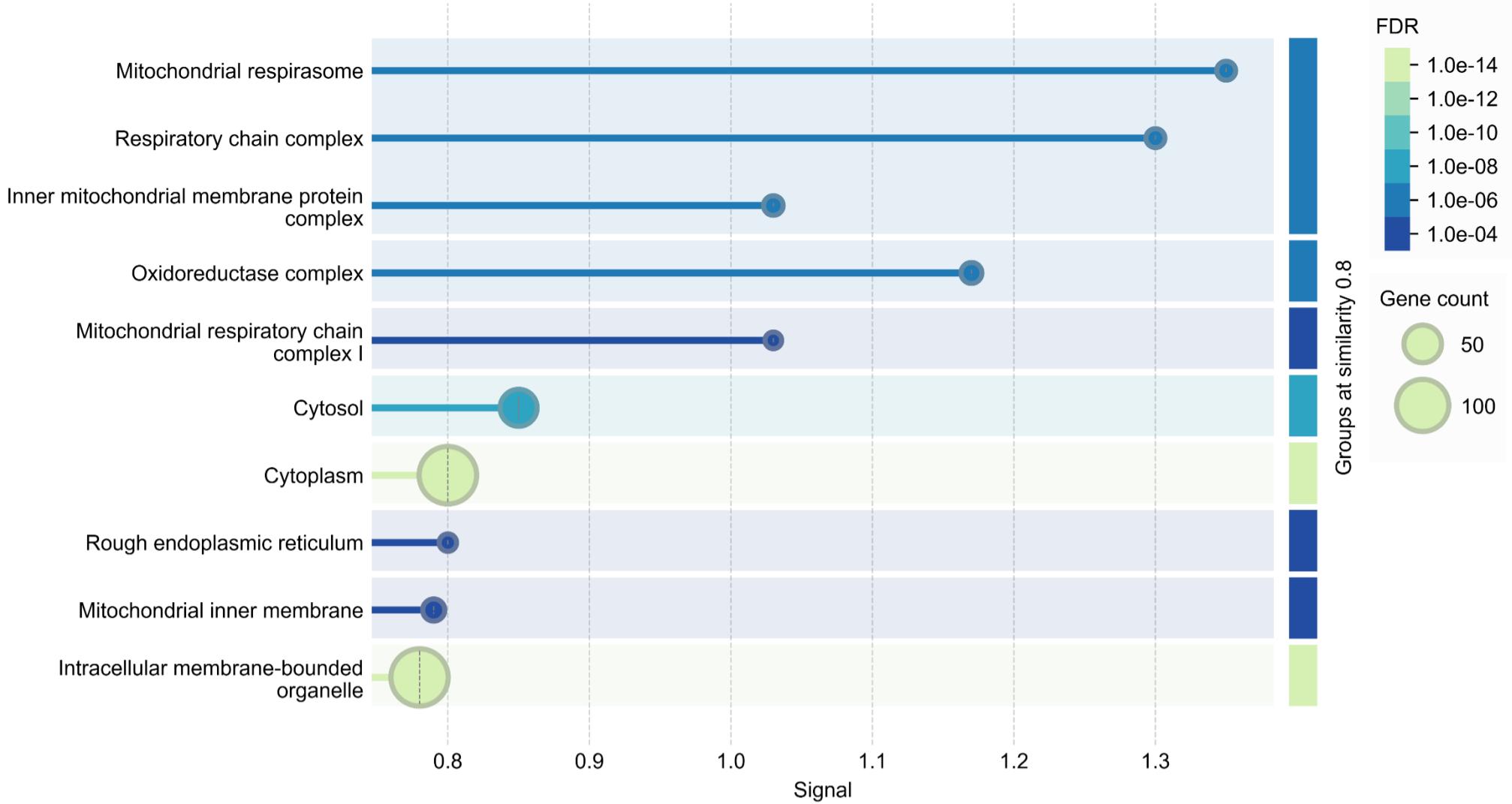
Molecular Function (Gene Ontology) enrichment



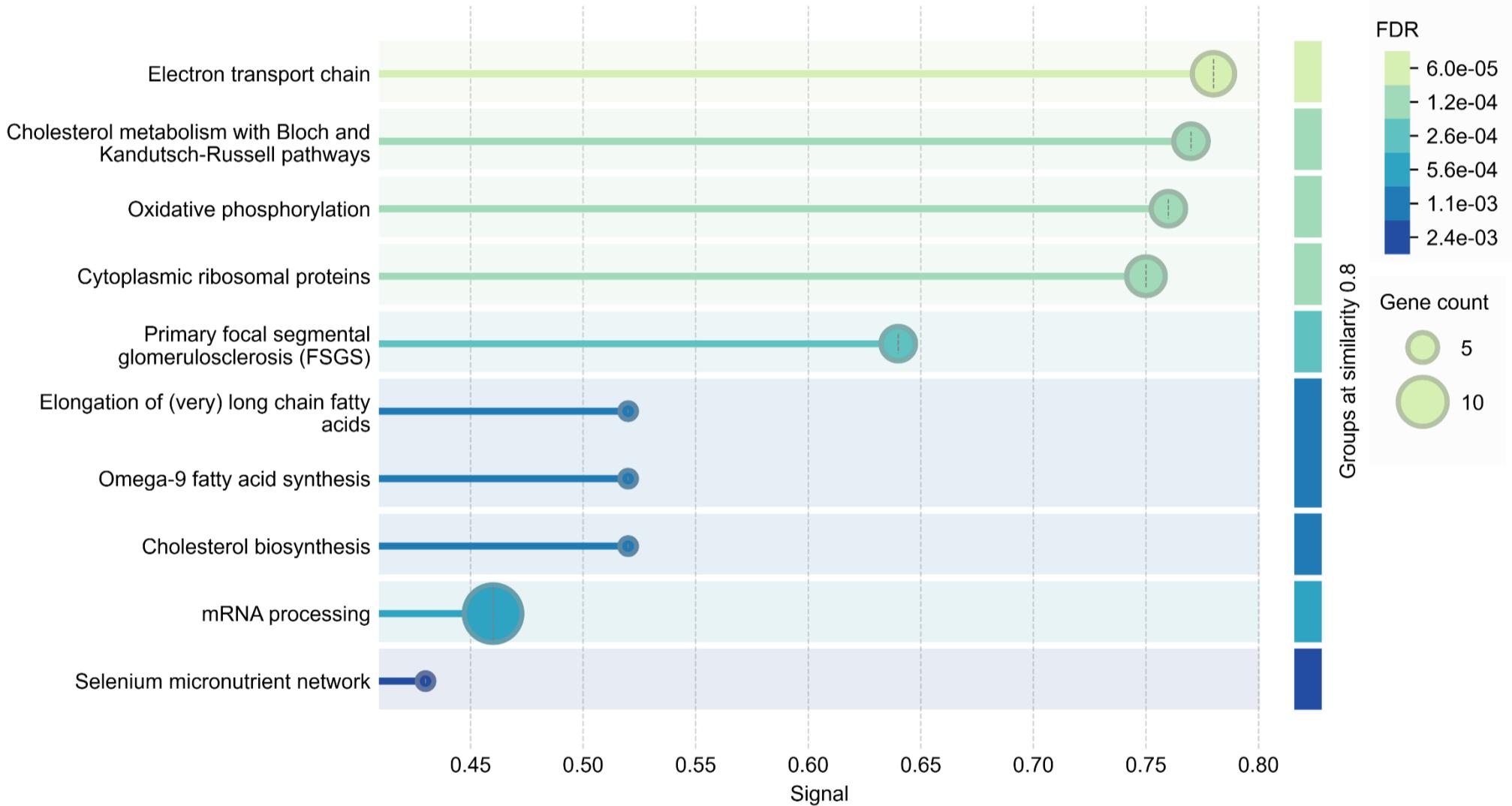
Cellular Component (Gene Ontology) enrichment



Subcellular Localization (COMPARTMENTS) enrichment

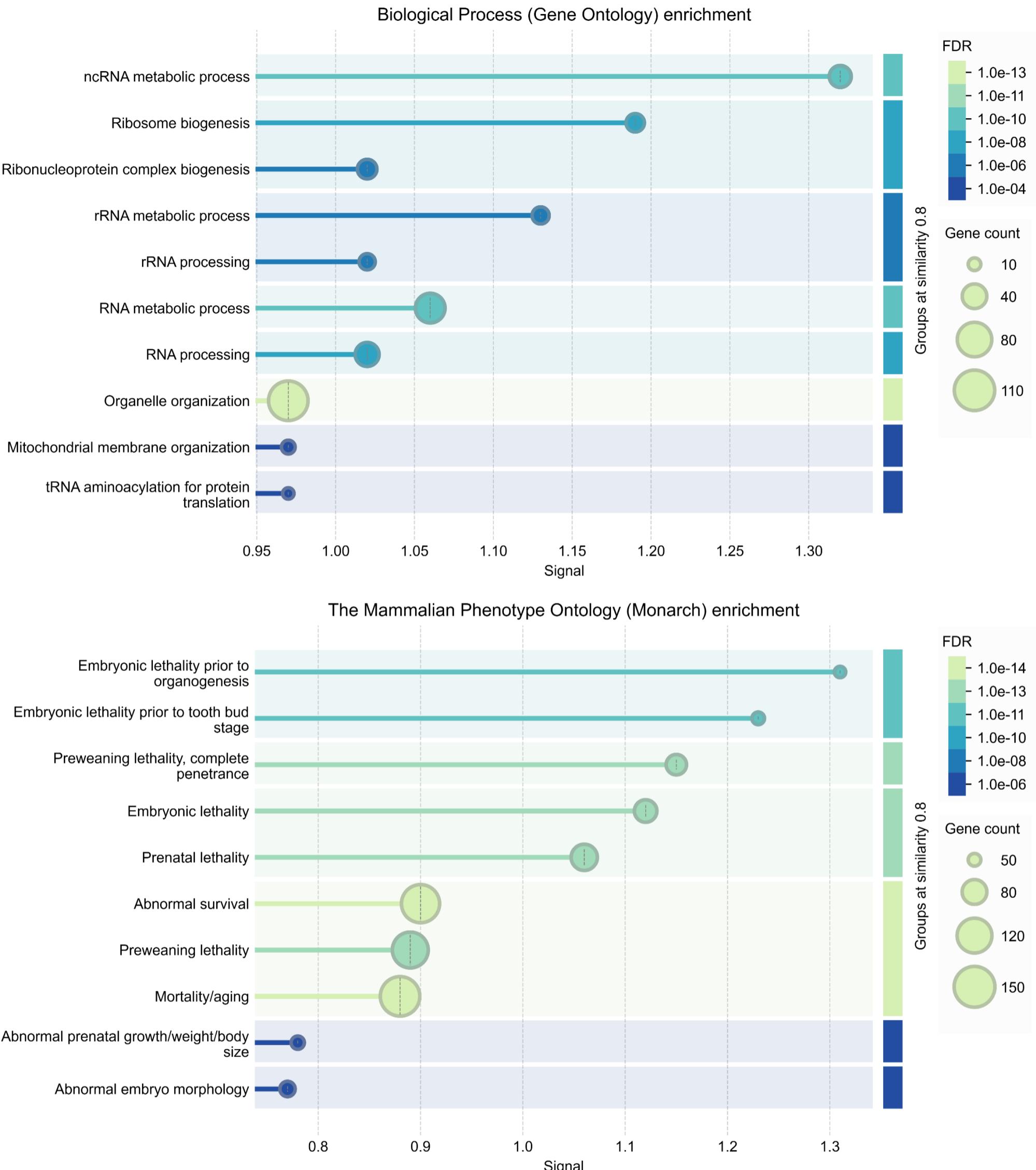


WikiPathways enrichment

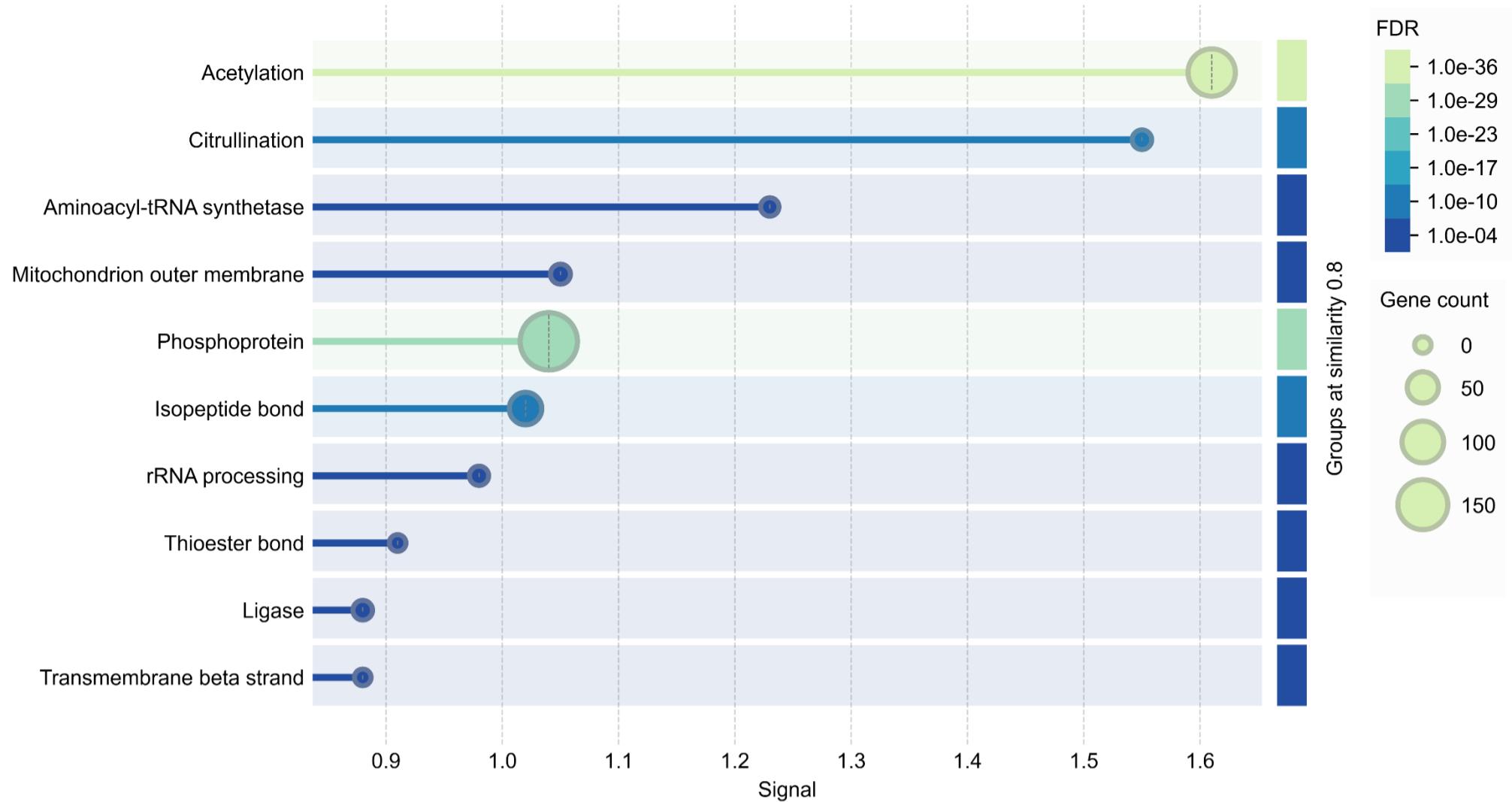


Comparison Injury2 (2DG) vs Injury0 (Control)

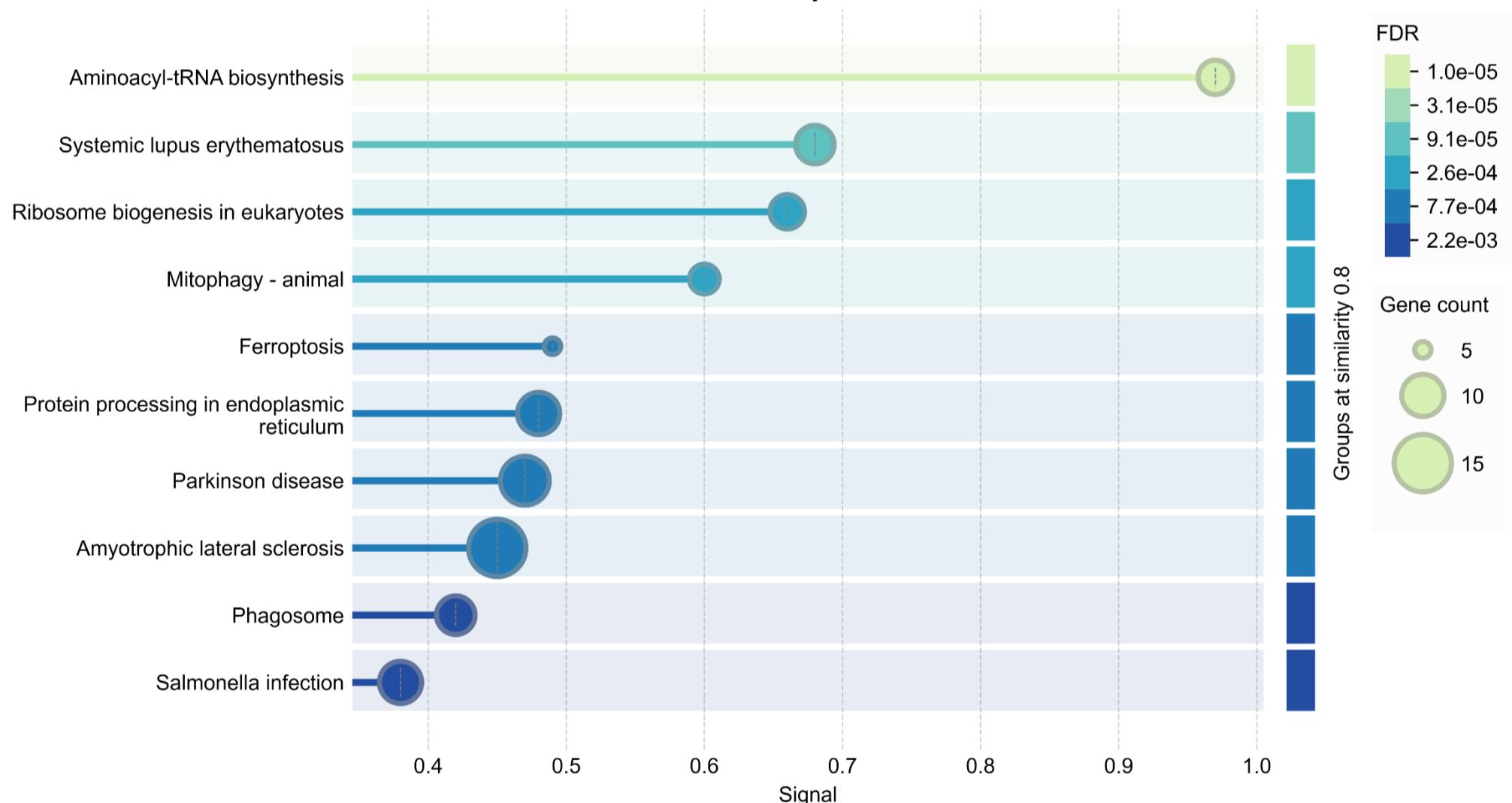
Upregulated



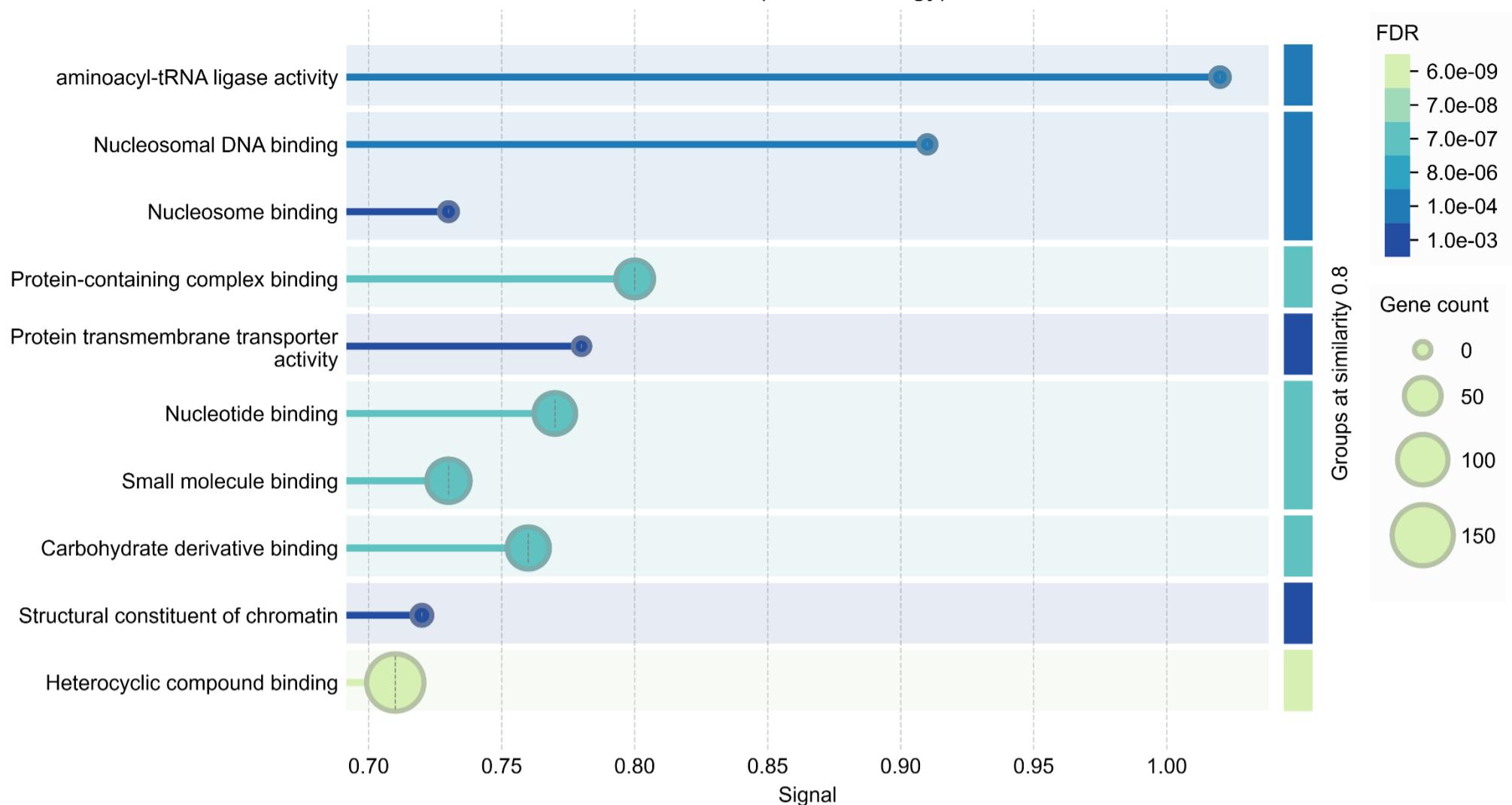
Annotated Keywords (UniProt) enrichment



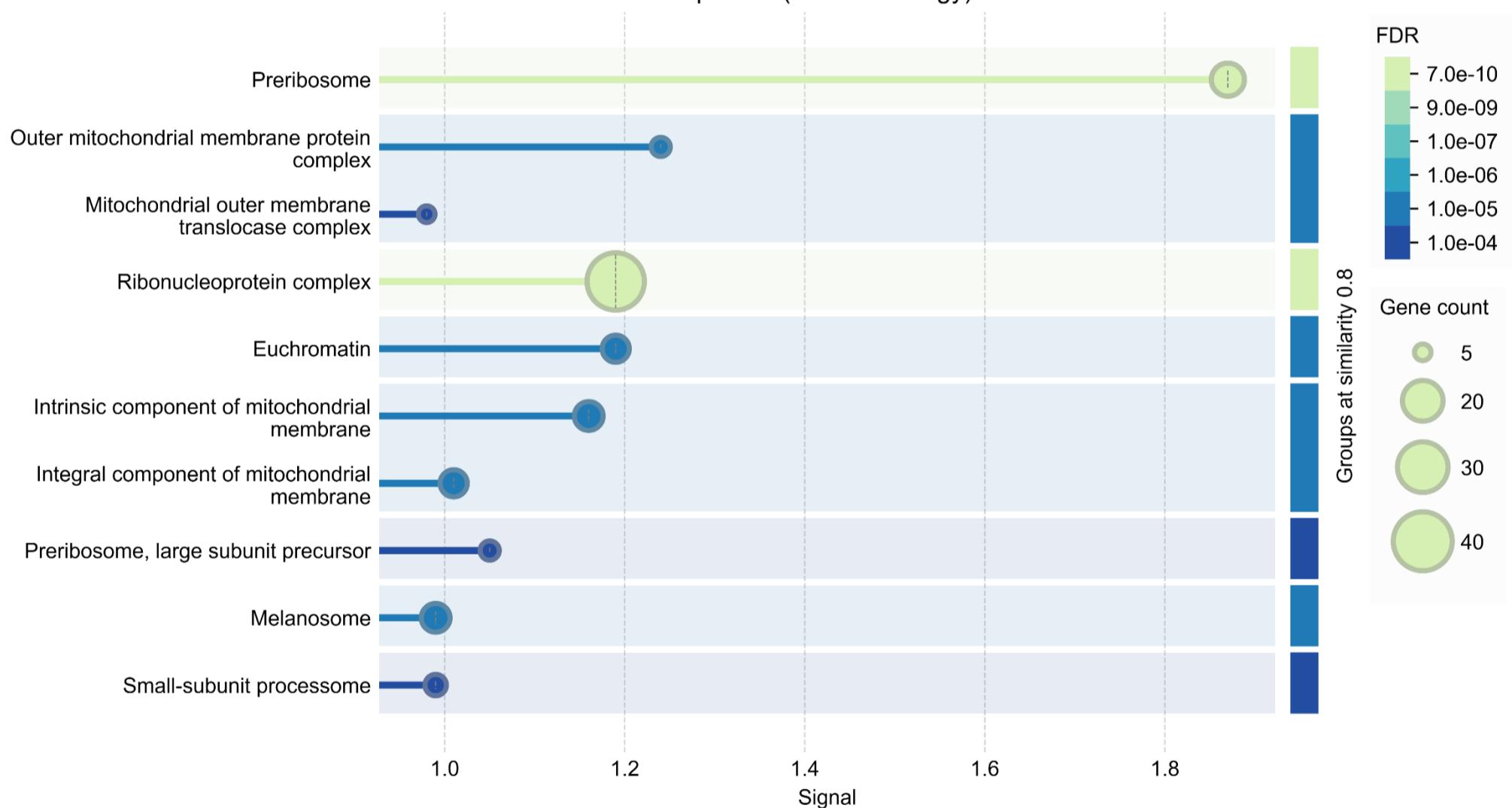
KEGG Pathways enrichment



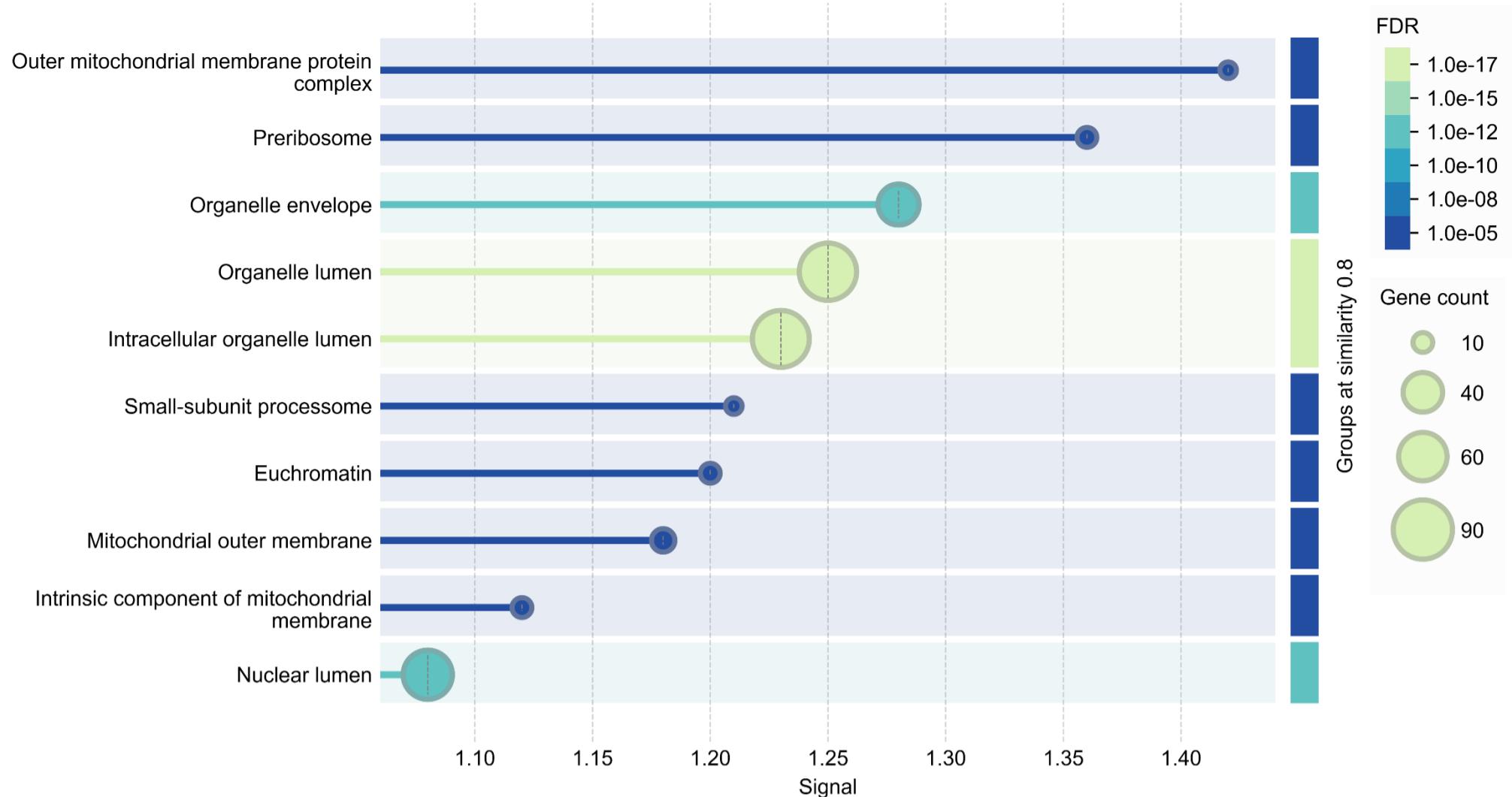
Molecular Function (Gene Ontology) enrichment



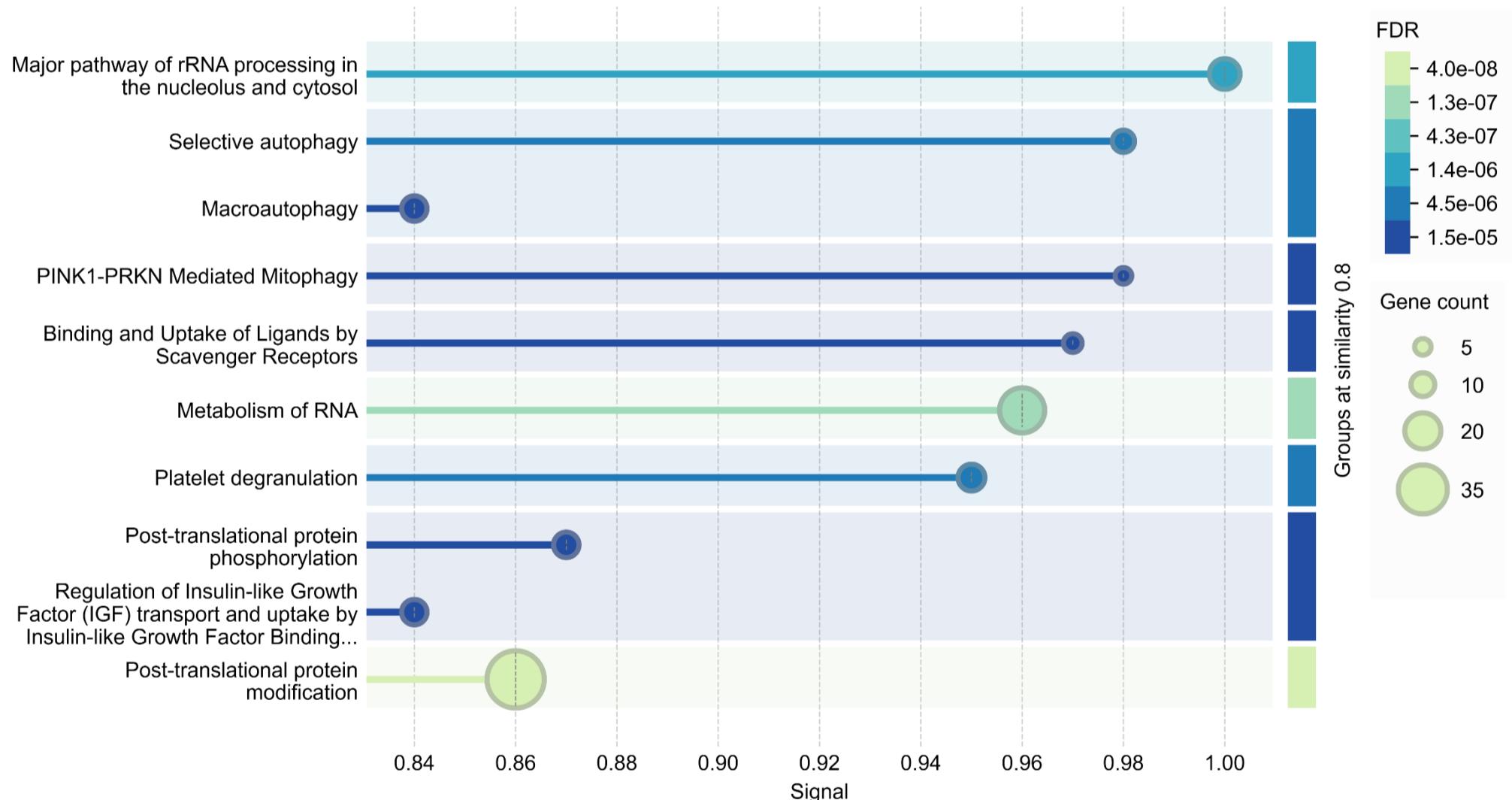
Cellular Component (Gene Ontology) enrichment



Subcellular Localization (COMPARTMENTS) enrichment



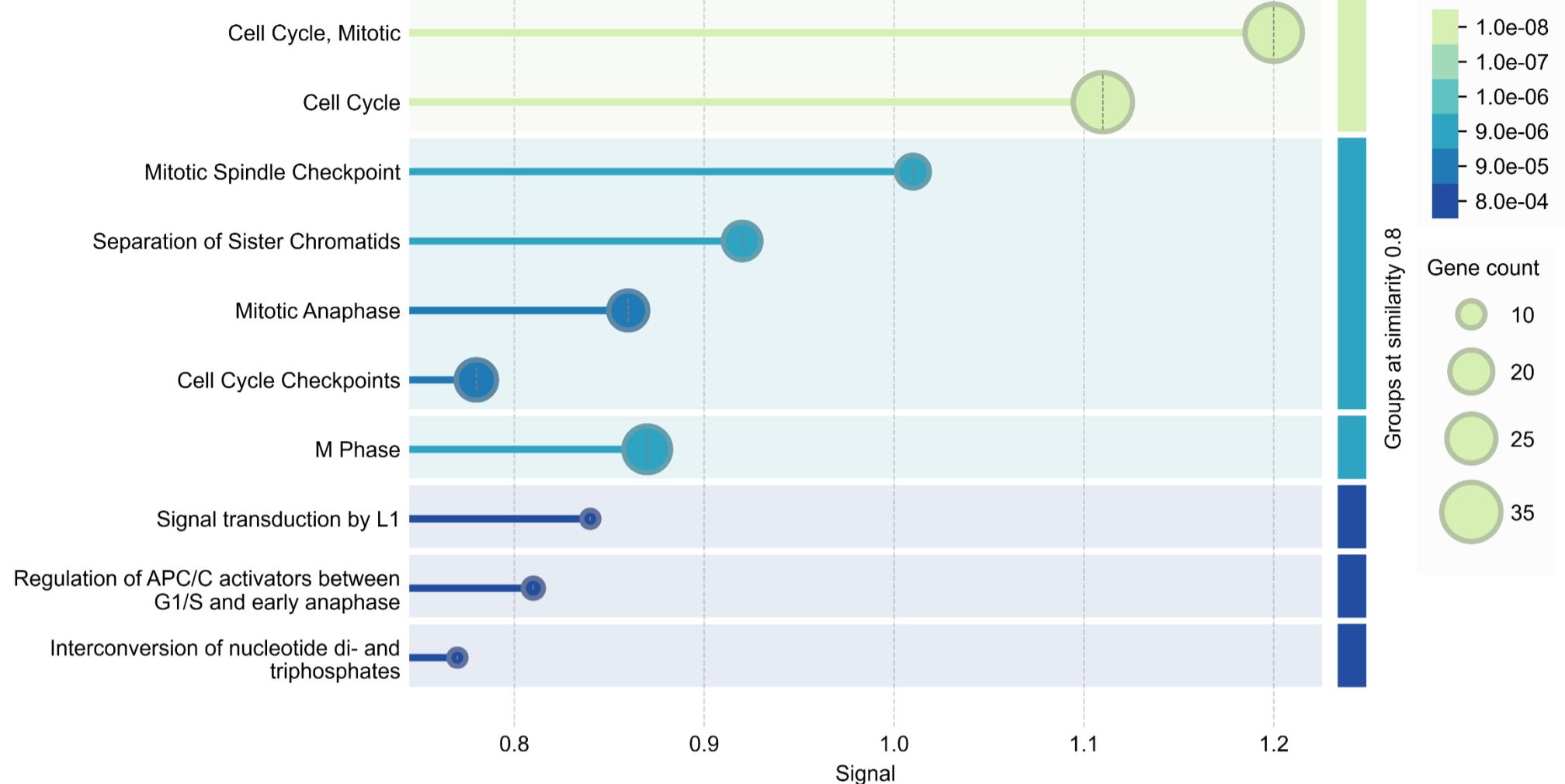
Reactome Pathways enrichment



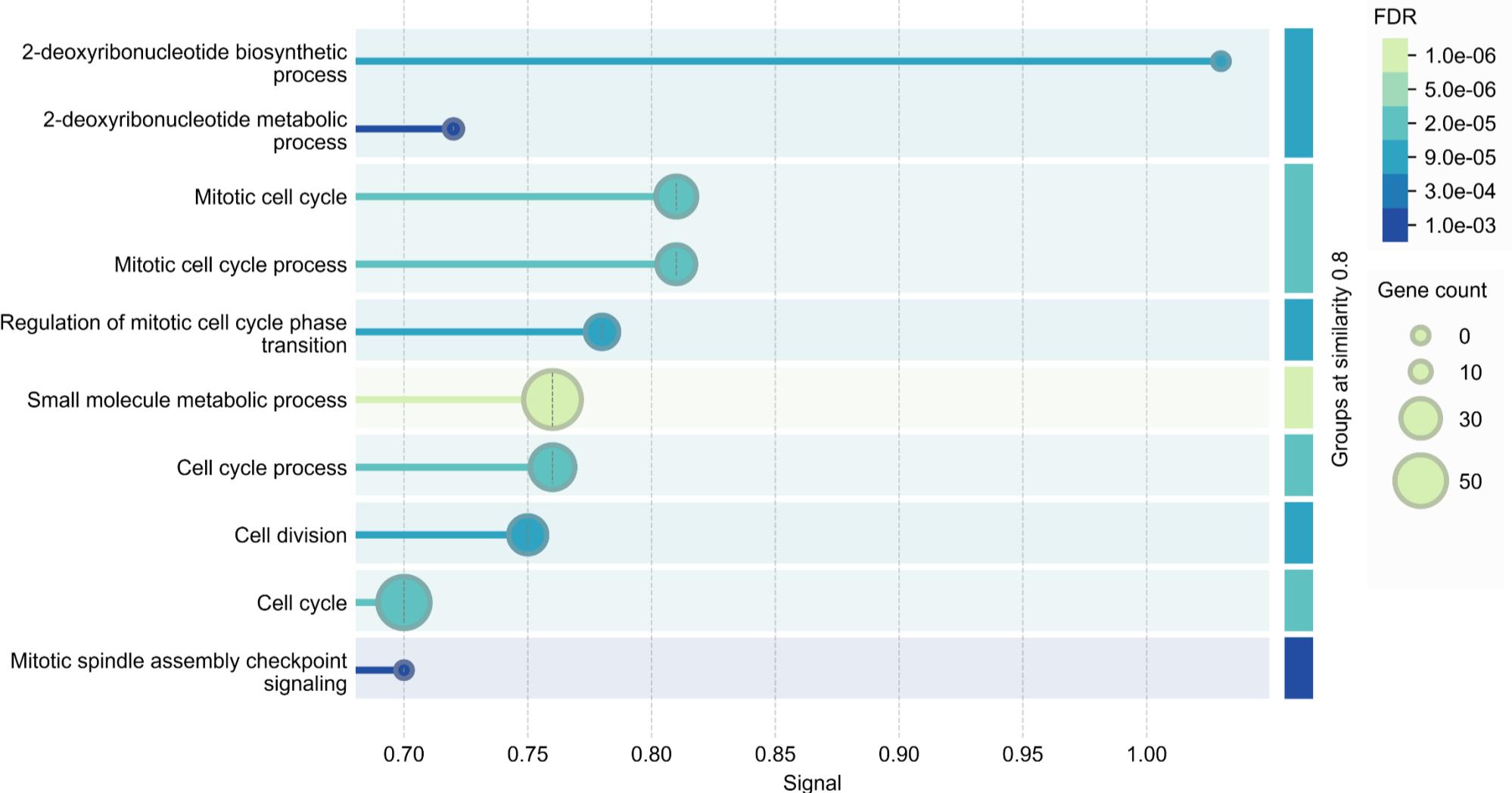
Comparison Injury2 (2DG) vs Injury0 (Control)

Downregulated

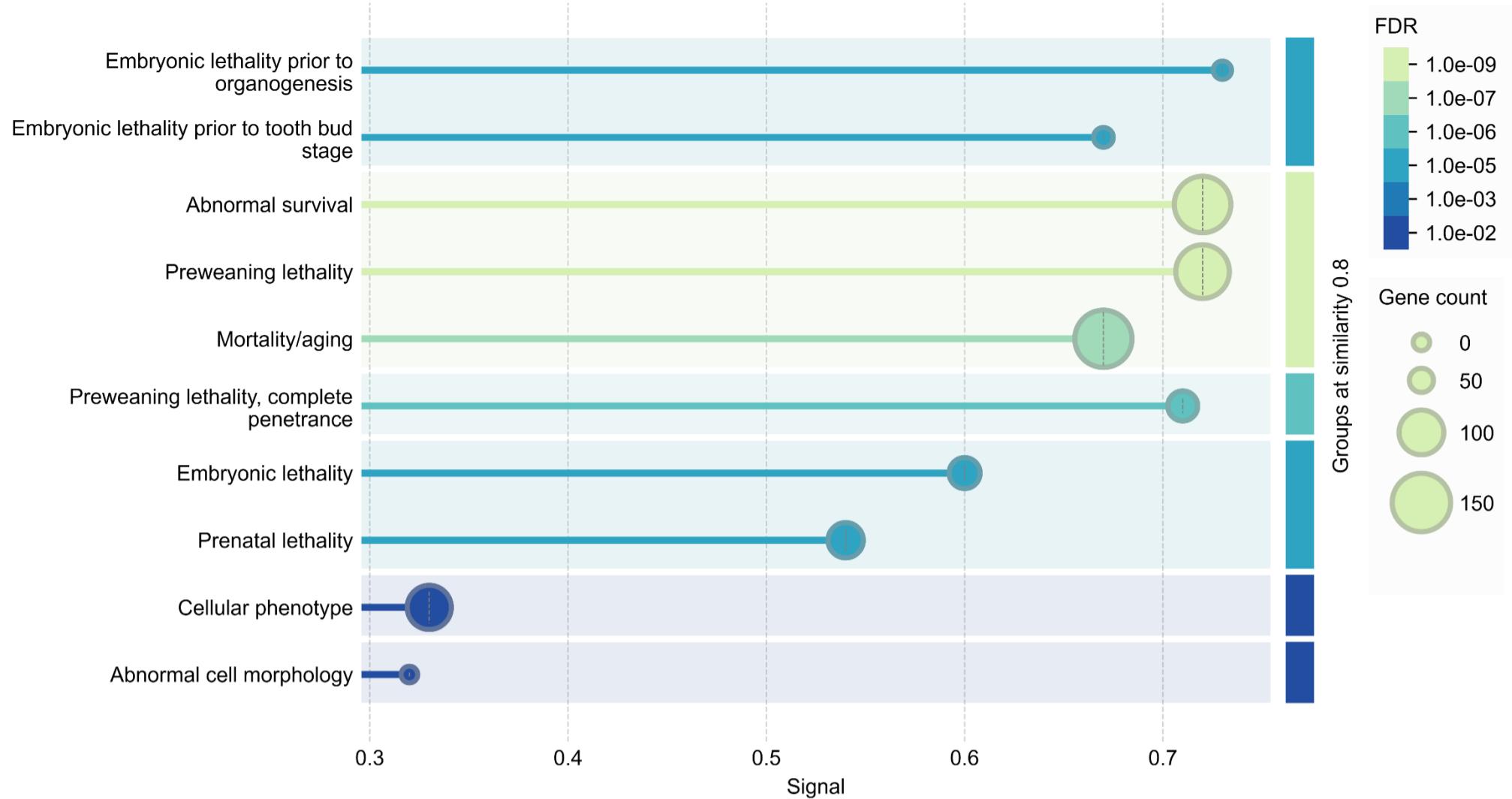
Reactome Pathways enrichment



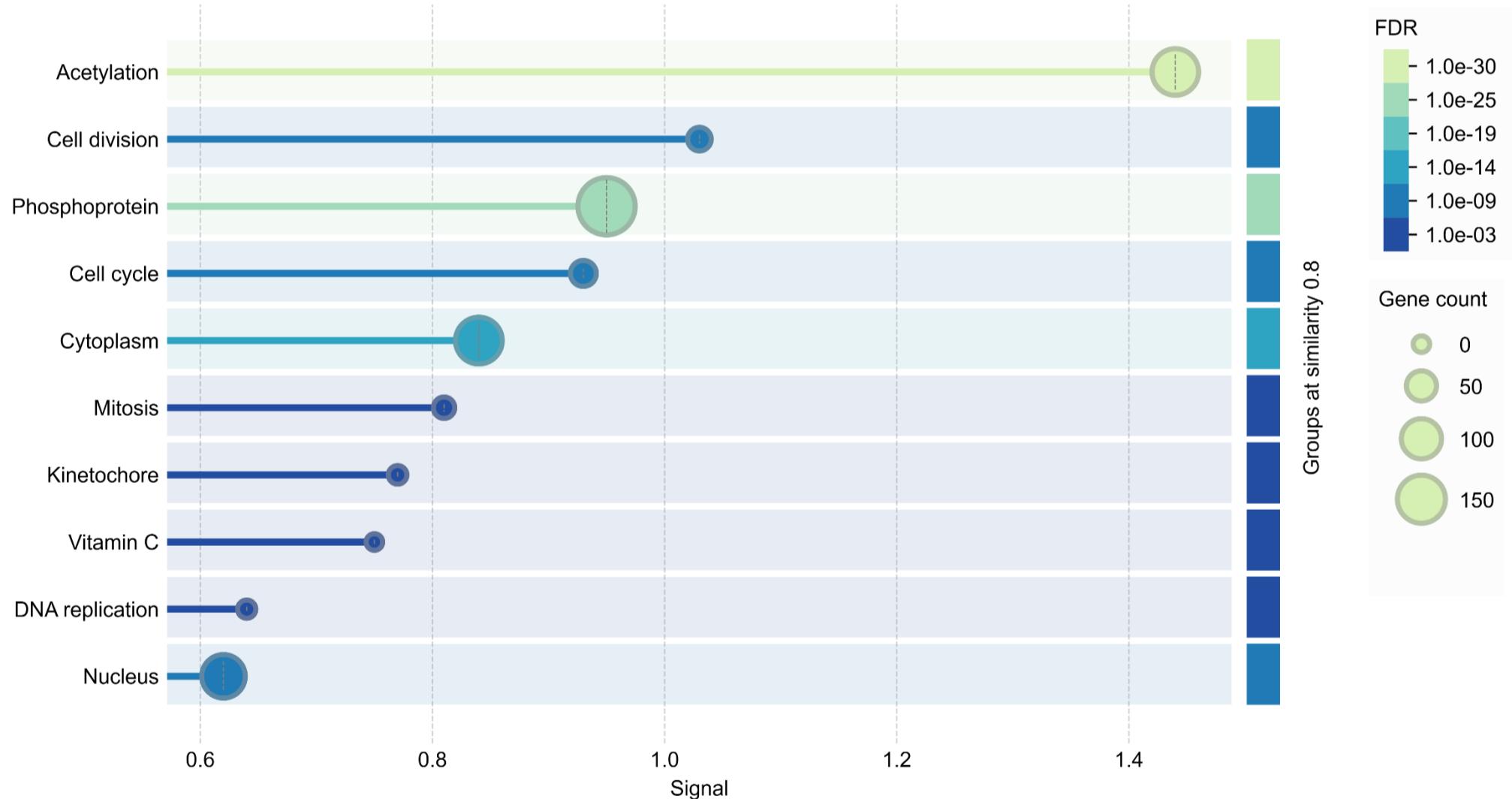
Biological Process (Gene Ontology) enrichment



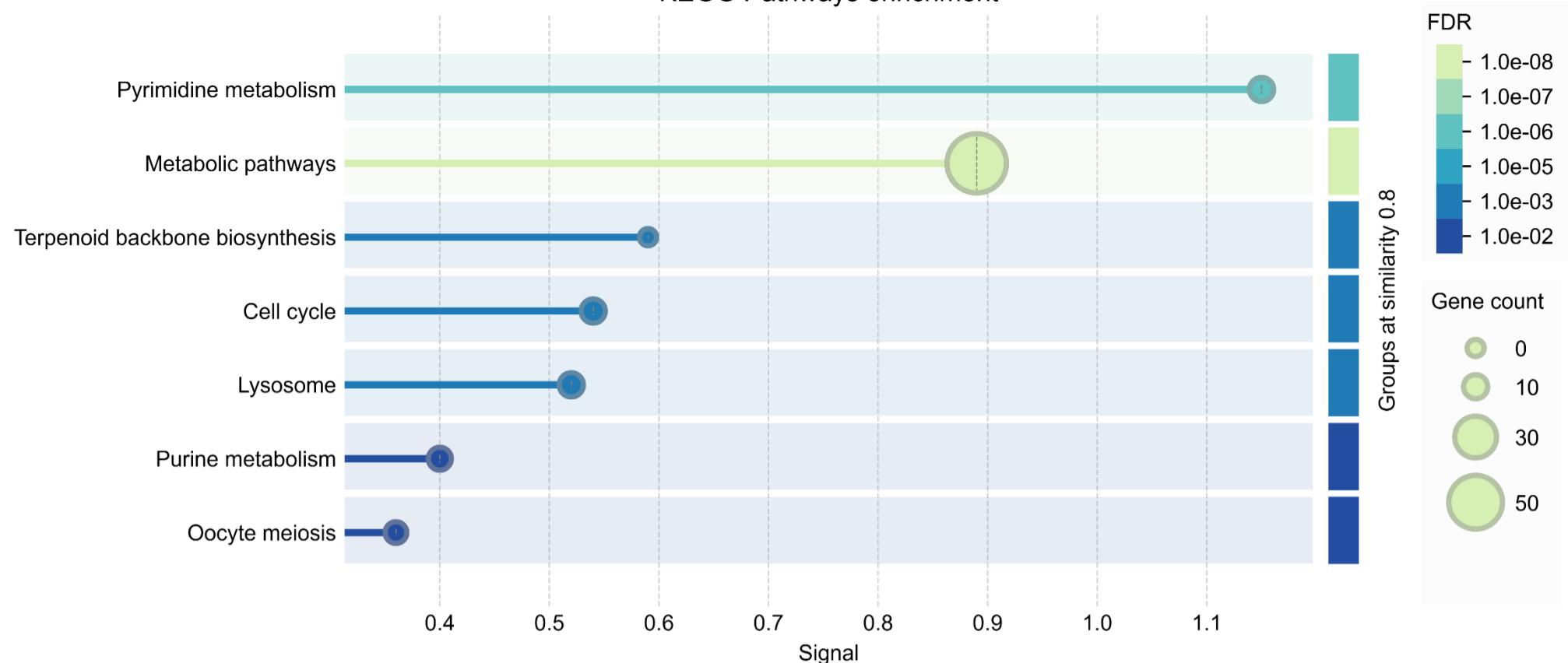
The Mammalian Phenotype Ontology (Monarch) enrichment



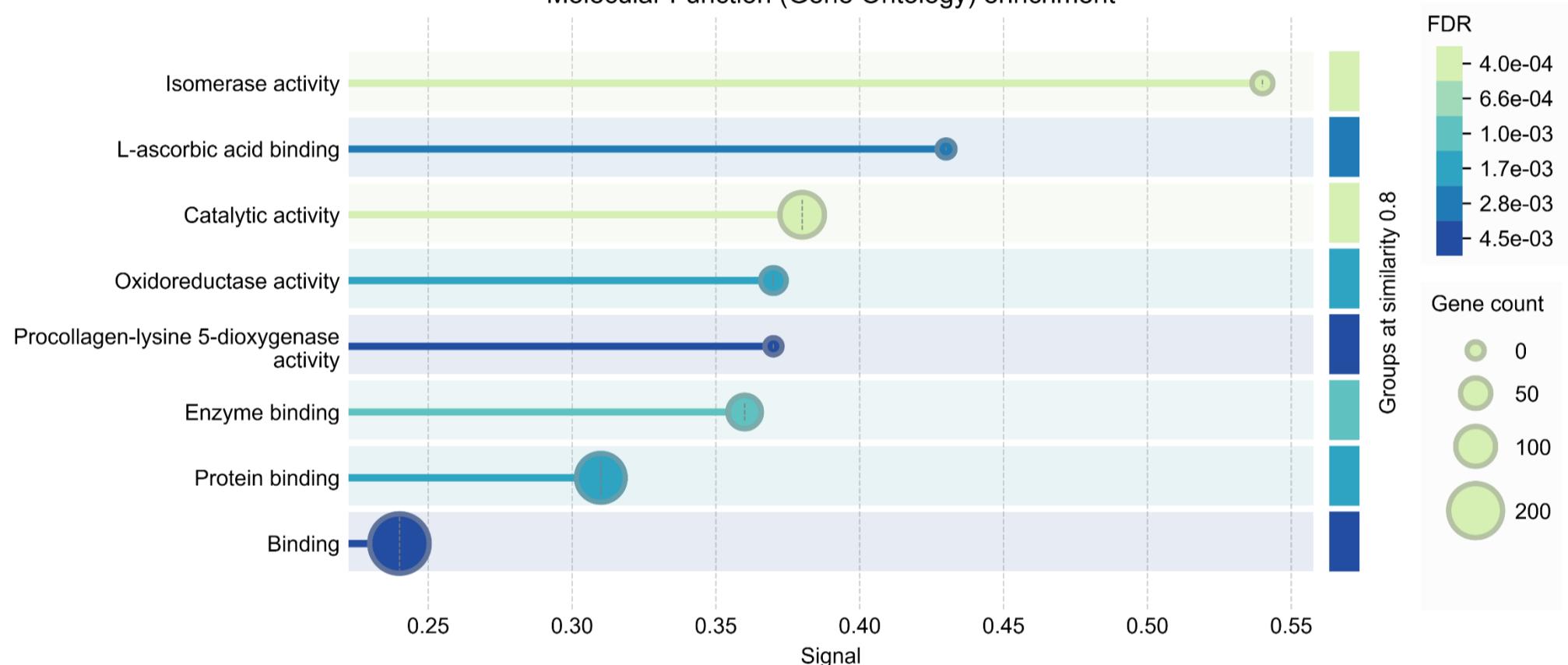
Annotated Keywords (UniProt) enrichment



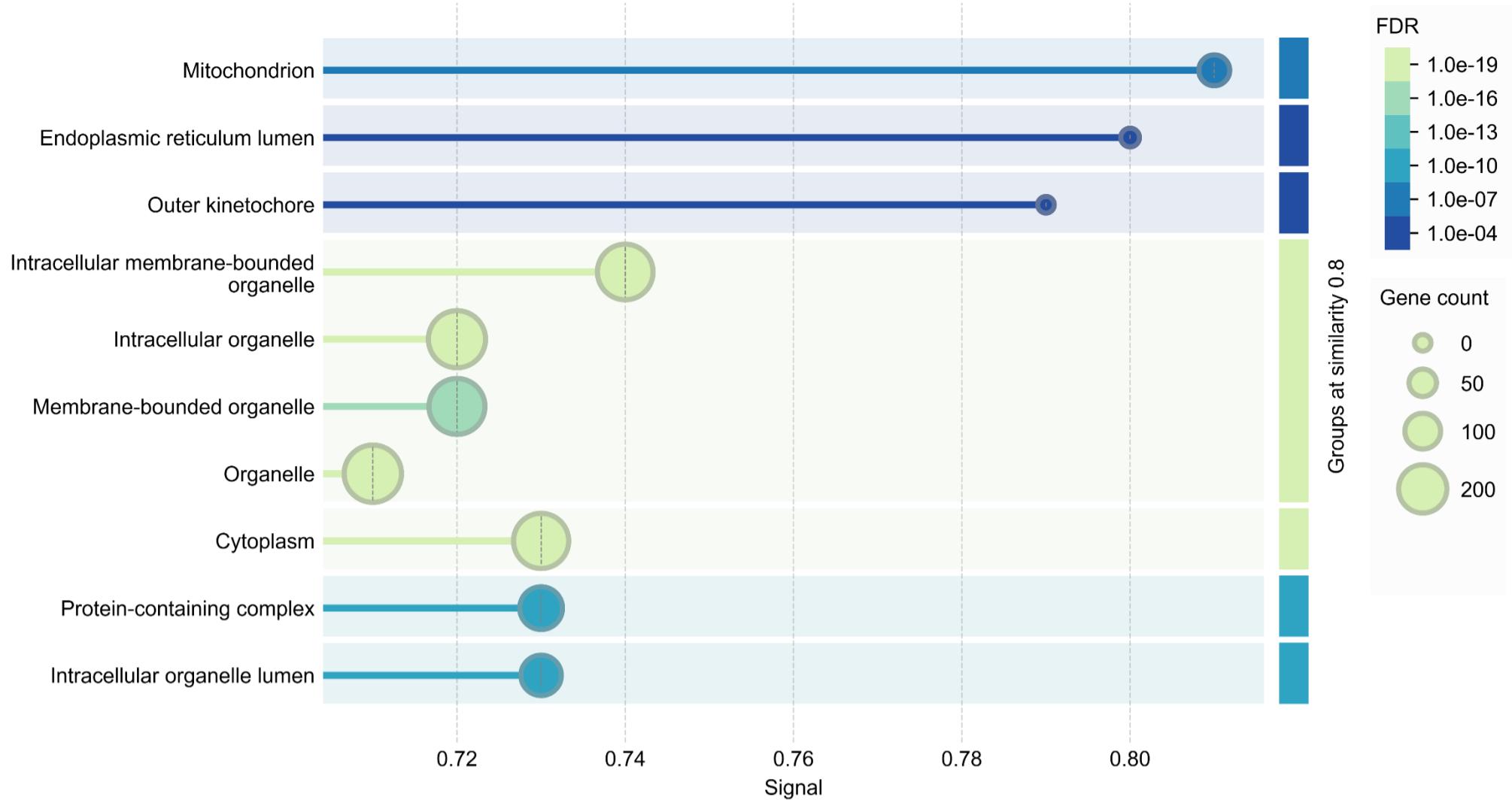
KEGG Pathways enrichment



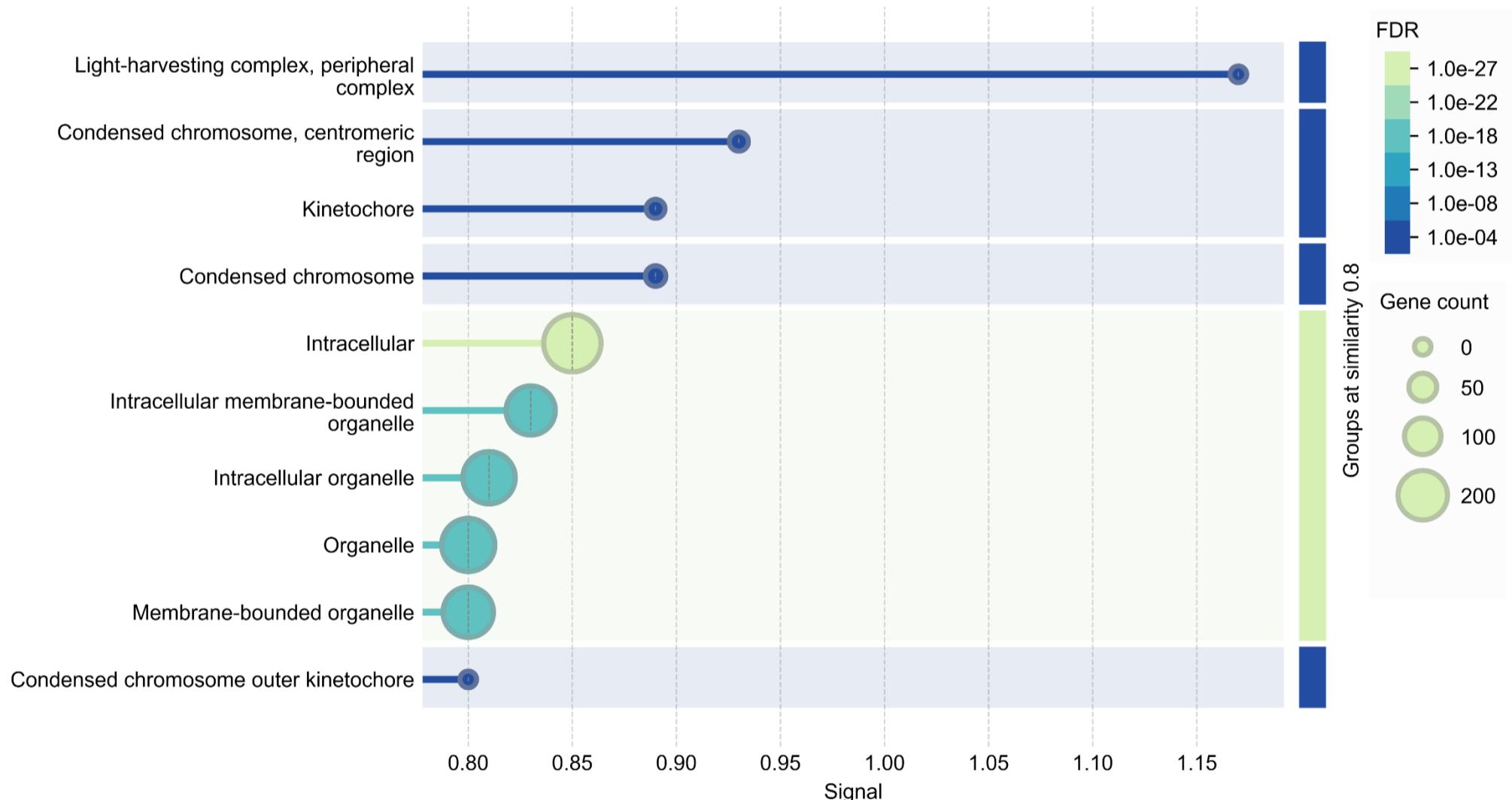
Molecular Function (Gene Ontology) enrichment



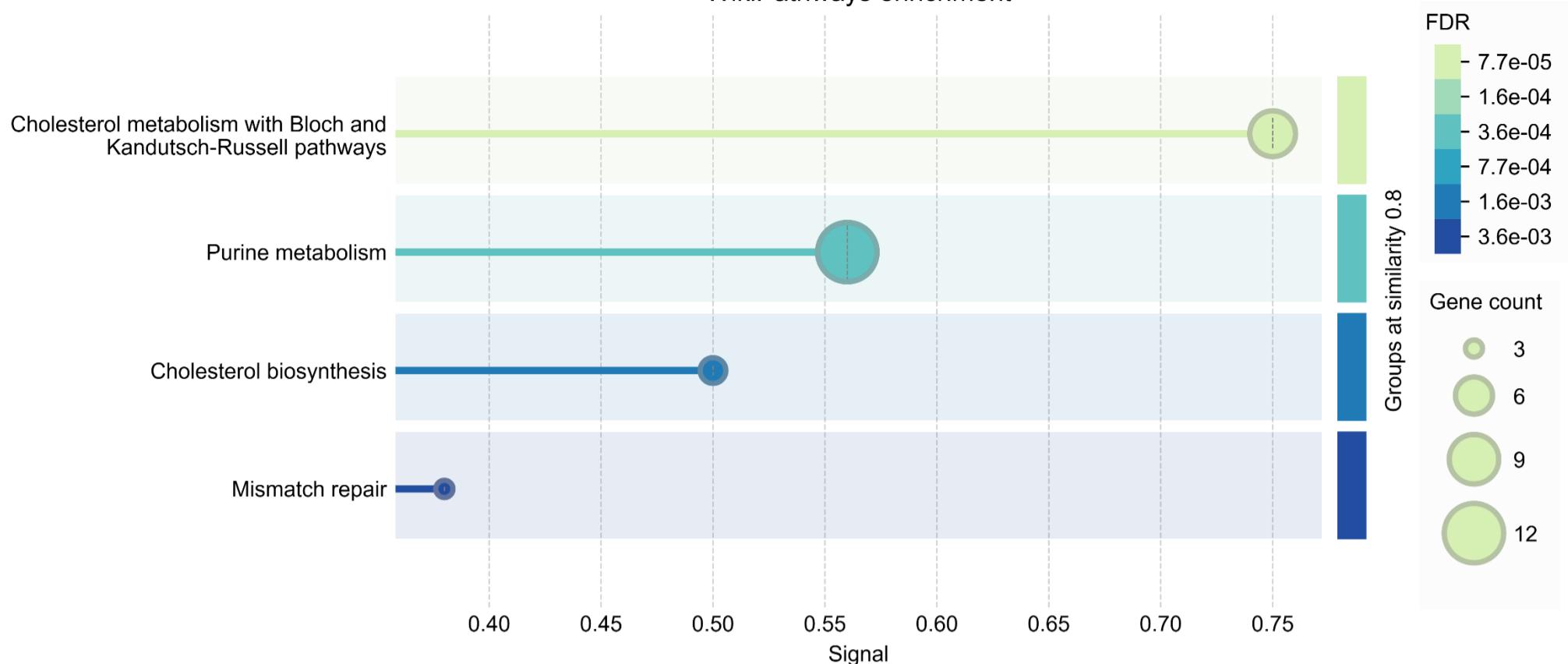
Cellular Component (Gene Ontology) enrichment



Subcellular Localization (COMPARTMENTS) enrichment



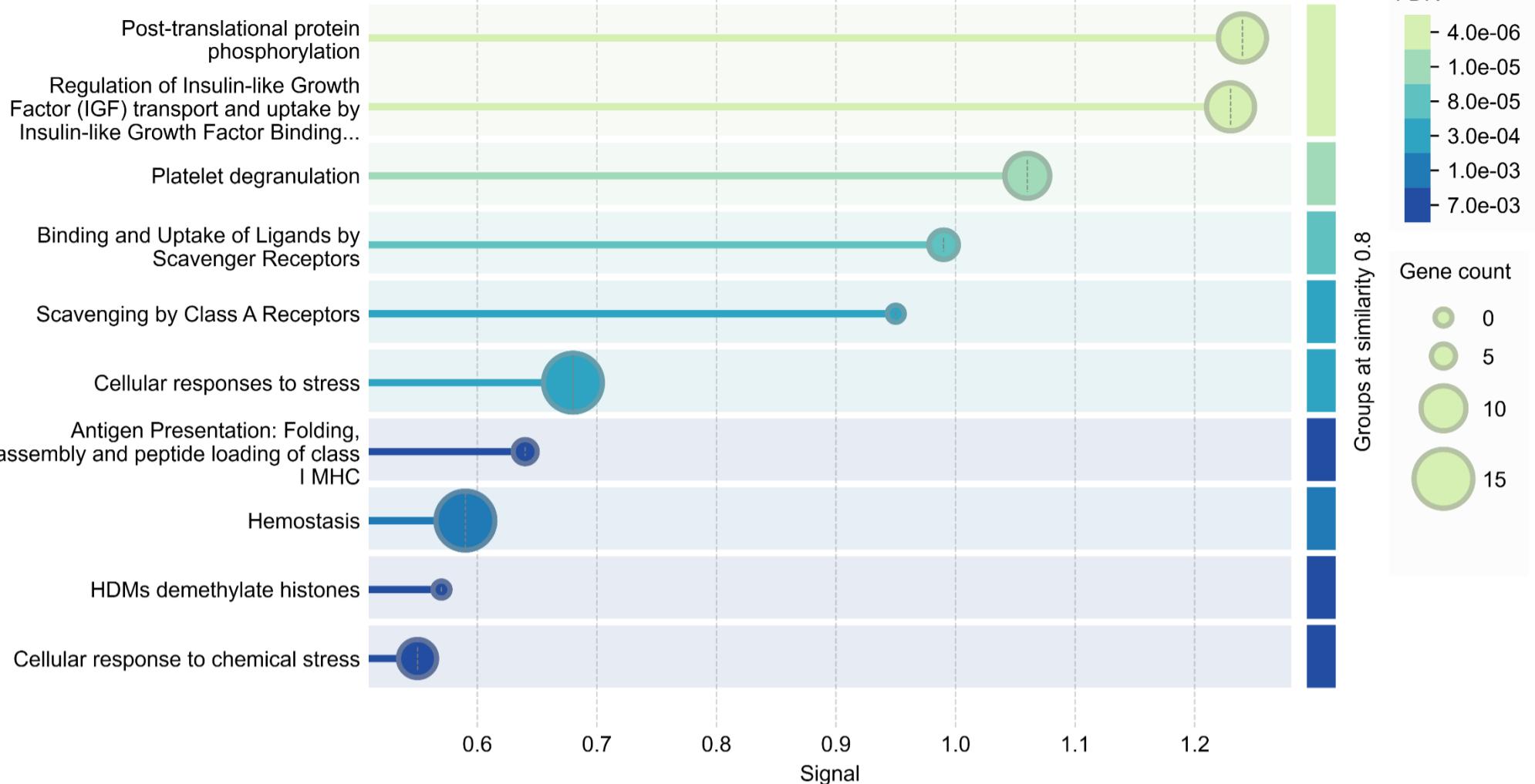
WikiPathways enrichment



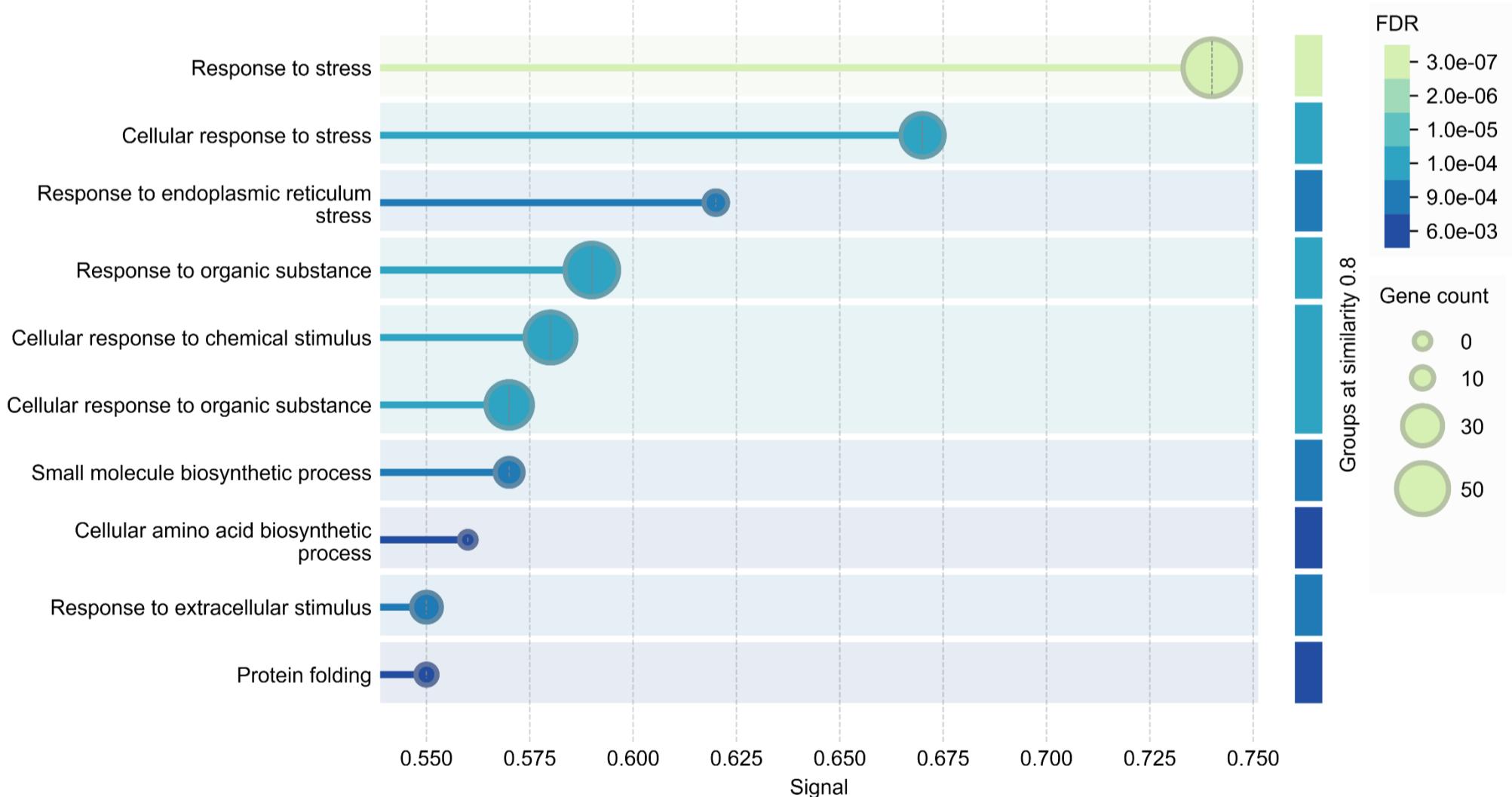
Comparison Injury2 (2DG) vs Injury1 (SDT)

Upregulated

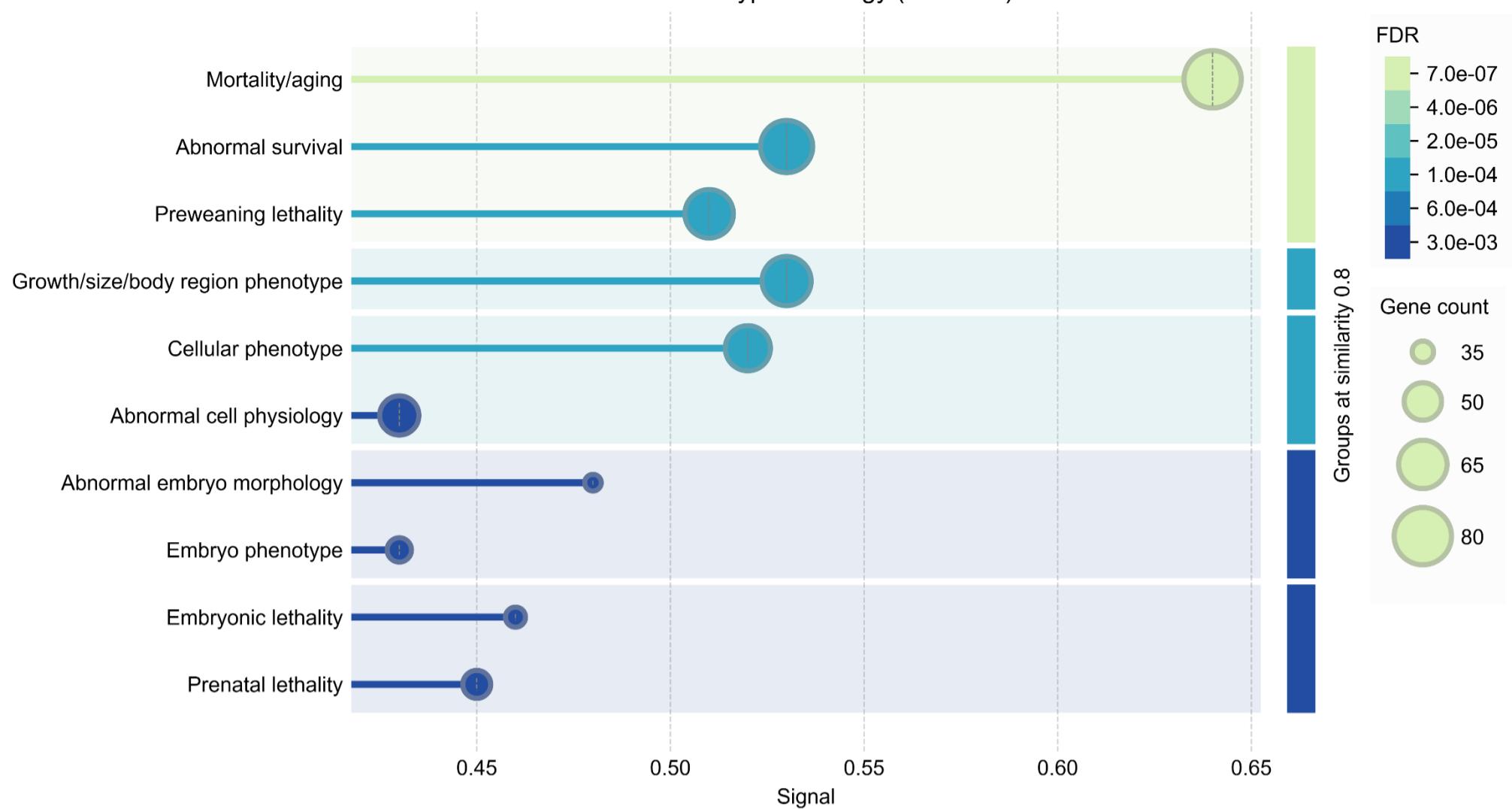
Reactome Pathways enrichment



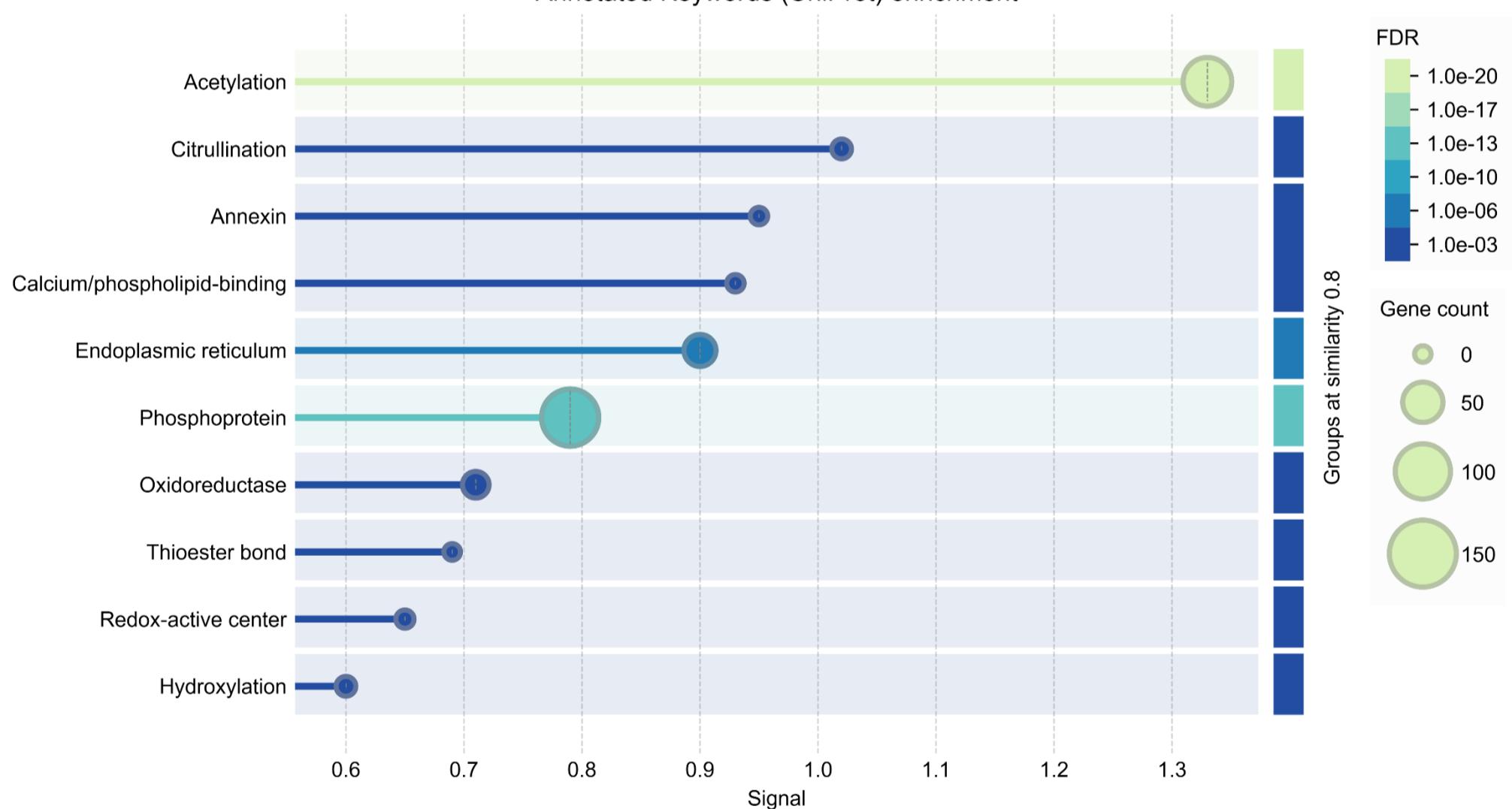
Biological Process (Gene Ontology) enrichment



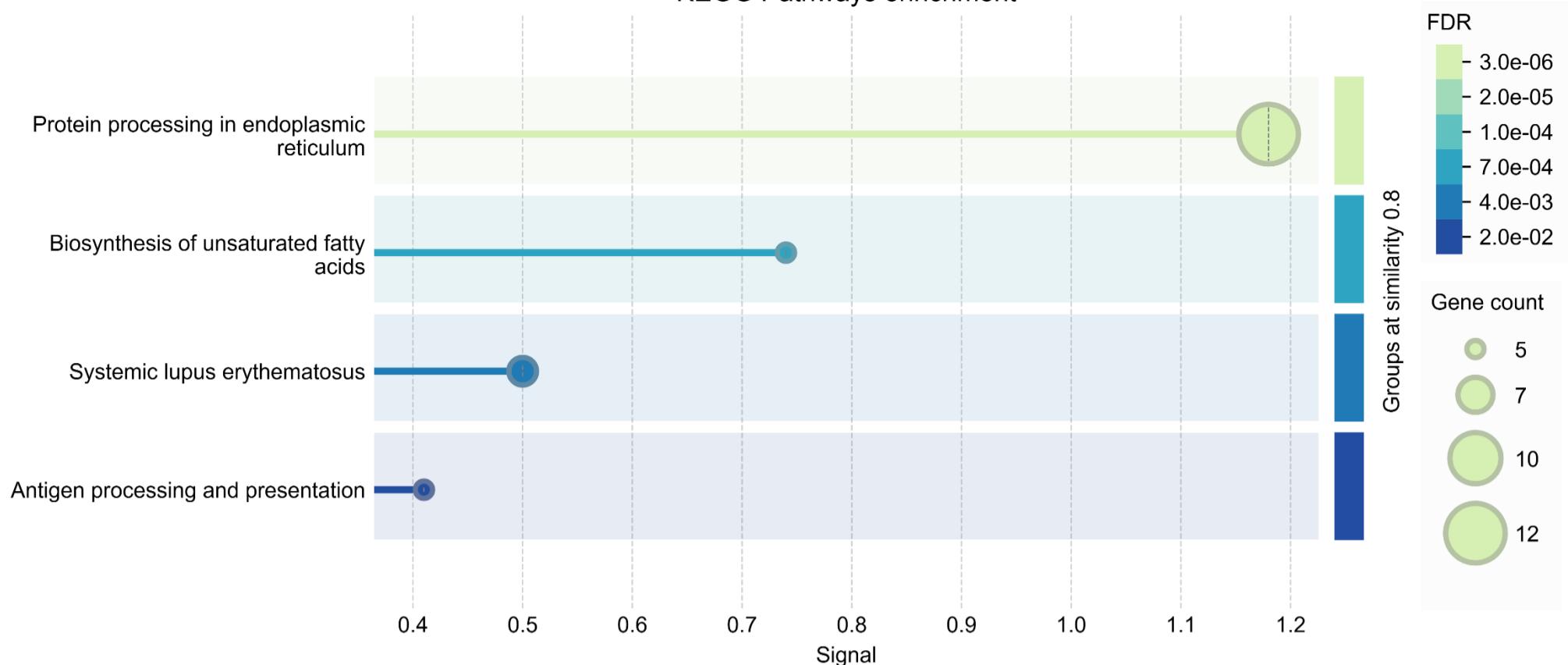
The Mammalian Phenotype Ontology (Monarch) enrichment



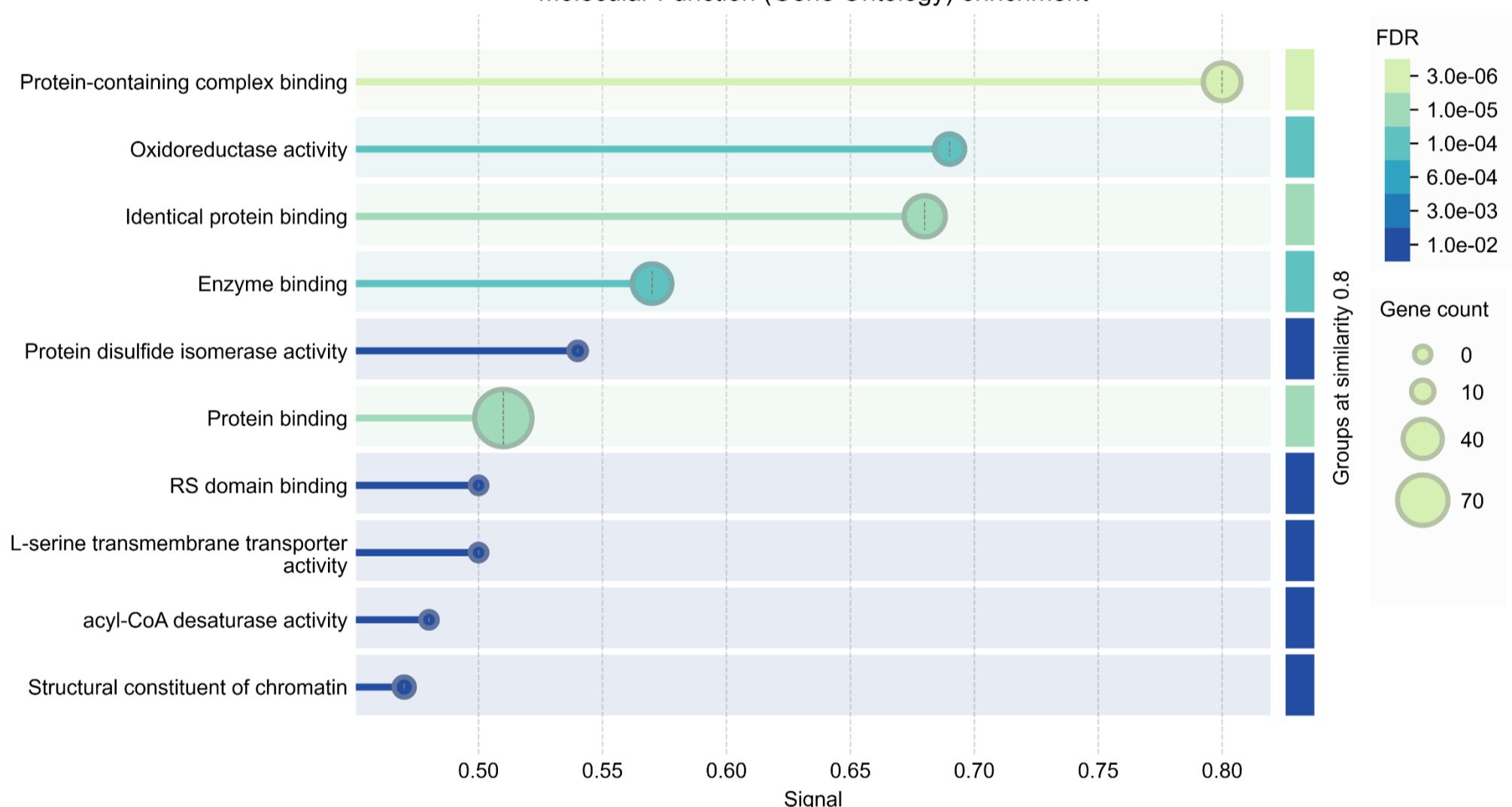
Annotated Keywords (UniProt) enrichment



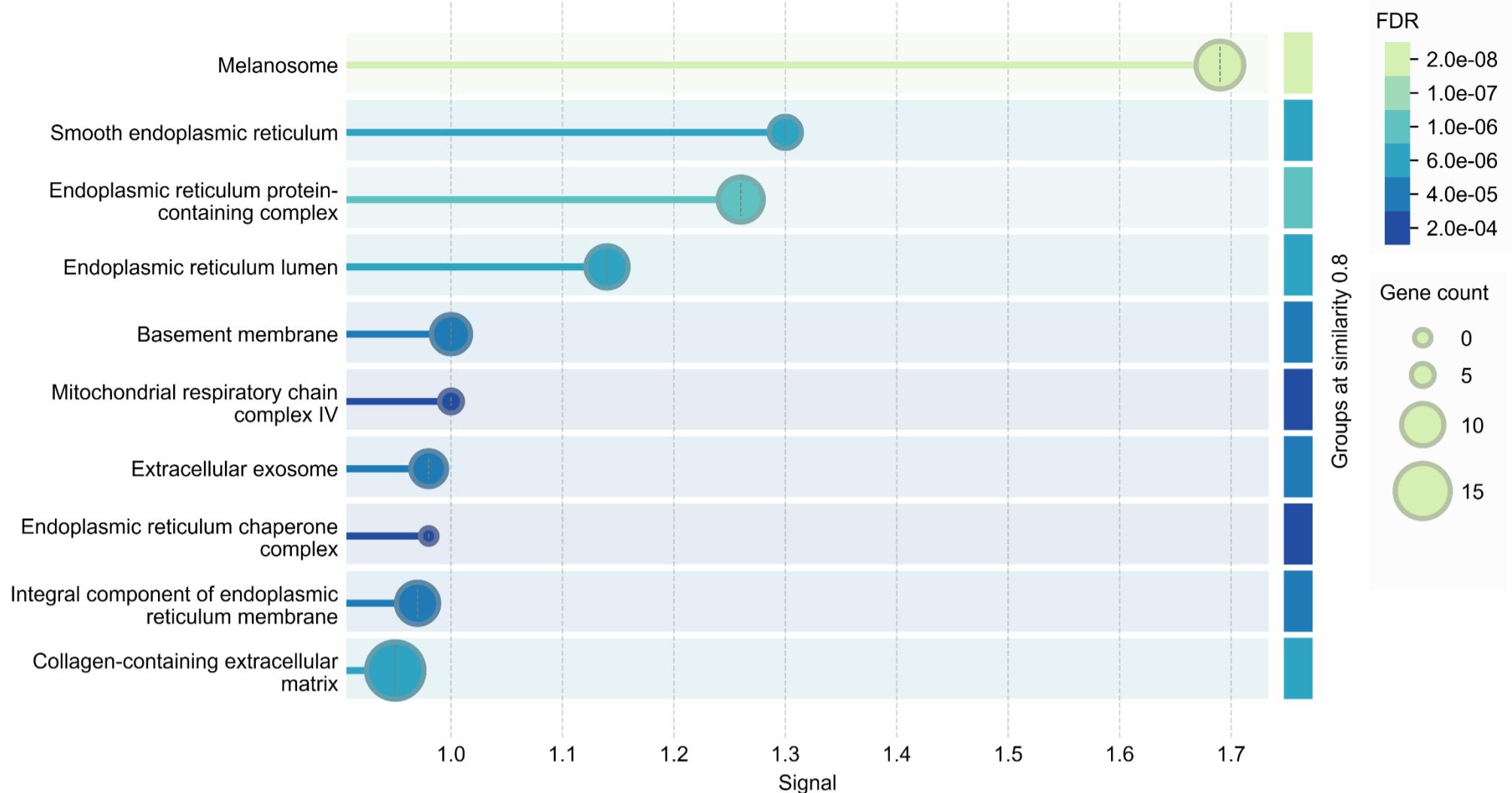
KEGG Pathways enrichment



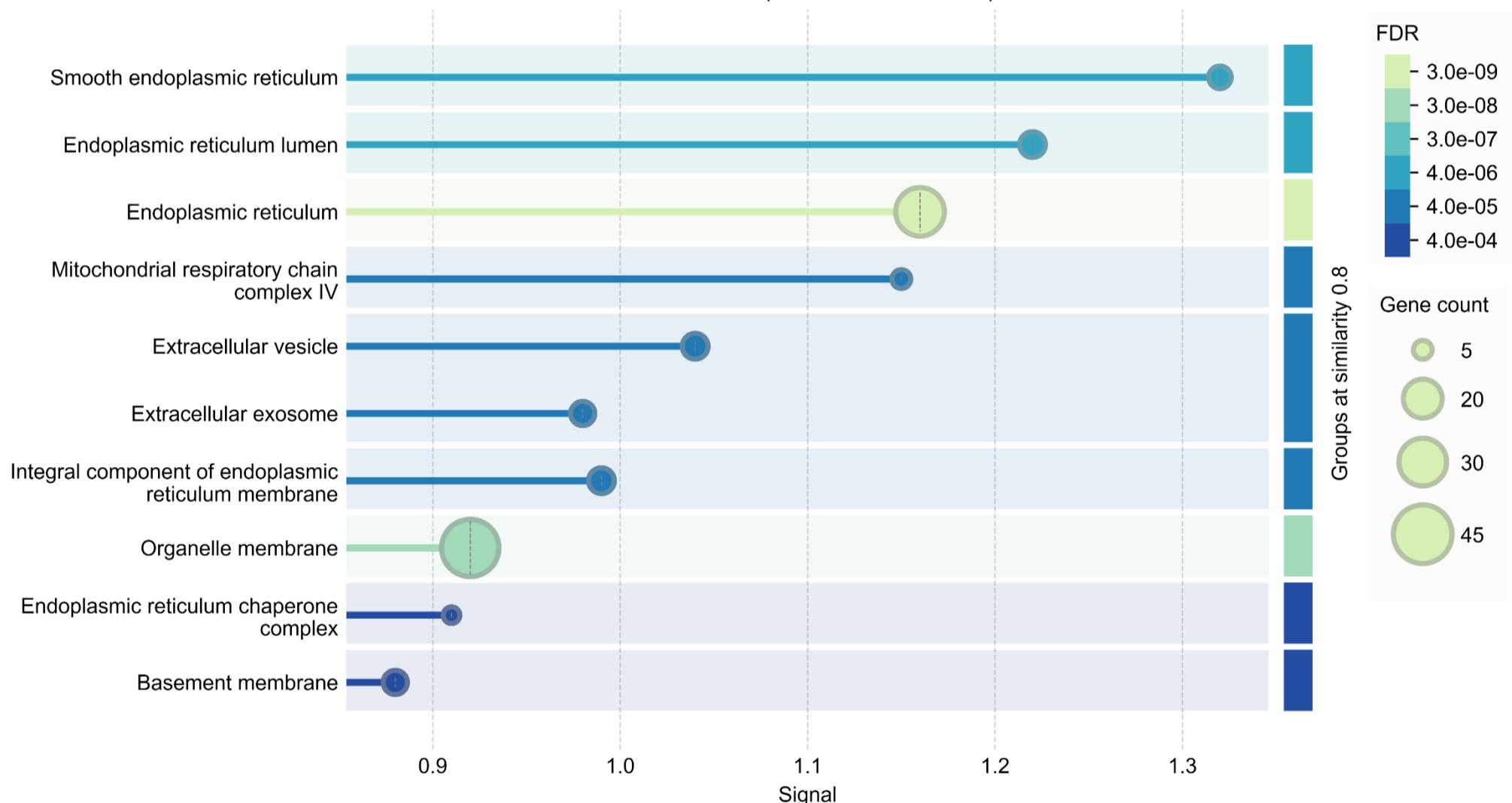
Molecular Function (Gene Ontology) enrichment



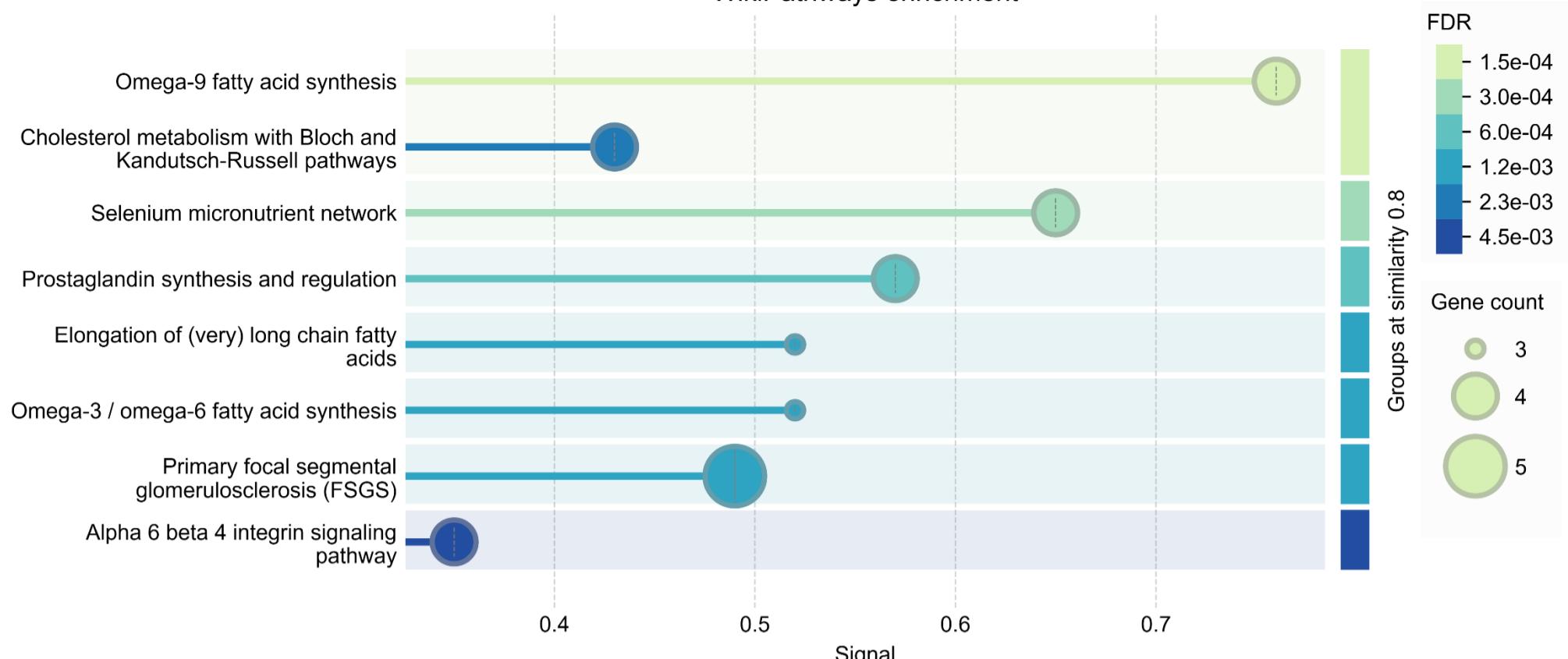
Cellular Component (Gene Ontology) enrichment



Subcellular Localization (COMPARTMENTS) enrichment

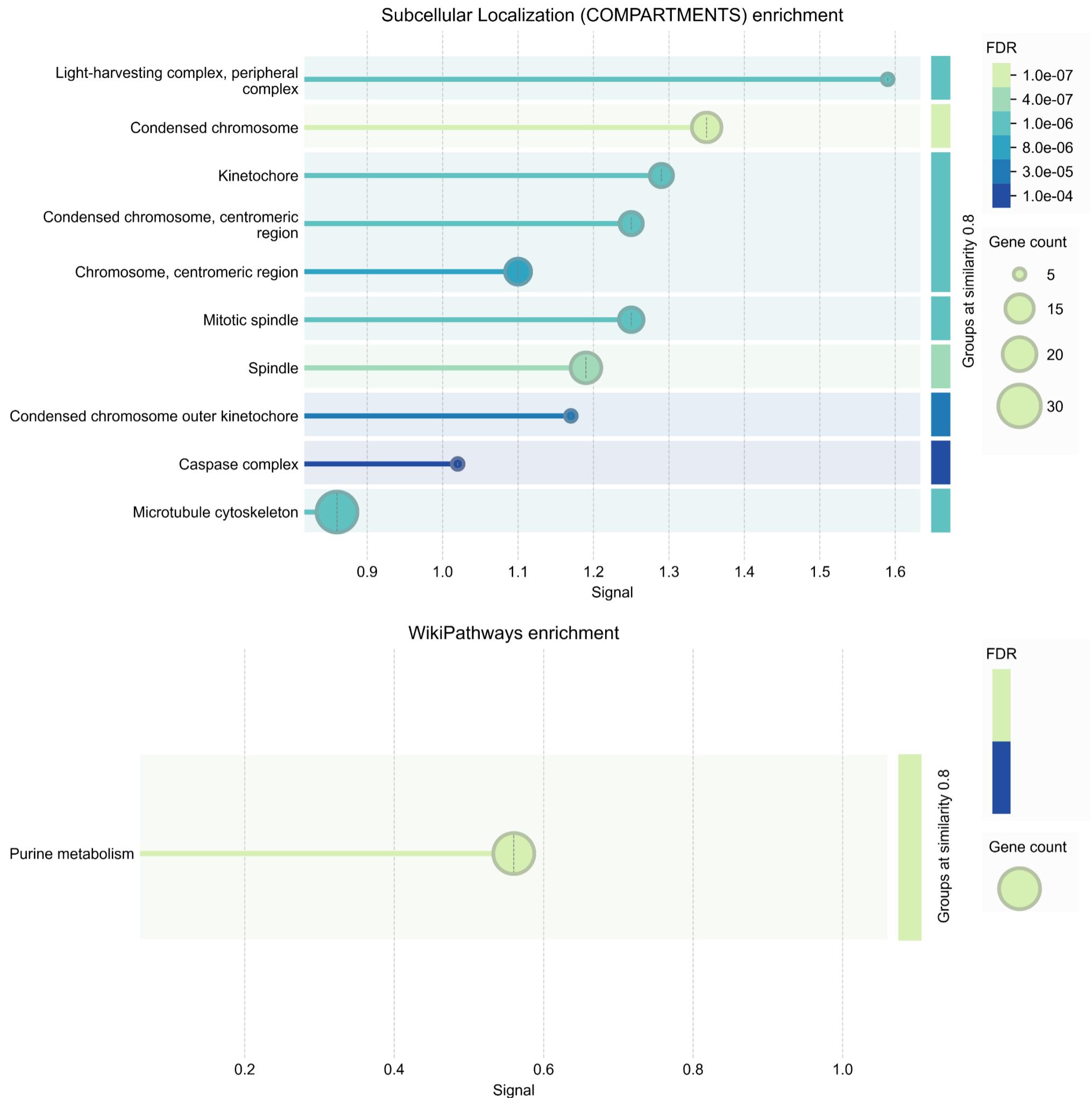


WikiPathways enrichment

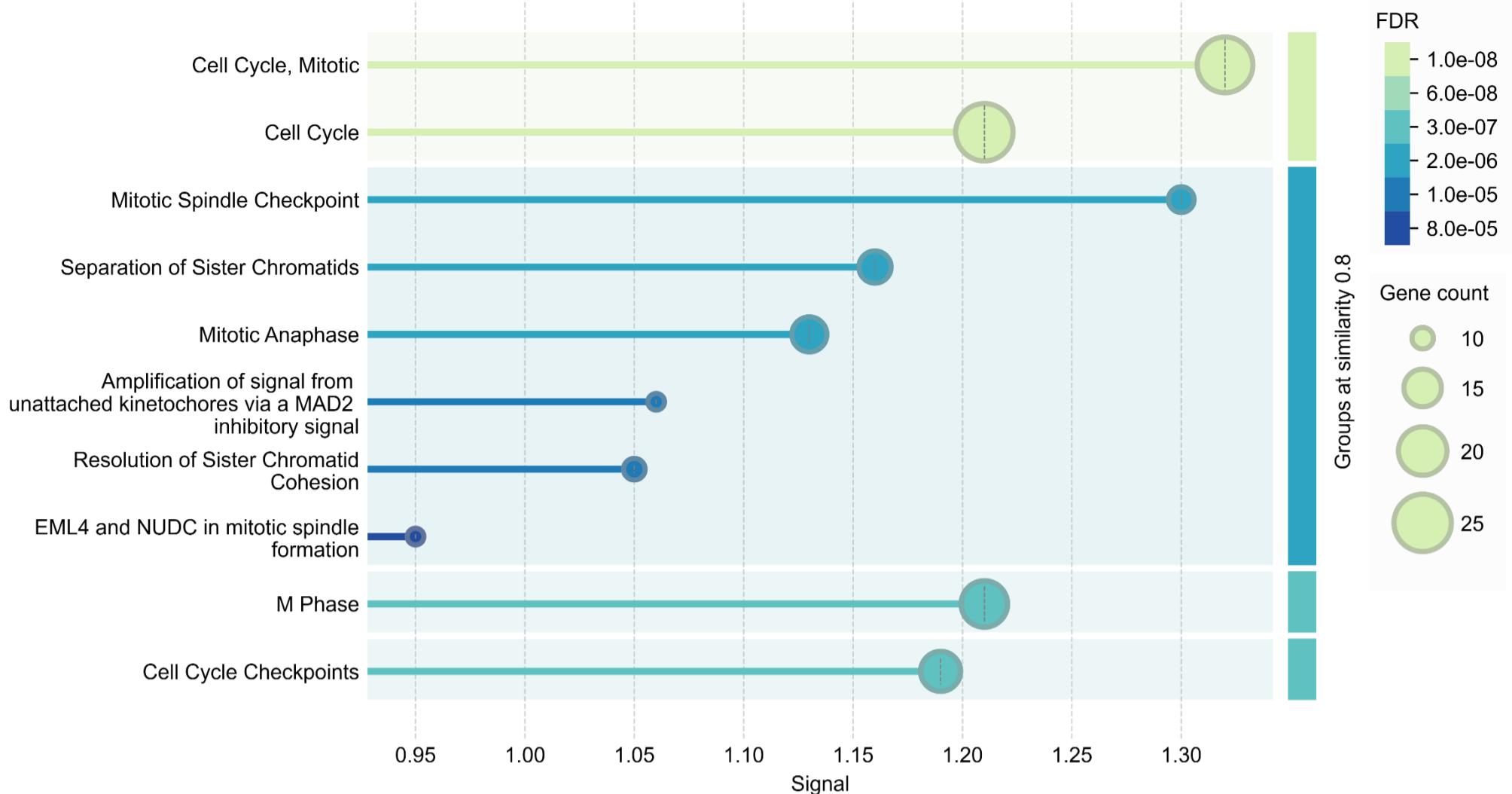


Comparison Injury2 (2DG) vs Injury1 (SDT)

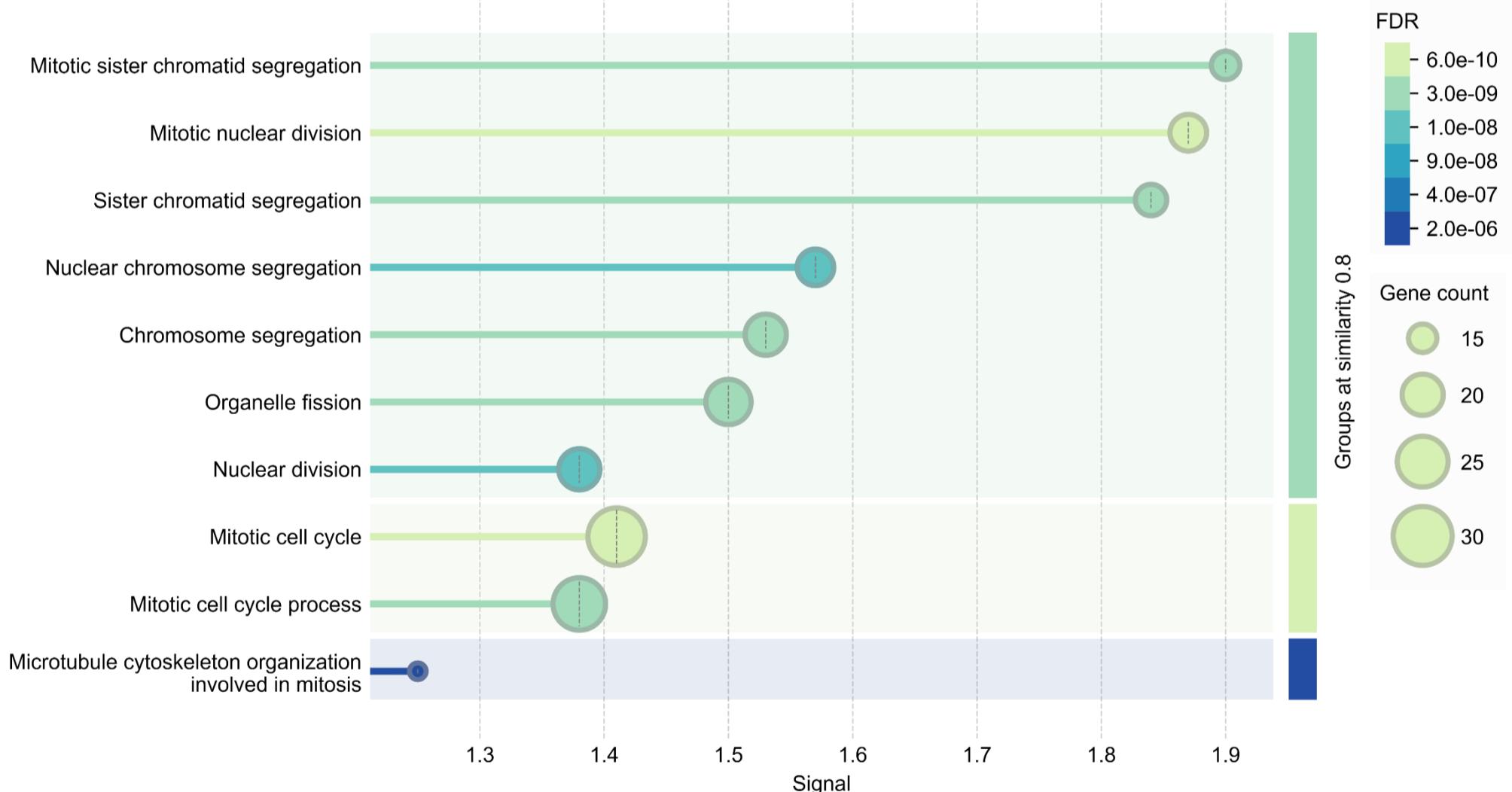
Downregulated



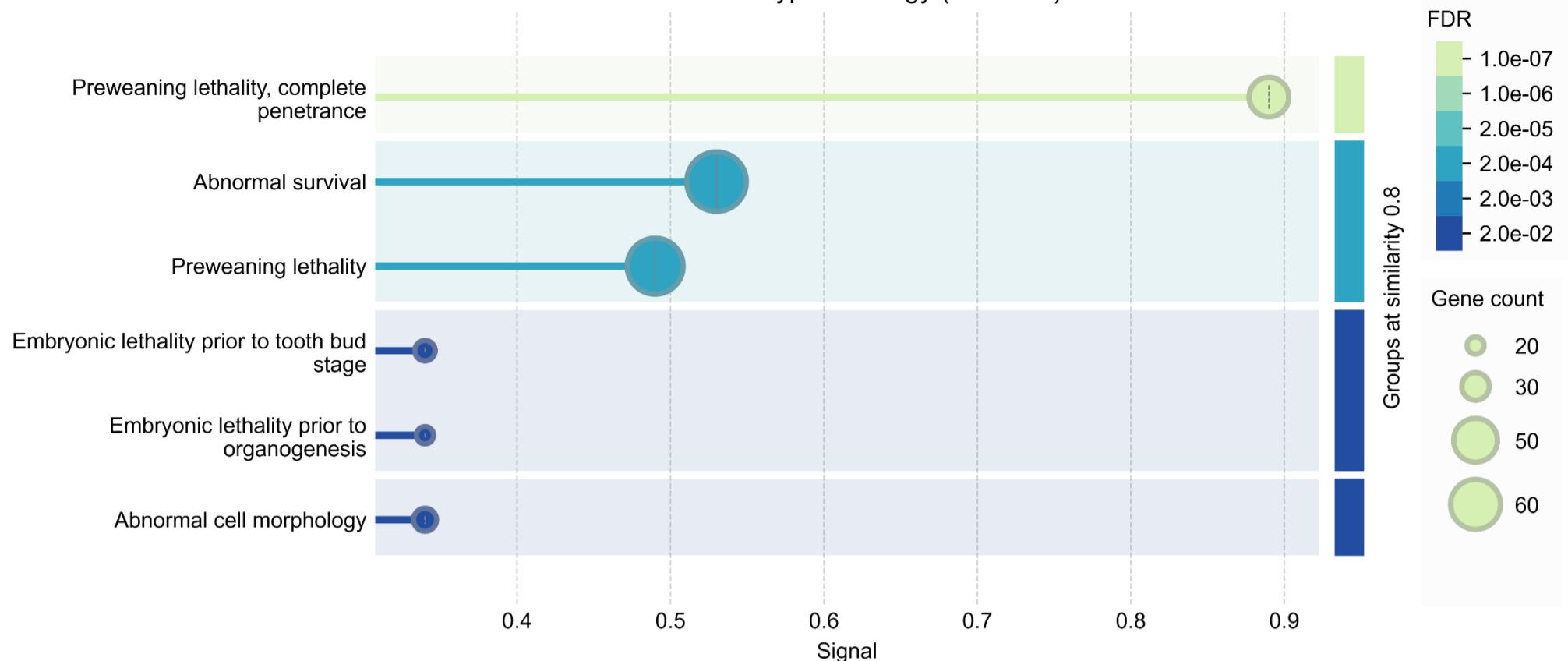
Reactome Pathways enrichment



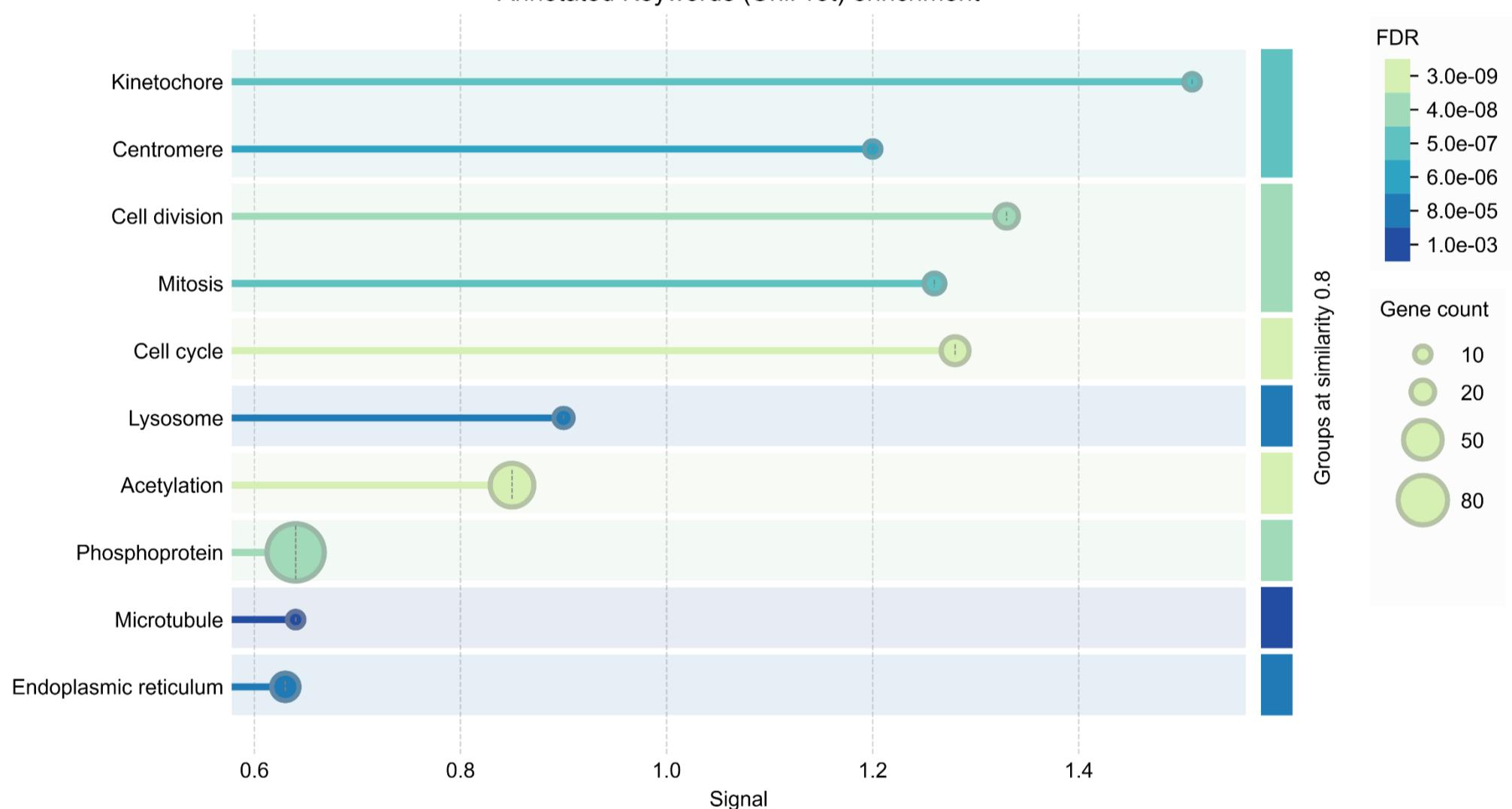
Biological Process (Gene Ontology) enrichment



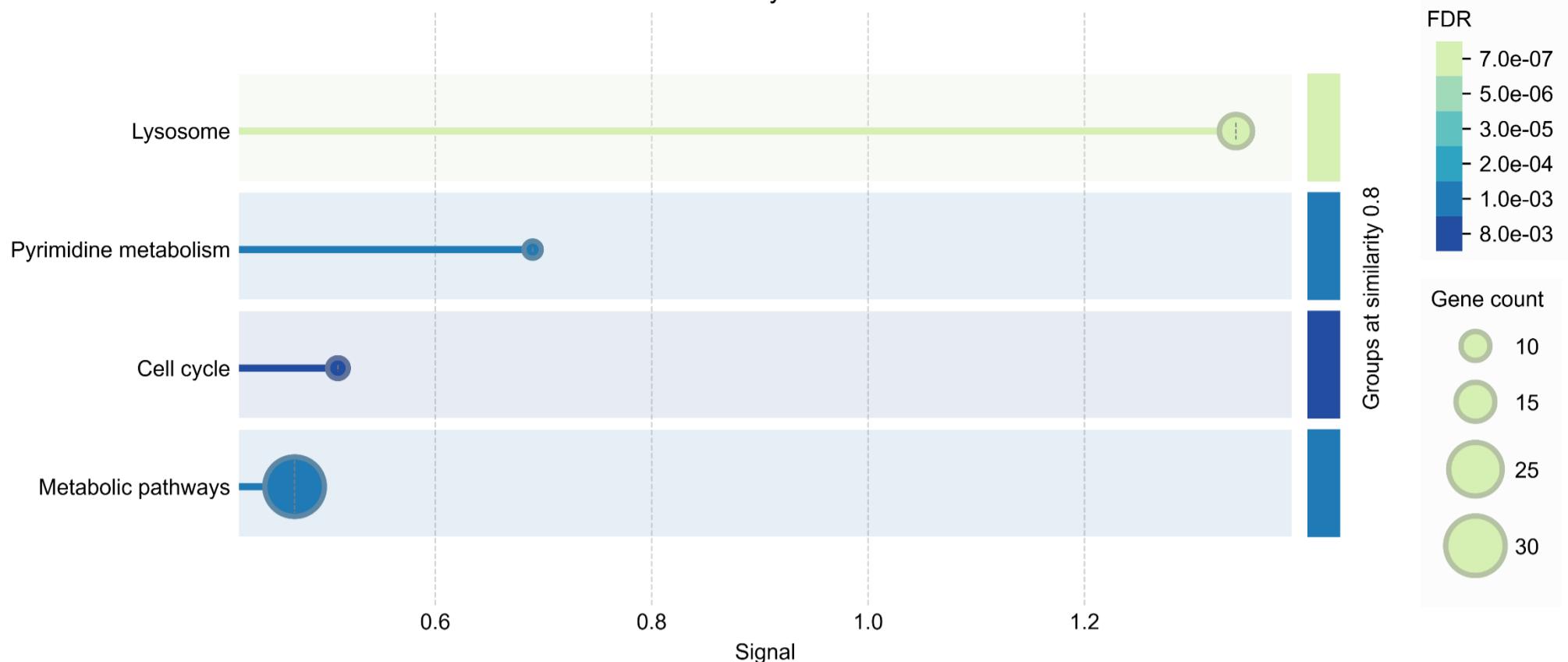
The Mammalian Phenotype Ontology (Monarch) enrichment



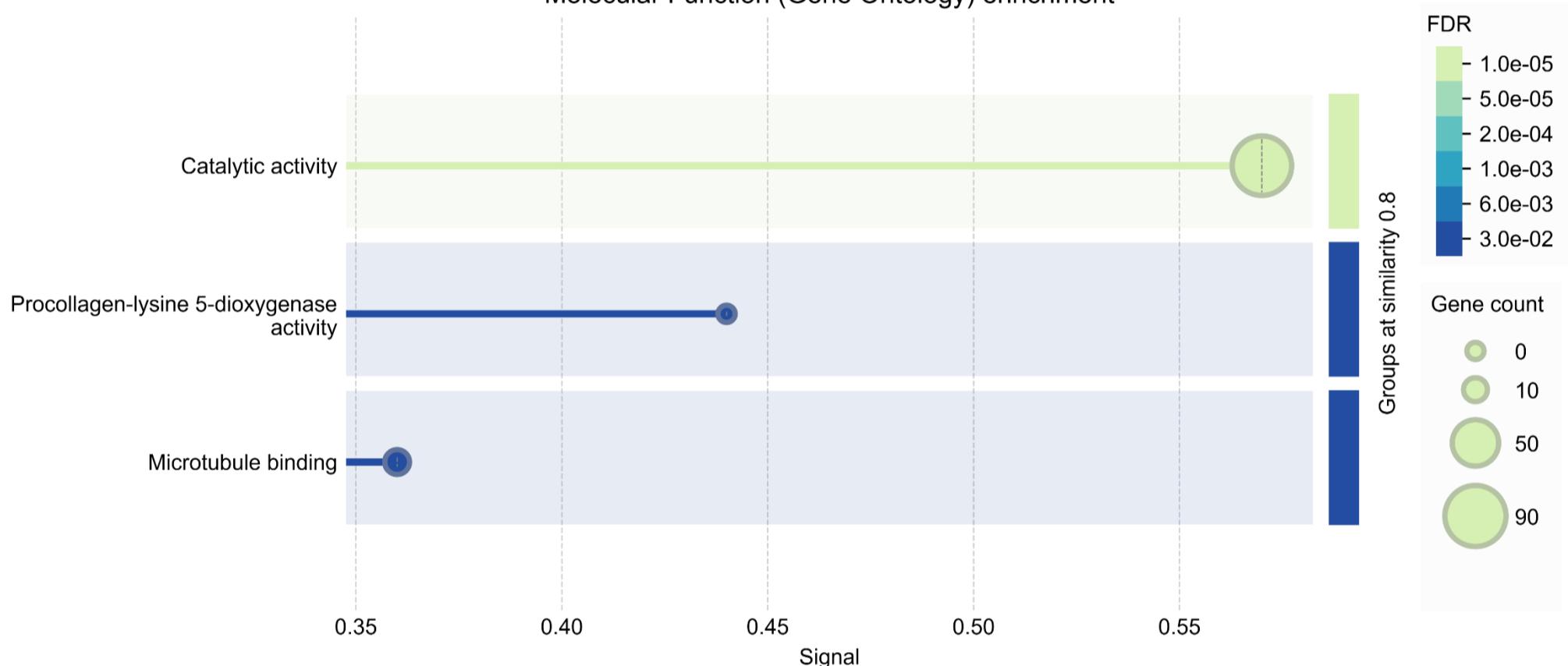
Annotated Keywords (UniProt) enrichment



KEGG Pathways enrichment



Molecular Function (Gene Ontology) enrichment



Cellular Component (Gene Ontology) enrichment

