

DUB Project

Mapping protein deubiquitinase substrates in health and disease

Barcelona
09/11/2018

Proteored: CNB, CNIO, CRG, UPV/EHU
BNLC: CMRB

Started 16th March 2017

Why?

- Failures on protein homeostasis are at the onset of many diseases
- The ubiquitin proteasome system is key to protein homeostasis
- DUB enzymes are reported to be feasible pharmaceutical targets

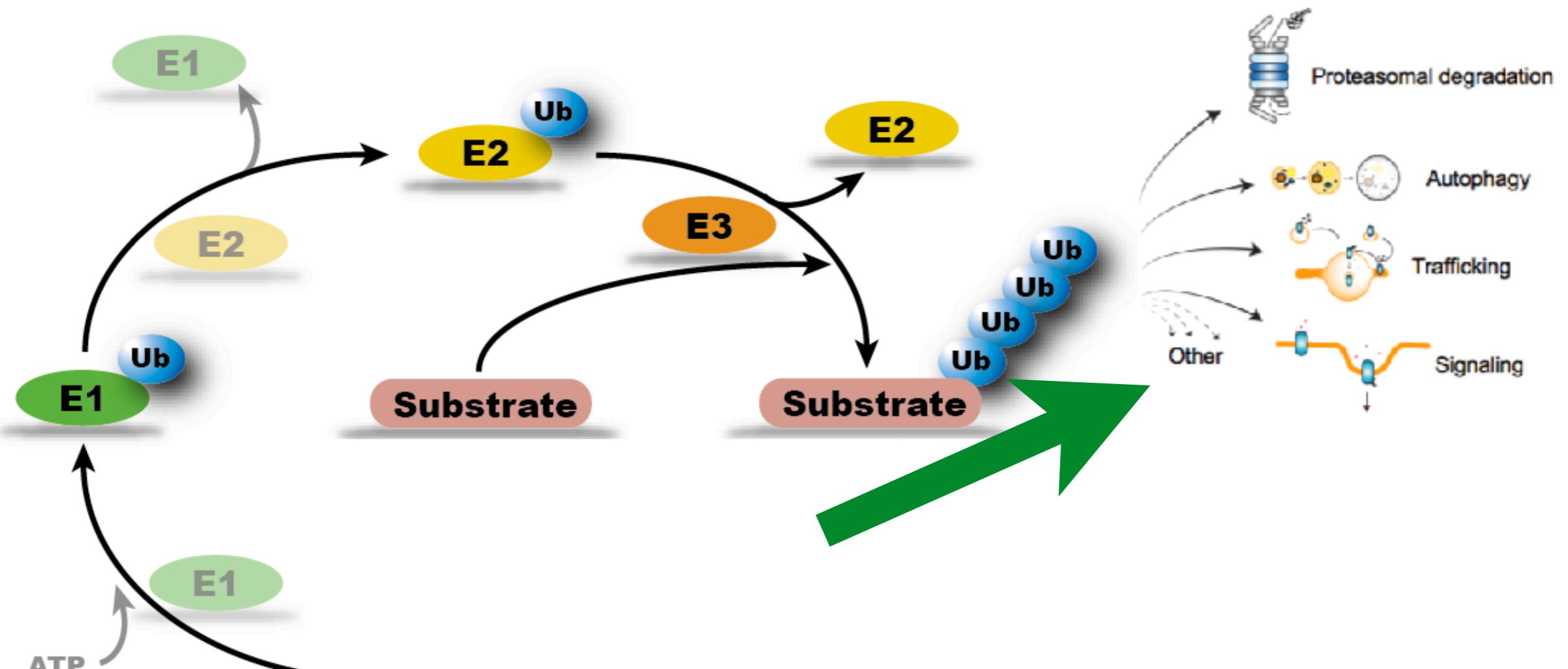
Mission

- Perform a pilot study to later identify substrates of all human DUBs
- Explore the application of the bioUb system in iPSC cell lines

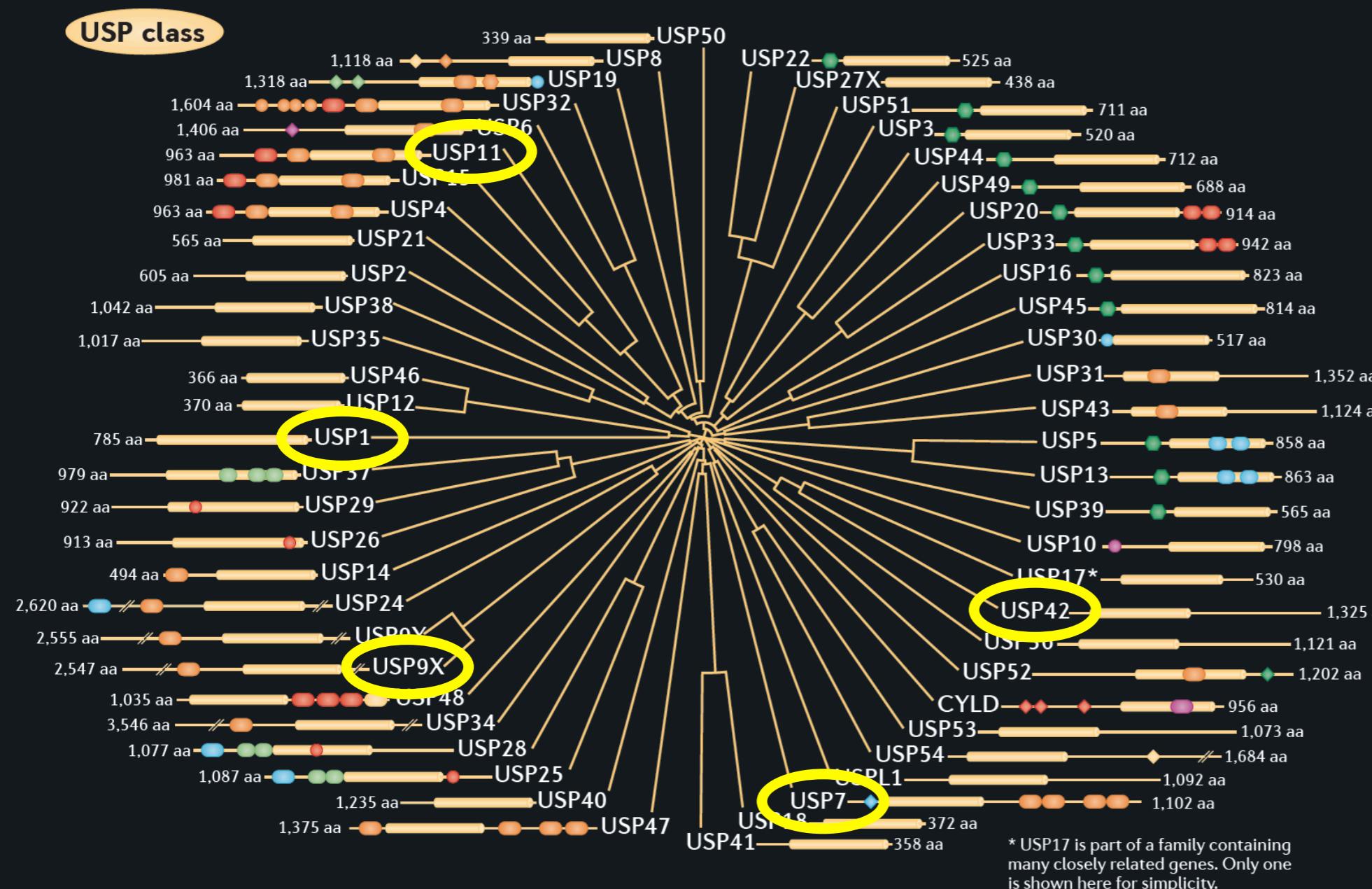
Aims for the pilot study

1. Optimize protocol and identify the substrates of at least 4 DUBs
2. Establish the bioUb expression on cells from the BNLC

The ubiquitin system

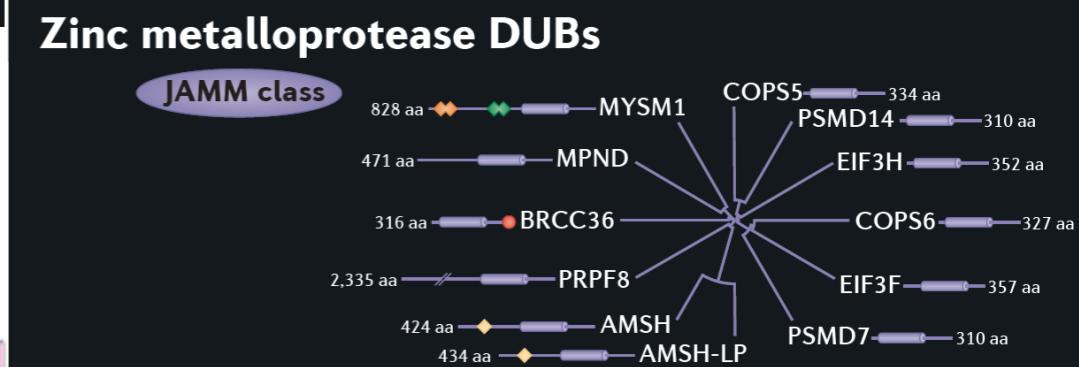
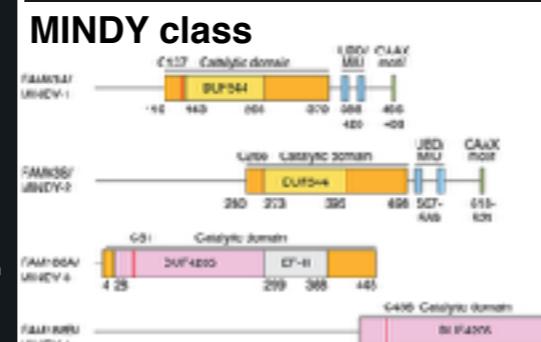
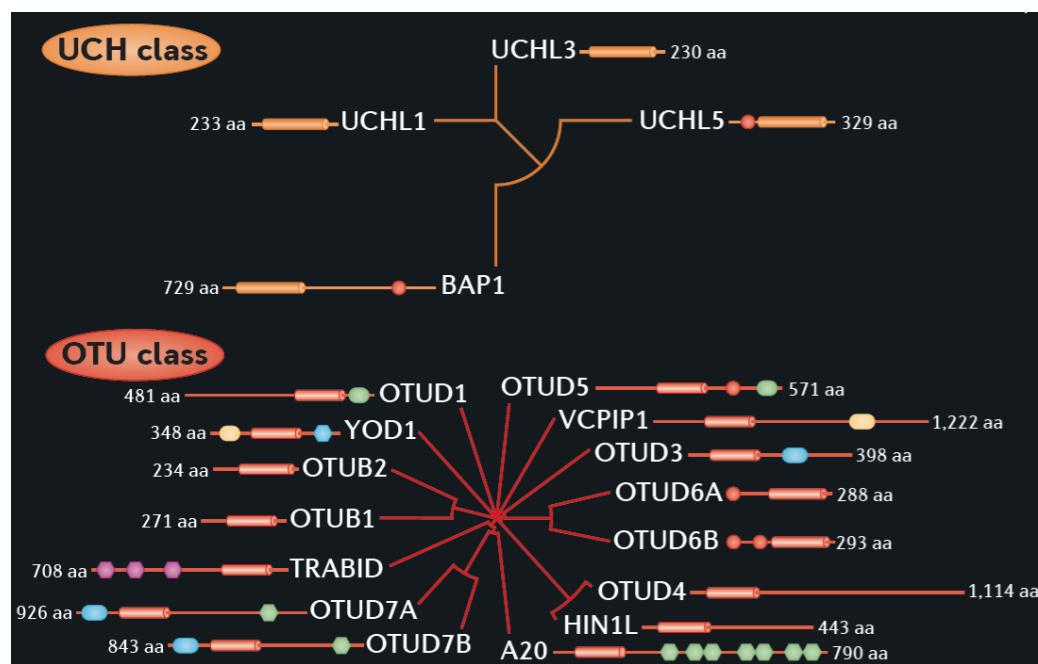


Cysteine protease DUBs

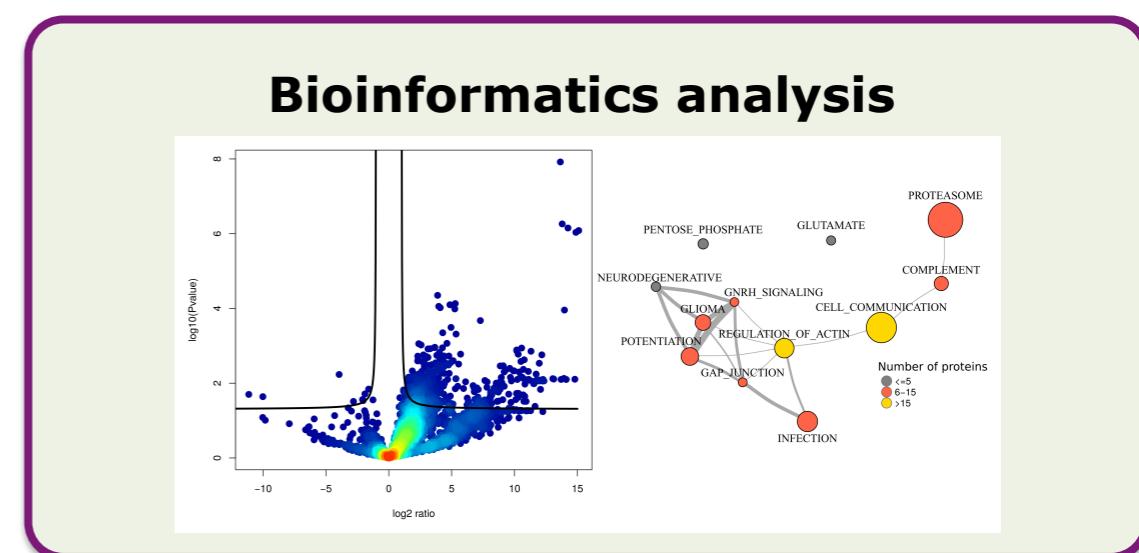
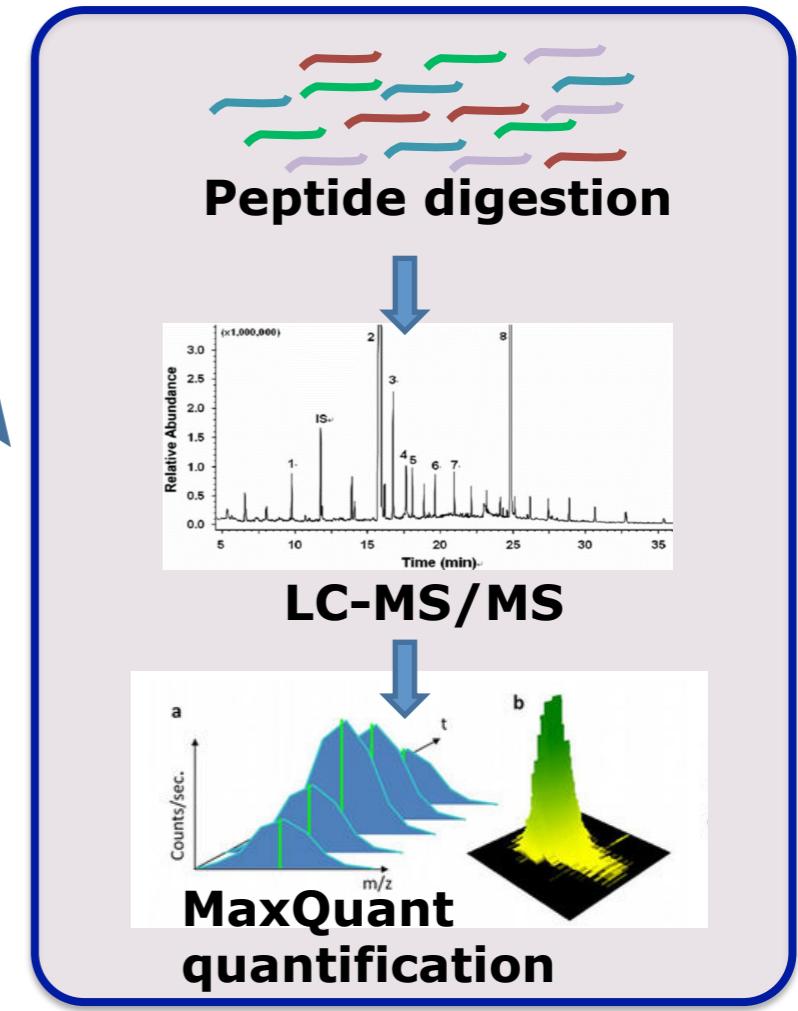
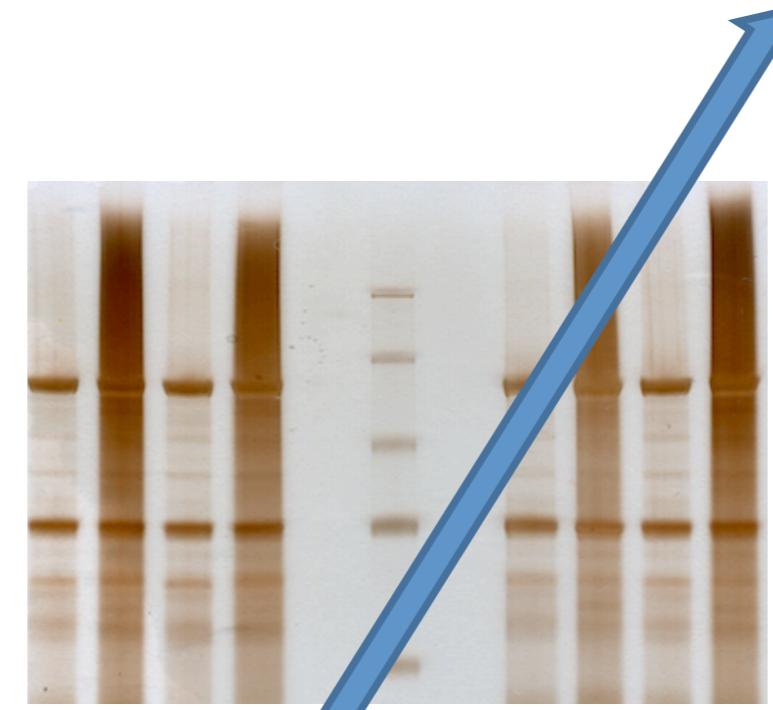
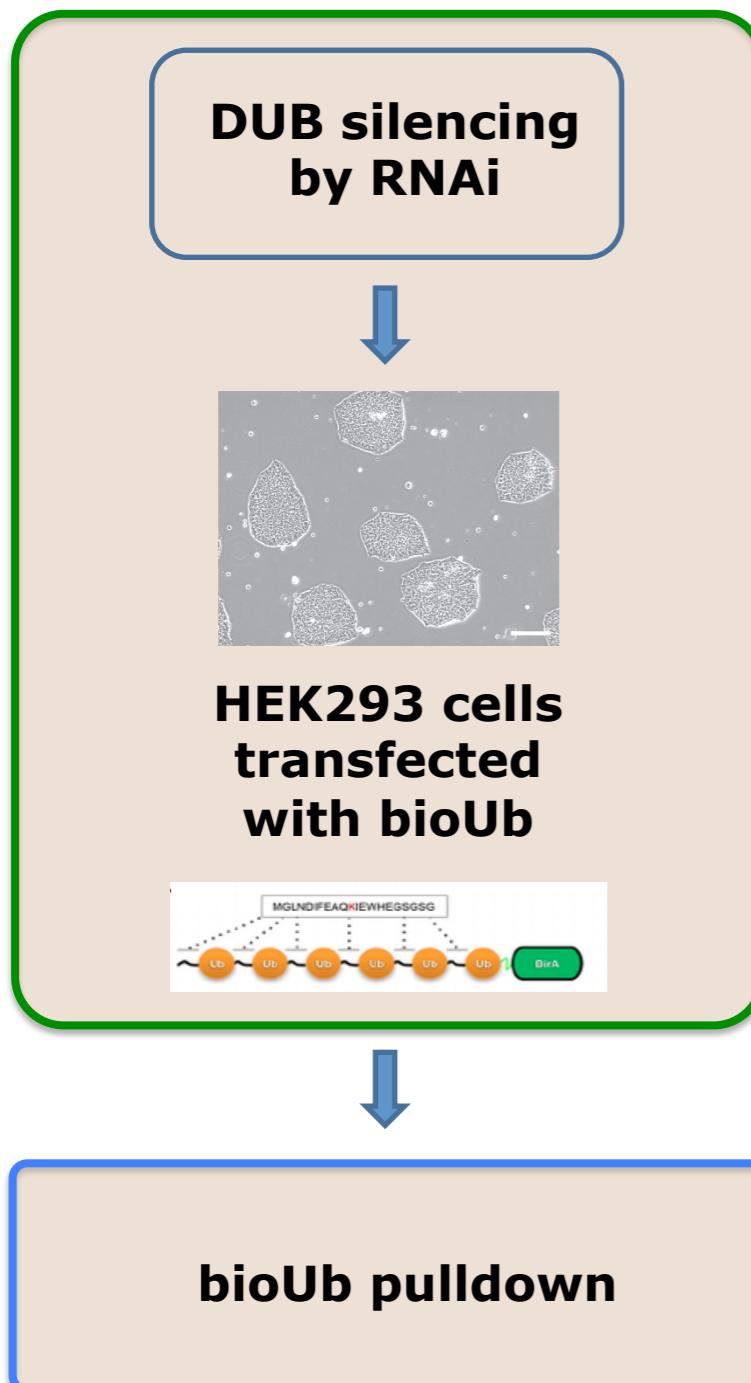


Domains
USP
UCH
OTU
Josephin
JAMM
ZnF-UBP
ZnF-MYND
ZnF-A20
NZF
ZnF-C2H2
B-box
UBL
DUSP
UBA
UIM
MIT
Rhodanese
Exonuclease
TBC-RabGAP
CAP-Gly
MATH
CS
Transmembrane
EF-hand
Ataxin 2C
Coiled-coil
SWIRM
SANT

Additional protein sequence not shown



Work-flow for Aim 1

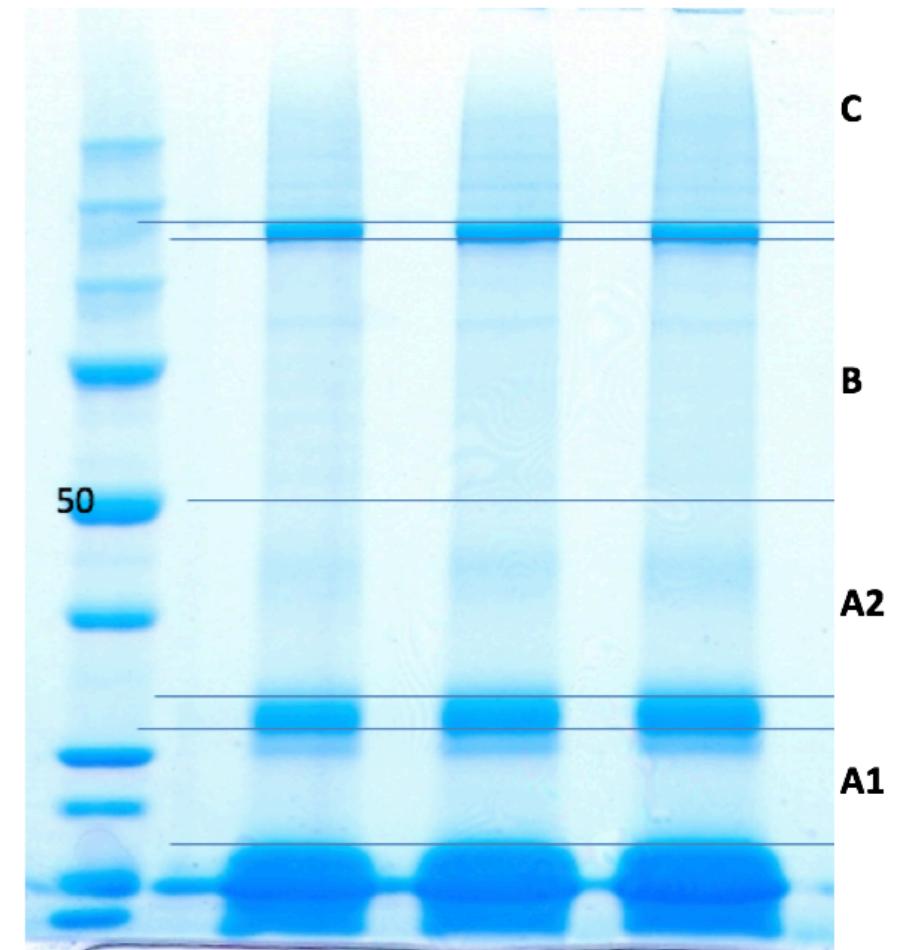


Optimizing the workflow

✓ Preparation of 4 replica at a time. Then run 3 on MS.

✓ In-Gel digestion better than FASP or In-bead digestion: also exclusion of Avidin and endogenous carboxylases

✓ 3 injections better than 1. Acquisition gradient: 120 min



Instruments used



Q Exactive

TripleTOF 5600

Q Exactive Plus

Q Exactive HF-X

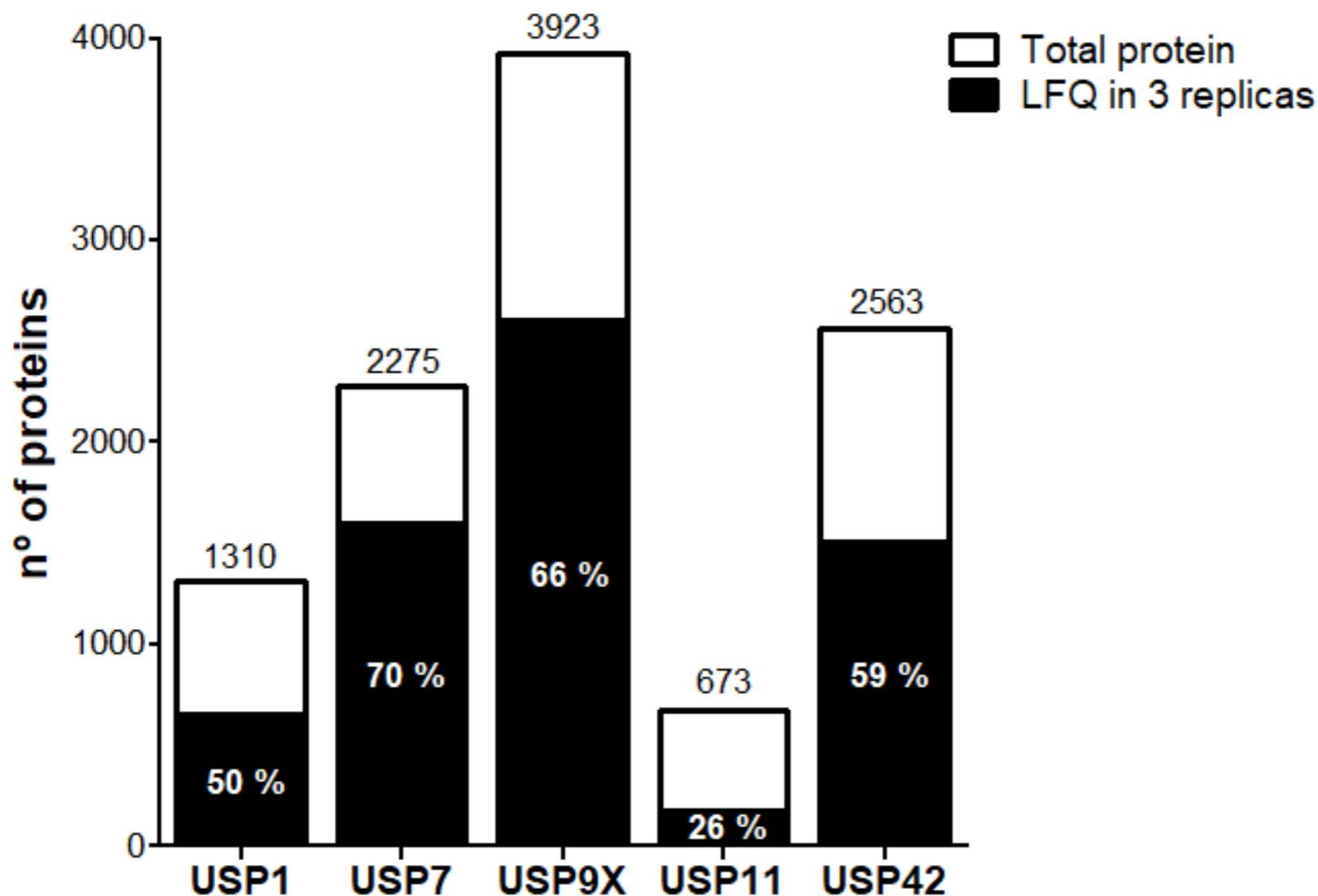
Orbitrap Fusion Lumos

RESULTS

Analysis tested

- MaxQuant + Perseus
- MaxQuant + Prostar (Limma)
- Proteobiotics

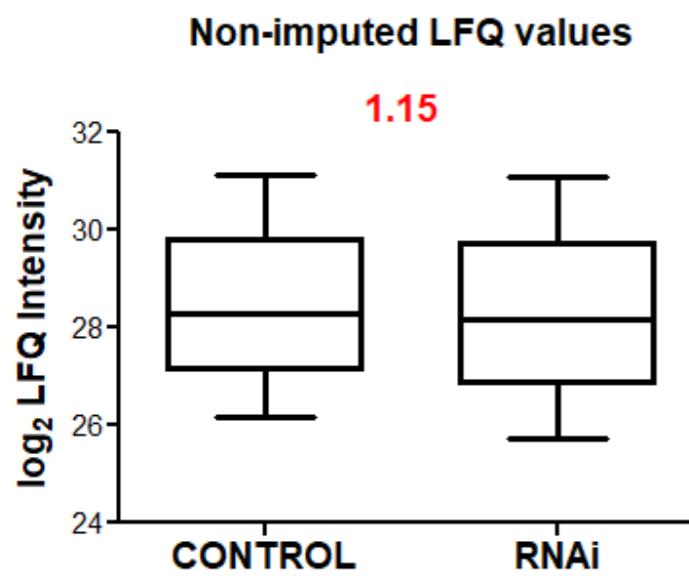
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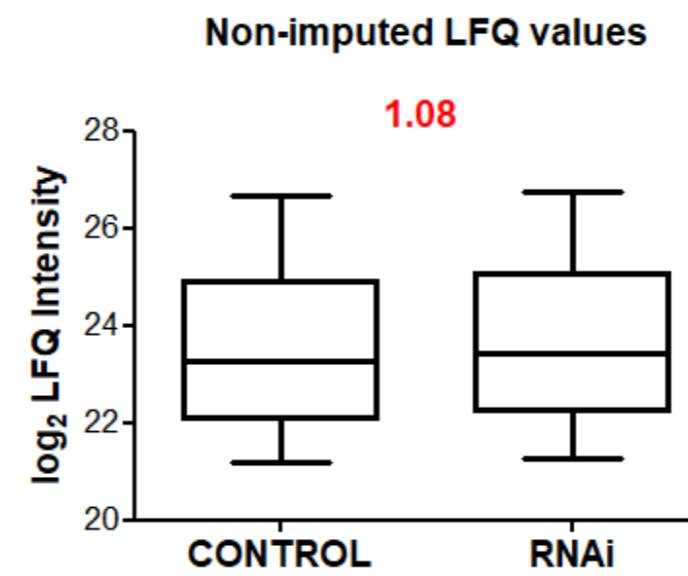
At least 1 unique peptide, and LFQ value in at least one sample

Imputed and non-imputed values

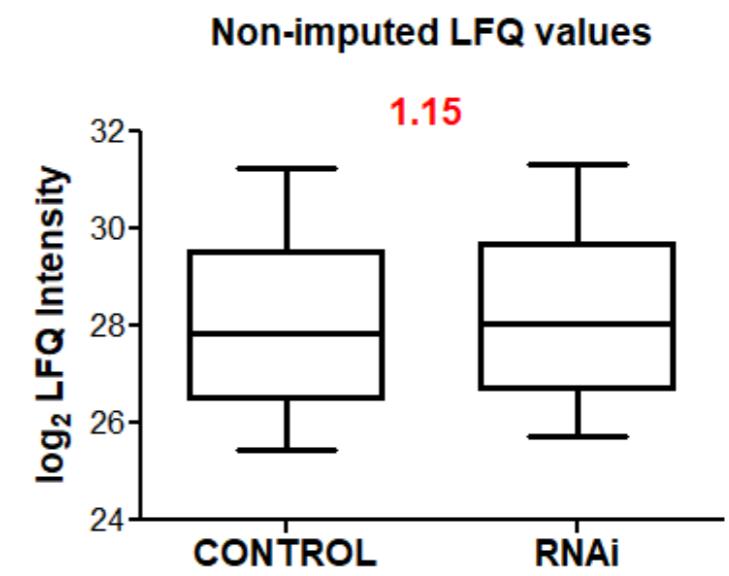
USP1



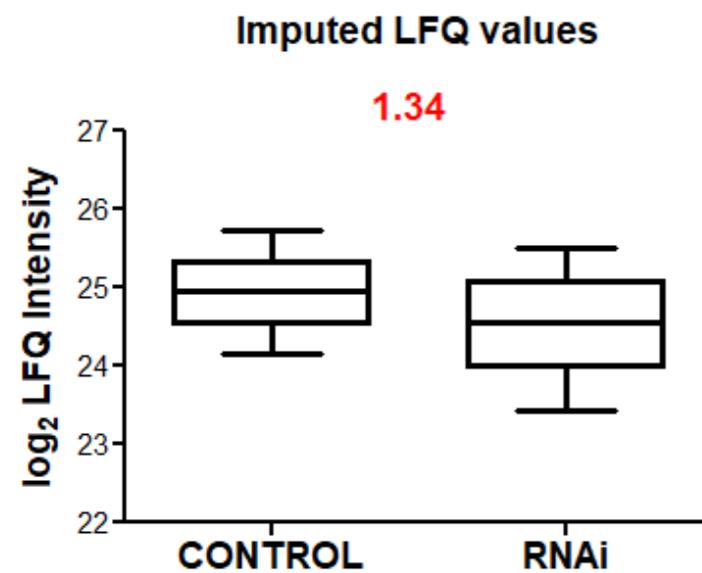
USP7



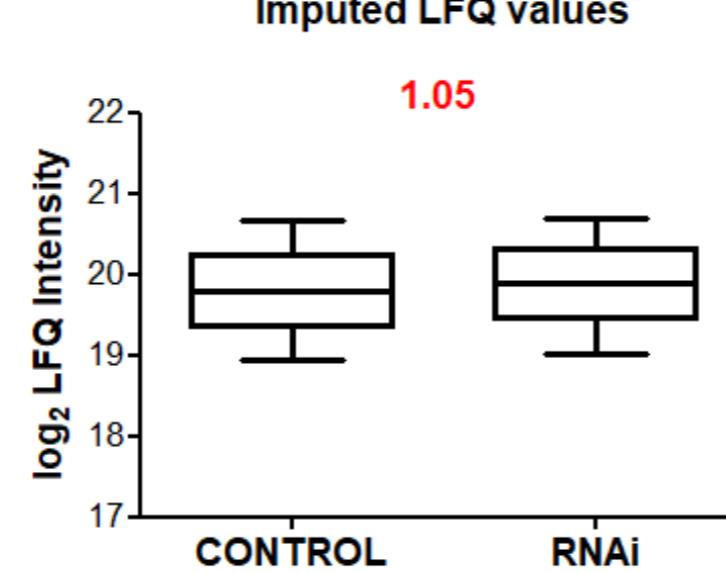
USP9X



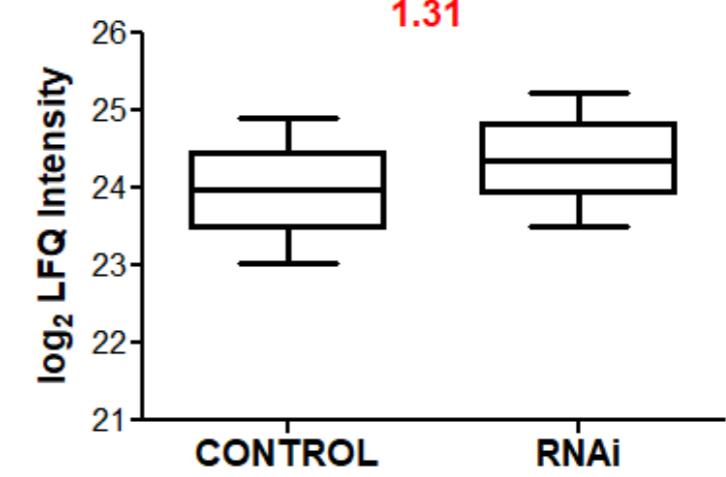
Imputed LFQ values



Imputed LFQ values

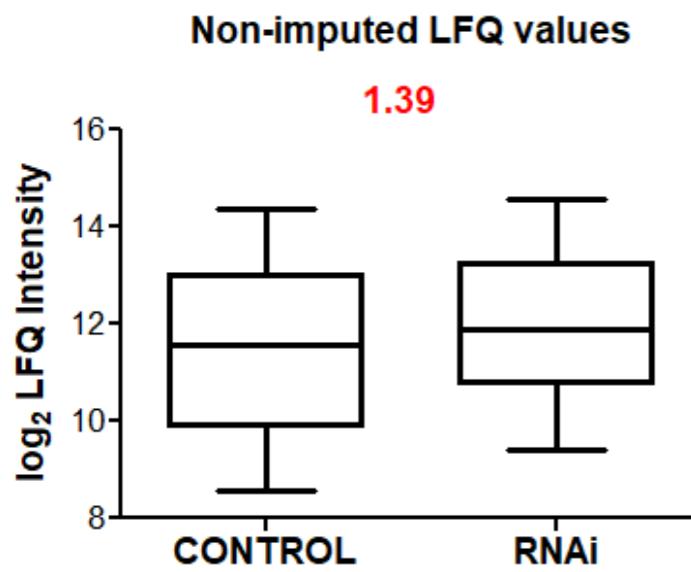


Imputed LFQ values

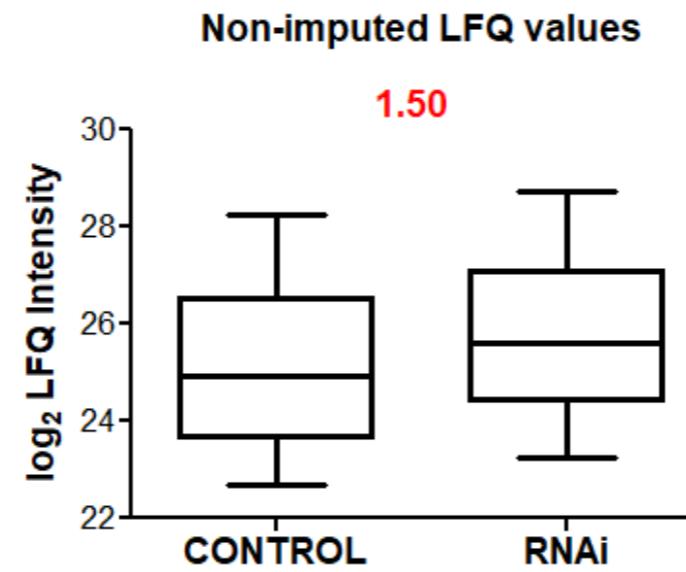


Imputed and non-imputed values

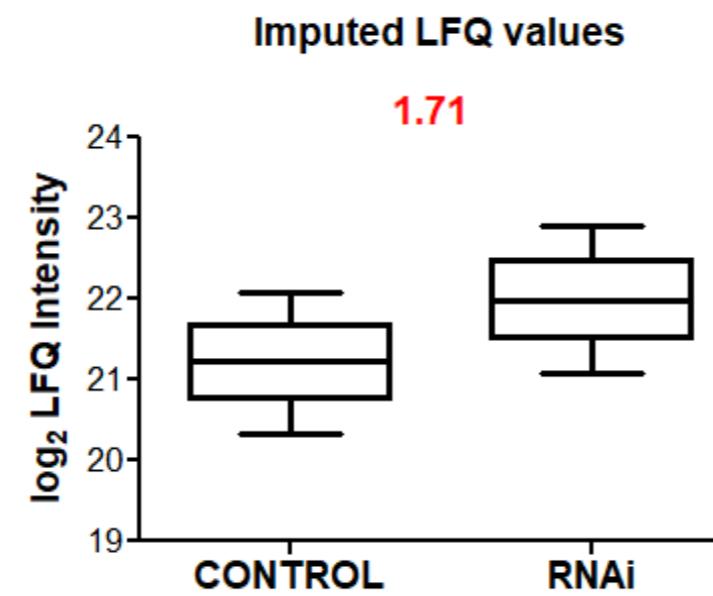
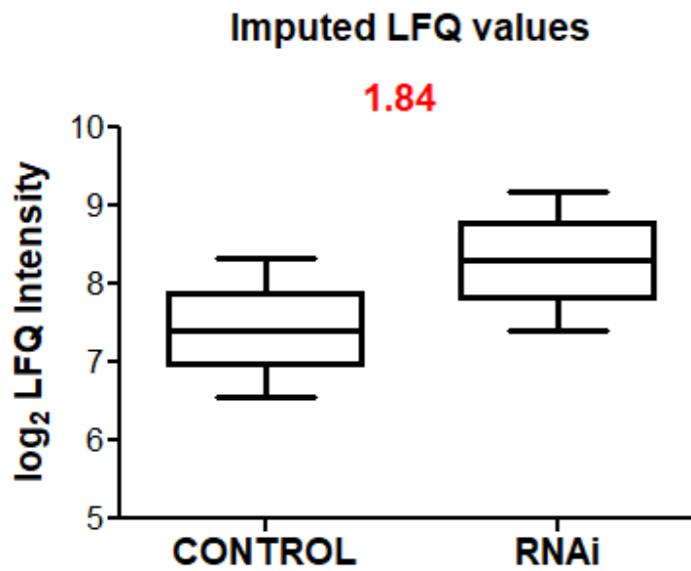
USP11



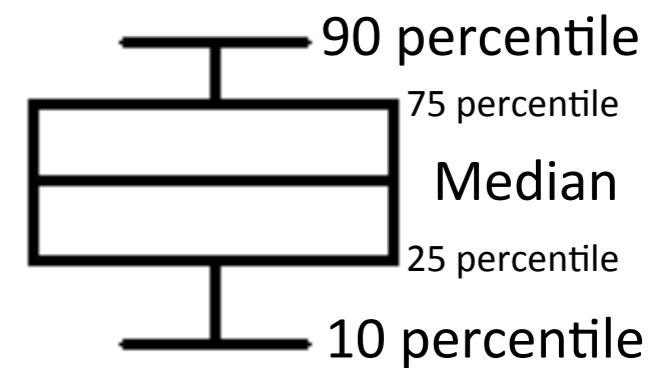
USP42



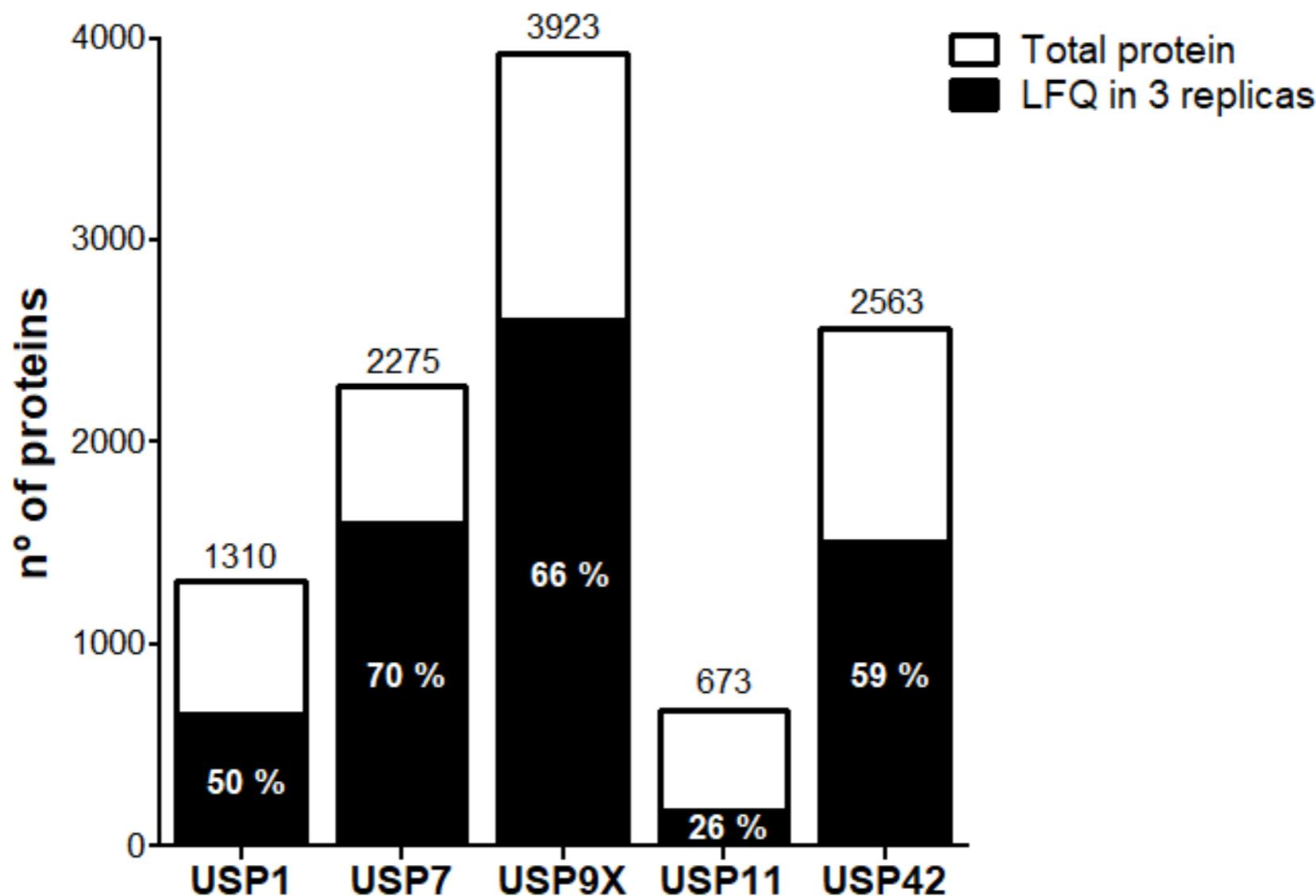
Ratio of Means



Box plot

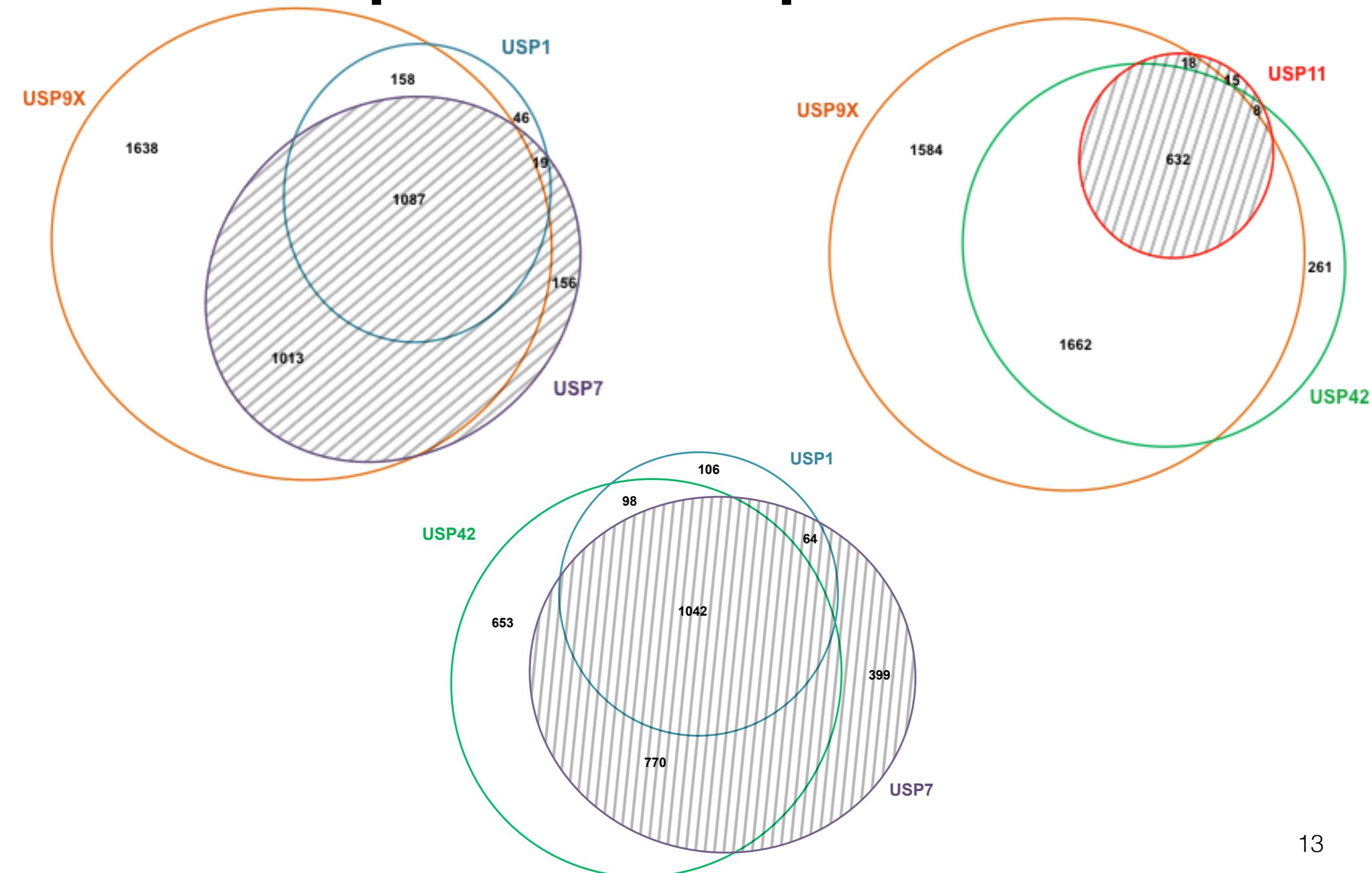


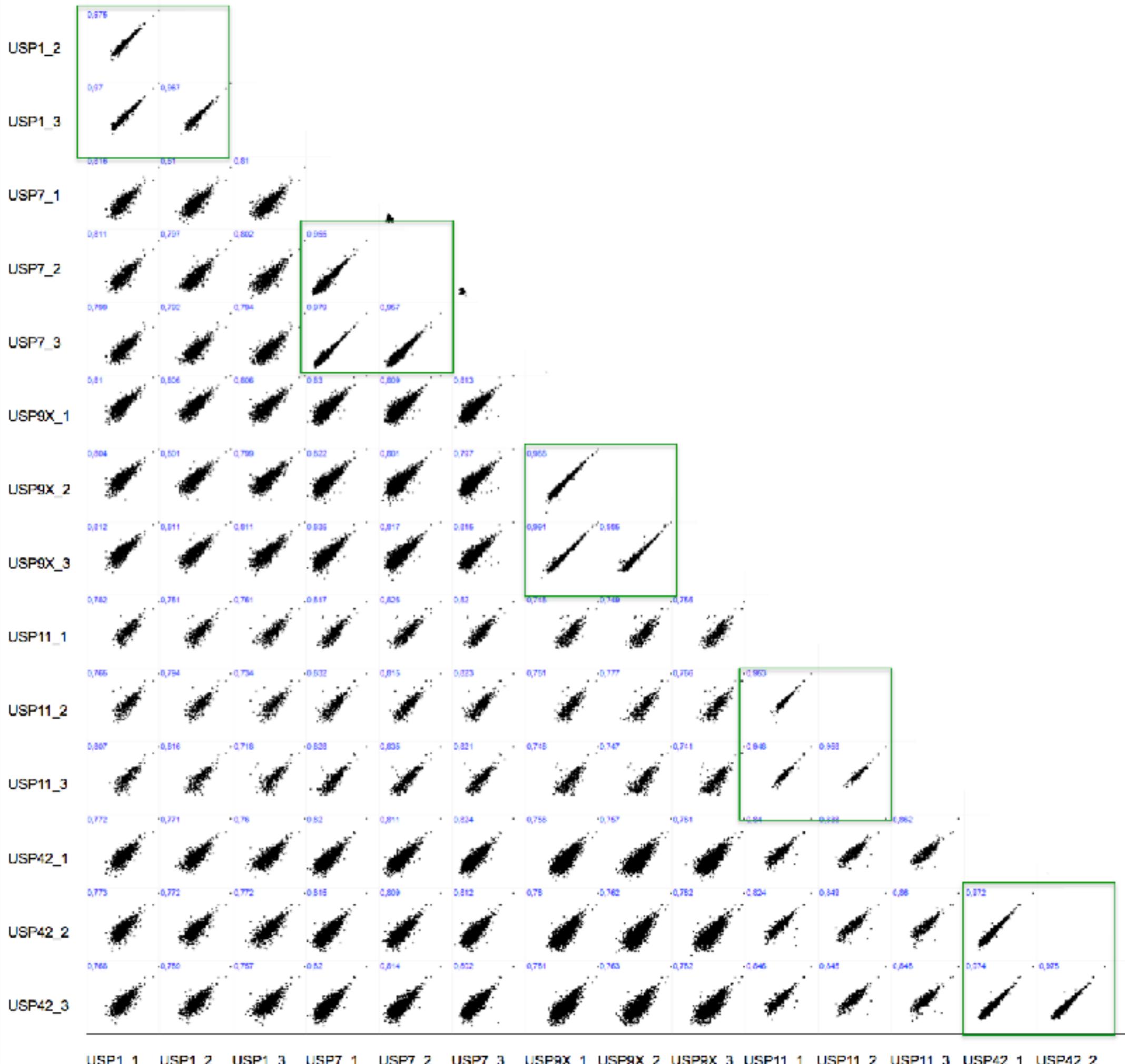
Landscape search depth: controls



At least 1 unique peptide, and LFQ value in at least one sample

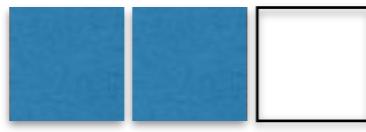
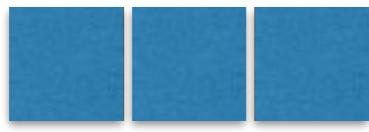
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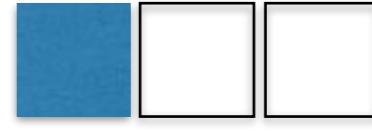
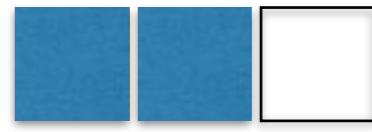
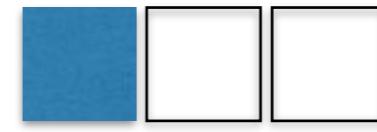
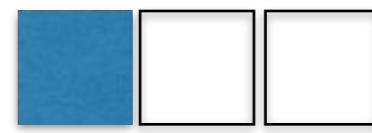
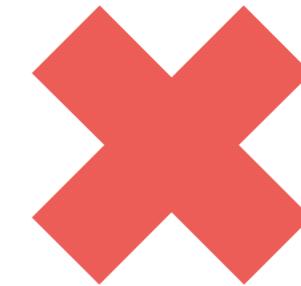


Stringent filters and Perseus analysis

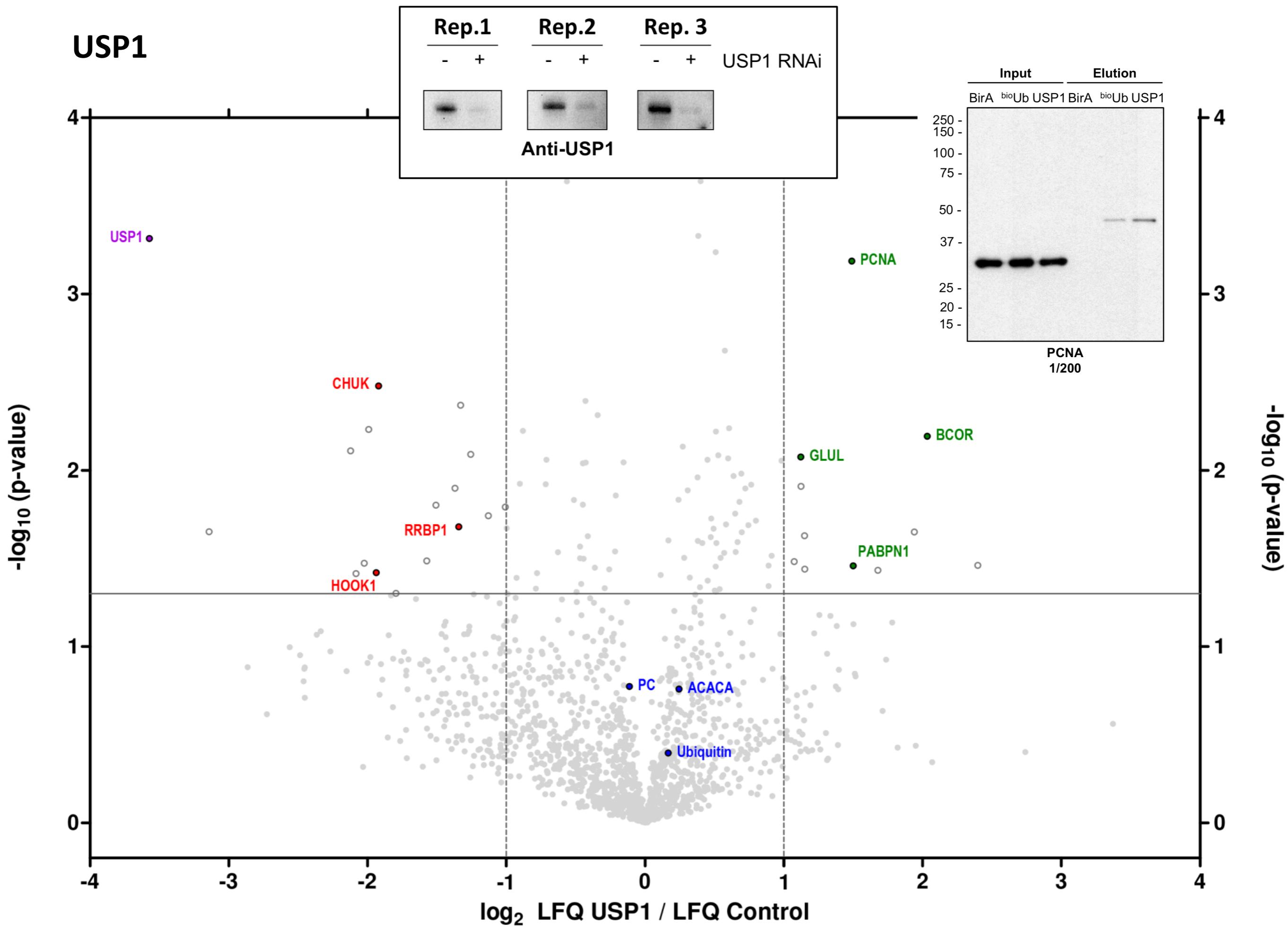
- **LFQ values**



- **Minimum 2 unique peptide**



USP1

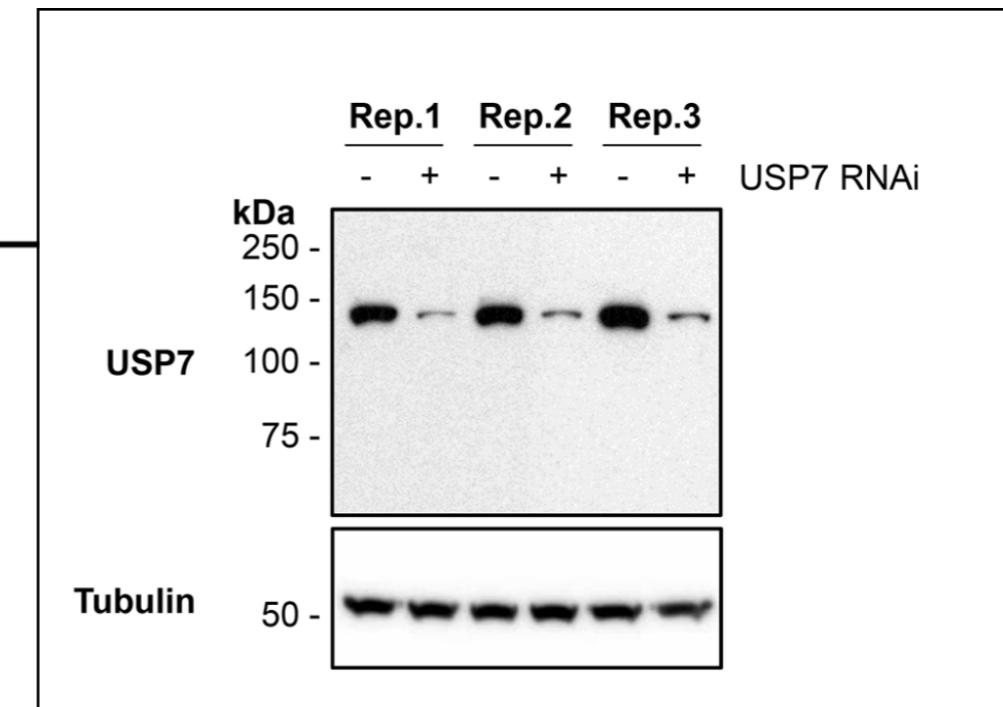
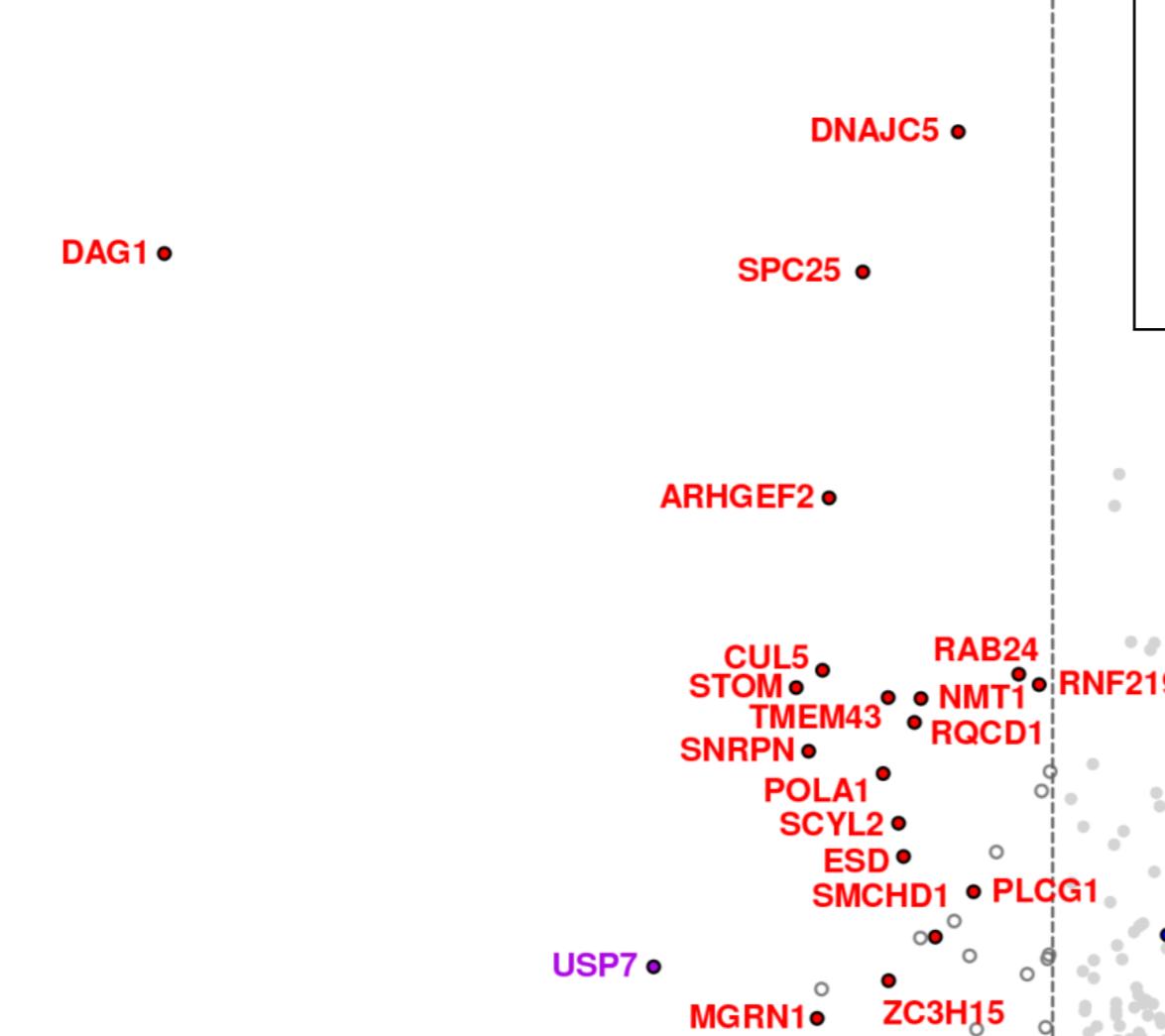


USP7

\log_{10} (p-value)

6
5
4
3
2
1
0

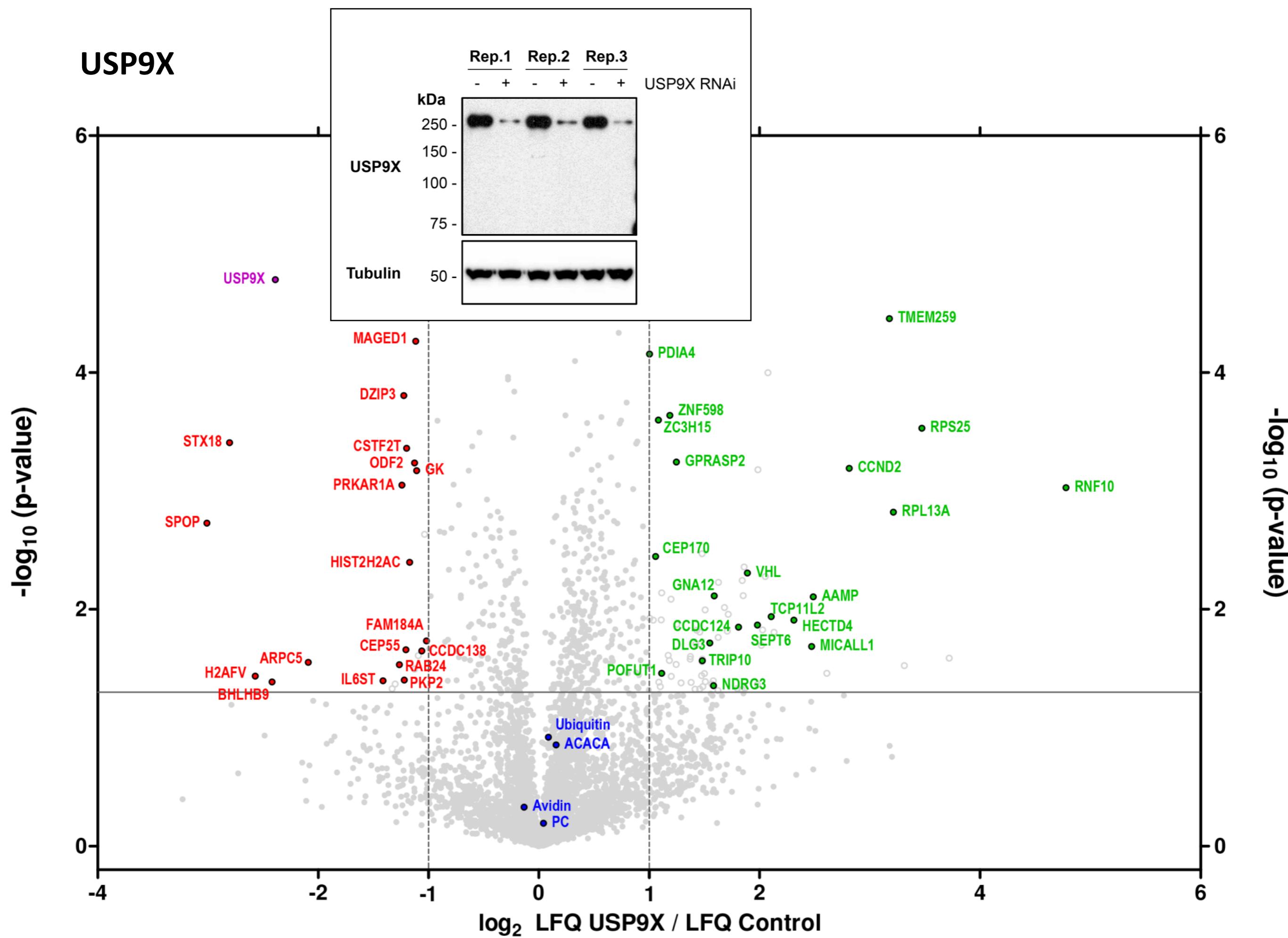
\log_2 LFQ USP7 / LFQ Control



\log_{10} (p-value)

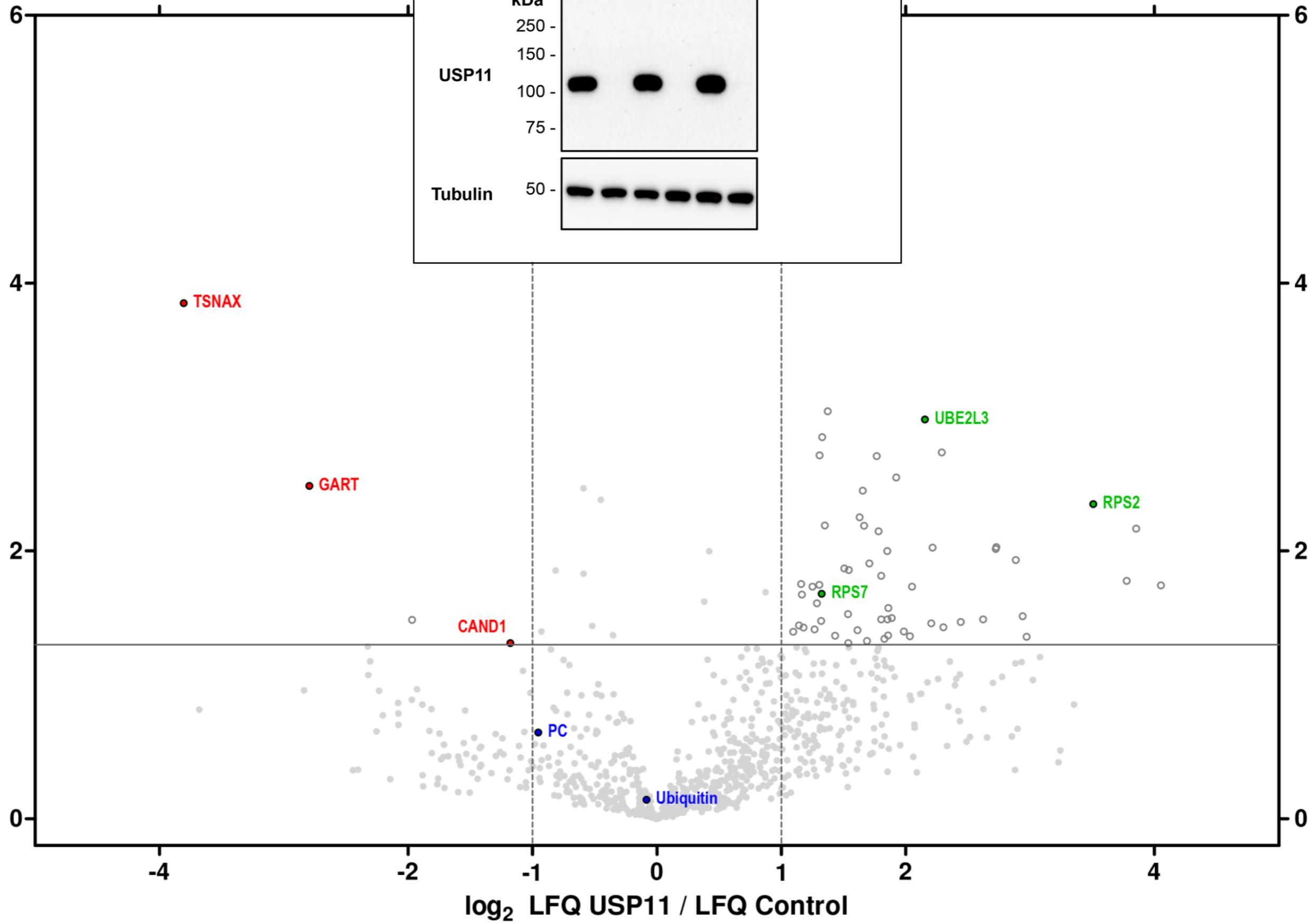
6
5
4
3
2
1
0

USP9X

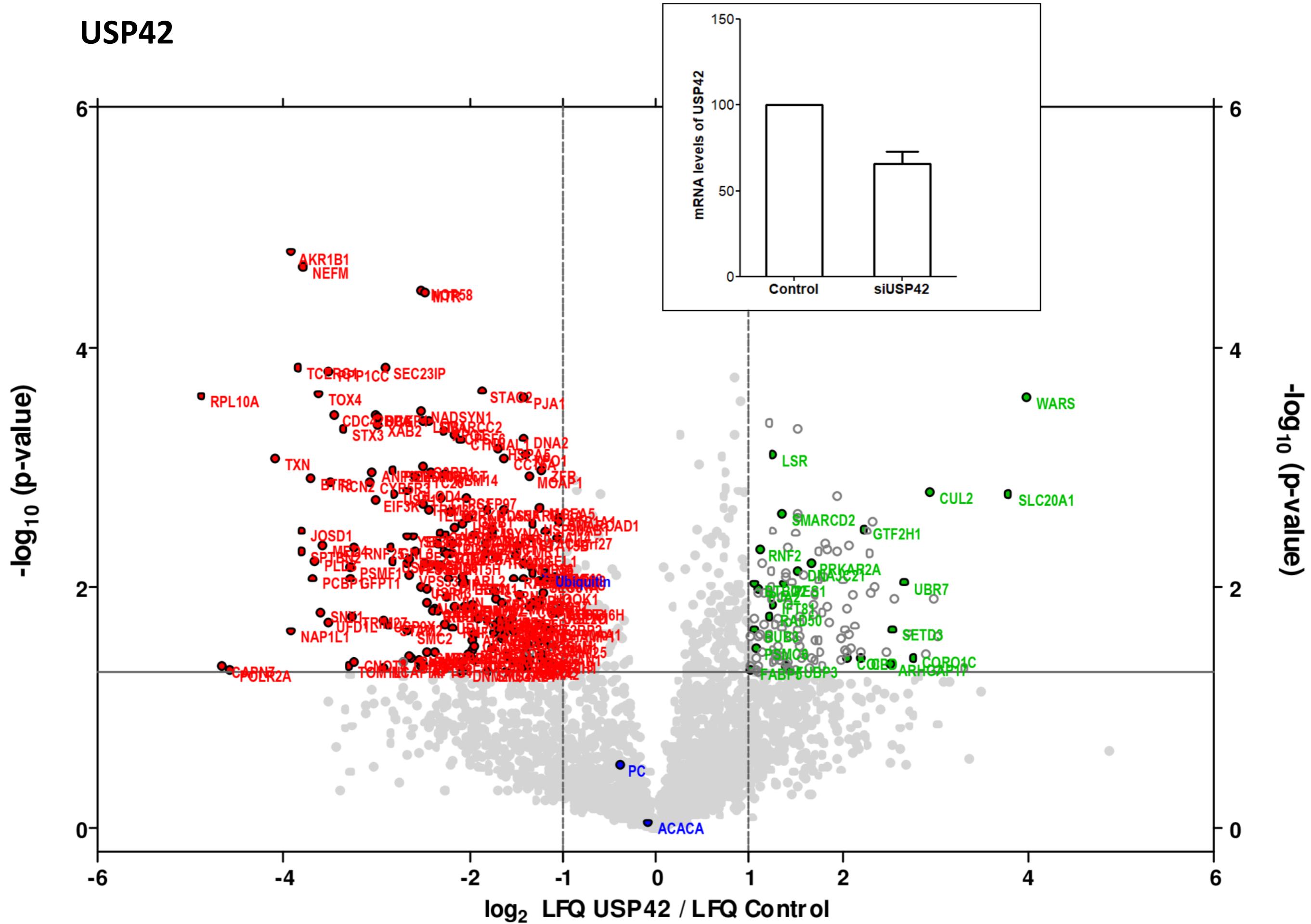


USP11

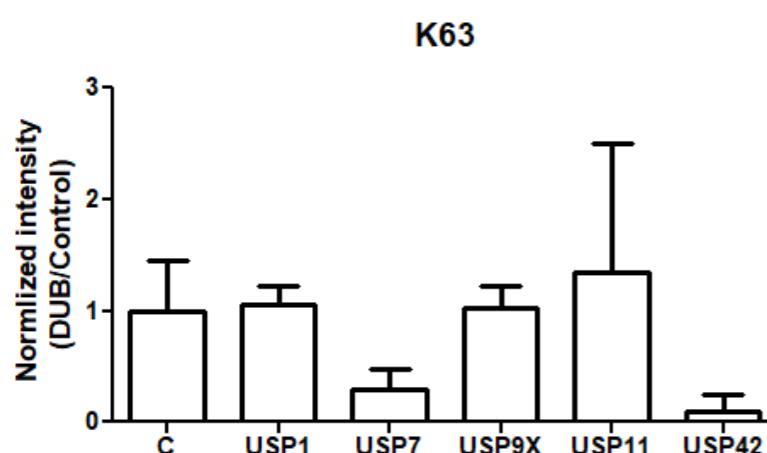
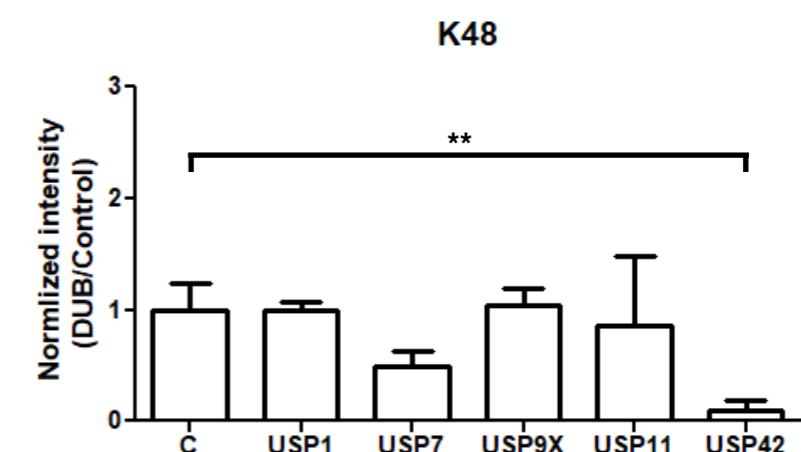
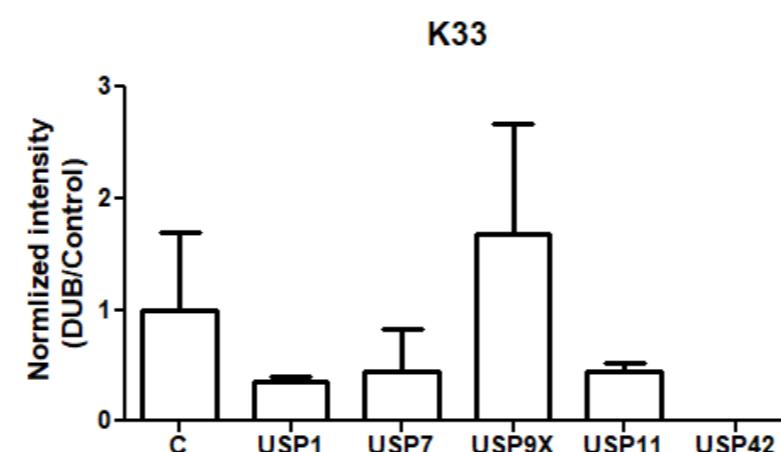
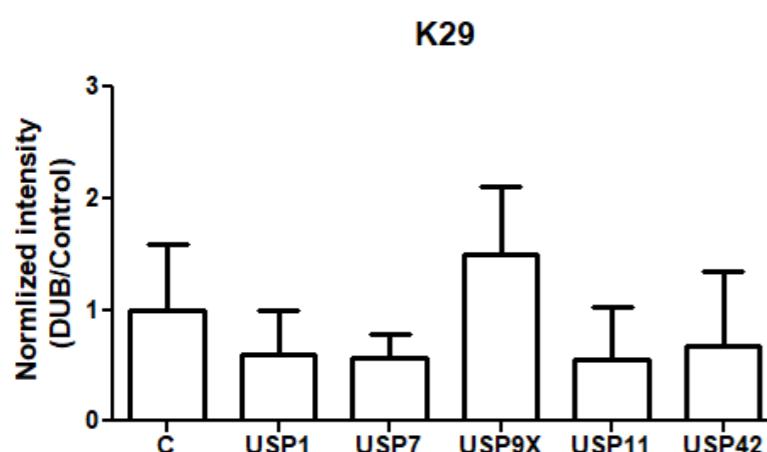
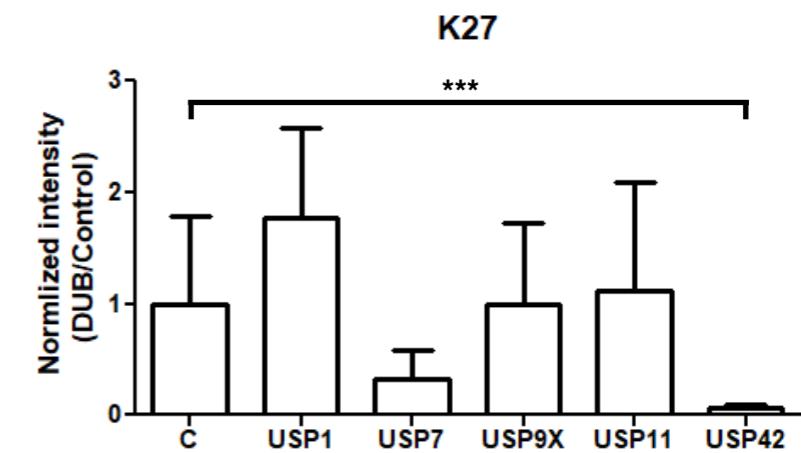
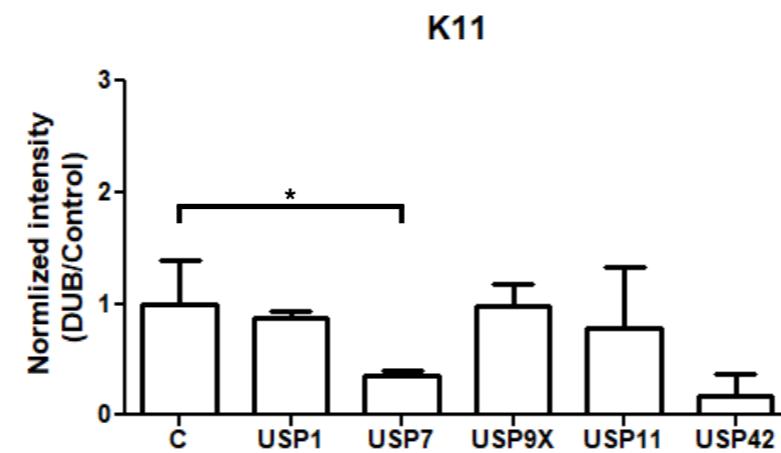
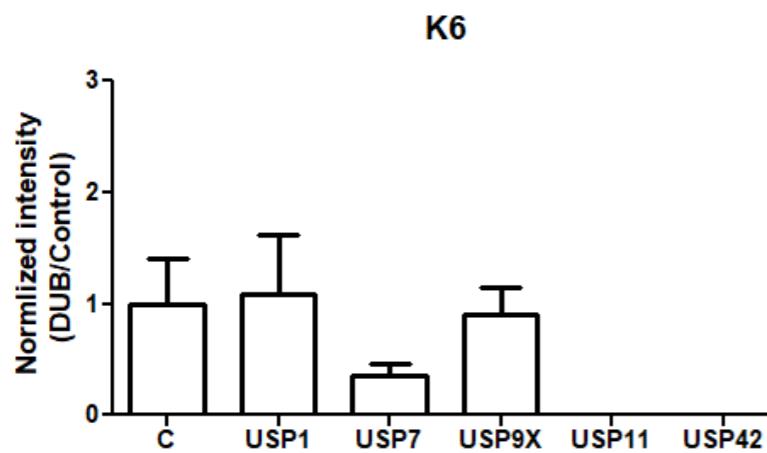
$-\log_{10}(\text{p-value})$



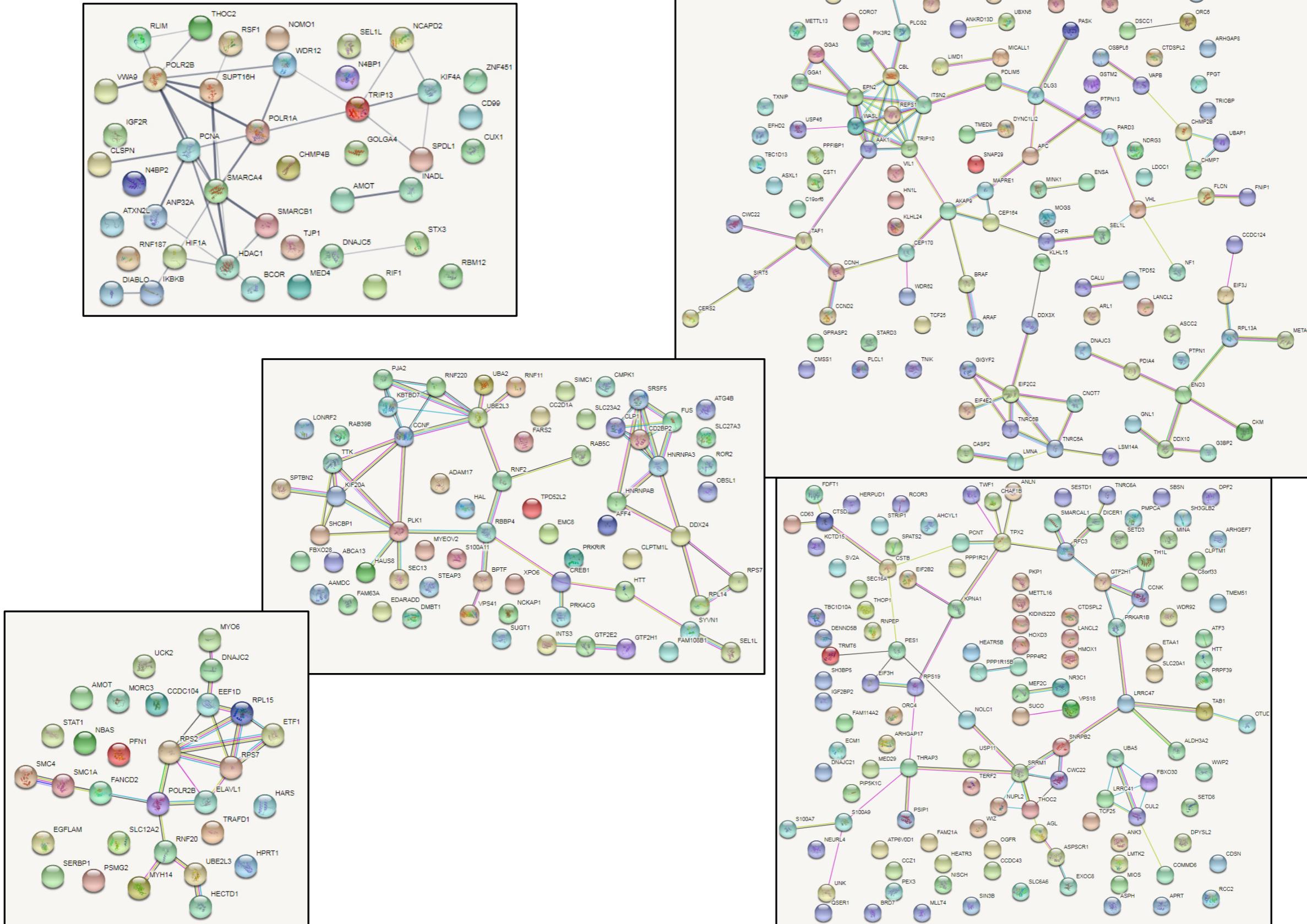
USP42



Ubiquitin chain analysis



Data analysis: ongoing



Thanks!



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