Suberoylanilide Hydroxamic Acid Treatment Reveals Crosstalks among Proteome, Ubiquitylome and Acetylome in Non-Small Cell Lung Cancer A549 Cell Line

Quan Wu[1], Zhongyi Cheng[2], Jun Zhu[3], Weiqing Xu[1], Xiaojun Peng[3], Chuangbin Chen[3], Wenting Li[1], Fengsong Wang[4], Lejie Cao[5], Xingling Yi[3], Zhiwei Wu[1], Jing Li[1], Pingsheng Fan[6]

[1] Central Laboratory of Medical Research Centre, Affiliated Provincial Hospital, Anhui Medical University, Hefei, 230001, China

[2] Institute for Advanced Study of Translational Medicine, Tongji University, Shanghai, 200092, China

[3] Jingjie PTM Biolab (Hangzhou) Co. Ltd, Hangzhou 310018, China

[4] School of Life science, Anhui Medical University, Hefei, 230032, China

[5] Department of Respiration, Affiliated Provincial Hospital, Anhui Medical University, Hefei, 230001, China

[6] Department of Oncology, Affiliated Provincial Hospital, Anhui Medical University, Hefei, 230001, China

Correspondence and requests for materials should be addressed to Q.W. (powerwoo01@ahmu.edu.cn, Ph: +86-551-62283574, fax: +86-551-62283292) or P.S.F. (fanpingsheng@csco.org.cn, Ph: +86-551-65897876, fax: +86-551-62283292)

Bioinformatic Analysis

Enrichment of Gene Ontology analysis. Proteins were classified by GO annotation into three categories: biological process, cellular compartment and molecular function. For each category, we used Functional Annotation Tool of DAVID Bioinformatics Resources 6.7 to identify enriched GO against the background of Homo sapiens. A two-tailed Fisher's exact test was employed to test the enrichment of the protein-containing IPI entries against all IPI proteins. Correction for multiple hypothesis testing was carried out using standard false discovery rate control methods. The GO with a corrected p-value < 0.05 is considered significant.

Enrichment of pathway analysis. Encyclopedia of Genes and Genomes (KEGG) database was used to identify enriched pathways by Functional Annotation Tool of DAVID against the background of Homo sapiens. A two-tailed Fisher's exact test was employed to test the enrichment of the protein-containing IPI entries against all IPI proteins. Correction for multiple hypothesis testing was carried out using standard false discovery rate control methods. The pathway with a corrected p-value < 0.05 was considered significant. These pathways were classified into hierarchical categories according to the KEGG website.

Enrichment of protein domain analysis. For each category proteins, InterPro (a resource that provides functional analysis of protein sequences by classifying them into families and predicting the presence of domains and important sites) database was researched using Functional Annotation Tool of DAVID against the background of Homo sapiens. A two-tailed Fisher's exact test was employed to

test the enrichment of the protein-containing IPI entries against all IPI proteins. Correction for multiple hypothesis testing was carried out using standard false discovery rate control methods and domains with a corrected p-value < 0.05 were considered significant.

Enrichment of complex analysis. Manually curated CORUM protein complex database for human was used for protein complex analysis. Overrepresented complexes were identified using hypergeometric test for each category proteins. A two-tailed Fisher's exact test was employed to test the enrichment of the protein-containing SwissProt entries against all SwissPort human proteins. Correction for multiple hypothesis testing was carried out using standard false discovery rate control methods and complexes with a corrected p-value < 0.05 were considered significant.

Enrichment-based clustering analysis. All the protein categories obtained after enrichment were collated along with their P values, and then filtered for those categories which were at least enriched in one of the clusters with P value<0.05. This filtered P value matrix was transformed by the function x = −log10 (P value). Finally these x values were z-transformed for each category. These z scores were then clustered by one-way hierarchical clustering (Euclidean distance, average linkage clustering) in Genesis. Cluster membership was visualized by a heat map using the "heatmap.2" function from the "gplots" R-package.

Protein-protein interaction network analysis. We analyzed protein-protein interaction for identified proteins using Cytoscape software.

Protein-protein interaction network obtained from STRING database. STRING defines a metric called “confidence score” to define interaction confidence; we fetched all interactions that had a confidence score ≥ 0.7 (high confidence). Interaction network form STRING was visualized in Cytoscape. A graph the oretical clustering algorithm, molecular complex detection (MCODE) was utilized to analyzed densely connected regions. MCODE is part of the plug-in tool kit of the network analysis and visualization software Cytoscape.

Figure Legends

Figure S1. Functional enrichment-based clustering analysis for the quantified proteome. A, cellular component analysis. B, biological process analysis. C, molecular function analysis. D, protein domain analysis. E, KEGG pathway analysis. F, protein complex analysis.

Figure S2. Functional enrichment-based clustering analysis for the quantified ubiquitylome. A, protein domain analysis. B, protein complex analysis.

Figure S3. Protein-protein interaction network of ubiquitylome established using Cytoscape.

Figure S4. Functional enrichment-based clustering analysis for the quantified acetylome. A, protein domain analysis. B, protein complex analysis.

Figure S5. Protein-protein interaction network of acetylome established using Cytoscape.

Figure S6. MS/MS spectra of HSPA8 K601 acetylation and ubiquitination. A, acetylation. B, ubiquitination. This site was both undergo acetylation and ubiquitination.

Figure S1

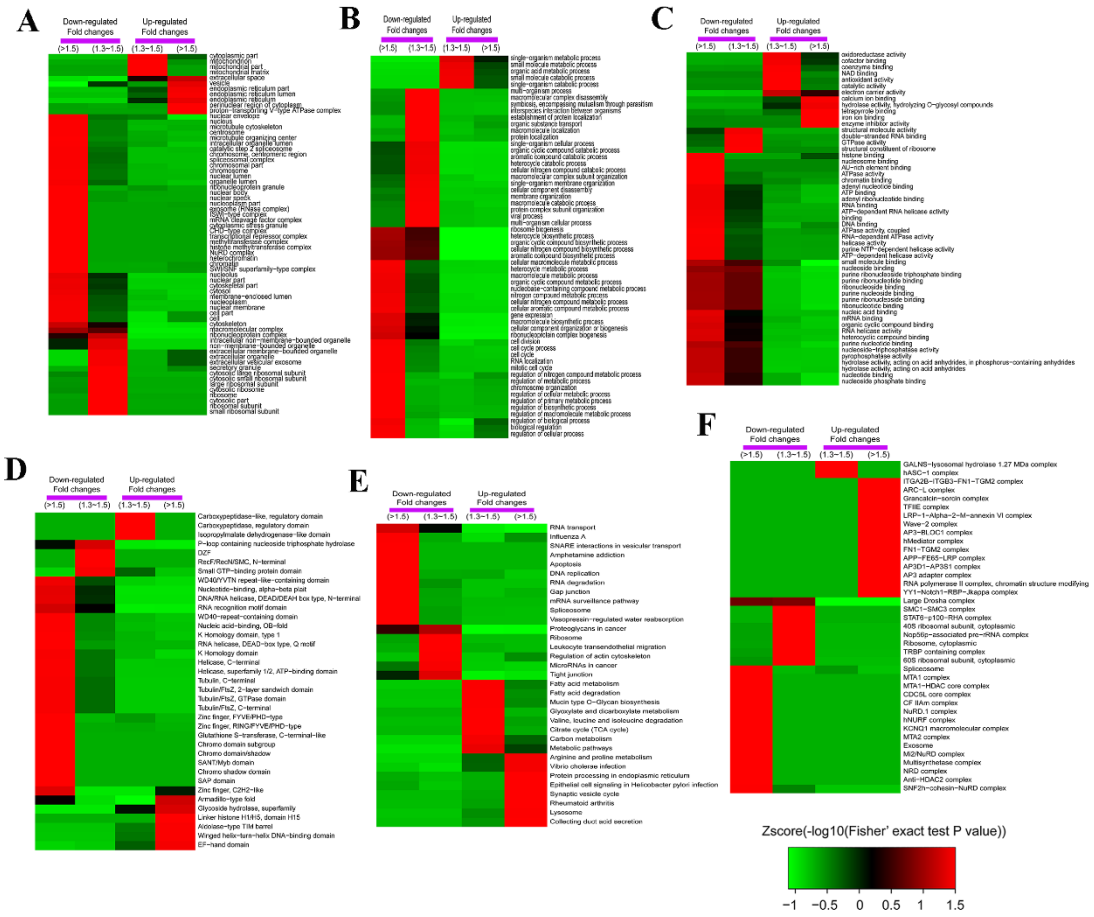
A
B
C
D
E
F
Down-regulated
Fold changes
Up-regulated
Fold changes
(>1.5)
(1.3~1.5)
(1.3~1.5)
(>1.5)
Zscore(-log10(Fisher' exact test P value))
−1
−0.5
0
0.5
1
1.5

Figure S2

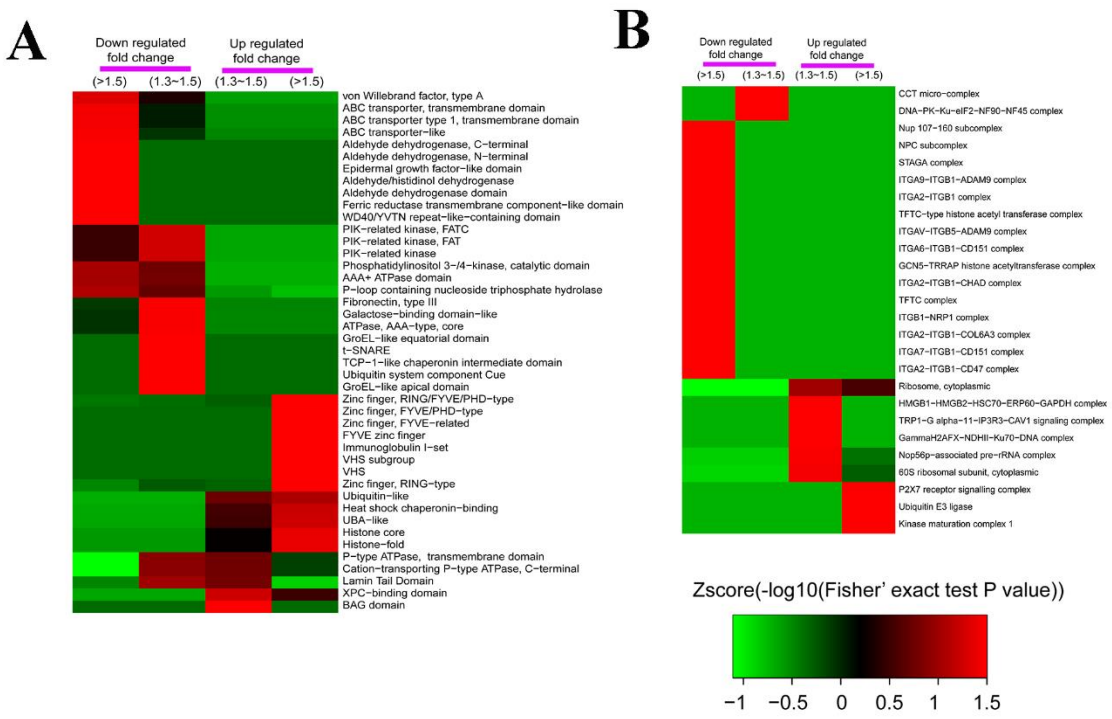
A
Down regulated fold change
Up regulated fold change
(>1.5) (1.3~1.5) (1.3~1.5) (>1.5)
von Willebrand factor, type A
ABC transporter, transmembrane domain
ABC transporter type 1, transmembrane domain
ABC transporter−like
Aldehyde dehydrogenase, C−terminal
Aldehyde dehydrogenase, N−terminal
Epidermal growth factor−like domain
Aldehyde/histidinol dehydrogenase
Aldehyde dehydrogenase domain
Ferric reductase transmembrane component−like domain
WD40/YVTN repeat−like−containing domain
PIK−related kinase, FATC
PIK−related kinase, FAT
PIK−related kinase
Phosphatidylinositol 3−/4−kinase, catalytic domain
AAA+ ATPase domain
P−loop containing nucleoside triphosphate hydrolase
Fibronectin, type III
Galactose−binding domain−like
ATPase, AAA−type, core
GroEL−like equatorial domain
t−SNARE
TCP−1−like chaperonin intermediate domain
Ubiquitin system component Cue
GroEL−like apical domain
Zinc finger, RING/FYVE/PHD−type
Zinc finger, FYVE/PHD−type
Zinc finger, FYVE−related
FYVE zinc finger
Immunoglobulin I−set
VHS subgroup
VHS
Zinc finger, RING−type
Ubiquitin−like
Heat shock chaperonin−binding
UBA−like
Histone core
Histone−fold
P−type ATPase, transmembrane domain
Cation−transporting P−type ATPase, C−terminal
Lamin Tail Domain
XPC−binding domain
BAG domain
B
Down regulated fold change
Up regulated fold change
(>1.5) (1.3~1.5) (1.3~1.5) (>1.5)
CCT micro−complex
DNA−PK−Ku−eIF2−NF90−NF45 complex
Nup 107−160 subcomplex
NPC subcomplex
STAGA complex
ITGA9−ITGB1−ADAM9 complex
ITGA2−ITGB1 complex
TFTC−type histone acetyl transferase complex
ITGAV−ITGB5−ADAM9 complex
ITGA6−ITGB1−CD151 complex
GCN5−TRRAP histone acetyltransferase complex
ITGA2−ITGB1−CHAD complex
TFTC complex
ITGB1−NRP1 complex
ITGA2−ITGB1−COL6A3 complex
ITGA7−ITGB1−CD151 complex
ITGA2−ITGB1−CD47 complex
Ribosome, cytoplasmic
HMGB1−HMGB2−HSC70−ERP60−GAPDH complex
TRP1−G alpha−11−IP3R3−CAV1 signaling complex
GammaH2AFX−NDHII−Ku70−DNA complex
Nop56p−associated pre−rRNA complex
60S ribosomal subunit, cytoplasmic
P2X7 receptor signalling complex
Ubiquitin E3 ligase
Kinase maturation complex 1
Zscore(-log10(Fisher' exact test P value))
−1 −0.5 0 0.5 1 1.5

Figure S3

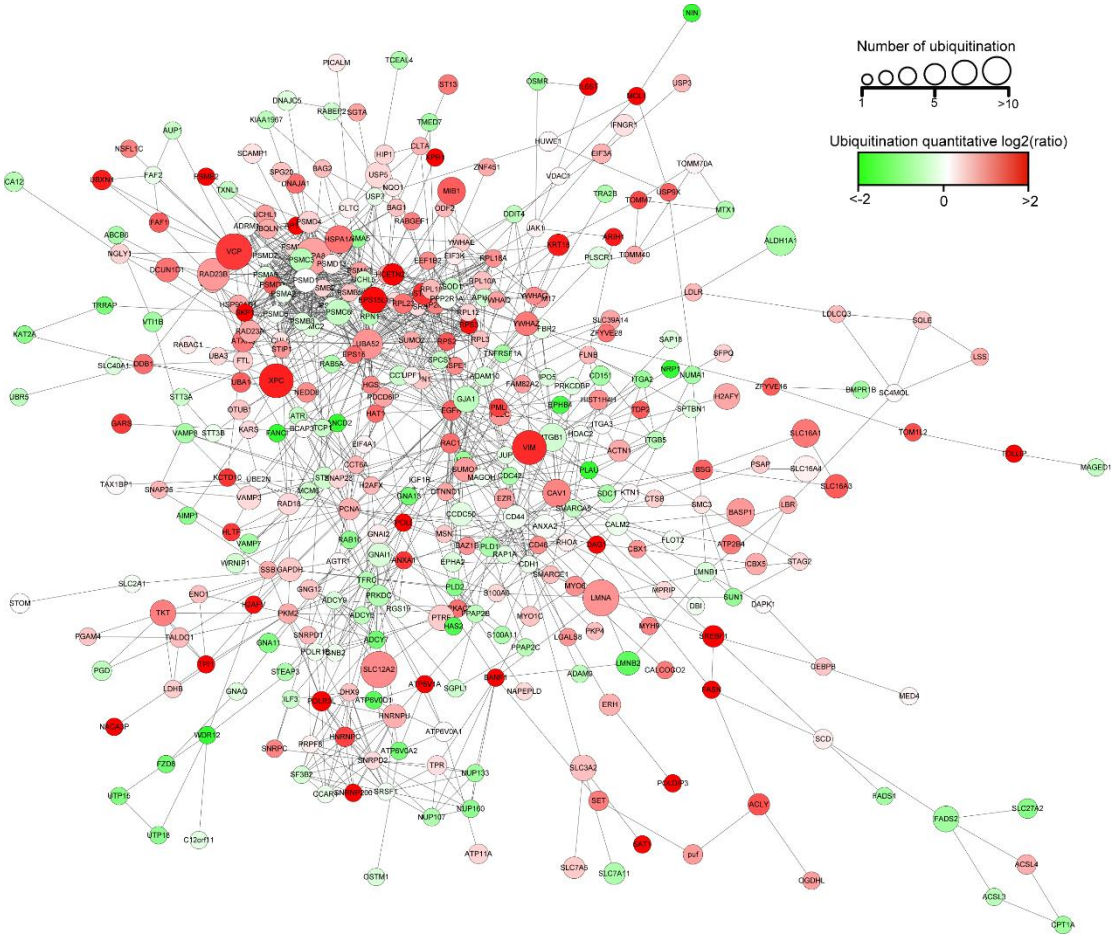
Number of ubiquitination
1
5
>10
Ubiquitination quantitative log2(ratio)
<-2
0
>2

Figure S4

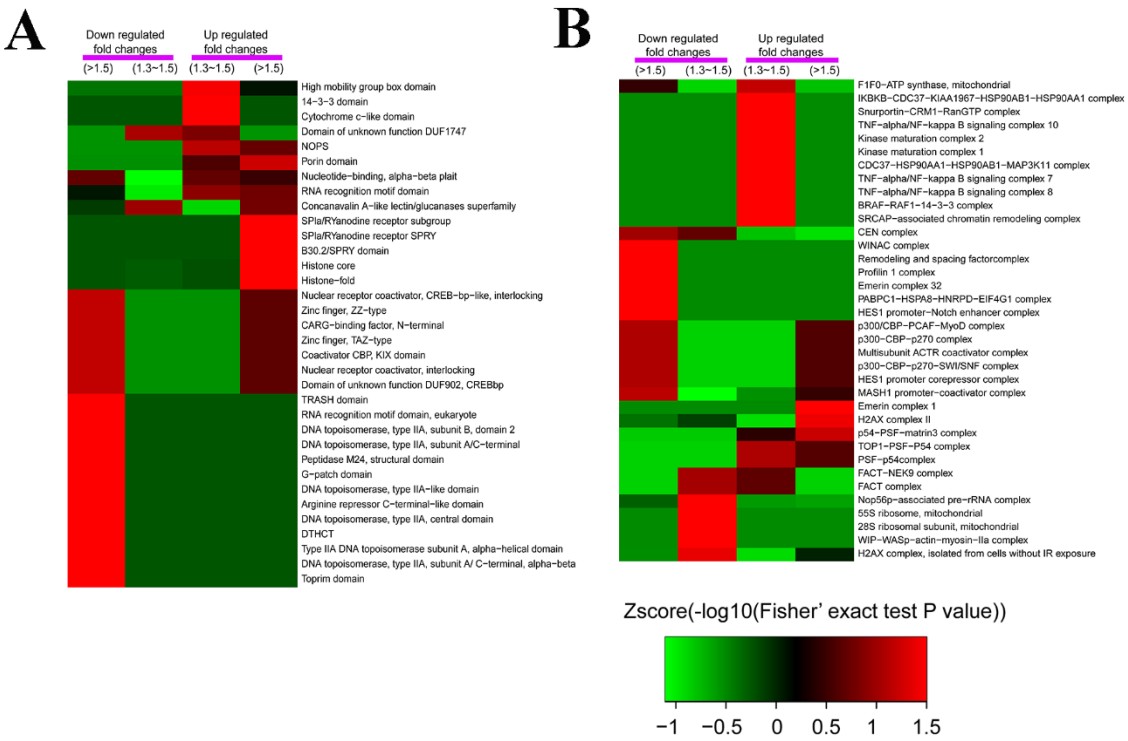
A
Down regulated fold changes
Up regulated fold changes
(>1.5) (1.3~1.5) (1.3~1.5) (>1.5)
High mobility group box domain
14−3−3 domain
Cytochrome c−like domain
Domain of unknown function DUF1747
NOPS
Porin domain
Nucleotide−binding, alpha−beta plait
RNA recognition motif domain
Concanavalin A−like lectin/glucanases superfamily
SPla/RYanodine receptor subgroup
SPla/RYanodine receptor SPRY
B30.2/SPRY domain
Histone core
Histone−fold
Nuclear receptor coactivator, CREB−bp−like, interlocking
Zinc finger, ZZ−type
CARG−binding factor, N−terminal
Zinc finger, TAZ−type
Coactivator CBP, KIX domain
Nuclear receptor coactivator, interlocking
Domain of unknown function DUF902, CREBbp
TRASH domain
RNA recognition motif domain, eukaryote
DNA topoisomerase, type IIA, subunit B, domain 2
DNA topoisomerase, type IIA, subunit A/C−terminal
Peptidase M24, structural domain
G−patch domain
DNA topoisomerase, type IIA−like domain
Arginine repressor C−terminal−like domain
DNA topoisomerase, type IIA, central domain
DTHCT
Type IIA DNA topoisomerase subunit A, alpha−helical domain
DNA topoisomerase, type IIA, subunit A/ C−terminal, alpha−beta
Toprim domain
B
Down regulated fold changes
Up regulated fold changes
(>1.5) (1.3~1.5) (1.3~1.5) (>1.5)
F1F0−ATP synthase, mitochondrial
IKBKB−CDC37−KIAA1967−HSP90AB1−HSP90AA1 complex
Snurportin−CRM1−RanGTP complex
TNF−alpha/NF−kappa B signaling complex 10
Kinase maturation complex 2
Kinase maturation complex 1
CDC37−HSP90AA1−HSP90AB1−MAP3K11 complex
TNF−alpha/NF−kappa B signaling complex 7
TNF−alpha/NF−kappa B signaling complex 8
BRAF−RAF1−14−3−3 complex
SRCAP−associated chromatin remodeling complex
CEN complex
WINAC complex
Remodeling and spacing factorcomplex
Profilin 1 complex
Emerin complex 32
PABPC1−HSPA8−HNRPD−EIF4G1 complex
HES1 promoter−Notch enhancer complex
p300/CBP−PCAF−MyoD complex
p300−CBP−p270 complex
Multisubunit ACTR coactivator complex
p300−CBP−p270−SWI/SNF complex
HES1 promoter corepressor complex
MASH1 promoter−coactivator complex
Emerin complex 1
H2AX complex II
p54−PSF−matrin3 complex
TOP1−PSF−P54 complex
PSF−p54complex
FACT−NEK9 complex
FACT complex
Nop56p−associated pre−rRNA complex
55S ribosome, mitochondrial
28S ribosomal subunit, mitochondrial
WIP−WASp−actin−myosin−IIa complex
H2AX complex, isolated from cells without IR exposure
Zscore(-log10(Fisher' exact test P value))
−1 −0.5 0 0.5 1 1.5

Figure S5

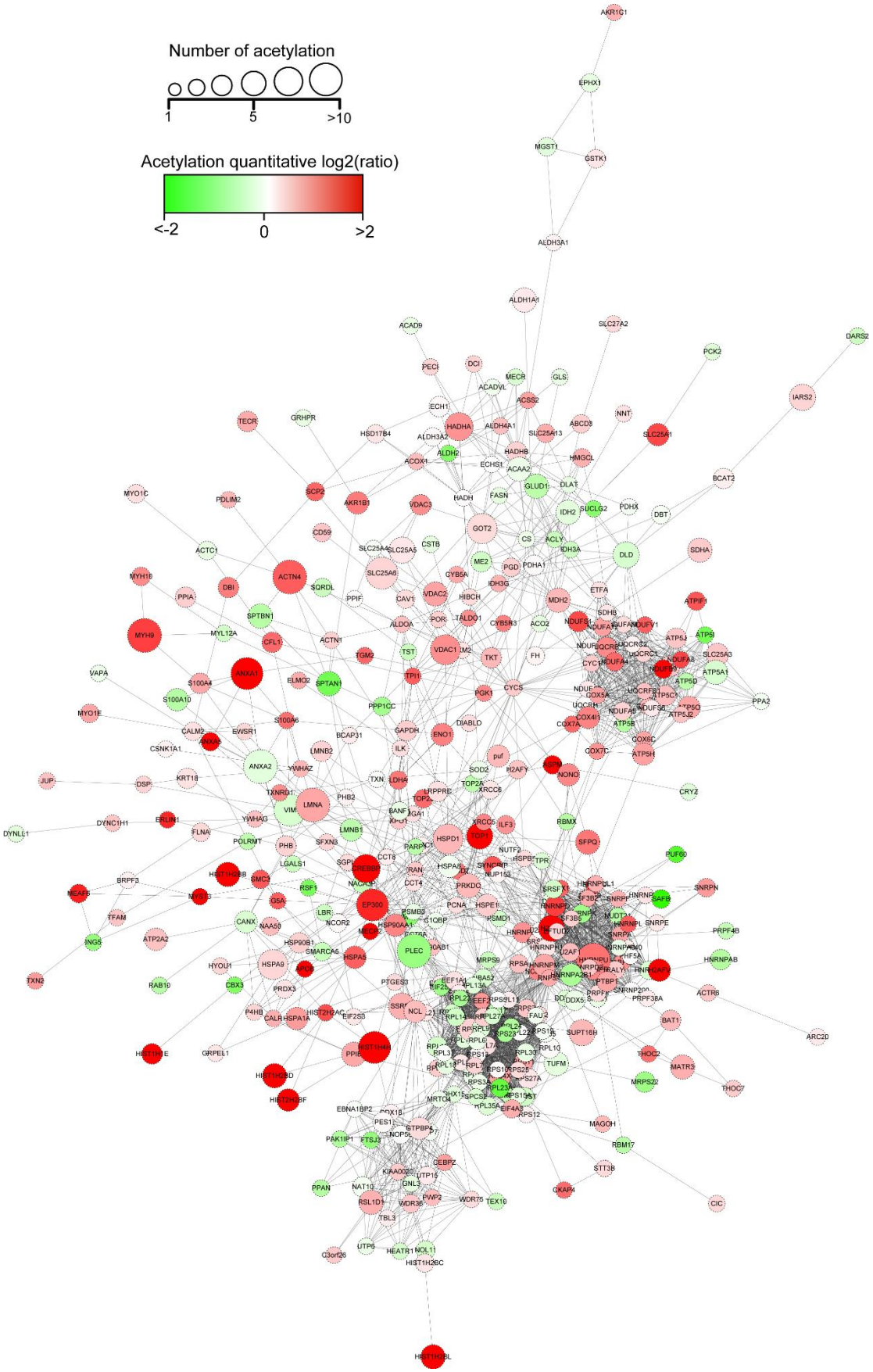
Number of acetylation
1
5
>10
Acetylation quantitative log2(ratio)
<-2
0
>2

Figure S6