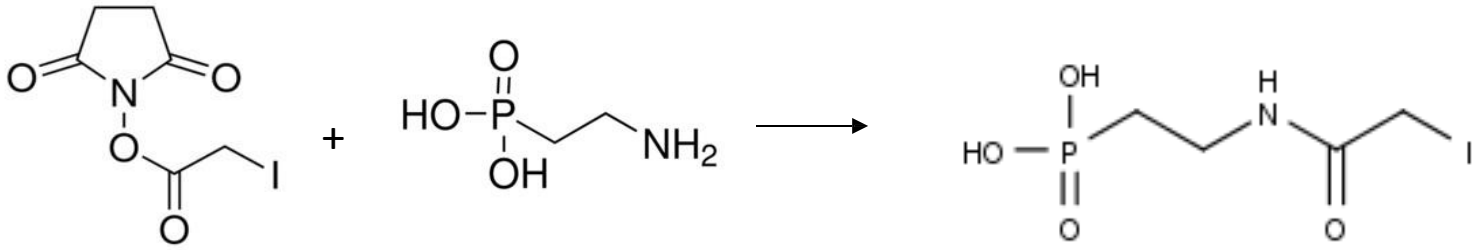
Figure 1

a)



Succinimidyl iodoacetate(SIA) MW: 283,02

2-aminoethyl)phosphonic acid (AEP) MW: 125,06

(2-(2-iodoacetamido)ethyl)phosphonic acid MW: 292,93

b)

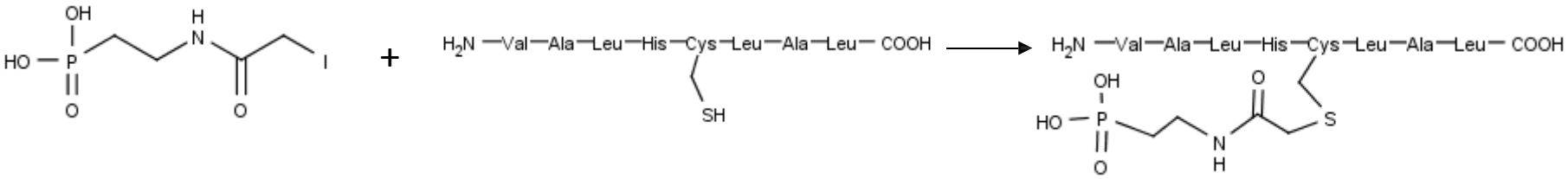


Figure 2

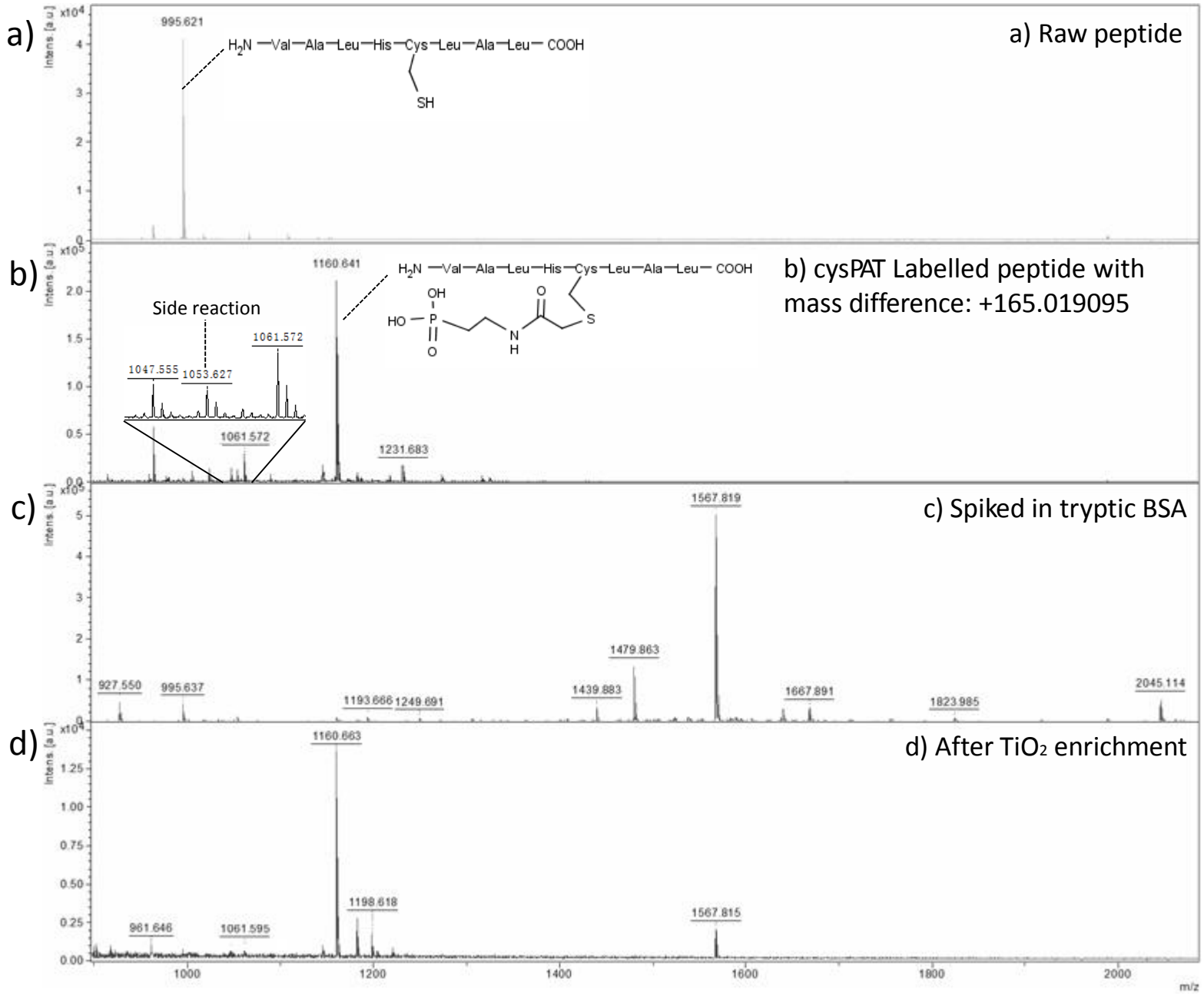

a)
995.621
H2N —Val—Ala—Leu—His—Cys—Leu—Ala—Leu— COOH
SH
a) Raw peptide
b)
1160.641
H2N —Val—Ala—Leu—His—Cys—Leu—Ala—Leu— COOH
Side reaction
1061.572
1047.555
1053.627
1061.572
1231.683
b) cysPAT Labelled peptide with mass difference: +165.019095
c)
1567.819
1479.863
927.550
995.637
1193.666
1249.691
1439.883
1667.891
1823.985
2045.114
c) Spiked in tryptic BSA
d)
1160.663
1198.618
961.646
1061.595
1567.815
d) After TiO2 enrichment
Intens. [a.u.]
m/z

Figure 3

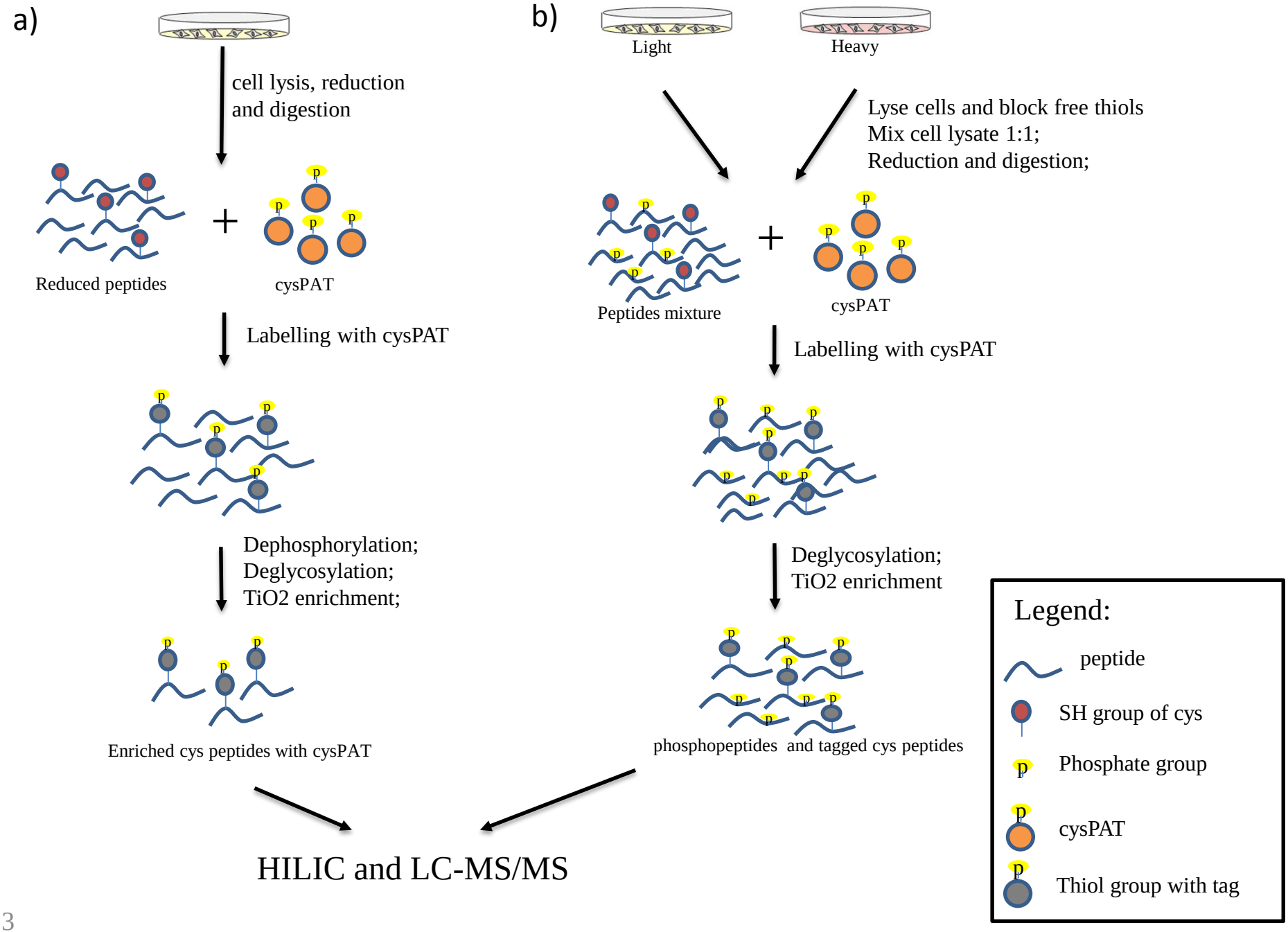
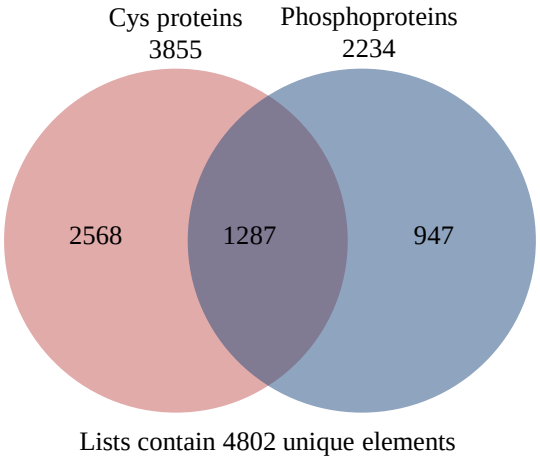
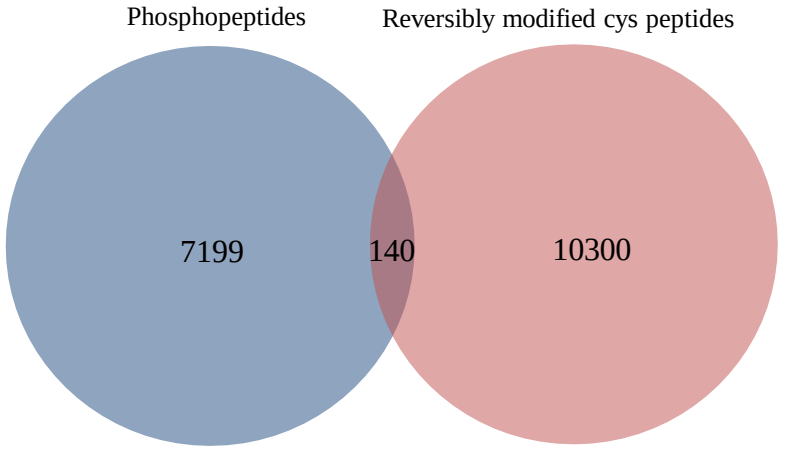

a)
cell lysis, reduction and digestion
Reduced peptides
cysPAT
Labelling with cysPAT
Dephosphorylation;
Deglycosylation;
TiO2 enrichment;
Enriched cys peptides with cysPAT
b)
control
5 min EGF
Light
Heavy
Lyse cells and block free thiols
Mix cell lysate 1:1;
Reduction and digestion;
Peptides mixture
cysPAT
Labelling with cysPAT
Deglycosylation;
TiO2 enrichment
phosphopeptides and tagged cys peptides
HILIC and LC-MS/MS
Legend:
peptide
SH group of cys
Phosphate group
cysPAT
Thiol group with tag

Figure 4

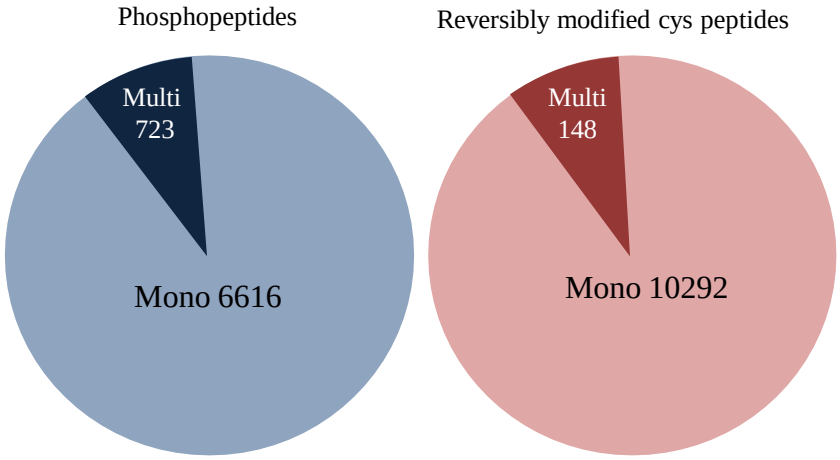
a) **Co-enriched proteins**



b) **Co-enriched unique peptides**



c) **PTM sites distribution**



d) **PTM sites summary**

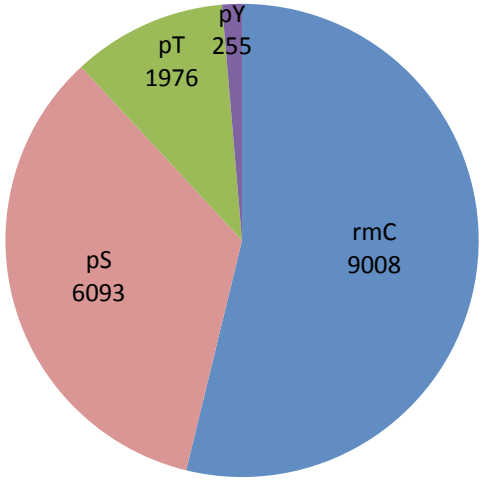


Figure 5

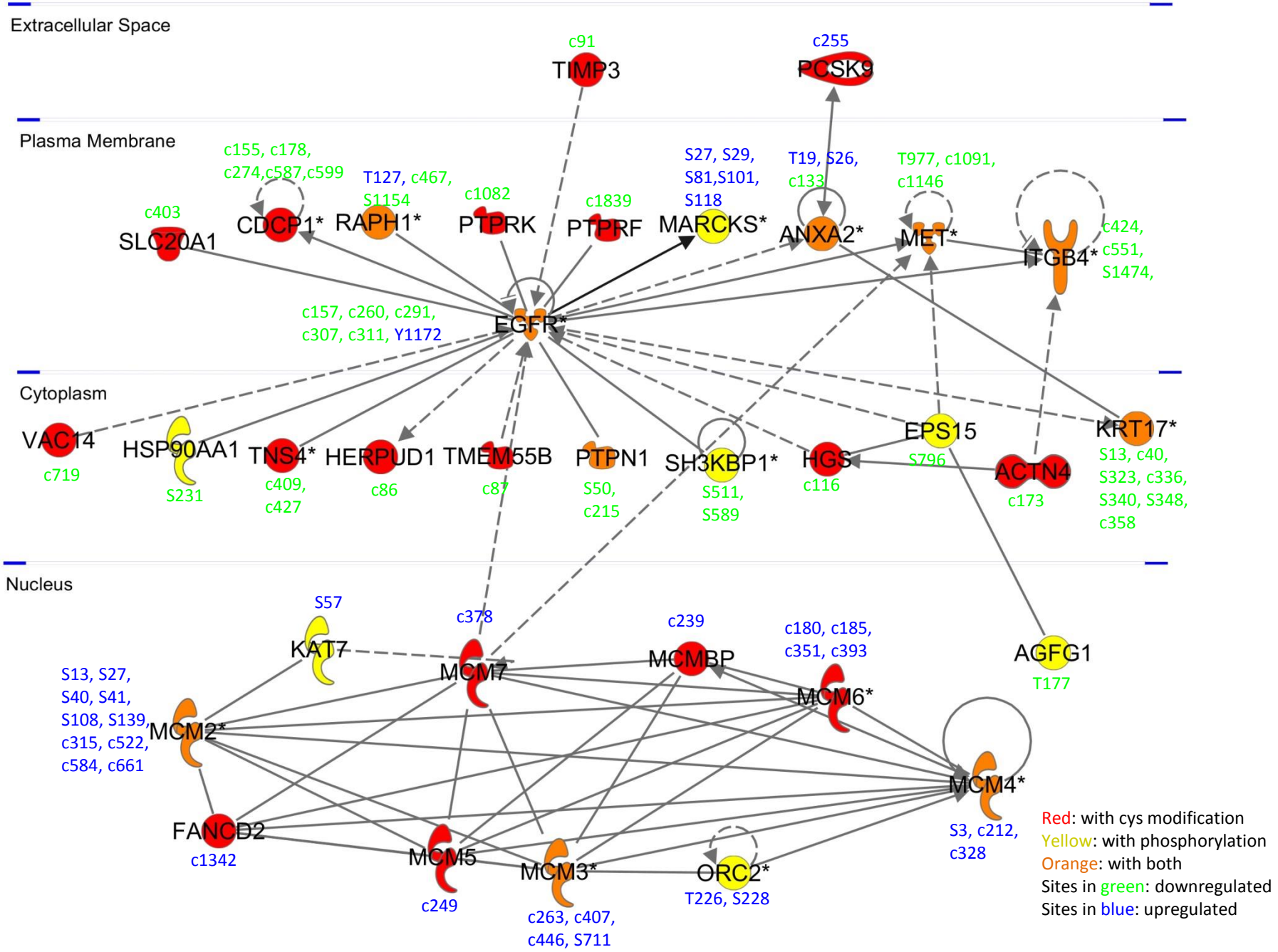
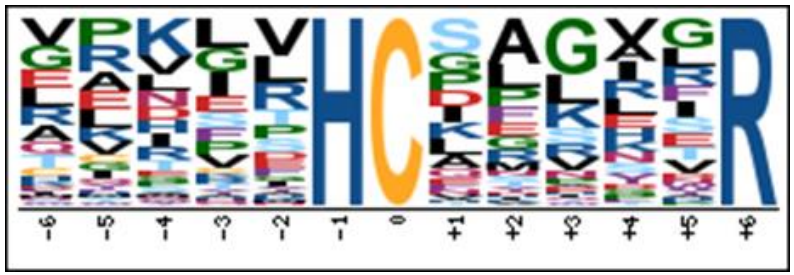

Extracellular Space
Plasma Membrane
Cytoplasm
Nucleus
TIMP3
c91
PCSK9
c255
SLC20A1
c403
CDCP1*
c155, c178, c274,c587,c599
RAPH1*
T127, c467, S1154
PTPRK
c1082
PTPRF
c1839
MARCKS*
S27, S29, S81,S101, S118
ANXA2*
T19, S26, c133
MET*
T977, c1091, c1146
ITGB4*
c424, c551, S1474,
EGFR*
c157, c260, c291, c307, c311, Y1172
VAC14
c719
HSP90AA1
S231
TNS4*
c409, c427
HERPUD1
c86
TMEM55B
c87
PTPN1
S50, c215
SH3KBP1*
S511, S589
HGS
c116
EPS15
S796
ACTN4
c173
KRT17*
S13, c40, S323, c336, S340, S348, c358
KAT7
S57
MCM7
c378
MCMBP
c239
MCM6*
c180, c185, c351, c393
AGFG1
T177
MCM2*
S13, S27, S40, S41, S108, S139, c315, c522, c584, c661
MCM4*
S3, c212, c328
FANCD2
c1342
MCM5
c249
MCM3*
c263, c407, c446, S711
ORC2*
T226, S228
Red: with cys modification
Yellow: with phosphorylation
Orange: with both
Sites in green: downregulated
Sites in blue: upregulated

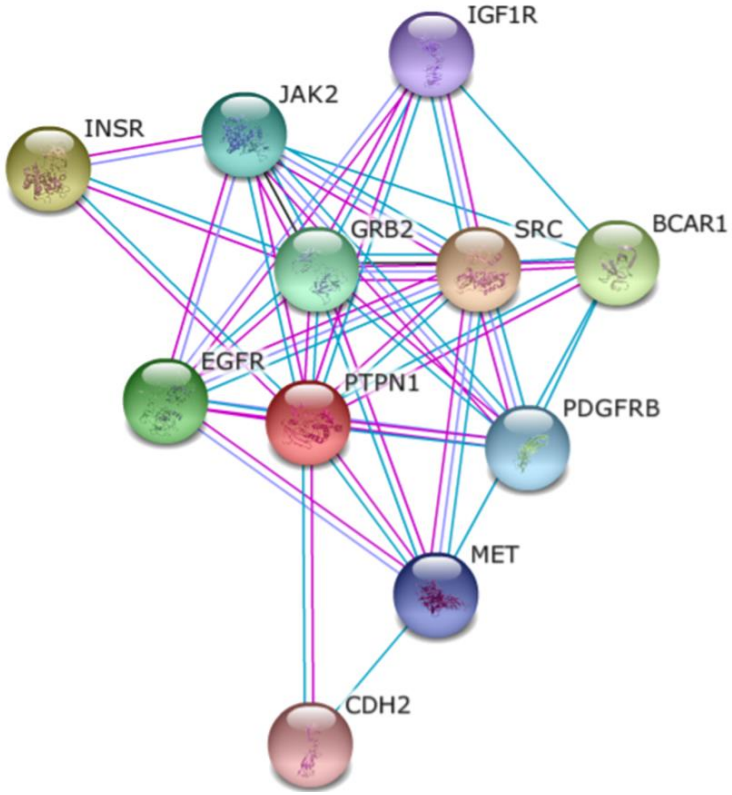
Figure 6

a)



Peptides	PTPs	Location	log2(H/L)	STD
ESGSLSPEHGPVVVH*CSAGIGR	**PTPN1**	**C215**	**-1.08**	**0.32**
EQFGQDGPITVH*CSAGVGR	**PTPRF**	**C1839**	**-2.37**	**0.32**
LSNPPSAGPIVVH*CSAGAGR	**PTPRK**	**C1082**	**-2.30**	**0.24**
GEAVGVH*CALGFGR	DUSP23	C95	-1.97	NA
ESGSLNPDHGPAVIH*CSAGIGR	PTPN2	C216	0.21	NA
VKACNPQYAGAIVVH*CSAGVGR	PTPRA	C442	-0.05	NA
TIIH*CLNGGGR	PTPRK	C1376	NA	NA

b)



c)

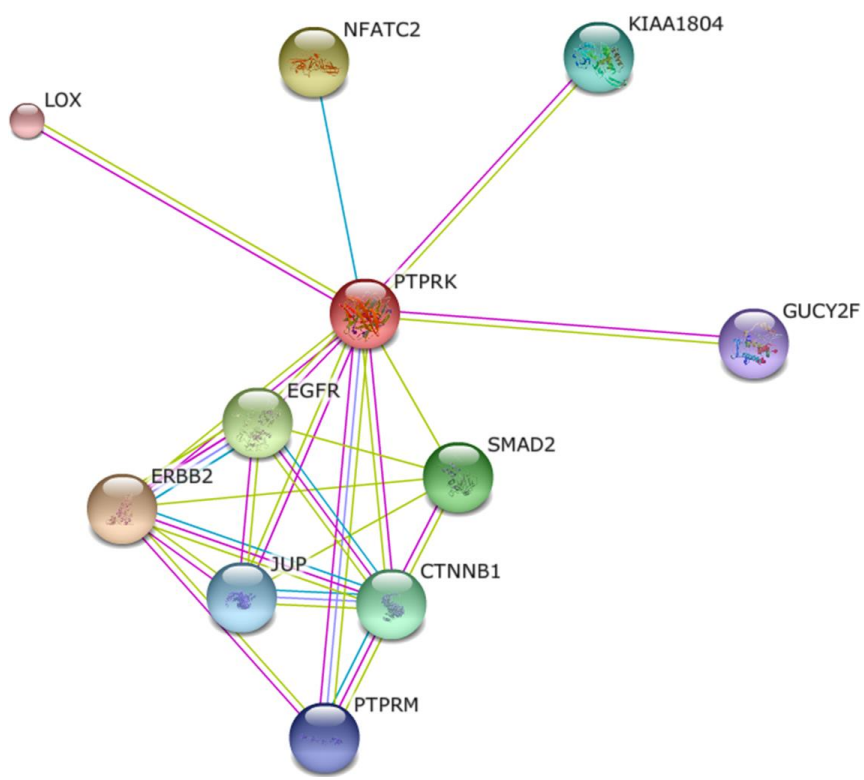
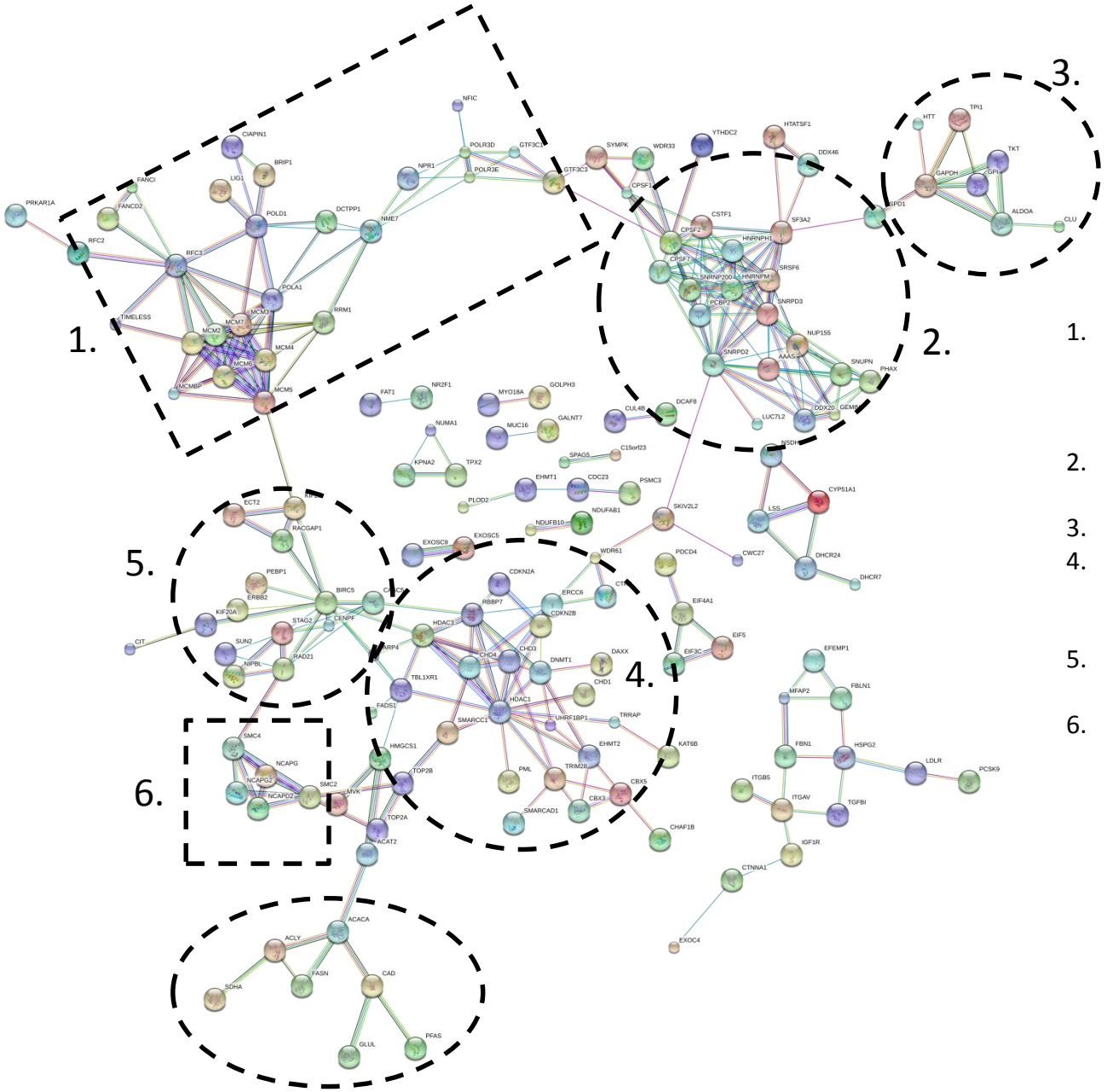


Figure 7

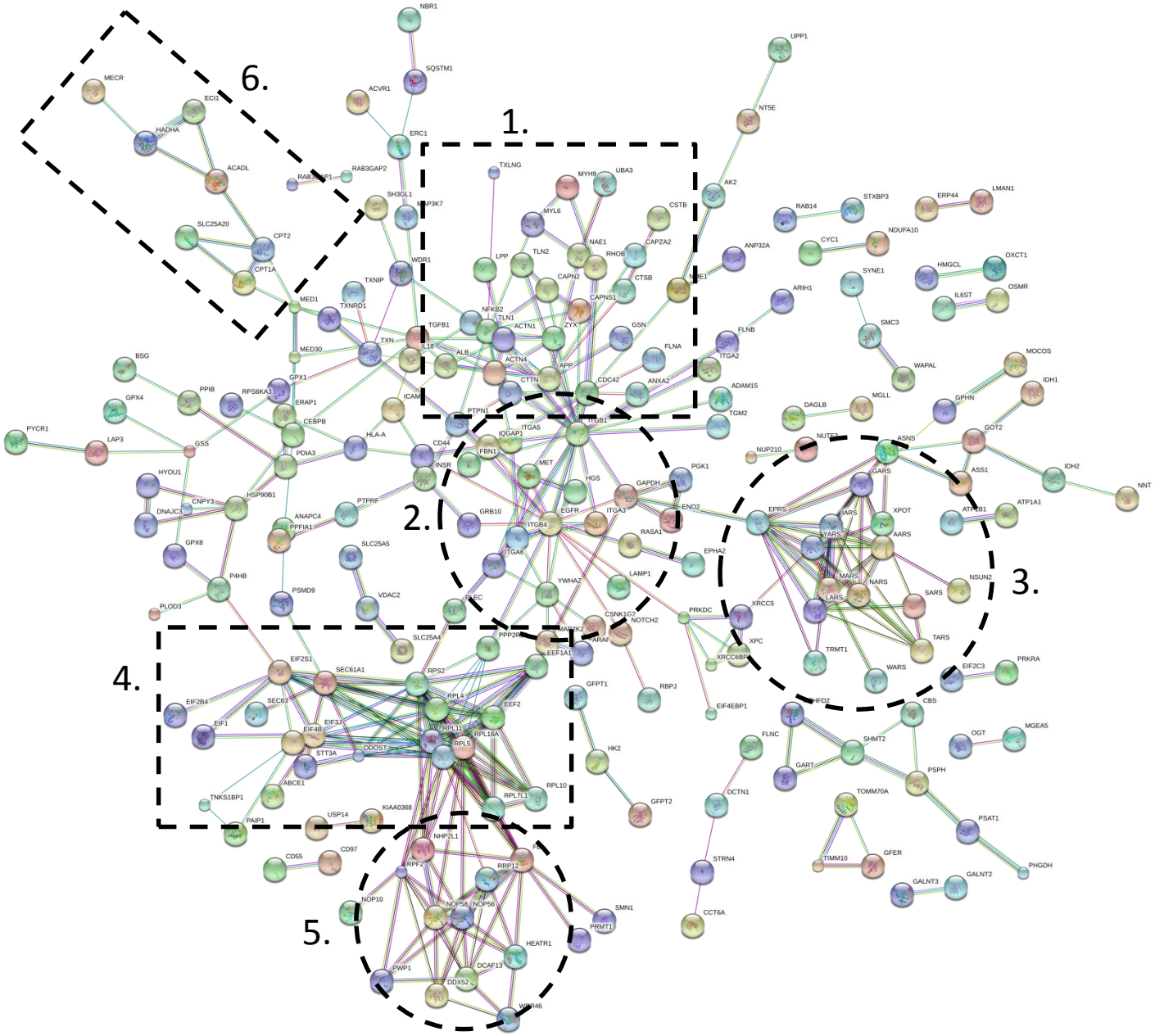
Up-regulated SIA proteins



1. DNA replication, incl transctiption factors, polymerases and the Minichromosome maintenance complex
2. Pre-mRNA processing transportation and splicing
3. Glycolysis
4. Chromatin remodeling, incl deacetylases and DNA methyltransferases
5. Cytokinesis and sister chromatid cohesion.
6. DNA condensin complex

Figure 8

Down-regulated SIA proteins



1. Integrin signaling with the cytoskeleton networks
2. EGFR signaling molecules
3. tRNA synthetases
4. Protein translation; ribosomes, initiation and elongation factors
5. Ribosomal biosynthesis and RNA splicing
6. fatty acid-oxidation