Proteomic snapshot of the EGF-induced ubiquitin network

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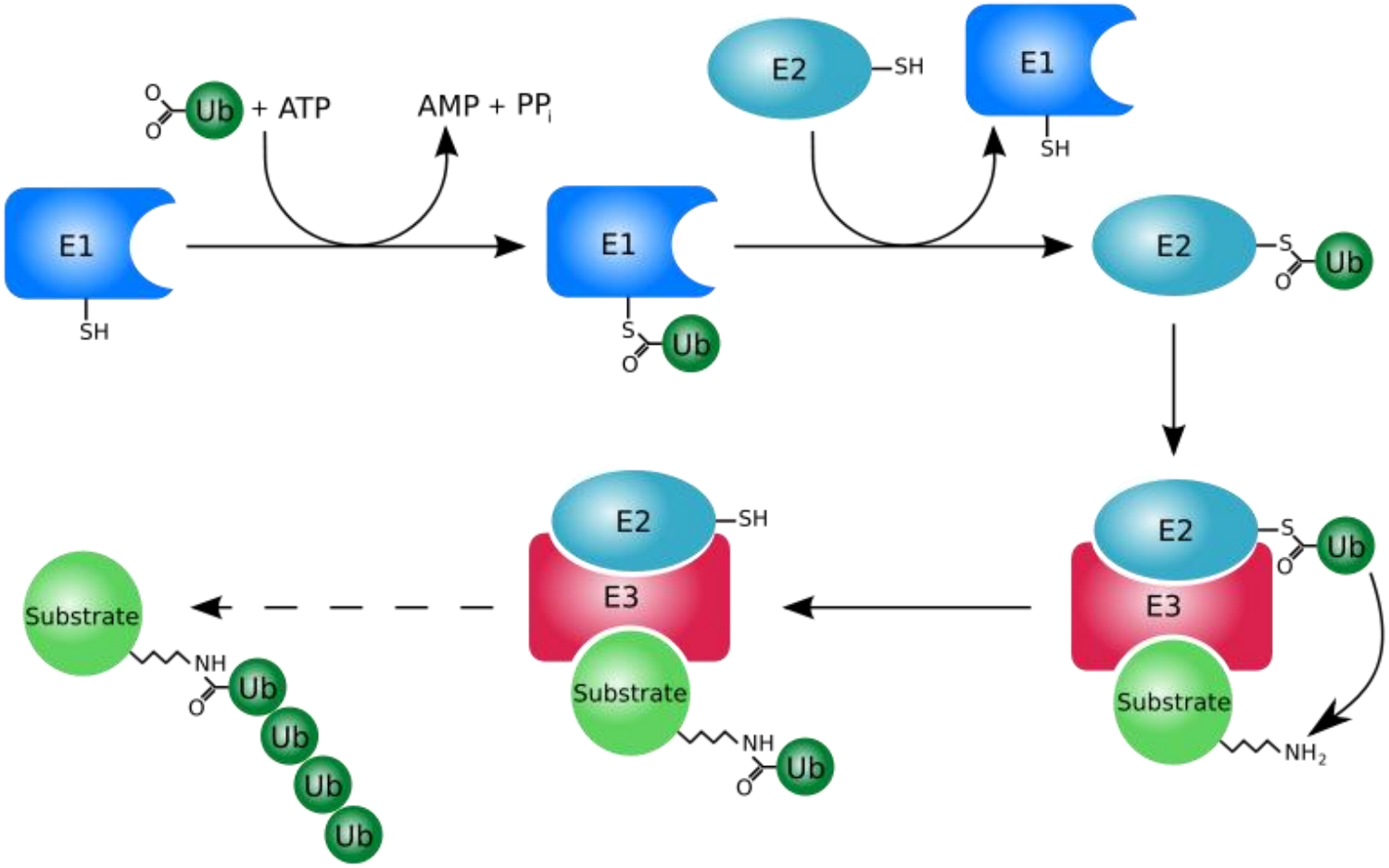
翻译后修饰
Post-translational modification（PTM）

- 加入官能团
- 乙酰化——通常于蛋白质的N末端加入乙酰。
- 烷基化——加入如甲基或乙基等烷基。
- 磷酸化——加入磷酸根至丝氨酸、酪氨酸、苏氨酸或组氨酸。
- 加入其他蛋白质或肽
- 泛素化——与泛素建立共价键。
- 改变氨基酸的化学性质
- 瓜氨化——将精氨酸转为瓜氨酸。
- 结构改变
- 二硫键——两个半胱氨酸间建立共价键。
- 分解蛋白质——将蛋白质的肽键剪开

泛素化
Ubiquitination



泛素化
Ubiquitination

- 调节蛋白质的功能
- 蛋白质的亚细胞定位
- 蛋白质与蛋白质之间相互作用

表皮生长因子
epidermal growth factor(EGF)

- 在EGF的激活下：
- 许多EGF受体会被泛素化修饰
- 对于泛素化后EGF受体在细胞内的去向，过去已有研究
- 但是目前依然缺乏一张高分辨率的泛素化图谱
- EGF-Ubiproteome

Purification of Ub-conjugated proteins

- “endogenous” approach
- FK2
- Hela cells
- Endogenous ub
- tandem affinity purification (TAP) approach
- FLAG-His-Ub
- B82L-BGFR cells
- TET-on inducible system
- Tagged ub

Identification of steady-state Ubiproteomes

- High resolution, high accuracy MS and stable isotope labeling with amino acids in cell culture (SILAC)
- Time point:EGF simulation 10min
- Filter

A

	Endogenous	TAP	NR	Overlap End/TAP	*P* (overlap)
Peptides raw	11722	3173			
Proteins raw	1765	744	2109	400	2.58×10^{-201}
Proteins after filtering	1175	582	1472	284	2.22×10^{-176}

The EGF-Ubiproteome

- SILAC data ⟶ MaxQuant ⟶ Protein quantitation
- Three-tiered selection process:
- Discard:P-value>0.1
- Discard:coefficent of variability>10
- The same trend of regulation in the experimental replicates

A

	Filter	Endog.	TAP	NR	Overlap	*P* (overlap)
Ubiproteome	None	1175	582	1472	284	2.22×10^{-176}
EGF-Ubiproteome	A	216	115	315	16	1.81×10^{-12}
	B	176	105	265	16	1.72×10^{-14}

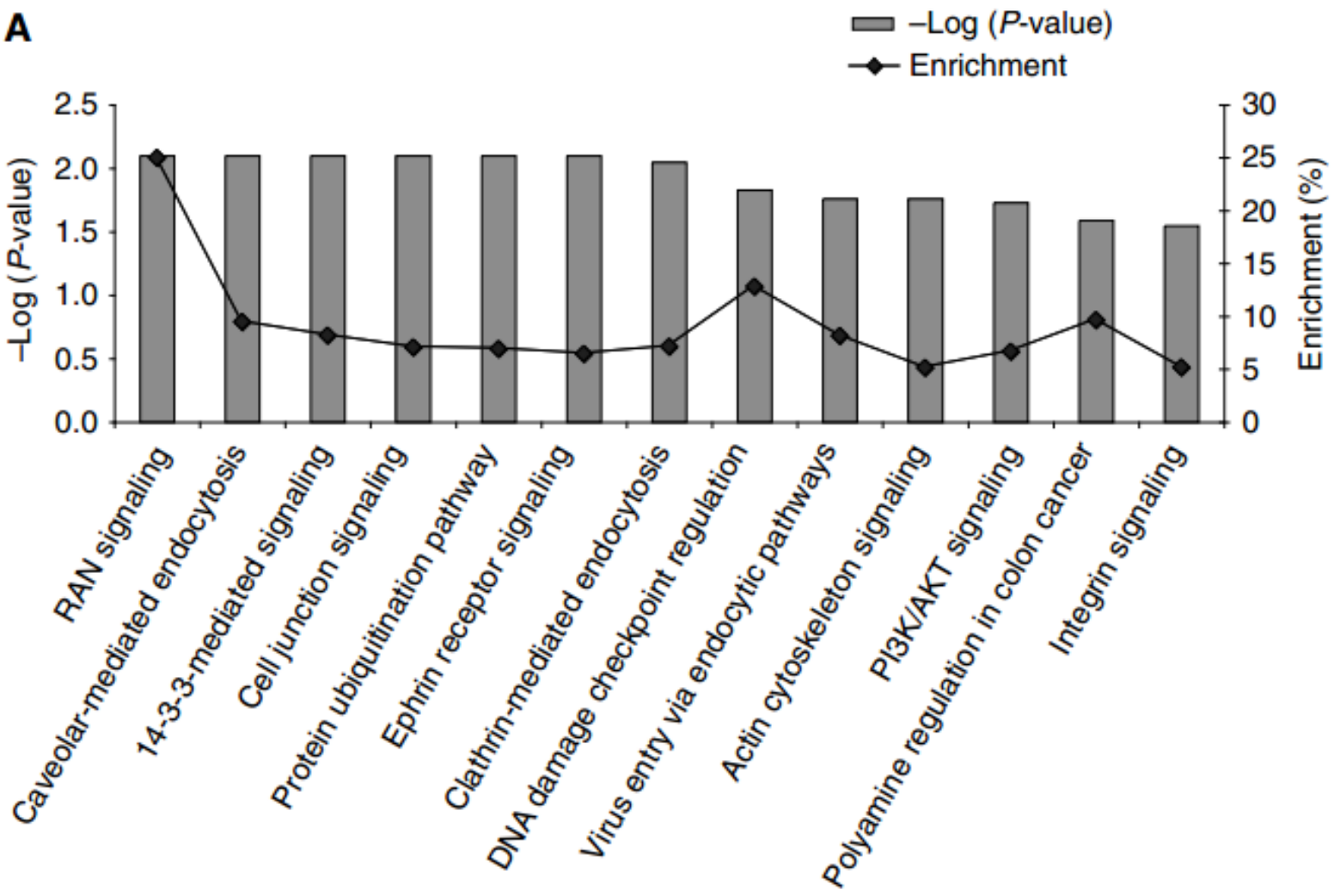
Chain topology of the EGF-regulated Ubiproteome

- SILAC ⟶ MS analysis ⟶ Ub “signature” peptides

- TAP approach: an increase in the K63-, K11- and K6-chain modifications after EGF stimulation
- Endogenous approach: only K63 linkages accumulated

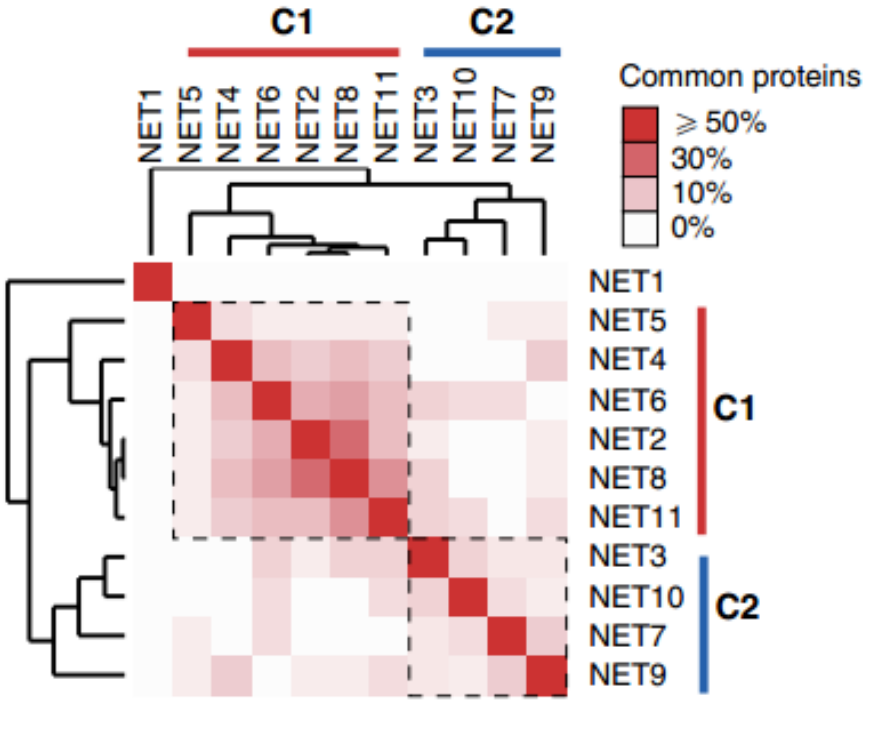
Network analysis of the EGF-regulated Ubiproteome

- NR-EGF-Ubiproteome
- Ingenuity Pathways Knowledge Software
- canonical pathways: endocytosis, virus entry pathways, many circuitries of intracellular signaling
- Distinct interaction networks
- 11 networks
- unsupervised clustering


A
–Log (P-value)
Enrichment
–Log (P-value)
Enrichment (%)
2.5
2.0
1.5
1.0
0.5
0.0
30
25
20
15
10
5
0
RAN signaling
Caveolar-mediated endocytosis
14-3-3-mediated signaling
Cell junction signaling
Protein ubiquitination pathway
Ephrin receptor signaling
Clathrin-mediated endocytosis
DNA damage checkpoint regulation
Virus entry via endocytic pathways
Actin cytoskeleton signaling
PI3K/AKT signaling
Polyamine regulation in colon cancer
Integrin signaling

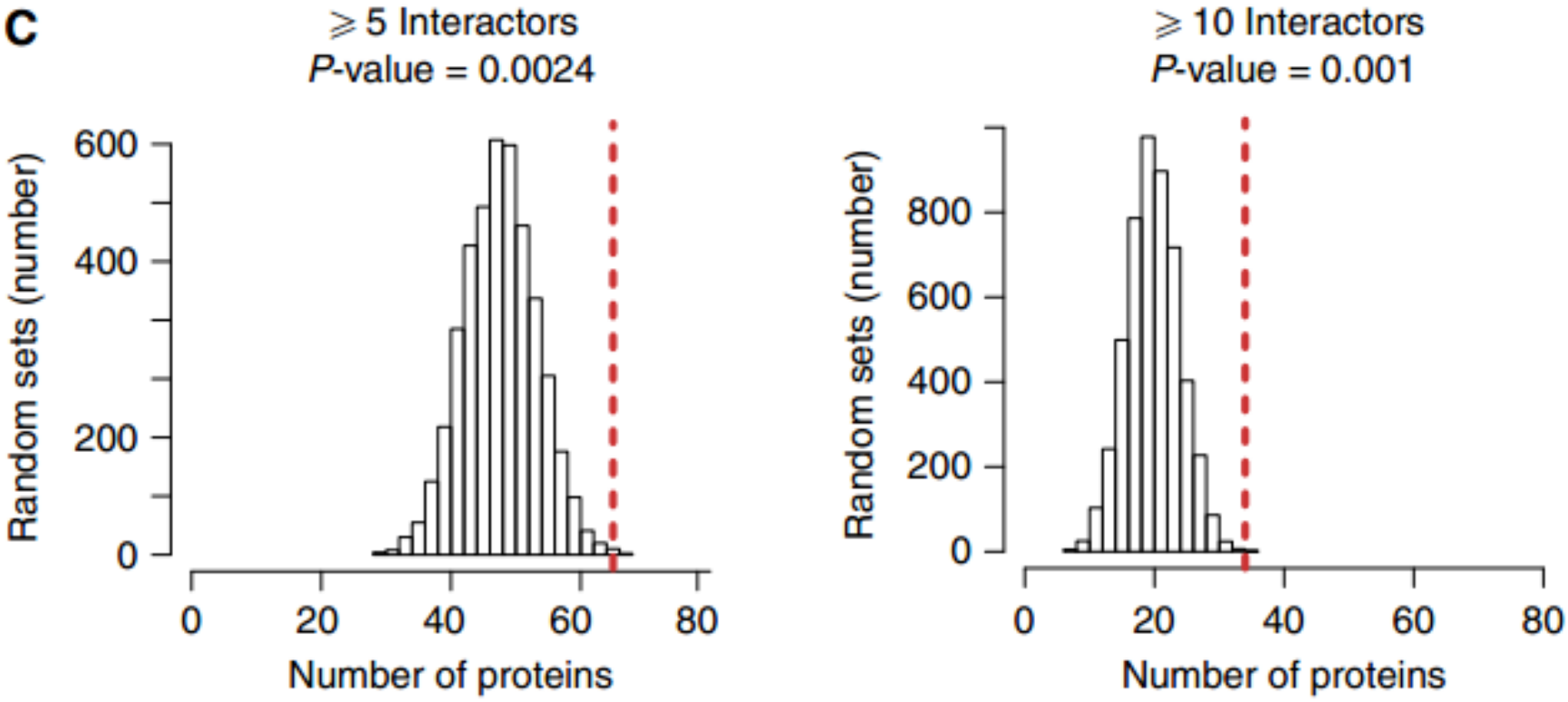
B

NET	Molecules	Description	Relevance
1	17/23	Ribosomes/translation	35%
2	17/35	Toll-like receptor signaling/RAS oncogenic signature/NFKB regulated/Hypoxia	49%
3	15/27	Calpains pathway/TID pathway/p53 signaling/HIF targets/Myc targets	41%
4	15/35	ERK pathway/EGFR signaling/IL6 pathway/focal adhesion/proliferation	51%
5	14/35	Insulin signaling/adipocytokine siganling/GCR signaling	34%
6	14/35	TGFb regulated/calcineurin signaling/inflammation	37%
7	14/35	Adherens junction/cell cycle	31%
8	13/35	Cytokine-cytokine receptor interaction/RAS oncogenic signature/NFKB regulated	34%
9	13/35	Adherens junction/ERBB signaling pathway/focal adhesion/CXCR4 pathway	49%
10	12/35	p53 signaling/apoptosis/cell cycle/drug resistance and metabolism	51%
11	12/35	Proliferation cell cycle/calcineurin -NF AT signaling/DNA damage	60%



Network analysis of the EGF-regulated Ubiproteome

- Cluster C1:proliferation and inflammation
- Cluster C2:apoptosis, adhesion and cell cycle
- Network 1:ribosomal proteins
- Hubs:
- Proteins with >=5 interactors or >=10 interactors
- 65 hubs

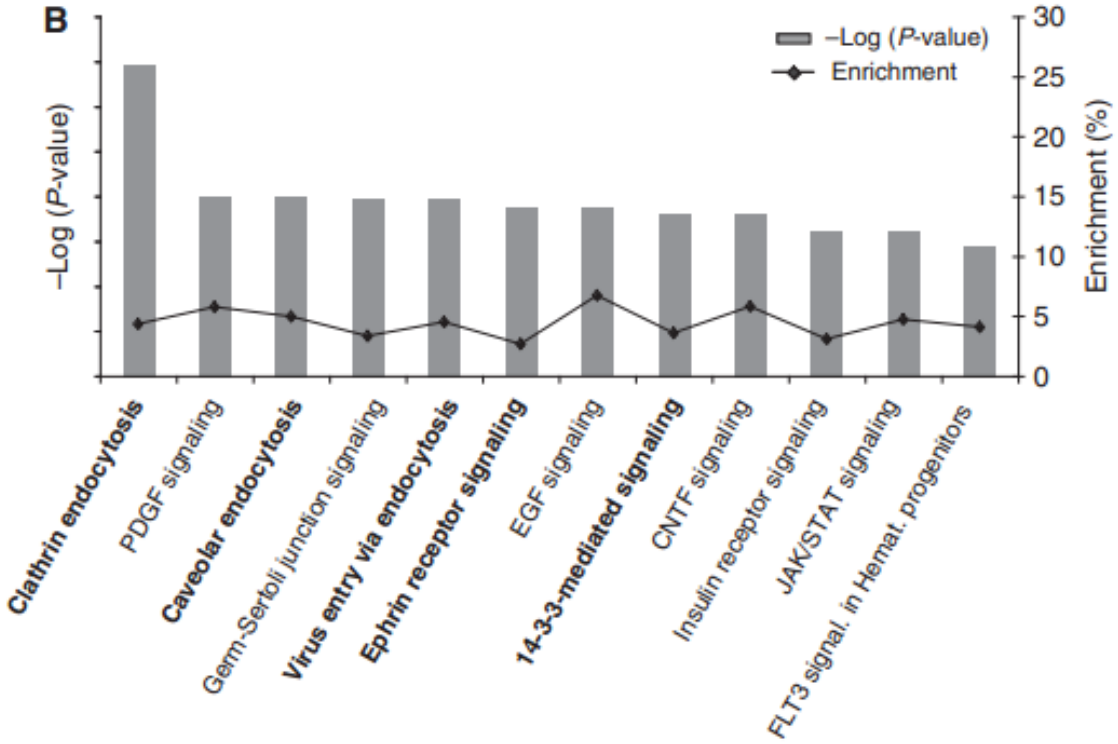

C
⩾ 5 Interactors
P-value = 0.0024
Random sets (number)
600
400
200
0
0
20
40
60
80
Number of proteins
⩾ 10 Interactors
P-value = 0.001
Random sets (number)
800
600
400
200
0
0
20
40
60
80
Number of proteins

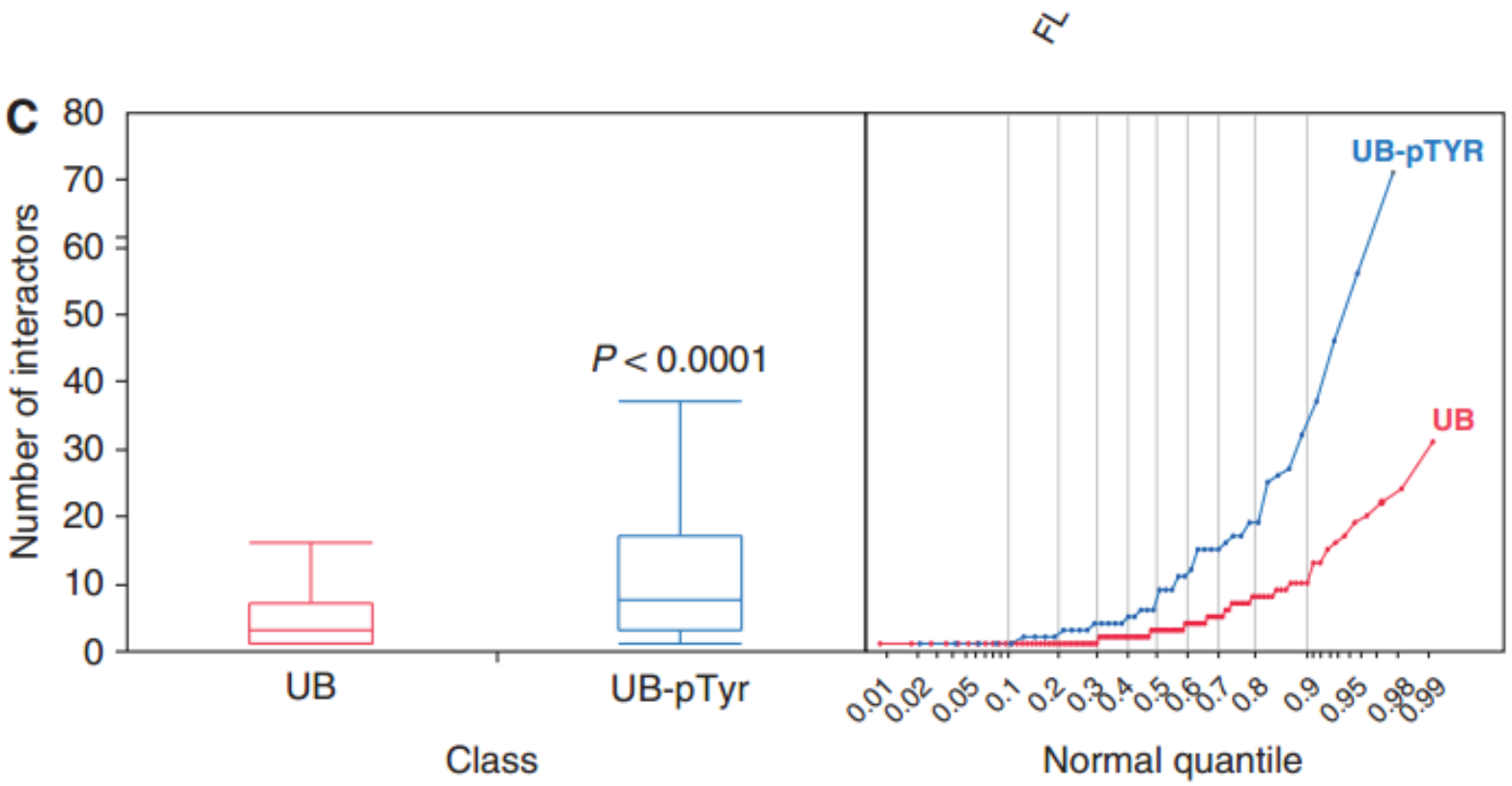
Intersection of the EGF-induced Ubi- and phosphotyrosine proteomes

- EGF-Ubiproteome and EGF-pY proteomes
- pY- phosphotyrosine
- 23% (61 of 265) of the EGF-Ubiproteome proteins are also tyrosine phosphorylated
- Pathway analysis
- endocytic and signal-transductio pathways
- Hub analysis

A

	EGF-Ubiproteome (265 proteins)	
	Overlap (*N*)	*P*
EGF-pY Blagoev (*N*=81)	18	4.6×10^{-16}
EGF-pY Oyama (*N*=136)	25	1.0×10^{-19}
EGF-pY Hammond (*N*=93)	18	6.2×10^{-15}
Phospho.ELM (*N*=687)	38	1.9×10^{-11}
All (*N*=900)	61	1.5×10^{-22}




FL
C
Number of interactors
80
70
60
50
40
30
20
10
0
P < 0.0001
UB
UB-pTyr
Class
UB-pTYR
UB
0.01
0.02
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0.95
0.98
0.99
Normal quantile