

Whole cell response to receptor stimulation involves many deep and distributed subcellular processes

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³ current address: Thermo Fisher Scientific, 1400 North Point Parkway, Suite 10, West Palm Beach, FL 33407, United States.

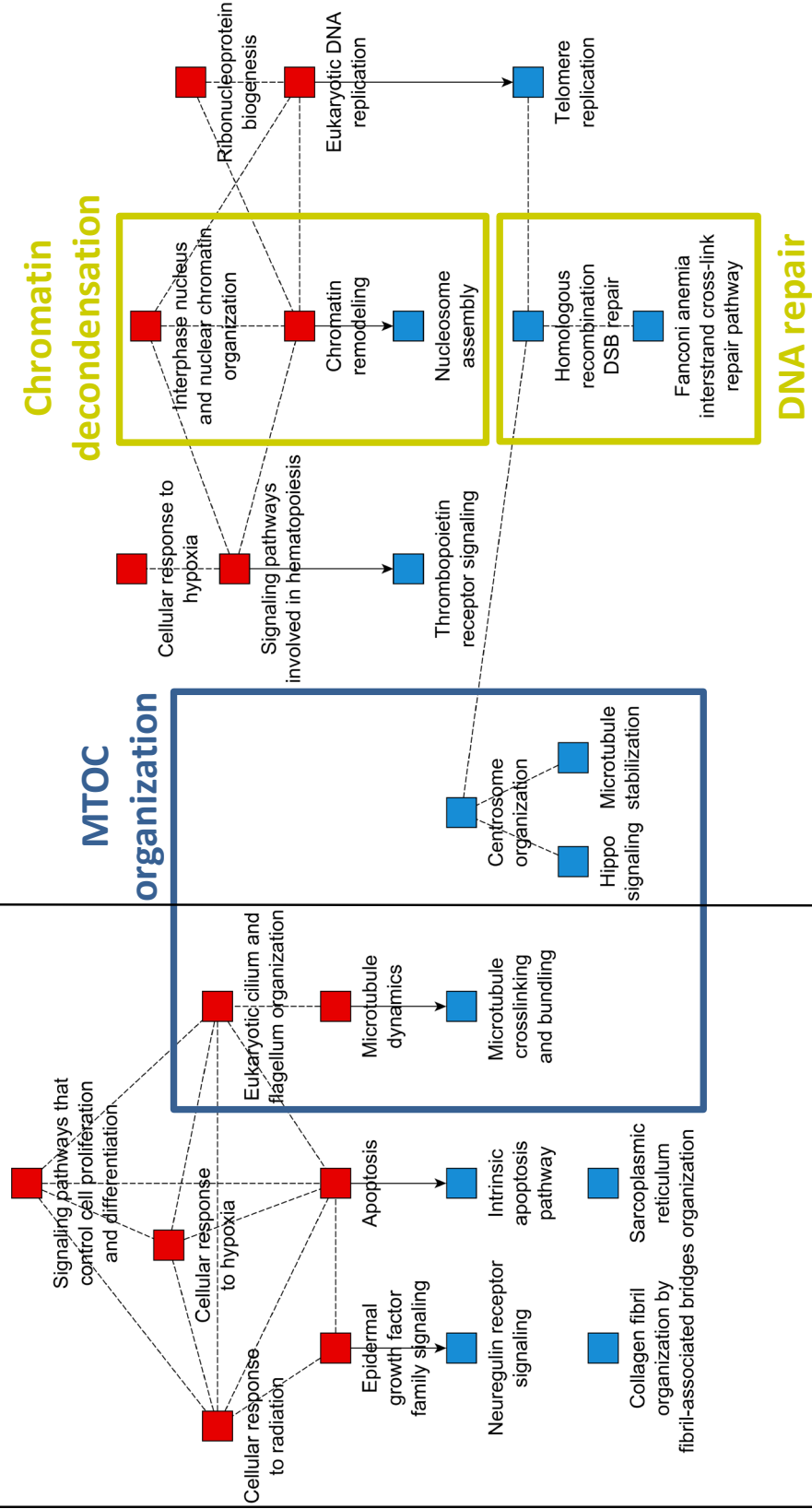
*To whom correspondence should be addressed: Ravi Iyengar, Department of Pharmacological Sciences, 1425 Madison, Rm 12-70, Icahn School of Medicine at Mount Sinai, New York NY 10029, United States, Voice: 212-659-1707, E-mail: ravi.iyengar@mssm.edu

Suppl. figure 1A

2h HU210: Dynamic enrichment results of DEGs

SCP-networks predicted based on up-regulated genes

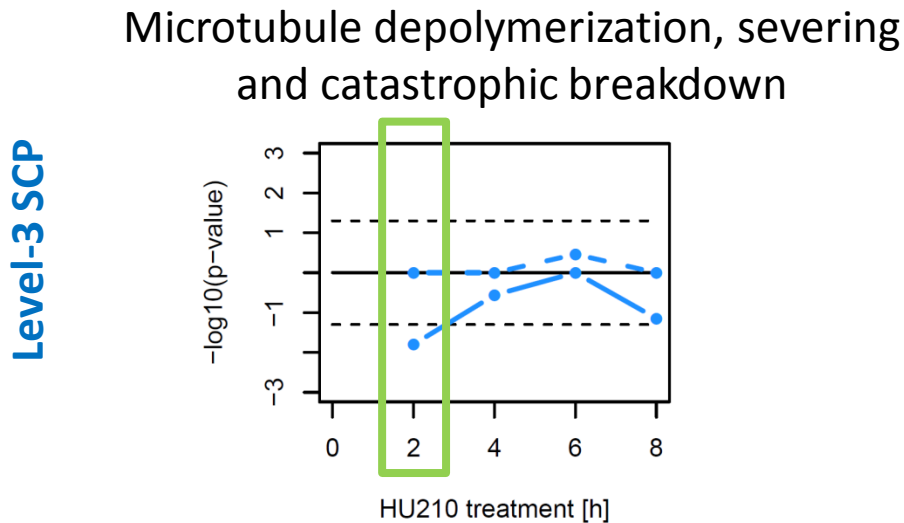
SCP-networks predicted based on down-regulated genes



Supplementary figure 1A: Dynamic enrichment analysis of DEGS at 2h. Up- and down-regulated genes after 2h HU210 (minimum $\log_2(\text{fold change})$ of $\pm \log_2(1.5)$) were submitted to dynamic enrichment analysis using the Molecular Biology of the Cell Ontology (MBCO). Dynamic enrichment analysis considers dependencies between SCPs based on computationally predicted horizontal SCP relationships that lie within and beyond the annotated hierarchy to predict SCP-networks that describe whole cell function. Briefly, the algorithm searches for all level-2 or level-3 SCPs that contain at least one input gene and combines them to SCP-unions, if they are strongly connected. The unions are added as function-specific SCPs to the original set of SCPs and the new function-specific MBCO is used for enrichment analysis of the input genes using Fisher's Exact Test. Top three level-2 and top five level-3 predicted SCP-unions or SCPs were used to generate indicated networks. Red rectangles: level-2 SCPs, blue rectangles: level-3 SCPs. Dashed lines: horizontal SCP interactions, arrows: hierarchical annotated interactions between level-2 parent SCPs and level-3 child SCPs. MTOC: Microtubule Organization Center.

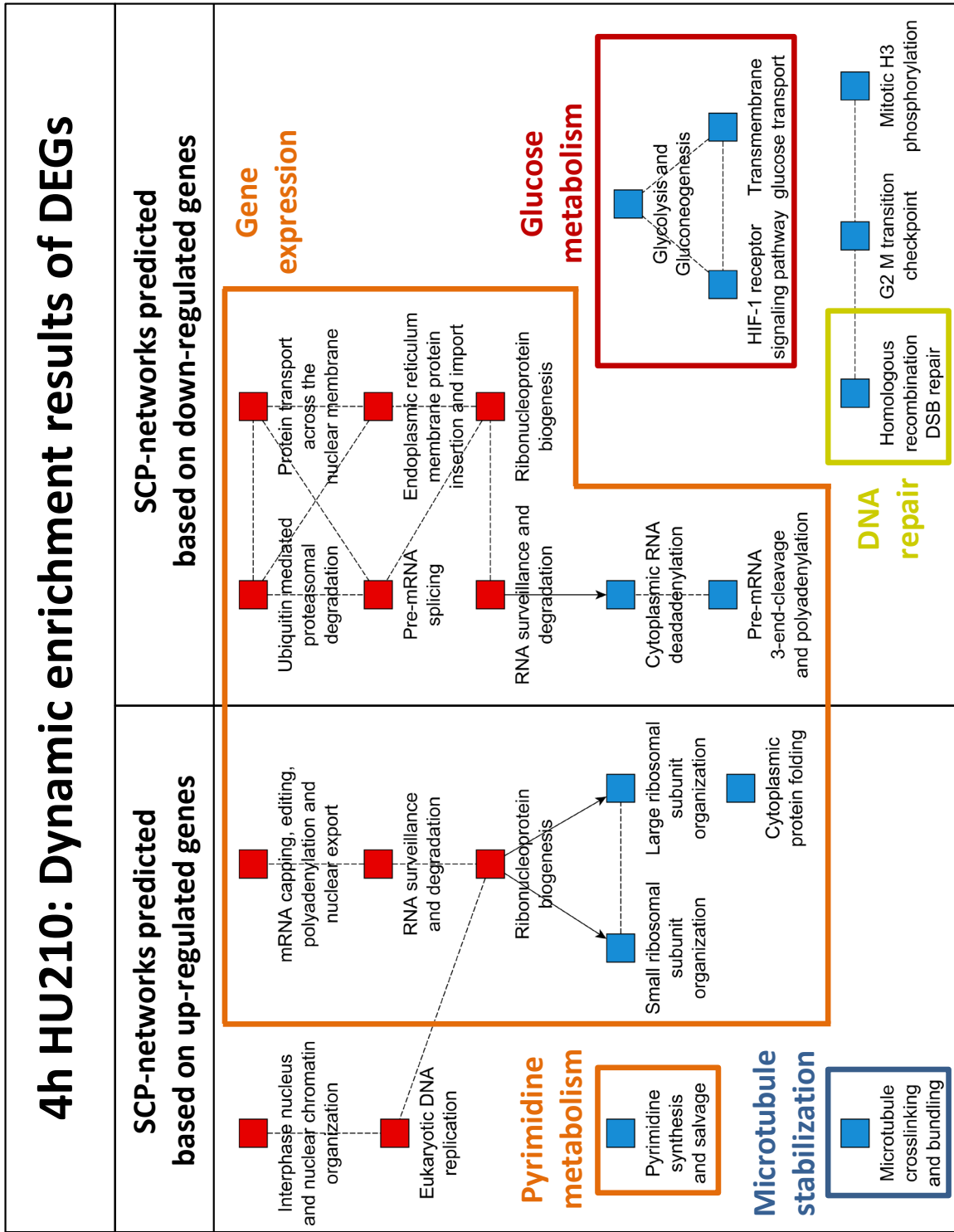
Suppl. figure 1B

2h HU210: Standard enrichment results of DEGs



Supplementary figure 1B: Selected SCP that was predicted by standard enrichment analysis of DEGs at 2h. Up- and down-regulated genes of each timepoint (2h, 4h, 6h, 8h) with a minimum $\log_2(\text{fold change})$ of $\pm \log_2(1.3)$ were submitted to standard (regular) enrichment analysis using the Molecular Biology of the Cell Ontology (MBCO) and Fisher's Exact test. Significance was defined by a maximum nominal p-value of 5%. Minus $\log_{10}(\text{p-values})$ of the indicated SCP were plotted above 0, if they are based on enrichment results of up-regulated genes, and below 0, if they are based on enrichment results of down-regulated genes. If up- or down-regulated genes of at least one timepoint significantly enrich for that particular SCP, the minus $\log_{10}(\text{p-values})$ are connected by a solid line and by a dashed line otherwise. Horizontal black solid line indicates minus $\log_{10}(\text{p-value})$ of 0 (p-value=1), black dashed line indicates nominal significance threshold (minus $\log_{10}(0.05)$). Green rectangle shows the minus $\log_{10}(\text{p-values})$ of the current timepoint. See supplementary figure 2 for all predicted SCPs.

Suppl. figure 1C

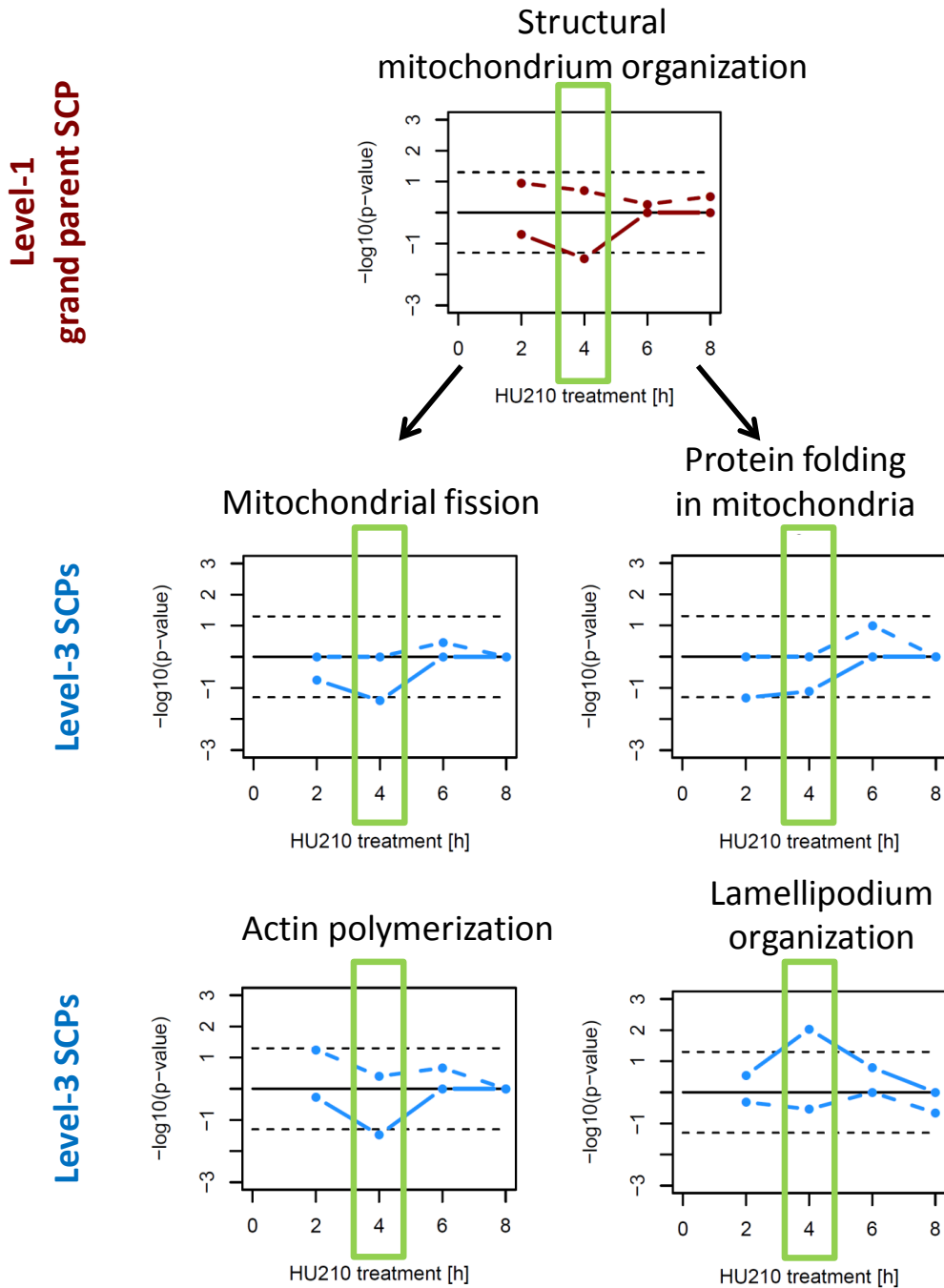


Supplementary figure 1C: Dynamic enrichment analysis of DEGs at 4h.

Suppl. figure 1D

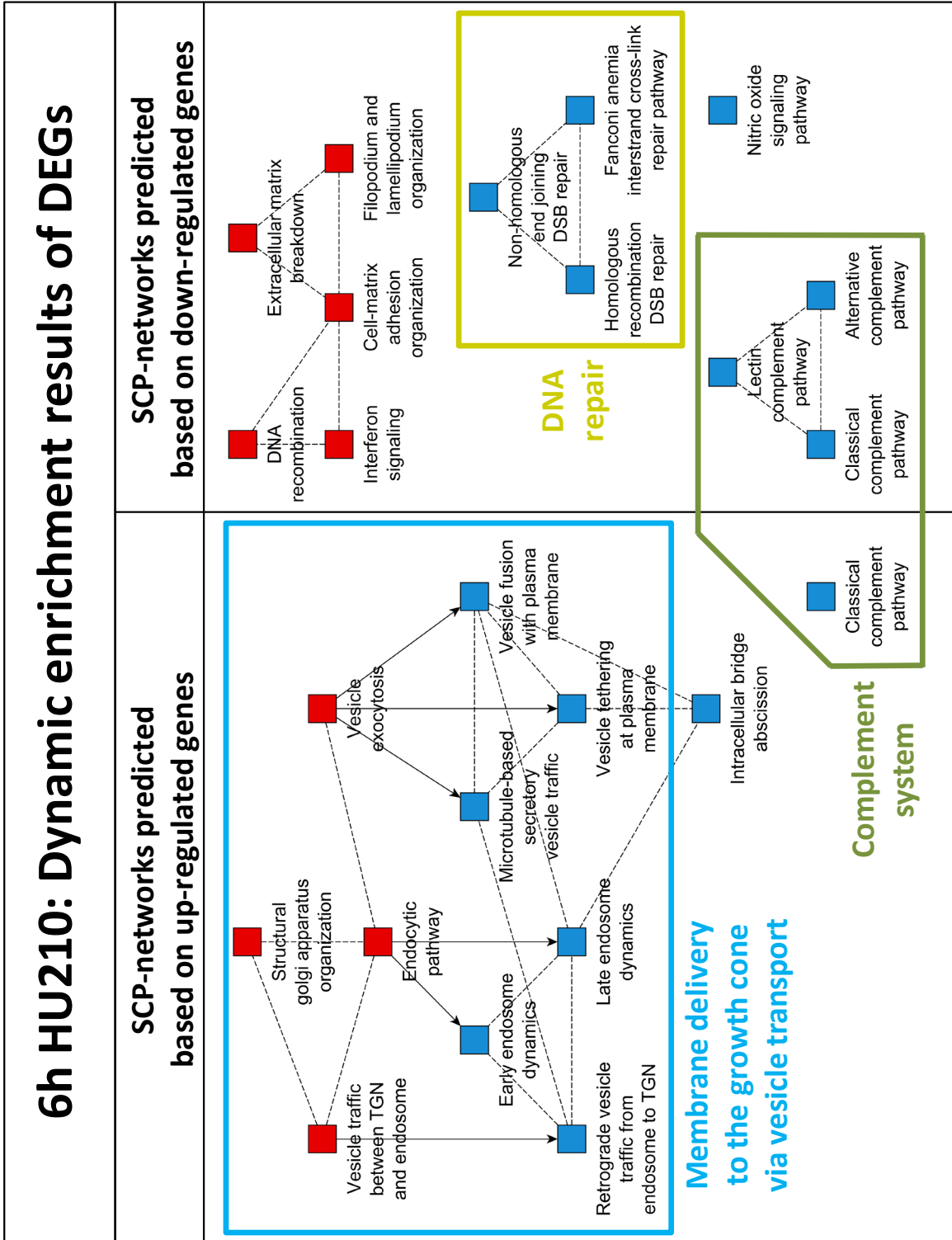
4h HU210:

Standard enrichment results of DEGs



Supplementary figure 1D: Selected SCPs that were predicted by standard enrichment analysis of DEGs at 4h.

Suppl. figure 1E

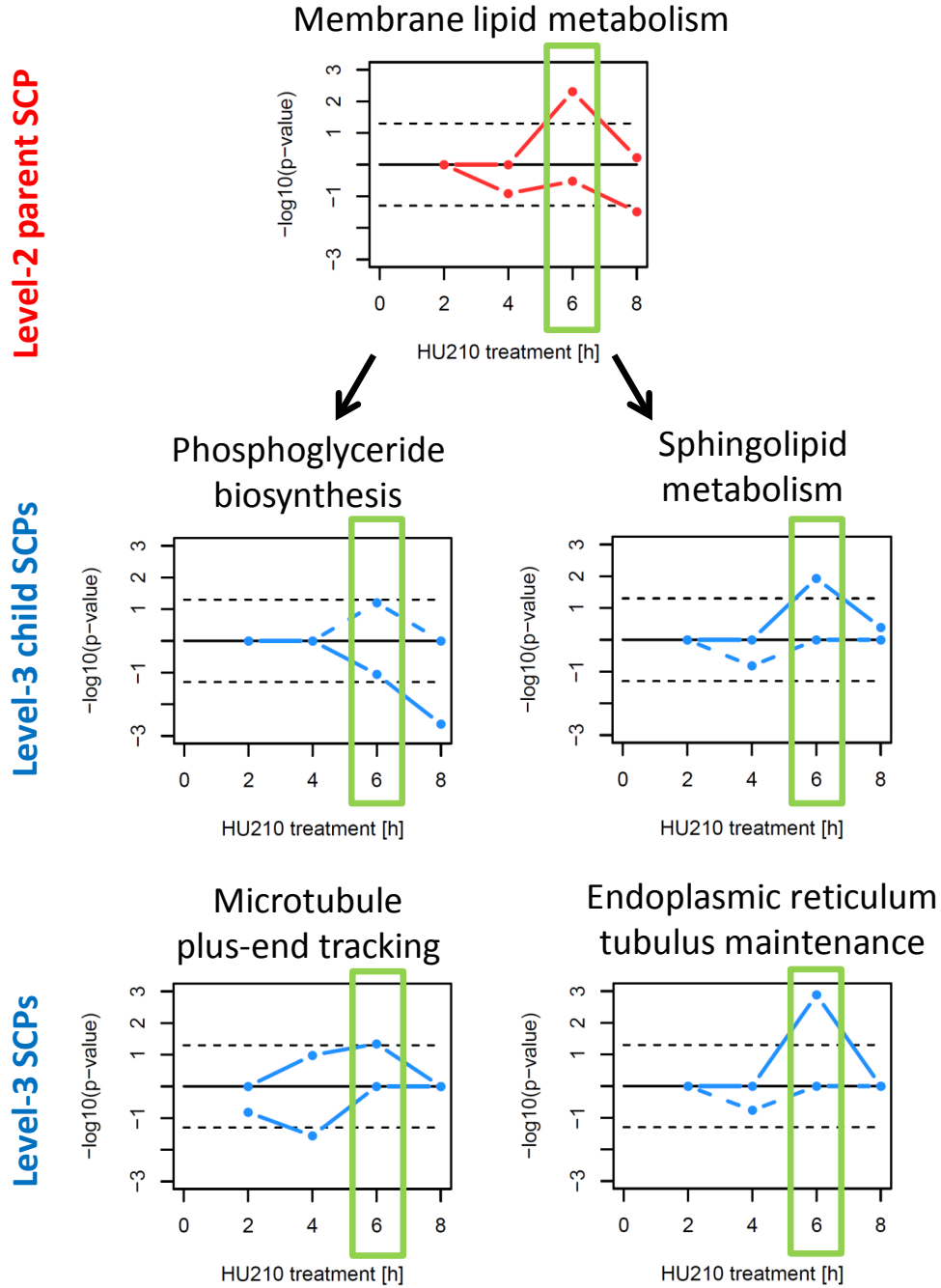


Supplementary figure 1E: Dynamic enrichment analysis of DEGs at 6h.

Suppl. figure 1F

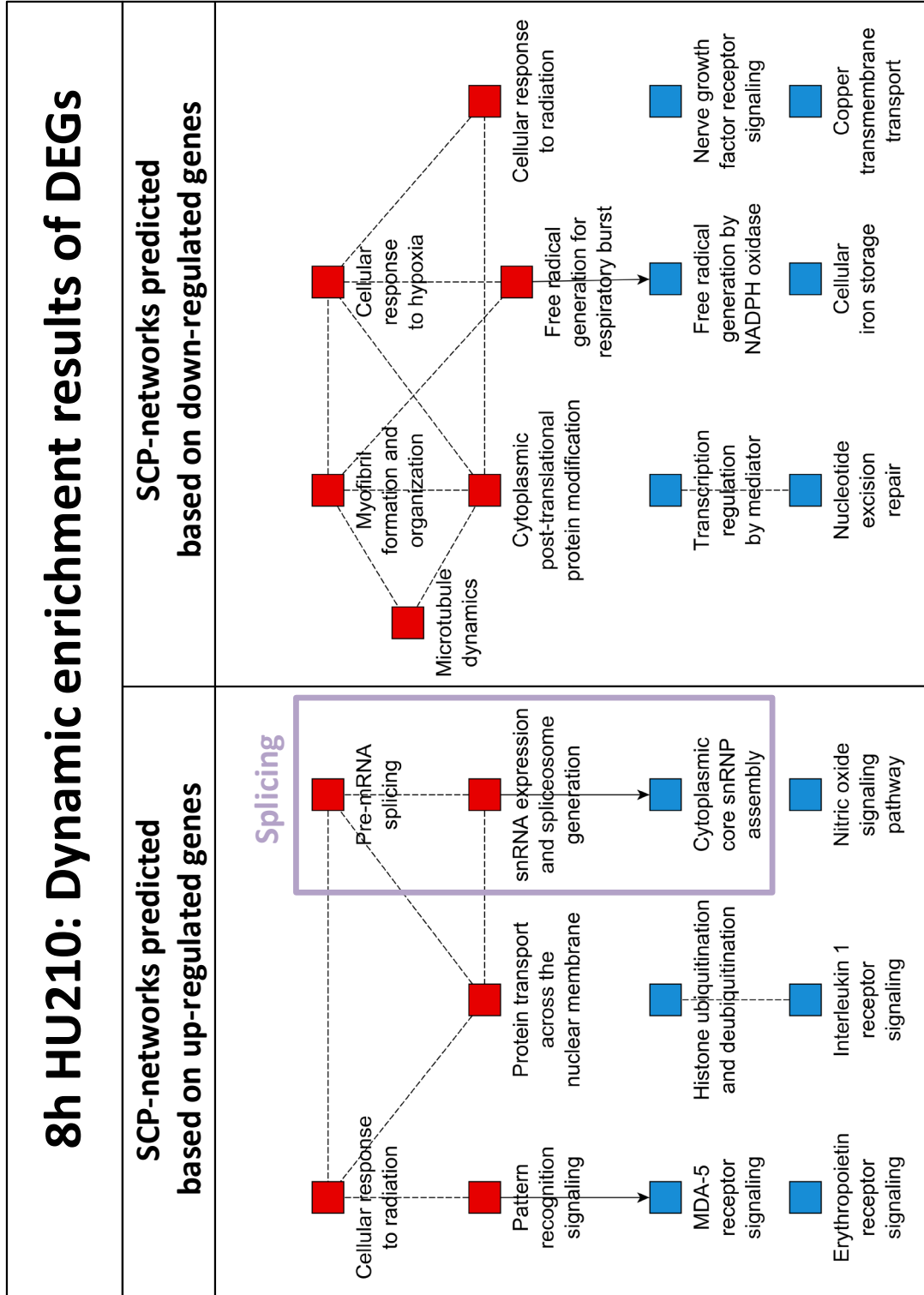
6h HU210:

Standard enrichment results of DEGs



Supplementary figure 1F: Selected SCPs that were predicted by standard enrichment analysis of DEGs at 6h.

Suppl. figure 1G

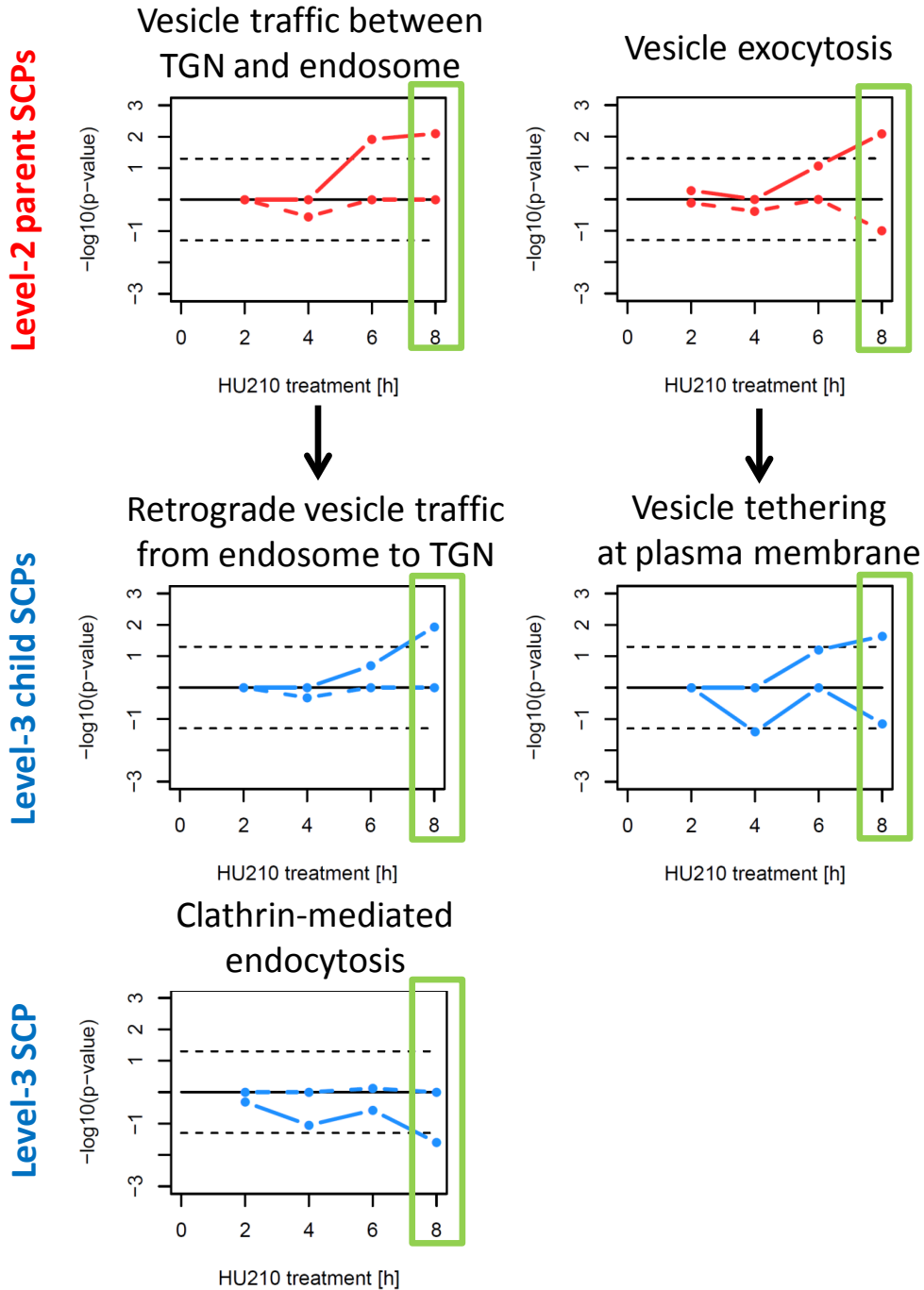


Supplementary figure 1G: Dynamic enrichment analysis of DEGs at 8h.

Suppl. figure 1H

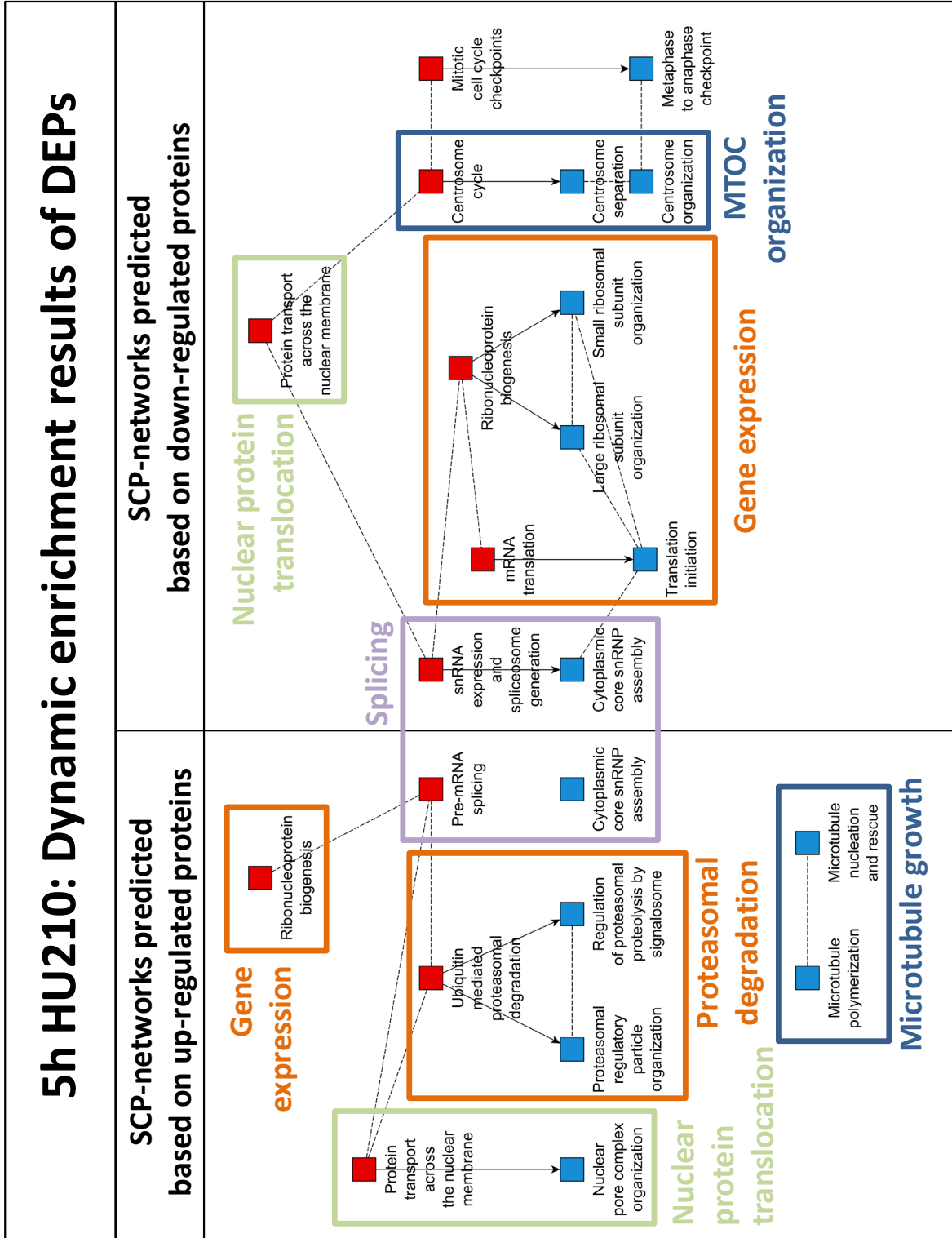
8h HU210:

Standard enrichment results of DEGs



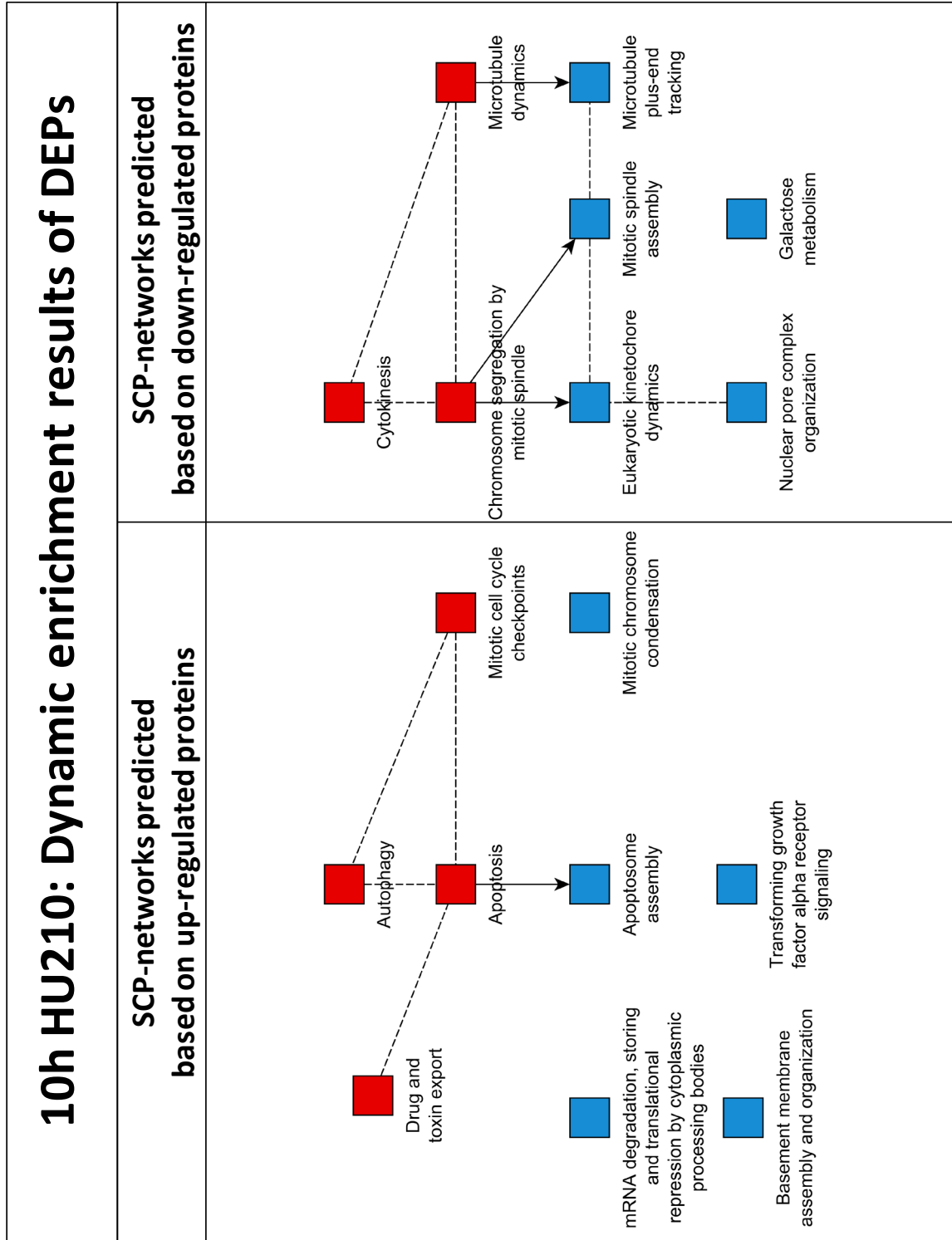
Supplementary figure 1H: Selected SCPs that were predicted by standard enrichment analysis of DEGs at 8h.

Suppl. figure 1I



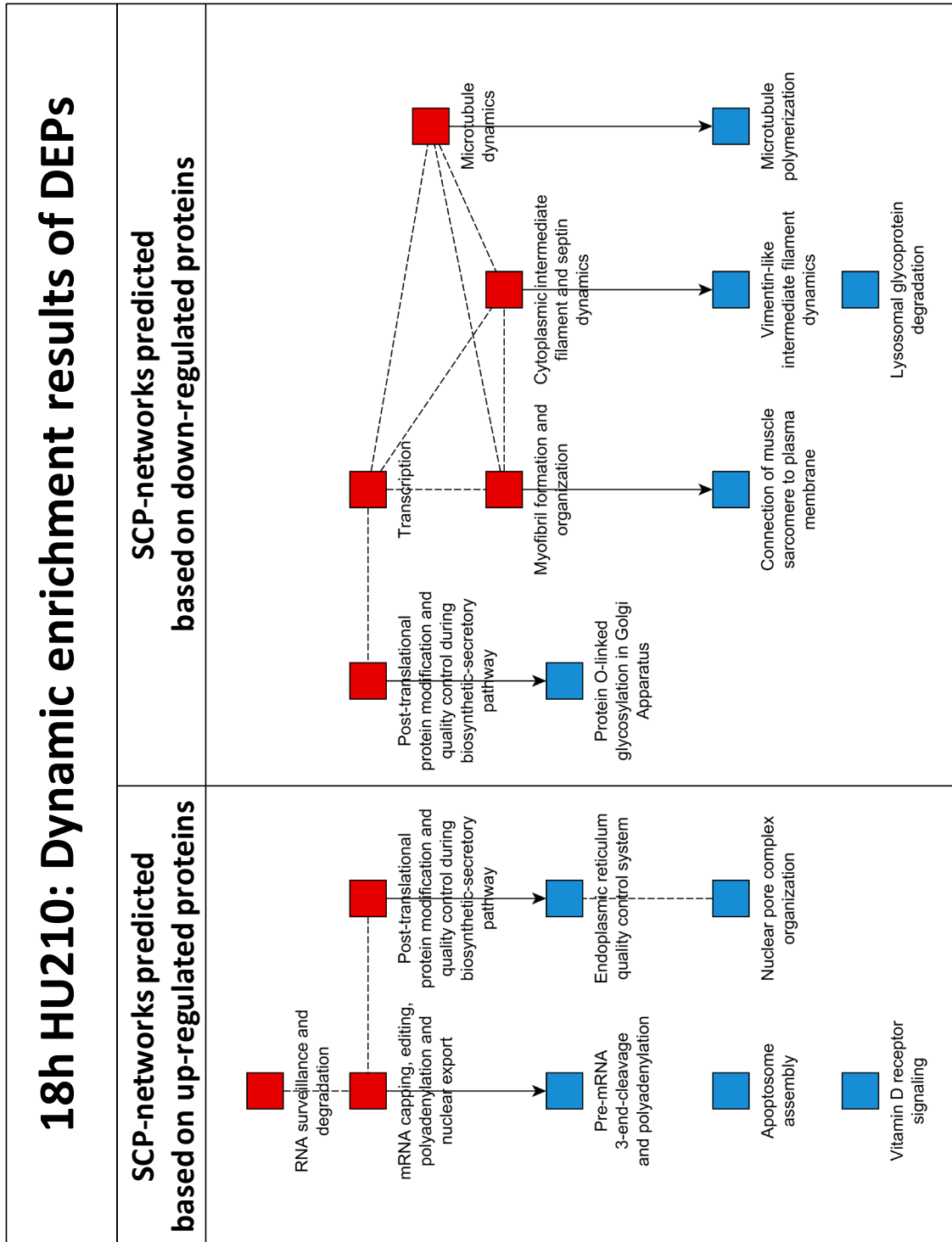
Suppl. figure 1I: Dynamic enrichment analysis of differentially expressed proteins (DEPs) at 5h.

Suppl. figure 1J



Suppl. figure 1J: Dynamic enrichment analysis of differentially expressed proteins (DEPs) at 10h.

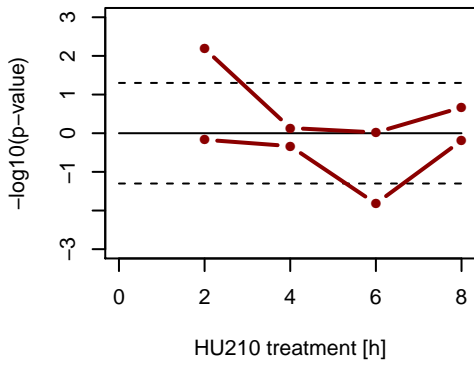
Suppl. figure 1K



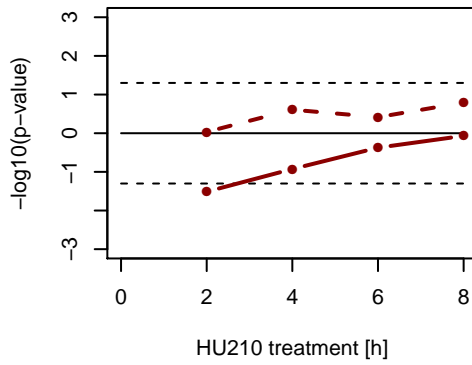
Suppl. figure 1K: Dynamic enrichment analysis of differentially expressed proteins (DEPs) at 18h.

Suppl. figure 2

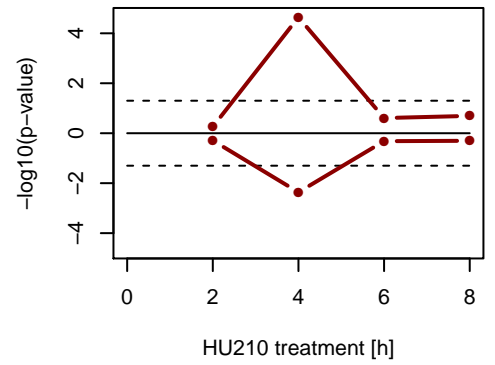
**Molecular Biology of the Cell:
Cellular communication**



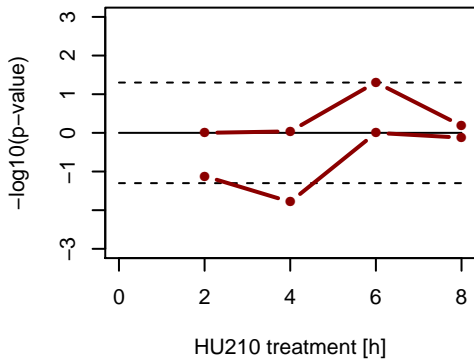
**Molecular Biology of the Cell:
Chromatin and histone dynamics**



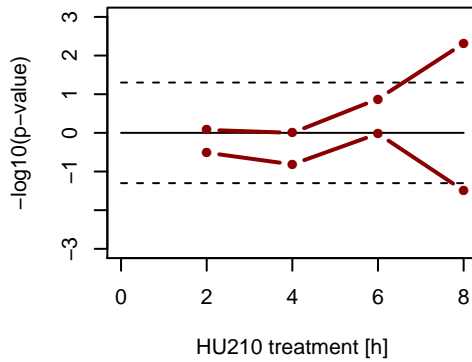
**Molecular Biology of the Cell:
Gene expression**



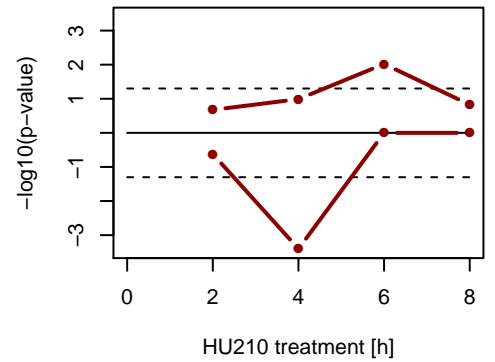
**Molecular Biology of the Cell:
Intracellular degradation pathways**



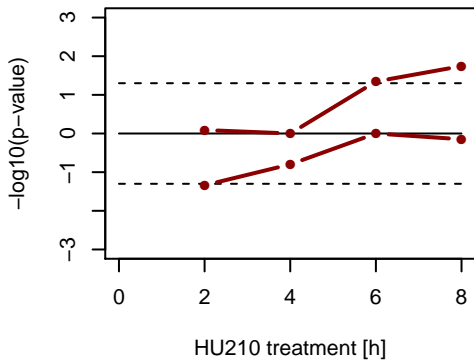
**Molecular Biology of the Cell:
Intracellular vesicle traffic**



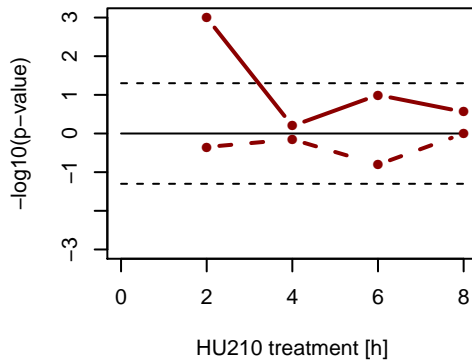
**Molecular Biology of the Cell:
Organelle organization**



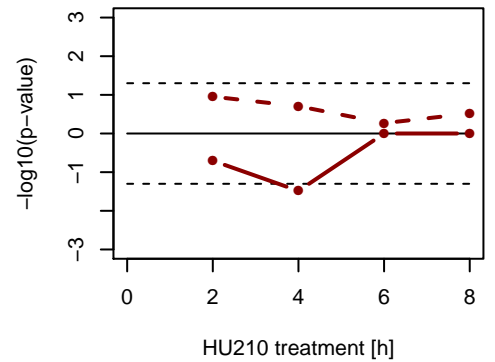
**Molecular Biology of the Cell:
Posttranslational protein modification**



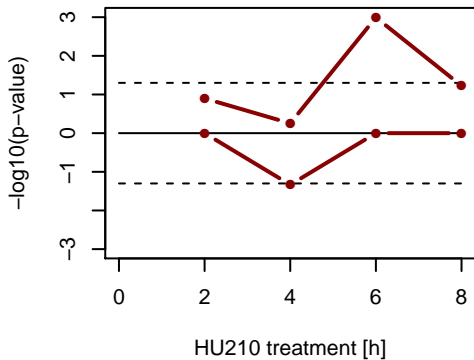
**Molecular Biology of the Cell:
Regulated cell death**



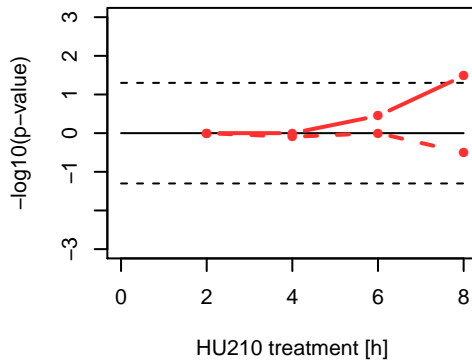
**Molecular Biology of the Cell:
Structural mitochondrion organization**



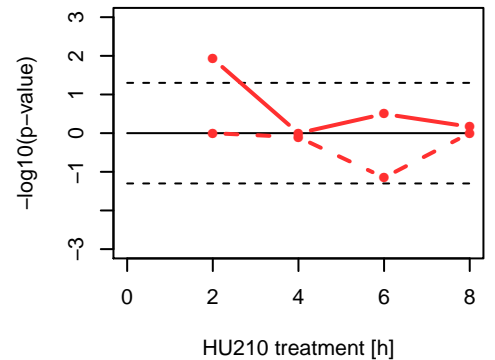
**Molecular Biology of the Cell:
Transmembrane protein import and
translocation**



**Cell cycle and cell division:
Cytokinesis**

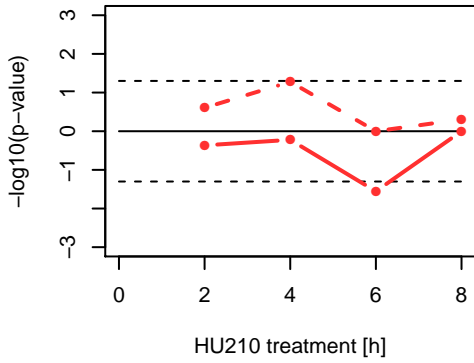


**Cellular communication:
Epidermal growth factor family signaling**

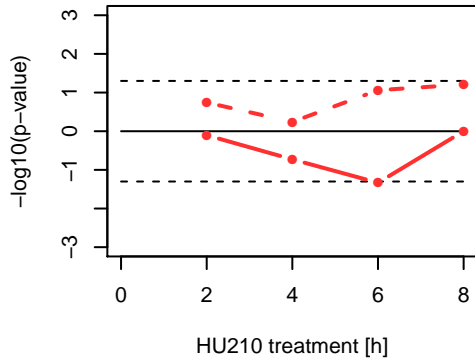


Suppl. figure 2

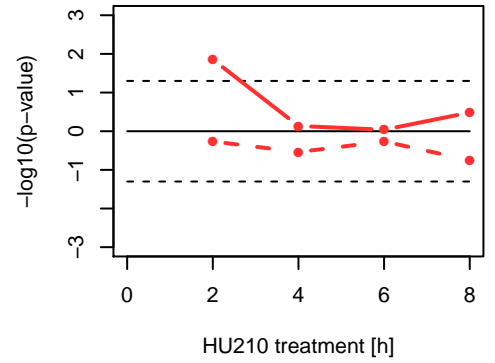
**Cellular communication:
Interferon signaling**



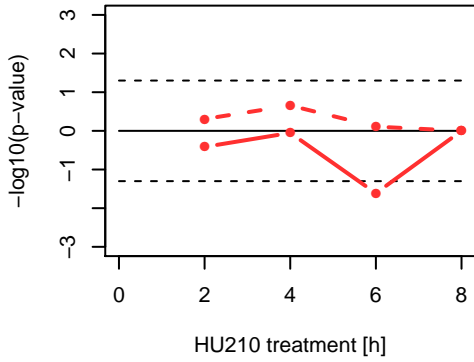
**Cellular communication:
Intracellular common signaling cascades of
multiple pathways**



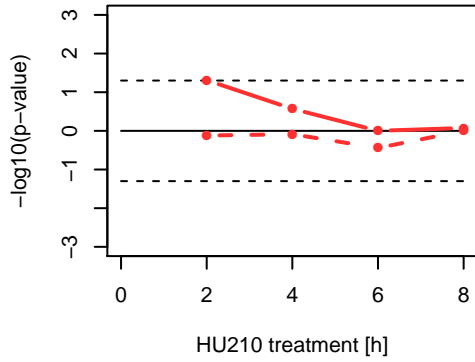
**Cellular communication:
Signaling pathways that control cell
proliferation and differentiation**



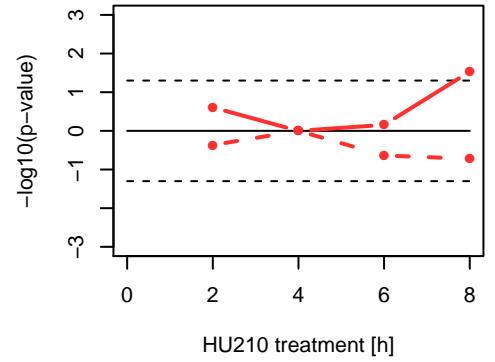
**Cellular communication:
TGF-beta superfamily signaling**



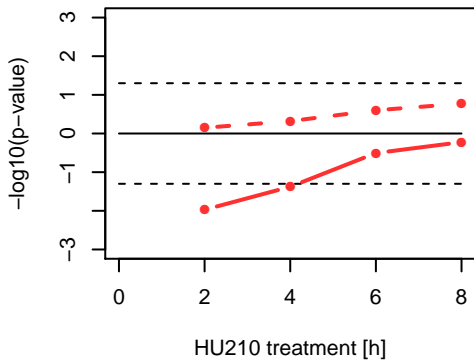
**Cellular protrusion organization:
Eukaryotic cilium and flagellum organization**



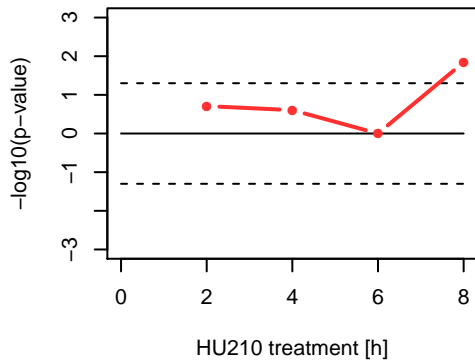
**Cellular response to stress:
Cellular response to radiation**



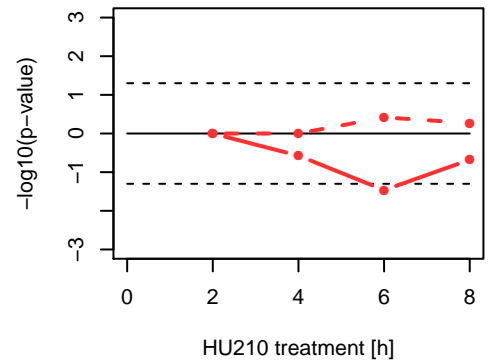
**Chromatin and histone dynamics:
Chromatin remodeling**



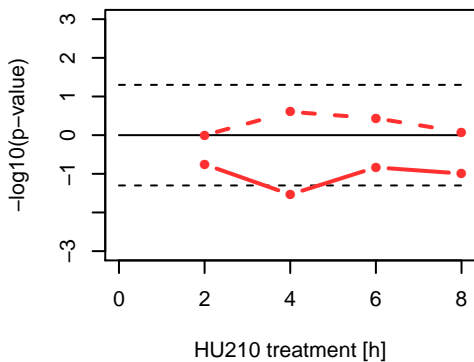
**Coagulation cascade, complement system and
blood protein dynamics:
Coagulation cascade and its regulation**



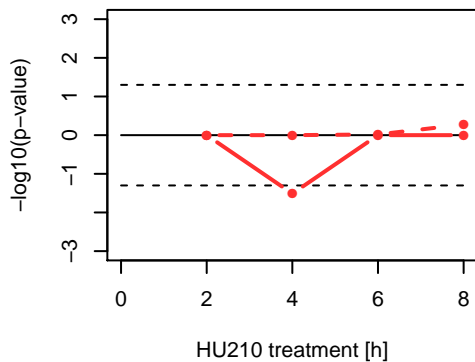
**Coagulation cascade, complement system and
blood protein dynamics:
Complement pathway and regulation**



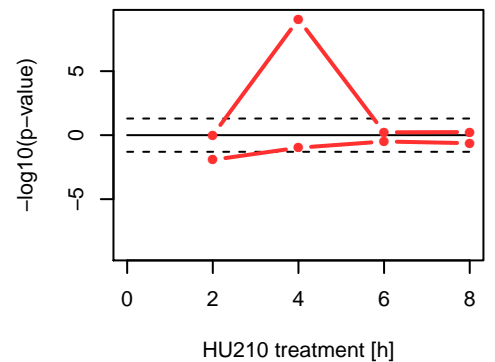
**DNA replication, recombination and repair:
DNA double-strand break repair**



**Gene expression:
Pre-mRNA splicing**

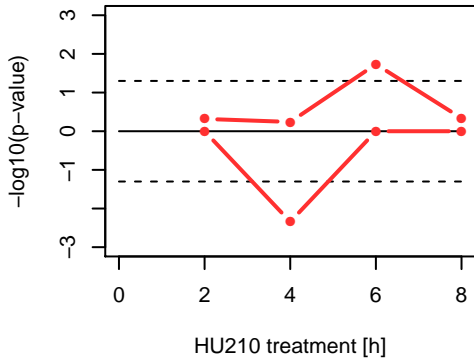


**Gene expression:
Ribonucleoprotein biogenesis**

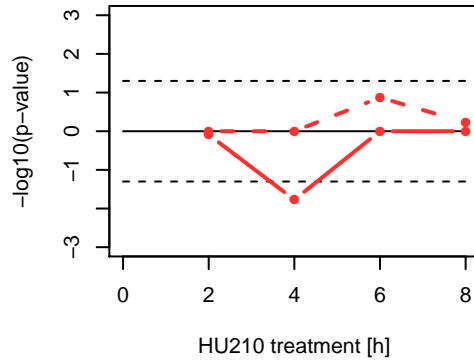


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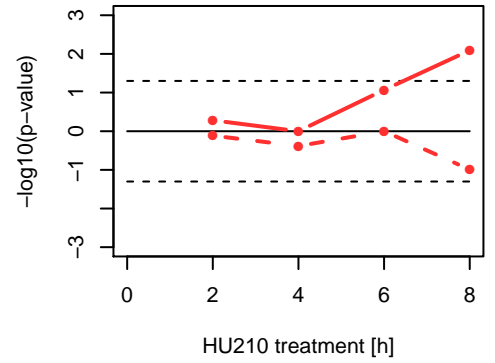
**Gene expression:
RNA surveillance and degradation**



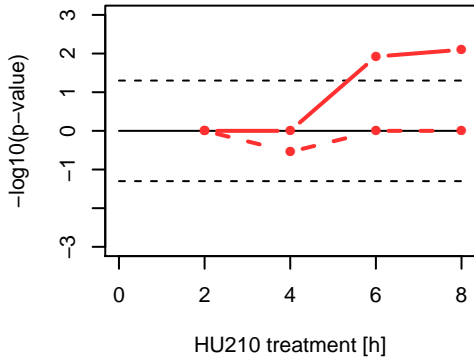
**Intracellular degradation pathways:
Ubiquitin mediated proteasomal degradation**



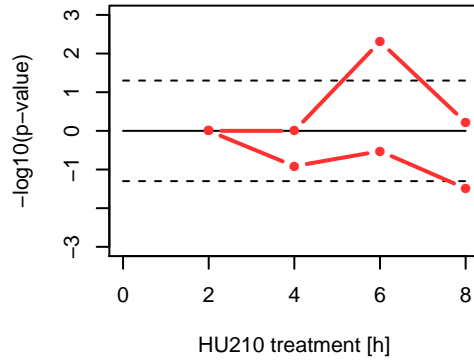
**Intracellular vesicle traffic:
Vesicle exocytosis**



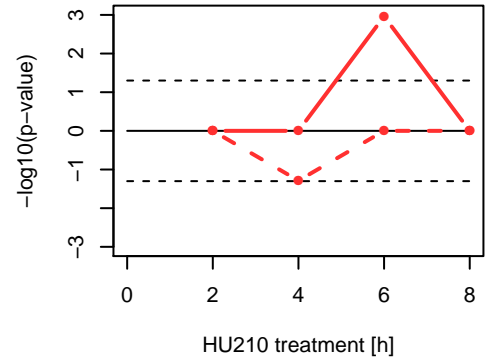
**Intracellular vesicle traffic:
Vesicle traffic between TGN and endosome**



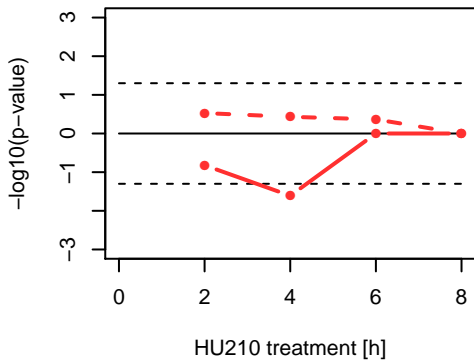
**Lipid metabolism:
Membrane lipid metabolism**



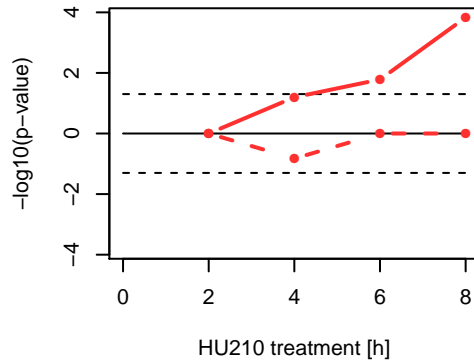
**Organelle organization:
Endoplasmic reticulum and nuclear envelope
organization**



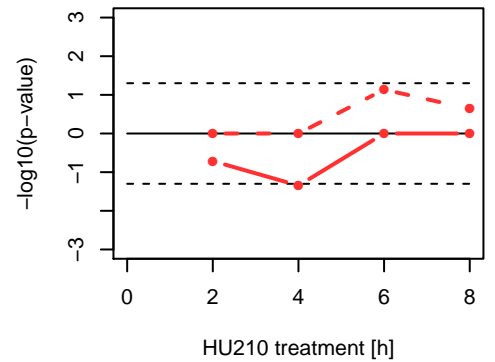
**Organelle organization:
Interphase nucleus and nuclear chromatin
organization**



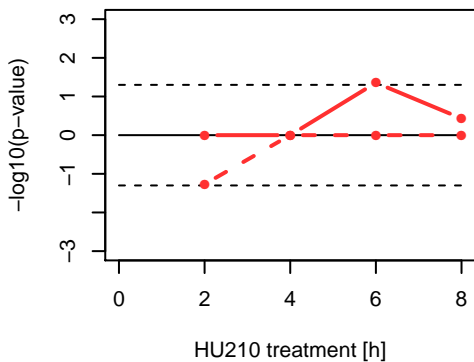
**Organelle organization:
Structural golgi apparatus organization**



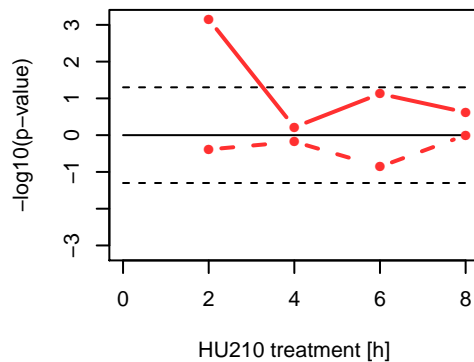
**Posttranslational protein modification:
Post-translational protein modification in
Mitochondria**



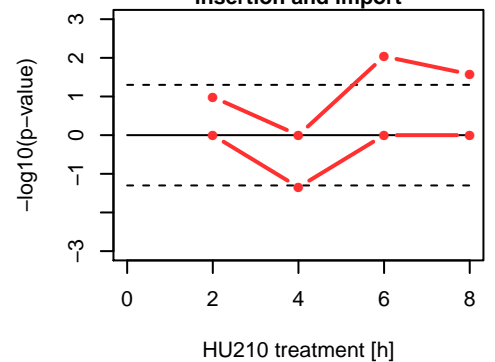
**Posttranslational protein modification:
Protein prenylation**



**Regulated cell death:
Apoptosis**

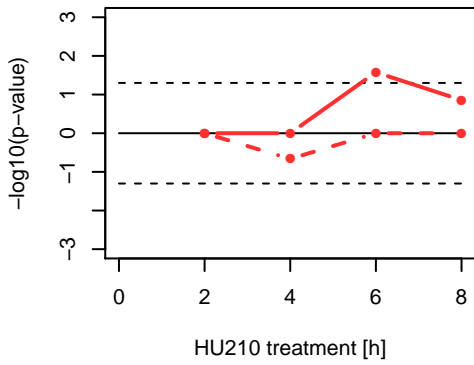


**Transmembrane protein import and
translocation:
Endoplasmic reticulum membrane protein
insertion and import**

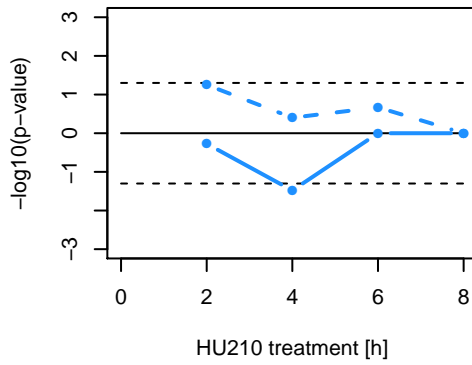


Suppl. figure 2

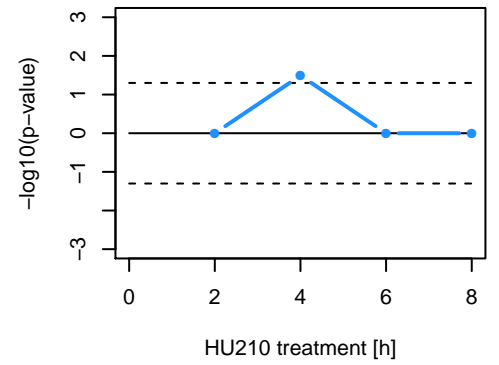
**Transmembrane protein import and translocation:
Protein transport across the nuclear membrane**



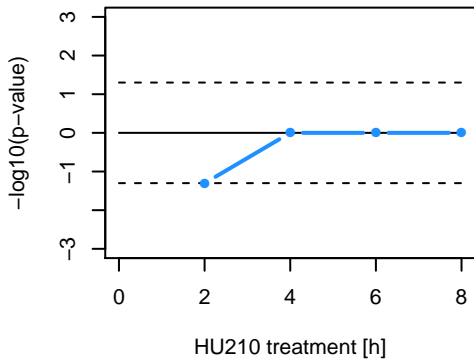
**Actin filament dynamics:
Actin polymerization**



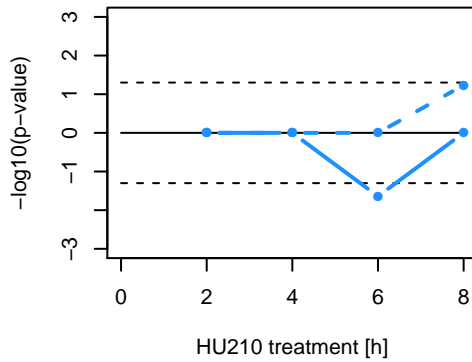
**Ammonium metabolism:
Amination of alpha-keto acids and deamination of amino acids**



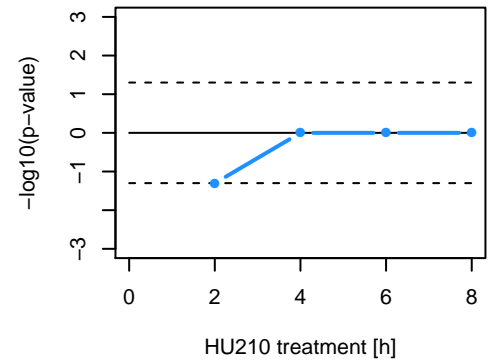
**Amyloid generation and aggregation:
Amyloid precursor protein cleavage**



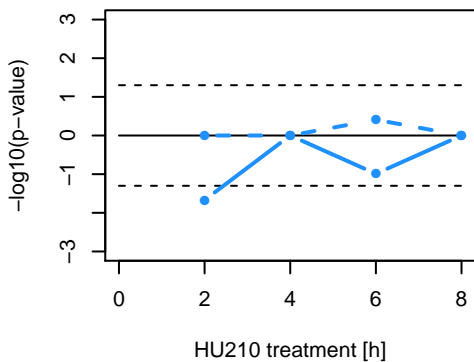
**Amyloid generation and aggregation:
Inhibition of amyloid aggregation, amyloid degradation and uptake**



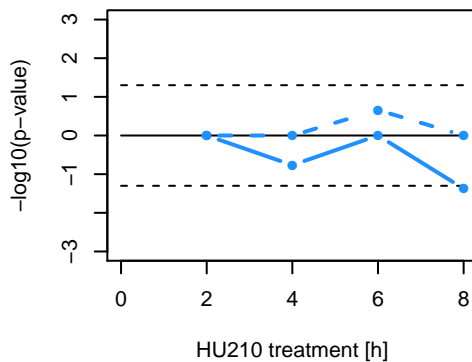
**Autophagy:
Macroautophagy**



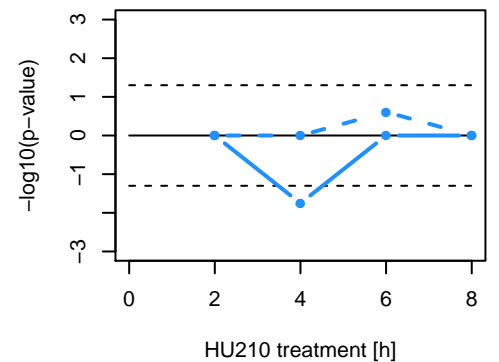
**Chromatin remodeling:
Nucleosome disassembly**



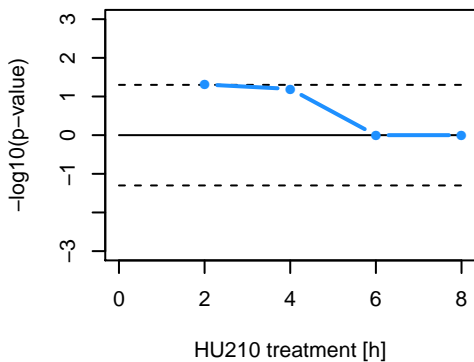
**Chromosome segregation by mitotic spindle:
Mitotic chromosome condensation**



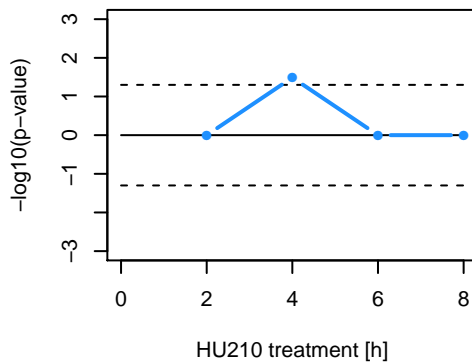
**Chromosome segregation by mitotic spindle:
Mitotic H3 phosphorylation**



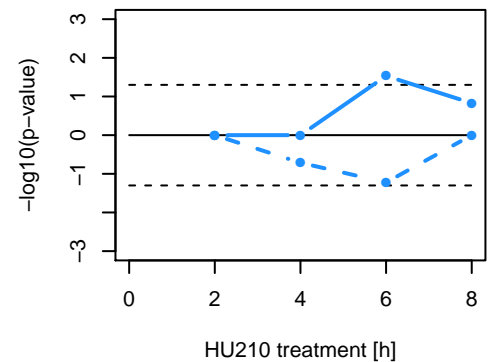
**Collagen biosynthesis:
Collagen fibril organization by fibril-associated bridges**



**Collagen biosynthesis:
Procollagen processing in the ER**

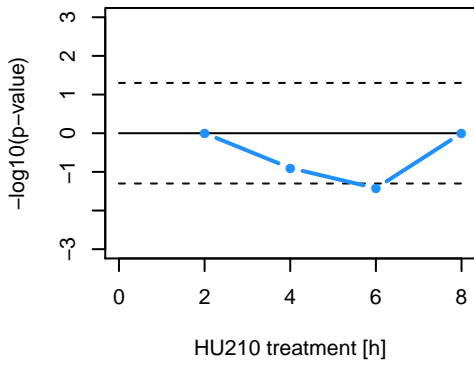


**Complement pathway and regulation:
Classical complement pathway**

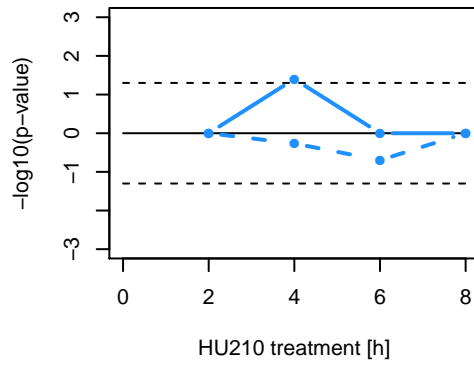


Suppl. figure 2

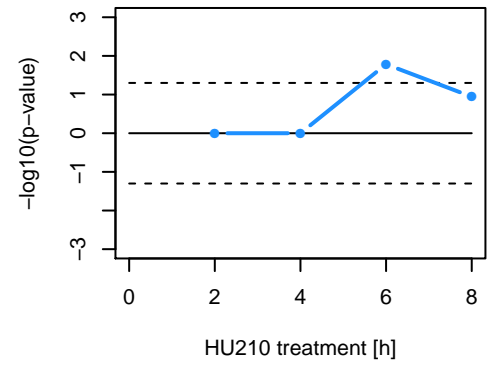
**Complement pathway and regulation:
Lectin complement pathway**



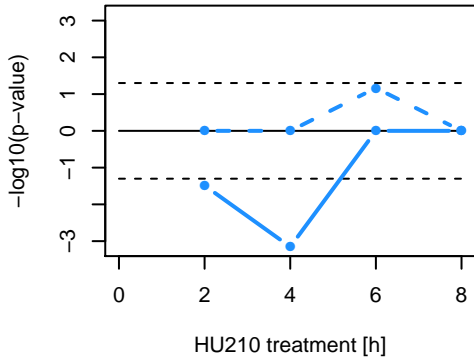
**Control of postsynaptic potential:
Glutamate-mediated control of postsynaptic potential**



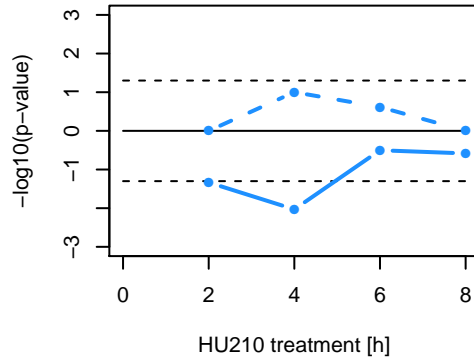
**Cytokinesis:
Equatorial RhoA activation**



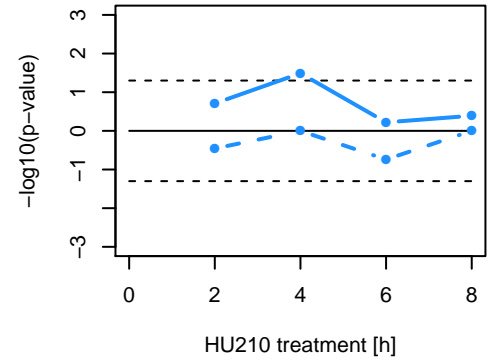
**Cytoplasmic post-translational protein modification:
Cytoplasmic protein folding**



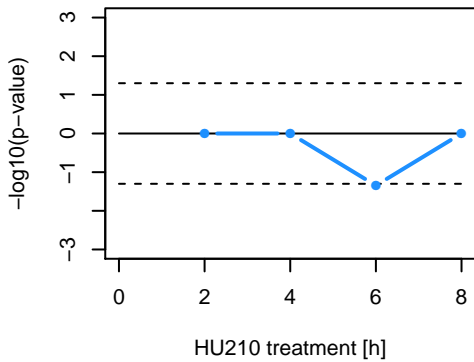
**DNA double-strand break repair:
Homologous recombination DSB repair**



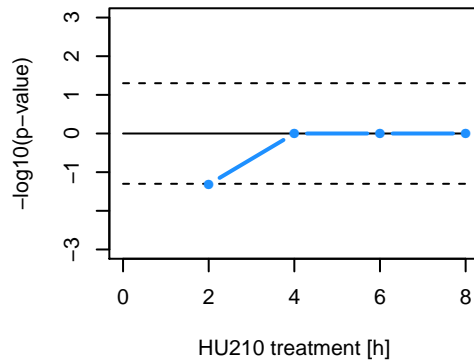
**DNA interstrand cross-links repair:
Fanconi anemia interstrand cross-link repair pathway**



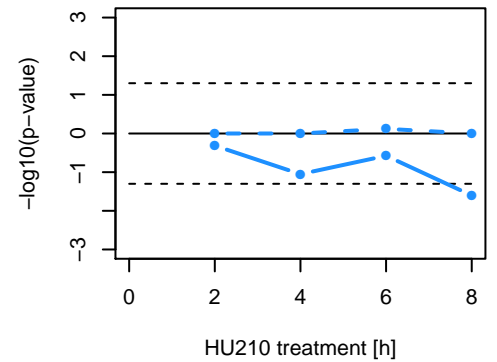
**DNA recombination:
VDJ recombination**



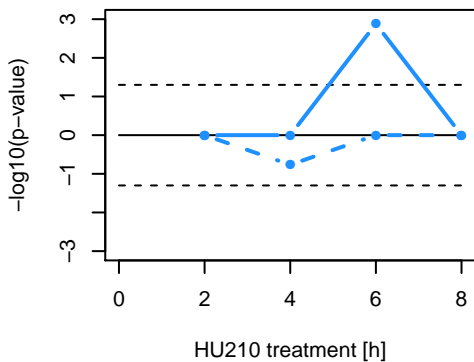
**Elastogenesis:
Elastin cross-linking and assembly**



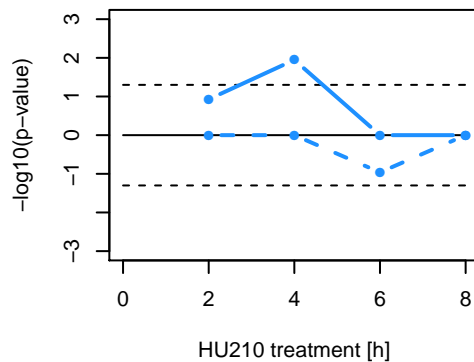
**Endocytic pathway:
Clathrin-mediated endocytosis**



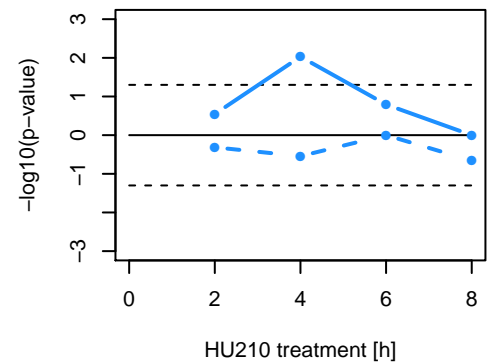
**Endoplasmic reticulum and nuclear envelope organization:
Endoplasmic reticulum tubulus maintenance**



**Eukaryotic DNA replication:
DNA cleavage**

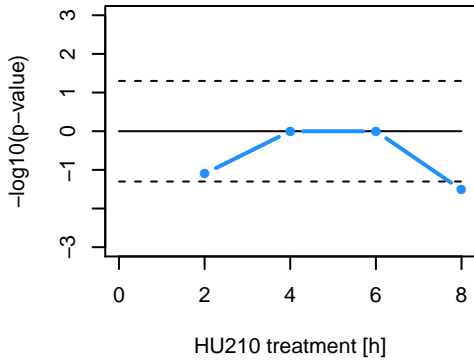


**Filopodium and lamellipodium organization:
Lamellipodium organization**

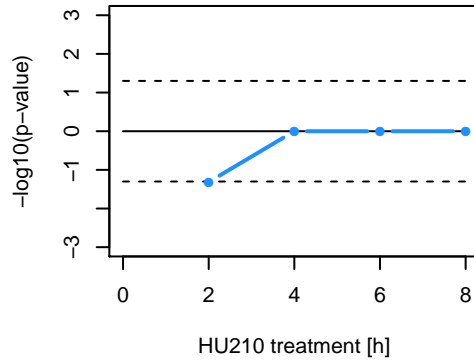


Suppl. figure 2

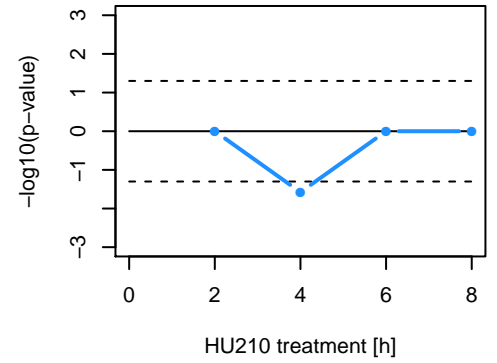
**Free radical generation for respiratory burst:
Free radical generation by NADPH oxidase**



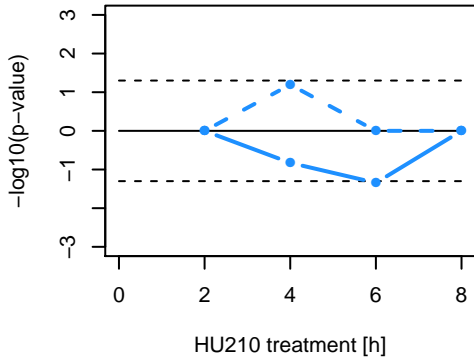
**Histone modification:
Histone phosphorylation and dephosphorylation**



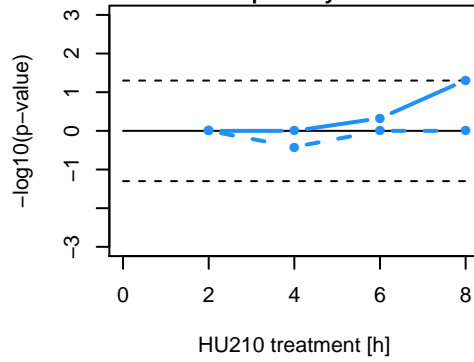
**Interleukin receptor signaling:
Interleukin 2 receptor signaling**



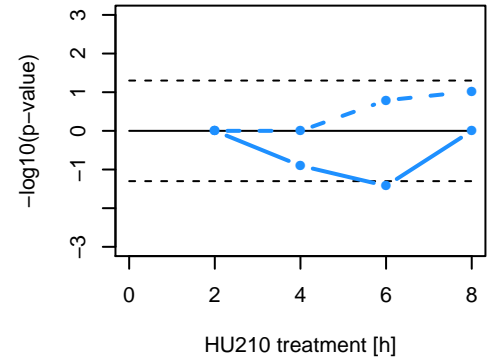
**Intracellular common signaling cascades of
multiple pathways:
CAM kinase signaling pathway**



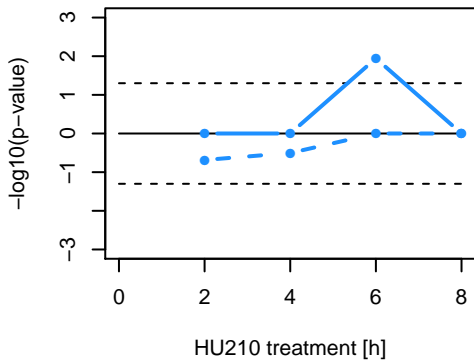
**Intracellular common signaling cascades of
multiple pathways:
Mammalian target of rapamycin signaling
pathway**



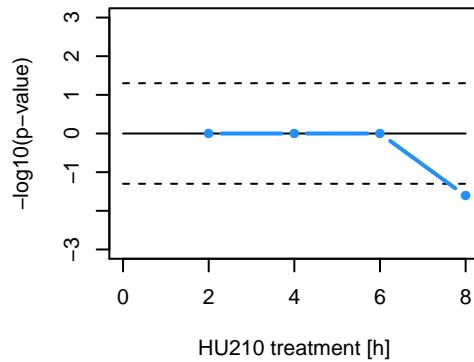
**Intracellular common signaling cascades of
multiple pathways:
Nitric oxide signaling pathway**



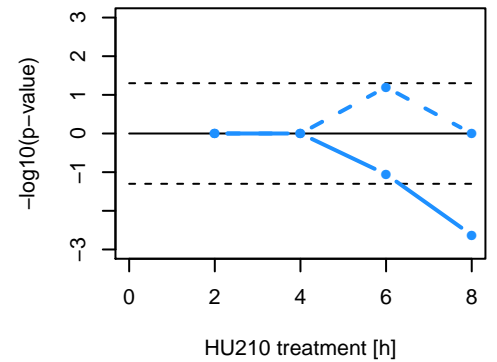
**Intracellular common signaling cascades of
multiple pathways:
PI3 kinase AKT signaling pathway**



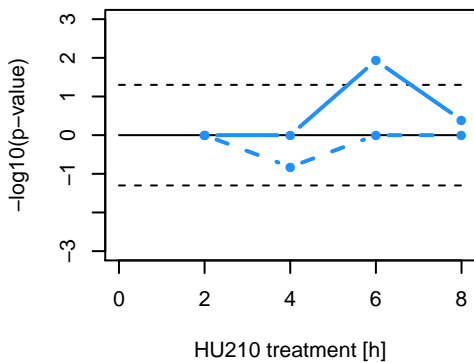
**Iron homeostasis:
Cellular iron storage**



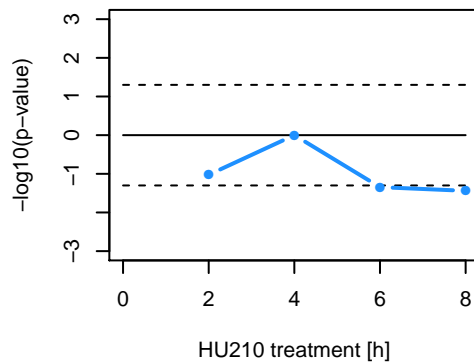
**Membrane lipid metabolism:
Phosphoglyceride biosynthesis**



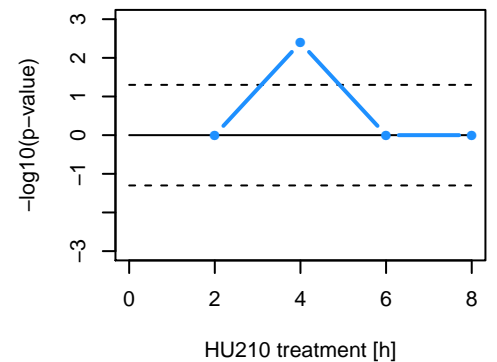
**Membrane lipid metabolism:
Sphingolipid metabolism**



**Metabolism of non-essential amino acids:
Proline metabolism**

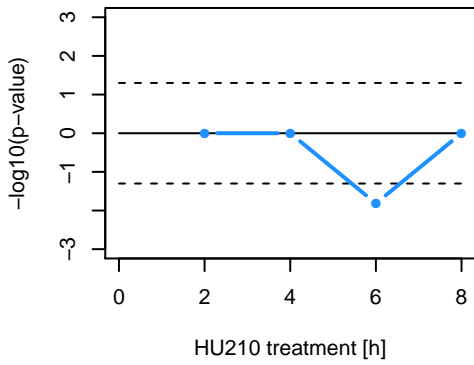


**Metabolism of purines and pyrimidines:
Pyrimidine synthesis and salvage**

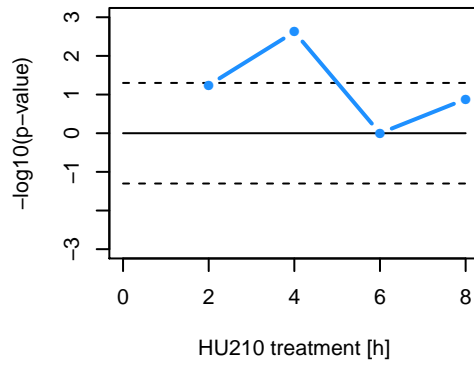


Suppl. figure 2

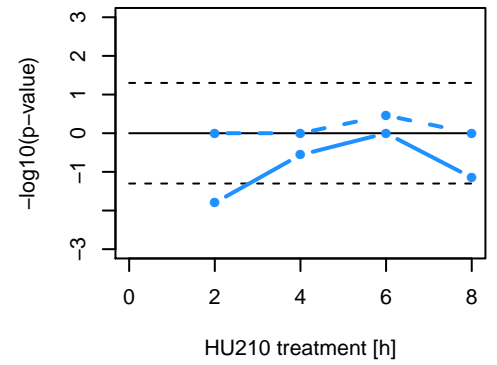
**Metabolism of water-soluble vitamins:
Vitamin C metabolism**



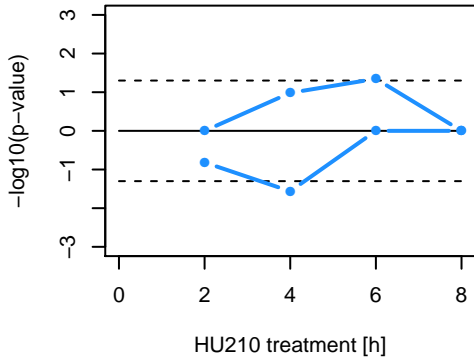
**Microtubule dynamics:
Microtubule crosslinking and bundling**



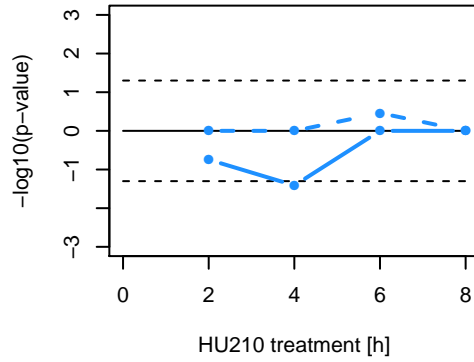
**Microtubule dynamics:
Microtubule depolymerization, severing and catastrophe**



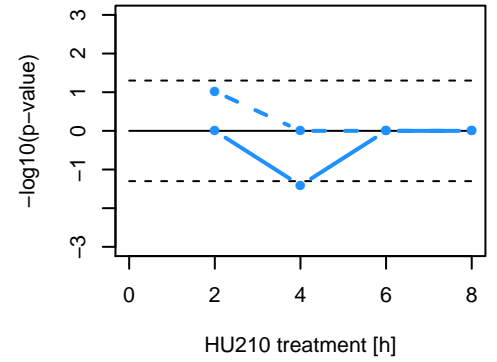
**Microtubule dynamics:
Microtubule plus-end tracking**



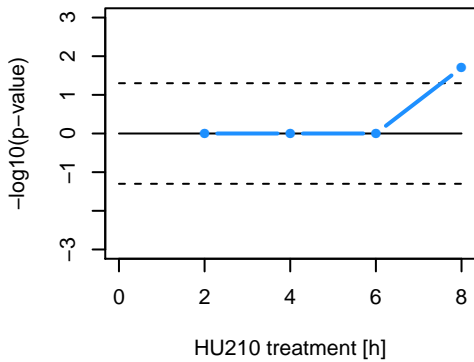
**Mitochondrial dynamics:
Mitochondrial fission**



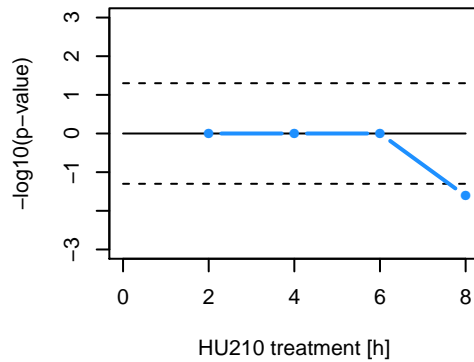
**Mitochondrial dynamics:
Mitochondrial fusion**



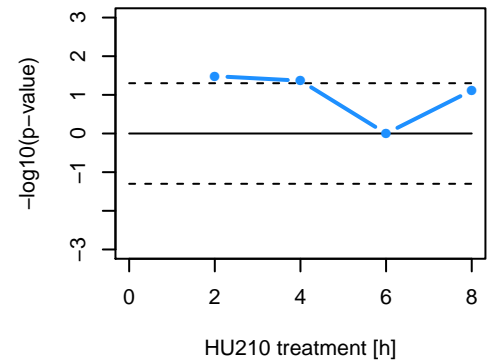
**Neuronal signaling pathways:
Brain derived neurotrophic factor receptor signaling**



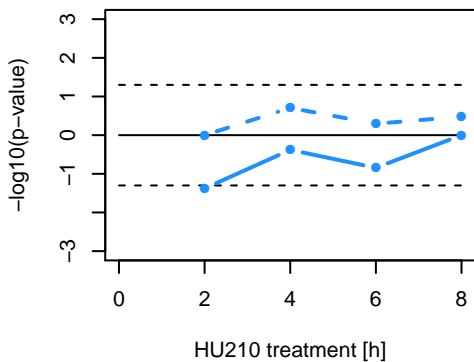
**Neuronal signaling pathways:
Nerve growth factor receptor signaling**



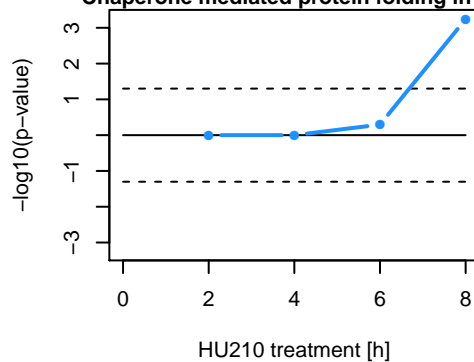
**Pattern recognition signaling:
MDA-5 receptor signaling**



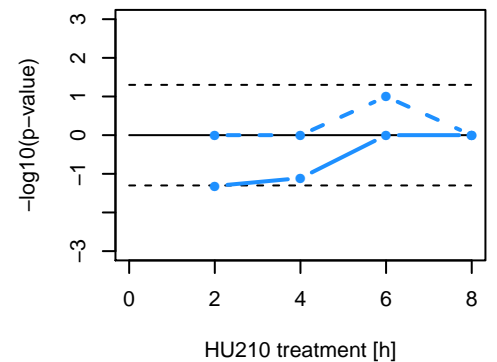
**Pattern recognition signaling:
Toll-like receptor signaling**



**Post-translational protein modification and quality control during biosynthetic-secretory pathway:
Chaperone mediated protein folding in ER**

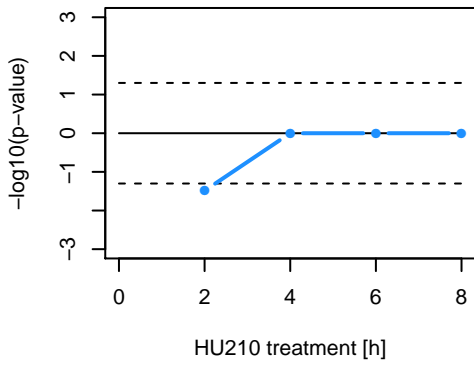


**Post-translational protein modification in Mitochondria:
Protein folding in Mitochondria**

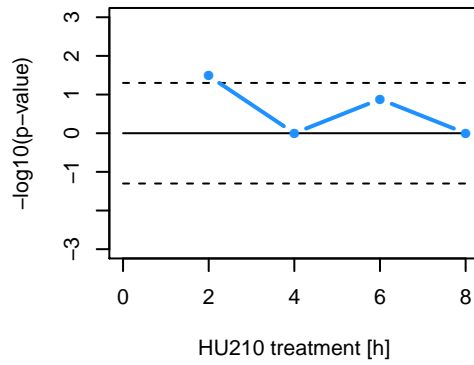


Suppl. figure 2

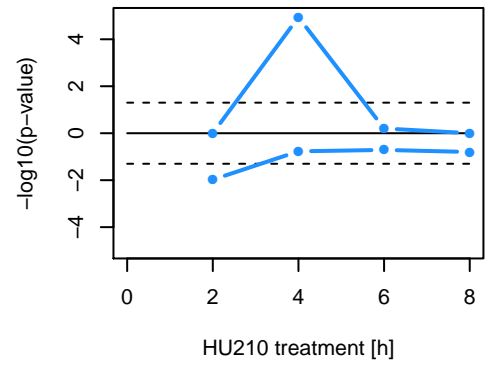
**Protein prenylation:
Protein myristoylation**



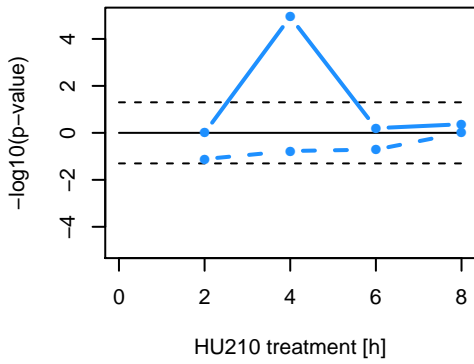
**Purinergic signaling:
Purinergic P1 receptor signaling**



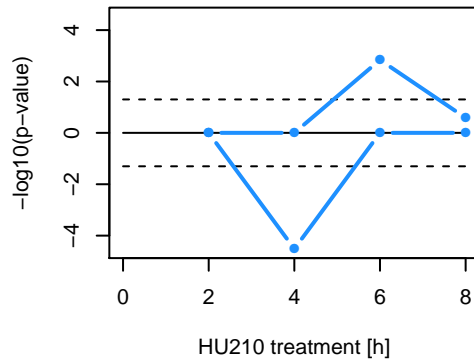
**Ribonucleoprotein biogenesis:
Large ribosomal subunit organization**



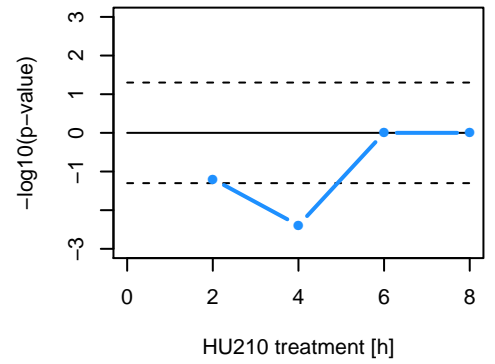
**Ribonucleoprotein biogenesis:
Small ribosomal subunit organization**



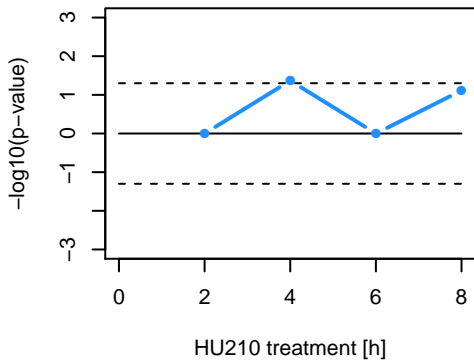
**RNA surveillance and degradation:
Cytoplasmic RNA deadenylation**



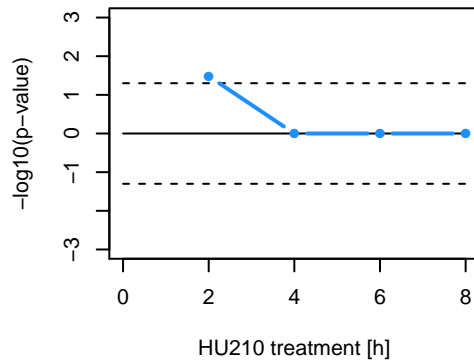
**Signaling by extracellular matrix components:
Integrin receptor signaling**



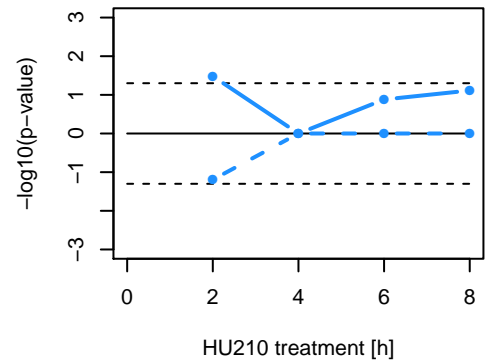
**Signaling pathways involved in hematopoiesis:
Erythropoietin receptor signaling**



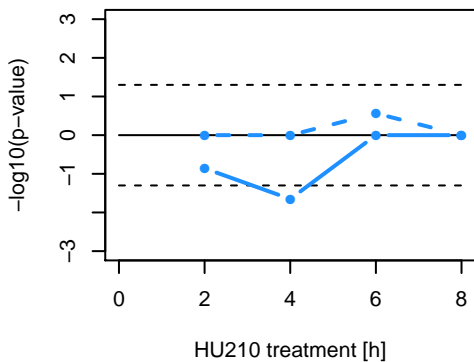
**Signaling pathways involved in hematopoiesis:
Granulocyte macrophage colony-stimulating
factor receptor signaling**



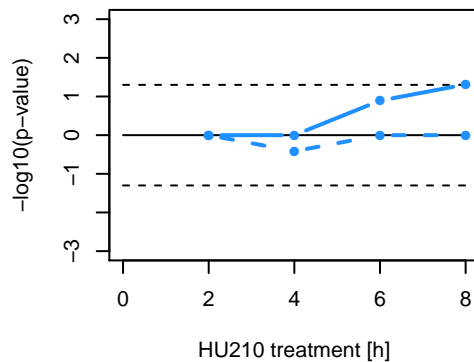
**Signaling pathways involved in hematopoiesis:
Thrombopoietin receptor signaling**



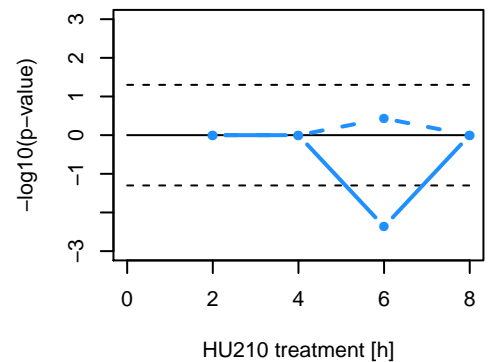
**Signaling pathways that control cell
proliferation and differentiation:
HIF-1 receptor signaling pathway**



**snRNA expression and spliceosome generation
Cytoplasmic core snRNP assembly**

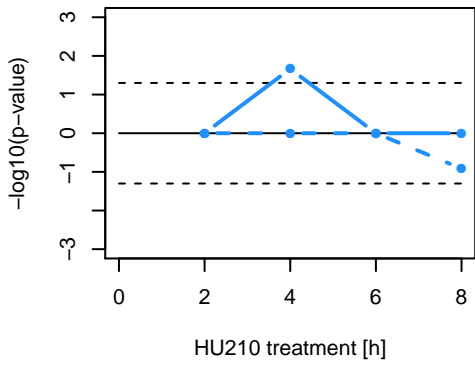


**TGF-beta superfamily signaling:
Activin receptor signaling**

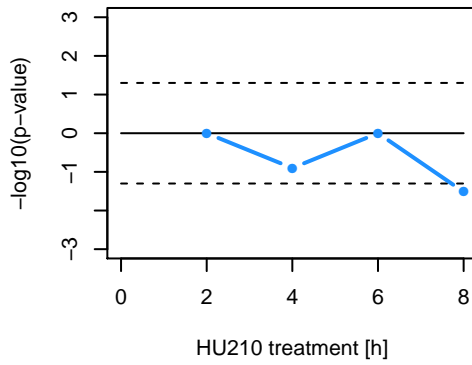


Suppl. figure 2

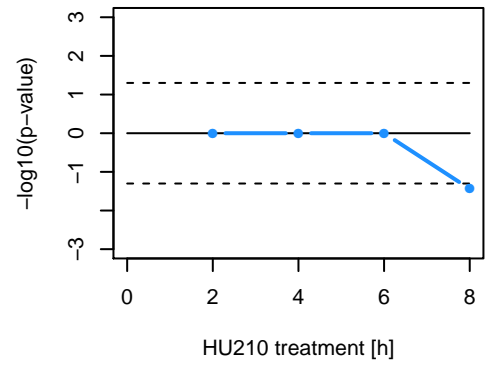
**Transmembrane ion transport involved in membrane potential generation:
Bicarbonate transmembrane transport**



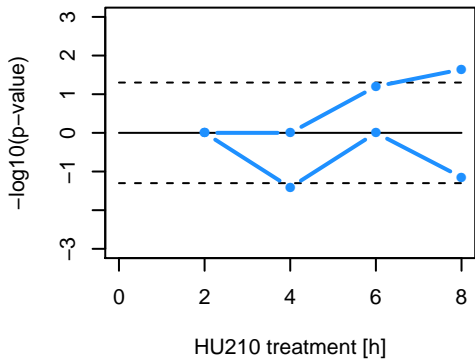
**Transmembrane water and ion transport not involved in membrane potential generation:
Copper transmembrane transport**



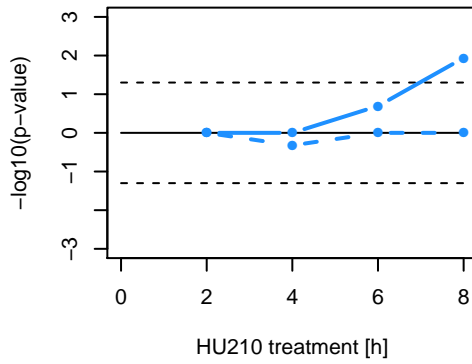
**Triacylglycerol metabolism and transport:
Triacylglycerol hydrolysis and mobilization**



**Vesicle exocytosis:
Vesicle tethering at plasma membrane**

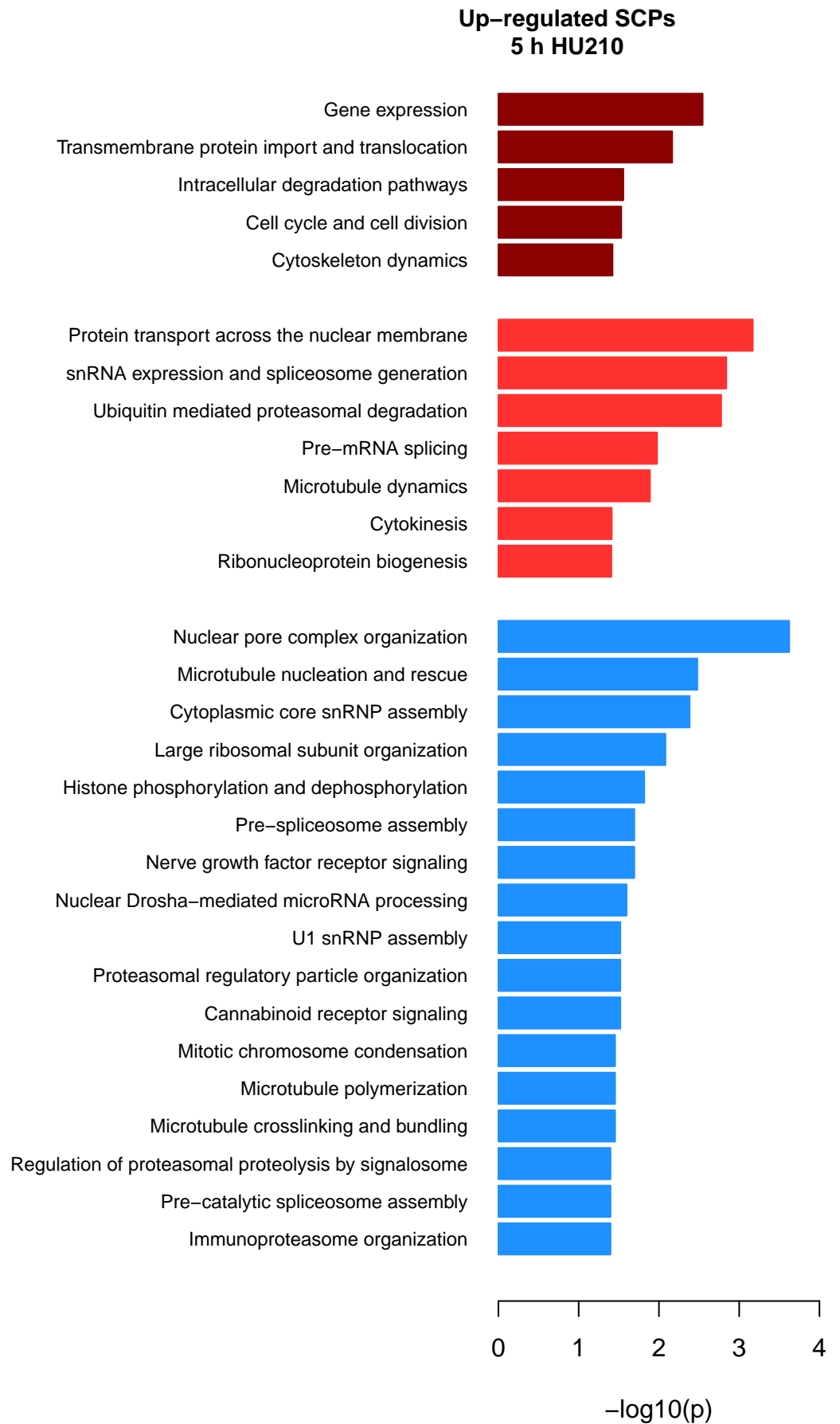


**Vesicle traffic between TGN and endosome:
Retrograde vesicle traffic from endosome to TGN**



Suppl. figure 2: Timeline of all SCPs that were predicted based on standard enrichment analysis. First headline: Parent SCP of predicted SCP, Second headline: predicted SCP. Dark red/light red/blue: predicted SCP is level-1/level-2/level-3 SCP. For details see supplementary figure 2B.

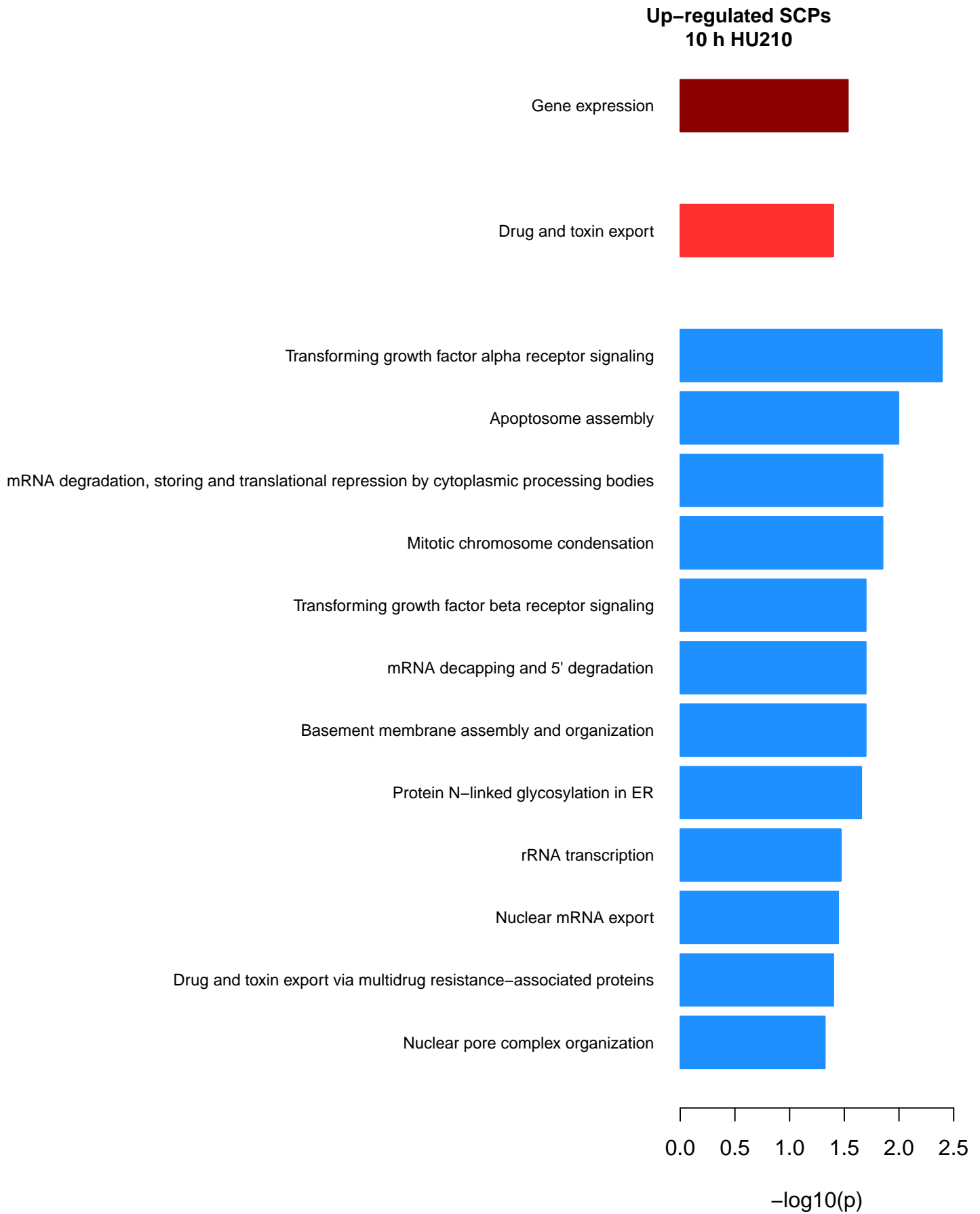
Suppl. figure 3



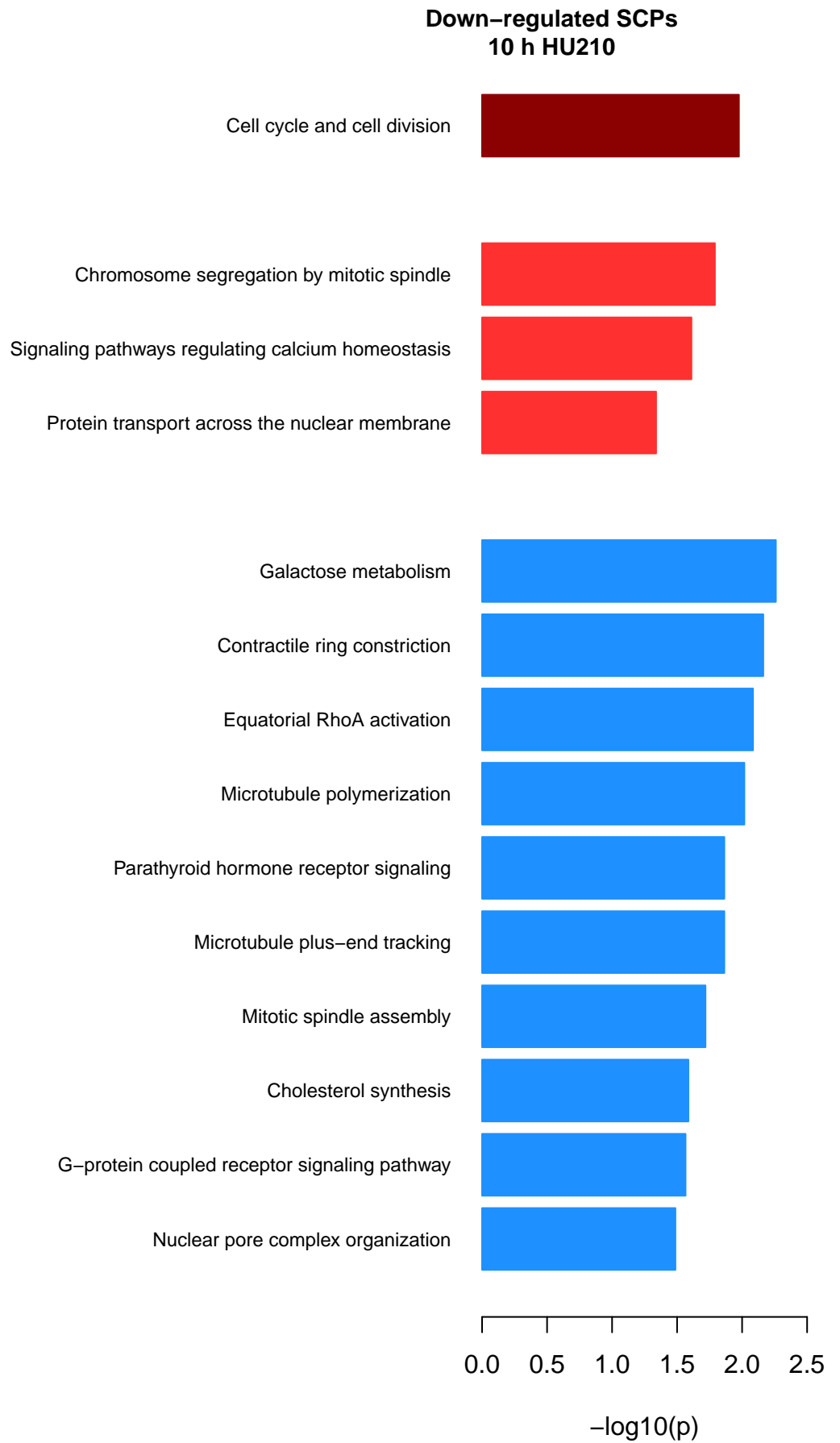
Suppl. figure 3



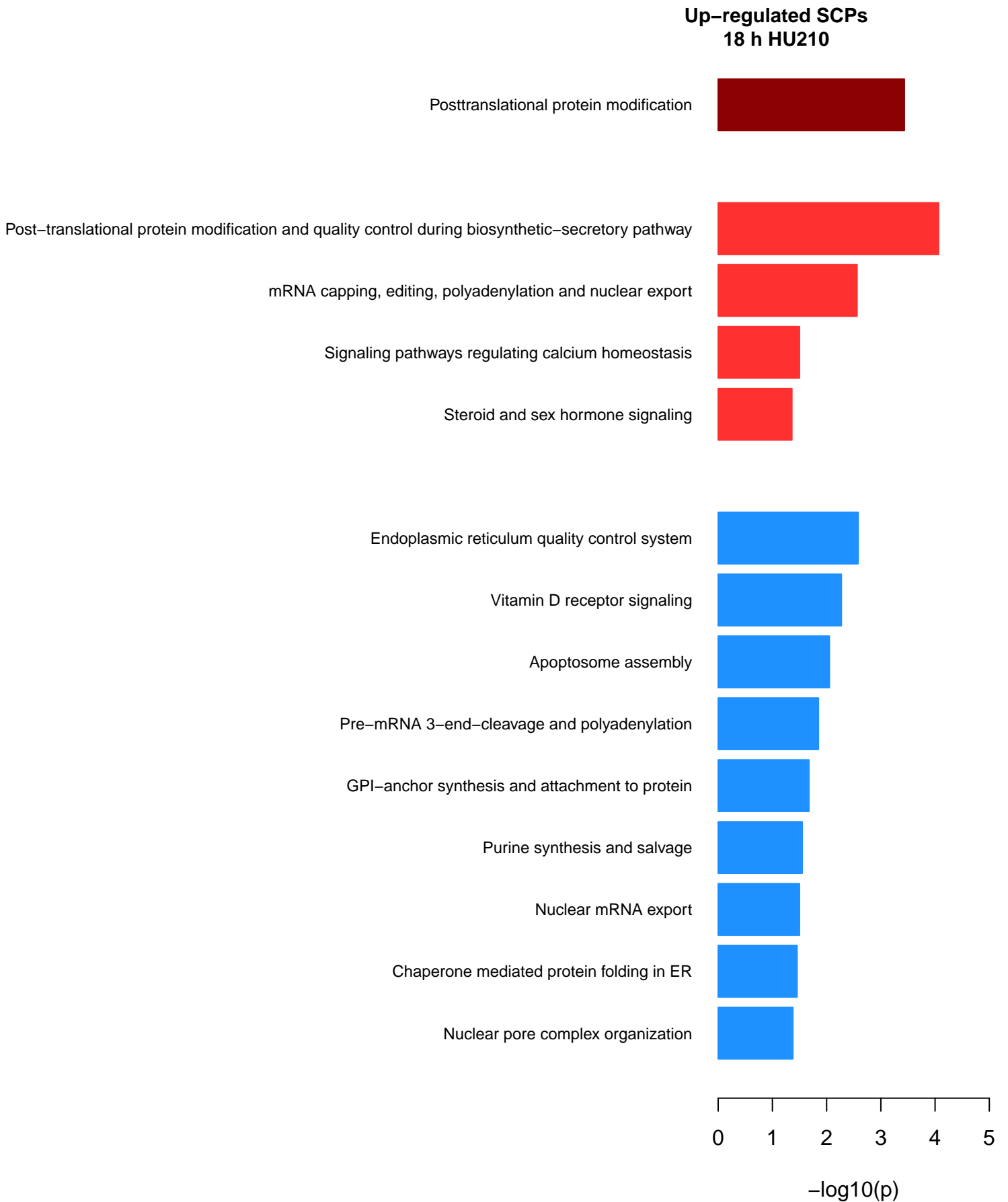
Suppl. figure 3



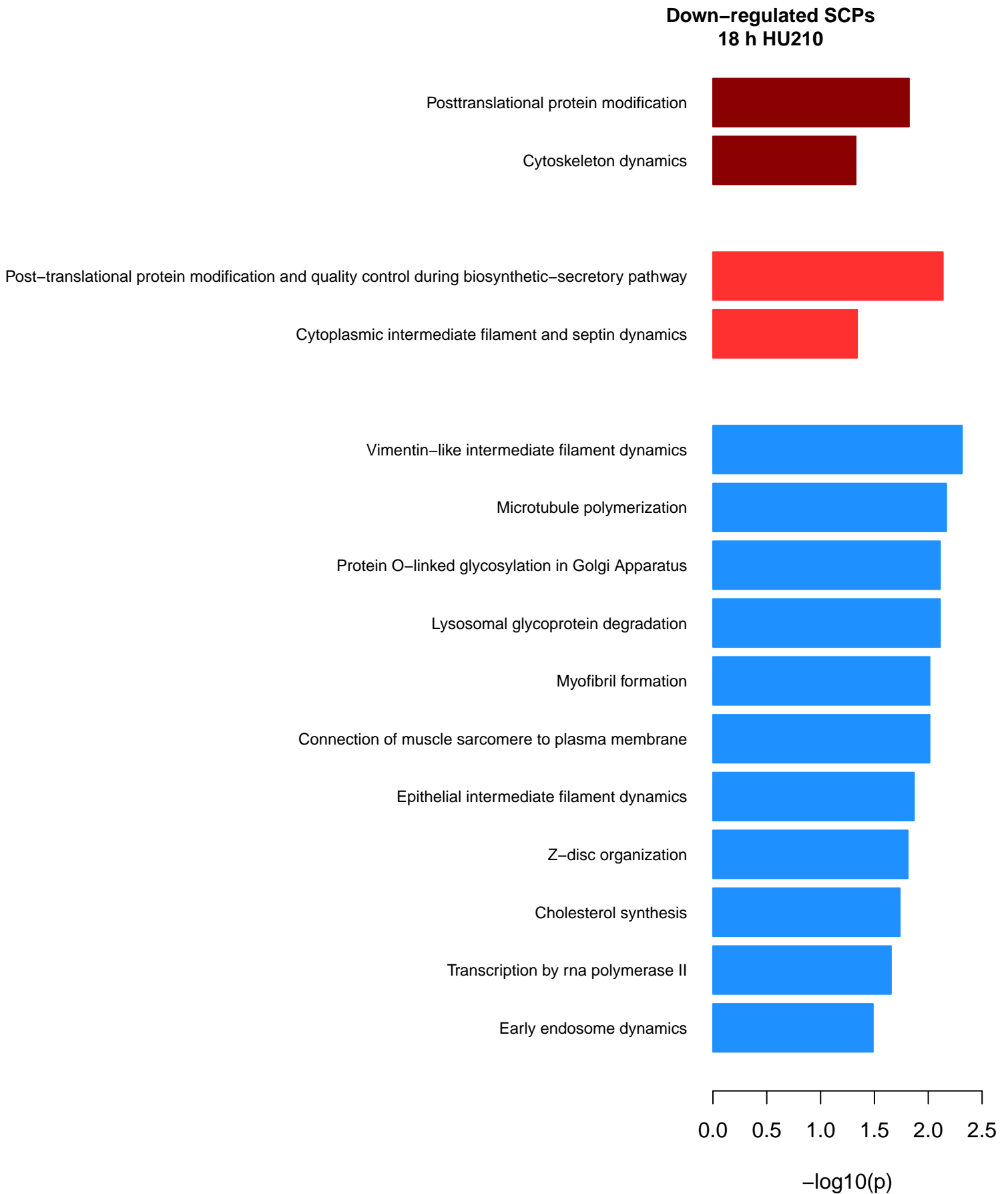
Suppl. figure 3



Suppl. figure 3



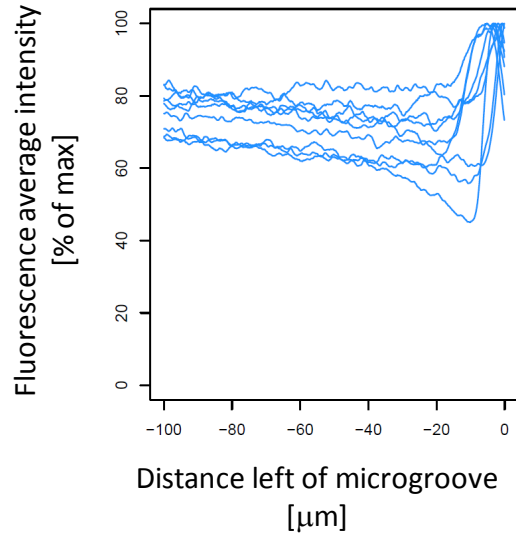
Suppl. figure 3



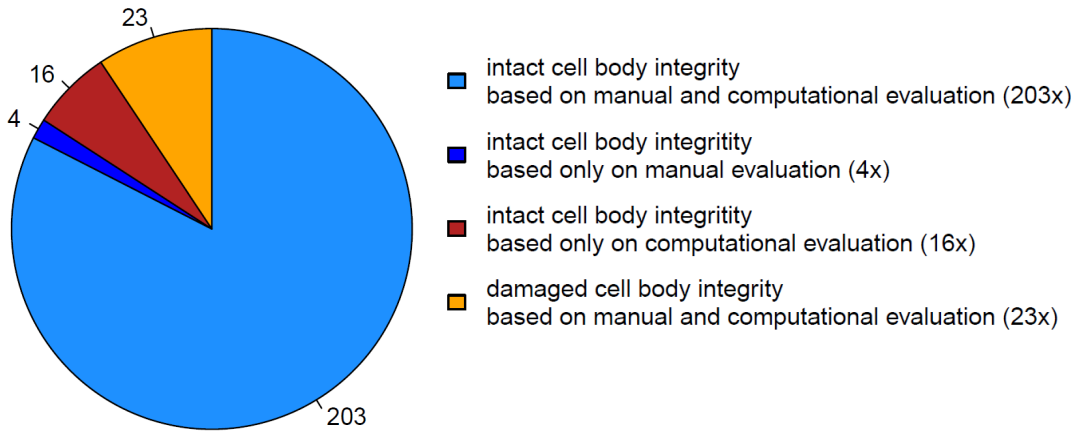
Suppl. figure 3: Standard enrichment analysis of differentially expressed proteins. Up- and downregulated proteins were subjected to standard enrichment analysis. Predicted level-1, level-2 and level-3 SCPs with nominal significance ($\alpha=0.05$) are visualized.

Suppl. figure 4

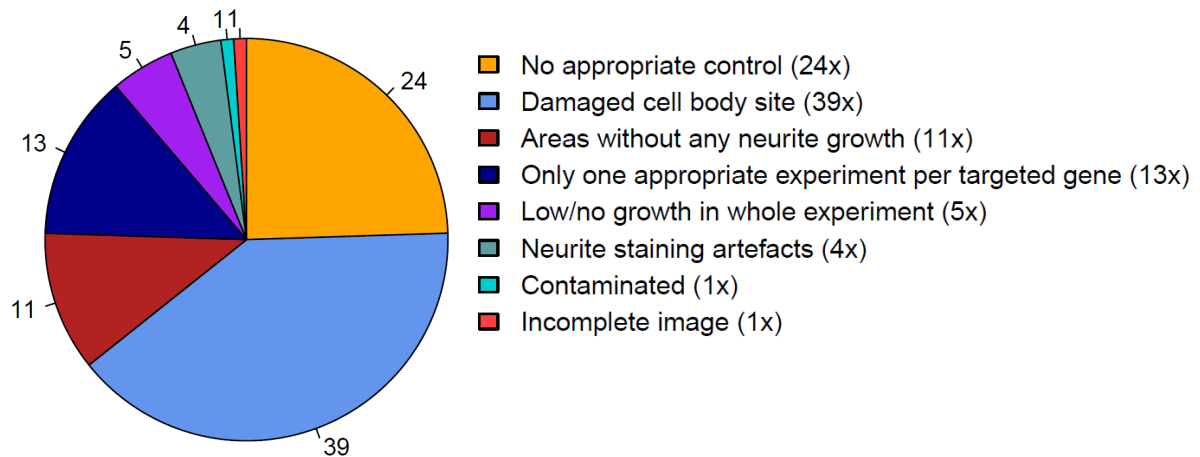
A



B

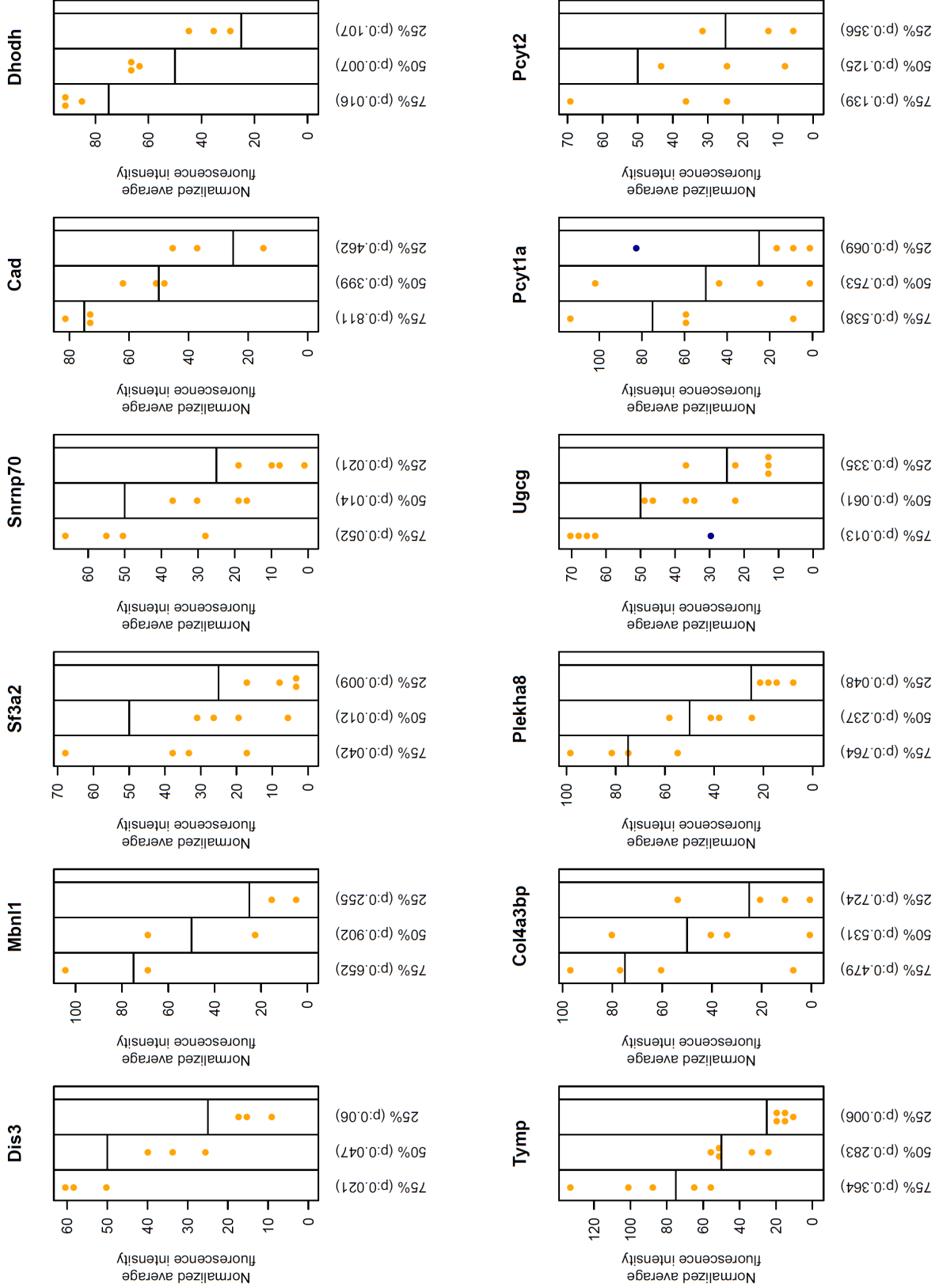


C

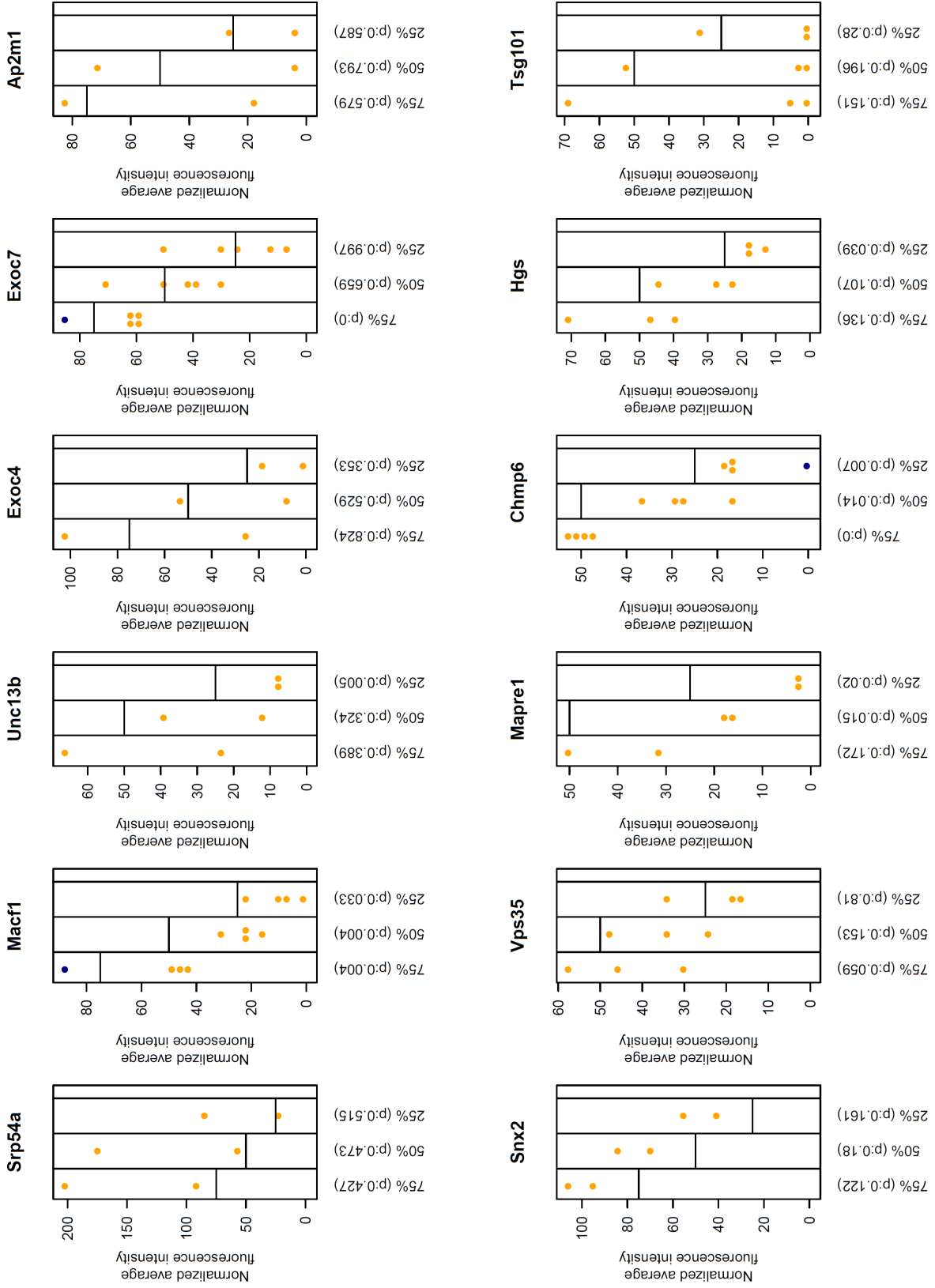


Suppl. figure 4: Quality control of neurite outgrowth assays. (A) During plating cells will occupy every space available within the cell body site of the chamber including those right in front of the microgroove. Microscopic analysis will document uniform tubulin staining and an intense line of tubulin staining right in front of the microgroove wall (see Figure 2A). In a few cases the cell body site was damaged during the axotomy, resulting in a removal of cells in front of the microgroove. Consequently, the staining will be less uniform and the intense line of tubulin staining will appear at an increased distance from the microgroove wall. To identify samples where the cell body site has been damaged during axotomy, we quantified fluorescence intensities on the cell body site and calculated the average intensity at each distance up to 100 μm left of the microgroove wall. All average intensities for one outgrowth chamber were normalized towards the highest intensity. Intact cell body sites show a fluorescence intensity peak right in front of the microgroove wall as shown for one typical experiment (each blue line represents one targeted or scramble siRNA treated sample). This peak corresponds to the intense line of tubulin staining. If the cell body site was damaged during axotomy, this peak will be farer away from the microgroove wall. Our algorithm was searching for the highest peak within the first 50 μm left to the microgroove wall (i.e. within a distance that corresponds to 1/3 of the microgroove wall width of 150 μm). If the normalized intensity of that peak was at least 85%, our algorithm labeled the cell body site as intact and as non intact otherwise. (B) Computational predictions of cell body integrity were manually verified. In 20 out of 246 manually assigned cell body integrity differed from the computationally predicted ones. In these cases we continued with the manual quantifications. (C) Any samples with a damaged cell body site as well as any experiments that did not contain controls with intact cell body sites were removed from any further analysis. Manual investigation also identified further exclusion criteria that lead to the removal of additional samples from the analysis.

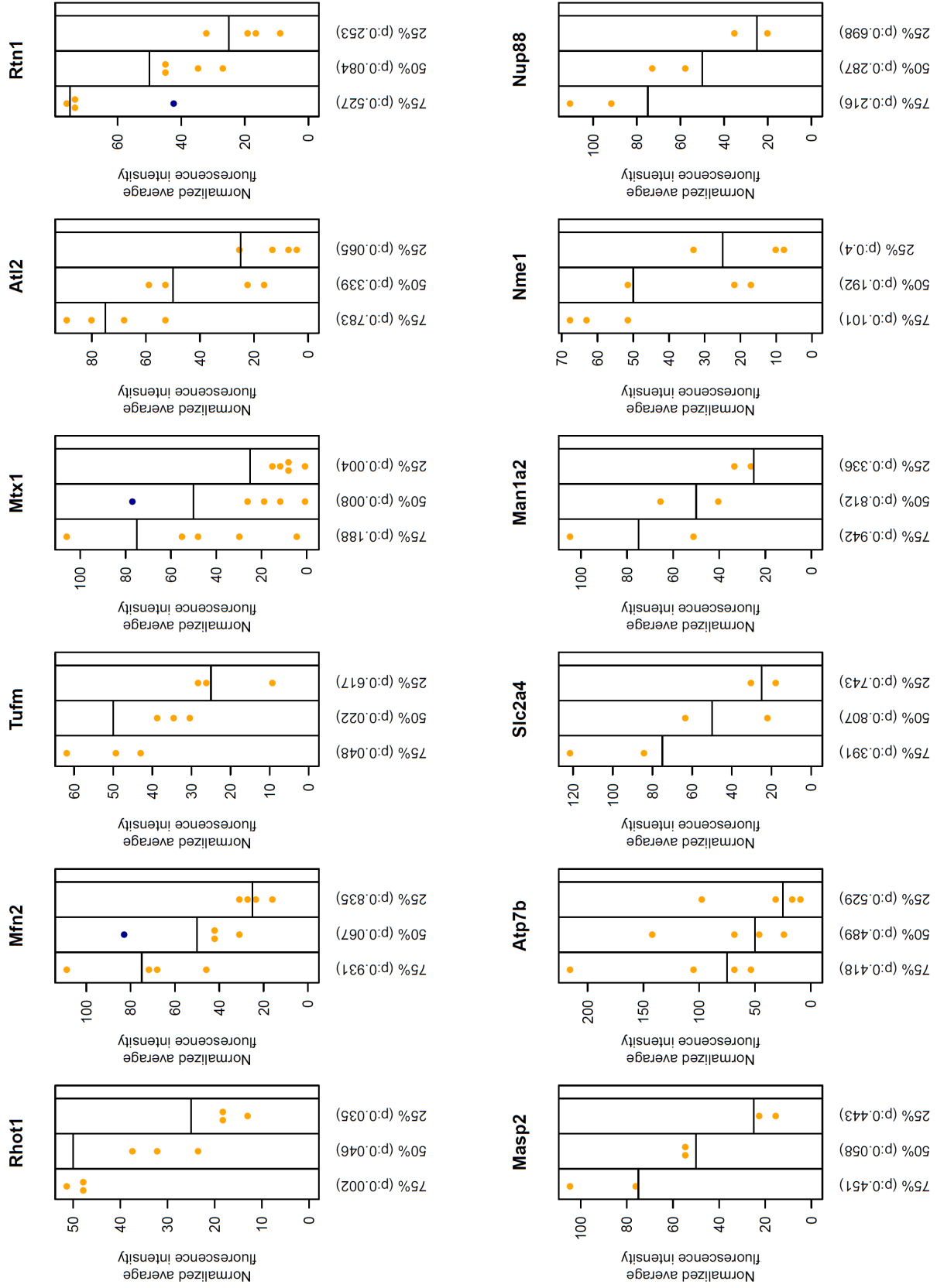
Suppl. figure 5



Suppl. figure 5



Suppl. figure 5



Suppl. figure 5: Neurite outgrowth results after siRNA mediated gene knock down. SiRNA knock down results were analyzed as described at figure 2. Orange and blue dots show the normalized outgrowth intensity obtained for the indicated gene in one experiment at the distance that showed indicated control intensity (75%, 50%, 25%). Blue dots are outliers that were identified using Dixon's Q-Test and removed before p-value calculation using one-sample two-tailed ttest. Horizontal lines show reference control intensities.

Suppl. table 1: Differentially expressed genes and proteins induced by Cb1r stimulation in Neuro 2A cells. FDR = 5%, minimum $\log_2(\text{fold change}) = \pm \log_2(1.3)$ for differentially expressed genes. Nominal p-value = 5% and minimum $\log_2(\text{fold change}) = \pm \log_2(1.1)$ for differentially expressed proteins.

Suppl. table 2: Dynamic enrichment results of differentially expressed genes. Differentially expressed genes (FDR = 5%, minimum $\log_2(\text{fold change}) = \pm \log_2(1.5)$) were submitted to dynamic enrichment analysis using MBCO. Seed supplementary figure 1A for details. The column 'Overlap symbols' lists those symbols that are part of the input gene list and the SCP. Numbers in brackets behind each symbols indicate $\log_2(\text{fold change})$ of that gene.

Suppl. table 3: Standard enrichment results of differentially expressed genes. Differentially expressed genes (FDR = 5%, minimum $\log_2(\text{fold change}) = \pm \log_2(1.5)$) were submitted to standard enrichment analysis. Seed supplementary figure 1B for details. The column 'Overlap symbols' lists those symbols that are part of the input gene list and the SCP. Numbers in brackets behind each symbols indicate $\log_2(\text{fold change})$ of that gene.

Suppl. table 4: Dynamic enrichment results of differentially expressed proteins.

Suppl. table 5: Standard enrichment results of differentially expressed proteins.