

**Title:** Lysine acetylation stoichiometry and proteomics analyses reveal pathways regulated by sirtuin 1 in human cells.

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**Supplemental tables:**

**Table S1.** List of proteins and peptides identified acetylated in the cell lines HaCaT, CaLo and SiHa. The degree of acetylation occupancy was determined for the number of acetylation sites in the peptides. Coefficient of variation (CV%) and relative errors (RE%) for those acetylated peptides identified/quantified using the three sample preparation methods.

**Table S2.** Pathway enrichment analyses performed with the online version of DAVID Bioinformatics software of the acetylated proteins identified in the three cell lines under study. Data are presented for each cell lines in different sheets.

**Table S3.** List of proteins identified with at least two razor-unique peptides by MS/MS that were used for label-free quantification of the cell lines HaCaT, CaLo and SiHa. The ratios of LFQ intensities between cells is provided.

**Table S4.** List of proteins and peptides that were found with less acetylation occupancy in SiHa cells in comparison with HaCaT and CaLo cells.

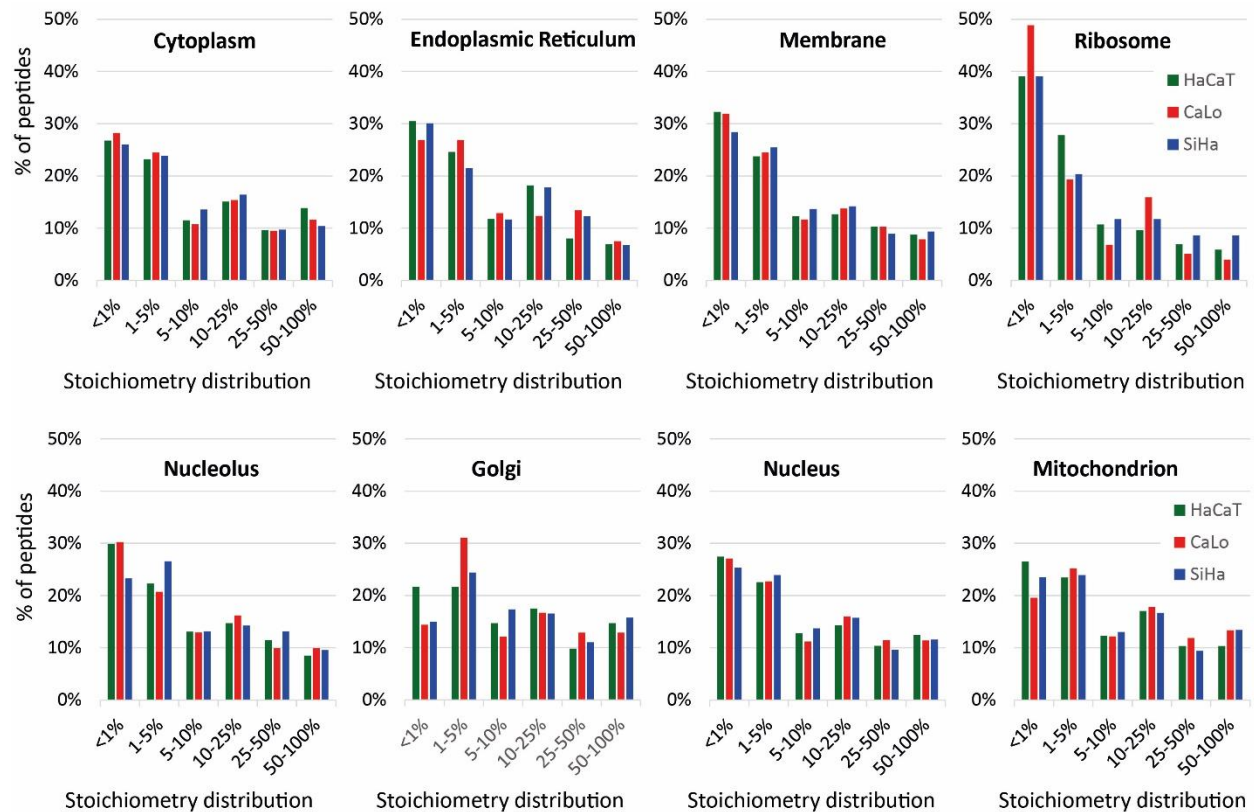
**Table S5.** List of proteins and peptides that their acetylation stoichiometry was calculated (included those with no endogenous acetylation detected), for each of the six experimental conditions, the three cell lines (HaCaT, CaLo and SiHa) untreated and treated with the SIRT1 inhibitor EX-527. Data are presented for each condition in different sheets.

**Table S6.** List of proteins and peptides that increased their acetylation occupancy in the three cell lines (HaCaT, CaLo and SiHa) after treatment with the SIRT1 inhibitor EX-527. The degree of acetylation for each condition was expressed in percentage of occupancy of at least one acetylation site.

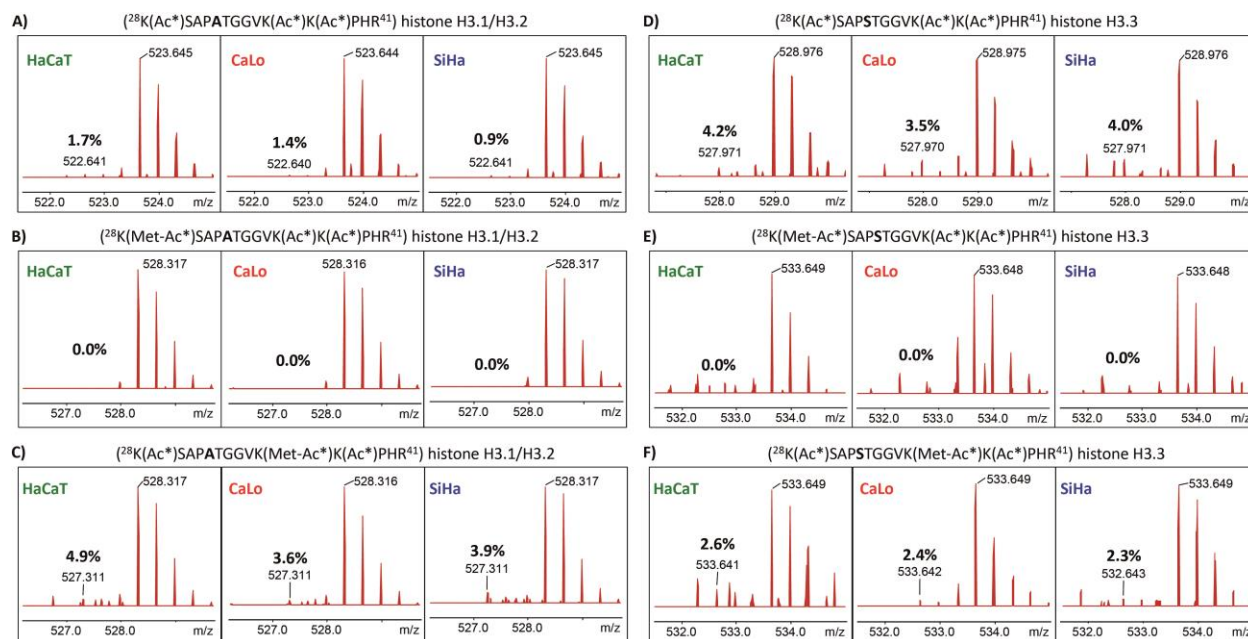
**Table S7.** List of peptides and proteins for each cell line that increased their acetylation occupancy by at least 5% after 24h treatment with the SIRT1 inhibitor EX-527. The LFQ intensities for each protein as well as the ratios of LFQ intensities between cells is provided. Data are presented for each cell lines in different sheets.

**Table S8.** List of proteins identified with at least two razor-unique peptides by MS/MS that were used for label-free quantification of the cell lines HaCaT, CaLo and SiHa, untreated and treated with the SIRT1 inhibitor EX-527. The ratio of LFQ intensities between conditions is provided.

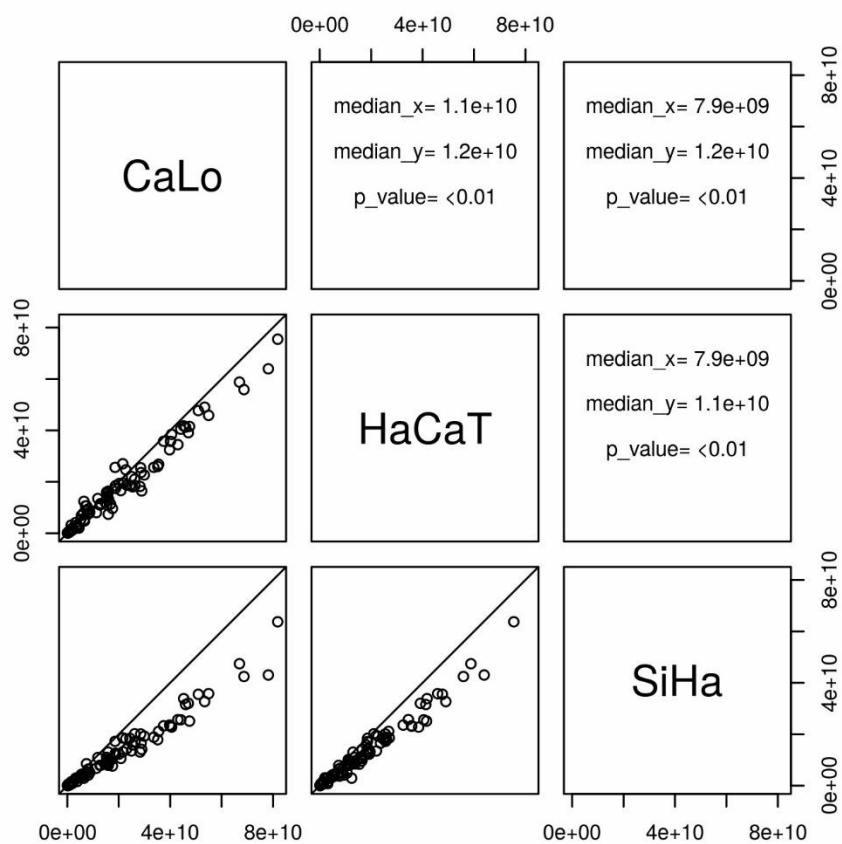
**Supplemental figures:**



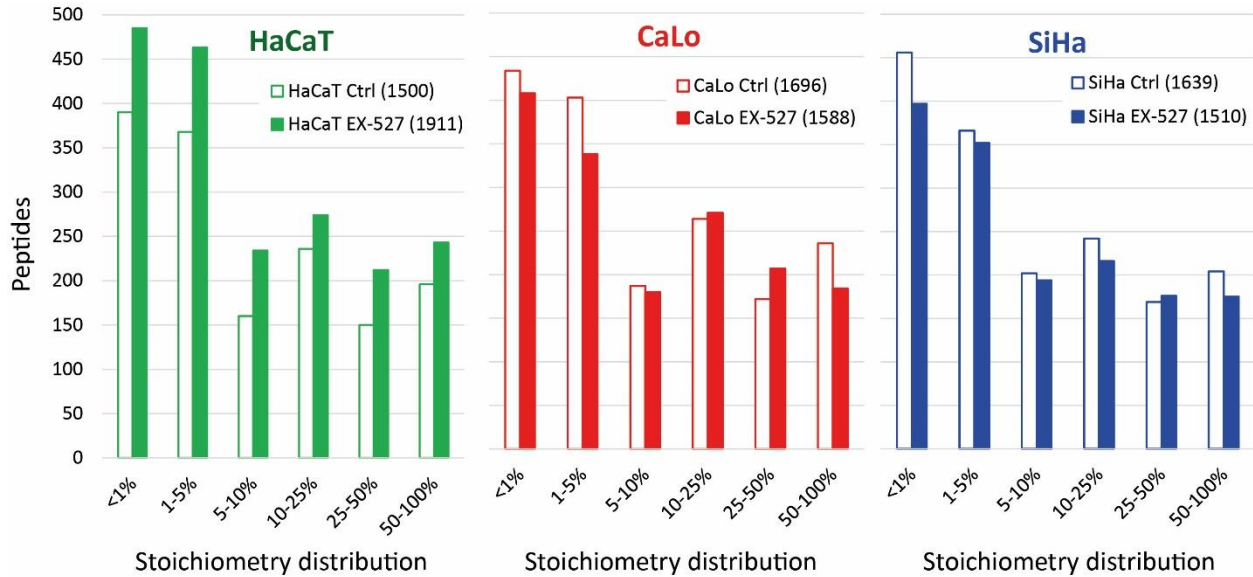
**Figure S1.** Distribution of peptides according to their acetylation stoichiometry. Each graph correspond to peptides from proteins of the represented cellular component or structure. All acetylated peptides were scattered in six quantiles corresponding to their acetylation occupancy, <1% corresponded to peptides with less than 1% of acetylation occupancy, 1-5%, 5-10%, 10-25% and 25-50% corresponded to peptide with a acetylation stoichiometry between the two values and 50-100% corresponded to peptides with acetylation occupancy from 50% to fully acetylated.



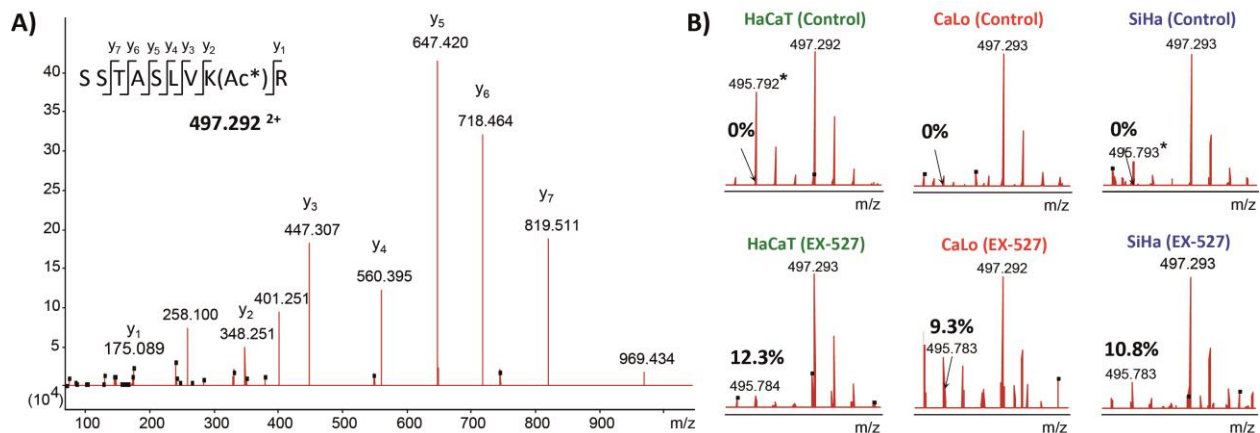
**Figure S2.** Survey scans showing the isotopic distributions of the peptide  $^{28}\text{KSAPATGGVKKPHR}^{41}$  from histone H3.1/H3.2 and the peptide  $^{28}\text{KSAPSTGGVKKPHR}^{41}$  from histone H3.3. A) Peptide from histone H3.1/H3.2 fully acetylated in the three cell lines, the values correspond to the degree of endogenous acetylation. B) and C) correspond to the peptide from histone H3.1/H3.2 with the residues K28 and K37 mono-methylated respectively. D) Peptide from histone H3.3 with their three lysine residues fully acetylated. The values correspond to the percentage of endogenous acetylation. E) and F) correspond to the peptide from histone H3.3 with the residues K28 and K37 mono-methylated respectively. All sequences were confirmed by MS/MS analysis.



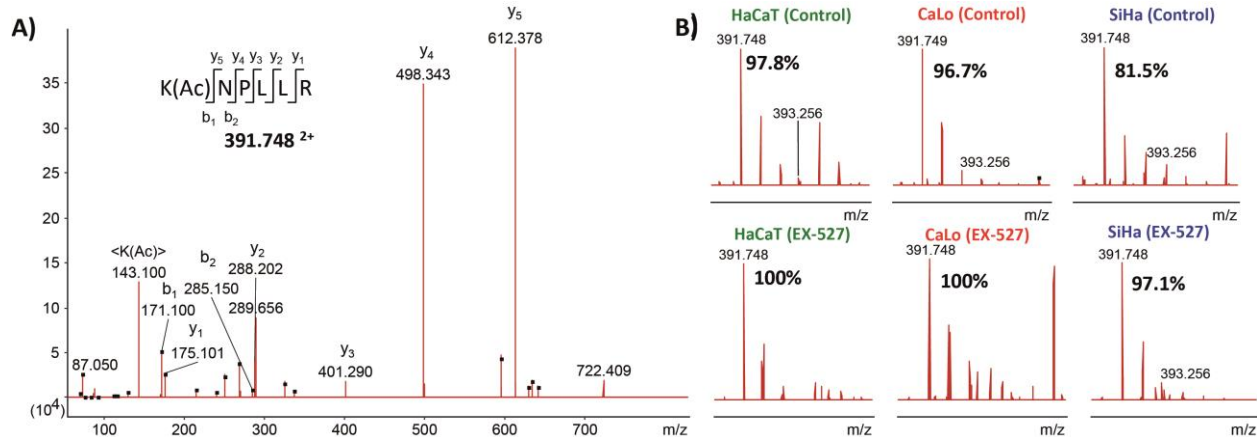
**Figure S3.** Statistical analysis to determine if the differences between the medians of the abundances of ribosomal proteins are significantly different between the three cell lines (HacaT, CaLo and SiHa). Each cycle correspond to the plot of the LFQ intensity of a ribosomal protein in two cells. The analysis determined with a p value <0.01 that the medians are different between the three cell lines.



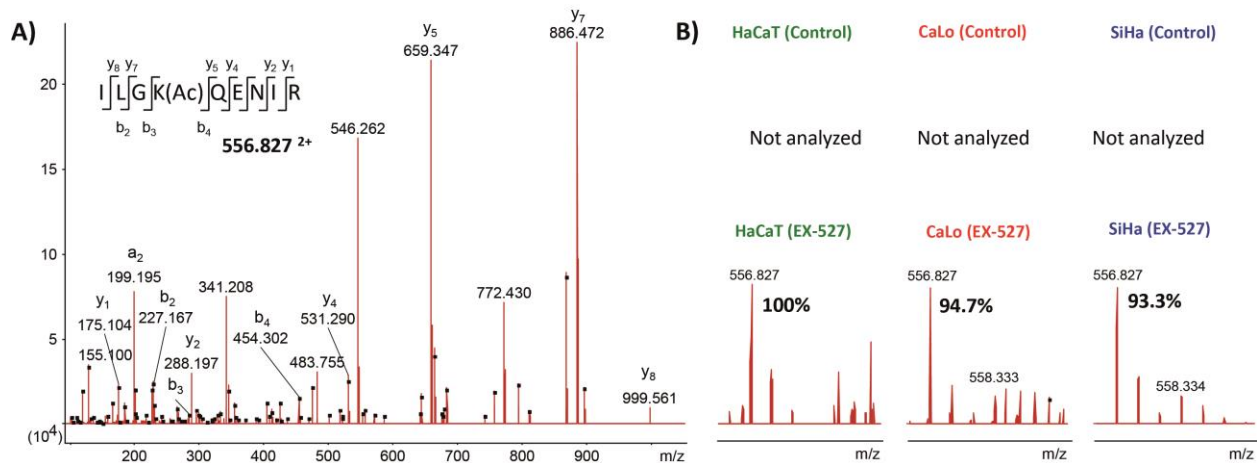
**Figure S4.** Distribution of peptides according to their acetylation stoichiometry. Each graph correspond to peptides from proteins of the represented cell line, filled bars correspond to the distribution of acetylation stoichiometry in cells treated with the SIRT1 inhibitor EX-527. All acetylated peptides were scattered in six quantiles corresponding to their acetylation occupancy, <1% corresponded to peptides with less than 1% of acetylation occupancy, 1-5%, 5-10%, 10-25% and 25-50% corresponded to peptide with a acetylation stoichiometry between the two values and 50-100% corresponded to peptides with acetylation occupancy from 50% to fully acetylated.



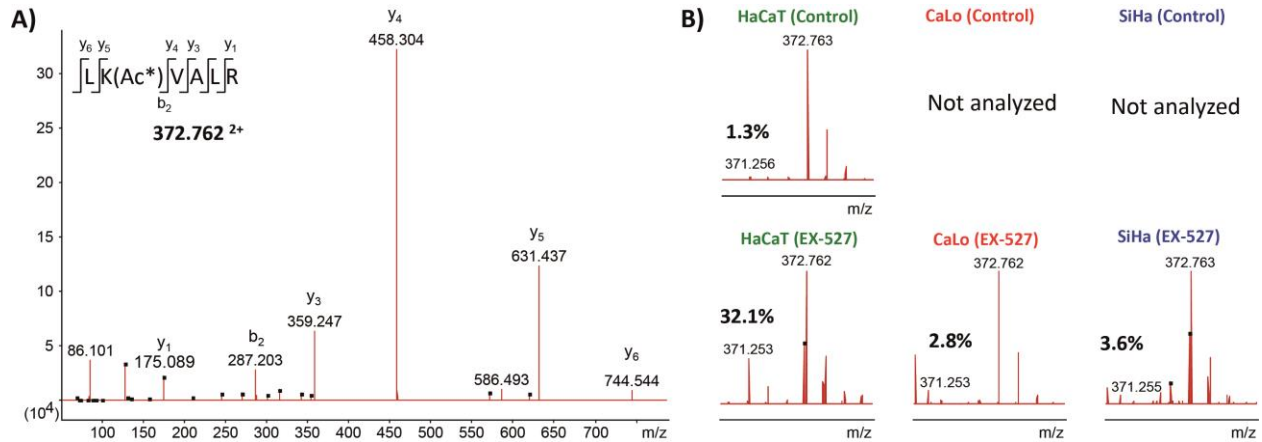
**Figure S5.** MS/MS and MS spectra of the peptide  $^{560}\text{SSTASLVKR}^{568}$  from WD repeat-containing protein 46 (WDR46), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac\*) represents the heavy acetyl group incorporated during acetylation reaction. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in K567 calculated with Pview software for each experimental condition.



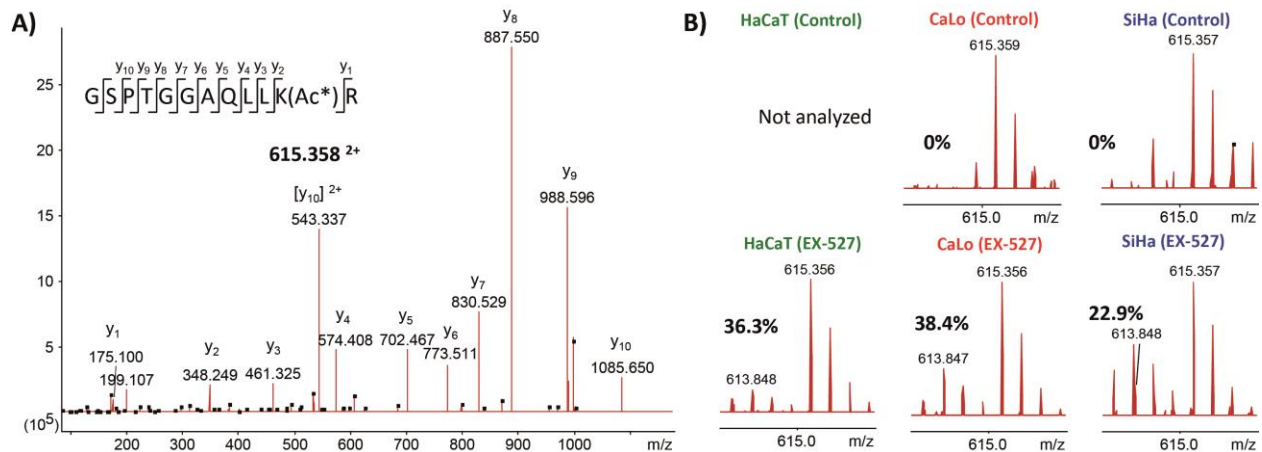
**Figure S6.** MS/MS and MS spectra of the peptide  $^{173}\text{KNPLLR}^{178}$  from Histone deacetylase 7 (HDAC7), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac) represents the endogenous acetyl group. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in K173 calculated with Pview software for each experimental condition.



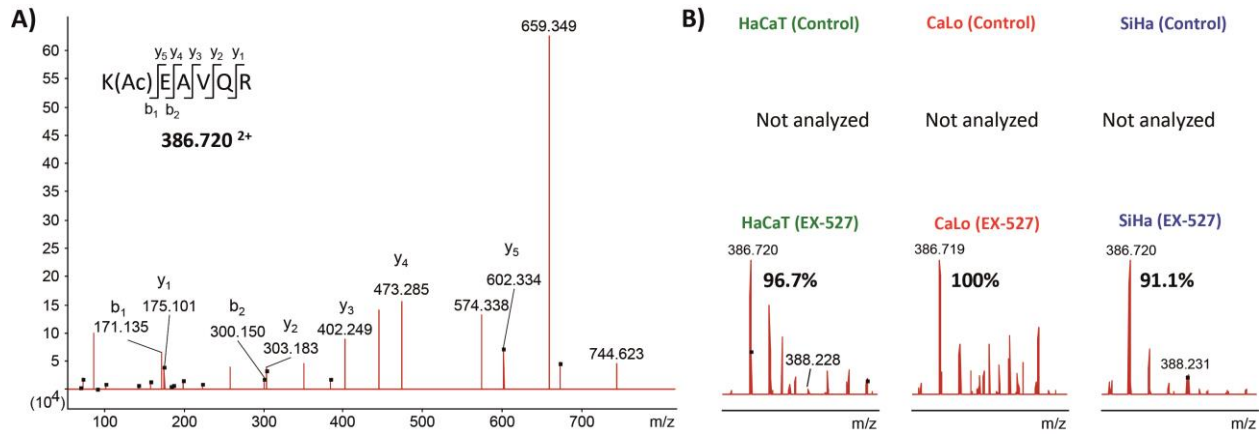
**Figure S7.** MS/MS and MS spectra of the peptide  $^{391}\text{ILGKQENIR}^{399}$  from Peptidylprolyl isomerase domain and WD repeat-containing protein 1 (PPWD1), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac) represents the endogenous acetyl group. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in K394 calculated with Pview software for each experimental condition. In untreated cells no signal corresponding to this peptide was selected for MS/MS analysis.



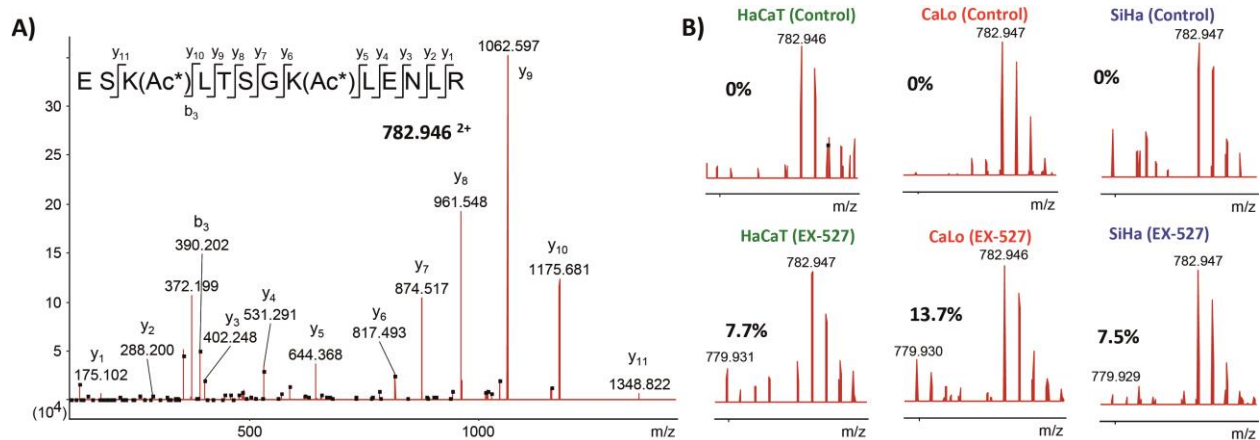
**Figure S8.** MS/MS and MS spectra of the peptide <sup>370</sup>LKVALR<sup>375</sup> from E3 ubiquitin-protein ligase BRE1B (RNF40), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac\*) represents the heavy acetyl group incorporated during acetylation reaction. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in K371 calculated with Pview software for each experimental condition. In untreated CaLo and SiHa cells no signal corresponding to this peptide was selected for MS/MS analysis.



**Figure S9.** MS/MS and MS spectra of the peptide <sup>512</sup>GSPTGGAQLLKR<sup>523</sup> from Ribosomal RNA processing protein 1 homolog B (RRP1B), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac\*) represents the heavy acetyl group incorporated during acetylation reaction. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in K522 calculated with Pview software for each experimental condition. In untreated HaCaT cells no signal corresponding to this peptide was selected for MS/MS analysis.

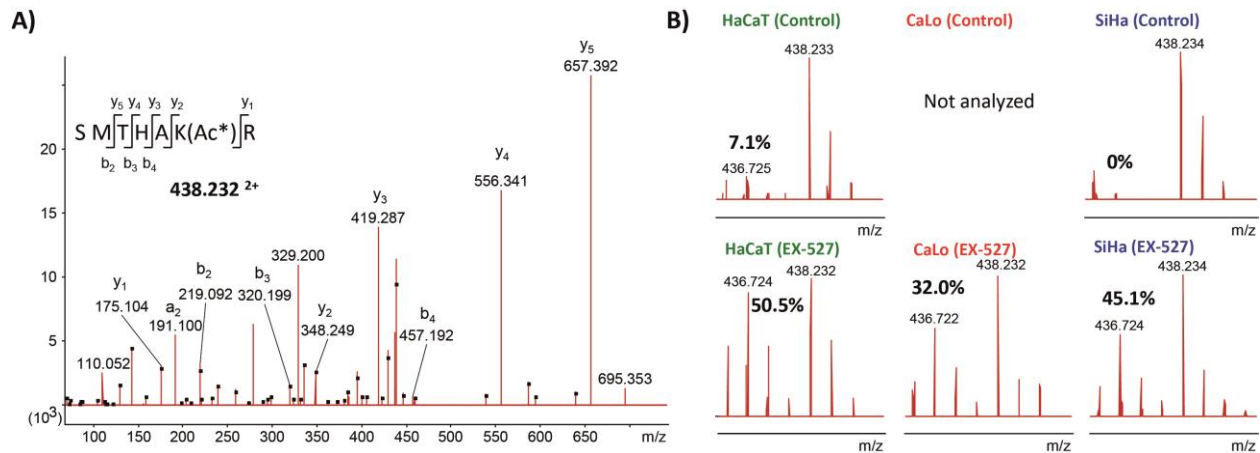


**Figure S10.** MS/MS and MS spectra of the peptide  $^{123}\text{KEAVQR}^{128}$  from Nuclear receptor subfamily 2 group F member 6 (NR2F6), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac) represents the endogenous acetyl group. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in K123 calculated with Pview software for each experimental condition. In untreated cells no signal corresponding to this peptide was selected for MS/MS analysis.

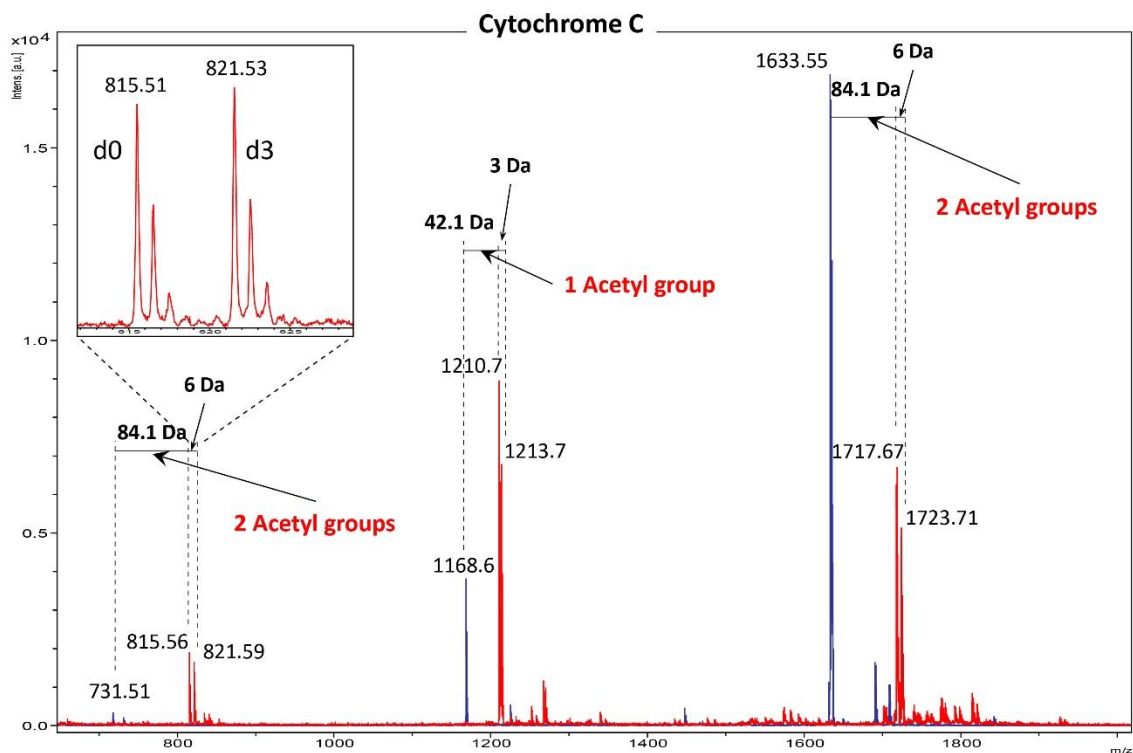


**Figure S11.** MS/MS and MS spectra of the peptide  $^{124}\text{ESKLTSGKLENLR}^{136}$  from Probable ATP-dependent RNA helicase DDX52 (DDX52), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac\*) represents the heavy acetyl group incorporated during acetylation reaction. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in both K residues (K126 and K131) calculated with Pview software for each experimental condition.





**Figure S12.** MS/MS and MS spectra of the peptide  $^{33}\text{SMTHAKR}^{39}$  from Anillin (ANLN), identified in the three cell lines under study. A) Representative MS/MS spectrum of the peptide. (Ac\*) represents the heavy acetyl group incorporated during acetylation reaction. B) Survey scans showing the isotopic distributions of the peptide chemically acetylated with NAS-d3 and with endogenous acetylation. The values reported correspond to the degree of endogenous acetylation in K38 calculated with Pview software for each experimental condition. In untreated CaLo cells no signal corresponding to this peptide was selected for MS/MS analysis.



**Figure S13.** MALDI-MS spectra from tryptic peptides from the model protein cytochrome C. Blue spectrum correspond to unmodified peptides, red spectrum correspond to a mixture of peptides with lysine residues chemically modified with NAS-d0 or NAS-d3. The zoomed spectrum correspond to a mixture 1:1 of acetylated peptides with NAS-d0 or NAS-d3.