Protein abundances from mass spectrometer were quantified by MaxQuant 1.6.0.16 (ref: Cox, 2008) using 2-plex labeling (Arg10 and Lys8).

The search was run against human UP000005640 proteome (version GCA_000001405.26) from UniProt (ref: The UniProt Consortium, 2017).

Search parameters were set up to allow variable oxidation on methionines and acetylation on N-termini as well as fixed carbamidomethylation on cysteines. Tryptic peptides with up to 2 missed cleavages were considered for analysis and tolerance settings were set to Orbitrap instrument.

Co-fragmented peptide identification and matching between runs has been enabled. For protein quantification, minimum ratio count was set to 1 and remaining settings left default.

The normalised H/L ratios in proteinGroups.txt file output by MaxQuant have been transformed to log2 scale for further processing.

Each protein in the dataset containing Ankyrin repeat-containing domain has been identified using information from InterPro superfamily IPR036770 (ref:Finn, 2017).

Twenty six proteins that were marked as either "Potential contaminant", "Reverse" or "Only identified by site" by MaxQuant were dropped from the dataset (i.e. 1.81% of data). Further 76 proteins (5.39% of the remaining data), have been dropped because they had only forward, or reverse ratio, but not complete pair.

Out of the remaining data points we were left with 12 ANK-repeat containing proteins: BCORL1 (2 distinct protein groups), BCOR, RFXANK, MPHOSPH8, EHMT1, EHMT2, TONSL, ANKRD32, ANKRD11, BARD1 and NFKBIL1.

Four protein families were then annotated manually:

- (1) BRCA1 family BRCA1, FAM175A, BARD1, BRE, UIMC1, FAM175A, BRCC3, BABAM1;
- (2) RAD18/SLF family RAD18, ANKRD32, FAM178A;
- (3) TONSL family TONSL;
- (4) ORC family ORC1, ORC2, ORC3, ORC4, ORC5, LRWD1

Proteins were labelled their gene names, except in the following cases where we substituted the following names to their synonyms for visualisation purposes:

(1) BRCA1 members:

BRE: BRCC45, UIMC1 RAP80, FAM175A: ABRAXAS1 BRCC3: BRCC36 BABAM1: MERIT40

(2) RAD18/SLF members:

RAD18: RAD18/RNF73 ANKRD32: SLF1/ANKRD32 FAM178A: SLF2/FAM178A

(3) Histones:

HIST1H2AD;HIST1H2AJ;HIST1H2AH;H2AFJ;HIST1H2AG;HIST2H2AC;HIST2H2AA3;H2AFX: H2A1

HIST3H2A;HIST1H2AB;HIST1H2AC: H2A²

H2AFV; H2AFZ: H2A3

HIST1H3A; HIST2H3A; HIST3H3; HIST2H3PS2: H3

HIST1H4A: H4

HIST1H2BK;H2BFS: H2B1

HIST1H2BC: H2B²

This data was then plotted on a scatter plot, with log2 forward ratio on x, and reverse ratio on the y axis.

Labels for BRCA1, RAD18, TONSL, ORC groups were automatically drawn and colour-coded. Proteins containing ANK repeat domain were highlighted in bold, as well as with an asterisk near their label.

Additional proteins that have either reverse or forward ratio above the threshold of 1.4 were also labelled along with LSM7, PAX6, TLX3, H2A², DNMT1, CPSF7, TTN, INO80E, E2F6, and FIZ1 which were included to the plot manually, as either they clustered visually close to proteins of interest, or were significant outliers.

Two versions of the plot were generated.

In the first version, the axes are cropped to the log2 range (-4.5, 4.5), which excluded the significant outliers SPTA1 (x=5.0295, y=-0.0470), H4 (x=-7.7283, y=-5.0210), H3 (x=-6.7843, y=-6.8198) and STAG2 (x=3.5792, y=7.8352)

The second version (intended for supplementary reference), was generated without the cropping of the axes.

We also provide an .xlsx file with the processed dataset. Raw data files (both the output from mass spectrometer and MaxQuant) will be uploaded to PRIDE.

References

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